A New Method for the Synthesis of Tri-*tert*-butyl Diethylenetriaminepentaacetic Acid and Its Derivatives

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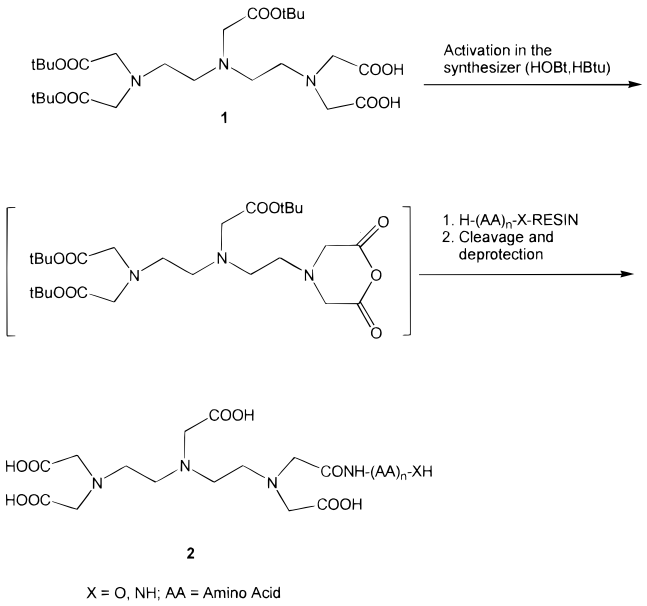
Diethylenetriaminepentaacetic acid (DTPA) ligands and other polyaminocarboxylates are widely used in fundamental research as chelating agents[1,2] and in the pharmaceutical industry for diagnostic purposes. In particular, they are useful chelators in magnetic resonance imaging (MRI),[3] nuclear medicine,[4] and most recently, in optical imaging[5] and radiation therapy with radioactive metals capable of destroying tumors.[6,7] Such applications are more effective when the radionuclide is delivered to tumors by specific targeting mechanisms.

Advances in molecular biology and peptide chemistry have facilitated the use of peptides to target tumors by receptor-mediated uptake. This approach necessitates the development of efficient methods for the synthesis of peptide−DTPA conjugates. Traditionally, these conjugates are formed by the reaction of DTPA dianhydride with peptides, which results in a mixture of the mono- and the bis-peptide−DTPA derivatives.[8,9] Studies have shown that metal complexes of bispeptide−DTPA conjugates have high levels of liver and kidney uptake[9] and are less stable in vivo[10,11] and in vitro[12] than the monopeptide−DTPA analogues. They must then be separated from the more stable monoconjugates and discarded. In view of the high cost of peptides, low yield of the desired monopeptide−DTPA conjugates is highly undesirable.

Scheme 1. Improved Procedure for the Synthesis of DTPA−Monopeptide Conjugates



Although Arano[13] described a method for the preparation of monofunctional DTPA intermediates, the procedure is cumbersome and gives a low yield (20%) of the final compound. Further, Arano's method is not suitable for selective functionalization of more than one DTPA carboxyl group, and it is not amenable to large-scale production due to the formation of ditrifluoroacetamides at higher reactant concentrations. Taking advantage of the procedure described by Rapoport[14] to prepare differentially protected DTPA derivatives, Srinivasan[15] developed an efficient procedure for the synthesis of DTPA−monopeptide conjugates, as shown in Scheme 1.

This method optimized the use of expensive peptides in the DTPA-conjugation step and simplified the purification process. However, it also introduced a different kind of problem. In the synthesis of tri-*tert*-butyl DTPA, **1**, from *tert*-butyl glycinate and *N*,*N*′-bis(*tert*-butylcarboxylmethyl)aminoethyl bromide, less than 15% of the desired product was isolated. This is due to lack of selectivity, resulting in the concomitant formation of **3**, lactams and some intermolecular amide side products, in addition to the target secondary amine, **4** (Figure 1).

Thus, an alternative method for the synthesis of this important ligand precursor is needed. We report a new method for the synthesis of tri-*tert*-butyl DTPA and its use in the preparation of monopeptide−DTPA derivatives.

Commercially available benzylethylenediamine **5** was alkylated with *tert*-butyl bromoacetate to give the tertiary amine **7**. Hydrogenolysis, followed by alkylation with the bromide **8**, gave orthogonally protected DTPA ester **9**. Subsequent hydrogenolysis of the benzyl ester gave tri-

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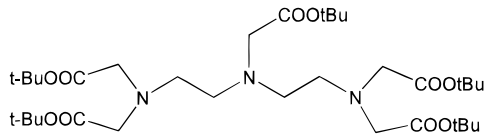

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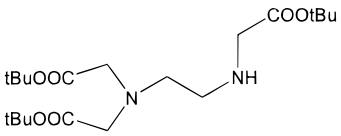

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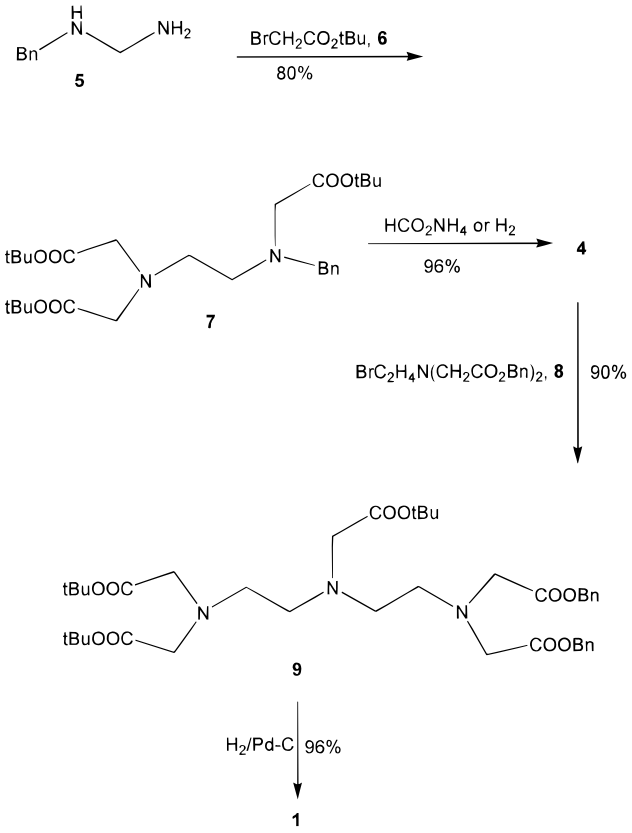
Figure 1. Major reaction intermediates.

Scheme 2. New Method for the Synthesis of Tri-*tert*-butyl-DTPA



tert-butyl DTPA **1** in about 70% overall yield. The new procedure is summarized in Scheme 2.

Two methods were evaluated for the removal of the benzyl protecting group of **7**. In method 1, we employed catalytic transfer hydrogenation with ammonium formate,[16] and the debenzylation was complete in 30 min. We observed that a tenth of the recommended Pd catalyst[16] equally removed the benzyl group when the benzylamine **7** and ammonium formate were refluxed in methanol for 40 min. However, this approach also gives side products that complicate purification of the secondary amine **4**. Although formic acid is widely used in catalytic transfer hydrogenation,[17] we chose not to deprotect **7** with this reagent in order to avoid premature removal of the acid-labile *tert*-butyl esters. In method 2, we used conventional catalytic hydrogenolysis of **7** under hydrogen pressure. Contrary to literature reports,[18,19] we observed that the debenzylation was complete in less than 2 h at room temperature and 3 atm of H_2. Comparison of our result with the catalytic hydrogenolysis of another tertiary benzylamine, *N*-benzyl-*N*,*N*-dipropylamine, showed that the debenzylation of the latter is sluggish, as expected, and required more than 12 h for complete hydrogenolysis. Hence, removal of the *N*-benzyl group may also be catalyzed by esters as was reported for acids.[19]

Unlike most monofunctional DTPA intermediates, compound **1** is a stable white powder at room temperature and is readily used in automated peptide synthesis without the need for cumbersome postsynthetic coupling reactions. Thus, reaction of **1** with peptides gave exclusively the desired monoamide−DTPA derivatives (Table 1). The peptides were prepared by standard automated Fmoc solid-phase peptide synthesis,[20a] and the DTPA conjugation was carried out with the peptides on solid support as described in the literature.[15]

The free carboxyl group of the monopeptide−DTPA **16** is derivable with a variety of nucleophiles capable of altering the physicochemical and biological properties of the metal chelate complex. For example, we successfully synthesized a somatostatin octapeptide analogue **18** on solid support from **1** in order to decrease the renal uptake of the tumor-targeting agent[20b] (Scheme 3).

A unique advantage of tri-*tert*-butyl DTPA over monofunctional DTPA intermediates[11,13] is the ease of using the former as both linker and ligand for a variety of applications. This is particularly useful for the introduction of different tumor specific agents on the same molecule. Toward this end, we synthesized a DTPA-bridged peptide derivative **19** (Figure 2) from the monocarboxylate **2** and monoprotected *N*-Fmoc ethylenediamine. This reaction demonstrates the potential to use tri-*tert*-butyl DTPA in the synthesis of a combinatorial library of compounds.

In summary, tri-*tert*-butyl DTPA is a versatile intermediate that can be used to synthesize a variety of compounds for fundamental research and industrial applications. It has several advantages over monofunctional DTPA intermediates, including the ease of selective functionalization of more than one carboxyl group, amenability to automatic Fmoc solid-phase synthesis without recourse to difficult postsynthetic coupling reactions, and versatility in the modification of the physicochemical and biological properties of the conjugates. We developed a new and high-yielding method for the synthesis of this ligand precursor and demonstrated its use as chelator and linker in the preparation of DTPA derivatives. These peptide conjugates are biocompatible as demonstrated by the in vivo stability of the monopeptide−DTPA metal chelate, ^{111}In-DTPA-octreotide.[21]

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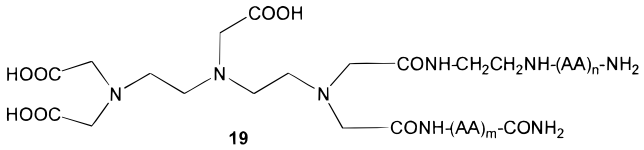
Table 1. Monoamide−DTPA Derivatives

compd	structure	*m/z*
10	DTPA-Ile-Gly-Gly-Gly-Leu-Gly-Gly-Gly-NH_2	960.4563
11	DTPA-Phe-cyclo(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH	1423.5156
12	α-DTPA-Lys-Phe-cyclo(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH	1551.6202
13	DTPA-Gly-Asp-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2	1543.6903
14	DTPA-Ala-Ala-Glu-Met-Ala-Pro-Trp-Lys-Thr-OH	1378.6358
15	DTPA-Gly-Pro-His-Trp-Ser-Tyr-OH	1120.4506



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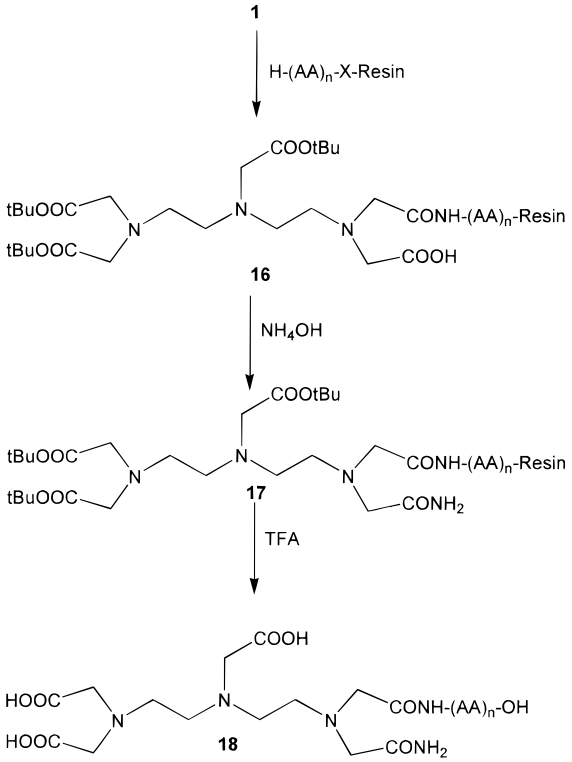
AA_m = Gln-Trp-Ala-Val-Gly-His-Leu-Met
AA_n= Gly-Phe-Asp

Figure 2. Bispeptide-linked DTPA.

Scheme 3. Selective Functionalization of DTPA−Peptide Conjugates



$(AA)_n$ = D-Phe-cyclo(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH

Experimental Section

General Comments. 2-[Bis-(benzyloxycarbonylmethyl)amino]ethyl bromide (**8**) was prepared as described in the literature.[14] Dry flash chromatography (DFC) was performed on TLC standard grade silica gel without binder as described elsewhere.[22] Electrospray ionization mass spectrometry experiments were performed on a triple quadruple mass spectrometer. The electrospray interface was operated in positive-ion mode with a spray voltage of 4.5 kV and a capillary temperature of 225 °C. Samples were introduced into the spectrometer by flow injection utilizing acetonitrile/water (7:3) containing 0.1%. trifluoroacetic acid. HRMS (MALDI-TOF) analyses were performed at the Washington University School of Medicine in St. Louis, MO. Combustion analyses were performed by Atlanta Microlab, Inc., Norcross, GA. Analytical (flow rate = 0.5 mL/min) and semi-preparative (flow rate = 10 mL/min) RP-HPLC were performed as described in the literature[23] using either of two gradient elution protocols: I (50 to 100% B in 30 min) and II (5 to 70% B in 30 min) where A is 5% CH_3CN in 0.1% aqueous TFA and B is10% H_2O in 0.1% TFA solution of CH_3CN. Peak detection was at 214 nm with a tunable absorbance detector.

***N*-Benzyl-*N,N′,N′*-tris(*tert*-butyloxycarbonylmethyl)ethylenediamine (7).** *N*-Benzylethylenediamine (5.0 g, 33.28 mmol) and K_2CO_3 (19.3 g, 139.64 mmol) were stirred in anhydrous acetonitrile (200 mL) under Ar atmosphere. *tert*-Butyl bromoacetate (39.2 g, 246.09 mmol) in 30 mL of anhydrous acetonitrile was added dropwise to the reaction mixture over 90 min. The progress of the reaction was monitored by TLC and was essentially complete in about 4 h but was left at room temperature overnight (12 h). The insoluble residue was filtered and washed with acetonitrile. The filtrate was evaporated to give 20 g of a yellow liquid that was triturated with hexane (100 mL), and the resulting white precipitate was filtered. Evaporation of the filtrate and purification of the crude product by DFC, eluting with 10% Et_2O in hexane, gave the pure triester **7** (13.8 g, 28.01 mmol, 85% yield; HPLC purity 100%, protocol II) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 7.28 (5H, m); 3.80 (2H, s); 3.44 (4H, s) 3.26 (2H, s); 2.84 (4H, m); 1.45 and 1.43 (27H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 170.9; 170.7; 139.1; 129.0; 128.2; 127; 80.8; 80.6; 58.3; 56.2; 55.0; 52.3; 52.0; 28.2; 28.1. MS (EI): 493.2 $(M + H)^+$. Anal. Calcd for $C_{27}H_{44}N_2O_6$: C, 65.83; H, 9.00; N, 5.69. Found: C, 66.04; H, 9.06; N, 5.71

***N,N′,N′*-Tris(*tert*-butyloxycarbonylmethyl)ethylenediamine (4) by Catalytic Transfer Hydrogenolysis.** *N*-Benzyl-*N,N′,N′*-tris(*tert*-butyloxycarbonylmethyl)ethylenediamine **7** (6 g, 12 mmol) was added to a heterogeneous mixture of 10% palladium on carbon (6 g, 1 weight equivalent) in methanol (100 mL). Anhydrous ammonium formate (3.8 g, 60.26 mmol) was added to the reaction mixture in one bulk, and the mixture was stirred for 2 h at room temperature. The product was filtered over Celite, and the residue was washed with chloroform. The filtrate was evaporated to dryness, and the residue was triturated in chloroform. The insoluble solid was filtered, and the filtrate was evaporated to give the pure compound (4.6 g, 11.43 mmol, 96% yield) as a pale yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 3.71 (2H, s); 3.49 (4H, s); 3.14 (2H, t, J = 2.5 Hz); 3.07 (2H, t, J = 2.5 Hz); 1.51 (9H, s); 1.46 (18H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 170.9; 171.1; 165.9; 83.2; 81.6; 56.4; 51.4; 48.6; 45.9; 27.7. MS (EI): 493.2 $(M + H)^+$.

***N,N′,N′*-Tris(*tert*-butyloxycarbonylmethyl)ethylenediamine (4) by Conventional Catalytic Hydrogenolysis.** *N*-Benzyl-*N,N′,N′*-tris(*tert*-butyloxycarbonylmethyl)ethylenediamine **7** (1 g; 2.0 mmol) was added to a heterogeneous mixture of 10% Pd/C (0.1 g) in methanol (50 mL). The mixture was hydrogenated at 3 atm for 2 h, and the product was filtered over Celite. After the cake was washed with MeOH, the solvent was evaporated to give **4** (740 mg, 1.84 mmol, 92% yield) as a pale yellow liquid. The spectral properties are shown above.

***N,N′,N′*-Tris(*tert*-butyloxycarbonylmethyl)-*N″,N″*-bis-(benzyloxycarbonylmethyl)diethylenetriamine (9).** A mixture of *N,N′,N′*-tris(*tert*-butyloxycarbonylmethyl)ethylenediamine **4** (4.4 g, 9.82 mmol), 2-[bis(benzyloxycarbonylmethyl)amino]ethyl bromide **8** (5.3 g, 12.76 mmol), and ethyldiisopropylamine (3.8 g, 29.45 mmol) in acetonitrile (100 mL) was stirred at reflux for 24 h under nitrogen atmosphere. After evaporation of the solvent, the residue was dissolved in dichloromethane and the solution was washed twice with water (100 mL). Evaporation

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of the organic solvent and subsequent purification of the crude product by DFC, eluting with 40% Et_2O in hexane, gave the tertiary amine **9** (6.5 g, 8.76 mmol, 90% yield; HPLC purity 100%, protocol I) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 7.35 (10H, m); 5.13 (4H, s); 3.65 (4H, s); 3.44 (4H, s); 3.32 (2H, s); 2.88 and 2.78 overlap (8H, m); 1.45 and 1.47 overlap (27H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 171.4; 171.0; 135.9; 128.7; 128.4, 80.8; 66.1; 56.0; 55.1; 52.6. MS (EI): 742.6 $(M + H)^+$. Anal. Calcd for $C_{40}H_{59}N_3O_{10}$: C, 64.76; H, 8.02; N, 5.66. Found: C, 64.54; H, 7.93; N, 5.66.

***N*,*N*′,*N*′-Tris(*tert*-butyloxycarbonylmethyl)-*N*″,*N*″-bis-(acetic acid)diethylenetriamine (1).** A mixture of *N*,*N*′,*N*′-tris(*tert*-butyloxycarbonylmethyl)-*N*″,*N*″-bis(benzyloxycarbonylmethyl)diethylenetriamine **9** (3.3 g, 4.45 mmol) and 10% Pd/C (0.21 g) in 50 mL of methanol was hydrogenated at 3 atm for 2 h. The mixture was filtered over Celite, and the residue was washed with methanol. Evaporation of the solvent gave **1** (2.4 g, 4.27 mmol, 96% yield; HPLC purity 100%, protocol II) as a white powder. Mp: 37 °C (uncorrected). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 4.13 (4H, s); 3.57 (6H, s); 3.44 (2H, m); 3.10 (2H, m); 2.93 (2H, m), 2.88 (2H, m); 1.45 (9H, s); 1.42 (18H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 170.7; 169.7; 169.1; 82.3; 81.6; 56.4; 55.6; 55.3; 52.6; 50.3; 48.7; 28.1; 28.0. MS (EI): 562.3 $(M + H)^+$. Anal. Calcd for $C_{26}H_{47}N_3O_{10}$: C, 55.60; H, 8.43; N, 7.48. Found: C, 54.70; H, 8.36; N, 7.44.

Synthesis of Peptide−DTPA Derivatives. All the peptides synthesized in this study follow the procedure described below. Exceptions are noted under specific peptides. The peptides were prepared on solid support by standard automated 9-fluorenylmethoxycarbonyl (Fmoc) procedures.[20a] Rink amide and Wang resins were used for the synthesis of C-terminal amide and carboxyl peptides, respectively. Initial loading of each Fmoc amino acid bound resin is 25 μmol. Automatic activation of the carboxyl group with a mixture of *N*-hydroxylbenzotriazole (HOBt) and 2-(1*H*-benzotriazole-1-yl)-1,1,1,3-tetramethyluronium hexafluorophosphate (HBTU) and coupling of subsequent Fmoc-protected amino acids (75 μmol) and the tri-*tert*-butyl DTPA **1** (placed as the last cartridge on the synthesizer) were carried out in situ. Either of the following reagent mixtures was used to cleave the peptide from the resin and remove the side-chain protecting groups, unless otherwise stated: reagent A (85% TFA/5% H_2O/5% PhOH/5% thioanisole) for 6 h or reagent B (85% TFA/5% H_2O/5% ethanedithiol/5% thioanisole) for 3 h. The crude peptides were precipitated with cold *tert*-butyl methyl ether and purified by RP-HPLC using gradient elution protocol II described in the general section above. All peptide-DTPA derivatives were characterized by analytical HPLC, electrospray spectrometric (EI), and HRMS (MALDI-TOF) analyses after lyophilization in 67% H_2O/33% CH_3CN. The HRMS data are presented in Table 1 above.

Peptide−DTPA 10 (DTPA-Ile-Gly-Gly-Gly-Leu-Gly-Gly-NH_2) was cleaved from the resin with 95% aqueous TFA (12.3 mg, 12.3 μmol, 49% yield; 100% HPLC purity; HRMS calcd for $C_{38}H_{64}N_{12}O_{17}$ 960.4512, found 960.4563).

Peptide−DTPA 11 (DTPA-Phe-cyclo(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH). After the synthesis was complete, the dithiol residue was cyclized with thallium trifluoroacetate (23 mg, 42 μmol in 2 mL of DMF for 90 min), and the product was cleaved from the solid support with reagent A (8.9 mg, 6.3 μmol, 25% yield; 100% HPLC purity; HRMS calcd for $C_{63}H_{85}N_{13}O_{21}S_2$ 1423.5424, found 1423.5156).

Peptide−DTPA 12 (α-DTPA-Lys-Phe-cyclo(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH) was prepared as described above (7.8 mg, 5.0 μmol, 20% yield; 100% HPLC purity; HRMS calcd for $C_{69}H_{97}N_{15}O_{22}S_2$ 1551.6374, found 1551.6202).

Peptide−DTPA 13 (DTPA-Gly-Asp-Gly-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2) was cleaved with reagent B (13.5 mg, 8.8 μmol, 35% yield; 100% HPLC purity; HRMS calcd for $C_{65}H_{97}N_{19}O_{23}S$ 1543.6725, found 1543.6903).

Peptide−DTPA 14 (DTPA-Ala-Ala-Glu-Met-Ala-Pro-Trp-Lys-Thr-OH) was cleaved with reagent B (13.1 mg, 9.5 μmol, 38% yield; 100% HPLC purity; HRMS calcd for $C_{59}H_{90}N_{14}O_{22}S$ 1378.6074, found 1378.6358).

Peptide−DTPA 15 (DTPA-Gly-Pro-His-Trp-Ser-Tyr-OH) was cleaved with reagent A (6.5 mg, 5.75 mmol, 23% yield; 100% HPLC purity; HRMS calcd for $C_{50}H_{64}N_{12}O_{18}$ 1120.4461, found 1120.4506).

Peptide−DTPA Monoamide 18 (DTPA(NH_2)-Phe-cyclo-(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-OH). The free carboxylic acid function of tri-*tert*-butyl-DTPA-octapeptide **11** on solid support (25 μmol) was activated with (HBTU)/(HOBt) in DMSO (0.2 M, 250 μL, 50 μmol) and *N*-ethyl-*N*,*N*-diisopropylamine in DMSO (0.2M, 250 μL, 100 μmol) for 30 min, and aqueous ammonia solution (density = 0.9 g ml^{-1}, 19.5 μL, 500 μmol) was added to the resin. The mixture was mixed for 3 h at room temperature, and the resin was washed with DMF, THF, and dichloromethane. Cleavage and removal of side chain protecting groups were carried out with reagent A (6.4 mg, 4.5 μmol, 18% yield; 100% HPLC purity; MS (EI) 1423.6 $(M + H)^+$).

DTPA-Bridged Bispeptide 19 (H_2N-Asp-Phe-Gly-NHC_2H_4NH-DTPA-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2). The free carboxylic acid function of tri-*tert*-butyl-DTPA-octapeptide **16** on solid support (25 μmol) was activated as described above and reacted with Fmoc$NHC_2H_4NH_2$ (24 mg, 75 μmol) for 2 h. After the resin was washed with DMF and THF, it was dried and returned to the synthesizer to complete the synthesis of the second peptide fragment. After reaction, the peptide was cleaved from the resin with reagent A (5.5 mg, 3.3 μmol, 13% yield; 93% HPLC purity; HRMS calcd for $C_{74}H_{110}N_{21}O_{22}S$ 1676.7855, found 1676.7808).

Supporting Information Available: ^{1}H NMR, ^{13}C NMR, HPLC, and mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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