# Synthesis of a Bifunctional Monophosphinate DOTA Derivative Having a Free Carboxylate Group in the Phosphorus Side Chain

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*Received 25 October 2007; revised 4 February 2008*

**Abstract:** A new bifunctional cyclen-based ligand, 3-[hydroxy({4,7,10-tris[(*tert*-butoxycarbonyl)methyl]-1,4,7,10-tetraazacyclododecan-1-yl}methyl)phosphoryl]propanoic acid, was synthesized by alkylation of tri-*tert*-butyl 1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (*t*-$Bu_3$DO3A) by ethyl 3-{ethoxy[(mesyloxy)methyl]phosphoryl}propanoate [$MsOCH_2P(O)(OEt)CH_2CH_2CO_2Et$]. The ethyl carboxylate group in the side chain was selectively deprotected to obtain the esterified bifunctional ligand. More efficient syntheses of some phosphinopropanoic acid derivatives were devised and the phosphorus alkylation reagent was prepared starting from hypophosphorus acid or its salt.

**Key words:** macrocycles, MRI contrast agents, phosphinate ligands, phosphorus, radiopharmaceuticals

Complexes of macrocyclic ligands with metal ions have been investigated as contrast agents for magnetic resonance imaging (MRI)[1] or as radiopharmaceuticals.[2] The most common macrocyclic ligand, which is also utilized in clinical practice, is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA, **1**, Figure 1). To improve chemical and pharmacological properties of the complexes many DOTA derivatives have been synthesized.[1,3] For the next generation of MRI contrast agents and for most of radiopharmaceuticals, targeting a specific organ or tissue is desired. Thus, the ligands or complexes are conjugated to biovectors such as oligopeptides or monoclonal antibodies. For such applications, the useable ligand derivatives are called bifunctional and must contain another suitable functional group (e.g., isothiocyanate, carboxylate, or amino groups) to achieve the conjugation. In the case of DOTA, its skeleton can be modified on the macrocycle itself or on the pendant arms. Currently, the most common modification of DOTA is the formation of an amide bond on one pendant acetate arm.

Another possible modification of the DOTA skeleton is the replacement of the pendant acetate arm(s) by methylphosphonic/phosphinic acid group(s). We have synthesized a series of monophosphonic/phosphinic acid derivatives of DOTA bearing different substituents on the phosphorus atom (Figure 1) and showed that the ligands and their complexes show some improved properties as possible MRI contrast agents.[4–7] In addition, the thermodynamic and kinetic properties of the complexes are similar to those of the DOTA complexes.[8,9] As a bifunctional ligand, the 4-aminobenzyl[5] derivative was prepared and its isothiocyanate was conjugated to polyamidoamine (PAMAM) dendrimers to obtain macromolecular MRI contrast agents (as the $Gd^{3+}$ complexes).[10] However, such amino/isothiocyanate derivatives are not suitable for peptide synthesis (e.g., for conjugation to oligopeptides). Biodistribution data for the complexes ($^{90}Y$, $^{111}In$) of the monophosphinic acid ligands in rats are comparable with those of DOTA **1**.[11]

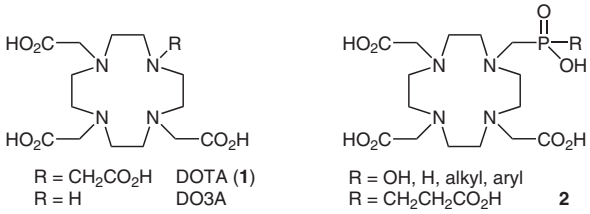


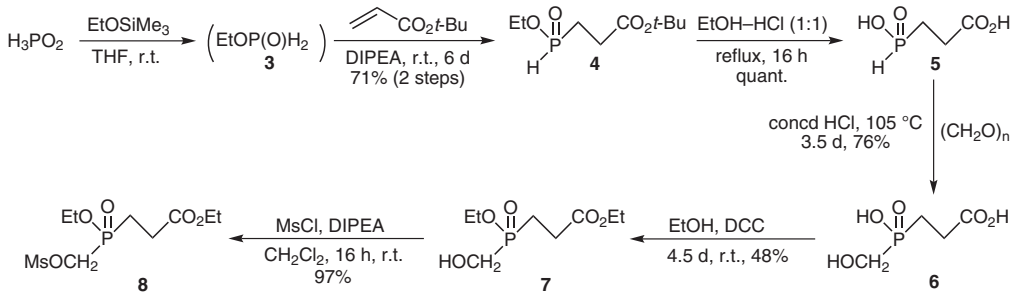

**Figure 1**

In this paper, we describe the synthesis of a DOTA monophosphinic acid derivative **12** which contains *tert*-butyl-protected azacycle carboxylates and a free carboxylate group in the phosphorus side chain that can be exploited for oligopeptide modifications. The fully deprotected ligand[9] **2** (Figure 1) was also prepared.

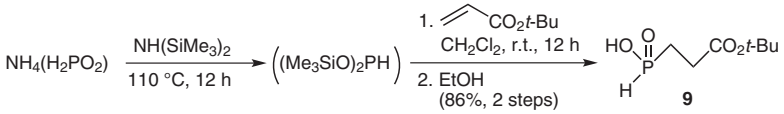
The most common method for introduction of the N–$CH_2$–P moiety is the Mannich reaction of a primary/secondary amine, formaldehyde, and an organophosphorus compound containing a P–H bond. If the reaction is performed under anhydrous conditions and with heating, the product mixture is generally difficult to separate and yields are variable, but low; the simplest (organo)phosphorus esters (e.g., dialkyl phosphites) are used for the reaction in organic solvents. The Mannich reaction in water at highly acidic pH (generally ~20% aq HCl) and with heating is more efficient. Unfortunately, these aqueous conditions are not compatible with the esters used as protecting groups of the acid functions. To obtain the target bifunctional ligand, we decided to use an alkylation approach. In the chemistry of azacycles, alkylation with organophosphorus reagents of the X–$CH_2$–P type (X = halogen, OMs, OTf) has not been used frequently.[12]

The phosphinic acid ester used for alkylation was prepared from hypophosphorus acid (Scheme 1). Initially, reaction of hypophosphorus acid with excess ethoxytrimethylsilane gave the known,[13] unstable ethyl

**SYNTHESIS** 2008, No. 9, pp 1431–1435
Advanced online publication: 27.03.2008
DOI: 10.1055/s-2008-1072571; Art ID: T16707SS




**Scheme 1**




**Scheme 2**

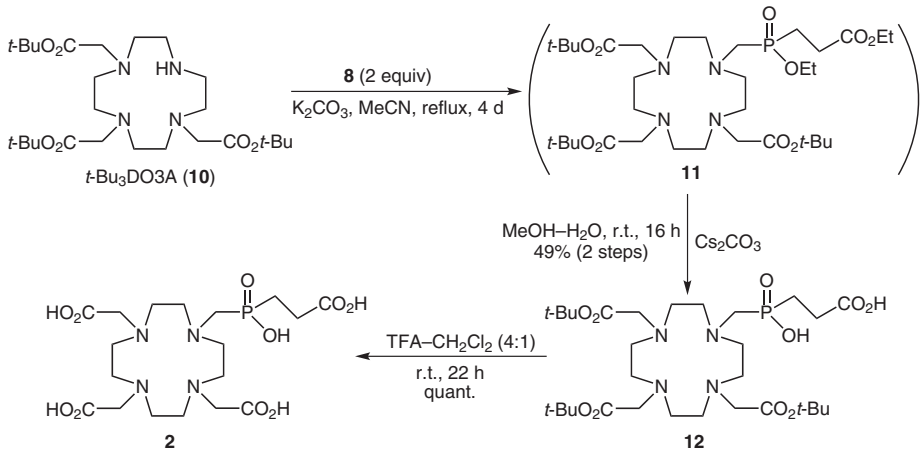
hypophosphinate (**3**). It was immediately used in nucleophilic addition to *tert*-butyl acrylate to give phosphinopropanoate **4**. Using a strong base (NaOEt)[14] accelerated the addition, but it also generated a much more complicated reaction mixture. *tert*-Butyl acrylate was used to facilitate aqueous treatment to remove the unreacted $H_3PO_2$ (the major phosphorus-containing impurity); the corresponding phosphinopropanoate diethyl ester is partially soluble in water. The ester **4** should be stored for extended periods at 4 °C; it slowly decomposes (over a number of weeks) at room temperature. After extractive treatment, the only byproduct detected was the bis(alkylated) product ($t$-$BuO_2CCH_2CH_2)_2P(O)(OEt)$ ($^{31}P$ NMR: $\delta = 55.1$, always <5%) arising from addition to both P–H bonds of **3** (despite using excess of the hypophosphinate ester). The bis(alkylated) byproduct was fully eliminated by silica gel chromatography.

In the recent years, the chemistry of the esters of hypophosphorus acid has been greatly developed by Montchamp et al.[15] For the production of $(RO)P(O)H_2$ esters, they used other silicon reagents, $(R^1O)_{4-n}SiR^2_n$ (n = 0–2; $R^1$, $R^2$ = alkyl, aryl), under analogous mild conditions.[16] Their silicon reagents gave the hypophosphorus acid esters similarly in almost quantitative yields, but the removal of excess organosilicon reagents and/or reaction mixture development can be problematic due to the formation of silica-like polymers. Our procedure is much easier to perform as excess monoalkoxytrimethylsilane and hydroxytrimethylsilane (the reaction product) is easily removed and we obtained a reasonable yield of ester **4**. In addition, preliminary results with other alkoxytrimethylsilanes ($ROSiMe_3$, R = alkyl) confirmed the generality of our reaction for the preparation of esters of hypophosphorus acid.

The reaction of ester **4** with paraformaldehyde under variable reaction conditions (various organic solvents, various bases/catalysts etc.) did not lead to a reasonable yield of the hydroxymethylated derivative and resulted in the formation of complicated reaction mixtures. Thus, we decided to test aqueous conditions for addition of the P–H bond to formaldehyde. The ester **4** was hydrolyzed to the known acid **5**[9,17] and this free acid was reacted with formaldehyde in aqueous hydrochloric acid to give hydroxymethyl acid **6**. Here, the phosphinopropanoic acid **5** was prepared by a much simple procedure than those previously published (see also below).[9,17] Again, the acid **6** is unstable at room temperature as it slowly forms the lactone and/or polymerizes with formation of hydroxymethylcarboxylate esters (giving multiple peaks in the $^{31}P$ NMR spectrum in a not fully reproducible way); the acid is obtained quantitatively after hydrolysis of such a mixture in refluxing ethanol–aqueous concentrated hydrochloric acid 1:1. Acid **6** was esterified in anhydrous ethanol in presence of *N*,*N*′-dicyclohexylcarbodiimide to give diethyl ester **7**. Mesylation with mesyl chloride gave the target organophosphorus reagent **8**. The mesyl derivative **8** should be used directly in the next step or can be stored below 4 °C. The multistep procedure was easily scaled up to a gram scale.

We realized that acid **5** has to be used in the synthetic sequence, hence we looked for an easier and faster procedure to give the pure intermediate **5**. Such a route (Scheme 2) can be based on the very easy addition of bis(trimethylsiloxy)phoshine[18] to an activated double bond. The siloxyphosphine was easily prepared from ammonium hypophosphite and its addition to *tert*-butyl acrylate (used again to facilitate the aqueous workup) led to a high yield of a pure monoester **9**.[18b] Using an excess of the very reactive siloxyphosphine completely eliminated the formation of any bis(alkylated) byproduct. The ester **9** was quantitatively hydrolyzed to acid **5**.

Synthesis of the macrocyclic derivatives **12** and **2** followed Scheme 3. The reaction of excess mesylate **8** with $t$-$Bu_3$DO3A **10** gave quantitative conversion of the macrocyclic reagent into intermediate **11**. The full ester **11** was not obtained in its pure form as it partially decomposed during chromatography (hydrolysis of the ethyl phoshinate ester bond) and, thus, the crude intermediate




**Scheme 3**

**11** was directly used in the next step. Removal of ethyl carboxylate ester together with very the labile ethyl phosphinate ester group was carried out with cesium carbonate according to a known procedure.[19] The protected bifunctional ligand **12** was obtained after simple chromatography. The ethyl phosphinate group in **11** is rather labile, however, unprotected phosphinates/phosphonates can be used in, e.g., peptide synthesis.[20] To test the stability of the protected ligand during a possible deprotection reaction, the acid **2** was prepared from ester **12**. Deprotection with trifluoroacetic acid was smooth giving a quantitative yield of **2**. The physical data of **2** prepared here were identical to those of ligand **2** previously prepared by the aqueous Mannich reaction between DO3A, formaldehyde, and acid **5**.[9] The multistep synthesis of ligand **2** presented here is not so inconvenient as it may appear, as the previously published procedure includes a tedious, time-consuming purification of ligand **2**.[9]

In conclusion, we describe a procedure for the preparation of a protected bifunctional DOTA derivative that is potentially useful in peptide syntheses. The hydrophilic phosphinic acid group should conveniently change physicochemical and pharmacological properties of possible conjugates similarly to the other free monophosphinate DOTA analogues, their conjugates and complexes as we have shown before. The syntheses of some organophosphorus intermediates were simplified and scaled up.

Paraformaldehyde was obtained by filtration of aged formaldehyde solns and dried over $P_2O_5$. *t*-$Bu_3$DO3A·HBr, **10**·HBr, was synthesized according to the published procedure.[21] Other chemicals were available from commercial sources. Crystalline $H_3PO_2$ was obtained by a careful vacuum evaporation (<30 °C bath temp.) of a commercial 50% aq soln followed by drying in a vacuum desiccator over $P_2O_5$ at 5 °C. Solvents were dried by established procedures.[22] TLC was performed on silica gel sheets (Merck TLC aluminum sheets silica gel 60 F254) and column chromatography was performed on silica gel (60–230 mesh, Merck). Elemental analyses were arranged at the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic. NMR spectra were recorded on a Varian Unity Inova 400 at 399.95 MHz for $^1$H, 100.58 MHz for $^{13}$C and 161.9 MHz for $^{31}$P. Internal references for $^1$H NMR: $CDCl_3$: TMS (δ = 0.00); $D_2O$: *t*-BuOH (δ = 1.25); $^{13}$C NMR: $CDCl_3$: $CDCl_3$ (δ = 77.0); $D_2O$: *t*-BuOH (δ = 32.8). External reference for $^{31}$P NMR: 85% $H_3PO_4$ (δ = 0.0). LR-MS (ESI) spectra were recorded on a Bruker Esquire 3000 with an ion-trap detector in positive or negative modes; HRMS (FAB) spectra were measured in positive mode on a ZAB-EQ (VG Analytical) in thioglycerol–glycerol matrix.

***Safety note***: $(Me_3SiO)_2PH$ is pyrophoric.

**Ethyl Hypophosphinate (3)**

Dry crystalline $H_3PO_2$ (10.0 g, 152 mmol), anhyd THF (10 mL), and $EtOSiMe_3$ (50 mL, 320 mmol) were stirred at r.t. for 1 h to form **3** in ca. 75% yield [based on $^{31}$P NMR: δ = 15.8 (t, $^1J_{PH}$ = 571 Hz)]. The mixture was used in the next reaction step without purification due to the instability of **3**.

***tert*-Butyl 3-[Ethoxy(hydro)phosphoryl]propanoate (4)**

To the mixture containing **3** from the previous step, *tert*-butyl acrylate (15.9 g, 124 mmol) was added and the mixture was stirred at r.t. Then DIPEA (19.6 g, 152 mmol) was added dropwise to the mixture over 2 h. When the addition was complete, the mixture was stirred for a further 6 d. The mixture was diluted with $CH_2Cl_2$ (100 mL) and successively washed with $H_2O$ (200 mL), 1.5% aq HCl (200 mL), and sat. aq $NaHCO_3$ (200 mL). The organic phase was dried (anhyd $Na_2SO_4$) and evaporated under reduced pressure. After chromatography (silica gel, EtOAc), ester **4** was obtained as a colorless oil (16.7 g, 71% over 2 steps).

$^1$H NMR ($CDCl_3$): δ = 1.38 (t, $^3J_{HH}$ = 7.3 Hz, 3 H,$CH_2CH_3$), 1.46 [s, 9 H, $C(CH_3)_3$], 2.04 (dtd, $^2J_{HP}$ = 15.3 Hz, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 1.8 Hz, 2 H, $PCH_2$), 2.57 (m, 2 H, $CH_2CO_2$), 4.09 (m, 1 H, $CH_2CH_3$), 4.19 (m, 1 H, $CH_2CH_3$), 7.21 (dt, $^1J_{HP}$ = 545 Hz, $^3J_{HH}$ = 1.8 Hz, 1 H, PH).

$^{13}$C NMR ($CDCl_3$): δ = 16.1 (d, $^3J_{CP}$ = 6.1 Hz, $CH_2CH_3$), 24.0 (d, $^1J_{CP}$ = 96.1 Hz, $PCH_2$), 27.0 (d, $^2J_{CP}$ = 3.1 Hz, $CH_2CO_2$), 27.9 [s, $C(CH_3)_3$], 62.4 (d, $^2J_{CP}$ = 6.5 Hz, $CH_2CH_3$), 81.2 [s, $C(CH_3)_3$], 171.0 (d, $^3J_{CP}$ = 13.0 Hz, $CO_2$).

$^{31}$P NMR ($CDCl_3$): δ = 37.4 (dm, $^1J_{PH}$ = 545 Hz).

**3-[Hydro(hydroxy)phosphoryl]propanoic Acid (5)**

Ester **4** (16.7 g, 75 mmol), EtOH (150 mL), and concd aq HCl (150 mL) were refluxed for 16 h. After evaporation and further co-evaporation with $H_2O$ under reduced pressure (3 ×), acid **5** was obtain as a colorless oil (10.4 g, 100%). If ester **4** was used without column chromatography, the product of this reaction, acid **5**, also contained a byproduct, the bis(derivative) $[HO_2CCH_2CH_2)_2PO_2H$ ($^{31}$P NMR: δ = 59.1, <5%).

$^1$H NMR ($D_2O$): δ = 2.07 (dtd, $^2J_{HP}$ = 15.3 Hz, $^3J_{HH}$ = 7.9 Hz, $^3J_{HH}$ = 1.8 Hz, 2 H, $PCH_2$), 2.68 (dt, $^3J_{HP}$ = 15.3 Hz, $^3J_{HH}$ = 7.9 Hz, 2 H, $CH_2CO_2$), 7.14 (dt, $^1J_{HP}$ = 557 Hz, $^3J_{HH}$ = 1.8 Hz, 1 H, PH).

$^{13}$C NMR ($D_2O$): δ = 30.4 (d, $^1J_{CP}$ = 91.6 Hz, $PCH_2$), 32.0 (s, $CH_2CO_2$), 182.6 (d, $^3J_{CP}$ = 13.0 Hz, $CO_2$).

$^{31}$P NMR ($D_2O$): δ = 38.2 (dquint, $^1J_{PH}$ = 557 Hz, $^2J_{PH}$ = $^3J_{PH}$ = 15.3 Hz).

MS (ESI): *m*/*z* = 138.9 $[M + H]^+$.

**3-[Hydroxy(hydroxymethyl)phosphoryl]propanoic Acid (6)**

Acid **5** (10.4 g, 75 mmol), $H_2O$ (160 mL), and concd aq HCl (80 mL) were refluxed 30 min. To the refluxing mixture, paraformaldehyde (7.2 g, 240 mmol) was added and mixture was stirred at 105 °C for 16 h in a bath. A further portion of paraformaldehyde (7.2 g, 240 mmol) was added and mixture was stirred at 105 °C for 3 d. After evaporation and further co-evaporation with $H_2O$ under reduced pressure (3 ×) and chromatography (Dowex 1×8, 200–400 mesh, $H_2O$ then 3% aq HCl) followed by further chromatography (silica gel, *i*-PrOH–concd aq $NH_3$, 3:2), the pure acid **6** was obtained as a colorless oil (9.6 g, 76%).

$^1$H NMR ($D_2O$): δ = 1.85 (m, 2 H, $PCH_2$), 2.44 (m, 2 H, $CH_2CO_2$), 3.63 (m, 2 H, $CH_2OH$), 7.22 (br s, 1 H, $CH_2OH$).

$^{13}$C NMR ($D_2O$): δ = 26.7 (d, $^1J_{CP}$ = 90.0 Hz, $PCH_2$), 32.0 (s, $CH_2CO_2$), 62.3 (d, $^1J_{CP}$ = 108.7 Hz, $CH_2OH$), 183.6 (d, $^3J_{CP}$ = 16.0 Hz, $CO_2$).

$^{31}$P NMR ($D_2O$): δ = 43.0 (m).

MS (ESI): *m*/*z* = 168.8 $[M + H]^+$.

**Ethyl 3-[Ethoxy(hydroxymethyl)phosphoryl]propanoate (7)**

To the soln of acid **6** (9.6 g, 57 mmol) in anhyd EtOH (250 mL) was added DCC (13.5 g, 65 mmol) and mixture was stirred at r.t. Further portions of DCC were added after 16 h (20 g, 97 mmol) and 3 d (20 g, 97 mmol). Finally, mixture was stirred at r.t. for 4 d. $H_2O$ (100 mL) was added and the mixture was filtered. The filtrate was evaporated under reduced pressure and chromatography (silica gel, $CH_2Cl_2$–MeOH, 96:4) gave the ester **7** (6.2 g, 48%) as a colorless oil.

$^1$H NMR ($CDCl_3$): δ = 1.27 (t, $^3J_{HH}$ = 7.2 Hz, 3 H, $CO_2CH_2CH_3$), 1.33 (t, $^3J_{HH}$ = 7.0 Hz, 3 H, $POCH_2CH_3$), 2.15 (m, 2 H, $PCH_2CH_2$), 2.67 (m, 2 H, $CH_2CH_2CO_2$), 3.89 (m, 2 H, $PCH_2OH$), 4.12 (m, 2 H, $POCH_2CH_3$), 4.16 (q, $^3J_{HH}$ = 7.2 Hz, 2 H, $CO_2CH_2CH_3$), 4.64 (br s, 1 H, $CH_2OH$).

$^{13}$C NMR ($CDCl_3$): δ = 14.1 (s, $CO_2CH_2CH_3$), 16.6 (d, $^3J_{CP}$ = 5.3 Hz, $POCH_2CH_3$), 21.0 (d, $^1J_{CP}$ = 90.7 Hz, $PCH_2CH_2$), 26.5 (d, $^2J_{CP}$ = 2.6 Hz, $CH_2CH_2CO_2$), 58.7 (d, $^1J_{CP}$ = 106.5 Hz, $PCH_2OH$), 61.1 (d, $^2J_{CP}$ = 7.6 Hz, $POCH_2CH_3$), 61.2 (s, $CO_2CH_2CH_3$), 172.5 (d, $^3J_{CP}$ = 13.8 Hz, $CO_2$).

$^{31}$P NMR ($CDCl_3$): δ = 52.7 (m).

MS (ESI): *m*/*z* = 246.9 $[M + Na]^+$.

**Ethyl 3-{Ethoxy[(mesyloxy)methyl]phosphoryl}propanoate (8)**

Ester **7** (6.2 g, 27 mmol) and DIPEA (4.2 g, 32.5 mmol) in anhyd $CH_2Cl_2$ (180 mL) were stirred at –5 °C. A soln of MsCl (3.4 g, 30 mmol) in anhyd $CH_2Cl_2$ (120 mL) was added dropwise over 1 h. When the addition was complete, the mixture was allowed to reach r.t. and stirred under argon for 16 h. The mixture was evaporated under reduced pressure and the solid residue was dissolved in $CH_2Cl_2$ (50 mL) and the soln was washed with 1.5% aq HCl (2 × 100 mL) and sat. aq $NaHCO_3$ (2 × 100 mL). The organic phase was dried (anhyd $Na_2SO_4$) and volatiles were evaporated under reduced pressure to give ester **8** (8.1 g, 97%) as a yellow oil.

$^1$H NMR ($CDCl_3$): δ = 1.28 (t, $^3J_{HH}$ = 7.2 Hz, 3 H, $CO_2CH_2CH_3$), 1.37 (t, $^3J_{HH}$ = 7.2 Hz, 3 H, $POCH_2CH_3$), 2.19 (m, 2 H, $PCH_2CH_2$), 2.68 (m, 2 H, $CH_2CH_2CO_2$), 3.14 (s, 3 H, $SO_3CH_3$), 4.17 (q, $^3J_{HH}$ = 7.2 Hz, 2 H, $CO_2CH_2CH_3$), 4.18 (m, 2 H, $PCH_2O$), 4.44 (m, 2 H, $POCH_2CH_3$).

$^{13}$C NMR ($CDCl_3$): δ = 14.1 (s, $CO_2CH_2CH_3$), 16.6 (d, $^3J_{CP}$ = 6.0 Hz, $POCH_2CH_3$), 21.7 (d, $^1J_{CP}$ = 99.6 Hz, $PCH_2CH_2$), 26.1 (d, $^2J_{CP}$ = 4.6 Hz, $CH_2CH_2CO_2$), 37.7 (s, $SO_3CH_3$), 61.1 (s, $CO_2CH_2CH_3$), 61.8 (d, $^2J_{CP}$ = 6.5 Hz, $POCH_2CH_3$), 62.2 (d, $^1J_{CP}$ = 104.5 Hz, $PCH_2O$), 171.8 (d, $^3J_{CP}$ = 14.1 Hz, $CO_2$).

$^{31}$P NMR ($CDCl_3$): δ = 44.9 (m).

MS (ESI): *m*/*z* = 302.9 $[M]^+$.

***tert*-Butyl 3-[Hydro(hydroxy)phosphoryl]propanoate (9)**

Under an argon atmosphere, dry $NH_4H_2PO_2$ (15.0 g, 178 mmol) was suspended in $(Me_3Si)_2NH$ (90 mL) and the mixture was heated at 110 °C (bath temperature) under a small flow of argon overnight. The mixture containing pure $(Me_3SiO)_2PH$ was cooled to r.t. and anhyd $CH_2Cl_2$ (150 mL) was added. The soln of *tert*-butyl acrylate (10.5 g, 82 mmol) in anhyd $CH_2Cl_2$ (60 mL) was added dropwise and the mixture was stirred at r.t. overnight. The resulting soln was added dropwise to EtOH (900 mL) to hydrolyze the silyl compounds. The volatiles were removed under reduced pressure and the crude product was dissolved in $CHCl_3$ (300 mL) and washed with 1% aq HCl (300 mL). The aqueous layer was re-extracted with $CHCl_3$ (2 × 300 mL) and the combined organic layers were dried (anhyd $Na_2SO_4$). The volatiles were removed under reduced pressure to give the pure ester **9** (13.5 g, 86%) as a colorless oil. The ester **9** was hydrolyzed under identical conditions as ester **4** to give acid **5** in a quantitative yield.

$^1$H NMR ($CDCl_3$): δ = 1.45 [s, 9 H, $C(CH_3)_3$], 2.02 (dt, $^3J_{HH}$ = 8.0 Hz, $^2J_{PH}$ = 16.0 Hz, 2 H, $CH_2P$), 2.55 (dt, $^3J_{PH}$ = 16.0 Hz, $^3J_{HH}$ = 8.0 Hz, 2 H, $CH_2CO_2$), 7.18 (d, $^1J_{PH}$ = 557.0 Hz, 1 H, PH).

$^{13}$C{$^1$H} NMR ($CDCl_3$): δ = 24.6 (d, $^1J_{PC}$ = 95.4 Hz, $CH_2P$), 27.5 (d, $^2J_{PC}$ = 84.7 Hz, $CH_2CO_2$), 28.0 [s, $C(CH_3)_3$)], 81.3 [s, $C(CH_3)_3$)], 171.1 (d, $^3J_{PC}$ = 13.4 Hz, $CO_2$).

$^{31}$P NMR ($CDCl_3$): δ = 35.5 (dquint, $^1J_{PH}$ = 557.0 Hz, $^2J_{PH}$ = $^3J_{PH}$ = 16.0 Hz).

**Tri-*tert*-butyl 10-({Ethoxy[2-(ethoxycarbonyl)ethyl]phosphoryl}methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate (11)**

Compound **8** (8.1 g, 27 mmol), anhyd MeCN (500 mL), macrocyclic ester **10**·HBr (7.8 g, 13.5 mmol) and $K_2CO_3$ (annealed, 22 g, 159 mmol) were refluxed for 4 d to form ester **11** in 100% yield (by $^{31}$P NMR: δ = 54.5). The mixture was filtered, the filtrate was evaporated under reduced pressure and used directly in the next reaction step without purification due to the instability of the ester **11**.

**3-[Hydroxy({4,7,10-tris[(*tert*-butoxycarbonyl)methyl]-1,4,7,10-tetraazacyclododecan-1-yl}methyl)phosphoryl]propanoic Acid (12)**

Above mixture containing compound **11**, MeOH (200 mL), $H_2O$ (100 mL), and $Cs_2CO_3$ (7.1 g, 22 mmol) was stirred at r.t. for 16 h. The mixture was evaporated under reduced pressure and purified by chromatography (silica gel, *i*-PrOH–MeOH–concd aq $NH_3$, 15:10:2) to obtain the acid **12** as a yellow oil (4.3 g, 49% over 2 steps based on **10**·HBr).

$^1$H NMR ($CDCl_3$): δ = 1.37 [s, 18 H, $C(CH_3)_3$], 1.39 [s, 9 H, $C(CH_3)_3$], 1.80–4.20 (m, 28 H, ring $CH_2$, $NCH_2CO_2$, $NCH_2PCH_2CH_2CO_2$).

$^{13}$C NMR ($CDCl_3$): δ = 25.1 (d, $^1J_{CP}$ = 88.1 Hz, $PCH_2CH_2$), 27.8 [s, $C(CH_3)_3$], 27.9 (s, 2 × $C(CH_3)_3$], 29.7 (d, $^2J_{CP}$ = 4.6 Hz, $PCH_2CH_2$), 48.8 (s, 2 × ring $CH_2$), 51.9 (s, 6 × ring $CH_2$), 52.9 (d, $^1J_{CP}$ = 103.8 Hz, $NCH_2P$), 55.5 (s, 2 × $NCH_2CO_2$), 55.6 (s, $NCH_2CO_2$), 81.5 [s, $C(CH_3)_3$], 81.8 [s, 2 × $C(CH_3)_3$], 172.1 (s, $CH_2CH_2CO_2$), 175.9 (s, 3 × $NCH_2CO_2$).

$^{31}$P NMR ($CDCl_3$): δ = 34.6 (m).

MS (ESI): *m/z* = 665.0 $[M]^+$.

HRMS (FAB): *m/z* $[M + H]^+$ calcd for $C_{30}H_{58}N_4O_{10}P$: 665.38906; found: 665.38957; *m/z* $[M + Na]^+$ calcd for $C_{30}H_{57}N_4NaO_{10}P$: 687.37100; found: 687.37061.

**10-{[Hydroxy(2-carboxyethyl)phosphoryl]methyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic Acid (2)**

Compound **12** (4.3 g, 6.5 mmol), anhyd $CH_2Cl_2$ (100 mL), and TFA (400 mL) were stirred at r.t. for 22 h. The mixture was evaporated and co-evaporated with $H_2O$ under reduced pressure (2 ×) to get the free ligand **2** as a slightly yellow oil (3.2 g, 100%).

NMR analysis ($D_2O$, 90 °C) was in full accordance with that of a previously prepared sample of the ligand.[9]

## Acknowledgment

We thank Bracco SpA (Italy) for a kind gift of cyclen and Dr. J. Cvačka (Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague) for HR-MS measurements). Support of the Grant Agency of the Czech Republic (No. 203/06/0467), Academy of Science of the Czech Republic (KAN 201110651) and Ministry of Education of the Czech Republic (MSM0021620857) is acknowledged. The work was done in the frame of COST D38 and EC-FP6 projects EMIL (LSHC-2004-503569) and DiMI (LSHB-2005-512146).

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