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No. 05-076616- -00010-0000
Fecha: 2011-01-31 14:36:31 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 444 RESPUESTAREQUE Folios: 54

Señor

Jefe de la División de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Comercio, Desarrollo, Industria y Turismo

E. S. D.

Ref.: Expediente No. 05.076.616

Solicitud de Patente de Invención para: **AMINO 1,3,5-TRIAZINAS
SUSTITUÍDAS EN N CON RADICALES BICICLICOS QUIRALES,
PROCEDIMIENTO PARA SU PREPARACIÓN, COMPOSICIONES DE
LAS MISMAS Y US USO COMO HERBICIDAS Y REGULADORES DEL
CRECIMIENTO DE LAS PLANTAS**

Solicitante: **BAYER CROPSCIENCE AG**

RESPUESTA AL EXAMEN DE BUSQUEDA DE ANTERIORIDADES

LUIS PATIÑO LEYVA, mayor de edad, identificado y domiciliado como aparece al pie de mi firma, en atención a su oficio No. 14106 Notificado el 29 de octubre de 2011, atentamente me permito dar repuesta al mismo de la siguiente manera:

Presento copia de los documentos requeridos por ese Despacho como resultado de la búsqueda de anterioridades:

- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262750, & ARCH. PHARM., VOL. 331, N° 2, 1998, pages 59-71.

- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262751, & ARCH. PHARM., VOL. 331, N° 2, 1998, pages 59-71.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262752, & ARCH. PHARM., VOL. 331, N° 2, 1998, pages 59-71.
- HYUN M H ET AL: *Liquid chromatographic resolution of racemic amines, amino alcohols and related compounds on a chiral crown ether stationary phase*, JOURNAL OF CHROMATOGRAPHY A, ELSEVIER SCIENCE, NL, VOL. 959, N° 1-2, June 14, 2002, pages 75-83, XP004360276, ISSN: 0021-9673, compounds 2s, 2t table 1.
- DATABASE CAPLUS, CHEMICAL ABSTRACT SERVICE, COLUMBUS OHIO, US, retrieved from STN accession No. 1988: 589967, Database accession No. 109: 189967, RNs: 117195-20-7; 117195-14-9, & TAIWAN YAOXUE ZAZHI, VOL. 39, N° 2, 1987, pages 131-133, XP002262753, abstract
- Patent abstract of Japan, VOL. 2002, N° 07, July 3, 2002, JP 2002 065286 A (NIPPON STEEL CHEM CO LTD), March 5, 2002, Formula (3), abstract.
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- LAWSON, B. W., ET AL, *Specificity in the alkylation of Serine at the active site of alpha-chymotrypsin by aromatic alpha-Bromo amides*, BIOCHEMISTRY, VOL. 9, N° 10, 1980, pages 2140-2144, XP002262749, compound 1.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262754, & ORG. LETT., VOL. 4, N° 10, 2002, pages 1695-1698, abstract.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262755, & FARMACO ED. SCI., VOL. 26, 1971, pages 474-486, abstract.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262756, Database accession No. BRN: 7122876, abstract, & J. AMER. CHEM. SOC., VOL. 88, N° 10, 1966, pages 2233-2240.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262757,

Database accession No. BRN: 8111842, abstract, & TETRAHEDRON LETT, VOL. 43, N° 32, 2002, pages 5541-5544.

- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262758, Database accession No. BRN: 9264314, abstract, & J. MED. CHEM. VOL. 45, N° 24, 2002, pages 5260-5279.
- DATABASE CROSSFIRE BEILSTEIN, Beilstein institute zur Förderung der chemischen Wissenschaften, Frankfurt am Main, DE; XP002262759, Database accession No. BRN: 8201255, abstract, & CHEM. EUROP. J., VOL. 6, N° 10, 2000, pages 1840-1846.
- PERRONE R ET AL: *1-aryl-4-(5-methoxy-1,2,3,4-tetrahydronaphtalen-1-yl)alkyl piperazines and their analogues*, J. MED. CHEM., VOL. 42, 1999, pages 490-496, XP002282274, schemes 2 and 3.

Estos documentos se presentan de acuerdo como fueron proporcionados por la autoridad internacional de búsqueda (oficina de patentes Europea), según fue considerado pertinente por estos.

Cumplido así con lo dispuesto, atentamente solicito a Ud. se continúe con el trámite correspondiente a esta solicitud de Patente de Invención.

Del Señor Jefe,



Luis Patiño Leyva

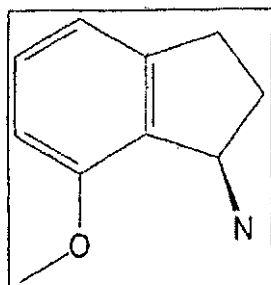
C.C. No. 2.930.616 de Bogotá

T.P.A. No. 2698 del C. Sup. de la Judicatura

Calle 94 A No. 7 A-09 – Bogotá, D.C.

Tel.: 7560770

Bogotá, D.C. **31 ENE. 2011**



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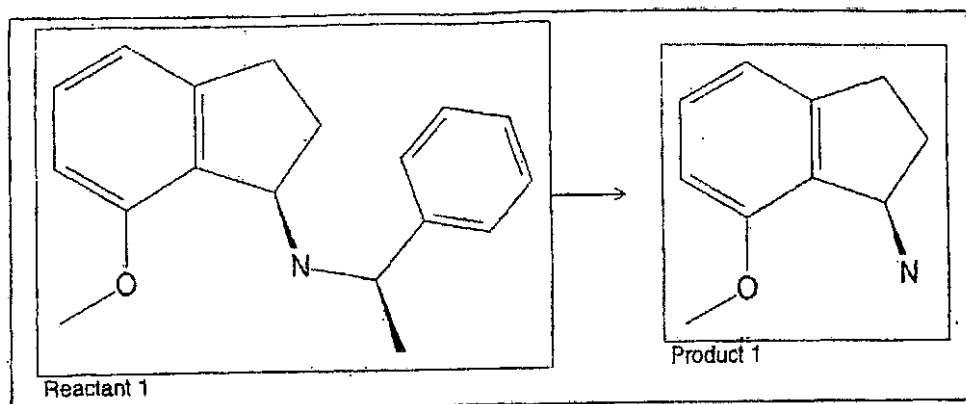
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Autoname	7-methoxy-indan-1-ylamine
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Molecular Weight	163.22
Lawson Number	14977, 289
Structure Keyword	Stereo compound
Compound Type	isocyclic
Constitution ID	6655880
Tautomer ID	7379485
Beilstein Reference	6-13
Entry Date	1998/04/30
Update Date	1998/05/04

Reaction

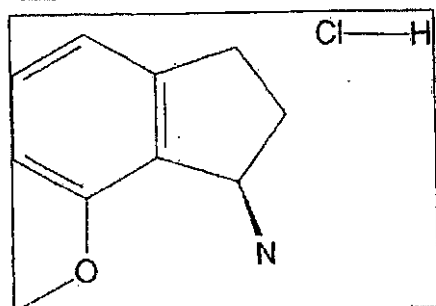
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No. of Reaction Details	1
Reaction Classification	Preparation
Yield	90 percent (BRN=7854896)
Reagent	H ₂
Catalyst	10percent Pd/C
Solvent	ethanol
Time	24 hour(s)
Temperature	50 C
Pressure	3750.3 Torr

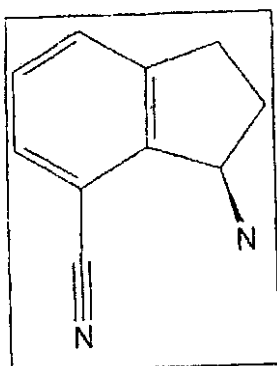
Ref. 1 6069423; Journal; Landsiedel-Maier, Dorothea; Frahm, August Wilhelm; ARPMAS; Arch.Pharm.(Weinheim Ger.); EN; 331; 2; 1998; 59-71;



Derivative

Derivative BRN	7862539 7-methoxy-indan-1-ylamine; hydrochloride
Ref. 1	6069423 ; Journal; Landsiedel-Maier, Dorothea; Frahm, August Wilhelm; ARPMAS; Arch.Pharm.(Weinheim Ger.); EN; 331; 2; 1998; 59-71;





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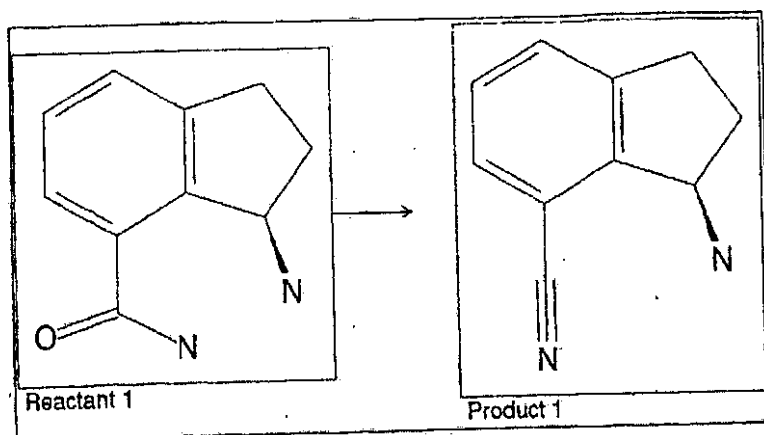
Substance

Beilstein Registry Number	7855080
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Autoname	3-amino-indan-4-carbonitrile
Molecular Formula	C ₁₀ H ₁₀ N ₂
Molecular Weight	158.20
Lawson Number	16090
Structure Keyword	Stereo compound
Compound Type	isocyclic
Constitution ID	6657621
Tautomer ID	7401999
Beilstein Reference	6-14
Entry Date	1998/04/30
Update Date	1998/05/04

Reaction

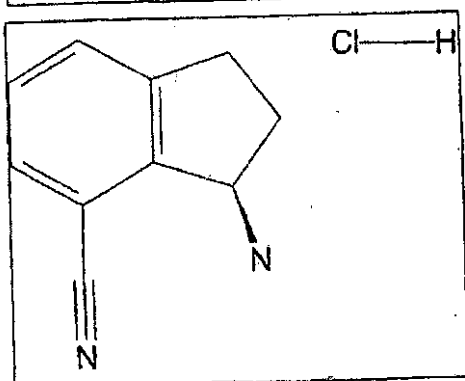
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Reactant BRN	7856079 3-amino-indan-4-carboxylic acid amide
Product BRN	7855080 (R)-(-)-7-cyano-1-aminoindan
No. of Reaction Details	1
Reaction Classification	Preparation
Yield	40 percent (BRN=7855080)
Reagent	P ₂ O ₅ , hexamethyldisiloxane
Solvent	CH ₂ Cl ₂
Time	24 hour(s)
Other Conditions	Heating

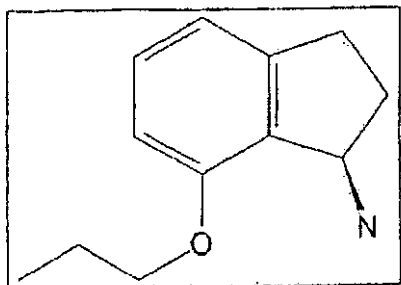
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Derivative

Derivative BRN	7862767 3-amino-indan-4-carbonitrile; hydrochloride
Ref. 1	6069423 ; Journal; Landsiedel-Maier, Dorothea; Frahm, August Wilhelm; ARPMAS; Arch.Pharm.(Weinheim Ger.); EN; 331; 2; 1998; 59-71;





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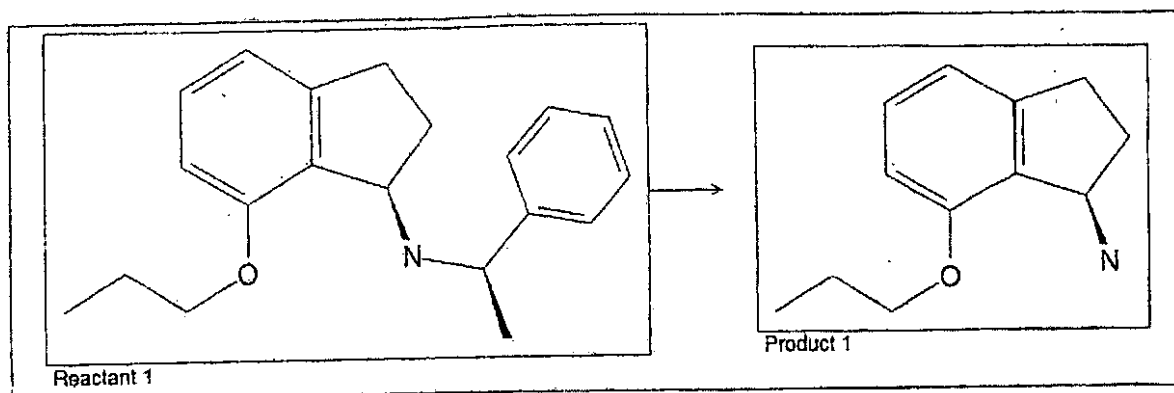
Substance

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Molecular Weight	191.27
Lawson Number	14977, 307
Structure Keyword	Stereo compound
Compound Type	isocyclic
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Tautomer ID	7383028
Beilstein Reference	6-13
Entry Date	1998/04/30
Update Date	1998/05/04

Reaction

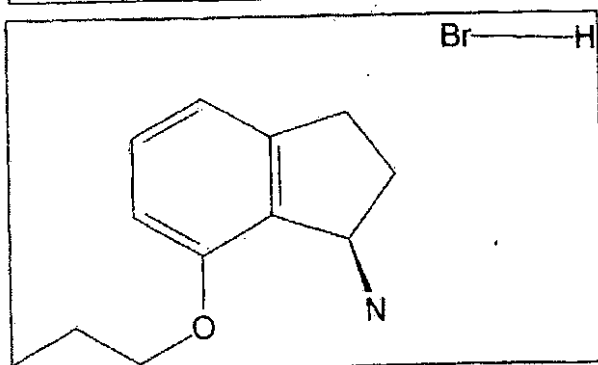
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Product BRN	7855360 (R)-(-)-7-propoxy-1-aminoindan
No. of Reaction Details	1
Reaction Classification	Preparation
Yield	85 percent (BRN=7855360)
Reagent	H ₂
Catalyst	10percent Pd/C
Solvent	ethanol
Time	24 hour(s)
Temperature	50 C
Pressure	3750.3 Torr

Ref. 1 6069423; Journal; Landsiedel-Maier, Dorothea; Frahm, August Wilhelm; ARPMAS; Arch.Pharm.(Weinheim Ger.); EN; 331; 2; 1998; 59-71;



Derivative

Derivative BRN	7863081 7-propoxy-indan-1-ylamine; hydrobromide
Ref. 1	6069423 ; Journal; Landsiedel-Maier, Dorothea; Frahm, August Wilhelm; ARPMAS; Arch.Pharm.(Weinheim Ger.); EN; 331; 2; 1998; 59-71;



Liquid chromatographic resolution of racemic amines, amino alcohols and related compounds on a chiral crown ether stationary phase

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Received 4 January 2002; received in revised form 10 April 2002; accepted 15 April 2002

Abstract

A chiral stationary phase (CSP) based on diphenyl-substituted 1,1'-binaphthyl crown ether was applied in resolving various racemic amines, amino alcohols and α -aminocarbonyl compounds including pharmaceutically important compounds such as amphetamine analogues, mexiletine, norepinephrine and norephedrine. The resolution was quite successful. In order to find out the effects of mobile phase additives on the chromatographic resolution behaviors, four selected racemic compounds were resolved on the CSP with the variation of the type and content of organic, acidic and cationic modifiers in aqueous mobile phase and with the variation of column temperature. The resolution behaviors were quite dependent on the type and the content of organic, acidic and cationic modifiers in aqueous mobile phase and on column temperature. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chiral stationary phases; LC; Enantiomer separation; Amines; Amino alcohols; Crown ethers

1. Introduction

Chiral stationary phases (CSPs) based on chiral crown ethers have been known to be very effective in resolving racemic compounds containing a primary amino group. For example, Cram and co-workers utilized bis-(1,1'-binaphthyl)-22-crown-6 immobilized on polystyrene or silica gel as CSPs in separating the enantiomers of racemic α -amino acids and their derivatives [1,2]. Disubstituted 1,1'-

binaphthyl-20-crown-6 dynamically coated on octadecyl silica gel has also been utilized as a CSP (a related CSP has been commercialized as CROWNPAK CR by Daicel Chemical Industries) in resolving racemic α -amino acids and other racemic compounds containing a primary amino group [3–5]. In recent years, CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid have also been developed and successfully utilized in resolving various racemic compounds containing a primary amino group [6–13].

Very recently, we reported a short communication paper concerning the development of a new CSP

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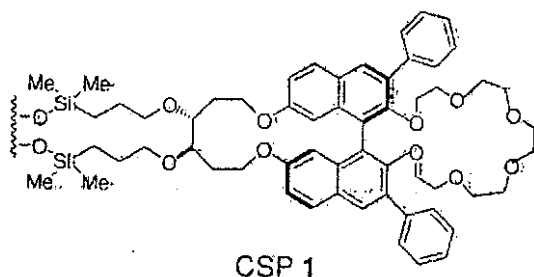


Fig. 1. Structure of CSP 1.

(CSP 1) (Fig. 1) [14]. The chiral-crown ether moiety of CSP 1 bears some similarity to the commercial CROWNPAK CR, but differs in that it is covalently bonded to silica gel. Consequently, covalently linked CSP 1 is useful in a variety of mobile phases. In the previous paper, we reported that CSP 1 is very effective in resolving various natural and unnatural α -amino acids. However, CSP 1 has not been utilized in resolving other racemic compounds containing a primary amino group. In this study, we wish to extend the use of CSP 1 to the resolution of racemic amines, amino alcohols and the related compounds.

2. Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20- μ l sample loop, a YoungLin M720 Absorbance detector (variable wavelength) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. The chiral column prepared by packing CSP 1 into a 250 $\times$ 4.6-mm I.D. stainless steel empty column was available from the previous study [14]. All analytes used in this study were available from previous study [10] or purchased from Aldrich. Injection samples were prepared by dissolving analytes in methanol at a concentration of 1.0 mg/ml and an injection size of 3 μ l was typically used. Elution orders were determined by injecting

configurationally known samples available from Aldrich.

3. Results and discussion

CSP 1 was applied in resolving various racemic amines (2), amino alcohols (3) and α -amino carbonyl compounds (4). The chromatographic resolution results are summarized in Table 1. The data shown in Table 1 were obtained under identical chromatographic conditions given in the footnote to Table 1. The chromatographic conditions utilized in obtaining data shown in Table 1 were not optimized for any racemate but generally quite useful. Sulfuric acid added to the mobile phase is necessary for protonation of the primary amino group of analytes to enhance the complexation of the ammonium ion ($R-NH_3^+$) inside the cavity of the crown ether ring of the CSP [15]. Under the mobile phase condition of the given pH (1.06) in Table 1, amino groups of all analytes seem to be fully protonated. Ammonium acetate added to the mobile phase is used to reduce the retention time of the two enantiomers on the chiral column, by competing with the ammonium ion of analytes for complexation inside the cavity of crown ether ring of the CSP [14]. Especially, it is interesting to note that pharmaceutically important compounds such as amphetamine analogues (2e, 2f), mexiletine (2g), norepinephrine (3b) and norephedrine (3f) were resolved with reasonable separation factors on CSP 1. While most of analytes contain aromatic functional groups, the non-aromatic analyte (4b) was also resolved very well. Consequently, the presence of aromatic functional groups in analytes seems to be not essential for the chiral recognition.

The organic and acidic modifiers and the cationic modifiers in mobile phase and the column temperature are expected to influence the enantioselectivity of CSP 1. In order to investigate the effects of mobile phase modifiers and the column temperature on the enantioselectivity, we selected four racemic compounds (2e, 2o, 3f and 4b), which are resolved well on CSP 1 as shown in Table 1 and Fig. 2, and tested their resolutions with the variation of the type and content of organic and acidic modifiers and cationic modifiers in aqueous mobile phase and with the variation of column temperature.

Table 1
Resolution of various anilines (2), amino alcohols (3) and α -amino carbonyl compounds (4) on CSP 1

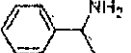
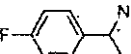
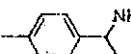
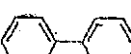
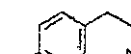
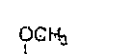
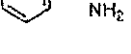
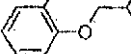
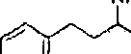
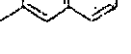
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2b 	0.83	1.84	2.23	3.39	
2c 	0.99	1.92	1.78	3.54	S
2d 	1.59	2.59	1.62	3.11	
2e 	0.73	1.09	1.49	2.27	
2f 	0.84	2.00	2.37	3.33	
2g 	0.34	0.40	1.16	0.44	
2h 	2.82	3.41	1.21	1.94	
2i 	3.31	3.86	1.16	1.33	
2j 	2.67	3.53	1.32	2.43	
2k 	0.32	0.47	1.47	1.60	

Table 1. Continued

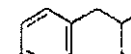
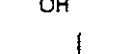
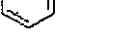
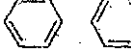
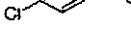
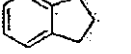
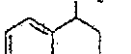
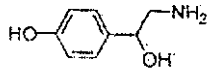
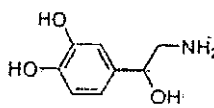
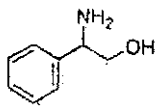
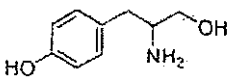
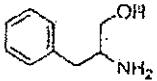
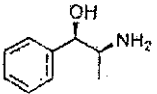
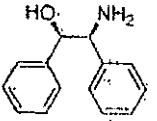
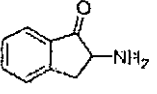
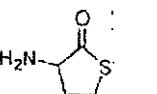
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2m 	1.73	2.30	1.33	1.85	S
2n 	2.69	3.28	1.22	1.47	
2o 	0.52	1.57	3.00	6.60	
2p 	0.65	0.77	1.20	0.71	
2q 	1.49	3.28	2.20	5.88	S
2r 	0.37	0.78	2.11	3.25	
2s 	0.67	1.64	2.45	4.67	
2t 	0.46	0.92	2.01	3.57	
2u 	2.75	4.45	1.62	4.30	

Table 1. Continued

Analytes	k_1^a	k_2^b	α^c	R_s^d	Conf. ^e
3a 	1.05	1.05	1.00		
3b 	0.89	0.97	1.09	0.44	
3c 	0.40	0.47	1.18	0.57	
3d 	0.31	0.36	1.16	0.62	
3e 	0.34	0.41	1.20	0.67	R
3f 	0.31	0.65	2.09	3.06	(1R,2S)
3g 	0.19	0.33	1.77	1.68	(1S,2R)
4a 	2.07	2.53	1.22	1.82	
4b 	0.66	1.02	1.55	2.14	

Mobile phase, 80% CH₃CN in H₂O+H₂SO₄ (10 mM)+CH₃COONH₄ (1 mM). Flow rate, 0.5 ml/min; detection, 225 nm UV; temperature, 20 °C.

^a Retention factor of the first eluted enantiomer.

^b Retention factor of the second eluted enantiomer.

^c Separation factor.

^d Resolution factor.

^e Absolute configuration of the second eluted enantiomer. For blanks, the elution orders were not determined.

First of all, the effects of organic modifiers on the enantioselectivity exerted by CSP 1 were investigated with the variation of the type and content of organic modifiers in aqueous mobile phase. In this study, we actually investigated two different organic modifiers such as methanol and acetonitrile. The chromatographic resolution results are summarized in Table 2. As shown in Table 2, the retention factors (k_1) generally decrease as the content of organic modifier in aqueous mobile phase increases except for the resolution of α -amino carbonyl compound, homocysteine thiolactone **4b**. In the resolution of homocysteine thiolactone **4b**, the retention factor (k_1) does not show any significant trends. The separation (α) and the resolution factors (R_s), in general, increase as the content of organic modifier in aqueous mobile phase increases. All of these trends with the variation of the content of organic modifier in aqueous mobile phase are consistent with those for the resolution of α -amino acids on CSP 1 [14].

The effects of acidic modifiers in aqueous mobile phase on the resolution of selected racemic compounds (**2c**, **2o**, **3f** and **4b**) on CSP 1 are summarized in Table 3. The retention factors (k_1) are significantly diminished as the concentration of acidic modifier in aqueous mobile phase increases. These trends are somewhat different from those for the resolution of α -amino acids on CSP 1. In the resolution of α -amino acids on CSP 1, the retention factors (k_1) were reported to show a maximum at a certain concentration of acidic modifier [14]. The initial increase in k_1 for the resolution of α -amino acids on CSP 1 was rationalized by increased complexation of the primary ammonium ion ($R-NH_3^+$) inside the cavity of the crown ether ring of the CSP, while the decrease in k_1 at higher acid concentrations was assumed to be a result of the greater ionic strength of the mobile phase. In this instance, the continuous decrease in k_1 with the increase in the acid concentration for the resolution of racemic compounds **2c**, **2o**, **3f** and **4b** shown in Table 3 might be rationalized to stem from the increased ionic strength of the mobile phase. Enantioselectivity (α) for the resolution of racemic compounds **2c**, **2o** and **3f** increases slightly as the concentration of acidic modifier in aqueous mobile phase increases and these trends are consistent with those for the resolution of

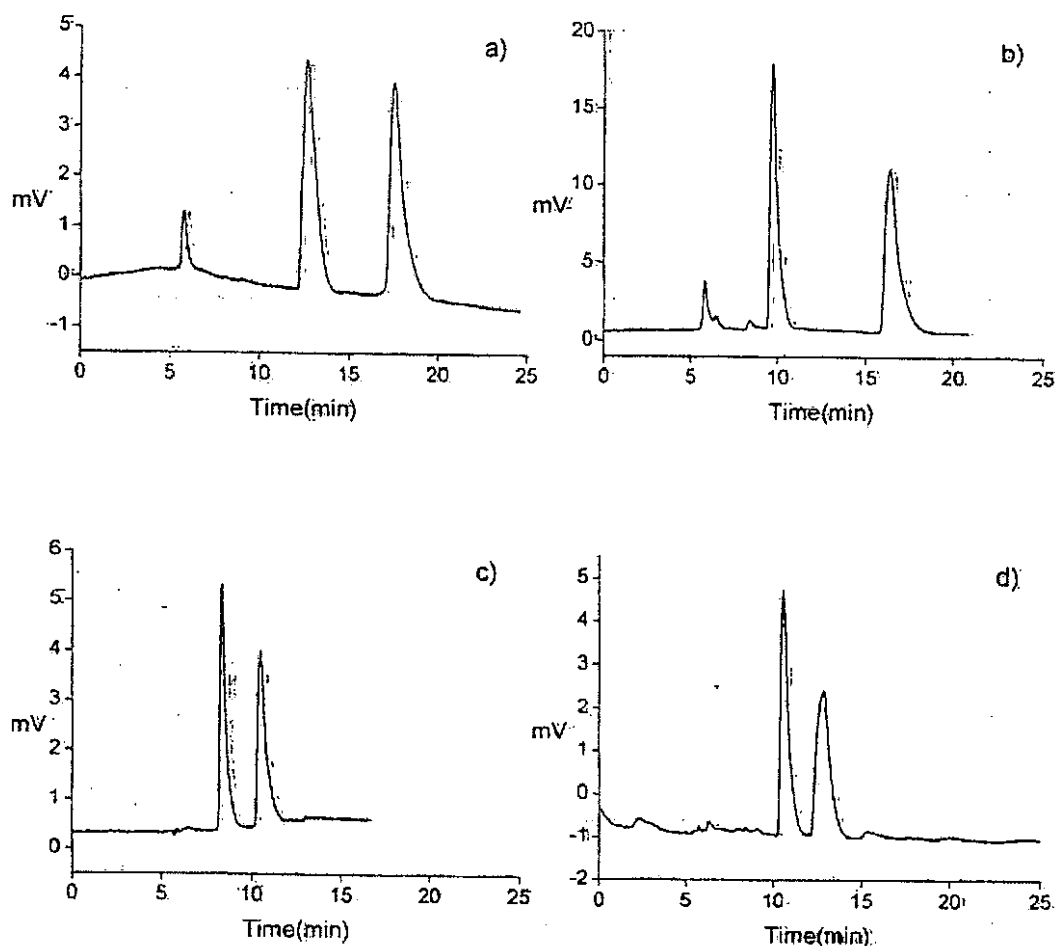


Fig. 2. Representative chromatograms for the resolution of (a) α -(4-methylphenyl)ethylamine **2c**, (b) 1,2-diphenylethylamine **2o**, (c) norephedrine **3f** and (d) homocysteine thiolactone **4h** on CSP 1. For chromatographic conditions, see the footnote to Table 1.

α -amino acids on CSP 1 [14]. On the contrary, enantioselectivity (α) for the resolution of homocysteine thiolactone **4h** shows a maximum at a certain concentration of acidic modifier. Resolution factors (R_s), however, do not show significant trends with the variation of the content of acidic modifier in aqueous mobile phase as shown in Table 3. The use of perchloric acid or trifluoroacetic acid as an acidic modifier in aqueous mobile phase was also effective for the resolution of racemic compounds **2c**, **2o**, **3f** and **4h**. However, sulfuric acid seems to be better as

an acidic modifier than perchloric or trifluoroacetic acid in terms of the resolution (R_s).

The effects of cationic modifiers in aqueous mobile phase on the resolution of four selected racemic compounds (**2c**, **2o**, **3f** and **4h**) on CSP 1 are summarized in Table 4. The cationic modifier added to mobile phase might compete with the ammonium ion of analytes ($R-NH_3^+$) for complexation inside the cavity of crown ether ring of the CSP. In this instance, the concentration of cationic modifier in mobile phase is expected to influence the retention of

Table 2
Resolution of selected racemic compounds (2c, 2o, 3f and 4b) on CSP 1 with the variation of the type and the content of organic modifier in aqueous mobile phase^a

Organic modifier (%)	2c			2o			3f			4b		
	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d
CH ₃ CN (20)	4.30	1.68	2.31	9.40	2.57	5.63	0.80	1.86	3.08	0.54	1.40	1.62
CH ₃ CN (50)	2.22	1.70	3.75	2.00	2.71	6.29	0.60	1.93	3.83	0.74	1.43	2.10
CH ₃ CN (80)	0.99	1.78	3.54	0.52	3.00	6.60	0.31	2.09	3.06	0.66	1.55	2.14
CH ₃ OH (50)	2.60	1.49	1.78	3.49	2.52	4.00	0.79	1.95	2.56	0.72	1.57	1.89
CH ₃ OH (80)	0.92	1.71	2.59	0.60	2.67	5.50	0.45	2.07	3.76	0.69	1.55	2.71

^a Mobile phase, organic modifier (%) in H₂O + H₂SO₄ (10 mM) + CH₃COONH₄ (1 mM); flow rate, 0.5 ml/min; detection, 225 nm UV; temperature, 20 °C.

^b Retention factor of the first eluted enantiomer.

^c Separation factor.

^d Resolution factor.

Table 3
Resolution of selected racemic compounds (2c, 2o, 3f and 4b) on CSP 1 with the variation of the type and the content of acidic modifier in aqueous mobile phase^a

Acidic modifier (mM)	2c			2o			3f			4b		
	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d
H ₂ SO ₄ (1)	1.49	1.60	4.00	0.85	2.48	7.11	0.65	1.75	3.81	1.21	1.34	2.50
H ₂ SO ₄ (5)	1.11	1.65	3.33	0.54	2.97	5.00	0.35	2.02	2.90	0.72	1.59	2.75
H ₂ SO ₄ (10)	0.99	1.78	3.54	0.52	3.00	6.60	0.31	2.09	3.06	0.66	1.55	2.14
H ₂ SO ₄ (20)	0.91	1.82	4.18	0.48	3.12	6.45	0.27	2.20	3.08	0.59	1.51	2.25
HClO ₄ (10)	1.06	2.58	3.30	0.69	3.26	5.85	0.42	2.21	2.57	0.93	1.57	1.81
CH ₃ COOH (10)	1.11	2.76	2.60	0.82	3.07	7.09	0.52	2.08	2.54	0.91	2.05	3.12

^a Mobile phase, CH₃CN (80%) in H₂O + Acidic modifier (mM) + CH₃COONH₄ (1 mM); flow rate, 0.5 ml/min; detection, 225 nm UV; temperature, 20 °C.

^b Retention factor of the first eluted enantiomer.

^c Separation factor.

^d Resolution factor.

Table 4
Resolution of selected racemic compounds (2c, 2o, 3f and 4b) on CSP 1 with the variation of the type and the content of the inorganic modifier in aqueous mobile phase^a

Inorganic modifier (mM)	2c			2o			3f			4b		
	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d	<i>k</i> ₁ ^b	α ^c	<i>R</i> _s ^d
CH ₃ COONH ₄ (1)	0.99	1.78	3.54	0.52	3.00	6.60	0.31	2.09	3.06	0.66	1.55	2.14
CH ₃ COONH ₄ (5)	0.64	1.69	2.13	0.29	3.24	4.78	0.17	2.35	2.15	0.40	1.68	2.31
NH ₄ Cl (1)	1.23	2.40	3.01	0.83	3.01	6.52	0.51	2.08	2.57	1.07	1.50	1.54
KCl (0.5)	0.67	1.72	1.56	0.39	2.74	3.70	0.25	2.08	2.19	0.54	1.54	1.96
KCl (1)	0.43	1.74	1.66	0.23	3.00	3.24	0.15	2.27	1.62	0.35	1.57	1.71

^a Mobile phase, CH₃CN (80%) in H₂O + H₂SO₄ (10 mM) + inorganic modifier (mM); flow rate, 0.5 ml/min; detection, 225 nm UV; temperature, 20 °C.

^b Retention factor of the first eluted enantiomer.

^c Separation factor.

^d Resolution factor.

analytes on the CSP. As expected, the retention (k_1) of analytes is decreased more significantly at the higher concentration of cationic modifier than at the lower concentration of cationic modifier. The retention (k_1) of analytes with the use of potassium chloride as an inorganic modifier is more significantly reduced than with the use of ammonium acetate as shown in Table 4. The stability constant of the complex between 18-crown-6 and cations has been known to be much greater with K^+ than with NH_4^+ ion [16]. In this instance, potassium chloride is expected to compete with the ammonium ion of analytes ($R-NH_3^+$) for complexation inside the cavity of crown ether ring of the CSP more effectively and consequently reduces the retention (k_1) more significantly than ammonium acetate. There was no much difference in enantioselectivity (α) when ammonium acetate or potassium chloride was used as a cationic modifier. However, the resolution (R_s) was generally greater when ammonium acetate was used as a cationic modifier than when potassium chloride was used. The use of ammonium chloride as a cationic modifier in aqueous mobile phase was observed to be equally effective as the use of ammonium acetate as shown in Table 4.

The effects of column temperature on the resolution of four selected racemic compounds **2c**, **2o**, **3f** and **4b** on CSP 1 are demonstrated in Table 5. As shown in Table 5, all three chromatographic parameters such as the retention (k_1), the separation (α) and the resolution factors (R_s) improve continuously as column temperature decreases. At lower temperature, the diastereomeric complexes formed between the individual enantiomers of an analyte and the chiral crown ether moiety of the CSP are expected to become energetically more favorable and this is

more significant with the more stable diastereomeric complex. Consequently, the retention (k_1) and the separation factors (α) improve as the column temperature decreases. The improved enantioselectivity (α) at lower temperature makes the distance wider between the two chromatographic peaks corresponding to the two enantiomers and consequently the resolution (R_s) is also expected to improve at lower temperature.

Finally, CSP 1 was demonstrated to be very useful in the determination of the optical purity of enantiomerically enriched samples. For example, the chromatogram for the resolution of a commercial sample of optically active **2c** (Aldrich, $R:S=1:99$, 98% ee) on CSP 1 in Fig. 3a shows the two peaks corresponding to the two enantiomers present in the sample. Another chromatogram for the resolution of a commercial sample of optically active norephedrine **3f** (Aldrich, optical purity is not known) shown in Fig. 3b demonstrated that the optical purity of the sample is also 98% ee. As shown in Fig. 3, the minor enantiomers present in the optically active samples are clearly shown and consequently their detection limit can be more lowered. However, enantiomerically more enriched samples are not available and the detection limit of the optical purity was not checked further.

In summary, in this study, we demonstrated that CSP 1 was quite successful for resolving various racemic amines (**2**), amino alcohols (**3**) and α -aminocarbonyl compounds (**4**). Especially, the resolution of pharmaceutically important compounds such as amphetamine analogues (**2e**, **2f**), mexiletine (**2g**), norepinephrine (**3b**) and norephedrine (**3f**) was noteworthy. In order to find out the effects of mobile phase additives on the chromatographic resolution

Table 5
Resolution of selected racemic compounds (**2c**, **2o**, **3f** and **4b**) on CSP 1 with the variation of column temperature^a

Column temp. (°C)	2c			2o			3f			4b		
	k_1^b	α^c	R_s^d	k_1^b	α^c	R_s^d	k_1^b	α^c	R_s^d	k_1^b	α^c	R_s^d
30	0.88	1.70	3.30	0.48	2.33	3.85	0.37	1.65	2.25	0.74	1.39	1.79
20	0.99	1.78	3.54	0.52	3.00	6.60	0.31	2.09	3.06	0.66	1.55	2.14
10	1.37	1.95	5.60	0.73	3.18	8.25	0.53	2.16	4.25	1.05	1.60	2.93

^a Mobile phase, CH_3CN (80%) in $H_2O+H_2SO_4$ (10 mM)+ CH_3COONH_4 (1 mM); flow rate, 0.5 ml/min; detection, 225 nm UV.

^b Retention factor of the first eluted enantiomer.

^c Separation factor.

^d Resolution factor.

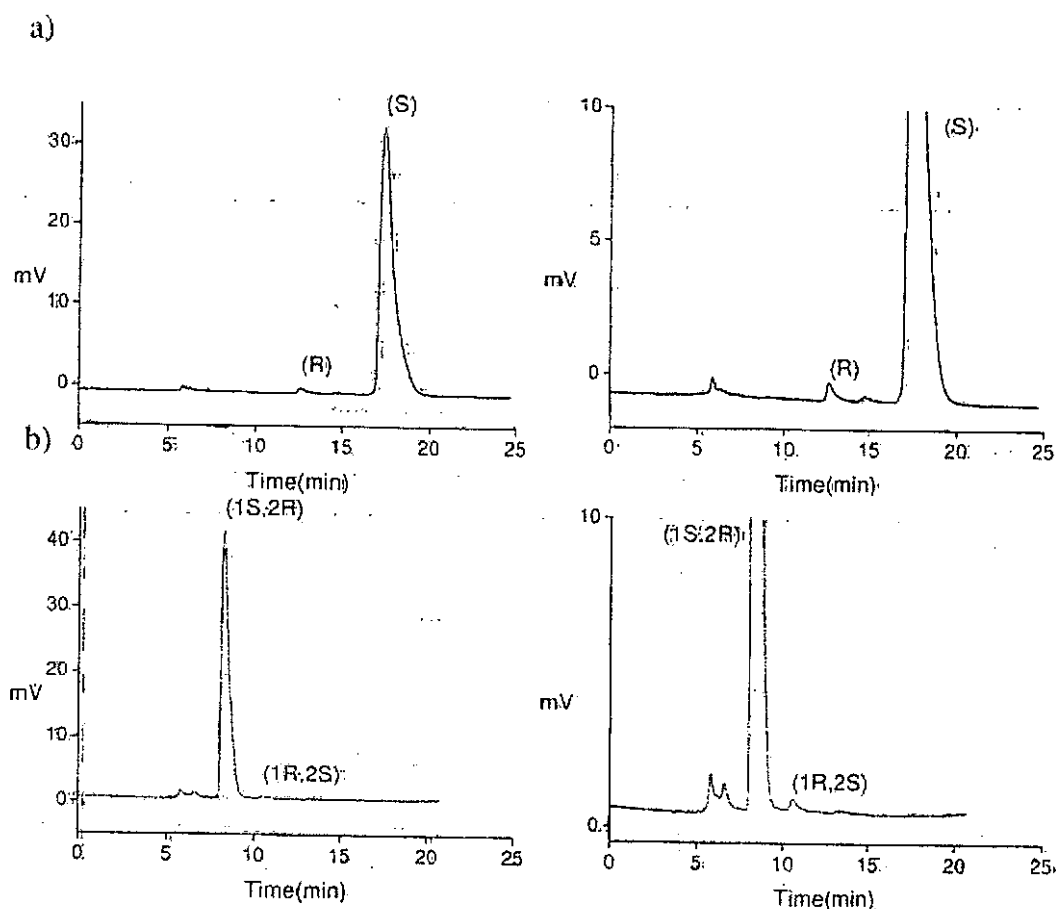


Fig. 3. Examples for the optical purity determination of enantiomerically enriched samples on CSP 1: (a) (left) the chromatogram for the resolution of a commercial sample of optically active 2e (Aldrich, $R:S=1:99$), (right) the expanded chromatogram and (b) (left) the chromatogram for the resolution of a commercial sample of optically active norephedrine 3f (Aldrich), (right) the expanded chromatogram. For the chromatographic conditions, see the footnote to Table 1.

behaviors, four selected racemic compounds (2c, 2o, 3f and 4b) were resolved on CSP 1 with the variation of the type and content of organic, acidic and cationic modifiers in aqueous mobile phase and with the variation of column temperature. All of the three chromatographic parameters such as retention (k_1), separation (α) and resolution factors (R_S) were found controllable to some extent by varying the type and content of organic, acidic or cationic modifiers in mobile phase or by varying column temperature.

CSP 1 was also demonstrated to be useful in the determination of optical purity of enantiomerically enriched samples. However, the chiral recognition mechanism is not clear except that the diastereoselective complexation of the primary ammonium group ($R-NH_3^+$) of analytes inside the chiral crown ether ring of the CSP might be responsible. Further studies are expected to be necessary to elucidate the more detailed chiral recognition mechanism.

Acknowledgements

This work was supported from the Ministry of Health and Welfare, Korea (Grant No. HMP-00-B-21500-0107).

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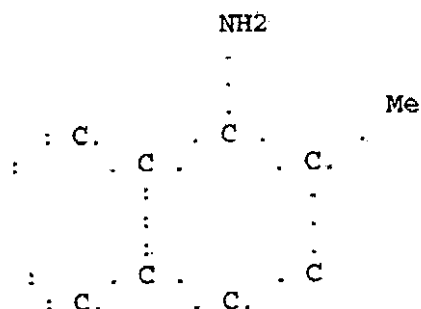
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 ketamine
 IN - Yang, David J.
 CS - Ann Arbor, MI, 48105, USA
 SO - Taiwan Yaoxue Zazhi (1987), 39(2), 131-3
 CODEN: JTPHAO; ISSN: 0368-4520
 DT - Journal
 LA - English
 IT - 6740-88-1, Ketamine
 RL: RCT (Reactant); RACT (Reactant or reagent)
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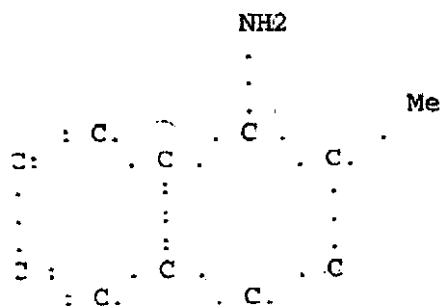
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OPTICALLY ACTIVE (R)-1-INDANEAMIDE DERIVATIVE AND METHOD FOR PRODUCING THE SAME

**Bibliographic
data**

Description

Claims

Mosaics

Original
document

INPADOC
LEGAL
status

Patent number: JP2002065286 (A)

Publication date: 2002-03-05

Inventor(s): KAWASHIMA HIROSHI; ASARI TORU; ISHIKAWA TADATAKA ±

Applicant(s): NIPPON STEEL CHEMICAL CO ±

Classification:

- international: C07C233/06; C12P13/02; C12P41/00; C12R1/72; C07C233/00;
C12P13/00; C12P41/00; (IPC1-7): C07C233/06; C12P13/02;
C12P13/02; C12P41/00; C12P41/00; C12R1/72; C12R1/72

- european:

Application number: JP20000259083 20000829

Priority number(s): JP20000259083 20000829

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Abstract of **JP 2002065286 (A)**

PROBLEM TO BE SOLVED: To provide an optically active (R)-1-indaneamide derivative which is a precursor of an optically active 1-aminoindane which is a compound useful as an asymmetric induction reagent and a synthetic intermediate for medicines and has high utility value as a medicine and a synthetic intermediate for liquid crystal materials. **SOLUTION:** Asymmetric amidation of racemic (±)-1-aminoindane is carried out in an organic solvent by using an enzyme such as lipase or esterase to produce the objective optically active (R)-1-indaneamide represented by the following formula (3) (wherein R1 is an alkyl group or an alkenyl group). The (R)-1-indaneamide of the formula (3) wherein R1 is an alkenyl group is a new compound.



(3)

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Highly Enantioselective Hydrogenation of Cyclic Enamides Catalyzed by a Rh-PennPhos Catalyst¹

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Received December 17, 1998

Chiral amines are often critical components of pharmaceutical agents. For example, about 15–25% of the single-enantiomer products in development contain this unit according to a recent analysis.¹ The development of practical methods for the synthesis of enantiomerically pure amines is therefore of great interest. Traditional resolution methods and enzymatic transaminase technology are frequent choices in industry for making chiral amines. However, recent attention has been devoted to asymmetric hydrogenation of imines and enamides as potentially more competitive synthetic routes.² A variety of transition-metal catalysts have been explored to date for asymmetric hydrogenation of imines, albeit with either low enantioselectivity, limited substrate scope, or low activity.³ Alternatively, asymmetric hydrogenation of simple enamides is a more useful strategy. Several Rh-bisphosphine compounds have been developed as efficient catalysts for asymmetric hydrogenation of acyclic enamides,⁴ and some can even tolerate *Z/E* mixtures of β -substituted enamides as substrates.^{4a,c} However, only limited success has been achieved in the hydrogenation of cyclic enamides despite the potential importance of this process for the synthesis of biologically active chiral aminotetralins and aminolindanes.⁵ Herein we report the highly enantioselective hydrogenation of cyclic enamides catalyzed by a Rh-PennPhos compound.

In our continuing work on catalytic asymmetric reactions, we have developed conformationally rigid chiral bisphosphines (e.g., BICP = bis(diphenylphosphino)dicyclopentane;⁶ phosphinobicyclo[2.2.1]heptanes;⁷ PennPhos = *P,P'*-1,2-

phenylenebis(*cis*-2,5-dialkyl-7-phosphabicyclo[2.2.1]heptane,⁸ Me-PennPhos, Figure 1) and are exploring their synthetic utility. High enantioselectivities have been achieved for the hydrogenation of acyclic enamides with a Rh-BICP catalyst.^{4c} However, only modest enantioselectivities (60–70% ee) were obtained for the hydrogenation of cyclic enamides derived from α -tetralone and α -indanone. Encouraged by the excellent results observed upon asymmetric hydrogenation of simple ketones catalyzed by a Rh-PennPhos complex,⁸ we have now extended our study of the asymmetric hydrogenation of enamides to include this unique catalytic system.

N-(3,4-Dihydro-1-naphthyl)acetamide was chosen as a typical enamide substrate. This enamide can be easily prepared by reduction of the corresponding oxime with iron powder in the presence of acetic anhydride (Scheme 1).^{9a,b} This facile synthesis of enamides⁹ combined with an efficient asymmetric hydrogenation step provides a practical protocol for the synthesis of chiral amines from ketones. Table 1 lists asymmetric hydrogenation results under different conditions and with a variety of chiral bisphosphine systems. The catalyst was generally prepared *in situ* by mixing a solution of a Rh precursor and a phosphine ligand. The reaction was carried out under an initial H₂ pressure of 40 psi at room temperature with a ratio of substrate/Rh/Me-PennPhos of 100:1:1.1. Different Rh catalytic precursors led to large variations in enantioselectivities (entries 1–3). Higher enantioselectivities were observed with cationic Rh precursors (entry 2, 97% ee; entry 3, 98% ee), while the ee is lower with a neutral Rh system (entry 1, 92% ee). Changing solvents and/or H₂ pressure have only a small effect on enantioselectivity, although reaction conversions vary to a large extent. The catalytic hydrogenation goes to completion in methylene chloride, methanol, and 2-propanol under 40 psi of H₂ in 20 h, while only 24% conversion was achieved under the same conditions in toluene. Reactions performed under 15 psi H₂ and 500 psi H₂ in MeOH give similar enantioselectivities (>88% ee) but gave different conversions (6% under 15 psi H₂ and 100% under 500 psi H₂ after 20 h).

Optimal reaction conditions with the Rh-Me-PennPhos catalyst use MeOH as the solvent and an initial H₂ pressure of 40 psi. Under these conditions, we have investigated the Rh-catalyzed asymmetric hydrogenation of *N*-(3,4-dihydro-1-naphthyl)acetamide with several commercially available chiral bisphosphines. Compared with the Rh-Me-PennPhos catalyst, significantly lower enantioselectivities (10% ee, entry 4; 24% ee, entry 5) were observed with Rh-DIOP and Rh-BINAP catalysts. The most surprising result is the low enantioselectivity (1% ee, entry 6) achieved with the Rh-Me-DuPhos complex, which is in sharp contrast to its effective asymmetric hydrogenation of acyclic enamides.^{4a} Our results are in agreement with the investigation by Burk,^{9b} who found that under similar conditions hydrogenation of *N*-(3,4-dihydro-1-naphthyl)acetamide gave 0% ee with a Rh-Me-DuPhos complex and 69% ee with a Rh-Me-BPE catalyst. However, up to 92% ee was reported with a Rh-Me-BPE catalyst if the reaction was done at 0 °C.^{9b} To the best of our knowledge, Rh-Me-PennPhos-catalyzed asymmetric hydrogenation of *N*-(3,4-dihydro-1-naphthyl)acetamide gives the highest enantioselectivity reported to date.

¹ Dedicated to Professor Xiyun Lu on the occasion of his 70th birthday.

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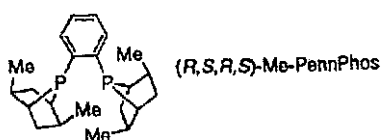
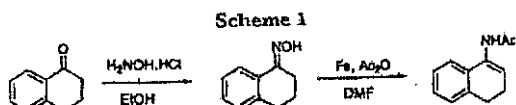


Figure 1.

Table 1. Rh-Catalyzed Asymmetric Hydrogenation of an Enamide^a

entry	Rh(II) species	ligand	% ee ^b
1	[Rh(COD)Cl] ₂	(<i>R,S,R,S</i>)-Me-PennPhos	92 (<i>R</i>)
2	Rh(COD)BF ₄	(<i>R,S,R,S</i>)-Me-PennPhos	97 (<i>R</i>)
3	Rh(COD)PF ₆	(<i>R,S,R,S</i>)-Me-PennPhos	98 (<i>R</i>)
4	Rh(COD)PF ₆	(+)-DIOP	10 (<i>S</i>)
5	Rh(COD)PF ₆	(<i>R</i>)-BINAP	24 (<i>R</i>)
6	Rh(COD)PF ₆	(<i>R,R</i>)-Me-DuPhos	1 ^c (<i>R</i>)

^a The reaction was complete in quantitative yield at room temperature under an initial hydrogen pressure of 40 psi after 20 h using MeOH as the solvent. The catalyst was made in situ by stirring a solution of Rh precursor and phosphine ligand in MeOH for 30 min ([substrate] (0.5 mmol, 0.17 M)/[Rh cat./ligand] = 1:0.01:0.011). ^b Enantiomeric excesses were determined by chiral GC using a Supelco Chiral Select 1000 (0.25 mm × 15 m) column. ^c 57% conversion.

To demonstrate that this is a potentially practical method for the synthesis of α-aminoaldehydes, we increased the ratio of *N*-(3,4-dihydronaphthalen-1-yl)acetamide/Rh-Me-PennPhos to 2000:1 and the reaction was still complete in 20 h with 98% ee.

Using these optimal conditions, several cyclic and acyclic enamides were investigated (Table 2). Hydrogenation of several cyclic enamides derived from α-tetralones and α-indanones (entries 1–5) gives high enantioselectivities (>97% ee) regardless of the substituents on the aromatic ring.¹⁰ Interestingly, a tetrasubstituted five-membered cyclic enamide gives high enantioselectivity (98% ee, entry 8). However, the ee with the corresponding six-membered cyclic enamide (73% ee, entry 9) is significantly lower. Only moderate ee was observed for the enamide made from β-tetralone (entry 7). Burk et al. reported excellent results (>98% ee) for the asymmetric hydrogenation of an indanone-derived enamide using a Rh-Me-DuPhos catalyst.^{2b} The profile of their catalytic systems has not yet been completely established for a variety of cyclic enamides. Using the Rh-Me-PennPhos catalyst, several acyclic enamides were hydrogenated with moderate to good ee's (entries 10–12).

(10) The absolute configuration was determined by comparing the GC trace and optical rotation of the acetamide derived from (*R*)-α-aminoindane and (*R*)-α-aminoindoline purchased from Lancaster, Inc.

Table 2. Asymmetric Hydrogenation of Enamides Catalyzed by a Me-PennPhos-Rh Complex

Entry	Substrate	% ee ^a	Entry	Substrate	% ee ^a
1		98	7		71
2		98	8		98
3		>99	9		73
4		>99	10		75
5		97	11		88
6		90	12		90

^a The reaction was complete in quantitative yield at room temperature under an initial hydrogen pressure of 40 psi after 20 h using MeOH as the solvent. The catalyst was made in situ by stirring a solution of [Rh(COD)PF₆] and Me-PennPhos in MeOH for 30 min ([substrate] (0.5 mmol, 0.17 M)/[Rh(COD)PF₆]/ligand = 1:0.01:0.011). ^b Enantiomeric excesses were determined by chiral GC using a Supelco Chiral Select 1000 (0.25 mm × 15 m) column.

However, these enantioselectivities are lower than those obtained with Rh-DuPhos, Rh-BICP, and other catalytic systems.⁴ Since there is as yet no universal catalyst to handle such a diverse set of substrates, the Rh-PennPhos complex serves to complement other efficient catalysts⁴ for hydrogenation of simple acyclic enamides.

In summary, we have developed a highly enantioselective Rh-PennPhos catalyst for asymmetric hydrogenation of cyclic enamides. The high reactivity and ease of operation combined with an efficient method for the synthesis of substrates make this reaction an attractive synthetic procedure for the synthesis of enantiomerically pure α-aminoaldehydes and α-aminoindanes.

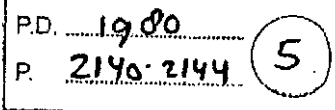
Acknowledgment. This work was supported by a Camille and Henry Dreyfus New Faculty Award and Teacher Scholar Award, an ONR Young Investigator Award, a DuPont Young Faculty Award, Catalytic Pharmaceuticals, and DuPont Agricultural Products. We acknowledge a generous loan of precious metals from Johnson Matthey, Inc. and a gift of chiral GC columns from Supelco and thank Dr. Albert L. Casalnuovo of DuPont Agricultural Products for useful suggestions for enamide synthesis.

Supporting Information Available: Spectroscopic data and experimental details.

JO9824605

Specificity in the Alkylation of Serine at the Active Site of α -Chymotrypsin by Aromatic α -Bromo Amides[†]

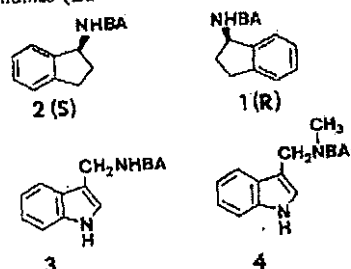
William B. Lawson



ABSTRACT: A number of aromatic α -bromo amides inactivate α -chymotrypsin by affinity labeling of either Met-192 or Ser-195. While the reaction with Met-192 is independent of pH [cf. Lawson, W. B., & Rao, G. J. S. (1980) *Biochemistry*, preceding paper in this issue], that with Ser-195 is governed by a group on the enzyme (presumably His-57) with a pK_a of about 7. Ser-195 is alkylated by those compounds in which the amide group and the aromatic ring are predominantly noncoplanar. Bromoacetyl-(*S*)-(-)-aminoindan was predicted from studies with models to have a higher reactivity toward Ser-195 than its *R*-(+)-enantiomer, and the prediction was confirmed by experiment. Further study with models led to the synthesis and testing of bromoacetyl-3-aminomethylindole

and -3-methylaminomethylindole. The latter compound proved to be the best to date for alkylation of Ser-195, both in regard to rate and to specificity. It is hypothesized that serine alkylation results from binding of an α -bromo amide in the active site with its amide carbonyl group positioned between the NH groups of Gly-193 and Ser-195. Further specificity and increased rates in the reaction with Ser-195 are gained by the use of *N*-(amino)methyl derivatives. The *N*-methyl group appears to prevent a nonproductive binding mode in which its NH analogue forms a hydrogen bond with the carbonyl group of Ser-214. The reactivity of Ser-195 is discussed in relation to the charge-transfer system, Asp-102:His-57:Ser-195.

During investigation of the stereochemistry of labeling of Met-192 at the active site of chymotrypsin by aromatic α -bromo amides, we observed that some compounds that did not react rapidly with Met-192 alkylated a serine residue, presumably Ser-195 (Lawson & Rao, 1980). In contrast to the alkylation of Met-192, the reaction displayed a marked pH dependence, similar to that observed in the alkylation of serine at the active site of trypsin by (α -bromoacetamide)alkylamines and -guanidines (Lawson et al., 1968).



Since two optical isomers, BA-(*R*)-(+)-1-aminoindan (1)[†] and BA-(*S*)-(-)-1-aminoindan (2), had been found to alkylate serine to some extent without a pH dependence (Lawson & Rao, 1980), it was decided to reinvestigate their reactions with the enzyme. A further impetus was provided by the active-site model of Clarke (1977). Examination of possible interactions of 1 and 2 with the active site led to a prediction that the *S*-(*-*) enantiomer, 2, should react more readily with Ser-195 than the *R*-(+)-enantiomer. Repetition of the earlier work confirmed the rate data but, more importantly, showed that the prediction was correct. Further study with models to improve selectivity in the alkylation of Ser-195 led to the synthesis of BA-3-aminomethylindole (3) and BA-3-methylaminomethylindole (4). The latter proved to be the best α -bromo

amide to date for selective alkylation of Ser-195 in chymotrypsin.

Materials and Methods

α -Chymotrypsin (3X recrystallized, salt free) was obtained from Worthington Biochemical Corp. Organic chemicals were products of Aldrich Chemical Co. or Eastman Organic Chemicals. Ches, Mes, Mops, and Taps were obtained from Calbiochem. α -Bromo amides, except for those described below, were the compounds used in the previous paper (Lawson & Rao, 1980). 3-(Aminomethyl)indole and 3-[(methylamino)methyl]indole were prepared as described by Gower & Leete (1963); these amines are rather unstable (cf. Putokhin & Davidova, 1932) and were stored under vacuum at 4 °C. *N*-(Bromoacetyloxy)succinimide was prepared according to Hora (1973). Silica Woelm TSC and nylon tubing for dry-column chromatography were obtained from ICN Pharmaceuticals. Eastman silica gel plates with fluorescent indicators were used for TLC.

Melting points were uncorrected. Ultraviolet spectra and enzyme assays were carried out with a Cary 118 spectrophotometer. A Perkin-Elmer 710A infrared spectrophotometer and a Varian EM 360 NMR spectrometer were used for recording infrared and NMR spectra. Elemental analyses were performed by Instranal Laboratory, Inc., Rensselaer, NY, and results obtained for the elements listed were within $\pm 0.3\%$ of the theoretical values. The active-site model was described previously (Clarke, 1977; Lawson & Rao, 1980).

BA-3-(aminomethyl)indole (3). A solution of 1.55 g (7.37 mmol) of *N*-(bromoacetyloxy)succinimide in 5 mL of dimethyl sulfoxide was added to an ice-cold solution of 1.03 g (7.37 mmol) of 3-(aminomethyl)indole in 3 mL of dimethyl sulfoxide, and the solution was allowed to warm to room temperature for 15 min. The reaction mixture was diluted with

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¹ Abbreviations used: BA, bromoacetyl; Ches, cyclohexylaminocitranesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; Mops, 3-(*N*-morpholino)propanesulfonic acid; Taps, 3-[[tris(hydroxymethyl)methyl]amino]propanesulfonic acid; CPK, Corey-Pauling-Koltun (models); CCS, color-coded-skeletal (models); Cm, carboxymethyl.

ethyl acetate and extracted with H₂O, 0.2 M HCl, H₂O, 0.2 M KHCO₃, and saturated NaCl. The organic layer was dried (MgSO₄) and evaporated in a rotary evaporator, and the residue was dried thoroughly under vacuum over Drierite to give 0.85 g of a frothy solid. TLC on silica gel in CHCl₃ showed an Ehrlich-positive spot (skatole) near the front, an Ehrlich-, Ellman- (Schloss & Hartman, 1977), and ninhydrin-positive spot near the origin, and a spot corresponding to the product, *R_f* ca. 0.3, that was slowly Ehrlich positive, Ellman and UV positive, and ninhydrin negative. The above solid (0.85 g) was dissolved in 5 mL of ethyl acetate, rotary evaporated with 5 g of Silica Woelm TSC, layered on a 100-g column of the same adsorbent in a nylon tube, and eluted with CHCl₃ until the middle (fluorescent) band was well separated from a band at the origin. The middle band was cut out and eluted with ethyl acetate. Evaporation of the solvent gave an oil that crystallized from ethyl acetate-cyclohexane to yield 230 mg (12%) of pale pink crystals, mp 100–102 °C. Further recrystallization gave fine white needles, mp 102–103 °C. The UV spectrum (in ethanol) was that of a typical indole. IR (in KBr) and NMR (in dimethyl-d₆ sulfoxide) spectra were in agreement with the structure of BA-3-aminomethylindole (3). Anal. C, H, N. This material should be kept dry in a freezer.

BA-3-[(methylamino)methyl]indole (4). 3-[(Methylamino)methyl]indole (3.0 g, 17.8 mmol) was converted to the bromoacetyl derivative in the same manner as 3; a similar purification on a dry column of Silica Woelm TSC gave 0.78 g of oil. This was crystallized from dry ether to yield 460 mg (9%), mp 84–95 °C, and recrystallized from benzene to give 170 mg (4%) of white crystals, mp 99–101 °C. Anal. C, H, N. This compound is somewhat less stable than its unmethylated analogue 3, and should be stored dry in a freezer.

Inactivation of Chymotrypsin and Analysis of the Inactivated Enzyme. Rates of inactivation were determined as in the preceding paper (Lawson & Rao, 1980) except that the buffer was 0.1 M Mops at pH 7.0. Amino acid analysis (Lawson et al., 1968; Lawson & Rao, 1980) was used to detect labeled amino acids eluting between cysteic acid and Asp. The shorter (50-cm) column for neutral amino acids now in use was operated between 50 and 60 °C for an optimum separation of methionine sulfone. The use of this column did not, however, permit an adequate separation of homoserine from Glu. Amounts of Ser and Met modification were estimated primarily on the determination of Cm-Ser and Met-sulfone and partially on the determination of S-Cm-homocysteine and Met.

pH Dependence of the Inactivation of Chymotrypsin by BA-N-methylbenzylamine (7). Rates of inactivation at 23 °C of 60 μM chymotrypsin were determined with a 20-fold molar excess of 7 in 10% ethanol and 0.1 M buffers, which were acetate (pH 5.0), Mes (pH 5.5–6.5), Mops (pH 7.0–7.5), Taps (pH 8.0–9.0), and Caes (pH 9.5–10.0). Enzyme blanks at pH 5, 6, and 7 were stable for 1 day; blanks at pH 8 and 9.5 lost 13% and 46%, respectively, of their activity. The instability of the enzyme at high pH probably does not affect the accuracy of the results appreciably, since the half-life of the enzymatic reaction is about 4–5 h from pH 8.0 to pH 9.5. The pH 10 value might be questioned, however, since no blank was run at that pH. Results were plotted as a function of reciprocal half-lives for inactivation vs. pH.

Results and Discussion

Inhibition of Chymotrypsin. Half-times for the inhibition of chymotrypsin under standardized conditions were determined by semilogarithmic plots and are given in Table I. The final activities were quite low in the faster reactions; but for

Table I: Rates of Inactivation of Chymotrypsin by α-Bromo Amides That Alkylate Ser-195

no.	compound	reaction with chymotrypsin, ^a half-time (min)	
		pH 5	pH 7
1	(R)-(+)-1-aminoinidan	4580	4980
2	(S)-(-)-1-aminoinidan	2580	2580
3	3-(aminomethyl)indole	1960	1580
4	3-[(methylamino)methyl]indole	420	156
5	N-methylaniline	7380	4320
6	benzylamine	3870	2640
7	N-methylbenzylamine	1390	430
8	N-methyl- <i>p</i> -phenethylamine	540	324

^a In 0.1 M acetate buffer, pH 5.0, and in 0.1 M Mops buffer, pH 7.0 (compounds 1, 2, 3, 4, and 7), or 0.1 M Tris buffer, pH 7.0 (compounds 5, 6, and 8; data taken from Table I of Lawson & Rao, 1980), 10% ethanol at 20-fold molar excess over chymotrypsin (60 μM). The half-times for loss of enzyme activity were obtained from pseudo-first-order plots.

Table II: Amino Acid Analyses for Constituents of the Active Site of Chymotrypsin after Reaction with Some Irreversible Inhibitors^a

compound	pH	Cm-Ser	MetO ₂	S-Cm	Met	His
1	7	0.27	1.30	0.17	0.13	1.94
	5	0.11	0.71	0.23	0.18	1.87
2	7	0.78	1.60	0.13	0.14	1.74
	5	0.36	1.37	0.09	0.17	1.82
3	7	0.64	1.42	0.03	trace	1.71
	5	0.13	0.91	0.27	0.16	1.97
4	7	1.18	1.48	trace	trace	1.67
	5	0.58	1.00	0.24	0.13	1.76
none	7		1.77			1.92

^a The inactivated enzyme preparation (<10% activity) were subjected to amino acid analysis as described under Materials and Methods after performic acid oxidation according to Hirs (1956), but at 25 °C for 2 h. Cm-Ser, *O*-(carboxymethyl)serine; MetO₂, methionine sulfone; S-Cm, *S*-(carboxymethyl)homocysteine.

practical reasons final activities were about 10% for the slower reactions (*t*_{1/2} > 2000 min). Amino acid analyses show that the compounds alkylate Met-192 and Ser-195, with methionine alkylation more pronounced at pH 5 and serine alkylation greater at pH 7 (Table II gives results for compounds 1–4). The analyses were carried out after performic acid oxidation of the inactivated enzyme. Under these conditions unmodified methionine is oxidized to methionine sulfone (MetO₂), while methionine alkylated with haloacetic acid derivatives is converted to a mixture of free methionine, *S*-(carboxymethyl)-homocysteine, homoserine, and homoserine lactone (Gundlach et al., 1959; Neumann et al., 1962; Lawson & Schramm, 1965). The latter two compounds were not resolved by our current analytical system, but the other products give a clear picture of the results. *O*-(Carboxymethyl)serine (Cm-Ser) is easily detected; the amounts listed in Table II are not corrected for the small quantities of glycolic acid produced by decomposition (Lawson et al., 1968). No evidence of histidine alkylation was noted, but a small amount, comparable to that previously observed in the alkylation of serine at the active site of trypsin (ibid.), cannot be excluded.

It can be safely assumed that the compounds discussed in this paper alkylate Ser-195 for the following reasons: (a) *O*-(carboxymethyl)serine is obtained on amino acid analysis of the inactivated enzyme, (b) chymotrypsin has never been shown to contain more than one uniquely reactive serine residue, and (c) the inhibitors were designed as affinity labels for the active site of chymotrypsin. The reactions of BA-N-

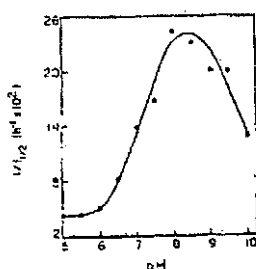


FIGURE 1. pH Dependence of the inactivation of chymotrypsin by BA-*N*-methylbenzylamine (7) under conditions described under Materials and Methods.

methylaniline (5) and BA-*N*-methylbenzylamine (7) were studied (Lawson & Rao, 1980) by the method of Kitz & Wilson (1962) and found to display the typical binding behavior expected of affinity labels (Lawson, 1978).

BA-(*S*)-(-)-aminoindan (2) is more selective than its enantiomer (1) in the alkylation of serine at the active site (Table I). The most effective and selective compound for the alkylation of serine in chymotrypsin at pH 7 is BA-3-[(methylanino)methyl]indole (4); the rate of inactivation (Table I) is the fastest of the series, and methionine alkylation is barely detectable (Table II).

The pH dependencies of the inactivations with compounds 3 and 4 are similar to those observed earlier with most of the compounds found to alkylate serine in chymotrypsin (Lawson & Rao, 1980). Repetition of the inhibitions with the aminoindan derivatives 1 and 2 gave similar half-times, but again no pH dependence could be observed (Table I). It seems likely that solvolysis of the compounds at the higher pH competes with the enzymatic reactions, and if this is true, the pH dependencies of the other slowly reacting inhibitors should be more pronounced than they were found to be. A more detailed study of the pH dependence was carried out with BA-*N*-methylbenzylamine (7). The reaction shows one pK_a of approximately 7 and another above pH 9 (Figure 1). The latter is attributable to deprotonation of the *N*-terminal Ile-16 and the consequent breakup of its hydrogen bond with Asp-194 (Himoe et al., 1967; Blow, 1971).

Stereochemistry of the Inhibitors and Their Interactions with the Active Site of Chymotrypsin. The stereochemistry of aromatic α -bromo amides that inactivate chymotrypsin by affinity labeling was discussed in the previous paper (Lawson & Rao, 1980). The ability of a number of these compounds to alkylate Met-192 correlated well with the likelihood that they exist to a significant extent in conformations with the amide group coplanar with the aromatic ring. Several compounds that should achieve coplanarity with difficulty were found to alkylate Met-192 slowly and to alkylate Ser-195 to some extent. This group of inhibitors is presented in Table I along with two additional ones, compounds 3 and 4.

All of these compounds should experience some difficulty in achieving coplanar conformations with the exception of BA-*N*-methyl- β -phenethylamine (8), which can exist in a coplanar extended conformation. The 1-aminoindan derivatives 1 and 2 can approximate (but not achieve) a coplanar conformation when the bromoacetamido substituent is equatorial to the 5-membered ring. The remainder of the series (3-7) should exist mostly in noncoplanar conformations due to steric hindrance between the amide groups and the aromatic rings (Lawson & Rao, 1980).

Possible interactions of the compounds with the active site were studied using CPK models and the CCS model of the

active site (Clarke, 1977). With the aromatic group in the specificity pocket, two orientations appear attractive for noncoplanar compounds. The first involves hydrogen bonding of the amide NH group (if any) to the carbonyl group of Ser-214. Such binding should be nonproductive for alkylation of Met-192 or Ser-195 because it places the back side of the CH_2Br group at some distance from and incorrectly oriented toward these nucleophiles. The second orientation involves hydrogen bonding of the amide CO group with the NH groups of Gly-193 and Ser-195, similar to the binding of the ester or carboxamide carbonyl group of typical substrates enroute to the tetrahedral intermediate (Blow, 1976, and references therein). In this binding mode the hydroxyl oxygen atom of Ser-195 has easy access to the back side of the CH_2Br group for a nucleophilic attack.

While studying possible interactions of the aminoindan derivatives 1 and 2 with the active site, it appeared that the indan ring of the *S*-(-) enantiomer 2 could fit into the specificity pocket in much the same way as the indole ring of a tryptophan residue while its carbonyl group makes contact with the NH groups of Gly-193 and Ser-195. This orientation allows an attack by the OH group of Ser-195 on the CH_2Br group. The proper conformation of 2 for this orientation can easily be made with a CPK model by placing the carbonyl oxygen of the *trans* amide group in a position *cis* to the hydrogen atom on the 1-position of the indan ring. Because of the opposite chirality of compounds 1 and 2, the same conformation of the *R*-(+) enantiomer 1 places its carbonyl oxygen in a direction opposite from the NH groups of Gly-193 and Ser-195 when the model is inserted into the active site. When the amide group of 1 is turned through 180° to bring the carbonyl group in the right direction, the resulting axial conformation encounters considerable steric hindrance in fitting into the site. These observations led to a prediction that the *S*-(-)-enantiomer 2 should be more effective than 1 in alkylation of Ser-195. The prediction was substantiated in regard to both rate (Table I) and specificity (Table II).

Although BA-(*S*)-(-)-1-aminoindan (2) is more rapid and selective in the alkylation of Ser-195 than the *R*-(+)-enantiomer 1, its rate of reaction with the enzyme is fairly slow ($t_{1/2} = 2580 \text{ min} = 1.5 \text{ days}$). The slow rate could be caused by (1) steric hindrance in the binding pocket due to the CH_3 groups of the indan ring and (2) nonproductive binding due to hydrogen bond formation between the NH group of the amide and the carbonyl group of Ser-214. The first possibility seemed somewhat unlikely since the amides of acetyl-L-phenylalanine and acetyl-L-hexahydrophenylalanine are hydrolyzed by chymotrypsin at very similar rates (Jennings & Niemann, 1953), as are their methyl esters (Jones & Niemann, 1963). It might have been possible to resolve this question with derivatives of 1-aminoindene. However, it seemed simpler to utilize the sterically similar 3-(aminomethyl)indole system, which is not asymmetric but should become so on binding to the active site. BA-3-(aminomethyl)indole (3) inactivates chymotrypsin somewhat faster than does BA-(*S*)-(-)-1-aminoindan (2) but in much the same manner (Tables I and II). The indole derivative 3 does not possess bulky CH_3 groups on the ring system but has an amide NH group that could bond to the carbonyl group of Ser-214. The *N*-methyl analogue of 3, BA-3-[(methylanino)methyl]indole (4), which cannot form such a hydrogen bond at the active site, proved to be the best inhibitor of this type to date in regard to both rate of inactivation and specificity for serine alkylation at pH 7 (Tables I and II and Figure 2).



FIGURE 2: BA-3-[(methylamino)methyl]indole (4) oriented for alkylation of Ser-195 of chymotrypsin. The *N*-methyl group prevents hydrogen bond formation with the CO group of Ser-214. Abbreviations used: CO 214, carbonyl group of Ser-214; NH 193 and NH 195, backbone NH groups of Gly-193 and Ser-195; OG 195, hydroxyl oxygen atom of Ser-195; SG 192, sulfur atom of Met-192; the other atoms belong to compound 4.

Our earlier work suggested that the primary effect of an *N*-methyl group in these compounds was to ensure noncoplanarity of the aromatic ring and the amide group and thus to decrease the rate of alkylation of Met-192 (Lawson & Rao, 1980). This view is still valid, especially in the case of BA-aniline (Lawson & Rao, 1980) and BA-*N*-methylaniline (5). But the second and no less important effect of an *N*-methyl group that emerged in the present work is that of directing the reaction to Ser-195, most likely by preventing nonproductive hydrogen bonding with Ser-214. The same contrast in rate observed between the indole derivatives 3 and 4 is seen also with the benzylamine derivatives 6 and 7 (Table I; Lawson & Rao, 1980).

Alkylation of Ser-195 and the Charge-Relay System. Perhaps the most interesting result of this study, in relation to the catalytic mechanism of chymotrypsin, is the alkylation of Ser-195 in a reaction that displays a pK_a of 7. In contrast to acylation reactions which could conceivably proceed via transfer of an acyl group from His-57 to Ser-195, alkylation of the serine hydroxyl group must be a direct process, namely, a nucleophilic attack on the CH_2Br group of an inhibitor by the serine anion or its equivalent. An intermediate alkyl-imidazole would be quite stable, even to acid hydrolysis. But since the pK_a of serine hydroxyl groups is usually around 14 (Bruice et al., 1962), the alkylation of Ser-195 must be mediated by the imidazole ring ($pK_a \sim 7$) of His-57. We pointed out some time ago in an analogous situation with trypsin "that the histidine-activated serine residue at the active site is indeed a powerful nucleophile, in contrast to normal serine residues" (Lawson et al., 1968). The present work confirms this conclusion.

Recently it has been argued that the serine need not be a powerful nucleophile and that it reacts with a tetrahedrally distorted carbonyl carbon atom because it is in an ideal position to do so (Kraut, 1977; Matthews et al., 1977). These conclusions are part of a continuing debate on the nature of the charge-relay system, Asp-102:His-57:Ser-195, which was first conceived when Blow and co-workers discovered that His-57 is hydrogen bonded to Asp-102 in the X-ray structure of chymotrypsin (for reviews see Blow, 1976; Kraut, 1977). The system as such exists in a number of serine proteases (Kraut, 1977). Major points of contention are whether the Asp-102

part of the system contributes to the nucleophilicity of Ser-195 or merely orients His-57 correctly with regard to Ser-195 and where the protons in the system are located both in the native enzymes and during substrate hydrolysis.

Hunkapiller et al. (1973) investigated α -lytic protease, enriched in ^{13}C on C-2 of its single histidine residue, by nuclear magnetic resonance. They concluded that the interior proton (located between Asp-102 and His-57) shifts from His-57 to Asp-102 as the imidazole is protonated externally by a decrease in pH (through the pK_a of 6.7) or by a transfer of a proton from Ser-195 during acylation. This interior proton shift to Asp-102 (the ultimate base) leaves the imidazole neutral. Only below pH 4.5 would the imidazole acquire a positive charge by introduction of a second interior proton. The result of this hypothesis is to reverse the pK_a 's normally assigned to Asp-102 and His-57.

Bachovchin & Roberts (1978) recently restudied this problem by using α -lytic protease enriched with ^{15}N in the imidazole group of its histidine. Their nuclear magnetic resonance study shows convincingly that the pK_a of the imidazole is 7.0. Consequently, the interior proton remains on His-57, and upon decreasing the pH or receiving a proton from Ser-195 during acylation, the imidazole becomes positively charged while Asp-102 remains as the anion down to its pK_a of about 4.5. Possible roles for the carboxylate anion of Asp-102 were thought to be orientation of the imidazole ring in its proper conformation and tautomer and stabilization of the imidazolium cation. At neutral pH the Asp-102:His-57 couple would remain as an integral unit and only be protonated on the exterior nitrogen of the imidazole.

Bachovchin & Roberts (1978) discussed the mechanistic implications of their findings in some detail. At the same time, they dismissed the possibility of activation of the serine hydroxyl group through hydrogen bonding on the basis that this concept, which involves what might be termed the problem of the exterior proton, is no longer supported by X-ray diffraction studies. Matthews et al. (1977; also see Kraut, 1977) had reexamined the X-ray coordinates of subtilisin, α - and γ -chymotrypsins, β -trypsin, and elastase and concluded that the hydroxyl oxygen (OG) of Ser-195 is too far from the exterior nitrogen (NE2) of His-57 for a hydrogen bond between the two atoms. This is the basis for their proposal that Ser-195 is not intrinsically a strong nucleophile but is reactive because it is precisely in the right place to react. The His-57:Asp-102 couple was assigned the role of transferring a proton from the serine (OG atom) to the leaving group of a substrate (amide nitrogen atom or ester oxygen atom) and was thus considered as a site for proton binding in the transition state of the reaction. Further support for this view was adduced from their previous work on tetrahedral boronic acid complexes of subtilisin, in which the exterior nitrogen of the imidazole is favorably situated for a hydrogen bond between it and the boronic acid oxygen atom that corresponds in position to that of the leaving group of a substrate (Matthews et al., 1975).

The essence of the exterior proton problem is not whether the imidazole plays a role in chymotrypsin-catalyzed reactions, since this concept is now universally accepted. The problem is reduced to a question of timing, namely, whether the imidazole of His-57 removes the proton from OG of Ser-195 before, during, or after its attack on the substrate. Matthews et al. (1977) cautioned against placing "too much reliance on details of the native structure when attempting to postulate mechanisms". In the same paper they speculated that the imidazole might undergo a "bistable flip-flop" between the

two oxygen atoms of the carbonyl group of Asp-102. Without taking a position on this, it seems quite reasonable to hypothesize that the OG of Ser-195 comes close enough to the external nitrogen of His-57 on binding of substrate to allow formation of a hydrogen bond. Subsequent proton removal might well be coordinated with the attack of the oxygen atom on the substrate. This concept is supported by X-ray crystallographic evidence that the hydrogen bond between His-57 and Ser-195 is "perfectly linear and of the expected length" in the complex of trypsin with pancreatic trypsin inhibitor, while it is "rather long" and "deviates appreciably from linearity" in trypsin itself (Bode et al., 1976).

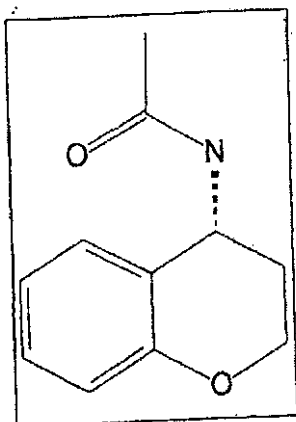
Assignment of an exact mechanism for the chymotryptic hydrolysis of amides (and the reverse reaction, the aminolysis of acyl-enzyme) is difficult because of the numerous tetrahedral intermediates at varying stages and locations of protonation that can occur during the reaction (Satterthwait & Jencks, 1974; Gresser & Jencks, 1977). Alkylation of serine by compounds containing a CH_2Br group is much less complicated, since the reaction should proceed by a bimolecular nucleophilic displacement mechanism ($\text{S}_{\text{N}}2$). A pH dependence is diagnostic of such reactions, exemplified by the solvolysis of α -bromoacetic acid (Hughes & Taher, 1940; Ingold, 1969), and it corresponds here to the increasing concentration of the nucleophile (serine anion or its equivalent) with increasing pH. Since the pK_a of the reaction is about 7, it is difficult to explain the alkylation without aid of the imidazole of His-57. The most probable route at present is analogous to mechanism 2 of Satterthwait & Jencks (1974), namely, concerted general base catalysis by imidazole of the attack of OG of Ser-195 on the alkylating agent. It is not necessary to postulate a free serine alkoxide ion to account for the pronounced nucleophilicity of Ser-195, since its equivalent can be generated with the cooperation of His-57. This mechanism might well serve as the first step in the hydrolysis of amides and esters by chymotrypsin.

Acknowledgments

I thank Edward Duchna for the amino acid analyses and Thomas J. Ryan and Bryan H. Landis for valuable discussions.

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XP-002262754

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P.	1-4	

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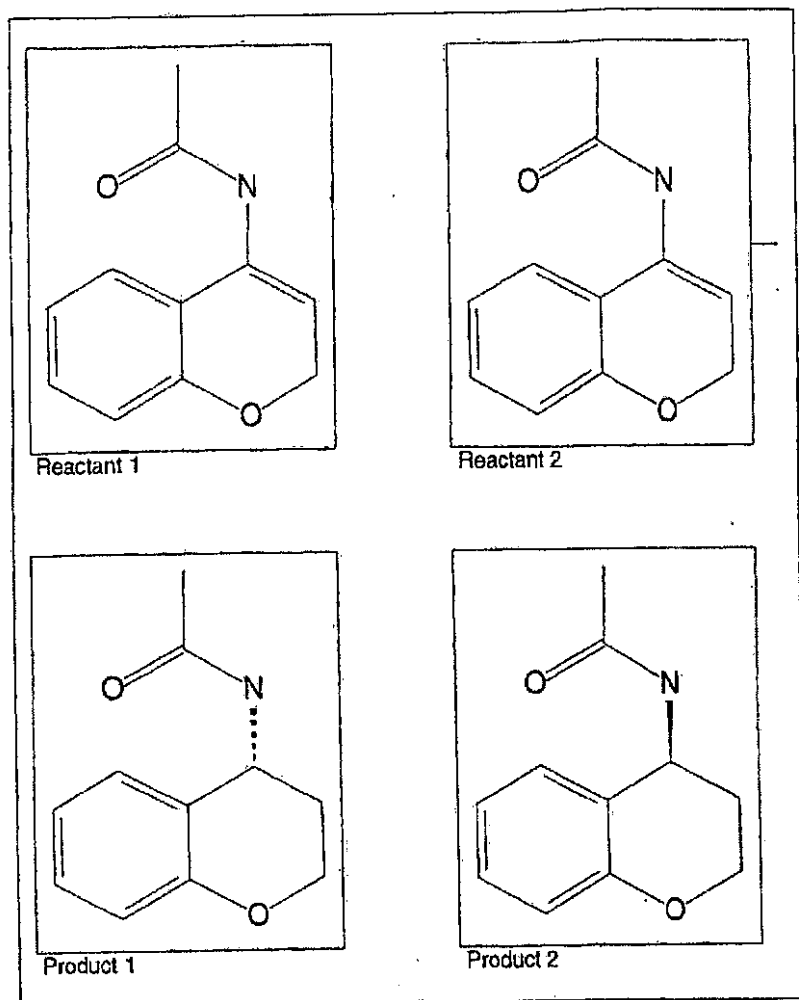
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Lawson Number	20444, 1155
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Constitution ID	6966393
Tautomer ID	7738375
Entry Date	2000/02/29
Update Date	2002/07/19

Reaction 1 of 3

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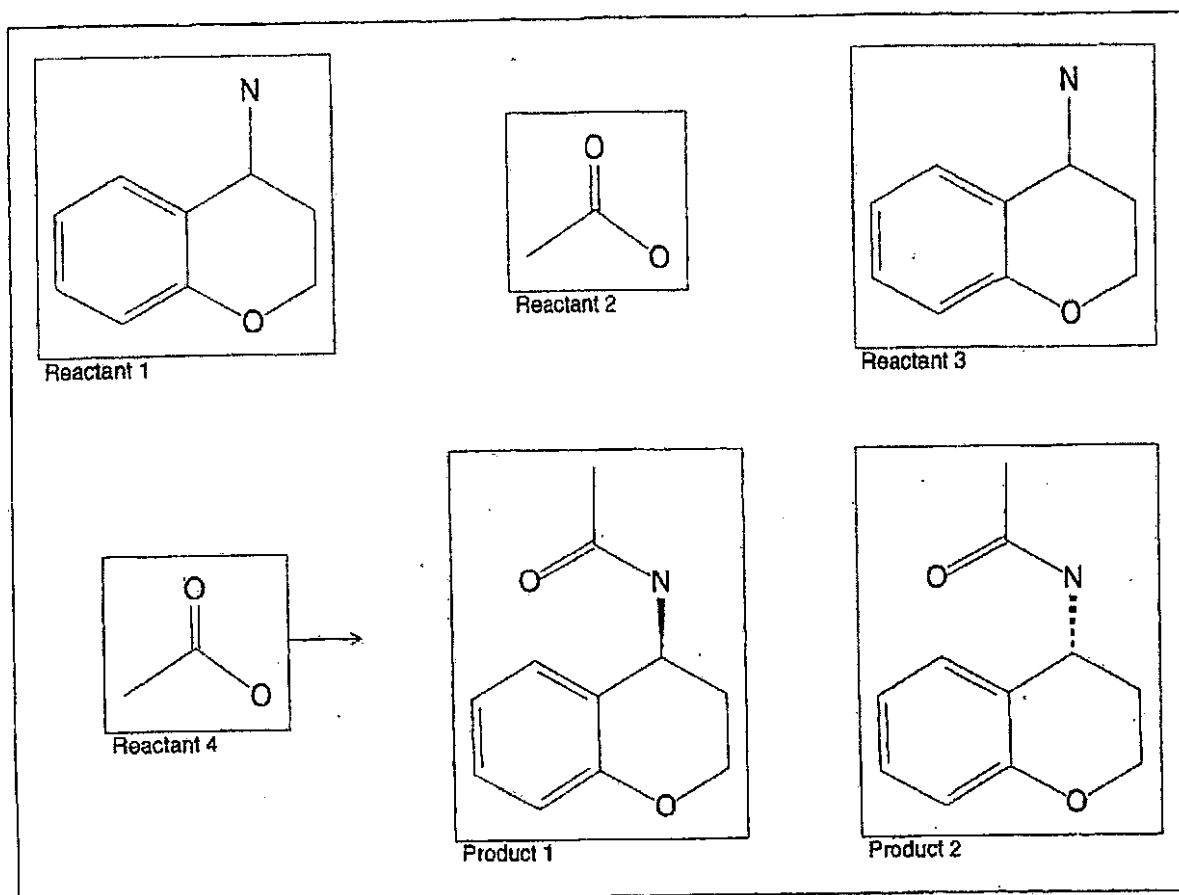
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Reaction 2 of 3

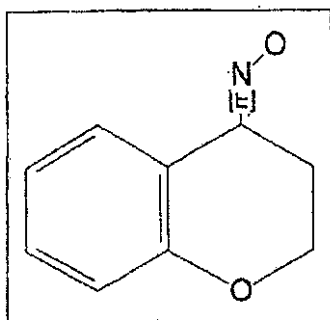
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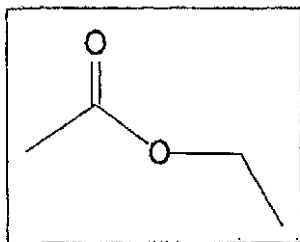
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	(i-Pr) ₂ NEt
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Catalyst	5 percent Pd/C
Solvent	toluene
Time	5 day(s)
Temperature	60 C

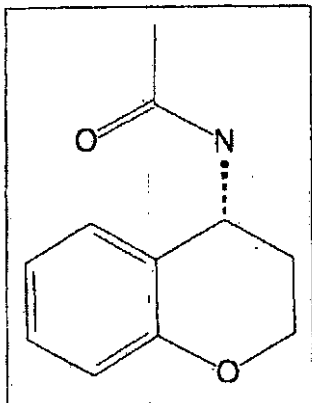
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Reactant 2

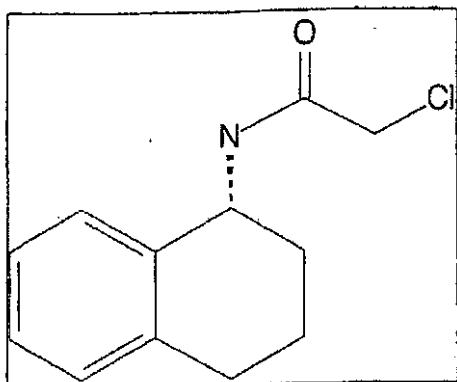


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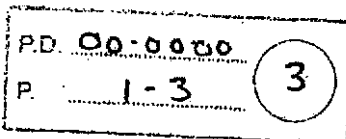
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XP-002262755



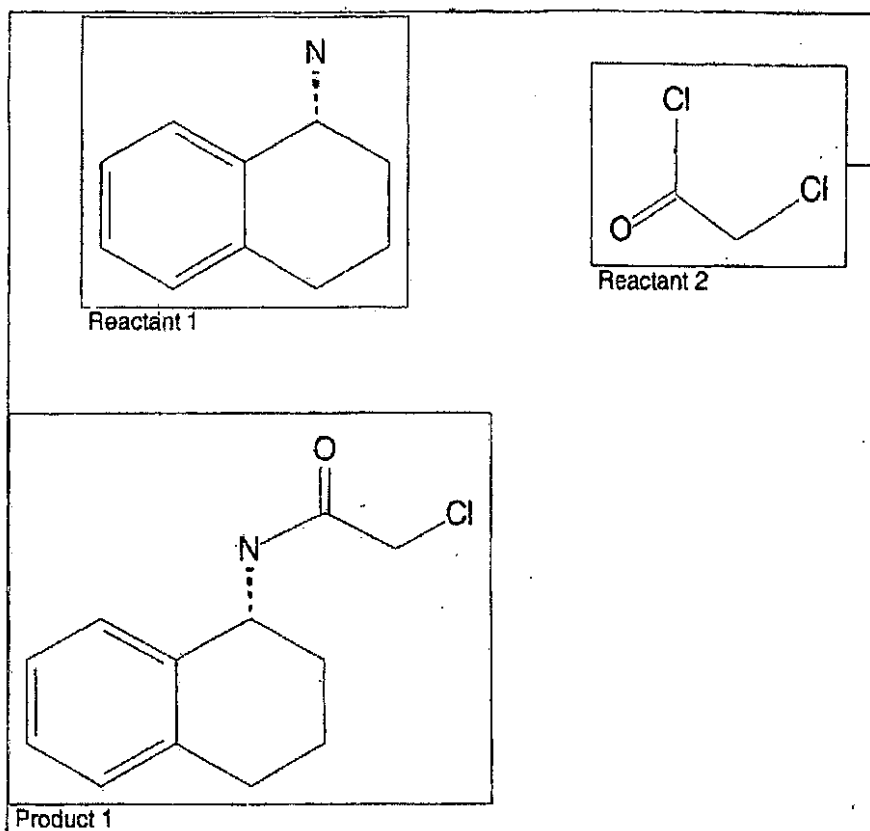
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Structure Keyword	Stereo compound
Compound Type	isocyclic
Constitution ID	2933026
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Entry Date	1994/04/25
Update Date	2000/03/03

Reaction

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Melting Point

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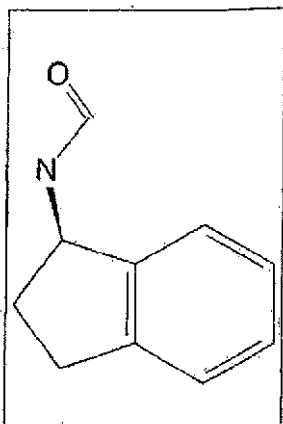
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Optical Rotatory Power 2 of 2

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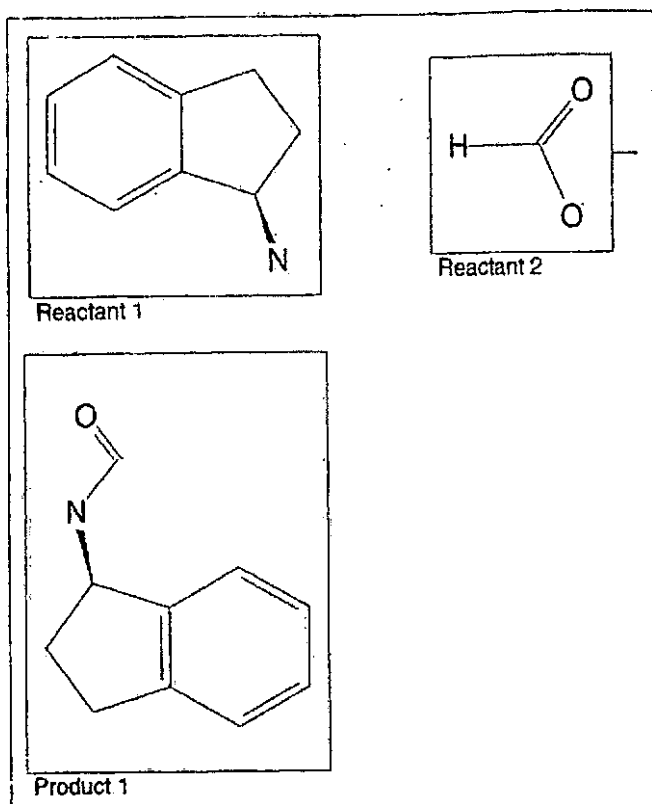
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Update Date	1995/08/14

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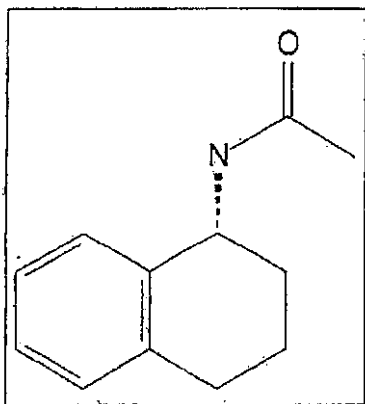
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Melting Point

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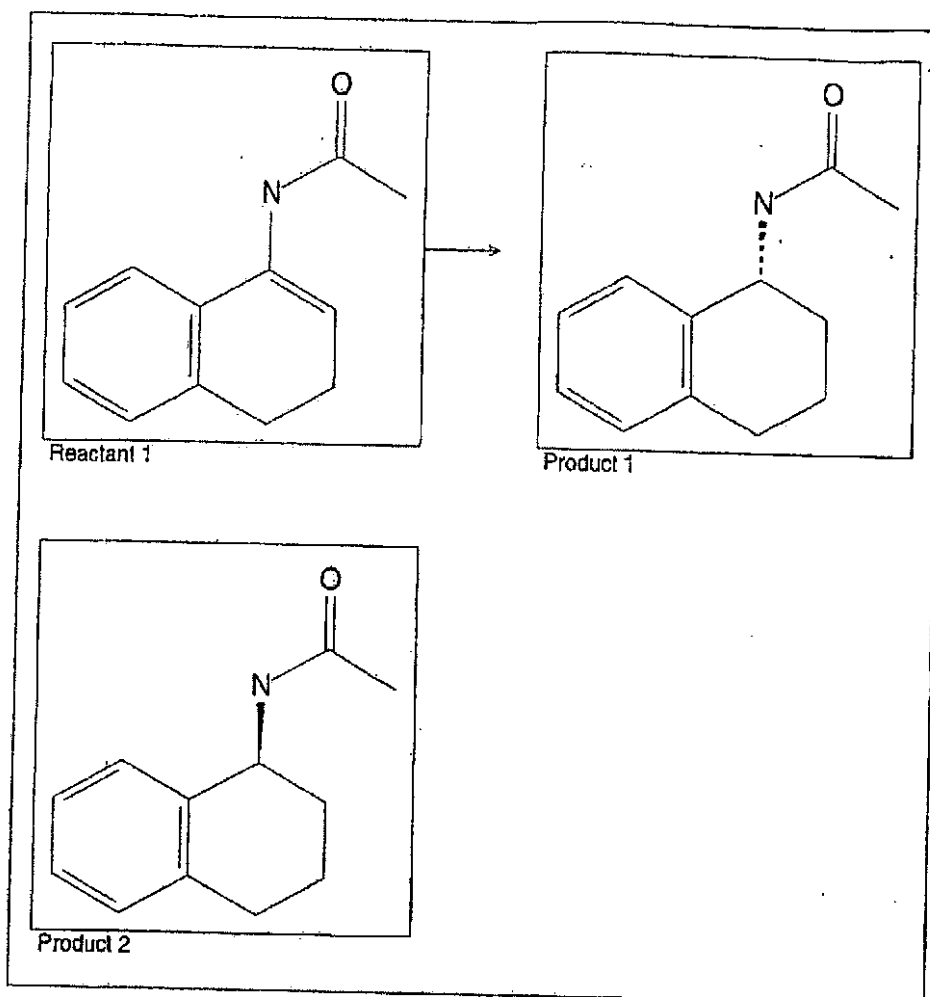
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Entry Date	1999/05/06
Update Date	2003/07/25

Reaction 1 of 2

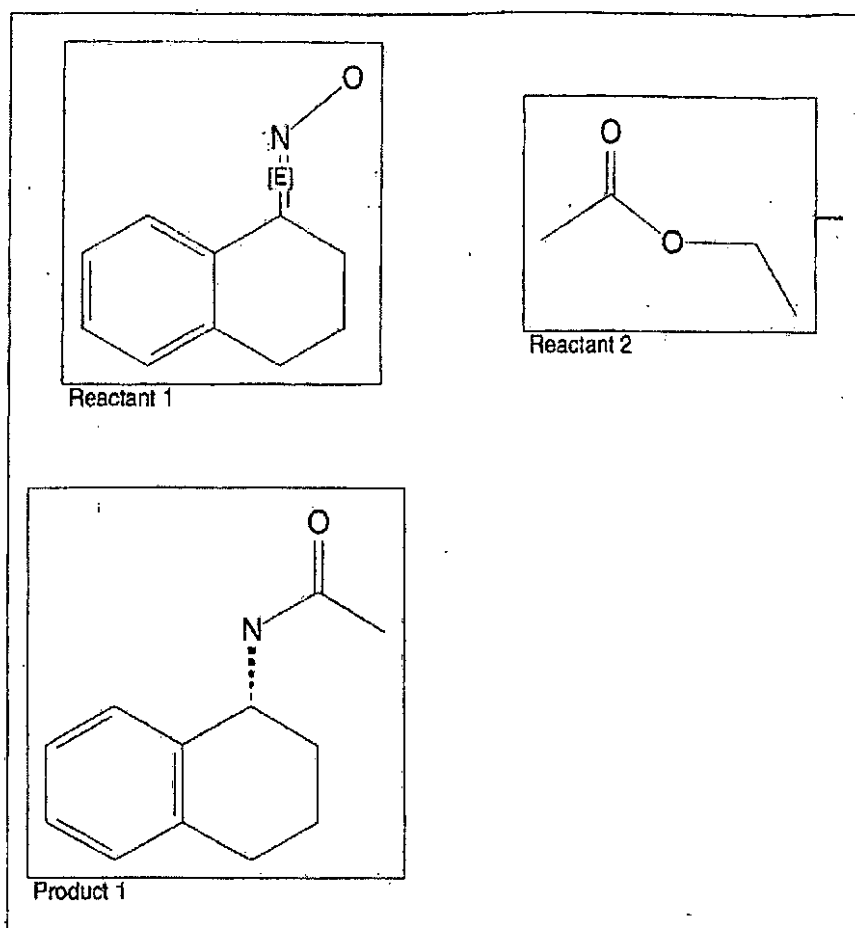
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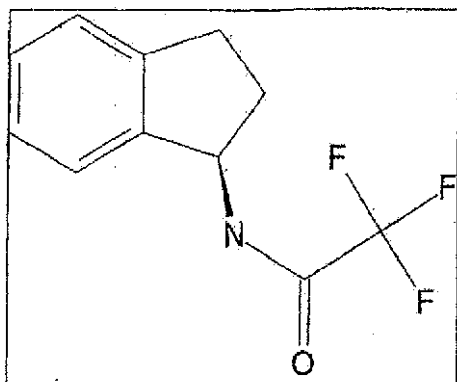
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Reactant BRN	1869333 3,4-dihydro-2H-naphthalen-1-one-(E)-oxime
Product BRN	506104 acetic acid ethyl ester
No. of Reaction Details	8111842 (R)-N-(1,2,3,4-tetrahydro-1-naphthyl)acetamide
Reaction Classification	1
Yield	Preparation
Reagent	70 percent (BRN=8111842)
	H2
	(i-Pr)2NEt
Catalyst	Candida antarctica lipase B
Solvent	5 percent Pd/C
Time	toluene
Temperature	5 day(s)
	60 C
Ref. 1	6321236; Journal; Choi, Yoon Kyung; Kim, Mi Jung; Ahn, Yangsoo; Kim, Mahn-Joo; ORLEF7; Org.Lett.; EN; 3; 25; 2001; 4099 - 4102;

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Optical Rotatory Power

Type	[alpha]
Concentration	0.1 g/100ml
Solvent	CHCl ₃
Optical Rotatory Power	83.6 deg
Wavelength	589 nm
Temperature	25 C
Ref. 1	<u>6321236</u> ; Journal; Choi, Yoon Kyung; Kim, Mi Jung; Ahn, Yangsoo; Kim, Mahn-Joo; ORLEF7; Org.Lett.; EN; 3; 25; 2001; 4099 - 4102;



XP-002262758

P.D.	00 00 00
P.	1-2

2

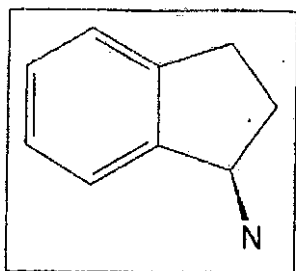
Substance

Beilstein Registry Number	9264314
Chemical Name	N-trifluoroacetyl-(R)-1-aminoindan
Antoname	2,2,2-trifluoro-N-indan-1-yl-acetamide
Molecular Formula	C ₁₁ H ₁₀ F ₃ NO
Molecular Weight	229.20
Lawson Number	14224, 1157
Structure Keyword	Stereo compound
Compound Type	isocyclic
Constitution ID	7822841
Tautomer ID	8700063
Entry Date	2003/04/17
Update Date	2003/04/17

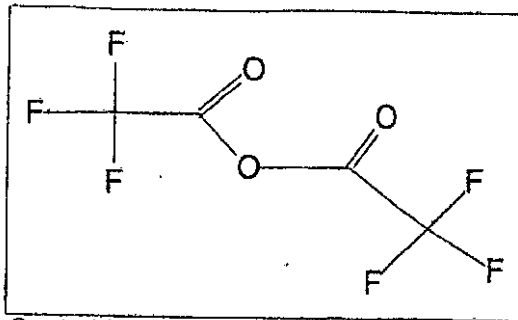
Reaction

Reaction ID	9210322
Reactant BRN	3196204 (R)-indanyl-(1)-amine
	746197 trifluoroacetic acid anhydride
Product BRN	9264314 N-trifluoroacetyl-(R)-1-aminoindan
No. of Reaction Details	1
Reaction Classification	Preparation
Yield	87 percent (BRN=9264314)
Reagent	aq. KOH
Solvent	toluene
Time	2 hour(s)
Temperature	20 C

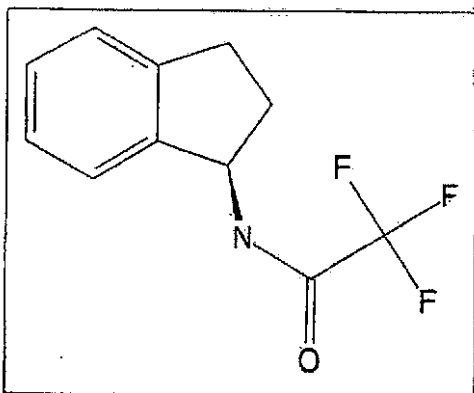
Ref. 1 6374004; Journal; Sterling, Jeffrey; Herzig, Yaacov; Goren, Tamar; Finkelstein, Nina; Lerner, David; Goldenberg, Willy; Miskolczi, Istvan; Molnar, Sandor; Rantal, Ferenc; Tamas, Tivadar; Toth, Gyorgy; et al.; JMC MAR; J. Med. Chem.; EN; 45; 24; 2002; 5260 - 5279;



Reactant 1



Reactant 2

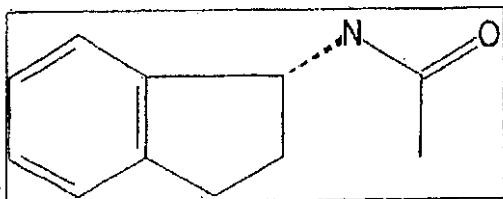


Product 1

Melting Point

VALUE (MP) C	Solvent (.SOL)	Note	Ref.
153 - 154			1

Ref. 1 **6374004**; Journal; Sterling, Jeffrey; Herzig, Yaacov; Goren, Tamar; Finkelstein, Nina; Lerner, David; Goldenberg, Willy; Miskolczi, Istvan; Molnar, Sandor; Rantal, Ferenc; Tamas, Tivadar; Toth, Gyorgy; et al.; JMC MAR; J.Med.Chem.; EN; 45; 24; 2002; 5260 - 5279;



XP-002262759

P.D.	00 00 00	4
P.	1-4	

Substance

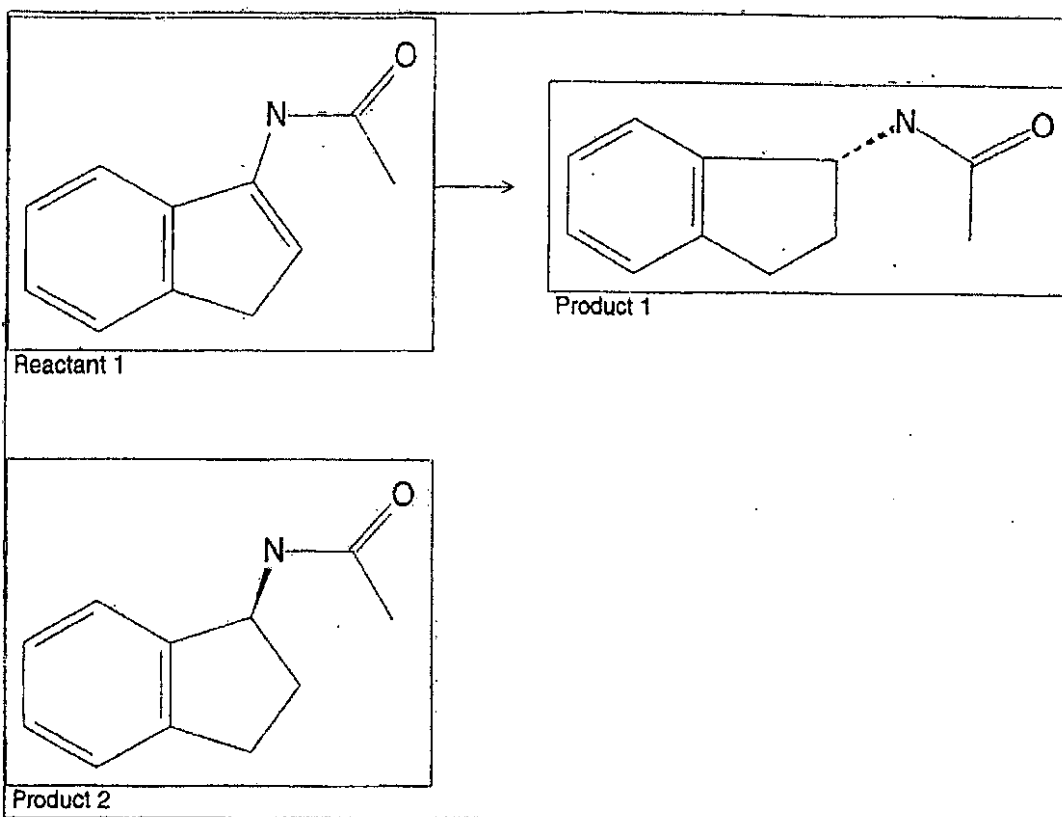
Beilstein Registry Number	8201255
Chemical Name	(R)-N-Acetyl-1-aminoindane
Autoname	N-indan-1-yl-acetamide
Molecular Formula	C ₁₁ H ₁₃ NO
Molecular Weight	175.23
Lawson Number	14224, 1155
Structure Keyword	Stereo compound
Compound Type	isocyclic
Constitution ID	2367769
Tautomer ID	7737653
Entry Date	2000/02/29
Update Date	2003/07/25

Reaction 1 of 3

Reaction ID	5114594
Reactant BRN	8052123 N-(3H-inden-1-yl)-acetamide
Product BRN	8052084 (S)-1-(N-acetylamido)indane 8201255 (R)-1-(N-acetylamido)indane
No. of Reaction Details	3
Reaction Classification	Preparation
Reagent	(((1R,2R)-1,3-Me2-2-(3,5-Me2-phenylthio)BuO)PPh2)Rh(COD)SbF6
	H2
Solvent	tetrahydrofuran
Time	18 hour(s)
Temperature	20 C
Pressure	5928 Torr

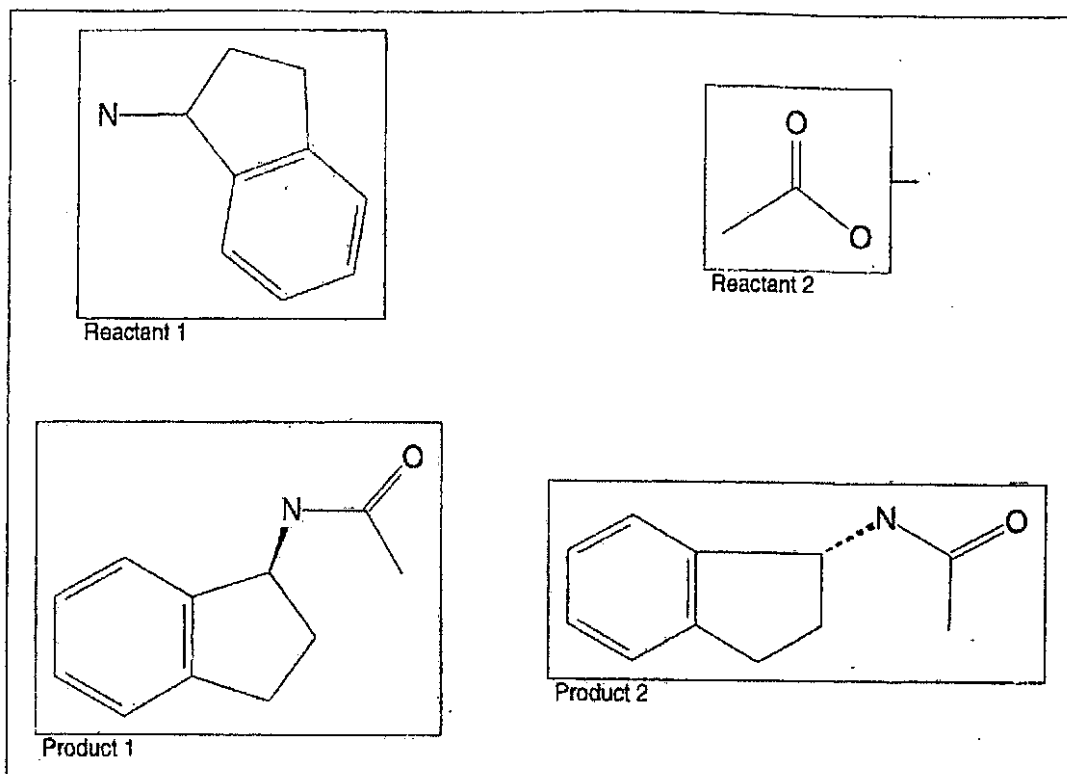
Note 1 Title compound not separated from byproducts

Ref. 1 6387399; Journal; Evans, David A.; Michael, Forrest E.; Tedrow, Jason S.; Campos, Kevin R.; JACSAT; J.Amer.Chem.Soc.; EN; 125; 12; 2003; 3534 - 3543;



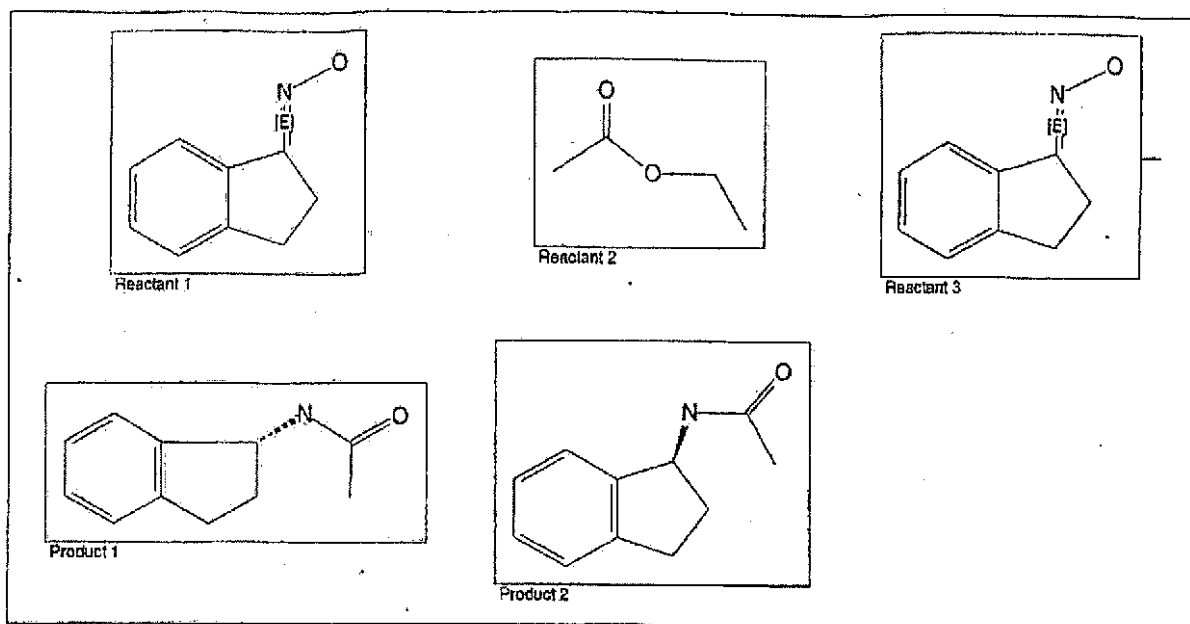
Reaction 2 of 3

Reaction ID	8630270
Reactant BRN	2206707 indan-1-ylamine
	506007 acetic acid
Product BRN	8052084 (S)-1-(N-acetylamido)indane
	8201255 (R)-1-(N-acetylamido)indane
No. of Reaction Details	1
Reaction Classification	Preparation
Reagent	1,1'-carbonyldiimidazole
Solvent	toluene
Reaction Type	Acetylation
Note 1	Title compound not separated from byproducts
Ref. 1	6255120 ; Journal; Fernandez, Elena; Maeda, Kenji; Hooper, Mark W.; Brown, John M.; CEUJED; Chem.Europ.J.; EN; 6; 10; 2000; 1840 - 1846;



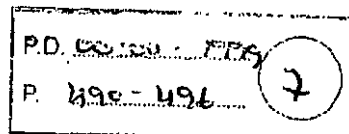
Reaction 3 of 3

Reaction ID	<u>8953773</u>
Reactant BRN	<u>1865148</u> indan-1-one-(<i>E</i>)-oxime
	<u>506104</u> acetic acid ethyl ester
Product BRN	<u>8201255</u> (R)-1-(N-acetylamido)indane
	<u>8052084</u> (S)-1-(N-acetylamido)indane
No. of Reaction Details	1
Reaction Classification	Preparation
Reagent	H ₂
	(i-Pr) ₂ NEt
	Candida antarctica lipase B
Catalyst	5 percent Pd/C
Solvent	toluene
Time	5 day(s)
Temperature	60 C
Note 1	Title compound not separated from byproducts
Ref. 1	<u>6321236</u> ; Journal; Choi, Yoon Kyung; Kim, Mi Jung; Ahn, Yangsoo; Kim, Mahn-Joo; ORLEF7; Org.Lett.; EN; 3; 25; 2001; 4099 - 4102;



Nuclear Magnetic Resonance

Description	Chemical shifts
Nucleus	¹ H
Solvents	CDCl ₃
Ref. 1	6158231; Journal; Zhu, Guoxin; Zhang, Xumu; JOCEAH; J.Org.Chem.; EN; 63; 25; 1998; 9590 - 9593;



1-Aryl-4-[(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)alkyl]piperazines and Their Analogues: Influence of the Stereochemistry of the Tetrahydronaphthalen-1-yl Nucleus on 5-HT_{1A} Receptor Affinity and Selectivity versus α_1 and D₂ Receptors. 5¹

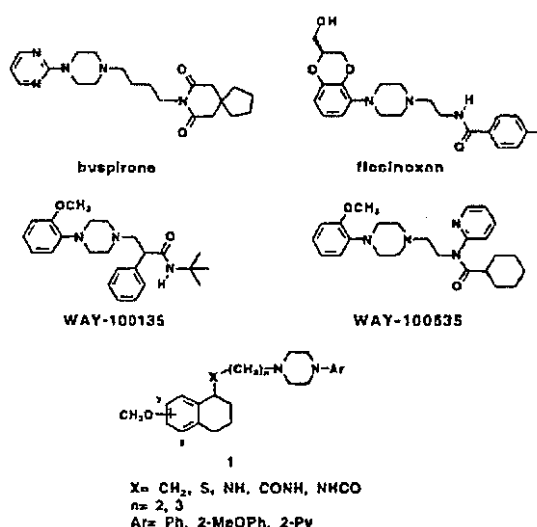
Roberto Perrone,* Francesco Berardi, Nicola A. Colabufo, Marcello Leopoldo, and Vincenzo Tortorella
Dipartimento Farmaco-Chimico, Università di Bari, via Orabona, 4, 70126 Bari, Italy

Received July 15, 1998

Some 1-aryl-4-[(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]piperazines and their alkylamino and alkylamido analogues, previously studied as 5-HT_{1A} ligands, were prepared in enantiomerically pure form, and their absolute configuration was determined by chemical correlation or by chiroptical properties. They were evaluated for in vitro 5-HT_{1A}, D₂, and α_1 receptor affinity by radioligand binding assays, to study the influence of the chiral carbon atom of the tetrahydronaphthalene nucleus on the 5-HT_{1A} affinity and selectivity. Results indicated that, as regarding the 5-HT_{1A} receptor affinity, there was no difference in affinity between (-) and (+)-enantiomers as well as the racemate of each compound. The stereochemistry, instead, influenced the selectivity: all (-)-enantiomers displayed affinity values higher than those of (+)-isomers at D₂ receptors, and conversely, all (+)-enantiomers displayed affinity values higher than those of (-)-isomers at α_1 receptors. As a result of this trend, it is not possible to predict the isomer with a better selectivity profile. However, compounds (S)-(+)-2, (S)-(+)-4, and (R)-(+)-6 displayed high affinity for the 5-HT_{1A} receptor (IC₅₀ values ranging between 7.0 and 2.3 nM) and good selectivity (≥ 250 -fold) versus both D₂ and α_1 receptors. Furthermore, compounds (S)-(+)-4 and (R)-(-)-4 were submitted to the [³⁵S]GTP γ S binding assay for a preliminary evaluation of their intrinsic activity on the 5-HT_{1A} receptor.

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter in the peripheral and central nervous system that mediates a wide variety of physiological responses by different receptors.²⁻⁴ Among these receptors, the 5-HT_{1A} receptor subtype is the most studied, and its involvement in psychiatric disorders such as depression^{5,6} and anxiety⁷ is generally accepted. Long-chain arylpiperazines represent one of the most important classes of 5-HT_{1A} receptor ligands: buspirone, flesinoxan, WAY-100135, and WAY-100635 are the most relevant members of this class. These compounds bind at the 5-HT_{1A} receptor with good or high affinity, but their selectivity versus dopaminergic D₂ and α_1 adrenergic receptors is not always remarkable. Indeed, it is well-known that these three receptors are members of the superfamily of G-protein-coupled receptors, and despite their completely distinct pharmacology, they show common features on their binding site.

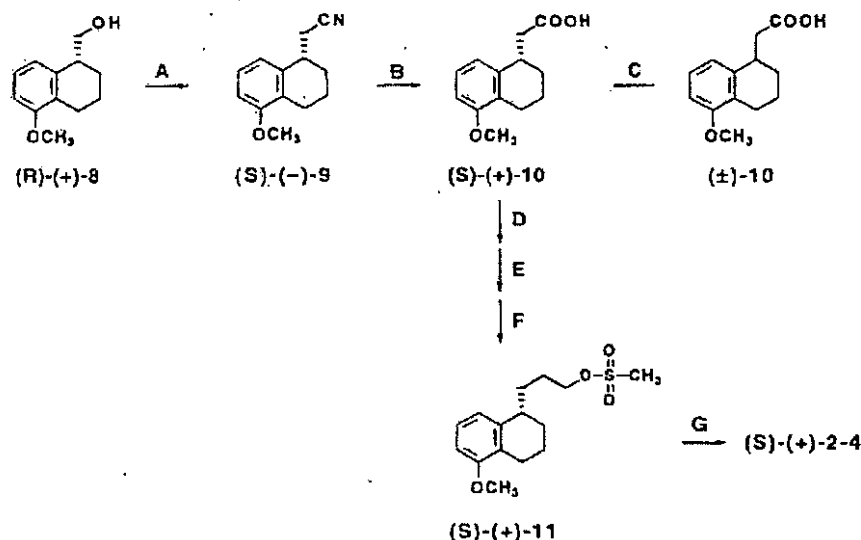
We recently reported structure-affinity relationship studies on a class of long-chain arylpiperazines of structure type 1⁸⁻¹⁰ with regards to the 5-HT_{1A} receptor affinity and selectivity versus D₂ and α_1 receptors. In particular we investigated the influence of the following features: (a) the nature of the aryl group on N-1 of the piperazine ring; (b) the presence or the absence of a heteroatom, an amide function, or a double bond in the intermediate alkyl chain and the length of the chain; (c) the position of the methoxy group on the tetralinyl nucleus. The obtained results indicated that only a few of the studied arylpiperazines (compounds 2-4) dis-



played high 5-HT_{1A} binding affinity (IC₅₀ < 1 nM) and also a good selectivity versus D₂ and α_1 receptors (≥ 100 -fold). Before performing further structural modifications on this class of compounds to improve the 5-HT_{1A} affinity and particularly the selectivity, we thought it would be interesting to investigate the role of the asymmetric carbon atom of the tetralinyl moiety on the affinity and selectivity for 5-HT_{1A} receptors. We were encouraged to carry out this program by the interesting results reported for other 1-arylpiperazine compounds,

* To whom correspondence should be addressed.

Scheme 1*



* Reagents: (A) (i) methanesulfonyl chloride, triethylamine, (ii) NaCN; (B) 50% H₂SO₄; (C) (i) (S)-(-)-1-phenylethylamine, (ii) 3 N HCl; (D) (i) SOCl₂, triethylamine, (ii) CH₂N₂, (iii) Ag⁺, MeOH; (E) LiAlH₄; (F) methanesulfonyl chloride, triethylamine; (G) 1-aryl piperazines.

acting at the 5-HT_{1A} receptor, which present an asymmetric center in their structure (i.e., flcsinoxan and WAY-100135). In particular, (S)-(+)-WAY-100135 displayed high stereoselectivity for the 5-HT_{1A} receptor, being markedly more active than the (R)-isomer in binding, functional, and behavioral assays.¹¹

In the present study we report the synthesis, the assignment of the absolute configuration, and the binding assay on 5-HT_{1A}, D₂, and α₁ receptors of optically active form of compounds 2–7, which were the most representative derivatives for each of the previously reported chemical classes.^{8–10} Our aim is to find the structural features, stereochemistry included, that account for affinity and selectivity on the 5-HT_{1A} receptor in order to design highly selective 5-HT_{1A} receptor ligands.

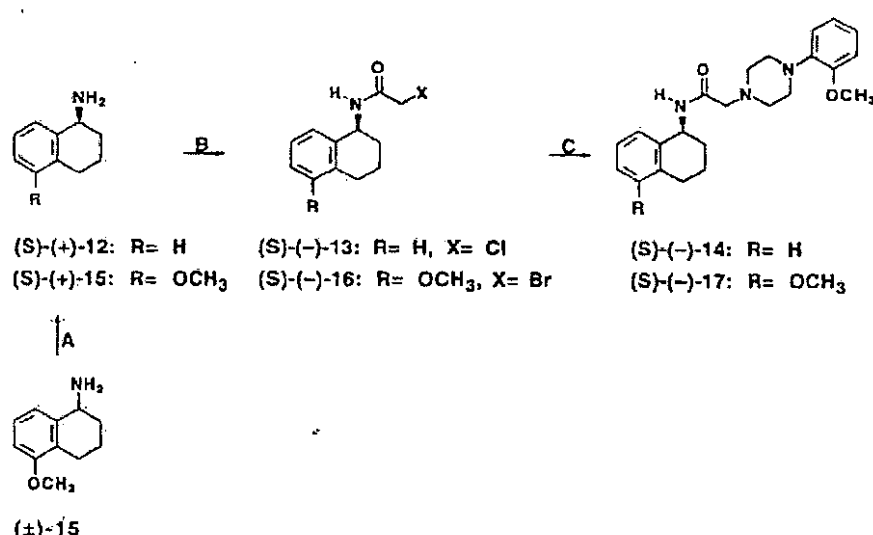
Chemistry

Several attempts to separate enantiomers of compounds 2–7 via fractional crystallization of their diastereomeric salts with optically active acids failed. Therefore, the synthesis of the target compounds was achieved starting from an easily accessible optically active intermediate. The synthesis of compounds (S)-(+)-2–4 is depicted in Scheme 1. The absolute configuration of the key intermediate (S)-(+)-10 was established by chemical correlation as follows: the optically active alcohol (R)-(+)-8¹² was activated for displacement as its mesylate ester and then displaced with NaCN to yield nitrile (S)-(-)-9; this compound was hydrolyzed to give (S)-(+)-5-methoxy-1,2,3,4-tetrahydro-1-naphthaleneacetic acid [(S)-(+)-10]. Because of the low yield and the moderate enantiomeric excess encountered, this synthetic pathway was not useful for preparative purposes, so acid (S)-(+)-10 was obtained in good yield by fractional crystallization of its diastereomeric salt with (S)-(-)-1-phenylethylamine. The multistep sequence previously reported¹³ afforded the mesylate derivative

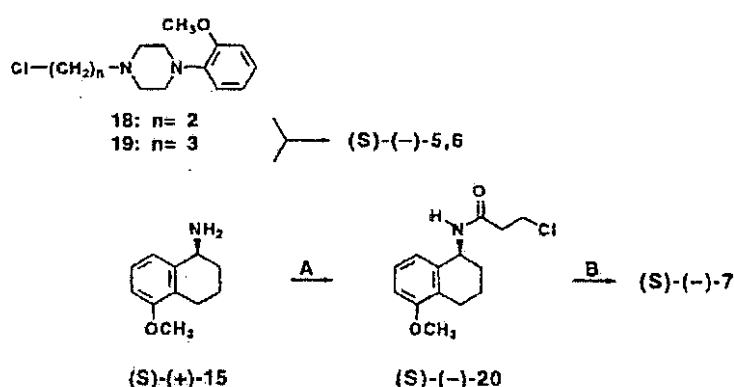
(S)-(+)-11 which was reacted with the appropriate 1-aryl piperazine to give final compounds (S)-(+)-2–4. Starting from (R)-(-)-5-methoxy-1,2,3,4-tetrahydro-1-naphthaleneacetic acid [(R)-(-)-10], the same synthetic procedure gave compounds (R)-(-)-2–4.

The absolute configuration of the key intermediate (±)-15 for the synthesis of tetrahydronaphthalenamine derivatives was unknown. We used the circular dichroism (CD) characteristics for its configurational assignment because the absolute configuration of (S)-(+)-1,2,3,4-tetrahydro-1-naphthalenamine [(S)-(+)-12] had been already established.¹⁴ Unfortunately, amine (S)-(+)-12 did not display the Cotton effect, so its derivatization was necessary (Scheme 2). Thus, amine (S)-(+)-12 was transformed with chloroacetyl chloride into the derivative (S)-(-)-13 which was reacted with 1-(2-methoxyphenyl)piperazine to give the derivative (S)-(-)-14. Similarly (R)-(-)-1,2,3,4-tetrahydro-1-naphthalenamine [(R)-(-)-12] gave derivative (R)-(+)-14. Racemic 5-methoxy-1,2,3,4-tetrahydro-1-naphthalenamine [(±)-15]¹⁵ was resolved by fractional crystallization of the diastereomeric salt with (S)-(+)-mandelic acid. Optically active amine (±)-15 was transformed with bromoacetyl chloride into derivative (±)-16, and the latter compound was aminated with 1-(2-methoxyphenyl)piperazine to give compound (±)-17. Similarly the amine (±)-15 furnished the amide (±)-17. Since both compounds (S)-(-)-14 and (±)-17 displayed a negative Cotton effect, it was stated that the absolute configuration of amine (±)-15 was S.

The synthesis of tetrahydronaphthalenamine derivatives (S)-(-)-5–7 is reported in Scheme 3. The amine (S)-(+)-15 was treated with chloro derivatives 18 and 19 to give the final amines (S)-(-)-5 and (S)-(-)-6, respectively. To prepare the amide (S)-(-)-7, amine (S)-(+)-15 was acylated with 3-chloropropionyl chloride to give chloro derivative (S)-(-)-20; the amination of the latter with 1-(2-methoxyphenyl)piperazine provided

Scheme 2^a

^a Reagents: (A) (i) (S)-(+)-mandelic acid, (ii) 5% NaOH; (B) haloacetyl chloride, NaHCO₃; (C) 1-(2-methoxyphenyl)piperazine.

Scheme 3^a

^a Reagents: (A) 3-chloropropionyl chloride, NaHCO₃; (B) 1-(2-methoxyphenyl)piperazine.

the target compound. Compounds (R)-(+)-5-7 were prepared in the same way starting from amine (R)-(-)-15.

Pharmacology

Target compounds in the form of hydrochloride salts (Table 1) were evaluated for in vitro affinity on serotonin 5-HT_{1A}, dopamine D₂, and adrenergic α₁ receptors by radioligand binding assays. The following specific radioligands and tissue sources were used: (a) serotonin 5-HT_{1A} receptors—³H-8-OH-DPAT, rat hippocampal membranes; (b) dopamine D₂ receptors—³H]spiroperidol, rat striatal membranes; (c) α₁ adrenergic receptors—³H]prazosin, rat brain cortex membranes. Concentrations required to inhibit 50% of radioligand specific binding (IC₅₀) were determined by using eight to nine different concentrations of the drug studied. Specific binding represents more than 80% of the total in all binding assays. The results were analyzed using the LIGAND program to determine IC₅₀ values. A preliminary and qualitative evaluation of the intrinsic activity at human

cloned 5-HT_{1A} receptors of compounds (+)-4 and (-)-4 was carried out using the [³⁵S]GTPγS binding method.¹⁶

Results and Discussion

Influence of the Stereochemistry in the Tetralin Moiety. First of all it should be noted that all (-)-isomers present the same relative position in the space of the four groups on the chiral center, but compounds (-)-2-4 are designated as *R*, whereas compounds (-)-5-7 are designated as *S*, according to the Cahn-Ingold-Prelog rule. Analogously, compounds (+)-2-4 are designated as *S*, and compounds (+)-5-7 are designated as *R*.

The results of an extended binding screening (Table 1) indicated that the chirality of the tetralin ring influenced the selectivity more than the affinity for 5-HT_{1A} receptors. In fact, the 5-HT_{1A} receptor affinity values presented no remarkable differences for racemates and (+)- and (-)-enantiomers. All the (-)-enantiomers, except compound (-)-5, showed lower affinity versus D₂ receptors than their (+)-isomers and

Table 1. Physical Properties and Binding Affinities

A=

B=

compd	type	X	Ar	formula ^a	mp, °C	IC ₅₀ , nM ^a			selectivity (IC ₅₀ ratio)	
						5-HT _{1A}	D ₂	α ₁	D ₂ /5-HT _{1A}	α ₁ /5-HT _{1A}
2 ^c	racemic	CH ₂	Ph			0.50	110	43	220	86
(R)-(-)-2	A	CH ₂	Ph	C ₂₄ H ₃₂ N ₂ O·2HCl	205–206	4.3	880	61	205	14
(S)-(+)-2	B	CH ₂	Ph	C ₂₄ H ₃₂ N ₂ O·2HCl	210–213	2.4	610	950	254	396
3 ^c	racemic	CH ₂	2-MeOPh			0.77	18	6.5	23	8
(R)-(-)-3	A	CH ₂	2-MeOPh	C ₂₅ H ₃₄ N ₂ O ₂ ·2HCl	240–242	1.6	150	0.8	94	0.5
(S)-(+)-3	B	CH ₂	2-MeOPh	C ₂₅ H ₃₄ N ₂ O ₂ ·2HCl·½H ₂ O	240–246	1.3	38	33	29	25
4 ^c	racemic	CH ₂	2-Py			0.54	140	66	259	122
(R)-(-)-4	A	CH ₂	2-Py	C ₂₅ H ₃₁ N ₂ O·2HCl·H ₂ O	157–163	4.5	880	85	196	19
(S)-(+)-4	B	CH ₂	2-Py	C ₂₅ H ₃₁ N ₂ O·2HCl	166–173	2.3	600	580	261	252
5 ^c	racemic	NH	2-MeOPh			7.7	380	220	49	29
(R)-(+)-5	B	NH	2-MeOPh	C ₂₄ H ₃₃ N ₂ O ₂ ·3HCl·½H ₂ O	178–179	10	3200	1000	320	100
(S)-(-)-5	A	NH	2-MeOPh	C ₂₄ H ₃₃ N ₂ O ₂ ·3HCl·H ₂ O	205–207	7	650	228	93	33
6 ^c	racemic	NHCH ₂	2-MeOPh			10	1050	881	106	88
(R)-(+)-6	B	NHCH ₂	2-MeOPh	C ₂₅ H ₃₅ N ₂ O ₂ ·3HCl·½H ₂ O	164–165	7	1900	1700	271	243
(S)-(-)-6	A	NHCH ₂	2-MeOPh	C ₂₅ H ₃₅ N ₂ O ₂ ·3HCl·H ₂ O	155–157	34	2300	649	68	19
7 ^c	racemic	NHCO	2-MeOPh			38	761	153	20	4
(R)-(+)-7	B	NHCO	2-MeOPh	C ₂₅ H ₃₃ N ₂ O ₃ ·2HCl·H ₂ O	192–193	320	870	770	3	2
(S)-(-)-7	A	NHCO	2-MeOPh	C ₂₅ H ₃₃ N ₂ O ₃ ·2HCl·½H ₂ O	187–189	89	2600	240	29	3
8-OH-DPAT						2.5				
buspirone						50				
spiroperidol							0.06			
phenotamine								12		

^a Data are the mean of three independent determinations (samples in triplicate) each with SEM < 10%. ^b Analyses for C,H,N; results were within ±0.4% of the theoretical values for the formulas given. ^c Formerly published data.¹⁰

racemates. Conversely, considering α₁ receptors the trend was opposite since all the (+)-enantiomers presented lower affinity than their (–)-isomers and racemates. Consequently, because of the opposite trend of the two enantiomers regarding D₂ and α₁ receptor affinity, it is impossible to predict the isomer with better selectivity, although (+)-enantiomers (+)-2, (+)-4, and (+)-6 displayed selectivity greater than 250-fold versus both α₁ and D₂. Among these compounds, (+)-4 distinguished itself for its 5-HT_{1A} receptor affinity and selectivity, and for this reason it was submitted to a preliminary and qualitative evaluation of its intrinsic activity using the [³⁵S]GTPγS binding assay method. In this assay the agonist 8-OH-DPAT (10 nM) produced about 100% increment of [³⁵S]GTPγS basal level, whereas compound (+)-4 did not increase the basal level of [³⁵S]GTPγS, displaying the same behavior as WAY-100635¹⁷ in this experimental condition. Compound (–)-4 underwent the same biochemical evaluation showing the same behavior as the (+)-isomer. These results indicated that the two enantiomers of compound 4 did not display 5-HT_{1A} agonistic property in this preliminary binding assay in the range of 10^{–4}–10^{–10} M.

Experimental Section

Chemistry. Column chromatography was performed with 1:30 ICN silica gel 60 Å (63–200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C,H,N) were performed on a Carlo Erba model 1106 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. Optical rotation was measured with Perkin-Elmer 241 MC polarimeter. CD curves were

measured in MeOH with a Cary 61 dichrograph using a 10-mm cell. High-performance liquid chromatography (HPLC) was performed on a Waters Associates liquid chromatograph model 600 equipped with a U6K model injector and a 481 model variable wavelength detector (λ = 254 nm). ¹H NMR spectra were recorded, using CDCl₃ as solvent, on a Varian EM-390 (TMS as internal standard) or a Bruker AM 300 WB instrument (where indicated 300 MHz); all values are reported in ppm (δ). Recording of mass spectra was done on a HP 5995C gas chromatograph/mass spectrometer, electron impact 70 eV, equipped with a HP59970A workstation; only significant *m/z* peaks, with their percent relative intensity in parentheses, are herein reported. All spectra were in accordance with the assigned structures. Spectral properties of compounds (S)- and (R)-2–7, (S)- and (R)-10, (S)- and (R)-16, and (S)- and (R)-20 were fully consistent with those already reported for their racemic mixture.^{9,10,12}

(S)-(-)-5-Methoxy-1,2,3,4-tetrahydro-1-naphthalene-acetonitrile [(S)-(-)-9]. Methanesulfonyl chloride (1.3 mL, 16.8 mmol) was added to a cooled solution of (R)-(+)-5-methoxy-1,2,3,4-tetrahydro-1-naphthalenemethanol [(R)-(+)-8] (1.52 g, 7.9 mmol) and triethylamine (4.2 mL, 30.1 mmol) in CH₂Cl₂. The resulting mixture was stirred for 1 h at room temperature; then it was washed with ice-cold H₂O, with 3 N HCl, and finally with saturated aqueous NaHCO₃. The separated organic layer was dried over Na₂SO₄ and the solvent evaporated under reduced pressure. The crude residue was chromatographed (CH₂Cl₂ as eluent) to give 1.84 g (86% yield) of mesylate derivative: [α]_D²⁰ = +11.9° (c = 3.4, CHCl₃). The mesylate (1.82 g, 6.7 mmol) was added to a mixture of NaCN (0.96 g, 19.6 mmol) and 20 mL of 90% ethanol. The mixture was refluxed for 7 h and then concentrated under reduced pressure. The residue was partitioned between 10% aqueous Na₂CO₃ and CH₂Cl₂. The separated organic layer was dried over Na₂SO₄ and concentrated in vacuo. The desired compound was chromatographed with petroleum ether/CH₂Cl₂, 1:1, as eluent (0.81 g, 60% yield): [α]_D²⁰ = –21.0° (c = 1.95, CHCl₃);

¹H NMR 1.65–2.05 (m, 4H, *endo* CH₂), 2.50–2.80 (m, 4H, CH₂-CN, benzyl CH₂), 3.05–3.30 (m, 1H, CH), 3.80 (s, 3H, CH₃), 6.65–7.30 (m, 3H, aromatic); GC/MS *m/z* 202 (M⁺ + 1, 4), 201 (M⁺, 30), 161 (100), 160 (21), 115 (22).

(S)-(+)-5-Methoxy-1,2,3,4-tetrahydro-1-naphthalene-acetic Acid [(S)-(+)-10]. A mixture of nitrile (S)-(-)-9 (0.80 g, 4.0 mmol) and 40 mL of 50% H₂SO₄ was refluxed for 4 h. Then the cooled mixture was extracted with CHCl₃ (2 × 40 mL). The collected organic layers were dried over Na₂SO₄ and concentrated under reduced pressure. The crude residue was chromatographed (CHCl₃ as eluent) to give 0.18 g of the expected acid (20% yield). HPLC analyses on a Daicel Chiralcel OD column using *n*-hexane/2-propanol/trifluoroacetic acid (98:2:0.1), flow rate 1.0 mL/min, indicated that the sample was 70% ee of the acid (S)-10. This acid displayed the same retention time of a pure sample of (-)-10 obtained by fractional crystallization of its diastereomeric salt with (S)-(-)-1-phenylethylamine.

(R)- and (S)-4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]-1-aryl piperazine Derivatives 2–4. General Procedure. A stirred suspension of the appropriate mesylate (2.0 mmol), 1-aryl piperazine (4.0 mmol), and K₂CO₃ (2.0 mmol) in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H₂O was added to the residue. The aqueous phase was extracted twice with CH₂Cl₂. The collected organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. The crude residue was chromatographed (CH₂Cl₂/MeOH, 19:1, as eluent) to yield target compounds as pale-yellow oils. All compounds displayed ee ≥ 98% (HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine, 9:1:0.1, as the mobile phase, flow rate 0.8 mL/min).

(S)-(+)-4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]-1-phenyl piperazine [(S)-(+)-2]. Starting from (S)-(+)-3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl methanesulfonate [(S)-(+)-11] and 1-phenyl piperazine, the title compound was obtained in 92% yield: [α]_D²⁰ = +8.3° (c = 1.3, CHCl₃).

(S)-(+)-1-(2-Methoxyphenyl)-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl] piperazine [(S)-(+)-3]. Starting from mesylate (S)-(+)-11 and 1-(2-methoxyphenyl) piperazine, the title compound was obtained in 85% yield: [α]_D²⁰ = +5.8° (c = 1.6, CHCl₃).

(S)-(+)-4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]-1-(2-pyridyl) piperazine [(S)-(+)-4]. Starting from mesylate (S)-(+)-11 and 1-(2-pyridyl) piperazine, the title compound was obtained in 80% yield: [α]_D²⁰ = +9.6° (c = 1.5, CHCl₃).

(R)-(-)-4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]-1-phenyl piperazine [(R)-(-)-2]. Starting from (R)-(-)-3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl methanesulfonate [(R)-(-)-11] and 1-phenyl piperazine, the title compound was obtained in 82% yield: [α]_D²⁰ = -8.6° (c = 1.2, CHCl₃).

(R)-(-)-1-(2-Methoxyphenyl)-4-[3-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl] piperazine [(R)-(-)-3]. Starting from mesylate (R)-(-)-11 and 1-(2-methoxyphenyl) piperazine, the title compound was obtained in 91% yield: [α]_D²⁰ = -5.5° (c = 1.85, CHCl₃).

(R)-(-)-4-[3-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-*n*-propyl]-1-(2-pyridyl) piperazine [(R)-(-)-4]. Starting from mesylate (R)-(-)-11 and 1-(2-pyridyl) piperazine, the title compound was obtained in 79% yield: [α]_D²⁰ = -9.7° (c = 1.5, CHCl₃).

(S)-(-)-N-(1,2,3,4-Tetrahydronaphthalen-1-yl) chloroacetamide [(S)-(-)-13]. Chloroacetyl chloride (0.23 mL, 2.9 mmol) in CH₂Cl₂ was added dropwise to a stirred mixture of the amine (S)-(+)-12 (0.33 g, 2.2 mmol) and NaHCO₃ (0.25 g, 3.0 mmol) in CH₂Cl₂ under cooling. The reaction mixture was stirred for 1 h, and then it was washed with brine. The separated organic phase was dried over Na₂SO₄ and evaporated under reduced pressure. The crude residue was chromatographed with CH₂Cl₂/ethyl acetate, 4:1, to give compound (S)-(-)-13 as a white semisolid in nearly quantitative yield:

[α]_D²⁰ = -77.2° (c = 1.0, CH₂OH); ¹H NMR 1.75–2.15 (m, 4H, *endo* CH₂CH₂), 2.65–3.00 (m, 2H, benzyl CH₂), 4.05 (s, 2H, CH₂Cl), 5.00–5.30 (m, 1H, CH), 7.00–7.30 (m, 4H, aromatic), 6.80 (br s, 1H, NH); GC/MS *m/z* 225 (M⁺ + 2, 1), 224 (M⁺ + 1, 1), 223 (M⁺, 4), 188 (23), 146 (37), 131 (21), 130 (100), 129 (43).

(R)-(+)-N-(1,2,3,4-Tetrahydronaphthalen-1-yl) chloroacetamide [(R)-(+)-13]. As above, starting from (R)-(-)-1,2,3,4-tetrahydronaphthalenamine [(R)-(-)-12] (0.34 g, 2.3 mmol) and chloroacetyl chloride (0.19 mL, 2.4 mmol), compound (R)-(+)-13 was obtained as a white semisolid in 93% yield: [α]_D²⁰ = +71.4° (c = 3.0, CH₂OH). ¹H NMR and GC/MS spectra of this compound were identical to those of its enantiomer.

(S)-(-)-4-(2-Methoxyphenyl)-N-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinoacetamide [(S)-(-)-14]. The derivative (S)-(-)-13 (0.43 g, 1.9 mmol) was refluxed overnight with 1-(2-methoxyphenyl) piperazine (0.73 g, 3.8 mmol) and a slight excess of NaHCO₃ in acetonitrile. After cooling, the mixture was concentrated under reduced pressure, and the residue was taken up with water and extracted with CHCl₃. The chloroform phase was dried over Na₂SO₄, the solvent was evaporated, and the crude residue was chromatographed (CH₂Cl₂/MeOH, 19:1, as eluent) to give (S)-(-)-14 as a white semisolid (0.60 g, 84% yield): [α]_D²⁰ = -13.8° (c = 2.35, CHCl₃). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (9:1:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated that the material was >98% ee: ¹H NMR (300 MHz) 1.68–1.88 and 2.04–2.11 (m, 4H, *endo* CH₂-CH₂), 2.70–2.88 (m, 6H, benzyl CH₂, CH₂N(CH₂)₂), 3.02 (br s, 4H, (CH₂)₂NAr), 3.13 (d, 2H, J = 3.0 Hz, CH₂N(CH₂)₂), 3.84 (s, 3H, CH₃), 5.17–5.24 (m, 1H, CH), 6.82–7.25 (m, 8H, aromatic), 7.42 (br d, 1H, NH, D₂O exchanged); GC/MS *m/z* 381 (M⁺ + 2, 1), 380 (M⁺ + 1, 7), 379 (M⁺, 26), 205 (100), 190 (26).

(R)-(+)-4-(2-Methoxyphenyl)-N-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinoacetamide [(R)-(+)-14]. Title compound was prepared as above starting from derivative (R)-(+)-13 (0.41 g, 1.8 mmol) and 1-(2-methoxyphenyl) piperazine (0.77 g, 4.0 mmol) in 84% yield: [α]_D²⁰ = +13.3° (c = 2.2, CHCl₃). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (9:1:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated the material was >98% ee. ¹H NMR and GC/MS spectra of this compound were identical to those of its enantiomer.

Resolution of 5-Methoxy-1,2,3,4-tetrahydro-1-naphthalenamine [(±)-15]. To a solution of racemic amine (±)-15 (7.62 g, 43.0 mmol) and (S)-(+)-mandelic acid (3.27 g, 21.5 mmol) in MeOH (60 mL) was added diethyl ether (30 mL). The solution was allowed to cool in the refrigerator until crystallization occurred. The crystals were filtered and recrystallized from MeOH/diethyl ether three times to afford 2.80 g of (+)-mandelate salt. The salt was dissolved in water, the ice-cooled solution made basic with 2 N NaOH, and the resulting mixture extracted with CH₂Cl₂ (40 mL). The organic phase was dried over Na₂SO₄, and the solvent was evaporated to dryness to give 1.48 g of pure (+)-15: [α]_D²⁰ = +15.7° (c = 12.8, MeOH). HPLC analyses on a Daicel Chiralcel OD-R column using MeOH/H₂O (9:1) as the mobile phase (flow rate 0.4 mL/min) indicated the material was >98% ee. The collected mother liquors containing the (-)-isomer of 15 were evaporated, dissolved in 40 mL of MeOH, and treated with (R)-(-)-mandelic acid (3.27 g, 21.5 mmol). The solution was treated with 30 mL of diethyl ether and kept in the refrigerator overnight. The crystals were filtered off and recrystallized from MeOH/diethyl ether three times to afford 2.50 g of (-)-mandelate salt which was treated as described above for its (+)-isomer to give 1.25 g of amine (-)-15: [α]_D²⁰ = -11.0° (c = 10.6, MeOH). HPLC analyses in the above condition indicated that the material was >98% ee.

(S)-(-)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl) bromoacetamide [(S)-(-)-16]. This compound was obtained as a semisolid in nearly quantitative yield from the amine (S)-(+)-15 (0.18 g, 1.1 mmol) and bromoacetyl chloride

(0.12 mL, 1.4 mmol) using the same reported procedure for the synthesis of compound (S)-(-)-13: $[\alpha]_D^{20} = -27.3^\circ$ ($c = 1.0$, CHCl_3).

(R)-(+)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)bromoacetamide [(R)-(+)-16]. As above, starting from amine (R)-(-)-15, compound (R)-(+)-16 was obtained in the same yield as a white semisolid: $[\alpha]_D^{20} = +31.2^\circ$ ($c = 1.0$, CHCl_3).

(S)-(-)-4-(2-Methoxyphenyl)-N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinoacetamide [(S)-(-)-17]. Using the procedure reported for the synthesis of compound (S)-(-)-14, title compound was obtained from (S)-(-)-16 and 1-(2-methoxyphenyl)piperazine in 84% yield as a white semisolid: $[\alpha]_D^{20} = -24.8^\circ$ ($c = 1.0$, CHCl_3). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (9:1:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated the material was >98% ee.

(R)-(+)-4-(2-Methoxyphenyl)-N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinoacetamide [(R)-(+)-17]. As above, title compound was prepared from (R)-(+)-16 and 1-(2-methoxyphenyl)piperazine in 85% yield as a white semisolid: $[\alpha]_D^{20} = +24.1^\circ$ ($c = 1.0$, CHCl_3). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (9:1:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated the material was >98% ee.

(R)- and (S)-1-(2-Methoxyphenyl)-4-[N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)aminoalkyl]piperazine Derivatives. General Procedure. A stirred suspension of the appropriate chloroalkyl derivative (4.4 mmol), the appropriate amine (4.0 mmol), and a slight excess of K_2CO_3 in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H_2O was added to the residue. The aqueous phase was extracted twice with CH_2Cl_2 . The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 19:1, as eluent) to yield pure target compound as pale-yellow oil. All compounds displayed ee $\geq 98\%$ (HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/2-propanol/diethylamine, 8:2:0.1, as the mobile phase, flow rate 0.8 mL/min).

(S)-(-)-1-(2-Methoxyphenyl)-4-[N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-2-aminoethyl]piperazine [(S)-(-)-5]. Starting from the amine (S)-(+)-15 and 4-(2-chloroethyl)-1-(2-methoxyphenyl)piperazine (18),¹⁸ title compound was obtained in 34% yield: $[\alpha]_D^{20} = -10.6^\circ$ ($c = 1.0$, CHCl_3).

(R)-(+)-1-(2-Methoxyphenyl)-4-[N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-2-aminoethyl]piperazine [(R)-(+)-5]. Starting from the amine (R)-(-)-15 and derivative 18, title compound was obtained in 34% yield: $[\alpha]_D^{20} = +14.1^\circ$ ($c = 1.0$, CHCl_3).

(S)-(-)-1-(2-Methoxyphenyl)-4-[N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-3-amino-*n*-propyl]piperazine [(S)-(-)-6]. Starting from the amine (S)-(+)-15 and 4-(3-chloropropyl)-1-(2-methoxyphenyl)piperazine (19),¹⁸ title compound was obtained in 41% yield: $[\alpha]_D^{20} = -12.0^\circ$ ($c = 1.1$, CHCl_3).

(R)-(+)-1-(2-Methoxyphenyl)-4-[N-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-3-amino-*n*-propyl]piperazine [(R)-(+)-6]. Starting from the amine (R)-(-)-15 and compound 19, title compound was obtained in 35% yield: $[\alpha]_D^{20} = +6.8^\circ$ ($c = 1.0$, CHCl_3).

(S)-(-)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-3-chloropropanamide [(S)-(-)-20]. As for compound (S)-(-)-13, title compound was prepared from the amine (S)-(+)-15 (2.50 g, 14.1 mmol) and 3-chloropropanoyl chloride (1.7 mL, 18.3 mmol) in nearly quantitative yield: $[\alpha]_D^{20} = -77.9^\circ$ ($c = 1.0$, CHCl_3).

(R)-(+)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-3-chloropropanamide [(R)-(+)-20]. As above, starting from amine (R)-(-)-15, title compound was obtained in 81% yield: $[\alpha]_D^{20} = +77.7^\circ$ ($c = 1.0$, CHCl_3).

(S)-(-)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-(2-methoxyphenyl)-1-piperazinoopropanamide [(S)-(-)-7]. This compound was obtained from (S)-(-)-20 (0.74 g,

2.8 mmol) and 1-(2-methoxyphenyl)piperazine (1.08 g, 5.6 mmol) as a white semisolid (1.00 g, 86% yield), as for compound (S)-(-)-14: $[\alpha]_D^{20} = -60.0^\circ$ ($c = 1.0$, CHCl_3). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (8:2:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated the material was >98% ee.

(R)-(+)-N-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-(2-methoxyphenyl)-1-piperazinoopropanamide [(R)-(+)-7]. As above, starting from the amide (R)-(+)-20, compound (R)-(+)-7 was obtained in 81% yield: $[\alpha]_D^{20} = +58.5^\circ$ ($c = 1.0$, CHCl_3). HPLC analysis on a Daicel Chiralcel OD using *n*-hexane/ethanol/diethylamine (8:2:0.1) as the mobile phase (flow rate 0.8 mL/min) indicated the material was >98% ee.

Hydrochloride Salts. General Procedure. The hydrochloride salts were prepared by adding an HCl ethereal solution to a methanolic solution of amine. All salts were recrystallized from $\text{MeOH}/\text{Et}_2\text{O}$ except for compounds (R)-(+)-6 and (S)-(-)-6 which were recrystallized from $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$. They were obtained as white to sand-yellow crystals or crystalline powders. Crystallization formulas and melting points are listed in Table 1.

Pharmacological Methods. 1. 5-HT_{1A} Serotonergic Binding Assay. Standard receptor binding methods were used to label 5-HT_{1A} receptors using [³H]-8-OH-DPAT as previously described.²⁰

2. D₂ Dopaminergic Binding Assay. Standard receptor binding methods were used to label D₂ receptors using [³H]-spiperidol as previously described.²⁰

3. α_1 Adrenergic Binding Assay. Standard receptor binding methods were used to label α_1 receptors using [³H]-prazosin as previously described.²⁰

4. [³⁵S]GTP γ S Binding Assay. This assay was performed according to Lazareno et al.²¹ with minor modifications. Each assay tube contained in a total volume of 1 mL: incubation buffer (50 mM Tris-HCl, 5 mM MgCl_2 , 100 mM NaCl, GDP 2 mM, pH 7.4), 8-OH-DPAT or compounds (+)-4 and (-)-4 or WAY-100635 with different concentrations (10^{-6} – 10^{-10} M), 0.1 nM [³⁵S]GTP γ S, and 1 unit of G-protein-coupled serotonergic 5-HT_{1A} receptor (human clone expressed in CHO cells; manufactured for NEN Life Science Products by BioSignal Inc., Montreal, Canada). The reaction was carried out for 30 min at 27 °C. The solution was filtered on Whatman glass fiber GF/A filters and washed two times with 2 mL of 50 mM Tris-HCl, pH 7.4. The nonspecific binding was determined with 10 mM GTP γ S. The stimulation by agonist was calculated as the percentage increase above basal level (dpm agonist – dpm no agonist/dpm no agonist $\times$ 100).

Acknowledgment. This study was supported by Research Grant No. 9703028183-022 from Università degli Studi di Bari and MURST (Italy) for the scientific program in CO7X Field – 1998–2000: "Design, synthesis and biological evaluation of new drugs: SAR studies on serotonergic agents with arylpiperazine structure".

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JM980420N