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4-AMINOBENZYL-SUBSTITUTED DIETHYLENETRIAMINEPENTAACETIC ACID PENTAMETHYL ESTER: A CONVENIENT INTERMEDIATE FOR ATTACHMENT OF A DTPA MOIETY TO AMINE-CONTAINING TARGET MOLECULES

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Abstract: 4-Aminobenzyl-substituted DTPA pentamethyl ester **2** is a versatile reagent for the attachment of a DTPA moiety to amine-containing target molecules.

Diethylenetriaminepentaacetic acid (DTPA) forms strong complexes with metal ions including the lanthanides. The incorporation of DTPA units is a crucial step in the preparation of a variety of agents useful for radioimmunodiagnostic,[1] radioimmunotherapeutic,[2] and especially magnetic resonance imaging (MRI)[3] applications. DTPA reagents containing a reactive aromatic isothiocyanate group have been introduced in recent years.[4-7] These compounds react with amino groups on target molecules such as proteins,[8] antibodies,[9] aminodextrans,[10-11] or cascade polymers[12] to form robust thiourea linkages. The modified molecules typically bear several DTPA residues which subsequently undergo complexation with a lanthanide ion.

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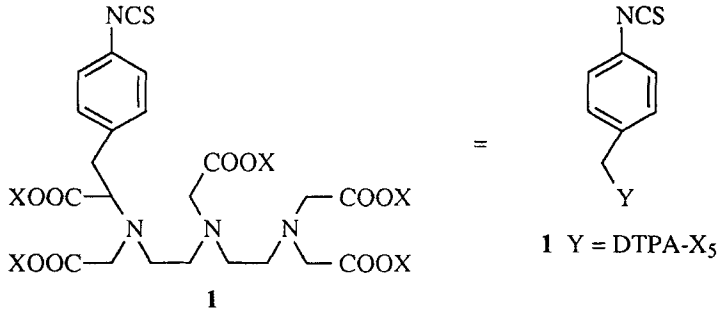
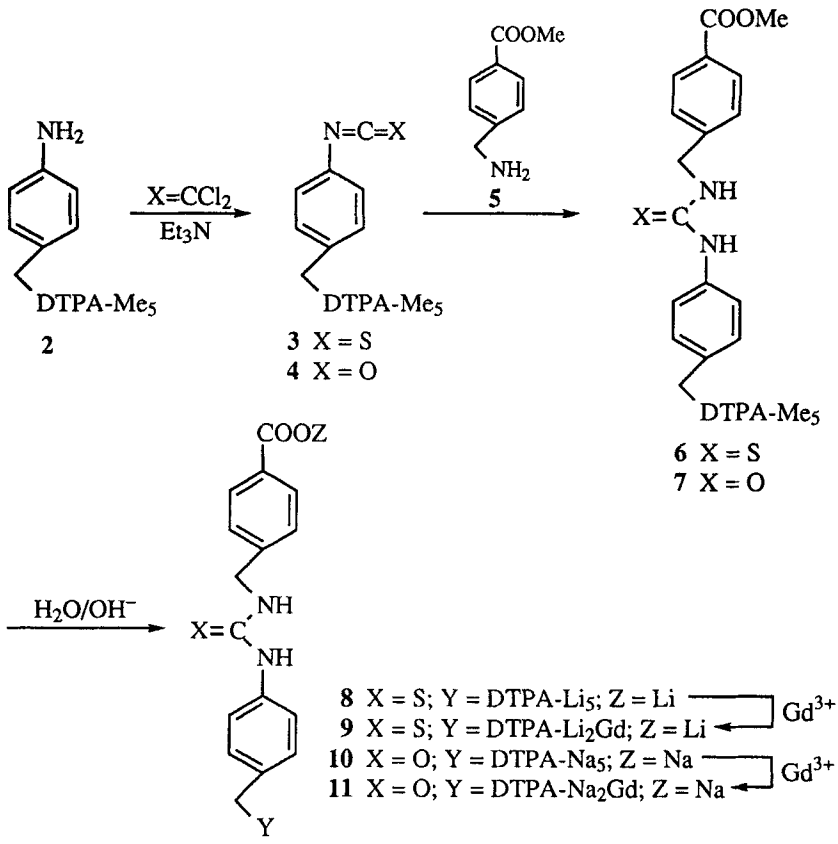
An alternative approach to MRI agents based on structurally well-defined compounds rather than labeled macromolecules was developed earlier in our laboratory.[7, 13] In a continuation of these studies we required a preparative method for the attachment of DTPA units to low molecular weight amines. The resulting DTPA derivatives were designed to serve as precursors of new Gd(III) chelates for MRI studies and also as intermediates in multistep syntheses of more complex MRI agents. Herein we report the synthesis of pentaester **3** and some reactions with mono- and diamino target molecules.

Initial experiments utilized isothiocyanate reagents in which the DTPA unit is in the form of a salt (**1**, X = Li or Na)[7] (note the abbreviated formula to the right). However, the ionic nature of both **1** and the product(s) made it difficult to employ conventional chromatography for monitoring the progress of the reaction and for separation and purification of the products.



1

Thus, we turned our attention to aniline derivative **2** in which the DTPA unit is in the form of the pentamethyl ester (Scheme 1). Pentaester **2**, easily available from DTPA-pentamethyl ester by a three-step sequence, was previously postulated as an intermediate in the preparation of a bifunctional Gd-DTPA labeling reagent.[7] We now find that **2** can be isolated in pure form and stored for several days at -10 °C.



Scheme 1

Pentaester **2** was activated for coupling by treatment with thiophosgene to give isothiocyanate derivative **3** (Scheme 1) which was purified by flash chromatography. Isothiocyanate **3** was unstable toward storage, therefore it was allowed to react immediately with amine **5**. The resulting thiourea pentaester **6** was purified by preparative TLC and characterized by NMR and HRMS. Selective hydrolysis of **6** with excess lithium hydroxide gave hexalithium salt **8**. The over-

all yield of **8** exceeded 100%, indicating that salt **8** contained inorganic material. However, ^{1}H NMR and HPLC data on **8** demonstrated the absence of other organic compounds. Salt **8** was converted into gadolinium chelate **9**. The analytical speciman was obtained by size-exclusion chromatography.

The successful activation of aniline **2** by the conversion to isothiocyanate **3** suggested an analogous activation with phosgene to give the more reactive isocyanate derivative **4**. Reaction of pentaester **2** with phosgene in dry THF gave the extremely moisture-sensitive isocyanate **4**. Without isolation or purification, isocyanate **4** was treated with amine **5** in THF solution to give urea hexaester **7**. Urea **7** was hydrolyzed to sodium salt **10** which was subsequently converted into Gd-complex **11**.

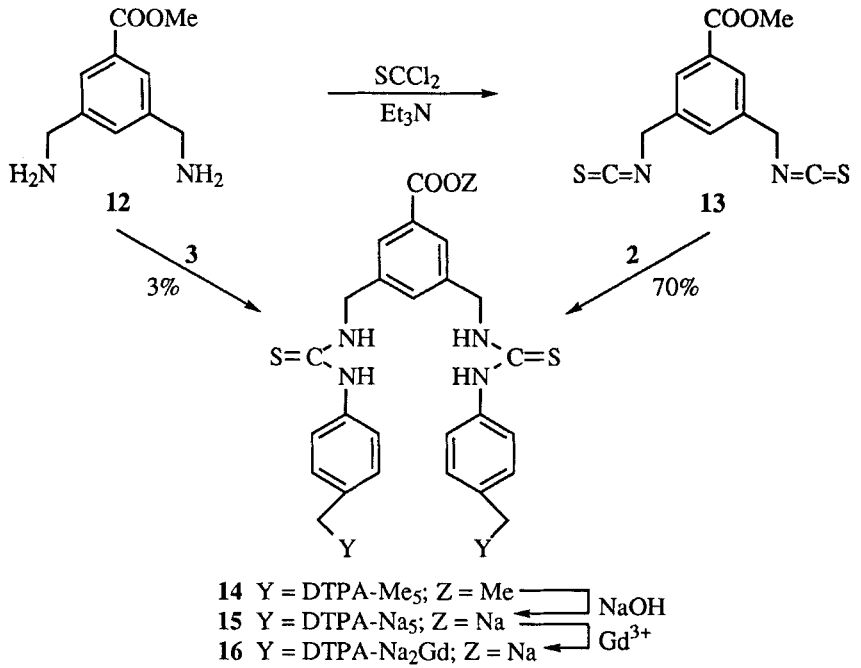
The coupling of isothiocyanate **3** to target diamine **12** gave the desired bis thiourea **14** in only 3% yield (Scheme 2).

Therefore, the roles of the two partners were reversed. Treatment of diamine **12** with thiophosgene in chloroform gave the stable diisothiocyanate **13**. Combination of diisothiocyanate **13** and amine **2** resulted in the formation of undecaester **14** as the major product which was purified by preparative TLC. Saponification to sodium salt **15**, followed by chelation with Gd(III) ion gave dichelate **16**.

In conclusion, we have found that 4-aminobenzyl DTPA pentaester **2** is a versatile neutral precursor for a family of DTPA derivatives.

Experimental Section

General. Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Analytical HPLC was performed on a Waters Resolve C_{18} 0.8 x 10 cm Radial-PAK cartridge; eluent, gradients A (H_2O + 0.2% TFA) – B (MeCN with 0.2% TFA) with UV-detection at 230 or 254 nm. The reported values are retention times and peak areas in relative percent. Size exclusion chromatography



Scheme 2

was performed on a Pharmacia-LKB Gradifrac system (UV-detection at 280 nm) on Sephadex G-10 and Sephadex G-25 Fine gels (bed 2.5 x 80 cm). All the reactions were performed under a nitrogen atmosphere.

Preparation of amine pentaester 2. A mixture of nitrobenzyl-DTPA[7] (2.70 g, 4.4 mmol) and anhydrous $SnCl_2$ (6 g, 30.1 mmol) in abs MeOH (100 mL) was refluxed for 5 h, evaporated to ~25 mL and poured into EtOAc (300 mL). Ethyl acetate was washed with sat. $NaHCO_3$ (10 x 50 mL), H_2O (50 mL), dried ($MgSO_4$), and evaporated. The crude product was chromatographed over silica gel (2.5 x 30 cm, eluent 2% MeOH in $CHCl_3$) to give 1.72 g (68%) of amine **2** as a yellow oil: ^1H NMR ($CHCl_3$) δ 3.00 (m, 8 H), 3.40-3.70 (m, 30 H), 6.58 (A_2B_2,

J = 9 Hz, 2 H), 6.95 (A_2B_2, J = 9 Hz, 2 H). HRMS Calcd for $C_{26}H_{40}N_4O_{10}$: 568.2744. Found: 568.2800.

Isothiocyanate 3. To a solution of amine **2** (0.250 g, 0.44 mmol) in $CHCl_3$ (10 mL), $CSCl_2$ (1 *N* in $CHCl_3$; 0.5 mL, 0.5 mmol) was introduced. The mixture was stirred for 2 h and then evaporated to dryness. The residue was purified by flash chromatography over silica gel (1 x 30 cm). Elution with $CHCl_3$ gave 0.150 g (56%) of isothoicyanate **3** as an orange oil which was immediately used in next step.

Thiourea-hexamethyl ester 6. A solution of isothiocyanate **3** (0.052 g, 0.085 mmol) and amine **5** (0.018 g, 0.102 mmol) in MeOH (2 mL) was stirred overnight. The mixture was evaporated and residue was purified on a silica gel TLC plate using a $CHCl_3$-MeOH (25:1) mixture as eluent to yield 0.047 g (71%) of ester **6** as an orange oil: ^{1}H NMR ($CDCl_3$) δ 2.55-2.80 (m, 8 H), 2.90 (m, 2 H), 3.13-3.70 (m, 24 H), 3.89 (s, 3 H), 4.94 (br s, 2 H), 7.13 (d, 2 H, J = 8 Hz), 7.20 (br s, 1 H, N-H), 7.26 (d, 2 H, J = 8 Hz), 7.36 (d, 2 H, J = 8 Hz), 7.97 (d, 2 H, J = 8 Hz). HRMS Calcd for $C_{36}H_{49}N_5O_{12}S$: 775.3098. Found: 775.3110.

Urea-hexamethyl ester 7. To a stirred solution of $COCl_2$ (1.9 M in toluene; 3.6 mL, 7.0 mmol) in dry THF (20 mL) a solution of amine **2** (0.400 g, 0.7 mmol) and Et_3N (0.29 mL, 2 mmol) in THF (5 mL) was added dropwise at -10 °C. The mixture was allowed to warm to room temperature. It was stirred for 0.5 h and then cooled to -50 °C. The solvent was evaporated under vacuum and the crude isocyanate **4** was kept at 0.05 torr for 10 min at room temperature to remove residual phosgene. Dry THF (20 mL) was added followed by a solution of amine **4** (0.246 g, 1.4 mmol) in THF (10 mL). The mixture was stirred for 4 h, then diluted with THF (50 mL), filtered from salts, and evaporated. The residue was chromatographed on a prep. TLC plate (40 x 30 cm x 3 mm, eluent 3% MeOH in

$CHCl_3$) to give 0.231 g (43%) of urea **7** as a yellow oil: IR (CCl_4) 1736, 1613, 1515, 1436 and 1283 cm^{-1}. ^1H NMR ($CDCl_3$) δ 2.58-2.80 (m, 8 H), 2.93 (m, 2 H), 3.37-3.70 (m, 24 H), 3.92 (s, 3 H), 4.47 (d, 2 H, J = 6 Hz), 7.13 (d, 2 H, J = 8 Hz), 7.25 (d, 2 H, J = 8 Hz), 7.37 (d, 2 H, J = 8 Hz), 7.97 (d, 2 H, J = 8 Hz). HRMS Calcd for $C_{36}H_{49}N_5O_{13}$: 759.3327. Found: 759.3320.

Saponification of Me-protected intermediates 6, 7, 14. A mixture of the methyl ester (0.25 mmol) and 1 *N* LiOH or NaOH (5 mL, 5 mmol) in MeOH (10 mL) was stirred for 16 h. The solvent was evaporated and the crude product (**8**, **10**, or **15**) was reprecipitated by addition of acetone to a MeOH solution. **Thiourea-hexalithium salt 8**: White powder; dec above 230 °C; IR (KBr) 1596, 1414, 1333 and 865 cm^{-1}; ^1H NMR (D_2O) δ 2.60 (br m, 8 H), 2.90-3.45 (m, 11 H), 7.13 (d, 2 H, J = 8 Hz), 7.30 (m, overlapping d's 4 H), 7.79 (d, 2 H, J = 9 Hz). HPLC (20-60% B over 15 min) 11.9 min (93%). **Urea-hexasodium salt 10**: White powder; dec above 270 °C; IR (KBr) 1601, 1552, 1512, 1400, 1320, 1240 and 936 cm^{-1}. ^1H NMR (D_2O) δ 2.62 (br m, 8 H), 2.80-3.48 (br, 11 H), 7.15 (d, 2 H, J = 6 Hz), 7.32 (m, overlapping d's 4 H), 7.82 (d, 2 H, J = 8 Hz). HPLC (10-90% B over 15 min): 10.8 min (99%). **Undecasodum salt 15.** Pale yellow solid; dec above 250 °C; IR (KBr) 1591, 1437, 1329, 1187, 1109 and 880 cm^{-1}. ^1H NMR (D_2O) δ 2.66 (m, 16 H), 2.80-3.50 (m, 22 H), 7.22 (d, 4 H, J = 6Hz), 7.32 (d, 4 H, J = 6 Hz), 7.36 (s, 1 H), 7.64 (s, 2 H). HPLC (10-90% B over 15 min): 12.2 (96%).

Gadolinium complex 9. A solution of salt **8** (0.147 g, 0.2 mmol) in 50% MeOH (20 mL) was acidified with 0.2 *N* HCl to pH 5.5 (pH meter). A solution of $GdCl_3 \cdot 6H_2O$ (0.074 g, 0.2 mmol) in H_2O (1 mL) was then added. The mixture was stirred for 0.5 h, then 0.1 *N* LiOH was added dropwise to gradually increase the pH to 9.5 over 2 h. The mixture was stirred for 2 h, filtered from some

insoluble material, and evaporated. The residue was redissolved in MeOH (10 mL) and precipitated with acetone to give the crude product which was chromatographed on a Sephadex G-10 column (2.5 x 60 cm). Complex **9** was eluted with H_2O in the void volume (monitored by HPLC) to give 0.121 g (69%) of complex **9** as a pale yellow solid: dec above 260 °C; IR (KBr) 1597, 1406, 1323, 1271, 1093 and 934 cm^{-1}. HPLC (10-90% B over 15 min): 11.3 (100%). Anal. Calcd for ($C_{30}H_{41}N_5O_{12}SLi_3Gd \cdot 6H_2O$): C, 37.08; H, 4.46; N, 7.21. Found: C, 37.01; H, 4.69; N, 7.02.

Urea derivative 11 was prepared similarly with final purification on a Sephadex G-25 column. Yield 74%; white powder, dec above 300 °C; IR (KBr) 1600, 1554, 1515, 1402, 1320, 1241, 1094 and 933 cm^{-1}. HPLC (10-90% B over 15 min): 10.9 min (98%). Anal. Calcd for ($C_{30}H_{31}N_5O_{13}Na_3Gd \cdot 6H_2O$): C, 35.89; H, 4.32; N, 6.98. Found: C, 35.50; H, 4.65; N, 6,71.

Digadolinium complex 16 was prepared similarly with final purification on a Sephadex G-25 column. Yield 66%; white powder, dec above 300 °C; IR (KBr) 1601, 1405, 1319, 1264, 1093, 1018 and 931 cm^{-1}. HPLC (10-90% B in 15 min): 12.0 min (98%). Anal. Calcd for ($C_{53}H_{57}N_{10}O_{22}S_2Na_5Gd_2 \cdot 14H_2O$): C, 32.95; H, 4.43; 7.25. Found: C, 33.26; H, 4.07; 7.01.

Reaction of isothiocyanate 3 with diamine 12. To a solution of isothiocyanate **3** (0.284 g, 0.5 mmol) in ethanol-free $CHCl_3$ (20 mL), a mixture of diamine **11**[14] (dihydrochloride, 0.067 g, 0.25 mmol) and Et_3N (0.14 mL, 1 mmol) was added. The mixture was stirred for 16 h, diluted with $CHCl_3$ (50 mL), washed with H_2O (3 x 20 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed over silica gel (1.5 x 20 cm, eluent $CHCl_3$), then on a prep. TLC plate (eluent MeCN-H_2O 9:1) to give 0.010 g (3%) of hexaester **14** as a red glassy solid: liquif. above 65 °C; ^1H NMR ($CDCl_3$) δ 2.53-2.83 (m, 16 H), 2.83-3.11 (m, 4 H), 3.40-3.80 (br, 48 H), 3.88 (s, 3 H), 4.91 (d, J = 6 Hz, 4 H), 7.24 (A_2B_2, 8 H, J = 8 Hz),

7.55 (s, 1 H), 7.80 (s, 2 H). FAB MS Calcd for ($C_{64}H_{90}N_{10}O_{22}S_2$ + H): 1415.6. Found: 1415.6.

Methyl (3,5-Bis-isothiocyanatomethyl)benzoate (13). To a vigorously stirred mixture of diamine **12** (dihydrochloride, 0.267 g, 1 mmol) and $CSCl_2$ (1 M in $CHCl_3$, 5 mL, 5 mmol) in ethanol-free $CHCl_3$ (10 mL), Et_3N (0.7 mL, 5 mmol) was added dropwise at -20 °C. The mixture was stirred for 30 min, diluted with $CHCl_3$ (20 mL), washed with 1 *N* HCl (2 x 10 mL), H_2O (2 x 10 mL), sat. $NaHCO_3$ (20 mL), dried ($MgSO_4$), and evaporated. The oily residue was chromatographed over silica gel (1 x 40 cm, eluent $CHCl_3$) to yield 0.195 g (71%) of diisothiocyanate **13** as a yellow solid: mp 77-79 °C (from hexane-EtOAc 5:1); IR (KBr) 2193, 2174, 2097, 1722 and 1223 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.95 (s, 3 H), 4.82 (3, 4 H), 7.49 (s, 1 H,), 7.97 (s, 2 H,); ^{13}C NMR ($CDCl_3$) δ 48.21, 52.49, 127.94 (2 C), 129.41 (1 C), 131.81 (1 C), 134.40 (br, 1 C, C=S), 135.87 (2 C), 165.75 (1 C, C=O). Anal. Calcd for $C_{12}H_{10}N_2O_2S_2$: C, 51.78; H, 3.62; N, 10.06. Found: C, 52.11; H, 3.52; N, 9.94.

Coupling of Diisocyanate 13 with Amine 2. A solution of amine **2** (0.057 g, 0.1 mmol) and diisocyanate **13** (0.013 g, 0.05 mmol) in ethanol-free $CHCl_3$ was stirred for 18 h and then evaporated. The crude product was purified on prep. TLC (eluent 3% MeOH in $CHCl_3$) to yield 0.049 g (70%) of hexaester **14** which was identical to that described above.

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