Manganese Phosphonate Complexes as MRI Probes for Bone Imaging Studies

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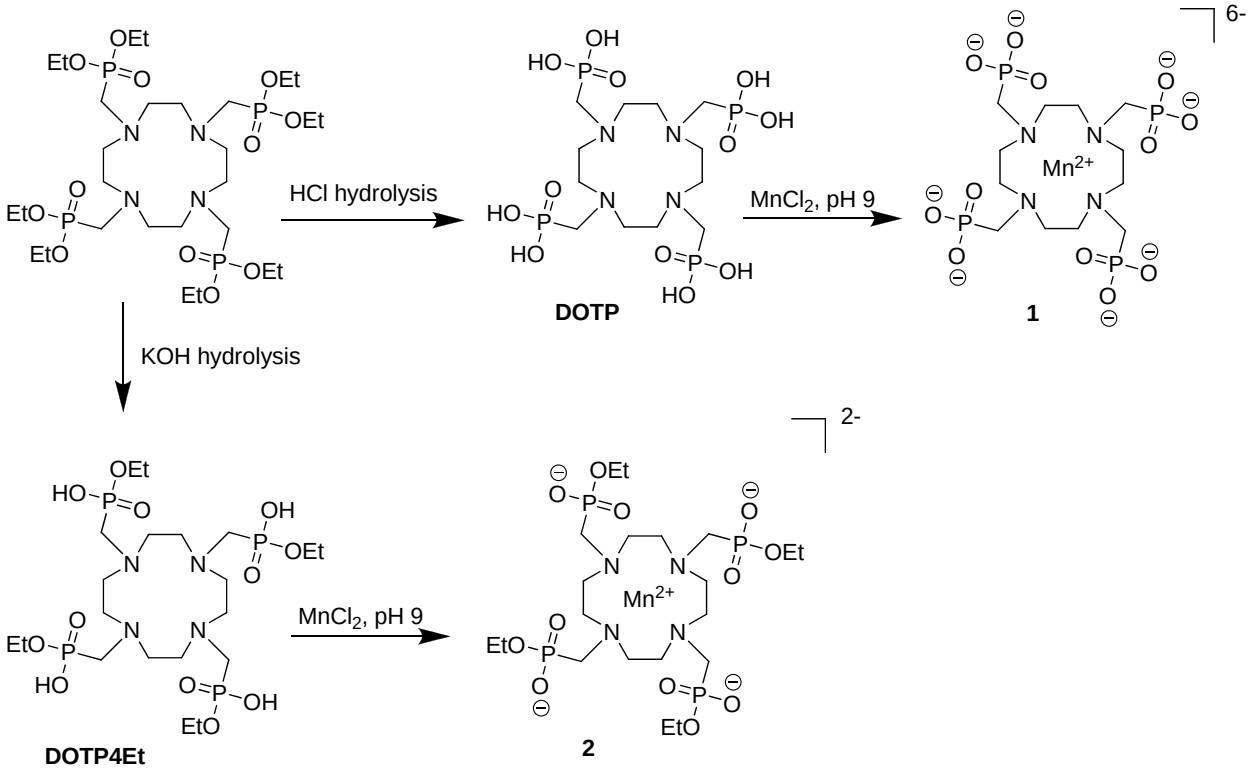
Background

Magnetic resonance imaging (MRI) is a common imaging modality, based on the abundance of water and fat protons in soft tissues, with contrast arising largely from inherent differences in proton densities and T_1 (spin-lattice) and T_2 (spin-spin) relaxation rates of these protons. The diagnostic accuracy of MRI can often be improved by administration of a paramagnetic contrast agent to alter either T_1 or T_2 of bulk water protons. The majority of contrast agents are based on Gd(III)-chelate complexes since this ion displays optimum properties for MRI, such as having seven unpaired electrons, long electronic relaxation times and an ability to form stable complexes with polyaminocarboxylate ligands. Manganese(II) with its five unpaired electrons may provide an alternative to gadolinium(III) although its cellular toxicity mandates strong sequestration by a chelating agent. This has proven difficult to achieve, and only one Mn(II) complex [dipridoxyl diphosphate (MnDPD)], a hepatobiliary contrast agent has been developed, and that has since been discontinued due to toxicological concerns. The current agents seek to deposit Mn(II) on bone surfaces after specific cleavage of phosphonate bonds at those surfaces, while maintaining a stable Mn(II) chelate conjugate away from the bone surface target.

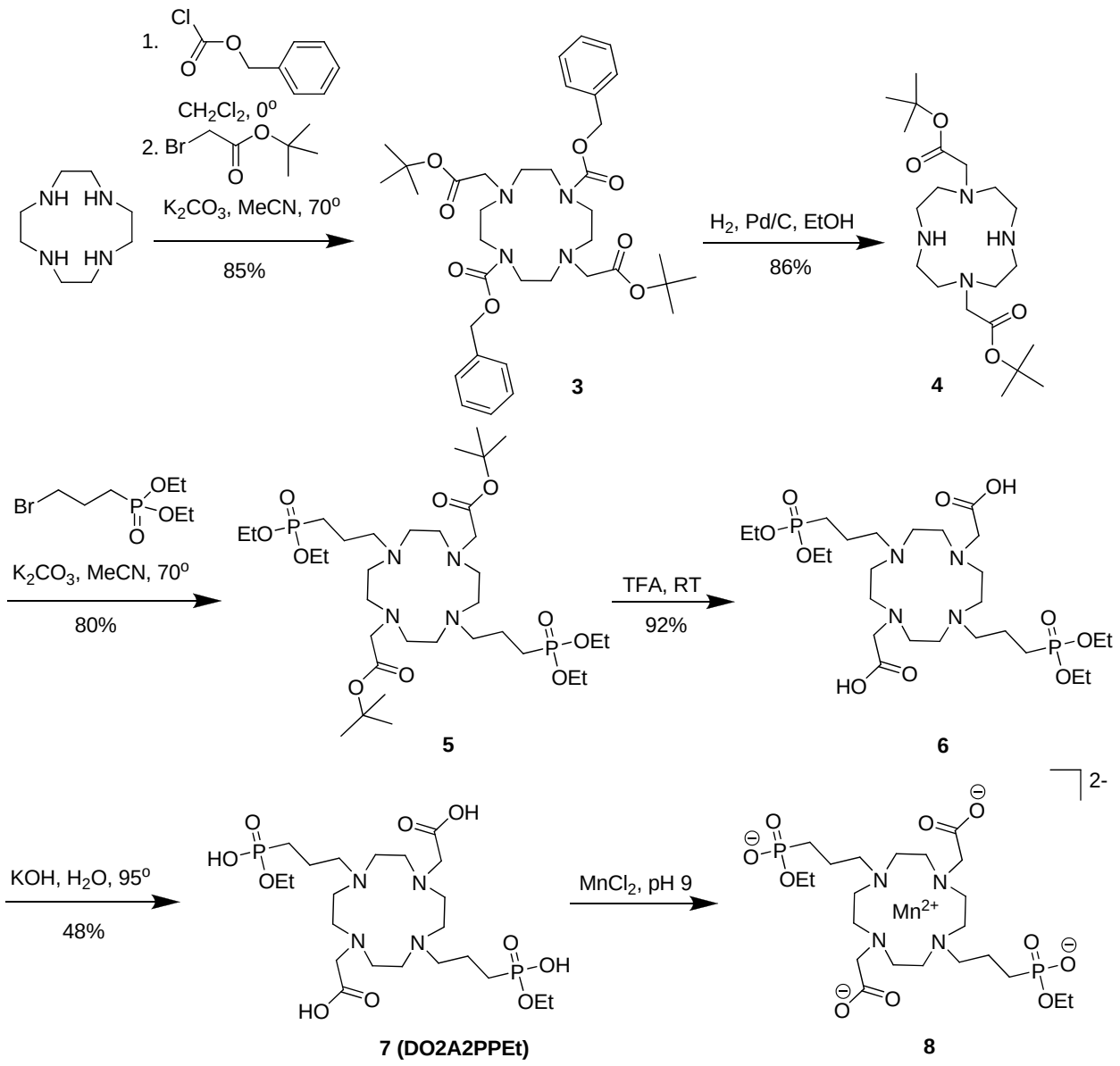
Chemistry

Over and above the task-in-hand of producing a Mn(II) bone seeking agent, a larger challenge is to obtain a more stable manganese complex. Herein we present the syntheses of more stable manganese complexes of phosphonic acid macrocycles as candidate Mn(II) chelating agents. These types of ligands have been shown in the past[1] to produce stable complexes with lanthanide metals and their structures and methods of syntheses are shown below in Schemes 1 and 2.

Scheme 1. Synthesis of manganese complexes of DOTP and DOTP4Et.



Scheme 2. Synthesis of DO2A2PPEt (**7**) and its manganese complex (**8**).



Experimental

General: All organic precursors and solvents were obtained from commercial sources and used as received. Hydrogenations were performed on a Parr hydrogenation apparatus. ^{1}H and ^{13}C NMR spectra were acquired using a Varian spectrometer operating at 400 and 100 MHz, respectively. Chemical shifts are reported in parts per million (δ) and referenced to tetramethylsilane (TMS). T_1 values were measured using a 400 MHz Varian spectrometer. Prior to data acquisition, samples were warmed to room temperature and equilibrated in the probe at

25˚C for at least 10 minutes before measurement. T_1 was measured by using an inversion-recovery sequence with 12 different delay times and 4 averaged. APCI mass spectrometry (APSI-MS) was performed on LC/MSD TrapXCl Agilent Technologies. 1,4,7,10-tetraazacyclo dodecane-1,4,7,10-tetramethyleneethyl phosphonate potassium salt (DOTP4Et) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethyl phosphonic acid (DOTP)[2], 1,4,7,10-tetraazacyclo dodecane-1,7-dicarboxylic acid dibenzyl ester (referred to as bis-protected cyclen)[3] and 1-bromo-3-(diethylphosphonate)propane[4] were prepared using published methods.

1,4,7,10-Tetraazacyclododecane-1,7-diacetic acid di-*tert*-butyl ester-4,10- dicarboxylic acid dibenzyl ester (3). Potassium carbonate (1.15 g, 8.3 mmol) was added to a solution of bis-protected cyclen (1.47 g, 3.34 mmol) in acetonitrile (20 mL) and the suspension was heated at 70˚C for 10 minutes. *tert*-Butyl bromoacetate (1.04 mL, 7.02 mmol) was then added in one portion. The reaction mixture was heated for 8 hours at 65-70˚C, filtered and the solvent was removed under reduced pressure. The resulting residue was adsorbed on 1.5 g silica gel and purified by flash chromatography CH_2Cl_2/MeOH 100:0 to 90:15. Solvents were removed to afford colorless oil (1.88 g, 84.5%). ^{1}H NMR (400 MHz, $CHCl_3$): δ = 1.43 (18H, s, CH_3), 2.88 (8H, br, CH_2), 3.21-3.42 (12H, m, ring CH_2, C$\underline{H}_2$COO), 5.12 (4H, C$\underline{H}_2$Ph), 7.34 (10H, m, Ph). ^{13}C NMR (100.56 MHz, $CHCl_3$): δ = 28.24, 46.96-47.32(br), 54.66-54.31 (br), 56.26, 67.26, 81.19, 128.12, 128.16, 128.70, 137.10, 156.71, 170.79. *m/z* (APCI MS) 669.6 ($[M+H]^+$).

1,4,7,10-Tetraazacyclododecane-1,7-diacetic acid di-*tert*-butyl ester (DO2A2*t*Bu) (4). 10% Palladium on carbon (0.2 g) was added to a solution of compound **3** (1.18 g, 1.77 mmol) in ethanol (30 mL). The reaction mixture was placed in a hydrogenation vessel for 12 h with the H_2 pressure set to 50 psi. The catalyst was filtered through Celite®545 (Aldrich) and the solvents were removed under reduced pressure to afford a colorless oil, which solidified under high vacuum (0.61 g, 86 % yield). ^{1}H NMR (400 MHz, $CHCl_3$): δ = 1.46 (18H, s, CH_3), 2.63 (8H, t, J= 5 Hz, CH_2 ring), 2.82 (8H, s br, CH_2 ring), 3.32 (4H, s, CH_2COO). ^{13}C NMR (100.53 MHz, $CHCl_3$) δ = 28.42, 46.03, 52.27, 57.83, 81.14, 171.89. *m/z* (APCI MS) 401.4 ($[M+H]^+$).

1,4,7,10-Tetraazacyclododecane-1,7-diacetic acid di-*tert*-butyl ester- 4,10-dipropylenediethyl phosphonate (5). Potassium carbonate (0.45 g, 3.3 mmol) was added to a solution of compound **4** (0.6 g, 1.5 mmol) in acetonitrile (20 mL) and the suspension was heated at 70˚C for 10 minutes. 1-Bromo-3-(diethylphosphonate)propane (0.796 g, 3.06 mmol) was then

added in one portion and the vial was washed with acetonitrile (2 mL). The reaction mixture was heated for 8 hours at 65-70˚C, filtered and the filtrate taken to dryness *in vacuo*. The residue was purified on alumina oxide 60 (EMD, Active Basic) $CH_3Cl/MeOH/Et_3N$ (89:10:1). Solvents were removed under high vacuum to afford a yellow oil (0.91 g, 80 %). ^{1}H NMR (400 MHz, $CHCl_3$): δ = 1.32 (12H, t, J = 7 Hz, $OCH_2C\underline{H}_3$), 1.46 (18H, s, CH_3), 1.74 (8H, m, CH_2), 2.48 (4H, t, J= 6 Hz, CH_2), 2.58 (8H, t, J = 5 Hz, CH_2 ring), 2.81 (8H, t, J = 5 Hz, CH_2 ring), 3.26 (4H, s, CH_2COO), 4.09 (8H, m, $OC\underline{H}_2CH_3$). ^{13}C NMR (100.53 MHz, $CHCl_3$) δ = 16.69, 16.75, 20.27, 20.32, 23.01, 24.42, 28.45, 52.42, 52.61, 56.22, 56.4, 56.71, 61.56, 61.62, 80.88, 171.24. ^{31}P NMR (161.85 MHz, $CHCl_3$) δ = 33.95. *m/z* (APCI MS) 757.6 ($[M+H]^+$).

1,4,7,10-Tetraazacyclododecane-1,7- diacetic acid-4,10-dipropylene diethyl phosphonate (6). Compound **5** (0.28 g, 0.37 mmol) was dissolved in trifluoroacetic acid (3 mL) and stirred at room temperature for 3 hours. The solvent was removed *in vacuo*, the residue dissolved in methanol, and the solvents were evaporated (3x 30 mL) to afford a highly hydroscopic oil (0.22 g, 92 %). ^{1}H NMR (400 MHz, CH_3OH): δ = 1.31 (12H, t, J = 7 Hz, $OCH_2C\underline{H}_3$), 1.87 (4H, m, CH_2), 2.03 (4H, br m, CH_2), 3.02 (4H, m), 3.13 (4H, m), 3.35 (4H, m), 3.42-3.48 (12H, m), 4.1 (8H, m, $OC\underline{H}_2CH_3$). ^{13}C NMR (100.53 MHz, CH_3OH) δ = 15.50, 15.55, 16.99, 17.04, 20.93, 22.36, 48.67, 50.30, 52.44, 54.27, 54.47, 62.44, 62.51, 174.23. *m/z* (APCI MS) 645.5 ($[M+H]^+$).

1,4,7,10-Tetraazacyclododecane-1,7- diacetic acid-4,10-dipropyleneethyl phosphonic acid (7). The diacid **6** (0.2 g, 0.31 mmol) was dissolved in water (1.5 mL) and potassium hydroxide (0.14 g, 2.5 mmol) was added. The reaction mixture was stirred for 16 hours at 95 ˚C. The reaction mixture was cooled to room temperature, filtered and passed through an ion-exchange resin (DOWEX 50 WX4-400). The resin was prepared by equilibration with 1M HCl followed by washing with water until the pH was 6.5. The compound was added to the top of a column of resin and eluted with water, the elution time being identified by a drop in the column eluate pH from 6.5 to 4-4.5. Fractions containing product were combined and lyophilized to obtain a light yellow solid (0.088 g, 48%).

General procedure for the preparation of manganese complexes (1, 2, 8). The ligands were dissolved in water and pH was adjusted to 9 using 1M sodium hydroxide. Manganese chloride solution (~10% excess) was added in one portion. The expected drop of the pH to 5.7-5.8 was observed in each instance, and was followed by a readjustment of the pH back to 9 using 1M

sodium hydroxide. The complexation reactions were stirred for 20 minutes at 65°C, cooled to room temperature, filtered, and the pHs of the solutions were adjusted to 7.5 using 1M hydrochloric acid. Complexes were then lyophilized and redissolved into deuterium oxide for T_1 measurements. Compounds: **1 =** T_1 0.2749 ± 0.0022 seconds; **2** = T_1 0.1382 ± 0.002 seconds; **7** = T_1 0.2328 ± 0.009 seconds.

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