An Efficient Synthesis of Glycoprotein IIb/IIIa Inhibitor DMP728. A Novel Synthesis of N^{α}-Methylarginine-Containing Peptide

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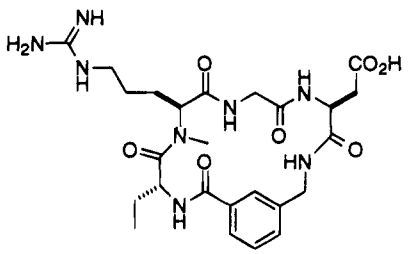
An efficient synthesis of an antithrombotic cyclic peptide antagonist of glycoprotein IIb/IIIa, cyclo-(D-Abu-N^{α}-methyl-Arg-Gly-Asp-*m*-(aminomethyl)benzoic acid) has been developed. In the course of this work an efficient and selective method has been developed for the synthesis of derivatives of N^{α}-methylarginine from Cbz-Gln. The carboxamide of Cbz-Gln is dehydrated to the corresponding nitrile, and the resulting compound is methylated providing Cbz-2-(methylamino)-4-cyanobutyric acid, a diprotected precursor of N^{α}-methylornithine. At a later point in the reaction sequence the nitrile is reduced to the amine and the resulting N^{α}-methylornithine derivative is guanylated, converting it to a derivative of N^{α}-methylarginine.

Glycoprotein IIb/IIIa[1] is a membrane-bound protein that projects from the surface of platelets where it plays an important role in hemeostasis. In response to a number of different agonists this protein undergoes a conformational change allowing it to bind to multiple sites on the serum protein fibrinogen, leading to crosslinking of the platelets, and ultimately to clot formation. The pathophysiological consequences of this process includes ischemic heart failure, myocardial infarction, and reocclusion after angioplasty. Thus, there has been considerable activity aimed at the design of compounds that compete with fibrinogen for binding to the IIb/IIIa receptor. It was early realized[2] that this protein is capable of binding to the tripeptide sequence, Arg-Gly-Asp, and a number of very potent cyclic Arg-Gly-Asp-containing peptides have recently been developed as potential antithrombotics.[3–6] Among these structures is cyclo(D-2-aminobutyryl-N^{α}-methyl-Arg-Gly-Asp-*m*-(aminomethyl)benzoic acid) (**1**), which is particularly attrac-



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tive because of its extremely high potency[7,8] (K_{diss} for binding to the receptor is approximately 100 pM) and oral activity.[5]

A key feature required for the potency of DMP728 is the N^{α}-methyl group of the arginine residue. This modification was first described in a series of cyclic, disulfide-containing peptides and has consistently increased the potency of our own series of peptides. This modification has been shown to increase the rigidity of the peptide macrocycle and also to restrict the number of low-energy rotomers of the arginine side chain.[9] It also presents a synthetic challenge to the development of a cyclic peptide product. Our original synthesis[5] of DMP728 began with the highly costly derivative N^{α}-Boc-N^{α}-methyl-N^{ω}-tosylarginine (Boc-*N*MeArg(Tos)), which is prepared in very low overall yield from N^{ω}-tosylarginine[3] by (*i*) benzylation of the α-amine using benzaldehyde and $NaCNBH_3$; (*ii*) monomethylation of the resulting secondary amine using formaldehyde and formic acid; (*iii*) hydrogenolysis of the benzyl group; (*iv*) addition of the Boc group using di-*tert*-butyldicarbonate. The reaction sequence requires chromatography and is not very attractive because of its length, the expense of N^{ω}-tosylarginine, and the very harsh acidic conditions required to remove the tosyl protecting group and the concomitant side reactions.[10] Preliminary experiments to replace the N^{ω}-tosylarginine with the less expensive N^{ω}-nitro-arginine derivative were largely unsuccessful (Tom Ma-

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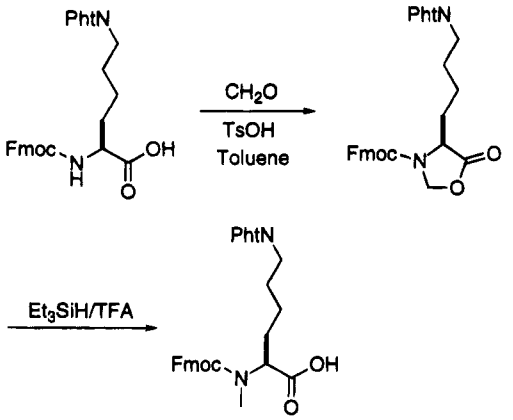
Scheme 1



duskuie, personal communication), suggesting that a new synthetic route to the compound should be investigated.

Our retrosynthetic analysis of DMP728 indicated that this compound might be best prepared via the corresponding N^{α}-methylornithine derivative, which could be guanylated at some point in the reaction sequence. Freidinger et al.[11] have developed a route to N^{α}-Fmoc-N^{α}-methyl-N^{ϵ}-phthalyl-L-lysine (Scheme 1), which could readily be adapted to ornithine. However, attempts to prepare derivatives of Lys in which the N^{ϵ}-group was monoprotected with a benzyloxycarbonyl (Cbz) group were unsuccessful,[11] and the harsh conditions required to deprotect the phthalyl group proved to be problematic in preliminary syntheses of DMP728. Freidinger et al., have also prepared N^{α}-methyllysine derivatives via α-aminopimellic acid, but the overall yield was low (15%), and the corresponding amino acid required for the synthesis of ornithine derivatives is not readily available in chiral form. We therefore explored the use of a nitrile as a masked form of the δ-nitrogen of ornithine. The dehydration of glutamine to the corresponding nitrile precursor has long been recognized as an undesirable side reaction that frequently accompanies peptide bond formation upon activation of N^{α}-protected amino acids.[12] Further, dehydration of glutamine derivatives, followed by reduction of the intermediate nitrile to a derivative of ornithine has been demonstrated.[13] Thus, it appeared that this procedure would provide an attractive route to the synthesis of derivatives of N^{α}-methylarginine (*N*MeArg) if the intermediate nitrile could be selectively methylated.

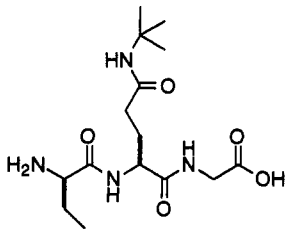
Results and Discussion

The overall schemes involved in the synthesis of DMP728 are illustrated in Schemes 2–4 below. The macrocycle was divided into a tripeptide and a dipeptide segment consisting of the D-Abu-*N*MeArg-Gly and Boc-Asp-Mamb (Mamb = *m*-(aminomethyl)benzoic acid). This parsing of the macrocycle provides a convergent approach to the synthesis and additionally avoids the possibility of epimerization of the *C*-terminal amino acid during segment coupling and cyclization. The guanidino group of *N*MeArg and the β-carboxylate of Asp were protected using the Cbz and benzyl groups, respectively, allowing convenient removal of both groups by catalytic hydrogenation at the completion of the synthesis.

D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-Arg-Gly (D-Abu-*N*MeArg(Cbz$_2$)-Gly; 6). This compound was prepared beginning with Cbz-Gln as described in Scheme 2.

Conversion of Cbz-Gln to the corresponding nitrile by treatment with phosgene[14] followed by selective methylation[15] at the N^{α}-group using CH_3I/NaH led to the key intermediate, N^{α}-Cbz-N^{α}-methyl-α-amino-γ-cyanobutyric acid (**2**) in 94% yield. Methylation of *N*-protected α-amino-γ-cyanobutyric acid using the current method has been demonstrated to retain over 99.8% optical purity in a separate study.[16] The next step in the reaction sequence was particularly convenient in that it involved simultaneous N^{α}-deprotection as well as C^{α}-carboxyl activation for the next coupling step; **2** was converted to the corresponding crystalline *N*-carboxy anhydride[17] **3** by treatment with phosphoros pentachloride.[18] The resulting derivative **3** was treated with glycine-*tert*-butyl ester yielding a dipeptide.[19] Without isolation of the dipeptide, it was coupled *in situ* with Boc-D-Abu. The coupling of Boc-D-Abu to the *N*-methyl dipeptide proved to be especially difficult. A variety of coupling agents (isobutyl chloroformate,[20] PyBrOP,[21] BOP-Cl,[22] DPP-Cl[23] and HBTU or TBTU[24])[29] were screened (Table 1), and diphenylphosphinic chloride (DPP-Cl)[23] was shown to be the best, giving a moderately good overall yield (75%) of tripeptide (**4**).

Initial attempts to remove the Boc and *tert*-butyl groups from **4** using TFA (trifluoroacetic acid) containing 10% anisole or a variety of other carbonium ion scavengers were only partially successful, and led to about 20% formation of the TFA salt of *tert*-butylated product **7**.



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This product appears to have been a product of *tert*-butylation of the nitrile, which hydrated upon workup. The reaction appeared to be partially intramolecular in

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Scheme 2

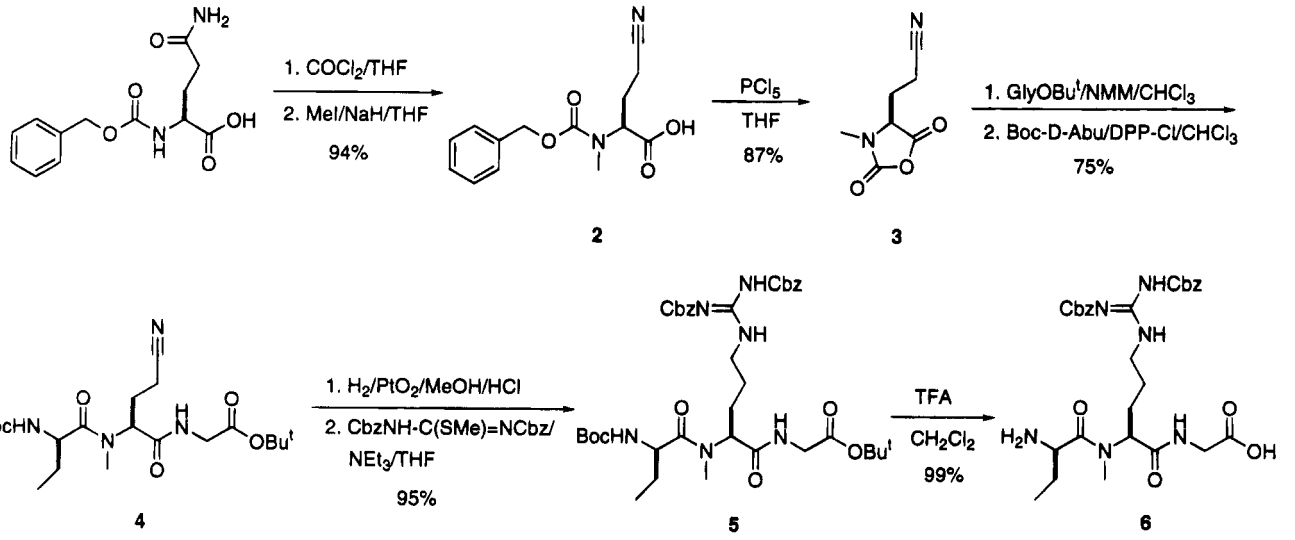


Table 1. Coupling Efficiency of Boc-d-Abu to *N*-Methyl Dipeptide Using Different Couplings Agents

entry	coupling agent	base	solvent	time (h)	yield (%)[a]
1	isobutyl chloroformate	NMM	THF	5	59
2	PyBrOP	DIEA	DMF	24	45
3	BOP-Cl	DIEA	DMF	24	27[b]
4	DPP-Cl	NMM	THF	4	64
5	HBTU	NMM	DMF	24	50
6	DPP-Cl	NMM	$CHCl_3$	24	70
7	DPP-Cl	NMM	$CHCl_3$	48	75

[a] Overall isolated yield for the ring opening of **3** with $GlyOBu^t$ and the coupling of the generated dipeptide with Boc-D-Abu. [b] The low yield was due to the low solubility of BOP-Cl.

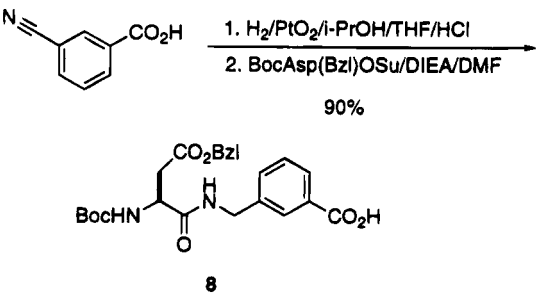
nature[25] as it could be only partially suppressed using carbonium ion scavengers such as anisole. Even addition of up to 50% acetonitrile failed to entirely suppress the reaction. Therefore, at this point, we opted to reduce the nitrile to the amine and introduce a diprotected guanyl derivative.

Reduction of the nitrile by catalytic hydrogenation at 55 psi could be accomplished in quantitative yield using PtO_2 as a catalyst, and the resulting product was treated with the bis-protected guanylating agent, *N,N'*-bis(benzyloxycarbonyl)-*S*-methylisothiourea[27] providing crystalline product **5** in excellent yield. The deprotection of the Boc and *tert*-butyl groups of compound **5** could then be accomplished using 50% TFA in methylene chloride to give compound **6** in 99% yield.

***N*-Boc-Asp(Bzl)-Mamb (8).** This half of the target molecule was synthesized from Boc-Asp(Bzl) and 3-cyanobenzoic acid as described in Scheme 3. Reduction of 3-cyanobenzoic acid to 3-aminomethyl benzoic acid appeared to proceed in quantitative yield and the resulting product was coupled with the hydroxysuccinimide ester of Boc-Asp(Bzl), yielding the desired crystalline segment (**8**) in 90% overall yield.

DMP728 (1). The synthetic transformations required to prepare (**1**) from the two protected segments **6** and **8** are illustrated in Scheme 4. Conversion of **8** to the corresponding pentafluorophenyl ester[26] and coupling of this activated derivative to tripeptide **6** provided the

Scheme 3



linear precursor of DMP728, **9**. Removal of the Boc group in compound **9** followed by cyclization using TBTU[24] resulted in the formation of the macrocycle **11** in excellent yield (90%). Final deprotection of the product by hydrogenation using palladium on charcoal as the catalyst in the presence of methanesulfonic acid gave the desired product **1** in 90% yield, corresponding to approximately a 43% overall yield starting with Cbz-Gln.

Synthesis of Prodrug Esters of DMP728. The above synthetic route could be modified to allow a rapid synthesis of numerous prodrug esters of DMP728 (Scheme 5).

To allow the efficient synthesis of these esters we wished to obtain a synthon with a protected guanidino group and a free carboxylate, which could be reacted with a variety of alkylating agents. This could be accomplished by substituting $Fmoc\text{-}Asp(OBu^t)$-Mamb (**12**) for Boc-Asp(OBzl)-Mamb (**8**) and coupling this dipeptide **12** with the above-described tripeptide fragment **6**. Removal of the Fmoc protecting group and closure of the macrocycle was accomplished in a single step using DMAP both as deprotecting agent as well as a base for acylation.[28] Removal of the *tert*-butyl group was accomplished in excellent yield, and the resulting carboxylate was alkylated with a variety of alkyl halides. Deprotection of the bis-Cbz groups by hydrogenation completed the synthesis of prodrug esters of DMP728, some of which (**16a–j**) are provided in Table 2.

Conclusion

This paper describes a novel and efficient method to prepare N^{α}-methylornithine and N^{α}-methylarginine de-

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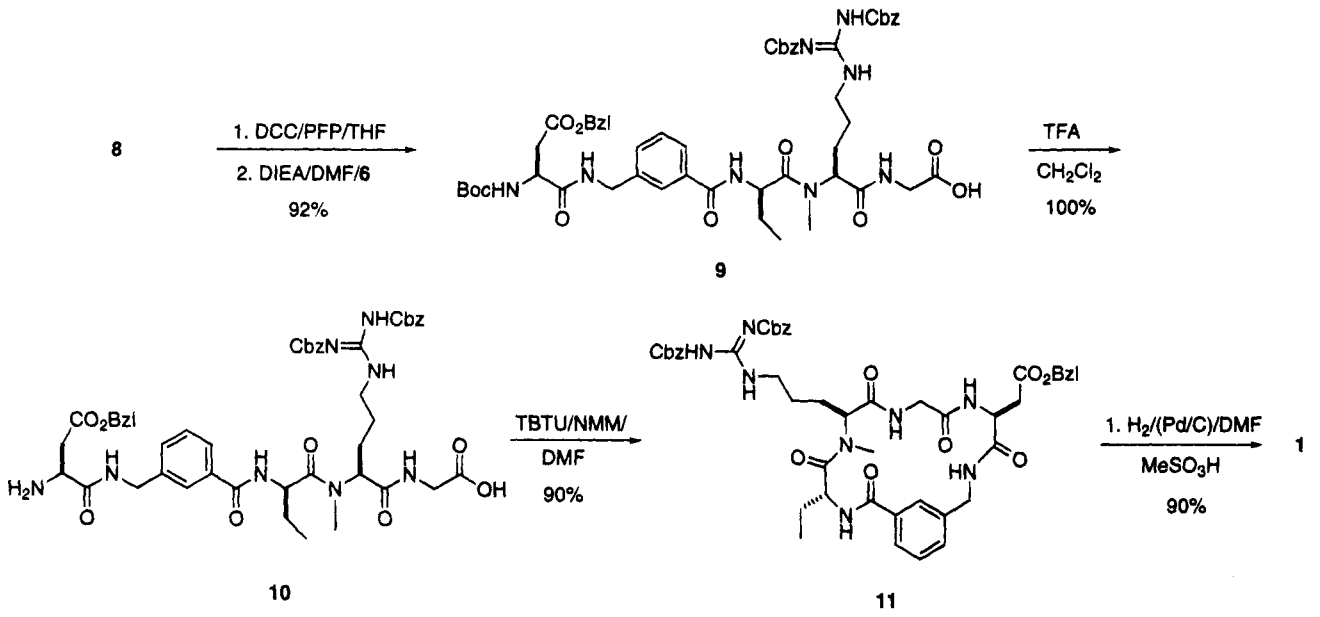
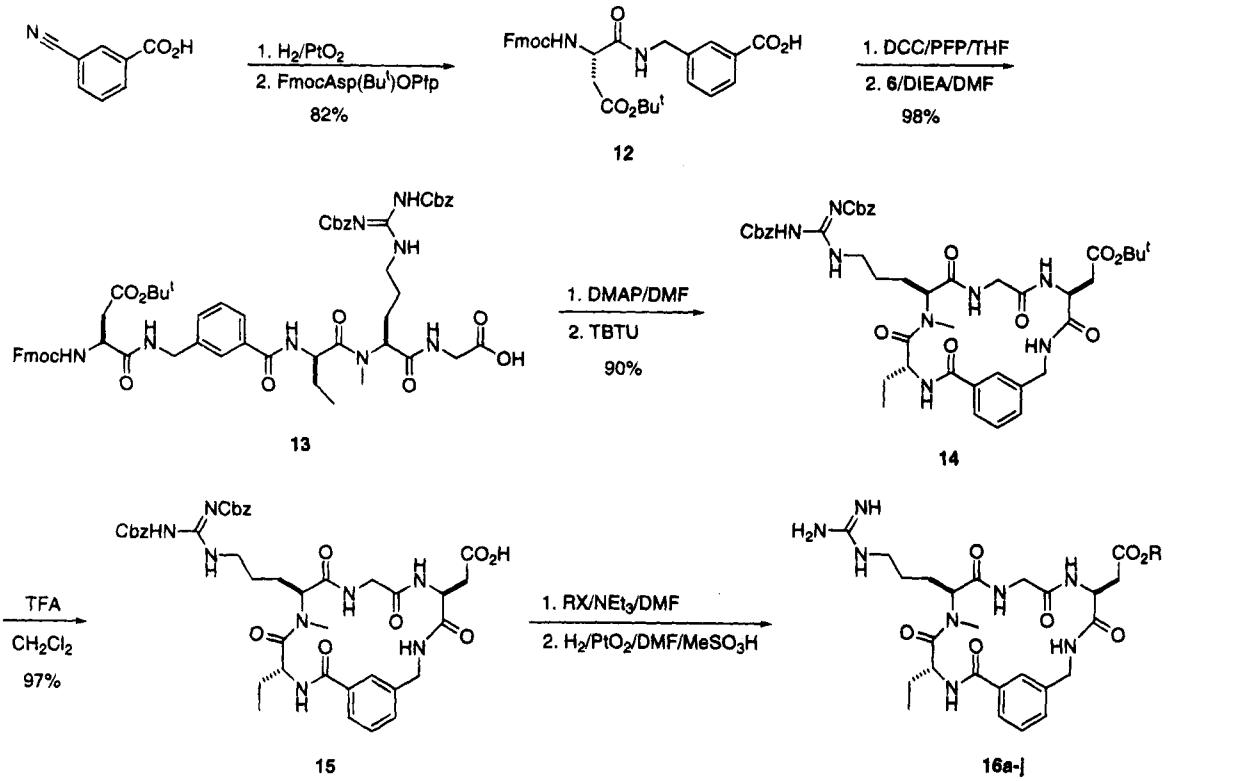
Scheme 4



Scheme 5



rivatives beginning with the inexpensive starting material, Cbz-Gln. The corresponding *N*-methyl derivative of 2,4-diaminobutyric acid could similarly be prepared beginning with Cbz-Asn. This improved procedure for the synthesis of derivatives of *N*MeArg has been incorporated into a very effective route to DMP728. In addition, this convergent route to DMP728 has allowed the convenient synthesis of numerous derivatives of the parent compound including prodrug esters.

Experimental Section[29]

General Methods. *N*-Methylmorpholine, triethylamine, and diisopropylethylamine were distilled from calcium hydride and stored over 4-A molecular sieves under nitrogen. DMF, THF, acetonitrile, and ether were dried over 4-A molecular sieves prior to use. All other chemicals and solvents (reagent grade) were used as supplied without further purification. Amino acid derivatives were purchased from Bachem California, Advanced ChemTech, or Bachem Bioscience and were used without further purification. Thin layer chromatography

(29) Abbreviations: D-Abu, D-2-aminobutyric acid; Boc, *tert*-butyloxycarbonyl; BOP-Cl, *N,N*-bis(2-oxo-3-oxozolidinyl)phosphorodiamidic chloride; Cbz, benzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DPP-Cl, diphenylphosphinic chloride; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; Mamb, *m*-(aminomethyl)benzoic acid; *N*MeArg, N^{α}-methylarginine; NMM, *N*-methylmorpholine; PfP, pentafluorophenol; Pht, phthalyl; PyBrOP, bromotris(pyrrolidino)phosphonium hexafluorophosphate; TBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; Tos, tosyl.

Table 2. Prodrug Esters of DMP728 Synthesized by Alkylation of Compound 15

entry	R	product	yield (%)[a]	FAM-MS Calcd	FAM-MS Found
1	acetoxymethyl	**16a**	51	633.3	633.2
2	pivaloyloxymethyl	**16b**	26	675.3	675.3
3	[(isopropyloxycarbonyl)oxy]methyl	**16c**	25	667.3	667.3
4	[(cyclohexyloxycarbonyl)oxy]methyl	**16d**	26	717.4	717.6
5	(*tert*-butyloxycarbonyl)methyl	**16e**	32	675.3	675.3
6	(ethyloxycarbonyl)methyl	**16f**	30	647.3	647.3
7	(cyclohexylcarbonyl)oxy)methyl	**16g**	25	701.4	701.6
8	1-(pivaloyloxy)ethyl	**16h**	20	689.4	689.4
9	1-[[(cyclohexyloxy)carbonyl]oxy]ethyl	**16i**	15	731.4	731.6
10	(4-*tert*-butylbenzoyloxy)methyl	**16j**	18	751.4	751.3

[a] Overall isolated yield after removal of the bis-Cbz protecting groups and purification of the product by HPLC.

(TLC) was performed on silica gel 60 F_{254} TLC plates (layer thickness 0.2 mm) which were purchased from EM Separations. TLC visualization was accomplished using UV light, iodine, and/or ninhydrin spray. Solvent system for eluting TLC plates was chloroform:methanol:acetic acid (85:10:5). HPLC analyses were performed on a Hewlett-Packard 1090 system using a Vydac C18 column (4.6 mm × 25 cm, 5 μm), and semipreparative HPLC was run on a Rainin system using a Vydac C18 column (22 mm × 25 cm, 10 μm). Water (0.05% TFA) and acetonitrile (0.05% TFA) were used as eluent. Melting points were determined using a Thomas Hoover or Electrothermal 9200 melting point apparatus and are uncorrected. NMR spectra were recorded on a 300 MHz General Electric QE-300, Varian 300, or Varian 400 spectrometer. Fast atom bombardment mass spectrometry (FAB-MS) was performed on a VG Zab-E double-focusing mass spectrometer using a Xenon FAB gun as the ion source or a Finnigan MAT 8230.

N^{α}-(Benzyloxycarbonyl)-N^{α}-methyl-4-cyano-L-2-aminobutyric Acid (2). Z-Gln (28.03 g, 100 mmol) was dissolved in 300 mL of THF with protection from moisture and was treated with 100 mL of 1.93 M phosgene in toluene (193 mmol). The solution was stirred at room temperature for 2 h and then concentrated at 30 °C to 200 mL. Water (200 mL) was added slowly with stirring. After stirring at room temperature for 2 h, the organic phase was seperated, and the water phase was extracted with ethyl acetate twice. The combined organic solution was washed with brine four times, dried ($MgSO_4$), and concentrated. The oily product was dried over KOH overnight.

The oily product was taken up in 300 mL of dry THF and then treated with 49.8 mL (800 mmol) of methyl iodide with protection from moisture, and the solution was cooled in an ice bath. To it was slowly added 10 g of sodium hydride (250 mmol, 60% dispersion in oil). The mixture was stirred in the ice bath for 1 h and then at room temperature for 22 h. Ethyl acetate (50 mL) was added, and after stirring for 10 min, 100 mL water was added slowly. The solution was acidified with a few drops of 4 N HCl to pH 8–9 and then concentrated at 30 °C to remove the organic solvents. Water (100 mL) was added followed by 10 mL of 0.1 N sodium thiosulfate, and the solution was extracted with ether twice. The water layer was cooled in an ice bath and to it was slowly added 4 N HCl to pH 3 with stirring. The product, which crystallized during the acidification, was filtered, washed with water several times, and dried. Yield 26.0 g (94%). Mp 81–83 °C. R_f = 0.51. ^{1}H-NMR ($CDCl_3$): δ 2.15 (m, 1H); 2.38 (m, 1H); 2.42 (m, 2H); 2.96 and 2.98 (2 s, *cis and trans* N-CH_3); 4.62 (m, 1H); 4.90 (b, 1H); 5.19 (s, 2H); 7.35 (m, 5H). $[\alpha]^{25}_D$ −43.11° (c = 0.740, methanol). FAB-MS $[M+H]^+$: calcd 277.2, found 277.2. Anal. Calcd for $C_{14}H_{16}N_2O_4$: C, 60.85; H, 5.84; N, 10.14. Found: C, 60.64; H, 5.88; N, 10.03.

N^{α}-Methyl-4-cyano-L-2-aminobutyric Acid N-Carboxy Anhydride (3). To a solution of **2** (11.05 g, 40 mmol) in 50 mL of dry THF cooled in an ice bath was added phosphorus pentachloride (15 g, 72 mmol), and the mixture was stirred for 2 h and concentrated to dryness. The residue was triturated with petroleum ether to give a solid which was filtered, washed with petroleum ether, and dissolved in dry acetonitrile. Insoluble material was filtered off, and the solution was concentrated. The solid was washed with cold ether and dried. Yield 5.86 g (87%). Mp 90–92 °C. ^{1}H-NMR ($CDCl_3$): δ 2.18 (m, 1H); 2.39 (m, 1H); 2.60 (m, 2H); 3.02 (s, 3H); 4.28 (m, 1H). $[\alpha]^{25}_D$ +16.99° (c = 0.618, DMSO). Anal. Calcd for $C_7H_8N_2O_3$: C, 49.99; H, 4.79; N, 16.43. Found: C, 49.62; H, 4.77; N, 16.66.

N-Boc-D-(2-Aminobutyryl)-N^{α}-methyl-4-cyano-L-(2-aminobutyryl)-glycine *tert*-Butyl Ester (4). To a solution of glycine *tert*-butyl ester hydrochloride (3.68 g, 22 mmol) in 40 mL of chloroform and 4.84 mL (44 mmol) of N-methylmorpholine cooled to −40 °C was added a solution of **3** (3.36 g, 20 mmol) in 20 mL dry acetonitrile, the solution was stirred at −20 °C for 1 h, and the solvent was reduced to about 10 mL *in vacuo* at a temperature below 30 °C.

To a solution of N-Boc-D-2-aminobutyric acid dicyclohexylamine salt (8.08 g, 21 mmol) in 30 mL of chloroform cooled to −10 °C was added diphenylphosphinic chloride (3.91 mL, 20.5 mmol), and the mixture was stirred at −5 to −10 °C for 1 h. To it was added the above prepared solution (10 mL) followed by 2.42 mL (22 mmol) of N-methylmorpholine. The mixture was stirred at 0 to −5 °C for 48 h and then concentrated. Ethyl acetate was added, and the insoluble material was removed by filtration. The filtrate was washed with $NaHCO_3$ four times and with brine three times, dried over $MgSO_4$, and concentrated to a small amount at which time the product crystallized. Petroleum ether was added, and after cooling, the solid was filtered, washed with petroleum ether, and dried. Yield 6.7 g (75%). Mp 90–92 °C. R_f = 0.68. ^{1}H-NMR (DMSO-d_6): δ 0.87 (t, 3H); 1.37 (s, 9H); 1.39 (s, 9H); 1.52 (m, 1H); 1.64 (m, 1H); 1.92 (m, 1H); 2.21 (m, 1H); 2.40 (m, 2H); 2.75 (20%) and 2.95 (80%) (s, 3H, *cis* and *trans* N-CH_3); 3.70 (m, 2H); 4.30 (m, 1H); 4.61 and 5.06 (m, 1H, *cis and trans* NMeArg α-CH); 7.08 (d, 1H); 7.94 (t, 1H). $[\alpha]^{25}_D$ −42.37° (c = 0.760, THF). FAB-MS $[M+H]^+$: calcd 441.3, found 441.3. Anal. Calcd for $C_{21}H_{36}N_4O_6$: C, 57.28; H, 8.24; N, 12.72. Found: C, 57.03; H, 8.21; N, 12.72.

N-Boc-D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycine *tert*-Butyl Ester (5). Compound **4** (4.63 g, 10.5 mmol) was dissolved in 70 mL of methanol in a Parr bottle and to it was added a cold solution of 1.2 mL of concentrated hydrochloric acid (38%) in 10 mL of methanol followed by 200 mg of platinum(IV) oxide. The mixture was hydrogenated at 55 psi for 1 h, the catalyst was filtered off, and 2.09 mL (15 mmol) of triethylamine was added. The solvent was removed under reduced pressure, and the residue was taken up in 20 mL of THF. To it was added N,N'-bis(benzyloxycarbonyl)-S-methylisothiourea (3.58 g, 10 mmol) followed by 2.09 mL (15 mmol) of triethylamine. The mixture was stirred overnight during which time the bottle was evacuated several times to remove the byproduct methanethiol. Ethyl acetate was added, and the solution was washed with 1% citric acid, brine, 5% $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. Crystallization from ethyl ether–petroleum ether gave 7.2 g (95%) product. Mp 110–111 °C. R_f = 0.89. ^{1}H-NMR (DMSO-d_6): δ 0.80 (t, 3H); 1.37 (s, 9H); 1.38 (s, 9H); 1.44 (m, 2H); 1.58 (m, 3H); 1.88 (m, 1H); 2.76 (15%) and 2.92 (85%) (s, 3H, *cis and trans* N-CH_3); 3.32 (m, 2H); 3.70 (m, 2H); 4.32 (m, 1H); 4.61 and 5.01 (m, 1H, *cis and trans* NMeArg α-CH); 5.02 (s, 2H); 5.20 (s, 2H); 6.97 (d, 1H); 7.39 (m, 10H); 7.92 (t, 1H); 8.44 (t, 1H); 11.56 (s, 1H). $[\alpha]^{25}_D$ −18.89° (c = 0.900, methanol). FAB-MS $[M + H]^+$: calcd

755.4, found 755.4. Anal. Calcd for $C_{38}H_{54}N_6O_{10}$: C, 60.49; H, 7.22; N, 11.14. Found: C, 60.25; H, 7.27; N, 11.06.

D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycine TFA Salt (6). A solution of **5** (9 g, 11.9 mmol) in 90 mL of 50% TFA in methylene chloride was stirred at room temperature for 2 h, and the solution was concentrated at 30 °C. Cold ether was added, and after standing, the solid was filtered, washed with ether, and dried. Yield 8.4 g (99%). ^{1}H-NMR (DMSO-d_6): δ 0.85 (t, 3H); 1.40 (m, 2H); 1.70 (m, 3H); 1.88 (m, 1H); 2.82 (23%) and 2.95 (77%) (s, 3H, *cis and trans* N-CH_3); 3.31 (m, 2H); 3.74 (m, 2H); 4.36 (m, 1H); 5.00 (m, 1H); 5.02 (s, 2H); 5.20 (s, 2H); 7.32 (m, 10H); 8.10 (s, 3H); 8.20 (t, 1H); 8.45 (t, 1H); 11.57 (s, 1H). $[\alpha]^{25}_D$ −26.73° (c = 0.520, methanol). FAB-MS [M + H]$^+$: calcd 599.3, found 599.3. Anal. Calcd for $C_{31}H_{39}N_6O_{10}F_3 \cdot H_2O$: C, 50.98; H, 5.66; N, 11.51. Found: C, 50.76; H, 5.37; N, 11.37.

***N*-Boc-L-Aspartyl(benzyl)-3-(aminomethyl)benzoic Acid (8).** 3-cyanobenzoic acid (3.38 g, 23 mmol) was dissolved in 30 mL of THF by warming and stirring. 2-Propanol (20 mL) was added and the solution was allowed to cool to room temperature. To it was added 2.5 mL of precooled (0 °C) concentrated HCl (38%) followed by 160 mg platinum(IV) oxide. The mixture was hydrogenated at 55 psi overnight. The product precipitated during the hydrogenation. Ether (100 mL) was added, and the mixture was stirred and then cooled. The precipitate was filtered, washed with cold ether, and dissolved in 40 mL of DMF. The catalyst was filtered off and rinsed with DMF. BocAsp(Bzl)OSu (8.4 g, 20 mmol) was added followed by 7.7 mL (44 mmol) of diisopropylethylamine. After stirring at room temperature for 5 h, the solution was added slowly to 200 mL of 3% citric acid with stirring. After cooling, the precipitate was filtered, washed with water and cold ether, and dried. Yield 8.2 g (90%). Mp 148−150 °C. R_f = 0.58. ^{1}H-NMR (DMSO-d_6): δ 1.38 (s, 9H); 2.62 (m, 1H); 2.80 (m, 1H); 4.32 (d, 2H); 4.40 (m, 1H); 5.07 (s, 2H); 7.20 (d, 1H); 7.36 (s, 5H); 7.44 (m, 2H); 7.81 (m, 2H); 8.46 (t, 1H); 12.90 (s, 1H). $[\alpha]^{25}_D$ −9.08° (c = 0.760, methanol). FAB-MS [M + H]$^+$: calcd 457.2, found 457.4. Anal. Calcd for $C_{24}H_{28}N_2O_7$: C, 63.14; H, 6.18; N, 6.14. Found: C, 62.77; H, 6.08; N, 6.08.

***N*-Boc-L-Aspartyl(benzyl)-3-(aminomethyl)benzoyl-D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycine (9).** To a solution of **8** (2.29 g, 5 mmol) and pentafluorophenol (1.01 g, 5.5 mmol) in 15 mL of THF was added DCC (1.03 g, 5 mmol), and the mixture was stirred overnight. Dicyclohexylurea was filtered off and rinsed with THF, and the solvent was removed under reduced pressure. To the residue was added a solution of **6** (3.56 g, 5 mmol) in 10 mL of DMF followed by 2.1 mL (12 mmol) of diisopropylethylamine. After stirring at room temperature for 6 h, 50 mL of 5% citric acid was added followed by 80 mL of ethyl acetate. The organic phase was separated, washed with 1% citric acid and brine, dried ($MgSO_4$), and concentrated. The residue was triturated with ether−petroleum ether to give 4.8 g (92%) product. R_f = 0.43. $[\alpha]^{25}_D$ −27.89° (c = 0.760, methanol). HRMS [M + H]$^+$: calcd for $C_{53}H_{65}N_8O_{14}$ 1037.4603, found 1037.4620.

L-Aspartyl(benzyl)-3-(aminomethyl)benzoyl-D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycine TFA Salt (10). A solution of **9** (5.7 g, 5.5 mmol) in 50 mL of 50% TFA in methylene chloride was stirred at room temperature for 1 h and concentrated. The residue was triturated with cold ether, and the solid was filtered, washed with ether, and dried. Yield 5.8 g (100%). R_f = 0.14. $[\alpha]^{25}_D$ −18.24° (c = 0.740, methanol). HRMS [M + H]$^+$: calcd for $C_{48}H_{57}N_8O_{12}$ 937.4079, found 937.4096.

Cyclo[L-aspartyl(benzyl)-3-(aminomethyl)benzoyl-D-(2-Aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycyl] (11). To a solution of TBTU (963 mg, 3 mmol) in 25 mL of DMF was added slowly a solution of **10** (3.15 g, 3 mmol) in 25 mL of DMF and 1 mL (9 mmol) of *N*-methylmorpholine over a period of 1.5 h, and stirring was continued for 2.5 h. The solution was added slowly to 200 mL of 1% citric acid cooled in an ice bath, and the precipitate was filtered, washed with water and ether, and dried. Yield 2.5 g (90%). R_f = 0.52. $[\alpha]^{25}_D$ −10.75° (c = 0.456, THF). HRMS [M + H]$^+$: calcd for $C_{48}H_{55}N_8O_{11}$ 919.3999, found 919.3990.

Cyclo[L-aspartyl-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-N^{α}-methyl-L-arginylglycyl] (1). A mixture containing **11** (919 mg, 1 mmol), methanesulfonic acid (71 mL, 1.1 mmol), and 150 mg 10% palladium on carbon in 5 mL of DMF was hydrogenated at atmospheric pressure for 4 h, and the catalyst was filtered off and rinsed with DMF. The solution was added to 50 mL of acetonitrile with stirring and the precipitate was filtered and washed with ether to give 590 mg (90%) methanesulfonic acid salt of the title compound. The material was dissolved in water and the pH of the solution was adjusted to pH 7.4 by addition of ammonium hydroxide. Acetone was added to give a precipitate. Crystallization from water gave pure zwitterion product. Mp > 245 °C dec. $[\alpha]^{25}_D$ −97.57° (c = 0.740, 0.1 N HCl). FAB-MS (M + H)$^+$: Calcd 561.3; found 561.3. Anal. Calcd for $C_{25}H_{36}N_8O_7 \cdot H_2O$: C, 51.92; H, 6.62; N, 19.38. Found: C, 51.80; H, 6.56; N, 19.08.

Fmoc-L-Aspartyl(*tert*-butyl)-3-(aminomethyl)benzoic Acid (12). 3-Cyanobenzoic acid (5.88 g, 40 mmol) was suspended in 50 mL of THF and the mixture was warmed up with stirring. After all solid went into solution, 50 mL of 2-propanol was added and the solution was allowed to cool to room temperature. To it was added 4.2 mL of precooled concentrated HCl followed by 300 mg of platinum(IV) oxide. The mixture was hydrogenated at 55 psi overnight. Ether (50 mL) was added, and the precipitate was filtered, washed with ether, and dissolved in 50 mL of DMF. The catalyst was filtered off and rinsed with DMF. FmocAsp(Bu^t)OPfp (17.33 g, 30 mmol) was added followed by 11.5 mL (66 mmol) diisopropylethylamine, and after stirring at room temperature for 5 h, 200 mL 5% citric acid was added and the solution was extracted with ethyl acetate twice. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated to give a solid which was washed with ether−petroleum ether and dried. Yield 16.3 g (100%). Mp 160−163 °C. R_f = 0.57. ^{1}H-NMR (DMSO-d_6): δ = 1.35 (s, 8H); 2.48 (dd, 1H); 2.70 (dd, 1H); 4.2−4.4 (m, 6H); 7.30 (t, 2H); 7.4−7.5 (m, 4H); 7.7−7.9 (m, 7H); 8.55 (t, 1H); 12.92 (s, 1H). $[\alpha]^{25}_D$ −14.27° (c = 0.820, methanol). FAB-MS [M + H]$^+$: calcd 545.3, found 545.4. Anal. Calcd for $C_{31}H_{32}N_2O_7$: C, 68.36; H, 5.92; N, 5.14. Found C, 67.91; H, 6.10; N, 5.14.

Fmoc-L-Aspartyl(*tert*-butyl)-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-N^{α}-methyl-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycine (13). A mixture containing **12** (10.89 g, 20 mmol), pentafluorophenol (4.05 g, 22 mmol) and DCC (4.13 g, 20 mmol) in 50 mL of THF was stirred at room temperature overnight. Dicyclohexylurea was filtered off and rinsed with THF, and the filtrate was concentrated. To it was added a solution of **6** (14.25 g, 20 mmol) in 40 mL of DMF followed by 7.32 mL (42 mmol) of diisopropylethylamine. The mixture was stirred at room temperature for 4 h, insoluble material was filtered off, and the filtrate was added to 200 mL of 3% citric acid with stirring. The solution was extracted with ethyl acetate twice, and the combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was triturated with ether−petroleum ether to give 22 g (98%) product. R_f = 0.52. $[\alpha]^{25}_D$ −20.25° (c = 0.780, methanol). HRMS [M + H]$^+$: calcd for $C_{60}H_{69}N_8O_{14}$ 1125.4927, found 1125.4933.

Cyclo[L-aspartyl(*tert*-butyl)-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycyl] (14). A solution of **13** (22.5 g, 20 mmol) and 4-(dimethylamino)pyridine (14.66 g, 120 mmol) in 100 mL of DMF was stirred overnight at room temperature and added slowly to a solution of TBTU (6.42 g, 20 mmol) in 200 mL of DMF over 3 h and stirring was continued for 1 h. Ethyl acetate (1000 mL) was added and the solution was washed with 1% citric acid two times and brine three times and concentrated to dryness. The residue was taken up in THF and after filtration, the solvent was removed under reduced pressure to give a solid which was washed with ether and dried. Yield 16 g (90%). R_f = 0.52. $[\alpha]^{25}_D$ −39.06° (c = 0.640, methanol). HRMS [M + H]$^+$: calcd for $C_{45}H_{57}N_8O_{11}$ 885.4140, found 885.4147.

Cyclo[L-aspartyl-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-N^{ω},$N^{\omega'}$-bis(benzyloxycarbonyl)-L-arginylglycyl] (15). A solution of **14** (16 g, 18 mmol) in 200 mL of 50%

TFA in methylene chloride was stirred at room temperature for 1.5 h and then concentrated. The residue was triturated with ether to give 14.5 g (97%) product. FAB-MS $[M + H]^+$: calcd 829.4, found 829.1.

Cyclo[L-aspartyl(acetoxymethyl)-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-L-arginylglycyl] (16a). A mixture containing **15** (1.42 g, 1.7 mmol), bromomethyl acetate (980 mL, 10 mmol), and triethylamine (976 mL, 7 mmol) in 10 mL of DMF was stirred at room temperature overnight. Ethyl acetate was added, and the solution was washed with brine three times, dried ($MgSO_4$), concentrated. The residue was taken up in 8 mL of DMF and to it was added 0.13 mL (2 mmol) of methanesulfonic acid followed by 150 mg of 10% palladium on carbon. The mixture was hydrogenated at atmospheric pressure for 2 h, the catalyst was filtered off, and the solution was diluted with water. Purification using semipreparative reversed phase HPLC gave 650 mg (51%) of pure product. FAB-MS $[M + H]^+$: calcd 633.3, found 633.2.

Cyclo[L-aspartyl(pivaloyloxymethyl)-3-(aminomethyl)benzoyl-D-(2-aminobutyryl)-L-arginylglycyl] (16b). A mixture containing **15** (4.14 g, 5 mmol), chloromethyl pivalate (4.3 mL, 30 mmol), triethylamine (2.8 mL, 20 mmol), and NaI (4.5 g, 30 mmol) in 10 mL of DMF was stirred at room temperature for 18 h. Ethyl acetate (100 mL) was added and the solution was washed with brine three times, dried ($MgSO_4$), and concentrated. The residue was taken up in 15 mL of ethyl acetate and passed through a silica gel column using ethyl acetate–THF (1:1) as eluent to give 1.5 g of pure product. The product was dissolved in 10 mL of DMF and hydrogenated at atmospheric pressure using 10% palladium on carbon (130 mg) in the presence of methanesulfonic acid (0.1 mL) for 2 h. The catalyst was filtered off and rinsed with DMF, and the solution was diluted with water. Purification using semi-preparative reversed phase HPLC gave 1 g (26%) of pure product. FAB-MS $[M + H]^+$: calcd 675.3, found 675.3.

Synthesis of Compound 16c–j. Compounds **16c–j** were synthesized using a procedure similar to that described above for the synthesis of compound **16b**.

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Supplementary Material Available: HPLC chromatograms for **9–11** and **13–15**, and proton NMR spectra for **1**, **9–11**, and **13–15** (13 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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