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Bogotá D.C., 11 de junio de 2019

Señores

DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Ciudad

REFERENCIA: Expediente No. NC2017/0008870
TÍTULO: “ANÁLOGOS DE AMILINA”
SOLICITANTE: **BOEHRINGER INGELHEIM INTERNATIONAL GMBH,**
ZEALAND PHARMA A/S
TRÁMITE: **Respuesta a Concepto Técnico notificado mediante Oficio**
No. 840 del 11 de marzo de 2019.

JAIME EDUARDO DELGADO VILLEGAS, en mi condición de apoderado de la sociedad **BOEHRINGER INGELHEIM INTERNATIONAL GMBH, ZEALAND PHARMA A/S**, dentro del término legal, presento respuesta a Concepto Técnico notificado mediante Oficio No. 840 del 11 de marzo de 2019.

1. DE LA SOLICITUD DE PATENTE.

TÍTULO

El Examinador objeto el título de la invención porque:

“El título no corresponde con el objeto de la solicitud, porque hace referencia a “Análogos de amilina” el cual es general y no limita el alcance de la solicitud, la cual se relaciona con análogos de amilina estables químicamente y que presentan



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delección de residuos en las posiciones 21 y 22. Por lo tanto, se sugiere al solicitante hacer la corrección necesaria.”.

R/: El solicitante de acuerdo a lo requerido por el Examinador procedió a modificar el título de la invención, el cual pone a consideración del Despacho, así:

NUEVO TITULO:

**“ANÁLOGO DE AMILINA QUE TIENE ACTIVIDAD AGONISTA EN
UNO O MÁS DE HAMR1, HAMR2 Y HAMR3, ESTABLES
QUÍMICAMENTE Y QUE PRESENTAN DELECCIÓN DE RESIDUOS
EN LAS POSICIONES 21 Y 22”**

REIVINDICACIONES (Art. 30 D 486, Art. 22 PCT)

El Examinador objeto el Capítulo Reivindicatorio de la invención porque:

“La reivindicación 1 no es clara, porque la referencia a la actividad agonista sobre hAMR1, hAMR2 y hAMR3 no se consideran como características esenciales de los polipéptidos. Se sugiere colocar esta característica en el preámbulo de la reivindicación o eliminar estas referencias de la reivindicación”.

“Las reivindicaciones 1, 2, 4 a 14 no están sustentadas en la descripción, porque mencionan las sales o solvatos farmacéuticamente aceptables y no se establece que tipo de sal, solvato, grado de solvatación, etc., lo anterior no permite establecer el o los compuestos reclamados, ya que surgen múltiples posibilidades cuyo desarrollo no se evidencia en el capítulo descriptivo. Se sugiere eliminar estas sales y solvatos de las reivindicaciones”.

R/: El solicitante de acuerdo a lo requerido por el Examinador procedió a:



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Modificar el preámbulo de la Reivindicación 1 de la siguiente manera “Un análogo de amilina que tiene actividad agonista en uno o más de hAMYR1, hAMYR2 y hAMYR3”. El resto de la Reivindicación 1 se ha modificado de manera acorde.

Se han eliminado de las Reivindicaciones las secuencias y compuestos que contienen los residuos N21-N22.

Además, se ha **eliminado** el término “**solvatos**” de todas las reivindicaciones.

En cuanto a la objeción a la expresión “*sales farmacéuticas aceptables*”, nuestro poderdante desea respetuosamente señalar que la **finalidad de las Reivindicaciones de una patente** es la de definir el ámbito de protección de la misma y no la de definir suficientemente una invención, para lo cual están la descripción y los ejemplos. Es decir, las Reivindicaciones cumplen en la patente el papel de definir el ámbito de protección y la descripción cumple el papel de definir suficientemente la invención. Por ello, las Reivindicaciones se interpretan a la luz de la descripción para no repetir innecesariamente información recogida en la parte de Descripción.

Una **Reivindicación debe ser clara y concisa por definición y debe contener todas las características esenciales para reproducir una invención**, pero no elementos superfluos fácilmente determinables por un experto con su conocimiento general común. Toda limitación innecesaria que contengan las Reivindicaciones servirá para que a un posible infractor le resulte más fácil reproducir una invención sin infringir las Reivindicaciones y eso con todo el respecto atenta simple y llanamente contra el sentido mismo



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de la ley de patentes ya que la protección conferida al solicitante resulta muy mermada a cambio de que éste divulgue su invención.

En este caso en particular, respetuosamente **consideramos que no da lugar a incluir en la Reivindicación un listado de las distintas sales farmacéuticamente aceptables**, ya que las mismas son ampliamente conocidas por el experto en la materia, en particular a la luz de la presente descripción en la que se cita un listado de dichas sales y se hace referencia al conocido Remington's Pharmaceutical Sciences (ver de la página 34, línea 23, a la página 35, línea 11). **Incluir el listado de sales en la reivindicación haría que la reivindicación fuese menos concisa que con la redacción actual, y limitaría extremadamente el ámbito de protección.** Por ello, consideramos que la expresión “**sales farmacéuticas aceptables**” debería ser aceptada por el Examinador.

Por último, la expresión “**sales farmacéuticas aceptables**” se utiliza en multitud de patentes ya concedidas, por ejemplo, en la patente proveniente de la solicitud en Colombia de número 16-089.238, que pertenece al mismo solicitante que esta y en la que en la descripción se da una definición de las sales farmacéuticamente aceptables prácticamente igual a la dada en la presente solicitud.

En cualquier caso, para intentar avanzar en la tramitación de esta solicitud, el solicitante **ha eliminado las sales farmacéuticamente aceptables de las Reivindicaciones 1-14 y ha incluido una nueva Reivindicación (Reivindicación 16) en la que define dichas sales.** Asimismo, **ha incluido una nueva Reivindicación 17 que define una composición farmacéutica**



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que comprende una sal farmacéuticamente aceptable según se define en la **Reivindicación 16**, en combinación con un portador, un excipiente o vehículo farmacéuticamente aceptables. La base para esta reivindicación se encuentra en la **Reivindicación 29** original (**Reivindicación 15** de las anteriores Reivindicaciones).

Si el Examinador prefiere que las sales se mantengan en las Reivindicaciones 1-14, como estaban antes de estas últimas modificaciones, el solicitante autoriza al Examinador a realizar dichas modificaciones si así se agiliza la concesión de la presente patente.

Teniendo en cuenta todo lo anterior, el solicitante respetuosamente considera que el **nuevo juego de Reivindicaciones** supera las objeciones emitidas por el examinador y no supone adición de materia.

Por lo anterior, el solicitante pone a consideración del Examinador un **NUEVO CAPÍTULO REIVINDICATORIO** el cual incluye **diecisiete (17) reivindicaciones** en dieciséis folios.

2. ANÁLISIS DE PATENTABILIDAD.

Nivel Inventivo (Art. 18 D486)

El Examinador objeto el **nivel inventivo** de las Reivindicaciones 1 a 15 con los documentos **D1 (US20130059770)** y **D2 (Moriarty D.F (1999))** porque:



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“El documento D1 se considera el estado de la técnica cercano al polipéptido análogo de amilina definido en la reivindicación 1, porque este documento divulga polipéptidos que comprenden una secuencia de aminoácidos que son análogos de pramlintide (análogo de amilina) que demuestran solubilidad incrementada y estabilidad física (Resumen, Pág. 49 Párr. 1085; Reivindicaciones)”.

“La diferencia entre los polipéptidos reclamados en la reivindicación 1 y los polipéptidos divulgados en el documento D1, consiste en que en la presente solicitud hace referencia a los análogos con Xaa21 y Xaa22: Deletados o Asn; mientras que el documento en esas posiciones menciona los aminoácidos Glu, Gln, Ser, Thr, Asn”.

“En consecuencia, la persona del oficio normalmente versada en la materia se vería motivada a realizar modificaciones en los análogos de amilina divulgados en D1 con los estudios realizados por el documento D2 principalmente en la región que involucra los aminoácidos de las posiciones 21 y 22 y que dichas modificaciones involucren deletar o variar dichos aminoácidos como una estrategia para mejorar las propiedades de estabilidad, solubilidad y tener una menor formación de agregados llegando de esa forma al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera que carece de nivel inventivo”.

“Puesto que las reivindicaciones dependientes 2-15 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas”.

R/: Nuestro poderdante se permite manifestar en cuanto a las objeciones de **falta de nivel inventivo de la invención** por parte del Examinador, que no está de acuerdo por las siguientes razones:

El Examinador considera el documento **D1** como el estado de la técnica más cercano, y sugiere que la diferencia entre los polipéptidos de la Reivindicación 1 y los revelados en el documento **D1** es la identidad de los residuos en las posiciones Xaa21 y Xaa22. Tal y como se ha explicado anteriormente, **los compuestos con estos residuos han sido eliminados de las Nuevas Reivindicaciones.**



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Además, el Examinador alega que no hay pruebas de que los compuestos reivindicados proporcionen una mejora en comparación con los del documento **D1**.

El solicitante respetuosamente considera que el Examinador **ha podido pasar por alto una serie de características clave en la presente invención**.

Es importante destacar que **todos los compuestos reivindicados tienen residuos N-metilado en las posiciones Xaa24 y Xaa26, que no se describen en el documento D1**. Todos los compuestos en el documento **D1** tienen residuos de prolina (Pro) en las posiciones 28 y 29, y típicamente también en la posición 25, **que no se encuentran en los compuestos de la presente invención**.

Esto se debe, como señala el Examinador, a que los compuestos del documento **D1** se basan en la pramlintida. Sin embargo, **los compuestos de la presente invención no se basan en pramlintida, sino en un compuesto distinto**, como se discute a continuación.

La página 2, líneas 13-17 de la presente solicitud explica que Yan et al (PNAS, 103(7), 2046-2051, 2006) describen el análogo de la amilina IAPP-GI, que posee residuos N-metilados en posiciones 24 (Gly(Me)) y 26 (Ile(Me)). Se adjunta una copia de dicho documento.

El resumen dice:



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"Mostramos que el análogo doble N-metilado de longitud completa IAPP [(N-Me)G24, (N-Me)I26]-IAPP (IAPP-GI) es una molécula altamente soluble, no amiloidógena y un imitador IAPP no citotóxico y un agonista del receptor de IAPP".

Por lo tanto, la incorporación de residuos N-metilado en las posiciones 24 y 26 elimina efectivamente la fibrilación vista con la amilina humana nativa (a pesar de retener los residuos de Asn en las posiciones 21 y 22) bajo las condiciones reportadas por Yan et al. (Fig. 2C). Por lo tanto, IAPP-GI representa un enfoque alternativo para tratar los problemas de estabilidad física asociados con la amilina nativa al proporcionado por pramlintida.

Sin embargo, IAPP-GI tiene menor actividad que la amilina nativa, siendo alrededor de 7 veces menos potente (ver página 2048, columna 2 (bajo "IAPP-GI es un agonista completo del receptor IAPP") y Figura 4).

Por lo tanto, el **objetivo principal de los inventores de la presente invención** no era abordar la fibrilación (ya que ese problema había sido abordado efectivamente mediante la introducción de los residuos N-metilados) sino más bien **aumentar la potencia de la molécula IAPP-GI**.

Sorprendentemente, **encontraron que la eliminación de los residuos de Asn en las posiciones 21 y 22 tiene el efecto de aumento de la actividad en los receptores hCTR2 y hAMYR3, en moléculas que tienen Gly(Me) y Ile(Me) en las posiciones 24 y 26** (véanse los datos del Tabla 6 de la presente solicitud, donde los compuestos 55, 57 y 89 tienen valores EC50 más bajos que los compuestos de comparación 98, 99 y 100). Sin embargo,

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la delección tiene poco o ningún efecto en compuestos con residuos de la cadena no metilados, como demuestran los compuestos 97 y NN96, que tienen valores EC50 similares. Este no podía predecirse a partir del estado de la técnica.

Por ello, la presente invención se refiere a aumentar la potencia de compuestos que tiene en las posiciones 24 y 26 residuos N-metilados. Todos los compuestos reivindicados tienen mayor potencia que la IAPPGI.

Esto puede verse en la Tabla 1, páginas 76 a 79, de la presente solicitud, la cual muestra que todos los compuestos de los ejemplos tienen mayor potencia que pramlintida en hCTR2. La propia pramlintida tiene una potencia comparable con la amilina humana nativa según WO93/10146 (citado en el informe de búsqueda internacional). La Tabla I en la página 28 de WO93/10146 muestra un EC50 para 25,28,29Pro-h-Amilina en el ensayo de músculo sóleo comparable al rango de 1-10 nM mencionado para la amilina humana nativa en página 27, líneas 20-23. Como ya se ha mencionado, Yan et al muestran que la amilina nativa tiene aproximadamente 7 veces más potencia que IAPP-GI. Por lo tanto, se deduce que **los compuestos de la presente invención deben ser todos más activos que la IAPP-GI.**

Por lo tanto, creemos que el artículo científico de Yan et al debe ser considerado como el estado de la técnica más cercano, y la actividad inventiva debe ser evaluada frente a dicho documento en lugar de frente al documento **D1. No hay ninguna sugerencia** en el estado de la técnica por la cual fuese esperable que **la eliminación de los residuos 21 y 22**



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aumentase la potencia de las moléculas que contienen residuos N-metilados en las posiciones 24 y 26.

El Examinador cita el documento **D2** (Moriarty y Raleigh, 1999) como prueba de que el experto en la materia consideraría la posibilidad de modificar estos residuos para mejorar la estabilidad y reducir la fibrilación. Sin embargo, como se ha indicado más arriba, el objetivo principal de los inventores de la presente invención no era abordar la fibrilación, ya que IAPP-GI y otras moléculas que contienen residuos N-metilados en las posiciones 24 y 26 son ya mucho menos susceptibles a la fibrilación que la amilina. Por el contrario, el **objetivo de la presente invención era aumentar la potencia de la molécula IAPP-GI.**

Incluso si el experto en la materia se hubiese visto motivado a considerar la modificación de esta región, nada en el documento **D2** le hubiese llevado a realizar eliminaciones (en vez de sustituciones con Pro) en las posiciones 21 y 22, y menos aún a eliminar ambos residuos juntos. De hecho, el documento **D2**, en el mejor de los casos, llevaría al experto en la materia a hacer sustituciones con prolina (Pro). Ese es el enfoque del trabajo experimental descrito en el documento **D2**, ya que la prolina es "un residuo que se espera que perturbe la estructura ordenada encontrada en el amiloide" potencialmente explicando la falta de formación de amiloide por la amilina de rata (ver la última frase de la página 1811, y la frase siguiente).

Además, WO93/10146 (mencionado anteriormente) muestra un alineamiento de secuencias de amilina de tipo silvestre en la Figura 2, reproducida a continuación para mayor claridad:

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Human:	KCNTATCATQRLANFLVHSSNFGAILSSSTNVGSNTY
Cat:	-----IR---L---P-----
Dog:	-----RT---L---P-----
Rat:	-----R---L-PV-PP-----
Mouse:	-----R---L-PV-PP-----
Hamster:	-----N---L-PV---P-----
Guinea pig:	-----T---R--H-L--A-LP-D-----

Se puede ver que los residuos N21-N22 están totalmente conservados a través de humanos, gatos, perros, ratas, ratones, y hámsteres, con únicamente teniendo las cobayas una sola sustitución. Por lo tanto, el experto creería que estos residuos muy probablemente sean funcionalmente importantes y ciertamente no consideraría eliminar ambos, menos aún con la expectativa de aumentar la potencia de las moléculas con residuos N-metilados en las posiciones 24 y 26.

Por todo lo anteriormente mencionado consideramos que, con las **modificaciones introducidas** en el **capítulo reivindicatorio propuesto**, los documentos **D1 y D2** mencionados por el Examinador el objetivo de la invención **no se deriva de manera evidente del estado de la técnica**, puesto que **no describen, ni revelan, ni enseñan, las características técnicas del ANÁLOGO DE AMILINA que tiene actividad agonista en uno o más DE HAMYR1, HAMYR2 Y HAMYR3 de la presente invención** de nuestro poderdante, además, Por lo tanto, la invención es **NOVEDOSA**, tiene **NIVEL INVENTIVO y APLICACIÓN INDUSTRIAL**, es decir cumple con los requisitos de exigidos por la Decisión 486 del Acuerdo de la Comisión de la Comunidad Andina; además, estamos dando respuesta al Concepto Técnico notificado mediante Oficio No. 840 del 11 de marzo de 2019.



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Finalmente, solicito respetuosamente se proceda a **CONCEDER** la Patente de Invención titulada: “**ANÁLOGO DE AMILINA QUE TIENE ACTIVIDAD AGONISTA EN UNO O MÁS DE HAMR1, HAMR2 Y HAMR3, ESTABLES QUÍMICAMENTE Y QUE PRESENTAN DELECCIÓN DE RESIDUOS EN LAS POSICIONES 21 y 22**” a nombre de la sociedad **BOEHRINGER INGELHEIM INTERNATIONAL GMBH, ZEALAND PHARMA A/S**, para las nuevas Reivindicaciones 1 a 17 inclusive.

Atentamente,

JAIME EDUARDO DELGADO VILLEGAS

C.C. No. 19.270.955 Bogotá

T.P. No. 43.308 C.S.J.

Anexo:

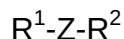
Nuevas Reivindicaciones (17) en dieciséis folios.

Artículo “Design of a mimic of nonamyloidogenic and bioactive human islet amyloid polypeptide (IAPP) as nanomolar affinity inhibitor of IAPP cytotoxic fibrillogenesis”, de Li-Mei Yan, Marianna Taterek-Nossol, Aleksandra Velkova, Athanasios Kazantzis, and Aphrodite Kapurniotu, en seis folios.

NUEVAS REIVINDICACIONES

1. Un análogo de amilina que tiene actividad agonista en uno o más de hAMYR1, hAMYR2 y hAMYR3 que es un compuesto que tiene la fórmula:

5



en donde

R^1 es [19-carboxinonadecanoilo]-isoGlu-;

R^2 es OH o NHR^3 , en donde R^3 es hidrógeno o alquilo C_{1-3} ; y

Z es una secuencia de aminoácidos seleccionada del grupo que consiste

10 en:

RCNTATCATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCNTATCATQRLANFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

15

RCGTATCATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

20

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-Hyp;

RCNTATCATQRLAHFLHRF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;

RCNTATCATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;

RCGTATCATERLANFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

25

RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCGTATCATERLAHFLKRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;

RCNTATCATQRLAHFLHRVSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCNTATCATQRLAHFLHRVSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

30

RCPTATCATDRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSNTP;

RCNTATCATQRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCPTATCATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSNTP;

RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP;

RCPTATCATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSNTP;

35

RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-Hyp;

RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-Hyp;

RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp;
 RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp;
 5 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCGTATCATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 10 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFTP;
 RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFTP;
 RCGTATCATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCGTATCATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp;
 RCGTATCATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 15 RCGTATCATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Orn-T-Hyp;
 RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Apr;
 RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Orn-TP;
 20 RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp;
 RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCNTATCATQRLAHFL-Aad-RSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Orn-T-Hyp;
 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 25 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Aad-T-Hyp;
 RCGTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCGTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCQTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 30 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCPTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSNT-Hyp;
 RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSDT-Hyp;
 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNTP;
 RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNTP;
 35 RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;
 RCPTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSNT-Hyp;

RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 RCNTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 RCPTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 RCNTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 5 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp;
 10 RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp;
 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSAad-T-Hyp;
 RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSAad-T-Hyp;
 RCGTATCATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 15 RCNTATCATQRLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP;
 RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTTP;
 RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp;
 20 RCNTATCATQRLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP;
 RCNTATCATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 RCNTATCATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp;
 RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 25 RCQTATCATDRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 RCQTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp;
 RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 RCQTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP;
 30 RCQTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP;
 RCQTATCATDRLA-Aad-FLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp;
 RCQTATCATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP; y
 RCNTATCATQRLADFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP.

35 2. Un análogo de amilina de conformidad con la Reivindicación 1, que se selecciona del grupo que consiste en:

[19CD]-isoGlu-RCNTATCATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLANFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

5 [19CD]-isoGlu-RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH-Typ-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH-Typ-NH₂ ;

10 [19CD]-isoGlu-RCGTATCATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH-Typ-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSH-Typ-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSH-Typ-NH₂ ;

15 [19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSH-Orn-T-Typ-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSH-Aad-T-Typ-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGH-Typ-NH₂ ;

20 [19CD]-isoGlu-RCNTATCATQRLAHFLHRF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSHNTP-NH₂ ;

25 [19CD]-isoGlu-RCNTATCATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSHNTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLANFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSHNTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSHNTP-NH₂ ;

30 [19CD]-isoGlu-RCGTATCATERLAHFLKRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSHNTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLHRSVF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

35 [19CD]-isoGlu-RCNTATCATQRLAHFLHRVSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCPTATCATDRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTDVGSDTP-NH₂ ;

5 [19CD]-isoGlu-RCNTATCATQRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCPTATCATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTDVGSNTP-NH₂ ;

10 [19CD]-isoGlu-RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSETP-NH₂ ;

[19CD]-isoGlu-RCPTATCATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTDVGSDTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

15 [19CD]-isoGlu-RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

20 [19CD]-isoGlu-RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGST-Hyp-NH₂ ;

25 [19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

30 [19CD]-isoGlu-RCGTATCATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

35 [19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

5 [19CD]-isoGlu-RCGTATCATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-
10 LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Apr-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSSTP-NH₂ ;

15 [19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-TP-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTT-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
20 LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFL-Aad-RSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-TP-NH₂ ;

25 [19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGT-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Aad-T-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-
30 LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

35 [19CD]-isoGlu-RCQTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSST-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCPTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTDVGSNH₂-Hyp-NH₂ ;

5 [19CD]-isoGlu-RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSDH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

10 [19CD]-isoGlu-RCNTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

[19CD]-isoGlu-RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCPTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH₂-Hyp-NH₂ ;

15 [19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

[19CD]-isoGlu-RCPTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

20 [19CD]-isoGlu-RCNTATCATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSH₂-Hyp-NH₂ ;

25 [19CD]-isoGlu-RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGH₂-Hyp-NH₂ ;

30 [19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTH₂-Hyp-NH₂ ;

[19CD]-isoGlu-RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTH₂-Hyp-NH₂ ;

35 [19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSAad-T-Hyp-NH₂ ;

[19CD]-isoGlu-RCPTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSAad-T-Hyp-NH₂ ;

[19CD]-isoGlu-RCGTATCATERLAHFLQRF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTHyp-NH₂ ;

5 [19CD]-isoGlu-RCGTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGTHyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
10 LSSTEVGSGTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGTHyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTTP-NH₂ ;

15 [19CD]-isoGlu-RCNTATCATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSTTHyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLERSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
20 LSSTEVGSSTP-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTHyp-NH₂ ;

[19CD]-isoGlu-RCNTATCATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSSTHyp-NH₂ ;

25 [19CD]-isoGlu-RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTHyp-NH₂ ;

[19CD]-isoGlu-RCQTATCATDRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTHyp-NH₂ ;

[19CD]-isoGlu-RCQTATCATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
30 LSSTEVGSGTHyp-NH₂ ;

[19CD]-isoGlu-RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSGTHyp-NH₂ ;

[19CD]-isoGlu-RCQTATCATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

35 [19CD]-isoGlu-RCQTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-
LSSTEVGSNTP-NH₂ ;

- [19CD]-isoGlu-RCQTATCATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-NH₂ ;
- [19CD]-isoGlu-RCQTATCATDRLA-Aad-FLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-T-Hyp-NH₂ ;
- 5 [19CD]-isoGlu-RCQTATCATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂; y
- [19CD]-isoGlu-RCNTATCATQRLADFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ ;
- en donde [19CD] representa [19-carboxinonadecanoilo].
- 10
3. Un análogo de amilina de conformidad con la Reivindicación 1 o 2, que comprende un puente disulfuro intramolecular formado entre los residuos de cisteína presentes en las posiciones 2 y 7 de la secuencia de aminoácidos.
- 15 4. Un análogo de amilina de conformidad con la Reivindicación 3, que se selecciona del grupo que consiste en:
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 1);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 2);
- 20 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLANFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 3);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-T-Hyp-NH₂ (Compuesto 6);
- 25 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-T-Hyp-NH₂ (Compuesto 7);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-T-Hyp-NH₂ (Compuesto 8);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSH-T-Hyp-NH₂ (Compuesto 9);
- 30 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSH-T-Hyp-NH₂ (Compuesto 10);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVG-Orn-T-Hyp-NH₂ (Compuesto 11);
- 35 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVG-Aad-T-Hyp-NH₂ (Compuesto 12);

- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp-NH₂ (Compuesto 13);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLHRF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 14);
- 5 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 15);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 16);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLANFLVRSSF-Gly(Me)-A-Ile(Me)-
- 10 LSSTEVGSNTP-NH₂ (Compuesto 17);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 18);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLKRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 19);
- 15 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLHRVSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 20);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLHRVSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 21);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-
- 20 LSSTEVGSETP-NH₂ (Compuesto 22);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATDRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSDTP-NH₂ (Compuesto 23);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 24);
- 25 [19CD]-isoGlu-R-C()-PTAT-C()-ATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSNTP-NH₂ (Compuesto 25);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSETP-NH₂ (Compuesto 26);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATDRLAHFLHRSSF-Gly(Me)-A-Ile(Me)-
- 30 LSSTDVGSDTP-NH₂ (Compuesto 27);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 28);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 29);
- 35 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 30);

- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 31);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp-NH₂ (Compuesto 32);
- 5 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp-NH₂ (Compuesto 33);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP-NH₂ (Compuesto 34);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
- 10 LSSTEVGSSSTP-NH₂ (Compuesto 35);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP-NH₂ (Compuesto 36);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP-NH₂ (Compuesto 37);
- 15 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLVRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP-NH₂ (Compuesto 38);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFTP-NH₂ (Compuesto 39);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-
- 20 LSSTEVGSSFTP-NH₂ (Compuesto 40);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 41);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLADFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSFT-Hyp-NH₂ (Compuesto 42);
- 25 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 43);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLA-Aad-FLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Orn-T-Hyp-NH₂ (Compuesto 44);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-
- 30 LSSTEVGSSST-Apr-NH₂ (Compuesto 45);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSSTP-NH₂ (Compuesto 46);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSS-Orn-TP-NH₂ (Compuesto 47);
- 35 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp-NH₂ (Compuesto 48);

- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 49);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFL-Aad-RSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 50);
- 5 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVG-S-Orn-T-Hyp-NH₂ (Compuesto 51);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp-NH₂ (Compuesto 52);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVG-S-Aad-T-Hyp-NH₂ (Compuesto 53);
- 10 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 54);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 55);
- 15 [19CD]-isoGlu-R-C()-NTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 56);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 57);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 58);
- 20 [19CD]-isoGlu-R-C()-PTAT-C()-ATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTDVGSN-T-Hyp-NH₂ (Compuesto 59);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSDT-Hyp-NH₂ (Compuesto 60);
- 25 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 61);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 62);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 63);
- 30 [19CD]-isoGlu-R-C()-PTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSN-T-Hyp-NH₂ (Compuesto 64);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 65);
- 35 [19CD]-isoGlu-R-C()-NTAT-C()-ATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 66);

- [19CD]-isoGlu-R-C()-PTAT-C()-ATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 67);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATERLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 68);
- 5 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 69);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSST-Hyp-NH₂ (Compuesto 70);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
- 10 LSSTEVGSGT-Hyp-NH₂ (Compuesto 71);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp-NH₂ (Compuesto 72);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp-NH₂ (Compuesto 73);
- 15 [19CD]-isoGlu-R-C()-PTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp-NH₂ (Compuesto 74);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSAad-T-Hyp-NH₂ (Compuesto 75);
- [19CD]-isoGlu-R-C()-PTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-
- 20 LSSTEVGSAad-T-Hyp-NH₂ (Compuesto 76);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 77);
- [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 78);
- 25 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp-NH₂ (Compuesto 79);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-NH₂ (Compuesto 80);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-
- 30 LSSTEVGSGT-Hyp-NH₂ (Compuesto 81);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTTP-NH₂ (Compuesto 82);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSTT-Hyp-NH₂ (Compuesto 83);
- 35 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLERSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSTP-NH₂ (Compuesto 84);

- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSTP-NH₂ (Compuesto 85);
- [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 86);
- 5 [19CD]-isoGlu-R-C()-NTAT-C()-ATQRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSSH-Hyp-NH₂ (Compuesto 87);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 88);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATDRLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 89);
- 10 [19CD]-isoGlu-R-C()-QTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGH-Hyp-NH₂ (Compuesto 90);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGH-Hyp-NH₂ (Compuesto 91);
- 15 [19CD]-isoGlu-R-C()-QTAT-C()-ATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 92);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 93);
- [19CD]-isoGlu-R-C()-QTAT-C()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGTP-NH₂ (Compuesto 94);
- 20 [19CD]-isoGlu-R-C()-QTAT-C()-ATDRLA-Aad-FLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 95); y
- [19CD]-isoGlu-R-C()-QTAT-C()-ATERLAHFLQRF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 96);
- 25 en donde los paréntesis "(" indican puentes de disulfuro intramoleculares entre las cadenas laterales de los residuos de cisteína presentes en las posiciones 2 y 7.

5. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:

- 30 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLNRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 6).

6. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:

- 35 [19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNH-Hyp-NH₂ (Compuesto 7).

7. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLTRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSGT-Hyp-NH₂ (Compuesto 13).
- 5
8. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-GTAT-C()-ATERLAHFLHRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-NH₂ (Compuesto 15).
- 10
9. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-NTAT-C()-ATQRLADFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 49).
- 15
10. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-GTAT-C()-ATERLA-Aad-FLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 55).
- 20
11. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-NTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 56).
- 25
12. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-QTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSNTP-Hyp-NH₂ (Compuesto 57).
- 30
13. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:
[19CD]-isoGlu-R-C()-NTAT-C()-ATERLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGSDT-Hyp-NH₂ (Compuesto 60).
- 35
14. Un análogo de amilina de conformidad con la Reivindicación 4, que es el compuesto:

[19CD]-isoGlu-R-C()-QTAT-C()-ATDRLAHFLQRSSF-Gly(Me)-A-Ile(Me)-LSSTEVGST-Hyp-NH₂ (Compuesto 88).

15. Una composición farmacéutica que comprende un análogo de amilina de
5 conformidad con una cualquiera de las Reivindicaciones anteriores, en
combinación con un portador, un excipiente o vehículo farmacéuticamente
aceptables.
16. Una sal farmacéuticamente aceptable de un análogo de amilina según se
10 define en una cualquiera de las Reivindicaciones 1 a 4 o de un compuesto según
se define en una cualquiera de las Reivindicaciones 5 a 14.
17. Una composición farmacéutica que comprende una sal farmacéuticamente
aceptable según se define en la Reivindicación 16, en combinación con un
15 portador, un excipiente o vehículo farmacéuticamente aceptables.

Design of a mimic of nonamyloidogenic and bioactive human islet amyloid polypeptide (IAPP) as nanomolar affinity inhibitor of IAPP cytotoxic fibrillogenesis

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Protein aggregation into cytotoxic oligomers and fibrils *in vivo* is linked to cell degeneration and the pathogenesis of >25 incurable diseases, whereas the high aggregation propensity and insolubility of several bioactive polypeptides and proteins *in vitro* prevent their therapeutic use. Aggregation of human islet amyloid polypeptide (IAPP) into pancreatic amyloid is strongly associated with the pathogenesis of type II diabetes. IAPP is a 37-residue polypeptide that acts as a neuroendocrine regulator of glucose homeostasis. However, IAPP misfolds and self-associates into cytotoxic aggregates and fibrils even at nanomolar concentrations. Because IAPP aggregation causes β -cell death and prohibits therapeutic application of IAPP in diabetes, we pursued a minimalistic chemical design approach to generate a molecular mimic of a nonamyloidogenic and bioactive IAPP conformation that would still be able to associate with IAPP and thus inhibit its fibrillogenesis and cytotoxicity. We show that the double N-methylated full length IAPP analog [(N-Me)G24, (N-Me)I26]-IAPP (IAPP-GI) is a highly soluble, nonamyloidogenic, and noncytotoxic IAPP molecular mimic and an IAPP receptor agonist. Moreover, IAPP-GI binds IAPP with low nanomolar affinity and completely blocks IAPP cytotoxic self-assembly and fibrillogenesis with activity in the low nanomolar concentration range. Importantly, IAPP-GI dissociates cytotoxic IAPP oligomers and fibrils and is able to reverse their cytotoxicity. Bifunctional soluble IAPP mimics that combine bioactivity with the ability to block and reverse IAPP cytotoxic self-assembly are promising candidates for the treatment of diabetes. Moreover, our amyloid disease inhibitor design concept may be applicable to other protein aggregation diseases.

amyloidogenesis inhibitor | chemical design | protein aggregation | diabetes | therapeutic compound

Folding, misfolding, and the aggregation and fibrillogenesis propensities of proteins or polypeptides appear to be intrinsic properties of the amino acid sequence (1). An increasing body of evidence suggests that aggregates that form during *in vivo* self-assembly and amyloidogenesis are cytotoxic. This offers a link between protein/polypeptide aggregation, cell degeneration, and the pathogenesis of a number of diseases called protein aggregation diseases (1, 2). In addition, the high aggregation and fibrillogenesis propensities of several bioactive polypeptides and proteins *in vitro* cause a major problem in their pharmaceutical use as therapeutics (3).

Compounds that block cytotoxic protein/polypeptide self-assembly and amyloidogenesis are therefore important targets of therapeutic intervention in disease (2, 4). However, the design of such compounds is a very demanding task (2, 4, 5). Short self-recognition regions of amyloidogenic polypeptides have been applied as scaffolds for low-molecular-weight peptidomimetics to disrupt amyloidogenesis (6–9). Although a number of such compounds have been generated, highly potent and pharmacologically tested agents are not yet available (2, 4–6). A potential hurdle in their development is to overcome the high

affinity and cooperativity of the interactions of cytotoxic self-assembly of the full length amyloidogenic sequences (2, 4, 5).

The extremely high propensity of the pancreatic polypeptide human islet amyloid polypeptide (IAPP) to misfold into cytotoxic aggregates and fibrils is strongly associated with β -cell degeneration in type II diabetes (10, 11). IAPP is a 37-residue polypeptide secreted by the β -cells that acts together with insulin as a regulator of glucose homeostasis (12, 13). Clinical studies suggest that the use of IAPP or IAPP receptor agonists as glucose regulators might be beneficial in diabetes treatment (13). However, low solubility (11, 13), high fibrillogenesis propensity, and the cytotoxicity of misfolded and aggregated IAPP preclude its pharmacological use (12, 13). IAPP is a conformationally flexible polypeptide that misfolds into cytotoxic β -sheet aggregates and fibrils via a multistep nucleation-dependent process (11, 14, 15). Soluble IAPP analogs that combine bioactivity with the ability to inhibit cytotoxic self-assembly of native IAPP are therefore of high biomedical interest. Such “bifunctional” analogs would be promising candidates for therapeutic application in diabetes (13) and tools for understanding IAPP amyloidogenesis and cytotoxicity.

Here, we use a minimalistic conformational restriction strategy to design a full length IAPP-derived molecular mimic of a nonamyloidogenic and bioactive IAPP conformation. According to our design concept, the mimic should have nearly the same sequence as IAPP but should be soluble, nonamyloidogenic, and noncytotoxic and would ideally exhibit agonistic activity. At the same time, the mimic should be able to bind IAPP and inhibit its cytotoxic amyloidogenesis process. Approaches to changing the biophysical properties of self-assembling proteins or polypeptides include redesigning self-recognition interfaces (16, 17). Our approach features the structure-based introduction of a minimum number of two N-methyl rests on the same side of the β -strand in the IAPP “amyloid core” sequence IAPP [22–27] or NFGAIL of full length IAPP (Fig. 1A) (9, 18). Constraining the conformation of a conformationally flexible and bioactive polypeptide is a known strategy to both modulate its biophysical properties and generate high-affinity agonists of biological function (17, 19). Further, amide bond N-methylation is a minimally invasive method toward inhibiting peptide self-assembly in β -sheets (17, 20).

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: IAPP, human islet amyloid polypeptide; IAPP-GI, [(N-Me)G24, (N-Me)I26]-IAPP; ThT, thioflavin T; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TEM, transmission EM; Fluos-IAPP, N^α-amino-terminal fluorescein-labeled IAPP; Fluos-IAPP-GI, N^α-amino-terminal fluorescein-labeled IAPP-GI; RIN 5fm, cultured pancreatic rat insulinoma cells; Dig-IAPP-GI, amino-terminal digoxigenin-labeled IAPP-GI; Biotin-IAPP, N^α-amino-terminal biotin-labeled IAPP.

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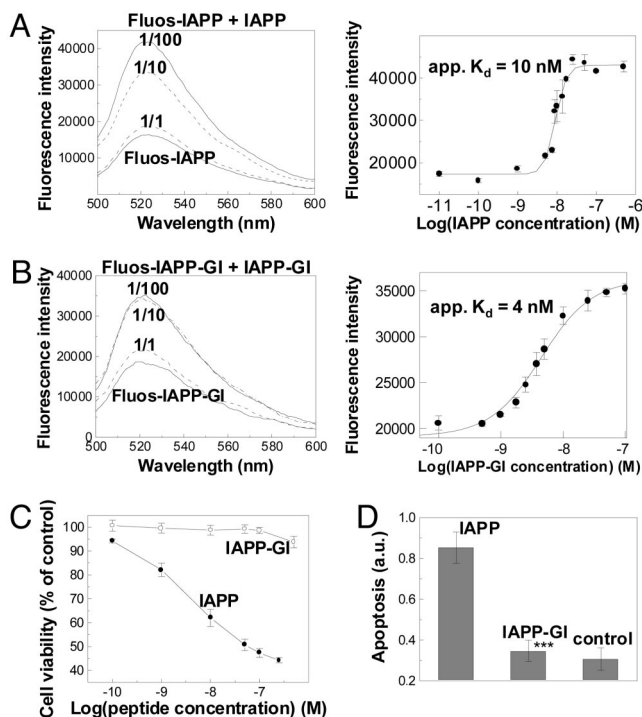


Fig. 3. Self-assembly and effects on β -cell viability of IAPP-GI and IAPP. (A and B) Self-association of IAPP (A) and IAPP-GI (B) studied by fluorescence spectroscopy: (A) Spectra of Fluos-IAPP (1 nM) alone and with IAPP (*Left*) and the fluorescence of Fluos-IAPP at 522 nm after titration with IAPP (*Right*). (B) Spectra of Fluos-IAPP-GI (1 nM) alone and with IAPP-GI (*Left*) and the fluorescence of Fluos-IAPP-GI at 522 nm after titration with IAPP-GI (*Right*). Fluos-IAPP/ligand or Fluos-IAPP-GI/ligand molar ratios are shown. Data are means ($\pm$ SEM) from three binding curves. (C and D) Effects of IAPP and IAPP-GI on β -cell viability. (C) MTT reduction assay. Data are means $\pm$ SEM of three to seven assays ($n = 4$ each). (D) Apoptosis assay. Data are means ($\pm$ SEM) of six determinations (***, $P < 0.001$ by ANOVA).

absence of fibrils (Fig. 2D *Insets*). These findings indicated that IAPP-GI was constrained in a nonamyloidogenic conformation.

IAPP-GI did have a pronounced self-association propensity. This was shown by TEM, size exclusion chromatography (SEC), and far-UV CD concentration dependence studies (not shown). TEM of IAPP-GI solutions (5–100 μM) revealed soluble spheroidal aggregates of different sizes (diameters up to 100 nm) (Fig. 2 *B* and *D*). SEC indicated that both IAPP-GI and IAPP formed monomers and dimers, when freshly dissolved (6.25 μM) in aqueous buffer, pH 7.4 (Fig. 7 *A* and *B*, which is published as supporting information on the PNAS web site). Kinetic follow-up of peptide association indicated that IAPP further associated into tetramers, various different oligomers, and large insoluble aggregates (Fig. 7*B*). IAPP-GI also formed soluble oligomers but no insoluble aggregates (Fig. 7*A*).

To quantify affinities of self-association, we used fluorescence spectroscopy (24). N α -amino-terminal fluorescein-labeled IAPP-GI (Fluos-IAPP-GI) and IAPP (Fluos-IAPP) were titrated at 1 nM with IAPP-GI and IAPP, respectively, in aqueous buffer, pH 7.4, and fluorescence emission spectra were recorded (9). Binding of IAPP to Fluos-IAPP and of IAPP-GI to Fluos-IAPP-GI at a 100-fold molar excess resulted in fluorescence enhancement of 260% and 190%, respectively (Fig. 3 *A* and *B*). Sigmoidal titration curves were obtained and were consistent with cooperative self-assembly processes (Fig. 3 *A* and *B*). The determined apparent affinities (app. K_d) of interactions were 9.7 (± 0.9) nM for Fluos-IAPP-IAPP and 4.2 (± 0.8) nM for Fluos-

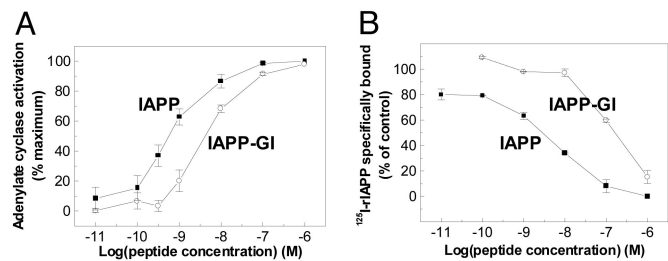


Fig. 4. Human IAPP receptor activation and binding potentials of IAPP-GI and IAPP in MCF-7 cells. (A) Stimulation of adenylate cyclase after incubation with IAPP or IAPP-GI. Adenylate cyclase activity above basal levels is plotted as a function of peptide concentration. Results are means ($\pm$ SEM) of three assays ($n = 2$ each). (B) Receptor-binding affinities as assessed by competitive inhibition of the specific receptor binding of ^{125} I-rat IAPP by IAPP and IAPP-GI. Specific radioligand binding is plotted as a function of IAPP or IAPP-GI concentration. Data are means ($\pm$ SEM) of two to three assays ($n = 2$ each).

IAPP-GI-IAPP-GI, demonstrating that both peptides self-associated with strong and nearly identical affinities.

We next studied the potential cytotoxic properties of IAPP-GI as compared with IAPP. Peptides were applied to cultured pancreatic rat insulinoma cells (RIN 5fm), and cell viability was determined by two different assays: first, by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay (Fig. 3C) and secondly by assessment of apoptosis (Fig. 3D) (11). Both assays showed that, whereas IAPP was strongly cytotoxic and caused apoptotic cell death in β -cells, IAPP-GI was completely devoid of any cytotoxicity (Fig. 3 C and D).

IAPP-GI Is a Full IAPP Receptor Agonist. IAPP exhibits several of its biological actions via binding to G protein-coupled receptors and activation of adenylate cyclase (12, 25, 26). To evaluate the receptor agonistic potency of IAPP-GI, we studied adenylate cyclase activation of high-affinity human IAPP receptors expressed on the human breast carcinoma cell line MCF-7 (25). Various concentrations of IAPP-GI or IAPP were incubated with the cells and generated cAMP was quantified. IAPP-GI exhibited the same maximum effect on adenylate cyclase stimulation as IAPP, which was consistent with IAPP-GI being a full agonist of the human IAPP receptor (Fig. 4A). However, the adenylate cyclase activation isotherms showed that IAPP-GI was a 7-fold less potent agonist than IAPP (IAPP, EC_{50} 631 pM; IAPP-GI, EC_{50} 4.4 nM). To determine the receptor-binding affinity of IAPP-GI, we next studied competitive inhibition of the specific receptor binding of radioactively labeled rat IAPP, the strongest ligand known, by IAPP-GI and IAPP on MCF-7 cells (Fig. 4B) (25). The binding isotherms showed that, whereas IAPP-GI was a potent IAPP receptor ligand, its receptor-binding affinity was significantly decreased as compared with IAPP (IAPP, IC_{50} 2.3 nM; IAPP-GI, IC_{50} 158.5 nM). Together, the above results showed that IAPP-GI is a full receptor agonist, albeit weaker than IAPP.

IAPP-GI Is a Nanomolar Affinity IAPP Ligand. To address the question whether IAPP-GI is able to bind IAPP, we performed a pull-down assay in combination with NuPAGE gel electrophoresis and Western blotting (27) (Fig. 5A). Amino-terminal digoxigenin-labeled IAPP-GI (Dig-IAPP-GI) (2.5 μ M) was incubated (1 h) with N $^{\alpha}$ -amino-terminal biotin-labeled IAPP (Biotin-IAPP) (2.5 μ M), and complexes were isolated by capturing Biotin-IAPP on streptavidin-coated beads. After dissociation, components were revealed by NuPAGE and Western blotting with antibiotin and antidigoxigenin antibodies (Fig. 5A). The antidigoxigenin

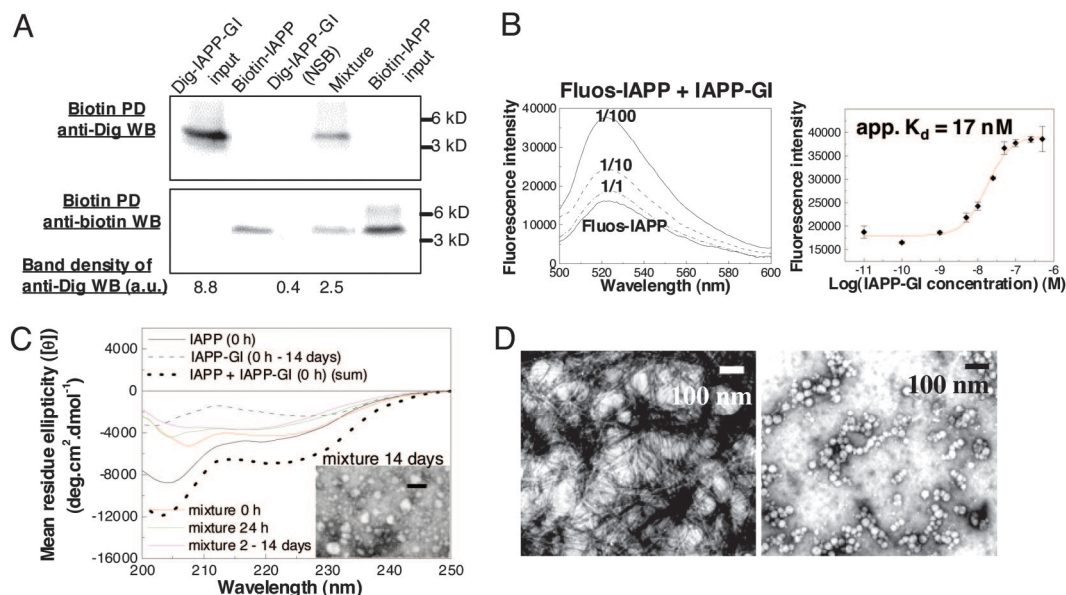


Fig. 5. Binding of IAPP-GI to IAPP and inhibition of IAPP misfolding and fibrillogenesis. (A) Binding of Dig-IAPP-GI to Biotin-IAPP assessed by a biotin pull-down assay. (Upper) Antidioxigenin Western blot analysis of a mixture of Dig-IAPP-GI with Biotin-IAPP (2.5 μ M) and suitable controls after pull-down and peptide dissociation. Lane Dig-IAPP-GI input: input (100%) [freshly dissolved (4 μ g) not incubated with beads]; lane Biotin-IAPP: Biotin-IAPP alone; lane Dig-IAPP-GI: Dig-IAPP-GI alone [nonspecific binding (NSB) of Dig-IAPP-GI for lane "mixture"]; lane Mixture, mixture of Dig-IAPP-GI and Biotin-IAPP-GI; lane Biotin-IAPP input: Biotin-IAPP input (100%, 4 μ g). (Lower) Antibiotin Western blot analysis of the same mixture as in Upper. Lanes are as in Upper. The band densities of the antidioxigenin western are also shown. NSB of Dig-IAPP-GI was 4% of input. (B) Binding of IAPP to IAPP-GI as assessed by fluorescence spectroscopy: Spectra of Fluos-IAPP (1 nM) alone and after titration with IAPP-GI (Fluos-IAPP/ligand molar ratios as indicated) are shown (Left). (Right) Fluorescence at 522 nm of Fluos-IAPP after titration with IAPP-GI. Data are means ($\pm$ SEM) from three binding curves. (C) Interaction of IAPP with IAPP-GI as followed by CD and TEM: The conformation of a mixture of IAPP and IAPP-GI (5 μ M each) was followed by CD over 14 days. CD spectra at various time points up to 14 days are shown. (Inset) TEM at 14 days (Scale bar, 100 nm). In parallel, CD spectra of IAPP and IAPP-GI incubations (5 μ M) were measured (Fig. 2D). These spectra (0 h) and their sum are also shown. (D) TEM of IAPP (Left) and the mixture of IAPP with IAPP-GI (1/1) (at 24 h) (incubation as in C) (Right).

Western blot showed the presence of significant amounts of Dig-IAPP-GI, demonstrating that IAPP-GI binds IAPP.

The interaction of IAPP-GI with IAPP was then characterized by fluorescence spectroscopy. Fluos-IAPP (1 nM) was titrated in aqueous buffer, pH 7.4, with IAPP-GI. Binding of a 100-fold molar excess of IAPP-GI resulted in a 240% increase of fluorescence emission (Fig. 5B). A sigmoidal binding curve was obtained and an app. K_d of 17.5 nM ($\pm$ 3.2) was determined (Fig. 5B). Of note, a similar affinity was obtained when Fluos-IAPP-GI (1 nM) was titrated with IAPP [app. K_d = 8.9 nM ($\pm$ 1.5)] (not shown). A directly proportional concentration dependence was found for the fluorescence of Fluos-IAPP within 1–4 nM (9). This finding and the 10 nM affinity of Fluos-IAPP - IAPP assemblies were consistent with Fluos-IAPP being mainly monomeric at 1 nM. We also titrated a 10 nM Fluos-IAPP solution, where both Fluos-IAPP monomers and oligomers were present, with IAPP-GI or IAPP (not shown). The app. K_d were in the same concentration range as above. These findings suggested that IAPP-GI bound IAPP monomers and oligomers with very low nanomolar affinity and showed that the affinity of the IAPP-GI-IAPP interaction is very similar to the affinity of IAPP self-association.

IAPP-GI Inhibits IAPP Misfolding into β -Sheets, Fibrillogenesis, and Cytotoxicity. To examine whether interaction of IAPP-GI with IAPP could inhibit IAPP misfolding and self-assembly into soluble β -sheets and fibrils, we next used far-UV CD spectroscopy and TEM. IAPP (5 μ M, pH 7.4) was incubated alone or in the presence of IAPP-GI (5 μ M), and CD spectra were measured at various time points (Figs. 2D and 5C). Aliquots were subjected to TEM, whereas UV spectra were measured to control for peptide loss because of insolubilization. IAPP aggregated into soluble β -sheets and insoluble fibrillar aggregates

precipitated at 2.5 h (Figs. 2D and 5D Left). In the presence of IAPP-GI, however, no insolubilization of IAPP occurred, and the spectrum did not change over 14 days (Fig. 5C). The spectrum of the mixture differed markedly from the sum of the spectra of IAPP (at 0 h) and IAPP-GI, which indicated that IAPP-GI had interacted with IAPP. TEM between 24 h and 14 days showed soluble round oligomers and chain-like assemblies of spherical oligomers (Fig. 5D Right), but no fibrillar aggregates were detected (Fig. 5C Inset).

IAPP fibrillization in the absence or presence of IAPP-GI was next followed by the ThT assay (Fig. 6A). Conversion of IAPP (6.25 μ M, pH 7.4) into fibrils was accomplished within 2–4 h. In the presence of IAPP-GI (1/1), however, fibril formation was completely suppressed (14 days) (Fig. 6A).

Next, IAPP or 1/1 mixtures of IAPP with IAPP-GI were added to RIN 5fm cells, and cytotoxicities were assessed by MTT reduction (Fig. 6B) and by quantification of apoptotic cell death (Fig. 6C). IAPP-GI strongly suppressed IAPP-mediated apoptosis, and IAPP cytotoxicity was nearly completely inhibited over a 1,000-fold IAPP concentration range. Of note, rat IAPP, which is nonamyloidogenic and differs from IAPP in six residues, did not affect IAPP cytotoxicity (not shown). Titration of cytotoxic IAPP (100 nM) with IAPP-GI gave a sigmoidal titration curve, and a 1/1 molar ratio of IAPP-GI to IAPP caused maximal inhibition of cytotoxicity (Fig. 6C Inset). An IC_{50} of 20 nM was determined, which correlated very well with the 17 nM nanomolar affinity of the IAPP-GI-IAPP interaction.

IAPP-GI Redissolves Cytotoxic IAPP Aggregates and Fibrils and Reverses Their Cytotoxic Effects. We next asked whether IAPP-GI could block or reverse already-started IAPP fibrillogenesis processes. Using the ThT assay, we compared fibrillization kinetics of a solution of IAPP alone (16.5 μ M, pH 7.4) with those

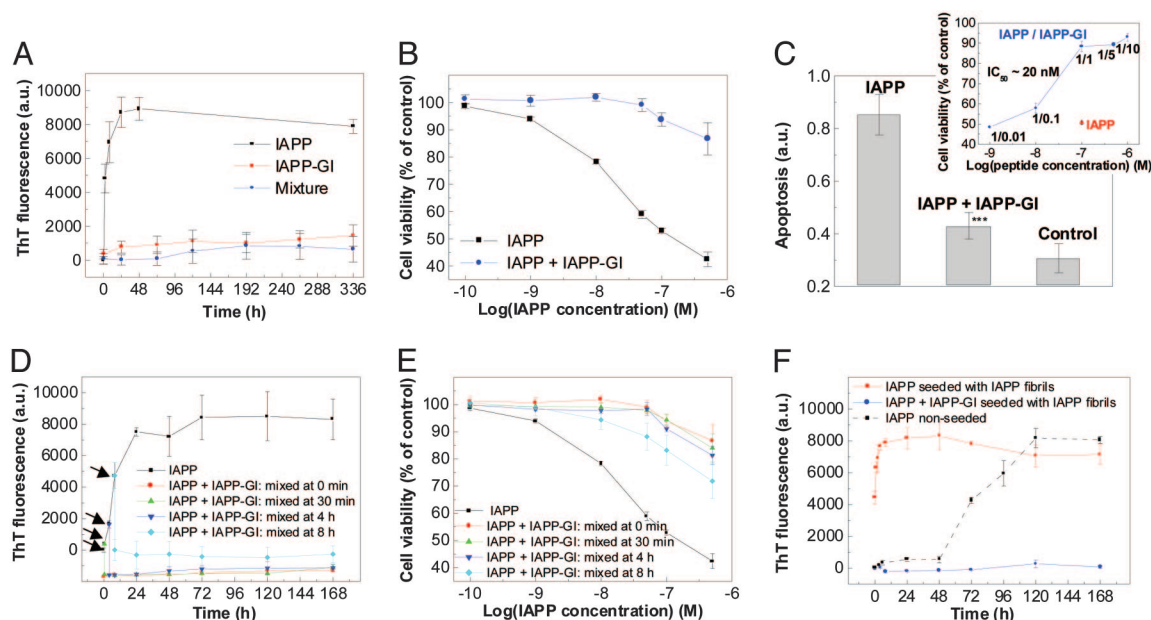


Fig. 6. Inhibition of IAPP fibrillogenesis and cytotoxicity, dissolution of preformed IAPP fibrils, and reversal of cytotoxic IAPP self-assembly and fibrillogenesis by IAPP-GI. (A) Fibrillogenesis of IAPP (6.25 μ M), a mixture of IAPP and IAPP-GI (6.25 μ M each), and IAPP-GI (6.25 μ M) alone as assessed by ThT binding. (B) Effects of IAPP (16.5 μ M) versus a mixture of IAPP and IAPP-GI (1/1) on β -cell viability as assessed by MTT reduction. Data are means ($\pm$ SEM) of three assays ($n = 3$ each). (C) Inhibition of IAPP (500 nM) mediated β -cell apoptosis by IAPP-GI and determination of the IC_{50} of the inhibitory effect of IAPP-GI on IAPP cytotoxicity (Inset). Data are means ($\pm$ SEM) of six determinations (***, $P < 0.001$ by ANOVA). (Inset) Titration of cytotoxic effect of IAPP (100 nM) with IAPP-GI using the MTT assay. Data are means ($\pm$ SEM) of eight determinations. (D) Dissociation of IAPP fibrils by IAPP-GI as followed by ThT binding. IAPP (16.5 μ M) was allowed to aggregate. At the indicated time points, aliquots were mixed with IAPP-GI (1/1), and fibrillogenesis of IAPP alone versus the mixtures was quantified. Data are means ($\pm$ SEM) of three assays. (E) Reversal of cytotoxicity of IAPP oligomers and fibrils by IAPP-GI. Solutions of D were added to RIN 5fm cells (at 24 h). Cell viability was assessed by the MTT assay. Data are means ($\pm$ SEM) of three assays ($n = 3$ each). (F) Inhibition of the nucleation effect of IAPP fibrillar seeds on IAPP fibrillogenesis by IAPP-GI as followed by the ThT assay. IAPP (6.25 μ M) with or without IAPP-GI (1/1) was seeded with IAPP fibrils. Results are means ($\pm$ SEM) of three to four assays.

of an identical solution to which IAPP-GI (16.5 μ M) was added at various time points (Fig. 6D). IAPP-GI completely interrupted IAPP fibrillization (at 1/1) regardless of the stage of the process, i.e., when added both before and after nucleation. Most importantly, IAPP-GI (1/1) redissolved IAPP fibrils and reversed already-started fibrillogenesis (Fig. 6D).

To address whether IAPP-GI-mediated dissolution of fibrils resulted in reversal of cytotoxicity, solutions of the ThT assays were applied to RIN 5fm cells (at 24 h), and cell viability was assessed (Fig. 6E). In fact, IAPP-GI was able to nearly quantitatively reverse the cytotoxic effects of already-formed IAPP aggregates and fibrils (Fig. 6E). Of note, the same cytotoxic effect of the IAPP-alone incubations was obtained when IAPP was incubated for 4–24 h before addition to the cells (Fig. 6F), whereas cytotoxicity was somewhat reduced in the 0- or 30-min incubations (not shown).

We also tested whether IAPP-GI was able to inhibit the nucleating effect of exogenously added fibrillar seeds on IAPP fibrillogenesis. Under assay conditions where IAPP fibrillogenesis (6.25 μ M) began after a lag of 48 h (9), seeding with preformed IAPP fibrils (10%) resulted in an immediate beginning of fibrillogenesis (Fig. 6F). In the presence of IAPP-GI (1/1), however, fibril formation was completely suppressed (Fig. 6F).

Discussion

We have designed a molecular mimic of a nonamyloidogenic and bioactive IAPP conformation. The mimic, IAPP-GI, is a 37-residue polypeptide that exhibits nearly the same amino acid sequence as the extremely amyloidogenic and cytotoxic polypeptide IAPP. However, IAPP-GI is at least 100-fold more soluble and less amyloidogenic than IAPP under physiological pH and has no cytotoxic properties. Our results provide evidence that

the gain-of-toxic activity of IAPP is directly linked to its ability to misfold and self-assemble in β -sheet aggregates and fibrils (1, 2, 11).

Reported inhibitors of IAPP fibrillogenesis so far include aromatic compounds and short peptides active in the micromolar or submicromolar concentration range, at best (7–9, 28). These inhibitors can merely delay or suppress amyloidogenicity and cytotoxicity and are unable to block or reverse IAPP cytotoxic self-assembly after nucleation. For example, the hexapeptide NF(N-Me)GA(N-Me)IL, a recently described submicromolar affinity IAPP ligand, exhibits its strongest inhibitory effects in the micromolar concentration range, is unable to completely block IAPP fibrillogenesis and cytotoxicity, and does not block or reverse cytotoxic self-assembly after nucleation (9). By contrast, IAPP-GI binds IAPP with a $\approx$ 10-fold higher affinity than NF(N-Me)GA(N-Me)IL, completely blocks IAPP cytotoxic misfolding and self-association with an IC_{50} of 20 nM both in pre- and postnucleation phase, and redissolves cytotoxic IAPP aggregates and fibrils. Because treatment of disease usually starts after the disease begins, these properties are very important with regard to a potential therapeutic use.

Our studies suggest that the high inhibitory potency of IAPP-GI might be mediated by the high affinity binding of IAPP-GI, (i) to IAPP monomers, their kinetic stabilization toward cytotoxic misfolding and self-assembly or their sequestering from the amyloid pathway and (ii) to IAPP fibrillogenesis intermediates and their sequestration from further fibrillogenesis. IAPP-GI binding to nontoxic IAPP monomers would shift self-assembly to the left and might underlie dissociation and reversal of cytotoxicity of oligomers and fibrils (2) (Fig. 1B).

Conclusion

IAPP-GI is a designed low nanomolar affinity IAPP ligand. Furthermore, IAPP-GI is the only known compound that com-

pletely blocks with low nanomolar activity and reverses IAPP cytotoxic self-assembly and fibrillogenesis. Finally, IAPP-GI is the only known IAPP amyloidogenesis inhibitor that is at the same time a soluble at physiological pH, nonamyloidogenic and noncytotoxic, full length IAPP analog and an IAPP receptor agonist. For comparison, the best-known soluble IAPP receptor agonist, a triproline analog of full length IAPP ([P25,P28,P29]-IAPP), precipitates above pH 5.5, and no amyloid-inhibitory properties have been reported (13). In addition, because large parts of the IAPP sequence are required for receptor binding and activation, other reported inhibitors would be devoid of receptor agonistic properties (7–9, 13, 28). These points demonstrate the efficiency of our amyloid inhibitor design strategy and underline the so-far-unique bifunctional nature of IAPP-GI, i.e., its function as both a highly potent IAPP amyloid inhibitor and a soluble IAPP receptor agonist. Bifunctional IAPP mimics, such as IAPP-GI, could therefore be promising drug candidates for the treatment of diabetes (13).

Our results offer a proof of principle of a concept for designing potent amyloid disease therapeutics and of a chemical engineering approach to redesign a natively amyloidogenic and bioactive polypeptide sequence into a soluble, noncytotoxic, bioactive, and highly potent inhibitor of its own cytotoxic self-assembly. Therefore, our amyloid inhibitor design approach may be applicable to other disease-related self-associating polypeptides (3, 4, 29).

Materials and Methods

Peptides. Synthetic IAPP (human and rat sequences) were obtained from Calbiochem, dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at 200–500 μ M, and filtered (human sequence) over 0.2- μ m filters (Millipore). Concentrations of stock solutions were determined by UV (9, 14). [^{125} I]Bolton/Hunter-labeled rat IAPP for receptor-binding studies was from Amersham Pharmacia Biosciences (25).

Peptide Synthesis. Peptides, including the mimic and the labeled analogs, were synthesized by using previously published fluorenylmethoxycarbonyl solid-phase synthetic protocols (9, 30) (see *Supporting Text*, which is published as supporting information on the PNAS web site).

Sedimentation Assays. Assays were performed as described in *Supporting Text*.

TEM. Aliquots of incubations {results shown in Figs. 2 and 5 are from incubations in 10 mM phosphate buffer [1% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP)], pH 7.4} were applied on carbon-coated grids, stained with uranyl acetate as described (14), and examined with a Philips (Eindhoven, The Netherlands) EM 400 T electron microscope at 60 kV.

ThT-Binding Assays. Assays were performed by using a recently published assay system (9) (see *Supporting Text*).

Far-UV CD Spectroscopy. CD spectra were measured as described in *Supporting Text*.

Size Exclusion Chromatography (SEC). SEC was performed with a TSK-GEL G2000SW_{XL} (TosoHaas, Montgomeryville, PA) (300 $\times$ 7.8 mm), as described in *Supporting Text*.

Adenylate Cyclase Activation and Receptor-Binding Assays. Cell culture (MCF-7 cells) and assays were performed as described in *Supporting Text*.

Pull-Down Assays. Assays were performed as described in *Supporting Text* (27).

Fluorescence Spectroscopic Titration Studies. Measurements were performed with a Spex Fluorolog 2 fluorescence spectrophotometer, as described (9, 31) (see *Supporting Text*).

Assessment of Cytotoxicity via the MTT Reduction Assay. The rat insulinoma cell line RIN 5fm was used and cells were plated as described (9, 22) (see *Supporting Text*).

Assessment of Apoptosis via ELISA. RIN 5fm cells were plated at a density of 5×10^5 cells/ml, and the assay was performed as described (9) (see *Supporting Text*).

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Patente PCT
ANÁLOGOS DE AMILINA

Detalle del pago

Id.	Rentístico	Concepto y Servicio	Cant.	Val. Unit.	Subtotal
1	50005-01-02	Pago unitario reivindicacion adicional patente de invencion (A partir de de undí½cima reivindicacion) (DESCUENTO INTERNET)	2	\$ 40,500	\$ 81,000
TOTAL					\$ 81,000