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Synthesis of bifunctional chelating agents based on mono and diphosphonic derivatives of diethylenetriaminepentaacetic acid

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ABSTRACT

Bifunctional chelating agents (BFCAs) are small molecules containing a chelating unit, able to strongly coordinate a metal ion, and a reactive functional group, devised to form a stable covalent bond with another molecule. BFCAs are widely employed since their conjugation to a suitable biomolecule (e.g., a peptide or an antibody) allows the synthesis of diagnostic or therapeutic agents that specifically target diseased tissue with metals or radiometals. For this reason, BFCAs find application in diagnostic imaging, molecular imaging, and radiotherapy of cancer. The synthesis of new BFCAs based on a diethylenetriaminepentaacetic acid (DTPA) structure in which one or two carboxylic groups are replaced with phosphonic units is described. The phosphonic group, aside from being a classical isostere of the carboxylic acid in coordination chemistry, allows to modulate the physico-chemical properties of the ligands and of the corresponding complexes.

1. Introduction

The use of metal or radiometal complexes in medicine as therapeutic or diagnostic agents is an area of growing interest.[1–3] For diagnostic purposes, gadolinium complexes are employed as contrast agents in magnetic resonance imaging (MRI)[4–6] while complexes of ^{111}In or ^{68}Ga, for example, find application in single photon emission computed tomography (SPECT) or positron emission tomography (PET), respectively.[7–10] For therapy, and in particular in the treatment of some kind of tumors and metastases, complexes of β or α emitters, such as ^{90}Y, ^{177}Lu or ^{225}Ac, are usually studied and administered to patients.[7–12]

All these metal ions need to be strongly coordinated with a suitable ligand to guarantee a safe administration in vivo and prevent an undesirable deposition of toxic metal ions in tissues or organs different from the desired target. Polyaminopolycarboxylic derivatives, such as diethylenetriaminepentaacetic acid **1a** (DTPA) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid **2** (DOTA) (Fig. 1), are the ligands of choice and are widely employed because they provide very stable complexes with a wide variety of metal ions.[13]

The metal complex is usually covalently bound to a targeting vector (e.g., peptides[14–16] or monoclonal antibodies[17,18]) that allows a specific delivery to particular type of cells by a recognition process. The easiest way of linking a vector to a metal complex is by direct conjugation to a bifunctional chelating agent (BFCA) followed by deprotection of eventual protective groups and final complexation of the metal ion of choice.[19–21]

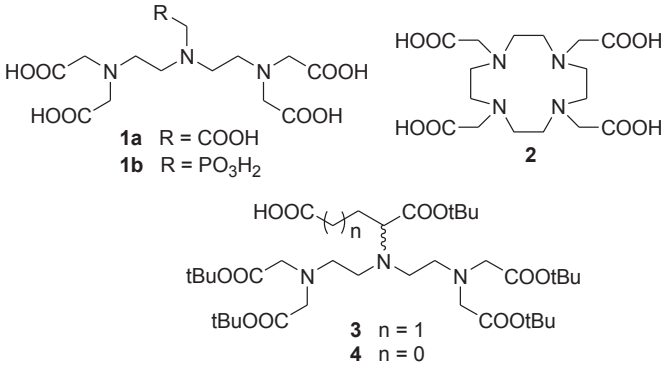

Fig. 1. Common chelating agents and bifunctional derivatives.

Among the huge number of polyaminopolycarboxylic bifunctional chelating agents reported in literature,[22] BFCA **3**[23] and **4**[24] (Fig. 1) found a broad spectrum of applications. For example, BFCA **3** has been conjugated to bile acids,[25,26] oxytocin,[27]

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aminoacids,[28] CCK8 peptides,[29] RGD (Arg-Gly-Asp) peptidomimetics,[30,31] while BFCA **4** has been exploited in the synthesis of a potential MRI contrast agent,[32] linked to a tamoxifen derivative,[33] and conjugated to a cyclic peptide for fibrin targeting.[34]

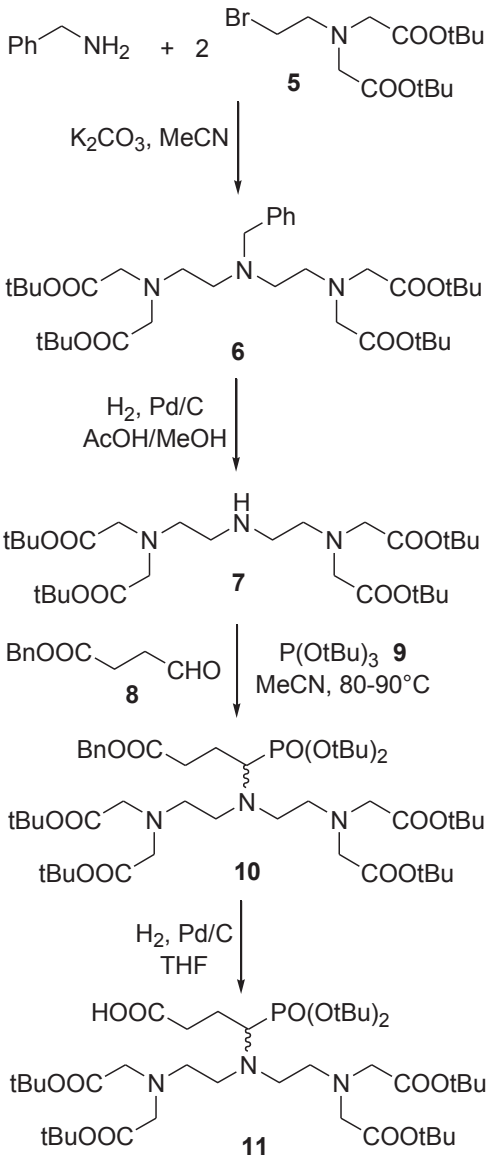
Efforts directed towards improvements of MRI contrast agents rely on different strategies involving structural variations of the ligand; among them the substitution of carboxylic groups with different acidic functionalities was extensively investigated, leading to the preparation of several derivatives, embodying more or less classical isosteres, such as phosphonic acid,[35,36] phosphinic acid,[37] acylsulphonamide,[38] and tetrazole.[39]

The phosphonic acid is particularly appealing as its size and acidic properties closely resemble the behavior of carboxylic groups.[40] Its tetrahedral deprotonated form ($-PO_3^{2-}$) occurring at physiological pH values offers multiple coordination modes, and residual anionic charges after complexation provide a beneficial effect on the solubility of the corresponding chelate, due to the establishment of an extended hydrogen bond network leading to improved solvation. Several examples of polydentate ligands embodying one[36] or more[41,42] phosphonic groups were reported for different purposes ranging from water corrosion inhibitors to diagnostic and therapeutic applications.

Phosphonic derivatives of the octadentate ligand DTPA (diethylenetriaminepentaacetic acid) are already known: the perphosphonated derivative (DTPMP)[43] is commercially available (Dequest® 2060), while the monophosphonic DTPA-analog **1b**[44] is reported to form thermodynamically very stable metal complexes, with stability constants comparable with the parent DTPA ligand.[45]

Despite the growing interest in phosphonic derivatives of polyaminocarboxylic ligands, it is surprising to observe only a few examples of bifunctional derivatives. A perphosphonic derivative (DOTP) of the macrocyclic ligand DOTA was decorated with different appendages, among them a *p*-aminophenyl residue amenable to use for conjugation purposes.[46] Moreover, two different cyclohexane-1,2-diamine tetraphosphonic ligands bearing ready-to-use isothiocyanate groups were designed for application in ^{153}Sm-based radioimmunotherapy.[47] To the best of our knowledge, the only BFCA with carboxylic and phosphonic moieties reported so far, is a triethylenetetraaminehexaacetic acid (TTHA) analog.[48]

We describe here the synthesis of two novel BFCA based on the DTPA structure but having one (Scheme 1, compound **11**) or two (Scheme 2, compound **18**) phosphonic group replacing the corresponding carboxylic moieties.



Scheme 1. Synthesis of BFCA **11**.

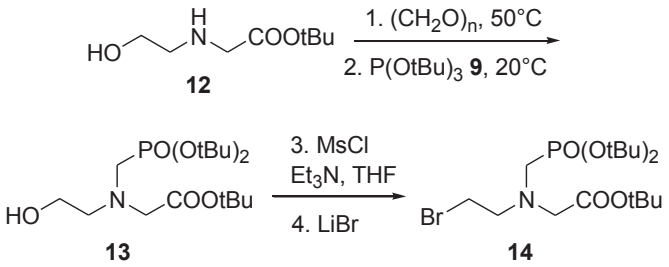
2. Results and discussion

The synthesis of the phosphonic derivatives **3** and **4** was realized adapting the original protocol of Rapoport,[49] widely applied for the easy assembly of DTPA derivatives and implying the double N-alkylation of a primary amine (representing the 'central' nitrogen atom of DTPA) with two molar equivalents of an *N*-(β-bromoethyl) iminodiacetate ester (representing the 'left' and 'right' wings of the DTPA).

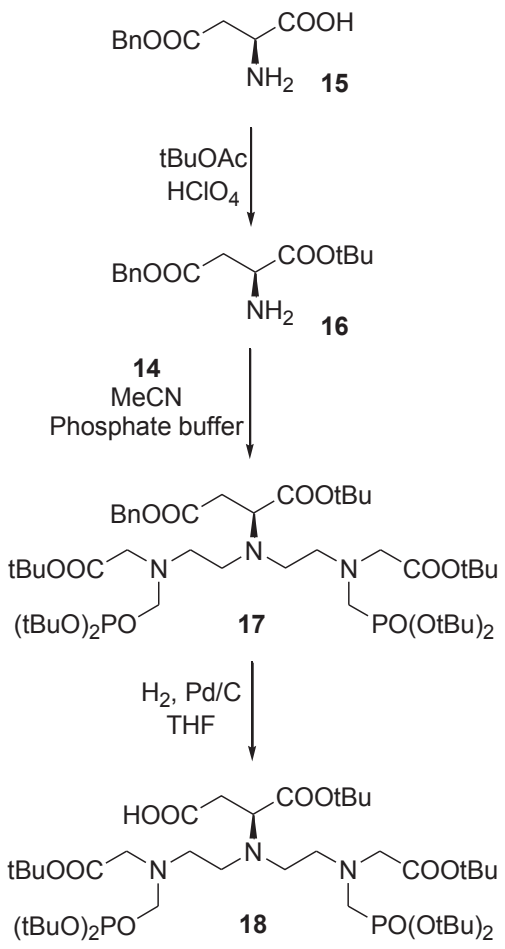
The synthesis of monophosphonic BFCA **11** is shown in Scheme 1. Benzylamine was bisalkylated with bromoderivative **5**[49] in acetonitrile and in the presence of micronized K_2CO_3 as base. Compound **6** was then hydrogenated to give in good yield the symmetrical diethylenetriaminetetraacetic acid tetra-*t*-butyl ester **7**,[50] with the central secondary amine available for further functionalization. Reaction of **7** with the aldehyde–ester **8**[51] and tri-*t*-butylphosphite **9**[52] in refluxing acetonitrile produced the monophosphonic derivative **10**. Selective deprotection of the carboxylic ester located on the side chain by hydrogenolysis afforded the protected BFCA **11**.

The synthesis of diphosphonic BFCA **18** relied on a similar strategy, necessarily implying the modification of the *N*-(β-bromoethyl)iminodiacetate ester alkylating agent to include a (protected) phosphonate group. The novel bromoderivative **14** (Scheme 2) was synthesized by phosphonomethylation of *N*-(2-hydroxyethyl)glycine 1,1-dimethylethyl ester **12**[53] with paraformaldehyde and tri-*t*-butylphosphite **9**[52] to give the aminoalcohol **13**. Mesylation of the primary alcohol with the combination methanesulfonyl chloride/triethylamine in THF and a prompt treatment with lithium bromide generated the desired 'mixed' alkylating agent **14**.

The assembly of the BFCA continues with the bis-alkylation of the nitrogen atom of a suitable protected aminoacid. Esterification of aspartic acid 4-phenylmethyl ester **15** into its *t*-butyl ester derivative **16** was easily performed in *t*-BuOAc with perchloric acid, as reported by Taschner.[54] Treatment of **16** with two molar equivalents of the bromoderivative **14** in a buffered system gave compound **17**. As for the previous example, selective hydrogenation of the benzyl ester located on the side chain released the corresponding free carboxylic acid completing the synthesis of the diphosphonic BFCA **18** (Scheme 3).



Scheme 2. Synthesis of bromoderivative **14**.



Scheme 3. Synthesis of BFCA **18**.

3. Conclusions

The preparation of two original bifunctional chelating agents formally based on mono and diphosphonic derivatives of diethylenetriaminepentaacetic acid, was designed and realized. For the first time, DTPA ligands with a mixed combination of carboxylic and phosphonic coordinating group are endowed with an additional remote carboxylic group, devised for direct conjugation to biomolecules or to be coupled to terminal amino groups or lysine ε-amine during solid phase synthesis.[55] The availability of a multigram synthesis of these BFCA will pave the way to the preparation of several conjugated derivative of the corresponding metal complexes, exploiting the potential of these mixed ligands in their diagnostic and therapeutic applications.

4. Experimental section

4.1. General

Reagent-grade chemicals and solvents were obtained from commercial sources and directly used without further purification. *N*-(2-Bromoethyl)-*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl] glycine 1,1-dimethylethyl ester **5**,[49] 4-oxobutanoic acid phenylmethyl ester **8**,[51] tri-*t*-butylphosphite **9**,[52] and *N*-(2-hydroxyethyl) glycine 1,1-dimethylethyl ester **12**[53] were synthesized as reported in literature. TLC was performed on Merck silica gel 60 TLC plates F_{254} and visualized by using UV or 1% $KMnO_4$ in 1 M NaOH. Column chromatography was performed by using silica gel 60 (70–230 mesh) while flash chromatography was carried out on silica gel 60 (230–400 mesh). The ^1H, ^{13}C, and ^{31}P spectra were recorded on a Bruker Avance 400 instrument and using $CDCl_3$ as solvent. Mass spectra were recorded with a ThermoFinnigan TSQ700 triple-quadrupole instrument equipped with an electrospray ionization source. Analytical HPLC was performed on a Merck KGaA apparatus with the following method: stationary phase: Lichrosorb RP-Select B 5 μm, 250×4 mm column packed by Merck KGaA; mobile phase: eluent A=0.01 M KH_2PO_4 and 0.017 M H_3PO_4 in H_2O, eluent B=MeCN, gradient elution: t=0 min (5% B), t=45 min (80% B); T=45 °C; flow rate: 1 mL min^{-1}; UV detection: 210 nm.

4.2. *N,N′*-[[(Phenylmethyl)imino]di-2,1-ethanediyl]bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine bis(1,1-dimethylethyl) ester (6)

Micronized K_2CO_3 (13.82 g; 100 mmol) was added to a solution of benzylamine (5.46 mL; 50 mmol) and *N*-(2-bromoethyl)-*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine 1,1-dimethylethyl ester **5**[49] (37.2 g; 100 mmol) in MeCN (100 mL). The mixture was stirred at room temperature and in a nitrogen atmosphere. After 72 h the mixture was filtered and the solvent evaporated. The residue was dissolved in CH_2Cl_2 (150 mL) and the solution was washed with water (3×100 mL). The organic phase was dried (Na_2SO_4) and evaporated. The residue was purified by column chromatography (EtOAc/petroleum ether, 1:1) to give **6** (28.0 g; 86%) as a yellow oil. Found: C, 64.4; H, 9.1; N, 6.4. $C_{35}H_{59}N_3O_8$ requires C, 64.69; H, 9.15; N, 6.47%; HPLC R_t=28 min, 97% (area %); ^1H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.32–7.20 (5H, m, Ph), 3.63 (2H, s, CH_2Ph), 3.41 (8H, s, CH_2COO^tBu), 2.86–2.82 (4H, m, NCH_2), 2.64–2.60 (4H, m, NCH_2), 1.46 (36H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 171.1 (CO), 140.0 (C), 129.2 (CH), 128.5 (CH), 127.2 (CH), 81.1 (C), 59.5 (CH_2), 56.5 (CH_2), 53.2 (CH_2), 52.4 (CH_2), 28.6 (CH_3); ν_{max} (KBr): 3086, 3063, 2978, 2933, 1741, 1603, 1455, 1393, 1255, 1219, 1152, 738, 700; ESI/MS m/z calcd for: $[C_{35}H_{59}N_3O_8+H]^+$ 650.87; found: 650.65.

4.3. *N,N′*-(Iminodi-2,1-ethanediyl)bis[*N*-[2-(1,1-dimethylethoxy)-2-oxoethyl]glycine bis(1,1-dimethylethyl) ester (7)

5% Palladium on charcoal (2.85 g) was added to a solution of **6** (28.49 g; 43.8 mmol) in AcOH (10 mL; 175.4 mmol) and MeOH (1250 mL) and the mixture was stirred under hydrogen atmosphere at room temperature. After 2.5 h the catalyst was filtered and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (150 mL) and the solution was washed with saturated solution of $NaHCO_3$ (3×100 mL) then with water (2×100 mL). The organic phase was dried (Na_2SO_4) and evaporated to give **7** (23.48 g; 96%) as a yellow oil. Found: C, 59.8; H, 9.4; N, 7.4. $C_{28}H_{53}N_3O_8$ requires C, 60.08; H, 9.54; N, 7.51; R_f=0.24 (MeOH/EtOAc, 1:4); ^1H NMR ($CDCl_3$, 400 MHz, 298 K): δ 3.35 (8H, s, CH_2COO^tBu), 2.79 (4H, t, J=6.3 Hz,

NCH_2), 2.61 (4H, t, J=6.3 Hz, NCH_2), 1.37 (36H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 171.2 (CO), 81.1 (C), 56.7 (CH_2), 54.7 (CH_2), 48.4 (CH_2), 28.4 (CH_3); ν_{max} (KBr): 3313, 2978, 2933, 1736, 1457, 1393, 1368, 1254, 1220, 1151; ESI/MS m/z calcd for: $[C_{28}H_{53}N_3O_8+H]^+$ 560.75; found: 560.63.

4.4. 9-[1-[Bis(1,1-dimethylethoxy)phosphinyl]-4-oxo-4-(phenyl methoxy)butyl]-6,12-bis[2-(1,1-dimethylethoxy)-2-oxoethyl]-2,2-dimethyl-4-oxo-3-oxa-6,9,12-triazatetradecan-14-oic acid, 1,1-dimethylethyl ester (10)

A solution of 4-oxobutanoic acid phenylmethyl ester **8** (7.7 g; 40 mmol)[51] in MeCN (50 mL) was added dropwise in 30 min to a solution of **7** (20.15 g; 36 mmol) in MeCN (200 mL) at 80 °C. The reaction mixture was stirred for further 30 min and then cooled to room temperature. A solution of tri-*t*-butylphosphite **9** (14.62 g; 40 mmol) was added dropwise over 20 min, then the mixture was stirred at room temperature for 4 days. After evaporation of the solvent, the residue was purified by flash chromatography (EtOAc/petroleum ether, 3:7→1:1) to give **10** (12.08 g, 36%) as a yellow oil. Found: C, 60.7; H, 8.9; N, 4.4; P, 3.1. $C_{47}H_{82}N_3O_{13}P$ requires C, 60.82; H, 8.91; N, 4.53; P, 3.34; R_f=0.45 (EtOAc/*n*-hexane, 1:1); HPLC R_t=34.5 min, 98% (area %); ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.35–7.28 (5H, m, Ph), 5.10 (2H, s, CH_2Ph), 3.39 (8H, s, CH_2COO^{t}Bu), 2.94–2.86 (5H, m, NCH_2 and CH), 2.74–2.69 (4H, m, NCH_2), 2.56 (2H, t, J=7.2 Hz, CH_2COOBn), 1.95 (1H, m, CH_2), 1.79 (1H, m, CH_2), 1.49 (18H, s, PO^{t}Bu), 1.42 (36H, s, COO^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 174.0 (CO), 170.9 (CO), 136.6 (C), 128.8 (CH), 128.6 (CH), 128.4 (CH), 82.9 (d, J_{CP}=12.1 Hz, C), 82.2 (d, J_{CP}=9.3 Hz, C), 81.0 (C), 66.3 (CH_2), 60.8 (d, J_{CP}=138.8 Hz, CH), 56.0 (CH_2), 54.2 (CH_2), 50.6 (CH_2), 31.3 (d, J_{CP}=12.4 Hz, CH_3), 31.0 (d, J_{CP}=3.4 Hz, C), 30.9 (d, J_{CP}=3.3 Hz, CH_3), 28.6 (CH_3), 24.3 (d, J_{CP}=6.5 Hz, CH_2); ^{31}P NMR ($CDCl_3$, 162 MHz, 298 K): δ 19.9 (s); ν_{max} (KBr): 3066, 2979, 2934, 2874, 1738, 1457, 1393, 1369, 1257, 1155, 978, 752, 698; ESI/MS m/z calcd for: $[C_{47}H_{82}N_3O_{13}P+Na]^+$ 951.14; found: 951.25.

4.5. 9-[1-[Bis(1,1-dimethylethoxy)phosphinyl]-3-carboxypropyl]-6,12-bis[2-(1,1-dimethyl ethoxy)-2-oxoethyl]-2,2-dimethyl-4-oxo-3-oxa-6,9,12-triazatetradecan-14-oic acid, 14-(1,1-dimethylethyl) ester (11)

5% Palladium on charcoal (3.6 g) was added to a solution of **10** (12 g; 13 mmol) in THF (75 mL) and the mixture was stirred under hydrogen atmosphere at room temperature. After 18 h the catalyst was filtered and the solution was evaporated to give **11** (10.2 g; 94%) as a viscous yellow oil. Found: C, 57.4; H, 9.1; N, 5.1; P, 3.7. $C_{40}H_{76}N_3O_{13}P$ requires C, 57.33; H, 9.14; N, 5.01; P, 3.70; R_f=0.41 (EtOAc/*n*-hexane, 4:1); ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 3.42 (8H, s, CH_2COO^{t}Bu), 3.27 (1H, bt, CH), 3.09–3.00 (4H, m, NCH_2), 2.92–2.83 (4H, m, NCH_2), 2.72–2.65 (2H, m, CH_2COOH), 1.95 (1H, m, CH_2), 1.85 (1H, m, CH_2), 1.51 (9H, s, PO^{t}Bu), 1.50 (9H, s, PO^{t}Bu), 1.44 (36H, s, COO^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 176.2 (CO), 170.8 (CO), 83.8 (d, J_{CP}=12.1 Hz, C), 83.1 (d, J_{CP}=9.0 Hz, C), 81.4 (C), 61.5 (d, J_{CP}=141.8 Hz, CH), 55.6 (CH_2), 52.8 (CH_2), 50.3 (CH_2), 34.2 (d, J_{CP}=14.1 Hz, CH_2), 31.0 (d, J_{CP}=4.0 Hz, CH_3), 30.9 (d, J_{CP}=3.0 Hz, CH_3), 28.5 (CH_3), 24.0 (d, J_{CP}=2.0 Hz, CH_2); ^{31}P NMR ($CDCl_3$, 162.0 MHz, 298 K): δ 17.4 (s); ν_{max} (KBr): 3441 (br), 2978, 2934, 1732, 1457, 1394, 1369, 1256, 1220, 1151, 981; ESI/MS m/z calcd for: $[C_{40}H_{76}N_3O_{13}P-H]^-$ 836.50; found: 836.37.

4.6. *N*-[[Bis(1,1-dimethylethoxy)phosphynyl]methyl]-*N*-(2-hydroxyethyl)glycine 1,1-dimethylethyl ester (13)

A mixture of paraformaldehyde (1.71 g; 57.07 mmol) and *N*-(2-hydroxyethyl)glycine 1,1-dimethylethyl ester **12**[52] (10 g; 57.07 mmol) was stirred at 50 °C. After 16 h CH_2Cl_2 (150 mL) was added, the solution was dried (Na_2SO_4) and the solvent was removed under reduced pressure. Tri-*t*-butylphosphite **9**[51] (14.28 g; 57.07 mmol) was added to the residue and the mixture was stirred overnight at room temperature, then evaporated in vacuo. The residue was purified by flash chromatography (Et_2O/CH_2Cl_2, 45:55) to give **13** (5.21 g; 24%) as a pale yellow oil. R_f=0.19 (Et_2O/CH_2Cl_2, 45:55); ^{1}H NMR ($CDCl_3$, 300.0 MHz, 298 K): δ 3.52 (2H, t, J=4.9 Hz, CH_2OH), 3.47 (2H, s, CH_2COO^{t}Bu), 2.95 (2H, d, J=8.3 Hz, CH_2P), 2.84 (2H, t, J=4.9 Hz, NCH_2), 1.45 (18H, s, PO^{t}Bu), 1.41 (9H, s, COO^{t}Bu); ^{13}C NMR ($CDCl_3$, 75.4 MHz, 298 K): δ 171.1 (CO), 82.7 (d, J_{CP}=9.2 Hz, C), 81.3 (C), 59.9 (CH_2), 58.8 (d, J_{CP}=9.2 Hz, CH_2), 57.2 (d, J_{CP}=3.4 Hz, CH_2), 52.3 (d, J_{CP}=165.7, CH_2), 30.5 (CH_3), 28.2 (CH_3); ^{31}P NMR ($CDCl_3$, 121.4 MHz, 298 K): δ 19.4 (s); ν_{max} (KBr): 3367 (br), 2973, 2931, 2877, 1732, 1668, 1457, 1387, 1368, 1232, 1155, 1061; ESI/MS m/z calcd for: $[C_{17}H_{36}NO_6P+Na]^+$: 404.21; found: 404.30.

4.7. *N*-[[Bis(1,1-dimethylethoxy)phosphynyl]methyl]-*N*-(2-bromoethyl)glycine 1,1-dimethylethyl ester (14)

Under a nitrogen atmosphere, methanesulfonyl chloride (2.8 mL; 36 mmol) was slowly added to a solution of compound **13** (12.72 g; 33.3 mmol) and triethylamine (6.5 mL; 46.6 mmol) in dried THF (500 mL) cooled at −15 °C. After 1.5 h lithium bromide (25.0 g; 288 mmol) was added and the slurry was vigorously stirred for 16 h allowing the temperature to rise spontaneously to 20 °C. The solvent was evaporated and EtOAc (300 mL), Et_2O (300 mL), and water (200 mL) were added to the residue. The organic phase was separated, washed with water (200 mL), brine (2×100 mL), and dried (Na_2SO_4). The solvents were evaporated and the residue was purified by flash chromatography (2-PrOH/Et_2O/*n*-hexane, 0.01:1:1) to give **14** (12.35 g; 83%) as a colorless oil, solidifying upon storage at −18 °C. Mp 50–51 °C; R_f=0.35 (EtOAc/^{i}Pr$_2$O, 1:4); ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K): δ 3.55 (2H, s, CH_2COO^{t}Bu), 3.41 (2H, t, J=7.5 Hz, BrCH_2), 3.15 (2H, t, J=7.5 Hz, NCH_2), 3.03 (2H, d, J=9.6 Hz, CH_2P), 1.45 (18H, s, PO^{t}Bu), 1.43 (9H, s, COO^{t}Bu); ^{13}C NMR ($CDCl_3$, 75.4 MHz, 298 K): δ 171.1 (CO), 82.4 (d, J_{CP}=9.0 Hz, C), 81.3 (C), 58.1 (d, J_{CP}=15.1 Hz, CH_2), 56.7 (CH_2), 54.2 (CH_2), 52.0 (CH_2), 30.6 (d, J_{CP}=4.3 Hz, CH_3), 28.3 (CH_3); ^{31}P NMR ($CDCl_3$, 121.4 MHz, 298 K): δ 17.3 (s); ν_{max} (KBr): 2974, 2933, 1735, 1454, 1389, 1370, 1250, 1150, 994, 731; ESI/MS m/z calcd for: $[C_{17}H_{35}BrNO_5P+Na]^+$: 466.13; found: 466.00.

4.8. L-Aspartic acid 1-(1,1-dimethylethyl) 4-(phenylmethyl) ester (16)

A 70% aqueous solution of $HClO_4$ (0.930 mL; 10.75 mmol) was slowly added to a suspension of aspartic acid 4-phenylmethyl ester **15** (2.0 g; 8.95 mmol) in *t*-butyl acetate (50 mL). The mixture was then stirred for 36 h at room temperature. The reaction mixture was diluted with H_2O (40 mL) and after separation the aqueous phase was extracted with EtOAc (3×15 mL). The combined organic phases were washed with 10% aq Na_2CO_3 (50 mL) and H_2O (50 mL), then dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give **16** (1.4 g; 56%) as a colorless oil. R_f=0.52 (EtOAc); HPLC R_t=14.4 min, 97% (area %); ^{1}H NMR ($CDCl_3$, 300 MHz, 298 K): δ 7.42–7.23 (5H, m, Ph), 5.12 (2H, br s, CH_2Ph), 3.73 (1H, dd, J_1=7.0 Hz, J_2=4.9 Hz, CH–N), 2.81 (1H, dd, J_1=16.5 Hz, J_2=4.9 Hz, CH_2), 2.73 (1H, dd, J_1=16.8 Hz, J_2=7.0 Hz, CH_2), 2.68 (2H, br s, NH_2),1.40 (9H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3$, 75.6 MHz, 298 K): δ 173.1 (C), 171.2 (C), 135.7 (C), 128.6 (CH), 128.4 (2×CH), 81.7 (C), 66.6 (CH_2), 51.8 (CH), 39.0 (CH_2), 28.0 (CH_3); $[\alpha]_D^{20}$ +16.0 (*c* 0.57, $CHCl_3$) (lit.[56] +10.9, *c* 0.57, $CHCl_3$); ν_{max} (KBr): 3350, 2974, 2942, 1728, 1736, 1667, 1368, 1360, 1250, 1151, 750, 736, 697; ESI/MS m/z calcd for: $[C_{15}H_{21}NO_4+H]^+$: 280.15; found: 280.05.

4.9. *N,N*-Bis[2-[[[2-(1,1-dimethylethoxy)-2-oxoethyl][bis(1,1-dimethylethoxy)phosphynyl]methyl]amino]ethyl]]-L-aspartic acid 1-(1,1-dimethylethyl) 4-(phenylmethyl) ester (17)

A mixture of **16** (0.947 g; 3.39 mmol) and **14** (3.32 g; 7.46 mmol) in MeCN (8 mL) and 2 M phosphate buffer (pH 8.0, 8.0 mL) was vigorously stirred at room temperature for 24 h.[49] The aqueous layer was separated and replaced with fresh buffer (8 mL) and the mixture was stirred for further 18 h. The organic phase was separated and the solvent was evaporated. The residue was dissolved in EtOAc (50 mL), washed with water (50 mL), brine (2×30 mL), and then dried (Na_2SO_4). The solvent was evaporated and the crude oil was purified by flash chromatography (2-PrOH/Et_2O/petroleum ether, 5:15:75) to give **17** (1.87 g; 55%) as a pale yellow oil. R_f=0.35 (2-PrOH/Et_2O/petroleum ether, 5:15:75); ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 7.36–7.30 (5H, m, Ph), 5.13 (1H, d, J=12.3 Hz, CH_2Ph), 5.07 (1H, d, J=12.3 Hz, CH_2Ph), 3.78 (1H, m, CH), 3.54 (4H, s, CH_2COO^{t}Bu), 2.98 (4H, d, J=9.8 Hz, CH_2P), 2.83 (1H, dd, J_1=15.9 Hz, J_2=8.5 Hz, CH_2COOBn), 2.75–2.67 (8H, m, NCH_2), 2.53 (1H, dd, J_1=15.9 Hz, J_2=6.6 Hz, CH_2COOBn), 1.49 (36H, s, PO^{t}Bu), 1.45 (18H, s, ^{t}Bu), 1.44 (9H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 171.6 (CO), 171.3 (CO), 171.1 (CO), 136.3 (C), 128.9 (CH), 128.7 (CH), 128.5 (CH), 82.4 (d, J_{CP}=10 Hz, C), 81.7 (C), 81.0 (C), 66.6 (CH_2), 61.6 (CH), 56.3 (CH_2), 53.1 (d, J_{CP}=166.0 Hz, CH_2), 51.7 (CH_2), 36.3 (CH_2), 30.9 (d, J_{CP}=3.0 Hz, CH_3), 28.7 (CH_3), 28.6 (CH_3); ^{31}P NMR ($CDCl_3$, 162 MHz, 298 K): δ 18.8 (s); ν_{max} (KBr): 2975, 2933, 2909, 2868, 1731, 1389, 1368, 1193, 1156, 1022, 951; ESI/MS m/z calcd for: $[C_{49}H_{89}N_3O_{14}P_2+Na]^+$: 1028.57; found: 1028.98.

4.10. *N,N*-Bis[2-[[[2-(1,1-dimethylethoxy)-2-oxoethyl][[bis(1,1-dimethylethoxy)phosphynyl]methyl]amino]ethyl]]-L-aspartic acid 1-(1,1-dimethylethyl) ester (18)

5% Palladium on charcoal (0.75 g) was added to a solution of **17** (1.87 g; 1.86 mmol) in THF (80 mL) and the mixture was stirred under a hydrogen atmosphere at room temperature. After 1.5 h the catalyst was filtered and the solution was evaporated to give **18** (1.57 g; 92%) as a yellowish oil. R_f=0.14 (2-PrOH/Et_2O/petroleum ether, 5:15:75); ^{1}H NMR ($CDCl_3$, 400 MHz, 298 K): δ 4.13 (1H, dd, J_1=11.5 Hz, J_2=4.2 Hz, CH), 3.55 (2H, d, J=17.7 Hz, CH_2COO^{t}Bu), 3.50 (2H, d, J=17.7 Hz, CH_2COO^{t}Bu), 3.10–2.93 (4H, m, CH_2P), 2.90–2.76 (9H, m, NCH_2 and CH_2COOH), 2.65 (1H, dd, J_1=15.8 Hz, J_2=4.1 Hz), 1.49 (36H, d, J=2.0 Hz, ^{t}Bu), 1.46 (9H, s, ^{t}Bu), 1.45 (18H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3$, 100.6 MHz, 298 K): δ 172.7 (CO), 171.8 (CO), 170.8 (CO), 83.0 (C), 82.2 (C), 81.3 (C), 61.4 (CH), 56.7 (d, J_{CP}=4.0 Hz, CH_2), 55.6 (d, J_{CP}=11.1 Hz, CH_2), 53.1 (d, J_{CP}=163.1 Hz, CH_2), 50.9 (CH_2), 35.8 (CH_2), 30.8 (CH_3), 28.7 (CH_3), 28.4 (CH_3); ^{31}P NMR ($CDCl_3$, 162 MHz, 298 K): δ 19.0; ν_{max} (KBr): 3432 (br), 2976, 2934, 1733, 1456, 1392, 1369, 1275, 1221, 1153, 951; ESI/MS m/z calcd for: $[C_{42}H_{83}N_3O_{14}P_2+H]^+$ 916.54; found: 916.65.

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