The 1,1-Dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc)† Amino-Protecting Group

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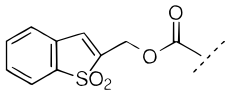
Full details are presented for use of the Bsmoc amino-protecting group for both solid phase and rapid continuous solution syntheses. Application to the latter methodology represents a significant improvement over the corresponding Fmoc-based method for rapid solution synthesis due to the opportunity to use water or saturated sodium chloride solution rather than an acidic phosphate buffer to remove all byproducts, with consequent cleaner phase separation and higher yields of the growing peptide. Comparison of the Bsmoc and Bspoc functions showed that the former, because of steric hindrance, does not suffer from the competitive or premature deblocking observed with the Bspoc system. Because of its incorporation of a styrene chromophore, resin loading of Bsmoc amino acids could be followed as has previously been shown for the Fmoc analogues. Applications of Bsmoc chemistry to peptide sequences incorporating the base sensitive Asp-Gly unit gave less contamination due to aminosuccinimide formation than comparable syntheses involving standard Fmoc chemistry because a weaker or less concentrated base could be used in the deblocking step. Experimental details are presented for building up peptides in solution via the continuous methodology. Deblockings involved the use of insoluble piperazino silica as well as the polyamine TAEA which simplified aqueous separation of the growing, but nonisolated peptide product, from excess acylating agent and other side products formed in the deblocking process. By the appropriate choice of base, one can act selectively at either site of a molecule which incorporates both β-elimination and Michael acceptor sites as protective units (Bsmoc vs Fm and Fmoc vs Bsm).

Introduction

Recently a new base-sensitive amino-protecting group, the 1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl (Bsmoc) function **1**, was described in a preliminary report.[1] In the present paper we provide new findings regarding the practical use of this system as well as full experimental details on the preparation of the key reagents needed for its application to both solid phase and solution peptide synthesis. We also update the previous lists (Tables 1 and 2) of Bsmoc-amino acids and amino acid fluorides synthesized to date and add detailed directions for the synthesis of three trifunctional acids, namely the Bsmoc derivatives of Asn and Gln which are side-chain-protected by the dimethyl(cyclopropyl)methyl residue[2] and the Pbf derivative of arginine. Finally, methods are described for the selective deblocking of systems built from 9-fluorenemethyl and benzothiophenesulfonemethyl residues.



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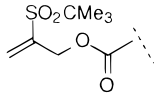

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The Question of Premature or Competitive Deblocking. The very high base-lability of the Bsmoc-relative to that of the Fmoc residue raised the question of possible premature deblocking during the coupling step. During a study of the "parent" 2-(*tert*-butylsulfonyl)-2-propenoxycarbonyl (Bspoc) residue **2**, such a reaction was noted during acylation of phenylalanine *tert*-butyl ester by means of Bspoc-Phe-Cl.[3] The more-hindered

† Common name: *b*enzo[*b*]thiophenesulfone-2-*m*ethyloxycarbonyl. Other abbreviations used: ACP = acyl carrier peptide; BOC = *tert*-butyloxycarbonyl; Bsm = 1,1-dioxobenzo[*b*]thiophene-2-ylmethyl; Bsmpip = 1,1-dioxobenzo[*b*]thiophene-2-ylmethylpiperidine; Bspoc = 2-*tert*-butylsulfonyl-2-propenoxycarbonyl; DCM = dichloromethane; DIEA = diisopropylethyl-amine; DMAP = 4-(dimethylamino)pyridine; DMDMAP = 2,6-dimethyl-DMAP; Dmcp = dimethyl(cyclopropyl)methyl; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EEDQ = 1-(ethyloxycarbonyl)-2-(ethyloxy)-1,2-dihydroquinoline; Fm = 9-fluorenylmethyl; Fmoc = 9-fluorenylmethyloxycarbonyl; HATU = N-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU = *N*[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HOAt = 7-aza-1-hydroxybenzotriazole; HOSu = *N*-hydroxysuccinimide; Marfey's reagent = 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide; Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; pGlu = pyroglutamic acid; PAC-PEG-PS = peptide acid linker on poly(ethylene glycol)/polystyrene support; TAEA = tris(2-aminoethyl)amine; TBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide; TFA = trifluoroacetic acid; TFFH = tetramethylfluoroformamidinium hexafluorophosphate; TLC = thin-layer chromatography; ToppipU = 2-(2-oxo-1(2H)-pyridyl)-1,1,3,3-bispentamethyleneuronium tetrafluoroborate; Trt = trityl = triphenylmethyl; Z = benzyloxycarbonyl.

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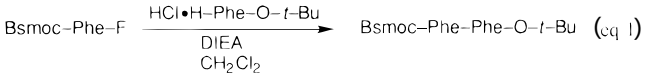
Table 1. Synthesis of Bsmoc-Amino Acids and Derivatives[a]

compound	prep method	recryst solvent[b]	specific rotation, $[\alpha_D]$, *t*, °C	mp (°C)	yield (%)[c]
Bsmoc-Gly-OH	1			168−169	88.7 (80.0)[3]
Bsmoc-Ala-OH	1		−17° (*c* 1.0 DMF), 23	98−100	86.0
Bsmoc-Val-OH	1	DCM/hexane	−9.4° (*c* 1.0 DMF), 20	155−156	89.0 (80.7)[3]
Bsmoc-Phe-OH	1	DCM/hexane	−0.37 (*c* 1.0 DMF), 20	175−176	94.0 (90.0)[3]
Bsmoc-D-Phe-OH	1	DCM/hexane	+0.37 (*c* 1.0 DMF), 20	172−173	85.0
Bsmoc-Leu-OH	1		−11.7 (*c* 1.0 EtOAc), 20	43−45	91.0
Bsmoc-Ile-OH	1		−5.0 (*c* 0.5 DMF), 23	104−106	82.0
Bsmoc-Thr(*t*-Bu)-OH	1		+3.0 (*c* 1.0 DMF), 20	163−164	93.0
Bsmoc-Aib-OH	1			149.5−151	82.8 (31)[3]
Bsmoc-Asp(O-*t*-Bu)-OH	2			foam	80.7
Bsmoc-Glu-(O-*t*-Bu)-OH	1		+6.2 (*c* 0.5 $CHCl_3$), 22	108−109	52.7
Bsmoc-Trp-OH	2		−40.4 (*c* 0.5 DMF), 22	amorphous	92.0
Bsmoc-Tyr(*t*-Bu)-OH	2	Et_2O/hexane	−33.4 (*c* 1.0 DMF), 22	133−134	92.0 (70)[1]
Bsmoc-Met-OH	2	CH_2Cl_2/hexane	+12.4 (*c* 0.5 $CHCl_3$), 22	109−111	58.0
Bsmoc-Pro-OH	2			oil	90.0
Bsmoc-Asn-OH	3	EtOH/H_2O		204−206 (dec)	90.4
Bsmoc-Gln-OH	3	EtOH		144−146	84.2
Bsmoc-Asn(Trt)-OH	2	DCM/hexane[d]	−20.0 (*c* 0.5 CMF), 20	166−167	82.8
Bsmoc-Gln(Trt)-OH	2	DCM/hexane[d]	−3.2 (*c* 0.5 DMF), 20	139 (dec)	91.0
Bsmoc-Phg-OH	1		+79.2 (*c* 0.5 DMF), 23	127.5−129	79.0 (80.0)[3]
Bsmoc-D-Phg-OH	1		−79.8 (*c* 0.5 DMF), 23	128−130	75.0
Bsmoc-Lys(Boc)-OH	1		−2.0 (*c* 1.0 EtOAc), 23	amorphous	80.0
Bsmoc-Orn(Boc)-OH	1			amorphous	80.0
Bsmoc-Ser(*t*-Bu)-OH	1			oil	75
Bsmoc-Asn-ONp	DCC	EtOH		159−160	50
Bsmoc-Gln-ONp	DCC	CH_3CN/Et_2O		144−147	63
Bsmoc-Asp(O-*t*-Bu)-OH·DCHA	−	Et_2O/Skelly B		90 dec	87
Bsmoc-Gly-Gly-OH	−	MeOH		200−203	83
Bsmoc-Gln-OPfp	DCC			115	85
Bsmoc-Asn-OPfp	DCC			138	74
Bsmoc-Gln(Dod)-OH	*e*	THF/hexane		96−98	71
Bsmoc-Asn(Dod)-OH	*e*	DCM/hexane		147−149	80
Bsmoc-Ala-NHDmcp	*f*	Et_2O/hexane		88−90	97

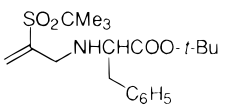
[a] All compounds were characterized on the basis of elemental analyses (±0.3%, C, H, N) and consistent IR and ^{1}H NMR spectral data. Most of these acids and intermediates are available from Oryza Laboratories, Inc., Chelmsford, MA 01824, FAX (978)-256-7434; Web: http://www.oryzalabs.com; e-mail: oryza@world.std.com. [b] All compounds were recrystallized from EtOAc/hexane unless otherwise indicated. [c] The numbers in parentheses refer to the yields of the same compounds obtained by the alternate technique indicated by the superscript. [d] In these cases a relatively small amount of hexane was used. [e] Via the acid, 4,4′-dimethoxybenzhydrol and a catalytic amount of H_2SO_4 in HOAc. [f] Via Bsmoc-Ala-F and the amine hydrochloride in the presence of DIEA in DCM. Chromatographed over SiO_2 with elution by EtOAc prior to recrystallization.

Bsmoc residue has been found not to undergo an analogous reaction. Thus HPLC examination of the reaction shown in eq 1 indicated that 98.5% of the dipeptide **4** was formed along with 1.3% of Bsmoc-Phe-OH.



3 4

Presumably the latter had been present in the original sample of **3** or had been formed by hydrolysis during the reaction or on workup of the reaction mixture. No other side product was observed whereas in the case of the Bspoc system there was evidence for the formation of 2.1% of **5** and 0.3% of **6**.[3] The best chance of observing such a side reaction would involve the least hindered amino acid



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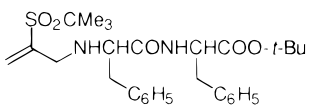

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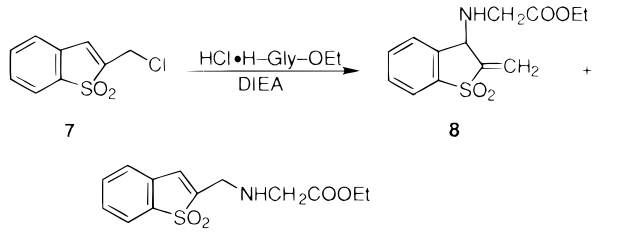
glycine. In fact, in a comparable Bsmoc test with glycine ethyl ester there was no evidence for the formation of either **8** or **9**. Authentic reference samples of these two compounds were synthesized according to eq 2. If not observed in the glycine case, such reactions are unlikely in the case of



7 8 9

(eq 2)

higher, more-hindered amino acids. Based on this result, general application of Bsmoc chemistry to solution and solid-phase peptide synthesis was deemed safe. The preliminary publication has outlined the results, and additional details are presented here.

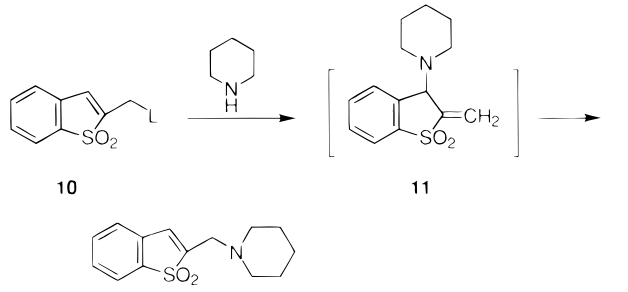
Application of Bsmoc Chemistry to Solid-Phase Syntheses. For solid-phase syntheses one could choose as a starting point a commercial resin already loaded with a particular amino acid. On the other hand it was considered of importance to learn whether Bsmoc amino acids could themselves be loaded onto normal resins by the usual techniques. In view of the stepwise nature of the deblocking process as evidenced by ^{1}H NMR examination,[4] one expects that UV analysis[5] of the deblocking by piperidine of a Bsmoc substrate will involve first a drop in absorption followed by a rise as the weak

Table 2. Synthesis of Bsmoc-Amino Acid Fluorides[a]

compound	recryst solvent[b]	mp (°C)	yield (%)
Bsmoc-Gly-F		132−133	82.7
Bsmoc-Ala-F		118−120	79.9
Bsmoc-Phe-F		foam or amorphous solid[c]	85.6
Bsmoc-D-Phe-F		foam or amorphous solid	80.0
Bsmoc-Val-F		114−115	81.7
Bsmoc-Asp(O-*t*-Bu)-F	Et_2O/hexane	107−110	79.7
Bsmoc-Glu-(O-*t*-Bu)-F		amorphous powder	75.0
Bsmoc-Lys(Boc)-F		foam	93.0
Bsmoc-Tyr(*t*-Bu)-F		foam	79.7
Bsmoc-Aib-F		126−127	66.7
Bsmoc-Leu-F		oil	90.0
Bsmoc-Ile-F		68−70	85.0
Bsmoc-Pro-F		oil	95.0
Bsmoc-Thr(*t*-Bu)-F		118−120	78.0
Bsmoc-Asn(Trt)-F		foam	88.9
Bsmoc-Gln(Trt)-F		foam	87.0
Bsmoc-Trp-F		foam	80.0
Bsmoc-Phg-F		foam	78.0
Bsmoc-D-Phg-F		foam	81.0
Bsmoc-Ser(*t*-Bu)-F		amorphous	82.0
Bsmoc-Met-F		amorphous	75.0
Bsmoc-Asn(Dmcp)-F		amorphous solid	87.8
Bsmoc-Gln(Dmcp)-F		amorphous solid	93.7

[a] All crystalline acid fluorides were characterized on the basis of elemental analyses (±0.3%, C, H, N) and consistent IR and ^{1}H NMR spectral data. Foams or amorphous solids were characterized as the methyl esters where necessary. [b] All acid fluorides were recrystallized from 20% hexane in DCM unless otherwise indicated. [c] Also obtained as a white solid, mp 132−133 °C (see Experimental Section).

chromophore of the intermediate **11** is converted to the strong styrene chromophore of the stable adduct **12**. Precisely this effect is observed as Bsmoc-Gly-O-*t*-Bu is



10 11 12 (eq 3)

treated with a low concentration, e.g., 2%, of piperidine in DCM (Figure 2a,b, Supporting Information). Because the isomerization of **11** to **12** is slow under these conditions, quantitative analysis is not possible. On the other hand if 20% piperidine in DMF is used, deblocking and subsequent isomerization to **12** is rapid. As an example, a PEG-PS resin loaded with Bsmoc-Leu-OH was treated with 20% piperidine in DMF for 30 min, leading to a calculated loading of 0.168 mequiv/g. The calculated loading did not change after the resin had been subjected to the same deblocking reagent for a period of 16 h. On the other hand, after treatment of the same resin with 2% piperidine in DMF for 5 min, the initial calculated loading was only 0.140 mequiv/g, but after the solution was allowed to stand for an additional 16 h, the loading was measured as 0.168 mequiv/g in agreement with the figure observed for the 20% piperidine run. Thus, for accurate analytical results, one must use appropriate deblocking conditions. Real time monitoring during a synthesis, without some modification of the methodology, would not be feasible in the case of deblockings carried out with low concentrations of piperidine.

Table 3. Extent of Loading and Level of Racemization for Attachment to a PAC-PEG-PS Resin[a]

coupling reagent	solvent	base (equiv)	loading (mequiv/g) (% D-form)
Bsmoc-Leu-F	DCM	3,4-lutidine (4)	0.17 (<0.1)
Bsmoc-Leu-F	DCM	collidine (4)	0.17 (<0.1)
Bsmoc-Leu-F	DCM	DIEA (4)	0.16 (0.21)
Bsmoc-Val-F	DCM	3,4-lutidine (4)	0.14 (<0.1)
Bsmoc-Val-F	DCM	collidine (4)	0.11 (<0.1)
Bsmoc-Phg-F	DCM	3,4-lutidine (4)	0.13 (13.7)
Bsmoc-Phg-F	DCM	collidine (4)	0.09 (9.5)
Bsmoc-Gly-F	DCM	collidine (4)	0.11
Bsmoc-Gly-F	DMF/DCM (1/9)	NMM (4)	0.13

[a] The resin (200 mg) was reported as having a loading of 0.24 mequiv of OH/g. All experiments were performed with 4 equiv of the amino acid derivative in 1.0 mL of solvent for reaction times of 3 h.

Table 4. Retention Times for Marfey's Adducts

adduct	retention time (min)[a]
Leu[b]	32.3 (22.8)
D-Leu	36.1 (22.7)
Phg	29.1 (22.5)
D-Phg	34.0 (22.5)
Val	26.8 (20.4)
D-Val[b]	31.5 (20.3)
piperidine	33.2 (21.8)
morpholine	24.4 (22.5)
reagent (after hydrolysis)	21.9
Asp	18.8 (21.6)
D-Asp	20.1 (21.8)
Ala	21.2 (21.1, coelutes)
D-Ala	24.4 (20.8)
Asn[c]	17.6 (20.9)
D-Asn[c]	17.6 (21.0)
Phe	32.2 (22.4)
D-Phe	35.3 (22.4)

[a] The t_R shown in parentheses is due to the hydrolysis product of Marfey's reagent which serves as a convenient t_R standard. [b] These amino acid adducts can be separated from the piperidine adduct by eluting with an isocratic system consisting of 10% MeCN/H_2O (0.1% TFA) for 5 min followed by a gradient system (10% to 60% over 55 min). [c] Asn diastereomers can be separated by isocratic elution with an aqueous buffer system involving 8% MeCN/0.02 M NaOAc, pH 4; retention time L,L = 14.8 min, D,L = 29.5 min.

Having established an analytical method to study the loading process, we examined the applicability of various bases and conditions in connection with the problem of racemization (Table 3). The levels of racemization given were determined by application of Marfey's reagent (Table 4).[6] Model studies of similar reactions carried out in solution have been published.[7] These studies showed

(4) Reference 1, Supporting Information.

(5) In view of the presence of a strong styrene chromophore in the Bsmoc residue, one would expect that methods similar to those used for the Fmoc group to follow resin loading, the coupling and deblocking steps, etc., could be used in this case as well. For the Fmoc methods, see Meienhofer, J.; Waki, M.; Heimer, E. P.; Lambras, T. L.; Makofske, R. C.; Chang, C.-D. *Int. J. Pept. Protein Res.* **1979**, *13*, 35.

(6) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591.

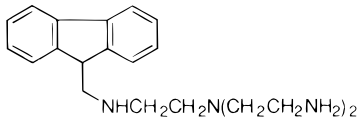
(7) Granitza, D.; Beyermann, M.; Wenschuh, H.; Haber, H.; Carpino, L. A.; Truran, G. A., Bienert, M. *J. Chem. Soc., Chem. Commun.* **1995**, 2223. It is not certain whether this technique is feasible in the case of Bsmoc amino acids, some of which, e.g., that of glycine, are sensitive to DMAP.

that both general base[8] and nucleophilic[9] catalysis were important. The importance of the latter is emphasized by the difference between 3,4-dimethyl- and 2,6-dimethylpyridine.

Having succeeded in loading appropriate resins with leucine, we first attempted the assembly of leucine enkephalin via Bsmoc chemistry. These syntheses proceeded well using *N*-[[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl]methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU)[10] as coupling reagent with a 15-min coupling time and deblocking conditions which varied from 20% piperidine in DMF for 10 min (standard Fmoc conditions) to 2% piperidine for 7 min. When the weaker base morpholine was substituted for piperidine in the deblocking step, a 5% solution in DMF gave very poor syntheses, whereas 20% morpholine in DMF gave products of acceptable purity. The ACP decapeptide, a common test sequence,[11] was assembled similarly with good results using either HATU or tetramethylfluoroformamidinium hexafluorophosphate(TFFH)[12] as coupling reagents.

Most interesting was the case of hexapeptide (H-Val-Lys-Asp-Gly-Tyr-Ile-OH) **13** [Toxin 2(1−6) of the scorpion *Androctonus australis Hector*].[13] In the preliminary communication of this work[1] it was described how the β-Asp form of this peptide[14] could be assembled by standard Fmoc and standard Bsmoc methodology with the result that the latter method gives a significantly smaller amount of the aminosuccinimide byproduct according to HPLC analysis. In the additional work described in the present paper, the α-analogue was assembled by the same two methods. Then, following Quibell et al.,[13] the Fmoc product, still attached to the resin, was treated with 20% piperidine in DMF for 19 h in order to simulate a longer synthesis (Figure 1c). The extended treatment leads to the formation of large amounts of the α- and β-piperidides and their D-isomers as well as the initial cyclization product, the aminosuccinimide derivative. Similar treatment with 20% morpholine/DMF for 19 h was unaccompanied by any such byproducts (Figure 1d). Using Bsmoc amino acid fluorides as coupling reagents with 2% piperidine/DMF, the deblocking reagent previously used in the synthesis of the β-Asp system, a small amount of the aminosuccinimide was formed (Figure 1a). Therefore Bsmoc chemistry using 20% morpholine for deblocking was examined for the assembly of **13**. The results were excellent (Figure 1b). On the other hand for the synthesis of leucine enkephalin via Bsmoc chemistry an attempt to use 2−5% morpholine/DMF gave little, if any, of the desired product. It was not determined whether the 20% morpholine system would provide a purer product than 2% piperidine when used with Bsmoc chemistry for the assembly of Field's β-isomer of **13**.[15]

Bsmoc-Based Rapid Continuous Solution Synthesis of Peptides. Previously we devised a method of using Fmoc chemistry in the rapid synthesis of short peptides in solution.[16] The key step involved removal of the TAEA deblocking byproduct **14** from the



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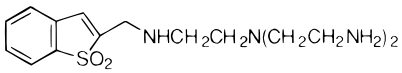

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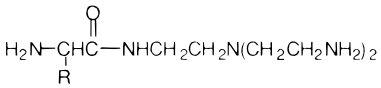
growing peptide by buffer extractions at pH 5.5 It has now been found that the process can be simplified by switching to Bsmoc chemistry since the byproduct adduct **15** formed in this case is soluble in water, thus avoiding the need for extraction with an acidic buffer. This results in fewer complications with emulsions and loss of growing peptide into the aqueous phase. A second byproduct, derived from excess acylating agent, is the amide **16** which must also be removed in the



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17

aqueous washings. In this case, solubility in water is determined by the nature of the R group. It was noted that for glutamine, if the hydrophobic trityl group is used for N-amide side chain protection, the resulting amide (**16**, R = $CH_2CH_2CONHC(C_6H_5)_3$) is not completely removed in the washing process.[17] Water solubility is enhanced, however, by switching to the lower-molecular-weight, less-hydrophobic dimethylcyclopropylcarbinyl function **17**[2] for side chain amide protection (**16**, R = $CH_2CH_2CONHC(C_3H_5)Me_2$). Results for the asparagine analogue (**16**, R = $CH_2CONHC(C_3H_5)Me_2$) are comparable, and similar solutions are being sought for the His

(8) (a) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1961**, *83*, 1743. (b) Bondarenko, L. I.; Kirichenko, A. I.; Litvinenko, L. M.; Dmitrenko, I. N.; Kobets, V. D. *Zh. Org. Khim.* **1981**, *17*, 2588.

(9) Fersht, A. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1970**, *92*, 5432.

(10) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397. (b) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201. (c) For a description of the current view of the structure of such coupling reagents, see: Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 3561.

(11) (a) For a brief review of the use of the acyl carrier protein decapeptide (65−74) as a test sequence for solid phase assembly, see: Adams, J. H.; Cook, R. M.; Hudson, D.; Jammalamaddaka, V.; Lyttle, M. H.; Songster, M. F. *J. Org. Chem.* **1998**, *63*, 3706, footnote 21. (b) Reference 10b.

(12) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401.

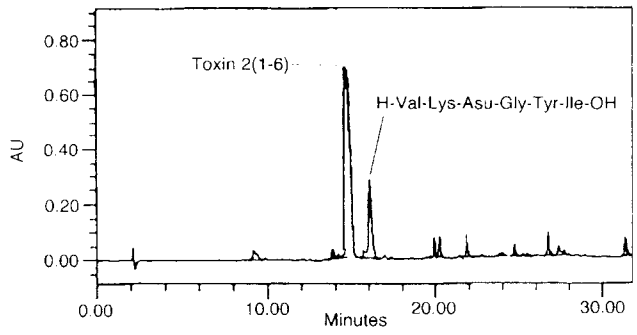
(13) Quibell, M.; Owen, D.; Packman, L. C.; Johnson, T. *J. Chem. Soc., Chem. Commun.* **1994**, 2343.

(14) Lauer, J.; Fields, C. G.; Fields, G. B. *Lett. Pept. Sci.* **1994**, *1*, 197.

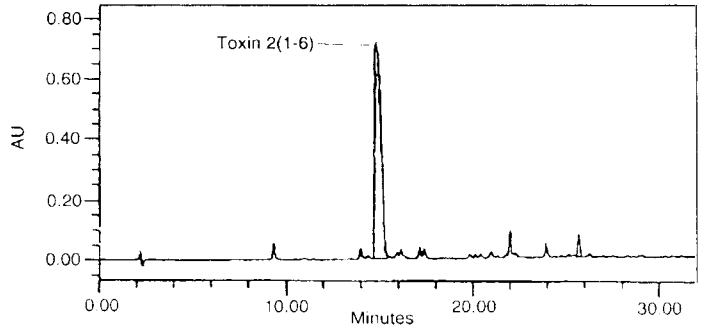
(15) For recent alternative approaches to minimize β-catalyzed aspartimide formation during Fmoc assembly and references to earlier work, see: (a) Karlström, A.; Undén, A. *Int. J. Pept. Protein Res.* **1996**, *48*, 305. (b) Zhang, H.; Wang, Y.; Voelter, W. *Chem. Lett.* **1995**, 1111. (c) Yang, Y.; Sweeney, W. V.; Schneider, K.; Thörquist, S.; Chait, B. T.; Tam, J. P. *Tetrahedron Lett.* **1994**, *35*, 9689. (d) Dölling, R.; Beyermann, M.; Haenel, J.; Kernchen, F.; Krause, E.; Franke. P.; Brudel, M.; Bienert, M. *J. Chem. Soc., Chem. Commun.* **1994**, 853 and ref 13.

(16) Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673.

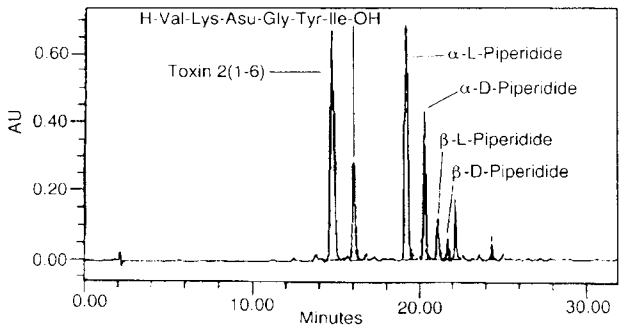
(17) On the other hand, Bsmoc-Gln(Trt)-OH has previously been used successfully in rapid solution syntheses (see refs 1 and 2 and the synthesis of heptapeptide **23** described herein), possibly because earlier syntheses have involved a greater excess of TAEA than needed or used in current work. The presence of excess TAEA modifies the solvent properties of the aqueous phase during the extraction process but may also serve to remove some of the desired growing peptide as well as the byproducts. It should be noted that structures **14**−**16** are assigned on the basis of analogy to the corresponding products derived from piperidine. None of these materials has been isolated and characterized unequivocally.



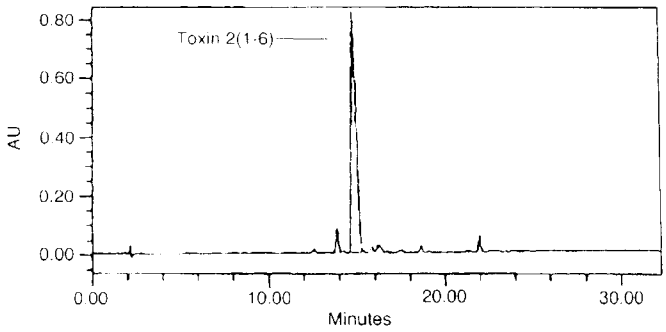
(a) Toxin #2 assembled via Bsmoc-AA-F after an additional 19-h treatment with 2% piperidine/DMF.



(b) Toxin #2 assembled via Bsmoc chemistry using 20% morpholine/DMF.



(c) Toxin #2 assembled via Fmoc/HATU after an additional 19-h treatment with 20% piperidine/DMF. α-Piperidides = H-Val-Lys-NHCH(CONC$_5$H$_{10}$)CH$_2$CO-Gly-Tyr(*t*-Bu)-Ile-OH, β-Piperidides = H-Val-Lys-NHCH(CH$_2$CONC$_5$H$_{10}$)CO-Gly-Tyr(*t*-Bu)-Ile-OH.



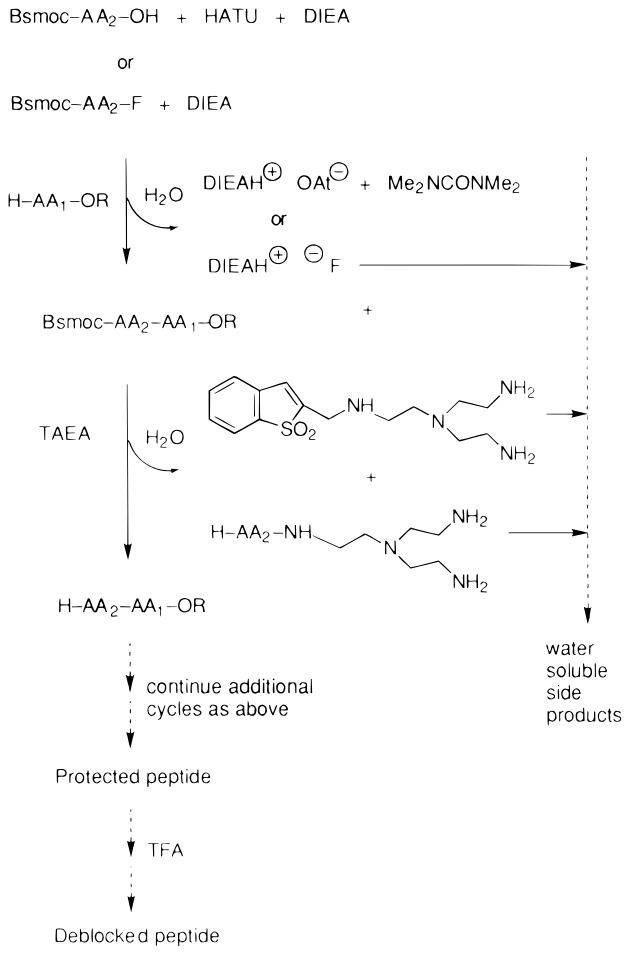
(d) Toxin #2 assembled via Fmoc/HATU after an additional 19-h treatment with 20% morpholine/DMF.

Figure 1.

and Cys cases. An outline of the Bsmoc rapid solution methodology is given in Scheme 1.

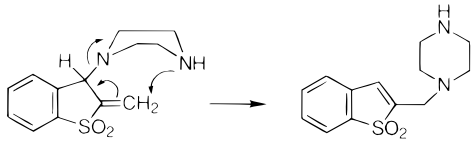
As noted earlier[1] the Bsmoc deblocking process is easily monitored by ^{1}H NMR techniques. The methylene pro-

Scheme 1. Bsmoc Rapid Continuous Solution Synthesis of Peptides



tons adjacent to the 2-position of the benzothiophene-sulfone ring show chemical shifts in the range 5.0−5.2 ppm. Following the initial deblocking step, conversion to the vinyl intermediate **11** is signaled by a shift of these protons to 6.3−6.5 ppm. As this intermediate is isomerized to **12**, the new methylene protons, being now adjacent to nitrogen, are shifted upfield to 3.4−3.6 ppm. Thus both deblocking and rearrangement steps are easily monitored separately by using a limited amount (e.g., 2 equiv) of the deblocking base. To gain a general picture of the ease of the deblocking process, a number of simple deblocking amines was examined, and the following reactivity order observed: piperidine > piperazine > morpholine ≈ ethanolamine.[18] The deblocking rates in DMF roughly parallel the pK_a values for these amines except in the case of ethanolamine (pK_a 9.51) which was only about as effective as morpholine (pK_a 8.33) despite its greater basicity. A possible explanation is the increased rate of addition to a Michael acceptor of a secondary vs a primary amine.[19] As previously suggested, this effect may be the result of steric effects due to increased hydrogen bonding in the case of the primary system.

The difference between piperidine and piperazine is particularly interesting in that the former is more basic and as expected causes deblocking at a significantly higher rate whereas just the opposite effect is seen for the rearrangement step. A possible rationale is that the piperazine adduct **19** is extremely insoluble in DMF, and its precipitation may drive the reaction to completion. A similar solubility effect has previously been observed for the corresponding piperazine adduct formed upon Fmoc deblocking.[20] In addition, or alternatively, conversion of the initial adduct **18** to **19** may be accelerated via the cyclic transition state implied in **18**.



18 19

Assembly of the Octapeptide LED-CC-II. Although the piperazine-induced deblocking/rearrangement process described above could be of special use for Bsmoc deblocking, initial studies were carried out with TAEA since good results had previously been obtained with this base in the Fmoc case. In the preliminary report[1] of this work, application of the TAEA deblocking technique to a number of short peptides was described. Here experimental details are presented for application to an octapeptide **20** (*p*Glu-Leu-Thr-Phe-Thr-Pro-Asn-Trp-NH_2), one among many related insect hormones.[21] Application of the Bsmoc protocol proceeded well except at the stage of the heptapeptide where unacceptable losses of the Bsmoc-deblocked, free-amino-containing intermediate into the aqueous phase occurred. To avoid the need for aqueous extractions at this step, an insoluble piperazino-substituted silica reagent[20] **24** was substituted for TAEA. In this way simple filtration and evaporation of solvent gave the free-amino segment **25** ready for the next, in this case final, step. The complete outline is presented in Scheme 2.

Since the octapeptide occurs as a peptide amide, the assembly was started with the C-terminal function of tryptophan amide modified as the *N*-dimethylcyclopropylmethyl (Dmcp) derivative[2] in order to maintain solubility of the growing peptide in the nonpolar, water-immiscible solvent dichloromethane used in the extraction procedures needed to effect byproduct removal. Two different methods were used to assemble the chain, each being equally satisfactory. In one case isolated amino acid fluorides[22] were used throughout for coupling. In a second run, the HATU reagent[10a,b] was used for the first seven coupling steps, and when the switch was made from TAEA to piperazino silica as deblocking agent, the appropriate acid fluoride was substituted. Prior to our extensive use of Bsmoc amino acid fluorides for coupling purposes it was considered important to determine whether any racemization might occur during the coupling process. Although many other urethane-protected amino acid fluorides (Fmoc, Z, Boc) have been used without any problems, the Bsmoc group is unique in incorporating a residue, the sulfone function, which

(18) The pK_a values, taken from Perrin, D. D. *The Dissociation Constants of Organic Bases*; Butterworths: London, 1965, are piperidine (11.12), piperazine (9.81, 5.55), morpholine (8.33), and ethanolamine (9.51).

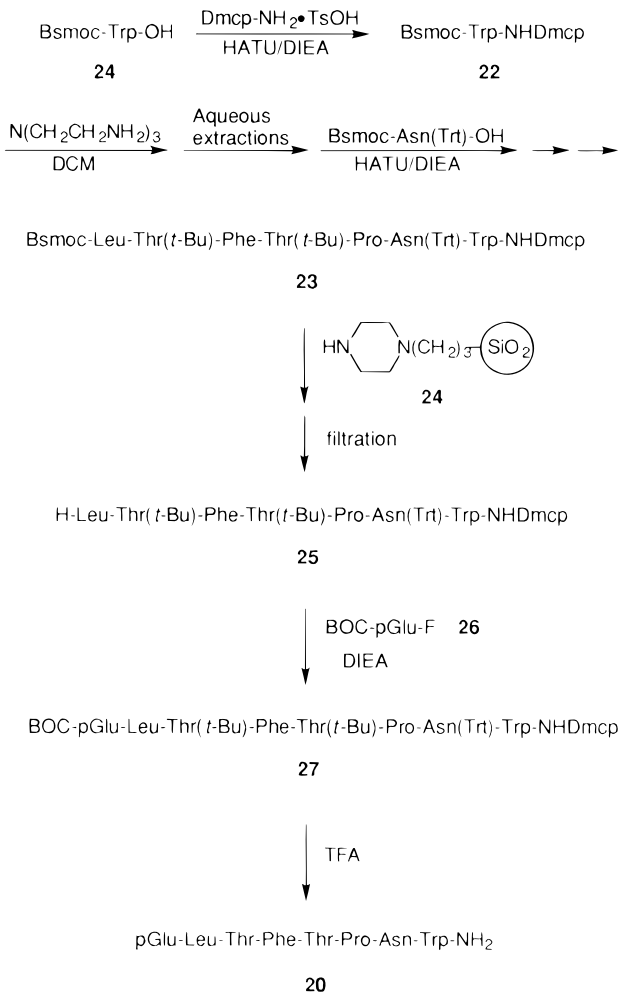
(19) McDowell, S. T.; Stirling, C. J. M. *J. Chem. Soc. (B)* **1967**, 343.

(20) Carpino, L.A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666.

(21) Gaede, G.; Keller, R. *Peptides* **1989**, *10*, 1287.

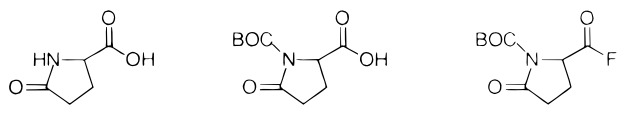
(22) Compare: (a) Carpino, L. A.; Sadat-Aalaee, D.; Chao, H.-G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651. (b) Carpino, L. A.; Mansour, E. M. E.; Sadat-Aalaee, D. *J. Org. Chem.* **1991**, *56*, 2611.

Scheme 2



exhibits a high electron-withdrawing inductive effect which might conceivably promote base-catalyzed abstraction of the proton α- to the carbonyl fluoride residue with consequent loss of configuration.[23] In fact, coupling of the highly sensitive amino acid, α-phenylglycine, as its acid fluoride with alanine methyl ester occurred without any loss of configuration according to ^{1}H NMR analysis of the methyl ester peaks of the two expected diastereomers. It is not expected therefore that other less-sensitive amino acids would be at risk. Loss of configuration is even less likely in the presence of coupling reagents such as HATU or HBTU.

The protected heptapeptide **23**, assembled in a single series of steps over a period of 7 h, was obtained in a yield of 37% for the purified peptide (column chromatography). Piperazino silica deblocking of **23** gave the free-amino segment **25**, ready for the final coupling. The first approach to introduce the N-terminal amino acid, pyroglutamic acid, involved prior conversion of the acid **28** to



28 29 26

its acid fluoride. Even though it is internally "protected" as a lactam, attempts to prepare the acid fluoride of **28** were unsuccessful. On the other hand the corresponding Boc derivative **29**[24] was easily converted to the acid fluoride **26** by a modification of the standard procedure. Following the directions of Schröder and Klieger, Boc-pyroglutamic acid was synthesized in the form of its crystalline dicyclohexylamine salt. Since the technique described for converting the salt to the free acid invariably gave an oil which could not be crystallized, direct conversion of the salt to the acid fluoride was examined. In fact, despite its potential, as a secondary amine, to destroy the acid fluoride, dicyclohexylamine, presumably because of its exceptionally hindered structure, did not in fact interfere in the reaction. Boc-pyroglutamic acid fluoride **26** was obtained in a yield of 85%. The same technique, using the dicyclohexylamine salt of Fmoc-Phe-OH, was shown to be applicable to Fmoc-Phe-F suggesting its probable generality. Treatment of the free-amino heptapeptide **25** with acid fluoride **26** gave somewhat unstable protected octapeptide **27** in 58% yield. For proof of structure, a freshly prepared sample of **27** was purified by silica gel chromatography and then subjected to trifluoroacetic acid deblocking. The free octapeptide **20** showed a similar instability although a freshly prepared sample could be characterized by HPLC, amino acid analysis, and mass spectral examination. Although only used in one step of the present synthesis, the technique involving resin-based deblocking promises to be generally applicable to every step, and if the method is modified to include resin-based coupling for each amino acid, a contemporary solution to the problem of clean "two polymer"[25] or inverse Merrifield synthesis of peptides could be contemplated.

An important aspect of the use of acid-sensitive amino protecting groups is the opportunity to distinguish some such functions on the basis of the acidity of the deblocking reagent. Thus, under appropriate conditions, one can easily remove trityl protection in the presence of a *tert*-butyl-based system.[26] In the preliminary description of the present work it was similarly demonstrated that two base-sensitive groups could be distinguished. Thus the Bsmoc group could be deblocked in the presence of an Fm ester residue. Full experimentatal details for assembly of Bsmoc-Leu-Leu-Leu-OFm from Bsmoc-Leu-F and H-Leu-OFm are appended here. For the dipeptide Bsmoc-Leu-Phe-OFm, experimental conditions are given for the selective deblocking of either the Bsmoc- or the Fm-residue.

Another example, not previously discussed, based on the reverse of the selectivity outlined in the preliminary report, namely removal of Fmoc protection in the presence of a Bsm ester residue, is presented here.

Preliminary experiments with various simple amines allowed the identification of hindered amines which could effect β-elimination of the Fmoc residue under conditions which avoided extensive Michael-like addition to the Bsm residue. The most effective reagents were *tert*-butylamine and *N*-methyl-*tert*-butylamine (see Table 5). The latter was more selective with less than 3% removal of the Bsm

(23) Compare: Carpino, L. A.; Ionescu, D.; El-Faham, A.; Henklein, P.; Wenschuh, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **1998**, *39*, 241. The increased reactivity of Bsmoc amino acids over the Fmoc analogues has been attributed to a similar effect.[1,4]

(24) Schröder, E.; Klieger, E. *Liebigs Ann. Chemie* **1964**, *673*, 196.

(25) Carpino, L. A.; Cohen, B. J.; Lin, Y.-Z.; Stephens, K. E., Jr.; Triolo, S. A. *J. Org. Chem.* **1990**, *55*, 251.

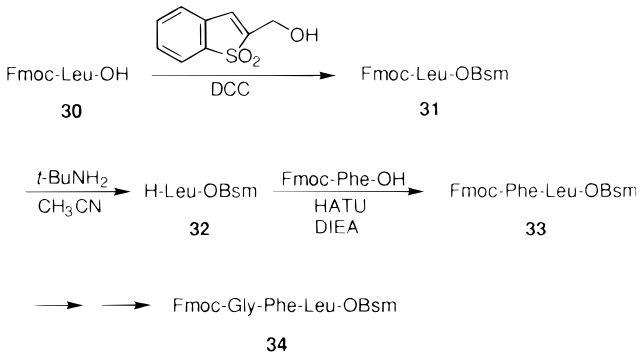
(26) Barlos, K.; Chatzi, O.; Gatos, D.; Stauropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513.

Table 5. Deblocking of Fmoc-Phe-Leu-OBsm to H-Phe-Leu-OBsm[a]

deblocking amine/solvent	time for complete Fmoc deblocking (min)	deblocking at both Fmoc and Bsm sites (%)
10% Et_2NH/CH_3CN	30	48
10% *N*-Methyl-*N*-cyclohexylamine/CH_3CN	30	10
20% Me_2NEt/DMF	90	26
20% Me_2NiPr/DMF	90	19
30% *t*-$BuNH_2/CH_3CN$[b]	8	5.8
20% *tert*-amylNH_2/CH_3CN	60	38
20% *t*-BuNHMe/DMF	5	2.6
30% *t*-BuNHMe/DMF	5	2.8

[a] To 0.5 mL of the base/solvent combination was added 34 mg of the protected dipeptide and the progress of the reaction followed by HPLC analysis, linear gradient 10/90 in 20 min with H_2O/CH_3CN/0.1% TFA, retention times: Fmoc-Phe-Leu-OBsm 20.1 min, Fmoc-Phe-Leu-OH 17.1 min, H-Phe-Leu-OBsm 11.9 min, H-Phe-Leu-OH 7.8 min. [b] The *t*-$BuNH_2$ was a sample from Aldrich Chemical Co. of 99.5% purity. Less pure samples (98%) gave a less sharp distinction between the two base-sensitive sites.

Scheme 3



residue occurring after the 5-min deblocking time which completely removed the Fmoc residue. Although potentially an inexpensive reagent, *N*-methyl-*tert*-butylamine is currently not widely available commercially. *tert*-Butylamine, on the other hand, is an inexpensive reagent and was used in the present studies, although somewhat less than 6% of the Bsm residue was removed during deblocking of the Fmoc residue. Other amines of greater selectivity are being sought. Unsuccessful were *N*,*N*-diethyl- and *N*,*N*-diisopropylamine as well as *tert*-amylamine.

As an example of the practical utility of this methodology, leucine enkephalin C-terminal tripeptide **34** can be assembled as outlined in Scheme 3.

During the synthesis of **34**, protected dipeptide **33** was isolated and purified before going to the next step. If one attempts to add a second glycine residue to **34** to give the corresponding tetrapeptide without purification of the tripeptide, because of the workup method used (see Experimental Section), some residual unreacted Fmoc-Gly-OH remains which then forms a nonvolatile salt with *tert*-butylamine, and upon addition of the coupling reagent some Fmoc-Gly-NH-*t*-Bu is formed to contaminate the desired tetrapeptide. A more hydrophobic Bsm derivative is being sought which would allow an aqueous workup step to remove byproducts and excess *tert*-butylamine, thus allowing a continuous synthesis without any isolation step.

Conclusion

This work has described a new base-sensitive protecting group, the Bsmoc residue, for which the deblocking step involves addition of an amine to a Michael acceptor site. Relative to the less-hindered Bspoc residue, introduced in the accompanying paper,[3] no premature deblocking has been noted. Applications of this protectant are described for both solid phase and rapid continuous solution synthesis. For the latter system the Bsmoc methodology shows advantages over the Fmoc system in that it is simpler, cleaner, and provides higher yields. Further advantages of Bsmoc chemistry are apparent in cases where byproduct formation is caused by base-catalyzed processes. Finally it is shown that Fm- and Bsm-based protecting groups can be orthogonally deblocked by appropriate choice of the deblocking base.

Experimental Section

Benzothiophene-2-methanol. Benzothiophene (45.5 g, 0.34 mol) was dissolved in 225 mL of dry THF and the solution cooled to −30 °C under an atmosphere of N_2. *n*-BuLi (330 mL, 1.6 M in hexane, 1.5 equiv) was added dropwise over 2 h with mechanical stirring. The dark blue solution was allowed to come to 0 °C and then recooled to −78 °C. Paraformaldehyde (72 g, 2.4 mol) was added in small portions over 20 min, and the mixture was allowed to warm to room temperature overnight. *Caution*! A considerable amount of gas is evolved on warming especially when the temperature reaches 10−20 °C. The apparatus should be well-ventilated and stirring maintained, otherwise the mixture may boil uncontrollably. The mixture was acidified to pH about 3 with 3 N hydrochloric acid, the layers were separated, and the aqueous phase was extracted twice with 100-mL portions of ether. The solid remaining in the flask was washed with three 150-mL portions of ether, all ether extracts were combined and dried over $MgSO_4$, and the solvent was removed by rotary evaporation. The residual oil was dissolved in 600 mL of DCM and the solution washed with saturated $NaHCO_3$ (2 × 100 mL), saturated NaCl (100 mL), and dried over $MgSO_4$. The solution was filtered, and the solvent was removed to give an oil which was recrystallized from DCM/hexane (4:1) to give 42.4 g (70%) of the alcohol as a white solid, mp 68−70 °C (lit.[27] mp 99−100 °C); IR (KBr): 3277, 1457 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.24 (s, 1), 4.88 (s, 2), 7.17−7.27 (m, 5). The same alcohol was obtained in 63% yield, mp 99−100 °C, by treatment of 2-lithiobenzothiophene with *N*,*N*-dimethylformamide followed by reduction with sodium borohydride in MeOH. Presumably the two forms, mp 68−70 °C and 99−100 °C, are polymorphic forms, as the two give identical IR and ^1H NMR spectra. The latter method was based on a literature procedure.[28]

Benzothiophenesulfone-2-methanol. The previous description of the oxidation of benzothiophene-2-methanol involved use of magnesium bismonoperoxyphthalic acid. More recently it has been shown that the method of McKillop and Kemp[29] involving sodium perborate is simpler and less expensive. A general procedure involves portionwise addition over a period of 20 min of 95.4 g (0.62 mol) of sodium perborate tetrahydrate to a stirred solution of 0.124 mol of benzothiophene-2-methanol in 500 mL of acetic acid held at 45−50 °C. Stirring was continued at this temperature until completion of the oxidation (TLC). The acetic acid was removed by evaporation under reduced pressure, and the residue was stirred with 150 mL of water. The precipitated sulfone alcohol was collected by filtration and recrystallized from chloroform/hexane or dichloromethane/hexane to give the sulfone alcohol in about 75% yield. Prior to the development of this general

(27) Blicke, F. F.; Sheets, D. G. *J. Am. Chem. Soc.* **1949**, *71*, 2856.
(28) Takeshita, H.; Mametsuka, H.; Motomura, H. *J. Heterocycl. Chem.* **1986**, *23*, 1211.
(29) McKillop, A.; Kemp, D. *Tetrahedron* **1989**, *45*, 3299.

method, a small scale test of a similar procedure, but without removal of acetic acid prior to workup, showed that the benzothiophenesulfone alcohol was obtained in a yield of about 60%. It is expected that the yield will be improved upon following the general procedure described. The alcohol was obtained after recrystallization from $CHCl_3$/hexane (4:1) in the form of white crystals, mp 113−114 °C; IR (KBr): 3358, 1289, 1148 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.86 (bs, 1), 4.69 (s, 2), 7.05−7.71 (m, 5). Anal. Calcd for $C_9H_8O_3S$: C, 55.09; H, 4.11; S, 16.33. Found: C, 54.86; H, 4.11; S, 16.21.

Benzothiophenesulfone-2-methyl Chloroformate. To a stirred solution of 30.8 g (0.16 mol) of benzothiophenesulfone-2-methanol in 400 mL of dry THF cooled to −30 °C in a dry ice−acetone bath was added in one portion 104 g (80 mL, 1.05 mmol) of phosgene which had been condensed into a graduated cylinder. The solution was allowed to come to room-temperature overnight. Excess phosgene and solvent were removed with the aid of a water aspirator, the process being repeated using additional solvent: 200 mL of THF, 200 mL of THF−ether (1:1), and 200 mL of ether. Recrystallization of the residue from dry ether gave 36.1 g of the chloroformate as white crystals, mp 76−77 °C; IR (KBr): 1772 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.3 (d, 2), 7.29 (bs, 1), 7.3−7.89 (m, 4). The chloroformate is unstable on TLC plates (SiO_2). Anal. Calcd for $C_{10}H_7ClO_4S$: C, 46.43; H, 2.73; Cl, 13.71. Found: C, 46.42; H, 2.69; Cl, 13.82.

***N*-(Benzothiophenesulfone-2-methyl)-*N*-succinimidyl Carbonate.** Bsmoc-Cl (8.98 g, 34.8 mmol) was dissolved in 100 mL of dry DCM. The dicyclohexylamine salt of *N*-hydroxysuccinimide[30] (10.3 g, 34.8 mmol) was added with stirring to the solution which was protected from moisture ($CaSO_4$). After stirring for 20 h, the solution was filtered and the amine salt washed with two 75-mL portions of DCM. The combined organic filtrates were washed with saturated $NaHCO_3$ (2 × 100 mL) and saturated NaCl (2 × 100 mL), dried ($MgSO_4$), filtered, and treated with decolorizing carbon. Filtration and evaporation of solvent gave 9.6 g (81%) of the crude carbonate which on recrystallization from DCM/hexane or EtOAc/hexane gave 8.6 g (74%) of the carbonate as white crystals, mp 170−172 °C; IR (KBr): 1818, 1790, 1742 cm^{-1}; ^1H NMR ($CDCl_3$ + TFA): 2.87 (s, 4), 5.45 (d, 2), 7.73 (m, 5). Anal. Calcd for $C_{14}H_{11}NO_7S$: C, 49.85; H, 3.28; N, 4.15. Found: C, 49.61; H, 3.28; N, 4.12.

Benzothiophenesulfone-2-methyl *N*-(*p*-Chlorophenyl)-carbamate. To a solution of 1 g (3.87 mmol) of Bsmoc-Cl in 20 mL of dry DCM was added at 0 °C portionwise 0.98 g (7.74 mmol) of *p*-chloroaniline. A white precipitate separated at once. After addition was complete, the mixture was stirred at 0 °C for 10 min and for 2 h at room temperature. The white precipitate was filtered and washed with DCM. The combined DCM solution was washed with two 25-mL portions of 5% HCl followed by 25 mL of water and dried over $MgSO_4$. After filtration, evaporation, and recrystallization from DCM/hexane there was obtained 1.15 g (85%) of the carbamate as a white solid, mp 165−166 °C; IR (KBr): 3339, 1700, 1305, 1151 cm^{-1}; ^1H NMR ($CDCl_3$/DMSO-d_6): δ 5.25 (s, 2), 7.22−7.76 (m, 9), 9.96 (bs, 1). Anal. Calcd for $C_{18}H_{12}ClNO_4S$: C, 54.94; H, 3.46; N, 4.00; Cl, 10.13. Found: C, 54.74; H, 3.35; N, 3.90; Cl, 10.35. The same compound was obtained in 88.6% yield from *p*-chlorophenyl isocyanate and benzothiophenesulfone-2-methanol in benzene after 20 h of refluxing.

***N*-(Benzothiophenesulfone-2-methoxy)succinimide.** 2-(Chloromethyl)benzothiophenesulfone (310 mg, 1.46 mmol) was dissolved in 4 mL of DMF and 0.3 mL (1.72 mmol) of DIEA, 0.23 g (1.96 mmol) of N-hydroxysuccinimide was added, and the mixture was stirred for 3.5 h. Dilution with 80 mL of water gave a solid which was filtered and washed with H_2O, MeOH−H_2O, and acetone to give 278 mg (65%) of the succinimide as white crystals, mp 234−235 °C, IR (KBr): 3490, 3049, 2991, 2951, 1710, 1451, 1373, 1305, 1210, 1190, 1148, 1108, 1076; ^1H NMR ($CDCl_3$ + TFA): δ 2.81 (s, 4), 5.05−5.2 (d, 2), 7.2−7.9 (m, 5). The analytical sample was recrystallized from nitromethane, mp 234−235 °C. Anal. Calcd for $C_{13}H_{11}NO_5S$: C, 53.32; H, 3.78; N, 4.78. Found: C, 53.13; H, 3.78; N, 4.85.

***N*-(Benzothiophenesulfone-2-methyl)piperidine (12).** To an ice-cold solution of Bsmoc-Cl (1.22 g, 5 mmol) in 15 mL of DMF was added piperidine (1.5 mL, 15 mmol) dropwise over several minutes. After stirring for 1 h, the mixture was poured slowly into 150 mL of cold water. The resulting precipitate was washed with water and recrystallized from MeOH to give 473 mg (36%) of the piperidine derivative as white crystals, mp 122.5−124.5 °C (lit.[31] mp 124−126 °C); ^1H NMR ($CDCl_3$): δ 1.3−1.9 (m, 6), 2.3−2.7 (m, 4), 3.4−3.6 (d, 2), 6.85−7.10 (t, 1), 7.15−7.9 (m, 4); UV (MeOH) 310.5 nm (log ϵ 3.484), (DCM) 311.5 nm (log ϵ 3.454).

General Procedures for the Preparation of Benzothiophenesulfone-2-methoxycarbonyl Amino Acids. For Availability See Footnote a, Table 1. (Mtd 1) Bolin Technique[32] via Bsmoc-Cl. To a suspension of 3.87 mmol of an amino acid in 20 mL of DCM was added in one portion 0.98 mL (7.73 mmol) of chlorotrimethylsilane. The mixture was then refluxed for 1 h and cooled in an ice bath. Diisopropylethylamine (1.3 mL, 7.31 mmol) was added slowly followed by 1 g (3.87 mmol) of benzothiophenesulfone-2-methyl chloroformate. The reaction mixture was allowed to stand at 0 °C for 20 min and then for 1−1.5 h at room temperature. The solvent was removed in vacuo and the resulting oil distributed between 40 mL of ether and 80 mL of 2.5% $NaHCO_3$ solution. The combined aqueous layers were acidified to pH 2 with concentrated HCl and extracted with three 30-mL portions of EtOAc. The extracts were combined and washed with 30 mL of saturated NaCl and 30 mL of water, dried over $MgSO_4$, and filtered, and solvent was evaporated. The white residue or oil was recrystallized from the appropriate solvent or solvent mixture to give the corresponding Bsmoc-protected amino acids. See Table 1.

(Mtd 2) Active Ester Technique via Bsmoc-OSu in Acetonitrile−Water in the Presence of Triethylamine. To a suspension of 2.96 mmol of the amino acid in 20 mL of acetonitrile/water (1/1) was added an amount of triethylamine to give an apparent pH of 9.0. The suspension became clear. The pH was kept at 8.5−9.0, after the addition of 1 g (2.96 mmol) of Bsmoc-OSu, by adding triethylamine. The uptake of base ceased after about 15 min. The reaction mixture was stirred at room temperature for 40−45 min, acidified to pH 5 with 0.1 N HCl or 10% $KHSO_4$, and concentrated in vacuo. The mixture was diluted with about 5 mL of water and acidified to pH 2 with 0.1 N HCl. The mixture was extracted with four 25-mL portions of ethyl acetate. The combined ethyl acetate extracts were washed several times with saturated NaCl solution, dried over $MgSO_4$, and filtered, the solvent was removed in vacuo, and the residue was treated as in method 1.

(Mtd 3) Active Ester Technique via Bsmoc-OSu in Acetone−Water in the Presence of Sodium Bicarbonate. To a solution of the amino acid (1.48 mmol) and $NaHCO_3$ (2.95 mmol) in 10 mL of water was added a solution of benzothiophenesulfone-2-methyl *N*-succinimidyl carbonate (1.48 mmol) in 10 mL of acetone. The reaction mixture was stirred overnight at room temperature, diluted with water, and extracted twice with DCM to remove a small amount of benzothiophenesulfone-2-methanol and unreacted Bsmoc-OSu. The aqueous layer was cooled in an ice bath and acidified with concentrated HCl to pH 2. The resulting white precipitate or oil was extracted with three 25-mL portions of ethyl acetate. The combined organic layer was washed with 30 mL of saturated NaCl solution and 30 mL of water and dried over $MgSO_4$, the solvent was removed in vacuo, and the residue was treated as in method 1.

General Procedure for Preparation of *N*-(Benzothiophenesulfone-2-methoxycarbonyl)amino Acid Fluorides. A solution (or suspension) of Bsmoc-amino acid (1 mmol) in dry DCM (10 mL) was treated under a nitrogen atmosphere

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with cyanuric fluoride (295 μL, 3.5 mmol) and pyridine (80.8 μL, 1 mmol). The reaction mixture was stirred at room temperature for 1−1.5 h. During the reaction, a white precipitate separated. The reaction mixture was extracted with two 20-mL portions of ice−water. The organic layer was dried over $MgSO_4$ and filtered and solvent removed to give a residue which was crystallized from DCM/hexane to give the corresponding Bsmoc-amino acid fluoride. The purity of the acid fluorides was easily checked by converting them to their methyl esters with dry methanol and checking the product by TLC. Exceptions to this general procedure were made in the cases of Bsmoc-Asn(Trt)-F and Bsmoc-Gln(Trt)-F. In both of these cases it was necessary to use 2 equiv of pyridine and a lower temperature (−20 °C). Unfortunately, some of the Bsmoc-amino acid fluorides were not easily crystallized. In such cases after removal of the DCM, dry ether was added, the ether removed in vacuo, and the resulting foam (oil) dried in a vacuum pump for 24 h prior to storage. Storage vials were flushed with dry N_2 and placed in capped bottles flushed with dry N_2 containing a desiccant ($CaSO_4$ or P_2O_5). Failure to dry these materials well leads to formation of the corresponding free acid on attempted long-term storage. See Table 2.

***N*-(Benzothiophenesulfone-2-methoxycarbonyl)phenylalanine *tert*-Butyl Ester.** A solution of 0.5 g (1.93 mmol) of benzothiophenesulfone-2-methyl chloroformate and 0.497 g (1.93 mmol) of *tert*-butyl phenylalaninate hydrochloride in 15 mL of DCM was stirred with 0.324 g of $NaHCO_3$ in 10 mL of water at room temperature for 3 h. The aqueous phase was separated, and the organic phase was washed with two 30-mL portions of 5% HCl. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed in vacuo to give a colorless oil which was recrystallized from MeOH/H_2O to give 0.708 g (82.8%) of the phenylalanine derivative as white crystals, mp 112.5−114 °C; IR (KBr): 3421, 1714, 1313, 1155 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.39 (s, 9), 3.06 (d, 2), 4.49 (m, 1), 5.07 (d, 2), 5.38 (d, 1), 7.07−7.70 (m, 10); [α] −15.2° (c = 1, DMF). Anal. Calcd for $C_{23}H_{25}NO_6S$: C, 62.29; H, 5.68; N, 3.16. Found: C, 62.34; H, 5.64; N, 3.13.

Deblocking of Bsmoc-Phe-OCMe$_3$. A solution of 1 g (2.25 mmol) of *tert*-butyl benzothiophenesulfone-2-(methoxycarbonyl)phenylalaninate in 10 mL of 50% DCM/TFA was stirred at room temperature for 4 h. Excess TFA and solvent were removed in vacuo from a water bath at 45 °C. The resulting oil was recrystallized from DCM to give 0.739 g (84.9%) of the colorless acid. The melting point and spectral data (IR, ^1H NMR) obtained for this material agreed with that obtained directly from phenylalanine (see Table 1).

Methyl Benzothiophenesulfone-2-(methoxycarbonyl)-phenylglycylalaninate. Prepared from Bsmoc-Phg-F as described in the Supporting Information for the Phe-Ala analogue in 90% yield, mp 179−180 °C; IR (KBr): 3327, 1736, 1654, 1307, 1152 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.37 (d, 3), 3.64 (s, 3), 4.5 (m, 1), 5.10 (d, 2), 5.25 (d, 1), 6.25 (two d, 2), 7.1−7.75 (m, 10); [α]+34.4° (c = 0.5, DMF). Examination of the region near δ 3.73 and 1.24 by 300 MHz ^1H NMR analysis of the crude sample prior to recrystallization gave no evidence for the presence of the corresponding DL-diastereomer described in the Supporting Information. The methodology and detection limits have been described previously.[33] Anal. Calcd for $C_{22}H_{22}N_2O_7S$: C, 57.63; H, 4.84; N, 6.11. Found: C, 57.54; H, 4.61; N, 6.04.

Bsmoc-Asn(Trt)-Trp-NH-Dmcp. There was added 0.4 g (0.93 mmol, 1.2 equiv.) of Bsmoc-Trp-F to a solution of 105 mg (0.78 mmol) of dimethylcyclopropylcarbinylamine hydrochloride in 5 mL of DCM in the presence of 267 μL of DIEA. The resulting mixture was stirred for 15 min and treated directly with tris(2-aminoethyl)amine (2.3 mL, 15 equiv). The mixture was stirred for 10 min. A white precipitate was formed which dissolved during subsequent extractions with saturated NaCl. The reaction mixture was diluted with 40 mL of DCM, extracted with three 30-mL portions of saturated NaCl, dried over $MgSO_4$, and filtered and the solvent removed in vacuo to give a pale yellow oil. A mixture of 511 mg (0.85 mmol) of Bsmoc-Asn(Trt)-OH and 324 mg (0.857 mmol) of Toppipu in 3 mL of DCM in the presence of 136 μL of DIEA was allowed to stand for 3 min. The resulting mixture was added to the oily tryptophan amide and the resulting mixture stirred at room temperature for 3 h. The solvent was evaporated in vacuo to give a yellow oil which was chromatographed on silica gel using EtOAc/$CHCl_3$ (75:25) as an eluent to give 538.5 mg (80%) of the desired product as an off-white solid. Recrystallization from Et_2O/CH_2Cl_2 (4:1) gave 444.2 mg (66%) of the dipeptide amide; mp 138−140 °C; IR (KBr): 3404, 1734, 1670, 1303, 1151 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.12 (m, 5), 0.95 (d, 6), 2.69−3.26 (m, 4), 4.47 (m, 2), 5.03 (dd, 2), 5.45 (s, 1), 6.54 (d, 1), 6.86−8.15 (m, 28); [α] −28° (c = 0.5, DMF). Anal. Calcd for $C_{50}H_{49}N_5O_7S$: C, 69.50; H, 5.72; N, 8.10. Found: C, 69.33; H, 5.49; N, 7.96.

Bsmoc-Phe-Phe-Val-Gly-Leu-Met-OBn. Bsmoc-Leu-F (260 mg, 0.73 mmol) was added to a solution of H-Met-OBn·TsOH (200 mg, 0.49 mmol) in 5 mL of dry DCM in the presence of 170 μL of DIEA. The resulting mixture was stirred for 15 min and then treated with tris(2-aminoethyl)amine (1.5 mL, 20 equiv). The solution was stirred for 10 min, diluted with 30 mL of DCM, extracted with three 30-mL portions of saturated NaCl, dried over $MgSO_4$, and filtered, and the solvent was removed in vacuo. The resulting oil was dissolved in 5 mL of DCM and a new cycle begun. The procedure was repeated four times using Bsmoc-Gly-F (175 mg, 0.58 mmol), Bsmoc-Val-F (200 mg, 0.5 mmol), and Bsmoc-Phe-F (twice with 292 mg, 0.58 mmol, in each cycle). After the last coupling, the solvent was removed, and the resulting oil was chromotagraphed on silica gel using $CHCl_3$/*t*-BuOH (6:1) to give 187 mg (37.2%) of the protected hexapeptide as a white solid, mp 224−225 °C; ^1H NMR ($CDCl_3$): δ 0.88 (m), 1.6−1.9 (m), 2.4−2.58 (m), 3.0 (m), 3.5 (m), 4.7 (m), 5.1 (bs), 7.1−7.6 (m); [α] −38.4° (c = 0.5, DMF). Anal. Calcd for $C_{53}H_{64}N_6O_{11}S_2$: C, 62.09; H, 6.29; N, 8.20. Found: C, 62.28; H, 6.49; N, 8.05.

Bsmoc-Tyr(*t*-Bu)-Gly-Gly-Phe-Leu-O-*t*-Bu. The rapid solution synthesis was carried out as described above using Fmoc amino acid fluorides as coupling reagents starting with 1 mmol of leucine *tert*-butyl ester hydrochloride. After the last coupling with Bsmoc-Tyr(*t*-Bu)-F, the solvent was removed in vacuo and the resulting oil chromatographed on silica gel using EtOAc as eluent to give 436.5 mg (49.1%) of the protected pentapeptide as a white solid, mp 133.5−135 °C; ^1H NMR ($CDCl_3$): δ 0.86 (d, 6), 1.3 (s, 9), 1.5 (m, 12), 3.0 (m, 4), 3.9 (m, 4), 4.6 (m, 2), 5.1 (s, 2), 6.6−7.7 (m, 17); [α] −27.2° (c = 0.5, DMF). Anal. Calcd for $C_{46}H_{59}N_5O_{11}S$: C, 62.07; H, 6.68; N. 7.87. Found: C, 61.88; H, 6.93; N, 7.73.

Bsmoc-Gln(Dmcp)-OH. To a solution of 15.6 g (42 mmol) of Z-Glu-OBn in 150 mL of acetonitrile there were added 12.53 g (46 mmol) of Dmcp-NH_2·TsOH, 13.5 g of TBTU (42 mmol), and 21.9 mL (125 mmol) of DIEA. The mixture was stirred overnight at room temperature, the solvent removed in vacuo, and the residue dissolved in 500 mL of EtOAc. The solution was washed with 5% $NaHCO_3$ and saturated NaCl solution and dried ($MgSO_4$), and the solvent was removed in vacuo to give 24 g of the ester as a colorless oil which without purification was dissolved in MeOH (150 mL) and 0.5 g of 5% Pd/C catalyst was added. The mixture was shaken under an atmosphere of H_2 (40 psi) for 24 h. Filtration and washing with MeOH followed by evaporation of solvent gave a residue which was triturated with ether and filtered to give 9.6 g (∼100%) of the crude deblocked acid (H-Gln(Dmcp)-OH). To a suspension of 10 g (43.8 mmol) of the crude acid in 300 mL of acetonitrile−H_2O (1:1) there was added an amount of triethylamine to give an apparent pH of 9.0. The suspension became clear. There was added 14.8 g (43.8 mmol) of Bsmoc-OSu and the pH kept at 9.0 by the addition of triethylamine. The uptake of base ceased after about 15 min. After stirring for 2 h, the reaction mixture was acidified to pH 5.0 with 10% citric acid or 0.1 N HCl and extracted with 3 100-mL portions of EtOAc. The combined organic extracts were washed with saturated NaCl solution (3 × 50 mL) and dried ($MgSO_4$), and the solvent

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was removed in vacuo. The residue was flash chromatographed (Merck Silica Gel 60) using a mixture of EtOAc−MeOH (1:1) as eluent to give 16.2 g (82.1%) of the Bsmoc acid as a white solid having an indistinct melting point. Examination of the NMR spectrum showed that the solid material had crystallized with 0.5 mol of H_2O and 0.5 mol of EtOAc. When attempts were made to dry the sample, the material became oily as the EtOAc and water were removed. The NMR and MS samples were analyzed as such with the EtOAc and water present. ^{1}H NMR ($CDCl_3$) δ 0.24 (br s, 5), 1.13 (s, 6), 2.1−2.3 (m, 4), 4.14 (m, 1), 4.8−5.3 (m, 2), 7.25−7.60 (m, 5): HRFABMS: calcd $(M + H)^+$ 451.1539; found: 451.1548.

Bsmoc-Asn(Dmcp)-OH. The asparagine derivative was obtained as described for the glutamine analogue. In this case also the solid acid was obtained (yield 87.8%) as a solvate containing 0.5 mol of water and 0.5 mol of EtOAc which was of indistinct melting point, ^{1}H NMR ($CDCl_3$) δ 0.22 (br s, 5), 0.94 (s, 6), 2.75 (br s, 2), 4.4 (br s, 1), 4.98 (m, 2), 7.1−7.45 (m, 5); HRFABMS: calc $(M + H)^+$ 437.1383; found 437.1370.

Bsmoc-Arg(Pbf)-OH. To a suspension of 2.33 g (5.46 mmol) of H-Arg(Pbf)-OH in 34 mL of acetonitrile−H_2O (1:1) an amount of triethylamine was added to give an apparent pH of 9.0. The suspension became clear and was treated with 1.83 g (5.46 mmol) of Bsmoc-OSu. While stirring, the pH was kept at 9.0 by the addition of triethylamine. The uptake of base ceased after about 15 min. The reaction mixture was stirred at room temperature for 2 h and then acidified to pH 5 with 10% citric acid. Acetonitrile was removed in vacuo at a bath temperature of 30−35 °C, the mixture cooled in an ice bath, and the pH brought to 2 with 10% citric acid. The mixture was extracted with EtOAc (3 × 30 mL), the extracts were washed with saturated NaCl (2 × 50 mL) and dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was recrystallized twice from EtOAc−ether. The acid separated first as an oil which solidified upon trituration with ether while cooling. There was obtained 3.3 g (93.1%) of the acid as a white solid, mp 118−126 °C. As in the cases of the Asn and Gln derivatives discussed above, the indistinct melting point was associated with loss of solvent. In this case the solid crystallized with 0.5 mol of ethyl ether. ^{1}H NMR ($CDCl_3$): δ 1.42 (s, 6), 1.43−2.0 (m, 4), 2.03 (s, 3), 2.45 (s, 3), 2.50 (s, 3), 2.9 (s, 2), 3.16 (s, 2), 4.24 (s, 1), 5.05 (t, 2), 7.18−7.64 (m, 5); HRFABMS: calc $(M + H)^+$ 649.2002; found: 649.1987.

Preparation of Bsmoc-Gly-Gly-OEt. A Test for Premature Deblocking. There was added 300 mg (1 mmol) of benzothiophenesulfone-2-(methoxycarbonyl)glycyl fluoride to a solution of 117 mg (0.83 mmol) of glycine ethyl ester hydrochloride in 7 mL of dry DCM in the presence of 0.3 mL of DIEA. The reaction mixture was stirred for 10 min and the solvent removed in vacuo to give a pale yellow oil which was purified by column chromatography using EtOAc with 1% of HOAc as eluent to give 276 mg (87%) of the desired dipeptide ester as a colorless oil; IR (neat): 3359 (NH), 1731 (CO, ester, urethane), 1679 (CO, amide), 1302, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3), 3.93 (d, 2), 3.99 (d, 2), 4.15 (q, 2), 5.15 (s, 2), 5.79 (bs, 1), 6.8 (bs, 1), 7.1−7.72 (m, 5). HPLC analysis of the crude reaction product showed, in addition to the dipeptide (84.8%, t_R = 15.45), 3.66% of Bsmoc-Gly-OH, presumably formed via hydrolysis of the acid fluoride and other minor unidentified impurities but none of the expected adducts **8** (t_R = 18.63 min) and **9** (t_R = 11.08) which would have been present had the amino acid ester caused deblocking during the coupling process. Anal. Calcd for $C_{16}H_{18}N_2O_7S$: C, 50.26; H, 4.74; N, 7.32. Found: C, 50.21; H, 4.88; N, 7.03.

Reaction of Bsmoc-OSu with *N*-Hydroxysuccinimide Anion. Bsmoc-OSu (337 mg, 1.0 mmol), HOSu (165 mg, 1.5 mmol), and DIEA (260 μL, 1.5 mmol) were added to 10 mL of MeCN/H_2O (10:1), and the mixture was stirred for 17 h. The resulting white solid was filtered from the reaction mixture and washed with water and acetone to give 143 mg (49%) of Bsm-OSu as a white solid, mp 234−235 °C. The solid was identified by comparison of its IR and ^{1}H NMR spectra with the spectra of authentic Bsm-OSu prepared from 2-(chloromethyl)benzothiophenesulfone as described above.

Treatment of 2-(Chloromethyl)benzothiophenesulfone with Ethyl Glycinate. A solution of 0.8 g (3.73 mmol) of 2-(chloromethyl)benzenethiophenesulfone, 1 g (7.17 mmol) of ethyl glycinate hydrochloride, and 0.93 g (7.17 mmol) of DIEA in 5 mL of DMF was stirred at room temperature for 3 h. The reaction mixture was poured into 100 mL of ice cold water and extracted with four 25-mL portions of EtOAc. The combined organic extracts were dried ($MgSO_4$), solvent was removed in vacuo, and the residual oil was purified by column chromatography using EtOAc/$CHCl_3$ (1:1) as eluent. The first fractions (R_f = 0.7) were combined, evaporated, and the residue recrystallized from EtOAc/hexane (1:1) to give 480 mg (45.8%) of adduct **9**, as white crystals, mp 78−79 °C; IR (KBr): 3336 (NH), 1734 (CO, ester), 1295, 1147 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.2 (t, 3), 3.45 (s, 2), 3.87 (s, 2), 4.15 (q, 2), 7.0−7.2 (m, 5). Anal. Calcd for $C_{13}H_{15}NO_4S$: C, 55.50; H, 5.37; N, 4.98. Found: C, 55.65; H, 5.18; N, 4.95.

A second fraction (R_f = 0.5) was collected and following recrystallization from Et_2O/hexane gave 100 mg of the unrearranged adduct **8**, mp 124−125 °C; IR (KBr): 3364 (NH), 1742 (CO, ester), 1636 (C=C), 1270, 1164 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 3), 3.64 (s, 1), 4.03 (s, 2), 4.19 (q, 2), 7.26−7.72 (m, 6). Anal. Calcd for $C_{13}H_{15}NO_4S$: C, 55.50; H, 5.37; N, 4.98. Found: C, 55.26; H, 5.32; N, 4.88.

Loading of Bsmoc Amino Acids onto Hydroxymethyl Resins. A sample of hydroxyl resin (170 or 340 mg) was weighed into a tared 5-mL polypropylene syringe fitted with a coarse polypropylene frit which was mounted on an adapter equipped with a Teflon stopcock connected to a vacuum source. The resin was swollen and washed three times with 4 mL of DMF and three times with 4 mL of DCM. Bsmoc-AA-F (4 eq based on the claimed functionality of resin used) or Bsmoc-AA-OH (4 equiv) and an appropriate coupling reagent were weighed into a 4-mL sample vial and dissolved in solvent (1.0 or 2.0 mL depending on the weight of resin used), and the calculated volume (liquids) or weight (solids) of base (4 equiv) was added to the solution. The contents of the vial were dissolved and added to the resin for a given time period. The resin was washed with 4-mL volumes of DMF three times, MeOH once, and DCM three times. The entire sample in the syringe was then transferred to a vacuum desiccator and dried overnight. Loadings and levels of racemization observed are given in Table 3.

UV Absorption Characteristics of Bsm-Pip. *N*-(Benzothiophenesulfone-2-methyl)piperidine (Bsm-pip) (263.35 mg, 1.0 mmol) was weighed into a tared 10-mL volumetric flask and diluted to volume with methanol. One milliliter of this solution was transferred to a 250-mL volumetric flask with a volumetric pipet, diluted to volume, and used as a stock solution (standard 0.4 mmol/L). From the stock solution, standards in the range of 0.08−0.4 mmol were prepared by serial dilution into 25-mL volumetric flasks. Standard curves obtained were linear (R^2 > 0.999) in absorbance vs concentration in the ranges of 0.15−1.6 and 0.08−0.4 (mmol/L), respectively. The average slope of three calibration curves (three separate weighings) was used to determine the absorption data: λ_{max} = 310.5 nm, log ϵ = 3.484 (MeOH), λ_{max} = 311.5 nm, log ϵ = 3.454 (DCM), R^2 > 0.999 for both solvents.

Method for Determination of Resin Loading. A thoroughly dried sample of loaded resin (20−45 mg depending on expected loading) was weighed into a fritted polypropylene syringe. The sample was deblocked with 1.00 mL of 2 or 20% piperidine (v/v) in DMF for 5 or 30 min, respectively. The 2% solution was used to check for completion of the deblocking/isomerization process using short reaction times at low base concentrations. The solution was drained into a 25-mL volumetric flask, the resin and syringe were washed with methanol (20 mL) into the flask, and the solution was diluted to volume. A solvent blank was prepared by diluting 1.0 mL of deblocking solution to volume in a 25-mL flask. Absorbance was determined at 310.5 nm (MeOH) and the loading calculated using the formula ($A/\epsilon \times 25$ mL)/1000 mL/L/wt of resin in g = loading in mmol/g (ϵ is taken as 3.048 for a concentration of c = mmol/L).

Extent of Racemization During Resin Loading of Bsmoc Amino Acids. Standard solutions of Marfey's adducts were prepared by mixing 0.4 mL of 10 mM Marfey's reagent[6] in acetone with 0.4 mL of 10 mM amino acid in 0.1 N $NaHCO_3$. The same samples of amino acid were used to prepare the Marfey's standards as those used for the preparation of the Bsmoc-protected amino acids and for the subsequent racemization studies. The mixtures were placed in 4-mL capped sample vials, sonocated in a 40 °C water bath for 1 h, neutralized with 0.2 mL of 0.2 N HCl, and stored at −5 °C. If a solid precipitated upon storage, the samples are warmed prior to use. The standards were diluted with 2 parts of acetonitrile prior to injection onto a Waters C^{18} Nova-pak column, 5-μL injection volume, sensitivity = 0.3, flow = 1.0 mL/min, using an acetonitrile/water gradient (0.1% in TFA) from 10% to 60% acetonitrile over 50 min, with detection at 340 nm. No D,L diastereomer was detected in any of the L,L standards. Standards of the deblocking base adducts (from piperidine and morpholine) were also prepared using the same method.

A weighed sample of loaded resin (20−50 mg, depending on the determined loading) in a fritted 5-mL syringe was deblocked for 30 min using 20% piperidine or 20% morpholine (v/v) in DMF (the retention times of both diasteriomers expected are checked in relation to the piperidine and morpholine standards as these peaks may overlap). The resin was washed with DMF, MeOH, DMF, MeOH, DCM, MeOH, and finally DCM twice. The N-deblocked amino acid was then cleaved from the resin using neat TFA for 90 min. The TFA solution of the free amino acid was drained into a small round-bottomed flask and the syringe and resin washed with several portions of TFA. The combined TFA filtrates were then evaporated to dryness, the residue was dissolved in 400 μL of 10 mM $NaHCO_3$, and the solution was diluted with 200 μL of acetone and treated with 200 μL of 10 mM Marfey's reagent in acetone. The mixture was sonocated and neutralized exactly as described for the standards above. The samples were injected directly with no prior dilution for examination by HPLC. The amount of racemization was determined by locating the retention times of the L,L- and D,L-diasteriomers by comparison with the standards and integrating the corresponding peaks from the resin samples followed by coinjection of the D,L-standard to confirm the position of the D,L-peak (see Table 4). If the piperidine adduct had the same or a close retention time relative to either diasteriomer, morpholine was substituted as deblocking base since the corresponding adduct has a considerably shorter retention time in the separation system used. Attempts to remove totally the deblocking base from the resin prior to cleavage by additional washes was not possible, a fact which has been noted in the literature previously for piperidine.[34] The amount of racemization is expressed as % D.

UV Monitoring of the Deblocking/Scavenging Process for Bsmoc-Gly-O-*t*-Bu with Piperidine. The extinction coefficient of Bsmoc-Gly-O-*t*-Bu (ϵ = 2611, DCM) at 310 nm was determined as described for Bsm-Pip. To three 25-mL volumetric flasks were added stock solutions of Bsmoc-Gly-O-*t*-Bu (5.00 mL, 1.97 mmol/L) in DCM. Thereafter, 5-mL portions of stock solution containing 1.9, 3.8, or 19 equiv of piperidine were added to each flask which were then diluted to volume. Using the corresponding concentration of piperidine solution as a reference blank, the solution was scanned from 245 to 320 nm to monitor the deblocking/scavenging process. Deblocking was noted by an initial decrease in absorbance in the region of 310 nm followed by a slow increase as the initial deblocking intermediate isomerized to the final product. The extent of isomerization was estimated by calculating the expected absorbance of a 0.3936 mmol/L solution of Bsm-Pip at λ = 311.5 nm (the initial reaction mixture is 0.3936 mmol/L in Bsmoc-Gly-O-*t*-Bu). For the results, see Figure 2a,b in the Supporting Information.

(34) Adamson, J. G.; Hoang, T.; Crivici, A.; Lajoie, G. A. *Anal. Biochem.* **1992**, *202*, 210.

General Procedure for the Solid-Phase Synthesis of Model Peptides. Manual syntheses were performed in 5-mL fritted polypropylene syringes. A 200-mg sample of resin was weighed into a syringe which was connected to a stoppered vacuum source. The resin was swollen and washed with DCM (3 × 4 mL) and DMF (3 × 4 mL). The solvent was removed by aspiration and the protecting group attached to the first amino acid deblocked by means of a solution of amine in DMF or DCM (4 mL). At no time during the deblocking process was the resin drained dry of deblocking solution. The deblocking solution and wash solvents are most conveniently handled in 500-mL wash bottles. The resin was washed five times with 4-mL portions of DMF, the duration of each wash being 1 min. The Bsmoc-AA-F or the combination of Bsmoc-AA-OH's and coupling reagent (4 equiv) were preweighed into 4-mL sample vials and then dissolved with a freshly prepared 1.0-mL stock solution of DMF containing the desired amount of tertiary amine catalyst. The contents of the vial were thoroughly mixed and pipetted into the syringe. If uronium salts were used as coupling reagents the coupling solution was allowed to preactivate in the vial prior to addition to the resin. The coupling was performed for a given time and the resin again was washed as described above. At this point the cycle of deblocking/washing/coupling/washing was repeated until the desired sequence of amino acids was obtained. The Bsmoc group on the amino terminus was then deblocked as described above. The resin was thoroughly washed with DMF (3 × 4 mL), MeOH (4 mL), DMF (3 × 4 mL), and DCM (4 × 4 mL), the duration of each wash being 1.5 min. The peptide was cleaved from the resin along with any side chain protecting groups by 90-min treatment with 10% TFA/H_2O. The TFA solution of the peptide was drained into a 50- or 100-mL round-bottomed flask and the syringe and resin washed with TFA (3 × 3 mL). The solvent was removed from the combined filtrate and washes in vacuo without heating. The concentrated peptide solution was cooled in a dry ice bath, and cold anhydrous ether was added to precipitate the TFA salt of the peptide. If no precipitate appeared the ether was evaporated and the precipitation process repeated. The precipitated peptide was separated from ether by centrifugation in a tared centrifuge tube and the resulting peptide pellet washed with several portions of fresh ether by breaking up the pellet with a Pasteur pipet in order to resuspend it in fresh ether. Finally the peptide was dried in a desiccator. The crude peptide was used directly for HPLC studies. No corrections were made for different epsilon values of the components in the crude HPLC profile (λ = 220 nm), although the HPLC of the crude peptide was also examined at longer wavelengths (λ = 280 or 310 nm) in order to search for the presence of residual protecting groups or premature deblocking products.

Comparison of the Bsmoc Deblocking/Rearrangement Process for Various Amines. Stock solutions of organic bases (piperidine, piperazine, morpholine, and ethanolamine) were prepared by weighing 0.733 mmol of base into tared 2.00-mL volumetric flasks and diluting to volume with $CDCl_3$ or DMF. Each solution (0.60 mL, 0.22 mmol base) was pipetted into a vial containing 44 mg (0.1 mmol) of Bsmoc-Phe-O-*t*-Bu and ^{1}H NMR analysis used to follow the deblocking/rearrangement process on a Hitachi R-1200 spectrometer at 60 MHz. In the case of runs in DMF the solvent proton peak at δ 8.05 was used as reference rather than TMS which was used in $CDCl_3$. Deblocking was considered complete when the methylene protons of the Bsmoc residue (δ 5.075, 5.095, d, 2) were no longer observed and rearrangement when the terminal vinyl protons (δ 6.3−6.5, m, 2) of the labile intermediate were no longer observed. The results are given qualitatively in the text.

Assembly of Toxin 2 of *A. androctonus Hector* (13). (1) Via Bsmoc Chemistry. A PAC-PEG-PS resin (2.5 g, 0.325 mmol) with a claimed functionality of 0.13 mmol/g was washed and swollen in DCM. A solution of 460 mg (1.3 mmol) of Bsmoc-Ile-F and collidine (0.17 mL, 1.3 mmol) dissolved in sufficient DCM to cover the swollen resin was added. The mixture was kept for 19 h with occasional agitation. The resin was washed with DCM (5 × 35 mL) and capped with DCM/

Ac_2O/pyridine 5/1/1, 30 mL) for 14 h. The capped resin was thoroughly washed with DCM (5 × 35 mL), MeOH (2 × 35 mL), and DCM (4 × 35 mL) and dried in a desiccator. Two samples (42.0 and 42.5 mg) of the resin were deblocked with 1.0 mL of 20% piperidine in DMF for 30 min, and the extent of loading was determined by UV analysis. The loadings observed were 0.096 mmol/g for both samples. Following the general procedure, a 700-mg sample of the above-described resin was used to assemble the hexapeptide under the following conditions: (a) 5-min deblocking with 2% piperidine/DMF; (b) 30-min coupling via Bsmoc-AA-F (4 equiv) without base; (c) lysine, tyrosine, and aspartic acid protected via Boc, *t*-Bu ether, and *t*-Bu ester groups, respectively. Before removal of the Bsmoc group from valine, the resin was divided in half, one portion being deblocked for 5 min, the other for 19 h with 2% piperidine in DMF. In the latter case (Figure 1a) the major impurity was the Asu-containing peptide (peak eluting near 16 min). In a second run the couplings were performed using 4 equiv of Bsmoc-AA-F and 1 equiv of collidine in DMF for 20 min. Deblockings were carried out as above except that 20% morpholine was used for deblocking. An acceptable synthesis resulted. See Figure 1b.

(2) Via Fmoc Chemistry. In this case the assembly modifications were as follows: (a) 10-min deblocking with 20% piperidine/DMF; (b) 15-min coupling via HATU(4)/Fmoc-AA-OH(4)/TMP(8) with a 3-min preactivation period. As before, two samples of the resin were deblocked for 5 min (not shown) and 19 h. The numerous side products seen after 19 h with retention times (min) in parentheses included aminosuccinimide (16.1), α-L-piperidide (19.2), α-D-piperidide (20.4), *β*-L-piperidide (21.3), and *β*-D-piperidide (22.3) (Figure 1c). A third sample treated for 19 h with 20% morpholine showed little effect (Figure 1d) in agreement with the successful use of these conditions for the Bsmoc synthesis.

Synthesis of Heptapeptide 23 via Bsmoc-AA-OH and HATU. To a solution of 67.75 mg (0.5 mmol) of (dimethylcyclopropylcarbinyl)amine hydrochloride were added 0.270 mL (3.1 equiv) of DIEA and 213.12 mg (0.5 mmol) of Bsmoc-Trp-OH dissolved in 10 mL of dry DCM. After cooling in an ice bath, 190.5 mg (0.5 mmol) of HATU was added in one batch. The course of the reaction was monitored by TLC and found to be complete within 30 min after which 1.5 mL (20 equiv) of TAEA was poured into the solution. After 10 min, deblocking was complete, and the mixture was washed with three 10-mL portions of saturated NaCl solution. After drying over $MgSO_4$ and evaporation of solvent a new coupling cycle was started. The remaining cycles were executed in the same way except that only 2.1 eq of DIEA were used considering that in step I 1 equiv of DIEA was used to neutralize the hydrochloride salt. The next three amino acids were added in the same manner; however, because of expected loss of material, for the fifth, sixth, and seventh amino acids, the amount of Bsmoc amino acid and HATU was progressively reduced to 0.475, 0.47, and 0.45 mmol, respectively. The amount of TAEA used was not changed.

In its fully protected form the heptapeptide was purified by column chromatography (40 g of SiO_2, 2 × 50 cm glass column). There was obtained 280 mg (37%) of the heptapeptide as a white solid, mp 143−145 °C. MS(FAB) *m*/*e* calcd 1557.9, found 1557.7 $(M + Na)^+$. A very intense peak at *m*/*e* 243.1 was observed for the stable trityl cation. A second independent MS determination showed *m*/*e* 1535.8, calcd 1535.9 $(M + H)^+$ along with the trityl cation at 243.1. Amino acid analysis: found (expected): Asp 0.65 (1.0), Thr 2.15 (2.0), Pro 1.17 (1.0), Leu 0.99 (1.0), Phe 1.04 (1), Trp not determined. Anal. Calcd for $C_{86}H_{106}N_{10}O_{14}S$: C, 67.25; H, 6.96; N, 9.12. Found: C, 67.00; H, 7.04; N, 8.91.

Boc-pyroglutamic Acid Fluoride 26. **Method A (via Boc-pGlu-OH).** 210.4 mg (0.918 mmol) of Boc-pGlu-OH was dissolved in 10 mL of dry DCM and treated with 0.077 mL (1.05 equiv) of pyridine and 0.234 mL (3 equiv) of cyanuric fluoride at −30 °C. After 3 h the mixture was washed with two 20-mL portions of ice cold water. Recrystallization attempts failed, the product being obtained as an oil in 89% yield; IR (neat) 2983 (CH), 1850 (COF), 1795 (CO), 1712 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) *δ* 1.62 (s, 9), 1.9−2.9 (m, 4 H), 4.85 (t, 1 H).

Method B (via BOC-pGlu-OH·DCHA). A solution of 201 mg (0.4 mmol) of BOC-pGlu-OH·DCHA was dissolved in 10 mL of dry DCM, and the solution was cooled to −30 °C and treated with 0.035 mL (1.1 equiv) of pyridine and 0.101 mL (3 equiv) of cyanuric fluoride. After 3 h the mixture was washed with two 10-mL portions of ice cold water. The fluoride was obtained as an oil in a yield of 85%, IR(neat): 1850 (COF), 1795 (CO), 1712 cm^{-1} (CO); ^1H NMR ($CDCl_3$): *δ* 1.62 (s, 9), 1.9−2.9 (m, 4), 4.85 (t, 1). The fluoride was not further purified but used as such in the acylation of the heptapeptide.

***N*-(Benzothiophenesulfone-2-methoxycarbonyl)phenylalanine Dicyclohexylammonium Salt.** A suspension of 0.5 g (1.29 mmol) of Bsmoc-Phe-OH in 20 mL of dry ether and 3 mL of dry THF was treated with 0.308 mL (1.2 equiv) of dicyclohexylamine dropwise. After a few min the solution became clear, and a few moments later a white precipitate separated. After stirring for 4 h at room temperature and storage in the refrigerator overnight filtration gave 690 mg (95%) of the salt as a white solid, mp 164 °C; ^1H NMR ($CDCl_3$) *δ* 1.1−2.35 (bm, 1), 3.2 (d, 2), 3.1 (t, 1), 5.1 (s, 2), 7.0−7.7 (m, 10). Anal. Calcd for $C_{31}H_{40}O_6N_2S$: C, 65.47; H, 7.09; N, 4.93. Found: C, 65.07; H, 7.03; N, 4.90.

***N*-(Benzothiophenesulfone-2-methoxycarbonyl)phenylalanine Fluoride via Bsmoc-Phe-OH·DCHA.** A solution of 300 mg (0.528 mmol) of Bsmoc-Phe-OH·DCHA in 20 mL of dry DCM was treated with 0.046 mL (1.1 equiv) of pyridine and 0.134 mL (3 equiv) of cyanuric fluoride. The reaction was found to be complete within 75 min. After washing with two 20-mL portions of ice cold water, the organic layer was dried over $MgSO_4$ and evaporated in vacuo and the residue recrystallized from DCM/hexane to give the fluoride in 97% yield as a white solid, mp 129 °C. The IR and NMR spectral data agreed with data obtained for a sample of the fluoride obtained from the free acid (mp 174 °C). In the latter case the acid fluoride was obtained either as a clear white foam, mp 50 °C or a white solid, mp 132−133 °C, IR (KBr): 3345 (NH), 1846 (COF), 1731 (CO), 1303, 1151 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$): *δ* 3.2 (d, 2), 4.8 (m, 1), 5.1 (s, 2), 5.3 (d, 1), 7.1−7.7 (m, 10). Anal. Calcd for $C_{19}H_{16}FNO_5S$: C, 58.60; H, 4.14; N, 3.60. Found: C, 58.82; H, 4.26; N, 3.64.

Fully Protected Octapeptide, BOC-pGlu-Leu-Thr(*t*-Bu)-Phe-Thr(*t*-Bu)-Pro-Asn(Trt)-Trp-NHDmcp (27). To a solution of 70 mg (0.0456 mmol) of the heptapeptide **23** in 20 mL of acetonitrile there was added 684 mg (15 equiv of NH, 1 mequiv/g of NH) of a piperazine-functionalized silica gel reagent. The reaction mixture was gently rotated on the shaft of a rotary evaporator while monitoring the course of the reaction by TLC analysis (elution with 1% HOAc in EtOAc). Deblocking was complete within 60 min. The silica gel was filtered and washed with 100 mL of DCM. Evaporation of the solvent gave a white solid, which was redissolved in 10 mL of dry DCM and treated with 0.0087 mL (1.1 equiv, 0.05 mmol) of DIEA and 5.3 mg (0.5 equiv) of freshly prepared BOC-pGlu-F. To avoid using an excess of the fluoride, which has not been obtained in a completely pure state, the needed full equivalent was approached stepwise. After further additions of 0.4, 0.2, and 0.2 equiv (total 1.3 equiv) of the fluoride, the coupling was found to be complete. The solvent was evaporated, the residue was redissolved in 1 mL of DCM, and the solution was applied to a preparative TLC plate. The first elution was made with DCM/acetone (2:1). The collected material still showed an impurity spot on TLC. A second preparative TLC plate was loaded and eluted with EtOAc/1% glacial acetic acid. Although a clear separation was made, the same impurity spot was observed again when the material was tested a few hours after the pure product had been collected. Evaporation of the solvent led to the isolation of 36.8 mg (53%) of the peptide as an off-white residue. HPLC analysis on a 4 *μ*m, 3.9 × 150 mm, 60 Å Waters Nova Pak C_{18} column using as eluent (A) CH_3CN/(B) H_2O 0.1% TFA, detection at 220 nm, flow rate 1 mL/min using the following gradient: (min/A/B) 1/10/90, 20/90/10, 25/10/90, showed a purity of 89%; t_R 26.10 min; MS/(FAB) *m*/*e* calcd 1546.9. Found: 1546.8 $(M + Na)^+$. Also observed were the

expected trityl cation peak at *m/e* 243.1 and an (M − BOC) peak at *m/e* 1446.7. Amino acid analysis: Found (expected): Asp 0.83 (1), Glu 1.17 (1), Thr 2.45 (2), Pro 1.35 (1), Leu 1.16 (1), Phe 1.32 (1).

Octapeptide 20 as TFA Salt. The protected octapeptide **27** (25 mg, 0.0157 mmol) was treated with a deblocking cocktail having the composition 88% TFA, 5% phenol, 5% water, 2% triisopropylsilane. The course of the reaction was followed by HPLC. Deblocking was complete after 1 h, the reaction mixture was evaporated in vacuo, and the residue was precipitated with excess ether and collected by centrifugation. The washing process was repeated three times. The light brown octapeptide salt was dried in a vacuum. HPLC conditions used for analysis were the same as described for the protected octapeptide. The t_R of the main peak was 17.45 min with integration giving an approximate purity of 70%. MS(FAB): calcd *m/e* 988.12; found *m/e* 988.4 $(M + Na)^+$. Amino acid analysis: found (expected): Asp 0.63 (1), Glu 1.10 (1), Thr 2.05 (2), Pro 1.13 (1), Leu 1.00 (1), Phe 1.08 (1).

Bsmoc-Leu-Leu-Leu-OFm. To a solution of 0.196 g (1 mmol) of FmOH and 0.178 mL (1 mmol) of DIEA in 10 mL of dry DCM was added 0.427 g (1.2 mmol) of Bsmoc-Leu-F. After the reaction mixture had been stirred at room temperature for 5 h, TLC analysis showed no trace of alcohol remaining. The solution was diluted with 20 mL of DCM and washed with 1 M HCl solution (2 × 10 mL), saturated $NaHCO_3$ solution (2 × 10 mL), and saturated NaCl solution (2 × 10 mL). The dried ($MgSO_4$) solution was rotoevaporated and the residual sticky residue dissolved in 10 mL of 2% TAEA in DCM. After stirring at room temperature for 0.5 h, TLC analysis showed the absence of starting material. Addition of 30 mL of DCM was followed by washing with saturated NaCl (2 × 15 mL), water (2 × 15 mL), and saturated NaCl (15 mL). The dried ($MgSO_4$) solution was filtered and rotavaped and the residue dissolved in 10 mL of dry DCM and treated with 0.178 g (1 mmol) of DIEA followed by 1.1 mmol of Bsmoc-Leu-F. The mixture was stirred for 1 h at room temperature, diluted with 20 mL of DCM, and worked up as described above. After removing the solvent, the resulting dipeptide was deblocked by means of 2% TAEA/DCM as described previously and coupled with Bsmoc-Leu-F, again as described above. Following the usual workup and removal of solvent, the crude tripeptide was purified by column chromatography on silica gel with elution by EtOAc/hexane (6/4) and final crystallization from DCM/hexane to give 0.51 g (67.4%) of the protected tripeptide as a white powder, mp 94−95 °C; IR (KBr) 3282 (NH), 1737 (CO, ester), 1707 (CO, urethane), 1643 cm^{-1} (CO, amide); ^1H NMR ($CDCl_3$) δ 0.8−1.05 (m, 18), 1.4−1.8 (m, 9), 4.1−4.3 (m, 3), 4.45−4.55 (m, 3), 5.2 (q, 2), 5.5 (d, 1), 6.7 (t, 2), 7.15−7.8 (m, 13); HPLC (linear gradient 40/90 CH_3CN/H_2O/0.1% TFA in 20 min, Nova Pak C-18 column, 4 μm, 60 Å, 3.9 × 150 mm, flow rate 1 mL/min, 254 nm): single peak at t_R = 15.05 min. Anal. Calcd for $C_{42}H_{51}N_3O_8S$: C, 66.55; H, 6.78; N, 5.54. Found: C, 66.55; H, 6.71; N, 5.51.

Bsmoc-Leu-Phe-OFm. Following the method described for the synthesis of Bsmoc-Leu-OFm, BOC-Phe-OFm, mp 131−132 °C, ^1H NMR ($CDCl_3$) δ 1.5 (s, 9), 3.1 (d, 2), 4.1−4.6 (m, 4), 6.9−7.8 (m, 13), was obtained from BOC-Phe-F and FmOH. The crude product was treated with 50% TFA/DCM at room temperature for 2 h to give after recrystallization from EtOH/ether the TFA salt of H-Phe-OFm, mp 151−152 °C, ^1H NMR ($CDCl_3$-DMSO-d_8) δ 3.3 (d, 2), 4.1−4.6 (m, 4), 6.1 (br s, 1), 7.2−8.1 (m, 13). To the crude TFA salt (5 mmol) in 30 mL of dry DCM was added 5 mmol of DIEA followed by 5 mmol of Bsmoc-Leu-F. After workup as described for the Leu-Leu analogue, chromatographic purification on silica gel with elution by EtOAc/hexane (6/4) gave in 73.4% yield the dipeptide as a yellowish white solid, mp 135−137 °C, IR (KBr) 3418 (NH), 1735 (CO, ester), 1678 (CO, amide); ^1H NMR ($CDCl_3$) δ 1.05 (d, 6), 1.3−1.5 (m, 3), 3.1 (d, 2), 4.1−4.6 (m, 5), 5.1 (s, 2), 5.4 (d, 1), 6.5 (d, 1), 7.0−7.9 (m, 18); HPLC (linear 10/90 CH_3CN/H_2O/0.1% TFA in 20 min followed by 5 min at 90% CH_3CN, Nova Pak C-18 column, 4 μm, 60 Å, 3.9 × 150 mm, flow rate 1 mL/min, 254 nm): single peak at t_R = 18.1 min. Anal. Calcd for $C_{39}H_{38}N_2O_7S$: C, 69.02; H, 5.60; N, 4.13. Found: C, 68.99; H, 5.66; N, 4.14.

Bsmoc-Leu-Phe-OH. Treatment of 2 mmol of H-Phe-$OCMe_3$·HCl with 4 mmol of DIEA and 2.2 mmol of Bsmoc-Leu-F in 20 mL of dry DCM in the normal manner followed by treatment of the crude protected dipeptide with 50% TFA/DCM at room temperature for 2 h gave in 78.5% yield after recrystallization from DCM/hexane the dipeptide acid as a white solid, mp 91−93 °C, ^1H NMR ($CDCl_3$) δ 1.05 (d, 6), 1.2−1.3 (m, 3), 3.05 (d, 2), 4.1−4.5 (m, 2), 5.08 (s, 2), 5.3 (s, 1), 7.3−8.1 (m, 10). Anal. Calcd for $C_{25}H_{28}N_2O_7S$: C, 60.00; H, 5.60; N, 5.60. Found: C, 59.64; H, 5.65; N, 5.54.

H-Leu-Phe-OFm·TFA. By treatment of H-Phe-OFm·TFA with Boc-Leu-F there was obtained in the normal manner the protected dipeptide which with 50% TFA/DCM gave the TFA salt in 79.3% yield as a white solid, mp 186−187 °C; ^1H NMR ($CDCl_3$−DMSO-d_6) δ 1.0 (d, 6), 1.2−1.4 (m, 3), 3.1 (d, 2), 4.1−4.6 (m, 5), 7.1−7.9 (m, 13). Anal. Calcd for $C_{31}H_{33}F_3N_2O_5 \cdot {}^1/_2 H_2O$: C, 64.25; H, 5.69; N, 4.84. Found: C, 64.68; H, 5.65; N, 4.84.

Selective Deblocking of Bsmoc-Leu-Phe-OFm. (A) Removal of the Fm Residue. A solution of 0.25 mmol of the protected dipeptide in 2 mL of 10% diisopropylamine in DMF was allowed to stand at room temperature with samples being removed from time-to-time, diluted with CH_3CN/H_2O, and injected onto an HPLC column using the conditions described above which gave the following retention times: Bsmoc-Leu-Phe-OFm, 18.1 min; Bsmoc-Leu-Phe-OH, 12.3 min; H-Leu-Phe-OFm, 14.8 min; H-Leu-Phe-OH, 9.2 min. After 5 min the starting material peak at 18.1 min began to drop and a new band at 17.1 due to dibenzofulvene began to increase along with the desired product at 12.3 min. These bands underwent gradual changes until after 60 min the starting dipeptide had disappeared completely with only two major peaks being present: that for product, Bsmoc-Leu-Phe-OH at 12.3 min and the dibenzofulvene band at 17.1 min. The HPLC picture did not change after 24 h, showing that the Bsmoc residue was not affected by diisopropylamine.

(B) Removal of the Bsmoc Residue. As already described in the case of the leucine trimer, 2% TAEA/DCM gave, after about 20 min, mainly H-Leu-Phe-OFm (t_R = 14.8 min) along with a peak at 17.6 min believed to be due to the TAEA/Bsmoc adduct. With 2% piperidine both Bsmoc and Fm groups were completely removed after 5 min.

Fmoc-Leu-OBsm. To a solution of 1.1 g (3 mmol) of Fmoc-Leu-OH and 0.59 g (3 mmol) of benzothiophenesulfone-2-methanol in 30 mL of dry THF was added 0.618 g (3 mmol) of DCC at 0 °C. After stirring at 0 °C for 1 h and at room-temperature overnight, the solvent was removed by means of a rotary evaporator, 10 mL of EtOAc added, the mixture filtered to remove DCU, the organic layer washed with 10% citric acid, saturated $NaHCO_3$, and saturated NaCl (2 × 20 mL each) and dried ($MgSO_4$), the solvent again evaporated, and the residue flash chromatographed using EtOAc/hexane (6/4) to give 1.23 g (77.3%) of the ester as a foamy white solid, mp 65−68 °C, IR(KBr) 3486 (NH), 1751 (CO, ester), 1720 cm^{-1} (CO, urethane); ^1H NMR ($CDCl_3$) δ 0.9 (d, 6), 1.6−0.18 (m, 3), 4.25−4.50 (m, 4), 5.16 (s, 2), 5.21 (d, 1), 7.1−7.7 (m, 13). Anal. Calcd for $C_{30}H_{29}NO_6S$: C, 67.80; H, 5.46; N, 2.64. Found: C, 67.89; H, 5.60; N, 2.50.

Fmoc-Phe-Leu-OBsm. A solution of 0.531 g (1 mmol) of Fmoc-Leu-OBsm in 10 mL of 30% *tert*-butylamine in CH_3CN was allowed to stand at room temperature for 10−15 min (a precipitate separated during the deblocking process). The solvent was removed with a rotary evaporator at room temperature and the oily residue dissolved in 10 mL of a mixture of DCM and DMF (1/1). The solution was cooled to 0 °C and 0.387 g (1 mmol) of Fmoc-Phe-OH, 0.35 mL (2 mmol) of DIEA, and 0.38 g (1 mmol) of HATU added. The reaction mixture was stirred at 0 °C for 30 min and at room temperature for 30 min. The solution was diluted with 50 mL of EtOAc, washed with 10% citric acid, saturated $NaHCO_3$, and saturated NaCl (2 × 10 mL each), and dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was dissolved in 3 mL of DCM and the solution diluted with 100 mL of hexane. The precipi-

tate was filtered and washed with 30 mL of hexane to remove DBF. Since TLC showed an extra spot due to unreacted Fmoc-Phe-OH, the product was flash chromatographed using EtOAc/hexane (6/4) as eluent. Following removal of solvent, recrystallization of the residue gave 0.49 g (72.3%) of the dipeptide as a white solid, mp 78 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (d, 6), 1.5−1.7 (m, 3), 3.1 (d, 2), 4.1−4.6 (m, 5), 5.12 (s, 2), 5.3 (d, 1), 6.4 (d, 1), 7.0−7.7 (m, 19). Anal. Calcd for $C_{39}H_{38}N_2O_7S$: C, 69.03; H, 5.61; N, 4.13. Found: C, 69.02; H, 5.62; N, 4.07.

Fmoc-Gly-Phe-Leu-OBsm. A solution of 0.339 g (0.5 mmol) of Fmoc-Phe-Leu-OBsm in 5 mL of 30% *tert*-butylamine in acetonitrile was allowed to stand at room temperature for 10 min. TLC and HPLC analysis showed that the Fmoc group had been completely deblocked at this point. Solvent was removed immediately using an efficient double-trapped oil pump. DCM was added twice and the solvent removed a second and a third time in the same manner. The residue was dissolved in 5 mL of DCM/DMF (1/1) and the mixture cooled to 0 °C and treated with 0.15 g (0.5 mmol) of Fmoc-Gly-OH, 0.174 mL (1 mmol) of DIEA, and 0.19 g (0.5 mmol) of HATU. After 30 min at 0 °C and 30 min at room temperature, 50 mL of EtOAc was added and the solution washed with 10% citric acid, saturated $NaHCO_3$, and saturated NaCl (2 × 10 mL each). The solvent was removed with a rotary evaporator in vacuo, the residue dissolved in 2 mL of DCM, and 50 mL of hexane added. The precipitate was filtered and washed with hexane (2 × 10 mL) to give 0.295 g (80.4%) of the tripeptide as a white solid for which HPLC analysis showed in addition to the tripeptide at t_R 18.5 min (89.6%), two extra peaks at t_R 13.2 min (8.5%) and 19.5 min (residual DBF not removed by the hexane washes, amount 1.9%). An analytical sample was obtained by flash column chromatography over silica gel using as eluent EtOAc/hexane (7/3). Following chromatography, recrystallization from DCM/hexane gave the pure tripeptide in 72.4% yield as a white solid, mp 108−109 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (d, 6), 1.5−1.8 (m, 3), 3.06 (d, 2), 3.8 (d, 2), 4.2 (t, 1), 4.36 (d, 2), 4.5 (m, 1), 4.7 (m, 1), 5.09 (s, 2), 5.6 (t, 1), 7.1 (m, 2), 7.0−7.8 (m, 13). Anal. Calcd for $C_{41}H_{41}N_3O_8S\cdot 1/2\ H_2O$: C, 66.13; H, 5.65; N, 5.65. Found: C, 66.13; H, 5.77; N, 5.62.

Selective Fmoc-Deblocking of Fmoc-Phe-Leu-OBsm. To a solution of 34 mg of the dipeptide dissolved in 0.5 mL of a solvent containing a specific deblocking amine HPLC analysis using a Nova Pak 4 μm, 60 Å, C-18 silica column (3.9 × 150 mm) using a PDA detector, 220 and 254 nm, flow rate 1 mL/min with a linear gradient 10/90 in 20 min (CH_3CN/H_2O/0.1% TFA). Under these conditions, the retention times in minutes observed for authentic samples of the starting material and the various reaction products were as follows: Fmoc-Phe-Leu-OBsm, 20.1; DBF, 19.7; Fmoc-Phe-Leu-OH, 17.1; H-Phe-Leu-OBsm, 11.9; H-Phe-Leu-OH, 7.8. Authentic samples of these various deblocking products were obtained as follows: (a) Fmoc-Phe-Leu-$OCMe_3$/TFA; (b) Boc-Phe-Leu-OBsm/TFA; (c) Fmoc-Phe-Leu-OH/Et_2NH/CH_3CN. For the results of the deblocking tests, see Table 5.

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Supporting Information Available: Model experimental procedures, stability studies, Figure 2, and confirmatory IR, NMR, MS, UV, and amino acid analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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