259
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SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

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Re: P1.-**ASTRAZENECA AB**.-Solicitud de Patente de Invención para "**NUEVAS FORMAS CRISTALINAS DE MELAGATRAN**".-Clase **A.61**.- Expediente número **00-048.529**.-

1. Yo, **ALICIA LLOREDA RICAURTE**, mayor, vecina de ésta ciudad, identificada con la cédula de ciudadanía número 39'690.713 de Usaquén, Representante Legal en Bogotá para todos los asuntos relativos a la Propiedad Industrial de la sociedad **ASTRAZENECA AB**, organizada y existente de conformidad con las leyes de Suecia, domiciliada en Södertälje, Suecia, atentamente manifiesto a usted que interpongo Recurso de Reposición y en subsidio de Apelación contra la Resolución número **34297**, dictada por el señor Superintendente de Industria y Comercio el 21 de Diciembre de 2005 y notificada por Edicto fijado el 11 de Enero de 2006, por medio de la cual se niega la presente solicitud de patente.

2. Fundamento mi recurso en los siguientes hechos:

(1) De la lectura de la Resolución número **34297** mediante la cual se niega el privilegio de patente para las reivindicaciones 1 a 17 de la presente solicitud, es evidente que de ninguna manera se consideró la importancia, alcance y objeto de la presente invención en su verdadera dimensión, como tampoco el aporte técnico que representan las novedosas formas cristalinas de melagatrán, caracterizadas por los patrones de rayos X ilustrados en las figuras que acompañan al capítulo descriptivo y reseñados en el capítulo reivindicatorio. De la misma manera, en la presente solicitud se describen por primera vez procesos para la preparación de dichas formas cristalinas de melagatrán, caracterizados por las etapas y condiciones prácticas que permiten su obtención.

La Resolución que se impugna se limita a desvirtuar el nivel inventivo de la invención argumentando que se trata de una derivación evidente del estado de la técnica, toda vez una persona versada en la materia, supuestamente está en capacidad de obtener dichas formas cristalinas de melagatrán de manera obvia a partir de las enseñanzas del estado de la técnica citado, que revela la forma amorfa de melagatrán y aspectos generales sobre el fenómeno del polimorfismo y los métodos de cristalización, lo que en combinación con su conocimiento lo llevaría a obtener las formas cristalinas reivindicadas. Adicionalmente, se afirma en la mencionada resolución que las formas cristalinas de melagatrán no

representan ninguna ventaja frente a la forma amorfa conocida en el estado de la técnica.

Sin embargo, atentamente me permito dirigir la atención de ese Despacho a los argumentos que se expondrán a continuación, mediante los cuales se demuestra que la presente invención sí es en efecto inventiva, toda vez que ni las formas cristalinas reivindicadas, ni sus propiedades, estructuras, métodos de obtención y ventajas, pueden ser predichos por una persona versada en la materia por ningún medio y que la simple existencia de dichos polimorfos cristalinos de melagatrán resulta sorprendente e inesperada. Además, a lo largo del presente recurso se demostrará que el examen de patentabilidad realizado se basó en una interpretación errada y subjetiva del objeto de la solicitud, en la falta de análisis de los argumentos y pruebas técnicas presentados por el solicitante a los requerimientos anteriores de ese Despacho y en una aplicación equivocada de los conceptos involucrados en el análisis del requisito de nivel inventivo.

(2) Con el fin de demostrar no solo que la presente solicitud sí cumple con el requisito de altura inventiva, sino además que el examen de patentabilidad realizado por el Examinador corresponde a una interpretación errada y subjetiva tanto de la invención como de los parámetros a tener en cuenta cuando se analiza este requisito, de la manera más atenta me permito dirigir la atención de ese Despacho a las consideraciones que sobre esta materia establece la Secretaría General de la Comunidad Andina en su publicación "**Manual Andino de Patentes**".

Es importante resaltar en este punto que tal como lo establece el Acuerdo de Cartagena de 25 de Junio de 1997 en su Artículo 30, son funciones de la Secretaría General de la Comunidad Andina, entre otras: "(...) a) Velar por la aplicación de este acuerdo y por el cumplimiento de las normas que conforman el ordenamiento jurídico de la Comunidad Andina".

En este sentido, es claro que los pronunciamientos emitidos por dicho organismo deben servir de guía para la aplicación e interpretación de las normas andinas y los países miembros no deben dejar de lado estos lineamientos al momento de estudiar una solicitud de patente.

En efecto, para una correcta apreciación del requisito de nivel inventivo en áreas específicas de la tecnología, el **Manual Andino de Patentes** bajo el numeral 10.8.1, titulado Química, establece lo siguiente:

"b) ¿Qué es necesario considerar para que un compuesto tenga nivel inventivo?

Respuesta:

1. Que el compuesto posea una ***estructura inesperada****; o*

2. Que el compuesto ***exhiba un uso o un efecto sorprendente.***

b.1. Estructura inesperada

Es un caso poco frecuente. ***La estructura del nuevo compuesto no hubiera podido ser deducida por el técnico medio en la materia. El examinador no necesita examinar si este compuesto posee o no un uso o efecto sorprendente puesto que la mera estructura ya le confiere nivel inventivo.***

b.2 Efecto o uso no esperado

b.2.1 ***La existencia de un efecto o uso sorprendente*** *es la manera más común de establecer altura inventiva para compuestos nuevos, particularmente cuando tienen estructuras muy próximas a las del estado de la técnica.*

b.2.2. Tipos de efecto sorprendente: (i) completamente diferente de los usos o efectos conocidos de compuestos descritos en el estado de la técnica; y (ii) una mejora sustancial de un efecto de la misma índole que exhiba un compuesto conocido del estado de la técnica más próximo"[1] *(resaltado fuera de texto).*

Teniendo en cuenta lo anteriormente expuesto resulta evidente, como ya se ha manifestado ampliamente en las respuestas a los Conceptos Técnicos presentadas ante ese Despacho, **que de ninguna forma es posible predecir la existencia de una forma cristalina, así como tampoco es posible predecir su estructura, propiedades y método de obtención**.

Así las cosas, es claro que ninguno de los documentos citados, ya sea solos o en combinación, pueden hacer que una persona versada en la materia deduzca la estructura de las nuevas formas cristalinas de

[1] COMUNIDAD ANDINA, Secretaría General, "Manual Andino de Patentes", Junio de 2004, Lima, Perú, p. 83.

melagatrán, toda vez que ni siquiera su existencia podía ser anticipada. Más aún, es un hecho plenamente reconocido en el campo del polimorfismo cristalino que a pesar de que se pueda especular acerca de la existencia de un nuevo polimorfo, no existe un camino o manera que pueda permitir la predicción de dicho polimorfo, mucho menos de sus características estructurales y propiedades químicas y físicas.

Sobre este particular, me permito dirigir la atención de ese Despacho al hecho de que la anterior afirmación fue suficientemente sustentada en la respuesta presentada ante esa Oficina con fecha de 10 de Octubre de 2005, mediante las enseñanzas de personas verdaderamente versadas en la materia que trabajan en el campo de la obtención de polimorfos, tal como el Dr. Desiraju, por lo que resulta claro que **de ninguna manera es posible predecir la existencia de una forma cristalina, así como tampoco es posible predecir su estructura, propiedades y método de obtención, por lo cual el objeto de la invención denegada no puede derivarse a partir de las enseñanzas de los documentos citados, toda vez que ninguno de ellos llega a sugerir o indicar la existencia de polimorfismo en la sustancia melagatrán, y mucho menos pueden llegar a enseñar la forma de preparar tales formas cristalinas**.

En este sentido, el pronunciamiento de la Secretaría General de la Comunidad Andina con respecto a qué es necesario considerar para reconocer la altura inventiva de un compuesto novedoso, es completamente claro: "***La estructura del nuevo compuesto no hubiera podido ser deducida por el técnico medio en la materia. El examinador no necesita examinar si este compuesto posee o no un uso o efecto sorprendente puesto que la mera estructura ya le confiere nivel inventivo***".

En este punto, es necesario resaltar que el Artículo 18 de la Decisión 486 establece claramente que el requisito de nivel inventivo de una solicitud se reconoce "si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia, ni se hubiese derivado de manera evidente del estado de la técnica".

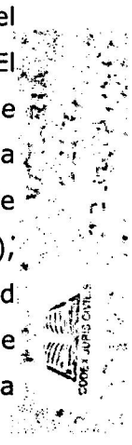
En el presente caso, el solicitante ya ha señalado en forma clara, y con base en las mismas enseñanzas de los documentos citados por el Examinador, que la **estructura de la nueva Forma cristalina de melagatrán** resulta completamente **impredecible e inesperada**, debido a la falta de indicaciones o sugerencias en los documentos pertenecientes al

283 263

estado de la técnica más cercano que evidencien el polimorfismo del melagatrán, y que es precisamente gracias a este concepto de "estructura inesperada o impredecible" que se cumple con el requisito fundamental para reconocer la existencia de nivel inventivo. En vista de lo anterior, la persona versada en la materia **no se encontraría en capacidad de predecir en forma obvia o evidente, a partir de las enseñanzas del estado de la técnica la novedosa estructura cristalina que caracteriza el objeto de la presente invención**.

Por lo tanto, de acuerdo con las enseñanzas del **Manual Andino de Patentes**, la novedosa forma cristalina de melagatrán cumple con el requisito de altura inventiva, exigido por la legislación Andina, debido a que resulta imposible anticipar la existencia y la disposición espacial de dicha estructura a partir de las enseñanzas de los documentos citados como el estado del arte más cercano (**D1** a **D4**), los cuales simplemente se limitan a enseñar el melagatrán amorfo, y algunas composiciones que contienen el mismo.

Sobre este particular, es conveniente resaltar que si bien el solicitante reconoce que el **Manual Andino de Patentes** no es un documento de carácter legal que obligue la actuación de la Autoridad Nacional Competente, también es necesario resaltar que este constituye una guía clara y significativa acerca de la manera como la misma Secretaría General de la Comunidad Andina interpreta y recomienda realizar el análisis de patentabilidad de una solicitud de patente de invención. El valor de esta publicación se ve además soportado por el hecho de que fue preparada por la Oficina Internacional de la Organización Mundial de la Propiedad Intelectual (*OMPI*) en colaboración con la Oficina Europea de Patentes (*OEP*), el Instituto Mexicano de la Propiedad Industrial (*IMPI*), contando además con la participación de las Oficinas de Propiedad Industrial de los Países Miembros de la Comunidad Andina y de funcionarios del Programa de Propiedad Intelectual de la Secretaría General.



(3) Por otra parte, con el fin de demostrar que la presente invención sí posee nivel inventivo, y además que el análisis realizado no fue objetivo, resulta imprescindible resaltar que, dados los comentarios expuestos en la Resolución impugnada, el Examinador carece de información suficiente respecto a la ciencia de los polimorfos (formas cristalinas).

En efecto, si bien diferentes formas cristalinas de compuestos particulares pueden ser conocidas, no existe predictibilidad alguna con respecto a la

existencia de nuevas formas cristalinas de un compuesto conocido. En forma análoga, tampoco existe predictibilidad con respecto al (los) proceso(s) por medio del (los) cual(es) una nueva forma cristalina puede ser preparada. Finalmente, y como consecuencia de lo anterior, no existe tampoco predictibilidad con respecto a las propiedades fisico-químicas y biológicas de las nuevas formas cristalinas de un compuesto conocido.

Por lo tanto, es correcto afirmar que el Examinador comete un error al asegurar una falta de nivel inventivo para las nuevas formas cristalinas reivindicadas en la presente invención, con base en los documentos citados, toda vez que ninguna de estas referencias puede sustentar adecuadamente sus argumentaciones, como se demostrará a continuación.

En efecto, luego de la lectura del *capítulo reivindicatorio de la solicitud* denegada, es posible concluir que el nivel inventivo del objeto allí revelado se desprende de las siguientes nociones, que pertenecen al conocimiento común general de una persona versada en el campo del polimorfismo de cristales en la fecha de prioridad de la solicitud, y que de hecho, se aplican también hoy en día:

(a) A partir de la existencia de una forma amorfa de melagatrán, la existencia de uno o más polimorfos cristalinos de la misma sustancia no puede ser predicha de ninguna manera;

(b) Aún sí una persona versada en la materia ha especulado acerca de la existencia de un polimorfo cristalino de melagatrán, habría sido imposible para esa persona predecir la estructura cristalina de tal polimorfo. Por lo tanto, si bien era conocido que la forma amorfa de melagatrán tiene ciertas propiedades químicas y físicas, era imposible predecir las propiedades químicas y físicas de un polimorfo cristalino de melagatrán hasta obtenerlo, toda vez que estas propiedades dependen de la estructura cristalina del polimorfo;

(c) Aún si la persona versada en la materia, como se ve en el punto anterior, ha especulado acerca de la existencia de un polimorfo cristalino de melagatrán, era imposible predecir el método de preparación de tal polimorfo adicional.

Con el fin de corroborar las anteriores afirmaciones, me permito presentar a ese Despacho la declaración rendida por una experta en la materia como lo es la Doctora Sofía Någård, quien además de poseer una amplia

experiencia en el campo de la cristalografía y el polimorfismo, es una de las inventoras de la solicitud denegada, a partir de la cual se puede apreciar claramente que la falta de predictibilidad es ampliamente reconocida en este campo. En efecto, la Doctora Någård expone en su declaración los siguientes puntos:

(i) "No creo que el arte anterior revele melagatrán en una forma sustancialmente cristalina. Por el contrario, el arte anterior más cercano en mi opinión, WO 94/29336, **revela un método para la obtención de melagatrán amorfo únicamente**".

(ii) "**No creo que sea posible para una persona versada en la materia predecir, con base solamente en la estructura molecular, cuál va a ser el comportamiento de la cristalización de un compuesto**".

(iii) "Adicionalmente, **no creo que exista motivación alguna** en el estado de la técnica **para que la persona versada en la materia aplique las técnicas de cristalización descritas en la presente solicitud al melagatrán**".

(iv) "**La presente invención proporciona además una ventaja inesperada, la cual creo que no podría haber sido predicha en forma alguna antes de la fecha de prioridad de la presente solicitud**".

Como bien lo podrá apreciar ese Despacho, todos los argumentos anteriormente expuestos confirman el nivel inventivo del objeto reivindicado, sobretodo frente al estado de la técnica citado, tomando como base la falta de predectibilidad de la existencia misma de las formas cristalinas reveladas por el solicitante, como es reconocido por una experto en el campo de la solicitud como lo es la Dra. Någård.

Como un sustento adicional a las afirmaciones anteriores, el solicitante se permite citar al Dr. Stephen Byrn, un reconocido experto en la química del estado sólido de fármacos. En un texto guía en el campo escrito por él donde se establece que "**no existen reglas que permitan la predicción de si un compuesto podrá exhibir polimorfismo**"[2] (copia anexa) (resaltado fuera del texto).

[2] Byrn, S. R., "*Solid-State Chemistry of Drugs*", Academic Press, Nueva York, 1982, p 7.

En otro artículo escrito por el Dr. Byrn et al., se establece que "quizás el principal reto en el manejo del fenómeno de las múltiples formas sólidas de los fármacos es nuestra incapacidad para predecir cuantas formas pueden ser esperadas en un caso dado"[3] (copia anexa).

Adicionalmente, y como complemento a las anteriores afirmaciones, de la manera más atenta me permito dirigir la atención de ese Despacho al artículo "*Disappearing Polymorphs*", escrito por el Dr. Dunitz y el Dr. Bernstein, ambos expertos ampliamente conocidos en el campo de la cristalografía química. En la página 194 de este documento, los autores señalan que "*algunos compuestos muy ampliamente estudiados no han mostrado evidencia de comportamiento polimórfico, aún cuando han sido cristalizados y manipulados por muchos años bajo una variedad muy extensa de condiciones; el naftaleno es un ejemplo que inmediatamente viene a la mente*"[4] (copia anexa). Se ha conocido por años que el naftaleno existe en una forma cristalina sencilla; y basados en la creencia de que otras formas cristalinas del naftaleno podrían existir, repetidos intentos han sido realizados con el fin de preparar esas otras formas cristalinas, pero ninguno ha sido exitoso.

En este orden de ideas, el artículo "*A New Polimorphic Modification of Tetrathiafulvalene*"[5] (copia anexa) enseña como el conocimiento general en el campo de los polimorfos cristalinos confirma la imposibilidad de predicción sobre la existencia de formas adicionales para un compuesto que muestra polimorfismo. De hecho, este documento revela que por 20 años solamente un polimorfo del compuesto tetratiafulvaleno era conocido a pesar del estudio extenso de este compuesto. Solo fue después de 20 años de estudio que un segundo polimorfo fue accidental e inesperadamente encontrado. No importa que tanto esfuerzo se haya colocado en el proyecto de obtener un polimorfo adicional del compuesto, tal resultado fue obtenido por casualidad y para sorpresa de los mismos investigadores.

Por lo tanto, es claro que **la literatura y los expertos en el campo enseñan y confirman que para la fecha de prioridad de la solicitud no existía una manera de predecir que las formas cristalinas**

[3] Byrn S. R., et. al., "*Solid-State Pharmacuetical Chemistry*", Chem. Mater., Vol. 6, 1148-1158 (1994), p 1148

[4] Dunitz J. D. et. al., "*Disappearing Polymorphs*", Acc. Chem. Res., 28, 193-200 (1995), p 194

[5] Ellern A., et. al. "A New Polymorphic Modification of Tetrathiafulvalene", Chem. Mater., 6, 1378-1385 (1994).

(hidrato, anhidrato e iso-propanolato/hidrato) de melagatrán reivindicadas podrían existir.

En consecuencia, me permito recalcar que la falta de posibilidad de predicción de la simple existencia de los polimorfos reivindicados confiere nivel inventivo a la materia revelada por el solicitante, **sin importar como sus propiedades farmacológicas se puedan comparar con aquellas de la forma amorfa ya conocida**.

(4) Además de la falta de posibilidad de predicción sobre la existencia de una forma polimórfica de un compuesto químico, también se reconoce la falta de posibilidad de predicción de las propiedades de esa posible nueva forma cristalina. En otras palabras, el conocimiento de las propiedades de la forma amorfa ya conocida de un compuesto dado no permite predecir las propiedades de una forma polimórfica nueva del mismo compuesto.

De manera más específica, el hecho de que un compuesto dado exhiba polimorfismo y que uno de sus polimorfos presente ciertas propiedades químicas o físicas farmacéuticamente adecuadas, de ninguna manera implica que un polimorfo adicional del mismo compuesto también poseerá propiedades similares.

En el presente caso, es completamente claro que el conocimiento general en el campo del polimorfismo cristalino, tal como se evidencia mediante un número notable de revelaciones específicas de la literatura, sugiere que incluso las diferencias polimórficas más pequeñas no pueden ser omitidas para propósitos de asegurar o predecir las propiedades de un polimorfo dado.

Nuevamente, el solicitante se remite a un experto en el campo del polimorfismo cristalino con el fin de sustentar las nociones fundamentales anteriores. En el numeral 8 de su Declaración, la Dra. Någård establece: "***La presente invención proporciona además una ventaja inesperada, la cual creo que no podría haber sido predicha en forma alguna antes de la fecha de prioridad de la presente solicitud***". En este sentido, la experta manifiesta en el numeral 10 de dicho documento que "Si bien, puede ser que espere que una o más formas cristalinas de una sustancia sean más estables desde un punto de vista **físico** que la correspondiente forma amorfa, **este significativo incremento en la estabilidad química para la forma cristalina se convierte en una sorpresa para mí y no podría haber sido predicha**

de ninguna forma a la luz de WO 94/29336 o cualquier otro arte anterior del cual esté enterada".

En igual sentido, el Dr. Byrn establece en su libro ya citado que "*debido a que tienen diferentes estructuras cristalinas, los polimorfos tienen diferentes propiedades químicas y físicas. Polimorfos de la misma sustancia muestran diferentes puntos de fusión, diferentes reactividades químicas, diferentes velocidades de disolución y diferentes biodisponibilidades*"[6].

De la misma manera, en el artículo "Pharmaceutical Solids: *A Strategic Approach to Regulatory Considerations*" (copia anexa), también escrito por el Dr. Byrn, se afirma que "*...las propiedades físicas variables de diferentes fármacos con diferentes polimorfos son reportadas en la literatura. Por ejemplo, los perfiles de disolución de los polimorfos de cloramfenicol son significativamente diferentes... Por equilibrio, es importante manifestar que también hay casos donde existen polimorfos pero tienen virtualmente propiedades de disolución idénticas*"[7]. En otras palabras, es conocido en el campo de los polimorfos cristalinos que diferentes formas del mismo compuesto no tienen necesariamente las mismas propiedades; además, no existe manera de predecir cuales formas compartirán propiedades y cuales no.

En resumen, es evidente que **las formas cristalinas reveladas por el solicitante son inesperada y químicamente más estables que la forma amorfa**, lo que resulta ser verdaderamente ventajoso, tal como lo manifiesta la misma experta en el numeral 11 de su declaración, en el cual establece que "Claramente, esta estabilidad química mejorada, que se observa en una forma cristalina de melagatrán proporciona una **significativa e inesperada ventaja desde el punto de vista de facilidad de manipulación, fabricación de composiciones farmacéuticas que incluyan la sustancia fármaco, y subsecuente almacenamiento en forma sólida** (por ejemplo, en la forma de tabletas orales) **antes de y durante la distribución, en farmacias y de hecho en los hogares de los pacientes**".

Adicionalmente, en vista de la gran cantidad de variables inesperadas e impredecibles involucradas en los procesos de formación de polimorfos cristalinos, que no corresponden al mismo proceso de "re-cristalización" como parece comprender el Examinador y de lo cual nos ocuparemos más

[6] Byrn, S. R., "*Solid-State Chemistry of Drugs*", Academic Press, Nueva York, 1982, p 7.
[7] Byrn S. R., et. al., "*Pharmaceutical Solids*", Pharm. Res., 12, 945-954 (1995), p 947.

adelante, y considerando que, en comparación, tales variables son muy favorables con respecto a las formas cristalinas reivindicadas, se considera que **dichas formas representan un alternativa no obvia y realmente ventajosa frente a la forma amorfa de melagatrán**, de tal manera que las reivindicaciones 1 a 7 de la presente invención cubren un objeto claramente inventivo.

Por lo tanto, se considera que tanto las propiedades superiores del hidrato, anhidrato e iso-propanolato/hidrato de melagatrán revelados, así como su naturaleza inesperada, han sido plenamente explicadas y demostradas, por lo que en opinión del solicitante el nivel inventivo de la solicitud que se estudia debe ser reconocido.

(5) En conexión con lo anterior, me permito dirigir ahora la atención de ese Despacho a la afirmación expuesta en la Resolución impugnada según la cual "*No se aprecia, entonces, nivel inventivo, porque el compuesto presenta las mismas propiedades anticoagulantes, inhibidoras de trombina* ***y no presenta propiedades sorprendentes o inesperadas que resuelvan algún problema en la técnica****, cuando se compara con el compuestos del estado del arte*" (resaltado fuera de texto).

Como respuesta a esta afirmación, aclaré suficientemente en mi memorial de 4 de Octubre de 2005, que no se había tenido en cuenta el problema técnico propuesto en la presente invención, ni la solución dada al mismo. En efecto, se llamó la atención a las páginas 1 y 2 de la descripción, en las que se enseña que existen problemas técnicos relacionados con la estabilidad química, estabilidad en estado sólido, y con la vida de almacenamiento de los ingredientes activos; y que, así mismo, los materiales amorfos (como el melagatrán revelado en los documentos **D1**, **D2** y **D3** del arte anterior citado), presentan problemas significativos debido a que son típicamente difíciles de manipular, suelen proporcionar una solubilidad no confiable, y con frecuencia se ha encontrado que son inestables y químicamente impuros. Al respecto, vale la pena resaltar una vez más que tales problemas son solucionados por la forma cristalina de melagatrán reivindicada en la presente solicitud.

Adicionalmente, y con el fin de demostrar que la forma cristalina de melagatrán reivindicada sí presenta **propiedades sorprendentes e inesperadas que brindan una solución efectiva a los problemas de estabilidad** del principio activo en las formulaciones medicamentosas, el solicitante presentó a ese Despacho con su memorial de 4 de Octubre de 2005 un estudio denominado como anexo A, en el cual se podía apreciar

la mayor estabilidad química presentada por **la forma cristalina de melagatrán** frente a la forma amorfa enseñada en el estado del arte (documentos **D1**, **D2** y **D3**).

En dicho estudio, el solicitante comparó la forma cristalina de los ejemplos de la presente invención, y la forma amorfa de melagatrán, tal y como es revelada en la solicitud internacional de patente **WO 94/29336**, la cual representa entre los cuatro documentos citados, el arte anterior más cercano, toda vez que trata sobre la misma sustancia química.

Las dos formas de melagatrán sobre las que se llevó a cabo el estudio, fueron almacenadas a 50°C/Humedad Relativa (HR) ambiente, y adicionalmente se llevo a cabo otro ensayo a 40°C/75% de HR. En ambos ensayos se determinó la cantidad de impurezas presentes con el transcurso del tiempo, mediante cromatografía líquida. Los resultados son presentados como la cantidad total (porcentaje) de melagatrán comparado con el área total del cromatograma en tiempos de almacenamiento de hasta seis semanas.

Los resultados obtenidos claramente **demuestran la estabilidad química significativamente mejorada** de la forma cristalina de melagatrán probada, frente a un material amorfo de la misma sustancia. Adicionalmente, se aprecia claramente que después de tres semanas, **el material amorfo presenta un incremento de 2 a 3 veces en la cantidad de impurezas químicas, mientras que para la forma cristalina hidrato, tan solo se aprecian aumentos insignificantes** después de 6 semanas de almacenamiento, sin importar que sea a 50°C y humedad relativa del ambiente, o a 45°C y 75% de humedad relativa.

En consecuencia, el estudio de estabilidad aportado por el solicitante demuestra que, efectivamente, sí se presentan ventajas sorprendentes e inesperadas que no se habrían podido predecir o anticipar a partir de las enseñanzas generales de los documentos citados por el Examinador, toda vez que ninguno de dichos documentos sugiere la existencia de polimorfismo para el principio activo melagatrán, y mucho menos son capaces de indicar un proceso o método adecuado para llevar a cabo la fabricación de dichos cristales. Por consiguiente, la presente invención reivindicada es completamente patentable.

No obstante el hecho de que el solicitante sí haya aportado una prueba experimental que demuestra la sorprendente estabilidad química de las

291 271

formas cristalinas reivindicadas, en la Resolución impugnada se contesta a este hecho manifestando simplemente que "el hecho de que la forma cristalina presente una mejor estabilidad mejorada, en comparación con el compuesto amorfo, **no es sorprendente, porque en general los cristales tienen más pureza, estabilidad y solubilidad que la forma amorfa**" (resaltado fuera de texto).

Una vez más, este tipo de afirmaciones demuestran el desconocimiento que el Examinador asignado al caso tiene acerca del campo de la cristalografía y el fenómeno del polimorfismo. En efecto, tal como ya se ha indicado en este mismo escrito, **no existe capacidad de predicción frente a las propiedades que una nueva forma cristalina pueda presentar, tal como lo afirman diversos expertos en el campo y se demuestra mediante la literatura aportada**, por lo que decir que en "general" los polimorfos cristalinos tienen, por ejemplo, "más pureza, estabilidad y solubilidad que la forma amorfa" constituye una simplificación que carece de sustento técnico que no puede ser considerada como una afirmación válida para desvirtuar los resultados experimentales, **específicos para el presente caso**, que fueron adjuntados por el solicitante y que demuestran el efecto sorprendente de los compuestos reivindicados.

En este sentido, la Secretaria de la Comunidad Andina se ha pronunciado sobre este aspecto en el ya mencionado **Manual Andino**, estableciendo que:

"b.2. Efecto o uso no esperado

*b.2.1 La existencia de un **efecto o uso sorprendente** es la manera más común de establecer altura inventiva para compuestos nuevos, particularmente cuando tienen estructuras muy próximas a las del estado de la técnica.*

*b.2.2 Tipos de efecto sorprendente: (i) completamente diferente de los usos o efectos conocidos de compuestos descritos en el estado de la técnica; y (ii) **una mejora sustancial** de un efecto de la misma índole que exhiba un compuesto conocido del estado de la técnica más próximo"*[8].

[8] COMUNIDAD ANDINA, Secretaría General, "**Manual Andino de Patentes**", Junio de 2004, Lima, Perú, p 83.

292272

Sobre este particular, me permito dirigir nuevamente la atención de ese Despacho a la declaración rendida por una experta en la materia como lo es la Dra. Någård, quien en los numerales 9 a 11 de su experticio manifiesta lo siguiente:

"(9) **Creo que los datos experimentales proporcionados por medio del Anexo A demuestran claramente una ventaja inesperada** al poner de manifiesto la estabilidad química mejorada de una forma cristalina de melagatrán descrita en la presente solicitud sobre la forma amorfa obtenida por medio de **WO 94/29336**.

(10) Si bien, puede ser que espere que una o más formas cristalinas de una sustancia sean más estables desde un punto de vista **físico** que la correspondiente forma amorfa, **este significativo incremento en la estabilidad química para la forma cristalina se convierte en una sorpresa para mí y no podría haber sido predicha de ninguna forma as la luz de WO 94/29336 o cualquier otro arte anterior del cual esté enterada**.

(11) Claramente, esta estabilidad química mejorada, que se observa en una forma cristalina de melagatrán proporciona una **significativa e inesperada ventaja desde el punto de vista de facilidad de manipulación, fabricación de composiciones farmacéuticas que incluyan la sustancia fármaco, y subsecuente almacenamiento en forma sólida** (por ejemplo, en la forma de tabletas orales) **antes de y durante la distribución, en farmacias y de hecho en los hogares de los pacientes**".

De acuerdo con lo anterior, ese Despacho podrá notar claramente que en la opinión de una verdadera experta en la materia, la presente invención sí enseña un **efecto sorprendente** (ver numeral b.2.1 anteriormente transcrito), **como lo es la estabilidad química mejorada totalmente inesperada**, en donde este efecto sorprendente constituye una **mejora sustancial** (ver numeral b.2.2) de un efecto de la misma índole, o en palabras de la propia experta "una **significativa e inesperada ventaja desde el punto de vista de facilidad de manipulación, fabricación de composiciones farmacéuticas que incluyan la sustancia fármaco, y subsecuente almacenamiento en forma sólida antes de y durante la distribución en farmacias y de hecho en los hogares de los pacientes**".

273

Por todo lo anterior, es completamente claro que el objeto de la solicitud denegada sí cumple con las consideraciones técnicas necesarias para que se le reconozca el nivel inventivo, tal y como han sido expuestos en una publicación de los méritos del **Manual Andino de Patentes**, y más aún, como se ha demostrado por medio de **datos experimentales y la opinión de una experta en la materia como la Dra. Någård, quien no duda en calificar de sorprendente e inesperada la estabilidad química de las formas cristalinas de melagatrán enseñadas por el solicitante.**

Es claro entonces, que la materia objeto de la presente invención no podría derivarse de manera obvia a partir del estado de la técnica disponible, toda vez que la simple existencia de las formas cristalinas de melagatrán no podrían ser predichas, teniendo en cuenta además, que fue obtenida en una forma que tampoco podría resultar obvia.

Por lo tanto, es preciso concluir que partiendo del hecho ya demostrado de que la simple existencia de dichos polimorfos de melagatrán, su estructura cristalina, su método de preparación y sus propiedades químicas y físicas no podrían ser predichas, las formas reivindicadas cumplen sin lugar a dudas con el requisito de nivel inventivo exigido por la Decisión 486.

(6) De otra parte, en la Resolución impugnada nuevamente se objeta el nivel inventivo del proceso descrito en la invención denegada para la obtención de las formas cristalinas reivindicadas, toda vez que los documentos citados "**sugieren las técnicas de re-cristalización**" y que por lo tanto: "***El proceso mencionado es una forma convencional para obtener una sal cristalizada, de modo que también carece de nivel inventivo***".

Sobre este particular, me permito manifestar una vez más que las afirmaciones empleadas para objetar el nivel inventivo del proceso reivindicado para la obtención de la forma cristalina específica de melagatrán, no son suficientes para desvirtuar la patentabilidad de dicho proceso, toda vez que el mismo no corresponde a un simple proceso convencional para obtener una sal cristalizada como erróneamente es argumentado en la Resolución impugnada.

Por el contrario, el proceso reivindicado emplea solventes completamente específicos, y **permite llegar a construir una estructura cristalina definida y diferente** a la estructura amorfa conocida para el melagatrán.

Lo anterior no equivale a simplemente cristalizar cualquier sal, a partir de una solución de la misma, toda vez que es del conocimiento de una persona medianamente versada en la química del estado sólido, que los términos "cristalización" o "re-cristalización", se emplean usualmente en forma general para denotar **precipitación o solidificación de un soluto a partir de una solución, aún cuando la morfología resultante del producto obtenido sea un sólido amorfo**.

Sobre este particular, es necesario aclarar a ese Despacho que el término "recristalización" empleado en la referencia **D1**, **no indica que se esta obteniendo una forma cristalina de un producto**, y mucho menos una de las novedosas formas cristalinas de melagatrán reveladas en la invención denegada, por el contrario, de acuerdo con la propia referencia citada (página 246), es claro que dicha expresión denota allí el uso de este tipo de técnica **para la separación de mezclas de isómeros**, tal como me permito transcribir a continuación "... y en aquellos casos donde la reacción resulte **en una mezcla de estereoisómeros, éstos son opcionalmente separados por técnicas cromatográficas estándar o de re-cristalización**, y si se desea se aísla un estereoisómero único" (resaltado fuera de texto, página 246 del documento **WO 94/29336** citado como **D1**).

El hecho de que el solicitante de la referencia **D1** emplee el término "recristalización" sin obtener un producto cristalino no es algo sorprendente, ya que como se ha manifestado en reiteradas oportunidades, dicho término es usualmente empleado en forma "genérica" para denotar precipitación o solidificación de un soluto a partir de una solución aun cuando la morfología resultante del producto obtenido sea un sólido amorfo. Para demostrar lo anterior, solicito a ese Despacho que examine las siguientes referencias de la literatura que se anexan al presente escrito, las cuales son ejemplos que demuestran el uso genérico de la palabra "recristalización":

Kohn, et al., J. Med. Chem. 1990, 33:919-926. En la página 923, en la columna izquierda, línea 1, se indica que el compuesto 2j fue **recristalizado** a partir de Acetato de Etilo/Hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) **produciendo un sólido amorfo**.

Poindexter, et al., J. Org. Chem., 1992, 57:6257-6265. En la página 6263, columna derecha, en el último ejemplo, se indica que la base libre fue subsecuentemente convertida a la sal oxalato para proporcionar un

295275

sólido **amorfo** blanco después de una recristalización a partir de Acetato de Etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes).

Crocker, et al., Bioconjugate Chem.;1990, 1:419-424. En la página 420, columna derecha, en el último ejemplo, se indica que se realizó una recristalización subsecuente a partir de Acetato de Etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) para producir 13 como un sólido **amorfo**.

La patente *U.S. 5,378,729.* En el ejemplo 24, se señala que el producto fue recristalizado a partir de acetato de etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) para dar un sólido **amorfo** ligeramente amarillo. En el ejemplo 76, se señala que el producto fue recristalizado a partir de acetato de etilo/hexano como un sólido coloreado Beige.

Con base en estas referencias, es claro que la palabra "recristalizado" empleada en los documentos citados por ese Despacho hace referencia a la obtención de un sólido amorfo y por lo tanto, es claro que la simple mención de este término no puede conducir, como erróneamente ha establecido el Examinador, a la revelación en el arte anterior de una forma cristalina de melagatrán, o a sugerir un método para llegar a alguna de ellas.

Como puede observarse en los procedimientos de preparación indicados o establecidos en los documentos citados por ese Despacho, las etapas y condiciones establecidas en dichos documentos son claramente diferentes del procedimiento establecido en la presente solicitud para obtener las formas cristalinas de melagatrán. Estas diferencias en los procedimientos claramente indican que se trata de productos distintos, esto es, que los procedimientos de los documentos citados conducen **invariablemente a la obtención de un compuesto amorfo**, mientras que las etapas y condiciones del procedimiento seguido en la presente invención, **conducen a obtener las formas cristalinas reivindicadas.**

Es decir, que **además del hecho de que los documentos citados por ese Despacho no indican la existencia de las formas cristalinas hidrato, anhidrato e iso-propanolato/hidrato de melagatrán, los procedimientos allí descritos no podrían de ninguna manera conducir a la obtención de dichas formas cristalinas reivindicadas en la presente solicitud.**

Por otra parte, me permito dirigir la atención de ese Despacho al hecho de que el método aplicado para la obtención de los polimorfos cristalinos de melagatrán, confiere altura inventiva a este nuevo polimorfo debido a los resultados inesperados alcanzados por tal método.

En verdad, es un hecho bien reconocido que incluso cuando se asume (sobre la base de una pura especulación) que un compuesto dado que exhibe una forma amorfa puede además existir como una nueva forma cristalina, es imposible predecir cual método de preparación específico podrá producir dicha nueva forma.

La imposibilidad de predicción del método de preparación que produciría una forma cristalina determinada, también es confirmada por el Dr. Bernstein en su artículo "*Crystal growth, polymorphism and structure-property relationship in organic crystals*" (copia anexa), en donde se establece que "*el rango de combinaciones de condiciones de crecimiento de cristales es virtualmente infinito, y no existe forma de garantizar la preparación de polimorfos adicionales de una sustancia, mucho menos de garantizar la generación de 'todos' ellos*"[9] (copia anexa). Este documento confirma entonces que incluso si una persona versada en la materia especulara acerca de la existencia de una nueva forma cristalina de un compuesto y, por lo tanto, decidiera preparar dicha nueva forma, no le sería posible predecir un proceso para la obtención de dicha forma novedosa.

Ahora bien, la imposibilidad de predecir si cualquier proceso dado produciría una forma cristalina nueva de un compuesto que no se sabe si exhibe polimorfismo y la imposibilidad de predecir cual proceso posiblemente produciría esta nueva forma cristalina, implica que los investigadores activos en el campo de la presente invención trabajan en la ausencia de cualquier expectativa de éxito. En su lugar, trabajan con el deseo de obtener resultados útiles sobre la base de una simple especulación.

Con base en lo anterior, es claro que ninguno de los documentos citados por el Examinador pueden hacer obvio el objeto de la presente invención, y menos cuando lo que revelan es una forma amorfa de melagatrán y el método de preparación de esa forma amorfa. Esto es debido a la imposibilidad de predecir, como se ha mencionado anteriormente, la

[9] Bernstein, J. "*Crystal Growth, polymorphism and structure-property relationships in organic crystals*", J. Phis. D:Appl. Phys., 26, B66-B76 (1993), p B67.

29 277

manera de obtener una forma cristalina de un compuesto dado, mucho menos cuando la revelación conocida de tal compuesto en el estado de la técnica indica su obtención únicamente como un sólido amorfo.

Por lo tanto, **la imposibilidad de predicción del método de preparación de una forma cristalina novedosa, incluso por analogía con los métodos conocidos para la preparación de otras formas cristalinas, hace de un polimorfo cristalino novedoso un objeto claramente inventivo. En particular, la imposibilidad de predicción del método de preparación de las novedosas formas cristalinas de melagatrán, máxime cuando los métodos conocidos indican la preparación de una forma amorfa de dicho compuesto, *hace de las formas cristalinas materia inventiva*.**

(7) Finalmente, y con el propósito de corroborar los argumentos que realzan el nivel inventivo de la presente invención, atentamente me permito dirigir la atención de ese Despacho al hecho de que la patentabilidad del objeto de la presente invención ha sido aceptada por las Oficinas de Patentes de Sur Africa, Estados Unidos de América, Singapur, Rusia, Filipinas, Nueva Zelanda, Indonesia y Australia. Más aún, también han sido aceptadas en Europa reivindicaciones correspondientes a las actuales reivindicaciones independientes 1, 3 y 6.

Por lo tanto, el hecho de que un número significativo de países a nivel mundial, hayan aceptado el nivel inventivo de las solicitudes extranjeras correspondientes al objeto de la presente invención, tiene gran significado y relevancia en el momento en que en Colombia se efectúa un estudio para determinar si la misma invención cumple con los requisitos de patentabilidad y puede ser privilegiada con el otorgamiento del derecho de patente.

Aún cuando lógicamente cada país es independiente de conceder o negar una solicitud, luego de la realización del estudio de fondo, los estándares de conocimiento de personas que laboran en un área particular de tecnología está siendo cada vez más uniforme y, por consiguiente, el hecho de que la altura inventiva de una invención haya sido reconocida por varias oficinas a nivel mundial con tantos expertos en la materia y con tantos años de desarrollo jurisprudencial y doctrinal, es **un indicio** de que la invención debe cumplir con el nivel de altura inventiva requerido en cualquier otra oficina de cualquier otro país.

298 273

Lo anterior es tan importante y relevante que ha sido considerado dentro del Artículo 46 de la Decisión 486 vigente actualmente. En dicho artículo, se establece que la Oficina Nacional competente podrá requerir informes de otras oficinas de propiedad industrial, para lo cual el solicitante podrá proporcionar copia de los resultados de exámenes de patentabilidad efectuados respecto a las solicitudes extranjeras correspondientes. En este sentido, de acuerdo con dicho Artículo 46, la Oficina Nacional competente podrá reconocer los resultados de los exámenes como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad de la invención.

En otras palabras, el propósito de citar las solicitudes correspondientes ya concedidas en otros países, es el de proporcionar una visión más amplia a ese Despacho acerca de la opinión de otros expertos sobre la patentabilidad del objeto de la presente solicitud.

Además, debe tenerse en cuenta que la independencia que posee ese Despacho para juzgar la patentabilidad de una invención, se refiere más a las excepciones de patentabilidad que lógicamente pueden variar de legislación a legislación o a las causales de nulidad o cancelación, respecto de las cuales cada país puede tener su propio criterio. Sin embargo, tratándose de la apreciación de la altura inventiva, el cual es un requisito *sine qua non* de toda patente, es evidente que los criterios de apreciación no varían de legislación a legislación y que una apreciación positiva acerca de la existencia de dichos requisitos por parte de diferentes Oficinas de Propiedad Industrial a nivel mundial, sí constituye un indicio de su existencia que debe ser entendido no como una intromisión en la soberanía de un país, sino como una herramienta más para ser utilizada en la evaluación de la patentabilidad de una solicitud bajo estudio.

En consecuencia, atentamente solicito a ese Despacho considerar estos hechos como relevantes para evaluar la patentabilidad de la presente invención concedida en otros países para las solicitudes correspondientes.

3. **CONCLUSIÓN**

El solicitante ha demostrado, con base en el conocimiento previo del estado de la técnica y en la abundante literatura aportada especializada en el tema, que existe en el estado actual de la ciencia la imposibilidad para predecir la **estructura cristalina** (**distribución específica en el espacio**) de una **nueva** forma cristalina, sin importar si se trata de un compuesto químico novedoso, o uno ya conocido. Así pues, lo realmente relevante en casos como el de la presente invención, es si esa estructura

299 279

cristalina puede ser predicha o no por el técnico de nivel medio a quien se encuentra dirigida la invención.

Por lo tanto, es precisamente por el hecho de encontrarse "en una conformación espacial diferente" que las formas cristalinas de melagatrán son novedosas e inventivas, toda vez que estas conformaciones no habían sido reveladas previamente en el estado de la técnica y su simple existencia no podría haber sido predicha por el técnico medio en la materia (nivel inventivo), tal como se ha demostrado a lo largo del presente escrito, con lo argumentos científicos que respaldan la falta de predicción en este campo y la dificultad que la misma representa para el investigador que tiene como meta la obtención de una nueva forma cristalina.

En el presente caso, la forma cristalina reivindicada es, **inesperada y químicamente más estable** que la forma amorfa conocida ampliamente en el estado de la técnica, situación que representa una ventaja técnica que soluciona los problemas de estabilidad conocidos para una sustancia con propiedades farmacéuticas (tal como el melagatrán), y que corresponde a un "efecto sorprendente" que claramente constituye un elemento indicador de la presencia de altura inventiva en una solicitud, tal y como lo afirma la Secretaria general de la Comunidad Andina en el citado **Manual Andino de Patentes**.

Adicionalmente, el solicitante ha presentado la declaración rendida por una experta en la materia, como lo es a Doctora Sofía Någård, quien además de poseer una amplia experiencia en el campo de la cristalografía y el polimorfismo, es una de las inventoras de la solicitud denegada, y quien concluye que "En resumen, no creo que existiera motivación alguna, antes de la fecha de prioridad, para que la persona versada en la materia llegara a cualquiera de las formas cristalinas de melagatrán tal como son descritas en la presente solicitud. Además, no existe ninguna motivación, antes de la fecha de prioridad, para llegar a las formas cristalinas específicas de melagatrán en la forma de un hidrato, anhidrato o *iso*-propanolato/hidrato mezclado según lo reseñado por el presente juego de reivindicaciones modificado. Claramente esto es indicativo de nivel inventivo. Además, no existe ninguna sugerencia de las ventajas inesperadas (en estabilidad química) de la invención, ventajas que son resaltadas por los datos experimentales presentados. Estos datos demuestran claramente evidencia de nivel inventivo, y por lo tanto, de patentabilidad".

Por lo tanto, es claro que tanto la forma cristalina de melagatrán, como su proceso de obtención y la composición farmacéutica que la contiene, no se pueden derivar en forma evidente del estado de la técnica. Es decir, no es posible para el técnico medio predecirlos, y por ende, son completamente **INVENTIVOS**,

cumpliendo así con las disposiciones legales contenidas en el Artículo 18 de la Decisión 486 de Régimen Común sobre Propiedad Industrial de la Comisión de la Comunidad Andina.

4. En consecuencia, atentamente solicito a ese Despacho considerar los argumentos expuestos anteriormente y revocar la Resolución número **34297** impugnada para conceder el privilegio de patente solicitado.

5. El documento que acredita mi calidad de Representante Legal en Bogotá para asuntos relativos a la Propiedad Industrial de la sociedad peticionaria, fue presentado con mi memorial de 31 de Agosto de 2004.

6. Anexo me permito presentar:

(i) Copia simple de la declaración rendida por la Doctora Sofía Någård, junto con su hoja de vida. La copia debidamente legalizada será presentada en los próximos días teniendo en cuenta que se encuentra en trámite;

(ii) Copia del capítulo introductorio del libro de Byrn, S. R., "*Solid-State Chemistry of Drugs*", Academic Press, Nueva York, 1982, en la que se resaltan los apartes citados, junto con traducción oficial de la información bibliográfica y del acápite "C. POLIMORFOS" que se encuentra en las páginas 6 y 7 de dicho texto;

(iii) Copia del artículo de Byrn S. R., et. al., "*Solid-State Pharmaceutical Chemistry*", Chem. Mater., Vol. 6, 1148-1158 (1994);

(iv) Copia del artículo de Dunitz J. D. et. al., "*Disappearing Polymorphs*", Acc. Chem. Res., 28, 193-200 (1995);

(v) Copia del artículo de Ellern A., et. al. "*A New Polymorphic Modification of Tetrathiafulvalene*", Chem. Mater., 6, 1378-1385 (1994);

(vi) Copia del artículo de Byrn S. R., et. al., "*Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations*", Pharm. Res., 12, 945-954 (1995);

(vii) Copia del Artículo de Kohn, et al., J. Med. Chem. 1990, 33:919-926, en cuya página 923, en la columna izquierda, línea 1, se indica que el compuesto 2j fue recristalizado a partir de Acetato de Etilo/Hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) produciendo un sólido amorfo;

301 281

(viii) Copia del artículo de Poindexter, et al., J. Org. Chem., 1992, 57:6257-6265, en cuya página 6263, columna derecha, en el último ejemplo, se indica que la base libre fue subsecuentemente convertida a la sal oxalato para proporcionar un sólido amorfo blanco después de una recristalización a partir de Acetato de Etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes);

(ix) Copia del artículo de Crocker, et al., Bioconjugate Chem.;1990, 1:419-424, en cuya página 420, columna derecha, en el último ejemplo, se indica que se realizó una recristalización subsecuente a partir de Acetato de Etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) para producir 13 como un sólido amorfo;

(x) Copia de la página frontal y de las columnas 29 y 30 de la patente U.S. 5,378,729, en cuyo ejemplo 24, se señala que el producto fue recristalizado a partir de acetato de etilo/hexano (como en el caso de las referencias citadas por ese Despacho que emplean los mismos solventes) para dar un sólido amorfo ligeramente amarillo; y

(xi) Copia del artículo de Bernstein, J. "*Crystal Growth, polymorphism and structure-property relationships in organic crystals*", J. Phis. D:Appl. Phys., 26, B66-B76 (1993).

7. Mi dirección es Calle 72 No. 5-83, Piso 6º., Bogotá D.C., Colombia.

Del Señor Superintendente,

ALICIA LLOREDA RICAURTE
T. P. 53.215

Lloreda Ricaurte

39690713
QP - 53214

ALICIA LLOREDA RICAURTE
C.C. No. 39.690.713 de Usaquén

382-082

TRADUCCION OFICIAL No. 2006-01-31-071
DE UN DOCUMENTO ESCRITO EN INGLES

Número de Solicitud de Colombia: 00-048.529
Declaración

Yo, Sofía Någård, declaro lo siguiente:

1. Recibí mi título de la Universidad de Gotemburgo. He sido empleada de AstraZeneca (previamente Astra Hässle AB) R&D Mölndal, S-431 83 Mölndal, Suecia, el solicitante de la solicitud anteriormente referenciada, desde 1993. Anexo mi Curriculum Vitae, el cual incluye una lista de mis publicaciones.

2. Como un inventor en el presente caso, estuve directamente comprometida en los estudios que llevaron a la presente invención y que se revelan en la descripción del presente. También estuve directamente relacionada con los experimentos que produjeron los datos que se incluyen a continuación en el Anexo A. Por lo tanto, estoy plenamente calificada para hacer esta declaración.

3. La presente solicitud se relaciona con formas sustancialmente cristalinas del compuesto HO_2C-CH_2-(*R*)Cgl-(*S*)Aze-Pab-H (donde Cgl representa ciclohexilglicina, Axe representa azetidina-2-carboxilato y Pab-H representa 4-aminometilamidinobenceno). El compuesto es también conocido como melagatrán. Este compuesto y otros han sido descritos en WO 94/29336 como inhibidores de trombina.

4. En vista de toda la técnica anterior estoy consciente de, y en particular de los documentos de AstraZeneca citados por el examinador (a saber WO 94/29336, WO 97/23499 y WO 98/16252), y creo que la presente invención es patentable.

5. No creo que la técnica anterior revele melagatrán en una forma sustancialmente cristalina. Más bien, la técnica anterior más cercana según mi punto de vista, WO 94/29336, revela un método para obtener únicamente melagatrán amorfo.

6. No creo que sea posible para una persona versada en la técnica predecir, únicamente de la estructura molecular, cuál será el comportamiento de cristalización de un compuesto. Además, no creo que haya ninguna motivación en la técnica anterior para la persona versada en la técnica para aplicar las técnicas de cristalización descritas en la presente solicitud al melagatrán.

7. Más específicamente, y con referencia a la reivindicación pendiente expresada en esta solicitud de Colombia, no creo que sea posible alegar que las revelaciones anteriores equivalen a cualquier clase de sugerencia para hacer una forma sustancialmente cristalina de melagatrán en la forma de un hidrato (presente Reivindicación 1), en la forma de un anhidrato (actual Reivindicación 3), o en la forma de un *iso*-propanolato/hidrato mezclado (actual Reivindicación 6).

8. La presente invención además proporciona una ventaja inesperada, que no creo que de ninguna manera pudiese haber sido predicha antes de la fecha de prioridad de la presente solicitud.

304-234

9. Creo que los datos experimentales proporcionados por medio del Anexo A claramente demuestran una ventaja inesperada al revelar la estabilidad química mejorada de una forma cristalina de melagatrán descrita en la presente solicitud sobre la forma amorfa obtenida por medio de WO 94/29336.

10. Aunque yo esperaría que una o más formas cristalinas de una sustancia fuesen más estables desde un punto de vista **físico** que una forma amorfa correspondiente, este aumento significativo en estabilidad **química** para la forma cristalina me llega como una sorpresa y no podría de ninguna manera haber sido predicho a la luz de WO 94/29336 o cualquier otra técnica anterior de la cual estoy consciente.

11. Claramente, esta estabilidad química mejorada, que se observa en una forma cristalina de melagatrán proporciona una ventaja significativa e inesperada desde el punto de vista de la facilidad de manejo, fabricación de formulaciones farmacéuticas incluyendo la sustancia fármaco y el subsiguiente almacenamiento en forma sólida (v.g. en la forma de tabletas orales) antes de y durante la distribución, en farmacias y de hecho en los hogares de los pacientes.

12. En resumen, no creo que haya habido ninguna motivación, antes de la fecha de prioridad, para la persona versada en llegar a formas cristalinas de melagatrán según se describen en la presente solicitud. Además, no hay absolutamente ninguna motivación, antes de la fecha de prioridad, para llegar a las formas cristalinas específicas de melagatrán en la forma de un hidrato, anhidrato o *iso*-propanolato/hidrato mezclado según se describe en

305285

el presente juego de reivindicaciones modificado. Esto es claramente indicativo de nivel inventivo.

13. Además, no hay absolutamente ninguna sugerencia de las ventajas inesperadas (en estabilidad química) de la invención, ventajas que se describen en los datos experimentales presentados. Estos datos claramente demuestran evidencia de paso inventivo, y por lo tanto, de patentabilidad.

14. Por el presente declaro que todas las afirmaciones hechas en el presente de mi propio conocimiento son verdaderas y todas las declaraciones sobre información y creencia se cree que son verdaderas.

Fecha: 25 / 01 / 2006
Día Mes Año

(firma: ilegible)
Firmado: Sofía Någård
Científica

Lugar: Mölndal, Suecia

Curiculum vitae

Nombre:

Mona Sofía Någård

Educación:

Gimnasio, Educación técnica con énfasis en química	1989
Maestría en Ciencias en Química en la Universidad de Gotemburgo	1994

Tesis de la Maestría en Ciencias:

Infección de grandes volúmenes de muestras usando isotacoforesis como una técnica de preconcentración en línea para electroforesis de zonas capilares.

Publicaciones:

Larsson M, Någård S: *On-column ITP focusing in CZE for the quantitation of low-molecular-weight basic solutes in biological samples.* Journal of Microcolumn Separations 6(2):107-113, 1994 Mar-Abr.

Witte DT, Någård S, Larsson M: *Improved sensitivity by on-line isotachophoretic preconcentration in the capillary zone electrophoretic determination of peptide-like solutes.* Journal of Chromatography A, 687 (1):155-166, 1994 Dic 9.

Gustafsson D, Nyström JE, Carlsson S, Bredberg U, Eriksson U, Gyzander E, Elg M, Antonsson T, Hoffmann KJ, Ungell AL, Sörensen H, Någård S, Abrahamsson A, Bylund R: *The direct thrombin inhibitor melagatran and its oral prodrug H 376/95: Intestinal absorption properties, biochemical and pharmacodymanic effects.* Thrombosis Research. 101(3):171-181, 2001 Feb 1.

237

307

Owens PK, Wikstrom H, Någård S, Karlsson L: *Development and validation of a capillary electrophoresis method for ximelagatran assay and related substance determination in drug substance and tablets.* Journal of Pharmaceutical & Biomedical Analysis. 27(3-4):587-598, 2002 Ene 15.

Empleo:

Análisis de Sustancias, Investigación & Desarrollo Farmacéutico & Analítico,
Astra Hässle AB (hoy AstraZeneca R&D Mölndal) 1993-

Experiencia Profesional:

1993-2005 Análisis de Sustancias representativas para melagatrán (-93 - -99) y ximelagatrán (-96 - -05)

El análisis de Sustancias representativo para un compuesto es responsable del desarrollo de procedimientos analíticos apropiados para la evaluación de las características de calidad y químicas del compuesto, tal como por ejemplo, identidad, pureza, ensayo y estabilidad, y el desempeño y documentación de los análisis. El análisis de sustancia representativo es el que lleva al equipo funcional de químicos sintéticos y químicos analíticos que expresan la especificación para el control de la calidad del compuesto. El papel exige conocimiento de química orgánica, cristalización, formas polimórficas y química analíticas.

1999 marzo a agosto, Gerente del equipo (reemplazo) dentro del Análisis de Sustancias.

Traducido según mi mejor saber y entender, este día 31 de enero de 2006, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 del 15 de agosto de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTORA OFICIAL
RES. 2177/89 MINJUSTICIA
ANITA ESCOBAR DE TAMAYO

Colombian Application Number: 00-048.529

Declaration

I, Sofia Någård, declare as follows:

1. I received my degree from Göteborg University. I have been employed by AstraZeneca (previously Astra Hässle AB) R&D Mölndal, S-431 83 Mölndal, Sweden, the applicant of the above-referenced application, since 1993. I enclose a CV, which includes a list of my publications.

2. As an inventor in the present case, I was directly involved in the studies that led to the present invention and which are disclosed in the instant specification. I was also directly involved with the experiments that yielded the data set forth in Exhibit A. I am therefore fully qualified to make this declaration.

3. The present application relates to substantially crystalline forms of the compound HO_2C-CH_2-(*R*)Cgl-(*S*)Aze-Pab-H (wherein Cgl represents cyclohexylglycine, Aze represents azetidine-2-carboxylate and Pab-H represents 4-aminomethylamidinobenzene). The compound is also known as melagatran. This compound and others have been described in WO 94/29336 as being thrombin inhibitors.

4. In view of all the prior art that I am aware of, and in particular the AstraZeneca documents cited by the examiner (namely WO 94/29336, WO 97/23499 and WO 98/16252), I believe that the present invention is patentable.

5. I do not believe that the prior art discloses melagatran in a substantially crystalline form. Rather, the closest prior art in my view, WO 94/29336, discloses a method of obtaining amorphous melagatran only.

6. I do not believe that it is possible for a person skilled in the art to predict, from molecular structure alone, what the crystallisation behaviour of a compound will be. Further, I do not believe that there is any motivation in the prior art for the person skilled in the art to apply the crystallisation techniques described in the present application to melagatran.

7. More particularly, and with reference to the pending claim set in this Colombian application, I do not believe that it could possibly be alleged that the prior disclosures amount to any kind of suggestion to make a substantially crystalline form of melagatran in the form of a hydrate (present Claim 1), in the form of an anhydrate (current Claim 3), or in the form of a mixed *iso*-propanolate/hydrate (current Claim 6).

8. The present invention further provides an unexpected advantage, which I believe could in no way have been predicted prior to the priority date of the present application.

9. I believe that the experimental data provided by means of Exhibit A clearly demonstrate an unexpected advantage in displaying enhanced chemical stability of a crystalline form of melagatran described in the present application over the amorphous form obtained by means of WO 94/29336.

10. Although, I might expect one or more crystalline forms of a substance to be more stable from a **physical** point of view than a corresponding amorphous form, this significant increase in **chemical** stability for the crystalline form comes as a surprise to me and could in no way have been predicted in the light of WO 94/29336 or any other prior art of which I am aware.

11. Clearly, this enhanced chemical stability, which is observed in a crystalline form of melagatran provides a significant and unexpected advantage from the point of view of ease of handling, manufacture of pharmaceutical formulations including the drug substance, and subsequent storage in solid form (e.g. in the form of oral tablets) prior to and during distribution, in pharmacies and indeed in the homes of patients.

12. In summary, I do not believe that there was any motivation, prior to the priority date, for the skilled person to arrive at any crystalline forms of melagatran as described in the present application. Further, there is absolutely no motivation whatsoever, prior to the priority date, to arrive at the specific crystalline forms of melagatran in the form of a hydrate, anhydrate or mixed *iso*-propanolate/hydrate as outlined by the present amended claim set. This is clearly indicative of inventiveness.

13. Further, there is absolutely no suggestion of the unexpected advantages (in chemical stability) of the invention, which advantages are outlined by the experimental data submitted. These data clearly demonstrate evidence of inventive step, and therefore patentability.

14. I hereby declare that all statements made herein of my own knowledge are true and all statements on information and belief are believed to be true.

Date: 25/01/2006
Day Month Year

Sofia Någård

Signed: Sofia Någård, MSc
Senior Scientist

Place: Mölndal, Sweden

3/05-29

Curriculum vitae

Name:
Mona Sofia Någård

Education:

Gymnasium, Technical education with chemistry speciality	1989
Master of Science in Chemistry at Göteborg University	1994

Master of science thesis:
Injection of large sample volumes using isotachophoresis as an on-line preconcentration technique for capillary zone electrophoresis.

Publications:
Larsson M, Någård S: *On-column ITP focusing in CZE for the quantitation of low-molecular-weight basic solutes in biological samples.* Journal of Microcolumn Separations 6(2):107-113, 1994 Mar-Apr.

Witte DT, Någård S, Larsson M: *Improved sensitivity by on-line isotachophoretic preconcentration in the capillary zone electrophoretic determination of peptide-like solutes.* Journal of Chromatography A, 687 (1):155-166, 1994 Dec 9.

Gustafsson D, Nyström JE, Carlsson S, Bredberg U, Eriksson U, Gyzander E, Elg M, Antonsson T, Hoffmann KJ, Ungell AL, Sörensen H, Någård S, Abrahamsson A, Bylund R: *The direct thrombin inhibitor melagatran and its oral prodrug H 376/95: Intestinal absorption properties, biochemical and pharmacodymanic effects.* Thrombosis Research. 101(3):171-181, 2001 Feb 1.

Owens PK, Wikstrom H, Någård S, Karlsson L: *Development and validation of a capillary electrophoresis method for ximelagatran assay and related substance determination in drug substance and tablets.* Journal of Pharmaceutical & Biomedical Analysis. 27(3-4):587-598, 2002 Jan 15.

Employment:

Substance Analysis, Pharmaceutical &Analytical R&D,	
Astra Hässle AB (today AstraZeneca R&D Mölndal)	1993-

Working experience:
1993-2005 Substance Analysis representative for melagatran (-93 - -99) and ximelagatran (-96 - -05).
The Substance analysis representative for a compound is responsible for the development of appropriate analytical procedures for the assessment of the quality and chemical characteristics of the compound, such as eg identity, purity, assay and stability, and the performance and documentation of the analyses. The substance analysis representative is the one leading the cross-functional team of synthetic chemists and analytical chemists that set the specification for the control of the quality of the compound. The role demands knowledge of organic chemistry, crystallisation, polymorphic forms and analytical chemistry.

1999 March toAug, Team manager (replacement) within Substance Analysis

Line manager for 5 people comprising of a trainee, two laboratory engineers, a Substance Analysis representative and a technique specialist.

2005- Substance Analysis representative for a new compound

Patents:
New Crystalline Forms; A2151

25/01/2006 Sofia Magnusson

Date Signature

312 292

Solid-State Chemistry of Drugs

STEPHEN R. BYRN

Department of Medicinal Chemistry and Pharmacognosy
School of Pharmacy and Pharmacal Sciences
Purdue University
West Lafayette, Indiana

ACADEMIC PRESS 1982

A Subsidiary of Harcourt Brace Jovanovich, Publishers

New York London
Paris San Diego San Francisco São Paulo Sydney Tokyo Toronto

1 Introduction

The branch of science termed solid-state chemistry of drugs is ur ally broad. It includes the areas of physical pharmacy and indus pharmacy (including powder technology and formulation). It also incl the areas of stability of solids, kinetics of solid-state reactions, and mc ular details of solid-state reactions.

Studies of the molecular details of solid-state reactions aim at provi an explanation of the products and rate of the reaction in ter s of molecular change (chemical reaction) that occurs. These stud. ust use the crystal structure and other molecular information to provide an explanation.

The aim of this book is to review the molecular details of solid-s reactions of drugs and to bring these details to bear on pharmaceu problems. It is thus an attempt to provide a molecular basis for un standing the solid-state chemistry of drugs. Related aims in the area biology and chemistry (i.e., attempts to put these areas on a molec basis) have led to the rapid development of these branches of science similar rapid development of the area of solid-state chemistry of drug also expected.

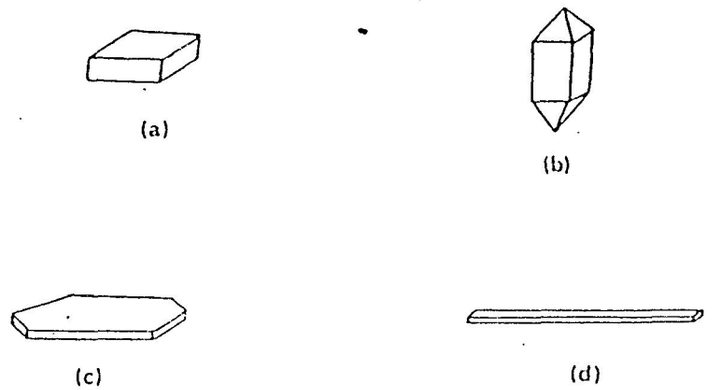


FIGURE 1. Different crystal habits of a drug: (*a*) tabular, (*b*) prismatic, (c) plate, and (*d*) needle.

Once the crystals obtained have been shown to have habits it is often necessary to characterize these habits. Habits are best characterized using instruments called reflecting or optical goniometers. A reflecting goniometer is shown in Chapter 2 in Figure 9 (p. 38).

Crystal habits influence several pharmaceutical characteristics related to the physical shape and nature of the crystal.

1. *Suspension syringeability.* This parameter is influenced by mostly mechanical factors. For example, a suspension of plate-shaped crystals may be injected through a small needle with greater ease than one of needle-shaped crystals.
2. *Tableting behavior.* Behavior upon compression of crystals is dependent upon the habit present; however, no generalization can be made. It is quite reasonable to expect platelike crystals to exhibit different tableting behavior from needles. However, a priori, it is difficult to predict the differences in behavior.

C. POLYMORPHS

Crystallization can also result in the formation of several different solvates and/or anhydrates, which have different crystal habits and different x-ray diffraction patterns. These different solvates and/or anhydrates will be referred to as *polymorphs*. Polymorphs are thus different crystal forms of the same compound, and are detected by x-ray diffraction. Polymorphs have different physical and chemical properties. They can be interconverted by phase transformations or a solvent-mediated process. Phase transformations can be induced by heat or mechanical stresses. Different

polymorphs have different dissolution rates and bioavailability. No rules exist that allow prediction of whether a compound will exhibit polymorphism; however, polymorphism is widespread in pharmaceuticals, particularly in steroids, sulfonamides, and barbiturates.

Two special types of polymorphism are termed *conformational* and *configurational* polymorphism. Conformational polymorphism occurs when a molecule adopts a significantly different conformation in different crystal polymorphs. The term "significantly different" is open to interpretation; however, it implies torsion or dihedral angles different by at least three standard deviations. For example, the Schiff's base *p*-(*N*-chlorobenzylidene)-*p*-chloroaniline crystallizes in two polymorphs. The stable form belongs to the triclinic crystal system, while the unstable form belongs to the orthorhombic crystal system. Both polymorphs are disordered but, strikingly, the conformation of the Schiff's base is different in the two polymorphs. Thus, these forms are termed *conformational polymorphs* (Bernstein and Hagler, 1978).

Conformational polymorphism involves the crystallization of different conformers in different crystalline forms, but this term does not adequately describe cases where different types of isomers crystallize in different forms. Thus a new term—*configurational polymorphism*—is needed. Configurational polymorphism exists when different configurations (i.e., cis–trans isomers or tautomers) crystallize in separate crystalline forms. Of course the crystallization of cis and trans isomers of the same compound in different crystalline forms is well known and occurs whenever the pure isomer is crystallized. Similarly, crystallization of a pair of pure tautomers in separate crystals leads to what may be called tautomerizational polymorphs. The crystallization of equilibrating isomers in configurational polymorphs is of significantly more interest. When this occurs, the phenomena of configurational polymorphism can be used to isolate and study the individual isomers.

Polymorphism is very common in the pharmaceutical area. Because they have different crystal structures, polymorphs have different chemical and physical properties. Polymorphs of the same substance show different melting points, different chemical reactivities, different dissolution rates, and different bioavailabilities.

D. CRYSTAL SOLVATES

Upon crystallization, drugs often entrap solvent in the crystal. This solvent can be in stoichiometric or nonstoichiometric amounts. Crystals that contain solvent of crystallization are termed *solvates*. If water is the solvent of crystallization, the solvates are called *hydrates*. Crystal solvates

based on the x-ray diffraction pattern of solvates and desolvated crystals appears to be the scheme of choice.

II. Properties of Other Solids

A number of other solids in addition to crystals are encountered in the study of solid pharmaceuticals. The properties of these solids are also of interest.

A. AMORPHOUS SOLIDS

Amorphous solids have no crystal shape and cannot be identified as either habits or polymorphs. The most common amorphous solid is a glass in which the atoms and molecules exist in a nonuniform array.

An *amorphous form* can also be understood in terms of the size of the crystallites. Starting with a crystalline material, the size of the crystals would be reduced in stages and an x-ray photograph could be taken at each stage. The lines on the photograph would become diffuse when the crystal size falls below about 10^{-5} cm. As the crystal size is reduced further, the lines would become increasingly diffuse until the limit is reached at about 10^{-6} cm, the region of atomic dimensions. At this point it is impossible for a crystal to exist since, by definition, it is no longer an ordered array of atoms or molecules. Amorphous forms give no diffraction pattern and thus have crystal sizes less than 10^{-5} Å.

Amorphous crystals can be prepared by rapid crystallization or by lyophilization. For example, rapid crystallization gave an amorphous form of chloramphenicol palmitate (Kimura and Hashimoto, 1960), and lyophilization gave the amorphous form of fluprednisolone (J. Haleblian *et al.*, 1971).

Amorphous solids are best characterized by x-ray powder diffraction, since these solids give very diffuse lines or no crystal diffraction pattern at all.

While amorphous solids often have desirable pharmaceutical properties such as rapid dissolution rates, they are not usually marketed because of their instability. Since they are noncrystalline, amorphous forms are an energetic form which tend to crystallize to a more stable form. In addition, amorphous forms are often more chemically reactive than their crystalline counterparts (Pikal *et al.*, 1977).

However, in some cases amorphous forms are used and are desirable. An excellent example is novobiocin (Mullins and Macek, 1960). Novobiocin exists in a crystalline and an amorphous form. The crystalline

BLE II

Plasma Levels (μg/ml) of Novobiocin in Dogs after Administration of Different Forms of Novobiocin[a]

	Hours after dose						
Form administered	0.5	1.0	2.0	3.0	4.0	5.0	6.0
Sodium novobiocin	0.5	0.5	14.6	22.2	16.9	10.4	6.4
Amorphous novobiocin acid	5.0	40.6	29.3	22.3	23.7	20.2	17.5
Crystalline novobiocin acid	Not detectable at any time						

[a] From Haleblian (1975).

form is poorly absorbed and does not provide therapeutically active blood levels; in contrast, the amorphous form is readily absorbed and is therapeutically active. Further studies show that the amorphous form is 70 times more soluble than the crystalline one in 0.1 *N* HCl at 25°C particles $<10\ \mu m$ are used.

Table II shows data for the plasma levels of novobiocin's amorphous and crystalline forms and for sodium novobiocin, which also gives detectable plasma levels but is chemically unstable in solution.

Unless special precautions are taken, the amorphous form is slowly converted to the crystalline form. Several additives have been developed to retard this conversion, with methylcellulose and several alginic acids being most effective.

B. HYGROSCOPIC SOLIDS

Unfortunately, there is no clear definition of hygroscopic solids. Instead, a solid is *hygroscopic* when it takes up moisture from the atmosphere. Hygroscopicity is determined by both a kinetic term and a thermodynamic term. For example, at equilibrium a solid may only take up a small amount of water but because the uptake is rapid the solid would be termed hygroscopic. Likewise, the solid may absorb a large amount of water at a very slow rate. Because the rate is slow, such a solid is not termed hygroscopic.

In addition, the relative humidity of the atmosphere plays an obviously important role in determining whether or not a solid is hygroscopic. In high relative humidities, many solids are hygroscopic. In atmospheres of low humidity, only a few solids will be hygroscopic.

A third factor influencing hygroscopicity is surface area. The larger the surface area of the solid, the more rapid the uptake of moisture. This is because solids with a larger surface area have more sites for condensation and adsorption of water molecules. In general, amorphous solids are often

component A is heated [illegible] point W. [illegible]ion then occurs completely in the solid state until 100% B is formed.

Thus only Case 4 depicts a true solid-state reaction. From this discussion it is clear that care must be taken to ensure that the reaction is being carried out at a temperature below the eutectic temperature of the reactants and products, to be sure that the reaction takes place in the solid state.

B. STEPS IN A SOLID-STATE REACTION

Once it has been established that the reaction is occuring in the solid state, the reaction is understood in terms of a four-step process (Paul and Curtin, 1973).

1. *Loosening of molecules at the reaction site.* It is reasonable to assume that the extent of loosening required depends on the distortion of the reaction cavity required to accomplish the next step.
2. *Molecular change.* This step is similar to the corresponding solution reaction where the reactants bonds are broken and the product bonds formed.
3. *Solid-solution formation.* During the early stages of the reaction, a solid solution of the product in the starting crystal is formed; however, after the product concentration reaches a certain point the product will separate.
4. *Separation of product.* This step often gives randomly oriented crystals or crystals with an orientation governed by the crystals of the starting material. This latter case is termed a topotactic reaction and will be discussed further.

Molecular Loosening. Solid-state reactions begin at one or more nucleation sites and spread through the crystal. In some cases, particularly in desolvations and some thermal reactions, a reaction begins at a nucleation site and spreads through the crystal in a front that advances at a more or less linear rate through the crystal.

Nucleation sites for reaction are developed during crystallization or can sometimes be produced by mechanical deformations such as pricking with a pin or cutting the crystal. Nucleation sites can also sometimes be produced by exposing the starting crystal to product crystals. In other cases, neither mechanical deformation nor exposure to product crystals nucleates the reaction. Obviously, this variability in nucleation and the random number of nucleation sites that are present in crystals can greatly complicate the kinetics of solid state reactions. In fact, the rates of some solid-state reactions can be explained entirely in terms of propagation of nucleation sites through the crystal.

For solid–gas reactions, this molecular loosening step also involves diffusion of the gas to the reactive site. Of course, diffusion of a gas into the crystal requires molecular loosening. Thus for solid–gas reactions the molecular loosening step actually involves simultaneous or sequential molecular loosening and diffusion.

Molecular Change. This step is most similar to the solution reaction of compounds: in it, the chemical reaction occurrs. If nucleation and the molecular loosening process can be separated from the molecular change, then the rate of solid-state reactions is explainable by the same factors that explain the rates of solution reactions. Unfortunately, in many cases the molecular loosening process cannot be separated from the molecular change. It is interesting to note that, except in rare instances of favorable orientation, solid-state reactions are usually much slower than their solution counterparts. This is probably due to the compactness of solids and the fact that large movements of functional groups are restricted in a rigid crystal.

Solid-Solution Formation. After a small amount of product is formed, it usually exists as a solid solution in the starting crystal lattice. In general the extent of solid solution formation and the energies required to form solid solutions probably do not greatly influence the rates of solid-state reactions. However, in cases where the energy of the product is [illegible] the energy barrier, these factors could play a role. The formation of a solid solution of product in the starting crystal does indicate that methods such as x-ray diffraction that measure the diffraction of the starting crystal may not be suitable for measuring rates of reaction, since during solid solution formation the diffraction pattern of the starting crystal would not change greatly or even predictably.

Separation of the Product Phase. After the limit of solubility of the product in the starting crystal lattice is reached, the product will crystallize. This step would not influence the rate of the reaction if the rate is measured by chemically measuring the amount of product formed or the disappearance of the reactant. However, if the rate is measured by measuring the diffraction from the crystal lattice of the product, then this step will contribute to the measured rate of the reaction. Thus rates measured using x-ray diffraction differ from those measured chemically.

It is clear from the above discussion that the rates of solid-state reactions depend on several factors, including nucleation and the molecular change involved.

It is important to realize that the crystal structure and crystal packing profoundly affect the molecular-loosening and molecular-change steps of a solid-state reaction. The crystal packing determines the extent of molecular loosening required for the molecules to get in an orientation to undergo the molecular change required to complete the reaction. The

The molecular loosening ... requires the nucleation of reaction. During this step, the molecules partially unpack from the original crystal lattice. This can in some cases be initiated by a pin-prick or other mechanical deformation. For example, Kitaigorodskii *et al.* (1965) showed that a pin-prick can initiate the transformation of α-*p*-dichlorobenzene to β-*p*-dichlorobenzene. The transformation of α- to β-*p*-dichlorobenzene is delineated by the spread of the reaction front from the nucleation site through the crystal.

A related process is the thermally induced rearrangement of the α to β form of *p*-nitrophenol (Coppens and Schmidt, 1965). In this reaction, needle-shaped single crystals rearrange, with the phase boundary moving approximately perpendicular to the needle axis.

Separation of the product phase involves crystallization of the new crystal form. The product appears as microcrystallites with little or no net orientation relative to the starting crystal. This is in contrast to *p*-dichlorobenzene, where the product is a single crystal.

E. DESOLVATION REACTIONS

Desolvation is related to polymorphic transformation, however the solvent exits the crystal. For polymorphic solvates, the product crystal separates into a new crystal form. For pseudopolymorphic solvates, loss of solvent does not lead to the formation of a new polymorph.

The desolvation of polymorphic solvates involves four steps:

1. Molecular loosening.
2. Breaking of the host–solvent hydrogen bonds (or other associations).
3. Solid solution formation.
4. Separation of the product phase.

The molecular-loosening step involves nucleation and loosening of the crystal packing so that the solvent can escape. Then the forces that hold the solvent to the host molecules in the crystal are broken. Usually this is the breaking of hydrogen bonds. The solvent then exits the crystal and the product separates. Extensive studies in our laboratory show that this reaction is quite similar in mechanism to other solid–state reactions.

Desolvation of pseudopolymorphic solvates involves two steps: (*a*) molecular loosening and (*b*) breaking of host–solvent hydrogen bonds or associations.

F. CHEMICAL REACTIONS

It is important to realize that many solid-state reactions of drugs involve drug degradation. These reactions are of interest because of a desire to prevent such degradation. Therefore in many respects the solid-st chemistry of drugs is synonymous with drug degradation.

Drug degradation has been studied mostly on the macroscopic level. fact, few studies aimed at determining the molecular aspects of the sol state chemistry of drugs have been published. Even for such comm drugs as vitamin D_2 and vitamin A, only the structures of a few of t degradation products have been published.

Table III summarizes some of the solid-state chemical reactions drugs, and is arranged according to the chemical reaction involved. Or solid-state reactions in which the chemical structure of the product(s) known are included. The classification scheme used in this table is us throughout this book, and each class of reaction is treated in a se[illegible] chapter or chapters.

IV. Stability Testing

One of the practical areas encompassed by the field of solid-s chemistry of drugs is the area of stability testing. Stability tests are co ducted on all marketed drugs in order to determine an expiration dat which the drug will not be sold.

Companies are required to ensure that the drugs distributed and ke keted are of the best possible quality. However, because the phrase "be possible quality" is vague the government has attempted to define thi idea in terms of good manufacturing practices. The most recent goo manufacturing practices were published in the "Federal Register" o September 29, 1978. They require, among other things, that

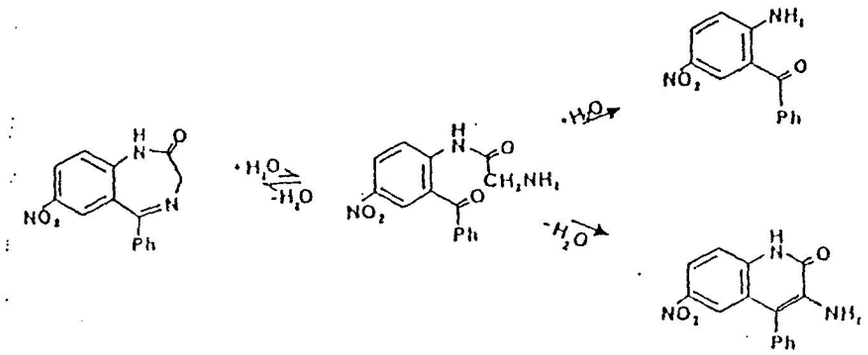
1. Essentially all products must bear an expiration date.
2. All products bearing this date must describe the storage condition under which this date applies.
3. The stability-testing program must be defined in writing.

An expiration date is required in hopes of assuring that drug product have the identity, purity, structure, and quality described on the label an package insert during their period of use. Obviously the expiration date is only for the storage conditions described. If the product is subjected to extremes, such as higher temperatures than those described, then the actual expiration date will be sooner than the expiration date on the label.

Generally, stability studies and expiration dates should be determined under conditions approximating normal storage conditions rather than under accelerated conditions. However, accelerated stability tests can be very important since they can be used to determine the best storage conditions. Using accelerated tests, a number of storage conditions can be

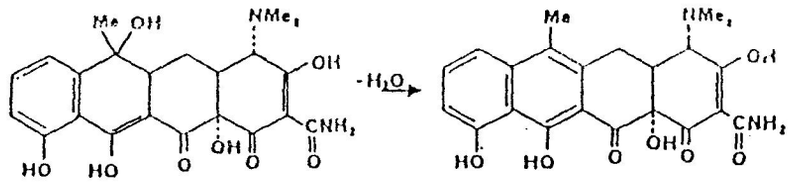
TABLE III. (*continued*)

2. Nitrazepam

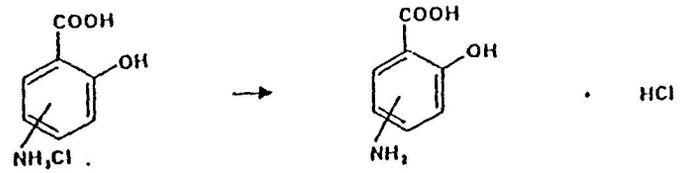


C. Solid-state decomposition reactions of the type A(solid) → B(solid) + C(gas) (Chapter 8)

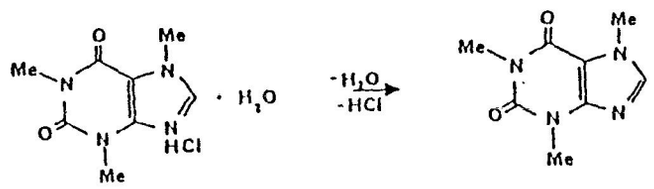
1. Dehydration of Tetracyclines



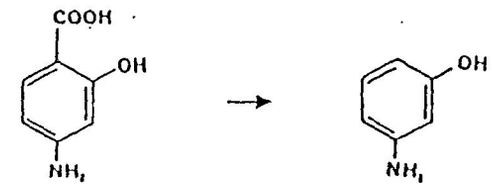
2. Dehydrochlorinations of aminosalicylic acid hydrochlorides



3. Dehydrochlorination of caffeine hydrochloride

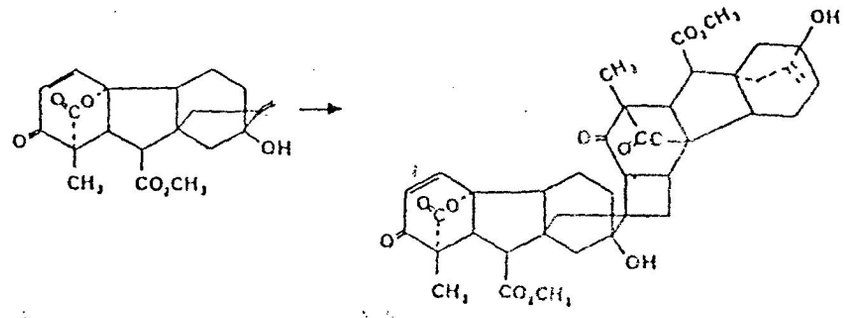


4. Decarboxylation of *p*-aminosalicylic acid



D. Solid-state photochemical reactions (Chapter 9)

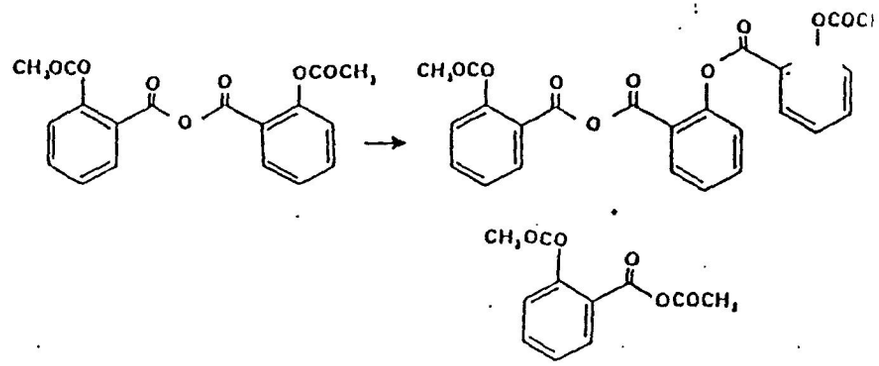
1. Gibberellins



2. Dieldrin

E. Solid-state thermal reactions (Chapter 10)

1. Rearrangement of aspirin anhydride



dosage form contai. between 9 a 110% of the labeled amount. T expiration date is the date when the amount will drop below 90% of the labeled amount. The expiration date can be lengthened by including 110% of the labeled amount in the dosage form. The excess over labeled amount is also sometimes called an *overage*. Overages may not always be cost-effective, since if very stable and very expensive drugs are being marketed the savings in increasing the shelf life may not be offset by the cost of the overage.

It is important to realize that stability testing does not require an understanding of the molecular details of the reaction. Instead, it requires a good analytical method to follow drug degradation and controlled conditions. An understanding of the molecular details of a solid-state reaction requires a much more extensive study of the process.

V. Summary

This chapter summarizes the scope of the area of solid-state chemistry of drugs. It is clear that this is a broad, relatively unexplored area involving an understanding of crystallization, the properties of crystals, the forces holding crystals together, the properties of other solids (i.e., amorphous solids), the chemical reaction involved, the criteria for solid-state reactions, the kinetics of solid-state reactions, and the broad field of stability testing.

There is a need to develop an understanding of solid-state reactions of drugs in terms of the molecular details of reactions. Of particular interest is a determination of the molecular parameters that can lead to retardation of the solid-state reactions of drugs and thus render drugs more stable.

It is the aim of the rest of this book to further illustrate the importance and value of molecular understanding of solid-state reactions.

References

Bernstein, J., and Hagler, A. T. (1978). *J. Am. Chem. Soc.* 100, 673.
Carstensen, J. (1974). *J. Pharm. Sci.* 63, 1.
Cohen, M. D. (1975). *Angew. Chem. Int. Ed.* 14, 386.
Cohen, M. D., and Cohen, R. (1976). *J. Chem. Soc., Perkin Trans. 2*, 1731.
Cohen, M. D., Cohen, R., Lahav, M., and Nie, P. L. (1973). *J. Chem. Soc., Perkin Trans. 2*, 1095.
Coppens, P., and Schmidt, G. M. J. (1965). *Acta Cryst.* 18, 62, 654.
Etter, M. C. (1976). *J. Am. Chem. Soc.* 98, 5326.
Haleblian, J. K., Koda, R. T., and Biles, J. A. (1971). *J. Pharm. Sci.* 60, 1485.
Haleblian, J. (1975). *J. Pharm. Sci.*, 64, 1269.
Hung, J. D., Lahav, M., Luwisch, M., and Schmidt, G. M. J. (1972). *Isr. J. Chem.* 10, 5
Kimura, T., and Hashimoto, S. (1960). *Jap. Patent* 60, 5798.
Kitaigorodskii, A. I. (1961). Organic Chemical Crystallography. Consultants Bureau, N York.
Kitaigorodskii, A. I., Mnyukh, Y. V., and Asadov, Y. G. (1965). *J. Phys. Chem. Solids* 463.
Morawetz, H. (1966). *Science*, 152, 705.
Paul, I. C., and Curtin, D. Y. (1973). *Acc. Chem. Res.* 7, 223.
Pfeiffer, R. R., Yang, K. S., and Tucker, M. J. (1970). *J. Pharm. Sci.* 59, 1809.
Pikal, M. J., Lukes, A. L., and Lang, J. E. (1977). *J. Pharm. Sci.* 66, 1312.
Schmidt, G. M. J. (1964). *J. Chem. Soc.*, 2014.
Schmidt, G. M. J. (1971). *Pure Appl. Chem.* 27, 647.
Sukenik, C. N., Bonapace, J. A. P., Mandel, N. S., Lau, P. Y., Wood, G., and Bergma R. G. (1977). *J. Am. Chem. Soc.* 99, 851.
United States Pharmacopeia, XX (1980). U.S. Pharmacopeial Convention, Inc., Rockvill Md. 20852.

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Solid-State Pharmaceutical Chemistry

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Solid-state pharmaceutical chemistry encompasses a wide range of studies on pharmaceutical solids including (1) determination of the physical properties of polymorphs and solvates, (2) physical transformations between polymorphs and solvates, (3) chemical reactions in the solid state, and (4) solid–solid reactions which occur in pharmaceutical preparations. Recent advances in this field include improved understanding of crystallization processes, improved understanding of the need for characterization of polymorphs and solvates for both control and regulatory purposes, and a better understanding of the mechanisms of solid-state degradations and solid–solid reactions. This review will briefly describe recent advances in the following areas: (1) crystallization and the properties of crystals of pharmaceutical solids; (2) characterizations of crystal forms of drugs using solid-state NMR spectroscopy.

The study of the solid-state chemistry of drugs not only encompasses many scientific disciplines but also impinges on virtually all phases of the pharmaceutical industry, from discovery to successful marketing. It is clear that an understanding of the molecular structure of the solid state can lead to better design and control of drug performance.

The mission of those working in the field of solid-state pharmaceutical chemistry is to provide each drug in a solid form that has optimum performance in a given application. Pursuit of this mission requires recognition of several general, interrelated points: (1) Drugs can exist in a number of solid forms, each having different properties of pharmaceutical importance, including stability and bioavailability; the number and properties of these forms are largely unpredictable and vary considerably from case to case. (2) The forms of a drug may interconvert under various conditions. (3) Once a solid form is chosen for a product, methods for analysis and control of the form must be devised.

Let us briefly review each of these points with regard to their status in current practice and to some associated scientific challenges that remain.

(1) The most common solid forms that may be found for a given drug substance are as follows: crystalline polymorphs, forms having the same chemical composition but different crystal structures and therefore different densities, melting points, solubilities, and many other important properties; solvates, forms containing solvent molecules within the crystal structure, giving rise to unique differences in solubility, response to atmospheric moisture, loss of solvent, etc. Sometimes a drug product may be a desolvated solvate, formed when solvent is removed from a specific solvate while the crystal structure is essentially retained—again, many important properties are unique to such a form; finally, amorphous solid forms that have no long-range molecular order (i.e., no crystallinity) and which tend to be more soluble, more prone to moistu uptake, and less chemically stable than their crystalli counterparts (pharmaceutical processing operations m produce solids of low crystallinity intermediate betwe that of a crystalline solid and an amorphous solid).

To be sure, differentiating among the various solid for of a substance is generally a routine matter. A numb of analytical methods, used together, make this possib Various familiar methods reveal the chemical compositi of the solid and reveal the presence of solvent. The physic form is further characterized by X-ray powder diffractic infrared and solid-state NMR spectroscopy, differenti scanning calorimetry, and microscopy. The single mc valuable piece of information about a crystalline soli although not always available, is the molecular structu determined by single-crystal X-ray diffraction.

Perhaps the chief challenge in managing the phenor enon of multiple solid forms of drugs is our inability predict how many forms can be expected in a given cas too often costly delays are encountered when a less solub solid form suddenly appears late in a developme program. Progress along these lines awaits analysis an quantification of the myriad intermolecular forces with any proposed crystal structure as well as the ability postulate the likely packing modes for a given molecu in all its configurations. Further research similar to th of Margaret Etter and co-workers, reviewed in section will doubtless lead to better success in predicting altern. tive solid forms of new drugs.

A second challenge relates to the strikingly differer reactivity of different solid forms of many substance whether it be oxidation, dehydration, decarboxylation, c other chemical reactions. Kinetics involving the solid stat in which specific contacts (or noncontacts) betwee reactive groups are dictated by the structure of a give solid form, are apt to be more complex than kinetics i solution, where the corresponding molecular encounter are much more random. Much the same can be said abou the relative difficulty of elucidating mechanisms in thes two states. Moreover, reactions in the solid state may b further complicated by other, often unknown factors suc

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type	functional group
reliable donor	-OH,[a] $-NH_2$, -NHR, $-CONH_2$, -CONHR, -COOH
occasional donor	-COH, -XH, -SH, -CH
reliable acceptors	-COOH, $-CONHCO-$, -NHCONH-, -CON< (1-3°), >P—O, >S—O, -O-
occasional acceptors	>O, $-NO_2$, -CN, -CO, -COOR, -N<, -

[a] Not COH.

Etter, MacDonald, and Bernstein developed a graph-theory-based approach to classifying and symbolically representing the different types of hydrogen bonds that can be formed.[5]

Etter also developed rules governing hydrogen bonding in solids. These rules require a classification of hydrogen bond donors and acceptors into "reliable" hydrogen-bond donors and acceptors and "occasional" donors and acceptors (Table 1). Using these classifications, three rules were devised: (1) All (or as many as possible) good proton donors and acceptors are used in hydrogen bonding. (2) Six-membered ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds. (3) The best proton donors and acceptors remaining after intramolecular hydrogen bond formation will form intermolecular hydrogen bonds. These rules apply quite well to hydrogen bonding of small molecules. However, in some larger molecules, e.g., erythromycins, steric factors make it impossible to satisfy all of the possible hydrogen bonded interactions, and some donors and acceptors are not involved in any hydrogen bonds.

Cocrystals. An important aspect of research into hydrogen bonding involves the realization that cocrystals can be obtained from certain solutions containing more than one molecular species.[6] Cocrystals can also be formed by mixing or grinding two solids together. Cocrystals are usually formed between a hydrogen-bond donor molecule and a hydrogen-bond acceptor molecule. The nature of hydrogen bonding in cocrystals can also be described using the above rules. The cocrystals observed by Etter's group include numerous ureas with ketones, carboxylic acids with 2-aminopyridine, and also adenine or cytosine combined with many acidic organic compounds[6] such as carboxylic acids and *N*-acylamino acids. Other classes of cocrystals investigated by Etter's group are

pyrimidine, pyridines: carboxylic acids

pyridine-N-oxides: acids, alcohols, amines

phosphine oxides: acids, amides, alcohols, ureas sulfonamides, amines, water

carboxylic acids: other carboxylic acide, amides

m-dinitroureas: acids, ethers, phosphine oxides, sulfoxides, nitroanilines

imides: other imides, amides

The formation of cocrystals may be quite important in explaining solid–solid interactions in many fields including those of pharmaceuticals.

(4) Etter, M. C.; Reutzel, S. M. *J. Am. Chem. Soc.* 1991, *113*, 2586–98.
(5) Etter, M. C.; MacDonald, J. C.; Bernstein, J. *Acta Crystallogr., Sect. B* 1990, *B46*, 256–62.
(6) Etter, M. C.; Reutzel, S. M. *J. Am. Chem. Soc.* 1991, *113*, 2586–98.

This elegant work of Etter and co-workers has greatly increased our understanding of the hydrogen-bonding interactions of molecules in both the solid state and in solution.

B. How Crystals Form

Crystallization and the factors controlling the formation of crystals is an extremely important area in solid-state pharmaceutical chemistry. Dr. Margaret Etter made an extremely important observation when she pointed out that molecules in solution often tend to form different types of hydrogen-bonded aggregates and hypothesized that these aggregate precursors are related to the crystal structures that form from the supersaturated solution.[2] This concept helps to explain the many different hydrogen-bonding motifs seen in different solids.

A number of factors can affect the crystal formed either by influencing the hydrogen-bonded aggregate precursors in solution or influencing one of the many other factors involved in crystallization. These include (1) solvent composition or polarity, (2) concentration or degree of supersaturation, (3) temperature including cooling rate and the cooling profile, (4) additives, (5) seeds and the presence of seeds, (6) pH (pH is important for crystallization of salts), and (7) agitation.

The composition of the solvent used is known to influence crystallizations either directly or by influencing the temperature at which the crystallization is initiated. For example, in the mannitol system, the α-polymorph is formed by evaporation of 100% ethanol while the β-polymorph is formed by crystallization from aqueous ethanol.[7] In a study of inosine, Suzuki showed that crystallization from water gave the α-form whereas crystallization from 70% DMSO gave the β-form.[8]

A second important factor influencing crystallization is the degree of supersaturation—the ratio of the concentration of the solution to that of a saturated solution. In his study of the polymorphism of cimetidine, Sudo showed[9,10] that in isopropyl alcohol at high supersaturation ratios (greater than 3.6) form A crystallized spontaneously or in the presence of seeds of either form A or form B, whereas in lower supersaturation ratios (less than 2) form A crystallized if there were A seeds and B crystallized if there were B seeds.

Temperature can have a very significant effect on the polymorph produced. Studies by Kitamura[11] on the crystallization of L-glutamic acid showed that at 45° the α-form nucleates slowly resulting in β-form growth, whereas at 25 °C the α-form nucleates rapidly causing α-form growth.

The effect of additives on crystallization has been of interest for many years. Early work by Simonelli indicated that polymeric additives could prevent the crystallization of certain phases.[12] Significantly, studies in recent years by Lahav and co-workers[13] have shown that additives (as

(7) Berman, H. M.; Jeffrey, G. A.; Rosenstein, R. D. *Acta Crystallogr.* 1968, *B24*, 442–449.
(8) Suzuki, Y. *Bull. Chem. Soc. Jpn.* 1974, *47*, 2551–2552.
(9) Sudo, S.; Sato, K.; Harano, Y. *J. Chem. Eng. Jpn.* 1991, *24*, 237–242.
(10) Sudo, S.; Sato, K.; Harano, Y. *J. Chem. Eng. Jpn.* 1991, *24*, 625–632.
(11) Kitamura, M. *J. Cryst. Growth* 1989, *96*, 541–546.
(12) Simonelli, A. P.; Mehta, S. C.; Higuchi, W. I. *J. Pharm. Sci.* 1970, *59*, 633.
(13) Weissbuch, I.; Addadi, L.; Lahav, M.; Leiserowitz, L. *Science* 1991, *253*, 637–646.

stirring gives mostly crystals [illegible] D. Investigation showed that [illegible] effect was due to the fact that once a particular crystal (either L or D) has formed, when that initial crystal collides with the stirrer and is broken into many small seeds which then nucleate further crystallization of that hand crystal (either L or D). This observation supports the idea that one nucleating seed can produce a single crystalline form. It also suggests that in this particular crystallization, if one added a seed of either L or D crystals then one would obtain entirely that crystal form. This is one of the best examples of the concept: one seed, one crystal.

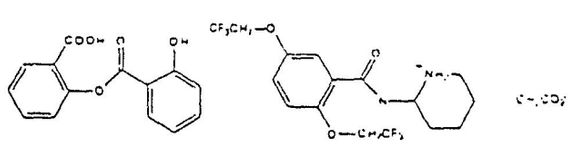
McCrone, in a letter to the editor of the *Journal of Applied Crystallography*, suggested that nucleation by seeding was the best explanation for the situation in which one unexpectedly obtains a new, more stable crystal form and then finds it is difficult or impossible to obtain the older less stable crystal form.[23] In response to this letter Jacewicz et al.[24] suggested that it is not impossible to produce the earlier form. They stated that the original crystal form should be capable of being produced but that the selection of the right conditions may require some time and trouble.

C. Disorder in Crystals of Pharmaceutical Solids

Another solid-state phenomenon which has important implications for pharmaceutical solids is disorder. Just as crystals of chiral and racemic drugs may have different physical properties, the presence of disorder in a crystal may also affect the physical properties of a crystal. One method of classifying disorder is to distinguish between cases involving static and dynamic disorder. Because single-crystal X-ray techniques give results which are averages over a long time period relative to molecular motion, X-ray crystallography at one temperature is usually unable to distinguish between static and dynamic disorder. Static and dynamic disorder can often be distinguished by comparing X-ray thermal parameters (ADPs) for two different temperatures. If the disorder results in observable resonance peaks in solid-state NMR spectra, then clearly this technique can be used to study this disorder.

In the crystallographic literature there are numerous examples of disorder which are caused by a molecule crystallizing in a single conformation but in a different orientation relative to other molecules in the crystal. There are also examples where the disorder is due to molecules crystallizing in different conformations.

Two such examples of conformational disorder are the crystal structures of nonsteroidal antiinflammatory (NSAID) drug salicylsalicylic acid (SALSALATE)[25,26] and the antiarrhythmic compound flecainide (TAMBOCOR).[27]



SALSALATE TAMBOCOR

(23) McCrone, W. C. *J. Appl. Crystallogr.* 1975, *8*, 342.
(24) Jacewicz, V. W.; Nayler, J. H. C. *J. Appl. Crystallogr.* 1979, *12*, 396–397.

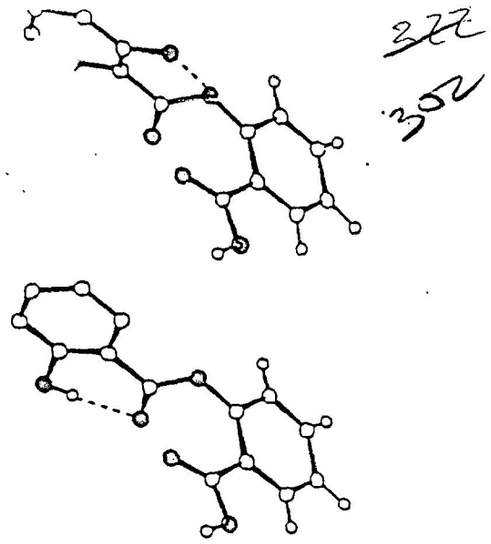


Figure 1. The two geometries of the SALSALATE mole which are incorporated into the disordered crystal.

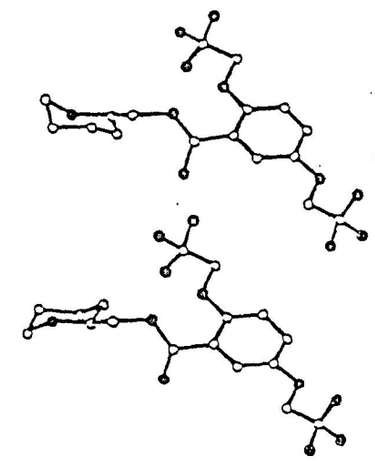


Figure 2. The two geometries of the TAMBOCOR which incorporated into the disordered crystal.

The salsalate molecule can crystallize in two differe conformations, each of which is intramolecularly hydrog bonded. Presumably, these two different intramolecu modes of hydrogen bonding are roughly equivale energetically and the gross shape of the molecule in bc conformations is similar so that both conformations c be incorporated into a single crystal as shown in Figu 1.

The other interesting example is the antiarrhythm compound flecainide which is disordered in the solid sta The form of the compound which exhibits this disorc is actually the acetate salt, which was originally used i therapeutic purposes. Here the piperidinium ring tak on two alternate chair conformations as shown in Figu 2.

What are the consequences of such disorder? At t. level of processing, the difficulty in reproducing mater with the same properties may be responsible for differe

(25) Frink, N.; Gleason, W. B.; Sweeting, L. M. In *IVth Midwe Organic Solid-State Chemistry Symposium*; Lincoln, NE. 1992.
(26) Frink, N.; Beaurline, J.; Gleason, W. B. In *Am. Cryst. Asso Annual Meeting*; Pittsburgh, PA. 1992.
(27) Gleason, W. B.; Bannit, E. H. In *Am. Cryst. Assoc., Annu Meeting*, 1987; p PE18.

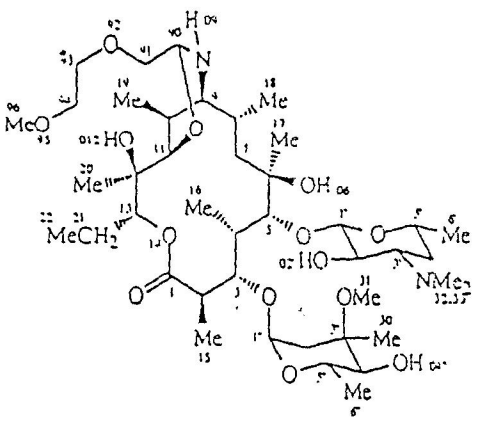
which a drug crystallizes ... to a drug ... improvement in understanding the stability, bioavailability, and ease of processing of pharmaceutical solids.

1. Infrared Spectroscopy of Solids. Infrared spectroscopy is quite useful for the analysis of solids, because it can be performed on small amounts of the solid substance. The method involves grinding the sample and measuring its infrared spectrum in one of several types of cells available. Infrared spectra of solids can also be obtained after suspending the sample in Nujol or grinding the sample with KBr and compressing this mixture into a disc. FT-IR microscopy can be used to measure the infrared spectrum of a single crystal or group of crystals. If care is taken to study only crystals of the same morphology, this method minimizes the possibility of obtaining infrared spectra of mixtures of crystal forms which is a distinct advantage over approaches that use powdered samples.

The infrared spectrum is extremely sensitive to the structure and conformation of a compound and, thus, can be used to compare the structure and conformation of the compound in different solids or in solid and solution. Infrared spectroscopy is thus a powerful method for the determination of polymorphs.

Cholerton et al.[29] reported the use of infrared spectroscopy to differentiate form 1 and form 2 of ranitidine hydrochloride (Figure 3). This is a classic example of the power of infrared analysis in polymorph studies.

Infrared spectroscopy is also useful for the investigation of solvates, as demonstrated by a study of erythromycin derivative (I).[30] Figure 4 shows the spectra of forms I and



II and the amorphous form of this compound. Clearly these three forms have different spectra especially in the carbonyl region. Note that form I shows two carbonyl absorptions, whereas the amorphous form and form II show only a single carbonyl absorption. Figure 5 shows the infrared spectra of three of the many solvates of this erythromycin derivative. These spectra are fairly similar except for the obvious C≡N stretching absorption in the acetonitrile-$3H_2O$ solvate. There are also slight differences among these three solvates in the fingerprint region. Figure 6 shows the infrared spectra of six solvates which are isomorphous (have the same crystallographic unit cell). These spectra are fairly similar as might be expected since they crystallize in the same unit cell. In cases where the solvents contain keto groups (e and f) the ketone absorption is obvious as a second carbonyl absorption in the spectrum.

(29) Cholerton, T. *J. Chem. Soc., Perkin Trans 2* 1984, 1761.
(30) Stephenson, G. Unpublished observations, 1993.

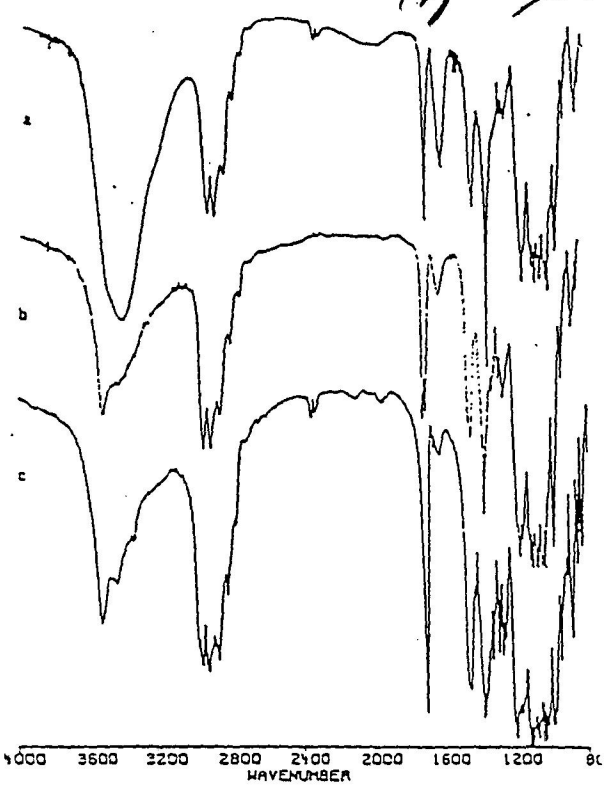


Figure 4. Infrared spectra of the amorphous form (a) and anhydrous forms, I and II, (b and c) of an erythromycin derivative.[30]

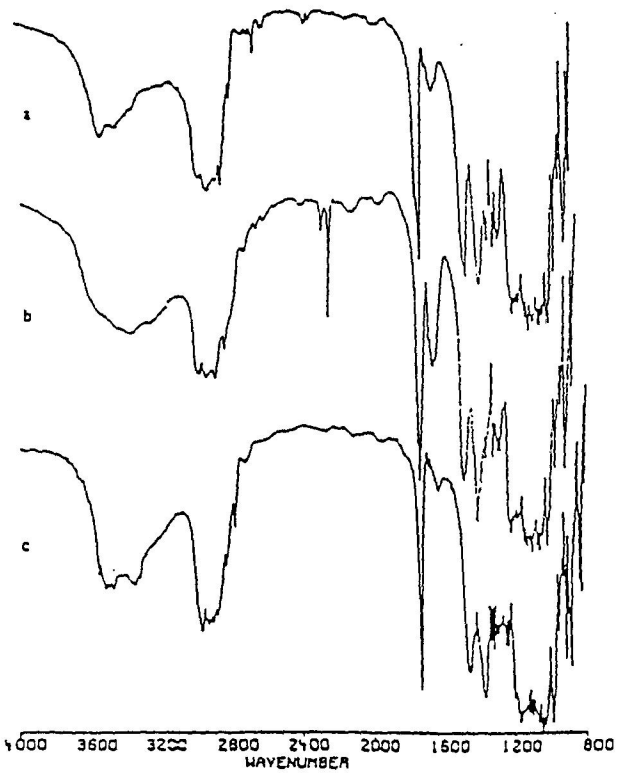


Figure 5. Infrared spectra of three nonisomorphic solvates an erythromycin derivative: (a) tricyclohexane, (b) acetonitrile-$3H_2O$, and (c) *tert*-butyl alcohol solvate.[30]

Figure 7 shows an enlarged view of the ester carbonyl regic of the spectrum in three of the crystal forms of th erythromycin derivative. These spectra suggest that th *n*-propanol solvate contains non-hydrogen-bonded ca: bonyl groups, that form II contains hydrogen-bonde carbonyl groups, and that form I contains both hydroger bonded and non-hydrogen-bonded carbonyl groups. Thes suggestions have been confirmed by the crystal structure

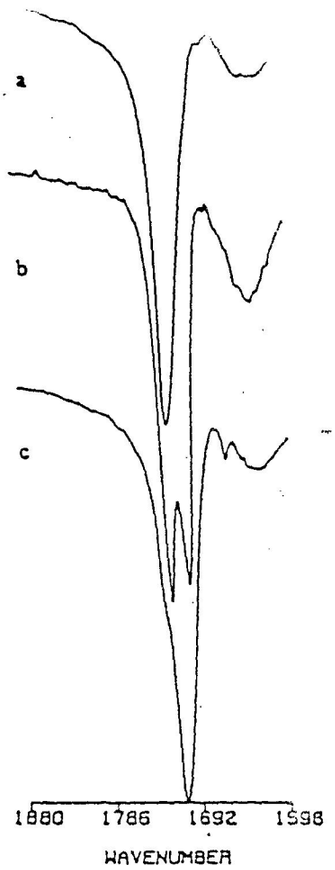
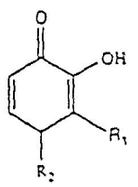


Figure 7. Ester carbonyl region of the *n*-propanol solvate (a), form I (b), and form II (c).[30]

where the stronger the hydrogen bonding the greater the chemical shift changes in ppm.

This type of correlation for different polymorphs and solvates shown in Figure 11 may also apply to members of a series of closely related compounds. Figure 12 shows that the $^{13}C(4)$ chemical shift difference between solid state and solution is a negative linear function of the crystallographic O(4)···O hydrogen bond distance in the crystal structures of a series of 1,2-dialkyl-3-hydroxy-4-pyridones (DAHPs) whose molecular structures are shown below:[34]



EMHP	$R_1 = CH_3$	$R_2 = C_2H_5$
PMHP	$R_1 = CH_3$	$R_2 = C_3H_7$
BMHP	$R_1 = CH_3$	$R_2 = C_4H_9$
MEHP	$R_1 = C_2H_5$	$R_2 = CH_3$
DEHP	$R_1 = C_2H_5$	$R_2 = C_2H_5$
EMHP	$R_1 = C_2H_5$	$R_2 = C_3H_7$

The DHAPs are iron chelators some of which may be promising drugs for oral administration in the treatment of diseases associated with iron overload. Although the

(34) Ghosh, S. Ph.D. Thesis, University of Minnesota, 1993.

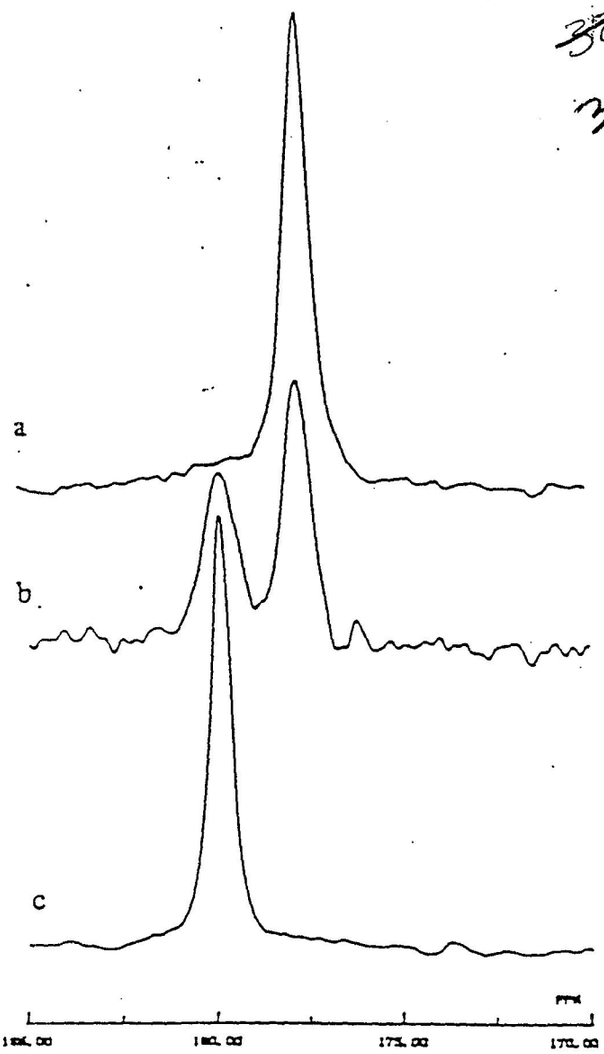


Figure 8. Solid-state NMR spectra of the ester carbonyl carbon: (a) *n*-propanol solvate, (b) form I, and (c) hydrogen-bonded form II.[30]

crystal structure of the compound BMHP could not be determined, this compound provided an excellent ^{13}C NMR spectrum. The measured $^{13}C(4)$ chemical shift of BMHP might enable the O(4)···O hydrogen bond distance to be predicted using Figure 12, if this correlation is generally applicable.

Solid-state NMR is also useful for the study of mobility in solids. The rate of relaxation of the carbon spins toward equilibrium is characterized by the spin lattice relaxation time, T_1.[35] In this process the excess energy from the spin system is transferred to "the lattice". Interactions of randomly fluctuating magnetic fields at the Larmor frequency of the nucleus stimulate these transitions. Such fields arise from motions of other nuclear magnetic moments such as protons. Spin–lattice relaxation is thus most efficient when these motions are near the Larmor frequency of the nucleus being observed. Studies of relaxation times can give valuable information about fast motions in the megahertz range such as methyl group rotations. Numerous studies of molecular motion using

(35) Fukushima, E.; Roeder, S. B. W. *Experimental Pulse NMR*; Addison Wesley: Reading, MA, 1988.

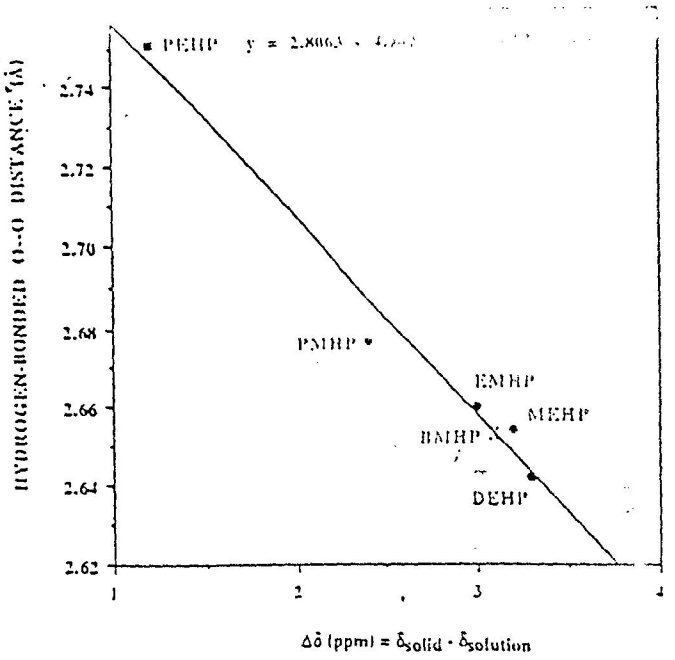


Figure 12. Plot of the hydrogen bonded O---O distances against the differences ($\Delta\delta$) between the ^{13}C chemical shifts of the pyridone carbonyl (O4) and the solid state (δ_{solid}) and the solution state ($\delta_{solution}$) of DAHPs. Although the crystal structure of BMHP is unknown, its hydrogen-bonded O---O distance can be estimated from the plot using the NMR chemical shift data (point X).[34]

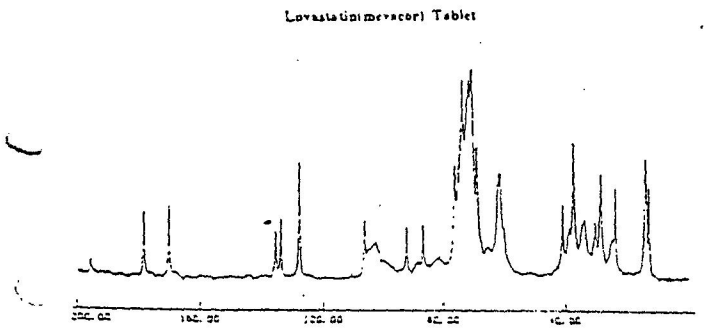
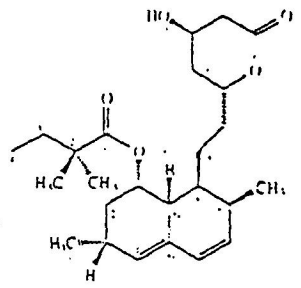


Figure 13. Solid-state CP/MAS NMR spectrum of a lovastatin tablet.

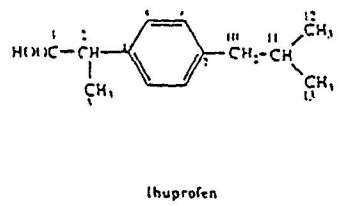
enhanced chemical reactivity of these materials in the presence of water.[46] Further study of the relationship between molecular mobility and solid-state reactivity requires the development of new approaches to determine molecular mobility especially in mixtures such as pharmaceutical dosage forms where single-crystal X-ray methods cannot be used.

Solid-state NMR offers several approaches to studying the molecular mobility of solids. These include (1) determination of the activation energies for T_1 relaxation of individual carbon atoms using variable-temperature solid-state NMR, (2) study of processes which result in peak coalescence of solid-state NMR resonances using variable-temperature solid-state NMR, (3) use of interrupted decoupling to detect methylene and possible methine groups with unusual mobility, and (4) comparison of solid-state MAS spectra measured with and without cross polarization.

Recent research at Purdue University is using all of these approaches but especially the first one to study the relative mobility of methyl groups in a number of drugs which contain multiple methyl groups including simvastatin, lovastatin, ibuprofen, and phenacetin.[47-49] The results of these studies will be published shortly.



Simvastatin



Ibuprofen

In addition, Maverick and Dunitz[50] have suggested that solid-state NMR activation energies and X-ray thermal parameters are related. Therefore, solid-state NMR appears to be a good method for study of the mobility of methyl groups in solids. Because solid-state NMR can be used to study pharmaceutical dosage forms and other mixtures,[31] this powerful method will find increased application in the determination of the molecular mobility of drugs in mixtures and dosage forms.

Surprisingly little research has been done on the solid-state NMR spectra of pharmaceutical dosage forms. To our knowledge, prior to the paper by Saindon et al.,[31] solid-state NMR has been used to study only two tablets both consisting almost entirely of pure drug: aspirin[51,52] and acetaminophen.[53] This paper shows that solid-state NMR is useful for the study of more complex pharmaceutical products.[31]

For example, Figure 13 shows the solid-state NMR spectrum of lovastatin tablets, which contain a rather large excess of excipients. The excipients, which have resonances in the 50–100 ppm range obscure resonances of lovastatin which appear at the same chemical shift but have little effect on resonances at other chemical shifts. Similar results were obtained for tablets of another closely related HMG-CoA reductase inhibitor, simvastatin.

E. Summary

In this review, areas of particular interest to Peggy Etter and her group were discussed including crystallization, hydrogen bonding, solvates and solid-state NMR. It is clear that her pioneering work has tremendously important implications for solid-state pharmaceutical chemistry.

(44) Byrn, S. R.; Lin, C. T. *J. Am. Chem. Soc.* 1976, *98*, 4004.
(45) Byrn, S. R.; Kessler, D. W. *Tetrahedron* 1987, *43*, 1335–43.
(46) Zografi, G. Z.; Ahlneck, K. V. *Int. J. Pharm.* 1990, *62*, 87.
(47) Cauchon, N. Ph.D. Thesis, Purdue University, 1992.
(48) Saindon, P. Ph.D. Thesis, Purdue University, 1992.
(49) Toma, P. Ph.D. Thesis, Purdue University, 1993.
(50) Maverick, E.; Dunitz, J. *Mol. Phys.* 1987, *62*, 451.
(51) Chang, C. J.; Diaz, L. E.; Morin, F.; Grant, D. *Magn. Reson. Chem.* 1986, *1986*, 768.
(52) Diaz, L. E.; Frydman, L.; Olivieri, A.; Frydman, B. *Anal. Lett.* 1987, *20*, 1657.
(53) Jagannathan, N. R. *Curr. Sci.* 1988, *56*, 827.

Disappearing Polymorphs

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Introduction

When a compound exhibits polymorphism—the existence of more than one crystal structure—it may be important to obtain a particular polymorph under controlled and reproducible conditions. However, this is not always easy to achieve. Tales of difficulties in obtaining crystals of a particular known form or in reproducing results from another laboratory (or even from one's own!) abound. Indeed, there are cases where it was difficult to obtain a given polymorphic form even though this had previously been obtained routinely over long time periods. Several monographs contain explicit or passing references to these problems,[1] but much of this lore has gone undocumented, especially in the last 30 years or so. In this Account we present and discuss old and new examples.

Crystallization is a process taken for granted by most practicing chemists; the majority of the techniques were developed long ago and are described in all standard laboratory textbooks. It is the standard method for purifying solid compounds, and chemists generally believe that they can control the process, at least when it yields the desired product. What is disturbing about the phenomenon of disappearing or elusive polymorphs is the apparent loss of control over the process: we did the experiment last week and got this result, and now we cannot repeat it! This kind of statement can lead to raised eyebrows or even to outspoken expressions of disbelief. We have ourselves experienced the frustration of not being able to reproduce an experimental result that was undoubtedly obtained earlier.

Crystallization: Nucleation and Growth

The process of crystallization of a compound from solution or from the melt is poorly understood. At least two stages must be distinguished: the formation of a critical nucleus and its subsequent growth. The first step is decisive in that it can be regarded as being associated with a free energy of activation and is therefore rate limiting. Under suitable conditions, that step may be delayed almost indefinitely. For instance, Faraday[2] observed that molten sulfur in a flask cooled to room temperature did not entirely solidify. When a drop of the fluid material was touched, it immediately crystallized; untouched, some drops were retained for a week in the fluid state. Faraday noted that this supercooled state of sulfur is analogous to that of water cooled below its freezing point, although the temperature difference is much greater (the freezing point of sulfur is 119 °C); De Coppet found that samples of salol (phenyl salicylate) could be kept in the liquid state at room temperature for periods of several years.[3] When nucleation is rapid, the formation of many nuclei leads to many crystals, whereas slow nucleation tends to produce a smaller number of larger crystals. Of course, stirring, shaking, or other disturbances of the liquid phase during the crystallization process can affect the outcome.

A striking case where nucleation was decisive in determining the result of a crystallization experiment has been described recently.[4] Sodium chlorate ($NaClO_3$) crystallizes in the chiral space group $P2_13$; that is to say, individual crystals of this substance may occur in enantiomorphic forms. Normally, crystallization from solution produces the enantiomorphs in roughly equal numbers. Kondepudi, Kaufman, and Singh[5] found, however, that stirring an aqueous solution of this substance leads to a predominance of

Jack D. Dunitz was born in Glasgow, Scotland, in 1923 and studied chemistry at Glasgow University. Following a decade of postdoctoral studies at Oxford, Caltech, NIH, and the Royal Institution, London, he moved to the ETH in Zurich as professor of chemical crystallography, a post he held until his retirement in 1990. He is the author of *X-Ray Analysis and the Structure of Organic Molecules* (1979) and (with E. Heilbronner) *Reflections on Symmetry in Chemistry... and Elsewhere* (1993).

Joel Bernstein was born in Cleveland, OH, in 1941. He received his B.A. in chemistry from Cornell University in 1962 and Ph.D. in physical chemistry from Yale in 1967. Following postdoctoral stints in chemical crystallography at UCLA with K. N. Trueblood and organic solid state chemistry at the Weizmann Institute with G. M. J. Schmidt he moved to the Ben-Gurion University of the Negev in Beer Sheva Israel, where he is now professor of chemistry. His research interests include a variety of aspects of the chemistry of the organic solid state, including polymorphism, structure–activity relationships, hydrogen bonding, and organic conductors.

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(1) Buckley, H. E. *Crystal Growth*; Wiley: New York, 1951. Tipson, R. S. Crystallization and Recrystallization. In *Technique of Organic Chemistry*; Weissberger, A., Ed.; Interscience Publishers, Inc.: New York, 1956; Volume III, Part I, Chapter III, pp 395–562. Holden, A.; Singer, P. *Crystals and Crystal Growing*; Doubleday: New York, 1960.

(2) Faraday, M. *Experimental Researches in Chemistry and Physics*; Taylor and Francis: London, 1853; p 212. On the following page, Faraday apologized for not having acknowledged observations along similar lines made earlier (in 1813) by M. Bellani: "I very fully join in the regret...that scientific men do not know more perfectly what has been done, or what their companions are doing; but I am afraid the misfortune is inevitable. It is certainly impossible for any person who wishes to spend a portion of his time to chemical experiment, to read all the books and papers that are published in connection with his pursuit; their number is immense, and the labour of winnowing out the few experimental and theoretical truths which in many of them are embarrassed by a very large proportion of uninteresting matter, of imagination, and of error, is such, that most persons who try the experiment are quickly induced to make a selection in their reading, and thus, inadvertently, at times, pass by what is really good." Since Faraday's times, these difficulties have multiplied out of all proportion, but we may still use his words to apologize to any scientists whose works we may similarly have overlooked.

(3) De Coppet, M. L.-C. *Ann. Chim. Phys.* **1907**, *10*, 457. "La surfusion dure donc depuis bientôt 6 ans." In another experiment, de Coppet reported that a sample of sodium sulfate, supersaturated with respect to the decahydrate, had still not crystallized after 25 years. In general, the higher the temperature to which the liquid was raised and the longer the time it was held at high temperature, the more resistant the liquid was to crystallization. Heating a liquid destroys residual order.

(4) McBride, J. M.; Carter, R. L. *Angew. Chem.* **1991**, *103*, 298; *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 293.

(5) Kondepudi, D. K.; Kaufman, R. J.; Singh, N. *Science* **1990**, *250*, 975.

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crystals of one handedness, sometimes right, sometimes left, but not depending on the direction of stirring. In checking this result, McBride and Carter[4] showed by video recording that a single nucleation event can produce almost all of the crystals formed: "...Crystals begin nucleating at random, but the first crystal to be struck by the stirrer clones hundreds or thousands of new nuclei. Growth of so many nuclei soon lowers the concentration of the solute below the threshold for spontaneous formation of primary nuclei, so that there is no way to begin crystallization of the enantiomer."

Seeding

One way of influencing the crystallization process is by seeding, and here we need to differentiate between what we may term intentional and unintentional seeding. Intentional seeding is a common practice among chemists who wish to coax crystallization of a compound from solution or from the melt; small crystals or crystallites of the desired material (seeds) are added to the system. In this way, the rate-limiting nucleation step, which may be extremely slow, is circumvented. For this method to be applied, it is of course necessary that a sample of the crystalline material is available; that is, the compound must have been already crystallized in a previous experiment. When polymorphic forms of a substance are known to occur, intentional seeding with one of the polymorphs is a useful and often the most successful way of preferentially producing it rather than the other.

Seeding may also occur if small amounts of the crystalline material are present as contaminants: unintentional seeding.[5] Unintentional seeding is often invoked as an explanation of phenomena which otherwise are difficult to interpret. We shall argue in favor of this explanation, although there is no consensus about the size and range of activity of such seeds, which have never actually been directly observed.[7] Estimates of the size of a critical nucleus range from a few tens of molecules to a few million molecules.[8] With a size of about a million molecules, even a speck (10^{-6} g) of a compound of molecular weight 100 contains approximately 10^{16} molecules, sufficient to make 10^{10} such nuclei. One can think of local seeding, where the contamination may apply to the experimentalist's clothing, a portion of a room, an entire room, a building, or even, with increasing degrees of implausibility, to a district, a town, a country, a continent, and so on. In the limit we have what has been proposed as universal seeding (planetary seeding would be a more accurate expression), where the whole planet is assumed to be contaminated.[9] A seed that promotes formation of a crystallization nucleus need not necessarily be composed of the same molecules as the compound that is to be crystallized. Specks of dust, smoke particles, and other small foreign bodies can act as seeds in promoting crystallization, which is the reason laboratory chemists often scratch the walls of a glass vessel with a glass rod to encourage a solute to crystallize.[10]

Polymorphism

We have mentioned the phenomenon of polymorphism, which is commonly understood as connoting the ability of a compound (or of an element) to crystallize in more than one distinct crystal structure. According to McCrone,[11] "A polymorph is a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state." Because polymorphs have different structures, they may differ greatly in density, hardness, solubility, and optical and electrical properties; e.g., diamond and graphite are two polymorphic forms (allotropes) of carbon. Many compounds are known to crystallize in polymorphic forms. In the inorganic and mineralogical fields, these sometimes have different names, e.g., ZnS, wurtzite and sphalerite; $CaCO_3$, calcite, aragonite, and vaterite; TiO_2, rutile, brookite, and anatase; but, more generally, different polymorphic forms are denoted by letters, A, B, C or α, β, γ, etc., or by Roman numerals, I, II, III, etc., depending on the preference of the discoverer. McCrone[11] has provocatively suggested that "every compound has different polymorphic forms, and that, in general, the number of forms known for a given compound is proportional to the time and money spent in research on that compound." In support of this, McCrone observes that many compounds of industrial importance (i.e., those on which a great deal of time and money are spent) are known to exhibit polymorphism: silica, iron, calcium silicate, sulfur, soap, pharmaceutical products, dyes, and explosives. Such compounds, unlike the vast majority of compounds that are isolated, are prepared and crystallized not just once but repeatedly, under conditions that may vary slightly from time to time. Similarly, in the biomolecular area, where much time and effort is invested in attempts to crystallize proteins under many slightly different conditions, polymorphism is frequently observed.[12] The universality suggested by McCrone's statement may, however, be considerably tempered by the fact that fewer than 5% of the compounds in the Cambridge Structural Database (CSD) are known to be polymorphic (although it must be admitted that crystallographers typically choose one crystal specimen from their sample and leave it at that). Moreover, some very widely studied compounds have shown no evidence of polymorphic behavior, even though they have been crystallized and handled for many years under a far-ranging variety of conditions; naphthalene is an example that immediately comes to mind.

Here we shall be concerned exclusively with molecular crystals, where the molecule may have the same shape in the two polymorphs or it may have a different shape, resulting in what has been termed "conforma-

(6) It is well-known that it is often difficult to crystallize a newly synthesized compound. Subsequent crystallizations may be easier, because of the presence of suitable seeds.

(7) Chemists and physicists have long become accustomed to postulating models as explanations for phenomena that cannot be directly observed. The existence of atoms is perhaps the classic example.

(8) Mullin, J. W. *Crystallization*, 3rd ed.; Butterworth-Heineman Ltd.: Oxford, 1993; pp 182–185.

(9) The claim for "universal seeding", taken literally, is obviously absurd. After all, the universe is estimated to contain about a millimole of stars, so one seed per star (per solar system)—not much—would need about 100 kg of the compound in question (MW ≈ 100).

(10) "Auch das Reiben mit einem Glasstab an der Wandung des Gefässes schafft Keime, an deren Vorhandensein die Kristallisation gebunden ist." *Organikum*; VEB Deutscher Verlag der Wissenschaften: Berlin, 1977; p 46.

(11) McCrone, W. C. Polymorphism In *Physics and Chemistry of the Organic Solid State*; Fox, D., Labes, M. M., Weissberger, A., Eds.; Interscience: New York, 1965; Vol. II, pp 726–767.

(12) For example, according to the Protein Data Bank (distributed by Brookhaven National Laboratory, Upton, NY), the extensively studied human hemoglobin is known in monoclinic, orthorhombic, and tetragonal modifications; lysozyme in triclinic, monoclinic, orthorhombic, trigonal, tetragonal, and hexagonal ones.

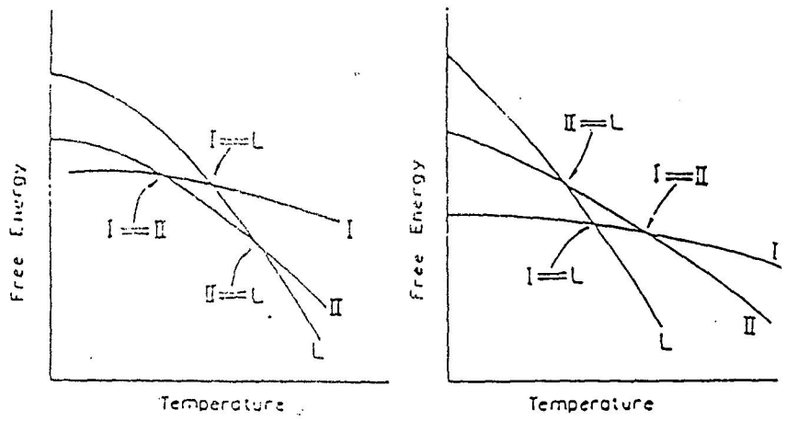


Figure 1. Free energy vs temperature diagrams for two polymorphs, with crossing points where their free energies cross: le: enantiotropic system: right, monotropic system.

tional polymorphism".[13] McCrone's criterion[11] is that polymorphs are different in crystal structure but identical in the liquid or vapor states. This implies that crystals containing molecules with different atomic arrangements are to be classed as polymorphs if the molecules concerned interconvert rapidly in the melt or in solution to give the same equilibrium mixture. Thus, this definition would encompass not only conformational isomers but all kinds of isomers in dynamic equilibrium. In phase-rule terminology,[14] the various polymorphs and the liquid obtained by melting them constitute a one-component system (or a two-component system if we consider solution of the polymorphs in a given solvent).

Clearly, this definition is not completely satisfactory and leaves several kinds of borderline cases open: are *syn*- and *anti*-oximes in the solid state to be classed as polymorphs or as separate compounds? What about the various molecular species involved in the complex equilibria among open-chain and cyclic forms of saccharides (constitutional and configurational polymorphs)? How long are we supposed to wait for equilibrium to be established? Should different hydrates or solvates of a given compound be classified as polymorphs? (The term pseudopolymorphism has been proposed to cover such cases.) Definitive answers to these and similar questions cannot be given; they depend on one's point of view. In the same way, there seems to be no unequivocal way of distinguishing between polymorphic transformations and solid-state chemical reactions. There are borderline cases that show characteristic features of both.

In molecular crystals, free energy differences between polymorphs are usually quite small, a matter of a few kilocalories/mole at most,[15] and depend on temperature, mainly because of the entropic contribution to the free energy. Because of the thermodynamic relation $G = H - TS$, the form with the higher entropy will tend to become the thermodynamically more stable form as the temperature is raised (Figure 1). Thus, over a small temperature range, and particularly between room temperature and the melting point, one polymorph or another can change from being the stable form to being metastable. If the thermodynamic transition temperature is below th melting point, the polymorphic system is known a enantiotropic (not to be confused with enantiotopic, term applied to atoms or groups in a molecule tha are related by an improper symmetry operation bu not by a proper one, e.g., the two methylene H atom in ethanol) and the transition is in principle reversible if the transition temperature is above the meltin point, then the system is monotropic and the transi tion can take place only in one direction. A metastabl form can persist for years, or it can undergo spontane ous transformation to the stable form.

Mechanisms of Polymorphic Transformations

The title of this section promises more than it can deliver, because the mechanisms of polymorphic trans formations in molecular crystals are largely unknown The one type of transformation for which some leve of understanding can be claimed is order–disorder transformations, where the high- temperature phase has essentially the same molecular arrangement as the low-temperature one and differs from it only by an increase in the crystallographic site symmetry of the structural units. This increase in apparent molecular symmetry is due to an increase in crystal disorder such that the space-averaged, time-averaged distribution of matter has a higher symmetry than the instantaneous distribution in an individual unit cell. The reverse transformation corresponds to the onset of an ordering process. Such transitions are usually classified as "second-order" from the thermodynamic point of view, and, since they are virtually the only ones that can be handled on a theoretical basis, they receive the most attention in textbooks. From reading, one might even get the impression that order–disorder transformations are the prototype of phase transitions in general, but this is not the case.

Presumably, as in the primary crystallization process, the mechanisms of most solid–solid transformations involve the formation of critical nuclei of the new phase, followed by their growth. According to Mynukh,[16] the nucleation step is critically dependent on the presence of "suitable" defects. Depending on the nature of these defects, nuclei of the new phase may be formed at different temperatures and grow at different rates. Thus, defects in the initial crystal structure may be necessary for initiating (or cata-

(13) Bernstein, J.; Hagler, A. T. *J. Am. Chem. Soc.* 1978, *100*, 673. Bernstein, J. Conformational Polymorphism In *Organic Solid State Chemistry*; Desiraju, G., Ed.; Studies in Organic Chemistry, Vol. 32; Elsevier: Amsterdam, 1987; pp 471–518.

(14) See, for example: Findlay, A.; Campbell, A. N.; Smith, N. *The Phase Rule and its Applications*, 9th ed.; Dover: New York, 1951.

(15) Kitaigorodskii, A. I. *Adv. Struct. Res. Diffr. Methods* 1970, *3*, 173.

(16) Mynukh, Yu. V. *J. Cryst. Growth* 1974, *38*, 284; *Mol. Cryst. Liq. Cryst.* 1979, *52*, 467, 505.

lyzing) nucleation of the new phas. Indeed, in some cases, the transformation can be induced by mechanically introducing defects, for example, by scratching the surface of the crystal with a pinpoint. On the other hand, there are also examples where the transformation is virtually instantaneous (and in one case even reversible), causing the crystals to "jump".[17]

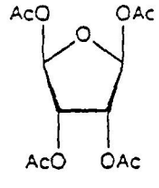
Solid-state transformations in molecular crystals often show a high degree of hysteresis. It may be necessary to heat the low-temperature form to a temperature well above the thermodynamic transition temperature before signs of phase transformation can be detected. Even when no solid–solid transformation of the low-temperature form occurs below the melting point, this is not sufficient proof that the system is monotropic; the transformation may simply be too sluggish to be observed. Similarly, transformations in the reverse direction, produced by cooling the high-temperature form, are also invariably accompanied by hysteresis. This can be so severe that a high-temperature form can sometimes be kept indefinitely at temperatures well below the transition point. Thus, X-ray structure analyses at 100 K have been made of crystal phases more than 200 K below their thermodynamic range of stability.[18]

Vanishing Polymorphs

Woodard and McCrone[19] described several cases where, after nucleation of a more stable crystal form, a previously prepared crystal form could no longer be obtained. Other examples were described by Webb and Anderson,[20] who wrote, "Within the fraternity of crystallographers anecdotes abound about crystalline compounds which, like legendary beasts, are observed once and then never seen again." In a sober comment on these views, Jacewicz and Nayler[21] criticized some of the more exaggerated claims. While admitting the role of seeding in promoting nucleation, they argue that the disappearance of the metastable form is a local and temporary phenomenon and conclude that "any authentic crystal form should be capable of being re-prepared, although selection of the right conditions may require some time and trouble."

In most of the examples cited by these authors, relevant questions are left unanswered. Many chemists remain skeptical about a subject that calls into question the criterion of reproducibility as a condition for acceptance of a phenomenon as being worthy of scientific inquiry. Nevertheless, there are well-documented cases of crystal forms that were observed over a period of time but not thereafter, having been apparently displaced by a more stable polymorph. The relevant literature is scattered and almost impossible to find by subject searches. In the remaining space we review published examples, present some new results, and try to put the subject into perspective. We begin with one of the best-studied examples.

1,2,3,5-Tetra-*O*-acetyl-β-D-ribofuranose (I). The early history of this compound reads like a mystery story. As first prepared in 1946 in Cambridge, England, by Howard, Lythgoe, and Todd,[22] the compound had melting point 58 °C.



I

Virtually the same melting point was measured for material prepared by a different method in Jena by Bredereck and Hoepfner.[23] When several batches of the same material were prepared soon afterward (1949) in a different laboratory on the other side of the Atlantic, in New York, by Davoll, Brown, and Visser,[24] the first three preparations had melting point 56–58 °C, but the fourth run yielded material with a distinctly higher melting point, 85 °C. Around the same time, in Jena, by direct acetylation of ribose, Zinner[25] obtained a mixture of two tetraacetyl derivatives, one the ribopyranose and the other the ribofuranose, with a melting point of 82 °C for the latter. The two high-melting compounds appeared to be identical, although the nature of the structural difference between them and the low-melting form was unknown. So far, so good; innumerable examples of polymorphism are known. The low-melting form can be called A, the high-melting one B:

After some time, however, the melting points of the early New York preparations had risen to 85 °C, and it was no longer possible to prepare the A form.[24] A sample of A was sent from Cambridge, but when it was exposed to the air in New York, in a laboratory that contained samples of B, the crystals of A rapidly became opaque and transformed to B. In the meantime, transformation of A to B was also found to have taken place in Cambridge. Since the A form could no longer be obtained in the New York laboratory, further experiments involving this form were moved to distant Los Angeles, where it was shown that when 1 g of A (melting point 57 °C) was inoculated with 1 mg of B (melting point 85 °C), the melting point of the sample was raised to 75–77 °C within 2 h and to 77–79 °C overnight.[24] Similar phenomena were observed in Manchester.[26] Low-melting A was first obtained, but when B was introduced into the laboratory, the whole of the material had the higher melting point and the low-melting form could no longer be prepared.[27]

The scene now changes to Philadelphia, where Patterson and Groshens[28] (the same Patterson as in the Patterson function used in crystallography) took on the task of measuring X-ray diffraction data for the two crystalline forms. Low-melting A was found to be monoclinic, space group $P2_1$, and the crystal was

(17) Gigg, J.; Gigg, R.; Payne, S.; Conant, R. *J. Chem. Soc., Perkin Trans. 1*, 1987, 2411. Ding, J.; Herbst, R.; Praefke, K.; Kohne, B.; Saenger, W. *Acta Crystallogr., Sect. B* 1991, *47*, 739. Steiner, T.; Hinrichs, W.; Saenger, W.; Gigg, R. *Ibid.*, in press. Zamir, S.; Bernstein, J.; Greenwood, D. *J. Mol. Cryst. Liq. Cryst.* 1994, *242*, 193. Etter, M. C.; Seidel, A. R. *J. Am. Chem. Soc.* 1983, *105*, 641. Kohne, B.; Praefke, K.; Mann, G. *Chimia* 1988, *42*, 139.

(18) For example, the white high-temperature modification of dimethyl 3,6-dichloro-2,5-dihydroxyterephthalate, unstable below about 340 K, crystal structure analysis at 98 K. Yang, Q.-C.; Richardson, M. F.; Dunitz, J. D. *Acta Crystallogr., Sect. B* 1989, *45*, 312. Richardson, M. F.; Yang, Q.-C.; Novotny-Bregger, E.; Dunitz, J. D. *Ibid.* 1990, *46*, 653.

(19) Woodard, G. D.; McCrone, W. C. *J. Appl. Crystallogr.* 1975, *8*, 342.

(20) Webb, J.; Anderson, B. *J. Chem. Educ.* 1978, *55*, 644.

(21) Jacewicz, V. W.; Nayler, J. H. C. *J. Appl. Crystallogr.* 1979, *12*, 396.

(22) Howard, G. A.; Lythgoe, B.; Todd, A. R. *J. Chem. Soc.* 1947, 1052.

(23) Bredereck, H.; Hoepfner, E. *Chem. Ber.* 1948, *81*, 51.

(24) Davoll, J.; Brown, B. B.; Visser, D. W. *Nature (London)* 1952, *170*, 64.

(25) Zinner, H. *Chem. Ber.* 1950, *83*, 153.

(26) Farrar, K. R. *Nature (London)* 1952, *170*, 896.

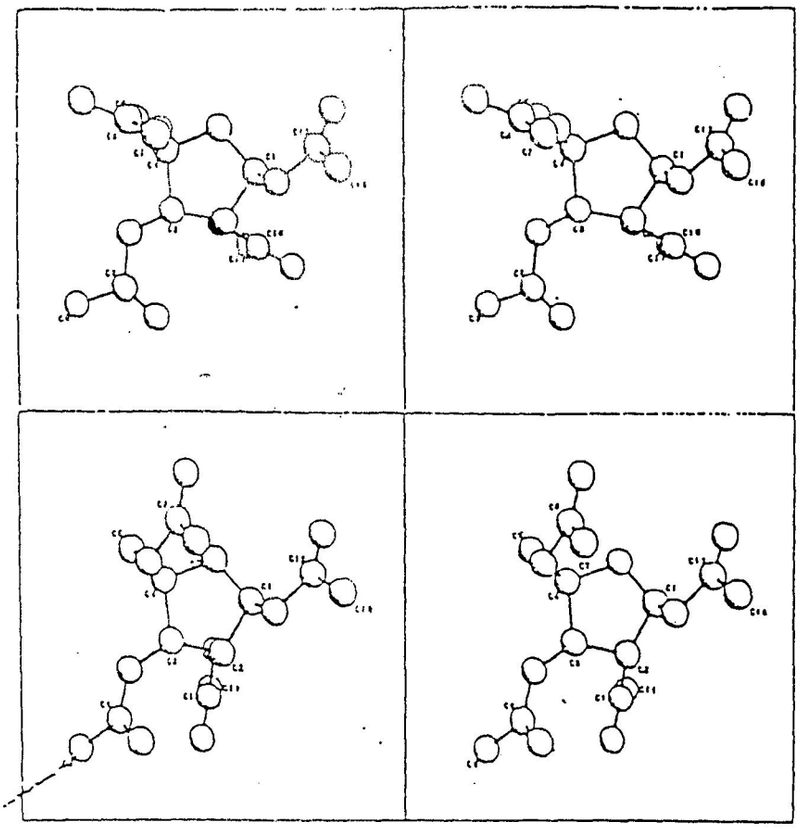


Figure 2. Stereoviews of the two forms of I. In both cases the view is on the plane of C1–O–C4 of the furanose ring: upper, monoclinic A form; lower, orthorhombic B form. For clarity, only carbon atoms are labeled.

sufficiently stable to last for 7 weeks. At the end of this time, crystals of B were introduced into the room. After three days, the A crystal was unchanged, but when powdered B was sprinkled over the A crystal, the latter transformed completely to B in a few minutes. The transformed material still had the external shape of the original A crystal, but it was opaque and polycrystalline with no preferred orientation of the crystallites. Crystals of B were found to be orthorhombic, space group $P2_12_12_1$, with quite different cell dimensions from A. Patterson and Groshens noted that the molecular volume increased by about 2% during the A to B transformation (A, 383.9 Å^3; B, 392.5 Å^3).

In the early 1950s it would have been a major undertaking to determine the atomic arrangement in these noncentrosymmmetric crystals by X-ray analysis, and it was only some 20 years later that the crystal structure of form B was determined.[29] The authors made no mention of the other polymorph. Essentially the same structure was found by Poppleton,[30] who commented that an attempt to prepare the "rare" A form by application of high pressure was unsuccessful.

Comparison of the structures of the two forms only became possible when the elusive A form was obtained in Budapest and its crystal structure determined.[31] There is no simple structural relationship between the two polymorphs; the crystal packing is quite different, and although the ribose ring and its directly attached atoms are nearly superimposable, the molecules adopt different conformations with respect to the orientations of the acetyl groups about the bonds C2–O2, C3–O3, and C5–O5 (Figure 2).

According to force-field calculations[31] the intramolecular nonbonded potential energy of the form A conformation is lower than that of the B conformation by 15.7 kJ mol^{-1}; that is, the more stable molecular structure is found in the low-melting polymorph. This is reasonable, because, as mentioned earlier, the thermodynamic stability of a high-temperature form must be due to its higher entropy rather than to its lower potential energy (see Figure 1). The increase in molecular volume on going from the A to the B form is consistent with this.

In spite of all the work done on this system, we still do not know the thermodynamic transition point, where the two free energy curves cross. From the many instances where A has been reported to transform spontaneously to B, we can infer that the transition point lies somewhat below normal laboratory temperature. Thus, form A is likely to have been present as a metastable species during most of its existence. In spite of its thermodynamic instability with respect to form B, it may have tended to crystallize first from solution because of a more rapid rate of nucleation, a kinetic factor. Once formed, the crystals of A may endure for a longer or shorter period, depending on the local temperature and other factors.

(27) The state of affairs was summarized by Brown et al. (Brown, G. B.; Davoll, J.; Lowy, B. A. *Biochem. Prep.* 1955, *4*, 70) as follows: "The form first reported melted at 58° or 56° and the form melting at 84° was initially termed the B form. A number of laboratories have observed the transformation of the low melting into the high melting form and once the latter is obtained the former is not encountered." For another contemporary account of the confusion, see: Overend, W. G.; Stacey, M. In *The Nucleic Acids*; Chargaff, E., Davidson, J. N., Eds.; Academic Press: New York, 1955; Vol. 1, p 44.

(28) Patterson, A. L.; Groshens, B. P. *Nature (London)* 1954, *173*, 396.

(29) James, V. J.; Stevens, J. D. *Cryst. Struct. Commun.* 1973, *2*, 609.

(30) Poppleton, B. J. *Acta Crystallogr., Sect. B* 1976, *32*, 2702.

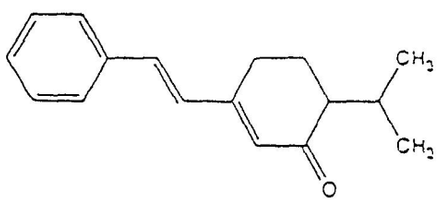
(31) Czugler, M.; Kálmán, A.; Kovács, J.; Pintér, I. *Acta Crystallogr., Sect. B* 1981, *37*, 172.

The solid-state transformation to B may take place spontaneously, or it may be catalyzed by the presence of seeds of B. In subsequent crystallization experiments in the same laboratory the presence of B seeds will circumvent the kinetic advantage of form A; once such seeds are present in the laboratory atmosphere, the lower solubility of thermodynamically stable B must tip the balance in its favor, resulting in the virtual "disappearance" of metastable A from laboratories "contaminated" by B.

It is also possible that, when first prepared, the A form was preferred thermodynamically as well as kinetically. Although normal laboratory temperature is nowadays taken as around 25 °C, this can by no means be taken as typical of former times.[32] Besides, the immediate postwar period was marked by severe fuel shortages in Europe. British and German laboratories at that time must often have been considerably colder than 25 °C, except in warm summer weather. Was form A obtained in Cambridge and Jena during cold weather conditions, when the ambient temperature fell below the thermodynamic transition point? After so many years it is difficult to find out.

There are clearly many questions left unanswered, and this is typical of the information that can be gathered today from the literature about these phenomena. The accounts of the optical rotation measurements are particularly confusing. For example, Havoll, Brown, and Visser[34] reported that when a methanolic solution of A was inoculated with a minute amount of B, the specific rotation $[\alpha]_D$ changed from about −3.5° (the normal value for solutions of A) to about −13.5° (the normal value for solutions of B). The authors were somewhat at a loss to explain this, since they considered α, β isomerism at the anomeric carbon atom to be unlikely (although we shall encounter examples later). In contrast, Farrar[26] found that solutions of the two forms in chloroform had nearly the same specific rotation $[\alpha]_D$ of about −12°, the value expected for B. From this result, correctly as we now know, Farrar considered the difference between the two forms to be merely one of dimorphism in the solid state; equilibrium among the various conformational states is attained rapidly in chloroform solution, regardless of whether the solution is prepared from the A or the B form. What about the different results in methanolic solutions of A and B? It seems most unlikely that interconversion would be slow enough to be observable from optical rotation measurements. Our tentative conclusion is that these measurements are unreliable.

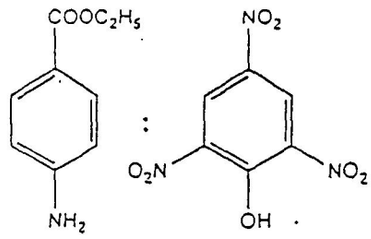
Benzylidene-*dl*-piperitone (II). The compound was first prepared in Sydney, Australia, in 1921 as large pale yellow prisms (mp 59−60 °C, α form). After a second form appeared (mp 63−64 °C, yellow rhombic prisms, β form), it was difficult to reproduce the original α form, and the conditions leading to each form were carefully determined.[33] Several years later, in St. Andrews, Scotland, only a third polymorph (mp 69−70 °C, faintly yellow small needles, γ form) could be obtained,[34] and it was found that α and β transform readily into γ "by inoculation" (seeding). Once seeds of γ were present, even intentional seeding of solutions or melts with α or β seeds yielded only the γ form. The authors wrote, "It seems clear, however, that the fortuitous presence of a nucleus of the γ form is sufficient to suppress the production of the α and β forms: such nuclei may have been carried from one building to another in the two sets of St. Andrews experiments—for example, on the clothes of the operators—in spite of precautions."



II

In 1987, the same compound was prepared in Bangalore, India,[35] by the same method as used in Sydney.[33] Only the α form was obtained and used in solid-state photochemical reactions. The authors wrote, "Innumerable attempts to obtain the β form were met with failure", and they did not even mention the γ form.

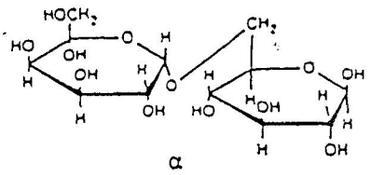
Benzocaine Picrate (III). A low-melting (132 °C) form has been used as a pharmacopeial standard.[36] A higher-melting (162−163 °C) form is obtained by drying this material at 105 °C for at least 1 h or by vacuum drying/sublimation (100 °C/ 0.1 mmHg).[37] Experiments in two laboratories showed that, once the latter form had been obtained, the lower-melting form could no longer be prepared. It was realized that drastic measures were called for. All samples were discarded, equipment and laboratory benches were washed, and, following a waiting period of 8−12 days, the low-melting form could again be obtained. This "purging" procedure was followed several times with reproducible results.



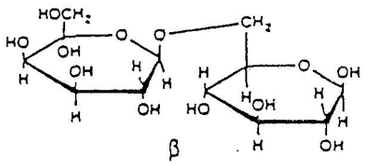
III

Melibiose (IV) and Mannose (V). The Pfanstiehl Chemical Company, in Waukegan, IL, specialized in isolating and purifying natural products. One of these was the disaccharide β-melibiose IV, with crystallization as the final purification step. The production ran into a problem:[38] "Then one day, for no apparent reason, the melibiose turned out to be of the α variety. Try as they might, the Pfanstiehl chemists could not get a batch of melibiose to crystallize in the β form. They finally concluded that mere traces of the α form in the air or on the equipment were enough to seed the solutions, but where it came from was never

(32) As an indication of earlier typical laboratory temperatures, it may be recalled that the calorie was defined as the amount of heat necessary to raise 1 g of water by 1 °C at 15 °C.
(33) Read, J.; Smith, G. S. *J. Chem. Soc.* 1921, *119*, 779.
(34) Dewar, J.; Morrison, D. R.; Read, J. *J. Chem. Soc.* 1936, 1598.
(35) Kamagapushna, D.; Ramamurthy, V.; Venkatesan, K. *Acta Crystallogr., Sect. C* 1987, *43*, 1128.
(36) *Pharmcopoea Nordica*. See also *The Merck Index*, 8th ed., mp 134 °C.
(37) Nielsen, T. K.; Borka, L. *Acta Pharm. Suec.* 1979, *9*, 503.
(38) *Ind. Eng. Chem.* December 1953, p 11a.

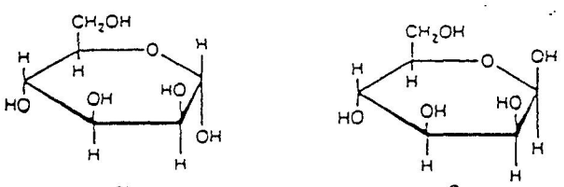


α



β

IV



α β

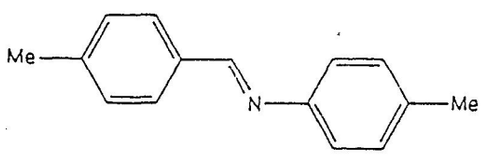
V

determined. They gave up the manufacture of that particular item, but are convinced that in some other locality in which there is not a trace of α-melibiose, it could be possible to crystallize the β sugar." [39]

Similar problems arise with mannose V. Once the α anomer was obtained, recrystallization from alcohol was no longer a suitable method for purifying the β anomer,[40] which could still be obtained from the mixture by extraction at 0 °C with 80% alcohol.[41]

We include these two cases involving epimerization at the anomeric carbon atom as examples of polymorphism because they fit McCrone's criterion; once dissolved, α- and β-melibiose equilibrate rapidly, and so do α- and β-mannose. Of course, each pair is usually regarded as two separate, isomeric compounds. We are here in one of those borderline areas where insistence on precise definitions may not be productive.

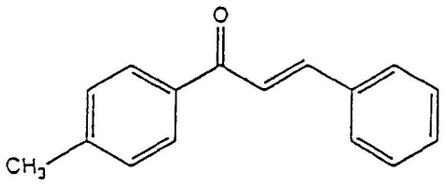
N-(4′-Methylbenzylidene)-4-methylaniline (VI). Over the years, we have been interested in benzylideneanilines. In 1968 J.D.D. reported the cell constants of several derivatives, among them the subtitle compound.[42] This work was repeated in J.B.'s laboratory in 1973.[43] After a break of about 8 months the original crystals did not diffract well, and recrystallization experiments were undertaken to prepare crystals suitable for structure determination. For



VI

nearly three years, many recrystallization experiments, often preceded by the synthesis of fresh batches of the compound, were undertaken, resulting in the discovery of two new polymorphic forms, but the original one could not be obtained.[44] Suspecting that our laboratory had been "infected" by seeds of the two newer polymorphs, we took advantage of the opening of a new laboratory about a kilometer away, to try again from scratch, using new reagents, virgin glassware, and a "new" student, whose contact with the old laboratory and its inhabitants was forbidden. The first attempt to prepare the original form under these conditions was successful.

3-Phenyl-1-*p*-tolylprop-2-enone (*p*′-Methylchalcone) (VII). Experiments made in 1988–89 at the ETH provide another example. Following earlier reports that *p*′-methylchalcone was polymorphic,[45] the compound was obtained with a melting point close to 55 °C (Weygand's β form).[46] This was the average



VII

onset temperature of the melting endotherm peak in a DSC apparatus, as observed for 21 crystalline samples, recrystallized from hexane and from ethanol. One sample, prepared by sublimation, had a higher melting point of 75.5 °C (Weygand's α form). Shortly afterward, it was noticed that the melting point of a sample that had been measured three weeks earlier had increased from 54.6 to 76 °C. From then on, only the high-melting form could be obtained from numerous recrystallization experiments from a variety of solvents. Only in the DSC apparatus could the low-melting form sometimes be obtained as microcrystals, by supercooling the molten liquid, but these reverted instantly to the high-temperature form when removed from the DSC cell (which is normally covered and shielded from the atmosphere). The most likely explanation is that after the high-melting α form had been prepared, the laboratory atmosphere was contaminated by seeds of this form, which acted as catalysts for the solid-state transformation and as critical nuclei in the subsequent crystallization experiments. The same explanation had been given many years earlier by Weygand:[45] "...Die Ruckverwandlung in die stabile Form erfolgt beim Berühren mit einer Spur α-Produkt fast augenblicklich, in Raümen, die mit α-Keimen infiziert sind, meist sehr bald beim blossen Stehen an der Luft."

Xylitol (VIII). Two crystalline forms of xylitol were reported in the early literature: a metastable, hygroscopic monoclinic form melting at 61 °C and a stable orthorhombic form melting at 94 °C.[47] When a sample of the monoclinic form was introduced into a laboratory where the orthorhombic form had been prepared, the crystals "changed in a few days into the high-

(39) The problem may still exist. From a survey of chemical catalogs, including those of Merck, Fluka, BDH, Aldrich, and Sigma, only the α form seems to be available.

(40) Levene, P. A. *J. Biol. Chem.* 1935, *108*, 419.

(41) Powers, H. E. C. *Z. Zuckerind.* 1971, *21*, 272.

(42) Bürgi, H.-B.; Dunitz, J. D.; Zust, C. *Acta Crystallogr., Sect. B* 1968, *21*, 463.

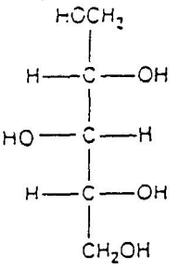
(43) Izak, I. Senior Thesis, Ben-Gurion University of the Negev, Beer Sheva, 1973.

(44) Bernstein, J.; Bar, I.; Christensen, A. *Acta Crystallogr., Sect. B* 1976, *32*, 1609. Bar, I.; Bernstein, J. *Ibid.* 1977, *33*, 1738; 1982, *38*, 121.

(45) Weygand, C.; Mathes, A. *Justus Liebigs Ann. Chem.* 1926, *449*, 29.

(46) Müller, R. Diplomarbeit, ETH-Zurich, 1989.

(47) Wolfram, M. L.; Kohn, E. J. *J. Am. Chem. Soc.* 1942, *64*, 1739.



VIII

melting stable form on exposure to the air of the laboratory."[48] The crystal structure of the orthorhombic form was determined by Kim and Jeffrey,[49] who wrote: "Attempts to obtain the lower melting monoclinic form from alcoholic solutions either at room temperature or close to 0 °C have hitherto been unsuccessful. We invariably grow the orthorhombic crystals. It is interesting to note that although xylitol was first prepared as a syrup in 1891 there was no report of crystallization until fifty years later, when it was the metastable hygroscopic form that was prepared first. Having now obtained the stable form, it is difficult to recover the metastable crystals.... The availability of appropriate nuclei in the laboratory is clearly a determining factor, as is well known to carbohydrate chemists."

Concluding Remarks

While we are far from being able to present a theory of disappearing polymorphs, we hope that we have at least taken the mystery out of the phenomenon. Prospects for gaining additional control over the nucleation and growth processes are good. With the use of "tailor-made" impurities, the growth of a particular crystal form can be suppressed and thereby the growth of other forms can be promoted.[50] Generally, interest in polymorphs is on the increase, and even becoming almost fashionable, as polymorphism can be regarded as a topic in supramolecular chemistry: supramolecular isomerism, in fact.

One regular feature can be discerned. All our examples of disappearing polymorphs involve molecules that can adopt different shapes: mostly molecules with conformational freedom but some with different configurations (epimers such as α and β sugars) or different arrangements of their parts (e.g., benzocaine picrate). In solution or in the liquid phase, all these conformations will be in dynamic equilibrium. There is no reason why the conformer present in the thermodynamically stable crystal form should be the most stable conformer in solution. One can imagine that interconversion barriers run up to about 20 kcal/mol, corresponding to interconversion rates up to a few seconds, certainly not immeasurably rapid compared with the rate of formation of critical nuclei. Thus, formation of nuclei of a stable crystal modification could easily be hampered by a low concentration of the particular conformer required, while another conformer could be incorporated in rapidly growing nuclei of a less stable crystal form. It is also possible that a metastable form (at room temperature and higher) was actually obtained as the thermodynamically stable form at somewhat lower temperature below the thermodynamic transition point. Unfortunately, we do not have the information that would be required to check all these points. In any case, we believe that once a particular polymorph has been obtained, it is *always* possible to obtain it again; it is only a matter of finding the right experimental conditions.

Postscript

A dramatic science-fictional implementation of the seeding phenomenon was presented over 30 years ago by Kurt Vonnegut, Jr., in his novel *Cat's Cradle*; the following is taken from the chapter entitled "Ice-Nine".[51]

> "There are several ways," Dr. Breed said to me, "in which certain liquids can crystallize—can freeze—several ways in which their atoms can stack and lock in an orderly, rigid way."
>
> That old man with spotted hands invited me to think of the several ways in which cannon balls might be stacked on a court-house lawn, of the several ways in which oranges might be packed into a crate.
>
> "So it is with atoms in crystals, too; and two different crystals of the same substance can have quite different physical properties."
>
> He told me about a factory that had been growing big crystals of ethylene diamine tartrate. The crystals were useful in certain manufacturing operations, he said. But one day the factory discovered that the crystals it was growing no longer had the properties desired. The atoms had begun to stack and lock—to freeze—in a different fashion. The liquid that was crystallizing hadn't changed, but the crystals it was forming were, as far as industrial applications went, pure junk.
>
> How this had come about was a mystery. The theoretical villain, however, was what Dr. Breed called "a seed". He meant by that a tiny grain of the undesired crystal pattern. The seed, which had come from God-only-knows where, taught the atoms the novel way in which to stack and lock, to crystallize, to freeze....

J.B. is grateful to the Israel Academy of Sciences and the U.S.–Israel Binational Science Foundation, who have supported his studies of polymorphism. We both acknowledge our debt to many colleagues who have piqued our curiosity and excitement by making us aware of examples of disappearing polymorphs.

AR9400756

(48) Carson, J. F.; Waisbrot, S. W.; Jones, F. T. *J. Am. Chem. Soc.* 1943, *65*, 1777.
(49) Kim, H. S.; Jeffrey, G. A. *Acta Crystallogr., Sect. B* 1969, *25*, 2607.
(50) Weissbuch, I.; Zbaida, D.; Leiserowitz, L.; Lahav, M. *J. Am. Chem. Soc.* 1987, *109*, 1869.
(51) *Cat's Cradle* Copyright 1963, Kurt Vonnegut, Jr., reprinted by permission of Donald C. Farber, Attorney for Mr. Vonnegut.

A New Polymorphic Modification of Tetrathiafulvalene. Crystal Structure, Lattice Energy and Intermolecular Interactions†

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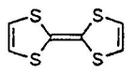
The molecular and crystal structure of the previously unknown modification of 2,2′-bi-1,3-dithiole (tetrathiafulvalene or TTF) has been determined by X-ray diffraction. The crystal is triclinic, at 298 K, a = 8.379(2), b = 12.906(3), c = 8.145(2) Å, α = 98.91(3), β = 96.62(3), γ = 100.44(3)°, V = 846.4(4), Z = 4, space group $P\bar{1}$. The molecular geometrical parameters of this modification (TTF2) do not differ from those found for the known modification (TTF1). However, the crystal structures are rather different. Only infinite chains formed by intermolecular interactions between S atoms of the closest molecules have been found in TTF2, in contrast to TTF1 monoclinic modification (space group $P2_1/c$), in which all molecules of the cell are stacked along the short b axis. The two structures have been used as the basis for a reexamination of the idea of a van der Waals radius for sulfur and the comparison and evaluation of various atom···atom potential energy forms and parameters which include interactions involving sulfur atoms.

Introduction

In an oft-quoted pseudoaphorism, McCrone suggested that virtually "every compound has different polymorphic forms... [and] the number of forms for a given compound is proportional to the time and energy spent in research on that compound".[1] While a great deal of time and energy have been spent studying tetrathiafulvalene (TTF) since its discovery in 1972, only one polymorph has been reported to date.[2] The combined experience of many people who deal with crystals and crystal growing is that polymorphic forms are more often than not discovered via a combination of serendipity and careful observation, rather than as a result of systematic searches and variation of experimental conditions.[3,4] In view of the fact that this issue of *Chemistry of Materials* is dedicated to the memory of M. C. Etter, it is fitting to mention here one case, the formation of crystalline complex of a cyanine dye molecule and an oxonol dye molecule, in which her systematic search for polymorphs and solvates did lead to a large number of identified and characterized different phases.[5]

Tetrathiafulvalene (TTF) is arguably the prototypical π-electron donor, and together with its derivatives, has



TTF

been widely used in the formation of charge transfer complexes and salts, especially those with potential as organic conductors and superconductors.[6] Recently, in the course of preparing tellurium derivatives of TTF[7] according to Scheme 1, we recovered one brown crystal of a material, which turned out to be a new polymorph of TTF, referred to herein as TTF2. The existence of two polymorphic structures of this symmetric prototypical molecule presents an excellent opportunity to examine in detail the nature and relative importance of various intermolecular interactions, especially those of the S···S

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* Abstract published in *Advance ACS Abstracts*, August 15, 1994.

(1) McCrone, W. C. In *Physics and Chemistry of the Organic Solid State*; Fox, D., Labes, M. M., Weissberger, A., Eds.; Interscience: New York, 1963; Vol. 1, p 725.

(2) Cooper, W. F.; Kenny, N. C.; Edmonds, J. W.; Nagel, A.; Wudl, F.; Coppens, P. *J. Chem. Soc., Chem. Commun.* 1971, 889. Cooper, W. F.; Edmonds, J. W.; Wudl, F.; Coppens, P. *Cryst. Struct. Commun.* 1974, *3*, 23.

(3) There are some notable exceptions to this generalization, namely the school of M. Kuhnert-Brandstatter and A. Burger in Innsbruck and of course W. McCrone himself, who make extensive use of the polarizing microscope and the hot stage to search for the existence of polymorphs [see, for instance: Kuhnert-Brandstatter, M. *Thermomicroscopy Analysis of Pharmaceuticals*; Pergamon Press: New York, 1971]. While these methods are extremely useful in identifying the existence of polymorphs, most laboratory and industrial procedures for obtaining organic crystals still involve crystallizations from solutions. Under these conditions a much narrower thermodynamic range is generally covered, resulting in the generation, and hence the discovery, of many fewer polymorphic forms.

(4) The lack of careful observation or suitable analytical techniques where no polymorphic forms are expected is also remarkable. For instance, it has been shown that the recently discovered polymorphs (more correctly *allotropes* of carbon) C_{60} and C_{70} fullerenes may be obtained from the soot generated in carbon arc lamps. Hundreds of thousands of such lamps existed around the world a century ago, and not a single scientist thought, or had the proper means, to analyze the soot for anything but "carbon black". Over the period during which such arcs were used for public lighting literally tons of fullerenes must have been produced without having been discovered.

(5) Etter, M. C.; Kress, T. B.; Bernstein, J.; Cash, D. J. *J. Am. Chem. Soc.* 1984, *106*, 6921.

(6) See, for instance: Bechgaard, K. In *Studies in Physical and Theoretical Chemistry*; Pierrot, M., Ed.; Elsevier: Amsterdam, 1990; Vol. 69, "Structure and Properties of Molecular Crystals", Chapter 5, p 235.

(7) Shahal, L. Ph.D. Thesis, Ben-Gurion University of the Negev, Beer Sheva, 1993.

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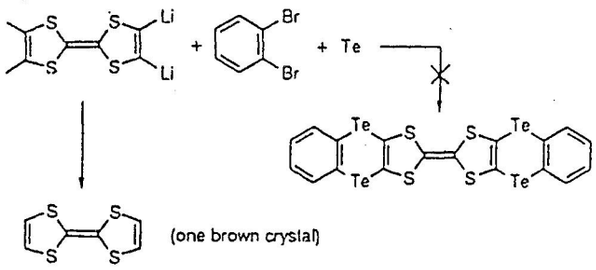
Scheme 1



ype considered to be of importance in determining the onductivity properties in the whole family of conductors ased on TTF. In addition, such a polymorphic system rovides an almost ideal testing ground for the efficacy of otential forms and potential parameters used in tom···atom lattice energy calculations, and the results of hose tests are presented here as well. Finally, since the rystal structure of TTF2 contains some unusual crystallographic aspects, we report on its determination and alysis in some detail.

Experimental Section

Preparation of TTF2. In the course of the workup at the nd of the attempted preparation shown in Scheme 1, the organic hase was separated from the aqueous phase, and the former vas evaporated to dryness under vacuum, leaving an oily mass vhich contained large amounts of *o*-bromobenzene. The vessel ontaining the oil was put in the refrigerator for 3–4 days, after vhic' there appeared one large crystal. Since the crystal was ro it was thought to contain tellurium. Rather than subject his small amount of material to an analysis requiring dissolution r grinding, it was subjected to a crystal structure analysis and roved to a second polymorph of TTF. The desired product was ot obtained, and attempts by other routes to prepare it have not een successful.

Crystal Structure Determination of TTF2. $C_6H_4S_4$, triclinic, space group $P\bar{1}$, at 298 K: a = 8.379(2), b = 12.906(3), c = 8.145(2) Å, α = 98.91(3), β = 96.62(3), γ = 100.44(3)°, V = 346.4(4), Z = 4, ρ = 1.603 Mg m^{-3}, μ(Mo Kα) = 1.039 mm^{-1}, F(000) = 416. 1885 unique reflections with $F_o > 4\sigma(F_o)$ were used out of a total of 2181 independent reflections collected with a Philips PW1100 diffractometer (Mo Kα = 0.710 69 Å, graphite monochromator, $\omega/2\theta$ scan, $2\theta < 45°$). The structure was solved by direct methods and refined by a full-matrix least-squares procedure using SHELX-86 and SHELX-76, respectively,[8] to R = 0.038, R_w = 0.055, GOF = 1.81. SCHAKAL92[9] was used for the graphic representations. Other details are given in Table 1. The final coordinates are given in Table 2. Tables of bond lengths, bond angles, and temperature factors have been deposited.

While the structure solution was quite routine, the crystal structure has a number of unusual properties. The first of these is the fact that the asymmetric unit in the triclinic $P\bar{1}$ cell is comprised of the halves of four molecules, each one lying about a center of symmetry, to complete the four molecules in the unit cell. Such cases are quite rare, and a number of those reported recently (often resulting from "black-box" solution space-group assignment and structure solution) have been shown to be incorrect.[10] Hence, considerable effort was expended in verifying the correctness of this assignment. It is worthy of note that these programs all give the corrected cells reported by Marsh.[11]

While cell reduction algorithms are by no means new,[12] the past few years have witnessed the development of increasingly sophisticated programs for checking space-group assignments, and the presence of unrecognized symmetry elements, including centering.[13] We have applied three of these programs to the cell of TTF2. All of them produced results indicating that the reported cell is the correct one. No other solution was produced.

Additional examples of cases such as the current one, in which the asymmetric unit is comprised of the halves of even *two* crystallographically independent molecules (each full molecule being generated by an inversion center) cannot be readily identified automatically with the current software of the Cambridge Structural Database. The ZPRIME parameter, which is extremely useful for identifying structures with multiple molecules in the asymmetric unit, would in the present case simply

Table 1. Details of Data Collection, Structure Solution and Refinement for TTF2

empirical formula	$C_6H_4S_4$
color	brown
crystal size (mm)	0.31 × 0.21 × 0.2
scan speed	variable 2.00–29.30°/min in ω
scan range	(ω) 1.8°
background measurement	stationary crystal and stationary counter at beginning and end of scan, each for 25.0% of total scan time
standard reflections	3 measured every 100 reflections
index ranges	$-9 < h < 8$, $-13 < k < 13$, $0 < l < 8$
Solution and Refinement	
program packages used	Shelx-76, Shelx-86[8]
structure solution	direct methods
refinement method	full-matrix least-squares
quantity minimized	$\sum w(F_o - F_c)^2$
hydrogen atoms	riding model, fixed isotropic U
weighting scheme	$w^{-1} = \sigma^2(F) + 0.0001F^2$
no. of parameters refined	181
final R indices	R = 0.0379, R_w = 0.0555
final R indices (all data)	R = 0.0536, R_w = 0.1958
goodness-of-fit	1.81
largest and mean Δ/σ	0.001, 0.000
data-to-parameter ratio	10.4:1
largest difference peak	0.22 e Å^{-3}
largest difference hole	−0.25 e Å^{-3}

Table 2. Atomic Coordinates (×10^4) and Equivalent Isotropic Displacement Coefficients (Å^2 × 10^3)

	x	y	z	U(eq)[a]
S(1)	5268(1)	8393(1)	−1136(2)	74(1)
S(2)	7540(1)	10167(1)	1164(2)	79(1)
S(3)	6161(2)	5027(1)	7567(1)	81(1)
S(4)	3820(2)	3358(1)	5134(1)	75(1)
S(5)	8982(2)	5480(1)	2294(1)	77(1)
S(6)	9447(1)	3386(1)	603(2)	71(1)
S(7)	319(1)	8337(1)	4464(1)	69(1)
S(8)	2340(1)	10377(1)	4059(2)	74(1)
C(1)	7233(5)	8224(4)	−511(6)	75(2)
C(2)	8241(5)	9008(4)	514(5)	72(2)
C(3)	5576(4)	9699(3)	9(4)	53(1)
C(4)	5436(5)	3834(3)	8190(5)	73(2)
C(5)	4404(5)	3085(3)	7116(5)	71(2)
C(6)	4996(4)	4669(3)	5553(4)	52(1)
C(7)	8502(6)	4398(4)	3281(6)	83(2)
C(8)	8689(5)	3465(4)	2513(6)	80(2)
C(9)	9678(4)	4769(3)	598(4)	53(1)
C(10)	2174(6)	8303(4)	3756(6)	82(2)
C(11)	3065(6)	9211(4)	3571(6)	84(2)
C(12)	550(4)	9735(3)	4702(4)	51(1)

[a] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

(8) Sheldrick, G. M. SHELX86, a Crystallographic Structure Solution Package; SHELX76, a Crystallographic Structure Solution and Refinement Program; University of Gottingen: Gottingen, 1986.
(9) Keller, E. SCHAKAL92, A Molecular and Crystallographic Plotting Package, Crystallographic Institute, University of Freiburg, 1993.
(10) Marsh, R. E.; Herbstein, F. H. *Acta Crystallogr.* 1983, *B39*, 280; 1988, *B44*, 77.
(11) Marsh, R. E. *Acta Crystallogr.* 1986, *C42*, 511; 1989, *C45*, 347; 1990, *C46*, 1761; 1989, *C45*, 2029.
(12) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, England, 1952; Vol. I, pp 530–533.
(13) For instance, the following are now available: (a) BLAF: Macicek, J. Bulgarian Academy of Sciences, Sofia, Bulgaria, 1991. (b) NIST*LATTICE: Karen, V. L. Reactor Radiation Division, U.S. National Institute of Standards and Technology, Gaithersburg, MD 20899, 1991. (c) Spek, A. L. *J. Appl. Crystallogr.* 1988, *21*, 578, based on the algorithm of LePage, Y. *J. Appl. Crystallogr.* 1982, *15*, 255.

Table 3. Some Representative Examples of Structures in Which the Asymmetric Unit Is Comprised of Molecules Lying in Both a General Position and on a Symmetry Element

compound	space group	Z	ZPRIME	contents of asymmetric unit	ref
9-cyanoanthracene dimer	$P\bar{1}$	4	2.0	$1 + 2 \times {}^1/_2$	*a*
2,5-dimethylhexane-2,5-diol	$P2_1/c$	6	1.5	$1 + {}^1/_2$	*b*
biphenylene	$P2_1/a$	6	1.5	$1 + {}^1/_2$	*c*
anthanthrone	$P2_1/c$	8	2.0	$1 + 2 \times {}^1/_2$	*d*
hexafluorobenzene	$P2_1/n$	6	1.5	$1 + {}^1/_2$	*e*
pinnacol	$C2/c$	16	2.0	$1 + 2 \times {}^1/_2$	*f*
2,3-dimethylthiirene	$A2/a$	12	1.5	$1 + {}^1/_2$	*g*

[a] Theocharis, C. R.; Jones, W. *J. Chem. Soc., Chem. Commun.* 1984, 369. [b] Helmholdt, R. B.; Reynaens, H. *Acta Crystallogr.* 1976, *B32*, 2243. [c] Fawcett, J. K.; Trotter, J. *Acta Crystallogr.* 1966, *20*, 87. [d] Edwards, I. A. S.; Stadler, H. P. *Acta Crystallogr.* 1971, *B27*, 946. [e] Boden, N.; Davis, P. P.; Stam, C. H.; Wesselink, G. A. *Mol. Phys.* 1973, *25*, 81. [f] Jeffrey, G. A.; Robbins, A. *Acta Crystallogr.* 1978, *B34*, 3817. [g] Ammon, H. L.; Fallon, L.; Planstas, L. A. *Acta Crystallogr.* 1976, *B32*, 2171.

give a value of 2 and would not hint at the fact that there are four half molecules in the asymmetric unit. While we are not aware of another example of a structure in which the asymmetric unit contains four half molecules, one classic case in which there are two crystallographically independent half molecules in the asymmetric unit is the pair of isostructural stilbene and azobenzene structures[14] which, however, are monoclinic $P2_1/a$, so the problem of unidentified symmetry elements does not exist. By examination of the individual cases for which ZPRIME > 1 in the three most commonly encountered centrosymmetric space groups ($P\bar{1}$, $P2_1/c$, $C2/c$),[15] we have identified a selection of additional examples (Table 3), including some in which the asymmetric unit consists of a whole molecule plus one or two halves of molecules, e.g., $(1 + {}^1/_2)$ or $(1 + 2 \times {}^1/_2)$ molecules in the asymmetric unit that result in a ZPRIME of 1.5 or 2.0, respectively. We have not found any other structures similar to TTF2, in which the asymmetric unit is comprised of the halves of four molecules, each lying at an inversion center.

Packing calculations were carried out with the widely used PCK83 program of Williams.[16,17] The majority of calculations using this program have been carried out on hydrocarbons or hydrocarbon compounds containing only first-row elements as additional substituents. Since the available parameters for sulfur have not been widely tested, either individually or in combination with other widely used parameters, we have employed three different sets of parameters in these calculations (Table 4). First, we have set all C–H bonds to 1.08 Å[18] and used the standard Williams hydrocarbon C, H potentials.[17] Second, we have employed the full set of Mirsky potentials for C and H and S,[19] The third set of potentials was taken from the recently published list of improved potentials for PCK83 (Buckingham potential form) by Filippini and Gavezzotti[20] that is based on a statistical analysis of intermolecular contacts found in a large sampling of crystal structures in the Cambridge Structural Database.[21] Although they appear to be quite similar, the two sulfur potentials (Figure 1) differ slightly both in the position and depth of the energy minimum, and it was of interest to examine whether these rather small differences would influence the results or the interpretation of them.

Table 4. Atom–Atom Potential Parameters in the Form $U = B\exp(-\alpha R_{ij}) - AR_{ij}^{-6}$ with A in kJ/mol Å^6, B in kJ/mol, and α in Å^{-1}

interaction	A	B	α	D_6	ϵ	parameter set
H···H	136.40	11 970.5	3.74	3.311	−0.0534	*W*[a]
C···H	576.90	66 527.8	3.67	3.606	−0.1434	
C···C	2439.40	369 737.0	3.60	3.898	−0.3978	
C···S[d]	4893.10	602 946.2	3.545	3.894	−0.7937	
S···S	9815.70	983 240.0	3.49	3.889	−1.5836	
S···H[d]	1157.10	108 489.9	3.615	3.592	−0.2898	
H···H	121.30	20 501.6	4.29	2.794	−0.1273	*M*[b]
C···H	493.70	77 822.4	3.94	3.297	−0.2067	
C···C	1761.50	299 574.4	3.68	3.805	−0.3319	
C···S	3543.80	415 889.6	3.54	3.876	−0.5880	
S···S	9815.70	983 240.0	3.49	3.889	−1.5836	
S···H	1091.20	141 978.8	3.89	3.275	−0.468	
H···H	109.20	24 158.4	4.01	3.363	−0.0419	*F/G*[c]
C···H	472.80	120 792.1	4.10	3.295	−0.2054	
C···C	2418.40	226 145.2	3.47	3.891	−0.3874	
C···S	6292.70	529 108.6	3.41	3.958	−0.9090	
S···S	10757.10	1 087 672.6	3.52	3.831	−1.8891	
S···H	1167.30	268 571.0	4.03	3.351	−0.4578	

[a] Average values with S···S of *M* set. [b] Williams potentials.[17] [c] Mirsky potentials.[19] [d] Filippini/Gavezzotti potentials.[20]

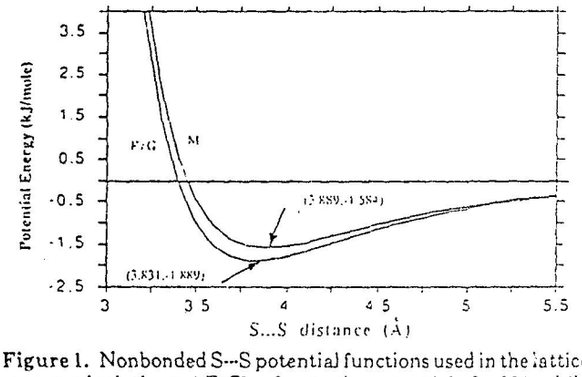


Figure 1. Nonbonded S···S potential functions used in the lattice energy calculations. "*F/G*" refers to the potential of ref 20, while "*M*" is that of ref 19. The first entry in parentheses is the S···S distance at the energy minimum, which is the second entry.

Comparison of Molecular Structures

While TTF is a rigid molecule and large variations in the features are not expected,[22] it is of interest to examine the internal consistency of the results obtained for the four independent molecules and to compare those features with those of TTF1. The average values of the bond lengths and angles are given in Figure 2. Virtually none of the out-of-plane deformation found recently in di-TTF derivatives linked by tellurium[23] is found in any of the four molecules, the largest deviation from the least-squares plane in TTF2 being 0.031 Å. On the other hand it is noteworthy that a recent electron diffraction study of TTF indicated that in the gas phase the TTF molecule not centrosymmetric but rather is distorted into a boat

(14) Hoekstra, A.; Meertens, P.; Vos, A. *Acta Crystallogr.* 1975, *B31*, 2813. Brown, *Acta Crystallogr.* 1966, *31*, 146.
(15) Donohue, J. *Acta Crystallogr.* 1985, *A41*, 203.
(16) Williams, D. E. *PCK83*, QCPE Program 548, Quantum Chemistry Program Exchange, Chemistry Department, Indiana University, Bloomington, IN.
(17) Williams, D. E. *Acta Crystallogr.* 1984, *B40*, 404.
(18) Allen, F. H.; Kennard, O.; Watson, D.; Brammer, L.; Orpen, G.; Taylor, R. *J. Chem. Soc., Perkin Trans. 2* 1987, S1.
(19) Mirsky, K. V. In *Computing in Crystallography*; Schenk, H., Olthof-Hazekamp, M., van Komingsveld, M., Bassi, G. C., Eds.; Delft University Press: Twente, 1978; pp 169–182.
(20) Filippini, G.; Gavezzotti, A. *Acta Crystallogr.* 1993, *B49*, 868.
(21) Allen, F. H.; Davies, J. E.; Galloy, J. J.; Johnson, O.; Kennard, O.; MacRae, C. F.; Mitchell, E. M.; Mitchell, G. F.; Smith, J. M.; Watson, D. G. *J. Inf. Comput. Sci.* 1991, *31*, 187.

(22) A noteworthy exception in the present context is the pair of polymorphic structure of tetramethylthiotetrathifulvalene. In the triclinic form [Endres, E. *Z. Naturforsch.* 1986, *41*, 1351] the molecule lies on a crystallographic inversion center and is essentially planar. In the monoclinic form [Katayama, C.; Honda, M.; Kumagai, H.; Tanaka, J.; Saito, G.; Inokuchi, H. *Bull. Chem. Soc. Jpn.* 1985, *58*, 2272] the molecule lies on a general position and adopts a boat conformation due to dihedral angels about the transannular S···S axes of 23.7° and 19.3°.
(23) Martin, J. D.; Canadell, E.; Becker, J. Y.; Bernstein, J. *Chem. Mater.* 1993, *5*, 1199.

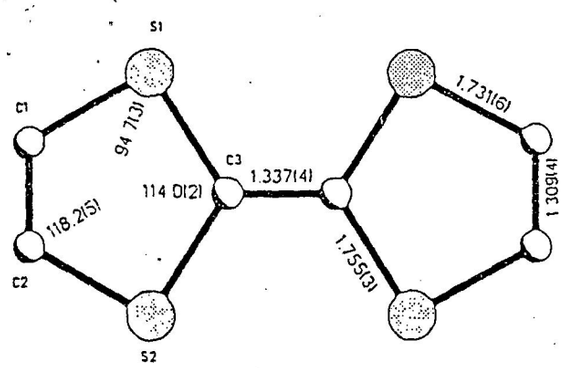


Figure 2. Molecular structure of TTF2 showing the values of the bond lengths (angstroms) and bond angles (degree) averaged over the four independent (half) molecules in the asymmetric unit. The numbers in parentheses represent the esd of the average value.

conformation as manifested in dihedral angles of 13.5° about transannular S···S axes.[24] In the TTF1 structure this angle is equal to 2.1°, in equal but opposite directions, nsistent with the molecule lying on a center of symmetry. r or the four molecules in TTF2 the distortion was found to be in the range 1.2–4.1°.

Packing and Intermolecular Interactions, Including a Brief Reconsideration of the Significance of a van der Waals Radius

: crystal packing of TTF and its derivatives is of particular interest, because many of the unique properties observed for ion-radical salts and charge-transfer complexes of these compounds (e.g., electrical conductivity and superconductivity) stem from the arrangement of mo' ·ules in the crystal. In particular, molecules in this fam...y tend to form networks of intermolecular S···S contacts, which have been considered an important structural element in facilitating electrical conductivity.[25,26] The crystal structures of the two polymorphic modifications of TTF are clearly different (Figures 3 and 4). The TTF1 crystal has a short *b* axis, and consequently all the molecules in the ceil are stacked along this axis. ɜ distance between parallel planar molecules of a stack ıs 3.63 Å, and the two symmetry-related S atoms have two contacts of 3.41 and 3.58 Å with S atoms of molecules in another stack (Figure 4a), which are shorter than twice the van der Waals radius of sulfur (3.6 or 3.7 Å, depending on the value chosen for the van der Waals radius[27,28]). This additional interaction leads to the two-dimensional net···ork shown in Figure 4b.

...e structure of TTF2, on the other hand, is probably best characterized as being composed of two chains of molecules, each chain consisting of alternating crystallographically independent molecules. The first chain contains the TTF molecules with S(1) and S(2) alternating wi· ·hat containing S(7) and S(8), while the second chain contains the TTF units with S(3) and S(4) alternating

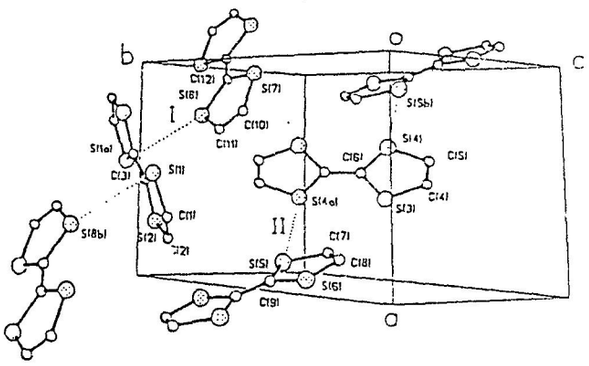


Figure 3. Partial view of the experimentally determined crystal structure of TTF2, showing the relationships between the four different molecules in the unit cell (each lying on the inversion center), and the formation of chains. The two S···S intermolecular distances <3.70 Å are indicated: I: S8···S1a (1 − *x*, 2 − *y*, − *z*), 3.65 Å. II: S5···S4a (1 − *x*, 1 − *y*, 1 − *z*), 3.69 Å.

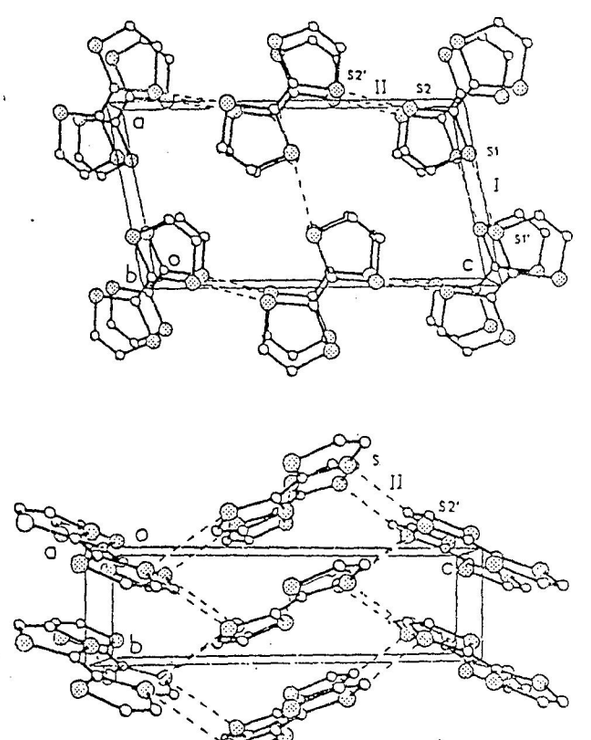


Figure 4. Two views of the experimentally determined crystal structure of TTF1. (a) View on the *ac* plane. (b) View on the *bc* plane. The intermolecular S···S contacts less than 3.7 Å are indicated: I: S1···S1′ (1 − *x*, − *y*, − *z*), 3.41 Å. II: S2···S2′ (− *x*, 0.5 + *y*, 0.5 − *z*), 3.58 Å.

with that containing S(5) and S(6). We are inclined to call these "chains" rather than stacks, since the angles between the planes of alternating molecules (defined as C(1)–C(2)–C(3) ∧ C(10)–C(11)–C(12) and C(4)–C(5)–C(6) ∧ C(7)–C(8)–C(9) are 50.8° and 56.1°, respectively. In contrast to the crystal structure of TTF1, the structure of TTF2 cannot be characterized by a small number of particularly short S···S intermolecular distances less than 3.9 Å. As can be seen in Table 5, there are no fewer than eight intermolecular distances varying almost continuously over the range 3.65–3.90 Å.

This observation raises some fundamental questions about the nature of the van der Waals radius and its use in understanding the "important" or "dominant" interac-

(24) Hargittai, I.; Brunvoll, J.; Kolonits, M.; Khodorkovsky, V. *Angew. Chem., Int. Ed. Engl.*, in press.
(25) Wudl, F. *Acct. Chem. Res.* 1984, *17*, 227.
(26) Williams, J.; Beno, M. A.; Wang, H. H.; Leung, P. C. W.; Enger, Y. J.; Geiser, U.; Carlson, K. D. *Acc. Chem. Res.* 1984, *17*, 227. Williams, Schultz, A. J.; Geiser, U.; Carlson, K. D.; Kini, A. M. J.; Wang, H. H.; Kwok, W.-K.; Whangbo, M. H.; Schirber, J. E. *Science* 1991, *252*, 1501.
(27) Bondi, A. *J. Phys. Chem.* 1964, *68*, 441.
(28) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960; Chapter 7.

Table 5. Summary of S···S Intermolecular Distances before and after Lattice Energy Minimization. TTF1 and TTF2

TTF1

atom I	atom II	symmetry operation[a]	X-ray[b]	distances in Å *F/G*	*W*	*M*	interaction no. (Figure 5)
S1	S1	I 0 1 0		3.63	3.76	3.64	IV
S1	S1A[c]	I -1 0 0	3.41		3.50		I
S2	S2	II 0 -1 0	3.58	3.51	3.52	3.52	II
S1	S2A[c]	I 0 -1 0		3.59	3.86	3.67	III
S2	S2	I 0 1 0		3.63	3.76	3.64	IV

TTF2

atom I	atom II	symmetry operation[d]	X-ray	distances in Å *F/G*	*W*	*M*	interaction no. (Figure 6)
S1A[c]	S4	I 0 -1 0	3.90	3.59	3.66	3.60	I
S1A[c]	S8	I 0 0 0	3.65	3.63	3.67	3.66	V
S2A[c]	S7	I 0 0 1	3.84	3.73	3.78	3.76	VI
S2A[c]	S8	I 0 0 0				3.89	
S2A[c]	S8	I 0 0 1		3.89	3.90	3.85	
S3	S6A[c]	I 0 0 -1	3.88	3.72	3.78	3.79	VII
S4A[c]	S5	I 0 0 0	3.67	3.64	3.66	3.66	IV
S4A[c]	S7	I -1 0 0	3.82	3.57	3.60	3.58	II
S4	S8	I 0 1 0	3.75	3.63	3.83	3.72	VIII
S5	S7	I -1 0 0	3.74	3.59	3.79	3.66	III

[a] For symmetry operations the roman numeral indicates either I: x, y, z or II: $-x, 0.5+y, 0.5-z$. The following three numbers indicate translations along the a, b, and c unit-cell axes, respectively. [b] For X-ray column distances equal to or less than 3.9 Å are listed. [c] The "A" designator indicates the atom generated through the inversion center. [d] For symmetry operations the roman numeral indicates either I: x, y, z. The following three numbers indicate translations along the a, b, and c unit-cell axes, respectively.

tions in molecular crystals. To the best of our knowledge, the idea of a van der Waals radius originated with Pauling,[28] and it represents a *characteristic, average value* for the closest intermolecular approach of two atoms in a variety of crystal structures. All two-body interactions can be described by some potential energy curve similar to those in Figure 1, and the implication of this definition is that the sum of the van der Waals radii is, or is very close to, the minimum energy value for that interaction. By the very nature of a potential energy curve, both shorter and longer values may very well represent higher energies, unless, of course, interactions other than those of the van der Waals type are being considered.

It is common practice in discussing the crystal structures of organic materials to tabulate and note the intermolecular distances that are shorter than the sum of the van der Waals radii for the two atoms in question. In the age of automation of crystal structure analysis, such a tabulation is a trivial matter. Analysis of the resulting data then forms the basis for the understanding of the "important" interactions in a particular crystal structure—indeed, in whole classes of crystals structures. A case in point is the family of compounds based on TTF and its derivatives.[25,26]

We believe that the definition of a van der Waals radius is still somewhat uncertain and arbitrary. Moreover, the *automatic* tabulation of "short" intermolecular distances based on van der Waals radii and the subsequent attribution of particular significance to the resulting "interactions" contains the ingredients on which misunderstandings and incorrect models and theories can be based, and if practiced at all, should be done so with caution. This idea is not new; the origin and use of the van der Waals radius has been recently reviewed and critically analyzed by Zefirov and Zorkii,[29] and they have expressed many of the ideas presented here. The danger of using a van der Waals radius as a criterion for defining hydrogen bonds was stated quite clearly by Jeffrey a. Saenger[30] in rejecting a strict geometric definition of hydrogen bonds, in particular the donor···acceptor or H···acceptor distance. Moreover, such notions were crucial in Etter's emphasis on focusing on and defining the *patterns* of hydrogen bonds and hydrogen-bond networks, rather than the strict geometric or symmetry relations of individual hydrogen bonds.[31]

The structure of TTF2 is a perfect case in point. The blind use of, say 1.85 Å as the van der Waals radius of sulfur (i.e. Pauling's value) would have led to the "recognition" of two "important" interactions in the structure. Are the two "interactions" of 3.74 and 3.75 Å so much less important that they can be ignored? Examination of any potential energy curve for the van der Waals S···S interaction (for example, see Figure 1) indicates that the energy is not changing rapidly at this distance. And why should these four distances, say, be chosen as "important", when there are four more very similar ones, with correspondingly similar (albeit lower, according to these potential energy curves) energies of interaction?

There is no simple formula for resolving this dilemma. Each structure must be examined carefully to determine the nature and relative importance of the intermolecular interactions. In TTF1 there are very clearly two short intermolecular S···S distances (Figure 4), and these can indeed be attributed to the dominant interactions with a fair degree of confidence. In TTF2 the S···S distances (X-ray column in Table 5) are spread over a distance continuum, and considerable caution must be exercised in attributing more or less particular significance to any one or even any small number of "interactions". For instance, three of the distances are greater than the minimum in *F/G* potential curve of Figure 1, and one is longer than the *M* potential curve in Figure 1. Depending on the curvature of those curves, these longer distances could, in fact, correspond to lower energies than some of the shorter distances.

Lattice Energy Calculations

Polymorphic systems provide unique opportunities for comparing and testing the parameters used in atom-atom potential energy calculations.[32-34] The only variable between polymorphs in the structural one, thus eliminating considerations of electronic, substitutional, and other variables. The relative stability of different polymorphic forms of a substance are generally known, even from considerations of crystallization conditions,[35] history of appearance,[36] etc., and this information may be used for the qualitative comparison with the relative stabilities obtained from the computed energies. In the present case, the fact that TTF2 did not appear for 20 years, and the unusual conditions under which it did appear, suggest that it is the less stable form. Also, it has been shown that in polymorphic systems the more dense polymorph is gener-

(29) Zefirov, Y. V.; Zorkii, P. M. *Russ. Chem. Rev.* 1989, *58*, 421 (translated from *Usp. Khim.* 1989, *58*, 713).
(30) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures*; Springer-Verlag: Berlin, 1991; pp 29–33.
(31) Etter, M. C. *Acc. Chem. Res.* 1990, *23*, 120. Etter, M. C. *J. Phys. Chem.* 1991, *95*, 4601. Etter, M. C.; MacDonald, J. C.; Bernstein, J. *Acta Crystallogr.* 1990, *B46*, 256.
(32) Bernstein, J.; Hagler, A. T. *J. Am. Chem. Soc.* 1978, *100*, 673.
(33) Hagler, A. T.; Bernstein, J. *J. Am. Chem. Soc.* 1978, *100*, 6349.
(34) Bar, I.; Bernstein, J. *J. Phys. Chem.* 1982, *86*, 3223.
(35) Bernstein, J. *J. Phys. D: Appl. Phys.* 1993, *26*, B66.
(36) Woodard, G. D.; McCrone, W. C. *J. Appl. Crystallogr.* 1975, *8*, 342. Jacewicz, V. W.; Nayler, J. H. C. *J. Appl. Crystallogr.* 1979, *12*, 396. Webb, J.; Anderson, B. *J. Chem. Educ.* 1978, *55*, 644.

y the more stable form.[37] The calculated density of TF1 is 1.679 and that of TTF2 1.603 Mg m^{-3}, which again consistent with the proposition that TTF1 is the more ible polymorph. The best measure of the validity of nputational procedures and parameters is the comrison with experimentally determined sublimation ergy, but the number of cases in which the sublimation ergy is available for different polymorphs of a particular bstance is very limited.[38] However, it is possible to make ough estimate of the expected energies of different lymorphs, since the energy differences among polyrphs of organic compounds usually do not exceed 10–kJ mol^{-1}.[39] Thus, knowledge of the sublimation energy one polymorph (usually the most stable one) does vide at least a semiquantitative basis for comparison h the other polymorphic structure(s).

TTF is also a particularly suitable molecule for the nparative calculations of polymorphic structures. In dition to its importance as a π-electron donor in charge-nsfer complexes and salts, it has become one of the hetypical molecules of organic chemistry. Its high ecular symmetry (mmm, D_{2h}) means that all four sulfur ms are chemically equivalent, as are the four hydrogens, four carbons to which they are bonded, and the two ntral carbon atoms. It is thus relatively easy to isolate d analyze the particular intermolecular interactions ich are principally responsible for the packing patterns the different polymorphic structures. Because of this h chemical symmetry on the molecular level, there is ery limited number of types of intermolecular interacns, which considerably simplifies the analysis of the king energies and the energetic comparison of the two uctures. This polymorphic system also provides a fertile ting ground and fairly stringent requirements for the ram rs used in the lattice energy calculations, since can require all of them to indicate that TTF1 is the re stable polymorph with a sublimation energy that proximates the experimental value, while the calculated tice energy for TTF2 should be higher by no more than proximately 10 kJ-mol^{-1}.[39]

We will examine simultaneously the packing patterns packing energy of the two forms of TTF. The packing TF1 is shown in Figure 4. The view down the b axis gure 4a) highlights the presence of plane-to-plane stacks h a repeat of 4.023 Å. Neighboring stacks are related the glide operation in $P2_1/c$, as can be seen clearly from ure 4b. Short intermolecular S···S distances have long n recognized as important factors in determining the uctures of TTF and its derivatives.[25,26] The two short tar of this type which dominate the structure of TF1 generate a three-dimensional network. Qualitative ervations of this type have traditionally led to the clusion that these short distances represent the minant interactions in stabilizing the crystal structure. th the increasing sophistication of computational tho or calculating the energetics of crystal structures, h correlations can be put to quantitative test.

The results of the lattice energy calculations on TTF1, ng the methods and parameters described in the Experimental Section, are summarized in Table 6. The lattice energy minimizes quite smoothly, and for all three sets of potential parameters the commonly known TTF1 calculates as the lower energy form, consistent with our earlier qualitative observations. Two values for ΔH_s have been reported: 92 ± 6.3 and 95.3 ± 1 kJ mol^{-1}.[40,41] While none of the computed values agrees precisely with either of the two experimental values, it is encouraging that all three give quite similar energy differences between the two forms and that these are within the expected range for such values. The closest match is for the M parameter set. This strengthens a point that we have been making for some time about these calculations—namely, that there can be quite large variations in absolute energies for different parameter sets, but *differences* in energies, which are of particular interest in regard to polymorphic systems, appear to be less sensitive to the potential function and parameters used.[42]

We note that for the F/G and M potentials the "R factor", which is a measure of the changes between the initial and minimized structures, is quite large (normal values are <100).[42] This indicates that there have been considerable, and perhaps significant, changes in the structure as a result of the minimization. The structure resulting from the minimization is shown in Figure 5. For all three minimizations there are now three S···S intermolecular distances less than 3.80 Å, and these are the three largest contributors to the minimized lattice energy (Table 7). In general, in molecular crystals which are dominated by van

Table 6

(a) Results of Lattice Energy Minimizations on the Two Forms of TTF

	form 1			form 2			
potential	E_i	E_f	R	E_i	E_f	R	$\Delta E^{a,b}$
W	−102.4	−105.9	51.7	−92.0	−96.6	70.5	9.3
M	−93.2	−102.4	404	−86.5	−94.9	117	7.5
F/G	−114.6	−123.4	589	−105.6	−112.4	109	11.0

(b) Cell Parameters and Shifts at Covergence for the Three Parameter Sets of the Two Forms of TTF

potential	W	M	F/G	X-ray
		Form I		
a (Å)	7.418	8.306	8.514	7.364
b (Å)	3.764	3.635	3.627	4.023
c (Å)	14.115	12.432	12.252	13.922
β (deg)	100.20	102.95	102.18	101.42
Δa (Å)	0.054	0.942	1.150	
Δb (Å)	−0.259	−0.388	−0.396	
Δc (Å)	0.193	−1.490	−1.670	
$\Delta\beta$ (deg)	−1.22	1.53	1.49	
		Form II		
a (Å)	8.232	8.078	8.221	8.379
b (Å)	13.048	12.782	12.865	12.906
c (Å)	7.791	7.653	7.733	8.145
α (deg)	100.70	99.47	100.94	98.91
β (deg)	95.75	97.14	94.98	96.62
γ (deg)	101.89	101.07	103.04	100.44
Δa (Å)	−0.147	−0.301	−0.158	
Δb (Å)	0.142	−0.124	−0.041	
Δc (Å)	−0.354	−0.492	−0.412	
$\Delta\alpha$ (deg)	1.79	0.56	2.03	
$\Delta\beta$ (deg)	−0.87	0.52	−1.64	
$\Delta\gamma$ (deg)	1.45	0.63	2.60	

[a] Energy in kJ mol^{-1}. [b] E_i and E_f indicate initial and final energies.

(37) Burger, A.; Ramburger, R. *Mikrochim. Acta* [Wien] 1979, 2, 259.

(38) Chickos, J. S. In *Molecular Structure and Energetics, Physical Measurements*; Liebman, J. F., Greenberg, A., Eds.; VCH Publishers, Inc.: New York, 1987; Vol. 2, Chapter 2.

(39) Kitaigorodsky, A. I. *Molecular Crystals and Molecules*; Academic Press: New York, 1973; Chapter 7.

(40) Sandman, D. J.; Epstein, A. J.; Chickos, J. S.; Ketchum, J.; Fu, J. S.; Scheraga, H. A. *J. Chem. Phys.* 1979, 70, 305.

(41) DeKruif, S. G.; Govers, H. A. J. *J. Chem. Phys.* 1980, 73, 533.

(42) Instruction manual for ref 16.

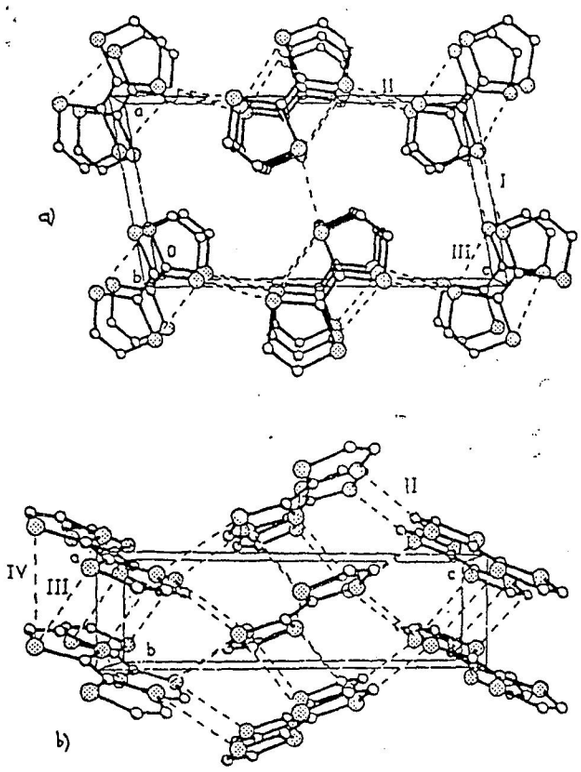


Figure 5. Two views of the crystal structure of TTF1 after lattice energy minimization: (a) View on the *ac* plane. (b) View on the *bc* plane. The intermolecular S···S distances indicated are discussed in the text and are given in Table 7. To minimize confusion only one interaction of the types S1···S1 (x, $1+y$, z) and S2···S2 (x, $1+y$, z) is shown (IV).

Table 7. Summary of Specific S···S Interactions for TTF1[a,b]

rank	potential function		
	W^c	M^d	F/G^e
1	−0.48	−0.62	−1.06
	S1···S1	S2···S2	S2···S2
	I −1 0 0	II 0 −1 0	II 0 −1 0
	3.501	3.520	3.509
2	−0.64	−1.21	−1.50
	S2···S2	S1···S1	S1A′···S2
	II 0 1 0	I 0 1 0	II 0 −1 0
	3.523	3.635	3.594
3	−1.51	−1.32	−1.62
	S1···S1	S1A′···S2	S1···S1
	I 0 1 0	II 0 −1 0	I 0 1 0
	3.764	3.669	3.627

[a] Down the columns entries are given in order of increasing distance. Each entry contains, respectively, the energy of the specific interaction (in kJ mol^{-1}), the atom pair involved in the interaction, the symmetry operation generating the atom pair interaction,[b] and the distance (in angstroms) between the two atoms in the pair. [b] For symmetry operations the roman numeral indicates either I: x, y, z or II: $-x$, $0.5+y$, $0.5-z$. The following three numbers indicate translations along the a, b, and c unit-cell axes, respectively. [c] Williams potentials.[17] [d] Mirsky potentials.[19] [e] Filippini/Gavezzotti potentials.[20] / The "A" designator indicates the atom generated through the inversion center.

der Waals interactions (i.e., in the absence of hydrogen bonds or stronger intermolecular interactions) the crystal structure is stabilized by the cooperative effect of many small contributions to the lattice energy.[43] In the present case the dominance of these three interactions simply reinforces the historically intuitive recognition by chemists and crystallographers of the dominance of S···S interactions in determining the packing of these structures.

Table 8. Summary of Specific S···S Interactions of TTF2[a]

rank	potential function		
	W^c	M^d	F/G^e
1	−0.97	−1.08	−1.40
	S7···S4A	S7···S4A	S7′···S4A
	I −1 0 0	I −1 0 0	I −1 0 0
	3.579	3.602	3.570
2	−1.05	−1.28	−1.48
	S4′···S1A	S4···S1A	S7···S5
	I 0 −1 0	I 0 −1 0	I −1 0 0
	3.595	3.656	3.587
3	−1.28	−1.28	−1.50
	S5···S7	S5···S4A	S4···S1A
	I −1 0 0	I 0 0 0	I 0 −1 0
	3.655	3.656	3.593
4	−1.29	−1.33	−1.63
	S5···S4A	S8···S1A	S8A···S4A
	I 0 0 0	I 0 0 0	I −1 0 0
	3.659	3.671	3.631
5	−1.30	−1.53	−1.66
	S8′···S1A	S3···S6A	S5···S4A
	I 0 0 0	I 0 0 −1	I 0 0 0
	3.660	3.776	3.639

[a] Down the columns entries are given in order of increasing distance. Each entry contains, respectively, the energy of the specific interaction (in kJ mol^{-1}), the atom pair involved in the interaction, the symmetry operation generating the atom pair interaction,[b] and the distance (in angstroms) between the two atoms in the pair. [b] For symmetry operations the roman numeral I indicates x, y, z. The following three numbers indicate translations along the a, b, and c unit-cell axes, respectively. [c] Williams potentials.[18] [d] Mirsky potentials.[19] [e] Filippini/Gavezzotti potentials.[18] / The "A" designator indicates the atom generated through the inversion center.

However, in the process of minimizing the lattice energy, the crystal structure of TTF1 has been shifted to maximize the interactions along the c axis and to decrease the contribution of the interaction along the a axis present in the experimentally determined structure. The short b axis has been reduced further, leading to an increase in the plane-to-plane stacking interactions (Table 6). While this result may appear surprising at first, upon consideration it is totally consistent with the computational process. The algorithm for minimizing the lattice energy attempts to adjust the crystal structure to bring the maximum number of interactions to the lowest possible energy. Both S···S potential energy curves employed in this case (Figure 1) have energy minima at distances which are *greater* than the two shortest contacts in the experimental crystal structure. Hence the minimization procedure will *lengthen* those two interactions and shorten others which exceed 3.83 or 3.89 Å in the experimental structure. In the M and F/G minimizations, the number of interactions and the corresponding energy can be maximized by lengthening the a axis and reducing that interaction, while at the same time decreasing the lateral interactions along the c axis and the interactions along the stack, which is exactly what happens.

While the use of lattice energy calculations is becoming increasingly common as a means for investigating and understanding the structure of organic crystals, the analysis of the results of such calculations is often limited to a comparison of the initial and final energies. In light of the significant changes in the nature and number of the interactions that stabilize the crystal structure as a result of the minimization, this procedure is not sufficient, and it is clearly necessary to examine carefully the crystal

(43) Bernstein, J. In *Organic Solid State Chemistry*; Desiraju, G. R., Ed.; Elsevier: Amsterdam, 1987; pp 471–518.

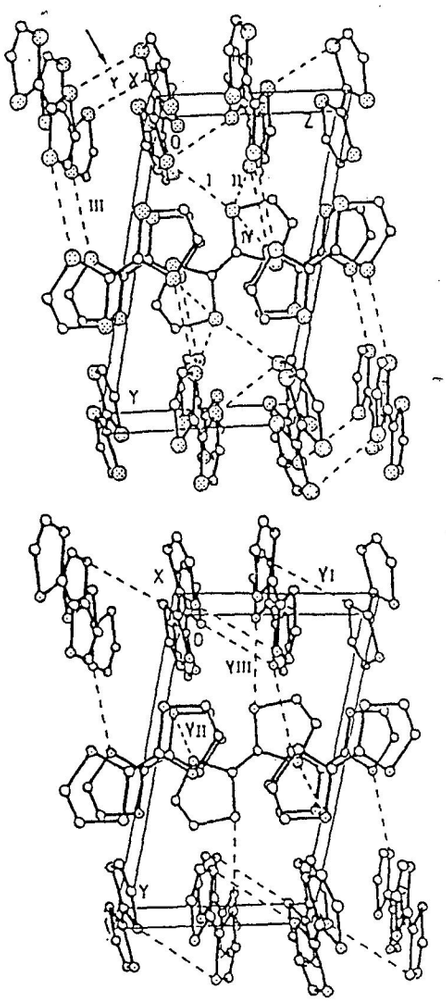


Figure 6. Two views of the crystal structure of TTF2 after lattice energy minimization. Both are views on the *bc* plane but indicate different intermolecular S···S distances described in the text and listed in Table 8.

tructures as well. In the case in point the procedure deed leads to a reasonable energy, but not a necessarily esentative structure. The source of the discrepancy s the S···S potential.

Similar results, but manifested in a different way, are btained for TTF2.[44] The S···S intermolecular distances 3.90 Å which have been discussed earlier for the xperimentally determined structure are shown in Figure and listed in Tables 6 and 7. The changes in the order f t' nteractions (as determined by the energy contribution and corresponding to the distance) are dependent on he potentials used in the calculation. Hence, in this case, s opposed to that of TTF1, in terms of the *kinds* of nteractions, the calculated structure accords with that of he e perimental one; however, their relative importance har s as a result of the energy minimization. In addition, for the *F/G* potential only, two additional (S(2)···S(8)) distances <3.90 Å appear in the list.

The structural differences between the experimental crystal structure and that following the minimization are visibly imperceptible. In either case, it is still clear that the S···S interactions are those which dominate the structure. Which are the "truly important" ones seems to be impossible to determine at this stage. As opposed to TTF1, where a few short intermolecular distances dominate the structure, TTF2 is a structure in which it does seem clear, however, that many nearly energetically equivalent S···S interactions serve to stabilize the crystal structure. Many TTF derivatives crystallize with plane-to-plane stacking which involves S···S distances of the magnitude of those observed here.[42] Also, transverse interactions are observed, leading to the formation of sheets. The geometry of nonbonded contacts to divalent sulfur has been surveyed, and appears to involve orbital interactions.[46] In TTF2 it appears that the inclination to form the stacks (chains, in the present case) is modulated by competition with the neighboring chain, leading to the unusual structure observed.

Concluding Remarks

The newly discovered second polymorph of TTF crystallizes in a very unusual manner, with four independent half molecules in the asymmetric unit, and exhibiting virtually none of the plane-to-plane stacking features characteristic of this family of compounds. The crystal structures of both polymorphs are characterized by intermolecular networks of S···S van der Waals interactions. We have shown that considerable care must be exercised in defining which of these interactions should be included in the determination of the nature of the network. The availability of two polymorphic structures also facilitated a comparison of the utility of three parameter sets for calculating lattice energies based on the atom···atom method. All three potentials indicate that TTF2 is less stable than TTF1, with differences in calculated lattice energies in the range expected for polymorphic structures. However, the calculated values for the lattice energy are all greater (i.e., more stable) than the experimental sublimation energy of TTF1. A more critical evaluation of these potential parameters, in particular those employed for interactions including sulfur, awaits the determination of the sublimation energy of TTF2 and other polymorphic sulfur-containing systems, as well as the appropriate crystal structure determinations and lattice energy calculations.

Acknowledgment. This work was supported in part by the Israel Ministry of Absorption and the Israel Ministry of Science and the Arts under a grant for new immigrant scientists (to A.E.). Additional financial support from the United States Army European Office of Research and the KFA Julich, Germany, is also appreciated. The authors are indebted to a referee whose careful reading and cogent remarks were particularly helpful in revising the manuscript.

Supplementary Material Available: Tables of anisotropic displacement coefficients and bond lengths and angles (2 pages); list of observed and calculated structure factors for TTF2 (12 pages). Ordering information is given on any current masthead page.

(44) A referee has correctly pointed out that the calculation of the minimized lattice energy is a 7-parameter optimization for TTF1 anmd 29-parameter optimization for TTF2 and that the performance of the optimization routine may differ in the two cases. A difference in the number of parameters to be optimized can be expected for any, indeed perhaps most, polymorphic systems, although it is quite large here. One of the hard tests for the lattice energy programs and parameters is the treatment of such polymorphic systems, and carrying out that test is one of our purposes here. We observed no evidence for any unusual behavior of the optimization procedure.

(45) Desiraju, G. R.; Halini, V. *J. Mater. Chem.* 1991, *1*, 201.

(46) Rosenfield, R. E.; Parthasarathy, R.; Dunitz, J. D. *J. Am. Chem. Soc.* 1977, *99*, 4860.

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322

Review

Pharmaceutical Solids: A Strategic Approach to Regulatory Considerations

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Purpose. This review describes a conceptual approach to the characterization of pharmaceutical solids. *Methods.* Four flow charts are presented: (1) polymorphs, (2) hydrates, (3) desolvated solvates, and (4) amorphous forms. *Results.* These flow charts (decision trees) are suggested as tools to develop information on pharmaceutical solids for both scientific and regulatory purposes. *Conclusions.* It is hoped that this review will lead to a more direct approach to the characterization of pharmaceutical solids and ultimately to faster approval of regulatory documents containing information on pharmaceutical solids.

KEY WORDS: polymorph; hydrate; amorphous form; desolvated solvate.

Interest in the subject of pharmaceutical solids stems in part from the Food and Drug Administration's (FDA's) drug substance guideline that states "appropriate" analytical procedures should be used to detect polymorphic, hydrated, or amorphous forms of the drug substance. These guidelines suggest the importance of controlling the crystal form of the drug substance. The guideline also states that it is the applicant's responsibility to control the crystal form of the drug substance and, if bioavailability is affected, to demonstrate the suitability of the control methods.

Thus, while it is clear that the New Drug Application (NDA) should contain information on solid state properties, particularly when bioavailability is an issue, the applicant may be unsure about how to scientifically approach the gathering of information and perhaps what kind of information is needed. This review is intended to provide a strategic approach to remove much of this uncertainty by presenting concepts and ideas in the form of flow charts rather than a set of guidelines or regulations. This is especially important because each individual compound has its own peculiarities which require flexibility in approach. The studies proposed herein are part of the Investigational New Drug (IND) process.

Solid drug substances display a wide and largely unpredictable variety of solid state properties. Nevertheless, application of basic physicochemical principles combined with appropriate analytical methodology can provide a strategy for scientific and regulatory decisions related to solid state behavior in the majority of cases. By addressing *fundamental* questions about solid state behavior at an early stage of drug development, both the applicant and the FDA are in a better position to assess the possible effects of any variations in the solid state properties of the drug substance. The resulting early interaction of the parties with regard to this area would not only tend to ensure uniformity of the materials used throughout the clinical trials but also fully resolve solid state issues before the critical stages of drug development. A further benefit of these scientific studies is the development of a meaningful set of solid state specifications which critically describe the solid form of the drug substance. These specifications would thus also facilitate the approval of a change in supplier or chemical process.

Our approach in this review is to suggest a sequence for collecting data on a drug substance that will efficiently answer specific questions about solid state behavior in a logical order. In "difficult" cases, perhaps where mixtures of forms must be dealt with, or other unusual properties are encountered, the suggested sequences would still have to be followed as a first stage in this investigation.

We have chosen to present this approach in the form of a series of decision trees, or flow charts (algorithms), one for each of the most common solid state forms. The charts are accompanied by examples from the literature representing the kind of data that would be useful in supporting the various decisions.

Decision trees provide conceptual frameworks for understanding how the justification for different crystal forms might be presented in the drug application. Industry may wish to use these decision trees as a strategic tool to organize the gathering of information early in the drug development process. Put another way, these decision trees provide a thought process that will lead to development of the most

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and further research is not needed. The work of Miyamae *et al.* serves as a good example of solid state studies of a drug substance which exists as polymorphs (1). Powder diffraction showed that there were two crystal forms (see Figure 2).

These workers also carried out single crystal analysis of the two crystal forms of the compound. The structures are shown in Figure 3. While such studies are not required, and indeed sometimes not possible, they provide an unequivocal confirmation of the existence of polymorphs. Moreover, once the single crystal structure of a phase has been determined, it is possible to calculate the corresponding X-ray powder pattern. This provides an irrefutable standard for identifying the phase by that method.

The DSC thermal curves of the two forms are slightly different, as shown in Figure 4 and thus may not be the preferred way of differentiating these polymorphs.

The IR spectra of the two polymorphs are quite similar(1), and IR does not appear to be a powerful method for differentiating the crystal forms in this case. Thus, for 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo(1,2-a)pyridine, powder diffraction appears to be the best method for differentiating the two forms.

Solid-state NMR is another powerful technique for analyzing different crystal forms (2,3). Figure 5 shows the solid-state C-13 NMR spectra of Forms I and II of prednisolone. Differences in the positions of the two resonances in the 120 ppm range clearly differentiate the two forms. In principle, solid state NMR is an absolute technique in which the signal intensity is proportional to the number of nuclei provided appropriate conditions are met. In addition, solid state NMR is a bulk technique which is not very sensitive to surface changes. This method appears to be very sensitive and will undoubtedly be used more often in the future as a tool to detect different crystal forms. However, with present technology, errors in solid-state quantitative studies may be rather large.

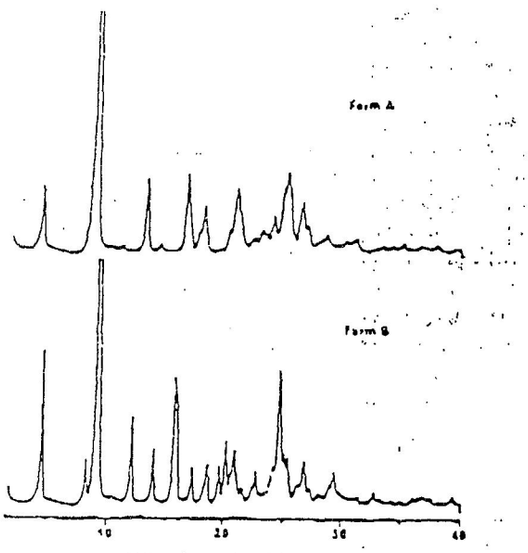

Figure 2. Powder X-ray diffraction patterns of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo(1,2-a)pyridine (1).

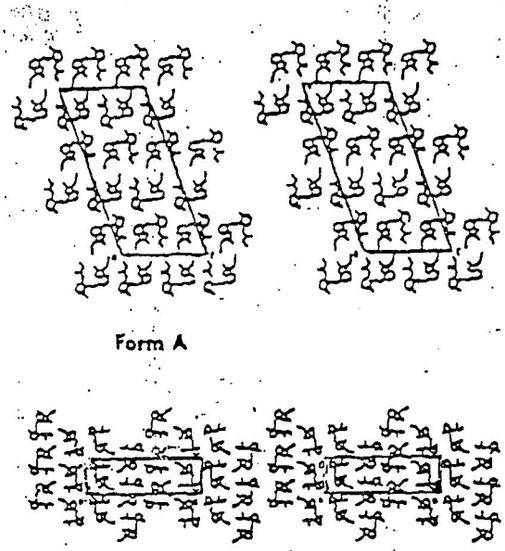

Figure 3. Stereoscopic drawings of the crystal packing of both polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo(1,2-a)pyridine viewed along the shortest axis (Form A, b-axis; Form B, a-axis) (1).

B. Do the Polymorphs Have Different Physical Properties?

If polymorphs exist then it is necessary to examine the physical properties of the different polymorphs that can affect dosage form performance (bioavailability and stability) or manufacturing reproducibility. The properties of interest are solubility profile (intrinsic dissolution rate, equilibrium solubility), stability (chemical and physical), and crystal morphology (including both shape and particle size), calorimetric behavior, and %RH profile. If there are no discernible differences between these physico-chemical properties, then the answer to the second question in the decision tree, "Different physical properties?" is "No."

The variable physical properties of several drugs with different polymorphs are reported in the literature. For example, the dissolution profiles of the polymorphs of chloramphenicol are significantly different (4). In addition, van't Hoff solubility analysis has been used to elucidate the dif-

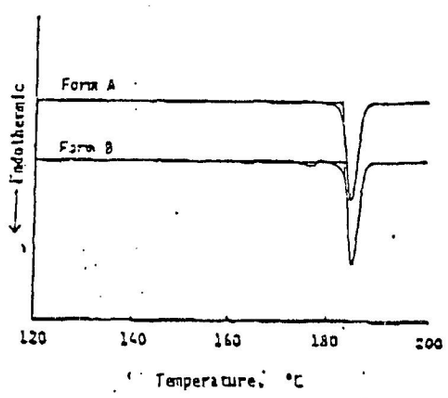

Figure 4. DSC thermal curves of the polymorphs of 8-(2-methoxycarbonylamino-6-methylbenzyloxy)-2-methyl-3-(2-propynyl)-imidazo(1,2-a)pyridine (1). These curves show that Form A melts whereas Form B undergoes a small endothermic transition and then melts at the same temperature as Form A.

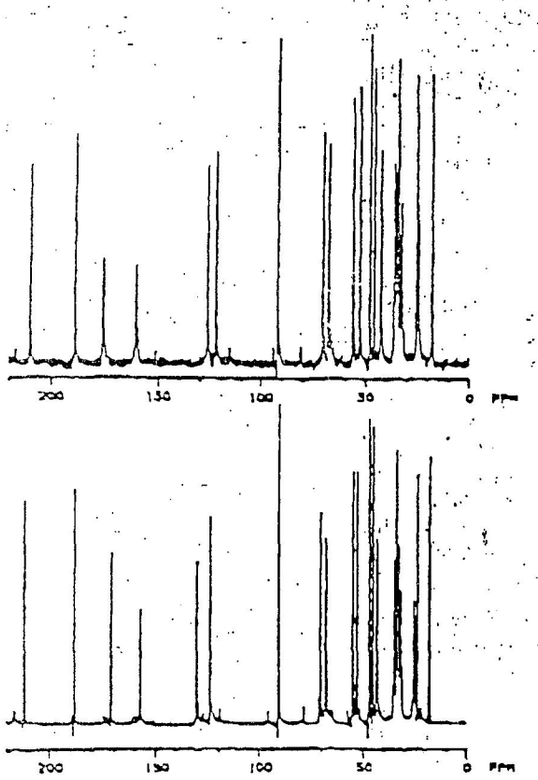

Figure 5. Solid state NMR of the two Crystal Forms of Prednisolone (2).

ferent solubilities of two polymorphs of methyl prednisolone(5). This method involves determining the equilibrium solubility of each polymorph at various temperatures. The log of the equilibrium solubility is then plotted vs 1/T. This should give straight lines for each polymorph and the temperature at which the curves intersect is the transition temperature. This technique does not work if the polymorphs interconvert.

For balance, it is important to point out that there are also cases where polymorphs exist but they have virtually identical dissolution properties(6).

C. Drug Substance Control

The important question lies in the properties that differ among polymorphs and whether those properties affect the dosage form performance (i.e., quality or bioavailability). If they do then from a regulatory standpoint it is appropriate to establish a specification/test (e.g. powder X-ray diffraction or IR) to ensure the proper form is produced. From a production standpoint, it is important to develop a process that reproducibly produces the desired polymorph.

If mixtures of forms cannot be avoided, then quantitative control is needed to ensure that a fixed proportion of forms is obtained. Furthermore, the method of analyzing for the proportion of forms would have to be validated. Also, the proportion of forms would have to remain within stated limits through the retest date of the drug substance and potentially throughout the shelf life of the product: a difficult requirement if the forms interconvert. Thus, the way to avoid a substantial amount of work in this area is to select a single solid form for production. Usually, this would be the most physically stable form when their bioavailabilities are not significantly different. Selection of the most stable from would, of course, insure that it there would be no conversion into other forms.

Powder diffraction is often a useful method to determine the percentages of polymorphs in a mixture; however, the detection limit is variable from case to case and can be as high as 15%. Matsuda (7) carried out a mixture analysis of phenylbutazone polymorphs. Diffraction lines disappear and appear as the ratio of the crystal forms change. Some of these calibration curves developed from this analysis are almost horizontal, meaning that any given mixture gives the same line intensity in this mixture range. However, other calibration curves are sloped and would appear to allow a reasonable analysis. It is fair (although Matsuda did not carry out an estimate) to estimate the errors in this analysis as ±15%.

Tanninen and Ylirussi (8) used computer curve fitting to carry out a mixture analysis of prazosin. In this particular case, they reported a highly accurate analysis, and, in fact, showed a calibration curve that could detect 0.5% of one form in another. This is obviously a highly accurate mixture analysis by powder diffraction and shows the power of this method for some applications. However, this analysis required extreme care in sample preparation and may be more difficult to carry out in a production setting where particle size may not be controlled. Similar comments apply to the analysis of mixtures by IR, where the accuracy and precision may also vary considerably from case to case. Given the analytical problems in dealing with mixtures of forms, it may generally be simpler to develop a method to prepare only one crystal form.

In summary, it is important to determine whether polymorphs are present and to solve any problems before pivotal clinical studies are initiated.

D. Determination of the Polymorph Present in the Drug Product

In cases where stability or bioavailability issues exist, the solid form present in the drug product should be investigated, if possible.

For bulk drug substances, X-ray powder diffraction and other techniques can identify the polymorph; however, solid state NMR appears to be the best method for the study of the drug substance in the dosage form (2, 3). Solid-state NMR study of three commercial products containing prednisolone showed that the products A and B contain Form I, whereas product C contains Form II. This analysis was possible even though these tablets contain approximately 95 mg of excipients and 5 mg of drug. There are numerous cases, often involving complex mixtures or low dose products, where solid state NMR (and, in fact, any technique) will not be sensitive enough to identify the polymorph present in the drug product. However, the safety and efficacy is, of course, controlled by the potency assays and by the physical tests (e.g., dissolution).

HYDRATES (SOLVATES)

The flow chart/decision tree for hydrates (solvates) is shown in Figure 6. It outlines investigations of the formation

Preparation and Anticonvulsant Activity of a Series of Functionalized α-Aromatic and α-Heteroaromatic Amino Acids

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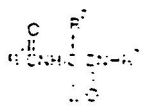
We recently reported the potent anticonvulsant activity of (*R,S*)-α-acetamido-*N*-benzyl-α-phenylacetamide (2b). Selectively substituted derivatives of this compound have now been prepared (23 examples) and evaluated in the maximal electroshock seizure (MES) and horizontal screen (tox) tests in mice. In several key cases, replacement of the α-phenyl substituent in 2b by a relatively small, electron-rich, heteroaromatic moiety led to a substantial improvement in the anticonvulsant potency of the drug candidate. The most active compounds were (*R,S*)-α-acetamido-*N*-benzyl-2-furanacetamide (2g) and (*R,S*)-α-acetamido-*N*-benzyl-2-pyrroleacetamide (2i). After ip administration, the MES ED_{50} values for 2g (10.3 mg/kg) and 2i (16.1 mg/kg) compared well with phenytoin (9.50 mg/kg). Evaluation of the two individual enantiomers of 2g demonstrated that the anticonvulsant activity resided in the *R* stereoisomer. The low ED_{50} value (3.3 mg/kg) for (*R*)-2g contributed to the large protective index (TD_{50}/ED_{50}) observed for this drug candidate, which approached that of phenytoin.

Recently we have reported the excellent anticonvulsant activities of functionalized amino acid derivatives 1.[1-5]



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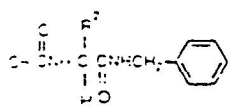


2 a $R^2 = CH_3$
2 b $R^2 = Ph$

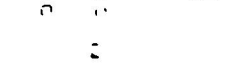
The pharmacological data suggested that these compounds comprised a new and important class of anticonvulsant agents.[6] The structure–activity profile for 1 indicated that stringent steric and electronic requirements existed for optimal anticonvulsant activity. Excellent protection against maximal electroshock seizures (MES) in mice was observed for functionalized amino acid racemates containing an *N*-benzylamide moiety, an acetylated amino group, and either a methyl (2a) or a phenyl (2b) substituent on the α-carbon. The median effective doses (ED_{50}) for 2a and 2b in mice (ip) were 76.5 and 20.3 mg/kg, respectively.[3] These values compared favorably with the corresponding ED_{50} value obtained for the proven antiepileptic phenobarbital (21.8 mg/kg).[7] Significantly, evaluation of the individual enantiomers of 2a and 2b showed that the anticonvulsant activity resided primarily in the *R* stereoisomers.[1,4,5] In both cases, the *R* isomer was over 10 times more effective in the MES test than the corresponding *S* enantiomer. This difference in activity represented the greatest eudismic ratio[8] reported to date for MES-selective anticonvulsants.

The pronounced activity observed for 2b prompted our investigation of the anticonvulsant properties of a select series of functionalized amino acids in which the α-substituent is either an aromatic or a heteroaromatic group. In this paper, the synthesis, physical properties, and anticonvulsant activities of these compounds are described. Evidence is presented that placement of a relatively small, electron-rich, heteroaromatic moiety at the α-site leads to a substantial enhancement in the anticonvulsant activity of the drug candidate and that the high eudismic ratio observed for 2a and 2b is preserved for the most active member of this series of compounds.

Selection of Compounds

(*R,S*)-α-Acetamido-*N*-benzyl-α-phenylacetamide[3] (2b) served as the parent compound in this study (Table I). In the first series of functionalized amino acid derivatives

Table I. Selected Physical and Pharmacological Data in Mice for *R,S*-α-Aromatic and α-Heteroaromatic Substituted Functionalized Amino Acid Derivatives 2[a]



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no.	R^2	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
2b[e]	C_6H_5	202–203	32.1 (27.5–40.2)	>40
2c	4-C_6H_4OH	232–235	>300	*f*
2d	4-$C_6H_4OCH_3$	196–198	>300	*f*
2e	2-OH-5-$CH_3C_5H_3$	183–185	>300	*f*
2f	2-$C_{10}H_7$	210–211	>300	>300
2g	2-furanyl	178–179	10.3 (9.1–11.6)	~40
2h	5-CH_3-2-furanyl	148–150	19.2 (16.4–23.5)	75.4
2i	2-pyrrolyl	174–175	16.1 (13.2–19.9)	>30, <100
2j	5-CH_3-2-pyrrolyl	167–168	36.5 (30.6–57.1)	*f*
2k	1-CH_3-2-pyrrolyl	179–181	~300	*f*
2l	2-thienyl	167–169	44.8 (38.9–51.4)	>30, <100
2m	3-thienyl	198–199	87.8 (69.9–150)	>100
2n	benzo[*b*]furan-2-yl	195–196	>100, <300	>100, <300
2o	indol-3-yl	213–214	>300	<300
2p	benzo[*b*]thien-2-yl	226–227	>100, <300	>100, <300
phenytoin[g]			9.5 (8.1–10.4)	65.5 (52.5–72.1)
phenobarbital[g]			21.8 (15.0–22.5)	69.0 (62.8–72.9)
valproate[e]			272 (247–338)	426 (369–450)

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) are uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Reference 3. [f] Not determined. [g] Reference 7.

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† University of Houston.
‡ Lilly Research Laboratories.

(1) Kohn, H.; Conley, J. D. *Chem. Br.* 1988, *24*, 231.
(2) Cortes, S.; Liao, Z.-K.; Watson, D.; Kohn, H. *J. Med. Chem.* 1985, *28*, 601.
(3) Conley, J. D.; Kohn, H. *J. Med. Chem.* 1987, *30*, 567.
(4) Kohn, H.; Conley, J. D.; Leander, J. D. *Brain Res.* 1988, *457*, 371.
(5) Conley, J. D.; Kohn, H., unpublished results.

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Table II. Selected Physical and Pharmacological Data in Mice for Fluoro-Substituted (*R,S*)-α-Acetamido-*N*-substituted-benzyl-2-furanacetamides 3

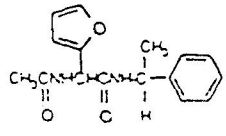
3

no.	Ar	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
2g	C_6H_5	178–179	10.3 (9.1–11.6)	~40
3a	2-FC_6H_4	193–195	40.0	[e]
3b	3-FC_6H_4	163–165	13.3 (11.5–15.3)	136 (115–162)
3c	4-FC_6H_4	188–190	12.7 (10.4–15.1)	144 (123–171)
3d	2,5-$F_2C_6H_3$	177–178	23.8 (20.2–28.4)	[e]
3e	2,6-$F_2C_6H_3$	237–239	>25, <100	[e]

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) and uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Not determined.

selected for synthesis, the α-substituent was systematically varied. Both aromatic (2c–f) and heteroaromatic (2g–p) moieties were incorporated into the amino acid backbone. In all cases, the functionalized amino acid racemates were prepared and tested.

The pharmacological properties observed for 2g warranted further investigation of this compound. Accordingly, two different types of structural modifications of the N-terminal benzyl moiety were made. First, a series of racemic fluorine-substituted benzylamides 3a–e were synthesized (Table II). Impetus for this study was provided by an earlier observation that a modest improvement of the overall activity of 2a in mice (ip) was obtained upon incorporation of a fluorine atom at the meta position of the aromatic ring.[3] The second structural modification examined for 2g involved the replacement of the *N*-benzylamide group by the corresponding *N*-α-methylbenzylamides. Use of (*R*)-α-methylbenzylamine and (*S*)-α-methylbenzylamine in the synthesis permitted the preparation and pharmacological evaluation of each of the four individual diastereomers of 4.



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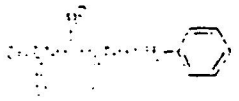
The final group of drug candidates synthesized were the individual *R*-(−) and *S*-(+) stereoisomers of 2g (Table III). The marked selectivity previously noted[1,4,5] for the individual enantiomers of 2a and 2b prompted this investigation.

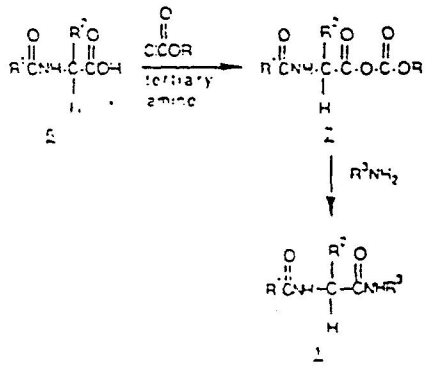
Table III. Selected Physical and Pharmacological Data in Mice for Functionalized Amino Acid Stereoisomers



no.	R^2	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
(*R,S*)-2g	2-furanyl	178–179	10.3 (9.1–11.6)	~40
(*R*)-2g	2-furanyl	196–197	3.3 (2.8–3.9)	23.8
(*S*)-2g	2-furanyl	196–197	>25	>200
(*R,S*)-2a′	CH_3	139–141	76.5 (66.6–89.0)	454 (417–501)
(*R*)-2a′	CH_3	139–141	54.8 (50.3–59.7)	214 (148–262)
(*S*)-2a′	CH_3	139–142	546 (463–741)	841 (691–954)
(*R,S*)-2b′	C_6H_5	202–203	32.1 (27.5–40.2)	>40
(*R*)-2b′	C_6H_5	219–221	26.4 (21.1–32.0)	>80
(*S*)-2b′	C_6H_5	221–222	>300	>100, <200

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) are uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Values determined at the Epilepsy Branch, NINCD, NIH; see ref 4. The median toxic dose (TD_{50}) was determined by using the rotorod test (see: Durham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc.* 1957, *46*, 208). [f] Reference 4.

Scheme I. Preparation of Compound 1 via the Mixed Carbonic Anhydride Method



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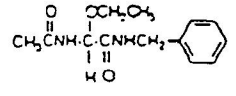
Chemistry

The strategies employed in the synthesis of the functionalized amino acid derivatives were patterned after procedures previously employed. The preparation of compounds 2c–e,g–i,k,l,n–p have been reported.[9] Introduction of the aromatic or heteroaromatic group at carbon 2 in compounds 2c–e,g–l,n–p was accomplished by an amidoalkylation reaction using 2-acetamido-*N*-benzyl-2-ethoxyacetamide (5), BF_3, and the appropriate aromatic substrate. The reactions proceeded in moderate yields (28–67%) with excellent regioselectivity.



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The remaining compounds in this study were prepared by the mixed carbonic anhydride method (Scheme I).[10] In

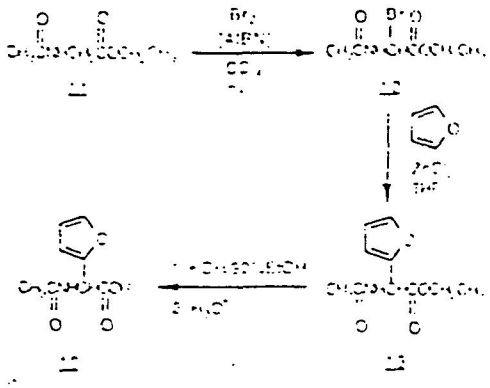
(6) For the pharmacological properties of the related α,α-dialkyl-α-phthalimidoacetamides and α,α-dialkyl-α-benzamidoacetamides, see: Upham, S. D.; Dermer, O. C. *J. Org. Chem.* 1957, *22*, 799.

(7) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. *Cleveland Clin. Q.* 1984, *51*, 293.

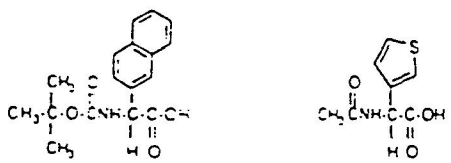
(8) Eudismic ratio = ratio of activities of the two enantiomers. See: Lehmann, P. A. *Trends Pharmacol. Sci.* 1982, *3*, 103.

(9) LeGall, P.; Sawhney, K. N.; Conley, J. D.; Kohn, H. *Int. J. Pept. Protein Res.* 1988, *32*, 279.

Scheme II. Improved Procedure for the Preparation of (*R,S*)-α-Acetamido-2-furanacetic Acid (10)



this procedure, the N-acylated amino acid 6 was treated with an alkyl chloroformate in the presence of a tertiary amine to generate the mixed N-acyl amino acid carbonic ester anhydride 7. This intermediate was not isolated but reacted in situ with the appropriate amine (R^3NH_2) to produce the N-acyl amino acid N-substituted amide 1. The starting N-acylated amino acid selected for the synthesis of 2f was *N*-*t*-Boc-(*R,S*)-2-naphthylglycine[11] (8).



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Subsequent removal (CF_3CO_2H) of the N-protecting group after the mixed carbonic anhydride coupling step, followed by acetylation with acetyl chloride and triethylamine, yielded 2f. In the synthesis of (*R,S*)-2g, (*R*)-2g, (*S*)-2g, 2m, 3, and 4 the appropriate N-acetyl amino acid was directly employed, thereby simplifying the experimental procedure. Synthesis of (*R,S*)-α-acetamido-3-thiopheneacetic acid (9) was readily accomplished beginning with (*R,S*)-α-amino-3-thiopheneacetic acid and acetic anhydride. An improved procedure (Scheme II) was developed for the synthesis of (*R,S*)-α-acetamido-2-furanacetic acid (10). This functionalized amino acid served as the starting material for compounds 2g, 3, and 4. The method adopted took advantage of the recent report on the employment of protected α-bromo amino acid derivatives as electrophilic glycine templates in amino acid synthesis.[12] Accordingly, ethyl acetamido-2-bromoacetate[13] (12) was prepared from ethyl acetamidoacetate (11) and then treated with furan and $ZnCl_2$ to give 13. Hydrolysis of the ethyl ester yielded 10, the necessary precursor for the mixed carbonic anhydride coupling procedure (Scheme I). The overall yield for 10 in this three-step sequence was 65%.

Several different alkyl chloroformates and tertiary amines were examined for the mixed carbonic anhydride reaction beginning with 10. Higher conversion rates were generally obtained with isobutyl chloroformate and 4-methylmorpholine.[10] The yields for the final coupling step

(10) For an excellent discussion and review of this method, see: Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.

(11) Kukolja, S.; Draheim, S. E.; Pfeil, J. L.; Cooper, R. D. G.; Graves, B. J.; Holmes, R. E.; Neel, D. A.; Huffman, G. W.; Webber, J. A.; Kinneck, M. D.; Vasileff, R. T.; Fosiur, B. J. *J. Med. Chem.* **1985**, *28*, 1886.

(12) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547.

(13) Kuber, R.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 599.

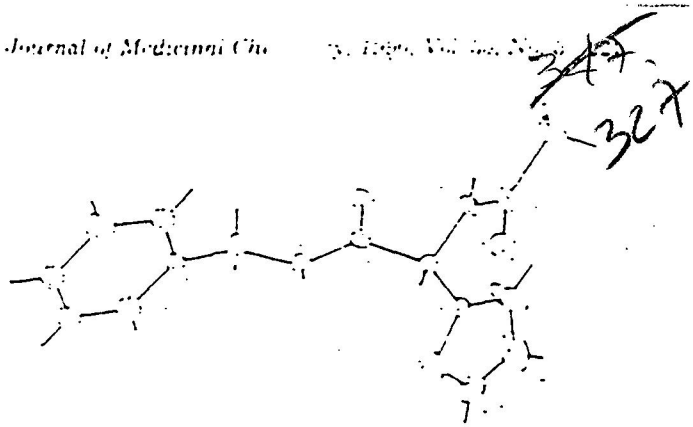
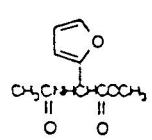


Figure 1. ORTEP view of compound (*R*)-2g with atom labeling scheme. The thermal ellipsoids are 20% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. Only one orientation of the disordered phenyl ring is shown.

for the preparation of the fluorine-substituted aryl amides 3 (Table II) ranged from 50 to 88%. A comparable synthetic protocol was adopted for methylbenzyl amides 4. The configuration at C-2 in each of the four individual diastereomers of 4 was not determined.

Synthesis of the two enantiomeric forms of 2g, (*R*)-2g, and (*S*)-2g (Table III) was achieved by resolution of racemic 10 via fractional recrystallization of the diastereomeric salts formed with (*R*)- and (*S*)-α-methylbenzylamine, respectively, and then coupling the individual stereoisomers with benzylamine. Use of isobutyl chloroformate and 4-methylmorpholine in the mixed carbonic anhydride coupling procedure did not lead to significant amounts of racemization of 2g. An X-ray crystallographic structural determination of (*R*)-2g was conducted to provide basic information concerning the solid-state structure of this compound, and the ORTEP view is presented in Figure 1. Thermal disorder limited the amount of data obtainable in this determination, but some useful observations concerning the structure of the molecular backbone of (*R*)-2g in the solid state can still be made. Significant double bond character in the C–N peptide linkages were indicated by unusually short N1–C2 (1.338 (9) Å) and N2–C4 (1.323 (8) Å) bond lengths and unusually long C1–C2 (1.509 (9) Å) and C3–C4 (1.534 (8) Å) bond lengths [for C(sp^2)–C(sp^3)]. Comparable observations have been previously noted in related compounds.[14] The torsion angles about N1–C2 and N2–C4 are essentially 0°, as would be expected in this highly conjugated system, and the sum of the angles about both nitrogens is 359°, indicating virtual planarity and substantial delocalization of the lone electron pairs.

The absolute configurations of the enantiomers of 10 were determined by converting an enriched sample of (*R*)-10 to the corresponding methyl ester (*R*)-14 with diazomethane. The optical rotation observed for this adduct [$[\alpha]^{25}_D$ = −95° (*c* = 1, MeOH)] was comparable to a sample obtained after treatment of racemic 14[9] with papain



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in aqueous DMF. This enzymatic system has been re-

(14) (a) Ishida, T.; Tanabe, N.; Inoue, M. *Acta Crystallogr.* **1983**, *C39*, 110. (b) Kojima, T.; Tanaka, I.; Ashida, T. *Ibid.* **1982**, *B38*, 221. (c) Hansen, L. K.; Hagen, E. A.; Loennechen, T.; Aasen, A. J. *Acta Chem. Scand.* **1982**, *B36*, 327. (d) Aubry, A.; Vitoux, B.; Boussard, G.; Marraud, M. *Int. J. Pept. Protein Res.* **1981**, *18*, 195 and references therein.

ported to selectively hydrolyze racemic N-protected furylglycine methyl esters to the free (S)-acids and unreacted (R)-esters in high enantiomeric excess.[15]

Several attempts were conducted to directly employ chiral (R)-13 or (R)-14 (obtained by papain-mediated hydrolysis of the corresponding racemic esters) for the preparation of (R)-2g. Unfortunately, treatment of either ester with benzylamine in the absence or presence of NaCN gave racemic 2g.[16]

Pharmacological Evaluation

The N-acetyl amino acid N-substituted amides 2–4 were tested for anticonvulsant activity by using the procedures described by Krall et al.[17] All compounds were administered intraperitoneally (ip) in mice. Tables I–III list the median effective dose (ED_{50}) values required to prevent seizures in the MES test by racemic 2, racemic 3, and the individual enantiomers of 2g, respectively. Included in these tables are the median toxic dose (TD_{50}) values determined for select compounds by using the horizontal screen test.[18]

Table I lists the pharmacological data for those compounds in which only the α-carbon moiety has been modified. Evaluation of this subset of results revealed several important observations. First, addition of electron-releasing hydroxy (i.e., 2c and 2e) or methoxy (i.e., 2d) groups to the α-substituted phenyl group in 2b or expansion of the aromatic ring from the phenyl group in 2b to the naphthyl residue in 2f led to a precipitous drop in anticonvulsant potency. Second, replacement of the α-phenyl ring in 2b with an electron-rich, five-membered heteroaromatic ring resulted in a substantial improvement in the potency of the compound in the MES test. Notable protection against seizures were observed for the racemates of 2g–j and 2l. The ED_{50} values for these compounds compared favorably with the reported data for phenytoin.[7] Third, placement of a methyl substituent on the five-membered heteroaromatic ring was accompanied by a decrease in the potency of the drug candidate versus the unsubstituted compounds (i.e., 2h versus 2g; 2j, 2k versus 2i). Fourth, replacement of the α-heteroaromatic substituent by the corresponding benzoheteroaromatic group led to a reduction in biological activity (i.e., 2n–p). This observation paralleled the results obtained for 2b versus 2f.

The second and third series of compounds tested for anticonvulsant activity were adducts in which the terminal N-benzylamide moiety in 2g was altered. Table II lists the comparative data obtained for five fluorine-substituted derivatives. All the compounds exhibited pronounced activity in the MES test. The meta (3b) and para (3c) fluoro adducts displayed activities comparable to that of 2g, while a small reduction in activity versus 2g was noted for ortho derivative 3a, and the two difluoro analogues 3d and 3e. These data contrasted with the pharmacological results secured from the α-methylbenzylamides 4a–d. Increasing the size of the benzylamide moiety by incorporation of an α-methyl group resulted in a significant decrease in the anticonvulsant potency of the drug candidate regardless of the stereochemical relationship between the two asymmetric centers. The MES ED_{50} values for these compounds were all greater than 100 mg/kg. A similar trend was previously noted in the 2-acetamido-N-benzylpropionamide (2a) series.[5]

Table III lists the pharmacological data for the two individual isomers of 2g along with the racemic mixture, as well as the corresponding data for 2a and 2b.[4] In all three series of compounds a pronounced improvement in anticonvulsant potency was noted for the R enantiomer versus either the S isomer or the racemate. Moreover, in each case little activity in the MES test was observed for the S enantiomer. The ED_{50} value of (R)-2g was 3.3 mg/kg, which was considerably lower than that reported for phenytoin[7] (ED_{50} = 9.5 mg/kg). The enhanced potency of (R)-2g contributed to the observed high protective index (TD_{50}/ED_{50} = 7.2) for this compound, which compared favorably with the value observed for phenytoin (TD_{50}/ED_{50} = 6.9).

Conclusions

The pharmacological data obtained in this investigation significantly extended the structure–activity profile previously reported for functionalized amino acid derivatives.[2,3] The observed data supported our hypothesis that stringent steric and electronic requirements exist for maximal anticonvulsant activity in this novel class of compounds.[3] The outstanding potencies noted for 2g and 2i in the MES test suggested that the placement of relatively small, electron-rich groups at the α-position in 1 was beneficial for anticonvulsant activity. Furthermore, our finding that the primary activity of 2g resided in the R enantiomer provided additional evidence for the marked stereospecificity exhibited in this new class of anticonvulsants. Additional studies are in progress investigating the generality of this class of compounds as well as their mode of action.

Experimental Section

Chemistry. General Methods. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on Perkin-Elmer 1330 and 283 spectrometers and calibrated against the 1601 cm^{-1} band of polystyrene. Absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on Nicolet NT-300 and General Electric QE-300 NMR instruments. Chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si and coupling constants (J values) are in hertz. Low-resolution mass spectra (MS) were recorded at an ionizing voltage of 70 eV with a Varian MAT CH-5 spectrometer at the Lilly Research Laboratories. Microanalyses were provided by the Physical Chemistry Department of the Lilly Research Laboratories. Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521) or silica gel GHLF (20 × 20 cm; Analtech 11187).

Preparation of (R,S)-2-Acetamido-N-benzyl-2-(5-methylpyrrolyl)acetamide (2j). 2-Acetamido-N-benzyl-2-ethoxyacetamide[9] (5, 2.00 g, 8 mmol) was suspended in anhydrous Et_2O (175 mL), and then BF_3-Et_2O (1.38 g, 9.7 mmol) was added and the resulting solution was stirred (15 min). 2-Methylpyrrole[19] (0.85 g, 10 mmol) was then added and the reaction mixture was stirred under N_2 (6 days), during which time the color of the reaction mixture turned reddish brown and a dark-brown deposit formed at the bottom of the flask. The clear solution was decanted and treated with an aqueous saturated $NaHCO_3$ solution containing ice (100 mL) for 30 min. The aqueous reaction mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The brown oily residue was purified by flash column chromatography using 2% MeOH/$CHCl_3$ as the eluent to yield 0.20 g (9%) of the

(15) Drueckhanmer, D. G.; Barbas, C. F.; Nozaki, K.; Wong, C.-H.; Wood, C. Y.; Ciufolini, M. A. *J. Org. Chem.* 1988, *53*, 1607.
(16) Hogberg, T.; Strom, P.; Ebner, M.; Ramsby, S. *J. Org. Chem.* 1987, *52*, 2033.
(17) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* 1978, *19*, 409.
(18) Coughenour, L. L.; McLean, R. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* 1977, *6*, 351.
(19) Castro, A. J.; Deck, J. F.; Hugo, M. T.; Marsh, J. P.; Pfiffer, R. J. *J. Org. Chem.* 1963, *28*, 857.

desired product. Compound 2j was recrystallized from ethyl acetate/hexane to give a light yellow amorphous solid: R_f 0.44 (95:5, $CHCl_3$/MeOH); mp 167–168 °C; IR (KBr) 3250, 1630, 1520, 1420, 1360, 1300, 1260, 1230, 1160, 1110, 1020 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.87 (s, 3 H), 2.13 (s, 3 H), 4.27 (br s, 2 H), 5.33 (d, J = 7.4 Hz, 1 H), 5.60 (s, 1 H), 5.77 (s, 1 H), 7.19–7.30 (m, 5 H), 8.22 (d, J = 7.4 Hz, 1 H), 8.45 (t, J = 5.5 Hz, 1 H), 10.38 (s, 1 H); ^{13}C NMR (DMSO-d_6) 12.74, 22.49, 42.11, 51.21, 105.09, 106.07, 126.16, 126.64, 126.85, 127.09 (2 C), 128.17 (2 C), 139.33, 168.88, 169.79 ppm. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

Preparation of (*R,S*)-2-Acetamido-*N*-benzyl-2-(2-naphthyl)acetamide (2f). ***N*-*t*-Boc-(*R,S*)-2-naphthylglycine *N*-Benzylamide.** *N*-*t*-Boc-(*R,S*)-2-naphthylglycine[11] (8, 7.53 g, 25 mmol) was combined with CH_3CN (100 mL) and the mixture was placed into an ice/salt water bath (−5 °C). Et_3N (2.53 g, 3.50 mL, 25 mmol) was added dropwise, followed by ethyl chloroformate (2.71 g, 2.40 mL, 25 mmol). All additions were done slowly so that the temperature of the mixture did not rise above 0 °C. The mixture was then stirred at −5 °C (20 min). Benzylamine (2.95 g, 3.0 mL, 27.5 mmol) in CH_3CN (10 mL) was added dropwise and the mixture was stirred at −5 °C (1 h) and then room temperature (18 h). The brown mixture was concentrated in vacuo and the residue was combined with hot THF and cooled in the freezer (3 h), resulting in the formation of a white precipitate. The mixture was filtered and the precipitate was collected, dried in vacuo, and identified as Et_3NHCl. The filtrate was concentrated in vacuo and the resulting solid was recrystallized from chloroform/hexane: yield 4.18 g (43%); mp 127–129 °C; IR (KBr) 3240, 1635, 1520, 1505, 1460, 1370, 720, 705 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (s, 9 H), 4.32 (s, 2 H), 5.42 (s, 1 H), 7.14–7.79 (m, 12 H), the N-H protons were not detected; ^{13}C NMR (DMSO-d_6) 28.2 (3C), 43.3, 58.3, 80.0, 124.6, 126.1, 126.2, 126.3, 127.1, 127.2 (2 C), 127.5, 127.9, 128.3 (2 C), 128.6, 133.0, 133.2, 135.9, 137.7, 155.3, 170.3 ppm. Anal. ($C_{24}H_{26}N_2O_3$) C, H, N.

(*R,S*)-2-Naphthylglycine *N*-Benzylamide Methanesulfonate. The Boc-protected amino acid *N*-benzylamide (3.91 g, 10 mmol) was dissolved in trifluoroacetic acid (25 mL) and was stirred at room temperature (30 min), during which time gas evolved. The solution was concentrated in vacuo and the residue was redissolved in MeOH (50 mL). Methanesulfonic acid (0.96 g, 0.65 mL, 10 mmol) was added dropwise and stirred (5 min). After concentrating the solution in vacuo, the residue was repeatedly dissolved in MeOH and the solvent was removed (3 × 50 mL). The residue was then dried under vacuum (18 h), leaving a yellow oil. Trituration with CH_2Cl_2 gave a white solid: yield 2.48 g (83%); mp 180–182 °C; IR (KBr) 3245, 1655, 1460, 1385, 730, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3 H), 4.33 (d, J = 5.5 Hz, 2 H), 5.18 (s, 1 H), 7.15–8.09 (m, 12 H), 8.78 (s, 1 H), 9.06 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 39.5, 42.3, 55.7, 124.8, 126.6, 126.7, 127.0, 127.5, 127.8, 128.0 (2 C), 128.3, 131.4, 132.4, 132.8, 138.3, 167.1 ppm. The resonances for the remaining aromatic carbons were not detected. Anal. ($C_{20}H_{22}N_2O_4S$) C, H, N.

(*R,S*)-2-Acetamido-*N*-benzyl-2-(2-naphthyl)acetamide (2f). (*R,S*)-2-Naphthylglycine *N*-benzylamide methanesulfonate (1.59 g, 4.1 mmol) was suspended in CH_3CN (25 mL) and was then cooled in an ice bath. Et_3N (0.83 g, 1.20 mL, 8.2 mmol) was added dropwise, followed by acetyl chloride (0.32 g, 0.30 mL, 4.1 mmol). The ice bath was removed and stirring was continued at room temperature (18 h). The solution was concentrated in vacuo and the residue was recrystallized from 1:1 95% EtOH/H_2O: yield 1.31 g (95%); mp 210–211 °C; IR (KBr) 3230, 1710, 1625, 1535, 1465, 760, 710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.94 (s, 3 H), 4.30 (d, J = 5.2 Hz, 2 H), 5.86 (d, J = 7.9 Hz, 1 H), 7.15–7.91 (m, 12 H), 8.63 (d, J = 7.9 Hz, 1 H), 8.33 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.5, 42.2, 56.6, 125.5, 126.0, 126.1, 126.3, 126.8, 127.1 (2 C), 127.5, 127.7, 127.9, 128.2 (2 C), 132.4, 132.8, 136.5, 139.1, 169.2, 170.0 ppm. Anal. ($C_{21}H_{22}N_2O_2$) C, H, N.

Preparation of (*R,S*)-α-Acetamido-*N*-benzyl-3-thiopheneacetamide (2m). **(*R,S*)-α-Acetamido-3-thiopheneacetic Acid (9).** (*R,S*)-α-Amino-3-thiopheneacetic acid (3.92 g, 25 mmol) was combined with H_2O (55 mL) and was cooled in an ice water bath. Solid NaOH (1.00 g, 25 mmol) was added in one portion and the reaction mixture was stirred until homogeneous. Ac_2O (5.10 g, 4.70 mL, 50 mmol) was added dropwise, followed by aqueous NaOH (5 M, 15 mL). The solution was stirred at a temperature below 25 °C (15 min). Acidification of the reaction solution with concentrated HCl (pH 1, 15 mL) led to the formation of a precipitate. The mixture was filtered and the collected white solid was recrystallized from 1:1 95% EtOH/H_2O, producing light yellow crystals: yield 3.55 g (75%); mp 190–192 °C; ^1H NMR (DMSO-d_6) δ 1.90 (s, 3 H), 5.42 (d, J = 7.6 Hz, 1 H), 7.13 (d, J = 5.0 Hz, 1 H), 7.50–7.55 (m, 2 H), 8.02 (d, J = 7.6 Hz, 1 H), 12.89 (s, 1 H); ^{13}C NMR (DMSO-d_6) 22.3, 52.2, 123.3, 126.5, 127.2, 137.3, 169.3, 171.8 ppm. Anal. ($C_8H_9NO_3S$) C, H, N.

(*R,S*)-α-Acetamido-*N*-benzyl-3-thiopheneacetamide (2m). With the procedure previously described for the preparation of *N*-*t*-Boc-(*R,S*)-2-naphthylglycine *N*-benzylamide, compound 9 (2.99 g, 15 mmol) was treated with Et_3N (1.51 g, 2.10 mL, 15 mmol) and ethyl chloroformate (1.63 g, 1.43 mL, 15 mmol) and benzylamine (1.77 g, 16.5 mmol). The filtrate upon workup was concentrated in vacuo and the resulting yellow solid was recrystallized from 1:1 95% EtOH/H_2O: yield 1.91 g (44%); mp 198–199 °C; IR (KBr) 3460, 1675, 1570, 1400, 720, 695 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.91 (s, 3 H), 4.29 (d, J = 5.2 Hz, 2 H), 5.61 (d, J = 7.9 Hz, 1 H), 7.14–7.50 (m, 8 H), 8.55 (d, J = 7.9 Hz, 1 H), 8.74 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.3, 42.0, 52.5, 122.4, 126.1, 126.7, 127.0 (3 C), 128.2 (2 C), 139.0, 139.2, 169.0, 169.8 ppm. Anal. ($C_{13}H_{16}N_2O_2S$) C, H, N.

Synthesis of (*R,S*)-Ethyl α-Acetamido-2-furanacetate (13). An ethereal solution of $ZnCl_2$ (1 N, 28.00 mL, 0.028 mol) was added to a stirred solution of 12[13] (4.40 g, 0.019 mol) and furan (11.23 g, 0.165 mol) in dry THF (100 mL), and allowed to stir at room temperature (5 h). The mixture was then treated with H_2O (50 mL), the organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined and dried (Na_2SO_4), and the volatile materials were removed by distillation in vacuo to give approximately 4.00 g (97%) of light-brown semisolid material. TLC analysis showed a major spot at R_f 0.30 (1% MeOH/$CHCl_3$). The desired compound was purified by flash column chromatography on silica gel using 1% MeOH/$CHCl_3$ as the eluent to give 3.60 g (87%) of a beige solid: mp 68–70 °C (lit.[9] mp 69–70 °C).

Preparation of (*R,S*)-α-Acetamido-2-furanacetic Acid (10). Compound 13 (4.00 g, 19 mmol) was dissolved in 90:10 EtOH/H_2O (150 mL) and then KOH (2.00 g, 35 mmol) was added and the resulting solution was stirred at room temperature (48 h). The reaction was concentrated in vacuo and the residue was diluted with H_2O and then washed with Et_2O (3 × 50 mL). The aqueous layer was then made acidic with 85% H_3PO_4 and extracted with EtOAc (3 × 150 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated to dryness in vacuo to give 10: yield 2.65 g (76%), mp 172–174 °C (lit.[9] mp 171–172 °C); R_f 0.37 (8:1:1 2-propanol/NH_4OH/H_2O).

Synthesis of (*R,S*)-α-Acetamido-*N*-benzyl-substituted-2-furanacetamides (2–4). General Procedure. 4-Methylmorpholine (1 equiv) was added to a solution of 10 (1 equiv) in dry THF (75 mL/10 mmol) at −10 to −15 °C under N_2. After stirring (2 min), isobutyl chloroformate (1 equiv) was added, leading to the precipitation of a white solid. The reaction was allowed to proceed for two additional minutes and then a solution of the substituted benzylamine (1 equiv) in THF (10 mL/10 mmol) was added over 5 min at −10 to −15 °C. The reaction mixture was allowed to stir at room temperature for 5 min and then the 4-methylmorpholine hydrochloride salt was filtered. The organic layer was concentrated in vacuo, the residue was triturated with EtOAc, and the remaining white solid was filtered. Concentration of the EtOAc layer led to additional amounts of the white solid. The desired product was purified by recrystallization or flash chromatography of the combined solid material.

Using this procedure the following compounds were prepared.

(*R,S*)-α-Acetamido-*N*-benzyl-2-furanacetamide (2g). Using benzylamine (0.27 g, 2.56 mmol) and racemic 10 (0.47 g, 2.56 mmol) gave 0.46 g (65%) of 2g. The product was recrystallized from EtOAc to give a white solid: mp 177–178 °C (lit.[9] mp 178–179 °C); R_f 0.30 (2% MeOH/$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.90 (s, 3 H), 4.31 (d, J = 5.0 Hz, 2 H), 5.58 (d, J = 8.1 Hz, 1 H), 6.27–6.33 (m, 1 H), 6.40–6.44 (m, 1 H), 7.20–7.36 (m, 5 H), 7.60–7.64 (m, 1 H), 8.57 (d, J = 8.1 Hz, 1 H), 8.73 (t, J = 6.0 Hz, 1 H).

(*R,S*)-α-Acetamido-*N*-(2-fluorobenzyl)-2-furanacetamide (3a). Using 2-fluorobenzylamine (1.13 g, 9.0 mmol) and racemic

acid 10 (1.50 g, 8.2 mmol) gave 1.20 g (50%) of 3a: mp 193–195 °C (recrystallized from EtOAc); R_f 0.36 (4% MeOH/$CHCl_3$); IR (KBr) 3270, 1630, 1520, 1440, 1360, 1220, 1180, 1140, 1100, 1000, 740 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.89 (s, 3 H), 4.33 (d, J = 5.5 Hz, 2 H), 5.58 (d, J = 8.0 Hz, 1 H), 6.28 (s, 1 H), 6.29 (s, 1 H), 7.13–7.35 (m, 4 H), 7.62 (s, 1 H), 8.61 (d, J = 8.0 Hz, 1 H), 8.76 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.35, 36.12 (d, J_{CF} = 6.6 Hz), 50.88, 107.64, 110.43, 115.04 (d, J_{CF} = 21.4 Hz), 124.29 (d, J_{CF} = 4.2 Hz), 125.64 (d, J_{CF} = 15.0 Hz), 128.94 (d, J_{CF} = 9.0 Hz), 129.27 (d, J_{CF} = 5.5 Hz), 142.66, 151.07, 159.99 (d, J_{CF} = 244.4 Hz), 168.17, 169.24 ppm. Anal. ($C_{15}H_{15}N_2O_3F$) C, H, N.

(*R,S*)-α-Acetamido-*N*-(3-fluorobenzyl)-2-furanacetamide (3b). Making use of 3-fluorobenzylamine (1.13 g, 9.0 mmol) and racemic 10 (1.50 g, 8.2 mmol) gave 1.90 g (80%) of 3b: mp 163–165 °C (recrystallized from EtOAc); R_f 0.30 (4% MeOH/$CHCl_3$); IR (KBr) 3230, 1630, 1540, 1440, 1360, 1220, 1140, 1000, 730 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.89 (s, 3 H), 4.31 (d, J = 5.5 Hz, 2 H), 5.55 (d, J = 7.8 Hz, 1 H), 6.31 (s, 1 H), 6.42 (s, 1 H), 6.98–7.37 (m, 4 H), 7.62 (s, 1 H), 8.61 (d, J = 7.8 Hz, 1 H), 8.70 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.35, 41.71, 51.01, 107.73, 110.59, 113.50 (d, J_{CF} = 21.6 Hz), 113.60 (d, J_{CF} = 22.3 Hz), 122.95, 130.18 (d, J_{CF} = 8.6 Hz), 142.21 (d, J_{CF} = 7.5 Hz), 142.66, 151.03, 162.28 (d, J_{CF} = 243.3 Hz), 168.23, 169.31 ppm. Anal. ($C_{15}H_{15}N_2O_3F$) C, H, N.

(*R,S*)-α-Acetamido-*N*-(4-fluorobenzyl)-2-furanacetamide (3c). Using racemic acid 10 (1.50 g, 8.2 mmol) and 4-fluorobenzylamine (1.13 g, 9.0 mmol) gave 2.10 g (88%) of 3c: mp 188–190 °C (recrystallized from EtOAc); R_f 0.30 (4% MeOH/$CHCl_3$); IR (KBr) 3230, 1620, 1500, 1360, 1320, 1260, 1210, 1140, 1000, 820, 780, 730 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.88 (s, 3 H), 4.27 (d, J = 5.5 Hz, 2 H), 5.55 (d, J = 8.0 Hz, 1 H), 6.27 (s, 1 H), 6.41 (s, 1 H), 7.09–7.15 (m, 2 H), 7.21–7.27 (m, 2 H), 7.61 (s, 1 H), 8.58 (d, J = 8.0 Hz, 1 H), 8.75 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.28, 41.51, 50.87, 107.52, 110.46, 114.90 (d, J_{CF} = 21.1 Hz), 129.48 (d, J_{CF} = 8.3 Hz), 135.23 (d, J_{CF} = 3.2 Hz), 142.53, 151.08, 161.12 (d, J_{CF} = 242.2 Hz), 167.95, 169.13 ppm. Anal. ($C_{15}H_{15}N_2O_3F$) C, H, N.

(*R,S*)-α-Acetamido-*N*-(2,5-difluorobenzyl)-2-furanacetamide (3d). Using 2,5-difluorobenzylamine (1.30 g, 9.0 mmol) and racemic acid 10 (1.50 g, 8.2 mmol) gave 1.60 g (64%) of 3d: mp 177–178 °C (recrystallized from EtOAc); R_f 0.38 (4% MeOH/$CHCl_3$); IR (KBr) 3230, 1620, 1520, 1480, 1360, 1260, 1230, 1180, 1140, 1000, 860, 810, 730, 710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.89 (s, 3 H), 4.31 (d, J = 5.5 Hz, 2 H), 5.55 (d, J = 7.7 Hz, 1 H), 6.32 (s, 1 H), 6.43 (s, 1 H), 7.22–7.25 (m, 3 H), 7.62 (s, 1 H), 8.62 (d, J = 7.7 Hz, 1 H), 8.78 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.30, 35.98 (d, J_{CF} = 5.8 Hz), 51.02, 107.81, 110.58, 115.06 (dd, J_{CF} = 19.5, 25.6 Hz), 115.16 (dd, J_{CF} = 15.6, 24.7 Hz), 116.52 (dd, J_{CF} = 10.1, 23.9 Hz), 127.98 (dd, J_{CF} = 9.2, 17.7 Hz), 142.69, 150.78, 155.89 (d, J_{CF} = 239.0 Hz), 158.18 (d, J_{CF} = 238.8 Hz), 168.38, 169.35 ppm. Anal. ($C_{15}H_{14}N_2O_3F_2$) C, H, N.

(*R,S*)-α-Acetamido-*N*-(2,6-difluorobenzyl)-2-furanacetamide (3e). Making use of 2,6-difluorobenzylamine (1.30 g, 9.0 mmol) and racemic acid 10 (1.50 g, 8.2 mmol) gave 1.90 g (73%) of 3e: mp 237–239 °C (recrystallized from EtOH); IR (KBr) 3230, 1620, 1530, 1460, 1360, 1320, 1260, 1220, 1160, 1140, 1030, 1000, 920, 780, 750, 740, 710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.86 (s, 3 H), 4.33 (d, J = 4.5 Hz, 2 H), 5.53 (d, J = 8.3 Hz, 1 H), 6.17 (s, 1 H), 6.38 (s, 1 H), 7.05–7.10 (m, 2 H), 7.36–7.41 (m, 1 H), 7.60 (s, 1 H), 8.52 (d, J = 8.3 Hz, 1 H), 8.66 (t, J = 4.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.33, 30.74 (t, J_{CF} = 4.4 Hz), 50.48, 107.24, 110.40, 111.61 (dd, J_{CF} = 8.0, 25.1 Hz), 113.67 (t, J_{CF} = 19.5 Hz), 129.98 (t, J_{CF} = 10.5 Hz), 142.50, 151.23, 160.93 (d, J_{CF} = 248.1 Hz), 161.10 (d, J_{CF} = 248.1 Hz), 167.59, 169.00 ppm. Anal. ($C_{15}H_{14}N_2O_3F_2$) C, H, N.

(*S*)-α-Acetamido-*N*-((*R*)-α-methylbenzyl)-2-furanacetamide and (*R*)-α-Acetamido-*N*-((*R*)-α-methylbenzyl)-2-furanacetamide (4a and 4b). Using (*R*)-α-methylbenzylamine (1.10 g, 9.0 mmol) and racemic 10 (1.50 g, 8.2 mmol) gave a crude mixture (2.00 g) which was purified by flash column chromatography on SiO_2 gel using 2% MeOH/$CHCl_3$ as the eluent to give 4a and 4b.

Compound 4a: yield 0.70 g (30%); mp 224–226 °C (recrystallized from EtOAc); $[\alpha]^{25}_D$ = +127.4° (*c* = 1, MeOH); R_f 0.32 (2% MeOH/$CHCl_3$); IR (KBr) 3250, 1620, 1520, 1440, 1360, 1300, 1270, 1220, 1140, 1000, 800, 740, 690 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (d, J = 6.9 Hz, 3 H), 1.86 (s, 3 H), 4.85–4.98 (m, 1 H), 5.61 (d, J = 8.2 Hz, 1 H), 6.27 (s, 1 H), 6.42 (s, 1 H), 7.22–7.32 (m, 5 H), 7.62 (s, 1 H), 8.51 (d, J = 8.2 Hz, 1 H), 8.70 (d, J = 7.9 Hz, 1 H), addition of (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of 4a gave only one signal for the acetyl methyl protons; ^{13}C NMR (DMSO-d_6) 22.27 (2 C), 48.17, 50.73, 107.22, 110.47, 126.01 (2 C), 126.67, 128.21 (2 C), 142.52, 144.08, 151.42, 166.96, 168.97 ppm. Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

Compound 4b: yield 0.72 g (32%); mp 216–218 °C (recrystallized from EtOAc); $[\alpha]^{25}_D$ = +0.3 ± 0.1° (*c* = 1, MeOH); R_f 0.27 (2% MeOH/$CHCl_3$); IR (KBr) 3250, 1620, 1520, 1440, 1360, 1270, 1230, 1140, 1000, 740, 690 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.36 (d, J = 6.9 Hz, 3 H), 1.90 (s, 3 H), 4.85–5.00 (m, 1 H), 5.64 (d, J = 8.2 Hz, 1 H), 6.16 (s, 1 H), 6.38 (s, 1 H), 7.20–7.29 (m, 5 H), 7.59 (s, 1 H), 8.53 (d, J = 8.2 Hz, 1 H), 8.72 (d, J = 7.8 Hz, 1 H), addition of (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of 4b gave only one signal for the acetyl methyl protons; ^{13}C NMR (DMSO-d_6) 22.32, 22.50, 48.03, 50.64, 107.20, 110.41, 125.75 (2 C), 126.59, 128.14 (2 C), 142.48, 144.19, 151.38, 166.92, 169.02 ppm. Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

(*R*)-α-Acetamido-*N*-((*S*)-α-methylbenzyl)-2-furanacetamide and (*S*)-α-Acetamido-*N*-((*S*)-α-methylbenzyl)-2-furanacetamide (4c and 4d). Making use of (*S*)-α-methylbenzylamine (1.00 g, 8.2 mmol) and racemic 10 (1.50 g, 8.2 mmol) gave a diastereomeric mixture of 4c and 4d (2.00 g). The two isomers were separated by flash column chromatography on SiO_2 gel using 2% MeOH/$CHCl_3$ as the eluent.

Compound 4c: yield 0.36 g (15%); mp 224–226 °C (recrystallized from EtOAc); $[\alpha]^{25}_D$ = −138 ± 2° (*c* = 1, MeOH); R_f 0.32 (2% MeOH/$CHCl_3$); IR (KBr) 3250, 1620, 1530, 1360, 1270, 1220, 1140, 1000, 740, 690 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (d, J = 6.8 Hz, 3 H), 1.85 (s, 3 H), 4.85–4.98 (m, 1 H), 5.60 (d, J = 8.2 Hz, 1 H), 6.28 (s, 1 H), 6.42 (s, 1 H), 7.22–7.32 (m, 5 H), 7.62 (s, 1 H), 8.51 (d, J = 8.2 Hz, 1 H), 8.70 (d, J = 7.8 Hz, 1 H), addition of (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of 4c gave only one signal for the acetamide methyl protons; ^{13}C NMR (DMSO-d_6) 22.30 (2 C), 48.18, 50.74, 107.26, 110.51, 126.03 (2 C), 126.71, 128.25 (2 C), 142.57, 144.12, 151.44, 166.98, 169.00 ppm. Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

Compound 4d: yield 0.45 g (19%); mp 216–218 °C (recrystallized from EtOAc); $[\alpha]^{25}_D$ = −2° (*c* = 1, MeOH); R_f 0.27 (2% MeOH/$CHCl_3$); IR (KBr) 3250, 1630, 1520, 1440, 1360, 1270, 1220, 1200, 1140, 1000, 740, 690 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.35 (d, J = 6.7 Hz, 3 H), 1.89 (s, 3 H), 4.85–4.99 (m, 1 H), 5.63 (d, J = 8.2 Hz, 1 H), 6.15 (s, 1 H), 6.38 (s, 1 H), 7.19–7.29 (m, 5 H), 7.58 (s, 1 H), 8.52 (d, J = 8.2 Hz, 1 H), 8.71 (d, J = 7.6 Hz, 1 H), addition of (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of 4d gave only one signal for the acetamide methyl protons; ^{13}C NMR (DMSO-d_6) 22.32, 22.53, 48.03, 50.63, 107.22, 110.42, 125.75 (2 C), 126.61, 128.16 (2 C), 142.50, 144.21, 151.38, 166.92, 169.04 ppm. Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

(*R*)-(−)-α-Acetamido-*N*-benzyl-2-furanacetamide ((*R*)-2g). Starting with (*R*)-10 (2.45 g, 13.38 mmol) and benzylamine (1.43 g, 13.38 mmol), 2.54 g (70%) of pure (*R*)-2g was obtained. The product was further recrystallized from EtOAc to give 2.30 g of (*R*)-2g: mp 196–197 °C; $[\alpha]^{25}_D$ = −78.3° (*c* = 1, MeOH), addition of (*R*)-(−)-mandelic acid to a $CDCl_3$ solution of (*R*)-2g gave only one signal for the acetamide methyl protons; mass spectrum, *m/e* (relative intensity) 272 (M^+, 2), 184 (2), 165 (2), 140 (8), 139 (88), 138 (34), 97 (46), 96 (100), 91 (63). Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

(*S*)-(+)-α-Acetamido-*N*-benzyl-2-furanacetamide ((*S*)-2g). Using (*S*)-10 (2.83 g, 15.46 mmol) and benzylamine (1.65 g, 15.46 mmol) gave 3.80 g of the *S* enriched 2g. ^1H NMR ($CDCl_3$) analysis with (*R*)-(−)-mandelic acid showed that it was greater than 80% enriched in the *S*-(-) enantiomer 2g. The pure *S* enantiomer (1.60 g) was obtained by recrystallization from absolute EtOH: mp 196–197 °C; $[\alpha]^{25}_D$ = +79.0° (*c* = 1, MeOH); mass spectrum *m/e* (relative intensity) 273 (M^+ + 1,3), 229 (2), 214 (2), 184 (1), 165 (7), 157 (4), 140 (33), 139 (100), 138 (95), 97 (98), 96 (100), 91 (98). Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

Resolution of (*R,S*)-α-Acetamido-2-furanacetic Acid (10) Using (*R*)-(+)-α-Methylbenzylamine and (*S*)-(−)-α-Methylbenzylamine. (*R*)-α-Methylbenzylamine (13.22 g, 0.11 mol) was added to an absolute EtOH solution (550 mL) of racemic 10 (20.00 g, 0.11 mol). The resulting solution was cooled in the freezer overnight. The white precipitate (12.00 g) which separated

upon cooling was filtered, and the mother liquor was evaporated to give a salt which was later used for obtaining (S)-10. The initial precipitate was recrystallized (3×) from absolute EtOH to yield 4.20 g of the pure diastereomeric salt (R)-(α-methylbenzyl)ammonium (R)-α-acetamido-2-furanacetate: mp 173–175 °C; $[\alpha]^{25}_D$ = −105° (c = 1, MeOH). Anal. ($C_{16}H_{20}N_2O_4$) C, H, N.

The diastereomeric salt was treated with 5% aqueous NH_4OH, extracted with Et_2O (3 × 50 mL), and then acidified with an 8.5% aqueous solution of H_3PO_4 and then extracted with EtOAc (3 × 100 mL) to yield 2.45 g (25%) of (R)-10: mp 169–171 °C; $[\alpha]^{26}_D$ = −184.2° (c = 1, MeOH). Anal. ($C_8H_9NO_4$) C, H, N.

The salt obtained after evaporation of the main mother liquor was hydrolyzed with 5% aqueous NH_4OH to give 10.10 g of the enriched (S)-10 [$[\alpha]^{26}_D$ = +47.7° (c = 1, MeOH)]. (S)-α-Methylbenzylamine (6.70 g, 0.055 mol) was added to a solution of enriched (S)-10 (10.10 g, 0.055 mol) in absolute EtOH (275 mL). The white precipitate of the diastereomeric salt (8.10 g) that separated upon cooling of the solution in the freezer (1 h) was filtered. The salt was recrystallized from absolute EtOH (3×) to yield 3.00 g of (S)-(α-methylbenzyl)ammonium (S)-α-acetamido-2-furanacetate: mp 172–174 °C; $[\alpha]^{26}_D$ = +106° (c = 1, MeOH). Anal. ($C_{16}H_{20}N_2O_4$) C, H, N.

The salt from the third recrystallization was treated with 5% aqueous NH_4OH, extracted with Et_2O (3 × 50 mL), then acidified with an 8.5% aqueous solution of H_3PO_4, and then extracted with EtOAc (3 × 100 mL) to give 1.63 g (32%) of (S)-10: mp 169–171 °C; $[\alpha]^{26}_D$ = +182° (c = 1, MeOH).

Enzymatic Resolution of (R,S)-(±) Methyl α-Acetamido-2-furanacetate (14). Racemic 14[9] (0.50 g, 2.54 mmol) was dissolved in DMF (4 mL) and the solution was diluted with H_2O (20 mL). Phosphate buffer (0.2 mL, 0.1 M) and β-mercaptoethanol (0.2 mL) were then added, and the pH of the solution was adjusted to 7.0 by addition of an aqueous 1 N HCl solution. Upon addition of papain (Sigma, Catalog Number P3375, activity 2.8, 50 mg) the pH of the solution dropped below 7.0. The pH of the solution was maintained at 7.0 by addition of aqueous 0.1 N NaOH solution with the aid of a pH stat instrument. The hydrolysis reaction was slow, and therefore, a second lot of papain (50 mg) was added to the reaction mixture. After 9.9 mL of alkali (78% of the theoretical amount) had been added (5 h), the pH of the solution was raised to 8.0 by addition of aqueous 0.1 N NaOH. The reaction mixture was extracted with EtOAc (3 × 30 mL), and the combined extracts were dried (Na_2SO_4). The solvent was removed in vacuo, giving an oily residue containing desired ester (R)-14, DMF, and β-mercaptoethanol. The DMF was removed by distillation under reduced pressure (40–45 °C, 0.8 Torr) and the residue was purified by flash chromatography on SiO_2 using 1% MeOH/$CHCl_3$ as the eluent. The compound obtained was triturated with H_2O and filtered to give 0.05 g of (R)-14: $[\alpha]^{26}_D$ = −115° (c = 1, MeOH). Extraction of the acidified aqueous layer with EtOAc gave an inseparable gel which did not permit isolation of (S)-10.

Enzymatic Resolution of (R,S)-(±) Ethyl α-Acetamido-2-furanacetate (13). Racemic 13 (0.50 g, 2.37 mmol) was dissolved in DMF (4 mL), H_2O (20 mL), and phosphate buffer (0.1 mL, 0.1 M). β-Mercaptoethanol (0.1 mL) was added to the above solution and the pH of the solution was adjusted to 7.0 by addition of aqueous 1 N HCl. Papain (Sigma, Catalog Number P3375, activity 2.8, 0.10 g) was added and the pH of the reaction was maintained between 6.90 and 7.35 by the manual addition of aqueous 0.1 N NaOH. After approximately 2.5 mL of the alkali solution had been added, the rate of pH change versus time slowed down. Additional papain (2 × 0.10 g) and β-mercaptoethanol (0.2 mL) were added. After a total of 10.3 mL of alkali had been added (87% of the theoretical amount) (7.5 h), the pH of the solution was raised to 8.0 and the alkaline solution was extracted with EtOAc (3 × 30 mL). The combined extracts were dried (Na_2SO_4) and the solvent was removed. The residue that was obtained contained DMF and β-mercaptoethanol. The volatile materials were removed by distillation under reduced pressure (40–45 °C, 0.2–0.8 Torr), and the residue was purified by flash chromatography on silica gel using 1% MeOH/$CHCl_3$ as the eluent to yield 0.20 g (80%) of (R)-13: mp 65–67 °C; $[\alpha]^{26}_D$ = −69.6° (c = 1, EtOH).

Synthesis of R-(−) Enriched Methyl α-Acetamido-2-furanacetate (14). A solution of R-(−) enriched 10 (0.10 g, 0.55 mmol, $[\alpha]^{26}_D$ = −176.5° (c = 1, MeOH)) in a minimum amount of MeOH (5 mL) was mixed with an ethereal solution of diazomethane at 0 °C. The reaction was allowed to stand (1 h) at this temperature and then the excess diazomethane was removed by flushing the reaction solution with N_2. The solvent was removed by distillation in vacuo to give a quantitative yield of R enriched 14: R_f 0.43 (3% MeOH/$CHCl_3$); $[\alpha]^{26}_D$ = −95° (c = 1, MeOH). ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3 H), 3.77 (s, 3 H), 5.77 (d, J = 7.8 Hz, 1 H), 6.36 (s, 2 H), 6.67 (br s, 1 H), 7.36 (s, 1 H); ^{13}C NMR ($CDCl_3$) 22.82, 50.14, 52.95, 108.65, 110.65, 142.74, 148.50, 169.37, 169.51 ppm.

X-ray Crystal Structure of (R)-(−)-α-Acetamido-N-benzyl-2-furanacetamide ((R)-2g). Colorless, orthorhombic, thin needles were grown by slow cooling of a sample dissolved in absolute EtOH. Data collection was performed with a Nicolet R3m/v automatic diffractometer using MoKα radiation. The unit-cell parameters are a = 4.788 (1) Å, b = 13.364 (6) Å, c = 22.580 (9) Å, and V = 1445 Å^3 in space group $P2_12_12_1$. The structure was solved using Nicolet's SHELXTL PLUS direct methods program, yielding coordinates for most of the non-hydrogen atoms in the asymmetric unit, which consisted of the complete molecule. The remaining non-hydrogen atoms were located in subsequent Fourier syntheses. The usual sequence of isotropic and anisotropic refinements were followed, after which all hydrogens were entered in ideal calculated positions. Only H_1 and H_2 on the nitrogens were allowed to refine independently, the rest being constrained to riding motion. Excessive "thermal motion" was noted in the phenyl ring, and it was decided to refine a disordered model at this site. There were no heavy atoms in the compound, and thus it was not possible to determine the correct absolute configuration. Therefore, the chirality was adjusted so as to make the C_3 absolute configuration R, which is consistent with (R)-14. For compound (R)-2g after all shift/esd ratios were less than 0.5, convergence was reached at R = 0.058 (R_w = 0.034).

Pharmacology. Initial anticonvulsant evaluation of these compounds was conducted with at least three dose levels (30, 100, and 300 mg/kg) administered intraperitoneally. All tests were performed with male CF-1 mice from Charles River Breeding Laboratories (Portage, MI). Test solutions of all compounds were dissolved in 30% polyethylene glycol 400. Four mice at each dose level were tested at 0.5, 1, and 4 h after administration to determine if there was protection against MES seizures.

MES seizures were elicited by electrical current (ac 60 cps, 50 mA, 0.2 s) applied via corneal electrodes. A drop of 0.9% saline was instilled on the eye prior to application of the electrodes to insure electrical contact. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

After optimal time period for activity and the approximate dose range were determined, a dose–response curve was generated with at least three or four doses and 10–12 mice per dose. The MES ED_{50} is the estimated dose from the dose–response data to protect 50% of the mice in the MES test. Neurological impairment was measured in mice by using the horizontal screen (tox) test.[11] Previously trained mice were dosed with the compound and then placed individually on top of a square (13 × 13 cm) wire screen (no. 4 mesh) which was mounted on a vertical rod. The rod was then rotated 180°, and the number of mice that returned to the top of the screen within 1 min was determined. To determine ED_{50}s, a dose–response curve was determined at the time of peak anticonvulsant activity with at least three to four doses and 10 mice per dose. The TD_{50} is the estimated dose from the dose response data to impair 50% of the mice.

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Registry No. 2c, 124421-21-2; 2d, 124421-22-3; 2e, 124421-23-4; 2f, 124421-24-5; 2g, 124421-25-6; (R)-2g, 124509-55-3; (S)-2g, 124509-56-4; 2h, 124421-26-7; 2i, 124421-27-8; 2j, 124421-28-9; 2k, 124441-50-5; 2l, 124421-29-0; 2m, 124421-30-3; 2n, 124421-31-4

2o, 124421-32-5; 2p, 124421-33-6; 3a, 124421-34-7; 3b, 124421-35-8; 3c, 124421-36-9; 3d, 124421-37-0; 3e, 124421-38-1; 4a, 124421-44-9; 4b, 124421-45-0; 4c, 124421-46-1; 4d, 124421-47-2; 5, 117422-80-7; 8, 33741-79-6; 9, 117422-79-4; 10, 117422-81-8; (*R*)-10, 124509-57-5; (*S*)-10, 124509-58-6; 12, 124421-42-7; 13, 124421-43-8; (*R*)-13, 124509-63-3; 14, 124421-48-3; (*R*)-14, 124509-62-2; (±)-(BOC)-NHCH(2-$C_{10}H_7$)CONHCH$_2$Ph, 124421-39-2; (±)-$H_2NCH(2\text{-}C_{10}H_7)CONHCH_2Ph\cdot MeSO_3H$, 124421-41-6; 2-$FC_6H_4CH_2NH_2$, 89-99-6; 3-$FC_6H_4CH_2NH_2$, 100-82-3; 4-$FC_6H_4CH_2NH_2$, 140-75-0; 2,5-$F_2C_6H_3CH_2NH_2$, 85118-06-5; 2,6-$F_2C_6H_3CH_2NH_2$, 69385-30-4; (*R*)-PhCHMeNH$_2$, 3886-69-9; (*S*)-PhCHMeNH$_2$, 2627-86-3; 2-methylpyrrole, 636-41-9; (±)-α-amino-32-thiopheneacetic acid, 38150-49-1; furan, 110-00-9; (*R*)-(α-methylbenzyl)ammonium (*R*)-α-acetamido-2-furanacetate, 124509-59-7; (*S*)-(α-methylbenzyl)ammonium (*S*)-α-acetamido-2-furanacetate, 124509-60-0; (*R*)-(α-methylbenzyl)ammonium (*S*)-α-acetamido-2-furanacetate, 124509-61-1.

Supplementary Material Available: Tables listing data collection and processing parameters (Table IV), atomic coordinates and equivalent isotropic displacement parameters (Table V), bond lengths (Table VI), bond angles (Table VII), and hydrogen-bonding parameters (Table VIII) (4 pages); observed and calculated structure factors (Table IX) for compound (*R*)-2g (3 pages). Ordering information is given on any current masthead page.

The Use of 2-Oxazolidinones as Latent Aziridine Equivalents. 2. Aminoethylation of Aromatic Amines, Phenols, and Thiophenols[*,1]

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The utility of 2-oxazolidinones 1 as latent, carboxylated aziridine functionalities was examined. Reaction of 2-oxazolidinone (1a), 3-methyl-2-oxazolidinone (1b), 3-(phenylmethyl)-2-oxazolidinone (1c), 3-phenyl-2-oxazolidinone (1d), 4,4-dimethyl-2-oxazolidinone (1e), and 5-ethyl-2-oxazolidinone (1f) with aromatic amine salts, phenol, or thiophenols at elevated temperatures (>130 °C) afforded aminoethylated adducts. The aminoethylation occurred with concomitant loss of carbon dioxide to furnish variously substituted N-aryl-1,2-ethanediamines 4, 1-(2-phenoxyethyl)-2-imidazolidinone (8), or 2-(arylthio)ethanamines 9 on reactions of 1 with aromatic amine salts, phenol, and thiophenols, respectively. Imidazolidinone 8 is believed to be a secondary reaction product resulting from the condensation of the initially formed 2-phenoxyethanamine with starting oxazolidinone 1a. The aminoethylation reaction did not proceed with aliphatic amine hydrochlorides or alkyl mercaptans. Preliminary mechanistic pathways for these ring openings were also investigated employing a specific, C-5 deuterium-labeled oxazolidinone 1b-d_2. Ring-opening experiments of 1b-d_2 with *N*-methylaniline hydrochloride suggest reaction can occur through either a dioxazolinium 5 and/or an aziridinium 6 intermediate. In contrast, reaction of 1b-d_2 with thiophenol suggests ring-opening to proceed only via the dioxazolinium pathway.

Introduction

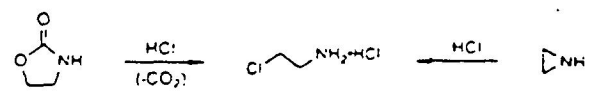
Aminoethylation transformations with amines constitute a fundamental preparative approach to myriad substituted ethylenediamine (1,2-ethanediamine) intermediates in organic synthesis.[3] These intermediates are typically prepared through use of variously substituted aziridine or substituted aziridine salts. However, over the past several decades, increasing evidence has accumulated on the toxic and carcinogenic properties of aziridines and aziridine salts which has severely limited their use for these applications.[4] We wish to describe the continuation of our efforts at the utilization of substituted 2-oxazolidinones for specific aminoethylation applications.[1,5]

The aminoethylation potential of 2-oxazolidinone was first described by Nemirowsky in 1885.[6] He reported that treatment of 2-oxazolidinone (1a) with HCl afforded 2-chloroethylamine hydrochloride and CO_2 (Scheme I). This result was followed by Gabriel's observation three years later that aziridine also furnished the same aminoethylation product on reaction with HCl.[7] As a probable result from Gabriel's findings with aziridine, the general utility of the oxazolidinone transformation lay dormant for over 65 years. Later investigators eventually corroborated Nemirowsky's earlier findings by employing other mineral acids and other oxazolidinones.[8] Similarly, more recent work has shown that 2-oxazolidinones can undergo decarboxylative ring-openings with carboxylic and sulfonic acids,[9] carboxylic acid chlorides,[1] and, under appropriate conditions, aromatic amines[10] and π-rich aromatics[11] to afford a wide variety of aminoethylated adducts.

In contrast to the ring opening of oxazolidinones at the C-5 position under electrophilic conditions, nucleophiles react exclusively at the C-2 position to give carbonyl addition products.[12] For example, reaction of oxazolidinones 1 with aliphatic or aromatic amines afford *N*-(2-hydroxyethyl)ureas 2[13] and imidazolidinones 3[14] via attack at the C-2 ring carbonyl position. Imidazolidinones 3 are observed only when aromatic amines are employed as a consequence of the higher temperatures which are required to promote the initial ring opening with 1. These higher temperatures subsequently promote the dehydration of 2 to 3 (Scheme II).

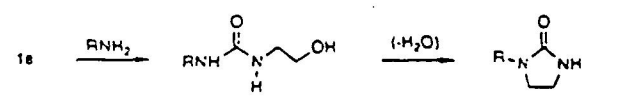
We wish to report in this account our findings on the general reaction of variously substituted 2-oxazolidinones

Scheme I



2-Oxazolidinone (1a) Aziridine

Scheme II



2 (R=aliphatic) 3 (R=aromatic)

1 with aromatic amines salts, phenols, and thiophenols to afford the corresponding 1,2-ethanediamine,[15] imidazolidinone, and 2-(arylthio)ethanamine products.[16] These

[*] Dedicated to Professor A. I. Meyers on the occasion of his sixtieth birthday.

(1) For the preceding paper in this series see: Poindexter, G. S. *J. Heterocycl. Chem.* 1983, *20*, 1431.

(2) Central Research, Dow Chemical Co., Midland, MI 06498.

(3) Dermer, O. C.; Ham, G. E. *Ethylenimine and Other Aziridines*; Academic Press: New York, 1969; pp 260–262 and references cited therein.

(4) (a) Fishbein, L. J. *Toxicol. Environ. Health (U.S.A.)* 1980, *6*, 1133. (b) Hata, Y.; Watanabe, M.; Shiratori, O.; and Takase, S. *Biochem. Biophys. Res. Commun.* 1978, *80*, 911.

(5) Poindexter, G. S. *U.S. Patent* 1983, 4,381,401.

(6) Nemirowsky, J. J. *J. Prakt. Chem.* 1885, *31*, 179.

(7) Gabriel, S. *Chem. Ber.* 1888, *21*, 1049.

(8) (a) McKay, A. F.; Braun, R. O. *J. Org. Chem.* 1951, *16*, 1829. (b) Viard, M. J. *Brit. Patent*, 1953, *693*, 325. (c) Jones, J. I. *Chem. Ind.* 1956, 1454. (d) Piper, J. R.; Elliot, R. D.; Stringfellow, C. R.; Johnson, T. P. *Ibid.* 1966, 2010. (e) Suto, M. J.; Stier, M. A.; Werbel, L. M. *J. Med. Chem.* 1991, *34*, 1207.

(9) (a) Najar, H.; Chambrier, P.; Guidicelli, R.; Menin, J. *Bull. Soc. Chim. Fr.* 1957, 1069. (b) Hsieh, H. H. *U.S. Patent* 1984, 4,444,694.

(10) (a) Yamanaka, T.; Shimizo, S. and Ikeda, S. *Jpn. Kokai Tokyo Koho* 1975, 75,111,089; *Chem. Abstr.* 1976, *84*, 44,207m. (b) Shimizu, S. Yamanka, T. *Jpn. Kokai Tokyo Koho* 1975, 101, 373; *Chem. Abstr.* 1976 *84*, 59,593h.

(11) Jouitteau, C.; Le Perchec, P.; Forestiere, A.; Sillion, B. *Tetrahedron Lett.* 1980, *21*, 1719.

(12) (a) Dyen, M. E.; Swern, D. *Chem. Rev.* 1966, *67*, 197. (b) Cornforth, J. W. *Heterocyclic Compounds*; Elderfield, R. C., Ed.; John Wiley and Sons: New York, 1957; Vol. 5, pp 396–403.

(13) Najar, H.; Chambrier, P.; Guidicelli, R.; Menin, J.; Duchemin, J. *Bull. Soc. Chim. Fr.* 1959, 1841.

(14) Gabriel, S.; Eschenbach, G. *Chem. Ber.* 1987, *30*, 2494.

(15) Analogous products have been reported for the reaction of aromatic amine salts and aziridine. For example, see: Spuenig, H.; Dokner, T.; Karn, H.; Frank, A. *Ger. Offen.* 1972, 2,057,744; *Chem. Abstr.* 1972 *77*, 61,523j.

(16) (a) Tomalia, D. A. in *Functional Monomers*; Yocum, R. H., Nyquist, E. B., Eds.; Marcel Dekker: New York, 1974; Vol. 2, Chapter 1, pp 55–71. (b) Pankratov, V. A.; Frenkel, Ts. M.; Fainleib, A. M. *Uspekhi Khimii*, 1983, *52*, 1018; English translation, *Russian Chem. Rev.* 1983, *52* 776.

Table I. Solvent and Temperature Dependence on the Reaction of 2-Oxazolidinone (1a) with Aniline Hydrochloride To Yield 4a[a]

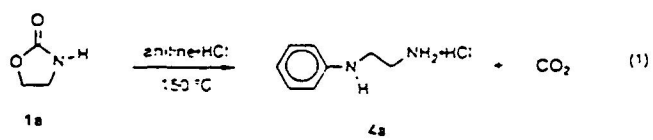
entry	solvent	temp[b] (°C)	time (h)	% yield 4a[c]
1	water	105	16	0
2	*n*-butanol	118	24	0
3	*n*-hexanol	156	72	85
4	2-(2-methoxyethoxy)ethanol	170	3	78
5	neat[d]	170	5	38

[a] All reactions were carried out using 0.10 mol quantities of 1a and aniline hydrochloride in 35 mL of the indicated solvents. [b] Experimentally determined reaction temperature. [c] Isolated crude yields. [d] Solid mixture became a partial melt at 160 °C.

results support those earlier findings of Nemirowsky's which demonstrate that under certain conditions 2-oxazolidinones can function as carboxylated aziridine equivalents.

Results and Discussion

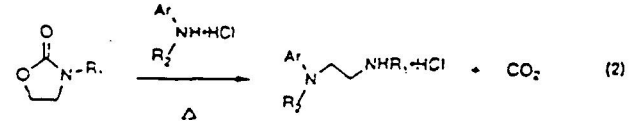
We initially observed that the reaction of 2-oxazolidinone (1a) with 1 equiv of aniline hydrochloride at 160 °C resulted in ring opening of 1a with concomitant evolution of CO_2. After basic workup, *N*-phenyl-1,2-ethanediamine (4a) was obtained as the only product in a 78% distilled yield. Products resulting from C-2 carbonyl addition (2 or 3) were not observed as was previously reported for the reaction of 1a with aniline.[12] Thus, the use of an amine salt in place of a free amine altered the course of the reaction and furnished an aminoethylated product instead of a urea or imidazolidinone (vide supra).



In order to assess the generality of the reaction, the effects of temperature and solvent were investigated. The results of these studies are summarized in Table I. The influence of reaction temperature on the ring-opening is shown in entries 1–4. No reaction occurred on heating equimolar quantities of 1a and aniline hydrochloride in either refluxing H_2O or *n*-BuOH. However, use of the higher boiling *n*-hexanol (156 °C) and 2-(2-methoxyethoxy)ethanol (170 °C) solvents promoted the ring-opening to give diamine 4a and CO_2. By incremental heating of 1a and aniline hydrochloride in *n*-hexanol, CO_2 evolution was initially observed at approximately 130 °C. Thus, this temperature probably represents the minimal temperature which is needed to initiate the reaction.

Reaction rate was also influenced by increasing the reaction temperature. Diamine 4a was obtained in an 85% yield after 72 h in refluxing *n*-hexanol (156 °C, entry 3). A 78% yield of 4a could be obtained after 3 h at 170 °C in the higher boiling 2-(2-methoxyethoxy)ethanol (entry 4). Neat reaction of 1a and aniline hydrochloride at 170 °C only gave diamine 4a in a 38% yield after 5 h. The lower reaction rate and yield observed in this latter case were probably due to inadequate mixing of the reactants since a homogeneous melt between the two reactants was not achieved.

The scope of the reaction was subsequently studied using variously substituted aromatic amine salts and other 2-substituted oxazolidinones 1. The results of these investigations are summarized in Table II. The reactions



1a, R_1=H
1b, R_1=Me
1c, R_1=CH_2Ph
1d, R_1=Ph

were carried out either at 170 °C in 2-(2-methoxyethoxy)ethanol solvent (method A) or at 160 °C as neat melts (method B). The progress of the ring-opening reactions could be conveniently monitored by observing carbon

Table II. 1,2-Ethanediamines 4 from Reaction of Oxazolidinones 1 with Aromatic Amine Hydrochloride Salts

entry	oxazolidinone	ArNHR'·HCl	method	product[b]	% yield[c]	mp/bp (°C (mmHg))
1	1a, R = H	$PhNH_2$	A	4a, $PhNHCH_2CH_2NH_2$[d]	78	95 (10 mm)
2	1a, R = H	$PhNH_2$	B	4a, $PhNHCH_2CH_2NH_2$[d]	38	95 (10 mm)
3	1a, R = H	4-$MePhNH_2$	A	4b, 4-$MePhNHCH_2CH_2NH_2$[e]	76	110 (1.5 mm)
4	1a, R = H	2-$ClPhNH_2$	A	4c, 2-$ClPhNHCH_2CH_2NH_2$[f]	50	90 (0.5 mm)
5	1a, R = H	3-$ClPhNH_2$	B	4d, 3-$ClPhNHCH_2CH_2NH_2$[f]	80	201–202[g]
6	1a, R = H	4-$MeOPhNH_2$	A	4e, 4-$MeOPhNHCH_2CH_2NH_2$[e]	45	130 (1 mm)
7	1a, R = H	4-$MeSPhNH_2$	A	4f, 4-$MeSPhNHCH_2CH_2NH_2$[f]	58	95 (0.1 mm)
8	1a, R = H	4-EtO_2CPhNH_2	A	4g, 4-$EtO_2CPhNHCH_2CH_2NH_2$[e]	61	130 (1 mm)
9	1a, R = H	3-O_2NPhNH_2	A	4h, 3-$O_2NPhNHCH_2CH_2NH_2$[h]	72	245 dec[i]
10	1a, R = H	4-H_2NPhNH_2	A	4i, 4-$H_2NPhNHCH_2CH_2NH_2$[f]	29	160 (0.1 mm)
11	1a, R = H	PhNHMe	A	4j, $PhN(Me)CH_2CH_2NH_2$[h]	79	95 (0.5 mm)
12	1a, R = H	PhNHMe	B	4j, $PhN(Me)CH_2CH_2NH_2$[h]	90	214–215[j]
13	1b, R = Me	$PhNH_2$	A	4k, $PhNHCH_2CH_2NHMe$[f]	81	105 (0.4 mm)
14	1b, R = Me	$PhNH_2$	B	4k, $PhNHCH_2CH_2NHMe$[f]	68	160–162[k]
15	1b, R = Me	PhNHMe	B	4l, $PhN(Me)CH_2CH_2NHMe$[m]	89	149–150[k]
16	1b, R = Me	$PhNHCH_2Ph$	B	4m, $PhN(CH_2Ph)CH_2CH_2NHMe$[n]	22	157–159[o]
17	1c, R = CH_2Ph	$PhNH_2$	B	4n, $PhNHCH_2NHCH_2Ph$[f]	63	172–178[j]
18	1c, R = CH_2Ph	PhNHMe	B	4o, $PhN(Me)CH_2CH_2NHCH_2Ph$	56	169–170[p]
19	1d, R = Ph	3-$MePhNH_2$	A	4p, 3-$MePHNHCH_2CH_2NHPh$	28	172–175[q]

[a] All reactions were carried out as described in the Experimental Section. Method A: 2-(2-methoxyethoxy)ethanol used as solvent. Method B: reactions carried out as neat melts. [b] All products displayed spectral properties (^1H NMR and ^{13}C NMR) which were consistent with the assigned structure. [c] Isolated yield. [d] Seto, E.; Kise, M.; Morita, I. Jpn. Kokai Tokkyo Koho 78 65,845, 1978; *Chem. Abstr.* 1978, *89*, P197,174b. [e] Fauran, C.; Douzon, C.; Raynaud, G.; Bailly, Y. Fr. Demande 2,204,407, 1974; *Chem. Abstr.* 1974, *81*, P169,539r. [f] Hiltman, R.; Wollweber, H.; Herman, G. Ger. Offen. 2,140,405, 1973; *Chem. Abstr.* 1973, *78*, 136,299x. [g] Reference 21b. [h] Lehman, D.; Femmer, K.; Faust, G. Ger. Offen. 2,844,497, 1979; *Chem. Abstr.* 1979, *91*, P123,552t. [i] Monohydrochloride salt. [j] Wotiz, J. H.; Kleopfer, R. D.; Barelski, P. M.; Hinckley, C. C.; Koster, D. F. *J. Org. Chem.* 1972, *37*, 1758. [k] Chapuis, C.; Gauvreau, A.; Klaebe, A.; Lattes, A.; Perie, J. J.; Tran Le Tran; D'Ali, A. *Bull. Soc. Chim. Fr.* 1973, 977. [l] Kliegel, W.; Franckenstein, G. H. *Liebigs Ann. Chem.* 1977, 956. [m] Kametani, T.; Kigasawa, K.; Hiiragi, M.; Aoyama, T. *J. Org. Chem.* 1972 *37*, 1450. [n] Van der Brink, F. G.; Lien, E. J. *Hand. Exp. Pharmacol.* (Histamine, Anti-Histaminics, Part 2), 1978, *18*, 333. [o] *n*-Hexanol used as solvent. [p] Dihydrochloride salt.

dioxide evolution or by TLC analysis. In contrast to the results obtained with aniline hydrochloride, good yields of diamines could be obtained by reaction of 1 and the amine hydrochlorides as neat mixtures (entries 5, 12, 14, 15, and 17). In these instances homogeneous melts were realized on heating. In general, if homogeneous melts were not obtained after heating, addition of a small amount of 2-(2-methoxyethoxy)ethanol led to dissolution of the reactants and subsequently higher yields of diamines 4.[17]

There was a casual relationship between relative amine salt acidities and yields of isolated products using the salts of π-deficient and π-rich aromatic amines. In general, the more acidic, π-deficient, amine salts gave higher isolated yields of diamine products as shown in Table II. For example, yields of aminoethylation products resulting from the reaction of 1a with the hydrochloride salts of 3-chloroaniline (entry 5), 3-nitroaniline (entry 9), or aniline itself (entry 1) were greater than those from the reaction of the more basic salts of 4-anisidine (entry 6), 4-(methylthio)aniline (entry 7), or 1,4-phenylenediamine (entry 10).

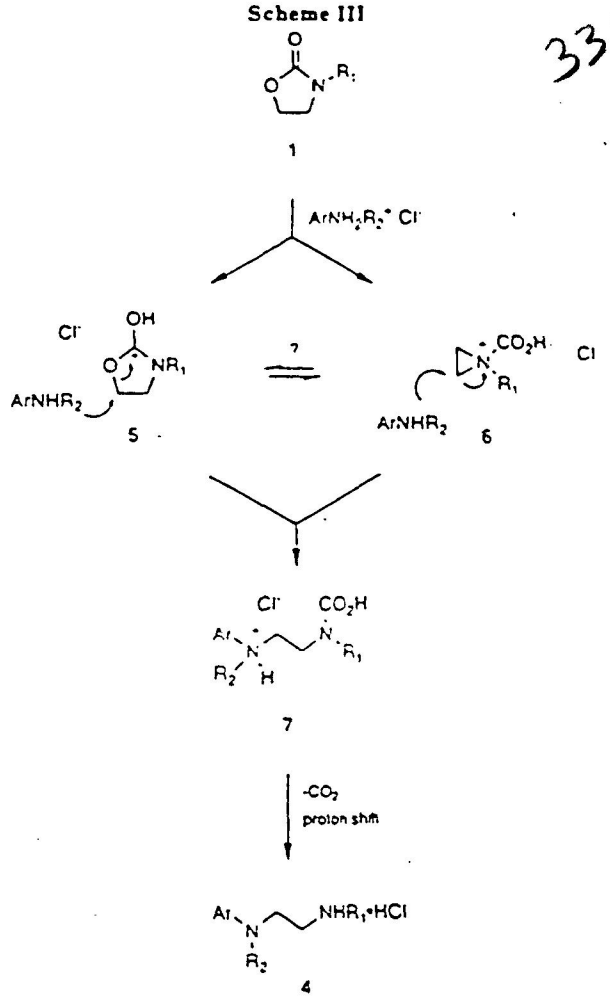
Steric interactions of both the oxazolidinone and amine salt were also examined. Addition of an *N*-methyl group to either the amine salt (entries 1 vs 11) or the oxazolidinone (entries 12 vs 15) had little effect on yield of isolated product. However, addition of the larger *N*-benzyl substituent to either oxazolidinone or amine salt (entries 15 vs 16, and 15 vs 18) dramatically lowered the isolated yields of product diamines 4 in comparison to the less sterically demanding methyl congeners. Debenzylation of 1c under the acidic conditions might also explain lowered product formation in the former example. *N*-Phenyl substitution also adversely affected yields with amine salts. Only low isolated yields of diamine product 4p were observed on the reaction of *m*-toluidine with 3-phenyl-2-oxazolidinone (1d, entry 19).

A more profound example of steric interactions adversely influencing the outcome of the ring-opening reaction was observed with use of either 4,4-dimethyl-2-oxazolidinone (1e) or 5-ethyl-2-oxazolidinone (1f). No ring-opening of either oxazolidinone with aniline hydrochloride could be detected to give any diamine products. It appears that in the ring openings with amines the oxazolidinone must be unsubstituted at the reacting or adjacent centers. Alkyl ring substituents at either the C-4 or C-5 positions of the oxazolidinone ring critically alters reactivity to preclude aminoethylation chemistry with aniline salts.

1e 1f

The ring-opening reaction did not proceed using the hydrochloride salts of aliphatic amines. Reaction of 1a and *n*-$BuNH_2$·HCl in 2-(2-methoxyethoxy)ethanol at 160 °C failed to give any diamine product after 12 h. Starting amine and oxazolidinone 1a were recovered unchanged. The ability of aromatic amine salts and reluctance of of aliphatic amine salts to promote ring-opening can presumably be explained as a function of the relative acidities of their respective conjugate acids. Aromatic amine salts (pK_a's 5) appear to be sufficiently acidic to promote the ring-opening reaction. In contrast, aliphatic amine salts (pK_a's 10) are considerably less acidic than their aromatic counterpoints and presumably not strong enough acids to initiate the reaction.

Scheme III



Although little is known about the mechanism of this amine salt mediated ring-opening, the results above are consistent with and support reaction through ionic intermediate 5 and/or 6 (Scheme III). Protonation of 1a by the amine salt at high temperatures could lead directly to ambident dioxazolinium intermediate 5. Ring-opening by nucleophilic attack at C-5 with aniline would furnish the carbamic acid 7.[18] Loss of CO_2 and prototropic shift to the more basic aliphatic nitrogen would afford the observed diamine product 4. Alternatively, ring-opening could perhaps occur through an aziridinium species such as 6. Nucleophilic attack at the C-2 position of 6 would furnish the carbamic acid 7 and ultimately diamine 4 after decarboxylation and prototropic shift. Whether the diazonium species is preceded by the formation of dioxazolinium intermediate 5 or is formed directly from starting oxazolidinone 1 is unknown at this time.

The mechanism is also attractive since it explains the absence of any polyaminoethylated amine and/or carbonyl addition products. Polyaminoethylated adducts (tri- and tetramines, etc.) are not formed since the primary aliphatic amine salts 4 are not sufficiently acidic to promote further

(17) In one particular instance, large-scale preparation of *N*-methyldiamine 4k using aniline–HCl and oxazolidinone 1b and employing 2-(2-methoxyethoxy)ethanol as solvent resulted in substantial formation of the *N*,*N*-dimethyldiamine 4l. 4l was believed to result from secondary reaction of 4k with the solvent at 170 °C under acidic conditions.

(18) Similar ambident species have been demonstrated to undergo nucleophilic attack at C-5. See: Hunig, S. *Angew. Chem., Int. Ed. Engl.* 1964, *3*, 548.

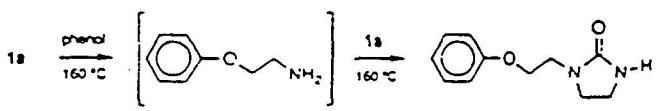
(19) (a) Oda, R.; Miyanoki, M.; Okano, M. *Bull. Chem. Soc. Jpn.* 1962, *35*, 1910. (b) Oda, R.; Miyanoki, M.; Okano, M. *Ibid.* 1962, *35*, 1309. (c) Bald, G.; Kretzschmer, K.; Markert, M.; Wimmer, M. *Angew. Makromol Chem.* 1975, *44*, 151. (d) Banks, M. R.; Cadogan, J. I. G.; Gosney, I.; Hodgson, P. K. G.; Thomson, D. E. *J. Chem. Soc., Perkin Trans. 1* 1991, 961.

Table III. Arylthioethanamines 9 from Reaction of Oxazolidinones 1 with ArSH[a]

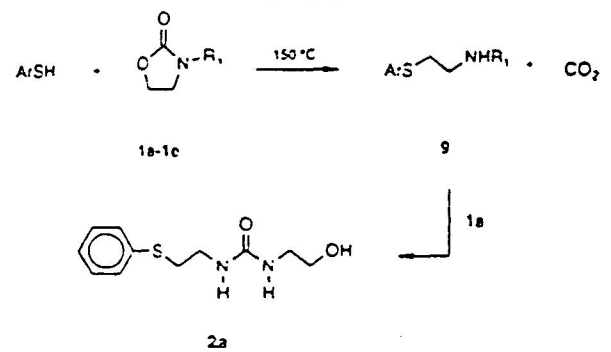
entry	oxazolidinone	ArSH	product[b]	% yield[c]	mp/bp (°C (mmHg))
1	1a, R = H	PhSH	9a, $PhSCH_2CH_2NH_2$[d]	60	130 (0.3 mm)
2	1a, R = H	2-H_2NPhSH	9b, 2-H_2NPhSCH$_2$CH$_2$NH$_2$[e]	66	130 (0.2 mm)
3	1a, R = H	4-*t*-Bu-2-MePhSH	9c, 4-*t*-Bu-2-MePhSCH$_2$CH$_2$NH$_2$	27	118 (0.2 mm)
4	1b, R = Me	PhSH	9d, PhSHCH$_2$CH$_2$NHMe[f]	86	95 (0.3 mm)
5	1b, R = Me	2-MeOPhSH	9e, 2-MeOPhSCH$_2$CH$_2$NHMe	78	101–103[g]
6	1b, R = Me	2-MePhSH	9f, 2-MePhSCH$_2$CH$_2$NHMe	82	103–104[g]
7	1b, R = Me	3-MePhSH	9g, 3-MePhSCH$_2$CH$_2$NHMe	70	56–57[g]
8	1b, R = Me	4-MePhSH	9h, 4-MePhSCH$_2$CH$_2$NHMe	76	104–106
9	1b, R = Me	4-HOPhSH	9i, 4-HOPhSCH$_2$CH$_2$NHMe	55	75–77
10	1b, R = Me	4-H_2NPhSH	9j, 4-H_2NPhSCH$_2$CH$_2$NHMe	94	212–215 dec[h]
11	1b, R = Me	4-*t*-Bu-2-MePhSH	9k, 4-*t*-Bu-2-MePhSHCH$_2$CH$_2$NHMe	76	115 (0.9 mm)
12	1c, R = CH_2Ph	PhSH	9l, PhSCH$_2$CH$_2$NHCH$_2$Ph[i]	75	120 (0.1 mm)
13	1d, R = Ph	PhSH	9m, PhSCH$_2$CH$_2$NHPh[j]	93	125 (0.1 mm)
14	1d, R = Ph	4-HO-3,5-(*t*-Bu)$_2$PhSH	9n, 4-HO-3,5-(*t*-Bu)$_2$PhSCH$_2$CH$_2$NHPh	63	138–140[g]
15	1e, (4,4-dimethyl)	PhSH	9o, PhSCH$_2$C(Me)$_2$NH$_2$[f]	64	159–160[g]
16	1e, (4,4-dimethyl)	2-MePhSH	9p, 2-MePhCH$_2$C(Me)$_2$NH$_2$	65	85 (0.4 mm)
17	1e, (4,4-dimethyl)	4-*t*-Bu-2-MePhSH	9q, 4-*t*-Bu-2-MePhSCH$_2$C(Me)$_2$NH$_2$	63	149–150[g]

[a] All reactions were carried out as neat solutions as described in the Experimental Section. [b] All products displayed spectral properties (^{1}H and ^{13}C NMR) which were consistent with assigned structure. [c] Isolated yield. [d] Reference 24. [e] Pagani, G.; Borgna, P.; Baruffini, A. *Farmaco. Ed. Sci.* 1967, *22*, 519; *Chem. Abstr.* 1968, *68*, 39,259v. [f] Wehrmeister, H. L. *J. Org. Chem.* 1963, *28*, 2589. [g] Monohydrochloride salt. [h] Dihydrochloride salt. [i] Brookes, R. F.; Godson, D. H.; Hams, A. F.; Weighton, D. M.; Wells, W. H. Aus. Patent 491,880, 1978; *Chem. Abstr.* 1978, *89*, 163,572q. [j] Grillot, G. F.; Schaffrath, R. E. *J. Org. Chem.* 1959, *24*, 1035. [k] Oxalate salt. [l] Meguerian, G.; Clapp, L. B. *J. Am. Chem. Soc.* 1951, *73*, 2121.

Scheme IV



Scheme V



ring-opening with 1. Carbonyl addition products (2 and/or 3) are not observed since the primary diamines 4 are initially formed as their hydrochloride salts and thus precluded from secondary carbonyl additions with the oxazolidinone.

In contrast to the reaction of aromatic amine hydrochlorides with oxazolidinones, the reaction of phenol with 1a was sluggish and did not afford the desired 2-phenoxyethanamine. Treatment of oxazolidinone 1a with 1 equiv of phenol at 160 °C afforded a viscous oil. Workup and purification of the oil yielded a small amount (10% yield) of a crystalline material as the only isolable product. Analysis of this material revealed it be imidazolidinone 8 (Scheme IV). Imidazolidinone 8 is the probable result of the reaction of the desired product 2-phenoxyethanamine with 1a at these elevated temperatures. The predominant products of the reaction were unreacted phenol and a polymeric material assumed to be polyethylenimine.[16a] No further attempts at optimizing the aminoethylation conditions were attempted.

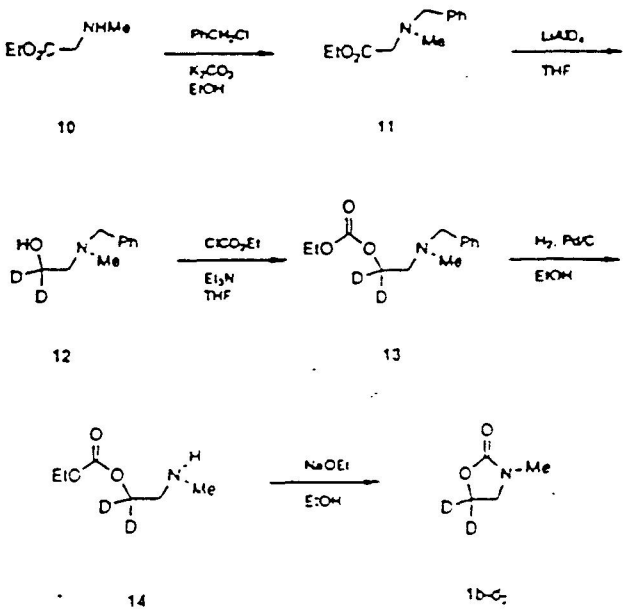
The reaction of oxazolidinones with thiophenols was also examined. In contrast to the reaction described above with phenols, thiophenols promote rapid reaction with oxazolidinones to give aminoethylated adducts in good yields. Treatment of 1a with 1 equiv of thiophenol in a 130 °C oil bath resulted in a vigorous evolution of CO_2. After 1 h the neat reaction mixture was cooled to ambient temperature where it solidified to a colorless solid. The solid was found to be a 5:1 mixture of the desired 2-(phenylthio)ethanamine (9a) and the urea 2a. Distillation afforded the pure amine 9a in 60% yield (Scheme V). Control reactions demonstrated that urea 2a was a secondary product from the condensation of amine 9a with unreacted 1a.

Other oxazolidinones and thiophenols were examined in this ring-opening reaction and the results summarized in Table III. No attempts were made to isolate any products other than the desired amines from these reactions. However, it can be seen that when other N-substituted oxazolidinones were used in the ring-opening process, the yields of the desired aminoethylation products 9 increased. Urea formation was only a problem when the N-unsubstituted 2-oxazolidinones (1a and 1e) were employed to give the more reactive primary amines (entries 1–3 and 15–17).

Yields of the desired 2-(arylthio)ethanamines 9 were very high when N-substituted 2-oxazolidinones were used. With the exception of entry 9, isolated yields of amines ranged from 70 to 94% for reaction with oxazolidinones 1b, 1c, and 1d. Thus reaction of thiophenol with *N*-methyl-, *N*-benzyl-, or *N*-phenyl-substituted oxazolidinones efficiently afforded the corresponding N-substituted amines 9d, 9l, and 9m in yields of 86%, 75%, and 93%, respectively. It was observed that some of the more sterically hindered thiophenols having substituents in the ortho position required longer reaction periods for the ring opening to go to completion. However, these examples still provided the desired amines in relatively high yield (entries 5, 6, 11, 16, and 17).

There was little influence of thiophenol acidity on ring-opening. For example, treatment of thiophenol (entry 4) or 4-aminothiophenol (entry 10) with *N*-methyloxazolidinone (1b) had little effect on product yields (86% yield for 9d vs 94% yield for 9j). The low isolated yield of amine 9i (entry 9) was probably the result of the chromatography which was required for isolation and purification. In contrast with the aromatic amines, ring-

Scheme VI



opening of the 4,4-dimethyloxazolidinone (1e) was easily accomplished to afford 2-(arylthio)propanamines 9o, 9p, and 9q in moderate yields (entries 15–17). Aminoethylations using oxazolidinone 1e generally required longer reaction times for completion due to the steric effect of the adjacent quaternary position on the ring. No reaction was observed with oxazolidinone 1f.

Aliphatic mercaptans did not promote the ring opening. Treatment of 1b with octanethiol at 160 °C for 8 h afforded no aminoethylation products. The oxazolidinone was recovered unchanged. This result is not surprising considering aliphatic amine salts also fail to effect ring opening. In this latter instance aliphatic mercaptans also appear too weakly acidic to promote the reaction.

In comparison to the analogous reactions of oxazolidinones with either amines or phenols, the use of thiophenols generally afforded higher yields of the desired amines as well as requiring shorter reaction times for completion. It appears thiophenols are sufficiently acidic to promote protonation while at the same time better nucleophiles than anilines or phenol to affect ring-opening. The mechanism of the ring opening can be envisioned to proceed through the same intermediates as illustrated in Scheme III with aromatic amines. Both a dioxazolinium intermediate 5 and/or an aziridinium species 6 can be postulated in this reaction. In order to distinguish between these possible modes of ring opening through either dioxazolinium intermediate 5 and/or aziridinium intermediate 6, we prepared the specifically labeled oxazolidinone 1b-d_2 to study deuterium distribution in the resulting diamine product. Ring-opening would either be expected to yield a specific C-2 labeled amine from reaction at the C-5 position of 5 or an amine product with both a C-1 and C-2 deuterium distribution pattern by virtue of ring-opening at the degenerate ring positions of aziridinium 6.

The preparation of 3-methyl-2-oxazolidinone 1b-d_2 having perdeuterio substitution at C-5 is depicted is Scheme VI. Treatment of ethyl sarcosine (10) with benzyl chloride gave the *N*-benzyl adduct 11 in a 72% yield. Reduction of 11 with $LiAlD_4$ in dry THF furnished amino alcohol 12 in quantitative yield. Direct reduction of 10 was precluded due to the appreciable water solubility and consequent low isolated yields of the resulting *N*-methylamino alcohol. High-field ^1H and ^{13}C NMR analysis of 12 revealed complete deuterium incorporation at the reduced position. Reaction of amino alcohol 12 with $ClCO_2Et$ gave carbonate 13 which was subsequently debenzylated on hydrogenolysis to yield amino carbonate 14 in an 80% overall yield. ^1H NMR analysis of 14 indicated no deuterium scrambling had occurred on debenzylation. Ring closure to oxazolidinone 1b-d_2 was accomplished by treatment with NaOEt in EtOH in a quantitative yield.

The integrity of the deuterium labeling in 1b-d_2 was proven by NMR analysis. The ^1H NMR spectrum of 1b-d_2 revealed no C-5 methylene absorption at 4.31 ppm indicating complete deuterium incorporation.[20] This was further supported by ^{13}C NMR analysis of the material which showed a small pentuplet for C-5 at 61.5 ppm which is also consistent for perdeuterio substitution at this position.

Ring-opening of oxazolidinone 1b-d_2 was carried out by reaction with *N*-methylaniline hydrochloride in the absence of solvent and at temperatures between 140 to 170 °C. The melts were maintained at those temperatures for 18 h to assure completeness of reaction. The deuterium substitution pattern in the product diamine 15a:16a ratios were determined by high-field ^1H NMR analysis (see Experimental Section). The results from these ring openings to yield either diamine 15a and/or 16a are summarized in Table IV.

Table IV. Deuterium Labeling Results from the Reaction of Oxazolidinone 1b-d_2 with *N*-Methylaniline Hydrochloride[a]

entry	temp (°C)	time (h)	% yield	15a:16a[b]
1	140	18	27	91:9
2	150	18	31	78:22
3	170	18	24	66:34

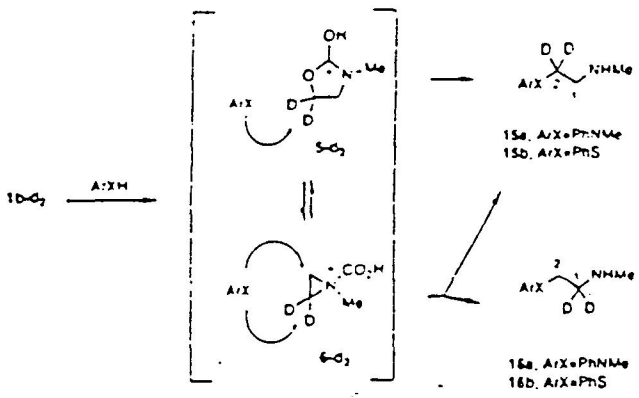
[a] Reactions were carried out on a 5 mmol scale described in the Experimental Section. [b] Deuterium distribution ratios determined by ^1H NMR analysis using a Bruker AM-300 spectrometer and are accurate to within ±5%.

Reaction of 1b-d_2 with *N*-methylaniline hydrochloride at 140 °C for 18 h gave the deuterium-substituted diamine in a 27% yield. ^1H NMR analysis showed a 15a:16a ratio of 91:9 (entry 1). This result suggests nucleophilic ring-opening proceeds in a greater than 80% fashion via dioxazolinium intermediate 5-d_2. The ratio, however, was not constant with reaction temperature. If the same reaction was carried out at 150 °C for 18 h, a 31% yield of diamine was isolated and found to have a 15a:16a ratio of 78:22 (entry 2). This temperature effect of lowering the 15a:16a ratio was further manifested on conducting the ring-opening reaction at 170 °C. In this manner the diamine was obtained in a 24% yield and found to have a 15a:16a ratio of 66:34. Control experiments using a 15a:16a hydrochloride salt mixture indicated no thermally-induced, secondary deuterium scrambling occurred on heating. The 15a:16a ratio of 91:9 was unchanged after this mixture was heated at 160 °C for 18 h. Additionally, the integrity of the deuterium positioning in starting oxazolidinone 1b-d_2 was maintained after heating at 170 °C for 18 h.

These results indicate that reaction temperature has an effect on the deuterium distribution ratios and suggest ring opening can occur through either of the postulated intermediates (Scheme VII). Lower temperatures promote reaction through dioxazolinium species 5-d_2 while higher temperatures lead to increasing ring-opening by way of aziridinum 6-d_2. Since the isotopic integrity of oxazoli-

(20) NMR spectral data and assignments for oxazolidinone 1b: ^1H NMR ($CDCl_3$) δ 4.31 (m, 2 H, CH_2O), 3.60 (m, 2 H, CH_2N), and 2.66 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) 158.8 (C-2), 61.7 (C-5), 46.8 (C-4), and 30.9 ppm (C-methyl).

Scheme VII



dinone 1b-d_2 and product diamines were not secondarily affected at these elevated temperatures, the results can possibly be explained by way of a thermally-induced 5-d_2 to 6-d_2 transformation. Oxazolidinone to aziridine rearrangements and the reverse have been reported to occur at elevated temperatures (>200 °C).[19] Under the electrophilic conditions reported here employing aniline salts, it appears this transformation can proceed at substantially lower temperatures (ca. 140 °C).

Reaction of 1b-d_2 with thiophenol at 140 °C for 5 h gave 2-(phenylthio)ethanamine 15b in an 82% yield after chromatography. ^{1}H NMR analysis of the free base revealed the C-2 methylene absorption 3.00 ppm was not present. Moreover, the ^{13}C NMR spectrum displayed a pentuplet at the C-2 position (33.2 ppm). None of the isomeric, C-1 deuterium-substituted product 16b was observed. The same reaction was also carried out at higher temperatures to determine whether any products resulting from the aziridinium pathway could be observed. Reaction of 1b-d_2 with thiophenol at 170 °C gave 15b in low yield after chromatography. Again none of the isomeric deuterium pattern in 16b was observed. In contrast to the aromatic amine hydrochlorides, it appears temperature has no influence on the ring-opening pathway with thiophenols.

In conclusion, appropriately substituted 2-oxazolidinones can function as latent carboxylated aziridine equivalents on reaction with aniline salts and thiophenols. This methodology allows the preparation of a wide variety of *N*-aryl-1,2-ethanediamines and 2-(arylthio)ethanamines. Phenols give only poor yields of aminoethylation products with oxazolidinones. In comparison to other aminoethylation methodologies using oxazolines[21] and/or aziridines,[3] the oxazolidinone method will not yield aminoethylation products with aliphatic amines or alkyl mercaptans. However, this oxazolidinone methodology can be conducted without the need for secondary hydrolysis as in the case of oxazolines or require the use of special equipment necessary for the handling of noxious and toxic aziridine intermediates. Carbon dioxide is the only byproduct. We are currently exploring reactions of oxazolidinones with other electrophiles and are attempting to extend the general methodology to other cyclic carbamates.

Experimental Section

General Methods. Distillations were performed using an Aldrich Kugelrohr oven (bulb-to-bulb), and thus the boiling points reported in Tables II and III are only approximate. Unless otherwise indicated, the aromatic amine hydrochlorides an thiophenols were obtained from commercial sources. If the salt were not commercially available, the free bases were converte to their hydrochloride salts by treatment with ethanolic HC collected by filtration, and then air dried. Starting 2-oxazolidinon (1a), 3-methyl-2-oxazolidinone (2b), and ethyl sarcosine (10) wer purchased from Aldrich Chemical Co., Milwaukee, WI. 3 Phenyl-2-oxazolidinone (1d, mp 121–122 °C), 4,4-dimethyl-2 oxazolidinone (1c, mp 54–56 °C), and 5-ethyl-2-oxazolidinone [1 bp 116 °C (0.3 mm)] were prepared following literature proce dures.[22]

3-(Phenylmethyl)-2-oxazolidinone (1c). To a stirred, col (ice-bath), N_2-blanketed suspension of NaH (28.0 g, 0.55 mol, 60% in mineral oil, hexane washed) in 100 mL of dry THF was slowl added by dropping funnel a solution of oxazolidinone 1a (43. g, 0.500 mol) dissolved in 500 mL of 10:1 THF/DMF. After th addition was complete (ca. 30 min) the resulting gray suspensio was allowed to warm to ambient temperature and stir for 24 h Benzyl bromide (66 mL, 0.56 mol) was then added to the whit suspension via syringe and the mixture stirred for 24 h. H_2O (2 mL) was then carefully introduced and the resulting solutio reduced in vacuo to approximately 200 mL and poured into 50 mL of H_2O. The aqueous mixture was extracted with thre portions of CH_2Cl_2, and then the combined organic layers we washed with H_2O and brine and dried over $MgSO_4$. After fi tration, the solution was concentrated in vacuo and the resultin yellow oil purified by Kugelrohr distillation [bp 110 °C (0.1 mm) to furnish 81.1 g (92% yield) of 1c as a clear oil which solidifie on standing at room temperature to a colorless solid: mp 76–7 °C (lit.[23] mp 80–81 °C); ^{1}H NMR ($CDCl_3$) δ 7.26 (s, 5 H), 4.37 (2 H), 4.21 (m, 2 H), and 3.63 (m, 2 H); ^{13}C NMR ($CDCl_3$) 158. 135.9, 128.8, 128.1, 127.0, 61.8, 43.3, and 44.0 ppm. Anal. Calc for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 68.02; H 6.30; N, 8.03.

General Method for the Preparation of 1,2-Ethaned amines 4. Method A. Equimolar quantities (0.20 mol) of th requisite 2-oxazolidinone 1 and aromatic amine hydrochloride we taken up in 35 mL of 2-(2-methoryethoxy)ethanol and then heate while being stirred in a 160–170 °C oil bath. During this tim the mixture generally became homogeneous and CO_2 evolutic began. After 4–24 h, the CO_2 evolution had ceased, the heati bath was removed, and the dark reaction solution was allowe to cool to room temperature. In some instances, the diamin hydrochloride 4 crystallized on standing and was collected b filtration and ether wash. When crystallization did not occu the solution was concentrated in vacuo and the resulting da residue dissolved into 100 mL of 10% aqueous NaOH (wt/vo and extracted with CH_2Cl_2. The combined organic extracts we washed with brine and then dried over anhydrous K_2CO_3. Aft filtration the solvent was removed in vacuo to furnish the cru diamines as dark liquids. Kugelrohr distillation at reduc pressures or use of flash chromatography afforded the purifi products as pale yellow liquids or low-melting solids (Table I.

Method B. Equimolar quantities (0.10 mol) of the requisi 2-oxazolidinone 1 and aromatic amine hydrochloride were heat neat while being stirred to 150–165 °C for 5–24 h or until all C evolution had ceased. The dark mixtures were then cooled to roo temperature and recrystallized from EtOH/Et_2O (Table II). T these procedures the following novel diamines were obtaine

N-[4-(Methylthio)phenyl]-1,2-ethanediamine hydr chloride (4f) was obtained as a yellow oil (method A). The fr base was then converted to its hydrochloride salt by treatme with 6.5 N ethanolic HCl and collected as a colorless white sol mp 231–232 °C dec; ^{1}H NMR (DMSO-d_6) δ 8.34 (br s, 3 H), 7. (m, 2 H), 6.60 (m, 2 H), 6.08 (br s, 1 H), 3.34 (m, 2 H), 2.95 2 H, J = 6.0 Hz), and 2.34 (s, 3 H); ^{13}C NMR (DMSO-d_6) 146 130.6, 122.5, 113.0, 40.3, 37.9, and 18.1 ppm. Anal. Calcd f $C_9H_{14}N_2S$·HCl: C, 49.42; H, 6.91; N, 12.81. Found: C, 49.22; 6.93; N, 12.86.

N-Methyl-*N*-phenyl-*N*'-(phenylmethyl)-1,2-ethanediami monohydrochloride (4o) was isolated as a creamy white so (method B): mp 169–170 °C; ^{1}H NMR (DMSO-d_6) δ 9.90 (br

(21) (a) Fazio, M. J. *J. Org. Chem.* 1984, *49*, 4889; (b) Poindexter, G. S. *Synthesis* 1981, 541. (c) Seelinger, W.; Aufdehaar, E.; Diepers, W.; Feinauer, R.; Nehring, R.; Thier, W.; Hellman, H. *Angew. Chem., Int. Ed. Engl.* 1966, *5*, 875. (d) Kormendy, K.; Sohar, P.; Vorford, J. *Ann. Univ. Sci. Budap. Rolando Eotvos Nominatae, Sect. Chim.* 1962, *4*, 61; *Chem. Abstr.* 1962, *58*, 12,505f.

(22) Homeyer, A. H. *U.S. Patent* 1946, 2,399,118.

(23) Maillard, J.; Vincent, M.; Rapin, M.; VanTri, V.; Remond. *Bull. Soc. Chim. Fr.* 1967, 2110.

2 H), 7.66 (m, 2 H), 7.35 (m, 3 H), 7.18 (m, 2 H), 6.75 (m, 3 H), 4.17 (s, 2 H), 3.80 (m, 2 H), 3.04 (m, 2 H), and 2.86 (s, 3 H); ^{13}C NMR (DMSO-d_6) 148.4, 132.0, 130.1, 129.0, 128.4, 116.3, 112.2, 50.0, 47.7, 42.7, and 37.9 ppm. Anal. Calcd for $C_{16}H_{20}N_2$·HCl: C, 69.43; H, 7.65; N, 10.12. Found: C, 69.48; H, 7.66; N, 10.21.

N-(3-Methylphenyl)-*N*'-phenyl-1,2-ethanediamine dihydrochloride (4p) was obtained as a yellow oil after purification by flash chromatography (SiO_2: EtOAc/hexane). The free base was converted to the hydrochloride salt and the salt isolated as a white crystalline solid after recrystallization from *i*-PrOH/H_2O: mp 172–175 °C; ^{1}H NMR (DMSO-d_6) δ 8.59 (br s, 2 H), 7.28 (m, 3 H), 7.07 (m, 6 H), 3.45 (s, 4 H), and 2.27 (s, 3 H); ^{13}C NMR (DMSO-d_6) 142.3, 140.1, 139.1, 129.5, 129.3, 125.3, 122.6, 119.7, 117.5, 116.4, 45.4, 43.7, and 21.1 ppm. Anal. Calcd for $C_{15}H_{18}N_2$·1.9 HCl: C, 60.95; H, 6.79; N, 9.48. Found: C, 60.69; H, 6.78; N, 9.36.

Reaction of 2-Oxazolidinone (1a) with Phenol. A mixture of phenol (9.60 g, 0.100 mol) and 2-oxazolidinone (1a, 8.70 g, 0.100 mol) was heated in a 160 °C oil bath for 3 h. After the mixture was cooled to room temperature, 250 mL of 10% aqueous NaOH (wt/vol) was added to the oil and the resulting solution extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O and brine and dried over $MgSO_4$. After filtration, the volatiles were removed in vacuo to give a colorless residue. The residue was taken up in *i*-PrOH and recrystallized to give 3.59 g (10.2 mmol, 10% yield) of 1-(2-phenoxyethyl)-2-imidazolidinone (8) as a colorless solid: mp 109–110 °C; IR (Nujol) 1695 and 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.21 (m, 2 H), 6.85 (m, 3 H), 5.58 (br s, 1 H), 4.05 (m, 2 H), and 3.50 (m, 6 H); ^{13}C NMR ($CDCl_3$) 163.1, 158.6, 129.5, 121.0, 114.6, 67.3, 46.7, 43.4, and 38.4 ppm. Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 65.87; H, 6.85; N, 13.32.

Reaction of 2-Oxazolidinone (1a) with Thiophenol. A mixture of thiophenol (13.2 g, 0.120 mol), 1a (8.71 g, 0.100 mol), and 0.45 g of LiCl was purged under N_2 for 5 min and then heated under N_2 for 1 h in a 130 °C oil bath. After cooling to ambient temperature, the resulting white solid was taken up in 10% aqueous HCl and heated on a steam bath for several min. The mixture was filtered, and the resulting solid was washed with H_2O. After recrystallization from EtOAc, 2.91 g (12.1 mmol, 12% yield) of the colorless white solid *N*-(2-hydroxyethyl)-*N*'-[2-(phenylthio)ethyl]urea (2a) was collected by filtration: mp 112–113 °C; IR (KBr) 1620, and 1590 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.33 (m, 5 H), 6.26 (t, 1 H, J = 5.7 Hz), 6.06 (t, 1 H, J = 5.5 Hz), 4.69 (br s, 1 H), 3.39 (m, 2 H), 3.20 (q, 2 H, J = 6.6 Hz), 3.10 (q, 2 H, J = 5.7 Hz), and 2.99 (t, 2 H, J = 7.0 Hz); ^{13}C NMR (DMSO-d_6) 158.0, 135.9, 128.9, 128.0, 125.5, 60.8, 42.1, 39.0, and 32.7 ppm. Anal. Calcd for $C_{11}H_{16}N_2O_2S$: C, 54.98; H, 6.72; N, 11.66. Found: C, 54.70; H, 6.80; N, 11.35.

The above filtrate was made basic to pH 10 with solid NaOH and then extracted with CH_2Cl_2. The combined extracts were washed with H_2O and brine and dried over K_2CO_3. After filtration and concentration in vacuo, Kugelrohr distillation (130 °C (0.3 mm)) gave 9.18 g (60.0 mmol, 60% yield) of 2-(phenylthio)-ethanamine (9a)[24] as a clear oil. The oil was treated with ethanolic HCl to give the salt as a colorless solid after trituration from Et_2O: mp 106–110 °C (sintered); ^{1}H NMR (DMSO-d_6) δ 8.41 (br s, 3 H), 7.28 (m, 5 H), 3.27 (t, 2 H, J = 7.0 Hz), and 2.94 (t, 2 H, J = 7.0 Hz). Anal. Calcd for $C_8H_{11}NS$·HCl: C, 50.66; H, 6.38; N, 7.39. Found: C, 50.59; H, 6.54; N, 7.66.

General Method for the Preparation of 2-(Arylthio)-ethanamines 9. Equimolar amounts (0.1 mol) of the appropriate oxazolidinone 1 and thiophenol (0.1 mol) were heated together while stirring under N_2 in a 130–150 °C oil bath. The stirred reaction mixtures were maintained at these temperatures for several h or until all CO_2 evolution had ceased. Generally small amounts (ca. 10 mg) of LiCl were added to reduce reaction times. The crude reaction mixtures were then cooled to room temperature and the resulting amines purified by either distillation, flash chromatography, or conversion to their hydrochloride salts and recrystallized. The following novel amines reported in Table III were obtained using this procedure.

2-[[4-(1,1-Dimethylethyl)-2-methylphenyl]thio]ethanamine (9c) was obtained as a pale yellow oil after distillation: ^{1}H NMR ($CDCl_3$) δ 7.19 (m, 3 H), 2.67 (m, 4), 2.40 (s, 3 H), 1.50 (br s, 2 H), and 1.27 (s, 9 H); ^{13}C NMR ($CDCl_3$) 148.9, 137.5, 131.8, 129.1, 127.1, 123.3, 41.1, 37.5, 34.1, 31.2, and 20.7 ppm. Anal. Calcd for $C_{13}H_{21}NS$·0.10 H_2O: C, 69.35; H, 9.49; N, 6.23; H_2O, 0.80. Found: C, 69.43; H, 9.68; N, 6.17; H_2O, 1.10.

2-[(2-Methoxyphenyl)thio]-*N*-methylethanamine hydrochloride (9e) was obtained as a pale tan solid after recrystallization from EtOH/Et_2O: ^{1}H NMR (DMSO-d_6) δ 9.50 (br s, 2 H), 7.46 (m, 1 H), 7.22 (m, 1 H), 7.05 (m, 2 H), 3.84 (s, 3 H), 3.30 (m, 2 H), 3.06 (m, 2 H), and 2.54 (s, 3 H); ^{13}C NMR (DMSO-d_6) 156.7, 128.5, 127.5, 122.1, 121.1, 111.1, 55.8, 47.2, 32.2, and 26.0 ppm. Anal. Calcd for $C_{10}H_{15}NOS$·HCl: C, 51.38; H, 6.90; N, 5.99. Found: C, 51.18; H, 6.96; N, 6.16.

N-Methyl-2-[(2-methylphenyl)thio]ethanamine hydrochloride (9f) was isolated as a colorless solid after recrystallization from EtOH/Et_2O: ^{1}H NMR (DMSO-d_6) δ 9.64 (br s, 2 H), 7.53 (m, 1 H), 7.22 (m, 3 H), 3.42 (m, 2 H), 3.10 (m, 2 H), 2.62 (s, 3 H), and 2.34 (s, 3 H); ^{13}C NMR (DMSO-d_6) 136.7, 133.3, 130.2, 127.5, 126.7, 126.0, 47.1, 32.1, 26.8, and 19.8 ppm. Anal. Calcd for $C_{10}H_{15}NS$·HCl: C, 55.16; H, 7.41; N, 6.43. Found: C, 55.51; H, 7.54; N, 6.38.

N-Methyl-2-[(3-methylphenyl)thio]ethanamine hydrochloride (9g) was obtained as a colorless, low-melting solid after trituration from Et_2O: ^{1}H NMR ($CDCl_3$) δ 2.31 (br s, 2 H), 7.24 (m, 3 H), 7.05 (m, 1 H), 3.38 (m, 2 H), 3.11 (m, 2 H), 2.68 (s, 3 H), and 2.31 (s, 3 H); ^{13}C NMR ($CDCl_3$) 139.2, 133.1, 130.9, 129.2, 128.1, 127.3, 48.5, 33.1, 29.2, and 21.3 ppm. Anal. Calcd for $C_{10}H_{15}NS$·HCl: C, 55.16; H, 7.41; N, 6.43. Found: C, 55.28; H, 7.34; N, 6.37.

N-Methyl-2-[(4-methylphenyl)thio]ethanamine hydrochloride (9h) was collected as a colorless solid after recrystallization from from EtOH/Et_2O: ^{1}H NMR (DMSO-d_6) δ 9.60 (br s, 2 H), 7.40 (d, 2 H, J = 8.2 Hz), 7.18 (d, 2 H, J = 8.2 Hz), 3.38 (m, 2 H), 3.09 (m, 2 H), 2.60 (s, 3 H), and 2.30 (s, 3 H); ^{13}C NMR (DMSO-d_6) 136.0, 130.3, 129.9, 129.3, 47.3, 32.1, 28.1, and 20.5 ppm. Anal. Calcd for $C_{10}H_{15}NS$·HCl: C, 55.16; H, 7.41; N, 6.43. Found: C, 55.26; H, 7.47; N, 6.49.

4-[[2-(Methylamino)ethyl]thio]phenol hydrochloride (9i) was obtained as a colorless white solid after purification by flash chromatography (SiO_2: MeOH/$CHCl_3$) and conversion to the HCl salt: ^{1}H NMR (DMSO-d_6) δ 9.82 (br s, 1 H), 9.26 (br s, 2 H), 7.32 (d, 2 H, J = 8.5 Hz), 6.84 (d, 2 H, J = 8.5 Hz), 3.13 (m, 2 H), 2.99 (m, 2 H), and 2.54 (s, 3 H); ^{13}C NMR (DMSO-d_6) 157.3, 133.4, 121.1, 116.3, 47.3, 32.1, and 30.2 ppm. Anal. Calcd for $C_9H_{13}NOS$·HCl·0.20 H_2O: C, 48.41; H, 6.50; N, 6.27; H_2O, 1.61. Found: C, 48.41; H, 6.45; N, 6.24; H_2O, 1.48.

4-[[2-(Methylamino)ethyl]thio]benzenamine dihydrochloride (9j) was obtained as a brown solid after recrystallization from EtOH/Et_2O: ^{1}H NMR (DMSO-d_6) δ 10.18 (br s, 2 H), 9.40 (br s, 3 H), 7.56 (d, 2 H, J = 8.6 Hz), 7.39 (d, 2 H, J = 8.6 Hz), 3.64 (m, 2 H), 3.07 (m, 2 H), and 2.57 (s, 3 H); ^{13}C NMR (DMSO-d_6) 133.3, 130.7, 129.3, 123.6, 46.9, 32.1, and 27.4 ppm. Anal. Calcd for $C_9H_{14}N_2S$·HCl: C, 42.36; H, 6.32; N, 10.98. Found: C, 42.25; H, 6.10; N, 10.73.

2-[[4-(1,1-Dimethylethyl)-2-methylphenyl]thio]-*N*-methylethanamine (9k) was isolated as a pale yellow liquid after Kugelrohr distillation: ^{1}H NMR ($CDCl_3$) δ 7.18 (m, 3 H), 3.00 (m, 2 H), 2.80 (m, 2 H), 2.42 (s, 3 H), 2.39 (s, 3 H), 1.42 (br s, 1 H), and 1.29 (s, 9 H); ^{13}C NMR ($CDCl_3$) 149.1, 137.6, 131.7, 129.0, 127.2, 123.4, 50.4, 36.0, 34.2, 33.5, 31.3, and 20.7 ppm. Anal. Calcd for $C_{14}H_{23}NS$: C, 70.83; H, 9.77; N, 5.90. Found: C, 70.77; H, 9.78; N, 5.68.

2,6-Bis(1,1-Dimethylethyl)-4-[[2-(phenylamino)ethyl]-thio]phenol oxalate (9n) was obtained as a brown oil after purification by flash chromatography (SiO_2: EtOAc/hexane). The free base was subsequently converted to the oxalate salt to afford a white amorphous solid after recrystallization from EtOAc/hexane: ^{1}H NMR (DMSO-d_6) δ 7.15 (s, 2 H), 7.00 (t, 2 H, J = 4.0 Hz), 6.49 (t, 1 H, J = 7.3 Hz), 6.45 (t, 2 H, J = 7.8 Hz), 3.15 (t, 2 H, J = 5.7 Hz), 2.92 (t, 2 H, J = 6.4 Hz), and 1.35 (s, 18 H); ^{13}C NMR (DMSO-d_6) 160.9, 153.4, 148.2, 140.2, 128.9, 127.9, 124.6, 115.9, 111.9, 42.5, 34.6, 33.9, and 30.2 ppm. Anal. Calcd for $C_{22}H_{31}NSO$·0.55 $C_2H_2O_4$: C, 68.16; H, 7.95; N, 3.44. Found: C, 68.14; H, 7.92; N, 3.72.

(24) Gabriel, S.; Colman, J. *Chem. Ber.* 1911, *44*, 3628.

1-[(2-Methylphenyl)thio]-2-methyl-2-propanamine (9p) was obtained as a pale yellow liquid after Kugelrohr distillation: ^{1}H NMR ($CDCl_3$) δ 7.32 (d, 1 H, J = 7.0 Hz), 7.10 (m, 3 H), 2.94 (s, 2 H), 2.39 (s, 3 H), 1.45 (br s, 2 H), and 1.19 (s, 9 H); ^{13}C NMR ($CDCl_3$) 137.6, 136.6, 130.2, 128.6, 126.3, 125.7, 50.5, 49.3, 29.8, and 20.5 ppm. Anal. Calcd for $C_{11}H_{17}NS$-0.10 H_2O: C, 67.03; H, 8.80; N, 7.11; H_2O, 0.91. Found: C, 66.76; H, 8.80; N, 7.03; H_2O, 1.15.

1-[[4-(1,1-Dimethylethyl)-2-methylphenyl]thio]-2-methyl-2-propanamine hydrochloride (9q) was isolated as a colorless white solid after trituration from Et_2O: ^{1}H NMR (DMSO-d_6) δ 8.58 (br s, 3 H), 7.25 (m, 3 H), 3.28 (s, 2 H), 2.37 (s, 3 H), 1.35 (s, 6 H), and 1.25 (s, 9 H); ^{13}C NMR (DMSO-d_6) 149.0, 136.9, 131.3, 129.1, 127.2, 123.5, 51.5, 42.1, 34.0, 31.0, 24.3, and 20.5 ppm. Anal. Calcd for $C_{15}H_{25}NS$·HCl: C, 62.58; H, 9.10; N, 4.87. Found: C, 62.48; H, 9.05; N, 5.00.

Preparation of Deuterium-Labeled Oxazolidinone 1b-d_2. Ethyl *N*-Methyl-*N*-(phenylmethyl)glycine (11).[25] A mixture of sarcosine ethyl ester (10, 50.0 g, 0.326 mol), benzyl chloride (49.9 g, 0.390 mol), micropulverized K_2CO_3 (53.8 g, 0.390 mol), and a catalytic amount (ca. 700 mg) of NaI in 150 mL of absolute EtOH was refluxed under N_2 for 20 h. The resulting mixture was cooled to room temperature and filtered and the filtrate concentrated in vacuo to give a crude oil. The oil was purified by flash chromatography (SiO_2: $CHCl_3$) to yield 48.8 g (72% yield) of 11 as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5 H), 4.17 (q, 2 H, J = 7.5 Hz), 3.68 (s, 2 H), 3.25 (s, 3 H), 2.38 (s, 3 H), and 1.24 (t, 3 H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) 170.6, 138.5, 129.0, 128.3, 127.2, 61.0, 57.5, 42.0, and 14.3 ppm. Anal. Calcd for $C_{12}H_{17}NO_2$: C, 68.22; H, 9.06; N, 6.63. Found: C, 67.95; H, 8.99; N, 6.42.

Ethyl [*N*-Methyl-*N*-(phenylmethyl)amino]ethanol-*1,1*-d_2 (12). To a stirred, room-temperature, N_2-atm suspension of $LiAlD_4$ (5.00 g, 0.150 mol) in 150 mL of THF was added a solution of 11 (30.5 g, 0.150 mol) in 150 mL of THF. The mixture was stirred for 20 h at ambient temperatures, and then H_2O (5 mL), 15% aqueous NaOH (5 mL, wt/vol), and H_2O (15 mL) were successively added. After filtration, the filtrate was concentrated in vacuo to yield 18.1 g (74% yield) of 12 as a colorless oil. A small portion of the oil was treated with oxalic acid to give the salt as a colorless solid: mp 109–111 °C; ^{1}H (DMSO-d_6) δ 10.0 (br s, 3 H), 7.58 (br s, 2 H), 7.44 (br s, 3 H), 4.37 (s, 2 H), and 2.75 (s, 3 H); ^{13}C NMR (DMSO-d_6) 165.3, 131.0, 130.4, 129.2, 128.6, 58.9, 56.2, 55.0 (CD_2), and 39.5 ppm. Anal. Calcd for $C_{10}H_{13}D_2NO$·$C_2H_2O_4$: C, 56.46; H, 6.71; N, 5.49. Found: C, 56.06; H, 6.66; N, 5.36.

Ethyl [*N*-Methyl-*N*-(phenylmethyl)amino]ethyl-*1,1*-d_2 Carbonate (13). A solution of 12 (8.10 g, 50.0 mmol), Et_3N (5.60 g, 55.0 mmol), and a catalytic amount of 4-(dimethylamino)-pyridine (DMAP) in 100 mL of dry THF was cooled to ice-bath temperatures and stirred under N_2. $ClCO_2Et$ (6.00 g, 55.0 mmol) in 100 mL of THF was added and the resulting solution warmed to room temperature and then refluxed for 20 h. After the solvent was removed under reduced pressure, the residue was subjected to flash chromatography (SiO_2: $CHCl_3$) to give 9.4 g (80%) of 13 as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 5 H), 4.16 (q, 2 H, J = 7.1 Hz), 3.54 (s, 2 H), 2.66 (s, 2 H), 2.25 (s, 3 H), and 1.28 (t, 3 H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) 155.1, 138.7, 128.8, 128.3, 127.0, 65.4 (CD_2), 63.8, 62.4, 55.2, 42.5 and 14.3 ppm. Anal. Calcd for $C_{13}H_{17}D_2NO_3$: C, 64.71; H, 8.77; N, 5.81. Found: C, 64.39; H, 8.71; N, 5.66.

Ethyl 2-(*N*-Methylamino)ethyl-*1,1*-d_2 Carbonate (14). A solution of 13 (12.0 g, 50.0 mmol) in 150 mL of absolute EtOH containing 1.0 g of 10% Pd on C was shaken under 60 psi of H_2 on a Parr hydrogenation apparatus for 20 h. The catalyst was removed by filtration through Celite and the filtrate concentrated to yield 7.50 g (quantitative) of 14 as as a clear oil. A small portion of the oil was converted to the HCl salt by treatment with ethanolic HCl and isolated as a colorless white solid: mp 106–109 °C; ^{1}H NMR ($CDCl_3$) δ 9.26 (br s, 2 H), 4.25 (q, 2 H, J = 7.1 Hz), 3.38 (s, 2 H), 2.81 (s, 3 H), and 1.31 (q, 3 H, J = 7.1 Hz); ^{13}C NMR ($CDCl_3$) 154.5, 64.7, 62.8 (CD_2), 47.6, 33.6, and 14.2 ppm. Anal. Calcd for $C_6H_{11}D_2NO_3$·HCl: C, 39.25; H, 7.69; N, 7.63. Found: C, 38.96; H, 7.60; N, 7.35.

3-Methyl-2-oxazolidinone-*5,5*-d_2 (1b-d_2). A solution of 14 (2.90 g, 20.0 mmol) in 75 mL of absolute EtOH containing a catalytic amount of NaOEt was refluxed 1 h under N_2. Removal of the volatiles in vacuo gave a crude residue which was purified by flash chromatography (SiO_2: $CHCl_3$) to furnish 2.0 g (quantitative yield) of oxazolidinone 1b-d_2 as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.59 (s, 2 H) and 2.89 (s, 3 H); ^{13}C NMR ($CDCl_3$) 158.6, 61.1 (p, CD_2, J = 21.6 Hz), 46.6, and 31.8 ppm. Anal. Calcd for $C_4H_5D_2NO_2$: C, 45.70; H, 8.63; N, 13.33. Found: C, 45.92; H, 8.89; N, 12.99.

Reactions of Oxazolidinone 1b-d_2 with N-Methylaniline Hydrochloride. A mixture of 1b-d_2 (500 mg, 4.85 mmol) and *N*-methylaniline hydrochloride (650 mg, 4.51 mmol) were heated in an oil bath for 18 h at the indicated temperatures (Table IV). The usually dark reaction mixtures were cooled to room temperature and then made basic by the addition of 50 mL of 1 N aqueous NaOH solution. After extraction of the basic solution with CH_2Cl_2, the combined organic portions were dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo to yield crude diamine product. Final purification was accomplished by flash chromatography (SiO_2: MeOH/$CHCl_3$) to furnish the diamines 15a and 16a as a clear oil. Deuterium positioning in 15a:16a was determined by careful ^{1}H NMR analysis at 300 MHz in $CDCl_3$. The C-1 methylene absorption at δ 2.80 was integrated against the C-2 methylene absorption at δ 3.45 to determine relative amounts of 15a and/or 16a as indicated in Table IV. These proton assignments were based on a substituent effect study using diamine 4l and its corresponding trifluoroacetamide derivative [300-MHz ^{1}H NMR spectrum for 4l free base: ($CDCl_3$) δ 7.21 (m, 2 H), 6.74 (m, 3 H), 3.45 (t, J = 6.5 Hz), 2.95 (s, 3 H), 2.80 (t, 2 H, J = 6.5 Hz), 2.46 (s, 3 H), and 1.28 (br s, 1 H)]. The C-1 methylene absorption of 4l at 2.80 ppm was shifted downfield to 3.58 ppm after formation as the trifluoroacetamide derivative. The C-2 methylene absorption was only slightly affected on derivatization (3.45–3.56 ppm).

Reaction of Oxazolidinone 1b-d_2 with Thiophenol. A mixture of thiophenol (1.10 g, 10.0 mmol), oxazolidinone 1b-d_2 (1.00 g, 9.90 mmol), and 10 mg of LiCl was heated under N_2 in a 140 °C oil bath for 5 h. After being cooled to room temperature, the yellow residue was taken up in $CHCl_3$ and purified by flash chromatography (SiO_2: MeOH/$CHCl_3$) to give 1.37 g (8.11 mmol, 82% yield) of *N*-methyl-2-(phenylthio)ethanamine-2,2-d_2 (16b) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 5 H), 2.81 (s, 2 H), 2.43 (s, 3 H), and 2.00 (br s, 1 H); ^{13}C NMR ($CDCl_3$) 136.0, 129.4, 128.3, 126.8, 58.3, 36.0, and 33.2 (p, CD_2, J = 22.6 Hz) ppm. The deuterium positioning in 16b was determined by the lack of any C-2 methylene ^{1}H NMR absorption of 9d. Both the C-1 and C-2 methylene signals were assigned after conversion of 9d to its corresponding trifluoroacetamide derivative [300-MHz ^{1}H NMR spectrum of 9d free base: ($CDCl_3$) δ 7.34 (m, 2 H), 7.25 (m, 2 H), 7.17 (m, 1 H), 3.04 (m, 2 H), 2.80 (m, 2 H), 2.41 (s, 3 H), and 1.33 (br s, 1 H)]. The C-1 methylene absorption of 9d at 2.80 ppm was shifted downfield to 3.56 ppm on derivatization. The C-2 methylene was only slightly affected after derivatization (3.04–3.07 ppm).

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Registry No. 1a, 497-25-6; 1b, 19836-78-3; 1b-d_2, 143745-73-7; 1c, 2510-33-0; 1d, 703-56-0; 1e, 26654-39-7; 1f, 25664-78-2; 2a, 66923-49-7; 4a, 108445-06-3; 4b, 143745-52-2; 4c, 53573-09-9; 4d, 143745-53-3; 4e, 143745-54-4; 4f, 143745-55-5; 4g, 143745-56-6; 4h, 84035-89-2; 4i, 143745-57-7; 4j, 141832-98-6; 4k, 138374-03-4; 4l, 33905-42-9; 4m, 143745-58-8; 4n, 143745-59-9; 4o, 143745-60-2; 4p, 143745-61-3; 9a, 2014-75-7; 9b, 60116-14-5; 9c, 143745-62-0; 9d, 2014-78-0; 9e, 143745-63-5; 9f, 115335-00-7; 9g, 115335-01-8; 9h, 115335-16-5; 9i, 143745-64-6; 9j, 143745-65-7; 9k, 114458-75-1; 9l, 67747-34-5; 9m, 92249-42-8; 9n, 143745-67-9; 9o, 56216-03-6;

(25) Maier, R.; Wentrup, E.; Wetzel, B.; Reuter, W.; Goeth, H.; Lechner, U. *Ger. Offen.* 1976, 2,515,076.

9p, 143745-68-0; 9q, 143745-69-1; 10, 13200-60-7; 11, 62004-76-6; 12, 143745-70-4; 13, 143745-71-5; 14, 143745-72-6; 15a, 143745-74-8; 15b, 143745-75-9; 16a, 143745-76-0; 16b, 143745-77-1; $PhNH_2$, 142-04-1; 4-$MePhNH_2$, 540-23-8; 2-$ClPhNH_2$, 137-04-2; 3-$ClPhNH_2$, 141-85-5; 4-$MeOPhNH_2$, 20265-97-8; 4-$MeSPhNH_2$, 39870-00-3; 4-EtO_2CPhNH_2, 23239-88-5; 3-O_2NPhNH_2, 33240-96-9; 4-H_2NPhNH_2, 55972-71-9; PhNHMe, 2739-12-0; $PhNHCH_2Ph$, 2290-89-3; 3-$MePhNH_2$, 638-03-9; PhSH, 12385-08-9; 2-H_2NPhSH, 137-07-5; 4-*t*-Bu-2-MePhSH, 15570-10-2; 2-MeOPhSH, 7217-59-6; 2-MePhSH, 137-06-4; 3-MePhSH, 108-40-7; 4-MePhSH, 106-45-6; 4-HOPhSH, 637-89-8; 4-H_2NPhSH, 1193-02-8; 4-HO-3,5-(*t*-Bu)$_2$PhSH, 950-59-4.

An Asymmetric Route to Enantiomerically Pure 1,2,3-Trisubstituted Cyclopropanes

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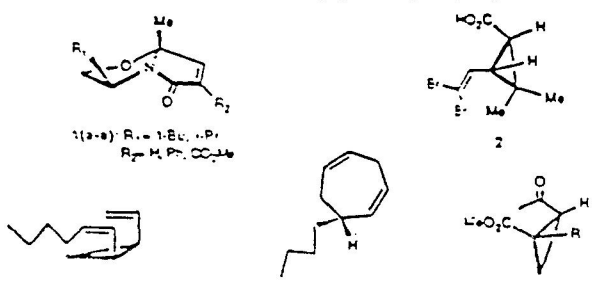
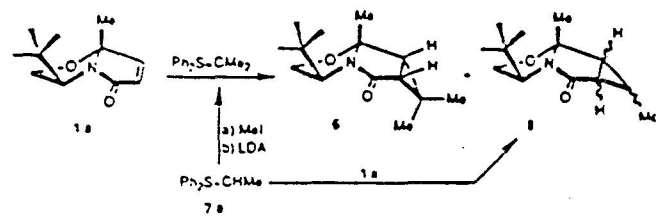
Cycloaddition of various sulfur ylides to the chiral unsaturated lactams 1a, 1b led to cyclopropanated products containing a monosubstituted appendage. The stereochemical outcome is such that all the products are mainly (or exclusively) the kinetically controlled *endo-syn*-8, -9, or *endo-anti*-10. The latter occurs by virtue of an epimerization to the thermodynamically favored product. Removal of the chiral auxiliary following Wittig reaction on the intermediate carbinol amines (11, 15) gave chiral, nonracemic 1,2,3-trisubstituted cyclopropanes containing various functionalities (13, 16).

Earlier cyclopropanations of unsaturated bicyclic lactams 1 have provided access to the potent insecticide precursor, *cis*-(1*S*,3*R*)-deltamethrinic acid (2), dictyopterene C (3), a proposed biogenic precursor to dictyopterene C′ (4), the potent seaweed sperm attractant, and various other enantiomerically pure cyclopropanes 5.[1] It

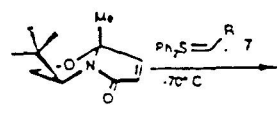


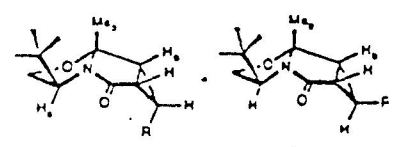
is obvious from their structures that these cyclopropyl systems possess only two stereogenic centers. We now wish to disclose extensions of this chiral cyclopropanation methodology which afford absolute stereocontrol of three centers leading to enantiomerically pure 1,2,3-trisubstituted cyclopropanes.[2]

In our earlier effort directed toward the asymmetric synthesis of deltamethrinic acid, 2, cyclopropanation of the unsaturated lactam 1a with diphenylsulfonium isopropylide gave mixtures of the desired *gem*-dimethylcyclopropane 6 and two diastereomeric monomethylcyclopropanes 8. The isopropylide was generated in situ and, as a result of incomplete alkylation of the ylide 7a, varying amounts of monomethylcyclopropanes 8 were produced. Interestingly, of the four possible stereoisomers (endo/exo and syn/anti) monomethylcyclopropanes, only two were produced, and more importantly they were produced in unequal amounts.



These findings prompted an investigation of cyclopropanation of the unsaturated lactam 1a with diphenylsulfonium ethylide 7 (R = Me).[3] When the reaction with 1a was performed at −70 °C followed by warming to −20 °C, the two *syn*- and *anti*-cyclopropyl adducts 8a were obtained in 95% yield as a 3.0:1 mixture as determined by VPC analysis. The diastereomeric ratio of 8a could be improved to 4.6:1 by maintaining the reaction temperature between −70 °C and −60 °C.





	syn	anti
8a R = Me	4.6	1
8b R = CH_2CH_3	3.1.0	1
8c R = Ph	7.0	1

The major diastereomer was determined to be the endo–syn adduct 8a by NOE experiments which showed

(1) For earlier studies on chiral bicyclic lactams, including cyclopropanation, see a review on this subject: Romo, D.; Meyers, A. I. *Tetrahedron* 1991, *46*, 9503–9569.

(2) For recent reports of cyclopropanations providing tri- and tetrasubstituted, enantiomerically pure cyclopropanes, see: (a) Winkler, J. D.; Gretler, E. A. *Tetrahedron Lett.* 1991, *41*, 5733. (b) Lowenthal, R. E.; Masamune, S. *Tetrahedron Lett.* 1991, *50*, 7373. (c) Evans, D. A.; Woerpel, K. A.; Hinman, M. M. *J. Am. Chem. Soc.* 1991, *113*, 726. (d) Suzimura, T.; Katagiri, T.; Tai, A. *Tetrahedron Lett.* 1992, *33*, 367.

(3) Corey, E. J.; Jautelat, M. *J. Am. Chem. Soc.* 1967, *89*, 3912.

Heterobifunctional Cross-Linking Agents Incorporating Perfluorinated Aryl Azides

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New heterobifunctional cross-linking reagents were developed that possess a photoactive tetrafluorinated phenyl azide as the photoactive terminus and a chemically reactive succinimidyl ester as the electrophilic terminus. These reagents, succinimidyl *N*-(4-azido-2,3,5,6-tetrafluorobenzoyl)tyrosinate (**9**) and succinimidyl 2-(4-azido-2,3,5,6-tetrafluorophenyl)thiazole-4-carboxylate (**15**), were designed to possess either an ^{125}I or ^{35}S radiolabel, respectively. In a biochemical study, the latter reagent was coupled to Lys-75 of calmodulin (CaM), and the radioiodinated monoadduct was photochemically cross-linked, in a calcium-dependent manner, to the porcine erythrocyte plasma membrane Ca^{2+},Mg^{2+}-ATPase. Densitometry scans of the gel indicated a reproducible 22% cross-linking of the CaM with one of the Ca^{2+},Mg^{2+}-ATPase bands. Since the purification of the Ca^{2+},Mg^{2+}-ATPase results in micelles having Ca^{2+},Mg^{2+}-ATPase with its CaM binding site oriented both to the inside and outside of the micelle, the amount of Ca^{2+},Mg^{2+}-ATPase available for cross-linking was reduced by approximately half, suggesting that the actual cross-linking efficiency was on the order of 40%.

INTRODUCTION

Interest in studying the interactions of the calcium-binding protein calmodulin (CaM) (*1*) with other proteins has led to the development of new heterobifunctional cross-linking reagents (*2*, *3*). An effective reagent for studying intermolecular interactions of this type should possess three features: a photoactive terminus that cross-links with high efficiency to the target protein, an electrophilic terminus that establishes the initial covalent link to CaM,[1] and a radiolabel that will facilitate the identification of cross-linked peptides (*4*). In order to secure primary sequence data, the radiolabel must be readily introduced, possess a convenient half-life, and survive both the photochemical event and the subsequent purification process.

Several heterobifunctional reagents fulfill most of these requirements by incorporating both an aryl azide and an ^{125}I radiolabel. However, the putative cross-linking species produced in the photolysis of an aryl azide is not a singlet nitrene but rather an electrophilic dehydroazepine (*5–8*) that scavenges selectively for nucleophilic amino acid residues. The absence of such residues in the immediate vicinity of the photochemical event dramatically affects cross-linking efficiency. Moreover, the photolysis of aryl azides that possess an iodine substituent in the *same* ring as the azide leads to rapid photodeiodination (*9*), a competitive process that further reduces the observed cross-linking efficiency. For example, the cross-linking of radioiodinated CaM adducts of succinimidyl *N*-[2-(4-azidosalicyl)ethyl]suberamate (*3*) with human erythrocyte plasma membrane Ca^{2+},Mg^{2+}-ATPase proceeded with only 8% cross-linking efficiency.

In order to improve upon this limited efficiency, other photoactive reporter groups that might display an enhanced level of cross-linking were introduced in place of the simple aryl azides. Although the photoactive 3-aryl-3-(trifluoromethyl)diazirine (*10–12*) group generates a reactive carbene that might provide efficient cross-linking, an investigation of the photoproducts of 3-aryl-3-(trifluoromethyl)diazirines revealed that these products may not, in some cases, be stable toward conditions necessary for protein sequencing.[2] The photolysis of perfluorinated aryl azides (*13–18*) generates a singlet nitrene that inserts efficiently into a variety of bond types and promises to provide a solution to *both* the cross-linking efficiency and product stability problems. It was necessary, therefore, to investigate whether the perfluorinated azides could be incorporated in heterobifunctional reagents that would possess either a photostable ^{125}I or ^{35}S radiolabel (*19*) and to compare the cross-linking efficiency of these new reagents with their nonfluorinated counterparts.

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[1] The abbreviations used are as follows: CaM, calmodulin; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMSO, dimethyl sulfoxide; DSO, disuccinimidyl oxalate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NHS, *N*-hydroxysuccinimidyl; Pth, phenylthiohydantoin; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TRIS, tris(hydroxymethyl)aminomethane.

[2] M. Platz and D. S. Watt, unpublished observations. For example, the photolysis of 3-(4-toluyl)-3-(trifluoromethyl)-diazirine in diethylamine generated a carbene possessing a low-lying singlet state and a triplet ground state. The photoinsertion of the singlet carbene into various substrates was examined. Photoinsertion into the N–H bond of diethylamine produced the adduct *N*,*N*-diethyl-*N*-[(2,2,2-trifluoro-1-(4-toluyl)ethyl]-amine. Rapid elimination of hydrogen fluoride from this adduct under the conditions of the photolysis experiment afforded an enamine, 1′-(diethylamino)-2′,2′-difluoro-4-methylstyrene. The subsequent hydrolysis of the enamine produced diethylamine and α,α-difluoro-4-methylacetophenone. This unexpected elimination and hydrolysis sequence had the net effect of reversing the photoinsertion process.

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EXPERIMENTAL PROCEDURES

General Chemical Procedures. Chemicals were purchased from Aldrich or Sigma. Infrared spectra were recorded on a Perkin-Elmer Model 357 spectrometer. Nuclear magnetic resonance spectra were determined on a Varian 400-MHz or Gemini 200-MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as an internal standard. Mass spectra were determined on a VG ZAB spectrometer. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Column chromatography using Macherey Nagel silica gel 60 is referred to as "chromatography on silica gel". Procedures for the preparation of the following compounds were developed concurrently in these laboratories but were recently published (*14*) elsewhere: methyl 4-amino-2,3,5,6-tetrafluorobenzoate (2), methyl 4-azido-2,3,5,6-tetrafluorobenzoate (3), 4-azido-2,3,5,6-tetrafluorobenzoic acid (4), and succinimidyl 4-azido-2,3,5,6-tetrafluorobenzoate (5).

Methyl *N*-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-tyrosinate (6). To a solution of 2.05 g (6.21 mmol) of 5 and 1.58 g (6.83 mmol) of Tyr(OCH_3)·HCl in 50 mL of DMSO was added 1.25 g (12.4 mmol) of Et_3N. The solution was stirred for 23 h at 25 °C and diluted with EtOAc. The organic solution was washed with 2 N HCl solution and brine and dried over anhydrous $MgSO_4$. The crude product was chromatographed on silica gel using 1:1 EtOAc–hexane to afford 2.33 g (91%) of 6: mp 155–157 °C dec; IR (KBr) 2112, 1717, 1649 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.80–3.08 (m, 2, CH_2), 3.67 (s, 3, CH_3), 4.55–4.66 (m, 1, CH), 6.68 and 7.05 (2 d, J = 8.5 Hz, 4, ArH), 9.27 (s, 1, OH), 9.39 (d, J = 7.9 Hz, 1, NH). Anal. Calcd for $C_{17}H_{12}F_4N_4O_4$: C, 49.52; H, 2.93. Found: C, 49.63; H, 2.99.

Methyl *N*-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-2,6-diiodotyrosinate (7). To a solution of 65 mg (0.16 mmol) of 6 in 2.4 mL of a 5:1 mixture of MeOH and TRIS buffer (pH 7.8) were added at 0 °C 60 mg (0.38 mmol) of NaI and 86 mg (0.38 mmol) of chloramine T hydrate. The mixture was stirred for 60 min at 0 °C and concentrated. The residue was dissolved in EtOAc, washed successively with 5% sodium thiosulfate solution, saturated $NaHCO_3$ solution, and brine, and dried over anhydrous $MgSO_4$. The product was chromatographed on silica gel using $CHCl_3$ to afford 85 mg (81%) of 7: mp 168–170 °C dec; IR (KBr) 2114, 1723, 1650 cm^{-1}; ^1H NMR (CD_3OD) δ 2.83–3.17 (m, 2, CH_2), 3.74 (s, 3, CH_3), 4.45–4.63 (m, 1, CH), 7.62 (s, 2, ArH). Anal. Calcd for $C_{17}H_{10}F_4I_2N_4O_4$: C, 30.75; H, 1.52. Found: C, 30.84; H, 1.56.

***N*-(4-Azido-2,3,5,6-tetrafluorobenzoyl)tyrosine (8).** To a solution of 1.15 g (2.79 mmol) of 6 in 33 mL of 10:1 MeOH–water was added 0.70 g (17 mmol) of LiOH·H_2O. The solution was stirred for 16 h at 25 °C and concentrated. The residual solid was acidified with 2 N HCl solution and extracted with EtOAc. The organic solution was washed with brine and dried over anhydrous $MgSO_4$. The product was crystallized from $CHCl_3$ to afford 576 mg (52%) of 8: mp 147–149 °C dec; IR (KBr) 2114, 1700, 1643 cm^{-1}; UV (95% EtOH) λ_{max} 256 nm (ϵ 19 000) (decay half-life was <10 s under 254 nm irradiation); ^1H NMR (acetone-d_6) δ 2.96–3.30 (m, 2, CH_2), 3.40 (br s, 1, CO_2H), 3.81 (s, 1, OH), 4.81–4.96 (m, 1, CH), 6.76 and 7.13 (2 d, J = 8.6 Hz, 4, ArH), 8.13 (d, J = 7 Hz, 1, NH). Anal. Calcd for $C_{16}H_{10}F_4N_4O_4$: C, 48.25; H, 2.53. Found: C, 48.41; H, 2.56.

Succinimidyl *N*-(4-Azido-2,3,5,6-tetrafluorobenzoyl)tyrosinate (9). To a solution of 274 mg (0.69 mmol) of 8 in 10 mL of anhydrous THF were added 114 mg (0.99 mmol) of *N*-hydroxysuccinimide and 170 mg (0.83 mmol) of DCC. After stirring for 22 h at 25 °C, the suspension was filtered. To the filtrate was added 3 mL of and the solution was concentrated. The resulting of the NHS ester 9 was used directly in coupling r with amines.

2-(4-Amino-2,3,5,6-tetrafluorophenyl)-4-ca 4,5-dihydrothiazole (11). A solution of 101 m mmol) of 4-amino-2,3,5,6-tetrafluorobenzonitrile mg (0.53 mmol) of L-cysteine, and 73 mg (0.53 m K_2CO_3 in 2.0 mL of 1:1 MeOH–water was heate 70 °C for 14 h. The MeOH was removed under pressure. The aqueous solution was extracted wi to remove any unreacted 10 (19% recovery). The was acidified to pH 3–4 with 2 N HCl soluti extracted with EtOAc while the pH was check adjusted between extractions to maintain a value The EtOAc fractions were combined, washed wit and dried over anhydrous $MgSO_4$. The crude prod recrystallized from EtOAc–hexane to yield 63 mg of 11 as a pale yellow solid: mp 184–186 °C dec; 76.8° (c = 0.95, acetone); IR (KBr) 3000 (br), 171 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.77 (d, J = 9.1 Hz, 5.34 (t, J = 9.3 Hz, 1, CH), 5.98 (br s, 2, NH_2). Ana for $C_{10}H_6F_4N_2O_2S$: C, 40.82; H, 2.06. Found: C H, 2.03.

2-(4-Azido-2,3,5,6-tetrafluorophenyl)-4-car bo dihydrothiazole (12). To a solution of 199 m mmol) of 11 in 3 mL of TFA at 0 °C was added (1.5 mmol) of $NaNO_2$. The solution was stirred for at 0 °C and 150 mg (2.3 mmol) of NaN_3 was adde solution was stirred an additional 1.5 h at 0 °C, to 25 °C, and concentrated. The residue was dilut 20 mL of water, and the pH was adjusted to ca. 4 wi Na_2CO_3. The solution was extracted with EtOA combined organic solutions were washed with bri dried over anhydrous $MgSO_4$. The EtOAc soluti concentrated to afford 198 mg (91%) of 12: IR (th 3000 (br), 2135 cm^{-1}; ^1H NMR (acetone-d_6) δ 3.87 9.2 Hz, 2, CH_2), 5.43 (t, J = 9.2 Hz, 1, CH), 6.97 (CO_2H). This material resisted efforts to prep analytically pure sample and was fully characterize methyl ester: IR ($CHCl_3$) 2135, 1738 cm^{-1}; ^1H ($CDCl_3$) δ 3.68–3.99 (m, 2, CH_2), 3.86 (s, 3, OCH_3), J = 9.5 Hz, 1, CH); exact mass spectrum ca $C_{11}H_6F_4N_4O_2S$ 334.0148, found 334.0146.

2-(4-Amino-2,3,5,6-tetrafluorophenyl)-4-carb azole (13). A suspension of 415 mg (1.4 mmol) of 413 mg (2.0 mmol) of 9,10-phenanthrenequinone 8.4 mL of 5% HOAc in $CHCl_3$ was refluxed for 15 solution was cooled, concentrated, and diluted with The solution was washed with several portions NaOH solution. The combined aqueous solutio extracted with $CHCl_3$ to remove much of th phenanthrenequinone/-quinol. The aqueous solut acidified to pH 3–4 and extracted with EtOAc w pH was checked to maintain it at 3–4. These extracts were combined, washed with brine, dri anhydrous $MgSO_4$, and concentrated to afford 43 13 that was used directly in the next reaction. I to secure an analytical sample, traces of 9,10-p threnequinone/-quinol were removed by triturati crude product with $CHCl_3$ and a subsequent rec zation from EtOAc–hexane to yield 13 as an am solid: mp >230 °C; IR (KBr) 3000 (br), 1704, 16 ^1H NMR (acetone-d_6) δ 3.50 (br s, 1, CO_2H), 6.04 NH_2), 8.60 (s, 1, CH). Anal. Calcd for $C_{10}H_4F_4$ C, 41.10; H, 1.38. Found: C, 41.17; H, 1.39.

2-(4-Azido-2,3,5,6-tetrafluorophenyl)-4-carb azole (14). To a solution of 415 mg (1.4 mmol)

8.0 mL of TFA at 0 °C was added 149 mg (2.2 mmol) of $NaNO_2$. The solution was stirred for 30 min at 0 °C, and 184 mg (2.8 mmol) of NaN_3 was added. The solution was stirred for 1.5 h at 0 °C, allowed to warm to 25 °C, and concentrated. The oil was diluted with EtOAc and washed with half-saturated $NaHCO_3$ solution to neutralize the residual TFA. The aqueous layer was separated, and the pH was adjusted to ca. 3. The aqueous solution was extracted with EtOAc. The combined organic solutions were washed with brine and dried over anhydrous $MgSO_4$. The crude product was recrystallized from EtOAc to yield 172 mg (38%) of 14: mp 172–174 °C dec; IR (KBr) 3105, 2800 (br), 2130, 1680, 1640 cm^{-1}; UV (95% EtOH) λ_{max} 280 nm (ϵ 17 000) (decay half-life was <10 s under 254 nm irradiation); ^1H NMR (acetone-d_6) δ 3.50 (br s, 1, CO_2H), 8.74 (s, 1, CH). Anal. Calcd for $C_{10}H_2F_4N_4O_2S$: C, 37.75; H, 0.63. Found: C, 37.83; H, 0.64.

Succinimidyl 2-(4-Azido-2,3,5,6-tetrafluorophenyl)-thiazole-4-carboxylate (15). To a suspension of 12 mg (0.037 mmol, 1.0 equiv) of 14, 10 μL (0.13 mmol, 3.5 equiv) of anhydrous pyridine, and 1.0 mL of anhydrous CH_3CN was added 14 mg (0.05 mmol, 1.4 equiv) of DSO (*21*). The solution was stirred for 18 h at 25 °C, concentrated, diluted with 10 mL of water, and extracted with EtOAc. The EtOAc solution was separated and washed successively with half-saturated $NaHCO_3$ solution, water, and brine, dried over anhydrous $MgSO_4$, and concentrated to afford 7.6 mg (49%) of 15. The crude product was sufficiently pure to use directly or could be recrystallized from EtOAc–hexane to provide an analytically pure sample: mp 175–177 °C dec; IR (KBr) 2130, 1775, 1724 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.94 (s, 4, CH_2CH_2), 8.68 (s, 1, CH). Anal. Calcd for $C_{14}H_5F_4N_5O_4S$: C, 40.48; H, 1.21. Found: C, 40.57; H, 1.22.

Preparation of Calmodulin. CaM was isolated from bovine testes according to the procedure of Jamieson and Vanaman (22).

Preparation of Porcine Erythrocyte Plasma Membrane Ca^{2+},Mg^{2+}-ATPase. The ATPase was isolated from fresh porcine blood according to the procedure of Niggli (23).

Functionalization of CaM with Succinimidyl 2-(4-Azido-2,3,5,6-tetrafluorophenyl)thiazole-4-carboxylate (15). CaM was treated with succinimidyl 2-(4-azido-2,3,5,6-tetrafluorophenyl)thiazole-4-carboxylate (15) in the presence of 2 mM $CaCl_2$ or 2 mM EGTA. Reagent 15 was dissolved in HPLC-grade acetonitrile at a concentration of 1 mg/mL immediately prior to use. The reagent solution was added to a final concentration of 20 μM to a solution of 10 μM CaM, 30 mM HEPES (pH 7.4), and 2 mM $CaCl_2$ (or EGTA). The mixture was incubated at 25 °C for 2 h, and the reaction was quenched by adding lysine to a final concentration of 10 mM. The mixture was then applied to a C-4 column (Vydac bulk resin, 25 cm × 0.4 cm) and progress of the reaction was monitored at 230 nm (protein) and 290 nm (tetrafluorinated phenyl azide) as shown in the HPLC traces in Figure 1.

The major calmodulin derivative observed in Figure 1 was prepared in quantities sufficient for further characterization and for use in photochemical cross-linking studies by increasing the volume of reaction mixtures and maintaining concentrations of all components to keep them identical with those used in Figure 1 (upper panel). For example, 3 mg of CaM was reacted with 133 μg of reagent 15 in 0.13 mL of acetonitrile in a total reaction volume of 18 mL. The mixture was then applied to a Pharmacia MONO Q anion-exchange FPLC column to separate the CaM–reagent 15 monoadduct from native and polymodified CaM. This was done with a gradient of buffer A [10 mM TRIS (pH 7.2) and 2 mM EGTA] and buffer B [10 mM TRIS (pH 7.2), 2 mM EGTA, and 0.8 M NaCl] from 45 to 60% B. The efficiency of the separation was monitored on a C-4 HPLC column.

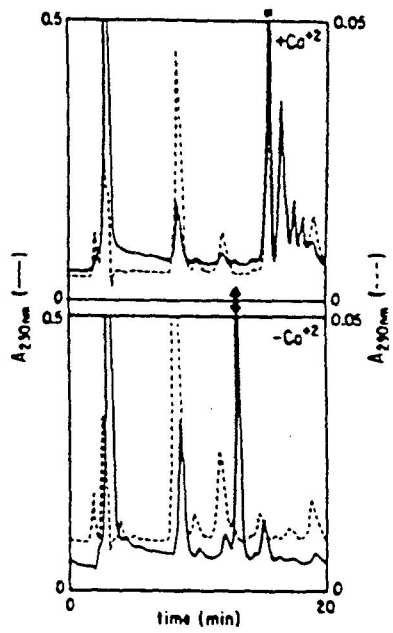


Figure 1. Reverse-phase HPLC profiles (C-4 column) of adducts of CaM and reagent 15 in the presence and absence of Ca^{2+} as monitored at 230 and 290 nm. The latter wavelength indicated the presence of the perfluoroaryl azide. Separation of the native CaM and its monoadducts was achieved with a 1% per min gradient of acetonitrile in 10 mM NaH_2PO_4 (pH 6) and 2 mM EGTA in which acetonitrile varied from 20% to 40%. The asterisk denotes the monoadduct at Lys-75. The arrow denotes the position at which native CaM would elute.

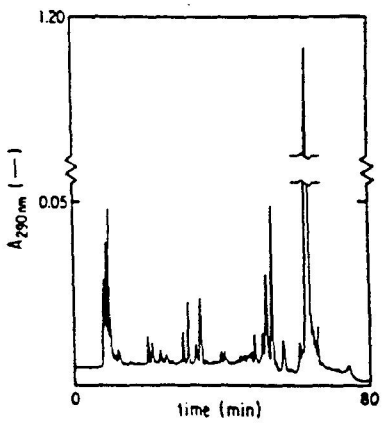


Figure 2. Phenyl μ Bondpak reverse-phase profiles of the tryptic digest of CaM–reagent 15 monoadduct as monitored at 290 nm. The separation was achieved with a gradient (starting at 5 min) of buffer A (10 mM NaH_2PO_4, 2 mM EGTA). Buffer B (5 mM NaH_2PO_4, 2 mM EGTA, 50% acetonitrile) was added from 0 to 50% (at 50% the gradient plateaus for 5 min before beginning a column wash cycle).

The CaM–reagent 15 monoadduct was then digested with trypsin and applied to a Phenyl μ Bondpak HPLC (Waters) according to the method of Vanaman (*1*) in order to separate the CaM tryptic peptides. The separation was monitored at 290 nm in order to detect the peptide bearing the tetrafluorinated phenyl azide group. The resulting HPLC trace is shown in Figure 2. The major peak was collected, desalted, and applied to an Applied Biosystems Model 477a protein sequencer equipped for on-line HPLC analysis.

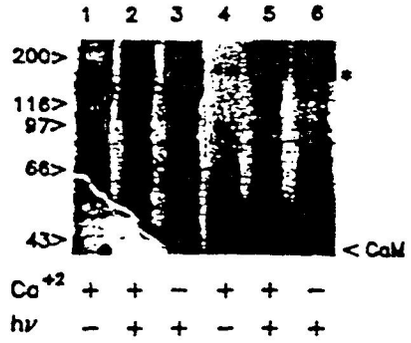


Figure 3. SDS–Polyacrylamide gel and autoradiogram of cross-linking experiments. Lanes 1–3 correspond to the silver-stained gel, and lanes 4–6 correspond to the autoradiogram of lanes 1–3, respectively. Molecular weight standards were run and their position is indicated by the numbers (kDa) at the left of lane 1. Lanes 2 and 5 correspond to the photolysis product of the ^{125}I-labeled monoadduct of CaM and Ca^{2+},Mg^{2+}-ATPase in the presence of Ca^{2+}. Lanes 1 and 4 correspond to the same mixture without photolysis. Lanes 3 and 6 correspond to the photolysis in the presence of 10 mM EGTA. The asterisk on the right indicates the position of the cross-linked species. The position of the non-cross-linked CaM is indicated at the lower right. The Ca^{2+},Mg^{2+}-ATPase runs as a doublet at 138 and 128 kDa, and the cross-linked species runs at 151 kDa.

Preparation of ^{125}I-Labeled Monoadduct of CaM and Succinimidyl 2-(4-Azido-2,3,5,6-tetrafluorophenyl)-thiazole-4-carboxylate (15). Radioiodination of 1 mg of purified, monomodified CaM was achieved with 100 μCi of [^{125}I]NaI and Enzymobeads (Bio-Rad) according to the procedure supplied by Bio-Rad.

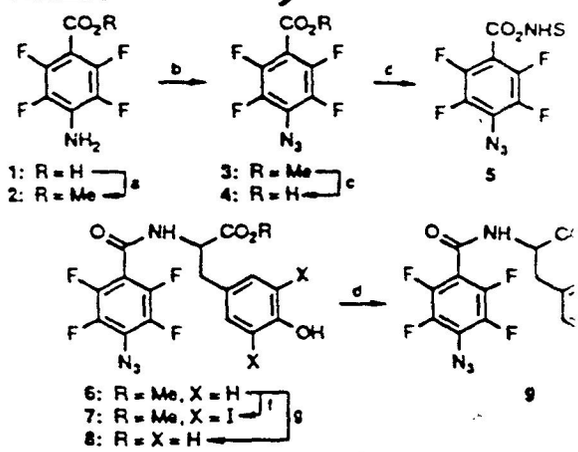
Photochemical Cross-Linking Experiments. A solution of 2 μg of Ca^{2+},Mg^{2+}-ATPase was incubated with 2 μg of ^{125}I-labeled CaM–reagent 15 monoadduct (80 mCi/μmol) in 15 μL of 30 mM HEPES (pH 7.4), 130 mM NaCl, 2 mM $MgCl_2$, 0.05% (v/v) Triton X-100, 5% (v/v) glycerol, 0.5 mg/mL phosphatidylcholine containing either 50 μM $CaCl_2$ *or* 10 mM EGTA. The solution was photolyzed for 1 min with a hand-held ultraviolet light with the glass faceplate removed (4600 μW/cm², Model UVS-11, Ultraviolet Products, Inc.) at a distance of 4 cm. The solution was suspended in a protein-solubilizing mixture of 65 mM Tris-HCl, 2% (v/v) SDS, 10% (v/v) glycerol, 5% (v/v) β-mercaptoethanol, and 0.001% bromophenol blue, and the solution was boiled for 5 min. The products were analyzed by 7.5% SDS–PAGE (*24*). The gel was dried, autoradiographed, resuspended, and silver stained. Autoradiography was performed for 4 h. Figure 3 shows the results of these experiments.

RESULTS

As shown in Scheme I, succinimidyl 4-azido-2,3,5,6-tetrafluorobenzoate (5) (*14*) was prepared from 4-amino-2,3,5,6-tetrafluorobenzoic acid (1) and coupled to methyl tyrosinate to obtain methyl ester 6. Methyl ester 6 underwent iodination under the usual conditions without any potential interference from nucleophilic aromatic substitution. Methyl ester 6 was converted to succinimidyl *N*-(4-azido-2,3,5,6-tetrafluorobenzoyl)tyrosinate (9) in the usual fashion.

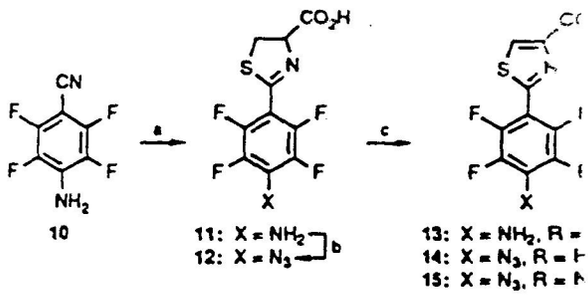
As shown in Scheme II, 2-(4-amino-2,3,5,6-tetrafluorophenyl)-4-carboxy-4,5-dihydrothiazole (11) was prepared in a condensation of 4-amino-2,3,5,6-tetrafluorobenzonitrile (10) with Cys. Although it was possible to convert thiazoline amine 11 to the corresponding thiazolinyl azide 12, the coupling of the NHS ester of 12 with various amines failed to produce the desired amides presumably as a consequence of competitive nucleophilic attack at the imino

Scheme I[a]



[a] Reagents: (a) HCl, MeOH; (b) (1) $NaNO_2$, TFA, 0 °C, (2) NaN_3; (c) LiOH·H_2O, 5:1 MeOH–H_2O; (d) NHS(OH), DCC; (e) Tyr(OCH_3)·HCl, Et_3N, DMSO; (f) NaI, chloramine T, TRIS buffer; (g) LiOH·H_2O, 10:1 MeOH–H_2O.

Scheme II[a]



[a] Reagents: (a) Cys, K_2CO_3, 1:1 MeOH–H_2O, 65 °C; (b) $NaNO_2$, TFA, 0 °C, (2) NaN_3; (c) 9,10-phenanthrenequinone, HOAc in $CHCl_3$, reflux; (d) DSO, Py, CH_3CN.

thioester carbon. Consequently, thiazoline amine oxidized to thiazole amine 13 with 9,10-phenanthrenone (*20*) and thiazole amine 13 was converted desired succinimidyl 2-(4-azido-2,3,5,6-tetrafluoropl thiazole-4-carboxylate (15).

Figure 1 shows reverse phase (C-4) HPLC separ performed as described in the legend, of the pr obtained when CaM (10 μM) was treated with a molar excess (20 μM) of reagent 15 in the presen mM Ca^{2+} (upper panel) or 2 mM EGTA (lower pane double arrow denotes the elution position of ur fied, native CaM as displayed in a separate analysi effluent was monitored for UV absorption at 230 detect protein and at 290 nm to detect the tetrafluc nyl azide moiety. Calmodulin shows negligib absorption at the latter wavelength.

In the absence of Ca^{2+}, a major peak displaying bance at 230 nm but lacking absorbance at 290 n obtained that possessed a retention time consister that of native CaM (Figure 1, lower panel). A nun early eluting peaks possessing both 230 and 2 absorption represented products of reagent hydroly reaction with lysine that was added after incuba quench further reaction with CaM. (This Lys addu is substantially reduced in the upper trace whe stantial CaM modification consumed reagent 15 p the addition of lysine.)

The reaction of CaM with reagent 15 in the pr of Ca^{2+} yielded a single major product denoted asterisk above the peak in Figure 1 (upper panel) component, which was present in only small amo the lower trace, eluted slightly later than native Ca possessed absorbance at both 230 and 290 nm.

CaM is nearly absent in the upper trace, indicating that chemical modification was nearly complete in the presence of Ca^{2+} under these conditions. A series of lesser peaks were also observed in the upper trace that eluted later than the major CaM monoadduct and that possessed a much higher ratio of the absorbance values at 290 and 230 nm, suggesting that these peaks were polymodified CaM adducts.

The modification reaction was successfully increased in scale. An anion-exchange FPLC separation yielded one major protein, again possessing both 230 and 290 nm UV absorption, that was isolated in a yield of 1.7 mg. An aliquot was further analyzed on C-4 reverse-phase HPLC (as in Figure 1) to yield a single peak identical in elution and UV absorbing properties with that labeled with the asterisk in Figure 1. Amino acid composition analysis confirmed that this material was a CaM adduct.

This adduct was digested with trypsin, and the resulting tryptic peptides were isolated by Phenyl μ Bondpak reverse-phase HPLC. Figure 2 shows the resulting elution profile obtained by monitoring the effluent at 290 nm. A single major peak (1.17 AU) was eluted at 65 min with 50% buffer B. Only minor peaks (<0.05 AU), probably representing Tyr-containing peptides, were observed earlier in the trace. It should be noted that the scale is magnified in the lower absorbance range. The 65-min peak was collected, desalted, and directly subjected to sequence analysis. The HPLC analysis of cycle 1 yielded no known Pth amino acid but had a major peak eluting at 10.95 min that presumably represented Pth-Lys modified by reagent 15 (vide infra). Cycle 2 gave 6.6 nmol of Pth-Met, and cycle 3 gave 4.8 nmol of Pth-Lys. The only sequence in CaM which could yield this tripeptide is residues 75–77, Lys-Met-Lys, with the ϵ-amino group of Lys-75 being the presumed site of modification by reagent 15.

Figure 3 shows the results of SDS-PAGE analysis of products obtained on photochemical cross-linking of the ^{125}I-labeled, Lys-75 CaM–reagent 15 monoadduct and a preparation of the porcine erythrocyte membrane Ca^{2+},-Mg^{2+}-ATPase. Lanes 1–3 represent the gel after silver staining, and lanes 4–6 display an autoradiogram of the same gel that was exposed prior to silver staining. Two major protein bands were observed at 128 and 138 kDa in lanes 1–3 representing the two different isoforms of the enzyme recently identified in erythrocytes (25). A major band of radioactivity is present in lane 5 with apparent M_r of 151 kDa (denoted by the asterisk), which is absent in a control sample run without photolysis (lane 4) and substantially diminished on photolysis of a sample that contained excess EGTA. Consequently, the cross-linking was both light- and calcium-dependent. The formation of this cross-linked species is also detectable in the silver-stained gel in lane 2. Densitometric scans of the silver-stained gel indicated that 22% of the Ca^{2+},Mg^{2+}-ATPase was cross-linked in the presence of excess CaM monoadduct under these conditions.

DISCUSSION

These studies describe a new family of heterobifunctional, tetrafluorophenyl azide reagents that are designed for improved cross-linking efficiently (particularly with hydrophobic amino acid side chains) and that are capable of bearing a photostable radiolabel. Among the radioisotopes of high specific activity and reasonable half-life, ^{125}I and ^{35}S are logical candidates for inclusion in heterobifunctional cross-linking reagents. As shown in Schemes I and II, succinimidyl *N*-(4-azido-2,3,5,6-tetrafluorobenzoyl)tyrosinate (9), a photoactive perfluorinated aryl azide that could bear an ^{125}I-radiolabel, and succinimidyl 2-(4-azido-2,3,5,6-tetrafluorophenyl)thiazole-4-carboxylate (15), a perfluorinated aryl azide that could bear an ^{35}S-radiolabel, were prepared in a straightforward series of operations. The ^{35}S-radiolabeled NHS ester 15 is particularly attractive since the radiolabel is photostable whereas the ^{125}I-radiolabel suffers some photodeiodination (9) under certain circumstances. Before applying the radiolabeled version of reagent 15 in a detailed biochemical study, it seemed prudent to demonstrate that the tetrafluorophenyl azide moiety, incorporated into a donor protein, was capable of efficient photochemical cross-linking to an appropriate target enzyme and to assess the efficiency of this photoactive group relative to other reagents bearing just a simple phenyl azide.

In previous studies (2, 3), we have used the calmodulin (CaM) system to test two related heterobifunctional reagents bearing the simple phenyl azide group. These and a number of other studies (26, 27) demonstrated that most hydrophobic acylating reagents modify CaM at Lys-75 in a calcium-dependent manner and that reagent 15 demonstrated similar behavior. The photochemical cross-linking of the ^{125}I-labeled, Lys-75 CaM–reagent 15 monoadduct to the porcine erythrocyte membrane Ca^{2+},Mg^{2+}-ATPase, shown in Figure 3, provided evidence for improved cross-linking efficiency for the tetrafluorinated phenyl azide. In our previous work with Lys-75 CaM monoadducts containing a simple phenyl azide, cross-linking to the human erythrocyte membrane Ca^{2+},Mg^{2+}-ATPase gave an 8% yield (3) of cross-linked material under the same conditions where the tetrafluorophenyl azide gave at least 20% cross-linking. It should be noted that these studies were performed with a purified membrane enzyme solubilized in a detergent (Triton X-100) micelle. Assuming random insertion of the enzyme into the micelle, simple geometry dictates that approximately 50% of the molecules would have their cytoplasmic domains (where CaM binds) facing the interior and unavailable for interaction with the ^{125}I-labeled CaM–reagent 15 probe. This suggests that the cross-linking efficiency for accessible molecules is approximately twice that observed in densitometry readings.

The utilization of the tetrafluorophenyl azides in cross-linking agents addresses two other problems encountered with simple phenyl azide based cross-linking reagents: the selectivity of the reactive intermediate for hydrophobic versus hydrophilic amino acid residues in the target protein and chemical stability of the covalent attachment generated in the cross-linking process. The photolysis of a simple phenyl azide generates a substituted dehydroazepine as the reactive intermediate (5–8) that *selectively* traps nucleophilic substituents in certain hydrophilic amino acids. However, protein–protein interactions are largely dictated by hydrophobic forces and by van der Waal contacts that involve aliphatic and aromatic groups in hydrophobic amino acid side chains (28) and it would be preferable to have a reactive intermediate capable of intercepting such residues. Just such a hydrophobic site lies adjacent to Lys-75 in CaM and appears to be responsible for the calcium-dependent interaction with target enzymes as well as small hydrophobic compounds including the interaction of reagent 15 with CaM. Thus, the agents that rely on dehydroazepine-mediated cross-linking are unlikely to make efficient probes of protein–protein interaction sites as reflected in the minimal cross-linking experienced with a mixture of monoadducts of CaM and succinimidyl *N*-[2-(4-azidosalicyl)ethyl]suberamate (3).

On the other hand, the photolysis of the tetrafluorophe-

nyl azide group generates a reactive nitrene capable of inserting into carbon–hydrogen bonds in hydrophobic amino acid side chains. Photochemical studies with model perfluorinated azides in organic solvents have led to similar conclusions (*13–18*). In addition, such carbon–hydrogen insertion reactions generate stable adducts that are more likely to survive adventitious hydrolysis during the digestion and purification process. The enhanced cross-linking efficiency observed in the present study is consistent with perfluorinated phenyl azides generating a more reactive intermediate capable of covalently linking a wider variety of amino acid residues in the target protein than the corresponding simple phenyl azides. This study also demonstrates that enhanced reactivity is seen with interacting proteins even though they are surrounded by water as a bulk medium. Clearly, further studies will be required to determine the structure of the photoinsertion products obtained in studies of proteins using these tetrafluorophenyl azide-based probes.

In conclusion, we have prepared heterobifunctional cross-linking reagents that incorporate perfluorinated phenyl azides as the photoactive component and have the capacity to bear either an ^{125}I or ^{35}S radiolabel. One of these reagents displayed a cross-linking efficiency that suggest these perfluorinated azides will prove effective in biochemical studies, and detailed studies along these lines are in progress.

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LITERATURE CITED

(1) Vanaman, T. C. (1983) Chemical approaches to the calmodulin system. *Methods Enzymol. 102*, 296–310.

(2) Imai, N., Kometani, T., Crocker, P. J., Bowdan, J. B., Demir, A., Dwyer, L. D., Mann, D. M., Vanaman, T. C., Watt, D. S. (1990) Photoaffinity heterobifunctional cross-linking reagents based on *N*-(azidobenzoyl)tyrosines. *Bioconjugate Chem. 1*, 138–143.

(3) Imai, N., Dwyer, L. D., Kometani, T., Ji, T., Vanaman, T. C., Watt, D. S. (1990) Photoaffinity heterobifunctional cross-linking reagents based on azide-substituted salicylates. *Bioconjugate Chem. 1*, 144–148.

(4) Bayley, H. (1983) *Photogenerated Reagents in Biochemistry and Molecular Biology*, Elsevier, New York.

(5) Torres, M. J., Zayas, J., Platz, M. S. (1986) A formal CH insertion reaction of an aryl nitrene into an alkyl CH bond. Implications for photoaffinity labelling. *Tetrahedron Lett. 27*, 791–794.

(6) Leyva, E., Platz, M. S., Persy, G., Wirz, J. (1986) Photochemistry of phenyl azide: the role of singlet and triple phenylnitrene as transient intermediates. *J. Am. Chem. Soc. 108*, 3783–3790.

(7) Shields, C. J., Chrisope, D. R., Schuster, G. B., Dixon, A. J., Poliakoff, M., Turner, J. J. (1987) Photochemistry of aryl azides: Detection and characterization of a dehydroazepine by time-resolved infrared spectroscopy and flash photolysis at room temperature. *J. Am. Chem. Soc. 109*, 4723–4726.

(8) Schuster, G. B., Liang, T.-Y. (1987) Photochemistry of 3- and 4-nitrophenyl azides: Detection and characterization of reactive intermediates. *J. Am. Chem. Soc. 109*, 7803–7810.

(9) Watt, D. S., Kawada, K., Leyva, E., Platz, M. S. Exploratory photochemistry of iodinated aromatic *Tetrahedron Lett. 30*, 899–902.

(10) Brunner, J., Senn, H., Richards, F. M. (1980) 3-(romethyl)-3-phenyldiazirine: A new carbene generatin for photolabeling reagents. *J. Biol. Chem. 255*, 331

(11) Baldini, G., Martoglio, B., Schachenmann, A., Zugl Brunner, J. (1988) Mischarging *Escherichia coli* tRNA L-4′-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenylalanin toactivatable analogue of phenylalanine. *Biochemi* 7951–7959.

(12) Frey, A. B., Kreibich, G., Wadhera, A., Clarke, L. man, D. J. (1986) 3-(Trifluoromethyl)-3-(*m*-[^{125}I]iodop diazirine photolabels a substrate-binding site of rat l cytochrome P-450 form PB-4. *Biochemistry 25*, 479

(13) Soundararajan, N., Platz, M. (1990) Descriptive chemistry of polyfluorinated azide derivatives of meth zoate. *J. Org. Chem. 55*, 2034–2039.

(14) Keana, J. F. W., Cai, S. X. (1990) New reagents f toaffinity labeling: Synthesis and photolysis of func ized perfluorophenyl azides. *J. Org. Chem. 55*, 3640–

(15) Keana, J. F. W., Cai, S. X. (1989) Functionalized p rophenyl azides: New reagents for photoaffinity label *Fluorine Chem. 43*, 151–154.

(16) Cai, S. X., Keana, J. F. W. (1989) 4-Azido-2-iodo trifluorophenylcarbonyl derivatives. A new class of fu alized and iodinated perfluorophenyl azide photo *Tetrahedron Lett. 30*, 5409–5412.

(17) Young, M. J. T., and Platz, M. S. (1989) Polyfluor aryl azides as photoaffinity labelling reagents; The temperature CH insertion reactions of singlet pentafluo nyl nitrene with alkanes. *Tetrahedron Lett. 30*, 2199

(18) Leyva, E., Munoz, D., and Platz, M. S. (1989) Photoche of fluorinated aryl azides in toluene solution and in froze crystals. *J. Org. Chem. 54*, 5938–5945.

(19) For an example of the use of [^{35}S]Cys in a radiolabel azolidine, see: Mitchell, R. N., Harrison, E. H., Bowe E. (1984) The use of radioactive cyteine methyl ester for l glycosylated molecules oxidized by periodate or neur dase plus galactose oxidase. *Arch. Biochem. Biophys. 22* 554.

(20) Barton, M. A., Kenner, G. W., Sheppard, R. C. (1966 tides. Part XXI. Dehydrogenation of some thiazolines d from cysteine. *J. Chem. Soc. C* 1061–1067.

(21) Takeda, K., Sawada, I., Suzuki, A., Ogura, H. (19 convenient synthesis of peptides using oxalates. *Tetrah Lett. 24*, 4451–4454.

(22) Jamieson, G. A., Vanaman, T. C. (1979) Calcium-dep affinity chromatography of calmodulin on immobilize nothiazine. *Biochem. Biophys. Res. Commun. 90*, 1055.

(23) Niggli, V., Zurini, M., Carafoli, E. (1987) Purific reconstitution, and molecular characteristics of the Ca^{2+} of plasma membranes. *Methods Enzymol. 139*, 791–8

(24) Laemmli, U. (1970) Cleavage of structural proteins the assembly of the head of Bacteriophage T4. *Natur* 680–685.

(25) Strehler, E. E., James, P., Fischer, R., Heim, R., Vo R., Filoteo, A. G., Penniston, J. R., Carafoli, E. (1990) tide sequence analysis and molecular cloning reveal two c pump isoforms in the human erythrocyte membrane. *J Chem. 265*, 2835–2842.

(26) Mann, D. M., Vanaman, T. C. (1988) Modification of odulin on Lys-75 by carbamoylating nitrosoureas. *J Chem. 263*, 11284–11290.

(27) Mann, D. M., Vanaman, T. C. (1989) Topographical ma of calmodulin-target enzyme interaction domains. *J Chem. 264*, 2373–2378.

(28) Janin, J., Chothia, C. (1990) The structure of pr protein recognition sites. *J. Biol. Chem. 265*, 16027–

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United States Patent [19]

Kohn et al.

[11] Patent Number: 5,378,729

[45] Date of Patent: Jan. 3, 1995

[54] **AMINO ACID DERIVATIVE ANTICONVULSANT**

[75] Inventors: **Harold L. Kohn**, Houston; **Darrell Watson**, Belton, both of Tex.

[73] Assignee: **Research Corporation Technologies, Inc.**, Tucson, Ariz.

[21] Appl. No.: **710,610**

[22] Filed: **Jun. 4, 1991**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 354,057, May 19, 1989, abandoned, and Ser. No. 392,870, Aug. 11, 1989, abandoned, which is a continuation of Ser. No. 80,528, Jul. 31, 1987, abandoned, which is a continuation-in-part of Ser. No. 916,254, Oct. 7, 1986, abandoned, which is a continuation-in-part of Ser. No. 702,195, Feb. 15, 1985, abandoned, said Ser. No. 354,057, is a continuation-in-part of Ser. No. 80,528, Feb. 15, 1985.

[51] **Int. Cl.**[6] **A61K 31/535**; A61K 31/445; C07D 211/72; C07D 261/04

[52] **U.S. Cl.** **514/231.2**; 514/315; 514/397; 514/406; 514/415; 514/424; 514/461; 514/468; 514/486; 546/292; 548/371.4; 548/245; 564/148; 564/152; 564/154

[58] **Field of Search** 564/148, 155, 154, 152; 548/616, 245, 371.4; 514/461, 548, 549; 546/292

[56] **References Cited**

U.S. PATENT DOCUMENTS

2,676,188	4/1954	Bruce et al.	564/155
2,721,197	10/1955	Sheehan	564/155
3,340,147	9/1967	Martin et al.	514/616
3,657,341	4/1972	Thorne et al.	260/558 A
3,707,559	12/1972	Mazur et al.	564/158
4,018,826	4/1977	Gless, Jr. et al.	564/215
4,260,684	4/1981	Schult	564/155
4,303,673	12/1981	Biedermann et al.	564/155
4,513,009	4/1985	Roques et al.	564/155
4,595,700	6/1986	Donald et al.	514/616
4,618,708	10/1986	Roques et al.	564/154
4,873,241	10/1989	Napier et al.	514/237.8

FOREIGN PATENT DOCUMENTS

0885303	3/1981	Belgium .
0194464	2/1980	European Pat. Off. .
0007441	10/1980	European Pat. Off. .
0038758	10/1981	European Pat. Off. .
0042626	12/1981	European Pat. Off. .
0046707	3/1982	European Pat. Off. .
0263506	10/1987	European Pat. Off. .
0400400	5/1990	European Pat. Off. .
1927692	12/1969	Germany .
0393355	10/1965	Switzerland .
1051220	12/1966	United Kingdom .

OTHER PUBLICATIONS

Chemical Abstracts, vol. 92; No. 7:51712r (Feb. 18, 1990).

Chemical Abstracts, vol. 96; No. 5:35710r (Feb. 1, 1982).

Chemical Abstracts, vol. 101; No. 9; 72124v (Aug. 27, 1984).

Chemical Abstracts, vol. 91; No. 21:175147; (Nov. 19, 1979).

(List continued on next page.)

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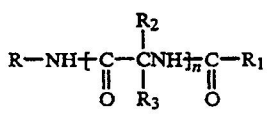
[57] **ABSTRACT**

The present invention relates to compounds exhibiting central nervous system (CNS) activity which are useful in the treatment of epilepsy and other CNS disorders. The compounds of this invention have the following general formula:



and pharmaceutically acceptable salts thereof.

150 Claims, No Drawings

Yield: 0.06 g (4%). R_f 0.32 (99:1 chloroform/methanol). mp 226°-227° C.

^1H NMR (DMSO-d_6) δ1.94 (s, CH_3CO), 4.34 (d, J=5.7 Hz, CH_2), 5.86 (d, J=8.1 Hz, CH), 7.20-7.38 (m, C_3·H, C_6·H, C_7H, Ph), 7.77-7.80 (m, C_4·H or C_5·H), 7.89-7.93 (m, C_4·H or C_5·H), 8.76 (d, J=8.1 Hz, NH), 8.97 (t,J=5.7 Hz, NH).

^{13}C NMR (DMSO-d_6) 22.34 (CH_3CO), 42.38 (CH_2), 52.70 (CH), 122.15 (C_4· or C_7·), 122.32 (C_4· or C_7·), 123.45 (C_3·), 124.37 (C_5· or C_6·), 124.41 (C_5· or C_6·), 126.89 (C_4··), 127.27 ($2C_2$·· or $2C_3$··), 128.27 ($2C_2$·· or $2C_3$··), 138.84 ($C_{3'a}$ or $C_{7'a}$), 138.95 ($C_{3'a}$ or $C_{7'a}$), 142.58 (C_1·), 168.65 (CH_3CO), 169.12 (CONH) ppm. [A distinct signal for the C_2· carbon was not detected and is presumed to coincide with the C_1· carbon at 142.58 ppm.].

IR (KBr) 3240, 1610 (br), 1510 (br), 1420, 1360, 1215, 1085, 885, 730, 710,685 cm^{-1}.

Mass spectrum, m/e (relative intensity) 338 (8), 295 (2), 205 (76), 162 (100), 135 (22), 108 (12), 91 (59).

Elemental Analysis: Calculated: 67.43% C; 5.36% H; 8.28% N. Found: 67.21% C; 5.37 %H; 8.12% N.

EXAMPLE 23

Preparation of (D,L)-α-Acetamido-N-benzyl-3-indoleacetamide

N-Acetyl-D,L-ethoxyglycine-N-benzylamide (0.69 g, 2.75 mmol) was suspended in anhydrous ethyl ether (20 mL) and then boron trifluoride etherate (0.63 g, 4.40 mmol) was rapidly added, and the resulting solution was stirred for 15 min. The indole (1.30 g, 11.00 mmol) was then added and the reaction was stirred at room temperature (22 h). Petroleum ether (35°-60° C.) was added to the reaction, and the resulting semisolid material filtered, and washed with petroleum ether (35°-60° C.). Purification of the reaction mixture was accomplished by flash chromatography (98:2 chloroform/methanol) to produce the title compound as a white solid.

Yield: 0.25 g (18%). R_f 0.14 (95:5 chloroform/methanol) mp 213°-214° C.

^1H NMR (DMSO-d_6) δ1.90 (s, CH_3CO), 4.36 (d,J=6.0 Hz, CH_2), 5.72 (d,J=7.2 Hz, CH), 6.90-7.37 (m, Ph, C_2·H), 7.02 (dd,J=7.5 Hz, J=7.5 Hz, C_5·H or C_6·H), 7.12 (dd,J=7.5 Hz, J=7.5 Hz, C_5·H or C_6·H), 7.39 (d,J=7.5 Hz, C_4·H or C_7·H), 7.65 (d J=7.5 Hz, C_4·H or C_7·H), 7.86 (d,J=7.2 Hz, NHCH), 8.13 (t,J=6.0 Hz, $NHCH_2$), 10.30-10.80 (br s, NH).

^{13}C NMR (DMSO-d_6) 22.32 (CH_3CO), 42.23 (CH_2), 49.98 (CH), 111.51 (C_7·), 112.08 (C_3·), 118.76 (C_4· or C_6·), 119.24 (C_4· or C_6·), 121.37 (C_5·), 123.94 (C_2·), 126.58 ($C_{3'a}$), 126.71 (C_{γ}), 127.33 ($2C_2$·· or $2C_3$··), 128.18 ($2C_2$·· or $2C_3$··), 136.28 ($C_{7'a}$), 139.44 (C_1··), 169.13 (CH_3CO), 170.81 (CONH) ppm.

IR (KBr) 3260, 1610 (br), 1515 (br), 1450, 1420, 1370, 1350, 1235, 1095, 895, 735, 715, 695, 600 cm^{-1}.

Mass spectrum, m/e (relative intensity) 321 (5), 278 (1), 264 (1), 233 (1), 214 (6), 187 (85), 171 (3), 145 (100), 118 (18), 91 (39).

Elemental Analysis: Calculated: 71.01% C; 5.96% H; 13.06% N. Found: 70.87% C; 6.15% H; 12.78% N.

EXAMPLE 24

Preparation of (D,L)-α-Acetamido-N-benzyl-2-(5-methylpyrrole)acetamide

N-Acetyl-D,L-ethoxyglycine-N-benzylamide (2.00 a, 8 mmol) was suspended in anhydrous ethyl ether (175 mL), and then boron trifluoride etherate (1.38 g, 9.7 mmol) was added and the resulting solution stirred (15 min). The 2-methylpyrrole (0.85 g, 10 mmol) was then added and the reaction mixture was stirred under N_2 (6 d), during which time the color of the reaction mixture turned reddish brown and a dark-brown deposit formed at the bottom of the flask. The clear solution was decanted and treated with an aqueous saturated $NaHCO_3$ solution containing ice (100 mL) for 30 min. The aqueous reaction mixture was extracted with ethyl acetate (3×30 mL). The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. The brown oily residue was purified by flash column chromatography using 98:2 chloroform/methanol as the eluent to yield the desired compound. The product was recrystallized from ethyl acetate/hexane to give a light yellow amorphous solid.

Yield 0.20 g (94%) R_f0.44 (95:5, chloroform/methanol). mp 167°-168° C.

^1H NMR (DMSO-d_6) δ1.87 (s, CH_3), 2.13 (s, $COCH_3$), 4.27 (br s, CH_2), 5.33 (d,J=7.4 Hz, CH), 5.60 (s, C_4H), 5.77 (s, C_3H), 7.19-7.30 (m, 5 PhH), 8.22 (d,J=7.4 Hz, NH), 8.45 (t,J=5.5 Hz, NH), 10.38 (s, NH).

^{13}C NMR (DMSO-d_6) 12.74 (CH_3), 22.49 ($COCH_3$), 42.11 (CH_2), 51.21 (CH), 105.09 (C_4), 106.07 (C_3), 126.16 (C_5), 126.64 (C_4·), 126.85 (C_2), 127.09 ($2C_2$· or $2C_3$·), 128.17 ($2C_2$· or $2C_3$·), 139.33 (C_1·), 168.88 ($COCH_3$), 169.79 (CONH) ppm.

IR (KBr) 3250, 1630, 1520, 1420, 1360, 1300, 1260, 1230, 1160, 1110, 1020 cm^{-1}.

Mass spectrum, m/e (relative intensity) 285 (M+,10), 178 (20), 152 (24), 151 (100), 110 (12), 109 (93), 108 (22), 107 (25), 94 (16), 91 (43).

Elemental Analysis: Calculated: 67.35% C; 6.71% H; 14.73% N. Found: 67.57% C; 6.90% H; 14.52% N.

Synthesis of Unsubstituted and Substituted-α-Acetamido-N-benzyl-2-furanacetamides

General Procedure. 4-Methylmorpholine (1 equiv) was added to a solution of α-acetamido-2-furanacetic acid (1 equiv) in dry tetrahydrofuran (75 mL/10 mmol) at −10 to −15° C. under N_2. After stirring (2 min), isobutyl chloroformate (1 equiv) was added leading to the precipitation of a white solid. The reaction was allowed to proceed for 2 additional minutes and then a solution of the substituted benzylamine (1 equiv) in tetrahydrofuran (10mL/10 mmol) was added over 5 min at −10 to −15° C. The reaction mixture was allowed to stir at room temperature for 5 min and then the 4-methylmorpholine hydrochloride salt filtered. The organic layer was concentrated in vacuo, and the residue was triturated with ethyl acetate, and the remaining white solid filtered. Concentration of the ethyl acetate layer led to additional amounts of the white solid. The desired product was purified by either recrystallization, or flash chromatography of the combined solid material. Examples 25-32 were prepared according to this procedure.

EXAMPLE 25

(D,L)-α-Acetamido-N-benzyl-2-furanacetamide

Using benzyl amine (0.27 g, 2.56 mmol) and racemic α-acetamido-2-furanacetic acid (0.47 g, 2.56 mmol) gave the desired compound. The product was recrystallized from ethyl acetate to give a white solid.

property, at least within the limitations of the structural [illegible] to the systematic search for and investigation [illegible]

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polymorphic forms of organic materials until the last twenty-five years or so†. The extent of the phenomenon is evident in the fact that over 3000 of the entries in the Cambridge Structural Database (Allen *et al* 1991) contain qualifying descriptions as being members of polymorphic systems.

While most examples of polymorphism are still discovered through serendipity, there are a number of areas of chemical research and development where full characterization of solid materials is critical in the determination of their ultimate use. These include pharmaceuticals (Haleblian and McCrone 1969, Haleblian 1975, Clements 1976), dyes (Walker *et al* 1972, Griffiths and Monahan 1976, Etter *et al* 1984, Tristani-Kendra *et al* 1983, Morel *et al* 1984) and explosives (Karpowicz *et al* 1983). Various aspects of the subject have been treated in books (Varma and Krishna 1966, Byrn 1983, Kuhnert-Brandstatter 1971) and a number of reviews (McCrone 1963, Haleblian and McCrone 1969, Haleblian 1975).

The proliferation of examples of polymorphism in these areas would seem to lend credence to the widely quoted statement by McCrone (1963) that 'Virtually all compounds are polymorphic and the number of polymorphs of a material depends on the amount of time and money spent in research on that compound'. However, it is still not possible to predict with any reasonable level of confidence the crystal structure of an organic material, much less the existence of polymorphism, although considerable progress is being made in these endeavours (Roberts 1992, Sato 1992, Desiraju 1991, Gavezzotti 1989, 1991, Maddox 1988, Scaringe and Perez 1987, Fagan and Ward 1992). Moreover, since scientists do not make a practice of reporting negative results, we are generally not aware of unsuccessful systematic searches for polymorphs. When required, a systematic search for polymorphic forms should be carried out on solid materials, involving a wide variety of techniques and conditions for growing crystals and enlisting a diversified armoury of analytical techniques to detect them. The range and combinations of crystal growth conditions are virtually infinite, and there is no way to guarantee the preparation of additional polymorphs of a substance, much less the generation of 'all' of them.

In spite of the considerable efforts and progress in understanding and controlling polymorphic behaviour (Weissbuch *et al* 1987) the phenomenon still seems to be at least partially shrouded in mystery and folklore, with tales of metastable crystal forms 'disappearing' in favour of more stable ones (Woodard and McCrone 1975, Jacewicz and Nayler 1979, Webb and Anderson 1978, Scheidt *et al* 1983). To fuel this debate there have been reports of successful attempts (Catti and Ferraris 1976, Czugler *et al* 1981, Bar and Bernstein 1982), after considerable efforts, to overcome the 'poisoning' of a laboratory or even vast areas of the earth (Woodard and McCrone 1975).

† One notable exception is the relatively obscure but very useful compilation by Deffet (1942) of polymorphic organic materials.

3. Comparing polymorphic crystal structures

The crystal structure representation containing most information is a stereo diagram of the packing, with a suitably chosen view of a central reference molecule and its immediate surroundings. Comparison of polymorphic structures is best made by preparing the same type of drawing on the same reference plane of the reference molecule for all of the polymorphs. While this pictorial approach is very helpful to a viewer it is difficult to translate the graphic information into a form which can be communicated to others. The traditional method of doing so involves the preparation of a listing of the 'short' intermolecular contacts (i.e. those less than the sum of the van der Waals radii) including the symmetry operations relating the neighbouring molecules. This representation falls at the opposite end of the information spectrum: it contains all the necessary analytical data, but is virtually impossible for the reader to visualize.

A solution to this dilemma may lie in the use of graph sets which have been developed recently to represent the patterns of hydrogen bonds (Etter 1990, Etter *et al* 1990), but which, in principle, may be applied to any type of intermolecular interaction. In this representation all hydrogen bonds may be classified as belonging to one of only four different patterns: intramolecular, finite dimeric, finite ring, or infinite chain. A complete definition of the pattern is given by specifying the number of donors (hydrogens) and the number of acceptors (basic atoms) as well as the total number of atoms contained in the pattern. The hydrogen-bond networks may then be summarized in a concise shorthand way which carries a great deal of information that may be used to compare readily the structures of polymorphic systems. Examples of such a treatment are given for the trimorphic system of iminodiacetic acid (Bernstein *et al* 1990) and the dimorphic L-glutamic acid (Bernstein 1991a).

Because of their highly directional nature, as compared with other types of interactions, this approach has been applied initially to hydrogen-bonded structures. Other intermolecular atom–atom interactions also exhibit characteristic patterns (Desiraju 1991) which are now much more amenable to study because of the ready accessibility of the structural data from the Cambridge Structural Database and the analytical and statistical software associated with it. The extension of the graph set approach for cataloguing structures from hydrogen-bond systems to other types of interaction is a natural one which should allow for much more facile understanding and comparison of polymorphic systems.

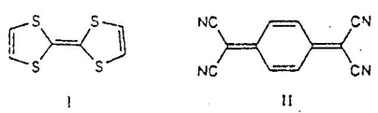
4. Examples of structure–property studies on polymorphic systems

4.1. Bulk properties

For historical reasons, the properties of organic materials have been considered in terms of the molecular structure. This was a natural result of the fact that most of the

chemistry of these materials was carried out in solution, where molecules might interact or react in a pairwise fashion. Even three-body interactions were considered quite unusual. The last two decades have witnessed a revolution in this point of view, as the wealth of possibilities for designing and utilizing the solid state properties of organic materials have become evident. These properties intrinsically depend on both the nature of the molecules and the way in which they interact with each other in the solid. Important information about the role of these solid state interactions can be obtained from the study of polymorphic materials, and variation of physical properties from one polymorph to another. Moreover, the recognition of the conditions required for the growth of the different polymorphs can provide additional information on the energetic relationships among them, and the crystallization conditions necessary to obtain the crystal architecture with the desired intermolecular interactions required for a particular physical property. Two examples from the realm of bulk properties will serve to illustrate these points.

4.1.1. Electrical conductivity. Organic materials are traditionally considered to be electrical insulators, but the discovery twenty years ago of metallic conductivity in crystals of the π molecular complex of tetrathiafulvalene I and tetracyanoquinodimethan II



I II

(Ferraris *et al* 1973, Coleman *et al* 1973) led to a revolution in thinking about these materials in particular and the potential for organic materials in general, as the basis for the next generation of electronic components (Wudl 1984, Williams *et al* 1985). In contrast to the vast majority of known π molecular complexes that crystallize with plane-to-plane stacks of alternating donors and acceptors (mixed stack, figure 1(*a*)) (Herbstein 1972), the complex of I and II crystallized with segregated stacks of molecules along the same crystallographic axis (but not mutually parallel), each stack containing only one type of molecule.

This structural feature has been shown to be a necessary condition for electrical conductivity in these materials, although the mixed mode of stacking is generally the thermodynamically preferred one (Shaik 1982). What means do chemists have at their disposal to overcome the tendency to form mixed stacks rather than segregated stacks? One method is to choose either a donor or acceptor with a completely different molecular shape. The principles of close packing in organic crystals (Kitaigorodskii 1973) then suggest that molecules will pack in segregated stacks in order to most efficiently fill the space. Such reasoning led, for instance, to the 2:1 'Bechgaard salts' (Bechgaard *et al* 1980, 1981) based on III as a donor, and a similar series based on IV ('ET') as a donor, in which the acceptors are

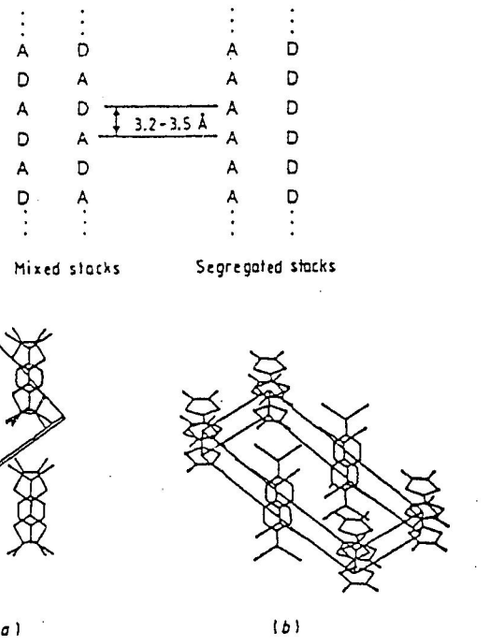
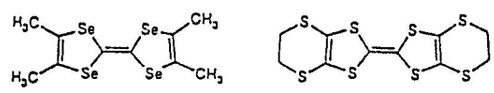


Figure 1. Upper part: schematic diagram of the mixed stack and segregated stack motifs for packing of molecular charge-transfer complexes; lower part: views of the two polymorphic structures of II:III. In both cases the view is on the plane of the TCNQ molecule (II). (*a*) The red, transparent, mixed stack complex, a semiconductor; (*b*) the black, opaque, segregated stack complex, a conductor. (From Bernstein (1991b) with permission.)

nearly spherical (i.e. octahedral or tetrahedral) or short linear anions that fill the voids between stacks of donors.



III IV

The 2:1 stoichiometry ensures the partial charge on the donor stack, another necessary condition for conductivity, and many of these salts are conductors or superconductors.

Proof of the relative stability of the mixed and segregated stack packing motifs and a recipe for obtaining crystals of the latter came with the discovery of a pair of polymorphic 1:1 complexes of II with III (Bechgaard *et al* 1977, Kistenmacher *et al* 1982). The red, transparent, mixed stack form of the complex is a semiconductor, while the black, opaque, segregated stack is a conductor (figure 1(*b*)). This finding demonstrated conclusively that the segregated stacks are a necessary condition for electrical conductivity. Reflecting the relative stabilities for the two stacking modes noted above, crystals of the red semiconductor form are obtained by a 'thermodynamic' or 'equilibrium' crystallization: equimolar solutions of the donor and acceptor in acetonitrile are mixed and allowed to evaporate slowly. Crystals of the black form are obtained by a 'kinetic' or nonequilibrium crystallization: hot equimolar solutions of the donor and acceptor in acetonitrile are mixed and cooled rapidly. Some microcrystals of the resulting black powder are then used

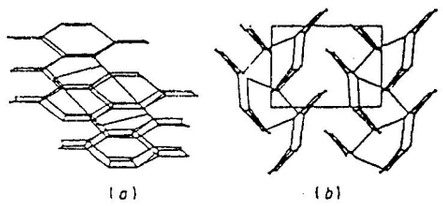


Figure 2. Network of ET (V) molecules in two phases of the salt $(ET)_2^+I_3^-$. The unit cell is included in each figure and thin lines indicate short intermolecular S···S contacts. (*a*) β form; (*b*) κ form. (From Williams *et al* (1991) with permission.)

as seeds to obtain larger crystals of the mixed stack black form. Non-equilibrium crystallization methods, in particular electrochemical techniques, have become standard procedure for obtaining crystals of organic conductors, in part because of the ability to control and reproduce the crystallization conditions. However, as we indicate below, control and reproducibility do not guarantee obtaining a single or unique crystal phase.

The role of polymorphism in understanding the connection between structure and properties is most poignantly represented by the ET_2X salts of IV. No fewer than 20 have been reported to be superconductors, having among them the highest known T_C values (Williams *et al* 1991). For $X=I_3^-$ alone, there are at least fourteen known phases, and learning to understand and control the crystal growth conditions in these polymorphic systems is one of the greatest challenges facing workers in this rapidly expanding field. As above, crystals are typically obtained by electrocrystallization methods, using Pt electrodes and an H tube, and a constant current of ~ 1 μA cm^{-2}. Crystal growth experiments can extend up to periods of months, with a number of polymorphs simultaneously appearing on the same electrode, in many cases with indistinguishable colours or crystal habits. For instance, Kobayashi *et al* (1987) identified the presence of the $\alpha, \beta, \gamma, \delta, \theta$ and κ polymorphs of $(ET)_2^+I_3^-$ in the same crystallization experiment.

Intense activity in this field has led to the definition of some conditions for preferentially obtaining one, or a limited number, or phases (Williams *et al* 1991), but very often the isolation and identification of the various phases requires characterization of each individual crystal (Kato *et al* 1987).

As an example of the type of variation observed, we can compare the β and κ phases of $(ET)_2I_3^-$ (figure 2). The former, apparently favoured by thermodynamic crystallization conditions (e.g. low current density) is a centrosymmetric triclinic structure with one formula unit of the salt in the unit cell. The symmetry arguments require that the anion lie on a crystallographic inversion centre, and that the donor molecules all be parallel, as shown in figure 2(*a*). The structure is thus characterized by stacks along the diagonal of the unit cell, with strong intermolecular S···S interactions between stacks. T_C for this phase is 1.4 K.

The κ phase may be obtained, together with the α and θ phases, in a THF solution under N_2 with a mixed supporting electrolyte of $(n\text{-}C_4H_9)_4NI_3$ and $(n\text{-}C_4H_9)_4NAuI_2$ at 20 °C and constant current of 1.0 μA (Kato *et al* 1987). It is characterized by the formation of dimers, but a number of different salts exhibit more variability in this type of structure than in the β phase (Williams *et al* 1991). κ-$(ET)_2^+I_3^-$ is centrosymmetric, with one layer per unit cell in a $P2_1/c$ structure (Kato *et al* 1987) (figure 2(*b*)) and has a T_C of 3.6 K.

Understanding the structure–property relationship in these materials is crucial to the rational development of organic conductors and superconductors with increasingly high T_C values. The plethora of polymorphic structures can easily lead the unwary investigator astray, but it provides an opportunity not available in many other systems for isolating the structural characteristics required for a very specific physical property. Williams *et al* (1991) have also pointed out that the isostructural series of salts are important for the information they can yield. In this case, the *structural* parameter is kept nearly fixed (or only slightly perturbed) and the effect of *chemical* perturbations can then be evaluated.

4.1.2. Aggregation of dyes. The aggregation of organic dye molecules has held the attention of dye chemists since its discovery over half a century ago (Smith 1974, Herz 1974). A lack of understanding of the phenomenon did not prevent extensive use being made of it, for instance, in the spectral 'tuning' of the response of photographic silver halide emulsions (Nassau 1983). Jelley (1936) found that when the solution concentration of many dyes is increased, the intensity of the characteristic molecular absorption band decreases in favour of the growth of a new intense, narrow absorption band on the long-wavelength side of the original molecular absorption band (figure 3). The new absorption, called a J band after its discoverer, is commonly attributed to the formation of aggregates of dye molecules, and results from the coupling of transition dipoles within the aggregate structure.

Since the aggregates are generally believed to contain only a few tens of molecules (Smith 1974) it has been difficult to study directly the relationship between their structure and spectral properties. While there is no certainty that a crystal structure represents the structure of the aggregate, the existence of polymorphic structure of a dye does allow one to probe directly the relationship between structure and spectral properties. For many years great difficulty was encountered in obtaining crystals of most dyes, to say nothing of polymorphic forms. However, as a result of the energy crises during the 1970s, considerable research effort was expended on many dyes with potential applications as photovoltaic materials (Morel *et al* 1978, Morel 1979, Forster and Hester 1982). True to McCrone's dictum some were found to be polymorphic, and one, the squarylium dye V was the subject of a combined crystallographic and spectroscopic study to investigate the relationship between the structure and spectral properties of such dyes.

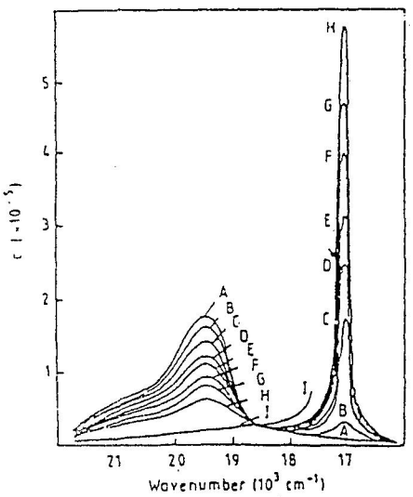
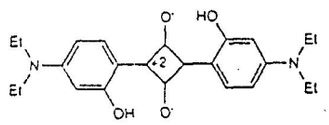


Figure 3. Solution spectra of a dye exhibiting J banding. Solutions numbered 1–9 range in concentration from 8.8×10^{-7} M to 1.4×10 M. The molecular absorption band is on the left; the J band on the right (see text). (From Bernstein (1991b) with permission.) A, 8.81×10^{-9} M; B, 1.76×10^{-6} M; C, 2.20×10^{-6} M, D, 2.64×10^{-6} M; E, 3.08×10^{-6} M; F, 3.53×10^{-6} M; G, 4.40×10^{-6} M; H, 6.16×10^{-6} M; I, 1.44×10^{-3} M.



V

he material is dimorphic, forming well developed crystals of both a violet triclinic structure and a green triclinic structure. Representative views of the crystal structures are given in figure 4. Here again, the crystal growth process is instructive in understanding the energetic relationships between the two observed phases. The two polymorphs appear simultaneously in the same beaker, and hence they grow under identical conditions, indicating that they are of very similar energies. The energetic similarity would appear to be coincidental since the difference in space groups and crystal structures indicated by figure 4(*a*) and (*b*) would suggest at least some difference in lattice energy and hence different crystallization conditions. The key to conundrum lies, in part at least, in the way the cr structures are viewed. When the reference plane is the molecular plane (figure 4(*c*) and (*d*)) it is clear that the plane-to-plane stacking in the two structures is essentially identical. This apparently is the dominant interaction, and the one that governs the crystallization pr s. The only difference between the triclinic and monoclinic structures, then, is the relationship between the stacks, a translation in the triclinic structure, and a screw axis in the monoclinic structure. These must be either subsidiary to the plane-to-plane stacking interaction or equal to each other for the crystals to appear simultaneously (Bernstein and Chosen 1988).

What are the spectroscopic manifestations of these structural differences? The molecule is essentially flat n both structures, so differences in the spectroscopic

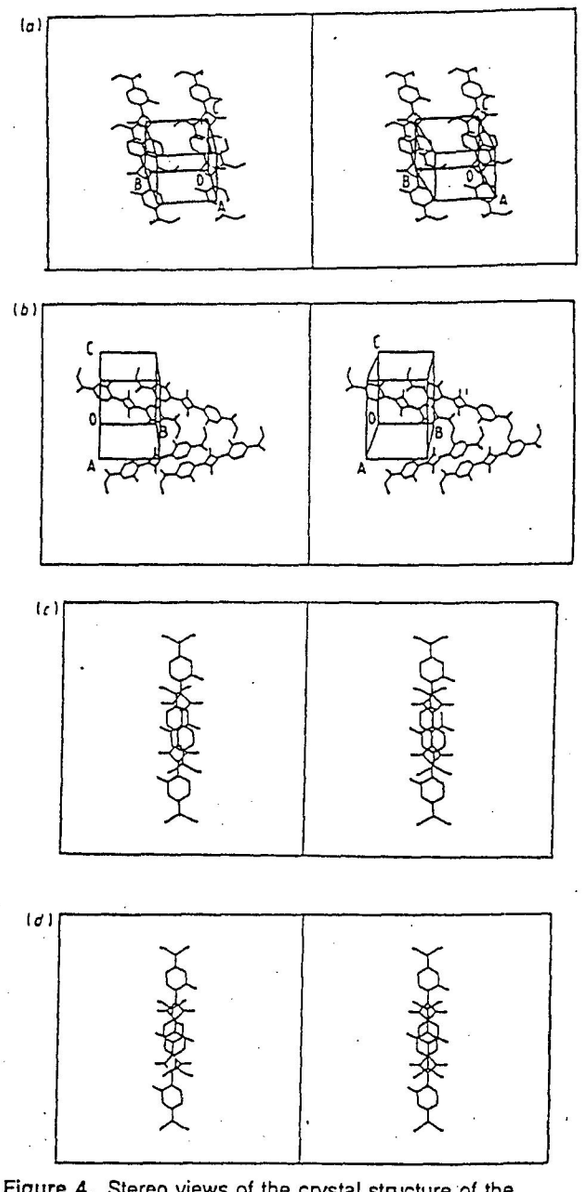


Figure 4. Stereo views of the crystal structure of the squarylium dye V: (*a*) triclinic structure, (100) face; (*b*) monoclinic structure, (100) face (from Tristani-Kendra *et al* (1983) with permission.); (*c*) view of the molecular plane, triclinic structure; (*d*) view of the molecular plane, monoclinic structure. (From Bernstein and Chosen (1988) with permission.)

properties must be an expression of the differences of intermolecular relationships in the two structures. The polarized normal incidence reflection spectra of the two crystals are given in figure 5, and it can be readily seen that the spectra are significantly different between the two forms for light polarized along the long axis of the molecules. This must be a consequence of the difference in the interaction of a single molecule with its surroundings. The reflection spectrum of the band in the monoclinic form has been interpreted as being composed of two oscillators, while that of the triclinic form contains three, or possibly four, oscillators

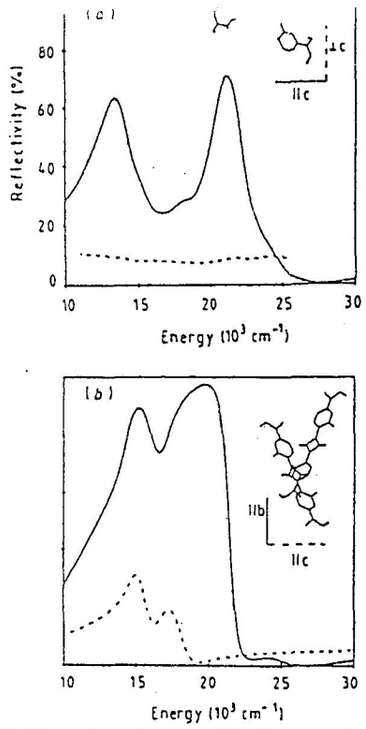


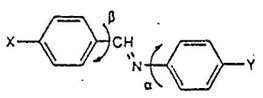
Figure 5. Normal incidence reflection spectra of the two forms of V. In each case there are two spectra measured with the light polarized along each of the two directions (the so-called principal directions), as indicated in the upper right-hand corner, which also shows the projection of the molecule(s) onto the crystal face studied. (*a*) Triclinic polymorph, (100) face; (*b*) monoclinic polymorph, (100) face. (From Tristani-Kendra *et al* (1983) with permission.)

(Tristani-Kendra and Eckhardt 1984, Tristani-Kendra *et al* 1985).

4.2. Molecular properties

At the other extreme in the range of interaction between molecules, organic crystals may be considered as simply a matrix for holding molecules in a specific juxtaposition with respect to each other. The interactions among molecules are ignored, at least to a first approximation, and the properties of the crystal reflect to a large extent the properties of the molecule, albeit with the three-dimensional ordering imposed by the very fact that it is a crystal.

When the molecule in question is conformationally flexible with a number of energetically accessible conformations (differing by less than ~ 2 kcal mol^{-1}) then different crystallization conditions may lead to conformational polymorphs. An almost classic example is the dichloro-benzylideneaniline VI (X=Y=Cl).



VI (X,Y = Cl, Br, CH_3)

The molecule has only two degrees of conformational freedom, designated α and β. The planar conformation of the molecule known to be more energetic than the conformation which $\alpha \simeq 50°$, $\beta \simeq 0°$ (Buergi and Dunitz 1971, Bernstein *et al* 1981) by about 1.5 kcal mol^{-1}. Two polymorphs are known: a triclinic form in which the molecule adopts a planar conformation ($\alpha = \beta \simeq 0°$) (Bernstein and Schmidt 1972), and an orthorhombic form in which the conformation is $\alpha \simeq 25°$, $\beta \simeq -25°$ (Bernstein and Izak 1976). Hence this system is an example of conformational polymorphism where the crystal environment of the triclinic form is sufficient to stabilize the higher-energy planar conformation of the molecule (Bernstein and Hagler 1978).

The treatment of the energetics of this system have been described in detail elsewhere (Bernstein and Hagler 1978, Bernstein 1987). However, in the present context it is of interest to review the crystallization behaviour. The triclinic form was discovered serendipitously in the course of a search for dichloro-substituted molecules which might have a 4 Å translation axis (Leser *et al* 1969, Green and Schmidt 1971, Desiraju 1989). A single oscillation photo of one crystal unambiguously indicated the presence of a 4 Å axis, but that crystal did not diffract on the second photo, and all subsequent attempts to obtain such crystals were unsuccessful. Recrystallizations carried out under equilibrium conditions yielded only the pale yellow orthorhombic form. The triclinic form was finally obtained under kinetic conditions by rapidly cooling a boiling saturated ethanolic solution in a desiccator freshly charged with $CaCl_2$. The white (actually colourless when viewed individually under the microscope) needles, characteristic of a structure with a 4 Å axis, proved to be metastable, and often upon standing for a few days would spontaneously revert to a pale yellow powder. The transformation could also be initiated by cleaving the crystals perpendicular to the needle axis, and sometimes could be followed visually by a gradual clouding of the crystal accompanied by a failure to extinguish polarized light. All of these phenomena are consistent with a metastable crystal form, and it is clear why somewhat unconventional methods were required to grow crystals of that form.

The trimorphic dimethyl derivative VI (X=Y=CH_3) represents an example of the 'disappearing' crystal form. Apparently unaware of the existence of polymorphism, Buergi *et al* (1968) reported cell constants for the compound (form I). We had no trouble preparing the compound and repeating their determination (Izak 1973). After an interim of about eight months these crystals did not diffract well. Over a three-year period, subsequent recrystallization experiments, often preceded by a fresh synthesis of the material, resulted in the discovery of two previously unknown polymorphs (forms II and III) (Bar and Bernstein 1977, 1982, Bernstein *et al* 1976), but failed to yield form I. Suspecting that our laboratory had been 'poisoned' by forms II and III we took advantage of the opening of a new laboratory about a kilometre away to try again from scratch, using new reagents, new glassware, and a 'new' student—this time with

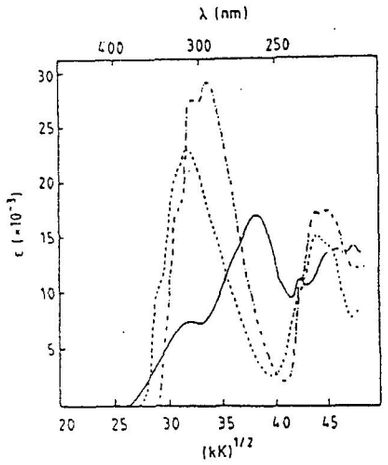
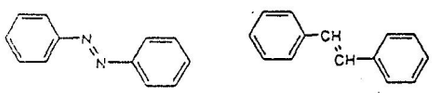


Figure 6. Solution absorption spectra of benzylideneaniline (VI, X=Y=H) (——), azobenzene VII (– – –) and stilbene VIII (— · —) in ethanolic solutions. (From Bernstein *et al* (1979) with permission.)

success. While the 'entire universe' was not poisoned by forms II and III, there clearly is some basis to the claims of McCrone and Woodard regarding the difficulties in crystallizing metastable polymorphic forms in an environment in which a more stable form has been crystallized.

The slight, but certainly visually distinguishable, difference in the colour of the two polymorphs of the dichloro-derivative suggested using the two forms to make a direct examination of a problem which had occupied spectroscopists for nearly three decades (Haselbach and Heilbronner 1968, and references therein). Benzylideneaniline VI (X=Y=H) is isoelectronic with azobenzene VII and stilbene VIII



VII VIII

but its solution absorption spectrum differs significantly from them (figure 6). The difference in the absorption spectra between stilbene and azobenzene on the one hand, and benzylideneaniline on the other, has been attributed to a difference in molecular conformation in solution: the former were believed to be essentially planar on the average, while the latter is not (Hasselbach and Heilbronner 1968). The non-planarity of VI is due to a repulsion between the hydrogen on the bridge and one of the *ortho*-hydrogens on the aniline ring. The bridge hydrogen is absent from VII and the increased length of the =C bond in VIII alleviates that steric effect. For benzylideneaniline, the tendency towards nonplanarity due to the hydrogen repulsion is balanced in part by the π-electron conjugation, leading to the minimum energy conformation of $\alpha \simeq 50°$.

The existence of the two polymorphic structures of VI (X=Y=Cl), and the corresponding planar and nonplanar conformations found therein, provides a unique opportunity to examine directly the relationship between molecular conformation and the electronic structure, as manifested in the absorption spectrum. Assuming that the two crystal structures merely serve to hold the molecule in the two different conformations, the absorption spectra should reflect the difference in conformation: that measured on the triclinic structure, with a planar molecular conformation, should closely resemble that of VII and VIII, while that for the orthorhombic structure, with the nonplanar molecular conformation, should retain the characteristics of VI in solution.

The measurement of the crystal absorption spectra of highly absorbing materials (in this case $\epsilon \simeq 20\,000$) would normally require the preparation of very thin samples. However, the use of polarized nearly normal incidence reflection spectroscopy on single crystals not only overcomes this problem but provides some additional advantages (Anex 1966, Pennelly and Eckhardt 1976). The use of polarized light allows one to gain information on the directional properties of the electronic transitions as well as information on the energies and intensities. Second, the fact that measurements using this technique are made on crystal surfaces rather than through the bulk allows one to choose a crystal face which provides a suitable projection of the molecules for study of a particular property.

This technique was applied to the dimorphic dichlorobenzylideneaniline (Bernstein *et al* 1979). Faces were chosen for study (figure 7) on the basis of a favourable projection of the long axis of the molecule, which was believed to be the polarization direction of the lowest-energy transition (Bally *et al* 1976). The reflection spectra with the light polarized nearly along the long axis of the molecule (figure 8) clearly indicate a significant difference, with that of the triclinic form even exhibiting vibronic structure reminiscent of the stilbene and azobenzene solution spectra. These reflection spectra may be converted into the corresponding absorption spectra using the Kramers–Kronig transform (Anex 1966, Anex and Fratini 1964), yielding the spectra shown in figure 9. These can be considered as the absorption spectra which would be obtained if the measurement could be made through that particular crystal face.

The absorption spectra of the two polymorphs differ in the same way as do the reflection spectra. The similarity to the expected solution spectra is indicative of weak interactions between molecules, hence confirming the assumption that these may simply be considered as 'oriented gas' crystals. The spectrum of the planar molecule exhibits a single low-energy band, which as noted above is punctuated by reproducible vibronic structure due to the superposition of the C=N stretching mode ($\sim$ 1350 cm^{-1}) on the long-axis transition. In the nonplanar conformation found in the orthorhombic structure the conjugation of the π system is broken, so the long-axis transition no longer exists and consequently no vibronic structure is expected or observed. These observations provide direct confirmation of the interpretations of spectroscopists on the origins of the

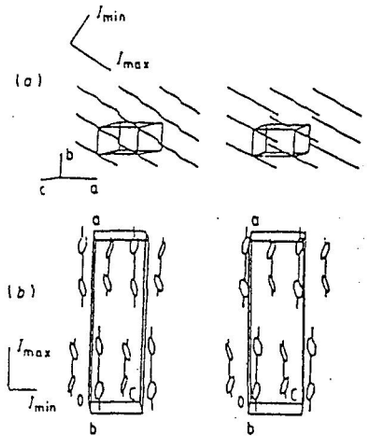


Figure 7. Projections of the crystal structures of VI, X=Y=Cl onto the faces studied spectroscopically: (*a*) the (001) face of the triclinic form; (*b*) the (110) face of the orthorhombic. I_{min} and I_{max} are the extinction directions which were oriented parallel to the electric vector of the incident light for the measurement of the reflection spectra. (From Bernstein *et al* (1979) with permission.)

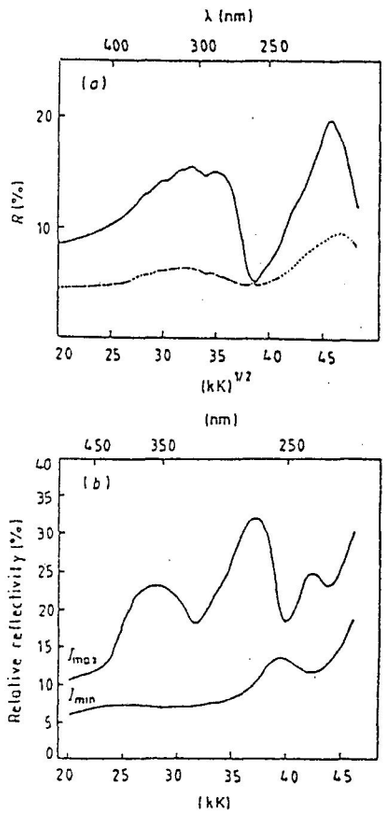


Figure 8. Normal incidence polarized reflection spectra at 298 K for the two forms of VI (X=Y=Cl): (*a*) the (001) face of the triclinic structure (—— and ····· are the spectra for light polarized parallel to the I_{max} and I_{min} directions respectively); (*b*) the (110) face of the orthorhombic form. (From Bernstein *et al* (1979) with permission.)

spectral differences among VI, VII, and VIII (Bally *et al* 1976).

In summary, the existence of conformational polymorphic structures, obtained in this case by very different crystal growth methods, provides an ideal system for investigating the relationship between molecular conformation and electronic structure. Additional examples can be found among those listed in the appendix and their numbers should increase as the number of known conformational polymorphic systems grows.

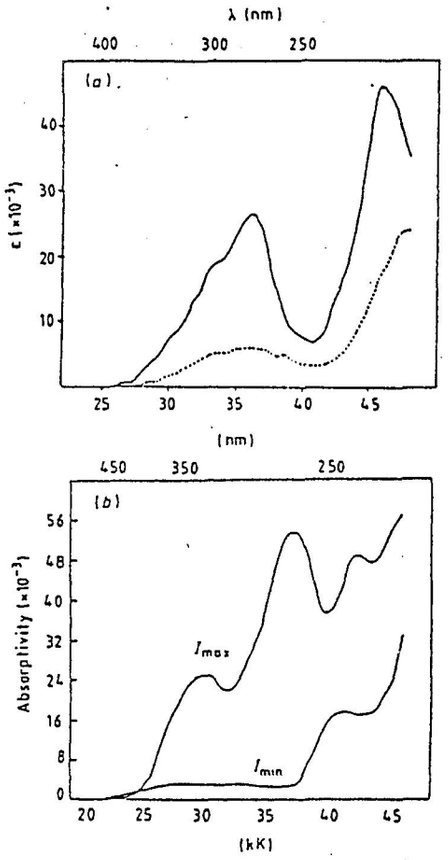


Figure 9. Kramers–Kronig transformed spectra of VI(X=Y=Cl), obtained from the reflection spectra in figure 7. The captions for (*a*) and (*b*) are as in figure 7. (From Bernstein *et al* (1979) with permission.)

5. Concluding remarks

Polymorphic systems are a potential source of detailed information on structure–property relations in organic solids. The constancy of chemical composition among polymorphs limits the variables to structural ones only. Differences in properties among polymorphs may then be directly related to differences in structure. We have discussed here but a few of the increasing number of polymorphic systems which have been studied to investigate structure–property differences. The appendix includes a table of a selection of other structure–property studies of this type which space limitations prevented our discussing in the body of the paper. Hopefully this will provide a source for those interested in pursuing similar studies.

Growing crystals is becoming one of the key steps in the development and analysis of new organic materials.

There has been impressive progress in understanding and controlling the crystal growth process, and it seems fair to say that the state of knowledge is such that it has matured from very much an art to a legitimate and important science. As in any science, there will always be a component of art, and that, perhaps is one of the main attractions of the field. One of the challenges to those trying to obtain polymorphs in general, or a specific polymorph in particular, is to be able to understand and control the crystallization conditions sufficiently is to meet this challenge successfully. The conditions under which different polymorphs are obtained exclusively or together can also provide very useful information about the relative stability of different phases and the methods and techniques that might be necessary to obtain similar structures of different chemical systems.

Appendix

There are, of course, many examples in the literature of the utilization of polymorphic materials to understand differences and similarities in physical and/or chemical properties as a function of structure. What follows is a very partial and biased list of some of these which were gleaned from this author's collection in addition to those papers cited above. Hopefully, it will provide a starting point and a source of material for further investigation.

Subject	References
Aggregation of surfactants	[7]
Anisotropy of intermolecular interactions	[55]
Bioavailability of drugs	[58]
Conformational polymorphism —organics	[23, 37–39]
Conformational polymorphism —organometallics	[2, 3, 16, 29, 48]
Control of polymorphic crystallization	[55]
Dyes	[54]
Electrical conductivity	[31, 51, 52]
Ferromagnetism (molecular)	[34]
High energy materials (explosives)	[39, 40]
IR, Raman studies	[4, 8, 10, 11], [13–15, 22], [23, 25, 27], [30, 32–34], [36–40, 43, 53, 56]
Mechanical properties	[9, 25, 26] [30, 47]
Nonlinear optical materials	[17, 18, 21, 46]
Nuclear quadrupole resonance	[20, 33, 41]
Pharmaceuticals—solution rate	[24, 25, 28]
Photoactivity	[5, 6, 8, 9]
Photochromism	[4]
Polymers	[12, 35]
Proton transfer processes	[10, 17, 21, 45]
Pyroelectric, dielectric, piezoelectric properties	[42]
Solid state NMR	[4, 11, 35]
Thermal reactivity of crystal	[44, 50]
Thermochromism	[1]
Triboluminescence	[19]
UV–Visible spectroscopy	[2, 9, 10, 26, 43], [45, 48, 49, 54, 57]

References for appendix

[1] Begley M J, Crombie L, Griffiths G L, Jones C F and Rahmani M 1981 *J. Chem. Soc. Chem. Commun.* 823
[2] Bernal I 1987 *J. Coord. Chem.* 15 337
[3] Boche G, Etzrodt H, Massa W and Baum G 1985 *Angew. Chem. Int. Ed. Eng.* 24 863
[4] Burns G R, Cunningham C W and McKee V 1988 *J. Chem. Soc. Perkin Trans. II* 1275
[5] Clarke B P, Thomas J M and Williams J O 1975 *Chem. Phys. Lett.* 35 251
[6] Cohen R, Ludmer Z and Yakhot V 1975 *Chem. Phys. Lett.* 34 251
[7] Coiro V M, Manigrasso M, Mazza F and Pochetti G 1987 *Acta Crystallogr.* C 43 850
[8] Craig D P and Mallett C P 1982 *Chem. Phys.* 65 129
[9] Dao L H and Perrier G 1986 *Chem. Lett.* 1259
[10] Dutta P K and Lee B S 1988 *J. Raman Spectrosc.* 19 175
[11] Fletton R A, Lancaster R W, Harris R K, Kenwright A M, Packer K J, Waters D N and Yeadon A 1986 *J. Chem. Soc. Perkin Trans. II* 1705
[12] Gidley M 1987 *Carbohydrate Res.* 161 301
[13] Grip J and Samuelsen E J 1984 *Physica Scripta* 29 556
[14] Grunenberg A and Bougeard D 1987 *J. Molec. Struct.* 160 27
[15] Haisa M, Kashino S, Ikejiri F, Ohno T and Teranishi K 1976 *Acta Crystallogr.* B 32 857
[16] Hall D, McKinnon and Waters T N 1965 *J. Chem. Soc.* 425
[17] Hall R C, Paul I C and Curtin D Y 1988 *J. Am. Chem. Soc.* 110 2848
[18] Hall S R *et al* 1986 *J. Cryst. Growth* 79 745
[19] Hardy G E, Zink J I, Kaska W C and Baldwin J C 1978 *J. Am. Chem. Soc.* 100 8001
[20] Hashimoto M and Mano K 1978 *J. Magnet. Res.* 30 431
[21] Herbstein F H, Kapon M, Reisner G M, Lehman M S, Kress R B, Wilson R B, Shiau- W-I, Deusler E N, Paul I C and Curtin D Y 1985 *Proc. R. Soc.* A 399 295
[22] Herranz J and Delgado J M 1975 *Spectrochim. Acta* A 31 1245
[23] Horn A, Klaeboe P, Myrvold B O and Nielsen C J 1986 *Spectrochim. Acta.* A 42 1141
[24] Huang M L and Niazi S 1977 *J. Pharm. Sci.* 66 609
[25] Ibrahim G, Pisano F and Bruno A 1977 *J. Pharm. Sci.* 66 669
[26] Kistenmacher T J and Destro R 1983 *Inorg. Chem.* 22 2104
[27] Kuhnert-Brandstatter M and Riedmann M 1987 *Mikrochim. Actas (Wien)* II 107
[28] Lagas M and Lerk C F 1981 *Int. J. Pharmaceutics* 8 11
[29] Lockhart T P and Manders W F 1986 *Inorg. Chem.* 25 583
[30] Lowes M M J, Caira M R, Lotter A P and vad der Watt J G 1987 *J. Pharm. Sci.* 76 744
[31] Magmedova N S and Zvonkova Z V 1978 *Sov. Phys. Crystallogr.* 23 155
[32] Mathieu J P 1973 *J. Raman Spectrosc.* 1 47
[33] Mido Y and Hashimoto M 1985 *J. Mol. Struct.* 129 253
[34] Miller J S, Glatzhofer D T, Calabrese J C and Epstein A J 1988 *J. Chem. Soc. Chem. Commun.* 322
[35] Moller M 1988 *Makromol. Chem. Rapid Commun.* 9 107
[36] Nash C P, Olmstead M M, Weiss-Lopez B and Musker W K 1985 *J. Am. Chem. Soc.* 107 7194
[37] Ogawa Y and Tasumi M 1978 *Chem. Lett.* 947
[38] Ogawa Y and Tasumi M 1979 *Chem. Lett.* 1411
[39] Oyumi Y, Brill T B and Rheingold A L 1986 *J. Phys. Chem.* 90 4686
[40] Oyumi Y, Brill T B and Rheingold A L 1987 *Thermochim. Acta.* 114 209
[41] Pies W and Weiss A 1980 *J. Mol. Struct.* 58 273
[42] Richter U and Haussuhl S 1987 *Cryst. Res. Technol.* 22 539

[43] Sakaino Y, Inouye Y, Kakis[illegible] and Takizawa T 1988 *Molec. Cryst. Liq. Cryst. Nonlinear Opt.* 161 255
[44] Sciesinska E and Sciesinska J 1986 *J. Molec. Struct.* 143 63
[45] Smulevich G, Guarini G G T and Marzocchi M P 1987 *Mol. Cryst. Liq. Cryst.* 142 173
[46] Staab E, Addadi L, Leiserowtiz L and Lahav M 1990 *Adv. Mater.* 2 40
[47] Summers M P, Enever R P and Carless J E 1977 *J. Pharm. Sci.* 66 1173
[48] Tellinghuisen P C, Robinson W T and Wilkins C J 1985 *J. Chem. Soc. Dalton Trans.* 1289
[49] Tristani-Kendra M, Eckhardt C J, Goldstein E and Bernstein J 1983 *Chem. Phys. Lett.* 98 57
[50] Uchida A, Danno M, Sasada Y and Ohashi Y 1987 *Acta Crystallogr.* B 43 528
[51] Uchida T, Ito M and Kozawa K 1983 *Bull. Chem. Soc. Japan* 56 577
[52] Uchida T, Sekhi H, Ito M, Nakano, Hoshizaki T and Kozawa K 1986 *Chem. Lett.* 999
[53] Ivan Wart H E and Scheraga H A 1986 *Proc. Natl. Acad. Sci. USA* 83 3064
[54] Walker M S, Miller R L, Griffiths C H and Goldstein P 1972 *Molec. Cryst. Liq. Cryst.* 16 203
[55] Weissbuch I, Zbaida D, Addadi L, Leiserowitz L and Lahav M 1987 *J. Am. Chem. Soc.* 109 1869
[56] Weiss-Lopez B E, Goodrow M H, Musker W K and Nash C P 1986 *J. Am. Chem. Soc.* 108 1271
[57] Wheeler G L and Colson S D 1976 *J. Chem. Phys.* 64 1227
[58] Yokoyama T, Umeda T, Kuroda K, Kuroda T and Asada S 1981 *Chem. Pharm. Bull.* 29 194

References

Allen F H, Davies J E, Galloy J J, Johnson O, Kennard O, Macrae C F, Mitchell E M, Mitchell G F, Smith J M and Watson D G 1991 *J. Inform. Comp. Sci.* 31 187
Anex B G 1966 *Mol. Cryst.* 1 1
Anex B G and Fratini A V 1964 *J. Mol. Spectrosc.* 14 1
Bally T, Haselbach E, Lanyiova S, Mardcher F and Rossi M 1976 *Helv. Chim. Acta* 59 486
Bar I and Bernstein J 1977 *Acta Crystallogr.* B 33 1738
—— 1982 *Acta Crystallogr.* B 38 121
Bechgaard K, Jacobsen C S, Mortensen K, Pedersen H J and Torup N 1980 *Solid State Commun.* 33 1119
Bechgaard K, Kistenmacher T J, Bloch A N and Cowan D O 1977 *Acta Crystallogr.* B 33 417
Bernstein J 1987 *Organic Solid State Chemistry* ed G Desiraju (Amsterdam: Elsevier) pp 471–518
—— 1991a *Acta Crystallogr.* B 47 1004
—— 1991b *Organic Crystal Chemistry* ed J B Garbarczyk and D W Jones (New York: International Union of Crystallography & Oxford Science) ch 2
Bernstein J, Anderson T E and Eckhardt C J 1979 *J. Am. Chem. Soc.* 101 541
Bernstein J, Bar I and Christensen A 1976 *Acta Crystallogr.* B 32 1609
Bernstein J and Chosen E 1988 *Mol. Cryst. Liq. Cryst.* 164 213
Bernstein J, Etter M C and MacDonald J C 1990 *J. Chem. Soc. Perkin Trans.* 2 695
Bernstein J and Hagler A T 1978 *J. Am. Chem. Soc.* 100 673
Bernstein J, Hagler A T and Engel M 1981 *J. Chem. Phys.* 75 2346
Bernstein J and Izak I 1976 *J. Chem. Soc. Perkin Trans. II* 429
Bernstein J and Schmidt G M J 1972 *J. Chem. Soc. Perkin Trans. II* 951
Buergi H-B and Dunitz J D 1971 *Helv. Chim. Acta* 54 1255
Buergi H-B, Dunitz J D and Zust C 1968 *Acta Crystallogr.* B 21 463
Byrn S R 1983 *Solid-State Chemistry of Drugs* (New York: Academic)
Catti M and Ferraris G 1976 *Acta Crystallogr.* B 32 359
Clements J A 1976 *Proc. Anal. Div. Chem. Soc.* 21
Coleman L B, Cohen M J, Sandman D J, Yamagishi F G, Garito A F and Heeger A J 1973 *Solid State Commun.* 12 1125
Czugler M, Kalman A, Kovacs J and Pinter I 1981 *Acta Crystallogr.* B 37 172
Deffet L 1942 *Repertoire des Composes Organiques Polymorphes* (Liège: Desoer)
Desiraju G T 1989 *J. Am. Chem. Soc.* 111 8725
—— 1991 *Crystal Engineering* (Amsterdam: Elsevier) ch 6
Etter M C 1990 *Acc. Chem. Res.* 23 120
Etter M C, Kress R B, Bernstein J and Cash D C 1984 *J. Am. Chem. Soc.* 106 6921
Etter M C, MacDonald J C and Bernstein J 1990 *Acta Crystallogr.* B 46 256
Fagan P J and Ward M D 1992 *Sci. Am.* 261 28
Ferraris J, Cowan D O, Walatka V and Perlstein J H 1973 *J. Am. Chem. Soc.* 95 948
Forster M and Hester R E 1982 *J. Chem. Soc. Faraday Trans.* 78 1847
Gavezzotti A 1989 *J. Am. Chem. Soc.* 111 1835
—— 1991 *J. Am. Chem. Soc.* 113 4622
Green B S and Schmidt G M J 1971 *Israel Chem. Soc. Annual Meeting Abstracts* p 190
Griffiths C H and Monahan A R 1976 *Mol. Cryst. Liq. Cryst.* 33 175
Haleblian J K 1975 *J. Pharm. Sci.* 64 1269
Haleblian J K and McCrone W C 1969 *J. Pharm. Sci.* 58 411
Haselbach E and Heilbronner E 1968 *Helv. Chim. Acta* 51 16
Herbstein F H 1971 *Perspectives in Structural Chemistry* ed J D Dunitz and J Ibers, vol 4 (New York: Wiley) pp 166–395
Herz A 1974 *Phot. Sci. Eng.* 18 441
Izak I 1973 *Senior Thesis* Ben-Gurion University of the Negev, Beer Sheva
Jacewicz V W and Nayler J H C 1979 *J. Appl. Crystallogr.* 12 396
Jelley E E 1936 *Nature* 138 1009
Karpowicz R J, Sergio S T and Brill T B 1983 *I & EC Prod. Res. Dev.* 22 363
Kato R, Kobayashi H, Kobayashi A, Moriyama S, Nishio Y, Kajita K and Sasaki W 1987 *Chem. Lett.* 507
Kistenmacher T J, Emge T J, Bloch A N and Cowan D O 1982 *Acta Crystallogr.* B 38 1193
Kitaigorodskii A I 1973 *Molecular Crystals and Liquid Crystals* (New York: Academic) ch I
Kobayashi A, Kato R, Kobayashi H, Moriyama S, Nishio Y, Kajita K and Sasaki W 1987 *Chem. Lett.* 459
Kuhnert-Brandstatter M 1971 *Thermomicroscopy in the Analysis of Pharmaceuticals* (New York: Pergamon)
Leser Y, Green B S and Schmidt G M J 1969 Personal communication
Maddox J 1988 *Nature* 335 201
McCrone W C 1963 *Physics and Chemistry of the Organic Solid State* ed D Fox *et al* vol I (New York: Interscience) p 725
Mitscherlich E 1822 *Ann. Chim. Phys.* 19 350
—— 1823 *Ann. Chim. Phys.* 24 264
Morel D L 1979 *Mol. Cryst. Liq. Cryst.* 50 127
Morel D L, Ghosh A K, Feng T, Stogryn E L, Shaw P E and Fishman C 1978 *Appl. Phys. Lett.* 32 495
Morel D L, Stogryn E L, Ghosh A K, Feng T, Purwin P E, Shaw R F, Fishman C, Bird G R and Piechowski A P 1984 *J. Phys. C: Solid State Phys.* 88 923
Nassau K 1983 *The Physics and Chemistry of Color* (New York: Wiley) ch 13
Pennely R R and Eckhardt C J 1976 *Chem. Phys.* 12 89
Roberts K J 1992 *J. Phys. D: Appl. Phys.* 26 B7–B21
Sato K 1992 *J. Phys. D: Appl. Phys.* 26 B77–B84

Boulfelfel S, Perez S 1987 *J. Phys. C: Solid State Phys.* **91** 2394
Bordner W R, Geiger D K, Hayes R G and Lang G 1983 *J. Am. Chem. Soc.* 105 2625
Etter M C 1982 *J. Am. Chem. Soc.* 104 5328
Garvin D 1974 *Phot. Sci. Eng.* 18 309
Haget-Kendra M, Delaney J and Eckhardt C J 1985 *J. Physique* 46 C7 425
Haget-Kendra M and Eckhardt C J 1984 *J. Chem. Phys.* **81** 1160
Haget-Kendra M, Eckhardt C J, Goldstein E and Bernstein J 1983 *Chem. Phys. Lett.* 98 57
Kitaigorodskii A R and Krishna P 1966 *Polymorphism and Polytypism in Crystals* (New York: Wiley)
Walker M S, Miller R L, Griffiths C H and Goldstein P 1972 *Mol. Cryst. Liq. Cryst.* 16 203
Webb J and Anderson B 1978 *J. Chem. Educ.* **55** 644
Weissbuch I, Zbaida D, Addadi L, Leiserowitz L and Lahav M 1987 *J. Am. Chem. Soc.* 109 1869
Williams J M, Beno M A, Wang H H, Leung P C W, Enge Y J, Geiser U and Carlson K D 1985 *Acc. Chem. Res.* **18** 261
Williams J M, Schultz A J, Geiser U, Carlson K D, Kini A M, Wang H H, Kwok W-K, Whangbo M H and Schirber J E 1991 *Science* 252 1501
Woodard G D and McCrone W C 1975 *J. Appl. Crystallogr.* 8 342
Wudl F 1984 *Acc. Chem. Res.* 17 227

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Scheidt W R, Geiger D K, Hayes R G and Lang G 1983 *J. Am. Chem. Soc.* 105 2625
Shaik S S 1982 *J. Am. Chem. Soc.* 104 5328
Smith D 1974 *Phot. Sci. Eng.* 18 309
Thistani-Kendra M, Delaney J and Eckhardt C J 1985 *J. Physique* 46 C7 425
Thistani-Kendra M and Eckhardt C J 1984 *J. Chem. Phys.* 81 1160
Thistani-Kendra M, Eckhardt C J, Goldstein E and Bernstein J 1983 *Chem. Phys. Lett.* 98 57
Vaidya A R and Krishna P 1966 *Polymorphism and Polytypism in Crystals* (New York: Wiley)
Walker M S, Miller R L, Griffiths C H and Goldstein P 1972 *Mol. Cryst. Liq. Cryst.* 16 203
Webb J and Anderson B 1978 *J. Chem. Educ.* 55 644
Weissbuch I, Zbaida D, Addadi L, Leiserowitz L and Lahav M 1987 *J. Am. Chem. Soc.* 109 1869
Williams J M, Beno M A, Wang H H, Leung P C W, Enge Y J, Geiser U and Carlson K D 1985 *Acc. Chem. Res.* 18 261
Williams J M, Schultz A J, Geiser U, Carlson K D, Kini A M, Wang H H, Kwok W-K, Whangbo M H and Schirber J E 1991 *Science* 252 1501
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

RESPUESTA AL RECURSO DE REPOSICIÓN

2020
Bogotá, D.C., Marzo 21 de 2006

Abogada
ALICIA LLOREDA RICAURTE

REFERENCIA:	Radicación No.	00 048 529
	Trámite	002
	Evento	001
	Actuación	430
	Oficio No.	
	Folios	00

TÍTULO "Nuevas Formas cristalinas de Melagatrán"

En atención al Recurso de Reposición interpuesto, se hacen las siguientes aclaraciones:

1. Documentos estudiados

Para el presente estudio se tuvieron en cuenta el capítulo descriptivo comprendido en los folios 5 – 35 y 123 – 145, el nuevo capítulo reivindicatorio de los folios 238 - 242 y las observaciones del solicitante de los folios 279 – 301 de la solicitud en estudio.

2. Objeto de la Invención

El objeto de la presente solicitud de patente de invención consiste en:

Las reivindicaciones 1 – 7 se refieren a una forma cristalina de melagatrán (anhidrato e isopropanolato/hidrato).

Las reivindicaciones 8 – 16 se refieren a un proceso para producir una forma cristalina de melagatrán que consiste en la cristalización en un sistema solvente (acetatos, alcoholes, hidrocarburos, éteres, cetonas, soluciones acuosas, alquilacetatos, alquilalcoholes, hidrocarburos aromáticos, dialquiléteres, como p. ej. acetato de etilo, etanol, isopropanol, isooctano, n-heptano, tolueno, diisopropil éter, metil isobutilcetona, agua ó mezcla de ellos) y un antisolvente (acetato de etilo, acetonitrilo).

La reivindicación 17 se refiere a una formulación farmacéutica que contiene un compuesto de las reivindicaciones 1 - 7 con un auxiliar ó vehículo farmacéuticamente aceptables.

El problema planteado en esta solicitud es la necesidad de proporcionar, posterior a la síntesis, una forma más pura y estable de melagatrán.

Para resolver este problema técnico la solicitud en estudio proporciona una forma cristalina de melagatrán (anhidrato e isopropanolato/hidrato) y la cristalización para obtenerla.

El objeto de la invención definido anteriormente se clasificó de acuerdo con la IPC: A 61 K 38/55.

También se reivindica prioridad de la Solicitud de Patente de Suecia 9902550-4 julio 2 de 1999, con respecto a la cual la fecha de presentación de la actual solicitud cumple el plazo máximo permitido.

3. Respuesta a los Argumentos del Solicitante

En atención a los argumentos del solicitante se hacen las siguientes aclaraciones:

1. Dice el solicitante en los folios 280 – 283 que el Manual Andino menciona que para que un compuesto se considere con nivel inventivo basta que con que tenga una estructura inesperada. Y considera que el caso actual corresponde a esa situación.

Se aclara al solicitante que esta no es la situación de esta solicitud. Porque lo que se habla en el Manual Andino es para casos en los cuales **el compuesto es nuevo.** Es decir que tiene **una estructura química nueva**, o dicho en otras palabras, es una **"nueva entidad química".** Parece que el error deriva del hecho que el solicitante olvidó transcribir la palabra "nuevo", en el párrafo b), el cual se presenta a continuación para poder hacer una nueva lectura, según el cual: "b) ¿Qué es necesario considerar para que un compuesto nuevo tenga nivel inventivo?". Se reitera que **no es nuevo** porque el compuesto N-((R)-(((2S)-2-((-p-amidobencil)carbamoil)-1-azetidinil) carbonil) ciclohexilmetil)glicina, de fórmula:

HOOC-CH2-(R)Cgl-Aze-Pab

también conocido como Melagatrán, se enseñaba tanto en D1 como en D2 y D3. Si la estructura química fuese nueva, tendría otro nombre químico. Y como tal, tendría nivel inventivo, y sería patentable. Pero ya es claro que no es este el caso, porque no es que tiene una estructura química "muy próxima a las del estado de la técnica", sino que es idéntica.

Del Manual Andino de Patentes:

10.8 Nivel inventivo en áreas específicas de la tecnología

10.8.1 Química

a) ¿Cuándo un compuesto químico o composición química tienen nivel inventivo?

- el compuesto tiene una estructura inesperada (caso poco frecuente); o,
- presenta un efecto inesperado, (es el caso más frecuente sobre todo si el compuesto es similar a otros del estado de la técnica. El efecto inesperado puede ser completamente diferente de los descritos para los compuestos similares conocidos, o bien ser igual pero con una mejora en los resultados).

En el caso de invenciones de selección, por ejemplo, la selección de un subgrupo de compuestos en una fórmula *Markush* que cumplen el requisito de novedad. En este caso tiene nivel inventivo si todos los compuestos en el subgrupo presentan un efecto o propiedad técnica no descrita en el arte anterior y que además es inesperada. Asimismo, es necesario que todos los compuestos que entran en la fórmula presenten nivel inventivo. Esto quiere decir que si el nivel inventivo se basa en un efecto técnico, todos los compuestos posibles de la fórmula *Markush* tienen que presentar nivel inventivo.

Por lo tanto, si el examinador puede demostrar que en una parte de la reivindicación no se presenta ese efecto (por ejemplo por el tipo de sustituyente que hace insoluble o tóxico el compuesto, porque el compuesto es inestable, etc.), entonces no habría nivel inventivo en todo el conjunto de compuestos de la fórmula *Markush* y el solicitante tendría que restringir a aquellos compuestos que sí presentan actividad.

En el caso de productos intermedios que se utilizan para la producción de compuestos inventivos y que no presentan por sí mismos un nivel inventivo, se consideran inventivos si contribuyen estructuralmente al producto final inventivo, y esa contribución es responsable al menos de una de las características que diferencian el producto final del estado de la técnica. Si el intermedio es un compuesto que se utiliza en un proceso inventivo, su nivel inventivo se puede derivar de su contribución al proceso.

Las composiciones suelen presentar nivel inventivo por la presencia de un efecto sinérgico. El efecto que presentan es superior al que se podría esperar si se sumaran los efectos o actividades de sus componentes tomados por separado.

b) ¿Qué es necesario considerar para que un compuesto nuevo tenga nivel inventivo?

Respuesta:

1. que el compuesto posea una estructura inesperada; o
2. que el compuesto exhiba un uso o un efecto sorprendente.

b.1. Estructura inesperada

Es un caso poco frecuente. La estructura del nuevo compuesto no hubiera podido ser deducida por el técnico medio en la materia. El examinador no necesita examinar si este compuesto posee o no un uso o efecto sorprendente puesto que la mera estructura ya le confiere el nivel inventivo.

b.2. Efecto o uso no esperado

b.2.1 La existencia de un efecto o uso sorprendente es la manera más común de establecer altura inventiva para compuestos nuevos, particularmente cuando tienen estructuras muy próximas a las del estado de la técnica.

b.2.2 Tipos de efecto sorprendente: (i) completamente diferente de los usos o efectos conocidos de compuestos descritos en el estado de la técnica; y (ii) una mejora sustancial de un efecto de la misma índole que exhiba un compuesto conocido del estado de la técnica más próximo.

b.2.3 Uso o efecto no descrito previamente: un uso o efecto se considerara sorprendente cuando para los compuestos descritos en el estado de la técnica no haya sido descrito ningún uso o efecto y éste no pueda ser derivable del conocimiento general.

2. Dice el solicitante en los folios 283 - 287 que no es posible predecir que el compuesto pueda estar en forma cristalina, a partir de la forma amorfa, que no se puede predecir la estructura cristalina de estos, ni su forma de preparación.

Se reitera al solicitante que de acuerdo con lo enseñado en D4, aunque no es posible predecir que el compuesto que se obtiene inicialmente en forma amorfa pueda estar en forma cristalina, una persona normalmente conocedora de la materia sigue como parte del proceso normal después de la síntesis, el paso de purificación que consiste en aislarlo en diferentes tipos de solventes, en los cuales lo que ocurre de manera espontánea es que estos cristalizan. Ahora bien, la forma de los cristales obtenidos depende de las impurezas de la solución, del pH, de la velocidad de agitación, de la de enfriamiento y del solvente. De manera que los cristales obtenidos no tienen el carácter de nivel inventivo, aunque no se pueda predecir su estructura cristalina porque no son fruto de la inventiva humana, propiamente. Y más aún, por ser tan conocido es paso de cristalización en solventes, no se puede considerar esta forma de preparación como inventiva.

3. Dice el solicitante en los folios 289 – 292 que la forma cristalina tiene nivel inventivo respecto de la forma amorfa porque tiene más estabilidad química, porque al cabo de 6 semanas presenta menos cantidad de impurezas que la forma amorfa.

Se aclara al solicitante que obviamente toda forma cristalina contiene menos impurezas que la forma obtenida después de la síntesis del compuesto. Y todo compuesto recién sintetizado debe aislarse del medio en el cual se obtuvo, y la forma, como ya se explicó, es mediante la cristalización en solventes. Se reitera que aunque las formas cristalinas no están expresamente mencionadas en las anterioridades, se considera que no tienen nivel inventivo, porque la disposición de las moléculas para formar uno u otro cristal es una característica intrínseca del compuesto denominada polimorfismo. Y el hecho de establecer cuáles son los polimorfos de un compuesto, su estabilidad, solubilidad, mejor forma de obtención de cada uno de ellos y su posterior integración con los demás componentes de la formulación, es un proceso rutinario y obligatorio, previo a la elaboración de

una preparación con el fin de que esta tenga un comportamiento reproducible. Además no se aprecia nivel inventivo porque el compuesto presenta las mismas propiedades anticoagulantes, inhibidoras de trombina y no presenta propiedades sorprendentes ó inesperadas que resuelvan algún problema en la técnica, cuando se compara con el compuesto del estado del arte. Puesto que no se observa una ventaja comparativa sorprendente con respecto al compuesto enseñado en la anterioridad, es claro que no hay nivel inventivo.

4. Conclusión

Teniendo en cuenta lo anterior, se recomienda ratificar la negación del privilegio solicitado para las reivindicaciones 1 - 17, folios 238 - 242.

Bogotá, D. C., Marzo 27 de 2006

CONCEPTO TÉCNICO 484	JEFE DIVISIÓN NUEVAS CREACIONES	EXAMINADOR TÉCNICO 202007

367

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: **0-48529** EDICTO No. **7796**

LA SECRETARÍA GENERAL AD-HOC
HACE SABER:

Que esta Superintendencia profirió **Resolución** No. **11063** de **28-ABR-2006**. Que el contenido de la parte resolutiva es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

ARTICULO PRIMERO: Confirmar la decisión contenida en la Resolución 34297 de 21 de diciembre de 2005 por medio de la cual se negó una patente de invención.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución a la doctora Alicia Lloreda Ricaurte, en su calidad de apoderada de ASTRAZENECA AB, o a quien haga sus veces, entregándole copia de la misma, advirtiéndole que contra ella no procede recurso alguno por encontrarse agotada la vía gubernativa.

NOTIFIQUESE Y CUMPLASE
Dada en Bogotá, D. C., a los 28 ABR. 2006

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,

JAIRO RUBIO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc **19-MAY-2006** Este edicto se desfija hoy **02-JUN-2006** a las cinco (5:00 p.m.)
Secretaria General Ad-Hoc
EDICTO No. **7796**

JOSE LLOREDA CAMACHO & CO.

Calle 72 No. 5-83 Piso 5, Bogotá, D.C. Tel: 6069700 Fax 6069701

266 368

Señor
SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. S. D

SUPERINDUSTRIA Y COMERCIO
Radicacion : 00048529 00000016 Folios: 13
Fecha (AMD): 2006-02-08 17:24:36
Tramite : 002 PATENTES D 1 REGISTRO/ 329 COMPLEMEN
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Re: P1.-**ASTRAZENECA AB**.-Solicitud de Patente de Invención para "**NUEVAS FORMAS CRISTALINAS DE MELAGATRAN**".-Clase **A.61**.-Expediente número **00-048.529**.-

1. Me refiero al memorial de Recurso de Reposición presentado el 31 de Enero de 2006 en contra de la Resolución No. 34297 por medio de la cual se niega el privilegio de patente solicitado.

2. Dando alcance al escrito arriba referido, atentamente me permito presentar a ese Despacho documento original consistente en la declaración rendida por la Dra. Sofía Nagard, debidamente apostillado y junto con su Traducción Oficial No. 2006-02-06-086.

3. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,

ALICIA LLOREDA RICAURTE
T.P. 53.215

TRADUCCION OFICIAL No. 2006-02-06-086
DE UN DOCUMENTO ESCRITO EN INGLES

Apostilla, documento escrito en español.

Número de Solicitud de Colombia: 00-048.529

Declaración

Yo, Sofía Någård, declaro lo siguiente:

1. Recibí mi título de la Universidad de Gotemburgo. He sido empleada de AstraZeneca (previamente Astra Hässle AB) R&D Mölndal, S-431 83 Mölndal, Suecia, el solicitante de la solicitud anteriormente referenciada, desde 1993. Anexo mi Curriculum Vitae, el cual incluye una lista de mis publicaciones.

2. Como un inventor en el presente caso, estuve directamente comprometida en los estudios que llevaron a la presente invención y que se revelan en la descripción del presente. También estuve directamente relacionada con los experimentos que produjeron los datos que se incluyen a continuación en el Anexo A. Por lo tanto, estoy plenamente calificada para hacer esta declaración.

3. La presente solicitud se relaciona con formas sustancialmente cristalinas del compuesto HO_2C-CH_2-(*R*)Cgl-(*S*)Aze-Pab-H (donde Cgl representa ciclohexilglicina, Axe representa azetidina-2-carboxilato y Pab-H representa

4-aminometilamidinobenceno). El compuesto es también conocido como melagatrán. Este compuesto y otros han sido descritos en WO 94/29336 como inhibidores de trombina.

4. En vista de toda la técnica anterior estoy consciente de, y en particular de los documentos de AstraZeneca citados por el examinador (a saber WO 94/29336, WO 97/23499 y WO 98/16252), y creo que la presente invención es patentable.

5. No creo que la técnica anterior revele melagatrán en una forma sustancialmente cristalina. Más bien, la técnica anterior más cercana según mi punto de vista, WO 94/29336, revela un método para obtener únicamente melagatrán amorfo.

6. No creo que sea posible para una persona versada en la técnica predecir, únicamente de la estructura molecular, cuál será el comportamiento de cristalización de un compuesto. Además, no creo que haya ninguna motivación en la técnica anterior para la persona versada en la técnica para aplicar las técnicas de cristalización descritas en la presente solicitud al melagatrán.

7. Más específicamente, y con referencia a la reivindicación pendiente expresada en esta solicitud de Colombia, no creo que sea posible alegar que las revelaciones anteriores equivalen a cualquier clase de sugerencia para hacer una forma sustancialmente cristalina de melagatrán en la forma de un hidrato (presente Reivindicación 1), en la forma de un anhidrato (actual Reivindicación 3), o en la forma de un *iso*-propanolato/hidrato mezclado (actual Reivindicación 6).

269371

8. La presente invención además proporciona una ventaja inesperada, que no creo que de ninguna manera pudiese haber sido predicha antes de la fecha de prioridad de la presente solicitud.

9. Creo que los datos experimentales proporcionados por medio del Anexo A claramente demuestran una ventaja inesperada al revelar la estabilidad química mejorada de una forma cristalina de melagatrán descrita en la presente solicitud sobre la forma amorfa obtenida por medio de WO 94/29336.

10. Aunque yo esperaría que una o más formas cristalinas de una sustancia fuesen más estables desde un punto de vista **físico** que una forma amorfa correspondiente, este aumento significativo en estabilidad **química** para la forma cristalina me llega como una sorpresa y no podría de ninguna manera haber sido predicho a la luz de WO 94/29336 o cualquier otra técnica anterior de la cual estoy consciente.

11. Claramente, esta estabilidad química mejorada, que se observa en una forma cristalina de melagatrán proporciona una ventaja significativa e inesperada desde el punto de vista de la facilidad de manejo, fabricación de formulaciones farmacéuticas incluyendo la sustancia del medicamento, y el subsiguiente almacenamiento en forma sólida (v.g. en la forma de tabletas orales) antes de y durante la distribución, en farmacias y de hecho en los hogares de los pacientes.

12. En resumen, no creo que haya habido ninguna motivación, antes de la fecha de prioridad, para la persona versada en llegar a formas cristalinas de melagatrán según se describen en la presente solicitud. Además, no hay

absolutamente ninguna motivación, antes de la fecha de prioridad, para llegar a las formas cristalinas específicas de melagatrán en la forma de un hidrato, anhidrato o *iso*-propanolato/hidrato mezclado según se describe en las presentes reivindicaciones modificadas. Esto es claramente indicativo de nivel inventivo.

13. Además, no hay absolutamente ninguna sugerencia de las ventajas inesperadas (en estabilidad química) de la invención, ventajas que se describen en los datos experimentales presentados. Estos datos claramente demuestran evidencia de paso inventivo, y por lo tanto, de patentabilidad.

14. Por el presente declaro que todas las afirmaciones hechas en el presente de mi propio conocimiento son verdaderas y todas las declaraciones sobre información y creencia se cree que son verdaderas.

Fecha: 25 / 01 / 2006
Día Mes Año

(firma: ilegible)
Firmado: Sofía Någård
Científica

Lugar: Mölndal, Suecia

Curiculum vitae

Nombre:

Mona Sofía Någård

Educación:

Gimnasio, Educación técnica con énfasis en química	1989
Maestría en Ciencias en Química en la Universidad de Gotemburgo	1994

Tesis de la Maestría en Ciencias:

Infección de grandes volúmenes de muestras usando isotacoforesis como una técnica de preconcentración en línea para electroforesis de zonas capilares.

Publicaciones:

Larsson M, Någård S: *On-column ITP focusing in CZE for the quantitation of low-molecular-weight basic solutes in biological samples.* Journal of Microcolumn Separations 6(2):107-113, 1994 Mar-Abr.

Witte DT, Någård S, Larsson M: *Improved sensitivity by on-line isotachophoretic preconcentration in the capillary zone electrophoretic determination of peptide-like solutes.* Journal of Chromatography A, 687 (1):155-166, 1994 Dic 9.

Gustafsson D, Nyström JE, Carlsson S, Bredberg U, Eriksson U, Gyzander E, Elg M, Antonsson T, Hoffmann KJ, Ungell AL, Sörensen H, Någård S, Abrahamsson A, Bylund R: *The direct thrombin inhibitor melagatran and its oral prodrug H 376/95: Intestinal absorption properties, biochemical and pharmacodymanic effects.* Thrombosis Research. 101(3):171-181, 2001 Feb 1.

27/2374

Owens PK, Wikstrom H, Någård S, Karlsson L: *Development and validation of a capillary electrophoresis method for ximelagatran assay and related substance determination in drug substance and tablets.* Journal of Pharmaceutical & Biomedical Analysis. 27(3-4):587-598, 2002 Ene 15.

Empleo:

Análisis de Sustancias, Investigación & Desarrollo Farmacéutico & Analítico, Astra Hässle AB (hoy AstraZeneca R&D Mölndal) 1993-

Experiencia Profesional:

1993-2005 Análisis de Sustancias representativas para melagatrán (-93 - -99) y ximelagatrán (-96 - -05)

El análisis de Sustancias representativo para un compuesto es responsable del desarrollo de procedimientos analíticos apropiados para la evaluación de las características de calidad y químicas del compuesto, tal como por ejemplo, identidad, pureza, ensayo y estabilidad, y el desempeño y documentación de los análisis. El análisis de sustancia representativo es el que lleva al equipo funcional de químicos sintéticos y químicos analíticos que expresan la especificación para el control de la calidad del compuesto. El papel exige conocimiento de química orgánica, cristalización, formas polimórficas y química analíticas.

1999 marzo a agosto, Gerente del equipo (reemplazo) dentro del Análisis de Sustancias.

Gerente de línea para 5 personas que incluyen a un aprendiz, dos ingenieros de laboratorio, un representante de Análisis de Sustancias y un especialista técnico.

2005- Representante de Análisis de Sustancias para un compuesto nuevo.

2733

Patentes:

Nuevas Formas Cristalinas; A2151

25 / 01 / 2006 (firma: ilegible)

Fecha Firma

Legalización del documento, documento escrito en español.

Traducido según mi mejor saber y entender, este día 6 de febrero de 2006, en la ciudad de Bogotá, D.C., por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTORA OFICIAL
2177/89 MINJUSTICIA
ANITA ESCOBAR DE TAMAYO

376
274

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Pais: Suecia

El presente documento oficial

2. ha sido firmado por Anne-Marie Bonde

3. **en su calidad de** Notario Público

4. y lleva el timbre/sello de Notario Público

en Esctocolmo

Lo cual certifica

5. en Estocolmo **6. el** 30.1.2006

7. por Björn Sandin

Notario Público

8. No 427

9. Timbre/Sello: **10. Firma:**

NOTARIUS PUBLICUS I STOCKHOLM
BJÖRN SANDIN

275377

Colombian Application Number: 00-048.529

Declaration

I, Sofia Någård, declare as follows:

1. I received my degree from Göteborg University. I have been employed by AstraZeneca (previously Astra Hässle AB) R&D Mölndal, S-431 83 Mölndal, Sweden, the applicant of the above-referenced application, since 1993. I enclose a CV, which includes a list of my publications.

2. As an inventor in the present case, I was directly involved in the studies that led to the present invention and which are disclosed in the instant specification. I was also directly involved with the experiments that yielded the data set forth in Exhibit A. I am therefore fully qualified to make this declaration.

3. The present application relates to substantially crystalline forms of the compound HO_2C-CH_2-(*R*)Cgl-(*S*)Aze-Pab-H (wherein Cgl represents cyclohexylglycine, Aze represents azetidine-2-carboxylate and Pab-H represents 4-aminomethylamidinobenzene). The compound is also known as melagatran. This compound and others have been described in WO 94/29336 as being thrombin inhibitors.

4. In view of all the prior art that I am aware of, and in particular the AstraZeneca documents cited by the examiner (namely WO 94/29336, WO 97/23499 and WO 98/16252), I believe that the present invention is patentable.

5. I do not believe that the prior art discloses melagatran in a substantially crystalline form. Rather, the closest prior art in my view, WO 94/29336, discloses a method of obtaining amorphous melagatran only.

6. I do not believe that it is possible for a person skilled in the art to predict, from molecular structure alone, what the crystallisation behaviour of a compound will be. Further, I do not believe that there is any motivation in the prior art for the person skilled in the art to apply the crystallisation techniques described in the present application to melagatran.

7. More particularly, and with reference to the pending claim set in this Colombian application, I do not believe that it could possibly be alleged that the prior disclosures amount to any kind of suggestion to make a substantially crystalline form of melagatran in the form of a hydrate (present Claim 1), in the form of an anhydrate (current Claim 3), or in the form of a mixed *iso*-propanolate/hydrate (current Claim 6).

8. The present invention further provides an unexpected advantage, which I believe could in no way have been predicted prior to the priority date of the present application.

NOTARIUS PUBLICUS I STOCKHOLM
ANNE-MARIE BONDE

9. I believe that the experimental data provided by means of Exhibit A clearly demonstrate an unexpected advantage in displaying enhanced chemical stability of a crystalline form of melagatran described in the present application over the amorphous form obtained by means of WO 94/29336.

10. Although, I might expect one or more crystalline forms of a substance to be more stable from a **physical** point of view than a corresponding amorphous form, this significant increase in **chemical** stability for the crystalline form comes as a surprise to me and could in no way have been predicted in the light of WO 94/29336 or any other prior art of which I am aware.

11. Clearly, this enhanced chemical stability, which is observed in a crystalline form of melagatran provides a significant and unexpected advantage from the point of view of ease of handling, manufacture of pharmaceutical formulations including the drug substance, and subsequent storage in solid form (e.g. in the form of oral tablets) prior to and during distribution, in pharmacies and indeed in the homes of patients.

12. In summary, I do not believe that there was any motivation, prior to the priority date, for the skilled person to arrive at any crystalline forms of melagatran as described in the present application. Further, there is absolutely no motivation whatsoever, prior to the priority date, to arrive at the specific crystalline forms of melagatran in the form of a hydrate, anhydrate or mixed *iso*-propanolate/hydrate as outlined by the present amended claim set. This is clearly indicative of inventiveness.

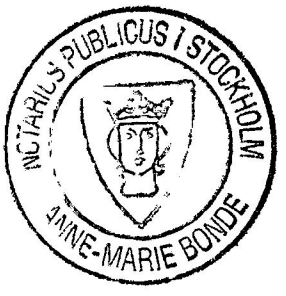
13. Further, there is absolutely no suggestion of the unexpected advantages (in chemical stability) of the invention, which advantages are outlined by the experimental data submitted. These data clearly demonstrate evidence of inventive step, and therefore patentability.

14. I hereby declare that all statements made herein of my own knowledge are true and all statements on information and belief are believed to be true.

Date: 25/01/2006
Day Month Year

Signed: Sofia Någård, MSc
Senior Scientist

Place: Mölndal, Sweden



2/4 379

Curriculum vitae

Name:
Mona Sofia Någård

Education:
Gymnasium, Technical education with chemistry speciality 1989
Master of Science in Chemistry at Göteborg University 1994

Master of science thesis:
Injection of large sample volumes using isotachophoresis as an on-line preconcentration technique for capillary zone electrophoresis.

Publications:
Larsson M, Någård S: *On-column ITP focusing in CZE for the quantitation of low-molecular-weight basic solutes in biological samples.* Journal of Microcolumn Separations 6(2):107-113, 1994 Mar-Apr.

Witte DT, Någård S, Larsson M: *Improved sensitivity by on-line isotachophoretic preconcentration in the capillary zone electrophoretic determination of peptide-like solutes*. Journal of Chromatography A, 687 (1):155-166, 1994 Dec 9.

Gustafsson D, Nyström JE, Carlsson S, Bredberg U, Eriksson U, Gyzander E, Elg M, Antonsson T, Hoffmann KJ, Ungell AL, Sörensen H, Någård S, Abrahamsson A, Bylund R: *The direct thrombin inhibitor melagatran and its oral prodrug H 376/95: Intestinal absorption properties, biochemical and pharmacodymanic effects.* Thrombosis Research. 101(3):171-181, 2001 Feb 1.

Owens PK, Wikstrom H, Någård S, Karlsson L: *Development and validation of a capillary electrophoresis method for ximelagatran assay and related substance determination in drug substance and tablets.* Journal of Pharmaceutical & Biomedical Analysis. 27(3-4):587-598, 2002 Jan 15.

Employment:
Substance Analysis, Pharmaceutical &Analytical R&D,
Astra Hässle AB (today AstraZeneca R&D Mölndal) 1993-

Working experience:
1993-2005 Substance Analysis representative for melagatran (-93 - -99) and ximelagatran (-96 - -05).
The Substance analysis representative for a compound is responsible for the development of appropriate analytical procedures for the assessment of the quality and chemical characteristics of the compound, such as eg identity, purity, assay and stability, and the performance and documentation of the analyses. The substance analysis representative is the one leading the cross-functional team of synthetic chemists and analytical chemists that set the specification for the control of the quality of the compound. The role demands knowledge of organic chemistry, crystallisation, polymorphic forms and analytical chemistry.

1999 March toAug, Team manager (replacement) within Substance Analysis

NOTARIUS PUBLICUS I STOCKHOLM
ANNE-MARIE BONDE

Line manager for 5 people comprising of a trainee, two laboratory engineers, a Substance Analysis representative and a technique specialist.

2005- Substance Analysis representative for a new compound

Patents:
New Crystalline Forms; A2151

25/01/2006 Sofia Nagård

Date Signature

La infrascrita Anne-Marie Bonde, Notario Público de Estocolmo, Suecia, con carácter oficial

SOFIA NÅGÅRD

ha otorgado y firmado ante mí en Estocolmo 200 - 30.1.2006

NOTARIUS PUBLICUS I STOCKHOLM ANNE-MARIE BONDE