An Efficient Synthesis of Cyclic RGD Peptides as Antithrombotic Agents

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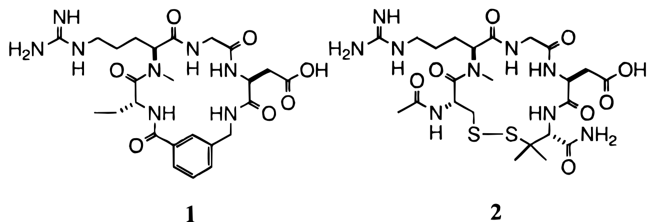
The use of glycoprotein (GP) IIb/IIIa antagonists as antithrombotic agents has been an active area of research in recent years.[1−7] When linear peptides containing the Arg-Gly-Asp (RGD) sequence bind to the IIb/IIIa receptor, their dissociation constants are about 10 mM. It has been shown that the conformation of the peptides has an important impact on the binding affinities. For instance, the binding was considerably improved when cyclic RGD peptides, such as SK&F 106760 (**2**), were studied as IIb/IIIa antagonists.[7,8] We found that the cyclic peptide linked with *d*-2-aminobutyric acid (D-Abu) and *m*-(aminomethyl)benzoic acid (Mamb) had an IC_{50} value of 20 nM. After an extensive study of structure−activity relationships, cyclic (D)-Abu-N-MeArg-Gly-Asp-Mamb (**1**), was selected as a development candidate. Compound **1** has a dissociation constant in the picomolar range when binding to GP IIb/IIIa and exhibits potent antiplatelet activities both *in vitro* and *in vivo*.[8]



In support of a clinical study of this antithrombotic agent, an effective large scale preparation of **1** was required. However, there was little known about preparations of cyclic peptides on larger than a laboratory scale.[9] The presence of arginine and aspartic acid in the peptide was expected to increase the difficulties of synthesis and purification.[10] This paper reports our successful large scale synthesis of cyclic peptide **1**.

The original 14-step synthesis, similar to Scheme 1, was employed to allow quick preparations of many structurally similar cyclic peptides. In this procedure, the linear peptide **7** was prepared on a polymeric oxime support then cleaved from the resin by an intramolecular aminolysis. The guanidino group of arginine was protected as the tosylate, and the carboxyl group of aspartic acid was protected as the cyclohexyl ester.[8] Both protecting groups were removed by anhydrous HF, and the product was purified by reverse-phase HPLC in 11% overall yield.

Scheme 1 represents our attempt to adapt the same 14-step reaction sequence in solution phase by substituting the 4-nitrobenzophenone oxime ester in place of the solid support. In this preparation, the cyclic peptide **8** must be prepared under dilute conditions to avoid intermolecular coupling of the linear peptide **7**. The tosyl and cyclohexyl protecting groups of **8** were removed by a combination of trifluoroacetic acid (TFA) and triflic acid, over 3−5 h, under carefully controlled temperature constraints (−6 to −8 °C). At higher temperatures (> −6 °C) ring-opened side products were formed and at lower temperatures (<−8 °C) the reaction was too slow to be practical. Furthermore, a large volume of anhydrous diethyl ether was used to precipitate the product from the acidic media. This synthesis provided sufficient material for safety assessment and early clinical studies; however, it was neither economical nor practical for large scale synthesis.

Since target **1** is a cyclic pentapeptide, retrosynthetic analysis indicated that any of the five peptide bonds provided the opportunity to form the cycle. We knew that successfully closing the cycle onto the secondary α-nitrogen of the arginine was unlikely. We also preferred a convergent synthesis that minimized the number of deprotection steps. Finally, we wanted to be able to use as many commercially available peptide derivatives as possible. The combination of these considerations directed us to the retrosynthetic analysis shown in Scheme 2. Models indicated that ring closure between the C-terminus of glycine and the N-terminus of aspartic acid was a good choice because of minimal steric hindrance and favorable conformation. Furthermore, after coupling of intermediates **10** and **11,** the orthogonality of the remaining four protecting groups permits unmasking of the cyclization sites in a single step.

The tripeptide **11** was produced in four steps without isolation of intermediates. The coupling of commercially available N-Boc-N-Me-N^g-*p*-tosyl-L-arginine (**5**) with glycine benzyl ester (**12**) was performed with HBTU and diisopropylethylamine (DIEA) as shown in Scheme 3. Using ethyl acetate, an atypical choice of solvent, produced a homogenous reaction and an easy aqueous workup.[11] The unreacted glycine was removed by aqueous citric acid washes. The organic portion was then concentrated followed by the addition of TFA to remove the Boc group and produce **13**.

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(11) THF or methylene chloride are typically used solvents.

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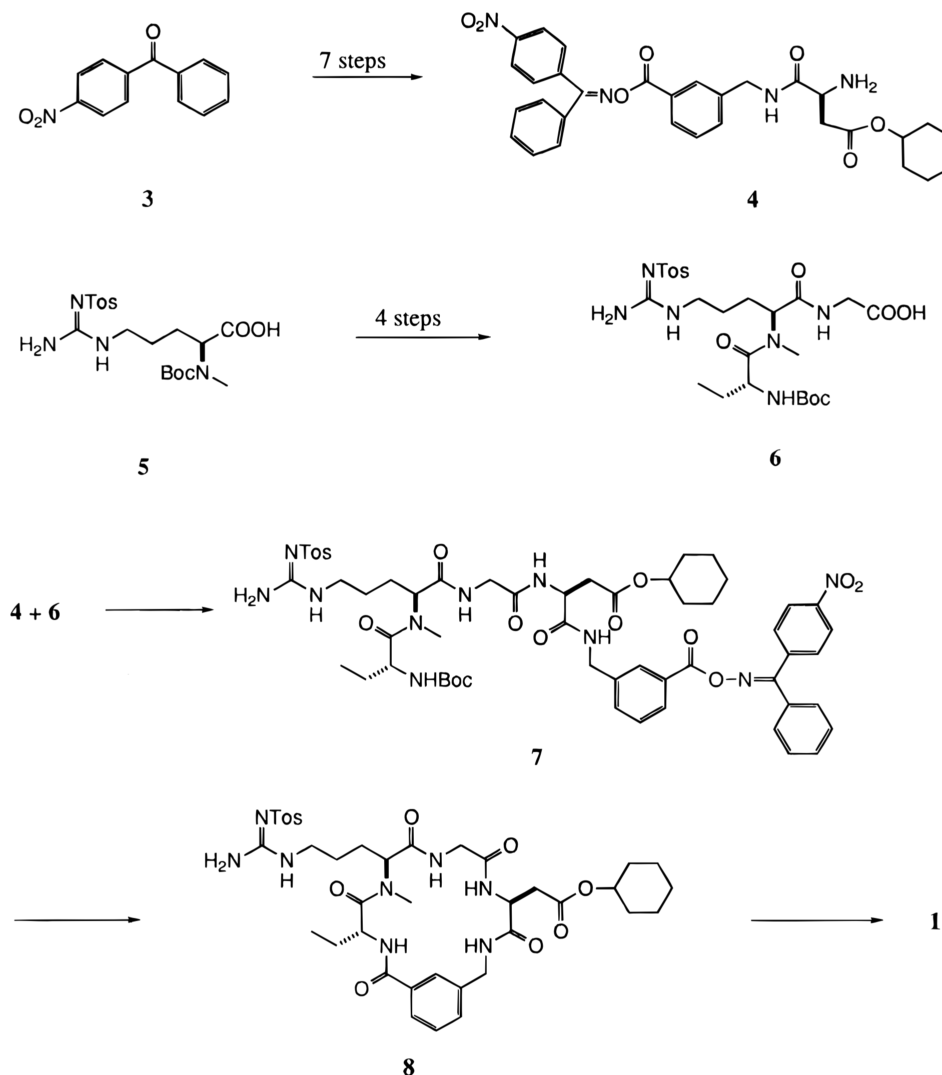
Scheme 1



The physical characteristics of **13** were not favorable, preventing its useful isolation. The product tended to be gummy and was very hygroscopic.[12] After concentration and workup, the crude product solution was used directly in the next step. Peptide coupling on a substituted nitrogen is known to be difficult,[9] but HBTU functioned well to add N-Boc-*d*-amino butyric acid (D-Abu) to the arginine. Accordingly, an ethyl acetate solution of the free acid of D-Abu was added to the solution containing **13** and dried via toluene azeotrope at reduced pressure. A small amount of acetonitrile was added to homogenize the solution, HBTU/DIEA were charged, and coupling was complete in about 4 h. At a kilogram scale, tripeptide **14** was deprotected and precipitated as the HCl salt of **11** using anhydrous HCl in dioxane.[13] The tripeptide prepared by this procedure was greater than 97% pure by HPLC analysis. We later learned that a similar deprotection/precipitation protocol could be used to isolate dipeptide **13**, as long as all solvent was replaced by dioxane.

The remaining portion (**10**) of the cyclopeptide was prepared by either the reaction of *N*-(carbobenzyloxy)-*tert*-butyl-L-aspartic acid **15** with 1,1-carbonyldiimidazole followed by the addition of *m*-(aminomethyl)benzoic acid (**16**), or by the reaction of *N*-(carbobenzyloxy)-*tert*-butyl-L-aspartic *N*-hydroxysuccinimide ester with the sodium salt of *m*-(aminomethyl)benzoic acid (Scheme 4). The yield was higher (82% versus 73%) for the latter method and the use of *N*-hydroxysuccinimide esters proved to be most suitable for our large scale preparations. Furthermore, the major byproduct from the reaction, *N*-hydroxysuccinimide, was water soluble and easily removed. The peptide **10** obtained by this approach had a purity of >96%.

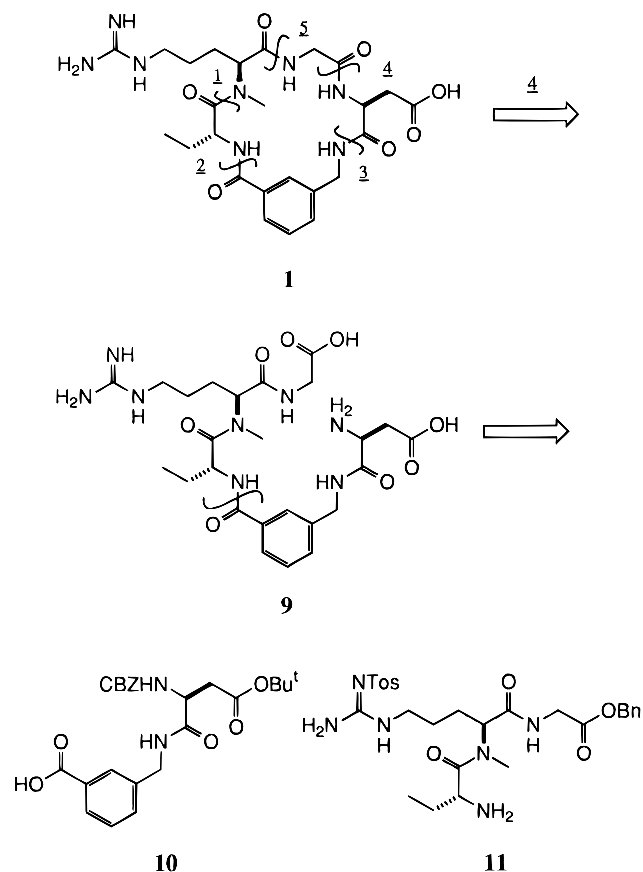
Coupling of intermediate **11** with **10** in the presence of HBTU and DIEA in acetonitrile provided the pentapeptide **17**. Because this linear peptide was oily and difficult to handle, it was best to replace ethyl acetate via the methanol azeotrope and proceed directly to the following deprotection step without isolation of **17**.

At this point, four protecting groups remain on the pentapeptide. Catalytic hydrogenation at 1 atm at 25 °C over wetted 10% palladium/carbon simultaneously removed both protecting groups from the ends of the linear pentapeptide to form **18**. The reduction proceeded in 2 h and was nonselective in rate between the two protecting groups. The product was precipitated as a

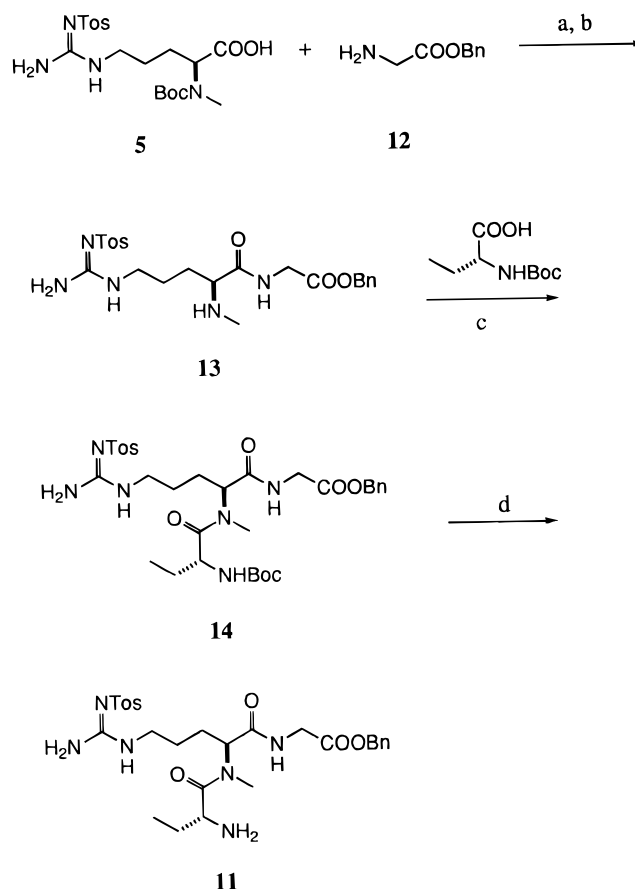
(12) Isolation of a dry solid was only possible by addition of an ethyl acetate solution of **13** to a large excess of *tert*-butyl methyl ether at low temperature (-15 to 20 °C).

(13) Initially, the HCl salt precipitation was unsuccessful in either dioxane or toluene due to the residual DCHA, because the free base of D-Abu had not been used. Once this was recognized and rectified, the HCl salt was easily produced as long as both water and ethyl acetate were removed *via* initial azeotropic distillation with dioxane.

Scheme 2



Scheme 3[a]



[a] (a) HBTU, DIEA. (b) TFA. (c) HBTU, DIEA. (d) HCl, 82% yield from **5**.

white solid by the addition of the filtered reaction solution into 1-butanol followed by the distillation of methanol and water.

Once again, cyclization of **18** made use of the coupling agent HBTU/DIEA.[14] Dicyclohexylcarbodiimide (DCC) in acetonitrile could be used but the yields were half that using HBTU. Remarkably, in spite of not using high dilution conditions, excellent yields of the cyclopentapeptide were obtained. The product was contaminated with trace quantities of impurities, tentatively characterized as oligomers by ^{1}H NMR spectroscopy. To minimize the putative oligomer formation, the solution of **18** and DIEA in acetonitrile was added to the solution of HBTU in acetonitrile over 5–6 h. However, even when the addition was shortened to 1 hour, minor, if any, additional impurities formed.

The cyclized product **19** precipitated as it formed, leaving in solution most of the impurities from the previous several steps. If insufficient base was initially charged to complete the reaction, a second charge completed the cyclization with no effect upon yield or purity. The only purification necessary was to wash the filtered solids with acetonitrile/DIEA.

The cyclic peptide **19** was sparingly soluble in most solvents. We could purify **19** by slow reprecipitation from acetonitrile/dimethylacetamide solution but found the following process to be more efficient. Pure (>98%) **19** was obtained by passing a methanolic chloroform solution through a pad of silica gel. Solids were precipitated by replacement of the solvent with acetonitrile via vacuum distillation. Occasionally, a gel would begin to form rather than dry solids, a known propensity of certain peptides.[15] Adding back sufficient methanol to break the gel and restarting the distillation always eventually resulted in solids. On a preparative scale, the yield of **19** was 58% from crude intermediate **11**. No further purification was required to use the cyclopeptide in the subsequent tandem deprotection step.

Typically, removal of tosyl protection from guanidine requires relatively forcing conditions.[16] There are a number of examples reported for the detosylation of arginine- and arginine-containing peptides.[17,18] However, these methods did not produce satisfactory results when applied to deprotection of **19**. In this case, we discovered that trifluoroacetic acid/triflic acid/anisole in combination was a good detosylation system. The *tert*-butyl group of aspartic acid was first removed by neat trifluoroacetic acid to give intermediate **20**. Subsequent addition of triflic acid and anisole cleaved the tosyl group. In contrast to the slow detosylation of the *N*-tosyl, *C*-cyclohexyl congener **8**, the tosyl group of cyclic peptide **20** was removed within 1 h between −20 to −6 °C. This

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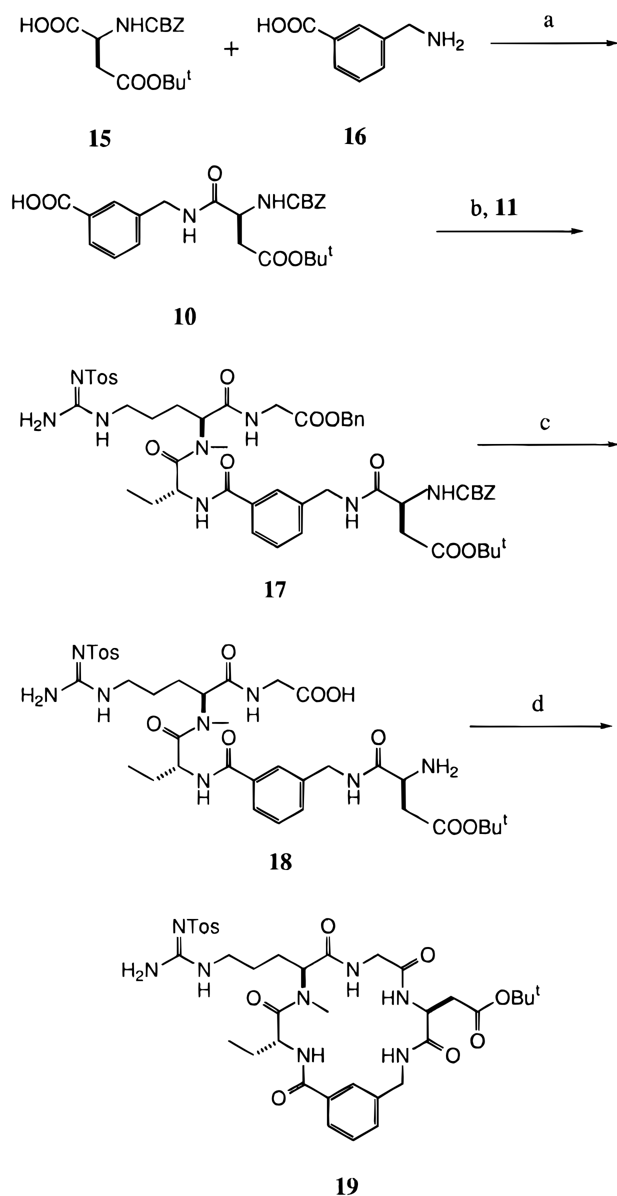
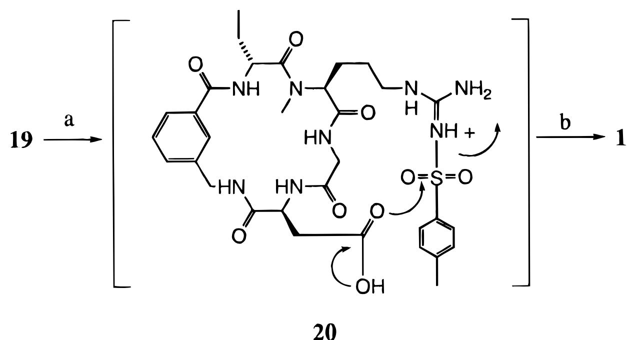
Scheme 4[a]



[a] (a) *N*-Hydroxysuccinimide ester of **15**, NaOH, 82%. (b) HBTU, DIEA. (c) H_2, Pd/C, >99%. (d) HBTU, DIEA, 58% from **11**.

Scheme 5[a]



[a] (a) TFA. (b) Triflic acid, anisole, 79% from **19**.

rate difference suggests that the free carboxylic acid function might facilitate the cleavage of the tosyl group by neighboring group participation (Scheme 5).

A variety of workup procedures were surveyed in order to determine conditions to isolate **1** before the strongly acidic medium degraded the cyclic peptide. Accordingly, **1** was precipitated out of solution by butyl ether dilution. However the particles were slow to filter, and as these solids warmed on the filter, the residual acids led to the formation of impurities. The use of large filter beds minimized this, but ultimately this problem was resolved by controlling the particle size via the stirring speed and the addition rate of butyl ether.

The crude solid was dissolved into aqueous acetone[19] and treated with an anion exchange resin until the pH reached 4.55−4.95. This neutralization was very efficient for the removal of any residual trifluoroacetic acid and trifluoromethanesulfonic acid.[20] Following evaporation of the solvent, recrystallization from warm aqueous acetone or simply stirring the solids in water would usually reduce the remaining impurities to acceptable levels. The isolated product **1** often contained appreciable water (up to 20%) which did not affect the subsequent step. On a large scale, the yield of the tandem deprotection was 79%.

The preparation of the methanesulfonic acid salt of **1** was done by the addition of 1 equiv of acid to a suspension of **1** in refluxing 2-propanol (IPA). Usually, no change in the appearance of the slurry was seen during the acid addition as the solids transformed from zwitterion to the salt. After cooling, filtration and an IPA wash of the white solids, clean drug was obtained in nearly quantitative yield by simply drying the powder on a Buchner funnel under nitrogen. The product prepared by this synthetic route has >99% optical purity based on chiral HPLC analysis and >98.5% weight percent purity exclusive of solvents.

In summary, we have developed an efficient route to cyclic peptide **1**, a potent antithrombotic agent. This is a nine-step synthesis from commercially available starting materials, involving no chromatographic purification. The economy of deprotection, efficiency of cyclization and the enhanced detosylation method provided an attractive route to manufacture cyclic peptide **1** in bulk.

Experimental Section

General. All melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.4 MHz, respectively, and are reported in DMSO-d_6 (except where noted). The coupling constants (*J*) of NMR spectra were reported in hertz. Mass spectra were recorded by the analytical laboratory, Chemical Process R&D, DuPont Merck Research Laboratories. The elemental analyses were determined by Quantitative Technologies Inc., Whitehouse, NJ. Solvents and reagents were used as purchased without further purification. The protected amino acids were purchased from Bachem Bioscience Inc.

HCl Salt of Boc-N-Me-Arg(Tos)-Gly-OBzl (13). Ethyl acetate (3.2 L), N-Boc-N-Me-N^g-*p*-tosyl-L-arginine (**5**) (500 g, 1.13 mol), glycine benzyl ester (**12**) toluenesulfonate salt (450 g, 1.33 mol), and HBTU (500 g, 1.32 mol) were combined under nitrogen and stirred at room temperature. To this was added diisopropylethylamine (480 mL) at <30 °C. The reaction was stirred at room temperature for 6 h. Solvent (2 L) was removed *in vacuo* at <30 °C, and trifluoroacetic acid (1.7 L) was added at <40 °C. The reaction was stirred for 6 h, and the solution was concentrated *in vacuo* until 1.1 L of solvent had been removed. This crude **13** in solution was used directly in the subsequent step. The spectral data of **13** were identical to those reported in the literature.[8]

(19) Occasionally it was necessary to dissolve **1** by the addition of a few drops of acetic acid to a water slurry prior to resin treatment.

(20) The extent of exchange could be determined by measuring the ^{19}F NMR spectrum of a portion of evaporated solution versus an internal standard of trifluoroethanol. In instances of incomplete removal, further treatment with more resin would remove the last traces.

Boc-D-Abu-N-MeArg(Tos)-Gly-OBzl (14). Ethyl acetate (2 L) and water (2 L) were added to the above reaction mixture, and its pH was adjusted to 7.6 with 25% NaOH. Additional water (4 L) and ethyl acetate (2 L) were added and the layers separated. The aqueous phase was extracted with ethyl acetate (2 × 4 L), and the combined organic layer was washed with saturated aqueous $NaHCO_3$ (2 × 3 L). The organic phase was concentrated *in vacuo* until 8.8 L of distillate was removed.

In a separate flask, Boc-*d*-aminobutyric acid dicyclohexylamine salt (551 g, 1.43 mol) was suspended in ethyl acetate (2.5 L) and 10% aqueous $KHSO_4$ (4 L). The suspension was stirred until all the solids dissolved (~40 min). The phases were separated, and the aqueous phase extracted with ethyl acetate (2 × 1 L). The combined ethyl acetate layers were washed with water (3 × 1 L) until the aqueous portion no longer gave a precipitate with 2% $BaCl_2$ solution.

This amine-free solution of Boc-*d*-aminobutyric acid was combined with the solution containing dipeptide **13** and toluene (4 L). The solution was concentrated *in vacuo* until 6 L had been removed (water content of solution = 0.05% at this point). Acetonitrile (1 L), HBTU (501 g, 1.32 mol), and DIEA (480 mL) were added successively at <30 °C. The reaction was stirred at room temperature overnight, the solution was concentrated *in vacuo* until 2.5 L of solvent had been removed, and the solution was diluted with ethyl acetate (2.5 L). The solution was washed with 5% citric acid solution (3 × 1.7 L) followed by saturated aqueous $NaCHO_3$ (2 × 800 mL). The solution was dried by azeotropic distillation with dioxane until 6.7 L had distilled and 5.5 L of dioxane had been added (water content = 0.16% at this point). This solution containing **14** was used directly in the next step. The spectral data of **14** were identical to those reported in the literature.[8]

D-Abu-N-MeArg(Tos)-Gly-OBzl (11). The above solution of **14** was combined with 4.0 M HCl in dioxane (2 L) at <40 °C. The solution was stirred over 12 h during which time the product precipitated. Acetonitrile (3 L) was added and the mixture cooled to <15 °C. The white solids were filtered under nitrogen. The wet weight of the product was 860.5 g (66.1 weight percent purity), corresponding to an overall yield over four steps of 82.4% from **5**. Preparation of an analytical standard of **11** was accomplished by a slurry of the crude product in THF. The product was collected by a filtration and dried: mp 120−121 °C; ^{1}H NMR δ 0.93 (t, 3H, *J* = 7.6), 1.9 (m, 2H), 1.6−2.0 (br m, 4H), 2.42 (s, 3H), 2.95 (s, 3H), 3.15 (br s, 2H), 3.87 (br m, 2H), 4.25 (br m, 1H), 5.05 (dd, *J* = 4.0, 11.2, 1H), 5.37 (s, 2H), 6.75 (br s, 1H), 7.30 (d, *J* = 7.8, 2H), 7.35 (m, 5H), 7.65 (d, *J* = 7.8, 2H), 8.28 (br s, 3H), 8.59 (t, *J* = 5.8, 1H). HRMS (M + H): Calcd for $C_{27}H_{39}N_6O_6S$: 575.2652. Found: 575.2635.

Z-Asp(OBu^t)-*m*-(Aminomethyl)benzoic Acid (Mamb) (10). A slurry of *N*-(carbobenzyloxy)-*β*-*tert*-butyl aspartic succinate (756.7 g, 1.8 mol) in acetonitrile (2.7 L) was prepared. To this slurry was added over 1−2 h a solution of the sodium salt of *m*-aminomethylbenzoic acid (327.3 g, 1.89 mol) at 20−25 °C. (This solution of the sodium salt was made from the HCl salt by dissolving *m*-(aminomethyl)benzoic acid hydrochloride (354.6 g, 1.89 mol) in water (2.4 L). The pH of this solution was adjusted to 8.8 ± 0.2 with 30% NaOH at 20−30 °C.) Sodium bicarbonate (151.2 g, 1.8 mol) was added in five portions, and small amounts of insolubles were removed by filtration. The reaction was stirred overnight and the pH of the solution was adjusted to 3.4−3.7 with 60% aqueous citric acid. The slurry was stirred at 20−25 °C for 2 h while maintaining this pH range. The slurry was cooled to 5−10 °C, held for 1 h, and filtered, and the wet cake washed successively with water (1.2 L) and a cold solution of 1/1 volume of acetonitrile/water (1.2 L). The yield of **10** after drying *in vacuo* at 40−50 °C, was 681 g (82.8%). Recrystallization of a sample from MeOH/water gave an analytical sample as a white solid: mp 149−151 °C dec; ^{1}H NMR δ 1.34 (s, 9H), 2.45 (dd, *J* = 16.1, 8.8; 1H), 2.68 (dd, *J* = 16.1, 5.5; 1H), 4.31 (d, *J* = 5.9; 2H), 4.44−4.34 (m, 1H), 5.07−4.99 (AB_q, *J* = 12.4; 2H), 7.36−7.26 (m, 5H), 7.41 (t, *J* = 7.7, 1H), 7.47 (dt, *J* = 7.7, 1.5; 1H), 7.60 (d, *J* = 8.4, 1H), 7.80 (dt, *J* = 7.7, 1.8; 1H), 7.84 (t, *J* = 1.8, 1H), 8.54 (t, *J* = 5.9, 1H), 12.91 (bs, 1H); ^{13}C NMR (DMSO-d_6, 75.4 MHz) 36.22, 46.29, 47.32, 47.59, 47.87, 48.15, 48.42, 48.70, 48.98, 50.61, 60.2, 74.12, 76.26, 86.67, 88.67, 128.42, 136.27, 136.33, 136.74, 136.85, 136.99, 139.36, 140.21, 145.48, 148.42, 164.32, 175.81, 177.87, 179.1; IR (nujol) 3307, 3248, 1713, 1660 cm-1; ms (CI) m/z 457.1 [M + H]$^+$. Anal. Calcd for $C_{24}H_{28}N_2O_7$: C, 63.14; H 6.18; N 6.13. Found C, 62.90; H, 6.31; N, 6.09.

Z-Asp(OBu^t)-Mamb-D-Abu-N-MeArg(Tos)-Gly-OBzl(17). (Carbobenzyloxy)aspartic acid(*tert*-butyl)-*m*-(aminomethyl)-benzoic acid **10** (305 g, 0.654 mol) and D-α-aminobutyric acid-*N*-methylarginine(tosyl)-glycine benzyl ester hydrochloride salt **11** (400 g, 0.654 mol) were mixed with HBTU (273 g, 0.720 mol) in acetonitrile (2.4 L) and chilled to 4 °C. DIEA (490 mL, 2.81 mol) was added slowly over 90 m at <9 °C. The mixture was stirred for 16 h at 25 °C. Ethyl acetate (4.8 L) was added, and the organic layer was washed sequentially with 5% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine. The solvent was azeotropically replaced with methanol and used as is in the next step (>99% yield). An analytical sample of **17** was purified by chromatography. ^{1}H NMR δ 0.97 (m, 1H), 1.20 (m, 1H), 1.30 (m, 2H), 1.42 (s, 5H), 1.66 (m, 1H), 1.80 (m, 3H), 2.05 (m, 1H), 2.41 (s, 1H), 2.77 (s, 1H), 2.81 (s, 1H), 2.99 (s, 1H), 3.12 (s, 1H), 3.39 (s, 1H), 3.99 (m, 1H), 4.40 (d, *J* = 6.0 Hz, 1H), 4.50 (m, 1H), 4.85 (m, 1H), 5.15 (m, 3H), 7.40 (m, 8H), 7.70 (m, 1H), 7.82 (m, 1H), 8.23 (m, 1H), 8.60 (m, 1H). MS (CI) m/z 1013 (M + H)$^+$. Anal. Calcd for $C_{51}H_{64}N_8O_{12}S$ (1013.2): C, 60.46; H, 6.37; N, 11.06. Found: C, 60.86; H, 6.31; N, 10.64.

Asp(OBu^t)-Mamb-D-Abu-N-MeArg(Tos)-Gly (18). A slurry of **17** (658g, 0.65 mol) in methanol (5.0 L) and 10% palladium on carbon (316 g of 50% aqueous mixture, 0.15 mol) was degassed, and hydrogen gas was sparged subsurface at atmospheric pressure for 2 h. The catalyst was filtered off over Celite. The filtrate was stripped to 1.2 L of viscous oil and added dropwise to 1-butanol (7.5 L) at 30 °C under 7-20 mm Hg of vacuum. The resulting slurry was cooled to 5 °C, filtered, and dried *in vacuo* to yield 721 g of **18**/1-butanol complex (~100% yield). An analytical sample was produced by flash chromatography. ^{1}H NMR (DMSO-d_6) δ 8.93 (br s, 1H), 8.63 (d, 1H), 7.85 (s, 1H), 7.71−7.81 (m, 2H), 7.63 (d, *J* = 8.9, 3H), 7.32−7.50 (m, 3H), 7.21−7.32 (m, 3H), 5.01(dd, *J* = 6.0, 9.0; 1H), 4.77 (q, *J* = 6.8, 1H), 4.33 (br s, 2H), 3.63 (dd, *J* = 5.2, 7.4; 2H), 3.31−3.57 (m, 12H), 3.06 (br s, 3H), 2.91 (s, 3H), 2.60 (dd, *J* = 3.0, 15.0; 2H), 2.34 (s, 3H), 2.29−2.47 (m, 2H), 1.47−1.91 (m, 6H), 1.38 (s, 9H), 1.05 (t, *J* = 6.9, 2H), 1.04 (d, *J* = 6.2, 1H), 0.90 (t, *J* = 7.7, 3H). HRMS (ESI) Calcd for $C_{36}H_{52}N_8O_{10}S$ 789.3597 (M + H)$^+$, found 789.3605 (M + H)$^+$.

Cyclo-D-Abu-N-MeArg(Tos)-Gly-Asp(OBu^t)-Mamb (19). A solution of **18** (393 g, ~0.35 mol), DIEA (100 mL, 0.574 mol), and acetonitrile (3.4 L) was added dropwise to a stirred solution of HBTU (136 g, 0.359 mol) in acetonitrile (3.0 L) over 5.5 h. The reaction mixture was stirred overnight. The mixture was cooled to 0 °C over 2 h, and the solids were collected by filtration. The solids were washed with a solution of acetonitrile/DIEA (400 mL/4 mL) and dried *in vacuo*. The crude **19** was dissolved into a solution of 18% methanolic chloroform (1215 mL) at 34 °C. Silica gel (527 g) was added to the solution. This slurry was filtered, and the filter cake was further extracted with 12% methanolic chloroform. The filtrate was concentrated *in vacuo*. The solvent was replaced with acetonitrile until GC analysis of the pot contents indicated the chloroform content was <0.5%. The slurry was stirred at 34 °C overnight, and the purified **19** was collected by filtration. The solids were washed with acetonitrile (200 mL) and dried under vacuum to 158.6 g (58% overall yield from **11**) of white solids: mp 200.6−202.3 °C; ^{1}H NMR δ 8.86 (br d, *J* = 5.1; 1H, N*H*), 8.61−8.46 (m, 2H, N*H*), 7.76−7.22 (m, 8H), 7.02 (v br s, N*H*), 6.82 (v br s, N*H*), 6.55 (v br s, N*H*), 5.11 (dd, *J* = 4.6,10.8; 1H), 4.66 (dd, *J* = 7.8,16.7; 1H), 4.60−4.48 (m, 2H), 4.19 (dd, *J* = 8.4,16.5; 1H), 4.00 (dd, *J* = 4.0,16.5; 1H), 3.68 (br s, N*H*), 3.65−3.54 (m, 1H), 3.14−2.96 (m, 2H), 2.89 (s, 3H), 2.68 (dd, *J* = 7.1,15.9; 1H), 2.33 (s, 3H), 2.02−1.84 (m, 1H), 1.84−1.62 (m, 3H), 1.60−1.42 (m, 1H), 1.38 (s, 9H), 1.34−1.16 (m, 2H), 0.93 (t, *J* = 7.3, 3H); ^{13}C NMR (DMF-d_7 + TFA) δ 174.27, 171.42, 170.33, 170.00, 169.89, 168.85, 157.92, 143.16, 141.86, 140.55, 134.59, 130.31, 129.61, 128.27, 126.34, 125.96, 125.83, 80.98, 56.65, 53.01, 51.66, 41.94, 41.90, 40.98, 37.34, 30.51, 28.00, 26.52, 24.83, 24.61, 21.05, 10.52. MS(ESI), m/z 771 (M + H)$^+$. Anal. Calcd for $C_{36}H_{50}N_8O_9S$: C, 56.09; H, 6.54; N, 14.53; S, 4.16. Found: C, 56.10; H, 6.71; N, 14.45; S, 4.00.

Cyclo-D-Abu-N-MeArg-Gly-Asp-Mamb (1). Trifluoroacetic acid (1043 g, 9.15 mol) was added to ice cooled **19** (140.0 g, 0.182 mol) with stirring. The ice bath was removed and stirring continued for 20 min after the solids dissolved. Trifluoro-

methanesulfonic acid (1196 g, 7.97 mol) was added to this solution at −18 to −13 °C. The solution was cooled to −30 °C, anisole (139 g, 1.3 mol) was added at −30 to −23 °C, and then the reaction was warmed to −14 °C. The deep purple mixture was held at −14 to −8 °C for 1 h and cooled to −30 °C. Butyl ether (4150 mL) was added at −30 to −12 °C to produce bright yellow solids. The slurry was cooled to −30 °C and held there without stirring for 30 min. The solids were collected on Whatman no. 24 filter paper using vacuum and under nitrogen. The resulting solids were washed with additional butyl ether and dried under a stream of nitrogen. The dry solids were dissolved into 1:1 aqueous acetone (2.0 L). Residual butyl ether as a top layer at this point was separated and extracted with water (2 × 100 mL). These extracts were added to the aqueous layer. Anion exchange resin (Biorad AG1 resin, acetate form; 1.03 kg) was added in portions until the pH entered the range 4.55−4.95. The slurry was stirred for an additional 40 min and filtered. The resin was washed with 1:1 aqueous acetone (1.6 L) and the filtrate evaporated to 117.8 g of white solids (crude yield 115%). These were slurried into water (950 mL), heated to 50 °C, and diluted slowly with acetone (160 mL). Most of the acetone was removed *in vacuo* at 45 °C, and **1** was allowed to further recrystallize overnight with stirring. The next day the slurry was cooled to 0 °C over 45 min, held 5 h, and collected by filtration. The solids were washed with enough water to cover the solids, allowed a 10-min soak, and dried under a nitrogen stream. These solids were further dried to constant weight *in vacuo* to produce 106.8 g of white solids (79% yield): mp 270−5 °C dec; ^{1}H NMR (D_2O/DCl with water repression, reference = TSP) δ 8.89 (m, N*H*), 8.00 (br dd, J = 2.4, 8.6; NH), 7.64 (br dt, J = 2.2, 6.2; 1H), 7.51−7.42 (m, 2H), 7.36 (br s, 1H), 5.38 (dd, J = 4.4, 11.4; 1H), 4.82−4.65 (m, 3H), 4.59 (dt, J = 4.4, 17.2; 1H), 4.31 (br d, J = 16.8, 1H), 3.84 (br d, J = 17.2, 1H), 3.23 (br t, J = 6.6, 2H), 3.17 (s, 3H), 2.99 (dd, J = 7.3,16.8, 1H), 2.84 (dd, J = 7.3,16.8, 1H), 2.26−2.08 (m, 1H), 2.04−1.74 (m, 3H), 1.70−1.42 (m, 2H), 1.02 (t, J = 7.3, 3H); ^{13}C NMR (CD_3OD + TFA) δ 176.29, 173.49, 173.42, 172.08, 171.15, 171.03, 158.70, 140.44, 134.89, 131.26, 129.31, 126.52, 126.11, 57.71, 54.01, 52.36, 42.76, 42.48, 41.90, 36.05, 31.28, 26.49, 25.22, 25.09, 10.68. MS (ESI) m/z 561 (M + H)$^+$. Anal. Calcd for $C_{25}H_{36}N_8O_7$: C, 53.56; H, 6.47; N, 19.99. Found: C, 53.31; H, 6.41; N, 19.85.

Cyclo-D-Abu-N-MeArg-Gly-Asp-Mamb, $MeSO_3H$ Salt. A solution of methanesulfonic acid (11.66g, 0.121 mol) in 2-propanol (0.2 L) was added to a refluxing suspension of **1** (80.0 g, 0.121 moles, 15% water by weight) in 2-propanol (3 L) over 16 m. The slurry was cooled to 1 °C over 29 min and stirred for 1 h. The salt was collected, washed with cold 2-propanol (600 mL), and dried to constant weight under a stream of dry nitrogen. The final weight was 79.0 g (99%) of white solids: mp 260−1 °C; ^{1}H NMR (400 MHz) δ 12.33 (br s, D_2O exchanged), 8.85 (d, J = 5.5, 1H), 8.52 (d, J = 7.5, 1H), 8.44 (dd, J = 7.6, 4.9; 1H), 7.75−7.66 (m, 1H), 7.63 (dd, J = 8.2, 3.1; 1H), 7.57 (br t, J = 5.6, 1H), 7.50 (br s, 1H), 7.40−7.32 (m, 2H), 5.15 (dd, J = 4.6, 11.0; 1H), 4.68−4.54 (m, 2H), 4.17 (dd, J = 16.7, 8.2; 1H), 4.05 (dd, J = 16.5, 4.9; 1H), 3.68 (dd, J = 16.7, 3.1; 1H), 3.31 (br s, D_2O exchanged), 3.18−3.04 (m, 2H), 2.95 (s, 3H), 2.71 (dd, J = 16.7, 6.6; 1H), 2.58-2.50 (m, 1H), 2.37 (s, 3H), 2.08−1.94 (m, 1H), 1.77 (dq, J = 7.5, 7.5; 2H), 1.66−1.52 (m, 1H), 1.44−1.26 (m, 2H), 0.95 (t, J = 7.5, 3H); ^{13}C NMR (100.6 MHz) δ 10.17, 23.52, 23.69, 25.11, 30.20, 35.35, 39.66, 40.27, 40.80, 41.04, 50.14, 51.99, 55.64, 125.18, 125.24, 127.46, 129.65, 133.23, 139.51, 156.64, 167.99, 168.40, 169.21, 170.61, 171.44, 173.43. MS (FAB) m/z 561.3 (M + H)$^+$. Anal. Calcd for $C_{26}H_{40}N_8O_{10}S$: C, 47.55; H, 6.14; N, 17.06; O, 24.36; S, 4.88. Found: C, 47.49; H, 6.27; N, 16.72; O, 24.51; S, 4.78.

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra of **1, 11, 10, 17, 18,** and **19** (12 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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