Synthesis of New Phosphonated Tripod Ligands as Putative New Therapeutic Agents in the Chelation Treatment of Metal Intoxications

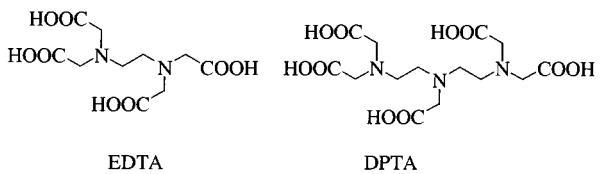
Ramon Burgada,*[a] Theodorine Bailly,[a] Marc Lecouvey,[a] Valery Larue,[a] and Yves Leroux[a]

Keywords: Tripodal ligands / Metal intoxication / P ligands / Chelation therapy / Actinides

In a quest for new chelation therapy drugs for the treatment of intoxication by actinides, some new phosphonated chelating tripod ligands have been synthesized. They have been obtained by means of a coupling reaction between a diethylphosphonoalkanoic acid activated with *N*,*N*′-disuccinimidyl carbonate (DSC) and a series of polyamines, with subsequent deprotection of the phosphonate groups.

Introduction

For many years, the quest for new therapeutic agents for the treatment of human metal intoxication has been the subject of numerous investigations.[1] Chelation therapy represents a promising means of combatting acute or chronic intoxication by divalent metal ions or other metal or metalloid compounds.[2] The chelating agent must be non-toxic and the entire complex must be easily removed from the body. Chelation therapy is the only known effective treatment[3] for accidental contamination with radionuclides such as plutonium and uranium. After entering the blood, these heavy metals are complexed by proteins such as transferrin or by small species such as citrate, carbonate, or phosphate. The major long-term effect of these heavy metals is the induction of cancer. On the other hand, in magnetic resonance imaging[4−6] or radiotherapy,[7,8] the chelated radionuclides are routinely used. For each application, the optimum choice for the chelator is complicated and is based on various physicochemical and chemical criteria[9,10] such as the denticity, stereocompatibility, and the stability constant, as well as on biological criteria such as toxicity, biodistribution, and solubility. Ethylenediamine tetraacetic acid (EDTA) and diethylenetriamine pentaacetic acid (DTPA), in the form of their Zn or Ca salts,[11] have been widely used as chelating agents in the treatment of intoxication by a number of heavy metals (Scheme 1). For example, DTPA is currently used to rid the body of the actinides plutonium and americium. However, it is ineffective in the case of uranium intoxication.



Scheme 1

With a view to increasing the chelating properties of these agents, some structural modifications have been described, such as the replacement of the carboxylic group by the more acidic phosphonic group[11,12] and the replacement of the linear polyamine chain by a cyclic polyamine moiety.[9] The search for new, more efficient, more specific, or more polyvalent chelating agents represents a considerable challenge and is of great interest with regard to human health. The purpose of the work described herein has been to investigate new tripodal structures of variable carbon chain length, having phosphonic acid, methylenebis(phosphonic acid), and hydroxymethylenebis(phosphonic acid) functions as terminal groups.

Results and Discussion

In the class of aminophosphonates, [bis(phosphomethylamino)methyl]phosphonic acid was the first tripod to be synthesized.[11] In 1971, Maier described the homologue [bis(phosphoethylamino)ethyl]phosphonic acid.[13] A tripodal phosphinate, namely tris[4-(phenylphosphinato)-3-methyl-2-azabutyl]amine, has previously been synthesized and used as a complexing agent for Al, Ga, and In.[14] More recently, Cristau and Virieux showed that the reaction of triethanolamine with excess diethyl vinylphosphonate followed by hydrolysis gives a new phosphonated tripod.[15]

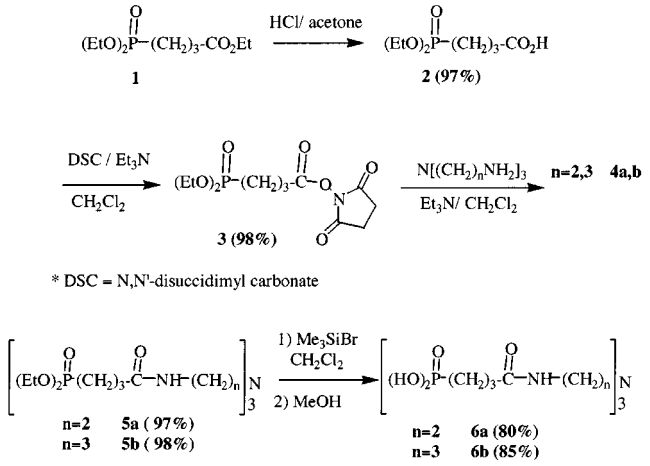
In this paper, we describe an efficient procedure that allows the synthesis of new phosphonated chelating agents. We present our first results relating to the phosphonate series (Scheme 2).

For the synthesis of these two tripods, the diethylphosphonocarboxylic ester **1** was used as the starting compound. Our synthesis commenced with a Michaelis−Arbusov reaction, in which ethyl 3-bromopropanoate was heated with triethyl phosphite to give the corresponding product in good yield.[16]

Selective hydrolysis of the carboxylic ester was then achieved using concentrated hydrochloric acid in acetone. After refluxing for 30 min, the carboxylic acid was obtained in 97% yield. No side reaction, such as phosphonate hydro-

[a] Laboratoire de Chimie Structurale et Spectroscopie Biomoléculaire, ESA CNRS 7031, Université Paris 13, 74, Rue Marcel Cachin, 93017 Bobigny Cedex, France
E-mail: r.burgada@smbh.univ-paris13.fr

  1434−193X/01/0102−0349 $ 17.50+.50/0



Scheme 2

lysis, was observed. The activation of diethylphosphonoalkanoic acids has been widely studied by Coutrot et al. in relation to the synthesis of phosphonopeptides. The BOP reagent was shown to be the best activator.[17] In the present case, however, *N*,*N*′-disuccinimidyl carbonate (DSC) was preferred as the activation agent. The diethylphosphonocarboxylic acid **2** was thus transformed into the active ester **3** by reaction with DSC in the presence of triethylamine at 20 °C. The desired product was obtained in quantitative yield in a very pure state and was characterized by NMR. The active ester **3** could be used immediately, but was found to be stable on storage for a week under nitrogen atmosphere.

The next step of our strategy was the coupling reaction. Treatment of the active ester **3** with polyamines **4a**,**b** in the presence of triethylamine at room temperature furnished compounds **5a**,**b** in yields of 97−98%. The desired phosphonated tripods **6a**,**b** were then obtained by dealkylation of the phosphonate groups using a large excess of trimethylsilyl bromide in dichloromethane followed by treatment with methanol.

Conclusion

In summary, the procedure described herein allows an efficient access to new tripod structures possessing phosphonic acid functions as terminal groups. Further studies on the scope and synthetic applications of this methodology are in progress.

Experimental Section

Ethanol (99.95%, analytical reagent, Prolabo), $CHCl_3$ (HPLC grade, Prolabo), CH_2Cl_2 (HPLC grade, Prolabo), and CH_3CN (HPLC grade, Prolabo) were distilled prior to use and were dried by means of 4 Å molecular sieves. Triethylamine was distilled from KOH and stored over 4 Å molecular sieves. Tris(3-aminopropyl)amine (**4b**) was synthesized as described by Chin et al.[18] Ethyl 4-(diethoxyphosphoryl)butyrate (**1**) was prepared as described previously.[16] − All NMR experiments were performed with a Varian Unity INOVA spectrometer (11.7 T) operating at a proton frequency of 500 MHz. Temperatures were calibrated with an ethylene glycol test sample. Proton spectra were referenced to external ([2,2,3,3-D_4])-3-trimethylsilylpropionic acid, sodium salt (TMSP). ^{13}C chemical shifts were calibrated using external iodomethane as a reference (δ = −22). ^{31}P NMR spectra were referenced to external phosphoric acid.

1D Experiments: 1D experiments were performed at 20 °C in HDO or $CDCl_3$. To suppress the water signal, its presaturation in the proton spectra was achieved with a delay of 1.5 s after relaxation. Over a spectral width of 6000 Hz, data were collected with a hard proton pulse of 8.2 μs, power 58 dB. − 1D ^{13}C spectra were recorded over a spectral width of 25000 Hz using a 90° non-selective carbon pulse of 31 μs, power 50 dB. During acquisition, proton decoupling (Waltz 16) was employed with a power of 35 dB and a duration of 0.4 s.

2D Experiments: In the 2D experiments, data were collected using the hypercomplex method. A recycle delay of 5 s was used to allow complete relaxation of all the proton and carbon magnetizations. − A COSY experiment in absolute value mode was performed with 512 t_1 increments of 8 transients each. The total experiment time was 1.3 h. The 90° non-selective proton pulses were of duration 8.2 μs at 58 dB and presaturation of the water signal was achieved with a power of 2 dB for 1.5 s before the first 90° non-selective proton pulse. Data were acquired with 2048 points. Zero-filling and sine-bell apodization were applied in both dimensions before Fourier transformation to generate a final 4 K*2 K matrix. − A $^{1}H,^{13}C$ gHSQC experiment[19] was performed on a ^{13}C natural abundance sample. Gradient pulses were applied in a ratio of 4:1 at a power level of 72 gauss/cm. The 90° non-selective proton pulses were of duration 8.2 μs at 58 dB, while the 90° non-selective carbon pulses lasted for 31 μs at 50 dB. The 1/4 $^{1}J_{CH}$ delay was set to a value of 1.8 ms, as optimized for a corresponding $^{1}J_{CH}$ of 140 Hz. GARP decoupling was applied during acquisition to increase the sensitivity. Spectral widths were 4000 Hz and 8000 Hz for the ^{1}H and ^{13}C nuclei, respectively. Zero-filling up to 2 K*1 K and squared sine-bell apodization were applied in both dimensions before Fourier transformation. A total of 256 experiments of 8 transients each were collected. The total experiment time was 1.5 h. − A $^{1}H,^{13}C$ gHMBC experiment[20] was performed on a ^{13}C natural abundance sample with gradient pulses in a ratio of 2:2:1. In the pulse sequence, the first delay of 1/2 $^{3}J_{CH}$ of 8 Hz was used to determine multiple bond connectivities (63 ms) and the second delay of 1/4 $^{1}J_{CH}$ was set to a value of 1.8 ms, as optimized for a corresponding $^{1}J_{CH}$ of 140 Hz. No decoupling was applied during acquisition. Spectral widths were 4000 Hz and 25000 Hz for the ^{1}H and ^{13}C nuclei, respectively. The 90° non-selective proton pulses were of duration 8.2 μs at 58 dB, while the 90° non-selective carbon pulses lasted for 31 μs at 50 dB. Zero-filling up to 2 K*4 K and squared sine-bell apodization were applied in both dimensions before Fourier transformation. A total of 512 experiments of 32 transients each were collected. The total experiment time was 3 h.

4-(Diethyloxyphosphoryl)butyric Acid (2): A solution of ethyl 4-(diethyloxyphosphoryl)butyrate (5.0 g, 19.8 mmol) in acetone (60 mL) was placed in a three-necked flask equipped with a magnetic stirrer, a dropping funnel, a thermometer, and a nitrogen inlet tube. H_2O (10 mL) followed by hydrochloric acid (10 mL) were quickly added to the stirred solution and the resulting mixture was stirred for 30 min under reflux. The volatiles were then evaporated in vacuo (10 Torr) to leave a colorless oil. Yield 4.3 g (97%). − $^{31}P\{^{1}H\}$ NMR ($CDCl_3$): δ = 33.2. − ^{1}H NMR ($CDCl_3$): δ = 1.21 (t, ^{3}J = 7.0 Hz, 6 H, 2 × CH_3CH_2OP), 1.80−1.93 (m, 4 H, CH_2CH_2P), 2.44 (t, ^{3}J = 6.6 Hz, 2 H, CH_2COOH), 4.00−4.15 (m, 4 H, 2 ×

CH_3CH_2OP), 10.10 (s, 1 H, COOH). – $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 16.3 (CH_3CH_2OP), 18.1 (CH_2CH_2P), 24.3 (d, $^1J_{C,P}$ = 141.5 Hz, CH_2CH_2P), 31.0 (CH_2COOH), 61.8 (CH_3CH_2OP), 171.2 (CH_2COOH).

Diethyl 4-[(2,5-Dioxopyrrolidin-1-yl)oxy]-4-oxobutylphosphonate (3): A solution of 4-(diethyloxyphosphoryl)butyric acid (**2**) (2.0 g, 8.9 mmol) in dry CH_3CN (20 mL) and triethylamine (3.5 g, 34.6 mmol) was placed in a three-necked flask equipped with a magnetic stirrer, a dropping funnel, a thermometer, and a nitrogen inlet tube. *N*,*N*′-Disuccinimidyl carbonate (3.0 g, 11.7 mmol) was quickly added to the stirred solution and the mixture was stirred for 12 h at room temperature. The solvent was then evaporated in vacuo. The residue was redissolved in $CHCl_3$ (50 mL), and the organic phase was washed with concentrated aq. $NaHCO_3$ solution (20 mL) and brine (20 mL), and dried with Na_2SO_4. The solvent was evaporated in vacuo and the residual pale-brown oil was used without purification. Yield 2.8 g (98%). – $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 31.4. – ^1H NMR ($CDCl_3$): δ = 1.30 (t, ^3J = 7.1 Hz, 6 H, 2 × CH_3CH_2OP), 1.79–1.88 (m, 2 H, CH_2CH_2P), 1.82–2.04 (m, 2 H, CH_2CH_2P), 2.73 (t, ^3J = 7.1 Hz, 2 H, CH_2CON), 2.80 (s, 4 H, $COCH_2CH_2CO$), 4.02–4.11 (m, 4 H, 2 × CH_3CH_2OP). – $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 16.4 (CH_3CH_2OP), 18.0 (CH_2CH_2P), 24.3 (d, $^1J_{C,P}$ = 141.8 Hz, CH_2CH_2P), 25.6 ($COCH_2CH_2CO$), 31.0 (CH_2COO), 61.7 (CH_3CH_2OP), 168.0 ($COCH_2CH_2CO$), 169.2 (CH_2CON).

General Procedure for the Coupling Reaction: A solution of compound **3** (8.6 mmol) in CH_2Cl_2 (25 mL) was placed in a three-necked flask (50 mL) equipped with a magnetic stirrer, a dropping funnel, a thermometer, and an argon inlet tube. A mixture of the appropriate polyamine **4a**,**b** (2.2 mmol) and triethylamine (8.6 mmol) was added dropwise, which led to a slightly exothermic reaction. After cooling and allowing the mixture to stand overnight, it was refluxed for 1 h and then concentrated in vacuo. The residue was redissolved in $CHCl_3$ (25 mL) and the organic phase was washed with concentrated aq. $NaHCO_3$ solution (20 mL), brine (20 mL), and H_2O (4 × 20 mL), and dried with Na_2SO_4. The chloroform was evaporated in vacuo.

Diethyl {3-[2-(Bis{2-[4-(diethyloxyphosphoryl)butyrylamino]ethyl}amino)ethylcarbamoyl]propyl}phosphonate (5a): Pale-brown oil. Yield 1.7 g (97%). – $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 32.0. – ^1H NMR ($CDCl_3$): δ = 1.25 (t, ^3J = 6.8 Hz, 18 H, 6 × CH_3CH_2OP), 1.71–1.76 (m, 6 H, 3 × CH_2CH_2P), 1.82–1.90 (m, 6 H, 3 × CH_2CH_2P), 2.29 (t, ^3J = 6.8 Hz, 6 H, 3 × CH_2CON), 2.53 (t, ^3J = 5.0 Hz, 6 H, 3 × NCH_2CH_2NHCO), 3.17–3.20 (m, 6 H, 3 × NCH_2CH_2NHCO), 3.97–4.00 (m, 12 H, 6 × CH_3CH_2OP), 7.20 (t, ^3J = 5.0 Hz, 3 H, 3 × CH_2NHCO). – $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 16.1 (CH_3CH_2OP), 20.6 (CH_2CH_2P), 27.1 (d, $^1J_{C,P}$ = 136 Hz, CH_2CH_2P), 36.1 (CH_2CON), 37.9 ($NCHCH_2NHCO$), 51.2 (NCH_2CH_2NHCO), 61.5 (CH_3CH_2OP), 176.0 (NCH_2CH_2NHCO). – $C_{30}H_{63}N_4O_{12}P_3$ (764.76): calcd. C 47.12, H 8.30, N 7.33, P 12.15; found C 46.92, H 8.23, N 7.11, P 12.19.

Diethyl {3-[3-(Bis{3-[4-(diethyloxyphosphoryl)butyrylamino]propyl}amino)propylcarbamoyl]propyl}phosphonate (5b): Pale-brown oil. Yield 1.8 g (98%). – $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ = 29.6. – ^1H NMR ($CDCl_3$): δ = 1.29 (t, ^3J = 6.8 Hz, 18 H, 6 × CH_3CH_2OP), 1.55–1.59 (m, 6 H, 3 × $CH_2CH_2CH_2N$), 1.68–1.74 (m, 6 H, 3 × CH_2CH_2P), 1.85–1.88 (m, 6 H, 3 × CH_2CH_2P), 2.24 (t, ^3J = 6.8 Hz, 6 H, 3 × CH_2CON), 2.34–2.36 (m, 6 H, 3 × $NCH_2CH_2CH_2NHCO$), 3.17–3.20 (m, 6 H, 3 × $NCH_2CH_2CH_2NHCO$), 3.96–4.00 (m, 12 H, 6 × CH_3CH_2OP), 7.20 (t, ^3J = 5.0 Hz, 3 H, 3 × CH_2NHCO). – $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ = 16.6 (CH_3CH_2OP), 21.6 (CH_2CH_2P), 27.5 (d, $^1J_{C,P}$ = 135 Hz, CH_2CH_2P), 28.2 ($CH_2CH_2CH_2N$), 35.6 (CH_2CON), 39.9 ($NCHCH_2CH_2NHCO$), 52.3 ($NCH_2CH_2CH_2NHCO$), 61.5 (CH_3CH_2OP), 175.7 (NCH_2CH_2NHCO). – $C_{33}H_{69}N_4O_{12}P_3$ (806.41): calcd. C 49.12, H 8.62, N 6.94, P 11.52; found C 48.92, H 8.58, N 6.73, P 11.58.

General Procedure for the Deprotection of the Phosphonic Esters: To a solution of **5a**,**b** (2.0 mmol) in anhydrous CH_2Cl_2 (10 mL) under nitrogen was added Me_3SiBr (4 mL). After stirring at room temperature for 4 d, the mixture was concentrated in vacuo. The residue was redissolved in methanol (10 mL) and then the solvent was removed in vacuo. This operation was repeated four times to finally afford a hygroscopic yellow solid. The sodium salt could be obtained by precipitation from a methanolic MeONa (24.0 mmol) solution. The mixture was filtered, and the collected solid was dried in vacuo at 100 °C to give a white solid. It proved difficult to obtain reliable elemental analytical data for these compounds because they are very hygroscopic.

[3-(2-{Bis[2-(4-phosphonobutyrylamino)ethyl]amino}ethylcarbamoyl)propyl]phosphonic Acid (6a): Yellow solid. Yield 1.0 g (80%). – $^{31}P\{^1H\}$ NMR (D_2O): δ = 31.5 (pH = 1), 22.0 (pH = 14). – ^1H NMR (D_2O, pH = 1): δ = 1.70–1.80 (m, 12 H, 3 × CH_2CH_2P; 3 × CH_2CH_2P), 2.39 (t, ^3J = 7.0 Hz, 6 H, CH_2CON), 3.42–3.47 (m, 6 H, 3 × NCH_2CH_2NHCO), 3.57–3.61 (m, 6 H, 3 × NCH_2CH_2NHCO). – $^{13}C\{^1H\}$ NMR (D_2O, pH = 14): δ = 22.6 (CH_2CH_2P), 30.1 (d, $J_{C,P}$ = 132 Hz, CH_2CH_2P), 38.1 (CH_2CON), 38.9 ($NCHCH_2NHCO$), 53.5 (NCH_2CH_2NHCO), 178.5 (NCH_2CH_2NHCO).

[3-(3-{Bis[3-(4-phosphonobutyrylamino)propyl]amino}propylcarbamoyl)propyl]phosphonic Acid (6b): Pale-brown oil. Yield 1.6 g (85%). – $^{31}P\{^1H\}$ NMR (D_2O, pH = 14): δ = 24.8. – ^1H NMR (D_2O, pH = 14): δ = 1.43–1.49 (m, 6 H, 3 × CH_2CH_2P) 1.71–1.74 (m, 6 H, 3 × CH_2CH_2P), 1.85–1.88 (m, 6 H, 3 × $CH_2CH_2CH_2N$), 2.25–2.29 (m, 6 H, 3 × CH_2CON), 2.95–3.15 (m, 6 H, 3 × $NCH_2CH_2CH_2NHCO$), 3.20–3.23 (m, 6 H, 3 × $NCH_2CH_2CH_2NHCO$). – $^{13}C\{^1H\}$ NMR (D_2O, pH = 14): δ = 23.3 (CH_2CH_2P), 26.4 ($CH_2CH_2CH_2N$), 30.1 (d, $J_{C,P}$ = 122 Hz, CH_2CH_2P), 39.3 (CH_2CON), 39.9 ($NCH_2CH_2CH_2NHCO$), 53.5 ($NCH_2CH_2CH_2NHCO$), 179.8 ($NCH_2CH_2CH_2NHCO$).

Acknowledgments

R. B. and T. B. thank the Radioprotection Department of EDF for financial support.

[1] R. A. Bulman, in *Structure and Bonding*, Springer Verlag, **1987**, Vol. 67, p. 93–141.
[2] O. Andersen, *Chem. Rev.* **1999**, *99*, 2683–2710.
[3] M. H. Henge-Napoli, M. Archimbaud, E. Ansoborlo, H. Metivier, P. Gourmelon, *Int. J. Radiat. Biol.* **1995**, *68*, 389–393.
[4] C. J. Anderson, M. J. Welch, *Chem. Rev.* **1999**, *99*, 2219–2234.
[5] S. S. Jurisson, J. D. Lydon, *Chem. Rev.* **1999**, *99*, 2205–2218.
[6] P. Caravan, J. J. Ellisson, T. J. McMurry, R. B. Lauffer, *Chem. Rev.* **1999**, *99*, 2293–2352.
[7] W. A. Volkert, T. J. Hoffman, *Chem. Rev.* **1999**, *99*, 2269–2292.
[8] V. Sharma, D. Piwnica-Worms, *Chem. Rev.* **1999**, *99*, 2545–2560.
[9] R. D. Hancock, A. E. Martell, *Chem. Rev.* **1989**, *69*, 1875–1914.
[10] F. I. Bel'skii, Y. M. Polilcarpov, M. I. Kabachnik, A. N. Nesmeyanaov, *Russ. Chem. Rev.* **1992**, *61*, 221–242.

[11] L. M. Shkol'nikova, M. A. Porai-Koshits, *Russ. Chem. Rev.* **1990**, *59*, 643−662.
[12] M. I. Kabachnik, T. Y. Medved′, N. M. Dyatlova, M. V. Rudomino, *Russ. Chem. Rev.* **1974**, *43*, 733−744.
[13] L. Maier, *Phosphorus* **1971**, *1*, 67−69.
[14] M. P. Lowe, S. J. Stewen, C. Orvig, *J. Am. Chem. Soc.* **1996**, *118*, 10446−10456.
[15] H. J. Cristau, D. Virieux, *Tetrahedron Lett.* **1999**, *40*, 703−706.
[16] J. P. Berry, A. F. Isbell, G. E. Hunt, *J. Org. Chem.* **1972**, *37*, 4396−4400.
[17] P. Coutrot, C. Grison, C. Charbonnier-Gerardin, *Tetrahedron* **1992**, *48*, 9841−9868.
[18] J. Chin, M. Banaszcyk, V. Jubian, X. Zou, *J. Am. Chem Soc.* **1989**, *111*, 186−190.
[19] W. Wilker, D. Leibfritz, R. Kerssebaum, W. Bermel, *Magn. Reson. Chem.* **1993**, *31*, 287−292.
[20] A. Bax, M. F. Summers, *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

Received June 5, 2000
[O00297]