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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
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Solicitud de Patente a nombre de **Novartis AG**

Yo, JIMENA ESCOBAR URIBE, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 No. 86-53 Piso 6, obrando como apoderada de la sociedad **Novartis AG**, domiciliada en Basilea, Suiza, solicitante de la patente de la referencia, en respuesta a su oficio No. 7029 notificado por fijación en lista el día 30 de mayo de 2008, me permito acompañar los siguientes documentos solicitados por ese despacho:

1. Y. KITA, ET AL.: "Novel and direct nucleophilic sulphenylation and thiocyanation of phenol ethers using a hypervalent iodine (III) reagent" JOURNAL OF ORGANIC CHEMISTRY, Vol. 60, No. 22, 3 Noviembre 1995 (1995-11-03), páginas 7144-7148, American Chemical Society, Washington, DC.
2. H.E. ZIEGER, ET AL.: "2-Chloro-3-dimethylamino-6-phenylsulphon- yl phenyllithium" JOURNAL OF ORGANIC CHEMISTRY, Vol. 27, No. 9, Septiembre 1962 (1962-09), páginas 3270-3273, American Chemical Society, Washington, DC.
3. G. SCHROETER: "Über Acylierung von Anilin-sulfosäuren" BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT, Vol. 39, 1906, páginas 1559-1570, Verlag Chemie, Weinheim, DE.

4. R. HUISIGEN, ET AL.: "Zur ThermoLyse des 2-Azido-4,6-dimethylpyrimidins; Reaktionen des Azens" TETRAHEDRON LETTERS, No. 30, Junio 1969 (1969-06), páginas 2595-2598, Elsevier Science Publishers, Amsterdam.
5. F. KURZER: "The conversion of arylamines into 'symmetrical diarylureas. The melting points of certain substituted ureas" JOURNAL OF THE CHEMICAL SOCIETY, 1949, páginas 2292-2295, Royal Society of Chemistry, Letchworth, G,B .
6. R. NEIDLEIN, ET AL.: "Heterocyclische 12-pi- und 14-pi-systeme, 34. Untersuchungen zum Reaktionsverhalten von 2-Phenyl-5H-naphtho[1,8-bc]furan-2-on (Oxapseudophenalenon)" LIEBIGS ANNALEN DER CHEMIE, No. 7, Julio 1979 (1979-07-30), páginas 959-964, Verlag Chemie, Weinheim, DE .
7. T. NAKAYMA, ET AL.: "Synthesis and structure-activity study of protease inhibitors. II. Amino- and guanidino-substituted naphthoates and tetrahydronaphthoates" CHEMICAL AND PHARMACEUTICAL BULLETIN, Vol. 32, No. 10, Octubre 1984 (1984-10), páginas 3968-3980, Pharmaceutical Society of Japan, Tokyo, JP .
8. G.O. DOAK, ET AL.: "Arsene oxides of naphthalene and biphenyl" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, Vol. 64, No. 5, Mayo 1942 (1942-05), páginas 1064-1066, American Chemical Society, Washington, DC.
9. W.A. DENNY, ET AL.: "Potential antitumour agents. 29. Quantitative structure-activity relationships for the antileukaemic bisquaternary ammonium heterocycles" JOURNAL OF MEDICINAL CHEMISTRY, Vol. 22, No. 2, Febrero 1979 (1979-02), páginas 134-150, American Chemical Society, Washington, DC.
10. W.F. MICHNE, ET AL.: "Novel inhibitors of potassium ion channels on human T lymphocytes" JOURNAL OF MEDICINAL CHEMISTRY, Vol. 11, No. 38, Mayo 26, 1995 (1995-0526), páginas 1877-1883, American Chemical Society, Washington, DC.

11. GANGJEE, ET AL.: "2,4-Diamino-5-deaza-6-substituted pyrido[2,3-d]pyrimidine antifolates as potent and selective nonclassical inhibitors of dihydrofolate reductases" JOURNAL OF MEDICINAL CHEMISTRY, Vol. 39, No. 7, Marzo 29 1996 (1996-03-29), páginas 1438-1446, American Chemical Society, Washington, DC.
12. GANGJEE, ET AL.: "6-Substituted 2,4-dia_mino-5-methylpyrido[2,3-d]pyrimidin es as inhibitors of dihydrofolate reductases from Pneumocystis carinii and Toxoplasma gondii and as antitumour agents" JOURNAL OF MEDICINAL CHEMISTRY, Vol. 38, No. 10, Mayo 12, 1995 (1995-05-12), páginas 1778-1785, American Chemical Society, Washington, DC.
13. D. TANIYAMA, ET AL.: "A facile and efficient asymmetric synthesis of (+)-salsolidine" TETRAHEDRON LETTERS, Vol. 41, No. 29, Julio 15, 2000 (2000-07-15), páginas 5533-5536, Elsevier Science Publishers, Amsterdam.
14. E. NOELTING, ET AL.: "Über Farbstoffe der Naphtyl-diphenyl-methan-, Dinaphtyldiphenyl-methan- und Trinaphtyl-methan-Reihen" BERICHTE DER DEUTSCHEN CHEMISCHEN GESELLSCHAFT, Vol. 37, 1904, páginas 1899-1920, Verlag Chemie, Weinheim, DE.
15. V.A. CHAUZOV: "Stereospecific transition from phenyl(1-naphthyl)phosphinothioic acid to S-ethyl phenyl(1-naphthyl)phosphinothioate, methyl phenyl(1-naphthyl)phosphinate, and N,N-diethyl-P-phenyl-P-(1-naphthyl)phosphi nic amide" JOURNAL OF GENERAL CHEMISTRY OF THE USSR, Vol. 51, No. 8, pt. 2, Agosto 1981 (1981-08), páginas 1650, Consultants Bureau, New York, US.
16. K.H. GRELLMANN, ET AL.: "Reactivity and decay pathways of photoexcited anilinonaphthalenes" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, Vol. 104, No. 23, 17 Noviembre 1982 (1982-11-17), páginas 6267-6272, American Chemical Society, Washington, DC, US.
17. C.J. CAVALLITO, ET AL.: "Choline acetyltransferase inhibitors. Styrylpyridine analogues with nitrogen-atom modifications" JOURNAL OF

MEDICINAL CHEMISTRY, Vol. 14, No. 2, Febrero 1971 (1971-02),
páginas 130-133, American Chemical Society, Washington, DC, US.

Espero con esto haber dado cabal cumplimiento a lo solicitado por ese despacho y de conformidad con lo indicado en los artículos 3°, inciso 3, 35 inciso 2 y 59 inciso 2 del CCA, al analizar este memorial, atentamente solicito se traten todas las cuestiones planteadas con motivo del mismo.

Señor Superintendente,


JIMENA ESCOBAR URIBE
C.C. 39.779.452 de Usaqué
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Novel and Direct Nucleophilic Sulfonylation and Thiocyanation of Phenol Ethers Using a Hypervalent Iodine(III) Reagent

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Novel and direct nucleophilic sulfonylation and thiocyanation of phenol ethers and related compounds (1) with the hypervalent iodine reagent, phenyliodine(III) bis(trifluoroacetate) (PIFA) have been developed. The reaction proceeded smoothly in 1,1,1,3,3,3-hexafluoro-2-propanol ((CF₃)₂CHOH), a poorly nucleophilic and polar solvent. Various unsymmetrical diaryl sulfides and aryl thiocyanates, which could be applicable to the synthesis of various types of sulfur-containing aromatic compounds, were prepared in good yields. These reactions could be performed under mild conditions without heating or the use of strong Lewis acid catalysts.

Introduction

The direct sulfonylation of aryl compounds is an important subject in synthetic organic chemistry.¹ Due to the useful transformations of the thiocyanato group into various sulfur functional groups² and sulfur-containing heterocycles,³ the direct thiocyanation of aryl compounds is also of prime importance. Several types of sulfonylation methods for aryl compounds have been reported involving (i) nucleophilic substitution of an aryl halide with aromatic thiols catalyzed by nickel(0) and nickel(II) complexes,⁴ (ii) Friedel-Crafts reaction using sulfonyl dihalides, arylsulfonyl halides, or diaryl disulfide catalyzed by iron or Lewis acids⁵ or under heating,^{6,7} and (iii) a modified Ziegler reaction using diazonium salts and aryl thiols.⁸ Most of these reactions, however, require high temperature, metals, or strong Lewis acid catalysts and long reaction times. Thiocyanation methods for aromatic compounds have also been reported, involving the reaction between aromatic compounds and thiocyanogen [(SCN)₂].^{3a} Thiocyanogen is, however, not active enough for direct thiocyanation of phenol ethers and usually needs to be activated by a Lewis acid.

In our continuing efforts to develop new methods for the direct substitution of para-substituted phenol ethers,⁹ we have reported a novel and direct aromatic azidation of para-substituted phenol ethers using phenyliodine(III)

bis(trifluoroacetate) (PIFA) in 1,1,1,3,3,3-hexafluoro-2-propanol ((CF₃)₂CHOH) followed by treatment with trimethylsilyl azide (TMSN₃)^{9a} and applied it to the direct aromatic substitution of phenol ethers by oxygen and carbon nucleophiles.^{9b} We have now examined the possibility of direct substitution of phenol ethers and related compounds by sulfur nucleophiles such as aryl thiols (ArSH) and trimethylsilyl isothiocyanate (TMSNCS).

Results and Discussion

At first, we examined the possibility of direct substitution of phenol ethers by various thiophenols using PIFA in (CF₃)₂CHOH.¹⁰ To a solution of *p*-isopropylanisole (1a) containing 2 equiv of *S*-(trimethylsilyl)thiophenol (TMSSPh) in (CF₃)₂CHOH was added PIFA to give the direct sulfonylation compound 2a in 62% yield. The use of thiophenol instead of TMSSPh gave 2a in nearly the same yield (61%). (Scheme 1).

The reaction proceeded successfully in (CF₃)₂CHOH, which is a polar and low-nucleophilic protic solvent, while in other solvents (CH₃CN, CH₂Cl₂, etc.) only a trace of the sulfonylation compound was detected. Furthermore, various types of thiophenols could be employed as sulfur nucleophiles. When 1b was treated with thiophenols having electron-withdrawing groups such as *p*-nitro and 2,3,5,6-tetrafluoro groups in the presence of PIFA under similar conditions, the corresponding arylthio compounds 3 and 4 were obtained in good yield. Thiophenols having electron-donating groups such as *p*-methoxy and *p*-methyl groups gave the corresponding sulfonylation products 5 and 6 in lower yield due to the generation of byproducts (such as diaryl disulfides). These results are summarized in Table 1.

Other aromatic compounds, alkoxybenzenes (1c-f) and methoxynaphthalenes (1g,h), similarly reacted with PIFA and PhSH in (CF₃)₂CHOH to give the corresponding unsymmetrical diaryl sulfides 2. Dialkoxybenzenes 1i-k, which are very reactive compounds,¹¹ yielded the diarylthio products 2i-k without formation of the monoarythio products (Table 2).

The mechanism of sulfonylation of phenol ethers with PIFA plausibly involves the formation of a CT complex

* Abstract published in *Advance ACS Abstracts*, October 15, 1995.

(1) Alkyl aryl sulfides are generally prepared by the condensation of aromatic thiols with alkyl halides (Oae, S. *Organic Chemistry of Sulfur*; Plenum Press: New York, 1977; p 231), but the type of aromatic thiols available are limited.

(2) A recent report: Toste, F. D.; Laronde, F.; Still, W. J. *Tetrahedron Lett.* 1995, 36, 2946.

(3) Reviews: (a) Wood, J. L. *Organic Reactions*; Adams, R., Ed.; John Wiley & Sons: New York, 1946; Vol. 3, Chapter 8. (b) Guy, R. G. *In the Chemistry of Cyanates and Their Thio Derivatives*; Patai, S., Ed.; John Wiley & Sons: New York, 1977; Part 2, Chapter 18. (c) Harusawa, S.; Shioiri, T. *Yuki Gosei Kagaku Kyokaishi* 1981, 39, 741.

(4) (a) Takagi, K. *Chem. Lett.* 1987, 2221. (b) Cristau, H.; Charaud, A.; Chene, A.; Chriatol, H. *Synthesis* 1982, 892.

(5) (a) Fujisawa, T.; Kobori, T.; Ohtsuka, N.; Tsuchihashi, G. *Tetrahedron Lett.* 1988, 10, 5073. (b) Mukaiyama, T.; Suzuki, K. *Chem. Lett.* 1993, 1.

(6) Ranken, D. F.; McKinnin, B. G. *J. Org. Chem.* 1989, 54, 2985.

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(9) (a) Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Yakura, T. *Tetrahedron Lett.* 1991, 32, 4321. (b) Kita, Y.; Tohma, H.; Hatanaka, K.; Takada, T.; Fujita, S.; Mitoh, S.; Sakurai, H.; Oka, S. *J. Am. Chem. Soc.* 1994, 116, 3684. (c) Kita, Y.; Tohma, H.; Takada, T.; Mitoh, S.; Fujita, S.; Gyoten, M. *Synlett* 1994, 427.

(10) For a preliminary communication of a portion of the studies described herein, see: Kita, Y.; Takada, T.; Mihara, S.; Tohma, H. *Synlett* 1995, 211.

(11) The oxidative potentials (*E*^o) of dialkoxybenzenes are lower than those of alkylbenzenes. (Sankaraman, S.; Haney, W. A.; Kochi, J. K. *J. Am. Chem. Soc.* 1987, 109, 7824.)

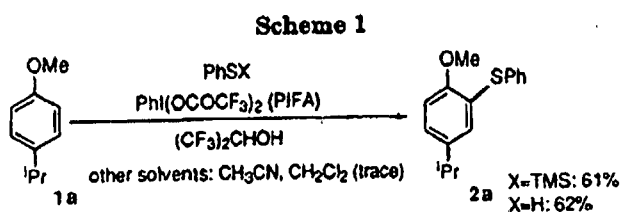


Table 1. Sulfenylation of 1b by Various Thiophenols

substrates	R	products	yields (%)
1b	4-nitrophenyl	3	87
	2,3,5,6-tetrafluorophenyl	4	72
	phenyl	2b	62
	4-methoxyphenyl	5	37
	4-methylphenyl	6	40

Table 2. Sulfenylation of Phenol Ethers and Alkoxy-naphthalenes

Substrates	Products	Yields (%)
 1a: R ¹ =Me, R ² = ⁱ Pr c: R ¹ =Et, R ² = ⁱ Pr d: R ¹ = ⁱ Pr, R ² = ⁱ Pr e: R ¹ =Me, R ² = ^t Bu	 2a c d e	62 67 70 67
 1f	 2f	88
 1b	 2b	62
 1g: R=Me h: R=Et	 2g h	81 86
 1i: R=Me j: R=Et	 2i j	68 75
 1k	 2k	88

of PIFA with 1 followed by a nucleophilic attack by the thiophenol on the cation radical generated by single electron transfer (SET) from the CT complex (mechanism A^{9b}). Product formation, on the other hand, can also be explained by the formation of a diaryl disulfide arising from the reaction of thiophenols with PIFA, followed by a PIFA-promoted Friedel-Crafts reaction of the diaryl disulfide with 1 (mechanism B¹²). The sulfenylation product, however, was not obtained when diaryl disul-

fides activated with PIFA were treated with 1. Therefore, we believe that the present sulfenylation proceeds via cation radicals as reactive intermediates formed by SET from the CT complex of 1 and PIFA (mechanism A) (Scheme 2).

The characteristic points of the present reactions are (i) thiophenols, which are easily available can be used as nucleophiles, (ii) the reaction can be performed under mild conditions, (iii) the hypervalent iodine reagent is simpler and safer than metal catalysts, and (iv) unsymmetric diaryl sulfides were directly and selectively obtained.

Next, we examined the possibility of direct thiocyanation of phenol ethers and alkoxy-naphthalenes by using TMSNCS as a nucleophile. To a solution of *p*-isopropylanisole 1a containing 2 equiv of TMSNCS in (CF₃)₂CHOH was added PIFA to give 4-isopropyl-2-thiocyanatoanisole 7a in 66% yield. Other aromatic compounds, alkoxybenzenes 1c-m and alkoxy-naphthalenes 1b,g,h, similarly reacted with PIFA and TMSNCS in (CF₃)₂CHOH to give the corresponding aryl thiocyanato compounds 7b-m.¹³ The thiocyanato group was selectively introduced at the ortho position of para-substituted phenol ethers and at the para position of ortho-substituted phenol ethers. These results are summarized in Table 3.

The reaction mechanism of thiocyanation of phenol ethers with PIFA is the same as that of sulfenylation of phenol ethers described above, that is, nucleophilic attack of TMSNCS on the cation radical generated by SET from the CT complex of PIFA and phenol ethers to give the corresponding aryl thiocyanato compounds. The present reaction is useful for the preparation of aryl thiocyanato compounds and proceeds smoothly without heating or the addition of Lewis acid catalysts.

In conclusion, novel and direct sulfenylation and thiocyanation of phenol ethers and related compounds have been developed using a hypervalent iodine reagent. The present methods could be applicable to the synthesis of various types of sulfur containing aromatic compounds.

Experimental Section

All melting points are uncorrected. Infrared (IR) absorption spectra were recorded with CHCl₃ as solvent. E. Merck silica gel 60 for column chromatography and E. Merck precoated TLC plates, silica gel F₂₅₄ for preparative thin layer chromatography (preparative TLC) were used. Organic layers were dried with anhydrous MgSO₄. PIFA is commercially available. Starting materials (1b,f,g,i-m) were purchased, and compounds (1a,c-e,h) were prepared by the reported methods.¹⁴ Thiophenols and TMSNCS were purchased and were used without further purification.

General Experimental Procedure. Nucleophilic Sulfenylation Reaction of Phenol Ethers and Related Compounds with PIFA. To a stirred solution of 1 (0.10 mmol) and aryl thiols (0.20 mmol) in 1,1,1,3,3,3-hexafluoro-2-propanol ((CF₃)₂CHOH) (1.0 mL) was added PIFA (0.12–0.15 mmol) at rt under nitrogen. The reaction mixture was stirred for 30 min and then was evaporated in vacuo. The residue was

(12) Kitamura, T.; Matsuyuki, J.; Taniguchi, H. *J. Chem. Soc., Perkin Trans. 1* 1991, 1607.

(13) No aryl isothiocyanato compounds (ArNCS) were generated in these reactions.

(14) Methyl ethers (1a,e): Vyas, G. N.; Shah, N. M. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, p 836. Ethyl ethers (1c,h): *Organic Syntheses*; Wiley: New York, 1941; Collect. Vol. 1, p 58. Isopropyl ether (1d): Sala, T.; Sargent, M. V. *J. Chem. Soc., Perkin Trans. 1* 1979, 2593.

Scheme 2

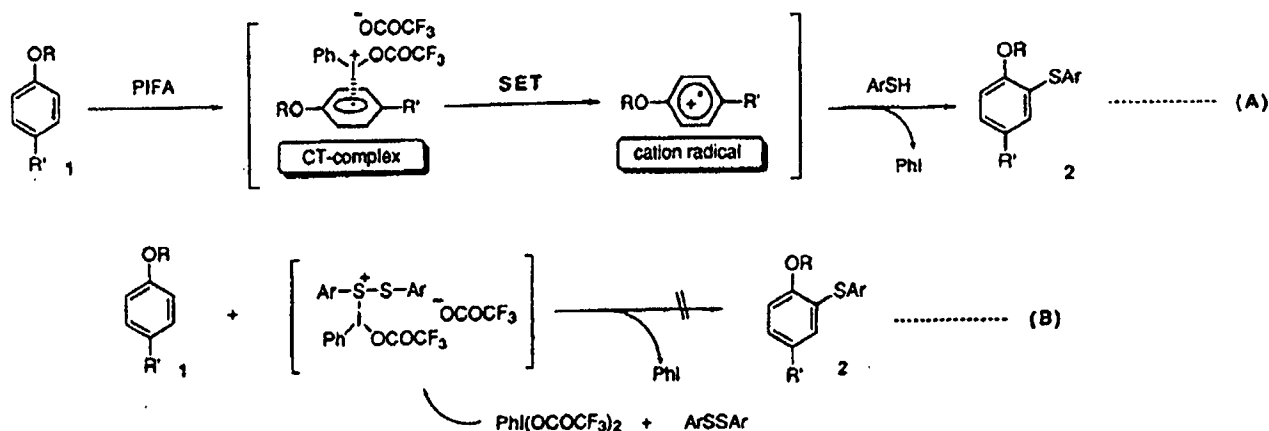


Table 3. Thiocyanation of Phenol Ethers and Alkoxynaphthalenes

Substrates	Products	Yields (%)	
	1a: R¹=Me, R²= ⁱ Pr c: R¹=Et, R²= ⁱ Pr d: R¹= ⁱ Pr, R²= ⁱ Pr e: R¹=Me, R²= ⁱ Bu f: R¹=Me, R²=OMe j: R¹=Et, R²=OEt l: R¹=Me, R²=Me	 7 7 7 7 7 7 7 7	71 94 91 59 66 90 55
		7f	73
		7k m	95 62
		7b	96
		7g h	78 57

purified by column chromatography or preparative TLC on silica gel to give the corresponding diaryl sulfides 2.

Nucleophilic Thiocyanation Reaction of Phenol Ethers and Related Compounds with PIFA. To a stirred solution of 1 (0.10 mmol) and TMSNCS (0.20–0.50 mmol) in (CF₃)₂CHOH (1.0 mL) was added PIFA (0.15–0.20 mmol) at rt under nitrogen. The reaction mixture was stirred for 30 min and then was evaporated in vacuo. The residue was purified by column chromatography or preparative TLC on silica gel to give the corresponding thiocyanato compounds 7.

4-Isopropyl-2-(phenylthio)anisole (2a). 1a (13.3 mg, 0.089 mmol), PhSH (0.018 mL, 0.177 mmol), PIFA (57.1 mg, 0.133 mmol), and (CF₃)₂CHOH (1.0 mL) gave 2a (14.5 mg, 62%) as an oil: IR (CHCl₃) ν 1580, 1490 cm⁻¹; ¹H NMR (200 MHz) (CDCl₃) δ 1.16 (d, *J* = 7.0 Hz, 6H), 2.78 (m, 1H), 3.83 (s, 3H), 6.85 (d, *J* = 8.4 Hz, 1H), 7.06 (d, *J* = 2.4 Hz, 1H), 7.12 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.20–7.30 (m, 5H); HRMS calcd for C₁₆H₁₈OS 258.1082, found 258.1077.

1,4-Dimethoxy-2-(phenylthio)naphthalene (2b). 1b (10.7 mg, 0.057 mmol), PhSH (0.012 mL, 0.114 mmol), PIFA (29.4 mg, 0.068 mmol), and (CF₃)₂CHOH (1.0 mL) gave 2b (10.4 mg, 62%) as an oil: IR (CHCl₃) ν 1590, 1460 cm⁻¹; ¹H NMR (250 MHz) (CDCl₃) δ 3.80 (s, 3H), 3.97 (s, 3H), 6.55 (s, 1H), 7.22–7.37 (m, 5H), 7.48 (m, 2H), 8.07 (d, *J* = 7.5 Hz, 1H), 8.20 (d, *J* = 7.5 Hz, 1H); HRMS calcd for C₁₈H₁₆O₂S 296.0883, found 296.0871. Anal. Calcd for C₁₈H₁₆O₂S: C, 73.25; H, 5.62. Found: C, 72.95; H, 5.44.

1,4-Dimethoxy-2-[(4-nitrophenyl)thio]naphthalene (3). 1b (11.4 mg, 0.061 mmol), 4-nitrothiophenol (18.8 mg, 0.121 mmol), PIFA (39.1 mg, 0.091 mmol), and (CF₃)₂CHOH (1.0 mL) gave 3 (18.1 mg, 87%) as crystals: mp 138–139 °C (from *n*-hexane); IR (CHCl₃) ν 1580, 1520 cm⁻¹; ¹H NMR (250 MHz) (CDCl₃) δ 3.94 (s, 6H), 6.75 (s, 1H), 7.23 (d, *J* = 9.0 Hz, 2H), 7.59–7.64 (m, 2H), 8.07 (d, *J* = 9.0 Hz, 2H), 8.11–8.15 (m, 1H), 8.27–8.31 (m, 1H); HRMS calcd for C₁₈H₁₆NO₄S 341.0722, found 341.0722. Anal. Calcd for C₁₈H₁₆NO₄S: C, 63.09; H, 4.48; N, 4.07; S, 9.42. Found: C, 63.33; H, 4.43; N, 4.10; S, 9.39.

1,4-Dimethoxy-2-[(2,3,5,6-tetrafluorophenyl)thio]naphthalene (4). 1b (36.3 mg, 0.193 mmol), 2,3,5,6-tetrafluorothiophenol (0.047 mL, 0.386 mmol), PIFA (124.5 mg, 0.290 mmol), and (CF₃)₂CHOH (1.0 mL) gave 4 (48.8 mg, 72%) as crystals: mp 102–103 °C (from CH₂Cl₂–*n*-hexane); IR (CHCl₃) ν 1590, 1490 cm⁻¹; ¹H NMR (200 MHz) (CDCl₃) δ 3.87 (s, 3H), 4.01 (s, 3H), 6.55 (s, 1H), 7.08 (m, 1H), 7.43–7.49 (m, 2H), 8.00 (dd, *J* = 7.6, 1.4 Hz, 1H), 8.19 (dd, *J* = 7.6, 1.4 Hz, 1H); HRMS calcd for C₁₈H₁₂O₂F₄S 368.0493, found 368.0493. Anal. Calcd for C₁₈H₁₂O₂F₄S: C, 58.69; H, 3.28. Found: C, 58.75; H, 3.52.

1,4-Dimethoxy-2-[(4-methoxyphenyl)thio]naphthalene (5). 1b (12.1 mg, 0.064 mmol), 4-methoxythiophenol (0.016 mL, 0.129 mmol), PIFA (55.3 mg, 0.129 mmol), and (CF₃)₂CHOH (1.0 mL) gave 5 (7.8 mg, 37%) as an oil: IR (CHCl₃) ν 1590, 1500, 1460 cm⁻¹; ¹H NMR (200 MHz) (CDCl₃) δ 3.74 (s, 3H), 3.83 (s, 3H), 3.99 (s, 3H), 6.35 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 2H), 7.43 (td, *J* = 7.0, 1.6 Hz, 1H), 7.44 (d, *J* = 9.0 Hz, 2H), 7.53 (td, *J* = 7.0, 1.6 Hz, 1H), 8.03 (dd, *J* = 8.2, 1.6 Hz, 1H), 8.15 (dd, *J* = 8.2, 1.6 Hz, 1H); HRMS calcd for C₁₉H₁₈O₃S 326.0977, found 326.0977.

1,4-Dimethoxy-2-[(4-methylphenyl)thio]naphthalene (6). 1b (11.6 mg, 0.062 mmol), 4-methylthiophenol (15.3 mg, 0.123 mmol), PIFA (53.0 mg, 0.123 mmol), and (CF₃)₂CHOH (1.0 mL) gave 6 (11.8 mg, 40%) as an oil: IR (CHCl₃) ν 1590, 1490, 1480 cm⁻¹; ¹H NMR (200 MHz) (CDCl₃) δ 2.34 (s, 3H), 3.78 (s, 3H), 3.97 (s, 3H), 6.49 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 7.42–7.58 (m, 2H), 8.05 (dd, *J* = 8.0, 1.6 Hz, 1H), 8.16 (dd, *J* = 8.0, 1.6 Hz, 1H); HRMS calcd for C₁₉H₁₈O₂S 310.1014, found 310.1025. Anal. Calcd for C₁₉H₁₈O₂S: C, 73.43; H, 5.89; S, 10.33. Found: C, 73.52; H, 5.84; S, 10.33.

1-Ethoxy-4-isopropyl-2-(phenylthio)benzene (2c). 1c (16.3 mg, 0.099 mmol), PhSH (0.020 mL, 0.198 mmol), PIFA (64.0 mg, 0.149 mmol), and (CF₃)₂CHOH (1.0 mL) gave 2c (18.2 mg, 67%) as an oil: IR (CHCl₃) ν 1580, 1480 cm⁻¹; ¹H NMR

(200 MHz) (CDCl_3) δ 1.15 (d, J = 7.0 Hz, 6H), 1.29 (t, J = 6.8 Hz, 3H), 2.76 (m, 1H), 4.02 (q, J = 6.8 Hz, 2H), 6.81 (d, J = 8.4 Hz, 1H), 7.05 (s, 1H), 7.20–7.32 (m, 6H); HRMS calcd for $\text{C}_{17}\text{H}_{20}\text{OS}$ 272.1253, found 272.1235.

1-Isopropoxy-4-isopropyl-2-(phenylthio)benzene (2d). 1d (10.7 mg, 0.060 mmol), PhSH (0.012 mL, 0.120 mmol), PIFA (38.7 mg, 0.090 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2d (12.1 mg, 70%) as an oil: IR (CHCl_3) ν 1580, 1480 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 1.15 (d, J = 7.0 Hz, 6H), 1.24 (d, J = 7.0 Hz, 6H), 2.77 (m, 1H), 4.49 (m, 1H), 6.82 (d, J = 8.2 Hz, 1H), 7.04 (s, 1H), 7.21–7.35 (m, 6H); HRMS calcd for $\text{C}_{18}\text{H}_{22}\text{OS}$ 286.1386, found 286.1389. Anal. Calcd for $\text{C}_{18}\text{H}_{22}\text{OS}$: C, 75.34; H, 7.71; S, 11.16. Found: C, 75.48; H, 7.74; S, 11.19.

4-tert-Butyl-2-(phenylthio)anisole (2e). 1e (15.6 mg, 0.095 mmol), PhSH (0.020 mL, 0.190 mmol), PIFA (61.3 mg, 0.143 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2e (17.3 mg, 67%) as an oil: IR (CHCl_3) ν 1580, 1490 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 1.22 (s, 9H), 3.83 (s, 3H), 6.85 (d, J = 8.4 Hz, 1H), 7.23–7.29 (m, 7H); HRMS calcd for $\text{C}_{17}\text{H}_{20}\text{OS}$ 272.1230, found 272.1232.

1,2,3-Trimethoxy-4-(phenylthio)benzene (2f). 1f (12.4 mg, 0.074 mmol), PhSH (0.015 mL, 0.147 mmol), PIFA (47.5 mg, 0.111 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2f (18.0 mg, 88%) as an oil: IR (CHCl_3) ν 1580, 1480 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 3.82 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 6.65 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 8.6 Hz, 1H), 7.18–7.27 (m, 5H); HRMS calcd for $\text{C}_{15}\text{H}_{16}\text{O}_3\text{S}$ 276.0826, found 276.0820. Anal. Calcd for $\text{C}_{15}\text{H}_{16}\text{O}_3\text{S}$: C, 65.42; H, 5.87; S, 11.60. Found: C, 65.19; H, 5.84; S, 11.60.

1-Methoxy-4-(phenylthio)naphthalene (2g). 1g (15.8 mg, 0.100 mmol), PhSH (0.021 mL, 0.200 mmol), PIFA (64.4 mg, 0.150 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2g (21.4 mg, 81%) as crystals: mp 88–89 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 1590 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 4.03 (s, 3H), 6.82 (d, J = 8.0 Hz, 1H), 7.01–7.24 (m, 5H), 7.49–7.53 (m, 2H), 7.79 (d, J = 8.0 Hz, 1H), 8.30–8.34 (m, 2H); HRMS calcd for $\text{C}_{17}\text{H}_{14}\text{OS}$ 266.0755, found 266.0762. Anal. Calcd for $\text{C}_{17}\text{H}_{14}\text{OS}$: C, 76.49; H, 5.37; S, 11.88. Found: C, 76.66; H, 5.30; S, 12.04.

1-Ethoxy-4-(phenylthio)naphthalene (2h). 1h (17.7 mg, 0.103 mmol), PhSH (0.021 mL, 0.206 mmol), PIFA (66.4 mg, 0.155 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2h (24.8 mg, 86%) as crystals: mp 106–107 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 1580 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 1.58 (t, J = 7.0 Hz, 3H), 4.25 (q, J = 7.0 Hz, 2H), 6.81 (d, J = 8.2 Hz, 1H), 6.99–7.15 (m, 5H), 7.48–7.53 (m, 2H), 7.78 (d, J = 8.2 Hz, 1H), 8.28–8.38 (m, 2H); HRMS calcd for $\text{C}_{18}\text{H}_{16}\text{OS}$ 280.0905, found 280.0920. Anal. Calcd for $\text{C}_{18}\text{H}_{16}\text{OS}$: C, 77.22; H, 5.75; S, 11.41. Found: C, 77.11; H, 5.75; S, 11.43.

1,4-Dimethoxy-2,5-bis(phenylthio)benzene (2i). 1i (10.8 mg, 0.078 mmol), PhSH (0.020 mL, 0.195 mmol), PIFA (83.9 mg, 0.195 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2i (18.9 mg, 68%) as crystals: mp 145–146 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 1580, 1480 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 3.65 (s, 6H), 6.65 (s, 2H), 7.29–7.35 (m, 10H); HRMS calcd for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{S}_2$ 354.0748, found 354.0748.

1,4-Diethoxy-2,5-bis(phenylthio)benzene (2j). 1j (12.3 mg, 0.074 mmol), PhSH (0.019 mL, 0.185 mmol), PIFA (79.6 mg, 0.185 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2j (21.2 mg, 75%) as crystals: mp 93–94 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 1590, 1470 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 1.23 (t, J = 6.8 Hz, 6H), 3.83 (q, J = 6.8 Hz, 4H), 6.63 (s, 2H), 7.25–7.39 (m, 10H); HRMS calcd for $\text{C}_{22}\text{H}_{22}\text{O}_2\text{S}_2$ 382.1072, found 382.1061. Anal. Calcd for $\text{C}_{22}\text{H}_{22}\text{O}_2\text{S}_2$: C, 69.04; H, 5.84. Found: C, 69.08; H, 5.80.

1,2-Dimethoxy-4,5-bis(phenylthio)benzene (2k). 1k (12.0 mg, 0.087 mmol), PhSH (0.018 mL, 0.174 mmol), PIFA (56.0 mg, 0.130 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 2k (27.2 mg, 88%) as crystals: mp 79–80 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 1580, 1480, 1460 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 3.73 (s, 6H), 6.83 (s, 2H), 7.19–7.29 (m, 10H); HRMS calcd for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{S}_2$ 354.0746, found 354.0744. Anal. Calcd for $\text{C}_{20}\text{H}_{18}\text{O}_2\text{S}_2$: C, 67.77; H, 5.12; S, 18.09. Found: C, 67.77; H, 5.20; S, 17.99.

4-Isopropyl-2-thiocyanatoanisole (7a). 1a (11.1 mg, 0.074 mmol), TMSNCS (0.021 mL, 0.148 mmol), PIFA (47.7

mg, 0.111 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7a (10.9 mg, 71%) as an oil: IR (CHCl_3) ν 2160, 1600, 1500, 1460, 1440 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 1.24 (d, J = 7.5 Hz, 6H), 2.90 (m, J = 7.5 Hz, 1H), 3.89 (s, 3H), 6.86 (d, J = 7.8 Hz, 1H), 7.21 (dd, J = 8.0, 1.8 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H); HRMS calcd for $\text{C}_{11}\text{H}_{13}\text{NOS}$ 207.0721, found 207.0718.

1,4-Dimethoxy-2-thiocyanatonaphthalene (7b). 1b (31.1 mg, 0.165 mmol), TMSNCS (0.047 mL, 0.330 mmol), PIFA (106.4 mg, 0.248 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7b (38.8 mg, 96%) as crystals: mp 95–96 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 2160, 1620, 1580, 1510, 1460, 1440 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 3.95 (s, 3H), 4.04 (s, 3H), 6.89 (s, 1H), 7.53 (td, J = 7.0, 1.6 Hz, 1H), 7.61 (td, J = 7.0, 1.6 Hz, 1H), 7.97 (dd, J = 7.2, 2.2 Hz, 1H), 8.25 (dd, J = 7.6, 2.2 Hz, 1H); HRMS calcd for $\text{C}_{13}\text{H}_{11}\text{NO}_2\text{S}$ 245.0509, found 245.0509. Anal. Calcd for $\text{C}_{13}\text{H}_{11}\text{NO}_2\text{S}$: C, 63.48; H, 4.54; N, 5.67. Found: C, 63.65; H, 4.52; N, 5.71.

1-Ethoxy-4-isopropyl-2-thiocyanatobenzene (7c). 1c (11.7 mg, 0.071 mmol), TMSNCS (0.040 mL, 0.285 mmol), PIFA (61.3 mg, 0.143 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7c (14.9 mg, 94%) as an oil: IR (CHCl_3) ν 2160, 1600, 1500, 1480, 1460 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 1.23 (d, J = 7.0 Hz, 6H), 1.44 (t, J = 7.0 Hz, 3H), 2.89 (m, J = 7.0 Hz, 1H), 4.11 (q, J = 7.0 Hz, 2H), 6.84 (d, J = 8.4 Hz, 1H), 7.17 (dd, J = 8.4, 2.0 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H); HRMS calcd for $\text{C}_{12}\text{H}_{14}\text{NOS}$ 221.0881, found 221.0874. Anal. Calcd for $\text{C}_{12}\text{H}_{14}\text{NOS}$: C, 64.81; H, 6.75; N, 6.22. Found: C, 65.12; H, 6.83; N, 6.33.

1-Isopropoxy-4-isopropyl-2-thiocyanatobenzene (7d). 1d (10.4 mg, 0.058 mmol), TMSNCS (0.033 mL, 0.233 mmol), PIFA (50.1 mg, 0.117 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7d (12.6 mg, 91%) as an oil: IR (CHCl_3) ν 2160, 1600, 1500, 1480, 1460 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 1.24 (d, J = 6.8 Hz, 6H), 1.37 (d, J = 6.0 Hz, 6H), 2.89 (m, J = 6.8 Hz, 1H), 4.58 (m, J = 6.0 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 7.16 (dd, J = 8.5, 1.8 Hz, 1H), 7.38 (d, J = 1.8 Hz, 1H); HRMS calcd for $\text{C}_{13}\text{H}_{17}\text{NOS}$ 235.1020, found 235.1028.

4-tert-Butyl-2-thiocyanatoanisole (7e). 1e (10.2 mg, 0.062 mmol), TMSNCS (0.018 mL, 0.124 mmol), PIFA (40.1 mg, 0.093 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7e (7.8 mg, 57%) as an oil: IR (CHCl_3) ν 2160, 1600, 1500, 1460, 1440 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 1.32 (s, 9H), 3.90 (s, 3H), 6.87 (d, J = 8.8 Hz, 1H), 7.38 (dd, J = 8.8, 2.4 Hz, 1H), 7.56 (d, J = 2.2 Hz, 1H); HRMS calcd for $\text{C}_{12}\text{H}_{15}\text{NOS}$ 221.0867, found 221.0872.

1,2,3-Trimethoxy-4-thiocyanatobenzene (7f). 1f (13.4 mg, 0.080 mmol), TMSNCS (0.045 mL, 0.320 mmol), PIFA (68.8 mg, 0.160 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7f (13.1 mg, 73%) as an oil: IR (CHCl_3) ν 2160, 1580, 1480, 1460 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 3.68 (s, 3H), 3.89 (s, 3H), 4.00 (s, 3H), 6.73 (d, J = 8.5 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H); HRMS calcd for $\text{C}_{10}\text{H}_{11}\text{NO}_3\text{S}$ 225.0459, found 225.0459. Anal. Calcd for $\text{C}_{10}\text{H}_{11}\text{NO}_3\text{S}$: C, 53.50; H, 5.00; N, 6.20. Found: C, 53.32; H, 4.92; N, 6.22.

1-Methoxy-4-thiocyanatonaphthalene (7g). 1g (22.1 mg, 0.139 mmol), TMSNCS (0.078 mL, 0.556 mmol), PIFA (119.5 mg, 0.278 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7g (23.4 mg, 78%) as crystals: mp 106–107 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 2150, 1620, 1590, 1570, 1510, 1460, 1440 cm^{-1} ; ^1H NMR (270 MHz) (CDCl_3) δ 4.03 (s, 3H), 6.80 (d, J = 8.2 Hz, 1H), 7.59 (td, J = 7.5, 1.0 Hz, 1H), 7.72 (td, J = 7.0, 1.0 Hz, 1H), 7.84 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.6 Hz, 1H), 8.34 (d, J = 8.3 Hz, 1H); HRMS calcd for $\text{C}_{12}\text{H}_9\text{NOS}$ 215.0406, found 215.0406. Anal. Calcd for $\text{C}_{12}\text{H}_9\text{NOS}$: C, 66.61; H, 4.31; N, 6.47. Found: C, 66.95; H, 4.21; N, 6.51.

1-Ethoxy-4-thiocyanatonaphthalene (7h). 1h (10.4 mg, 0.060 mmol), TMSNCS (0.017 mL, 0.121 mmol), PIFA (39.0 mg, 0.091 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave 7h (7.9 mg, 57%) as crystals: mp 74–76 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 2150, 1590, 1570, 1510 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 1.58 (t, J = 6.8 Hz, 3H), 4.24 (q, J = 6.8 Hz, 2H), 6.80 (d, J = 8.5 Hz, 1H), 7.59 (td, J = 7.5, 1.0 Hz, 1H), 7.72 (td, J = 7.5, 1.0 Hz, 1H), 7.84 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.38 (d, J = 8.0 Hz, 1H); HRMS calcd for $\text{C}_{13}\text{H}_{11}\text{NOS}$ 229.0563, found 229.0562.

1,4-Dimethoxy-2-thiocyanatobenzene (7i). **1i** (16.2 mg, 0.117 mmol), TMSNCS (0.099 mL, 0.702 mmol), PIFA (100.6 mg, 0.234 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave **7i** (15.0 mg, 66%) as crystals: mp 68–69 °C (from CH_2Cl_2 -*n*-hexane); IR (CHCl_3) ν 2160, 1590, 1490, 1460, 1440 cm^{-1} ; ^1H NMR (250 MHz) (CD_3OD) δ 3.79 (s, 3H), 3.87 (s, 3H), 6.94 (dd, $J = 9.3$, 2.5 Hz, 1H), 7.01 (d, $J = 9.3$ Hz, 1H), 7.08 (d, $J = 2.5$ Hz, 1H); HRMS calcd for $\text{C}_9\text{H}_9\text{NO}_2\text{S}$ 195.0359, found 195.0354. Anal. Calcd for $\text{C}_9\text{H}_9\text{NO}_2\text{S}$: C, 55.06; H, 4.63; N, 7.11. Found: C, 55.37; H, 4.65; N, 7.17.

1,4-Diethoxy-2-thiocyanatobenzene (7j). **1j** (10.4 mg, 0.063 mmol), TMSNCS (0.035 mL, 0.250 mmol), PIFA (53.8 mg, 0.125 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave **7j** (12.5 mg, 90%) as an oil: IR (CHCl_3) ν 2160, 1580, 1500, 1470 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 1.40 (t, $J = 7.3$ Hz, 3H), 1.42 (t, $J = 7.5$ Hz, 3H), 4.00 (q, $J = 7.0$ Hz, 2H), 4.06 (q, $J = 7.0$ Hz, 2H), 6.84 (s, 2H), 7.09 (s, 1H); HRMS calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_2\text{S}$ 223.0662, found 223.0665. Anal. Calcd for $\text{C}_{11}\text{H}_{13}\text{NO}_2\text{S}$: C, 59.20; H, 5.86; N, 6.33; S, 14.22. Found: C, 59.17; H, 5.87; N, 6.27; S, 14.36.

1,2-Dimethoxy-4-thiocyanatobenzene (7k). **1k** (20.8 mg, 0.151 mmol), TMSNCS (0.085 mL, 0.604 mmol), PIFA (129.9 mg, 0.302 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave **7k** (28.1 mg, 95%) as crystals: mp 49–50 °C (from CH_2Cl_2 -*n*-

hexane); IR (CHCl_3) ν 2150, 1590, 1510, 1460, 1440 cm^{-1} ; ^1H NMR (250 MHz) (CDCl_3) δ 3.91 (s, 3H), 3.92 (s, 3H), 6.89 (d, $J = 8.5$ Hz, 1H), 7.05 (d, $J = 2.5$ Hz, 1H), 7.16 (dd, $J = 8.5$, 2.5 Hz, 1H); HRMS calcd for $\text{C}_9\text{H}_9\text{NO}_2\text{S}$ 195.0330, found 195.0354. Anal. Calcd for $\text{C}_9\text{H}_9\text{NO}_2\text{S}$: C, 55.22; H, 4.61; N, 7.14; S, 16.35. Found: C, 55.37; H, 4.65; N, 7.17; S, 16.42.

4-Methyl-2-thiocyanatoanisole (7l). **1l** (12.6 mg, 0.103 mmol), TMSNCS (0.058 mL, 0.412 mmol), PIFA (88.6 mg, 0.206 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave **7l** (10.2 mg, 55%) as an oil: IR (CHCl_3) ν 2160, 1600, 1500 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 2.32 (s, 3H), 3.88 (s, 3H), 6.82 (d, $J = 8.2$ Hz, 1H), 7.14 (dd, $J = 8.2$, 2.0 Hz, 1H), 7.36 (d, $J = 2.0$ Hz, 1H); HRMS calcd for $\text{C}_9\text{H}_9\text{NOS}$ 179.0411, found 179.0405.

2-Methyl-4-thiocyanatoanisole (7m). **1m** (22.9 mg, 0.187 mmol), TMSNCS (0.110 mL, 0.748 mmol), PIFA (160.8 mg, 0.374 mmol), and $(\text{CF}_3)_2\text{CHOH}$ (1.0 mL) gave **7m** (20.8 mg, 62%) as an oil: IR (CHCl_3) ν 2150, 1590, 1570, 1490, 1460, 1440 cm^{-1} ; ^1H NMR (200 MHz) (CDCl_3) δ 2.22 (s, 3H), 3.85 (s, 3H), 6.84 (d, $J = 8.5$ Hz, 1H), 7.37 (d, $J = 2.5$ Hz, 1H), 7.38 (dd, $J = 8.5$, 2.5 Hz, 1H); HRMS calcd for $\text{C}_9\text{H}_9\text{NOS}$ 179.0385, found 179.0404.

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hydroxide²⁵ and the other with Bial's orcinol reagent.²⁶ Three ketose bands were detected. Two of these were close to each other and were situated in the 1,3-dihydroxy-2-propanone region; the third was in the region usually occupied by hexoses. The similar bands from the four chromatograms were combined and eluted. Fraction A, the effluent from the slowest band, on concentrating to dryness, weighed 0.7 g. This sirupy material was identified as containing 4-C-(hydroxymethyl)-D,L-glycero-pentulose (dendroketo-²⁶) from its similarity in behavior to the product prepared by the action of dilute alkali on compound I. The dendroketo-²⁶ from the two sources showed similar chromatographic behavior, and both gave a gray color with the orcinol spray.

5,7-O-Ethylidene-D-manno-heptulose (III).—The material in fraction B, which reacted with the orcinol spray to give a blue-green color, weighed 4.2 g. on drying. Compound III crystallized from methanol; weight 0.41 g., m.p. 200.5–201.5°, $[\alpha]_D^{20} +4.4^\circ$ (c 3, water). No mutarotation was observed.

Anal. Calcd. for $C_8H_{16}O_7$: C, 45.8; H, 6.8. Found: C, 45.6; H, 6.8.

A solution of 0.05 g. of compound III in 2 ml. of water containing 0.5 ml. of Amberlite IR-120(H⁺) was agitated with a gentle stream of nitrogen and hydrolyzed by heating for 0.5 hr. in a boiling water bath. On removal of the resin, D-manno-heptulose¹⁸ was obtained; it was identified by its infrared spectrum,²⁷ m.p. 151–152°, mixed m.p. with

authentic material 151–152°, and $[\alpha]_D^{20} +29^\circ$. Compound III and the parent sugar were found to produce a green color in the orcinol test, in accordance with observations by Richtmyer.²⁸

D-gluc-Heptulose (IV).—The mother liquor (from which III had crystallized) was hydrolyzed as described above and, from the hydrolyzate, was crystallized 1.025 g. of compound IV,²⁸ identified by its infrared spectrum, m.p. 171–174°, mixed m.p. 171–174°, $[\alpha]_D^{20} +67.6^\circ$, and a blue color²⁸ with the orcinol reagent.

5,7-O-Ethylidene- α -D-altro-heptulose (V).—A sirupy residue resulted on concentrating the effluent from band C; wt. 1.9 g. Crystalline compound V, wt. 0.98 g., was obtained from a 2-propanol solution of the material, m.p. 149–151°, $[\alpha]_D^{20} +55^\circ$ (c 1; water), $[\alpha]_D^{20} +50^\circ$ (1 min.) $\rightarrow +40^\circ$ (7 min.; c 1; methanol). From the mutarotation in methanol, an α -D configuration is assigned to the crystalline acetal.

Anal. Calcd. for $C_8H_{16}O_7$: C, 45.8; H, 6.8. Found: C, 45.7; H, 6.9.

A sample (0.05 g.) of compound V, on hydrolysis as described above, yielded 2,7-anhydro-D-altro-heptulose hydrate,²⁹ which was identified by its infrared spectrum,²⁷ m.p. 99–101°, mixed m.p. with authentic material 99–101°, and $[\alpha]_D^{20} -134^\circ$.

All melting points are uncorrected.

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2-Chloro-3-dimethylamino-6-phenylsulfonylphenyllithium

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By the metalation of 3-chloro-4-dimethylaminodiphenyl sulfone (I) using *n*-butyllithium, 3-chloro-4-dimethylamino-6-phenylsulfonylphenyllithium (II) was prepared. At temperatures higher than -10° , II eliminates lithium chloride forming 3-dimethylamino-6-phenylsulfonylbenzyne (III). This intermediate reacts readily with furan yielding 5-dimethylamino-8-phenylsulfonyl-1,4-dihydronaphthalene 1,4-endoxide (V). The consumption of the organolithium compound II with time was used to measure the rate of formation of III. The elimination of lithium chloride by II was shown to be a first-order process.

An elimination-addition mechanism has been discussed to explain reactions of *o*-halophenyllithium compounds proceeding through a dehydrobenzene² intermediate.³ It has been shown that

o-halophenyllithium compounds can be prepared and are stable at temperatures ranging from -60 to -110° .⁴ For example, *o*-chlorophenyllithium is stable at -90° . We hoped to modify the properties of both the halophenyllithium compounds and the short-lived benzyne intermediate by the introduction of the dimethylamino and the phenylsulfonyl groups at positions 3 and 6. These groups were chosen because of their respective mesomeric and inductive effects. The expected stabilization of this 3,6-disubstituted *o*-chlorophenyllithium compound was observed.

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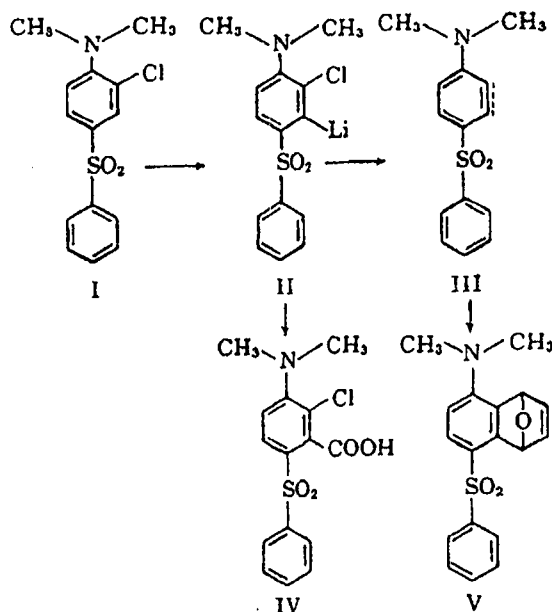
(2) The authors feel that dehydrobenzene is a more general name than benzyne, because the name benzyne implies the presence of a triple bond in the symmetrical intermediate. Experimental evidence is not available to substantiate this implication. For the sake of conformity with the current usage of this journal, we shall use the term benzyne to mean dehydrobenzene.

(3) For the reviews of the field see G. Wittig, *Naturwissenschaften*, **30**, 896 (1942); G. Wittig, *Angew. Chem.*, **69**, 245 (1957); E. F. Jenney, M. C. Caserio, and J. D. Roberts, *Experientia*, **14**, 349 (1958); R. Huisgen in H. Zeiss, "Organometallic Chemistry," Reinhold Publishing Corp., New York, 1960, pp. 36–87.

(4) H. Gilman and R. D. Gorsich, *J. Am. Chem. Soc.*, **79**, 2625 (1957); *ibid.*, **78**, 2217 (1956); H. Gilman and T. S. Soddy, *J. Org. Chem.*, **22**, 1715 (1957).

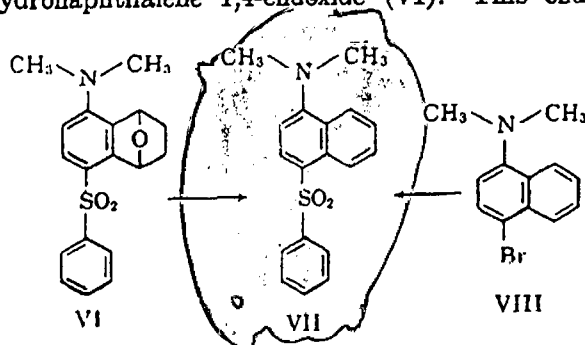
Results

3-Chloro-4-dimethylaminodiphenyl sulfone (I) was metalated rapidly and completely at -80° with *n*-butyllithium yielding 3-chloro-4-dimethylamino-6-phenylsulfonylphenyllithium (II). When II was treated with freshly crushed carbon dioxide at temperatures below -60° , 3-chloro-4-dimethylaminodiphenyl sulfone-2-carboxylic acid (IV, 83-90%) was formed. Regardless of whether carboxylation was carried out shortly after the addition of butyllithium or twenty-four hours later, the results were the same as long as a temperature below -60° was maintained throughout.



Upon warming an ether solution of II gradually to room temperature in the presence of furan (1.0-6.0 moles), 5-dimethylamino-8-phenylsulfonyl-1,4-dihydronaphthalene 1,4-endoxide (V) was secured in 50-61% yields. With a somewhat larger excess of furan (12.0 moles), yields were only slightly increased (68%).

In order to confirm the structure of V, hydrogenation was carried out over palladium to form 5-dimethylamino-8-phenylsulfonyl-1,2,3,4-tetrahydronaphthalene 1,4-endoxide (VI). This end-



(5) These are more strenuous reaction conditions than were required to effect removal of the oxygen atom in 1,2,3,4-tetrahydronaphthalene 1,4-endoxide [G. Wittig and L. Pohmer, *Ber.*, 89, 1334 (1956)].

oxide was converted to 1-dimethylamino-4-phenylsulfonylnaphthalene (VII) by heating with hydrochloric acid in acetic anhydride on the steam bath.⁶

For an alternate synthesis of VII, 1-dimethylamino-4-lithionaphthalene was prepared from 1-bromo-4-dimethylaminonaphthalene⁶ (VIII) by halogen-metal interconversion using *n*-butyllithium. Upon treatment of this intermediate with benzenesulfonyl fluoride,⁷ 1-dimethylamino-4-phenylsulfonylnaphthalene was obtained. This sample of VII was identical with that obtained from endoxide VI as evidenced by mixture melting point and infrared spectra comparison.

In order to determine the temperature at which II eliminated lithium chloride to form III, the extent of reaction was followed as the temperature of the system was elevated. The number of equivalents of organolithium reagent present at the indicated temperatures was determined after hydrolysis with water by titration using 0.1000 *N* hydrochloric acid. The data of Table I were obtained by pipetting aliquots (1.0 ml. $\pm$ 0.15 ml.) of a reaction mixture (0.10 *N*) and titrating to the phenolphthalein end point. In six titrations which were obtained between -65 and -10° , the standard deviation was 0.90 mmole of RLi (II) or $\pm 9.1\%$. The data of Table I were interpreted to indicate that the elimination of lithium chloride from II was proceeding with a measureable rate at 0° .

TABLE I

Time, min.	T, $^{\circ}$ C.	Mmoles II	Mmoles consumed	% Reaction
0	-65	9.88	...	...
294	-9	9.87	0.01	0.1
320	0.0	6.85	3.03	30.6
353	$+21.0$	5.53	4.35	44.0
∞	$+25.0$	0.15	9.73	98.5

Preliminary kinetic studies using the acidimetric titration method for a reaction indicate that the half-life of II is about seven minutes ($k_1 = 0.094$ min.⁻¹).⁸ The data of Table II were secured at 0° .

In order to check this result, four runs similar to

TABLE II

Time, min.	(II), mole/l.
0	0.1835
9.0	.0420
10.0	.0360
19.0	.0206
21.0	.0156
28.0	.0066
30.0	.0056

(6) H. H. Snyder and F. W. Wyman, *J. Am. Chem. Soc.*, 70, 231 (1948).

(7) G. Kobrich, *Ber.*, 92, 2981 (1959).

(8) The extent of reaction with time was followed by acidimetric titration of the amount of II remaining (using 5.0-ml. aliquots). The data of Table II were obtained using 20.7 mmoles of I and 22.0 mmoles of *n*-butyllithium. When the logarithm of the organolithium concentration minus a correction for excess butyllithium and non-organometallic bases was plotted against time, a linear relationship was secured.

that in Table II were conducted in the presence of furan (5.0 ml., 0.0674 mole). Reaction was terminated at different times by adding excess carbon dioxide. The results are shown in Table III. A plot of the logarithm of per cent IV was linear with time; the slope was determined and a value for k_1 of 0.064 min.⁻¹ was found ($t_{1/2} = 10.9$ min.)

TABLE III

Mmoles, I	% IV	% V	Time, min.
5.115	65.9	17.9	5.0
5.094	57.3	32.3	15.0
5.077	30.1	41.9	26.0
5.084	12.5	56.3	39.0

Discussion

The data indicate that the elimination of lithium chloride from *o*-chlorophenyllithium compounds is a first-order process. Secondly, the temperature at which this process occurs has been sharply modified by the dimethylamino and phenylsulfonyl substituents. The increased acidity of the proton at position two in 3-chloro-4-dimethylaminodiphenyl sulfone (I) is believed to be caused by the presence of the phenylsulfonyl group in the *ortho* position.

Recently, *o*-bromo-*N,N*-dimethylaniline was studied in the lithium piperidide-piperidine system.⁹ The conclusion drawn from this work was that the metalation step prior to lithium halide elimination is the rate-determining step. This conclusion is based upon the assumption that the lithium halide elimination from the substituted *o*-halophenyllithium compounds is an extremely rapid process. This assumption—that elimination is not rate determining at +20°—is probably valid in the lithium piperidide-piperidine system.

Although efforts were made to trap all of the intermediate III as the endoxide V with various concentrations of furan, III undergoes competitive side reactions. One of these side reactions was emphasized in the absence of furan to yield a crystalline product which is believed to be a dimer of III. The reactions of III form the nucleus for continued investigations in this laboratory.

Experimental

Throughout this study freshly prepared solutions of *n*-butyllithium in ether (1.2–1.3 *N*) were used. All operations were carried out in a dry, oxygen-free nitrogen atmosphere. Infrared spectra of IV and V were obtained in matching 0.30-cm. cells with a Perkin-Elmer Model 21 double beam apparatus. The endoxide absorption of V at 10.03 μ was measured at various concentrations from 0.025 *M* to 0.10 *M*. Slight deviation from linearity was observed at concentrations greater than 0.138 *M* (absorbance = 1.02). The carbonyl absorption at 5.75 μ of the acid IV was studied over the concentration range 0.0222 to 0.0457 *M*. No difficulty was experienced in analyzing mixtures of IV and V.

(9) R. Huisgen, W. Mack, K. Herbig, N. Ott, and E. Anneser, *Ber.*, **93**, 412 (1960).

The data of Table III were obtained using the chloro sulfone (0.00509 mole I), ether (75.0 ml.), furan (5.0 ml., 0.0674 mole, freshly distilled from sodium), and *n*-butyllithium (4.15 ml., 0.00510 mole). After reaction was ended with carbon dioxide, hydrolysis was accomplished with water. Solid endoxide (V contaminated with IV) was separated from the water and ether phases, dried, and weighed. This solid was dissolved in chloroform and was analyzed by infrared spectroscopy using the endoxide and carbonyl absorptions as described above. The aqueous and ether phases were separated and the latter was dried over sodium sulfate. The ether was replaced with chloroform and the samples were analyzed by infrared spectroscopy as described. Endoxide, V, was present in the ether phase in amounts ranging from 10–15% based on the amount of starting material. Chloro acid (IV) was present in percentages from 2.8–4.4. The bulk of the chloro acid was isolated after acidification of the aqueous phase. Since the chloro acid is slightly hygroscopic, it was dried *in vacuo* before its identity was checked by melting point and neutralization equivalent.

3-Chloro-4-dimethylaminodiphenyl Sulfone.—In a 1-l. three-necked flask equipped with a stirrer were placed 4-dimethylaminodiphenyl sulfone¹⁰ (65.2 g., 0.250 mole), a crystal of ferric chloride, and dry chloroform (600 ml.). To this system, maintained at 0°, was added chlorine (20.8 g., 0.294 mole) which had previously been dissolved in chloroform (250 ml.) at -80°. This solution became colorless after 1 hr. of stirring at 0°; after 3 hr., the chloroform solution was shaken twice with 100-ml. portions of water. Phases were separated and chloroform was replaced with methanol (200 ml.). Crystallization of 3-chloro-4-dimethylaminodiphenyl sulfone (53.3 g., 71.0%, m.p. 65.0–65.8°) occurred slowly at room temperature during several days. Recrystallization from methanol yielded a purified product, m.p. 65.8–66.0°.

Anal. Calcd. for C₁₄H₁₄ClNO₂S: C, 56.85; H, 4.77; Cl, 11.99; N, 4.73. Found: C, 56.55; H, 4.78; Cl, 12.19; N, 5.46.

3-Chloro-4,4-dimethylaminodiphenylsulfone-2-carboxylic Acid.—In a nitrogen-filled Schlenk tube were placed 3-chloro-4-dimethylaminodiphenylsulfone (2.980 g., 0.0101 mole) and absolute ether (100 ml.). To this solution maintained at -80° was added *n*-butyllithium (8.90 ml., 0.0111 mole). The solution became yellow immediately and a white precipitate was noticed after a short time. The tube was sealed in a nitrogen atmosphere and was maintained at -80° for 24.0 hr. The tube was opened under dry nitrogen and freshly crushed carbon dioxide was added. Hydrolysis was accomplished with water, the phases were separated, and the aqueous phase was warmed gently to expell excess carbon dioxide and ether. Following careful acidification with hydrochloric acid, 2.980 g. of the acid IV was isolated, m.p. 165–170°. After purification by recrystallization from water (65 mg. per 100 ml.) or a water-methanol pair (10:1), 2.82 g. of purified acid was obtained, m.p. 172–173.0°. An analytical sample was obtained after one additional recrystallization from water, m.p. 173.2–174.0°.

Anal. Calcd. for C₁₅H₁₄ClNO₃S: C, 53.02; H, 4.15; Cl, 10.43; N, 4.12; S, 9.44. Found: C, 52.80; H, 4.23; Cl, 10.52; N, 4.27; S, 9.38.

Neut. equiv.: Calcd: 339.8. Found 338.0, 339.1.

An analogous run having excess furan present (4.0 ml., 0.055 mole) yielded 2.85 g. (83.2%) of acid IV, m.p. 171.0–173.0°, after a reaction time of 1.25 hr. Neither 4-dimethylaminodiphenyl sulfone-3-carboxylic acid (the product one would expect if halogen-metal interconversion had occurred) nor the endoxide V could be isolated.

5-Dimethylamino-8-phenylsulfonyl-1,4-dihydronaphthalene 1,4-Endoxide.—In a three-necked flask equipped with stirrer were placed 3-chloro-4,4-dimethylaminodiphenyl-

(10) Prepared by the method of A. Gebauer-Fullneg, *Ber.*, **61**, 1307 (1928), in 63% yield using chlorobenzene as solvent.

sulfone (6.053 g., 0.02045 mole), furan (16.3 g. 0.248 mole, freshly distilled from sodium), and absolute tetrahydrofuran¹¹ (50 ml.). The resulting solution was cooled to -65° and *n*-butyllithium (21.2 ml., 0.254 mole) was added dropwise during 15 min. The resulting pale yellow solution was stirred with gradual warming to room temperature. Hydrolysis of the reaction mixture was accomplished with a saturated ammonium chloride solution. 5-Dimethylamino-8-phenylsulfonyl-1,4-dihydronaphthalene 1,4-endoxide (3.248 g., 48.7%, m.p. 166–170°) was separated from the ether and aqueous phases. The ether was separated from the aqueous phase and the solvent was replaced with methanol yielding additional crude endoxide (1.250 g., 18.6%, m.p. 165–170°). Recrystallization of these materials from methanol yielded a purified product (3.73 g., m.p. 172.5–174.0°).

Anal. Calcd. for $C_{18}H_{17}O_2NS$: C, 66.03; H, 5.23; N, 4.28; Found: C, 66.32; H, 5.39; N, 4.30.

5-Dimethylamino-8-phenylsulfonyl-1,2,3,4-tetrahydronaphthalene 1,4-Endoxide.—Palladium oxide (0.2073 g., 0.00168 mole) in 25 ml. of methanol was reduced with hydrogen in a conventional glass hydrogenation vessel (250 ml. volume). To this freshly prepared catalyst-suspension was added 5-dimethylamino-8-phenylsulfonyl-1,4-dihydronaphthalene 1,4-endoxide (0.5260 g., 0.00161 mole) in 25 ml. of methanol. During 35 min., 40.3 ml. of hydrogen was consumed at 29° and 761 mm. (0.00156 mole, 97.5% of theory). The catalyst was removed by filtration and was extracted with boiling methanol. Concentration of the methanol solution was followed by crystallization of 5-dimethylamino-8-phenylsulfonyl-1,2,3,4-tetrahydronaphthalene 1,4-endoxide (0.470 g., 0.00144 mole, 89.5%, m.p. 171.4–172.2°). A mixed melting point with the starting material was undepressed! The infrared spectrum, however, was noticeably different having a strong absorption at 2980-cm^{-1} characteristic for alicyclic carbon-hydrogen bond stretching motions.

Anal. Calcd. for $C_{18}H_{19}O_2NS$: C, 65.63; H, 5.81; N, 4.25; S, 9.73. Found: C, 65.90; H, 5.70; N, 4.23; S, 9.58.

1-Dimethylamino-4-phenylsulfonylnaphthalene.—A mixture of 5-dimethylamino-8-phenylsulfonyl-1,2,3,4-tetrahydronaphthalene 1,4-endoxide (0.1708 g., 0.000516 mole), acetic anhydride (5.0 ml.), and concentrated hydrochloric acid (1.0 ml.) was heated on the steam bath for 16.5 hr. Neutralization was accomplished with excess 2*N* ammonium hydroxide (55 ml.); the organic materials were extracted

with ether and separated from the aqueous phase. Replacement of the ether with methanol was followed by crystallization of 1,1-dimethylamino-4-phenylsulfonylnaphthalene (0.1321 g., 82.1%, m.p. 149–153°). Three further recrystallizations from methanol raised the m.p. to 157.2–158.2°.

For structure proof, 1-dimethylamino-4-phenylsulfonylnaphthalene (VII) was synthesized from 1-bromo-4-dimethylaminonaphthalene⁸ (VIII). In a three-necked flask equipped with a stirrer were placed a solution of 1-bromo-4-dimethylaminonaphthalene⁸ (8.80 g., 0.0352 mole) in absolute diethyl ether (25 ml.) To this solution maintained at -65° was added *n*-butyllithium (37.8 ml., 0.0363 mole) during 30 min. This new solution was added dropwise during 12 min. to a cold solution (-70°) of benzenesulfonyl fluoride¹² (5.42 g., 0.0351 mole) in ether (25 ml.). The resulting reaction mixture was stirred for 2 hr. at -70° , warmed to room temperature during 2 hr., and then hydrolyzed with water (50 ml.). Excess ether was used to dissolve the organic reaction products; phases were separated and the ether phase was dried over sodium sulfate. After concentration to a volume of 50 ml., the ether solution deposited two different kinds of crystals: (a) rhombohedrons, m.p. 154–157° and (b) monoclinic prisms, m.p. 135–138°. These materials were separated mechanically. Purified 1,1-dimethylamino-4-phenylsulfonylnaphthalene (1.755 g., 16.0%, m.p. 157.5–158.5°) was isolated after three recrystallizations of the former compound from methanol. A mixed melting point of this material with the sulfone obtained by cleavage of water from 5,5-dimethylamino-8-phenylsulfonyl-1,2,3,4-tetrahydronaphthalene 1,4-endoxide (VI) was undepressed. The infrared spectra of these two compounds were superimposable.

Anal. Calcd. for $C_{18}H_{17}NO_2S$: C, 69.42; H, 5.50; N, 4.50; S, 10.30. Found: C, 69.65; H, 5.52; N, 4.28; S, 10.19.

Acknowledgment.—The authors would like to thank the Deutschen Forschungsgemeinschaft for funds which supported in part this investigation, Dr. H. Harle for the valuable results collected during his exploratory investigations, and Dr. Kobrich for assistance in obtaining the infrared data. One of us¹ is indebted to The Fulbright Program for a stipend.

(11) The results were the same in diethyl ether as in THF.

(12) W. Davies and J. H. Dick, *J. Chem. Soc.*, 2104 (1931).

170

(5)

(3)

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P: 1559-1570



240. G. Schroeter: Ueber Acylierung von Anilin-sulfosäuren.
 (Mittheilung aus dem chemischen Institut der Universität Bonn.)
 (Eingegangen am 14. April 1906.)

Die folgenden Versuche sind aus Betrachtungen über den Process des Färbens²⁾ hervorgegangen:

Da ein grosser Theil der technischen Farbstoffe Sulfosäuren oder Carbonsäuren sind, die Faserstoffe aber alkoholische Hydroxyl- oder Amin Gruppen enthalten, hatte ich den Gedanken, diese Farbstoffe durch Ester- oder Amid-Bildung mit den Faserstoffen zu vereinigen. Diese Methode würde besonders für Baumwollfärbungen von Interesse sein, indem man etwa die Chloride der sauren Farbstoffe mit mercerisirter

¹⁾ Journ. für prakt. Chem. [2] 31, 474.

²⁾ Vergl. A. Binz und G. Schroeter, diese Berichte 35, 4225 [1902]; 36, 3005 [1903]; 37, 727 [1904].

Cellulose zur Umsetzung bringen und auf diese Weise auch »adjective« Farbstoffe direct auf Baumwolle fixiren könnte.

Für Darstellung der Chloride erschienen mir die von Phenolen, sowie von primären und secundären Aminen derivirenden Sulfo- oder Carbon Säuren von Farbstoffen an sich ungeeignet, weil das als allgemeinstes Chlorierungsmittel in Betracht kommende Phosphorpentachlorid gleichzeitig mit den Säuregruppen zumeist auch die phenolischen Hydroxyl- und Amin-Gruppen angreifen wird. Geeigneter erscheinen die Sulfo- und Carbon-Säuren tertiärer Anilinfarbstoffe. In der ersteren Gruppe von Farbstoffen konnte man aber daran denken, die phenolischen Hydroxyl- und die Amin-Gruppen durch Einführung von Acylgruppen vor dem Angriff des Phosphorpentachlorids zu schützen.

Der Versuch zeigte indessen, dass weder acetyliertes Säuregelb (Acetaminoazobenzolsulfosäure) noch Heliantbin (Dimethylaminoazobenzolsulfosäure) mit Phosphorpentachlorid unter Chloridbildung reagieren; Reaction tritt erst bei höherer Temperatur ein und führt dann zur Verkohlungs der Producte.

Einen besseren Erfolg hatte eine Methode, die auf folgender Erwägung beruhte: Zur Darstellung von Azofarbstoffen auf der Faser, z. B. Baumwolle, konnte erst die eine (saure) Componente des Azofarbstoffs esterartig fixirt und die so vorbehandelte Faser alsdann mit Diazolösungen combinirt werden.

Es ergaben sich darans zunächst Studien über die Chloridbildung aus Anilinsulfosäuren, in welchen die Amingruppen durch Acylierung oder Alkylierung geschützt sind.

Die Literatur über die Sulfosäuren acylierter aromatischer Amino ist aber lückenhaft, und das Studium dieser Körper hat mich daher etwas weiter vom Wege abgeführt. Die Versuche hierüber hat z. Th. Hr. Dr. Georg Rösing¹⁾ unter meiner Leitung ausgeführt; sie ergaben folgende Resultate:

1. Die Acetylierung des Natriumsalzes der Sulfanilsäure mit Essigsäureanhydrid verläuft im Gegensatz zu bisherigen Angaben hierüber spontan unter starker Wärmeentwicklung; ebenso vollzieht sich die Acetylierung der Naphtionsäure und 1.5-Naphtylaminsulfosäure. Auch die Einführung von Benzoyl- und Benzolsulfonyl-Resten in die Natrium-Sulfonate aromatischer Amine geht leicht von Statten.

2. Die freie Acetsulfanilsäure lässt sich entgegen den bisherigen Angaben aus ihren Salzlösungen durch concentrirte Mineralsäuren ausfällen und ist relativ beständig; auffallender Weise verläuft ihre Selbstverseifung in concentrirter alkoholischer Lösung schneller als in concentrirter wässriger Lösung.

¹⁾ Inauguraldissertation, Bonn 1906.

Auch die anderen Acylaminosulfosäuren lassen sich in ähnlicher Weise aus ihren Salzlösungen ausfällen und sind, soweit untersucht, beständig.

3. Die Chloride dieser Säuren bilden sich sehr leicht und glatt beim Verreiben der Natriumsalze mit Phosphorpentachlorid und sind sehr beständige, wohlcharakterisirte Körper.

4. Acetnaphthionsäure und 1.5-Acetnaphthylaminsulfosäure kuppeln mit den Diazolösungen aus den Nitranilinen, α -Naphthylamin, Benzidin zu gefärbten, wohlkrystallisirenden, relativ beständigen Körpern, welche aber beim Kochen mit Wasser Stickstoff entwickeln, daher Diazoniumsalze sind.

Benzolsulfon-naphthionsäure und Benzolsulfon-sulfanilsäure verhalten sich gegen die Diazoniumlösungen aus Benzidin, α -Naphthylamin, *o*- und *m*-Nitranilin u. a. ebenso; nur mit diazotirtem *p*-Nitranilin kuppeln diese Körper zu echten Azofarbstoffen. Dieses Resultat ist deshalb auffallend, weil ein solcher Unterschied in dem Kuppelungsvermögen von Diazolösungen nahe verwandter Körper gegenüber ein und derselben Componente meines Wissens bisher nicht beobachtet wurde.

5. Behandelt man Baumwolle mit Lösungen der Chloride von Benzolsulfon Naphthionsäure oder Sulfanilsäure, so wird die Faser beim Nachbehandeln mit *p*-Nitrophenyldiazoniumsalzen orangebraun bezw. gelb angefärbt. Die Technik dieser Färbeversuche muss jedoch noch besser durchgearbeitet und an weiterem Material geprüft werden.

Specieller Theil

Acet-sulfanilsäure, $\text{CH}_3\text{CO.NH}[4]\text{C}_6\text{H}_4[1]\text{SO}_3\text{H}$.

Ueber Acetylsulfanilsäure berichten Nietzki und Benkiser¹⁾, Junghahn²⁾, sowie eine Patentschrift von Hofmann-La Roche & Co., Basel³⁾. Nietzki und Benkiser erhitzen sulfanilsaures Natrium mit überschüssigem Essigsäureanhydrid auf 140°, bis alles in Lösung gegangen ist; der beim Erkalten gewonnene Brei wird von überschüssigem Anhydrid befreit, das so gewonnene Salz der acetylrten Säure ist jedoch den Analysen zufolge noch nicht rein. Die freie Acetsulfanilsäure vermochten Nietzki und Benkiser aus dem Salz auf keine Weise darzustellen, sodass sie an der Existenzfähigkeit der freien Säure zweifeln. Junghahn berichtet, diese Säure durch Behandeln von Acetanilid mit rauchender Schwefelsäure in verfilzten Nadeln gewonnen zu haben, theilt jedoch keine Analysen mit und macht

¹⁾ Diese Berichte 17, 707 [1884]. ²⁾ Diese Berichte 33, 1366 [1900].

³⁾ Friedländer, Fortschritte der Theerfarbenindustrie 4, 1152.

keine näheren Angaben ausser der, dass die Säure nicht gut haltbar sei. Hoffmann-La Roche & Co. kochen Natriumsulfanilat 6—8 Stunden mit Eisessig, destilliren die überschüssige Essigsäure ab, lösen den Rückstand in wenig heissem Wasser und fällen mit Alkohol; Analysen und nähere Angaben fehlen auch hier; jedoch soll das Salz antipyretische Eigenschaften haben.

Ich fand nun zu meiner Ueberraschung Folgendes: Werden gleiche Theile trocknes, feingepulvertes Natriumsulfanilat und Essigsäureanhydrid gemischt, so tritt nach wenigen Sekunden Wärmeentwicklung ein, die sich bis über 100° steigert, und die Acetylierung ist so nach kurzer Zeit vollendet, der Gleichung: $(\text{CH}_3\text{CO})_2\text{O} + \text{NH}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{SO}_3\text{Na} = \text{CH}_3\text{CO} \cdot \text{NH} \cdot \text{C}_6\text{H}_4 \cdot \text{SO}_3\text{Na} + \text{CH}_3 \cdot \text{COOH}$ entsprechend. Das pulvrige, mit trockenem Aether gründlich gewaschene und im Vacuum über Natrockalk getrocknete Acetylierungsproduct muss zur völligen Reinigung ca. 10 Stunden im trocknen Luftstrom auf 150—160° erhitzt werden, da Essigsäure hartnäckig zurückgehalten zu werden scheint. Ehe ich diesen Umstand erkannt hatte, schloss ich aus verschiedenen Natrium-, Schwefel- und Stickstoff Bestimmungen, dass das Product diacetsulfanilsaures Natrium sei. Die Natriumbestimmung des in oben angegebener Weise bis zur Gewichtconstanz getrockneten Productes ergab jedoch die für monoacetsulfanilsaures Natrium stimmende Zahl:

0.2952 g Sbst.: 0.0874 g SO_4Na_2 .

$\text{C}_8\text{H}_8\text{NSO}_4\text{Na}$. Ber. Na 9.70. Gef. Na 9.63.

Das Salz¹⁾ ist sehr leicht löslich in Wasser, schwer löslich in Alkohol und unlöslich in Aether. Versetzt man die concentrirte wässrige Lösung desselben mit concentrirter Salzsäure unter Kühlung, so erstarrt nach kurzer Zeit die ganze Flüssigkeit zu einem steifen Brei von feinen Nadelchen. Dies ist die freie Acet-sulfanilsäure; sie wird abgesogen, mit etwas concentrirter Salzsäure und alsdann mit Aceton und Aether gewaschen. Das so gewonnene Product ist in Wasser sehr leicht löslich, wird aber aus dieser Lösung durch concentrirte Salzsäure wieder ausgeschieden, auch in Alkohol löst die Säure sich im Gegensatz zu der unlöslichen Sulfanilsäure leicht auf. Um Spuren von mitgerissenem Chlornatrium oder von Sulfanilsäure aus der Acetsulfanilsäure zu entfernen, löst man sie in Alkohol, filtrirt eventuell und füllt die alkoholische Lösung mit Aether; sie scheidet sich dabei zunächst ölig aus, wird aber bald pulvrig. Sie enthält dann noch

¹⁾ Das Salz scheint nach Versuchen, die Hr. Privatdocent Dr. Hilbert angestellt hat, die Brennbarkeit hatte, physiologisch unwirksam zu sein (vergl. oben Hoffmann-La Roche).

2 Mol. Krystallwasser: $\text{CH}_3\text{CO.NH.C}_6\text{H}_4.\text{SO}_3\text{H} + 2 \text{H}_2\text{O}$, wie die Titration mit $\frac{1}{10}$ -Natronlauge und die Schwefelbestimmung ergaben:
 0.4593 g Säure brauchten 18.75 ccm $\frac{1}{10}$ -NaOH. — 0.2621 g Subst.:
 0.2354 g SO_4Ba .

$\text{C}_8\text{H}_9\text{NSO}_4 + 2 \text{H}_2\text{O}$. Ber. NaOH 15.94, S 12.75.

Gef. » 16.33, » 12.54.

Die Acetsulfanilsäure ist in verdünnter wässriger Lösung in der Kälte beständig. Der Titer einer 0.5-procentigen Lösung in Wasser und einer 1-procentigen in Alkohol war nach tagelangem Stehen unverändert geblieben. Auch beim Kochen erleiden verdünnte wässrige Lösungen nur langsam Hydrolyse; nach 2-stündigem Kochen einer 0.5-procentigen Lösung waren erst ca. 18 pCt. verseift. Schneller verläuft die Selbstverseifung in stark concentrirten Lösungen und zwar merkwürdiger Weise schneller in alkoholischer als in wässriger Lösung:

0.6024 g Acetsulfanilsäure, in 2 ccm Wasser gelöst, hatte nach ca. 70-stündigem Stehen Krystalle von Sulfanilsäure ausgeschieden; die Titration ergab, dass 35.37 pCt. der Acetsäure in Sulfanilsäure und Essigsäure gespalten waren.

0.7964 g Acetsulfanilsäure, in 4 ccm 99-procentigem Alkohol gelöst, hatte schon nach 24 Stunden erhebliche Mengen Sulfanilsäure ausgeschieden, deren Gewicht nach 3 Tagen ca. 0.28 g betrug, d. i. 50 pCt. der Theorie; die Flüssigkeit roch stark nach Essigester, sodass die Spaltung der Acetsulfanilsäure in Alkohol nach der Gleichung:

$\text{CH}_3\text{CO.NH.C}_6\text{H}_4.\text{SO}_3\text{H} + \text{C}_2\text{H}_5.\text{OH} = \text{CH}_3.\text{COOC}_2\text{H}_5 + \text{H}_2\text{N.C}_6\text{H}_4.\text{SO}_3\text{H}$
 zu verlaufen scheint.

Acet-sulfanilsäurechlorid, $\text{CH}_3\text{CO.NH.C}_6\text{H}_4.\text{SO}_2\text{Cl}$.

10 g trocknes, acetsulfanilsäures Natrium werden mit 10 g Phosphor-pentachlorid im Mörser verrieben; das Gemisch verflüssigt sich erst und nimmt dann wieder festere Consistenz an; es wird jetzt in kaltes Wasser eingetragen (zur Lösung von Chlornatrium und Phosphoroxychlorid) und so das wasserbeständige Acetsulfanilsäurechlorid als weisses, phosphor-freies Pulver gewonnen; Ausbeute 7—8 g. Das Chlorid ist in Aether, Essigester und Alkohol leicht löslich, aus alkoholischer Lösung wird es durch Wasser unverändert ausgefällt. Durch Umkrystallisiren aus heissem Benzol wird es in kleinen Nadelchen erhalten, die bei 149° schmelzen.

0.2915 g Subst.: 0.4390 g CO_2 , 0.0983 g H_2O . — 0.3466 g Subst. gaben nach Zerstörung durch Kochen mit Natronlauge 0.2154 g AgCl .

$\text{C}_8\text{H}_8\text{NO}_3\text{SCL}$. Ber. C 41.11, H 3.42, Cl 15.20.

Gef. » 41.07, » 3.74, » 15.37.

Mit Natriumalkoholat setzt sich die alkoholische Lösung des Chlorids zu dem Acet-sulfanilsäureäthylester, $\text{CH}_3\text{CO.NH.C}_6\text{H}_4.\text{SO}_2.\text{OC}_2\text{H}_5$, um, der, aus Benzol umkrystallisirt, weisse Nadelchen vom Schmp. 115° bildet.

0.2212 g Sbst.: 0.4020 g CO₂, 0.1060 g H₂O. — 0.4012 g Sbst.: 0.851 g SO₄Ba.

C₁₀H₁₂NSO₄. Ber. C 49.39, H 5.35, S 13.17.
Gef. » 49.56, » 5.27, » 13.28.

Mit Phenetidin setzt sich das Chlorid in alkoholischer Lösung leicht zu Acet-sulfanilsäure-phenetidid, CH₃CO.NH.C₆H₄.SO₂.NH.C₆H₄.[4]OC₂H₅, um, das, aus wässrigem Aceton umkrystallisiert, weisse, glänzende Blättchen vom Schmp. 204° bildet.

0.2380 g Sbst.: 0.5036 g CO₂, 0.1167 g H₂O. — 0.2108 g Sbst.: 0.4675 g SO₄Ba.

C₁₆H₁₈N₂SO₄. Ber. C 57.49, H 5.39, S 9.58.
Gef. » 57.70, » 5.44, » 9.55.

Acet-naphthionsäure, CH₃CO.NH[4].C₁₀H₆.[1]SO₂H.

Trocknes, feingepulvertes Natriumnaphthionat wird mit dem gleichen Gewicht Essigsäureanhydrid vermischt, auch hier erwärmt sich das Gemisch spontan bis 60° und darüber; nach völliger Umsetzung sicher zu sein, wurde schliesslich noch einige Zeit auf dem Wasserbade erhitzt. Das Product wurde dann mit absolutem Alkohol gründlich gewaschen und bei 120° getrocknet. Das so erhaltene weisse Pulver wurde analysirt.

0.1961 g Sbst.: 0.0493 g SO₄Na₂. — 0.4105 g Sbst.: 0.3420 g SO₄Ba.
C₁₂H₁₀O₄NSNa. Ber. S 11.15, Na 8.02.
Gef. » 11.44, » 8.14.

Es liegt demnach reines Acetylnatriumnaphthionat vor, das in Wasser sehr leicht, in Alkohol und Aether schwer oder nicht löslich ist. Die concentrirte, wässrige Lösung des Salzes giebt wie beim Acetylnatriumsulfanilat auf Zusatz von concentrirter Salzsäure einen steifen Brei von Krystallnadelchen, die aus der freien Acetnaphthionsäure besteht. Auch diese Säure ist in Wasser und Alkohol leicht löslich, unlöslich in Aether und schwer löslich in concentrirter Salzsäure. Beim Kochen mit Wasser wird sie in Essigsäure und Naphthionsäure gespalten, die sich ausscheidet. Die aus alkoholischer Lösung mit Aether gefällte Säure scheint einer Krystallwasserbestimmung zufolge 1/2 Mol. Wasser zu enthalten. Das bei 120° getrocknete Product gab auf wasserfreie Säure stimmende Zahlen:

0.2280 g Sbst.: 0.4553 g CO₂, 0.0392 g H₂O. — 0.4486 g Sbst.: 0.665 g SO₄Ba.

C₁₂H₁₁NO₄S. Ber. C 54.34, H 4.15, S 12.09.
Gef. » 54.46, » 4.31, » 12.45.

Acet-naphthionsäurechlorid, CH₃CO.NH[4].C₁₀H₆.[1]SO₂Cl.

10 g trocknes Acetylnaphthionat werden mit dem gleichen Gewicht Phosphoryenchlorid im Mörtel verrieben; nach etwa 15 Minuten

bringt man die halbfeste Masse in Wasser und saugt das nach Zerstörung der Phosphorchloride als weissgelbes Pulver sich absetzende Chlorid ab. Es wird durch Umkrystallisiren aus Essigester in schönen, durchsichtigen Krystallen erhalten, die beim Erhitzen ohne zu schmelzen verkohlen. Ausbeute ca. 9 g. <

0.1339 g Sbst.: 0.3439 g CO₂, 0.0586 g H₂O. — 0.2348 g Sbst.: 0.1175 g AgCl.

C₁₂H₁₀O₃NSCl. Ber. C 50.79, H 3.52, Cl 12.52.
Gef. » 51.01, » 3.52, » 12.45. D.

Durch Umsetzen des Chlorids mit Natriumäthylatlösung entsteht der Acet-naphthionsäureäthylester, CH₃CO.NH[4].C₁₀H₆.[1]SO₂.OC₂H₅, welcher, aus Benzol umkrystallisirt, weisse Nadeln vom Schmp. 148° bildet. Mit Anilin (2 Mol.) entsteht Acet-naphthionsäureanilid, CH₃CO.NH[4].C₁₀H₆.[1]SO₂.NH.C₆H₅, das aus Alkohol in Blättchen vom Schmp. 231° erhalten wird.

1.5-Acetamino-naphtalin-sulfosäure,
CH₃CO.NH[5].C₁₀H₆.[1]SO₃H.

Das aus technischer 1.5-Aminonaphtalinsulfosäure gewonnene rohe Natriumsalz wurde durch Umkrystallisiren aus Alkohol gereinigt, für die Behandlung mit Essigsäureanhydrid bei 120° getrocknet und gepulvert. Eine so starke, spontane Temperaturerhöhung wie Natriumsulfanilat und Natriumnaphthionat zeigt dieses Salz beim Behandeln mit Essigsäureanhydrid nicht, jedoch wird auch hier durch einstündiges Erwärmen des Gemisches auf dem Wasserbade die Acetylierung leicht zu Ende geführt; das mit Alkohol und Aether gewaschene Rohproduct ergab stimmende Analysenzahlen:

0.1674 g Sbst.: 0.043 g SO₄Na₂. — 0.3925 g Sbst.: 16.6 ccm N (22°, 764 mm).

C₁₂H₁₀O₄NSNa. Ber. Na 8.02, N 4.88.
Gef. » 8.32, » 4.82.

Das Salz lässt sich in gleicher Weise, wie das Natrium-acetylnaphthionat, in 1.5-Acetylamino-naphtoesäurechlorid, CH₃CO.NH[5].C₁₀H₆.SO₂Cl, überführen.

Benzoyl-sulfanilsäure, C₆H₅CO.NH[4].C₆H₄.[1]SO₃H.

20 g sulfanilsaures Natrium werden in wenig Wasser gelöst und im Verlauf einer Stunde mit 28 g Benzoylchlorid und mit Natronlauge versetzt und geschüttelt. Die alkalische Flüssigkeit erstarrt dann durch ausgeschiedenes benzoylsulfanilsaures Natrium zu einem Brei, das, abgesogen und aus Wasser umkrystallisirt, in weissen Nadeln erhalten wird.

0.2315 g Subst.: 0.0544 g SO_4Na_2 . — 0.4200 g Subst.: 0.3240 g SO_4Ba .

$\text{C}_{13}\text{H}_{10}\text{NO}_4\text{SNa}$. Ber. Na 7.68, S 10.70.
Gef. » 7.61, » 10.59.

Beim Verreiben mit Phosphorpentachlorid ergibt das Salz Benzoyl-sulfanilsäurechlorid, $\text{C}_6\text{H}_5\text{CO}\cdot\text{NH}\cdot\text{C}_6\text{H}_4\cdot\text{SO}_2\text{Cl}$, welches, aus Benzol umkrystallisiert, gelbe Nadelchen vom Schmp. 176° bildet.

Benzoyl-naphthionat, $\text{C}_6\text{H}_5\text{CO}\cdot\text{NH}[4]\cdot\text{C}_{10}\text{H}_6\cdot\text{SO}_3\text{Na}$, wurde durch Erhitzen von Naphthionat mit Benzoesäureanhydrid dargestellt, die Benzoesäure durch Auskochen mit Aether entfernt und das Product aus Wasser umkrystallisiert.

Benzolsulfon-sulfanilsäure, $\text{C}_6\text{H}_5\text{SO}_2\cdot\text{NH}[4]\cdot\text{C}_6\text{H}_4\cdot[1]\text{SO}_3\text{H}$.

10 g sulfanilsaures Natrium, in möglichst wenig Wasser gelöst, werden mit 16 g Benzolsulfchlorid und Natronlauge in kleinen Portionen versetzt und geschüttelt; das benzolsulfonsulfanilsaure Natrium scheidet sich hiernach als weisses Magma ab, das abgesogen und aus Alkohol umkrystallisiert wird. Zur Analyse wird mehrere Stunden bei 140° getrocknet.

0.2800 g Subst.: 0.0565 g SO_4Na_2 . — 0.8877 g Subst.: 31.8 ccm N (14° , 748 mm).

$\text{C}_{12}\text{H}_{10}\text{NS}_2\text{O}_3\text{Na}$. Ber. Na 6.86, N 4.18.
Gef. » 6.54, » 4.14.

Versetzt man eine gesättigte, wässrige Lösung des Salzes unter Kühlung mit concentrirter Schwefelsäure, so scheidet sich die Benzolsulfon-sulfanilsäure als allmählich erstarrendes Oel ab. Die abgesogenen Krystalle schmelzen bei 78° und sind in Wasser leicht löslich. Verreibt man das Salz mit dem gleichen Gewicht Phosphorpentachlorid, zersetzt die Phosphorchloride mit Wasser und krystallisiert das abgesogene Pulver aus Essigester um, so erhält man Benzolsulfon-sulfanilsäurechlorid, $\text{C}_6\text{H}_5\cdot\text{SO}_2\cdot\text{NH}[4]\cdot\text{C}_6\text{H}_4\cdot[1]\text{SO}_2\text{Cl}$, in weissen Nadeln vom Schmp. 177° :

0.4024 g Subst.: 0.1721 g AgCl. — 0.2471 g Subst.: 0.3911 g CO_2 , 0.0769 g H_2O .

$\text{C}_{12}\text{H}_{10}\text{NS}_2\text{O}_4\text{Cl}$. Ber. Cl 10.70, C 43.43, H 3.01.
Gef. » 10.58, » 43.16, » 3.50.

Benzolsulfon-naphthionsäure, $\text{C}_6\text{H}_5\text{SO}_2\cdot\text{NH}[4]\cdot\text{C}_{10}\text{H}_6\cdot[1]\text{SO}_3\text{H}$.

Das Natriumsalz dieser Säure wird aus Naphthionat, Benzolsulfchlorid und Natronlauge gewonnen und scheidet sich als röthlich gefärbter, schleimiger Niederschlag ab, der in kaltem Wasser schwer löslich ist, aus heissem Wasser oder besser aus Alkohol umkrystallisiert in Blättchen gewonnen wird. Zur Trocknung wird 4—5 Stdn. im Vacuum auf 80° erhitzt.

0.1956 g Sbst.: 0.0355 g SO_4Na_2 . — 0.3679 g Sbst.: 0.4378 g SO_4Ba . —
0.6175 g Sbst.: 19.7 ccm N (25°, 764 mm).

$\text{C}_{16}\text{H}_{12}\text{O}_5\text{NS}_2\text{Na}$. Ber. Na 5.98, N 3.63, S 16.62.

Gef. » 5.88, » 3.57, » 16.34.

Die freie Säure wird aus der concentrirten, wässrigen Lösung des Salzes durch concentrirte Salzsäure in Nadelchen ausgefällt. Durch Umsetzung mit Phosphorchlorid in der mehrfach angegebenen Weise erhält man aus dem Salz das Benzolsulfon-naphthionsäurechlorid, $\text{C}_6\text{H}_5\text{SO}_2\text{NH}[\text{C}_{10}\text{H}_6\text{SO}_2\text{Cl}]$, das, aus Benzol umkrystallisirt, schöne Krystalle vom Schnmp. 171° bildet:

0.1957 g Sbst.: 0.3603 g CO_2 , 0.0624 g H_2O . — 0.3361 g Sbst.: 0.1263 g AgCl.

$\text{C}_{16}\text{H}_{12}\text{O}_4\text{NS}_2\text{Cl}$. Ber. C 50.32, H 3.12, Cl 9.30.

Gef. » 50.21, » 3.54, » 9.29.

Umsetzungen der aromatischen Acylamino-sulfosäuren mit Diazoniumsalzen.

Ueber die Combination von acylirten Anilinen mit Diazoniumsalzen findet sich eine Arbeit von Witt und Schmitt¹⁾, derzufolge die aus *p*-Toluolsulfochlorid und α - und β -Naphthylamin entstehenden Amide, $\text{C}_7\text{H}_7\text{SO}_2\text{NH}\cdot\text{C}_{10}\text{H}_7$, mit Diazolösungen zu Azofarbstoffen kuppeln.

Versetzt man die wässrige Lösung von Natriumacetonaphthionat, $\text{CH}_3\text{CO}\cdot\text{NH}\cdot\text{C}_{10}\text{H}_6\text{SO}_3\text{Na}$, mit äquimolaren Mengen von Diazoniumlösungen aus α -Naphthylamin, *o*-, *m*- und *p*-Nitranilin oder Benzidin, so krystallisiren aus den zunächst klaren Lösungen alsbald die Kupplungsproducte aus: aus Naphthylamin gelbbraune, seidenglänzende Blättchen, aus *p*- und *o*-Nitro-anilin dunkelrothe, glänzende Nadeln, aus *m*-Nitro-anilin hellgelbrothe Blättchen, aus Benzidin stark glänzende, rothe Nadeln. Diese Producte sind aus mässig heissem Wasser umkrystallisirbar, in Natronlauge löslich und bei vorsichtiger Behandlung mit Säure wieder fällbar; durch Ammoniak werden sie sämmtlich aus den wässrigen Lösungen als dunkel violette Niederschläge gefällt. Beim Erhitzen auf dem Platinbleche zeigen die Producte nur schwache Verpuffung. Beim Kochen mit Wasser aber entwickeln sie Stickstoff und zwar überall da, wo es geprüft wurde quantitativ 1 oder 2 Mol. Aehnlich verhalten sich die Producte aus 1.5-acetylamino-naphthalinsulfosaurem Natrium mit diazotirtem *p*-Nitro-anilin (rothbraune Blättchen) und Benzidin (gelbbraune Blättchen.) Wegen ihrer Zersetzung beim Kochen mit Wasser halte ich alle diese Producte für Diazoniumsalze der allgemeinen Formel $\text{CH}_3\text{CO}\cdot\text{NH}\cdot\text{C}_{10}\text{H}_6\text{SO}_3\cdot\text{N}_2\text{Ar}$.

¹⁾ Diese Berichte 27, 2372 [1894].

Der durch Kochen mit Wasser abspaltbare Stickstoff, sowie der Gesamt-Stickstoff-, Kohlenstoff- und Wasserstoff-Gehalt wurden bestimmt bei den Producten aus Acetnaphthionat (I) und 1,5-Acetnaphthylaminsulfonat (II) mit *p*-Nitro-anilin und mit Benzidin:

I.

0,2641 g Sbst. (beim Kochen mit Wasser): 15,8 ccm N (20°, 766 mm). — 0,3012 g Sbst. (beim Verbrennen): 35,2 ccm N (15°, 764 mm). — 0,2021 g Sbst.: 0,5875 g CO₂, 0,0661 g H₂O.

C₁₃H₁₁N₄O₆S. Ber. N₂ 6,76, N₁ 13,52, C 52,17, H 3,38.
Gef. » 6,89, » 13,36, » 52,29, » 3,63.

0,2861 g Sbst. (beim Kochen mit Wasser): 20 ccm N (20°, 750 mm). — 0,2842 g Sbst. (beim Verbrennen): 29 ccm N (22°, 762 mm). — 0,2121 g Sbst.: 0,4562 g CO₂, 0,0681 g H₂O.

C₁₃H₁₂N₄O₆S₂. Ber. N₂ 7,61, N₁ 11,41, C 58,69, H 3,80.
Gef. » 7,87, » 11,53, » 58,65, » 3,56.

II.

0,1954 g Sbst. (beim Kochen mit Wasser): 12,2 ccm N (24°, 758 mm). — 0,1198 g Sbst. (beim Verbrennen): 14 ccm N (18°, 770 mm). — 0,2341 g Sbst.: 0,4472 g CO₂, 0,0742 g H₂O.

C₁₃H₁₁N₄O₆S. Ber. N₂ 6,76, N₁ 13,52, C 52,17, H 3,38.
Gef. » 6,98, » 13,67, » 52,08, » 3,51.

0,2013 g Sbst. (beim Kochen mit Wasser): 12,4 ccm N (19°, 766 mm). — 0,1340 g Sbst. (beim Verbrennen): 12,2 ccm N (15°, 758 mm). — 0,2387 g Sbst.: 0,4773 g CO₂, 0,0889 g H₂O.

C₁₆H₁₂N₆O₈S₂ + 3 H₂O. Ber. N₂ 7,09, N₁ 10,63, C 54,68, H 4,30.
Gef. » 7,13, » 10,64, » 54,54, » 4,09.

Während diesen Resultaten zufolge die Acetnaphthylaminsulfosäure mit Diazoniumlösungen durchweg zu allerdings stark gefärbten und auffallend beständigen Diazoniumsalzen kuppelt, geben die oben beschriebene Benzolsulfonaphthionsäure und die Benzolsulfonsulfanilsäure mit allen anderen untersuchten Diazoniumlösungen zwar auch Diazoniumsalze, mit *p*-Nitrophenyldiazoniumsalzen aber echte Azofarbstoffe:

Benzolsulfon-sulfanilsaures Natrium (3,5 g) + diazotirtes Benzidin (1,8 g) scheidet zunächst Schmierchen ab, nach mehreren Stunden hatten sich jedoch 3 g gelbbraune Krystalle abgesondert, welche in Natronlauge mit brauner Farbe löslich sind, mit Ammoniak einen violetschwarzen Niederschlag geben, beim trocknen Erhitzen verkohlen, beim Kochen mit Wasser 2 Mol. Stickstoff entwickeln:

0,2350 g Sbst.: 13,8 ccm N (22°, 762 mm).

C₁₆H₁₂N₆O₁₀S₄. Ber. N₂ 6,76. Gef. N₂ 6,66.

Benzolsulfon-sulfanilsaures Natrium (3,5 g) + diazotirtes *o*-Nitro-anilin (1 g): nach einiger Zeit orangegelbe Nadelchen, in

Natronlauge roth löslich, entwickeln beim Kochen mit Wasser 1 Mol. Stickstoff:

0.1379 g Subst.: 10 ccm N (22°, 752 mm).

$C_{18}H_{14}O_7S_2N_4$. Ber. N₂ 6.06. Gef. N₂ 5.90.

Benzolsulfon-sulfanilsaures Natrium (3.5 g) + diazotirtes *m*-Nitro-anilin (1 g): gelbes Pulver, in Natronlauge roth löslich, entwickelt beim Kochen mit Wasser 1 Mol. Stickstoff:

0.3075 g Subst.: 15.8 ccm N (27°, 762 mm).

$C_{18}H_{14}O_7S_2N_4$. Ber. N₂ 6.06. Gef. N₂ 6.09.

Benzolsulfon-sulfanilsaures Natrium (7 g) + diazotirtes *p*-Nitro-anilin (2 g): eigelber Niederschlag, nach dem Umkrystallisiren aus Wasser schöne, rothgelbe Nadeln; das Product ist in Natronlauge, wie in Ammoniak mit rother Farbe löslich, spaltet beim Kochen mit Wasser keinen Stickstoff ab, färbt Wolle in saurer Lösung gelb:

0.2312 g Subst.: 0.3960 g CO₂, 0.0636 g H₂O. — 0.2081 g Subst.: 22 ccm N (18°, 744 mm).

$C_{18}H_{14}N_4S_2O_7$. Ber. C 46.67, H 3.24, N 12.09.

Gef. » 46.71, » 3.29, » 11.94.

Diazoniumsalze wurden ferner noch erhalten aus diazotirtem «-Naphthylamin und Pseudocumidin mit Benzolsulfonsulfanilsäure.

Benzolsulfon-naphthionsaures Natrium + diazotirtes Benzidin: braungelbes Pulver, giebt beim Kochen mit Wasser 2 Mol. Stickstoff:

0.2400 g Subst.: 12.8 ccm N (23°, 762 mm).

$C_{24}H_{12}N_6S_2O_{10}$. Ber. N₄ 6.01. Gef. N₄ 6.02.

Benzolsulfon-naphthionsaures Natrium + diazotirtes *m*-Nitro-anilin: gelbes Pulver, spaltet beim Kochen mit Wasser 1 Mol. Stickstoff ab:

0.2198 g Subst.: 14.9 ccm N (22°, 762 mm).

$C_{22}H_{16}N_4S_2O_7$. Ber. N₂ 5.46. Gef. N₂ 5.26.

Benzolsulfon-naphthionsaures Natrium + diazotirtes *p*-Nitro-anilin scheidet nach einiger Zeit rothe Krystalle ab, die, aus Alkohol umkrystallisirt, in schönen, rothen Nadelchen erhalten werden; wird durch Kochen mit Wasser nicht zersetzt, färbt Wolle orange.

0.2646 g Subst.: 0.5021 g CO₂, 0.0821 g H₂O. — 0.2682 g Subst.: 25.7 ccm N (22°, 762 mm).

$C_{22}H_{16}N_4S_2O_7$. Ber. C 51.56, H 3.12, N 10.94.

Gef. » 51.75, » 3.45, » 10.83.

Es wurde nun versucht, die Farbstoffe: *p*-Nitrobenzolazo-Benzolsulfon-sulfanilsäure und -Benzolsulfon-naphthionsäure auf der Baumwolle als Celluloseester: $\text{Cell.O.SO}_2.\text{C}_6\text{H}_5 \left\{ \begin{array}{l} \text{NH.SO}_2.\text{C}_6\text{H}_5 \\ \text{N:N.C}_6\text{H}_4.\text{NO}_2 \end{array} \right.$ bzw. $\text{Cell.O.SO}_2.\text{C}_{10}\text{H}_7 \left\{ \begin{array}{l} \text{NH.SO}_2.\text{C}_6\text{H}_5 \\ \text{N:N.C}_6\text{H}_4.\text{NO}_2 \end{array} \right.$ zu erzeugen:

Baumwolle wurde mit der Chloroformlösung des Benzolsulfon-sulfanilsäurechlorids (s. oben) unter Zusatz von Pyridin einige Zeit am Rückflusskühler gekocht, aus der Flüssigkeit entfernt, mit Chloroform und mit Wasser gründlich gewaschen. Die Baumwolle wies alsdann einen Schwefelgehalt von 2.77 pCt. auf. Die so behandelte Faser wurde nun in eine Lösung von *p*-Nitrobenzoldiazoniumacetat getaucht und einige Zeit darin belassen; die Faser färbte sich gelb an.

Mercerisirte Baumwolle wurde mit Chloroformlösung von Benzolsulfonnaphthionsäurechlorid (s. oben) mehrere Stunden geschüttelt und die so behandelte Faser in *p*-Nitrophenyldiazoniumlösung getaucht; sie hatte sich nach einiger Zeit orangebraun angefärbt.

Die Untersuchungen werden in der eingangs angedeuteten Richtung fortgeführt.

241. E. Erlenmeyer jun. und C. Barkow: Ueber stereo-isomere Zimmtsäuren.

[Mitgetheilt von E. Erlenmeyer jun.]

(Eingeg. am 10. April 1906; mitgeth. i. d. Sitzung v. Hrn. W. Markwald.)

Die Theorie über geometrische Isomerie verlangt, dass Körper von der Constitution der Zimmtsäure in zwei und nicht mehr isomeren Modificationen existiren.

Nachdem nun durch die Untersuchungen von Liebermann, Erlenmeyer sen. und die meinigen festgestellt wurde, dass es mindestens vier isomere Zimmtsäuren giebt, war der Beweis erbracht, dass die Anschauung über die Isomerie bei Aethylenderivaten erweitert werden muss.

Die nächstliegende Möglichkeit, in möglichster Anlehnung an die bewährte van't Hoff'sche Theorie zu einer Erweiterung der Anschauungen über die Isomerie bei Aethylenderivaten zu gelangen, bot die Annahme, dass ausser den geometrisch isomeren Aethylenderivaten, bei denen die vier Gruppen in einer Ebene liegen, noch solche denkbar sind, bei denen dieselben im Raume vertheilt sind.



DEUTSCHES PATENTAMT

INTERNATIONALE KL. C 07 c; d

AUSLEGESCHRIFT I 016 717

ANMELDETAG: 30. APRIL 1956
BEKANNTMACHUNG DER ANMELDUNG UND AUSGABE DER AUSLEGESCHRIFT 3. OKTOBER 1957

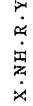
Verfahren zur Herstellung aromatischer Amine mit sekundärer Aminogruppe

Anmelder:

Kalle & Co. Aktiengesellschaft, Wiesbaden-Biebrich, Rheinstr. 25

Dr. Oskar Süs, Dr. Hans Heiss, Dipl.-Chem. Dr. Karl Möller und Dr. Wilhelm Neugebauer, Wiesbaden-Biebrich, sind als Erfinder genannt worden

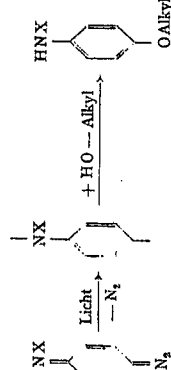
Gegenstand der vorliegenden Erfindung ist ein Verfahren zur Herstellung aromatischer Amine mit sekundärer Aminogruppe und einem zu dieser Aminogruppe paraständigen Substituenten, die der allgemeinen Formel



entsprechen, in welcher X Aryl oder Acyl oder Sulfonyl, R ein aromatisches Ringsystem, Y einen zur Aminogruppe p-ständigen Alkoxy- oder Aryl- oder aromatischen heterocyclischen Rest bedeutet. Unter aromatischen heterocyclischen Resten oder Verbindungen werden heterocyclische Reste oder Verbindungen mit aromatischem Charakter verstanden.

Das Verfahren zur Herstellung dieser aromatischen Amine entsprechend obiger allgemeiner Formel besteht darin, daß man Imminochinon-(1,4)-diäzide, in denen das Wasserstoffatom der Imminogruppe durch X = Aryl oder Acyl oder Sulfonyl ersetzt ist, in Gegenwart von aromatischen Alkoholen oder aromatischen, aber wenigstens ein reaktionsfähiges, an einem Ringkohlenstoff sitzendes Wasserstoffatom verfügend aromatischen oder aromatisch-heterocyclischen Verbindungen in Abwesenheit von Wasser mit Lichtstrahlen des ultravioletten bis sichtbaren Spektralbereichs behandelt. Als besonders wirksam haben sich Lichtstrahlen erwiesen, in denen neben sichtbarem Licht ein hoher Anteil von ultraviolettem Licht enthalten ist.

Das Verfahren wird vorteilhaft so ausgeführt, daß man Lösungen oder Suspensionen der oben definierten, als Ausgangsmaterial dienenden Imminochinon-(1,4)-diäzide in aliphatischen Alkoholen, denen gegebenenfalls noch indifferenten, organische Lösungsmittel zugesetzt sein können, in Abwesenheit von Wasser so lange belichtet, bis kein Imminochinondiazid mehr nachweisbar ist. Die Umsetzung verläuft wahrscheinlich nach folgendem Reaktionsschema, das an Hand eines Imminobenzochinon-(1,4)-diäzids dargestellt wird:



Die Umsetzung verläuft analog, wenn man dabei an Stelle der aliphatischen Alkohole aromatische oder aromatisch-heterocyclische Verbindungen mit wenigstens einem reaktionsfähigen Wasserstoffatom an einem Ringkohlenstoff verwendet, beispielsweise Benzol, Toluol, Naphthalin, Pyridin.

2

Die nach dem Verfahren gemäß der Erfindung hauptsächlich hergestellten Verbindungen sind Zwischenprodukte, die hauptsächlich für die Herstellung von Farbstoffen praktische Bedeutung besitzen. Sie sind auf anderem Wege größtenteils nur schwer und unter Bildung von erheblichen Mengen von Nebenprodukten erhältlich. Das vorliegende Verfahren dagegen gestattet ihre Herstellung in glatt verlaufender, einfach durchzuführender Reaktion.

Beispiel 1

Eine Suspension von 4 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 400 ccm Methanol wird im Sonnenlicht belichtet. Sobald das Imminochinondiazid völlig in Lösung gegangen und keine Diazoverbindung mehr nachzuweisen ist, wird das Methanol abgedampft. Das ölig anfallende 4-(Benzolsulfonyl-imino)-1-methoxybenzol erstarrt beim Erkalten kristallin. Es hat den Schmelzpunkt 88 bis 89°. Die Ausbeute beträgt 3,55 g.

Die in analoger Weise vorgenommene Belichtung von 4 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in Gegenwart von 400 ccm n-Propyl-alkohol ergibt 2,9 g 4-(Benzolsulfonyl-imino)-1-(n-propyloxy)-benzol vom Schmelzpunkt 136 bis 137°. Man erhält in analoger Weise bei Verwendung von Glykoldimethylether an Stelle von Methanol 4-(Benzolsulfonyl-imino)-1-(β-methoxy-äthoxy)-benzol. Der beim Eindampfen des Reaktionsproduktes im Vakuum hinterbleibende rotbraune ölige Rückstand kristallisiert bei Zusatz von Wasser, 3 g des angewendeten Imminobenzochinondiazids ergeben 2,12 g Reaktionsprodukt vom Schmelzpunkt 98°.

Die Lösung von 10 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in einem Gemisch aus 1000 ccm Dimethylformamid und 3000 ccm Äthylalkohol wird

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belichtet und anschließend der Alkohol verdampft. Bei langsame Wassergabe zur zurückbleibenden Dimethylformamidlösung kristallisiert 4-(Benzolsulfonyl-imino)-1-äthoxybenzol aus. Es besitzt den Schmelzpunkt 127°, die Ausbeute beträgt 4,5 g.

Beispiel 2

Die Lösung von 5 g 4-(p-Toluolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 1250 ccm Äthanol wird belichtet und im Vakuum bei 40° zur Trockne eingedampft. Das 4-(p-Toluolsulfonyl-imino)-1-äthoxybenzol bildet farblose Nadeln, Schmelzpunkt 99 bis 101°. Die Ausbeute beträgt 2,75 g.

Beispiel 3

Eine Lösung von 8 g 2,5-Dimethyl-4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 200 ccm Dimethylformamid und 800 ccm Methanol wird belichtet. Nach dem Verdampfen des Methanols wird die zurückbleibende Dimethylformamidlösung mit Wasser versetzt und ausgeschüttelt. Das beim Verdampfen des Äthers hinterbleibende Rohprodukt wird aus Äther-Gasolin-Gemisch umkristallisiert. Das gereinigte 4-(Benzolsulfonyl-imino)-2,5-dimethyl-1-methoxybenzol hat den Schmelzpunkt 123 bis 124°. Die Ausbeute beträgt 3,3 g. Analog wird die Lösung von 5 g 2,5-Dichlor-4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 200 ccm Dimethylformamid und 500 ccm Methanol belichtet. Nach dem Verdampfen des Alkohols wird die zurückbleibende Lösung mit Wasser versetzt und ausgeschüttelt. Das aus Ätherlösung gewonnene 4-(Benzolsulfonyl-imino)-2,5-dichlor-1-methoxybenzol wird zur Reinigung aus Benzol-Gasolin-Gemisch umkristallisiert und schmilzt bei 148°. Die Ausbeute beträgt 2 g.

Beispiel 4

Die Lösung von 5 g 4-(Benzolsulfonyl-imino)-naphthochinon-(1,4)-diäzid-(I) in einer Mischung aus 250 ccm Dimethylformamid und 1000 ccm Methanol wird belichtet und der beim Verdampfen des Lösungsmittels im Vakuum hinterbleibende Rückstand aus Benzol-Gasolin-Gemisch umkristallisiert. Das gebildete 4-(Benzolsulfonyl-imino)-1-methoxy-naphthalin hat den Schmelzpunkt 132 bis 133°. Die Ausbeute beträgt 3,6 g.

Beispiel 5

Eine Lösung von 2,5 g 4-(Benzolsulfonyl-imino)-di-phenyl-chinon-(4,4')-diäzid-(II) in 125 ccm Dimethylformamid und 625 ccm Äthanol wird belichtet. Nach dem Verdampfen des Lösungsmittels im Vakuum wird der ölige Rückstand mit Methanol aufgenommen und die Lösung über Kohle filtriert. Das Filtrat wird eingedampft, beim Abkühlen kristallisiert 4-(Benzolsulfonyl-imino)-4-äthoxy-diphenyl aus. Es schmilzt bei 172 bis 173°, die Ausbeute beträgt 0,75 g.

Beispiel 6

5 g 2,5-Dimethoxy-4-benzoylimino-benzochinon-(1,4)-diäzid-(I) werden in 2500 ccm Methanol gelöst, und die Lösung wird wie in den vorhergehenden Beispielen belichtet. Das beim Verdampfen des Lösungsmittels hinterbleibende 4-Benzoylimino-1,2,5-trimethoxybenzol wird aus Methanol umkristallisiert und schmilzt bei 137 bis 138°. Ausbeute 1,75 g.

Beispiel 7

Die Lösung von 10 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 1000 ccm Dimethylformamid und 3000 ccm Benzol wird belichtet. Nach dem Verdampfen des Benzols wird das Reaktionsprodukt aus der

4

verbleibenden Lösung durch Zugabe von Wasser ausgefällt. Das gebildete 4-(Benzolsulfonyl-imino)-diphenyl bildet nach dem Umkristallisieren aus Methanol farblose Kristalle vom Schmelzpunkt 147 bis 148°. Die Ausbeute beträgt 4,5 g.

Beispiel 8

In analoger Weise wie im Beispiel 7 werden aus einer Lösung von 5 g 2,5-Dichlor-4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 200 ccm Dimethylformamid und 500 ccm Benzol 3,7 g 2,5-Dichlor-4-(Benzolsulfonyl-imino)-diphenyl in Form farbloser Kristalle und dem Schmelzpunkt 175° (aus Methanol) erhalten. Die Darstellung des Imino-chinondiazids geschieht analog der des im Beispiel 1 verwendeten.

Beispiel 9

Aus einer Lösung von 8 g 2,5-Dimethyl-4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 200 ccm Dimethylformamid und 800 ccm Benzol erhält man in analoger Weise wie im Beispiel 7 durch Belichten, Verdampfen des Benzols, Versetzen des Rückstandes mit Wasser und Ätherextraktion ein Rohprodukt, das durch Auflösen in Methanol und Wiederausfällen durch Wassergabe gereinigt wird. Das 2,5-Dimethyl-4-(Benzolsulfonyl-imino)-diphenyl bildet nach dem Umkristallisieren aus Methanol farblose Kristalle vom Schmelzpunkt 170 bis 171°. Die Ausbeute beträgt 5,3 g.

Beispiel 10

Eine Suspension von 2,6 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 400 ccm wasserfreiem Aceton und 150 ccm absolutem Toluol wird belichtet. Das Reaktionsgemisch wird mit Kohle behandelt, filtriert, und das Filtrat wird eingedampft. Der hinterbleibende Rückstand (2,5 g) erstarrt beim Erkalten zu einer kristallinen Masse. Durch fraktioniertes Umkristallisieren aus verdünntem Methanol gelingt die Trennung der beiden bei der Umsetzung in Licht entstandenen isomeren Verbindungen. Das schwerer lösliche, in Form farbloser Rhomben ausfallende 4-(Benzolsulfonyl-imino)-2-methyl-diphenyl schmilzt bei 155°. Die leichter lösliche para-Verbindung 4-(Benzolsulfonyl-imino)-4-methyldiphenyl wird in Form farblos, bei 147° schmelzender Nadeln erhalten.

Beispiel 11

Eine Suspension von 2,5 g 4-(Benzolsulfonyl-imino)-benzochinon-(1,4)-diäzid-(I) in 500 ccm Anisol wird belichtet und die Lösung im Vakuum eingedampft. Der hinterbleibende ölige Rückstand erstarrt beim Erkalten kristallin. Durch fraktionierte Kristallisation aus Methanol werden die beiden bei der Umsetzung in Licht gebildeten Isomeren getrennt. Das schwerer lösliche 4-(Benzolsulfonyl-imino)-4'-methoxy-diphenyl kristallisiert in Form derber, bei 189 bis 190° schmelzender Spieße aus. Die leichter lösliche isomere o-Verbindung 4-Benzolsulfonyl-amino-2'-methoxy-diphenyl wird in Form feiner, bei 142 bis 143° schmelzender Nadeln erhalten.

Beispiel 12

Die Lösung von 10 g 4-Phenylimino-benzochinon-(1,4)-diäzid-(I) in 400 ccm Dimethylformamid und 1500 ccm Benzol wird belichtet, das Benzol verjagt und die übrigebleibende Dimethylformamidlösung mit 1000 ccm Wasser verdünnt. Das als dunkles Öl ausfallende Reaktionsprodukt wird mit Äther extrahiert und der beim Verdampfen des getrockneten Äthers hinterbleibende Rückstand ausgekocht. Beim Einengen der Gasolinlösung kristallisiert das p-Phenyl-diphenylamin in Form

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ZUR THERMOLYSE DES 2-AZIDO-4.6-DIMETHYL-PYRIMIDINS;

REAKTIONEN DES AZENS

Rolf Huisgen und Karl v. Fraunberg

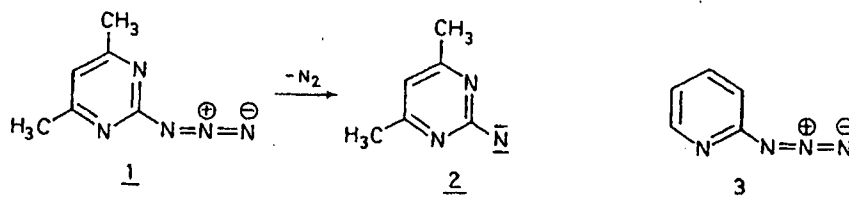
Institut für Organische Chemie der Universität München

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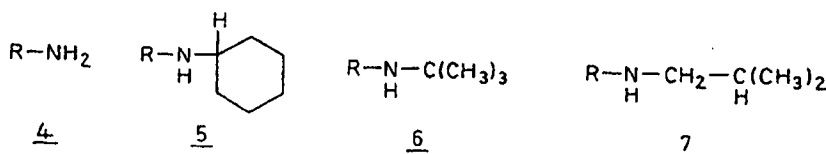
Die kinetische Verfolgung der Stickstoff-Entwicklung aus 4.6-Dimethyl-pyrimidyl-(2)-azid (1), das im Gleichgewicht mit 5.7-Dimethyl-tetrazolo[1.5-a]-pyrimidin vorliegt, gestattet es, zwischen "induziertem" Zerfall (primäre Cycloaddition) und 1-Thermolyse zu unterscheiden (1).

A. Thermolyse in Alkanen und Alkenen

Der Zerfall von 1 in Cyclohexan bei 185° lieferte, wohl über das Azen 2, 48% 2-Amino-4.6-dimethyl-pyrimidin (4) und 11% des Insertionsprodukts 5. Das



analoge 2-Pyridyl-azid (3) - das Gleichgewicht liegt auf der Seite des Tetrazolo-[1.5-a]pyridins (1) - gab bei 195° in Cyclohexan außer 1% 2-Amino-pyridin nichts Flüchtliges.



R = 4.6-Dimethyl-pyrimidyl-(2)

Aus der 1-Thermolyse in Isobutan bei 175° gingen 48% 4 und 5.4 bzw. 1.6% der Einschleppungsprodukte 6 und 7 hervor. Das entspricht einem Vorzug des tert. vor dem prim. CH von ≈ 30 ; für Phenyl-azen wurde 200 (2), für $C_2H_5O_2C-N$ 30 (3) gefunden.

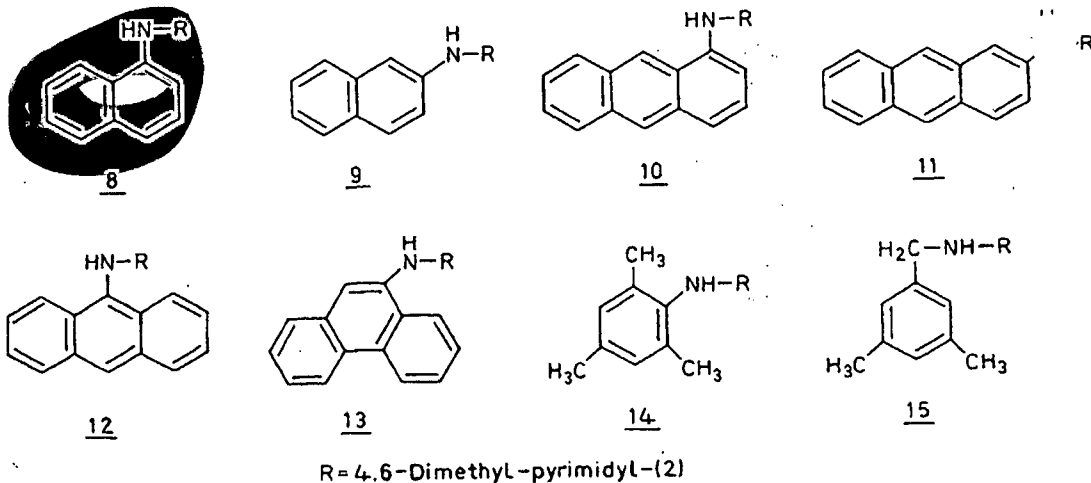
B. Thermolyse von 1 in Aromaten

Nur das Azen 2, nicht das 2-Pyridyl-azen aus 2, trat mit Aromaten unter formaler Substitution zusammen (Tab. 1); Aromaten beschleunigen den 1-Zerfall nicht. Die Produkte wurden spektral charakterisiert und meist mit Präparaten unabhängiger Synthese identifiziert.

Tab. 1. Thermolyse von 1 in aromatischen Verbindungen bei 180-220°

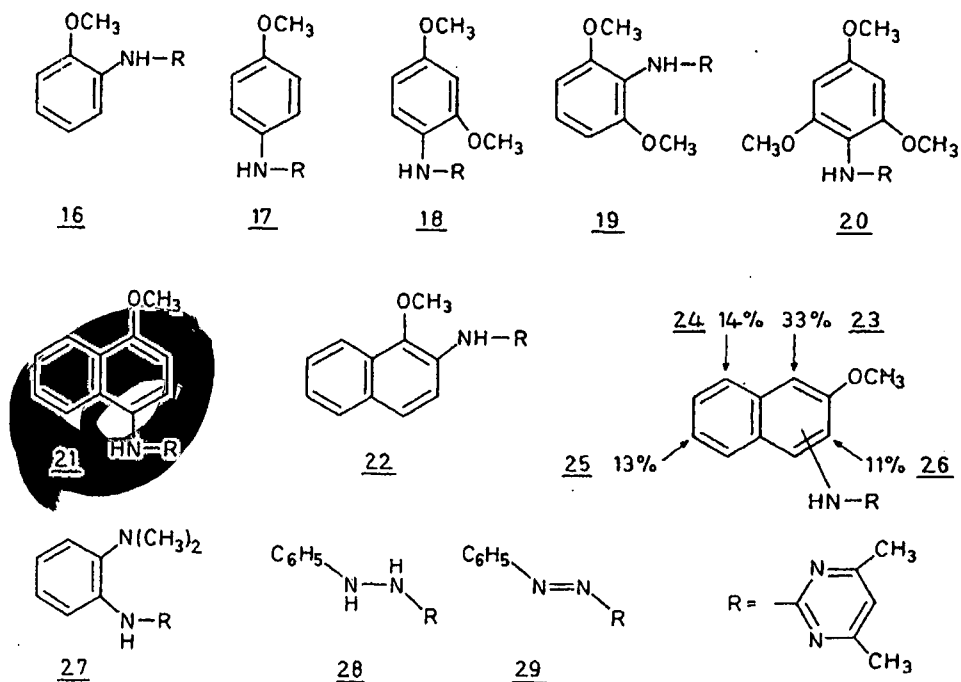
Aromat	% Produkte [Schmp.]
Benzol	46 <u>4</u> [150°]
Naphthalin	35 <u>8</u> [126-127°], 3.3 <u>2</u> [135-136°]
Anthracen	15 <u>10</u> [133-134°], 4 <u>11</u> [204-206°], 20 <u>12</u> [232-233°]
Phenanthren	14 <u>13</u> [181.5-182.5°], 12% weitere Isomere
Mesitylen	34 <u>14</u> [135.5-136.5°], 3 <u>15</u> [89-90.5°], 45 <u>4</u>
Anisol	9 <u>16</u> [106.5-108°], 5 <u>17</u> [86-88°], 50 <u>4</u>
1.3-Dimethoxy-benzol	33 <u>18</u> [116-118°], 11 <u>19</u> [196-198°], ca. 5 <u>4</u>
1.3.5-Trimethoxy-benzol	50 <u>20</u> [208-210°], ca. 5 <u>4</u>
1-Methoxy-naphthalin	17 <u>21</u> [211-213°], 15 <u>22</u> [129-130°], 5+3% weitere Isomere
2-Methoxy-naphthalin	33 <u>23</u> [197-199°], 14 <u>24</u> [137-139°], 13 <u>25</u> [151.5-152.5°], 11 <u>26</u> [174.5-176°]
N.N-Dimethylanilin	6.5 <u>27</u> [66-67°], 9% weitere Isomere, ca. 5 <u>4</u>
Anilin	32 <u>28</u> [166-167°], 5 <u>29</u> [72-74°], 27 <u>4</u>
1.3-Dichlorbenzol	33 <u>4</u>

In Benzol wird nur Wasserstoffabstraktion beobachtet. Dagegen traten in Naphthalin die sek. Amine 8 und 9 zu 38% im 11:1-Verhältnis auf. Anthracen



lieferte 39% der Stellungsisomeren 10-12 unter Bevorzugung des 9-Derivats 12. Bei Phenanthren wurde nur 13 isoliert, aber die vier weiteren Isomeren wahrscheinlich gemacht.

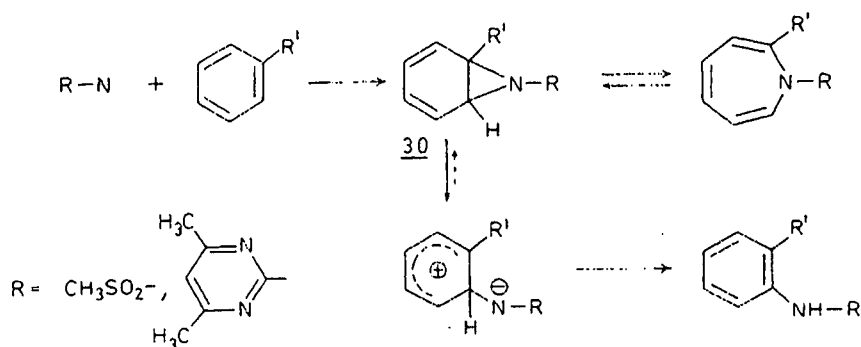
Bei Mesitylen war die arom. Substitution zu 14 vor der aliphatischen zu 15 bevorzugt; Hauptreaktion war die H-Abstraktion 2→4. Letzteres gilt auch für Anisol, bei dem sich die Substitution auf 9% o- (16) und 5% p-Produkt 17 beschränkte. Bei 1,3-Dimethoxy-benzol stiegen die Ausbeuten auf 33% 18 und 11% 19 und beim 1,3,5-Trimethoxy-benzol auf 50% 20. Besonders eindrucksvoll ist die Orientierung bei 2-Methoxy-naphthalin; 71% Substitutionsprodukte 23-26 verteilen sich auf die vier Stellungen, die vom mesomeren Effekt des Methoxyls erreicht werden. Allerdings ist die Unterscheidung zwischen diesen Stellungen - wie übrigens bei allen untersuchten Aromaten (Tab. 1) - viel geringer als bei elektrophilen arom. Substitutionen.



Während Dimethylanilin wenig 27 erbrachte, reagierte Anilin unter Substitution an der Aminogruppe zu 28; die Hydrazoverbindung 28 wurde teilweise zum Azokörper 29 dehydriert. 1,3-Dichlorbenzol gab keine Substitutionsprodukte.

Beim Zerfall des Phenylazids in aromatischen Solventien kommt es nicht zur Substitution; die Bildung des 2-Anilino-3H-azepins aus Phenylazid und Anilin (4-6) erfolgt über eine Ringerweiterung des Phenyl-azens. Das Verhalten des elektronenarmen heterocyclischen Azids 1 bzw. seines Spaltstücks 2 schließt sich mehr an das der Sulfonylazide an, deren Zerfall in Aromaten bekanntlich zu Sulfonaniliden (7) führt.

Gehen 8-27 auf eine elektrophile aromatische Substitution mit normaler σ -Zwischenstufe zurück? Konkurrenzversuche von Benzol mit monosubstituierten Abkömmlingen um Methansulfonyl-azen (aus $\text{CH}_3\text{-SO}_2\text{-N}_3$) lieferten erstaunlich kleine Konkurrenzkonstanten (8), die denen der Azepinbildung aus Benzol-Derivaten und Äthoxycarbonyl-azen (9) sehr ähnlich sind. Daß auch das $\text{CH}_3\text{SO}_2\text{-N}$ zunächst eine 2+1-Cycloaddition mit den Aromaten zu 30 eingeht, wurde von Abramovitch und Uma (10) elegant bewiesen. Die für das heterocyclische Azen 2 vorliegenden Daten erscheinen uns mit dem gleichen Mechanismus im Einklang.



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197

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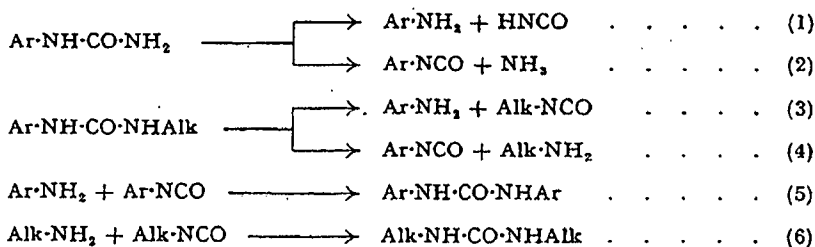
P: 2292-2295

484. The Conversion of Arylureas into Symmetrical Diarylureas.
The Melting Points of Certain Substituted Ureas.

By FREDERICK KURZER.

The preparation of 1-ureido-2- and 1-ureido-4-alkoxynaphthalenes is described and their conversion into the corresponding symmetrical bisalkoxynaphthylureas examined. The conversion into the *s*-diarylureas occurs particularly rapidly in the *para*-substituted series and accounts for the occurrence of indefinite melting points in certain substituted arylureas. The analogous behaviour of 2- and 4-diphenylureas is also recorded.

THE conversion of arylureas above their melting points into symmetrical diarylureas has often been observed (e.g., Sell, *Annalen*, 1863, 126, 157). Davis *et al.* (*J. Amer. Chem. Soc.*, 1922, 44, 2595; 1923, 45, 1816) showed that arylureas dissociated in two ways, forming on the one hand isocyanic acid and an aromatic amine (equation 1), and on the other ammonia and aryl isocyanate (equation 2); recombination of the aromatic dissociation products according to equation 5 accounted for the production of the symmetrical diarylureas. The thermal rearrangement of 1-aryl-3-alkylureas into symmetrical diarylureas was accounted for similarly (see equations 3—6).



1-Ureido-2- and 4-ureido-1-naphthol were prepared by existing methods from the corresponding aminonaphthols, and were converted into the *methyl* and *ethyl ethers*. Prolonged fusion of 1-amino-2-naphthol hydrochloride with urea gave *di-2-hydroxy-1-naphthylurea*, also produced by heating 1-ureido-2-naphthol at 250° for one hour. *Di-2-alkoxy-1-naphthylureas* were obtained by heating 1-ureido-2-alkoxynaphthalenes above their melting points, or by boiling their aqueous solutions for several hours, and were identical with specimens prepared by the alkylation of *di-2-hydroxy-1-naphthylurea*. The rearrangement of 1-ureido-4-alkoxy-naphthalene to symmetrical *di-4-alkoxy-1-naphthylureas* at elevated temperatures occurred with particular ease: conversion took place almost instantaneously and was completed by brief heating near the melting point of the starting material.

The ureidoalkoxynaphthalenes described are closely related to the intensely sweet 4-ureido-phenetole ("Dulcin"). 1-Ureido-4-methoxy- and 1-ureido-4-ethoxy-naphthalene were found to be tasteless or very faintly bitter probably owing to the change of a benzene for a naphthalene nucleus and to their very limited solubility in water at ordinary temperatures. These results agree with the observation, that introduction of even a single alkyl group into the "Dulcin" molecule (as, for example, in 4-ureido-3-methylphenetole) suppresses the sweet taste. 4-Ureido-1-naphthol, produced, after a bitter, burning sensation, a persistent sweet taste; similar, but very faint effects were observed with the *ortho*-isomer.

The behaviour of arylureas on fusion recalls the frequent discrepancies in the reported melting points of certain urea derivatives, particularly asymmetric aryl-, diaryl-, and arylalkylureas (cf. Beilstein's *Handbuch*, 1929, 12, 801, 941, 1292). The decomposition temperature of 4-bromophenylurea, for example, has variously been given at points between 220° and 296° (see experimental). Wheeler (*J. Amer. Chem. Soc.*, 1929, 51, 3653), although noticing a difference

20

in the behaviour of this compound on slow and rapid heating, was unable to account for these observations. Van Gelderen (*Rec. Trav. chim.*, 1933, 52, 976), describing the preparation of a series of substituted *p*-diphenylureas of the type $\text{Ph}\cdot\text{C}_6\text{H}_4\cdot\text{NH}\cdot\text{CO}\cdot\text{NR}'\text{R}''$, has pointed out that the melting points of the majority of compounds studied were not sharp, and ascribed this to the changes represented by equations (3—6). The observation that the decomposition ranges of these derivatives depended on the rate of heating, the nature of the melting-point apparatus used, etc., led to the conclusion that melting points cannot be used for the characterisation of substituted ureas.

The results obtained in the present investigation have shown that the thermal conversion of aryl- into the corresponding diaryl-ureas, proceeding according to equations (1), (2), and (5), takes place extremely rapidly, often below the melting point, with arylureas containing certain substituents, particularly in the position *para* to the ureido-group, and is responsible for the occurrence of indefinite melting points. Thus, for example, whilst 2-diphenylurea melted sharply at 158—160°, 4-diphenylurea, heated under the same conditions, sintered at 180—190° and re-melted at 295—300°. This behaviour was accounted for by experiments which showed that 4-diphenylurea was completely converted into bis-4-diphenylurea, m. p. 316°, by heating at 210° for 10 minutes. The corresponding conversion of the *ortho*-isomer into bis-2-diphenylurea, however, was incomplete after 1 hour's heating; a sample of the melt withdrawn after 10 minutes' fusion showed a nitrogen content corresponding to a mixture of 84% of starting material, and 16% of bis-2-diphenylurea. On prolonged heating, small quantities of 2-diphenylbiuret were obtained in this conversion, but not in the others. The formation of biurets is readily accounted for by the following extension of Davis's mechanism:



In determining the melting points of substituted ureas which undergo the above rearrangement rapidly it seems desirable to carry out the measurements under such conditions that conversion into the symmetrical diarylurea is avoided as far as possible. Reproducible values have now been determined by the following simple procedure: after the approximate softening temperature or melting range of the urea had been found, the bath temperature of the apparatus was raised 10—20° above that point, and samples inserted into the slowly cooling bath, until a temperature was reached at which a specimen just failed to liquefy. Insertion of another specimen at a temperature one degree higher caused instantaneous fusion, and could be taken as the "melting point" of the urea derivative under examination. Under these conditions, practically no conversion into diarylurea occurs, and samples withdrawn immediately after fusion showed nitrogen contents corresponding to the original arylurea. If left in the melting-point apparatus, the fused arylurea normally resolidified after a few seconds and then showed a second melting point, that of the diarylurea. This procedure gives satisfactorily reproducible physical constants for urea derivatives whose properties preclude slow heating. Using this method it has been found that melting points recorded in the literature for arylureas are indeed often those of the corresponding diarylureas, or mixtures of both. Thus, for example, 4-diphenylurea melts sharply at 197—198°, whilst the "decomposition temperature," 210°, reported by van Gelderen (*loc. cit.*), is that of a mixture with bis-4-diphenylurea. Similar considerations apply to the constants recorded by Herrmann (*Annalen*, 1922, 429, 163) for 1-ureido-4-ethoxynaphthalene ("sintering at 205—207°, decomposition at 264—265°"). The compound was found to melt at 221—222°; short heating above this melting point rapidly converted it into *di*-4-ethoxy-1-naphthylurea, m. p. 266—267°. The analogous behaviour of 4-bromophenylurea is also described.

EXPERIMENTAL.

All m.p.s are uncorrected. Analyses are by Drs. Weiler and Strauss. M.p.s of *p*-substituted arylureas were determined by the method described in the theoretical section.

1-Ureido-2-naphthol.—(a) *By the action of urea.* 1-Amino-2-naphthol hydrochloride (20 g., 1 mol.), dissolved in a mixture of water (150 ml.) and glacial acetic acid (2 ml.), was treated with urea (40 g., 6.5 mols.) and the whole heated under reflux with mechanical stirring for one hour. After cooling of the solution to room temperature, the product was filtered off and freed from coloured impurities by boiling briefly under reflux with ethanol (40 ml.). The residue in sodium hydroxide solution (500 ml.; 2% w/v), was treated with sodium dithionite (1 g.), and stirred with charcoal at 60° for $\frac{1}{2}$ hour. After the removal of the carbon, the product was precipitated by passage of sulphur dioxide. A second reprecipitation, and cooling to 0°, gave 1-ureido-2-naphthol (15—18 g.; 60—72%) in white micro-crystalline plates, m. p. 224—225° (decomp.) (Found: C, 65.4; H, 5.0; N, 13.6. $\text{C}_{11}\text{H}_{10}\text{O}_2\text{N}_2$ requires C, 65.35; H, 4.95; N, 13.85%).

(b) *By the action of sodium cyanate.* To a solution of 1-amino-2-naphthol hydrochloride (10 g., 1 mol.) in water (150 ml.) at 30°, sodium cyanate (10 g., 3 mols.; saturated aqueous solution) was added

with stirring. The product separated immediately as a greyish-purple solid. After two reprecipitations with sulphur dioxide 1-ureido-2-naphthol, m. p. 224–225° (decomp.), was obtained as a white micro-crystalline powder (8–9 g.; 63–71%) (Found: N, 13.9%). 1-Ureido-2-naphthol is difficultly soluble in hot methanol, ethanol, and acetone, and almost insoluble in benzene and light petroleum. Crystallization from ethanol yields shiny needles, m. p. 225–226° (decomp.).

1-Ureido-2-methoxynaphthalene.—1-Ureido-2-naphthol (4 g., 1 mol.), dissolved in sodium hydroxide solution (100 ml.; 5% w/v) was treated at 50° with methyl sulphate (12 g., 5 mols.) in two portions. The reaction mixture, from which the crude product separated immediately, was kept at 100° for 30 minutes, and maintained alkaline by the addition of more sodium hydroxide solution. After being cooled to 0° and filtered, the product was crystallized from dilute ethanol (200 ml.; 80%) and gave 1-ureido-2-methoxynaphthalene, m. p. 236–238°, in colourless lustrous plates (3.5 g.; 75%), which recrystallized from a large volume of boiling water as needles (Found: C, 66.9; H, 5.5; N, 11.8. $C_{13}H_{10}O_2N_2$ requires C, 66.7; H, 5.6; N, 13.0%). Tasteless.

1-Ureido-2-ethoxynaphthalene.—This product, obtained by the procedure used for the methoxy-compound in 60% yield, formed shiny needles, m. p. 194–196° (Found: C, 67.5; H, 6.0; N, 12.2. $C_{13}H_{12}O_2N_2$ requires C, 67.85; H, 6.1; N, 12.2%). It was sparingly soluble in water, producing a faint bitter taste.

1-Acetamido-2-naphthyl Acetate.—1-Ureido-2-naphthol (6 g., 1 mol.), in pyridine (50 ml.), was heated with acetic anhydride (30 g., 10 mols.) at 100° for two hours. The resulting liquid was poured into water (250 ml.), and well-developed prisms separated overnight (4.5 g.; 60%). After two crystallizations from ethanol (150 ml., 120 ml.) 1-acetamido-2-naphthyl acetate, m. p. 204–206°, was obtained in lustrous colourless prisms (Found: C, 68.7; H, 5.4; N, 5.5, 5.9. Calc. for $C_{14}H_{12}O_4N$: C, 69.1; H, 5.35; N, 5.8%). The m. p. was not depressed on admixture with authentic material (Michel and Grandmougin, *Ber.*, 1892, 25, 3432). Alkaline hydrolysis gave lustrous plates of 1-acetamido-2-naphthol, m. p. 235–236° (decomp.).

Di-2-hydroxy-1-naphthylurea.—(a) *From 1-amino-2-naphthol hydrochloride.* An intimate mixture of 1-amino-2-naphthol hydrochloride (20 g., 1 mol.) and urea (24 g., 4 mols.) was fused at 160° in nitrogen. The temperature of the orange melt was raised slowly and maintained at 200° for 1 hour, while strong evolution of ammonia took place. The solidified product was broken up, and its alkaline solution (4% w/v sodium hydroxide solution) treated at 40° with charcoal, followed by precipitation with sulphur dioxide. After two reprecipitations di-2-hydroxy-1-naphthylurea, m. p. 214–216°, was obtained as a white micro-crystalline powder (14–15 g.; 81–87%) (Found: C, 73.0; H, 4.4; N, 7.9. $C_{21}H_{16}O_3N_2$ requires C, 73.3; H, 4.65; N, 8.1%).

(b) *From 1-ureido-2-naphthol.* 1-Ureido-2-naphthol (1 g.) was melted and maintained at 240° for 1 hour. Isolation of the product as described above gave di-2-hydroxy-1-naphthylurea (0.75 g.), m. p. 214–215° (Found: N, 8.7%).

Di-2-methoxy-1-naphthylurea.—(a) *From di-2-hydroxy-1-naphthylurea.* Treatment of the symmetrical hydroxy-compound with methyl sulphate as previously described (but under somewhat more drastic conditions—80°; 30 minutes' shaking) and two crystallizations from aqueous acetic acid, gave micro-crystalline needles of di-2-methoxy-1-naphthylurea, m. p. 268–270° (decomp.), in excellent yield (Found: C, 73.8; H, 5.5; N, 7.25. $C_{23}H_{20}O_3N_2$ requires C, 74.2; H, 5.4; N, 7.5%).

(b) *From 1-ureido-2-methoxynaphthalene.* 1-Ureido-2-methoxynaphthalene (1 g.) was heated under reflux for 2 hours with water (250 ml.), in which it dissolved very gradually. The solution was slowly evaporated to small bulk (50 ml.) during 2 hours and cooled to 0°, and then deposited a product which gave di-2-methoxy-1-naphthylurea (0.5 g.), m. p. 268–270° (decomp.), from aqueous acetic acid. There was no depression of m. p. in admixture with material obtained by method (a) (Found: N, 7.35%).

(c) 1-Ureido-2-methoxynaphthalene (1 g.) was heated above its melting point in nitrogen for 30 minutes; ammonia was evolved and partial re-solidification occurred. Crystallization of the residue from aqueous acetic acid, with the addition of decolorizing carbon, gave di-2-methoxy-1-naphthylurea (0.4 g.), m. p. 268–270° (decomp.) not depressed on admixture with material obtained by method (a) (Found: N, 7.9%).

Di-2-ethoxy-1-naphthylurea was prepared by methods (a) and (c), and formed colourless needles, m. p. 246–247° (Found: N, 7.3, 7.5. $C_{25}H_{22}O_3N_2$ requires N, 7.0%).

4-Ureido-1-naphthol.—1-Amino-4-naphthol hydrochloride (10 g., 1 mol.) in water (100 ml.) was added dropwise within 15 minutes, with mechanical stirring, to a solution of sodium cyanate (10 g., 3 mols.) in water (120 ml.), cooled externally to 0°. The temperature of the reaction mixture was kept below 5° by the addition of ice. The pale purple solid obtained was dissolved in sodium hydroxide solution (200 ml.; 2% w/v), treated with charcoal at 50° for 30 minutes, and reprecipitated by the slow addition of excess of dilute sulphuric acid to the filtrate, previously cooled to 0°. After two further reprecipitations, 4-ureido-1-naphthol, m. p. 184–185° (decomp.), was obtained as a white micro-crystalline powder (8–9 g.; 80–90%) (Found: C, 64.9; H, 4.8; N, 13.3. $C_{11}H_{10}O_2N_2$ requires C, 65.35; H, 4.95; N, 13.85%). It turns pale purple on storage, is readily soluble in methanol, ethanol, and acetone and can be crystallized from ethanol-light petroleum. An aqueous solution of this compound produced, after a bitter burning sensation, a persistent sweet taste. Attempts to prepare 4-ureido-1-naphthol by boiling a solution of 1-amino-4-naphthol hydrochloride with urea failed.

1-Ureido-4-methoxynaphthalene.—Methylation of 4-ureido-1-naphthol as previously described gave a product (72% yield) which crystallized from ethanol-water (2:1) as white micro-crystalline needles, m. p. 224–225° (Found: C, 65.95; H, 5.45; N, 12.9. $C_{12}H_{12}O_2N_2$ requires C, 66.7; H, 5.6; N, 13.0%). The compound was very sparingly soluble in water and produced a faint bitter taste.

1-Ureido-4-ethoxynaphthalene.—This was prepared analogously. It was twice crystallized from ethanol and formed a silky mass of white micro-crystalline needles, m. p. 221–222° (Found: C, 67.6; H, 5.6; N, 11.9. Calc. for $C_{13}H_{14}O_2N_2$: C, 67.85; H, 6.1; N, 12.2%). Herrmann (*loc. cit.*) reports sintering at 205–207°, followed by decomposition at 264–265°. The bitter taste reported by this author for the compound is very faint and appears to be confined to its alcoholic solution.

Di-4-hydroxy-1-naphthylurea.—A mixture of finely ground 1-amino-4-naphthol hydrochloride (12 g., 1 mol.) and urea (12 g., 3 mols.) was put into a paraffin bath at 250°, and the temperature rapidly raised

199

to 270°. The vigorous reaction accompanied by evolution of ammonia slackened after 15 minutes and heating was discontinued. The powdered melt (21 g.) was dissolved in warm sodium hydroxide solution (250 ml.; 3% w/v) containing a little sodium dithionite, and purified with charcoal, and the product precipitated from the filtrate at 0° with dilute hydrochloric acid. Two further reprecipitations gave di-4-hydroxy-1-naphthylurea as a pale-grey powder, m. p. 208–210° (decomp.) (Found: N, 8.6, $C_{21}H_{16}O_3N_2$ requires N, 8.1%). Fusion of the corresponding unsymmetrical urea derivative above the m. p. was unsuitable for the preparation of this compound.

Di-4-methoxy-1-naphthylurea.—1-Ureido-4-methoxynaphthalene (1 g.), was heated to 240° for 10 minutes. After rapid fusion, the material resolidified immediately and formed a white mass (0.9 g.; m. p. 306–307°), a small specimen of which was crystallized from ethanol and gave white micro-crystalline di-4-methoxy-1-naphthylurea, m. p. 310–312° (decomp.) (Found: N, 7.6. $C_{23}H_{20}O_3N_2$ requires N, 7.5%).

Di-4-ethoxy-1-naphthylurea.—This was prepared similarly and formed a micro-crystalline mass, m. p. 276–278° (decomp.) (Found: N, 7.4. $C_{25}H_{22}O_3N_2$ requires N, 7.0%).

Bis-2-diphenylurea.—2-Diphenylurea (1 g.) (Morgan and Walls, *J.*, 1932, 2225) was heated to about 200° for 1½ hours. The brown melt began to solidify after ½ hour and had set completely at the end of 1 hour. The powdered material was boiled with three successive portions of ethanol (3 × 20 ml.), giving an insoluble powdery residue of 2-diphenylbiuret, m. p. 258–260° (0.2–0.3 g.) (Found: C, 65.6; H, 5.1; N, 16.3, 16.4. $C_{14}H_{13}O_2N_2$ requires C, 65.9; H, 5.1; N, 16.5%). The pale violet filtrates were decolorized with carbon and concentrated to small volume and then deposited clusters of lustrous needles (0.4–0.5 g.) of bis-2-diphenylurea, m. p. 185–186° (Found: N, 7.95. $C_{25}H_{20}ON_2$ requires N, 7.7%).

In separate experiments, 2-diphenylurea was fused at 200° for 5 and 10 minutes. The melts, which were completely soluble in ethanol (indicating absence of 2-diphenylbiuret) were finely powdered, and extracted with a little cold water to remove traces of urea. The specimens showed nitrogen contents (12.6 and 12.3%) corresponding to the presence of 90 and 84% of the original unsymmetrical urea, respectively.

Bis-4-diphenylurea.—4-Diphenylurea (2.12 g.) (van Gelderen, *loc. cit.*) was maintained at 200–210° for 10 minutes. After 5 minutes, the substance softened and melted partially, but solidified on continued stirring. The cooled material was finely powdered, boiled with ethanol (30 ml.), filtered, and dried (1.6 g.; 87%). Crystallization of a specimen from a large volume of ethanol gave white micro-crystalline bis-4-diphenylurea, m. p. 316–318° (Found: C, 82.3; H, 5.7; N, 7.65. Calc. for $C_{25}H_{20}ON_2$: C, 82.4; H, 5.5; N, 7.7%).

Di-4-bromophenylurea.—The m. p. of 4-bromophenylurea (reported as approximately 260° (decomp.) (Pinnow, *Ber.*, 1891, 24, 4172), above 270° (decomp.) (Dains and Wertheim, *J. Amer. Chem. Soc.*, 1920, 42, 2303), above 265° (decomp.) (Scott and Cohen, *J.*, 1922, 121, 2034), at 220° or 296° (decomp.) (Wheeler, *J. Amer. Chem. Soc.*, 1929, 51, 3653), and at 278° (Desai *et al.*, *J.*, 1934, 1186)) was found consistently to be 225–227° when determined by the technique described in this paper. 4-Bromophenylurea (1.5 g.) was heated to 240° for 8 minutes, and the white, amorphous, partially sublimed mass crystallized from glacial acetic acid (250 ml.), yielding lustrous needles of di-4-bromophenylurea, m. p. 314–316° (decomp.) (Found: N, 7.75. Calc. for $C_{15}H_{10}ON_2Br_2$: N, 7.6%). [The following m. p.s have been recorded for this compound: 320–330° (decomp.) (Biltz, *Annalen*, 1909, 368, 227), 218–219° (Sah *et al.*, *J. Chinese Chem. Soc.*, 1936, 4, 193), and 275–277° (Zetzsche *et al.*, *Ber.*, 1938, 71, 1088).]

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485. Preparation of spiroPyrans Derived from 9-Methyl- and 9-Ethyl-thiaxanthylum and 9-Benzylxanthylum Salts. The Action of Grignard Solutions on Thermochromic spiroPyrans.

By AHMED MUSTAFA.

(a) spiroPyrans (IIa–IIc) derived from 9-methyl- and 9-ethyl-thiaxanthylum and 9-benzylxanthylum salts have been prepared. These spiroPyrans are compared with benzo- β -naphthospirodipyrans (I).

(b) The action of various Grignard solutions on thermochromic spiroPyrans (IIa and V) leads to formation of 2:3-dihydrofuran derivatives (IV and VI).

(a) DICKINSON and HEILBRON (*J.*, 1927, 1699) have shown that only spiroPyrans having at least one of the pyran rings attached to a naphthalene nucleus form coloured ions when heated; further, that the 3'-carbon atom in the naphthopyran ring must be unsubstituted (cf. I). A study has now been made of the condensation of 9-methyl- and 9-ethyl-thiaxanthylum chloride and 9-benzylxanthylum chloride with 2-naphthol-1-aldehyde and of the spiroPyrans produced on hydrolysis (cf. Irving, *J.*, 1929, 1093), in order to compare the latter compounds with benzo- β -naphthospirodipyrans (I). β -Naphthothiaxanthospiropyran (IIa, A = S) fulfils the above conditions and, as was expected, develops a pink colour when boiled with diphenyl ether and

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959

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Heterocyclische 12- π - und 14- π -Systeme, 34¹⁾

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P: 959-964 6
Untersuchungen zum Reaktionsverhalten von 2-Phenyl-5H-naphtho[1,8-bc]furan-5-on (Oxapseudophenalenon)

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Eingegangen am 13. November 1978

Die Reaktionen von 2-Phenyl-5H-naphtho[1,8-bc]furan-5-on (1) mit Perchlorsäure in Alkoholen führen zu den (4-Alkoxy-8-hydroxy-1-naphthyl)(phenyl)ketonen 3a–3c, die auch aus (4,8-Dihydroxy-1-naphthyl)(phenyl)keton (2) in sauren alkoholischen Lösungen zugänglich sind. Mit Diphenylketen, Naphthylphenylketen, *tert*-Butylcyanketen, Cyanmethylketen, Cyanphenylketen werden die 2-Phenyl-oxapseudophenafulvene 4–8 erhalten, mit dem durch Thermolyse von 2,5-Diazido-3,6-dioxo-1,4-cyclohexadien-1,4-dicarbonitril (9) in situ erzeugten Dicyanketen (10) wird unter Eliminierung von CO₂ das auf anderen Wegen nicht zugängliche (2-Phenyl-oxapseudophenafulven-5-yliden)malonodinitril 11 synthetisiert.

Heterocyclic 12- π - and 14- π -Systems, 34¹⁾. – Experiments on the Reactivity of 2-Phenyl-5H-naphtho[1,8-bc]furan-5-one (Oxapseudophenalenone)

Reactions of 2-phenyl-5H-naphtho[1,8-bc]furan-5-one (1) with perchloric acid in alcohols yield the 4-alkoxy-8-hydroxy-1-naphthyl phenyl ketones 3a–3c which are also obtained from 4,8-dihydroxy-1-naphthyl phenyl ketone (2) in acidic alcoholic solutions. Diphenylketene, naphthylphenylketene, *tert*-butylcyanoketene, cyanomethylketene, cyanophenylketene react with 1 to give the 2-phenyl-oxapseudophenafulvenes 4–8; thermolysis of 2,5-diazido-3,6-dioxo-1,4-cyclohexadiene-1,4-dicarbonitrile (9) yields in situ the dicyanoketene (10) which reacts with the ketone 1 via elimination of CO₂ to give the (2-phenyl-oxapseudophenafulven-5-ylidene)malonodinitrile 11. Compound 11 was not obtained by another route.

Im Rahmen unserer Untersuchungen über heterocyclische 12- π - und 14- π -Molekülsysteme interessierte das Reaktionsverhalten jener Tricyclen, welche sich vom Phenalenon dadurch ableiten, daß eine Vinylgruppe formal durch Sauerstoff ersetzt und dadurch diese iso- π -elektronische Systeme sind. Wir haben als Vertreter solcher Oxapseudophenalenone zunächst das bekannte 2-Phenyl-5H-naphtho[1,8-bc]furan-5-on – ein Oxapseudophenalenon – untersucht, welches als Nebenprodukt bei Versuchen zur Totalsynthese von Tetracyclinen durch Barton und Mitarbeiter³⁾ erhalten worden ist.

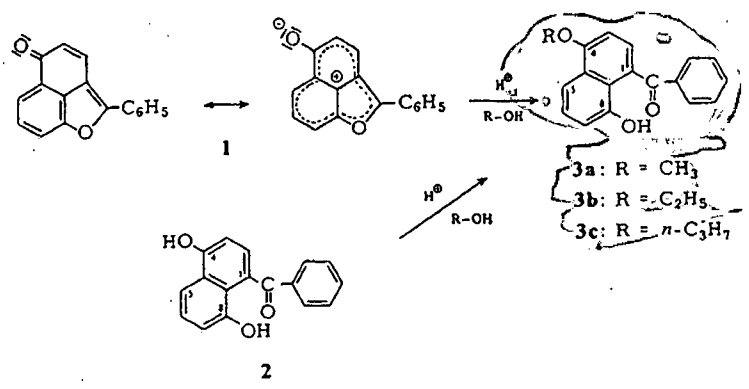
Wird das Oxapseudophenalenon 1 in alkoholischer Lösung mit Perchlorsäure behandelt, so erfolgt unter Ringöffnung die Bildung von 3a–3c.

¹⁾ Korrespondenz bitte an diesen Autor richten.

²⁾ Herrn Professor Dr. Dr. h. c. *Heinrich Hellmann* mit den besten Wünschen zum 65. Geburtstag gewidmet.

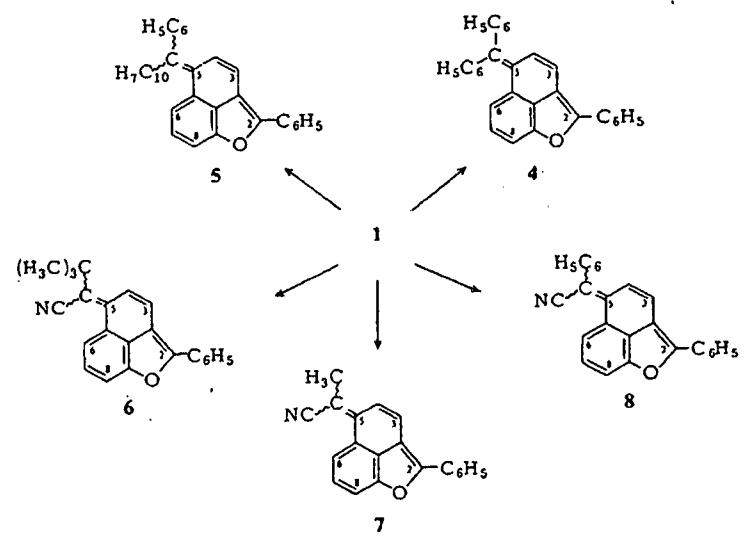
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Im IR-Spektrum von 3b ist eine breite OH-Bande bei 2800–3350 cm⁻¹, die auf eine intramolekulare „Chelat“-Wasserstoffbrückenbindung hinweist, sowie eine C=O-Absorptionsbande bei 1640 cm⁻¹ zu erkennen. Im ¹H-NMR-Spektrum von 3b in [D₆]DMSO als Lösungsmittel zeigt sich ein Triplet bei δ = 1.46 (t; 3H für CH₃), ein Quartett bei δ = 4.22 (q; 2H für –CH₂–, J_{AB} = 7 Hz); zusätzlich zu dem den einzelnen Aromatenprotonen nicht zuzuordnenden Multipl. bei δ = 6.78–7.80 (10H) tritt noch ein einem OH-Proton zuzuschreibendes Singulett bei δ = 10.18 auf. – Die Röntgenstrukturanalyse⁴⁾ von 3b bestätigte ebenfalls die angegebene Struktur.

Daß bei der Reaktion 1→3 die Zwischenstufe 2 durchlaufen wird, konnte dadurch gezeigt werden, indem 2 aus dem Reaktionsgemisch isoliert und dieses anschließend durch Erhitzen beispielsweise mit Propanol in 3c übergeführt werden konnte.

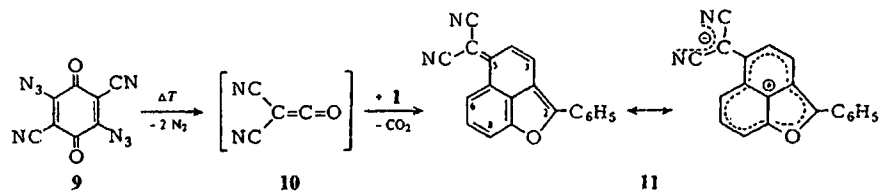


Oxapseudophenafulvene waren aus **1** in Eisessig^{5,6,8-10)} und entsprechenden C-Nukleophilen¹¹⁾ nicht zugänglich, so daß zu deren Darstellung die Ketenmethode eingesetzt wurde, wie dies im Falle des carbocyclischen Phenalenons von *Gompper* und Mitarbeitern¹¹⁾ bereits gezeigt wurde. Die zu unseren Untersuchungen verwendeten Alkylcyan- bzw. Arylcyanketene – nämlich *tert*-Butylcyanketen, Cyanmethylketen, Cyanphenylketen – wurden nach *Moore* und *Weyler*¹²⁾ aus den entsprechenden Dialkyl- bzw. Diaryldiazidobenzochinonen in wasserfreier benzolischer Lösung durch Thermolyse in situ erzeugt.

Werden Diphenylketen, Naphthylphenylketen sowie die in situ hergestellten *tert*-Butylcyanketen und Cyanphenylketen unter Schutzgas mit **1** zur Reaktion gebracht, so resultieren die Oxapseudophenafulvene **4**–**8**. Chromatographische Trennungen der in den Fällen **5**–**8** möglichen Isomerengemische waren trotz mehrfacher Versuche nicht realisierbar.

Im ¹H-NMR-Spektrum von **4** entspricht das bei $\delta = 6.93$ liegende Singulett zwei Protonen; aufgrund der nahezu gleichen chemischen Umgebung ist für H³ und H⁴ dieselbe chemische Verschiebung anzunehmen. Die beiden Dubletts von Dubletts bei $\delta = 6.47$ und $\delta = 7.15$ entsprechen den beiden Protonen H⁶ und H⁸ mit einer *meta*-Kopplungskonstanten von 0.8 Hz. Die starke diamagnetische Verschiebung von H⁶ ist auf den Einfluß des auf H⁶ diamagnetisch wirkenden Ringstroms des über H⁶ liegenden, aus sterischen Gründen verdrehten Phenylrestes zurückzuführen. Das Triplett bei $\delta = 6.88$ entspricht dem Proton H⁷ mit einer gleichgroßen doppelten *ortho*-Kopplungskonstanten von 7.9 Hz. Die angegebene Zuordnung der drei Protonen H⁶, H⁷ und H⁸ wurde auch durch Doppelresonanzexperiment bewiesen: Bei einer Einstrahlung bei $\delta = 6.47$ (H⁶) entartet das Triplett von H⁷ zum Dublett, bei einer Einstrahlung bei $\delta = 6.88$ (H⁷) entarten die beiden Dubletts von Dubletts bei $\delta = 6.47$ und $\delta = 7.15$ (H⁶ und H⁸) zum Singulett (die *meta*-Kopplungskonstante konnte hier nicht mehr gemessen werden). Neben dem Multiplett bei $\delta = 7.30$ bis 7.54 findet man ein Dublett von Dubletts bei $\delta = 7.86$ mit einem Integrationswert für zwei Protonen, einer *ortho*-Kopplung von 7.8 Hz und einer *meta*-Kopplung von 1.9 Hz, das den beiden *ortho*-Protonen der zu H⁴ *cis*-ständigen Phenylgruppe zuzuordnen ist.

Die Reaktionen von 1-Carbonylfluoren¹³⁾ sowie von Indenketen¹⁴⁾ mit dem Keton **1** führten nicht zu den entsprechenden Oxapseudophenafulvalenen sondern zu Zersetzungsprodukten, während aus **1** mit dem aus 2,5-Diazido-3,6-dioxo-1,4-cyclohexadien-1,4-dicarbonitril¹⁵⁾ (**9**) leicht zugänglichen Dicyanketen¹⁵⁾ (**10**) unter Eliminierung von CO₂ das (2-Phenyl-5*H*-naphtho[1,8-*bc*]furan-5-yliden)malonodinitril (**11**) – (2-Phenyl-oxapseudophenafulven-5-yliden)malonodinitril – zugänglich ist; **11** war andererseits aus **1** und Malonsäuredinitril in Acetanhydrid/Eisessig nicht zu erhalten.



204

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Experimenteller Teil

Schmelzpunkte: nicht korrigiert. Schmelzpunktmikroskop der Fa. Reichert. – Massenspektren: Gerät Varian MAT 311, dessen inverse Nier-Johnson-Geometrie die Aufnahme der DADI-Spektren (direct analysis of daughter ions) ermöglichte. ^1H -NMR- und ^{13}C -NMR-Spektren: Geräte Bruker-HX 90 E und teilweise auch T-60-A (TMS als interner Standard). IR-Spektren: Gerät Perkin-Elmer 177. UV-Spektren: Gerät DMR 10 der Fa. Carl Zeiss. – Die Elementaranalysen wurden mit dem CHN-Analysator der Fa. Heraeus ausgeführt. Säulenchromatographische Trennungen und Reinigungen wurden auf einer mit Kieselgel 60 (Korngröße 0.063 – 0.20 mm) der Fa. Merck beschickten Säule von 80 cm Länge und einem Innendurchmesser von 2.5 cm durchgeführt, präparative schichtchromatographische Untersuchungen auf mit Kieselgel 60 GF₂₅₄ (Fa. Merck) bestrichenen Glasplatten (20 × 20 cm). Die Lösungsmittel und Reagenzien wurden nach den üblichen Methoden getrocknet und gereinigt.

(8-Hydroxy-4-methoxy-1-naphthyl)(phenyl)keton (3a): Zu einer in der Hitze hergestellten Lösung von 1.00 g (4.06 mmol) 1-Phenyl-5H-naphtho[1,8-bc]furan-5-on (I) in 25 ml Methanol, gibt man 5 Tropfen 70proz. Perchlorsäure und erhitzt 2 h unter Rückfluß. Nach dem Abkühlen erhält man blaßgelbe Nadeln mit Schmp. 250 – 253 °C (aus Acetonitril); Ausb. 1.11 g (98%). – IR (KBr): 3400 – 2850 (OH), 1643 cm^{-1} (C = O). – UV (Methanol): λ_{max} (lg ϵ) = 300 (3.9), 315 (3.8), 329 nm (3.7). – ^1H -NMR (60 MHz, $[\text{D}_6]\text{DMSO}$): δ = 4.01 (s; 3H, CH_3), 6.75 – 7.75 (m; 10H, Aromaten-H), 10.18 (s; 1H, OH). – MS (70 eV): m/e (%) = 278 (100, M^+), 262 (33), 261 (65), 247 (10), 246 (36), 235 (8), 231 (12), 218 (10), 207 (8), 201 (93), 190 (10), 189 (33), 188 (43), 185 (21), 163 (7), 149 (23), 115 (26), 105 (32), 100 (28), 83 (20), 77 (48).

$\text{C}_{18}\text{H}_{14}\text{O}_3$ (278.31) Ber. C 77.68 H 5.07 Gef. C 77.57 H 5.10

(4-Ethoxy-8-hydroxy-1-naphthyl)(phenyl)keton (3b): Es wird analog der Vorschrift für 3a aus 1.00 g (4.06 mmol) I in 25 ml Ethanol und 5 Tropfen 70proz. Perchlorsäure durch 2stdg. Erhitzen unter Rückfluß dargestellt. Man erhält gelbe Nadeln mit Schmp. 198.5 – 199 °C (aus Nitromethan); Ausb. 1.14 g (96%). – IR (KBr): 3350 – 2800 (OH), 1640 cm^{-1} (C = O), 1585 cm^{-1} (C = C). – UV (Methanol): λ_{max} (lg ϵ) = 300 (3.7), 318 (3.7), 328 nm (3.6). – ^1H -NMR (60 MHz, $[\text{D}_6]\text{DMSO}$): δ = 1.46 (t, J = 7 Hz; 3H, CH_2CH_3), 4.22 (q, J = 7 Hz, 2H, CH_2CH_3), 6.78 – 7.80 (m; 10H, Aromaten-H), 10.18 (s; 1H, OH). – MS (70 eV): m/e (%) = 292 (100, M^+), 275 (26), 264 (6), 247 (36), 246 (39), 235 (16), 231 (9), 218 (13), 215 (38), 207 (12), 202 (10), 189 (23), 187 (51), 178 (7), 163 (5), 138 (10), 131 (13), 105 (13), 98 (20), 77 (23), 64 (41), 56 (14), 42 (39).

$\text{C}_{19}\text{H}_{16}\text{O}_3$ (292.33) Ber. C 78.08 H 5.48 Gef. C 78.03 H 5.55

(8-Hydroxy-4-propyloxy-1-naphthyl)(phenyl)keton (3c): Es wird analog der Vorschrift für 3a aus 1.00 g (4.06 mmol) I in 40 ml Propanol und 10 Tropfen 70proz. Perchlorsäure durch 2stdg. Erhitzen unter Rückfluß dargestellt. Man erhält gelbe Kristalle mit Schmp. 185 – 187 °C (aus Nitromethan); Ausb. 765 mg (61%). – IR (KBr): 3350 – 2800 (OH), 1642 cm^{-1} (C = O). – UV (Methanol): λ_{max} (lg ϵ) = 304 (3.8), 314 (3.8), 325 nm (3.7). – ^1H -NMR (60 MHz, $[\text{D}_6]\text{DMSO}$): δ = 1.10 (t, J = 7 Hz; 3H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 4.15 (t, J = 7 Hz; 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$), 6.74 – 7.78 (m; 10H, Aromaten-H), 10.14 (s; 1H, OH). – MS (70 eV): m/e (%) = 306 (34, M^+), 290 (9), 289

37

(5), 264 (14), 247 (40), 246 (100), 235 (6), 231 (7), 218 (37), 207 (10), 190 (25), 189 (97), 187 (41), 186 (18), 163 (25), 131 (8), 123 (10), 109 (12), 105 (15), 94.5 (50), 87 (13), 77 (37).

$C_{20}H_{18}O_3$ (306.36) Ber. C 78.41 H 5.92 Gef. C 78.78 H 5.61

5-Diphenylmethylen-2-phenyl-5H-naphtho[1,8-bc]furan (4): Zu einer Lösung von 0.5 g (2.03 mmol) **1** in 25 ml wasserfreiem Benzol werden 0.4 g (2.06 mmol) Diphenylketen gegeben, anschließend wird 2 h bei Raumtemp. gerührt. Die Hauptmenge des Benzols wird i. Vak. entfernt, die Reaktionslösung mit Benzol als Elutionsmittel säulenchromatographisch gereinigt und das Elutionsmittel entfernt. Man erhält gelborange Nadeln mit Schmp. 184–185°C (aus Ether/Methanol 1:5); Ausb. 614 mg (76%). – IR (KBr): 3040 (CH), 1585 cm^{-1} (C=C). – UV (Acetonitril): λ_{max} (lg ϵ) = 238 (4.1), 293 (3.9), 397 nm (4.4). – ^1H -NMR (90 MHz, $CDCl_3$): δ = 6.47 (dd, $J_{6,7}$ = 7.9 Hz, $J_{6,8}$ = 0.8 Hz; 1H, H^6), 6.88 (t, $J_{6,7}$ = 7.9 Hz, $J_{7,8}$ = 7.9 Hz; 1H, H^7), 6.93 (s; 2H, H^1 , H^4), 7.15 (dd, $J_{6,8}$ = 0.8 Hz, $J_{7,8}$ = 7.9 Hz; 1H, H^8), 7.30–7.54 (m; 13H, Aromaten-H), 7.86 (m; 2H, Aromaten-H). – MS (70 eV): m/e (%) = 396 (100, M^+), 366 (5), 363 (8), 319 (10), 318 (13), 291 (10), 290 (14), 289 (38), 276 (4), 263 (3), 231 (5), 198 (12), 165 (8), 159 (7), 105 (15), 78 (10), 77 (8).

$C_{30}H_{30}O$ (396.49) Ber. C 90.87 H 5.09 Gef. C 90.83 H 5.20

5-[(1-Naphthyl)(phenyl)methylen]-2-phenyl-5H-naphtho[1,8-bc]furan (5): Zu einer Lösung von 0.25 g (1.02 mmol) **1** in 20 ml wasserfreiem Benzol tropft man 0.33 g (1.35 mmol) 1-Naphthylphenylketen – gelöst in 10 ml Petrolether. Die über Nacht bei Raumtemp. gerührte Reaktionslösung wird eingengt und über Kieselgel 60 (Fa. Merck) mit Chloroform säulenchromatographisch gereinigt. Man erhält dunkelrote Kristalle mit Schmp. 168–169°C (aus Ligroin); Ausb. 260 mg (57%). – IR (KBr): 3060, 2920 (CH), 1590 cm^{-1} (C=C). – UV (Methanol): λ_{max} (lg ϵ) = 248 (4.0), 279 (4.1), 392 nm (4.6). – ^1H -NMR (90 MHz, $CDCl_3$): δ = 5.89–8.05 (m; Aromaten-H); das Verhältnis der *E/Z*-Isomeren ist aufgrund der Komplexität des ^1H -NMR-Spektrums nicht zu bestimmen. – MS (70 eV): m/e (%) = 446 (100, M^+), 369 (16), 368 (15), 341 (7), 340 (9), 339 (21), 318 (4), 289 (6), 223 (25), 169.5 (9), 169 (8), 168.5 (14), 105 (29), 77 (8).

$C_{34}H_{22}O$ (446.55) Ber. C 91.45 H 4.97 Gef. C 91.07 H 4.91

3,3-Dimethyl-2-(2-phenyl-5H-naphtho[1,8-bc]furan-5-yliden)butyronitril (6): Eine Lösung von 0.25 g (1.02 mmol) **1** und 170 mg (0.56 mmol) 2,5-Diazido-3,6-di-*tert*-butyl-1,4-benzochinon in 25 ml wasserfreiem Benzol wird 3 h unter Stickstoff und Rückfluß erhitzt. Die eingengte Reaktionslösung wird über Kieselgel 60 (Fa. Merck) mit Chloroform als Elutionsmittel säulenchromatographiert. Man erhält orangefarbene Kristalle mit Schmp. 139–141°C (aus Methanol); Ausb. 170 mg (51%). – IR (KBr): 2980 (CH), 2185 (C $\equiv$ N), 1605, 1590, 1565 cm^{-1} (C=C). – UV (Methanol): λ_{max} (lg ϵ) = 240 (4.1), 292 (4.1), 406 (Schulter), 422 nm (4.5). – ^1H -NMR (360 MHz, $CDCl_3$): δ = 1.53 [s; 9H, C(CH₃)₃], ABB'XX'-Signal (δ_A = 7.36, *p*-H; δ_B = 7.51, *m*-H; δ_X = 7.94, *o*-H; J_o = 7.1 Hz, J_m = 1.3 Hz, 5H, 2-Phenyl-H), 7.45–7.46 (m, 2H, H^7 , H^8), 8.88 (mc, 1H, H^6). – MS (70 eV): m/e (%) = 325 (21, M^+), 311 (28), 310 (100, 105 (10), 77 (10), 57 (40), 43 (28).

$C_{23}H_{19}NO$ (325.41) Ber. C 84.89 H 5.88 N 4.31 Gef. C 84.16 H 5.73 N 4.53

2-(2-Phenyl-5H-naphtho[1,8-bc]furan-5-yliden)propiononitril (7): Es wird analog der Vorschrift für **6** aus 0.5 g (2.03 mmol) **1** und 250 mg (1.15 mmol) 2,5-Diazido-3,6-dimethyl-1,4-benzochinon in 50 ml wasserfreiem Benzol durch 5stdg. Erhitzen unter Rückfluß dargestellt. Man erhält gelbe Kristalle mit Schmp. 158–160°C (aus Methanol); Ausb. 263 mg (45%). – IR (KBr): 2190 (C $\equiv$ N), 1595 cm^{-1} (C=C). – UV (Methanol): λ_{max} (lg ϵ) = 241 (3.7), 251 (3.7), 281 (4.0), 395 (Schulter), 407 nm (4.6). – ^1H -NMR (90 MHz, $CDCl_3$): δ = 2.20 (s; 0.35H, 3H, CH₃, *E*-Isomer), 2.40 (s; 0.65H, 3H, *Z*-Isomer), 6.83 (d, J = 10 Hz; 0.65H, 1H, H^4 , *Z*-Isomer),

7.20–7.95 (m; 8.7H, Aromaten-H), 8.60 (mc, 0.65H, 1H, H⁶, Z-Isomer). – MS (70 eV): *m/e* (%) = 283 (100, M⁺), 282 (96), 256 (19), 231 (32), 230 (27).

C₂₀H₁₃NO (283.33) Ber. C 84.78 H 4.63 N 4.95 Gef. C 84.51 H 4.98 N 4.73

2-Phenyl-2-(2-phenyl-5H-naphtho[1,8-b]furan-5-yliden)acetoneitril (8): Es wird analog der Vorschrift für 6 aus 0.5 g (2.03 mmol) 1 und 370 mg (1.08 mmol) 2,5-Diazido-3,6-diphenyl-1,4-benzochinon in 50 ml wasserfreiem Benzol durch 3stg. Erhitzen unter Rückfluß dargestellt. Man erhält orangerote Kristalle mit Schmp. 190–192°C (aus Eigroin); Ausb. 284 mg (40%). – IR (KBr): 2920 (CH), 2190 cm⁻¹ (C≡N). – UV (Methanol): λ_{max} (lg ε) = 246 (3.9), 286 (4.0), 419 nm (4.6). – ¹H-NMR (90 MHz, CDCl₃): δ = 6.66 (dd, J_{6,7} = 7.7 Hz, J_{6,8} = 1 Hz; 0.1H, 1H, H⁶, E-Isomer), 6.92–8.00 (m; 14H, Aromaten-H), 8.88 (mc; 0.9, 1H, H⁶, Z-Isomer). – MS (70 eV): *m/e* (%) = 345 (100, M⁺), 316 (10), 289 (18), 105 (11), 77 (17).

C₂₂H₁₅NO (345.41) Ber. C 86.94 H 4.38 N 4.06 Gef. C 86.87 H 4.39 N 3.77

(2-Phenyl-5H-naphtho[1,8-b]furan-5-yliden)malonodinitril (11): Eine Lösung von 61.5 mg (0.25 mmol) 1 und 90 mg (0.375 mmol) 2,5-Diazido-3,6-dioxo-1,4-cyclohexadien-1,4-dicarbonitril (9) in 25 ml wasserfreiem Benzol wird unter Stickstoff 4h unter Rückfluß erhitzt. Die abgekühlte Reaktionslösung wird eingedampft und der Rückstand auf einer PSC-Fertigplatte Kieselgel 60 F₂₅₄ (Fa. Merck) mit Dichlormethan entwickelt. Die rote Fraktion wird mit Dichlormethan im Soxhlet-Apparat extrahiert, das Elutionsmittel entfernt und der Rückstand aus Acetonitril umkristallisiert. Man erhält rote Kristalle mit Schmp. 274–276°C; Ausb. 33.0 mg (45%). – IR (KBr): 2210 (C≡N), 1610, 1585 cm⁻¹ (C=C). – UV (Methanol): λ_{max} (lg ε) = 229 (3.8), 280 (3.7), 304 (3.8), 471 nm (4.6). – ¹H-NMR (90 MHz, CDCl₃): δ = 7.85 (d, J_{3,4} = 9.7 Hz; 1H, H³), 7.47 (d, J_{3,4} = 9.7 Hz; 1H, H⁴), 8.84 (dd, J_{6,7} = 7.3 Hz, J_{6,8} = 1.2 Hz; 1H, H⁶), 7.55–7.71 (m; 5H, H⁷, H⁸, p-H, 2 m-H). – MS (70 eV): *m/e* (%) = 294 (100, M⁺), 265 (10), 238 (13), 105 (3), 77 (5).

C₂₀H₁₀N₂O Ber. Molmasse 294.0792 Gef. Molmasse 294.0779 (MS)

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[161/78]

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Synthesis and Structure-Activity Study of Protease Inhibitors. II.^{1,2)}
Amino- and Guanidino-Substituted Naphthoates
and Tetrahydronaphthoates

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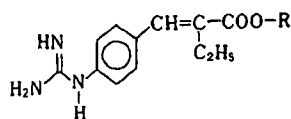
Various amino- and guanidino-substituted naphthoates and tetrahydronaphthoates were synthesized and evaluated for inhibitory activities against trypsin, plasmin, kallikrein, thrombin and Cl esterase. Among these compounds, phenyl 4-guanidino-1-naphthoate (IIIj) and phenyl 6-guanidino-1-naphthoate (IIIi) exhibited potent and selective trypsin inhibition (IC_{50} : 4×10^{-7} and 5×10^{-8} M, respectively) and phenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vb) and *p*-ethoxycarbonylphenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vg) had selective inhibitory activities against thrombin (IC_{50} : 4×10^{-5} and 1×10^{-5} M, respectively).

Keywords—enzyme inhibition; trypsin; plasmin; kallikrein; thrombin; Cl esterase; guanidinonaphthoate; guanidinotetrahydronaphthoate; aminonaphthoate; aminotetrahydronaphthoate

Serine-proteases play important roles in the homeostasis of the living body, but anomalous activation is known to be involved in the pathogenesis of various diseases. Because of these facts, the development of synthetic serine-protease inhibitors has been a goal of many investigators.^{3a-e)} Phenyl *p*-guanidinobenzoate^{1,3a)} and *p*-nitrophenyl *p*-guanidinobenzoate^{3b)} are well-known as potent serine-protease inhibitors and trypsin titrants. Moreover, Tamura *et al.*^{3c)} reported that *N,N*-dimethylamino *p*-(*p*'-guanidinobenzoyloxy)benzylcarbonyloxy glycolate has strong inhibitory activity against trypsin and Muramatsu *et al.*^{3d)} reported that *trans*-4-(guanidinomethyl)cyclohexanecarboxylic acid esters inhibit the esterolytic activity of chymotrypsin.

We have also been interested in synthetic serine-protease inhibitors, and we have synthesized various guanidino ester derivatives and evaluated their effects on serine-proteases. In the previous paper,¹⁾ we reported that aryl esters (Ia, b) of α -ethyl-*p*-guanidinocinnamic acid inhibited Cl esterase potently and selectively. As a continuation of this work, we have synthesized various aminonaphthoates (IIa—h), guanidinonaphthoates (IIIa—l), aminotetrahydronaphthoates (IVa—g) and guanidinotetrahydronaphthoates (Va—g) and examined their inhibitory activities against trypsin, plasmin, kallikrein, thrombin, and Cl esterase. It was found that phenyl 4-guanidino-1-naphthoate (IIIj) and phenyl 6-guanidino-1-naphthoate (IIIi) exhibited potent and selective trypsin inhibition (IC_{50} : 4×10^{-7} and 5×10^{-8} M, respectively) and phenyl 7-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vb) and *p*-ethoxycarbonylphenyl 7-guanidino-1,2,3,4-tetrahydronaphthoate (Vg) had selective inhibitory activities against thrombin (IC_{50} : 4×10^{-5} and 1×10^{-5} M, respectively).

In this paper, we describe the synthesis and serine-protease inhibitory activities of these compounds, and discuss the structure-activity relationships.



Ia, b

a : R = C₆H₅

b : R = 6-methyl-3-pyridyl

Chart 1

IIa-h		IIIa-l		IVa-g			Va-g		
NH ₂	R	Gu	R	NH ₂	COOR	R	Gu	COOR	R
a 3	C ₂ H ₅	a 3	H	a 5	1	C ₆ H ₅	a 5	1	C ₆ H ₅
b 4	C ₂ H ₅	b 4	H	b 7	1	C ₆ H ₅	b 7	1	C ₆ H ₅
c 5	C ₂ H ₅	c 5	H	c 6	2	C ₆ H ₅	c 6	2	C ₆ H ₅
d 6	C ₂ H ₅	d 6	H	d 7	5	C ₆ H ₅	d 7	5	C ₆ H ₅
e 3	C ₆ H ₅	e 3	C ₂ H ₅	e 8	5	C ₆ H ₅	e 8	5	C ₆ H ₅
f 4	C ₆ H ₅	f 4	C ₂ H ₅	f 5	1	ECP	f 5	1	ECP
g 5	C ₆ H ₅	g 5	C ₂ H ₅	g 7	1	ECP	g 7	1	ECP
h 6	C ₆ H ₅	h 6	C ₂ H ₅						
		i 3	C ₆ H ₅						
		j 4	C ₆ H ₅						
		k 5	C ₆ H ₅						
		l 6	C ₆ H ₅						

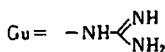
ECP = *p*-ethoxycarbonylphenyl

Chart 2

Synthesis

As shown in Chart 2, we synthesized various ethyl and phenyl esters of amino- and guanidino-substituted naphthoic and tetrahydronaphthoic acids as well as free guanidino-naphthoic acids and *p*-ethoxycarbonylphenyl esters of amino- and guanidinotetrahydronaphthoic acids. In principle, as illustrated in Chart 3, nitro acids were converted to esters, followed by reduction with hydrogen and palladium on a carbon (Pd-C) catalyst to obtain amino-esters and these were further converted to guanidino-esters by reaction with cyanamide.

Nitro-acids (VIb, d, VIIa-f)^{4b-f)} were prepared by nitration of the corresponding naphthoic acid (IX) and tetrahydronaphthoic acids (X-XII), except for VIa, c,^{4a)} which were obtained as a mixture by hydrolysis of 3-nitro-1,8-naphthalic anhydride (VIII) followed by decarboxylation. Separation of the mixtures of nitro-acids to obtain each isomer was performed either with or without esterification. Thus, VIb and VI d were separated directly from the mixture by recrystallization, while the mixtures of VIa, c and of VI d-f were each converted to esters, followed by chromatographic separation of the components on a silica-gel column, and VIIa and VIIb were obtained by conversion of the mixture to *p*-methoxycarbonylphenyl esters (XIIIa, b), which were separated by recrystallization and then hydrolyzed.

Esterification was achieved either with EtOH/H₂SO₄ to obtain ethyl nitro-esters (VIe-g) or by means of acid chloride formation with PCl₅ to prepare phenyl or *p*-ethoxycarbonylphenyl nitro-esters (VIh-j, VIIg-n). Esterification of 8-nitro-1-naphthoic acid (VI d) by these procedures was unsuccessful. An attempt to prepare a corresponding amino-ester from phenyl 6-nitro-1,2,3,4-tetrahydro-5-naphthoate (VIIj) by reduction with hydrogen on

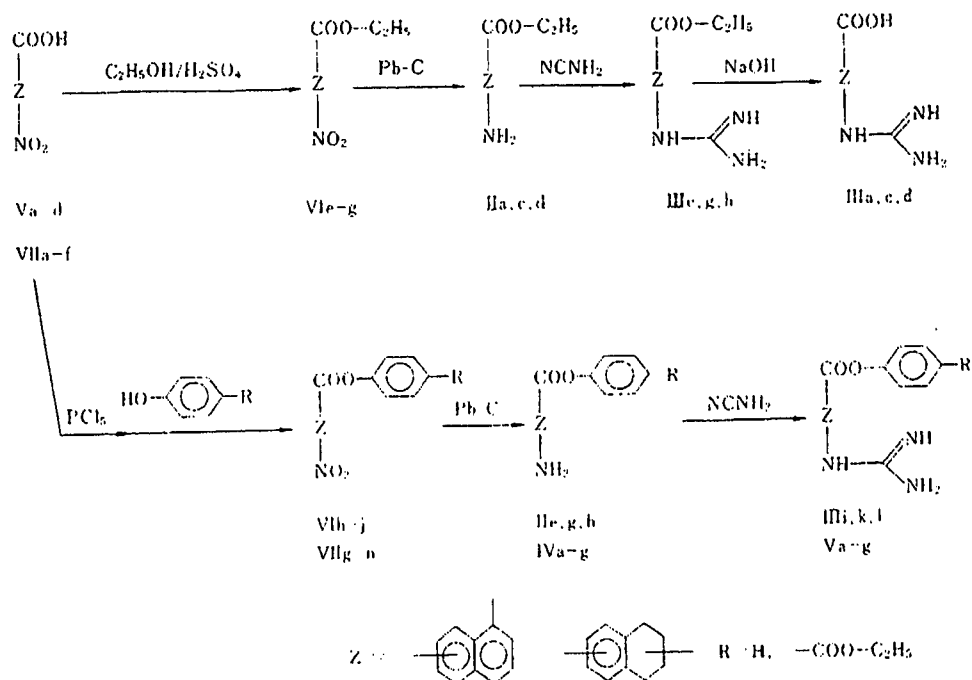


Chart 3

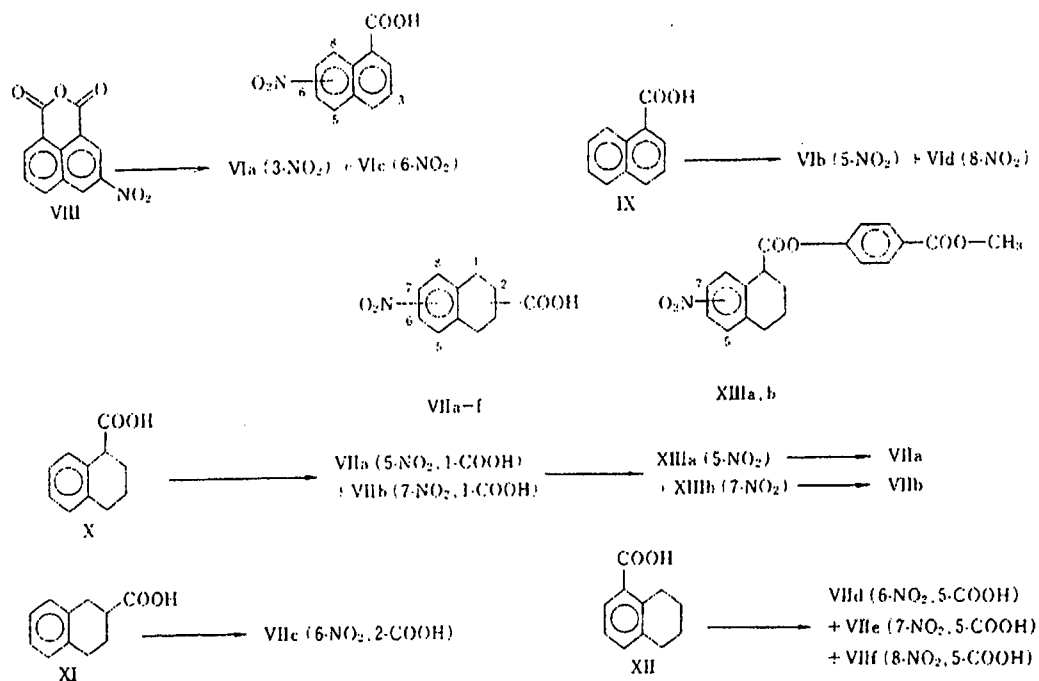


Chart 4

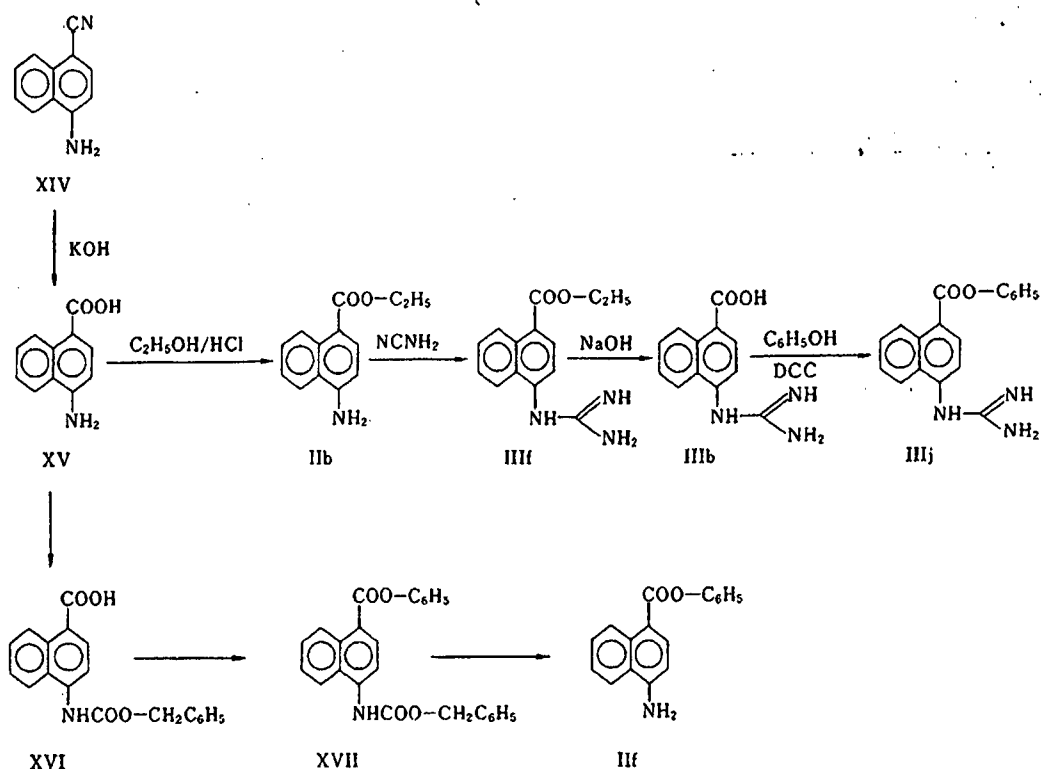


Chart 5

Pd-C also failed.

Free guanidino-acids (IIIa, c, d) were obtained from the corresponding ethyl guanidino-esters (IIIe, g, h) by hydrolysis in aqueous NaOH.

Derivatives of 4-amino and 4-guanidino-1-naphthoic acid were synthesized by a separate route starting from 4-amino-1-naphthalenecarbonitrile (XIV). Compound XIV was hydrolyzed in aqueous KOH to give 4-amino-1-naphthoic acid (XV) which was converted, in turn, to the ethyl amino-ester (IIb) and the ethyl guanidino-ester (IIIi) by reaction with cyanamide, to the free acid (IIIb) by hydrolysis and to the phenyl guanidino-ester (IIIj) by treatment with dicyclohexylcarbodiimide (DCC). The phenyl amino-ester (IIf) was obtained by benzyloxycarbonylation (XVI) of XV with benzyloxycarbonyl chloride, conversion of XVI to the phenyl ester (XVII) via the acid chloride and subsequent reduction with hydrogen on Pd-C.

Protease Inhibitory Activities

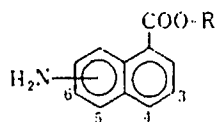
The inhibitory activities against trypsin, plasmin, kallikrein and thrombin were determined as described in the previous paper and expressed similarly in terms of IC_{50} , the molar concentration inhibiting 50% of the activity of enzymes to hydrolyze the designated substrate.

The potency of compounds whose inhibitory activities correspond to IC_{50} of less than $1 \times 10^{-3} \text{ M}$ is expressed as the percent inhibition at a concentration of $1 \times 10^{-3} \text{ M}$.

Results and Discussion

The results are shown in Tables I—IV. It can be concluded that the ester linkage is

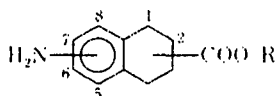
TABLE I. Enzyme Inhibition by Aminonaphthoates



Compd. No.	NH ₂	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IIa	3	C ₂ H ₅	< 10 ^{a)}	< 10	< 10	< 10	
IIb	4	C ₂ H ₅	3 × 10 ^{-4 b)}	< 10	< 10	11	
IIc	5	C ₂ H ₅	11	< 10	< 10	11	
IId	6	C ₂ H ₅	19	< 10	32	< 10	
IIe	3	C ₆ H ₅	8 × 10 ⁻⁴	< 10	< 10	< 10	< 10
IIf	4	C ₆ H ₅	2 × 10 ⁻⁴	< 10	< 10	< 10	< 10
IIg	5	C ₆ H ₅	9 × 10 ⁻⁴	< 10	< 10	< 10	< 10
IIh	6	C ₆ H ₅	5 × 10 ⁻⁵	< 10	< 10	31	< 10

a) % inhibition at a concentration of the compound of 1 × 10⁻³ M. b) IC₅₀.

TABLE II. Enzyme Inhibition by Aminotetrahydronaphthoates



Compd. No.	NH ₂	COOR	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IVa	5	1	C ₆ H ₅	38 ^{a)}	< 10	< 10	22	
IVb	7	1	C ₆ H ₅	1 × 10 ^{-3 b)}	< 10	< 10	3 × 10 ⁻⁴	
IVc	6	2	C ₆ H ₅	8 × 10 ⁻⁵	< 10	< 10	24	
IVd	7	5	C ₆ H ₅	4 × 10 ⁻⁴	< 10	< 10	< 10	< 10
IVe	8	5	C ₆ H ₅	2 × 10 ⁻⁴	< 10	< 10	< 10	< 10
IVf	5	1	ECP ^{c)}	8 × 10 ⁻⁵	< 10	11	22	< 10
IVg	7	1	ECP	3 × 10 ⁻⁵	29	< 10	5 × 10 ⁻⁵	< 10

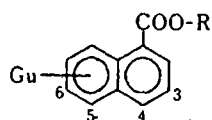
a) % inhibition at a concentration of the compound of 1 × 10⁻³ M.
b) IC₅₀. c) ECP = *p*-ethoxycarbonylphenyl.

necessary for compounds to show protease inhibitory activities; free guanidinonaphthoic acids studied were practically devoid of activity. In comparison with amino derivatives, guanidino esters, in both the naphthoate and tetrahydronaphthoate series, were more potent in terms of both the activity and the range of protease inhibitory effects. More precisely, the inhibitory effects against trypsin were limited in the case of amino-esters of both series, whereas the inhibitory effectiveness of guanidino-esters was higher against trypsin and extended to proteases other than trypsin.

In a series of tetrahydronaphthoates, the introduction of an ethoxycarbonyl group in the *p*-position of the ester phenyl moiety increased the inhibitory action in terms of both the activity and the range.

Comparison of a series of congeners, *e.g.* phenyl 5-guanidino-1-naphthoate (IIIk), phenyl 5-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Va) and *p*-ethoxycarbonylphenyl 5-guanidino-1,2,3,4-tetrahydro-1-naphthoate (Vf), revealed that IIIk and Vf showed practically the same

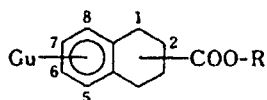
TABLE III. Enzyme Inhibition by Guanidinonaphthoates



Compd. No.	Gu ^{a)}	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
IIIa	3	H	<10 ^{b)}	<10	<10	<10	<10
IIIb	4	H	19	<10	19	<10	<10
IIIc	5	H	47	<10	<10	14	<10
IIId	6	H	16	<10	<10	<10	<10
IIIe	3	C ₂ H ₅	42	<10	<10	16	41
IIIf	4	C ₂ H ₅	42	47	<10	13	7 × 10 ⁻⁴
IIIg	5	C ₂ H ₅	1 × 10 ^{-3 c)}	<10	<10	<10	22
IIIh	6	C ₂ H ₅	2 × 10 ⁻⁴	48	<10	<10	2 × 10 ⁻⁴
IIIi	3	C ₆ H ₅	31	31	1 × 10 ⁻⁴	8 × 10 ⁻⁴	42
IIIj	4	C ₆ H ₅	4 × 10 ⁻⁷	5 × 10 ⁻⁴	28	1 × 10 ⁻⁴	7 × 10 ⁻⁴
IIIk	5	C ₆ H ₅	6 × 10 ⁻⁵	30	18	15	4 × 10 ⁻⁴
IIIl	6	C ₆ H ₅	5 × 10 ⁻⁸	1 × 10 ⁻⁵	48	7 × 10 ⁻⁶	4 × 10 ⁻⁵

a) Gu = -NH-C(=NH)-NH₂.b) % inhibition at a concentration of the compound of 1 × 10⁻³ M.c) IC₅₀.

TABLE IV. Enzyme Inhibition by Guanidinotetrahydronaphthoates



Compd. No.	Gu ^{a)}	COOR	R	Trypsin	Plasmin	Kallikrein	Thrombin	Cl esterase
Va	5	1	C ₆ H ₅	<10 ^{b)}	<10	18	<10	18
Vb	7	1	C ₆ H ₅	7 × 10 ^{-4 c)}	19	43	4 × 10 ⁻⁵	4 × 10 ⁻⁴
Vc	6	2	C ₆ H ₅	40	1 × 10 ⁻³	26	4 × 10 ⁻⁴	3 × 10 ⁻⁴
Vd	7	5	C ₆ H ₅	7 × 10 ⁻⁴	<10	2 × 10 ⁻⁴	2 × 10 ⁻⁴	30
Ve	8	5	C ₆ H ₅	3 × 10 ⁻⁴	<10	<10	39	24
Vf	5	1	ECP ^{d)}	3 × 10 ⁻⁴	20	7 × 10 ⁻⁴	38	5 × 10 ⁻⁴
Vg	7	1	ECP	7 × 10 ⁻⁵	7 × 10 ⁻⁵	2 × 10 ⁻⁴	1 × 10 ⁻⁵	6 × 10 ⁻⁴

a) Gu = -NH-C(=NH)-NH₂.b) % inhibition at a concentration of the compound of 1 × 10⁻³ M.c) IC₅₀.d) ECP = *p*-ethoxycarbonylphenyl.

inhibitory effectiveness whereas Va was almost ineffective.

Among the compounds studied, the 1,6-substituted compound (IIh) in the amino-naphthoates was a weak but selective inhibitor of trypsin, and the 1,4- and 1,6-substituted compounds (IIIj, l) in the guanidino-naphthoates showed particularly strong inhibition of trypsin with IC₅₀ values of 4 × 10⁻⁷ and 5 × 10⁻⁸ M, respectively, together with moderate inhibition on other proteases including Cl esterase and thrombin, while the 1,7-substituted compounds (IVb, g) in the amino-tetrahydronaphthoates showed moderate inhibitions of

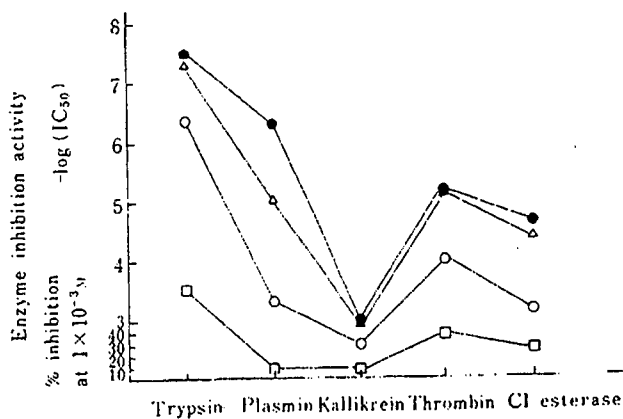
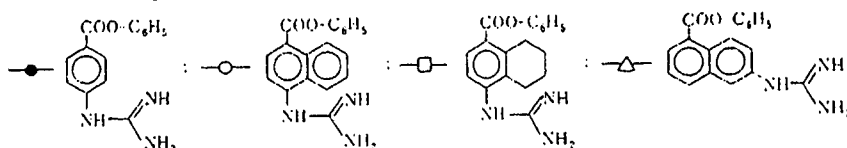


Fig. 1. Enzyme Inhibition Spectrum of Guanidino Phenyl Esters



both trypsin and thrombin. Among guanidino-tetrahydronaphthoates, the 1,7-substituted compounds (Vb, g) showed particularly potent inhibition of thrombin, the inhibition spectrum of *p*-ethoxycarbonylphenyl compound (Vg) being particularly broad.

Figure 1 compares the spectrum of protease inhibitory activities of 4 representative phenyl-esters which can be regarded as analogues of phenyl *p*-guanidinobenzoate reported in the previous paper. It seems likely that the inhibitory effectiveness may be determined by many factors, such as the distance between the guanidino group and the ester linkage, the electron atmosphere of the molecules and, particularly, the steric configuration of the carrier moiety of the guanidino and ester groups.

Experimental

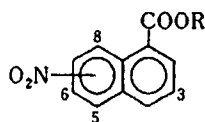
Melting points were determined on a Yamato MP-21 melting point apparatus and are uncorrected. The infrared (IR) spectra were determined on a Shimadzu IR-430, proton-nuclear magnetic resonance (¹H-NMR) on a JEOL FX-60Q or Varian T-60 and carbon-13 nuclear magnetic resonance (¹³C-NMR) on a JEOL FX-60Q (with tetramethylsilane as an internal standard). The physical data for the compounds are summarized in Tables V–X.

3-Nitro-1-naphthoic Acid (VIa) and 6-Nitro-1-naphthoic Acid (VIc) (Method A)—3-Nitro-1,8-naphthalic anhydride (50 g) and then a solution of 49.1 g of mercuric oxide in 120 ml of water and 40 ml of acetic acid were added to a solution of 27 g of NaOH in 1000 ml of water with stirring. The reaction mixture was refluxed for 98 h, then cooled, and the precipitates were collected and washed with water. The precipitates were suspended in 420 ml of conc. HCl and the mixture was refluxed for 3 h, then cooled. The precipitates were collected and recrystallized from acetic acid to give 25.4 g (57%) of VIa⁴¹ as pale yellow needles. The filtrate was concentrated to dryness *in vacuo* to give a mixture of VIa and VIc. The mixture was used for the next reaction without purification.

5-Nitro-1-naphthoic Acid (VIb) and 8-Nitro-1-naphthoic Acid (VIc) (Method B)—1-Naphthoic acid (10 g) was added to 28 ml of conc. HNO₃ (*d* = 1.38) under ice cooling and the mixture was warmed for 4 h in a boiling water bath. The mixture was then poured into ice water and the precipitates were collected, washed with water, and dissolved in 100 ml of boiling 8% Na₂CO₃ solution. After filtration, the solution was acidified with 10% HCl. The precipitates formed were collected and recrystallized from EtOH to give 4.6 g (37%) of VIb⁴² as pale yellow needles. The filtrate was concentrated and the residue was recrystallized from EtOH to give 5.5 g (44%) of VIc⁴² as pale yellow prisms.

5-Nitro-1,2,3,4-tetrahydro-1-naphthoic Acid (VIIa) and 7-Nitro-1,2,3,4-tetrahydro-1-naphthoic Acid (VIIb) (Method C)—1,2,3,4-Tetrahydro-1-naphthoic acid (30 g) was added to 300 ml of conc. HNO₃ (*d* = 1.38) at room

TABLE V. Physical Data



Compd. No.	NO ₂	COOR	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd	Found	
								C	H	N
VIa	3	1	H	260—261 ^{a)}	A	1700				
VIb	5	1	H	232—234.5 ^{b)}	B	1690				
VIc	8	1	H	208—209 ^{c)}	B	1685				
VIe	3	1	C ₂ H ₅	87—88 ^{d)}	H	1720	C ₁₃ H ₁₁ NO ₄	63.67 (63.64)	4.52 (4.43)	5.71 (5.66)
VIg	5	1	C ₂ H ₅	94—94.5 ^{e)}	H	1725	C ₁₃ H ₁₁ NO ₄	63.67 (63.55)	4.52 (4.44)	5.71 (5.71)
VIh	6	1	C ₂ H ₅	113—114 ^{f)}	H, F	1720	C ₁₃ H ₁₁ NO ₄	63.67 (63.62)	4.52 (4.43)	5.71 (5.69)
VIi	3	1	C ₆ H ₅	168—169	E	1733	C ₁₇ H ₁₁ NO ₄	69.62 (69.60)	3.78 (3.74)	4.78 (4.78)
VIj	5	1	C ₆ H ₅	143—144	E	1720	C ₁₇ H ₁₁ NO ₄	69.62 (69.55)	3.78 (3.72)	4.78 (4.77)
VIk	6	1	C ₆ H ₅	135—135.5	F	1722	C ₁₇ H ₁₁ NO ₄	69.62 (69.52)	3.78 (3.72)	4.78 (4.79)

a) Lit.,⁴⁰ 270—271.5°C. b) Lit.,⁴⁰ 234—235°C. c) Lit.,⁴⁰ 215°C.
d) Lit.,⁴⁰ 87.5—88.5°C. e) Lit.,⁴⁰ 92—93°C. f) Lit.,⁴⁰ 111.5—112°C.

temperature with stirring and the mixture was stirred for 10 h at room temperature, then poured into water. The precipitates were collected, washed with water and air-dried at room temperature to give 23.9 g (63%) of a mixture of VIIa, b. A suspension of the mixture of VIIa, b in 200 ml of EtOAc was treated with 25 g of PCl₅. The reaction mixture was stirred for 3 h at room temperature, then concentrated *in vacuo*. The residue was washed with hexane and dissolved in 300 ml of EtOAc. To this solution, 16.5 g of *p*-methoxycarbonylphenol and 13 g of triethylamine were added with stirring. The reaction mixture was stirred for 3 h at room temperature, then washed with 10% HCl, 5% NaOH, and satd. NaCl, dried over MgSO₄ and concentrated *in vacuo*. Recrystallization from EtOAc gave 12.6 g of *p*-methoxycarbonylphenyl 7-nitro-1,2,3,4-tetrahydro-1-naphthoate (XIIIb) as a colorless powder, mp 178—179.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1745 (C=O), 1712 (C=O). Anal. Calcd for C₁₉H₁₇NO₆: C, 64.22; H, 4.82; N, 3.94. Found C, 64.13; H, 4.74; N, 3.90.

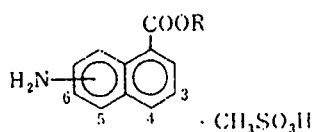
The filtrate was concentrated to dryness *in vacuo* and the residue was recrystallized from EtOH to give 21.4 g of *p*-methoxycarbonylphenyl 5-nitro-1,2,3,4-tetrahydro-1-naphthoate (XIIIa) as a colorless powder, mp 101—101.5°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1745 (C=O), 1714 (C=O). Anal. Calcd for C₁₉H₁₇NO₆: C, 64.22; H, 4.82; N, 3.94. Found C, 64.11; H, 4.73; N, 3.86.

XIIIa (10 g) was added to a solution of 40 ml of 20% NaOH and 10 ml of EtOH, and the mixture was stirred for 1 h at room temperature, then acidified with 10% HCl. The precipitates were collected and washed with water. Recrystallization from EtOAc gave 5 g of VIIa⁴⁰ as pale yellow prisms. VIIb⁴⁰ was obtained from XIIIb by the same procedure.

6-Nitro-1,2,3,4-tetrahydro-2-naphthoic Acid (VIc), 6-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIId), 7-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIe) and 8-Nitro-1,2,3,4-tetrahydro-5-naphthoic Acid (VIIf) (Method D)—VIc was prepared by nitration of 1,2,3,4-tetrahydro-2-naphthoic acid with conc. HNO₃ ($d=1.38$) at 40°C and VIId, e, f were prepared by nitration of 1,2,3,4-tetrahydro-5-naphthoic acid with fum. HNO₃ ($d=1.52$) at 0°C according to method C. The mixture of VIId, e, f, was further subjected to esterification without purification. Analytical samples of VIe, f were prepared by hydrolysis of their phenyl esters (VIIf, I) with 10% NaOH.

Phenyl 3-Nitro-1-naphthoate (VIh) (Method E)—A suspension of 13.8 g of VIa in 100 ml of EtOAc was treated with 17.3 g of PCl₅. The mixture was stirred overnight at room temperature, then concentrated to dryness *in vacuo*. The residue was washed with hexane to give the acid chloride. A solution of the acid chloride in 50 ml of EtOAc was added to a solution of 6 g of phenol and 8.4 g of triethylamine in 200 ml of EtOAc. The whole was stirred overnight at

TABLE VI. Physical Data



Compd. No.	NH ₂	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
							Calcd (Found)		
							C	H	N
IIa	3	C ₂ H ₅	155—156 ^b	I	1719	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.92)	5.50 (5.50)	4.50 (4.43)
IIb	4	C ₂ H ₅	217—220 (d) ^c	K	1718	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.51)	5.50 (5.48)	4.50 (4.48)
IIc	5	C ₂ H ₅	213 214.5 (d) ^d	I	1706	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	54.01 (53.89)	5.50 (5.50)	4.50 (4.40)
IIId	6	C ₂ H ₅	204 205	I	1688	C ₁₃ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.08)	4.77 (4.77)	3.90 (3.80)
IIe	3	C ₆ H ₅	200 200.5	I	1740	C ₁₉ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (59.67)	4.77 (4.81)	3.90 (4.02)
IIIf	4	C ₆ H ₅	233 236	O	1738	C ₁₉ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.23)	4.77 (4.75)	3.90 (3.83)
IIg	5	C ₆ H ₅	243 (d)	I	1730	C ₁₉ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.23)	4.77 (4.75)	3.90 (3.83)
IIh	6	C ₆ H ₅	240 <	I	1730	C ₁₉ H ₁₃ NO ₂ ·CH ₄ O ₃ S	60.16 (60.12)	4.77 (4.72)	3.90 (4.01)

- a) The structure of IIId was confirmed by the following spectral data: IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3450, 3360, 3100—2500 (NH), 1688 (COO), ¹H-NMR (DMSO-*d*₆): 1.40 (3H, t, *J* = 7.1 Hz, CH₃-CH₂), 2.48 (3H, s, CH₃-SO₃-), 4.43 (2H, q, *J* = 7.1 Hz, CH₂-CH₃), 7.45—9.01 (6H, m, aromatic H), 9.29 (3H, br, NH₂).
- b) Lit.^{6a)} free base bp 170—175 °C (0.5 mmHg).
- c) Lit.^{6a)} free base 80 °C. d) Lit.^{6a)} free base 92 °C.

room temperature, then washed with 10% HCl, 5% NaOH and satd. NaCl, dried over anhyd. MgSO₄ and concentrated to dryness *in vacuo*. Recrystallization from EtOH-acetone gave 17 g (92%) of VIh as pale yellow needles.

Phenyl 6-Nitro-1-naphthoate (VIj) (Method F)—A mixture of VIh, j was prepared from VIa, c by reaction with phenol according to method E and the mixture was chromatographed on a silica gel column to separate VIh and VIj, the former being obtained from the first eluate with benzene:hexane = 1:1 and the latter from the second eluate.

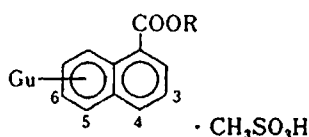
Phenyl 6-Nitro-1,2,3,4-tetrahydro-5-naphthoate (VIIj), Phenyl 7-Nitro-1,2,3,4-tetrahydro-1-naphthoate (VIIk) and Phenyl 8-Nitro-1,2,3,4-tetrahydro-5-naphthoate (VIIf) (Method G)—A mixture of VIIj, k, l was prepared from VIId, e, f and phenol by method F. The isomers, VIIi, VIIk and VIIj, were obtained from the first, second and third eluates, respectively, with benzene:hexane = 1:1.

Ethyl 5-Nitro-1-naphthoate (VIIf) (Method H)—A mixture of 15 g of 5-nitro-1-naphthoic acid and 3 ml of conc. H₂SO₄ in 150 ml of anhyd. EtOH was refluxed overnight, then concentrated to dryness *in vacuo* and the residue was dissolved in EtOAc. This solution was washed with satd. NaHCO₃ and satd. NaCl, dried over anhyd. MgSO₄ and concentrated to dryness *in vacuo*. Recrystallization of the residue from EtOH gave 14.8 g (88%) of VIIf as colorless needles.

Methanesulfonic Acid Salt of Phenyl 5-Amino-1-naphthoate (IIg) (Method I)—Methanesulfonic acid (2.4 g) and 10% Pd-C (2 g) were added to a solution of 6 g of VIIi in 60 ml of dimethylformamide (DMF). The mixture was hydrogenated with stirring at room temperature under atmospheric pressure, then filtered. Ethyl ether was added to the filtrate and the precipitates were collected. Recrystallization from EtOH gave 6.3 g (88%) of IIg as colorless needles.

Methanesulfonic Acid Salt of Phenyl 5-Guanidino-1-naphthoate (IIIk) (Method J)—A mixture of 3.6 g of IIg and 0.84 g of cyanamide in 30 ml of MeOH was stirred overnight at 50 °C, then filtered. The filtrate was concentrated to half the initial volume, then poured into satd. NaHCO₃ solution. The precipitates were collected and washed with water and acetone. A suspension of the precipitates in DMF was treated with 1.2 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate, and the precipitates were collected. Recrystallization from EtOH gave 1.9 g (46%) of IIIk as colorless prisms.

TABLE VII. Physical Data



Compd. No.	Gu ^{a)}	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
							Calcd	(Found)	
							C	H	N
IIIa	3	H	232.5—233	M	1715	C ₁₂ H ₁₁ N ₃ O ₂ ·CH ₄ O ₃ S	47.99 (47.95)	4.65 4.71	12.92 12.84
IIIb	4	H	260—261.5	M	1705	C ₁₂ H ₁₁ N ₃ O ₂ ·CH ₄ O ₃ S	47.99 (47.87)	4.65 4.65	12.92 12.91
IIIc	5	H	264—265	M	1700	C ₁₂ H ₁₁ N ₃ O ₂ ·CH ₄ O ₃ S	47.99 (47.89)	4.65 4.62	12.92 12.79
IIId	6	H	224—225	M	1710	C ₁₂ H ₁₁ N ₃ O ₂ ·CH ₄ O ₃ S	47.99 (47.78)	4.65 4.73	12.92 12.80
IIIe	3	C ₂ H ₅	186—187	L	1690	C ₁₄ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	50.98 (50.86)	5.42 5.44	11.89 11.88
IIIf	4	C ₂ H ₅	175.5—176	L	1710	C ₁₄ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	50.98 (50.83)	5.42 5.40	11.89 11.86
IIIg	5	C ₂ H ₅	186.5—187	L	1690	C ₁₄ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	50.98 (50.81)	5.42 5.45	11.89 11.87
IIIh	6	C ₂ H ₅	183.5—184	L	1706	C ₁₄ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	50.98 (50.85)	5.42 5.41	11.89 11.93
IIIi	3	C ₆ H ₅	206.5—207	J	1722	C ₁₈ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	56.85 (56.58)	4.77 4.81	10.47 10.50
IIIj ^{b)}	4	C ₆ H ₅	219.5—220	N	1722	C ₁₈ H ₁₅ N ₃ O ₂ ·C ₇ H ₈ O ₃ S	62.88 (62.44)	4.85 4.93	8.80 8.92
IIIk	5	C ₆ H ₅	187—187.5	J	1720	C ₁₈ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	56.85 (56.35)	4.77 4.84	10.47 10.47
IIIl	6	C ₆ H ₅	235—237	J	1732	C ₁₈ H ₁₅ N ₃ O ₂ ·CH ₄ O ₃ S	56.85 (56.60)	4.77 4.74	10.47 10.50

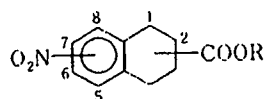
a) Gu = $\text{—NH—}\begin{array}{c} \text{NH} \\ \diagup \quad \diagdown \\ \text{NH}_2 \end{array}$ b) *p*-Toluenesulfonate.

Methanesulfonic Acid Salt of Ethyl 4-Amino-1-naphthoate (IIb) (Method K)—A mixture of 8.4 g of 4-amino-1-naphthalenecarbonitrile in 100 ml of 50% KOH was refluxed overnight. After cooling, the mixture was diluted with 200 ml of water and filtered. The filtrate was acidified with conc. HCl and the precipitates were collected and air-dried at room temperature. Dry HCl gas was passed into a suspension of the precipitates in 200 ml of EtOH with stirring at room temperature until saturation. The reaction mixture was stirred overnight at room temperature, then the reaction mixture was concentrated to dryness *in vacuo*, and satd. NaHCO₃ and EtOAc were added to the residue. The separated organic layer was washed with 5% NaOH and with satd. NaCl, dried over anhyd. MgSO₄ and concentrated to dryness *in vacuo*. The residue was dissolved in ethyl ether and the solution was filtered. The filtrate was treated with 5.8 g of methanesulfonic acid and the precipitates were collected and recrystallized from EtOH–ethyl ether to give 8.4 g (54%) of IIb as colorless needles.

Methanesulfonic Acid Salt of Ethyl 4-Guanidino-1-naphthoate (IIIf) (Method L)—A mixture of 9.2 g of IIb and 3.6 g of cyanamide in 50 ml of MeOH was stirred overnight at 50 °C. The mixture was concentrated to half the initial volume, and the concentrate was poured into satd. NaHCO₃. The precipitates were collected and washed with water and acetone. A suspension of the precipitates in 15 ml of EtOH was treated with 4.5 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected and recrystallized from EtOH–ethyl ether to give 3.6 g (35%) of IIIf as a colorless powder.

Methanesulfonic Acid Salt of 4-Guanidino-1-naphthoic Acid (IIIb) (Method M)—IIIf (3.5 g) was added to a solution of 1.2 g of NaOH in 10 ml of EtOH and 10 ml of water, and the mixture was stirred overnight at room temperature. The mixture was neutralized with 10% HCl and the resulting precipitates were collected. A suspension

TABLE VIII. Physical Data



Compd. No.	NO ₂	COOR	R	mp (°C)	Method	IR $\nu_{\text{max}}^{\text{KBr}}$ cm ⁻¹	Formula	Analysis (%)		
								Calcd	Found	
								C	H	N
VIIa	5	1	H	145—146 ^{a)}	C	1700				
VIIb	7	1	H	161—162 ^{b)}	C	1700				
VIIc	6	2	H	194—195 ^{c)}	D	1698				
VIIe	7	5	H	219—221 ^{d)}	D	1683				
VIIIf	8	5	H	155—157 ^{e)}	D	1690				
VIIg	5	1	C ₆ H ₅	98—99	E	1742	C ₁₇ H ₁₅ NO ₄	68.68 (68.56)	5.09 5.10	4.71 4.71)
VIIh	7	1	C ₆ H ₅	94—94.5	E	1740	C ₁₇ H ₁₅ NO ₄	68.68 (68.48)	5.09 5.05	4.71 4.69)
VIIi	6	2	C ₆ H ₅	123—124	E	1742	C ₁₇ H ₁₅ NO ₄	68.68 (68.61)	5.09 5.06	4.71 4.65)
VIIj	6	5	C ₆ H ₅	99—99.5	G	1752	C ₁₇ H ₁₅ NO ₄	68.68 (68.63)	5.09 5.13	4.71 4.64)
VIIk	7	5	C ₆ H ₅	101—102	G	1737	C ₁₇ H ₁₅ NO ₄	68.68 (68.72)	5.09 5.10	4.71 4.64)
VIII	8	5	C ₆ H ₅	109—110	G	1734	C ₁₇ H ₁₅ NO ₄	68.68 (68.72)	5.09 5.11	4.71 4.64)
VIIIm	5	1	ECP ^{f)}	58—59	E	1750, 1712	C ₂₀ H ₁₉ NO ₆	65.03 (64.81)	5.18 5.19	3.79 3.71)
VIIIn	7	1	ECP	154—155	E	1755, 1720	C ₂₀ H ₁₉ NO ₆	65.03 (64.76)	5.18 5.16	3.79 3.71)

a) Lit.^{4a)} 143°C. b) Lit.^{4a)} 163°C. c) Lit.^{4f)} 196.5—197.0°C.
d) Lit.^{4a)} 212—214°C. e) Lit.^{4a)} 161—162°C. f) ECP = *p*-ethoxycarbonylphenyl.

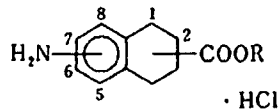
of the precipitates in 25 ml of DMF was treated with 1.4 g of methanesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected and recrystallized from EtOH to give 2.3 g (71%) of IIIb as a colorless powder.

***p*-Toluenesulfonic Acid Salt of Phenyl 4-Guanidino-1-naphthoate (IIIj) (Method N)**—A mixture of 1.3 g of IIIb, 1.2 g of phenol and 1.0 g of dicyclohexylcarbodiimide in 20 ml of dry pyridine was stirred overnight at room temperature, then filtered. The filtrate was concentrated to half the initial volume and the concentrate was poured into satd. NaHCO₃. The precipitates were collected and washed with water and acetone. A suspension of the precipitates in 10 ml of MeOH was treated with 0.7 g of *p*-toluenesulfonic acid. The mixture was filtered, ethyl ether was added to the filtrate and the precipitates were collected. Recrystallization from MeOH–ethyl ether gave 0.4 g (21%) of IIIj as a colorless powder.

4-(Benzyloxycarbonyl)amino-1-naphthoic Acid (XVI)—Benzyloxycarbonyl chloride (3.6 g) was added to a solution of 3.8 g of 4-amino-1-naphthoic acid in 50 ml of 0.5 N KOH under ice cooling with stirring. The mixture was stirred for 3 h at room temperature, then washed with ethyl ether and neutralized with 10% HCl. The precipitates were collected and recrystallized from DMF–water to give 3.5 g (54%) of XVI as a colorless powder, mp 245—246°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3260 (NH), 1682 (C=O), 1532, 1233. ¹H-NMR (*d*₆-DMSO) δ : 5.26 (2H, s, -CH₂-), 7.01—9.24 (11H, m), 10.00 (1H, s, NH), 13.01 (1H, br, COOH). Anal. Calcd for C₁₉H₁₅NO₄: C, 71.02; H, 4.70; N, 4.36. Found: C, 71.02; H, 4.69; N, 4.34.

Phenyl 4-Benzyloxycarbonylamino-1-naphthoate (XVII)—A mixture of 2.0 g of XVI in 20 ml of SOCl₂ was refluxed for 0.5 h, then concentrated *in vacuo*. The residue was added to a solution of 1 g of phenol in 20 ml of pyridine. The mixture was stirred for 3 h at room temperature, then poured into water. The precipitates were collected and recrystallized from EtOAc–hexane to give 2.0 g (81%) of XVII as a colorless powder, mp 141—142°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3300 (NH), 1725 (ester C=O), 1690 (amide C=O), 1530, 1225. ¹H-NMR (*d*₆-DMSO) δ : 5.27 (2H, s, -CH₂-), 7.04—9.18 (16H, m), 10.14 (1H, s, NH). Anal. Calcd for C₂₅H₁₉NO₄: C, 75.55; H, 4.82; N, 3.52. Found C,

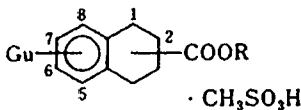
TABLE IX. Physical Data



Compd. No.	NH ₂	COOR	R	mp (°C)	Method	IR ν _{max} ^{KBr} cm ⁻¹	Formula	Analysis (%)		
								Calcd	(Found)	
								C	H	N
IVa	5	1	C ₆ H ₅	210—213 (d)	I	1744	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (67.10)	5.97 5.96	4.61 4.60
IVb	7	1	C ₆ H ₅	149—150	I	1741	^{b)}			
IVc	6	2	C ₆ H ₅	218—222 (d)	I	1735	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.90)	5.97 5.96	4.61 4.64
IVd	7	5	C ₆ H ₅	179—181	I	1734	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.89)	5.97 5.97	4.61 4.69
IVe	8	5	C ₆ H ₅	>270	I	1734	C ₁₇ H ₁₇ NO ₂ ·HCl	67.21 (66.91)	5.97 6.05	4.61 4.51
IVf	5	1	ECP ^{a)}	206—209 (d)	I	1742, 1715	C ₂₀ H ₂₁ NO ₄ ·HCl	63.91 (63.84)	5.90 5.87	3.73 3.72
IVg	7	1	ECP	173—175	I	1765, 1712	^{c)}			

a) ECP = *p*-ethoxycarbonylphenyl.
b, c) The structures were confirmed as the guanidino derivatives (obtained in the next step).

TABLE X. Physical Data



Compd. No.	Gu ^{a)}	COOR	R	mp (°C)	Method	IR ν _{max} ^{KBr} cm ⁻¹	Formula	Analysis (%)		
								Calcd	(Found)	
								C	H	N
Va	5	1	C ₆ H ₅	156—158	J	1742	^{c)}			
Vb	7	1	C ₆ H ₅	189—190	J	1763	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.16)	5.72 5.74	10.36 10.33
Vc	6	2	C ₆ H ₅	184—185	J	1751	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.30)	5.72 5.74	10.36 10.39
Vd	7	5	C ₆ H ₅	197—198	J	1731	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.25)	5.72 5.71	10.36 10.35
Ve	8	5	C ₆ H ₅	161—162	J	1722	C ₁₈ H ₁₉ N ₃ O ₂ ·CH ₄ O ₃ S	56.28 (56.22)	5.72 5.70	10.36 10.35
Vf	5	1	ECP ^{b)}	177—178	J	1763, 1705	C ₂₁ H ₂₃ N ₃ O ₄ ·CH ₄ O ₃ S	55.34 (55.29)	5.70 5.70	8.80 8.87
Vg	7	1	ECP	159—160	J	1760, 1718	C ₂₁ H ₂₃ N ₃ O ₄ ·CH ₄ O ₃ S	55.34 (55.02)	5.70 5.66	8.80 8.77

a) Gu = $\text{--NH--}\begin{matrix} \text{NH} \\ \diagup \quad \diagdown \\ \text{NH}_2 \end{matrix}$ b) ECP = *p*-ethoxycarbonylphenyl.
c) The structure of Va was confirmed by the following spectral data: IR ν_{max}^{KBr} cm⁻¹: 3320, 3150 (–NH), 1742 (C=O), ¹H-NMR (d₆-DMSO) δ: 1.47—3.00 (6H, br m), 2.42 (3H, s, CH₃), 4.18 (1H, br, H-1), 6.66—8.01 (12H, m), 9.30 (1H, s, NH); ¹³C-NMR (d₆-DMSO): 19.4, 23.8, 25.5 (–CH₂–CH₂–CH₂), 44.0 (–CH<).

75.48; H, 4.81; N, 3.55.

Methanesulfonic Acid Salt of Phenyl 4-Amino-1-naphthoate (IIc) (Method O)—Hydrogen gas was passed into a mixture of 1 g of XVII and 0.5 g of 10% Pd/C in 30 ml of DMF containing 0.3 g of methanesulfonic acid with stirring for 5 h at room temperature. The mixture was filtered, then ethyl ether was added to the filtrate to give an amorphous powder. Recrystallization from MeOH-ethyl ether gave 0.7 g (77%) of IIc as a colorless powder.

Enzyme Inhibition—Bovine trypsin was purchased from Sigma Chemical Co., St. Louis, USA, and dissolved in 0.1 M borate buffer containing 0.01 M CaCl₂, pH 8.5. Human plasmin was purchased from Green Cross Co., Osaka, Japan, and porcine kallikrein from Bayer, and they were each dissolved in 0.1 M borate buffer, pH 8.5. Bovine thrombin was purchased from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan, and dissolved in 0.02 M phosphate buffer, pH 7.4. Human Cl esterase was prepared by the method of Sumi *et al.*^{5a)} The rates of hydrolysis of TAME by trypsin, plasmin, kallikrein, and thrombin were determined as described by Muramatsu *et al.*^{5b)} and that of ATEE by Cl esterase was assayed as described by Tamura *et al.*^{5c)} at a substrate concentration of 10 mM.

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TABLE I
 PHYSICAL CONSTANTS OF THE NITROALCOHOLS

Name of compound	B. p., °C., and pressure	Mm.	Sp. gr., d ₄ ²⁰	Refr. ind., n _D ²⁰	Formula	% Nitrogen Calcd.	% Nitrogen Found ^b
3-Methyl-3-nitro-2-hexanol	97	5	1.0512	1.4522	C ₇ H ₁₅ O ₂ N	52.17 ^{a,c}	50.47
3-Methyl-3-nitro-4-nonanol	99-101	1.5	0.9835	1.4540	C ₁₀ H ₂₁ O ₂ N	59.11 ^a	58.73
3-Methyl-3-nitro-4-octanol	90-94	2.5	.9734	1.4568	C ₉ H ₁₉ O ₂ N	57.14 ^a	56.92
1-Nitro-2-octanol ^d	120	2		1.4500	C ₈ H ₁₇ O ₂ N	8.00	7.81
2-Nitro-3-nonanol	110	1.5	.9937	1.4508	C ₉ H ₁₉ O ₂ N	7.42	6.93
2-Methyl-2-nitro-3-nonanol	109	1	.9854	1.4525	C ₁₀ H ₂₁ O ₂ N	6.90	6.82
4-Nitro-5-hendecanol	135	2	.9550	1.4550	C ₁₁ H ₂₃ O ₂ N	6.47	6.40
3-Methyl-3-nitro-4-decanol	128	1.3	.9477	1.4578	C ₁₁ H ₂₃ O ₂ N	60.83 ^a	60.67
1-Nitro-2-nonanol	120-121	1	.9838	1.4508	C ₉ H ₁₉ O ₂ N	7.42	7.33
2-Nitro-3-decanol	125	2	.9761	1.4506	C ₁₀ H ₂₁ O ₂ N	6.90	6.61
3-Nitro-4-hendecanol	128	2	.9636	1.4509	C ₁₁ H ₂₃ O ₂ N	6.47	6.29
2-Methyl-2-nitro-3-decanol	124-125	1.2	.9784	1.4533	C ₁₁ H ₂₃ O ₂ N	6.47	6.30
4-Nitro-5-dodecanol	130	1.2	.9666	1.4522	C ₁₂ H ₂₅ O ₂ N	6.06	5.73
3-Methyl-3-nitro-4-hendecanol	111	1.5	.9521	1.4569	C ₁₁ H ₂₃ O ₂ N	62.28 ^a	62.44
2-Nitro-3-hendecanol	128	1.8	.9640	1.4513	C ₁₁ H ₂₃ O ₂ N	6.47	6.20
3-Nitro-4-dodecanol	138-140	2.2	.9622	1.4517	C ₁₂ H ₂₅ O ₂ N	6.06	5.97
2-Methyl-2-nitro-3-hendecanol	125	3	.9240	1.4524	C ₁₂ H ₂₅ O ₂ N	6.06	5.83
1-Nitro-2-hendecanol	140	2	.9587	1.4548	C ₁₁ H ₂₃ O ₂ N	6.47	6.23
2-Nitro-3-tridecanol	153-155	2	.9496	1.4618	C ₁₃ H ₂₇ O ₂ N	5.72	5.30
3-Nitro-4-tetradecanol	150-155	1.5	.9480	1.4638	C ₁₄ H ₂₉ O ₂ N	5.41	5.02

^a % Carbon (not nitrogen), by "Wet Method," Pollard and Forsee, *Ind. Eng. Chem., Anal. Ed.*, 7, 77 (1935). ^b "Kjeldahl," Harte, *ibid.*, 7, 432 (1935). ^c Low carbon analysis is due to the presence of 1-nitrobutane in the nitropentanes. ^d Not a new compound (2).

the nitroparaffin and aldehyde followed by dilution and hydrolysis with dilute acetic acid, and (c) by the mixing of a dilute solution of the sodium salt of the nitroparaffin with an aqueous suspension of the sodium bisulfite addition product of

the aldehyde and subsequent heating of the mixture.

2. Some of the physical constants of these nitroalcohols have been measured.

LAFAYETTE, INDIANA

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[CONTRIBUTION FROM THE SYPHILIS DIVISION OF THE DEPARTMENT OF MEDICINE, JOHNS HOPKINS MEDICAL SCHOOL, AND THE UNITED STATES PUBLIC HEALTH SERVICE]

Arsine Oxides of Naphthalene and Biphenyl¹

By G. O. DOAK, HARRY EAGLE AND H. G. STEINMAN

Arsine oxides, derived from naphthalene and biphenyl, have been prepared by us in order that their treponemicidal activity might be investigated.

Reduction of α -naphthylarsonic acid with sulfur dioxide and potassium iodide yielded an arsine oxide identical with that obtained by Michaelis,² and by Blicke and Smith³ by other methods.

The β -naphthylarsonic acid, used by Michaelis to prepare the corresponding oxide, was shown by Brown⁴ to be a mixture of α - and β -naphthyl-

arsonic acids. β -Naphthylarsonic acid, prepared by Brown's method, yielded an arsine oxide on reduction which did not melt below 360°.

Saunders, Hamilton and Sweet⁵ have described the preparation of a number of nitronaphthylarsonic acids. We have discovered that the more convenient Scheller-Bart reaction⁶ is equally applicable for the preparation of these compounds. Some of these nitronaphthylarsonic acids were reduced catalytically to the corresponding amino compounds by the use of Raney nickel. The yields were comparable to those ob-

(1) Paper V in the series entitled "The Preparation of Phenylarsenoxides."

(2) Michaelis, *Ann.*, 820, 271 (1902).

(3) Blicke and Smith, *This Journal*, 53, 3479 (1929).

(4) Brown, *Trans. Kansas Acad. Sci.*, 42, 209 (1939).

(5) (a) Saunders and Hamilton, *This Journal*, 54, 636 (1932);

(b) Sweet and Hamilton, *ibid.*, 56, 2408 (1934).

(6) Scheller, French Patent 624,028, *Chem. Zentr.*, 98, II, 229 (1927); Doak, *This Journal*, 52, 167 (1940).

tained by Saunders and Hamilton,¹⁰ who used ferrous hydroxide.

The substance described by Adler and Adler⁷ as 1-hydroxy-4-naphthylarsonic acid may be 1-hydroxy-2-naphthylarsonic acid according to Brown and Hamilton.⁸ Although Brown⁹ was unable to synthesize the former compound by the customary methods, and we were unable to prepare it from 4-amino-1-naphthol by the Scheller-Bart procedure, it was finally obtained, in small yield, by a series of reactions which leave no doubt as to its identity.

The only biphenylarsine oxides prepared previously are those described by Worrall¹⁰ and by Oneto and Way.¹¹ We have obtained three new biphenylarsine oxides.

In an attempt to find a procedure for the synthesis of 4-amino-4'-arsonobiphenyl, simpler than that published by Bauer and Adams,¹² we employed the Bart reaction and diazotized only one amino group of benzidine by following Tauber's directions.¹³ Instead of the desired product, a small amount of 4,4'-diarsonobiphenyl, formed from some tetrazotized benzidine, and a large quantity of an unidentified substance were obtained. 4-Amino-4'-arsonobiphenyl was produced finally by another synthesis, and converted into the acetamido and carboxy derivatives; all three products yielded arsine oxides on reduction.

Although an attempt to obtain 3-amino-4-hydroxy-4'-arsonobiphenyl by a process which involved diazotization of 3-nitrobenzidine¹⁴ was unsuccessful, several interesting new compounds were isolated.

Experimental Part

4-Nitro-1-naphthyl Benzoate.—This substance was obtained in 40% yield by the Schotten-Baumann reaction, and in 92% yield by the procedure of Hazlet.¹⁵ It crystallized from acetone in pale yellow needles; m. p. 176° (cor.).

Anal. Calcd. for $C_{17}H_{11}O_4N$: N, 4.78. Found: N, 4.9.

4-Amino-1-naphthyl Benzoate Hydrochloride.—The above nitro compound, suspended in acetone, was reduced catalytically employing Raney catalyst. After filtration, the addition of hydrochloric acid to the filtrate precipitated

the amine hydrochloride. Recrystallization from alcohol-acetone mixture gave needles; m. p. 258–263° (cor.) with decomposition. The yield was 89%.

4-Hydroxy-1-naphthylarsonic Acid.—Although the customary Bart reaction with the above amine hydrochloride failed to give an arsonic acid, the desired compound was obtained in a 2.5% yield with the Scheller-Bart reaction. It crystallized from water as needles which did not melt below 380°. The acid gave a deep green color with ferric chloride solution, and coupled with benzenediazonium chloride to give a bright red dye.

6-Carboxy-2-naphthylarsonic Acid.—The Sandmeyer reaction, employing nickelous cyanide, was applied to 6-amino-2-naphthylarsonic acid, and the resulting nitrile isolated through the magnesium salt. Hydrolysis with alkali followed by acidification gave the desired acid.

6-Carbamyl-2-naphthylarsine Oxide.—A suspension of the preceding compound in chloroform was treated with phosphorus trichloride and pentachloride and the mixture poured into ammonium hydroxide. The arsine oxide separated as an amorphous precipitate, insoluble in all common solvents.

Reaction Product of Diazotized Benzidine and Sodium Arsenite.—A solution of diazotized benzidine, prepared according to Tauber,¹³ was added to a solution of sodium arsenite containing a little cupric sulfate. A copious brown precipitate formed immediately. After standing several days the mixture was filtered. The filtrate yielded 3.4% of 4,4'-diarsonobiphenyl after acidification. The brown precipitate, which constituted most of the reaction product, could not be positively identified.

4-Amino-4'-arsonobiphenyl.—4-Amino-4'-nitrobiphenyl by the Scheller-Bart reaction gave 4-nitro-4'-arsonobiphenyl, which gave the desired amine by catalytic reduction employing Raney catalyst.

4-Carbamyl-4'-arsenosobiphenyl.—The Sandmeyer reaction, employing nickelous cyanide, was applied to the above amine, and the resulting nitrile hydrolyzed in solution without isolation. The resulting 4-carboxy-4'-arsonobiphenyl was converted to 4-carbamyl-4'-arsenosobiphenyl in the usual manner.

3-Nitro-4-amino-4'-arsonobiphenyl and 3-Nitro-4,4'-diarsonobiphenyl.—The Bart reaction when applied to 3-nitrobenzidine gave a mixture of the above compounds. This mixture was dissolved in ammonium hydroxide, cooled, treated with magnesia mixture, and filtered after standing for one hour. The precipitate was triturated with dilute hydrochloric acid, again dissolved in ammonium hydroxide and the process repeated. In this manner, a pure sample of 3-nitro-4,4'-diarsonobiphenyl was obtained. On boiling the filtrate from the first separation, the magnesium salt of 3-nitro-4-amino-4'-arsonobiphenyl precipitated, which was similarly purified. We have found it to be a general rule that diarsonic acids give insoluble magnesium salts in the cold, whereas the magnesium salts of monoarsonic acids usually precipitate only on heating.

3-Nitro-4-hydroxy-4'-arsonobiphenyl.—3-Nitro-4-amino-4'-arsonobiphenyl was boiled in a 25% potassium hydroxide solution for several hours. The impure acid, which separated upon acidification, was purified by dissolving in sodium bicarbonate solution, followed by treatment with Darco and then again acidifying. Reduction of the nitro

(7) Adler and Adler, *Ber.*, 41, 931 (1908); German Patent 205,775, *Chem. Zentr.*, 30, 1, 881 (1909).

(8) Brown and Hamilton, *THIS JOURNAL*, 56, 151 (1934).

(9) Brown, Thesis, University of Nebraska, 1933.

(10) Worrall, *THIS JOURNAL*, 53, 664 (1930).

(11) Oneto and Way, *ibid.*, 63, 3068 (1941).

(12) Bauer and Adams, *ibid.*, 46, 1925 (1924).

(13) Tauber, *Ber.*, 27, 2627 (1894).

(14) Le Fèvre and Turner, *J. Chem. Soc.*, 245 (1928).

(15) Hazlet, *THIS JOURNAL*, 59, 287 (1937).

TABLE I
ARSONIC ACIDS AND ARSINE OXIDES DERIVED FROM NAPHTHALENE AND BIPHENYL

Compound, R = Arsono	Description	Yield, %	Formula	As analyses, %		N analyses, %	
				Calcd.	Found	Calcd.	Found
6-R-2-naphthoic acid	White powder	22	C ₁₁ H ₉ O ₄ As	25.3	25.5		
4-R-1-naphthol	White needles	2.5	C ₁₀ H ₇ O ₄ As	27.9	27.9		
3-Nitro-4,4'-di-R-biphenyl ^a	Yellow powder	19.4	C ₁₂ H ₁₁ O ₄ NAs	33.5	33.6	3.1	3.3
3-Nitro-4-amino-4'-R-biphenyl	Brown powder	14.9	C ₁₂ H ₁₁ O ₄ N ₂ As	22.2	22.3	8.3	8.0
3-Nitro-4-hydroxy-4'-R-biphenyl	Red powder	75	C ₁₂ H ₁₀ O ₅ NAs	22.1	22.0	4.1	4.3
4-Nitro-4'-R-biphenyl	Pale yellow needles	34	C ₁₂ H ₁₀ O ₄ NAs	23.2	22.9	4.3	4.4
4-Amino-4'-R-biphenyl	White needles	80	C ₁₂ H ₁₂ O ₄ NAs	25.6	25.4	4.8	4.8
4-Acetamido-4'-R-biphenyl	White needles	100	C ₁₂ H ₁₄ O ₄ NAs	22.4	22.6	4.2	4.1
4-Carboxy-4'-R-biphenyl	White powder	14	C ₁₂ H ₁₁ O ₆ As	23.3	23.4		
4,4'-Di-R-biphenyl	White prisms	3.4	C ₁₂ H ₁₂ O ₆ As ₂	37.3	36.9		
R = Arsenoso							
2-R-naphthalene	White powder	90	C ₁₀ H ₇ OAs	34.2	34.0		
4-Acetamido-1-R-naphthalene ^b	White needles	71	C ₁₂ H ₁₀ O ₂ NAs	27.2	27.2		
2-Acetamido-1-R-naphthalene ^c	White powder	65	C ₁₂ H ₁₀ O ₂ NAs	27.2	27.3		
6-R-2-naphthamide	White powder	91	C ₁₁ H ₉ O ₂ NAs	28.7	28.3	5.4	5.4
4-Carbamyl-4'-R-biphenyl ^d	White powder	85	C ₁₂ H ₁₀ O ₂ NAs	26.1	26.0	4.9	4.7
4-Amino-4'-R-biphenyl ^e	White powder	100	C ₁₂ H ₁₀ ONAs·2H ₂ O	25.4	25.3	4.8	4.8
4-Acetamido-4'-R-biphenyl ^f	White powder	100	C ₁₄ H ₁₂ O ₂ NAs·H ₂ O	23.4	23.5	4.4	4.4

^a M. p. 249.5–250.5° (all melting points are corrected). ^b M. p. 272°. ^c We have been unable to obtain satisfactory nitrogen analyses on many naphthalene arsonic acids and arsine oxides containing nitrogen attached directly to the ring. Kjeldahl determinations were entirely unsatisfactory. Using the Dumas procedure, even with the addition of potassium chlorate, the results were erratic and we were never able to obtain the theoretical value. ^d M. p. 256.5°. ^e M. p. 271–273°. ^f M. p. 221–222°. ^g M. p. 297.5–298.5°.

group with ferrous hydroxide, or catalytically, using Raney catalyst, was unsatisfactory.

Table I lists the new compounds, or compounds prepared by a new procedure. Except as noted in the text, the arsine oxides were prepared by sulfur dioxide reduction of the corresponding arsonic acids in the usual manner. They were insoluble in water and the usual organic solvents. 4-Arsono-1-naphthol was recrystallized from hot water. The remaining crys-

talline compounds were crystallized from alcohol.

Summary

Several new arsonic acids derived from naphthalene and biphenyl have been prepared, and more convenient methods have been described for the preparation of other known compounds. The arsonic acids have been reduced to the corresponding arsine oxides.

BALTIMORE, MD.

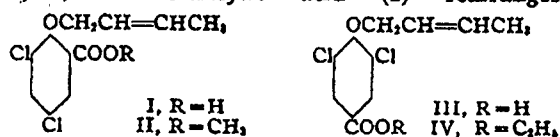
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER]

The Rearrangement of 4-Crotyloxy-3,5-dichlorobenzoic Acid

By D. S. TARBELL AND J. W. WILSON¹

In a previous paper² it was shown that O-crotyl-3,5-dichlorosalicylic acid (I) rearranges



smoothly at 125° with loss of carbon dioxide to give 2,4-dichloro-6-(α -methylpropenyl)-phenol. This reaction therefore goes with inversion

(1) Sherman Clarke Fellow, 1941–1942.

(2) Tarbell and Wilson, *THIS JOURNAL*, **64**, 607 (1942).

(attachment of the γ -carbon atom) just as the ordinary Claisen rearrangement does. The present paper reports a study of a similar compound, 4-crotyloxy-3,5-dichlorobenzoic acid (III), in which the substituted allyl group migrates to the para position.

The crotyl ether of ethyl 3,5-dichloro-4-hydroxybenzoate (IV) was obtained using aqueous sodium hydroxide-acetone as the reaction medium. Saponification of the ester yielded 4-crotyloxy-3,5-dichlorobenzoic acid (III), which was

Potential Antitumor Agents. 29. Quantitative Structure-Activity Relationships for the Antileukemic Bisquaternary Ammonium Heterocycles

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Quantitative relationships between physicochemical drug properties and antileukemic (L1210) efficacy have been examined for a series of bisquaternary ammonium heterocycles employing multiple variable regression analysis. Three measures of biologic response were examined: ILS_{max} , the percentage increase in mean life span of leukemic animals at the LD_{10} dose; D_{40} , the drug dose necessary to provide 40% increase in life span; and $CI (=LD_{10}/D_{40})$, the chemotherapeutic index. A cross correlation matrix between these three measures and the LD_{10} values demonstrates ILS_{max} and CI to be independent of toxicity. D_{40} is highly inversely correlated with LD_{10} and positively correlated with ILS_{max} , suggesting that this parameter measures a composite of both drug selectivity and toxicity. Superior regression equations resulted at all stages employing ILS_{max} as a measure of antitumor selectivity. Acceptable equations modeling LD_{10} could not be obtained. There was a parabolic relationship between agent lipophilic-hydrophilic balance, measured as chromatographic R_m values, and ILS_{max} . To reduce residual variance in the L1210 screening data, not accepted by this parabolic equation, measures of agent-DNA interaction were investigated as possible indices of site fit. Relative levels of drug-DNA interaction were obtained by spectrofluorimetric quantitation of drug displacement of DNA-bound ethidium. Addition to regression equations of agent C_{50} values for calf thymus DNA, those micromolar drug concentrations necessary to displace 50% of the ethidium bound to that DNA, provided a significant reduction in the screening data variance. C_{50} values for drug interactions with poly[d(A-T)] and poly[d(G-C)] were also investigated as possible indicators of drug selectivity toward different DNA sites. Marked differences were observed in the C_{50} values for the two synthetic nucleic acids, with those for calf thymus DNA and poly[d(G-C)] proving highly covariant. A regression equation containing a positive term in $C_{50}[\text{poly}[\text{d}(\text{G-C})]]$ and a negative term in $C_{50}[\text{poly}[\text{d}(\text{A-T})]]$ provided the greatest acceptance of the variation in the biologic data. The term in $C_{50}[\text{poly}[\text{d}(\text{A-T})]]$ is the single most important equation term, alone accounting for 35% of the variance in the data. This best equation provides both an adequate summary of the L1210 screening data for the 174 active compounds considered and a physical explanation for the antitumor selectivity for these agents. The major factor influencing antitumor selectivity in these drugs is their ability to distinguish alternating adenine-thymine sequences in DNA.

Following demonstration of significant antitumor (L1210) activity with a bisquaternary ammonium heterocycle,¹ a large number of congeners have been synthesized and screened as part of this laboratory's program to develop new antitumor agents.¹⁻⁹ This particular series of compounds is remarkable for both the high percentage which prove L1210 active and the extremely high experimental activity shown by certain members. For one example, 221 (Table I, NSC176319), pharmacologic and toxicologic examination has been completed as a prelude to clinical trial.

Employing as a measure of antitumor efficacy the percentage increase in mean life span (ILS), in L1210 screening tests, qualitative structure-activity relationships (SAR) have been developed.⁶ The rise and then fall in antileukemic activity, seen on homologation within series of bisquaternary salts (e.g., 89-93 and 100-103; Table I), suggest that agent lipophilic-hydrophilic balance plays a dominant role in antitumor selectivity. Additionally, certain structural requirements necessary for activity are evident. These include the following: limitations on the separation of the two cationic charges; a need for a close approach to overall planarity, coupled with a certain degree of molecular rigidity;⁹ and a molecular outline permitting binding to an annular site limited by radii of 20 and 25 Å.⁶ All these features appear compatible with the hypothesis that the minor groove of twin-helical DNA provides the site of drug action.⁶

When derivation of quantitative SAR for these agents is attempted, the marked diversity of structural types precludes the use of usual extrathermodynamic parameters that are appropriate with series of simply substituted drug congeners. From the viewpoint that DNA is a likely site of action for these agents, drug-DNA association constants should then provide measures of site fit and therefore be directly incorporable into correlation equations. Relative drug-DNA binding constants have been measured utilizing

drug competition with ethidium for DNA sites. The possible utility of these constants in regression analysis of L1210 screening data for the bisquaternary salts is examined in this study.

Chemistry. With the exception of the acridine congeners, synthesis of the quoted bisquaternary salts employed terminal quaternization of the necessary heterocyclic bisbases. In most cases construction of the bisbase required essentially the assembly of a polyamide framework. For ease of handling of intermediates, attention is drawn to our earlier comments² on the high crystallinity and ease of purification of the methyl esters of acid components, in contrast with the sometimes difficultly purifiable, microcrystalline acids.

In cases where the terminal base units were identical and there was a central diacid component (e.g., 303 and 306; Table II), reaction of the acid chloride from the diacid, with the necessary amine-bearing base unit, as before¹⁻⁴ provided the necessary bisbase in essentially quantitative yield. With asymmetric bisbases, stepwise construction of the amide skeleton was necessary and the phosphorazo reaction^{2,10} was the one of choice for coupling acid and amine components. In the asymmetric bisbases containing a 4-aminoquinoline unit this component was appended terminally. Quaternization, except when there was marked steric shielding of the ring nitrogen to be alkylated, employed the reaction conditions and precautions detailed earlier.⁸ Formerly 8-substituted quinolines such as 307 and 309 proved difficult to completely quaternize.⁷ In subsequent work it appeared that one factor contributing to incomplete quaternization was the continually slow breakdown of the initially formed quaternary salt and also the quaternizing agent during the extended reaction times necessary with these sterically hindered bases, acidic byproducts being formed. The salts resulting from these acidic products and the input bisbase were much more slowly quaternized. By carrying out such reactions in the presence of 2 mol equiv of *N,N*-diisopropylethylamine,¹¹

acid byproducts were scavenged and more complete quaternization resulted.

When terminal reduction of nitro groups was necessary, use of Fe/H^+ , as detailed before,² provided high yields of the corresponding amines without appreciable overreduction involving the heterocyclic quaternary functions.

All congeners of the 3-amino-9-anilino-10-methylacridinium salts were generated by coupling 3-(trifluoroacetamido)-9-chloro-10-methylacridinium chloride¹² with the requisite aniline component. Hydrolytic removal of the protecting trifluoroacetyl group employed 4 N NH_3 in a suitable solvent.

1,4-Bis(4-nirostyryl)benzene, conveniently prepared in moderate quantities by condensation of (4-nitrophenyl)acetic acid and terephthal dicarboxaldehyde, could be readily reduced ($\text{SnCl}_2\text{-HCl-HOAc}$) to the corresponding diamine. Application of Zincke's pyridinium salt synthesis,¹³ interacting *N*-(2,4-dinitrophenyl)pyridinium 4-toluenesulfonate and 1,4-bis(4-aminostyryl)benzene, readily afforded agent 299.

Lipophilic-Hydrophilic Balance. The most usually employed measure of such balance is the logarithm of the partition coefficient (P) of the agents in 1-octanol-water. Agent R_m values [$R_m = \log (1/R_f - 1)$], obtained from partition chromatography employing a particular solvent system, are linearly related to $\log P$ values in that solvent system. From Hansch's study¹⁴ of the interrelationships between the partition coefficients obtained when different solvents are employed, it is possible to select those solvents which should provide R_m values highly covariant with $\log P_{\text{oct}}$ values. In particular, with very hydrophilic compounds such as the quaternary salts discussed here, R_m values are considerably more readily measured than $\log P_{\text{oct}}$ values. By employing a range of neutral compounds, whose $\log P_{\text{oct}}$ values had been previously measured¹⁴ and provided an acceptable spread and range (-2.92 to 1.64), R_m values in the partition chromatographic system employed were found linearly related to those $\log P$ values by the equation

$$\log P = 2.41(\pm 0.34)R_m - 1.31(\pm 0.24) \quad (1)$$

$$n = 25, r = 0.95, s = 0.15, F_{1,23} = 205$$

C_{50} Values for Drug-DNA Interaction. Earlier we reported the development of a method of obtaining measures of the relative DNA-binding level of intercalating agents by spectrofluorimetric quantitation of drug displacement of DNA-bound ethidium.^{15,16} The micromolar concentration of drug necessary to displace 50% of the ethidium from DNA sites (C_{50} value) can be shown inversely proportional to the drug-DNA association constant.¹⁶ The ethidium displacement technique provides a readily reproducible, rapid measure of relative DNA binding and requires only milligram quantities of agent and micrograms of DNA. This technique is therefore eminently suitable for rapid examination of a large series of agents such as in the present study. While accurate measures of drug-DNA association constants can be obtained for intercalating agents,¹⁶ it must be emphasized that the bulk of the bisquaternary salts considered here does not bind by intercalation. Examination of representative examples (e.g., 152, 216, 221 and 229; Table I) shows that these provide no unwinding of PM2 bacteriophage DNA by hydrodynamic criteria.¹⁷ Since these agents appear to bind equally well to the DNA of T₂ bacteriophage, in which the major groove is occluded by bulky glycosyl residues,¹⁸ as to other DNA samples of comparable base-pair composition, it appears that they also do not bind in the major groove of the nucleic acid.¹⁹ Lodgment of the bisquaternary salts in the DNA minor

groove is compatible with both these observations and the earlier developed qualitative SAR.

Competition between the intercalating agent ethidium and a bisquaternary salt, for DNA, then utilizes two different site types. On intercalation into DNA, ethidium produces distortion in the helical structure and the phenyl ring at the 9 position of this drug protrudes into the minor groove.²⁰ A bisquaternary salt lodged in the minor groove of a DNA sample prevents ethidium approach to the occluded intercalation sites. Site sizes for the two agent types also differ. Ethidium lodges between two adjacent base pairs and effectively prevents entry of further intercalating molecules into the nearest neighboring potential sites.²¹ From model fitting, examples of the bisquaternary salts in the minor groove could mask potential intercalation sites between from three up to seven base pairs. There is no simple proportionality between C_{50} values and association constants which is applicable to the full range of bisquaternary salts. By employing considerably more detailed and laborious techniques, ethidium displacement can provide DNA association constants for members of the bisquaternary salt series.¹⁹ However, for the purposes of this study C_{50} values are best considered as semiempirical parameters defined by and derived from drug-ethidium competition for DNA samples.

The antibiotic distamycin has also been more recently suggested to lodge in the minor groove of DNA samples.²² The penchant for this antibiotic to bind to adenine-thymine (A-T) rich DNA samples, as shown by more usual binding methods,²² can be also demonstrated by measuring C_{50} values for the agent, employing as substrates synthetic, homopolymeric DNA samples of defined base-pair sequence (Table I).

Measures of Biologic Response. A prerequisite for regression analysis of biologic data is that the measures be of acceptable form. Two alternate measures of response are considered acceptable: (a) that obtained at a constant dose and (b) that dose necessary to provide a constant biologic response.²³ The latter alternative is almost invariably considered when pharmacodynamic agents are under examination. The desired end point of such research is usually the development of more dose-potent material; the smaller the dose of agent necessary to provide the required response the more active the agent is considered. The needs of the research inevitably dictate the nature of the response measure employed. In contrast, with cancer, and to a certain extent with antiparasitic chemotherapy, more selective agents are sought, those which will provide greater therapeutic responses at permissible doses. Dose potency is of secondary importance. We suggest that in such cases the most important, and pertinent, end point for use in regression analyses is response at a constant dose. Further, when data from animal assays are employed, the "constant" dose must be considered in biologic terms rather than in absolute magnitude, i.e., in terms of toxicity to the animals employed. From this viewpoint a constant dose to animals would be a standard fraction of the LD₁₀ or LD₅₀ dose. We have recently conducted regression analyses of screening data for several groups of cancer chemotherapeutic agents,^{1,24} employing as input the percentage increases in life span [$\text{ILS} = 100[(T - C)/C]$] obtained in L1210 leukemia assays. From a full dose profile of antileukemic activity significant life extensions observed at and below the measured LD₁₀ dose were linearly correlated with the logarithms of the corresponding doses. The ILS specified by the linear correlation at the LD₁₀ dose (ILS_{max}) was utilized as a measure of anti-leukemic activity and as input for regression analyses.

Table I

entry no.	type	substituent groups				link groups			C ₅₀ data ^c				log IL _{S_{max}} ^f					
		R	R'	R ²	R ³	A	B	C	count ^e	R _m ^b	ct ^d	d(A-T) ^e	d(G-C) ^f	LD ₅₀ ^g	D ₅₀ ^h	obsd	calcd ⁱ	diff
54 ^k	1	CH ₃ CH ₃	H			NHCO	CH	CONH	TsO ⁻	-0.21				5	1	<1.4	<1.4	
55 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.03				4	1	1.43	1.58	-0.15
56 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.3				21	15	1.89	1.88	0.01
57 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.67				59	25	2.04	1.81	0.23
58 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.92				57	55	1.62	1.46	0.16
59 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	1.03				27	1	<1.4	<1.4	
60 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.40				17	11	2.04	1.92	0.13
61 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.8				20	1	<1.4	1.67	>-0.27
62 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.94				24	1	<1.4	1.42	>-0.02
63 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	-0.01				6	1	<1.4	1.51	>-0.11
64 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.25				10	3.9	1.92	1.85	0.07
65 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.60				18	9	1.72	1.87	-0.15
66 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.85				16	15	1.51	1.58	-0.07
67 ^k	1	CH ₃	H			NHCO	CH	CH=	TsO ⁻	-0.2				3	1	<1.4	<1.4	
68 ^k	1	CH ₃ CH ₃	H			NHCO	CH	CHCONH	TsO ⁻	0.11				9	5	1.70	1.69	0.01
69 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CHCONH	TsO ⁻	0.50				20	4.3	2.00	1.91	0.09
70 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CHCONH	TsO ⁻	0.9				25	25	1.40	1.50	-0.10
71 ^k	1	CH ₃	H			NHCO	CH	CHCONH	TsO ⁻	-0.3				0.6	1	<1.4	<1.4	
72 ^k	1	CH ₃ CH ₃	H			NHCO	CH	CONH	TsO ⁻	-0.11				4	4	1.40	<1.4	
73 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.15				8.3	6.3	1.59	1.75	-0.16
74 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.41				210	80	1.96	1.91	0.05
75 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.78				150	100	1.53	1.69	-0.16
76 ^k	1	CH ₃	H			NHCO	CH	CONH	TsO ⁻	0.85				270	1	<1.4	1.58	>-0.18
77 ^k	1	CH ₃ CH ₃	H			NHCO	CH	CONH	TsO ⁻	1.10				1.6	1	<1.4	<1.4	
78 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	CONH	TsO ⁻	1.25				2.0	1	<1.4	<1.4	
79 ^k	1	CH ₃	H			NHCOCH ₃	CH	CONH	TsO ⁻	0.07				15	1	<1.4	1.64	>-0.24
80 ^k	1	(CH ₃) ₂ CH ₃	H			NHCOCH ₃	CH	CH ₃ CONH	TsO ⁻	0.92				1.0	1	<1.4	1.50	>-0.10
81 ^m	1	CH ₃ CH ₃	H			NHCO	CH	NHCO	TsO ⁻	0.02				4	1	<1.4	1.56	>-0.16
82 ^m	1	(CH ₃) ₂ CH ₃	H			NHCO	CH	NHCO	TsO ⁻	0.59				200	1	<1.4	1.87	>-0.47
83 ^m	1	CH ₃	H			NHCO	N	CONH	TsO ⁻	1.10				300	1	<1.4	<1.4	
84 ^k	1	CH ₃ CH ₃	H			NHCO	N	CONH	TsO ⁻	-0.4				4.0	1	<1.4	<1.4	
85 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	N	CONH	TsO ⁻	-0.15				6.0	6.0	1.42	1.4	<-0.02
86 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	N	CONH	TsO ⁻	0.05				16	14	1.51	1.61	-0.01
87 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	N	CONH	TsO ⁻	0.31				175	120	1.87	1.88	-0.01
88 ^k	1	(CH ₃) ₂ CH ₃	H			NHCO	N	CONH	TsO ⁻	0.60				250	1	<1.4	1.87	>-0.46
89 ^m	2	CH ₃	H			CONH	N	CONH	TsO ⁻	-0.25				27	12	2.03	2.06	-0.03
90 ^m	2	CH ₃ CH ₃	H			CONH	N	CONH	TsO ⁻	0.09				9	2.3	2.20	1.99	0.21
91 ^m	2	(CH ₃) ₂ CH ₃	H			CONH	N	CONH	TsO ⁻	0.74				20	10.2	2.17	1.82	0.35
92 ^m	2	(CH ₃) ₂ CH ₃	H			CONH	N	CONH	TsO ⁻	0.44				20	10.2	2.17	1.82	0.35
93 ^m	2	(CH ₃) ₂ CH ₃	H			CONH	N	CONH	TsO ⁻	0.85				20	10.2	2.17	1.82	0.35

Table 1 (Continued)

entry no.	type	substituent groups			link groups			C ₁₀ data ^c	LD ₅₀ ^g	D ₅₀ ^h	log ILS _{max} ⁱ			
		R	R'	R''	A	B	C				obsd	calcd ^j	diff	
143 ^a	22	CH ₃	H					5.4	12	19	14.7	1.65	1.65	0.00
144 ^a	23	CH ₃	H					1.1	2.7	140	54.5	1.84	1.78	0.06
145 ^a	24	CH ₃ CH ₃	H					5.3	11	24	4.3	2.00	1.74	0.26
146 ^a	24	(CH ₂) ₂ CH ₃	H					(5.3)	(11)	27	18	1.65	1.57	0.08
147 ^a	24	(CH ₂) ₂ CH ₃	H					(5.3)	(11)	30	1	1.54	1.44	0.10
148 ^a	25	CH ₃ CH ₃	H					1.2	1.9	35	25	1.85	1.86	-0.01
149 ^a	25	CH ₃ CH ₃	H					(1.2)	(1.9)	12	12	1.60	1.69	-0.09
150 ^a	26	CH ₃	H					2.3	2.6	75	72	1.65	1.82	-0.16
151 ^a	27	CH ₃ CH ₃	H					0.41	0.91	56	5.9	2.13	2.02	0.11
152 ^a	27	(CH ₂) ₂ CH ₃	H					0.40	0.90	33	9.8	2.24	2.05	0.19
153 ^a	27	(CH ₂) ₂ CH ₃	H					0.39	0.90	27	12.9	2.06	2.00	0.16
154 ^a	27	(CH ₂) ₂ CH ₃	H					0.41	0.93	58	30	1.76	1.77	0.01
155 ^a	27	(CH ₂) ₂ CH ₃	H					0.42	0.92	70	1	<1.4	1.47	>-0.07
156 ^a	27	CH ₃ CH ₃	H					1.0	2.4	14	5.7	2.09	1.97	0.12
157 ^a	27	CH ₃ CH ₃	H					(1.2)	(2.4)	26	9.6	1.85	1.83	0.02
158 ^a	27	(CH ₂) ₂ CH ₃	H					(1.0)	(2.4)	30	1	<1.4	1.57	>-0.17
159 ^a	27	CH ₃ CH ₃	H					0.64	1.51	18	10.3	1.72	1.94	-0.22
160 ^a	27	CH ₃ CH ₃	H					(0.64)	(1.51)	20	18	1.85	1.76	0.09
161 ^a	27	(CH ₂) ₂ CH ₃	H					(0.64)	(1.51)	22	22	1.60	1.55	0.05
162 ^a	27	CH ₃ CH ₃	H					0.37	0.93	20	3.9	2.17	2.05	0.12
163 ^a	27	CH ₃ CH ₃	H					(0.37)	(0.93)	47	1	<1.4	1.99	>-0.59
164 ^a	27	(CH ₂) ₂ CH ₃	H					(0.37)	(0.93)	14	2.4	2.13	2.09	0.04
165 ^a	27	CH ₃ CH ₃	H					1.0	2.0	30	5.6	2.11	1.96	0.15
166 ^a	27	CH ₃ CH ₃	H					(1.0)	(2.0)	29	22	1.70	1.80	-0.10
167 ^a	27	(CH ₂) ₂ CH ₃	H					0.15	0.54	9	1.4	2.32	2.13	0.19
168 ^a	27	CH ₃ CH ₃	H					0.12	0.39	44	2.4	2.35	2.14	0.21
169 ^a	27	CH ₃ CH ₃	H					(0.12)	(0.39)	10	3.2	2.18	2.16	0.02
170 ^a	27	(CH ₂) ₂ CH ₃	H					(0.12)	(0.39)	30	5	2.02	2.19	-0.17
171 ^a	27	CH ₃ CH ₃	H					0.30	0.35	41	15	1.78	2.01	-0.23
172 ^a	27	CH ₃ CH ₃	H					(0.30)	(0.35)	40	24	1.76	1.87	-0.11
173 ^a	27	(CH ₂) ₂ CH ₃	H					(0.30)	(0.35)	37	22	1.79	1.69	0.10
174 ^a	27	CH ₃ CH ₃	H					0.99	2.1	26	1	<1.4	1.90	>-0.50
175 ^a	27	CH ₃ CH ₃	H					(0.08)	(0.48)	14	2.6	2.29	2.14	0.15
176 ^a	27	(CH ₂) ₂ CH ₃	H					0.08	0.48	10	2.2	2.10	2.27	-0.17
177 ^a	28	CH ₃ CH ₃	H					0.14	1.8	3.1	0.3	2.33	2.30	0.03
178 ^a	28	CH ₃ CH ₃	H					(0.14)	(1.8)	2.5	0.5	2.24	2.11	0.13
179 ^a	28	(CH ₂) ₂ CH ₃	H					(0.14)	(1.8)	4.1	0.6	1.99	1.84	0.15
180 ^a	28	CH ₃ CH ₃	H					0.40	1.15	110	22	2.28	2.11	0.17
181 ^a	28	CH ₃ CH ₃	H					(0.40)	(1.15)	14	1.2	2.23	2.04	0.19
182 ^a	28	(CH ₂) ₂ CH ₃	H					(0.40)	(1.15)	10	4.6	2.03	1.86	0.17
183 ^a	29	CH ₃	H					0.37	3.01	2.6	0.6	2.28	2.18	0.10
184 ^a	30	CH ₃ CH ₃	H					0.08	1.5	6.7	1.4	2.30	2.39	-0.09
185 ^a	30	CH ₃ CH ₃	H					(0.08)	(1.5)	7.5	1.4	2.30	2.26	0.04
186 ^a	30	CH ₃ CH ₃	H					0.14	0.51	11	4.2	2.28	2.21	0.07
187 ^a	30	CH ₃ CH ₃	H					(0.14)	(0.51)	8	1.6	2.20	2.17	0.03
188 ^a	31	CH ₃ CH ₃	H					(0.14)	(0.51)	10	5.1	1.88	2.17	-0.29
189 ^a	31	CH ₃ CH ₃	H					(0.11)	(0.38)	32	9	1.88	2.19	-0.31
190 ^a	31	(CH ₂) ₂ CH ₃	H					(0.11)	(0.38)	25	13	1.85	1.93	-0.12
191 ^a	32	CH ₃	H					(0.11)	(0.38)	6	1.4	2.34	2.23	0.11
192 ^a	32	CH ₃	H					0.10	0.38	30	1	<1.4	1.60	>-0.20
193 ^a	33	CH ₃	H					22	77	20	1	<1.4	1.46	>-0.06
194 ^a	33	CH ₃ CH ₃	H					(22)	(77)	20	1	<1.4	1.46	>-0.06

BNSDOCID: <XP_2139936A_1_>

195 ^u	34	CH ₃ CH ₃	H	NH	Br ⁻	0.59	40	20	75	30	1	<1.4	1.55	>-0.15
196 ^u	34	CH ₃ CH ₃	H	NH	Br ⁻	0.83	(40)	(20)	(75)	10	1	<1.4	<1.4	>-0.15
197 ^r	35	CH ₃ CH ₃		CONH	Br ⁻	-0.42	(0.84)	(0.45)	(2.1)	12	1.5	2.22	2.16	0.06
198 ^r	35	CH ₃ CH ₃		CONH	Br ⁻	-0.19	0.84	0.45	2.1	15	3.5	2.17	2.16	0.01
199 ^r	35	(CH ₃) ₂ CH ₃		CONH	Br ⁻	-0.08	(0.84)	(0.45)	(2.1)	50	7	2.15	2.14	0.01
200 ^o	35	CH ₃		CH=	Br ⁻	-0.62	0.40	0.11	0.57	50	10.7	2.20	2.24	-0.04
201 ^o	35	CH ₃ CH ₃		CHCONH	Br ⁻	-0.35	(0.40)	(0.11)	(0.57)	27	5.1	2.23	2.27	-0.04
202 ^r	36	CH ₃ CH ₃	H	CHCONH	Br ⁻	-0.64	0.45	0.29	0.64	75	8.6	2.20	2.07	0.13
203 ^r	36	CH ₃ CH ₃			Br ⁻	-0.3	(0.45)	(0.29)	(0.64)	112	10	2.06	2.10	-0.04
204 ^r	36	CH ₃ CH ₃	CH ₃		Br ⁻	-0.71	0.5	0.4	0.8	250	75	2.12	2.02	0.10
205 ^r	36	CH ₃ CH ₃	CH ₃		Br ⁻	-0.4	(0.5)	(0.4)	(0.8)	200	69	2.09	2.07	0.02
206 ^k	37	CH ₃ CH ₃			Br ⁻	0.11	(2.2)	(0.83)	(2.9)	10	1	<1.4	1.97	>-0.57
207 ^k	37	CH ₃ CH ₃			Br ⁻	0.26	(2.2)	(0.83)	(2.9)	10	1	<1.4	2.01	>-0.61
208 ^k	37	(CH ₃) ₂ CH ₃			Br ⁻	0.40	2.2	0.83	2.9	11	1	<1.4	1.87	>-0.47
209 ^r	38	CH ₃ CH ₃			Br ⁻	0.13	5.6	2.8	8.8	16	1	<1.4	1.91	>-0.51
210 ^r	38	CH ₃ CH ₃			Br ⁻	0.53	(5.6)	(2.8)	(8.8)	16	1	<1.4	1.70	>-0.30
211 ^r	38	(CH ₃) ₂ CH ₃			Br ⁻	0.71	(5.6)	(2.8)	(8.8)	100	1	<1.4	1.57	>-0.17
212 ^m	39	CH ₃ CH ₃			Br ⁻	0.11	ν	ν	ν	7	1	<1.4	ν	
213 ^m	39	CH ₃ CH ₃			Br ⁻	0.57	ν	ν	ν	13	1	<1.4	ν	
214 ^m	39	(CH ₃) ₂ CH ₃			Br ⁻	0.73	ν	ν	ν	100	1	<1.4	ν	
215 ^r	40	CH ₃			Br ⁻	-0.15	0.49	0.15	0.75	25	3.4	2.39	2.23	0.16
216 ^r	41	CH ₃ CH ₃			Br ⁻	0.29	1.4	0.16	3.0	13	2.0	2.23	2.28	-0.05
217 ^r	41	CH ₃ CH ₃			Br ⁻	0.54	2.0	0.19	3.1	15	2.8	2.07	2.06	-0.01
218 ^r	41	(CH ₃) ₂ CH ₃			Br ⁻	0.74	2.3	0.20	3.0	15	8.8	1.81	1.90	-0.09
219 ^r	41	(CH ₃) ₂ CH ₃			Br ⁻	1.14	2.1	0.19	2.8	11	1	<1.4	1.50	>-0.10
220 ^r	41	CH ₃	3-CH ₃		Br ⁻	0.60	4.9	4.5	15.0	5	1	<1.4	1.76	>-0.36
221 ^r	41	CH ₃ CH ₃	6-NH ₂		Br ⁻	0.07	0.66	0.09	1.05	12	1.2	2.35	2.31	0.04
222 ^r	41	CH ₃ CH ₃	6-NH ₂		Br ⁻	0.22	0.69	0.10	1.04	10	0.9	2.25	2.20	0.05
223 ^r	41	(CH ₃) ₂ CH ₃	6-NH ₂		Br ⁻	0.46	0.68	0.10	1.10	10	1.8	2.07	2.11	-0.04
224 ^r	41	CH ₃	6-Cl		Br ⁻	0.57	0.79	0.24	1.76	40	14	1.81	1.93	-0.12
225 ^r	41	CH ₃	6-CH ₃		Br ⁻	0.51	1.05	0.17	2.28	15	1.0	2.17	2.07	0.10
226 ^r	41	CH ₃	6-OCH ₃		Br ⁻	0.46	0.68	0.19	1.15	50	1.5	2.20	2.00	0.20
227 ^r	41	CH ₃	7-NH ₂		Br ⁻	0.08	0.68	0.23	1.4	11	2	2.11	2.17	-0.06
228 ^r	41	CH ₃	7-NO ₂		Br ⁻	0.15	0.69	0.20	1.3	27	3	2.02	2.14	-0.12
229 ^r	41	CH ₃	7-Cl		Br ⁻	0.31	1.0	0.21	2.27	39	12	1.97	2.15	-0.18
230 ^o	41	CH ₃	8-NH ₂		Br ⁻	-0.10	0.85	0.20	1.43	60	0.9	2.48	2.25	0.23
231 ^r	41	CH ₃	8-Cl		Br ⁻	0.51	1.1	0.29	1.4	55	50	1.70	1.91	-0.21
232 ^r	41	CH ₃	8-CH ₃		Br ⁻	0.61	0.87	0.31	2.01	60	12	2.02	1.94	0.12
233 ^r	41	CH ₃	8-OCH ₃		Br ⁻	0.30	0.76	0.16	1.62	6	0.3	2.14	2.16	-0.02
234 ^r	41	CH ₃	3-Ph		Br ⁻	0.65	0.39	0.15	0.63	22	10	1.78	1.84	-0.06
235 ^r	41	CH ₃	6-NH ₂		Br ⁻	0.27	1.6	0.21	3.0	7.5	2.2	2.29	2.20	0.09
236 ^r	41	CH ₃ CH ₃	H	NHCO	Br ⁻	0.45	(1.6)	(0.21)	(3.0)	10	0.6	2.16	2.10	0.06
237 ^r	41	CH ₃	6-NH ₂		Br ⁻	0.05	0.60	0.12	1.20	11	1.2	2.30	2.28	0.02
238 ^r	41	CH ₃ CH ₃	6-NH ₂		Br ⁻	0.19	(0.60)	(0.12)	(1.20)	9	0.7	2.23	2.23	0.00
239 ^r	41	CH ₃	7-NH ₂		Br ⁻	-0.04	0.43	0.12	1.04	25	1.5	2.34	2.29	0.05
240 ^r	41	CH ₃	7-NHCO		Br ⁻	-0.10	0.34	0.13	0.53	97	10	2.26	2.21	0.05
241 ^o	41	CH ₃	CH ₃		Br ⁻	0.07	0.31	0.10	0.61	56	1.1	2.33	2.23	0.10
242 ^r	41	CH ₃	6-NH ₂		Br ⁻	0.17	0.51	0.28	1.2	6	3	1.74	2.08	-0.34
243 ^r	41	CH ₃	8-CH ₃		Br ⁻	0.61	0.34	0.13	0.67	23	12	1.89	1.90	-0.01
244 ^r	41	CH ₃	6-NH ₂		Br ⁻	0.31	0.35	0.15	0.53	16	1.8	2.19	2.04	0.15
245 ^r	41	CH ₃	6-NH ₂		Cl ⁻	0.96	1.7	0.9	2.6	5	1	2.22	2.11	0.11

Table I (Continued)

entry no.	type	substituent groups				link groups			C ₁₀ data ^c		LD ₁₀ ^f	D ₅₀ ^h	log ILS _{max} ⁱ	
		R	R'	R'	R'	A	B	C	d(A-T) ^e	d(G-C) ^f			obsd	calcd ^j
246 ^x	41	CH ₃	H	H	H	CH=CHCONH			0.15	0.71	22	1.3	2.22	2.09
247 ^x	41	CH ₃	6-NH ₂	H	H	CH=CHCONH			0.09	0.41	40	1.6	2.24	2.21
248 ^y	41	CH ₃	H	NH ₂	H	CONH			0.22	2.9	29	1.1	2.18	2.20
249 ^y	41	CH ₃ CH ₂ CH ₃	H	NH ₂	H	CONH			(0.22)	(2.9)	53	11.9	1.95	1.95
250 ^y	41	(CH ₃) ₂ CH ₂	H	NH ₂	H	CONH			(0.22)	(2.9)	24	24	1.60	1.84
251 ^y	41	CH ₃ CH ₂ CH ₂	H	H	NH ₂	CONH			0.24	4.76	8	2.8	1.85	2.14
252 ^y	41	CH ₃ CH ₂ CH ₂	H	H	NH ₂	CONH			0.56	(4.76)	10	5.1	1.69	1.95
253 ^y	41	(CH ₃) ₂ CH ₂	H	H	NH ₂	CONH			0.76	(4.76)	4.4	1	1.43	1.80
254 ^y	41	CH ₃ CH ₂ CH ₂	H	NH ₂	NH ₂	CONH			0.08	3.17	18	15	1.78	2.04
255 ^y	41	(CH ₃) ₂ CH ₂	H	NH ₂	NH ₂	CONH			0.35	(3.17)	20	1	<1.4	1.92
256 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.63	(3.17)	15	1	<1.4	1.74
257 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.01	1.1	20	2.9	2.45	2.32
258 ^y	41	(CH ₃) ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.37	(1.1)	17	1.4	2.33	2.17
259 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.52	(1.1)	20	5.9	2.03	2.08
260 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	H	NH ₂	CONH			-0.03	1.3	3	0.7	2.18	2.19
261 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	H	NH ₂	CONH			0.15	(1.3)	6.5	1.5	2.10	2.14
262 ^y	41	(CH ₃) ₂ CH ₂	6-NH ₂	H	NH ₂	CONH			0.41	(1.3)	10	3.6	1.95	2.01
263 ^y	41	CH ₃ CH ₂ CH ₂	7-NH ₂	NH ₂	H	CONH			-0.12	(1.11)	20	1.2	2.45	2.30
264 ^y	41	CH ₃ CH ₂ CH ₂	7-NH ₂	NH ₂	H	CONH			0.07	1.11	11	1.4	2.39	2.25
265 ^y	41	CH ₃ CH ₂ CH ₂	2-CH ₃	NH ₂	H	CONH			0.16	(1.17)	110	8.4	2.20	2.06
266 ^y	41	CH ₃ CH ₂ CH ₂	2-CH ₃	NH ₂	H	CONH			0.34	1.17	20	7.2	1.86	1.98
267 ^y	41	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	NHCO			0.64	2.05	7	1	<1.4	1.91
268 ^x	42	CH ₃ CH ₂ CH ₂	H	NH ₂	H	CONH			0.30	3.0	15	2.2	2.08	2.11
269 ^x	42	CH ₃ CH ₂ CH ₂	H	H	H	CONH			(0.30)	(3.0)	10	2.7	1.98	1.97
270 ^x	42	(CH ₃) ₂ CH ₂	H	H	H	CONH			(0.30)	(3.0)	4.8	4.8	1.60	1.77
271 ^x	43	CH ₃ CH ₂ CH ₂	H	H	H	CONH			-0.01	2.73	20	3.5	2.27	2.29
272 ^x	43	CH ₃ CH ₂ CH ₂	H	H	H	CONH			0.45	(2.73)	32	3.3	2.11	2.08
273 ^x	43	(CH ₃) ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.68	(2.73)	40	15.8	1.85	1.92
274 ^y	43	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			-0.25	0.62	75	4.4	2.41	2.34
275 ^y	43	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.33	(0.62)	100	8.6	2.28	2.17
276 ^y	43	(CH ₃) ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			0.56	(0.62)	50	13.7	2.03	2.03
277 ^x	44	CH ₃ CH ₂ CH ₂	H	H	H	CONH			-0.07	1.04	33	13	1.91	2.08
278 ^x	44	CH ₃ CH ₂ CH ₂	6-NH ₂	H	H	CONH			0.40	0.50	28	2.1	2.31	2.27
279 ^x	44	CH ₃ CH ₂ CH ₂	7-NH ₂	H	H	CONH			-0.36	0.53	31	4	2.20	2.23
280 ^x	44	CH ₃ CH ₂ CH ₂	7-NH ₂	H	H	CONH			-0.24	0.2	56	5.9	2.18	2.27
281 ^x	44	CH ₃ CH ₂ CH ₂	6-NH ₂	H	H	NHCO			0.13	0.74	30	2.6	2.37	2.23
282 ^x	44	CH ₃ CH ₂ CH ₂	7-NH ₂	H	H	NHCO			0.12	0.52	40	3.0	2.28	2.20
283 ^x	44	CH ₃ CH ₂ CH ₂	6-NH ₂	H	H	NHCONH			0.17	0.60	165	18.5	2.20	2.09
284 ^y	44	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			-0.15	0.53	150	17	2.25	2.21
285 ^y	44	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			-0.41	0.21	40	6.0	2.32	2.18
286 ^y	44	(CH ₃) ₂ CH ₂	6-NH ₂	NH ₂	H	CONH			(0.21)	(0.78)	50	10	2.20	2.12
287 ^o	45	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	NHCO			(0.21)	(0.78)	60	7	1.90	2.00
288 ^o	46	CH ₃ CH ₂ CH ₂	6-NH ₂	H	H	NHCO			0.35	0.87	7	2	2.00	2.00
289 ^x	47	CH ₃ CH ₂ CH ₂	6-NH ₂	H	H	NHCO			-0.47	0.34	140	22.1	1.95	2.07
290 ^y	48	CH ₃ CH ₂ CH ₂	6-NH ₂	NH ₂	H	NHCO			0.13	1.7	3	1	<1.4	2.03
291 ^y	48	CH ₃ CH ₂ CH ₂	6-NH ₂	H	NH ₂	NHCO			0.50	1.7	9	1.1	2.03	2.29
292 ^y	48	CH ₃ CH ₂ CH ₂	7-NH ₂	H	NH ₂	NHCO			0.25	2.68	20	1.0	2.43	2.27
293 ^y	48	CH ₃ CH ₂ CH ₂	7-NH ₂	OCH ₃	H	NHCO			0.15	0.91	20	1.7	2.38	2.24
									0.14	1.18	14	3	2.23	2.21

294 ^o	49	CH ₃	3-NH ₂	NHCO	Cl ⁻	0.11	0.06	0.16	37	2	2.23	2.13	0.10
295 ^o	49	CH ₃	3-NH ₂	NHCO	ClO ₄ ⁻	0.10	0.07	0.12	35	5	2.18	1.84	0.34
296 ^o	50	CH ₃	3-NH ₂	NHCONH	Cl ⁻	0.13	0.09	0.20	22	0.3	2.55	1.76	0.78
297 ^o	51	CH ₃	3-NH ₂	NHCONH	Cl ⁻	0.09	0.06	0.13	200	8	2.48	1.95	0.54
298 ^o	52	CH ₃	2-NH ₂	CONH	Cl ⁻	0.17	0.09	0.25	55	9	2.36	1.97	0.39
299 ^o	53	CH ₃			TsO ⁻	0.76	0.20	2.1	7	1.6	1.95	2.25	-0.30
distamycin						0.68	0.07	2.4					

^a TsO⁻ = *p*-toluenesulfonate. ^b Relative measure of lipophilic-hydrophilic balance from partition chromatography; see Experimental Section. ^c The C₅₀ value is the micromolar drug concentration necessary to displace 50% of DNA-bound ethidium; see Experimental Section and ref 15 and 16. ^d cl = calf thymus DNA. ^e d(A-T) = poly[d(A-T)] - leukemic animals. ^f ILS_{max} = poly[d(G-C)] - poly[d(G-C)] - LD₅₀ lethal dose for 10% of the animals in mg/kg. ^g D₅₀ dose in mg/kg providing 40% increase in the life span of compounds 89-299 using eq 23. ^h Reference 1. ⁱ ILS of 40% not reached at the LD₅₀ dose; see text. ^j Log ILS values for compounds 54-88 were calculated using eq 2 and for measured for their homologous counterparts; see text. ^k New compounds; analytical data provided in Table II. ^l C₅₀ values in parentheses were assumed to be the same as those in Reference 3. ^m Reference 9. ⁿ Compound too water insoluble to measure C₅₀ values. ^o Calculation not possible. ^p Reference 7. ^q Reference 8. ^r Reference 32.

Table II. Structural and Analytical Details for Previously Unreported Bisquaternary Heterocycles

entry no. ^a	type	substituents			link groups			mp, °C	formula	analyses ^b
		R	R'	R''	A	B	C			
300	3	- ^c			CONH			>360	C ₁₂ H ₁₈ N ₂ O ₂	C, H, N
94	3	CH ₂ CH ₂			CONH			196-198	C ₁₂ H ₁₈ N ₂ O ₂ ·1.3H ₂ O	C, H, N; ^f
95	3	(CH ₂) ₃ CH ₂			CONH			265-266	C ₁₃ H ₂₀ N ₂ O ₂	C, H, N
111	7	CH ₂						308-309	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N, S
301	19	CH ₂			NHCO	NHCO	CONH	348-350	C ₁₂ H ₁₈ N ₂ O ₂	C, H, N
135	19	CH ₂			NHCO	NHCO	CONH	317-318	C ₁₂ H ₁₈ N ₂ O ₂ ·I ₂	C, H, N
302	26	CH ₂						>360	C ₁₂ H ₁₈ N ₂ O ₂ ·0.5H ₂ O	C, H, N
150	26	CH ₂						330-331	C ₁₂ H ₁₈ N ₂ O ₂ ·0.5H ₂ O	C, H, N
303	30	CH ₂	NO ₂		NH			332-324	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ·0.5H ₂ O ^e	C, H, N
304	30	CH ₂	NO ₂		NH			192-193	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N
305	30	CH ₂	NO ₂		NH			228-230	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N
185	30	CH ₂	NH ₂		NH			248-250	C ₁₂ H ₁₈ N ₂ O ₂ ·Br ₂	C, H, N, Br
306	35	CH ₂	NH ₂		CH=CHCONH			300-301	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ·1.5H ₂ O	C, H, N
200	35	CH ₂			CH=CHCONH			347-349	C ₁₂ H ₁₈ N ₂ O ₂ ·0.5H ₂ O	C, H, N
201	35	CH ₂			CH=CHCONH			>360	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N
307	41	CH ₂	8-NO ₂		CONH			216-218	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ·1.5H ₂ O ^e	C, H, N, S
308	41	CH ₂	8-NO ₂	H	CONH			274-275	C ₁₂ H ₁₈ N ₂ O ₂ ·H ₂ O	C, H, N
230	41	CH ₂	8-NH ₂	H	CONH			235-237	C ₁₂ H ₁₈ N ₂ O ₂ ·Br ₂	C, H, N, Br
309	41	CH ₂	6-NO ₂ , 8-OCH ₃	H	NHCO			224-225	C ₁₂ H ₁₈ N ₂ O ₂	C, H, N, I
310	41	CH ₂	6-NO ₂ , 8-OCH ₃	H	NHCO			209-211	C ₁₂ H ₁₈ N ₂ O ₂ ·H ₂ O	C, H, N, S
241	41	CH ₂	6-NO ₂ , 8-OCH ₃	H	NHCO			207-208	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N, S
311	45	CH ₂	6-NO ₂ , 8-OCH ₃	H	NHCO			176-178	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ·H ₂ O ^e	C, H, N, S
287	45	CH ₂	6-NO ₂	H	NHCO			284-286	C ₁₂ H ₁₈ N ₂ O ₂ ·Cl ₂	C, H, N, Cl
312	46	CH ₂	6-NH ₂	H	NHCO			321-322	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N, S
313	46	CH ₂	6-NO ₂	H	NHCO			>360	C ₁₂ H ₁₈ N ₂ O ₂ ·0.5H ₂ O	C, H, N, S
288	46	CH ₂	6-NH ₂	H	NHCO			203-205	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ·H ₂ O ^e	C, H, N, S
294 ^o	49	CH ₂	3-NH ₂	H	NHCO			256-257	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N, S
295	49	CH ₂	3-NH ₂	H	NHCO			277-282	C ₁₂ H ₁₈ N ₂ O ₂ ·5H ₂ O	C, H, N, Cl
296	50	CH ₂	3-NH ₂	H	NHCONH			195-197	C ₁₂ H ₁₈ N ₂ O ₂ ·SO ₂ ·Cl ₂ ·H ₂ O	C, H, N, Cl
297	51	CH ₂	3-NH ₂	H	NHCONH			269 dec	C ₁₂ H ₁₈ N ₂ O ₂ ·Cl ₂	C, H, N, Cl
299	53	CH ₂	3-NH ₂	H	NHCONH			306-309	C ₁₂ H ₁₈ N ₂ O ₂ ·Cl ₂	C, H, N, Cl
								371-372	C ₁₂ H ₁₈ N ₂ O ₂ ·S ₂ ^e	C, H, N, S

^a Entries below number 300 refer to formerly unreported compounds for which biophysical and biological test data have been provided in Table I. ^b Elementary analyses for the elements indicated gave results within ±0.4% of those required for the formula provided, unless otherwise indicated. ^c (-) denotes no ring nitrogen substituent; that is, details are for the unquaternized parent bisbase. ^d l: calcd, 29.8; found, 31.1. ^e Anion -toluenesulfonate. ^f N: calcd, 15.5; found, 14.8.

Such a data manipulation method utilizes the totality of the screening data, thereby assisting to minimize biologic variation, and also limits acceptance of unrealistic "best ever" ILS values which can result from successful employment of a dose exceeding the LD₁₀ or other chance event. Measures of the dose necessary to provide a constant biologic response, for example, that to provide an ILS of 40% (*D*₄₀), are also available from the linear correlations. The same data processing method has been used in the present work; where necessary to meet the stringencies of this procedure,²⁴ additional animal test data have been obtained. The agents previously examined^{24,25} consisted primarily of homologous series wherein, from current views,^{26,27} there should be close to parabolic relationships between measures of agent lipophilic-hydrophilic balance and ILS_{max}. The excellent agreement found between the binomial correlation equations derived and the observed biologic data^{24,25} suggests that further investigation of the use of ILS_{max} in regression analysis is clearly warranted.

The present bisquaternary salt series also contains many homologous series and provides an extensive data base (Tables I and II) which would permit further examination of the applicability of our methods. Further, analysis of the large data base using the two alternate types of response assessment—ILS_{max}, representing response at constant toxicity, and *D*₄₀, a measure of the dose necessary to provide a constant response—might provide information on which particular measure was more appropriate in such studies. An additional measure of drug selectivity, the chemotherapeutic index (LD₁₀/*D*₄₀ = CI), apparently unexamined in regression analyses, is readily available from the screening data and has also been included in this comparative study.

Results and Discussion

Adequate biological test data were available for 246 bisquaternary salts, 198 of which were active and 48 inactive. This data set embraces all active bisquaternary salts previously reported¹⁻⁹ and most inactive examples as well. Many of the inactive compounds excluded were nitro group substituted derivatives which were merely prepared as intermediates to the corresponding, extremely active amino compounds⁸ and were accordingly not screened. A small number of agents, containing six aromatic rings, proved so exceedingly insoluble that *C*₅₀ values could not be determined. Formerly unreported compounds are listed in Table II. The generic formulas for the agents are 1-53 (Chart I), and all necessary parameter values are provided in Table I.

The first group of compounds in Table I (54-88), all represented by formula 1, includes the 18 active compounds described in the first paper of this series.¹ Certain of these earliest prepared analogues had been completely used in screening so that *C*₅₀ values for all members could not be measured without resynthesis. As the activities of these closely related congeners could be adequately summarized by a simple parabolic equation in *R*_m alone (eq 2), it was decided that resynthesis would not be

$$\log \text{ILS}_{\text{max}} = -2.00(\pm 0.64)R_m^2 + 1.77(\pm 0.56)R_m + 1.53 \quad (2)$$

$$n = 18, r = 0.86, s = 0.14, F_{2,15} = 25.6$$

warranted. Attention would instead be concentrated on the remaining examples (89-293) which form a better sample set for our aims since there is a considerably greater structural variation in the group and there is a much higher level of data variance which is not accepted by equations in *R*_m alone (see later). In fact, inclusion of data for

Table III. Correlation Matrix for Interrelationships of Measures of Activity Employed

	$\log (1/D_{40})$	$\log \text{LD}_{10}$	$\log \text{CI}$
$\log \text{ILS}_{\text{max}}$	0.597	-0.067	0.780
$\log (1/D_{40})$		-0.711	0.696
$\log \text{LD}_{10}$			0.011

compounds 54-88, for which there is relatively high correlation coefficient for equations in *R*_m alone, into the total data set might serve to attenuate differences between any alternate regression equations developed.

The remaining major group of 204 agents (89-293; Table I) of diverse structure (formulas 2-47) contained 174 L1210 active examples. Five of these (142, 147, 245, 250 and 253) showed levels of activity between 25 and 40% ILS, and, accordingly, *D*₄₀ values for these could not be derived. For the remaining 169 compounds, all three measures of biologic activity of interest were available, and it was clearly desirable to examine the interrelationship of these and the possible correspondence with toxicity as provided by the LD₁₀ values. The cross correlation matrix for the logarithms of these four parameters (Table III) supports the contention that ILS_{max} and CI are measures of drug selectivity since the very low coefficients associated with LD₁₀ terms suggest that these two factors are unrelated to toxicity. ILS and CI are quite highly correlated. In contrast, *D*₄₀ is highly inversely correlated with toxicity (LD₁₀) and directly correlated with ILS_{max}; it appears that this parameter is a composite of measures of both selectivity and toxicity.

Equations in *R*_m Alone. Earlier work⁶ had demonstrated that the lipophilic-hydrophilic balance of these agents was critical for display of biologic activity. As a rise and then fall in L1210 activity were seen as the alkyl quaternary function, on a particular structural variant, was progressively lengthened, parabolic relationships between *R*_m and biologic activity were confidently expected. In agreement, equations linking the various measures of biologic activity and *R*_m values alone (eq 3 and 4) were

$$\log \text{ILS}_{\text{max}} = -0.24(\pm 0.07)R_m + 2.05 \quad (3)$$

$$n = 174, r = 0.45, s = 0.21, F_{1,172} = 43.2$$

$$\log \text{CI} = -0.21(\pm 0.14)R_m + 0.66 \quad (4)$$

$$n = 169, r = 0.23, s = 0.37, F_{1,167} = 9.3$$

$$\log \text{ILS}_{\text{max}} = -0.44(\pm 0.14)R_m^2 - 0.23(\pm 0.06)R_m + 2.14 \quad (5)$$

$$n = 174, r = 0.59, s = 0.19, F_{2,171} = 46$$

$$\log \text{CI} = -0.60(\pm 0.29)R_m^2 - 0.23(\pm 0.13)R_m + 0.76 \quad (6)$$

$$n = 169, r = 0.37, s = 0.36, F_{2,166} = 13.6$$

$$\log (1/D_{40}) = -0.74(\pm 0.42)R_m^2 - 0.02(\pm 0.19)R_m + 5.32 \quad (7)$$

$$n = 169, r = 0.26, s = 0.53, F_{2,166} = 6.0$$

significantly improved by incorporation of terms in *R*_m² (eq 5-7). In fact, the coefficient associated with the *R*_m term in eq 7 is not appreciably different from zero. No significant equation linking *D*₄₀ or LD₁₀ with *R*_m alone could be computed. Similarly, no parabolic equation in *R*_m could be derived for LD₁₀. Stepwise development of these various equations is detailed in Table IV.

Even with the best of these equations (eq 5) 65% (1 - *r*²) of the variance in the biologic data remains unaccounted for.

For the diverse range of structural types in the data base (89-293) it is clearly impossible to employ usual extra-

1

2 X =  3 X = 

4 X =  5 X = 

6

7

8

9 Y = H, X =  10 Y = H, X = 

11 Y = H, X =  12 Y = H, X = 

13 Y = H, X =  14 Y = H, X = 

15 Y = H, X =  16 Y = H, X = 

17 X = H, Y =  18 X = H, Y = 

19 X = H, Y = 

20 A =  21 A = 

22 A =  23 A = 

24 A =  25 A = 

26

27

28 B =  29 B = 

30 B =  31 B = 

32 B =  33 B = 

34 B =  35

36 X = Y =  37 X = Y = 

38 X = Y =  39 X = Y = 

40 X =  Y = 

41 X =  42 X = 

43 X =  44 X = 

45 X =  46 X = 

47

48

49 X =  50 X = 

51 X =  52 X = 

53

$$n = 169, r = 0.28, s = 0.52, F_{3,165} = 4.8$$

Table IV. Steps in the Development of the Equations Discussed^a

eq no.	intercept	R_m	R_m^2	ct	r	s	$F_{1,X}^b$		
3	2.05	-0.24			0.46	0.21	43.2 (172)		
5	2.14	-0.23	-0.44		0.59	0.19	39.3 (171)		
8	2.11	-0.18	-0.45	-0.11	0.61	0.19	5.0 (170)		
		R_m^2	R_m		r	s	$F_{1,X}$		
7	5.31	-0.74			0.26	0.53	12.1 (167)		
	5.32	-0.74	-0.02		0.26	0.53	0.2 (166)		
		R_m^2	R_m	ct	r	s	$F_{1,X}$		
6	0.75	-0.59			0.29	0.38	15.0 (167)		
	0.76	-0.60	-0.22		0.37	0.36	11.9 (166)		
9	0.74	-0.62	-0.19	0.11	0.38	0.36	1.2 (165)		
		R_m^2	ct	R_m	r	s	$F_{1,X}$		
10	5.31	-0.74			0.26	0.53	12.1 (167)		
	5.34	-0.71	-0.15		0.28	0.52	1.3 (166)		
	5.35	-0.71	-0.20	-0.08	0.28	0.52	0.5 (165)		
		A-T	R_m^2	R_m	G-C	r	s	$F_{1,X}$	
12	1.86	0.34				0.58	0.19	88.0 (172)	
	1.94	0.33	-0.44			0.70	0.17	50.6 (171)	
	1.97	0.29	-0.43	-0.16		0.76	0.15	35.5 (170)	
	1.88	0.42	-0.39	-0.27	-0.28	0.81	0.14	40.8 (169)	
		A-T	R_m^2	R_m	G-C	r	s	$F_{1,X}$	
13	0.36	0.50				0.47	0.35	47.2 (167)	
	0.47	0.51	-0.64			0.56	0.33	23.2 (166)	
	0.50	0.47	-0.66	-0.16		0.59	0.32	7.3 (165)	
	0.36	0.69	-0.60	-0.32	-0.43	0.65	0.30	20.0 (164)	
		A-T	G-C	R_m^2	R_m	r	s	$F_{1,X}$	
14	4.91	0.49				0.34	0.51	21.6 (167)	
	4.63	0.96	-0.82			0.55	0.46	45.0 (166)	
	4.75	0.94	-0.76	-0.63		0.59	0.44	12.3 (165)	
	4.72	0.99	-0.97	-0.062	-0.27	0.62	0.43	8.5 (164)	
3	2.05	R_m	A-T/G-C	R_m^2		r	s	$F_{1,X}$	
18	1.81	-0.24				0.45	0.21	43.2 (172)	
	1.90	-0.35	0.42			0.71	0.16	108.5 (171)	
		-0.33	0.39	-0.38		0.79	0.14	50.1 (170)	
		A-T/G-C	R_m	R_m^2		r	s	$F_{1,X}$	
19	0.38	0.45				0.37	0.37	27.0 (167)	
	0.28	0.64	-0.41			0.55	0.33	40.3 (166)	
	0.39	0.62	-0.42	0.56		0.62	0.31	19.3 (165)	
		R_m^2	R_m	A-T/G-C		r	s	$F_{1,X}$	
20	2.16	-0.64				0.49	0.23	12.7 (39)	
	2.18	-0.50	-0.24			0.61	0.21	7.4 (38)	
	1.97	-0.40	-0.36	0.31		0.73	0.18	12.9 (37)	
		R_m^2	R_m	(A)(T)/G-C		r	s	$F_{1,X}$	
21	2.16	-0.64				0.49	0.23	12.7 (39)	
	2.18	-0.50	-0.24			0.61	0.21	7.4 (38)	
	2.09	-0.47	-0.28	0.08		0.62	0.21	1.2 (37)	
		A-T	R_m^2	R_m	G-C	I	r	s	$F_{1,X}$
22	1.85	0.35					0.60	0.19	99.2 (177)
	1.94	0.35	-0.42				0.70	0.17	44.6 (176)
	1.96	0.32	-0.40	-0.14			0.74	0.16	25.1 (175)
	1.90	0.41	-0.37	-0.19	-0.16		0.76	0.15	13.6 (174)
23	1.88	0.42	-0.38	-0.27	-0.27	0.46	0.81	0.14	37.7 (173)

^a Terms employing C_{50} values for a particular DNA species have been abbreviated; e.g., $\log [1/C_{50}(\text{ct})]$ is provided as ct. The same applies for those of the other DNAs employed [A-T; (A)(T); G-C]. ^b The X value pertaining to a particular F test is provided in parentheses.

the third variable being 5.0 although there is only a small increase (0.02) in correlation coefficient. However, when modeling CI and D_{50} such added terms were not significant (eq 9 and 10). Again no significant equation could be derived for LD₁₀. Steps in the development of the above equations are listed in Table IV.

The minor groove of DNA consists essentially of two poly(deoxyribose phosphate) chains. Intuitively it might then be expected that minor-groove binding agents would show similar association constants for all DNA samples of similar topology, regardless of base-pair sequence. Such is clearly not the case. Agents such as distamycin,²² claimed to bind at this site, show a marked selectivity for

adenine-thymine (A-T) rich DNAs when conventional methods of examining binding are employed. C_{50} values for distamycin-ethidium competition for binding sites in poly[d(A-T)] [$C_{50}(\text{A-T})$] and poly[d(G-C)] [$C_{50}(\text{G-C})$] clearly display the preference for the A-T rich polymer (Table I). Similarly, examples of the bisquaternary salts provided markedly different [$C_{50}(\text{G-C})$]/[$C_{50}(\text{A-T})$] ratios, some examples (e.g., 185 and 216) providing figures as high as 20. The absolute values of such ratios are of course dependent on the relative binding affinities of ethidium to the two homopolymers; in this case the binding affinities of ethidium to poly[d(A-T)] ($9.5 \pm 0.9 \times 10^6 \text{ M}^{-1}$) and to poly[d(G-C)] ($9.9 \pm 0.5 \times 10^6 \text{ M}^{-1}$) are identical within

Table V. Squared Correlation Matrix for the Independent Variables Associated with Equations 12-19

	R_m^2	$\log [1/C_{50}(\text{ct})]$	$\log [1/C_{50}(\text{A-T})]$	$\log [1/C_{50}(\text{G-C})]$	$\log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\}$
R_m^2	0.003	0.263	0.059	0.314	0.115
R_m^2		0.001	0.002	0.001	0.005
$\log [1/C_{50}(\text{ct})]$			0.464	0.863	0.053
$\log [1/C_{50}(\text{A-T})]$				0.401	0.237
$\log [1/C_{50}(\text{G-C})]$					0.135

experimental error.¹⁶ Hesitations in accepting the equating of C_{50} values and absolute drug-DNA association constants are of lesser consequence when the ratios $[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]$ are considered. Most modifying factors considered, site size, etc., should effectively cancel in such ratios and these should then correlate with the ratio of the drug association constants for the two different DNAs.

Within the DNA of a mammalian cell it is known that certain regions are redundant whereas others, acting as promoters or repressors controlling the synthesis of critical macromolecules, are vital to the continued functioning of the cell.¹⁸ The importance of different regions of DNA to the continued functioning of a cell varies. Drugs capable of distinguishing different regions, i.e., sequences of DNA, are then likely to vary in their ability to disrupt the continued functioning of a cell. It was then clearly of interest to examine if C_{50} values for binding to different DNA samples could be employed to distinguish possibly more drug-sensitive sequences of tumor cells. Accumulated C_{50} values for interaction of the bisquaternary salts with poly[d(A-T)] and poly[d(G-C)] are listed in Table I. The cross correlation matrix relating to use of these values as independent variables in regression equations is provided in Table V.

As seen from this matrix $C_{50}(\text{A-T})$ and $C_{50}(\text{G-C})$ proved to be reasonably independent variables, whereas $C_{50}(\text{G-C})$ and $C_{50}(\text{ct})$ were highly covariant (eq 11).

$$\log [1/C_{50}(\text{ct})] = 0.83(\pm 0.05) \log [1/C_{50}(\text{G-C})] + 0.21 \quad (11)$$

$$n = 174, r = 0.93, s = 0.14, F_{1,172} = 1085$$

By employing the C_{50} values for drug interaction with the two homopolymeric synthetic DNAs, eq 12-14

$$\log \text{ILS}_{\text{max}} = -0.39(\pm 0.10)R_m^2 - 0.27(\pm 0.06)R_m + 0.42(\pm 0.07) \log [1/C_{50}(\text{A-T})] - 0.28(\pm 0.08) \log [1/C_{50}(\text{G-C})] + 1.88 \quad (12)$$

$$n = 174, r = 0.81, s = 0.14, F_{4,169} = 81.9, R_m(\text{optimum}) -0.35 (-0.23 \text{ to } -0.47)$$

$$\log \text{CI} = -0.60(\pm 0.16)R_m^2 - 0.32(\pm 0.13)R_m + 0.69(\pm 0.16) \log [1/C_{50}(\text{A-T})] - 0.43(\pm 0.19) \log [1/C_{50}(\text{G-C})] + 0.36 \quad (13)$$

$$n = 169, r = 0.65, s = 0.31, F_{4,164} = 29.3, R_m(\text{optimum}) -0.27 (-0.13 \text{ to } -0.41)$$

$$\log (1/D_{40}) = -0.62(\pm 0.36)R_m^2 - 0.27(\pm 0.18)R_m + 0.99(\pm 0.22) \log [1/C_{50}(\text{A-T})] - 0.97(\pm 0.28) \log [1/C_{50}(\text{G-C})] + 4.72 \quad (14)$$

$$n = 169, r = 0.62, s = 0.43, F_{4,164} = 25.5, R_m(\text{optimum}) -0.22 (-0.03 \text{ to } -0.41)$$

could be derived. Stepwise development of these equations

is detailed in Table IV. Again, no significant equation could be derived for LD_{10} .

For all three measures of biologic activity, clearly superior regression equations result on inclusion of $C_{50}(\text{A-T})$ and $C_{50}(\text{G-C})$ values; compare eq 5 and 12, 6 and 13, 7 and 14. Equation 12 highlights the importance of $C_{50}(\text{A-T})$ for antitumor selectivity; the term in this function is the single most important variable, alone accounting for 35% of the variance in the biologic data. Equation 12 provides both a reasonable summary of the experimental data and an explanation of this in physical terms.

Within the confidence limits computed, the coefficients of the terms in $C_{50}(\text{A-T})$ and $C_{50}(\text{G-C})$ in eq 12 are identical and opposite in sign. Rearrangement of eq 12-14 furnishes eq 15-17. Equation 15, employing the ratio of the C_{50}

$$\log \text{ILS}_{\text{max}} = -0.39(\pm 0.10)R_m^2 - 0.27(\pm 0.06)R_m + 0.28(\pm 0.08) \log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\} + 0.15(\pm 0.07) \log [1/C_{50}(\text{A-T})] + 1.88 \quad (15)$$

$$\log \text{CI} = -0.60(\pm 0.16)R_m^2 - 0.32(\pm 0.13)R_m + 0.43(\pm 0.19) \log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\} + 0.26(\pm 0.16) \log [1/C_{50}(\text{A-T})] + 0.36 \quad (16)$$

$$\log (1/D_{40}) = -0.62(\pm 0.36)R_m^2 - 0.27(\pm 0.18)R_m + 0.97(\pm 0.28) \log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\} + 4.72 \quad (17)$$

values to the two homopolymers, may be a more satisfactory one than the equivalent eq 12 for several reasons. First, $\log [1/C_{50}(\text{A-T})]$ is less highly correlated with $\log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\}$ ($r^2 = 0.237$) than with $\log [1/C_{50}(\text{G-C})]$ ($r^2 = 0.402$), and the former two variables then better meet desirable criteria for use in regression analysis. Second, it emphasizes the importance of the differential binding of the bisquaternary salts to different DNA sequences in determining the level of biologic activity measured as ILS_{max} . In eq 15 the coefficient associated with the term in $[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]$ is of higher significance (computed $T = 6.4$) than that in $1/C_{50}(\text{A-T})$ (computed $T = 4.3$). In fact, omission of the latter term from eq 15 provides eq 18 of almost equal usefulness.

$$\log \text{ILS}_{\text{max}} = -0.38(\pm 0.10)R_m^2 - 0.33(\pm 0.06)R_m + 0.39(\pm 0.07) \log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\} + 1.90 \quad (18)$$

$$n = 174, r = 0.79, s = 0.14, F_{3,170} = 93.4$$

Similarly when the same term is deleted from eq 16, eq 19 results.

$$\log \text{CI} = -0.56(\pm 0.25)R_m^2 - 0.42(\pm 0.12)R_m + 0.62(\pm 0.16) \log \{[C_{50}(\text{G-C})]/[C_{50}(\text{A-T})]\} + 0.39 \quad (19)$$

$$n = 169, r = 0.62, s = 0.31, F_{3,165} = 33.7$$

Equation 18 also provides a novel perspective—it suggests that the absolute magnitude of DNA binding may be of minor importance; it is the ability of the drugs to distinguish particular DNA sequences which is the critical feature. Other DNA binding drugs, particularly cancer chemotherapeutic agents, might repay investigation employing this perspective. As noted earlier, when ratios of C_{50} values are successfully employed, as in eq 18, there is less concern about the influence of changing site size on C_{50} values as molecular dimensions of the agents change.

Within a subset of 43 compounds, selected to include representatives of the major structural classes and hopefully as representative of the whole data base as possible, C_{50} values for a further synthetic homopolymeric DNA, poly[d(A)]-poly[d(T)] [(A)(T)], were measured. The C_{50} values for the compounds of the subset to all four DNA polymers are listed in Table VI. As a check that these

Table VI. DNA Interaction Data for a Subset of the Bisquaternary Salts

compd no.	C ₅₀ (ct) ^a	C ₅₀ (A-T) ^b	C ₅₀ (G-C) ^c	C ₅₀ (A)(T) ^d
100	(0.30) ^d	(0.18) ^d	(0.61) ^d	(0.075) ^d
101	0.30	0.18	0.61	0.075
102	(0.30)	(0.18)	(0.61)	(0.075)
103	(0.30)	(0.18)	(0.61)	(0.075)
111	61	63	80	3.8
112	11.2	7.7	22	0.325
121	0.29	0.22	0.37	0.16
122	(0.29)	(0.22)	(0.37)	(0.16)
123	(0.29)	(0.22)	(0.37)	(0.16)
136	11.7	6.0	16.7	0.35
145	6.8	5.3	11	1.6
146	(6.8)	(5.3)	(11)	(1.6)
151	0.42	0.41	0.91	0.13
152	0.48	0.40	0.90	0.11
153	0.44	0.39	0.90	0.12
154	0.46	0.41	0.93	0.14
172	0.40	0.30	0.35	0.18
173	(0.40)	(0.30)	(0.35)	(0.18)
174	(0.40)	(0.30)	(0.35)	(0.18)
178	1.30	0.14	1.8	0.043
179	(1.30)	(0.14)	(1.8)	(0.043)
180	(1.30)	(0.14)	(1.8)	(0.043)
209	5.6	2.8	8.8	0.15
216	1.4	0.16	3.0	0.036
217	(1.4)	(0.16)	(3.0)	(0.036)
218	(1.4)	(0.16)	(3.0)	(0.036)
221	0.66	0.09	1.05	0.048
222	(0.66)	(0.09)	(1.05)	(0.048)
223	(0.66)	(0.09)	(1.05)	(0.048)
230	0.85	0.20	1.43	0.056
231	1.1	0.29	1.4	0.038
251	2.25	0.43	4.76	0.052
252	(2.25)	(0.43)	(4.76)	(0.052)
253	(2.25)	(0.43)	(4.76)	(0.052)
254	1.76	0.79	3.17	0.12
257	0.49	0.10	1.1	0.039
258	(0.49)	(0.10)	(1.1)	(0.039)
259	(0.49)	(0.10)	(1.1)	(0.039)
260	0.71	0.23	1.3	0.067
261	(0.71)	(0.23)	(1.3)	(0.067)
262	(0.71)	(0.23)	(1.3)	(0.067)
264	0.52	0.13	1.11	0.054
281	0.40	0.16	0.52	0.057

^a See footnote d, Table I. ^b See footnote e, Table I.
^c See footnote f, Table I. ^d See footnote n, Table I.

Table VII. Correlation Matrix for the Activity Parameters of the Entries of Table VI

	log (1/D ₅₀)	log LD ₁₀	log CI
log ILS _{max}	0.675	-0.111	0.805
log (1/D ₅₀)		-0.761	0.707
log LD ₁₀			-0.093

43 agents did provide a representative subset, cross correlation matrices for the biologic parameters (Table VII) and the independent variables (Table VIII) were constructed.

Comparison of the values in Table VII and VIII with those of Tables III and V shows that essentially similar

relationships exist between the parameters considered. Similar germinal steps to those followed earlier provide regression eq 20 for these 43 compounds, which can be

$$\log \text{ILS}_{\max} = -0.40(\pm 0.31)R_m^2 - 0.36(\pm 0.16)R_m + 0.31(\pm 0.19) \log \{ [C_{50}(\text{G-C})] / [C_{50}(\text{A-T})] \} + 1.97 \quad (20)$$

$$n = 41, r = 0.73, s = 0.18, F_{3,37} = 14.0$$

directly compared with that derived from the full data base (eq 18). From the similar relationships seen in Tables VII, and III, VIII and V and eq 18 and 20 it appears that the chosen subset is a realistic representation of the whole. Additionally, it can be noted from Table VIII that C₅₀(A)(T) is not related to C₅₀(G-C) although it is reasonably covariant with C₅₀(A-T).

The ratio [C₅₀(G-C)]/[C₅₀(A)(T)], when employed as an independent variable, provided eq 21. The coefficient

$$\log \text{ILS}_{\max} = -0.47(\pm 0.35)R_m^2 - 0.28(\pm 0.19)R_m + 0.08(\pm 0.14) \log \{ [C_{50}(\text{G-C})] / [C_{50}(\text{A})(\text{T})] \} + 2.09 \quad (21)$$

$$n = 41, r = 0.62, s = 0.21, F_{3,37} = 7.8$$

associated with the C₅₀ ratio term in eq 21 is not significantly different from zero and provides no improvement over a two-variable equation in R_m and R_m² alone.

The equations developed demonstrate clearly that the selective antitumor properties of the bisquaternary salts result from their lipophilic-hydrophilic balance and ability to distinguish certain DNA sites. Further, such sites presumably have an excess of alternating A-T residues and a lesser number of G-C base pairs. These appear to be quite remarkable conclusions to reach from attempted modeling of screening results from intact animal studies.

It is not known if there is a single critical DNA site of action, several, or many. Since there is a residuum of as yet unexplained variance in the screening data, it might prove possible to further delineate the sequence of a single critical site or that of a group of highly complementary sites. If sufficient synthetic DNAs of defined sequence were available, then measurement of the C₅₀ values for these, with, for example, the compounds of the subset of Table VI, could be readily carried out. Following regression analysis of these data might allow selection of a synthetic DNA which most closely corresponds in sequence to that of the critical site(s). The preliminary work of this paper provides an indication that such may well be possible. Pinpointing of critical site features, in this fashion, by employing data from animal screening tests, would constitute a novel demonstration of the remarkable power of regression analytical techniques currently available.

Judging from the goodness of fit of the various equations derived for the three measures of biologic activity, ILS_{max}, D₄₀, and CI, it is apparent that ILS_{max} is the superior parameter. It is noteworthy that no significant correlation equation could be derived for LD₁₀. Possibly this reflects the diversity of compounds in the data set and presumably their quite different rates of metabolism and excretion, etc.,

Table VIII. Squared Correlation Matrix for the Independent Variables Associated with the Entries of Table VI

	R _m ²	log [1/C ₅₀ (ct)]	log [1/C ₅₀ (A-T)]	log [1/C ₅₀ (G-C)]	log [1/C ₅₀ (A)(T)]	log { [C ₅₀ (G-C)] / [C ₅₀ (A)(T)] }
R _m ²	0.094	0.154	0.003	0.130	0.015	0.150
log [1/C ₅₀ (ct)]		0.000	0.001	0.000	0.000	0.000
log [1/C ₅₀ (A-T)]			0.468	0.927	0.074	0.332
log [1/C ₅₀ (G-C)]				0.407	0.684	0.216
log [1/C ₅₀ (A)(T)]					0.039	0.440
						0.360

which will influence what fraction of administered dose will ultimately reach critical sites. It may not then be surprising, in view of the covariance seen between LD_{10} and D_{40} (Table II), that the latter has proved less effective as a measure of biologic activity. Employing the LD_{10} as a constant dose, at which measurement of biologic response is made, must compensate in reasonable measure for the diversion of administered drug by metabolism and other causes.

The ILS_{max} values of most active compounds are reasonably well predicted by eq 15, as are the inactivities of the bulk of those lacking biologic activity. Importantly, markedly divergent examples can be immediately noted by scrutiny of the residuals listed in Table I. Thus, compounds 189–191 comprise a small homologous series wherein activities are quite depressed. These three molecules possess the greatest charge separation of all the compounds in the data base, and it is possible that an upper limit to such separation is being reached. Additionally, the series 251–253 and 254–256 are two where activities are also well depressed. Since both groups contain a common 2''- NH_2 group, it may be surmised that this function is in some way responsible for the depression seen. The inactivity of all 2-substituted pyridinium compounds examined (206–214) is not in accord with the predictions of eq 15. It would clearly be of interest to examine the C_{50} values for these less well predicted compounds with a more extensive range of synthetic DNAs.

Intercalating Variants. Compounds 294–298 constitute a small group of highly active compounds which were not included in the derivation of eq 15. Although there is apparently a close relationship to the quinoline quaternary salts (e.g., 290) in two-dimensional representations of structure, there is one important difference. In progressing from a 4-anilinoquinoline to a 9-anilinoacridine system, steric interactions, stemming from the additional fused ring in the latter, force the plane of the appended 9-anilino ring system well away from that of the acridine ring plane.²⁹ Bisquaternary salts based on the 9-anilinoacridine system (294–298) then do not have, overall, close to planar structures. Representatives of the 4-anilinoquinoline system (216, 221, and 229) do not remove superhelical turns in bacteriophage PM2 DNA¹⁷ and, to this criterion, are not intercalating agents. However, examples of the acridine bisquaternary salts do provide unwinding of this DNA¹⁷ and can then be classified as intercalating agents. Presumably it is the acridine nucleus of these agents which intercalates into the nucleic acid. These compounds (294–298) distinguish between the A-T and G-C polymers to a much greater extent than do the simple 9-anilinoacridines.¹⁵ On the basis of later discussion it can be hypothesized that these agents have the acridine nucleus intercalated into the DNA and the remainder of the molecules lodged in the minor groove. These compounds then act as conceptual bridges between the minor groove binding bisquaternary salts and the extensive series of 9-anilinoacridines (AMSA agents)³⁰ which bind to DNA predominantly by intercalation.³¹ In fact, the genesis of the AMSA series lay in the initial lead provided by the bisquaternary salts, followed through the series of quinoline variants (e.g., 216–293)^{7,8} and a group of dibasic acridines akin to 292–298.³²

All the acridine bisquaternary compounds (294–298) have exceptional antitumor activity and provide long-term survivors in standard L1210 assays. They are poorly predicted by eq 12, being much more active than expected. Recalculation of eq 12 including these five acridine

compounds provides the significantly less well fit eq 22.

$$\log ILS_{max} = -0.37(\pm 0.12)R_m^2 - 0.19(\pm 0.06)R_m + 0.41(\pm 0.07) \log [1/C_{50}(A-T)] - 0.16(\pm 0.09) \log [1/C_{50}(G-C)] + 1.90 \quad (22)$$

$$n = 179, r = 0.76, s = 0.15, F_{4,174} = 61.4$$

Addition of an indicator variable denoting the presence ($I = 1$) or absence ($I = 0$) of an acridine ring system provided the improved eq 23. Comparison of eq 12 and

$$\log ILS_{max} = -0.38(\pm 0.10)R_m^2 - 0.27(\pm 0.09)R_m + 0.42(\pm 0.07) \log [1/C_{50}(A-T)] - 0.27(\pm 0.09) \log [1/C_{50}(G-C)] + 0.46(\pm 0.15)I + 1.88 \quad (23)$$

$$n = 179, r = 0.81, s = 0.14, F_{5,173} = 67.1$$

23 shows these to be virtually identical except for the indicator variable in the latter. To the extent of the small number of acridine compounds available these must follow the same quantitative SAR as the bisquaternary salts, with a $\log ILS_{max}$ increment of 0.46 conferred by the acridine system. From the discussion above it is tempting to attribute this considerable increment in activity to the ability of these compounds to intercalate their acridine nuclei into DNA. If the findings embodied in eq 12 are correct, the acridine compounds related to 294–298, which are bet- able to distinguish between poly[d(A-T)] and poly[d(G-C)], should possess even greater antitumor selectivity.

Structural Features Modifying C_{50} Values. There is little doubt that a considerable proportion of the binding interaction of the bisquaternary salts with DNA stems from the aromatic ring components. Thus, loss of aromatic rings from 151 to give progressively 112 and 111 results in a progressive increase in C_{50} values [$C_{50}(A-T) = 0.41, 7.7, \text{ and } 63 \mu M$, respectively]. Similarly, compounds with aliphatic link units (193–196) have high C_{50} values in comparison with those containing aromatic ring systems in the same relative position (178–192). From model fitting, and by considering the likely order of the relative magnitudes of the possible binding forces involved, it can be suggested that the DNA binding contributions provided by the aromatic ring components probably result from ion ($-PO_2^-$)–dipole interactions as well as the associated van der Waals contact forces.

The sequence selectivity of DNA binding, observed with the bisquaternary salts, invariably favors better binding to poly[d(A-T)]. Similar binding distinctions have been observed with distamycin and it has been suggested that there is selective hydrogen-bond formation between polar functions of the drug and the A-T pairs distinguished. However, fitting of space-filling models (Courtald) of the bisquaternary salts to that of twin-helical DNA shows that most of the polar link structures employed in these agents are unlikely to be able to form reasonably dimensioned H bonds with base-pair functionality due to steric limitations imposed by the aromatic ring units employed. The models do, however, show that the observed distinction for poly[d(A-T)] might not result from a positive discrimination for this polymer but rather from an inhibition of binding to poly[d(G-C)]. The models suggest that a dominant steric inhibition of drug binding would be exerted by the 2- NH_2 group of guanine, particularly exacerbated if this function has associated H-bonded water molecule(s). On such steric grounds, the binding distinction between d(A-T) and d(G-C) would depend primarily on how far the skeletal framework between the two charged functions of any drug extended into the minor

groove, thus impinging against the guanine amino groups. For example, in the 3-aminopyridine quaternary salt 151, when cationic charges are matched to the exterior phosphates of the DNA, the skeletal framework of the agent lies along the outside of the minor groove and is, in consequence, only mildly discriminatory against the G-C polymer [$[C_{50}(G-C)]/[C_{50}(A-T)] = 2.2$]. In a variant utilizing the same skeletal framework but containing highly angular 4-anilinoquinolinium basic functions (178), similar matching of cationic drug charges to anionic site phosphates results in the drug framework being forced deeper into the minor groove and an apparently high A-T selectivity results [$[C_{50}(G-C)]/[C_{50}(A-T)] = 12.9$]. Such considerations, coupled with judicious model building, appear in qualitative agreement with the A-T/G-C selectivities observed. Quantitative ordering of such results appears a somewhat more formidable task.

If the above views are correct it should prove possible to construct a bisquaternary salt which contains no polar functions capable of H bonding to specific base-pair functionality, yet would demonstrate selectivity of binding to poly[d(A-T)], and, providing it was of suitable lipophilic-hydrophilic balance, should also then prove tumor active. Of the quaternary salt functions investigated, which contain no peripheral H-bond donor or acceptor groups, the *N*-arylpyridinium salts prove the most hydrophilic. A distyryl-linked bisquaternary salt, utilizing this basic function (299), has no polar groups which can H bond with base-pair functionality but nevertheless binds selectively to poly[d(A-T)] in relation to poly[d(G-C)] [$[C_{50}(G-C)]/[C_{50}(A-T)] = 10.5$]. From the measured R_m value of 299 and the C_{50} values for poly[d(A-T)] and poly[d(G-C)], it proved possible to predict from eq 15 that this compound would be L1210 active, and the absolute magnitude of such activity was quite reasonably predicted—before the screening in animals was undertaken.

Experimental Section

Melting points were determined in open capillaries on an Electrothermal melting point apparatus with the makers' stem corrected thermometer and are as read. Quaternary salts as crystallized from aqueous solvents are often extensively hydrated. For analysis, samples have been dried in vacuo over silica gel at room temperature. Attempts to dry thoroughly at elevated temperatures gave extremely hygroscopic samples and, in some cases, a loss of crystallinity. Melting points have been determined on the samples dried and ready for analysis.

UV spectra were determined on a Shimadzu UV 200. To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2 , F₂₅₄) was used. Homogeneity of the bisquaternary salts was best monitored by employing the top phase of a mixture of *n*-BuOH-HOAc-H₂O (5:1:4; v/v) as solvent.

R_m Values. The top phase of a mixture of *i*-BuOH, HOAc, H₂O, and DMF (30:6:24:2.25; v/v) was used with Merck DC cellulose F₂₅₄. Agents were detected by UV scanning and spraying with Dragendorff's reagent. Quaternary salts were applied as solutions in the lower phase of the solvent mixture. Applied spots should not be dried on the cellulose sheets by the application of heat; under such conditions certain quaternary salts appear to complex firmly with the cellulosic support and then fail to move satisfactorily on subsequent development. Immediately after each sheet was spotted, it was transferred to a developing tank whose walls were hung with filter paper soaked in the lower phase of the solvent mixture. An equilibration time of 17 h was employed before development with the top phase. Values are the mean of four determinations. The standard errors found associated with such measurements were normally inside the range of ± 0.03 .

C_{50} Values. The fluorimetric method, earlier detailed in full,^{15,16} was employed with substitution of the requisite nucleic acid for the calf thymus DNA then used. With selected agents multiple determinations of C_{50} values provided figures which have lain within an extreme range of $\pm 9\%$ of the mean value.

6-[4-(Methoxycarbonyl)benzamido]quinoline was prepared by the usual phosphorazo coupling² of equimolecular quantities of 6-aminoquinoline and methyl potassium terephthalate² in pyridine solution. Pure product (92% yield) separated as colorless needles from DMF-MeOH: mp 222–223 °C. Anal. ($C_{18}H_{14}N_2O_3$) C, H, N. The ester function in the latter was saponified by stirring with excess 1 N KOH in 85% aqueous MeOH until dissolved and then standing at room temperature for a further 1 h. Precipitation from the clarified solution, by addition of a volume of 1 N HCl equivalent to the KOH employed, afforded the corresponding acid as a microcrystalline, TLC homogeneous precipitate.

3-[(4-Nitrophenyl)carbamoyl]pyridine was similarly prepared by phosphorazo coupling of 4-nitroaniline and nicotinic acid. The product crystallized as yellow needles from EtOH: mp 259–260 °C (87% yield). Anal. ($C_{12}H_9N_3O_3$) C, H, N.

3-[(4-Aminophenyl)carbamoyl]pyridine was prepared by Fe/H⁺ reduction² of the preceding compound and separated from an EtOH-H₂O solution as colorless needles of mp 186–187 °C (83% yield). Anal. ($C_{12}H_{11}N_3O$) C, H, N.

300. Phosphorazo coupling of 6-(4-carboxybenzamido)quinoline and 3-[(4-aminophenyl)carbamoyl]pyridine in dry *N*-methyl-2-pyrrolidone (NMePy)-pyridine solution provided bisbase 300 (Table II) in 71% yield.

3-(3-Nitrobenzamido)pyridine resulted from phosphorazo coupling of 3-nitrobenzoic acid and 3-aminopyridine. Pure product was obtained from EtOH-H₂O as pale yellow needles of mp 149–150 °C. Anal. ($C_{12}H_9N_3O_3 \cdot H_2O$) C, H, N.

3-(3-Aminobenbamido)pyridine was obtained by Fe/H⁺ reduction of the aforementioned product and crystallized from EtOH-H₂O as colorless needles of mp 161–162 °C. Anal. ($C_{12}H_{11}N_3O$) C, H, N.

3-[3-(4-(Methoxycarbonyl)benzamido)benzamido]pyridine was prepared by phosphorazo coupling of 3-(3-aminobenbamido)pyridine and methyl potassium terephthalate. Pure product separated from DMF-MeOH solution as colorless needles of mp 231–232 °C (87%). Anal. ($C_{22}H_{17}N_5O_4$) C, H, N.

3-[3-(4-Carboxybenzamido)benzamido]pyridine resulted from saponification of the methyl ester function in the preceding compound, essentially as before, but with added DMF to increase solubility. Pure acid crystallized from DMF-H₂O as colorless needles of mp 325–326 °C. Anal. ($C_{20}H_{15}N_5O_4$) C, H, N.

301. Phosphorazo coupling of the aforementioned acid and 3-(4-aminobenbamido)pyridine² in NMePy-pyridine solution provided crude product. Crystallization from DMF-MeOH provided the TLC homogeneous product (72% yield; Table II).

3-(4-Nitro-1-naphthamido)pyridine resulted from standard coupling of 4-nitro-1-naphthoic acid and 3-aminopyridine. Pure product separated from DMF-H₂O as pale yellow crystals of mp 168–169 °C (82%). Anal. ($C_{18}H_{11}N_3O_3 \cdot 0.5H_2O$) C, H, N.

3-(4-Amino-1-naphthamido)pyridine was obtained by Fe/H⁺ reduction of the preceding product and crystallized from EtOH-H₂O as colorless needles of mp 203–205 °C (91%). Anal. ($C_{16}H_{13}N_3O \cdot 0.5H_2O$) C, H, N.

302. Phosphorazo coupling of 3-(4-amino-1-naphthamido)pyridine [dried in a vacuum oven at 120 °C (15 mm)] and 3-[4-(4-carboxybenzamido)benzamido]pyridine provided the desired product which separated from DMF-MeOH as colorless crystals (83% yield; Table II).

307 was prepared by reacting 4-chloro-8-nitroquinoline and 4-[4-(4-aminobenbamido)anilino]pyridine⁶ in acid media as before.^{6–8} The pure bisbase separated from DMF-H₂O as pale yellow crystals (83% yield; Table II).

309 was similarly prepared in 87% yield by reacting 4-chloro-6-nitro-8-methoxyquinoline and 4-[4-(4-aminophenyl)carbamoyl]anilino]pyridine⁷ by the standard method. Pure product crystallized readily from DMF-MeOH (Table II).

312 (Table II) resulted from similar interaction of 4-chloro-6-nitroquinoline and 3-[4-[(4-aminophenyl)carbamoyl]benzamido]benzamido]pyridine.⁴

2-(4-Carboxyphenyl)-1,3-dioxolane. 4-Carboxybenzaldehyde (15.1 g, 0.1 mol) was suspended in C₆H₆ (150 mL) containing ethylene glycol (28 mL) and 4-toluenesulfonic acid hydrate (50 mg), and the mixture was heated to reflux under a Dean-Stark water entrainment head until no further H₂O was removed (2 h). Following removal of C₆H₆ in vacuo a solution of NaOH (8 g) in H₂O (100 mL) was added, and the mixture was warmed until

homogeneous and then allowed to stand at room temperature for 1 h. The latter step saponifies ester functions also generated in the initial condensation. Following cooling of the solution to 0 °C crude product was precipitated by addition of HOAc (12 mL). Product was collected in CHCl₃ and the washed (H₂O) and dried (Na₂SO₄) solution evaporated to a small volume. Petroleum ether was then added to turbidity at the boil, and cooling then provided TLC homogeneous product as colorless plates of mp 177–178 °C (11.0 g; 57%). Anal. (C₁₀H₁₀O₄) C, H.

2-[(4-Nitrophenyl)carbamoyl]phenyl]-1,3-dioxolane was prepared by phosphorazo coupling of the preceding product and 4-nitroaniline. Pure product separated as pale yellow needles from DMF–MeOH, mp 267–268 °C (73% yield). Anal. (C₁₆H₁₄N₂O₅) C, H, N.

4-[(4-Nitrophenyl)carbamoyl]benzaldehyde. To a suspension of the preceding dioxolane (6.8 g, 0.021 mol) in boiling dioxane (110 mL) was added in one portion 12 N HCl (7.5 mL), the starting material promptly dissolving, and then the desired product crystallized from the solution. Following thorough cooling, product was collected and recrystallized from DMF–H₂O, separating as pale yellow needles of mp 284–286 °C (87%). Anal. (C₁₄H₁₀N₂O₄) C, H, N.

2-[4-[(4-Nitrophenyl)carbamoyl]styryl]-1,4,5,6-tetrahydropyrimidine. The preceding aldehyde (5.42 g, 0.02 mol) was suspended in a mixture of pyridine (20 mL), ethyl formate (20 mL), and DMF (10 mL), then 2-methyl-1,4,5,6-tetrahydropyrimidine (2.5 g, 0.026 mol) added, and the mixture heated on a steam bath for 18 h. After evaporation in vacuo, EtOH (30 mL) and 12 N HCl (3 mL) were added and the solution was re-evaporated. The residue was extracted with boiling 0.01 N HCl (660 mL) and NaCl (100 g) dissolved in the hot clarified solution. On cooling, crude product hydrochloride crystallized from the solution. Multiple crystallizations from EtOH–H₂O–HCl provided TLC homogeneous product hydrochloride as colorless needles of mp 328–329 °C (27% yield). Anal. (C₁₉H₁₉N₄O₄Cl·1.5H₂O) C, H, N, Cl.

2-[4-[(4-Aminophenyl)carbamoyl]styryl]-1,4,5,6-tetrahydropyrimidine was prepared by Fe/H⁺ reduction of the aforementioned product and was conveniently purified as the bishydrochloride: colorless needles from boiling 1 N HCl; mp 339 °C dec (79% yield). Anal. (C₁₉H₂₂N₄OCl₂·H₂O) C, H, N, Cl.

1-Methyl-1,4-dihydro-6-nitroquinol-4-one was prepared in 72% yield by the method formerly used to prepare the corresponding 1-ethyl analogue.⁷ Pure compound crystallized from pyridine–H₂O as bronze needles of mp 236–237 °C. Anal. (C₁₀H₈N₂O₃) C, H, N.

311. 1-Methyl-4-chloro-6-nitroquinolinium chloride was prepared in situ, as before,⁷ by the action of SOCl₂–DMF on 1-methyl-1,4-dihydro-6-nitroquinol-4-one. This quinolinium salt was reacted with 2-[4-[(4-aminophenyl)carbamoyl]styryl]-1,4,5,6-tetrahydropyrimidine bishydrochloride in 65% EtOH–H₂O solution as before⁷ to provide the desired product (Table II).

294. 1-Methyl-4-[4-[(4-aminophenyl)carbamoyl]anilino]pyridinium bromide⁷ (0.02 mol) and 3-(trifluoroacetamido)-9-chloro-10-methylacridinium chloride (0.02 mol) were reacted in 65% aqueous EtOH (125 mL) as before¹² until TLC monitoring demonstrated complete reaction. Following cooling to 25 °C sufficient 12 N NH₃ was added to provide a 4 N concentration and the solution allowed to stand until TLC demonstrated complete removal of the trifluoroacetyl group. Adjusting the pH to below 4 with HCl, removal of EtOH in vacuo, and addition of an equal volume of saturated aqueous NaCl precipitated crude, crystalline 294 chloride salt. Further crystallization from EtOH–H₂O–NaCl provided TLC homogeneous agent (68%) as vermilion-colored needles (Table II).

4-[4-[(4-Nitrophenyl)sulfamoyl]anilino]pyridine was prepared from 4-[(4-nitrophenyl)sulfamoyl]aniline and N-pyridyl-4-pyridinium chloride hydrochloride by the methods derived earlier.⁴ Pure product crystallized from EtOH–H₂O as yellow prisms of mp 261–262 °C (67% yield). Anal. (C₁₇H₁₄N₄O₄S) C, H, N, S.

1-Methyl-4-[4-[(4-nitrophenyl)sulfamoyl]anilino]pyridinium Chloride. Attempted preparation of this compound by direct quaternization of the preceding product afforded multiple products, presumably due to cooccurring alkylation of the sulfonamide function. Vigorous acetylation, employing re-

fluxing Ac₂O containing NaOAc for 3 h, provided a TLC homogeneous acetyl derivative which crystallized from HOAc. This product, on treatment with 1 N NH₃–EtOH at room temperature, slowly returned the starting sulfonamide; at reflux temperature deprotection was complete in 15 min. To a solution of the above acetyl derivative (18.2 g) in the minimum necessary volume of hot (120 °C) nitrobenzene, methyl *p*-toluenesulfonate (14.9 g) was added in one portion. After 15 min at this temperature the mixture was cooled and the deposited quaternary salt washed with dry *i*-Pr₂O, dried in vacuo, and dissolved in EtOH (75 mL). After addition of 12 N aqueous NH₃ (11 mL) the solution was heated to reflux for 15 min, then solvent removed in vacuo, and 2 N HCl (50 mL) added to the residue. Crude quaternary salt was collected from the cooled mixture and crystallized from boiling 0.01 N HCl until homogeneous to TLC. Pure product was obtained as colorless needles of mp 180–182 °C (11.2 g). Anal. (C₁₈H₁₇N₄SO₂Cl) C, H, N, S, Cl.

1-Methyl-4-[4-[(4-aminophenyl)sulfamoyl]anilino]pyridinium chloride was prepared by Fe/H⁺ reduction of the corresponding nitro compound. Pure product (76% yield) separated from H₂O–NaCl as colorless needles of mp 193–195 °C. Anal. (C₁₈H₁₉N₄SO₂Cl) C, H, N, S, Cl.

295. Reaction of the preceding product with 3-(trifluoroacetamido)-9-chloro-10-methylacridinium chloride by the standard method and following deprotection with aqueous NH₃ and workup in the usual manner provided crude agent. Crystallization from EtOH–H₂O provided TLC homogeneous product as red needles (76% yield; Table II).

296. 4-[(Aminophenyl)carbamido]acetophenone⁷ and 3-[(trifluoroacetyl)amino]-9-chloro-10-methylacridinium chloride were coupled by the standard method, and the protecting trifluoroacetyl function was removed as in earlier examples. The crude chloride salt resulting from this sequence (81%), TLC demonstrating contamination with 3-amino-10-methyl-9(10*H*)-acridone, was dissolved in the minimum necessary volume of boiling 60% EtOH–H₂O which was 2 N in HCl. After addition of a twofold excess of aminoguanidine hydrochloride the solution was heated on a steam bath for 1 h, the desired product crystallizing progressively from the solution. A further crystallization from EtOH–H₂O–NaCl provided TLC homogeneous product (Table II).

N¹-[4-[(4-Nitrophenyl)carbamido]phenyl]biguanide Hydrochloride. 4-[(4-Nitrophenyl)carbamido]aniline (0.019 mol) and dicyanodiamide (0.028 mol) in dry NMePy (35 mL) containing methanesulfonic acid (0.19 mol) were heated together at 140 °C, with stirring, for 20 min. Dilution of the cooled mixture with C₆H₆ (250 mL) precipitated an oil which solidified on trituration with petroleum ether. The dried solid was dissolved in boiling H₂O (550 mL) containing 12 N HCl (1 mL), decolorizing charcoal added, and then hot 20% NaCl–H₂O added to the clarified solution until crystallization initiated. Recrystallization from NaCl–H₂O provided TLC homogeneous product as yellow prisms of mp 241–242 °C (73%). Anal. (C₁₅H₁₇N₈O₃Cl) C, H, N, Cl.

297. Fe/H⁺ reduction of the preceding nitro compound by the usual method provided a very readily autoxidized amine which rapidly furnished colored oxidation products on exposure to air. Accordingly, following demonstration of homogeneity by TLC and employing minimal further manipulation, this product was immediately coupled with 3-(trifluoroacetamido)-9-chloro-10-methylacridinium chloride, as before. Terminal hydrolytic removal of the trifluoroacetyl group in the usual way provided the desired product (Table II).

1,4-Bis(4-nitrostyryl)benzene has been prepared by a variety of methods^{33–35} but moderate quantities can be simply prepared by the following route employing commercially available starting materials. To (4-nitrophenyl)acetic acid (54 g, 0.3 mol) and terephthalaldehyde (20.1 g, 0.15 mol) dissolved in NMePy (60 mL) was added piperidine (60 mL), and the resulting mixture was heated on a steam bath for 3 h. C₆H₆ (100 mL) was then added and the mixture heated to reflux (oil bath) under a Dean-Stark H₂O entrainment head for 12 h. Solvent was then distilled until an internal temperature of 160 °C was reached and this temperature maintained for 1 h. Following cooling, MeOH (120 mL) was added and the mixture briefly boiled and then thoroughly cooled. The collected crystals were recrystallized once from DMF–MeOH and then DMF. TLC homogeneous product

was obtained as glistening orange plates of mp 290–291 °C (24% yield). Anal. ($C_{22}H_{18}N_2O_4$) C, H, N. Quoted melting points for this compound are 262–264,³³ 280,³⁴ and 290 °C.³⁵

1,4-Bis(4-aminostyryl)benzene. To the above dinitro compound (8.9 g, 0.024 mol) suspended in boiling HOAc (600 mL) was added a solution of $SnCl_2 \cdot 2H_2O$ (65 g, 0.29 mol) in 12 N HCl (65 mL) and the mixture stirred vigorously while boiling. After ca. 0.5 h of heating a clear solution resulted, and after a further 0.5 h the volume was reduced in vacuo to 90 mL and 12 N HCl (30 mL) added. After thorough cooling the crystals were collected, washed well with saturated brine, and then suspended in a solution of NaOH (45 g) in H_2O (450 mL), and the whole mixture was stirred until of homogeneous consistency. The insoluble diamine was collected, washed well with H_2O , dried, and crystallized from DMF-EtOH. Pure product was obtained as off-white needles of mp 297–298 °C; λ_{max} (20% DMF-EtOH) 385.5 nm ($\log \epsilon$ 4.71), lower peaks were obscured by the solvent necessary for solution. Anal. ($C_{22}H_{20}N_2$) C, H, N.

299. In the preparation of simple N-substituted pyridines Zincke¹³ could employ N-(2,4-dinitrophenyl)pyridinium chloride for reaction with primary amines. However, the marked insolubility of this pyridinium chloride in solvents in which 1,4-bis(4-aminostyryl)benzene could be dissolved prevented successful reaction. A simple change of anion, to 4-toluenesulfonate, provided a suitably soluble salt. Addition of excess 20% aqueous sodium 4-toluenesulfonate, acidified to pH below 4 with 4-toluenesulfonic acid, to a saturated aqueous solution of N-(2,4-dinitrophenyl)pyridinium chloride precipitated the required salt. A single crystallization from a small volume of boiling water provided pure N-(2,4-dinitrophenyl)pyridinium 4-toluenesulfonate as massive prisms of mp 255–257 °C. Anal. ($C_{18}H_{15}N_3O_7S$) C, H, N, S.

A suspension of 1,4-bis(4-aminostyryl)benzene (5 g, 0.016 mol) and N-(2,4-dinitrophenyl)pyridinium 4-toluenesulfonate (26.75 g, 0.064 mol) in anhydrous dimethylacetamide (80 mL) was heated while stirring until a solution resulted. The reaction mixture was then boiled for 30 min and cooled, and crude product was precipitated with $CHCl_3$ (375 mL). The solid was washed by alternately suspending in boiling $CHCl_3$ and filtering. This process was continued until all yellow 2,4-dinitroaniline, a further product of the reaction, was completely removed. The solid residue was suspended in H_2O (1100 mL) and the suspension boiled for 15 min, decolorizing charcoal added, and the solution clarified. Overnight cooling of the filtrate provided crystalline product. Further crystallization from H_2O (100 mL/g) and then EtOH- H_2O containing sodium 4-toluenesulfonate provided TLC homogeneous 299 (Table II) as yellow needles (3.35 g; 32%); λ_{max} (H_2O) 372.5 nm ($\log \epsilon$ 4.79), shoulders at 219 (4.63) and 248 (4.31).

L1210 Screening. The L1210 leukemia was maintained by serial, weekly transfer (10^5 cells ip in DBA₂ mice) for periods of no longer than 3 months. At three monthly intervals tumor was refurnished with a sample withdrawn from liquid N_2 storage.

Antitumor tests were performed in $C_3H_9 \times DBA_{2F_1}$ hybrid mice produced in the animal colonies of this laboratory. Animals were implanted with 10^5 L1210 cells ip on day 0. Drug treatment was ip once daily on days 1–5. Animal deaths were recorded twice daily. Every attempt was made to obtain solutions of the quaternary salts in H_2O so that the dose for a 20-g mouse was contained in a 0.2-mL volume. Animals in the 18.5–22.5-g weight range were employed but any drug-treated group contained weight matched animals varying by no more than 1 g. No significant difference in the mean life span of groups of control animals within this weight range could be detected. There were a minimum of six animals per treatment group and one control group was employed with every six treatment groups.

A dose-response profile of antileukemic activity was accumulated employing doses separated by 0.09 log dose intervals. In determination of the LD_{10} the doses employed were separated by 0.05 log dose intervals and animals observed for a 50-day period. The LD_{10} dose was derived by linear correlation of percent probit mortalities and the logarithms of the corresponding drug doses employed, as before.²⁴ Significant life extensions in leukemia L1210 assays, obtained at and below the LD_{10} dose, were linearly correlated with the logarithms of the corresponding doses employed. The percentage increase in life span (ILS) specified by the regression line at the measured LD_{10} dose was employed as

ILS_{max}. The dose providing 40% increase in life span in L1210 tests (D_{40}) was also obtained from this regression line.

While the confidence limits on ILS_{max} are not centrosymmetric, those for log ILS_{max} are more nearly so.²⁴ From the 95% confidence limits about the ILS/log dose regression line and the LD_{10} dose it can be predicted that log ILS_{max} should be duplicable within the limits of ± 0.15 .²⁴ Replicate screening by the methods described has provided values within these limits.

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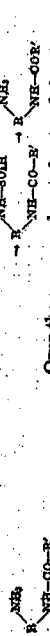
UNITED STATES PATENT OFFICE

WILHELM KREMLER OF LEVERHUSEN-ON-THIE-RIEHE, AND WILHELM LAMERZ OF WIESBADEN, GERMANY, ASSIGNORS TO GENERAL ANILINE WORKS, INC., OF NEW YORK, N. Y. A CORPORATION OF DELAWARE

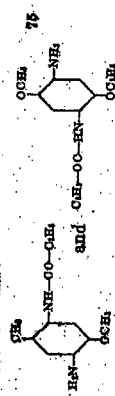
AROMATIC MONOAMINO DIAMINES

No Drawing. Application filed January 8, 1931, Serial No. 604,884, and in Germany January 15, 1930.

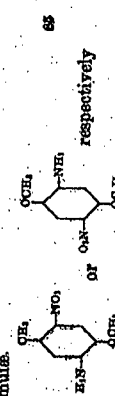
The present invention relates to a process of preparing monoaryl-diamines of the benzene and naphthalene series, more particularly it relates to compounds which may be represented by the probable general formula:



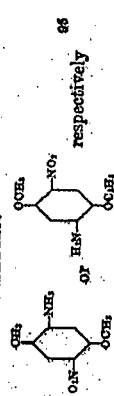
Over the processes heretofore used for the manufacture of monoaryl-diamine compounds of the benzene or naphthalene series, the present process has the advantage that apart from the first step the process can be carried out in aqueous solution due to the presence of the sulfamic acid group, and that iron which is the cheapest reducing agent can be used. The process also renders possible in many cases the manufacture of bases which in consequence of the difficulty of obtaining the nitroamine compounds could only be produced by other methods in a tedious or cumbersome manner. Thus the compounds of the formula:



which in accordance with this invention are obtainable from the compounds of the formula:



were only to be obtained from the nitroamines of the formula:



these nitroamines being obtainable only with difficulty.

The following examples will illustrate our invention, without restricting it thereto:

Example 1.—4-amino-2-benzoylaminobenzene-1-methoxybenzene

268 kgs. of the sodium salt of 6-nitro-1,3-dimethylbenzene-4-sulfamic acid (obtained by sulfonating in the amino group of 6-nitro-4-amino-1,3-dimethylbenzene with chlorosulfonic acid in the presence of a tertiary base) are reduced in the usual manner at 60-70° C. with iron and formic acid. When the reaction is complete, the mass is rendered alkaline and the amino compound present in the solution is pressed out from the iron oxide sludge. Then there are gradually added to the filtrate at 25-30° C. with good stirring, 148 kgs. of benzoylchloride, and the mixture is kept weakly alkaline by means of sodium carbonate. Then it is heated to 70-90° C. by means of hydrochloric acid, and at the same time, the temperature is raised to 80-90° C. After a short time the saponification is complete, the hydrochloric acid salt of the 4-amino-2-benzoylaminobenzene-1-methoxybenzene is precipitated, while still hot, in white lustrous needles, by the addition of common salt. It is separated nearly quantitatively. Then after filtering by suction, it is again dissolved in hot water and filtered; the base is precipitated with sodium carbonate. White needles, melting point 176° C. Calculated on the nitroarylsulfamic acid employed the yield is 88%.

On acrylating instead of benzoylchloride for example, with the chloride of the phenylacetic acid, the corresponding phenacetyl compound is obtained. White needles, melting point 177° C.

Example 2.—5-amino-2-benzoylaminobenzene-1-methoxybenzene

284 kgs. of 2-nitro-1-methyl-4-methoxybenzene-5-sulfamic acid (sodium salt), obtained by sulfonating in the amino group of 1-methyl-5-amino-4-methoxy-2-nitrobenzene as described in Example 1. The reduction liquor freed from iron is acrylated with 148 kgs. of benzoyl chloride with the addition of sodium carbonate, and the sulfonic group is split off by means of dilute hydrochloric acid at 70-80° C. When the saponification is complete, the hydrochloric acid salt of 5-amino-2-benzoylaminobenzene-1-methoxybenzene is separated, while still hot, in white lustrous needles, and by dissolving in hot water, filtering and precipitating with sodium carbonate, the free base is obtained. White needles, melting point 183° C. Calculated on the nitroarylsulfamic acid used the yield is 94%. On employing instead of benzoylchloride, for example, cinnamic acid chloride or o-chlorophenoxycetic acid chloride, the corre-

sponding compounds are obtained.

Example 3.—2-amino-5-benzoylaminobenzene-1-methoxybenzene

314 kgs. of 1-methoxy-4-ethoxy-5-nitrobenzene-2-sulfamic acid (sodium salt) obtained by sulfonating in the amino group of 1-methoxy-4-ethoxy-2-amino-5-nitrobenzene are reduced as described in Example 1. Acrylated in aqueous soda solution with 148 kgs. of benzoylchloride, and the sulfonic group is saponified with hydrochloric acid. By redissolving the hydrochloric acid salt with hot water and precipitating with sodium carbonate, the free base is obtained. White needles of the melting point 117° C. Calculated on the nitroarylsulfamic acid used the yield is 80%.

Example 4.—4-benzoylaminobenzene-1-methoxybenzene

320 kgs. of the sodium salt of 4-nitronaphthalene-1-sulfamic acid (produced by sulfonating in the amino group of 4-nitro-1-naphthylamine) are reduced as described in Example 1. Acrylated in aqueous soda solution with 148 kgs. of benzoylchloride, and the sulfonic group is split off by means of hydrochloric acid salt with hot water and precipitated with sodium carbonate, the free base is obtained. White needles of the melting point 183° C. Calculated on the sodium salt of the 4-nitronaphthalene-1-sulfamic acid employed the yield is 76%.

Example 5.—2-amino-5-naphthylaminobenzene-1-methoxybenzene

270 kgs. of 1-methoxy-5-nitrobenzene-2-sulfamic acid (sodium salt), (produced by sulfonating in the amino group of 1-methoxy-2-amino-5-nitrobenzene), are reduced as described in Example 1 and acrylated in aqueous soda solution by means of 200 kgs. of benzoylchloride. The sulfonic group is split off with hydrochloric acid. By redissolving the hydrochloric acid salt with hot water and precipitating with sodium carbonate, the free base is obtained. White prisms, melting point 191-192° C. Calculated on the sodium salt of 1-methoxy-5-nitrobenzene-2-sulfamic acid used the yield is 55%.

We claim:

1. The process which comprises sulfonating

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Novel Inhibitors of Potassium Ion Channels on Human T Lymphocytes

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The in vitro biological characterization of a series of 4-(alkylamino)-1,4-dihydroquinolines is reported. These compounds are novel inhibitors of voltage-activated n-type potassium ion (K⁺) channels in human T lymphocytes. This series, identified from random screening, was found to inhibit [¹²⁵I]charybdotoxin binding to n-type K⁺ channels with IC₅₀ values ranging from 10⁻⁶ to 10⁻⁸ M. These analogs also inhibit whole cell n-type K⁺ currents with IC₅₀ values from 10⁻⁵ to 10⁻⁷ M. The preparation of a series of new 4-(alkylamino)-1,4-dihydroquinolines is described. Structure-activity relationships are discussed. Naphthyl analog 7c, the best compound prepared, exhibited >100-fold selectivity for inhibition of [¹²⁵I]charybdotoxin binding to n-type K⁺ channels compared with inhibition of [³H]dofetilide binding to cardiac K⁺ channels. These compounds represent a potent and selective series of n-type K⁺ channel inhibitors that have the potential for further development as anti-inflammatory agents.

Introduction

The most prominent and best characterized ion channel in human T lymphocytes is the voltage-activated n-type potassium ion (K⁺) channel.^{1,2} The channel is selective for K⁺, activates at -40 mV, shows cumulative inactivation, and is blocked by both peptide and non-peptide antagonists.^{1,3,4} Charybdotoxin (ChTX), a 37-amino acid peptide purified from the venom of the scorpion *Leiurus quinquestriatus*,⁵ is a potent antagonist of n-type K⁺ channels. ChTX is active at low nanomolar concentrations^{3,4,6} and, when radiolabeled, binds to human T lymphocytes with characteristics indicative of binding to n-type K⁺ channels.⁷ Nonpeptide antagonists are significantly less potent (20 μM < IC₅₀'s < 10 mM) and include the nonselective K⁺ blockers quinine, 4-aminopyridine, and tetraethylammonium and the Ca²⁺ channel antagonists verapamil and nifedipine.^{1,6}

Numerous studies using both peptide and nonpeptide antagonists have indicated that n-type K⁺ channels are specifically involved in T lymphocyte proliferation. For example, antagonists of this K⁺ conductance inhibit both mitogen- and antigen-stimulated proliferation with similar rank order of potency.^{1,6,8-10} Since the production of activated T lymphocytes is essential for initiating and supporting the immune response, T lymphocyte K⁺ channels represent a novel therapeutic target for the discovery of anti-inflammatory agents. However, the development of T lymphocyte K⁺ channel inhibitors as anti-inflammatory agents will require the discovery of potent and selective nonpeptide antagonists. Reference nonpeptide antagonists of n-type K⁺ channels, such as classical K⁺ and Ca²⁺ channel blockers, are neither potent nor selective.

In order to identify more potent and selective inhibitors of n-type K⁺ channels, high throughput screening of our compound files using [¹²⁵I]ChTX binding to T lymphocytes was performed. These efforts have resulted in the identification of a novel series of n-type K⁺ channel antagonists, the 4-(alkylimino)-1,4-dihydroquinolines. The effects of compounds on both [¹²⁵I]-ChTX binding and n-type K⁺ currents (I_{Kn}) as measures of n-type K⁺ channel inhibition were examined. In addition, the effects of compounds on [³H]dofetilide binding to cardiac myocytes as a measure of cardiac K⁺ channel (I_{Kr}) inhibition and as a determinant of the selectivity of the (alkylimino)-1,4-dihydroquinolines for the lymphocyte versus cardiac K⁺ channels were examined.^{11,12} This paper describes the structure-activity relationships (SAR) for this series of analogs and the combination of classical medicinal and computational techniques used for new inhibitor design.

Chemistry

The synthesis of (alkylimino)-1,4-dihydroquinolines is illustrated in Scheme 1. The key synthetic precursors, 4-chloroquinolines 12, were prepared from commercially available anilines according to the well-established route of Price and Roberts.¹³ Conversion into the hexyl ether analog 13 was accomplished by treatment of 4-chloroquinoline with hexanol in the presence of base and phase transfer catalyst tris[2-(2-methoxyethoxy)ethyl]amine, TDA-1. The alkylamine group was installed using a modified procedure of the Surrey method,¹⁴ whereby the 4-chloroquinoline derivative 12 is heated with an excess of alkylamine to furnish 4-(alkylamino)quinoline 14. Alkylation on the quinoline nitrogen was performed by treatment of the 4-substituted quinolines 13 and 14 with the appropriate alkyl halide or benzylic halide to afford target molecules 6-10.

The synthesis of 4-benzyl-1-(hexyloxy)naphthalene (11) is illustrated in Scheme 2. Commercially available 1-naphthol was first O-alkylated and subsequently acylated through the action of benzoyl chloride/AlCl₃ to furnish intermediate 15. Mild reduction with sodium

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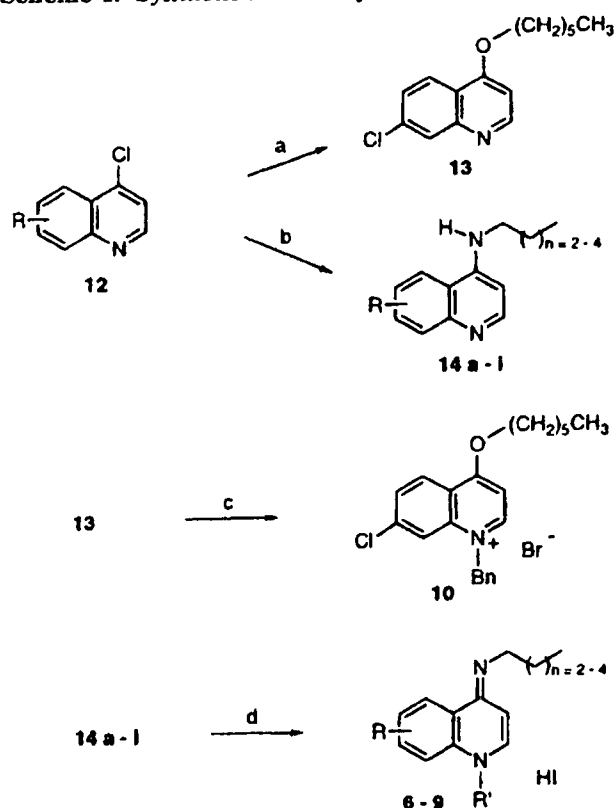
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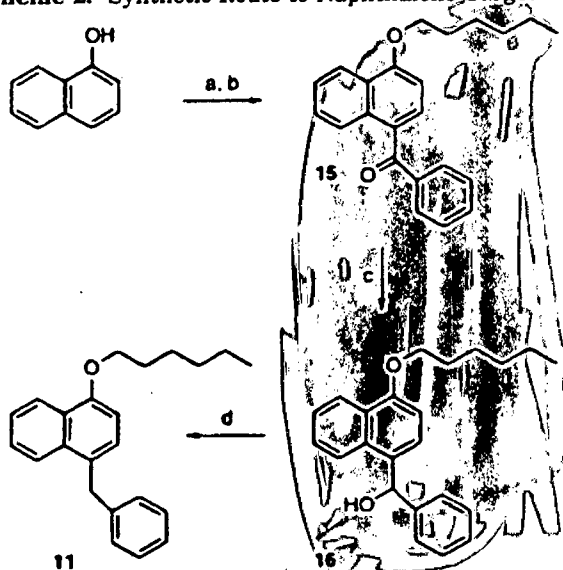
[¶] Department of Biochemistry.

^{||} All research prior to manuscript submission was carried out at the Sterling Winthrop Pharmaceuticals Research Division.

[¶] Abstract published in *Advance ACS Abstracts*, May 1, 1995.

Scheme 1. Synthetic Route to Quinoline Derivatives^a

^a Reagents: (a) hexanol, TDA-1, KOH, K₂CO₃; (b) alkylamine, Δ; (c) BnBr, Δ; (d) RX.

Scheme 2. Synthetic Route to Naphthalene Target 11^a

^a Reagents: (a) 1-iodohexane, K₂CO₃, Δ; (b) BnCOCl, AlCl₃; (c) 10% NaBH₄/Al₂O₃, 0 °C; (d) NaBH₄, CF₃CO₂H.

borohydride on alumina provided the secondary alcohol 16, which was further reduced with sodium borohydride in the presence of trifluoroacetic acid to target molecule 11.

Results and Discussion

High throughput screening of our chemical files disclosed the activity of compound 1f, 1-Benzyl-7-chloro-4-(*n*-hexylimino)-1,4-dihydroquinoline hydrochloride, as one of the most potent and selective compounds from

Table 1. Affinities for [¹²⁵I]ChTX, *n*-Type K⁺ Current (IKn), and [³H]Dofetilide

compd	R	IC ₅₀ (nM)		
		ChTX ^a	IKn ^b	dofetilide ^a
1a	H	>8000		
1b	Me	>5000		^c
1c	Pr	544	3160	
1d	<i>n</i> -Bu	198	2620	^c
1e	<i>n</i> -pentyl	65	250	1300
1f	<i>n</i> -hexyl	32	335	820
1g	<i>n</i> -heptyl	117	249	2500
1h	<i>n</i> -octyl	280	351	2400
1i	<i>i</i> -Bu	206		2000
1j	1,5-dimethylhexyl	817	1676	175
1k	1-adamantyl	1170		157
2a	<i>n</i> -hexyl	190	216	780
2b	(CH ₂) ₂ CO ₂ H	>10 000		
2c	(CH ₂) ₃ NHCH ₃	>30 000		
2d	(CH ₂) ₃ N(CH ₃) ₃	>30 000	>10 000	
2e	(CH ₂) ₂ N(Et) ₂	7680		57
3a	2,4-dichlorophenyl	>30 000	>10 000	>30 000
3b	2,4-dichlorobenzyl	550	259	2380
4	<i>n</i> -hexyl	100		3230
5	<i>n</i> -hexyl	310	231	>350 ^a

^a Mean IC₅₀, *n* = 2 determinations. ^b IC₅₀ from *n* > 9 data points. ^c 24% at 400 nM.

this series.¹⁵ Analog searching for other 4-(alkylimino)-1,4-dihydroquinolines resulted in the identification of a small number of analogs. Activity for these analogs is particularly sensitive to changes at the 4-imino position. The data in Table 1 illustrate that lengthening the alkyl chain results in an increase in primary assay activity from >10 μM for 1a (no substitution) to 0.032 μM for 1f ((CH₂)₅CH₃). The isobutyl derivative 1i is equivalent to the butyl derivative, whereas bulky alkyl derivatives such as 1j,k are much less potent. Other variations on the imino nitrogen provided further SAR at this position. The incorporation of an acidic group, 2b, or permanently charged amine, 2d, into the alkyl chain abolishes all primary assay activity. Similarly, the direct attachment of a phenyl group (3a) to this position exhibited a 60-fold drop in activity compared to a similarly substituted benzyl analog (3b). Thus, in general, potency increases with alkyl chain length to an optimum length of six and then decreases with added length, and an increase in bulk of the alkyl chain or the incorporation of hydrophilic substituents was not well tolerated.

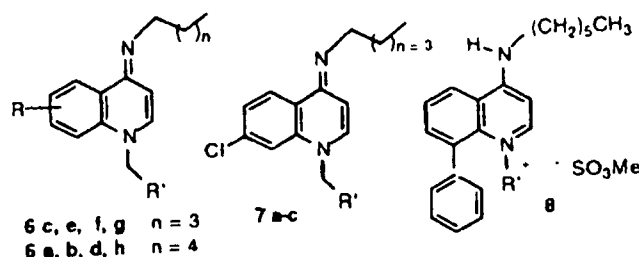
Additional data, including secondary electrophysiological assay (IKn) and cardiac K⁺ selectivity [³H]-dofetilide binding, were generated for selected examples from the existing quinolines in our library. The data for IKn was found to correlate closely with the ChTX activity trend, albeit in slightly weaker activity. The [³H]dofetilide binding data illustrate a lack of response toward alkyl chain lengthening, analogs 1e–h, whereas branching of the alkyl chain, analogs 1j,k, resulted in compounds that were >10-fold more potent. Additionally, the incorporation of a permanently charged amine (2e) was found to be favorable for [³H]dofetilide binding. Substitution with chlorine in either the 4- or 3,4-positions, analogs 4 and 5, respectively, was found to greatly diminish dofetilide activity without affecting ChTX binding activity.

Overall, the ChTX and IKn data for this set of analogs were consistent with a region around N-4 binding to a size-restricted hydrophobic pocket. However, the [³H]-dofetilide binding data for this same region were found to be consistent with a bulky hydrophilic pocket. The data from this series show that there are different SAR at the lymphocyte and cardiac K⁺ channels. As a result of these two opposing SAR trends, only a modest selectivity favoring lymphocyte K⁺ channels versus cardiac K⁺ channels was evident. The *n*-pentyl analog 1e exhibited the most favorable activity profile from the historical series, wherein a 20-fold difference in activity was found.

One of the primary goals of this study was to identify additional SAR that would more strongly support a structure-based mechanism. Our approach was based upon developing pharmacophoric hypotheses for the two separate K⁺ channels. This approach identified unexplored conformational space and areas where a divergent SAR between the two channels would exist. The substitutional areas around the quinoline ring positions 6–8 and the pendant benzyl group were initially identified as areas for further investigation. A small number of substituents (OMe, CF₃, and F) at positions 6–8 were investigated, wherein the N-1 and N-4 groups were identical to those for the pre-existing quinolines in Table 1. Analog 6b–h were found to possess equivalent or decreased binding activity for ChTX and generally poorer selectivity against dofetilide binding (with the exception of 6h) when compared to the 7-chloro analogs 1e,f or 4 (Table 2). Conformational restriction of the benzyl group into a 1-indanyl analog, 7a, greatly reduced the ChTX binding with no effect on selectivity. Enlargement of the benzyl aromatic into the 1- and 2-naphthylmethyl analogs 7b,c resulted in a slight loss of ChTX activity for the 1-naphthylmethyl analog and little change on the ChTX binding for the 2-naphthylmethyl analog. However, the 2-positional isomer 7c was found to be vastly weaker in dofetilide binding. 7c represents >100-fold selectivity for the lymphocyte K⁺ channel over the cardiac K⁺ channel.

In an effort to provide further information toward the development of a pharmacophoric hypothesis, analogs were prepared to test the dependence of activity on physical properties. Butylamine analog 9 when compared to hexylamine analog 1f illustrates that changing structure while holding physical properties essentially constant resulted in a noticeable loss in activity (Table

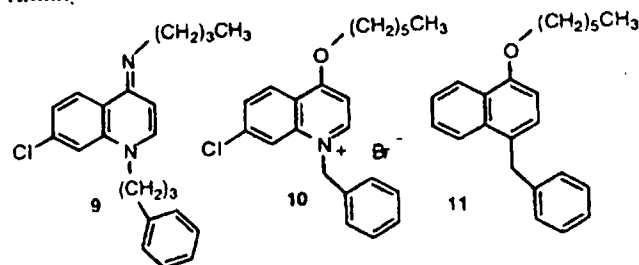
Table 2. Affinities for [¹²⁵I]ChTX, n-Type K⁺ Current (IKn), and [³H]Dofetilide



compd	R	R'	IC ₅₀ (nM)		
			ChTX ^a	IKn ^a	dofetilide ^a
6a	H	Ph	164	1053	
6b	6-MeO	Ph	47	267	680
6c	6-MeO	<i>p</i> -ClPh	275		1990
6d	8-MeO	Ph	475		210
6e	6-CF ₃	<i>p</i> -ClPh	340	213	140
6f	7-CF ₃	<i>p</i> -ClPh	239		90
6g	8-F	<i>p</i> -ClPh	227		310
6h	7-Cl	<i>p</i> -MeOPh	88	329	
7a	7-Cl	1-indanyl	2300		870
7b	7-Cl	1-naphthyl	280	279	900
7c	7-Cl	2-naphthyl	75	434	8000
8		Me	240	602	223

^a Mean IC₅₀, minimum of two determinations. ^b IC₅₀ from *n* > 9 data points.

Table 3. Comparative Physical Properties and [¹²⁵I]ChTX Affinity



compd	log <i>P</i> ^a	p <i>K</i> _a	ChTX IC ₅₀ (nM) ^b
1f	3.81	11.52	32
5	4.64	11.53	310
7b	4.01	11.43	280
7c	4.16	11.57	75
8a	3.89	9.58	930
8b	3.73		240
9	3.82	11.37	471
10	3.62	11.17	828
11			>5000

^a HPLC method of Kraak et al.¹⁶ ^b Mean IC₅₀, minimum of two determinations.

to hexyl ether analog 10 resulted in a noticeable loss in ChTX activity while holding physical properties essentially constant. The further modification of analog 9 physical properties, to the naphthalene ether analog 11, resulted in a profound loss of activity in the primary assay. The dramatic loss in activity for analog 11 could be a result from the gross change in a physical parameter.

Molecular Modeling

Excluded volume maps¹⁷ were generated for both the lymphocyte and cardiac channels to test the hypothesis that selectivity is based on a volume (shape) pharmacophoric model. The ChTX and dofetilide binding data were used to generate these maps. Strongly bound



Figure 1. Volume exclusion maps for the lymphocyte (left map) and cardiac channels: green regions, strong binding; red, weak binding; and blue, common volume.

compounds ($IC_{50} < 150$ nM) of the lymphocyte channel or the cardiac channel were designated as the active set for each respective channel. Weakly bound compounds ($IC_{50} > 1000$ nM) were designated as the inactive set for each respective channel. The active and inactive volume maps for both the cardiac and lymphocyte channels were created by generating the minimum volume occupied by a low-energy conformation for each of the members of the respective sets. The final volume exclusion maps for the lymphocyte and cardiac channels were generated by superimposing the active and inactive volume maps from their respective channels (Figure 1). The green and red areas represent strong and weak binding regions; the blue areas represent the regions common to both.

The utility of the volume exclusion maps is represented by the following examples. Binding data for the 6-methoxy analogs **6b,c** exhibited a 14- and 7-fold selectivity for the lymphocyte channel, respectively. The 6-position area is green in the lymphocyte volume exclusion map and red in the cardiac volume exclusion map corresponding to a ChTX selective region. For these analogs, the volume exclusion maps illustrate where a divergent SAR exists between the two channels. Analog **11** is an example of a compound that was prepared to test these maps. All low-energy conformations of analog **11** were found to project into the weak binding (red) areas of both the lymphocyte and cardiac volume exclusion maps. Binding data for **11** showed it to be inactive (> 5000 μ M) in both the lymphocyte and cardiac assays, in agreement with the volume exclusion map prediction. The volume exclusion maps have followed us to visualize where unexplored volume exists and have generated a three-dimensional picture of where the differences in binding occur for lymphocyte and cardiac channels.

Conclusions

A series of 4-(alkylimino)-1,4-dihydroquinolines were identified as inhibitors of [^{125} I]charybdotoxin binding to n-type K^+ channels in human T lymphocytes with IC_{50} values ranging from 10^{-6} to 10^{-8} M and to inhibit whole cell n-type K^+ currents with IC_{50} values from 10^{-6} to 10^{-7} M. These compounds were also evaluated for binding to cardiac K^+ channels via [^{3} H]dofetilide binding assay. A series of new 4-(alkylimino)-1,4-dihydroquinolines was prepared and evaluated for their ability to inhibit binding to both lymphocyte and cardiac channels. Naphthyl analog **7c**, the best compound prepared, exhibited > 100 -fold selectivity for inhibition of [^{125} I]charybdotoxin binding to n-type K^+ channels compared with inhibition of [^{3} H]dofetilide binding to cardiac K^+ channels. Additional studies will determine for this series, if the [^{3} H]dofetilide binding assay relates into a functional selectivity versus cardiac channels. These 4-(alkylimino)-1,4-dihydroquinolines represent a potent and selective series of n-type channel inhibitors that have potential for further development as anti-inflammatory agents.

Experimental Section

General Procedures. All solvents were HPLC grade and used as received with the exception of THF which was freshly distilled from sodium benzophenone ketyl. Melting points were determined using a Thomas Hoover apparatus and uncorrected. Infrared spectra were run as 1% KBr pellets on a Nicolet 20-SX spectrometer or obtained by Onieda Research Services, Inc., Oneida, NY. Mass spectra were recorded as follows: electron impact spectra were obtained on a Hewlett-Packard 5971 GC/MS at 70 eV; desorption chemical ionization spectra were obtained on a Nermag R-10-10C spectrometer; fast atom bombardment spectra were obtained on a Kratos Profile HV-2 spectrometer; high-resolution spectra were obtained by M-Scan, Inc., West Chester, PA. ^{1} H and ^{13} C NMR spectra were recorded on a Varian Unity 300 or General Electric QE 300 spectrometer. Chemical shifts are reported

in parts per million (δ) relative to tetramethylsilane. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ.

Iminoquinolines 1–6a. These compounds were previously prepared as described by Surrey.¹⁴

7-Chloro-4-(*n*-hexyloxy)quinoline (13). 4,7-Dichloroquinoline (10.0 g, 0.050 mol), K₂CO₃ (6.97 g, 0.050 mol), KOH (11.3 g, 0.20 mol), TDA-1 (1.63, 0.005 mol), and hexanol (7.73 g, 0.075 mol) were combined in a round bottom flask equipped with condenser under N₂ and heated to reflux for 55 h. The mixture was cooled, diluted with EtOAc (300 mL), washed with 0.1 M HCl (3 × 200 mL), dried (Na₂SO₄), and concentrated in vacuo. The resulting orange solid was recrystallized with ether/EtOAc to give 13 (6.76 g, 51%); mp 71–73 °C; ¹H NMR (DMSO) δ 8.74 (d, *J* = 5.2 Hz, 1H), 8.13 (d, *J* = 8.8 Hz, 1H), 7.97 (d, *J* = 2.1 Hz, 1H), 7.58 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.04 (d, *J* = 5.4 Hz, 1H), 4.23 (t, *J* = 6.4 Hz, 2H), 1.89–1.80 (m, 2H), 1.51–1.44 (m, 2H), 1.37–1.28 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 264 (MH⁺). Anal. (C₁₅H₁₈ClNO) C, H, N.

General Method A: Preparation of 4-Alkylquinolines 14a–j. A mixture of amine (1.1 equiv) and 4-chloroquinoline 12 (1.0 equiv) was refluxed for 18 h in ethanol (0.5 M). The solution was cooled to room temperature, and the solvents were removed in vacuo. The oily residue was diluted with NaOH (1 N) and extracted with EtOAc (3X). The combined organics were washed with brine, dried over K₂CO₃, and evaporated. Purification was carried out using silica gel and eluting the compounds with EtOAc/Hex.

4-(*n*-Hexylamino)quinoline (14a). Compound 14a was synthesized according to the method of Surrey,¹⁴ and the ¹H NMR and MS spectra were in agreement with its chemical structure.

6-Methoxy-4-(*n*-pentylamino)quinoline (14b). Compound 14b was synthesized according to general method A: mp 143–146 °C; ¹H NMR (CDCl₃) δ 8.45 (d, *J* = 5.3 Hz, 1H), 7.92 (d, *J* = 9.2 Hz, 1H), 7.30 (dd, *J* = 9.3, 2.8 Hz, 1H), 7.00 (d, *J* = 2.6 Hz, 1H), 6.43 (d, *J* = 5.4 Hz, 1H), 4.93 (bs, 1H), 3.92 (s, 3H), 3.56–3.29 (m, 2H), 1.84–1.74 (m, 2H), 1.49–1.38 (m, 4H), 0.95 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 245 (MH⁺). Anal. (C₁₅H₂₀N₂O) C, H, N.

6-Methoxy-4-(*n*-hexylamino)quinoline (14c). Compound 14c was synthesized according to general method A: mp 126–128 °C; ¹H NMR (CDCl₃) δ 8.46 (d, *J* = 5.4 Hz, 1H), 7.92 (s, 1H), 7.32 (d, *J* = 2.6 Hz, 1H), 6.42 (d, *J* = 5.3 Hz, 1H), 4.8 (bs, 1H), 3.9 (s, 3H), 3.35 (d of t, *J* = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (EI) *m/e* 258 (M⁺). Anal. (C₁₆H₂₂N₂O) C, H, N.

8-Methoxy-4-(*n*-hexylamino)quinoline (14d). Compound 14d was synthesized according to general method A: mp 146–148 °C; ¹H NMR (CDCl₃) δ 8.58 (d, *J* = 5.3 Hz, 1H), 7.35 (d of d, *J* = 7.2, 7.8 Hz, 1H), 7.26 (d, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 7.2 Hz, 1H), 6.46 (d, *J* = 5.3 Hz, 1H), 4.9 (bs, 1H), 4.06 (s, 3H), 3.35 (d of t, *J* = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (EI) *m/e* 258 (M⁺); HRMS (LSIMS) C₁₆H₂₂N₂O (MH⁺) requires 259.18104, found 259.18090; Δ = 0.55 ppm. Anal. (C₁₆H₂₂N₂O·4H₂O) C, H, N.

6-(Trifluoromethyl)-4-(*n*-pentylamino)quinoline (14e). Compound 14e was synthesized according to general method A: ¹H NMR (DMSO) δ 8.75 (s, 1H), 8.49 (d, *J* = 6.5 Hz, 1H), 8.92 (d, *J* = 8.5 Hz, 1H), 7.82 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 6.56 (d, *J* = 6.5 Hz, 2H), 3.39 (m, 2H), 1.78–1.61 (m, 2H), 1.48–1.29 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H); MS (EI) *m/e* 283.

7-(Trifluoromethyl)-4-(*n*-pentylamino)quinoline (14f). Compound 14f was synthesized according to general method A: ¹H NMR (DMSO) δ 8.50–8.47 (m, 2H), 8.06 (s, 1H), 7.65 (dd, *J* = 8.7, 1.7 Hz, 1H), 7.45 (bs, 1H), 6.54 (d, *J* = 5.5 Hz, 2H), 3.26 (q, *J* = 6.5 Hz, 1H), 1.68–1.61 (m, 2H), 1.40–1.29 (m, 4H), 0.87 (t, *J* = 7.1 Hz, 3H); MS (EI) *m/e* 283.

7-Chloro-4-(*n*-pentylamino)quinoline (14g). Compound 14g was synthesized according to general method A: mp 119–121 °C; ¹H NMR (CDCl₃) δ 8.53 (d, *J* = 5.4 Hz, 1H), 7.96 (s, 1H), 7.64 (d, *J* = 8.9 Hz, 1H), 7.35 (d, *J* = 8.9 Hz, 1H), 6.41 (d, *J* = 5.3 Hz, 1H), 5.0 (bs, 1H), 3.25 (m, 2H), 1.76 (m, 2H), 1.43

(m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 249 (MH⁺). Anal. (C₁₄H₁₇N₂Cl) C, H, N.

8-Fluoro-4-(*n*-pentylamino)quinoline (14h). Compound 14h was synthesized according to general method A: mp 103–105 °C; ¹H NMR (CDCl₃) δ 8.63 (d, *J* = 5.4 Hz, 1H), 7.52 (m, 1H), 7.31 (m, 2H), 6.48 (d, *J* = 5.3 Hz, 1H), 5.0 (bs, 1H), 3.25 (m, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 233 (MH⁺). Anal. (C₁₄H₁₇N₂F) C, H, N.

8-Phenyl-4-(*n*-hexylamino)quinoline Hydrochloride (14i). Compound 14i was synthesized according to general method A: mp 189–191 °C; ¹H NMR (DMSO) δ 9.81 (s, 1H), 8.75 (d, *J* = 5.4 Hz, 1H), 8.24 (d, *J* = 7.2 Hz, 1H), 7.77 (d of t, *J* = 6.1, 7.4 Hz, 2H), 7.5 (m, 5H), 6.87 (d, *J* = 7.2 Hz, 1H), 3.55 (d of t, *J* = 6.6 Hz, 2H), 1.77 (m, 2H), 1.41 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (EI) *m/e* 303 (M⁺). Anal. (C₂₁H₂₄N₂HCl) C, H, N.

7-Chloro-4-(*n*-butylamino)quinoline (14j). Compound 14j was synthesized according to general method A: mp 130–135 °C; ¹H NMR (DMSO) δ 8.37 (d, *J* = 5.4 Hz, 1H), 8.26 (d, *J* = 9.0 Hz, 1H), 7.76 (dd, *J* = 2.2 Hz, 1H), 7.43 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.28 (bs, 1H), 6.44 (d, *J* = 5.5 Hz, 1H), 3.24 (q, *J* = 7.0 Hz, 2H), 1.68–1.58 (m, 2H), 1.43–1.36 (m, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); MS (CI, CH₄) *m/e* 235 (MH⁺); HRMS (LSIMS) C₁₃H₁₆N₂Cl (MH⁺) requires 235.10020, found 235.10047; Δ = 1.16 ppm.

General Method B: Preparation of 4-(*n*-Hexylimino)-1,4-dihydroquinolines 6–9. A mixture of benzyl halide (1.1 equiv), 4-chloroquinoline 14 (1.0 equiv), and sodium iodide (1.2 equiv) was refluxed for 18 h in acetone (0.5 M). The solution was cooled to room temperature, and the resulting hydroiodide precipitate was collected and washed repeatedly with acetone. The hydroiodide salt was dissolved in hot ethanol (60 mL) and treated with NaOH (35%). The mixture was cooled and the ethanol removed in vacuo. The residue was diluted with H₂O and extracted with EtOAc (3X). The combined organics were washed with brine, dried over K₂CO₃, and evaporated. Purification was carried out by diluting the oil with various ethanolic acids and cooling the solution overnight. The precipitates were collected and washed repeatedly with ethanol.

1-Benzyl-4-(*n*-hexylimino)-1,4-dihydroquinoline (6a). Compound 6a was synthesized according to the method of Surrey,¹⁴ and the ¹H NMR and MS spectra were in agreement with its chemical structure.

1-Benzyl-6-methoxy-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydroiodide (6b). Compound 6b was synthesized according to general method B: mp 212–215 °C; ¹H NMR (CDCl₃) δ 8.33 (s, 1H), 8.06 (d, *J* = 7.4 Hz, 1H), 7.92 (s, 1H), 7.51 (d, *J* = 9.5 Hz, 1H), 7.31 (m, 4H), 7.11 (m, 2H), 6.48 (d, *J* = 7.5 Hz, 1H), 5.60 (s, 2H), 4.17 (s, 3H), 3.35 (d of t, *J* = 7.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 349 (MH⁺). Anal. (C₂₃H₂₈N₂O·HI) C, H, N.

1-(*p*-Chlorobenzyl)-6-methoxy-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (6c). Compound 6c was synthesized according to general method B: mp 164–167 °C; ¹H NMR (CDCl₃) δ 8.58 (d, *J* = 2.6 Hz, 1H), 8.40 (d, *J* = 7.5 Hz, 1H), 7.44 (d, *J* = 9.7 Hz, 1H), 7.26–7.20 (m, 3H), 7.06 (d, *J* = 8.5 Hz, 2H), 6.42 (d, *J* = 7.4 Hz, 1H), 5.73 (s, 2H), 4.08 (s, 3H), 3.56–3.49 (m, 2H), 1.80–1.76 (m, 2H), 1.39–1.34 (m, 4H), 0.86 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 369 (MH⁺); HRMS (LSIMS) C₂₂H₂₅N₂OCl (MH⁺) requires 369.17337, found 369.17373; Δ = 0.99 ppm. Anal. (C₂₂H₂₅N₂OCl·HCl·1/4H₂O) C, H, N.

1-Benzyl-8-methoxy-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydrochloride (6d). Compound 6d was synthesized according to general method B: mp 192–194 °C; ¹H NMR (CDCl₃) δ 8.73 (s, 1H), 7.82 (d, *J* = 7.7 Hz, 1H), 7.51 (t, *J* = 9.5 Hz, 1H), 7.31 (m, 4H), 7.16 (d, *J* = 8.2 Hz, 1H), 7.01 (d, *J* = 7.5 Hz, 2H), 6.43 (d, *J* = 7.7 Hz, 1H), 5.81 (s, 2H), 3.73 (s, 3H), 3.55 (d of t, *J* = 7.4 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 349 (MH⁺); HRMS (LSIMS) C₂₃H₂₈N₂O (MH⁺) requires 349.22799, found 349.22803; Δ = 0.13 ppm.

1-(*p*-Chlorobenzyl)-6-(trifluoromethyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydroiodide (6e). Compound

6e was synthesized according to general method B: mp 230–231 °C; ¹H NMR (DMSO) δ 9.82 (bs, 1H), 9.09 (s, 1H), 8.89 (d, *J* = 7.5 Hz, 1H), 8.25 (d, *J* = 8.6 Hz, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 7.45 (d, *J* = 8.4, 2H), 7.30 (d, *J* = 8.5, 2H), 7.16 (d, *J* = 7.5, 1H), 5.89 (s, 2H), 3.68–3.55 (m, 2H), 1.81–1.65 (m, 2H), 1.49–1.29 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 407 (MH⁺). Anal. (C₂₂H₂₂N₂F₃Cl₃·HI) C, H, N.

1-(*p*-Chlorobenzyl)-7-(trifluoromethyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydroiodide (6f). Compound 6f was synthesized according to general method B: mp 235–240 °C; ¹H NMR (DMSO) δ 9.76 (bs, 1H), 8.92 (d, *J* = 7.5 Hz, 1H), 8.83 (d, *J* = 8.8 Hz, 1H), 8.28 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.18 (d, *J* = 7.6 Hz, 1H), 5.98 (s, 2H), 3.60 (bs, 2H), 1.80–1.65 (m, 2H), 1.49–1.25 (m, 4H), 0.89 (t, *J* = 7.0 Hz, 3H); MS (FAB, NBA) *m/e* 407 (MH⁺). Anal. (C₂₂H₂₂N₂F₃Cl₃·HI) C, H, N.

1-(*p*-Chlorobenzyl)-8-fluoro-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (6g). Compound 6g was synthesized according to general method B: mp 221–223 °C; ¹H NMR (DMSO) δ 8.76 (d, *J* = 7.5 Hz, 1H), 8.66 (d, *J* = 8.2 Hz, 1H), 7.81 (m, 1H), 7.70 (m, 1H), 7.38 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.08 (d, *J* = 7.4 Hz, 1H), 5.86 (bs, 2H), 3.65 (d of t, *J* = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 357 (MH⁺). Anal. (C₂₁H₂₂N₂FCI·HCl) C, H, N.

7-Chloro-1-(*p*-methoxybenzyl)-4-(*n*-hexylimino)-1,4-dihydroquinoline Hydrochloride (6h). Compound 6h was synthesized according to general method B: mp 239–241 °C; ¹H NMR (CDCl₃) δ 9.43 (d, *J* = 9.2 Hz, 1H), 8.15 (d, *J* = 7.4 Hz, 1H), 7.63 (s, 1H), 7.46 (d, *J* = 9.0 Hz, 1H), 7.15 (d, *J* = 8.7 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 6.44 (d, *J* = 7.6 Hz, 1H), 5.54 (s, 2H), 3.79 (s, 3H), 3.53 (d of t, *J* = 6.2 Hz, 2H), 1.77 (m, 2H), 1.48 (m, 2H), 1.36 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 383 (MH⁺). Anal. (C₂₃H₂₇N₂ClO·HCl·0.5H₂O) C, H, N.

7-Chloro-1-indanyl-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (7a). Compound 7a was synthesized according to general method B: mp 79–80 °C; ¹H NMR (CDCl₃) δ 8.47 (d, *J* = 8.4 Hz, 1H), 7.38–7.21 (m, 4H), 7.20–7.13 (m, 2H), 6.58 (d, *J* = 7.5 Hz, 1H), 5.86 (d, *J* = 8.4 Hz, 1H), 5.79 (t, *J* = 6.6 Hz, 1H), 3.27 (t, *J* = 7.4 Hz, 2H), 3.15–2.96 (m, 2H), 2.75–2.66 (m, 1H), 2.12–2.08 (m, 1H), 1.75–1.65 (m, 2H), 1.45–1.31 (m, 4H), 0.91 (t, *J* = 6.9 Hz, 3H); MS (CI, CH₄) *m/e* 365 (MH⁺). Anal. (C₂₃H₂₅N₂Cl·HCl) C, H, N.

7-Chloro-1-(1-methylnaphthyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrochloride (7b). Compound 7b was synthesized according to general method B: mp 298–300 °C; ¹H NMR (DMSO) δ 8.91 (d, *J* = 7.5 Hz, 1H), 8.61 (d, *J* = 8.2 Hz, 1H), 8.15 (d, *J* = 7.5 Hz, 1H), 8.07 (s, 1H), 8.01 (d, *J* = 8.2 Hz, 1H), 7.91 (d, *J* = 8.3 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.65 (m, 2H), 7.38 (m, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.72 (d, *J* = 7.4 Hz, 1H), 6.35 (bs, 2H), 3.55 (d of t, *J* = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 389 (MH⁺). Anal. (C₂₅H₂₅N₂Cl·HCl) C, H, N.

7-Chloro-1-(2-methylnaphthyl)-4-(*n*-pentylimino)-1,4-dihydroquinoline Hydrobromide (7c). Compound 7c was synthesized according to general method B: mp 277–279 °C; ¹H NMR (DMSO) δ 8.88 (d, *J* = 7.5 Hz, 1H), 8.65 (d, *J* = 9.1 Hz, 1H), 8.15 (s, 1H), 7.77–7.97 (m, 5H), 7.51 (m, 2H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.10 (d, *J* = 87.6 Hz, 1H), 6.05 (bs, 2H), 3.55 (d of t, *J* = 7.7 Hz, 2H), 1.76 (m, 2H), 1.43 (m, 4H), 0.95 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 389 (MH⁺). Anal. (C₂₅H₂₅N₂Cl·HBr) C, H, N.

4-(*n*-Hexylimino)-8-phenyl-1-methyl-1,4-dihydroquinoline Methanesulfonate (8). Compound 8 was synthesized according to general method B: mp 239–240 °C; ¹H NMR (CDCl₃) δ 9.60 (m, 1H), 8.80 (m, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 7.64 (d, *J* = 5.1 Hz, 2H), 7.43 (m, 3H), 7.24 (m, 3H), 6.60 (d, *J* = 7.4 Hz, 1H), 3.51 (d of t, *J* = 6.2 Hz, 2H), 2.84 (s, 3H), 2.04 (s, 3H), 1.77 (m, 2H), 1.43 (m, 2H), 1.28 (m, 4H), 0.85 (t, *J* = 7.2 Hz, 3H); MS (CI, CH₄) *m/e* 319 (MH⁺); HRMS (LSIMS) C₂₂H₂₇N₂ (MH⁺) requires 319.21742, found 319.21745; Δ = 0.07 ppm. Anal. (C₂₃H₃₀N₂O₃S·H₂O) C, H, N.

4-(*n*-Butylimino)-7-chloro-1-(3-phenylpropyl)-1,4-dihydroquinoline Hydrochloride (9). Compound 9 was synthesized according to general method B: mp 195–197 °C; ¹H

NMR (DMSO) δ 10.05 (bs, 1H), 8.89 (d, *J* = 9.2 Hz, 1H), 8.6 (d, *J* = 7.6 Hz, 1H), 8.16 (d, *J* = 1.9 Hz, 1H), 7.77 (dd, *J* = 9.1 Hz, 2H), 7.27–7.13 (m, 5H), 6.88 (d, *J* = 7.6 Hz, 1H), 4.5 (t, *J* = 7.5 Hz, 2H), 3.51 (q, *J* = 69 Hz, 2H), 2.70 (t, *J* = 7 Hz, 2H), 2.12–2.07 (m, 2H), 1.68–1.60 (m, 2H), 1.42–1.35 (t, 2H), 0.92 (t, *J* = 7.3 Hz, 3H); MS (CI, CH₄) *m/e* 353 (MH⁺); HRMS (LSIMS) C₂₂H₂₅ClN₂ (MH⁺) requires 353.17845, found 353.17710; Δ = -3.83 ppm.

1-Benzyl-7-chloro-4-(*n*-hexyloxy)-1,4-dihydroquinoline Bromide (10). Compound 10 was synthesized according to general method B: mp 136–138 °C; ¹H NMR (DMSO) 9.57 (d, *J* = 7.2 Hz, 1H), 8.48–8.45 (m, 2H), 7.96 (dd, *J* = 9.1 Hz, 1H), 7.80 (d, *J* = 7.2 Hz, 1H), 7.43–7.31 (m, 4H), 6.1 (s, 2H), 4.62 (t, *J* = 6.3 Hz, 2H), 1.98–1.90 (m, 2H), 1.55–1.4 (m, 2H), 1.40–1.30 (m, 4H), 0.89 (t, *J* = 7.0 Hz, 3H); MS (FAB, NBA) *m/e* 354 (MH⁺). Anal. (C₂₂H₂₅ClNO·Br) C, H, N.

4-Phenacyl-1-(hexyloxy)naphthalene (15). 1-Naphthol (10.0 g, 0.069 mol), 1-iodohexane (22.0 g, 0.104 mol), and K₂CO₃ were dissolved in DMF (70 mL) and heated to 60 °C under N₂ for 18 h. The reaction mixture was cooled to room temperature, diluted with Et₂O (500 mL), washed successive with H₂O (3 × 500 mL) and NaOH (1 N, 500 mL), dried (Na₂SO₄), and evaporated to dryness. The sample was taken up in hexane (250 mL), washed with H₂O (3 × 400 mL), dried (Na₂SO₄), and evaporated to dryness. The product was purified by flash filtration through silica gel, eluting with hexane to give 1-(hexyloxy)naphthalene as a yellow oil (11.3 g). The 1-(hexyloxy)naphthalene (1.0 g, 4.38 mmol) was then dissolved in nitrobenzene (5 mL) and added dropwise to a mixture of benzoyl chloride (0.616 g, 4.38 mmol) and AlCl₃ (0.584, 4.38 mmol), in nitrobenzene (5 mL), at 0 °C under N₂. After 18 h at room temperature, the nitrobenzene was removed by steam distillation and the residue was diluted with EtOAc (50 mL), washed with H₂O (50 mL), dried (Na₂SO₄), and concentrated in vacuo. The resulting yellow oil was purified by flash filtration on silica gel with hexane/EtOAc (9:1) as eluent, give 15 as a yellow oil (263 mg, 18%); ¹H NMR (CDCl₃) δ 8.36 (s, 2H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.58–7.44 (m, 4H), 7.55–7.43 (m, 2H), 6.76 (d, *J* = 8.0 Hz, 1H), 4.19 (t, *J* = 7 Hz, 2H), 1.97–1.93 (m, 2H), 1.69–1.52 (m, 2H), 1.40–1.37 (t, 4H), 0.94 (t, *J* = 7.0 Hz, 3H); MS (EI) *m/e* 332. Anal. (C₂₃H₂₄O₂) C, H.

4-(1-Hydroxy-1-phenylmethyl)-1-(hexyloxy)naphthalene (16). To a chilled (0 °C) methanol solution (30 mL) of naphthalene 15 (1.0 g, 3.0 mmol) was slowly added 10 NaBH₄/Al₂O₃ (3.4 g, 9.0 mmol) with stirring. The ice bath was removed and the mixture allowed to return to room temperature overnight. The mixture was filtered, dried (Na₂SO₄), and evaporated. The product was purified by filtration through silica gel with hexane/EtOAc (9:1) as eluent to give 16 (0.5 g, 72%) as a waxy yellow solid: mp 54–55 °C; ¹H NMR (CDCl₃) δ 8.37–8.32 (m, 1H), 8.06–8.00 (m, 1H), 7.49–7.40 (m, 5H), 7.36–7.25 (m, 3H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.47 (s, 1H), 4.1 (t, *J* = 6.4 Hz, 2H), 2.26 (bs, 1H), 1.98–1.89 (m, 2H), 1.63 (s, 2H), 1.45–1.38 (m, 4H), 0.93 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 334 (MH⁺). Anal. (C₂₃H₂₆O₂) C, H.

4-Benzyl-1-(hexyloxy)naphthalene (11). The naphthalene 16 (0.24 g, 0.70 mmol) dissolved in CF₃CO₂H (7 mL) was added dropwise, simultaneously with NaBH₄ (0.27 g, 7 mmol), to a vigorously stirred solution of CF₃CO₂H under N₂ at 0 °C. After complete addition, stirring was continued for 1 min. The CF₃CO₂H was evaporated and the resulting solution quenched with H₂O (25 mL) and saturated NaHCO₃ (25 mL). The mixture was extracted with EtOAc (3 × 20 mL); the combined extracts were washed with H₂O (50 mL), dried (Na₂SO₄), and concentrated in vacuo. The product was purified by filtration through silica gel with hexane followed by hexane/EtOAc (9:1) to give a yellow oil. Additional drying at 0.1 Torr gave 11 (0.75 g, 34%) as a waxy yellow solid: mp 66–68 °C; ¹H NMR (CDCl₃) δ 8.20–8.17 (m, 1H), 7.96–7.93 (m, 1H), 7.50–7.43 (m, 1H), 7.30–7.11 (m, 6H), 6.90 (d, *J* = 7.8 Hz, 1H), 4.32 (s, 2H), 4.12 (t, *J* = 6.5 Hz, 2H), 1.90–1.79 (m, 2H), 1.60–1.43 (m, 2H), 1.38–1.29 (m, 4H), 0.88 (t, *J* = 7.0 Hz, 3H); MS (CI, CH₄) *m/e* 319 (MH⁺); HRMS (LSIMS) C₂₃H₂₆O (M) requires 318.19837, found 318.19783; Δ = -1.70 ppm.

[¹²⁵I]Charybdotoxin Binding Assay. [¹²⁵I]Charybdotoxin was obtained from New England Nuclear. Charybdotoxin was obtained from Peptides International. All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Jurkat T lymphocytes were grown in suspension in RPMI 1640 tissue culture medium supplemented with 10% bovine fetal calf serum, 2 mM glutamine, and 0.5 mg/mL gentamicin. Cells were routinely seeded at 0.25×10^6 cells/mL in 150 cm² falcon tissue culture flasks and incubated at 37 °C in an atmosphere of 95% O₂/5% CO₂.

[¹²⁵I]ChTX binding to T lymphocytes was based on the methodology of Deutsch et al.⁷ Jurkat T lymphocytes were resuspended in incubation buffer (NaCl, 5 mM; KCl, 5 mM; sucrose, 320 mM; HEPES, 10 mM; glucose, 6 mM, pH 8.4 with trizma base) to give a cell concentration of 1×10^7 cells/mL. Cells (2×10^6 /tube) were incubated in poly(propylene) 1 mL deep well plates in the presence of 25 pM [¹²⁵I]ChTX test agents for 20 min at 22 °C. Nonspecific binding was determined in the presence of 10 nM ChTX. At the end of the incubation period, samples were filtered through GF/C glass fiber filters that had been presoaked on 0.6% poly(ethyleneimine), using a Tomtec Mach II harvester (Orange, CT). Samples were washed with 2×1 mL of ice cold wash buffer (NaCl, 200 mM; 20 mM Hepes, pH 8.0 with trizma base). Radioactivity bound to filters was measured in a β -plate liquid scintillation counter (Wallac, Gaithersburg, MD). Initial experiments showed that the characteristics of [¹²⁵I]ChTX binding under these conditions are identical to that described by Deutsch et al.⁷ for isolated peripheral T lymphocytes and are indicative of [¹²⁵I]ChTX binding to n-type K⁺ channels.

[³H]Dofetilide Binding Assay. [³H]Dofetilide binding to guinea pig cardiac myocytes was determined as previously described.¹¹

Electrophysiology. Solutions and recording systems used were previously described.¹⁵ Whole cell recording modes were established using standard techniques.¹⁶ Membrane potential was held at -80 mV, and n-type K⁺ channel current was measured by giving 150 ms voltage steps to 30 mV once every minute. Cells were allowed to stabilize for >6 min in all cases. Stability was obtained when currents elicited by more than three consecutive voltage steps were superimposable. Pharmacological inhibition was assessed by obtaining peak outward current values during the voltage step described above. Test agent was then continuously perfused at a fixed concentration for 6 min. Following this period, current amplitudes were measured every minute with the same test pulse as above. Amplitudes for the first pulse following the 6 min exposure period were measured to assess block of closed channels. Amplitudes from the subsequent pulse were used to assess open channel block. Amplitudes in the presence of drug were compared to predrug data to determine the percentage of current blocked. A single drug concentration was tested on each cell (i.e., cumulative dose-response curves were not obtained). Initial experiments showed that the physiological and pharmacological profiles of the currents measured under these conditions are consistent with those previously reported to be n-type K⁺ channels.¹³

Computational Methods. All compounds were modeled using SYBYL 6.04¹⁹ software. The structures were minimized using default conditions. The lowest energy conformation was determined using the CEDD²⁰ conformational searching technique followed by minimization. All bonds attached to the 1-position and the heteroatom in the 4-position of the ring system were rotated 360° in 10° increments. A low-energy conformation was defined as any conformation within 3 kcal of the lowest energy conformation. The volume maps were created within SYBYL 6.04 by superimposing the quinoline ring system of the low-energy conformations for all the members of the respective sets.

Supplementary Material Available: Elemental analysis data (table). Ordering information is given on any current

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2,4-Diamino-5-deaza-6-Substituted Pyrido[2,3-*d*]pyrimidine Antifolates as Potent and Selective Nonclassical Inhibitors of Dihydrofolate Reductases^{1,2}Aleem Gangjee,^{*1} Anil Vasudevan,¹ Sherry F. Queener,² and Roy L. Kisliuk³

Division of Medicinal Chemistry, Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, Indiana 46202, and Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts 02111

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Fifteen novel nonclassical and two classical 2,4-diamino-6-(benzylamino)pyrido[2,3-*d*]pyrimidine antifolates were synthesized as potential inhibitors of *Pneumocystis carinii* (pc) *Toxoplasma gondii* (tg) rat liver (rl), and human (h) recombinant dihydrofolate reductases (DHFR). These analogues lack a 5-methyl substitution which has been shown to be important for increased hDHFR inhibitory activity. In addition, they contain a reversal of the C9-N10 bridge present in folates and most antifolates. The synthesis of the compounds involved the reaction of 2,4,6-triaminopyrimidine with the sodium salt of nitromalonaldehyde to afford the key intermediate 2,4-diamino-6-nitropyrido[2,3-*d*]pyrimidine (7), in a single step. Reduction of 7 to the 2,4,6-triaminopyrido[2,3-*d*]pyrimidine (8), followed by reductive amination with the appropriate benzaldehydes or phenylacetaldehydes afforded the target compounds. N9 methylation of these analogues was carried out using formaldehyde and sodium cyanoborohydride. The analogues demonstrated significant inhibition of pcDHFR and tgDHFR. N9 methylation significantly increased DHFR inhibitory potency. Compound 11, the 3',4',5'-trimethoxy-substituted analogue with a selectivity ratio of 9.4 for tgDHFR (compared to rlDHFR) was the most selective analogue of the nonclassical series. Compound 22, the N9 methyl 2',5'-dimethoxy-substituted analogue was the most potent analogue against tgDHFR (IC₅₀ = 6.3 nM) and was the second most selective analogue for tgDHFR (compared to rlDHFR) in the nonclassical series. The naphthyl-substituted analogues 23-25 were generally more potent against rlDHFR than against pcDHFR and tgDHFR. Selected analogues were also evaluated against *Streptococcus faecium* (sf) DHFR, *Escherichia coli* (ec) DHFR, *Lactobacillus casei* (lc) DHFR and tgDHFR with hDHFR as the mammalian reference, under slightly different assay conditions than those employed for rlDHFR. Analogues 11 and 22 had selectivity ratios of greater than 100 for tgDHFR (compared to hDHFR). Analogue 22 in particular, was the most selective analogue of the nonclassical series against tgDHFR (selectivity ratio = 303.5) with excellent potency (28 nM). Analogue 11, also displayed significant selectivity for sfDHFR (selectivity ratio = 4902). Compound 22 was evaluated *in vivo* for the inhibition of the growth of *T. gondii* trophozoites in mice, where at 50 mg/kg orally, it demonstrated distinct prolongation of survival without toxicity. Compounds 11, 12, and 21-23 were evaluated as antitumor agents in the National Cancer Institutes preclinical *in vitro* screening program. Compounds 12, 22, and 23 showed GI₅₀s for tumor growth inhibition in the 10⁻⁶-10⁻⁷ M range.

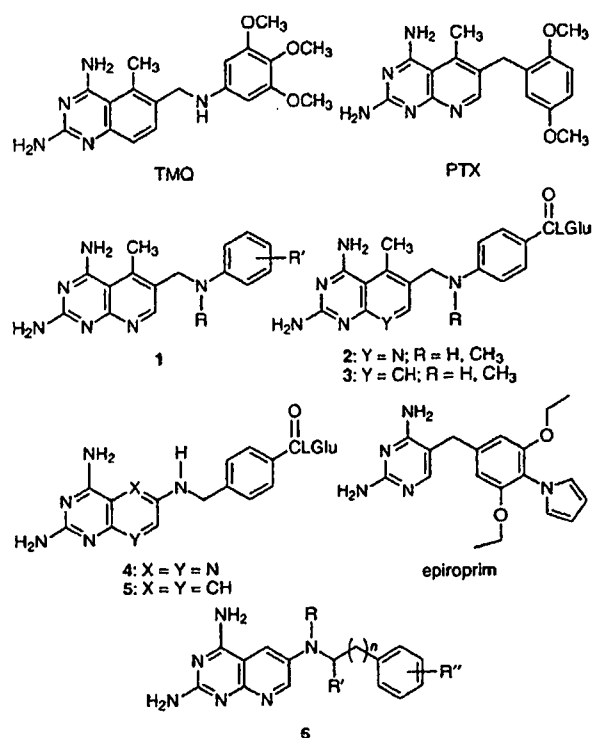
Infections with *Pneumocystis carinii* and *Toxoplasma gondii* are the principal cause of death in patients with acquired immunodeficiency syndrome (AIDS).³ One approach to treat infections with these organisms is the inhibition of the enzyme, dihydrofolate reductase (DHFR).⁴ Trimetrexate (TMQ) and piritrexim (PTX) are potent nonclassical DHFR inhibitors.⁵ TMQ has recently been approved for the treatment of *P. carinii* infections,⁶ while PTX has been evaluated in clinical trials for the treatment of these opportunistic infections. However, neither of these drugs are selective for *P. carinii* (pc) or *T. gondii* (tg) DHFR, and hence their use alone is associated with considerable host toxicity. Thus, TMQ is administered with the reduced folate leucovorin⁷ to rescue host cells. Since *P. carinii* and *T. gondii* lack the carrier mediated uptake mechanism(s) necessary for classical folates, they do not benefit from

the coadministration of leucovorin, which is selectively taken up by the host cells. Recently,⁸ epiroprim, a trimethoprim analogue, has been reported to be 200-fold selective for pcDHFR (against human DHFR). However, its potency is low and in the micromolar range.

We^{9,10} along with others¹¹ have reported the syntheses and biological activities of a number of nonclassical pyrido[2,3-*d*]pyrimidine antifolates of general structure 1 as inhibitors of pcDHFR and tgDHFR. These analogues contain variations of both the substituents on the side chain as well as the N10 nitrogen to optimize hydrophobic interactions with the enzyme(s). These studies afforded several analogues which display high selectivity or high potency against pcDHFR and/or tgDHFR. However, compounds with high selectivity and potency that are able to significantly penetrate *P. carinii* and *T. gondii* cells have remained a challenge.

The 5-methyl moiety of 5-deaza folates has been shown to be important for potent inhibition of human DHFR and of tumors and contributes favorably to the

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inhibition of bacterial DHFRs as well. Piper *et al.*¹² have synthesized 5-alkyl-5-deaza analogues of aminopterin (AMT) and methotrexate (MTX) represented by the general structure 2 and reported that the introduction of a 5-methyl substituent resulted in a decrease in the K_i (3.7 to 2.9 nM) for inhibition of DHFR from L1210 cells, indicating that a methyl group at the 5-position was beneficial to binding to DHFR. The 5-methyl-substituted analogues (IC_{50} 0.1–0.3 nM) of AMT were also reported to be superior to AMT (IC_{50} 0.72 nM) against the growth of L1210 cells in culture. The K_m values for unidirectional influx of these 5-deaza folates were identical to those of AMT and MTX. This, along with the fact that the 5-deaza analogues were poor substrates for polyglutamylation, and hence have no improved cellular retention over MTX or AMT, indicated that the sole reason for the high potency of the 5-methyl analogues is their increased ability to inhibit DHFR. Hynes *et al.*¹³ have synthesized 5-methyl-5,8-dideaza analogues of aminopterin and methotrexate of general structure 3. These workers similarly reported that 5-methyl-5,8-dideaza-AMT is 4 times as potent as the 5-desmethyl analogue against rat liver (rl) DHFR. Bertino *et al.*¹⁴ also reported nonclassical 2,4-diaminoquinazolines as DHFR inhibitors and of MTX resistant murine leukemia cell lines and found that 2,4-diamino-5-methyl-6-[N-(9-phenanthryl)amino]quinazoline (IC_{50} = 1.5 nM) was 60 times more potent than the 5-desmethyl analogue (IC_{50} = 94 nM). Similarly, 2,4-diamino-5-methyl-6-[N-(2-fluoryl)amino]quinazoline (IC_{50} = 1.7 nM) was 88 times more potent than its 5-desmethyl congener (IC_{50} = 150 nM), demonstrating further the importance of the 5-methyl group for potency against mammalian DHFR. We^{9,10,15} have also reported that 5-methyl-substituted nonclassical antifolates are more potent as inhibitors of rIDHFR compared to their 5-desmethyl analogues.

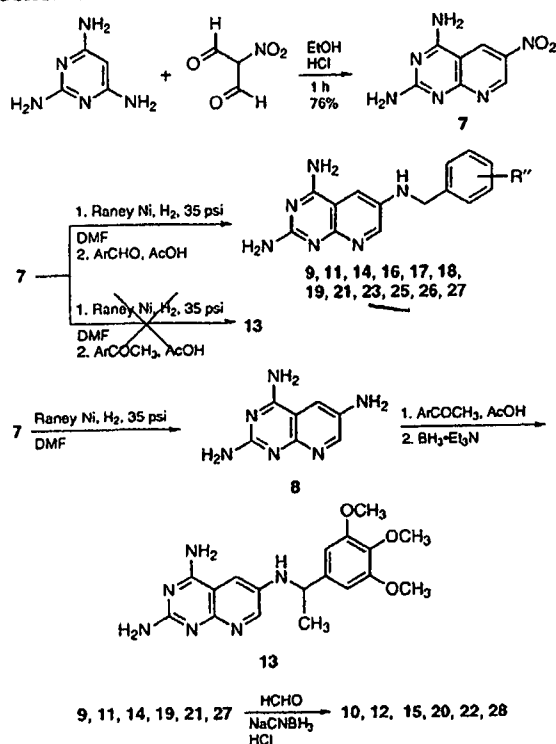
On the basis of the demonstrated importance of the 5-methyl group for inhibition of DHFR from mammalian

Table 1. Structures of 2,4-Diamino-6-(benzylamino)pyrido[2,3-d]pyrimidines

analogue no.	n	R	R'	R''
9	0	H	H	H
10	0	CH ₃	H	H
11	0	H	H	3',4',5'-OMe
12	0	CH ₃	H	3',4',5'-OMe
13	0	H	CH ₃	3',4',5'-OMe
14	0	H	H	2',3',4'-OMe
15	0	CH ₃	H	2',3',4'-OMe
16	0	H	H	2',4',6'-OMe
17	0	H	H	2',4',5'-OMe
18	0	H	H	3',4'-OMe
19	0	H	H	3',5'-OMe
20	0	CH ₃	H	3',5'-OMe
21	0	H	H	2',5'-OMe
22	0	CH ₃	H	2',5'-OMe
23	0	H	H	2',3'-C ₆ H ₄
24	0	H	H	4'-OMe, 2',3'-C ₆ H ₄
25	0	H	H	6'-OMe, 2',3'-C ₆ H ₄
26	0	H	H	4'-O-C ₆ H ₅
27	1	H	H	H
28	1	CH ₃	H	H
29	0	H	H	4'-CONH-L-glutamic acid
30	0	CH ₃	H	4'-CONH-L-glutamic acid

sources, we decided to synthesize compounds of general structure 6 which lack the 5-methyl group. The design of these analogues was based on the premise that since the 5-methyl moiety was important for the inhibition of rIDHFR, L1210 DHFR, and hDHFR and its absence, in certain instances, resulted in analogues with significantly decreased potency for mammalian DHFRs, such 5-desmethyl analogues would be less toxic to mammalian cells. Removal of the 5-methyl group was expected to also result in a decrease in potency against pcDHFR and tgDHFR. However, if this decrease in potency against pcDHFR and tgDHFR was less than the decrease in the inhibition of mammalian DHFR, selective and nontoxic inhibitors of pcDHFR and/or tgDHFR would be at hand. To investigate this hypothesis, we synthesized a series of 5-desmethyl-6-substituted, 2,4-diaminopyrido[2,3-d]pyrimidines 9–28 (Table 1), in which the usual C9–N10 bridge of folates and most antifolates was reversed to also determine the effect of substitutions at the 9-position of 5-deazapyrido[2,3-d]pyrimidines, a feature which has not been investigated in the 5-deaza series. There is precedence in the literature for enhanced DHFR inhibition of 9-substituted antifolates. DeGraw *et al.*¹⁶ reported the synthesis and biological activity of 9-methyl-10-deaza AMT, which was as potent as MTX against DHFR. Interestingly, it was 20 times more potent than MTX against the growth of L1210 cells in culture. The choice of the side chain substituents of compounds 9–28 was based, in part, on the results obtained from our previous studies with other nonclassical analogues.^{9,10,17–19} The nonclassical analogues 9–28 were evaluated as inhibitors of pcDHFR, tgDHFR, and rIDHFR.⁵ Selected analogues were also evaluated³³ against *Streptococcus faecium* (sf) DHFR, *Escherichia coli* (ec) DHFR, *Lactobacillus casei* (lc) DHFR, and recombinant tgDHFR and hDHFR under slightly different assay conditions. Davoll *et al.*²⁰ have reported 2,4-diamino-6-(benzylamino)-pyrido[2,3-d]pyrimidine, 2,4-diamino-6-[(3',4'-dichlo-

Scheme 1



robenzyl)amino]pyrido[2,3-*d*]pyrimidine, and its N9 nitroso derivative as antimalarial agents. However, no DHFR inhibitory activity was reported for these analogues nor were they evaluated against pcDHFR or tgDHFR. Hence, we resynthesized one of these analogues **9** and evaluated it along with all the other analogues against pcDHFR, tgDHFR, rIDHFR, and hDHFR.

The synthesis and DHFR inhibitory activity of the classical analogues **29** and **30** (Table 1) of the 5-deaza series with a N9-C10 bridge were of particular interest in light of the potent activity of isoaminopterin **4** against L1210 DHFR reported by Nair and Baugh²¹ and of the N9 methyl analogue of 5,8-dideazaisoaminopterin **5**, synthesized by Hynes *et al.*,²² which was not only a potent inhibitor of DHFR ($IC_{50} = 4.6$ nM) but also a potent inhibitor of thymidylate synthase ($IC_{50} = 33$ nM).

Chemistry

The synthesis of the desired compounds was achieved by the reaction of 2,4,6-triaminopyrido[2,3-*d*]pyrimidine (**8**), with appropriately substituted benzaldehydes or phenylacetaldehyde. The precursor to this compound, 2,4-diamino-6-nitropyrido[2,3-*d*]pyrimidine **7** (Scheme 1), had been reported by Davoll²⁰ in 1972 via a three-step procedure which involved the reaction of nitromalonaldehyde with cyanoacetamide followed by chlorination and cyclization with guanidine (62% overall yield). Since **7** was to serve as the key intermediate for the entire series, it was important to devise a more efficient synthesis of **7**. We, along with others, have previously reported the reaction of a variety of bis-electrophiles such as β -keto esters,^{23,24} β -keto aldehydes,²⁵ and β -dialdehydes^{26,27} with 2,4,6-triaminopyrimidine to yield pyrido[2,3-*d*]pyrimidines. Thus, we envisioned that **7** could similarly be synthesized via the condensation of nitromalonaldehyde (bis-electrophile)

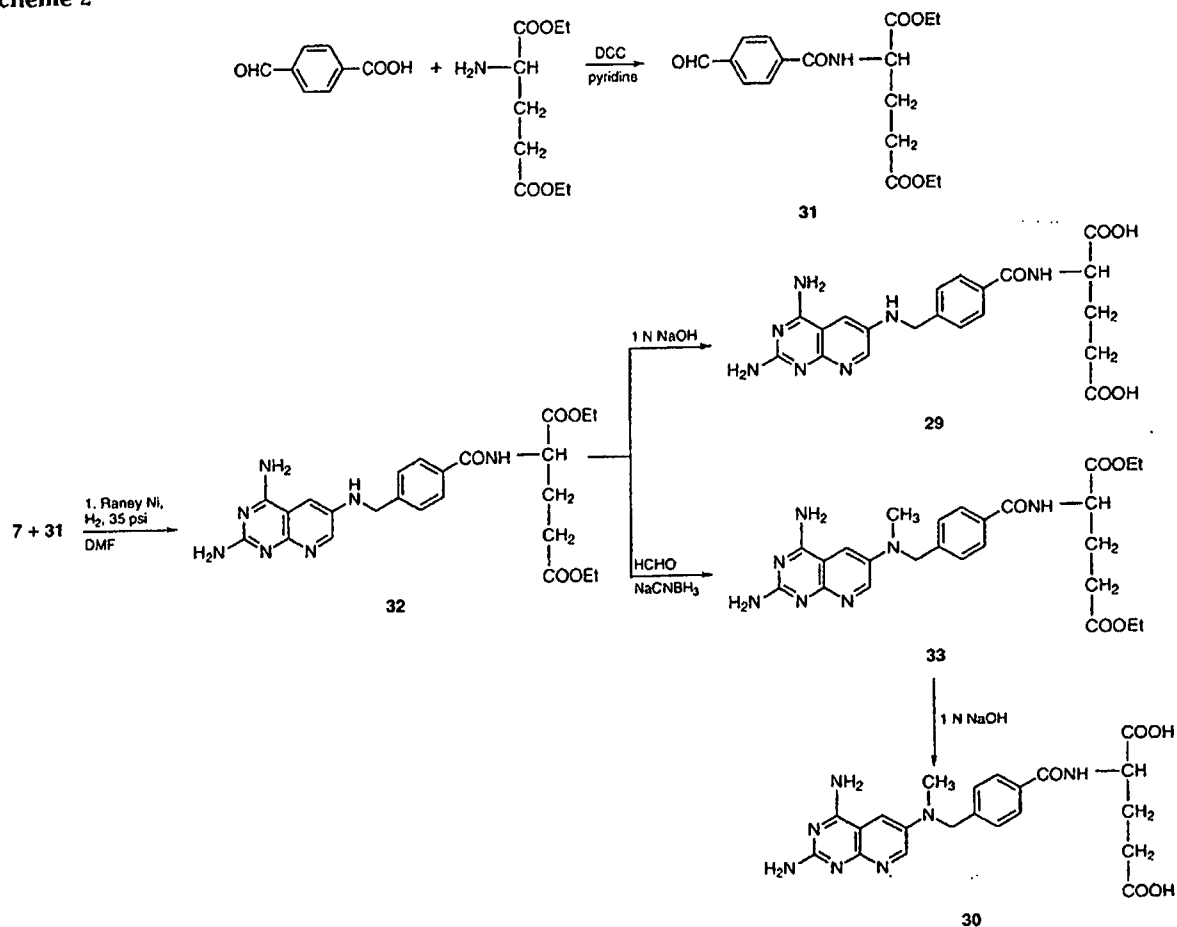
with 2,4,6-triaminopyrimidine. The nitromalonaldehyde was synthesized according to the method of Fanta²⁸ by the reaction of mucobromic acid and sodium nitrite to afford the desired β -dialdehyde in 65% yield as a sodium salt which was used immediately following its preparation. Thus, condensation of nitromalonaldehyde and 2,4,6-triaminopyrimidine in absolute ethanol at reflux, containing sufficient concentrated HCl to solubilize the pyrimidine, afforded a thick red suspension (Scheme 1). Due to the susceptibility of the 4-amino group of folates to hydrolysis under similar conditions,²⁹ the cyclocondensation reaction was closely monitored by TLC. The ¹H NMR of the product corresponded to that expected of **7**. Thus, **7** was obtained in a single step starting from 2,4,6-triaminopyrimidine in 76% yield. A similar methodology, involving base-catalyzed cyclization of 2,6-diamino-4-hydroxypyrimidine and nitromalonaldehyde, has been reported by Price *et al.*³⁰ for the synthesis of the 2-amino-4-hydroxy-6-nitropyrido[2,3-*d*]pyrimidine.

Compound **7** was reduced to the corresponding 6-amino analogue **8** using Raney Ni in DMF at 35 psi. Complete reduction was obtained in 3 h, as indicated by TLC. This intermediate was not isolated but reacted directly with the appropriately substituted benzaldehydes or phenylacetaldehyde in acetic acid and hydrogenated at 35 psi. The duration of the reductive aminations varied from 3 to 6 h and was predicated on the nature of the substitution on the aldehyde. This procedure afforded analogues **9**, **11**, **14**, **16**–**19**, **21**, **23**, and **25**–**28** in 60–70% yield following chromatographic purification. These analogues displayed an intense green fluorescence at 254 nm, probably due to the presence of the exocyclic amino moiety. Similar attempts with 3,4,5-trimethoxyacetophenone and **7** for the synthesis of **13** did not afford the desired C10 methyl analogue. Prolonged reaction times and increased pressure conditions resulted in recovery of unreacted **8**. As a result, **8** had to be isolated, and reductive amination with 3,4,5-trimethoxyacetophenone was carried out in acetonitrile using a borane–triethylamine complex to reduce the intermediate Schiff base which afforded **13**.

N-Methylation of these analogues was carried out by reductive methylation, using HCHO and NaCNBH₃ as reported by us earlier.⁹ It was observed that carrying out the methylations in acetonitrile using sufficient concentrated HCl to effect solution resulted in high isolated yields of the pure N9 methylated products.⁹ The position of the alkylation was confirmed by the ¹H NMR in deuterated dimethyl sulfoxide which indicated the absence of the N9 H and the presence of a sharp singlet at around δ 2.8 ppm corresponding to the N9 CH₃ group.

For the synthesis of the classical analogues **29** and **30**, 4-carboxybenzaldehyde was coupled with diethyl L-glutamate in anhydrous pyridine using diethylcarbodiimide by the method of Hynes and Garrett³¹ to afford (4-formylbenzoyl)-L-glutamate, **31** (Scheme 2). Compound **7** was reduced to **8** using Raney Ni and then reductively aminated with **31**, as described above for the nonclassical analogues, to afford the diester **32**. N9 methylation of **32** with HCHO, NaCNBH₃, and HCl afforded **33** in 72% yield. Both **32** and **33** were saponified with 1 N NaOH to afford the desired classical analogues **29** and **30**.

Scheme 2



Biological Activity and Discussion

The nonclassical analogues were evaluated^{5,32} as inhibitors of pcDHFR, tgDHFR, and r1 DHFR, and the results (IC₅₀) are reported in Table 2. Selectivity ratios (IC₅₀ r1DHFR/IC₅₀ pcDHFR and IC₅₀ r1DHFR/IC₅₀ tgDHFR) determined using r1DHFR as the mammalian source are also reported in Table 2. The classical analogues along with selected nonclassical analogues were also evaluated against ecDHFR, lcDHFR, sfDHFR, and hDHFR³³ as well as recombinant tgDHFR under slightly different assay conditions, and the results are reported in Table 3. In the nonclassical analogues evaluated, N9 methylation consistently resulted in a substantial increase in inhibition of pcDHFR, tgDHFR, r1DHFR, and hDHFR. In some cases, N9 methylation also provided for increased selectivity against pcDHFR and tgDHFR compared to the N9 H analogues. As indicated in Table 2, comparison of the unsubstituted phenyl analogues 9 and 10 indicates that N9 methylation increases potency 15–40-fold against all three DHFRs and also increases selectivity for both pcDHFR and tgDHFR (vs r1DHFR). This increase on N9 methylation in both potency and selectivity is significantly greater for pcDHFR than tgDHFR. For the N9 H trimethoxy-substituted analogues (11, 14, 16, and 17), the effect was predicated on the position of the methoxy groups. For the 3',4',5'- and 2',3',4'-trimethoxy analogues 11 and 14, potency decreased compared to 9, the phenyl-unsubstituted analogue, for all DHFRs except tgDHFR. Selectivity for pcDHFR in all of the tri-

Table 2. Inhibition Concentrations (IC₅₀, in nM) of Dihydrofolate Reductases from *P. carinii*, *T. gondii*, and Rat Liver and Selectivity Ratios^a

no.	pcDHFR	tgDHFR	r1DHFR	r1/pc	r1/tg
9	2700	520	2100	0.8	4.0
10	68	32	140	2.1	4.4
11	14100	350	3300	0.23	9.4
12	61	14	33	0.5	2.4
13	9200	194	1270	0.14	6.5
14	15300	670	3240	0.2	4.8
15	79	26	30	0.4	1.2
16	20700	230	1200	0.06	5.2
17	5500	480	1100	0.2	2.3
18	4800	730	1500	0.3	2.1
19	5700	1200	3400	0.6	2.8
20	76	31	72	0.9	2.3
21	3800	310	350	0.09	1.1
22	84	6.3	57	0.7	9.0
23	3900	980	240	0.06	0.2
24	8200	380	430	0.05	1.1
25	15400	710	370	0.02	0.5
26	24300	3700	2900	0.12	0.8
27	1940	4450	280	0.14	0.06
28	1800	920	1400	0.8	1.5
TMP	12000	2700	133000	11.1	49
TMQ	42	10	3.0	0.07	0.3
epir.	2600	470	73000	28.1	155
MTX	1.3	14	2.5	1.9	0.2

^a These assays were carried out at 37 °C under conditions of substrate (90 μM dihydrofolic acid) and cofactor (119 μM NADPH) in the presence of 150 mM KCl.^{5,32}

methoxy N9 H analogues was less than for 9. However for tgDHFR, the selectivity increases with trimethoxy substitution (except for 17) with compound 11 being the

Table 3. Inhibition Concentrations (IC₅₀, in nM) and Selectivity Ratios of Selected Analogues against DHFRs from Various Sources^a

no.	hDHFR	tgDHFR	h/tg	ecDHFR	h/ec	sfDHFR	h/sf	lcDHFR	h/lc
9	6200	nd	nd	nd	nd	nd	nd	nd	nd
10	700	35	20	nd	nd	nd	nd	nd	nd
11	76000	1300	192	760	329	51	4902	15000	17
15	530	26	20.4	nd	nd	nd	nd	nd	nd
19	160000	nd	nd	1400	114.2	86	1860	17000	9.4
20	2900	29	100	nd	nd	nd	nd	nd	nd
21	13000	500	26	130	100	15	867	5100	2.5
22	8500	28	304	140	60.7	28	304	5700	1.5
29	320	nd	nd	13	24.6	10	32	67	4.8
30	650	nd	nd	13	50	2.2	295	170	3.8
MTX	38	22	1.4	2.2	17	2.2	17	55	0.7

^a Methotrexate was purchased from Sigma, St. Louis, MO. Recombinant hDHFR was provided by Dr. J. H. Freisheim. Recombinant tgDHFR was provided by Dr. D. V. Santi. Recombinant ecDHFR was provided by Dr. R. L. Blakley. DHFR assay conditions:³³ all enzymes were assayed spectrophotometrically in a solution containing 50 μ M dihydrofolate, 80 μ M NADPH, 0.05 M Tris HCl, 0.001 M 2-mercaptoethanol, and 0.001 M EDTA at pH 7.4 and 30 °C. The reaction was initiated with an amount of enzyme yielding a change in o.d. at 340 nm of 0.015/min.

most selective tgDHFR inhibitor in the entire series. N9 methylation of 11 and 14 to afford 12 and 15, respectively, significantly increased potency from 25- to 231-fold and from 25- to 194-fold, respectively, against all three DHFRs and was similar to that observed for 9 and 10. Thus, while the 2',4',5'-trimethoxy analogue 17 was the most potent of the N9 H trimethoxy series against pcDHFR, the 2',4',6'-trimethoxy-substituted analogue 16 was the most potent against tgDHFR. The 3',4',5'-trimethoxy analogue 11 on the other hand was the most selective against tgDHFR. It was 31 times more selective than TMQ against tgDHFR and was the most tgDHFR selective compound (vs rIDHFR). N9 methylation of 11 (analogue 12) resulted in a 230-fold increase in potency against pcDHFR and a 25-fold increase against tgDHFR inhibition. However the selectivity ratio for tgDHFR decreased 4-fold. These differential inhibitory effects of N9 methylation on pcDHFR and tgDHFR underscore the fact that the active site of pcDHFR and tgDHFR are different and that separate structure-activity/selectivity relationships need to be developed for each enzyme. C10 methylation of 11 to afford 13 provided unexpected results. This analogue though more potent than the nonmethylated analogue 11 is still a poor inhibitor of pcDHFR, tgDHFR, and rIDHFR with IC₅₀s in the micromolar range. This was surprising since in the C9-N10 series previously reported by us,¹⁵ methylation of N10 afforded an extremely potent inhibitor of pcDHFR, tgDHFR, and rIDHFR, with IC₅₀s in the 10⁻⁸ M range. The low inhibitory activity of 13 could be attributed, in part, to the intolerance of the enzyme(s) to the N9 H as a H-bond donor or a hydrophilic substitution at the 9-position and is reflected in the low activity of all the N9 H analogues. Further studies are currently underway in our laboratory to determine the cause of this apparent anomaly in the SAR. The N9 methyl 2',3',4'-trimethoxy analogue 15 displayed increased activity against all the three DHFRs to a similar extent as did 12. Thus, 15 was 195-fold and 25-fold more active than the N9 H analogue 14 against pcDHFR and tgDHFR, respectively. As a result, the pcDHFR selectivity for 15 increased 2-fold, and decreased 4-fold for tgDHFR.

In the dimethoxy-substituted N9 H analogues 18, 19 and 21, the compounds have decreased potency compared to the phenyl unsubstituted analogue 9 against all three DHFRs (except 21 for rIDHFR). Moving the 2'-methoxy group of 21 to the 3'-position as in 19, results in a 10-fold decrease in inhibition of rIDHFR. N9 methylation in the dimethoxy series to give compounds

20 and 22 allows for a significant increase in potency for all three DHFRs. N9 methylation of 19 results in a 75-fold increase in pcDHFR inhibition and a 47-fold increase in tgDHFR inhibition. Selectivity for pcDHFR is somewhat increased for 20 with a slight decrease in selectivity for tgDHFR. The 2',5'-dimethoxy substitution pattern, as we have previously reported for the 5-CH₃, C9-N10 analogues,⁹ afforded the highest potency against all three DHFRs. N9 methylation of 21 to afford the N9 methyl 2',5'-dimethoxy analogue 22 resulted in the most potent inhibitor of tgDHFR of all the analogues tested. Compound 22 is 429 times more potent than TMP against tgDHFR. It is also more potent than TMQ against tgDHFR and is 30-fold more selective. Analogue 22 afforded a 10-fold increase in selectivity relative to TMQ against both pcDHFR and tgDHFR. With its high potency of 6.3 nM (better than TMQ) against tgDHFR along with the second highest selectivity against tgDHFR of the nonclassical series, 22 was an excellent lead analogue. Its potency and selectivity against pcDHFR was noteworthy and ranked it among the most potent and selective analogues of the nonclassical series. The significantly different effects of N9 methylation on pcDHFR and tgDHFR inhibition observed for the trimethoxy analogues was absent in the dimethoxy analogues. Thus, deletion of a methoxy group in the trimethoxy series provides for an increase in potency and selectivity against both pcDHFR and tgDHFR.

Analogues 23-25 were designed to evaluate the role of a naphthalene moiety on the side chain in place of a phenyl ring. Piper et al.³⁴ have shown that the active site of human DHFR is large enough to accommodate such a substitution. Further, we⁹ have shown that naphthalene substitution provides analogues which have excellent cell penetration, which is an important attribute for clinical viability. Comparison of the activities of 23 and 9 indicate that the additional hydrophobic contacts provided by the naphthalene ring are conducive to rIDHFR inhibition, but are detrimental to pcDHFR and tgDHFR inhibition. Compound 23 is 10 times more potent than 9 against rIDHFR and is the most potent of all the N9 H analogues against rIDHFR. Incorporation of a methoxy group at either the *ortho* or the *para* position of the naphthalene ring results in analogues with decreased affinity for pcDHFR and rIDHFR and a slight increase in tgDHFR inhibition. Substitution of a phenoxy group at the *para* position of the phenyl ring of compound 9 affords 26 which is an

extremely poor inhibitor of all three DHFRs reflecting a lack of bulk tolerance at the 4'-position.

Insertion of an extra methylene between the N9 and the side chain phenyl ring affords the three-atom-bridged analogue 27. A similar bridge extension has been reported by us in the tetrahydroquinazoline series.¹⁹ This analogue 27 is more potent than 9 against pcDHFR and rDHFR but is 10-fold less active against tgDHFR. Interestingly N9 methylation of 27 to afford 28 results in a significant increase in potency against tgDHFR and a slight increase in inhibition of pcDHFR, but the rDHFR inhibition is decreased 5-fold.

From Table 2, the most important criteria for high activity against pcDHFR and tgDHFR was N9 methylation as seen in compounds 10, 12, 15, 20, and 22. The substitution on the side chain phenyl ring or their positions were relatively unimportant except for 12 and 22 with respect to tgDHFR. Compound 12 was also the most potent inhibitor of pcDHFR. Thus the 3',4',5'-trimethoxy analogue 12 and the 2',5'-dimethoxy analogue 22 provided the most potent analogues of the series. In addition compounds 12 and 22 were the most potent analogues against tgDHFR, and 11 and 22 were the most selective. Thus in the dimethoxy-substituted compounds, N9 methylation was important for both pcDHFR and tgDHFR selectivity.

The nonclassical analogues 9–11, 15, and 19–22 were also evaluated against ecDHFR, lcDHFR, sfDHFR, recombinant tgDHFR, and recombinant hDHFR, and the results are listed in Table 3. These results were obtained under different assay conditions than that reported in Table 2. The tgDHFR inhibitory data in Tables 2 and 3 are remarkably similar despite different assay conditions. However, a significant difference in the inhibition of rDHFR compared to hDHFR was observed. As a result, the selectivity ratios of these analogues for tgDHFR vs hDHFR were quite different and are also reported in Table 3. The corresponding selectivity ratios for MTX are also listed for comparison and are different for hDHFR compared to rDHFR in Table 2. Thus 20, which has a selectivity ratio of 2.3 (vs rDHFR) for tgDHFR (Table 2), displays a 100-fold selectivity for tgDHFR compared to hDHFR (Table 3). Similarly, compound 11 which has a selectivity ratio of 9.4 (vs rDHFR) for tgDHFR displays a 192-fold selectivity for tgDHFR compared to hDHFR. This analogue also displayed more than a 4000-fold selectivity for sfDHFR vs hDHFR. Compound 19 was also extremely selective for sfDHFR with a selectivity ratio of 1860. The N9 methyl analogue of 19, compound 20, showed an increase in inhibition of hDHFR by 2 orders of magnitude compared to the N9 H analogue and displayed excellent tgDHFR selectivity, compared to hDHFR, of 100.

The N9 methyl 2',5'-dimethoxy-substituted analogue 22 showed astounding results with respect to potency and selectivity against tgDHFR. Compound 22 had a selectivity ratio of 300 for tgDHFR (Table 3) and is one of the most selective tgDHFR inhibitors reported to date. The high inhibitory potency against tgDHFR of 22 and its outstanding selectivity for tgDHFR suggests that this analogue could be a clinical candidate for the treatment of *T. gondii* infection.

The spectacular selectivity ratios obtained with 11, 20, and 22 for tgDHFR; 11, 19, and 21 for ecDHFR; and 11, 19, 21, 22, and 30 for sfDHFR (Table 3) validates

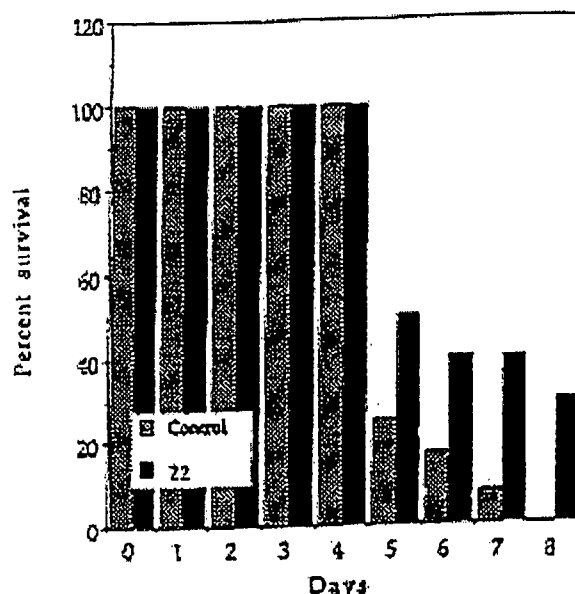


Figure 1. Percent survival of mice inoculated with *T. gondii* trophozoites. Each mouse received 5000 *T. gondii* trophozoites from culture (DCD13); the strain was derived from a clinical isolate from a patient at Indiana University Medical Center. The daily dose of drug was 50 mg/kg administered in food (compounded daily, 1.8 g of powdered rodent chow + 10 mg of drug + 3.2 g of peanut butter). There were 10 mice in this group. The control group consisted of 12 mice which received no drug. The amounts of drug were reduced proportionately as the mice died.

our initial premise that selective inhibitors of a variety of DHFRs can be generated by removal of the 5-methyl moiety and that this results in significantly greater decrease in potency against hDHFR, which translates into remarkably selective analogues against tgDHFR, ecDHFR, and sfDHFR.

Compound 22 on the basis of its potency and selectivity was identified for further development. A pilot study against *T. gondii* infection in the mouse model was carried out. The data for eight days is shown in Figure 1. The data indicates that after 7 days only one mouse survived in the control group (8.3% survival), while four mice survived in the drug group (40% survival), and after 8 days, all the mice in the control group had died and three mice were alive in the drug group, providing for a 30% survival. This data strongly indicates that 22 is active orally and allows a prolongation of life. Further, the arbitrarily chosen dose produced no obvious toxicity.

The classical analogues 29 and 30 were evaluated against lcDHFR, ecDHFR, sfDHFR, and hDHFR. These results are reported in Table 3. Both the N9 H and the N9 methyl analogues were moderate inhibitors of hDHFR with IC₅₀s in the 10⁻⁷ M range. N9 methylation decreased activity against hDHFR and lcDHFR, while there was no change against ecDHFR inhibition. Against sfDHFR, 30 was as potent as MTX.

Selected analogues were also evaluated as antitumor agents in the National Cancer Institute preclinical *in vitro* screening program.³⁵ The improved DHFR inhibition observed on N9 methylation was corroborated by the inhibitory activity observed against the growth of nonsmall lung cancer cell lines, colon cancer cell lines, melanoma cell lines, and breast cancer cell lines. Thus, while 11 was an extremely poor inhibitor of all the cell

lines evaluated with GI_{50} s $> 10^{-4}$ M, the N9 methyl congener displayed GI_{50} s in the 10^{-6} – 10^{-7} M range against several of the cell lines. Similarly, a 1–2 orders of increase in the inhibition of tumor growth was observed for 22 compared to 21. The higher rDHFR inhibition observed with 23, the naphthyl analogue, was corroborated by its tumor growth inhibition (GI_{50} s in the 10^{-6} – 10^{-7} M range), which was much better than any of the other N9 H analogues tested.

Remarkably potent and selective inhibitors of various DHFRs compared to hDHFR have been achieved by the removal of the 5-methyl moiety and the reversal of the C9–N10 bridge of nonclassical 6-substituted 2,4-diaminopyrido[2,3-*d*]pyrimidine antifolates. Whether the selectivities obtained are solely attributable to the removal of the 5-methyl moiety, the reversal of the C9–N10 bridge, or both factors will be the subject of future reports. Preliminary animal studies indicate that at 50 mg/kg orally, compound 22 is active in the prolongation of life in mice infected with *T. gondii* without toxicity. Analogue 22 with its impressive potency and selectivity against tgDHFR is currently undergoing further studies under the auspices of The National Institute of Allergy and Infectious Diseases, Division of AIDS. In addition, the crystal structures of this analogue with pcDHFR and hDHFR is in progress and will be the subject of future communications.

Experimental Section

Melting points were determined on a Mel-Temp II melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Perkin-Elmer Model 1430, in Nujol mulls. Nuclear magnetic resonance spectra for proton (^1H NMR) were recorded on a Bruker WH-300 (300 MHz) with internal standard TMS; s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Low-resolution mass spectra were obtained on an LKB-9000 instrument. Thin layer chromatography was performed on silica gel plates with fluorescent indicator and were visualized with light at 254 and 366 nm. Column chromatography was performed with 230–400 mesh silica gel purchased from Aldrich Chemical Company, Milwaukee, WI. All anhydrous solvents were purchased from Aldrich Chemical Co. and were used without further purification. Samples for microanalysis were dried *in vacuo* over phosphorus pentoxide at 70 °C or 110 °C. Microanalyses were performed by Atlantic Microlabs, Norcross, GA, and are within $\pm 0.4\%$ of the theoretical value.

2,4-Diamino-6-nitropyrido[2,3-*d*]pyrimidine, 7. To a suspension of 2,4,6-triaminopyrimidine (2.00 g, 0.02 mol) in refluxing ethanol was added concentrated HCl dropwise to effect solution. To this solution was added the sodium salt of nitromalonalddehyde (2.24 g, 0.02 mol) all at once, and the reaction mixture was stirred at reflux under nitrogen. Within 15 min, a thick red precipitate started to form. After 1 h, TLC analysis indicated trace amounts of starting material and a major spot for the product. The suspension was cooled, basified to a pH of 8, and filtered. The precipitate was washed with 100 mL of cold water to remove unreacted triaminopyrimidine and the precipitate dried to yield 2.51 g of pure 7 as a red solid (76%). A small amount was crystallized from water to yield analytically pure compound: mp > 370 °C; ^1H NMR (Me_2SO-d_6) δ 7.25 (br s, 2H, 2-NH₂), 8.23 (br s, 2H, 4-NH₂), 9.32 (m, 2H, H-5 and H-7). Anal. Calcd for ($C_7H_6N_6O_2 \cdot H_2O$) C, H, N.

General Procedure for the Synthesis of Compounds 9, 11, 14, 16–19, 21, and 23–27. To a solution of 7 in 50 mL of warm (40 °C) *N,N*-dimethylformamide (DMF) was added Raney Ni. The mixture was hydrogenated in a Parr shaker at 35 psi for 3 h. TLC analysis ($CHCl_3$:MeOH, 5:2) indicated the presence of a blue fluorescent spot ($R_f = 0.27$). At the end of this period, the appropriate aldehyde (substituted or unsubstituted benzaldehyde, substituted or unsubstituted naph-

thaldehyde, or phenylacetaldehyde) was added followed by 15 mL of acetic acid, and the mixture was hydrogenated for a further 3.5 h. The reaction mixture was treated with Norit and filtered through a Celite mat. Silical gel (1.0 g) was added to the filtrate which was then evaporated to dryness to afford a dry plug. This plug was chromatographed on a 1.05 in. $\times$ 23 in. column using $CHCl_3$:MeOH (10:1) as the eluant. Fractions containing the pure product, which showed an intense green fluorescence, were pooled and evaporated to yield a powder.

2,4-Diamino-6-(*N*-benzylamino)pyrido[2,3-*d*]pyrimidine, 9. Compound 9 was synthesized from 7 (0.49 g, 2.42 mmol), Raney Ni (3.0 g) and benzaldehyde (0.26 g, 2.42 mmol) to afford 0.39 g (61%) of 9: mp 267 °C; ^1H NMR (Me_2SO-d_6) δ 4.28 (d, 2 H, CH₂), 6.09 (br s, 2 H, 2-NH₂), 6.29 (t, 1 H, NH), 7.23 (m, 1 H, 4'-H), 7.33 (m, 2 H, 3'-H and 5'-H), 7.39 (m, 2 H, 2'-H and 6'-H), 7.44 (d, 1 H, H-5), 8.33 (d, 1 H, H-7). Anal. Calcd for ($C_{14}H_{14}N_6 \cdot H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(3',4',5'-trimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 11. Compound 11 was synthesized from 7 (0.50 g, 2.43 mmol), Raney Ni (3.0 g), and 3,4,5-trimethoxybenzaldehyde (0.48 g, 2.43 mmol) to afford 0.54 g (62%) of 11: mp 250 °C; ^1H NMR (Me_2SO-d_6) δ 3.62 (s, 3 H, 4'-OCH₃), 3.76 (s, 6 H, 3'-OCH₃ and 5'-OCH₃), 4.20 (d, 2 H, CH₂), 6.31 (br s, 3 H, 2-NH₂ and NH), 6.77 (s, 2 H, 2'-H and 6'-H), 7.50 (d, 1 H, H-5), 7.63 (s, 2 H, 4-NH₂), 8.32 (d, 1 H, H-7). Anal. Calcd for ($C_{17}H_{20}N_6O_3 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(2',3',4'-trimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 14. Compound 14 was synthesized from 7 (0.49 g, 2.42 mmol), Raney Ni (3.0 g), and 2,3,4-trimethoxybenzaldehyde (0.48 g, 2.42 mmol) to afford 0.52 g (61%) of 14: mp 234 °C; ^1H NMR (Me_2SO-d_6) δ 3.76 (s, 3 H, 2'-OCH₃), 3.77 (s, 3 H, 4'-OCH₃), 3.83 (s, 3 H, 3'-OCH₃), 4.18 (d, 2 H, CH₂), 5.933 (t, 1 H, NH), 6.02 (br s, 2H, 2-NH₂), 6.75 (d, 1 H, 5'-H), 7.05 (d, 1 H, 6'-H), 7.44 (m, 3 H, 4-NH₂ and 5-H), 8.34 (d, 1 H, H-7). Anal. Calcd for ($C_{17}H_{20}N_6O_3 \cdot H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(2',4',6'-trimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 16. Compound 16 was synthesized from 7 (0.75 g, 3.64 mmol), Raney Ni (4.00 g), and 2,4,6-trimethoxybenzaldehyde (0.71 g, 3.64 mmol) to afford 0.82 g (63%) of 16: mp 213 °C; ^1H NMR (Me_2SO-d_6) δ 3.78 (s, 3 H, 4'-OCH₃), 3.79 (s, 6 H, 2'-OCH₃ and 6'-OCH₃), 4.04 (d, 2 H, CH₂), 5.39 (br s, 1 H, NH), 6.12 (br s, 2 H, 2-NH₂), 6.27 (s, 2 H, 3'-H and 5'-H), 7.46 (br s, 3 H, 4-NH₂ and 5-H), 8.30 (d, 1 H, H-7). Anal. Calcd for ($C_{17}H_{20}N_6O_3 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(2',4',5'-trimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 17. Compound 17 was synthesized from 7 (0.45 g, 2.18 mmol), Raney Ni (3.0 g), and 2,4,5-trimethoxybenzaldehyde (0.43 g, 2.18 mmol) to afford 0.50 g (65%) of 17: mp 235 °C; ^1H NMR (Me_2SO-d_6) δ 3.66 (s, 3 H, 2'-OCH₃), 3.78 (s, 3 H, 4'-OCH₃), 3.81 (s, 3 H, 5'-OCH₃), 4.17 (d, 2 H, CH₂), 6.00 (t, 1 H, NH), 6.49 (br s, 2 H, 2-NH₂), 6.71 (s, 1 H, 3'-H), 7.03 (s, 1 H, 6'-H), 7.55 (d, 1 H, H-5), 7.79 (s, 2 H, 4-NH₂), 8.34 (d, 1 H, H-7). Anal. Calcd for ($C_{17}H_{20}N_6O_3 \cdot 0.75H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(3',4'-dimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 18. Compound 18 was obtained from 7 (0.50 g, 2.43 mmol), Raney Ni (3.0 g), and 3,4-dimethoxybenzaldehyde (0.40 g, 0.50 mmol) to afford 0.46 g (58%) of 18: mp 257 °C; ^1H NMR (Me_2SO-d_6) δ 3.72 (s, 3 H, 4'-OCH₃), 3.75 (s, 3 H, 3'-OCH₃), 4.20 (d, 2 H, CH₂), 6.36 (br s, 3 H, 2-NH₂ and H), 6.91 (m, 2 H, 5'-H and 6'-H), 7.05 (s, 1 H, 2'-H), 7.51 (d, 1 H, H-5), 7.74 (br s, 2 H, 4-NH₂), 8.32 (d, 1 H, H-7). Anal. Calcd for ($C_{16}H_{18}N_6O_2 \cdot H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(3',5'-dimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 19. Compound 19 was obtained from 7 (0.35 g, 1.70 mmol), Raney Ni (1.50 g), and 3,5-dimethoxybenzaldehyde (0.28 g, 1.70 mmol) to afford 0.33 mg (60%) of 19: mp 264 °C; ^1H NMR (Me_2SO-d_6) δ 3.71 (s, 6 H, 3',5'-OCH₃), 4.24 (d, 2 H, CH₂), 5.72 (s, 1 H, 4'-H), 5.86 (s, 2H, 2',6'-H), 6.01 (t, 1 H, NH), 6.14 (br s, 2 H, 2-NH₂), 7.14 (s, 2 H, 4-NH₂), 7.91 (d, 1 H, H-5), 8.31 (d, 1 H, H-7). Anal. Calcd for ($C_{16}H_{18}N_6O_2 \cdot 0.5H_2O$) C, H, N.

2,4-Diamino-6-[*N*-(2',5'-dimethoxybenzyl)amino]pyrido[2,3-*d*]pyrimidine, 21. Compound 21 was obtained from 7 (0.49 g, 2.42 mmol), Raney Ni (3.0 g), and 2,5-dimethoxy-

benzaldehyde (0.40 g, 2.42 mmol) to afford 0.54 g (68%) of 21: mp 216 °C; ¹H NMR (Me₂SO-*d*₆) δ 3.66 (s, 3 H, 2'-OCH₃), 3.71 (s, 3 H, 5'-OCH₃), 4.22 (d, 2 H, CH₂), 5.97 (br s, 3H, 2-NH₂ and NH), 6.79 (m, 1H, 6'-H), 6.92 (m, 2H, 3'-H and 4'-H), 7.32 (s, 2 H, 4-NH₂), 7.43 (d, 1 H, 5-H), 8.35 (d, 1 H, 7-H). Anal. Calcd for (C₁₆H₁₈N₆O₂·H₂O) C, H, N.

2,4-Diamino-6-[N-(1'-naphthylmethylene)amino]pyrido[2,3-d]pyrimidine, 23. Compound 23 was synthesized from 7 (0.25 g, 1.21 mmol), Raney Ni (3.0 g), and 1-naphthaldehyde (0.19 g, 1.21 mmol) to afford 0.23 g (61%) of 23: mp 259 °C; ¹H NMR (Me₂SO-*d*₆) δ 4.61 (d, 2 H, CH₂), 6.11 (t, 1 H, NH), 6.71 (br s, 2 H, 2-NH₂), 7.41 (m, 2 H, 2'-H and 3'-H), 7.56 (m, 2 H, 4'-H and 5'-H), 7.75 (d, 1 H, H-5), 7.90 (m, 5-H, 6'-H, 7'-H, 8'-H and 4-NH₂), 8.33 (d, 1 H, H-7). Anal. Calcd for (C₁₈H₁₆N₆·H₂O) C, H, N.

2,4-Diamino-6-[N-(4'-methoxy-1'-naphthyl)methylene]amino]pyrido[2,3-d]pyrimidine, 24. Compound 24 was obtained from 7 (0.49 g, 2.42 mmol), Raney Ni (3.0 g), and 4-methoxy-1-naphthaldehyde (0.45 g, 2.42 mmol) to afford 0.49 g (59%) of 24: mp 297 °C; ¹H NMR (Me₂SO-*d*₆) δ 3.91 (s, 3 H, 4'-OCH₃), 4.61 (d, 2 H, CH₂), 6.11 (t, 1 H, NH), 6.60 (br s, 2 H, 2-NH₂), 7.31 (d, 1 H, 3'-H), 7.37 (d, 1 H, 2'-H), 7.56 (m, 1 H, 5'-H), 7.79 (d, 1 H, H-5), 7.96 (m, 5 H, 6', 7', 8'-H and 4-NH₂), 8.37 (d, 1 H, H-7). Anal. Calcd for (C₁₉H₁₈N₆O·0.5H₂O) C, H, N.

2,4-Diamino-6-[N-(2'-methoxy-1'-naphthyl)methylene]amino]pyrido[2,3-d]pyrimidine, 25. Compound 25 was obtained from 7 (0.60 g, 2.91 mmol), Raney Ni (3.0 g), and 2-methoxy-1-naphthaldehyde (0.54 g, 2.91 mmol) to afford 0.54 g (54%) of 25: mp 288 °C; ¹H NMR (Me₂SO-*d*₆) δ 3.98 (s, 3 H, 2'-OCH₃), 4.58 (d, 2 H, CH₂), 6.03 (t, 1 H, NH), 6.65 (br s, 2 H, 2-NH₂), 7.39 (m, 1 H, 3'-H), 7.52 (m, 2 H, 4' and 5'-H), 7.75 (d, 1 H, H-5), 7.90 (m, 5 H, 6', 7', 8'-H and 4-NH₂), 8.33 (d, 1 H, H-7). Anal. Calcd for (C₁₉H₁₈N₆O·H₂O) C, H, N.

2,4-Diamino-6-[N-(4'-phenoxybenzyl)amino]pyrido[2,3-d]pyrimidine, 26. Compound 26 was synthesized from 7 (0.40 g, 1.94 mmol), Raney Ni (3.0 g), and 4-phenoxybenzaldehyde (0.36 g, 1.94 mmol) to afford 0.38 g (55%) of 26: mp 290 °C; ¹H NMR (Me₂SO-*d*₆) δ 4.26 (d, 2 H, CH₂), 5.87 (br s, 2 H, 2-NH₂), 6.23 (t, 1 H, NH), 6.38 (s, 2 H, 4-NH₂), 6.96–7.4 (m, 5 H), 7.95 (d, 2 H, 2'-H), 8.31 (d, 1 H, H-7), 8.69 (d, 1 H, H-7). Anal. Calcd for (C₂₀H₁₈N₆O·H₂O) C, H, N.

2,4-Diamino-6-[N-(2-phenylethyl)amino]pyrido[2,3-d]pyrimidine, 27. Compound 27 was synthesized from 7 (0.60 g, 2.91 mmol), Raney Ni (3.0 g), and phenylacetaldehyde (0.35 g, 2.91 mmol) to afford 0.49 g (60%) of 27: mp 251 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.86 (t, 2 H, CH₂CH₂), 3.24 (t, 2 H, CH₂CH₂), 5.80 (t, 1 H, NH), 5.87 (br s, 2 H, 2-NH₂), 7.32 (m, 5 H, C₆H₅), 7.37 (d, 1 H, H-5), 8.25 (d, 1 H, H-7). Anal. Calcd for (C₁₅H₁₆N₆·0.5H₂O) C, H, N.

General Procedure for the Synthesis of Compounds 10, 12, 15, 20, 22, and 28. To a suspension of the corresponding N9 H compound in 15 mL of acetonitrile were added HCHO and NaCNBH₃, followed by concentrated HCl dropwise to effect solution. TLC analyses after 1 h indicated the presence of a new spot corresponding to the product. The reaction mixture was stirred for 3 h, and the acetonitrile was evaporated. The residue was dissolved in water and brought to pH of 8 with concentrated NH₄OH. The resulting precipitate was filtered and washed with 25 mL of cold water to afford the pure N9 methyl compound.

2,4-Diamino-6-(N-benzyl-N-methylamino)pyrido[2,3-d]pyrimidine, 10. Compound 10 was synthesized from 9 (0.10 g, 0.38 mmol), NaCNBH₃ (0.07 g, 1.12 mmol), and HCHO (0.10 mL) to afford 0.07 g (72%) of 10: mp 255 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.97 (s, 3 H, N9 CH₃), 4.31 (s, 2 H, CH₂), 6.17 (br s, 2-H, 2-NH₂), 7.32 (m, 1 H, 4'-H), 7.36 (m, 2 H, 3'-H and 5'-H), 7.43 (m, 2 H, 2'-H and 6'-H), 7.59 (d, 1 H, H-5), 8.41 (d, 1 H, H-7). Anal. Calcd for (C₁₅H₁₆N₆·0.5H₂O) C, H, N.

2,4-Diamino-6-[N-(3',4',5'-trimethoxybenzyl)-N-methylamino]pyrido[2,3-d]pyrimidine, 12. Compound 12 was synthesized from 11 (0.10 g, 0.28 mmol), HCHO (0.10 mL), and NaCNBH₃ (0.05 g, 0.84 mmol) to afford 0.08 g (75%): mp 279 °C; ¹H NMR (Me₂SO-*d*₆) δ 3.03 (s, 3 H, N9 CH₃), 3.62 (s, 3 H, 4'-OCH₃), 3.71 (s, 6 H, 3'OCH₃ and 5'-OCH₃), 4.55 (s, 2 H, CH₂), 6.57 (s, 2 H, 2'H and 6'H), 6.88 (br s, 2 H, 2-NH₂),

7.82 (d, 1 H, 5-H), 8.25 (s, 2 H, 4-NH₂), 8.46 (d, 1 H, 7-H). Anal. Calcd for (C₁₈H₂₂N₆O₃·0.5H₂O) C, H, N.

2,4-Diamino-6-[N-(2',3',4'-trimethoxybenzyl)-N-methylamino]pyrido[2,3-d]pyrimidine, 15. Compound 15 was obtained from 14 (0.09 g, 0.25 mmol), HCHO (0.10 mL), and NaCNBH₃ (0.05 g, 0.76 mmol) to afford 0.07 g (80%) of 15: mp 236 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.81 (s, 3 H, CH₃), 3.75 (s, 3 H, 2'-OCH₃), 3.77 (s, 3 H, 4'-OCH₃), 3.81 (s, 3 H, 3'-OCH₃), 4.21 (s, 2 H, CH₂), 6.11 (br s, 3 H, 2 NH₂ and NH), 6.77 (d, 1 H, H-5), 7.11 (d, 1 H, 6'-H), 7.53 (m, 3 H, 4-NH₂ and H-5), 8.36 (d, 1 H, H-7). Anal. Calcd for (C₁₈H₂₂N₆O₃·H₂O) C, H, N.

2,4-Diamino-6-[N-(3',5'-dimethoxybenzyl)-N-methylamino]pyrido[2,3-d]pyrimidine, 20. Compound 20 was obtained from 19 (0.12 g, 0.37 mmol), NaCNBH₃ (0.06 g, 0.92 mmol), and HCHO (0.12 mL) to afford 0.09 g (79%) of 20: mp 259 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.94 (s, 3 H, N9-CH₃), 3.71 (s, 6 H, 3',5'-OCH₃), 4.26 (s, 2 H, CH₂), 5.75 (s, 1 H, 4'-H), 5.84 (s, 2 H, 2',6'-H), 6.19 (br s, 2 H, 2-NH₂), 7.24 (s, 2 H, 4-NH₂), 7.95 (d, 1 H, H-5), 8.37 (d, 1 H, H-7). Anal. Calcd for (C₁₇H₂₀N₆O₂·H₂O) C, H, N.

2,4-Diamino-6-[N-(2',5'-dimethoxybenzyl)-N-methylamino]pyrido[2,3-d]pyrimidine, 22. Compound 22 was obtained from 21 (0.08 g, 0.25 mmol), NaCNBH₃ (0.05 g, 0.74 mmol), and HCHO (0.08 mL) to afford 0.06 g (69%) of 22: mp 240 °C; ¹H NMR (Me₂SO-*d*₆) δ 3.04 (s, 3 H, N9 CH₃), 4.60 (s, 2 H, CH₂), 6.54 (d, 1 H, 3'-H), 6.79 (d, 1 H, 4'-H), 6.96 (m, 3 H, 2-NH₂ and 6'-H), 7.30 (br s, 2 H, 4-NH₂), 7.87 (d, 1 H, H-5), 8.36 (d, 1 H, H-7). Anal. Calcd for (C₁₇H₂₀N₆O₂·0.75H₂O) C, H, N.

2,4-Diamino-6-[N-(2-phenylethyl)-N-methylamino]pyrido[2,3-d]pyrimidine, 28. Compound 28 was synthesized from 27 (0.20 g, 0.71 mmol), NaCNBH₃ (0.14 g, 2.14 mmol), and HCHO (0.20 mL) to afford 0.14 g (66%) of 28: mp 258 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.86 (t, 2 H, CH₂CH₂), 2.92 (s, 3 H, CH₃), 3.21 (t, 2 H, CH₂CH₂), 5.89 (br s, 2 H, 2-NH₂), 7.39 (m, 5 H, C₆H₅), 7.47 (d, 1 H, H-5), 8.01 (br s, 2 H, 4-NH₂), 8.33 (d, 1 H, H-7). Anal. Calcd for (C₁₆H₁₈N₆·H₂O) C, H, N.

2,4-Diamino-6-[N-(3',4',5'-trimethoxybenzyl)methylamino]pyrido[2,3-d]pyrimidine, 13. Compound 7 (0.45 g, 2.18 mmol) was dissolved in 40 mL of warm DMF, Raney Ni (2.5 g) added, and the mixture hydrogenated for 4 h. The reaction mixture was filtered through a Celite mat and the DMF evaporated under reduced pressure (40 °C) to yield a brown solid. This solid was dissolved in 15 mL of methanol, and 3,4,5-trimethoxyacetophenone (0.47 g, 2.18 mmol) was added all at once. Following this, 2–3 mL of acetic acid was added followed by BH₃·Et₂N (0.48 g, 6.54 mmol). After 2 h, another 2–3 mL of acetic acid was added and the reaction mixture stirred overnight at room temperature. Water (15 mL) was then added, and all the solvents were evaporated. The residue was dissolved in methanol (30 mL), 1.0 g of silica gel added, and the methanol evaporated to yield a dry plug. This plug was eluted with CHCl₃:MeOH (5:1), and fractions containing the product were pooled and evaporated to dryness to yield pure 13 as a yellow powder (0.09 g, 12%): mp 283 °C; ¹H NMR (Me₂SO-*d*₆) δ 1.3 (d, 3 H, CH₃), 3.62 (s, 3 H, 4'-OCH₃), 3.71 (s, 6 H, 3' and 5'-OCH₃), 4.17 (m, 1 H, CH), 6.29 (m, 3 H, 2-NH₂ and NH), 6.61 (s, 2 H, 2'-H and 6'-H), 7.91 (d, 1 H, H-5), 8.12 (br s, 2 H, 4-NH₂), 8.41 (d, 1 H, H-7). Anal. Calcd for (C₁₈H₂₂N₆O₃·H₂O) C, H, N.

Diethyl 4-(Formylbenzoyl)-L-glutamate, 31. A solution of 4-carboxybenzaldehyde (3.38 g, 0.02 mol), diethyl L-glutamate ester hydrochloride (5.40 g, 0.02 mol), and DCC (4.64 g, 0.02 mol) in anhydrous pyridine was stirred for 7 days. At the end of this period, the pyridine was evaporated, and the oil was chromatographed on a silica gel column using hexanes:ethyl acetate (10:1) as the eluant. Fractions containing the desired product (TLC) were pooled and evaporated to yield 3.0 g (40%) of a white solid: mp 75 °C (lit.³¹ mp 74–76 °C).

5-Deazaisoaminopterin, 29. To a solution of 7 (0.50 g, 2.42 mmol) in warm DMF was added 3.0 g of Raney Ni, and the mixture was hydrogenated for 3 h at 35 psi. At the end of this period, 4-(formylbenzoyl)-L-glutamate diethyl ester 31 (0.81 g, 2.42 mmol) was added followed by 15 mL of acetic acid, and the mixture was hydrogenated for a further 3.5 h. The

reaction mixture was filtered through Celite, and the filtrate was evaporated to dryness. Workup and purification was carried out as described above for 16 to yield the diethyl ester, 32 (0.69 g, 58%).

The diester was dissolved in 5 mL of DMSO and saponified using 10 mL of 1 N NaOH at room temperature over a period of 6 h to afford 29 as a yellow powder (0.47 g, 77%): mp 280 °C; ¹H NMR (Me₂SO-*d*₆) δ 1.93–1.97 (m, 2 H, Glu β-CH₂), 1.99–2.03 (m, 2 H, Glu δ-CH₂), 4.36 (overlapping m, 3 H, CH₂ and Glu α-CH), 6.54 (br m, 3 H, 2-NH₂ and NH), 7.49 (m, 3 H, H-5 and 3'- and 5'-H), 7.67 (br s, 2 H, 4-NH₂), 7.83 (d, 2 H, 2'- and 6'-H), 8.32 (overlapping m, 2 H, H-7 and CONH). Anal. Calcd for (C₂₀H₂₁N₇O₅·1.5H₂O) C, H, N.

9-Methyl-5-deazaaminopterin, 30. To a suspension of 32 (0.5 g, 1 mmole) in 30 mL of acetonitrile was added NaCNBH₃ (0.06 g, 3 mmol) and HCHO (0.5 mL) followed by concentrated HCl dropwise to effect solution. The reaction mixture was stirred for 3 h, and the acetonitrile was evaporated. The residue was dissolved in water and brought to pH 8 with concentrated NH₄OH. The resulting precipitate was filtered and washed with cold water to afford pure 33. Saponification of 33 was carried out in a manner similar to that described for 32 (in the synthesis for 29 above) to yield 30 (0.24 g, 54%): mp 276 °C; ¹H NMR (Me₂SO-*d*₆) δ 1.94–1.98 (m, 2 H, Glu β-CH₂), 1.99–2.03 (m, 2 H, Glu δ-CH₂), 2.89 (s, 3 H, CH₃), 4.41 (overlapping m, 3 H, CH₂ and Glu α-CH), 6.60 (br s, 2 H, 2-NH₂), 7.52 (m, 3 H, H-5 and 3'- and 5'-H), 7.71 (br s, 2 H, 4-NH₂), 7.87 (d, 2 H, 2'- and 6'-H), 8.34 (overlapping m, 2 H, H-7 and CONH). Anal. Calcd for (C₂₁H₂₃N₇O₅·2.0H₂O) C, H, N.

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6-Substituted 2,4-Diamino-5-methylpyrido[2,3-d]pyrimidines as Inhibitors of Dihydrofolate Reductases from *Pneumocystis carinii* and *Toxoplasma gondii* and as Antitumor Agents¹

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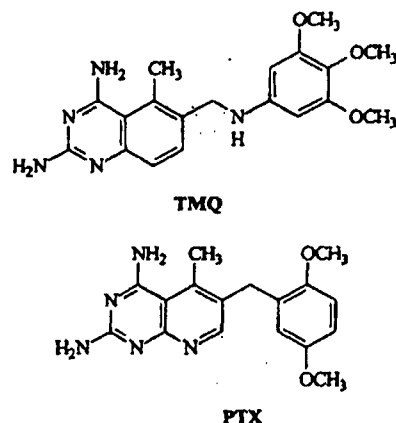
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The synthesis and biological activity of 15 6-substituted 2,4-diamino-5-methylpyrido[2,3-d]pyrimidines are reported. These compounds were synthesized in improved yields by modifications of procedures previously reported by us. Specifically, dimethoxyphenyl-substituted compounds with H and CH₃ at the N-10 position and trimethoxyphenyl-substituted compounds with N-10 ethyl, isopropyl, and propargyl moieties were synthesized. These compounds were evaluated as inhibitors of dihydrofolate reductases (DHFR) from *Pneumocystis carinii*, *Toxoplasma gondii*, and rat liver, and selected analogues were evaluated as inhibitors of the growth of *T. gondii* and tumor cells in culture. All the compounds showed increased selectivity (vs rat liver DHFR) for *T. gondii* DHFR compared to trimetrexate. In general, for the trimethoxy-substituted analogues, increasing the size of the N-10 substituent from a methyl group to larger groups resulted in a decrease in selectivity and potency for both *P. carinii* and *T. gondii* DHFR. For the dimethoxy-substituted analogues, N-10 methylation in general decreased potency but increased selectivity for *T. gondii* DHFR. In an attempt to improve the cell penetration of these analogues, the N-10 naphthyl-substituted analogues were also synthesized. These analogues displayed excellent cell penetration and inhibition of *T. gondii* cells in culture. Further, these analogues were potent inhibitors of the growth of tumor cells in the preclinical *in-vitro* screening program of the National Cancer Institute with IC₅₀s in the nanomolar range.

Infections with *Pneumocystis carinii* and *Toxoplasma gondii* remain the principal cause of death in patients afflicted with acquired immunodeficiency syndrome (AIDS).² Currently available treatments are beset with high cost, lack of selectivity, or host toxicity.³ Trimethoprim (TMP) and pyrimethamine, both of which are weak inhibitors of *P. carinii* dihydrofolate reductase (pcDHFR) and *T. gondii* dihydrofolate reductase (tgDHFR),⁴ are currently used in combination with sulfonamides in the treatment of *P. carinii* and *T. gondii* infections, respectively.

Lipophilic, nonclassical, bicyclic antifolates such as trimetrexate (TMQ), a quinazoline, and piritrexim (PTX), a pyrido[2,3-d]pyrimidine, have been reported as potent inhibitors of pcDHFR and tgDHFR.^{5,6} TMQ (NeuTrexin) has recently been approved as an agent for the treatment of *P. carinii* infections.⁷ These nonclassical antifolates enter cells *via* passive diffusion, circumventing the need for the folate transport system(s) necessary for the uptake of classical antifolates like methotrexate (MTX), and are able to penetrate *P. carinii* and *T. gondii* cells which lack the active transport system(s) for folates. However, TMQ and PTX lack selectivity for pcDHFR and tgDHFR compared to mammalian DHFR. Thus TMQ has been approved for clinical use against *P. carinii* infections only with leucovorin rescue.

We⁸ have previously reported the synthesis and



biological activities of a series of nonclassical pyrido[2,3-d]pyrimidine antifolates as inhibitors of pcDHFR and tgDHFR. Several of these compounds were more potent and selective than TMQ and PTX. Compound 1b, for example, was 5 times as potent and 8 times as selective for pcDHFR as TMQ.⁸ Against tgDHFR, 1b was 12 times as potent and 31 times as selective as TMQ. However, despite their potent DHFR inhibition and selectivity, compounds 1a,b were poorly active against the growth of these organisms in culture.⁸ In an attempt to extend our⁹⁻¹¹ efforts aimed at developing lipophilic, nonclassical antifolates with both high potency and selectivity for pcDHFR and/or tgDHFR, and to establish a structure-activity/selectivity relationship

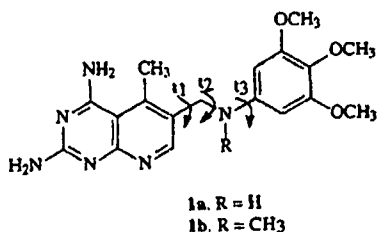
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for 6-substituted 2,4-diamino-5-methylpyrido[2,3-*d*]-pyrimidines, we synthesized compounds **2a-e**, **3a-e**, **4a-c**, and **5a,b**. Compounds **2a-e** were designed to investigate the importance of the third methoxy group of **1a,b** toward selectivity and potency against pcDHFR and tgDHFR as well as to the inhibition of the growth of these organisms in culture. Sequential removal of one of the trimethoxy groups of TMP results in a significant decrease in inhibition of bacterial DHFR.¹² We were interested in determining if a similar structure-activity relationship exists for **1a,b** against pcDHFR and tgDHFR. The effects on biological activity of altering the substitution pattern of the methoxy groups on the phenyl ring were of interest and were also addressed by compounds **2a-e**. Since N-10 methylation in the trimethoxy series resulted in significant increase in the selectivity and potency of **1b** toward both pcDHFR and tgDHFR compared to the nonmethylated analogue **1a**,⁸ compounds **3a-e** were synthesized with the idea that a similar increase in selectivity and potency of the dimethoxy-substituted analogues could be achieved via N-10 methylation. Compounds **4a-c** were designed to evaluate the inhibitory effects of different N-10 substitutions. These substituents serve to partially restrain the conformational flexibility around *t*₂ and *t*₃ and perhaps provide for additional interactions of the N-10 substituent with the enzyme-cofactor complex which could result in greater selectivity and/or potency as was observed for **1b** compared to **1a**.⁸ In spite of the



significant inhibitory activity of **1a,b** against tgDHFR, both compounds were considerably less potent against the growth of *T. gondii* cells in culture (Table 2), which could be attributed in part to their lack of sufficient lipid solubility. Compounds **5a,b** with N-10 naphthyl and N-10 4-methoxynaphthyl substitutions were synthesized to increase lipophilicity and the cell penetration of these analogues.

Chemistry

The syntheses of the target compounds are shown in Scheme 1 and required the intermediate 2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine-6-carbonitrile (**6**). One of the several methods of synthesizing the pyrido[2,3-*d*]pyrimidine ring system is by the reaction of *o*-amino nitriles with guanidine.¹³ The synthesis of **6** was achieved according to the method of Elslager and Davoll,¹⁴ later adapted by Piper *et al.*¹⁶ for the synthesis of classical 5-substituted pyrido[2,3-*d*]pyrimidine antifolates. This method involves the dechlorination of 2-chloro-3,5-dicyano-4-methyl-6-aminopyridine to the amino nitrile followed by cyclization with guanidine hydrochloride. We carried out the dechlorination in dioxane, a lower boiling solvent than DMF,¹⁶ which facilitated workup of the reaction. In addition, we found that the use of 5% Pd/C, which is less expensive than

Pd/BaCO₃, resulted in a slight improvement in the yield of the dechlorinated product compared to the literature methods (90%; lit.¹⁵ 86%). A significant improvement in the yield of the guanidine cyclization step to compound **6** was achieved by increasing the reaction time from 5 to 8 days and the amount of guanidine hydrochloride from 1 to 4 mol equiv (74%; lit.¹⁵ 58%). Reductive condensation of **6** with the appropriately substituted dimethoxyanilines and aminonaphthylenes afforded target compounds **2a-e** and **5a,b**. The yields of these condensations were generally low (25–40%). In an attempt to improve the overall yield of the target compounds, the intermediate **6** was converted to the 6-carboxaldehyde **7** with 97% formic acid and either Raney nickel or a Ni–Al alloy for 2 h to yield the crude aldehyde **7** (75–80%). Reductive amination of 2,5-dimethoxyaniline with **7** in 5 N HCl/MeOH and NaCNBH₃ at pH 5 afforded target compound **2a**. The use of 4 Å molecular sieves resulted in slight improvement in yields. The low yield of this reaction is presumably due to the poor solubility of **7** in MeOH. The overall yield of **2a** from the nitrile via the aldehyde was inferior to that obtained via the direct reductive condensation of 2,5-dimethoxyaniline with the nitrile.

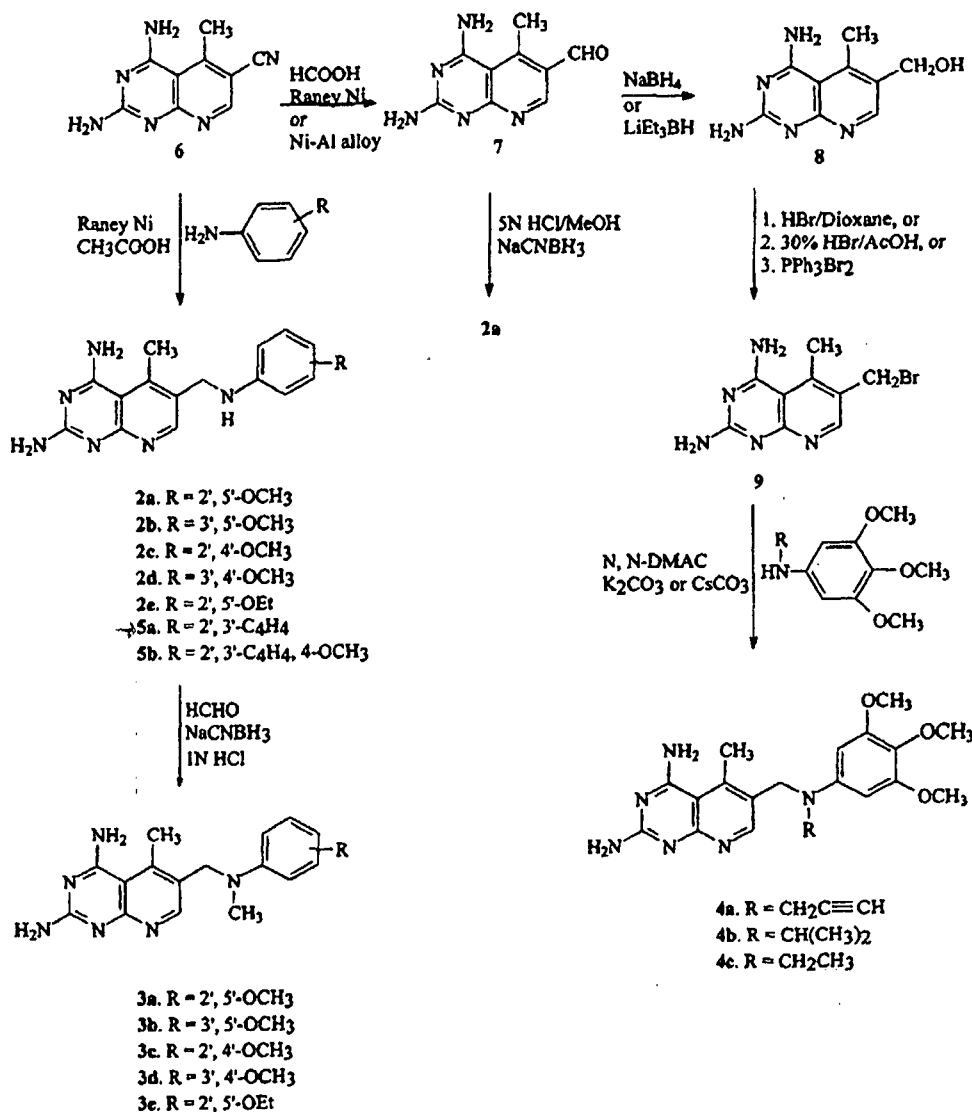
N-10 methylation of compounds **2a-e** to provide **3a-e** was carried out by a modification of an earlier procedure developed by us.⁸ Reductive methylations in acetonitrile with NaCNBH₃ and HCHO was facilitated by the dropwise addition of sufficient 1 N HCl to effect solution (usually at a pH of 3–4). This methodology affords high yields (70–80%) of pure N-10-methylated product without the need for chromatographic purification of the products.

Compound **7** was reduced to the alcohol **8** with NaBH₄ in methanol. Long reaction times result in over-reduction of the aldehyde to the 6-methyl compound which has been shown by Taylor *et al.*¹⁶ to be recalcitrant to any further transformations. The use of lithium triethylborohydride (Super Hydride) afforded **8** in yields comparable to that obtained with NaBH₄. The 6-hydroxymethyl compound **8** was then brominated using one of three methods: (a) triphenylphosphine dibromide in acetonitrile,¹⁵ (b) 30% HBr in acetic acid,¹⁷ (c) anhydrous HBr in dioxane.¹⁸ In our hands, method b afforded the highest yield (91%) of the 6-bromomethyl compound **9**. This compound was very reactive as its hydrobromide salt and was used immediately in the next step. The *N*-ethyl- and *N*-propargyl-3,4,5-trimethoxyanilines were obtained by alkylating 3,4,5-trimethoxyaniline with the appropriate alkyl halide. The reaction was also carried out using a hindered base¹⁹ to prevent dialkylation of the aniline. The best yield (63%) of the monoalkylated product was obtained with Hunigs base. *N*-Isopropyltrimethoxyaniline was obtained by the reductive amination of acetone with trimethoxyaniline.

Biological Results and Discussion

Compounds **2a-e**, **3a-e**, **4a-c**, and **5a,b** were evaluated as inhibitors of DHFRs from *P. carinii* (pc), *T. gondii* (tg), and rat liver (rl), and the results are listed in Table 1. Selectivity ratios were determined using rIDHFR as the mammalian source and are also listed in Table 1. Replacement of the three methoxy groups with two methoxy groups at various positions on the

Scheme 1



phenyl ring in an attempt to assess the contribution of the third methoxy substituent toward the selectivity observed for 1b⁸ afforded varied results. Substitution of a methoxy group at the *meta* position was important for potency toward both pcDHFR and tgDHFR. Thus, 2b, which has both *meta* positions substituted, was the most potent in this series and was 4 and 2 times as potent as 1a against pcDHFR and tgDHFR, respectively, and its selectivity for tgDHFR matches that of 1b. Compounds 2a,d, both of which have one *m*-methoxy substituent, had similar inhibitory activities against pcDHFR and tgDHFR. Compound 2a was 140-fold more selective than 1a and 4.5-fold as selective as 1b as an inhibitor of pcDHFR. It was as selective as 1b for tgDHFR. Compounds 2d, however, was significantly more potent against rDHFR and hence was not selective for pcDHFR or tgDHFR. Comparison of the biological activity of 2b,d and 2a,c defines the role of a *p*-methoxy group toward selectivity of the dimethoxy-substituted analogues. Compound 2b, which differs from 2d in the position of one methoxy group, was almost 12-fold more selective for pcDHFR and more

than 10-fold more selective for tgDHFR than 2d. Similarly, 2a was more selective and potent than 2c, which lacks a *meta* substituent and was the least potent analogue of the dimethoxy series. Clearly, the di-*m*-methoxy substitution in the dimethoxy series was conducive to both potency and selectivity toward pcDHFR and tgDHFR. In an attempt to increase the conformational restriction around *t*₃ and the size of the 2',5'-substituents, the 2',5'-diethoxy analogue 2e was synthesized. Comparison of the activity of 2e with 2a indicated no significant difference in potency toward either pcDHFR or tgDHFR. However, there was a loss in selectivity of 2e due to a 7-fold increase in the activity against rDHFR.

The ¹H NMR data for analogues 2a-e in deuterated dimethyl sulfoxide (Table 3) indicates, as anticipated, that the chemical shift of the N-10 H is strongly influenced by the position of the methoxy groups. It was interesting to note that the activity against pcDHFR and tgDHFR of analogues 2a-e was inversely dependent on the mesomeric effect of the methoxy groups and hence the electron density on the N-10 nitrogen; ana-

Table 1. Inhibitory Concentrations (IC_{50} , nM) and Selectivity Ratios against pcDHFR, tgDHFR, rDHFR, and lcDHFR

compd	pcDHFR	rDHFR	r/pc	tgDHFR	r/tg	lcDHFR ^{2a}
1a	86	2.1	0.02	7.4	0.28	22
1b	13.2	7.6	0.58	0.85	8.94	200
2a	46	128	2.8	16	8.0	39
2b	22.9	42.5	1.9	4.8	8.85	
2c	316	214	0.68	56.5	3.8	
2d	44	7.6	0.17	8.8	0.86	83
2e	76.7	17.4	0.23	17.0	1.0	
3a	216	407	1.9	30.1	13.5	
3b	130	170	1.3	58	2.9	
3c	510	330	0.6	38	8.7	
3d	320	44	0.14	29	1.5	
3e	3100	3000	1.0	100	30.0	
4a	53.5	11.8	0.22	7.7	1.5	
4b	13.4	17.5	1.3	6.7	2.6	210
4c	49.7	10.5	0.21	2.7	3.9	
5a	573	29.6	0.05	14.5	2.0	
5b	41	54	1.3	23	2.3	
TMQ	42	3.0	0.07	10	0.3	27
PTX	38	1.5	0.04	11	0.14	
TMP	12 000	133 000	11.1	2700	49	103

^a This value was incorrectly reported as 0.58 nM in our previous publication.⁶

Table 2. Comparison of the Inhibition (IC_{50} , μ M) of *T. gondii* Cells in Culture vs the Inhibition of tgDHFR

compd	tgDHFR	tg culture	culture/enzyme
1a	0.0074	23.0	3108
1b	0.00085	4.7	5529
2a	0.016	1.5	94
2b	0.0048	0.6	125
2c	0.017	0.32	19
3a	0.0301	1.05	35
3c	0.038	1.73	46
5a	0.0145	0.10	7
5b	0.02	0.04	2

Table 3. Correlation of Chemical Shifts (δ) of the N-10 H and the Inhibition (IC_{50} , nM) of pcDHFR and tgDHFR

compd	¹ H NMR (N-10 H)	pcDHFR	tgDHFR
2b	5.91	22.9	4.8
2d	5.53	44.0	8.8
2a	5.18	46.0	16.0
2e	5.21	76.7	17.0
2c	4.61	316	56.5

logue 2b, in which the N-10 H is not affected by the mesomeric effect of the methoxy groups, was the most potent and selective analogue against both pcDHFR and tgDHFR in the dimethoxyphenyl series. Similarly, the N-10 H of 2c was the most upfield of those of the N-10 H analogues due to the mesomeric effects of the *o*- and *p*-methoxy groups, and 2c was the least potent. We are currently investigating 4'-monosubstituted phenyl moieties with electron-withdrawing and -donating groups to further investigate this observation. This effect was not observed against rDHFR, nor was it observed with the N-10-substituted analogues.

N-10 methylation, in general, resulted in a decrease in activity against both pcDHFR and tgDHFR compared to the N-10 H analogues 2a–e (with the exception of 2c). This is in sharp contrast to the 3,4,5-trimethoxy-substituted series⁹ where N-10 methylation significantly increased activity. Further, the selectivity of 3a–d toward pcDHFR was decreased compared to that of 2a–d, again in contrast to the 3,4,5-trimethoxy analogues. This was surprising and suggests that extrapolation of results obtained in the 3,4,5-trimethoxy-substituted series to the dimethoxy-substituted series was inap-

propriate. One redeeming feature of N-10 methylation in the dimethoxy series was the increase in selectivity toward tgDHFR observed for all the compounds in this series (except 2b). Compounds 3a,e have greater selectivity for tgDHFR than 1b, and compound 3e in particular was 200-fold more selective for tgDHFR than TMQ and was the most selective inhibitor of the dimethoxy series.

In the 3,4,5-trimethoxy series, increasing the size of the N-10 substituent from a methyl group to larger alkyl groups in an attempt to increase the degree of conformational restriction around *t*₂ and *t*₃ and/or provide for additional interactions with the enzyme afforded varying results. Compound 4c, the N-10 ethyl analogue, was less potent against all the three DHFRs compared to the N-10 methyl analogue 1b. Similarly, introducing a propargyl group as in 4a results in a decrease in activity toward all three DHFRs. Both 4a,c were more potent and selective than 1a, the N-10 H analogue, against pcDHFR. Compound 4a was equipotent with 1a, and both 4a,c had greater selectivity than 1a against tgDHFR, indicating that substitution on the N-10 nitrogen is conducive to selective inhibition of pcDHFR and tgDHFR. Compound 4b was 4-fold more potent than 4a,c against pcDHFR, and its potency parallels that of 1b. Further 4b was 65-fold more selective than 1a and more than 2 times as selective as 1b for pcDHFR. However its potency and selectivity toward tgDHFR were similar to those of 4a,c. These results further substantiate the differences between DHFRs from *P. carinii*, *T. gondii*, and rat liver which should yield analogues selective for pcDHFR and/or tgDHFR.

Inhibition of *Lactobacillus casei* DHFR (lcDHFR) by compounds 2a,d and 4b is included in Table 1. Compound 2a was more potent than TMP; however, it lacks the selectivity for lcDHFR vs rDHFR seen with TMP. Removal of the 5-methoxy group of 1a to give 2d decreases lcDHFR inhibition by 4-fold and indicates that this modification is detrimental to lcDHFR inhibition. The inhibitory activity observed for 4b, together with that of 1b,⁸ indicates that N-10 alkylation is also detrimental to the inhibition of lcDHFR in the 3,4,5-trimethoxy series.

The significant selectivity and/or potency for tgDHFR of compounds 2a,b,e and 3a,c prompted their evaluation as inhibitors of the growth of *T. gondii* cells in culture. All five compounds showed greater inhibition of cell growth than 1a,b, despite having lower or comparable IC_{50} s against isolated tgDHFR (Table 2). Clearly, the dimethoxyphenyl- and diethoxyphenyl-substituted compounds have a greater degree of cell penetration compared to the trimethoxyphenyl analogues. The ratios of IC_{50} values in isolated enzyme (tgDHFR) and cell culture are also shown in Table 2. The trimethoxy analogue 1b had a ratio >5000, indicating poor cell penetration. The dimethoxy analogues had ratios which were 44–250 times better than the trimethoxy analogues and indicate significantly improved cell penetration. In an attempt to further increase the cellular penetration, we synthesized analogues with bicyclic N-10 side chains containing a naphthylene and a 4-methoxynaphthylene (5a,b, respectively) as an extension of our previously reported bicyclic side chain-containing analogues.^{20,21} The goal of significantly improving cell penetration with the naphthyl analogues

Table 4. Cytotoxicity Evaluation (GI_{50} , M) against Selected Tumor Cell Lines²³

	2c	3a	3b	5b
Leukemia				
CCRF-CEM	4.29×10^{-6}	7.15×10^{-6}	5.36×10^{-7}	7.66×10^{-8}
K-562	3.05×10^{-7}	4.99×10^{-7}	2.09×10^{-7}	1.61×10^{-8}
MOLT-4	1.22×10^{-6}	3.16×10^{-6}	7.42×10^{-7}	9.11×10^{-8}
SR	3.53×10^{-6}	7.45×10^{-6}	5.41×10^{-7}	7.60×10^{-8}
Non-small-cell Lung Cancer				
A549/ATCC	6.35×10^{-7}	7.32×10^{-7}	4.42×10^{-7}	3.84×10^{-8}
NCI-H322M	5.06×10^{-6}	5.56×10^{-6}	ND	8.50×10^{-8}
NCI-H460	4.81×10^{-7}	ND	5.44×10^{-7}	4.91×10^{-8}
Colon Cancer				
HCT-116	1.73×10^{-7}	3.97×10^{-6}	4.10×10^{-7}	1.17×10^{-8}
HCT-15	2.20×10^{-6}	2.54×10^{-6}	2.12×10^{-6}	4.56×10^{-8}
HT29	2.38×10^{-6}	3.22×10^{-6}	5.84×10^{-6}	7.23×10^{-8}
SW-620	2.24	5.04×10^{-6}	5.50×10^{-6}	7.96×10^{-8}
CNS Cancer				
SF-268	4.17×10^{-6}	6.91×10^{-6}	7.17×10^{-7}	6.04×10^{-8}
SF-295	1.61×10^{-6}	3.41×10^{-6}	5.15×10^{-6}	8.32×10^{-8}
U251	5.81×10^{-6}	4.21×10^{-6}	5.29×10^{-7}	7.29×10^{-8}
Melanoma				
LOX IMVI	3.35×10^{-6}	5.82×10^{-6}	1.99×10^{-6}	6.94×10^{-8}
SK-MEL-5	1.16×10^{-6}	1.56×10^{-6}	5.96×10^{-7}	5.78×10^{-8}
UACC-257	1.00×10^{-4}	2.00×10^{-6}	6.20×10^{-7}	8.68×10^{-8}
UACC-62	1.26×10^{-4}	1.15×10^{-6}	6.28×10^{-7}	4.32×10^{-8}
Ovarian Cancer				
IGROV1	6.27×10^{-6}	5.45×10^{-6}	9.56×10^{-7}	8.68×10^{-8}
Renal Cancer				
786-0	5.88×10^{-7}	8.43×10^{-7}	6.30×10^{-7}	5.50×10^{-8}
ACHN	2.89×10^{-6}	3.75×10^{-6}	8.16×10^{-7}	4.79×10^{-8}
Prostate Cancer				
PC-3	8.52×10^{-7}	7.83×10^{-7}	5.94×10^{-7}	7.98×10^{-8}
DU-145	2.83×10^{-6}	2.71×10^{-6}	4.36×10^{-6}	5.21×10^{-8}
Breast Cancer				
MDA-MB-231/ATCC	1.91×10^{-6}	7.64×10^{-7}	9.22×10^{-8}	1.88×10^{-8}
MDA-MB-435	3.53×10^{-6}	4.18×10^{-6}	6.93×10^{-6}	6.21×10^{-8}
MDA-N	3.59×10^{-7}	6.62×10^{-6}	5.27×10^{-7}	4.25×10^{-8}

was realized as indicated in Table 2. Thus, the IC_{50} ratios for 5a,b were 7 and 2, respectively. While this work was in progress, Piper *et al.*²² reported excellent *T. gondii* cell penetration of compound 5a.

Compounds 2c, 3a,b, and 5b were selected for evaluation as antitumor agents by the National Cancer Institute in the preclinical *in-vitro* screening program²³ (Table 4). Compounds 2c and 3a displayed reasonable antitumor activity with IC_{50} s in the range of 10^{-4} – 10^{-7} M against various cell lines. The cytotoxicity of 3b was approximately 1 order of magnitude better than that of 1b and 3a in various cell lines including colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, and renal cancer. This corroborates the potent inhibition of mammalian DHFR (rDHFR) of 3b ($IC_{50} = 1.7 \times 10^{-7}$ M). However, its inhibition of rDHFR was less than that of 1b, suggesting possibly an enhanced cellular uptake compared to 1b. The excellent cell penetration of 5b was further demonstrated by its potent inhibition ($GI_{50} = (1-9) \times 10^{-8}$ M) of the growth of 26 cell lines.

On the basis of this report and our previous study,⁸ it is apparent that N-10 side chain substituents play a pivotal role in the activity and selectivity of nonclassical pyrido[2,3-d]pyrimidine antifolates against DHFRs from *P. carinii* and *T. gondii* and that extrapolations of structure–activity/selectivity relationships from one series to another are inappropriate and need to be carried out separately for each series. In addition, cell penetration, which is an important attribute for a clinically viable agent, can be significantly improved by

incorporating a naphthyl side chain. The challenge however remains to develop highly potent and selective DHFR inhibitors with good cell penetration ability as clinical agents against *P. carinii* and *T. gondii* infections.

Experimental Section

Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Perkin Elmer Model 1430 spectrometer, in Nujol mulls. Nuclear magnetic resonance spectra for proton (^1H NMR) were recorded on a Bruker WH-300 (300 MHz) spectrometer. The data were accumulated by 16K size with 0.5 s delay time and 70° tip angle with internal standard TMS; s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Low-resolution mass spectra were obtained on an LKB-9000 instrument. Thin layer chromatography was performed on silica gel plates with fluorescent indicator, and the plates were visualized with light at 254 and 366 nm. Column chromatography was performed with 230–400 mesh silica gel purchased from Aldrich Chemical Co., Milwaukee, WI. All anhydrous solvents were purchased from Aldrich Chemical Co. and used without further purification. Samples for microanalysis were dried *in vacuo* over phosphorus pentoxide at 70 or 110 °C. Microanalyses were performed by Atlantic Microlabs, Norcross, GA.

2,4-Diamino-5-methyl-6-[(2,5'-dimethoxyanilino)methyl]pyrido[2,3-d]pyrimidine (2a). To a solution of the nitrile 6 (1.50 g, 7.50 mmol) in 200 mL of 85% acetic acid was added 5.00 g of Raney nickel followed immediately by 2,5-dimethoxyaniline (1.74 g, 11.34 mmol), and the mixture was hydrogenated at atmospheric pressure and room temperature for 5 h. The mixture was then filtered (Norit, Celite) and the filtrate concentrated *in vacuo* to yield a gummy residue. To a solution of this residue in warm ethanol was added a solution of 1 N

Na_2CO_3 dropwise, and the pH of the mixture was adjusted to 8–8.5. The precipitate obtained was filtered and the residue washed with copious amounts of acetone and ethyl acetate until the washings were colorless. To a solution of the residue in 200 mL of warm (45 °C) methanol, containing 1 mL of 1 N HCl, was added 1.0 g of silica gel, and the solvents were evaporated using a pump to yield a solid plug which was spread evenly on the surface of the silica gel in a 1.05 in. $\times$ 23 in. column. The column was eluted with CHCl_3 : CH_3OH (6:1), and 10 mL fractions were collected. Fractions 12–36 were combined, and the solvent was evaporated to yield 0.80 g (30%) of **2a** as a yellow solid. Mp > 250 °C dec. IR (Nujol): 3210, 3000 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.66 (s, 3 H, 5- CH_3), 3.62 (s, 3 H, 2'- OCH_3), 3.72 (s, 3 H, 5'- OCH_3), 4.27 (d, 2 H, CH_2NH), 5.18 (t, 1 H, CH_2NH), 6.07 (s, 1 H, 6'-H), 6.13 (d, 1 H, 4'-H), 6.20 (br s, 2 H, 4-NH₂), 6.70 (d, 1 H, 3'-H), 6.98 (br s, 2 H, 2-NH₂), 8.43 (s, 1 H, 7-H). Anal. ($\text{C}_{17}\text{H}_{20}\text{N}_6\text{O}_2 \cdot 0.5\text{HCl} \cdot 1.0\text{H}_2\text{O}$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3',5'-dimethoxyanilino)methyl]pyrido[2,3-d]pyrimidine (2b). To a solution of the nitrile **6** (1.50 g, 7.50 mmol) in 250 mL of 85% acetic acid was added 5.00 g of Raney nickel followed immediately by 3,5-dimethoxyaniline (1.74 g, 11.38 mmol). The mixture was hydrogenated at room temperature and atmospheric pressure for 5 h. The reaction mixture was then treated with Norit and filtered through Celite and the filtrate concentrated to yield a red gummy residue. This residue was taken up in 35 mL of warm ethanol and added with constant stirring to 50 mL of a 1 N solution of Na_2CO_3 to deposit crude **2b**. The precipitate was washed repeatedly with ethyl acetate and acetone to remove the unreacted aniline. The residue was then dissolved in 200 mL of methanol, 1.00 g of silica gel added, and the solution evaporated to yield a dry plug. Column chromatography as described previously for the purification of **2a** afforded 0.98 g (37% yield) of **2b** as a yellow solid in fractions 14–36. Mp > 250 °C dec. IR (Nujol): 3300, 3100 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.65 (s, 3 H, 5- CH_3), 3.64 (s, 6 H, 3',5'- OCH_3), 4.16 (d, 2 H, CH_2NH), 5.74 (s, 1 H, 4'-H), 5.82 (s, 2 H, 2',6'-H), 5.91 (t, 1 H, CH_2NH), 6.19 (br s, 2 H, 4-NH₂), 6.96 (br s, 2 H, 2-NH₂), 8.46 (s, 1 H, 7-H). Anal. ($\text{C}_{17}\text{H}_{20}\text{N}_6\text{O}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2,4-Diamino-5-methyl-6-[(2',4'-dimethoxyanilino)methyl]pyrido[2,3-d]pyrimidine (2c). To a solution of the nitrile **6** (1.20 g, 6.00 mmol) in 200 mL of 80% acetic acid was added 2,4-dimethoxyaniline (1.37 g, 9.00 mmol) followed by 4.50 g of Raney nickel. The mixture was hydrogenated at atmospheric pressure and room temperature for 5 h, treated with Norit, and filtered through a Celite mat. The filtrate was concentrated to yield a red residue. This residue was dissolved in 100 mL of warm ethanol and neutralized with potassium carbonate. A dry plug for column chromatography was prepared as before and applied to the surface of a 1.05 in. $\times$ 23 in. column packed with silica gel and eluted with CHCl_3 : CH_3OH (10:1) to yield 0.63 g (31%) of **2c** as a yellow solid. Mp = 230–235 °C dec. IR (Nujol): 3300, 3100 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.66 (s, 3 H, 5- CH_3), 3.64 (s, 3 H, 2'- OCH_3), 3.75 (s, 3 H, 4'- OCH_3), 4.21 (d, 2 H, CH_2NH), 4.61 (br s, 1 H, CH_2NH), 6.23 (br s, 2 H, 2-NH₂), 6.33 (m, 1 H, 6'-H), 6.48 (m, 2 H, 3',5'-H), 6.8 (s, 2 H, 4-NH₂), 8.40 (s, 1 H, 7-H). Anal. ($\text{C}_{17}\text{H}_{20}\text{N}_6\text{O}_2 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2,4-Diamino-5-methyl-6-[(3',4'-dimethoxyanilino)methyl]pyrido[2,3-d]pyrimidine (2d). To a solution of **6** (1.50 g, 7.50 mmol) in 200 mL of 85% acetic acid was added 4.50 g of Raney nickel followed immediately by 3,4-dimethoxyaniline (1.74 g, 11.38 mmol), and the mixture was hydrogenated at room temperature and atmospheric pressure for 5 h. TLC analysis (CHCl_3 : CH_3OH , 5:1) showed the absence of **6** and the presence of a new spot (R_f = 0.4). Workup and purification as described for **2a** afforded 0.69 g (34%) of **2d** as an HCl salt. Mp > 250 °C dec. IR (Nujol): 3290, 3830 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.67 (s, 3 H, 5- CH_3), 3.61 (s, 3 H, 4'- OCH_3), 3.68 (s, 3 H, 3'- OCH_3), 4.16 (d, 2 H, CH_2NH), 5.53 (br t, 1 H, CH_2NH), 6.10 (d, 1 H, 5'-H), 6.35 (s, 1 H, 2'-H), 6.42 (br s, 2 H, 4-NH₂), 6.71 (d, 1 H, 6'-H), 7.00 (br s, 2 H, 2-NH₂), 8.47 (s, 1 H, 7-H). Anal. ($\text{C}_{17}\text{H}_{20}\text{N}_6\text{O}_2 \cdot 0.5\text{HCl} \cdot 0.5\text{H}_2\text{O}$) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2',5'-diethoxyanilino)methyl]pyrido[2,3-d]pyrimidine (2e). To a solution of **6** (1.10 g, 5.50

mmol) in 200 mL of 85% acetic acid was added 2,5-diethoxyaniline (1.49 g, 8.25 mmol) followed by 4.00 g of Raney nickel. The mixture was hydrogenated for 4.5 h, at the end of which TLC analysis indicated the absence of **6**. The reaction mixture was filtered (Norit, Celite) and the filtrate evaporated to yield crude **2e**. The red residue was dissolved in 30 mL of methanol and chromatographed on a 1.05 in. $\times$ 23 in. silica gel column eluted with CHCl_3 : CH_3OH (10:1) to yield 0.60 g (32%) of **2e** as an acetate salt. Mp > 250 °C dec. IR (Nujol): 3300, 3130 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 1.21 (t, 3 H, OCH_2CH_3), 1.30 (t, 3 H, OCH_2CH_3), 2.67 (s, 3 H, 5- CH_3), 3.66 (q, 2 H, OCH_2CH_3), 3.91 (q, 2 H, OCH_2CH_3), 4.27 (d, 2 H, CH_2NH), 5.25 (t, 1 H, CH_2NH), 6.03 (d, 1 H, 4'-H), 6.07 (s, 1 H, 6'-H), 6.68 (d, 1 H, 6'-H), 6.89 (br s, 2 H, 4-NH₂), 7.6 (br s, 2 H, 2-NH₂), 8.45 (s, 1 H, 7-H). Anal. ($\text{C}_{19}\text{H}_{24}\text{N}_6\text{O}_2 \cdot 0.5\text{CH}_3\text{COOH}$) C, H, N.

2,4-Diamino-5-methyl-6-[(1'-naphthylamino)methyl]pyrido[2,3-d]pyrimidine (5a). To a solution of the nitrile **6** (1.20 g, 6.00 mmol) in 80% acetic acid was added 1-aminonaphthalene (1.29 g, 9.00 mmol) followed by Raney Ni (5.00 g). The reaction mixture was stirred at room temperature and atmospheric pressure in a hydrogenator for 7 h and filtered, the solvent evaporated, and the residue dissolved in 10 mL of warm methanol. The solution was then chromatographed on silica gel in a 1.05 in. $\times$ 23 in. column and eluted with CHCl_3 : MeOH (7:1). Fractions 15–39 were pooled and evaporated to yield 0.63 g (32%) of **5a** as a brown solid. Mp > 220 °C dec. IR (Nujol): 3300, 3150 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.73 (s, 3 H, 5- CH_3), 4.45 (d, 2 H, CH_2NH), 6.45 (br s, 1 H, CH_2NH), 6.51 (m, 3 H, 2-NH₂, 2'-H), 7.12 (d, 1 H, 4'-H), 7.27 (m, 3 H, 4-NH₂, 3'-H), 7.42 (m, 2 H, 6',7'-H), 7.76 (d, 1 H, 8'-H), 8.21 (d, 1 H, 5'-H), 8.50 (s, 1 H, 7-H). Anal. ($\text{C}_{19}\text{H}_{18}\text{N}_6 \cdot 0.5\text{CH}_3\text{COOH} \cdot 1.0\text{H}_2\text{O}$) C, H, N.

2,4-Diamino-5-methyl-6-[(4'-methoxy-1'-naphthyl)aminomethyl]pyrido[2,3-d]pyrimidine (5b). 4-Methoxy-1-nitronaphthalene (1.22 g, 6.00 mmol) was dissolved in 10 mL of absolute ethanol, Raney Ni (3.00 g) added, and the mixture hydrogenated at 35 psi for 3 h. To this was added the nitrile **6** (0.80 g, 4.00 mmol) dissolved in 125 mL of 80% acetic acid followed by another 6.00 g of Raney Ni, and the reaction mixture was hydrogenated at room temperature and atmospheric pressure for 6 h and filtered and the solvents evaporated. The residue was dissolved in 50 mL of ethanol and cooled and 1 N Na_2CO_3 added with stirring to precipitate crude **5b**. The solid was collected, washed repeatedly with acetone and ether until the washings were colorless, and then dissolved in 1 L of warm methanol, 1.00 g of silica gel was added, and the methanol was evaporated to yield a dry plug. This plug was placed on a silica gel column, eluted with CHCl_3 : MeOH (6:1), and fractions 21–38 were combined, evaporated, and dried to yield 0.36 g (25%) of pure **5b** as a yellow solid. Mp > 230 °C. IR (Nujol): 3320, 3180 (NH_2) cm^{-1} . ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.72 (s, 3 H, 5- CH_3), 3.85 (s, 3 H, 4'- OCH_3), 4.37 (d, 2 H, CH_2NH), 6.01 (t, 1 H, CH_2NH), 6.19 (br s, 2 H, 2-NH₂), 6.45 (d, 1 H, 1'-H), 6.78 (d, 1 H, 3'-H), 6.99 (br s, 2 H, 4-NH₂), 7.46 (m, 2 H, 6',7'-H), 8.08 (d, 1 H, 5'-H), 8.18 (d, 1 H, 8'-H), 8.49 (s, 1 H, 7-H). Anal. ($\text{C}_{20}\text{H}_{20}\text{N}_6\text{O} \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2,4-Diamino-5-methylpyrido[2,3-d]pyrimidine-6-carboxaldehyde (7). To a solution of **6** (5.00 g) in 125 mL of warm formic acid was added Raney nickel or a Ni–Al alloy (20.00 g), and the mixture was heated at 90 °C under nitrogen for 1.5 h. TLC analysis indicated the absence of **6** and the appearance of a major spot corresponding to the aldehyde (positive (2,4-dinitrophenyl)hydrazine) and a minor spot. The reaction mixture was filtered hot (Norit, Celite) and the filtrate evaporated to dryness to afford an orange residue. This residue was dissolved in 100 mL of boiling water, treated with Norit, and filtered and the filtrate neutralized with concentrated NH_4OH at 0–5 °C to yield 4.20 g of crude **7**. Due to the instability of this compound it was used directly in the next step without purification. ^1H NMR ($\text{Me}_2\text{SO}-d_6$): δ 2.96 (s, 3 H, 5- CH_3), 8.85 (s, 1 H, 7-H), 10.16 (s, 1 H, CHO).

2,4-Diamino-5-methyl-6-(hydroxymethyl)pyrido[2,3-d]pyrimidine (8). The crude aldehyde **7** (2.00 g) was stirred overnight in anhydrous methanol (1 L) under nitrogen. To this suspension was added NaBH_4 (0.50 g) in four separate

portions at 25 °C (exothermic). After the final addition, the reaction mixture was stirred for a further 6–7 h. (It was found that allowing the reaction mixture to stir longer resulted in over-reduction of the alcohol to the 6-methyl compound.) At the end of 6–7 h, the reaction mixture was filtered, water (30 mL) was added to the filtrate, which was concentrated to 20 mL and refrigerated, and the yellow solid obtained was collected by filtration to yield 1.10 g (54%) of **8**. ¹H NMR (Me₂SO-*d*₆): δ 2.66 (s, 3 H, 5-CH₃), 4.51 (s, 2 H, CH₂OH), 8.45 (s, 1 H, 7-H). Anal. (C₉H₁₁N₅O·1.0H₂O) C, H, N.

2,4-Diamino-5-methyl-6-(bromomethyl)pyrido[2,3-*d*]-pyrimidine Hydrobromide (9). Method A. To freshly prepared triphenylphosphine dibromide (1.39 g, 3.29 mmol) in 20 mL of *N,N*-dimethylacetamide was added **8** (225 mg, 1.09 mmol) with stirring. After 2 h, 20 mL of toluene was added which precipitated a brown solid. The clear liquid was decanted, the remaining solid was washed with toluene and ethyl ether, and then sufficient 48% HBr was added to dissolve the solid. This solution was added to cold acetonitrile (ice bath) to deposit a brown solid which was chilled in the refrigerator, filtered, washed with ethyl ether, and dried at 40 °C to yield 0.23 g (77%) of **9**.

Method B. To 0.15 g (0.72 mmol) of the alcohol **8** in round-bottomed flask was added 20 mL of glacial acetic acid, and the reaction mixture was heated to 80 °C to dissolve **7**. The solution was cooled to room temperature, 25 mL of 30% HBr in acetic acid added, and the mixture then stirred at room temperature for 48 h. TLC analysis indicated that most of the starting material had been consumed. The acetic acid was evaporated, and ethyl ether was added to precipitate 0.95 g (91%) of **9**.

Method C. The alcohol **8** (0.25 g, 1.21 mmol) was stirred in anhydrous dioxane and the suspension saturated with dry HBr gas. The reaction mixture was then stirred at room temperature in a tightly sealed flask for 24 h. Some undissolved solid that remained was filtered and the filtrate concentrated to a small volume (approximately 10 mL). To this was added anhydrous ether to precipitate 0.26 g (80%) of **9**. MS: *m/e* 268 and 270 (*M* + 1)⁺. ¹H NMR (Me₂SO-*d*₆): δ 2.77 (s, 3 H, 5-CH₃), 4.93 (s, 2 H, CH₂Br), 8.25 (br s, 2 H, 4-NH₂), 8.86 (s, 1 H, 7-H), 9.40 (br s, 2 H, 2-NH₂).

***N*-Isopropyl-3,4,5-trimethoxyaniline (10).** To a solution of 3,4,5-trimethoxyaniline (0.50 g, 2.73 mmol) in 25 mL of acetonitrile was added acetone (0.24 g, 4.10 mmol) followed by NaCNBH₃ (0.52 g, 8.19 mmol). Acetic acid (0.50 mL) was added to the reaction mixture at 0.5 h intervals over a 2 h period. The solution was allowed to stir at room temperature for a further 24 h, a small amount of water was added to the reaction mixture, and the solvents were evaporated. The residue was dissolved in 5 mL of CHCl₃ and chromatographed on a silica gel column using CHCl₃:diethyl ether (1:1) as the eluant. Fractions 18–29 were pooled together and evaporated to dryness to yield 0.33 g (55%) of *N*-isopropyltrimethoxyaniline as a brown solid. ¹H NMR (CDCl₃): δ 1.21 (d, 6 H, CH(CH₃)₂), 2.91 (br s, 1 H, NH), 3.56 (m, 1 H, CH(CH₃)₂), 3.76 (s, 3 H, 4-OCH₃), 3.82 (s, 6 H, 3,5-OCH₃), 5.84 (s, 2 H, 2,6-H). Anal. (C₁₂H₁₆NO₃) C, H, N.

***N*-Ethyl-3,4,5-trimethoxyaniline (11).** To a solution of 3,4,5-trimethoxyaniline (2.50 g, 13.60 mmol) in 50 mL of warm toluene (60 °C) was added iodoethane (2.13 g, 13.60 mmol) followed by *N,N*-diisopropylethylamine (1.76 g, 13.60 mmol) and the mixture refluxed for 24 h. At the end of this period, the solvent was evaporated and the residue dissolved in ~5 mL of chloroform and chromatographed on a 1.5 in. × 23 in. column using CHCl₃:diethyl ether (5:1) as the eluant. Fractions containing pure monoalkylated product were pooled and evaporated to yield an oil which solidified on refrigerating (1.53 g, 53%). ¹H NMR (CDCl₃): δ 1.23 (t, 3 H, CH₃), 2.80 (br s, 1 H, NH), 3.15 (m, 2 H, CH₂), 3.76 (s, 3 H, 4-OCH₃), 3.78 (s, 6 H, 3,5-OCH₃), 5.86 (s, 2 H, 2,6-H). Anal. (C₁₁H₁₇NO₃) C, H, N.

***N*-Propargyl-3,4,5-trimethoxyaniline (12).** A solution of 3,4,5-trimethoxyaniline (3.00 g, 16.00 mmol), propargyl bromide (1.94 g, 16.00 mmol), and *N,N*-diisopropylethylamine (2.10 g, 16.00 mmol) in toluene was heated at 60 °C (bath temperature) for 24 h. Workup and purification were carried

out in a manner similar to that mentioned previously for the *N*-ethyl analogue to yield 1.87 g (53%) of pure monoalkylated product. ¹H NMR (CDCl₃): δ 2.65 (t, 1 H, NH), 4.17 (s, 3 H, 4-OCH₃), 4.23 (s, 6 H, 3,5-OCH₃), 4.31 (d, 2 H, CH₂), 3.21 (br s, 1 H, CH), 6.34 (s, 2 H, 2,6-H). Anal. (C₁₂H₁₆NO₃) C, H, N.

2,4-Diamino-5-methyl-6-[(2',5'-dimethoxy-*N*-methyl-anilino)methyl]pyrido[2,3-*d*]pyrimidine (3a). To a suspension of **2a** (0.12 g, 0.35 mmol) in 15 mL of acetonitrile was added formaldehyde (0.12 mL) followed by NaCNBH₃ (0.07 g, 1.06 mmol). The mixture was stirred for 5 min, and 1 N HCl was added dropwise to the suspension until the reaction became a clear solution (usually at a pH of 3–4). TLC analysis at that time indicated that the *N*-methylated product had already begun to form. Within 10–15 min, a yellow solid began to fall out of solution. The reaction mixture was stirred for a total of 4 h at the end of which the solvent was removed, a small amount of water added to the residue, and the suspension filtered to yield 0.10 g of analytically pure **3a** (79%). Mp > 250 °C dec. IR (Nujol): 3350, 3190 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 2.54 (s, 3 H, 5-CH₃), 2.68 (s, 3 H, N-10-CH₃), 3.68 (s, 3 H, 2'-OCH₃), 3.74 (s, 3 H, 5'-OCH₃), 4.17 (s, 2 H, CH₂N), 6.45 (br s, 2 H, 4-NH₂), 6.50 (d, 1 H, 4'-H), 6.53 (s, 1 H, 6'-H), 6.87 (d, 1 H, 3'-H), 7.19 (br s, 2 H, 2-NH₂), 8.46 (s, 1 H, 7-H). Anal. (C₁₈H₂₂N₆O₂·0.5HCl·0.5H₂O) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3',5'-dimethoxy-*N*-methyl-anilino)methyl]pyrido[2,3-*d*]pyrimidine (3b). To a suspension of **2b** (0.13 g, 0.37 mmol) in CH₃CN was added formaldehyde (0.13 mL) followed by NaCNBH₃ (0.07 g, 1.10 mmol), and the suspension was allowed to stir for 5–10 min after which 1 N HCl was added dropwise until a clear solution was obtained. The reaction mixture was allowed to stir for an additional 3–4 h after which a small amount of water was added to the reaction mixture and the acetonitrile evaporated. The suspension was refrigerated overnight (0–5 °C), filtered, and dried to yield 0.10 g (76%) of analytically pure **3b** as an orange solid. Mp > 250 °C dec. IR (Nujol): 3290, 3120 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 2.62 (s, 3 H, 5-CH₃), 2.84 (s, 3 H, N-10-CH₃), 3.58 (s, 6 H, 3',5'-OCH₃), 4.49 (s, 2 H, CH₂-N), 5.86 (s, 3 H, 2',4',6'-H), 6.33 (br s, 2 H, NH₂), 7.07 (br s, 2 H, NH₂), 8.27 (s, 1 H, 7-H). Anal. (C₁₈H₂₂N₆O₂·0.5HCl) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2',4'-dimethoxy-*N*-methyl-anilino)methyl]pyrido[2,3-*d*]pyrimidine (3c). To a suspension of **2c** (0.15 g, 0.43 mmol) in acetonitrile was added 37% formaldehyde (0.15 mL) followed by NaCNBH₃ (0.08 g, 1.28 mmol). The reaction mixture was stirred for 10 min, and 1 N HCl was added dropwise to adjust the pH to 4. The resulting clear solution was stirred at room temperature for 3 h, and a small amount of water was added to the reaction mixture, concentrated to a small volume (aspirator) and refrigerated, to deposit 0.1 g (65%) of **3c** as a yellow solid. Mp > 270 °C dec. IR (Nujol): 3300, 3120 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 2.68 (s, 3 H, 5-CH₃), 2.71 (s, 3 H, N-10-CH₃), 3.65 (s, 3 H, 2'-OCH₃), 3.70 (s, 3 H, 4'-OCH₃), 4.23 (s, 2 H, CH₂N), 6.42–6.56 (m, 5 H, 3',5',6'-H, 4-NH₂), 7.24 (br s, 2 H, 2-NH₂), 8.44 (s, 1 H, 7-H). Anal. (C₁₈H₂₂N₆O₂·0.5HCl·1.0H₂O) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3',4'-dimethoxy-*N*-methyl-anilino)methyl]pyrido[2,3-*d*]pyrimidine (3d). To a suspension of **2d** (0.12 g, 0.36 mmol) in CH₃CN was added formaldehyde (0.12 mL) followed by NaCNBH₃ (0.07 g, 1.08 mmol), and HCl was added dropwise to adjust the pH to 4. After 4 h, a small amount of water was added to the reaction mixture, the acetonitrile evaporated, and the suspension refrigerated to afford 0.09 g (75%) of **3d** as a white solid. Mp > 270 °C dec. IR (Nujol): 3310, 3190 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 2.67 (s, 3 H, 5-CH₃), 2.81 (s, 3 H, N-10-CH₃), 3.66 (s, 3 H, 4'-OCH₃), 3.72 (s, 3 H, 3'-OCH₃), 4.41 (d, 2 H, CH₂N), 6.36 (br s, 2 H, 2',6'-H), 6.78 (d, 1 H, 5'-H), 7.12 (br s, 4 H, 2,4-NH₂), 8.24 (s, 1 H, 7-H). Anal. (C₁₈H₂₂N₆O₂·1.0HCl) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(2',5'-diethoxy-*N*-methyl-anilino)methyl]pyrido[2,3-*d*]pyrimidine (3e). To a suspension of **2e** (0.11 g, 0.3 mmol) in CH₃CN was added HCHO (0.11 mL) followed by NaCNBH₃ (0.06 g, 0.9 mmol), and the pH of the reaction mixture was adjusted to 4 by the dropwise

addition of 1 N HCl. The reaction mixture was stirred at 25 °C for 4 h. At the end of that time a small amount of water was added to the reaction mixture, and the acetonitrile was removed via an aspirator. The suspension was refrigerated overnight to afford 0.08 g (72%) of analytically pure **3e** as a white solid. IR (Nujol): 3320, 3160 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 1.27 (t, 6 H, OCH₂CH₃), 2.51 (s, 3 H, 5-CH₃), 2.68 (s, 3 H, N-10-CH₃), 3.95 (m, 4 H, OCH₂CH₃), 4.17 (s, 2 H, CH₂N), 6.21 (br s, 2 H, 4-NH₂), 6.43–6.50 (m, 4 H, 4',6'-H, 2-NH₂), 6.82 (d, 1 H, 3'-H), 8.47 (s, 1 H, 7-H). Anal. (C₂₀H₂₆N₆O₂·1.0HCl·0.5H₂O) C, H, N, Cl.

2,4-Diamino-5-methyl-6-[(3',4',5'-trimethoxy-N-propargylanilino)methyl]pyrido[2,3-d]pyrimidine (4a). To a solution of compound **9** (0.21 g, 0.78 mmol) in 15 mL of anhydrous *N,N*-dimethylacetamide was added *N*-propargyltrimethoxyaniline (0.19 g, 0.86 mmol) followed by potassium carbonate (0.11 g, 0.78 mmol). The mixture was stirred under an atmosphere of nitrogen for 48 h. TLC analysis in CHCl₃:CH₃OH (5:1) indicated the presence of a major spot (corresponding to the product, *R*_f = 0.5), excess aniline, and some impurity at the base. The reaction mixture was filtered and the solvent evaporated *in vacuo* at 60 °C. The residue was dissolved in 30 mL of methanol, 0.25 g of silica gel added, and the methanol evaporated to yield a dry plug. This plug was applied to the surface of a 1.05 in. × 23 in. column packed with silica gel and eluted with CHCl₃:CH₃OH (5:1). Fractions containing pure product were pooled and evaporated to yield 0.22 g (67%) of **4a** as a tan solid. Mp > 250 °C dec. IR (Nujol): 3500 (CH stretch), 3250, 3100 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 2.57 (s, 3 H, 5-CH₃), 3.17 (s, 1 H, C=CH), 3.58 (s, 3 H, 4'-OCH₃), 3.73 (s, 6 H, 3',5'-OCH₃), 3.97 (s, 2 H, CH₂-C=CH), 4.42 (s, 2 H, CH₂N), 6.24 (s, 4 H, 2',6'-H, 4-NH₂), 6.99 (br s, 2 H, 2-NH₂), 8.40 (s, 1 H, 7-H). Anal. (C₂₁H₂₄N₆O₃·0.5H₂O), C, H, N.

2,4-Diamino-5-methyl-6-[(3',4',5'-trimethoxy-N-isopropylanilino)methyl]pyrido[2,3-d]pyrimidine (4b). To a solution of compound **9** (0.25 g, 0.93 mmol) in 15 mL of anhydrous *N,N*-dimethylacetamide was added *N*-isopropyltrimethoxyaniline (0.23 g, 1.02 mmol) followed by cesium carbonate (0.20 g, 1.02 mmol). The brownish solution was stirred at room temperature under nitrogen for 60 h. At the end of this period, TLC analysis in CHCl₃:CH₃OH (5:1) showed the presence of a major spot (corresponding to the product, *R*_f = 0.48). The reaction mixture was filtered and the solvent removed *in vacuo* using a pump (bath temperature ~60 °C). A small amount of acetic acid (~2 mL) was added to the residue followed by 30 mL of methanol and 0.50 g of silica gel. Column chromatography as described above for the purification of **4a** yielded 0.24 g (61%) of **4b** as a tan solid. Mp > 270 °C dec. IR (Nujol): 3300, 3060 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 1.16 (d, 6 H, CH(CH₃)₂), 2.69 (s, 3 H, 5-CH₃), 3.52 (s, 3 H, 4'-OCH₃), 3.62 (s, 6 H, 3',5'-OCH₃), 4.14 (m, 1 H, CH(CH₃)₂), 4.31 (s, 2 H, CH₂N), 5.96 (s, 2 H, 2',6'-H), 6.15 (br s, 2 H, 4-NH₂), 6.95 (br s, 2 H, 2-NH₂), 8.26 (s, 1 H, 7-H). Anal. (C₂₁H₃₂N₆O₃·1.0H₂O), C, H, N.

2,4-Diamino-5-methyl-6-[(3',4',5'-trimethoxy-N-ethyl-anilino)methyl]pyrido[2,3-d]pyrimidine (4c). To a solution of **9** (0.18 g, 0.66 mmol) in 15 mL of anhydrous *N,N*-dimethylacetamide was added *N*-ethyltrimethoxyaniline (0.15 g, 0.73 mmol) followed immediately by cesium carbonate (0.14 g, 0.73 mmol). The reaction mixture was stirred under nitrogen for 48 h. TLC analysis indicated the presence of a major spot at *R*_f = 0.46. Workup and purification as described above for **4a** yielded 0.19 g (71%) of pure **4c**. Mp > 280 °C dec. IR (Nujol): 3300, 3140 (NH₂) cm⁻¹. ¹H NMR (Me₂SO-*d*₆): δ 1.06 (t, 3 H, CH₂CH₃), 2.64 (s, 3 H, 5-CH₃), 3.36 (q, 2 H, CH₂CH₃), 3.54 (s, 3 H, 4'-OCH₃), 3.68 (s, 6 H, 3',5'-OCH₃), 4.45 (s, 2 H, CH₂N), 5.99 (s, 2 H, 2',6'-H), 6.61 (br s, 2 H, 4-NH₂), 7.37 (br s, 2 H, 2-NH₂), 8.30 (s, 1 H, 7-H). Anal. (C₂₀H₂₆N₆O₃·1.0H₂O) C, H, N.

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A facile and efficient asymmetric synthesis of (+)-salsolidine

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Abstract

A three-key step methodology involving a highly selective asymmetric addition of an organolithium reagent to an *N*-naphthalenylimine, cyclization and oxidative removal of the *N*-naphthalenyl group provided a facile and efficient synthetic way to (+)-salsolidine. © 2000 Elsevier Science Ltd. All rights reserved.

Biological activity, especially that related to the pathogenesis of Parkinson's disease, has recently renewed interest in tetrahydroisoquinolines (TIQs) and related natural isoquinoline alkaloids.^{1,2} Since the enantiomers were observed to vary in activity,³ the synthesis of chiral 1-substituted TIQ is the current focus in medicinal organic chemistry.⁴ We have previously reported an approach to asymmetric synthesis of chiral 1-methyl and 1-phenyl TIQs based on a chiral external ligand-mediated asymmetric 1,2-addition reaction of organolithium reagents with the corresponding *N*-*p*-methoxyphenyl (PMP) imines.⁵ Extension of the method to an alkaloid natural product, salsolidine **1**, incurred several crucial problems such as moderate enantioselectivity and poor efficiency in oxidative removal of the *N*-PMP group.⁶ We describe herein a facile asymmetric synthesis of (+)-salsolidine **1** by employing highly efficient asymmetric addition of an organolithium reagent to *N*-1-naphthalenylimine and a new procedure for oxidative removal of the *N*-aryl group.^{7,8}

At first, we examined asymmetric addition reaction of methyllithium with imines **2** bearing substituted phenyl and naphthalenyl groups as an *N*-substituent, Ar (Fig. 1). The reaction was conducted in the presence of a stoichiometric amount of **3** in toluene at –95°C for 0.5 h to give the corresponding amines **4** in reasonably high chemical yields (Table 1). The enantioselectivity was not very high for **2a,b** bearing substituted phenyl groups as Ar (entries 1 and 2). We were very pleased to find that **2d–f** bearing substituted 1-naphthalenyl groups were converted to the amines **4d–f** (R = Me) in quite high yields, 97–94% (entries 4–6). It is interesting to note that enantioselectivity rises from 73 to 97% in the reverse order of electronegativity of the 4-substituent on the naphthalene, Cl, H, NMe₂ and OMe. This indicates that loss of electrophilicity of the imine gives rise to higher enantioselectivity, probably due to formation of a tight, three-component

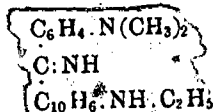
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1906

Ammoniak unter Zusatz von genügend Alkohol wieder gefällt; der Alkohol hält das Methylanilin in Lösung. Das Auramin scheidet sich so in hellgelblichen Flocken ab. Durch Krystallisation aus Alkohol wird dasselbe rein erhalten.

Es krystallisirt in grossen, schwach gelblichen Prismen vom Schmp. 199—200°. Es ist in Alkohol, Benzol und Toluol in der Wärme ziemlich leicht löslich, in der Kälte schwer, leicht löslich in Salzsäure und Essigsäure mit gelber Farbe. Seide, tannirte Baumwolle und Wolle werden intensiv orange angefärbt. Gegen Säuren, besonders aber gegen Wasser, ist dieses Auramin weit beständiger als das typische. Alkalien hingegen spalten es leicht zum Keton.

Seiner Bildung entsprechend hat es die Formel:



$\text{C}_{21}\text{H}_{23}\text{N}_3$. Ber. C 79.49, H 7.26, N 13.25.
Gef. » 79.22, » 7.35, » 13.33.

Das Chlorhydrat wurde dargestellt durch Auflösen der freien Base in Benzol und Fällen mit Salzsäuregas als ziegelrotes Pulver. Durch Umkrystallisiren aus Alkohol, in welchem es in der Kälte ziemlich schwer löslich ist, erhält man rothe, prismatische Kryställchen. In Wasser ist es ziemlich löslich. Es entspricht der Formel $\text{C}_{21}\text{H}_{23}\text{N}_3 \cdot \text{HCl}$. (Ber. Cl 10.04. Gef. Cl 10.14.)

Das Auramin mit Phenyl-naphtylamin wird in ähnlicher Weise erhalten.

Die erhaltene Rohschmelze wird nach dem Zerkleinern in absolutem Alkohol gelöst, filtrirt und in der Kälte trocknes Ammoniak eingeleitet. Die Spaltung geht etwas langsamer als bei dem soeben beschriebenen Product. Nachdem die tiefrothe Lösung grüngelb geworden ist, wird noch einige Zeit stehen gelassen und filtrirt. Das zurückbleibende Product wird gut auf Thon abgepresst und sodann mit Wasser behandelt. Das Auramin bleibt als orange gefärbte Masse zurück. Die alkoholische Lösung wird durch Verdunstenlassen auf ein kleineres Volumen gebracht, wodurch wieder ein guter Theil des Auramins ankrystallisirt. Die Mutterlaugen werden mit verdünnter Salzsäure versetzt und mit Wasser verdünnt. Dabei fällt das salzsaure Salz in hellgelben Flocken aus. Das Chlorhydrat wird in 50-proc. Essigsäure gelöst und in kaltes Wasser gegossen. Mit verdünntem Ammoniak fällt zuerst etwas harziges Product aus, von welchem abfiltrirt wird; alsdann kann durch weiteren Ammoniakzusatz die Base in schwach gelblichen Flocken erhalten werden. Durch zweimaliges Umkrystallisiren gewinnt man das Product rein in grossen, schwach gelben Prismen vom Schmp. 186°.

In Alkohol ist es in der Wärme mit gelber Farbe löslich, in Benzol löst es sich schwerer; die Lösung ist kaum gefärbt. Mit

1907

Essigsäure geht das Auramin mit intensiv grüngelber Farbe in Lösung. Seide, tannirte Baumwolle und Wolle werden sehr ausgiebig orange angefärbt.

Wir treffen auch hier die grössere Beständigkeit gegen verdünnte Säuren und Wasser in der Wärme. Alkalien aber spalten leicht und glatt in das entsprechende Keton.

Die Formel ist analog der vorherigen: $C_6H_4.N(CH_3)_2.C(=NH).C_{10}H_6.NH.C_6H_5$.

$C_{25}H_{23}N_3$. Ber. C 82.19, H 6.30, N 11.5.
Gef. » 82.43, » 6.52, » 11.5.

Das Chlorhydrat, wie bei dem vorherigen dargestellt, bildet carminrothe Prismen, die in Wasser äusserst schwer, in Alkohol leichter löslich sind.

$C_{25}H_{23}N_3.HCl$. Ber. Cl 8.84. Gef. Cl 8.86.

Für das Auramin $N(CH_3)_2.C_6H_4.C(=NH).C_{10}H_6.NH.C_6H_5$ wird in ganz analoger Weise verfahren. Das Auramin krystallisiert aus Benzol in hellgelben Prismen vom Schmp. 164—165°.

$C_{26}H_{25}N_3$. Ber. C 82.32, H 6.6, N 11.08.
Gef. » 82.03, » 6.6, » 11.25.

Das Chlorhydrat bildet rote Prismen, die in Wasser fast unlöslich sind, sich aber gut in Alkohol lösen.

$C_{26}H_{25}N_3.HCl$. Ber. Cl 8.54. Gef. Cl 8.44.

Durch den Ersatz eines Benzolkerns im gewöhnlichen Auramin durch einen Naphtalinkern wird also in allen Fällen die Nuance von gelb nach orange verschoben und die Säurebeständigkeit erheblich erhöht.

Behandelt man die Diamidonaphtylphenylketone, analog dem D. R.-P. 27789, mit tertiären, fettaromatischen Aminen (Dimethylanilin etc.), so erhält man die Farbstoffe der Victoriablaugruppe, welche man aber natürlich zweckmässiger aus Michler'schem Keton darstellt. Lässt man secundäre Naphtylamine bei Gegenwart von Phosphoroxchlorid einwirken, so resultiren die bislang noch nicht bekannten Farbstoffe der Dinaphtyl-phenyl-methan-Reihe.

Die von mir und meinen Mitarbeitern untersuchten Farbstoffe und Leukobasen sind in den folgenden Tabellen zusammengestellt.

Die Darstellungsmethoden sind die typischen, bekannten; ich glaube deshalb, dass es zu weit führen würde, sie bei jedem einzelnen Körper genau anzugeben. Ich werde mich demnach begnügen, sie an einigen Beispielen zu erläutern.

268

1908

Diphenyl-Naphtyl-Methan-Derivate.

No.	Leukobasen	Darstellung	Eigenschaften und Analyse	Mitarbeiter
1	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH_2$ $C_6H_4.N(CH_3)_2$	Condensation von Tetramethyldiamidobenzhydrol mit α -Naphtylamin in alkoholischer Lösung bei Gegenwart von Salzsäure	Schmp. 221—222°, glänzende Blättchen, sehr schwer löslich in Alkohol leichter in Benzol und Chloroform. Durch Oxydation geht der Körper leicht in einen blauen Farbstoff über. $C_{27}H_{29}N_3$ ber. gef. C 82.03 82.07 pCt. H 7.34 7.61 » N 10.63 10.90 »	Vergl. B. A. S. F. Patent No. 27032 Friedl. I, 75. Freyss
2	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.COCH_3$ $C_6H_4.N(CH_3)_2$	Durch Acetylieren der obigen Verbindung	Schmp. 228—229°, kleine, farblose Krystalle, sehr schwer löslich in Alkohol, leichter in Essigester, durch Oxydation grüner Farbstoff. $C_{29}H_{31}ON_3$ ber. gef. C 79.63 79.67 pCt. H 7.10 7.48 » N 9.61 9.88 »	Freyss
3	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.CH_3$ $C_6H_4.N(CH_3)_2$	1. Durch Reduction des entsprechenden Farbstoffes mit Zink und Essigsäure. 2. Durch Condensation von Tetramethyldiamidobenzhydrol mit Methyl- α -Naphtylamin	Schmp. 201—202°, glänzende, farblose Blättchen, luftbeständig, durch Oxydation blauer Farbstoff. $C_{28}H_{31}N_3$ ber. gef. C 82.15 81.93 pCt. H 7.58 7.72 » Farbstoff No. 19	Bourry
4	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_2H_5$ $C_6H_4.N(CH_3)_2$	1. Durch Condensation von Tetramethyldiamidobenzhydrol mit Aethyl- α -Naphtylamin. 2. Durch Reduction des entsprechenden Farbstoffes	Schmp. 172—173°, farblose Prismen, in Lösung stark fluorescirend, durch Oxydation resultirt ein blauer Farbstoff. $C_{29}H_{33}N_3$ ber. gef. C 82.26 81.94 pCt. H 7.80 7.97 » N 9.94 10.11 » Farbstoff No. 20	1. Serra. 2. Dreyfus

No.	Leukobasen	Darstellung	Eigenschaften und Analyse	Mitarbeiter
5	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_2H_5$ $C_6H_4.N(CH_3)_2$	1. Reduction von Victoriablau. 2. Reduction des Farbstoffes, erhalten durch Condensation des gemischten Ketons mit Dimethylanilin. 3. Condensation von Hydrol mit Phenyl-naphtyl-amin, D. R.-P. 66792.	Schmp. 167—168°, farblose Krystalle, durch Oxydation blauer Farbstoff. $C_{32}H_{33}N_3$ ber. gef. C 84.07 83.68 pCt. H 7.01 7.41 » Farbstoff No. 21	Serra. Vergl. auch Nathanson und Müller, diese Berichte 22, 1891 [1889]
Das von Nathanson und Müller erhaltene Product, Schmp. 125°, muss sehr unrein gewesen sein.				
6	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_7H_7$ $C_6H_4.N(CH_3)_2$	1. Durch Reduction des Farbstoffes, erhalten durch Condensation des gemischten Ketons mit Dimethylanilin. 2. Durch Condensation von Tetramethyldiamidobenzhydrol mit p-Tolyl- α -naphtylamin. 3. Reduction des Farbstoffes aus Michler's Keton mit p-Tolyl- α -naphtyl-amin	Schmp. 193—194°, kleine, weisse, lichtbrechende Prismen, schwer löslich in Alkohol, leichter löslich in Essigester mit blauer Fluorescenz. Durch Oxydation blauer Farbstoff. $C_{34}H_{35}N_3$ ber. gef. C 84.12 84.22 pCt. H 7.22 7.39 » N 8.66 8.60 » Farbstoff No. 22	Serra
7	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6(NH_2)_2$ $C_6H_4.N(CH_3)_2$	Condensation von Tetramethyldiamidobenzhydrol mit o-Naphtylen-diamin	Schmp. 233—234°, kleine, weisse Nadeln aus Benzol. Oxydation führt zu einem grünblauen Farbstoff. $C_{27}H_{20}N_4$ ber. gef. C 79.02 79.11 pCt. H 7.32 7.50 » N 13.66 13.32 »	Freyss

1910

No.	Leukobasen	Darstellung	Eigenschaften und Analyse	Mitarbeiter								
8	$\begin{array}{c} \text{C}_6\text{H}_4 \cdot \text{N}(\text{CH}_3)_2 \\ \text{H} \cdot \text{C} \cdot \text{C}_{10}\text{H}_5(\text{NH} \cdot \text{COCH}_3)_2 \\ \text{C}_6\text{H}_4 \cdot \text{N}(\text{CH}_3)_2 \end{array}$	Durch Acetylieren des obigen Körpers	Schmp. 258—259°, kleine, weisse Kryställchen, an der Luft nach längerem Stehen färbensie sich grün, schwer löslich in Alkohol, leichter in Essigester; durch Oxydation resultirt ein grüner Farbstoff. $\text{C}_{31}\text{H}_{34}\text{O}_2\text{N}_4$ <table><tr><td>ber.</td><td>gef.</td></tr><tr><td>C 75.30</td><td>75.21 pCt.</td></tr><tr><td>H 6.39</td><td>7.45 »</td></tr><tr><td>N 11.34</td><td>11.37 »</td></tr></table>	ber.	gef.	C 75.30	75.21 pCt.	H 6.39	7.45 »	N 11.34	11.37 »	Freyss
ber.	gef.											
C 75.30	75.21 pCt.											
H 6.39	7.45 »											
N 11.34	11.37 »											
9	$\begin{array}{c} \text{C}_6\text{H}_4 \cdot \text{N}(\text{CH}_3)_2 \\ \text{H} \cdot \text{C} \cdot \text{C}_{10}\text{H}_5 < \begin{array}{l} \text{N} : \text{C} \cdot \text{C}_6\text{H}_4 \\ \text{N} : \text{C} \cdot \text{C}_6\text{H}_4 \end{array} \\ \text{C}_6\text{H}_4 \cdot \text{N}(\text{CH}_3)_2 \end{array}$	Durch Condensation des Diaminderivates mit Phenanthrenchinon	Schmp. oberhalb 336°, hellgelbe, mikroskopische Nadelchen aus Benzol, fast unlöslich in Alkohol, Oxydation erzeugt einen grünen Farbstoff. $\text{C}_{41}\text{H}_{34}\text{N}_4$ <table><tr><td>ber.</td><td>gef.</td></tr><tr><td>C 84.54</td><td>84.44 pCt.</td></tr><tr><td>H 5.74</td><td>6.09 »</td></tr><tr><td>N 9.62</td><td>9.98 »</td></tr></table>	ber.	gef.	C 84.54	84.44 pCt.	H 5.74	6.09 »	N 9.62	9.98 »	Freyss
ber.	gef.											
C 84.54	84.44 pCt.											
H 5.74	6.09 »											
N 9.62	9.98 »											

Dinaphtyl-Phenyl-Methan-Derivate.

10	$\begin{array}{c} \text{C}_6\text{H}_4 \cdot \text{N}(\text{CH}_3)_2 \\ \text{H} \cdot \text{C} \cdot \text{C}_{10}\text{H}_6 \cdot \text{NH} \cdot \text{CH}_3 \\ \text{C}_{10}\text{H}_6 \cdot \text{NH} \cdot \text{CH}_3 \end{array}$	1. Condensation von Dimethyl-<i>p</i>-amidobenzaldehyd mit Methyl-α-Naphtylamin. 2. Durch Reduc-tion des entsprechenden Farbstoffes	Schwach gelbliches, amorphes Pulver, zeigt keinen scharfen Schmelzpunkt, leicht in organischen Lösungsmitteln löslich, durch Oxydation resultirt ein blauer Farbstoff. $\text{C}_{31}\text{H}_{31}\text{N}_3$ <table border="0"> <tr> <td>ber.</td> <td>gef.</td> </tr> <tr> <td>C 83.58</td> <td>83.34 pCt.</td> </tr> <tr> <td>H 6.97</td> <td>7.17 »</td> </tr> <tr> <td>N 9.44</td> <td>9.67 »</td> </tr> </table> Farbstoff No. 23	ber.	gef.	C 83.58	83.34 pCt.	H 6.97	7.17 »	N 9.44	9.67 »	1. Demant. 2. Bourry.
ber.	gef.											
C 83.58	83.34 pCt.											
H 6.97	7.17 »											
N 9.44	9.67 »											

Obgleich es nicht gelang, die Base krystallinisch zu erhalten, ergab die Analyse doch gut stimmende Zahlen. Der dieser Leukobase entsprechende Farbstoff ist nicht analysirt worden.

1911

No.	Leukobasen	Darstellung	Eigenschaften und Analyse	Mitarbeiter
11	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_2H_5$ $C_{10}H_6.NH.C_2H_5$	1. Condensation von Dimethyl-p-amidobenzaldehyd mit Aethyl- α -naphthylamin. 2. Reduction des entsprechenden Farbstoffes	Schmp. 220°, farblose, derbe Prismen, schwer löslich, leicht in Pyridin, Oxydation erzeugt einen blauen Farbstoff. $C_{33}H_{35}N_3$ ber. gef. C 83.72 83.48 pCt. H 7.40 7.46 " N 8.88 8.92 " Farbstoff No. 24	Demant
12	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_6H_5$ $C_{10}H_6.NH.C_6H_5$	1. Condensation von Dimethyl-p-amidobenzaldehyd mit Phenyl- α -naphthylamin. 2. Reduction des entsprechenden Farbstoffes	Weisses, amorphes Pulver, zeigt keinen scharfen Schmelzpunkt, in organischen Lösungsmitteln mit blauer Fluorescenz löslich, durch Oxydation blauer Farbstoff. $C_{41}H_{35}N_3$ ber. gef. N 7.38 N 7.46 pCt. Farbstoff No. 25	Demant
13	$C_6H_4.N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_6H_4.CH_3$ $C_{10}H_6.NH.C_6H_4.CH_3$	1. Condensation von Dimethyl-p-amidobenzaldehyd mit p-Tolyl- α -naphthylamin. 2. Reduction des entsprechenden Farbstoffes	Aehnliche Eigenschaften wie obiger Körper. $C_{43}H_{39}N_3$ ber. gef. C 86.43 86.20 pCt. H 6.53 6.62 " N 7.04 7.15 " Farbstoff No. 26	Demant
14	$C_6H_3(NO_2).N(CH_3)_2$ $H.C.C_{10}H_6.NH.CH_3$ $C_{10}H_6.NH.CH_3$	Condensation von 3-Nitro-4-dimethylamido-benzaldehyd mit Methyl- α -naphthylamin	Gelb-oranges, amorphes Pulver ohne scharfen Schmelzpunkt, leicht in organischen Lösungsmitteln löslich, Oxydationsmittel führen die Leukobase nur langsam in einen violettstichig blauen Farbstoff über. $C_{31}H_{30}O_2N_4$ ber. gef. N 11.42 N 11.25 pCt.	Demant
15	$C_6H_3(NO_2).N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_2H_5$ $C_{10}H_6.NH.C_2H_5$	Condensation von 3-Nitro-4-dimethylamido-benzaldehyd mit Aethyl- α -naphthylamin	Schmp 200°, rothe Prismen, schwer löslich in Alkohol und Benzol, leichter löslich in Eisessig, Pyridin und Anisol, Oxydation giebt ein violettstichiges Blau. $C_{33}H_{34}O_2N_4$ ber. gef. C 76.44 76.10 pCt. H 6.56 6.65 " N 10.81 10.65 "	Demant

1912

No.	Leukobasen	Darstellung	Eigenschaften und Analyse	Mitarbeiter
16	$C_6H_3(NO_2).N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_6H_5$ $C_{10}H_6.NH.C_6H_5$	Condensation von 3-Nitro-4-dimethylamido-benzaldehyd mit Phenyl- α -naphtylamin	Orangefarbenes, amorphes Pulver ohne scharfen Schmp., leicht löslich in Benzol und Eisessig, schwerer dagegen in Alkohol und Ligroin, durch Oxydationsmittel nur langsam angreifbar, giebt ein violettstichiges Blau. $C_{41}H_{34}O_2N_4$ ber. gef. C 80.13 79.80 pCt. H 5.54 5.70 » N 9.12 9.24 »	Demant
17	$C_6H_3(NO_2).N(CH_3)_2$ $H.C.C_{10}H_6.NH.C_6H_4.CH_3$ $C_{10}H_6.NH.C_6H_4.CH_3$	Durch Condensation von 3-Nitro-4-dimethylamidobenzaldehyd mit p-Tolyl- α -naphtylamin	Orangefarbenes, amorphes Pulver ohne scharfen Schmp., leicht in Benzol, Eisessig löslich, schwer in Alkohol, Ligroin, Oxydation wie vorhergehender Körper. $C_{43}H_{38}O_2N_4$ ber. gef. C 80.37 80.14 pCt. H 5.92 6.20 » N 8.72 8.65 »	Demant
Trinaphtyl-Methan-Derivate.				
18	$H.C(C_{10}H_6.NH.C_2H_5)_2$	1. Durch Reduction des Farbstoffes. 2. Condensation von Orthoameisensäureäthylester mit Aethyl- α -naphtylamin in Gegenwart von Chlorzink	Schmp. oberhalb 300°, farblose, glänzende Nadeln, an der Luft sich grün färbend, in heissem Alkohol ziemlich leicht löslich, sublimirbar. Oxydationsmittel greifen schwer an und führen in den blauen Farbstoff über. $C_{37}H_{37}N_3$ ber. gef. C 84.89 84.99 pCt. H 7.08 7.23 » N 8.03 7.23 » Farbstoff Nr. 27	Demant
No.	Farbstoffe	Darstellung	Eigenschaften und Analyse	Mitarbeiter
Diphenyl-Naphtyl-Methan-Derivate.				
19	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.CH_3$ $C_6H_4.N(CH_3)_2$	1. Condensation von Michler's Keton mit Methyl- α -naphtylamin. 2. Oxydation der Leukobase	Grünschillernde Krystalle, blauer Farbstoff. $C_{28}H_{20}N_2Cl$ ber. gef. C 75.76 74.76 pCt. H 6.76 6.89 » N 9.47 9.31 » Leukobase No. 3.	Bourry

1913

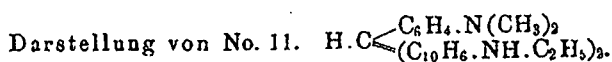
No.	Farbstoffe	Darstellung	Eigenschaften und Analyse	Mitarbeiter
20	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_2H_5$ $C_6H_4.N(CH_3)_2$	1. Condensation von Michler's Keton mit Aethyl- α -naphthylamin. 2. Condensation des Ketons No. II mit Dimethylanilin. Der nach diesem Verfahren erhaltene Farbstoff unterscheidet sich nicht von dem nach 1. dargestellten und gibt bei der Reduction dieselbe Leukobase No. 4. 3. Oxydation der Leukobase	Grün-schillernde Krystalle, blauer Farbstoff, als Neuvictoriablau bekannt Leukobase Nr. 4	Serra, Demant. Entdecker Nastvogel, (Farbenfabrik Bayer)
Die Farbbase, Schmp. 183—184°, bildet rothbraune, glänzende Kryställchen, leicht löslich in Alkohol, Benzol, schwer in Ligroin.				
21	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_6H_5$ $C_6H_4.N(CH_3)_2$	1. Condensation von Michler's Keton mit Phenyl- α -naphthylamin. 2. Condensation des gemischten Ketons mit Dimethylanilin. 3. Oxydation der Leukobase	Kupferbronzeglänzende Blättchen, in heissem Wasser und Alkohol leicht löslich, schwer löslich in Benzol und Aether. Victoriablau Leukobase Nr. 5	Serra. Vergl. Patent 27789 Friedl. I, 85 u. Nathanson und Müller, loc. cit.
22	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_6H_4.CH_3$ $C_6H_4.N(CH_3)_2$	1. Condensation von Michler's Keton mit <i>p</i> -Tolyl- α -naphthylamin. 2. Condensation des gemischten Ketons mit Dimethylanilin. 3. Oxydation der Leukobase	Goldglänzende Kryställchen, schwer löslich in Wasser, leichter in Alkohol, blauer Farbstoff. $C_{24}H_{24}N_2Cl$ ber. gef. C 78.61 78.11 pCt. H 6.55 6.98 » N 8.09 8.06 » Cl 6.75 6.32 » Leukobase No. 6	Serra
Dinaphthyl-Phenyl-Methan-Derivate.				
23	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.CH_3$ $C_{10}H_6.NH.CH_3$	1. Condensation des gemischten Ketons mit Methyl- α -naphthylamin. 2. Oxydation der Leukobase	Blaues Pulver, in Wasser nicht allzu schwer löslich. Wurde nicht krystallisiert erhalten. Leukobase No. 10.	

No.	Farbstoffe	Darstellung	Eigenschaften und Analyse	Mitarbeit
24	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_2H_5$ $C_{10}H_6.N.H.C_2H_5$	Condensation des gemischten Ketons mit Aethyl- α -naphthylamin. 2. Oxydation der Leukobase	Grünblaue, metallglän- zende Nadeln, schwer löslich in Wasser, leichter löslich in Alkohol und Essigsäure, blauer Farb- stoff. $C_{33}H_{34}N_3Cl$ ber. gef. N 8.27 8.03 pCt. Cl 6.99 6.82 » Leukobase No. 11	Demant
25	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_6H_5$ $C_{10}H_6.NH.C_6H_5$	1. Condensation des gemischten Ketons mit Phe- nyl- α -naphthyl- amin. 2. Oxydation der Leukobase	Grünblaue, metallglän- zende Nadeln, fast unlös- lich in Wasser, löslich in Alkohol und Eisessig, blauer Farbstoff. $C_{41}H_{34}N_3Cl$ ber. gef. N 6.96 6.93 pCt. Cl 5.88 5.98 » Leukobase No. 12	Demant
26	$C_6H_4:N(CH_3)_2Cl$ $C.C_{10}H_6.NH.C_6H_4.CH_3$ $C_{10}H_6.NH.C_6H_4.CH_3$	1. Condensation des gemischten Ketons mit p- Tolyl- α -naphthyl- amin. 2. Oxydation der Leukobase	Dunkelblau metallglän- zende Nadeln, unlöslich in Wasser, leicht in Al- kohol und Eisessig lös- lich, blauer Farbstoff. $C_{43}H_{38}N_3Cl$ ber. gef. N 6.65 6.65 pCt. Cl 5.62 5.80 » Leukobase No. 13	Demant
Trinaphthyl-Methan-Derivate.				
27	$C_{10}H_6:N.C_2H_5.HCl$ $C.C_{10}H_6.NH.C_2H_5$ $C_{10}H_6.NH.C_2H_5$	1. Condensation von Tetrachlor- kohlenstoff mit Aethyl- α -naphthylamin in Gegenwart von Chlor- aluminium. 2. Oxydation der Leukobase	Blaue, metallglänzende Nadeln, unlöslich in Wasser, schwer löslich in Alkohol, leichter in Eis- essig, blauer Farbstoff. $C_{37}H_{32}N_3Cl$ ber. gef. N 7.53 7.77 pCt. Cl 6.37 6.03 » Leukobase No. 18	Demant

Ueber die Darstellung der Leukobasen ist nicht viel zu bemerken.
Wenn das mit dem Hydrol zu condensirende Amin in wässriger Salz-
säure nicht löslich ist (z. B. Tolyl-naphthylamin), so arbeitet man in
alkoholischer Lösung. Dass man beim Ausfällen der Leukobasen aus

ihier sauren Lösung Erwärmung vermeiden muss, ist genügend bekannt.

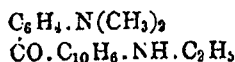
Die Condensation von Dimethylamino- und Nitro-*p*-dimethylamido-Benzaldehyd mit den secundären Naphtylaminen wird in alkoholisch-salzsaurer Lösung ausgeführt.



Ein Mol.-Gew. Dimethyl-*p*-amidobenzaldehyd, etwas mehr als zwei Mol.-Gew. Aethyl- α -naphtylamin und ein Mol.-Gew. concentrirte Salzsäure wurden in alkoholischer Lösung 7—10 Stdn. am Rückflusskühler erhitzt. Das Condensationsproduct scheidet sich zum guten Theile schon in der Wärme in krystallinischer Form ab. Man lässt die Lösung erkalten, verdünnt das Filtrat mit Wasser und fällt den Rest mit Ammoniak. Man presst auf Thon ab und krystallisirt aus Pyridin, in welchem der gelbliche Körper ziemlich leicht löslich ist.

Das Rohproduct wird in einer genügenden Menge reinen Pyridins gelöst, mit soviel Alkohol versetzt, dass es eben noch in Lösung bleibt, bis zur eintretenden Trübung mit Wasser vermischt und hierauf erkalten gelassen. Die Leukobase scheidet sich in farblosen, derben Prismen aus, welche zur Entfernung anhaftenden Pyridins mit Alkohol gewaschen werden; Schmp. 220°.

Die Leukobase ist in Alkohol, Benzol und Toluol ziemlich schwer löslich, mit Mineralsäuren und Essigsäure bildet sie lösliche Salze. Mit Oxydationsmitteln, am bequemsten in eisessigsaurer Lösung mit Chloranil, tritt nach kurzem Erwärmen intensive Blaufärbung ein, unter Bildung eines Farbstoffes, welcher sich in seinen Eigenschaften als identisch erwies mit demjenigen, der aus dem Keton:



mit $\text{C}_{10}\text{H}_6.\text{NH.C}_2\text{H}_5$ und POCl_3 erhalten wurde (No. 23).

Bei den Reduction dieses Farbstoffes in essigsaurer Lösung entsteht dieselbe Leukobase, die durch Vergleich der Eigenschaften und des Schmelzpunktes (Mischprobe) identificirt wurde.

Die Leukobasen 10, 12 und 13 wurden in analoger Weise dargestellt; es gelang aber nicht, sie krystallinisch zu erhalten. Nach nochmaligem Lösen in Alkohol und Ausfällen mit Wasser bilden sie weisse, amorphe Flocken, die aber doch sehr rein sein müssen, da sie bei der Analyse gute Zahlen liefern. Die phenylirte und tolylirte Base oxydiren sich sehr schlecht mit Bleisuperoxyd, gut mit Chloranil

1916

Darstellung von No. 15. $\text{H.C} \begin{matrix} \text{C}_6\text{H}_3(\text{NO}_2).\text{N}(\text{CH}_3)_2 \\ \text{(C}_{10}\text{H}_5.\text{NH.C}_2\text{H}_5)_2 \end{matrix}$.

10 g Nitrodimethylparamidobenzaldehyd, 19 g Aethyl-naphtylamin und 5 g concentrirter Salzsäure werden in alkoholischer Lösung auf dem Wasserbade erhitzt.

Die Reaction erfolgt rasch und ist schon nach einigen Stunden beendet. Das gelbbraune Product wird nach dem Erkalten abfiltrirt und auf Thon gepresst. Man löst es in Pyridin, verdünnt mit Alkohol und giebt Wasser zu bis zur eintretenden Trübung. Beim Erkalten krystallisirt die Leukoverbindung in rothen Prismen vom Schmp. 200° aus. Sie ist in den gewöhnlichen Lösungsmitteln sehr schwer löslich. Eine Lösung in concentrirter Schwefelsäure oder Salzsäure lässt beim Verdünnen mit Wasser die unveränderte Base wieder ausfallen. In Eisessig in der Wärme ist die Verbindung ziemlich leicht löslich. Chloranil oxydirt den Leukokörper ziemlich leicht zu dem entsprechenden Farbstoff, indem die Lösung eine violettstichig blaue Farbe annimmt, während das nicht nitrirte Product eine rein blaue Färbung zeigt.

Die Basen 14, 16 und 17, in analoger Weise dargestellt, wurden nicht krystallinisch erhalten. Zur Analyse wurden sie durch mehrmaliges Lösen in Benzol und Ausfällen mit Ligroin gereinigt.

Die phenylirten und tolylirten Basen werden selbst durch Chloranil nur sehr schwer oxydirt. Bemerkenswerth ist die intensiv rothe Farbe dieser Leukobasen; sie haben durch die Einführung der Nitrogruppe sogar einen gewissen Farbstoffcharakter erhalten, denn sie färben Seide und tannirte Baumwolle orange.

Leukobase No. 18. $\text{H.C}(\text{C}_{10}\text{H}_5.\text{NH.C}_2\text{H}_5)_3$.

Durch Condensation von Orthoameisensäureester, $\text{CH}(\text{OC}_2\text{H}_5)_3$, mit Aethyl-naphtylamin bei Gegenwart von Chlorzink lässt sich diese Base darstellen, aber es gelang nicht, das auf diese Weise erhaltene Product in krystallinische Form zu bringen. Stellt man sie dagegen durch Reduction des Farbstoffes (No. 27) dar, so gelingt es unschwer, sie zu krystallisiren.

Es empfiehlt sich, von vollständig reinem Material auszugehen, da sonst das Reductionsproduct in harziger, schwer zu reinigender Form erhalten wird. Den Farbstoff löst man in Eisessig, verdünnt mit etwas Essigsäure (50 pCt.) und trägt Zinkstaub bis zur Entfärbung ein. Nun filtrirt man und versetzt mit Wasser. Die Lösung trübt sich milchig und setzt beim längeren Stehen die Leukobase in farblosen Prismen ab. Behufs vollständiger Fällung der Base wird die Lösung mit Chlorammonium und Ammoniak versetzt, wobei sich das Reductionsproduct in weissen Flocken abscheidet. Dieselben werden am bequemsten aus Alkohol umkrystallisirt.

Man erhält so silberglänzende, farblose Nadeln, welche sich am Lichte schwach grünlich färben. Bei 280° bräunt sich der Körper sehr stark und ist bei 290° noch nicht geschmolzen. Bei sehr hoher Temperatur schmilzt er und sublimirt. Chloranil in Eisessig oxydirt langsam und nach längerem Erhitzen zu einem blauen Farbstoff.

Was den Farbstoff (No. 27) selbst anbetrifft, so erhält man ihn am besten nach der Friedel-Crafts'schen Reaction.

11 g Tetrachlorkohlenstoff, 10 g Aluminiumchlorid und 45 g Aethyl- α -naphthylamin wurden während 6 Stunden am Rückflusskühler erhitzt. Es ist zweckmässig, einen ziemlichen Ueberschuss an Amin zu verwenden, da hierdurch eine wesentlich bessere Ausbeute erzielt wird. Die Reaktionsmasse färbt sich allmählich dunkelblau und ballt sich schliesslich zu einer harten, metallglänzenden, dunkelblauen Masse zusammen. Sie wird zerkleinert und während ungefähr einer Stunde mit verdünnter Salzsäure ausgekocht, wobei der Farbstoff, der von flüssiger Consistenz ist, von Chloraluminium und Amin befreit wird.

Es wurde nun versucht, den nach dem Erkalten erstarrten Farbstoff zu krystallisiren, jedoch ohne Erfolg. Ebenso wenig gelang es, die Farbbase zu krystallisiren, jedoch war es möglich, den Farbstoff daraus rein zu erhalten. Das Rohproduct, welches in einer Ausbeute von ungefähr 70—80 pCt. erhalten wird, löst man in Alkohol und versetzt mit Alkali. Die Base scheidet sich beim Verdünnen mit Wasser vollständig in ziegelrothen Flocken aus, welche auf Thon scharf getrocknet werden. Sie wurde zur weiteren Reinigung in Benzol gelöst und mit Ligroin wieder gefällt. Bemerkenswerth ist, dass dieselbe nicht farblos, sondern ziegelroth ist. Aus derselben wird der Farbstoff regenerirt. Zu diesem Zwecke wird sie in Benzol gelöst und trocknes Salzsäuregas eingeleitet. Das salzsaure Salz, wahrscheinlich ein Di- und Tri-Chlorhydrat fällt in grau-grünen Flocken aus, welche abfiltrirt, mit Benzol gewaschen und auf Thon abgepresst werden.

Löst man die sehr hygroscopische Masse in Alkohol oder übergiesst sie damit, so erstarrt dieselbe zu einem Brei von tiefblauen Nadeln mit prächtig grünem Reflex. Dieselben sind in Wasser vollständig unlöslich, schwer löslich in Alkohol, leichter in Eisessig und werden am bequemsten aus demselben umkrystallisirt. Man sieht aus diesem Beispiel zur Genüge, welchen Schwierigkeiten man oft begegnet, um Körper aus diesem Gebiete in krystallisirtem Zustande zu erhalten.

Der Farbstoff färbt Seide, Wolle und tannirte Baumwolle blau an, jedoch kann man zu keiner befriedigenden Nuance gelangen, weil er in Wasser unlöslich ist. Es ist von Vortheil, dem Färbebade etwas Acetin zuzusetzen.

In Acetin gelöst und mit Tannin und Verdickungsmittel versetzt, lässt sich der Farbstoff dagegen ganz gut aufdrucken. Er ist ausserordentlich schwer verbrennlich, sodass keine gut stimmenden Kohlenstoffzahlen

1918

erhalten wurden, während die Leukobase zufriedenstellende Resultate ergab.

Ein qualitativer Versuch zeigte, dass auch aus Tetrachlorkohlenstoff und Phenyl-naphtylamin der entsprechende Farbstoff erhalten werden kann.

Was die Farbstoffe der Naphtyl-diphenyl-methan-Reihe. (No. 19, 20, 21 und 22) anbelangt, so kann man sie erhalten 1. nach Analogie des D. R.-P. 27789, durch Condensation des Michler'schen Ketons mit den secundären Naphtylaminen, 2. aus den gemischten Ketonen mit Dimethylanilin, 3. durch Oxydation der Leukobasen. Methode 1 ist bei weitem die vorteilhafteste und bequemste, auch liefert sie sofort krystallisierte Producte. Die Farbstoffe der Dinaphtyl-phenyl-methan-Reihe werden aus den gemischten Ketonen dargestellt.

Die Arbeitsweise ist folgende:

No. 24: 5 g Keton II wurden mit 5 g Aethyl- α -naphtylamin und 5 g Phosphoroxychlorid einige Stunden auf dem Wasserbade erwärmt. Es tritt sofort intensive Blaufärbung ein, und nach einiger Zeit erstarrt die Schmelze zu einer dunkelblaugrünen, metallglänzenden, spröden Masse. Dieselbe wird zerkleinert, mit verdünnter Salzsäure einige Zeit aufgekocht, filtrirt und aus etwas salzsäurehaltigem Alkohol umkrystallisirt. Der Farbstoff wird in prachtvollen, grünblauen, metallisch schimmernden Nadelchen erhalten, welche nach nochmaligem Umkrystallisiren rein sind.

Der Farbstoff ist unlöslich in Wasser, löslich in Alkohol und Essigsäure und färbt Wolle, Seide und tannirte Baumwolle blau. No. 23 wurde in der gleichen Weise dargestellt, konnte aber nicht krystallisirt werden.

No. 25: 5 g Keton III werden mit 5 g Phenyl- α -naphtylamin innig vermischt und 5 g Phosphoroxychlorid zugefügt. Die Reaction erfolgt schon in der Kälte unter beträchtlicher Erwärmung und intensiver Blaufärbung. Zur Beendigung der Condensation erhitzt man 3–5 Stdn. auf dem Wasserbade. Die harte, grünblaue, metallglänzende Masse wird zerkleinert, mit salzsäurehaltigem Wasser während einer Stunde ausgekocht und der unlösliche Farbstoff aus Alkohol mit etwas Salzsäure umkrystallisirt. Man erhält denselben in prachtvoll schillernden grünblauen Nadeln. Er ist unlöslich in Wasser, in Alkohol schwerer löslich als das äthylirte Product, leicht löslich in Eisessig. Dieses Dinaphtyl-phenyl-methanderivat färbt Seide, Wolle und tannirte Baumwolle mit tiefblauer Nuance.

No. 26: 7 g Keton IV wurden mit 5 g *p*-Tolyl- α -naphtylamin innig vermischt und 7 g Phosphoroxychlorid zugegeben. Nach 3–4-stündigem Erhitzen auf dem Wasserbade ist die Reaction beendet. Die dunkelblaue, metallglänzende Schmelze wird pulverisirt und einige

Zeit mit salzsäurehaltigem Wasser ausgekocht. Man nimmt den unlöslich zurückgebliebenen Farbstoff in Eisessig auf und fügt heisses, salzsäurehaltiges Wasser zu. Beim Erkalten erstarrt die Flüssigkeit zu einem Krystallblei von dunkelblauen, schimmernden Nadelchen. Der Körper ist unlöslich in Wasser, ziemlich schwer löslich in Alkohol, jedoch leicht in Eisessig. Seide, Wolle und tannirte Baumwolle werden dunkelblau angefärbt. Die Nuance unterscheidet sich kaum von der des entsprechenden Phenylderivates. Der Farbstoff zieht schwer; wegen seiner geringen Löslichkeit ist es deshalb empfehlenswerth, dem Färbegut Acetin zuzusetzen. Im Druck erzielt man wesentlich zufriedenstellendere Effecte.

Die Farbstoffe aus den äthylirten Ketonen V—VIII sind denjenigen aus den methylirten ganz analog, zeichnen sich aber vor denselben durch etwas grössere Löslichkeit aus.

Vom praktischen Standpunkte betrachtet, besitzen die Farbstoffe der Dinaphtyl-phenyl- und Trinaphtyl-Methan-Reihe wegen ihrer Schwerlöslichkeit und ihres verhältnissmässig hohen Herstellungspreises keine Bedeutung. In farbenchemischer Rücksicht haben sie jedoch ein gewisses Interesse. Es ist bemerkenswerth, dass der Ersatz eines Benzolkernes im Krystallviolet durch einen Naphtalinkern zu einem Blau führt, und dann die Einführung eines zweiten und dritten Naphtalinkernes die Nuance nicht mehr so erheblich verändert, sondern sie nur grünstichiger macht. Am grünsten ist der Trinaphtylfarbstoff.

Behandelt man die Naphtyldiphenylmethanfarbstoffe mit Acetanhydrid und Natriumacetat oder mit Acetanhydrid und einem Tropfen Schwefelsäure, so wird die secundäre Amidonaphtylgruppe acetyliert, und man erhält grüne Farbstoffe, welche in Wasser erheblich leichter löslich sind als die Ausgangsmaterialien. Durch Hydrolyse mit Säuren oder Alkalien werden sie wieder in die Letzteren übergeführt. Analysen derselben wurden nicht gemacht, aber nach ihrer Bildungsweise und ihren Eigenschaften unterliegt es keinem Zweifel, dass sie Acetyl-

derivate:
$$\begin{array}{l} \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2\text{Cl} \\ \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2 \\ \text{C}_{10}\text{H}_6:\text{N}(\text{C}_2\text{H}_5)\cdot\text{C}_2\text{H}_5\text{O} \end{array}$$
 u. s. w. sind.

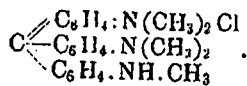
Die Leukobasen, z. B. $\text{C} \begin{array}{l} \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2 \\ \text{C}_{10}\text{H}_6:\text{NH}\cdot\text{C}_2\text{H}_5 \end{array}$, acetyliert und oxydirt, ergeben ebenfalls grüne Farbstoffe, die zweifellos mit den obigen identisch sind.

Dass das Nitrosamin des Victoriablaues, $\text{C} \begin{array}{l} \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2\text{Cl} \\ \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2 \\ \text{C}_{10}\text{H}_6:\text{N}(\text{NO})\cdot\text{C}_6\text{H}_5 \end{array}$, ein grüner Farbstoff ist, wurde von den Farbenfabriken vorm. F. Bayer & Co. schon in dem Patente 66792 gezeigt. Die gleiche

1920

Farbe besitzen, wie ich mich überzeugt habe, die Nitrosamine der drei anderen Naphtyldiphenylmethanfarbstoffe.

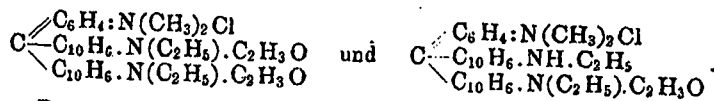
Wir haben also in diesen beiden Fällen, Acetylierung und Nitrosierung, vollkommene Analogie mit dem Pentamethylviolet,



Die Dinaphtylphenylmethanfarbstoffe, welche zwei secundäre Amidogruppen enthalten, geben bei der Acetylierung mit Acetanhydrid und etwas Schwefelsäure rothe Farbstoffe, welche in Wasser leicht löslich sind und tannirte Baumwolle und Seide roth färben, ganz ähnlich wie das Chlorid des Dimethylmonamidotriphenylcarbinols.

Acetyliert man sie in eisessigsaurer Lösung mit Acetanhydrid, so entstehen lösliche, grüne Farbstoffe, welche von gleichzeitig gebildeten kleinen Mengen der rothen durch die grössere Löslichkeit der Letzteren getrennt werden können.

Durch Hydrolyse mit Säure lassen sich aus allen beiden die blauen Ausgangsmaterialien wiedergewinnen. Die rothen Farbstoffe sind demnach ohne Zweifel die Diacetyl-, die grünen die Monoacetyl-Verbindungen, z. B.



Der Trinaphtylmethanfarbstoff lässt sich ebenfalls in eine grüne (Mono-) und rothe (Di-) Acetylverbindung verwandeln.

Mit einem Mol.-Gew. Nitrit in saurer Lösung behandelt, geben die Dinaphtylmethanfarbstoffe ebenfalls grüne, mit zwei Mol.-Gew. Nitrit dagegen rothe Farbstoffe. Aus beiden lassen sich durch Hydrolyse bei Gegenwart von Harustoff, um die salpetrige Säure, die sonst oxydirend wirken würde, zu zerstören, die blauen Farbstoffe regenerieren.

Mit einem, bzw. zwei Mol.-Gew. Nitrit lassen sich aus dem Trinaphtylderivat ebenfalls ein grüner und ein rother Farbstoff erhalten.

Mülhausen i/E. Chemieschule.

NO DRAWINGS

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P3B19C P3B19D P3B19E P3B20 P3B21 P6 P7 P7

(72) Inventor PETER TAVS



(54) METHOD OF PRODUCING ARYL AND VINYL PHOSPHONIC ACID ESTERS, ARYL AND VINYL PHOSPHONIC ACID ESTERS OR ARYL AND VINYL PHOSPHONIC OXIDES

(71) We, SUELLI INTERNATIONAL RESEARCH MATSCHAFF N.V., a company organised under the laws of The Netherlands, of 30 Carel van Bylandtlaan, The Hague, The Netherlands, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a method of producing aryl or vinyl phosphonic acid esters, aryl or vinyl phosphonic acid esters, or aryl or vinyl phosphine oxides, or for the production of mixtures which contain one or more of such phosphorus compounds.

The method according to the invention makes the phosphorus compounds to be produced more readily accessible than was hitherto the case.

Aromatic phosphonic acid esters could hitherto be obtained only to a limited extent by multistage methods. Thus they can be produced from aromatic hydrocarbons by reacting with halogen phosphines, phosphorus trichloride being utilised in addition to aluminium chloride as catalyst as a modification of the Friedel-Crafts reaction (Journal J. Am. Chem. Soc. 69 (1947), page 310), or by reacting aryl-magnesium halides having steric hindrance in the *ortho* position with dialkylchlorophosphonates (J. Org. Chem. 16, page 1250 and Vol. 16, page 207), or by phosphorylation of aromatic hydrocarbons with hexagonal phosphoric acid anhydride (U.S.A. Patent Specification 2,717,906) or with phosphorus pentasulphide (U.S.A. Patent Specification 2,847,458) at elevated temperatures. All these processes have in common a hydrolysis step which leads to reaction mixtures in which in addition to di- and higher phosphorylation products

in which Hal represents a halogen atom, R an optionally substituted aryl or vinyl group, and *n* designates 1 or 2, with a trialkyl phosphite, an alkyl alkylene phosphite, a dialkylphosphonite, or an alkylphosphinite, optionally in the presence of a solvent, at temperatures between 50 and 250°C in the presence of catalytic quantities of a nickel halide and in an atmosphere of an inert gas.

The starting materials preferably used as aromatic halides for the method according to the invention are:

R (Hal),

98%, $n_D^{20}=1.493$. Fraction 1 consisted of 19.7 g triethylphosphite and 18.2 g of phosphate/phosphonate in the ratio of 1:1.

EXAMPLE 35

Isopropylene-1,2-diphosphonic acid tetraethyl ester:

The isopropylene-1,2-diphosphonic acid tetraethyl ester was used as starting material for this experiment was obtained in accordance with the method of German Federal Patent No. 1,159,443 from isopropylene-phosphonic acid diethyl ester by bromination in tetrahydrofuran, followed by splitting off of HBr by means of sodium methylate in ethanol. 25.7 g (0.1 mol) of isopropylene-1,2-diphosphonic acid tetraethyl ester and 0.05 g (0.005 mol) of nickel chloride were heated to 160°C with the introduction of nitrogen and thereupon 0.65 g (0.05 mol) of nickel chloride dissolved in 25.0 g (0.15 mol) of triethylphosphite was added slowly with stirring. The reaction set in instantaneously. 10.0 g (93% of theory) of ethyl chloride were obtained after termination of the reaction.

The reaction mixture was allowed to cool, filtered off from nickel chloride, and then distilled.

Fraction 1: BP₁₀ 40–140°C 6.7 g
Fraction 2: BP₁₀ 140–150°C 23.2 g

After rectification of Fraction 2 it was possible to obtain 20.7 g (65% of theory) of isopropylene-1,2-diphosphonic acid tetraethyl ester, BP₁₀ 130–137°C ($n_D^{20}=1.4516$). When nickel bromide was used in a corresponding experiment, 38% of isopropylene-1,2-diphosphonic acid tetraethyl ester, BP₁₀ 150°C were obtained.

$C_{12}H_{24}P_2O_8$, calculated:

C 62.0; H 7.6; P 19.7

found:

C 61.8; H 7.6; P 19.7

EXAMPLE 36

Diphenyl phosphonate acid ethylester:

21.8 g (0.1 mol) of benzene phosphonous acid diethyl ester were added a drop at a time at 160°C with stirring, over a period of 30 minutes, to a suspension of 2.2 g (0.01 mol) of NiBr₂ in 15.7 g (0.1 mol) of bromobenzene, in an atmosphere of nitrogen. The temperature was kept at 160°C for 1 more hour. 8.7 g (79% of theory) of ethyl bromide had then collected in the cold trap. Distillation of the reaction mixture yielded 15.9 g (65% of theory) of diphenyl phosphonous acid ethylester, boiling point 148–152°C/0.01 mm.

Analysis: $C_{18}H_{18}P_2O_6$ (346.2)

calculated: C 68.3; H 5.11; P 12.6

found: C 68.1; H 5.9; P 12.7

In the same manner as in Example 36 4.4 g (0.02 mol) of NiBr₂, 18.7 g (0.1 mol) of α -bromoisobutyrate and 21.8 g (0.1 mol) of benzene phosphonous acid diethyl ester were reacted in an atmosphere of nitrogen, whereby 10.6 g (92%) of ethyl bromide were collected. The product was phenyl-p-methoxy phenyl phosphonous ethylester and its structure was confirmed as follows. The reaction mixture was boiled with reflux for 3 hours with 6 g of NaOH in 200 ml of methanol. The methanol was then drawn off, the residue taken up in water, filtered, extracted with ether, treated with activated charcoal, filtered, and acidified. Yield of phenyl-p-methoxyphenyl-phosphonic acid: 22.5 g (91%) colourless crystals

Flashpoint 182–184°C

Analysis: $C_{17}H_{18}P_2O_6$ (348.2)

calculated: C 62.9; H 5.28; P 12.5

found: C 62.9; H 5.2; P 12.7

EXAMPLE 38

In the same manner as in Example 36 2.2 g (0.01 mol) NiBr₂ in 16.3 g (0.1 mol) of 2-bromothiophene were reacted with 21.8 g (0.1 mol) of benzene phosphonous acid diethyl ester in an atmosphere of nitrogen, whereby 8.4 g (77%) of ethyl bromide were collected. The reaction product was phenyl-thiophenyl phosphonous ethylester and its structure was confirmed as follows. The reaction mixture was saponified and worked up in the same manner as in Example 37. Yield of phenyl-thiophenyl phosphonic acid: 18.2 g (82%) of colourless crystals, flashpoint 166–168°C after recrystallisation from methanol, flashpoint 172–174°C.

Analysis: $C_{11}H_8P_2O_6$ (224.2)

calculated: C 53.6; H 4.08; P 13.8; S 14.3

found: C 52.9; H 3.9; P 13.4; S 14.3

EXAMPLE 39

In the same way as in Example 36 2.2 g (0.01 mol) of NiBr₂ in 20.7 g (0.1 mol) of α -bromo-naphthalene were reacted with 21.8 g (0.1 mol) of benzene phosphonous acid diethyl ester in an atmosphere of nitrogen, whereby 9.9 g (91%) of ethyl bromide were collected. The product was phenyl- α -naphthyl phosphonous ethylester and its structure was confirmed as follows. The product was saponified and worked up as in Example 37. Yield of phenyl- α -naphthyl phosphonic acid: 25.3 g (94%) of colourless crystals, flashpoint 162–170°C, after recrystallisation from methanol, flashpoint 180–183°C. Analysis: $C_{19}H_{14}P_2O_6$ (328.2)

calculated: C 71.7; H 4.38; P 11.6

found: C 70.3; H 4.9; P 11.2

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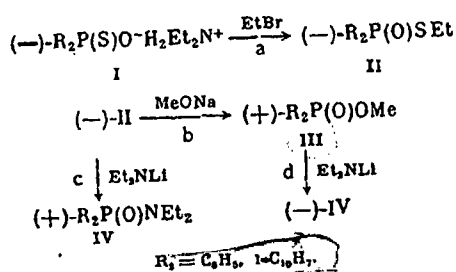
STEREOSPECIFIC TRANSITION FROM PHENYL(1-NAPHTHYL)PHOSPHINOTHIOIC
ACID TO S-ETHYL PHENYL(1-NAPHTHYL)PHOSPHINOTHIOATE, METHYL
PHENYL(1-NAPHTHYL)PHOSPHINATE, AND N,N-DIETHYL-P-PHENYL-P-
(1-NAPHTHYL)PHOSPHINIC AMIDE

V. A. Chauzov

P.D. 00-08-1981
P. 1650

UDC 541.653+547.241

We have previously [1] described the synthesis of phenyl(1-naphthyl)phosphinothioic acid and its resolution into its enantiomers. In this communication we give data on the preparation from it of enantiomeric S-ethyl phenyl(1-naphthyl)phosphinothioate, methyl phenyl(1-naphthyl)phosphinate, and N,N-diethyl-P-phenyl-P-(1-naphthyl)phosphinic amide by the following scheme.



By the action of ethyl bromide on diethylammonium phenyl(1-naphthyl)phosphinothioate (I), $[\alpha]_D -64.6^\circ$ (a, benzene-DMF 1:1, $90^\circ C$, 3 h) we obtained S-ethyl phenyl(1-naphthyl)phosphinothioate (II), $[\alpha]_D -40.5^\circ$ (benzene), mp $101-102^\circ C$, yield 75%. Found %: C 69.45; H 5.67; P 10.29; S 10.49. $C_{18}H_{17}POS$. Calculated %: C 69.25; H 5.49; P 9.91; S 10.26. The treatment of the phosphinothioate (II), $[\alpha]_D -40.5^\circ$, with sodium methoxide (b, toluene, $20^\circ C$, 0.5 h) led to the methyl ester (III), $[\alpha]_D +36.4^\circ$ (benzene), mp $90-91^\circ C$, yield 72%. Found %: C 72.50; H 5.25; P 10.86. $C_{17}H_{15}PO$. Calculated %: C 72.30; H 5.37; P 10.97. When the phosphinothioate (II), $[\alpha]_D -40.5^\circ$, was treated with (diethylamino)lithium (c, ether-toluene 1:2, $20^\circ C$, 20 h), we obtained the diethylamide (IV), $[\alpha]_D +13.7^\circ$ (benzene), mp $99-100^\circ C$, yield 60%. Found %: C 74.20; H 6.93; P 9.16. $C_{20}H_{23}NOP$. Calculated %: C 74.90; H 6.86; P 9.54. The enantiomeric amide (IV), $[\alpha]_D -12.6^\circ$, was formed in 56% yield by the action of (diethylamino)lithium on the phosphinate (III), $[\alpha]_D +36.4^\circ$ (d, ether-toluene 1:2, $20^\circ C$, 3 days).

On the basis of data in [2] it may be supposed that the stages (b), (c), and (d) go with predominating inversion of configuration, in view of which the compound (I)-(IV) of the same sign must have the same configuration of the fragment $R_2P(O)$.

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Translated from Zhurnal Obshchei Khimii, Vol. 51, No. 8, pp. 1921-1922, August, 1981.
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$A(4a) = 46.8$ G, $A(4b) = 45.8$, given the assumptions in section V. Although this difference is small (2%), we believe it to be real. Analyses of NADH spectra obtained with other dyes give similar results.²⁹ A slightly nonplanar geometry of the nicotinamide ring in NADH⁺ brought about by a stacking interaction with the adenine ring could be responsible.

(29) Hore, P. J.; Kaptein, R., unpublished results.

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Registry No. NADH, 58-68-4; 7,8,10-trimethyl-3-carboxymethylisoxazine, 20227-26-3.

XP-002206013

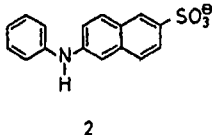
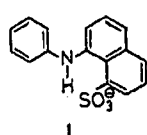
Reactivity and Decay Pathways of Photoexcited Anilidonaphthalenes

K. H. Grellmann* and U. Schmitt

Contribution from the Max-Planck-Institut für biophysikalische Chemie, Abteilung Spektroskopie, D 3400 Göttingen, West Germany. Received March 1, 1982

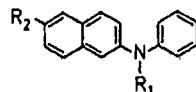
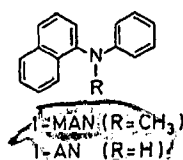
Abstract: *N*-Methyl-2-anilidonaphthalene (2-MAN) is converted by light into 7-methyl-7*H*-benzo[*c*]carbazole (7-MBC) and 5-methyl-6,7-dihydro-5*H*-benzo[*c*]carbazole (9). The reaction takes place only in the absence of oxygen and is regioselective; i.e., the isomeric 5-methyl-5*H*-benzo[*b*]carbazole (5-MBC) is not formed. Ring closure takes place from the excited triplet state of 2-MAN and a zwitterionic intermediate was found in flash experiments. *N*-Methyl-2-anilino-6-naphthalenesulfonate (2-MANS) behaves similarly. Caution should therefore be exercised if such compounds are used as fluorescence probes. *N*-Methyl-1-anilidonaphthalene (1-MAN) is photochemically more stable than 2-MAN.

Anilidonaphthalenes, e.g., 1 and 2, have been widely used as



fluorescence probes for structure studies in biochemistry¹ because the quantum yield and the spectral distribution of the fluorescence of such compounds depend strongly on solvent polarity.

Since diphenylamines are by light effectively converted into carbazoles,² it is to be expected that anilidonaphthalenes undergo similar photoinduced ring-closure reactions forming benzo-carbazoles. Such photoproducts which strongly fluoresce could cause artifacts if the parent compounds are used as fluorescence labels. We therefore investigated the photochemical properties of *N*-methyl-1-anilidonaphthalene (1-MAN) and *N*-methyl-2-

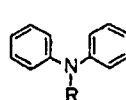


2-MAN ($R_1 = \text{CH}_3, R_2 = \text{H}$)
2-AN ($R_1 = R_2 = \text{H}$)
2-MANS ($R_1 = \text{CH}_3, R_2 = \text{SO}_3^-$)

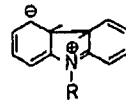
anilidonaphthalene (2-MAN) in some detail by means of steady-state and flash experiments. Similar to 1 and 2 the fluorescence spectra and the fluorescence quantum yields of 1-

MAN and 2-MAN depend strongly on solvent polarity.

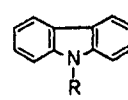
We began our studies with the *N*-methyl compounds in order to avoid complications due to side reactions which were observed in the case of diphenylamines. For instance, in the presence of oxygen *N*-methyldiphenylamine (3) is converted by light³ into



3 ($R = \text{CH}_3$)
4 ($R = \text{H}$)



5 ($R = \text{CH}_3$)
6 ($R = \text{H}$)



7 ($R = \text{CH}_3$)
8 ($R = \text{H}$)

N-methylcarbazole (7) via the zwitterionic dihydrocarbazole intermediate⁴ 5. In the case of 4 side reactions occur⁵ due to hydrogen abstraction from the N-H group of the corresponding zwitterion 6, which reduce the quantum yield of the formation of 8.

Results

***N*-Methyl-2-anilidonaphthalene (2-MAN). Steady-State Photochemistry and Product Analysis.** Illumination of degassed solutions of 2-MAN in acetonitrile, ethanol, or methylcyclohexane (MCH) results in the formation of 7-methyl-7*H*-benzo[*c*]carbazole (7-MBC) and 5-methyl-6,7-dihydro-5*H*-benzo[*c*]carbazole (9) with a total quantum yield of 5–10%. The concentration ratio 7-MBC:9 is about 2:1. Both compounds were identified by comparison with authentic samples (mp, UV and NMR spectra). The UV spectra of 2-MAN before and after illumination together with the spectrum of 7-MBC, all in acetonitrile, are given in Figure 1. In MCH the absorption spectrum of 7-MBC is similar to that in acetonitrile but the absorption peaks between 350 and 370 nm

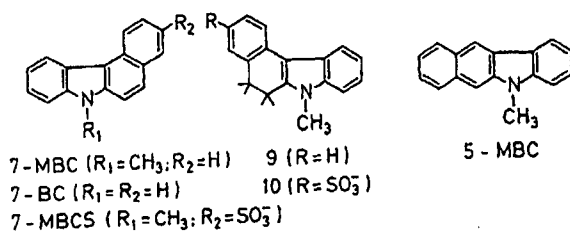
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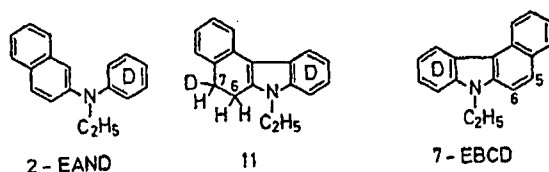
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are sharper in MCH (cf. Figure 3).

In air-saturated solution 2-MAN is much more stable. The quantum yield of 2-MAN disappearance is 10–20 times smaller than in degassed solutions. Only small amounts of 7-MBC are formed. The main part of the reaction mixture consists of other photoproducts which were not identified.

The ring-closure reaction of 2-MAN is remarkably regioselective. The isomer 5-methyl-5H-benzo[b]carbazole (5-MBC) could not be detected in the reaction mixture. We estimate that the NMR detection limit is 0.5% of 5-MBC contained in 7-MBC (cf. Figure 2). Some static illumination experiments were carried out with the partially deuterated amine *N*-ethyl-2-(penta-deuterioanilino)naphthalene (2-EAND). (The *N*-ethylamine was



used because it is easier to synthesize than the *N*-methyl derivative.) The NMR spectra of the photoproducts 11 and 7-EBCD show that the dihydrocarbazole 11 contains three protons at C-6 and C-7 and one deuterium either at C-6 or at C-7. We were not able to deduce from the NMR spectra whether the D atom is located at C-7 (as shown in the formula) or at C-6.

The carbazole 7-EBCD contains only hydrogen atoms in the naphtho group and only deuterium atoms in the benzo group. It does, therefore, not arise from 11 (via dehydrogenation) because in this case part of the 7-EBCD molecules should contain deuterium at C-5. More than 5–10% of such a compound (or of any other isomer) would have been detected in the NMR spectra.

The dihydrocarbazole 9 is in degassed as well as air-saturated solutions converted by light into 7-MBC without side reactions (Figure 3). In air-saturated solution the quantum yield of this reaction is 5–7 times higher than in the absence of oxygen.

Flash Photolysis. Two transient absorptions ($\lambda_{\text{max}} = 500$ nm and $\lambda_{\text{max}} = 620$ nm) were observed after flash excitation of 2-MAN dissolved in degassed MCH. In Figure 4 the absorption spectra of the two transients are shown. The decay of the transient with $\lambda_{\text{max}} = 500$ nm is first order and at 294 K its lifetime $\tau_1 = 120$ μs . An Arrhenius plot of $1/\tau_1$ between 344 and 153 K shows (cf. Figure 5) that two processes with rate constants $k_2 = (2.3 \times 10^5 \text{ s}^{-1}) \exp(-8.4 \text{ kJ mol}^{-1}/RT)$ and $k_3 = (6.1 \times 10^{11} \text{ s}^{-1}) \exp(-49.2 \text{ kJ mol}^{-1}/RT)$ are responsible for the decay of the transient. At low temperatures solutions of 2-MAN in MCH-isopentane strongly phosphoresce ($\lambda_{\text{max}} = 494, 505, 532, 548$ (shoulder), 577, 625 (weak shoulder) nm). At 81 K the phosphorescence lifetime $\tau_p = 1.4$ s. At higher temperatures (100–160 K) τ_p was determined in flash experiments and found to be in excellent agreement with τ_1 . We therefore assign the transient with $\lambda_{\text{max}} = 500$ nm to the triplet state of 2-MAN. The low-frequency factor ($T_1 \rightarrow S_0$ transition) and the low activation energy (adventitious quenching) of k_2 corroborate this assignment. (The decay process with rate constant k_3 will be discussed below.) The yield of triplet formation ($\phi_t(T)$) was found to be constant between 150 and 300 K (cf. Figure 6). The yield of the second transient ($\lambda_{\text{max}} = 620$ nm) decreases strongly at lower temperatures (cf. Figure 6). Its decay could therefore only be analyzed above 280 K. It is of mixed first and second order. Only the rate constant of the first-order process, $k_3 = [(4 \pm 2) \times 10^9 \text{ s}^{-1}] \exp(-60 \pm 3$

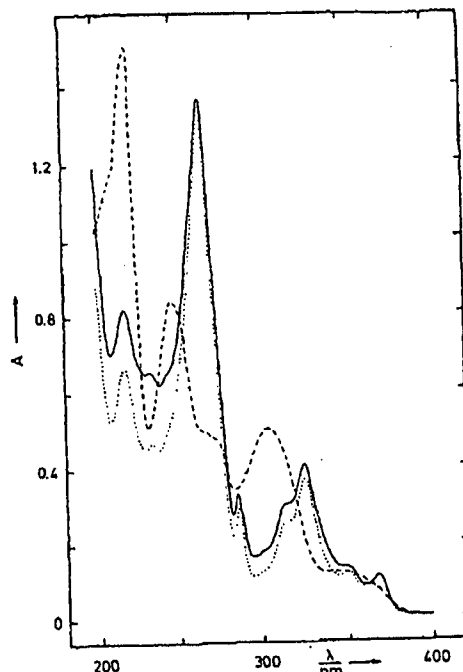


Figure 1. Absorption spectrum of 3×10^{-3} M 2-MAN in degassed MCH before (dashed line) and after (solid line) illumination with a medium-pressure Hg lamp through a 345-nm cutoff filter. For comparison the spectrum of an authentic sample of 7-MBC (dotted line) is also shown.

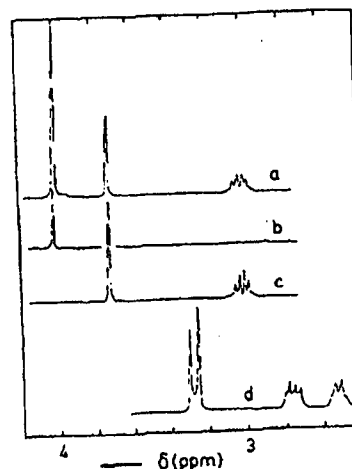


Figure 2. NMR (270 MHz) spectra. Solvents: CD_3CN (a–c), D_2O (d). Standards: Me_4Si (a–c), TSP- d_4 (d). a: Spectrum fraction of the reaction mixture obtained after illumination of 2-MAN in degassed MCH. (Before the spectrum was taken, unreacted 2-MAN was removed from the photoproducts by column chromatography.) b: Spectrum fraction of an authentic sample of 7-MBC. c: Spectrum fraction of an authentic sample of the dihydrocarbazole 9. d: Spectrum fraction of the reaction mixture obtained after illumination of 2-MANS in degassed ethanol.

$\text{kJ mol}^{-1}/RT$), could be determined fairly accurately from an Arrhenius plot because the accurate determination of the second-order rate constant required a better signal-to-noise ratio⁶ than we were able to obtain in our flash experiments. In air-equilibrated MCH solutions ($[\text{O}_2] = 2.3 \times 10^{-3}$ M) the triplet state is quenched by oxygen with a quenching constant $k_q = 8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (295 K); the second transient is not at all observed in air-equilibrated solutions.

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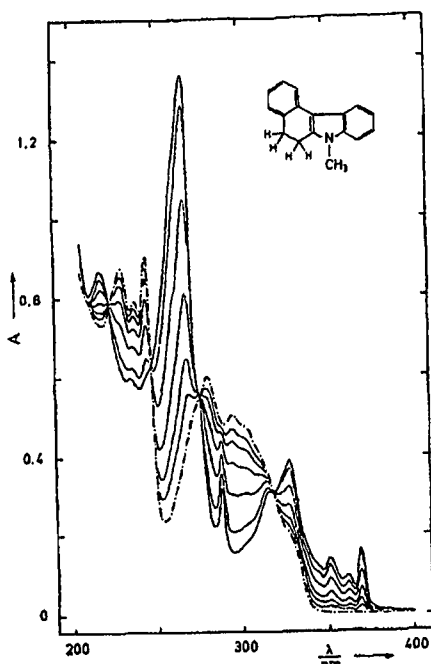


Figure 3. Absorption spectrum of 3.5×10^{-5} M 9 in air-equilibrated MCH before (dashed-dotted line) and after (solid lines) a number of consecutive illumination periods (medium-pressure Hg lamp through a 280-nm cutoff filter).

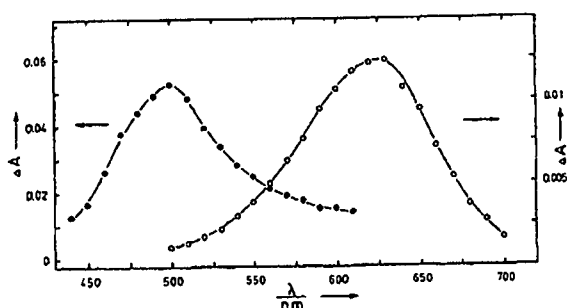


Figure 4. Transient absorption spectra observed after excitation of a solution of 3×10^{-5} M 2-MAN in degassed MCH with a microsecond flash (half-width of the flash $\sim 3 \mu\text{s}$); optical path length, 10 cm. Full circles: Triplet-triplet absorption of 2-MAN at 153 K. The change in absorbance, ΔA (left-hand scale), was determined from first-order decay curves extrapolated to time zero. Open circles: Spectrum of transient Z at 293 K. ΔA (right-hand scale) was determined from decay curves 80 ms after flash excitation.

N-Methyl-1-anilino-naphthalene (1-MAN). 1-MAN is photochemically much more stable than 2-MAN in both degassed and air-saturated solutions. The quantum yield of decomposition is 1×10^{-3} – 2×10^{-3} . Traces of the corresponding benzocarbazole are formed but the main fraction of the photoproducts consists of unidentified compounds with unstructured UV spectra.

In flash experiments carried out in degassed MCH as solvent we observed only one transient which decays in a first-order reaction (k_t) and which we assign to the triplet state of 1-MAN. Its lifetime is $\tau_t = 2$ ms in degassed MCH and $\tau_t' = 120$ ns in air-equilibrated MCH at 296 K; i.e., the oxygen quenching constant is $k_q = 4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. From an Arrhenius plot of k_t we obtained $k_t = (2 \times 10^4 \text{ s}^{-1}) \exp(-9.1 \text{ kJ mol}^{-1}/RT)$. The absorption spectrum of the transient is shown in Figure 7. At 81 K solutions of 1-MAN in isopentane-MCH (5:1) phosphoresce ($\lambda_{\text{max}} = 535, 568, 610$ (weak shoulder) nm). The phosphorescence lifetime at this temperature is $\tau_p = 560$ ms. In acetonitrile no phosphorescence is observed.

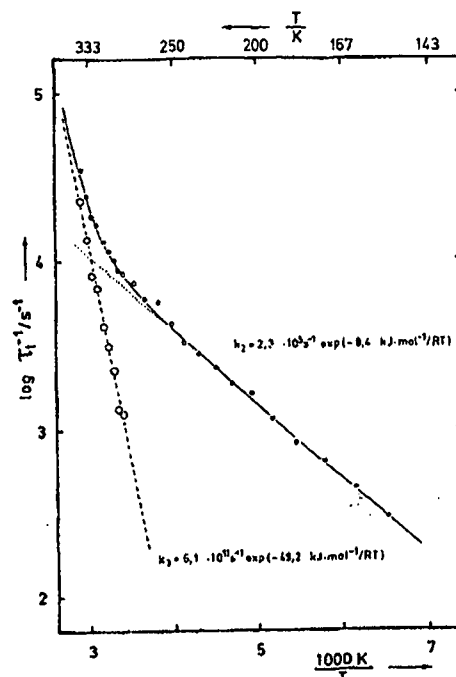


Figure 5. Arrhenius plot of the decay of the 2-MAN triplet state in degassed MCH, monitored at 490 nm. The rate constant $k_2(T)$ was calculated from the difference $k_2(T) = 1/\tau_1(T) - k_1(T)$.

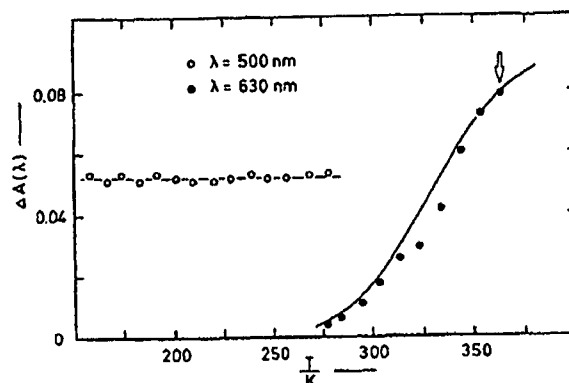


Figure 6. 2-MAN (1.3×10^{-4} M) in degassed MCH. Absorbance change, $\Delta A(\lambda)$, at different temperatures after flash excitation, caused by the formation of the triplet state of 2-MAN (open circles, monitoring wavelength $\lambda = 500$ nm) and of Z (full circles, $\lambda = 630$ nm). The solid line (right-hand curve) was calculated from rate constants (see text) and normalized at 363 K (arrow in Figure 6).

Other Anilino-naphthalenes. 2-Anilino-naphthalene (2-AN). 2-AN is in degassed solution converted by light into 7H-benzo-[c]carbazole (7-BC). The quantum yield of this reaction is lower than in the case of 2-MAN due to side reactions and it is completely suppressed in air-equilibrated solutions. The side reactions are presumably due to hydrogen abstraction from the N-H group of the primary ring-closure product.

N-Methyl-2-anilino-6-naphthalenesulfonate (2-MANS). 2-MANS is not soluble in MCH. Under illumination in air-equilibrated ethanol it decomposes slowly into unidentified products with unstructured absorption spectra. Illumination in degassed ethanol leads to the formation of two photoproducts which could not be separated by means of TLC or GC. The NMR spectrum of the reaction mixture shows at high field singlets of two different N-CH₃ groups with an intensity ratio of about 2:1 (cf. Figure 2). The absorption spectrum of the reaction mixture (Figure 8) is very similar to the photoproduct spectrum shown

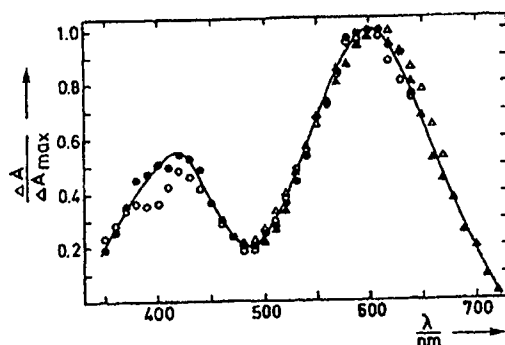


Figure 7. 1-MAN (3×10^{-5} M) in MCH degassed and air equilibrated. Triplet-triplet absorption observed after excitation with a nitrogen laser (flash half-width, 3 ns). Open circles: air-equilibrated solution, 50 ns after excitation. Full circles, open and full triangles: degassed solution 1, 50, and 80 μ s after excitation, respectively.

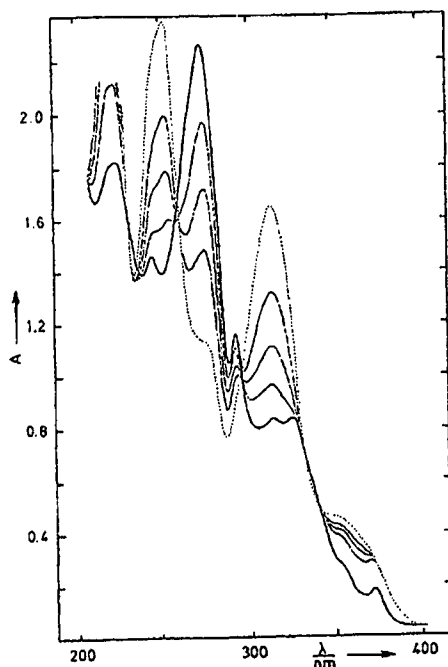


Figure 8. Absorption spectrum of 1×10^{-4} M 2-MANS in degassed ethanol before (dotted line) and after (solid lines) a number of consecutive illumination periods (medium-pressure Hg lamp through a 320-nm cutoff filter).

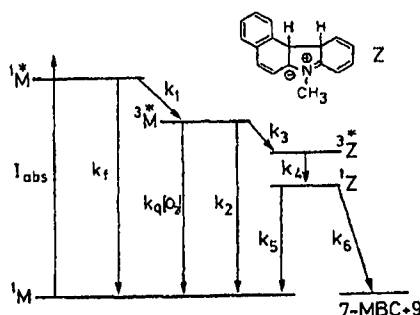
in Figure 1. We therefore conclude that the main photoproduct ($\sim 70\%$) is 7-methyl-7H-benzo[c]carbazole-3-sulfonate (7-MBCS). The minor product is presumably the corresponding dihydrocarbazole 10. The total quantum yield is 2–5%.

Flash experiments carried out with 2-MANS in degassed ethanol gave results which are quite similar to those obtained with 2-MAN in degassed MCH. We observed two transient species. One has an absorption maximum at 500 nm and decays in a first-order reaction with a lifetime of 200 μ s at 293 K. We assign it to the triplet state of 2-MANS. Yield and lifetime of the second transient ($\lambda_{\text{max}} = 630$ nm) depend strongly on temperature as in the case of 2-MAN. At 310 K its half-life is about 1 s and its decay is of mixed first and second order. An Arrhenius plot of the first-order process gave the rate constant $k = [(4 \pm 2) \times 10^8 \text{ s}^{-1}] \exp(-51 \pm 3 \text{ kJ mol}^{-1}/RT)$.

Discussion

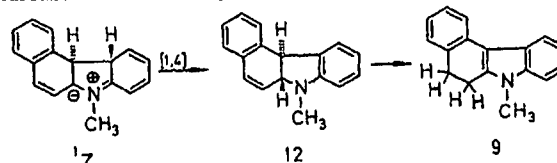
The experimental results presented in this paper indicate that the photochemical conversion of 2-MAN into 7-MBC proceeds in close similarity to the ring-closure reaction of diphenylamines.⁴

Scheme 1



We therefore propose the following reaction mechanism (Scheme 1). After intersystem crossing from the first excited singlet state $1M^*$ of 2-MAN (rate constant k_1) into its excited triplet state $3M^*$ (which competes with the radiative process (k_f)), ring closure occurs to the zwitterionic intermediate, Z, in its singlet ground state, $1Z$ (k_2 and k_4), with the quantum yield ϕ_2 . One of the resonance structures of Z is inserted in Scheme 1. Competing with the ring-closure reaction (k_2) is the "normal" triplet deactivation (k_3) and quenching ($k_4[O_2]$) if oxygen is present. According to Scheme 1 the temperature dependence of the quantum yield ϕ_2 in degassed solution is given by $\phi_2(T) = \phi_1 k_2(T)/(k_2(T) + k_3(T))$. Since the quantum yield of 2-MAN triplet formation, ϕ_1 , is temperature independent (cf. Figure 6), the relative yields $\phi_2(T)/\phi_2(363 \text{ K})$ can be calculated from the rate constants $k_2(T)$ and $k_3(T)$ (solid line in Figure 6) and compared with the relative yields, $\Delta A_{630}(T)/\Delta A_{630}(363 \text{ K})$ determined directly from the change of absorbance at 630 nm (ΔA_{630}) immediately after flash excitation at different temperatures, T (full points in Figure 6). The satisfactory agreement of the data shows that the assignment of the process with low activation energy (cf. Figure 5) to the normal triplet decay (k_3) is correct and that the process with high activation energy (k_2) leads to the formation of Z. The large frequency factor of k_2 indicates that the ring-closure reaction takes place adiabatically in the triplet manifold. In contrast to *N*-methylidiphenylamine where the formation and the decay of the zwitterionic intermediate in its excited triplet state, $3Z^*$, could be seen in nanosecond flash experiments^{4b} (in this case $k_3 = 2 \times 10^7 \text{ s}^{-1}$ at 293 K), we did not find such an intermediate in the case of 2-MAN. This is presumably due to the relatively small rate constant k_3 (for instance, $k_3 = 1 \times 10^3 \text{ s}^{-1}$ at 293 K) which might be the rate-determining step of the $3Z^*$ decay. If $k_3 < k_4$, no measurable concentration of $3Z^*$ can accumulate and the transient Z can only be observed in its singlet ground state $1Z$. Since below 300 K $1Z$ is formed only with low yield (cf. Figure 6), the growing-in of the Z spectrum can only be observed at higher temperatures where $k_3 > k_2$. At 340 K and above the buildup of the $1Z$ absorption (monitored at 620 nm) was observed. It matches exactly the decay of the amine triplet absorption (monitored at 480 nm).

The decay of $1Z$ is a rather complicated process. Part of $1Z$ reverts by ring opening (k_3) to the starting material. This is presumably the first-order process with 60 kJ/mol activation energy. A small fraction evidently rearranges into the dihydrocarbazole 9. Presumably, the first step of the rearrangement is



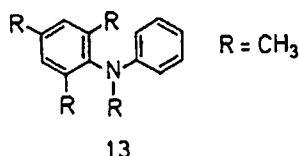
a sigmatropic 1,4 hydrogen shift, in analogy to the rearrangement reactions of enamines.⁷ The resulting dihydrocarbazole 12 is

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apparently not stable and rearranges further to yield 9.

The mechanism of the dehydrogenation reaction $^1Z \rightarrow 7\text{-MBC}$ is not known. We have no experimental evidence that the second-order part of the ^1Z decay is a disproportionation reaction as it was observed with *N*-methyl-diphenylamine⁸ because in the mass spectra of the reaction mixture we could not find a tetrahydrocarbazole, i.e., a mass two units higher than that of 2-MAN. Since tetrahydrocarbazoles are quite often very labile compounds and since low-temperature experiments cannot be carried out with 2-MAN because of the decreasing yield ϕ_2 (cf. Figure 6), it will be very difficult to investigate this part of the reaction in more detail.

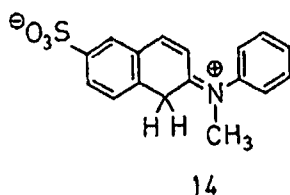
The relatively long lifetime of the amine triplet state is the reason that 7-MBC formation is not observed in air-equilibrated solutions because $k_4[\text{O}_2] \gg k_3$. For instance, at 293 K the ring-closure rate constant $k_3 = 1 \times 10^3 \text{ s}^{-1}$, but $k_4[\text{O}_2] = 1.6 \times 10^7 \text{ s}^{-1}$. Recently, it has been suggested⁹ that the angle of twist between the naphthyl and phenyl rings of 2-MAN is responsible for the nonreactivity of this compound and the triplet quenching by oxygen was considered to be unimportant, which is not correct. One could, perhaps, argue that a large angle of twist is the reason for the low value of k_3 . However, with the trimethyl derivative 13 of *N*-methyl-diphenylamine in which the two phenyl rings are



certainly twisted the ring closure to the corresponding zwitterionic transient proceeds even faster⁸ (about 10 times) than with the parent amine.

We feel that the electron density distribution in the excited triplet state governs this reaction and causes also its regioselectivity. This interesting aspect of the reaction mechanism will be discussed in more detail elsewhere together with the photoreactions of other relevant diphenylamine derivatives.

The results obtained with the sulfonated compound 2-MANS are very similar to those reported for 2-MAN. Therefore, the reaction mechanism outlined above for 2-MAN can also be applied to 2-MANS. In particular, the great similarity of the absorption spectra of the photoproducts (cf. Figures 1 and 8) leaves little doubt that the main photoproduct of 2-MANS irradiation is the benzocarbazole 7-MBCS. Dodiuk and Kosower published a photoproduct spectrum¹⁰ obtained from irradiation of 2-MANS in ethanediol which is practically identical with the absorption spectrum in Figure 8. Relying on NMR data these authors proposed for the photoproduct the imine structure 14. In our



experiments carried out in degassed ethanol two products were formed which could not be separated on chromatographic columns, presumably due to the highly polar sulfogroup. Therefore, the NMR spectrum of the reaction mixture is rather complicated. It can, however, be compared with the spectra of the authentic samples of 7-MBC and 9 a small part of which is shown in Figure 2. We see no reason to assume that in ethanol compound 14 is

formed and we are convinced that 7-MBCS is the main photoproduct. Since the absorption spectra of the photoproducts in ethanol and in ethanediol are identical, there remains little doubt that the assignment of Dodiuk and Kosower is not correct. We also suspect that in a kinetic study¹¹ the properties of 7-MBCS rather than of 14 were investigated.

1-MAN does not form 5-MBC upon illumination. Consequently, one observes in flash experiments only the triplet state of 1-MAN but no zwitterionic intermediate. Only one position in the naphthalene moiety of this molecule is available for a ring-closure reaction. Its nonreactivity is presumably due to an unfavorable electron density distribution in the excited state.

Kinetic studies in the nanosecond¹² and, especially, in the picosecond^{12b} time range clearly demonstrated that 1-AN and its derivatives can be used as fluorescence probes only with caution. Obviously, 2-AN derivatives are even worse candidates for such studies because additional problems may arise due to their facile conversion into benzocarbazoles under anaerobic conditions.

Experimental Section

Materials. *N*-Methyl-1-anilino-naphthalene (1-MAN). 1-Anilino-naphthalene (6.67 g, 0.03 mol) and 5.7 mL (0.06 mol) of dimethylsulfate were kept for 1 h at 100 °C. Then 7.6 g (0.135 mol) of KOH in 40 mL of H₂O was added and refluxed for 2 h. The reaction mixture was extracted with 2 × 50 mL of diethyl ether, and the extract dried with Na₂SO₄ and evaporated. The residue was chromatographed on Al₂O₃ with cyclohexane-CCl₄ 1:1: yield 0.68 g (0.003 mol); mp 43 °C (lit.¹³ 52–53 °C); correct elemental analysis; UV_{max} (log ε) in methyleyclohexane (MCH) 337 (3.46), 288 (3.81), 250 (4.23) nm. The lit.¹³ mp for 1-MAN and 2-MAN (see below) seem doubtful because the elemental analyses reported in ref 13 deviate strongly from the calculated values.

N-Methyl-2-anilino-naphthalene (2-MAN). 2-MAN was prepared from 2-anilino-naphthalene like 1-MAN: mp 53 °C (lit.¹³ 88–90 °C); correct elemental analysis; UV_{max} (log ε) in MCH 347 (3.61), 306 (4.20), 277 (4.15), 248 (4.41) nm; NMR (Me₂SO-*d*₆) δ 7.78 (d), 7.75 (d), 7.75 (d), 7.42 (t), 7.38 (s), 7.33 (t), 7.33 (t), 7.31 (t), 7.17 (d), 7.11 (d), 7.11 (d), 7.02 (t), 3.38 (s, 3 H).

3,4-Dihydro-1*H*-naphthalen-2-one Phenylhydrazone (15). 15 was prepared from phenylhydrazine and 3,4-dihydro-1*H*-naphthalen-2-one (β-tetralone) in 50% acetic acid, mp 109–110 °C (lit.¹⁴ 109 °C).

6,7-Dihydro-5*H*-benzo[*c*]carbazole (16). 15 (5 g, 0.021 mol) was dissolved in 75 mL of ethanol and under stirring 2.5 mL of concentrated H₂SO₄ in 25 mL of ethanol was slowly added. To the red solution 400 mL of H₂O was added and the solution was neutralized with 30% NaOH. A dark resin precipitated. Recrystallization from petrol ether (bp 60–80 °C) afforded 1.7 g (0.0078 mol) of colorless crystals, mp 100.5–101 °C (lit.¹⁵ 100 °C).

5-Methyl-6,7-dihydro-5*H*-benzo[*c*]carbazole (9). 16 (0.5 g, 0.0023 mol) was dissolved in 10 mL of acetone and 0.5 g (0.0089 mol) of ground KOH added. After 10 min 0.5 g (0.0035 mol) of CH₃I and after 15 min 20 mL of H₂O were added. The reaction mixture was extracted with diethyl ether, and the extract dried with Na₂SO₄ and evaporated. Recrystallization from petrol ether (bp 40–60 °C) afforded 0.27 g (0.0016 mol) of colorless crystals: mp 133.5 °C; NMR (CD₃CN) δ 8.00 (d), 7.82 (d), 7.43 (d), 7.27 (t), 7.26 (m), 7.20 (m), 7.06 (t), 3.72 (s, 3 H), 3.02 (m, 4 H).

N-Ethyl-2-(pentadeuterioanilino)naphthalene (2-EAND). *N*-Acetyl-2-naphthylamine (3 g, 0.016 mol), 10 g (0.06 mol) of bromobenzene-*d*₅, 1.5 g (0.01 mol) of K₂CO₃, a trace of Cu powder, and KI were heated to 210 °C for 1 h. Water and unreacted bromobenzene were evaporated under vacuum, and the residue was dissolved in 150 mL of ethyl ether, filtered, dried with Na₂SO₄, and reduced with LiAlH₄. The product was purified on a Al₂O₃ column with cyclohexane-diethyl ether 1:1: yield 0.5 g (0.002 mol); UV_{max} in MCH 350, 310, 278 nm; NMR (CD₃CN) δ 7.75 (d), 7.69 (d), 7.69 (d), 7.4 (t), 7.36 (s), 7.3 (t), 7.14 (d), 3.88 (q, 2 H), 1.23 (t, 3 H).

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7-Methyl-7H-benzo[c]carbazole (7-MBC). 2-MAN (0.233 g, 0.001 mol) was dissolved in 1 L of UV-grade cyclohexane. The solution was illuminated in a Rayonet RPR-100 reactor for 15 h with "350-nm" lamps. Thirty minutes before and during illumination purified N₂ was bubbled through the solution. After evaporation of the solvent the residue was chromatographed on a SiO₂ column with cyclohexane-toluene 1:1. Two fractions (starting material and photoproduct) were collected. The photoproduct (7-MBC) was recrystallized twice from methanol and afforded 17 mg (7.4 × 10⁻³ mol) pale yellow crystals: mp 118 °C (lit.¹⁷ 118-119 °C); UV_{max} (log ε) in MCH 369 (3.90), 362 (3.58), 350 (3.77), 327 (4.21), 315 (4.06), 288 (4.09), 267 (4.77) nm; NMR (Me₂SO-d₆) δ 8.80 (d), 8.62 (d), 8.09 (d), 8.01 (d), 7.93 (d), 7.77 (d), 7.72 (t), 7.52 (t), 7.48 (t), 7.36 (t), 4.02 (s, 3 H).

Irradiation. Spectroscopic irradiations were performed with about 5 × 10⁻³ M solutions in 1 × 1 cm² quartz cells. The solutions were degassed on a high-vacuum line, using the freeze-pump-thaw technique, and sealed off.

Solvents. Methylcyclohexane and isopentane (Fluka purum) were chromatographed.¹⁶ Acetonitrile and ethanol (Uvasol Merck) were used without further purification.

Spectra. UV spectra were recorded on a Perkin-Elmer Model 320 recording spectrophotometer. Mass spectra were measured on a Varian MAT Model CH 7 instrument at 70 eV and NMR spectra on a Bruker WH 270 pulse Fourier transform (270 MHz) instrument.

Flash Photolysis. The flash apparatus has been described elsewhere.^{4c} The cooling technique of Fischer¹⁸ was applied for experiments at low temperatures. The optical path length of the flash cells was 100 mm.

Acknowledgment. We are most grateful to Professor M. Zander, Rütgerswerke AG, Castrop-Rauxel, for a sample of 7-BC and to Dr. H. Dodiuk, Tel-Aviv University, for a sample of 2-MANS. The technical assistance of Mr. J. Plotzki is gratefully acknowledged. We are greatly indebted to Dr. H. Weller for measuring the NMR spectra.

Registry No. 1-MAN, 34160-17-3; 2-EAND, 83160-30-9; 2-AN, 135-88-6; 2-MAN, 6364-05-2; 2-MANS, 64032-78-6; 7-EBCD, 83160-31-0; 7-BC, 205-25-4; 7-MBC, 29103-83-1; 7-MBCS, 83160-32-1; 9, 54903-06-9; 10, 83160-33-2; 11, 83160-34-3; 15, 83160-35-4; 16, 5425-53-6; N-acetyl-2-naphthylamine, 581-97-5; bromobenzene-d₅, 4165-57-5.

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Torsional Potential Function of *n*-Butane. Correlation Effects in the Quantum-Mechanical Prediction of the Syn-Anti Energy Difference

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Abstract: Hartree-Fock-Roothaan (HFR) calculations have been carried out by using a split-valence (6-31G) basis set for the title conformations of *n*-butane. The calculated energy difference was 6.36 kcal/mol, compared to 5.99 kcal/mol reported earlier for an STO-3G basis set. (Both calculations used molecular mechanics (MM2) optimized geometries for which the energy difference is 4.73 kcal/mol.) Inclusion of polarization functions at the HFR level leaves the energy difference virtually unchanged, 6.48 kcal/mol. Configuration interaction calculations over 11 665 of 33 234 possible singly and doubly excited valence configurations reduce the energy difference to 4.58 kcal/mol.

The *n*-butane molecule is a fundamental unit, an understanding of which is required for conformational analysis of hydrocarbons. There are four important torsional extrema: 0° (syn or cis), ~60° (gauche), ~120° (eclipsed), and 180° (anti or trans). While the latter three conformations are reasonably well understood, the syn form has presented some difficulties.² Theoretical estimates of the syn-anti energy difference have been derived on several occasions from Hartree-Fock-Roothaan (HFR) calculations,³ but no direct experimental values are available (vide infra). The most reliable (in our judgement) HFR value for this difference is 5.99 kcal/mol (STO-3G),² but this is significantly higher than the molecular mechanics value of 4.73 kcal/mol (MM2).⁴ We do not see how the HFR value could be utilized in molecular mechanics to give reasonable results. We, therefore, concluded earlier² that the HFR value must be in error by ~1 kcal/mol. Such an error might be ascribed to basis set limitations, but the STO-3G basis set has seemed to be adequate for hydrocarbons in the past.²

Inclusion of electron correlation effects will give a lower calculated energy than the HFR formalism for a given molecule and choice of basis set. It is expected,⁵ and generally found, that the energy lowering is similar for different conformations of the same molecule. It has been suggested, however, that this discrepancy between the MM2 and HFR values is due to correlation effects.² The near-constancy of correlation effects is a necessary condition for the validity of HFR conformational studies. This is usually assumed to hold for "simple" alkanes. Even so, recent studies of 1,2-difluoroethene have demonstrated the importance of correlation effects in accounting for the cis-trans energy difference.⁶ There is reason to believe that a similar effect is operative for the syn and anti forms of *n*-butane. In the former conformation the methyl groups approach each other quite closely, well within their van der Waals radii. Their is substantial van der Waals repulsion, but masked by this is a van der Waals attraction previously estimated to be about 1 kcal/mol.² This dispersion energy is essentially a correlation effect, accommodated within the molecular

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The acidified aq soln was extd with EtOAc. These extracts were washed with H₂O and dried before evapn. The residue was treated with Et₂O, and the solid obtd weighed 210 mg (15%). Analysis by tlc (SGH, solvent K) indicated the two solids were of nearly identical compn and of excellent purity. Their repn from Et₂O-EtOAc did not yield material having satisfactory elemental analyses, and the remaining product was subjected to column chromatography (SGH, 20 g; developed with solvent K). The best fractions were combined: 339 mg, 24% yield; tlc (SGH, solvent K) indicated slight impurity; uv max (0.1 N NaOH) 256 (ϵ 33,900), 351 (7440). Anal. (C₁₄H₁₂N₂O₆) C, H, N.

B. Acetylation of the Product of Reductive Condensation of 2 and 4.—Compd 4 (3.55 g, 11 mmoles), 2 (2.33 g, 10 mmoles), and *p*-toluenethiol (8.7 g, 70 mmoles) in 300 ml of anhyd methoxyethanol were utilized in the reductive condensation procedure previously described (B, synthesis of 10). Celite (10 g) was added to the product suspended in Et₂O, and the total filter cake was air-dried before acetylation as previously described (A, synthesis of 3c). The solid phase was removed by filtration and washed with Ac₂O and Me₂CO. The filtrates were combined and evapd, and the residue was triturated with Et₂O. The insol fraction was extd with warm EtOAc several times, and the combined exts were washed with H₂O and dried. Evapn yielded 3.3 g of solid (57% crude yield).

This solid was chromatographed on a column of SGH (150 g) by development with solvent K. Product-containing fractions were rechromatographed (SGH, 75 g, solvent K). The best fractions were combined and evapd, and the residue obtd was triturated with Et₂O. The product was isolated by filtration: 740 mg, 13% yield; tlc was identical with that of the analytical sample; uv max (0.1 N NaOH) 256 (ϵ 33,700), 351 (7680); pmr (DMSO-*d*₆) δ 2.22 (s, 3 H, COCH₃), 2.24 (s, 3 H, COCH₃), 5.36 (s, 2 H, NCH₂), 7.87 (d, 1 H, C₂-H), 8.32 (d of doublets, 1 H, C₄-H), 8.88 (d, 1 H, CONH of glutamic acid), 8.89 (d, 1 H, C₂-H), 8.95 (s, 1 H, C₂-H), 12.10 (m, 2 H, CONH). Other pmr spectral data obtd for this sample were equiv to those given for 4. Anal. (C₂₄H₂₂N₂O₆) H; C: calcd, 53.60; found, 52.27; N: calcd, 19.24; found, 18.76.

N-(6-{[(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]-amino}nicotinoyl)glutamic Acid (3'-Azafolic Acid).—Compd 8b (759 mg, 1.3 mmoles) was dissolved in 390 ml of deaerated 0.1 N

NaOH, and the soln was allowed to stir at room temp under N₂. At the end of 16 hr, the soln was acidified to pH 3.5, and the ppt was isolated and washed with dil HCl by centrifugation. The gelatinous mass was dried (P₂O₅) *in vacuo*, and the solid obtd was dissolved in H₂O contg 2 equiv of NH₄OH. This soln was diluted to 1 l. and was made 0.1 M in mercaptoethanol; the resulting soln (pH <7) was applied to a DEAE-cellulose column (22 g, std capacity, phosphate form). The column was eluted with a linear NaCl gradient (1000 ml of 0.7 M NaCl in the reservoir and 1000 ml of 0.0 M NaCl in the mixing bottle; both soln were 0.1 M in mercaptoethanol). Elution of the fractions was monitored by uv absorption, and the desired fractions were combined and acidified to pH 3.5 with HCl.

The ppt was collected and washed with dil HCl by centrifugation. The gelatinous mass was dried (P₂O₅) *in vacuo*, and the product was pulverized before final drying (P₂O₅) for 24 hr at room temp and 0.5 mm. The yellow solid weighed 384 mg, 63% yield (8% yield from 2); a comparable reaction had previously afforded 53% yield (13% from 2); tlc: 6 appeared homogeneous (uv detection) on SGH (solvent A), Cell-A (solvent A), Cell-B (solvents C, D, E). On Cell-B (solvent A), 6 produced a pattern of 2 overlapping spots; repetition of this same pattern from each of the original 2 spots on 2-dimensional tlc indicated the pattern was a chromatographic artifact. On Cell-DEAE (solvent F), 6 appeared to contain two blue-fluorescent impurities. Side-by-side comparison of 6 with 10 in this tlc system indicated that the amount of 11 present in 6 was much less than 1%; the amount of the second impurity appeared comparable; uv max (0.1 N HCl) 261 (ϵ 24,800), 316 (15,500); uv max (pH 7) 277 (32,000), 347 (8020); uv max (0.1 N NaOH) 258 (28,200), 275 (29,000), 365 (8900); pmr (CF₃CO₂D) δ 2.62 (m, 4 H, CH₂CH₂), 5.13 (m, 1 H, NCH₂), 5.18 (s, 2 H, NCH₂), 7.42 (d, 1 H, C₂-H), 8.58 (d of doublets, 1 H, C₄-H), 8.84 (d, 1 H, C₂-H), 9.05 (s, 1 H, C₂-H). Anal. (C₁₁H₁₀N₄O₆ · 1.5H₂O) C, H, N.¹⁰

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Choline Acetyltransferase Inhibitors. Styrylpyridine Analogs with Nitrogen-Atom Modifications

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Among steric and electronic features of styrylpyridine analogs previously associated with their activity as inhibitors of choline acetylase were molecular coplanarity and the π -electron-deficient cationic pyridinium moiety. Azomethines, capable of assuming, but presumably not preferring, molecular coplanarity, are represented by both active and inactive trans isomer types. The active inhibitor is less potent than the styrylpyridine analog. An aminomethylstilbene had no inhibitory activity; the cationic and steric features are believed favorable, but the absence of a π -electron-deficient moiety is not. 2-Pyridoneimines were intermediate in potency and ionization between corresponding pyridine and pyridinium analogs. These observations are consistent with previously reported structure-activity relationships and proposed enzyme receptor interactions.

In previous articles,^{2,3} a variety of steric and electronic parameters of analogs of styrylpyridines were assessed in relation to influences on potency and speci-

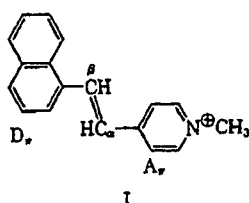
ficity as inhibitors of choline acetylase (ChA) (choline acetyltransferase). A simple, potent prototype is the *trans*-*N*-methyl-4-(1-naphthylvinyl)pyridinium salt, I. There are now described analogs that include azomethines, in which the α and β carbon atoms are selectively replaced by N, 2-pyridoneimine (1,2-dihydro-2-imino-pyridine) species, and *trans*-4-aminomethylstilbene. For the reasons next outlined, these have been designed

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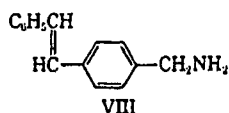
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to permit more critical evaluation of projected hypotheses.^{2,3}

Earlier evidence pointed to molecular coplanarity as a feature favoring ChA inhibitory activity among the styrylpyridine analogs. The *trans* isomers of stilbene, azobenzene, styrylpyridine, and their unhindered derivatives energetically favor coplanarity. Azomethines (Schiff bases), on the other hand, have been described as nonplanar.⁴⁻⁷ In benzylideneanilines, the phenyl ring attached to N has been proposed as existing at an angle of from 30° to 40-60° from the plane of the PhCH=N unit.^{5,6} These azomethines are stated to be more stable as *trans* isomers and to photoisomerize to *cis* forms.⁸ Although an unhindered *trans*-azomethine in solution may not preferentially assume coplanarity, there appears to be no barrier to existence of the coplanar form. Assuming electronic factors remain favorable, an azomethine should be less potent than styrylpyridine but not necessarily inert as a ChA inhibitor if coplanarity favors potency.

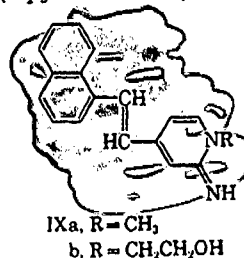
Previous studies showed that ChA inhibitory potency was favored by a cationic charge on the pyrido component of I. Such a structural feature endows this part of the molecule with both π -electron-acceptor (A_{π}) qualities and ionic-bonding potential. Earlier variations, such as styrylpyridine *N*-oxide and *trans*-stilbene-4-carboxylic acid, are neither cationic nor π -electron deficient, and their inactivity as inhibitors cannot be unequivocally associated with only one of these variables. There is considerable evidence that little specificity is associated with the nature of substituents in the vicinity of the pyrido-N.^{2,3,9} A compound with dimensional, hydrophobic and conjugation features comparable to *trans*-styrylpyridine, and possessing a basic group in the structurally nonspecific locus, is *trans*-4-aminomethylstilbene (VIII). It should be at least as potent as the



pyridine base if cationic charge alone is sufficient, but inactive if π -electron-acceptor properties are required.

The quaternized pyridinium derivatives, such as I, are considerably more potent inhibitors of ChA than are the weakly basic pyridine forms. A 2-pyridoneimine analog of I would be a much stronger base¹⁰ than a pyridine, presumably a π -electron acceptor in the ionized state, and predicted as intermediate in potency be-

tween the pyridine and its quaternized derivative. Compounds IXa and IXb were prepared and, consistent with spectral and other physical characteristics and evidence in the literature,¹¹ are assigned the 1,2-dihydro-2-iminopyridine (2-pyridoneimine) structure.



Discussion and Results

Two isomeric pairs of azomethines were compared with the styrylpyridine analogs, I and II. Although I and II are only about three times as potent as the corresponding azomethine pair III and IV, the isomeric azomethines V and VI are essentially inert. Anils as a group are more susceptible to hydrolytic cleavage,¹² than are styrylpyridines. Aqueous solutions of IV and VI at pH 7.4 in the dark showed, respectively, no change in the uv spectrum of VI after 3 hr, and a progressive change in IV measured from 0.5 to 4 hr. Inactive VI is more stable than inhibitory IV, so the difference in potency cannot be attributed to hydrolysis. Both IV and VI were photolabile, and uv spectral characteristics changed after 30-min exposure to fluorescent light. This is characteristic of *trans* isomers of azomethines⁸ as well as of styrylpyridines. The uv spectra of IV and VI are markedly different from one another and may reflect electronic differences between this pair of structural isomers which also may influence their relative tendency to favor molecular coplanarity. The isomer VI is about as inert as the nonconjugated, noncoplanar VII. The differences between IV and VI which influence ChA inhibitory activity may be steric as well as electronic.

The *trans*-4-aminomethylstilbene, VIII, not only was inactive as a ChA inhibitor, but was found to be slightly stimulating for this enzyme.¹³ This strongly supports the contention that the π -electron-deficient (acceptor) moiety in the styrylpyridine analogs is important for ChA inhibitory activity. If cationic charge were sufficient, VIII should have a ChA I_{50} value between that of *trans*-4-styrylpyridine (6×10^{-4} M) and its *N*-Me quaternary derivative (1.5×10^{-5}).² A much lower rate of photoisomerization was found for VIII than for the *trans*-styrylpyridines under ionizing conditions, further supporting the view¹⁴ that the π -electron-acceptor character of the pyridinium moiety contributes to *cis* isomer stabilization by intramolecular charge transfer interaction.

In contrast to the noninhibitory nature of the stilbene derivative, VIII, the two 2-pyridoneimines, IXa and IXb,

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TABLE I
CHOLINE ACETYLASE INHIBITORY ACTIVITIES

No.	x-y ^a	RX	Mp, °C	% ^b yield	ChA I ₅₀ , M	Formula
I		HCl			2.5 × 10 ⁻⁵	C ₁₇ H ₁₅ ClN
II		MeI			4.7 × 10 ⁻⁷	C ₁₈ H ₁₅ IN
III		MeI	111-112	87	7.5 × 10 ⁻⁸	C ₁₈ H ₁₅ N ₂
IV		MeI	209-212 dec	27	1.5 × 10 ⁻¹⁰	C ₁₇ H ₁₅ IN ₂
V		MeI	65-67	75	15%/10 ⁻⁴	C ₁₈ H ₁₅ N ₂
VI		MeI	187-189	12	15%/10 ⁻⁴	C ₁₇ H ₁₅ IN ₂
VII		MeI	193-195		10%/10 ⁻⁴	C ₁₈ H ₁₅ IN

^a Where x-y is a double bond link, the configuration is trans. ^b Yield as analytically satisfactory material. ^c Compound reported earlier,^{2,3} included to facilitate comparisons. ^d Molar I₅₀ against crude rat brain acetylcholinesterase is 3.8 × 10⁻⁴.

had molar I₅₀ values against ChA of 5 × 10⁻⁶ and 8 × 10⁻⁶, respectively. These values, as was predicted, are intermediate between those of the more ionized I and weaker base II. The relative nonspecificity of substituents in the pyridine-N region is again confirmed. The hydroxyethyl derivative, IXb, is more water soluble than IXa and may be particularly appropriate for animal pharmacology studies.

Recently, a variety of anthelmintic 1-arylvinylpyridinium derivatives have been described.¹⁵ One of these, 1-(phenylvinyl)pyridinium bromide, in our tests showed less than 20% inhibition of ChA at 10⁻⁴ M. This is relatively inactive compared with the (4-phenylvinyl)-1-methylpyridinium derivatives.^{2,3} This may result from an unfavorable localization of the cationic charge or from deviation from coplanarity, or both, in the 1-arylvinylpyridinium system.

Experimental Section

The final compds usually were dried *in vacuo* at 80°. Yields were of secondary importance to purity. Melting points were taken with a Fisher-Johus apparatus; reported values are corrected. Analyses were performed by M-H-W Laboratories, Garden City, Mich. Found anal. conform to within 0.3% of the calcd values.

Inhibition measurements against rat brain ChA and AChE enzyme preps were conducted as described previously.³ The ChA values also were checked by a second method.¹⁴ Subdued or pink light illumination was used during experimental exposure of solns of the test compds.

N-(1-Naphthyl)-1-(4-pyridyl)methylenimine (III) and Its Methiodide (IV).—A mixture of 2.8 g (0.02 mole) of 1-naphthylamine and 2.1 g (0.02 mole) of 4-pyridinecarboxaldehyde in 40 ml of EtOH was refluxed for 5 hr. The solvent was evapd off, and the crude material recrystd from hot *i*-PrOH to yield 4.0 g of product (III). Anal. (C₁₈H₁₅N₂) C, H.

A soln of 0.46 g (0.002 mole) of III in 5 ml of MeI was allowed to stand at room temp for 3 hr. The crude reaction material which pptd on addn of Et₂O was collected by filtration and recrystd from hot EtOH to yield 0.2 g of IV. Anal. (C₁₈H₁₅IN₂) C, H.

1-(1-Naphthyl)-N-(4-pyridyl)methylenimine (V) and Its Methiodide (VI).—A mixture of 4.7 g (0.05 mole) of 4-aminopyridine and 7.8 g (0.05 mole) of 1-naphthaldehyde in 70 ml of dry PhMe was heated to reflux for approx 30 hr with a catalytic amount of PhSO₃H, and the H₂O formed was distd off azeotropically. The solvent then was distd off, and the residue was crystd by dissolving in hot ligroin and cooling to -5° to yield V. Anal. (C₁₈H₁₅N₂) C, H.

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A soln of 2.3 g (0.01 mole) of V in 10 ml of MeI was allowed to stand at room temp overnight. The MeI was evapd off, the residue was triturated with warm EtOH, the soln was filtered, and the filtrate was cooled to yield product. Recrystn from hot EtOH was repeated to yield pure VI. Anal. (C₁₇H₁₅IN₂) C, H, N.

4-(1-Naphthylethyl)pyridine Methiodide (VII).—4-(1-Naphthylvinyl)pyridine was reduced with 50% HI¹⁶ to the Et analog, purified as the HCl salt. The liberated base treated with MeI yielded VII, recrystd from hot *i*-PrOH. Anal. (C₁₈H₁₅IN) C, H.

trans-4-Aminomethylstilbene Hydrochloride (VIII).—A soln of 4.2 g (0.02 mole) of stilbene-4-carbonitrile¹⁷ in Et₂O was treated with LAH.¹⁸ The product was pptd from Et₂O by addn of ethereal HCl. Two recrystns from *i*-PrOH gave 1.6 g of white solid: mp 268-269.5°; ir (KBr), 2800-3400 (NH₂⁺), 1610 (stilbene C=C), 975 cm⁻¹ (trans RC(H)=C(H)CR); uv, λ_{max} (H₂O), 308 mμ (ε 30,000), 298 (30,100), 216 (14,100); nmr (DMSO-d₆, Me₄Si) s, 2, τ 5.98 (C), m, 5, 2.48-2.68 (A), s, 6, 2.35 (b), b, 3, -1.0 (D). Anal. (C₁₅H₁₅ClN) C, H.

A melting point of 300-305° has been reported¹⁹ for VIII and is not in agreement with our finding. The ir, uv, and nmr spectra of our product are consistent with the structure.

Photoisomerization of VIII.—The uv spectrum of a sample of VIII in H₂O was determined using a Cary Model 15 spectrophotometer. The sample in the cuvette was then irradiated with a 275-W Westinghouse sun lamp at a distance of 1 m for 1-, 6-, and 66-min total exposures. The uv spectrum was determined after each exposure. The two bands at 308 and 298 mμ were replaced by one band at 283 mμ after 66-min exposure (Table II). This

TABLE II
INFLUENCE OF IRRADIATION ON UV SPECTRUM OF
trans-4-AMINOMETHYLSTILBENE HYDROCHLORIDE IN WATER

Exposure, min	Absorbance		
	308 mμ	298 mμ	216 mμ
0	0.75	0.74	0.34
1	0.67	0.67	0.34
6	0.35	0.39	0.40
		283 mμ	
66		0.26	0.40

rate of photoisomerization is considerably lower than that observed with the styrylpyridines under identical conditions.

1-Methyl-2-imino-4-(1-naphthylethenyl)-1,2-dihydropyridine (IXa) and HI Salt.—A soln of 2- amino-4-methylpyridine in MeI was refluxed for 10 min, excess MeI evapd off, and the residue recrystd from hot *i*-PrOH to yield 2-imino-1,4-dimethyl-1,2-dihydropyridine·HI.²⁰ To 2.5 g (0.01 mole) of this product in 30

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ml of MeOH was added 1.6 g (0.01 mole) of 1-naphthaldehyde and 3 drops of piperidine. After 30-hr refluxing, solvent was evapd off, and the residue was washed with Et₂O and then recrystd from hot 1:1 water-MeOH to yield 2.8 g (75%) of IXa-HI, mp 278-281°, dec. Anal. (C₁₈H₁₇IN₂) C, H.

Treatment of the HI salt at room temp with 10% aq NaOH yielded the base; recrystd from hot CH₃CN, mp 200-203°. Treatment of the base with HI regenerated the original salt (same mp and ir spectrum).

1-Hydroxyethyl-2-imino-4-(1-naphthylethenyl)-1,2-dihydropyridine (IXb) and HBr Salt. A soln of 5.3 g (0.05 mole) of 2-amino-4-methylpyridine and 6.2 g (0.05 mole) of bromoethanol in 30 ml of *i*-PrOH was refluxed overnight. Crude product sepd on cooling and was recrystd from hot MeCN to yield 9.2 g (80%) of 1-hydroxyethyl-2-imino-4-methyl-1,2-dihydropyridine-HBr, mp 132-134°. This was treated with 1-naphthaldehyde and the product recrystd as described for IXa, mp 214-217°. Anal. (C₁₉H₁₉BrN₂O) C, H.

Treatment of the HBr salt with 10% aq NaOH at room temp

yielded the base; recrystd from hot *i*-PrOH, mp 162-163. Anal. (C₁₉H₁₉N₂O) C, H, N.

Acknowledgments.—The choline acetyltransferase and AChE inhibition studies at Montefiore Hospital and Medical Center were supported in part by U. S. Public Health Service Grants MHI3331 and NBO5541. The research at the University of North Carolina was supported in part by U. S. Public Health Service Grant NBO7932. Compound VIII was prepared by M. L. Edwards while supported by a fellowship from Bristol Laboratories, Syracuse, New York. We are indebted to Pfizer Medical Research Laboratories, Groton, Conn., for a sample of 1-(phenylvinyl)pyridinium bromide.

Inhibitors of Blood Platelet Aggregation. 1. Biphenyloxyalkylamines

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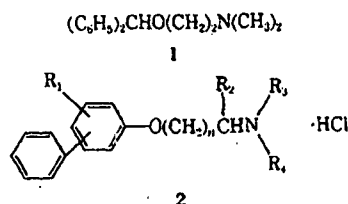
Certain biphenyloxyalkylamines (2) inhibit ADP-induced platelet aggregation *in vitro*. The most active compounds, 5-(4-chloro-2-phenylphenoxy)-*N*-ethyl-*N*-(2-hydroxyethyl)amylamine-HCl (14) and the homologous butylamine-HCl (11), afford 50% inhibition of ADP-induced platelet aggregation at 4.8×10^{-6} M and 7.1×10^{-6} M, respectively. The structural requirements for activity are rather general. Thus, a compound having a large lipophilic moiety separated from a basic N by 2-10 elements will usually inhibit, to some degree, this type of platelet aggregation. The activity of these compounds appears to be related to that exhibited by "membrane-active" drugs such as the tricyclic antidepressants and certain antihistamines. The mechanism of action appears to be mediated through a nonspecific adsorption of these lipophilic amines onto the platelet membranes, causing a general disruption of membrane function.

One of the earliest events in the formation of an intravascular thrombus is the aggregation of blood platelets.¹ This intravascular aggregation has been described as a pathological exaggeration of the normal role of platelets in hemostasis and repair.² It is generally conceded that an agent which would reduce abnormally high platelet adhesiveness to, but not below, that level required for adequate hemostasis may be useful in the treatment and prophylaxis of thrombotic diseases.

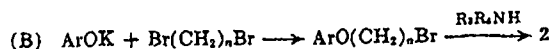
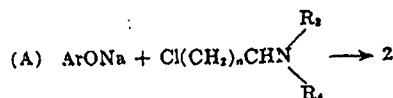
The role of adenosine diphosphate (ADP) as an important and biologically omnipresent initiator of platelet aggregation has been well documented.³ Thus, agents that will inhibit ADP-induced platelet aggregation are of interest as potential drugs.

A number of lipophilic amines have been reported to inhibit ADP-induced platelet aggregation *in vitro*. Among these are tricyclic antidepressants,⁴ antihistamines,^{4c,5} and phenothiazine antipsychotic agents.^{5a,c,6}

We explored the structure-activity relationship of a series of biphenyloxyalkylamines. The general structure (2) of this series can be derived from 1 by moving the side chain to one of the rings and eliminating the methylene bridge. Two methods were used to prepare



the compounds listed in Tables I and II.



Both methods involve a Williamson ether synthesis for coupling phenols and side chains. In method A the amine moiety was introduced using an aminoalkyl halide, whereas in method B an ω -bromoalkoxybiphenyl was prepared and used to alkylate the appropriate amine.

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complex between imine **2**, MeLi, and the chiral ligand **3** (entries 3–6). Enantioenrichment was possible by recrystallizing **4** to afford optically pure amines in high overall yield (entries 4 and 5). The imine **2f** bearing a 4-methoxynaphthalenyl group is also a suitable substrate for addition of butyl- and phenyllithiums to afford the corresponding amines **4f** (R = Bu, Ph) in 86 and 92% ees, respectively (entries 7–8).

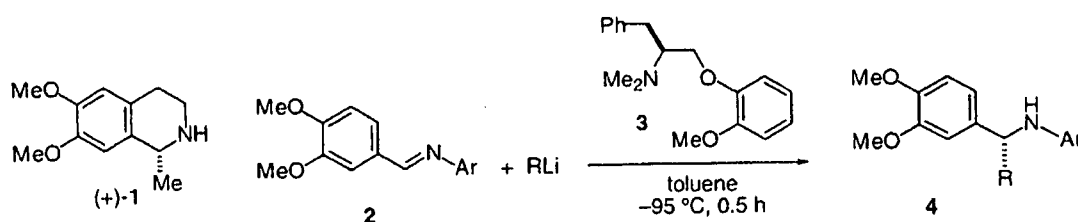


Figure 1. Asymmetric addition of organolithium reagents to **2** in the presence of **3**

Table 1
Asymmetric addition of RLi to **2** in the presence of **3** producing **4**

Entry	2	Ar	RLi	yield/% ^a	ee/% ^a	confign
1	a	<i>p</i> -MeOPh	Me	99	78	<i>R</i>
2	b	<i>p</i> -Me ₂ NPh	Me	99	88	<i>R</i> ^b
3	c	4-ClNaph	Me	95	73	<i>R</i> ^b
4	d	1-Naph	Me	99 (71)	94 (>99)	<i>R</i> ^b
5	e	4-Me ₂ NNaph	Me	99 (74)	97 (>99)	<i>R</i>
6	f	4-MeONaph	Me	97	97	<i>R</i>
7	f	4-MeONaph	Bu ^c	97	86	<i>R</i> ^b
8	f	4-MeONaph	Ph	99	92	<i>R</i> ^b

a) Numbers in parentheses are yield and ee for recrystallized compounds. Ee was determined by HPLC using a chiral stationary phase column (Daicel Chiralpak AD and OD-H) eluted with *i*-PrOH/hexane. b) Configurations were predicted by analogy. c) The reaction was conducted in a mixture of ether-toluene (2/1).

The next problem to be solved was oxidative removal of the *N*-aryl group. Treatment of **4f** (Ar = 4-MeONaph, R = Me) with ammonium cerium(IV) nitrate (CAN) in aqueous acetonitrile under standard conditions⁵ failed to provide the corresponding primary amine **5** in a detectable yield (Fig. 2). To avoid further Michael-type addition reaction of the primary amine **5** with naphtho-1,4-quinone **6** produced, sodium borohydride was added to the mixture to reduce **6** to naphthalene-1,4-diol **7**. To our delight, under these conditions the primary amine **5** was isolated in 47% yield. Substantial improvement in yield was realized by further treatment with acetic anhydride to afford a mixture of the primary amine **5** and its acetoamide **8** in 75% combined yield.

This new procedure for CAN oxidation is applicable to **4a** (Ar = PMP, R = Me) to afford a mixture of the amine **5** and amide **8** in 89% combined yield. Isolation of diacetate of benzene-1,4-diol **9** in 67% yield indicates that treatment with sodium borohydride reduces benzoquinone to

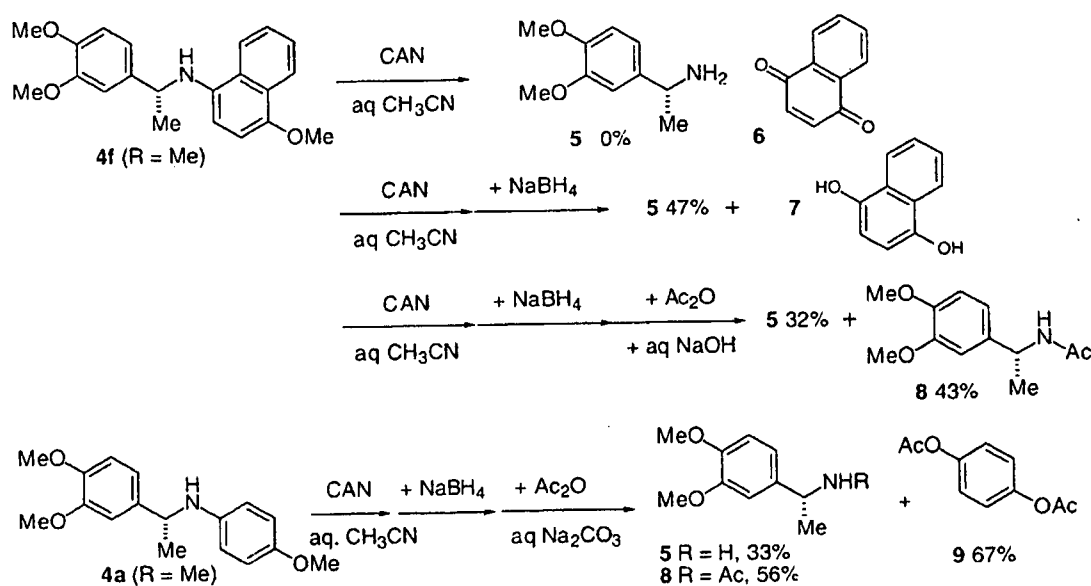


Figure 2. Successful treatment of **4** with CAN-NaBH₄-Ac₂O for oxidative removal of *N*-aryl group

benzene-1,4-diol, and further treatment with acetic anhydride acetylates diol to diacetate **9**, which process avoids back-oxidation to benzoquinone (Fig. 2).

Having established a new procedure for asymmetric alkylation and oxidative removal of the *N*-aryl group, we then focused on asymmetric synthesis of (+)-salsolidine **1**. Condensation of **10** and **11**⁹ gave an imine **12**, which was subjected asymmetric alkylation with MeLi in the presence of a stoichiometric amount of **3** to give an amine **13** in 93% ee and quantitative yield (Fig. 3).

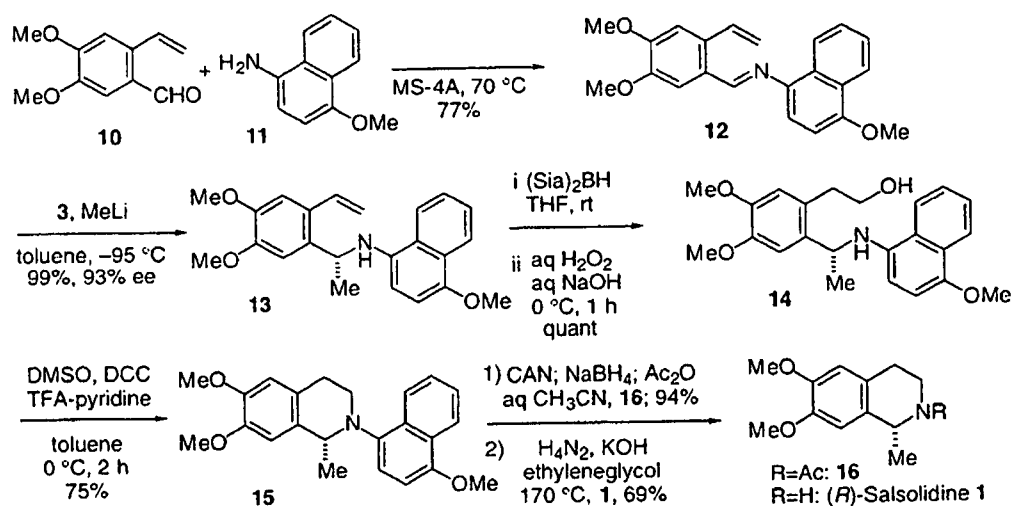


Figure 3. Asymmetric synthesis of (+)-salsolidine **1** from **10**

Hydroboration and subsequent oxidation gave an alcohol **14**, which was then subjected to cyclization to afford **15** in 75% overall yield from **13**. New CAN procedure gave **16** in 94% yield. Hydrolysis of **16** afforded the target (+)-**1** in 69% yield. Specific rotation of synthetic (+)-**R-1** ($[\alpha]_D^{25} +54.0$ (c 0.63, EtOH)) is indistinguishable from that of the reported (–)-**S-1** ($[\alpha]_D -59.5$ (c 4.39, EtOH)).¹⁰

The presented three-step procedure for the highly selective asymmetric synthesis of salsolidine is applicable to other biologically potent TIQ and related natural alkaloids. Further studies directed toward more efficient asymmetric alkylation of imines are in progress in our laboratories.

Acknowledgements

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1901

dessen Resultate ich jetzt im Zusammenhang mittheilen möchte. Der Antheil, den die HHrn. Freyss, Serra, Dreyfus, Bourry und Demant an diesen Arbeiten genommen, geht aus den weiter unten befindlichen Tabellen hervor.

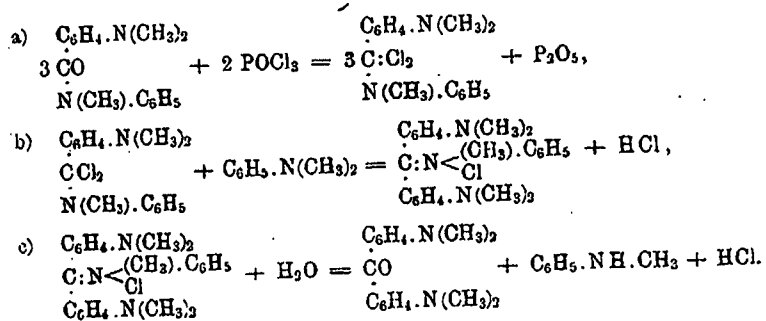
Zur Herstellung von Dinaphtylphenylmethanderivaten habe ich einerseits wie Friedländer und Wellmans Dimethyl-*p*-amidobenzaldehyd (und seine Nitroderivate) mit substituirten Naphtylaminen condensirt, aber nicht mit tertiären, sondern mit den sehr reactionsfähigen secundären ($C_{10}H_7.N<\begin{smallmatrix} CH_3 \\ H \end{smallmatrix}$, $C_{10}H_7.N<\begin{smallmatrix} C_2H_5 \\ H \end{smallmatrix}$, $C_{10}H_7.N<\begin{smallmatrix} C_6H_5 \\ H \end{smallmatrix}$, $C_{10}H_7.N<\begin{smallmatrix} C_7H_7 \\ H \end{smallmatrix}$), und die so erhaltenen Leukobasen oxydirt; andererseits habe ich gemischte Diamido-naphtyl-phenylketone dargestellt und diese mit secundären Naphtylaminen condensirt. (Mit tertiären Aminen der Benzolreihe geben diese Ketone Naphtyldiphenylmethanfarbstoffe.) Die Farbstoffe resp. Leukobasen der Trinaphtylmethanreihe endlich wurden aus secundären Naphtylaminen und Tetrachlorkohlenstoff, bezw. *o*-Ameisensäureester erhalten.

Darstellung der Diamido-phenyl-naphtyl-ketone.

In dem D. R.-P. Nr. 44077 vom 27. Oct. 1887 beschreiben die Farbwerke Höchst eine Methode zur Darstellung substituierter aromatischer Ketone, welche auf folgenden Reactionen beruht:

Dimethyl-*p*-amidobenzomethylanilid wird mit Phosphoroxychlorid behandelt, wodurch es in das Dichlorid übergeht. Durch Umsetzung dieses Letzteren mit tertiären Aminen entstehen Aurammoniumverbindungen, welche durch Wasser in Ketone, durch Ammoniak in Auramine umgewandelt werden.

Die Reaction mit Dimethylanilin z. B. findet nach folgendem Schema statt:



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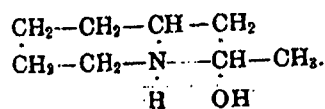
Silberchlorid erhält man das entsprechende Chlorid, das mit Platinchlorid bei geeigneter Concentration kleine, stark glimmernde Kristalle liefert. Sie sind den aus der activen Base gewonnenen sehr ähnlich, doch kleiner ausgebildet. Es zersetzt sich ebenfalls, bevor es schmilzt, und zwar liegt der Zersetzungspunkt bei derselben Temperatur wie bei dem activen Salz, 209—211°.

Es ist in Alkohol schwerer löslich als in Wasser.

0.2891 g Subst.: 0.0785 g Pt.

$(C_8H_{15}N.C_2H_5Cl)_2PtCl_3$. Ber. Pt 27.21. Gef. Pt 27.15.

Da die Base tertiär und gesättigt ist, kommt ihr mithin dieselbe Constitution zu wie der aus dem Jodid des Pipecolylmethylalkins durch Abspaltung von Jodwasserstoff gewonnenen, um 8° niedriger siedenden Base:



Sie ist daher mit jener stereoisomer und als die zweite inactive, spaltbare Form der tertiären Base aufzufassen und, so weit die Untersuchung der aus dem leichter löslichen Jodhydrat gewonnenen, bei derselben Temperatur siedenden Base ergeben haben, mit dieser identisch. Sie ist aber auch als die inactive Form des von Hofmann aus dem Conhydrin durch Abspaltung von Wasser gewonnenen α -Coniceins zu betrachten.

Die diesbezüglichen Versuche werden fortgesetzt.

University College, London.

285. E. Noelting: Ueber Farbstoffe der Naphtyl-diphenyl-methan-, Dinaphtyl-phenyl-methan- und Trinaphtyl-methan-Reihen.

(Eingegangen am 2. März 1904.)

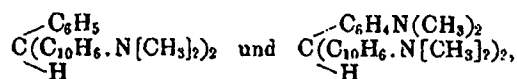
Die Farbabkömmlinge der Naphtyl-diphenyl-methanreihe sind durch die Patente 27032 und 27789 der Badischen Anilin- und Soda-Fabrik bekannt geworden. In dem ersten derselben werden durch Condensation des Tetramethyldiamidobenzhydrols mit α -Naphtylamin, Dimethyl- und Diäthyl-Naphtylamin Leukobasen dargestellt, welche

durch Oxydation Farbstoffe liefern; in dem zweiten werden durch Condensation von Tetramethyldiamidobenzophenon (und dem analogen Aethylderivate) mit Phenyl- α -naphtylamin, Methyl-phenyl- α -naphtylamin und Paratolyl- α -naphtylamin direct Farbstoffe erhalten, die unter dem Namen Victoriablau B, 4R und Nachtblau eine grosse technische Bedeutung erlangt haben. Die Farbenfabriken vorm. F. Bayer & Co. haben später (D. R.-P. 66712) das Hydrol mit Phenyl- α -naphtylamin und anderen secundären, aromatischen α -Naphtylaminen condensirt. Die so erhaltenen Leukobasen wurden dann in Form ihrer Nitrosamine der Oxydation unterworfen und hierauf die Nitrosogruppe abgespalten, wodurch dieselben Farbstoffe resultirten. Nach den Tabellen von Schultz-Julius stellen sie auch, sowohl nach der Keton- wie nach der Hydrol-Methode, den Farbstoff $\begin{matrix} \text{C}_6\text{H}_4:\text{N}(\text{CH}_3)_2\text{Cl} \\ \text{C}_6\text{H}_4.\text{N}(\text{CH}_3)_2 \\ \text{C}_{10}\text{H}_6.\text{NH}.\text{C}_2\text{H}_5 \end{matrix}$, Neuvictoria-blau, dar (4. Auflage 1902, Nr. 462).

Eine Anzahl weiterer Patente verschiedener Firmen, deren Aufzählung zu weit führen würde, behandelt die Condensation von Keton und Hydrol mit Naphtalin, Naphtolen, Dioxynaphtalinen und den Sulfosäuren dieser Verbindungen.

Die einzige wissenschaftliche Untersuchung über die Farbstoffe der Naphtyl-diphenylmethanreihe rührt von Nathanson und Müller¹⁾ her, welche die verschiedenen Victoriablau einem eingehenden Studium unterwarfen.

Verbindungen der Dinaphtyl-phenyl-methanreihe sind nur von Friedländer und Wellmans²⁾ dargestellt worden, und zwar durch Condensation von Benzaldehyd und Dimethylparaamidobenzaldehyd mit Dimethyl- α -naphtylamin. Diese Forscher erhielten die Basen:



konnten aber aus denselben nicht in glatter Weise die entsprechenden Farbstoffe erhalten, sodass die Dinaphtyl-phenyl-methanfarben bis jetzt noch als unbekannt angesehen werden können.

Ueber Trinaphtylmethanderivate endlich weiss man bis jetzt absolut nichts.

Ich habe seit einigen Jahren, in Gemeinschaft mit mehreren meiner Schüler, dieses Gebiet einem eingehenden Studium unterworfen,

¹⁾ Nathanson und Müller, diese Berichte 22, 1875 [1889].

²⁾ Friedländer und Wellmans, diese Berichte 21, 3123 [1888].

	Ketone	Eigenschaften	Mitarbeiter	Verwendung
I	$C_6H_4.N(OH)_2$ CO $C_{10}H_6.NH.OH_3$	Hellgelbe Krystalle, Schmp. 211—212°. $C_{20}H_{20}N_2O$ Ber. C 78.94, H 6.57, N 9.21. Gef. » 78.75, » 6.57, » 9.51.	Bourry	Giebt mit $C_{10}H_7.NH.OH_3$ und $POCl_3$ einen blauen Farbstoff analog dem No. 23, der aber schlecht kryscallisierte und deshalb nicht analysirt wurde. Bei der Reduktion giebt derselbe die Leukobase No. 10.
II	$C_6H_4.N(CH_3)_2$ CO $C_{10}H_6.NH.O_2H_5$	Grünliche, prismatische Krystalle, Schmp. 162°. Leicht löslich in Benzol, Alkohol, Aether, fast unlöslich in Ligroin. $C_{20}H_{22}N_2O$ Ber. C 79.25, H 6.92, N 8.80. Gef. » 79.36, » 7.27, » 8.92.	Serra Demant Entsprechendes Auramin: Demant	Giebt mit $C_{10}H_7.NH.O_2H_5$ und $POCl_3$ den Farbstoff No. 23.
III	$C_6H_4.N(CH_3)_2$ CO $C_{10}H_6.NH.C_6H_5$	Hellgelbe Nadeln, Schmp. 201—202°. Fast unlöslich in Alkohol, löslich in Toluol. $C_{23}H_{22}N_2O$ Ber. C 81.94, H 6.22, N 7.91. Gef. » 81.53, » 6.23, » 7.84.	Serra Auramin: Demant	Giebt mit $C_{10}H_7.NH.C_6H_5$ und $POCl_3$ den Farbstoff No. 24, mit $C_6H_5.N(CH_3)_2$ den Farbstoff No. 21.
IV	$C_6H_4.N(CH_3)_2$ CO $C_{10}H_6.NH.C_6H_4.CH_3$	Hellgelbe Nadeln (aus Benzol) Schmp. 221°. $C_{26}H_{24}N_2O$ Ber. C 82.10, H 6.31, N 7.36. Gef. » 81.93, » 6.49, » 7.51. » 81.82, » 6.5, » 7.5. Das entsprechende Hydrol löst sich in Essigsäure mit grüner Farbe und färbt Seide und taunirte Baumwolle grün.	Serra Dreyfus Auramin: Demant	Giebt mit $C_{10}H_7.NH.C_6H_4$ und $POCl_3$ den Farbstoff No. 25, mit $C_6H_5.N(CH_3)_2$ den Farbstoff No. 22.

1903

	Ketone	Eigenschaften	Mitarbeiter	Verwendung
V	$C_6H_4.N(C_2H_5)_2$ CO $C_{10}H_6.NH.C_2H_5$	Aus Alkohol grosse, gelbe Krystalle, Schmp. 149°. $C_{27}H_{24}N_2O$. Ber. C 79.51, H 7.28, N 8.43. Gef. » 79.79, » 7.34, » 8.60.	Bourry	Diese Ketone liefern unter genau den- selben Bedingungen Farbstoffe wie die Ketone I–IV. Dieselben sind leichter löslich und weniger gut krystallisierend, sonst in ihren Eigenschaften den Ersteren sehr ähnlich. Sie sind nicht analysirt worden.
VI	$C_6H_4.N(C_2H_5)_2$ CO $C_{10}H_6.NH.C_2H_5$	Aus Alkohol in zwei Formen erhältlich (grün oder gelb), Schmp. 133.5°. $C_{23}H_{20}N_2O$. Ber. C 79.76, H 7.51, N 8.09. Gef. » 79.57, » 7.65, » 8.22.	Bourry	
VII	$C_6H_4.N(C_2H_5)_2$ CO $C_{10}H_6.NH.C_6H_5$	Aus Alkohol, in welchem es schwer löslich ist, gelbe, derbe Krystalle, Schmp. 146–147°. $C_{27}H_{26}N_2O$. Ber. N 7.11. Gef. N 7.09.		
VIII	$C_6H_4.N(C_2H_5)_2$ CO $C_{10}H_6.NH.C_6H_4.OH_3$	Grünlich-gelbe Nadeln, Schmp. 176–177°, sehr schwer lös- lich in Alkohol und Aether, leicht in Benzol. $C_{28}H_{28}N_2O$. Ber. C 82.35, H 6.86, N 6.86. Gef. » 82.21, » 7.08, » 6.95.	Serra	

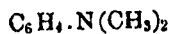
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Ich habe nun gefunden, dass nicht nur tertiäre fettaromatische Amine einer derartigen Umsetzung fähig sind, sondern dass auch secundäre fettaromatische und aromatische Amine, welche einen Naphtylkern enthalten, sich ähnlich verhalten, z. B. Methyl-, Aethyl-, Phenyl- und Paratolyl- α -Naphtylamin.

Die Darstellung dieser gemischten Naphtyl-phenyl-ketone bildet den Gegenstand der jetzt erloschenen Patente No. 79390 und 84655. (Friedlaender IV, S. 221 und 223.)

Die vorstehende Tabelle (S. 1902 u. 1903) fasst die acht auf diese Weise erhaltenen Ketone zusammen.

Die Arbeitsweise ist in allen Fällen so ziemlich die gleiche, so dass ich mich begnüge, sie an zwei Fällen zu erläutern.

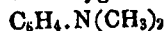


Zur Darstellung von CO

erwärmt man 25 g feinge-



pulvertes Dimethyl-*p*-amidobenzomethylanilid mit 17 g Aethyl- α -naphtylamin einige Zeit auf dem Wasserbade, um eine innige Mischung zu erzielen. Nach dem Erkalten giebt man 15 g Phosphoroxychlorid hinzu und erwärmt nun 3—5 Stdn. weiter. Die Schmelze erstarrt allmählich zu einer festen, rothbraunen, metallglänzenden Masse, welche fein zerrieben ein ziegelrotes, sehr hygroskopisches Pulver



darstellt. Diese Aurammoniumverbindung, $\text{C}:\text{N}(\text{C}_6\text{H}_4)(\text{CH}_3)_2.\text{Cl}$, ist in



Wasser wenig, in Alkohol leicht löslich; sie färbt Seide und tannirte Baumwolle orange. Die Lösung entfärbt sich aber nach einiger Zeit unter Bildung des Ketons; immerhin ist die Verbindung etwas beständiger als die analogen der Benzolreihe. Zur Darstellung des Ketons wird die gepulverte Rohschmelze in Alkohol gelöst und nach dem Filtriren mit concentrirter Natronlauge versetzt. Beim Schütteln tritt nach kurzer Zeit Entfärbung ein, und das Keton scheidet sich in harten, gelb gefärbten Klumpen ab. Man filtrirt, wäscht mit Wasser und etwas Alkohol und krystallisirt aus Alkohol um. In einem anderen Falle, bei dem Keton aus Phenyl- α -naphtylamin, haben wir auch so gearbeitet, dass wir die Rohschmelze in concentrirter Schwefelsäure lösten, nach einiger Zeit in Wasser gossen und dass ausgefällte Keton aus Benzol oder Toluol umkrystallisirten.

Die Ketone haben schwach-basische Eigenschaften, schwächer als das Michler'sche Keton; sie lösen sich in concentrirten Säuren, werden aber durch Wasser schon grösstentheils wieder ausgefällt. Sie färben schwach gelb, ein wenig stärker als das Michler'sche Keton,

1905

können aber ebensowenig wie dieses als wirkliche Farbstoffe bezeichnet werden.

Durch Behandeln mit Natriumamalgam in alkoholischer Lösung lassen sie sich leicht zu den entsprechenden Hydrolen reduciren. Das Michler'sche Hydrol färbt bekanntlich Seide und tannirte Baumwolle violettstichig blau. Die Nuance, welche das Trimethyldiamidonaphtylphenylhydrol ergiebt, ist derselben ausserordentlich ähnlich. Das Aethyldimethyl- und das Phenyl dimethyl-Hydrol färben dagegen ein sehr grünstichiges Blau, oder richtiger gesagt, ein blautichiges Grün, das Tolyldimethyl, färbt noch grüner und das Tolyldiäthyl-Hydrol endlich ganz rein grün.

Die Hydrole zeigen also unter sich grössere Unterschiede im Ton als die aus den entsprechenden Ketonen mit Dimethylanilin erhaltenen Farbstoffe.

Es wurde auch versucht, durch Einwirkung von Dimethyl- und Diäthyl- α -Naphtylamin auf das Anilid in ähnlicher Weise Ketone mit tertiär-substituierter Amidonaphtalinsgruppe zu erhalten. Die Reaction ist aber in diesem Falle so wenig glatt und die Ausbeute so schlecht, dass von einer weiteren Verfolgung dieses Gegenstandes vorläufig Abstand genommen wurde.

Durch Erhitzen mit Chlorammonium und Chlorzink liefern die gemischten Ketone, ähnlich wie das Michler'sche Keton, Auramine, welche gelb-orange färben, sich aber sonst den gewöhnlichen Auraminen analog verhalten, nur mit dem Unterschied, dass sie säurebeständiger sind. Dieselben Auramine kann man aber einfacher direct aus den Aurammoniumverbindungen mit Ammoniak erhalten.

Behandelt man die Rohschmelzen mit wässrigem Ammoniak, oder löst sie in Alkohol und setzt Ammoniak hinzu, so erhält man die Auramine, aber nicht ganz rein, sondern meist mit etwas durch das Wasser gebildetem Keton verunreinigt. Besser ist es, in die alkoholische Lösung, z. B. des mit Aethyl- α -Naphtylamin erhaltenen Condensationsproductes, unter Kühlung Ammoniakgas einzuleiten. Die tiefrothe Lösung nimmt unter Abscheidung von Chlorammonium eine immer gelbere Farbe an, und nach ungefähr $\frac{1}{4}$ Stunde ist dieselbe gelbgrün geworden. Man lässt noch einige Zeit stehen, wobei ein grosser Theil des Auramins auskrystallisirt. Nach dem Filtriren wird mit Wasser gewaschen und das zurückbleibende Auramin gut auf Thou abgepresst. Die alkoholische Lösung lässt man verdunsten, wobei sich weiter eine beträchtliche Menge in schön gelben Kryställchen abscheidet. Die ebenfalls noch Product enthaltenden Mutterlaugen werden am besten mit verdünnter Salzsäure versetzt und mit