Synthesis of Enantiomerically Pure Diethylenetriaminepentaacetic Acid Analogues. L-Phenylalanine as the Educt for Substitution at the Central Acetic Acid

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Received September 28, 1992

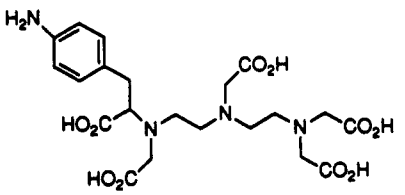
The enantiospecific synthesis of diethylenetriaminepentaacetic acid (DTPA) analogues from L-phenylalanine via a bis N-alkylation strategy is described. N-Alkylation of *p*-nitrophenylalanine benzyl ester (**4**) occurs readily with dibenzyl and di-*tert*-butyl [*N*-(bromoethyl)amino]diacetates **5a** and **5b** using a phosphate buffer/acetonitrile reaction medium. N,N-Dialkyl (**2a, 2b**) and N-monoalkyl (**3a, 3b**) derivatives of L-*p*-nitrophenylalanine thus are obtained directly in a single operation. Subjecting the monoalkylated material, *N*-(ethylamino)diacetic acid di-*tert*-butyl ester **3b**, to a second alkylation with dibenzyl bromoethylaminediacetate **5a** affords mixed pentaester **2c** in which the terminal carboxyl groups are differentiated as benzyl esters at one end and *tert*-butyl at the other. Both complete and selective deprotections of the differentiated carboxyl groups in pentaesters **2a, 2b,** and **2c** are possible, allowing specific control of the carboxyl functionality at each terminus. The enantiomeric composition of monoalkyl amino acids **3a** and **3b** was evaluated by derivatizing with (+)-α-methylbenzyl isocyanate to afford the corresponding ureas. Analysis of the ureas by HPLC established the enantiomeric purity in each case as >99%, thereby also establishing the enantiomeric purity of dialkyl compounds as >99%. Pentaacid **2d** readily forms an optically active metal chelate with yttrium(III) as a single diastereomer.

Introduction

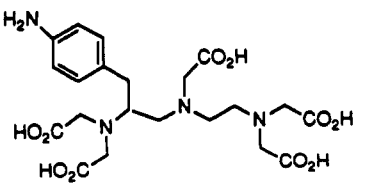
The site-specific delivery of metal ions via metal chelate–biomolecule conjugates has potential biological applications in the areas of radiodiagnostics, radiotherapy,[1,2] and magnetic resonance imaging.[3] Synthesis of functionalized ligands based on the ethylenediaminetetraacetic acid[4] (EDTA) or diethylenetriaminepentaacetic acid[4b,c,d,5] (DTPA) chelating moiety have been reported. These modified chelators possess an additional functional group that allows their covalent attachment to biomolecules. Traditionally, the covalent linkage of DTPA to proteins was accomplished through one of the carboxyl groups.[6] This, however, can compromise the stability of the metal–ligand complex by removing a carboxyl group as a metal coordinating site on the ligand. Also, introduction of an additional functional group in DTPA requires branching either at one of the five methylenes of the acetic acid residues or at one of the four carbons of the backbone. Such branching might assert a conformational consequence and, in turn, influence the properties of the metal–ligand complex.

A functional group commonly utilized in the preparation of DTPA and EDTA bioconjugates has been a *p*-isothiocyanatobenzyl group, which generally originates in the synthesis from a *p*-nitrophenylalanine moiety.[4a] Reduction of the *p*-nitro group to the *p*-amino followed by subsequent modification offers several options in addition to isothiocyanates for covalent attachment to biomolecules.

Prior syntheses of functionalized DTPA analogues have placed the *p*-nitrobenzyl group at either the methylene of a terminal acetic acid group[4b,c] (**1a**) or a methylene of the diethylenetriamine backbone[4d,5] (**1b**). As has been pointed out,[4b] the position of this substituent may have significant effects on the conformation and stability of the resulting metal chelate. In addition, these previously reported DTPA analogues, derived from *p*-nitrophenylalanine, have not been prepared in enantiomerically pure form.



1a



1b

We projected that optically pure DTPA analogous could be readily prepared from amino acids using the bis N-alkylation strategy shown in Scheme I. In this manner, the central acetic acid group of the parent DTPA structure

(1) Parker, D. *Chem. Brit.* 1990, 942.
(2) Marx, J. L. *Science* 1982, *216*, 283.
(3) Lauffer, R. B. *Chem. Rev.* 1987, *87*, 901.
(4) (a) Sundberg, M. W.; Meares, C. F.; Goodwin, D. A.; Diamanti, D. I. *J. Med. Chem.* 1974, *17*, 1304. (b) Westerburg, D. A.; Carney, P. L.; Rogers, P. E.; Kline, S. J.; Johnson, D. K. *J. Med. Chem.* 1989, *32*, 236. (c) Keana, J. F. W.; Mann, J. S. *J. Org. Chem.* 1990, *55*, 2868. (d) Brechbiel, M. W.; Gansow, O. A.; Atcher, R. W.; Schlom, J.; Esteban, J.; Simpson, D. E.; Colcher, D. *Inorg. Chem.* 1986, *25*, 2772.
(5) (a) Cummins, C. H.; Rutter, E. W.; Fordyce, W. A. *Bioconjugate Chem.* 1991, *2*, 180. (b) Brechbiel, M. W.; Gansow, O. A. *Bioconjugate Chem.* 1991, *2*, 187. (c) Mirzadeh, S.; Brechbiel, M. W.; Atcher, R. W.; Gansow, O. A. *Bioconjugate Chem.* 1990, *1*, 59.
(6) (a) Krejcarek, G. E.; Tucker, K. L. *Biochem. Biophys. Res. Commun.* 1977, *77*, 581. (b) Hnatowich, D. J.; Layne, W. W.; Childs, R. L.; Lanteign, D.; Davis, M. A.; Griffin, T. W.; Doherty, P. W. *Science* 1983, *220*, 613.

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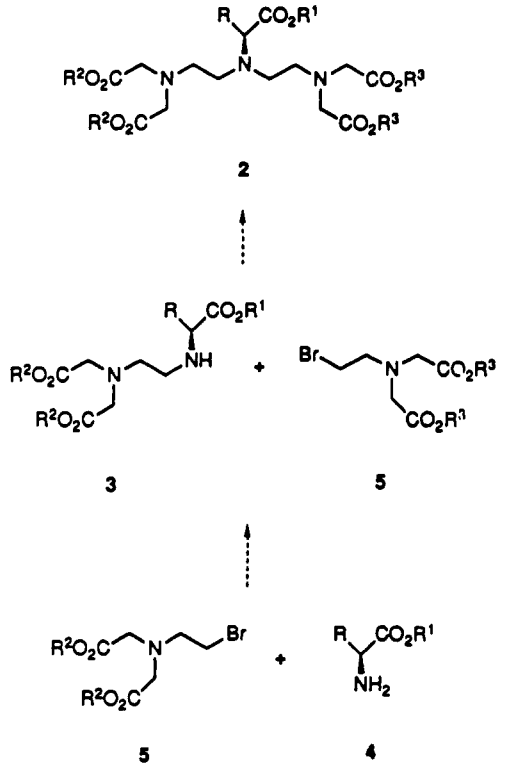
Scheme I. Plan for Preparation of Unsymmetrical Phenylalanine Analogues of DTPA

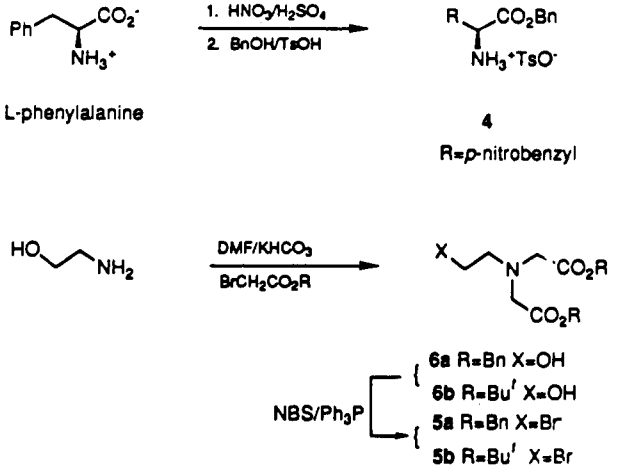


would bear the functional group. By placing the branching group on this central acetic acid, a certain symmetry would be retained in the ligand arms. In addition, since this group originates from the amino acid, access to a variety of functional groups is possible, depending on the amino acid chosen. Furthermore, a stepwise alkylation-based assembly of **2** allows control over the choice of carboxyl protecting groups of the five acetic acid groups, a feature which will facilitate selective deprotection and further modification.[7] We present successful application of this method and report the synthesis of DTPA analogues in >99% enantiometric purity from L-phenylalanine.

Results and Discussion

Preparation of Amino Acid Ester 4 and Bromides 5a and 5b. The synthetic strategy depicted in Scheme I required preparation of two separate components: a protected amino acid and a protected bis-carboxymethylated amino ethyl bromide. Protection of all carboxyl groups was invoked from the beginning, allowing conventional chromatographic purification of the ligand prior to final deprotection. Additionally, the carboxyl protecting groups were chosen such that deprotection would not introduce intractable impurities. A recent synthesis of DTPA analogues also noted this advantage of carboxyl group protection.[5a] Previous synthetic methods required purification of the final polyaminepolycarboxylic acids by ion-exchange chromatography and reversed-phase HPLC. In view of the convenience of deprotecting benzyl esters by catalytic hydrogenolysis, an initial synthetic target was pentabenzyl ester **2a** (Scheme I, $R^1 = R^2 = R^3$ = Bn) necessitating protection of all carboxyl groups in the amino acid and bromoethylamine components as

Scheme II



benzyl esters. *tert*-Butyl esters also conform to this strategy, and [*N*-(bromoethyl)amino]diacetic acid ester **5b** (R = *t*-Bu) was also prepared, thus allowing synthesis of DTPA analogues possessing differentiated carboxyl groups at the east and west termini.

Amino acid ester **4** was readily prepared from L-phenylalanine as shown in Scheme II. Nitration as reported[8] afforded the pure para isomer in 40% yield after several recrystallizations. Protection of the carboxyl group as the benzyl ester was achieved under Dean–Stark conditions (BnOH, *p*-TsOH, benzene)[9] in 96% crude yield, and recrystallization of **4** provided analytically pure material. It was essential to ensure that the optical purity of this material was >99%. This point will be further addressed in the section on enantiomeric purity.

As indicated, control of carboxyl group protection was achieved by preparation of [*N*-(bromoethyl)amino]diacetic acid esters **5a** and **5b**, outlined in Scheme II. Treatment of ethanolamine, with either benzyl bromoacetate or *tert*-butyl bromoacetate in DMF with $KHCO_3$, gave the bis N-alkylated alcohols **6a** and **6b**, respectively. While *tert*-butyl ester **6b** was isolated in 84% yield, the corresponding benzyl ester incurred significant lactone formation upon purification by silica gel chromatography. In practice, it was more convenient to isolate the alcohols by liquid–liquid extraction and then directly treat the crude product with NBS/Ph_3P in dichloromethane to afford bromides **5a** and **5b** in 70% and 86% yield, respectively, for the two steps. Benzyl and *tert*-butyl ester bromides **5a** and **5b** are stable and could be readily purified by chromatography. Upon standing at room temperature for extended times, however, these bromides were slowly converted to a mixture of products including the corresponding aziridinium salts.

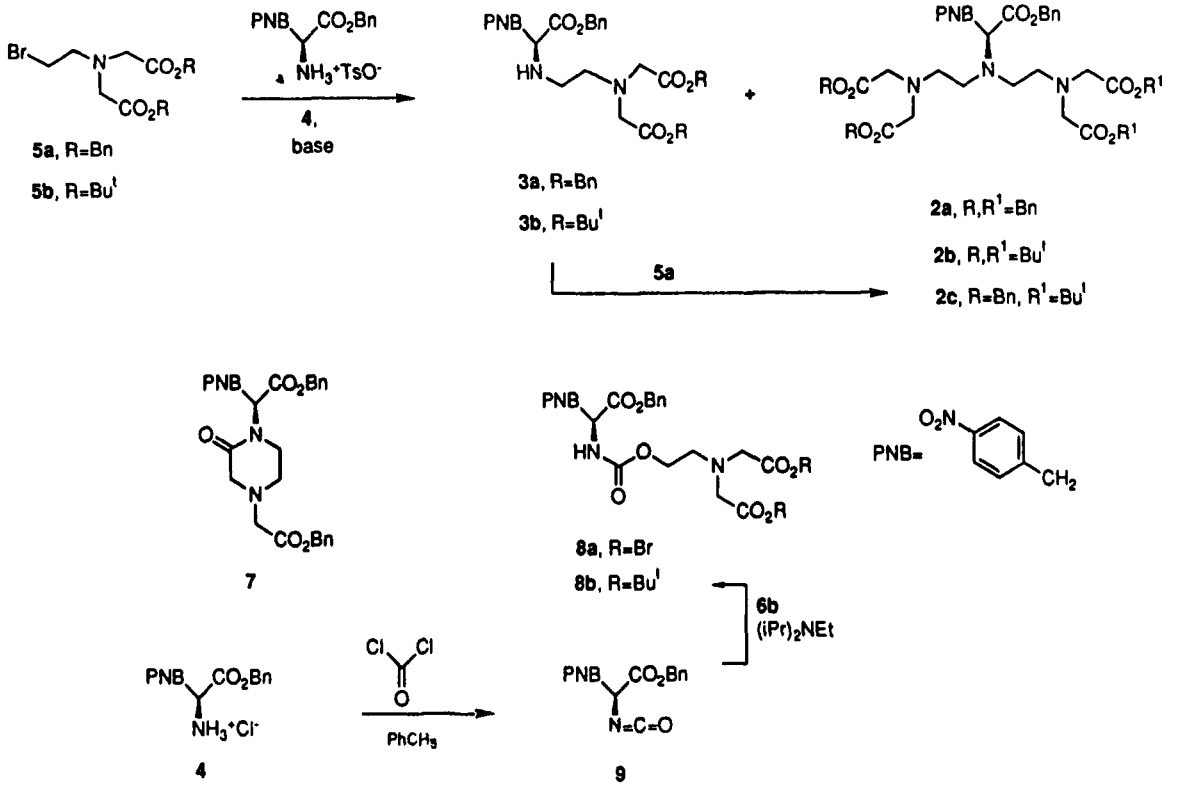
N-Alkylation of *p*-Nitrophenylalanine Benzyl Ester (4). We sought to obtain both monoalkylated and dialkylated derivatives of *p*-nitrophenylalanine as shown in Scheme III. Reaction conditions initially explored for the dialkylation of **4** utilized an excess of bromide **5** in a polar solvent (CH_3CN, DMSO, DMF) with potassium carbonate or bicarbonate as base. Although the dialkylated products were isolated, yields were poor and side products were produced. The failure of these conditions to effi-

(7) Maisano, F.; Gozzini, L.; de Haën, C. *Bioconjugate Chem.* **1992**, *3*, 212 and references cited therein.

(8) Houghten, R. A.; Rapoport, H. *J. Med. Chem.* **1974**, *17*, 556.

(9) Zervas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515.

Scheme III. N-Alkylation of *p*-Nitrophenylalanine Esters 4



ciently effect dialkylation was attributed in part to the insolubility of tosylate salt **4** and the use of an insoluble base.

A significant improvement in the alkylation reactions, however, was accomplished by using a 1/1 mixture of CH_3CN/water as the reaction medium with an excess of $KHCO_3$. Dialkyl *p*-nitrophenylalanines **2a** and **2b** were isolated in 42% and 61% yield, respectively. These aqueous alkylation conditions also afforded an interesting 1/1 amino acid ester/bromide adduct, which was not the monoalkyl product, but rather carbamate **8** (R = Bn, R = Bu^t). The structure of **8b** was confirmed by its independent synthesis via isocyanate **9** which was prepared in 77% yield from the hydrochloride salt of **4** and phosgene in toluene.[10] Treatment of **9** with alcohol **5b** and a catalytic amount of base gave carbamate **8b** in 79% yield, identical in every respect to the material isolated from the alkylation reactions.

The formation of carbamates **8a** and **8b** can be rationalized by considering the known behavior of amino acid derived carbamic acid salts. Amino acid esters combine readily with CO_2 in anhydrous ether to afford the *N*-carboxy-α-amino acid ester salts as labile compounds which decompose readily in the presence of moisture.[11] The apparent lability of these adducts, however, does not seem to preclude their formation under the aqueous alkylation conditions. Reversible addition of CO_2, generated from bicarbonate used as acid scavenger, to the free amine of amino acid ester **4**, followed by O-alkylation with either bromide **5a** or **5b** (or their corresponding aziridinium salts), affords carbamates **8a** and **8b**, respectively. When alkylations of the free amine derived from **4** were performed with $KHCO_3$ in dry DMF or DMSO at 60 °C, carbamate was the major product, although the yield of carbamates **8a** and **8b** isolated from the aqueous bicarbonate alkylation reaction was typically 10–15%.

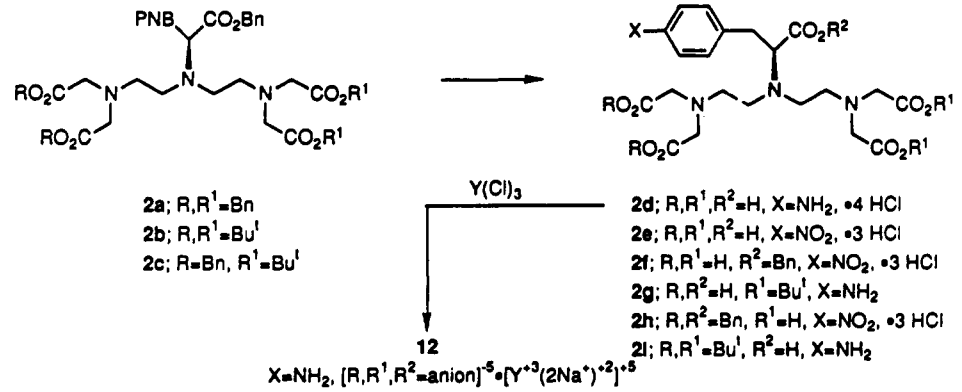
Carbamate formation was prevented by substituting 2 M, pH 8 phosphate buffer for the aqueous bicarbonate. Reaction of phenylalanine ester **4** with bromoethylamine **5a** (225 mol %) using these conditions (1/1 CH_3CN/pH 8 aqueous phosphate buffer, 24 h) gave dialkyl product **2a** in 54% yield and a 4/1 mixture of monoalkyl product **3a** and lactam **7**. Similarly, **4** and bromide **5b** gave a 59% yield of dialkylated material **2b** and 26% of monoalkyl product **3b**.

A particular advantage of this alkylation process was demonstrated in the two-step preparation of *N,N*-dialkyl-*p*-nitrophenylalanine **2c**, in which the terminal carboxyl groups are no longer symmetrically protected. Monoalkyl derivative **3a** (dibenzyl ester) was not suitable for this purpose, due to competitive formation of lactam **7**. However, treatment of di-*tert*-butyl ester **3b** with bromide **5a** in 1/1 CH_3CN/pH 8 buffer gave unsymmetrical dialkyl product **2c** in 42% yield after 42 h at room temperature. The monoalkyl starting material (46%) as well as bromide (50%) were recovered.

Carboxyl Group Deprotection. A completed synthesis of a functional metal chelator required the final deprotection of all carboxyl groups. The total and selective deprotections of DTPA's **2a**, **2b** and **2c** are summarized in Scheme IV. Cleavage of the benzyl protecting groups in pentaester **2a** by catalytic hydrogenolysis was particularly convenient since the nitro group would also be reduced under these conditions. Attempts to isolate the zwitterionic amino acid product by direct hydrogenation were not fruitful in the absence of acid. Incomplete cleavage of the benzyl esters was the result, even after prolonged exposure to 10% Pd/C in methanol at 50 psi hydrogen. However, when pentaester **2a** was subjected to the same hydrogenolysis conditions with sufficient aqueous HCl present to protonate the existing amino groups as

(10) (a) Morel, A. *Compt. Rend.* **1906**, *143*, 119. (b) Goldschmidt, S.; Wick, M. *Liebigs Ann. Chem.* **1952**, *575*, 217.
(11) Frankel, M.; Katchalski, E. *J. Am. Chem. Soc.* **1943**, *65*, 1670.

Scheme IV. Deprotection of DTPA Analogues



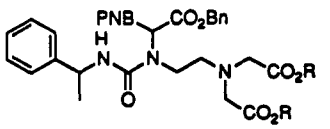
well as the nascent aniline, analytically pure pentaacid **2d** was isolated as its tetrahydrochloride salt in quantitative yield.

The ability to differentiate carboxyl groups in pentaesters **2b** and **2c** would only be realized if selective deprotection were possible. Benzyl and *tert*-butyl esters were chosen for this reason, since they should be readily and selectively removed. We first focused on the selective removal of the *tert*-butyl esters. Treatment of tetra *tert*-butyl ester **2b** with a large excess of trifluoroacetic acid gave incomplete deprotection; however, rapid and quantitative deprotecion of the *tert*-butyl esters was achieved with aqueous HCl. When **2b** was subjected to 6 M HCl for 2 h at 23 °C with methanol added as a cosolvent, a 98% yield of the fully deprotected tetraacid **2f** was obtained. Use of more vigorous acidic conditions also removed the benzyl ester in **2b** while preserving the nitro group. For example, treatment of **2b** with 6 M HCl at 50 °C to effect *tert*-butyl ester cleavage, followed by heating at reflux for 1 h to cleave the benzyl ester, gave pentaacid **2e** in quantitative yield. Reaction conditions that were successful in the removal of four *tert*-butyl esters, **2b** to **2f**, however, gave incomplete de-*tert*-butylation when applied to mixed di-*tert*-butyl ester **2c**, due to the heterogeneity of the reaction mixture. An effective combination of 1/1, 12 M aqueous HCl and dioxane gave the desired deprotected diacid **2h** in 98% yield as its trihydrochloride salt.

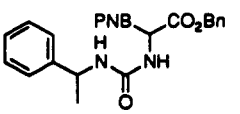
Removal of the benzyl esters in analogues **2b** and **2c** was anticipated to occur readily under catalytic hydrogenation conditions, as was the case. Both were quantitatively debenzylated, as shown by NMR analysis, upon treatment with hydrogen and 10% Pd/C in methanol, affording monoacid **2i** and triacid **2g**.

Determination of Enantiomeric Purity. A primary objective of this synthesis was the preparation of DTPA analogues as pure enantiomers. It still remained to be determined whether any racemization of the asymmetric center had occurred at any point during the synthesis. The most direct approach was to verify the optical integrity of either dialkyl derivative **2a** or **2b**; however, efforts to derivatize the central carboxyl group of dialkyl compound **2b** followed by determination of diastereomer composition by HPLC could not be effected. More suitable substrates to assay for enantiomeric purity were monoalkyl *p*-nitrophenylalanines **3a** and **3b**, which contain an available secondary amine for derivatization. By determining the enantiomeric purity of the monoalkyl products, the enantiomeric purity of the dialkyl products can be inferred, since the dialkyl products are derived from the monoalkyl. Isolation of the mono- and dialkyl products from the same reaction ensures both have been exposed to identical conditions. While the mono- and dialkyl products conceivably could behave differently in the reaction medium with respect to racemization, it appeared highly unlikely that any base-catalyzed epimerization incurred in the alkylation process would be significantly greater for the more sterically hindered dialkyl product than the monoalkyl.

Alkylation of *p*-nitrophenylalanine benzyl ester (**4**) with **5a** and **5b** was conducted as described previously using the CH_3CN/pH 8 buffer protocol, and the monoalkyl derivatives were isolated by silica gel chromatography. Optical antipodes of **3a** and **3b** were prepared from D-phenylalanine. The monoalkyl products were then treated with an excess (120–130 mol %) of α-methylbenzyl isocyanate, affording the corresponding ureas **10a** and **10b**. Analysis of the crude reaction by HPLC indicated the diastereomeric ratio in each case to be about 97/3. Therefore, approximately 3% of enantiomeric impurity was present in the monoalkyl products.



10a, R = Bn
10b, R = Bu^t



11

To determine whether the loss of stereochemical integrity occurred at or before the alkylation stage, amino acid ester **4** was then derivatized in order to determine its enantiomeric purity. Analysis of α-methylbenzyl isocyanate adduct **11** by HPLC determined the diastereomeric ratio to be also about 97/3, thus indicating racemization had ensued during the nitration of phenylalanine or the subsequent benzyl ester formation and not at the alkylation stage. The esterification of *p*-nitrophenylalanine was confirmed as the source of epimerization by allowing the esterification process to continue for longer time periods.[12] Using recrystallized **11** in subsequent alkylations gave monoalkyl products in which the enantiomeric purity was ascertained to be >99%. Hence, dialkyl compounds **2a** and **2b** were presumed also to be >99% enantiomerically pure. Although mixed ester **2c** was obtained by a two-step process, in which the monoalkyl product was isolated and resubjected to alkylation, the level of enantiomeric

(12) Benzyl ester prepared over a 6-h reflux time was found to be >99% enantiomerically pure, while benzyl ester isolated after a 24-h reflux period had an enantiomeric ratio of 93/7.

purity should be the same as for the dialkyl derivatives obtained by a single-step operation under the same conditions.

Partial racemization of **4** under the acidic conditions of the esterification caused some concern of similar racemization in the final products **2e**, **2f**, and **2h** which were exposed to acid during the deprotection step. To examine this possibility, **2f** was converted to **2d** by catalytic hydrogenation. This material was compared with a sample of **2d** prepared by the catalytic hydrogenation of penta benzyl ester **2a**. Both samples of **2d** had identical rotations, suggesting that **2f** had been unaffected by the acid deprotection conditions used to convert mixed ester **2b** to diacid **2f** (6 M HCl, rt). The acidic conditions employed in the conversion of tetra-*tert*-butyl ester **2b** to tetra acid **2e** (6 M HCl, reflux) resulted in racemic material. Furthermore, subjecting **2d** to refluxing 6 M HCl for 1 h gave optically inactive material. Inductive effects of the *p*-nitro and *p*-amino substituents are clearly operative and significant when compared to the absence of any racemization of L-phenylalanine in refluxing 6 M HCl over a 20-h period.[13]

Yttrium Chelate. A previous synthesis of a related DTPA analogue **1b** reported chelation chemistry with yttrium.[5a] That analogue, prepared from D,L-*p*-nitrophenylalanine, was demonstrated to form a diastereomeric mixture upon chelation with yttrium. Diastereomeric metal complexes may behave differently in vivo with respect to their stability, biodistribution, and pharmacokinetics. Assessing the biological properties of a metal–ligand complex would be simplified when a single chelate species is evaluated. We wanted to test whether our ligand **2d** would form a stable, isolable chelate and whether a single species would be formed.

Preparation of the yttrium chelate was accomplished as described.[5a] Pentaacid tetrahydrochloride salt **2d** was treated with YCl_3 to afford yttrium chelate **12** after purification by gel filtration chromatography on Sephadex. ^{13}C NMR data of the crude and purified chelate **12** indicated that chelation was quantitative and confirmed the formation of a single optically active ligand–metal complex.

Summary

The N-dialkylation of *p*-nitrophenylalanine benzyl ester with bromoethylamines **5a** and **5b** is a direct and concise method for the construction of protected DTPA analogues. Alkylation conditions are mild and provide the DTPA's **2a** and **2b** with little or no racemization of the stereogenic center. The methodology should be amenable to other amino acids, offering a variety of functional groups for subsequent covalent attachment to biomolecules. Assembly of polyaminepolycarboxylates by this route allows differentiation of the carboxyl groups depending on the carboxyl protection used in the amino acid and bromide components. Carboxyl group modification of the bromide could also be performed prior to alkylation of the amino acid. Ultimately, either approach will allow specific manipulation of the carboxyl groups, providing access to novel and important ligands.

(13) Jacobson, S. J.; Willson, C. G.; Rapoport, H. *J. Org. Chem.* **1974**, *39*, 1074.

Experimental Section

General. ^1H NMR spectra were obtained in $CDCl_3$ unless indicated otherwise and are referenced to internal tetramethylsilane, 3-(trimethylsilyl)propionate-d_4 was the reference for those in D_2O; coupling constants, J, are reported in Hz. ^{13}C NMR spectra obtained in the solvents listed were referenced as follows unless indicated otherwise: chloroform-d (δ = 77.0 ppm), DMSO-d_6 (δ = 39.5 ppm), methanol-d_4 (49.0 ppm), D_2O (internal dioxane δ = 69.0 ppm). Melting points are uncorrected. Evaporation of solvents was performed on a Berkeley rotary evaporator (35–40 °C) at aspirator pressure after drying over Na_2SO_4. High-pressure liquid chromatography (HPLC) was conducted on a 4.6- × 250-mm 5-μM Microsorb Si normal-phase silica column. Low-pressure chromatography (LPC) was performed with silica gel 60, 230–400 mesh (EM Science). TLC analysis was performed on aluminum-backed silica gel 60 F_{254}, 0.2-mm plates (MCB Reagents). All reactions were performed under a nitrogen atmosphere unless indicated otherwise. Dioxane and THF were distilled from sodium and benzophenone. Diisopropylethylamine and toluene were distilled from calcium hydride.

4-Nitro-L-phenylalanine Benzyl Ester Tosylate Salt (4). 4-Nitro-L-phenylalanine monohydrate (4.3 g, 18.84 mmol)[8] was finely powdered and combined with *p*-toluenesulfonic acid monohydrate (4.3 g, 22.6 mmol, 120 mol %) in benzyl alcohol (40 mL, 38.6 mmol, 2000 mol %) and benzene (30 mL). The reaction mixture was heated at reflux for 10 h during which time ~2 mL of water was collected in a Dean-Stark trap, the homogeneous yellow solution was cooled, ether (90 mL) was added, and the solid was collected by filtration. The solid was rinsed with ether (3 × 60 mL) and dried under vacuum to afford crude benzyl ester **4** (8.56 g, 96% yield). Recrystallization from ethanol gave **4** as fine white needles: mp 179–181 °C; ^1H NMR (DMSO-d_6) δ 2.29 (s, 3 H), 3.21 (dd, 1 H, J = 8.1, 14.0), 3.32 (dd, 1 H, J = 6.1, 14.0), 4.49 (app q, 1 H), 5.10 (d, 1 H, J_{AB} = 12.3), 5.16 (d, 1 H, J_{AB} = 12.3), 7.13 (d, 2 H, J = 8.2), 7.21–7.36 (m, 5 H), 7.48 (d, 2 H, J = 8.8), 7.54 (d, 2 H, J = 8.2), 8.09 (d, 2 H, J = 8.8); ^{13}C NMR (DMSO-d_6) δ 20.9, 35.7, 52.9, 67.3, 123.5, 125.6, 128.4, 128.3, 130.8, 134.7, 138.4, 142.9, 144.7, 146.7, 168.5. Anal. Calcd for $C_{23}H_{24}N_2O_7S$: C, 58.5; H, 5.1; N, 5.9. Found: C, 58.8; H, 4.9; N, 5.8.

***N,N*-Bis[(benzyloxycarbonyl)methyl]-2-bromoethylamine (5a).** To benzyl bromoacetate (16.0 g, 69.8 mmol, 225 mol %) dissolved in DMF (50 mL) was added $KHCO_3$ (7.81 g, 78.0 mmol, 250 mol %). The suspension was cooled to 0 °C, ethanolamine (1.89 g, 31.0 mmol) was added over a 5-min period, and the reaction mixture was stirred at 0 °C for 30 min and then for 22 h at rt. After partitioning between ether (150 mL) and saturated $NaHCO_3$ (100 mL), the organic phase was washed again with saturated $NaHCO_3$ (100 mL). The combined aqueous washes were extracted with ether (100 mL), and the combined ether extracts were washed with brine (100 mL), dried, and evaporated to give the crude product (12.4 g) as an oil. The crude dialkylated ethanolamine was dissolved in CH_2Cl (100 mL), Ph_3P (8.91 g, 34 mmol) was added, the solution was cooled to 0 °C, and solid NBS (6.05 g, 34.0 mmol) was added portionwise over 5 min. After the solution was stirred at 0 °C for 1.5 h, evaporation of the solvent gave a semisolid residue which was triturated with ether (200 mL) and the resulting solid was separated. The ether phase was concentrated and passed through a short column of silica, eluting with ether. Evaporation of the ether gave a pale yellow oil which was purified by silica gel chromatography (2/1, hexanes/ether) to afford bromide **5a** (9.10 g, 70% yield) as a light yellow oil: R_f 0.26 (2/1 hexanes/ether); IR (thin film) 3050, 3020, 2940, 1730 cm^{-1}; ^1H NMR δ 3.19 (t, 2 H, J = 7.4), 3.42 (t, 2 H, J = 7.4), 3.68 (s, 4 H), 5.16 (s, 4 H), 7.37 (s, 10 H); ^{13}C NMR δ 55.1, 56.2, 66.1, 128.0, 128.2, 135.2, 170.5. Anal. Calcd for $C_{20}H_{22}NO_4Br$: C, 57.2; H, 5.3; H, 3.3. Found: C, 57.3; H, 5.4; N, 3.3.

***N,N*-Bis[(*tert*-butoxycarbonyl)methyl]-2-bromoethylamine (5b).** Bromide **5b** was prepared in an analogous procedure as described for **5a**, from ethanolamine (1.85 mL, 30.7 mmol), *tert*-butyl bromoacetate (13.5 g, 69.2 mmol, 225 mol %), and $KHCO_3$ (7.68 g, 76.8 mmol, 250 mol %) in DMF (50 mL) followed by treatment of the crude product (9.58 g) with NBS (6.0 g, 33.8 mmol) and Ph_3P (8.87 g, 33.8 mmol) in CH_2Cl_2 (100 mL). Isolation of the product was performed as described above,

and the crude bromide was purified by chromatography on silica gel (5/1, hexanes/ether) to afford bromide **5b** (9.29 g, 86%) as a colorless oil: R_f 0.29 (5/1 hexanes/ether); IR (thin film) 2980, 2940, 1735 cm^{-1}; ^{1}H NMR δ 1.46 (s, 18 H), 3.13 (t, 2 H, J = 7.7), 3.44 (t, 2 H, J = 7.7), 3.48 (s, 4 H); ^{13}C NMR δ 27.6, 29.7, 55.8, 56.1, 80.4, 169.7. Anal. Calcd for $C_{14}H_{26}NO_4Br$: C, 47.7; H, 7.4; N, 4.0. Found: C, 48.1; H, 7.5; N, 3.9.

***N,N*-Bis[2-[*N',N'*-bis[(benzyloxycarbonyl)methyl]amino]-ethyl]-4-nitro-L-phenylalanine Benzyl Ester (2a).** In a 100-mL Morton flask equipped with mechanical stirring were combined bromide **2a** (7.6 g, 18.1 mmol, 225 mol %), tosylate salt **4** (3.8 g, 8.1 mmol, >99% optically pure) in CH_3CN (20 mL), and phosphate buffer (20 mL, 2 M, pH 8). The reaction mixture was vigorously stirred for 2 h, and the lower buffer layer (pH 7) was removed and was extracted with CH_3CN (10 mL). The CH_3CN extract was added to the reaction mixture along with new buffer solution (20 mL, pH 8), and stirring was continued for an additional 22 h. The CH_3CN phase was separated, and the solvent was evaporated to afford a residue which was partitioned between pH 8 buffer (100 mL) and ethyl acetate (100 mL). The organic phase was washed with brine (2 × 100 mL), dried, and evaporated to leave a crude oil. Chromatography on silica gel (gradient, 1% methanol in 3/1, 2/1, hexanes/ethyl acetate) afforded pure dialkylated product **2a** (4.40 g, 55% yield) as a viscous yellow oil: HPLC (5-μm silica, 4% CH_3CN/0.05% concd NH_4OH in CH_2Cl_2, 1 mL/min) t_R = 11 min; $[\alpha]^{23}_D$ −31° (c 1.5, $CHCl_3$); IR (neat) 3070, 3040, 2960, 2860, 1730 cm^{-1}; ^{1}H NMR δ 2.57–2.87 and 2.88 (dd overlapping m, 9 H, J = 7.8, 14), 3.05 (dd, 1 H, J = 7.7, 14), 4.95 (t, 1 H, J = 7.7), 5.04 (s, 2 H), 5.09 (s, 8 H), 7.27 and 7.31 (s overlapping m, 27 H), 7.97 (d, 2 H, J = 8.8); ^{13}C NMR δ 35.7, 49.4, 52.6, 54.8, 63.9, 66.0, 123.0, 128.1, 128.2, 128.3, 128.4, 130.1, 135.4, 146.2, 146.2, 170.6, 171.4. Anal. Calcd for $C_{58}H_{58}N_4O_{12}$: C, 68.7; H, 6.0; N, 5.7. Found: C, 68.9; H, 6.0; N, 5.6.

Further elution (1% MeOH in 1/1 hexanes/ethyl acetate) gave a 4/1 mixture of lactam **7** and ***N*-[2-[*N',N'*-Bis[(benzyloxycarbonyl)methyl]amino]ethyl]-4-nitro-L-phenylalanine Benzyl Ester (3a).** Rechromatography on silica gel (2/1 hexanes/acetone) gave the pure components.

3a: IR (neat) 3320, 3040, 2945, 1730 cm^{-1}; ^{1}H NMR δ 2.46–3.05 (series of m, 6 H), 3.56 (dd and CH, 5 H), 4.99 (d, 1 H, J = 12), 5.09 (d, 1 H, J = 12), 5.12 (s, 4 H), 7.19–7.34 (s overlapping m, 17 H), 7.99 (d, 2 H, J = 8.8); ^{13}C NMR δ 39.1, 45.4, 53.7, 54.8, 62.3, 66.1, 66.3, 123.2, 128.0, 128.1, 128.3, 128.4, 129.9, 135.1, 135.5, 145.0, 146.5, 170.9, 173.4. Anal. Calcd for $C_{36}H_{37}N_3O_8$: C, 67.6; H, 5.8; N, 6.6. Found: C, 67.5; H, 5.8; N, 6.7.

7: IR (neat) 3070, 3040, 2950, 1740, 1655 cm^{-1}; ^{1}H NMR δ 2.75 (m, 2 H), 3.10–3.30, 3.28, 3.34 (m overlapping two s, 9 H), 3.46 (dd, 1 H, J = 6.3, 14.5), 5.14 (s, 2 H), 5.16 (s, 2 H), 5.26 (dd, 2 H), 7.35 (m, 12), 8.11 (d, 2 H, J = 8.8); ^{13}C NMR δ 34.0, 44.5, 48.6, 55.9, 56.8, 57.2, 66.4, 67.1, 128.1, 128.2, 128.4, 128.4, 129.6, 135.0, 135.1, 144.4, 146.7, 166.7, 169.1, 169.2.

***N,N*-Bis[2-[*N',N'*-bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl]-4-nitro-L-phenylalanine Benzyl Ester (2b).** To bromide **5b** (2.8 g, 7.95 mmol, 200 mol %) dissolved in CH_3CN (8 mL) was added 2 M pH 8 phosphate buffer (16 mL) and tosylate salt **4** (1.9 g, 3.97 mmol, >99% optically pure). After the mixture was stirred for 2 h, the buffer layer was removed and replaced with fresh buffer (16 mL), the mixture was stirred for 20 h at rt, and the crude product was isolated as described for **2a**. The crude product was purified by chromatography on silica gel (gradient, 1% methanol in 3/1, 2/1, hexanes/ethyl acetate) affording a small amount of recovered **5b** and dialkylated tetra-*tert*-butyl ester **2b** (1.97 g, 59% yield) as a yellow oil: $[\alpha]^{23}_D$ −35° (c 1.5, $CHCl_3$); IR (neat) 2970, 2930, 1730 cm^{-1}; ^{1}H NMR δ 1.44 (s, 36 H), 2.60–2.87 (m, 8 H), 3.01 (dd, 1 H, J = 7.9, 13.8), 3.17 (dd, 1 H, J = 7.4, 13.8), 3.35 (s, 8 H), 4.01 (app t, 1 H, J = 7.5, 7.8), 5.06 (d, 1 H, J = 12), 5.12 (d, 1 H, J = 12), 7.23–7.32 (m, 5 H), 7.44 (d, 2 H, J = 8.8), 8.05 (d, 2 H, J = 8.8); ^{13}C NMR δ 28.0, 39.1, 45.5, 53.5, 55.7, 62.5, 66.3, 80.7, 123.1, 128.3, 128.4, 129.9, 135.2, 145.0, 146.4, 170.5, 173.3. Anal. Calcd for $C_{44}H_{66}N_4O_{12}$: C, 62.7; H, 7.9; N, 6.7. Found: C, 63.1; H, 8.1; N, 6.9.

Further elution (1% MeOH in 1/1 hexanes/ethyl acetate) afforded ***N*-[2-[*N',N'*-Bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl]-4-nitro-L-phenylalanine Benzyl Ester (3b,** 602 mg, 26% yield) as a yellow oil: $[\alpha]$ −9.3° (c 1.5, $CHCl_3$); ^{1}H NMR δ 2.48–2.92 (series of m, 4 H), 3.02 (dd, 1 H, J = 7.3, 13.5), 3.09 (dd, 1 H, J = 6.5, 13.5), 3.34 (d, 1 H, J = 17), 3.42 (d, 1 H, J = 17), 3.60 (t, 1 H, J = 6.5), 5.02 (d, 1 H, J = 12), 5.11 (d, 1 H, J = 12), 7.20–7.35 (m, 7 H), 8.01 (d, 2 H, J = 8.8); ^{13}C NMR δ 28.1, 36.0, 49.9, 53.0, 55.8, 64.3, 66.1, 80.8, 123.0, 128.2, 128.3, 128.4, 130.3, 135.5, 146.3, 146.6, 170.3, 171.8. Anal. Calcd for $C_{30}H_{41}N_3O_8$: C, 63.0; H, 7.2; N, 7.3. Found: C, 63.2; H, 7.5; N, 7.3.

Formation of *N*-[[2-[*N',N'*-Bis[(benzyloxycarbonyl)methyl]amino]ethoxy]carbonyl]-4-nitro-L-phenylalanine Benzyl Ester (8a) and N-[[2-[*N',N'*-Bis[(*tert*-butoxycarbonyl)-methyl]amino]ethoxy]carbonyl]-4-nitro-L-phenylalanine Benzyl Ester (8b). Alkylation of **4** with bromides **5a** and **5b** (225 mol %) was performed with $KHCO_3$ (500 mol %) in 1/1 CH_3CN/H_2O for 20 h at room temperature. Carbamates **8a** and **8b** were separated from the mono- and dialkylated products by chromatograhy on silica gel.

8a: ^{1}H NMR δ 3.00 (t, 2 H, J = 5.4), 3.08 (dd, 1 H, J = 6.1, 13.8), 3.18 (dd, 1 H, J = 6.1, 13.8), 3.64 (s, 4 H), 4.18 (m, 2 H), 4.66 (q, 1 H), 5.05 (d, 1 H, J = 11.8), 5.12 (s, 2 H), 5.19 (d, 1 H, J = 11.8), 5.30 (d, 1 H, J = 7.8), 7.12 (d, 2 H, J = 8.8), 7.27–7.39 (m, 15 H), 7.98 (d, 2 H, J = 8.8); ^{13}C NMR δ 37.8, 52.9, 54.3, 55.3, 63.4, 66.2, 67.3, 123.3, 128.1, 128.1, 128.4, 128.4, 128.6, 130.0, 134.6, 135.4, 143.3, 146.8, 155.2, 170.4, 170.4.

8b: ^{1}H NMR δ 1.45 (s, 18 H), 2.99 (t, 2 H, J = 5.7), 3.19 (m, 2 H), 3.47 (s, 4 H), 4.19 (m, 2 H), 4.7 (m, 1 H), 5.07 (d, 1 H, J = 11.9), 5.19 (d, 1 H, J = 11.9), 5.39 (br d, 1 H, J = 7.8), 7.16 (d, 2 H, J = 8.6), 7.24–7.39 (m, 5 H), 8.01, (d, 2 H, J = 8.6); ^{13}C NMR δ 28.1, 38.1, 52.8, 54.4, 56.3, 63.6, 67.4, 81.0, 123.5, 128.6, 128.7, 128.8, 130.2, 134.6, 143.4, 146.9, 155.4, 170.4. Anal. Calcd for $C_{31}H_{41}N_3O_{10}$: C, 60.5; H, 6.7; N, 6.8. Found: C, 60.7; H, 6.8; N, 6.8.

***N*-[2-[*N',N'*-Bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl]-*N*-[2-[*N'',N''*-bis[(benzyloxycarbonyl)methyl]amino]-ethyl]-4-nitro-L-phenylalanine (2c).** To monoalkylated phenylalanine **3b** (527 mg, 0.92 mmol) was added bromide **5a** (462 mg, 1.10 mmol, 120 mol %) dissolved in CH_3CN (2 mL) followed by pH 8 phosphate buffer (4 mL, 2 M). The two-phase reaction mixture was stirred at rt for 42 h at which time TLC (3/1 hexanes/ethyl acetate) indicated monoalkyl starting material still remained. The reaction mixture was partitioned between ethyl acetate (40 mL) and brine (40 mL), and the organic phase was separated, dried, and evaporated to give an orange oil. The crude product was chromatographed on silica gel (1% MeOH in 3/1, 2/1 hexanes/EtOAc) to afford **5a** (230 mg, 50% recovered yield) and dialkyl product **2c** (350 mg, 42%) as a yellow oil: $[\alpha]^{23}_D$ −30° (c 1.5, $CHCl_3$); R_f 0.32 (hexanes/ethyl acetate (2/1)); IR (neat) 3070, 3040, 2980, 1730 cm^{-1}; ^{1}H NMR δ 1.47 (s, 18 H), 2.58–2.86 (m, 8 H), 2.94 (dd, 1 H, J = 7.8, 13.8), 3.11 (dd, 1 H, J = 7.6, 13.8), 3.33 (s, 4 H), 3.52 (s, 4 H), 3.98 (t, 1 H, J = 7.6), 5.06 (d, 2 H), 5.10 (s, 4 H), 7.20–7.37 (m, 17 H), 8.00 (d, 2 H, J = 8.7); ^{13}C NMR δ 28.0, 35.8, 49.5, 49.7, 52.7, 52.9, 54.8, 55.7, 64.1, 66.0, 66.0, 80.7, 122.9, 128.1, 128.2, 128.3, 128.3, 130.2, 135.4, 146.2, 146.3, 170.2, 170.7, 171.5. Anal. Calcd for $C_{50}H_{62}N_4O_{12}$: C, 65.9; H, 6.9; N, 6.2. Found: C, 65.6; H, 6.8; N, 6.1.

Further elution gave **3b** (242 mg, 46% recovered yield).

Benzyl (*S*)-2-Isocyanato-3-(4-nitrophenyl)propionate (9). The free amine of tosylate salt **4** was prepared by treatment with saturated $NaHCO_3$ and extraction into CH_2Cl_2, the CH_2Cl_2 extracts were dried and concentrated, and the residue of the amine was used directly. A solution of this amine (1.12 g, 3.7 mmol) in toluene (30 mL) was saturated with HCl gas, precipitating the HCl salt, and then phosgene was slowly bubbled into the reaction for several min. The reaction flask was then lowered into an oil bath at 55 °C while continuing the flow of phosgene, holding the temperature at 55 °C for 1.5 h and then increasing it to 65 °C for 2 h and 75 °C for 3 h to afford a homogeneous solution. The reaction mixture was cooled to room temperature, and the solvent was removed by distillation under reduced pressure (water aspirator) to afford a solid residue which was recrystallized from $CHCl_3$/hexanes, providing isocyanate **9** (943 mg, 77% yield, two crops) as fine white needles: $[\alpha]^{23}_D$ −132 (c 1.0, $CHCl_3$); mp 72–73 °C; IR ($CHCl_3$) 2260, 1750 cm^{-1}; ^{1}H NMR δ 3.12 (dd, 1 H, J = 7.0, 13.8), 3.20 (dd, 1 H, J = 5.1, 13.8), 4.37 (dd, 1 H, J = 5.1, 7.0), 5.17 (d, 1 H, J = 11.8), 5.29 (d, 1 H, J = 11.8), 7.21 (d, 2 H, J = 8.7), 7.38 (m, 5 H), 8.05 (d, 2 H, J = 8.7); ^{13}C NMR δ 38.9, 57.6, 68.4, 123.4, 127.2, 128.6, 128.8, 134.3, 142.7,

147.0, 169.7. Anal. Calcd for $C_{17}H_{14}N_2O_5$: C, 62.6; H, 4.3; N, 8.6. Found: C, 62.7; H, 4.2; N, 8.3.

Carbamate 8b was prepared from alcohol **6b** (200 mg, 0.69 mmol) dissolved in THF (2 mL) and isocyanate **9** (225 mg, 0.69 mmol) plus diisopropylethylamine (5 μL, 4 mol %). The solution was stirred at room temperature for 36 h. Evaporation gave a yellow oil which was purified by chromatography on silica gel (1/1, hexanes/ethyl acetate) to afford carbamate **8b** (334 mg, 79%) as a pale yellow solid whose ^1H and ^{13}C NMR spectra and TLC behavior were identical to the compound isolated from alkylation reactions performed in the presence of aqueous bicarbonate.

***N,N*-Bis[2-[*N',N'*-bis(carboxymethyl)amino]ethyl]-4-amino-L-phenylalanine Tetrahydrochloride (2d).** Pentabenzyl ester (**2a**, 4.37 g, 4.46 mmol) was slowly dissolved in methanol (80 mL) while adding 4 M HCl (4.5 mL). To this solution was added 10% Pd on carbon (450 mg) which had been previously washed with 1 M HCl (2 × 10 mL) and several portions of MeOH. The reaction was shaken on a Parr hydrogenator at 50 psi hydrogen for 2 h, the catalyst was removed by filtration through a Millipore Mitex filter (5 μm, 45-mm diameter), the filtrate was evaporated, and the residue was redissolved in 4 M HCl (10 mL) and evaporated to dryness (2X). The solid material was dried (40 °C, 0.04 Torr, 16 h) to afford pentaacid **2d** (2.79 g, 97% yield) as a white powder: $[\alpha]^{22}_D$ −5.2° (*c* 2.0, H_2O); ^1H NMR (D_2O) δ 3.09–3.30 (m, 6 h), 3.48 (m, 4 H), 3.89 (t, 1 H, *J* = 7.2), 4.19 (s, 8 H), 7.40 (d, 1 H, *J* = 8.4), 7.50 (d, 1 H, *J* = 8.4); ^{13}C NMR (D_2O, pH 1.4) δ 36.1, 48.4, 56.4, 57.6, 66.9, 126.0, 130.9, 133.3, 141.5, 170.6, 177.5; ^{13}C NMR (D_2O, pH 6.0) δ 37.7, 48.2, 55.7, 59.7, 70.2, 120.2, 132.5, 133.5, 144.2, 172.8, 181.1. Anal. Calcd for $C_{21}H_{34}N_4O_{10}Cl_4$: C, 39.1, H, 5.3; N, 8.7. Found: C, 39.1, H, 5.5; N, 8.4.

***N,N*-Bis[2-[*N',N'*-bis(carboxymethyl)amino]ethyl]-4-nitro-L-phenylalanine Benzyl Ester Trihydrochloride (2f).** Tetra-*tert*-butyl ester **2b** (355 mg, 0.42 mmol) was dissolved in methanol (1 mL), 6 M HCl (7 mL) was added, the solution was stirred at rt for 1 h, and the solvents were evaporated. The residue was dried (40 °C, 0.01 Torr, 12 h) to afford trihydrochloride salt **2f** (299 mg, 98%) as a pale yellow powder: ^1H NMR (DMSO-d_6) δ 3.01–3.27 (m, 10 H), 4.04 (t, 1 H, *J* = 7.8), 4.18 (s, 8 H), 5.05 (d, 1 H, *J* = 12.4), 5.14 (d, 1 H, *J* = 12.4), 7.22–7.33 (m, 5 H), 7.57 (d, 1 H, *J* = 8.7), 8.10 (d, 1 H, *J* = 8.7); ^{13}C NMR (DMSO-d_6) δ 35.1, 46.3, 53.9, 54.8, 64.5, 66.2, 123.4, 128.2, 128.5, 130.7, 135.6, 146.3, 146.5, 167.5, 171.4. Anal. Calcd for $C_{28}H_{37}N_4O_{12}Cl_3$: C, 46.2; H, 5.1; N, 7.7. Found: C, 46.6; H, 5.0; N, 7.3.

***N,N*-Bis[2-[*N',N'*-bis(carboxymethyl)amino]ethyl]-4-nitro-L-phenylalanine Trihydrochloride (2e).** Compound **2b** (400 mg, 0.47 mmol) was stirred with 6 M HCl (10 mL) and gently heated (50 °C) until solution was achieved which was followed by the precipitation of a white solid. An additional 5 mL of 5 M HCl and 3 mL water were added, and the pale yellow solution was refluxed for 1 h. The solution was cooled to rt, the aqueous HCl was evaporated, and the pale yellow solid was further dried (Kugelrohr, 40 °C, 0.01 Torr, 16 h) to afford **2e** (303 mg, 100% yield) as the trihydrochloride salt: $[\alpha]^{23}_D$ −1.3 (*c* 1.0, H_2O); ^1H NMR (D_2O) δ 3.10–3.27 (m, 5 H), 3.43 (dd, 1 H, *J* = 7.3, 14.0), 3.48 (br t, 4 H, *J* = 6.6), 3.93 (t, 1 H, *J* = 7.4), 4.21 (s, 8 H), 7.56 (d, 2 H, *J* = 8.7), 8.20 (d, 2 H, *J* = 8.7); ^{13}C NMR (D_2O) δ 33.9, 45.9, 54.1, 55.1, 64.1, 123.8, 130.2, 146.1, 146.2, 167.9, 174.6. Anal. Calcd for $C_{21}H_{31}N_4O_{12}Cl_3$: C, 39.5; H, 4.9; N, 8.8. Found: C, 39.8; H, 5.0; N, 8.7.

***N*-[2-[*N',N'*-Bis(carboxymethyl)amino]ethyl]-*N*-[2-[*N',N'*-bis[(benzyloxycarbonyl)methyl]amino]ethyl]-4-nitro-L-phenylalanine Benzyl Ester (2h).** To a solution of **2c** (210 mg, 0.23 mmol) in dioxane (4 mL) was added 12 N HCl (4 mL), and the homogeneous solution was stirred at rt for 40 min at which time a white solid precipitated. The solvents were evaporated, and the residual white powder was further dried (Kugelrohr, 40 °C, 0.01 Torr, 12 h) to provide diacid **2h** (205 mg, 98% yield) as the trihydrochloride salt: $[\alpha]^{23}_D$ −3.0 (*c* 1.0, MeOH); ^1H NMR (CD_3OD) δ 3.23 (br m, 6 H), 3.53 (br s, 4 H), 3.96 (br s, 1 H), 4.31 (s, 4 H), 4.43 (s, 4 H), 5.06 (d, 1 H, *J* = 12.0, the other benzylic d is obscured by the solvent peak at δ 4.9), 5.27 (d, 2 H, *J* = 12), 5.32 (d, 2 H, *J* = 12), 7.10–7.46 (m, 17 H), 8.43 (d, 2 H, *J* = 8.4); ^1H NMR (DMSO-d_6) δ 3.01–3.35 (m, 10 H), 4.13, 4.16 (two s overlapping amino acid α-proton, total 9 H), 5.03 (d, 1 H, *J* = 12.4), 5.11 (d, 1 H, *J* = 12.4), 5.19 (s, 4 H), 7.18–7.45 (m, 15 H), 7.55 (d, 2 H, *J* = 8.7), 8.07 (d, 2 H, *J* = 8.7); ^{13}C NMR (CD_3OD) δ 35.3, 47.3, 55.1, 55.7, 56.0, 56.2, 65.6, 68.1, 69.5, 124.4, 129.4, 129.6, 129.7, 129.7, 131.6, 135.9, 136.4, 146.7, 147.9, 167.3, 168.5, 172.9. Anal. Calcd for $C_{42}H_{49}N_4O_{12}Cl_3$: C, 55.5; H, 5.4; N, 6.2. Found: C, 55.9; H, 5.7; N, 5.6.

***N,N*-Bis[2-[*N',N'*-bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl]-4-amino-L-phenylalanine (2i).** To a solution of **2b** (649 mg) in methanol (25 mL) was added 10% Pd on carbon (120 mg), hydrogen was slowly bubbled through the stirred solution for 1.5 h, the catalyst was filtered off (Celite), and the solvent was evaporated to provide monoacid **2i** (530 mg, 95% yield) as a yellow foam: ^1H NMR δ 1.45 (s, 18 H), 2.75–3.15 (m, 10 H), 3.41 (s, 8 H), 4.02 (m, 1 H), 5.1 (br s, 3 H), 6.61 (d, 2 H, *J* = 8.0), 7.11 (d, 1 H, *J* = 8.0); ^{13}C NMR δ 27.9, 32.0, 49.5, 51.2, 55.4, 66.1, 81.1, 115.1, 127.0, 129.6, 144.8, 170.0, 172.3.

***N*-[2-[*N',N'*-Bis(carboxymethyl)amino]ethyl]-*N*-[2-[*N',N'*-bis[(*tert*-butoxycarbonyl)methyl]amino]ethyl]-4-amino-L-phenylalanine (2g).** Compound **2c** (102 mg, 0.10 mmol) was dissolved in MeOH (10 mL), and to the solution was added 10% Pd–C (10 mg) as a suspension in 1 mL of MeOH. Hydrogenation was conducted in a Parr apparatus at 50 psi H_2 for 3 h, the catalyst was removed by filtration through a Millipore filter (Mitex, 4-mm diameter), and the solvent was evaporated to afford the triacid **2g** (66 mg, 97%) as a yellow powder. ^1H NMR indicated the nitro group was totally reduced and all benzyl esters were cleaved: ^1H NMR (DMSO-d_6) δ 1.41 (s, 18 H), 2.60–2.85 (m, 10 H), 3.36 (s, 4 H), 3.43 (s, 4 H), 3.60 (m, 1 H), 6.45 (m, 2 H), 6.90 (m, 2 H).

Enantiomeric Purity of Monoalkyl Derivatives 3a and 3b. Monoalkyl products **3a** or **3b** as well as their optical antipodes were derivatized as follows. To a solution of each monoalkyl product (100 mol %) in THF was added (+)-α-methylbenzyl isocyanate (120 mol %) as a neat liquid. The solution was stirred at room temperature for 20 h. An aliquot (100–200 μL) was removed and shaken with 1 mL of pH 7 phosphate buffer for 5 min. The product was extracted into ethyl acetate (1 mL). The ethyl acetate phase was separated and was filtered through a small plug of silica in a pipet eluting with ethyl acetate. The solvent was evaporated, and the residue, **10a** or **10b**, was dissolved in CH_2Cl_2 for subsequent HPLC analysis (HPLC conditions: mobile phase 4% CH_3CN, 0.05% NH_4OH (30%) in CH_2Cl_2; column: 4.6- × 250-mm, 5-μm silica; flow rate: 1 mL/min; detector: 275 nm). An aliquot was checked after stirring 5 days to reveal no change in the diastereomer composition. For diastereomer **10a**, t_R = 9 min (from L-phenylalanine); t_R = 10 min (from D-phenylalanine). For diastereomer **10b**, t_R = 10 min (from L-phenylalanine); t_R = 11 min (from D-phenylalanine). The detection limit for the presence of any diastereomer was <1%.

Enantiomeric Purity of 4-Nitrophenylalanine Benzyl Ester (4). To a suspension of tosylate salt **4** (100 mg, 0.21 mmol, 100 mol %) in THF (2 mL) in an ice–water bath was added diisopropylethylamine (35 μL, 0.20 mmol, 95 mol %) via syringe. The cloudy solution was stirred for 5 min, (+)-α-methylbenzyl isocyanate (40 μL, 0.28 mmol, 130 mol %) was added as the neat liquid, the cooling bath was removed, and the reaction mixture was stirred for 20 h at rt, poured into 0.5 M HCl (10 mL), and extracted with CH_2Cl_2 (15 mL). The organic phase was separated and washed with additional 0.5 M HCl (10 mL), the combined acid washes were extracted with CH_2Cl_2 (5 mL), and the combined CH_2Cl_2 extracts were washed with saturated $NaHCO_3$ (15 mL) and dried. An aliquot of the dried CH_2Cl_2 solution was removed and further diluted with CH_2Cl_2 for HPLC analysis (HPLC conditions: mobile phase: 4% CH_3CN, 0.05% NH_4OH (30%) in CH_2Cl_2; column: 4.6- × 250-mm, 5-μm silica; flow rate: 1 mL/min; detector: 275 nm). For the diastereomers of **11**, t_R = 18 min (from L-phenylalanine); t_R = 15 min (from D-phenylalanine). The detection limit for the presence of one diastereomer in the other was < 1%. Pure diastereomer **11** was isolated by evaporation of the CH_2Cl_2 and recrystallization of the solid residue from ethyl acetate: R_f 0.32 (hexanes/ethyl acetate (1/1)); mp 163–164 °C; ^1H NMR δ 1.4 (d, 3 H, *J* = 6.8), 3.02 (dd, 1 H, *J* = 5.4, 13.7), 3.10 (dd, 1 H, *J* = 5.4, 13.7), 4.63 (br t, 1 H), 4.81 (m, 1 H), 5.04 (d, 1 H, *J* = 11.9), 5.14 (br s) overlaps with 5.17 (d, total 2 H, *J* = 11.9), 6.84 (d, 2 H, *J* = 8.4), 7.22–7.37 (m, 10 H), 7.84 (d, 2 H), *J* = 8.4); ^{13}C NMR ($CDCl_3$) δ 23.5, 38.2, 50.4, 53.5, 67.3, 123.2,

125.6, 127.3, 128.6, 128.7, 130.0, 134.7, 143.7, 143.8, 146.5, 156.6, 171.4. Anal. Calcd for $C_{25}H_{25}N_3O_5$: C, 67.1; H, 5.6; N, 9.4. Found: C, 67.1; H, 5.6; N, 9.3.

Yttrium(III) *N,N*-Bis[2-[*N',N'*-bis(carboxymethyl)amino]ethyl]-4-amino-L-phenylalanine Disodium Salt (12). Chelation was performed as described.[5a] To a solution of **2a** (400 mg, 0.62 mmol) in water (20 mL) was added a solution of $YCl_3 \cdot 6H_2O$ (206 mg, 0.68 mmol, 110 mol %) in water (10 mL) to afford a solution of pH 1.6. The pH was slowly adjusted over a period of 45 min by the addition of 0.10 N NaOH (~50 mL) to a final pH of 6.0. Evaporation of the water afforded a pale yellow powder. The crude product was dissolved in H_2O (10 mL) and applied in 2-mL portions to a column of Sephadex-G15 (2.5 × 30 cm) eluting with H_2O at a flow rate of 1.5 mL/min with detection of the chelate at 254 nm. Chelate fractions free of NaCl (as determined by adding several drops to an $AgNO_3$ solution) were combined, the solvent was evaporated, and the residue was dried (45 °C, 0.01 Torr, 12 h) to afford yttrium chelate **12** (326 mg) as a yellow solid. An analytical sample was prepared by further drying (78 °C, 0.01 Torr, 16 h): $[\alpha]^{22}_D$ −64° (*c* 1.1, H_2O); ^1H NMR (D_2O) δ 2.44 (m, 3 H), 2.74 (br s, 3 H), 2.85–3.20 (m, 4 H), 3.13–3.46 (m, 9 H), 3.78 (t, 1 H, J = 6.9), 3.96 (s, 0.5 H), 4.02 (s, 0.5 H), 6.93 (d, 2 H, J = 8.4), 7.23 (d, 2 H, J = 8.4); ^{13}C NMR (D_2O, pH 6.0) δ 32.7, 51.7, 57.7, 58.5, 60.2, 64.8, 65.8, 66.2, 66.7, 74.4, 120.6, 132.4, 135.2, 142.8, 182.6, 182.7, 183.0, 183.6, 184.1. Anal. Calcd for $C_{21}H_{25}N_4O_{10}Na_2Y \cdot H_2O$: C, 39.0; H, 4.2; N, 8.7. Found: C, 39.2; H, 4.5; N, 8.4.