(51) International Patent Classification 6:
A61K 31/66, 31/675, C07F 9/40

(21) International Application Number: PCT/IB98/00967
(22) International Filing Date: 10 April 1998 (10.04.98)
(30) Priority Data:
08/912,574 18 August 1997 (18.08.97) US
60/074,445 11 February 1998 (11.02.98) US

(71) Applicant (for all designated States except US): QUEEN’S UNIVERSITY AT KINGSTON [CA/CA]; Kingston, Ontario K7L 3N6 (CA).


(74) Agents: FRITZ, Joachim, T. et al.; Scott & Aylen, 1000-60 Queen Street, Ottawa, Ontario K1P 5Y7 (CA).

(54) Title: PHOSPHONO-CARBOXYLATE COMPOUNDS FOR TREATING AMYLOIDOSIS

(57) Abstract

Therapeutic compounds and methods for modulating amyloid deposition in a subject, whatever its clinical setting, are described. Amyloid deposition is modulated by the administration to a subject of an effective amount of a therapeutic compound comprising a phosphonate group and a carboxylate group, a congener thereof, or a pharmaceutically acceptable salt or ester thereof. In preferred embodiments, an interaction between an amyloidogenic protein and a basement membrane constituent is modulated.

Published
With international search report.
With amended claims.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| AL  | Albania       | ES | Spain       | LS | Lesotho |
| AM  | Armenia      | FI | Finland     | LT | Lithuania |
| AT  | Austria       | FR | France      | LU | Luxembourg |
| AU  | Australia     | GA | Gabon       | LV | Latvia |
| AZ  | Azerbaijan    | GB | United Kingdom | MC | Monaco |
| BA  | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova |
| BB  | Barbados      | GH | Ghana       | MG | Madagascar |
| BE  | Belgium       | GN | Guinea      | MK | The former Yugoslav Republic of Macedonia |
| BF  | Burkina Faso  | GR | Greece      | ML | Mali |
| BG  | Bulgaria      | HU | Hungary     | MN | Mongolia |
| BJ  | Benin         | IE | Ireland     | MR | Mauritania |
| BR  | Brazil        | IL | Israel      | MW | Malawi |
| BY  | Belarus       | IS | Iceland     | MX | Mexico |
| CA  | Canada        | IT | Italy       | NR | Niger |
| CF  | Central African Republic | JP | Japan         | NL | Netherlands |
| CG  | Congo         | KE | Kenya       | NO | Norway |
| CH  | Switzerland   | KG | Kyrgyzstan  | NZ | New Zealand |
| CI  | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland |
| CM  | Cameroon      | KR | Republic of Korea | PT | Portugal |
| CN  | China         | KZ | Kazakhstan  | RO | Romania |
| CU  | Cuba          | LC | Saint Lucia | RU | Russian Federation |
| CZ  | Czech Republic | LI | Liechtenstein | SD | Sudan |
| DE  | Germany       | LK | Sri Lanka   | SE | Sweden |
| DK  | Denmark       | LR | Liberia     | SG | Singapore |
| EE  | Estonia       |    |            |    |         |
| SI  | Slovenia      |    |            |    |         |
| SK  | Slovakia      |    |            |    |         |
| SN  | Senegal       |    |            |    |         |
| SZ  | Swaziland     |    |            |    |         |
| TD  | Chad          |    |            |    |         |
| TG  | Togo          |    |            |    |         |
| TJ  | Tajikistan    |    |            |    |         |
| TM  | Turkmenistan  |    |            |    |         |
| TR  | Turkey        |    |            |    |         |
| TT  | Trinidad and Tobago |    |            |    |         |
| UA  | Ukraine       |    |            |    |         |
| UG  | Uganda        |    |            |    |         |
| US  | United States of America |    |            |    |         |
| UZ  | Uzbekistan    |    |            |    |         |
| VN  | Viet Nam      |    |            |    |         |
| YU  | Yugoslavia    |    |            |    |         |
| ZW  | Zimbabwe      |    |            |    |         |
PHOSPHONO-CARBOXYLATE COMPOUNDS FOR TREATING
AMYLOIDOSIS

Background of Invention

Amyloidosis refers to a pathological condition characterized by the presence of amyloid. Amyloid is a generic term referring to a group of diverse but specific extracellular protein deposits which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g., Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common x-ray diffraction and infrared spectra.

Amyloidosis can be classified clinically as primary, secondary, familial and/or isolated. Primary amyloidosis appears de novo without any preceding disorder. Secondary amyloidosis is that form which appears as a complication of a previously existing disorder. Familial amyloidosis is a genetically inherited form found in particular geographic populations. Isolated forms of amyloidosis are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as AScr or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by congophilic angiopathy, neuritic plaques and neurofibrillary tangles, all of which have the characteristics of amyloids. In this case, the plaque and blood vessel amyloid is formed by the beta protein. Other systemic or localized diseases such as adult-onset diabetes, complications of long-term hemodialysis and sequelae of long-standing inflammation or plasma cell dyscrasias are characterized by the accumulation of amyloids systemically. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

Summary of the Invention

This invention provides methods and compositions which are useful in the treatment of amyloidosis. The methods of the invention involve administering to a subject a therapeutic compound which inhibits amyloid deposition. Accordingly, the compositions and methods of the invention are useful for inhibiting amyloidosis in disorders in which amyloid deposition occurs. The methods of the invention can be used therapeutically to treat amyloidosis or can be used prophylactically in a subject susceptible to amyloidosis. Without wishing to be bound by theory, it is believed that
the methods of the invention are based, at least in part, on inhibiting an interaction between an amyloidogenic protein and a constituent of basement membrane to inhibit amyloid deposition. The constituent of basement membrane can be a glycoprotein or proteoglycan, preferably heparan sulfate proteoglycan. In certain embodiments, a therapeutic compound used in the method of the invention preferably can interfere with binding of a basement membrane constituent to a target binding site on an amyloidogenic protein, thereby inhibiting amyloid deposition.

The invention relates to phosphonocarboxylate compounds, i.e., compounds which include a phosphonate group and a carboxylate group, or a pharmaceutically acceptable salt or ester thereof. In one embodiment, the method of the invention involves administering to a subject an effective amount of a therapeutic compound having the formula (Formula I):

\[
\begin{align*}
  X \begin{array}{c} P \end{array} \begin{array}{c} (CY^1Y^2)_n C(X)XR^3 \end{array} \\
  R^1X^* \begin{array}{c} \text{Z} \end{array}
\end{align*}
\]

in which \( Z \) is \( XR^2 \) or \( R^4 \), \( R^1 \) and \( R^2 \) are each independently hydrogen, a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain; or an unsubstituted or substituted cyclic aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring; preferred aliphatic and cyclic aliphatic groups are alkyl groups, more preferably lower alkyl), an aryl group, a heterocyclic group, or a salt-forming cation; \( R^3 \) is hydrogen, lower alkyl, aryl, or a salt-forming cation; \( R^4 \) is hydrogen, lower alkyl, aryl or amino (including alkylamino, dialkylamino (including cyclic amino moieties), arylamino, diarylamino, and alkylarylamino); \( X \) is, independently for each occurrence, \( O \) or \( S \); \( Y^1 \) and \( Y^2 \) are each independently hydrogen, halogen (e.g., \( F, Cl, Br, \) or \( I \)), alkyl (preferably lower alkyl), amino, hydroxy, alkoxy, or arylxoy; and \( n \) is an integer from 0 to 12 (more preferably 0 to 6, more preferably 0 or 1); such that amyloid deposition is modulated.

In preferred embodiments, therapeutic compounds of the invention prevent or inhibit amyloid deposition in a subject to which the therapeutic compound is administered. Preferred therapeutic compounds for use in the invention include compounds in which both \( R^1 \) and \( R^2 \) are pharmaceutically acceptable salt-forming cations. It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) and the charge of the counterion. In a particularly preferred
embodiment, R¹, R² and R³ are each independently a sodium, potassium or calcium cation. In certain embodiments in which at least one of R¹ and R² is an aliphatic group, the aliphatic group has between 1 and 10 carbons atoms in the straight or branched chain, and is more preferably a lower alkyl group. In other embodiments in which at least one of R¹ and R² is an aliphatic group, the aliphatic group has between 10 and 24 carbons atoms in the straight or branched chain. In certain preferred embodiments, n is 0 or 1; more preferably, n is 0. In certain preferred embodiments of the therapeutic compounds, Y¹ and Y² are each hydrogen.

In certain preferred embodiments, the therapeutic compound of the invention can be represented by the formula (Formula II):

\[ \text{R}^1 \text{X} \text{P}^-(\text{CY}^1\text{Y}^2)\text{mC(O)OR}^3 \]

in which R¹, R², R³, Y¹, Y², X and n are as defined above. In more preferred embodiments, the therapeutic compound of the invention can be represented by the formula (Formula III):

\[ \text{R}^1\text{O}^+ \text{P}^-(\text{CY}^1\text{Y}^2)\text{mCH(NR}_a\text{R}_b)\text{C(O)OR}^3 \]

in which R¹, R², R³, Y¹, Y², and X are as defined above, Rₐ and Rₐ are each independently hydrogen, alkyl, aryl, or heterocyclyl, or Rₐ and Rₐ, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring, and n is an integer from 0 to 6. In certain preferred embodiments, Rₐ and Rₐ are each hydrogen. In certain preferred embodiments, a compound of the invention comprises an α-amino acid (or α-amino acid ester), more preferably a L-α-amino acid or ester.

In another embodiment, the compounds of the invention can be represented by the formula (Formula IV):

\[ \text{G} - \text{O} - \text{C} - \text{P} - \text{O} - \text{L} \]

in which G represents hydrogen or one or more substituents on the aryl ring (e.g., alkyl, aryl, halogen, amino, and the like) and L is a substituted alkyl group (in certain
embodiments, preferably a lower alkyl), more preferably a hydroxy-substituted alkyl or an alkyl substituted with a nucleoside base.

The therapeutic compounds of the invention are administered to a subject by a route which is effective for modulation of amyloid deposition. Suitable routes of administration include oral, transdermal, subcutaneous, intravenous, intramuscular and intraperitoneal injection. A preferred route of administration is oral administration. The therapeutic compounds can be administered with a pharmaceutically acceptable vehicle.

The invention also provides methods for treating a disease state associated with amyloidosis by administering to a subject an effective amount of a therapeutic compound having the formula described supra, such that a disease state associated with amyloidosis is treated.

The invention provides methods for modulating amyloid deposition characterized by interaction between an amyloidogenic protein and a constituent of a basement membrane by administering to the subject an effective amount of a therapeutic compound having the formula described supra, such that modulation of amyloid deposition characterized by interaction between an amyloidogenic protein and a constituent of a basement membrane occurs.

The invention further provides pharmaceutical compositions for treating amyloidosis. The pharmaceutical compositions include a therapeutic compound of the invention in an amount effective to modulate amyloid deposition and a pharmaceutically acceptable vehicle.

The invention also provides packaged pharmaceutical compositions for treating amyloidosis. The packaged pharmaceutical compositions include a therapeutic compound of the invention and instructions for using the pharmaceutical composition for treatment of amyloidosis.

**Detailed Description of Invention**

This invention pertains to methods and compositions useful for treating amyloidosis. The methods of the invention involve administering to a subject a therapeutic compound which modulates amyloid deposition. "Modulation of amyloid deposition" is intended to encompass prevention of amyloid formation, inhibition of further amyloid deposition in a subject with ongoing amyloidosis and reduction of amyloid deposits in a subject with ongoing amyloidosis. Modulation of amyloid deposition is determined relative to an untreated subject or relative to the treated subject prior to treatment. In certain embodiments, amyloid deposition can be modulated by modulating an interaction between an amyloidogenic protein and a constituent of basement membrane.
"Basement membrane" refers to an extracellular matrix comprising glycoproteins and proteoglycans, including laminin, collagen type IV, fibronectin chondroitan sulfate, and/or heparan sulfate proteoglycan (HSPG). In one embodiment, amyloid deposition is modulated by interfering with an interaction between an amyloidogenic protein and a sulfated glycosaminoglycan such as HSPG. Sulfated glycosaminoglycans are known to be present in all types of amyloids (see Snow, A.D. et al. (1987) Lab. Invest. 56:120-123) and amyloid deposition and HSPG deposition occur coincidentally in animal models of amyloidosis (see Snow, A.D. et al. (1987) Lab. Invest. 56:665-675). In preferred embodiments of the methods of the invention, molecules which have a similar structure to a sulfated glycosaminoglycan are used to modulate interaction between an amyloidogenic protein and basement membrane constituent. In particular, the therapeutic compounds of the invention preferably comprise at least one phosphonate group (or phosphonic ester), or a functional equivalent thereof (including phosphorus-containing anionic groups including, but not limited to, phosphates, phosphate esters, phosphinates, and the like), and a carboxylate group or carboxylic ester (or a congener such as a thioacid, thiolester, or thionoester), provided that the compound includes, or is capable of having after reaction in vivo, at least one anionic group. The anionic groups(s) can optionally be covalently bound to a carrier (e.g., an aliphatic group, peptide or peptidomimetic, or the like). In addition to functioning as a carrier for the anionic functionality, the carrier molecule can enable the compound to traverse biological membranes and to be biodistributed without excessive or premature metabolism.

In one embodiment, the method of the invention includes administering to the subject an effective amount of a therapeutic compound which has at least one phosphonate group or phosphonic ester group. The therapeutic compound is preferably capable of modulating interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane to thus modulate amyloid deposition. The therapeutic compound has the formula (Formula I):

\[
\begin{align*}
R1X & \quad P \quad (C\ Y^2)_{n} C(X)X R^3 \\
Z &
\end{align*}
\]

in which Z is XR₂ or R₄, R₁ and R₂ are each independently hydrogen, a substituted or unsubstituted aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms in the chain; or an unsubstituted or substituted cyclic
aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring; preferred aliphatic and cyclic aliphatic groups are alkyl groups, more preferably lower alkyl), an aryl group, a heterocyclic group, or a salt-forming cation; R³ is hydrogen, lower alkyl, aryl, or a salt-forming cation; X is, independently for each occurrence, O or S; R⁴ is hydrogen, lower alkyl, aryl or amino; Y¹ and Y² are each independently hydrogen, halogen (e.g., F, Cl, Br, or I), lower alkyl, amino (including alkylamino, dialkylamino, arylamino, diarylamino, and alkylarylamino), hydroxy, alkoxy, or aryloxy; and n is an integer from 0 to 12 (more preferably 0 to 6, more preferably 0 or 1); such that amyloid deposition is modulated.

In preferred embodiments, therapeutic compounds of the invention prevent or inhibit amyloid deposition in a subject to which the therapeutic compound is administered. Preferred therapeutic compounds for use in the invention include compounds in which both R¹ and R² are pharmaceutically acceptable salt-forming cations. It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) and the charge of the counterion. In a particularly preferred embodiment, R¹, R² and R³ are each independently a sodium, potassium or calcium cation. In certain embodiments in which at least one of R¹ and R² is an aliphatic group, the aliphatic group has between 1 and 10 carbons atoms in the straight or branched chain, and is more preferably a lower alkyl group. In other embodiments in which at least one of R¹ and R² is an aliphatic group, the aliphatic group has between 10 and 24 carbons atoms in the straight or branched chain. In certain preferred embodiments, n is 0 or 1; more preferably, n is 0. In certain preferred embodiments of the therapeutic compounds, Y¹ and Y² are each hydrogen.

In certain preferred embodiments, the therapeutic compound of the invention can be represented by the formula (Formula II):

\[
\begin{align*}
\text{R}^1 & \quad \text{X} \\
\text{P} & \quad \text{(CY}^1\text{Y}^2_\text{O})_\text{nC(O)}\text{OR}^3 \\
\text{XR}^2 & \quad \text{XR}^2
\end{align*}
\]

in which R¹, R², R³, Y¹, Y², X and n are as defined above. In more preferred embodiments, the therapeutic compound of the invention can be represented by the formula (Formula III):

\[
\begin{align*}
\text{R}^1 & \quad \text{X} \\
\text{P} & \quad \text{(CY}^1\text{Y}^2_\text{O})_\text{nCH(NR}^a\text{R}^b\text{)}\text{C(O)}\text{OR}^3 \\
\text{OR}^2 & \quad \text{OR}^2
\end{align*}
\]
in which R₁, R₂, R₃, Y¹, Y², and X are as defined above, Rₐ and Rₜ are each independently hydrogen, alkyl, aryl, or heterocyclyl, or Rₐ and Rₜ, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring, and n is an integer from 0 to 6. In certain preferred embodiments, Rₐ and Rₜ are each hydrogen. In certain preferred embodiments, a compound of the invention comprises an α-amino acid (or α-amino acid ester), more preferably a L-α-amino acid or ester.

The Z, Q, R₁, R₂, R₃, Y¹, Y² and X groups are each independently selected such that the biodistribution of the compound for an intended target site is not prevented while maintaining activity of the compound. For example, the number of anionic groups (and the overall charge on the therapeutic compound) should not be so great as to inhibit traversal of an anatomical barrier, such as a cell membrane, or entry across a physiological barrier, such as the blood-brain barrier, in situations where such properties are desired. For example, it has been reported that esters of phosphonoformate have biodistribution properties different from, and in some cases superior to, the biodistribution properties of phosphonoformate (see, e.g., U.S. Patent Nos. 4,386,081 and 4,591583 to Helgstrand et al., and U.S. Patent Nos. 5,194,654 and 5,463,092 to Hostetter et al.). Thus, in certain embodiments, at least one of R₁ and R₂ is an aliphatic group (more preferably an alkyl group), in which the aliphatic group has between 10 and 24 carbons atoms in the straight or branched chain. The number, length, and degree of branching of the aliphatic chains can be selected to provide a desired characteristic, e.g., lipophilicity. In other embodiments, at least one of R₁ and R₂ is an aliphatic group (more preferably an alkyl group), in which the aliphatic group has between 1 and 10 carbons atoms in the straight or branched chain. Again, the number, length, and degree of branching of the aliphatic chains can be selected to provide a desired characteristic, e.g., lipophilicity or ease of ester cleavage by enzymes. In certain embodiments, a preferred aliphatic group is an ethyl group.

It has also been reported that certain thiophosphate compounds have in vivo activity as anti-viral agents which is equal to or greater than the activity of the corresponding oxy-phosphate compounds (possibly due to differences in bioavailability of the compounds). Accordingly, in certain preferred embodiments, the therapeutic compound includes a moiety selected from the group consisting of -P(S)(OR₁)(OR₂), -P(S)(SR₁)(OR₂), or -P(S)(SR₁)(SR₂).

In another embodiment, compounds useful in the methods of the invention can be represented by the formula (Formula IV):

In another embodiment, the compounds of the invention can be represented by the formula (Formula IV):
in which G represents hydrogen or one or more substituents on the aryl ring (e.g., alkyl, aryl, halogen, amino, and the like) and L is a substituted alkyl group (in certain embodiments, preferably a lower alkyl), more preferably a hydroxy-substituted alkyl or an alkyl substituted with a nucleoside base. In certain embodiments, G is hydrogen or an electron-donating group. In embodiments in which G is an electron-withdrawing group, G is preferably an electron withdrawing group at the meta position. The term "electron-withdrawing group" is known in the art, and, as used herein, refers to a group which has a greater electron-withdrawing than hydrogen. A variety of electron-withdrawing groups are known, and include halogens (e.g., fluoro, chloro, bromo, and iodo groups), nitro, cyano, and the like. Similarly, the term "electron-donating group", as used herein, refers to a group which is less electron-withdrawing than hydrogen. In embodiments in which G is an electron donating group, G can be in the ortho, meta or para position.

In certain preferred embodiments, L is a moiety selected from the group consisting of (Formulas IVa-IVg):

IVa

IVb

IVc

IVd

IVe

IVf

IVg
Table 1 lists data pertinent to the characterization of these compounds using art-recognized techniques.

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>$^31$P NMR</th>
<th>$^{13}$C NMR</th>
<th>FAB-MS(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td>-6.33(DMSO-d$_6$)</td>
<td>60.97 CH$_2$OH(d, J=6Hz) 66.76 CH$_2$OH(d, J=7.8Hz) 121.65, 121.78, 121.99, 125.71, 129.48, 129.57, 126.43 Aromatic CH 134.38 Aniline C-N 150.39 Phenyl C-O(d, J=7Hz) 171.57 P-C=O(d, J=234Hz)</td>
<td>245.2</td>
</tr>
<tr>
<td>IVb</td>
<td>-6.41(DMSO-d$_6$)</td>
<td>13.94 CH$_3$ 22.11, 24.40, 28.56, 28.72, 28.99, 29.00, 31.30, 33.43, -(CH$<em>2$)$</em>{10}$ 65.03 CH$_2$-OC(O) 66.60 CH$_2$-OP(d, J=5.6Hz) 67.71 CH$_2$-OH(d, J=6 Hz) 121.73, 121.10, 125.64, 126.57, 129.40, 129.95, Aromatic CH 134.04 Aniline C-N 150.31 Phenyl C-O 171.44 P-C=O(d, J=6.7 Hz) 172.83 O-C=O</td>
<td>456</td>
</tr>
<tr>
<td>IVc</td>
<td>-6.46(DMSO-d$_6$)</td>
<td>13.94 CH$_3$ 22.11, 25.10, 28.68, 28.72, 28.85, 29.00, 30.76, 31.31, 32.10, -(CH$<em>2$)$</em>{10}$ 43.36 CH$_2$-S 68.43 CH$_2$-OH 68.43 CH-OH(d, J=6.3 Hz) 68.76 P-O-CH$_2$-9d, J=5.8 Hz) 121.75, 122.03, 125.62, 126.37, 129.30, 129.53, Aromatic CH 134.23 Aniline C-N 150.37 Phenyl C-O(d, J=6.7 Hz) 171.47 P-C=O(d, J=234.0 Hz) 198.47 S-C=O</td>
<td>471</td>
</tr>
<tr>
<td>COMPOUND</td>
<td>$^{31}$P NMR</td>
<td>$^{13}$C NMR</td>
<td>FAB- MS( )</td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>IVd</td>
<td>-6.61(DMSO-d$_6$)</td>
<td>13.94 CH$_3$ 22.06, 25.14, 28.24, 28.35, 31.09, 32.14 -CH$_2$)$_6$- 43.40 CH$_2$-S 68.50 P-O-CH$_2$-(d, J=5.8 Hz) 68.77 CH-OB(d, 6.4 Hz) 121.78, 122.59, 125.69, 127.06, 129.43, 129.59 Aromatic CH 133.39 Aniline C-N 150.38 Phenyl C-O(d, J=6.7 Hz) 171.47 P-C=O(d, J=234.4 Hz) 198.54 S-C=O</td>
<td>416</td>
</tr>
<tr>
<td>IVe</td>
<td>-5.76(D$_2$O)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>IVf</td>
<td>-7.00(DMSO-d$_6$)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>IVg</td>
<td>-6.60(DMSO-D$_6$)</td>
<td>70.84 CH$_2$-OH 72.17 CH-OB 121.68, 121.79, 121.85, 125.71 127.10, 127.92, 129.36, 129.50, 129.59 Aromatic CH 134.51 Aniline C-N 142.34 Aromatic C-CH 150.37 Phenyl C-O(d, J=6.2 Hz) 171.59 P-C=O(d, J=232.6 Hz)</td>
<td>321</td>
</tr>
</tbody>
</table>

In another aspect, the invention includes novel compounds useful for inhibiting amyloidosis, and/or compounds having antiviral activity. The compounds of the invention can be represented by the structures of Formula IV, e.g., a compound of Formula IV in which G is hydrogen (e.g., the phenyl ring is unsubstituted) and L is any of the moieties of Formulas IVa-IVg. A more preferred compound is the compound of Formula IVc.

In another aspect, the invention provides a method for preparing esters of phosphonates, e.g., phosphono-carboxylate compounds of the invention, e.g., a compound of Formula IV in which G is hydrogen and L is a moiety of Formula IVa-IVg. Illustratively, the method includes the step of reacting a phosphonodichloridate (or...
other phosphonate diacid halide) with a disilylated diol under conditions such that a compound of Formula IV is formed (see Example 2, infra).

Thus, in one embodiment, the invention provides a method for preparing a compound represented by the Formula (Formula V):

\[
\begin{align*}
&\text{RO} - \text{C} - \text{P} - \text{O} - \text{O} - \text{OH} \\
&\qquad \text{R'}
\end{align*}
\]

in which R is alkyl or aryl, and R' is hydrogen, alkyl, or aryl (including heteroaromatic groups such as nucleosides). The method includes the step of reacting an ester of a carboxylphosphono diacid halide (e.g., ROOC-P(O)(A)(A'), in which R is as described in Formula V, and A and A' are both halogen or other good leaving groups, e.g., chloro, iodo, bromo, pentafluorophenyl, and the like, which can be the same or different) with a disilylether of a vicinal diol, under conditions such that the compound of Formula V is prepared.

An anionic group (i.e., a phosphonate or carboxylate group) of a therapeutic compound of the invention is a negatively charged moiety that, in certain preferred embodiments, can modulate interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane to thus modulate amyloid deposition.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers (e.g., enantiomers and diastereomers) arising from such asymmetry are included within the scope of this invention. Such isomers can be obtained in substantially pure form by classical separation techniques and by sterically controlled synthesis. For the purposes of this application, unless expressly noted to the contrary, a compound shall be construed to include both the R or S stereoisomers at each chiral center.

The ability of a therapeutic compound of the invention to modulate interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane can be assessed by an in vitro binding assay, such as that described in the Exemplification or in U.S. Patent No. 5,164,295 by Kisilevsky et al. Briefly, a solid support such as a polystyrene microtiter plate is coated with an amyloidogenic protein (e.g., serum amyloid A protein or \(\beta\)-amyloid precursor protein (\(\beta\)-APP)) and any residual hydrophobic surfaces are blocked. The coated solid support is incubated with various concentrations of a constituent of basement membrane, preferably HSPG, either in the presence or absence of a compound to be tested. The solid support is washed extensively to remove unbound material. The binding of the basement membrane
constituent (e.g., HSPG) to the amyloidogenic protein (e.g., β-APP) is then measured using an antibody directed against the basement membrane constituent which is conjugated to a detectable substance (e.g., an enzyme, such as alkaline phosphatase) by detecting the detectable substance. A compound which modulates an interaction between an amyloidogenic protein and a glycoprotein or proteoglycan constituent of a basement membrane will reduce the amount of substance detected (e.g., will inhibit the amount of enzyme activity detected).

Preferably, a therapeutic compound of the invention interacts with a binding site for a basement membrane glycoprotein or proteoglycan in an amyloidogenic protein and thereby modulates the binding of the amyloidogenic protein to the basement membrane constituent. Basement membrane glycoproteins and proteoglycans include laminin, collagen type IV, fibronectin and heparan sulfate proteoglycan (HSPG). In a preferred embodiment, the therapeutic compound inhibits an interaction between an amyloidogenic protein and HSPG.

In certain embodiments, a therapeutic compound of the invention comprises a cation (i.e., in certain embodiments, at least one of R1, R2 or R3 is a cation). If the cationic group is hydrogen, H+, then the compound is considered an acid, e.g., phosphonoformic acid. If hydrogen is replaced by a metal ion or its equivalent, the compound is a salt of the acid. Pharmaceutically acceptable salts of the therapeutic compound are within the scope of the invention. For example, at least one of R1, R2 or R3 can be a pharmaceutically acceptable alkali metal (e.g., Li, Na, or K), ammonium cation, alkaline earth cation (e.g., Ca2+, Ba2+, Mg2+), higher valency cation, or polycationic counter ion (e.g., a polyammonium cation). (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", J. Pharm. Sci. 66:1-19). It will be appreciated that the stoichiometry of an anionic compound to a salt-forming counterion (if any) will vary depending on the charge of the anionic portion of the compound (if any) and the charge of the counterion. Preferred pharmaceutically acceptable salts include a sodium, potassium or calcium salt, but other salts are also contemplated within their pharmaceutically acceptable range.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared in situ during the final isolation and purification of the compounds or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent; either of which are methods known to those skilled in the art.

Carboxylic acids and phosphonic acids can be converted into esters according to methods well known to one of ordinary skill in the art, e.g., via treatment with an
alcohol in the presence of a catalyst. A preferred ester group (e.g., when R^3 is lower alkyl) is an ethyl ester group.

The term "alkyl" refers to the saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_30 for straight chain, C_3-C_30 for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 4-10 carbon atoms in their ring structure, and more preferably have 4-7 carbon atoms in the ring structure.

The term "lower alkyl" refers to alkyl groups having from 1 to 6 carbons in the chain, and to cycloalkyls having from 3 to 6 carbons in the ring structure.

Moreover, the term "alkyl" (including "lower alkyl") as used throughout the specification and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkyloxy, aryloxy, alkoxyalkoxy, aryloxyalkoxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, aminocarbonyl, diarylamino, and alkylaminocarbonyl), acylamino (including alkyloxy, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfate, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclic, aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "aralkyl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)).

The term "alkoxy", as used herein, refers to a moiety having the structure -O-alkyl, in which the alkyl moiety is described above.

The term "aryl" as used herein includes 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, unsubstituted or substituted benzene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. The aromatic ring can be substituted at one or more ring positions with such substituents, e.g., as described above for alkyl groups. Preferred aryl groups include unsubstituted and substituted phenyl groups.
The term "aryloxy", as used herein, refers to a group having the structure -O-aryl, in which the aryl moiety is as defined above.

The term "amino," as used herein, refers to an unsubstituted or substituted moiety of the formula -NR_aR_b, in which R_a and R_b are each independently hydrogen, alkyl, aryl, or heterocyclyl, or R_a and R_b, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring. Thus, the term "amino" is intended to include cyclic amino moieties such as piperidinyl or pyrrolidinyl groups, unless otherwise stated. An "amino-substituted amino group" refers to an amino group in which at least one of R_a and R_b, is further substituted with an amino group.

In a preferred embodiment of the compounds of Formulas I-III, R^1 or R^2 can be (for at least one occurrence) a long-chain aliphatic moiety. The term "long-chain aliphatic moiety" as used herein, refers to a moiety having a straight or branched chain aliphatic moiety (e.g., an alkyl or alkenyl moiety) having from 10 to 24 carbons in the aliphatic chain, e.g., the long-chain aliphatic moiety is an aliphatic chain of a fatty acid (preferably a naturally-occurring fatty acid). Representative long-chain aliphatic moieties include the aliphatic chains of stearic acid, oleic acid, linolenic acid, and the like.

The therapeutic compound of the invention can be administered in a pharmaceutically acceptable vehicle. As used herein "pharmaceutically acceptable vehicle" includes any and all solvents, excipients, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like which are compatible with the activity of the compound and are physiologically acceptable to the subject. An example of a pharmaceutically acceptable vehicle is buffered normal saline (0.15 molar NaCl). The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the therapeutic compound, use thereof in the compositions suitable for pharmaceutical administration is contemplated. Supplementary active compounds can also be incorporated into the compositions.

In certain embodiments, the therapeutic compound of the invention can be represented by the formula:

```
  O
R\text{I}O-\textsc{P}(\textsc{CY}^1\textsc{Y}^2)_n\textsc{COOR}^3
    \text{OR}^2
```
in which $R^1$ and $R^2$ are each independently hydrogen, an aliphatic group (preferably a branched or straight-chain aliphatic moiety having from 1 to 24 carbon atoms, more preferably 10-24 carbon atoms, in the chain; or an unsubstituted or substituted cyclic aliphatic moiety having from 4 to 7 carbon atoms in the aliphatic ring), an aryl group, a heterocyclic group, or a salt-forming cation; $R^3$ is hydrogen, lower alkyl, aryl, or a salt-forming cation; $Y^1$ and $Y^2$ are each independently hydrogen, halogen (e.g., F, Cl, Br, or I), lower alkyl, hydroxy, alkoxy, or aryloxy; and $n$ is an integer from 0 to 12; such that amyloid deposition is modulated. In one preferred embodiment, therapeutic compounds of the invention prevent or inhibit amyloid deposition in a subject to which the therapeutic compound is administered. Preferred therapeutic compounds for use in the invention include compounds in which both $R^1$ and $R^2$ are pharmaceutically acceptable salt-forming cations. In a particularly preferred embodiment, $R^1$, $R^2$, and $R^3$ are each independently a sodium, potassium or calcium cation, and $n$ is 0. In certain preferred embodiments of the therapeutic compounds, $Y^1$ and $Y^2$ are each hydrogen. Particularly preferred therapeutic compounds are salts of phosphonoformate. Trisodium phosphonoformate (foscarnet sodium or Foscavir®) is commercially available (e.g., from Astra), and its clinical pharmacology has been investigated (see, e.g., "Physician's Desk Reference", 51st Ed., pp. 541-545 (1997)).

A further aspect of the invention includes pharmaceutical compositions for treating amyloidosis. The therapeutic compounds in the methods of the invention, as described hereinbefore, can be incorporated into a pharmaceutical composition in an amount effective to modulate amyloidosis in a pharmaceutically acceptable vehicle.

The invention further contemplates the use of prodrugs which are converted in vivo to the therapeutic compounds of the invention (see, e.g., R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chp. 8). Such prodrugs can be used to alter the biodistribution (e.g., to allow compounds which would not typically cross the blood-brain barrier to cross the blood-brain barrier) or the pharmacokinetics of the therapeutic compound. For example, an anionic group, e.g., a phosphonate or carboxylate, can be esterified, e.g., with an ethyl group or a fatty group, to yield a phosphonic or carboxylic ester. When the phosphonic or carboxylic ester is administered to a subject, the ester can be cleaved, enzymatically or non-enzymatically, to reveal the anionic group. Such an ester can be cyclic, e.g., a cyclic phosphonate, or two or more anionic moieties may be esterified through a linking group. In a preferred embodiment, the prodrug is a phosphonate or carboxylate. An anionic group can be esterified with moieties (e.g., acyloxyethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound. Furthermore, an anionic moiety (e.g., a phosphonate or carboxylate) can be esterified to
a group which is actively transported in vivo, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the therapeutic moieties to particular organs, as described below for carrier moieties. In certain embodiments, as described above, compounds of the invention can have more than one phosphonic or carboxylic ester moiety, e.g., one phosphonic ester and one carboxylic ester, or a phosphonic diester. In such embodiments, the parent compound may include an anionic group and may be active; however, cleavage of any or all ester functionalities may result in an active compound. It will be appreciated that in a compound having multiple esterified moieties, the ester groups can be selected to permit selective cleavage of one or more ester functionalities, to unveil one or more anionic groups. The relative ease of cleavage of ester groups is well known; for example, a tert-butyloxy ester is generally cleaved more slowly than an ethyl ester under certain conditions. Selection of appropriate moieties to provide a desired rate or order of ester cleavage will be routine to the ordinarily-skilled artisan. Thus, the number of anionic functionalities can be controlled to provide for a selective activity of a compound of the invention according to the rate or order of ester cleavage.

Carrier or substituent moieties useful in the present invention may also include moieties which allow the therapeutic compound to be selectively delivered to a target organ or organs. For example, if delivery of a therapeutic compound to the brain is desired, the carrier molecule may include a moiety capable of targeting the therapeutic compound to the brain, by either active or passive transport (a "targeting moiety"). Illustratively, the carrier molecule may include a redox moiety, as described in, for example, U.S. Patents 4,540,564 and 5,389,623, both to Bador. These patents disclose drugs linked to dihydropyridine moieties which can enter the brain, where they are oxidized to a charged pyridinium species which is trapped in the brain. Thus, drug accumulates in the brain. Other carrier moieties include compounds, such as amino acids or thyroxine, which can be passively or actively transported in vivo. Such a carrier moiety can be metabolically removed in vivo, or can remain intact as part of an active compound. Structural mimics of amino acids (and other actively transported moieties), including peptidomimetics, are also useful in the invention. As used herein, the term "peptidomimetic" is intended to include peptide analogs which serve as appropriate substitutes for peptides in interactions with e.g., receptors and enzymes. The peptidomimetic must possess not only affinity, but also efficacy and substrate function. That is, a peptidomimetic exhibits function(s) of a peptide, without restriction of structure. Peptidomimetics, methods for their preparation and use are described in Morgan et al., "Approaches to the discovery of non-peptide ligands for peptide receptors and peptidases," In Annual Reports in Medicinal Chemistry (Virick, F.J., ed.) pp. 243-
253, Academic Press, San Diego, CA (1989), the contents of which are incorporated herein by reference. Many targeting moieties are known, and include, for example, asialoglycoproteins (see, e.g. Wu, U.S. Patent 5,166,320) and other ligands which are transported into cells via receptor-mediated endocytosis (see below for further examples of targeting moieties which may be covalently or non-covalently bound to a carrier molecule). Furthermore, the therapeutic compounds of the invention may bind to amyloidogenic proteins in the circulation and thus be transported to the site of action.

The targeting and prodrug strategies described above can be combined to produce a compound that can be transported as a prodrug to a desired site of action and then unmasked to reveal an active compound.

In the methods of the invention, amyloid deposition (e.g., deposition of β-amyloid) in a subject is modulated by administering a therapeutic compound of the invention to the subject. The term "subject" is intended to include living organisms in which amyloidosis can occur. Examples of subjects include humans, monkeys, cows, sheep, goats, dogs, cats, mice, rats, and transgenic species thereof. Administration of the compositions of the present invention to a subject to be treated can be carried out using known procedures, at dosages and for periods of time effective to modulate amyloid deposition in the subject. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the amount of amyloid already deposited at the clinical site in the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compound to modulate amyloid deposition in the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention (e.g., phosphonoformic acid, trisodium salt) is between 0.5 and 500 mg/kg of body weight/per day. In an aqueous composition, preferred concentrations for the active compound (i.e., the therapeutic compound that can modulate amyloid deposition) are between 5 and 500 mM, more preferably between 10 and 100 mM, and still more preferably between 20 and 50 mM.

The therapeutic compounds of the invention can be effective when administered orally. Accordingly, a preferred route of administration is oral administration. Alternatively, the active compound may be administered by other suitable routes such as subcutaneous, intravenous, intramuscular or intraperitoneal administration, and the like (e.g. by injection). Depending on the route of administration, the active compound may be coated in a material to protect the compound from the action of acids and other natural conditions which may inactivate the compound.
The compounds of the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs ("targeting moieties"), thus providing targeted drug delivery (see, e.g., V.V. Ranade (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, e.g., U.S. Patent 5,416,016 to Low et al.); mannosides (Umezawa et al., 1988) Biochem. Biophys. Res. Commun. 153:1038; antibodies (P.G. Bloeman et al. (1995) FEBS Lett. 357:140; M. Owais et al. (1995) Antimicrob. Agents Chemother. 39:180); surfactant protein A receptor (Briscoe et al. (1995) Am. J. Physiol. 1233:134); gp120 (Schreier et al. (1994) J. Biol. Chem. 269:9090); see also K. Keinanen; M.L. Laukkanen (1994) FEBS Lett. 346:123; J.J. Killion; I.J. Fidler (1994) Immunomethods 4:273. In a preferred embodiment, the therapeutic compounds of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety.

Delivery and in vivo distribution can also be affected by alteration of an anionic group of compounds of the invention. For example, anionic groups such as phosphonate or carboxylate can be esterified to provide compounds with desirable pharmacokinetic, pharmacodynamic, biodistributive, or other properties. Exemplary compounds include phosphonoformate trisodium salt (Foscarnet, Foscavir), phosphonoacetate, trisodium salt, and pharmaceutically acceptable salts or esters thereof.

To administer the therapeutic compound by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. For example, the therapeutic compound may be administered to a subject in an appropriate carrier, for example, liposomes, or a diluent. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejen et al., 1984) J. Neuroimmunol. 7:27).

The therapeutic compound may also be administered parenterally (e.g., intramuscularly, intravenously, intraperitoneally, intraspinally, or intracerebrally). Dispersions can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the
extemporaneous preparation of sterile injectable solutions or dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some cases, it will be preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

Sterile injectable solutions can be prepared by incorporating the therapeutic compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the therapeutic compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., the therapeutic compound) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The therapeutic compound can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic compound and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the therapeutic compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the therapeutic compound in the compositions and preparations may, of course, be varied. The amount of the therapeutic compound in such therapeutically useful compositions is such that a suitable dosage will be obtained.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be
treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the treatment of amyloid deposition in subjects.

Therapeutic compositions can be administered in time-release or depot form, to obtain sustained release of the therapeutic compounds over time. The therapeutic compounds of the invention can also be administered transdermally (e.g., by providing the therapeutic compound, with a suitable carrier, in patch form).

Active compounds are administered at a therapeutically effective dosage sufficient to modulate amyloid deposition (or amyloid load) in a subject. A "therapeutically effective dosage" preferably modulates amyloid deposition by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to modulate amyloid deposition can be evaluated in model systems that may be predictive of efficacy in modulating amyloid deposition in human diseases, such as animal model systems known in the art (including, e.g., the method described in PCT Publication WO 96/28187) or by in vitro methods, e.g., the method of Chakrabartty, described in PCT Publication WO 97/07402, or the assay described in Example 1, infra. Alternatively, the ability of a compound to modulate amyloid deposition can be evaluated by examining the ability of the compound to modulate an interaction between an amyloidogenic protein and a basement membrane constituent, e.g., using a binding assay such as that described hereinabove. Furthermore, the amount or distribution of amyloid deposits in a subject can be non-invasively monitored in vivo, for example, by use of radiolabelled tracers which can associate with amyloid deposits, followed by scintigraphy to image the amyloid deposits (see, e.g., Aprile, C. et al., Eur. J. Nucl. Med. 22:1393 (1995); Hawkins, P.N., Baillieres Clin. Rheumatol. 8:635 (1994); and references cited therein). Thus, for example, the amyloid load of a subject can be evaluated after a period of treatment according to the methods of the invention and compared to the amyloid load of the subject prior to beginning therapy with a therapeutic compound of the invention, to determine the effect of the therapeutic compound on amyloid deposition in the subject.

It will be appreciated that the ability of a compound of the invention to modulate amyloid deposition or amyloid load can, in certain embodiments, be evaluated by observation of one or more symptoms or signs associated with amyloid deposition or
amyloid load in vivo. Thus, for example, the ability of a compound to decrease amyloid deposition or amyloid load may be associated with an observable improvement in a clinical manifestation of the underlying amyloid-related disease state or condition, or a slowing or delay in progression of symptoms of the condition. Thus, monitoring of clinical manifestations of disease can be useful in evaluating the amyloid-modulating efficacy of a compound of the invention.

The method of the invention is useful for treating amyloidosis associated with any disease in which amyloid deposition occurs. Clinically, amyloidosis can be primary, secondary, familial or isolated. Amyloids have been categorized by the type of amyloidogenic protein contained within the amyloid. Non-limiting examples of amyloids which can be modulated, as identified by their amyloidogenic protein, are as follows (with the associated disease in parentheses after the amyloidogenic protein): β-amyloid (Alzheimer's disease, Down's syndrome, hereditary cerebral hemorrhage amyloidosis [Dutch], cerebral angiopathy); amyloid A (reactive [secondary]

amyloidosis, familial Mediterranean Fever, familial amyloid nephropathy with urticaria and deafness [Muckle-Wells syndrome]); amyloid κ L-chain or amyloid λ L-chain (idiopathic [primary], myeloma or macroglobulinemia-associated); Aβ2M (chronic hemodialysis); ATTR (familial amyloid polyneuropathy [Portuguese, Japanese, Swedish], familial amyloid cardiomyopathy [Danish], isolated cardiac amyloid, systemic senile amyloidosis); AIAPP or amylin (adult onset diabetes, insulinoma); atrial naturetic factor (isolated atrial amyloid); procalcitonin (medullary carcinoma of the thyroid); gelsofin (familial amyloidosis [Finnish]); cystatin C (hereditary cerebral hemorrhage with amyloidosis [Icelandic]); AApoA-I (familial amyloidotic polyneuropathy [Iowa]); AApoA-II (accelerated senescence in mice); fibrinogen-associated amyloid; lysozyme-associated amyloid; and AScr or PrP-27 (Scrapie, Creutzfeldt-Jacob disease, Gerstmann-Straussler-Scheinker syndrome, bovine spongiform encephalitis).

Compounds for use in the methods of the invention are commercially available and/or can be synthesized by standard techniques known in the art. In general, phosphonic esters can be prepared from the corresponding phosphonic acid by standard methods. Similarly, carboxylic esters can be prepared from the free carboxylic acid by standard techniques (for a reference to esterification techniques, see, e.g., R. Larock, "Comprehensive Organic Transformations," VCH Publishers (1989)). Carboxylic esters can be converted to thioesters by known reactions, e.g., by treatment with Lawesson's reagent (2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide, which is commercially available, e.g., from Aldrich Chemical Co., Milwaukee, WI). Compounds of the present invention also can be prepared as described below. The following
Examples further illustrate the present invention and are not intended to be further limiting in anyway.

**Example 1**

It is known that amyloidogenic peptides or proteins which have formed amyloid deposits or plaques have a significant amount of \( \beta \)-sheet secondary structure, while the unaggregated peptide or protein generally has less \( \beta \)-sheet structure. It is believed that the ability of a candidate therapeutic compound to prevent the formation of \( \beta \)-sheet secondary structure *in vitro* may be correlated with the ability of the compound to inhibit amyloidogenesis *in vivo*. Accordingly, phosphonate compounds were assayed for ability to prevent the formation of \( \beta \)-sheet secondary structure in assay systems including an *in vitro* circular dichroism (CD) assay.

\( \text{A}\beta \) is a 40 amino acid protein associated with Alzheimer's disease. \( \text{A}\beta \) peptide was prepared and purified as described in Fraser, P.E. et al., *Biochemistry* 31, 10716 (1992). Briefly, the peptide was synthesized by standard solid-phase techniques and purified by HPLC according to well known procedures.

All CD experiments were performed on a commercially available instrument. The cell was maintained at 25°C using a circulating water bath. Computer-averaging of traces was performed to improve signal-to-noise ratios. The solvent signal was subtracted. CD experiments were performed for each test compound according to the following procedure:

A stock solution of purified peptide was made by dissolving the peptide in phosphate-buffered saline (PBS) to a concentration of 2 mg/ml. A test solution was made for each potential therapeutic agent (test compound) as shown below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>A( \beta ) stock solution</td>
<td>25 µl</td>
</tr>
<tr>
<td>Test compound (20 mg/ml)</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>10 mM Tris-HCl buffer, pH 7</td>
<td>370 µl</td>
</tr>
</tbody>
</table>

The control sample had no test compound, and a total of 5 µl distilled water was added. The test solution was incubated for either 0 or 24 hours at 37°C before CD measurement. The size minimum in the CD spectrum at 218 nm is believed to be diagnostic of the presence of \( \beta \)-pleated sheet. Comparison of the minimum at 218 nm of a candidate compound, compared to the minimum of a control sample, is believed to be indicative of the ability of the candidate compound to inhibit the formation of \( \beta \)-pleated sheet.
Using this assay, several candidate compounds were tested. Phosphonoformate sodium salt (foscarnet sodium) was found to significantly and reproducibly reduce the amount of β-sheet formation, as measured by the CD assay. Phosphonoacetate was also found to be active in this assay. Thus, phosphonoformate and phosphonoacetate are believed to be inhibitors of amyloid deposition. 2-carboxyethylphosphonic acid had a lower ability to prevent β-pleated sheet formation in this model system.

In a preliminary result in a different assay system (in which the candidate compound and amyloid peptide were incubated together overnight, followed by centrifugation and determination of the amount of soluble peptide), phosphonoformate trisodium salt was found to have little effect on amyloid peptide solubility; it is believed that the buffer composition may have interfered with the ability of the compound to inhibit amyloid deposition.

The neurotoxicity of phosphonoformate trisodium salt was investigated in cortical/hippocampal neuronal cultures; no significant toxicity was noted at concentrations ranging from 10^{-7}M to 10^{-4} M.

**Example 2**

The procedure described below is further described in Gorin et al., Tet. Lett. 1997, 38:2791-2794, incorporated herein by reference. The procedure has the advantage that the reactivity of the nucleophile (e.g., the hydroxyl groups of a diol which react with the phosphonic acid chloride) is attenuated by use of a silyl ether (e.g., a trimethylsilyl ether), which can improve selectivity.

To a solution of (phenoxy carbonyl)phosphonodichloridate (5 mmol) in 10 ml dry THF cooled in an ice water bath under argon was added a vicinal bis-trimethylsilyl ether (5 mmol) (prepared from the vic-diol, e.g., by treatment with trimethylsilylchloride (TMSCl) or trimethylsilyltriflate (TMSOTf), available from Aldrich Chemical Co., Milwaukee, WI) in 10 mL dry THF. After addition was complete, the reaction mixture was stirred for one hour at room temperature, and the solvent was evaporated under reduced pressure. The residue was taken up in dioxane containing 90 mg water (5 mmol), neutralized by adding 5 mmol aniline in 10 mL dioxane, and the product precipitated by pouring into 200 mL 1:1 diethyl ether:hexanes. The solid product was filtered and washed with 1:1 diethyl ether:hexanes.

Compounds IVa - IVg were prepared by the above procedure using the corresponding diols, which are commercially available and/or can be readily prepared by one of ordinary skill in the art using no more than routine experimentation.
**Example 3**

The compounds of Formula IVa, IVc and IVd (in salt forms, e.g., methylpyridinium salts and/or anilinium salts) were tested in at least one assay for their ability to inhibit amyloidosis. It was found that these compounds showed activity in at least one assay system indicative of their ability to be an inhibitor of amyloidosis *in vivo* in both free or salt forms.

**Equivalents**

The contents of all references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.
What is claimed is:

1. A method for modulating amyloid deposition in a subject, comprising administering to a subject an effective amount of a therapeutic compound such that modulation of amyloid deposition occurs, wherein the therapeutic compound has the formula:

   \[
   \begin{array}{c}
   X \\
   R^1X \\
   R^2(Y^1Y^2)_nC(X)XR^3 \\
   Z \\
   \end{array}
   \]

   in which
   
   \( R^1 \) and \( R^2 \) are each independently hydrogen, a substituted or unsubstituted aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation;
   
   \( R^3 \) is hydrogen, lower alkyl, aryl, or a salt-forming cation;
   
   \( R^4 \) is hydrogen, lower alkyl, aryl or amino;
   
   \( X \) is, independently for each occurrence, O or S;
   
   \( Y^1 \) and \( Y^2 \) are each independently hydrogen, halogen, alkyl, amino, hydroxy, alkoxy, or aryloxy;
   
   \( Z \) is \( XR^2 \) or \( R^4 \); and
   
   \( n \) is an integer from 0 to 12.

2. The method of claim 1, wherein \( Z \) is \( XR^2 \).

3. The method of claim 2, wherein \( R^1 \) and \( R^2 \) are each a pharmaceutically acceptable salt-forming cation.

4. The method of claim 3, in which \( R^1 \), \( R^2 \) and \( R^3 \) are each independently a sodium, potassium or calcium cation.

5. The method of claim 4, wherein \( n \) is 0.

6. The method of claim 1, wherein at least one of \( R^1 \) and \( R^2 \) is a long-chain aliphatic moiety.
7. The method of claim 6, wherein R³ is a lower alkyl group.

8. The method of claim 1, wherein Y¹ and Y² are each hydrogen.

9. The method of claim 1, wherein the therapeutic compound is administered orally.

10. The method of claim 1, further comprising administering the therapeutic compound in a pharmaceutically acceptable vehicle.

11. The method of claim 1, wherein administering the therapeutic compound to the subject inhibits amyloid deposition in the subject.

12. The method of claim 1, wherein X is, for each occurrence, O.

13. The method of claim 1, wherein the compound is represented by the formula:

\[
\text{R}^1\text{X} \quad \text{P} \quad (\text{CY}^1\text{Y}^2)_n\text{C}(\text{O})\text{OR}^3 \\
\text{XR}^2
\]

14. The method of claim 1, wherein the compound is represented by the formula:

\[
\text{R}^1\text{O} \quad \text{P} \quad (\text{CY}^1\text{Y}^2)_n\text{CH}(\text{NR}_a\text{R}_b)\text{C}(\text{O})\text{OR}^3 \\
\text{OR}^2
\]

in which

Rₐ and Rₐ are each independently hydrogen, alkyl, aryl, or heterocyclic, or Rₐ and Rₐ, taken together with the nitrogen atom to which they are attached, form a cyclic moiety having from 3 to 8 atoms in the ring.

15. The method of claim 14, in which Rₐ and Rₐ are each hydrogen.

16. A method for treating a disease state associated with amyloidosis, comprising:
administering to a subject an effective amount of a therapeutic compound such that a disease state associated with amyloidosis is treated, wherein the therapeutic compound has the formula

\[
\begin{array}{c}
\text{O} \\
\text{R}^1 \text{O} \\
\text{P} \\
\text{C(} \text{Y}^1 \text{Y}^2 \text{)}_n \text{COOR}^3 \\
\text{OR}^2
\end{array}
\]

in which

- \( R^1 \) and \( R^2 \) are each independently hydrogen, an aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation;
- \( R^3 \) is hydrogen, lower alkyl, aryl or a salt-forming cation;
- \( Y^1 \) and \( Y^2 \) are each independently hydrogen, halogen, lower alkyl, amino, hydroxy, alkoxy, or aryloxy; and
- \( n \) is an integer from 0 to 12.

17. The method of claim 16, wherein said disease state is amyloid deposition associated with Alzheimer's disease.

18. The method of claim 16, wherein \( R^1 \) and \( R^2 \) are each a pharmaceutically acceptable salt-forming cation.

19. The method of claim 18, in which \( R^1, R^2 \) and \( R^3 \) are each independently a sodium, potassium or calcium cation.

20. The method of claim 19, wherein \( n \) is 0.

21. The method of claim 16, wherein at least one of \( R^1 \) and \( R^2 \) is a long-chain aliphatic moiety.

22. The method of claim 21, wherein \( R^3 \) is a lower alkyl group.

23. The method of claim 16, wherein \( Y^1 \) and \( Y^2 \) are each hydrogen.

24. The method of claim 16, wherein the amino group is \(-\text{NH}_2\).
25. The method of claim 16, wherein the therapeutic compound is administered orally.

26. The method of claim 16, further comprising administering the therapeutic compound in a pharmaceutically acceptable vehicle.

27. A method for modulating amyloid deposition in a subject in which said amyloid deposition is characterized by interaction between an amyloidogenic protein and a constituent of a basement membrane, the method comprising administering to the subject an effective amount of a therapeutic compound such that modulation of amyloid deposition characterized by interaction between an amyloidogenic protein and a constituent of a basement membrane occurs, wherein the therapeutic compound has the formula:

\[ \text{R}^1\text{O}^\gamma\text{P}-(\text{CY}^1\text{Y}^2)\text{nCOOR}^3\text{OR}^2 \]

in which

- R\(^1\) and R\(^2\) are each independently hydrogen, an aliphatic group, an aryl group, a heterocyclic group, or a salt-forming cation;
- R\(^3\) is hydrogen, lower alkyl, aryl or a salt-forming cation;
- Y\(^1\) and Y\(^2\) are each independently hydrogen, halogen, lower alkyl, amino, hydroxy, alkoxy, or aryloxy; and
- n is an integer from 0 to 12.

28. The method of claim 27, wherein R\(^1\) and R\(^2\) are each a pharmaceutically acceptable salt-forming cation.

29. The method of claim 28, in which R\(^1\), R\(^2\) and R\(^3\) are each independently a sodium, potassium or calcium cation.

30. The method of claim 29, wherein n is 0.

31. The method of claim 27, wherein at least one of R\(^1\) and R\(^2\) is a long-chain aliphatic moiety.
32. A method for preparing a compound represented by the formula:

\[
\text{RO} - \overset{\text{C}}{\overset{\text{P}}{\overset{\text{O}}{\overset{\text{O}}{\overset{\text{OH}}{\overset{\text{R'}}{\overset{\text{O}^-}{\text{R'}}}}}}}}
\]

in which R is alkyl or aryl, and R' is hydrogen, alkyl, or aryl; the method comprising:

reactions an ester of a carbonylphosphono diacid halide with a disilyether of a vicinal diol, under conditions such that the compound of Formula V is prepared.

33. A compound represented by the formula (Formula IV):

\[
\text{G} - \overset{\text{O}}{\overset{\text{C}}{\overset{\text{P}}{\overset{\text{O}}{\overset{\text{L}}{\overset{\text{O}^-}{\text{L}}}}}}}
\]

wherein G is hydrogen or one of more substituents on the aryl ring and the L is a substituted alkyl group.
32. A method for preparing a compound represented by the formula:

\[
\text{RO-\text{C-P-OR'}}
\]

in which R is alkyl or a substituted aryl group in which the aryl substituent(s) is/are H, an electron-donating group, or an electron-withdrawing substituent at the m-position; R' is alkyl, substituted alkyl or aryl; and R'' is hydrogen, sodium, potassium, calcium or a pharmaceutically acceptable salt-forming cation; the method comprising:

reacting an ester of a carbonylphosphono diacid halide with a silylether of an alcohol under conditions such that said compound is prepared.

33. A compound represented by the formula (Formula IV):

\[
\text{G-O-C-P-O-L}
\]

wherein G is hydrogen or one or more electron-donating substituents on the aryl ring, or one or more electron-withdrawing substituents at the m-position; and the L is a substituted alkyl group or C₆-C₂₄ alkyl group.

34. The method of claim 32 wherein R is an alkyl or aryl group, R' is a substituted alkyl group, and the silylether of an alcohol is a disilylether of a vicinal diol, such that the compound of Formula V is prepared.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

| IPC | A61K31/66 | A61K31/675 | C07F9/40 |

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

| IPC | A61K | C07F |

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>WO 98 25938 A (GORIN BORIS ;THATCHER GREGORY (CA); PRING BRIAN (SE); ASTRA AB (SE) 18 June 1998 see page 9, line 5 - page 10, line 4; claims 7-10; examples 9-12,18</td>
<td>32, 33</td>
</tr>
<tr>
<td>P,X</td>
<td>WO 98 13046 A (GUILFORD PHARM INC) 2 April 1998 see abstract see page 57, line 15 - page 69, line 1; claims; table II</td>
<td>1-13, 16-31</td>
</tr>
<tr>
<td>X</td>
<td>WO 98 11923 A (BAYLOR COLLEGE MEDICINE ;GIULIAN DANA J (US)) 26 March 1998 see figures 26B,32; examples 10,12,13</td>
<td>16-19, 24-26</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of box C.

**Date of the actual completion of the international search**

8 October 1998

**Date of filing of the international search report**

22.10.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. +31-70 340-2040, Tx. 31 651 epo nl, Fax: +31-70 340-3016

Authorized officer

Hoff, P
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>WO 94 27602 A (CORTEX PHARMA INC) 8 December 1994 see abstract see claims 15-17; example 16 ---</td>
<td>1-4, 9-19, 24-29</td>
</tr>
<tr>
<td>X</td>
<td>EP 0 434 173 A (SCHERING AG) 26 June 1991 see abstract see page 3, line 53 - line 58 see page 4, line 1 - line 4 see page 4, line 33 - line 36; claim 1 ---</td>
<td>1-4, 9-19, 24-29</td>
</tr>
<tr>
<td>X</td>
<td>A. COPANI ET AL.: &quot;ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS PROTECTS CULTURED NEURONS AGAINST APOPTOSIS INDUCED BY beta-AMYLOID PEPTIDE&quot; MOLECULAR PHARMACOLOGY, vol. 47, no. 5, 1995, pages 890-897, XP002079923 see the whole document ---</td>
<td>16-19, 24-26</td>
</tr>
<tr>
<td>A</td>
<td>see the whole document ---</td>
<td>1-31</td>
</tr>
<tr>
<td>X</td>
<td>BRIGGS A D ET AL: &quot;Acylxymethyl and 4-Acylxoybenzyl Diester Prodrugs of Phosphonoformate&quot; TETRAHEDRON, vol. 52, no. 47, 18 November 1996, page 14937-14950 XP004105086 see the whole document, in particular the compounds 1-1c ---</td>
<td>33</td>
</tr>
<tr>
<td>A</td>
<td>WO 94 22437 A (UNIV KINGSTON) 13 October 1994 see abstract see page 4, line 33 - page 5, line 7; claims 1-4 ---</td>
<td>1-31</td>
</tr>
</tbody>
</table>

Form PCT/ISA/210 (continuation of second sheet) (July 1992)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>EP 0 003 275 A (ASTRA LAEKEMEDEL AB) 8 August 1979 see the whole document</td>
<td>1-33</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claims Nos.:  
   because they relate to subject matter not required to be searched by this Authority, namely:  
   Although claims 1-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:  
   because they relate to parts of the international Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4151897 A</td>
<td>17-04-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9813044 A</td>
<td>02-04-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 3590697 A</td>
<td>07-01-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9748399 A</td>
<td>24-12-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 3513597 A</td>
<td>07-01-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9748400 A</td>
<td>24-12-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WO 9427602 A</td>
<td>08-12-1994</td>
<td>AU 6836394 A</td>
<td>20-12-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5622981 A</td>
<td>22-04-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2031433 A</td>
<td>05-06-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 3209335 A</td>
<td>12-09-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 96074 A</td>
<td>30-09-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2159649 A</td>
<td>13-10-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9422885 A</td>
<td>13-10-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0691844 A</td>
<td>17-01-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0691976 A</td>
<td>17-01-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 8508260 T</td>
<td>03-09-1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5643562 A</td>
<td>01-07-1997</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5728375 A</td>
<td>17-03-1998</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP 0003275 A</td>
<td>08-08-1979</td>
<td>AT 369017 B</td>
<td>25-11-1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AT 921778 B</td>
<td>15-04-1982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4268178 A</td>
<td>28-06-1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 530031 B</td>
<td>30-06-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4268278 A</td>
<td>28-06-1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1140049 A</td>
<td>25-01-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1144937 A</td>
<td>19-04-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1156651 A</td>
<td>08-11-1983</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 564178 A,B</td>
<td>23-06-1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 564278 A,B</td>
<td>23-06-1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0003807 A</td>
<td>11-07-1979</td>
</tr>
</tbody>
</table>