# Synthesis of Conformationally Constrained DTPA Analogues. Incorporation of the Ethylenediamine Units as Aminopyrrolidines

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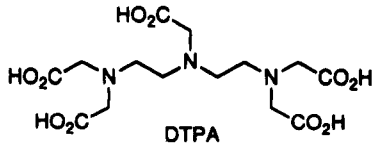
The synthesis of conformationally constrained diethylenetriaminepentaacetic acid (DTPA) analogues is an effort to probe the relationship between ligand structure and metal complex stability. In the pursuit of this objective, diastereomerically and enantiomerically pure mono- and bis-pyrrolidine analogues of DTPA have been prepared from *trans*-4-hydroxy-L-proline. The mono-pyrrolidine chelator **1** was constructed from a single hydroxyproline unit and an ethylenediamine moiety while two hydroxyproline-derived fragments **4e** or **14b** and **9b** were coupled by *N*-alkylation of a triflate to afford the core bis-pyrrolidine structures: optically active **10** and *meso*-**15**. Deprotection of the triamine pentaesters **12** and **17** afforded the triamine pentaacetic acids **2** and **3** as their hydrochloride salts. The stereochemical homogeneity of precursor esters **12** and **17** was determined by HPLC using authentic epimeric standards to establish that essentially no racemization of the original amino acid α-center had occurred. Some loss of stereochemical homogeniety was encountered in the synthesis of **10** and **15** by *N*-alkylation of aminoproline **9b** with a hydroxyproline-derived triflate, which had proceeded with some retention of configuration. The diastereomeric impurities were removed by crystallization of the respective benzyl carbamates. Bis-pyrrolidine pentaacids **2** and **3** formed isolable chelates with gadolinium and lutetium. A comparision of the lutetium chelates of **2** and **3** by NMR revealed significant differences which were reflective of a rigid structure with **2**, while metal complexation with **3** was structurally less defined.

## Introduction

Organic ligand-complexed paramagnetic metals are important contrast agents for magnetic resonance imaging (MRI).[1] The metal complex provides image enhancement by influencing proton relaxation in surrounding tissue. This method of visualization for use *in vivo* requires a complexed metal which is kinetically stable to dissociation, resisting transchelation with proteins or naturally occurring anions such as carbonate or phosphate.[1] Often the free metal is toxic so that the rate of decomplexation of the metal is a critical factor in evaluating the utility of a particular complex for MRI. Other factors which may be relevant to the selection of a metal complex depending on the application include rate of complexation, solubility, lipophilicity, and ionic charge.

Ligands which have seen frequent use as complexing agents for metals of interest as contrast agents are the polyamine polycarboxylate diethylenetriaminepentaacetic acid (DTPA) and its derivatives. While DTPA forms stable




DTPA

complexes with a variety of metals, efforts to enhance the stability of these complexes under physiological conditions have afforded new analogues. In the design of bifunctional DTPA analogues for use as radiopharmaceuticals, the addition of branching groups to the ethylenediamine backbone has been reported to enhance complex stability.[2] Branching also can be provided by the introduction of rings into the ligand structure which conformationally constrain specific portions of the DTPA skeleton.[3] The purpose of these constraints is to afford a preorganization of the ligand's chelating groups as they are presented to the metal thereby reducing some of the entropic cost required in forming the metal complex, while at the same time providing a steric barrier to decomplexation. Although macrocyclic ligands possess perhaps the greatest degree of conformational constraint, their structure may not allow sufficient flexibility to accommodate a variety of metals, as they possess a cavity of fixed dimension. Thus selective constraint of DTPA could provide a ligand with enhanced stability while retaining advantageous flexibility of the ligand.

Constrained analogues DTPA have been prepared from (±)-*trans*-1,2-diaminocyclohexane[4a] and 2,6-bis(aminomethyl)piperidine.[4b] Of the many possible structural modifications of DTPA, we chose to add an additional carbon bridge between an internal methylene and one of the acetic acid residues of the parent DTPA skeleton, generating structures **1**–**3**. As a result of the introduction of such constraints, the pyrrolidine-based ligands now contain two or four stereocenters. Control of these stereocenters is of obvious importance to the eventual disposition of the coordinating carboxyl and amino groups around the metal, affecting the overall stability of the complex. Thus, it is critical in the synthesis of such target ligands to control the stereochemistry and prepare the ligands as single diastereomers. In order to meet this

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(1) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901.

(2) (a) Brechbiel, M. W.; Gansow, O. A. *Bioconjugate Chem.* **1991**, *2*, 187. (b) Kozak, R. W.; Raubitschek, A.; Mirzadeh, S.; Brechbiel, M. W; Junghaus, R.; Gansow, O. A.; Waldmann, T. A. *Cancer Res.* **1989**, *49*, 2639. (c) Cummins, C. H.; Rutter, E. W., Jr.; Fordyce, W. A. *Bioconjugate Chem.* **1991**, *2*, 180.

(3) A theoretical treatment of structure–stability relationships of several DTPA analogues is given in: Fossheim, R.; Dugstad, H.; Dahl, S. G. *J. Med. Chem.* **1991**, *34*, 819.

(4) (a) Brechbiel, M. W.; Gansow, O. A. *J. Chem. Soc. Perkin Trans. 1* **1992**, 1173. (b) Gries, H.; Renneke, F-J.; Weinmann, H.-J. Eur. Pat. 1987, 0 250 358 A2, June 15, 1987.

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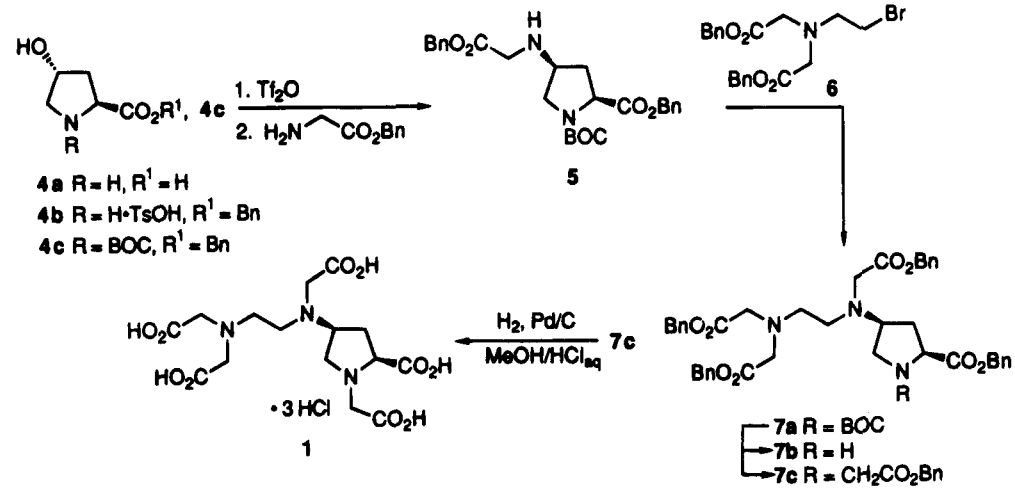
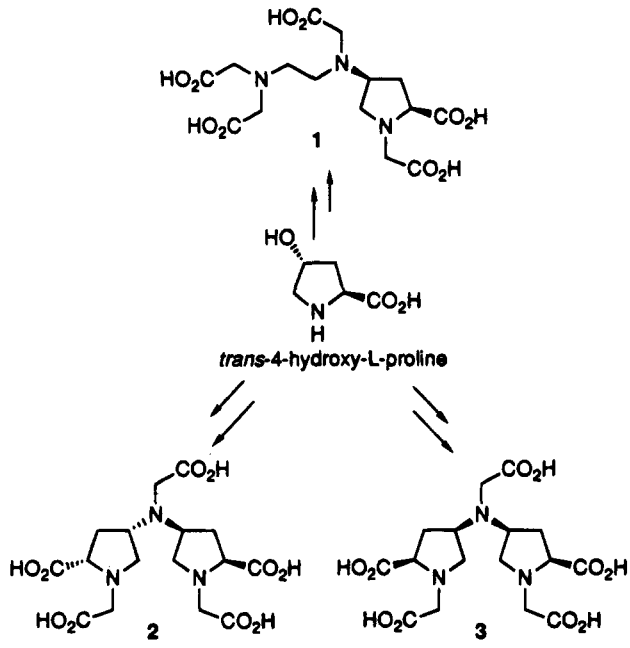
**Scheme 1. Synthesis of Mono-Pyrrolidine 1**




criteria, we chose to use *trans*-4-hydroxy-L-proline as the chiral educt for the preparation of mono-pyrrolidine **1** and bis-pyrrolidines **2** and **3**.




## Results and Discussion

**Synthesis of Mono-Pyrrolidine 1.** In the synthesis of mono-proline ligand **1** the requirement for a *cis*-amino group at C-4 of the pyrrolidine ring directed the initial stages of the synthesis, shown in Scheme 1. Starting from *trans*-4-hydroxy-L-proline, protection of the carboxyl group as the benzyl ester (87%) and the amino group as the *tert*-butyl carbamate (94%) afforded **4c**. The initial plan was to introduce the C-4 *cis*-amino group by azide displacement of a mesylate or tosylate derivative of the C-4 hydroxyl, followed by reduction and alkylation. The same transformation, however, was accomplished with the direct displacement of the triflate of **4c** by benzyl glycinate to provide *cis*-**5** in 79% yield. It was essential to use the isolated free amine of benzyl glycinate in the triflate displacement; benzyl glycinate generated in situ from the corresponding amine tosylate salt gave products in which a tosyl group was incorporated. In addition, the reaction was allowed to proceed at 0 °C for 20 h to minimize competitive elimination of the triflate. With these precautions, the triflate was superior to the less-reactive mesylate derivative of **4c**.

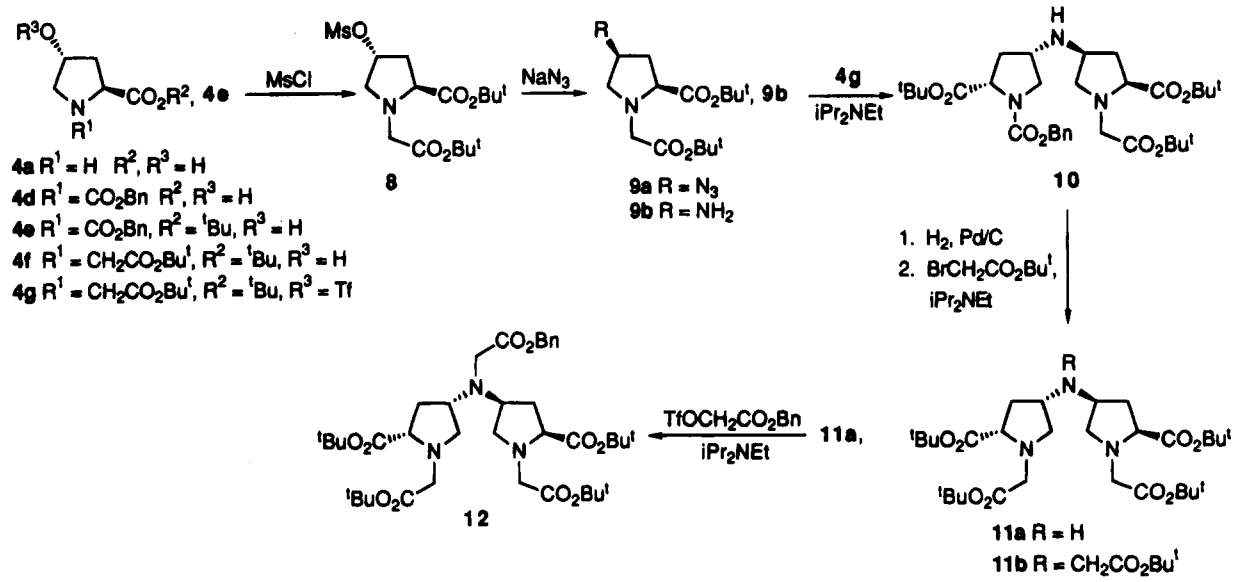
Direct displacement of **4c** with benzyl glycinate not only introduces the amino group, but also installs a requisite acetic acid residue. The remainder of the diethylenetriamine skeleton of ligand **1** was added as a single fragment. Alkylation of aminoproline **5** with known bromide **6**[5] in a mixed solvent system ($CH_3CN/H_2O$) at 50 °C gave triamine **7a** in 78% yield. The remaining acetic acid residue was added in a two-step sequence. Deprotection of the ring nitrogen with TFA to **7b** was followed by alkylation with benzyl 2-bromoacetate to provide pentaester **7c** in 90% yield. Removal of all benzyl protecting groups using conditions previously described for a DTPA analogue[5] gave a quantitative yield of pentaacid **1** as the trihydrochloride salt.

**Synthesis of Bis-Pyrrolidines 2 and 3.** The syntheses of meso and *C*-2 symmetric bis-pyrrolidines **2** and **3** were accomplished by coupling two fragments derived from *trans*-4-hydroxy-L-proline as presented in Scheme 2. *N*-(Carboxymethyl)-*cis*-4-aminoproline **9b** was a common key intermediate. The remaining halves of bis-pyrrolidines **2** and **3** were appended to **9b** by alkylation with a triflate of protected *trans*-4-hydroxy-L-proline or *trans*-4-hydroxy-D-proline, respectively. Protected proline **4e** was prepared in 76% yield from amino acid **4a** via CBZ derivative[6] **4d** and *tert*-butyl ester formation. Proline derivative **4e** was the left-side fragment in the bis-pyrrolidine synthesis and served as an intermediate in the synthesis of right-side fragment **9b**. Thus removal of the CBZ group by hydrogenolysis and direct alkylation with *tert*-butyl bromoacetate gave **4f** in 88% overall yield from **4e**. A three step sequence was then required to establish a *cis*-amino group at C-4 of the proline ring. Conversion of **4f** to mesylate **8** (92% yield) followed by displacement of the mesylate with sodium azide in DMF gave *cis*-azide **9a** in 95% yield.[7] The overall conversion of **4e** to **9a** could be performed on a multigram scale without intermediate isolation in 85% yield. Finally, the right-side fragment was completed by hydrogenation of azide **9a** to *cis*-amine **9b** in quantitative yield.

The coupling of primary amine **9b** with left-side fragment **4e** was accomplished through a triflate alkyl-

(5) Williams, M. A.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 1151.
(6) Patchett, A. A.; Witkop, B. *J. Am. Chem. Soc.* **1957**, *79*, 185.
(7) Azide **9a** was verified by HPLC as >99% diastereomerically pure using an authentic standard of the *trans*-azide prepared from *cis*-alcohol **13b** by the same sequence of reactions.

**Scheme 2. Synthesis of *C*-2 Symmetric Pentaester**




ation (Scheme 2). Conversion of **4e** to its triflate **4g** was conducted at 0 °C with triflic anhydride and diisopropylethylamine and, without isolation, amine **9b** was added. Maintaining the reaction at low temperature (0 °C, 40 h) gave an 80% yield of triamine **10** after chromatographic purification. Again, a competing process in this alkylation again was elimination to afford 3,4- and 4,5-dehydroprolines whose $^1$H NMR spectra correlated well with those of similar reported compounds.[8] Examination of both crude and purified **10** revealed the presence of a diastereomer which could not be separated by column chromatography. Fortunately, repeated recrystallization from hexane gave **10** which was >99% diastereomerically pure by HPLC analysis. The nature of this diastereomer formation is addressed in a following section on stereochemical purity.

Proceeding with the diastereomerically pure **10**, the synthesis was completed by appending the two remaining acetic acid residues. Hydrogenolysis of the CBZ group followed by alkylation with *tert*-butyl bromoacetate (−5 °C, $CH_3CN$) provided tetraester **11a** in 87% yield. Although the alkylation of the liberated pyrrolidine nitrogen was conducted at low temperature to minimize the formation of polar byproducts, alkylation of the central nitrogen also occurred, albeit in low (3−4%) yield, to give pentaester **11b**. An effective method for alkylation of the relatively hindered central nitrogen utilized the triflate of benzyl glycolate in $CH_3CN$ at −5 °C. Under these conditions, a 72% yield of pentaester **12** was realized. Benzyl ester protection for the central carboxyl group was chosen to introduce a chromophore for HPLC analysis of the final ligand for stereochemical homogeneity as well as to provide a differentiated carboxyl group for selective functionalization.

The synthetic strategy for the preparation of meso pentaester **17** was identical to that performed for **12** with the exception that *trans*-4-hydroxy-D-proline was needed for the left-side fragment of the bis-pyrrolidine structure. *trans*-4-Hydroxy-D-proline was prepared from the L-enantiomer by inversion of both stereocenters. Conversion of *trans*-4-hydroxy-L-proline to *cis*-4-hydroxy-D-proline **13a** (53%) was accomplished by modification of the reported method.[9] Although the inversion of the C-2 center was reported to afford pure cis product, the process for determining such purity was not described.[9b] Though we were concerned about diastereomeric contamination, we elected to ascertain stereochemical purity at a later more-convenient stage. Thus *cis*-4-hydroxy-D-proline (**13a**) was converted to protected proline **13b** in 75% yield (Scheme 3) using the sequence of reactions described in the preparation of **4e**. Inversion of the C-4 hydroxyl configuration of **13b** proceeded via Mitsunobu reaction (78%) and hydrolysis of the resulting *trans*-acetate (99%) and gave protected *trans*-4-hydroxy-D-proline **14b**. HPLC analysis (established detection limits <1%) of *trans*-**14b** using *cis*-**13b** as an authentic standard indicated *trans*-**14b** contained none of the cis material. Furthermore, crude **13b** was examined by HPLC and was found to be free of trans isomer, indicating that the initial synthesis and isolation of *cis*-4-hydroxy-D-proline had yielded diastereomerically pure material.

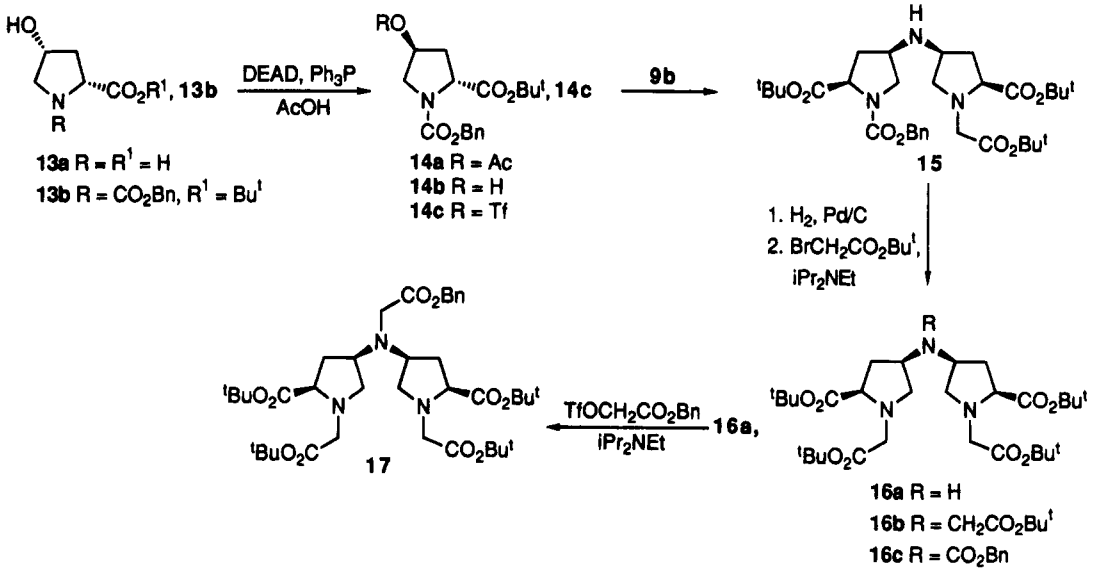
Protected hydroxyproline **14c** was coupled with **9b** as previously described to give triamine **15** (80%) again as a mixture of diastereomers. The level of contamination was approximately 5−6%. Since preparative chromatographic separation was not effective and **15** was not crystalline, the mixture was carried on with the expectation of achieving a separation at a later point. Triamine **15** was converted to tetraester **16a** (85% yield), and a partial separation of the contaminating diastereomer was possible at this stage; however, repeated chromatography gave a residual diastereomeric impurity of 3−4%. When **16a** was transformed to CBZ derivative **16c** in 81% yield, a crystalline product was obtained. A single recrystallization of **16c** gave diastereomerically pure material as established by HPLC analysis. An authentic standard for the HPLC analysis was prepared from the pure diastereomer isolated at the tetraester stage by reaction with benzyl chloroformate. This material was not detected in the HPLC of recrystallized **16c**.

Deprotection of **16c** by hydrogenolysis of the CBZ group and alkylation with the triflate of benzyl glycolate gave

(8) (a) Dormoy, J.-R.; Castro, B.; Chappuis, G.; Fritschi, U. S.; Grogg, P. *Angew. Chem. Int. Ed. Engl.* **1980**, 742. (b) Rüeger, H.; Benn, M. H. *Can. J. Chem.* **1982**, *60*, 2918.

(9) (a) Robinson, D. S.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *195*, 383. (b) Baker, G. L.; Fritschel, S. J.; Stille, J. R.; Stille, J. K. *J. Org. Chem.* **1981**, *46*, 2954.

**Scheme 3. Synthesis of Meso Pentaester**



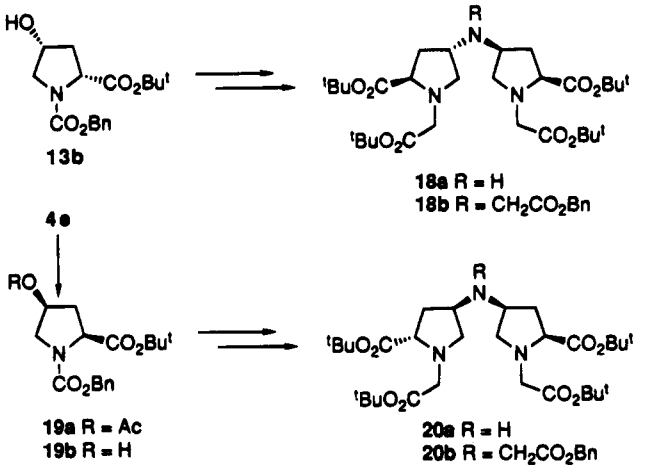

17 in 66% yield from **16c**. It is interesting to note that triamine pentaester isomers **12** and **17** give very similar $^1H$ and $^{13}C$ NMR spectra, such that it is difficult to differentiate between the two diastereomers. As expected, meso pentaester **17** was devoid of any optical activity.

The choice of protecting groups for pentaesters **12** and **17** was dictated by the need for clean, high-yielding deprotection of the carboxyl groups with minimal requirement for purification of the final products. Deprotection of the carboxyl groups in pentaesters **12** and **17** was performed in a two-step sequence. Thus hydrogenolysis of the benzyl ester followed by cleavage of the *tert*-butyl esters with aqueous HCl in dioxane furnished **2** and **3** as the trihydrochloride salts in 94 and 91% yields, respectively.

**Stereochemical Analysis of Final Products.** The presence of diastereomers during the synthesis of pentaesters **12** and **17** emphasized the requirement for developing a method to validate the final stereochemical purity of our target ligands. Epimerization of the C-2 center of the proline ring would give rise to diastereomers. Athough diastereomerically pure triamine **10** and **16c** were subsequently exposed only to mild reaction conditions ($iPr_2NEt$, low or ambient temperatures), the potential for racemization existed. Therefore the syntheses of authentic diastereomers **18b** and **20b** were undertaken to enable the unambiguous determination of stereochemical purity (Scheme 4).

Of the four stereocenters present in pentaesters **12** and **17**, only the centers at C-2 are prone to epimerization. Even though both of these centers may epimerize, only one diastereomer need be prepared for comparison. The absence of a diastereomer with a single epimerized center would exclude the formation of the diastereomer with two epimerized centers. Thus two diastereomers were prepared: **18b** as the epimer of **12**, and **20b** as the epimer of **17**. The synthesis of diastereomers **18b** and **20b** proceeded directly from amine **9b** and alcohols **13b** and **19b** by the previous process. Alcohol **19b** was obtained from **4e** by Mitsunobu inversion (DEAD, $Ph_3P$, AcOH) followed by hydrolysis of the acetate ester. The triflates of **13b** and **19b** were generated in-situ and coupled to amine **9b**. Hydrogenolysis of the CBZ group and alkylation gave tetraesters **18a** and **20a**. At the tetraester stage (without the presence of rotational isomers resulting

**Scheme 4. Bis-Pyrrolidine Diastereomers**




from the carbamate protecting group) these two diastereomers were compared to the contaminating diastereomer isolated in the synthesis of tetraester **16a**. Careful examination of 500-MHz $^1H$ NMR spectra revealed this diastereomer to be epimer **18a**, the formation of this diastereomer arising through retention of configuration in the triflate coupling rather than epimerization of the proline ring at C-2. Whether this process occurs through a pure carbocation intermediate or by participation of the benzyl carbamate, the superior leaving group ability of triflates facilitated the formation of this retention product in the synthesis of **15**.[10] Presumably amounts of diastereomer formed in the synthesis of epimers **18a** or **20a** would be less than that encountered with **11a** or **16a** since retention of configuration in the triflate coupling would confer a cis substituent rather than the trans orientation offered by an $S_N2$ displacement.

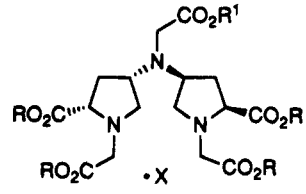
Conversion of **18a** and **20a** to the corresponding pentaesters was accomplished with the triflate of benzyl glycolate,[11] providing **18b** and **20b**. HPLC analysis of **12**

(10) An example of retention of configuration with 4-(tosyloxy)prolines is presented in: Thottathil, J. K.; Moniot, J. L. *Tetrahedron Lett.* **1986**, *27*, 151.

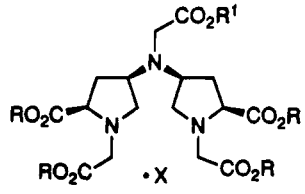
(11) Shiosaki, K.; Fels, G.; Rapoport, H. *J. Org. Chem.* **1981**, *46*, 3230. General procedures for the preparation of triflates of α-hydroxy esters: Vedejs, E.; Engler, D. A.; Mullins, M. J. *J. Org. Chem.* **1977**, *42*, 3109.

with a 1% spike of **20b** revealed that the diastereomeric purity of **12** was >99%. A comparable check of pentaester **17** established a similar level of purity. Thus, diastereomers resulting from retention of configuration at the triflate coupling were efficiently removed through the recrystallization of CBZ adducts **10** and **16c**, while little if any epimerization of the C-2 proline ring stereocenters occurred in the final steps of the synthesis.

**Metal Complexation.** To investigate the chelation of pentaacids **2** and **3** with lanthanide metals, particularly gadolinium, and demonstrate the viable application of these ligands as metal complexing agents, meso pentaacid **3** was reacted with an excess of $GdCl_3$ in water. After neutralization and Sephadex desalting, Gd-chelate **21** was isolated as a 1/1 metal/ligand complex. While elemental analysis established the composition of this chelate, the structure of gadolinium chelates cannot be observed by NMR spectroscopy due to inherently long metal-induced proton relaxation times—a feature which makes them ideal as image contrast agents.



**12** R = Bu$^t$ R$^1$ = Bn
**2** R = R$^1$ = H, X = 3HCl•$H_2O$
**23** R = R$^1$ = anion, X = $Lu^{3+}$, $2Na^+$



**17** R = Bu$^t$ R$^1$ = Bn
**3** R = R$^1$ = H, X = 3HCl•$H_2O$
**21** R = R$^1$ = anion, X = $Gd^{3+}$, $2Na^+$, $H_2O$
**22** R = R$^1$ = anion, X = $Lu^{3+}$, $2Na^+$, $2H_2O$

Lutetium has been proposed as a spectroscopically useful surrogate for gadolinium due to similar ionic radii in the $M^{3+}$ state.[12] Chelation of **3** with $LuCL_3$ again yielded an isolable metal complex in a 1/1 metal/ligand ratio. Both $^1H$ and $^{13}C$ NMR spectra of the crude and purified chelate contain features which are indicative of more than a single rigid metal complex. The $^1H$ NMR of Lu-chelate **22** possessed a complex region from $\delta$ 1.9–4.2 ppm with little resolution of the signals one would expect from the pyrrolidine ring protons and the acetic acid methylenes if present in a fixed conformation. Although the $^{13}C$ spectra revealed 5 distinct carbonyl resonances, the remaining signals were broad and complex. Such signal broadening could be the result of exchange between free and complexed ligand or the occurrence of conformational mobility.[12,13]

The preceding observations were even more significant when compared to the Lu-complex derived from *C*-2 symmetric ligand **2**. As shown in Figure 1, the $^1H$ NMR spectra of this chelate was significantly more resolved and defined. Furthermore, the $^{13}C$ spectrum for complex **23** indicated 5 carbonyl signals and the expected number of signals (11) for two differentiated proline rings and five acetic acid methylenes. On the basis of the spectroscopic information, Lu-chelate **23** appears to be a single complex. Although both ligands have the same number of chelating groups (amino and carboxyl) and the substituents on the proline rings are in a cis arrangement, clearly **2** and **3** have very different chelating properties.

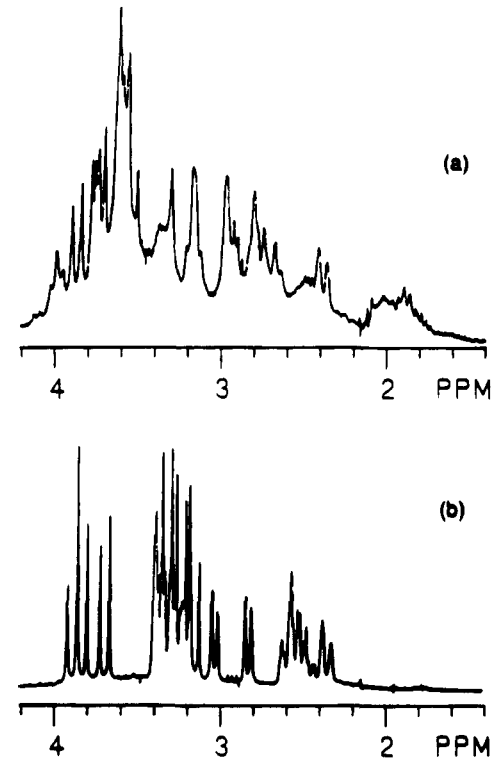



**Figure 1.** Partial $^1H$ NMR spectra of lutetium chelates in $D_2O$: (a) lutetium chelate (**22**) of meso pentaacid **3**, (b) lutetium chelate (**23**) of *C*-2 symmetric pentaacid **2**.

## Conclusion

In summary, the synthesis of several conformationally constrained DTPA analogues from *trans*-4-hydroxy-L-proline has been described. The preparation of *C*-2 symmetric analogue **2** and meso analogue **3** as pure diastereomers was confirmed by HPLC analysis of their pentaester precursors with authentic diastereomer standards. NMR analysis of the complexes formed with $Lu^{3+}$ indicate that these diastereomers behave quite differently in their ligation with the metal. The chelation behavior of *C*-2 symmetric ligand **2** is noteworthy and may be useful for applications requiring an optically active metal complex.

## Experimental Section

**General.** $^1H$ NMR spectra were obtained in $CDCl_3$ and were referenced to internal tetramethylsilane or 3-(trimethylsilyl)-propionate-$d_4$ in $D_2O$, unless indicated otherwise. $^{13}C$ NMR spectra were obtained in $CDCl_3$ (reference $\delta$ = 77.0 ppm) unless otherwise stated: methanol-$d_4$ (49.0 ppm), $D_2O$ (internal dioxane $\delta$ = 69.0 ppm). Melting points were determined on a Buchi melting point apparatus and are uncorrected. All organic

(12) White, D. H. Presented at Contrast Media Research 89, Sidney, Australia, 1989.
(13) Peters, J. A. *Inorg. Chem.* **1988**, *27*, 4686.

solutions from extractive isolation of products were dried over $Na_2SO_4$, filtered, and evaporated with a Berkeley rotary evaporator (35–40 °C) at aspirator pressure. Elemental analyses were determined by the Microanalytical Laboratories, University of California, Berkeley. High-pressure liquid chromatography (HPLC) was conducted on a 4.6 × 250 mm 5-μm Microsorb Si normal-phase silica column monitoring at 254 nm. 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) and visualized with iodine, UV light (254 nm), or ethanolic phosphomolybdic acid followed by heating. All reactions were performed under a nitrogen atmosphere unless indicated otherwise. Anhydrous THF and dioxane was distilled from sodium and benzophenone. Diisopropylethylamine and $CH_3CN$ were distilled from calcium hydride. Trifluoromethanesulfonic anhydride was prepared as reported.[14] Water used for the preparation and purification of metal chelates was distilled–deionized quality.

**4-Hydroxy-L-proline Benzyl Ester *p*-Toluenesulfonate (4b).** To a suspension of *trans*-4-hydroxy-L-proline (15 g, 114 mmol) in benzyl alcohol (50 mL) and benzene (100 mL) was added *p*-toluenesulfonic acid (23.8 g, 125 mmol). The mixture was heated at reflux while azeotropically removing water. After 4.5 h the solution was cooled to room temperature and $Et_2O$ (200 mL) was slowly added. The crystals formed were collected and rinsed with $Et_2O$ (3 × 100 mL). The crude product was recrystallized from EtOH/$Et_2O$ in the cold (0 °C) overnight and the crystals were collected and rinsed with $Et_2O$ (100 mL). Further drying under vacuum gave benzyl ester **4b** (37.8 g, 87%) as white needles (mp 125–127 °C). An analytical sample was prepared with additional drying (78 °C, 0.05 torr, 12 h) for which an identical mp was obtained: mp 125–127 °C [lit.[15] mp 107–109 °C for monohydrate]; $[\alpha]^{22}_D$ −25° (*c* 1.0, MeOH); $^1$H NMR ($CD_3OD$) δ 2.15 (ddd, 1H, *J* = 4.2, 10.8, 14.0), 2.35 (s, 3H), 2.41 (ddt, 1H, *J* = 1.6, 7.7, 13.7), 3.35 (m, 1H), 3.42 (dd, 1H, *J* = 3.7, 12.2), 4.55 (br t, 1H, *J* = 3.8), 4.61 (dd, 1H, *J* = 7.7, 10.7), 5.23 (d, 1H, *J* = 12.1), 5.30 (d, 1H, *J* = 12.1), 7.19 (d, 2H, *J* = 8.2), 7.36 (m, 5H), 7.7 (d, 2H, *J* = 8.2); $^{13}$C NMR ($CD_3OD$) 21.3, 38.4, 55.0, 59.4, 69.2, 70.5, 126.8, 129.4, 129.58, 129.59, 129.8, 136.2, 141.7, 143.2, 169.9. Anal. Calcd for $C_{19}H_{23}NO_6S$: C, 58.0; H, 5.9; N, 3.6. Found: C, 57.9; H, 6.0; N, 3.5.

***N*-(*tert*-Butoxycarbonyl)-4-Hydroxy-L-proline Benzyl Ester (4c).** To a suspension of tosylate **4b** (10 g, 25.4 mmol) in $CH_3CN$ (50 mL) at 0 °C was added $Et_3N$ (3.54 mL, 25.4 mmol). To the solution was added di-*tert*-butyl dicarbonate (8.3 g, 38.1 mmol) in $CH_3CN$ (10 mL). The resulting cloudy solution was stirred 30 min at 0 °C and then for 2 h at room temperature. Evaporation of the solvent gave an oily residue which was partitioned between $Et_2O$ (150 mL) and 0.1 M HCl (100 mL). The organic phase was washed with additional 0.1 M HCl (100 mL), saturated $NaHCO_3$ (100 mL), and brine (100 mL) and was dried ($MgSO_4$). Evaporation of the solvent gave an oil which was chromatographed on silica gel (1/1, hexane/EtOAc) to afford **4c** (7.3 g, 94%) as a colorless oil: $[\alpha]^{22}_D$ −65° (*c* 1.5, $CHCl_3$); $^1$H NMR (rotamer mixture[16]) δ 1.33 and 1.45 (s, 9H), 2.10–2.10 (m, 1H), 2.20–2.35 (br m, 3H), 3.46 (br dd, 1H), 3.62 (dd, 1H, *J* = 4.3, 11.6), 4.46 (m, 2H), 5.08 (d, 0.4H, *J* = 12.5), 5.15 (d, 1.2H), 5.26 (d, 0.4H, *J* = 12.5), 7.35 (m, 5H); $^{13}$C NMR δ 27.9, 28.1, 38.0, 38.7, 54.3, 54.4, 57.5, 57.8, 66.5, 68.6, 69.4, 79.9, 80.18, 127.7, 127.9, 128.0, 128.1, 128.2, 128.3, 135.1, 135.3, 153.8, 154.3, 172.5, 172.7. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.5; H, 7.2; N, 4.4. Found: C, 63.5; H, 7.1; N, 4.6.

***N*-[(2*S*,4*S*)-4-[2-(Benzyloxycarbonyl)-1-(*tert*-butoxycarbonyl)pyrrolidinyl]]glycine Benzyl Ester (5).** The free amine of benzyl glycinate *p*-toluenesulfonate[17] was prepared from 10 g of the salt which was partitioned between $CH_2Cl_2$ (25 mL) and 0.5 M $Na_2CO_3$ (100 mL). The phases were separated and the aqueous phase was extracted with $CH_2Cl_2$ (2 × 25 mL). The combined organic extracts were washed with 0.5 M $Na_2CO_3$ (100 mL) and brine, dried, and evaporated (high vacuum, ~30 min) to afford 4.49 g of the free amine as a pale yellow oil which was used directly in the next step.

A solution of alcohol **4c** (6 g, 18.7 mmol) in $CH_2Cl_2$ (30 mL) was cooled to −20 °C (bath, dry ice–$CCl_4$) and $iPr_2NEt$ (6.8 mL, 39.3 mmol) was added followed by the addition of triflic anhydride (3.3 mL, 19.6 mmol) over a period of several minutes. The resulting dark orange solution was stirred 45 min at −20 °C, at which time benzyl glycinate (4.49 g, 27.2 mmol, 146 mol %) in $CH_2Cl_2$ (10 mL) was added. The reaction flask was removed from the −20 °C bath and placed in a cold room (0 °C) where stirring was continued for 20 h, and the mixture was diluted with $CH_2Cl_2$ (150 mL) and washed with 0.5 M $Na_2CO_3$ (2 × 100 mL) and brine (100 mL). After drying, filtering, and evaporating the solvent, a crude oil was obtained which was purified by chromatography on silica gel (3/1, $CH_2Cl_2$/EtOAc) to afford alkylated pyrrolidine **5** (6.93 g, 79%) as a pale yellow oil: $[\alpha]^{22}_D$ −38° (*c* 1.6, $CHCl_3$); $^1$H NMR δ 1.33 and 1.40 (s, 9H), 1.95 (m, 1H), 2.28 and 2.36 (m, 1H), 2.23–4.42 (m, 4H), 3.61 (dd, 0.4H, *J* = 5.9, 10.8), 3.68 (dd, 0.6H, *J* = 5.3, 10.1), 4.27 (dd, 0.6H, *J* = 5.3, 8.6), 4.39 (dd, 0.4H, *J* = 4.7, 8.7), 5.05 (d, 0.4H, *J* = 12.4), 5.08–5.15 (5s, 3.2H), 5.25 (d, 0.4H, *J* = 12.4) 7.32 (m, 10H); $^{13}$C NMR δ 27.8, 28.1, 34.9, 36.1, 48.4, 48.5, 51.5, 52.2, 55.2, 56.0, 57.4, 57.7, 66.24, 66.35, 66.40, 79.6, 79.7, 127.69, 127.75, 127.97, 128.03, 128.10, 128.15, 128.21, 135.1, 135.2, 135.4, 153.3, 153.8, 171.43, 171.49, 172.0, 172.2. Anal. Calcd for $C_{26}H_{31}N_2O_6$: C, 66.8; H, 6.7; N, 6.0. Found: C, 66.5; H, 6.9; N, 6.0.

***N*-[[2-Bis[(benzyloxycarbonyl)methyl]amino]ethyl]-*N*-[(2*S*,4*S*)-4-[2-(benzyloxycarbonyl)-1-(*tert*-butoxycarbonyl)pyrrolidinyl]]glycine Benzyl Ester (7a).** To a solution of pyrrolidine **5** (5 g, 10.7 mmol) and bromide **6** (6.7 g, 16.0 mmol) in $CH_3CN$ (20 mL) was added $K_2HPO_4$ (5.58 g, 32 mmol, 300 mol %) in 20 mL of water. The two-phase reaction was vigorously stirred at 50 °C (bath temp) for 22 h. Separation of the organic phase and evaporation of the solvent gave a residue which was taken up in $Et_2O$ (100 mL) and washed with 0.5 M $Na_2CO_3$ (2 × 100 mL) and brine (100 mL), dried ($Na_2SO_4$), filtered, and evaporated. The residue was purified by chromatography on silica gel (1% MeOH in 3/1, hexanes/EtOAc) to afford alkylated product **7a** (6.75 g, 78%) as a viscous pale yellow oil: $[\alpha]^{22}_D$ −32° (*c* 1.6, $CHCl_3$); $^1$H NMR δ 1.32, 1.44 (2s, 9H), 1.74 (m, 2H), 2.40 (m, 1H), 2.79 (m, 1H), 3.15 (m, 1H), 3.46, 3.50, 3.56, (m, 9H), 3.68 (m, 0.4H), 3.82 (m, 0.6H), 4.18 (m, 0.6H), 4.26 (m, 0.4H), 5.10 (m, 10H), 7.33 (m, 20H); $^{13}$C NMR δ 27.8, 28.1, 33.5, 34.9, 49.6, 49.8, 51.7, 51.85, 51.93, 54.91, 54.95, 57.5, 57.9, 59.1, 59.9, 65.8, 65.9, 66.4, 77.4, 79.8, 127.71, 127.77, 127.78, 127.79, 127.82, 127.84, 127.94, 127.97, 128.07, 128.12, 128.2, 135.1, 135.29, 135.32, 135.41, 153.0, 153.7, 170.47, 170.67, 170.73, 171.8, 172.0. Anal. Calcd for $C_{46}H_{52}N_3O_{10}$: C, 68.5; H, 6.5; N, 5.2. Found: C, 66.5; H, 6.7; N, 5.3.

***N*-[[2-Bis[(benzyloxycarbonyl)methyl]amino]ethyl]-*N*-[(2*S*,4*S*)-4-[2-(benzyloxycarbonyl)-1-[(benzyloxycarbonyl)methyl]pyrrolidinyl]]glycine Benzyl Ester (7c).** *N*-BOC-pyrrolidine **7a** (6 g, 7.4 mmol) was dissolved in $CH_2Cl_2$ (15 mL), cooled to 0 °C, and treated with TFA (11 mL, 142 mmol, ~1900 mol %). After 10 min at 0 °C, the reaction was warmed to room temperature and was stirred for 2 h, at which time reaction was complete (TLC). Evaporation of the solvents gave a yellow residue which was dissolved in $CH_2Cl_2$ (50 mL) and washed with cold (0 °C) 1 M NaOH (100 mL). The basic aqueous phase was extracted with $CH_2Cl_2$ (50 mL) and the combined organic phases were washed with brine (100 mL), dried, filtered, and evaporated. After high-vacuum (0.05 torr, 12 h) drying, deprotected pyrrolidine **7b** (5.22 g, 99%) was obtained as a yellow oil which was used directly without any further purification: $^1$H NMR δ 1.76 (app dt, *J* = 6.6, 10.3), 2.24–2.35 (m, 3H), 2.78–2.89 (m, 5H), 3.06 (dd, 1H, *J* = 5.1, 8.1), 3.49 (s, 2H), 3.63 (s, 4H), 3.85 (t, 1H, *J* = 6.5), 5.15, 5.16, 5.19, 5.20 (s, 8H), 7.41 (m, 20H); $^{13}$C NMR δ 34.0, 50.4, 50.5, 52.1, 52.4, 55.0, 58.4, 62.4, 65.8, 65.9, 66.4, 127.9, 128.0, 128.1, 128.3, 135.33, 135.34, 135.44, 170.6, 171.0, 174.1.

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To a solution of pyrrolidine **7b** (5.22 g, 7.4 mmol) in THF (15 mL), cooled to 0 °C, was added $iPr_2NEt$ (1.41 mL, 8.14 mmol) followed by benzyl 2-bromoacetate (1.78 g, 7.8 mmol) dissolved in THF (2 mL). After stirring 2 h at 0 °C, the reaction was warmed to room temperature and stirred for 15 h. The precipitated salt was removed by filtration and the solvent was evaporated to afford a crude oil which was passed through a short column of silica gel eluting with EtOAc. Evaporation of the EtOAc gave a yellow oil which was purified by chromatography on silica gel (1% MeOH in 2/1, hexanes/EtOAc) affording pentabenzyl ester **7c** (5.73 g, 90% from **7a**) as a pale yellow oil: $[\alpha]^{22}_D$ −26° (*c* 1.5, $CHCl_3$); $^1H$ NMR δ 1.88 (m, 1H), 2.29 (m, 1H), 2.71−2.87 (m, 5H), 3.10 (m, 1H), 3.44−3.62 (series of s, 9H), 3.70 (t, 1H, *J* = 8.1), 5.10 and 5.11, and 5.08 (s, 10H), 7.31 (m, 25H); $^{13}C$ NMR δ 33.5, 50.3, 52.1, 52.1, 52.6, 55.0, 55.6, 60.0, 62.5, 65.79, 65.87, 65.97, 66.07, 127.77, 127.81, 127.89, 127.97, 128.19, 128.22, 128.24, 135.31, 135.38, 135.42, 135.53, 170.1, 170.7, 171.4, 172.5. Anal. Calcd for $C_{50}H_{53}N_3O_{10}$: C, 70.2; H, 6.2; N, 4.9. Found: C, 70.5; H, 6.5; N, 4.8.

***N*-[[2-Bis(carboxymethyl)amino]ethyl]-*N*-[(2*S*,4*S*)-4-[2-carboxy-1-(carboxymethyl)pyrrolidinyl]]glycine Trihydrochloride Monohydrate (1).** Pentabenzyl ester **7c** (1.04 g, 1.21 mmol, 100 mol %) was dissolved in MeOH (30 mL) as 4 M HCl (950 μL) was slowly added. After washing 10% Pd/C (120 mg) with several portions of 1 M HCl, distilled water, and then methanol, the catalyst was added to the methanol solution of the pentabenzyl ester and shaken on a Parr apparatus at 50 psi $H_2$ for 2 h. Removal of the catalyst by filtration through a Millipore 5-μm Mitex filter disc followed by evaporation of the filtrate gave a thick residue. The residue was dissolved in 4 M HCl (4 mL) and evaporated to dryness to afford a pale orange solid which was pulverized to a fine powder. Further drying (40 °C, 0.01 torr, 17 h) gave pentaacid **1** (649 mg, 100%) as the trihydrochloride monohydrate: $[\alpha]_D^{22}$ −16° (*c* 1.5, $H_2O$); $^1H$ NMR ($D_2O$) δ 2.15 (m, 1H, 2.78 (m, 1H), 3.16 (br t, 2H), 3.59−3.64 (m, 5H), 3.69 (dd, 1H, *J* = 3.7, 8.7), 4.15 (m, 1H), 4.22 (d, 1H, *J* = 17.1), 4.33 (s, 4H), 4.44 (d, 1H, *J* = 17.1), 4.56 (dd, 1H, *J* = 6.8, 11.2); $^{13}C$ NMR ($D_2O$) δ 31.9, 50.7, 52.9, 56.6, 57.7, 58.2, 61.0, 68.1, 170.2, 170.6, 171.4, 176.8. Anal. Calcd for $C_{15}H_{26}N_3O_{10}Cl_3 \cdot H_2O$: C, 33.8; H, 5.3; N, 7.9. Found: C, 33.7; H, 5.3; N, 7.5.

**(2*S*,4*R*)-1-(Benzyloxycarbonyl)-4-hydroxyproline *tert*-Butyl Ester (4e).** To a 100-mL Morton flask equipped with mechanical stirring was added *trans*-4-hydroxy-L-proline (**4a**) (7.0 g, 53.4 mmol, 100 mol %) and $H_2O$ (25 mL) followed by $NaHCO_3$ (11 g, 134 mmol). To this vigorously stirred suspension was added benzyl chloroformate (11 g, >98% purity, 65 mmol) as a neat liquid in four equal portions over a period of 30 min. After the addition of the CBZCl, stirring was continued for 2 h, the reaction mixture was diluted with $H_2O$ (50 mL) and was extracted with ether (50 mL). The aqueous phase was cooled to 0 °C and the pH was adjusted to 2 by the slow addition of 6 N HCl. The precipitated oil was extracted into EtOAc (100 mL), and the organic phase was washed with brine (50 mL), dried, filtered, and evaporated. The crude **4d**, isolated as a viscous oil (13 g, 92%), was dissolved in THF (100 mL), and to this solution was added O-*tert*-butyl-*N*,*N*′-diisopropylisourea (9.8 g, 49 mmol) dropwise over a period of 10 min at room temperature. The slightly exothermic reaction was stirred for 30 min at rt and then was heated to 55 °C for 4 h. At this time an additional 100 mol % of isourea was added and the stirring was continued for 16 h. After this period, 25 mol % more isourea was added; no starting acid remained after 30 min, ~1 mL of acetic acid was added, and the mixture was stirred for 1.5 h. The reaction was cooled to 0 °C to further precipitate the urea which was removed by filtration. Evaporation of the solvent gave crude **4e** as an oil which was purified by silica gel chromatography (2/1, EtOAc/hexane) affording initial fractions of pure **4e** with later fractions being contaminated with urea. Combination of all fractions gave **4e** (13 g, 76% from **4a**) as an oil which solidified upon storage at 0 °C, and contained ~1−2% of *N*,*N*-diisopropylurea. An analytical sample of **4e** was prepared by recrystallization from ether/hexanes as white needles: mp 51−53 °C; $R_f$ 0.33 (1/2, hexanes/EtOAc); $[\alpha]^{22}_D$ −68° (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 1.32, 1.45 (2s, 9H), 2.05 (m, 1H), 2.10−2.35 (m, 2H), 3.51, 3.55 (2s, 0.5H), 3.64, 3.65, 3.68, 3.70 (4s, 1.5H), 4.37 (m, 1H), 4.47 (br s, 1H), 5.10, 5.11, 5.14 (3s, 2H), 7.32 (m, 5H); methyl signals of the urea impurity are found at δ 1.12, 1.14 ppm; $^{13}C$ NMR δ 27.6, 27.8, 38.2, 39.0, 54.4, 55.0, 58.3, 58.7, 66.9, 67.0, 68.8, 69.6, 81.3, 81.4, 127.5, 127.7, 127.9, 128.1, 128.2, 128.3, 136.0, 136.3, 154.6, 154.8, 171.7, 171.8. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.5; H, 7.2; N, 4.4. Found: C, 63.6; H, 7.2; N, 4.4.

**(2*S*,4*R*)-1-[(*tert*-Butoxycarbonyl)methyl]-4-hydroxyproline *tert*-Butyl Ester (4f).** A mixture of **4e** (6.5 g, 20 mmol) in MeOH (50 mL) and 10% Pd/C (850 mg) was stirred under $H_2$ (balloon) for 2 h. The catalyst was removed by filtration (Celite), the filtrate was evaporated to afford the amino alcohol as a solid which was dissolved in $CH_3CN$ (75 mL), and the solution was cooled to 0 °C, reprecipitating the amino alcohol. To the stirred suspension was added $iPr_2NEt$ (3.9 mL, 22 mmol) followed by the dropwise addition of *tert*-butyl bromoacetate (4.3 g, 22 mmol). After complete addition of the bromoacetate, the solution was stirred at 0 °C for 30 min and then at room temperature for 9 h. The solvent was evaporated, and to the residue was added ether (100 mL) followed by filtration of the precipated salts. Evaporation of the ether gave an oil which was purified by chromatography on silica gel (1/1, hexanes/EtOAc) affording **4f** (5.38 g, 88%) as a pale yellow oil: $R_f$ 0.38 (1/2, hexanes/EtOAc); $[\alpha]^{22}_D$ −55° (*c* 1.3, $CHCl_3$); $^1H$ NMR δ 1.46, 1.47 (2s, 18H), 2.02 (ddd, 1H, *J* = 4.5, 10.0, 13.1), 2.21 (dddd, 1H, *J* = 1.6, 1.7, 6.7, 13.1), 2.82 (ddd, 1H, *J* = 0.9, 1.7, 11), 3.52 (d, 1H, *J* = 18.4), 3.55 (dd, 1H, *J* = 4, 11), 3.60 (d, 1H, *J* = 18.4), 3.81 (dd, 1H, *J* = 6.8, 10), 4.26 (m, 1H); $^{13}C$ NMR δ 27.86, 27.91, 39.9, 52.7, 60.6, 62.2, 70.8, 80.6, 81.7, 171.7, 172.8. Anal. Calcd for $C_{15}H_{27}NO_5$: C, 59.8; H, 9.0; N, 4.6. Found: C, 59.5; H, 9.0; N, 4.6.

**(2*S*,4*R*)-1-[(*tert*-Butoxycarbonyl)methyl]-4-[(methylsulfonyl)oxy]proline *tert*-Butyl Ester (8).** To a solution of alcohol **4f** (5.34 g, 17.7 mmol) in $CH_2Cl_2$ (40 mL) at 0 °C was added $iPr_2NEt$ (4.6 mL, 26.5 mmol) followed by methanesulfonyl chloride (1.7 mL, 22.1 mmol). After stirring 2.5 h at 0 °C the solvent was evaporated and the residue was partitioned between ether (100 mL) and 1 M $Na_2CO_3$ (100 mL). The organic phase was washed with additional 1 M $Na_2CO_3$ (100 mL) and brine (100 mL), dried, filtered, and evaporated; the residue solidified under high vacuum. Recrystallization from ether/hexane at 0 °C gave 5.11 g of mesylate **8** as pale yellow needles (mp 67.5−69 °C). The filtrate was evaporated and chromatographed on silica gel (1/1, hexane/EtOAc) to afford an additional 1.7 g of pure mesylate, giving a combined yield of 6.18 g (92%) of **8**. An analytical sample was prepared by recrystallization from ether/hexanes affording **8** as white needles: mp 68−69 °C; $R_f$ 0.34 (1/1, hexanes/EtOAc); $[\alpha]^{22}_D$ −32° (*c* 1.0, $CHCl_3$); $^1H$ NMR δ 1.46, 1.47 (2s, 18H), 3.17 (m, 2H), 3.03 (s, 3H), 3.17 (dd, 1H, *J* = 3.5, 10.7), 3.43 (d, 1H, *J* = 17.2), 3.49 (d, 1H, *J* = 17.2), 3.53 (dd, 1H, *J* = 6.2, 10.7), 3.80 (t, 1H, *J* = 7), 5.25 (m, 1H); $^{13}C$ NMR δ 27.8, 27.9, 36.3, 38.2, 53.1, 57.6, 62.4, 78.6, 81.0, 81.3, 169.2, 171.1. Anal. Calcd for $C_{16}H_{29}NO_7S$: C, 50.6; H, 7.7; N, 3.7. Found: C, 50.8; H, 7.6; N, 3.6.

**(2*S*,4*S*)-4-Azido-1-[(*tert*-butoxycarbonyl)methyl]proline *tert*-Butyl Ester (9a).** To a solution of mesylate **8** (24.39 g, 64.3 mmol) in DMF (125 mL) was added $NaN_3$ (16.7 g, 257 mmol). The stirred suspension was placed in a preheated oil bath (75 °C) for 8 h. The reaction mixture was partitioned between $Et_2O$ (250 mL) and $H_2O$ (200 mL), the organic phase was separated and washed with $H_2O$ (2 × 100 mL), the combined aqueous phases were extracted with $Et_2O$ (100 mL), and the combined organic phases were then washed with brine (100 mL), dried, and evaporated. The crude yellow oil thus obtained was chromatographed on silica gel (8/1, hexanes/EtOAc) to afford azide **9a** (19.6 g, 95%) as a colorless oil: $R_f$ 0.40 (3/1 hexanes/EtOAc); IR (thin film) 2100 1725 $cm^{-1}$; $[\alpha]^{22}_D$ −23° (*c* 1.2, $CHCl_3$); $^1H$ NMR δ 1.46, 1.48 (2s, 18H), 2.17 (ddd, 1H, *J* = 3.5, 4.9, 14.3), 2.50 (dddd, 1H, *J* = 1.7, 6.9, 9.2, 14.3), 3.11 (dd, 1H, *J* = 2.8, 9.5), 3.22 (dd, 1H, *J* = 5.8, 9.5), 3.51 (d, 1H, *J* = 17.7), 3.58 (d, 1H, *J* = 17.7), 3.70 (dd, 1H, *J* = 4.9, 9.2), 4.08 (m, 1H); $^{13}C$ NMR ($CDCl_3$) δ 27.9, 28.0, 35.2, 52.4, 56.9, 59.1, 62.0, 81.0, 81.1, 169.9, 171.8. Anal. Calcd for $C_{15}H_{26}N_4O_4$: C, 55.2; H, 8.0; N, 17.2. Found: C, 55.4; H, 8.3; N, 17.0.

**(2*S*,4*S*)-4-Amino-1-[(*tert*-butoxycarbonyl)methyl]proline *tert*-Butyl Ester (9b).** Azide **8** (7 g, 21.4 mmol) in MeOH

(200 mL) and 10% Pd–C (700 mg) were shaken under $H_2$ at 50 psi. The Parr bottle was evacuated (aspirator) and repressurized to 50 psi, and the hydrogenation was continued for an additional 30 min. The reaction solution was purged with $N_2$ and was filtered through a pad of Celite which was prewashed with first 10% $Et_3N$ in MeOH then MeOH. Evaporation of the filtrate and removal of residual solvent under high vacuum (0.1 torr, 12 h) gave amine **9b** (6.4 g, 99%) as an oil, used directly in the next step: $^1$H NMR $\delta$ 1.46, 1.47 (2s, 18H), 1.69–1.76 (m, 3H), 2.46 (ddd, 1H, $J$ = 7.1, 9.4, 13.3), 2.86 (ddd, 1H, $J$ = 0.06, 3.2, 8.9), 2.98 (dd, 1H, $J$ = 5.8, 8.9), 3.45, 3.46, 3.51 (2s, m, 3H), 3.58 (dd, 1H, $J$ = 4.9, 9.4); $^{13}$C NMR $\delta$ 27.8, 27.9, 39.5, 50.8, 53.2, 61.6, 63.0, 80.6, 80.7, 169.9, 173.3.

***N*-[(2*S*,4*S*)-4-[2-(*tert*-Butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[1-(benzyloxycarbonyl)-2-(*tert*-butoxycarbonyl)pyrrolidinyl]]amine (10).** To a solution of alcohol **4e** (8.0 g, 25 mmol) in $CH_2Cl_2$ (40 mL) at −30 °C was added $iPr_2NEt$ (8.67 mL, 50 mmol) followed the dropwise addition of $Tf_2O$ (4.4 mL, 26 mmol) over a period of 15 min. The orange triflate solution was stirred at −30 °C for 45 min and then at 0 °C for 15 min and amine **9b** (6.0 g, 20 mmol) in $CH_2Cl_2$ (10 mL) was added. The reaction mixture was stirred at 0 °C for 40 h and then was diluted with $CH_2Cl_2$ (75 mL), and the organic phase was washed with 0.5 M $K_3PO_4$ (200 mL) and brine and dried. Evaporation of the solvent gave a dark orange oil (15.6 g) which was passed through a column of silica gel (5 × 22 cm) eluting with EtOAc. Fractions containing product were combined and evaporated to afford 11 g. On addition of petroleum ether (100 mL) the product crystallized to provide 6 g of **10**. The filtrate was evaporated and chromatographed (20/1, $CHCl_3$/2-propanol) to afford an additional 3.6 g for a combined yield of 9.6 g (80%) of **10** as a ~95:5 mixture of diastereomers. Recrystallization four times from hexane (5 mL hexane/g of **10**), allowing the product to crystallize at rt overnight gave 5.4 g of **10**, >99% diastereomerically pure by HPLC: mp 73–75 °C; $R_f$ 0.16 (1/1, hexanes/EtOAc); $[\alpha]^{22}_D$ −57° ($c$ 1.1, $CHCl_3$); $^1$H NMR $\delta$ 1.34, 1.44, 1.45, 1.46 (4s, 27H), 1.75–1.92 (m, 3H), 2.37 (m, 2H), 2.90–3.05 (m, 2H), 3.10–3.34 (m, 3H), 3.40 (d, 1H, $J$ = 17.7), 3.46 (d, 1H, $J$ = 17.7), 3.57 (m, 1H), 3.76 (dd, 0.4H, $J$ = 5.9, 10.1), 3.83 (dd, 0.6H, $J$ = 5.7, 10.2), 4.22 (m, 1H), 5.08, 5.10, 5.15, 5.17 (4d, 2H, $J$ = 12.4), 7.33 (m, 5H). Anal. Calcd for $C_{32}H_{49}N_3O_8$: C, 63.7; H, 8.2; N, 7.0. Found: C, 63.8; H, 8.0; N, 7.0.

***N,N*-Bis[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]amine (11a).** A mixture of **10** (5.2 g, 8.6 mmol) dissolved in MeOH (6 mL) and 10% Pd/C (300 mg) was stirred for 1.5 h under $H_2$ (balloon). The catalyst was removed by filtration through Celite (prewashed with 10% $Et_3N$ in MeOH followed by MeOH) and the filtrate was evaporated. The residue was dissolved in $CH_3CN$ (20 mL) and cooled to −10 °C. To this solution was added $iPr_2NEt$ (1.65 mL, 9.5 mmol) followed by the dropwise addition of *tert*-butyl bromoacetate (1.76 g, 9.0 mmol) over a period of 5 min. The solution was stirred at −5 to −10 °C for 3.5 h and then at rt for 1 h. The solvent was evaporated and the residue was partitioned between $Et_2O$ (100 mL) and 0.5 M $K_3PO_4$ (100 mL). The organic phase was washed with brine (100 mL), dried, and evaporated to afford a crude residue (5.5 g) which was chromatographed on silica gel (25/1, EtOAc/MeOH) to give tetraester **11a** (4.4 g, 87%) as a pale yellow oil which solidified slowly to a white solid: $[\alpha]^{22}_D$ −55° ($c$ 1.1, $CHCl_3$); $^1$H NMR $\delta$ 1.45, 1.46 (2s, 36H), 1.79 (ddd, 2H, $J$ = 5.4, 6.2, 12.9), 1.81 (br s, 1H), 2.38 (ddd, 2H, $J$ = 7.2, 8.3, 12.9), 2.93 (dd, 2H, $J$ = 4.8, 8.9), 3.06 (dd, 2H, $J$ = 6.6, 8.9), 3.37 (m, 2H), 3.45 (s, 4H), 3.62 (dd, 2H, $J$ = 6.3, 8.3); $^{13}$C NMR $\delta$ 27.8, 27.9, 36.6, 53.0, 54.8, 58.2, 62.6, 80.4, 80.5, 169.9, 172.7. Anal. Calcd for $C_{30}H_{53}N_3O_8$: C, 61.7; H, 9.2; N, 7.2. Found: C, 62.1; H, 9.2; N, 7.2.

Eluting first from the column in the purification of **11a** was penta-*tert*-butyl ester **11b**, isolated as a pale yellow oil: $[\alpha]^{22}_D$ −48° ($c$ 1.1, $CHCl_3$); $^1$H NMR $\delta$ 1.43, 1.449, 1.453 (3s, 45H), 1.77 (dt, 2H, $J$ = 8.8, 12.7), 2.29 (dt, 2H, $J$ = 7.5, 12.7), 2.87 (t, 2H), 3.03 (dd, 2H, $J$ = 6.6, 9.3), 3.31 (d, 2H, $J$ = 17.2), 3.41, 3.43 (2s, 2H), 3.46 (d, 2H, $J$ = 17.2), 3.55 (dd, 2H, $J$ = 7.4, 8.7), 3.63 (m, 2H); $^{13}$C NMR $\delta$ 27.9, 28.0, 28.1, 34.3, 49.1, 53.6, 55.2, 59.9, 63.0, 80.2, 80.7, 80.8, 170.0, 172.4. Anal. Calcd for $C_{36}H_{63}N_3O_{10}$: C, 61.96; H, 9.10; N, 6.02. Found: C, 61.72; H, 9.04; N, 5.84.

**Triflate of benzyl glycolate** was prepared using a slight modification of the reported literature methods. To a solution of benzyl glycolate[18] (4 g, 24 mmol) and 2,6-lutidine (2.95 mL, 25.3 mmol) in $CH_2Cl_2$ (50 mL) at −20 °C was added triflic anhydride (6.8 g, 24.1 mmol) over a period of 5–10 min. After complete addition, the reaction mixture was stirred for 30 min and was then warmed to rt, stirring an additional 30 min. The reaction mixture was evaporated and rapidly passed through a short column of silica gel eluting with $CH_2Cl_2$. The fractions containing triflate were combined, the solvent was evaporated, and the residue was subjected to a second rapid chromatography (1/1, $CH_2Cl_2$/hexane) to provide 5.6 g of triflate as a pale yellow oil which solidified at 0 °C. This material was suitable for use directly without any further purification: $^1$H NMR $\delta$ 4.93 (s, 2H), 5.28 (s, 2H), 7.38 (s, 5H); $^{13}$C NMR $\delta$ 68.0, 68.8, 118.3 (q, $J_{CF}$ = 319 Hz), 128.4, 128.5, 128.7, 134.2, 164.4.

***N,N*-Bis[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]glycine Benzyl Ester (12).** To a solution of **11a** (3.85 g, 6.6 mmol) in $CH_3CN$ (15 mL) cooled to −20 °C was added $iPr_2NEt$ (1.26 mL, 7.3 mmol). The benzyl glycolate triflate (2.06 g, 6.9 mmol) was then added to the solution over a period of 10 min and it was stirred at −15 to −20 °C for 4 h and warmed to rt, stirring an additional 1 h. The solvent was evaporated, the residue was partitioned between $Et_2O$ (100 mL) and saturated $NaHCO_3$ (100 mL), and the organic phase was washed with additional saturated $NaHCO_3$ (100 mL) and brine and dried. Evaporation of the solvent gave a crude residue which was purified by chromatography on silica gel (1% MeOH in 3/1, hexane/EtOAc); pentaester **12** (3.5 g, 72%) was obtained as a pale yellow oil: $[\alpha]^{22}_D$ −49° ($c$ 1.0, $CHCl_3$); $^1$H NMR $\delta$ 1.43, 1.45 (2s, 36H), 1.79 (dt, 2H, $J$ = 8.4, 12.8), 2.30 (dt, 2H, $J$ = 7.8, 12.8), 2.87, dd, 2H, $J$ = 8.2, 9.3), 3.03 (dd, 2H, $J$ = 6.3, 9.3), 3.27 (d, 2H, $J$ = 17.1), 3.43 (d, 2H, $J$ = 17.1), 3.53 (dd, 2H, $J$ = 7.8, 8.4), 3.59, 3.65, 3.69 (d, $J$ = 18.0, m, d, $J$ = 18.0, total 4H); $^{13}$C NMR $\delta$ 27.8, 27.9, 33.9, 48.2, 53.3, 55.2, 59.7, 62.9, 66.0, 80.65, 80.67, 127.8, 128.0, 128.2, 135.7, 169.7, 172.1, 173.0. Anal. Calcd for $C_{39}H_{61}N_3O_{10}$: C, 64.0; H, 8.4; N, 5.7. Found: C, 64.2; H, 8.2; N, 5.7.

**cis-1-(Benzyloxycarboxyl)-4-hydroxy-D-proline *tert*-butyl ester (13b)** was prepared by modification of the reported method.[9] To a solution of AcOH (300 mL) and $Ac_2O$ (300 mL) was added *trans*-4-hydroxy-L-proline (25 g, 0.19 mol), the mixture was refluxed for 5.5 h, and the solvents were evaporated. The residue was dissolved in 2 *N* HCl (300 mL) and refluxed for 2 h. The resulting brown solution was treated with decolorizing carbon (1 g) while hot, allowed to cool, and filtered through Celite, and the filtrate was evaporated to 100 mL. Crystallization began at rt and was completed overnight at 0 °C. The crystals were filtered off and the filtrate was concentrated until additional crystals formed and a second crop was obtained. The combined crops afforded 22.35 g of **13a** which was dissolved in 1 *N* HCl (40 mL) and cooled to 0 °C. Filtration of the crystalline product followed by concentration of the filtrate to obtain a second crop, gave *cis*-4-hydroxy-D-proline (**13a**, 17 g, 53%) as the hydrochloride salt. This material was used directly in the next step. A mixture of **13a** (10 g, 60 mmol) and $NaHCO_3$ (17.6 g, 210 mmol) in $H_2O$ (60 mL) was treated with benzyl chloroformate (11.26 g, 95% purity, 66 mmol) as described in the preparation of **4e**. After isolation of the product by extraction, 11 g of the benzyl carbamate was otained. This crude material was dissolved in THF (80 mL) and treated initially with *O*-*tert*-butyl-*N,N'*-diisopropylisourea (8.3 g, 42 mmol). Additional isourea was added as described in the preparation of **4e**. The product was isolated as before and the crude residue was chromatographed (1/1, hexane/EtOAc) to provide ***cis*-1-(benzyloxycarbonyl)-4-hydroxy-D-proline *tert*-butyl ester (13b**, 10g, 75%) as an oil: $^1$H NMR $\delta$ 1.35, 1.50 (2s, 9H), 2.07 (dd, 1H), $J$ = 5.3, 13.9), 2.30 (m, 1H), 3.52–3.79 (m, 3H), 4.36 (m, 2H), 5.13, 5.16 (s, 2d, 2H, $J$ = 12.5), 7.34 (m, 5H); $^{13}$C NMR $\delta$ 27.5, 27.7, 37.5, 38.4, 55.4, 55.7, 58.4, 58.8, 66.9, 67.0, 69.7, 70.6, 82.2, 82.3, 127.56, 127.60, 127.7, 128.1, 128.2,

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136.0, 136.2, 154.2, 154.6, 173.3, 173.4. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.5; H, 7.2; N, 4.4. Found: C, 63.2; H, 7.3; N, 4.3.

***trans*-4-Acetoxy-1-(benzyloxycarbonyl)-D-proline *tert*-Butyl Ester (14a).** To a solution of alcohol **13b** (11.7 g, 36.4 mmol), $Ph_3P$ (11.45 g, 4.7 mmol), and AcOH (2.5 mL, 43.7 mmol) in THF (100 mL) at 0 °C was added diethyl azodicarboxylate (7.61 g, 43.7 mmol) as a neat liquid. The reaction mixture was stirred for 1.5 h at 0 °C and then at rt for 18 h. Methanol (10 mL) was added, the solvents were evaporated, the residue was dissolved in $Et_2O$ (200 mL), and hexane (100 mL) was added to further precipitate the $Ph_3PO$. After cooling to 0 °C, the mixture was filtered and the filter cake was washed with 2/1, $Et_2O$/hexane (75 mL). Evaporation of the solvents gave a residue which was passed through a column of silica, eluting with 1/1, hexane/EtOAc. The solvents were evaporated to give 12.4 g of material which was chromatographed (3/1, hexane/EtOAc) to afford *trans*-acetate **14a** (10.3 g, 78%) as an oil which solidified upon standing at 0 °C: mp 44–46 °C; $[\alpha]^{22}_D$ +49° (*c* 1.0, $CHCl_3$); $^1H$ NMR $\delta$ 1.33, 1.46 (2s, 9H), 2.04, 2.05 (2s, 3H), 2.21 (m, 1H), 2.41 (m, 1H), 3.62, 3.66 (2s, 0.5H), 3.76 (m, 2H), 4.36 (m, 1H), 5.14, 5.15 (2s, 2H), 5.27 (m, 1H); $^{13}C$ NMR $\delta$ 20.76, 20.78, 27.5, 27.7, 35.3, 36.4, 51.9, 52.4, 58.0, 58.4, 66.9, 67.0, 71.6, 72.4, 81.40, 81.45, 127.6, 127.7, 128.1, 128.2, 135.9, 136.2, 154.0, 154.3, 170.0, 170.05, 170.8, 171.0 Anal. Calcd for $C_{19}H_{25}NO_6$: C, 62.8; H, 6.9; N, 3.9. Found: C, 63.1; H, 7.2; N, 4.0

**(2*R*,4*S*)-1-(Benzyloxycarbonyl)-4-hydroxyproline *tert*-Butyl Ester (14b).** *trans*-Acetate **14a** (10.1 g, 28 mmol) was dissolved in MeOH (65 mL), the solution was cooled to 0 °C, $K_2CO_3$ (5.76 g, 42 mmol) in $H_2O$ (50 mL) was added, and the mixture was stirred 1.75 h. The solution was neutralized at 0 °C to pH 7 with 1 M $H_3PO_4$ (38 mL) and the methanol was evaporated. The remaining aqueous solution was diluted with $H_2O$ (50 mL) and was extracted with EtOAc (150 mL). The organic phase was washed with brine (100 mL), dried, and evaporated to afford alcohol **14b** (8.9 g, 99%) as an oil which slowly solidified at 0 °C (mp 49–52 °C). The product had identical NMR spectral data as reported for enantiomer **4e**. An analytical sample was recrystallized from ether/hexane: mp 51–53 °C. Anal. Calcd for $C_{17}H_{23}NO_5$: C, 63.5; H, 7.2; N, 4.4. Found: C, 63.7; H, 7.5; N, 4.5.

***N*-[(2*R*,4*R*)-4-[1-(Benzyloxycarbonyl)-2-(*tert*-butoxycarbonyl)pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1[(butoxycarbonyl)methyl]pyrrolidinyl]]amine (15).** Alcohol **14b** (7.0 g, 21.8 mmol) was converted to the triflate by treatment with $iPr_2NEt$ (7.6 mL, 55 mmol) and triflic anhydride (6.46 g, 23 mmol) in $CH_2Cl_2$ (50 mL) and then was treated with amine **9b** (5.22 g, 17.4 mmol) according to the procedure described for preparation of **10**. After isolation and purification by chromatography (20/1, $CHCl_3$/2-propanol), bispyrrolidine **15** (8.43 g, 80%) was obtained as a pale yellow oil which was a 95/5 mixture of diastereomers by HPLC (eluting with 3% iPrOH, 0.05% $NH_4OH$ in $CH_2Cl_2$; flow rate 0.5 mL/min). For diastereomer **15**, major isomer, $t_R$ 9.3 min; for minor isomer, $t_R$ 11.3 min: $[\alpha]^{22}_D$ +7.8° (*c* 1.06, $CHCl_3$); $^1H$ NMR $\delta$ 1.34, 1.44, 1.45 (3s, 27H), 1.75–1.92 (m, 3H), 2.35 (m, 2H), 2.90 (dd, 1H, *J* =3.8, 9.0), 3.15–3.19 (m, 3H), 3.43 (s, 2H), 3.58 (m, 1H), 3.80 (m, 1H), 4.22 (m, 1H), 5.11 (m, 2H), 7.32 (m, 5H); $^{13}C$ $\delta$ 27.4, 27.6, 27.8, 35.5, 35.8, 35.9, 36.5, 52.2, 52.5, 52.9, 53.7, 54.5, 54.7, 54.8, 57.9, 58.1, 58.5, 62.4, 66.5, 66.6, 80.5, 80.6, 80.8, 127.5, 127.9, 128.0, 136.0, 136.3, 153.9, 154.2, 169.59, 169.62, 170.8, 171.0, 172.8. Anal. Calcd for $C_{32}H_{49}N_3O_8$: C, 63.7; H, 8.2; N, 7.0. Found: C, 63.8; H, 8.3; N, 7.1.

***N*-[(2*R*,4*R*)-4-[2-(*tert*-Butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]amine (16a).** The *N*-CBZ triester **15** (8.0 g, 13.3 mmol) dissolved in MeOH (150 mL) was added 10% Pd/C (800 mg) and the mixture was hydrogenated (balloon). After 3 h, the catalyst was removed by filtration through a bed of Celite which was prewashed with 10% $Et_3N$ in MeOH followed by MeOH. Evaporation of the filtrate gave 6.04 g (96%) of the secondary amine as a pale yellow oil which was dissolved in $CH_3CN$ (25 mL) and to this solution at −10 °C was added $iPr_2NEt$ (2.5 mL, 14.2 mmol) followed by the dropwise addition of *tert*-butyl bromoacetate (2.52 g, 12.9 mmol) over a 15-min period. The solution was stirred at −5 to −10 °C for 5 h, an additional 5 mol % of bromoacetate was added, and the stirring was continued for 30 min at −5 to −10 °C and then for 1 h at rt. The solvent was evaporated and the residue was partitioned between $Et_2O$ (100 mL) and 0.5 M $K_3PO_4$ (100 mL). The organic phase was washed with brine (100 mL), dried, filtered, and evaporated. Chromatography of the residue on silica gel (25/1, EtOAc/MeOH) afforded alkylated product **16a** (6.6 g, 85%) as a mixture of diastereomers. Anal. Calcd for $C_{30}H_{53}N_3O_8$: C, 61.7; H, 9.2; N, 7.2. Found: C, 61.7; H, 9.2; N, 7.2.

***N*-(Benzyloxycarbonyl)-*N*-[(2*R*,4*R*)-4-[2-(*tert*-butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1-(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]amine (16c).** To a solution of **16a** (4.7 g, 8.1 mmol, as a mixture of diastereomers), DMAP (235 mg, 1.9 mmol), and $iPr_2NEt$ (2.12 mL, 12.2 mmol) in $CH_2Cl_2$ (25 mL) at 0 °C was added benzyl chloroformate (1.73 g, 95% purity, 9.6 mmol). The reaction mixture was stirred at 0 °C for 2.5 h and warmed to rt, additional benzyl chloroformate (230 $\mu$L) was added, and stirring was continued for 16 h. The solvent was evaporated and the residue was partitioned between $Et_2O$ (150 mL) and saturated $NaHCO_3$ (100 mL). The organic phase was washed with brine, dried, and evaporated. Chromatography of the residue (2/1, hexane/EtOAc) gave **16c** (4.7 g, 81%) containing 2–3% of the contaminating diastereomer. The diastereomeric mixture was suspended in petroleum ether (75 mL) and heated to boiling while EtOAc was slowly added until solution was achieved. Cooling afforded **16c** (4.04 g, 70%) as colorless prisms. HPLC analysis indicated the diastereomeric purity was >99% when compared to **18a** which was converted to the N-CBZ adduct using the procedure above: mp 107–109 °C; $^1H$ NMR $\delta$ 1.45 (s, 36H), 2.10–2.39 (m, 4H), 2.91 (app t, 2H, *J* = 9), 3.28 (dd, 2H, *J* = 6.6, 8.8), 3.34 (d, 2H, *J* = 17), 3.52 (d, 2H, *J* = 17), 3.59 (app t, 2H, *J* = 8), 4.61 (br m, 2H), 5.16 (s, 2H), 7.26–7.45 (m, 5H); $^{13}C$ $\delta$ 27.9, 28.0, 34.3, 52.5, 53.7, 55.3, 63.2, 66.7, 80.66, 80.71, 127.5, 127.9, 128.2, 136.5, 155.0, 169.8, 171.7. Anal. Calcd for $C_{38}H_{59}N_3O_8$: C, 63.6; H, 8.3; N, 5.8. Found: C, 63.4; H, 8.2; N, 5.8.

***N*-[(2*R*,4*R*)-4-[2-(*tert*-Butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[2-(*tert*-butoxycarbonyl)-1-[(*tert*-butoxycarbonyl)methyl]pyrrolidinyl]]glycine Benzyl Ester (17).** To a solution of **16c** (4.0 g, 5.6 mmol) in MeOH (80 mL) was added 10% Pd/C (400 mg), and the mixture was hydrogenated (balloon). After stirring for 30 min, the catalyst was removed by filtration through a bed of Celite (prewashed with 10% $Et_3N$ in MeOH followed by MeOH) and the filtrate was evaporated to afford 3.23 g of **16a** which was used directly in the next step.

To a solution of tetraester **16a** (3.0 g, 5.14 mmol) in $CH_3CN$ (20 mL) was added $iPr_2NEt$ (985 $\mu$L, 5.65 mmol) and benzyl glycolate triflate (1.61 g, 5.4 mmol) at −20 °C, and the mixture was stirred for 3 h and then for 2 h at rt. The product was isolated and purified in the same manner as described for pentaester **12**, to afford **17** (2.48 g, 66%) as a pale yellow oil: $^1H$ NMR $\delta$ 1.43, 1.45 (2s, 36H), 1.79 (dt, 2H, *J* = 8.4, 12.8), 2.30 (dt, 2H, *J* = 7.8, 12.8), 2.87 (dd, 2H, *J* = 8.2, 9.3), 3.03 (dd, 2H, *J* = 6.3, 9.3), 3.27 (d, 2H, *J* = 17.1), 3.43 (d, 2H, *J* = 17.1), 3.53 (dd, 2H, *J* = 7.8, 8.4), 3.59, 3.65, 3.69 (d, *J* = 18.0; m, 4H, *J* = 18.0); $^{13}C$ NMR $\delta$ 27.7, 27.8, 33.7, 48.1, 53.3, 55.2, 55.2, 59.5, 62.9, 65.9, 80.45, 80.48, 127.7, 127.8, 128.0, 135.5, 169.5, 172.0, 172.8. Anal. Calcd for $C_{39}H_{61}N_3O_{10}$: C, 64.0; H, 8.4; N, 5.7. Found: C, 63.7; H, 8.4; N, 5.7.

***N*,*N*-Bis[(2*S*,4*S*)-4-[2-carboxy-1-(carboxymethyl)pyrrolidinyl]]glycine (2).** To pentaester **12** (2.83 g, 2.4 mmol) in MeOH (80 mL) was added 10% Pd/C (280 mg) and the mixture was hydrogenated (balloon) with stirring for 2 h. Filtration and evaporation of the filtrate gave 2.45 g (99%) of the monocarboxylic acid which was dissolved in dioxane (8 mL) and to this solution was added 4 M HCl (30 mL) in 10-mL portions. The mixture was diluted with $H_2O$ (10 mL) and heated in a water bath at 50 °C for 1 h until solution was obtained. The resulting dark solution was stirred at rt 1 h and concentrated to a volume of 15 mL. After filtration through a plug of glass wool, the solution was evaporated (bath temperature, 40 °C). Further drying of the brown residue under high vacuum (40 °C, 0.10 torr) gave 2.15 g of the trihydrochloride salt. The product was decolorized with activated carbon (1 g, 50% w/w,

boiled with 1 M HCl and two successive portions of distilled water) by dissolving the hydrochloride salt in distilled water (25 mL) and the pretreated decolorizing carbon was added to this solution in 10–15 mL of distilled water. After the mixture was stirred 15 min, the carbon was removed by filtration through a millipore filter and evaporated. Drying of the residue (40 °C, 0.05 torr, 12 h) gave a glassy solid which was pulverized to a tan powder, affording **2** (1.98 g, 94%) as the trihydrochloride monohydrate: $[\alpha]^{22}_D$ −34° (*c* 1.1, $H_2O$); $^1H$ NMR ($D_2O$) δ 2.16 (m, 2H), 2.69 (m, 2H), 3.61 (dd overlapping $AB_q$, 4H, $J_{AB}$ = 19.5), 3.75 (dd, 2H, *J* = 8.1, 12.6), 4.14 (m overlapping d, 4H, *J* = 17.1), 4.39 (d, 2H, *J* = 17.1), 4.50 (dd, 2H, *J* = 7.1, 10.4); $^{13}C$ NMR ($D_2O$) δ 33.5, 49.4, 58.1, 58.5, 60.8, 68.1, 170.3, 171.7, 178.1. Anal. Calcd for $C_{16}H_{26}N_3O_{10}Cl_3 \cdot H_2O$: C, 35.3; H, 5.2; N, 7.7. Found: C, 35.5; H, 5.1; N, 7.8.

***N*-[(2*R*,4*R*)-4-[2-Carboxy-1-(carboxymethyl)pyrrolidinyl]]-*N*-[(2*S*,4*S*)-4-[2-carboxy-1-(carboxymethyl)pyrrolidinyl]]glycine (3).** To pentaester **17** (1.77 g, 2.4 mmol) dissolved in MeOH (50 mL) was added 10% Pd/C (180 mg) and the mixture was stirred for 1.5 h under hydrogen (balloon) and then filtered. Evaporation of the filtrate gave 1.52 g (99%) of the monocarboxylic acid which was dissolved in dioxane (5 mL) and to this solution was added 4 M HCl (15 mL). The solution was stirred at room temperature for 1 h then evaporated (bt, 40 °C). Further drying of the brown residue under high vacuum (40 °C, 0.10 torr) gave 1.28 g (97%) of the deprotected trihydrochloride salt which was analytically pure by C, H, N analysis. The product was decolorized with activated carbon (640 mg, 50% w/w) as described for **2**. The resulting glassy solid was pulverized to a tan powder, affording **3** (1.20 g, 91%) as the trihydrochloride monohydrate: $^1H$ NMR ($D_2O$) δ 2.14 (m, 2H), 2.69 (m, 2H), 3.61 (dd overlapping s, 4H, *J* = 8.2, 12.6), 3.78 (dd, 2H, *J* = 8.3, 12.6), 4.10 (m overlapping d, 4H, *J* = 17.0), 4.37 (d, 2H, *J* = 17.0), 4.46 (dd, 2H, *J* = 7.1, 10.6); $^{13}C$ NMR ($D_2O$) δ 33.7, 49.7, 58.1, 58.5, 60.6, 67.9, 170.3, 171.6, 178.2. Anal. Calcd for $C_{16}H_{26}N_3O_{10}Cl_3 \cdot H_2O$: C, 35.3; H, 5.2; N, 7.7. Found: C, 35.2; H, 5.1; N, 7.7.

**Diastereomeric Purity of Pentaesters 12 and 17.** Diastereomeric purities of pentaesters **12** and **17** were determined by HPLC using diastereomers **18b** and **20b** as standards. HPLC conditions: mobile phase, 1.5% PrOH, 0.05% $NH_4OH$ in $CH_2Cl_2$; flow rate, 0.5 mL/min. For pentaester **12**, $t_R$ = 16 min; diastereomer **20b**, $t_R$ = 14.5 min. For pentaester **17**, $t_R$ = 15.5 min; for diastereomer **18b**, $t_R$ = 14.3 min. The detection limit for the presence of any diastereomer was <1%.

**Gadolinium Complex 21.** To a solution of pentaacid **3** (212 mg, 0.39 mmol, 100 mol %) in $H_2O$ (2 mL) was added a solution of $GdCl_3 \cdot 6H_2O$ (162 mg, 0.44 mmol, 113 mol %) in $H_2O$ (2 mL). The pH of the resulting solution was 1.4 and 0.1 N NaOH was slowly added. When a pH of 2.9 was reached, a cloudy solution was obtained which was stirred for 3 h. The addition of base was resumed resulting in further precipitation of a white solid. At pH 5.0 the solid dissolved and base was added until a final pH of 6.7. Evaporation of the water gave 440 mg of the crude product. The metal chelate was dissolved in $H_2O$ (~2 mL) and passed through a column of sephadex G-10 (20 × 300 mm) eluting with $H_2O$ at a flow rate of 1 mL/min. Fractions containing metal chelate were detected by spotting on silica plates and staining with 2% phosphomolybdic acid in EtOH followed by heating. The fractions containing chelate were combined, filtered through a millipore filter to remove insoluble material, and evaporated to afford 200 mg of a solid. After a second sephadex desalting, Gd chelate **21** (170 mg, 69%) was obtained as a pale yellow solid. An analytical sample was prepared by further drying (77 °C, 0.02 torr, 12 h) affording the product as the disodium salt monohydrate. Anal. Calcd for $C_{16}H_{18}N_3O_{10}Na_2Gd \cdot H_2O$: C, 30.3; H, 3.2; N, 6.6. Found: C, 30.5; H, 3.3; N, 6.8.

**Lutetium Complex 22.** To a solution of pentaacid **3** (150 mg, 0.28 mmol) in $H_2O$ (2 mL) was added a solution of $LuCl_3 \cdot 6H_2O$ (116 mg, 0.30 mmol, 108 mol %) in $H_2O$ (2 mL). The pH of the resulting solution was 1.4 and 0.1 N NaOH was slowly added over a period of 30 min, until a final pH of 6.8 was obtained. (At pH 3 a slight cloudiness was imparted to the solution which disappeared as the pH was increased to 4.5.) Evaporation of the water gave a residue which was dissolved in $H_2O$ and filtered through a millipore filter (10 μm) and evaporated to afford 280 mg of a white solid. The metal chelate was dissolved in $H_2O$ (2 mL) and passed through a column of sephadex G-10 (20 × 300 mm) eluting with $H_2O$ at a flow rate of 1 mL/min. The fractions containing chelate were combined and evaporated to give 180 mg of a solid which was subjected to a second sephadex desalting to afford Lu chelate **22** (155 mg, 87%) as a white powder. An analytical sample was prepared by further drying (77 °C, 0.02 torr, 14 h) affording **22** as the disodium salt dihydrate: $^1H$ NMR ($D_2O$) δ 1.80–4.20 (br complex region), 5.13 (br d, 1H); $^{13}C$ NMR ($D_2O$) δ all signals were broad 35.1, 35.7, 37.1, 53.4, 56.7, 57.7, 59.8, 60.6, 60.7, 60.9, 62.3, 68.5, 70.0, 70.2, 70.7, 75.7, 181.4, 182.7, 183.3, 184.3, 185.9. Anal. Calcd for $C_{16}H_{18}N_3O_{10}Na_2Lu \cdot 2H_2O$: C, 28.7; H, 3.3; N, 6.3. Found: C, 28.7; H, 3.2; N, 6.2.

**Lutetium Complex 23.** The lutetium complex of **2** was prepared in the same manner as described for **3**. Crude chelate **23** was isolated as a white powder and was directly examined by NMR without desalting on sephadex: $^1H$ NMR (400 MHz, $D_2O$) δ 2.34 (br d, 1H, *J* = 15.6), 2.42–2.60 (m, 3H), 2.84 (dd, 1H, *J* = 1.2, 9.6), 3.02 (d, 1H, *J* = 9.6), 3.12–3.38 (series of m and AB d, 9H), 3.68 (d, 1H, *J* = 16.9), 3.82 (d, 1H, *J* = 17.1), 3.88 (d, 1H, *J* = 17.7); $^{13}C$ ($D_2O$) δ 36.7, 38.7, 61.2, 62.0, 62.2, 62.7, 65.0, 65.3, 70.5, 70.8, 73.7, 182.7, 183.5, 184.2, 184.7, 185.3.