

Señor Director

NUEVAS CREACIONES

Superintendencia de Industria y Comercio

E. S. D.

Referencia: Expediente No. 15-197.748

Asunto: Solicitud de patente para: DIACILHIDRAZINA CRISTALINA,
COMPOSICIONES FARMACÉUTICAS Y KITS DE LA MISMA

Solicitante: INTREXON CORPORATION

Fecha de solicitud: 24 de agosto de 2015

RESPUESTA A PRIMER EXAMEN DE PATENTABILIDAD

ANDRÉS RINCÓN USCÁTEGUI, identificado como aparece al pie de mi firma, en mi condición de apoderado de la sociedad solicitante de la patente de la referencia, doy respuesta al Oficio 3303, notificado por fijación en lista el 08 de marzo de 2017.

I. MODIFICACIONES AL CAPÍTULO REIVINDICATORIO

En respuesta al examen de patentabilidad apporto un Capítulo Reivindicatorio modificado conformado por 13 reivindicaciones en las que se han incluido las siguientes modificaciones:

1. Se eliminó la Reivindicación 5;
2. En la Reivindicación 1, se añadieron más picos distintivos del patrón de difracción de rayos X de polvo a las formas VI y IX. Esta modificación está debidamente soportada en el Capítulo Descriptivo, párrafo [0054], página 6 y párrafo [0057], página 7, respectivamente;

“EXCELENCIA LEGAL EN UN MUNDO SIN FRONTERAS”

BOGOTÁ - Edificio Siski - Carrera 4 No. 72 - 35 - Código Postal 110221 - Tel. (57-1) 347 3611 - Fax (57-1) 211 8650 - Fax in U.S.A: (1-305) 675 7743 - COLOMBIA
MEDELLÍN - San Fernando Plaza - Carrera 42 A No. 1 - 25 - Torre 4 - Oficina 303 - Código Postal 050021 - Tel: (57-4) 520 4760 - COLOMBIA
BARRANQUILLA - Calle 64 No. 50-22 - Código Postal 080002 - Barrio El Prado - Tel. (57-5) 3 51 64 40 - COLOMBIA

3. Las reivindicaciones, así como sus relaciones de dependencia, fueron reenumeradas de acuerdo con las modificaciones previamente enunciadas.

Estas modificaciones se ajustan al Artículo 34 de la Decisión 486 de 2000, ya que no constituyen una ampliación de la protección que correspondería a la divulgación contenida en la solicitud inicial.

II. EN CUANTO A LA CLARIDAD

1. El Examinador señala que el Capítulo Reivindicatorio no es claro ni conciso debido a la siguientes razones:

- la Reivindicación 1 caracteriza las formas VI y IX por un número muy bajo de picos de difracción de rayos X, los cuales son insuficientes para caracterizar de manera inequívoca el sólido cristalino de acuerdo con Bhattacharya;¹
- las Reivindicaciones 6 y 7 caracterizan las formas cristalinas por el tamaño de partícula, lo cual corresponde a un resultado a alcanzar.

2. En primer lugar, llamo la atención del Examinador sobre el Capítulo Reivindicatorio modificado adjunto, en el cual se caracterizan ahora las formas VI y IX por un número mayor a 10 picos de difracción de rayos X. Particularmente, se caracteriza la forma VI por 40 picos y la forma IX por 20 picos; teniendo un debido soporte en el Capítulo Descriptivo (ver párrafo [0054], página 6 y párrafo [0057], página 7). Por consiguiente, considero superada cualquier objeción relacionada con el número de picos asociados a las formas cristalinas reclamadas.

3. En segundo lugar, respecto a las Reivindicaciones 6 y 7, quisiera aclarar que el tamaño de partícula de los cristales de un compuesto no corresponde a resultados a alcanzar sino a una característica estructural de los cristales. Una forma cristalina puede estar definida por sus características estructurales microscópicas (como los picos de difracción de rayos X) así como por sus características estructurales macroscópicas (como el tamaño de partícula). Por lo tanto, este tipo de expresiones no solo deben ser aceptadas sino que además permiten definir con mayor claridad la invención en cuestión.

¹ Harry G. Brittain, ed., *Polymorphism of Pharmaceutical Solids*, 2. ed, Drugs and the Pharmaceutical Sciences 192 (New York: Informa Healthcare, 2009). Páginas 334 y 335

4. Con base en todo lo anterior, considero que el Capítulo Reivindicatorio modificado adjunto cumple con todos los requisitos de claridad y concisión, ajustándose así a los lineamientos del Artículo 30 de la Decisión 486, ya que cualquier persona medianamente versada en la materia está en completa capacidad de reproducir la invención sin ambigüedad alguna.

III. EN CUANTO AL TÍTULO

1. El Examinador señala que el título hace referencia a usos, por lo cual sugiere hacer la corrección necesaria.

2. En respuesta a la amable sugerencia del Examinador, se ha modificado el título de tal manera que ahora lee:

“DIACILHIDRAZINA CRISTALINA, COMPOSICIONES FARMACÉUTICAS Y KITS DE LA MISMA”

3. Esta modificación se ajustan al Artículo 34 de la Decisión 486 de 2000, ya que no constituyen una ampliación de la protección que correspondería a la divulgación contenida en la solicitud inicial.

IV. ESTADO DE LA TÉCNICA

El Examinador determinó dos documentos como relevantes para realizar el análisis de patentabilidad de la presente patente de invención:

1. D1, referido al documento US2009/163592 titulado *“Chiral diacylhydrazine ligands for modulating the expression of exogenous genes via an ecdysone receptor complex”* que se relaciona con el compuesto *N*-(1-ter-butil-buti)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico (Reivindicación 17), el cual puede encontrarse en diversas formas cristalinas (párrafo [0047]). D1 también se revela un proceso para obtener cristales del compuesto de interés mediante recristalización, que pueden ser posteriormente micronizados (párrafos [0113] y [0721], y Figura 4).

2. D2, referido al libro de referencia H.G. Brittain, Segunda Edición, Informa Healthcare, NY, 2009 titulado “*Polymorphism of Pharmaceutical Solids*” que se relaciona con enseñanzas generales relacionadas con formas cristalinas.²

V. EN CUANTO A LA NOVEDAD

1. El Examinador señala que la **Reivindicación 5** no cumple con el requisito de novedad basándose en que el compuesto de dicha reivindicación había sido divulgado previamente en D1 (Reivindicación 17, compuesto 5, Reivindicaciones 18 y 19; párrafos [0047], [0048], [0235] a [0237] y [0313]). En consecuencia, indica que la Reivindicación 5 no es nueva, según lo divulgado en D1.

2. Llamo la atención del Examinador sobre el Capítulo Reivindicatorio modificado adjunto, del cual se ha eliminado la antigua Reivindicación 5. Por lo tanto, considero superada la objeción relacionada con la novedad de dicha reivindicación.

VI. EN CUANTO AL NIVEL INVENTIVO

1. El Examinador señala que las Reivindicaciones 1 a 4 y 6 a 14 no cumplen con el requisito de nivel inventivo basándose en la siguiente línea argumentativa:

D1 se considera el estado de la técnica cercano a la invención definida en la **Reivindicación 1** porque enseña de manera específica el compuesto *N*-(1-terbutil-buti)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico (Reivindicación 17, compuesto 5, Reivindicaciones 18 y 19; Párrafos [0047], [0048], [0235] a [0237], [0313]), la anterioridad indica que este compuesto puede encontrarse en diversas formas cristalinas (Párrafo [0047]) y da a conocer las condiciones específicas del proceso de recristalización que permite obtener las diversas formas cristalinas o polimórficas para este compuesto (Párrafos [0113] y [0721]). El producto obtenido puede ser micronizado lo que permite uniformizar el tamaño de partícula del mismo (Figura 4). De igual forma, D1 enseña que el compuesto se puede formular mediante una composición farmacéutica que comprende el activo o sus cristales junto con uno o más excipientes tales como un vehículo farmacéuticamente aceptable, y donde dicha formulación puede estar en forma de solución, suspensión, tabletas, cápsulas, ungüentos, elixires, o composiciones inyectables

² Ibid.

(Reivindicaciones 20 y 21, Párrafo [0528]). Entre los excipientes útiles la anterioridad menciona el miglyol 812, fosfolipon 90G y succinato de tocoferil polietilenglicol 1000 (Párrafos [0532], [0755], [0533]).

La diferencia entre la invención, definida en la Reivindicación 1 y D1 consiste en que la anterioridad no divulga el espectro de difracción de rayos X y demás propiedades físicas de los cristales reivindicados en la presente solicitud.

El efecto técnico que logran las configuraciones cristalinas reivindicadas consiste en una adecuada estabilidad de las formas II, III, IV y V (Ejemplo 13) en una dispersión en solventes a diversas temperaturas. Sin embargo, de acuerdo con los resultados la forma V es la más estable a partir de una mezcla de las formas II, III, IV y V a temperaturas de 25°C, 45°C y 55°C (ver Tabla 10). Por otra parte, de acuerdo con los resultados del Ejemplo 15, la forma IV del compuesto es inestable a temperaturas entre 60-65°C y se convierte a una mezcla de las formas II y III después de 10 días. La solicitud carece de estudios comparativos e información que permita establecer la ventaja de estos polimorfos frente a la materia divulgada en el arte previo que ya enseña de manera explícita el agente *N*-(1-ter-butil-buti)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico (Reivindicación 17) como favorito y para el cual se había evidenciado su actividad como inductor de la expresión del gen basado en el receptor de ecdisona, por lo que los resultados presentados por la solicitante en los Ejemplos 16 y 17 no son sorprendentes.

El problema técnico objetivo de esta solicitud consiste en “¿Cómo obtener polimorfos alternativos del agente *N*-(1-ter-butil-buti)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico (Reivindicación 17) previamente divulgado en D1?”

La solución al problema técnico planteado por la invención reclamada se considera obvia porque D2 divulga que muchos sólidos con actividad farmacológica pueden exhibir polimorfismo, la existencia de diferentes estructuras cristalinas de los diversos polimorfos de una sustancia a menudo produce que estos sólidos exhiban una variedad de propiedades fisicoquímicas diferentes, muchas de las cuales se encuentran listadas en la Tabla 2 (Página 3) y entre las que están la higroscopicidad, entalpía, entropía, actividad termodinámica y solubilidad. Debido a las diferencias en las dimensiones, forma, simetría, capacidad (número de moléculas), y volúmenes de vacíos de sus celdas unitarias, los

diferentes polimorfos de una sustancia dada tienen diferentes propiedades físicas que surgen de las diferencias en el empaquetamiento molecular.

La solicitante no presenta estudios relacionados con cada una de las formas reclamadas y las composiciones que los contienen ni tampoco de los kits reclamados. Los resultados presentados por la solicitante corresponden a la caracterización de las propiedades fisicoquímicas de los sólidos reclamados mediante el espectro de difracción de rayos X y el estudio de estabilidad (Tablas 9, 10 y 11), lo cual no se considera un efecto técnico inesperado o sorprendente ya que es una identificación física básica de un sólido cristalino. En cuanto a la ventaja del interruptor génico del compuesto 1 (Ejemplo 17), estos ensayos están relacionados directamente con el agente previamente divulgado en D1 y no con las formas polimórficas reivindicadas.

En cuanto a los resultados de estabilidad presentados por la solicitante junto con este radicado, su Despacho encuentra que estas diferencias en las propiedades físicas entre las formas cristalinas de un sistema polimórfico y la forma amorfa son extremadamente interesantes para los científicos debido a que sus manifestaciones pueden algunas veces llevar a diferencias observables que poseen implicaciones para el procesamiento, formulación, y en la disponibilidad del agente activo. Sin embargo, aclara que los resultados presentados por la solicitante no pueden considerarse sorprendentes o inesperados toda vez que D2 indica que luego de que las fases espaciales de una sustancia han sido determinadas, y se ha establecido el alcance de las posibles formas polimórficas y solvatomórficas, se convierte en algo crítico el determinar los límites de estabilidad de las diferentes formas y la manera como estas pueden ser interconvertidas. Por lo menos, se debe determinar cuál forma cristalina es la más estable, y determinar la mejor forma a ser elegida para continuar con los desarrollos (Página 2). En consecuencia, estos estudios son ampliamente conocidos y hacen parte del desarrollo farmacotécnico tradicional en preformulación. De igual forma, D2 presenta un listado de las propiedades fisicoquímicas que usualmente difieren entre formas cristalinas (Tabla 2), y explica que cada forma polimórfica de una sustancia tendría un diagrama de energía similar al de la Figura 1 (Página 7):

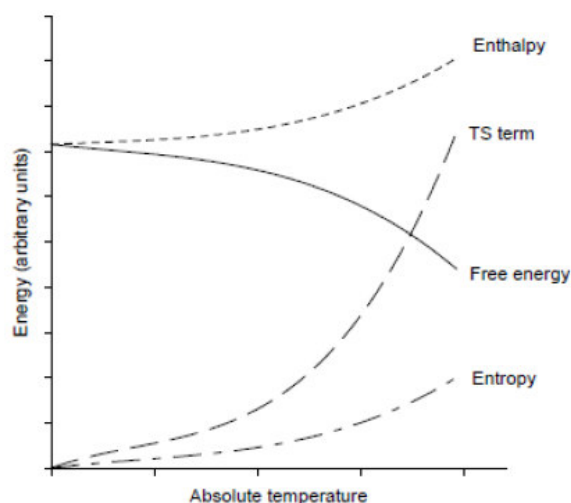


FIGURE 1 Temperature dependence of various thermodynamic functions.

Debido a que cada polimorfo tiene su propio enmallado cristalino, se puede anticipar que los valores de entalpía, entropía y energía libre dados a una temperatura pueden variar entre diversos polimorfos, y obviamente entre la forma cristalina y la forma amorfa. En discusión de la estabilidad relativa de los polimorfos y la fuerza impulsora para las trasformaciones polimórficas a temperatura y presión constantes, la diferencia en energía libre entre las formas es un factor decisivo, ya que las formas que presentan la menor energía libre son más estables (Figura 1, Página 7). En consecuencia, los resultados presentados por la solicitante en el estudio de estabilidad no son sorprendentes o inesperados, ya que es conocido que las formas cristalinas de una sustancia poseen menor energía libre que la forma amorfa, y en consecuencia son obviamente termodinámicamente más estables que esta última (Página 44 a 45, Desolvated Solvates).

Las diferencias en las propiedades físicas entre las formas cristalinas de un sistema polimórfico son de gran interés para los científicos farmacéuticos debido a que sus manifestaciones pueden algunas veces llevar a diferencias observables que poseen implicaciones para el procesamiento, formulación, y en la disponibilidad. Luego de que las fases espaciales de una sustancia han sido determinadas, y se ha establecido el alcance de las posibles formas polimórficas, se convierte en algo crítico el determinar los límites de estabilidad de las diferentes formas y la manera como estas pueden ser interconvertidas. Por lo menos, se debe determinar cuál forma cristalina es la más estable, y determinar la mejor forma a ser elegida para continuar con los desarrollos (Página 2). En consecuencia, estos estudios son ampliamente conocidos y hacen parte de la rutina del desarrollo farmacotécnico.

Adicionalmente, D2 enseña diferentes métodos útiles en la determinación de la presencia de diferentes estructuras cristalinas de un polimorfo, entre los que se encuentran la

termogravimetría para estudiar la cinética de transición de fase de los polimorfos, el XRD que permite obtener información fundamental sobre la estructura cristalina de la sustancia, XRD en monocristal que se considera la descripción más fundamental de un polimorfo y ayuda a explicar sus propiedades físicas, la espectroscopia de absorción de infrarrojo, la espectroscopia NIR, la espectroscopia de terahertz y la espectroscopia Raman (Página 318). De igual forma, D2 divulga las técnicas de obtención de polimorfos comúnmente utilizadas por la persona versada en la materia e indica que la cristalización es una de las más comunes para la purificación de un producto farmacéutico de los productos de reacción y para la consolidación del producto en una forma cristalina específica con propiedades físicas bien caracterizadas y consistentes (Páginas 88 a 93).

En consecuencia, es evidente que se pretende protección para nuevos estados cristalinos del agente N-(1-ter-butil-buti)-N'-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (R)-3,5-dimetil-benzoico, que, aunque no se encontraban divulgados los espectros de difracción de rayos X, se derivan de la naturaleza del estado sólido de estas moléculas, propiedad alrededor de la cual se tiene amplio conocimiento, de acuerdo con la materia técnica que D1 y D2 ya enseñaban al momento de la presentación de la solicitud y que se discutió anteriormente.

Por lo anterior, una persona medianamente versada en la materia, encontraría la sugerencia o motivación suficiente aplicando los métodos y conocimientos que se tiene sobre polimorfismo revelados en D2, al compuesto N-(1-ter-butil-buti)-N'-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (R)-3,5-dimetil-benzoico, con la finalidad de determinar las posibles formas cristalinas del mismo como se había sugerido en D1, lo cual también hace parte de las tareas básicas de preformulación.

Puesto que las Reivindicaciones 2 a 4 y 6 a 14 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas. Las Reivindicaciones 2 a 4 y 6 a 14 cumplen el requisito de novedad (Artículo 16), pero no cumplen con el requisito de nivel inventivo (Artículo 18).

2. En relación con el nivel inventivo, respetuosamente difiero de la opinión del Examinador. Con el fin de demostrar el nivel inventivo de la presente invención, y basándome en la Guía para Examen de Solicitudes de Patente de Invención y Modelo de Utilidad (Guía de la SIC) sobre el método problema-solución, a continuación expongo

cómo el arte previo citado por el Examinador no llevaría a la persona medianamente versada en la materia a la invención reclamada.

Identificar el estado de la técnica cercano a la invención reivindicada

D1 puede ser considerado como el arte previo más cercano en tanto divulga el compuesto de interés relacionado con la presente invención y menciona de manera general sus correspondientes formas cristalinas. Particularmente, D1 revela un proceso para obtener una forma cristalina del enantiómero (R) del compuesto, que comprende un proceso rápido de recristalización/precipitación a partir de metanol/agua, tolueno/heptano o metanol, en donde los sólidos obtenidos en las diferentes condiciones tienen el mismo patrón de difracción de rayos X (no se muestran los resultados), y tienen esencialmente el mismo punto de fusión (Párrafo [0721], D1).

Determinar la diferencia entre la invención y estado de la técnica cercano

D1 difiere de la presente invención principalmente en que D1 no revela cómo obtener específicamente las formas cristalinas reclamadas. Es decir, D1 sugiere que las formas cristalinas reclamadas podrían existir pero no enseña cómo obtenerlas y sus propiedades fisicoquímicas tampoco se sugieren o describen. Además, D1 no menciona el patrón de difracción de rayos X y, por lo tanto, no revela los picos característicos que definen las formas cristalinas.

Definir el efecto técnico causado y atribuible al elemento diferencial

Respecto al efecto técnico atribuible a dichas diferencias, llamo la atención del Examinador sobre el hecho de que las formas cristalinas reclamadas muestran estabilidad en mezclas de agua/metanol y n-heptano/etanol a diferentes temperaturas (Ejemplos 13 y 14). Las formas reclamadas también demuestran estabilidad entre 60 y 65 °C (Ejemplo 15). Estas propiedades son ventajosas en cuanto a propósitos comerciales, pues permiten la producción de composiciones usando un reducido número de excipientes para mejorar su estabilidad, así como también permiten un incremento en el tiempo de anaquel y una reducción en los costos de producción, procesamiento y almacenamiento.

Deducir el problema técnico objetivo

Según la Guía de la SIC, el problema técnico debería plantearse como: “¿cómo modificar o adaptar el estado de la técnica cercano para obtener el efecto técnico que la invención proporciona?”³ En el presente caso, el Examinador está obviando el efecto técnico logrado en realidad por la invención. El problema técnico objetivo resuelto por la invención debe ser correctamente planteado como: “Cómo obtener formas polimórficas estables del compuesto *N-(1-ter-butil-buti)-N’-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (R)-3,5-dimetil-benzoico, previamente divulgado en D1?*”

Evaluar si la invención reivindicada, partiendo del estado de la técnica cercano y del problema técnico objetivo, habría sido obvia para la persona medianamente versada en la materia

En esta etapa de la evaluación de nivel inventivo, la Guía de la SIC indica que para evaluar si la invención resulta obvia y que para ellos la Examinadora debe buscar si el estado de la técnica en su conjunto existe alguna indicación (no sólo que podría indicar, sino que indicaría) a la persona medianamente versada en la materia, enfrentada al problema técnico, cómo modificar o adaptar el estado de la técnica más cercano para resolver el problema, de la forma reivindicada, sin realizar una actividad inventiva⁴.

Al analizar si una persona medianamente versada en la materia es capaz de resolver el problema técnico, de una manera obvia, basándose en lo revelado en el arte previo, considero que tal declaración es desacertada. Lo anterior a la luz de las enseñanzas específicas de D1 y D2.

D2 se refiere a enseñanzas generales de formas cristalinas de uso farmacéutico. A pesar de que, hipotéticamente, un compuesto puede estar presente potencialmente en diferentes formas farmacéuticas, es impredecible la existencia de las disposiciones cristalinas particulares. D2 no enseña de manera obvia cómo obtener específicamente las formas cristalinas reclamadas, ni sus correspondientes propiedades fisicoquímicas del compuesto de la presente invención. De hecho, D2 se refiere únicamente a conocimiento

³ Superintendencia de Industria y Comercio, *Guía para Examen de Solicitudes de Patente de Invención y Modelo de Utilidad* (Bogotá, Colombia, 2014).

⁴ Ibid

general en el campo técnico. A pesar de que enseña que los disolventes y la temperatura afectan la formación de los cristales, no enseña que con las condiciones particulares de la presente invención es posible obtener las formas cristalinas reclamadas. Además, vale la pena resaltar que aunque un compuesto puede estar presente en diferentes formas polimórficas, las disposiciones cristalinas y el empaquetamiento son imposibles de predecir. En otras palabras, D2 enseña que asegurar un proceso de preparación de cristales reproducible es importante para un compuesto farmacéutico dados los problemas que pueden surgir a partir de la variabilidad de los procesos de recristalización.

De acuerdo con Braga *et al.*, a pesar de que se ha estudiado a profundidad el tema de las formas cristalinas, el fenómeno de polimorfismo en sí representa un reto científico.⁵ Es difícil predecir si una molécula específica formará cristales y si presentará una o más de una forma cristalina.⁶ Por lo tanto, la persona medianamente versada en la materia no tendría razonablemente la expectativa de obtener las formas cristalinas como se reclaman al momento de radicar la solicitud, a la luz de D1 y D2.

Fue necesario producir las formas cristalinas reclamadas de tal manera que permitieran la formulación del compuesto de interés cumpliendo con los requerimientos y especificaciones farmacéuticas. Más aún, el proceso por el cual se producen los cristales debe ser ajustable a gran escala. Se busca que el producto sea fácilmente procesable así como el beneficio en el aspecto comercial de que el producto sea estable por largos periodos sin la necesidad de condiciones especiales de almacenamiento; lo cual no se enseña en D1 ni D2, pero es resuelto por la presente invención.

El fenómeno de polimorfismo denota la existencia de más de una forma cristalina de una sustancia particular. Esta habilidad de una sustancia de recristalizar en más de una forma cristalina tiene un amplio efecto en su tiempo de anaquel, solubilidad, propiedades de formulación y propiedades de procesamiento de un fármaco. La aparición de una forma cristalina desconocida durante la manufactura puede, entonces, tener un impacto significativo. Entender y controlar el polimorfismo tiene, por lo tanto, la ventaja de llevar nuevos medicamentos al comercio. Principalmente, predecir cualquiera de los polimorfos de un fármaco puede utilizarse para disminuir la contaminación por parte de otra forma

⁵ Dario Braga et al., "Crystal Polymorphism and Multiple Crystal Forms", en *Molecular Networks*, ed. Mir Wais Hosseini, vol. 132 (Berlin, Heidelberg: Springer Berlin Heidelberg, 2009), 25–50, doi:10.1007/430_2008_7. p. 27

⁶ Ibid. p. 31

polimórfica durante la manufactura del medicamento o su almacenamiento. Es más, entender cuáles formas cristalinas de un fármaco son posibles de obtener en algunos casos permite a los investigadores maximizar las propiedades deseadas de un compuesto, como se ha mencionado anteriormente, su solubilidad, propiedades de formulación, propiedades de procesamiento y tiempo de anaquel. Conocer estos factores en etapas tempranas del desarrollo de un nuevo medicamento puede conllevar a la obtención de un medicamento más efectivo, más estable o más económico de fabricar. Las formas cristalinas proveen propiedades físicas susceptibles de explotación para obtener nuevas propiedades farmacológicas, que pueden utilizarse en el desarrollo del medicamento que contenga las formas farmacéuticas reclamadas. Considerando todo lo anterior, es imposible para la persona medianamente versada en la materia predecir que una nueva forma cristalina puede existir, particularmente a la luz de las enseñanzas de D1 y D2.

Finalmente, quisiera señalar que los polimorfos reivindicados en la presente solicitud son novedosos, y que los cristales no son una propiedad inherente del compuesto, incluso si se mencionan de manera general en el arte previo. Sin duda alguna, las técnicas analíticas no pueden predecir si existen polimorfos o, en caso de existir, si son separables, estables y menos aún cuáles serán sus características físico-químicas o biológicas puntualmente. Los argumentos que sostienen que los materiales polimórficos son meramente el resultado de tareas superficiales y rutinarias son inaceptables, ya que estas afirmaciones indican en otras palabras que las formas polimórficas no son materia patentable como tal. Por el contrario, los polimorfos de la presente invención son productos novedosos e inventivos, ya que la intervención humana y la creatividad fueron necesarias para producirlos. Además, el arte previo no hace obvias las etapas y condiciones necesarias para obtenerlos o sus beneficios específicos.

Teniendo en cuenta lo anteriormente expuesto, el Manual para el Examen de Solicitudes de Patente (MESPI) establece que las invenciones tienen nivel inventivo cuando tienen una estructura inesperada (no derivable del arte previo) o presentan un efecto técnico inesperado o mejorado impredecible del arte previo.⁷ En el presente caso, es evidente que las características técnicas esenciales de la invención no se podían prever a partir de las enseñanzas del arte previo citado por el Examinador.

⁷ Oficina Internacional de la OMPI, *Manual para el Examen de Solicitudes de Patente de Invención en las Oficinas de Propiedad Industrial de los Países de la Comunidad Andina* (Quito, Ecuador, 2004).

3. Quisiera añadir que el Examinador está aparentemente utilizando un tercer documento en su examen de patentabilidad pues la referencia “*páginas 44 a 45 Desolvated Solvates*” no corresponde a ningún capítulo de D2.

De hecho, el Examinador incluye dicha referencia como cita adicional porque la evaluación de nivel inventivo solo puede estar basada en la combinación particular de dos documentos al tiempo. Por lo cual, el Examinador incluye dicho documento como cita adicional en un esfuerzo para incluir más arte previo del permitido. En este aspecto, deseo aclarar que la Guía de la SIC establece claramente que uno de los indicios de la existencia de nivel inventivo es la necesidad de más de dos documentos para examinar el arte previo.⁸ Por consiguiente, la postura del Examinador sobre la inclusión de la cita adicional es incorrecta desde la perspectiva del conocimiento general de la persona medianamente versada en la materia, ya que incluye pasajes específicos de arte previo diferente a los dos documentos requeridos para hacer la evaluación del nivel inventivo.

Quisiera hacer énfasis en que para la persona medianamente versada en la materia debe ser evidente derivable la invención basándose únicamente en el arte previo citado por el Examinador (D1 y D2, en el presente caso), para hacer de la invención carente de nivel inventivo. De hecho, el MESPI claramente indica que solo se deben usar dos documentos para estudiar el nivel inventivo.⁹

4. Acorde al Artículo 18 de la Decisión 486, una invención tiene nivel inventivo cuando no hubiese resultado obvia ni se hubiera derivado de manera evidente del estado de la técnica para la persona medianamente versada en la materia. El análisis de nivel inventivo no puede entonces basarse en si la invención era -posible de desarrollarse, pues esto haría de todas las invenciones obvias y, tal y como se evidencia, existe. En otras palabras, lo que debería hacer parte del análisis no es lo que la persona medianamente versada en la materia podría haber hecho en la fecha de prioridad sino lo que habría hecho teniendo en cuenta de sus conocimientos en el área y lo enseñado en el arte previo. Además, no puede basarse el análisis tampoco en si la invención es -mejor que el arte previo o si presenta efectos mejorados, o si existe un documento que invite al científico a llevar a cabo investigación, sino únicamente en si la invención resulta obvia o

⁸ Guía de la Sic. *Op. Cit.* Sección 2.13.5.3.1

⁹ Oficina Internacional de la OMPI, *Manual de la Comunidad Andina*.

evidentemente derivable del arte previo, lo cual, como se explicó anteriormente, no aplica para el presente caso.

5. Por las razones expuestas anteriormente, considero que las nuevas Reivindicaciones 2 a 13 también son inventivas, pues al ser dependientes de la Reivindicación 1, presentan todas y cada una de sus características técnicas y, por lo tanto, presentan nivel inventivo.

6. En conclusión, según la aproximación problema-solución para el análisis del nivel inventivo de la presente invención, éste se reconoce claramente, ya que cualquier persona medianamente versada en la materia reconocería que la presente invención es inventiva, sumado al hecho que el arte previo en efecto no es suficiente para brindar indicaciones acerca de la posibilidad de desarrollar la invención de manera específica, por lo que la presente invención reviste de nivel inventivo.

7. Por último, deseo añadir que la presente invención ha sido concedida en Estados Unidos (Patente No. US9604913). Ahora bien, a pesar que las concesiones en otras jurisdicciones no tienen un carácter vinculante que obligue a la Superintendencia de Industria y Comercio (SIC) a conceder una patente en Colombia, es importante resaltar que los estándares de patentabilidad son semejantes a nivel mundial y las concesiones en otros países sí da luces e indicios de la patentabilidad de una invención, como sucede en el presente caso.

VII. PETICIÓN

1. Puesto que se ha dado respuesta a todas las objeciones formuladas por la Dirección de Nuevas Creaciones, se solicita proceder al otorgamiento de la solicitud de patente.

2. De manera subsidiaria, y en caso de que la Dirección de Nuevas Creaciones tenga observaciones adicionales a la patentabilidad del invento, solicito emitir un nuevo requerimiento en el marco del Artículo 45 de la Decisión 486 de 2000.

VIII. ANEXOS

1. Capítulo Reivindicatorio modificado conformado por 13 reivindicaciones;
2. Patente No. US9604913 concedida en Estados Unidos;
3. Dario Braga et al., “Crystal Polymorphism and Multiple Crystal Forms”, en *Molecular Networks*, ed. Mir Wais Hosseini, vol. 132 (Berlin, Heidelberg: Springer Berlin Heidelberg, 2009), 25–50, doi:10.1007/430_2008_7.

Señor Director,

ANDRÉS RINCÓN USCÁTEGUI

C.C. 79.780.910 de Bogotá

T.P. 114.908

CAPÍTULO REIVINDICATORIO

1. Las formas IV, V, VI, VII, VIII o IX o una mezcla de los mismos de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida de ácido (*R*)-3,5-dimetil-benzoico cristalina que, donde:

la forma IV se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 6.83, 10.31, 11.30, 12.18, 12.98, 13.69, 15.11, 16.23, 17.60, 17.99, 20.70, 21.15, 21.68, 22.71, 23.79, y 24.86 grados 2θ ;

la forma V se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 9.38, 12.22, 13.18, 14.98, 17.32, 18.40, 22.41, 23.40, 23.55, 24.63, 24.79, 25.61, 28.02, y 31.77 grados 2θ ;

la forma VI se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 6.09, 6.82, 8.57, 9.38, 11.26, 12.23, 13.25, 14.27, 15.05, 15.54, 15.95, 17.48, 18.41, 18.79, 19.54, 19.76, 20.79, 21.48, 22.02, 22.41, 23.42, 24.07, 24.34, 24.64, 24.83, 25.34, 25.67, 26.74, 26.87, 27.24, 27.99, 28.56, 28.93, 29.47, 30.04, 30.98, 31.75, 32.34, 32.96, y 33.84 grados 2θ ;

la forma VII se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 8.18, 9.71, 13.30, 16.22, 17.73, 20.98, 21.20, 22.76, 24.68, 26.72, y 29.39 grados 2θ ;

la forma VIII se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 10.05, 10.77, 14.06, 16.76, 18.11, 18.32, 18.43, 20.89, 21.71, 21.87, 24.07, 24.90, y 28.71 grados 2θ ; y

la forma IX se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 7.06, 9.93, 12.22, 14.13, 15.74, 17.28, 18.71, 19.96, 21.18, 22.39, 23.51, 24.54, 25.58, 27.52, 28.48, 29.33, 30.18, 31.01, 31.82, y 32.73 grados 2θ .

2. El cristal de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida de ácido (*R*)-3,5-dimetil-benzoico que comprende la mezcla de la forma III y la forma IV, donde:

la forma III se caracteriza por tener un patrón de difracción de rayos X de polvo con picos de 8.14, 8.52, 9.62, 11.02, 11.90, 12.16, 14.02, 14.62, 17.00, 17.88, 18.56, 19.02, 19.24, 20.51, 20.93, 22.19, 22.73, 23.22, 24.31, 24.53, 25.91, 26.22, 27.36, 27.73, 28.70, 30.84, 31.52, 32.30, 33.19, y 34.39 grados 2θ ; y

la forma IV se caracteriza por tener un patrón de difracción de rayos X de polvo con picos en 6.83, 10.31, 11.30, 12.18, 12.98, 13.69, 15.11, 16.23, 17.60, 17.99, 20.70, 21.15, 21.68, 22.71, 23.79, y 24.86 grados 2θ .

3. La *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de la Reivindicación 1, donde dicha *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida de ácido (*R*)-3,5-dimetil-benzoico es sustancialmente la forma IV pura.

4. La *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de la Reivindicación 3, donde dicha *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico es la forma IV pura.

5. La forma o las formas de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de acuerdo con cualquiera de las Reivindicaciones 1-4 que tienen una distribución de tamaño de partícula promedio de alrededor de 10 μm o menos.

6. La forma o las formas de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de acuerdo con la Reivindicación 5 que tienen una distribución de tamaño de partícula promedio de alrededor de 1 μm o menos.

7. Una composición que comprende la forma o las formas de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de acuerdo con cualquiera de las Reivindicaciones 1-6 y uno o más excipientes.

8. La composición de la Reivindicación 7, donde dichos uno o más excipientes comprenden acetona o dimetil-sulfóxido.

9. La composición de la Reivindicación 7, donde dichos uno o más excipientes comprenden uno o más excipientes farmacéuticamente aceptables.

10. La composición de la Reivindicación 9, donde dichos uno o más excipientes farmacéuticamente aceptables comprenden Miglyol 812, fosfolipon 90G, o succinato de tocoferil polietilenglicol 1000, o una mezcla de los mismos.

11. La composición de la Reivindicación 10, donde dicho uno o más excipientes farmacéuticamente aceptables consisten esencialmente de Miglyol 812, fosfolipon 90G, y succinato de tocoferil polietilenglicol 1000.

12. Un método para hacer una composición, en donde el método comprende mezclar la forma o las formas de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico de acuerdo con cualquiera de las Reivindicaciones 1-6 y un excipiente.

13. Un kit que comprende la composición de la Reivindicación 7, en donde la composición comprende de 0.01 a 99% en peso de dicha forma o formas de *N*-(1-ter-butil-butil)-*N'*-(2-etil-3-metoxi-benzoil)-hidrazida del ácido (*R*)-3,5-dimetil-benzoico.



US009604913B2

(12) **United States Patent**
Hormann et al.

(10) **Patent No.:** **US 9,604,913 B2**
(45) **Date of Patent:** **Mar. 28, 2017**

(54) **CRYSTALLINE DIACYLHYDRAZINE AND THE USE THEREOF**

(71) Applicant: **Intrexon Corporation**, Blacksburg, VA (US)

(72) Inventors: **Robert E. Hormann**, Elkins Park, PA (US); **Inna Shulman**, Langhorne, PA (US); **Eva Rödel**, Basel (CH); **Rolf Hilfiker**, Allschwil (CH); **Susan M. De Paul**, Zürich (CH)

(73) Assignee: **Intrexon Corporation**, Blacksburg, VA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/519,408**

(22) Filed: **Oct. 21, 2014**

(65) **Prior Publication Data**

US 2015/0045441 A1 Feb. 12, 2015

Related U.S. Application Data

(62) Division of application No. 13/606,444, filed on Sep. 7, 2012, now Pat. No. 8,946,294.

(60) Provisional application No. 61/532,368, filed on Sep. 8, 2011.

(51) **Int. Cl.**

A01N 37/18 (2006.01)

A61K 31/16 (2006.01)

C07C 243/38 (2006.01)

A01N 37/28 (2006.01)

A01N 37/40 (2006.01)

A61K 31/166 (2006.01)

(52) **U.S. Cl.**

CPC **C07C 243/38** (2013.01); **A01N 37/28** (2013.01); **A01N 37/40** (2013.01); **A61K 31/166** (2013.01); **C07B 2200/07** (2013.01); **C07B 2200/13** (2013.01); **C12N 2710/10341** (2013.01); **C12N 2830/002** (2013.01); **Y10T 428/2982** (2015.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

7,304,161 B2	12/2007	Hormann et al.
7,531,326 B2	5/2009	Kapitskaya et al.
7,563,879 B2	7/2009	Palli
7,563,928 B2	7/2009	Hormann et al.
7,919,269 B2	4/2011	Zhang et al.
7,935,510 B2	5/2011	Palli et al.
8,076,517 B2 *	12/2011	Hormann C07D 317/68 564/310
8,946,294 B2 *	2/2015	Hormann C07C 243/38 514/615
2002/0110861 A1	8/2002	Dhadialla et al.
2006/0100416 A1	5/2006	Palli et al.
2009/0123441 A1	5/2009	Braugher et al.

OTHER PUBLICATIONS

Braga, D. et al., "Crystal Polymorphism and Multiple Crystal Forms," *Structure and Bonding* 132:25-50, Springer-Verlag Berlin Heidelberg (2009).

Lee, T. et al., "Solubility, Polymorphism, Crystallinity, and Crystal Habit of Acetaminophen and Ibuprofen by Initial Solvent Screening," *Pharmaceutical Technology* 30:72, Advanstar Communications, United States (Oct. 2, 2006).

Caira, M.R., "Crystalline Polymorphism of Organic Compounds," *Topics in Current Chemistry* 198:163-208, Springer Verlag Berlin Heidelberg (1998).

Polymorphism in Pharmaceutical Solids, Second Edition, Brittain, H.G., ed., vol. 192, pp. 1-23 and 288-480, CRC Press, United States (2009).

* cited by examiner

Primary Examiner — Jared D Barsky

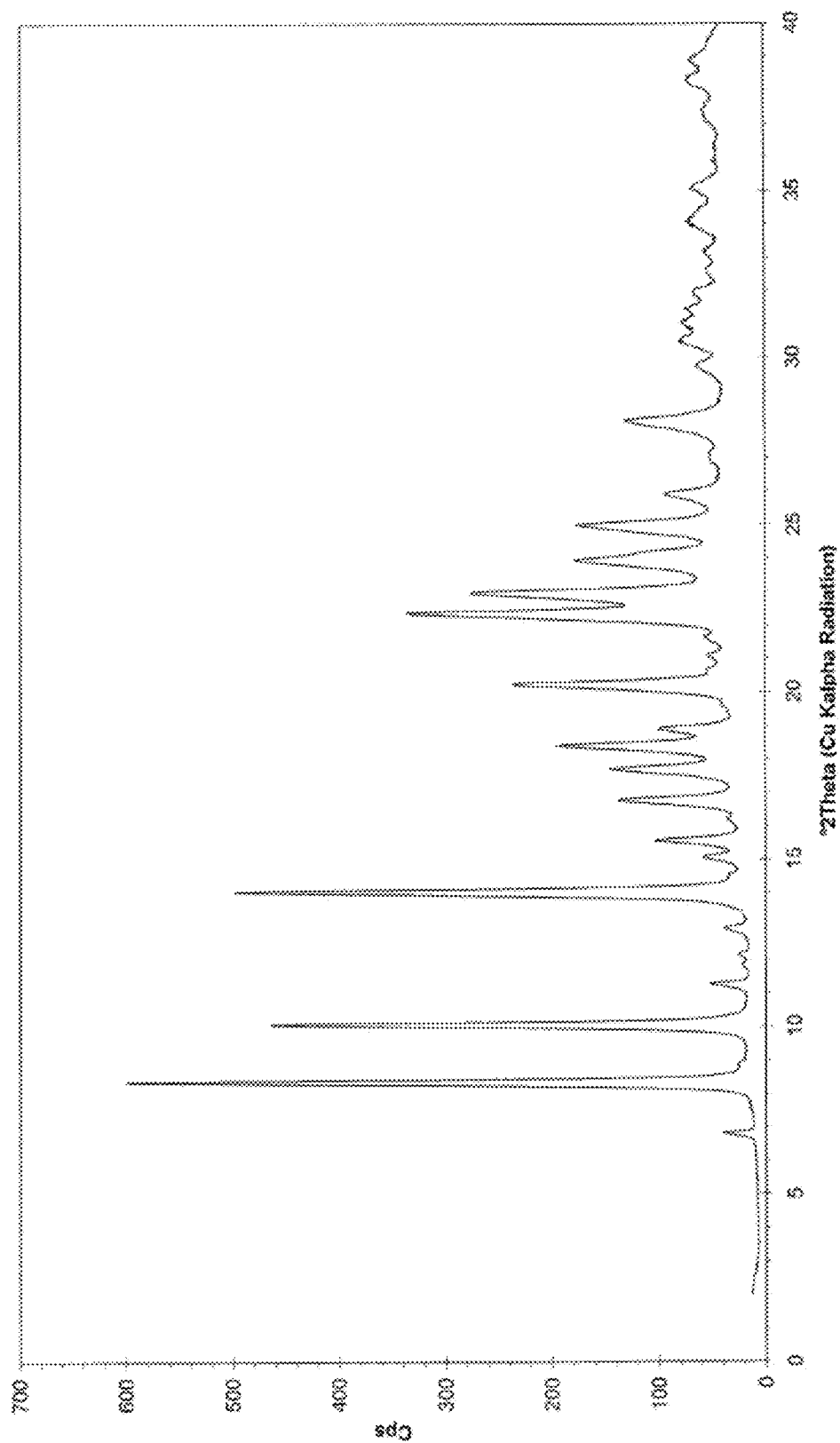
(74) *Attorney, Agent, or Firm* — Sterne, Kessler, Goldstein & Fox P.L.L.C.

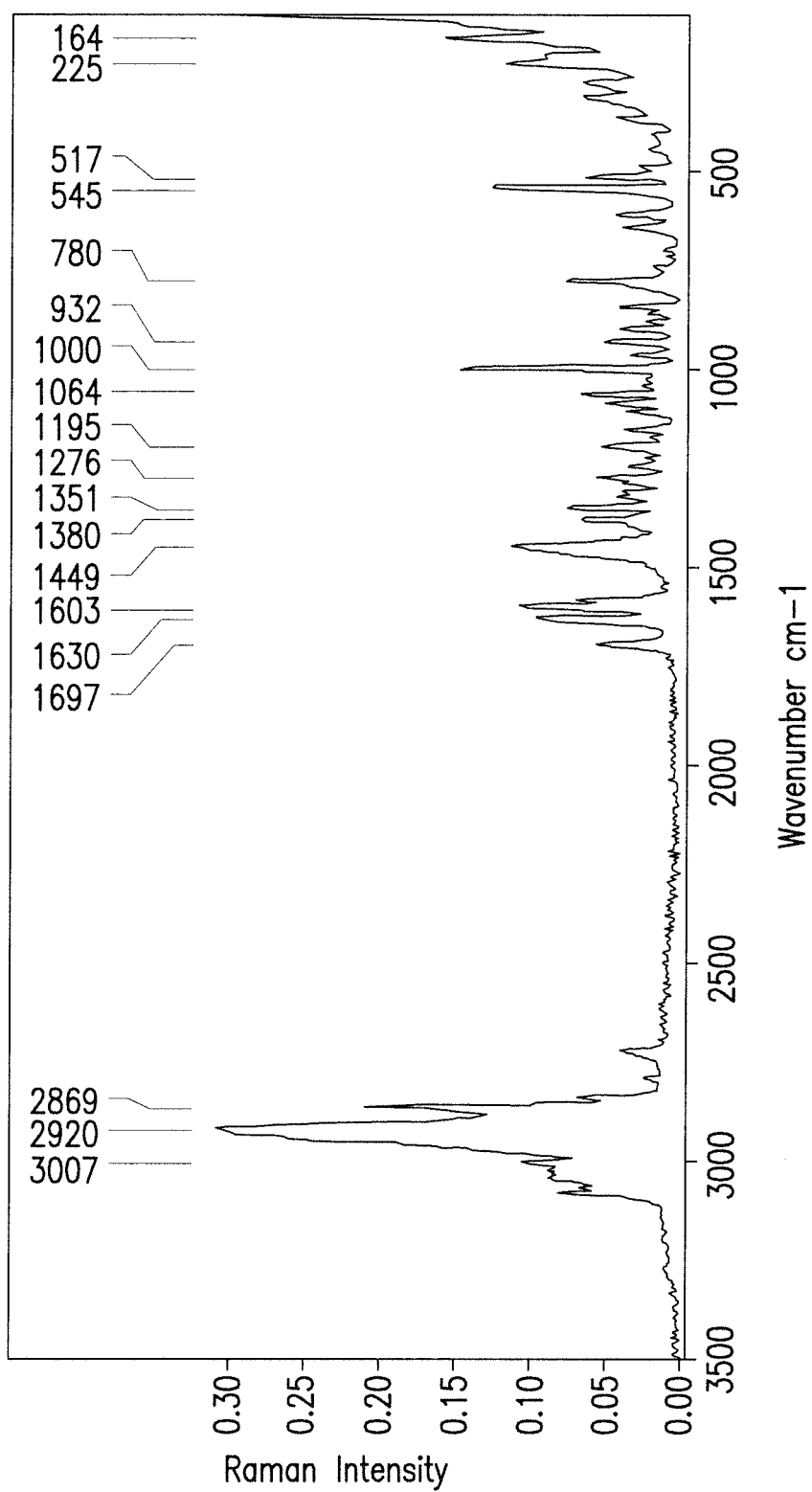
(57) **ABSTRACT**

The present disclosure provides crystalline polymorphic and amorphous forms of (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide (Compound 1) or (S)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide (Compound 2). The present disclosure further provides compositions comprising crystalline polymorphic and amorphous forms of Compound 1 or Compound 2 and an excipient, methods of making crystalline polymorphic or amorphous forms of Compound 1 or Compound 2, and methods of using crystalline polymorphic or amorphous forms of Compound 1 or Compound 2 to regulate gene expression in a cell or in a subject.

8 Claims, 32 Drawing Sheets

Fig. 1





— ta_fk 12700 28283_2
- - - ta_fk 12700 28283_1

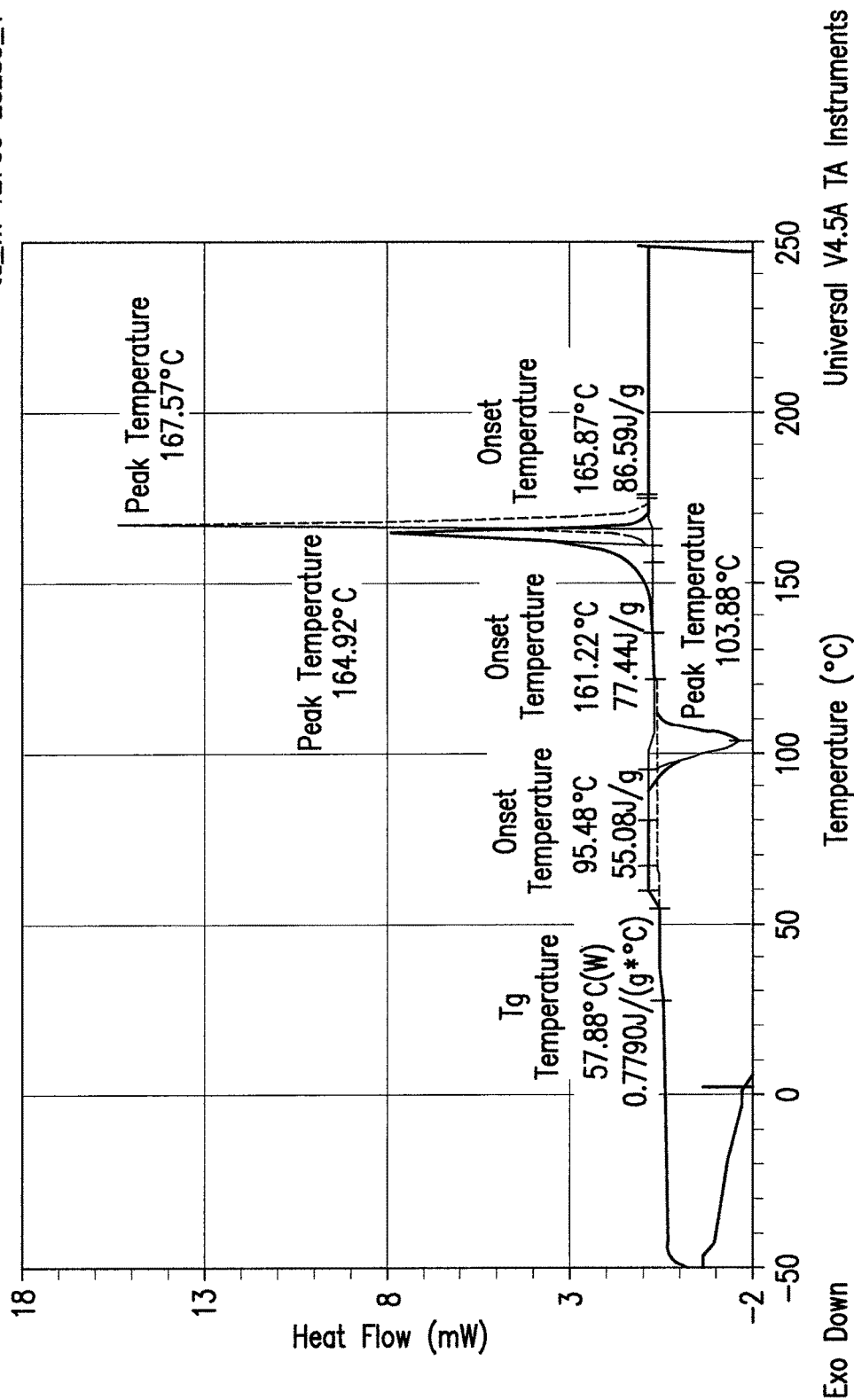


FIG.3

Fig. 4

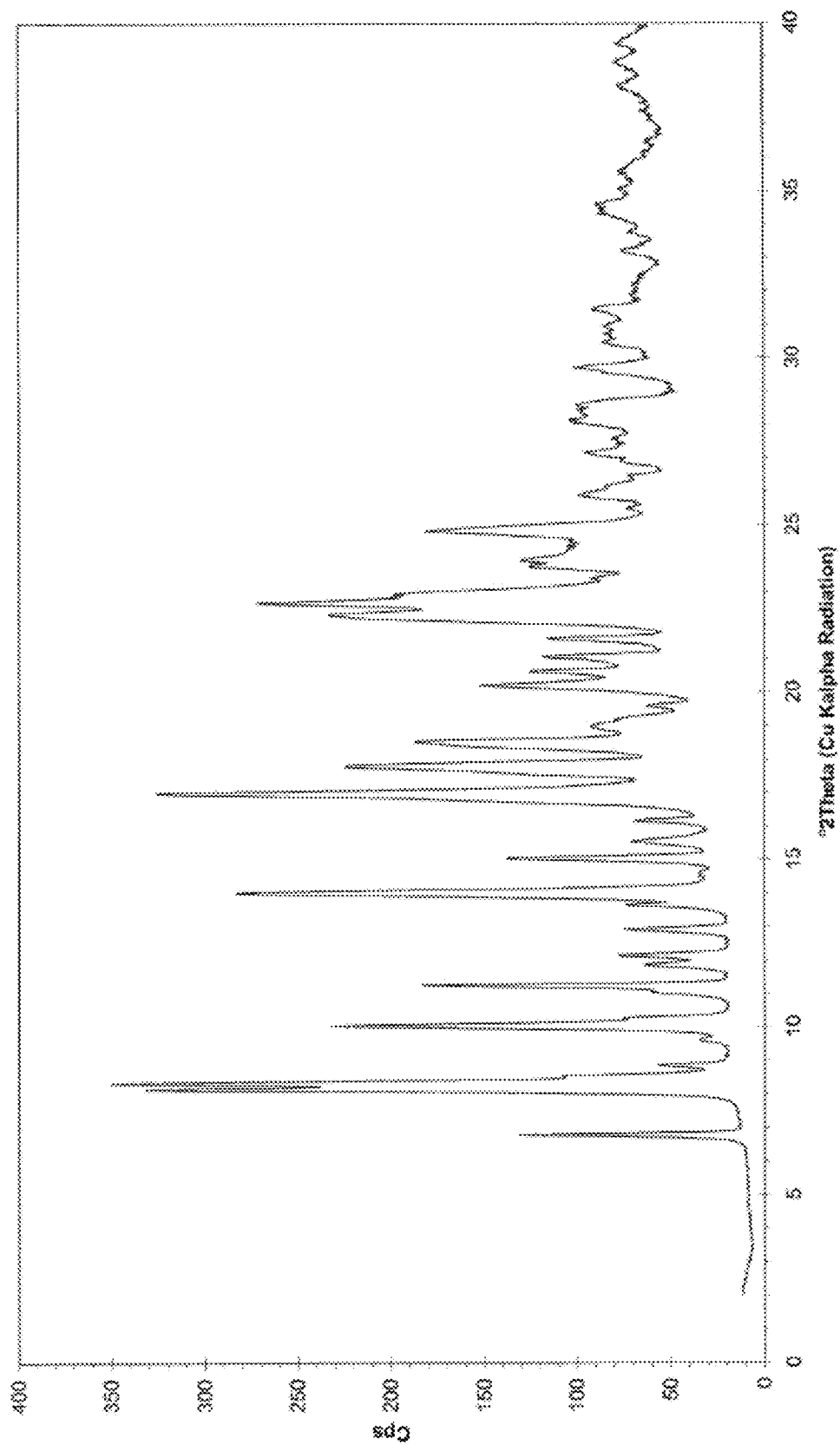


Fig. 5

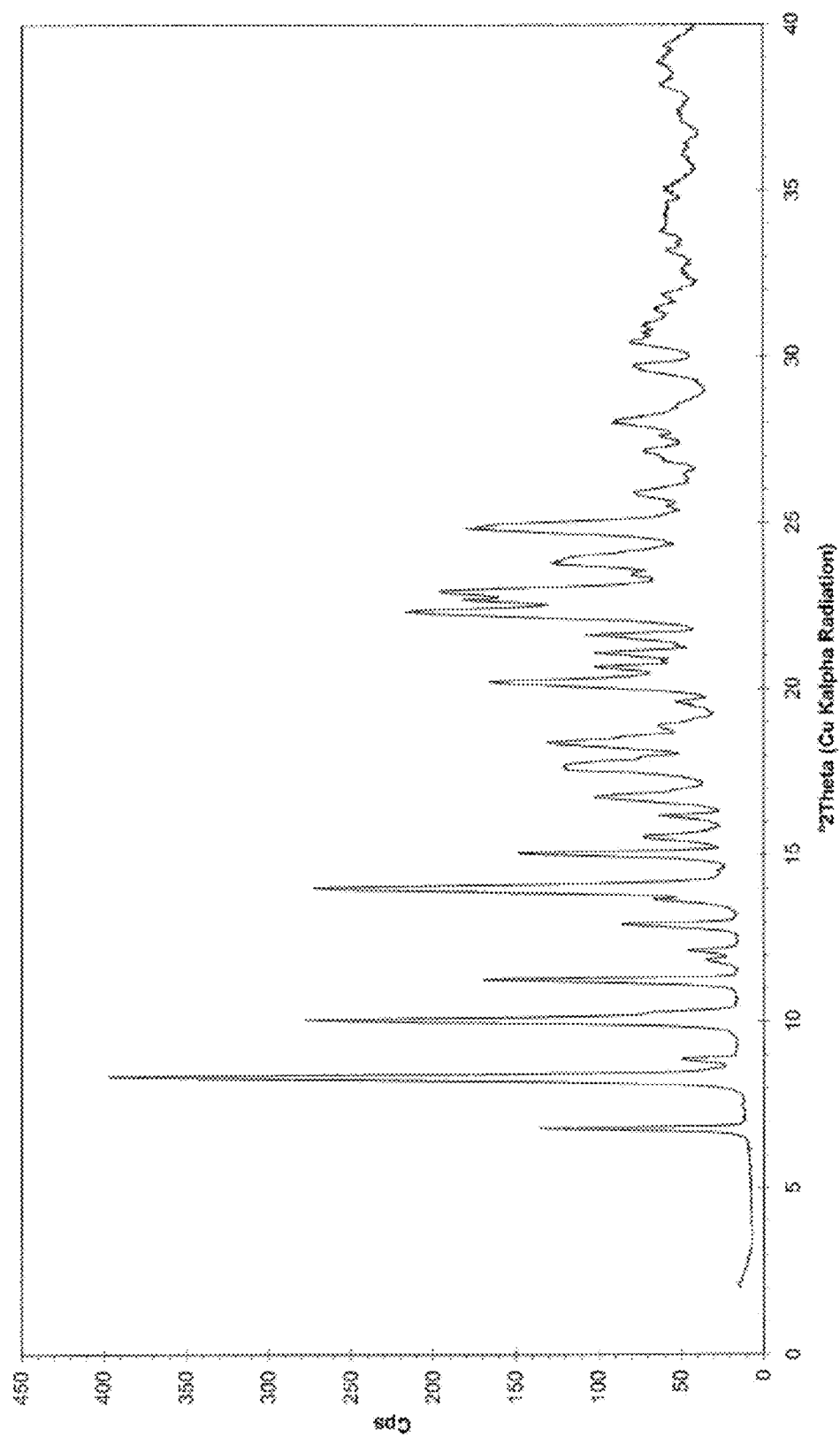


Fig. 6

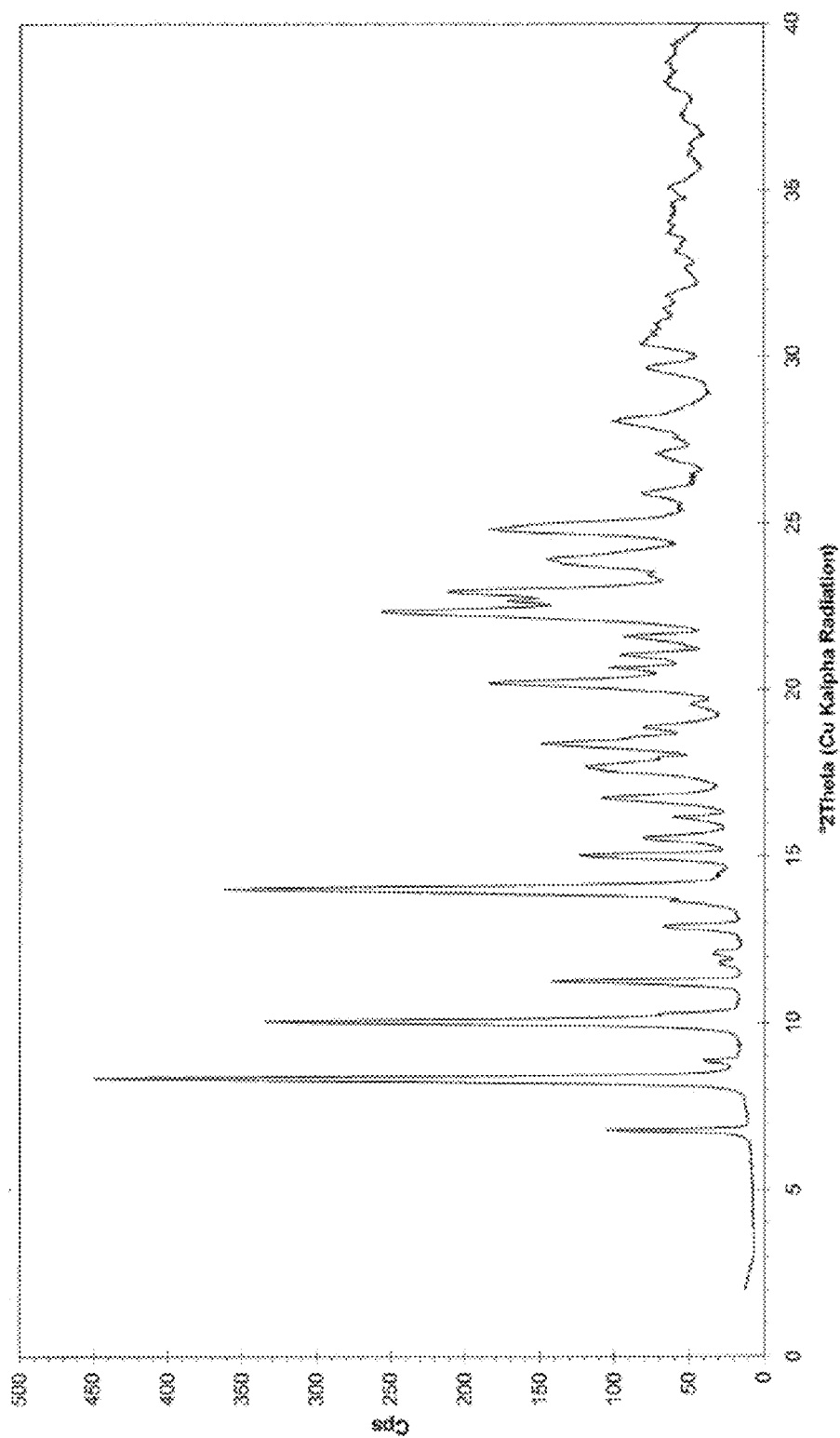
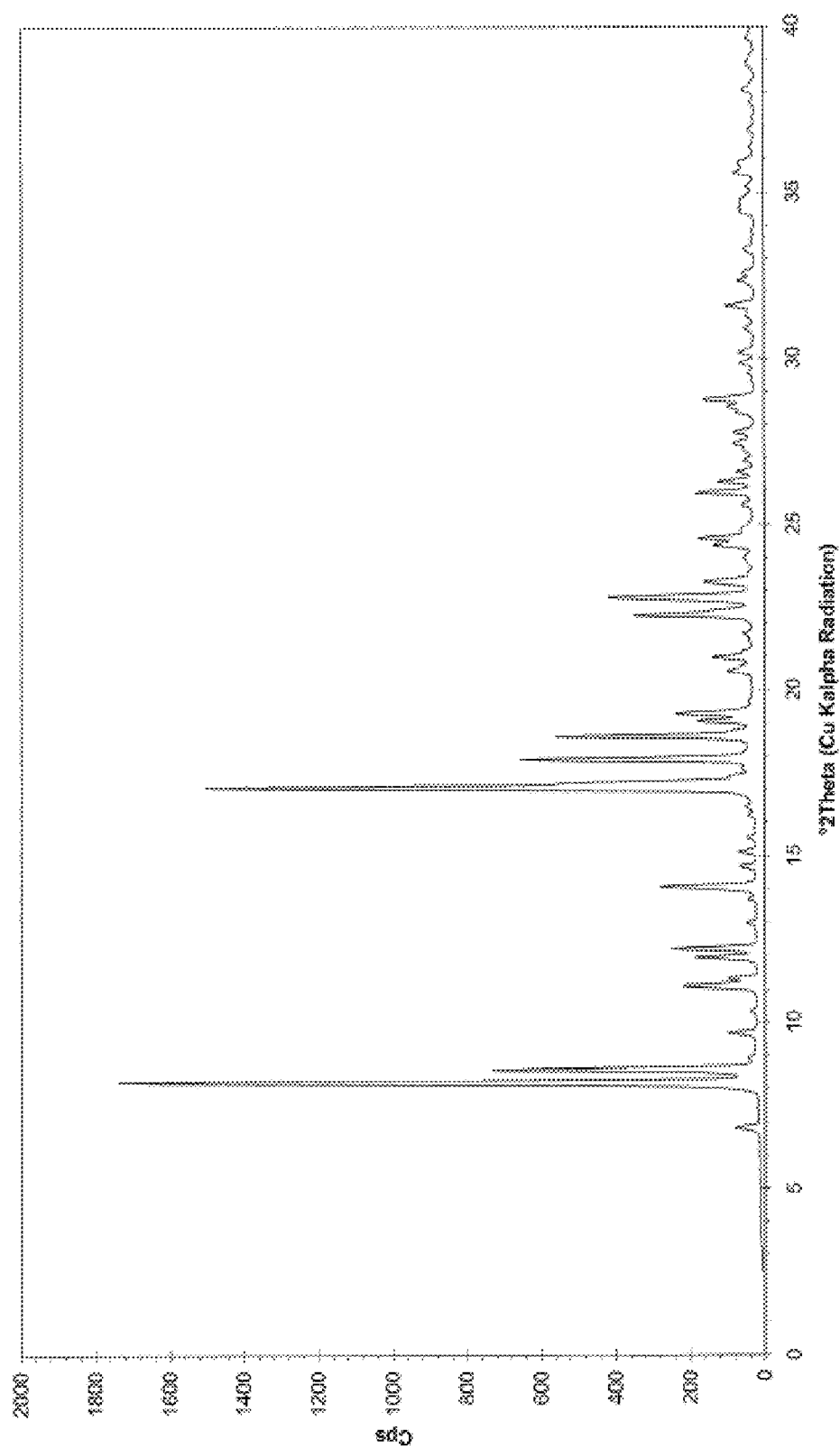


Fig. 7



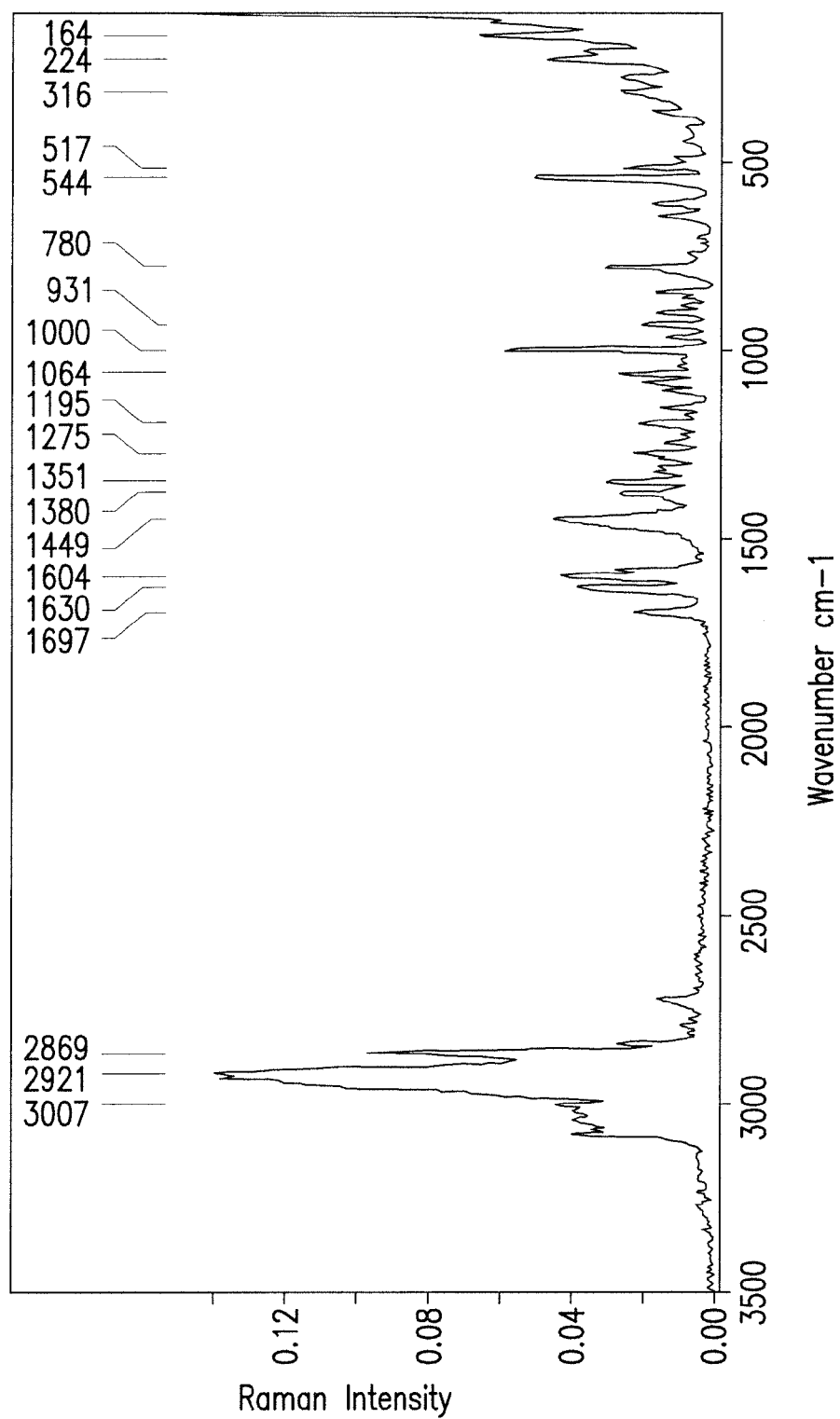


FIG. 8

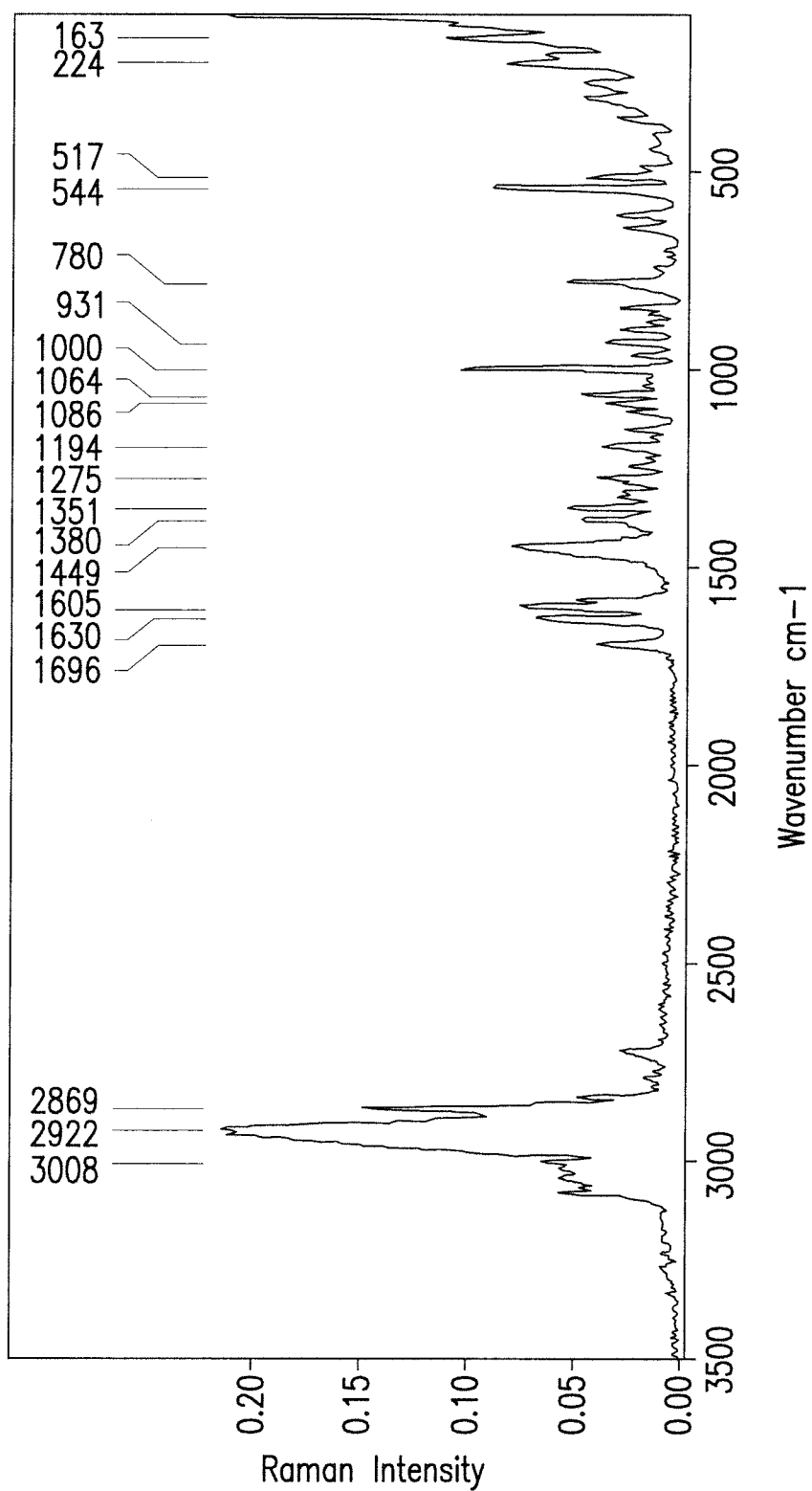
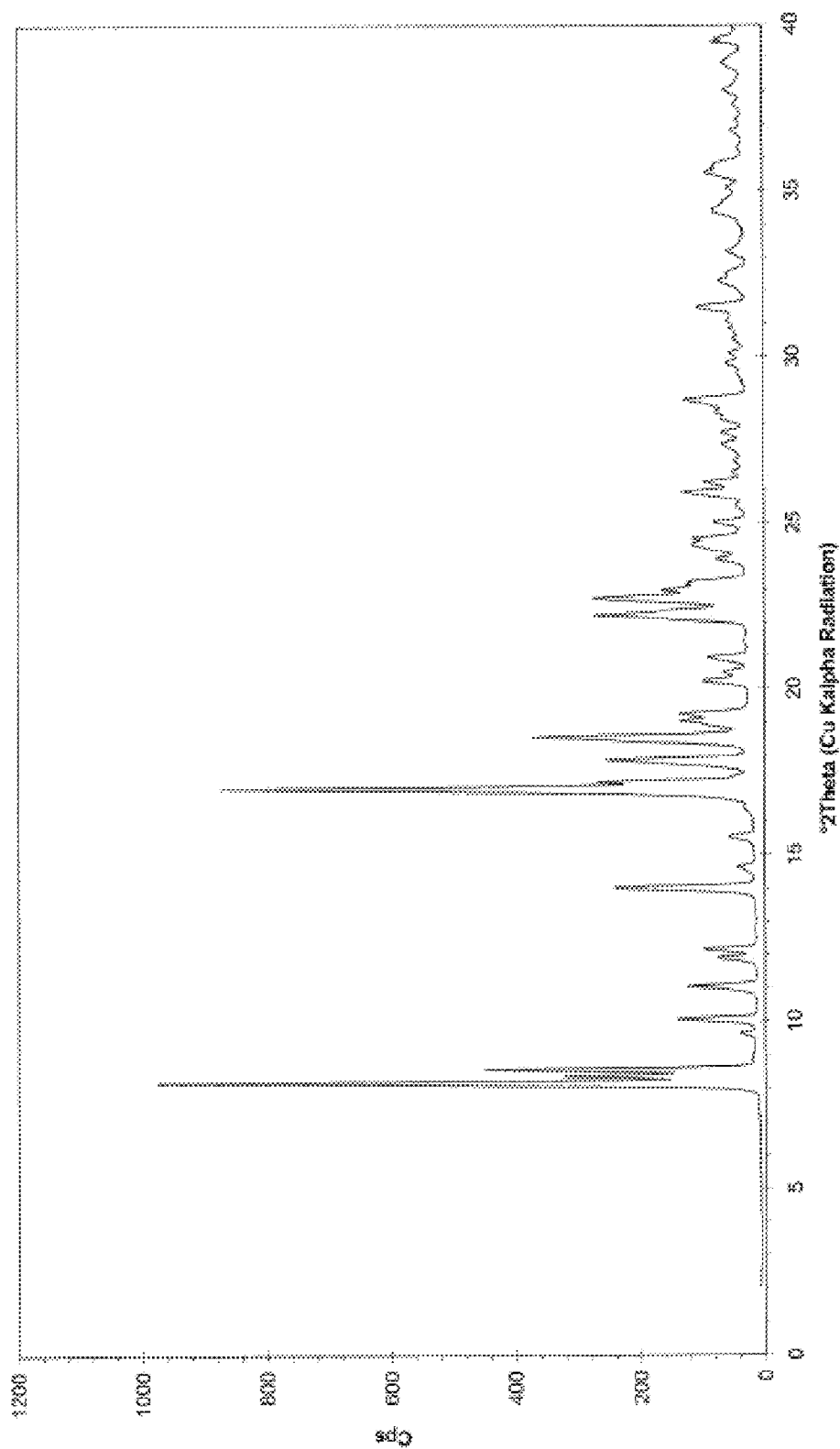
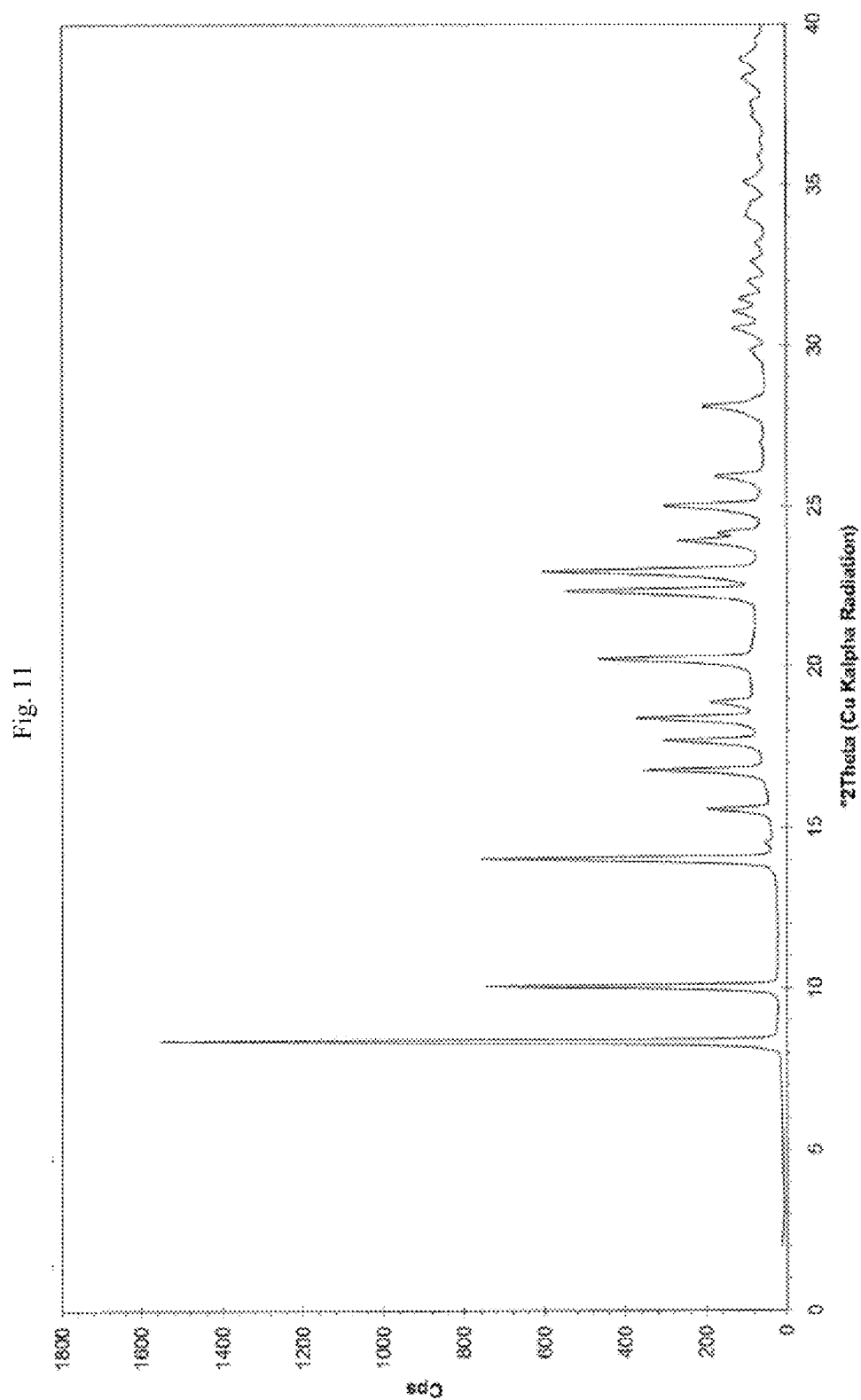
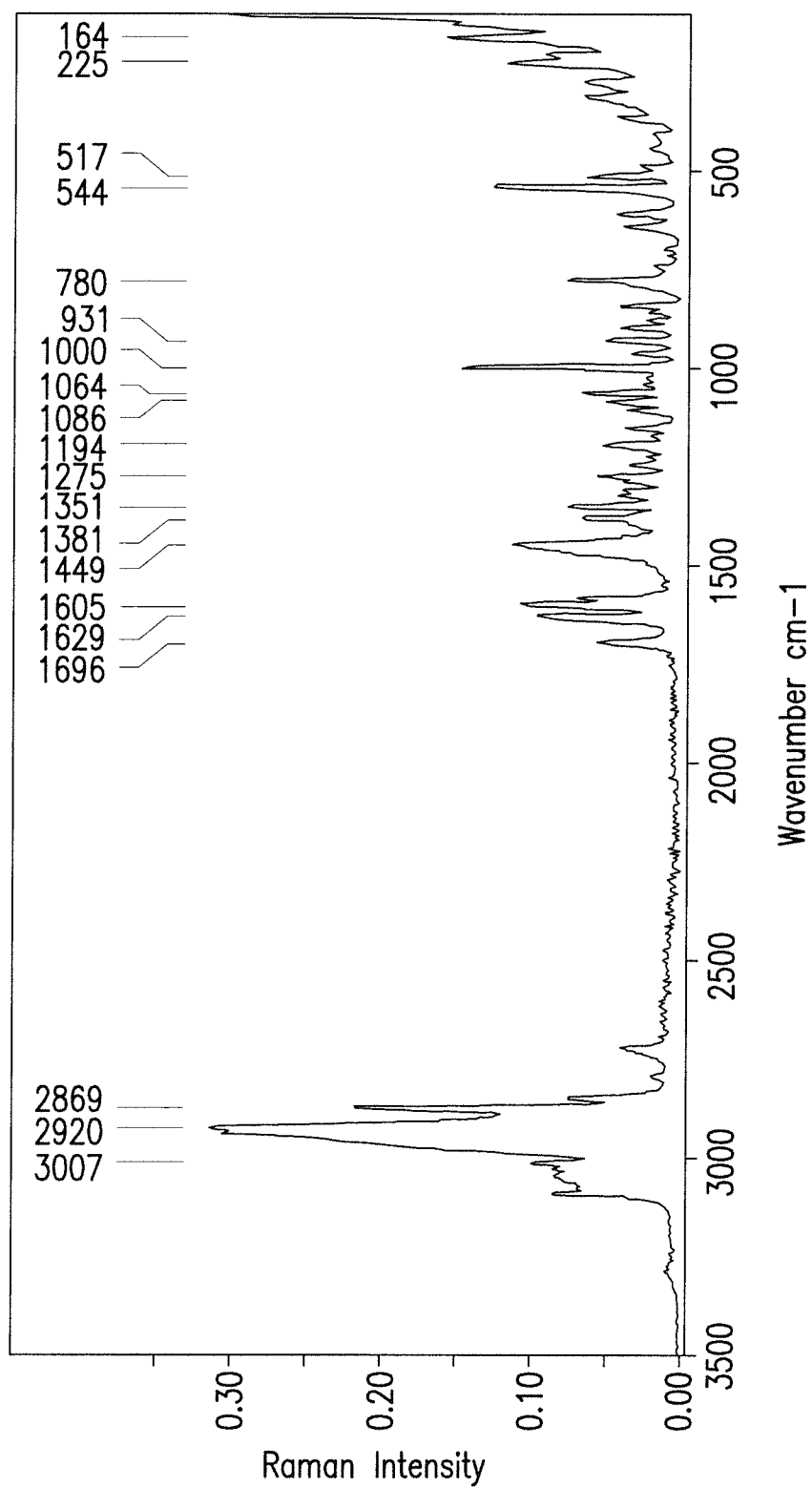


FIG. 9

Fig. 10







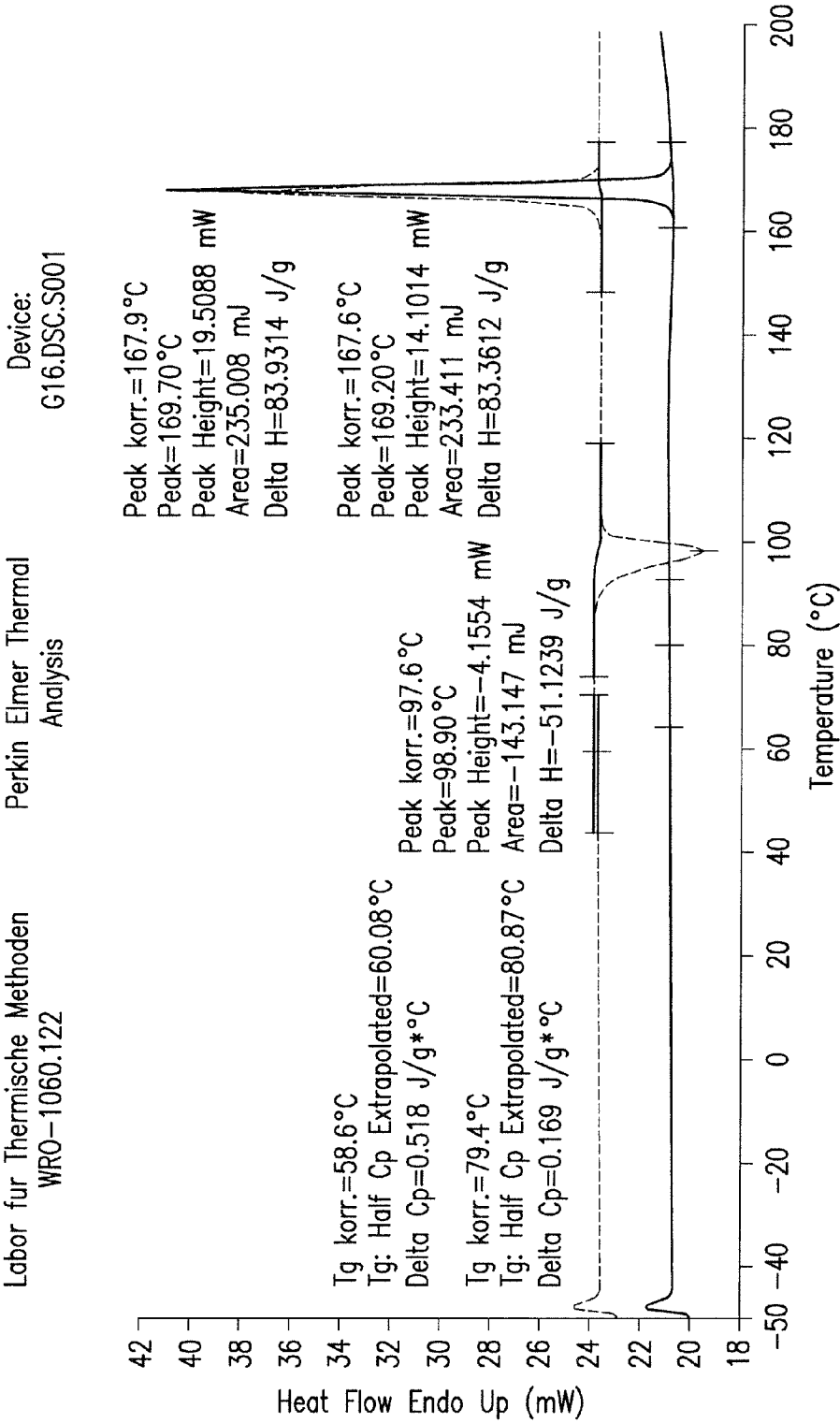
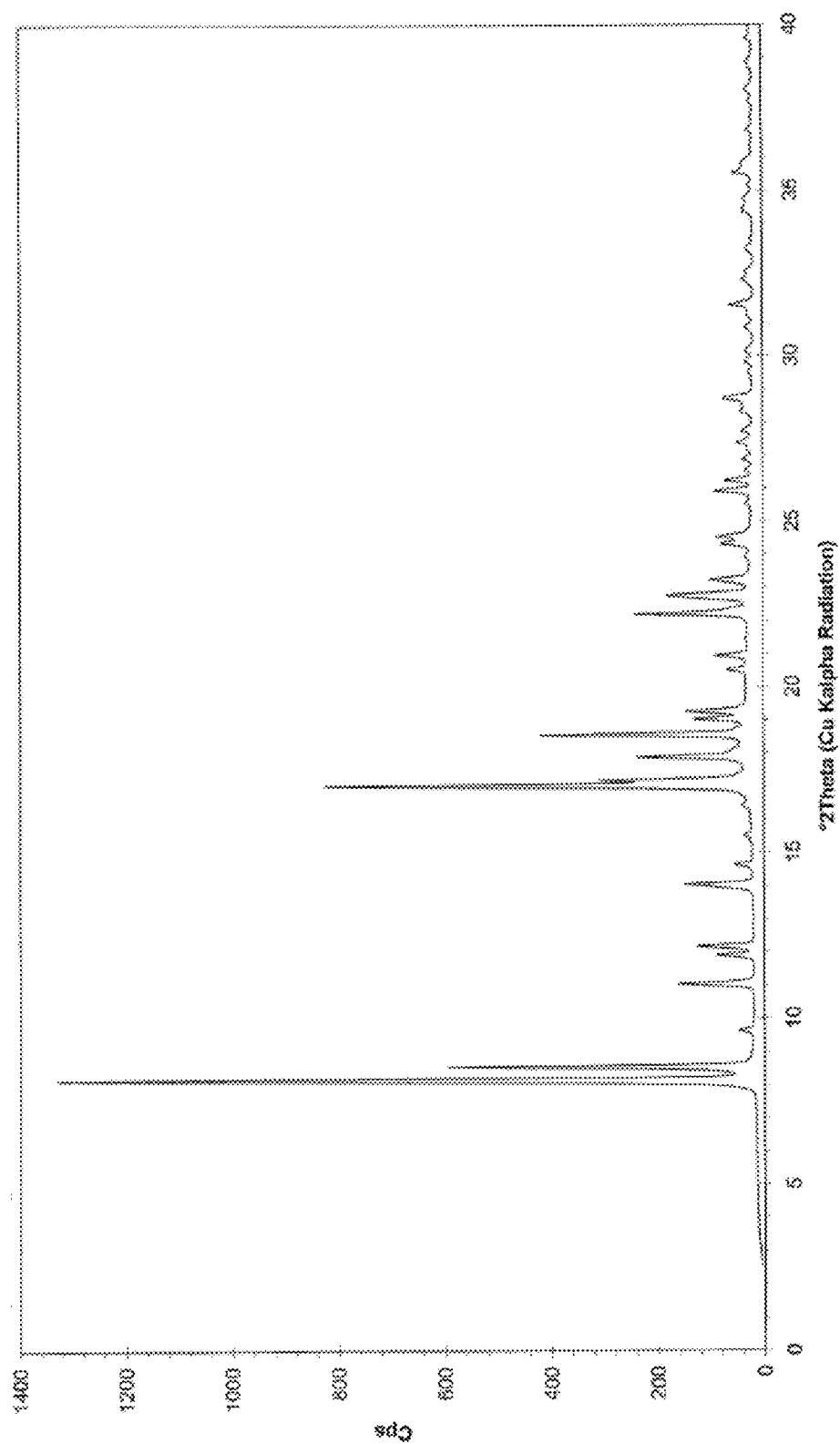


FIG.13

Fig. 14



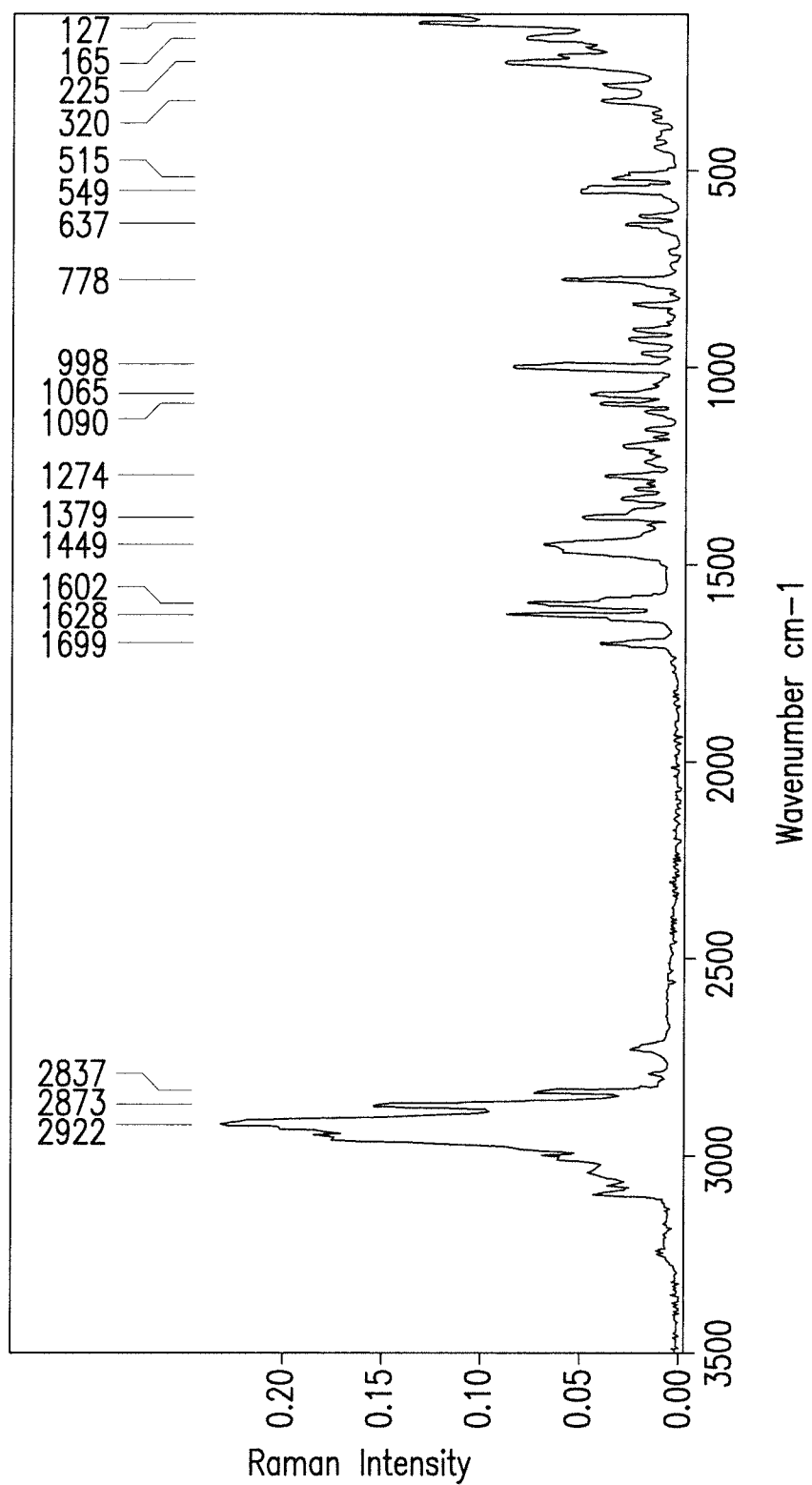


FIG. 15

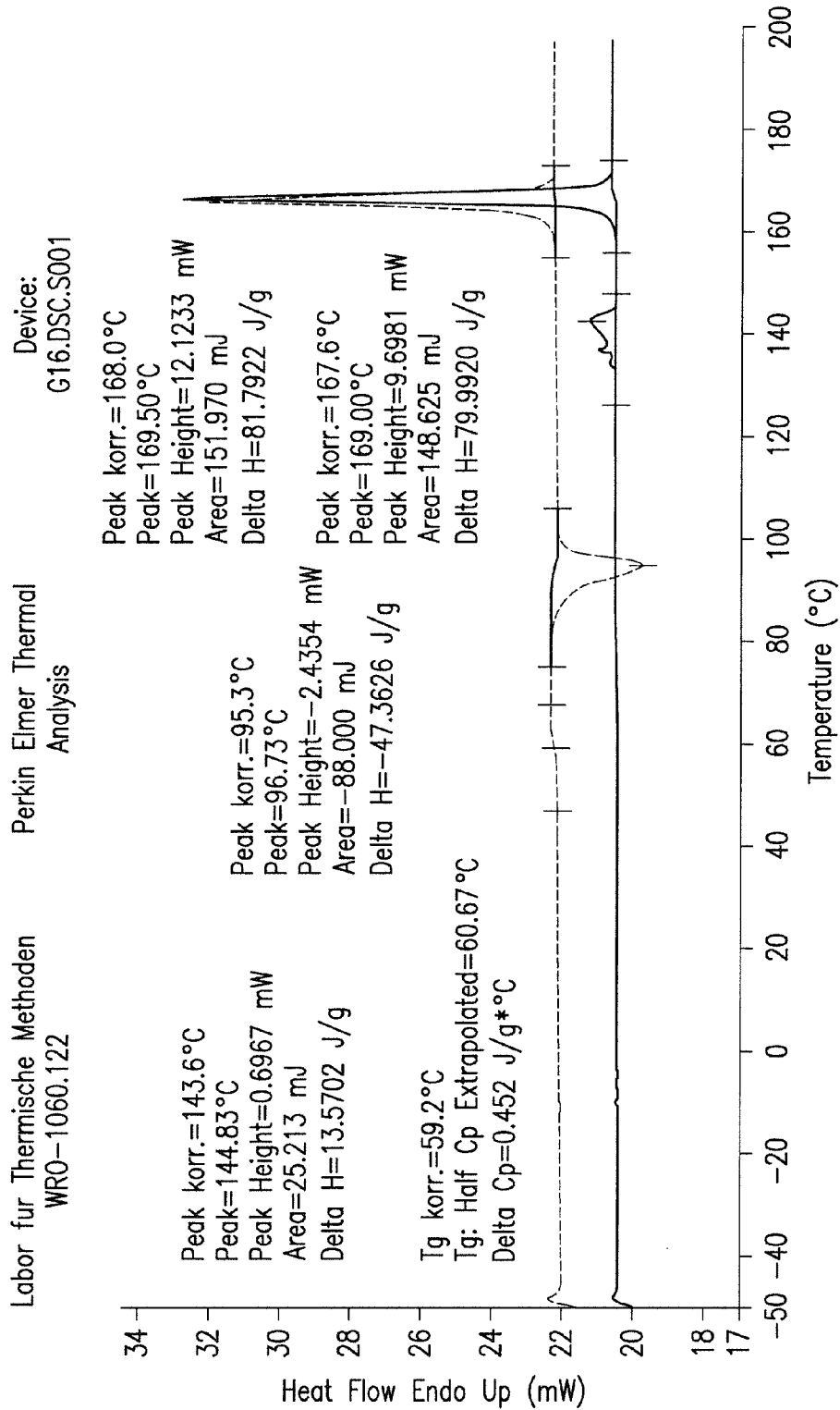
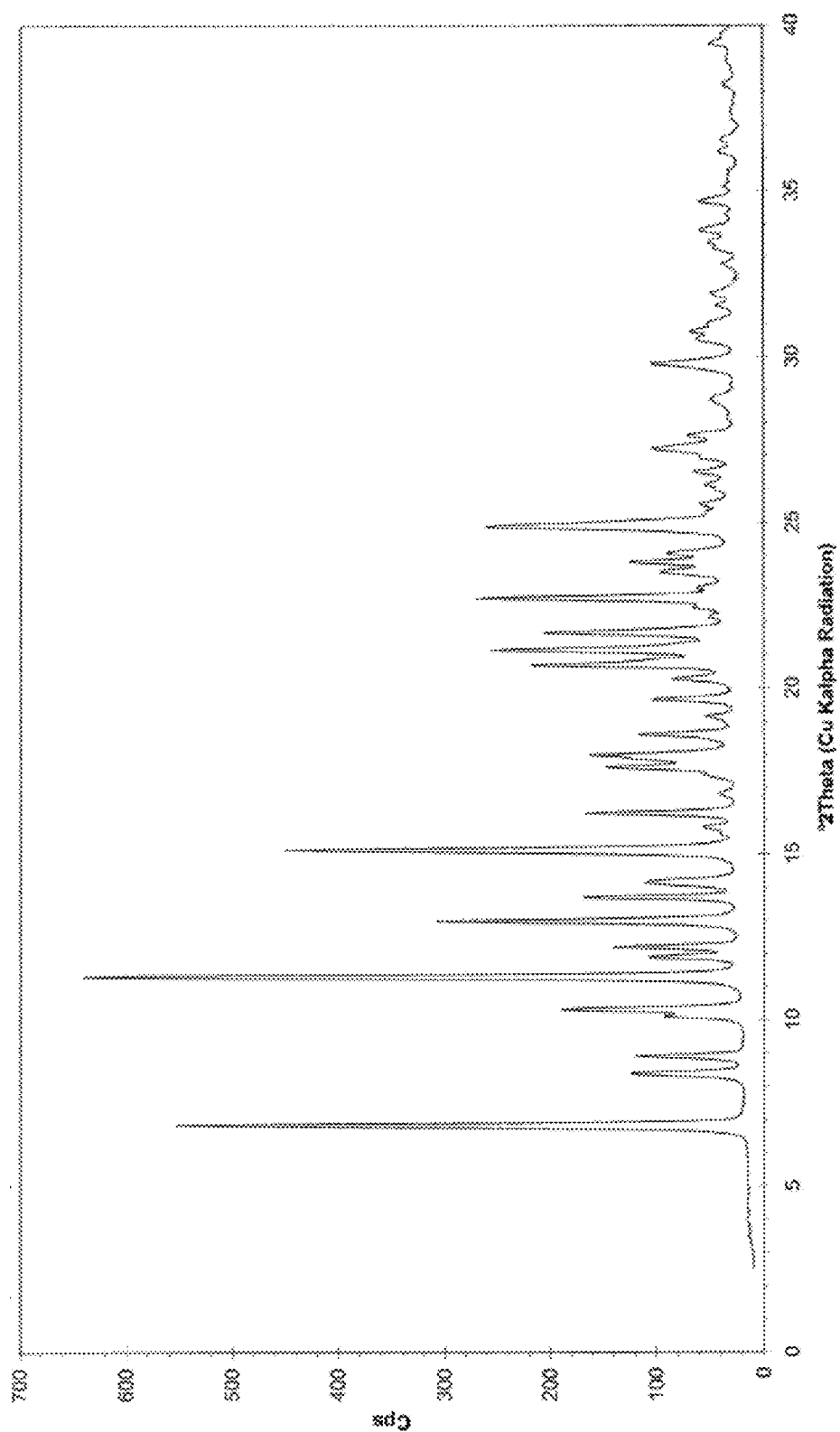


FIG.16

Fig. 17



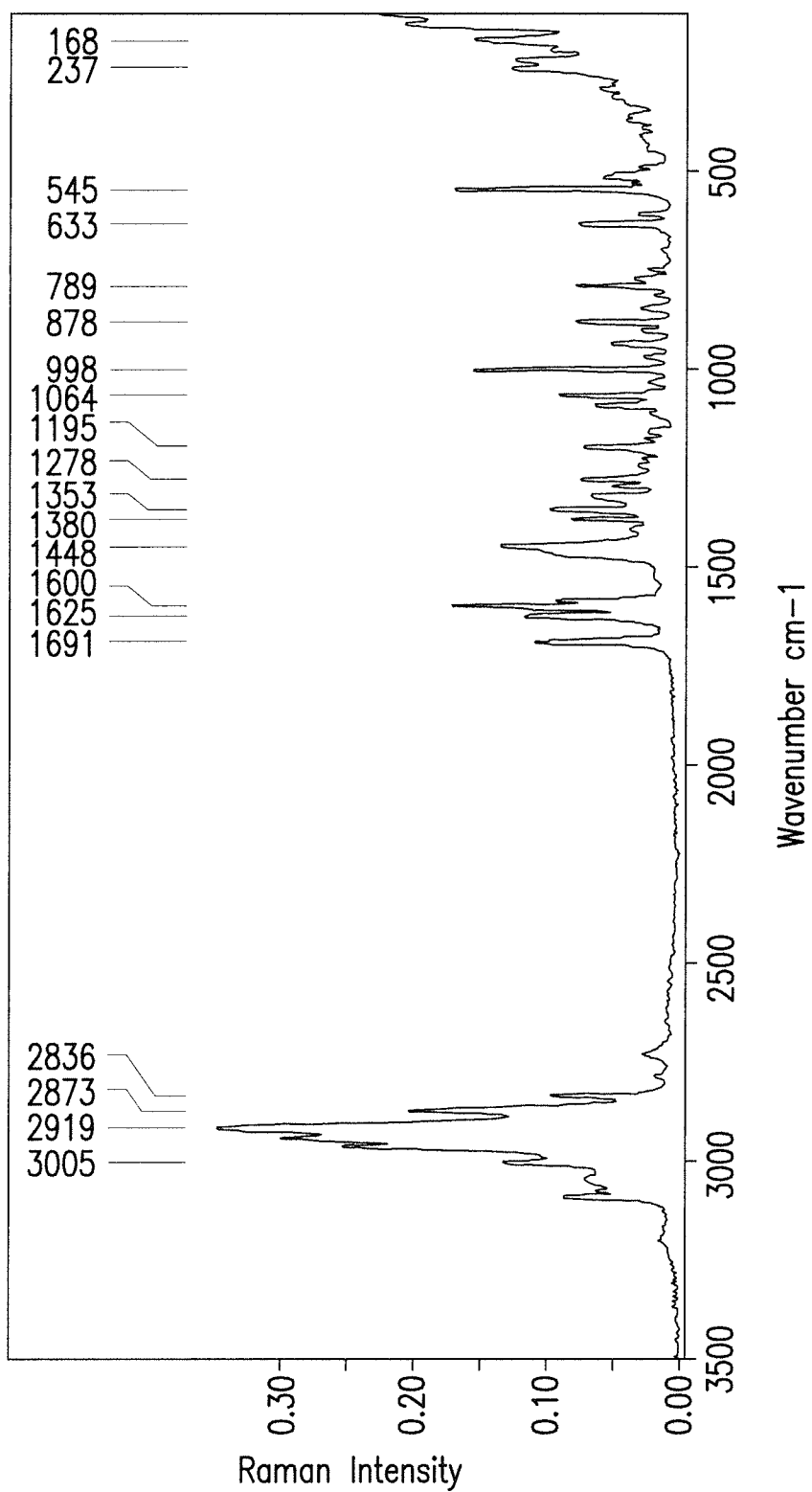


FIG. 18

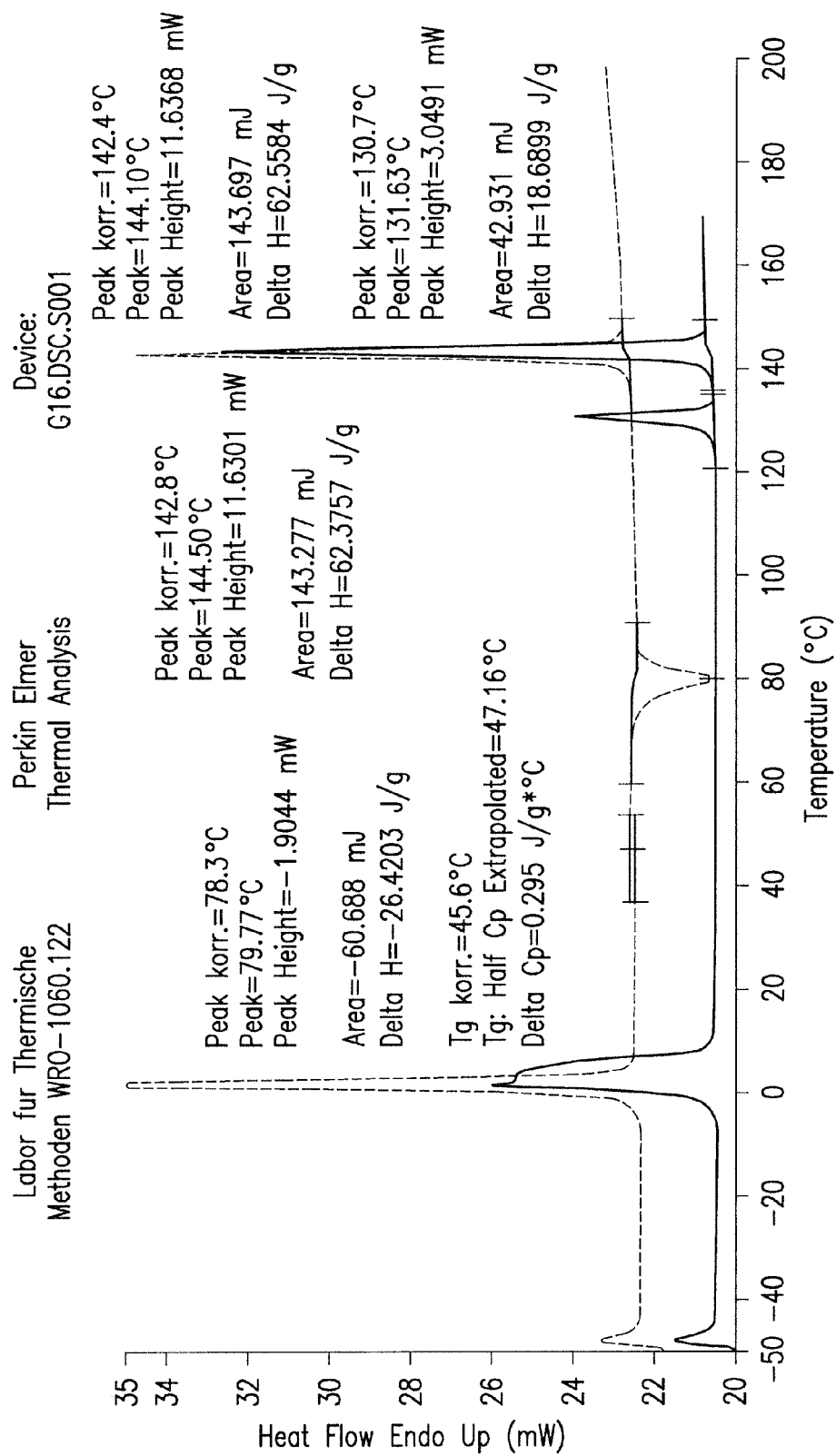


FIG.19

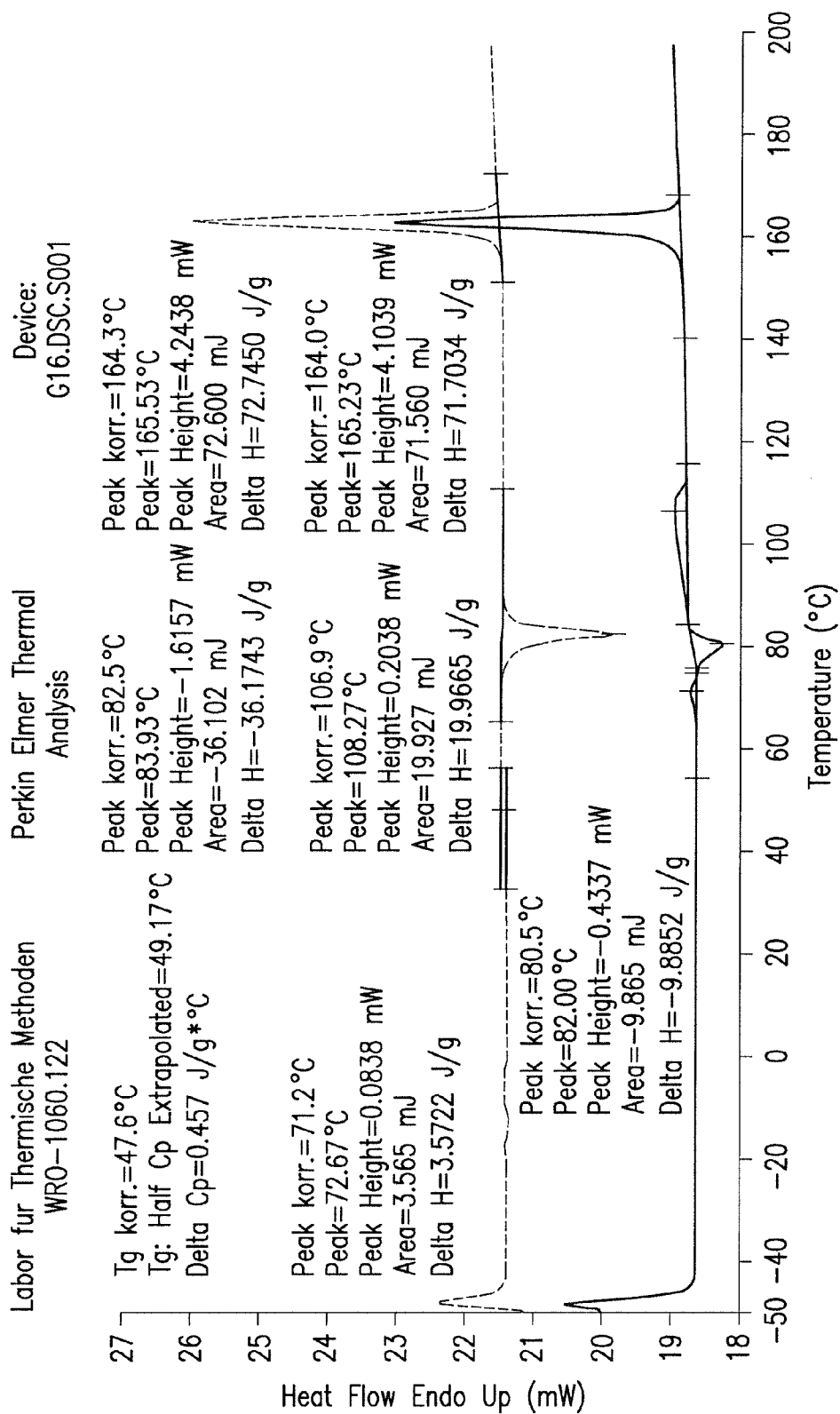
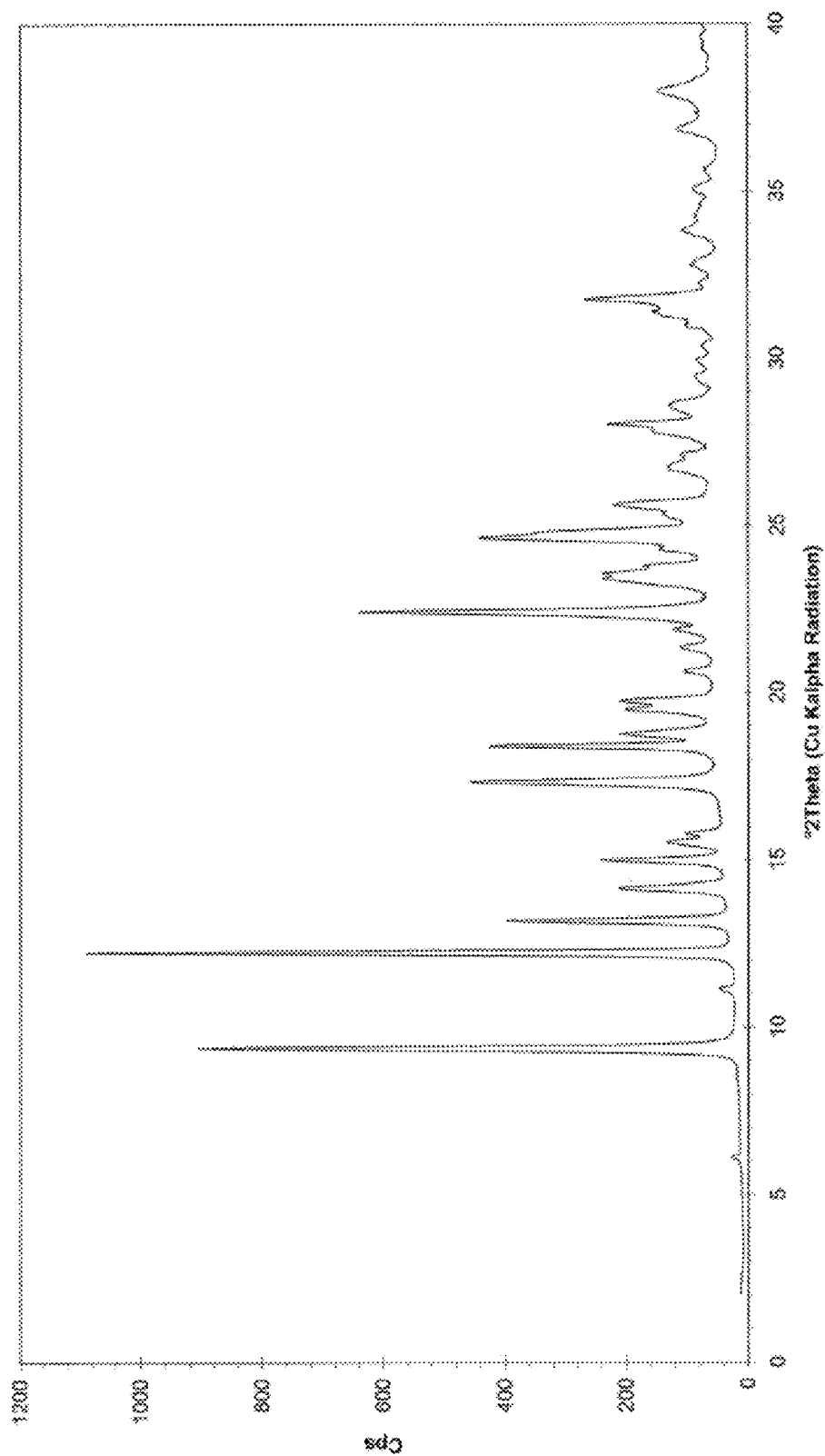


FIG. 20

Fig. 21



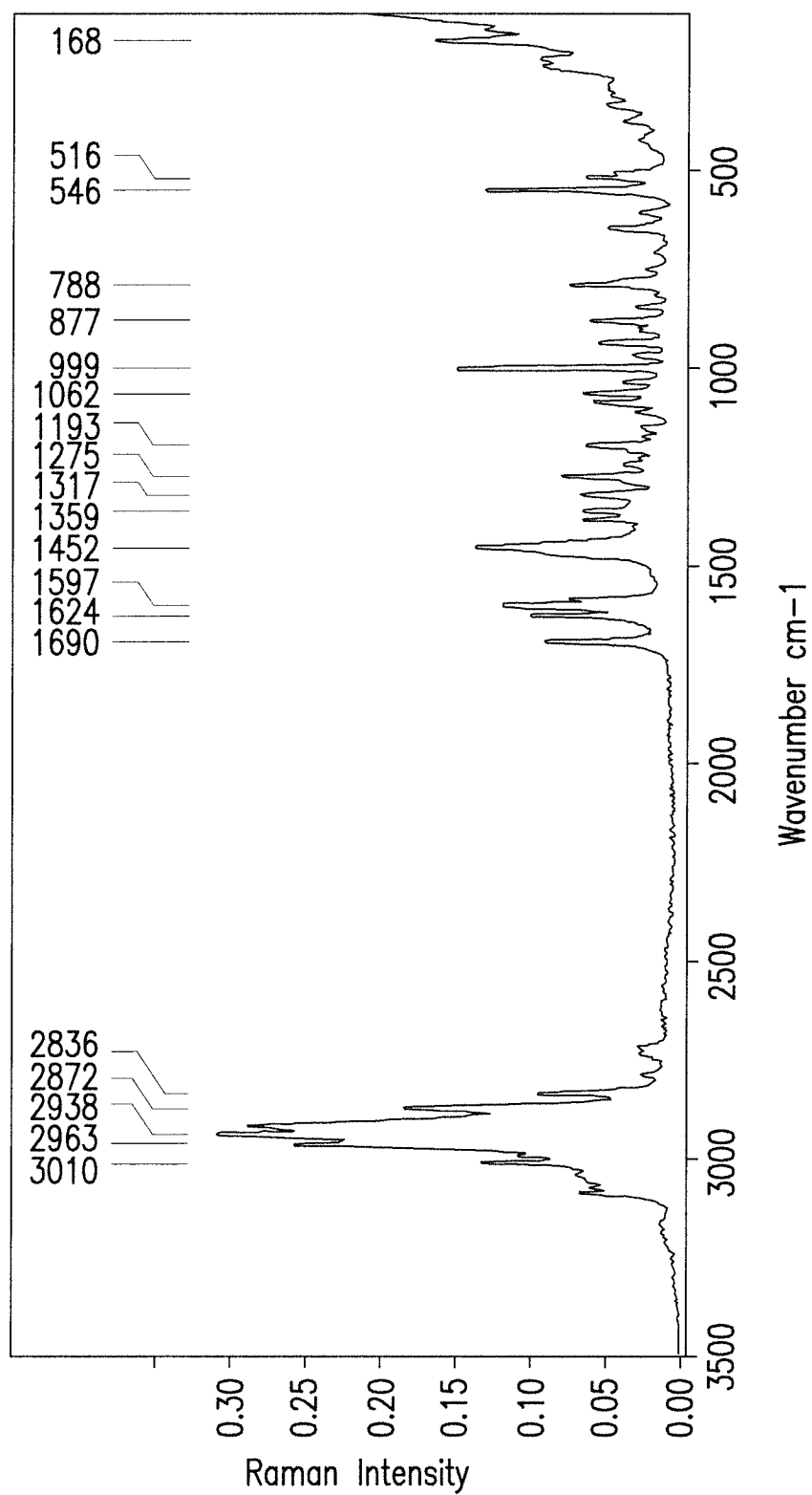
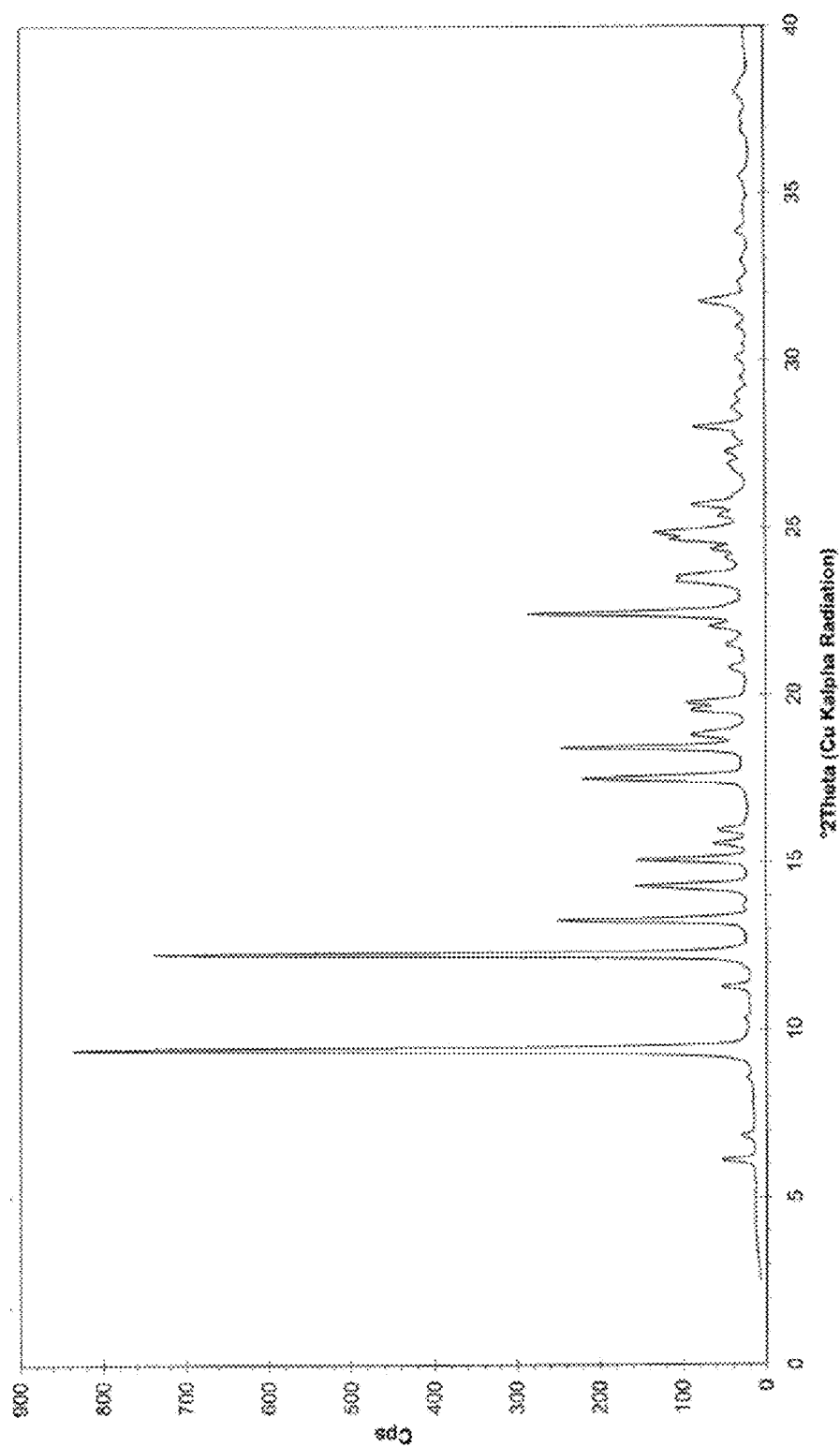


FIG. 22

Fig. 23



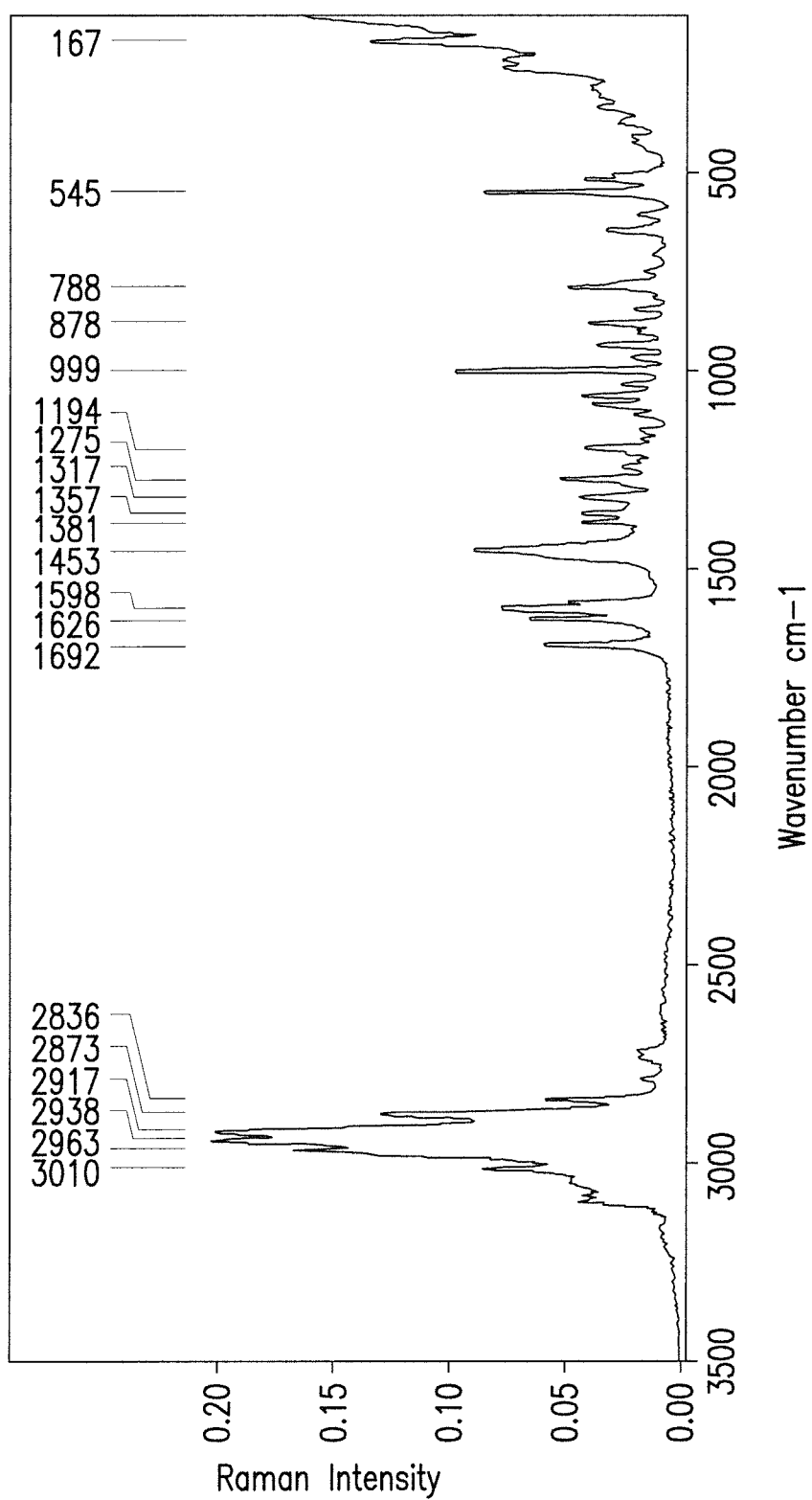
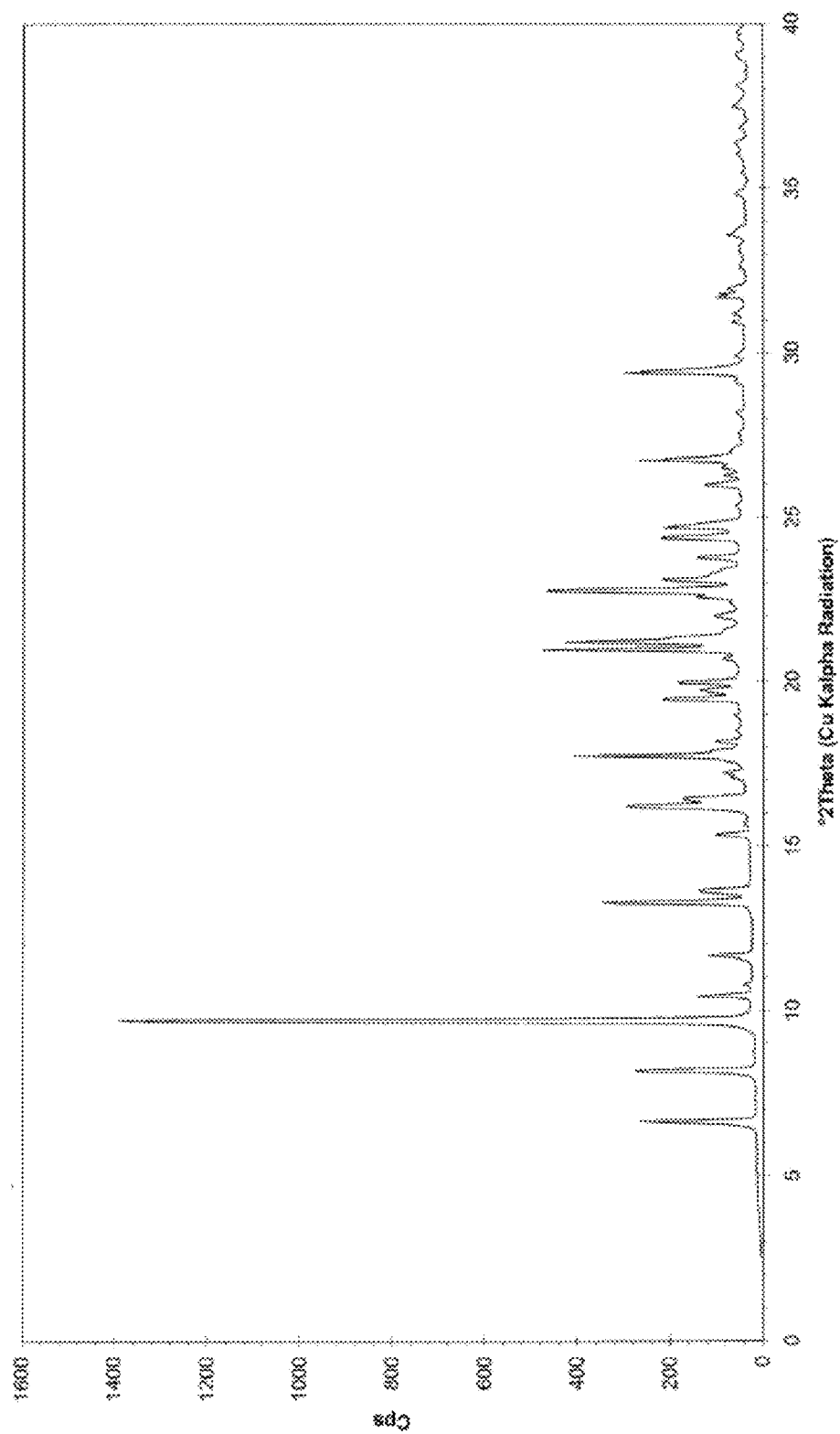


FIG. 24

Fig. 25



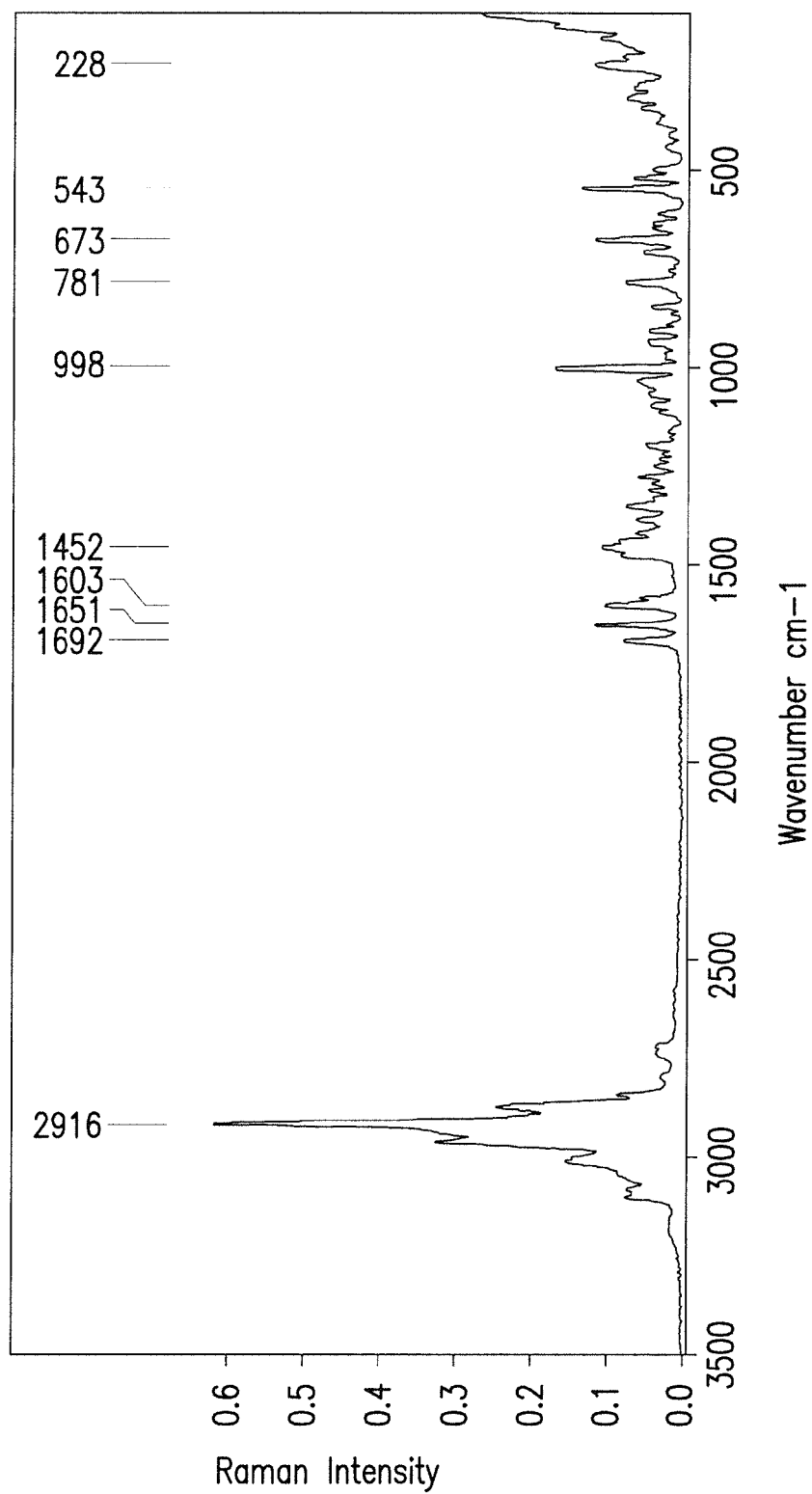
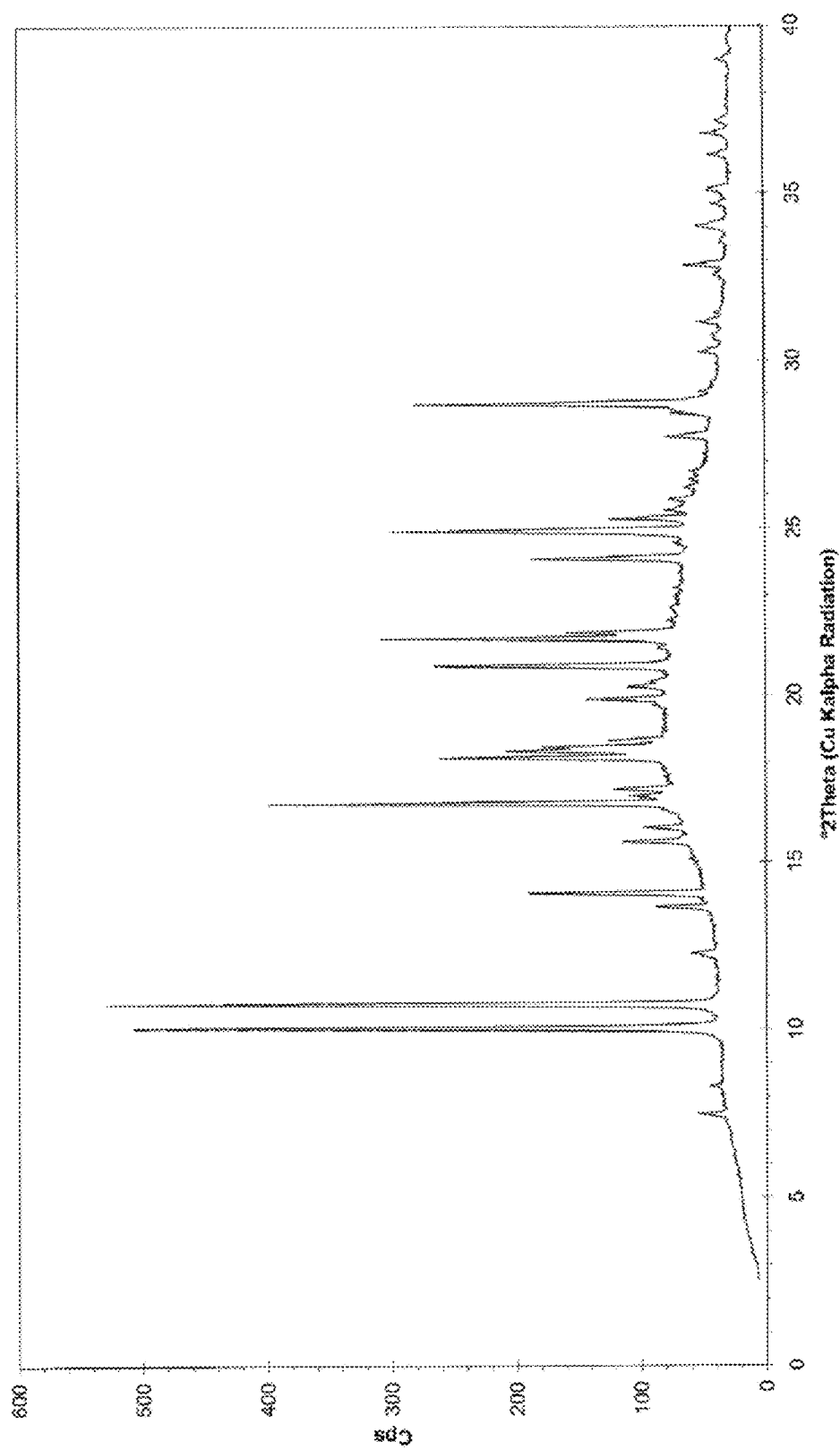


FIG. 26

Fig. 27



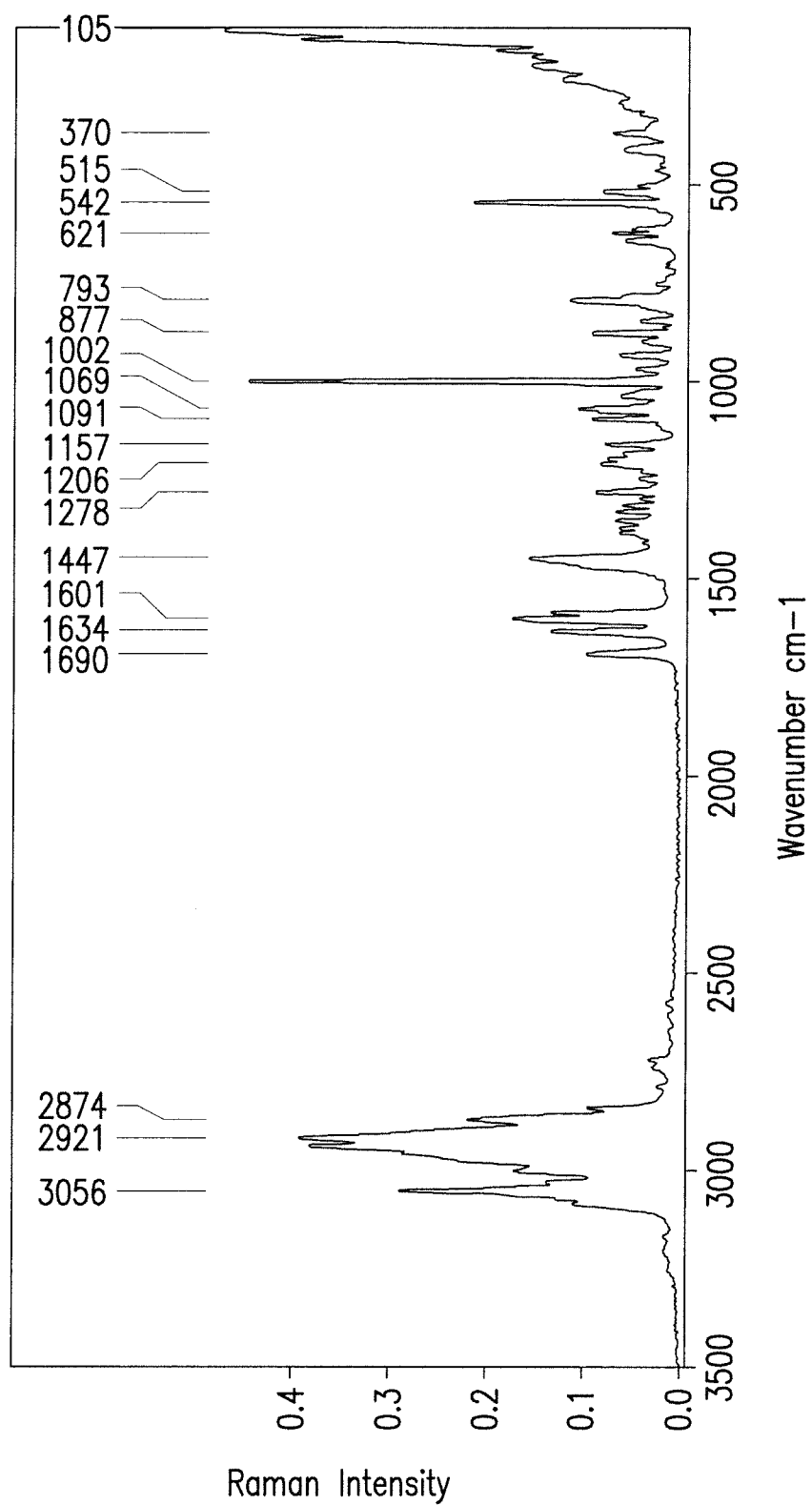
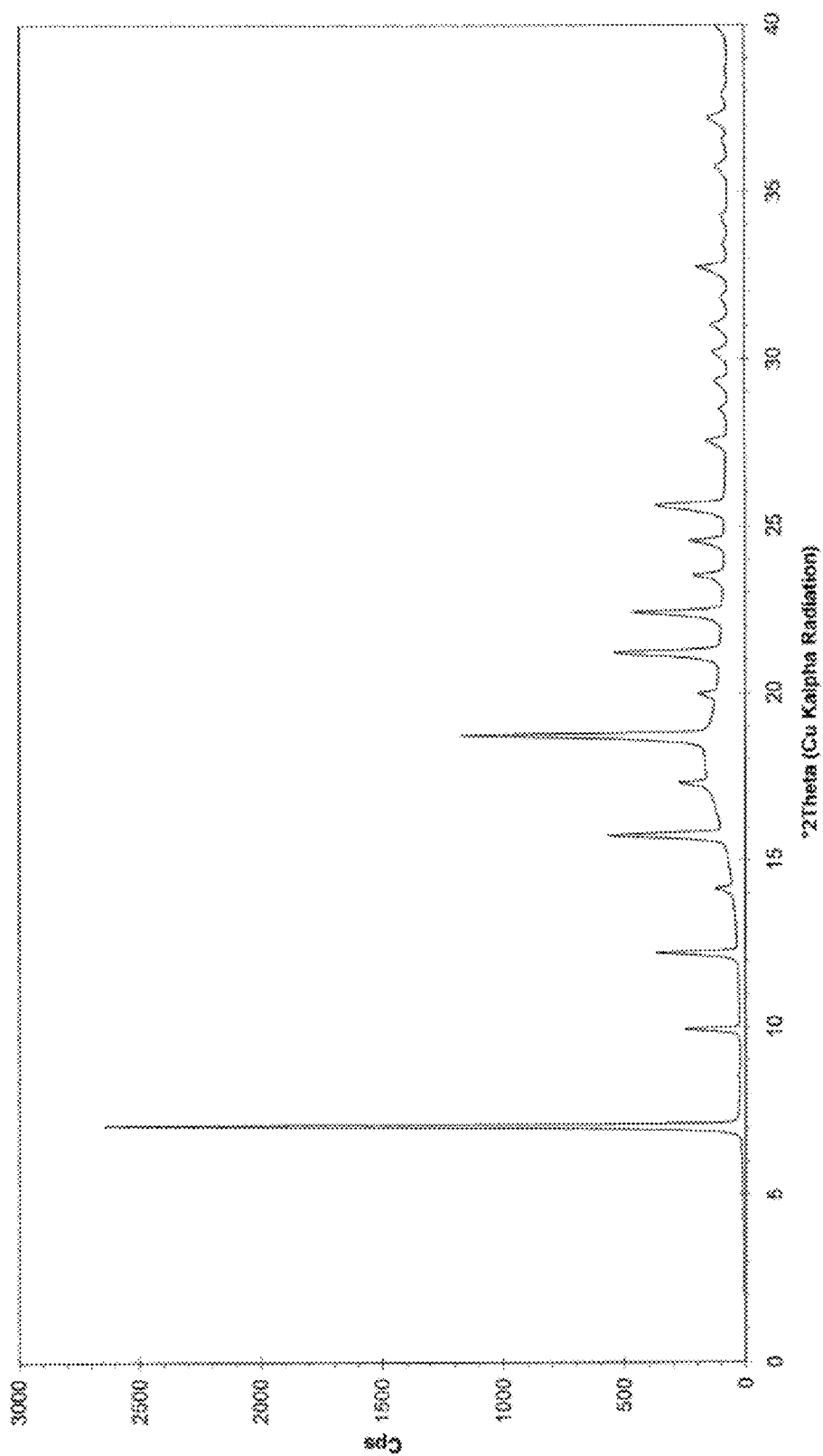


FIG. 28

Fig. 29



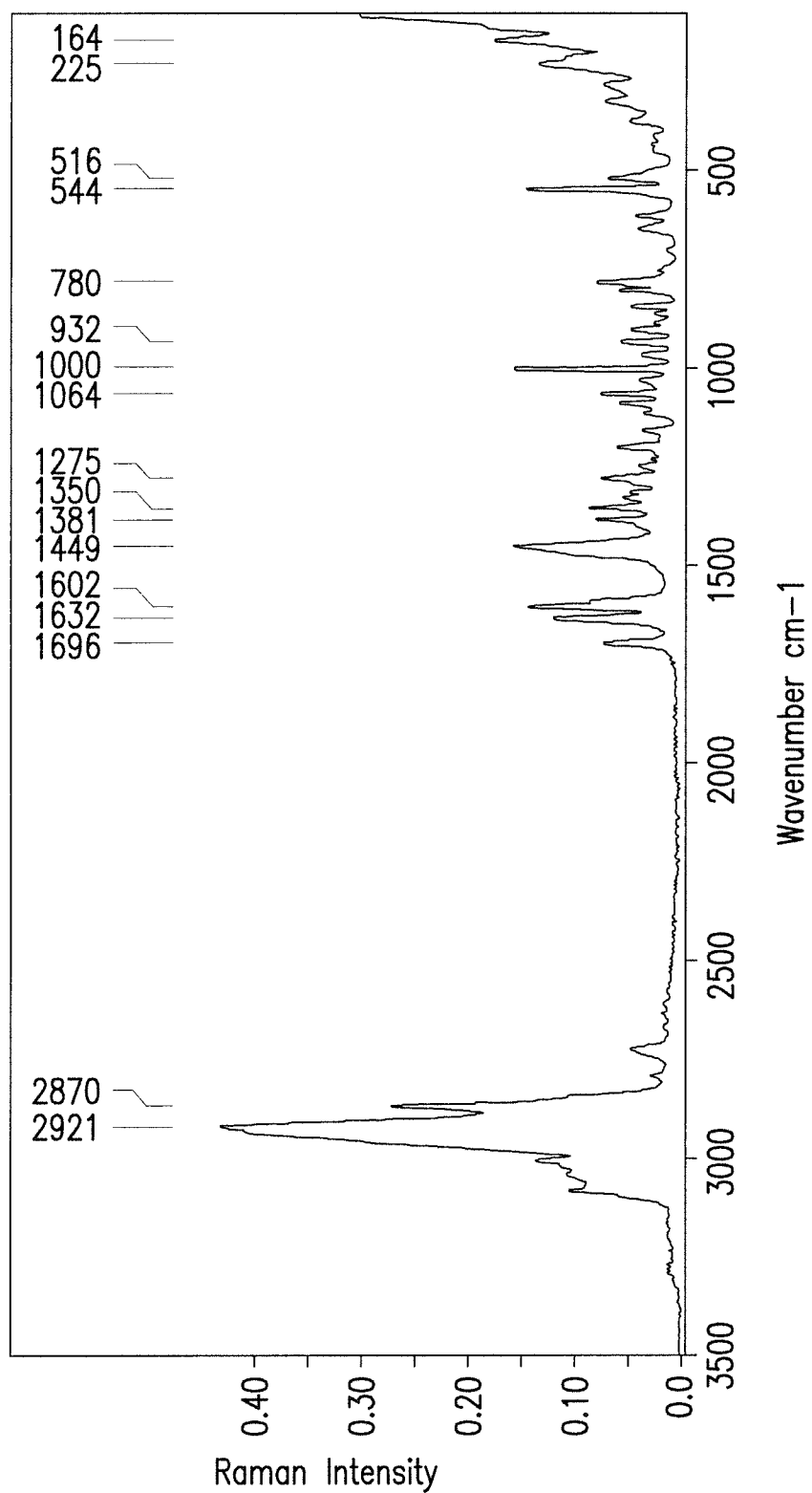


FIG. 30

Fig. 31

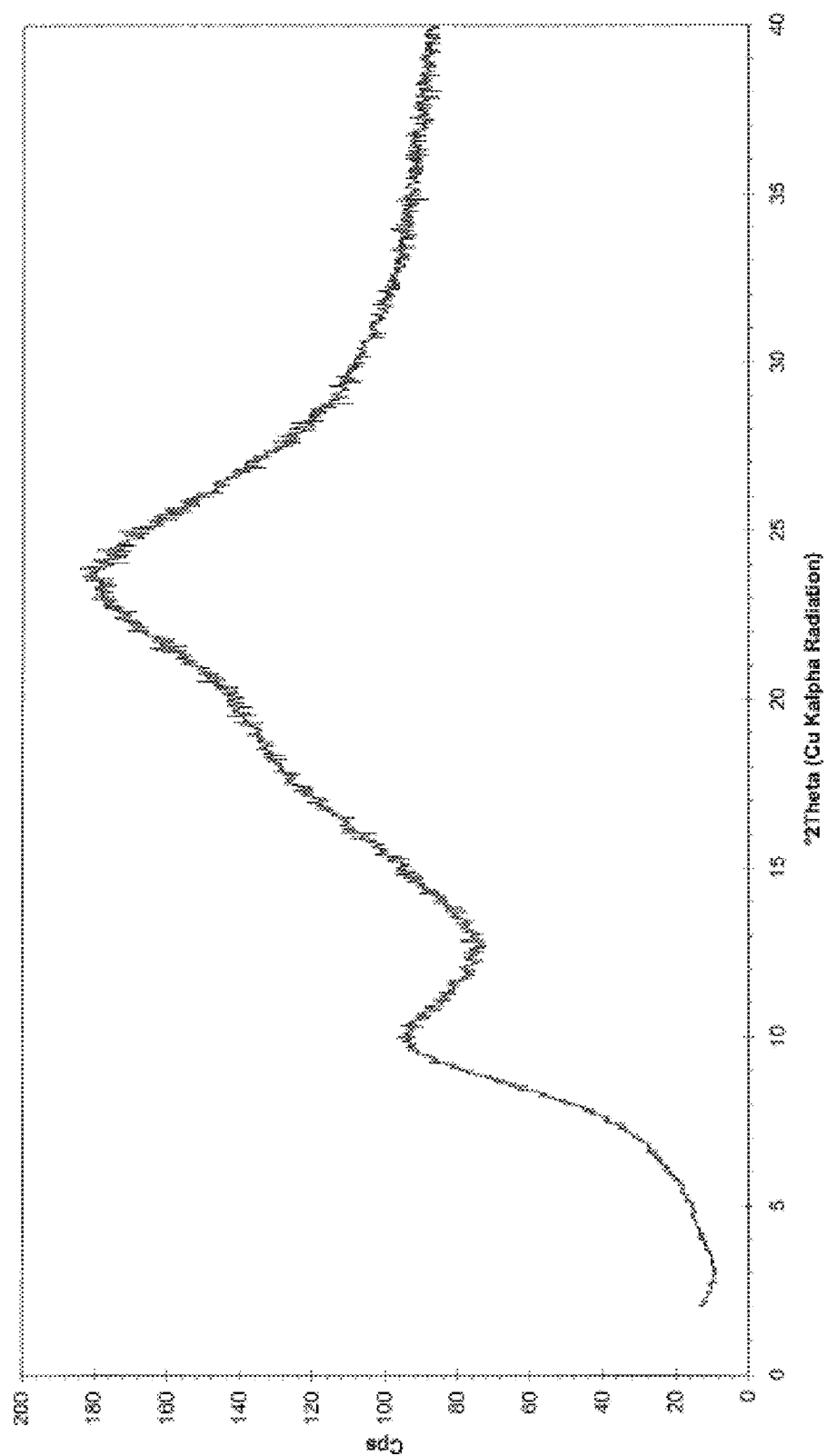
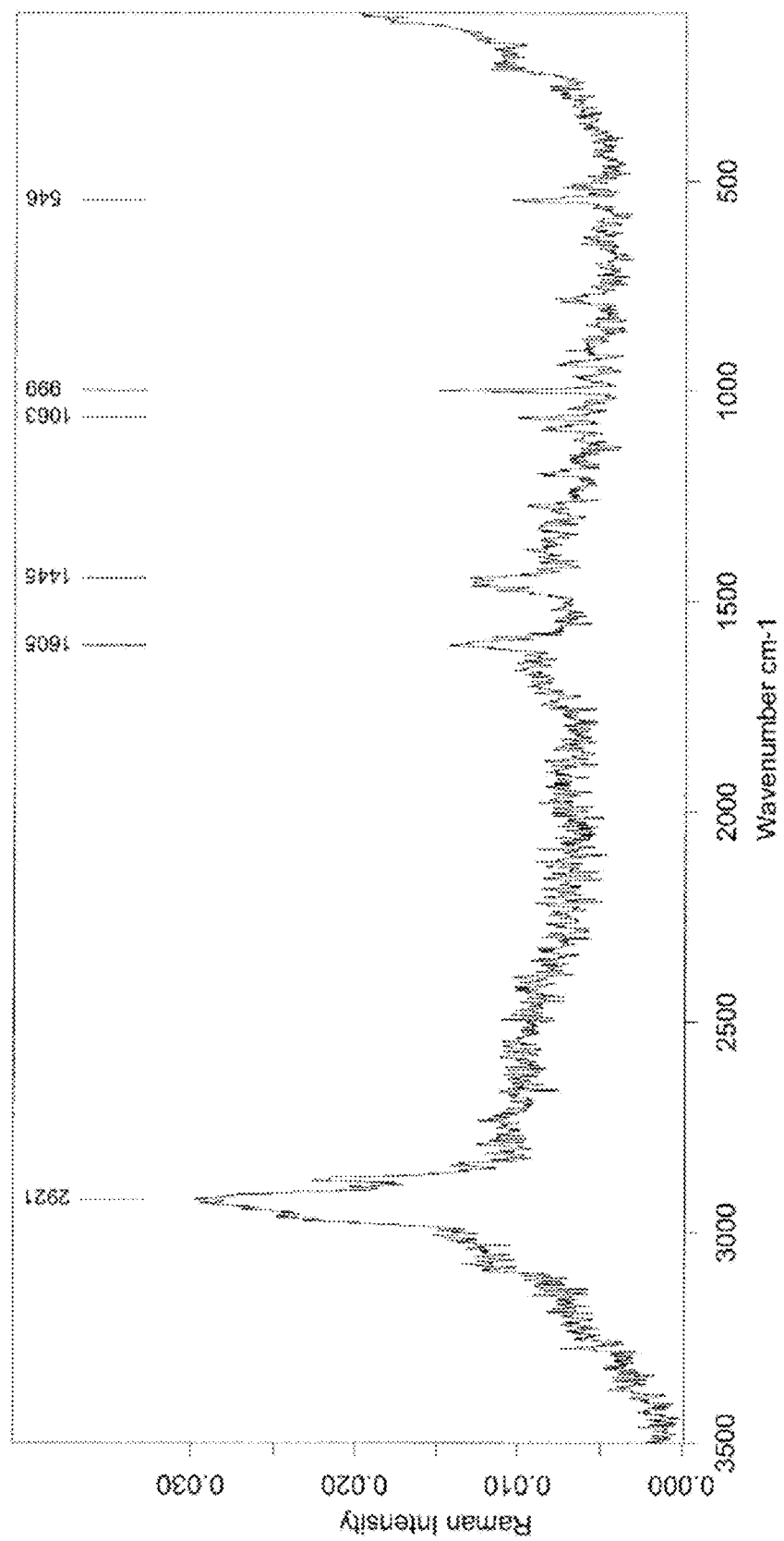


Fig. 32



1

CRYSTALLINE DIACYLHYDRAZINE AND THE USE THEREOF

BACKGROUND OF THE INVENTION

(R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide (referred to herein as "Compound 1") is a diacylhydrazine ligand used in ecdysone receptor-based inducible gene expression systems to regulate in vitro and in vivo gene expression, and treat diseases such as cancer.

US 2009/0163592 A1 discloses Compound 1, methods of making Compound 1, compositions comprising Compound 1, and methods of using Compound 1 to modulate in vitro or in vivo therapeutic gene expression in a host cell. For example, murine IL-12 expression, under the control of the RheoSwitch Therapeutic System® (RTS®) technology, is induced by administration of Compound 1 to mice. US 2009/0163592 A1 also discloses (S)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide (referred to herein as "Compound 2").

BRIEF SUMMARY OF THE INVENTION

There exists a need for stable crystalline polymorphic forms of Compound 1 or Compound 2, and methods to reproducibly make them, for use in regulating gene expression in ecdysone receptor-based inducible gene expression systems. The present disclosure provides crystalline polymorphic forms of Compound 1 or Compound 2, including anhydrous, hydrated, and solvated forms. The present disclosure also provides amorphous forms of Compound 1 or Compound 2.

In another aspect, the present disclosure provides methods of making crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2.

In another aspect, the present disclosure provides compositions comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more excipients.

In another aspect, the present disclosure provides methods of making compositions comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more excipients.

In another aspect, the present disclosure provides in vitro methods of regulating gene expression in a host cell, comprising contacting the cell with one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more excipients.

In another aspect, the present disclosure provides in vivo methods of regulating gene expression in a subject for the treatment of disease, comprising administering to the subject one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a pharmaceutically acceptable composition comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more pharmaceutically acceptable excipients.

In another aspect, the present disclosure provides methods of controlling insects, comprising contacting insects or their habitat with one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof.

2

In another aspect, the present disclosure provides kits comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a PXRD diffractogram of Form I-A of Compound 1.

FIG. 2 is a FT-Raman spectrum of Form I-A of Compound 1.

FIG. 3 DCS thermogram of Form I-A of Compound 1.

FIG. 4 is a PXRD diffractogram of Form I-B of Compound 1.

FIG. 5 is a PXRD diffractogram of Form I-C of Compound 1.

FIG. 6 is a PXRD diffractogram of Form I-D of Compound 1.

FIG. 7 is a PXRD diffractogram of Form I-E of Compound 1.

FIG. 8 is a FT-Raman spectrum of Form I-F of Compound 1.

FIG. 9 is a FT-Raman spectrum of Form I-G of Compound 1.

FIG. 10 is a PXRD diffractogram of Form I-H of Compound 1.

FIG. 11 is a PXRD diffractogram of pure Form II of Compound 1.

FIG. 12 is a FT-Raman spectrum of pure Form II of Compound 1.

FIG. 13 is a DCS thermogram of pure Form II of Compound 1.

FIG. 14 is a PXRD diffractogram of pure Form III of Compound 1.

FIG. 15 is a FT-Raman spectrum of pure Form III of Compound 1.

FIG. 16 is a DCS thermogram of pure Form III of Compound 1.

FIG. 17 is a PXRD diffractogram of pure Form IV of Compound 1.

FIG. 18 is a FT-Raman spectrum of pure Form IV of Compound 1.

FIG. 19 is a DCS thermogram of pure Form IV of Compound 1.

FIG. 20 is a DCS thermogram of pure Form IV (dried) of Compound 1.

FIG. 21 is a PXRD diffractogram of pure Form V of Compound 1.

FIG. 22 is a FT-Raman spectrum of pure Form V of Compound 1.

FIG. 23 is a PXRD diffractogram of pure Form VI of Compound 1.

FIG. 24 is a FT-Raman spectrum of pure Form VI of Compound 1.

FIG. 25 is a PXRD diffractogram of pure Form VII of Compound 1.

FIG. 26 is a FT-Raman spectrum of pure Form VII of Compound 1.

FIG. 27 is a PXRD diffractogram of pure Form VIII of Compound 1.

FIG. 28 is a FT-Raman spectrum of pure Form VIII of Compound 1.

FIG. 29 is a PXRD diffractogram of pure Form IX of Compound 1.

FIG. 30 is a FT-Raman spectrum of pure Form IX of Compound 1.

FIG. 31 is a PXRD diffractogram of Form X of Compound 1.

FIG. 32 is a FT-Raman spectrum of Form X of Compound 1.

DETAILED DESCRIPTION OF THE INVENTION

In one aspect, the present disclosure provides crystalline polymorphic forms of Compound 1, or mixtures thereof, or crystalline polymorphic forms of Compound 2, or mixtures thereof.

In another aspect, the present disclosure provides Compound 1 comprising Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or a mixture thereof, or Compound 2 comprising Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or a mixture thereof.

In another aspect, the present disclosure provides Compound 1 consisting essentially of Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or Compound 2 consisting essentially of Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX.

In another aspect, the present disclosure provides Compound 1 consisting of Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or Compound 2 consisting of Form II, Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX.

In another aspect, the present disclosure provides Compound 1 comprising Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or a mixture thereof, or Compound 2 comprising Form III, Form IV, Form V, Form VI, Form VII, Form VIII, or Form IX, or a mixture thereof.

In another aspect, the present disclosure provides Compound 1 comprising Form II, Form III, or Form IV, or a mixture thereof, or Compound 2 comprising Form II, Form III, or Form IV, or a mixture thereof.

In another aspect, the present disclosure provides Compound 1 comprising Form III, or Form IV, or a mixture thereof, or Compound 2 comprising Form III, or Form IV, or a mixture thereof.

In another aspect, the present disclosure provides Compound 1 Form II or

Compound 2 Form II. In one embodiment, Form II is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 8.34, 10.06, 14.01, 16.77, 17.70, 18.40, 20.23, 22.36, 22.97, and 25.00 degrees 2θ . In another embodiment, Compound 1 Form II is characterized as having a PXRD pattern with peaks at 8.34, 10.06, 14.01, 14.51, 15.55, 16.77, 17.70, 18.40, 18.88, 20.23, 22.36, 22.97, 23.91, 24.15, 25.00, 25.92, 26.96, 28.09, 28.33, 29.84, 30.52, 31.05, 31.45, 31.97, 32.61, 33.17, 34.02, 34.45, and 35.07 degrees 2θ . In another embodiment, Form II is characterized as having a PXRD pattern that is essentially the same as FIG. 11. In another embodiment, Form II is characterized as having a FT-Raman spectrum with peaks at 3007, 2920, 2869, 1696, 1629, 1605, 1449, 1381, 1351, 1275, 1194, 1086, 1064, 1000, 931, 780, 544, 517, 225, 164 cm^{-1} . In another embodiment, Form II is characterized as having a FT-Raman spectrum essentially the same as FIG. 12. In another embodiment, the present disclosure provides substantially pure Form II. In another embodiment, the present disclosure provides pure Form II. In another embodiment, the present disclosure provides pure Compound 1 Form II.

In another aspect, the present disclosure provides Compound 1 Form III or Compound 2 Form III. In one embodiment, Form III is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 8.14, 8.52, 17.00, 18.56, and 22.19 degrees 2θ . In another embodiment, Form III is characterized as having a PXRD pattern with peaks at 8.14, 8.52, 9.62, 11.02, 11.90, 12.16, 14.02, 14.62, 17.00, 17.88, 18.56, 19.02, 19.24, 20.51, 20.93, 22.19, 22.73, 23.22, 24.31, 24.53, 25.91, 26.22, 27.36, 27.73, 28.70, 30.84, 31.52, 32.30, 33.19, and 34.39 degrees 2θ . In another embodiment, Form III is characterized as having a PXRD pattern that is essentially the same as FIG. 14. In another embodiment, Form III is characterized as having an FT-Raman spectrum with peaks at 2922, 2873, 2837, 1699, 1628, 1602, 1449, 1379, 1274, 1090, 1065, 998, 778, 637, 549, 515, 320, 225, 165, and 127 cm^{-1} . In another embodiment, Form III is characterized as having an FT-Raman spectrum essentially the same as FIG. 15. In another embodiment, the present disclosure provides substantially pure Form III. In another embodiment, the present disclosure provides pure Form III. In another embodiment, the present disclosure provides pure Compound 1 Form III.

In another aspect, the present disclosure provides Compound 1 Form IV or Compound 2 Form IV. In one embodiment, Form IV is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 6.83, 10.31, 11.30, 12.18, 12.98, 13.69, 15.11, 16.23, 17.60, 17.99, 20.70, 21.15, 21.68, 22.71, 23.79, and 24.86 degrees 2θ . In another embodiment, Form IV is characterized as having a PXRD pattern with peaks at 6.83, 8.38, 8.91, 10.11, 10.31, 11.30, 11.89, 12.18, 12.98, 13.69, 14.14, 15.11, 15.81, 16.23, 17.60, 17.99, 18.60, 19.15, 19.66, 20.28, 20.70, 21.15, 21.68, 22.44, 22.71, 23.50, 23.79, 24.06, 24.86, 25.55, 26.53, 26.94, 27.21, 27.60, 28.67, 29.79, 30.50, 30.75, 31.55, 31.89, 32.78, 33.25, 33.48, 33.81, and 34.68 degrees 2θ . In another embodiment, Form IV is characterized as having a PXRD pattern that is essentially the same as FIG. 17. In another embodiment, Form IV is characterized as having a FT-Raman spectrum with peaks at 3005, 2919, 2873, 2836, 1691, 1625, 1600, 1448, 1380, 1353, 1278, 1195, 1064, 998, 878, 789, 633, 545, 237, and 168 cm^{-1} . In another embodiment, Form IV is characterized as having a FT-Raman spectrum essentially the same as FIG. 18. In another embodiment, the present disclosure provides substantially pure Form IV. In another embodiment, the present disclosure provides pure Form IV. In another embodiment, the present disclosure provides pure Compound 1 Form IV.

In another aspect, the present disclosure provides Compound 1 Form V or Compound 2 Form V. In one embodiment, Form V is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 9.38, 12.22, 13.18, 14.98, 17.32, 18.40, 22.41, 23.40, 23.55, 24.63, 24.79, 25.61, 28.02, and 31.77 degrees 2θ . In another embodiment, Form V is characterized as having a PXRD pattern with peaks at 6.11, 9.38, 11.13, 12.22, 13.18, 14.14, 14.98, 15.52, 15.78, 17.32, 18.40, 18.75, 19.48, 19.74, 20.63, 21.33, 21.88, 22.41, 23.40, 23.55, 23.76, 24.27, 24.63, 24.79, 25.61, 26.66, 27.10, 27.81, 28.02, 28.58, 29.91, 30.35, 30.95, 31.32, 31.77, 32.77, 33.81, and 34.98 degrees 2θ . In another embodiment, Form V is characterized as having a PXRD pattern that is essentially the same as FIG. 21. In another embodiment, Form V is characterized as having a FT-Raman spectrum with peaks at 3010, 2963, 2938, 2872, 2836, 1690, 1624, 1597, 1452, 1359, 1317, 1275, 1193, 1062, 999, 877, 788, 546, 516, and 168 cm^{-1} . In another embodiment, Form V is characterized as having an FT-Raman spectrum essentially the same as FIG. 22. In another

5

embodiment, the present disclosure provides substantially pure Form V. In another embodiment, the present disclosure provides pure Form V. In another embodiment, the present disclosure provides pure Compound 1 Form V.

In another aspect, the present disclosure provides Compound 1 Form VI or Compound 2 Form VI. In one embodiment, Form VI is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 9.38, 12.23, 13.25, 17.48, 18.41, and 22.41 degrees 2θ . In another embodiment, Form VI is characterized as having a PXRD pattern with peaks at 6.09, 6.82, 8.57, 9.38, 11.26, 12.23, 13.25, 14.27, 15.05, 15.54, 15.95, 17.48, 18.41, 18.79, 19.54, 19.76, 20.79, 21.48, 22.02, 22.41, 23.42, 24.07, 24.34, 24.64, 24.83, 25.34, 25.67, 26.74, 26.87, 27.24, 27.99, 28.56, 28.93, 29.47, 30.04, 30.98, 31.75, 32.34, 32.96, and 33.84 degrees 2θ . In another embodiment, Form VI is characterized as having a PXRD pattern that is essentially the same as FIG. 23. In another embodiment, Form VI is characterized as having a FT-Raman spectrum with peaks at 3010, 2963, 2938, 2917, 2873, 2836, 1692, 1626, 1598, 1453, 1381, 1357, 1317, 1275, 1194, 999, 878, 788, 545, and 167 cm^{-1} . In another embodiment, Form VI is characterized as having an FT-Raman spectrum essentially the same a FIG. 24. In another embodiment, the present disclosure provides substantially pure Form VI. In another embodiment, the present disclosure provides pure Form VI. In another embodiment, the present disclosure provides pure Compound 1 Form VI.

In another aspect, the present disclosure provides Compound 1 Form VII or Compound 2 Form VII. In one embodiment, Form VII is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 8.18, 9.71, 13.30, 16.22, 17.73, 20.98, 21.20, 22.76, 24.68, 26.72, and 29.39 degrees 2θ . In another embodiment, Form VII is characterized as having a PXRD pattern with peaks at 6.64, 8.18, 9.71, 10.44, 10.80, 11.69, 13.30, 13.64, 15.35, 16.22, 16.44, 17.23, 17.73, 18.16, 19.46, 19.72, 19.97, 20.70, 20.98, 21.20, 21.52, 21.98, 22.57, 22.76, 23.09, 23.75, 24.37, 24.68, 25.31, 25.97, 26.25, 26.49, 26.72, 28.13, 29.39, 29.88, 30.92, 31.17, 31.70, 31.96, 33.57, and 34.83 degrees 2θ . In another embodiment, Form VII is characterized as having a PXRD pattern that is essentially the same as FIG. 25. In another embodiment, Form VII is characterized as having a FT-Raman spectrum with peaks at 2916, 1692, 1651, 1603, 1452, 998, 781, 673, 543, and 228 cm^{-1} . In another embodiment, Form VII is characterized as having a FT-Raman spectrum essentially the same a FIG. 26. In another embodiment, the present disclosure provides substantially pure Form VII. In another embodiment, the present disclosure provides pure Form VII. In another embodiment, the present disclosure provides pure Compound 1 Form VII.

In another aspect, the present disclosure provides Compound 1 Form VIII or Compound 2 Form VIII. In one embodiment, Form VIII is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 10.05, 10.77, 14.06, 16.76, 18.11, 18.32, 18.43, 20.89, 21.71, 21.87, 24.07, 24.90, and 28.71 degrees 2θ . In another embodiment, Form VIII is characterized as having a PXRD pattern with peaks at 7.48, 8.31, 10.05, 10.77, 12.27, 13.65, 14.06, 15.60, 16.03, 16.76, 16.96, 17.16, 18.11, 18.32, 18.43, 18.65, 19.89, 20.28, 20.89, 21.71, 21.87, 24.07, 24.90, 25.28, 25.54, 25.86, 26.22, 26.66, 27.74, 28.44, 28.71, 29.08, 30.26, 31.16, 32.59, 32.85, 34.01, 34.68, and 35.09 degrees 2θ . In another embodiment, Form VIII is characterized as having a PXRD pattern that is essentially the same as FIG. 27. In another embodiment, Form VIII is characterized as

6

having a FT-Raman spectrum with peaks at 3056, 2921, 2874, 1690, 1634, 1601, 1447, 1278, 1206, 1157, 1091, 1069, 1002, 877, 793, 621, 542, 515, 370, and 105 cm^{-1} . In another embodiment, Form VIII is characterized as having an FT-Raman spectrum essentially the same a FIG. 28. In another embodiment, the present disclosure provides substantially pure Form VIII. In another embodiment, the present disclosure provides pure Form VIII. In another embodiment, the present disclosure provides pure Compound 1 Form VIII.

In another aspect, the present disclosure provides Compound 1 Form IX or Compound 2 Form IX. In one embodiment, Form IX is characterized as having a powder x-ray diffraction (PXRD) pattern with peaks at 7.06, 15.74, and 18.71 degrees 2θ . In another embodiment, Form IX is characterized as having a PXRD pattern with peaks at 7.06, 9.93, 12.22, 14.13, 15.74, 17.28, 18.71, 19.96, 21.18, 22.39, 23.51, 24.54, 25.58, 27.52, 28.48, 29.33, 30.18, 31.01, 31.82, and 32.73 degrees 2θ . In another embodiment, Form IX is characterized as having a PXRD pattern that is essentially the same as FIG. 29. In another embodiment, Form IX is characterized as having a FT-Raman spectrum with peaks 2921, 2870, 1696, 1632, 1602, 1449, 1381, 1350, 1275, 1064, 1000, 932, 780, 544, 516, 225, and 164 cm^{-1} . In another embodiment, Form IX is characterized as having an FT-Raman spectrum essentially the same a FIG. 30. In another embodiment, the present disclosure provides substantially pure Form IX. In another embodiment, the present disclosure provides pure Form IX. In another embodiment, the present disclosure provides pure Compound 1 Form IX.

In another aspect, the present disclosure provides Compound 1 Form X or Compound 2 Form X. Form X is an amorphous form of Compound 1 or Compound 2. In one embodiment, Form X is characterized as having a PXRD pattern that is essentially the same as FIG. 31. In another embodiment, Form X is characterized as having an FT-Raman spectrum essentially the same as FIG. 32. In another embodiment, the present disclosure provides substantially pure Form X. In another embodiment, the disclosure provides pure Form X. In another embodiment, the present disclosure provides pure Compound 1 in amorphous form.

In another aspect, the present disclosure provides micronized crystalline polymorphic forms or amorphous forms of Compound 1, or micronized crystalline polymorphic forms or amorphous forms of Compound 2. In one embodiment, the average particle size distribution of the micronized form of Compound 1 or Compound 2 is about 20 μm or less, e.g., about 19 μm , about 18 μm , about 17 μm , about 16 μm , about 15 μm , about 14 μm , about 13 μm , about 12 μm , or about 11 μm , or less. In another embodiment, the average particle size distribution is about 10 μm or less, e.g., about 9 μm , about 8 μm , about 7 μm , about 6 μm , or about 5 μm , or less. In another embodiment, the average particle size distribution is about 5 μm or less, e.g., about 4 μm , about 3 μm , about 2 μm , or about 1 μm , or less. In another embodiment, the average particle size distribution is about 1 μm or less, e.g., about 0.9 μm , about 0.8 μm , about 0.7 μm , about 0.6 μm , about 0.5 μm , about 0.4 μm , about 0.3 μm , about 0.2 μm , about 0.1 μm , about 0.09 μm , about 0.08 μm , about 0.07 μm , about 0.06 μm , about 0.05 μm , about 0.04 μm , about 0.03 μm , about 0.02 μm , or about 0.01 μm or less. In another embodiment, the present disclosure provides micronized crystalline Compound 1 Form III having an average particle size of about 20 μm or less, e.g., about 19 μm , about 18 μm , about 17 μm , about 16 μm , about 15 μm , about 14 μm , about 13 μm , about 12 μm , about 11 μm , about 10 μm , about 9 μm , about 8 μm , about 7 μm , about 6 μm , about 5 μm , about 4 μm , about 3

μm, about 2 μm, about 1 μm, about 0.9 μm, about 0.8 μm, about 0.7 μm, about 0.6 μm, about 0.5 μm, about 0.4 μm, about 0.3 μm, about 0.2 μm, about 0.1 μm, about 0.09 μm, about 0.08 μm, about 0.07 μm, about 0.06 μm, about 0.05 μm, about 0.04 μm, about 0.03 μm, about 0.02 μm, or about 0.01 μm, or less. In another embodiment, the present disclosure provides micronized amorphous Compound 1 Form X having an average particle size of about 20 μm or less, e.g., about 19 μm, about 18 μm, about 17 μm, about 16 μm, about 15 μm, about 14 μm, about 13 μm, about 12 μm, about 11 μm, about 10 μm, about 9 μm, about 8 μm, about 7 μm, about 6 μm, about 5 μm, about 4 μm, about 3 μm, about 2 μm, about 1 μm, about 0.9 μm, about 0.8 μm, about 0.7 μm, about 0.6 μm, about 0.5 μm, about 0.4 μm, about 0.3 μm, about 0.2 μm, about 0.1 μm, about 0.09 μm, about 0.08 μm, about 0.07 μm, about 0.06 μm, about 0.05 μm, about 0.04 μm, about 0.03 μm, about 0.02 μm, or about 0.01 μm, or less.

In another aspect, the present disclosure provides methods of making crystalline polymorphic forms or amorphous forms of Compound 1 or crystalline polymorphic forms or amorphous forms of Compound 2. Methods of making crystalline polymorphic forms or amorphous forms of Compound 1 are described in the Examples provided herein below. Other methods used to make crystalline polymorphic forms of Compound 1 include sublimation and pressurization, e.g., under CO₂ (*J. Am. Chem. Soc.* 133:1399 (2011)). Similar methods can be used to make crystalline polymorphic forms or amorphous forms of Compound 2.

In one embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in one or more non-solvate forming solvents, and isolated, e.g., by filtration or centrifugation, to give substantially pure Form III. In another embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in one or more non-solvate forming solvents, and isolated to give pure Form III. In another embodiment, the equilibration of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is repeated more than once, e.g., two, three, four, or five times, or more, to give pure Form III. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated for a period of time, e.g., about 15 minutes to about 24 hours, about 1 hour to about 8 hours, or about 1 hour to about 4 hours. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated for about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 15 hours, about 18 hours, about 20 hours, about 21 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about a month, or longer, until complete conversion to Form III is observed. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated at about 45° C. or less, e.g., at about 44° C., about 43° C., about 42° C., about 41° C., about 40° C., about 39° C., about 38° C., about 37° C., about 36° C., about 35° C., about 34° C., about 33° C., about 32° C., about 31° C., about 30° C., about 29° C., about 28° C., about 27° C., about 26° C. (i.e., about room temperature), about 25° C., about 24° C.,

about 23° C., about 22° C., about 21° C., about 20° C., about 19° C., about 18° C., about 17° C., about 16° C., about 15° C., about 14° C., about 13° C., about 12° C., about 11° C., about 10° C., about 9° C., about 8° C., about 7° C., about 6° C., about 5° C., about 4° C., about 3° C., about 2° C., about 1° C., or about 0° C., or less. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated at about room temperature, or less. In another embodiment, the one or more non-solvate forming solvents are n-heptane, cumene, diethyl ether, toluene, ethyl acetate, tert-butyl methyl ether, or n-dodecane. In another embodiment, the one or more non-solvate forming solvents are n-heptane, toluene, ethanol, or isopropanol. If two non-solvate forming solvents, e.g., heptane/toluene, are used, the ratio of solvents is about 50:1, e.g., about 25:1, about 20:1; about 10:1, about 9:1, about 8:1, about 7:1; about 6:1; about 5:1, about 4:1, about 3:1, about 2:1, or about 1:1.

In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/toluene, and isolated to give substantially pure Form III. In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/toluene, and isolated to give pure Form III. In another embodiment, the ratio of heptane:toluene is about 10:1 to about 1:10, e.g., about 5:1 to about 1:5, about 3:1 to about 1:3, or about 2:3 to about 3:2. In another embodiment, the ratio of heptane:toluene is about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 2:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10. In another embodiment, the ratio of heptane:toluene is about 9:1. In another embodiment, the ratio of heptane:toluene is about 2:3. In another embodiment, the suspension in heptane/toluene is equilibrated at about 25° C. In another embodiment, the suspension in heptane/toluene is equilibrated at about 5° C. In another embodiment, the suspension in heptane/toluene is equilibrated for about 2, about 3, about 4, or about 5 hours. In another embodiment, the suspension in heptane/toluene is equilibrated for about 20 hours. In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/toluene, isolated, re-equilibrated in heptane/toluene, or in another non-solvate forming solvent or mixture of non-solvate forming solvents, and re-isolated to give pure Form III.

In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/isopropanol or in heptane/ethanol, and isolated to give substantially pure Form III. In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/isopropanol or in heptane/ethanol, and isolated to give pure Form III. In another embodiment, the ratio of heptane:isopropanol or heptane:ethanol is about 25:1 to about 1:25, about 20:1 to about 1:20, about 10:1 to about 1:10, about 5:1 to about 1:5, or about 2:3 to about 3:2. In another embodiment, the ratio of heptane:isopropanol or heptane:ethanol is about 20:1, about 19:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 2:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, or about 1:10. In another embodiment, the ratio of heptane:isopropanol is about 3:2. In another embodiment, the ratio of heptane:ethanol is about 19:1. In another embodiment, the suspension in heptane/isopropanol or heptane/ethanol is equilibrated at about 25° C. In another embodiment, the

suspension in heptane/isopropanol or heptane/ethanol is equilibrated at about 5° C. In another embodiment, the suspension in heptane/isopropanol or heptane/ethanol is equilibrated for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, or more. In another embodiment, the suspension in heptane/isopropanol or heptane/ethanol is equilibrated for about 20 hours. In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/isopropanol, isolated, re-equilibrated in heptane/isopropanol, or in another non-solvate forming solvent or mixture of non-solvate forming solvents, and re-isolated to give pure Form III. In another embodiment, a suspension of one or more crystalline polymorphic forms of Compound 1 is equilibrated in heptane/ethanol, isolated, re-equilibrated in heptane/ethanol, or in another non-solvate forming solvent or mixture of non-solvate forming solvents, and re-isolated to give pure Form III.

In another embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in methanol/water and isolated to give substantially pure Form V. In another embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in methanol/water and isolated to give pure Form V. In another embodiment, the methanol content in the methanol/water mixture is greater than 60% by volume. In another embodiment, the equilibration of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is repeated in methanol/water more than once, e.g., two, three, four, or five times, or more, to give pure Form V. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in methanol/water for a period of time, e.g., for about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 10 hours, about 12 hours, about 15 hours, about 18 hours, about 20 hours, about 21 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about a month, or longer, until complete conversion to Form V is observed. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in methanol/water at about 65° C. or less, e.g., at about 60° C., at about 50° C., at about 45° C., at about 44° C., about 43° C., about 42° C., about 41° C., about 40° C., about 39° C., about 38° C., about 37° C., about 36° C., about 35° C., about 34° C., about 33° C., about 32° C., about 31° C., about 30° C., about 29° C., about 28° C., about 27° C., about 26° C. (i.e., about room temperature), about 25° C., about 24° C., about 23° C., about 22° C., about 21° C., about 20° C., about 19° C., about 18° C., about 17° C., about 16° C., about 15° C., about 14° C., about 13° C., about 12° C., about 11° C., about 10° C., about 9° C., about 8° C., about 7° C., about 6° C., about 5° C., about 4° C., about 3° C., about 2° C., about 1° C., or about 0° C., or less. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in methanol/water at about room temperature, or less.

In another embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in water and isolated to give substantially pure Form IV. In another embodiment, a suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in water

and isolated to give pure Form IV. In another embodiment, the equilibration of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is repeated in water more than once, e.g., two, three, four, or five times, or more, to give pure Form IV. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in water for a period of time, e.g., for about 15 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 10 hours, about 12 hours, about 15 hours, about 18 hours, about 20 hours, about 21 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 1 week, about 2 weeks, about 3 weeks, about a month, or longer, until complete conversion to Form IV is observed. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in water at about 65° C. or less, e.g., at about 60° C., at about 50° C., at about 45° C., at about 44° C., about 43° C., about 42° C., about 41° C., about 40° C., about 39° C., about 38° C., about 37° C., about 36° C., about 35° C., about 34° C., about 33° C., about 32° C., about 31° C., about 30° C., about 29° C., about 28° C., about 27° C., about 26° C. (i.e., about room temperature), about 25° C., about 24° C., about 23° C., about 22° C., about 21° C., about 20° C., about 19° C., about 18° C., about 17° C., about 16° C., about 15° C., about 14° C., about 13° C., about 12° C., about 11° C., about 10° C., about 9° C., about 8° C., about 7° C., about 6° C., or about 5° C. In another embodiment, the suspension of one or more crystalline polymorphic forms or amorphous forms of Compound 1 is equilibrated in water at about room temperature.

In another aspect, the present disclosure provides a method of preparing substantially pure Compound 1 Form III, the method comprising: a) combining a mixture of one or more polymorphic or amorphous forms of Compound 1 and two or more non-solvate forming solvents at about 26° C. to obtain a slurry; and b) filtering the slurry to give substantially pure Compound 1 Form III. In another embodiment, the two or more non-solvate forming solvents comprise heptane/toluene, heptane/isopropanol, or heptane/ethanol. In another embodiment, pure Compound 1 Form III is prepared according to steps a) and b).

In another aspect, the present disclosure provides a method of preparing substantially pure Compound 1 Form III, the method comprising: a) combining a mixture of one or more polymorphic or amorphous forms of Compound 1 and two or more non-solvate forming solvents at about 26° C. to obtain a slurry; b) heating the slurry to obtain a solution; c) cooling the solution to about 26° C. or less to form a precipitate; and d) filtering the precipitate to give substantially pure Compound 1 Form III. In another embodiment, the two or more non-solvate forming solvents comprise heptane/toluene, heptane/isopropanol, or heptane/ethanol. In another embodiment, the slurry is heated to about 45° C. or more, e.g., about 50° C., about 55° C., about 60° C., about 65° C., about 70° C., about 75° C., about 80° C., or more. In another embodiment, the solution is held at room. In another embodiment, pure Compound 1 Form III is prepared according to steps a), b), c), and d).

In another aspect, the present disclosure provides a method of preparing substantially pure Compound 1 Form III, the method comprising: a) combining a mixture of one or more crystalline polymorphic or amorphous forms of Compound 1 and two or more non-solvate forming solvents at about 26° C. to obtain a slurry; b) heating the slurry to obtain a solution; c) cooling the solution to about 40° C. to

about 30° C. (which may or may not cause some precipitation); d) adding about 0.5 weight % or less of pure Compound 1 Form III; and e) filtering the precipitate to give substantially pure Compound 1 Form III. In another embodiment, the two or more non-solvate forming solvents comprise heptane/toluene, heptane/isopropanol, or heptane/ethanol. In another embodiment, the slurry is heated to about 50° C. or more, e.g., about 55° C., about 60° C., about 65° C., about 70° C., about 75° C., about 80° C., or more. In another embodiment, pure Compound 1 Form III is prepared according to steps a), b), c), d), and e).

In another aspect, the present disclosure provides compositions comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more excipients. In one embodiment, compositions comprising Compound 1 Form III and one or more excipients are provided. In another embodiment, compositions comprising amorphous Compound 1 Form X and one or more excipients are provided. In one embodiment, the excipient comprises dimethyl sulfoxide or acetone. In one embodiment, the composition comprises a pharmaceutically acceptable excipient, i.e., a "pharmaceutically acceptable composition." In another embodiment, the composition comprises micronized crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2. In another embodiment, the composition comprises micronized crystalline Compound 1 Form III. In another embodiment, the pharmaceutically acceptable excipient comprises Miglyol 812, phospholipon 90G, or tocopheryl polyethylene glycol 1000 succinate, or a mixture thereof. In another embodiment, the pharmaceutically acceptable excipient consists essentially of Miglyol 812, phospholipon 90G, and tocopheryl polyethylene glycol 1000 succinate. In another embodiment, the pharmaceutically acceptable excipient comprises Labrasol®. In another embodiment, the pharmaceutically acceptable excipient comprises sorbitan monolaurate, hydroxypropylmethylcellulose acetate succinate, sodium taurocholate, Ethocel™ or palmitoyl-oleoyl-phosphatidylcholine, or a mixture thereof. In another embodiment, the pharmaceutically acceptable excipient comprises hydrogenated soy lecithin. Crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 can be admixed with one or more excipients using method well known to those of ordinary skill in the art.

Compositions may contain from 0.01% to 99% by weight of one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, e.g., about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95%. The amount in any particular composition will depend upon the effective dose, that is, the dose required to elicit the desired level of gene expression. In one embodiment, the composition comprises from 0.01 to 99% by weight of crystalline Compound 1 Form III. In another embodiment, the composition comprises from 0.01 to 99% by weight of amorphous Compound 1 Form X.

In another aspect, the present disclosure provides methods of making a composition, comprising admixing one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 with one or more excipients. In one embodiment, the excipient is a pharmaceutically acceptable excipient. In one embodiment, methods of making a composition comprising admixing Compound 1 Form III and one or more pharmaceutically acceptable excipient are pro-

vided. In another embodiment, methods of making a composition comprising admixing amorphous Compound 1 Form X and one or more pharmaceutically acceptable excipient are provided.

In another aspect, the present disclosure provides methods of regulating gene expression of a gene of interest in a host cell, comprising contacting the host cell with one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof. In one embodiment, methods of regulating gene expression of a gene of interest in a host cell, comprising contacting the host cell with a composition comprising crystalline Compound 1 Form III are provided. In another embodiment, methods of regulating gene expression of a gene of interest in a host cell, comprising contacting the host cell with a composition comprising amorphous Compound 1 are provided. In one embodiment, the host cell comprises a polynucleotide encoding a gene switch comprising a ligand binding domain that binds Compound 1 or Compound 2, wherein the level of expression of the gene of interest is increased, relative to the level of expression of the gene of interest in the absence of Compound 1 or Compound 2, respectively. In another embodiment, the host cell is an isolated host cell. In another embodiment, the host cell is in a subject, e.g., an animal, e.g., a human. In another embodiment, one or more crystalline polymorphic forms of Compound 1 are administered to a subject as a pharmaceutically acceptable composition. In another embodiment, the gene switch comprises an ecdysone receptor ligand binding domain. In another embodiment, the gene switch further comprises a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2. In another embodiment, the ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 is a retinoic X receptor ligand binding domain. In another embodiment, the ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 is a wild-type insect USP (Ultraspiracle protein). In another embodiment, the retinoic X receptor ligand binding domain is a chimeric retinoic X receptor ligand binding domain. In another embodiment, the host cell further comprises a polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch.

In another aspect, the present disclosure provides methods of treating a disease, disorder, injury, or condition in a subject, comprising administering to the subject one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof. In one embodiment, a host cell within the subject comprises a polynucleotide encoding a gene switch that comprises a ligand binding domain that binds Compound 1 or Compound 2. In another embodiment, the subject is human. In another embodiment, the disease, disorder, injury, or condition is selected from the group consisting of cancer, metabolic-related disorder, kidney disease, anemia, autoimmune disorder, ocular disorder, blood disorder, neurological disorder, lung disorder, rheumatologic disorder, and infectious disease. In another embodiment, the disease, disorder, injury, or condition is cancer. In another embodiment, the cancer is melanoma. In another embodiment, the gene switch comprises an ecdysone receptor ligand binding domain. In another embodiment, the gene switch further comprises a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1. In another embodiment, the ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or

Compound 2 is a retinoic X receptor ligand binding domain. In another embodiment, the retinoic X receptor ligand binding domain is a chimeric retinoic X receptor ligand binding domain. In another embodiment, the host cell further comprises a polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch. In another embodiment, the gene switch regulates the expression of a polynucleotide encoding IL-12 or a subunit thereof (See, for example, WO 2010/042189 A2).

In another embodiment, the present disclosure provides one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, for use in treating a disease, disorder, injury, or condition in a subject.

In another embodiment, the present disclosure provides one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, for use in the manufacture of a medicament for treating a disease, disorder, injury, or condition in a subject.

In another aspect, the present disclosure provides kits comprising one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or kits comprising a composition of one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and one or more excipients. In one embodiment, the kit further comprises instructions for administering the one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 to an isolated host cell or a subject. In another embodiment, the kit further comprises the RheoSwitch® Therapeutic System (see, for example, the Instruction Manual for "RheoSwitch® Mammalian Inducible Expression System," New England BioLabs® Inc., Version 1.3, November 2007; Karzenowski, D. et al., *BioTechniques* 39:191-196 (2005); Dai, X. et al., *Protein Expr. Purif.* 42:236-245 (2005); Palli, S. R. et al., *Eur. J. Biochem.* 270:1308-1515 (2003); Dhadialla, T. S. et al., *Annual Rev. Entomol.* 43:545-569 (1998); Kumar, M. B. et al., *J. Biol. Chem.* 279:27211-27218 (2004); Verhaegent, M. and Christopoulos, T. K., *Anal. Chem.* 74:4378-4385 (2002); Katalam, A. K., et al., *Molecular Therapy* 13:S103 (2006); and Karzenowski, D. et al., *Molecular Therapy* 13:S194 (2006)).

The crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 described herein may be administered to a subject in conjunction with other pharmaceutically active compounds. It will be understood by those skilled in the art that pharmaceutically active compounds to be used in combination the crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 will be selected in order to avoid adverse effects on the recipient or undesirable interactions between the compounds. Examples of other pharmaceutically active compounds which may be used in combination with one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 include, for example, AIDS chemotherapeutic agents, amino acid derivatives, analgesics, anesthetics, anorectal products, antacids and antiflatulents, antibiotics, anticoagulants, antidotes, antifibrinolytic agents, antihistamines, anti-inflammatory agents, antineoplastics, antiparasitics, anti-protozoals, antipyretics, antiseptics, antispasmodics and anticholinergics, antivirals, appetite suppressants, arthritis medications, biological response modifiers, bone metabolism regulators, bowel evacuates, cardiovascular agents, central nervous system stimulants, cerebral metabolic enhancers, cerumenolytics, cholinesterase inhibitors, cold and cough preparations, colony stimulating factors, contraceptives, cytoprotective agents, dental preparations, deodor-

ants, dermatologicals, detoxifying agents, diabetes agents, diagnostics, diarrhea medications, dopamine receptor agonists, electrolytes, enzymes and digestants, ergot preparations, fertility agents, fiber supplements, antifungal agents, galactorrhea inhibitors, gastric acid secretion inhibitors, gastrointestinal prokinetic agents, gonadotropin inhibitors, hair growth stimulants, hematinics, hemorrheologic agents, hemostatics, histamine H₂ receptor antagonists, hormones, hyperglycemic agents, hypolipidemics, immunosuppressants, laxatives, leprostatics, leukapheresis adjuncts, lung surfactants, migraine preparations, mucolytics, muscle relaxant antagonists, muscle relaxants, narcotic antagonists, nasal sprays, nausea medications nucleoside analogues, nutritional supplements, osteoporosis preparations, oxytocics, parasympatholytics, parasympathomimetics, Parkinsonism drugs, Penicillin adjuvants, phospholipids, platelet inhibitors, porphyria agents, prostaglandin analogues, prostaglandins, proton pump inhibitors, pruritus medications psychotropics, quinolones, respiratory stimulants, saliva stimulants, salt substitutes, sclerosing agents, skin wound preparations, smoking cessation aids, sulfonamides, sympatholytics, thrombolytics, Tourette's syndrome agents, tremor preparations, tuberculosis preparations, uricosuric agents, urinary tract agents, uterine contractants, uterine relaxants, vaginal preparations, vertigo agents, vitamin D analogs, vitamins, and medical imaging contrast media. In some cases Compound 1 may be useful as an adjunct to drug therapy, for example, to "turn off" a gene that produces an enzyme that metabolizes a particular drug.

For agricultural applications, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 may be used to control the expression of pesticidal proteins such as *Bacillus thuringiensis* (Bt) toxin. Such expression may be tissue or plant specific. In addition, particularly when control of plant pests is also needed, one or more pesticides may be combined with crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, thereby providing additional advantages and effectiveness, including fewer total applications, than if the pesticides are applied separately. When mixtures with pesticides are employed, the relative proportions of each component in the composition will depend upon the relative efficacy and the desired application rate of each pesticide with respect to the crops, pests, and/or weeds to be treated. Those skilled in the art will recognize that mixtures of pesticides may provide advantages such as a broader spectrum of activity than one pesticide used alone. Examples of pesticides which can be combined in compositions with crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 include fungicides, herbicides, insecticides, miticides, and microbicides.

Ecdysone receptors in insects are naturally responsive to the ecdysone steroid hormone (molting hormone) and other steroidal compounds such as ponasterone A and muristerone A. (Graham et al., *Insect Biochemistry and Molecular Biology* 37:611-626 (2007); Dinan and Hormann, "Ecdysteroid Agonists and Antagonists," *Comprehensive Molecular Insect Science*, 1st ed.:197-242, (2005)). Diacylhydrazines having ecdysone receptor agonist activity have been described as insecticides. (See U.S. Pat. No. 5,530,028).

In another aspect, the present disclosure provides a method of controlling, e.g., reducing or preventing the spread of, or killing, insects comprising contacting the insects or their habitat with an insecticidally effective amount of one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof. In another embodiment, one or more

crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are insecticidally active against:

(1) insects from the order of the lepidopterans (Lepidoptera), for example, *Agrotis ypsilon*, *Agrotis segetum*, *Alabama argillacea*, *Anfarsia gemmatilis*, *Argyresthia conjugella*, *Autographa gamma*, *Bupalus piniarius*, *Cacoecia murinana*, *Capua reticulana*, *Chematomia brumata*, *Choristoneura fumiferana*, *Choristoneura occidentalis*, *Cirphis unipuncta*, *Cydia pomonella*, *Dendrolimus pini*, *Diaphania nitidalls*, *Dlatraea grandiosella*, *Earias insulana*, *Elasmopalpus lignosellus*, *Eupoecilia ambiguella*, *Evetria bouliana*, *Feltia subterranea*, *Galleria mellonella*, *Grapholitha funebrana*, *Grapholitha molesta*, *Heliothis armigera*, *Heliothis virescens*, *Heliothis zea*, *Hellula undalis*, *Hibernia defoliaria*, *Hyphantria cunea*, *Hyponomeuta malinellus*, *Keiferia lycopersicella*, *Lambdina fiscellaria*, *Laphygma exigua*, *Leucoptera coffeella*, *Leucoptera scitella*, *Lithocolletis blancardella*, *Lobesia botrana*, *Loxostege sticticalis*, *Lymantria dispar*, *Lymantria monacha*, *Lyonetia clerkella*, *Malacosoma neustria*, *Mamestra brassicae*, *Orgyia pseudotsugata*, *Ostrinia nubilalis*, *Panolis flammea*, *Pectinophora gossypiella*, *Peridroma saucia*, *Phalera bucephala*, *Phthorimaea operculella*, *Phyllocnistis citrella*, *Pieris brassicae*, *Plathypena scabs*, *Plutella xylostella*, *Pseudoplusia includens*, *Rhyacionia frustrana*, *Scrobipalpula absoluta*, *Sitotroga cerealella*, *Sparganothis pilleriana*, *Spodoptera frugiperda*, *Spodoptera littoralis*, *Spodoptera litura*, *Thaumatopea pityocampa*, *Tortrix viridana*, *Trichoplusia ni* and *Zeiraphera Canadensis*;

(2) beetles (Coleoptera), for example, *Agrilus sinuatus*, *Agriotes lineatus*, *Agriotes obscurus*, *Amphimallus solstitialis*, *Anisandrus dispar*, *Anthonomus grandis*, *Anthonomus pomorum*, *Aphthona euphoridae*, *Athous haemorrhoidalis*, *Atomaria linearis*, *Blastophagus piniperda*, *Blitophaga undata*, *Bruchus rufimanus*, *Bruchus pisorum*, *Bruchus lentis*, *Byctiscus betulae*, *Cassida nebulosa*, *Ceratomya trifurcata*, *Cetonia aurata*, *Ceuthorrhynchus assimilis*, *Ceuthorrhynchus napi*, *Chaetocnema tibialis*, *Conoderus vespertinus*, *Crioceris asparagi*, *Ctenicera ssp.*, *Diabrotica longicornis*, *Diabrotica semipunctata*, *Diabrotica 12-punctata*, *Diabrotica speciosa*, *Diabrotica virgifera*, *Epilachna varivestis*, *Epitrix hiirtpennis*, *Eutinobothrus brasiliensis*, *Hylobius abietis*, *Hypera brunneipennis*, *Hypera postica*, *Ips typographus*, *Lema bilineata*, *Lema melanopus*, *Leptinotarsa decemlineata*, *Limonioides californicus*, *Lissorhoptrus oryzophilus*, *Melanotus communis*, *Meligethes aeneus*, *Melolontha hippocastani*, *Melolontha melolontha*, *Oulema oryzae*, *Otiorrhynchus sulcatus*, *Otiorrhynchus ovatus*, *Phaedon cochleariae*, *Phyllobius pyri*, *Phyllotreta chrysocephala*, *Phyllophaga sp.*, *Phyllopertha horticola*, *Phyllotreta nemorum*, *Phyllotreta striolata*, *Popillia japonica*, *Sitona lineatus* and *Sitophilus granaria*;

(3) flies, mosquitoes (Diptera), for example, *Aedes aegypti*, *Aedes albopictus*, *Aedes vexans*, *Anastrepha ludens*, *Anopheles maculipennis*, *Anopheles crucians*, *Anopheles albimanus*, *Anopheles gambiae*, *Anopheles freeborni*, *Anopheles leucophyrus*, *Anopheles minimus*, *Anopheles quadrimaculatus*, *Calliphora vicina*, *Ceratitis capitata*, *Chrysomya bezziana*, *Chrysomya hominivorax*, *Chrysomya macellaria*, *Chrysops discalis*, *Chrysops silacea*, *Chrysops allanticus*, *Cochliomyia hominivorax*, *Contarinia sorghicola*, *Cordylobia anthropophaga*, *Culicoides furens*, *Culex pipiens*, *Culex nigripalpus*, *Culex quinquefasciatus*, *Culex tarsalis*, *Culiseta inornata*, *Culiseta melanuria*, *Dacus cucurbitae*, *Dacus oleae*, *Dasineura brassicae*, *Delia antiqua*, *Delia coarctata*, *Delia platana*, *Della radicum*,

Dermatobia hominis, *Fannia canicularis*, *Geomyza tripunctata*, *Gasterophilus intestinalis*, *Glossina morsitans*, *Glossina palpalis*, *Glossina fuscipes*, *Glossina tachinoides*, *Haematobia irritans*, *Haplodiplosis equestris*, *Hippelates spp.*, *Hylemyia platana*, *Hypoderma lineata*, *Leptoconops torrens*, *Liriomyza sativae*, *Liriomyza trifolii*, *Lucilia caprina*, *Lucilia cuprina*, *Lucilla sericata*, *Lycoria pectoralis*, *Mansonina titillans*, *Mayetiola destructor*, *Musca domestica*, *Muscina stabulans*, *Oestrus ovis*, *Oponomyza forum*, *Oscinella fit*, *Pegomya hysocyami*, *Phorbia antiqua*, *Phorbia brassicae*, *Phorbia coarctata*, *Phlebotomus argentipes*, *Psorophora columbiana*, *Psila rosae*, *Psorophora discolor*, *Prosimullum mixtum*, *Rhagoletis cerasi*, *Rhagoletis pomonella*, *Sarcophaga haemorrhoidalis*, *Sarcophaga sp.*, *Simulium vittatum*, *Stomoxys calcitrans*, *Tabanus bovinus*, *Tabanus atratus*, *Tabanus lineola*, and *Tabanus similis*, *Tipula oleracea*, and *Tipulapaludosa*;

(4) thrips (Thysanoptera), for example, *Dichromothrips corbetti*, *Dichromothrips ssp.*, *Frankliniella fusca*, *Frankliniella occidentalis*, *Frankliniella tritici*, *Scirtothrips citri*, *Thrips olyaee*, *Thrips palmi* and *Thrips tabaci*,

(5) termites (Isoptera), for example, *Calotermes flavicollis*, *Leucotermes flavipes*, *Heterotermes aureus*, *Reticulitermes flavipes*, *Reticulitermes virginicus*, *Reticulitermes lucifugus*, *Termes natalensis*, and *Coptotermes formosanus*,

(6) cockroaches (Blattaria-Blattodea), for example, *Blattella germanica*, *Blattella asahinae*, *Periplaneta americana*, *Periplaneta japonica*, *Periplaneta brunnea*, *Periplaneta fuliginosa*, *Periplaneta australasiae*, and *Blatta orientalis*;

(7) true bugs (Hemiptera), for example, *Acrosternum hilare*, *Blissus leucopterus*, *Cyrtopeltis notatus*, *Dysdercus cingulatus*, *Dysdercus intermedius*, *Eurygaster integriceps*, *Euschistus impictiventris*, *Leptoglossus phyllopus*, *Lygus illeolaris*, *Lygus pratensis*, *Nezara viridula*, *Piesma quadrata*, *Solubea insularis*, *Thyanta perditor*, *Acyrtosiphon onobrychis*, *Adelges laricis*, *Aphidula nasturtii*, *Aphis fabae*, *Aphis forbesi*, *Aphis pomi*, *Aphis gossypii*, *Aphis grossulariae*, *Aphis schneideri*, *Aphis spiraeicola*, *Aphis sambuci*, *Acyrtosiphon pisum*, *Aulacorthum solani*, *Bemisia argentifolii*, *Brachycaudus cardui*, *Brachycaudus helichrysi*, *Brachycaudus persicae*, *Brachycaudus prunicola*, *Brevicoryne brassicae*, *Capitophorus horni*, *Cerosiphia gossypii*, *Chaetosiphon fragaefolii*, *Cryptomyzus ribis*, *Dreyfusia nordmanniana*, *Dreyfusia piceae*, *Dysaphis radicola*, *Dysaulacorthum pseudosolani*, *Dysaphis plantaginea*, *Dysaphis pyri*, *Empoasca fabae*, *Hyalopteris pruni*, *Hyperomyzus lactucae*, *Macrosiphum avenae*, *Macrosiphum euphorbiae*, *Macrosiphum rosae*, *Megoura viciae*, *Melanaphis pyramis*, *Metopolophium dirhodum*, *Myzus persicae*, *Myzus ascalonicus*, *Myzus cerasi*, *Myzus varians*, *Nasonovia ribis-nigri*, *Nilaparvata lugens*, *Pemphigus bursarius*, *Perkinsiella saccharicida*, *Phorodon humuli*, *Psylla mali*, *Psylla pini*, *Rhopalomyzus ascalonicus*, *Rhopalosiphum maidis*, *Rhopalosiphum padi*, *Rhopalosiphum inserfum*, *Sappaphis mala*, *Sappaphis mali*, *Schizaphis graminum*, *Schizoneura lanuginosa*, *Sitobion avenae*, *Trialeurodes vaporariorum*, *Toxoptera aurantiand*, *Viteus vitifolii*, *Cimex lectularius*, *Cimex hemipterus*, *Reduvius senilis*, *Triatoma spp.*, and *Arilus critatus*;

(8) ants, bees, wasps, sawflies (Hymenoptera), for example, *Athalia rosae*, *Atta cephalotes*, *Atta capiguara*, *Atta cephalotes*, *Apha laevigata*, *Atta robusta*, *Atta sexdens*, *Atta texana*, *Crematogaster spp.*, *Hoplocampa minuta*, *Hoplocampa testudinea*, *Monomorium pharaonis*, *Solenopsis geminata*, *Solenopsis invicta*, *Solenopsis richteri*, *Solenopsis xyloni*, *Pogonomyrmex barbatus*, *Pogonomyrmex californicus*, *Pheidole megacephala*, *Dasymutilla occidentalis*,

Bombus spp. *Vespula squamosa*, *Paravespula vulgaris*, *Paravespula pennsylvanica*, *Paravespula germanica*, *Dolichovespula maculata*, *Vespa crabro*, *Polistes rubiginosa*, *Camponotus floridanus*, and *Linepithema humile*;

(9) crickets, grasshoppers, locusts (Orthoptera), for example, *Acheta domestica*, *Gryllotalpa gryllotalpa*, *Locusta migratoria*, *Melanoplus bivittatus*, *Melanoplus femurrubrum*, *Melanoplus mexicanus*, *Melanoplus sanguinipes*, *Melanoplus spretus*, *Nomadacris septemfasciata*, *Schistocerca americana*, *Schistocerca gregaria*, *Dociostaurus maroccanus*, *Tachycines asynamorus*, *Oedaleus senegalensis*, *Zonozerus variegatus*, *Hieroglyphus daganensis*, *Kraussaria angulifera*, *Calliptamus italicus*, *Chortioctes terminifera*, and *Locustana pardalina*;

(10) Arachnoidea, such as arachnids (Acarina), for example, of the families Argasidae, Ixodidae and Sarcopitidae, such as *Amblyomma americanum*, *Amblyomma variegatum*, *Amblyomma maculatum*, *Argas persicus*, *Boophilus annulatus*, *Boophilus decoloratus*, *Boophilus microplus*, *Dermacentor silvarum*, *Dermacentor andersoni*, *Dermacentor variabilis*, *Hyalomma truncatum*, *Ixodes ricinus*, *Ixodes rubicundus*, *Ixodes scapularis*, *Ixodes holocyclus*, *Ixodes pacificus*, *Ornithodoros moubata*, *Ornithodoros hermsi*, *Ornithodoros turicata*, *Ornithonyssus bacoti*, *Otobius megnini*, *Dermanyssus gallinae*, *Psoroptes ovis*, *Rhipicephalus sanguineus*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi*, *Sarcoptes scabiei*, and *Eriophyidae* spp. such as *Aculus schlehtendali*, *Phyllocoptata oleivora* and *Eriophyes sheldoni*, *Tarsonemidae* spp. such as *Phytonemus pallidus* and *Polyphagotarsonemus latus*; *Tenuipalpidae* spp. such as *Brevipalpus phoenicis*; *Tetranychidae* spp. such as *Tetranychus cinnabarinus*, *Tetranychus kanzawai*, *Tetranychus pacificus*, *Tetranychus telarius* and *Tetranychus urticae*, *Panonychus ulmi*, *Panonychus citri*, and *Oligonychus pratensis*; *Araneida*, e.g., *Lafroductus mactans*, and *Loxosceles reclusa*,

(11) fleas (Siphonaptera), for example, *Ctenocephalides felis*, *Ctenocephalides canis*, *Xenopsylla cheopis*, *Pulex irritans*, *Tunga penefrans*, and *Nosopsyllus fasciatus*;

(12) silverfish, firebrat (Thysanura), for example, *Lepisma saccharins* and *Thermobia domestica*;

(13) centipedes (Chilopoda), for example, *Scutigera coleoptrata*,

(14) millipedes (Diplopoda), for example, *Narceus* spp.,

(15) Earwigs (Dermaptera), for example, *Forficula auricularia*; and/or

(16) lice (Phthiraptera), for example, *Pediculus humanus capitis*, *Pediculus humanus corporis*, *Phthirus pubis*, *Haematopinus eurysternus*, *Haematopinus suis*, *Linognathus vituli*, *Bovicola bovis*, *Menopon gallinae*, *Menacanthus stramineus* and *Solenopotes capillatus*.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are insecticidally active against insects of the order Diptera, Hemiptera, and/or Lepidoptera. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are insecticidally active against insects of the order Lepidoptera. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are insecticidally active against insects of the order Hemiptera.

The crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 described herein can be applied to plant foliage as aqueous sprays by methods commonly employed, such as conventional high-liter

hydraulic sprays, low-liter sprays, air-blast, and aerial sprays. The dilution and rate of application will depend upon the type of equipment employed, the method and frequency of application desired, and the ligand application rate. It may be desirable to include additional adjuvants in the spray tank. Such adjuvants include surfactants, dispersants, spreaders, stickers, antifoam agents, emulsifiers, and other similar materials described in *McCutcheon's Emulsifiers and Detergents*, *McCutcheon's Emulsifiers and Detergents/Functional Materials*, and *McCutcheon's Functional Materials*, all published annually by McCutcheon Division of MC Publishing Company (New Jersey). Crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 can also be mixed with fertilizers or fertilizing materials before their application. Crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 and solid fertilizing material can also be admixed in mixing or blending equipment, or they can be incorporated with fertilizers in granular formulations. Any relative proportion of fertilizer can be used which is suitable for the crops and weeds to be treated. The crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 described herein will commonly comprise from 5% to 50% of the fertilizing composition. These compositions provide fertilizing materials which promote the rapid growth of desired plants, and at the same time control gene expression.

As used herein, the term "Compound 1" refers to (R)-3, 5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide. In one embodiment, Compound 1 comprises about 10% or less, i.e., about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.5%, or less, of (S)-3, 5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide, by weight. The stereoisomeric purity of Compound 1 can be determined using conventional analytical methods such as chiral HPLC.

As used herein, the term "Compound 2" refers to (S)-3, 5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide. In one embodiment, Compound 2 comprises about 10% or less, i.e., about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about 1%, or about 0.5%, or less, of (R)-3, 5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide, by weight. The stereoisomeric purity of Compound 2 can be determined using conventional analytical methods such as chiral HPLC.

As used herein, the term "substantially pure" with reference to a particular crystalline polymorphic form of Compound 1 or Compound 2 means that the polymorphic form comprises about 10% or less, i.e., about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1%, or less, by weight of any other physical forms of Compound 1 or Compound 2, respectively.

As used herein, the term "substantially pure" with reference to amorphous Compound 1 or Compound 2 means that the amorphous form comprises about 10% or less, i.e., about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, or about 1%, or less, by weight of any crystalline forms of Compound 1 or Compound 2, respectively.

As used herein, the term "pure" with reference to a particular crystalline polymorphic form of Compound 1 or Compound 2 means that the polymorphic form comprises about 1% or less, i.e., about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, or about 0.1%, or less, by weight of any other physical forms of Compound 1 or Compound 2, respectively. In one

embodiment, a “pure” polymorphic form contains no PXRD-detectable amount of any other physical forms of Compound 1.

As used herein, the term “pure” with reference to amorphous Compound 1 or Compound 2 means that the amorphous form comprises about 1% or less, i.e., about 0.9%, about 0.8%, about 0.7%, about 0.6%, about 0.5%, about 0.4%, about 0.3%, about 0.2%, or about 0.1%, or less, by weight of any crystalline forms of Compound 1 or Compound 2, respectively.

As used herein, the term “amorphous” refers to a solid form of Compound 1 or Compound 2 that lacks the long-range order characteristic of a crystal, i.e., the solid is non-crystalline.

As used herein, the term “essentially the same” with reference to PXRD peak positions and relative intensities means that peak position and intensity variability are taken into account when comparing PXRD diffractograms. Likewise, the term “essentially the same” with reference to FT-Raman peak positions means that peak position variability are taken into account when comparing FT-Raman spectra. For example, PXRD peak positions can show inter-apparatus variability, e.g., as much as 0.2°. Relative peak intensities can also show inter-apparatus variability due to degree of crystallinity, orientation, prepared sample surface, and other factors known to those skilled in the art, and should be taken as qualitative measures only.

As used herein, the term “micronization” refers to a process or method by which the size of a population of particles is reduced, typically to the micron scale.

As used herein, the term “micron” or “ μm ” refer to “micrometer,” which is 1×10^{-6} meter.

As used herein, the term “therapeutically effective amount,” refers to the amount of Compound 1 or Compound 2 sufficient to treat one or more symptoms of a disease, condition, injury, or disorder, or prevent advancement of disease, condition, injury, or disorder, or cause regression of the disease, condition, injury, or disorder. For example, with respect to the treatment of cancer, in one embodiment, a therapeutically effective amount will refer to the amount of Compound 1 or Compound 2 that decreases the rate of tumor growth, decreases tumor mass, decreases the number of metastases, increases time to tumor progression, or increases survival time by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 100%.

As used herein, the term “insecticidally effective amount” refers to the amount of Compound 1 or Compound 2 sufficient to control, e.g., reduce or prevent the spread of, or kill, insects. For example, an insecticidally effect amount will refer to the amount of Compound 1 or Compound 2 that induces premature molting and death in an insect.

The terms “a” and “an” refer to one or more than one.

The term “about,” as used herein, includes the recited number $\pm 10\%$. Thus, “about 10” means 9 to 11.

As used herein, the term “average particle size distribution” or “ D_{50} ” is the diameter where 50 mass-% of the particles have a larger equivalent diameter, and the other 50 mass-% have a smaller equivalent diameter as determined by laser diffraction in Malvern Master Sizer Microplus equipment or its equivalent, or other suitable techniques.

As used herein, the term “non-solvate forming solvent” refers to a solvent that does not form a solvate or hydrate,

with Compound 1 or Compound 2. Non-solvate forming solvents include, but are not limited to, hexane, heptane, cumene, diethyl ether, toluene, ethyl acetate, tert-butyl methyl ether, n-dodecane, ethanol, and isopropanol.

As used herein, the term “excipient” refers to any ingredient in a composition other than the one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2. An excipient is typically an inert substance added to a composition to facilitate processing, handling, administration, etc. of the one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2. Useful excipients include, but are not limited to, adjuvants, antiadherents, binders, carriers, disintegrants, fillers, flavors, colors, diluents, lubricants, glidants, preservatives, sorbents, solvents, surfactants, and sweeteners.

Conventional pharmaceutical excipients are well known to those of skill in the art. In particular, one of skill in the art will recognize that a wide variety of pharmaceutically acceptable excipients can be used in admixture with crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, including those listed in the *Handbook of Pharmaceutical Excipients*, Pharmaceutical Press 4th Ed. (2003), and *Remington: The Science and Practice of Pharmacy*, Lippincott Williams & Wilkins, 21st ed. (2005). In one embodiment, the composition comprises one or more of the following excipients: water, Labrasol, Lauroglycol 90, Phosal 53 MCT, Miglyol, Cremophor EL, polysorbate 80, Crillett 1 HP, Isopropyl myristate, Oleic acid, and/or PEG 400 NF. In another embodiment, the composition comprises a lipid.

Pharmaceutically acceptable carriers include fillers such as saccharides, for example, trehalose, lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, disintegrating agents may be added such as the above-mentioned starches and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. In one embodiment, dragee cores are provided with suitable coatings which, if desired, are resistant to gastric juices. For this purpose, concentrated saccharide solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethyl-cellulose phthalate, are used. Dye stuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules or nanoparticles which may optionally be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In one

embodiment, the is dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin, optionally with stabilizers.

Fatty oils may comprise mono-, di- or triglycerides. Mono-, di- and triglycerides include those that are derived from C₆, C₈, C₁₀, C₁₂, C₁₄, C₁₆, C₁₈, C₂₀ and C₂₂ acids. Exemplary diglycerides include, in particular, diolein, dipalmitolein, and mixed caprylin-caprin diglycerides. Preferred triglycerides include vegetable oils, fish oils, animal fats, hydrogenated vegetable oils, partially hydrogenated vegetable oils, synthetic triglycerides, modified triglycerides, fractionated triglycerides, medium and long-chain triglycerides, structured triglycerides, and mixtures thereof. Exemplary triglycerides include: almond oil; babassu oil; borage oil; blackcurrant seed oil; canola oil; castor oil; coconut oil; corn oil; cottonseed oil; evening primrose oil; grapeseed oil; groundnut oil; mustard seed oil; olive oil; palm oil; palm kernel oil; peanut oil; rapeseed oil; safflower oil; sesame oil; shark liver oil; soybean oil; sunflower oil; hydrogenated castor oil; hydrogenated coconut oil; hydrogenated palm oil; hydrogenated soybean oil; hydrogenated vegetable oil; hydrogenated cottonseed and castor oil; partially hydrogenated soybean oil; partially soy and cottonseed oil; glyceryl tricaproate; glyceryl tricaprilate; glyceryl tri-caprate; glyceryl triundecanoate; glyceryl trilaurate; glyceryl trioleate; glyceryl trilinoleate; glyceryl trilinolenate; glyceryl tricaprilate/caprate; glyceryl tricaprilate/caprate/laurate; glyceryl tricaprilate/caprate/linoleate; and glyceryl tricaprilate/caprate/stearate.

In one embodiment, the triglyceride is the medium chain triglyceride available under the trade name LABRAFAC CC. Other triglycerides include neutral oils, e.g., neutral plant oils, in particular fractionated coconut oils such as known and commercially available under the trade name MIGLYOL, including the products: MIGLYOL 810; MIGLYOL 812; MIGLYOL 818; and CAPTEX 355. Other triglycerides are caprylic-capric acid triglycerides such as known and commercially available under the trade name MYRITOL, including the product MYRITOL 813. Further triglycerides of this class are CAPMUL MCT, CAPTEX 200, CAPTEX 300, CAPTEX 800, NEOBEE M5 and MAZOL 1400.

Pharmaceutical compositions comprising triglycerides may further comprise lipophilic and/or hydrophilic surfactants which may form clear solutions upon dissolution with an aqueous solvent. One such surfactant is tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS). Examples of such compositions are described in U.S. Pat. No. 6,267, 985.

In another embodiment, the pharmaceutically acceptable carrier comprises LABRASOL (Gattefosse SA), which is PEG-8 caprylic/capric glycerides. In another embodiment, the pharmaceutically acceptable carrier comprises PL90G, vitamin E TPGS, and Miglyol 812N.

As used herein, the term "treat," "treating," or "treatment" is meant to encompass administering to a subject one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, for the purposes of amelioration or cure of a disease, disorder, injury, or condition, including preemptive treatment.

As used herein, the term "subject" refers to an intact insect, plant, algae, or animal, e.g., human or veterinary animal, e.g., cow, sheep, pig, horse, dog, or cat. In one embodiment, a host cell of the subject comprises a polynucleotide encoding a gene switch that comprises a ligand binding domain that binds Compound 1. In another embodiment, a host cell of the subject comprises a polynucleotide

encoding a gene switch that comprises a ligand binding domain that binds Compound 2.

As used herein, the term "gene of interest" is any gene that one wishes to express that encodes a peptide, protein, or polypeptide.

As used herein, the term "gene expression" refers to the transcription of DNA to messenger RNA (mRNA), and/or the translation of mRNA to amino acid sequence.

As used herein, the term "regulating gene expression" refers to increasing the level of gene expression in response to contact of Compound 1 with the ligand binding domain that binds Compound 1, relative to the level of gene expression in the absence of contacting the ligand binding domain that binds Compound 1 with Compound 1.

As used herein, the term "gene switch" refers to peptide, protein or polypeptide complex that functions to (a) bind Compound 1 or Compound 2, i.e., the ligand, and (b) regulate the transcription of a gene of interest in a ligand-dependent fashion. Gene switches are useful for various applications such as gene therapy, production of proteins in cells, cell based high throughput screening assays, functional genomics, and regulation of traits in transgenic plants and animals.

In one embodiment, the polynucleotide encoding a gene switch is a recombinant polynucleotide, i.e., a polynucleotide, that has been engineered, by molecular biological manipulation, to encode the gene switch. In another embodiment, the recombinant polynucleotide is a synthetic polynucleotide.

As used herein, the term "gene" refers to a polynucleotide comprising nucleotides that encode a functional molecule, including functional molecules produced by transcription only (e.g., a bioactive RNA species) or by transcription and translation (e.g. a polypeptide). The term "gene" encompasses cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific RNA, protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and/or coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. A chimeric gene may comprise coding sequences derived from different sources and/or regulatory sequences derived from different sources. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" or "exogenous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

In one embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 are administered to an isolated host cell or a subject as a composition. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 are administered to an isolated host cell or a subject as a pharmaceutically acceptable composition.

As used herein, the term “dimerizes with the ligand binding domain that binds Compound 1” refers to a selective protein-protein interaction.

In one embodiment, the gene switch efficacy or “EC₅₀” of Compound 1 is about 100 nM or less, e.g., about 75 nM, about 50 nM, about 25 nM, about 15 nM, about 10 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 0.5 nM, or less in a cellular gene switch assay. Examples of in vitro assays for measuring gene switch-regulated gene expression are well known to those of ordinary skill in the art. See, for example, Karzenowski et al., *BioTechniques* 39: 191-200 (2005). In another embodiment, the gene switch efficacy of Compound 2 is about 100 nM or less, e.g., about 75 nM, about 50 nM, about 25 nM, about 15 nM, about 10 nM, about 9 nM, about 8 nM, about 7 nM, about 6 nM, about 5 nM, about 4 nM, about 3 nM, about 2 nM, about 1 nM, about 0.5 nM, or less in a cellular gene switch assay. As used herein, the “EC₅₀” is the “half maximal effective concentration,” which refers to the concentration of Compound 1 or Compound 2 that induces a gene switch-regulated change in expression of a polynucleotide encoding a gene of interest that is halfway between the baseline level of expression and the maximum level of expression after a specified exposure time.

As used herein, the term “ligand binding domain that binds Compound 1” refers to an amino acid sequence that selectively binds Compound 1. In the methods disclosed herein, Compound 1 binds to a ligand binding domain, e.g., an ecdysone receptor ligand binding domain, that is part of a ligand-dependent transcriptional activation complex that regulates the expression of a polynucleotide sequence that encodes a gene of interest. Hence, the expression of the gene of interest is regulated in a ligand (Compound 1) dependent fashion. Likewise, the term “ligand binding domain that binds Compound 2” refers to an amino acid sequence that selectively binds Compound 2.

In one embodiment, the ligand binding domain that binds Compound 1, e.g., an ecdysone receptor ligand binding domain, dimerizes with another ligand binding domain, e.g., a retinoid X receptor ligand binding domain, to form a protein-protein complex.

In one embodiment, the expression of the gene of interest is regulated by Compound 1 or Compound 2 in an on/off fashion that is independent of the concentration or dosage of Compound 1 or Compound 2, respectively. In another embodiment, the expression of the gene of interest is regulated by Compound 1 in a concentration (or dosage)-dependent fashion, i.e., there is a dose-response relationship between the concentration (or dosage) of Compound 1 and the level of gene expression of the gene of interest. See, e.g., US 2009/0123441.

The term “operably linked” refers to the association of polynucleotide sequences on a single polynucleotide so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

In one embodiment, the host cell is an isolated host cell. In one embodiment, an “isolated” host cell refers to a cell that is not present in a subject. In one embodiment, an “isolated” host cell refers to one or more host cells in a cell culture apparatus or in a cell culture preparation.

In one embodiment, the host cell is within a subject, and the host cell is contacted by Compound 1 or Compound 2 by administering one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, to the subject. In another embodiment, the host cell is contacted with one or more crystalline polymorphic forms of Compound 1, or a composition thereof, in vitro. In another embodiment, the host cell is contacted with one or more crystalline polymorphic forms of Compound 1, or a composition thereof, ex vivo. In another embodiment, the host cell is in a human subject. In another embodiment, the host cell is in an animal subject. In another embodiment, the host cell is in a plant subject. In another embodiment, the host cell is in an algae subject. In another embodiment, the host cell is contacted by crystalline Compound 1 Form III, or a composition comprising crystalline Compound 1 Form III and one or more excipients.

In one embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject. In one embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject orally. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject parenterally. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered subcutaneously, intramuscularly, intravenously, intraperitoneally or intratumorally. In another embodiment, crystalline Compound 1 Form III, or a composition thereof, is administered to a subject. In another embodiment, amorphous Compound 1 Form X, or a composition thereof, is administered to a subject.

In addition to or together with the above modes of administration, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, can be added to food consumed by a subject. In one embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, is combined, blended, or admixed with food material to provide a “food product.” The term “food material” is used in its broadest possible sense, and includes any form, e.g., solid, emulsion, liquid, of ingestible materials consumed by an animal, e.g., a human. Food products may be formulated so the subject takes in an appropriate quantity of one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2 with its diet. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, is formulated as a premix for addition to food material. In one embodiment, the food product or premix comprises one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, and one or more lipids.

In one embodiment, the ligand binding domain in the gene switch that binds Compound 1 or Compound 2 is a Group H nuclear receptor ligand binding domain, or a mutant thereof that binds Compound 1 or Compound 2, respectively. In another embodiment, the Group H nuclear receptor ligand binding domain is selected from the group consisting of an ecdysone receptor ligand binding domain, a ubiquitous receptor ligand binding domain, an orphan receptor-1 ligand binding domain, an NER-1 ligand binding domain, a receptor-interacting protein-15 ligand binding domain, a liver X

25

receptor-3 ligand binding domain, a steroid hormone receptor-like protein ligand binding domain, a liver X receptor ligand binding domain, a liver X receptor ligand binding domain, a farnesoid X receptor ligand binding domain, a receptor-interacting protein-14 ligand binding domain, and a farnesol receptor ligand binding domain ligand binding domain, or a mutant thereof that binds Compound 1.

In another embodiment, the Group H nuclear receptor ligand binding domain is an ecdysone receptor ligand binding domain, or a mutant thereof that binds Compound 1 or Compound 2. In another embodiment, the ecdysone receptor ligand binding domain is selected from the group consisting of an *Arthropod* ecdysone receptor ligand binding domain, a *Lepidopteran* ecdysone receptor ligand binding domain, a *Dipteran* ecdysone receptor ligand binding domain, an *Orthopteran* ecdysone receptor ligand binding domain, a *Homopteran* ecdysone receptor ligand binding domain and a *Hemipteran* ecdysone receptor ligand binding domain, a spruce budworm *Choristoneura fumiferana* ecdysone receptor ligand binding domain, a beetle *Tenebrio molitor* ecdysone receptor ligand binding domain, a *Manduca sexta* ecdysone receptor ligand binding domain, a *Heliothis virescens* ecdysone receptor ligand binding domain, a midge *Chironomus tentans* ecdysone receptor ligand binding domain, a silk moth *Bombyx mori* ecdysone receptor ligand binding domain, a squinting bush brown *Bicyclus anynana* ecdysone receptor ligand binding domain, a buckeye *Junonia coenia* ecdysone receptor ligand binding domain, a fruit fly *Drosophila melanogaster* ecdysone receptor ligand binding domain, a mosquito *Aedes aegypti* ecdysone receptor ligand binding domain, a blowfly *Lucilia capitata* ecdysone receptor ligand binding domain, a blowfly *Lucilia cuprina* ecdysone receptor ligand binding domain, a blowfly *Calliphora vicina* ecdysone receptor ligand binding domain, a Mediterranean fruit fly *Ceratitis capitata* ecdysone receptor ligand binding domain, a locust *Locusta migratoria* ecdysone receptor ligand binding domain, an aphid *Myzus persicae* ecdysone receptor ligand binding domain, a fiddler crab *Celula pugilator* ecdysone receptor ligand binding domain, an ixodid tick *Amblyomma americanum* ecdysone receptor ligand binding domain, a whitefly *Bemisia argentifolii* ecdysone receptor ligand binding domain, a leafhopper *Nephotetix cincticeps* ecdysone receptor ligand binding domain, or a mutant thereof that binds Compound 1 or Compound 2. In another embodiment, the ecdysone receptor ligand binding domain is a spruce budworm *Choristoneura fumiferana* ecdysone receptor ligand binding domain, for which the amino acid sequence is:

Leu Thr Ala Asn Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Leu Arg Val Ala Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val (position 107) Leu Phe Ala Asn Asn Gln Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr (position 127) Val Ile Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Pro Phe Leu

26

Glu Glu Ile Trp Asp Val (SEQ ID NO: 1), which is also set forth as SEQ NO: 1 in U.S. Patent Publication No. 2006/0100416 A1.

In another embodiment, the ecdysone receptor ligand binding domain is a mutant of the spruce budworm *Choristoneura fumiferana* ecdysone receptor ligand binding domain that binds Compound 1.

Suitable ecdysone receptor ligand binding domains include those disclosed, for example, in U.S. Pat. Nos. 7,935,510; 7,919,269; 7,563,879; and in U.S. Patent Publication No. 2006/0100416 A1.

In one embodiment, the gene switch comprises a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2. In one embodiment, the ligand binding domain that dimerizes with the ligand binding domain that binds the Compound 1 or Compound 2 is a Group B nuclear receptor ligand binding domain. In another embodiment, the Group B nuclear receptor ligand binding domain is selected from the group consisting of a retinoid X receptor ligand binding domain, an H-2 region II binding protein ligand binding domain, a nuclear receptor co-regulator-1 ligand binding domain, an ultraspiracle protein ligand binding domain, a 2C1 nuclear receptor ligand binding domain, and a chorion factor 1 ligand binding domain. In another embodiment, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 is not an ecdysone receptor ligand binding domain.

In one embodiment, the ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 is a retinoic X receptor ligand binding domain. In another embodiment, the retinoic X receptor ligand binding domain is a vertebrate retinoic X receptor ligand binding domain. In another embodiment, the retinoic X receptor ligand binding domain is a *Homo sapiens* retinoic X receptor ligand binding domain. In another embodiment, the retinoic X receptor ligand binding domain is a retinoic X receptor α isoform. In another embodiment, the retinoic X receptor ligand binding domain is a retinoic X receptor β isoform. In another embodiment, the retinoic X receptor ligand binding domain is a retinoic X receptor γ isoform.

In another embodiment, the retinoic X receptor ligand binding domain is an invertebrate retinoic X receptor ligand binding domain. In another embodiment, the invertebrate retinoic X receptor ligand binding domain is a *Locusta migratoria* retinoic X receptor ligand binding domain.

In another embodiment, the invertebrate retinoic X receptor ligand binding domain is a non-Lepidopteran, non-Dipteran retinoic X receptor ligand binding domain.

In one embodiment, the retinoid receptor ligand binding domain is a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, an ultraspiracle protein ligand binding domain, or a chimeric retinoid X receptor ligand binding domain.

In one embodiment, the chimeric retinoid X receptor ligand binding domain comprises two polypeptide fragments, wherein the first polypeptide fragment is from a vertebrate retinoid X receptor ligand binding domain, an invertebrate retinoid X receptor ligand binding domain, or an ultraspiracle protein ligand binding domain, and the second polypeptide fragment is from a different vertebrate retinoid X receptor ligand binding domain, a different invertebrate retinoid X receptor ligand binding domain, or a different ultraspiracle protein ligand binding domain.

In another embodiment, the chimeric retinoid X receptor ligand binding domain is one that is disclosed in U.S. Pat. No. 7,531,326, which is hereby incorporated by reference in its entirety.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-6, helices 1-7, helices 1-8, helices 1-9, helices 1-10, helices 1-11, or helices 1-12 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 7-12, helices 8-12, helices 9-12, helices 10-12, helices 11-12, helix 12, or F domain of a second species of retinoid X receptor, respectively.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-6 of a first species RXR according to the disclosure, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 7-12 of a second species of retinoid X receptor.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-7 of a first species retinoid X receptor according to the disclosure, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 8-12 of a second species retinoid X receptor.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-8 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 9-12 of a second species of retinoid X receptor.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-9 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 10-12 of a second species of retinoid X receptor.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-10 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 11-12 of a second species of retinoid X receptor.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-11 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helix 12 of a second species of retinoid X receptor.

In another preferred embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-12 of a first species of retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises an F domain of a second species of retinoid X receptor.

In one embodiment, the first polypeptide fragment in the chimeric retinoid X receptor ligand binding domain is human retinoid X receptor sequence, and the second polypeptide fragment in the chimeric retinoid X receptor ligand binding domain is invertebrate retinoid X receptor sequence. In another embodiment, the invertebrate retinoid X receptor sequence is *Locusta migratoria* retinoid X receptor sequence.

In another embodiment, the first polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 1-8 of a human retinoid X receptor, and the second polypeptide fragment of the chimeric retinoid X receptor ligand binding domain comprises helices 9-12 of *Locusta migratoria* retinoid X receptor.

In one embodiment, the gene switch further comprises a DNA binding domain ("DBD"). In another embodiment, the DBD is selected from the group consisting of a GAL4 DBD, a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, and a yeast DBD.

In one embodiment, the gene switch further comprises a transactivation domain ("TD"). In another embodiment, the transactivation domain is selected from the group consisting of a VP16 TD, a GAL4 TD, an NF- κ B TD, a BP64 TD, and a B42 acidic TD.

In one embodiment, a DNA binding domain, the ligand binding domain that binds Compound 1, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1, and a transactivation domain are encoded by polynucleotide sequences that are contained in the same polynucleotide.

In another embodiment, a DNA binding domain, a ligand binding domain that binds Compound 1, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1, and a transactivation domain are encoded by polynucleotide sequences that are contained in one or more separate polynucleotide sequences.

In another embodiment, a DNA binding domain, a ligand binding domain that binds Compound 1 or Compound 2, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2, and a transactivation domain are encoded by polynucleotide sequences that are contained in two separate polynucleotide sequences.

In another embodiment, a DNA binding domain and a ligand binding domain that binds Compound 1 or Compound 2 are encoded by polynucleotide sequences that are contained in a first polynucleotide sequence, and a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 and a transactivation domain are encoded by polynucleotide sequences that are contained in a second polynucleotide sequence.

In another embodiment, a DNA binding domain and a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2 are encoded by polynucleotide sequences that are contained in a first polynucleotide sequence, and a ligand binding domain that binds Compound 1 or Compound 2 and a transactivation domain are encoded by polynucleotide sequences that are contained in a second polynucleotide sequence.

In embodiments in which one or more of the DNA binding domain, a ligand binding domain that binds Compound 1 or Compound 2, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2, and a transactivation domain are encoded by polynucleotide sequences that are contained in one or more separate polynucleotide sequences, then the one or more separate polynucleotide sequences is operably linked to one or more separate promoters. In another embodiment, the one or more separate polynucleotide sequences are operably linked to one or more separate enhancer elements. In another embodiment, the promoter(s) and/or the enhancer(s) are

constitutively active. In another embodiment, the promoter(s) and/or the enhancer(s) are tissue specific promoters and/or enhancers.

In one embodiment, the gene switch comprises a DNA binding domain, an ecdysone receptor ligand binding domain, a ligand binding domain that dimerizes with the ecdysone receptor ligand binding domain, and a transactivation domain.

In another embodiment, the gene switch comprises a DNA binding domain, an ecdysone receptor ligand binding domain, a retinoid X receptor ligand binding domain, and a transactivation domain.

In another embodiment, the gene switch comprises a DNA binding domain, an ecdysone receptor ligand binding domain, a chimeric vertebrate/invertebrate retinoid X receptor ligand binding domain, and a transactivation domain.

In another embodiment, the gene switch comprises a GAL4 DNA binding domain, a *Choristoneura fumiferana* ecdysone receptor ligand binding domain that is engineered to contain the mutations V107I and Y127E of the *Choristoneura fumiferana* ecdysone receptor sequence set forth in SEQ ID NO: 1, a chimeric *Homo sapiens/Locusta migratoria* retinoid X receptor ligand binding, and a VP16 transactivation domain.

The term "V107I" means that the valine amino acid residue at position 107 in SEQ ID NO: 1 is changed to isoleucine. The term "Y127E" means that the tyrosine amino acid residue at position 127 in SEQ ID NO: 1 is changed to glutamate.

In another embodiment, the host cell further comprises a polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch. A promoter that binds the gene switch complex is operably linked to the polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch.

In another embodiment, the polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch is contained in the same polynucleotide as a polynucleotide that encodes one or more of a DNA binding domain, the ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2, and a transactivation domain. Such constructs are disclosed, for example, in U.S. Patent Publication No. 2009/0123441.

In another embodiment, the polynucleotide encoding a peptide, protein or polypeptide whose expression is regulated by the gene switch is contained in a different polynucleotide than a polynucleotide that encodes one or more of a DNA binding domain, the ligand binding domain that binds Compound 1 or Compound 2, a ligand binding domain that dimerizes with the ligand binding domain that binds Compound 1 or Compound 2, and a transactivation domain.

In one embodiment, the gene switch is more sensitive to Compound 1 or Compound 2 than to a steroid hormone. In another embodiment, the gene switch is more sensitive to Compound 1 than to another diacylhydrazine compound (including Compound 2). In another embodiment, the gene switch is more sensitive to Compound 2 than to another diacylhydrazine compound (including Compound 1).

The sensitivity of a gene switch to Compound 1 or Compound 2, relative to another ligand, can readily be determined in an in vitro assay, for example, an in vitro assay that employs a reporter gene, such as firefly luciferase. Examples of such in vitro assays are well known to those of ordinary skill in the art. See, for example, Karzenowski et al., *BioTechniques* 39: 191-200 (2005).

In one embodiment, the polynucleotide encoding the gene switch is contained in a vector. In one embodiment, the vector selected from the group consisting of a plasmid, an expression vector, a replicon, a phage vector, a cosmid, a viral vector, a liposome, an electrically charged lipid (e.g., a cytofectin), a DNA-protein complex, and a biopolymer.

In another embodiment, the vector is a retroviral vector. In another embodiment, the vector is selected from the group consisting of an adeno-associated viral vector, a pox viral vector, a baculoviral vector, a vaccinia viral vector, a herpes simplex viral vector, an Epstein-Barr viral vector, an adenoviral vector, a gemini viral vector, and a caulimo viral vector.

In one embodiment, the host cell is a prokaryotic host cell. In another embodiment, the host cell is a eukaryotic host cell.

In another embodiment, the host cell is a vertebrate host cell. In another embodiment, the host cell is an invertebrate host cell.

In another embodiment, the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a nematode cell, an insect cell, a fish cell, a plant cell, an avian cell, an algae cell, an animal cell, and a mammalian cell.

In another embodiment, the host cell is selected from the group consisting of a zebrafish cell, a chicken cell, a hamster cell, a mouse cell, a rat cell, a rabbit cell, a cat cell, a dog cell, a bovine cell, a goat cell, a cow cell, a pig cell, a horse cell, a sheep cell, a simian cell, a monkey cell, a chimpanzee cell, and a human cell.

In another embodiment, the host cell is selected from the group consisting of an *Aspergillus* cell, a *Trichoderma* cell, a *Saccharomyces* cell, a *Pichia* cell, a *Candida* cell, a *Hansenula* cell.

In another embodiment, the host cell is selected from the group consisting of a *Synechocystis* cell, a *Synechococcus* cell, a *Salmonella* cell, a *Bacillus* cell, a *Acinetobacter* cell, a *Rhodococcus* cell, a *Streptomyces* cell, an *Escherichia* cell, a *Pseudomonas* cell, a *Methylobacter* cell, a *Alcaligenes* cell, a *Synechocystis* cell, a *Anabaena* cell, a *Thiobacillus* cell, a *Methanobacterium* cell and a *Klebsiella* cell.

In another embodiment, the host cell is selected from the group consisting of an apple cell, an *Arabidopsis* cell, a bajra cell, a banana cell, a barley cell, a bean cell, a beet cell, a blackgram cell, a chickpea cell, a chili cell, a cucumber cell, an eggplant cell, a favabean cell, a maize cell, a melon cell, a millet cell, a mungbean cell, an oat cell, an okra cell, a *Panicum* cell, a papaya cell, a peanut cell, a pea cell, a pepper cell, a pigeonpea cell, a pineapple cell, a *Phaseolus* cell, a potato cell, a pumpkin cell, a rice cell, a sorghum cell, a soybean cell, a squash cell, a sugarcane cell, a sugarbeet cell, a sunflower cell, a sweet potato cell, a tea cell, a tomato cell, a tobacco cell, a watermelon cell, a mushroom cell, and a wheat cell.

In another embodiment, the host cell is selected from the group consisting of a hamster cell, a mouse cell, a rat cell, a rabbit cell, a cat cell, a dog cell, a bovine cell, a goat cell, a cow cell, a pig cell, a horse cell, a sheep cell, a monkey cell, a chimpanzee cell, and a human cell.

Host cell transformation is well known in the art and may be achieved by a variety of methods including but not limited to electroporation, viral infection, plasmid (or vector) transfection, non-viral vector mediated transfection, *Agrobacterium*-mediated transformation, particle bombardment, and the like. Expression of desired gene products involves culturing the transformed host cells under suitable conditions and inducing expression of the transformed gene.

Culture conditions and gene expression protocols in prokaryotic and eukaryotic cells are well known in the art. Cells may be harvested and the gene products isolated according to protocols specific for the gene product.

In addition, a host cell may be chosen which modulates the expression of the inserted polynucleotide, or modifies and processes the polypeptide product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage (e.g., of signal sequence)) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce a non-glycosylated core protein product. However, a polypeptide expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of a heterologous protein. Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting, the polypeptide's activity. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.

In one embodiment, the host cell comprises two or more orthogonal gene switches. Two or more individually operable gene regulation systems are said to be "orthogonal" when (a) modulation of each of the given gene switches by its respective ligand results in a measurable change in the magnitude of expression of the gene that is regulated by that gene switch, and (b) the change is statistically significantly different than the change in expression of all other gene switches that are in the host cell. In one embodiment, regulation of each individually operable gene switch system effects a change in gene expression at least 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, 50-fold, 70-fold, 100-fold, 200-fold, 300 fold, 400-fold or 500-fold greater than all of the other operable gene switches in the host cell. Non-limiting examples of orthogonal gene switch systems are set forth in U.S. Patent Publication No. US 2002/0110861 A1.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat cancer in the subject, for example, a cancer selected from the group consisting of myelodysplasia, breast cancer, prostate cancer, lymphoma, skin cancer, pancreatic cancer, colon cancer, melanoma, malignant melanoma, ovarian cancer, brain cancer, primary brain carcinoma, head-neck cancer, glioma, glioblastoma, liver cancer, bladder cancer, non-small cell lung cancer, head or neck carcinoma, breast carcinoma, ovarian carcinoma, lung carcinoma, small-cell lung carcinoma, Wilms' tumor, cervical carcinoma, testicular carcinoma, bladder carcinoma, pancreatic carcinoma, stomach carcinoma, colon carcinoma, prostatic carcinoma, genitourinary carcinoma, thyroid carcinoma, esophageal carcinoma, myeloma, multiple myeloma, adrenal carcinoma, renal cell carcinoma, endometrial carcinoma, adrenal cortex carcinoma, malignant pancreatic insulinoma, malignant carcinoid carcinoma, choriocarcinoma, mycosis fungoides, malignant hypercalcemia, cervical hyperplasia, leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, chronic granulocytic leukemia, acute granulocytic leukemia, hairy cell leukemia, neuroblastoma, rhabdomyosarcoma, Kaposi's sarcoma, polycythemia vera, essential thrombocytosis, Hodgkin's disease, non-Hodg-

kin's lymphoma, soft-tissue sarcoma, mesothelioma, osteogenic sarcoma, primary macroglobulinemia, and retinoblastoma, and the like.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a metabolic-related disorder in the subject, for example, a metabolic disorder selected from the group consisting of dyslipidemia, atherosclerosis, insulin resistance, diabetes (e.g., diabetes type I, diabetes type II, MODY, and gestational diabetes), obesity, impaired glucose tolerance, atheromatous disease, hypertension, heart disease (which includes, but is not limited to, coronary heart disease, stroke, cardiac insufficiency, coronary insufficiency, and high blood pressure), hyperlipidemia, glucose intolerance, insulin resistance, hyperglycemia, hyperinsulinemia, metabolic syndrome X (or syndrome X, or insulin resistance syndrome, or Reaven's syndrome, or the metabolic cardiovascular risk syndrome), hypertension, chronic fatigue, accelerated aging, degenerative disease, endocrine deficiencies of aging, G_{m1} gangliosidosis, Morquio-B disease, Krabbe's disease, Fabry's disease, Gaucher's disease, Tay-Sachs disease, Sandhoff disease, fucosidosis, disorders of carbohydrate metabolism (e.g., glycogen storage disease), disorders of amino acid metabolism (e.g., phenylketonuria, maple syrup urine disease, glutaric acidemia type 1), disorders of organic acid metabolism (e.g., alcaptonuria), disorders of fatty acid oxidation and mitochondrial metabolism (e.g., medium chain acyl dehydrogenase deficiency), disorders of porphyrin metabolism (e.g., acute intermittent porphyria), disorders of purine or pyrimidine metabolism (e.g., Lesch-Nyhan syndrome), disorders of steroid metabolism (e.g., congenital adrenal hyperplasia), disorders of mitochondrial function (e.g., Kearns-Sayre syndrome), and disorders of peroxisomal function (e.g., Zellweger syndrome).

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat kidney disease in the subject. In one embodiment, the kidney disease is renal failure. In another embodiment, the kidney disease is chronic renal failure.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat anemia in the subject. In one embodiment, the anemia is anemia associated with kidney disease, for example, renal failure or chronic renal failure. In another embodiment, the anemia is associated with cancer therapy with, for example, one or more chemotherapeutic agents. In another embodiment, the anemia is associated with advanced age. In another embodiment, the anemia is associated with impaired lung function. In another embodiment, the anemia is associated with myelodysplasia. In another embodiment, the anemia is associated with radiation therapy. In another embodiment, the anemia is associated with a critical illness. In another embodiment, the anemia is associated with cardiac disease. In another embodiment, the anemia is not a cardiac disease. Nonlimiting types of "cardiac disease" are congestive heart failure, hypoxia, ischemic heart disease, hypertensive heart disease, coronary artery disease, peripheral vascular disease and ischemic cardiac events, e.g., myocardial infarction, heart attack, heart failure, arrhythmia, myocardial rupture, pericarditis, cardiogenic shock, thrombosis, embolism, atherosclerosis, and arterial stenosis.

In another embodiment, the one or more crystalline polymorphic forms or amorphous forms of Compound 1 or

Compound 2, or a composition thereof, are administered to a subject to treat an autoimmune disorder in the subject, for example, an autoimmune disorder selected from the group consisting of Achlorhydra Autoimmune Active Chronic Hepatitis, Acute Disseminated Encephalomyelitis, Acute hemorrhagic leukoencephalitis, Addison's Disease, gamma-globulinemia, Agammaglobulinemia, Alopecia areata, Amyotrophic Lateral Sclerosis, Ankylosing Spondylitis, Anti-GBM/TBM Nephritis, Antiphospholipid syndrome, Antisynthetase syndrome, Arthritis, Atopic allergy, Atopic Dermatitis, Aplastic Anemia, Autoimmune cardiomyopathy, Autoimmune hemolytic anemia, Autoimmune hepatitis, Autoimmune inner ear disease, Autoimmune lymphoproliferative syndrome, Autoimmune peripheral neuropathy, Autoimmune pancreatitis, Autoimmune polyendocrine syndrome Types I, II, & III, Autoimmune progesterone dermatitis, Autoimmune thrombocytopenic purpura, Autoimmune uveitis, Balo disease/Balo concentric sclerosis, Behcet's Syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, Bullous Pemphigoid, Castleman's disease, Chronic Fatigue Immune Dysfunction Syndrome, chronic inflammatory demyelinating polyneuropathy, Chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome, Cicatricial Pemphigoid, Coeliac Disease, Cogan syndrome, Cold agglutinin disease, Complement component 2 deficiency, Cranial arteritis, CREST syndrome, Crohn's Disease, Cushing's Syndrome, Cutaneous leukocytoclastic angitis, Dego's disease, Dermatitis herpetiformis, Dermatomyositis, Diabetes mellitus type 1, Diffuse cutaneous systemic sclerosis, Dressler's syndrome, Discoid lupus erythematosus, eczema, Entesitis-related arthritis, Eosinophilic fasciitis, Epidermolysis bullosa acquisita, Erythema nodosum, Essential mixed cryoglobulinemia, Evan's syndrome, Fibrodysplasia ossificans progressiva, Fibromyositis, Fibrosing aveolitis, Gastritis, Gastrointestinal pemphigoid, Giant cell arteritis, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome (GBS), Hashimoto's encephalitis, Hashimoto's thyroiditis, Hemolytic anaemia, Henoch-Schönlein purpura, Herpes gestationis, Hughes syndrome (or Antiphospholipid syndrome), Hypogammaglobulinemia, Idiopathic Inflammatory Demyelinating Diseases, Idiopathic pulmonary fibrosis, Idiopathic thrombocytopenic purpura, IgA nephropathy (or Berger's disease), Inclusion body myositis, ory demyelinating polyneuropathy, Juvenile idiopathic arthritis, Juvenile rheumatoid arthritis, Lambert-Eaton myasthenic syndrome, Leukocytoclastic vasculitis, Lichen planus, Lichen sclerosus, Linear IgA disease (LAD), Lou Gehrig's Disease, Lupoid hepatitis, Lupus erythematosus, Majeeed syndrome, Ménière's disease, Microscopic polyangiitis, Miller-Fisher syndrome, Mixed Connective Tissue Disease, Mucha-Habermann disease, Muckle-Wells syndrome, Multiple Myeloma, Myasthenia gravis, Myositis, Narcolepsy, Neuromyelitis optica (also Devic's Disease), Ocular cicatricial pemphigoid, Ord thyroiditis, Palindromic rheumatism, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus*), Paraneoplastic cerebellar degeneration, Paraneoplastic cerebellar degeneration, Parry Romberg syndrome, Parsonnage-Turner syndrome, Pars planitis, Pemphigus, Pemphigus vulgaris, Pernicious anaemia, Perivascular encephalomyelitis, POEMS syndrome, Polyarteritis nodosa, Polymyalgia rheumatica, Polymyositis, Primary biliary cirrhosis, psoriasis, psoriatic arthritis, Pyoderma gangrenosum, pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, Relapsing polychondritis, Reiter's syndrome, Retroperitoneal fibrosis, Rheumatoid arthritis, Rheumatoid fever, Schmidt syndrome, Schnitzler syndrome, Scleritis, Sjögren's syn-

drome, Spondyloarthropathy, sticky blood syndrome, Still's Disease, Subacute bacterial endocarditis (SBE), Susac's syndrome, Sweet syndrome, Sydenham Chorea, Sympathetic ophthalmia, Takayasu's arteritis, Temporal arteritis, Tolosa-Hunt syndrome, Transverse Myelitis, Ulcerative Colitis, Undifferentiated connective tissue disease, Undifferentiated spondyloarthropathy, vasculitis, Wegener's granulomatosis, Wilson's syndrome, and Wiskott-Aldrich syndrome.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat an ocular disorder in the subject, for example, an ocular disorder selected from the group consisting of glaucoma including Open Angle Glaucoma (e.g., Primary Open Angle Glaucoma, Pigmentary Glaucoma, and Exfoliative Glaucoma, Low Tension Glaucoma), Angle Closure Glaucoma (also known clinically as closed angle glaucoma, narrow angle glaucoma, pupillary block glaucoma, and ciliary block glaucoma) (e.g., Acute Angle Closure Glaucoma and Chronic Angle Closure Glaucoma), Aniridic Glaucoma, Congenital Glaucoma, Juvenile Glaucoma, Lens-Induced Glaucoma, Neovascular Glaucoma (e.g., using vectors composed of Vascular Endothelial Growth Factor (VEGF) decoy, Pigment Derived Growth Factor (PDGF), Endostatin, Angiostatin, or Angiopoietin-1), Post-Traumatic Glaucoma, Steroid-Induced Glaucoma, Sturge-Weber Syndrome Glaucoma, and Uveitis-Induced Glaucoma, diabetic retinopathy (e.g., using vectors composed of VEGF decoy, PDGF, Endostatin, Angiostatin, or Angiopoietin-1), macular degeneration (e.g., vectors composed of VEGF decoy, PDGF, Endostatin, Angiostatin, Angiopoietin-1, ATP Binding Cassette Subfamily A Member 4), macular degeneration (e.g., using vectors composed of VEGF decoy, PDGF, Endostatin, Angiostatin, Angiopoietin-1, ATP Binding Cassette Subfamily A Member 4), choroidal neovascularization, (e.g., using vectors composed of VEGF decoy, PDGF, Endostatin, Angiostatin, or Angiopoietin-1), vascular leak, and/or retinal edema, bacterial conjunctivitis, fungal conjunctivitis, viral conjunctivitis, uveitis, keratic precipitates, macular edema (e.g., using vectors composed of VEGF decoy, PDGF, Endostatin, Angiostatin, or Angiopoietin-1), inflammation response after intra-ocular lens implantation, uveitis syndromes (for example, chronic iridocyclitis or chronic endophthalmitis), retinal vasculitis (for example, as seen in rheumatoid arthritis, juvenile rheumatoid arthritis, systemic lupus erythematosus, progressive systemic sclerosis, polyarteritis nodosa, Wegener's granulomatosis, temporal arteritis, Adamantiades Bechcet disease, Sjorgen's, relapsing polychondritis and HLA-B27 associated spondylitis), sarcoidosis, Eales disease, acute retinal necrosis, Vogt Koyanaki Harada syndrome, ocular toxoplasmosis, radiation retinopathy, proliferative vitreoretinopathy, endophthalmitis, ocular glaucomas (for example, inflammatory glaucomas), optic neuritis, ischemic optic neuropathy (e.g., vectors composed of Allotopic NADH dehydrogenase Unit 4), thyroid associated orbitopathy, orbital pseudotumor, pigment dispersion syndrome (pigmentary glaucoma), scleritis, episcleritis choroidopathies (for example, "White-dot" syndromes including, but not limited to, acute multifocal posterior placoid), retinopathies (for example, cystoid macular edema, central serous choroidopathy and presumed ocular histoplasmosis syndrome (e.g., vectors composed of Glial Cell Derived Neurotrophic Factor, Peripherin-2)), retinal vascular disease (for example, diabetic retinopathy, Coat's disease and retinal arterial macroaneurysm), retinal artery occlusions, retinal vein occlusions, retinopathy of prematu-

35

rity, retinitis pigmentosa (e.g., vectors composed of Retinal Pigment Specific 65 kDa protein), familial exudative vitreoretinopathy (FEVR), idiopathic polypoidal choroidal vasculopathy, epiretinal macular membranes and cataracts.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a blood disorder in the subject, for example, a blood disorder selected from the group consisting of a blood disorder selected from the group consisting of anemia, bleeding and clotting disorders (e.g., disseminated intravascular coagulation (DIC), hemophilia, Henoch-Schönlein Purpura, hereditary hemorrhagic telangiectasia, thrombocytopenia (ITP, TTP), thrombophilia, Von Willebrand's disease), leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia, chronic lymphocytic leukemia, chronic myelocytic leukemia), lymphomas (e.g., Hodgkin lymphoma, non-Hodgkin lymphoma), myeloproliferative disorders (e.g., myelofibrosis, Polycythemia Vera, thrombocytopenia), plasma cell disorders (e.g., macroglobulinemia, monoclonal gammopathies of undetermined significance, multiple myeloma), spleen disorders, white blood cell disorders (e.g., basophilic disorder, eosinophilic disorder, lymphocytopenia, monocyte disorders, neutropenia, neutrophilic leukocytosis), thrombosis, deep vein thrombosis (DVT), hemochromatosis, menorrhagia, sickle cell disease, and thalassemia.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a neurological disorder in the subject, for example, a neurological disorder selected from the group consisting of Gaucher disease, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), Huntington's disease, Friedrich's ataxia, Mild Cognitive Impairment, Cerebral Amyloid Angiopathy, Parkinsonism Disease, Lewy Body Disease, Frontotemporal Dementia (FTD) Multiple System Atrophy (MSA), Progressive Supranuclear Palsy, and movement disorders (including ataxia, cerebral palsy, choreoathetosis, dystonia, Tourette's syndrome, kernicterus) and tremor disorders, and leukodystrophies (including adrenoleukodystrophy, metachromatic leukodystrophy, Canavan disease, Alexander disease, Pelizaeus-Merzbacher disease), neuronal ceroid lipofuscinoses, ataxia telangiectasia, Rett Syndrome, alpha-synucleinopathy (e.g., Lewy Body Disease, Multiple System Atrophy, Hallervorden-Spatz disease, or Frontotemporal Dementia), Niemann-Pick Type C disease (NPCD), spinocerebellar ataxia Type 1, Type 2, and Type 3, and dentatorubral pallidolysian atrophy (DRLPA).

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a lung disorder in the subject, for example, a lung disorder selected from the group consisting of asthma, atelectasis, bronchitis, COPD (chronic obstructive pulmonary disease), emphysema, Lung cancer, mesothelioma, pneumonia, asbestosis, Aspergilloma, Aspergilliosis, Aspergilliosis—acute invasive, bronchiectasis, bronchiolitis obliterans organizing pneumonia (BOOP), eosinophilic pneumonia, necrotizing pneumonia, pleural effusion, pneumoconiosis, pneumothorax, pulmonary actinomycosis, monary alveolar proteinosis, pulmonary anthrax, pulmonary arteriovenous malformation, pulmonary fibrosis, pulmonary embolus, pulmonary histiocytosis X (eosinophilic granuloma), pulmonary hypertension, pulmonary edema, pulmonary hemorrhage, pulmonary nocardiosis, pulmonary tuberculosis,

36

pulmonary veno-occlusive disease, rheumatoid lung disease, sarcoidosis, radiation fibrosis, hypersensitivity pneumonitis, acute respiratory distress syndrome (ARDS), infant respiratory distress syndrome, idiopathic pulmonary fibrosis, idiopathic interstitial pneumonia, lymphangioleiomyomatosis, pulmonary Langerhans' cell histiocytosis, pulmonary alveolar proteinosis, sinusitis, tonsillitis, otitis media, pharyngitis, laryngitis, Pulmonary hamartoma, pulmonary sequestration, congenital cystic adenomatoid malformation (CCAM), and cystic fibrosis.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a rheumatologic disorder in the subject, for example, a rheumatologic disorder selected from the group consisting of systemic lupus erythematosus, dermatomyositis, scleroderma, systemic necrotizing arteritis, cutaneous necrotizing vasculitis, rheumatoid arthritis, Sjögren's Syndrome, Raynaud's phenomenon, Reiter's syndrome, arthritis, psoriatic arthritis, seronegative spondyloarthropathies, Sjögren's syndrome, systemic sclerosis, dermatomyositis/polymyositis, mixed connective tissue disease, and ankylosing spondylitis.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat an infectious disease in the subject, for example, an infectious disease selected from the group consisting of fungal diseases such as dermatophytosis (e.g., trichophytosis, ringworm or tinea infections), athlete's foot, paronychia, pityriasis versicolor, erythrasma, intertrigo, fungal diaper rash, candida vulvitis, candida balanitis, otitis externa, candidiasis (cutaneous and mucocutaneous), chronic mucocandidiasis (e.g., thrush and vaginal candidiasis), cryptococcosis, geotrichosis, trichosporosis, aspergilliosis, penicilliosis, fusariosis, zygomycosis, sporotrichosis, chromomycosis, coccidioidomycosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, pseudallescheriosis, mycetoma, mycotic keratitis, otomycosis, pneumocystosis, and fungemia, *Acinetobacter* infections, Actinomycosis, African sleeping sickness, AIDS (Acquired immune deficiency syndrome), Amebiasis, Anaplasmosis, Anthrax, *Arcanobacterium haemolyticum* infection, Argentine hemorrhagic fever, Ascariasis, Aspergilliosis, atrovirus infection, Babesiosis, *Bacillus cereus* infection, Bacterial pneumonia, Bacterial vaginosis (BV), *Bacteroides* infection, Balantidiasis, *Baylisascaris* infection, BK virus infection, Black piedra, *Blastocystis hominis* infection, *Borrelia* infection, Botulism (and Infant botulism), Brazilian hemorrhagic fever, Brucellosis, *Burkholderia* infection, Buruli ulcer, Calicivirus infection (Norovirus and Sapovirus), Candidiasis, Cat-scratch disease, Cellulitis, Chagas Disease (American trypanosomiasis), Chancroid, Chickenpox, Chlamydia, Cholera, Chromoblastomycosis, Clonorchiasis, *Clostridium difficile*, Coccidioidomycosis, Colorado tick fever (CTF), Common cold (Acute viral rhinopharyngitis; Acute coryza), Creutzfeldt-Jakob disease (CJD), Cryptococcosis, Cryptosporidiosis, ous larva migrans (CLM), Dengue fever, Dientamoebiasis, Diphtheria, Diphyllobothriasis, Diphyllobothriasis, Dracunculiasis, Ebola hemorrhagic fever, Echinococcosis, Ehrlichiosis, Enterobiasis (Pinworm infection), *Enterococcus* infection, Enterovirus infection, Epidemic typhus, Erythema infectiosum, Exanthema subitum, Fasciolopsiasis, Fasciolosis, Fatal familial insomnia (FFI), Filariasis, *Fusobacterium* infection, Gas gangrene (Clostridial myonecrosis), Geotrichosis, Gerstmann-Sträussler-Scheinker syndrome (GSS), Giardiasis Glanders, Gnathostomia-

sis, Gonorrhea, Granuloma inguinale (Donovanosis), Group A streptococcal infection, Group B streptococcal infection, *Haemophilus influenzae*, Hand, foot and mouth disease (HFMD), Hantavirus Pulmonary Syndrome (HPS) *Helicobacter pylori* infection, ic-uremic syndrome (HUS), Hemorrhagic fever with renal syndrome (HFRS), Hepatitis A, B, C, D, E, Herpes simplex, Histoplasmosis, Hookworm infection, n bocavirus infection, Human ewingii ehrlichiosis, Human granulocytic anaplasmosis (HGA), Human monocytic ehrlichiosis, Human papillomavirus (HPV) infection, Human parainfluenza virus infection, Hymenolepiasis, Epstein-Barr Virus Infectious Mononucleosis (Mono), Influenza (flu), Isosporiasis, Kawasaki disease, Keratitis, *Kingella kingae* infection, Kuru, Lassa fever, Legionellosis (Legionnaires' disease), Legionellosis (Pontiac fever), Leishmaniasis, Leprosy, Leptospirosis, Listeriosis, Lyme disease (Lyme borreliosis), Lymphatic filariasis (Elephantiasis), Lymphocytic choriomeningitis, Malaria, Marburg hemorrhagic fever (MHF), Measles, Melioidosis (Whitmore's disease), Meningitis, Meningococcal disease, Metagonimiasis, Microsporidiosis, *Molluscum contagiosum* (MC), Mumps, Murine typhus (Endemic typhus), *Mycoplasma pneumoniae*, Mycetoma, Myiasis, Neonatal conjunctivitis (Ophthalmia neonatorum), (New) Variant Creutzfeldt-Jakob disease (vCJD, nvCJD), Nocardiosis, Onchocerciasis (River blindness), Paracoccidioidomycosis (South American blastomycosis), Paragonimiasis, Pasteurellosis, Pediculosis capitis (Head lice), Pediculosis corporis (Body lice), Pediculosis pubis (Pubic lice, Crab lice), Pelvic inflammatory disease (PID), Pertussis (Whooping cough), Plague, Pneumococcal infection, *Pneumocystis pneumonia* (PCP), Pneumonia, Poliomyelitis, Poliomyelitis, *Prevotella* infection, many amoebic meningoencephalitis (PAM), Progressive multifocal leukoencephalopathy, Psittacosis, Q fever, Rabies, Rat-bite fever, Respiratory syncytial virus infection, Rhinosporidiosis, ino-virus infection, Rickettsial infection, Rickettsialpox, Rift Valley fever (RVF), Rocky mountain spotted fever (RMSF), Rotavirus infection, Rubella, Salmonellosis, SARS (Severe Acute Respiratory Syndrome), Scabies, Schistosomiasis, Sepsis, Shigellosis (Bacillary dysentery), Shingles (Herpes zoster), Smallpox (Variola), Sporotrichosis, Staphylococcal food poisoning, Staphylococcal infection, Strongyloidiasis, Syphilis, Taeniasis, tanus (Lockjaw), *Tinea barbae* (Barber's itch), *Tinea capitis* (Ringworm of the Scalp), *Tinea corporis* (Ringworm of the Body), *Tinea cruris* (Jock itch), *Tinea manuum* (Ringworm of the Hand), *Tinea nigra*, *Tinea unguium* (Onychomycosis), *Tinea versicolor* (Pityriasis versicolor), Toxocariasis (Visceral Larva Migrans (VLM)), Toxoplasmosis, Trichinellosis, Trichomoniasis, Trichuriasis (Whipworm infection), Tuberculosis, Tularemia, *Urea-plasma urealyticum* infection, Venezuelan equine encephalitis, Venezuelan hemorrhagic fever, viral pneumonia, West Nile Fever, White piedra (*Tinea blanca*), *Yersinia pseudotuberculosis* infection, Yersiniosis, Yellow fever, and Zygomycosis.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat angioedema in the subject. In another embodiment, the angioedema is hereditary angioedema.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a disease, condition or disorder selected from the group consisting of sepsis, hypercoagulability, pulmonary dysfunction, hypoxemia, hemorrhagic pancreatitis,

myocardial infarction, lung transplantation, trauma, thermal injury and vascular leak in the subject.

In another embodiment, one or more crystalline polymorphic forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a disease, condition or disorder in which inhibition of kallikrein provides a therapeutically beneficial effect. Examples of such diseases, conditions or disorders include, but are not limited to, disease, conditions or disorders of the contact system. See e.g., Shariat-Madar et al., *Innate Immunity*, vol. 10, no. 1, 3-13 (2004) and Frick, et al., *EMBO J.*, (2006) 25, 5569-5578 (2006). In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a disease, condition or disorder selected from the group consisting of atherothrombosis, coronary artery disease, Alzheimer's Disease, inflammatory bowel disease (for example, Crohn's Disease), vascular leak, acute respiratory distress syndrome and bradykinin-mediated inflammation in the subject.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a disease, condition or disorder in which inhibition of bradykinin B2 receptor provides a therapeutically beneficial effect. In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat a disease, condition or disorder selected from the group consisting of glomerulosclerosis, Alzheimer's Disease, cerebral edema, vascular leak, acute respiratory distress syndrome, pain, inflammation, trauma, burns, shock, allergy, and cardiovascular disease in the subject.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1 or Compound 2, or a composition thereof, are administered to a subject to treat an infectious disease in the subject, for example, an infectious disease selected from the group consisting of Bovine respiratory disease, Porcine respiratory disease, Avian influenza, Avian infectious bronchitis, Bovine spongiform encephalopathy, Canine leishmaniasis, Chronic wasting disease, human immune deficiency virus (HIV), hepatitis, hepatitis A, hepatitis B, hepatitis C, Classical swine fever, Echinococcus, Enzootic pneumonia, FIP, Foot-and-mouth disease, Jaagsiekte, Maedi-Visna, Mastitis in animals, *Microsporum canis*, Orf (animal disease), Peste des petits ruminants, Pox diseases, Psittacine beak and feather disease, Rabies, Mediterranean fever (Brucellosis) or Bang's disease or undulant fever, Malta fever, contagious abortion, epizootic abortion, *Salmonella* food poisoning, enteric paratyphosis, Bacillary dysentery, Pseudotuberculosis, plague, pestilential fever, Tuberculosis, Vibrios, Circling disease, Weil's disease (Leptospirosis) or canicola fever, Hemorrhagic jaundice (*Leptospira icterohaemorrhagiae*), dairy worker fever (*L. hardjo*), Relapsing fever, tick-borne relapsing fever, spirochetal fever, vagabond fever, famine fever, Lyme arthritis, Bannworth's syndrome (lime disease), tick-borne meningopolyneuritis, erythema chronicum migrans, Vibriosis, Colibacteriosis, colitoxemia, white scours, gut edema of swine, enteric paratyphosis, Staphylococcal alimentary toxicosis, staphylococcal gastroenteritis, Canine Corona Virus (CCV) or canine parvovirus enteritis, feline infectious peritonitis virus, transmissible gastroenteritis (TGE) virus, Hagerman Redmouth Disease (ERMD), Infectious Hematopoietic necrosis (IHN), porcine *Actinobacillus* (*Haemophilus*) pleuropneumonia, Hansen's disease,

Streptotrichosis, Mycotic Dermatitis of Sheep, Pseudoglanders, Whitmore's disease, Francis' disease, deer fly fever, rabbit fever, O'Hara disease, Streptobacillary fever, Haverhill fever, epidemic arthritic erythema, sodoku, Shipping or transport fever, hemorrhagic septicemia, Ornithosis, Parrot Fever, Chlamydiosis, North American blastomycosis, Chicago disease, Gilchrist's disease, Cat Scratch Fever, Benign Lymphoreticulosis, Benign nonbacterial Lymphadenitis, Bacillary Angiomatosis, Bacillary Peliosis Hepatis, Query fever, Balkan influenza, Balkan grippé, abattoir fever, Tick-borne fever, pneumorickettsiosis, American Tick Typhus, Tick-borne Typhus Fever, Vesicular Rickettsiosis, Kew Gardens Spotted Fever, Flea-borne Typhus Fever, Endemic Typhus Fever, Urban Typhus, Ringworm, Dermatophytosis, Tinea, Trichophytosis, Microsporiosis, Jock Itch, Athlete's Foot, Sporothrix schenckii, dimorphic fungus, Cryptococcosis and histoplasmosis, Benign Epidermal Monkeypox, BEMP, Herpesvirus simiae, Simian B Disease, Venezuelan equine encephalitis, Type C lethargic encephalitis, Yellow fever, Black Vomit, hantavirus pulmonary syndrome, Korean Hemorrhagic Fever, Nephropathia Epidemica, Epidemic Hemorrhagic Fever, Hemorrhagic Nephrosonephritis, lymphocytic choriomeningitis, California encephalitis/La crosse encephalitis, African Hemorrhagic Fever, Green or Vervet Monkey Disease, Hydrophobia, Lyssa, Infectious hepatitis, Epidemic hepatitis, Epidemic jaundice, Rubeola, Morbilli, Swine and Equine Influenza, Fowl Plague, Newcastle disease, Piroplasmosis, toxoplasmosis, African Sleeping Sickness, Gambian Trypanosomiasis, Rhodesian Trypanosomiasis, Chagas's Disease, Chagas-Mazza Disease, South American Trypanosomiasis, Entamoeba histolytica, Balantidial dysentery, cryptosporidiosis, giardiasis, Cutaneous leishmaniasis: Chiclero ulcer, espondia, pianbols, uta, and bubo (in the Americas); oriental sore, Aleppo boil (in the Old World); Bagdad boil, Delhi boil, Baum ulcer, Visceral leishmaniasis: kala-azar, Microsporidiosis, Anisakiasis, Trichinosis, Angiostrongylosis, eosinophilic meningitis or meningoencephalitis (*A. cantonensis*), abdominal angiostrongylosis (*A. costaricensis*), Uncinariasis, Necatoriasis, Hookworm Disease, Capillariasis, Brugiasis, Toxocariasis, Oesophagostomiasis, Strongyloidiasis, Trichostrongylosis, Ascariasis, Diphylllobothriasis, Sparganosis, Hydatidosis, Hydatid Disease, Echinococcus granulosis, Cystic hydatid disease, Tapeworm Infection, and Schistosoma.

In another embodiment, one or more crystalline polymorphic forms or amorphous forms of Compound 1, or Compound 2 or a composition thereof, are administered to a subject to treat chronic renal disease, osteoarthritis, oncology, viral upper respiratory infection, feline plasma cell stomatitis, feline eosinophilic granulomas, feline leukemia virus infection, canine distemper infection, systemic fungal infections, cardiomyopathy, and mucopolysaccharidosis VII in the subject.

In the methods of the present disclosure, the gene switch regulates the expression of a polynucleotide encoding a peptide, protein, or polypeptide of interest. In one embodiment, gene switch regulates the expression of a polynucleotide encoding a peptide, protein, or polypeptide of therapeutic interest for the treatment of a disease, condition, or disorder in a subject, e.g., a human. In another embodiment, the peptide, protein or polypeptide of interest is selected from the group consisting of Her-2/neu (ERBB2/c-erbB-2), Osteocalcin, stromelysin-1, prostate specific antigen, human sodium-iodide symporter, H19, IF-1, IGF-2, thymosin β 15, T cell factor, cartilage-derived retinoic acid-sensitive protein, Prostatein, telomerase catalytic subunit, cyclin-A, mid-

kine; c-erbB-2, prostate-specific membrane antigen, p51, telomerase RNA, prostatic acid phosphatase, PCA3dd3, DF3/MUC1, hex II, cyclooxygenase-2, super PSA, skp2, PRL-3, CA125/M17S2, IAI.3B, CRG-L2, TRPM4, RTVP, TARP, telomere reverse transcriptase, A4 amyloid protein, amyloid β -protein precursor, precursor of the Alzheimer's Disease A4 amyloid protein, neuropeptide FF, endoplasmic reticulum stress elements, urocortin II, tyrosine hydroxylase, complement factor 3; serum amyloid A3, tissue inhibitor of metalloproteinase-3 (TIMP-3), p75 tumor necrosis factor receptor, tumor necrosis factor- α , TRPM4, RTVP, TARP, telomere reverse transcriptase, A4 amyloid protein, amyloid β -protein precursor, precursor of the Alzheimer's Disease A4 amyloid protein, neuropeptide FF, endoplasmic reticulum stress elements, urocortin II, tyrosine hydroxylase, complement factor 3; serum amyloid A3, tissue inhibitor of metalloproteinase-3 (TIMP-3), p75 tumor necrosis factor receptor, tumor necrosis factor- α , peroxisome proliferator activated receptor/IIA-1 nonpancreatic secreted phospholipase A2, SOCS-3, SR-BI, Ob, site-1 protease, TIGR, VL30, excitatory amino acid transporter-2, MDT59, LIM, pyrroline 5-carboxylate reductase, SIM2, Bax, Fas, bbc3, PINK-1, troponin T, myoD, Actin, smooth muscle 22 α , Utrophin, Myostatin, smooth muscle myosin heavy chain, cardiac ankyrin repeat protein, MLP, Smoothelin, MYBPC3, T α 1 α -tubulin, intercellular adhesion molecule-4 (ICAM-4), γ -aminobutyric acid type A receptor β 1 subunit, neuronal nicotinic acetylcholine receptor β 2-subunit, presenilin-1, calcium-calmodulin-dependent kinase II α , CRF2 α receptor, nerve growth factor, GLP-2 receptor, type I transglutaminase, K14, stearoyl-CoA desaturase, Megsin, Prolactin, GDF-9, PSP94, NRL, NGAL, long whey acidic protein, mammary associated amyloid A, endothelin-1, Serglycin, platelet-endothelial cell adhesion molecule-1 (PECAM-1), Tie receptor tyrosine kinase, KDR/flk-1, Endoglin, CCR5, CD11d, platelet glycoprotein IIb, preproendothelin-1, interleukin-18 binding protein, CD34, Tec tyrosine kinase, MLH1, MSH2, MSH6, PMS1, APC, LEF-1, F2 receptor, TGF- β type II receptor, EYA4, PCA3, K2, PROST 03, PCAM-1, PCADM-1, PCA3dd3, PCAV, PACP, ATB α , CSA-1, SYG972, Urb-ctf, BCU399, TBX2, Cyr61, DIAPH3, BEHAB, IL-8, BLSA, BP1, DAP-kinase, HOXA9, ARP, Nbk, CD43, β 7-hcG, β 6-hcG, β 6e-hcG, β 5-hcG, β 8-hcG, β 3-hcG, MTA1s, Old-35, Old-64, LAGE-1, CIF150/hTAFII150, P65 oncofetal protein, Telomerase, CYP1B1, 14-3-3 σ , NES1, CAR-1, HMG1, MAG, ELL2, Ephrin B2, WAF1, CIF130, C35, BMP2, BUB3, Polymerase kappa, EAG1, EAG2, HMG I, HLTF, Barx2, Pp 32r1, BMP4, TS10q23.3, Nuclear spindle-associating protein, PFTAIRe, SEMA3B, MOGP, Fortilin, IGFBP-3, Polyhomeotic 2, PNQALRE, SCN5A, miR15, miR16, Headpin, PAOh1/SMO, Hippo, Mst2, PSMA-like, JAB1, NF-AT, P28ING5, MTG16, ErbB-2, HDAC9, GPBP, MG20, KLF6, ARTS1, Dock 3, Annexin 8, MH15, DELTA-N p73, RapR6, StarD10, Ciz1, HLJ1, RapR7, A34, Sef, Killin, SGA-1M, TGF β Type II receptor, GCA-associated genes, PRV-1, Vezf1, MLP, VEGI, PRO256, AOP2, Remodelin, Phosphodiesterase 4D, Prostaglandin receptor subtype EP3, CARP, HOP, PLTP, UCP-2, FLJ11011, Codanin-1, Resistin, Archipelin, Neuronatin, Ncb5 or, 7B2, PTHrP, PEX, KChIP1, SLIT-3, CX3CR1, SMAP-2, IC-RFX, E2IG4, UCP2, Ob receptor, Ob, Dpl, NRG-1, Synapsin III, NRG1AG1, AL-2, Proline dehydrogenase, MNR2, ATM, Ho-1, CON202, Ataxin-1, NR3B, NIPA-1, DEPP, adrenomedullin, csdA, Inf-20, EOPA, SERT, FRP-1, Serum amyloid A, BMP2, BMPRIa, ACLP, Resistin-like molecule β , Dlg5, TRANCE, Matrilin-3, Synoviolin, HIV LTR, SHIVA, EBI 1,

41

EBI 2, EBI 3, NM23, Eps8, Beta-10, Hair follicle growth factor, Corneodesmosin, GCR9, Bg, FGF23, BBSR, MIC-1, MIA-2, IL-17B, Formylglycine generating enzyme, LPLA2, CXCL10, HFE2A, IL-1, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10R DN or a subunit thereof, IL-15, IL-18, IL-21, IL-23, IL-24, IL-27, GM-CSF, IFN-alpha, IFN-gamma, IFN-alpha 1, IFN alpha 2, IL-15-R-alpha, CCL3 (MIP-1a), CCL5 (RANTES), CCL7 (MCP3), XCL1 (lymphotactin), CXCL1 (MGSA-alpha), CCR7, CCL19 (MIP-3b), CXCL9 (MIG), CXCL10 (IP-10), CXCL12 (SDF-1), CCL21 (6Ck-ine), OX40L, 4-1BBL, CD40, CD70, GITRL, LIGHT, b-Defensin, HMGB1, Flt3L, IFN-beta, TNF-alpha, dnFADD, BCG, TGF-alpha, PD-L1 RNAi, a PD-L1 anti-sense oligonucleotide, TGFbRII DN, ICOS-L, S100, CD40L, p53, survivin, p53-survivin fusion, MAGE3, myelin basic protein, PSA and PSMA.

In another embodiment, the gene switch regulates the expression of a polynucleotide encoding an IL-12 or a subunit thereof. In another embodiment, the IL-12 or subunit thereof is human IL-12 or subunit thereof.

In another embodiment, the gene switch regulates the expression of a polynucleotide encoding a C1 esterase inhibitor (for example, a human C1 esterase inhibitor), a kallikrein inhibitor, or a bradykinin B2 receptor antagonist.

Examples of kallikrein inhibitors include, but are not limited to, ecallantide and those kallikrein inhibitors set forth U.S. Patent Publication Nos. 2010/0034805, 2009/0264350, 2009/0234009, 2008/0221031, 2007/0213275, 2006/0264603 and 2005/0089515.

Examples of bradykinin B2 receptor inhibitors include, but are not limited to, helokinesstatin and anti-bradykinin B2 receptor antibodies. The amino acid sequence of helokinesstatin is Gly-Pro-Pro-Tyr-Gln-Pro-Leu-Val-Pro-Arg (SEQ ID NO: 2) (Kwok, H. F. et al., *Peptides* 29I 65-72 (2008), which is incorporated by reference in its entirety). Nonlimiting examples of anti-bradykinin B2 receptor antibodies are set forth in Alla, S. A. et al., *J. Biol. Chem.* 271: 1748-1755 (1996).

In another embodiment, the gene switch regulates the expression of a polynucleotide encoding an IL-12 or a subunit thereof for the treatment of cancer, e.g., melanoma, in a subject, e.g., a human.

In another embodiment, a polynucleotide encodes (a) a gene switch that comprises a GAL4 DNA binding domain, the *Choristoneura fumiferana* ecdysone receptor ligand binding domain having the mutations V107I and Y127E (relative to SEQ ID NO: 1), a chimeric RXR ligand binding domain consisting of helices 1-8 of *Homo sapiens* RXR and helices 9-12 of *Locusta migratoria* RXR, the VP16 trans-activation domain, and (b) human IL-12, and the gene switch encoded by the polynucleotide regulates the expression of human IL-12 when the ecdysone receptor ligand binding domain in the gene switch binds Compound 1 or Compound 2. In a further embodiment, the polynucleotide is administered to a subject having a cancer such as melanoma. The polynucleotide may be administered intratumorally either in a pharmaceutically acceptable carrier, or contained by an immune cell such as a dendritic cell. In one embodiment, the polynucleotide is administered to a subject followed by administration of one or more polymorphic forms of Compound 1, or composition thereof. In another embodiment, one or more polymorphic forms of Compound 1, or composition thereof, is administered to a subject followed by administration of the polynucleotide. For example, one or more polymorphic forms or amorphous forms of Compound 1, or composition thereof, may be administered to the

42

subject on day -1, 0, +1, +2, +3, +4, +5, +6, +7, or more, relative to the day the polynucleotide is administered to the subject.

In another embodiment, the gene switch regulates the expression of a polynucleotide encoding a transcription factor, e.g., GATA-1, friend of GATA (FOG-1), EKLF (a Kruppel-like transcription factor), p45/nuclear factor-erythroid 2 (NF-E2), stem cell leukemia (SCL) or T-cell acute lymphocytic leukemia-1, OCT4, or Sry-related high-mobility group box transcription factor (Sox6), or growth factor, e.g., IGFII, bFGF, Flt3, stem cell factor (SCF), thrombopoietin (TPO), bone morphogenetic protein 4 (BMP4), recombinant human vascular endothelial growth factor (VEGF-A165), interleukin-3 (IL-3) interleukin-6 (IL-6), or interleukin-11 (IL-11), or erythropoietin, for use in regenerative medicine, e.g., differentiation, trans-differentiation, reprogramming, self-renewal, or expansion of hematopoietic stem cells, haematopoietic progenitor cells, or induced pluripotent stem cells in the process of blood pharming, i.e., production of red blood cells or other blood products, in a subject.

EXAMPLES

Instrumentation—Typical Measurement Conditions

Powder X-Ray Diffraction (PXRD)

Bruker D8 Advance diffractometer in Bragg-Brentano reflection geometry; Cu K α radiation, 40 kV/40 mA; variable divergence slit; LynxEye detector with 3° window; step size, 0.02° 2 θ ; step time, 37 s; scanning range 2-50° in 2 θ . The samples were rotated (0.5 rps) during the measurement and prepared without any special treatment other than slight pressure to get a flat surface. The measurement was performed under ambient laboratory conditions in a silicon single crystal sample holder, 0.1- or 1-mm deep.

FT-Raman Spectroscopy:

Bruker RFS 100 FT-Raman system; FT-Raman spectra were recorded with a NIR Nd:YAG laser operating at 1064 nm and a liquid-nitrogen cooled germanium detector; 300 mW nominal laser power; 64 scans with resolution 2 cm⁻¹; sample measured in an aluminum sample holder under ambient laboratory conditions.

Differential Scanning Calorimetry (DSC):

Perkin Elmer DSC7; closed gold crucibles filled under nitrogen atmosphere; heating rate 10° C./min.

Dynamic Vapor Sorption (DVS):

Projekt Messtechnik SPS 11-100n multi-sample water vapor sorption analyzer or Surface Measurement Systems Ltd. DVS-1 water vapour sorption analyzer. The sample was allowed to equilibrate at 50% r.h. before starting a pre-defined humidity program.

Program: 50% r.h.→0% r.h.→95% r.h.→50% r.h., Ar.h.=5%/h

Hygroscopicity was classified as follows:

deliquescent sufficient water is absorbed to form a liquid very hygroscopic increase of the mass is $\geq 15\%$

hygroscopic increase of the mass is less than 15% and greater than or equal to 2%

slightly hygroscopic increase of the mass is less than 2% and greater than or equal to 0.2%

not hygroscopic increase of the mass is less than 0.2%

43

Thermogravimetry Coupled to Fourier-Transform Infrared Spectroscopy (TG-FTIR):

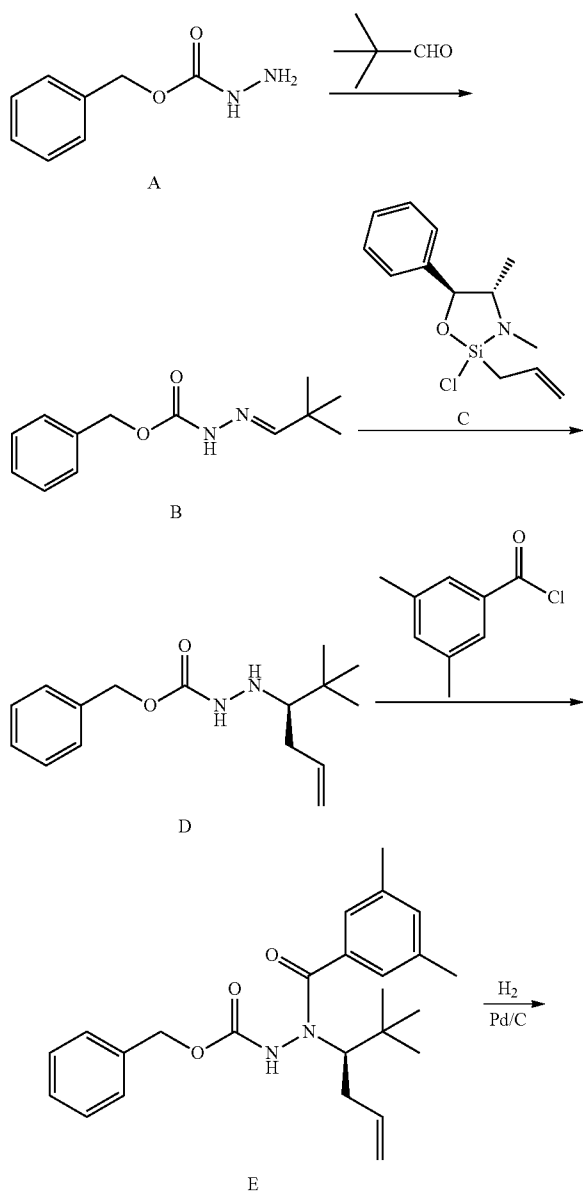
Netzsch Thermo-Microbalance TG 209 coupled to a Bruker Vector 22 FTIR spectrometer; aluminum crucible with a pinhole, measurement under N₂ atmosphere, heating rate 10° C./min.

Example 1

(R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-but-3-enyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide (Compound 1)

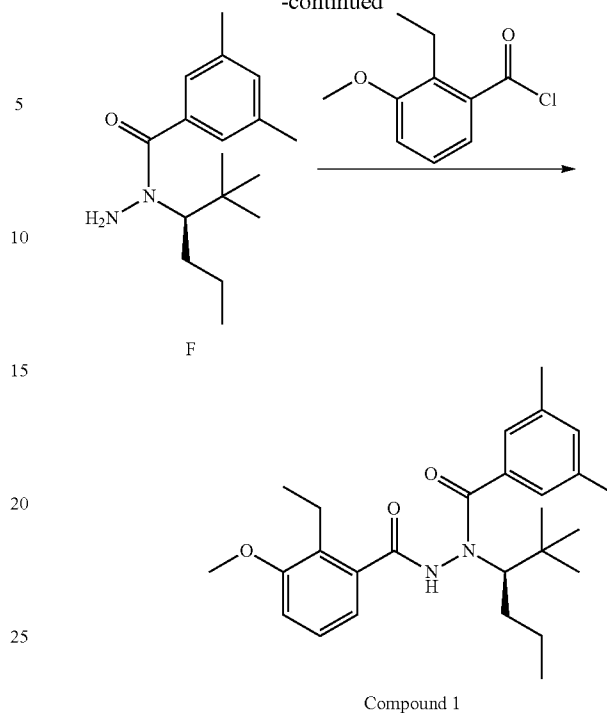
Compound 1 was prepared according to Scheme 1 as described in US 2009/0163592 (see Example 1).

Scheme 1



44

-continued



Briefly, benzyl carbazate (compound A) was reacted with pivaldehyde to give (E)-N'-(2,2-dimethyl-propylidene)-hydrazinecarboxylic acid benzyl ester (compound B). Compound B was reacted with (S,S)-2-allyl-2-chloro-3,4-dimethyl-5-phenyl-[1,3,2]oxazasilolidine (compound C; see Leighton et al., *J. Am. Chem. Soc.* 125:9596 (2003) and WO 03/074534) to give (R)-N'-(1-tert-butyl-but-3-enyl)-hydrazinecarboxylic acid benzyl ester (compound D). Compound D was reacted with 3,5-dimethyl benzoyl chloride to give (R)-N'-(1-tert-butyl-but-3-enyl)-N'-(3,5-dimethyl-benzoyl)-hydrazinecarboxylic acid benzyl ester (compound E). Compound E was hydrogenated to give (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-but-3-enyl)-hydrazide (compound F). Compound F was reacted with 2-ethyl-3-methoxybenzoyl chloride to give Compound 1. The crude material was triturated first with ether (2% ethanol) and then with 1:1 hexanes:ether in a fritted glass Büchner funnel. The product was then washed with deionized water with thorough mixing and allowed to dry in air. Compound 1 was isolated as a white powder.

Compound 2 was prepared in similar fashion using (R,R)-2-allyl-2-chloro-3,4-dimethyl-5-phenyl-[1,3,2]oxazasilolidine as disclosed in US 2009/0163592.

Compound 1 regulates in vitro and in vivo therapeutic gene expression via ecdysone receptor-based inducible gene expression systems as disclosed in US 2009/0163592 Examples 66, 67, 72, and 74.

Example 2

Preparation of Mixtures of Crystalline Polymorphic Forms of Compound 1

For the purpose of the present disclosure, mixtures of crystalline polymorphic forms of Compound 1 are designated Forms I-A, Form I-B, Form I-C, Form I-D, Form I-E, Form I-F, Form I-G, and Form I-H.

Method 1

Compound 1 obtained using the methodology described in Example 1 was recrystallized from toluene/heptane, filtered, and dried under vacuum. This crystalline solid obtained was subjected to a second recrystallization from methanol/water, filtered, and dried under vacuum. The crystalline solid obtained was micronized to give crystalline Compound 1 Form I-A as a mixture of polymorphic forms (Form II with traces of Form IV), as characterized by PXRD, FT-Raman spectroscopy, and DSC (FIGS. 1-3, respectively). Micronized Compound 1 Form I-A was used as the starting material to prepare pure Forms II, III, IV, V, VI, VII, VIII, and IX, and mixtures thereof, in Methods 2-40 herein below.

Using basically the same procedure as described in Method 1 without micronization, Compound 1 Form I-B (mixture of Form II, Form III, and Form IV), Form I-C (mixture of Form II and Form IV), and Form I-D (mixture of Form II and Form IV) were obtained (FIGS. 4-6, respectively).

Method 2

102 mg of Compound 1 Form I-A was suspended in 2.0 mL of n-hexane and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form I-E (mixture of Form III with traces of Form IV, FIG. 7).

Method 3

103 mg of Compound 1 Form I-A was dissolved in 0.25 mL of isopropyl acetate. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Form I-F (mixture of Form II with traces of Form IV, FIG. 8).

Method 4

100 mg of Compound 1 Form I-A was dissolved in 0.15 mL of tetrahydrofuran. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give a mixture of Form I-G (mixture of Form II with traces of Form IV, FIG. 9).

Method 5

108 mg of Compound 1 Form I-A was dissolved in 3.7 mL of diisopropylether at 60° C. and the solution was heated to 65° C. The solution was cooled at 3° C./hour to 5° C. to give a suspension. The solid was collected by filtration and dried under vacuum for 1 hour to give Form I-H (mixture of Form II and Form III, FIG. 10).

Example 3

Preparation of Compound 1 Form II

Pure crystalline Compound 1 Form II was prepared according to the following methods:

Method 6

101 mg of Compound 1 Form I-A was suspended in 1.0 mL of cyclohexane and sonicated. After stirring for 17 days at 60° C., the solid was collected by filtration and dried under vacuum for 1 hour to give Form II.

Method 7

101 mg of Compound 1 Form I-A was suspended in 0.1 mL of 1:1 (v/v) benzyl alcohol/cyclohexane and sonicated. After stirring for 17 days at 60° C., the solid was collected by filtration and dried under vacuum for 1 hour to give Form II.

Method 8

99 mg of Compound 1 Form I-A was suspended in 0.1 mL of 3:2 (v/v) n-heptane/toluene and sonicated. After stirring for 17 days at 60° C., the solid was collected by filtration and dried under vacuum for 1 hour to give Form II.

Method 9

100 mg of Compound 1 Form I-A was suspended in 0.2 mL of 10:1 (v/v) n-heptane/toluene and sonicated. After stirring for 17 days at 60° C., the solid was collected by filtration and dried under vacuum for 1 hour to give Form II.

Method 10

105 mg of Compound 1 Form I-A was suspended in 0.1 mL of 1:10 (v/v) n-heptane/toluene and sonicated. After stirring for 17 days at 60° C., the solid was collected by filtration and dried under vacuum for 1 hour to give Form II.

Method 11

105 mg of Compound 1 Form I-A was suspended in 0.2 mL of 3:2 (v/v) n-heptane/toluene and sonicated. After stirring for 5 days at 90° C., an additional 0.1 mL of solvent was added. After stirring 2 more days at 90° C., the solid was collected by filtration and dried under vacuum for 20 minutes to give Form II.

Method 12

203 mg of Compound 1 Form I-A was dissolved in 0.2 mL of methyl ethyl ketone, and 2.0 mL of n-dodecane was added to give a precipitate. The precipitate was collected by filtration and dried under vacuum for 25 minutes to give Form II.

Method 13

105 mg of Compound 1 Form I-A was dissolved in 0.25 mL of acetonitrile. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Form II.

Method 14

105 mg of Compound 1 Form I-A was dissolved in 0.25 mL of isopropyl acetate. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Form II.

Method 15

112 mg of Compound 1 Form I-A was dissolved in 0.15 mL of dichloromethane. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Form II.

PXRD, FT-Raman, and DSC characterization of Compound 1 Form II is provided in FIGS. 11-13, respectively. Table 1 lists the PXRD peak positions, peak intensities, and d values of Compound I Form II. TG-FTIR showed that it is a non-solvated, i.e., anhydrous form. DVS showed that it is non- to slightly-hygroscopic as a water content of 0.1 wt.-% is gained from 0% relative humidity (r.h.) to 85% r.h.

TABLE 1

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
8.34	10.6	1033	100
10.06	8.8	506	49
14.01	6.3	522	51
14.51	6.1	37	4
15.55	5.69	139	14
16.77	5.28	246	24
17.70	5.01	217	21
18.40	4.82	260	25
18.88	4.70	130	13
20.23	4.39	317	31
22.36	3.97	384	37
22.97	3.87	428	42
23.91	3.72	192	19
24.15	3.68	122	12
25.00	3.56	215	21
25.92	3.43	126	12
26.96	3.30	44	4
28.09	3.17	148	14
28.33	3.15	59	6

TABLE 1-continued

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
29.84	2.99	60	6
30.52	2.93	95	9
31.05	2.88	93	9
31.45	2.84	84	8
31.97	2.80	67	7
32.61	2.74	60	6
33.17	2.70	55	5
34.02	2.63	74	7
34.45	2.60	61	6
35.07	2.56	72	7

In a separate experiment, 86.9 mg of Compound 1 Form II was stored in an open container in an autoclave under 1 bar of CO₂ atmosphere and over saturated NaCl solution (75-76% relative humidity) for 1 month to give unchanged Form II. There was no indication of hydrate (Form IV) or CO₂ adduct formation.

Two types of experiments elucidated the mechanical stability of Compound 1 Form II. First, about 200 mg of Form II was pressed in an IR press with a force of 10 metric tons (13 mm diameter of pellet) for 30 min. The resulting pellets were analyzed by PXRD. Second, about 150 mg of Form II was vigorously ground and analyzed by PXRD. The PXRD patterns of the samples after mechanical treatment show no change in crystalline form after grinding or pressurizing.

Example 4

Preparation of Compound 1 Form III

Pure crystalline Compound 1 Form III was prepared according to the following methods:

Method 16

109 mg of Compound 1 Form I-A was suspended in 2.0 mL of cyclohexane and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 17

113 mg of Compound 1 Form I-A was suspended in 2.0 mL of n-heptane and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 18

106 mg of Compound 1 Form I-A was suspended in 2.0 mL of cumene and sonicated. After stirring for 1 day at room temperature, an additional 28 mg of Compound 1 Form I-A was added. After stirring for 13 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 19

109 mg of Compound 1 Form I-A was suspended in 1.0 mL of diethyl ether and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 20

165 mg of Compound 1 Form I-A was suspended in 0.2 mL of ethyl acetate and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 21

105 mg of Compound 1 Form I-A was suspended in 1.0 mL of tert-butyl methyl ether (TBME) and sonicated. After

stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 22

103 mg of Compound 1 Form I-A was suspended in 0.2 mL of toluene and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 23

101 mg of Compound 1 Form I-A was suspended in 2.0 mL of n-dodecane and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 24

133 mg of Compound 1 Form I-A was suspended in 0.2 mL of 1:1 (v/v) n-hexane/ethanol and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 25

178 mg of Compound 1 Form I-A was suspended in 0.2 mL of 1:1 (v/v) n-octane/acetone and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 26

111 mg of Compound 1 Form I-A was suspended in 0.7 mL of 3:2 (v/v) n-heptane/toluene and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 2.5 hours to give Form III.

Method 27

97 mg of Compound 1 Form I-A was suspended in 0.8 mL of 10:1 (v/v) n-heptane/toluene and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 2.5 hours to give Form III.

Method 28

100 mg of Compound 1 Form I-A was suspended in 0.5 mL of 1:10 (v/v) n-heptane/toluene and sonicated. After stirring for 1 day at room temperature, 30 mg of Compound 1 Form I-A was added. After stirring for 13 days at room temperature, the solid was collected by filtration and dried under vacuum for 2.5 hours to give Form III.

Method 29

105 mg of Compound 1 Form I-A was suspended in 0.7 mL of 3:2 (v/v) n-heptane/toluene and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 30

102 mg of Compound 1 Form I-A was suspended in 0.7 mL of 10:1 (v/v) n-heptane/toluene and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

Method 31

115 mg of Compound 1 Form I-A was suspended in 0.7 mL of 1:10 (v/v) n-heptane/toluene and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form III.

49

These experiments show that at 5° C. and room temperature Form III is more stable than Form II.

PXRD, FT-Raman, and DSC characterization of Compound 1 Form III is provided in FIGS. 14-16, respectively. Table 2 lists the PXRD peak positions, peak intensities, and d values of Compound I Form III. TG-FTIR investigation shows that it is a non-solvated, i.e., anhydrous form. DVS showed that it is non- to slightly-hygroscopic as a water content of 0.1 wt.-% is gained from 0% relative humidity (r.h.) to 85% r.h.

TABLE 2

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
8.14	10.9	899	100
8.52	10.4	402	45
9.62	9.2	34	4
11.02	8.0	116	13
11.90	7.4	63	7
12.16	7.3	86	10
14.02	6.3	105	12
14.62	6.1	38	4
17.00	5.21	611	68
17.88	4.96	172	19
18.56	4.78	321	36
19.02	4.66	100	11
19.24	4.61	106	12
20.51	4.33	50	6
20.93	4.24	68	8
22.19	4.00	194	22
22.73	3.91	129	14
23.22	3.83	69	8
24.31	3.66	61	7
24.53	3.63	67	8
25.91	3.44	71	8
26.22	3.40	58	6
27.36	3.26	39	4
27.73	3.21	30	3
28.70	3.11	57	6
30.84	2.90	24	3
31.52	2.84	52	6
32.30	2.77	28	3
33.19	2.70	22	3
34.39	2.61	24	3

In a separate experiment, 52.4 mg of Compound 1 Form III was stored in an open container in an autoclave under 1 bar of CO₂ atmosphere and over saturated NaCl solution (75-76% relative humidity) for 1 month to give unchanged Form III. There was no indication of hydrate (Form IV) or CO₂ adduct formation.

Two types of experiments elucidated the mechanical stability of Compound 1 Form III. First, about 200 mg of Form III was pressed in an IR press with a force of 10 metric tons (13 mm diameter of pellet) for 30 min. The resulting pellets were analyzed by PXRD. Second, about 150 mg of Form III was vigorously ground and analyzed by PXRD. The PXRD patterns of the samples after mechanical treatment show no change in crystalline form after grinding or pressurizing.

Example 5

Preparation of Compound 1 Form IV

Pure crystalline Compound 1 Form IV was prepared according to the following methods:

Method 32

106 mg of Compound 1 Form I-A was suspended in 2.0 mL of water and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried

50

under vacuum for 30 minutes to give Form IV. TG-FTIR investigation shows Form IV is a hydrate with a loss of 3.5 wt.-% water from 25° C.-150° C. (3.9 wt.-% would correspond to a monohydrate). According to TG-FTIR, the hydrate releases water near 100° C.

Method 33

100 mg of Compound 1 Form I-A was suspended in 5.0 mL of 1:1 (v/v) water:ethanol and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form IV.

Method 34

109 mg of Compound 1 Form I-A was suspended in 5.0 mL of 1:1 (v/v) water:2-propanol and sonicated. After stirring for 16 days at 5° C., the solid was collected by filtration and dried under vacuum for 30 minutes to give Form IV.

PXRD, FT-Raman, and DSC (2 thermograms) characterization of Compound I Form IV is provided in FIGS. 17-20, respectively. Table 3 lists the PXRD peak positions, peak intensities, and d values of Compound I Form IV.

TABLE 3

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
6.83	12.9	364	83
8.38	10.5	83	19
8.91	9.9	82	19
10.11	8.7	63	14
10.31	8.6	129	30
11.30	7.8	437	100
11.89	7.4	74	17
12.18	7.3	97	22
12.98	6.8	214	49
13.69	6.5	119	27
14.14	6.3	77	18
15.11	5.86	306	70
15.81	5.60	39	9
16.23	5.46	118	27
17.60	5.03	98	22
17.99	4.93	116	27
18.60	4.77	83	19
19.15	4.63	38	9
19.66	4.51	75	17
20.28	4.38	60	14
20.70	4.29	156	36
21.15	4.20	178	41
21.68	4.10	148	34
22.44	3.96	47	11
22.71	3.91	196	45
23.50	3.78	68	16
23.79	3.74	91	21
24.06	3.70	62	14
24.86	3.58	187	43
25.55	3.48	41	9
26.53	3.36	46	10
26.94	3.31	45	10
27.21	3.28	72	17
27.60	3.23	50	12
28.67	3.11	35	8
29.79	3.00	75	17
30.50	2.93	43	10
30.75	2.91	51	12
31.55	2.83	32	7
31.89	2.80	35	8
32.78	2.73	27	6
33.25	2.69	33	8
33.48	2.68	38	9
33.81	2.65	44	10
34.68	2.59	46	11

51

Example 6

Preparation of Compound 1 Form V

Method 35

126 mg of Compound 1 Form I-A was dissolved in 0.55 mL of methanol. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Form V. TG-FTIR investigation showed that Form V was a methanol monosolvate with a loss of 6.7 wt.-% of methanol from 50-150° C. (6.8 wt.-% would correspond to a monosolvate).

PXRD and FT-Raman characterization of Compound I Form V is provided in FIGS. 21 and 22, respectively. Table 4 lists the PXRD peak positions, peak intensities, and d values of Compound I Form V.

TABLE 4

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
6.11	14.5	18	2
9.38	9.4	607	81
11.13	7.9	33	4
12.22	7.2	746	100
13.18	6.7	271	36
14.14	6.3	143	19
14.98	5.91	160	22
15.52	5.71	90	12
15.78	5.61	70	9
17.32	5.12	310	42
18.40	4.82	309	42
18.75	4.73	145	19
19.48	4.55	139	19
19.74	4.49	142	19
20.63	4.30	74	10
21.33	4.16	75	10
21.88	4.06	85	11
22.41	3.97	444	60
23.40	3.80	163	22
23.55	3.78	164	22
23.76	3.74	118	16
24.27	3.67	103	14
24.63	3.61	314	42
24.79	3.59	232	31
25.61	3.48	150	20
26.66	3.34	91	12
27.10	3.29	77	10
27.81	3.21	104	14
28.02	3.18	170	23
28.58	3.12	87	12
29.91	2.99	58.5	8
30.35	2.94	51.2	7
30.95	2.89	74.9	10
31.32	2.85	100	14
31.77	2.82	198	27
32.77	2.73	64.4	9
33.81	2.65	74.2	10
34.98	2.56	61.4	8

Example 7

Preparation of Compound 1 Form VI

Method 36

101 mg of Compound 1 Form I-A was suspended in 5.0 mL of 1:1 (v:v) water:methanol and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form VI. The PXRD pattern of the dried sample is different from a superposition of Form IV (hydrate) and Form V (methanol monosolvate). Form VI is probably a mixed methanol solvate/hydrate. In this form, the methanol/water

52

ratio can affect the solid structure, and this would likely result in PXRD peak position shifts. TG-FTIR investigation shows a loss of 3.8 wt.-% methanol (and some water) from 25° C.-90° C. and 2.1 wt.-% water (and some methanol) from 90-130° C. (3.5 wt.-% methanol would correspond to a hemi-solvate; 2.0 wt.-% water would correspond to a hemi-hydrate).

PXRD and FT-Raman characterization of Compound I Form VI is provided in FIGS. 23 and 24, respectively. Table 5 lists the PXRD peak positions, peak intensities, and d values of Compound I Form VI.

TABLE 5

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
6.09	14.5	34	6
6.82	12.9	21	4
8.57	10.3	16	3
9.38	9.4	566	100
11.26	7.9	36	6
12.23	7.2	513	91
13.25	6.7	174	31
14.27	6.2	108	19
15.05	5.88	107	19
15.54	5.70	45	8
15.95	5.55	39	7
17.48	5.07	156	28
18.41	4.82	177	31
18.79	4.72	63	11
19.54	4.54	62	11
19.76	4.49	68	12
20.79	4.27	29	5
21.48	4.13	33	6
22.02	4.03	47	8
22.41	3.96	213	38
23.42	3.80	73	13
24.07	3.69	33	6
24.34	3.65	47	8
24.64	3.61	78	14
24.83	3.58	90	16
25.34	3.51	40	7
25.67	3.47	63	11
26.74	3.33	25	4
26.87	3.32	31	5
27.24	3.27	35	6
27.99	3.19	64	11
28.56	3.12	27	5
28.93	3.08	25	4
29.47	3.03	21	4
30.04	2.97	26	5
30.98	2.88	24	4
31.75	2.82	56	10
32.34	2.77	22	4
32.96	2.72	19	3
33.84	2.65	25	4

Example 8

Preparation of Compound 1 Form VII

Method 37

101 mg of Compound 1 Form I-A was suspended in 0.5 mL of DMSO and sonicated. After stirring for 14 days at room temperature, the solid was collected by filtration and dried under vacuum for 30 minutes to give Form VII. TG-FTIR investigation showed that Form VII was a DMSO solvate with a loss of 9.9 wt.-% of DMSO from 25° C.-150° C. (8.2 wt.-% DMSO would correspond to a hemi-solvate; 15.1 wt.-% would correspond to a monosolvate) indicating the Form VII is a DMSO solvate. PXRD and FT-Raman characterization of Compound I Form VII is provided in

53

FIGS. 25 and 26, respectively. Table 6 lists the PXRD peak positions, peak intensities, and d values of Compound I Form VII.

TABLE 6

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
6.64	13.3	175	19
8.18	10.8	185	20
9.71	9.1	928	100
10.44	8.5	114	12
10.80	8.2	30	3
11.69	7.6	81	9
13.30	6.7	236	26
13.64	6.5	93	10
15.35	5.77	71	8
16.22	5.46	201	22
16.44	5.39	117	13
17.23	5.14	58	6
17.73	5.00	290	31
18.16	4.88	70	8
19.46	4.56	178	19
19.72	4.50	90	10
19.97	4.44	147	16
20.70	4.29	60	7
20.98	4.23	416	45
21.20	4.19	309	33
21.52	4.13	59	6
21.98	4.04	75	8
22.57	3.94	105	11
22.76	3.90	361	39
23.09	3.85	161	17
23.75	3.74	107	12
24.37	3.65	173	19
24.68	3.60	188	20
25.31	3.52	42	5
25.97	3.43	93	10
26.25	3.39	65	7
26.49	3.36	67	7
26.72	3.33	226	24
28.13	3.17	40	4
29.39	3.04	271	29
29.88	2.99	46	5
30.92	2.89	52	6
31.17	2.87	50	5
31.70	2.82	88	10
31.96	2.80	60	6
33.57	2.67	59	6
34.83	2.57	45	5

Example 9

Preparation of Compound 1 Form VIII

Method 38

199 mg of Compound 1 Form I-A was dissolved in 0.32 mL of benzyl alcohol and 2.0 mL of methylcyclohexane was added. After stirring at 5° C. for 4 h a turbid solution was obtained. The solvent was evaporated at 5° C. The solid was collected by filtration and dried under vacuum for 45 minutes to give Form VIII. (FIGS. 27 and 28). Table 7 lists the PXRD peak positions, peak intensities, and d values of Compound I Form VIII. TG-FTIR investigation showed a loss of 63.0 wt.-% benzyl alcohol from 25° C.-250° C. The sample was likely incompletely dried but might also correspond to a solvate. The sample was investigated again after 2 weeks. It was found to be still in a wet state and to correspond to the same form. Then the sample was dried for 1 day under nitrogen flow and subsequently for 6 days under vacuum at room temperature. It then appeared to be a dry powder, the FT-Raman spectrum corresponded to Form VIII, and TG-FTIR investigation showed a loss of 8.9 wt.-% of benzyl alcohol (not all solvent released).

54

TABLE 7

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
7.48	11.8	16	5
8.31	10.6	9	3
10.05	8.8	332	96
10.77	8.2	346	100
12.27	7.2	15	4
13.65	6.5	30	9
14.06	6.3	106	31
15.60	5.68	44	13
16.03	5.53	25	7
16.76	5.29	264	76
16.96	5.23	31	9
17.16	5.16	40	12
18.11	4.90	147	43
18.32	4.84	100	29
18.43	4.81	79	23
18.65	4.75	38	11
19.89	4.46	56	16
20.28	4.38	28	8
20.89	4.25	149	43
21.71	4.09	193	56
21.87	4.06	68	20
24.07	3.69	105	30
24.90	3.57	200	58
25.28	3.52	61	18
25.54	3.49	20	6
25.86	3.44	18	5
26.22	3.40	10	3
26.66	3.34	5	2
27.74	3.21	31	9
28.44	3.14	30	9
28.71	3.11	208	60
29.08	3.07	11.6	3
30.26	2.95	15	4
31.16	2.87	18.3	5
32.59	2.75	7.95	2
32.85	2.72	31.4	9
34.01	2.63	18.7	5
34.68	2.59	11.9	3
35.09	2.56	15.7	5

Example 10

Preparation of Compound 1 Form IX

Method 39

198 mg of Compound 1 Form I-A was dissolved in 0.2 mL acetone and 2.0 mL of cyclohexane. The solution was stirred at 5° C. for 4 hours then evaporated at 5° C. for 2 hours to obtain a precipitate. The precipitate was collected by filtration and dried under vacuum for 1 hour to give Form IX (FIGS. 29 and 30). Table 8 lists the PXRD peak positions, peak intensities, and d values of Compound I Form IX. TG-FTIR investigation showed a loss of 51.2 wt.-% of cyclohexane from 25° C.-150° C. The sample was likely incompletely dried but might also correspond to a cyclohexane solvate. Reinvestigation of the sample by PXRD and FT-Raman spectroscopy shows that the sample had spontaneously transformed into Form II.

TABLE 8

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
7.06	12.5	1799	100
9.93	8.9	160	9
12.22	7.2	230	13
14.13	6.3	53	3
15.74	5.63	326	18
17.28	5.13	98	6

55

TABLE 8-continued

Angle (2-Theta °)	d value (Angstrom)	Intensity (Cps)	Intensity (%)
18.71	4.74	741	41
19.96	4.44	53	3
21.18	4.19	290	16
22.39	3.97	260	15
23.51	3.78	90	5
24.54	3.63	105	6
25.58	3.48	188	11
27.52	3.24	57	3
28.48	3.13	26	1
29.33	3.04	36	2
30.18	2.96	42	2
31.01	2.88	51	3
31.82	2.81	15	1
32.73	2.73	95	5

Example 11

Preparation of Compound 1 Form X

Method 40

128 mg of Compound 1 Form I-A was dissolved in 0.2 mL of formic acid. The solvent was evaporated under a stream of nitrogen for 1 day. The solid was dried under vacuum for 35 minutes to give Compound 1 as an amorphous solid (Form X) based on PXRD characterization (FIG. 31) and FT-Raman spectroscopy (FIG. 32).

Example 12

Solvent-Based Stability Tests in n-Heptane/Toluene

The thermodynamic stability of Form II and Form III as a function of temperature was investigated by suspension equilibration experiments.

Method 41

54.6 mg of Compound 1 Form III and 61.2 mg of Compound 1 Form II were suspended in 0.8 mL of 3:2 (v/v) n-heptane:toluene and sonicated. After stirring at 45° C. for 6 days the solid was collected by filtration and dried under vacuum for 1 hour to give a mixture of Form III and Form IV. In this experiment the starting material likely took up ambient humidity to give Form IV.

Method 42

18.5 mg of Compound 1 Form III and 28.0 mg of Compound 1 Form II were suspended in 0.4 mL of 3:2 (v/v) n-heptane:toluene and sonicated. After stirring at 60° C. for 6 days the solid was collected by filtration to give Form II.

Method 43

35.4 mg of Compound 1 Form III and 46.1 mg of Compound 1 Form II were suspended in 0.4 mL of 3:2 (v/v) n-heptane:toluene and sonicated. After stirring at 50° C. for 6 days the solid was collected by filtration to give a mixture of Form II and Form III.

Method 44

40.5 mg of Compound 1 Form III and 45.7 mg of Compound 1 Form II were suspended in 0.4 mL of 3:2 (v/v) n-heptane:toluene and sonicated. After stirring at 55° C. for 6 days the solid was collected by filtration to give Form II.

The solvent-based experiments are in principle thermodynamic stability tests between Compound 1 Forms II and III because mixtures of both forms were suspended. Below 45° C. Form III is the more stable form in 3:2 (v/v) n-heptane:toluene. Above 55° C. Form II is the more stable form in 3:2 (v/v) n-heptane:toluene. At 50° C. a mixture of

56

both Form II and Form III was obtained in 3:2 (v/v) n-heptane:toluene. This is not unexpected. Close to the transition temperature, the thermodynamic driving force would be expected to be very small leading to slow transition rates. Possibly the kinetics of transformation were too slow for the transformation to occur on the timescale of the experiment. According to these suspension equilibration experiments, Form II and Form III are enantiotropic polymorphs. The thermodynamic transition temperature between Form II and Form III is between 45° C. and 55° C.

Example 13

Solvent-Based Stability Tests in n-Heptane/Ethanol

Compound 1 Form II, Compound 1 Form III, or a mixture of Compound 1 Forms II and III were slurried in 95:5 heptane:ethanol (anhydrous). After one day at various temperatures, a portion of the slurry was filtered and analyzed by XRPD. The results are presented in Table 9. A solution mediated transition occurs at 45-47° C. in 95:5 heptane:ethanol. Below 45° C. Compound 1 Form III is more stable; above 47° C. Compound 1 Form II is more stable.

TABLE 9

Initial Form	Temperature (° C.)	Final Form
II	25	III
II and III	40	III
II and III	45	III
II and III	47	II
II and III	48	II
II and III	49	II
II and III	50	II
II and III	55	II
III	60	II

Example 14

Solvent-Based Stability Tests in Water/Methanol

A mixture of Compound 1 Forms II, III, IV, and V was slurried in various compositions of methanol/water, or in water, for at least three days at different temperatures. The results are presented in Table 10. At a methanol content of more than 60% volume, Form V is the most stable polymorphic form of Compound 1 at all three temperatures. At a methanol content of less than 60% volume, Form IV is the most stable polymorphic form of Compound 1 at all three temperatures.

TABLE 10

Solvent	25° C. Form	45° C. Form	55° C. Form
8:2 MeOH:water	V	V	V
7:3 MeOH:water	V	V	V
6:4 MeOH:water	V	IV and V	IV and V
5:5 MeOH:water	IV	IV	IV
4:6 MeOH:water	IV	IV	IV
2:8 MeOH:water	IV	IV	IV
water	IV	IV	IV

57

Example 15

Solid State Stability of Compound 1 Forms II, III, and IV at 60-65° C.

Compound 1 Form II, Compound 1 Form III, and Compound 1 Form IV were stored at 60-65° C. in a closed cap vial for various lengths of time. The results are presented in Table 11. Compound 1 Forms II and III remained unchanged under these conditions. Compound 1 Form IV is converted to a mixture of Forms II and III after 10 days at 60-65° C.

TABLE 11

Initial Form	3 days	10 days	18 days
II	II	II	II
III	III	III	III
IV	II, III, and IV	II and III	II and III

Example 16

Gene Switch Efficacy of Compound 1

Cellular gene-switch assays were performed by transfecting the following constructs in mouse embryonic fibroblast cells (NIH3T3). The wild-type D-, E-, and F-domains from a) *C. fumiferana* EcR (CfEcR-DEF), and b) *C. fumiferana* EcR with a E274V/V390I/Y410E mutation (VY-CfEcR-DEF), were fused to a GAL4-DBD and placed under the control of the CMV promoter in a pBIND vector (Promega Corporation, Madison, Wis., USA). A chimeric RXR from *Homo sapiens* RXR β and *Locusta migratoria* RXR fused to VP16-AD and under the control of an SV40e promoter has previously described. (See, Kothapalli et al., *Dev. Genet.* 17:319-330 (1995); Palli, et al., *FEBS J.* 272:5979-5990 (2005); and U.S. Pat. No. 7,935,510 B2). The inducible luciferase reporter plasmid, pFRLuc, (Stratagene Cloning Systems, La Jolla, Calif., USA) contains five copies of the GAL4 response element and a synthetic minimal promoter.

Under standard assay conditions, Compound 1 was tested at 8 doses from 0.01-33 μ M and the final DMSO concentration was 0.33% in both control and treatment wells. When necessary, Compound 1 was tested at lower concentrations. After a 48-hour post-treatment and transfection incubation, the cells were assayed for luciferase activity using the Bright-Glo™ Luciferase Assay System (Promega Corporation, Madison, Wis., USA) following the manufacturer's instructions. Assays were performed minimally in duplicate and definitive assays as many as six times. Data was fitted to a sigmoidal dose-response curve. Rel Max FI=maximum fold induction relative to a positive control. Results are presented in Table 12.

TABLE 12

Assay	Result Type	Value
WT-Cf EcR	EC ₅₀	1.8 nM
	Rel Max FI	0.99
VY-Cf EcR	EC ₅₀	0.133 nM
	Rel Max FI	0.88

Example 17

Clinical Study

The safety, tolerance, transgene function, and immunological effects of intratumoral injection(s) of adenoviral

58

transduced autologous dendritic cells engineered to express hIL-12 and one or more other immunomodulators under control of the RTS®, in human subjects with stage III and IV melanoma is evaluated through procedures such as those described below.

A study involving study human subjects with stage III and IV melanoma is conducted in 4 cohorts (groups) of subjects each subject receiving a single intratumoral injection (into a melanoma tumor) of adenoviral transduced autologous (re-inserted into the same subject that they came from) dendritic cells (DCs) engineered to express human interleukin-12 (hIL-12), and one or more other immunomodulators, at a dose of 5×10^7 in combination with daily oral doses of one or more crystalline polymorphic forms of Compound 1, or a composition thereof (referred to collectively in this example as the “activator drug”). The study will use injections of dendritic cells transduced ex vivo (after the cells are removed from the subjects) with adenoviral vector for inducible expression of human IL-12 and one or more other immunomodulators. The production of IL-12 and the one or more or other immunomodulators is “turned on” (induced) from the injected DCs through the activation of the RTS® by the oral administration of the activator drug. Safety and tolerance is assessed through physical examinations (including ECOG performance status), vital signs measurements, serum chemistry, urinalysis, hematology, adverse events “side-effects”, and antibodies and cellular immune response to the adenovirus, components of RTS®, and the activator drug. To evaluate progress, single dose and steady-state pharmacokinetics/ADME of the activator drug, analysis of hIL-12 levels, other immunomodulator levels, and cellular immune response (T cells) in biopsies of the target tumors, draining lymph nodes, and peripheral circulation, as well as a serum cytokine profile is measured.

For instance, 16 subjects with stage III and IV melanoma are divided into four cohorts with cohorts 1 and 2 containing three subjects and cohorts 3 and 4 containing 5 subjects. All subjects will receive a single intratumoral injection of 5×10^7 autologous DC transduced with adenoviral vector encoding human IL-12 and one or more other immunomodulators under the RTS® control. For example, the subjects are administered an intratumoral injection of autologous DC transduced with adenoviral vector encoding human IL-12 under the RTS® control and an immunomodulator such as IL-15 or IL-21.

The subjects will receive a single daily oral dose of the activator drug (cohort 1: 0.01 mg/kg, cohort 2: 0.1 mg/kg, cohort 3: 1.0 mg/kg or cohort 4: 3 mg/kg) the first dose starting approximately 3 hours prior to the DC injection on day 1 and continuing for 13 more consecutive days. Additional injection(s) of adenovirally transduced autologous dendritic cells in combination with 14 single (once) daily oral doses of activator drug may be administered to eligible subjects who meet the criteria for retreatment. Safety, tolerance, and dendritic cell function are assessed for all subjects in each group of cohort 1 for up to one month after injection of the in vitro engineered dendritic cells before enrolling subjects to receive the next highest dose of the activator drug. The safety assessment will continue in all subjects for 3 months after the initial injection of the engineered dendritic cells with the possibility of extending the follow-up period to a total of six months to monitor subject safety if toxicity is observed or the subject receives additional injection(s) of the dendritic cells.

Such a study demonstrates the safety and tolerance of a single or multiple intratumoral injection(s) of adenoviral transduced autologous dendritic cells in combination with

the oral activator drug in subjects with melanoma. The study provides steady-state pharmacokinetics/ADME of the oral activator drug. The study demonstrates functionality of the RTS® in subjects by measuring hIL-12 expression and the expression of the one or more other immunomodulators of adenovirus transduced autologous dendritic cells in target tumor and/or draining lymph nodes in response to the activation of the RTS® by the oral administration of the activator drug. Furthermore, the study demonstrates the immunological effects of the adenoviral transduced autologous dendritic cells in terms of the cellular immune response in the target tumor, draining lymph nodes, and peripheral circulation following oral administration of the activator drug.

Melanoma is selected as an exemplary cancer. Melanoma in particular among solid tumors has been shown to respond

to immunotherapy approaches, and melanoma tumors are readily accessible for intratumoral injection and biopsy. The subjects included in the study have unresectable stage III or IV melanoma, which has at least 0.5 cm in diameter, any tumor thickness, any number of lymph node involvement, in-transit metastases, or distant metastases.

It is to be understood that the foregoing described embodiments and exemplifications are not intended to be limiting in any respect to the scope of the disclosure, and that the claims presented herein are intended to encompass all embodiments and exemplifications whether or not explicitly presented herein

All patents and publications cited herein are fully incorporated by reference in their entirety.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 2

<210> SEQ ID NO 1

<211> LENGTH: 240

<212> TYPE: PRT

<213> ORGANISM: *Choristoneura fumiferana*

<400> SEQUENCE: 1

Leu Thr Ala Asn Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln
1 5 10 15

Asp Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln
20 25 30

Thr Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe
35 40 45

Arg Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu
50 55 60

Phe Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln
65 70 75 80

Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val
85 90 95

Ala Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn
100 105 110

Gln Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val
115 120 125

Ile Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu
130 135 140

Asp Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp
145 150 155 160

Arg Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr
165 170 175

Tyr Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser
180 185 190

Ala Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu
195 200 205

Leu Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys
210 215 220

Leu Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val
225 230 235 240

<210> SEQ ID NO 2

<211> LENGTH: 10

-continued

<212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Helokinestatin

<400> SEQUENCE: 2

Gly Pro Pro Tyr Gln Pro Leu Val Pro Arg
 1 5 10

What is claimed is:

1. Crystalline (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV,

characterized as having a powder x-ray diffraction pattern with peaks at 6.83, 10.31, 11.30, 12.18, 12.98, 13.69, 15.11, 16.23, 17.60, 17.99, 20.70, 21.15, 21.68, 22.71, 23.79, and 24.86 degrees 2 θ .

2. The (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV of claim 1 having an average particle size distribution of about 10 μ m or less.

3. A composition comprising the (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV of claim 1 and one or more excipients.

4. The composition of claim 3, wherein said one or more excipients comprise one or more pharmaceutically acceptable excipients.

5. The composition of claim 4, wherein said one or more pharmaceutically acceptable excipients comprise Miglyol 812, phospholipon 90G, or tocopheryl polyethylene glycol 1000 succinate, or a mixture thereof.

6. A method of making a composition, the method comprising admixing the (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV of claim 1 and an excipient.

7. A kit comprising the (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV of claim 1.

8. The crystalline (R)-3,5-dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N'-(2-ethyl-3-methoxy-benzoyl)-hydrazide hydrate Form IV of claim 2 having an average particle size distribution of about 1 μ m or less.

* * * * *

Crystal Polymorphism and Multiple Crystal Forms

Dario Braga, Fabrizia Grepioni, Lucia Maini, and Marco Polito

Abstract This chapter discusses the phenomenon of polymorphism in organic and organometallic compounds. Polymorphism is first introduced and then, to give the work some context, background information is given concerning properties and techniques for characterizing the solid phases. In particular, desolvation and interconversion are examined, and the gas–solid reactions are presented as a successful route to obtaining new crystalline phases. Co-crystal definition is then described and the problem in distinguishing co-crystals and salts is evaluated.

Keywords: Co-crystal · Hydrogen bond · Organic salt · Polymorphism · Solid-gas reaction · Solid-state NMR · Solvate · X-ray diffraction

Contents

1	Introduction	26
2	Definition of Crystal Forms	26
3	Properties of Crystal Forms	27
4	Characterization of Multiple Crystal Forms	29
5	Examples of Crystal Form Identification and Characterization	31
6	Examples of Solvate Crystal Forms and Interconversion	34
7	Solid–Gas Reactions: A Route to New Crystal Forms	35
8	Co-Crystals and Salts, Co-Crystals or Salts?	39
9	Conclusions	46
	References	47

1 Introduction

The isolation, identification and characterization of different crystal forms (polymorphs, solvates, salts and co-crystals) of the same molecule or of aggregates of the same molecule with other molecules represents one of the most active areas of modern solid state chemistry. The investigation of crystal forms impacts on fundamental science as well as on utilitarian objectives because different crystal forms may display a range of different physico-chemical properties, which may affect application and utilization of the solid materials.

Polymorphism [1] is one of the most fascinating phenomena of solid state chemistry and indeed is a “difficult” phenomenon, studied for many decades mainly, and separately, in the fields of organic and inorganic chemistry. In spite of the huge efforts of many researchers our knowledge of the phenomenon is still embryonic, and the relationship between growth of a crystalline phase and nucleation of the first crystallites is often mysterious. It is a fact that, despite the ambitions of the scientist, crystal construction is not yet strictly under human control. Many examples exist of *appearing* and *disappearing* polymorphs, some of which have had great practical consequences. The reader is addressed to the review by Dunitz and Bernstein for a number of intriguing examples [2].

2 Definition of Crystal Forms

Although the existence of three different crystal forms of calcium carbonate (calcite, vaterite and aragonite) was identified by Klaproth in 1788 [3], formal recognition of the phenomenon of *crystal polymorphism* is attributed to the work of Mitscherlich in 1823 [4]. In more general terms, the solid phase of a material, whether formed of organic molecules, inorganic ions or extended covalent networks, can exhibit different structures which, although possessing the same chemical composition, may manifest different properties. In addition to these polymorphic modifications the material may also lack long-range order and appear as an amorphous solid, which can be considered as another polymorph.

A number of authors have suggested that polymorphism is ubiquitous in crystal chemistry. McCrone stated long ago 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 energy spent in research on that compound” [5]. In 1951 Findlay noted in his book that polymorphism: “...is now recognized as a very frequent occurrence indeed” [6]. Buerger and Bloom stated: “...polymorphism is an inherent property of the solid state and it fails to appear only under special conditions” [7]. Similarly, Sirota wrote in 1982: “Polymorphism is now believed to be characteristic of all substances, its actual non-occurrence arising from the fact that a polymorphic transition lies above the melting point of the substance or in the area of as yet unattainable values of external equilibrium factor or other conditions providing for the transition” [8].

In 1965 Kuhnert-Brandstätter investigated by hot stage microscopy three common classes of drug: 70% of barbiturates, 60% of sulphonamides and 23% of steroids exist in various polymorphic or solvate forms [9, 10].

A number of statistical estimates of the extent of polymorphism have also been carried out. A search of the Cambridge Structural Database on the keywords “polymorph”, “form”, “modification” and “phase” indicated that about 3.5% of the ~350,000 entries fall into this category. Approximately 25% of the entries are either solvates or hydrates. Other studies based on different selection criteria reveal a much larger occurrence [11–13]. For instance, Griesser and Burger have collected information on about 600 polymorphic forms and solvates (including hydrates) of pharmaceutical compounds that are solid at 25°C [14].

Even though crystal forms have been and are the subject of intense investigations, polymorphism as a *phenomenon* still represents a substantial scientific challenge. Indeed it is hard to predict whether a given molecule will crystallize in one or several crystal forms, whether it will form solvates with different stoichiometries or will ever be “happy” to link up with other molecules and form stable co-crystals. Such variability and unpredictability have been taken by some scientists as an intrinsic drawback of being able to construct desired crystal structures (and obtain relevant properties) from a purposeful choice of the molecular components, which is the paradigm of molecular crystal engineering [15–19].

In recent times the concept of crystal polymorphism has expanded beyond its original boundaries to encompass crystal forms of the same molecule with different molecular partners. These may be solvent molecules in solvates [20], or counterions if the molecule can be made non-neutral (by say proton or electron transfer [21]), or other molecules in co-crystals [22]. It is worth noting that the formation of solvate and hydrated forms is commonly observed during polymorph screening, therefore the use of the term “pseudopolymorphism” to describe solvate forms of a given molecular crystal ought to be discouraged, at least because solvates may, in turn, be polymorphic [23–25].

In this paper we shall discuss all four different types of crystal forms of the same molecule: polymorphs of the mere molecule, solvates and hydrates, molecular salts and co-crystals (when the same molecule can be co-crystallized with different co-crystal formers). All these crystal forms are summarized in Fig. 1.

3 Properties of Crystal Forms

From thermodynamic principles, under specified conditions only one polymorph is the stable form (except at a transition point) [26]. In practice, however, due to kinetic considerations, metastable forms can exist or coexist in the presence of more stable forms. The relative stability of the various crystal forms and the possibility of interconversion between crystal forms, between crystals with different degree of solvation, and between an amorphous phase and a crystalline phase can have very

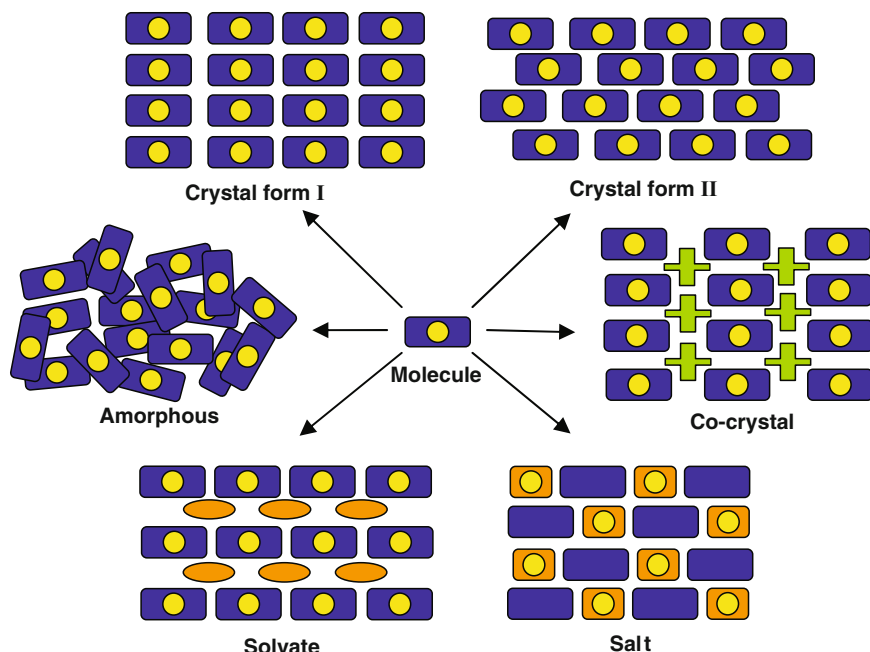


Fig. 1 Schematic representation of the structural relationship between “true” polymorphs, solvates, co-crystals, salts and the amorphous phase

serious consequences on the life and effectiveness of a polymorphic product and the persistence over time of the desired properties. The variety of phenomena related to polymorphism requires a thorough mapping of the “crystal space” of a substance that is ultimately intended for some specific applications (e.g. drugs, pigments, agrochemicals and food additives, explosives, etc.).

The industrial production and marketing lines need to know not only the exact nature of the material in the process, but also its stability with time, the variability of its chemical and physical properties as a function of the crystal form, etc. The search for and characterization of crystal forms is therefore a crucial step in the development of a new chemicals with relevant consequences on intellectual property issues [1, 27–29].

Different crystal forms are often recognized by differences in the colour and shape of crystals. A striking example of these two properties is provided by the differences in colour and form of the crystal forms of ROY (ROY = red, orange, yellow polymorphs of 5-methyl-2-[(2-nitrophenyl)amino]-3-thiophene carbonitrile) [30, 31]. Colour and shape are only some of the possible differences and Table 1 summarizes some major possible differences in chemical and physical properties between crystal forms and solvates of the same substance. In addition, one has to consider that new and different properties may derive from a change in the nature and chemical composition of the same molecule as a consequence of salt formation or co-crystallization. For each new crystal form obtained, a full characterization

PHYSICAL AND THERMODYNAMIC PROPERTIES	density and refractive index, thermal and electrical conductivity, hygroscopicity, melting points, free energy and chemical potential, heat capacity, vapor pressure, solubility, thermal stability
SPECTROSCOPIC PROPERTIES	electronic, vibrational and rotational properties, nuclear magnetic resonance spectral features
KINETIC PROPERTIES	rate of dissolution, kinetics of solid state reactions, stability
SURFACE PROPERTIES	surface free energy, crystal habit, surface area, particle size distribution
MECHANICAL PROPERTIES	hardness, compression, thermal expansion
CHEMICAL PROPERTIES	chemical and photochemical reactivity

Fig. 2 Examples of chemical and physical properties that can differ among crystal forms and solvates of the same substance

should be done since the new form can have different properties, such as higher solubility or higher melting point (Fig. 2).

4 Characterization of Multiple Crystal Forms

The exploration of the “crystal form space” of a substance is the search of polymorphs and solvates in order to identify the most stable form and the existence of unstable forms that interconvert (enantiotropism) or do not (monotropism) as a function of the temperature. This also applies to amorphous and solvate forms. The relationships between the various phases and commonly used industrial and research laboratory processes are schematically illustrated in Fig. 3.

It is worth stressing that polymorph assessment in industrial research and development as well as during processing is part of the system of quality control. Therefore it is necessary to make sure that the scale-up from laboratory preparation to industrial production does not introduce variations in crystal form. Polymorph assessment also guarantees that the product conforms to the guidelines of the appropriate regulatory agencies and does not infringe the intellectual property protection that may cover other crystal forms [32].

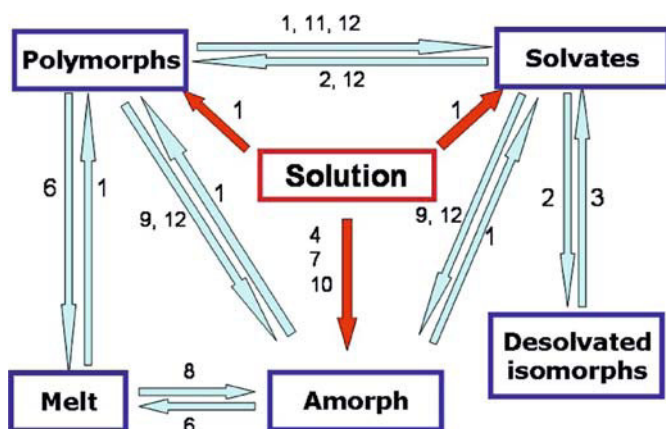


Fig. 3 Some general relationships between polymorphs, solvates and amorphous phases and the type of research laboratory or industrial or process for preparation and interconversion: 1 crystallization; 2 desolvation; 3 exposure to solvent/vapour uptake; 4 freeze drying; 5 heating; 6 melting; 7 precipitation; 8 quench cooling; 9 milling; 10 spray drying; 11 kneading; 12 wet granulation. Analogous relationships apply to polymorphic modifications of solvate forms. Note that the figure represents general trends rather than every possible transformation; the presence or absence of an arrow or number does not represent the exclusive existence or absence of a transformation

The screening of crystal forms is best achieved by the combined use of several solid-state techniques, such as hot stage microscopy, differential scanning calorimetry, thermogravimetric analysis and X-ray diffraction. Powerful complementary tools are also provided by solid-state spectroscopic techniques such as solid state nuclear magnetic resonance spectroscopy [33] and Raman spectroscopy [1]. Of great relevance is the possibility of determining the molecular and crystal structure of a crystal form by means of single crystal X-ray diffraction. This technique, although much more demanding than powder diffraction in terms of experiment duration and data processing, has the great advantage of providing detailed structural information on the molecular geometry. Furthermore, it affords insight into the factors controlling the packing of the molecules in the crystal and the nature and structural role of solvent molecules. Knowledge of the single-crystal structure allows a direct comparison between the X-ray powder diffraction pattern, calculated on the basis of the structure, with the one measured on the polycrystalline sample as it will be diffusely used in the following description of specific cases.

Importantly, the calculated diffraction pattern is not affected by the typical sources of errors of experimental powder diffraction (preferential orientation, mixtures, presence of amorphous phase) that often complicate or render uncertain the interpretation of measured powder diffractograms; hence the calculated powder pattern is often referred to as the “gold standard” pattern for a crystal form.

The search for various crystal forms requires that the behaviour of a solid phase is investigated as a function of the variables that can influence or determine the outcome of the crystallization process, e.g. temperature, choice of solvents,

crystallization conditions, rate of precipitation, interconversion between solid forms (from solvate to un-solvate and vice versa), pressure and mechanical treatment, absorption and release of vapour, etc. [24]. Techniques such as hot stage microscopy, DSC and micro-DSC can be used to obtain a semiempirical energy–temperature diagram [34, 35] that can be helpful in designing protocols for screening for crystal forms.

Another powerful tool for the investigation of the structural relationship between crystal forms is variable temperature X-ray diffraction that provides information on phase transitions and on adsorption/desorption of solvent molecules (and chemical reactivity, of course, but this is of less concern in this context).

Beside the traditional thermal and solution methods for obtaining new crystal forms, many other methods are being used or developed in the quest for multiple crystal forms. Some of these methods are based on the knowledge of crystal structures in the literature [16], the application of crystal engineering principles (based on hydrogen-bonding patterns) to the preparation of new multicomponent solids [36,37], the induction of crystal forms by using polymeric substrates [38], the development of high-throughput crystallization technology [39], the utilization of solid–solid and solid–gas reactions [40], solvent-free synthesis [41,42], the desolvation of solvated crystals [43] and crystallization from a supercritical solvent [44,45]. These methods, combined with the development of new technology [39], the attempts to design and control crystal structure [46], combined with some spectacular encounters with new (and undesired) crystal forms [47] (see Sect. 5) and some high profile pharmaceutical patent litigations [1], have led to many new techniques for exploring the crystal form space of any particular substance.

5 Examples of Crystal Form Identification and Characterization

There is no protocol of polymorph screening that can guarantee the identification of *all* crystal forms of a given molecule. As pointed out in the previous section, the understanding of a system exhibiting multiple crystal forms is best achieved by studying the system with a wide variety of analytical tools. We have chosen to illustrate this aspect by means of two recent examples coming from our own work.

The first example is that of cinchomeronic acid, which has been known for almost a century [48]. The cinchomeronic acid 3,4-dicarboxypyridine is one of the six isomers of the acid pyridinedicarboxyl; all isomers are widely utilized in the construction of coordination networks [49–51]. The presence of two of cinchomeronic forms is reported in the PDF-2 [52], while the crystal structure has been determined only for one form [53]. We have investigated the structural relationship between these two crystal forms by a combined use of single-crystal X-ray diffraction, IR and Raman spectroscopy, and solid state NMR spectroscopy [54]. Form I crystallizes in an acentric orthorhombic group, while form II crystallizes in a centric monoclinic group (Fig. 4), due to a static disorder for the nitrogen position (Fig. 5). The zwitterionic nature of the acid in both forms has been confirmed by ^1H MAS,

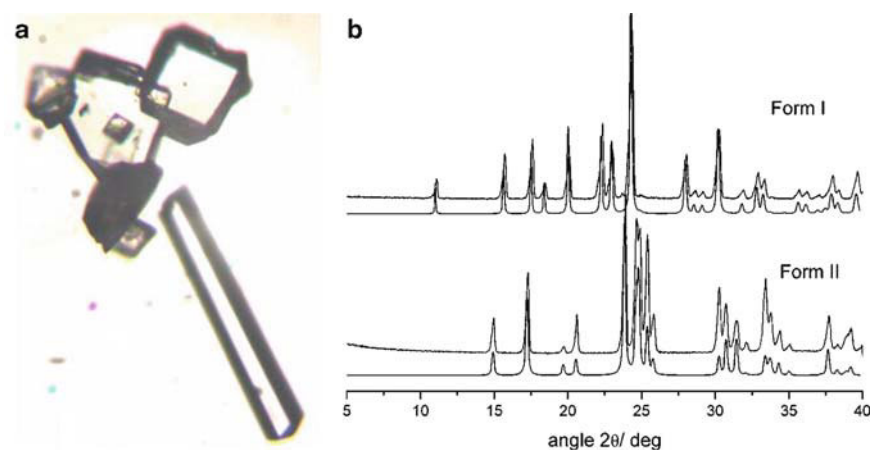


Fig. 4 Concomitant polymorphs obtained from an ethanol/water solution: *rods* form I, *blocks* form II **a** Comparison between the experimental and calculated powder diffractograms of form I and form II **b**

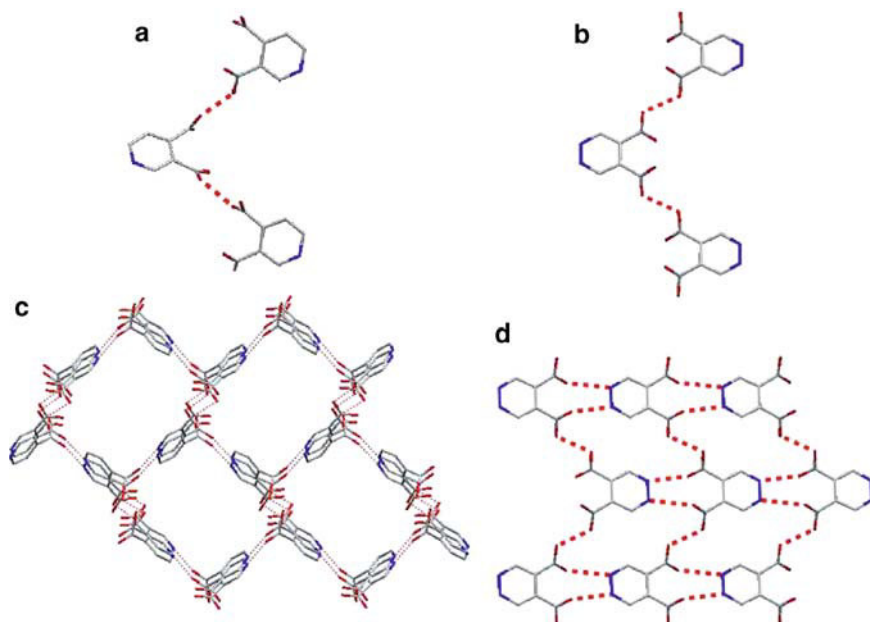


Fig. 5 Chains present in form I **a** and form II **b**; the adamantoid network formed by $\text{N-H} \cdots \text{O}$ —hydrogen bonds among the chains and the overall crystal structure formed by the three interpenetrating networks in form I **c**; interactions $\text{N-H} \cdots \text{O}$ — and $\text{C-H} \cdots \text{O}$ — among the chains and the 2D-network present in form II **d**

^{13}C CPMAS and ^{15}N CPMAS (Fig. 6). The single crystal structures give us a view of the hydrogen bonds formed by the molecule of cinchomeronic acid in the solid state; in both structures the molecules of cinchomeronic acid form infinite chains via

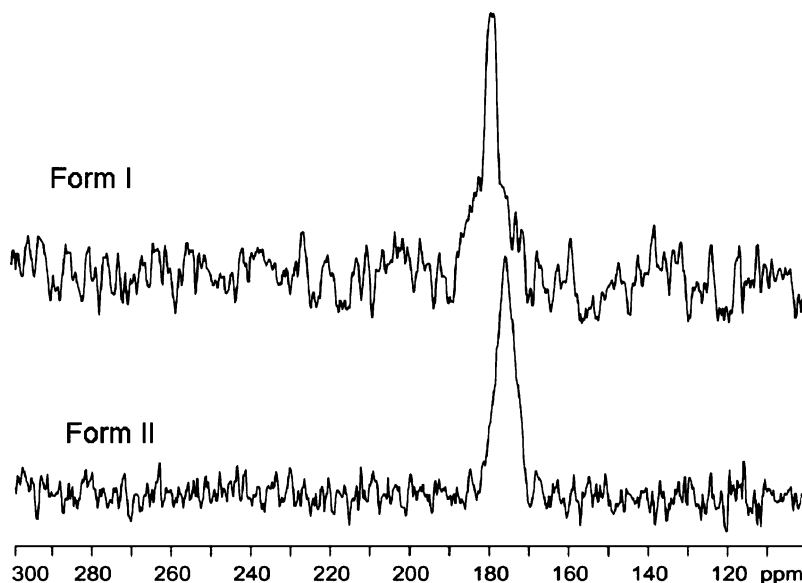


Fig. 6 ^{15}N CPMAS spectra of cinchomeronic acid: form I (*top*) and form II (*bottom*) recorded at 27.2 MHz with a spinning speed of 5 kHz which confirm the zwitterionic nature of cinchomeronic acid in both crystal phases

short O—H—O hydrogen bonds between the carboxylic and carboxylate groups (see Fig. 5). In form I the zwitterion chains are ordered and are connected via N—H—O interactions to form a threefold interpenetrating networks. In form II, on the other hand, the presence of static disorder leads to crystallographic equivalence between the —COO and —COOH groups (see Fig. 5) and the nitrogen atom is disordered over two positions, which leads to a longer and weaker N—H—O hydrogen bond consistent with the IR and Raman spectra. Both forms decompose before melting without any interconversion, which suggests the presence of a monotropic system, while the slurry experiment indicates that form I is the thermodynamically stable form. The crystal structure and spectroscopic analysis indicate that the difference in stability can be ascribed to the strength of the hydrogen bonding patterns established by the protonated N-atom and the carboxylic/carboxylate O-atoms. The possibility of optimizing the hydrogen bond interaction in form I, with respect to the somewhat *looser* interactions allowed by the packing in form II might be responsible for the stabilization of the crystal structure of form I.

Another example is provided by the two different crystal forms of the salt $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{NH}] [\text{OOC}(\text{CH}_2)\text{COOH}]_2$ obtained depending on preparation technique (grinding or solution) and crystallization speed. Form I, containing mono-hydrogen malonate anions forming conventional intramolecular O—H $\cdots$ O hydrogen bonds and inter-ionic N—H $\cdots$ O hydrogen bonds, is obtained by solid-state co-grinding or by rapid crystallization, while form II, containing *both* intermolecular and intramolecular O—H $\cdots$ O hydrogen bonds, is obtained by slow crystallization

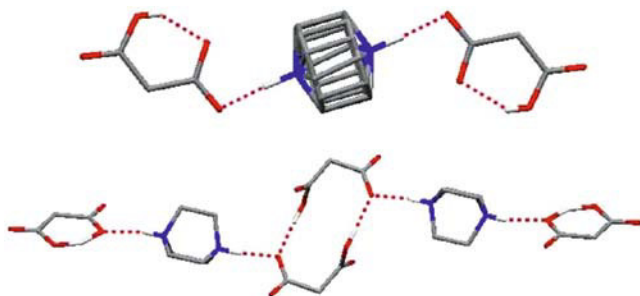


Fig. 7 Form I (*top*) and II (*bottom*) of $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{NH}][\text{OOC}(\text{CH}_2)\text{COOH}]_2$ and the hydrogen bonded anion $\cdots$ cation chains present in their crystals. Form I is obtained by solid-state co-grinding or by rapid crystallization, while form II is obtained by slow crystallization

(see Fig. 7). Form I and II do not interconvert, while form I undergoes an order–disorder phase transition on cooling. One can envisage the two crystalline forms as *hydrogen bond isomers* of the same *solid supermolecule* [55].

6 Examples of Solvate Crystal Forms and Interconversion

An intriguing case of interconversion between unsolvate and solvate crystals is observed when $[\text{N}(\text{CH}_2\text{CH}_2)_3\text{N}]$ is reacted with maleic acid $[\text{HOOC}(\text{HC}=\text{CH})\text{COOH}]$. The initial product is the anhydrous salt $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{N}][\text{OOC}(\text{HC}=\text{CH})\text{COOH}]$, which contains chains of $(^+)\text{N}-\text{H}\cdots\text{N}^{(+)}$ bonded cations $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{N}]^+$ and “isolated” $[\text{OOC}(\text{HC}=\text{CH})\text{COOH}]^-$ anions [56].

Upon exposure to humidity the anhydrous salt converts within few hours into the hydrated form $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{N}][\text{OOC}(\text{HC}=\text{CH})\text{COOH}] \cdot 0.25\text{H}_2\text{O}$, which contains more conventional “charge-assisted” $(^+)\text{N}-\text{H}\cdots\text{O}^{(-)}$ hydrogen bonds between anion and cation (see Fig. 8). This latter form can also be obtained by co-grinding.

In a similar process, crystals of $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2][\text{BF}_4]_2$ have been crystallized from nitromethane as the solvate form $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2][\text{BF}_4]_2 \cdot \text{MeNO}_2$. These solvate crystals, if exposed to air, rapidly convert to the unsolvate form $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2][\text{BF}_4]_2$. The nature of this latter compound was established from single crystals obtained from water in the presence of seeds of the powder material obtained from desolvated crystals $[\text{Ru}(\eta^6\text{-C}_6\text{H}_6)_2][\text{BF}_4]_2 \cdot \text{MeNO}_2$ [57]. The opposite process, namely solvent uptake, can often be activated by mechanical treatment of unsolvate crystals. There are several reports that even gentle grinding of a powder product may lead to the formation of a hydrated product [58–60].

Another example is provided by the hydrated salt $[\text{Co}(\eta_5\text{-C}_5\text{H}_5)_2]^+[\text{Fe}(\eta_5\text{-C}_5\text{H}_4\text{COOH})(\eta_5\text{-C}_5\text{H}_4\text{COO})]^- \cdot \text{H}_2\text{O}$, which is obtained by simply grinding in air the crystalline powder of $[\text{Co}(\eta_5\text{-C}_5\text{H}_5)_2]^+[\text{Fe}(\eta_5\text{-C}_5\text{H}_4\text{COOH})(\eta_5\text{-C}_5\text{H}_4\text{COO})]^-$ that precipitates from THF or nitromethane on reacting $[\text{Co}(\eta_5\text{-C}_5\text{H}_5)_2]$ with $[\text{Fe}(\eta_5\text{-C}_5\text{H}_4\text{COOH})_2]$ [61]. Once $[\text{Co}(\eta_5\text{-C}_5\text{H}_5)_2]^+[\text{Fe}$

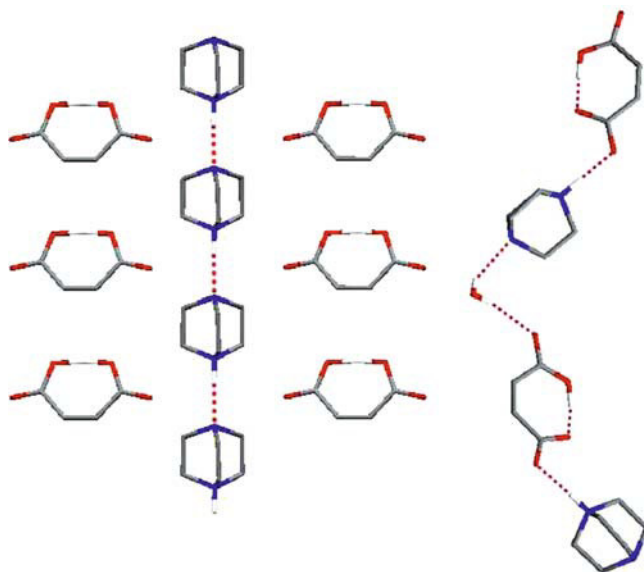


Fig. 8 Views of the packing and hydrogen bonding in the anhydrous salt $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{N}][\text{OOC}(\text{HC}=\text{CH})\text{COOH}]$ (*left*) and of the hydrated salt $[\text{HN}(\text{CH}_2\text{CH}_2)_3\text{N}][\text{OOC}(\text{HC}=\text{CH})\text{COOH}]\text{H}_2\text{O}_{0.25}$ (*right*)

$(\eta_5\text{-C}_5\text{H}_4\text{COOH})(\eta_5\text{-C}_5\text{H}_4\text{COO})]^- \cdot \text{H}_2\text{O}$ has been obtained by grinding, its single crystals can be grown from water or nitromethane, while crystals of the anhydrous form are no longer observed. However, on heating, the hydrated form loses water at 373 K and reverts to the starting material.

7 Solid–Gas Reactions: A Route to New Crystal Forms

Solid–gas reactions have been known for a long time and have recently attracted a renewed interest in the strive to find environmentally friendly processes. Since the reactant is a crystalline solid, the issue of whether different crystal forms would react in the same or a different way towards a gaseous reactant is of interest to the discussion. We have tackled this question by exploiting solid–gas reactions between crystal polymorphs of the same acidic substance and vapours of volatile bases, investigating in turn the inverse process, i.e. removal of the vapours from the crystal structures by thermal treatment.

The possibility of different chemical behaviour of polymorphic modifications has been explored before only in the case of indomethacine amorphous and crystal forms reacted with ammonia by Stowell, Griesser, Byrn et al. [62]. We have investigated two rather different systems, namely the polymorphic forms of barbituric acid [63] and of ferrocene dicarboxylic acids [64], which have been reacted with

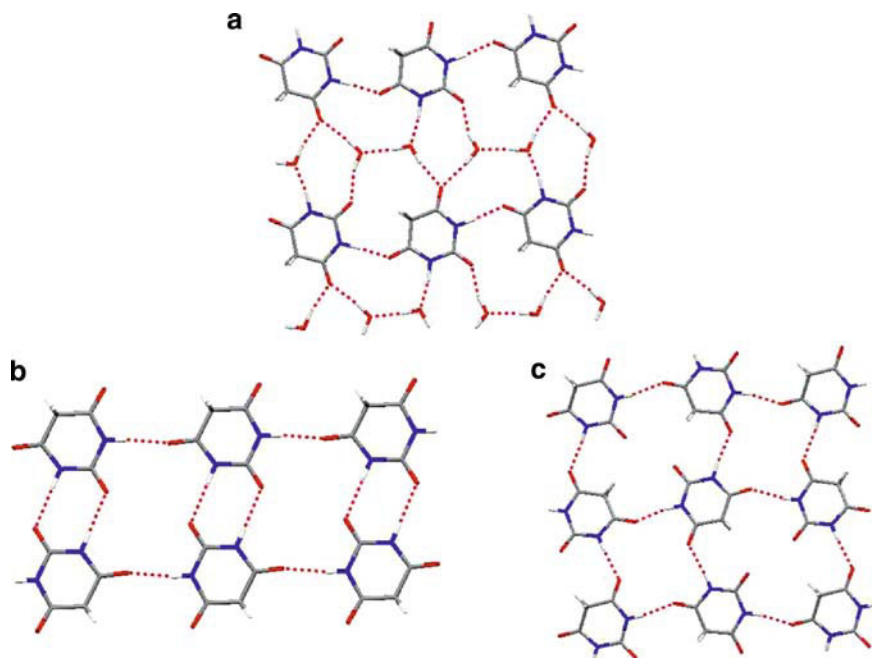


Fig. 9 Hydrogen bond networks present in the dihydrate form **a**, form I **b** and form II **c** of barbituric acid

volatile bases. Solid barbituric acid is known as two polymorphic forms (forms I and II) and as a dihydrate form for which single crystal data are available allowing computation of the theoretical powder diffractograms [65–68]. Views of the packings of the three forms are shown in Fig. 9. De-hydration of the dihydrate form has also been investigated, showing that it releases water to yield exclusively crystals of form II. We have been able to show that forms I, II and the dihydrate form of barbituric acid react with ammonia leading to the same crystalline ammonium barbiturate salt $\text{NH}_4(\text{C}_4\text{H}_3\text{N}_2\text{O}_3)$, while the gas–solid reactions of form II with methylamine and dimethylamine yield the corresponding crystalline salts $\text{CH}_3\text{NH}_3(\text{C}_4\text{H}_3\text{N}_2\text{O}_3)$ and $(\text{CH}_3)_2\text{NH}_2(\text{C}_4\text{H}_3\text{N}_2\text{O}_3)$, respectively. The processes are shown in Fig. 10.

Thermal desorption of the bases at ca. 200°C leads to formation of a new crystal form of barbituric acid, form III, as confirmed by H^1 -NMR spectroscopy and by chemical behaviour. Unfortunately several attempts to crystallize compound III yielded only the formation of the dihydrate form (Fig. 11).

Similarly, the reactions between solid 1–3 dimethylbarbituric acid (dmb) with vapours of NH_3 and of the volatile amines $\text{NH}_2(\text{CH}_3)$, and $\text{NH}(\text{CH}_3)_2$ have been investigated [69]. The barbiturate salts $[\text{NH}_4]\text{dmb}$, $[\text{NH}_3(\text{CH}_3)]\text{dmb}$ and $[\text{NH}_2(\text{CH}_3)_2]\text{dmb}$ have been characterized by X-ray powder diffraction (XRPD), differential scanning calorimetry and thermogravimetric analysis. The solid–gas reactions were monitored by UV–Vis spectroscopy in the solid state. In the case of $[\text{NH}_2(\text{CH}_3)_2]\text{dmb}$, recrystallization from methanol yields the salt

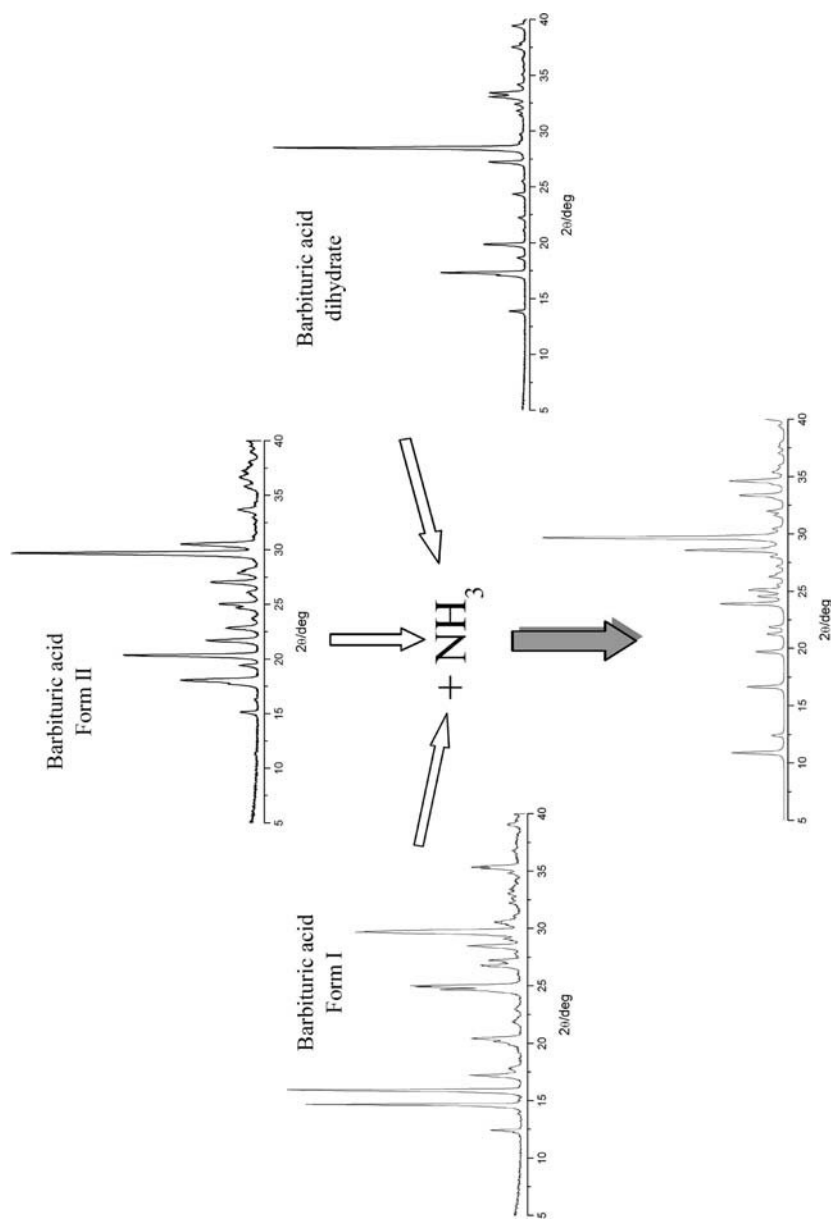


Fig. 10 Schematic representation of the XRPD patterns of the polymorphic forms of barbituric acid I and II and that of the dihydrate form. The interaction with NH_3 leads to the formation of the same product, $\text{NH}_4(\text{C}_4\text{H}_3\text{N}_2\text{O}_3)$

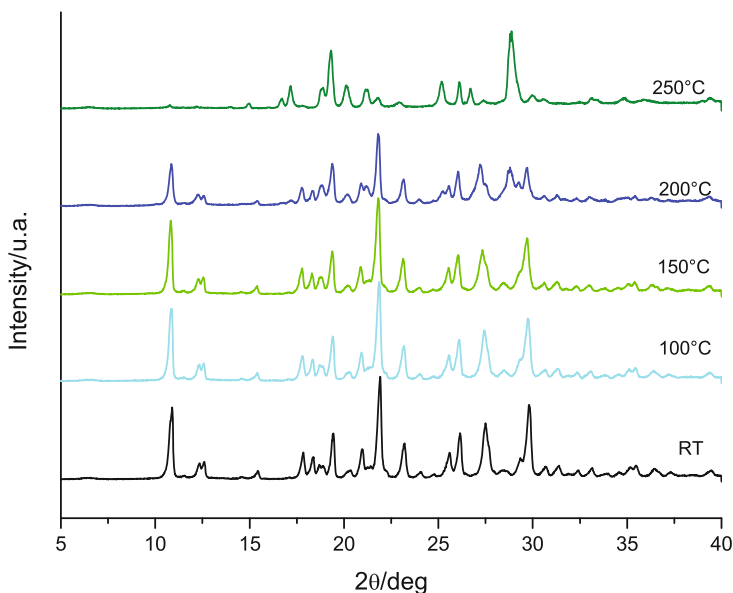


Fig. 11 XRPD recorded at different temperatures from room temperature to 250°C on crystalline $\text{NH}_4(\text{C}_4\text{H}_3\text{N}_2\text{O}_3)$ showing the presence of new reflections at 200°C

$[\text{NH}_2(\text{CH}_3)_2][\text{DMB}] \cdot 2[\text{DMBA}]$, while recrystallization of $[\text{NH}_3(\text{CH}_3)]\text{dmb}$ yields both the dimerization product $[\text{NH}_3(\text{CH}_3)][(\text{C}_6\text{O}_3\text{N}_2\text{H}_6)_2\text{OH}] \cdot 2\text{H}_2\text{O}$ and the hydurate salt $[\text{NH}_3(\text{CH}_3)]_2[(\text{C}_6\text{O}_3\text{N}_2\text{H}_6)_2]$, i.e. the product $[\text{NH}_3(\text{CH}_3)]\text{dmb}$ is only accessible via the solid–gas reaction.

In an analogous study, crystalline form I (monoclinic) and form II (triclinic) of ferrocene dicarboxylic acid $[\text{Fe}(\eta^5\text{-C}_5\text{H}_4\text{COOH})_2]$ have been reacted at room temperature with the gaseous bases NH_3 , $\text{NH}_2(\text{CH}_3)$ and $\text{NH}(\text{CH}_3)_2$ [64]. The two crystal forms behave in exactly the same way in the solid–gas reaction generating the same products, identified as the anhydrous crystalline salts $[\text{NH}_4]_2[\text{Fe}(\eta^5\text{-C}_5\text{H}_4\text{COO})_2]$, $[\text{NH}_3\text{CH}_3]_2[\text{Fe}(\eta^5\text{-C}_5\text{H}_4\text{COO})_2]$, and $[\text{NH}_2(\text{CH}_3)_2]_2[\text{Fe}(\eta^5\text{-C}_5\text{H}_4\text{COO})_2]$. Interestingly though, all these crystals revert via vapour release exclusively to the metastable crystalline form I, as shown in Fig. 12.

This is not surprising per se, because there is no reason why, if the reaction is quantitative, the crystalline salt product should “remember” which crystal form it comes from. It is instead of relevance that forms I and II of $[\text{Fe}(\eta^5\text{-C}_5\text{H}_4\text{COOH})_2]$ are not known to interconvert via a solid–solid phase transition, hence they constitute a monotropic system. The ammonia absorption/release process, therefore, can be seen as a solid state way to convert the thermodynamically stable form II into the metastable form I. The process is schematically represented in Fig. 13.

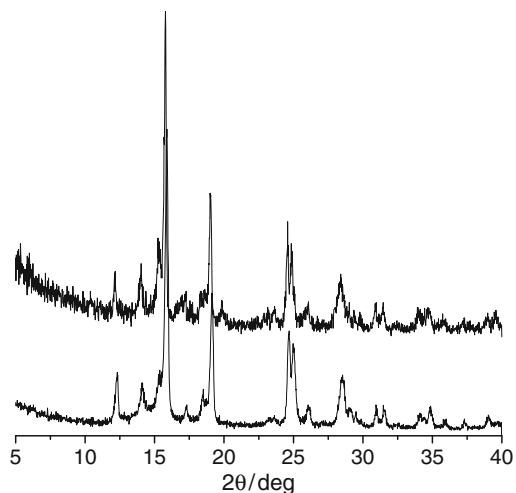


Fig. 12 Comparison between the experimental powder diffractograms of compound 1, obtained from the solid–gas reaction with ammonia of form I (*top*) and form II (*bottom*), respectively

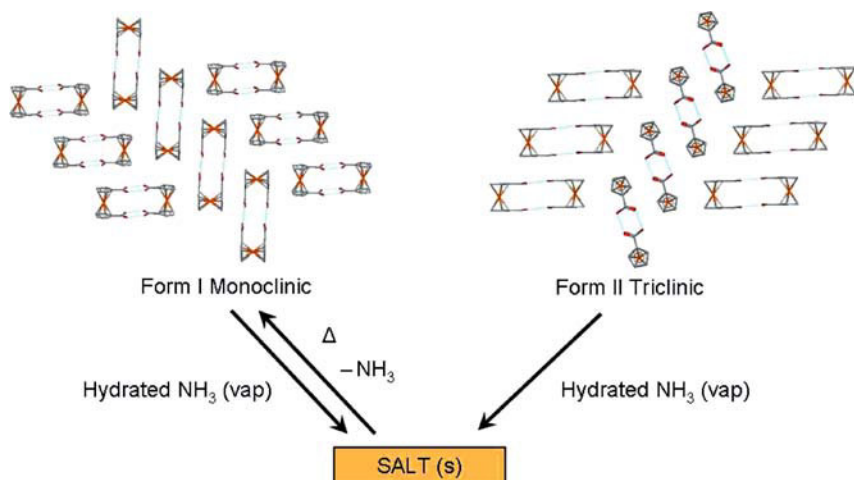


Fig. 13 Reaction of crystalline forms I and II with vapours of NH_3 yields the same salt, which upon heating and removal of NH_3 generates only the monoclinic form I

8 Co-Crystals and Salts, Co-Crystals or Salts?

In this last section we focus on co-crystals. Although co-crystals, i.e. multicomponent crystals containing chemically different molecular units in the asymmetric unit have long been known [70], they have recently become an important field of new discoveries and innovation under the strive to find new drugs and the potential of extension of IP protection on existing ones [71–73].

The definition of a co-crystal is still matter of debate [74, 75]. The definition initially put forward by Aakeroy focused on the aggregation state: “co-crystals are made from reactants that are solids at ambient conditions” [76] and has also been taken up by others [71, 77]. This definition, however, is not without ambiguity (see below). We prefer to take up Dunitz’ more liberal view of co-crystals as “encompassing molecular compounds, molecular complexes, solvates, inclusion compounds, channel compounds, clathrates, and other types of multi-component crystals.” This view has been echoed recently by Stahly [78] who wrote that: “co-crystals consist of two or more components that form a unique crystalline structure having unique properties.” At the bottom line, these multicomponent systems ought to be looked at as crystals of supermolecules whereby the component units interacting via non-covalent interactions generate collective physico-chemical properties that are different from those of the homo-molecular crystals formed by the components.

One may wonder whether solvates would fall under the same broad definition. We would argue in favour, not only because solvent molecules themselves form their own crystalline materials at appropriate temperature (ice!) but also because, as we have argued above, solvate crystals do not necessarily come from solutions since vapour or any other volatile molecule can be taken up from the ambient atmosphere and become incorporated into the crystal. Since we are dealing with crystal forms, it is useful to also remind the reader that the term pseudopolymorphism is not appropriate to refer to solvate forms of a given molecular crystal [23, 79, 80] because solvate crystals can themselves be polymorphic!

In a recent paper, Bond listed a number of interesting controversial examples of co-crystals [81]. For example, dioxane, $C_4H_8O_2$, forms isostructural 1:1 two-component crystals with I_2 , Br_2 or Cl_2 , which at room temperature and 1 bar pressure are solid, liquid, and gas, respectively [82–84]. Clearly a distinction based on the aggregation state of one component has little significance.

The crystal structures of 13 co-crystals formed by *n*-alkylcarboxylic acids and pyrazine in 2:1 stoichiometry have been reported [85, 86]. The acids include formic acid, which is liquid at room temperature, up to the tridecandioic acid which is solid. Pyrazine is also a solid. Clearly, the early members of this series cannot be called solvates only because the co-former is liquid, while from decanoic to tridecandioic the acid/pyrazine systems are co-crystals. Another example is the two-component crystals formed by picric acid forms and benzene, naphthalene and anthracene (amongst others), which contain stacks of alternating molecular components that are clearly comparable in all three structures [87–89]. All of these crystals are stable at room temperature.

Not only it is difficult to distinguish between a co-crystal and a solvate (as a matter of fact all molecules are solid and form crystals at sufficiently low temperature) but it is also often difficult to distinguish between a crystal and a salt. As discussed above in the case of hydrogen-bonded systems between acids and bases the *transition* between a salt and a co-crystal may be a very semantic issue depending exclusively on the position of a proton along a $N \cdots O$ supramolecular link [90].

Proton transfer along a hydrogen bond poses an interesting question about polymorph definition. In fact, proton mobility along a hydrogen bond (say from

$\text{O}-\text{H}\cdots\text{N}$ to $(^-)\text{O}\cdots\text{H}-\text{N}(^+)$) may not be associated with a phase transition, even though it implies the formal transformation of a molecular crystal into a molecular salt. This situation has been observed, for instance, for the proton migration along an $\text{O}-\text{H}\cdots\text{O}$ bond in a co-crystal of urea–phosphoric acid (1:1) as a function of temperature [91,92].

Mootz and Wiechert have isolated two co-crystals of pyridine and formic acid: in the 1:1 co-crystal, proton transfer from formic acid to pyridine does not take place, while in the 1:4 co-crystal $\text{N}-\text{H}(^+)\cdots\text{O}$ interactions are present (see Fig. 14) [93]. Examples of this kind are rare, but serve to stress how the phenomenon of polymorphism can be, at times, full of ambiguity.

In order to tackle the problem of the co-crystal or salt nature of acid–base adducts we have used the same approach illustrated above in the case of the polymorphs of barbituric and cinchomeric acid, namely a combined use of diffraction and spectroscopic methods in the investigations. We have shown that mechanical mixing of a di-nitrogen base $[\text{N}(\text{CH}_2\text{CH}_2)_3\text{N}]$ (dabco) and of dicarboxylic acid $\text{HOOC}(\text{CH}_2)_n\text{COOH}$ ($n = 1-7$) affords a series of hydrogen-bonded adducts of general formula $[\text{N}(\text{CH}_2\text{CH}_2)_3\text{N}]-\text{H}-[\text{OOC}(\text{CH}_2)_n\text{COOH}]$ ($n = 1-7$, that is to say **1C3**, **1C4**, **1C5**, **1C6**, **1C7**, **1C8**, and **1C9**) (see Fig. 15) [21].

These compounds can be classified as co-crystals or salts depending on whether proton transfer from oxygen to nitrogen takes place or not along the $\text{O}-\text{H}\cdots\text{N}$ bonds [94]. In order to address this issue we have compared the results of an X-ray investigation carried out on single crystals grown from the solid-state products by seeding the corresponding methanol solutions with those obtained from the polycrystalline powder by solid-state ^1H and ^{15}N and ^{13}C spectroscopy.

For example, it has been possible to correlate the isotropic ^1H and ^{15}N chemical shift data with the $\text{N}\cdots\text{O}$ distances of the atoms involved in the hydrogen bond interaction in a series of solid adducts of formula $[\text{N}(\text{CH}_2\text{CH}_2)_3\text{N}]-\text{H}-[\text{OOC}(\text{CH}_2)_n\text{COOH}]$ ($n = 1-7$) (Fig. 16). The ^1H MAS and ^{15}N CPMAS NMR data are in agreement with the X-ray data and allow discrimination between the proton transfers for the **1C3** and **1C5** adducts and the strong $\text{N}\cdots\text{H}-\text{O}$ interactions

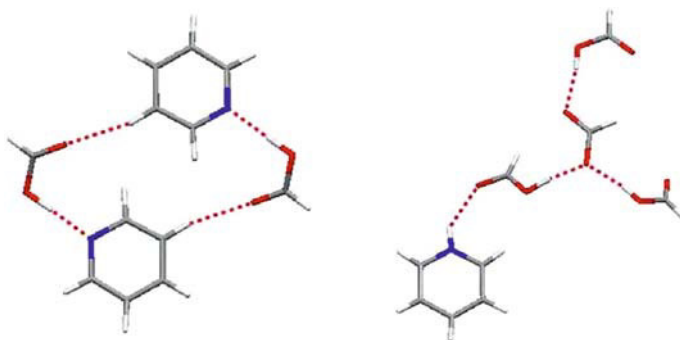


Fig. 14 The 1:1 co-crystal of pyridine and formic acid where the proton transfer does not occur (*left*) and the 1:4 co-crystal of pyridine and formic acid where the formate anion is present (*right*)

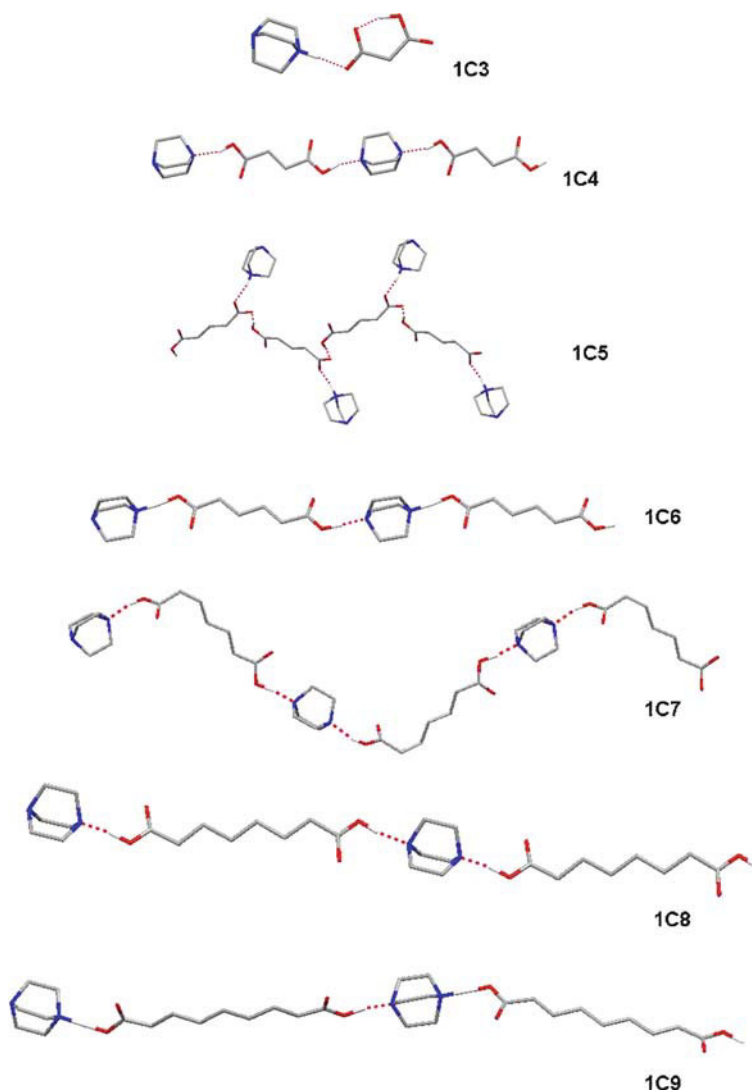


Fig. 15 Schematic representation of the packing motives in $[N(CH_2CH_2)_3N]\cdot H-[OOC(CH_2)_nCOOH]$ ($n = 1-7$, i.e. **1C3**, **1C4**, **1C5**, **1C6**, **1C7**, **1C8**, and **1C9**)

(without proton transfer) for the **1C4**, **1C7**, **1C8** and **1C9** co-crystals. **1C6** represents an intriguing case in which one of the nitrogen atoms of dabco is intermediate between the protonated and non-protonated forms. Density functional theory, applied to explore changes upon hydrogen bonding in the ^1H and ^{15}N shielding parameters, is in agreement with the experimental values found by solid-state NMR spectroscopy [95,96].

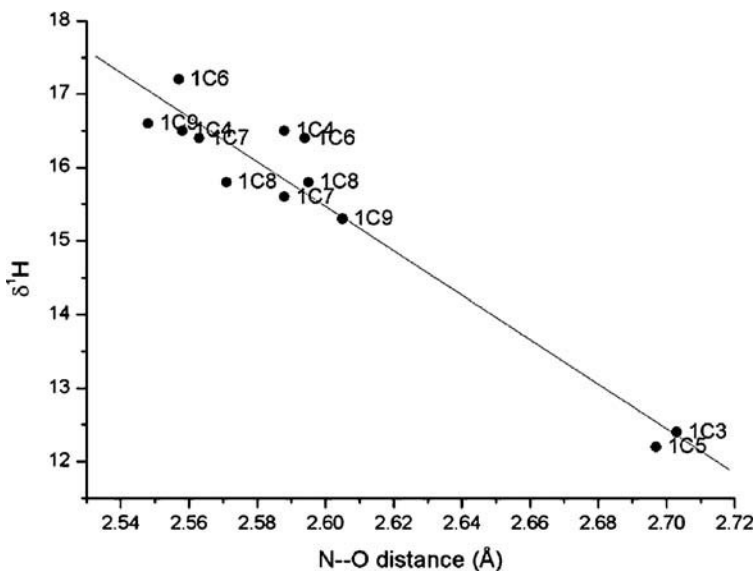


Fig. 16 Compact view of ^1H chemical shifts as a function of $\text{N}\cdots\text{O}$ distance

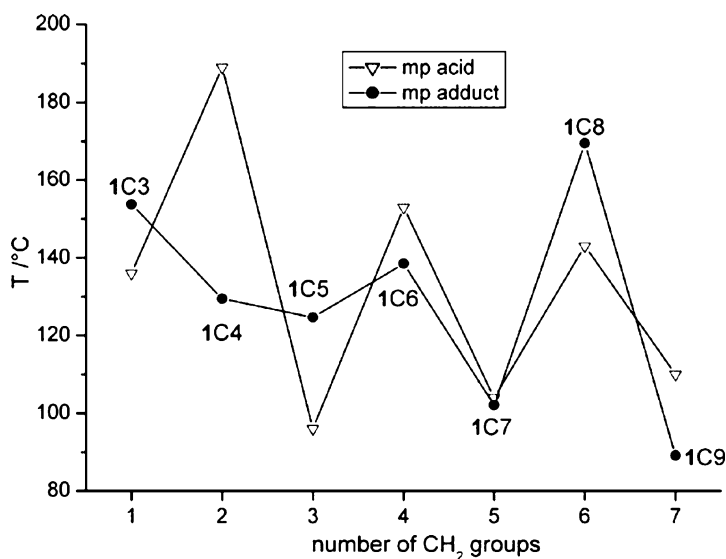


Fig. 17 Melting point alternation in neutral acids and in compounds **1C3–1C9**

Comparison of melting point data of the neutral organic acids and of the co-crystals and salts discussed herein yields another interesting observation. As shown in Fig. 17, the melting points of compounds **1C3–1C9** follow a trend very similar to that of the acids, i.e. they show an alternation of melting points as a function of the even–odd carbon chain length.

In summary, at room temperature malonic and glutaric adducts are salts; adipic is ambiguous; succinic, pimelic, suberic and azelaic adducts are co-crystals. Diffraction data and solid-state NMR data are in agreement except in the case of compound **1C6**, which shows proton motion on the NMR time scale. Salt or co-crystals – does it matter? The melting points of compounds **1C3–1C9** do not correlate with the salt-like or co-crystal nature of the adducts, but rather with the even–odd carbon chain length in spite of the substantial differences in supramolecular arrangements in the crystals of the adducts with respect to those of the parent diacids.

On closing this discussion on co-crystal systems, it is useful to cite few examples (selected from the many available in a rapidly expanding literature) of the importance of co-crystals in the pharmaceutical field. Piracetam, (2-oxo-1-pyrrolidiny) acetamide), has been co-crystallized by Zaworotko et al. with gentisic acid and *p*-hydroxybenzoic acid (see Fig. 18) [22].

Co-crystals of 4-hydroxybenzoic acid (4HBA) and 2,3,5,6-tetramethylpyrazine (TMP) (2:1) have been reported as a case of supramolecular synthon polymorphism in a co-crystal (see Fig. 19); in fact the two forms exhibit different hydrogen bond interactions. The co-crystal form I does not follow the hierarchy of hydrogen bonding, and converts into the stable form II, which follows the hierarchy of hydrogen bonding [97].

In yet another example, a co-crystal of caffeine and adipic acid [98] has been isolated by a co-crystallization methods based on a suspension/slurry containing both components of the co-crystal system. This approach provides an optimal environment for the putative co-crystal formation because the activity values of both

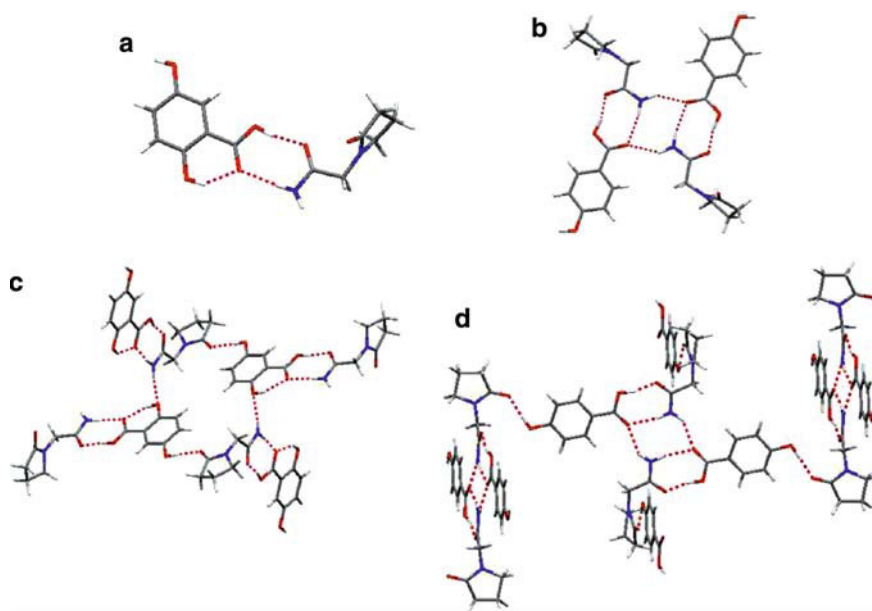


Fig. 18 Co-crystals of piracetam, (2-oxo-1-pyrrolidiny) acetamide with gentisic **a** and **c**, and *p*-hydroxybenzoic acids **b** and **d**

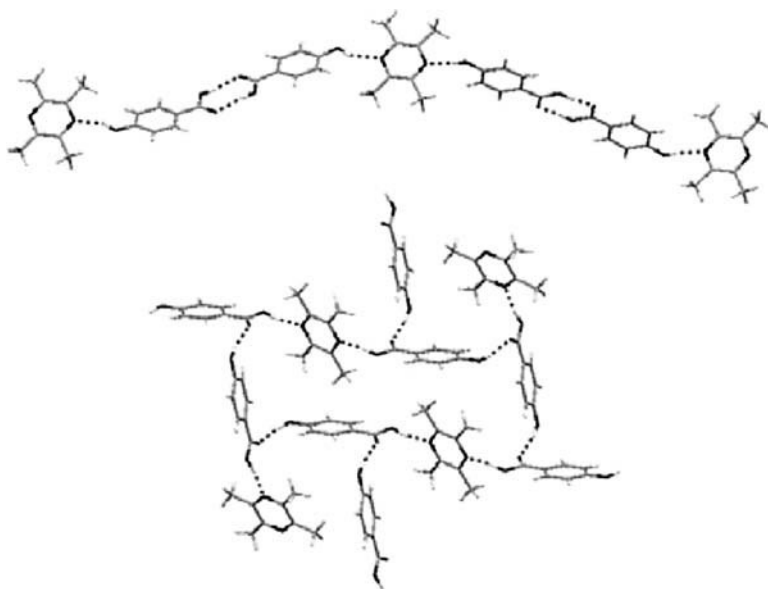


Fig. 19 Linear chain formed through carboxylic acid dimer and hydroxyl O-H $\cdots$ N hydrogen bonds in form I (*top*). Herringbone network in form II (*bottom*)

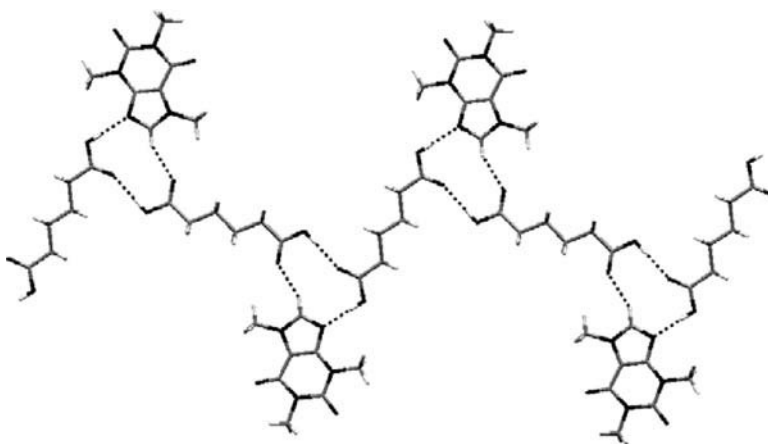


Fig. 20 View of caffeine–adipic acid trimers along the crystallographic plane (1 -2 0). The trimers are linked via hydrogen-bonded adipic acid molecules into molecular tapes

components are held at one. This method was applied to 16 pharmaceutically related co-crystal systems and was found to be 100% successful [99]. In the case of the co-crystal caffeine/adipic acid, a suspension of caffeine and adipic acid (1:1 molar ratio) in acetonitrile was prepared and equilibrated overnight at ambient temperature. The powder X-ray diffraction pattern of the solid after equilibration indicated formation of a new solid phase, which was then characterized as shown in Fig. 20.

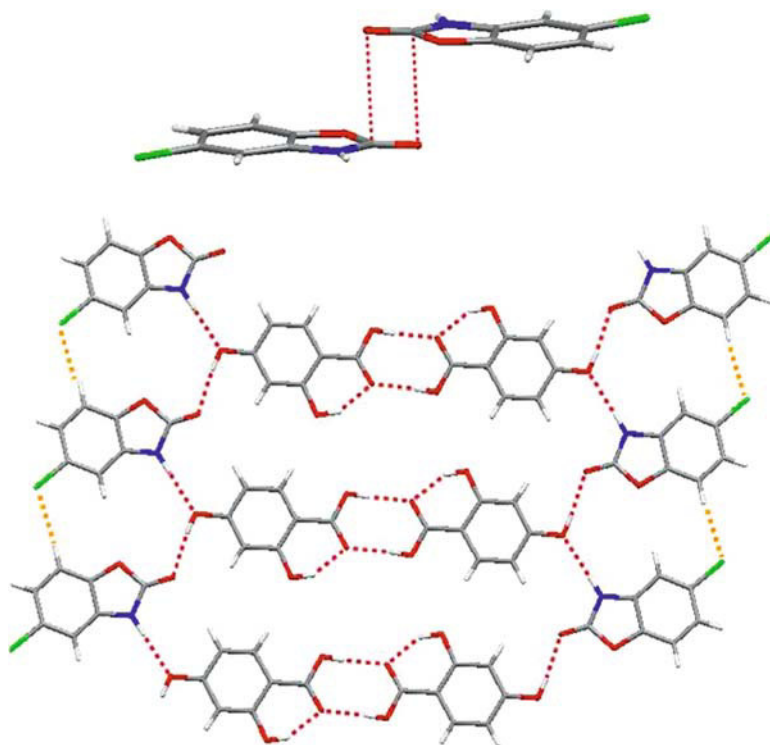


Fig. 21 Anti-parallel centrosymmetric carbonyl–carbonyl interaction between two molecules (*top*) and the packing motif (*bottom*) of chlorzoxazone in co-crystal form 1. Repulsive $\text{C-H}\cdots\text{Cl-C}$ interactions are in *light grey*

Evaporative crystallization experiments containing equimolar ratios of the API and guest has been instead used to obtain co-crystals of chlorzoxazone with carboxylic acids [100]. Two polymorphs of a chlorzoxazone:2,4-dihydroxybenzoic acid co-crystal and one form of a chlorzoxazone:4-hydroxybenzoic acid co-crystal have been characterized and the crystal structure of chlorzoxazone:2,4-dihydroxybenzoic acid (form 1) reported and analyzed, revealing an uncommon carbonyl–carbonyl interaction and a destabilizing $\text{C-H}\cdots\text{Cl-C}$ interaction (see Fig. 21).

9 Conclusions

The scope of this review has not been that of providing the reader with a comprehensive coverage of the topic of crystal forms, rather we have aimed at an introductory view of the phenomena of multiple crystals forms in general, and polymorphism in particular. For reasons of space, many problems have not been addressed (e.g. theo-

retical methods to study polymorphism), in particular the computational exploration of the possible crystalline structures of a given molecule. The interested reader will find in the necessarily limited number of references a good starting point for further reading.

The question on whether a crystalline material contains only molecules A in different arrangements (e.g. polymorphs), or molecules A *and* molecules B, and whether this latter association ought to be considered a co-crystal or a solvate is somewhat semantic. It is unquestionable, however, that crystals of different composition not only have different crystal structures but also may possess different physical properties, such as solubility, thermal behaviour, resistance to mechanical stress, gas-absorption/release capacity, colour, melting point etc. These differences may be relevant, and carry economical and practical implications, when considering, for instance, the bio-availability of a drug or the thermal stability of a pigment.

Reproducibility and predictability are paradigms in the exact sciences. This is why, beside all the utilitarian reasons associated with the marketing of solid state materials, we would like to learn how to *make* polymorphs, or, which is the same, how to effectively *prevent* polymorph formation. If seeds of a polymorphic modification can be obtained from non-solution methods (i.e. mechanical, thermodynamical, perhaps solid state reactions) these can be used in the seeding process, which may allow growth of less kinetically favoured crystal forms. Seeding may be valuable not only to obtain the desired crystalline form, but also to prevent crystallization of undesired forms. One could argue that polymorphism, with its high degree of serendipity, could be the *nemesis* of crystal engineering because polymorphism is in logical contrast with a discipline that aims to control and reproduce univocally a given crystal structure. This is not true (rather the investigation of crystal forms) of the way a molecule recognizes another molecule, whether the same or a different one, and links to form stable or metastable supramolecular arrays, it is rather a way *to tune* synthetic and assembly strategies to obtain the desired crystal structure, which is the paradigm of crystal engineering.

References

1. Bernstein J (2002) Polymorphism in molecular crystals. Oxford University Press, Oxford, p 352
2. Dunitz JD, Bernstein J (1995) *Acc Chem Res* 28:193–200
3. Klaproth MH (1798) *Bergmannische J I*:294–299
4. Mitscherlich E (1823) *Abhl Akad Berlin* 43
5. McCrone WC (1965) In: Fox D, Labes MM, Weissenberg A (eds) *Polymorphism in physics and chemistry of the organic solid state*, vol 2. Interscience, New York, p 726
6. Findlay AF (1951) *The phase rule and its applications*, 9th edn. Dover, New York, pp 7–16
7. Buerger MJ, Bloom MC (1937) *Z Kristallogr* 96:182–200
8. Sirota NN (1982) *Cryst Res Technol* 17:661–691
9. Kuhnert-Brandstatter M (1965) *Pure Appl Chem* 10:133–144
10. Kuhnert-Brandstatter M (1971) *Thermomicroscopy in the analysis of pharmaceuticals*. International series of monographs in analytical chemistry, vol 45. Pergamon, Oxford

11. Sarma JARP, Desiraju GR (1999) In: Seddon KR, Zaworotko M (eds) *Crystal engineering: the design and application of functional solids*. Kluwer, Dordrecht, pp 325–356
12. Griesser UJ (2002) *Acta Crystallogr A* 58(suppl):c241
13. Gorbitz CH, Hersleth HP (2000) *Acta Crystallogr B* 56:526–534
14. Griesser UJ, Burger A (1999) *Acta Crystallogr A* 55(suppl):400
15. Desiraju GR (1989) *Crystal engineering: the design of organic solids*. Elsevier, Amsterdam
16. Braga D, Grepioni F, Orpen AG (eds) (1999) *Crystal engineering: from molecules and crystals to materials*. In: *Proceedings of the NATO Advanced Study Institute*. 12–23 May 1999, Erice, Italy, p 500
17. Braga D, Grepioni F, Desiraju GR (1998) *Chem Rev* 98:1375–1405
18. Blake AJ, Champness NR, Hubberstey P, Li W-S, Withersby MA, Schroder M (1999) *Coord Chem Rev* 183:117–138
19. Moulton B, Zaworotko MJ (2001) *Chem Rev* 101:1629–1658
20. Griesser UJ (2006) In: Hilfiker R (ed) *The importance of solvates*. Wiley, Weinheim, pp 211–233
21. Braga D, Maini L, de Sanctis G, Rubini K, Grepioni F, Chierotti MR, Gobetto R (2003) *Chem Eur J* 9:5538–5548
22. Vishweshwar P, McMahon JA, Peterson ML, Hickey MB, Shattock TR, Zaworotko MJ (2005) *Chem Commun*, pp 4601–4603
23. Seddon KR (2004) *Cryst Growth Des* 4:1087–1087
24. Bernstein J (2005) *Chem Commun*, pp 5007–5012
25. Nangia A (2006) *Cryst Grow Des* 6:2
26. Burger A (1983) *Topics in pharmaceutical sciences*. Elsevier, Amsterdam, p 347
27. Cabri W, Ghetti P, Pozzi G, Alpegiani M (2007) *Org Process Res Dev* 11:64–72
28. Lara-Ochoa F, Espinosa-Perez G (2007) *Cryst Growth Des* 7:1213–1215
29. Raw AS, Yu LX (2004) *Adv Drug Delivery Rev* 56:235–236
30. Yu L, Stephenson GA, Mitchell CA, Bunnell CA, Snorek SV, Bowyer JJ, Borchardt TB, Stowell JG, Byrn SR (2000) *J Am Chem Soc* 122:585
31. Chen S, Xi H, Yu L (2005) *J Am Chem Soc* 127:17439–17444
32. Braga D, Bernstein J (2007) In: Dario B, Grepioni F (eds) *Crystal polymorphism: challenges at crossroads of science and technology*. Wiley, Weinheim, pp 293–314
33. Gobetto R (2007) In: Braga D, Grepioni F (eds) *Solid state NMR*. Wiley, Weinheim, pp 266–292
34. Grunenberg A, Henck JO, Siesler HW (1996) *Int J Pharm* 129:147–158
35. Henck J-O, Kuhnert-Brandstatter M (1999) *J Pharm Sci* 88:103–108
36. Almarsson O, Zaworotko MJ (2004) *Chem Commun*, p 1889
37. Aakeröy CB, Beatty AM, Helfrich BA (2001) *Angew Chem Int Ed* 40:3240–3242
38. Price CP, Grzesiak AL, Matzger AJ (2005) *J Am Chem Soc* 127:5512–5517
39. Morissette SL, Almarsson O, Peterson ML, Remenar JF, Read MJ, Lemmo AV, Ellis S, Cima MJ, Gardner CR (2004) *Adv Drug Delivery Rev* 56:275–300
40. Braga D, Grepioni F (2005) *Chem Commun*, pp 3635–3645
41. Trask AV, Shan N, Motherwell WDS, Jones W, Feng S, Tan RBH, Carpenter KJ (2005) *Chem Commun*, pp 880–882
42. Braga D, Grepioni F (2004) *Angew Chem Int Ed* 43:4002–4011
43. Griesser UJ, Burger A (1991) *Eur J Pharm Biopharm* 37:118
44. York P (2005) *Chem World* 2:50
45. Shekunov YB, York P (2001) *J Cryst Growth* 211:122
46. Desiraju GR (2003) *Crystal design: structure and function*. Wiley, Chichester, p 420
47. Bauer J, Spanton S, Henry R, Quick J, Dziki W, Porter W, Morris J (2001) *Pharm Res* 18:859
48. Kirpal A (1907) *Monatsh Chem* 28:439–445
49. Tong ML, Wang J, Hu S (2005) *J Solid State Chem* 178:1518–1525
50. Tong ML, Wang J, Hu S, Batten SR (2005) *Inorg Chem Commun* 8:48–51
51. Senevirathna MKI, Pitigala P, Perera VPS, Tennakone K (2005) *Langmuir* 21:2997–3001
52. International Centre for Diffraction Data (2005) Powder diffraction file PDF-2. ICDD, Newton Square, PA

53. Takusagawa F, Hirotsu K, Shimada A (1973) *Bull Chem Soc Jpn* 46:2669–2675
54. Braga D, Maini L, Fagnano C, Taddei P, Chierotti MR, Gobetto R (2007) *Chem Eur J* 13:1222–1230
55. Braga D, Maini L (2004) *Chem Commun*, pp 976–977
56. Braga D, Rubini K, Maini L (2004) *Cryst Eng Commun* 6:236–238
57. Braga D, Cojazzi G, Abati A, Maini L, Polito M, Scaccianocce L, Grepioni F (2000) *J Chem Soc Dalton Trans*, pp 3969–3975
58. Caira MR (1998) *Top Curr Chem* 198:163–208
59. Caira MR, Nassimbeni LR, Timme M (1995) *J Pharm Sci* 84:884–888
60. De Villiers MM, Van der Watt JG, Lotter AP (1991) *Drug Dev Ind Pharm* 17:1295–1303
61. Braga D, Maini L, Grepioni F (1999) *Chem Commun*, pp 937–938
62. Chen X, Morris KR, Griesser UJ, Byrn SR, Stowell JG (2002) *J Am Chem Soc* 124:15012–15019
63. Braga D, Cadoni M, Grepioni F, Maini L, Rubini K (2006) *Cryst Eng Commun* 8:756–763
64. Braga D, Grepioni F, Polito M, Chierotti MR, Ellena S, Gobetto R (2006) *Organometallics* 25:4627–4633
65. Lewis TC, Tocher DA, Price SL (2004) *Cryst Grow Des* 4:979–987
66. Bolton W (1963) *Acta Cryst* 16:166–173
67. Jeffrey GA, Ghose S, Warwicker JO (1961) *Acta Cryst* 14:881–887
68. Al-Karaghoulis AR, Abdul-Wahab B, Ajaj E, Al-Asaff S (1977) *Acta Crystallogr Sect B Struct Sci* B33:1655–1660
69. Braga D, Cadoni M, Grepioni F, Maini L, Streek JVD (2007) *New J Chem* 31:1935–1940 doi: 10.1039/b704772j
70. Etter MC, Reutzel SM, Choo CG (1993) *J Am Chem Soc* 115:4411–4412
71. Zaworotko MJ (2007) *Cryst Grow Des* 7:4–9
72. Hickey MB, Peterson ML, Scoppettuolo LA, Morrisette SL, Vetter A, Guzman H, Remenar JF, Zhang Z, Tawa MD, Haley S, Zaworotko MJ, Almarsson O (2007) *Eur J Pharm Biopharm* 67:112–119
73. Trask AV (2007) *Mol Pharm* 4:301–309
74. Desiraju GR (2003) *Cryst Eng Commun* 5:466–467
75. Dunitz JD (2003) *Cryst Eng Commun* 5:506
76. Aakeroy CB, Salmon DJ (2005) *Cryst Eng Commun* 7:439
77. Vishweshwar P, McMahon JA, Bis JA, Zaworotko MJ (2006) *J Pharm Sci* 95:499–516
78. Stahly GP (2007) *Cryst Growth Des* 7:1007–1026
79. Bernstein J (2005) *Cryst Growth Des* 5:1661–1662
80. Nangia A (2006) *Cryst Growth Des* 6:2–4
81. Bond AD (2007) *Cryst Eng Commun* 9:833–834
82. Bock H, Holl S (2001) *Z Naturforsch B* 56:111
83. Hassel OJH (1954) *Acta Chem Scand* 8:873
84. Hassel O, Stromme KO (1959) *Acta Chem Scand* 13:1775
85. Bond AD (2003) *Chem Commun*, pp 250–251
86. Bond AD (2006) *Cryst Eng Commun* 8:333–337
87. Takayanagi H, Toubai Y, Goto M, Yamaguchi S, Ogura H (1991) *Chem Pharm Bull* 39:2491
88. Banerjee A, Brown CJ (1985) *Acta Crystallogr Sect C Cryst Struct Commun* 41:82
89. Herbststein FH, Kaftory M (1976) *Acta Crystallogr Sect B Struct Sci* 32:387
90. Childs SL, Stahly GP, Park A (2007) *Mol Pharm* 4:323–338
91. Wilson CC (2001) *Acta Crystallogr Sect B Struct Sci* B57:435–439
92. Steiner T, Majerz I, Wilson CC (2001) *Angew Chem Int Ed* 40: 2651–2654
93. Wiechert D, Mootz D (1999) *Angew Chem Int Ed* 38:1974–1976
94. Braga D, Maini L, Polito M, Grepioni F (2004) *Struct Bond* 111:1–32
95. Gobetto R, Nervi C, Chierotti MR, Braga D, Maini L, Grepioni F, Harris RK, Ghi PY (2005) *Chem Mater* 17:1457–1466
96. Gobetto R, Nervi C, Chierotti MR, Braga D, Maini L, Grepioni F, Harris RK, Hodgkinson P (2005) *Chem Eur J* 11:7461–7471
97. Sreekanth BR, Vishweshwar P, Vyas K (2007) *Chem Commun*, pp 2375–2377

98. Bucar D-K, Henry RF, Lou X, Borchardt TB, Zhang GGZ (2007) *Chem Commun*, pp 525–527
99. Zhang GGZ, Henry RF, Borchardt TB, Lou X (2007) *J Pharm Sci* 96:990–995
100. Childs SL, Hardcastle KI (2007) *Cryst Eng Commun* 9:364–367

Molecular Networks

(Ed.) M. W. Hosseini

2009, XI, 165p. 116 illus., 58 illus. in color., Hardcover

ISBN: 978-3-642-01366-9