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Synthesis of New Potential Chelating Agents: Catechol–Bisphosphonate Conjugates for Metal Intoxication Therapy

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ABSTRACT: *In a quest for better chelating therapy drugs for the treatment of intoxication by Fe, Al, or actinides, three new series of bisphosphonates conjugated with catechol were synthesized.* © 2004 Wiley Periodicals, Inc. Heteroatom Chem 15:251–257, 2004; Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/hc.20013

INTRODUCTION

In recent years, a great deal of attention has been focused on the development of more satisfactory chelating agents for the treatment of human metal intoxication. Accumulation of metal ions usually causes serious diseases. For example, a number of inherited diseases result from iron overloading [1]. Aluminum poisoning has been associated with neurological dysfunction [2,3] and renal dialysis related encephalopathy [4].

The purpose of the present investigation was to synthesize new chelating agents **4, 8**, and **13** having mixed functional groups such as bisphosphonic acid and catechol in order to enhance binding affinity with concerned metal ions.

Bisphosphonates are widely used in the treatment of various diseases of bone mineral metabolism disorders [5]. Bisphosphonic acid ligand possesses well-known strong chelating properties owing to the formation of a three-dimensional structure capable of binding divalent metals ions such as Ca(II), Mg(II), and Fe(II) in a bidentate manner, and the affinity for calcium can be increased if hydroxyl group (—OH) is attached to the geminal carbon atom [6]. Some studies have demonstrated that bisphosphonates also have very high affinity for metal ions such as Cu(II) [7], Al(III), and Fe(III) [8].

Catechol ligand is commonly found in the siderochromes, which are low-molecular-weight compounds produced by microbes and involved in their cellular iron transports [9]. Catecholate ligands incorporating a variety of electron-withdrawing substituents have been extensively studied for their extraordinary high affinity with high oxidation state metals such as Fe(III) [10] and actinides such as uranium(IV) and thorium(IV) [11].

In this paper, we report the synthesis of new chelating agents containing functional groups of catechol and bisphosphonate.

RESULTS AND DISCUSSION

For the synthesis of new ligands **4**, polybenzyloxybenzoyl chlorides **1** were used as starting materials. They were prepared as previously described [12] from polyhydroxy benzoic acids (Scheme 1). The aminoalkylenebisphosphonic acid sodium salts **2** were prepared according to Kieczykowski's method [13].

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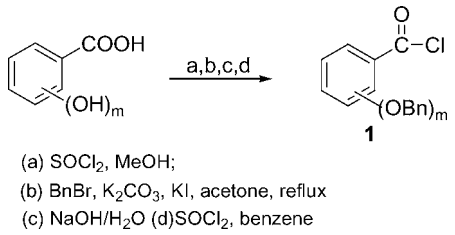


SCHEME 1

The benzyl-protected polyhydroxybenzoyl chloride in THF was added to a solution of monosodium salt of aminoalkylenebisphosphonic acid in aq NaOH. After completion of the reaction, the pH value of the solution was adjusted to acidic. The monosodium salt of bisphosphonic acid was obtained when the pH was lower than 2, while disodium salt **3** was obtained when the pH was between 3 and 4 (Scheme 2). The hydrogenolysis of benzyl-protected polyhydroxybenzoylaminoalkanebisphosphonates **3** was carried out in water with Pd/C as catalyst. The product bisphosphonates **4** were precipitated from aqueous solution by addition of alcohol (Scheme 2). The length of the methylene chain and the number of phenolic groups can be varied as desired with stable yields. This method avoided the acid breakage of the amide bond in the synthesis of phosphonate [14] and bisphosphonate derivatives [15].

We next directed our efforts to the synthesis of ligand **8**, and the desired 3,3-bis(dibenzyloxyphosphoryl)propanoic acid **5** [16] was prepared from tetraethyl methylenebisphosphonate (Scheme 3).

Coupling reaction of amine **6** with tetrabenzyl bisphosphonate **5** was carried out in THF at room temperature, using isobutyl chloroformate and *N*-methylmorpholine (NMM) as coupling agents in good yields. The amides **7** formed were then hydrogenated in methanol at atmospheric pressure using Pd/C as catalyst (Scheme 4). The free bisphosphonic acids are very hydroscopic while the sodium salts are very stable at ambient temperature.

For a comparative study, we have synthesized another series of nitrogen-containing bisphosphonic acids **13** incorporated with catechol linked by non-amide bond (Schemes 5 and 6).

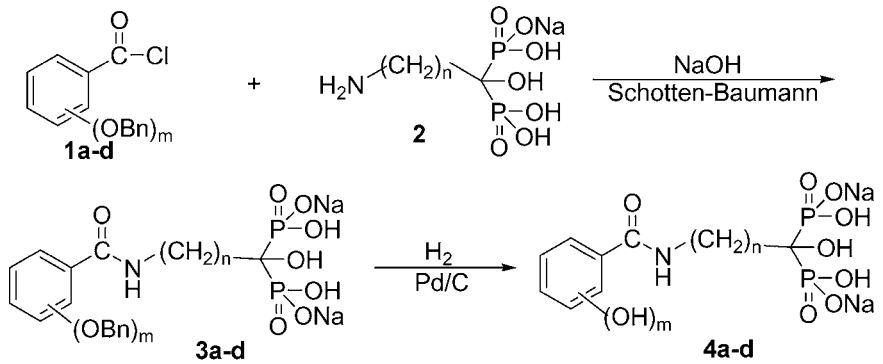
Compounds **12a,b** were obtained by condensation of tetraethyl aminomethylbisphosphonate **10** and dihydroxybenzaldehyde **9** followed by reduction in the presence of acetic acid with $NaBH(OAc)_3$ prepared in situ [17]. For the synthesis of **13c**, benzyl-protected dihydroxy cinnamaldehyde prepared from caffeic acid by several steps [18,19] was used as starting material. Reduction of **11c** gave **12c** and hydrogenolysis product **15**.

Dealkylation of bisphosphonic ester functions was carried out by using bromotrimethylsilane followed by methanolysis. In this way, the free bisphosphonic acids were obtained in satisfactory yields and good purity.

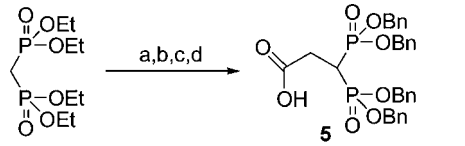
In summary, three types of 1,1-bisphosphonic acids bearing catechol moiety were efficiently prepared in satisfactory yields. Data on the chelating potency of the new bisphosphonates will be published elsewhere.

EXPERIMENTAL SECTION

Melting points were determined using a Büchi 510 melting point apparatus and are uncorrected. NMR, ^1H (400.13 MHz), ^{13}C (100.61 MHz), and ^{31}P (161.99 MHz), spectra were recorded on a Bruck-400 NMR spectrometer with TMS as an internal standard (^1H and ^{13}C) and 85% H_3PO_4 as an external standard



SCHEME 2



SCHEME 3

(^{31}P). Mass spectra were obtained on a MAT-95 spectrometer. Microanalysis were carried out on a Leco CHN-2000 elemental analyzer.

*4-N-(2,3-Dibenzyloxybenzoyl)aminobutane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**3a**)*

2,3-Dibenzyloxybenzoyl chloride (23.5 g, 66.7 mmol) in THF (24 ml) was added dropwise to a stirred solution of (4-amino-1-hydroxybutylidene)bisphosphonic acid monosodium salt (10.0 g, 30.8 mmol) in aq NaOH (3.5%, 180 ml, 158 mmol) at 5°C. After the addition was complete (30 min), the resulting mixture was allowed to warm to room temperature (rt), and stirred for an additional 6 h; aq. HCl (1.0 mol/l) was added until the pH of the solution reached 4. The precipitate was filtered with suction, washed with acetone (450 ml), water (150 ml), and acetone (450 ml), and the pure product was obtained by recrystallization from EtOH/H_2O, dried at 60°C for 24 h to give 16.58 g (81.2% yield) of white solid of **3a**. mp > 300°C, ^{1}H NMR (NaOD/D_2O): δ 1.78–1.82 (m, 2H, CH_2), 1.88–1.92 (m, 2H, CH_2CP_2), 3.19 (t, $J = 7.2$ Hz, 2H, CH_2N), 5.01 (s, 2H, $PhCH_2O$), 5.21 (s, 2H, $PhCH_2O$), 7.05–7.52 (m, 13H, Ph-H); ^{31}P NMR (D_2O): δ 19.12; Anal. calcd for $C_{25}H_{27}NNa_2O_{10}P_2 \cdot 3H_2O$: C, 45.26; H, 5.01; N, 2.11. Found: C, 45.21; H, 4.97; N, 2.20. Compounds **3b–d** were prepared using the method described for the preparation of **3a**.

*6-N-(3,4-Dibenzyloxybenzoyl)aminohexane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**3b**)*

80.3%, White powder, mp > 300°C, ^{1}H NMR (NaOD/D_2O): δ 1.22 (m, 2H, CH_2), 1.40–1.47 (m, 4H, $CH_2CH_2CP_2$), 1.70–1.80 (m, 2H, $C\underline{H}_2CH_2N$), 3.19 (t, $J = 7.0$ Hz, 2H, CH_2N), 5.04 (s, 2H, $PhCH_2O$), 5.06 (s, 2H, $PhCH_2O$), 6.96–7.28 (m, 13H, Ph-H); ^{31}P NMR (D_2O): δ 18.87; Anal. calcd for $C_{27}H_{31}NNa_2O_{10}P_2 \cdot 2.5H_2O$: C, 47.51; H, 5.32; N, 2.05. Found: C, 47.44; H, 5.44; N, 2.19.

*5-N-(2,3-Dibenzyloxybenzoyl)aminopentane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**3c**)*

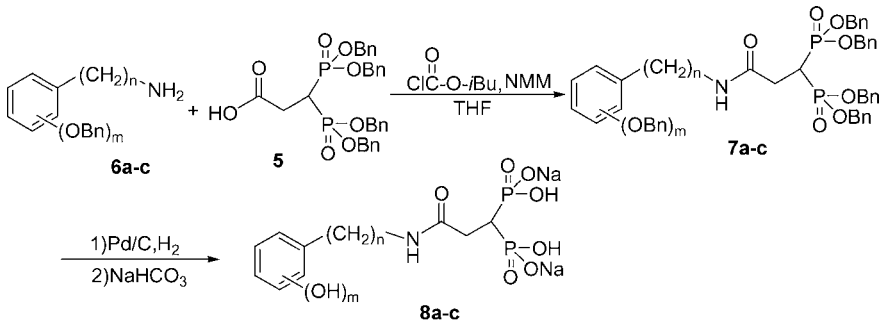
73.2%, White powder, mp > 300°C, ^{1}H NMR (NaOD/D_2O): δ 1.41 (m, 2H, CH_2), 1.48–1.56 (m, 2H, CH_2CP_2), 1.72–1.82 (m, 2H, $C\underline{H}_2CH_2N$), 3.11 (t, $J =$ 7.7 Hz, 2H, CH_2N), 4.93 (s, 2H, $PhCH_2O$), 5.13 (s, 2H, $PhCH_2O$), 6.96–7.44 (m, 13H, Ph-H); ^{31}P NMR (D_2O): δ 19.32; Anal. calcd for $C_{26}H_{29}NNa_2O_{10}P_2 \cdot 3H_2O$: C, 46.09; H, 5.21; N, 2.07. Found: C, 45.89; H, 5.34; N, 2.01.

*3-N-(3,4,5-Tribenzyloxybenzoyl)aminopropane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**3d**)*

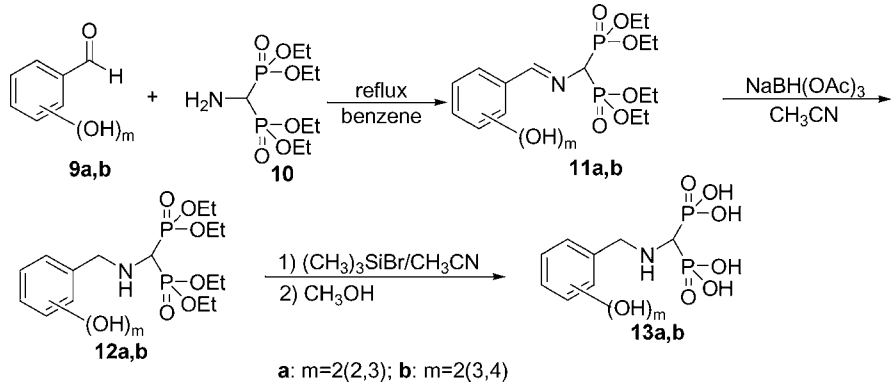
40.5%, White needle, mp > 300°C, ^{1}H NMR (NaOD/D_2O): δ 2.00–2.08 (m, 2H, CH_2), 3.45 (t, $J = 7.8$ Hz, 2H, CH_2N), 4.95 (s, 2H, $PhCH_2O$), 5.23 (s, 4H, $PhCH_2O$), 7.27–7.60 (m, 17H, Ph-H); ^{31}P NMR (D_2O): δ 19.96; Anal. calcd for $C_{31}H_{31}NNa_2O_{11}P_2 \cdot 4H_2O$: C, 48.13; H, 5.08; N, 1.81. Found: C, 48.21; H, 4.97; N, 1.89.

*4-N-(2,3-Dihydroxybenzoyl)aminobutane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**4a**)*

A suspension of **3a** (8.42 g, 12.8 mmol) in water (50 ml) was combined with Pd/C (10%, 4.5 g) and



SCHEME 4



SCHEME 5

hydrogenated at rt for 2 days; the mixture was then vacuum filtered from catalyst. The debenzylated product **4a** was purified by precipitating it from aq solution by addition of ethanol (450 ml); the white precipitate after drying in vacuo at 40°C for 24 h weighed 5.16 g (87.4% yield). mp > 300°C, ^{1}H NMR (D_2O): δ 1.88–2.02 (m, 4H, $CH_2CH_2CP_2$), 3.38 (t, $J = 6.8$ Hz, 2H, CH_2N), 6.83 (t, $J = 8.1$ Hz, 1H, Ph-H), 7.03 (d, $J = 8.1$ Hz, 1H, Ph-H), 7.22 (d, $J = 8.1$ Hz, 1H, Ph-H); ^{13}C NMR (D_2O): δ 26.1, 33.7, 42.7 (3s, CH_2), 76.3 (t, $J_{CP} = 135$ Hz, CP_2), 118.4, 120.8, 121.4, 121.6, 146.6, 149.4 (6s, Ar-C), 172.2 (C(O)N); ^{31}P NMR (D_2O): δ 18.9; Anal. calcd for $C_{11}H_{15}NNa_2O_{10}P_2{\cdot}2H_2O$: C, 28.40; H, 4.12; N, 3.01. Found C, 28.37; H, 4.14; N, 2.92; Compounds **4b–d** were prepared using the method described for the preparation of **4a**.

*6-N-(3,4-Dihydroxybenzoyl)aminohexane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**4b**)*

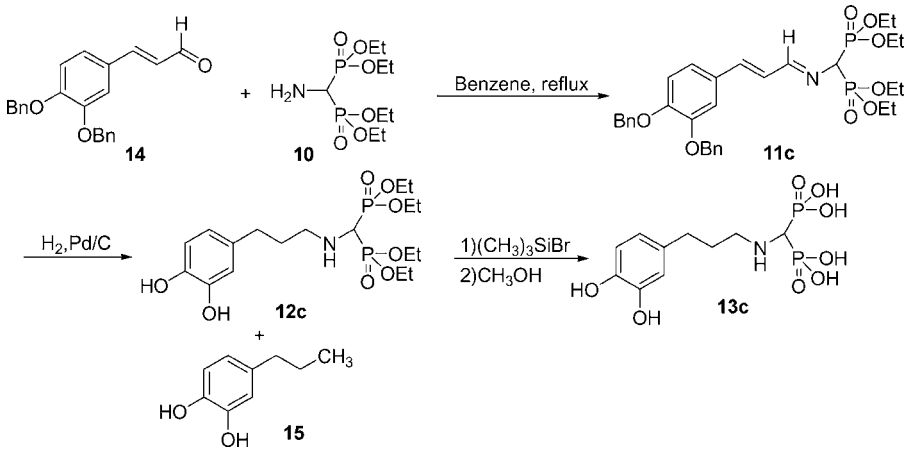
83.6%, White powder, mp > 300°C, ^{1}H NMR (D_2O): δ 1.43 (m, 2H, CH_2), 1.64–1.69 (m, 4H, $CH_2CH_2CP_2$), 1.94–2.04 (m, 2H, CH_2CH_2N), 3.40 (t, $J = 6.6$ Hz, 2H, CH_2N), 7.00 (d, $J = 8.4$ Hz, 1H, Ph-H), 7.28 (d, $J = 8.4$ Hz, 1H, Ph-H), 7.33 (s, 1H, Ph-H); ^{31}P NMR (D_2O): δ 18.12; Anal. calcd for $C_{13}H_{19}NNa_2O_{10}P_2$: C, 34.15; H, 4.19; N, 3.06. Found: C, 34.39; H, 4.39; N, 3.24.

*5-N-(2,3-Dihydroxybenzoyl)aminopentane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**4c**)*

84.3%, White powder, mp > 300°C, ^{1}H NMR (D_2O): δ 1.58–1.62 (m, 4H, $CH_2CH_2CP_2$), 1.93–1.96 (m, 2H, CH_2), 3.36 (t, $J = 6.6$ Hz, 2H, CH_2N), 6.81 (t, $J = 8.1$ Hz, 1H, Ph-H), 7.01 (d, $J = 8.1$ Hz, 1H, Ph-H), 7.17 (d, $J = 8.1$ Hz, 1H, Ph-H); ^{31}P NMR (D_2O): δ 18.54; Anal. calcd for $C_{12}H_{17}NNa_2O_{10}P_2{\cdot}1.5H_2O$: C, 30.65; H, 4.29; N, 2.98. Found: C, 30.87; H, 4.20; N, 2.89.

*3-N-(3,4,5-Trihydroxybenzoyl)aminopropane-1-hydroxy-1,1-bisphosphonic Acid Disodium (**4d**)*

88.8%, White powder, mp 255°C (decom.), ^{1}H NMR (D_2O): δ 2.20–2.28 (m, 2H, CH_2), 3.68 (t, $J = 7.0$ Hz, 2H, CH_2N), 6.96 (s, 2H, Ph-H); ^{31}P NMR (D_2O): 19.44;



SCHEME 6

Anal. calcd for $C_{10}H_{13}NNa_2O_{11}P_2 \cdot H_2O$: C, 26.74; H, 3.37; N, 3.12. Found: C, 26.79; H, 3.44; N, 2.98.

*Tetrabenzyl 3-Oxo-3-[(2,3-dibenzyloxyphenyl)-amino]propane-1,1-bisphosphonate (**7a**)*

To a solution of 3,3-bis(dibenzyloxyphosphoryl)-propanoic acid (355 mg, 0.597 mmol) in dry THF (5.5 ml) was added *N*-methylmorpholine (70 mg, 0.693 mmol), followed by isobutyl chloroformate (94.6 mg, 0.693 mmol) at −10°C; a sticky solid formed, and the mixture was left at −8°C for 20 min. To the mixture was added 210 mg of 2,3-dibenzyloxy-benzamine (0.688 mmol) and stirring was continued at room temperature for 30 min. Ice water (25 ml) was added, extracted with chloroform (25 ml), the organic phase was washed with saturated $NaHCO_3$ (2 × 5 ml), aq HCl (2.0 mol/L, 2 × 5 ml), water (2 × 10 ml), and dried with Na_2SO_4. After removal of solvent, the residue gum was purified by chromatography on a silica-gel column (mobile phase PE/EtOAc from 3:2 to 1:1) to provide 300 mg (57.0%) of colorless oil of **7a**. ^{1}H NMR($CDCl_3$): δ 2.63 (td, 2H, $J = 5.6$, 16.6 Hz, CH_2), 3.54 (tt, 1H, $J = 5.6$, 24.0 Hz, CH), 4.82 (s, 2H, $PhCH_2O$), 4.96–5.09 (m, 8H, 4 × $POCH_2Ph$), 5.16 (s, 2H, $PhCH_2O$), 6.75–7.88 (m, 33H, Ph-H); ^{31}P NMR ($CDCl_3$): δ 13.87; Anal. calcd for $C_{51}H_{49}NO_9P_2$: C, 69.46; H, 5.60; N, 1.59. Found C, 69.33; H, 5.91; N, 2.01. Compounds **7b** and **7c** were prepared using the method described for the preparation of **7a**.

*Tetrabenzyl 3-Oxo-3-[(3,4-dibenzyloxyphenyl)-amino]propane-1,1-bisphosphonate (**7b**)*

Colorless oil, 63.3%, ^{1}H NMR($CDCl_3$): δ 2.91 (td, 2H, $J = 5.9$, 16.8 Hz, CH_2), 3.42 (tt, 1H, $J = 5.9$, 24.2 Hz, CHP_2), 4.93–5.11 (m, 12H, $PhCH_2O$, 4 × $P(O)CH_2Ph$), 7.18–7.43 (m, 33H, Ph-H); ^{31}P NMR ($CDCl_3$): δ 14.32; Anal. calcd for $C_{51}H_{49}NO_9P_2$: C, 69.46; H, 5.60; N, 1.59. Found C, 69.12; H, 5.13; N, 1.44.

*Tetrabenzyl 3-Oxo-3-[2-(3,4-dibenzyloxyphenyl)-ethylamino]propane-1,1-bisphosphonate (**7c**)*

Colorless oil, 70.1%, ^{1}H NMR ($CDCl_3$): δ 2.53(t, $J = 6.9$ Hz, 2H, $PhCH_2$), 2.64 (td, $J = 5.9$, 16.5 Hz, 2H, CH_2CHP_2), 3.27 (q, 2H, CH_2NH), 3.41 (tt, $J = 5.9$, 24.3 Hz, 1H, CHP_2), 4.93–5.07 (m, 8H, 4 × $POCH_2Ph$), 5.13 (s, 4H, 2 × $PhCH_2O$), 6.60 (d, $J = 8.2$ Hz, 1H, Ph-H), 6.71 (s, 1H, Ph-H), 6.82 (d, $J = 8.2$ Hz, 1H, Ph-H), 7.22–7.46 (m, 30H, Ph-H); ^{31}P NMR ($CDCl_3$): δ 14.56; Anal. calcd for $C_{53}H_{53}NO_9P_2$: C, 69.96; H, 5.87; N, 1.54. Found C, 69.56; H, 6.18; N, 1.63.

*3-Oxo-3-[(2,3-dihydroxyphenyl)amino]propane-1,1-bisphosphonic Acid Disodium (**8a**)*

To a solution of compound **9a** (170 mg, 0.193 mmol) in absolute methanol (8 ml) was added 10% Pd/C (110 mg) and the resulting suspension was hydrogenated for 48 h at rt; the catalyst was removed by filtration and washed thoroughly with methanol (ca 2 ml), the combined solution was evaporated in vacuo, and the residue was triturated with aq $NaHCO_3$ (0.5%, 3.24 ml) to give 54.2 mg (71.3% yield) of white solid. mp > 300°C, ^{1}H NMR (D_2O): δ 2.78 (tt, 1H, $J = 6.8$, 21.8 Hz, CHP_2), 2.94 (td, $J = 6.8$, 15.0 Hz, 2H, CH_2), 6.84–6.91 (m, 3H, Ph-H); ^{13}C NMR (D_2O): δ 35.2 (s, CH_2), 38.4 (t, $J_{CP} = 105$ Hz, CP_2,), 117.0, 120.4, 122.5, 126.6, 141.6, 147.3 (6s, Ph-C), 175.9 (NHC═O); ^{31}P NMR (D_2O): δ 19.6; Anal. calcd. For $C_9H_{11}NNa_2O_9P_2 \cdot 0.5H_2O$: C, 27.43; H, 3.07; N, 3.55. Found: C, 27.81; H, 3.37; N, 3.44. Compounds **8b** and **8c** were prepared using the method described for the preparation of **8a**.

*3-Oxo-3-[(3,4-dihydroxyphenyl)amino]propane-1,1-bisphosphonic Acid Disodium (**8b**)*

mp > 300°C, White powder, 74.1%, ^{1}H NMR (D_2O): δ 2.82 (tt, 1H, $J = 6.6$, 20.8 Hz, CHP_2), 3.07 (td, 2H, $J = 6.6$, 15.6 Hz, CH_2), 7.08 (d, $J = 8.4$ Hz, 1H, Ph-H), 7.14 (d, $J = 8.4$ Hz, 1H, Ph-H), 7.30 (s, 1H, Ph-H); ^{31}P NMR (D_2O): δ 19.3; Anal. calcd for $C_9H_{11}NNa_2O_9P_2 \cdot 1.5H_2O$: C, 26.23; H, 3.42; N, 3.40. Found C, 26.51; H, 3.59; N, 3.11.

*3-Oxo-3-[2-(3,4-dihydroxyphenyl)ethylamino]-propane-1,1-bisphosphonic Acid Disodium (**8c**)*

mp > 300°C, White powder, 76.8%, ^{1}H NMR (D_2O): δ 2.53 (tt, 1H, $J = 5.6$, 22.1 Hz, CHP_2), 2.70 (td, 2H, $J = 5.6$, 16.8 Hz, CH_2CHP_2), 2.75 (t, $J = 7.1$ Hz, 2H, $PhCH_2$), 3.43 (t, $J = 7.1$ Hz, 2H, CH_2N), 6.78 (d, $J = 8.1$ Hz, 1H, Ph-H), 6.88 (s, 1H, Ph-H), 6.92 (d, $J = 8.1$ Hz, 1H, Ph-H); ^{31}P NMR (D_2O): δ 19.7; Anal. calcd for $C_{11}H_{15}NNa_2O_9P_2 \cdot H_2O$: C, 30.64; H, 3.97; N, 3.25. Found: C, 30.59; H, 4.30; N, 3.02.

*Tetraethyl (E)-N-(2,3-dihydroxybenzylidene)-aminomethylbisphosphonate (**11a**)*

2,3-Dihydroxybenzaldehyde (345 mg, 2.5 mmol) was added to a solution of tetraethyl aminomethylbis-phosphonate (758 mg, 2.5 mmol) in dry benzene (25 ml) and the mixture was heated at reflux in a Dean-stark for 4 h. The solvent was evaporated at reduced pressure and the crude product was purified by crystallization from ethyl acetate and petroleum

ether (1:1) to provide the pure compound as yellow crystal (81.6% yield). mp 131–133.5°C, ^{1}H NMR (DMSO-d_6): δ 1.19 (m, 12H, $4 \times CH_3$), 4.07 (m, 8H, $4 \times CH_2$), 4.83 (t, $J = 19.0$ Hz, 1H, CHP_2), 6.73–6.92 (m, 3H, Ph-H), 8.60 (t, 1H, $^4J_{PH} = 3.9$ Hz, CH=N), 9.2 (s, 1H, Ph-OH), 12.53 (s, 1H, Ph-OH). ^{31}P NMR (DMSO-d_6): δ 18.09, Anal. calcd. For $C_{16}H_{27}NO_8P_2$: C, 45.39; H, 6.43; N, 3.31. Found: C, 45.59; H, 6.31; N, 3.29. Compounds **11b** and **11c** were prepared using the method described for the preparation of **11a**.

*Tetraethyl (E)-N-(3,4-dihydroxybenzylidene)-aminomethylbisphosphonate (**11b**)*

Orange crystal, 88.4%, mp 140.5–142°C, ^{1}H NMR (DMSO-d_6): 1.20 (m, 12H, $4 \times CH_3$), 4.06 (m, 8H, $4 \times CH_2$), 4.52 (t, 1H, $J = 18.6$ Hz, CHP_2), 6.78–7.24 (m, 3H, Ph-H), 8.19 (t, 1H, $^4J_{PH} = 4.03$ Hz, CH=N); ^{31}P NMR (DMSO-d_6): δ 18.77; Anal. calcd. For $C_{16}H_{27}NO_8P_2$: C, 45.39; H, 6.43; N, 3.31. Found: C, 45.67; H, 6.43; N, 3.17.

*Tetraethyl (E)-N-[3-(3,4-dibenzyloxyphenylprop-2-enylidene)amino]methylbisphosphonate (**11c**)*

White crystal, 71.7%, mp 93–95°C, ^{1}H NMR (DMSO-d_6): δ 1.20 (m, 12H, $4 \times CH_3$), 4.06 (m, 8H, $4 \times CH_2$), 4.60 (t, 1H, $J = 18.8$ Hz, CHP_2), 5.17 (s, 2H, $PhCH_2O$), 5.19 (s, 2H, $PhCH_2O$), 6.91–7.47 (m, 15H, Ph-H, CH=CH), 8.10 (m, 1H, CH=N), ^{31}P NMR (DMSO-d_6): δ 18.62; Anal. calcd. For $C_{32}H_{41}NO_8P_2$: C, 61.04; H, 6.56; N, 2.22. Found: C, 61.20; H, 6.56; N, 2.10.

*Tetraethyl N-(2,3-dihydroxyphenylmethyl)-aminomethylbisphosphonate (**12a**)*

Glacial acetic acid (2.96 ml) was added dropwise to $NaBH_4$ (128 mg, 3.39 mmol) under N_2 atmosphere and the resulting solution was stirred at rt until evolution of hydrogen gas ceased. Then dry acetonitrile (5 ml) was added and cooled to 0°C, the imine **11a** (529 mg, 1.2 mmol) was added in one portion and stirred for 1 h at 0°C. Acetic acid and acetonitrile were removed at reduced pressure and the crude product was purified by flash chromatography eluting with ethyl acetate/methanol (25:1–15:1) to provide the pure compound **11a** as pale yellow crystal (377 mg, 70.9%). mp 115–117.5°C, ^{1}H NMR ($CDCl_3$): δ 1.22 (m, 12H, $4 \times CH_3$), 3.54 (t, 1H, $J = 22.1$ Hz, CHP_2), 3.88 (s, 2H, CH_2N), 4.05 (m, 8H, $4 \times CH_2$), 6.55–6.67 (m, 3H, Ph-H), ^{31}P NMR (DMSO-d_6): δ 17.33; MS: m/z 166 ($NH_2CHP(O)(OEt)_2^+$, 100), 138 ($(OH)_2PhCH_2NH^+$, 76). Anal. calcd. For $C_{16}H_{29}NO_8P_2$: C, 45.18; H, 6.87; N, 3.29. Found: C, 45.77; H, 6.88; N, 3.25. Compound **12b** was prepared using the method described for the preparation of **12a**.

*Tetraethyl N-(3,4-dihydroxyphenylmethyl)-aminomethylbisphosphonate (**12b**)*

Pale yellow crystal, 70.2%, mp 89–92°C, ^{1}H NMR ($CDCl_3$): δ 1.32 (m, 12H, $4 \times CH_3$), 3.46 (t, 1H, $J = 21.4$ Hz, CHP_2), 3.94 (s, 2H, CH_2N), 4.18 (m, 8H, $4 \times CH_2$), 6.66 (d, $J = 8.2$ Hz, 1H, Ph-H), 6.80 (d, $J = 8.2$ Hz, 1H, Ph-H), 7.00 (s, 1H, Ph-H), ^{31}P NMR (DMSO-d_6): δ 17.64; MS: m/z 425 ($M^+ - 1$, 13), 138 ($(OH)_2PhCH_2NH^+$, 47); Anal. calcd. For $C_{16}H_{29}NO_8P_2$: C, 45.18; H, 6.87; N, 3.29. Found: C, 45.73; H, 6.75; N, 3.16.

*Tetraethyl N-[3-(3,4-dihydroxyphenylpropyl)-amino]methylbisphosphonate (**12c**)*

To a solution of tetraethyl (*E*)-*N*-[3-(3,4-dibenzyloxyphenylprop-2-enylidene) amino]methylbisphosphonate (300 mg, 0.477 mmol) in ethyl acetate was added Pd/C (10%, 150 mg), the mixture was conducted in a hydrogen steam at rt under atmospheric pressure for 4 h. The catalyst was removed by filtration and the filtrate was evaporated under vacuum. The crude residue was purified by flash chromatography eluting with ethyl acetate/methanol 60:1 to yield 170 mg (78.8%) of **12c** as a pale yellow oil. ^{1}H NMR (DMSO-d_6): δ 1.22 (m, 12H, $4 \times CH_3$), 1.57 (m, 2H, $CH_2C\underline{H_2}CH_2$), 2.38 (t, $J = 7.4$ Hz, 2H, $PhCH_2$), 2.67 (t, $J = 7.1$ Hz, 2H, CH_2N), 3.33 (t, 1H, $J = 20.2$ Hz, CHP_2), 4.03 (m, 8H, $4 \times CH_2$), 6.39 (d, $J = 8.0$ Hz, 1H, Ph-H), 6.53 (s, 1H, Ph-H), 6.59 (d, $J = 8.0$ Hz, 1H, Ph-H), 8.58 (s, 1H, Ph-OH), 8.66 (s, 1H, Ph-OH); ^{31}P NMR(DMSO-d_6): δ 17.60; Anal. calcd. For $C_{18}H_{33}NO_8P_2$: C, 47.68; H, 7.34; N, 3.09. Found: C, 47.73; H, 7.55; N, 2.98.

*N-(2,3-Dihydroxyphenylmethyl)aminomethylbisphosphonic Acid (**13a**)*

Trimethylsilyl bromide (0.65 ml, 4.92 mmol) was added dropwise to a solution of bisphosphonate **12a** (147 mg, 0.346 mmol) in dry acetonitrile (4 ml) at 5°C under an N_2 atmosphere. The mixture was stirred at rt for 2 days and the solvent was evaporated off to give a yellow oil. Methanol (20 ml) was added to give a white precipitate, the purified product as a white powder was obtained by recrystallization from methanol (106 mg, yield 95.1%), mp 217–219°C (decom.), ^{1}H NMR(D_2O): δ 3.49 (t, 1H, $J = 18.1$ Hz, CHP_2), 4.60 (s, 2H, CH_2N), 6.90 (t, $J = 8.0$ Hz, 1H, Ph-H), 6.96 (d, $J = 7.7$ Hz, 1H, Ph-H), 7.03 (d,

$J = 7.7$ Hz, 1H, Ph-H); ^{13}C NMR(NaOD/D_2O): δ 54.1 (s, CH_2), 56.3 (t, $J_{CP} = 113$ Hz, CP_2), 118.8, 119.9, 125.2, 125.8, 146.5, 147.4 (6s, Ph-C); ^{31}P NMR (D_2O): δ 8.26; Anal. calcd. For $C_8H_{13}NO_8P_2 \cdot 0.5H_2O$: C, 29.83; H, 4.38; N, 4.35. Found: C, 29.93; H, 4.74; N, 4.09. Compounds **13b,c** were prepared using the method described for the preparation of **13a**.

*N-(3,4-Dihydroxyphenylmethyl)aminomethylbisphosphonic Acid (**13b**)*

89.1%, White powder, mp 190°C (decom.), ^{1}H NMR (D_2O): δ 3.58 (t, $J = 18.7$ Hz, 1H, CHP_2), 4.42 (s, 2H, CH_2N), 6.94–7.04 (m, 3H, Ph-H); ^{31}P NMR (D_2O): δ 8.06; Anal. calcd. For $C_8H_{13}NO_8P_2 \cdot 1.5H_2O$: C, 28.25; H, 4.74; N, 4.12. Found: C, 28.02; H, 5.00; N, 3.97.

*N-[3-(3,4-Dihydroxyphenylpropyl)amino]methylbisphosphonic Acid (**13c**)*

84.3%, White powder, mp 229–231°C (decom.), ^{1}H NMR (D_2O): δ 2.05 (m, 2H, $CH_2C\underline{H_2}CH_2$), 2.67 (t, $J = 7.5$ Hz, 2H, $PhCH_2$), 3.42 (t, $J = 7.9$ Hz, 2H, CH_2N), 3.55 (t, 1H, $J = 18.5$ Hz, CHP), 6.78 (d, $J = 8.2$ Hz, 1H, Ph-H), 6.88 (s, 1H, Ph-H), 6.91 (d, $J = 8.2$ Hz, 1H, Ph-H); ^{13}C NMR (NaOD/D_2O): 30.2, 33.6, 51.0 (3s, CH_2), 57.1 (t, $J_{CP} = 120$ Hz, CP_2), 118.6, 118.7, 123.0, 136.0, 144.4, 146.2 (6s, Ph-C); ^{31}P NMR (D_2O): δ 8.22; Anal. calcd. For $C_{10}H_{17}NO_8P_2$: C, 35.20; H, 5.02; N, 4.11. Found: C, 35.00, H, 5.21, N, 4.15.

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