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SUPERINTENDENCIA DE I NDUSTRIAY COMERCIO

No. 01-0188 7- -00009-0000
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Seæora Tra. 2 PATENTES Eve: I REODEPOSITO

i Act. 444 RESPUESTAREQUE Folios: 27
JEFE DE DIVISION DE NUEVAS CRECIONE
SUPERINTENDENCIA DE INDUSTRIA V COMERCIO
E. S. D.

REF: Expediente No. 01 018 857 - soilcitud do patente do invención a favor de BRISTOL-MYERS SQUIBB COMPANY

Respuesta a oflcio No. 3431 del 15 de marzo de 2007

JUAN PABLO CADENA SARMIENTO, portador de Is tarjeta profesional No. 67.492 del C.S.J., obrando como apoderado do Is sociedad BRISTOL-MYERS SQUIBB COMPANY segIn to acredita el docurnento do poder anexo me permito dar respuesta a las observaciones do carÆcter tØcnico y juridico, relacionadas con Is patentabilidad, para Is concesión de Is patente do acuerdo con la Decision 486
segn el concepto tØcnico No. 0398 notlficado fijaciOn en lista do fecha 15 de
.. .. marzo de 2007.

En atenciOn a las objeciones del seæor examinador, so presents un nuevo capitulo
- reivindicatorio anexo a la presente con I - 12 reMndicaciones, las cuales no adicionan materia nueva ni extienden el alcance de Is solicitud como so presentO originalmente, y reemplazan completamente el capItulo reivifldicatorio presentado anteriormente.

El nuevo capitulo reivindlcatorio subsaria las objecione8 presentadas por el seæor examinador-reiacionadas con Is claridad del mismo. El seæor exarninador objeta Ia excesiva amplitud do 10 divulgado par dicha anterioridad, Is nueva reMndicaciOn I subsana dicha objeción especificando la naturaleza do los sustituyentes respecto al nmero de carbonos o anillos presentes en dichos sustituyentes.

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Con esta correcciOn so supera tamblØn la objeción presentada por el seæor examinador relacionada con la extrema sonciUez de la formula I en relaciOn con el grado de complejidad quo podia alcarizar la molØcula at realizar las sustituciones mØs complejas posibles, ya que los sustituyentes ahora no pueden exceder un grado do complejidad definido.

En cuanto a la objeciOn del seæorexaminador relacionada con la falta de unidad de invención de la presente solicitud, originada do la infinidad de posible sustituyentes reivindicados en la reivindicaciOn 1, dicha objeciOn queda subsanada con la nueva reivindicaciOn 1 en la cual, como se dijo anteriormente, se realiza un delimitaciOn mÆs especifica delos sustituyentes propuestos.

En cuanto a las objeciones de altura inventiva, quislØramos hacer las siguientes observaciones. En primer lugar el seæor Examinador objeta la alturainventiva do la solicitud en base a lo divulgado por las anterioridades WO 99/47545 (Dl), US 6.011.155 (D2), DE 3.234.263 (D3) y EP 0007652 (D4).

De acuerdo con et seæor examinador la anterioridad Dl afecta la alturainventiva del grupo de invenciones que corresponde a los ’anfllos de cinco miembros condensados con un anillo de ciclopropano en (C), particularmente los de la anterior reivindicación 8, ahora renumerada como nueva reivindicaciOn 3. El compuesto de la anterioridad Dl tiene la formula.general:

0

RZ

N

R1" ’X N

de acuerdo a la misma anterioridad, Y puede ser:

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donde m es 0 o 1; la reivindicaciôn 2 de dicha anterioridad establece que Y puede

ser:

cuando forma un anillo con R6 y el Ætomo denitrôgeno al que se encuentran unidos, m es Un 0 o 1. La reivindicación 5 sugiere que Y es:

donde V3 cuando se selecciona apropiadamente de una lista de mÆs de 10 sustituyentes, puede ser:

Dado 10 anterior no es claro en que se basa el seæorexaminador para afirmar que la fOrmula estructural de Y:

en la reivindicaciôn 5 de la anterioridad Dl puede convertirse en:

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cuando dichas formulas corresponden a un sustituyente en Y, y no pueden ser Y de ninguna forma porque aun hay sustituyentes adicionales airededor. No as muy claro en que se basa el seæorexaminador para asegurar qua el tØcnico con habilidad en la materia puede derivar:

como el sustituyente resultante de la fusion de R1 y R2 como se divulga en la presente solicitud.,PorquØ razOn particular tomaria el tØcnico con habilidad en la materia esos dos sustituyentes particulares de Pa anterioridad de una lista qua comprende mÆsde 10, cuando dicha anterioridad no enseæao sugiere las ventajas de estos dos por sobre los demÆs? Asi mismo, al analizar cuidadosamente 10 divulgado por la anterioridad Dl, el compuesto cuya fOrmula estructural se acerca mÆs ala fOrmula de la presente solicitud as:

HN

qua corresponde a [as formulas obtenidas en el ejemplo 23. No es claro en qua se basa el seæor examinadorpara afirmar categOricamente qua asta fOrmula afecta la altura inventiva de los compuestos de fOrmula:

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o incluso, en donde divulga dicha anterioridad Dl las modificaciones necesarias, o las posibles ventajas de realizar las modificaciones necesarias, para pasar de esa fOrmula a la fOrmula:

cuando la fOrmula general divulgada en Dl es:

y ni las reivindicaciones ni Ia descripción enseæan 0 sugieren los sustituyentes necesarios para que X y R4 formen:

cuando segCin la misma anterioridad, este grupo corresponde a una parte de Y, y se encuentra ubicado en el extremo opuesto de la fOrmula. No hay enseæanza alguna en Dl que sugiera o motive al tØcnico con ablildad en (a materia a obtener los compuestos de (a presente solicitud, a menos que se haga uso del conocimiento adquirido de antemano en la presente solicitud. Las modificaciones necesarias para Ilegar de:

Ft2 Re
IN N

x H:1010

0
a

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no se divulgan ni se sugieren en Dl. De hecho, Dl no hace referenda alguna a la posibilidad de realizar dichos cambios que motive al tØcnico con habilidad en la materia, puesto que no hay una expectativa razonable de Øxito. Ni siquiera tomando la imagen especular de la formula de Dl Se puede Ilegar a la fOmiula de la presente solicitud a partir de lo ensetado par la anterioridad; y no es razonable suponer que los conocimientos ordinarios del tØcnico con habilidad en la materia y su actividad investigativa diana, Is permitirian ilegar de manera obvia a los cambios necesarios tanto en los sustituyentes coma en el proceso de obtenciOn. Niriguno de los ejemptos de (a anterioridad Dl sugieren un mØtodo para Hegar a los compuestos de la presente solicitud o a una molØcula que se aproxime, en su fOrmula estructural, a las molecules de La presents solicitud coma para que se pueda afirmar categOnicamente que de derivan de manera obvia los compuestos de la presents solicitud. El qua Dl enseæeuna molecule con un anillo de cinco miembros condensados con un anillo de ciclopropano no enseæa automØticamente la materia de la presents solicitud, a menos que se emplee el conocimiento adquirido de antemano de la tectura de la presente solicitud al estudiar el ante previo. Si este anØlisis fuera vØlido, cualquler sistema de un anillo de cinco miembros fusionado a un ciclopropano volverla obvia cualquier invenciOn que comprendiera un sistema similar, independiente de su aplicaciôni o ubicaciOn en (a molecule, o del proceso pare obtenier la misma.

AdemØs, los compuestos an Dl Se relacionan con inhibidores de caspaza, los cuales son completamente diferentes a los inhibidores de DP-4 divulgados en la presents solicitud. Una vez mâs, no es claro quØ en Dl motivanla at tØcnico con habilidad en la materia a tomar un inhibidor de caspaza para convertirlo en un inhibidor de DP-4. El examinador parece haber omitido este pequeæodetalle en su afØn injustificado por negar ía altura inventiva de ía solicitud, no proporciona argumentos que sustenten su punto de vista, y toda su objeciOn parece basarse en el hecho que la anterioridad divulga un anillo de cinco miembros fusionado con un anillo de ciclopropano. Esto es claramente una violación flagrante del derecho del peticioniario a que se Is concede una patente, puesto que este tipo de objeciones solo demuestran que el seæorexaminador no tiene intención alguna de conceder una patents claramente inventiva por sobre Ia divulgado en el ante previo, y esta dispuesto a emplear argumentos tanto dØbiles como vacios pare sustentan su opiniOn sesgada y su visiOn limitada de la solicitud y el ante previo.

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Mora, en cuanto a D2, el examinador argumenta que los compuestos de los ejemplos 28 y 29, para nombrar solo algunos, presentan analogias y similitudes qulmicas y funcionales con los compuestos de la presente invenciôn. De acuerdo con el seæor examinador lafOrmula:

de D2 es un anÆlogo qulmicofuncionalmente similar a:

de la presente solicitud; asi como tambiØn la fOrmula:

N)L%4.
es un anâlogo qulmico funcionalmente similar a:

de la presente solicitud. El tØcnico con habilidad ordinaria puede apreciar fÆcilmente que la Unica analogla existente entre los compuestos de D2 y los de la presente solicitud es su aplicaciOn como inhibidores de DP-4. Es fÆcil, para el tØcnicocon habilidad en la materia, darse cuenta que, en el caso del ejemplo 28 de Ia anterioridad D2, el anillo de 6 miembros en el extremo izquierdo de la formula se encuentra unido al Ætomode nitrOgeno por medio de un enlace C-N en tanto que la fOrmula de la presente solicitud es un anillo heterociclico de 5 miembros. No es claro, a partir de los escasos argumentos presentados por el seæor examinador,

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apreciar en que punto la anterioridad D2 enseæa o sugiere la posibilidad de transformer ese aniHo de ciclohexano en un anillo heterociclico de cinco miembros, menos aun como puede un tØcnico con habilidad Ordinaria en la materia, en su diario trasegar, Ilegar de manera obvia al compuesto de Ia presente solicitud. Un anÆlisis similarpuede realIzarse respecto al compuesto del ejemplo 29, donde ni siquiera tomando el estereoisOmero del compuesto se puede Hegar al compuesto de Is presente solicitud sin realizar modificaciones a la estructura que son de naturaleza compleja y no caen dentro del trajinar y la experimentación diana del tØcnico con habilidad en Ia materia, y que no son enseæados o sugeridos por la anterioridad.

La totalidad del argumento proporcionado por el seæor examinador parecebasarse en la misma teonia de la anterioridad Dl: son "similares", por 10 tanto es fÆcH pasar del uno al otro y, entonces, los de la presente solicitud carecen de altura inventive. El seæorexaminador simplemente tomó dos compuestos al azar en la anterioridad D2 y luego so puso a la tarea de encontrarles similitudes inexistentes con los de Is presente solicitud. Si un documento he do afectar la altura inventive, dicho documento debe proporcionar ideas quo, combinadas con los conocimientos del tØcnico con habilidad en Is materia, permitan Ilegar do manera obvia a los compuestos do la solicitud bajo estudio. En caso de falter lo anterior, debe haber algUn tipo do divulgaciôn en la anterioridad que le sugiera al tØcr,ico con habilidad ordinaria en la materia que realizar los cambios necesanios es viable y tiene posibilidades razonables do Øxito. Ninguno de los dos casos se da en Is anterioridad citada por el seæor examinadorquien, al parecer, nuevamente, en su afÆnde negar Is solicitud bajo estudio decidió ignorer do piano los principios bÆsicosde la qulmica organica y baser su argumento en quo son similares y es posible Ilegar de un compuesto al otro, por lo tanto los pnimeros afectan la altura inventive de los compuestos de la presente solicitud. Esto es un claro ejemplo del uso incorrecto del conocimiento adquirido do antemano por et seæor examinador al leer la presente solicitud, el anâlisis retrospectivo no es un anÆlisis vÆlido para el estudio de laaltura inventiva.

Por otro lado, el tØcnico con habilidad ordinania en la materia verla quo al sustituir la pirrolidona monocIctica do la anterioridad D2 con el sistema bicIclico de Is presente solicitud afectarla en gran medida la rigidez del sistema. El tØcnico con habilidad ordinania en Is materia sabe que los inhibidores actüan unlØridose a la enzima, impidiendo su funcionamiento normal. Dada la complejidad qulmica do las enzimas

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dicho tØcnico con habilidad ordinaria en la materia tambiØn sabe que cualquier modiflcaciôn que se le realice a un inhibidor puede anular 0 potenciar su actividad inhibidora. La anterioridad 02 no enseæa, en ningün aparte, las modificaciones riecesarias para Ilegar a los compuestos de (a presente solicitud, ni en la estructura
ni el procedimiento de obtención, ni mucho menos que tales sustituciones permitan obtener un compuesto que tambiØn actCia como inhibidor DP-4. Como se dijo arriba, se ha sustituido una pirrolidona moniciclica por un sistema biciclico, se ha alterado (a rigidez de [a estructura, y se ha modificado]a misma Jo suficiente como para asegurar que, si bien ambas son inhibidoras de DP-4, no se entazan de manera similar a la enzima. Dado 10 anterior, no es claro en que Se basa el seæor examinador para estabtecer categOricamente, por segunda vez consecutiva, qua los compuestos de la anterioridad D2 anticipan de manera obvia los compuestos de la presente solicitud.

Por Ciltimo, respecto a D3 y 04, la situación es la misma. Ninguna de las anterioridades citadas enseæa o sugiere compuestos inhibidores de DP-4. De hecho, la anterioridad 03 enseæacompuestos con aplicaciones en tratamientos de hipertensión e inhibidores de ACE, y 04 ertseæacompuestos intermedios utilizados en la formaciôn de compuestos similares a los de 03. De 10 anterior es mÆsque evidente que el seæor examinador ni siquiera se tomo la molestia de leer las anterioridades antes de volverlas a citar sosteniendo que afectan la altura inventiva de la solicitud. Ni siquiera Ilevando a cabo las sustituciones mÆs complejasen (a molØcula de la presente solicitud se pueden obtener los compuestos de las anterioridades, y viceversa. Nada en 03 0 04 enseæao sugiere que los compuestos allI obtenidos pueden ser utilizados coma inhibidores de DP-4, y ninguna de las enseæanzasde dichas anterioridades le permite al tØcnico con habilidad ordinaria en la materia derivar los compuestos de la presente solicitud,; principalmente porque, como se dice arriba, (a aplicación de los mismos es en campos diferentes, y el tØcnico con habilidad ordinaria en la materia sabe, con certeza, que un inhibidor diseæado especIficamente para inhibir enzimas ACE no puede ser utilizado para inhibir enzimas DP-4. Lo anterior es mÆsevidente aun cuando se pasa a considerar el campo de acciOn de cada enzima, las primeras se relacionan con el funcionamiento del sistema circulatorio central, en tanto que las segundas se enfocan en (a modulaciOn de los niveles de glucosa en la sangre.

Queda entonces claro que el seæor examinador simplemente se limitó a buscar documentos del arte previo qua, a su parecer, mostraban fOrmula que podrian

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"guiar" al "tØcnico con habilidad en la materia" a los compuestos de la presente solicitud. En el proceso de "estudiar" la solicitud, el seæor examinador paso por alto las teorias bÆsicasde la quimica orgÆnica, la inhibiciOn enzimÆticay la aplicaciOn del compuesto propuesto por la presente solicitud. De los 4 documentos citados por el seæor examinador solamente uno enseæa inhibidores DP-4, D2, y dicha anterioridad posse propiedades estructurales diferentes a las de la presente solicitud; propiedades que afectan la actividad inhibidora y determinan la forma como dicha molØcula Se une a la enzima que estÆ inhibiendo y la estabilidad de dicho enlace y del compuesto mismo al ser metabolizado. Los argumentos del seæor examinador son endebles, carentes de sustento tØcnico y, evidentemente, impulsados por un anÆlisis retrospectivo dela solicitud a la luz de 10 divulgado por el arte previo, sin consideraciOn alguna a to que dicho arte previo en reatidad estaba divulgando, menos aun a to que la presents solicitud estaba divulgando.

Al parecer es necesarlo hacer caer en cuenta at seæorexaminador que el anÆlisis retrospectivo de la altura inventiva no es apropiado. No basta con tomar las fOrmulas de la solicitud y buscarles "semejanzas" con documentos encontrados al azar en el arte previo. La altura inventiva exige quo el arts previo enseæe o sugiera mØtodosy composiciones que, at combinarse con los conocimientos del tØcnico con habilidad ordinaria en la materia, permitan derivar de manera obvia los compuestos de la solicitud en estudlo. Cabe entonces preguntarse iQuØ tØcnico con habilidad en Ia materia tomaria un inhibidor de caspaza para convertirlo en un inhibidor de DP-4? ,Que aparte de la anterioridad Dl le sugiere al tOcnico con habilidad ordinaria en la materia tal posibilidad? LEn que punto especIfico de las anterioridades D3 o D4 se motiva al tØcnico con habilidad ordinaria en la materia a tomar compuestos para el tratamiento de la hipertensiOn, modificarlos sustancialmente, y emplearlos como inhibidores DP-4? LEn que punto especifico de la antenoridad D2 se le sugiere al tØcnico con habilidad que el cambio de una pirrolidona monocIclica por un sistema bicIclico permite obtener un inhibidor DP-4? 40 que dicho inhibidor DP-4 va a unirse de manera mØs afectiva a la enzima?

Para corroborar las mejoras obtenidas por los compuestos de la presente solicitud en el campo de la inhibición de enzimas DP-4, se anexa un estudio realizado recientemente con los compuestos de la presente solicitud, donde el tØcnico con habilidad ordinaria en la materia puede apreciar claramente las ventajas, en estabilidad y potencia inhibitoria, que se pueden lograr con los compuestos de la presente solicitud.

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En vista de lo anterior, respetuosamente solicitamos el retiro de las objeciones a la presente solicitud, en base al nuevo capitulo reivindicatorio presentado y a los argumentos anteriores ys que, en opinion del solicitante, Pa presente invención cumple con los requisitos de claridad y ademÆs los de novedad, altura inventiva y aplicabilidad industrial de la DecisiOn 486.

De la Seæora Jefe,

Atentamente,

JUAN PABLO CADENA SARMIENTO
T.P. No. 57.492 del G,SJ.
C.C. 80.41 0.337 de UsaquØn
Carrera 7 No. 71-21, Torre B, Of. 402

Bogota, Junio 7 de 2007

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0 Un compuesto inhibidor de DP4 que tiene la estructura

caracterizado porque:
n es 0;
XesCN;
R1 es H, aiquilo, el cual contiene de I a 8 Ætomos de carbono, ciaIquilo, bicicloalquilo, tricicloalquilo, alquilcic!oalquilo, hidroxialquilo, hidroxialquilcicloalquilo, hidroxicicloalquilo hidroxibicicloalquió hidroxitricicloalquilo,en donde el cicloalquilo tiene un anillo que contiene de3a 10 âtomosdecarbono, y el bicicloalquilo y el tricicloalquilo tienen un mÆximo de l0Ætomosde carbonoenlos aniHos;
R2 es H oalquio, que contiene del a8âtomosde carbono;
R3esH; S
y una sal de los mismos farmaceuticamente aceptable, o un ester profÆrmacode los mismos, y todos los estereoisômeros de los mismos.

Itlaestructura:

El compuesto de acuerdo con la reivindicaciôn 1, que tiene

caracterizado porque R1 es aiquilo, el cual contiene de I a 8 Ætomos de carbono, cicloalquilo, bicicloalquilo, tricicloalquilo, alquilcicloalquilo, hidroxialquilo, hidroxialquilcicloalquilo, hidroxicicloalquilo hidroxibicicloalquilo, o hidroxitricicloalquilo, en donde el cicloalquilo tiene un anillo que contiene de 3 a 10 Ætomos de carbono, yel bicicloalquilo y el tricicloalquilo tienen un mÆximode 10 Ætomosde carbono en los anillos.

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El compuesto de conforrnidad con la reivindicaciOn 1, caracterizado porque tiene la estructura:

TII

o una sat farmacØuticamente aceptable de Is mismos.

iAI El compuesto de conformidad con la reivindicaciOn 1, caracterizado porque tiene la estructura:

o una sat farmacØuticamente aceptable del mismo.

5

El compuesto de conformidad con Is relvindicación 4,
do.. porque. Is sal farmacØuticamente aceptable es Is sal de
0 Is Sal del Æcido trifluoroacØtico. .

6.

Una cornposiciôn farrnaoeutioa caracterizada porque
comprende un compuesto de conformidad con cualqulera de las reMndicaclones I a 4y un portador del mismo farmacØutic.rnente aceptabte.

Una combinaciOn farmacØutica caracterizada porque
comprende un compuesto lnhibidor de DP4 como se define en cualquierade las reivndicaciones I a 4, un agente antidiabetico diferente de un inhlbidor de 0P41 pare el tratarniento de Is diabetes y enfermedades relacionadas, un agente anti-obesidad y/o un agente modulador de lipidos.

(llt8.) La cornbinación farrnacØutica de. conformIdad con Is reivindicaciôn 7, caracterizada porque comprende dicho compuesto inhibidor de DP4 y un agente antidIabticc.

9. La ccrnbinaciôn de conformidad con la reMndicaciôn 8,
caracterizada porque el agente antidiabØtico as uno a meg de un segundo ingredients qua es una biguanida, una sulfonilurea, y/o un sintetizador de insulina.

10. La combinación de conformidad con Is reMndicación 8,
caracterizada porque el agente antidlabØtico es uno o rnÆs de un segUndo ingredients qua as metformina, glibunda, glimepinda, ghpinda, glipizda, pioghtazona, y/o rosigbtazona

11. La cornbinaciôn de conformidad con Is reivindicaciôn 8
caracterizada porque el inhibidor de DP4 estÆpresente en una proporclôn en peso al agente antldiabØtico dentro del rango de 0.01 a. 100:1.

I2 La combinación de conformidad con Ia reivindicaclôn 7,
caracterizada porque el agente anti-obesidad es orlistat, ATL-962, AJ9671,

- S S

J. Med. Chew. 2004 47,2587-2598 25,87

Syntbsis of Novel Potent iMpeptidyl Peptidase IV Inhibitors with Enhanced Chem cal Stability interplay between the NTerrnIna1 Amino Acid Alkyl Side
Chal and the Cyclopropyl Group of
ct-Am noacyI-t-cis-45-rnetbanoproliuienItrile-Based Inhibitors

David. Magnth,t Jeffrey A. RabL* Richard B. Sulsky,t David J. Augeri,t. Yanting Huang,t Llgaya ivL Simpkins, Prakesh C. Taunk,t David A. etebeTrner,i,# James G. Robertson,* Benoni B. Abboa-Offei,
Alying ang, Michael Cap,* LI Xin,* Li Tao, Doree F. Sltkoff, Mary F. Mal.ley,° Jack Z. Gougoutas,°
Ashish thanna. QI Huang, Song-Ping Han, Rex A. Parker,*and Lawrence G. Hamann*,i

Pepaza4eats ofDiscovery Chernitry Metabolic Research, Exploratory Pharmaceutics, Computer-Assisted Drug Design, Solid Chemistry, and Pharmaceutical Candidate Optimizadom 13ri.toJ-Myers Squibb, Phannaceutical Research Institute,

P.O. 5400, Pi-inceton, New Jersey 08543.5400

of

drug

January27, 2004

A series of metharioprollnenitrile-contsinlng dipeptide mimetics were synthesized and as.

as Inhibitors ofthe NterminaL sequence-specific serine protease dipeptidyl peptidase IV (DPP- I\’). The -ctalyUc action of DP?-IV is the principle means of degradation of glucagon-like

peptide-1, a key mediator of glucose-stimulated Insulin secretion, and DPP-IV inhibition shows Clinical benefit as a novel mechanism for treatment of type 2 diabetes. However, many of the
reversible inhibitors to date suffer from chemical instability stemming from an amine to nitrile

intramolecular cyclizatlon. Installation ofa cyclopropyl moiety at e1thei the 3,4- or 4,5-position of traditional 2-cyanopyrrolidide praline mimetics led to compounds with potent inhibitory

activity against the enzyme. Additlpnally, cis-4,5-methanoprolinenitriles with fl-branching in

the N-terminal amino acid provided enhanced chemical stability and high’inhibitory potency. This class of Inhibitors also exhibited the ability to suppress prandial glucose elevations after

an oral glucose challenge in male Zucker rats. S

diuction - GLP-1 (9-36) by cleavageof the N-terminaldipeptide
the spread ofWestern lifestyles, the prevalence residues. The truncated metabolite has antagonist
2 diabetes in thoworld’s population is rising.’ activity against the GLF-. I receptor both in vitro arid
treatment strategies Include reducing insulin in v1vo9 The principle enzyme responsible for the
glltazones,2 Insulin of GLP-1 (7-36) trnide to GLP-1 (9-36)
with Increasing lnulin
nn with sulforzylureas,4 reducing hepatic glucose nonclassica, sequence-specific serine protease that
with blguanldes,5 and limiting glucose absorption catalyzes the cleavage of dipeptides from the N-term!-
targets proteins

t is the pharmacology surrounding the Incretin Inhibition of DPP-IV has been shown to be effective at

i to function as a mediator of glucose-stimulated therefore offers a new therapeutic approach for the
secretion, and several clinical studies have treatment of type 2 dIabetes.11

that administration of the’ peptide or Its ana- Early reports of DPP-IV inhibitors Included proline- results in antidiabetic action in subjects with type based dipeptide mimics bearing boronic acid" (1) or etes.8 Although CLP-i is secreted as GLP- I (7’ diphenyl phosphonate subtituents (2).13 These corn-
* rrespozldence should be addressed. For JAR.:.. g4eratlon dipeptide surrogates have been disclosed as

ene,6OS-S1’om8.5O48; tax. 688-350: reversible inhibitors of 2DPP-W, including both C-

maxnebms.tom.trIle

telephone 609 8I8-5328 6O9-818-35SO*m&l: substituted (3) and N-substItuted (4) glycinyiprolinenl-

dipeptide analogues.’4’15 These compound classes of Discory Chemistry.
11? esent address: Lexicon Pharmaceuticals, 350 Carter Rd, Include many potent inhibitors of the enzyme, but all

lncçori. NJ 03540. - ’ sufferfrom chemical instability whereby the N-terminal 0Pesent address: Pharmaceuticai Discovery Division, Abbott

bortories, Abbott amine lntrainolecttiarly cycUzes onto the nitrile, forming. 1at1c, IL 60064.

Departxneot of xioratory iarmaceutics. Inactive cyclic Imidates and/or their diketopiperazine Dpartment of Computer-Assisted Drug Design, hydrolysis products. However, more recent publications °t

partpxnent of Pharmaceutical CandidAte Optimization. have of Solid State Chenstry. 1D have disclosed a series ofmore hindered N.alkylamines
tDpartrnent of Metabolic Research. (5) that have much greater chemical stability.18 This-

Z588.74o-nal of Iviedicina! Chemistry, 2004, Vol. 47. No..10

Nq NI

P03Ph2 1N

NH CNNQ HOMR HaNflCD

H /a CN 0

be

a

5
7XCH2

1. Known praline-derived dipeptidyl peptidase IV
S

1idiie (6) and pyrralidide (7) based Inhibitors have also en disclosed that lack the nitrile moiety and thus ’ssess greater stability, though at the cost of reduced

itercy1711 with a - fevr very recent notable excep- 5!7d4 These compounds are shown in Figure 1.
We endeavored to synthesize novel dipeptide sur-

gates containing a praline mimic linked to an N- rn’inal amino acid that would act as reversible

hiltors of DPP-IV with maximum potency and en- need solution stability. Hanesslan has reported that
stMiation of a 4,5-methana moiety into a proline silue has the effect of flattening the five-membered one might extend a similar conformational g.1ment to other cyclopropanated prolines as well. By rrence, the cyclopropane bridge may occupy the ae-filutng region that would normally be given to the hylene group in the puckered or ’envelope" confor-on of a typical five-member ring. With this prece-tht we sought to establish whether a methanoproline ’1vative could serve as a viable praline surrogate in $’p Inhibitor. Our strategy was to prepare dipep-e surrogates containing a cyclopropanated prolineni-e derived from L-proline. The reglo- and stereachem-LI disposition of the cyclopropane bridge was varied order W identify a compound with maximal stability d potency. Herein, we report the discovery of L-cis--methanoprolinenitrile dipeptides as patent inhibi-’s of DPP-IV with increased chemical stability and h potency. in addition, we also present data demon-’ating that these novel DPP-IV Inhibitors effectively ver plasma glucose after a glucose challenge in rodent

Dipeptides (12-15) composed of N-terminal isoleucine ppended to cyclopropyiproithearnides or nitriles derived ram L-proline (8-11) were targeted to probe the otential for this type of inhibitor scaffold; Isoleucine as selected as the N-terminal residue because it was the most potent natural amino acid reported In the 2-cyanopyrrolidide series)4a These inhibitors were ex-pected to provide a dependable inhibitory benchmark

for the differing methanoprollne structures. The syn- thetic routes used to prepare the dipeptides derived from

L-cis- and L-trans-4,5-rnethanoprolthe, 18b L-cIs-3,4-meth-

Scheme j

Magnin et*193a,
LH

C0NH2 CONH2 COMM2 ON
B B10 11

f_2

t4

M2NA H2NkrNcH H2Nl)_N’T
0 CM 0 CM 0 CM N

12 13 U 15

8(a))VBoc-amino acid, EDAC, DMAP or PyBop, 50-90% (b) (for intermediates 8-10) F003. pyridine, Imidazole, 70-90%; (c)

anoproline,° and L-2,3-methanoproline2° are shown in Scheme 1.

Standard conditions were employed to couple the enantlomericafly pure L-cis-4,5-cyclopropylprolineamlde nucleus (8) to give the corresponding Boc-protecte’d dipeptides in good to excellent yield.;’ Dehydration’ 4,22 (FOC13, pyridine, imidazole) ofthe amide and removal23 of the N-terminal Boc protecting group (TEA or HCI) gave the praline dtpeptide (12) in high yield. Similar protocols were used for the L-cis-3.4-methanoprqline fragment 10 and the L-4.5-trans’-methanoproline com-pound 9. The known (-)-(2S.3R)-methanoprolinenitrile
(11) was prepared according to the method ofHereout20a In this Instance, the dehydration reaction was per-formed prior to introduction of the isoleucine fragment. Preparation of a library was undertaken to probe structure-activity relationships between the N-termi-nal amino acid residue and the cis-4,5-methanopro-

linenitriles. These inhibitors were generated in a three- step sequence in parallel array format In a manner

similar to that described in Scheme 1. Initial reaction of the Boc-protected amino acid with methanoprolinea-mlde, EDAC, and DMAP In dichloromethane, and subsequent purification through an SCX ion exchange cartridge, gave good to excellent yields of coupled dipeptides. Dehydration and acid-promoted deprotection (TFA in dichioromethane) yielded the inhibitors as TFA salts. Further purification of the final products was easily accomplished by preparative reverse-phase HPLC

Holmberg has prepared tert-alkylmalonlc acid deriva-tives through a T1C14-mediated Knoevenagel process and subsequent copper-assisted conjugate addition of a Grignard reagent or conjugate reduction. Following this method (Scheme 2), dlesters can be converted to the protected amino acids In three steps.24 This methodology provided an approach to the cycloalkyiglycine amino acid derivatives 20. First, the malonic acid diesters 17 were subjected to conjugate addition of a methyl group or hydride and then hydrolyzed to the Corresponding monoacids 18. Subsequent Curtius rearrangement of 18 by treatment with diphenyiphosphoryl azide, followed

by trapping of the intermediate isocyanate with benzyl alcohol, provided the Cbz adducts 19, Finally, the esters

were hydrolyzed to give the amino acids 20 In racemic form. The racemic Boc-protected acids 20 were then coupled to the L-cis-4,5-methanoprolineamide group.

I!

Dpep yl Peptidase IV Inhibitors

Schene 2

a (b,e

 1 atooc 000Et ZH

OH3 CH3
HO0c’HBoc1 g NHCbZ
19

il() iiCk THP, CC14, dithy1 tnalonate, 0 C, then pyridine, 0 "C. to rom temp. 88%; (b) MeMgL CuCI, Et20, 0 T, 89%; (c) NaC)H, ’HF. EtOH, 78%; (ci) (I) diphenylphosphorylazide, NEt3. Ph}t retux; (11) BnOI-L retlux, 18 h, 100%; (a) H, Pd(01-i) EtQA, 100%; (t (Bec)20, K2C08. ’11W, H20. 92%, two steps; (g) NaOH, MeOH, HF, room tamp, then aqueous HC!,

?roces1ug as previously described (Scheme 1) gave a mlxtur4 of diastereomers that were separated at the nitrtle 5tage by silica gel flash chromatography. The later lting Isomer In each case was consistently 1deritffld as the desired Lx-dlpepride.

: and Discussion

PAll c mpounds were tested in vitro against purified porcine PP-IS. Inhibition was determined against the substra e H-Al-FropJA. Production of p-nitroanlflne
(PNA) as measured at 405 nm over 15 mm. A cot-
parisori of methanoproline-derived lsoleucthe N-termnl-mis! dipe tides shows that the enzyme Inhibitory activity
Is criti y dependent on both the position and the
orlentat on of the methano bridge(Table 1). The pres-ence of the methsrio bridge on the trans side.of the (e.g., 22, 23) resulted In a significant loss
of activi relative to the unsubstituted proBnepltrile
21. ThIs result was consistent with early reports where
Irisertlo of a methyl group at the 2-postion of 2-yan-
opytroli ides resulted In a 2Q00-foId decrease in activ-
1ty.In ntrast, when the methano bridge was oriented
cis to e nitrile at either the 4,5- or 3,4-p6sition,
inhibito activity was only slightly diminished. This
is clearl Illustrated with cfs4,5-methano inhibitors 24 and 28 a d cis-3,4-metbio inhibitors 25 and 27, where toiy potency resides In the 20 uM range..A more
plet exploration of N-terminal, no acids reveals that Incz$sing the degree o1 -branching to that of tart-Leu (e.g, 29) further Increases potency in the 4,5-methano series to that ofthe prolinŁnItrile version (28). Replace emit of these alkyl substituents with aromatic residues uch as Phe (1) Trp (32) in the N-terminal position Ignificantly eroded potency. Alkyl substitution
on the to ned amine was generally ill-tolerated in this
series (e.., 33-35), though it would appear that the more Ore accessible N-terminus found in proline derlvativ 34 Is capable of restoring a modest degree.f
inh.lbito activity. Replacement ofLeu and tei’t-Leu side
chains Ith medium-ring cycloalkyl and methylcy-
cloalkyl (7-42) generally led to Inhibitors with com-parable. slightly improved activity, though potency dropped. teadily with increasing larger ring size (data

not show).The Cp-Gly and Me-Cp-Gly derived ana-

logues 38. ci 41 were among the most potent compound prepared In this series, exhibiting Rj values of 4 and 7 nM. resp ctively.

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Table 1. In Vitro Inhibition Constants for Porcine DPP-IV and Solution Stability Half-Lives for Proilnenitrile DPP-4 Inhibitors

R7 011-N? R’N91.
N CN ON ON

cls-4,5 cIs-3,4 tmhs-4,5 !ishs-2,3

N-terminal substituted . stability,
compd amino prolInenitrile J<1 (nM)b t (h)’
21 lie prolinenitrile. * 0.5 5
22 lie trans-4,5- 1620+ 80
23 lie trans2,3- 7500–200
24 Ile cls4,5- 25 – I 22
25 Be cis-3,4- 15 *1 4
26 Val cis-4,6- Z0.+1 28
27 Val cls-3.4- 12 – 1 2
28 tort-Lou proftnerzittile 84-0.5
29 ter&Leu ds-4,5- 7 – 0.5 42

33 fMeVaI ds-4,- 1940:k 80

36 Met ds-4,5- 135:L- 10

dl (I-Me-Cp-1-yl)-Gly cf.v.4,5- 7 – 0.5 24

Cb = cyclubutyl; Cp = cyclopentyl; Ch = cyclohexyLbValues represent the mean * SEM and are at least triplicate determina-tions. c Solution stability data are measured at 39.5 "C and pH

syn amIda rOtamer anti amkie rotamer
COsyntoON COanULoCN X-NH,O

Solution Stability, After the discovery that the L-CIs-

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cis-3.4-mcthanoprolinenitrile Or the unadorned pro-linenitrile compounds.. For instance, in the case where the N-terminal amino acid is isoleucine, the isomeric 4,5-methano-substituted compound 24 has a solution half-life of 22 h, which is 5.5-fold longer than the analogous 3,4-methano-substituted compound 25 and appoximate1y 4.5-fold longer than the unadorned pro-linenitrile analogue 21. The second feature is that there is a strong correlation between steric size at the,8-posi-tion of the N-terminal amino acid and relative solution stability. Increasing the degree of branching at the -position of the alkyiglycine substituent increases solution stability. The most stable compounds within their respective prolinenitrile series have the tent-Leu N-terminal amino acid fragment in common. For ex-ample, unadorned prolinenitrile-28 (tja = 27 h) is more table than its less branched isomer 21 (t112 h). ixniiarly, cis-4,5-.methanoprolinenitrile 29 (t112 = 42 h) s more stable than the less branched dipeptidenitriles 4 and 3.9 (run = 22 and 19 h, respectively), though the agnitude of the effect of Increased fl-branching in this eries appears to be blunted by the Inherent baseline $ability imparted by the metharia bridge. This observa-lion is in agreement with data reported by Coutesl2a 4nd Snow27 where the rates of cyclization for Xaa-iporoprollne dipeptides were shown to be Gly-BoroPro Ala-BoroPro> Val-BoroPro.

Computational Analysis. Computational analysis was undertaken to more fully understand the relative stabilities of the metharioproithenitrlles. Ground-state cnformattons were generated for methanoprolinenitrile aid prolinenitrile forms of the N-terminal tert-leucine dpeptide compounds. The calculated ground-state struc-ture for the tert-leuclne dipeptidenitrile is identical to tle conformation observed through single-crystal X-ray structurai analysis of the TFA salt of 29 (rms 0.1 A fo calculated and observed heavy atoms: see Figure 2, lover structure). Both have the same syn conformation aound the amine bond, characterized by a small C (2) N-C(8)=O torsional angle (50 and +20 for the TFA stucture). It is of additional interest that a similar
co formation has been observed in several recently
di closed cocrystal structures of DPP-TV/inhibitor corn-

- n addition to the syn conformation, there is a caculated local low-energy minimum where the reactive anine and nitrile are close to each other; in this anti)coiiformation (Figure 2, upper structure), the C(2)-N-- C()O torsional angle i 180*. Moreover, the angle ,.beiween the amine N and the CN group is 1090 – 10
and the distance between the these reactive partners Is.95 A. It is therefore reasonable to assume that the observed intramolecular cyclization is initiated from this conorrnation, The value of 109* between the amine groip and the nitrie is in close agreement with the hypothetical angle of attack of at least 1080 reported by axter and Conner.30 It was envisioned that the re1tive energetic differences between the global mini-mum and the reactive local minimum would represent a mans to evaluate the relative stabilities of compounds In solution.
The calculated conformations and their relative ener-gicscan be used to examine the basis for two aspects of the experimental compound stability: the increase in

Magnin et a].

Figure Z. The upper structure depicts the local low-energy minimum for 29 where the reactive amine is close to the nitrile (anti conformation). The lower structure is the solid-state conformation observed In the X-ray crystallographic structure of the TFA salt of compound 29 (syn conformation). Two Independent observed in the crystal structure, though because these have identical conformations, only one is shown for purposes of clarity. Carbon atoms C(2) and C(S) are labeled.

stability due to side chain bulk (specifically 9-branching) and the increase In stability upon conversion from prolinenitrile to cis-4,5-methanoprolinenitrtle. Calcu-lated relative energies between the ground-state con-formation and the local low-energy minimum confor-mation tha: brings the reactive amine and nitrile in proximity ara presented in Table 2 for the methanopro-iirienitriie end prolinenitrile forms of N-terminal tart-ieucine and alanine dlpeptidenitriles, as well as for the. same prolinenitriles with a simple acetamide cap. Conformational Stability Due to Side Chain Bulk, The values in the first column ofTable 2 compare the conformational energy differences between the ground state and the geometry where internal cycliza-tion could occur for the unsubstituted prolinenitrile series. The ab initlo (G98) results are expected to be considerably more. accurate than the force field values and indicate that the energy required to assume the anti conformation increases with side chain bulk (e.g., 0.3, 1.91 2.8 kcal/mnol for no side chain, the.alanlrje side chain, and the tei-t4eucine side chain; respectively). The. force field eziergy results agree qualitatively wiih the ab initio values and suggest that the primary contribu-tion is due to van der Waals interactions. Examination

of the structures reveals extremely close contacts be-tween two of the side chain methyl groups and the carbonyl oxygen in the anti conformation (approxi-mately 3 A each) that would Increase the energy barrier for internal cyclization and thus lead to greaterstability. A similar trend is observed for increasing side chain bulk In the methano-substituted series (column 2), and these values are in good agreement with the solution data where increased fl-branching enhances stability. Conformational Stability Due to Methano Sub-stitution. As shown in the third column of Table 2, the ab lnitio (G98) energy calculations indicate that the

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mpept(dyl.Peptldase IV Inhibitors

Table 2, Calculated Differences between the
Calculated Ground State and the Local Low-Energy llAlninium Required for Cyclization

AN?

CN A
2Ate,t-Leu CN
43AAle 29A=tert-LŁu
45AAo 44A=Ala
46 A Ac

28 (Prolinenitrile) and 29 (Methanoprolinenitrlie)
(A = tert-Leu)
method lnteraŒtion l.H(28)b AH(29) b EAH(29 - 28)
C98 eb irtltlo 2.8 3.4 0.6
Insight CFF force field
total nonbond 3.5 4.2 0.7
van der Weals 3.3 3.9 0.6
electrostatic 0.2 0,3 0.1

43 (Prolinenitrile) and 44 (Methanoprolinenitrile)
(A Ala)
method interaction tH(43) b AH(44) &H(44 43)

ab InItlo 1.9 2.6 0.6
nsight CFF force field
total nonbond 3.3 3.9 0.8
van der Weals 2.9 3.4 0.6
electrostatIc. 0.4 0.4 0.0

45 (Pro1inenItrl1e) and 46 (MethanoproIinenitrl1e
(A= Ac)
method interaction MJ(45)" &H(46) AAH (46 -, 45)
ab initlo 0.3 0.9 0.6
Ixsight CFF force field
total nonbond -02 0.0 0.2
van der Weala -0.4 -02 0.2
electrostatic 0.2 0.3 0.1

Energies are for the cis-4,5-methanoprolinenitrlle and pro-1111

ro-.

linnitrfle versions of the compounds and are given in kcallmol. b Eergiü are for the anti conformation (Scheme 3 and Figure 2

uper structure) relative to the global minimum conformation, wh)ch corresponds to the syn geometry in Scheme 3 and Figure 2 Ioer structure.

addition of the methano bridge increases the energy barrier toward adopting the conformation required for cyc1zatlon by approximately 0.6 kcal/ml for the N-ter4ninal tert-Ieucine dipeptide compound as well as for the alanthe dipeptide and acetamide compounds. The

V6

of dependence on the nature of the side chain ugests that the observed increase in stability of the cis-,5-methano compounds is due to a local interaction bet*een the methano bridge and the N-terminal amino acid that favors the ground state relative to the anti confrniatIon where cyclization is initiated. The force fiek energy analysis (Insight CFF) results also agree qualitatively, implying that van der Waals interactions are primarily responsible for the increase in energy. Thee conclusions are supported by the cyclization rate diffeences between the isomeric isoleucthe derivatives

24 (Li2 22 Ii) and 21 (r1,2 = 5 h), where a 4.5-fold incrase in stability is observed for the methanopro-1inentriJe. This increase in stability is in fair. agreement with he calculated value of 0.6 kcal/mol. The calculated H and observed solution half-life results support contributions to stability from both 8-.branching and the preseice of the 4,5-rnethano bridge on the pyrrolidine ring.
In Vivo Activity. In viva evaluation of DPP-IV inhibitors has supported the connection between DPP-

Journal of1*didna1 Chemisby, 2004, Vol. 47, No. JO 2591

pao1.vs VOW* conlroi

211 p0.001.vhecooInI
41
400

300

200

100

o4---------r--------------’--.-’--------,
- 30 0 30 60 120

’Pine

Figure 3, Effects of inhibitors 29 () and 41 (W) dosed at 3 ,umol/kg pa versus vehicle control (0) on plasma p?P-IV

IV inhibition, increases in plasma insulin levels, and an improvement in glucose tolerance.31 Compounds 29 and 41 were potent inhibitors of DPP-IV in vitro and demonstrat.d excellent solution stability. As such, these inhibitors were selected to determine the effects of DPP-IV inhibition in viva on glucose tolerance and insulin secretion ir. Zucke011fa rats. An oral glucose tolerance test (oGTT) In the Zurkee-11ta rat is a frequently used model of hyperglycethia in type 2 diabetes and obesity research. Zucker’ rats are severely hyperphagic, extremely obese, markedly insulin-resistant, and mildly hyperglycemic because of a mutation and loss of function of the leptin receptor gene.32’33 Fasted male Zuckert rats were dosed orally with water or with inhibitors 29 and 41 (3 uno1Jkg), and an oGIT was conducted 0.5 h after the dosing. Plasma DPP-IV activity, glucose, and Insulin levels were then monitored over a 2 h period. Figures 3-5 show the ax vivo plasma DPP-IV activity, Insulin response, and glucose excursion curves in re-sponse to an oral glucose challenge (2 g/kg). Animals in the control group reached peak plasma glucose levels 60 min after glucose administration, at which point the - drug-treated animals exhibited a 30-35% decrease in glucose levels compared to controls (control animals, 358 mg/dL; compound 29 treated animals, 226 mg/dL; compound 41 treated animals, 245 mgfdL). Glucose levels were significantly reduced in the drug-treated animals from 30 min onward, with maximal reductions In glucose observed at 90 mm (-34% to -’38%). Plasma DPP-IV activity was maximally suppressed (60%) 30 min after dosing (Figure 3), and the effects of these - Inhibitors were sustained throughout the course of the experiment (60-35%). The insulin response to oral glucose was also enhanced by treatment with DPP-IV inhibitors (Flgur 4), demonstrating the link between the glucose-Lowering effects and DPP-IV inhibition of these compounds.
Interestingly, a significant decrease in plasma glucose levels occurred when DPP-IV activity in plasma was inhibited on-’y by 35-60%. This finding suggests that It may not be necessary to completely suppress plasma DPP-IV activity in order to achieve antihyperglycemic efficacy in type 2 diabetics. However, differences may exist in the inhibition of the turnover of native sub-strates su& as GLP-1 compared with that of the pseudosubstrate used in this assay, and potential assay

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so

5O

40

30

20

10

0

p.O5.
".p<0.O1,vsveecnt.I

4. Effects of inhibitors 29 (0) and 41 (a) dosed at 3 po versus, vehicle control (0) on plasma glucose after In Zuckeeolfa rats.

 0Vehicle

41
26

20

10

- 30 0 30 120

Thee (min)

re 5 Effects of inhibitors 29 (0) and 41 dosed at 3
g po versus vehicle control (0) on plasma Insulin after
TT in Zucker rats.

acts relating to the kinetic aspects of certain inhibi-
cannot be ruled out at present.

We have demonstrated that the prolinenitrile frag-
-- nent of previously reported DPP-1V inhibitors can be rplaced with either a cis-3,4-methano- or a cis-4,5-ntiethanoprollnenitrile ring system to provide novel and
’ghly potent DPP-IV inhibitors. Solution stability
udies demonstrate that Introduction of either a steri-

clly bul1y amino acid side chain on the N-terminal $rnirio acid or a cis-4.5-methano bridge to the prolirieni-t’ile moiety significantly enhances solution stability, minimizing a known intramolecular cyclization path-
fray. The greatest improvement In stability is observed
hen both of these structural features are present and t work in concert. This added stability has the potential o beneficially impact the chemical and formulation stability of cyanopyrrolidide-based pharmaceuticals. In nany cases, the presence of a p8-branched amino acid kIso provides Increased inhibitory potency as well as

5hutiori stability. This class of inhibitors has been own to be effective In suppression of prandial glucose vations after an oral glucose challenge. These Initial findings are being used as the basis for the development of DPP-IV Inhibitors with even greater stability and in vivo efficacy for the treatment of diabetes and impaired glucose homeostasis through potentiation of meal-induced levels of GLP-1 (7’-’36) and insulin.

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Expthnental Section

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AAM
General Chemical Procedures. All reactions were carried out using oven-dried or flame-dried round-bottomed (rb) flask and glassware under a static atmosphere of argon or nitrogen,
and the mixtures were stirred magnetically unless oth4rwise noted. All reagents used were of commercial quality and were obtained from Aldrich Chemical Co., Sigma Chemical Co., Lancaster Chemical Co., or Acros Chemical Co. All reactions were carrie i out using’ commercially available anhydrous solvents from Aldrich Chemical Co. or EM Science Chemical Co. unless therwise noted. Brine" refers to a saturated aqueous soii1on ofNaCl. Unless otherwise specified, solutions of common inorganic salts used In workups are aqueous solutions. 1H (500 MHz) and 13C (125 MHz) NMR spectra were recorded onJEOL JNM-ECPSOO spectrometer, and 1H (400 MHz) and nC (100 MHz) spectra’were recorded on a JEOL GSX400 spectrometer. Chemical shifts are given In parts per million (ppm) dpwafleld from internal reference tetramethyl-silane in 6 units, and coupling constants (I values) are given In hertz (Hz). Selected data are reported In the following manner: chemical shift; multiplicity; coupling constants; assignment. Elemental analyses were performed by the Ana-lytical Chemistry department at Bristol-Myers Squibb. Melting points were taken on a Hoover Un-melt melting point ap-paratus and are uncorrected. Boiling points are reported uncorrected. KOgelrohr distillations were performed using,a BOth! GKR-51 apparatus, and reported boiling points cor-respond to uncorrected oven air bath temperatures. Optical rotations were obtained a Perkin-Elmer 241 polarimeter using a cell 1 dm in length and are reported as follows: [ai’he (concentration in gIlOO niL, solvent). All flash chromatographic separations were performed using E. Merck silica gel (particle size. 0.040-0.063 mm). Reactions were monitored by TLC using 0.25 mm B. Merck silica gel plates (60 F) and were visualized using UV light and 5% phospho-molybdic acid in 95% BtOH, or by sequential treatment with I N HCI In CHOH followed by ninhydrin staining. LC/MS data were recorded on a Shlmadzu LC-IOAT equipped with an SIL-IDA injector, an SPD-10AV detector normally operating at 220 nm and interfaced to a Micromass ZMD mass spec-trometer. LC/MS or l-IPLC retention times are reported using a Phenomenex Luna C-lB 4.8 mm x 50 mm column, with elution with a 4 gradient of 0-100% solvent B. where solvent A is 10:90:0.1 CH30H-1-120--TFA and solvent B Is 90: 10:0.1 CH3OHHOTFA (1-IPLC, method 1). Other HPLC methods include the following: method 2, YMC S-5 C-lB 4.6 mm x 50 mm, 0-400% B, elution with a 4 min gradient of 0-100% solvent B, where solvent A is 10:90:0.1 CHiCNHO TFA and solvent B is 90:10:0.1 CH3CNH20TFA; method 3, Zorbax SB C-18 4.6 mm x 75 mm column, elution with an 8 nUn gradient of 0-100% solvent B, where solvent A Is 10: 90:0.1 CHOHH20H3PO4 and solvent B is 90:10:0.1 C143-OHH20HBP04, All solvents were removed by rotary evapo-ration under vacuum using a standard rotary evaporator equipped with a dry ice condenser. All filtrations were performed using vacuum unless, otherwise noted. Representative Example of Preparation: General Method A. N-Boc-L.cis-4,5-rnethanoprolineanhide. To a solution of NBoc4,5methancprolin&8b (1.20 g, 5.28 mmol) In THP (20 mL) at 15 C was added 4-methylmorpholine (0.71 ml,, 6.50 mmol) and then isobutyl chlorotormate (0.78 mL,, 6.00 mmol) over 5 The reaction mixture was stirred at 15 ’C for 30 ruin, cooled to 30’C, and then treated with a solution of NH3in dioxarie (50 ruL, 25 mmol). The reaction mixture was stirred at 30 ’C for 0.5 h, warmed to room

temperature, and stirred overnight. The reaction mixture was quenched with citric acid solution (pH 4) and extracted with
Et-,O (3 x 50 niL). The combined organic fractions were washed

with brine, dried (NaSO4), and concentrated. The residue was purified by flash column chromatbgraphy on silica gel with
EtOAc to give 1.00 g (84%) of the title compound. ’H NMR and 13C NMR signals were very broad and poorly defined as a

[,-A

Peptidase IV Inhibitors

t-cis4,5-Methanoprolinearnide TPA Salt (8). To a solu-don of N’Boc-4;5-methanoprolineomide (0.90 g, 4.00 mrnol) in
12 (3 mL) at C) C was added T’FA (3 niL). The reaction ntiixture was stirred at 0 C for 18 Ii and concentrated under rduced pressure to give 0.98 g (100%) of the title compound an oil thatsolidified upon prolonged standing. Alternatively, the protected material can be treated with HCI(g) in EtOAc to give a white powder. Data for HCI salt: ’H NMR (CD30D, 490 MHz) 6 0.78 (m, 111), 0.95 (q, J= 8.0, IH), 1,85 (m, 1H), 227 (dd, J= 14.1, 3.0, 11-1), 2.75 (m, IH), 3.42 (m, 1H), 4.57 (4d, J 11.1,3.0, 1H); "C NMR (CD300, 100 MHz) 610.7,
1.8, N. 38.3, 62.2, 170.3. Anal. (Ce4,0N20’i.03HCI’
0.10H2O) C,

H.
N, Cl.
Amides Prepared frorw Known Carboxylic Acids by Method A. 1-trans-45-Methanopro1ineamide TFA Salt Z}..j NMR (CD30D, 400 MHz) 6 0.89 (g, J= 6.0, IH), 0.95 (ni, IH), 1.90 (m, 1H), 2.18 (td, J= 5.0, 11.4, 1H),,59 (dd, J =81, 13.2, IH), 3.47 (td, J= 2.5, 6.6, 11-0, 4.01 (dd, J- 8,1, 10.8, 1H);C NMR (CD3OD, 100 MHz) 67.1, 17.0, 32.0, 36.1, 5.3, 118,6 (q, J = 263.5), 163.0 (q, 1 = 35.4), 172.0. Anal. (CH,0N2O’1.00TFA) C H, N. F.
L-Cis-3,4-Methanoprollfleanhlde TFA Salt (10), ’H NMR (CP3OD,400 MHz) 6 0.60 (m, lI-I), 0.75 (q. J 7.5, 11-1), 1.85 (n, 114), 2.10 (it, 114), 3.45 (d, J= 10.9, IH),.3.49 (dd, J 110, 3,5, 114), 4.44 (d, J= 4.3, 11-4); 13C NMR (CD30D, 100 M44 6 5.3, 17.5, 20.4, 62,5, 170.4 (TFA not observed). Anal. (C,H,0NaO’1.30TFA) C, H, N. F.
L-2,3-Methanoprollncnitrile HC1 Salt (11). ’H NMR .(C)OD, 400 MHz) 6 1.55 (ni. 114), 1.70(m, 114), 2.10 (in, 11-1), 2.5 (m, 11-fl, 2.37 (m, 114), 2.67 (m. 114), 3.15 (in, IH). epresentative Example of Preparation. General Mthod B. Ltert-Leucinyl-L-cis-4,5-methanoprolinenl-

trl4e TFA Salt (29). An oven-dried 15 ml. test tube was chrged with the L-cis-4,5-niethanoprollne.ainlde TFA salt (56
mg 0.22 rnrnol), N-tertbuty1oycarbony1-L-tert-leucfne (53 mg,

0.23 mmml), 4-(dimethylamtno)pyridine (0.11 g, 0.88 mmol), an CH2Cl (4 niL). The mixture was treated with 1-((3

(diinethy1),raino)propyl]-3-ethykarbodlimide (84 ing. 0.44 ,nno1), and the tube was sealed under a nitrogen atmosphere. Th tube was placed in a shalcer and shaken overnight. The prosluct was purified by solid-phase extraction using a United Tecino1ogy SCX column (2 g of sorbent In a 6 ml. column) by loaqirig the material on an SCX ion exchange column and sücessive1y washing with C}12Cl2 (5 niL), 1:3 CI-130H-CH2-Cl2 5 ml.), 1:2 CH3OH-CHC12 (5 niL), and 1:1 C}-1,OT-1-CH2-

Cl2 (10 niL). The product-containing fractions were concen- tratd under reduced pressure to give the desired amide.
Further P1 purification by reverse-phase preparative HPLC on YI4C 85 ODS 20 mm x 250 mm column gave the desired

am1i2e (50 mg, 68% yield). Purification conditions were the

PLOwtng: gradient elation from 30:70:0.1 CFI3OH-H20-TFA

9q:1o:o. I C143014-J-120.-TFA over 15 nun; flow rate, 20 tnt] inun; detection wavelength. 220 am; retention time, 14 min. ’H I4lvfR (CD3OD, 500 MHz) 6 0.87 (in, 11-1), 1.08 (a + in, 1014), l.44(s, 9H), 1.75 Cm, 11-4), 2.01 (dd, J- 13.7, 3.5, 114), 2.58 (in, H), 3.87 (in, 114), 4.53 (s, 114), 4.77 (dd, J= 11,8, 3.2, 11-fl; ’3C NMI (CD30D, 125 MHz) 6 14.6, 18.4,27.1, 28.7,31.4, 36.4, 39.9, 50.4, 62.7, 80.7, 157.9, 172.4, 176.7.
Ar ovendriØd 15 niL test tube was charged with the NBoc-tert-1ucine-cis-4,5-methai-iopro1hneamide (50 mg, 0.15 nunol),

lrnidzole (31 rag, 0.46 nui’iol), and pyridine (1 niL). The tube was 4caled under nitrogen atmosphere and cooled to -30 0C.
Slow addition of POCI3 (141 mg, 88L, 0.92 mmmi) gave after mixlxig a thick slurry. The tube was mixed at -30 °C for I h, and the volatiles were evaporated. The product was purified by slId-phase extraction using a United Technology silica e,ctration column (2 g of sorbent in a mL column) by loading

the material on a silica column and successively washing with C112c12 (5 niL), 5:95 CH30H-CH2C12 (5 ml.), 7:93 Cl-{30H-- CH202 (5 niL), and 12:88 CH30H-CH2Cl2 niL). The

product-containing fractions were pooled and concentrated undeij reduced pressure to give the IVfloc-protected nitr’Ile compund (46 rag, 96%). ’H NMR (CD2OD, 500 MHz) 6 0.88 (m, 1i-l), 1.08 (a + m. 1014), 1.43 (s, 9HJ, 1.94 (in, 1H), 2.32

(dd, 1= 11.5, 2.5, lI-fl, 2.65 (m, 11-), 3.95 (in, 1H), 4,51 (s, 14, 5.09 (dd, J= 11.2, 2.2, 114).
An oven-dried 15 ml. test tube was charged with the N-Six-terD.leuclne-4,5-metharaiproltnenitrile (45 mg 0.14 mxnol), CHr Cl2 (1 niL). and TFA (1 mL). The reaction mixture was vortexed for 40 min at room temperature, diluted with toluene (4 ml.), and concentrated under reduced pressure to a thick oil. The product was purified by reverse-phase preparative HPLC on a YMC S5 OS 20 min x 250 mm. column to.give 14 mg (35%) of the title ci.mpound. Purification conditions were the follow-ing: gradient elution from 10:90:0.1 Cl-IOH-H20-TFA to 90: 10:0.1 CH30H-1420-TFA over 18 min,: flow rate 20 rnLmmin; detection wavelength, 220 am; retention tlme, 10 min. ’H NMR (DMSQ-d5, 400 MHz) 6 0.73 (m, IH), 14)5 (a + m, 1014). 1.99 Cm, 11-1), 2.26 (dd, 1= 13.5, 2.2, 114), 2.50 Cm, 11-1), 4.11 (m, 114), 4.37 (a, 14, 5.25 (dd, J= 10.5, 2.2, 114), 8.20 (a, ZH); 12C NMR (DMSO-d6. IOU MHz) 6 12.7, 17.3, 25.9, 29.8, 34.2, 37.6, 45.0, 57.6, 118.0 (q, J = 280), 119.5, 157.5 (q, J = 38, 166.7. L-tert.Leucinyl-L-cis-4,5--metllanoprolinenitrile HCI Salt (29). An oven-dried 15 xnL test tube was charged with JVBoc-tez-r-ieuchie-4,5-methanoprolfnenitrile (0.45 trig, 0.14 mmml) and Et20 (1 ml.) and was treated with a 2 N HCI solution in Et20 (1 ml.). The reaction mixture was srortexed for 15 into at room temperature and concentrated under reduced pressure to a white powder. The product was purified by trituration with Et20, collected, and dried under vacuum to give the title compound (25 mg, 85%). ’H NMR (CD30D, 400 MHz) 6 0.91 (in, 11-fl, 1.13 (a + in, 1014), 2.00 (m, 1H), 2.32 (dd. J= 14.0, 2.2, 114). 2.65 (m, 11-4), 3.97 (in, 114), 4.37 (a, 1H), 5.26 (dd, J= 10.5, 2.2, 114); 12C NMR (CO30D, 100 MHz) 6 14.1, 19.1, 26.7,

t?ipeptlde Nitriles Prepared, by General Method B. L-IsoIeuclne--L-prolinenitrile HCI Salt (21). ’H NMR (400 MHz, CD30D) 6 0.99 t, 1- 7.2, 314), 1.10 (d, 1 = 6,6, 3H), 1.20 (in, 214), 1.70 (iii, 11-0. 1.90-2.45 (iii, 414), 3.70’ (in, 2H),

L-Isoleucinyl-L-ci.9-4,5-methanopr6llnenftrile TFA Salt (24), ’H NMR (D20, 500 MHz) 0 0.90 (m, 11-), 0.95 (t, J= 7.3, 31-I), 1,12 Jtt 1.27 (m, 114), 1.25 (m, IH), 1.47 (iii,

2.78 Cm, 114), 3,73 (td, 1= 6.4, 2.8, 114), 4.46 (d, J= 9. 1, 114), 5.32 (dd, 1= 11, 2.8, IH); 13C NMR (D20, 125 MHz) 6 10.9,

290), 119.8, 163.2 (q, 1= 35), 165.4. HPLC: method 3; tk = 2.49 3.03 mm. HRMS (FAB) m"zz [M + Wcalcd for C,21-120N20,

222.1606; found, 222.1606.
L-ValIflyl-L-cis-4,5-metharioprollnenitrile TFA Salt (26). ’HNMR (CD’OD, 500 MHz) 5 0.93 (rn 114), 1,07 (d, J 7.1,

(dd, J= 13.8, 3.0, 114), 2.50 (m, 1H) 2.65 (in, 114), 3.82 (td, I 6.0, 2.5, IH), 4.39 (d. J= 5.0,11-), 5.16 (dd, J= 11.0, 2.2,

38.7, 47.6. 58.5, 120.8, 168.9. HPLC: method 2; t 0.89 mm. HRMS (FAB) m’z [M + 1-Ir calcd for C,,H,7N30, 208.1450;
L-tert-Leuelnyl-L-prolinenitrile TPA Salt (28). ’1-! NMR’

OD, 100 Mi-la) 26,7, 26.8, 31.2, 35.9, 48.2, 60.5, 119.4 (q, ’J

t-Pbenyla)aninyl-L.-cis-4,5-methanoprolinenitrile TPA Salt (31). ’14 NMR (CD30D, 500 MHz) 6 0.31 (m, 114), 0.66 (dd, J= 8.5, 6,5, 11-1), 1.84 (m, IH), 2.25 (dd, J- 13.8, 2.5,

4.73 (t.J 6.6, 114), 5.10 (dd, J= 10.2, 2.2, 114), 7.32 (in, 514);

(q, J- 43), 128.0. HPLC: method 2; tR = 1.33 min. HRMS (FAB) 1n1z IM + H) calcd for C,51-I,7N30, 256.1450; found,.

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L-Tryptophanyl-L-cis-45-methanopi’olinenitriie TPA Salt (32). IH NMR (CDQD, 500 MHz) 6 0.00 (in, 2H), 1.64 (in, 11-), 2.11 (dd, J’ 13.8, 2.2 Hz, 11-1), 2.44 (in, 11-1), 3.23 (s,
21-1), 3.35 (m, 114), 4.71 Ct, J 6.6, 1H), 5,00 (dd, J" 10.5, 22, 114), 7.00 (t, J= 7.2, 11-1), 7.07 (t, J= 7.7, 11-1), 7.10
7.32 (d, J= 8.2, 11-I), 7.56 (d, J= 7.7, 11-1); 13C NMR (CD30D, 100 MHz) 6 13.4, 18.6, 28.8,31.8, 38.9, 47.4, 53.4, 107.7, 113. 1, 119.2, 120.5, 120.9, 123.3, 126.2, 128.9, 138.9, 169.5. HPLC: method 2; tg = 1.39 mm. HRMS (FAB) m/z [M + H]4 calcd l’or C,7H,8N40, 295.1559; found, 295.1558, L-N-Met.hylvalinyl.-L-cis4,5-methanoprollnenitrile TFA

Salt (33). ’H NMR (CD3OD, 500 MHz) 6 0.94 (in, 114), 1.11 m + d, J= 7.2, 4H). 1.18 (d J= 7.2, 3H), 2.02 (m, 11-1), 2.35 dd, J= 13,8, 2.3, IH), 2.50 Cm, 11-1), 2.65 (in, fl-I), 2.70 (s, 314), 3.85 (td, J= 6.0, 2.7. 1H), 4.42 d, J’ 5.0, 114), 5.20 (dd,

J- 10.5, 2.2, 114); ’C NMR (CDOD, 100 MHz) 6 14.4, 18.3, 19.0, 19.5, 31.4, 31.8, 33.8,’38.8, 47.6, 66.8, 120.7, 167.5.

-IPLC: method 2; tR 0.87 min. HRMS (FAB) m/ EM + H]4;alcd for C,3HN30, 222.1606; found, 222.1604,
L-ProIlnyl-L-cis-4,5-methanoprolinenitrile TFA Salt

34. H NMR CD3OD, 400 MJ-Tz 6 0.93 cm, 114), 1.10 cm, 11-1),.00 (m, 1H), 2.11 (m, 3M), 2.38 (dd. J’ 13.8, 2.2, IH), 2.66

rn, 2H), 3.43 (in, 21-1), 3.75 (td, J= 6.0, 2.7, 11-1), 4.85 (t, J tz 3.8. IH), 5.11 (dd, J- 12.6, 2.2, IH); ’C NMR (CDOD, 100 vIH 6 14.1, 19.2, 25.4, 29.8.30.0,32.0,38.3, 47.8, 61.1, 120.7,

168.4. HPtC: method 2; tp = 0.48 mm. HRMS (FAB) jn’z [M f- H]4 calcd for C,,H,sN3O, 206.1296; found, 206.1293.
L-Pipecolinyl-L-cis-4,5-methanopx-oflneiiftrile TFA Salt :35). ’H NMR.(CDOD, 500 MHz) a 0.93 (in, IH), 1.12 (q, J 1.5, IH), 1.20 Cm, 11-1), 1.60-2.10 Cm, 314), 2.35 (dd, J= 14.0, .5, 114), 2.65 (in, 11-1), 3.10 (m, 114), 2.66 (m,.IH), 3.45 Cd, J 1,0, lI-I), 3.56 (t, J= 5.0, 11-1), 3.68 (t, J- 4.5, 11-1) 3.76 (in,- 1-1), 4.48 (dd, J= 13.5, 3,5, 11-1), 5.06 (dd, J- 10.5, 2.5, 14; C NMR (CD30D, 125 MHz) 6 13.5, 16.5, 21.0, 21.7, 25.5, 29.1, 4.2, 40.5, 47.8, 61.4, 118.5 (q, J= 289), 120.7, 166.3 (q, J 6), 171.1. HPLC: method I; 0.85 min. MS in1z220 EM +

L-Methonlny1-Lcis-4,5-methanoprollnenjtrj1e TFA Salt :36). ’H NMR (CD30D, 500 MHz) 6 0.93 Cm, 114), 1.13 (q, J= r.1, 114), 2.03 (in, 114), 2.25 (s. 3M), 2.29 (m, 21-3), 2.36 (dd, J 13.8, 2.0, 14, 2.66 (in, 3H), 3.79 (td, J= 6.0, 3.0, 1H), 4.68 dd, J- 7,0, 4.5, iN), 5.15 (dd, J= 10.5, 2.0, 11-1); J3C NMR CD3OD, 100 MHz) 6 13.9, 15.2, 19.0, 29.6, 30.3,31.4,38.1, 17.4, 52.4, 120.4, 168.4 HPLC: method 1; tg = 1.18 min. MS niz 240 [M + H]4.

(5)’Cyc1obuty1glycmnyl-LcJs4,5:rnethanoprounenj. rile TFA Salt (37). ’H NMR (1)0, 400 MHz) 6 0.85 (in, IH),.05 (in, 114), 1.73 (in, IH), 1.92 (in, 71-1), 2,30 (dd, J- 14. 1,

.2, IH), 2.56 Cm,]H), 2.89 (m, 11-), 3,70 (in, 114), 4.45 (d, J"’ L4, 11-0, 5.06 (dd, J= 10.8, 2.4, 114); ’C NMR (1D0, 400 MHz) 13.8, 17.8, 18.3, 24,5, 24.7,30.1,38.0, 46.8, 55.1, 119.9, 168.3. RMS (ESI),m’z EM + H]4 calcd for C,2H,7N30, 220,145; ound, 220.144, HPLC: method 1: -.tR = L26 min. Anal. C,2H,7N30’1.00TFA’0.671-1z0). C, H, N. F.

(5)-Cyclopenty1g1ycinyl-L-cJs-4,5methanopro11neziJ.

rile TA Salt (38). ’H NMR (DO, 400 MHz) 60.86 (in, 114),.04 (q, J- 1.1, 11-1), 1.25’-L80 (in, 8H), 1.93 Cm, 114), 2.32

id, J= 11,5, 2.8, 1H), 2,40 (in, 114), 2.59 (in, IN), 3.70 (td, J 6,1, 2.6, 114), 4.43 Cd, J"7,5, 114), 5.09 (dd, J- 11.0, 2.6, H); 13C NMR (DiO, 100 MHz) 6 13.5, 18.2, 24.3, 24.7, 27.9,

.8,4, 30.2, 37.9, 41,3, 46.8, 55.1, 119.6, 169.1. HRMS (FAB) rifz [M + H]4 calcd for C13H,gN30, 234.1602; found, 234.1606.

nat. (C,3H,N0’1.23TFA) C, H, N.
($)-Cyclohexy1g1yciny1.Lc1$4,5methanoproj1nenj.

rile TFA Salt (39). ’H NMR (400 MHz, CD30D) 6 0.90 (in, H), 1.05-1.35 (in, 61-), 1.70-1.90 (m, 514), 2.00 (in, 1H), 2.15

n, 114), 2.37 (dd, j= 13.6, 1.8, 114), 2.63 (in, 11-1), 3.83 (in. H), 4.35 (d, J = 5.7, IH),. 5.17 (dd, J= 10.6, 2.2, 1H); ’C IMR (D20, 100 MHz) 6 13.9, 19.1, 26.7, 26.9, 27.1, 28.5, 30.0, 1.4, 38,4, 40.3, 47.1, 57.2, 120.5, 168.5. FIRMS (FAB) rn/z [M J-I] calcd for C141-321N30, 248.1768; found, 248.1763. Anal. H2,N30’1.00TFA) C, H, N.
(S)-1-Methylcyclobut-1-ylglycinyl-L. cis-4,5-methano-roluneriltrile TFA Salt (40). ’1-1 NMR (020, 400 MHz) 6

Ldop,..ZA’?

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0.99 (in, IH), 1.19 (q, J= 8.8, 6.6, 11-0.1.37 (s, 31-), 1.89 (in, 31-), 2.12 (m, 214), 2,24 (in, 11-1), 2.47 (dd, J= 13.6, 2,6, 11-), 2.54 (in, 11-1), 2.72 (m, IH), 3.90 (in, 11-1), 4.82 (s, IH), 5.27 (dd, J= 10.8, 2.4, 114); 13C NMR (020,400 MHz) 6 14.1, 15.1, 18.5, 21.1, 30.0, 30.9, 31.0, 38.6, 40. 1, 46.8, 59.0, 120.0, 167.8. HRMS (ESI) mIz [M + H] calcd for C13H,,,N30, 234,1607; found, 234.1592. i-IPLC: method 3; 4, = 3.55 mm. Anal., (C13H,9N20’l.00TFA) C, 1-I, N, F.

(5)-1Methylcyclopent-1.yIglyciny1-L-cis-4,5-inethano- prolinenitrile TFA Salt (41). ’H NMR (D20,.400 MHz) 6
0.82 (in, 1H), 0,95 (s, 31-), 1.00 (q, J" 7.1, 1H), 1.25-1.75 (in.

8H), 1.90 (in, 11-3). 2,28 (dd, J= 14.0, 2.7, 11-), 2.54 (m, 11-1), 3.10 td, J- 6.1.3.L IH), 4,35 s, 114), 5.08 dd, J 8.5, 2.6,

11-3); 13C NMR (1)20. 100 MHz) 6 13.8, 14,5, 21.3, 23.2, 23.4,

30.0, 36.2, 36.7, 45.7, 46.7, 58,8, 119.8, 168.2. HRMS (FAB) nilz EM + H]4 calcd for C14HN30, 248.1756; found, 248.1749.
Anal. (C,4J-32N30’i.00TFA’0.89H20) C, H, N. (5)-l-MethyIcycIohex-1-.ylglycinyl-L-cis-4,5-methano-. prohnenitrile TFA Salt (42). ’H NMI (D0, 400 MHz) 6. 0.86 (m, 114), 1.03t, 314), 1.07 (iii, ZH), 1.39 (in, 91-), 1.95 (in, 114), 2,31 (dd, J = 13.6, 2.6, 114), 2,58 (in, IH), 3.76 (in, 114), 4,26 (s, 1H), 5.12 (dd, J= 11, 2.6, iN); 13C NMR (1)20, 400 lvfl-Iz) 6 13.8, 1&2,18.5, 30.9, 21.0, 25.3, 300, 33.5, 34.1, 37.9,. 38.9, 46.7, 60,0, 119.8, 168.0, HRMS (ESI) m’z EM + H14 calcd

for C,5H23N30, 262.1920; found, 262.1904. HPLC: method 3; 4, = 4.77 mm. Anal. (C,5,H23N30.1.00TFA.0.50H20) C. H, N,

F.

Representative Example of Preparation: General

Method C. 1-tert-Leucinyl-L-cis-3,4-methanoprollnenl- trlle TFA S1t (30). An oven-dried rb flask was charged with
cls-3,4-methanoprc1J.neamide TFA salt (50 mg, 0.21 mmol), Nteributy1oxycarbonyl-L-tert-leuc1ne (48 mg, 0.21 mnmol), benzotriazol-1-yloxytripyrrolidlnophosphcnjum hexaflucro-phosphate (164 mg, 0.32 mmol), 4-methylmnorpholine (70 aL, 0.63 mmol), and CH2C12 (4 mL). The flask was sealed under

nitrogen atmosphere and stirred overnight. The product was purified by solid-phase extraction using a United Technology silica extraction column (2 g ofsorbent in a 6 mL column) by loading the material on a silica column and successively washing with CH2Cl2 (5 ml,), 5:95 CH30H-CH202 (5 mL), 7:93 CH30H-CH2C12 (5 mL), and 12:88 0420H-CH202 (10 mL). The product-containing fractions were pooled and con- centrated under reduced pressure to give the desired amide

compound (64 mg, 90%). ’H NMR (CD30D, 400 MHz) 6 0.69 (in, 11-1), 0.78 (q, J= 4.1, IH), 1.00 (s, 9H), 1.42 (s, OH), 1.74 (in, 114), 1.93 (in, 1H), 3.82 (d, J= 9.2, 1H), 3.98 (in, 11-1), 4,16 (a, 11-I), 4.61 (ci, J= 5.2, 114); ’C NMR (Ct)0D, 100 MHz) 6 8.8, 17.1, 21.0, 26.8, 28.7, 36.1, 52.5, 60.2, 62.5, 80.7, 158,0, 174.0, 175.0. MS rn1z 340 [M + H]4.

An oven-dried flask was charged with N-Boc-rert-leuclnyl- ds-4,5-rnethanoprolineamlde (65 mg, 0.19 mmol), Imidazole

(31 mg, 0.46 mmol), and pyridine (2 mL). The flask was sealed under nitrogen atmosphere and cooled to -30 C. Slow addition of POd3 (132 mg, 81 yL, 0.86 mmol) gave, after mixing, a thick slurry that was mixed at -30 C for 1 h and concentrated. The product was purified by silica gel column

chromatography using 1 5 EtOAc-’CH2C12. The product- containing fractions were pooled and concentrated under
reduced pressure to give the desired N-Boc-protected title

compound (50 mg, 8096). ’H NMR (COd3, 400 MHz) 6 0.65 (q, J= 4.7, 114), 0.99 (a, 914), 1.00 (in, 1H), 1.42 (s, 9H), 1.89 (in, 11-1), 2.00 (m, H-I), 3.80 (d, J= 9.2, 1H), 3.99 (in, IN), 4.15 (d, J- 9.2, iN), 4.72 (ci, J 4.9, IH), 5.18 (d, J= 9.6, IN); 13C

NMR (COd13, 100 MHz) 6 11.1, 18.5, 19.1, 26.2, 28.3, 35.3,

50.0, 50.7, 58,5, 79.9, 116.9, 156.0, 173.0. MS n7/z322 [M + H]4.
An oven-dried flask was charged with N-Boc-tcrt-leuclnyl. -

3,4-methanoprolinenitriie (0.45 mg, 0.14 mmol), CH2C12 (1 mL), and TFA (1 mL). The reaction mixture was stirred for 1
h at room temperature, diluted with toluene (4 mL) and

concentrated under reduced pressUre. The product was puri- fied by reverse-phase preparative HPLC on a YMC S5 01)3 20 mm x 250 nm column to give the title compound (20 mg, 45%). Purification conditions were the following: gradient

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elul
OH
wa

5 0.

(m,
NM

3H),
(m.
5,5,
10.7

(q,J
tR =

1.05

IH),
3,90
611

(q, -

, L(25)
3H),
IH),
11-0,
10,8
118.

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bn from 10:90:0.1 CH30H-H20-TFA to 90:10:0.1 CH3 H20-TFA over 1.8 flow rate, 20 thL/xnln; detection length, 220 rim; t, 10 min. ’H NMR (CD30D, 400 MHz)
S (q. J’’ S.7,114), 1.10 (s + m,. 10H), 1.95 (in, 11-1), 2.13
1-1), 3.80 Cm, 21-1), 3.93 (s, 114), 4.84 (d, Jr" 5,0, 11-1); "SC (CD20D, 100 MHz) 611.3, 19.1, 20.1, 26.8, 35.8, 52.1 60.5, 117.3 (q, J- 289), 119.2, 161.6 (q, J= 38), 170.5.
- (C,iH19N20’1.00TFA) C, H, N, F.
peptide Nitriles Prepared by General Method C. leucinyl-L-trans-4,5--methanoprollnenitrile TFA Salt ’H NMR (1)20, 500 MHz) 5 0.72 (in, 1H), 0.92 Ct, J= 7.3, 1.07 (d+m, J
H), 2.43 (in, 1H), 2.60 Cm, 1H), 3.63 (in, 1H), 4.39 (d,.1= 1H), 4.82 (q, J 9. 1, IH); 13C NMR DzO, 125 MHz) 6 14.6, 18.2, 18.8, 23.7, 31.6, 36.4, 37.1, 50.9, 56.6, 116.6 =290), 118.5, 163.2 (q,J35), 170.2; HPLC: method 3; 2.89 min. HRMS (FAB) m/z EM + 1-1j calcd for [11430, 222,1606; found, 222.1601.
(soleuclnyl-L-trans-2,3-inethanoprolinenitrlle TFA (23). ’H NMR (D20, 500 MHz) 6 0.84 (t, J= 7.3, 3H), (d,J7.3,3}f), 1.14--1.30(m2}l), 1.64 (in, IH), 1.76Cm, 2.10 (m, 21-1), 2.27 (in, 11-1), 2.75 Cm, 11-1), 3.08 (m, 1H), (in, 111), 4.30 (d, J 2.3, IH); ’3C NMR (D20,125 MHz) 5, 15.0, 22.4, 23.6, 24.0, 28.1, 36.5, 42.0, 44.8, 60.7, 116.6
- 290), 117.7, 163.2 (q, J- 35), 165.6. HPLC: method 3; 2.49 HRMS (FAB) m/z (M + HJ calcd for 19N30, 222,1606; found, 222.1611.
[soleucfnyl-L-cis-3,4-methanoprolirlenitrfle TFA Salt ’H NMR (500 MHz, 1)20) 5 0.53 (in, 1H), 0.90 Ct, J= 7.3, 1.00 (d, J= 6.9, 3H), 1.08 (in, IN), 1.18 (in, 11-I), 1.48 (in, 2.00 (in, 21-1), 2.20 (rn, 1H), 3,83 (s, 21-1), 4.10 (d, J= 5.5, 4.89 (d, J= 5.0, IH); 13C NMR 020, 125 MHz) 6 10.1, 14.6, 18.5, 23.8, 36.4, 50.7, 51.2, 56.4, 116.6 (q. J= 290), 1, 163.2 (q, J= 35), 170.4. HPLC: method 3; t, = 3.15 HRMS (FAB) inlz [M + H]" calcd for C,2H,N30, 1606; found, 222.1605.
Vaflnyl-L-cis-3,4-methanoprollnerdtrile TFA Salt (27). MR (CD30D, 400MHz) 6 0.55 (q,J- 5.7, 1H), 1.03 (d, J L 3H), 1.08 (d + in, J= 7.01"41-1), 1.95 (in, 11-I), 2.10 (in, 2.20 Cm, IH), 3.80 (d, J= 9.5, 11-0, 3.86 (dd, J= 9.5.4.8, 4.00 (d, J’ 5.7, IH), 4.84 Cd, J= 4.8, IH); ’3C NMR (CD3-100MHz) 6 11.5. 17.8, 19.2, 19.8, 20.2, 31.7, 51.6, 52.1, 118.4, 118.8 (q,)’ 289). 162.0 (q, J-’ 37), 171.0. Anal. 127N30’1.30TFA) C, H.N, F.
,presentative Example ofPreparation ofIntermedi-19 and 20: General Method D. 2-Cyclopentyliden-tonic Acid Diethyl Ester (jib). A mixture of dry THF nit.) and dry CCI,, (25 mL) was cooled to 0 ’C and treated T1C14 (11.0 mL, 0.1 mol). The resulting yellow suspension stirred at 0 oC for 5 mm, treated sequentially with pentanone (4.4 mL, 0.05 mol) and freshly distilled diethyl

nate (7.6 niL, 0.05 mol), and then stirred at 0 ’C for 0.5 ae reaction mixture was then treated with a solution of
yridine (16 mL, 0.20 mol) in dry THF (30 mL) and stirred ’C for 1.0 1-i and then at room temperature for 72 h. The ion mixture was quenched with water and extracted with

(2 x 100 mL). The combined organic extracts were ad with brine and saturated NaHCO3, dried (MgSO4), and intrated under reduced pressure. The residue (12,1 g) was led by flash column chromatography (EtOAc-hexanes,
to give the title compound (5.25 g, 46%). ’H NMR (CDC13, 4:r-rz) 6 1.30 (in, 6H), 1.73 (nn, 41-1), 2.68 (m, 414), 4.24 (m, LCIMS m/z 227 EM + H]"’.
1-Methylcycloperit-1-yJ)malonic Acid Monoethyl Es-lSb). A mixture of 3.0 M meihymmagnesium iodide (3.1 .4 mmol) and cuprous chloride (10.6 mg) was stirred at treated with a solution of 2"ryclopentylldenemalonlc acid

yl ester (17b) (1.41 g. 8.24 mmol) in dry Et20 (2 mL) over andtirred at 0 ’C for I h and then at room temperature

h. The reaction was then quenched by the dropwlse Ion of Ice-water (15 mL) followed by 3 M HC! (3.7 niL). nixture was then extracted with EtOAc (3 x 25 mL). The med organic extracts were washed with 1% NaiSzOs and dried (MgSO,,), and evaporated (1.57 g). The residue was

purified by flash column chromatography on silica gel (5:95 Et20-hexanes) to give the diethyl ester compound (1.09 g, 68.7%). ’H NMR (CDC13, 400 MHz) 61.15 (s, 3H), 1.26 (t, J-7.0, 611), 1.7 (m, 2H), 1,55-1.70 (m, 6H), 3.34 (s, 1H), 4,20 (q, J= 7.0, 41-1); Ir NMR (CDC13, 100 MHz) 6 14.1, 23.7,24,0, 38.6, 43.9, 50.7, 60.8, 168.7. LC/MS zn/z 243 fM + H)". A solution of i:-(l-niethylcyclopenty!)rnalonlc acid diethyl ester (736 mg 3.14 mmol) in a mixture of EtOH (12 niL) and TI-IF (6 niL) was ireated with 1.0 M NaOH (3.04 mL, 3.04 minc,1) and stirred a; room temperature for 24 h. The reaction mixture was evapor&ted to a syrup. dissolved in water (10 mL), and extracted with Et20 (15 nnL)..The aqueous phase was acidified with 1 M HCI to pH 2.0 and extracted with EtOAc (3 x 25 ant.). The combined organic extracts were washed with brine, and evaporated to give the compound
g. 95%). R, 0.56 (CH2Cl2-CH30H, 9:1; FMA). ’H NMR (CDC13, 400 MHz) 6 1.15 (a. 311), 1.28 (t, J= 7.0, 3H), 1.55-1.70 (in, 8H), 3.75 (s. 111), 4.20 (q, J = 7.0, 214); "C NMR (CDC13, 100MHz) 6 14.0, 23.2, 23.9, 38.5, 44.5, 60.4, 61.4, 169.7, 173.5, LC/MS rn/z 215 EM + 1-11+.

enzyIoxycarbony1amino(i-rnethy1cyclopent-1-yI)ace-tic Acid Ethyl Ester (19b). A solution of 2-(I-methylcyclo-pentyl)malonic acid monoethyl ester (0.48 g. 2.26 mmol) In dry benzene (2,85 mL) was treated with triethylanulne (0.3 mL, 2.26 mmol)and diphenyiphosphoryl azide (0.5 mL, 2.28 mmol), refluxed for 1. h. and cooled- to room temperature. The solution was treated with benzyl alcohol (0.35 mL, 3.39 mmd), refluxed for 17 h, cooled, then partitioned between aqueous 10% citric acid solution (3.8 mL) and EtOAc (2 x 25 niL). The combined organic extracts were washed with 5% NaHCO3 solution, dried (MgSO,,). and &iaporated. The remainder was purified by flash column chromatography (1:9 EtOAc-hexanes) to give the title compound (1.20 g, 1001/6). ’H NMR (CDC13, 400 MHz) 6 0.92 (a + t, J= 7.0, 611), 1.20-1,80 (in, 8H), 4.20 (in, 311), 5.11 (s, 21-1), 5.38 (in, 111), 7.30-7.50 (an. 511). LC/MS in/z 342 [M +

tert-Btztyloxycarbonylamino(1-mnethylcyclopent-1-yl)- acetic Acid (20b). A solution of benzyloxycarbonylaminu(1-methylcyclopentyl)acetic acid ethyl ester (0.798 g. 2,28 mmol) In EtOAc (35 mL) was treated with 10% palladium hydroxide on carbon (190 mg) under an atmosphere of H2 at room temperature for 20 h. The mixture was diluted with EtOAc and filtered through a Celite pad, washing the pad well with EtOAc (2 x 35 mL). The filtrate5 was evaporated to dryness to give the crude amine (0.42 g, 100%). The crude amine (0.42 g, 2.25 mmol) in a mixture of THF (9 mt.) and water (9 rnL) was treated with dl-tert-butyl dicarbonate (0.73 g, 3.35 mmol) and K2CO3 (0.68 4.54 mmol) and stirred at room temperature for 36 h. The reaction mixture was partitioned between water and Et20. The aqueous fraction was then extracted with Et20 (3 x 30 mL), and the combined organic extracts were washed with brine, dried (MgSO), and evaporated. The remainder was purified by fksh column chromatography (1:9 EtOAc-"-hexane) to give the desired N-Boc-protected ester (0.57 g; 92%). ’H NMR (CDC13, 400 MHz) 6 0.95 (s, 311), 1.28 (t, J= 7.0, 3H), 1.30 (in, 2H), 1.44 (a, 91-1), 1.55-1.80 (rn, 6H), 4.20 (q, J- 7.0, 211), 5.11 (d,.i= 6.0, 111); 13C NMR (CDC13, 100 MHz) 6 14.2, 225, 23.9, 243, 28.3, 36.8, 45.8, 60.5, 80.0, 155.7, 172.5; LC/MS rn/z 286 [M + H]". A solution of tert-butyloxycarbony-lamino(1 -methylcyclopentyl)acetfc acid ethyl ester (0.55 g 1.97 mmol) in MeOH (6.9 niL) and THF (6.9 mL) was treated with 1,0 M NaOH 2.9 mL, 2.9 mmol) and stirred at room temper-ature for 24 h. The reaction mixture was evaporated, dissolved

in water (10 niL), and extracted with Et20. The aqueous phase was acidified to pH 2 with 1.0 M HC1 (2.9 mL) and extracted with EtOAc (3 x 30 mL). The combined organic extracts were washed with brine, dried (MgSO4, and evaporated to give the
title compound (0.81 g. 96%): mp = 151-152 ’C, ’H NMR

111-1), 1.55-1.80 (in, 611), 4,20 (d, J- 7.0, 211), 5.11 (d, J-

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amino acids prepared according to this method were rerted to the corresponding clipeptlde nitrites using general

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ornpatatlona1 Assessment Methods. Conformations generated for both methanoproithenitrile and prolineni-forms ofthe N-terminal tert-Ieucine dipeptide and alanine ptides using Macromodel with OPLSAA parameters and SA salvation model.-4Two conformations were selected for ’getic comparisons: the global minimum, which has the amide rotamer (Figure 2, lower structure), and an anti ormation, which brings the reactive amine and nitrile close icli other. (Figure 2, upper structure). These conformations reminimized using ab initlo quantum mechanics with the

YP DFT method and the 6.31+G** basis set lxi Gaussian. To approximate van dot- Waals and electrostatic con- ztios, relative energies of the conformations were also

1ated using Insight’s CFF force fie1d. Ab energetic ntizations and force field calculations were also performed ie praline and metharioproline compounds with a simple amide cap in both conformations.

Vitro Assays, All compounds were tested In vitro against àed porcine DPP-IV as previously published.31 Inhibition
etermined using the substrate H-Gly-Pro-PNA. Produc-of p-nitroariiltne was measured at 405 rim at 8s Intervals 15 Reactions were initiated by addition of the and data collection was started Immediately. The

tItons were run at 11 substrate concentrations (ranging

0 to 3000 AM) and 7 inhibitor concentrations (ranging 1000 nM). Enzynieeactions contained a final volume /L, ATE buffer (100 mM Aces, 52 mM Tris, 52 mM n1amthe, pH 7.4), 4.5 riM porcine DPP-W, and 1% DMSO.

’Vivo Assay Methods. Male Zucker rats (Harlan) hfrtg between 400 and 450 g were housed in a room that

mintairted on a 12 h light--dark cycle and were allowed acess to. normal rodent chow and tap water. The day -e he experiment, the rats were weighed and divided into
ol and treated groups ofsix. Rats were fasted 17 h prior o start of the study. On the day of the experiment, animals

dosed orally with vehicle (water) or DPP-IV inhibitors nolkg) at -30 rnfri. Two blood samples were collected at and 0 min by tail bleed. Glucose (2 glkg) was administered ya 0 Additional blood samples were collected at 15,
0, 00, and 120 mm. Blood samples were collected into

A cnita1nilng tubes from Starstedt. Plasma glucose was ’nzThped by Cobas Mire (Roche Diagnostics) by the glucose

oxidation method.

Rat.plasma insulin was assayed using an ELISA kit from

Crystal Chem Inc.. Properly diluted plasma samples were d4ed t the ELISA inicroplate. Insulin was then detected

ing guriea pig anti-Insulin set-urn. DPP-1V activity In rat asm as assayed ex viva using Ala-Pro.AFC’TFA, a fiuorescexice-generating substrate from Enzyme Systems Prod-

ucts. A C1 aL plasma sample was mixed with 200 4aL of reaction buffer, 50 mM Hepes, and 140 thM NaCl. The buffer contained 0.1 mM Ala-Pro-AFC’TFA. Fluorescence was then read for 2l mm on a Perseptive I3iosystem Cytofluor-LT at 360

rim excitation wavelength and 530 nrn emission wavelength. The initial rate ofDPP-1V enzyme activity was calculated over the fIrst 20 mini of the reaction, with imits/rnL defined as the rate of Inc. ease offluorescence Intensity (arbitrary units) per milliliter o plasma. All In viva data presented are the mean SE (n 6). Data analysis was performed. using ANOVA

followed b Fisher Post-hoc.

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SUPERINTENDENCIA DE INDUSTRIA V COMERC1O DIVISION DE NUEVAS CREACIONES

2020/
Bogota, D.C.,

Doctor
Juan Pablo Cadena Sarmlento

MYERS SQUIBB COMPANY

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mo resultado del examen de patentabilidad de la solicitud de patents de invención 0118857, realizado segn el artIculo 46 de la decision 486 de la comisión do la munidad Andina, se notifica:

DE SUMINISTRO DE INFORMACIÔN

art. 46 do la DecisiOn 486 establece quo La Oficina Nec/anal competente padre uerir el informe do oxpertos o do organismos cientificos a tecnológicos quo so nsideren idOneos pare quo emitan opinion Sabre ía patentabilidad de la invención. imismo, cuando Jo estime conveniente, padre requeririnformes do otras oficinas do ,piedad industrial, be ser necesario, a efectos dol examen de patentabilidad y a iuerimiento do ía oficina nacional competente, el solicitante proporcionarO, en un ?zo quo no excederÆdo 3 meses, uno a,viÆs documentosrelativos a una o mØs ilcitudes extranjeras referidas total a parc/a/monte a Ia misma invención quo so Imine:..b,) copia do los resultados do exØmenes do novedad a do patentabilidad ctuados respecto a esa soicitud extranjera. Si el solicitante no presentara los umentos requeridos dentro del plaza seflalado en el presente articulo Ia oficina cionai competente donegara ía patente"

Como consocuencia de la bósqueda do anterioridades do materia relacionada con la de ia presente solicitud, se determinO que exists en el estado de la tØcnica los siguientes docurnentos que eventualmente afectarlan los requisitos de patentabilidad de Østa solicitud:

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2) TVEREZOVSKY V V ET AL: "Synthesis of(2S3R, 48)-3,4-methanoproline and analogues by cyclopropylidene Insertion" TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 53, no. 43,27 october 1997 (1997-10-27), pages 14773-14792, XP004I 06307. ISSN: 0040-4020.

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ndo que las publicaciones referenciadas hacen parte del resultado del examen de squeda realizado para la solicitud WO 0168603, equivalente a la solicitud lombiana aqul estudiada, y no siendo viable la consecución de dicho articulo por to despacho, se requiere que el solicitante, en virtud de to estabtecido on of art. 46 la Decision 486 de la ComisiOn de la Comurildad Andina, proporcione la blicación completa, con lo que podrâ realizarse un anÆllsis absoluto de la tentabilidad de la invención correspondiente a la presente solicitud.

se el presente oficlo corIfornfle a lo dispuesto on el numeral 5.2, literal e), del
5 del tFtulo rimero d.9’ la CWcular Unica NO 10 de 2001.

Alx Carmenza Csedes de VergeS
Jefe de la DivisiOfi de Nuevas Creaciones

El interior requerimiento fue notificado porfijaclOn en lista, de fecha

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