Synthesis of New Macrocyclic Amino-Phosphinic Acid Complexing Agents and Their C- and P-Functionalised Derivatives for Protein Linkage

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Received 27 August 1991

The synthesis of various macrocyclic complexing agents with alkyl- and arylphosphinic substituents is reported together with their C- and N-functionalised analogues as active esters suitable for antibody conjugation.

The development of macrocyclic metal complexes that are kinetically stable in vivo has accelerated a study of their use in diagnostic and therapeutic medicine. 1 Kinetically stable complexes of the imaging isotopes 111In (γ, t1/2 2.81 d) and 67Ga (γ, t1/2 3.25 d) have been reported using hexadentate ligands based on a triazacyclononane skeleton. 2 Octadentate ligands designed to bind the therapeutic radioisotope 90Y (β, t1/2 64 h) and the paramagnetic Gd 3+ ion (for use in magnetic resonance imaging as a contrast agent 3) have also been developed using a tetraazacyclododecane skeleton. 4, 5 Until recently such ligands incorporated carboxymethyl groups to act as donor groups almost exclusively to satisfy the nuclear charge on the bound metal ion. An attractive alternative to the carboxylic acid donor group is a phosphinic acid. It is a stronger acid so that protonation not only of the free ligand but also of the phosphorus oxygen double bond in the metal complex is inhibited. The pentavalency of phosphorus means that an alkyl, aryl or other functionality may be introduced readily, permitting not only control over complex lipo-

philicity but also the introduction of a remote electrophilic site as is required if such ligands are to be used as bifunctional complexing agents in protein conjugation. 1, 2, 4 The synthesis of examples of hexa- and octadentate complexing agents incorporating alkyl and arylphosphinic acids is reported, together with their C- and P-functionalised analogous bearing remote active esters for conjugation to proteins.

Condensation of 1,4,7-triazacyclononane (1) or 1,4,7,10-tetraazacyclododecane (2) with anhydrous paraformaldehyde in THF yielded an intermediate hydroxymethyl species which could be trapped with various dialkoxyphosphines to give the corresponding polymethylene phosphinite esters 3–7 (Scheme 1). Exclusion of moisture was essential to prevent formation of the hydroxymethyphosphinite esters, HOCH2P(OR)OR', and molecular sieves were used to scavenge any water. Attempts to promote the Arbusov reaction by deliberate addition of anhydrous tetrapentylammonium chloride or bromide failed to improve the yield of ester. In the case of triazacyclononane 1, competitive formation of a bicyclic aminal monophosphinite ester, 13 (Scheme 2), occurred. The yield of this bicyclic compound was increased when the concentration of MeP(OEt)2 was reduced. Related aminals have been reported previously with macrocyclic

\[
\begin{align*}
\text{Compound} & \quad R^1 & \quad R^2 & \quad \text{Compound} & \quad R^1 & \quad R^2 \\
3 & \quad \text{Ph} & \quad \text{Me} & \quad 8 & \quad \text{Ph} & \quad \text{H} \\
4 & \quad \text{Me} & \quad \text{Et} & \quad 9 & \quad \text{Me} & \quad \text{H} \\
5 & \quad \text{Me} & \quad \text{Et} & \quad 10 & \quad \text{Me} & \quad \text{H} \\
6 & \quad \text{Ph} & \quad \text{Me} & \quad 11 & \quad \text{Ph} & \quad \text{H} \\
7 & \quad \text{Bu} & \quad \text{Me} & \quad 12 & \quad \text{Bu} & \quad \text{H} \\
\end{align*}
\]

Scheme 1
Acidic hydrolysis of the amidine yielded the monosubstituted tetradentate ligand 14, thereby permitting the synthesis of mixed donor ligands. Similarly, hydrolysis (6 M HCl, reflux, 16 h) of the esters 3–7 afforded the phosphinic acids 8–12 in essentially quantitative yield.

Scheme 2

This synthetic scheme was also used in the preparation of the C-aminoalkyl substituted analogues 19 and 21. The 2-substituted benzamidobutyl polyamines 15 and 16 were accordingly converted to the acids 17 and 18 in moderate yield. Again in the triazacyclononane series competitive formation of one of the two constitutionally isomeric bicyclic amines occurred (10–20%). In order to conjugate these bifunctional complexing agents to a protein (or nucleotide), the pendant primary amine was reacted with a bifunctional linker molecule, such as a bis(p-nitrophenyl) succinate or N-hydroxysuccinimidyl-3-maleimidopropionate (Scheme 3). The resultant active esters 20 and 22 for example may be used directly to acylate lysine residues on an antibody (pH 8, phosphate buffer), with minimal protein aggregation.

The synthesis of the enantiopure precursor amines 15 and 16 from (2S)-lysine methyl ester involves a six-step procedure and a shorter route to a functionalised ligand was sought using the parent polyamides as starting materials. With this in mind the mesylate 29 was prepared. It incorporates a protected amine group to allow subsequent protein conjugation. Radical addition of hypophosphorus acid across the olefinic bond of N-benzoylallylamine (24) (Scheme 4) followed by trapping of the intermediate alkylphosphonous acid with formaldehyde gave the hydroxymethylphosphinic acid 26, isolated as its ammonium salt. Following ion exchange to the acid (Dowex 50 W, H+), esterification with triethyl orthoformate yielded the ethyl ester 27.

Competitive formation of mixed orthoformate 27 was observed, and this mixed ester could be separated by column chromatography on alumina and easily transesterified to the desired ester 28 in acidic ethanol. Mesylation of 28 in THF, rather than dichloromethane, afforded the mesylate 29 which was used to alkylate the [9]- and [12]-membered polyamines directly (Scheme 5), in moderate yield. Higher yields of 31 have been obtained by protection of three of the four nitrogens with a Mo(CO)3 moiety prior to alkylation. Transformation of the monosubstituted amines 30 and 31, via the amino-acids 33 and

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>H</td>
</tr>
<tr>
<td>20</td>
<td>CO(CH2)2CO2C6H4NO2-p</td>
</tr>
<tr>
<td>21</td>
<td>H</td>
</tr>
<tr>
<td>22</td>
<td>CO(CH2)2CO2C6H4NO2-p</td>
</tr>
</tbody>
</table>

Scheme 3
35 to suitable active esters such as 36 could be achieved using the previously established methods.

Column chromatography was carried out using either ‘gravity’ silica (Merck 7734), ‘flash’ silica (Merck 9385), or neutral

![Scheme 4]

**Table 1. Synthesis of Parent and C- and P-Functionalised Phosphinate Esters 4-7, 17, 18, 32 and 34**

<table>
<thead>
<tr>
<th>Starting Material Product (%)</th>
<th>Yield δ</th>
<th>31P NMR (CDCl3) δ (ppm)</th>
<th>1H NMR (CDCl3) δ (ppm)</th>
<th>13C NMR (CDCl3) δ (ppm)</th>
<th>Molecular Formula*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>54.2, 54.13 (5:2)</td>
<td>1.32 (t, 9H), 1.54 (d, 9H, J = 13.2, CH3, N), 3.0 (m, 12H, CH2N), 4.08 (dq, 6H)</td>
<td>13.33 (d, J = 91, CH3P), 16.61, 16.64, 16.66 (CH3CH2), 57.3, 57.4, 57.6, 57.7, 57.8, 58.4, 58.5 (CH3N), 60.1, 60.2 (CH2O)</td>
<td>C21H22N2O4P3 (489.2)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>51.9, 51.8, 51.6 (diastereomers)</td>
<td>1.31 (t, 12H), 1.57 (d, 12H, J = 13.7), 2.64–3.07 (m, 24H, CH3N), 4.07 (dq, 8H, CH2O)</td>
<td>2.1–2.9 (br m, 24H, CH2N), 3.56 (d/d + d/d, 12H, CH2O isomers), 7.45 (m, 12H), 7.78 (m, 8H, α-Ar)</td>
<td>13.44 (J = 91, CH3P), 16.42 (d, J = 5), 54.18, 54.30 (d, J = 110), 59.82 (d, J = 6)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>41.5</td>
<td>0.97 (t, 12H), 1.24 (t, 1H), 1.35 (dt, 8H, CH2), 1.50 (m, 8H, CH2C), 1.7 (br m, 8H, CH2P), 2.6–2.95 (br, 24H, CH3N), 4.05 (dq, 8H, CH2O)</td>
<td>14.49 (CH3CH2C), 16.70 (d, J = 4.4), 23.75, 23.88, 24.04 (CH2C), 27.35 (d, J = 87, CH3P), 53.28 (d, J = 104, CH3N), 53.95, 54.02, 54.10 (CH3N), 60.01 (d, J = 4, CH2O)</td>
<td>C24H24N6O4P4 (900.4)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>46</td>
<td>53.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>17</td>
<td>50.3, 50.3, 49.9 (2 diastereomers)</td>
<td>1.20 (t+t, 9H), 1.30 (m, 9H), 1.55–1.42 (m, 4H, CH2CH2), 1.63 (m, 2H, CH2CH2NCH2O), 3.50–2.60 (m, 19H, CHN + CH2N), 4.02 (m, 6H), 7.45–7.39 (m, 3H, α- and P-Ar), 7.85 (br t, 1H NHCCH), 7.91 (dd, 2H, α-Ar)</td>
<td>12.95 + 12.80 (d/d, J = 90, J = 89) major isomer, 16.40, 16.36 (CH3), 24.0, 23.9 (CH2C), 28.6 (CH2), 39.4, 39.35 (CH2NCH2O), 52.7, 53.0, 53.1, 57.0, 57.8, 57.9, 58.15, 58.2, 59.0, 59.9 (CH3N), 63.5 (CH2O), 127.1, 121.9, 130.7, 134.75, 167.4</td>
<td>C29H29N5O4P3 (664.3)</td>
</tr>
<tr>
<td>16</td>
<td>18</td>
<td>50.4, 50.4</td>
<td>1.18–1.23 (m, 12H, CH3), 1.25–1.50 (m, 18H, CH2P + CH2C), 2.23–3.82 (m, 25H), 3.93–4.05 (dq + dq + dq, 8H, CH2O), 7.34 (m, 3H), 7.66 (br t, 1H, NHCCH), 8.07 (dd, 2H, α-Ar)</td>
<td>16.1 + 15.9 (d + d, J = 91, CH3P), 19.2 (CH2C), 26.4, 29.5, 29.6 (CH2C), 40.9 (CH2NCH2O), 49.7, 51.0, 51.5, 51.7, 54.7, 55.0, 55.9, 56.3, 56.8, 58.0, 58.1, 58.2, 59.2 (CH2N), 60.5 (CH2O), 124.7, 127.5, 128.3 (Ar, C=H), 132.5 (s), 164.8 (NHCCH)</td>
<td>C33H29O4P3 (827.4)</td>
</tr>
<tr>
<td>30</td>
<td>32</td>
<td>50.4, 50.4, 50.0</td>
<td>1.30 (t+t, 9H), 1.51 (d, 6H, CH3P), 1.98 (brm, 4H, CH2CH2COEt), 2.85–3.05 (m, 20H, CH3N), 4.07 (dq + dq + dq, 6H, CH2CH2O), 7.45 (m, 3H), 7.91 (dd, 2H), 8.10 (br t, 1H, NHCCH)</td>
<td>1.30 (t, 12H, J = 7.2), 1.49 (d/d + d/d, 9H, PCH3), 1.80–3.70 (br m, 30H, CH3N + CH2P + CH2C), 4.05 (dq, 8H, CH2O), 7.39 (m, 3H), 7.92 (dd, 2H, α-Ar), 8.35 (br t, 1H, NHCCH)</td>
<td>C27H15N4O4P3 (636.3)</td>
</tr>
<tr>
<td>31</td>
<td>34</td>
<td>50.5, 50.5</td>
<td>1.30 (t, 12H, J = 7.2), 1.49 (d/d + d/d, 9H, PCH3), 1.80–3.70 (br m, 30H, CH3N + CH2P + CH2C), 4.05 (dq, 8H, CH2O), 7.39 (m, 3H), 7.92 (dd, 2H, α-Ar), 8.35 (br t, 1H, NHCCH)</td>
<td>C27H15N4O4P4 (799.4)</td>
<td></td>
</tr>
</tbody>
</table>

* Accurate masses were obtained (DCI, MeOH) with ±0.0007 amu, except for 34 and 18 ±0.0009 amu.
Table 2. Synthesis of Phosphinic Acids 9–12, 19, 21, 33 and 39

<table>
<thead>
<tr>
<th>Starting Ester</th>
<th>Product Yield (%)</th>
<th>Product (pD as stated)</th>
<th>δ (H NMR (D₂O), J (Hz))</th>
<th>δ (P NMR (D₂O), J (Hz))</th>
<th>δ (C NMR (D₂O))</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9</td>
<td>pD 0.0: 50.0</td>
<td>1.42 (d, 9H, J = 14), 3.32 (br d + s, 18H, CH₂N)</td>
<td>15.43 (d, J = 93), 51.60, 55.06 (d, J = 92)</td>
<td>C₁₃H₂₀N₅O₃P₃</td>
<td>(405.1)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>pD 14: 39.2</td>
<td>3.37 (br, 24H, CH₂CN)</td>
<td>50.70 (CH₃N), 51.64 (d, J = 118)</td>
<td>C₁₆H₂₆N₅O₃P₄</td>
<td>(542.2)</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>pD 14: 28.0</td>
<td>2.26 (br, 8H, CH₂N), 7.25 (m, 12H), 7.46 (m, 8H, J = Ar)</td>
<td>128.3 (br), 131.0 (d, J = 118)</td>
<td>C₁₈H₂₈N₅O₃P₄</td>
<td>(782.2)</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>46.63</td>
<td>0.97 (t, 12H, 1.4–1.7 (m, 16H, CH₂C), 1.95 (dt, 2H, CH₂CH₃P), 3.4–3.8 (br, m, 24H, CH₂N))</td>
<td>13.95 (CH₃), 24.11, 24.16, 24.79, 24.95 (CH₃C), 29.61 (d, J = 96, CH₃C), 52.4 (br, d), 52.9 (br, CH₂N)</td>
<td>C₂₃H₄₆N₆O₅P₄</td>
<td>(708.4)</td>
</tr>
</tbody>
</table>

- Molecular ions were observed using negative ion FAB mass spectrometry (3-nitrobenzyl alcohol).
- Conversion was quantitative as deduced by 400 MHz 1H NMR (Varian VXR-400).
- Isolated yield of the hydrochloride salts, analyses were C ± 0.35, H ± 0.27, N ± 0.31.
- Solvent: MeOD.

Scheme 5
alumina (Merck Art 1077) which had previously been treated with ErOAc. Analytical and semi-preparative HPLC was performed with a Varian V650/Polychrom 9060 instrument fitted with either cation exchange (Synchropak CM 300), anion exchange (‘Synchropak’ AX 100) or reverse phase columns (‘Spherisorb’ 5 ODS2). Flow rates of 1.4 mL min−1 and 4.0 mL min−1 were used for analytical and semi-preparative columns respectively. Column and gradient elution conditions were as follows: cation exchange, t = 0 min, 80% H2O, 0% aqueous NH4OAc (1.0 M, pH 5.6), 20% MeCN; t = 5 min, 60% H2O, 20% aqueous NH4OAc, 20% MeCN; t = 10 min, 0% H2O, 80% aqueous NH4OAc, 20% MeCN. For anion exchange: t = 0 min, 70% H2O, 10% aqueous NH4OAc, 20% MeCN; t = 20 min, 0% H2O, 80% aqueous NH4OAc, 20% MeCN. For reverse phase: t = 0 min, 95% H2O, 0% aqueous NH4OAc, 5% MeCN; t = 20 min, 5% H2O (0.1% trifluoroacetic acid) 0% NH4OAc, 95% MeCN (0.1% trifluoroacetic acid). Solvents used were dried from an appropriate drying agent, and water was purified by the Milli Q system. [R] spectra were recorded with a Perkin Elmer 577 spectrometer.

1H, 13C and 31P NMR spectra were obtained with a Bruker AC 250 operating at 250.13, 62.90 and 101.1 MHz, respectively. Mass spectra were recorded with a VG 7070E spectrometer operating in CI, DCl or FAB modes with DCl samples presented as dilute CH2Cl2 or MeOH solutions and ammonium as the m-Netrobenzyl alcohol or glycerol was the matrix for FAB analyses.

4-Ethoxy(methyl)phosphoryl)methyl-1,4,7-triazacyclononane[5,2.1]nonane (13): This compound was formed during the synthesis of 4, and was prepared in higher yield using an analogous procedure from triazacyclononane and diethoxy(methyl)phosphine in equal molar amounts; yield 51%.

MS (DCI): m/z = 262 (M + H); calc. for C11H12N4O2P: 261.1601, found: 261.1602.
C11H12N4O2P calc. C 50.54 H 9.19 N 16.08 (261.2) found 50.31 9.01 15.84

1H NMR (CDCl3): δ = 1.33 (t, 3 H, OCH3, CH3), 1.53 (d, 3 H, CH3P), 2.80 (m, 4 H, CH2N), 3.05 (m, 8 H, CH2N), 3.24 (dd, 2 H, CH2P), 4.09 (dq, 2 H, CH2O), 4.16 (dd, 2 H, J = 10.4 Hz, NCH2N).

13C NMR (CDCl3): δ = 12.69 (d, J = 89, CH3P), 16.53 (d, J = 5 Hz, CH3), 49.10, 49.05 (CH3N, 5 ring), 54.02, 54.00 (CH3N, 8 ring), 55.71 (d, J = 5.3 Hz, CH2NCH3), 55.94 (d, J = 6.1 Hz, NCH2CH3), 55.97 (d, J = 114 Hz, CH3P), 60.10 (d, J = 6.8 Hz, CH3O), 76.16 (NCH2N).

1,4-Triazacyclononane-1-yl-methylene(methylphosphonic Acid) (14): This was prepared from 13 as described for the preparation of 8, and was isolated as the hydrochloride salt as a colourless glass; yield 98%.

MS (FAB): m/z = 222 (M + H+).


1H NMR (CDCl3, d 1): δ = 0.70 (m, 12 H, CH2N), 2.99 (d, 6 H, J = 7 Hz, CH3P), 3.65 (d, 9 H, J = 11 Hz, OCH3).

13C NMR (CDCl3): δ = 29.15 (m, J = 7 Hz, OCH3), 52.95 (m, J = 7 Hz, CH2N), 62.95 (m, J = 11 Hz, NCH2N), 129.6 (d, J = 118 Hz, CH2N), 128.6 (d, J = 12 Hz, o-C), 131.6 (d, J = 10 Hz, m-C), 132.2 (p-C).

31P NMR (CDCl3): 40.74 (s).

Data for other esters prepared in a similar manner are given in Table 1.

1,4,7-Triazacyclononane-1,4,7-trityltris[methylene(phenylphosphinate)] (8): Typical Procedure: The trimethyl ester 3 (0.4 g, 0.63 mmol) was dissolved in hydrochloric acid (6 M, 15 mL) and the solution was heated to reflux for 16 h. Concentration of the solution to small volume and adjustment of the pH (KOH solution) to 2, led to formation of 8 as a colourless solid which was filtered and dried (25 °C, 10 mbar); yield: 0.34 g (77%).

C11H12N4P2O72HCl2H2O calc. C 46.29 H 5.57 N 6.00 Cl 10.19 (696.1) found 46.01 5.75 6.07 9.09.
MS (FAB): m/z: 588 (M), 587 (M-1).

1H NMR (D2O, d 0.5): δ = 3.10 (br, 12 H, CH2N), 3.25 (d, 6 H, J = 7 Hz, CH2P), 7.5 (br, 9 H, m- p-C6H4), 7.60 (m, 6 H, o-C6H4).

13C NMR (D2O, d 0.5): δ = 51.6 (CH2N), 55.4 (d, J = 98 Hz, CH3P), 129.9 (d, J = 100 Hz, s-o-C6H4), 133.8 (br, p-Ar), 133.8 (br, o-Ar).

31P NMR (D2O, d 0.5): δ = 27.2.

Data for other acids prepared in a similar manner are given in Table 2.

3-Benzamidopropyl(hydroxymethyl)phosphonic acid (26): To a solution of N-benzoylaluminium 24 (7.47 g, 46.4 mmol) in dioxane (100 mL) was added hypophosphorous acid (8.66 g, 50% aque sol) and tert-butyl peroxide (0.3 g) and the mixture was heated to reflux for 18 h. After removal of solvent under reduced pressure, 1H NMR analysis of the crude residue revealed the disappearance of the olefinic resonances. The residue was redissolved in dioxane (50 mL) and excess paraformaldehyde (20 g) was added and the mixture heated to reflux for 68 h. After removal of solvent, the residue was purified by chromatography on silica gel (eluant 70% CH2Cl2: 28–25% MeOH, 2 → 5%aqueous NH4OAc) to yield the ammonium salt of the acid 26 as a hygroscopic colourless glass, yield: 9.12 g (69%).

MS (FAB): m/z = 257 (M), 256 (M-1).

13C NMR (D2O): δ = 22.03 (CH2CH2P), 25.12 (d, J = 8 Hz, CH2O), 41.01 (CH2NHCO), 59.73 (d, J = 59 Hz, PCH2OH), 127.22, 128.98, 132.28 (m, 134.0 (s), 170.04 (CONH).

31P NMR (D2O): δ = 41.17 (s).

Ethyl 3-Benzamidopropyl(hydroxymethyl)phosphinate (27): To a solution of the ammonium salt of 26 (5 g) in water (25 mL) was added a strong acid ion-exchange resin (Dowex 50W, H+ form, 30 g) and after filtration and evaporation, the residue was treated with triethyl orthoformate (25 mL) and the mixture heated to reflux for 96 h. After evaporation, the residue was purified by silica gel chromatography (5 to 10% MeOH/CH2Cl2) to yield a mixture of the desired ester 27 and its mixed orthoformate ester 27[31P NMR (CDCl3): δ = 51.68; MS(CI): m/z = 387 (M)]. Transesterification of this mixture was effected by boiling in EtOH (100 mL) in the presence of cone. H2SO4 (1 mL) for 36 h. Evaporation and purification by silica gel chromatography yielded 27 as a pale yellow oil; overall yield: 4 g (79%).
Ethyl 3-Benzamidopropyl(2-methoxyethyl)methylphosphinate (29):

To a suspension of the alcohol 27 (0.57 g, 2 mmol) in dry THF (40 mL) at 0°C was added Et$_3$N (1 g, 10 mmol) and MsCl (1.14 g, 10 mmol). After stirring for 2 h, EtOH (5 mL) was added and solvent removed under reduced pressure and the residue redissolved in EtOAc (20 mL), filtered, evaporated and the residue purified by flash chromatography on silica gel (2 to 5% MeOH/CH$_2$Cl$_2$) to yield 29 as a colourless oil; yield: 392 mg (61%).

C$_9$H$_{18}$NO$_5$PS calc. C 46.26 H 6.06 N 3.85 P 8.54 (363.1) found 46.41 5.92 3.91 8.19

MS (DCI): m/z = 364 (M + 1).

1-[3-Benzamidopropyl][ethoxyphosphorylmethyl]-1,4,7,10-tetraazaacyclododecan-1-ol (31):

To a solution of 1,4,7,10-tetraazaacyclododecan-1-ol (2, 0.16 g, 0.92 mmol) in dry DMF (20 mL) at 60°C was added K$_2$CO$_3$ (0.13 g, 0.92 mmol) and a solution of the mesylate 29 (0.167 g, 0.46 mmol) in DMF (15 mL) over a period of 4 h under nitrogen. After 6 h, HPLC analysis (CM 300 cation exchange) revealed that the reaction was not progressing further, solvent was removed under reduced pressure and the residue redissolved in CH$_2$Cl$_2$ (10 mL). Filtration and evaporation yielded a residue which was purified by cation-exchange HPLC to yield 30 as a colourless gummy solid, t$_R$ = 8.2 min (CM 300), yield: 52 mg (25%).

MS (DCI): m/z = 440 (M + 1).

1-[3-Benzamidopropyl][ethoxyphosphorylmethyl]-1,4,7,10-tetraazaacyclododecan-1-ol (30):

This was prepared starting from 1 as described for the preparation of 31; t$_R$ = 6.8 min (CM 300); yield 28%.

MS (DCI): m/z = 398 (M + 2), 397 (M + 1), 256, 142.

(+)-(2S)-2-[4-[(p-Nitrophenoxy)carbonyl]propionamidobutyryl]-1,4,7,10-tetraazaacyclododecan-1-ol, 1,4,7,10-tetraazaacyclododecan-1-ol, 1,4,7,10-tetraytetrakis[methylene-(methylene- by HPLC analysis revealed complete reaction, solvent was removed under reduced pressure and the residue purified by reverse-phase HPLC (Dynamax C-18, 60 Å; A = 0.1% TFA/H$_2$O, C = 0.1% TFA/MeCN; t = 0.90% A, 10% C; t = 20 min A = 25%, C = 75%; λ = 278 nm); t$_R$ = 12.7 min. The product was isolated by lyophilisation as the triis(trifluoroacetate) salt; yield: 26 mg (38%).

MS (FAB): m/z = 833 (M + 1), 855 (M + Na).

1H NMR (D$_2$O); δ = 1.45 (m, 13H, CH$_3$P + CH$_2$C), 1.70 – 1.90 (m, 2H, CH$_2$CO), 2.65 (t, 2H, CH$_2$CO), 2.94 (t, 2H, CH$_2$CO), 3.10 – 3.85 (m, 25H, CH$_2$N + CH$_3$P + CH$_2$NHCOC + CHN), 7.35 (d, 2H), 8.32 (d, 2H).

The following compounds were prepared in a similar manner:

We thank SERC and MRC for support.