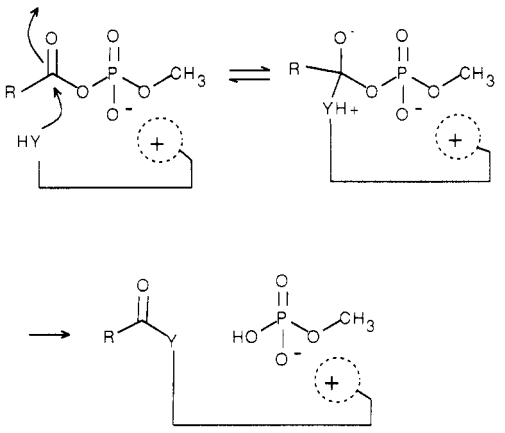
Scheme III. Mechanism of Acylation Reaction



formed. Detals of that work will be published when that study is completed.

Generalization. The reaction of an acyl chloride and dimethyl phosphate followed by ester cleavage provides a convenient means of preparing monomethyl esters of a wide variety of acyl phosphates. Since the coupling reaction is independent of the alkyl group, it is likely that the method can be extended to give materials in which other alkyl and or aryl ester functions are present. In addition, cleavage of a single ester group by the reaction with sodium iodide can be accomplished with other primary alkyl or benzyl side chains. Since the reagents appear to recognize a general class of site on a protein and react with amino groups in that site, they also should function as general inactivators of enzymes with similar active sites. A preliminary survey reveals that fumaroyl bis(methyl phosphate) irreversibly inactivates enzymes that bind NADH.[29]

Mechanism of the Reaction of Acyl Phosphates with Nucleophiles. The addition of a nucleophile to an acyl phosphate ester is expected to form a tetrahedral addition intermediate that can revert to reactants or proceed to give the acylated nucleophile (Scheme III). The initial tetrahedral adduct can revert to starting materials or expel methyl phosphate. Since the leaving group, methyl phosphate, is moderately basic, it will leave less readily than very weakly basic groups such as water, alcohols, or halide ions, and the reagent will manifest its selectivity even after adduct formation has occurred.

The half-time for hydrolysis of phenyl acetyl phosphate is approximately 80 h at 39 °C ($k = 2.50 \times 10^{-6}$ s^{-1}) while the second-order rate constant for the reaction of phenyl acetyl phosphate and glycine is 41 M^{-1} s^{-1} (at 39 °C).[4] Thus, in contrast to their stability in water, acyl phosphate monoesters react rapidly with amine nucleophiles and are selective. When these molecules associate with a protein, they should react with adjacent nucleophiles, as is observed. On the other hand, dialkyl acyl phosphates are extremely reactive toward nucleophiles and are unstable in water.[17] The leaving group (dimethyl phosphate in the case of dimethyl acetyl phosphate) is the conjugate base of a strong acid and thus leaves readily from the tetrahedral intermediate.

Conclusions

We have shown that acyl phosphate monomethyl esters can be prepared by a route that should permit almost any carboxylic acid to be converted to the corresponding acyl phosphate monomethyl ester or diester. The combination of a negative charge and electrophilic reactivity will make these materials candidates for trials as site-directed reagents for protein modification. The difunctional analogues can be tested as site-directed cross-linking agents. Combination of the acyl phosphate monoester functional group with other selective electrophiles in a heterodifunctional molecule should provide reagents that will give further types of specificity.

Acknowledgment. We thank Professor R. T. Jones for helpful discussions and for communicating unpublished results. Our work has been supported by grants from the Bickell Foundation and the Natural Sciences and Engineering Research Council of Canada (NSERC) to Ronald Kluger. Andrew Grant is the recipient of a NSERC postdoctoral fellowship, and Stephen Bearne is the recipient of a NSERC postgraduate fellowship. Marcel Trachsel received a fellowship from the Schweizerische Stiftung auf dem Gebiete der Chemie.

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Chelating Ligands Functionalized for Facile Attachment to Biomolecules. A Convenient Route to 4-Isothiocyanatobenzyl Derivatives of Diethylenetriaminepentaacetic Acid and Ethylenediaminetetraacetic Acid

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The title compounds were synthesized by alkylation of the monoenolates of DTPA and EDTA permethyl esters **3** and **4** with benzyl bromide. The benzylated esters **5** and **6** were nitrated, hydrogenated, and converted to their isothiocyanate derivatives **13** and **14** in good yields. The Gd(III) complex of 4-(isothiocyanatobenzyl)-DTPA **16**, a potential contrast-enhancing agent for magnetic resonance imaging (MRI), was prepared.

Poly(amino carboxylate) chelates of metal ions are widely used as probes of protein structure,[1] as contrast-enhancing agents for magnetic resonance imaging (MRI),[2] and as radiopharmaceuticals.[3] Recent novel applications of these chelates involve their covalent attachment to drugs, antibodies, or other biomolecules. For example, Meares and co-workers[4] have prepared a bleomycin–EDTA

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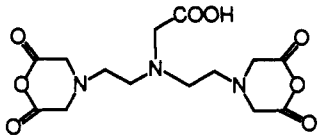
0022-3263/90/1955-2868$02.50/0

derivative, which, after chelation of the radioisotope $^{111}In^{3+}$ has proven promising for the diagnosis of cancer in humans. Metal chelates have also been attached to tumor-seeking antibodies for scintographic imaging of tumors in mice.[5] Efforts are currently being directed toward the synthesis of new chelating agents functionalized to allow this attachment.[5-7]

Chelates of paramagnetic metal ions are also useful as MRI contrast-enhancing agents[2] and in general they have substantially lower biotoxicities than the corresponding free ions.[8] The most useful of these chelates, Gd-DTPA, has been attached as its amide derivative to molecules such as bovine serum albumin (BSA).[9] Attaching the chelate to a large, slowly tumbling molecule such as BSA augments the chelate's ability to effect contrast enhancement[9] by increasing its rotational correlation time (τ_R).[10] In other work, amphipathic derivatives of Gd-DTPA have been prepared and then incorporated into liposomes by Kabalka and co-workers.[11] These compounds are preferentially taken up by the liver and yield a 150% enhancement in the proton T_1 signal of that organ a few minutes after injection.

The most commonly used method for attaching DTPA or EDTA chelates to other molecules involves the reaction between the cyclic dianhydride **1**[12] or the carboxy carbonic mixed anhydride derivative **2**[13] of the chelating agent and an amine residue on the target molecule. The stability



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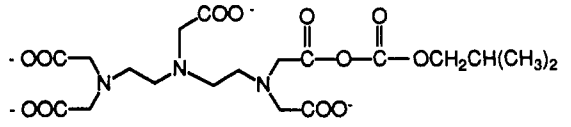

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constant, K, of the resulting carboxamide-derivatized metal chelate can be lower than that of the parent carboxylate by several orders of magnitude. For example, Gd-DTPA linked to BSA, through a carboxamide,[9] has a stability constant of 10^{19} compared to a stability constant of 10^{22} for Gd-DTPA complexing through five carboxylates.[14] The toxicity of the free Gd(III) ion[8] makes any reduction in complex stability undesirable. Another disadvantage inherent in both the mixed and dianhydride derivatives is their reactivity toward water in the aqueous solutions in which the anhydride and biomolecule are frequently coupled. To circumvent this problem large excesses of the anhydride must be used. Further, the dianhydride contains two identical reactive groups and must be viewed as a potential cross-linking reagent.[15]

One solution to these difficulties is a well-characterized metal chelate bearing a reactive functional group, such as an isothiocyanate, through which the chelate, with its full complement of carboxylates, may be attached to another molecule. The recent report by Westerberg and co-workers[16] of the synthesis of 4-(isothiocyanatobenzyl) derivatives of EDTA and DTPA has prompted us to describe a convenient route we have developed which leads to functionalized chelates isomeric to those of Westerberg et al.

Discussion

Our initial approach to an isothiocyanate derivatized DTPA analogue paralleled the procedure by which Meares and co-workers[17] synthesized *p*-isothiocyanatobenzyl EDTA. While our work was in progress, a paper appeared by Brechbiel et al.[5] describing the synthesis of a *p*-isothiocyanatobenzyl-derivatized DTPA by the above route. The route described by Brechbiel et al. began with *p*-nitrophenylalanine, a moderately expensive starting material, and proceeded with a relatively low overall yield. Owing to difficulties with their route in our hands, we sought to develop a route beginning with the inexpensive chelating agents themselves, i.e. DTPA, EDTA.

The work of Rathke and co-workers[18] on the low-temperature alkylation of ester enolates constituted a promising route to α-benzylated DTPA and EDTA permethyl esters (Scheme I). The permethyl DTPA ester **3** and EDTA ester **4** were synthesized in high yield by the action of $MeOH/SOCl_2$ on the corresponding chelating agents. Attempts to introduce the nitrobenzyl functionality by alkylation of a THF solution of the enolate at −78 °C with 3- or 4-nitrobenzyl bromide/HMPA consumed the starting ester but gave rise to brightly colored, intractable mixtures, possibly through a radical anion intermediate.[19] Benzylation of the enolate was accomplished in THF at −78 °C with a mixture of benzyl bromide and HMPA in 30–40% yield after optimization. Nitration of the benzylated methyl esters **5** and **6** with HNO_3/H_2SO_4 took place predominantly at the para position and, after reesterification and silica gel chromatography, gave the *p*-nitrobenzyl-substituted esters **7** and **8** in 80–86% yield. The nitro compounds were reduced to the corresponding amines **9** and **10** with H_2 and Pd/C. While attempts to isolate the intermediate amino permethyl esters **9** and **10** were not rewarding, the aminocarboxylates **11** and **12** could be isolated in pure form after saponification of their methyl esters **9** and **10** with aqueous LiOH. Rapid and quantitative formation of the isothiocyanates **13** and **14** was achieved by the action of $SCCl_2$ on the amines **11** and **12**. The metal complexes **15** and **16** were prepared as the disodium salts by the addition of 1 equiv of a methanolic solution of either $BiCl_3$ or $GdCl_3$ to a solution of the isothiocyanate-functionalized ligand in MeOH followed by

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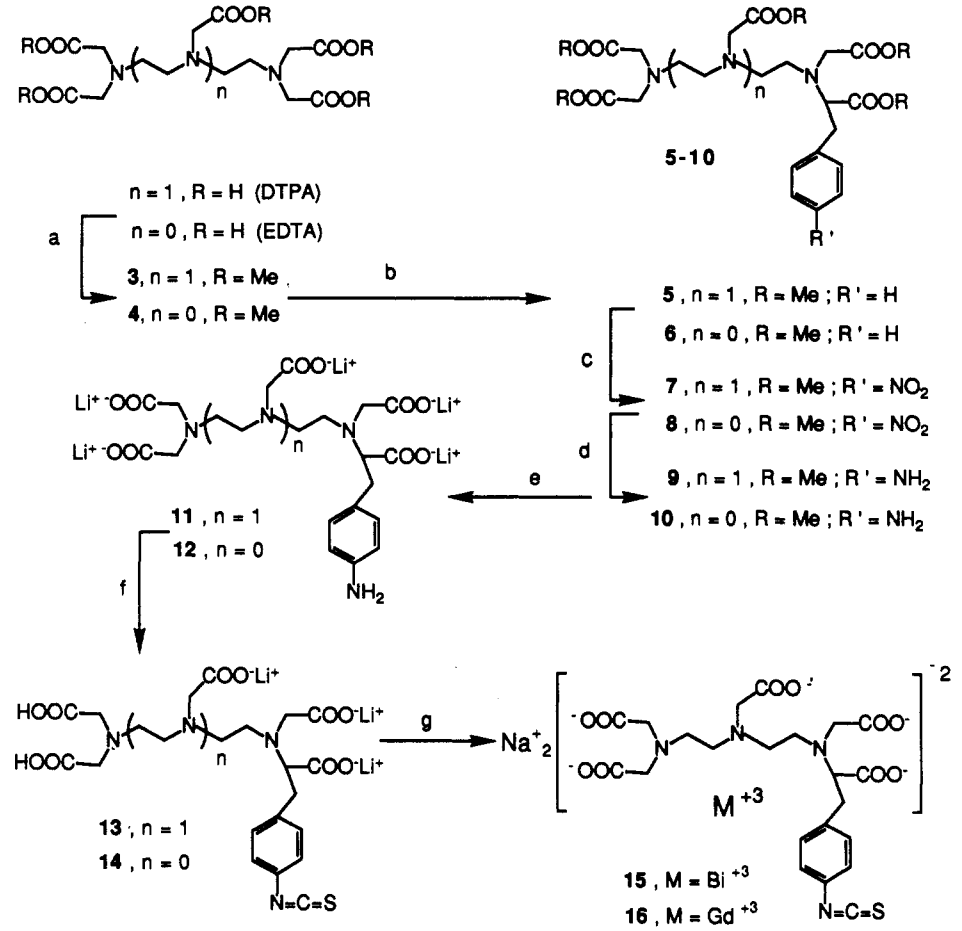
Scheme I[a]



[a] (a) $SOCl_2$/MeOH; (b) LDA, benzyl bromide/HMPA; (c) HNO_3/H_2SO_4; (d) H_2/Pd; (e) LiOH/H_2O; (f) $SCCl_2$/MeOH; (g) MCl_3, 2 equiv of NaOH.

the addition of 2 equiv of methanolic NaOH. The pure complexes were separated from the byproduct LiCl by taking advantage of the solubility of the latter in dry acetone.

The route described herein provides analytically pure monofunctionalized ligands which are synthesized in a few steps from the inexpensive chelating agents themselves.

Experimental Section

Diethylenetriaminepentaacetic acid (DTPA, Aldrich), ethylenediaminetetraacetic acid (EDTA, Sigma), and $GdCl_3$ (Aldrich) were used without further purification. Diisopropylamine (Aldrich), hexamethylphosphoric triamide (HMPA, Aldrich), benzyl bromide (Aldrich), and thionyl chloride (J. T. Baker) were all purified by standard methods[20] prior to use. *n*-Butyllithium was purchased from Aldrich as a 2.5 M solution in hexanes. Anhydrous tetrahydrofuran (THF) was obtained by distillation under N_2 from sodium benzophenone ketyl. Lithium diisopropylamide (LDA) was freshly prepared for each reaction.[21] Proton NMR spectra were obtained using a GE QE 300 spectrometer. The samples were dissolved in $CDCl_3$ (7.26 ppm), CD_3OD (3.30 ppm), or D_2O (4.80 ppm), and the chemical shifts were reported in ppm on the δ scale using the residual proton absorbances of these solvents as references. Infrared spectra were obtained as KBr pellets on a Nicolet 5DXB FTIR spectrometer. Flash chromatography was performed on Davisil grade 643 silica gel (mesh 200–425, Aldrich). Preparative thin-layer chromatography was performed on Analtech 1000 μm silica gel plates. All reactions were performed under a dry N_2 atmosphere.

[[(Methoxycarbonyl)methyl]imino]bis(ethylenenitrilo)-tetraacetic Acid Tetramethyl Ester (3). To a stirred suspension of DTPA (20.6 g, 52.4 mmol) in dry MeOH (600 mL) at 0 °C was added dropwise neat $SOCl_2$ (62.3 g, 524 mmol). The resulting clear solution was stirred for 16 h, and then the volatiles were stipped off by rotary evaporation. The white solid residue was suspended in Et_2O (300 mL) at 0 °C as saturated aqueous $NaHCO_3$ (200 mL) was slowly added. The organic layer was removed, and the aqueous layer was extracted with Et_2O (3 × 100 mL). The ether solutions were combined, dried (K_2CO_3), evaporated to dryness, and then maintained for 12 h at 0.1 mmHg and 25 °C over P_2O_5, affording pentamethyl ester **3** (20.4 g, 84%) as a colorless oil: NMR ($CDCl_3$) δ 2.76–2.82 (m, 8), 3.47–3.70 (m, 25). Anal. Calcd for $C_{19}H_{33}N_3O_{10}$: C, 49.24; H, 7.18; N, 9.07. Found: C, 48.96; H, 7.00; N, 9.36.

(Ethylenedinitrilo)tetraacetic Acid Tetramethyl Ester (4). Tetramethyl ester **4** was prepared in the same manner as **3** from EDTA (10.6 g, 36.3 mmol) and $SOCl_2$ (41.0 g, 343 mmol) in MeOH (400 mL) and isolated as a colorless oil in 78% yield: NMR ($CDCl_3$) δ 2.92 (s, 4), 3.63 (s, 8), 3.70 (s, 12). Anal. Calcd for $C_{14}H_{24}N_2O_8$: C, 48.27; H, 6.94; N, 8.04. Found: C, 48.08; H, 6.96; N, 7.93.

***N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-DL-phenylalanine Pentamethyl Ester (5).** LDA was prepared by adding *n*-butyllithium (13.2 mL, 33.0 mmol) to a stirred THF solution (125 mL) of diisopropylamine (3.30g, 33.0 mmol, 1.20 eq) at 0 °C. This colorless solution was maintained at 0 °C for 15 min and then cooled to −78 °C, and tetramethyl ester **3** (13.04 g, 27.77 mmol) in THF (200 mL) was added dropwise via cannula over 20 min. The resulting yellow solution was stirred an additional 20 min and then a THF solution (150 mL) of benzyl bromide (5.64 g, 33.0 mmol) and HMPA (1.4 g, 8.0 mmol) was added dropwise over 30 min. The solution was stirred for 12 h during which time it slowly

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warmed to 25 °C. The resulting yellow suspension was evaporated to dryness, and the residue was dissolved in EtOAc (50 mL) and added to cracked ice/H_2O (15 mL). After the organic layer was removed, the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, dried (K_2CO_3), and evaporated to dryness, affording crude **5** as a yellow oil (15.0 g). Flash chromatography over silica gel (500 g) (3:7 EtOAc–hexanes) gave the monobenzylated pentamethyl ester (5.5 g, 36%) as a pale yellow oil; R_f = 0.48. An analytical sample was obtained as a pale yellow oil by preparative TLC (3:1 Et_2O–THF; R_f = 0.62), followed by drying for 16 h at 25 °C and 0.1 mmHg: NMR ($CDCl_3$) δ 2.64–2.91 (m, 9), 3.39–3.60 (m, 10), 3.63–3.66 (m, 15), 7.15–7.23 (m, 5). Anal. Calcd for $C_{26}H_{39}N_3 \cdot 1.5H_2O$: C, 53.80; H, 7.29; N, 7.24. Found: C, 53.92; H, 7.04; N, 7.35.

***N*-[2-[Bis(carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-DL-phenylalanine Tetramethyl Ester (6).** Benzylated tetramethyl ester **6** was prepared similarly to **5** from tetramethyl ester **4** (5.47 g, 15.7 mmol), in THF (50 mL), LDA (18.8 mmol) in THF (100 mL), and an alkylation mixture consisting of benzyl bromide (3.21 g, 18.8 mmol) and HMPA (1.5 g, 8.6 mmol) in anhydrous THF (40 mL). The resulting yellow oil was chromatographed on silica gel (150 g) (2:8 EtOAc–hexanes), giving the desired product (2.2 g, 32%) as a pale yellow oil; R_f = 0.62. An analytical sample was obtained as a pale yellow oil by preparative TLC (Et_2O): NMR ($CDCl_3$) δ 2.88 (s, 4), 3.61–3.68 (m, 21), 7.18–7.33 (m, 5). Anal. Calcd for $C_{21}H_{30}N_2O_8$: C, 57.52; H, 6.89; N, 6.39. Found: C, 57.27; H, 6.69; N, 6.41.

***N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-nitro-DL-phenylalanine Pentamethyl Ester (7).** Concentrated HNO_3 (275 mg, 3.12 mmol) was added dropwise via micropipet to a stirring solution of benzylated ester **5** (1.43 g, 2.65 mmol) in 97% H_2SO_4 (5 mL) at 0 °C. The resulting brown solution was stirred for 3 h, and then it was poured onto cracked ice (10 mL), cooled, and carefully taken to pH 5 with 7 N NaOH. The yellow suspension was evaporated to dryness, and the residue was repeatedly suspended in MeOH and evaporated (3 × 10 mL). The residue was resuspended in MeOH (50 mL) and cooled to 0 °C as $SOCl_2$ (4.6 g, 39 mmol) was added dropwise. The pale yellow suspension was stirred for 14 h. A workup similar to that of ester **3** gave crude **7** as a dark yellow oil (1.30 g), which was purified by flash chromatography (3:1 Et_2O–THF), giving the desired 4-nitro derivative (1.25 g, 81%) as a pale yellow oil: R_f = 0.52; NMR ($CDCl_3$) δ 2.61–2.91 (m, 8), 3.0–3.21 (m, 2), 3.55–3.70 (m, 24), 7.45 (d, 2), 8.16 (d, 2). Anal. Calcd for $C_{26}H_{38}N_4O_{12}$: C, 52.17; H, 6.37; N, 9.36. Found: C, 52.09; H, 6.40; N, 9.11.

***N*-[2-[Bis(carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-nitro-DL-phenylalanine Tetramethyl Ester (8).** Compound **8** was prepared similarly to **7** from benzylated tetramethyl ester **6** (176 mg, 0.401 mmol) and concentrated HNO_3 (30 μL, 0.5 mmol) in 97% H_2SO_4 (1.5 mL) and isolated, after preparative TLC (3:1 Et_2O–THF; R_f = 0.62), as a light yellow oil (156.0 mg, 80%): NMR ($CDCl_3$) δ 2.65–2.89 (m, 4), 2.98–3.14 (m, 2), 3.41–3.69 (m, 19), 7.42 (d, 2), 8.13 (d, 2). Anal. Calcd for $C_{21}H_{29}N_3O_{10}$: C, 52.17; H, 6.05; N, 8.69. Found: C, 52.54; H, 6.14; N, 8.44.

***N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-amino-DL-phenylalanine Pentamethyl Ester (9).** A methanolic solution (20 mL) of **7** (306.2 mg, 0.5115 mmol) in a Parr shaker bottle was treated with 30% Pd/C (47.2 mg) and shaken under 60 psi of H_2 for 3 h. TLC (3:1 Et_2O–THF) showed complete conversion of the starting material. The suspension was filtered through Celite, and the colorless solution was used immediately without further purification.

***N*-[2-[Bis(carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-amino-DL-phenylalanine Tetramethyl Ester (10).** The nitro derivative **8** (93.1 mg, 0.193 mmol) in MeOH (15 mL) was reduced to the amino compound **10** in a manner similar to **7** with 30% Pd/C (15 mg). The suspension was filtered through Celite, and the nearly colorless solution was used without further concentration or purification.

***N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-amino-DL-phenylalanine Pentalithium Salt (11).** The methanolic solution of amine **9** was transferred to a 100-mL round-bottomed flask and stirred as a solution of LiOH (105.0 mg, 2.502 mmol) in H_2O (2 mL) was added. The resulting pale yellow solution was stirred for 3 h and then evaporated to dryness. The residue was dissolved in MeOH and evaporated (3 × 10 mL). The pale yellow powder (270.0 mg, 100%) was pure by NMR: NMR (CD_3OD) δ 2.25–2.80 (m, 9), 3.10–3.15 (m, 2), 3.41–3.50 (m, 8), 6.61 (d, 2), 7.03 (d, 2).

***N*-[2-[Bis(carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-amino-DL-phenylalanine Tetralithium Salt (12).** In a manner similar to **9** the methanolic solution of amine **10** was treated with LiOH (32.3 mg, 0.770 mmol) in H_2O (2 mL). The pure (by NMR) product was isolated as a pale yellow powder (82.6 mg, 98%): NMR (CD_3OD) δ 2.98–3.14 (m, 4), 3.16–3.18 (m, 9), 6.50 (d, 2), 6.93 (d, 2).

***N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-isothiocyanato-DL-phenylalanine Trilithium Salt (13).** A solution of **11** (174.3 mg, 0.3122 mmol) in MeOH (5 mL) was treated with a 0.20 N solution of thiophosgene in chloroform (1.7 mL, 0.34 mmol) and stirred for 1 h. The solution was then evaporated to dryness, giving a very pale yellow powder (215.0 mg). Pure ligand **13** (172.1 mg, 99%) was obtained as an off-white solid by precipitating the ligand from MeOH (3 mL) with dry acetone (30 mL). The solid was collected by filtration, washed with acetone (3 × 5 mL), and then dried at 35 °C and 0.1 mmHg for 12 h: NMR (D_2O) δ 2.98–3.32 (m, 17), 3.53–3.60 (m, 2), 7.10 (d, 2), 7.30 (d, 2); IR 2121.6 cm^{-1}. Anal. Calcd for $C_{22}H_{25}N_4O_{10}SLi_3 \cdot H_2O$: C, 45.85; H, 4.72; N, 9.72. Found: C, 46.09; H, 4.69; N, 9.28.

***N*-[2-[Bis(carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-isothiocyanato-DL-phenylalanine Dilithium Salt (14).** In a manner similar to **11** a solution of **10** (82.6 mg, 0.196 mmol) in MeOH (10 mL) was treated with thiophosgene (0.20 mmol) and stirred for 1 h. The volatiles were removed, and pure ligand **14** was isolated as a cream-colored powder (81.4 mg, 95%) by precipitation from MeOH (2 mL) with dry acetone (30 mL): NMR (D_2O) δ 2.90–3.48 (m, 13), 7.18 (d, 2), 7.24 (d, 2); IR 2114.1 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_3O_8SLi_2 \cdot 2H_2O$: C, 44.36; H, 4.76; H, 8.62. Found: C, 44.36; H, 4.72; N, 8.36.

Bismuth(III) *N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-isothiocyanato-DL-phenylalanine Disodium Salt (15). A solution of **13** (30.3 mg, 5.43 × 10^{-2} mmol) in methanol (10 mL) was treated with $BiCl_3$ (16.7 mg, 5.43 × 10^{-2} mmol) dissolved in MeOH (5 mL) and stirred for 30 min. The resulting suspension was treated with 1.10 mL (0.110 mmol) of a 0.100 M solution of NaOH in MeOH. The solution was stirred an additional 30 min and then evaporated to dryness. The residue was taken up in MeOH (2 mL), and an off-white solid was precipitated by the addition of dry acetone (25 mL). The suspension was filtered, and the solid was washed with acetone (3 × 10 mL), dissolved in H_2O (5 mL), filtered, and lyophilized, giving the product as a white powder (37.7 mg, 90%): IR 2118.0 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_4O_{10}SBiNa_2 \cdot H_2O$: C, 30.63; H, 3.62; N, 6.49. Found: C, 30.77; H, 3.29; N, 6.51.

Gadolinium(III) *N*-[2-[[2-[Bis(carboxymethyl)amino]ethyl](carboxymethyl)amino]ethyl]-*N*-(carboxymethyl)-4-isothiocyanato-DL-phenylalanine Disodium Salt (16). Methanolic solutions (5 mL, each) of **13** (200.0 mg, 0.3581 mmol) and $GdCl_3$ (133.1 mg, 0.3581 mmol) were combined and stirred for 30 min, and the resulting suspension was treated with 7.20 mL (0.720 mmol) of a 0.100 M solution of NaOH in MeOH. The resulting solution was stirred for 30 min and then evaporated to dryness. The residue was redissolved in dry methanol (2 mL), and a pale yellow solid precipitated by the addition of dry acetone (25 mL). The suspension was filtered, and the solid was washed with dry acetone (3 × 10 mL). The resulting solid was dissolved in water (10 mL), filtered, and lyophilized. The product was isolated as a white powder (243.3 mg, 92%): IR 2116.2 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_4O_{10}SGdNa_2 \cdot 3H_2O$: C, 33.31; H, 3.69; N, 7.07. Found: C, 33.47; H, 3.83; N, 6.64.

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