

G. HOLLMANN RESTREPO
ABOGADOS LTDA.

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SUPERINDUSTRIA Y COMERCIO
Radicación: 03046600 00000002 Folios: 94
Fecha (FOLIO): 2004-10-20 16:06:10
Tramite: 011 PCI-PRIME 379 FASE NÚM. 023 COMPLETA
Dependencia: 0200 DIVISION DE NUEVAS CREACIONES

54223

SUPERINTENDENCIA DE INDUSTRIA Y COMERC.

DIVISION DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. 03 046600

Solicitud de patente PCT fase nacional

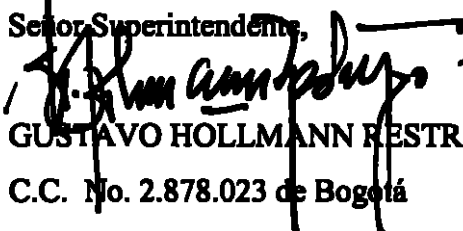
Solicitante: SCHERING AKTIENGESELLSCHAFT

Yo, GUSTAVO HOLLMANN RESTREPO, vecino de Bogotá D.C., portador de la tarjeta profesional No. 5903, obrando como apoderado en este negocio, digo a usted que acompaño al presente memorial, para que sean agregados al expediente de la referencia los siguientes documentos:

- Con carácter de anexión voluntaria, copia certificada de la solicitud de patente No. 60/246,299 presentada en Estados Unidos el 6 de noviembre de 2000, con su correspondiente traducción al español de la carátula y Reivindicaciones y que ha servido de base para la reivindicación de prioridad de la solicitud internacional No. 12607.

Los documentos de cesión de los inventores al solicitante, debidamente traducidos y legalizados.

Señor Superintendente,


GUSTAVO HOLLMANN RESTREPO

C.C. No. 2.878.023 de Bogotá

T.P. No. 5903

mm.

**DOCUMENTO DE CESIÓN
ASSIGNMENT DOCUMENT**

Los suscritos,
(We, the undersigned)

CHRISTOPH-STEPHAN HILGER
con domicilio en Berlín, Alemania

BERND JOHANNSEN
con domicilio en Dresden, Alemania

JÖRG STEINBACH
con domicilio en Bischofswerda, Alemania

PETER MÄDING
con domicilio en Dresden, Alemania

MEREDITH HALKS-MILLER
con domicilio en Woodside, Estados Unidos de América

RICHARD HORUK
con domicilio en Lafayette, Estados Unidos de América

HARALD DINTER
con domicilio en San Rafael, Estados Unidos de América

RAJU MOHAN
con domicilio en Moranga, Estados Unidos de América

JOSEPH E. HESSELGESSER
con domicilio en San Francisco, Estados Unidos de América

declaramos que somos los inventores de la invención que lleva el título
(declare that we are the inventors of the invention titled)

RADIOPHARMACEUTICALS FOR DIAGNOSING ALZHEIMER'S DISEASE

**PRODUCTOS RADIOFARMACÉUTICOS PARA EL DIAGNÓSTICO DE LA
ENFERMEDAD DE ALZHEIMER**

y que hemos cedido el derecho sobre esta invención en Colombia, junto con los
privilegios

(and that we have assigned our rights over this invention in Colombia, together with its
privileges)

y anexidades a favor de
(and attachments, to)

SCHERING AKTIENGESELLSCHAFT
Berlín, Alemania

- 1) *Christoph Stephan Hilger*
.....
HILGER, Christoph-Stephan
- 2)
JOHANNSEN, Bernd
- 3)
STEINBACH, Jörg
- 4)
MÄDING, Peter
- 5)
HALKS-MILLER, Meredith
- 6)
HORUK, Richard
- 7)
DINTER, Harald
- 8)
MOHAN, Raju
- 9)
HESSELGESSER, Joseph E.

4
268
258

Ich habe das Mitwirkungsverbot nach § 3 Abs. 1 Nr. 7 BeurkG erläutert. Meine Frage, ob eine Vorbefassung im Sinne der Vorschrift vorliege, wurde verneint.

Die vor mir geleistete Unterschrift

des Herrn Dr. Christoph Stephan Hilger,
geboren am 29. Juni 1955,
geschäftsansässig in 13353 Berlin, Müllerstraße 178,

- ausgewiesen durch Vorlage seines Reisepasses Nr. 2471693259 -

beglaubige ich.

Berlin, 20. Mai 2003

Notar

Kostenberechnung gem. §§ 141, 154 KostO

Geschäftswert: 12.000,00 €

Beglaubigungsgebühr §§ 32, 45 I	15,00 €
Wegegebühr gem. § 58	15,00 €
Schreibauslagen §§ 136, 152	1,00 €
Postauslagen §§ 137, 152	1,44 €
	<u>32,44 €</u>
16 % Mehrwertsteuer	5,19 €
	<u>37,63 €</u>

Berlin, 20. Mai 2003

Notar

Apostille
(Convention de La Haye du 5 octobre 1961)

1. Land: Bundesrepublik Deutschland

Diese öffentliche Urkunde

2. ist unterschrieben von Dimitri R e m p e n

3. in seiner Eigenschaft als Notar

4. sie ist versehen mit dem Siegel
des Notars

Bestätigt

5. in Berlin

6. am 23. Mai 2003

7. durch den Präsidenten des Landgerichts in Berlin

8. unter Nr. 9101 a E - F. 1531/2003

9. Siegel

10. Unterschrift

Im Auftrage

Bünning
(Bünning)

Vorsitzende Richterin am Landgericht

260 Z
270

Traducción oficial del alemán de las autenticaciones de firmas y las apostillas adjuntas al documento de cesión mediante el cual la invención PRODUCTOS RADIOFARMACEUTICOS PARA EL DIAGNOSTICO DE LA ENFERMEDAD DE ALZHEIMER es cedida por sus inventores a Schering Aktiengesellschaft de Berlín, Alemania.

Registro Notarial No. R 275 para el año 2003

Yo expliqué la prohibición de participación según el Artículo 3 inciso 1 No. 7 de la Ley de Notarización. Mi pregunta sobre si existía algún impedimento de acuerdo con la mencionada disposición fue contestada negativamente.

Autentico la firma que aparece anteriormente, ejecutada en mi presencia por el

Señor Dr. Christoph Stephan Hilger,
nacido el 29 de Junio de 1955,
domicilio comercial 13353 Berlín, Müllerstraße 178,
quien se identificó presentando su pasaporte No. 2471693259.

Berlín, 20 de Mayo del 2003

Dimitri Rempen, Notario en Berlín
(Firma y Sello Oficial)

Nota de gastos Artículos 141,154 Disposición de Impuestos

Valor del negocio: 12.000,00 EUROS

Impuesto Artículos 32, 45I	15,00 EUROS
Impuesto Artículo 58	15,00 EUROS
Impuesto Artículos 136, 152	1,00 EUROS
Impuesto Artículos 137, 152	1,44 EUROS
16% IVA	5,19 EUROS
Total	37,63 EUROS

Berlín, 20 de Mayo del 2003

Dimitri Rempen, Notario en Berlín (Firma)

Apostilla

(Convención de La Haya del 5 de Octubre de 1961)

1. País: República Federal de Alemania

Este documento público

2. Ha sido firmado por: Dimitri Rempen

3. En su calidad de: Notario

4. Y lleva el sello: Del Notario

Confirmado

5. En: Berlín

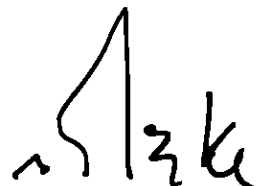
6. El: 23 de Mayo del 2003

7. Por: El Presidente del Tribunal Regional en Berlín

8. Bajo el No.: 9101 a E-F. 1531/2003

9. Sello: EL PRESIDENTE DEL TRIBUNAL REGIONAL EN BERLIN

10. Firma: Por encargo: Bünning, Jueza Presidenta en el
Tribunal Regional


ADOLF WATZKE
Traductor Oficial
Res. 2433 Minjusticia

MINISTERIO DE
RELACIONES EXTERIORES

Adolfo Watzke
COTA MIA A. A. C. EN EL
DOCUMENTO DESEMPA
LAS FUNCIONES INDICADAS

MINISTERIO DE
NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2003 OCT - 8 P 12: 33 5
Jefe de Legación A. A. C. EN EL
DOCUMENTO DESEMPA
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2003 OCT - 8 P 12: 40
Jefe de Legación A. A. C. EN EL
DOCUMENTO DESEMPA
LAS FUNCIONES INDICADAS

15
279
262

Nummer **0648** der Urkundenrolle des Jahrgangs 2003 der
Notarin Bettina Körting mit Amtssitz in 01309 Dresden, Haydnstraße 21

Vorstehende, vor mir der unterzeichnenden Notarin vollzogene Unterschriften von

1. Herrn Prof. Dr. Bernd Johannsen,
geboren am 27. Mai 1939,
wohnhaft in 01324 Dresden, Lausitzer Straße 5,
2. Herrn Dr. Jörg Steinbach,
geboren am 28. November 1952,
wohnhaft in 01877 Bischofswerda, Geschwister-Scholl-Straße 14,
3. Herrn Peter Mäding,
geboren am 10. Juni 1955,
wohnhaft in 01277 Dresden, Voglerstraße 19,

ausgewiesen durch amtlichen Personalausweis Nr.
zu 1. 7881142227,
zu 2. 7985126141,
zu 3. 7658603993,

beglaubige ich.

Dresden, am 29. August 2003



Körting, Notarin



Apostille

(Convention de la Haye du 5 octobre 1961)

1.) Land: Bundesrepublik Deutschland

Diese öffentliche Urkunde

2.) ist unterschrieben von	Bettina Körting
3.) in ihrer Eigenschaft als	Notarin in Dresden
4.) sie ist versehen mit dem Siegel der	Notarin Bettina Körting in Dresden

Bestätigt

5.) in Dresden	6.) am 02.09.2003
7.) durch den Präsidenten des Landgerichts Dresden	
8.) unter Nummer E 910a-247/03	

9.) Siegel



10.) Unterschrift

Gerd Halfar
Gerd Halfar



**DOCUMENTO DE CESIÓN
ASSIGNMENT DOCUMENT**

Los suscritos,
(We, the undersigned)

CHRISTOPH-STEPHAN HILGER
con domicilio en Berlín, Alemania

BERND JOHANNSEN
con domicilio en Dresden, Alemania

JÖRG STEINBACH
con domicilio en Bischofswerda, Alemania

PETER MÄDING
con domicilio en Dresden, Alemania

MEREDITH HALKS-MILLER
con domicilio en Woodside, Estados Unidos de América

RICHARD HORUK
con domicilio en Lafayette, Estados Unidos de América

HARALD DINTER
con domicilio en San Rafael, Estados Unidos de América

RAJU MOHAN
con domicilio en Moranga, Estados Unidos de América

JOSEPH E. HESSELGESSER
con domicilio en San Francisco, Estados Unidos de América

declaramos que somos los inventores de la invención que lleva el título
(declare that we are the inventors of the invention titled)

RADIOPHARMACEUTICALS FOR DIAGNOSING ALZHEIMER'S DISEASE

**PRODUCTOS RADIOFARMACÉUTICOS PARA EL DIAGNÓSTICO DE LA
ENFERMEDAD DE ALZHEIMER**

y que hemos cedido el derecho sobre esta invención en Colombia, junto con los
privilegios

(and that we have assigned our rights over this invention in Colombia, together with its
privileges)

y anexidades a favor de
(and attachments, to)

SCHERING AKTIENGESELLSCHAFT
Berlín, Alemania

- 1)
HILGER, Christoph-Stephan
- 2) *Bernd Johannsen*
JOHANNSEN, Bernd ✓
- 3) *Jörg Steinbach*
STEINBACH, Jörg ✓
- 4) *Peter Mäding*
MÄDING, Peter ✓
- 5)
HALKS-MILLER, Meredith
- 6)
HORUK, Richard
- 7)
DINTER, Harald
- 8)
MOHAN, Raju
- 9)
HESSELGESSER, Joseph E.

275
266

Traducción oficial del alemán de las autenticaciones de firmas y las apostillas adjuntas al documento de cesión mediante el cual la invención PRODUCTOS RADIOFARMACEUTICOS PARA EL DIAGNOSTICO DE LA ENFERMEDAD DE ALZHEIMER es cedida por sus inventores a la compañía Schering Aktiengesellschaft domiciliada en Berlín, Alemania.

Registro Notarial No. 0648 para el año 2003

Notaria Bettina Körting en 01309 Dresden, Haydnstraße 21

Autentico las firmas que aparecen anteriormente, ejecutadas en mi presencia por

1. Señor Prof. Dr. Bernd Johannsen,
nacido el 27 de Mayo de 1939,,
domicilio 01324 Dresden, Lausitzer Straße 5,
2. Señor Dr. Jörg Steinbach,
nacido el 28 de Noviembre de 1952,
domicilio 01877 Bischofswerda, Geschwister-Scholl-Str. 14,
3. Señor Peter Mäding,
nacido el 10 de Junio de 1955,
domicilio 01277 Dresden, Voglerstraße 19,

cada uno de ellos identificado respectivamente mediante su cédula de identidad

1. No. 7881142227,
2. No. 7965126141,
3. No. 7658603993.

Dresden, 29 de Agosto del 2003

BETTINA KÖRTING, NOTARIA EN DRESDEN

(Firma y Sello Oficial)

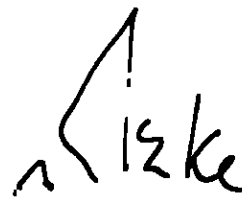
Apostilla

(Convención de La Haya del 5 de Octubre de 1961)

1. País: República Federal de Alemania
Este documento público
2. Ha sido firmado por: Bettina Körting
3. En su calidad de: Notaria en Dresden
4. Y lleva el sello: De la Notaria Bettina Körting en Dresden

Confirmado

5. En: Dresden
6. El: 02.09.2003
7. Por: El Presidente del Tribunal Regional en Dresden
8. Bajo el No.: E 910a - 247/03
9. Sello: EL PRESIDENTE DEL TRIBUNAL REGIONAL EN DRESDEN
10. Firma: Gerd Halfar



ADOLF WATZKE
Traductor Oficial
Res. 3438 Minjusticia

MINISTERIO DE
RELACIONES EXTERIORES

3/3/83
Cuya firma aparece en el
documento desempeña
las funciones indicadas

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

26/09/83 12:40

SELECCION DE CALIFICACIONES
1983-10-03

51783A

J-V 223 13
277
268

COLOMBIA

DOCUMENTO DE CESIÓN ASSIGNMENT DOCUMENT

Los suscritos,
(We, the undersigned)

CHRISTOPH-STEPHAN HILGER
con domicilio en Berlín, Alemania

BERND JOHANNSEN
con domicilio en Dresden, Alemania

JÖRG STEINBACH
con domicilio en Bischofswerda, Alemania

PETER MÄDING
con domicilio en Dresden, Alemania

MEREDITH HALKS-MILLER
con domicilio en Woodside, Estados Unidos de América

RICHARD HORUK
con domicilio en Lafayette, Estados Unidos de América

HARALD DINTER
con domicilio en San Rafael, Estados Unidos de América

RAJU MOHAN
con domicilio en Moranga, Estados Unidos de América

JOSEPH E. HESSELGESSER. ✓
con domicilio en San Francisco, Estados Unidos de América

declaramos que somos los inventores de la invención que lleva el título
(declare that we are the inventors of the invention titled)

RADIOPHARMACEUTICALS FOR DIAGNOSING ALZHEIMER'S DISEASE

PRODUCTOS RADIOFARMACÉUTICOS PARA EL DIAGNÓSTICO DE LA
ENFERMEDAD DE ALZHEIMER

y que hemos cedido el derecho sobre esta invención en Colombia, junto con los
privilegios
(and that we have assigned our rights over this invention in Colombia, together with its
privileges)

y anexidades a favor de
(and attachments, to)

SCHERING AKTIENGESELLSCHAFT
Berlín, Alemania

COLOMBIA

14
278
269

- 1)
HILGER, Christoph-Stephan
- 2)
JOHANNSEN, Bernd
- 3)
STEINBACH, Jörg
- 4)
MÄDING, Peter
- 5)
HALKS-MILLER, Meredith
- 6)
HORUK, Richard
- 7)
DINTER, Harald
- 8)
MOHAN, Raju
- 9)
HESSELGESSER, Joseph E.

SEE ATTACHED
ACKNOWLEDGEMENT

270 LF
279

State of California

SECRETARY OF STATE

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country: *United States of America*
This public document
2. *has been signed by RICHARD DAVID DUPLER*
3. *acting in the capacity of Notary Public, State of California*
4. *bears the seal/stamp of RICHARD DAVID DUPLER, Notary Public,
State of California*

CERTIFIED

5. *At Sacramento, California*
6. *the 6th day of June 2003*
7. *by Deputy Secretary of State, State of California*
8. *No. 158062*
9. *Seal/Stamp:*



10. Signature

Kevin Shelley
KEVIN SHELLEY
Secretary of State



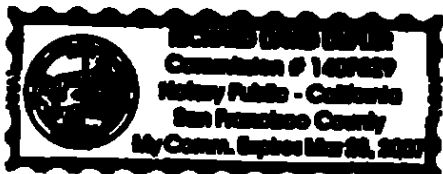
BY *M. O'Leary*

27.1 ¹⁶
280

CALIF RNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California
County of San Francisco } ss.

On MAY 17, 2003 before me, Richard David Dupler, Notary Public
Date Name and Title of Officer (e.g., "Jane Doe, Notary Public")
personally appeared JOSEPH E. HESSELBESSER
Name(s) of Signer(s)



☐ personally known to me
☒ proved to me on the basis of satisfactory evidence

to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.

WITNESS my hand and official seal.

Signature of Notary Public

Richard David Dupler, Notary Public

OPTIONAL

Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

Description of Attached Document

Title or Type of Document: _____

Document Date: _____ Number of Pages: _____

Signer(s) Other Than Named Above: _____

Capacity(ies) Claimed by Signer

Signer's Name: _____

- ☐ Individual
☐ Corporate Officer — Title(s): _____
☐ Partner — ☐ Limited ☐ General
☐ Attorney-in-Fact
☐ Trustee
☐ Guardian or Conservator
☐ Other: _____

Signer is Representing: _____



281
272

TRADUCCION OFICIAL DEL INGLES de la autenticación de firmas y la apostilla adjunta al documento de cesión mediante el cual la invención PRODUCTOS RADIOFARMACEUTICOS PARA EL DIAGNOSTICO DE LA ENFERMEDAD DE ALZHEIMER es cedida por sus inventores a la compañía Schering Aktiengesellschaft de Berlín, Alemania.

RECONOCIMIENTO HECHO EN CALIFORNIA PARA TODO PROPOSITO

Estado de California, Condado de San Francisco

El día 17 de Mayo del 2003 ante Richard David Dupler, Notario Público, compareció personalmente Joseph E. Hesselgesser, quien sobre la base de evidencia satisfactoria demostró ser una de las personas cuyo nombre aparece suscrito en el instrumento adjunto y reconoció haber ejecutado dicho instrumento en calidad de persona autorizada y que mediante su firma en dicho instrumento entonces ese instrumento fue ejecutado por la persona o por la entidad en nombre de la cual él actuaba.

En testimonio de lo cual coloco mi firma y mi sello oficial.

RICHARD DAVID DUPLER, Notario Público
(Firma y Sello Oficial)

Estado de California
SECRETARÍA DE ESTADO

Apostilla

(Convención de La Haya del 5 de Octubre de 1961)

1. País: Estados Unidos de América
Este documento público
2. Ha sido firmado por: RICHARD DAVID DUPLER
3. En su calidad de: Notario Público, Estado de California
4. Y lleva el sello: De RICHARD DAVID DUPLER, Notario Público,
Estado de California

Confirmado

5. En: Sacramento, California
6. El: 6 de Junio del 2003
7. Por: El Secretario Suplente del Estado de California
8. Bajo el No.: 158062
9. Sello: GRAN SELLO DEL ESTADO DE CALIFORNIA
10. Firma: KEVIN SHELLEY, Secretario de Estado

M. Méndez S.
JOSE MIGUEL MENDEZ
Traductor Oficial
Reg. 911 de Mayo 1988 MINJUSTICIA

MINISTERIO DE
RELACIONES EXTERIORES

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

200 OCT - 1972 12:39

733628
Cuya firma aparece en el
RECIBO de EMPEÑA
LAS FUNCIONES INDICADAS

OFI DE LEGALIZACIONES
RECIBO D.E.

51783A

COLOMBIA

14225
18
273
282

DOCUMENTO DE CESIÓN ASSIGNMENT DOCUMENT

Los suscritos,
(We, the undersigned)

CHRISTOPH-STEPHAN HILGER
con domicilio en Berlín, Alemania

BERND JOHANNSEN
con domicilio en Dresden, Alemania

JÖRG STEINBACH
con domicilio en Blechfswarda, Alemania

PETER MÄDING
con domicilio en Dresden, Alemania

MEREDITH HALKS-MILLER,
con domicilio en Woodside, Estados Unidos de América

RICHARD HORUK,
con domicilio en Lafayette, Estados Unidos de América

HARALD DINTER,
con domicilio en San Rafael, Estados Unidos de América

RAJU MOHAN,
con domicilio en Moranga, Estados Unidos de América

JOSEPH E. HESSELGESSER,
con domicilio en San Francisco, Estados Unidos de América

declaramos que somos los inventores de la invención que lleva el título
(declare that we are the inventors of the invention titled)

RADIOPHARMACEUTICALS FOR DIAGNOSING ALZHEIMER'S DISEASE

**PRODUCTOS RADIOFARMACÉUTICOS PARA EL DIAGNÓSTICO DE LA
ENFERMEDAD DE ALZHEIMER**

y que hemos cedido el derecho sobre esta invención en Colombia, junto con los
privilegios

(and that we have assigned our rights over this invention in Colombia, together with its
privileges)

y anexidades a favor de
(and attachments, to)

SCHERING AKTIENGESELLSCHAFT
Berlín, Alemania

- 1)
HILGER, Christoph-Stephan
- 2)
JOHANNSEN, Bernd
- 3)
STEINBACH, Jörg
- 4)
MÄDING, Peter
- 5)
HALLS-MILLER, Meredith
- 6)
HORUK, Richard
- 7)
DINTER, Harald
- 8)
MOHAN, Raju
- 9)
HESSELGESSER, Joseph E.

20
275 284

State of California



SECRETARY OF STATE

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country: *United States of America*

This public document

2. *has been signed by CINDY LOU STEWART*

3. *acting in the capacity of Notary Public, State of California*

4. *bears the seal/stamp of CINDY LOU STEWART, Notary Public,
State of California*

CERTIFIED

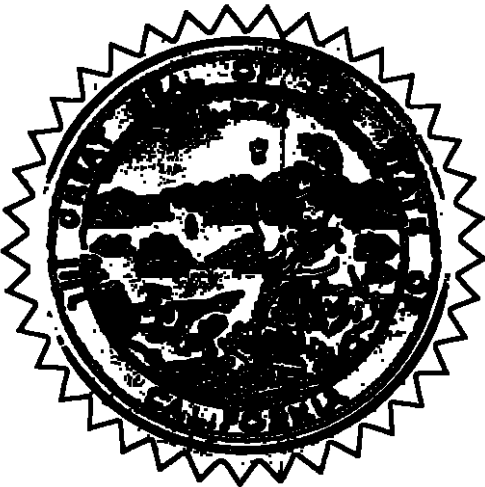
5. *At Sacramento, California*

6. *the 6th day of June 2003*

7. *by Deputy Secretary of State, State of California*

8. *No. 158063*

9. *Seal/Stamp:*



10. *Signature*

Kevin Shelley
KEVIN SHELLEY
Secretary of State



BY *M. Joyhan*

27
285
276

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California

County of

San Diego

ss.

On

May 14, 2013

before me,

Cindy Lou Stewart, Notary Public

Name and Title of Officer (e.g., Jane Doe, Notary Public)

personally appeared

Name(s) of Signer(s)

- ☒ personally known to me
☐ proved to me on the basis of satisfactory evidence

to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.



WITNESS my hand and official seal.

Cindy Lou Stewart
Signature of Notary Public

OPTIONAL

Though the information below is not required by law, it may prove valuable to persons relying on the document and could prevent fraudulent removal and reattachment of this form to another document.

Description of Attached Document

Title or Type of Document:

Document Date:

Number of Pages:

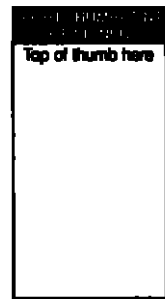
Signer(s) Other Than Named Above:

Capacity(ies) Claimed by Signer

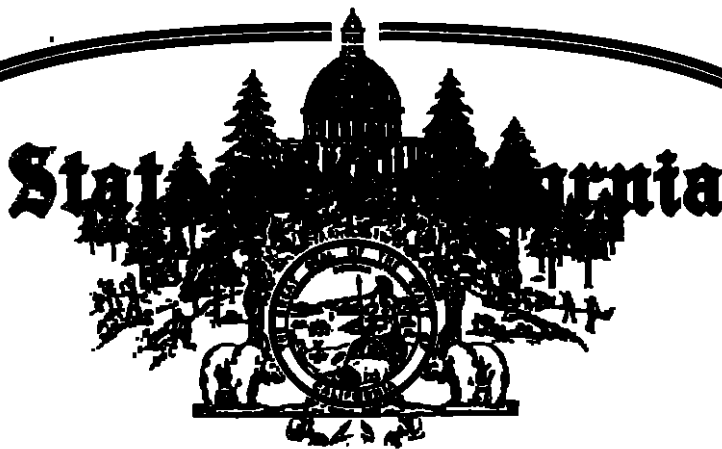
Signer's Name:

- ☐ Individual
☐ Corporate Officer — Title(s):
☐ Partner — ☐ Limited ☐ General
☐ Attorney-in-Fact
☐ Trustee
☐ Guardian or Conservator
☐ Other:

Signer Is Representing:



DATE 3/



SECRETARY OF STATE

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country: *United States of America*
This public document
2. *has been signed by Carole J. Smith*
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5. *At Sacramento, California*
6. *the 28th day of June 2003*
7. *by Deputy Secretary of State, State of California*
8. *No. 160559*
9. *Seal/Stamp:*

10. Signature



Kevin Shelley
KEVIN SHELLEY
Secretary of State



BY *Staci McElyea*

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278 287

CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

State of California

County of

Contra Costa

ss.

On

May 16, 2003

before me,

Carole J. Smith, Notary Public

Name and Title of Officer (e.g., "Jane Doe, Notary Public")

personally appeared

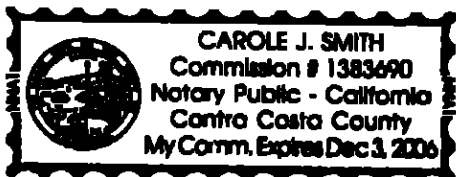
Harold Dinter

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☐ personally known to me

☒ proved to me on the basis of satisfactory evidence

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WITNESS my hand and official seal.

Carole J. Smith
Signature of Notary Public

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Assignment Document Columbia 51783

Document Date:

Number of Pages:

2

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☐ Corporate Officer — Title(s):

☐ Partner — ☐ Limited ☐ General

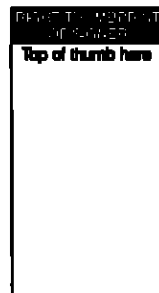
☐ Attorney-In-Fact

☐ Trustee

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State of California



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Kevin Shelley
KEVIN SHELLEY
Secretary of State



BY *Staci McElyea*

280 25

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State of California

County of

Contra Costa

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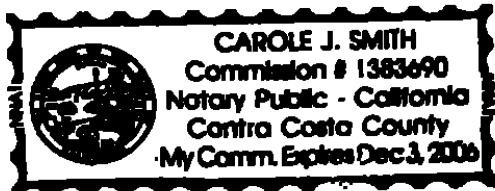
On May 21, 2003 before me, Carole J. Smith Notary Public
personally appeared Meredith Halden-Miller

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Carole J. Smith
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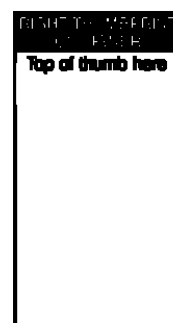
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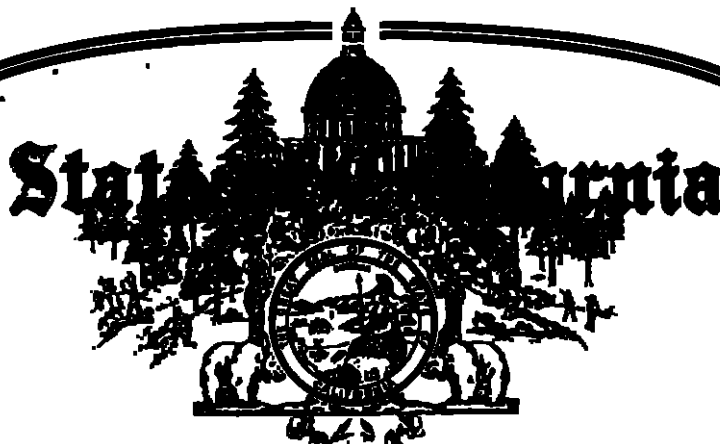
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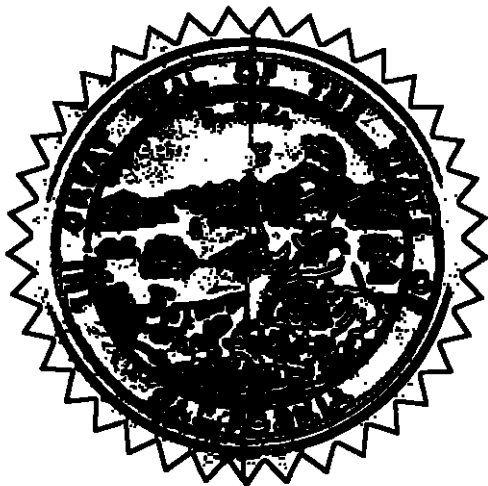
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5. *At Sacramento, California*
6. *the 28th day of June 2003*
7. *by Deputy Secretary of State, State of California*
8. *No. 160561*
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10. Signature



Kevin Shelley
KEVIN SHELLEY
Secretary of State

BY *Staci McElyea*



CALIFORNIA ALL-PURPOSE ACKNOWLEDGMENT

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State of California

County of

Contra Costa

ss.

On May 22, 2003

Date

before me,

Carole J. Smith, Notary Public

Name and Title of Officer (e.g., "Jane Doe, Notary Public")

personally appeared

Richard Hersh

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to be the person(s) whose name(s) is/are subscribed to the within instrument and acknowledged to me that he/she/they executed the same in his/her/their authorized capacity(ies), and that by his/her/their signature(s) on the instrument the person(s), or the entity upon behalf of which the person(s) acted, executed the instrument.



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TRADUCCION OFICIAL DEL INGLES de las autenticaciones de firmas y las apostillas adjuntas al documento de cesión mediante el cual la invención PRODUCTOS RADIOFARMACEUTICOS PARA EL DIAGNOSTICO DE LA ENFERMEDAD DE ALZHEIMER es cedida por sus inventores a la compañía Schering Aktiengesellschaft de Berlín, Alemania.

RECONOCIMIENTO HECHO EN CALIFORNIA PARA TODO PROPOSITO

Estado de California

Condado de San Diego

El día 14 de Mayo del 2003 ante Cindy Lou Stewart, Notaria Pública, compareció personalmente Raju Mohan, conocido personalmente por la suscrita Notaria, y reconoció ser una de las personas cuyo nombre aparece suscrito en el instrumento adjunto y haber ejecutado dicho instrumento en calidad de persona autorizada, y que mediante su firma en dicho instrumento entonces ese instrumento fue ejecutado por la persona o por la entidad en nombre de la cual él actuaba.

En testimonio de lo cual coloco mi firma y mi sello oficial.

CINDY LOU STEWART, Notaria Pública
(Firma y Sello Oficial)

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Confirmado

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6. El: 6 de Junio del 2003
7. Por: El Secretario Suplente del Estado de California
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9. Sello: GRAN SELLO DEL ESTADO DE CALIFORNIA
10. Firma: KEVIN SHELLEY, Secretario de Estado
[Firma Ilegible del Secretario Suplente de Estado]

M. Méndez S.
JOSE MIGUEL MENDEZ

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RECONOCIMIENTO HECHO EN CALIFORNIA PARA TODO PROPOSITO

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Condado de Contra Costa

El día 16 de Mayo del 2003 ante Carole J. Smith, Notaria Pública, compareció personalmente Harald Dinter, quien sobre la base de evidencia satisfactoria demostró ser una de las persona, cuyo nombre aparece suscrito en el instrumento adjunto y reconoció haber ejecutado dicho instrumento en calidad de persona autorizada, y que mediante su firma en dicho instrumento entonces ese instrumento fue ejecutado por la persona o por la entidad en nombre de la cual él actuaba.

En testimonio de lo cual coloco mi firma y mi sello oficial.

Carole J. Smith, Notaria Pública
(Firma y Sello Oficial)

Descripción del Documento Adjunto

Tipo de documento: Documento de Cesión para Colombia 51783

Número de páginas: 2

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6. El: 28 de Junio del 2003
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8. Bajo el No.: 160559
9. Sello: GRAN SELLO DEL ESTADO DE CALIFORNIA
10. Firma: KEVIN SHELLEY, Secretario de Estado
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M. Méndez S.
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RECONOCIMIENTO HECHO EN CALIFORNIA PARA TODO PROPOSITO

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El día 21 de Mayo del 2003 ante Carole J. Smith, Notaria Pública, compareció personalmente Meredith Halks-Miller, quien sobre la base de evidencia satisfactoria demostró ser una de las personas cuyo nombre aparece suscrito en el instrumento adjunto y reconoció haber ejecutado dicho instrumento en calidad de persona autorizada, y que mediante su firma en dicho instrumento entonces ese instrumento fue ejecutado por la persona o por la entidad en nombre de la cual él actuaba.

En testimonio de lo cual coloco mi firma y mi sello oficial.

Carole J. Smith, Notaria Pública
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286**RECONOCIMIENTO HECHO EN CALIFORNIA PARA TODO PROPOSITO**

Estado de California

Condado de Contra Costa

El día 22 de Mayo del 2003 ante Carole J. Smith, Notaria Pública, compareció personalmente, Richard Horuk, conocido personalmente por la suscrita notaria y reconoció ser una de las personas cuyo nombre aparece suscrito en el instrumento adjunto y haber ejecutado dicho instrumento en calidad de persona autorizada, y que mediante su firma en dicho instrumento entonces ese instrumento fue ejecutado por la persona o por la entidad en nombre de la cual él actuaba.

En testimonio de lo cual coloco mi firma y mi sello oficial.

Carole J. Smith, Notaria Pública

(Firma y Sello Oficial)

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Tipo de documento: Cesión de Patente 51783 Colombia

Número de páginas: 2

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6. El: 28 de Junio del 2003
7. Por: El Secretario Suplente del Estado de California
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9. Sello: GRAN SELLO DEL ESTADO DE CALIFORNIA
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Stacy McElyea, Secretaria Suplente de Estado

M. Méndez S.
JOSE MIGUEL MENDEZ

Traductor Oficial

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Ricardo Wenz
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THE UNITED STATES OF AMERICA

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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

October 10, 2001

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APPLICATION NUMBER: 60/246,299

FILING DATE: November 06, 2000



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

L. Edelen

**L. EDELEN
Certifying Officer**

11/06/00
35943 U.S. PTO

11-08-00

Docket Number 51783AUST1

PATENT A/prov 285
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35943 U.S. PTO
60/246299
11/06/00

I hereby certify that this correspondence and the documents referred to as attached therein are being deposited with the United States Postal Service on November 6, 2000, in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10, Mailing Label Number EL414874025US addressed to Box Provisional Patent Application, Assistant Commissioner for Patents, Washington, DC 20231

By: Carol J. Smith By: Carol J. Smith
Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

For: Radiopharmaceuticals for Diagnosing Alzheimer's Disease

Box Provisional Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 C.F.R. §1.51(c)(1)(i).

The following comprises the information required by 37 C.F.R. §1.51(c)(1):

The name(s) of the inventor(s) are:

Given Name (first and middle, if any)	Family Name Surname	Residence
1. Harold	Dinter	San Rafael, California
2. Meredith	Halke-Miller	Woodside, California
3. Joseph E.	Hesselgesser	San Francisco, California
4. Christoph-Stephan	Hilger	Berlin, Germany
5. Richard	Horst	La Jolla, California
6. Bernd	Johannsen	Dresden, Germany
7. Peter	Mäding	Dresden, Germany
8. Raju	Mohan	Merced, California
9. Jörg	Steinbach	Bielefeld, Germany

The title of the invention is: Radiopharmaceuticals for Diagnosing Alzheimer's Disease

The name, registration, and telephone number of the attorney or agent:

Carol J. Roth
Reg. No. 32,783
Telephone Number: 510 858-4483

The docket number used to identify this application is: 51783AUST1

The correspondence address for this application is:

Carol J. Roth
Biorix Biosciences
16049 San Pablo Avenue
P.O. Box 4089
Richmond, California 94804-0089

Telephone: 510 858-4483
Facsimile: 510 282-7086

This invention was not made by an agency of the U.S. Government or under a contract with an agency of the U.S. Government

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Identification of documents accompanying this cover sheet

Documents required by 37 C.F.R. §§1.51(c)(2)-(3): Specification No. of Pages 80 (includes Abstract and Drawings, Drawings
No. of Pages 10, Additional documents: Claims 15, Power of Attorney _____, Assignment _____, Other _____

Fee

The filing fee for the provisional application, as set in 37 C.F.R. §1.16(x) is \$150.00 for other than a small entity.

Fee payment

Fee payment in the amount of \$150.00 is being made at this time.

Method of fee payment

The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account No. 02-2117.

A duplicate of this cover sheet is attached.

Respectfully submitted,


Carol J. Roth
Reg. No. 32,783
Attorney for Applicants

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16049 San Pablo Avenue
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Richmond, California 94804-0089

Telephone: 510 669-4453
Facsimile: 510 262-7085
carol_roth@Berlex.com

009011-66294209

products
Radiopharmaceuticals for Diagnosing Alzheimer's Disease

Field of the Invention

5 The present invention relates to novel radiopharmaceuticals useful for the diagnosis of Alzheimer's disease.

Brief description of the background art

10 Alzheimer's disease is a severe neurodegenerative disorder, and currently about 4 million Americans suffer from this disease. As the aging population continues to grow, this number could reach 14 million by the middle of next century unless a cure or prevention is found. At present, there is no sensitive and specific premortem test for early diagnosis of this disease. Alzheimer's disease is currently diagnosed based on the clinical observation of cognitive decline, coupled with the systematic elimination of other possible causes of those symptoms. The confirmation of the clinical diagnosis of "probable Alzheimer's disease" can only be made by examination of the postmortem brain. The Alzheimer's disease brain is characterized by the appearance of two distinct abnormal proteinaceous deposits in regions of the brain responsible for learning and memory (e.g., cerebral cortex and hippocampus).
15 These deposits are extracellular amyloid plaques, which are characteristic of Alzheimer's disease, and intracellular neurofibrillary tangles (NFTs), which can be found in other neurodegenerative disorders as well. Amyloid peptides are typically either 40 or 42 amino acids in length ("A β ¹⁻⁴⁰" or "A β ¹⁻⁴²", respectively) and are formed from abnormal processing of a larger membrane-associated protein of unknown function, the amyloid precursor protein
20 ("APP"). Oligomeric aggregates of these peptides are thought to be neurotoxic, eventually resulting in synaptic degeneration and neuronal loss. The amount of amyloid deposition roughly correlates with the severity of symptoms at the time of death.

 In the past, there have been several attempts for the design of radiopharmaceuticals that could be used as diagnostic agents for a premortem diagnosis of Alzheimer's disease.

30 Bornebroek *et al.* showed that the amyloid-associated protein serum amyloid P

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component (SAP), labeled with ^{123}I , accumulates at low levels in the cerebral cortex, possibly in vessel walls, of patients with cerebral amyloidosis (Bornebroek, M., *et al.*, *Nucl. Med. Commun.* (1996), Vol. 17, pp. 929-933).

Saito *et al.* proposed a vector-mediated delivery of ^{125}I -labeled $\text{A}\beta^{1-40}$ through the blood-brain barrier. It is reported that the iodinated $\text{A}\beta^{1-40}$ binds $\text{A}\beta$ amyloid plaque in tissue sections (Saito, Y., *et al.*, *Proc. Natl. Acad. Sci. USA* 1995, Vol. 92, pp.10227-10231).

U.S. Patent No. 5,231,000 discloses antibodies with specificity to A4 amyloid polypeptide found in the brain of Alzheimer's disease patients. However, a method to deliver these antibodies across the blood-brain barrier has not been described.

Zhen *et al.* described modifications of the amyloid-binding dye known as "Congo Red™", and complexes of these modified molecules with technetium and rhenium. The complexes with radioactive ions are purported to be potential imaging agents for Alzheimer's disease (Zhen *et al.*, *J. Med. Chem.*(1999), Vol. 42, pp. 2805-2815). However, the potential of the complexes to cross the blood-brain barrier is limited.

A group at the University of Pennsylvania in the U.S.A. (Skovronsky, M., *et al.*, *Proc. Natl. Acad. Sci.* 2000, Vol. 97, pp.7609-7614) has developed a fluorescently labeled derivative of Congo Red that is brain permeable and that non-specifically binds to amyloid materials (that is, peptides in β -pleated sheet conformation). This compound would need to be radiolabeled and then run through pre-clinical screens for pharmacokinetics and toxicity before clinical testing.

Klunk *et al.* reported experiments with a derivative of Congo Red™, Chrysamine G (CG). It is reported that CG binds synthetic β -amyloid well *in vitro*, and crosses the blood-brain barrier in normal mice (Klunk *et al.*, *Neurobiol. Aging* (1994), Vol. 15, No. 6, pp. 691-698).

Bergström *et al.* presented a compound labeled with Iodine-123 as a potential radioligand for visualization of M_1 and M_2 muscarinic acetylcholine receptors in Alzheimer's disease (Bergström *et al.*, *Eur. J. Nucl. Med.* (1999), Vol. 26, pp. 1482-1485).

Recently, it has been discovered that certain specific chemokine receptors are upregulated in the brains of patients with Alzheimer's disease (Horuk, R. *et al.*, *J. Immunol.* (1997), Vol. 158, pp. 2882-2890); Xia *et al.*, *J. NeuroVirol.* (1999), Vol. 5, pp. 32-41). In

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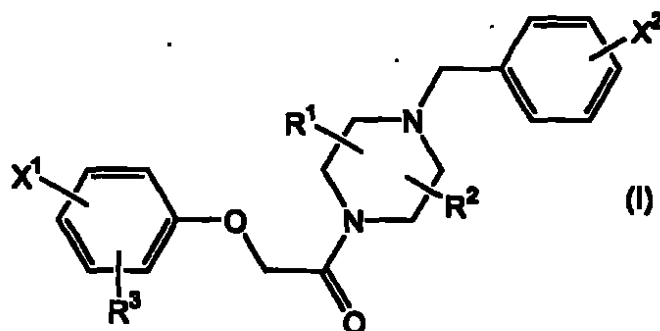
addition, it has been shown recently that the chemokine receptor CCR1 is upregulated in the brains of patients with advanced Alzheimer's disease and absent in normal-aged brains (Halks-Miller *et al*, CCR1 Immunoreactivity in Alzheimer's Disease Brains, Society for Neuroscience Meeting Abstract, #767.6, Volume 24, 1998). Antagonists to the CCR1
5 receptor and their use as anti-inflammatory agents are described in the PCT Published Patent Application, WO 98/56771.

None of the above described proposals have resulted in a clinical development of an imaging agent for the early diagnosis of Alzheimer's disease. Accordingly, there is still a clinical need for a diagnostic agent that could be used for a reliable and early diagnosis of
10 Alzheimer's disease.

SUMMARY OF THE INVENTION

The present invention is directed to radiopharmaceuticals that bind to the CCR1
15 receptor and are able to pass through the blood-brain barrier, and are therefore useful in diagnosing Alzheimer's disease, preferably at an early stage of the disease.

Accordingly, in one aspect, the invention is directed to compounds of formula (I):



wherein:

- 20 X¹ and X² are each independently halo;
R¹ and R² are each independently hydrogen or alkyl; and
R³ is hydrogen, amino, monoalkylamino, dialkylamino, monoaralkylamino,
alkylcarbonylamino, alkenylcarbonylamino, haloalkylcarbonylamino,
arylcabonylamino, alkoxyalkylcarbonylamino, alkoxycarbonylalkylcarbonylamino,

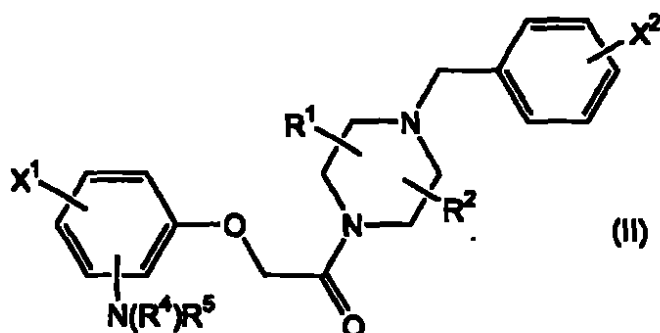
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glycinamido, monoalkylglycinamido, arylcarbonylglycinamido, aminocarbonylglycinamido, (aminocarbonyl)(alkyl)glycinamido, (alkoxyalkylcarbonyl)glycinamido, ureido, monoalkylureido, monoarylureido, monoaralkylureido, or alaninamido;

- 5 and wherein either one of X^1 or X^2 is selected from the group of ^{123}I , ^{125}I , ^{128}I , ^{131}I , ^{76}Br , ^{78}Br , ^{80}Br and ^{18}F ; or wherein one of the carbon atoms in the compound is ^{11}C ; or a pharmaceutically acceptable salt thereof.

In another aspect, the invention is directed to compounds of formula (II):



wherein

X^1 and X^2 are each independently halo;

R^1 and R^2 are each independently hydrogen or alkyl; and

R^4 is hydrogen; and

- 15 R^5 comprises a chelator capable of binding a radioactive metal atom chosen from the group of ^{99m}Tc , ^{186}Re and ^{188}Re ;

or as a complex with ^{99m}Tc , ^{186}Re and ^{188}Re ;

or a pharmaceutically acceptable salt thereof.

- 20 In another aspect, this invention is directed to a method of diagnosing Alzheimer's disease in a human which comprises administering to a human in need of such diagnosis a compound of formula (I) or formula (II), as described above and herein, and measuring the radioactivity arising from the administration of the compound to the human either by using a gamma camera or by positron emission tomography (PET).

In another aspect, the invention is directed to a method of using a compound of the

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invention for the manufacture of a radiopharmaceutical for the diagnosis of Alzheimer's disease in a human.

In another aspect, the invention is directed to a method of preparing compounds of the invention.

5

Brief Description of the Figures

Figure 1 shows the expression of CCR1 in Alzheimer's disease brain tissue .

Figure 2 shows CCR1 antibody specificity in Alzheimer's disease brain tissue.

10 Figure 3 shows CCR1-A β ¹⁻⁴⁰ double-labeled tissue sections

Figure 4 shows neuritic plaques double-labeled for CCR1 and A β ¹⁻⁴².

Figure 5 shows diffuse A β ¹⁻⁴² staining in Alzheimer's disease brain.

Figure 6 is a graph showing the relationship between CCR1 and A β ¹⁻⁴⁰ by CDR score.

Figure 7 is a graph showing the relationship between CCR1 and A β ¹⁻⁴² by CDR score.

15 Figure 8 is a graph demonstrating the decrease in total brain radioactivity over time.

Figure 9 is a graph showing the percent of injected dose in brain and plasma.

Figure 10 are graphs showing CCR1 expression in other neurodegenerative diseases.

Detailed Description of the Invention

20 Definitions

As used in the specification and appended claims, unless specified to the contrary, the following terms have the meaning indicated:

"Alkyl" refers to a straight or branched chain monovalent or divalent radical consisting solely of carbon and hydrogen, containing no unsaturation and having from one to eight carbon atoms, e.g., methyl, ethyl, *n*-propyl, 1-methylethyl (*iso*-propyl), *n*-butyl, *n*-pentyl, 25 1,1-dimethylethyl (*t*-butyl), *n*-heptyl, and the like.

"Alkylcarbonylamino" refers to a radical of the formula -N(H)-C(O)-R_a where R_a is an alkyl radical as defined above, e.g., acetylamino, ethylcarbonylamino, *n*-propylcarbonylamino, and the like.

30 "Alkenyl" refers to a straight or branched chain monovalent or divalent radical

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consisting solely of carbon and hydrogen, containing at least one double bond and having from two to eight carbon atoms, e.g., ethenyl, prop-1-enyl, but-1-enyl, pent-1-enyl, penta-1,4-dienyl, and the like.

5 "Alkenylcarbonylamino" refers to a radical of the formula $-N(H)-C(O)-R_c$ where R_c is an alkenyl radical as defined above, e.g., ethenylcarbonylamino, prop-2-enylcarbonylamino, but-2-enylcarbonylamino, and the like.

"Alkoxy" refers to a radical of the formula $-OR_a$ where R_a is an alkyl radical as defined above, e.g., methoxy, ethoxy, *n*-propoxy, 1-methylethoxy (*iso*-propoxy), *n*-butoxy, *n*-pentoxy, 1,1-dimethylethoxy (*t*-butoxy), and the like.

10 "Alkoxycarbonylalkylcarbonylamino" refers to a radical of the formula $-N(H)-C(O)-R_d-C(O)OR_a$ where each R_a is independently an alkyl radical as defined above, e.g., ethoxycarbonylmethylcarbonylamino, methoxycarbonylmethylcarbonylamino, (2-ethoxycarbonylethyl)carbonylamino, (2-methoxycarbonylethyl)carbonylamino, and the like.

15 "(Alkoxyalkylcarbonyl)glycinamido" refers to a radical of the formula $-N(H)-C(O)-CH_2-N(H)-C(O)-R_a-O-R_a$ where each R_a is independently an alkyl radical as defined above, e.g., (methoxyacetyl)glycinamido, (ethoxyacetyl)glycinamido, and the like.

"Amino" refers to the radical $-NH_2$.

"Aminocarbonyl glycinamido" refers to a radical of the formula $-N(H)-C(O)-CH_2-N(H)-C(O)-NH_2$.

20 "(Aminocarbonyl)(alkyl)glycinamido" refers to a radical of the formula $-N(H)-C(O)-CH_2-N(R_a)-C(O)-NH_2$ where R_a is an alkyl radical as defined above.

25 "Aryl" refers to a phenyl or naphthyl radical. Unless stated otherwise specifically in the specification, the term "aryl" or the prefix "ar-" (such as in "aralkyl") is meant to include aryl radicals optionally substituted by one or more substituents selected from the group consisting of halo, alkyl, alkoxy, haloalkyl, haloalkoxy, nitro, amino, monoalkylamino, and dialkylamino, as defined herein.

"Arylcarbonylamino" refers to a radical of the formula $-N(H)-C(O)-R_b$ where R_b is an aryl radical as defined above, e.g., (4-methoxyphenyl)carbonylamino, (4-fluorophenyl)carbonylamino, (4-chlorophenyl)carbonylamino, and the like.

30 "Arylcarbonyl glycinamido" refers to a radical of the formula

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-N(H)-C(O)-CH₂-N(H)-C(O)-R₆ where R₆ is an aryl radical as defined above, e.g., phenylcarbonylglycinamido, (4-fluoro-3-trifluoromethylphenyl)carbonylglycinamido, (4-fluorophenyl)carbonylglycinamido, and the like.

5 "Aralkyl" refers to a radical of the formula -R_aR_b where R_a is an alkyl radical as defined above and R_b is an aryl radical as defined above, e.g., benzyl, and the like.

"Alkoxyalkylcarbonylamino" refers to a radical of the formula -N(H)-C(O)-R_a-O-R_a where each R_a is an alkyl radical as defined above, e.g., methoxymethylcarbonylamino, ethoxyethylcarbonylamino, methoxyethylcarbonylamino, and the like.

"Alaninamido" refers to a radical of the formula -N(H)-C(O)-C(CH₃)H-NH₂.

10 "BenzyI" refers to a radical of the formula -CH₂-R_h where R_h is a phenyl radical optionally substituted by one or more substituents selected from the group consisting of halo, alkyl, haloalkyl, alkoxy, nitro, amino, monoalkylamino, and dialkylamino.

15 "Dialkylamino" refers to a radical of the formula -N(R_a)R_a where each R_a is independently an alkyl radical as defined above, e.g., dimethylamino, methylethylamino, diethylamino, dipropylamino, ethylpropylamino, and the like.

"Glycinamido" refers to a radical of the formula -N(H)-C(O)-CH₂-NH₂.

"Halo" refers to bromo, chloro, iodo or fluoro.

20 "Haloalkyl" refers to an alkyl radical, as defined above, that is substituted by one or more halo radicals, as defined above, e.g., trifluoromethyl, difluoromethyl, trichloromethyl, 2-trifluoroethyl, 1-fluoromethyl-2-fluoroethyl, 3-bromo-2-fluoropropyl, 1-bromomethyl-2-bromoethyl, and the like.

"Haloalkylcarbonylamino" refers to a radical of the formula -N(H)-C(O)-R_i where R_i is an haloalkyl radical as defined above, e.g., trifluoromethylcarbonylamino, trifluoromethylcarbonylamino, 2-bromoethylcarbonylamino, and the like.

25 "Monoalkylamino" refers to a radical of the formula -N(H)R_a where R_a is an alkyl radical as defined above, e.g., methylamino, ethylamino, propylamino, and the like.

"Monoaralkylamino" refers to a radical of the formula -N(H)R_d where R_d is an aralkyl radical as defined above, e.g., benzylamino, (3,4,5-trimethoxybenzyl)amino, (4-chlorobenzyl)amino, and the like.

30 "Monoalkylglycinamido" refers to a radical of the formula -N(H)-C(O)-CH₂-N(H)R_a

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where R_a is an alkyl radical as defined above.

"Monoalkylureido" refers to a radical of the formula $-N(H)-C(O)-N(H)R_a$ or a radical of the formula $-N(R_a)-C(O)-NH_2$ where R_a is an alkyl radical as defined above.

"Monoarylureido" refers to a radical of the formula $-N(H)-C(O)-N(H)R_b$ or a radical of the formula $-N(R_b)-C(O)-NH_2$ where R_b is an aryl radical as defined above

"Monoaralkylureido" refers to a radical of the formula $-N(H)-C(O)-N(H)R_d$ or a radical of the formula $-N(R_d)-C(O)-NH_2$ where R_d is an aralkyl radical as defined above.

"Optional" or "optionally" means that the subsequently described event of circumstances may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not. For example, "optionally substituted aryl" means that the aryl radical may or may not be substituted and that the description includes both substituted aryl radicals and aryl radicals having no substitution.

"Pharmaceutically acceptable salt" includes both acid and base addition salts.

"Pharmaceutically acceptable acid addition salt" refers to those salts which retain the biological effectiveness and properties of the free bases, which are not biologically or otherwise undesirable, and which are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, pyruvic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluenesulfonic acid, salicylic acid, and the like. Particularly preferred salts of compounds of the invention are the monochloride salts and the dichloride salts.

"Pharmaceutically acceptable base addition salt" refers to those salts which retain the biological effectiveness and properties of the free acids, which are not biologically or otherwise undesirable. These salts are prepared from addition of an inorganic base or an organic base to the free acid. Salts derived from inorganic bases include, but are not limited to, the sodium, potassium, lithium, ammonium, calcium, magnesium, zinc, aluminum salts and the like. Preferred inorganic salts are the ammonium, sodium, potassium, calcium, and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, such as isopropylamine,

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trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, 5 N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic bases are isopropylamine, diethylamine, ethanolamine, trimethylamine, dicyclohexylamine, choline and caffeine.

"Urldo" refers to a radical of the formula $-N(H)-C(O)-NH_2$.

10 It is understood from the above definitions and examples that for radicals containing a substituted alkyl group any substitution thereon can occur on any carbon of the alkyl group.

The compounds of the invention, or their pharmaceutically acceptable salts, may have asymmetric carbon atoms in their structure. The compounds of the invention and their pharmaceutically acceptable salts may therefore exist as single enantiomers, diastereoisomers, racemates, and mixtures of enantiomers and diastereomers. All such 15 single enantiomers, diastereoisomers, racemates and mixtures thereof are intended to be within the scope of this invention. Absolute configuration of certain carbon atoms within the compounds, if known, are indicated by the appropriate absolute descriptor R or S.

Utility and Administration

20 The compounds of the invention as described herein are antagonists to the chemokine receptor known as CCR1 and have the ability to pass the blood-brain barrier. The compounds are therefore suited as *in vivo* diagnostic agents for imaging of Alzheimer's disease. The detection of radioactivity is performed according to well-known procedures in the art, either by using a gamma camera or by positron emission 25 tomography (PET).

Preferably, the free base or a pharmaceutically acceptable salt form, e.g. a monochloride or dichloride salt, of a compound of the invention used in a galenical formulation as diagnostic agent. The galenical formulation containing the compound of the invention optionally contains adjuvants known in the art, e.g. buffers, sodium chloride, 30 lactic acid, surfactants etc. A sterilization by filtration of the galenical formulation under

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sterile conditions prior to usage is possible.

The radioactive dose should be in the range of 1 to 100 mCi, preferably 5 to 30 mCi, and most preferably 5 to 20 mCi per application.

5 Testing

The suitability of the compounds as imaging agents for Alzheimer's disease can be demonstrated by experimental protocols known to those of ordinary skill in the art. For example, the upregulation of CCR1 receptors in Alzheimer's disease brains can be demonstrated in immunohistochemical staining experiments of autopsy brain tissue collected from Alzheimer's disease patients as described in detail below in the Examples. The ability of the compounds of the invention to bind to the CCR1 receptor and their ability to pass through the blood-brain barrier, can also be assessed in known *in vitro* and *in vivo* assays as described below in the Examples. In particular, Example 10 describes a large study that was undertaken to address the degree of CCR1 expression in different stages of Alzheimer's disease. Brain tissue from 50 autopsy cases showed a correlation between degree of clinical severity (dementia) in Alzheimer's disease and CCR1 expression in dystrophic neurites. CCR1 expression in plaque-like structures within the brains of clinically normal individuals is rare. Also, CCR1 expression is not found in the brains of individuals with other neurodegenerative diseases unless there is a concomitant Alzheimer's disease pathology (specifically, A β ¹⁻⁴² in plaques).

Preferred Embodiments

Of the various aspects of the invention, certain compounds of formula (I) are preferred. In particular, compounds of formula (I) wherein X¹ is a chloro at the 4-position of the phenyl ring and X² is a ¹⁸F atom at the 4-position of the phenyl ring are preferred. Especially preferred are such compounds for use as diagnostic agents in positron emission tomography (PET).

Even more preferred are those compounds of formula (I) wherein R³ is in the 2-position of the phenyl ring and R¹ is a methyl at the 2-position of the piperaziny ring and R² is a methyl at the 5-position of the piperaziny ring. Equally preferred are those

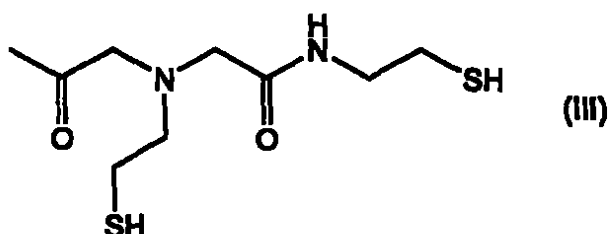
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compounds of formula (I) wherein R^3 is in the 2-position of the phenyl ring and R^1 is a methyl in the 2-position of the piperaziny ring and R^2 is hydrogen.

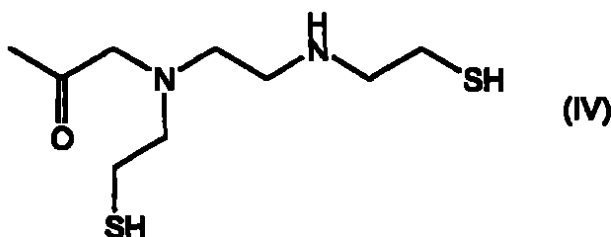
Further preferred are these preferred compounds in their mono- or dichloride salt form.

5 Of the various aspects of the invention, certain compounds of formula (II) are preferred. In particular, compounds of formula (II) wherein $-N(R^4)R^5$ is in the 2-position of the phenyl ring and R^1 is in the 2-position of the piperaziny ring and R^2 is in the 5-position of the piperaziny ring. Equally preferred are those compounds of formula (II) wherein R^3 is in the 2-position of the phenyl ring and R^1 is a methyl in the 2-position of the piperaziny ring and R^2 is hydrogen.

10 Even more preferred are those compounds of formula (II) wherein R^5 comprises a chelator according to formula (III):



or formula (IV):



15 as well as their complexes with ^{99m}Tc , ^{188}Re and ^{186}Re . Of these preferred compounds, even more preferred are those compounds of formula (II) wherein R^5 comprises a chelator according to formula (III) or (IV), and wherein the chelator is bound to the nitrogen in the $-N(R^4)R^5$ group of the non-radioactive compound of formula (II) via a linker moiety comprising

20 an alkyl radical having one to ten carbon atoms, wherein the alkyl radical optionally contains one to ten $-C(O)-$ groups, one to ten $-C(O)N(R)-$ groups, one to ten $-N(R)C(O)-$ groups, one to ten $-N(R)-$ groups, one to ten $-N(R)_2$ groups, one to ten hydroxy groups, one to ten $-C(O)OR-$

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groups, one to ten oxygen atoms, one to ten sulfur atoms, one to ten nitrogen atoms, one to ten halogen atoms, one to ten aryl groups, and one to ten saturated or unsaturated heterocyclic rings wherein R is hydrogen or alkyl. A preferred linker moiety is $-C(O)-CH_2-N(H)-$.

- 5 Of the compounds of the invention, the most preferred compounds of formula (I) are those compounds selected from the group consisting of the following:

1-(5-chloro-2-[2-[(2R)-4-(4-fluoro- ^{18}F -benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy]phenyl)urea;

10 N' -(mercaptoeth-1-yl)- N' -(5-mercapto-3-aza-2-oxopent-1-yl)- N -(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2R)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide, technetium-99m-complex;

1-(2-[2-[(2R)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy]-5-iodo- ^{125}I -phenyl)urea;

15 N' -(2-mercaptoeth-1-yl)- N' -(5-mercapto-3-aza-2-oxopent-1-yl)- N -(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2R)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, technetium-99m-complex;

N -(2-mercaptoeth-1-yl)- N' -(5-mercapto-3-azapent-1-yl)- N -(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2R)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, technetium-99m-complex;

20 2-(2-amino-4-chlorophenoxy)-1-[(2SR,5RS)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

2-(2-amino-4-chlorophenoxy)-1-[(2RS,5RS)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

2-(2-amino-4-chlorophenoxy)-1-[(2SR,5SR)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

25 2-(2-amino-4-chlorophenoxy)-1-[(2RS,5SR)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

2-[4-chloro-2-(diethylamino)phenoxy]-1-[(2SR,5RS)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

30 2-[4-chloro-2-(diethylamino)phenoxy]-1-[(2RS,5RS)-4-(4-fluoro- ^{18}F -benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

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2-[4-chloro-2-(diethylamino)phenoxy]-1-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

2-[4-chloro-2-(diethylamino)phenoxy]-1-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

5 1-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(2,4-dichlorophenyl)urea;

1-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(2,4-dichlorophenyl)urea;

10 1-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(2,4-dichlorophenyl)urea;

1-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(2,4-dichlorophenyl)urea;

1-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

15 1-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

20 1-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

25 1-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

30 1-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-(2-isopentylamino-4-chlorophenoxy)ethan-1-one;

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1-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-(2-isopentylamino-4-chlorophenoxy)ethan-1-one;

1-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-(2-isopentylamino-4-chlorophenoxy)ethan-1-one;

5 1-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-(2-isopentylamino-4-chlorophenoxy)ethan-1-one;

N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-methylpropanamide;

10 N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-methylpropanamide;

N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-methylpropanamide;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-methylpropanamide;

15 N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxy)acetamide;

N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxy)acetamide;

20 N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxy)acetamide;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxy)acetamide;

(E)-N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-butanamide;

25 (E)-N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-butanamide;

(E)-N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-butanamide;

(E)-N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-butanamide;

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methyl *N*-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

methyl *N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

5 methyl *N*-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

methyl *N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

10 ethyl *N*-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

ethyl *N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

ethyl *N*-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

15 ethyl *N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)succinamate;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

N-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

N-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

25 *N*-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)propanamide;

N-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)propanamide;

N-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)propanamide;

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N-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)propanamide;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-fluorobenzamide;

5 *N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-fluorobenzamide;

N-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-fluorobenzamide;

10 *N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-fluorobenzamide;

1-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(*p*-tolyl)urea;

1-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(*p*-tolyl)urea;

15 1-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(*p*-tolyl)urea;

1-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(*p*-tolyl)urea;

1-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-ethylurea;

1-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-ethylurea;

1-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-ethylurea;

25 1-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-ethylurea;

1-benzyl-3-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-benzyl-3-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸*F*-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

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1-benzyl-3-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

1-benzyl-3-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

5 1-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(4-nitrophenyl)urea;

1-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(4-nitrophenyl)urea;

10 1-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(4-nitrophenyl)urea;

1-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-3-(4-nitrophenyl)urea;

2-(2-benzylamino-4-chlorophenoxy)-1-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

15 2-(2-benzylamino-4-chlorophenoxy)-1-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

2-(2-benzylamino-4-chlorophenoxy)-1-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

20 2-(2-benzylamino-4-chlorophenoxy)-1-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]ethan-1-one;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide;

N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide;

25 N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide;

N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide;

30 1-(5-chloro-2-{2-[(2R)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

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1-(5-chloro-2-{2-[(2S)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethyl-piperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methylamino)acetamide;

5 *N*-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethyl-piperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methylamino)acetamide;

N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethyl-piperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methylamino)acetamide;

N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethyl-piperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methylamino)acetamide;

10

2-bromo-*N*-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

2-bromo-*N*-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

15

2-bromo-*N*-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

2-bromo-*N*-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)acetamide;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(ureido)acetamide;

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N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(ureido)acetamide;

N-(5-chloro-2-{2-[(2RS,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(ureido)acetamide;

25

N-(5-chloro-2-{2-[(2SR,5RS)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(ureido)acetamide;

N-(5-chloro-2-{2-[(2RS,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(1-methylureido)acetamide;

N-(5-chloro-2-{2-[(2SR,5SR)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(1-methylureido)acetamide;

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N-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(1-methylureido)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(1-methylureido)acetamide;

5 (2*RS*)-*N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*SR*)-*N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*RS*)-*N*-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

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(2*SR*)-*N*-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*RS*)-*N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*SR*)-*N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*RS*)-*N*-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

(2*SR*)-*N*-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-aminopropanamide;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2,4-difluorobenzoylamino)acetamide;

N-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2,4-difluorobenzoylamino)acetamide;

25 *N*-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2,4-difluorobenzoylamino)acetamide;

N-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2,4-difluorobenzoylamino)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxyacetylamino)acetamide;

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N-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxyacetylamino)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxyacetylamino)acetamide;

5 *N*-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(methoxyacetylamino)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2-iodobenzoylamino)acetamide;

10 *N*-(5-chloro-2-{2-[(2*RS*,5*RS*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2-iodobenzoylamino)acetamide;

N-(5-chloro-2-{2-[(2*SR*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2-iodobenzoylamino)acetamide;

N-(5-chloro-2-{2-[(2*RS*,5*SR*)-4-(4-fluoro-¹⁸F-benzyl)-2,5-dimethylpiperazin-1-yl]-2-oxoethoxy}phenyl)-2-(2-iodobenzoylamino)acetamide;

15 *N*-(5-chloro-2-{2-[(2*R*)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide; and

N-(5-chloro-2-{2-[(2*S*)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)glycinamide;

as well as the mono- and dichloride salts thereof.

Preparation of the Compounds of the Invention

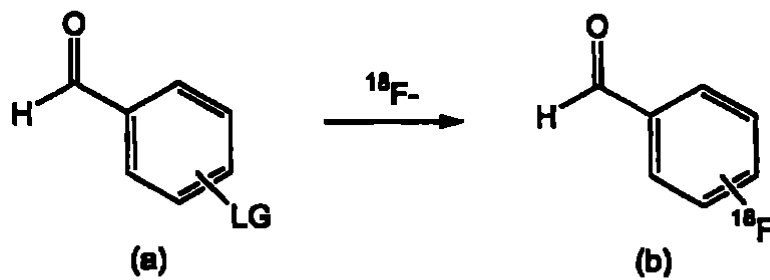
A. Preparation of Compounds of Formula (I)

In general, the radioactive imaging agents of formula (I) of the present invention are prepared by reacting radioactive 4-halobenzyl derivatives with piperazine derivatives.

25 Preferred are ¹⁸F-labeled 4-fluorobenzyl derivatives for PET-imaging. A general method for the preparation of 4-fluoro-¹⁸F-benzyl halides is described in Iwata *et al.*, *Applied Radiation and Isotopes* (2000), Vol. 52, pp. 87-92.

The ¹⁸F-labeled 4-fluorobenzyl derivatives are prepared by reaction of a benzaldehyde compound of formula (a) with ¹⁸F⁻ ions to obtain a benzaldehyde compound of formula (b):

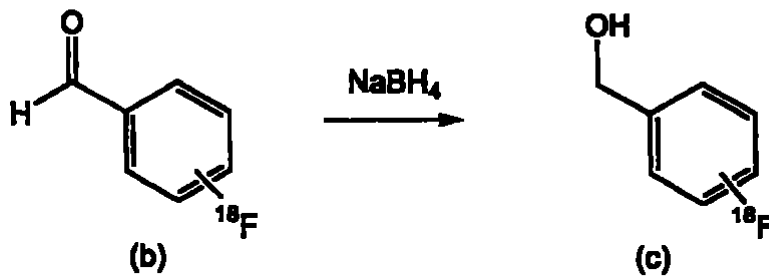
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wherein LG is a leaving group, for example, bromo, chloro, iodo, nitro, or $\text{N}(\text{R})_3^+ \text{X}^-$ (where R is alkyl and X is a halo ion, such as Br^- , Cl^- , or I^- ; an ion of an alkanolic acid, such as an acetate ion ($\text{CH}_3\text{C}(\text{O})\text{O}^-$); or an ion of an alkylsulfonic acid or haloalkylsulfonic acid, such as triflate

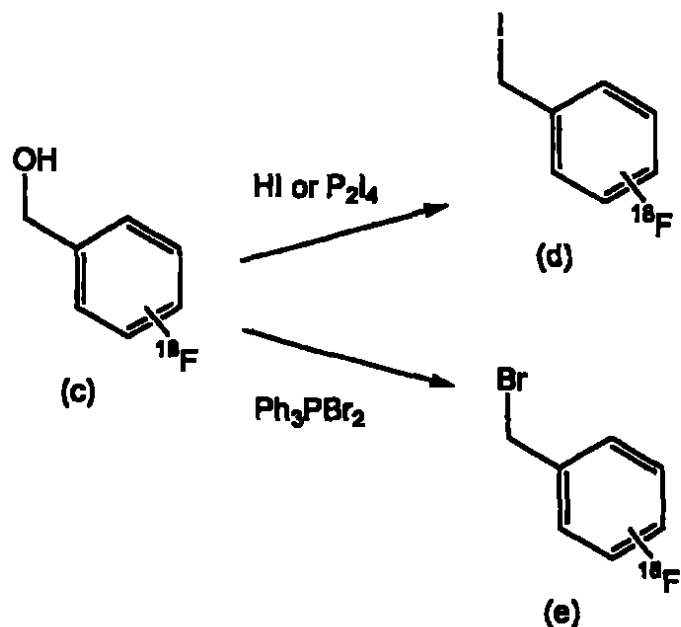
5 (CF_3SO_3^-)). Preferably LG is triflate. Starting from the compound of formula (b), several synthetic pathways are possible in preparing the compounds of formula (I).

In a first synthetic pathway, a compound of formula (b) is reduced with NaBH_4 to obtain a compound of formula (c):



The compound of formula (c) is then reacted with HI , P_2I_4 or Ph_3PBr_2 to obtain an iodo- or bromo-substituted compound of formulae (d) or (e):

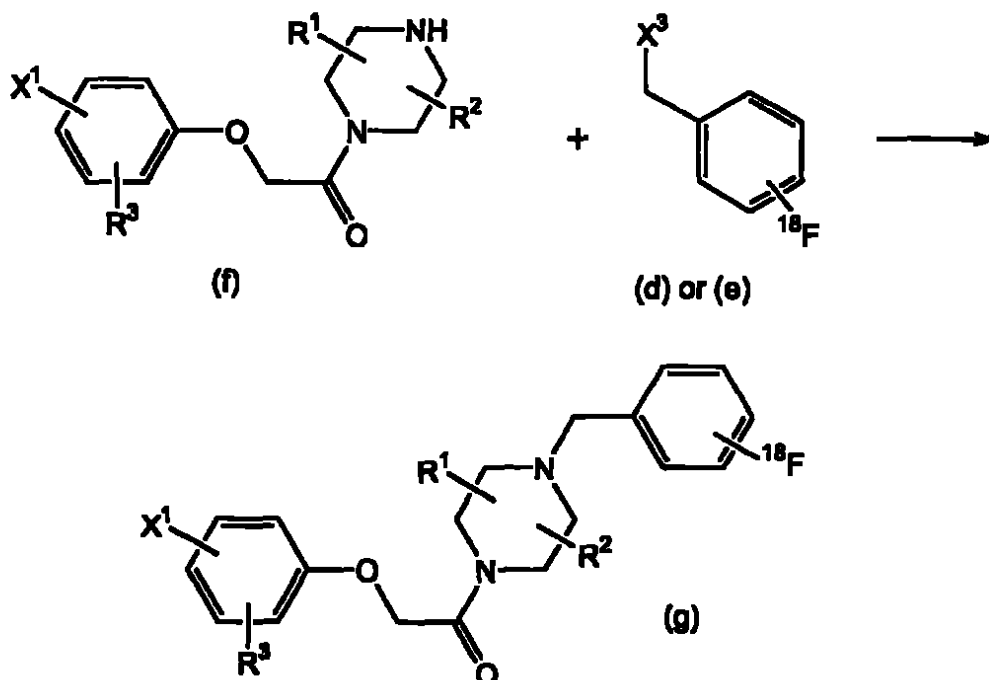
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The compounds of formulae (d) and (e) can be obtained with a radiochemical yield of 50 to 60%. The radiochemical purity is greater than 95%. The specific activity of the compounds is 5 mCi per 1 nmol.

A compound of formulae (d) or (e) can then be reacted with a piperazine derivative (f) to obtain a compound of formula (g) (a compound of formula (l)):

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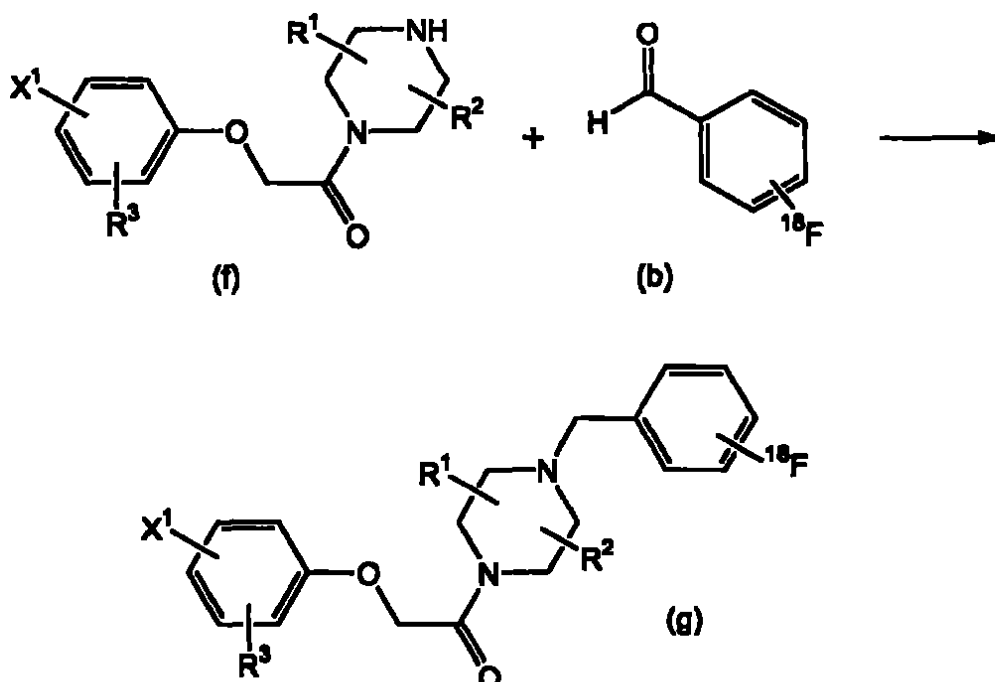


Compounds of formula (f) may be prepared according to methods known to those of ordinary skill in the art and is described in detail in PCT Published Patent Application, WO 98/56771.

5 In a second synthetic pathway, a compound of formula (b) is directly reacted with a compound of formula (f) using a reducing agent, for example, formic acid, ammonium formate, NaBH₄, or NaBH₃CN, to obtain a compound of formula (g) (a compound of formula (I)):

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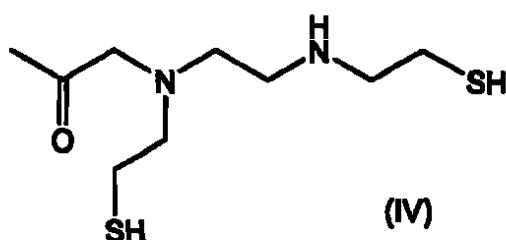
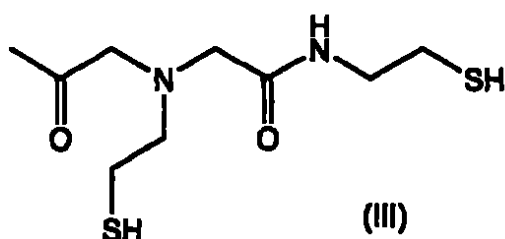


B. Preparation of Compounds of Formula (II)

For Single Photon Emission Computed Tomography ("SPECT"), ^{99m}Tc -labeled compounds are preferred. Those compounds are compounds of formula (II). A general synthetic pathway for these compounds starts with non-radioactive analogues of compounds of formula (II) that are reacted with ^{99m}Tc -binding chelators, e.g. N_2S_2 -Chelators. Preparation of the non-radioactive analogs of the compounds of formula (II) is described in detail in PCT Published Patent Application, WO 98/56771. The synthesis of the chelators follows standard procedures, for example, the procedures described in A. Mahmood *et al.*, *A N_2S_2 -Tetradentate Chelate for Solid-Phase Synthesis: Technetium, Rhenium in Chemistry and Nuclear Medicine* (1999), Vol. 5, p. 71, or in Z.P. Zhuang *et al.*, *Bioconjugate Chemistry* (1999), Vol. 10, p. 159.

Preferred chelators are chelators of formulae (III) or (IV):

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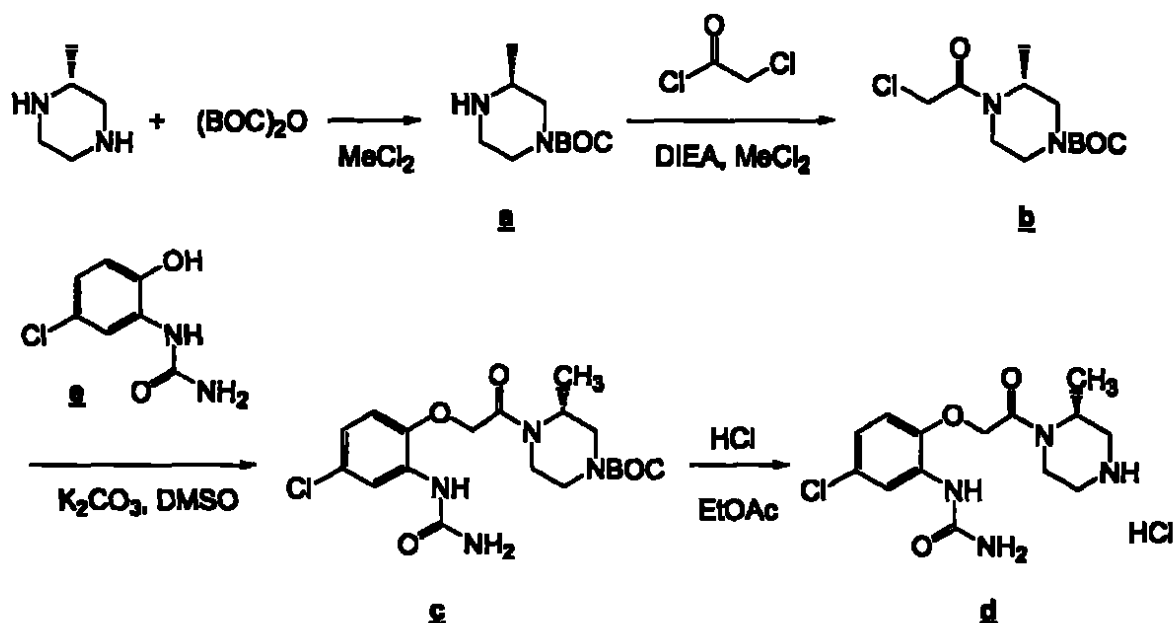
One of the chelators is either bound directly to the nitrogen in the $-N(R^4)R^5$ group of the non-radioactive compound of formula (II), or via a linker moiety comprising an alkyl radical having one to ten carbon atoms, wherein the alkyl radical optionally contains one to ten $-C(O)-$ groups, one to ten $-C(O)N(R)-$ groups, one to ten $-N(R)C(O)-$ groups, one to ten $-N(R)-$ groups, one to ten $-N(R)_2$ groups, one to ten hydroxy groups, one to ten $-C(O)OR-$ groups, one to ten oxygen atoms, one to ten sulfur atoms, one to ten nitrogen atoms, one to ten halogen atoms, one to ten aryl groups, and one to ten saturated or unsaturated heterocyclic rings wherein R is hydrogen or alkyl. A preferred linker moiety is $-C(O)-CH_2-N(H)-$.

The following specific examples are provided as a guide to assist in the practice of the invention, and are not intended as a limitation on the scope of the invention.

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Example 1

Preparation of 1-(5-Chloro-2-{2-[(2*R*)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea

A. DIEA (19.10 mL, 110 mmol) was added to a solution of (*R*)-(-)-2-methylpiperazine (10 g, 100 mmol) in 250 mL of methylene chloride. The solution was cooled to -10°C. The BOC anhydride was dissolved in 250 mL of methylene chloride and this solution was added to the chilled piperazine solution over 1 hour. The reaction was allowed to warm to ambient temperature over 16 hours. The reaction mixture was filtered to remove the solids and the filtrate washed with 500 mL of water, dried over magnesium sulfate, filtered and evaporated to an oil. The oil was purified by flash column chromatography to afford 11.0 g of the compound of formula (a).

B. The compound of formula (a) (11.0 g, 55 mmol) and DIEA (10.5 mL, 60.4 mmol) were dissolved in 100 mL of methylene chloride. The resulting solution was chilled to -10°C. Chloroacetyl chloride (4.37 mL, 55 mmol) was added dropwise to the solution maintaining the temperature at -10°C. After stirring for 1 hour the reaction mixture was washed with 100 mL of water, dried over magnesium sulfate, filtered and evaporated to an oil. The oil was purified by flash column chromatography to afford 14.8 g of the compound of

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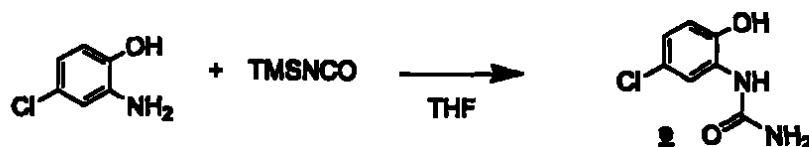
formula (b).

C. To a solution of the compound of formula (b) (14.8 g, 53.5 mmol) and the compound of formula (e) (9.98 g, 53.5 mmol) in 75 mL of DMSO was added potassium carbonate (18.48 g, 133.7 mmol). The resulting mixture was heated to 50°C for 3 hours. The mixture was cooled to 30°C and poured into 700 mL of water. The water was extracted three times with 200 mL of ethyl acetate. The ethyl acetate extracts were combined and washed with 200 mL of 1N KOH followed by brine. The organic layer was dried over magnesium sulfate, filtered and evaporated to a foam. The foam was purified by flash column chromatography to afford 19.6 g of the compound of formula (c).

D. The compound of formula (c) (7.68 g, 18 mmol) was dissolved in 40 mL of ethyl acetate. The resulting solution was chilled in an ice bath and anhydrous HCl gas was bubbled through the solution for 5 minutes. The product precipitated while the mixture was allowed to sit at ambient temperature for 1 hour. The product was collected by filtration, washed on the filter with fresh ethyl acetate, and dried under vacuum at ambient temperature to constant weight to afford 5.9 g of 1-(5-chloro-2-{2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea, the compound of formula (d), as white solid; NMR (2 rotomers); (400 MHz, DMSO) 9.7 (m, 0.5H), 9.2 (m, 0.5H), 8.16 (s, 1H), 8.13 (s, 0.5H), 6.8 (s, 2H), 4.9 (m, 2H), 4.4 (m, 5H), 3.8 (bs, 0.5H), 3.4, (bs, 0.5H), 3.2, (m, 2.5H), 3.0 (m, 2 H) 1.2-1.4 (m, 3H) ppm.

Example 2

Preparation of the Compound of Formula (e)



To a solution of 2-amino-4-chlorophenol (10 g, 69.7 mmol) in 100 mL of anhydrous THF at ambient temperature was added trimethylsilyl isocyanate (18.8 mL, 139.4 mmol) in one portion. The solution was heated to 60°C and remained at this temperature for 22 hours at which time water (1.3 mL, 76.7 mmol) was added. After 30 minutes the solution was

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cooled to ambient temperature and concentrated to a brown oil. This oil was dissolved in ethyl acetate, treated with activated carbon, dried over magnesium sulfate and filtered. The filtrate was concentrated to a pink solid which was crystallized from 10:1, toluene/methanol to give 5.4 g of the compound of formula (e) as tan powder.

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Example 3

Preparation of 1-(5-Chloro-2-{2-[(2R)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea

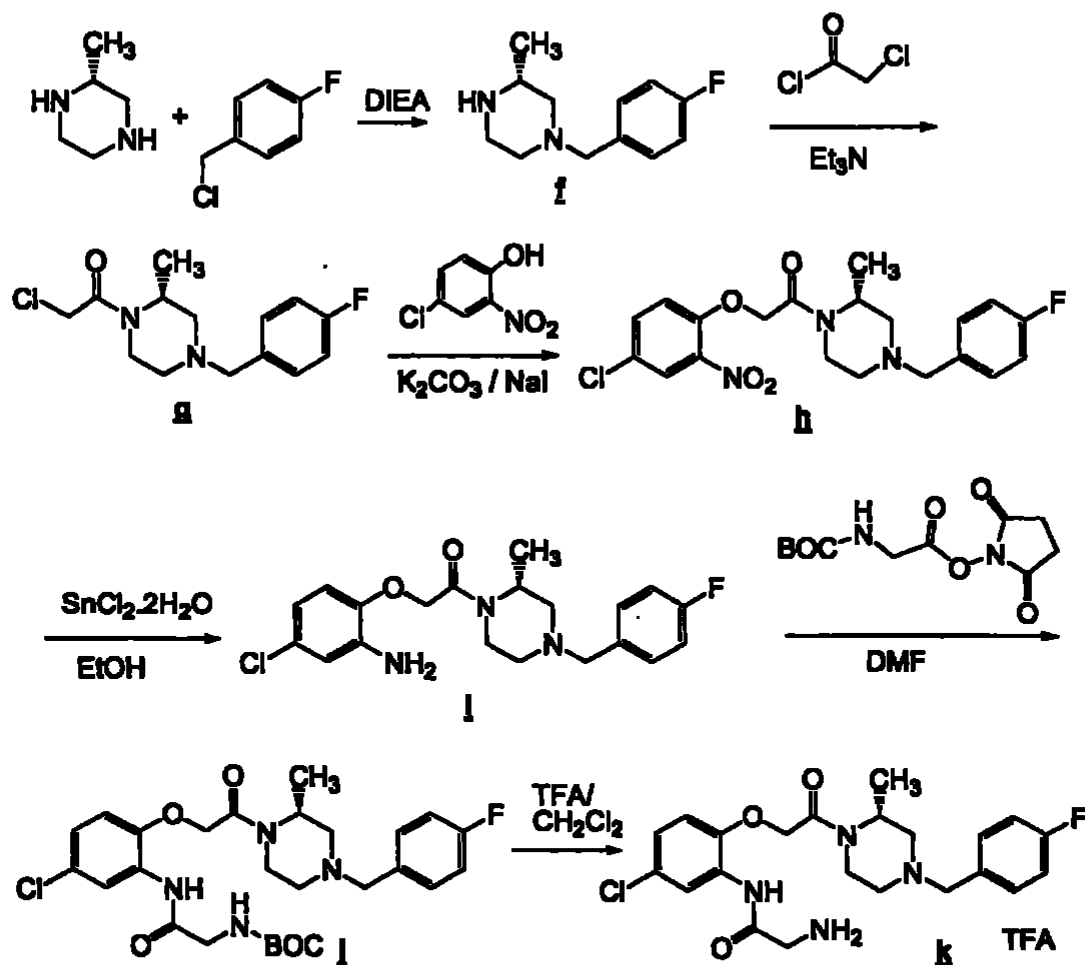
10 A. Hydrogen fluoride-¹⁸F was prepared in a cyclotron by bombardment of H₂O-¹⁸O with protons. The resulting hydrogen fluoride-¹⁸F was adsorbed on an anion-exchange cartridge. The hydrogen fluoride-¹⁸F was eluted with a solution of Kryptofix 222 (15 mg, 40 μmol) and K₂CO₃ (2.77 mg, 20 μmol) in aqueous acetonitrile (1.5 mL, 88%). The radioactive fractions were evaporated to dryness in a nitrogen gas stream. This procedure was repeated three times with dry acetonitrile (1 mL). After addition of a solution of 4-trimethylammonium-benzaldehyde-triflate (2 mg, 6.4 μmol) in dry DMF (250 μl) the resulting reaction mixture was heated for 5 minutes to 100°C. After cooling to ambient temperature a solution of 1-(5-chloro-2-{2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea, a compound of formula (d), (3 mg, 8.3 μmol) in 50 μl acetic acid and a solution of sodium-cyano-borohydride (4 mg, 63.7 μmol) in 100 μl dry DMF were added. The reaction mixture was heated to 120°C for 10 minutes. After addition of 5 mL of water the mixture was filtered over a polystyrol-cartridge. The adsorbed product was washed with 2 mL of water to afford the title compound, 1-(5-chloro-2-{2-[(2R)-4-(4-fluoro-¹⁸F-benzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea, which was eluted with 1.5 mL acetonitrile and purified by HPLC.

25 B. In a similar manner, other compounds of formula (I) containing a ¹⁸F atom are prepared.

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Example 4

Preparation of *N*-(5-Chloro-2-[(2*R*)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy)phenyl)glycinamide

A. To a solution of (*R*)-(-)-2-methylpiperazine (2.0 g, 20 mmol) in 20 mL CH_2Cl_2 was added DIEA (5.2 g, 40 mmol) and 4-fluorobenzyl chloride (2.39 mL, 20 mmol). The resulting mixture was stirred at ambient temperature for 15 hours. After the reaction was completed, the reaction mixture was washed with water (3X20 mL) and brine, then dried over Na_2SO_4 , filtered and concentrated in vacuo to afford the compound of formula (I) (2.2 g) as a white solid.

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B. To a solution of the compound of formula (f) (2.2 g, 10 mmol) in 50 mL CH_2Cl_2 was added chloroacetyl chloride (0.84 mL, 10 mmol). The resulting mixture was stirred at ambient temperature for 10 minutes and then triethylamine (3 mL, 21 mmol) was added. After 30 minutes, the mixture was washed with water (3x20mL) and brine, then dried over

5 Na_2SO_4 , filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded the compound of formula (g) (2.5 g).

C. To a solution of the compound of formula (g) (2.4 g, 8.4 mmol) in 50 mL DMF was added K_2CO_3 (2.5 g, 17 mmol), NaI (0.2 g) and 4-chloro-2-nitrophenol (1.3 g, 8.4 mmol). The resulting mixture was heated at 70-80°C. After 1 hour, the mixture was concentrated in
10 vacuo, then taken up in ethyl acetate (150 mL) and washed with water (3X100mL) and then brine. The organic layer was separated, dried over Na_2SO_4 , filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded the compound of formula (h) (3.1 g).

D. To a solution of the compound of formula (h) (1.1 g, 2.6 mmol) in 10 mL ethanol was added a solution of tin(II) chloride dihydrate (3.0 g, 13 mmol) in 5 mL ethanol. The resulting mixture was heated at 75°C. After 1 hour, the reaction was concentrated in vacuo, then taken in ethyl acetate (100 mL), washed with 1N NaOH solution in water (3X100mL) and brine. The organic layer was separated, dried over Na_2SO_4 , filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded the compound
15 of formula (i) (0.75g).

E. To a solution of the compound of formula (i) (0.7 g, 1.78 mmol) in 10 mL DMF was added BOC-Gly-OSU (0.58 g, 2.13 mmol). The resulting mixture was heated at 50-60°C. After 24 hours, the mixture was concentrated in vacuo, then taken up in ethyl acetate (150 mL) and washed with water (3X100mL) and then brine. The organic layer was
25 separated, dried over Na_2SO_4 , filtered and concentrated in vacuo to afford an oil.

Purification by flash column chromatography afforded the compound of formula (j) (0.7 g).

F. To a solution of the compound of formula (j) (0.6 g, 1.1 mmol) in 10 mL CH_2Cl_2 was added TFA (5 mL). The resulting mixture was heated at ambient temperature. After the reaction was completed in 1 hour, the reaction was concentrated in vacuo, then taken up in
30 ethyl acetate (100 mL), washed with 1N NaOH solution in water (2X100mL) and brine. The

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organic layer was separated, dried over Na_2SO_4 , filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded *N*-(5-chloro-2-[2-[(2*R*)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy]phenyl)glycinamide, the compound of formula (k), (0.45 g) as a white solid; NMR (CDCl_3) 10.2 (s, 1), 8.5 (s, 1), 7.3 (m, 2), 7.0 (m, 3), 6.8 (d, 1), 4.7 (m, 2), 3.4-3.6 (m, 5), 3.0 (m, 1), 2.8 (m, 1), 2.6 (d, 1), 2.2 (m, 1), 2.0 (m, 2), 1.2-1.4 (m, 3) ppm.

Example 5

N'-(mercaptoeth-1-yl)-*N'*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide, Technetium-99m-complex

A. To a stirred solution of *N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide (175.5 mg, 0.4 mmol) (the compound of formula (k) as prepared above in Example 4), *N*-(*S*-trityl-2-mercaptoeth-1-yl)-*N*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)glycine (291.4 mg, 0.4 mmol) (which can be synthesized according to A. Mahmood *et al.*, *A N_2S_2 -Tetradentate Chelate for Solid-Phase Synthesis: Technetium, Rhenium in Chemistry and Nuclear Medicine* (1999), Vol. 71), and *N*-hydroxysuccinimide (45.5 mg, 0.4 mmol) in 5 mL dichloromethane was added dropwise a solution of dicyclohexylcarbodiimide (81.4 mg, 0.4 mmol) in 3 mL dichloromethane. The resulting suspension was stirred over night at ambient temperature. After filtration, the resulting solution was evaporated under reduced pressure. The desired product, *N*-(*S*-trityl-2-sulfanyleth-1-yl)-*N*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide (340 mg, 72.8%) was isolated after silica gel chromatography (eluent: dichloromethane/methanol, 98:2) as a white powder.

Elemental Analysis:

Calc.:	C 69.93	H 5.87	N 7.20	O 6.85	S 5.49
Found:	C 69.69	H 6.05	N 7.01	O	S 5.32

B. *N*-(*S*-Trityl-2-sulfanyleth-1-yl)-*N*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-

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yl)glycylglycinamide (116.8 mg, 0.1 mmol), as prepared above, was dissolved in 5 mL trifluoroacetic acid. After addition of triethyl silane (48 μ l, 0.3 mmol) the resulting suspension was stirred for 15 minutes at ambient temperature. The filtrate was evaporated under reduced pressure and the residue was triturated with 7 mL diethyl ether. The precipitate was stirred for 1 hour at ambient temperature and filtered off, yielding the desired product, *N*-(mercaptoethyl-1-yl)-*N'*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide, bis-trifluoroacetic acid salt (87 mg, 95.5%), as a white solid.

Elemental Analysis:

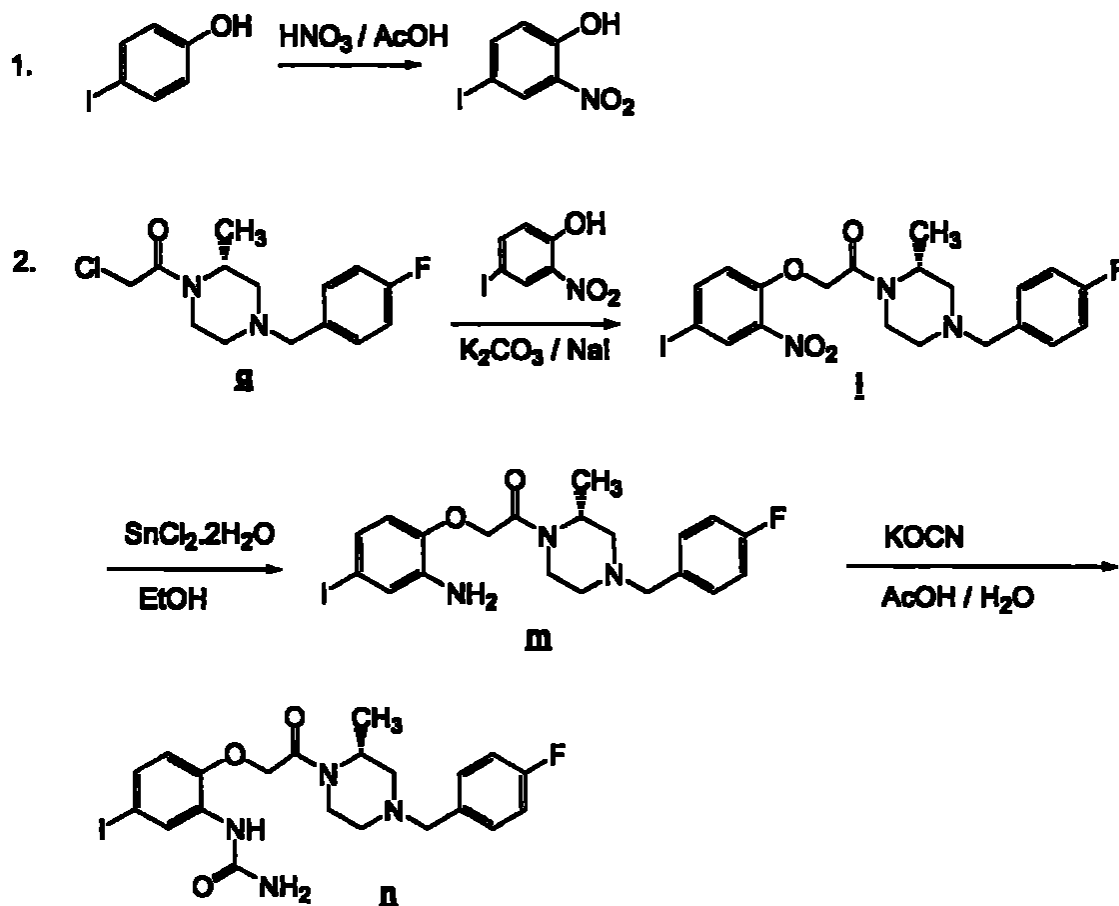
Calc.:	C 44.81	H 4.65	N 9.22	O 15.80	S 7.04
Found:	C 44.54	H 4.91	N 8.99	O	S 6.80

C. Disodium tartrate (1 mg) and *N*-(mercaptoethyl-1-yl)-*N'*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide, bis-trifluoroacetic acid salt (100 μ g), as prepared above, were dissolved in 500 μ l sodium phosphate buffer (0.1M, pH=8.5). After addition of 37MBq ^{99m}Tc-generator eluate, 5 μ l tin(II) chloride solution was added and the mixture was heated for 10 min to 100°C. HPLC analysis showed a major peak indicating that the desired product, *N*-(mercaptoethyl-1-yl)-*N'*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycylglycinamide,

Technetium-99m-complex, was synthesized with a RCP > 90%.

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Example 6

Preparation of 1-(2-{2-[(2*R*)-4-(4-Fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}-5-iodophenyl)urea

A. To a solution of *p*-iodophenol (2.2 g, 10 mmol) in 18 mL acetic acid was added a solution of HNO₃ (0.7 mL, 70%) in 5 mL acetic acid dropwise over 10 minutes. The resulting mixture was stirred at ambient temperature. After 30 minutes, the reaction mixture was diluted by 100 mL ice-water. The precipitate was collected, washed with 100 mL water. Purification by flash column chromatography afforded 1.2 g of 2-iodo-4-nitrophenol.

B. To a solution of the compound of formula (g) (0.3 g, 1.05 mmol) (as prepared herein) in 10 mL DMF was added K₂CO₃ (0.45 g, 3.2 mmol), NaI (0.01 g) and 2-iodo-4-

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Iodophenol (0.28 g, 1.05 mmol). The resulting mixture was heated at 70-80°C. After 1 hour, the mixture was concentrated in vacuo, then taken up in ethyl acetate (150 mL) and washed with water (3X100mL) and then brine. The organic layer was separated, dried over Na₂SO₄, filtered and concentrated in vacuo to afford an oil. Purification by flash column

5 chromatography afforded 0.28 of the compound of formula (l).

C. To a solution of the compound of formula (l) (0.28 g, 0.55 mmol) in 5 mL ethanol was added a solution of Tl₂(Cl)₂ dihydrate (0.616 g, 2.73 mmol) in 5 mL ethanol. The resulting mixture was heated at 75°C. After 1 hour, the reaction was concentrated in vacuo, then taken up in ethyl acetate (100 mL), washed with 1N NaOH solution in water (3X100mL) and brine. The organic layer was separated, dried over Na₂SO₄, filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded the compound of formula (m) (0.25g).

D. To a solution of the compound of formula (m) (0.25 g, 0.52 mmol) in 3 mL AcOH was added water (6 mL). The resulting mixture was stirred at ambient temperature for 10 minutes, then a solution of KOCN (0.085 g, 1.0 mmol) in 1 mL water was added dropwise. The reaction mixture was stirred at ambient temperature for 10 minutes, then heated at 55°C for 5 minutes. After the reaction was completed, the reaction mixture was concentrated in vacuo, then taken up in CH₂Cl₂ (50 mL), washed with 2N NaOH solution in water (2x100mL) and brine. The organic layer was separated, dried over Na₂SO₄, filtered and concentrated in vacuo to afford an oil. Purification by flash column chromatography afforded the compound of formula (n) (0.16 g) as a white solid; NMR (CDCl₃) 9.0 (s, 1), 8.6 (s, 1), 7.3 (m, 2), 7.0 (t, 3), 6.6 (d, 1), 4.9 (s, 2), 4.7 (m, 2), 4.4 (m, 1), 3.4-3.6 (m, 3), 3.0 (m, 1), 2.8 (m, 1), 2.6 (d, 1), 2.2 (m, 1), 2.0 (m, 1), 1.2-1.4 (m, 3) ppm.

Example 7

Preparation of 1-(2-{2-[(2R)-4-(4-Fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}-5-iodo-¹²³I-phenyl)urea

A. To a solution of the compound of formula (n) (1 mg), as prepared above, and 10 µg copper-(II)-sulfate in 300 µl DMF was added 1 mCi sodium Iodine [¹²³I] solution. The resulting reaction mixture was heated over night to 100°C. After adding of 1 mL half-

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saturated aqueous NaHCO_3 solution the product was extracted with 2 mL CH_2Cl_2 . The organic layer was evaporated to dryness in a nitrogen gas stream. The desired product, 1-(2-{2-[(2*R*)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}-5-iodo- ^{123}I -phenyl)urea, was purified using a RP-cartridge [eluant: EtOH/water (2:1)].

- 5 B. In a similar manner as described above, other compounds of formula (I) are prepared.

Example 8

***N'*-(2-Mercaptoeth-1-yl)-*N'*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, Technetium-99m-complex**

10 A. To a stirred solution of 156.7 mg (0.4 mmol) 5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]aniline (compound of formula (I)) (291.4 mg, 0.4 mmol), *N*-(*S*-trityl-2-mercaptoeth-1-yl)-*N*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)glycine (synthesized according to A. Mahmood *et al.*, *A N₂S₂-Tetradentate Chelate for Solid-Phase Synthesis: Technetium, Rhenium in Chemistry and Nuclear Medicine* (1999), Vol. 5, p. 71) and *N*-hydroxysuccinimide (45.5 mg, 0.4 mmol) in 5 mL dichloromethane was added dropwise a solution of dicyclohexylcarbodiimide (81.4 mg, 0.4 mmol) in 3 mL dichloromethane. The resulting suspension was stirred over night at ambient temperature. After filtration, the resulting solution was evaporated under reduced pressure. The desired product, *N'*-(*S*-trityl-2-mercaptoeth-1-yl)-*N'*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide (352 mg, 79.2%), was isolated after silica gel chromatography (eluent: dichloromethane/methanol, 98:2) as a white powder.

25 Elemental Analysis:

Calc.:	C 71.36	H 5.90	N 6.30	O 5.76	S 5.77
Found:	C 71.08	H 6.13	N 6.05	O	S 5.52

30 B. *N'*-(*S*-trityl-2-mercaptoeth-1-yl)-*N'*-(*S*-trityl-5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide (111.1 mg, 0.1 mmol) was dissolved in 5 mL trifluoroacetic acid. After addition

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of 48 μ l (0.3 mmol) triethyl silane the resulting suspension was stirred for 15 min at ambient temperature. The filtrate was evaporated under reduced pressure and the residue was triturated with 7 mL diethyl ether. The precipitate was stirred for 1 hour at ambient temperature and filtered off, yielding the desired product, *N*-(2-mercaptoeth-1-yl)-*N*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, bis-trifluoroacetic acid salt (75 mg, 87.8%) as a white solid.

Elemental Analysis:

Calc.: C 44.99 H 4.60 N 8.20 O 14.98 S 7.51

Found: C 44.71 H 4.89 N 8.03 O S 7.22

C. Disodium tartrate (1 mg) disodium tartrate and *N*-(2-mercaptoeth-1-yl)-*N*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, bis-trifluoroacetic acid salt (100 μ g) were dissolved in 500 μ l sodium phosphate buffer (0.1M, pH=8.5). After addition of 37.5MBq ^{99m} Tc-generator eluate, 5 μ l tin(II) chloride solution was added and the mixture was heated for 10 minutes to 100°C. HPLC-analysis showed a major peak indicating that the desired product, *N*-(2-mercaptoeth-1-yl)-*N*-(5-mercapto-3-aza-2-oxopent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, technetium-99m-complex was synthesized with a RCP > 91%.

Example 9

N-(2-Mercaptoeth-1-yl)-*N*-(5-mercapto-3-azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide,
Technetium-99m-complex

A. To a stirred solution of 5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]aniline (156.7 mg, 0.4 mmol) and triethylamine (40.5 mg, 0.4 mmol) in 5 mL dichloromethane was added dropwise a solution of α -bromoacetyl chloride (63 mg, 0.4 mmol) in 3 mL dichloromethane. The resulting solution was stirred over night at ambient temperature. The solvent was evaporated under reduced pressure. The desired product, *N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)-2-

bromoacetamide (175 mg, 85.3 %), was isolated after silica gel chromatography (eluent: dichloromethane/methanol, 99:1) as a white powder.

Elemental Analysis:

Calc.: C 51.53 H 4.72 N 8.19 O 5.76

Found: C 51.28 H 4.99 N 7.92 O

B. A stirred solution of *N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)-2-bromoacetamide (165 mg, 0.42 mmol) and *N,N*-bis-(*S*-(4-methoxybenzyl)-2-mercaptoeth-1-yl)ethylenediamine (841.3 mg, 2 mmol) (synthesized according to Z.P. Zhuang et al., *Bioconjugate Chem.* (1999), Vol. 10, p. 159) in 1,4-dioxane (5 mL) was heated under reflux for 24 hours. The resulting reaction mixture was evaporated under reduced pressure. The residue was dissolved in 15 mL dichloromethane and washed with saturated aqueous sodium carbonate solution. After drying over MgSO_4 the solvent was evaporated under reduced pressure. The desired product, *N*-(*S*-(4-methoxybenzyl)-2-mercaptoeth-1-yl)-*N'*-(*S*-(4'-methoxybenzyl)-5-mercapto-3-azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide (154 mg, 43%), was isolated after silica gel chromatography (eluent: dichloromethane/methanol, 9:1) as a white powder.

Elemental Analysis:

Calc.: C 61.99 H 6.50 N 8.22 O 9.38 S 7.52

Found: C 61.71 H 6.58 N 8.03 O S 7.28

C. *N*-(*S*-(4-methoxybenzyl)-2-mercaptoeth-1-yl)-*N'*-(*S*-(4'-methoxybenzyl)-5-mercapto-3-azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide (140 mg, 0.164 mmol) was dissolved at 0°C in 5 mL trifluoroacetic acid. After addition of $\text{Hg}(\text{OAc})_2$ (104.5 mg, 0.328 mmol) the resulting mixture was stirred for 30 minutes at 0°C and saturated for 15 min with H_2S . After filtration the solvent was evaporated under reduced pressure. The resulting yellow oil was triturated with 3 mL diethyl ether. The desired product, *N'*-(2-mercaptoeth-1-yl)-*N'*-(5-mercapto-3-azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, tri-trifluoroacetic acid salt (126 mg (80.5%)), was isolated after filtration as a white powder.

Elemental Analysis:

Calc.:	C 42.79	H 4.44	N 7.34	O 15.09	S 6.72
Found:	C 42.48	H 4.73	N 7.02	O	S 6.76

D. Disodium tartrate (1 mg) and *N*-(2-mercaptoeth-1-yl)-*N*-(5-mercapto-3-

- 5 azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, tris-trifluoroacetic acid salt (100 µg) were dissolved in 500 µl sodium phosphate buffer (0.1M, pH=8.5). After addition of 37.5MBq ^{99m}Tc-generator eluate, 5 µl tin(II) chloride solution was added and the mixture was heated for 10 minutes to 100°C. HPLC-analysis showed a major peak indicating that the desired product, *N*-(2-mercaptoeth-
- 10 1-yl)-*N*-(5-mercapto-3-azapent-1-yl)-*N*-(5-chloro-2-[2-[4-(4-fluorobenzyl)-2-(2*R*)-methylpiperazin-1-yl]-2-oxoethoxy]phen-1-yl)glycinamide, Technetium-99m-complex, was synthesized with a RCP > 95%.

Example 10

Immunohistochemical Localization of CCR1 In Human Brains

Materials and Methods

A first group of tissue samples from brains that had been obtained at autopsy within 12 hours of death was used for immunohistochemical staining of the chemokine receptor CCR1 in Alzheimer's disease and control brains. Paraffin-embedded, 5-µm-thick, unstained sections from frontal cortex and hippocampus were used for all immunohistochemical and histochemical stains.

- Slides were de-paraffinized in xylene and hydrated to phosphate-buffered saline containing 0.005% Triton X100 (PBS-T). For light microscopy (using DAB as chromogen) endogenous peroxidase activity was blocked by incubating the slides with 0.01% H₂O₂ in methanol for 30 minutes. Non-specific binding was reduced by blocking in 10% normal goat serum (NGS) in PBS for 30 minutes. The primary antibodies [Rabbit anti-CCR1, (N-terminal peptide); Mouse anti-Neurofilament; mouse anti-GFAP; mouse anti-Tau (AT8); mouse anti-CD68 (clone KP1); mouse anti-β-amyloid (Boehringer Mannheim, #1 381 431); Rabbit anti-CCR8, (N-terminal peptide)] were diluted in PBS-T and slides were incubated overnight at room temperature. Staining was completed using a Biotogenex ABC™ kit. All

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washes used PBS-T. After the DAB reaction was completed, slides were lightly counterstained with Gill's Hematoxylin, dehydrated and coverslipped with Permount. Before coverslipping, some slides were re-stained with antibodies against A β peptide using identical methods except that the chromogen was True Blue™. For immunofluorescent double-labeling studies the slides were de-paraffinized, blocked with 10% NGS, and incubated with a cocktail of both primary antibodies overnight. The slides were washed with PBS-T and then incubated with a cocktail of goat secondary antibodies, each at 1/50 dilution. To avoid confusion from endogenous (yellow-green) tissue fluorescence, the secondary antibodies were conjugated to either Cy3 (ϵ_{max} = 565 nm; goat-anti-mouse, Amersham) or Cy5 (ϵ_{max} =700 nm; goat-anti-rabbit, Amersham). Slides were viewed on a confocal microscope with a krypton-argon laser (model 2010, Molecular Dynamics, Sunnyvale, CA).

Subsequently, a second group of brain tissues composed of 10 cases from cognitively normal elderly and 40 cases from Alzheimer's disease patients who had been assessed for clinical dementia rating (CDR) were obtained and evaluated for immunohistochemical expression of CCR1. The samples were stained for CCR1 and other markers as described above except that monoclonal antibodies (clone #6D5) specific for A β ¹⁻⁴² (a marker for diffuse, early amyloid deposits as well as for more mature neuritic plaques) were obtained from Dr. Ursula Moenning. These antibodies were visualized with Vector Red™ (Vector Labs). Dr Moenning also provided monoclonal antibodies specific for A β ¹⁻⁴⁰ (clone #13E9), a marker for plaques found in late-stage disease, that was visualized with True Blue™.

Results

In both sets of brains, areas with Alzheimer's disease pathology showed a characteristic staining pattern. CCR1 immunoreactivity was found in association with neuritic (i.e., senile) plaques in both the hippocampal formation and the cerebral cortex. The immunostained structures were round to ovoid and were not usually associated with cell bodies. They varied in size and appeared to be filled with a punctate granular material. These structures were found to form "coronas" around the amyloid deposits in

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senile plaques, but were distinct from the A β itself (Figures 1-4). The top panel in Figure 1 shows a neuritic plaque with a corona of CCR1-positive processes. Neuronal cell bodies of some CA1 and CA3 neurons in Alzheimer's disease cases were sometimes stained by CCR1 antibodies. The bottom panel in Figure 1 illustrates this finding. This staining was distinct from neurofibrillary tangles (NFT) or granulovacuolar bodies, although it was commonly present in the somata of neurons with these degenerative changes.

Pre-incubation of antibodies with a 16-mer polypeptide from the extracellular (N-terminus) region of the CCR1 receptor protein completely blocked all tissue staining, as illustrated in Figure 2. In particular, the top image in Figure 2 shows a lack of staining in Alzheimer's disease brain with CCR1 antibodies that were pre-incubated with the polypeptide while the bottom image in Figure 2 shows many CCR1-positive structures in a sister section stained with un-incubated antibodies.

Double-labeling studies showed that nearly all CCR1-positive structures were associated with neuritic plaques containing A β^{1-42} as shown in Figure 4. A β^{1-42} in diffuse plaques (Figure 5) was not typically associated with CCR1 nor with any significant cellular responses. In more advanced cases of Alzheimer's disease, many more A β^{1-40} -positive plaques were seen. Some, but not all of these were associated with CCR1 staining as shown in Figure 3. In some cases CCR1-positive plaques were completely free of A β^{1-40} staining.

In cases of severe Alzheimer's disease where significant neuronal loss and gliosis were seen, reactive astrocytes in the subiculum and entorhinal cortex were also CCR1 positive. In less severe cases, CCR1-positive reactive astrocytes were uncommon.

Confocal microscopy of double-labeled sections showed that CCR1 immunoreactivity co-localized with neurofilament-positive processes. The macrophage/microglial marker, CD68, was not associated with CCR1 staining, nor was the AT8 antibody against abnormally phosphorylated tau protein. As expected from light microscopic studies, CCR1 immunoreactivity did co-localize with GFAP in areas of astrogliosis.

Leukocytes present within cerebral vessels were strongly CCR1 positive in both Alzheimer's disease and control brain tissues, serving as positive internal controls for staining methods. In Figure 5 note the CCR1-positive staining of two intravascular cells

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(arrows). Some CCR1-positive material is also seen at asterisk, but in general, diffuse plaques are not associated with CCR1.

Using hippocampal tissue from the second study (where patients were grouped into known clinical categories by CDR score), a quantitative evaluation of the number of CCR1-positive plaque-like structures in the hippocampus was undertaken. Histologic evaluations of individual sections were performed under blinded conditions with respect to clinical (CDR) status. The volume analyses were conducted using unbiased computerized methods.

Figure 3 demonstrates the histologic relationship between CCR1-positive dystrophic neurites (brown stain) and a neuritic plaque containing $A\beta^{1-40}$ (blue stain). Note in that in Figure 3 the CCR1-positive processes are distinct from the amyloid. Plaques containing $A\beta^{1-40}$ were discrete and easily counted, as were the clusters of CCR1-positive neurites. Slides of hippocampus were evaluated for the number of CCR1-positive coronas, the number of $A\beta^{1-40}$ positive plaques, and the number of plaques containing both markers. The structures were counted by region (e.g., CA3, CA1, and subiculum) within the entire hippocampal formation, including entorhinal cortex and then totaled. The values are relative estimates of the number of structures in the hippocampus as represented by 5- μ m thick cross-sections of the hippocampal formation. Figure 6 shows the relationship between CCR1 and $A\beta^{1-40}$ by CDR score.

Quantification of the amount of $A\beta^{1-42}$ required a different technique because $A\beta^{1-42}$ was more abundant and formed "diffuse" plaques that could not be easily enumerated (see Figure 5). For these slides a computer-assisted method (C.A.S.T. stereology system) was used to estimate the area of entorhinal cortex occupied by $A\beta^{1-42}$. This area was expressed as a percent of the total area of cortex on the slide. The areas occupied by neuritic and diffuse plaques were counted separately. The computer-driven microscope stage assured random (unbiased) evaluation of the tissue sections. The volume % of entorhinal cortex occupied by $A\beta^{1-42}$ (both diffuse and neuritic) is compared to the number of CCR1-positive plaques in a sister section of entorhinal cortex in Figure 7.

Figure 6 shows that the average number of CCR1-positive dystrophic neurites in the hippocampus increases as a function of CDR score in Alzheimer's disease. The number

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of positive structures in early Alzheimer's disease (CDR 0.5) is increased above control (CDR 0) levels. Although the differences between control and Alzheimer's disease groups did not become statistically significant until group CDR 2, these expression patterns support the conclusion that CCR1 is upregulated in dystrophic neuronal processes even at very early stages of Alzheimer's disease. Note that the number of $A\beta^{1-40}$ plaques do not rise until late in the disease. CCR1 expression in brain tissue may thus be considered a relatively early indicator of Alzheimer's disease.

The correlation between number of CCR1-positive plaques in entorhinal cortex and the amount of $A\beta^{1-42}$ is shown in Figure 7. In general, CCR1 levels in entorhinal cortex rise as the disease state increases; however, the area sampled is much smaller than the area sampled in Figure 6. Note, however, that $A\beta^{1-42}$ levels rise early in the disease.

Example 11

Assessment of brain availability, using a ^{14}C -labeled tracer

A ^{14}C analogue of 1-(5-chloro-2-{2-[(2*R*)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea was prepared and used for pharmacokinetic studies of brain availability. Mice were injected i.v. through cannulated jugular veins with the analogue at 36 mg/kg containing 334,000 dpm per dose. Mice (in groups of four) were sacrificed at 1, 15, 30, 120, and 1440 minutes after injection by CO_2 inhalation followed by intra-cardiac puncture for removal of whole blood. The chest was then opened and mice were perfused through the heart with phosphate buffered saline for five minutes to remove residual radioactive drug from the blood compartment. The brains were then removed, weighed, and sampled for amount of radioactivity in two separate pieces of cerebral cortex. The radioactivity levels per mg of tissue in the two samples were averaged and the data were normalized to total brain weight, as illustrated in Figure 8. The bars represent the standard error ($n=4$ mice per time point). In particular, the graph in Figure 8 shows that the total number of disintegrations per minute (DPM) for ^{14}C analogue in whole mouse brain decreases to negligible levels by two hours after injection. At the 1-minute time point, calculations show that, on average, about 3% of the injected dose is found in whole mouse brain (average weight of 450 g).

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Plasma samples prepared from the whole blood removed from the mice were also analyzed for radioactive content. The amount of radioactivity in equal volumes of brain and plasma was compared over time as a percent of injected dose, as illustrated in Figure 9. The % of injected dose (i.d.) found per mL of plasma also declines over time, reaching negligible levels after two hours. For comparison to brain levels, the total number of DPM per brain were normalized to a gram of brain weight and expressed as a percentage of the i.d. per gram of brain tissue. Note that mouse brains weigh on average about 450 mg so that the % of i.d. in 1 g of mouse brain shown in the graph overestimates the real value by about two-fold. It is clear that the levels of the analogue in the brain fall in concert with plasma levels. Although the analogue is lipophilic, normal mouse brain does not appear to be a depot site for the CCR1 antagonist.

Example 12

CCR1 Expression In Other Neurodegenerative Diseases

Materials and Methods

Histologic samples of autopsy brain tissue from a total of 29 cases from seven different neurodegenerative diseases (other than pure Alzheimer's disease) were obtained and studied for CCR1 content. Slides were immunostained with antibodies against CCR1 as described in Example 10 above and reviewed under blinded conditions with respect to the specific neurodegenerative disease. Subsequently, sister sections were double-labeled with antibodies against CCR1 (DAB as chromogen) and A β ¹⁻⁴² (Vector Red™ as chromogen).

The diseases examined are listed below:

Parkinson's disease (PD)	6 cases
Parkinsonian dementia of Guam	3 cases
Congophilic angiopathy	4 cases
Multi-Infarct dementia (MID)	4 cases
Diffuse Lewy body dementia (DLBD)	4 cases
Pick's disease	4 cases
Progressive supranuclear palsy (PSP)	4 cases

The stained slides were graded for CCR1 and A β ¹⁻⁴² content using a histologic scale

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of 0 to 4, with 0 being absence of staining, 0.5 indicating rare expression, and grades 1 through 4 indicating increasing levels of expression with 4 being highly abundant.

Results

5 All six cases of Parkinson's disease and all three cases of Parkinsonian dementia of Guam were negative for CCR1. CCR1-positive plaque-like structures, similar to those found in Alzheimer's disease, were observed in all 4 cases of congophilic angiopathy, in 3 of the 4 cases of DLBD, in 2 out of the 4 cases of PSP, and in 1 out of the 4 cases of both MID and Pick's disease.

10 Double-labeling studies confirmed the assumption that these CCR1-positive plaque-like structures were associated with $A\beta^{1-42}$, as was found in pure cases of Alzheimer's disease (Example 10). Figure 10 shows the results of the pathological evaluations in graphic form for all the diseases except for Parkinson's (which was negative for CCR1 in brain). Basically, CCR1 expression was never found in brain tissue samples unless $A\beta^{1-42}$ -positive
15 neuritic plaques were also present.

After the code was broken, it was found that the one case of Pick's disease showing CCR1 expression also carried the diagnosis of Alzheimer's disease. The two PSP cases with CCR1 were also diagnosed with concurrent Alzheimer's disease. Congophilic angiopathy and DLBD are diseases that are closely associated with Alzheimer's disease pathology.

20 Therefore, it is not surprising that they would also have high levels of CCR1 expression in association with $A\beta^{1-42}$. In elderly populations it is often the case that Alzheimer's disease pathology will overlay other disease processes. This was found in one of the four MID cases. These results suggest that CCR1 is a marker that is closely associated with Alzheimer's disease pathology (specifically, $A\beta^{1-42}$ -positive neuritic plaques) regardless of other
25 concurrent pathological processes that may be present in brain. As such, it is likely to be highly specific for Alzheimer's disease pathology and therefore may be useful as a diagnostic surrogate marker of disease progression.

30 While the present invention has been described with reference to the specific

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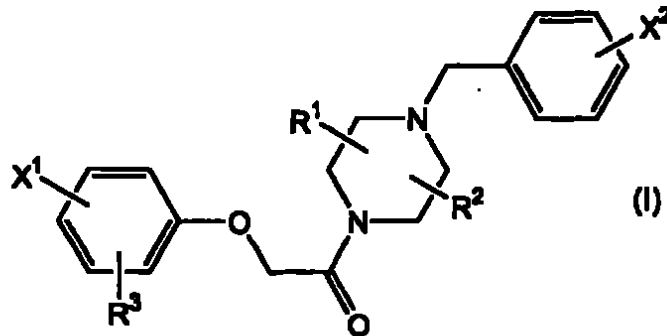
embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the
5 objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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What is claimed is:

1. A compound of formula (I):



wherein:

X^1 and X^2 are each independently halo;

R^1 and R^2 are each independently hydrogen or alkyl; and

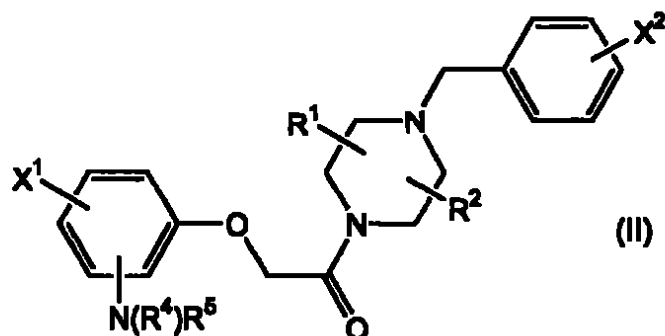
R^3 is hydrogen, amino, monoalkylamino, dialkylamino, monoaralkylamino, alkylcarbonylamino, alkenylcarbonylamino, haloalkylcarbonylamino, arylcarbonylamino, alkoxyalkylcarbonylamino, alkoxyaralkylcarbonylamino, glycinamido, monoalkylglycinamido, arylcarbonylglycinamido, aminocarbonylglycinamido, (aminocarbonyl)(alkyl)glycinamido, (alkoxyalkylcarbonyl)glycinamido, ureido, monoalkylureido, monoarylureido, monoaralkylureido, or alaninamido;

and wherein either one of X^1 or X^2 is selected from the group of ^{123}I , ^{125}I , ^{129}I , ^{131}I , ^{75}Br , ^{76}Br , ^{80}Br and ^{18}F ; or wherein one of the carbon atoms in the compound is ^{11}C ; or a pharmaceutically acceptable salt thereof.

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2. A compound of formula (II):



wherein

X^1 and X^2 are each independently halo;

R^1 and R^2 are each independently hydrogen or alkyl; and

R^4 is hydrogen; and

R^5 comprises a chelator capable of binding a radioactive metal atom chosen from the group of ^{99m}Tc , ^{188}Re and ^{186}Re ;

or as a complex with ^{99m}Tc , ^{188}Re and ^{186}Re ;

or a pharmaceutically acceptable salt thereof.

3. The compound of Claim 1 or Claim 2 wherein said compound binds to chemokine receptor CCR1 and passes the blood-brain barrier.

4. The compound of Claim 1 or Claim 2 wherein R^1 is methyl at the 2-position of the piperazinyl radical and R^2 is methyl at the 5-position of the piperazinyl radical.

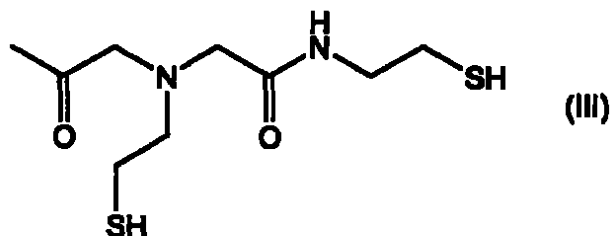
5. The compound of Claim 1 or Claim 2 wherein R^1 is methyl at the 2-position of the piperazinyl radical and R^2 is hydrogen.

6. The compound of Claim 1 wherein X^1 is chloro at the 4-position of the phenyl radical and X^2 is a ^{18}F atom at the 4-position of the phenyl radical.

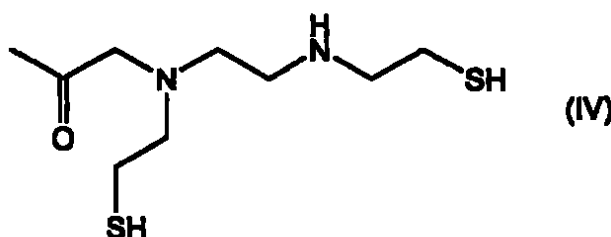
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7. The compound of Claim 2 wherein R^5 is a chelator of formula (III):



8. The compound of Claim 2 wherein R^5 is a chelator of formula (IV):



9. The compound of Claim 7 or Claim 8 wherein R^5 further comprises a linker moiety comprising an alkyl radical having one to ten carbon atoms, wherein the alkyl radical optionally contains one to ten -C(O)- groups, one to ten -C(O)N(R)- groups, one to ten -N(R)C(O)- groups, one to ten -N(R)- groups, one to ten -N(R)₂ groups, one to ten hydroxy groups, one to ten -C(O)OR- groups, one to ten oxygen atoms, one to ten sulfur atoms, one to ten nitrogen atoms, one to ten halogen atoms, one to ten aryl groups, and one to ten saturated or unsaturated heterocyclic rings wherein R is hydrogen or alkyl.

10. The compound of Claim 9 wherein the linker moiety is -C(O)-CH₂-N(H).

11. The monochloride salt of a compound of Claim 1 to Claim 10.

12. The dichloride salt of a compound of Claim 1 to Claim 10.

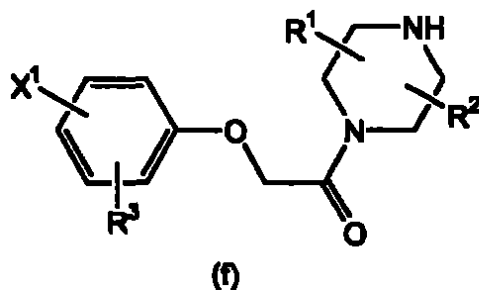
13. A method of diagnosing Alzheimer's disease in a human which comprises administering to a human in need of such diagnosis a compound according to Claim 1 to 12 and measuring the radioactivity arising from the administration of the compound to the human either by using a gamma camera or by positron emission tomography (PET).

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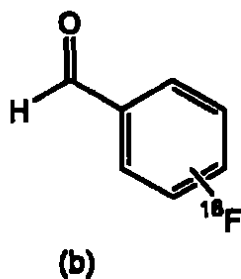
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14. A method of using a compound according to Claim 1 to 12 for the manufacture of a radiopharmaceutical for the diagnosis of Alzheimer's disease in a human.

15. A process for the production of a compound of formula (I) according to Claim 1 comprising the reaction of a compound of formula (f):



wherein R^1 , R^2 , R^3 , and X^1 are as defined in Claim 1,
with a compound of formula (b):



in the presence of a reducing agent.

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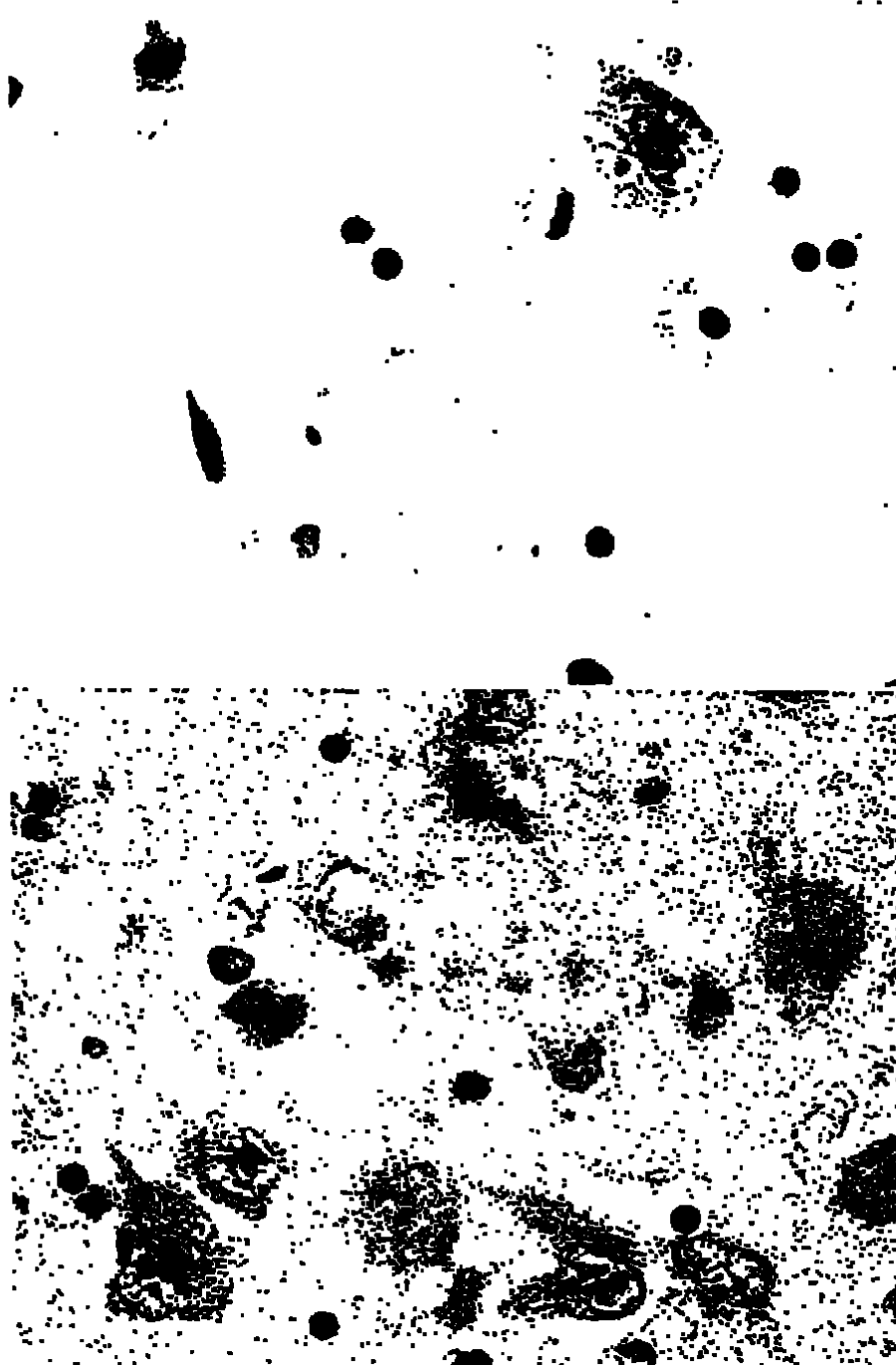
Figure 1



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Figure 2

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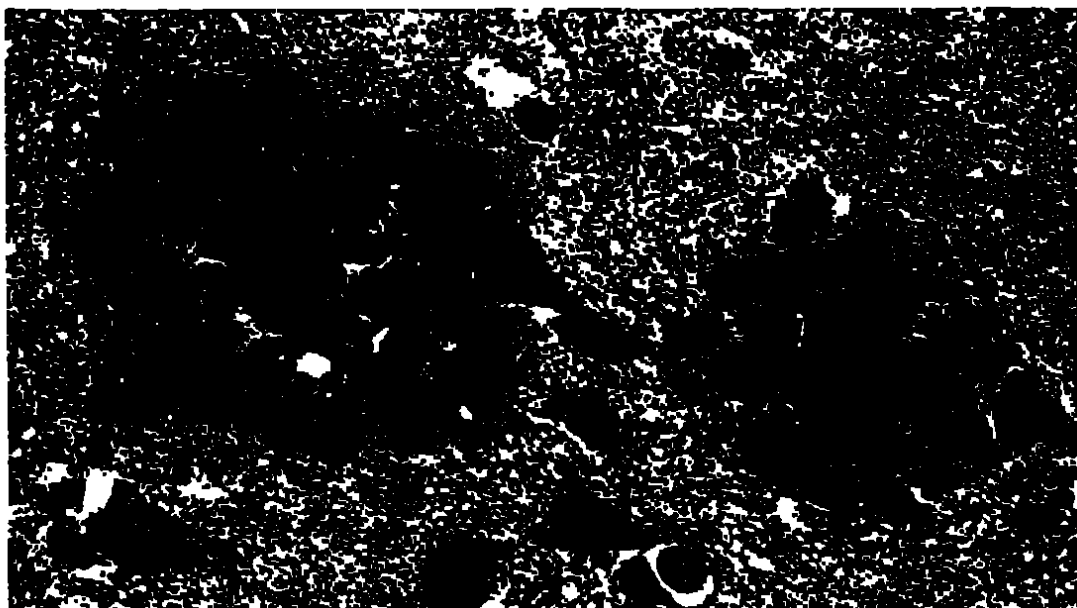
Figure 3



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Figure 4

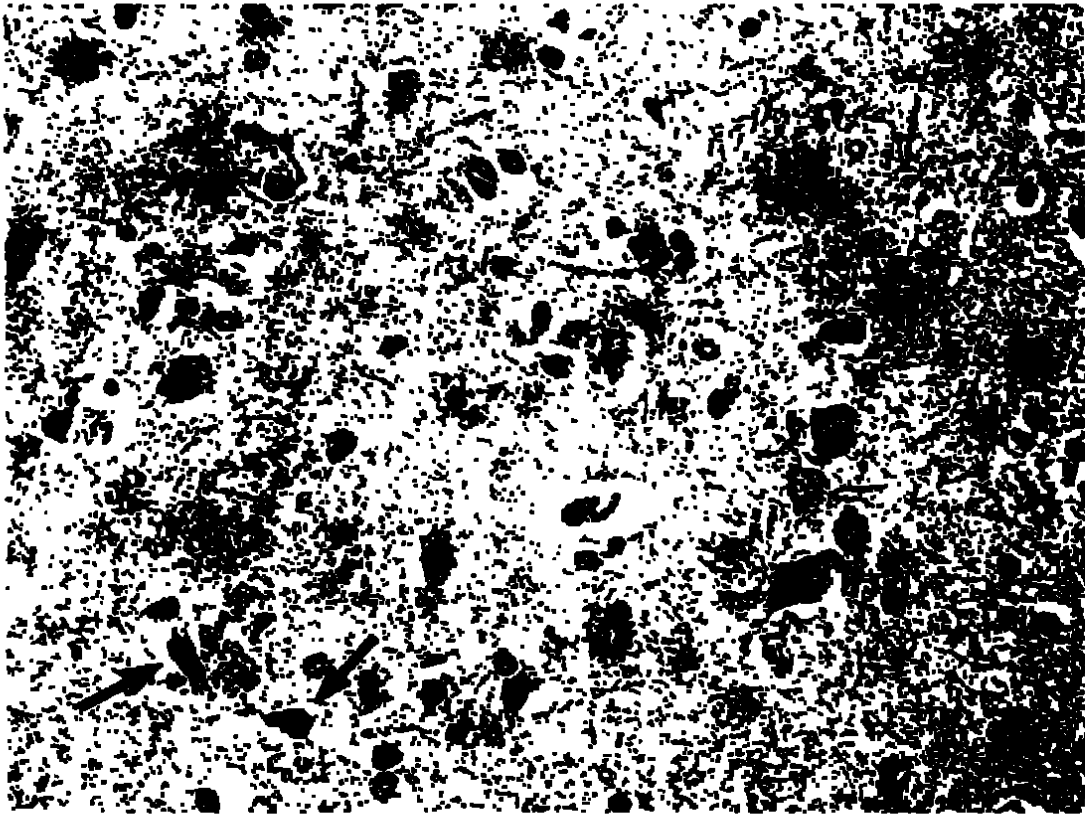


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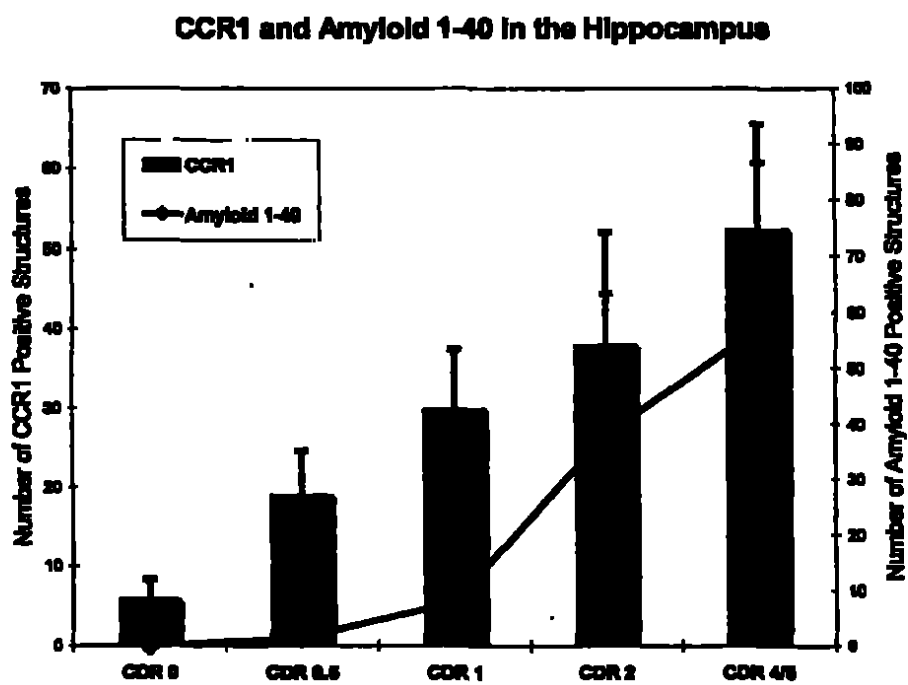
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Figure 5



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Figure 6

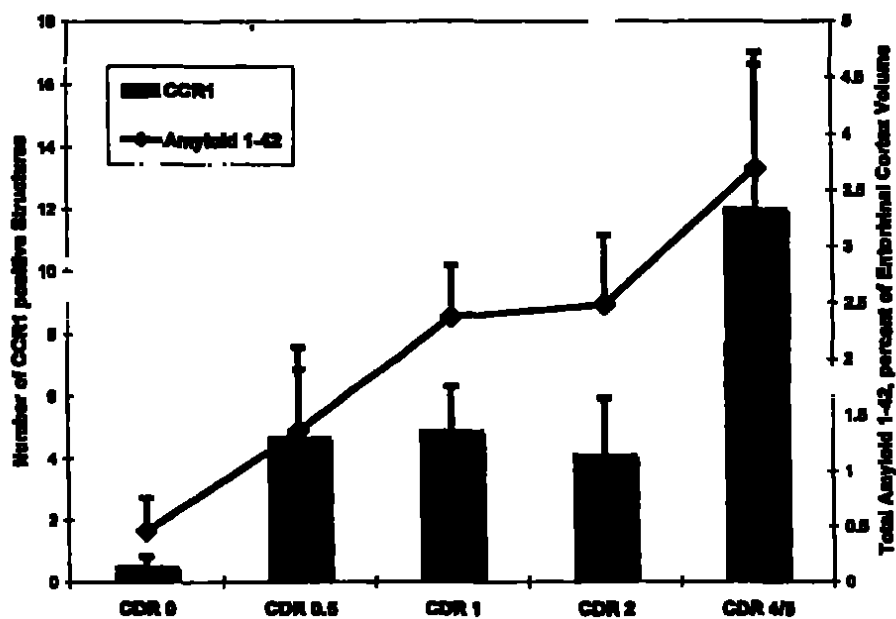


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Figure 7

GCR1 and Amyloid 1-42 In the Entorhinal Cortex

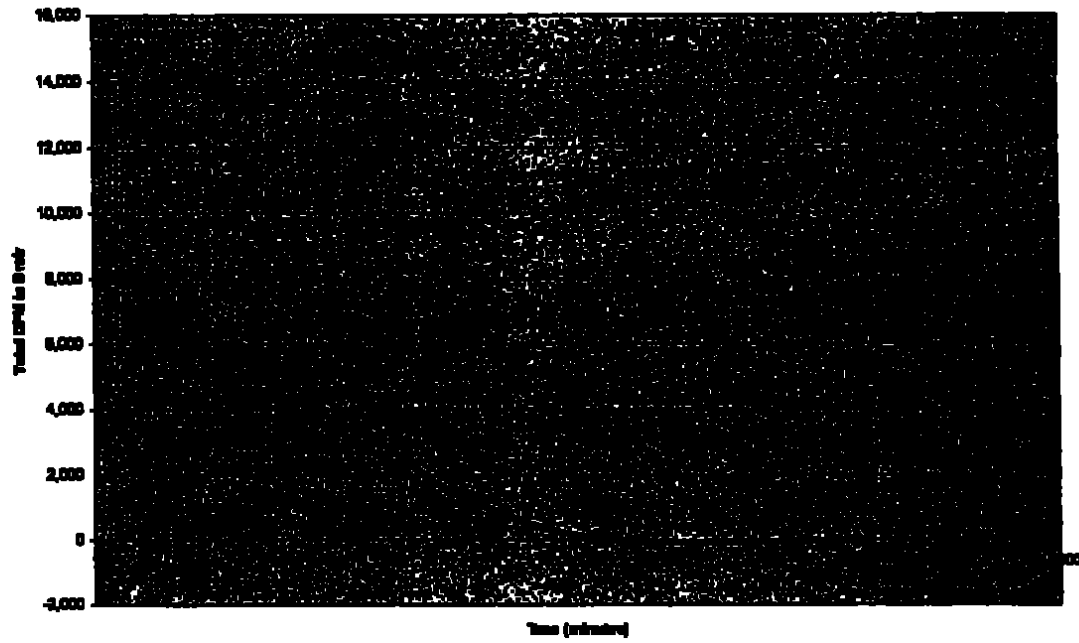


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Figure 8

Decrease in Total Brain DPM over Time

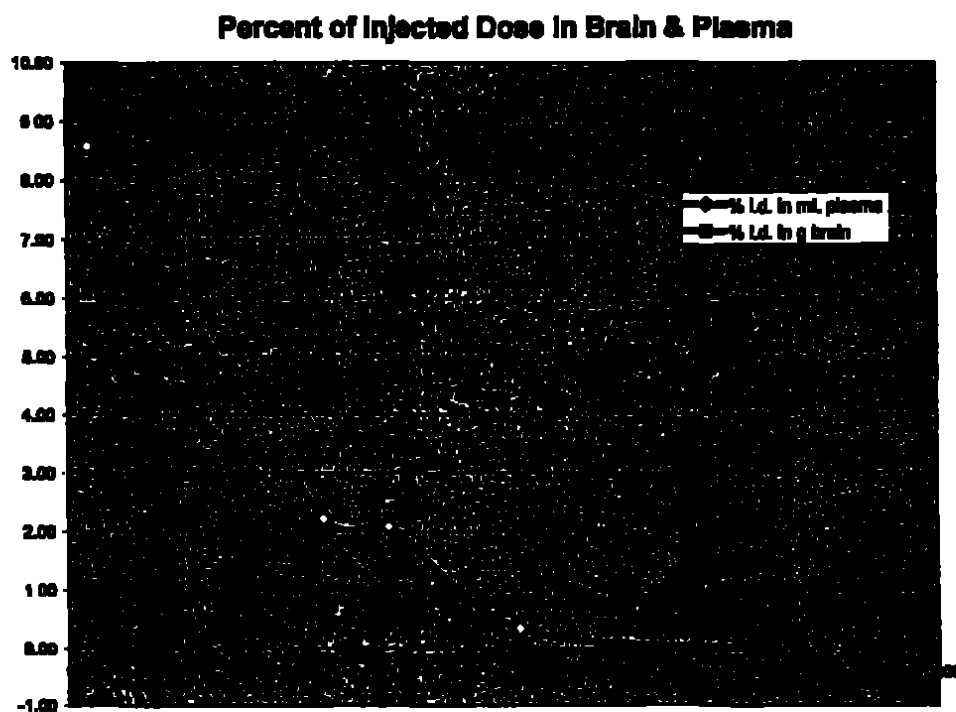


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Figure 9



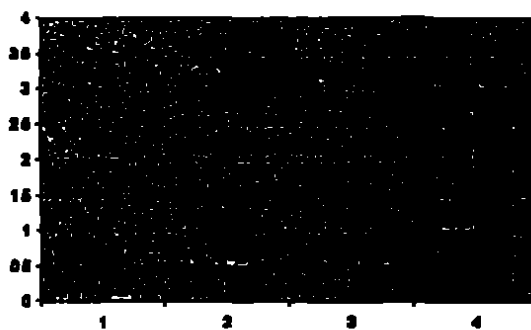
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Figure 10

Congophilic Angiopathy



Multi-Infarct Dementia



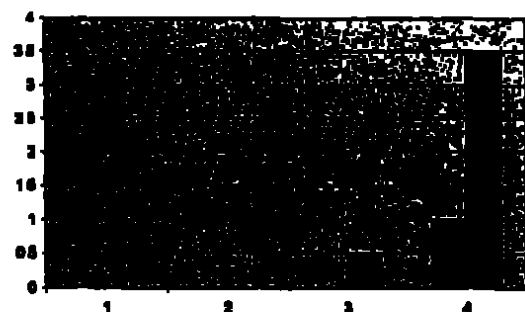
Diffuse Lewy Body Dementia



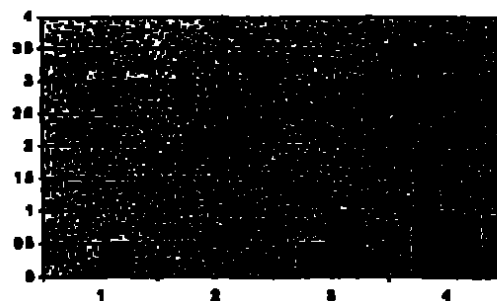
Parkinsonian Dementia of Guam



Pick's Disease



Progressive Supranuclear Palsy



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ABSTRACT

This invention is directed to the use of radiopharmaceuticals in diagnosing Alzheimer's disease. In particular the radiopharmaceuticals of the invention are able to pass through the blood-brain barrier and bind to the CCR1 receptor present in brain tissue of patients having Alzheimer's disease.

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LOS ESTADOS UNIDOS DE AMERICA

A TODOS CUANTOS LAS PRESENTES VIEREN:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas de los Estados Unidos

Octubre 10 del 2001

SE CERTIFICA QUE ADJUNTO SE ENCUENTRA UNA COPIA FIEL Y EXACTA DE LOS REGISTROS EXISTENTES EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS RESPECTO A LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA ABAJO LA CUAL CUMPLIO LOS REQUISITOS PARA QUE SE LE OTORGARA UNA FECHA DE PRESENTACION DE CONFORMIDAD CON 35 USC 111.

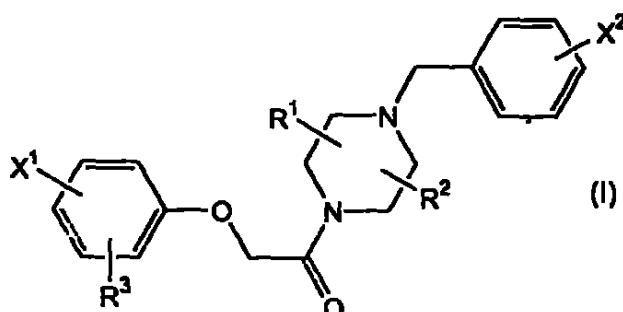
NUMERO DE SOLICITUD: 60/246,299

FECHA DE PRESENTACION: *Noviembre 06 del 2000*

Por Autoridad del
COMISIONADO DE PATENTES Y MARCAS
L. EDELEN, Funcionario de Certificaciones
(Firma y Sello Oficial)

Reivindicaciones

1 Un compuesto de la fórmula (I)



en la cual

X^1 y X^2 independientemente entre sí son halo,

R^1 y R^2 independientemente entre sí son hidrógeno o alquilo, y

R^3 es hidrógeno, amino, monoalquilamino, dialquilamino, monoaralquilamino, alquilcarbonilamino, alquenilcarbonilamino, haloalquilcarbonilamino, arilcarbonilamino, alcoxialquilcarbonilamino, alcoxicarbonilalquilcarbonilamino, glicinamido, monoalquilglicinamido, arilcarbonilglicinamido, aminocarbonilglicinamido, (aminocarbonil)(alquil)glicinamido, (alcoxialquilcarbonil)glicinamido, ureido, monoalquillureido, monoarilureido, monoaralquillureido o alaninamido,

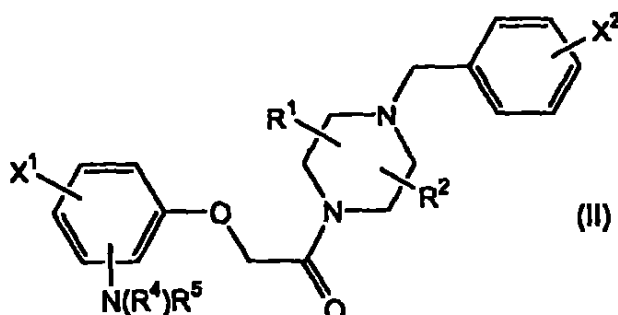
y en la cual ya sea X^1 o X^2 se selecciona entre el grupo de

^{123}I , ^{125}I , ^{128}I , ^{131}I , ^{75}Br , ^{76}Br , ^{80}Br y ^{18}F , o en la cual uno

de los átomos de carbono en los compuestos es ^{11}C ,

o una sal farmacéuticamente aceptable de éste

2 Un compuesto de la fórmula (II)



en la cual

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R^1 y R^2 independientemente entre sí son hidrógeno o alquilo, y R^4 es hidrógeno, y R^5 comprende un quelator capaz de enlazar un átomo de metal radioactivo escogido entre el grupo ^{99m}Tc , ^{186}Re y ^{188}Re , o como un complejo con ^{99m}Tc , ^{186}Re y ^{188}Re , o una sal farmacéuticamente aceptable de éste

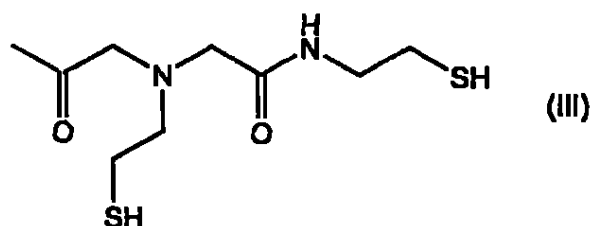
3 El compuesto de la reivindicación 1 o la reivindicación 2, en donde el mencionado compuesto se enlaza al receptor de quimiocina CCR1 y pasa la barrera sangre-cerebro

4 El compuesto de la reivindicación 1 o la reivindicación 2, en donde R^1 es metilo en la 2ª posición del radical piperazini-
lo y R^2 es metilo en la 5ª posición de radical piperazini-
lo

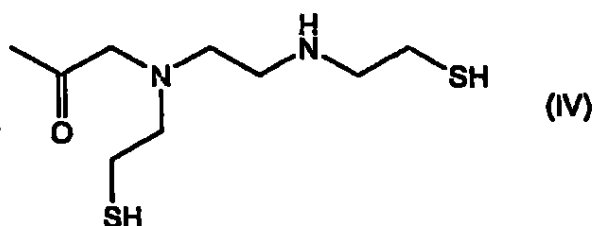
5 El compuesto de la reivindicación 1 o la reivindicación 2, en donde R^1 es metilo en la 2ª posición del radical piperazini-
lo y R^2 es hidrógeno

6 El compuesto de la reivindicación 1, en donde X^1 es cloro en la 4ª posición del radical fenilo y X^2 es un átomo ^{18}F en la 4ª posición del radical fenilo

7 El compuesto de la reivindicación 2, en donde R^5 es un quelator de la fórmula (III)



8 El compuesto de la reivindicación 2, en donde R^5 es un quelator de la fórmula (IV)



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9 El compuesto de la reivindicación 7 o la reivindicación 8, en donde R^5 comprende además una mitad enlazadora que comprende un radical alquilo que tiene de uno a diez átomos de carbono, en donde el radical alquilo contiene opcionalmente de uno a diez grupos $-C(O)-$, de uno a diez grupos $-C(O)N(R)-$, de uno a diez grupos $-N(R)C(O)-$, de uno a diez grupos $-N(R)-$, de uno a diez grupos $-N(R)_2-$, de uno a diez grupos hidroxil, de uno a diez grupos $-C(O)OR-$, de uno a diez átomos de oxígeno, de uno a diez átomos de azufre, de uno a diez átomos de nitrógeno, de uno a diez átomos de halógeno, de uno a diez grupos arilo y de uno a diez anillos heterocíclicos saturados o insaturados en los cuales R es hidrógeno o alquilo

10 El compuesto de la reivindicación 9, en donde la mitad enlazadora es $-C(O)-CH_2-N(H)$

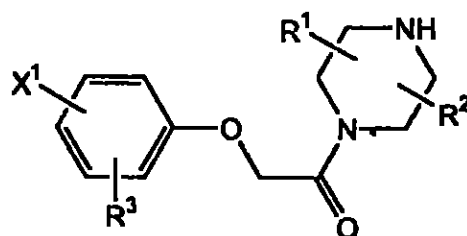
11 La sal de monocloruro de un compuesto de la reivindicación 1 a la reivindicación 10

12 La sal de dicloruro de un compuesto de la reivindicación 1 a la reivindicación 10

13 Un método para diagnosticar la enfermedad de Alzheimer en un ser humano, método que comprende en administrar al ser humano que necesita tal diagnóstico un compuesto de acuerdo con la reivindicación 1 a 12 y medir la radiactividad que surge de la administración del compuesto al ser humano ya sea utilizando una cámara gama o mediante tomografía de emisión de positrones (PET)

14 Un método para usar un compuesto de acuerdo con la reivindicación 1 a 12 para la manufactura de un producto radiofarmacéutico para el diagnóstico de la enfermedad de Alzheimer en un ser humano

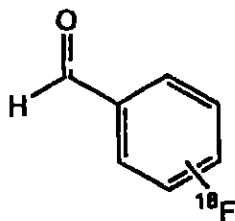
15 Un procedimiento para la producción de un compuesto de la fórmula (I) de acuerdo con la reivindicación 1, procedimiento que comprende la reacción de un compuesto de la fórmula (f)

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(f)

en donde R^1 , R^2 , R^3 y X son como se definió en la reivindicación 1,

con un compuesto de la fórmula (b)



(b)

en presencia de un agente reductor



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2020
Bogotá, D.C.,

Doctor:
Gustavo Hollmann Restrepo
Apoderado
Shering Aktiengesellschaft

Oficio N°

09708

ASUNTO:

Radicación N°	03-046800
Solicitud internacional	PCT/EP2001/12607
Fecha inicio fase nacional	08/06/2003
Trámite	011
Evento	379
Actuación	430
Folios	001

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Única de la Superintendencia de Industria y Comercio, se le comunica que:

☐ La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 28-k) Decisión 486).

En cumplimiento de lo dispuesto por el artículo 39 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única N° 10 de 2001.


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones.

07 NOV. 2003

El anterior requerimiento fue notificado por fijación en lista de fecha _____.