Peptide Synthesis by Prior Thiol Capture. 6. Rates of the Disulfide Bond Forming Capture Reaction and Demonstration of the Overall Strategy by Synthesis of the C-Terminal 29-Peptide Sequence of BPTI†

Nader Fotouhi, Nicholas George Galakatos, and D. S. Kemp*

Department of Chemistry, Room 18-584, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Peptide bond formation by prior thiol capture involves as a first step formation of a disulfide bond between two S-functionalized peptide fragments, one bearing a 4-(acyloxy)-6-mercaptodibenzofuran at its C-terminus, the other bearing an S-activated cysteine residue at its N-terminus. The Scm procedure (Scm MeO-CO-S) of Brois[8] and others is used to generate disulfides of general structure -Cys(S-S-Ar)- by reaction of suitable arene thiols with -Cys(S-Scm)- derivatives. Mixtures of hexafluoroisopropyl alcohol (HFIP) with water and acetonitrile facilitate this reaction, which is markedly accelerated by traces of tertiary amines, by electron-withdrawing groups near the Scm function, and by an increase in the fraction of water in the mixture. A 94% yield in 5 min was seen for reaction of the trifluoroacetate salt of H-L-Cys(S-Scm)-OMe (5×10^{-4} M) with 4-mercaptodibenzofuran (5×10^{-4} M) in 9:1 HFIP–MeCN. The scope of the thiol capture strategy is demonstrated by a four-fragment, three-stage assembly of the 29-peptide sequence 30–58 of the protein basic pancreatic trypsin inhibitor (BPTI).

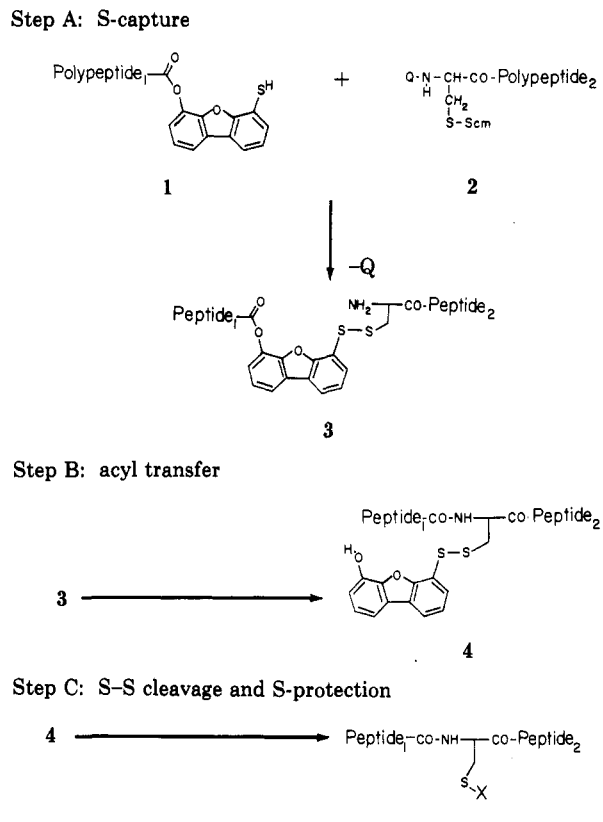
In previous papers in this series we have described a new approach for forming the amide bonds of polypeptides by a strategy of thiol capture. As noted in Scheme I, the following steps are involved. First, by using a new form of solid-phase peptide synthesis, a peptide fragment is prepared that bears a C-terminal phenolic ester linkage to a thiol-functionalized template, which is optimally a 4,6-disubstituted dibenzofuran. Second, the template thiol is joined by an unsymmetrical disulfide bond to the sulfur of a cysteine residue that appears at the N-terminus of a second peptide fragment. Amide bond formation then occurs in an intramolecular *O,N*-acyl transfer, facilitated by the fit of the template linkage to the transition state for the acyl-transfer reaction. In a final step, spent template is removed by reduction of the disulfide bond. Scheme I also implicitly summarizes a series of practical problems which must be solved before these concepts can be applied to practical peptide synthesis.

Two critical issues are the design and synthesis of a template that permits efficient intramolecular acyl transfer (i.e., effective molarity > 1 M) and a convenient and reliable means by which a phenolic ester linkage can be formed between the template and a medium-sized peptide fragment. Solutions to these problems have been reported in the first two papers of this series,[1] and elsewhere we have given a detailed discussion of the logic of our procedure for template design and optimization.[2] The intramolecular acyl-transfer step has been shown to be compatible with the amino acid side chains,[3] and we have also shown that for the thiol capture reaction sequence, racemization lies below the limits of detectability[4] and that disulfide interchange during the acyl-transfer step can be efficiently and cleanly controlled by the addition of traces of silver ion.[5]

In this paper we deal with two remaining fundamental problems of Scheme I, the capture step A, in which an unsymmetrical disulfide linkage is formed, and the application of the methodology to practical peptide synthesis.

The Thiol Capture Step (1 + 2) → 3. The thiol capture strategy is a highly specialized amide-forming process that has been designed as a solution for the important problem of coupling relatively large peptide fragments

Scheme I



(>10 amino acid residues) reliably and in reasonable yield (>50%). Its major application is expected to be fragment condensation synthesis of large peptides and proteins, for which conventional methodology is highly unreliable. It offers no advantage over existing methodology for the synthesis of small or medium-sized peptides and presum-

†This paper is dedicated to Professor Frederick D. Greene in appreciation of his years of service as Editor of *The Journal of Organic Chemistry*.

(1) Kemp, D. S.; Galakatos, N. G. *J. Org. Chem.* **1986**, *51*, 1821. Kemp, D. S.; Galakatos, N. G.; Bowen, B.; Tan, K. *J. Org. Chem.* **1986**, *51*, 1829.
(2) Kemp, D. S. *Biopolymers* **1981**, *20*, 1793. Kemp, D. S.; Carey, R.; Dewan, J.; Galakatos, N. G.; Kerkman, D.; Leung, S.-L. *J. Org. Chem.* **1989**, *54*, 1589.
(3) Kemp, D. S.; Galakatos, N. G.; Drangenis, S.; Ashton, C. P.; Fotouhi, N.; Curran, T. *J. Org. Chem.* **1986**, *51*, 3320.
(4) McBride, B.; Kemp, D. S. *Tetrahedron Lett.* **1987**, *28*, 3435.
(5) Kemp, D. S.; Fotouhi, N. *Tetrahedron Lett.* **1987**, *28*, 4637.

0022-3263/89/1954-2803$01.50/0

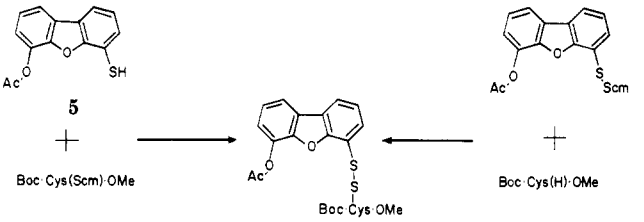
ably will never be used for this purpose, outside of model studies that demonstrate feasibility. The key to the practicality of the overall strategy is the capture step, in which a sulfur-sulfur bond must be formed by reaction of a highly nucleophilic thiolate anion with a sulfenyl derivative bearing a good leaving group. This reaction must meet stringent conditions if the thiol capture strategy is to provide an improvement over conventional amide-forming processes. A brief review of these conditions is appropriate.

Conventional amide bond forming reactions have three fundamental characteristics that cause problems for coupling reactions of large peptide fragments.[6] First, the affinity between a weakly basic peptide amine and an active derivative is often insufficient to allow rapid, unimolecular side-reaction-free coupling at the low concentrations encountered with fragments of molecular weight greater than 10^3 Da. Second, lack of selectivity of the acylating agent toward peptide nucleophiles necessitates complete blocking of side-chain functions and requires that a large number of protective groups must be removed cleanly at the final step of a synthesis. Third, competing solvolysis of the acylating agent at low amine concentrations requires use of dipolar aprotic solvents, for which solvent power for peptides is often marginal and in which coupling-inhibiting association effects within or between peptides are maximal. To represent an improvement over conventional amide-forming methods, the thiol capture reaction must occur rapidly at submillimolar concentrations in protic solvents that show a high capacity for solubilizing peptides and for breaking unproductive inter- and intramolecular associations. Thiol capture should also be compatible either with maximally or minimally blocked peptides.

Reliable procedures for forming specific sulfur–sulfur bonds between cysteine residues of peptides were first explored systematically by Hiskey and co-workers.[7] The introduction of the sulfenylcarbalkoxy function by Brois et al.[8] as an activating agent for thiols was a major advance, and application of this function to peptides by Kamber[9] and by Hiskey[10] provided the foundation for the Ciba-Geigy synthesis of insulin in which the three disulfide bonds are formed selectively.[11] These results provide practical evidence that the potentially labile disulfide bond can withstand the routine bond-forming steps and physical manipulations of peptide synthesis. The decisive evidence on this point was provided by Zahn and co-workers[12] with their studies of relatively large insulin-derived disulfides.

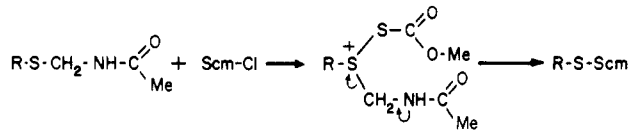
Our initial objective was the demonstration that the Scm (-S-CO-O-Me; sulfenylcarbomethoxy) methodology could be applied not just to the synthesis of cystine derivatives but also to aryl cysteinyl disulfides such as **3**.

Of the two possible routes to aryl cysteinyl disulfides, only the combination of an arenethiol (e.g., **5**) with an Scm-functionalized cysteine gives the desired disulfide **6** cleanly and in good yield. The alternative route involving the Scm derivative of the arenethiol **7** combined with a cysteine thiol was found to give only 38% of the disulfide **6**. More generally the reaction of **9** with Scm derivatives of a series of para-substituted benzene thiols gave the unsymmetrical disulfides in yields of 60–80%, contaminated by symmetrical arenedisulfides which are most likely generated by S–S exchange of the Scm group. An exception was *p*-nitrobenzenethiol, which gave almost exclusively the symmetrical disulfide. By contrast, the reaction of **5** with **8** generates **6** quantitatively (HPLC assay), without byproduct formation (87% yield by isolation).

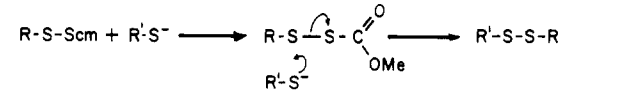


8 6 9

Mixtures of methanol with polar aprotic solvents such as chloroform appear to be the most widely used media for conversion of an *S*-trityl or *S*-Acm to an *S*-Scm derivative and for reactions of the latter with thiols. Although suitable for many protected peptide derivatives of low molecular weight, these solvents lack the solubilizing capacity required for larger peptides. The literature and our own experience suggested a requirement of protic character for both the formation of *S*-Scm derivatives and for their reactions with thiols, a finding which is consistent with the likely mechanisms for **10** → **11** and **11** → **12**, which involve polar intermediates and transition states.



10 11



11 12

If the mechanism of **11** → **12** proceeds as written, then this reaction belongs to the extensively studied class of nucleophilic displacements at bivalent sulfur by thiolate anions.[13] Substituent effects on the rates of these reactions are known to be large, and in the case of the 2,2′-dipyridyl disulfide dication, rate constants have been shown to reach the diffusion-controlled limit.[14] Since the thiolate anion ranks among the most reactive simple nucleophiles and is by far the most nucleophilic species that can be derived from a polypeptide under natural conditions, the proposed thiol capture reaction **11** → **12** has the potential for maximizing both rate and selectivity for any reaction in dilute solution that combines a pair of polyfunctional molecules of high molecular weight, one of which is a normal peptide. Because this point is pivotal, we sought experimental confirmation for the expected solvent effect and mechanism.

Fluorinated alcohols have occasionally been used in peptide synthesis, owing primarily to their effect on the selectivity of acidolysis of protective groups.[15] Shortly

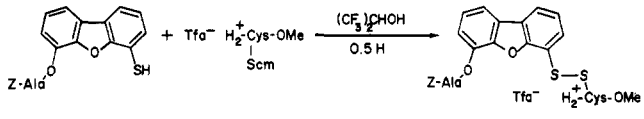
(6) Meienhofer, J. *Biopolymers* **1981**, *20*, 1761. Fanchere, J. L.; Schwyzer, R. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; pp 203–245.
(7) Hiskey, R. G.; Ward, B. F. *J. Org. Chem.* **1970**, *35*, 1118. Hiskey, R. G.; Sparrow, J. T. *J. Org. Chem.* **1970**, *35*, 215.
(8) Brois, S. J.; Pilot, J.; Barnum, H. *J. Am. Chem. Soc.* **1970**, *92*, 7629.
(9) Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 13701.
(10) Hiskey, R. G.; Li, C.; Vunnam, R. *J. Org. Chem.* **1975**, *40*, 3697. Hiskey, R. G.; Mithukumaraswamy, N.; Vunnam, R. *J. Org. Chem.* **1975**, *80*, 950.
(11) Sieber, P.; Kamber, B.; Eisler, K.; Hartmann, A.; Riniken, B.; Rittel, W. *Helv. Chim. Acta* **1974**, *57*, 2617.
(12) Lunkenheimer, W.; Zahn, H. *Justus Liebigs Ann. Chem.* **1970**, *740*, 1.
(13) Ciuffarin, E.; Fava, A. *Prog. Phys. Org. Chem.* **1968**, *6*, 81–111.
(14) Grimshaw, C. E.; Whistler, R. L.; Cleland, W. W. *J. Am. Chem. Soc.* **1979**, *101*, 1521.

Table I. Rate Constants for Disulfide Bond Formation in HFIP, 22 °C (17 + 8) → 18

solvent	[17]	base	[base]	k_2, h^{-1} M^{-1}	$t_{1/2}$, h
		1. Effect of the Structure of Base			
HFIP–MeCN (9:1)	9×10^{-4}	–	–	2.5	15
	4×10^{-4}	Et_3N	4×10^{-4}	31	2.5
		iPr_2NEt		37	2.1
		4-Me-2,6-di-*t*-Bupyridine		36	2.2
		2. Effect of [Base]			
HFIP–MeCN (9:1)	4×10^{-4}	Et_3N	4×10^{-4}	31	2.5
			16×10^{-4}	160	0.53
			40×10^{-4}	large	≤0.1
		3. Effect of Water and Amine			
HFIP–MeCN–H_2O (82:9:9)	9×10^{-4}	–	–	36	2
HFIP–MeCN–H_2O (55:9:36)		–	–	large	≤0.01
HFIP–MeCN–H_2O (78:13:9)		Et_3N	4.4×10^{-4}	large	≤0.05

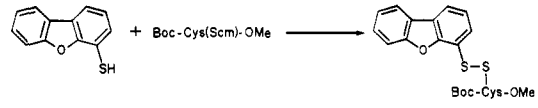
after its introduction by Middleton at Du Pont,[16] hexafluoroisopropyl alcohol (HFIP) was noted by Phillips[17] to dissolve ribonuclease and by Blout and co-workers to have an extraordinarily high and general solubilizing power for polypeptides and proteins.[18] It has been widely used subsequently for conformational studies of oligopeptides. Since HFIP is a suitable solvent for both disulfide bond formation and cleavage, we selected it as our first choice for studies of the thiol capture step.

A variety of simple reaction in this solvent between Scm-functionalized cysteine thiols and dibenzofuran thiols were found to be rapid and clean, products being isolated in all cases in yields greater than 85%. For example **13** reacts with **8** to give the corresponding unsymmetrical disulfide **14** in an isolated yield of 90%. Particularly striking was the reaction of the alanine-functionalized template **13** at 0.007 M concentration with 1 equiv of the trifluoroacetic acid salt of H-L-Cys(Scm)-OMe **15** to yield **16** as an isolated, crystalline solid in 96% yield. From these experiments the capture reaction was found to proceed nearly quantitatively at low concentrations in HFIP, using cysteine derivatives either with or without N-terminal blocking groups.



13 **15** **16**

A detailed knowledge of the important rate-controlling factors for this key reaction would clearly have great practical value, and therefore we carried out a kinetic study. The reaction (8 + 17) → 18 was studied, usually



17 **8** **18**

under pseudo-first-order conditions with a thiol concentration of 4×10^{-4} M, in mixtures of HFIP with acetonitrile and water, with or without tertiary amines as catalysts. Results are reported in Table I.

Two dramatic effects are evident from the data of the Table. First, consistent with a mechanism that involves the thiolate anion of **17** as the reactive species, there is a pronounced catalytic effect of tertiary amines, even at very low concentrations. Strikingly the catalytic efficiency is independent of either the base strength or the degree of steric hindrance, suggesting that at low concentrations the three amines react in this acidic solvent to form lyate anion. Second, there is a dramatic increase in reaction rate—roughly 4 orders of magnitude—when the solvent polarity is increased by the addition of water. In similar mixtures of simple alcohols with water, a comparable rate increase is seen for other polar reactions such as the solvolysis of tertiary halides.[19] Evidently the transition state for the disulfide bond forming reaction has a substantial polar character, owing most likely to a combination of leaving group effects and the participation of the anionic thiolate as nucleophile. Polar effects were also strikingly apparent when the urethane-blocked cysteine derivative **8** was replaced with the salt $Tfa^-H_2^+$-L-Cys(Scm)OMe **15** in the reaction with **17**. In a solvent mixture containing HFIP–MeCN (9:1) and under second-order conditions in the absence of basic catalyst, the salt reacts to completion (94% yield) with an equivalent of **17** at 4.5×10^{-4} M concentration within 5 min. A comparison with the first entry of Table I suggests a rate-accelerating effect of greater than 4 orders of magnitude for the change of a (*tert*-butoxycarbonyl)amine to an ammonium salt. A through-bond electron withdrawal experienced at the cysteine sulfur, a salt effect on the equilibrium constant for the formation of thiolate anions, and a stabilization of a thiolate–ammonium ion pair are all likely contributors to this striking rate increase.

As a result of these studies, practical conditions are demonstrated for the thiol capture reaction at submillimolar concentrations in solvents that have unusually large capacities for solubilizing peptides and proteins. The results of this section are epitomized by the following observation: when equal concentrations of **17** and **8** (4.4×10^{-4} M) are allowed to react in HFIP–MeCN–water (55:9:36), a half-life of 20 min is seen at 25 °C, and after 10 half-lives 91% of **18** is observed, containing 1% of the symmetrical disulfide of **17**.

Together with results of previous model studies the above data now define the practical conditions that must be used to achieve thiol capture ligation of peptide fragments reliably and in good yield. The stage was now set for a pilot application of this methodology to the coupling of fragments of a small protein. In the following sections of this paper, we report synthesis by thiol capture of the

(15) Moroder, L.; Gemeiner, M.; Göhring, W.; Jaeger, E.; Wünsch, E. In *Peptides 1980*; Bronfeldt, K., Ed.; Scriptor: Copenhagen, 1981; pp 121–126. See also: Hiskey, R. G. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1981; pp 145–147.

(16) Middleton, W. J.; Lindsey, K. V. *J. Am. Chem. Soc.* **1964**, *86*, 4948.

(17) "Hexafluoroisopropanol", Product Information Sheet, Du Pont Co., Polymer Products Department, Elastomers Division, Wilmington, DE, 1988.

(18) Parrish, J. R.; Blout, E. R. *Biopolymers* **1971**, *10*, 1491; **1972**, *11*, 1001.

(19) Olson, A. R.; Halford, R. S. *J. Am. Chem. Soc.* **1937**, *59*, 2644. Grunwald, E.; Winstein, S. *J. Am. Chem. Soc.* **1948**, *70*, 846.

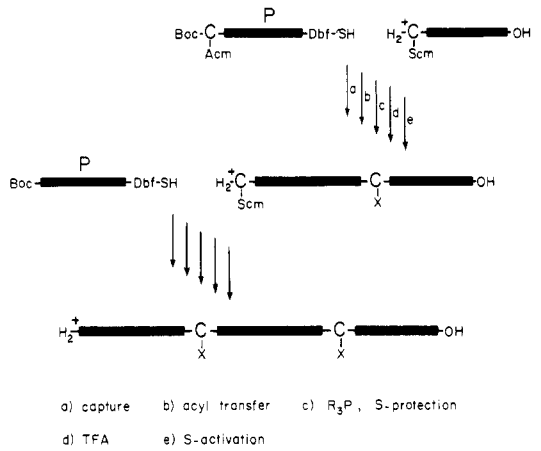


Figure 1. Assembly tactic of the thiol capture strategy. The presence of P indicates that the side chains of the peptide segment are protected; the protective groups are tBu for D, E, S, T; Boc for K; Ans or Pmc for R; Dnp for Y. X represents the protective group of the nascent cysteine thiol; i.e. acetyl or Dnp. Scm: methoxycarbonylsulfenyl, Acm: acetamidomethyl.

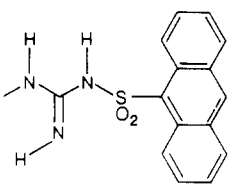
C-terminal half of the 58-peptide, basic pancreatic trypsin inhibitor, BPTI.

Synthesis of Fragment 30–58 of BPTI: A Practical Demonstration of the Thiol Capture Strategy. A. Preliminary Methodology. A variety of different tactics for applying thiol capture to protein synthesis are under development, but in this first report we rely exclusively on the linear assembly tactic summarized in Figure 1, in which fully blocked N-terminal peptide fragments are prepared and added serially from right to left to a growing C-terminal fragment, which is fully deblocked, except for cysteine protection. Each of the sets of multiple arrows of Figure 1 thus represents the chemical steps of capture, acyl transfer, template cleavage and S-protection, deblocking, and S-activation, together with purification. This tactic thus provides a critical first test of the feasibility of using the thiol capture strategy to N-α-acylate unprotected peptides bearing an Scm-functionalized N-terminal cysteine residue.

A preliminary to the thiol capture strategy is the solid-phase synthesis of side-chain-blocked peptide fragments bearing a suitably functionalized cysteine residue at the N-terminus and a 4-(acyloxy)-6-mercaptodibenzofuran at the C-terminus. Modern solid-phase synthesis offers three major alternatives for N-α-blocking and side-chain blocking.[20] Of these we were led to choose the combination of *tert*-butyl functions at most of the side-chain sites and *N*-α-Bpoc at the N-terminus of the growing chain.[21] (Bpoc ≡ 2-(*p*-biphenylyl)-2-propyloxycarbonyl; a glossary of abbreviations appears in the Experimental Section.) The obligatory use of secondary amines as deblocking reagents in the *N*-α-Fmoc strategy is incompatible with our phenyl ester linkage, and we sought to avoid the use of HF or other strong acids that are required for removal of side-chain benzyl functions required for the popular *N*-α-Boc strategy. Elsewhere we have reported detailed synthetic protocols for the preparation of chromatographically homogeneous *N*-α-Bpoc amino acid derivatives that are required for these syntheses.[22]

Within the thiol capture strategy every choice of tactic for fragment combination places particular demands on the three types of protective groups required for the thiol function of cysteine. The demands created by the linear tactic of Figure 1 are the simplest. First, an *activatable* blocking group must withstand the requirements of solid-phase synthesis and one cycle of thiol capture ligation, including purification. It must also be capable of undergoing clean S-activation. Although we will report elsewhere on the utility of the *N*-α-Boc-2,2-dimethylthiazolidinyl-5-carboxamido function for this purpose, for the work here described we have relied exclusively on the *S*-acetamidomethyl (Acm) function developed by the Merck group.[23] Second, an *activated* group must be formed from the Acm group and must permit clean thiol capture. Because of its kinetic properties discussed above, we have used the Scm group of Brois[8] throughout this study. Third, a *passive* group must be selectively, conveniently, and quantitatively introduced after the template cleavage step (Step C of Scheme I). It then must withstand conditions of S-activation, synthesis, and purification, yet be cleanly and selectively removable under mild conditions at the final step of the synthesis. In this study we have explored both *S*-acetyl and *S*-2,4-dinitrophenyl.[24] We have also used the 2,4-dinitrophenyl group to block the phenolic oxygen of tyrosine.[25]

BPTI lacks tryptophan and histidine, two of the amino acids that require special side-chain protection. However the presence of six arginine residues in the BPTI sequence has caused us to examine carefully two blocking groups for the guanidino function of arginine, the Ans group **19** which we have previously described[26] and the Pmc group **20** recently reported by Ramage and co-workers.[27]



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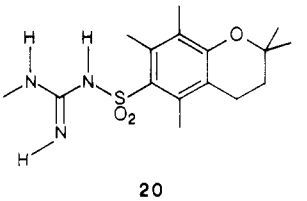

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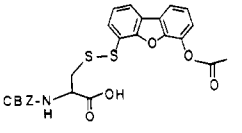
Solid-phase peptide synthesis on a disulfide-linked, resin-bound dibenzofuran template requires a convenient and reliable means of achieving resin attachment. We have explored several improved alternatives to the method we first reported,[1] including use of a PAM resin spacer,[28] but have found the most satisfactory to be coupling of the functionalized cysteine derivative **21** to purified aminomethyl polystyrene,[29] followed by hydroxylamine treat-



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(20) Barany, G.; Kneib-Cordonier, N.; Mullen, D. G. *Int. J. Peptide Protein Res.* **1987**, *30*, 705. Merrifield, R. B. *Science* **1986**, *232*, 341.
(21) Wang, S.; Merrifield, R. B. *Int. J. Peptide Protein Res.* **1969**, *1*, 235. Feinberg, R. S.; Merrifield, R. B. *Tetrahedron* **1972**, *28*, 5865. Mojsov, S.; Merrifield, R. B. *Biochemistry* **1981**, *20*, 2950.
(22) Kemp, D. S.; Fotouhi, N.; Boyd, J. G.; Carey, R. I.; Ashton, C. P.; Hoare, J. *Int. J. Peptide Protein Res.* **1988**, *31*, 359.
(23) Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschman, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456.
(24) Zahn, V. H.; Traumann, K. *Z. Naturforsch.* **1954**, *9B*, 518. Shaltiel, S. *Isr. J. Chem.* **1974**, *12*, 403. Shaltiel, S.; Soria, M. *Biochemistry* **1969**, *8*, 4411.
(25) Fridkin, M.; Hazum, E.; Tauer-Finkelstein, M.; Shaltiel, S. *Arch. Biochem. Biophys.* **1977**, *178*, 517.
(26) Arzeno, H.; Kemp, D. S. *Synthesis* **1987**, *1*, 32.
(27) Ramage, R.; Green, J. *Tetrahedron Lett.* **1987**, *28*, 2287.
(28) Mitchell, A. R.; Kent, S. B. H.; Engelhard, M.; Merrifield, R. B. *J. Org. Chem.* **1978**, *43*, 2845.
(29) Gisin, B. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1972**, *94*, 6165.

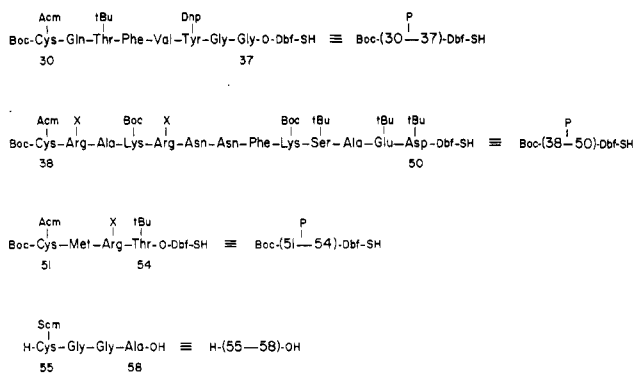


Figure 2. Protected peptide fragments of BPTI. The numbers under the amino acids indicate the position of the particular residue in BPTI.

ment to cleave the acetoxy function, and acylation of the resulting phenol during the first step of the solid-phase synthesis. Yields in solid-phase synthesis of 99.5% or greater per acylation step are routine with this (acyloxy)dibenzofuran-functionalized resin preparation.

The following sections report synthesis by solid phase of the four side-chain-blocked BPTI sequences shown in Figure 2. Together, these four fragments encompass the C-terminal region 30–58 of the BPTI molecule.

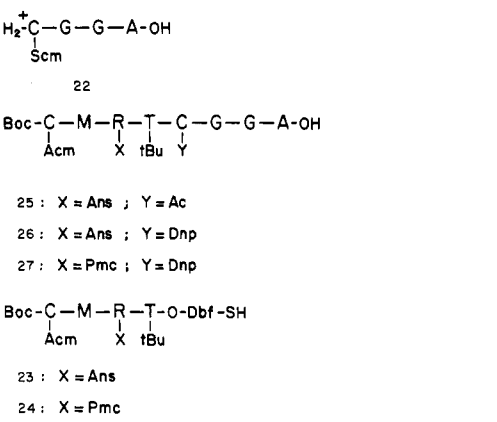
B. Synthesis and Coupling of BPTI Fragments 51–54 and 55–58. Selection of the 2,4-Dinitrophenyl (Dnp) Protective Group for Cysteine and the Pentamethylchromanesulfonyl (Pmc) Group for Arginine. When applied to the BPTI molecule, the tactics of Figure 1 dictate a first thiol capture coupling of Boc-C(Acm)-(52–54)-O-DBF-SH + H-C(Scm)-(56–58)-OH. The simplicity of this coupling allowed its use for testing and optimization of many reaction conditions and variables. The choices of the inert protective group for the cysteine thiol and of the guanidino blocking group for arginine were primary objectives of these studies.

As reported in the Experimental Section the required C-terminal tetrapeptide fragment H-Cys(Scm)-Gly-Gly-Ala-OH, **22**, was generated in pure form (>99.5%) by standard solution synthesis in 66% yield based on Z-Gly-Gly-OH. Uneventful solid-phase synthesis generated the functionalized tetrapeptide derivatives Boc-Cys-(Acm)-Met-Arg(Ans)-Thr(tBu)-O-DBF-SH, **23**, and Boc-Cys(Acm)-Met-Arg(Pmc)-Thr(tBu)-O-DBF-SH, **24**, in respective weight yields of 94% and 99% and respective HPLC purities of 97% and 98%.

Three thiol capture syntheses of the octapeptide corresponding to residues 51–58 of the BPTI sequence were carried out, generating as final intermediates the blocked peptides Boc-Cys(Acm)-Met-Arg(Ans)-Thr(OtBu)-Cys-(Ac)-Gly-Gly-Ala-OH, **25**, Boc-Cys(Acm)-Met-Arg(Ans)-Thr(OtBu)-Cys(Dnp)-Gly-Gly-Ala-OH, **26**, and Boc-Cys-(Acm)-Met-Arg(Pmc)-Thr(OtBu)-Cys(Dnp)-Gly-Gly-Ala-OH, **27**.

The four-step reaction sequence of the Ans derivative **23** + **22** of thiol capture, acyl transfer, HO-4,6-DBF-SH cleavage, and thiol trapping with acetic anhydride generated the (Ans, Ac) derivative **25** in an overall yield of 45–50% after purification. Side-chain deprotection with retention of S-blocking was next addressed.

The *S*-acetyl protective group offered advantages of low molecular weight, simple NMR marker characteristics, potential for radiolabeling, and ease of final removal. These attractive features led us to give it serious study despite its lability under basic or nucleophilic conditions. Model experiments had shown that simple *S*-acetylcysteine derivatives are sufficiently inert to TFA and the mildly

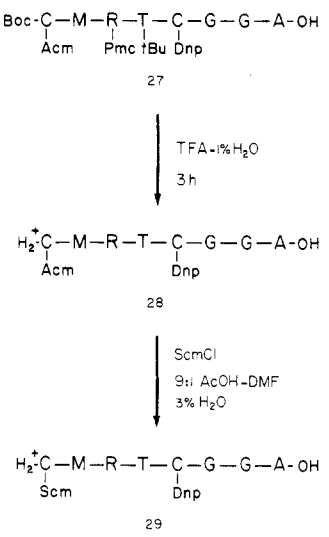


acidic aqueous conditions of HPLC separations to be worth initial scrutiny. Unfortunately the more stringent conditions required for deblocking of **25** revealed problems. Clean removal of the Ans group was found to require treatment with TFA for 7 h in the presence of thioanisole as scavenger, and under either these conditions or the milder ones employed for Pmc removal the *S*-acetyl function of **25** rapidly undergoes complex transformations that render it unusable.

Despite its bulk and lipophilicity the 2,4-dinitrophenyl group has much to recommend it as the inert blocking group for the thiols of cysteine residues. Model studies with proteins have demonstrated the clean and selective introduction and cleavage of this group under mild conditions,[24] and its simple and distinctive ^{1}H NMR resonances and characteristic long-wavelength UV absorption permit detection and quantitative assay. Model experiments established that the *S*-Dnp group resists TFA treatment in the presence of thioanisole for many hours as well as treatment with phosphines, DMSO, and thiols in the absence of base. In accord with literature observations it is rapidly and cleanly cleaved by 2-mercaptoethanol under protic conditions at pH 8.[24] Repetition with **23** + **22** of the four-step reaction sequence of capture, acyl transfer, HO-4,6-DBF-SH cleavage followed by trapping with 2,4-dinitrofluorobenzene Dnp-F formed the (Ans, Dnp) derivative **26** in 50% yield after purification. This product was cleanly converted to H-Cys(Acm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH (**28**) by reaction with TFA/thioanisole for 14 h at 25 °C and purification by partition chromatography and preparative HPLC gave material in 83% yield that was homogeneous by ^{1}H NMR and analytical HPLC.

As described in the next section, problems encountered during the synthesis and deblocking of the BPTI fragment 38–58 led us to replace the guanidino blocking group Ans with the Pmc group recently reported by Ramage and co-workers. The template-functionalized Pmc derivative **24** was subjected to capture with **22**, acyl transfer, HO-4,6-DBF-SH cleavage, and thiol trapping with Dnp-F to generate the (Pmc,Dnp) derivative **27** in 70% yield after purification. As indicated in Scheme II, cleavage of the Pmc group, Boc group, and *tert*-butyl ether function in the absence of thioanisole but in TFA containing a trace of water for 3 h at 25 °C proceeded cleanly to yield H-Cys(Acm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH (**28**) in 87% yield after purification by preparative HPLC. Conversion of this substance to the Scm derivative H-Cys(Scm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH (**29**) was carried out by reaction with Scm-Cl in moist 9:1 acetic acid–dimethylformamide at 0 °C to yield **29** in 86% yield after purification by preparative HPLC. The disappearance of the ^{1}H NMR resonances associated with the

Scheme II



Cys(Acm) function and the appearance of the Scm singlet at δ 3.95, together with the integrity of the Dnp resonances established that selective S-activation of a Cys(Acm) function in the presence of an inert Cys(Dnp) function had occurred. The purity of this product was shown by analytical HPLC and ^{1}H NMR to be greater than 99.5%.

Careful scrutiny of the reaction of **28** with Scm-Cl in dry acetic acid by HPLC and ^{1}H NMR revealed the formation of an intermediate, which is presumably the result of an initial reaction of the methionine thioether function with the reagent. Clean, concentration-dependent conversion of this species to **29** was observed, and an increase in the solvent polarity by the addition of 3% of water resulted in clean transformation of **28** to **29** without detectible intermediates.

Synthesis of BPTI Fragment 38–50 and Thiol Capture Coupling with 51–58 To Form BPTI Fragment 38–58. The 13-peptides Boc-Cys(Acm)-Arg(X)-Ala-Lys(Boc)-Arg(X)-Asn-Asn-Phe-Lys(Boc)-Ser(tBu)-Ala-Glu(tBu)-Asp(tBu)-O-4,6-DBF-SH, **30a** (X = Ans) and **30b** (X = Pmc), that comprise alternative blocking patterns for the third BPTI fragment are heavily functionalized with an unusual density of side-chain-blocked amino acid derivatives. The resulting lipophilic character, the difficulty of carrying out Arg couplings to completion, the presence of the Asn-Asn sequence, and the extended conformation that this sequence exhibits in the native conformation of BPTI all may contribute to the difficulties that were encountered during solid-phase synthesis of this species.

Addition of the first six C-terminal amino acids of fragment 38–50 to the resin-bound template proceeded uneventfully in an average yield of 99.8% per coupling step. Difficulties began with the first asparagine coupling reaction, for which an optimal coupling procedure was found to involve pregeneration of the active ester Bpoc-Asn-OBt by reaction of 0.5 M Bpoc-Asn-OH with equivalents of DCC and HOBt in a mixture of dichloromethane and DMF for 15 min at 0 °C. (Contrary to literature reports[30] no decomposition of 0.5 M Bpoc-Asn-OH was observed over 24 h in this solvent mixture.) Two successive Asn couplings generated the C-terminal 8-peptide sequence of 38–50, accompanied by 5% of HOBt-induced liberation of the template phenol, which was capped by acetylation with acetic anhydride.

(30) Mojsov, S.; Mitchell, A. R.; Merrifield, R. B. *J. Org. Chem.* **1980**, *45*, 555.

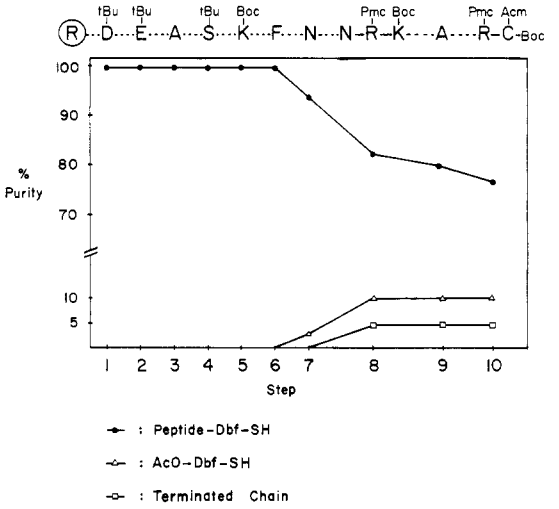


Figure 3. Solid-phase synthesis of fragment 38–50, **30b**. The dotted line indicates the formation of a peptide bond. Terminated chain, Ac-N-F-K(tBu)-S(tBu)-A-E(tBu)-D(tBu)-O-Dbf-SH; Ac-O-Dbf-SH: 4-acetoxy-6-mercaptodibenzofuran. The percent purity is taken directly from the integration of the corresponding peak on the HPLC trace.

Attempts to introduce the 42-Arg function by acylation with Bpoc-Arg(Ans)-OH and DCCI in dichloromethane resulted in an extremely slow coupling rate, and the resin was found to contain amine function that could not be completely acetylated with acetic anhydride over 2 h. Although coupling rates returned to normal after the addition of the 41-Lys(Boc) residue, the resulting peptide was obtained as a mixture and could only be isolated in relatively low yield. Difficulties of this type have been documented by others[31] and have been remedied by solvent changes which we were not able to realize in this case. A satisfactory synthesis of this material was achieved by introducing the troublesome Arg(Ans)-Asn sequence as a performed dipeptide, but coupling with the 51–58 fragment followed by TFA deblocking for 7 h in the presence of thioanisole led to a complex mixture, apparently owing to difficulties associated with the Ans removal.

Replacement of the troublesome Ans with the Pmc group, which had met preliminary model studies including the successful synthesis of the 51–58 fragment described above, led to the hope that the less bulky Pmc group would lessen the rate problem encountered in the solid-phase synthesis. In fact, no improvement in rate or purity of product was observed during a conventional solid-phase synthesis if Bpoc-Arg(Ans)-OH was replaced with Bpoc-Arg(Pmc)-OH. In this example variation of the bulk and lipophilicity of a side chain blocking group in the region of reduced coupling rate and yield had no effect on the association phenomenon that results in resin shrinkage and rate retardation. This result is consistent with a dominant role of the Asn-Asn sequence in yield-reducing association effects, or alternatively it is consistent with a threshold lipophilicity value for the Arg side chain that both protective groups unfortunately meet.

A successful synthesis of the fragment 38–50 was achieved by a hybrid linear/fragment condensation solid-phase tactic as outlined as Figure 3 using two classically synthesized peptide fragments, the tripeptide derivative

(31) Westall, F. C.; Robinson, A. B. *J. Org. Chem.* **1970**, *35*, 2842. Hancock, W. S.; Prescott, D. J.; Marshall, G. R.; Vangelos, P. R. *J. Biol. Chem.* **1972**, *247*, 6224; *J. Org. Chem.* **1973**, *38*, 774. Atherton, E.; Woolley, V.; Sheppard, R. C. *J. Chem. Soc. Chem. Commun.* **1980**, 971. Sarin, V. K.; Kent, S. B. H.; Merrifield, R. B. *J. Am. Chem. Soc.* **1980**, *102*, 5463.

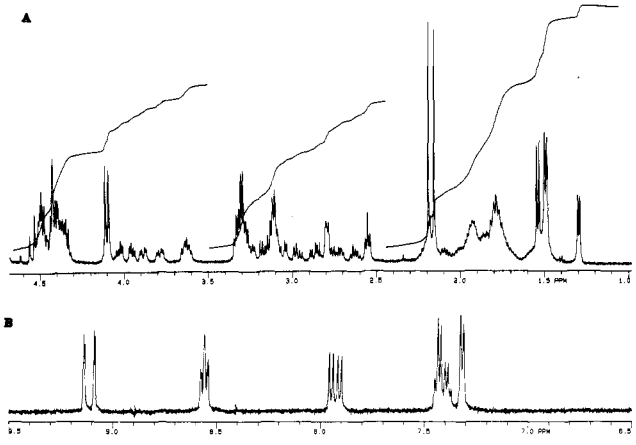


Figure 4. 500-MHz ^1H NMR spectra of **31** in D_2O, at 22 °C. (A) 1.0–5 ppm region; (B) 6.5–9.5 ppm region.

Bpoc-Lys(Boc)-Arg(Pmc)-Asn-OH and the dipeptide derivative Boc-Cys(Acm)-Arg(Pmc)-OH. Coupling with the latter avoided a troublesome double acylation during the introduction of 39-Arg. Unacylated peptide chains were capped with acetic anhydride after the seventh and eighth steps shown in the figure, and included in it are yields based on the amount of internal standard, the 4-acetoxydibenzofuran-6-thiol released with the peptide after phosphine cleavage.

Liberation of **30b** from the resin deserves special comment. Previously we have noted that for inductive reasons the aspartyl ester linkage to the dibenzofuran template is highly activated, and excess phosphine in the disulfide reduction step must be avoided to prevent hydrolysis or alcoholysis of the unusually reactive template linkage. Moreover, careful choice of the cleavage solvent was necessary to solubilize this highly associating peptide and extract it from the interior space of the resin. Confirming an earlier observation of Li and Blake[32] on the remarkable swelling effect of dichloromethane–trifluoroethanol mixtures, we consistently obtained 95% weight recovery of **30b** using a 4:1 mixture of DCM–TFE containing 1 equiv of triethylphosphine for 15 min. By analytical HPLC the purity of **30b** isolated in this manner was found to be 75–80%.

The released peptide **30b** was combined without purification in a thiol capture step with the 51–58 fragment **29**, then subjected to acyl transfer, HO-4,6-DBF-SH cleavage, Dnp-F thiol blocking, TFA cleavage (2 h, trace of water), and purification by Sephadex G-25 gel filtration and HPLC chromatography on a preparative reverse-phase Vydac column to yield the 21-peptide H-Cys(Acm)-Arg-Ala-Lys-Arg-Asn-Asn-Phe-Lys-Ser-Ala-Glu-Asp-Cys-(Dnp)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH ≡ H-C-(Acm)-(39–50)-C(Dnp)-(52–54)-C(Dnp)-(56–58)-OH (**31**) in an overall yield of 52%. The purified product was homogeneous (>99%) by HPLC and showed the correct amino acid sequence and the correct molecular mass by FAB mass spectrometry; as noted in Figure 4, **31** also exhibited a clean 500-MHz ^1H NMR spectrum. Conversion to the 38-Cys(Scm) derivative **32** was carried out uneventfully in 90% isolated yield as described for **29**. The FAB mass spectrum of **32** showed all the expected C^{12} and C^{13} molecular ions in the ratios expected from natural abundances as noted in Figure 5.

Synthesis of BPTI Fragment 30–37 and Thiol Capture Coupling with 38–58 To Form BPTI Fragment

(32) Yamashiro, D.; Blake, J.; Li, C. H. *Tetrahedron Lett.* **1976**, *18*, 1469.

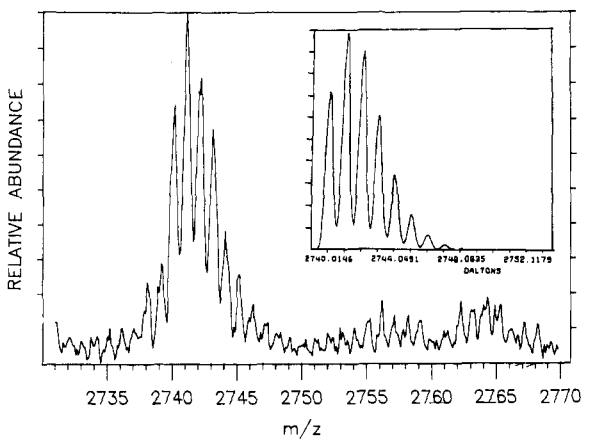
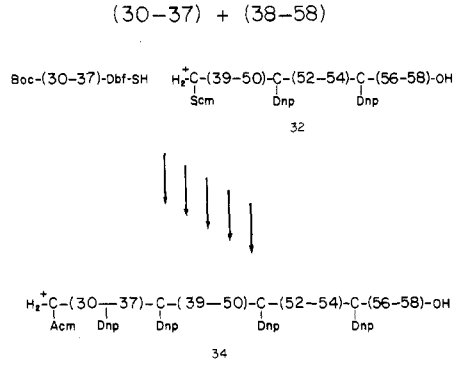


Figure 5. Fast atom bombardment spectrum of **32** in a *m*-nitrobenzyl alcohol matrix. The theoretical isotopic mass distribution for $C_{104}H_{159}N_{38}O_{40}S_2$ is shown in the box.

Scheme III[a]



[a] (a) Capture in HFIP–H_2O (3:1) for 45 min; (b) acyl transfer: 10^{-4} M in DMSO, DIEA, 1 equiv, under N_2, at 25 °C, in the dark, 4 h; (c) 2 equiv of Et_3P in dioxane–water (7:3), 15 min; Dnp-F (4 equiv) in 1:1 TFE–pH 7.5 $NaHCO_3$ buffer; (d) TFA with 1% H_2O for 1.5 h. Overall yield: 60%.

30–58. The third amide ligation by thiol capture was initiated by the uneventful solid-phase synthesis of the template-functionalized fragment Boc-Cys(Acm)-Gln-Thr(tBu)-Phe-Val-Tyr(Dnp)-Gly-Gly-O-DBF-SH in a yield per coupling step of 99.5%. As summarized in Scheme III, this fragment was combined without purification in a thiol capture step with the 38–58 fragment **32**, and the product **33** of capture was isolated by preparative HPLC as the TFA salt in 86% yield. The desired 29-peptide **34** was generated when **33** (90 μM) was treated under standard conditions with 1.1 equiv of diisopropylethylamine in purified DMSO at 25 °C in the dark for 4 h, followed by solvent removal, phosphine-induced HO-4,6-DBF-SH cleavage, Dnp-F thiol blocking, and TFA treatment (1.5 h). Purification by preparative HPLC yielded the desired **34**, 70%, characterized by amino acid analysis and sequencing, and FAB mass spectrometry. The purity of high molecular weight peptides prepared by thiol capture ligation is apparent from the chromatograms of Figure 6, which shows the analytical HPLC traces for **31** and **34**, each isolated after a single preparative HPLC purification.

The formation of **34** provides the first evidence of clean amide bond formation by a thiol capture sequence involving an unprotected peptide fragment that bears potentially reactive side chain functionalities. However, it is significant that in the presence of a large excess of tertiary amine (10–20 equiv) a competing intramolecular acylation involving the ε-amino group of 41-lysine is also observed. No acylation of the ε-amino group 46-lysine can

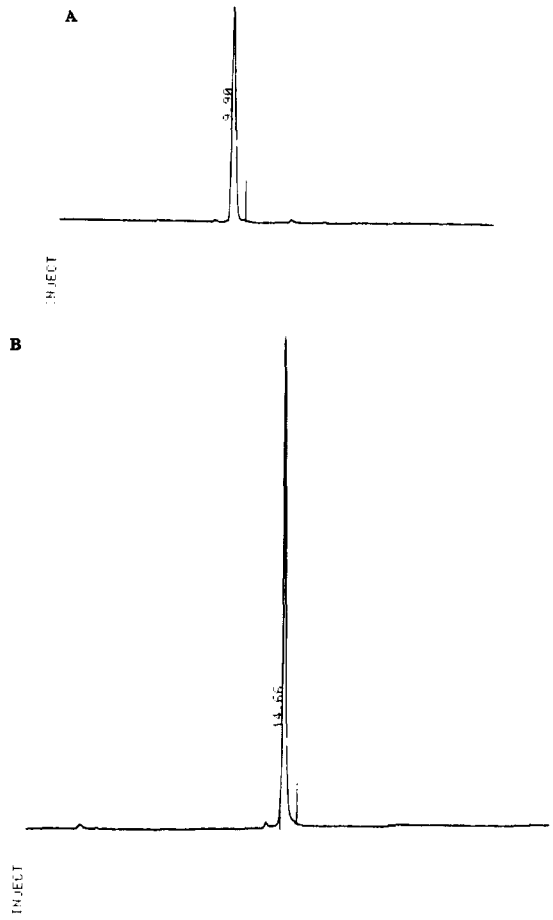


Figure 6. (A) HPLC trace of **31**; 25% MeCN in 0.1% TFA to 35% MeCN over 20 min; 254 or 214 nm; Vydac 218TP54 C-18 reverse-phase column; 1 mL/min. (B) HPLC trace of **34**; 30–55% MeCN in 0.1% TFA over 20 min; 254 or 214 nm; Vydac 218TP54 C-18 reverse-phase column; 1 mL/min.

be detected, and we believe that this interesting and unusual result which can be suppressed by proper amine stoichiometry is highly dependent on both lysine position and neighboring amino acid sequence. Results of experiments to define the scope of the intramolecular nucleophilicity of lysine ϵ-amino functions will be reported subsequently.

Summary and Conclusions

The first step of the thiol capture strategy for ligating peptide fragments involves disulfide bond formation. Optimal conditions for this reaction combine an Scm derivative of a protonated N-terminal cysteine residue with a 4-(acyloxy)-6-mercaptodibenzofuran in a mixture of hexafluoroisopropyl alcohol and water. Under these conditions disulfide bond formation is complete in minutes at submillimolar reactant concentrations.

A linear tactic of thiol capture requires three types of cysteine S-functionalization, and the set consisting of Acm (S-acetamidomethyl), Scm (*S*-thiocarbomethoxy), and Dnp (*S*-2,4-dinitrophenyl) has been shown to be functional. A working example of the thiol capture strategy is provided by the synthesis in pure form of the C-terminal 29-peptide from the sequence of bovine pancreatic trypsin inhibitor BPTI by a linear sequence of three thiol capture-mediated amide ligations. Yields of purified material from each five-step cycle of thiol capture lie in the range of 50–75% and are acceptable for the last steps of a highly convergent synthetic protocol. Alternative choices for S-protection may allow a more convergent tactical alternative to the linear assembly protocol used in this report, and these are under study. Completion of the BPTI synthesis as well as application of thiol capture ligation to the synthesis of other small proteins are in progress and will be reported subsequently.

Experimental Section

High-resolution ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker WM-250, a Bruker WM-270, a Varian XL-300, or a Varian VXR-500 instrument. Chemical shifts are reported in ppm downfield from Me_4Si and splitting patterns are designated as s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet; b, broad. Low-resolution, high-resolution and field desorption mass spectra were recorded on a Varian MAT-44, CEC-110, and a Finnigan MAT-731 mass spectrometers, respectively. UV spectra were taken on a Perkin-Elmer Model 330 UV–vis spectrophotometer. Fast atom bombardement spectra of high molecular weight peptides were obtained on a Finnigan MAT-8200 mass spectrometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Amino acid sequences were obtained on an Applied Biosystems 470A gas-phase sequencer. Amino acid analyses were conducted on the Pico Tag system developed at Waters Associates, Inc.;[33] hydrolysates were prepared either in solution phase at 110 °C for 30 h in 6 N HCl, or in the gas phase at 108 °C for 24 h in the presence of 6 N HCl.

Analytical thin-layer chromatography was performed on glass precoated silica gel 60 plates (Merck F-254). Compounds were visualized by UV absorption (254 nm), phosphomolybdic acid, 1% ninhydrin in a 9:1 EtOH–CF_3CO_2H mixture (primary and secondary amines), and Ellman's reagent (thiols). Preparative layer chromatography was performed on Analtech GF 1000 μm and GF 2000 μm silica gel plates, and flash chromatography on silica gel 60 (230–400 mesh).

Analytical HPLC was performed on a Waters system consisting of two Model 6000–A pumps, a Model 680 automated gradient controller, a Model U6K injector, a Model 440 dual channel UV detector (280, 254 nm), a Model 490 programmable multiwavelength detector, a Model 730 data module, and a Vydac 218TP54 reverse phase, C-18 column. Preparative HPLC was performed on a system including a Waters Associates Model 590 pump fitted with preparative heads, a Rheodyne injector, Autochrome OPG/S prepump mixer, a Waters Associates Model 450 variable-wavelength detector, and a Vydac 218TP1022 reverse-phase, C-18 column.

Abbreviations: DMF, dimethylformamide; DMAc, dimethylacetamide; DMSO, dimethyl sulfoxide; DCM, dichloromethane; $CHCl_3$, chloroform; DME, dimethoxyethane; ether, diethyl ether; EtOAc, ethyl acetate; MeCN, acetonitrile; MeOH, methanol; EtOH, ethanol; TFE, trifluoroethanol; HFIP, hexafluoroisopropyl alcohol; HOAc, acetic acid; TFA, trifluoroacetic acid; THF tetrahydrofuran; CHA, cyclohexylamine; DCHA, dicyclohexylamine; DEA, diethylamine; DIEA, diisopropylethylamine; TEA, triethylamine; DCCI, dicyclohexylcarbodiimide; ScmCl, methoxycarbonylsulfenyl chloride; Dnp, dinitrophenyl; Dnp-F, dinitrofluorobenzene; Acm, acetamidomethyl; Ans, anthracenesulfenyl; Pmc, pentamethylchromane; Dbf, dibenzofuran.

4-Mercaptodibenzofuran (17). To a solution of dibenzofuran (33.6 g, 0.20 mol) in anhydrous diethyl ether under N_2 (400 mL) was added dropwise (20 min) at 0 °C a solution of *n*-butyllithium (0.22 mol) in *n*-hexane (92.0 mL), and the mixture was heated to reflux for 20 h. The resulting yellow-green suspension was cooled to 0 °C, elemental sulfur (Baker, sublimed, N.F.; 7.04 g, 0.22 mol) was added in 4 portions (15 min total), and the mixture was allowed to warm up to room temperature and then was heated to reflux for 2 h. The mixture was then cooled to 0 °C and cautiously acidified to pH 1 with 20% HCl (80 mL). The aqueous phase was extracted with ether, and the organic layers were combined, washed with 1 N HCl and water, and extracted with 1 N NaOH (3×). The alkaline phases were combined, washed with ether (2×), and acidified to pH 1 with concentrated HCl, and the aqueous mixture was extracted with ether. The organic layers were evaporated. The resulting dark yellow oily residue (32.8 g, 82%) was distilled under reduced pressure (160 °C, 0.4

(33) Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. *J. Chromatogr.* 1984, *336*, 93.

Torr) to afford 17 as a light yellow-green oil that solidified on standing (28.6 g, 71%), mp 46.0–47.5 °C (lit.[34] 33%, mp 50.5–52.0 °C).

Anal. Calcd for $C_{12}H_8OS$: C, 71.97; H, 4.03; S, 16.01. Found: C, 71.98; H, 3.97; S, 15.97.

4-(Methoxycarbonyldithio)-6-acetoxydibenzofuran (7). As reported by Kemp and Galakatos,[1] reaction of ScmCl with 4-hydroxy-6-mercaptodibenzofuran gave 4-(methoxycarbonyldithio)-6-hydroxydibenzofuran. To a solution of this substance (176 mg, 0.575 mmol) in acetic anhydride (2.50 mL) was added a catalytic amount of concentrated sulfuric acid (5 μL), and the clear mixture was stirred at room temperature under N_2 for 4 h. The solution was then poured into 5% $NaHCO_3$–ice and extracted with DCM (3×). The organic layers were combined, back-washed with 5% $NaHCO_3$, washed with water and brine, dried ($MgSO_4$), and evaporated. The oily residue (208 mg) was azeotroped with MeCN (2×) and dried under high vacuum to afford pure 7 as a white powder (194 mg, 97%), mp 95–6 °C.

^1H NMR (250 MHz, $CDCl_3$): δ 2.50 (3 H, s, Ar:OAc), 3.90 (3 H, s, Ar:SScm), 7.25–7.38 (3 H, m), 7.73 (1 H, dd, J = 8, 1 Hz), 7.79 (1 H, dd, J = 8, 1 Hz), 7.91 (1 H, dd, J = 8, 1 Hz, C_1-H). High-resolution mass spectrum calcd for $C_{16}H_{12}O_5S_2$ 348.0126, found 348.0115.

4-Mercapto-6-acetoxydibenzofuran (5). A solution of 350 mg of 7 (1.00 mmol) in 5 mL of 4:1 dioxane–water was treated at 25 °C under N_2 with 0.25 mL (1.05 mmol) of Bu_3P. Lyophilization yielded a white solid that was recrystallized from DCM–pentane to yield 5, 225 mg (87%) of pale yellow prisms, mp 115–116 °C. ^1H NMR (250 MHz, $CDCl_3$): δ 2.48 (3 H, s, Ar:OAc), 3.94 (1 H, s, Ar:SH), 7.22–7.40 (4 H, m), 7.76 (1 H, dd, J = 8, 1 Hz), 7.81 (1 H, dd, J = 8, 1 Hz).

Anal. Calcd for $C_{14}H_{10}O_3S$: C, 65.10; H, 3.90; S, 12.41. Found: C, 65.33; H, 4.05; S, 12.44.

Methyl *N*-(*tert*-Butoxycarbonyl)-*S*-(methoxycarbonylsulfenyl)-L-cysteinate (Boc-L-Cys(Scm)-OMe, 8). The title substance was obtained in 85% yield, via the procedure of Hiskey et al.[7] by the reaction of ScmCl with Boc-L-Cys-OMe, as white needles from hexane, mp 76.5–78.0 °C (lit. mp 78 °C). ^1H NMR (250 MHz, acetone-d_6): δ 1.42 (9 H, s, Boc), 3.21–3.40 (2 H, m, methylene), 3.72 (3 H, s, Cys:OMe), 3.89 (3 H, s, Cys:Scm), 4.51–4.63 (1 H, m, Cys:methine), 6.47 (1 H, b d, J = 8 Hz, urethane). UV^{HFIP} λ_{max}: 270 nm (ε 823).

Anal. Calcd for $C_{11}H_{19}O_6NS_2$: C, 40.60; H, 5.88; N, 4.30. Found: C, 40.79; H, 6.00; N, 4.25.

Methyl *N*-(*tert*-Butoxycarbonyl)-*S*-(6-acetoxy-4-dibenzofuranylthio)-L-cysteinate (6). To a solution of 5 (22.5 mg, 87.1 μmol) in 6.5 mL of MeOH was added 2 μL of TEA and 28.3 mg of 8 (87.1 μmol) in 1:1 $CHCl_3$–MeOH. The solution was stirred at 25 °C under N_2 for 0.5 h and then evaporated. A solution of the residue in DCM was washed with cold 0.1 N HCl, water, and brine, dried, and evaporated. After preparative layer chromatography (1000 μm; 9:1 $CHCl_3$–EtOAc) 6 was obtained as an oil that solidified on standing, 37.4 mg, 87%, mp 63–64 °C. An identical product was obtained in 33% yield by reaction of 7 and 9. ^1H NMR (250 MHz, $CDCl_3$): δ 1.43 (9 H, s, Boc), 2.48 (3 H, s, Ar-OAc), 3.29–3.48 (2 H, m, Cys:methylene), 3.70 (3 H, s, Cys:OMe), 3.75 (3 H, s, Ar:Scm), 4.62–4.72 (1 H, m, Cys:methine), 5.47 (1 H, b d, J = 8 Hz, urethane), 7.26 (1 H, dd, J = 8, 1 Hz, C_7-H), 7.35 (2 H, tm, C_2-H and C_8-H), 7.70 (1 H, dd, J = 8, 1 Hz), 7.81 (1 H, dd, J = 8, 1 Hz), 7.89 (1 H, dd, J = 8, 1 Hz, C_1-H). High-resolution mass spectrum calcd for $C_{23}H_{25}O_7NS_2$ 491.1073, found 491.1081.

Anal. Calcd for $C_{23}H_{25}O_7NS_2$: C, 56.20; H, 5.13; N, 2.85; S, 13.04. Found: C, 55.99; H, 5.31; N, 2.77; S, 13.20.

Trifluoroacetate Salt of Methyl *S*-(Methoxycarbonylsulfenyl)-L-cysteinate ($Tfa^-H_2^+$-L-Cys(Scm)-OMe, 15). A solution of Boc-L-Cys(Scm)-OMe 8 (69.4 mg, 0.21 mmol) in 0.4 mL of TFA and 2.2 μL of anisole was stirred at 0 °C for 1 h and then evaporated. The residue was triturated with toluene and evaporated, three times. After drying the residual gum (67 mg, 100%) was used directly for the preparation of 16. ^1H NMR (250 MHz, $CDCl_3$): δ 3.47 (2 H, b s, Cys:methylene), 3.88 (3 H, s, Cys:Ome), 3.95 (3 H, s, Cys:Scm), 4.17–4.36 (1 H, m, Cys:methine), 8.33 (3 H, b s, ammonium salt).

Methyl *N*-(*tert*-Butoxycarbonyl)-*S*-[6-(*N*-benzyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthio]-L-cysteinate (14). To a suspension of 4-(methoxycarbonyldithio)-6-(*N*-benzyloxycarbonyl-L-alaninyloxy)dibenzofuran[1] (40.8 mg, 79.8 μmol) in a 4:1 dioxane–water mixture (2.00 mL) was added in one portion at room temperature under N_2 Bu_3P (20 μL, 80.3 μmol); the mixture was warmed to 40 °C and stirred under N_2 for 0.5 h. The resulting clear solution was then lyophilized, and the white solid residue (50.8 mg) 13 was dissolved in a 14:1 HFIP–$CHCl_3$ mixture (1.50 mL) and added in one portion to a solution of Boc-L-Cys(Scm)-OMe (8) (26.1 mg, 80.3 μmol) in HFIP (0.70 mL). The clear mixture was stirred at room temperature under N_2 for 0.5 h, the solvent was then evaporated, and the residue was dried under high vacuum to yield 83 mg of a white solid. Preparative layer chromatography (1000 μm; eluent 9:1 $CHCl_3$–EtOAc) afforded the pure title substance as a white solid (46.7 mg, 90%), mp 133–135 °C. An analytical sample was obtained by recrystallization from EtOAc, mp 133.5–134.5 °C. ^1H NMR (270 MHz, $CDCl_3$): δ 1.40 (9 H, s, Boc), 1.80 (3 H, d, J = 7 Hz, Ala:methyl), 3.29–3.44 (2 H, m, Cys:methylene), 3.67 (3 H, s, Cys:OMe), 4.63–4.75 (1 H, m, Cys:methine), 4.78–4.90 (1 H, m, Ala:methine), 5.18 (2 H, m, benzyl), 5.42 (1 H, b d, J = 8 Hz, Cys:urethane), 6.01 (1 H, b d, J = 8 Hz, Ala:urethane), 7.28–7.42 (8 H, m), 7.70 (1 H, dd, J = 8, 1 Hz), 7.83 (1 H, dd, J = 8, 1 Hz), 7.92 (1 H, dd, J = 8, 1 Hz, C_1-H).

Anal. Calcd for $C_{32}H_{34}N_2O_9S_2$: C, 58.70; H, 5.23; N, 4.28. Found: C, 58.68; H, 5.41; N, 4.17.

Trifluoroacetate Salt of Methyl *S*-[6-(*N*-Benzyloxycarbonyl-L-alaninyloxy)-4-dibenzofuranylthio]-L-cysteinate (16). Method A. A solution of 27.9 mg (42.7 μmol) of 14 in 0.6 mL of TFA was treated as described for 15. The residue was triturated with 1:1 ether–petroleum ether to give 16 as a tan solid, mp 85–88 °C (23 mg, 81%). ^1H NMR (270 MHz, DMSO-d_6): δ 1.57 (3 H, d, J = 7 Hz, Ala:methyl), 3.15–3.49 (2 H, m, Cys:methylene), 3.67 (3 H, s, Cys:OMe), 4.45 (2 H, m, Cys and Ala methines), 5.07 (2 H, s, benzyl), 7.14–7.56 (8 H, m), 7.78 (1 H, d, J = 8 Hz), 8.18 (2 H, m), 8.22 (1 H, d, J = 8 Hz), 8.68 (3 H, b s, ammonium salt). Field desorption mass spectrum: m/e 555 (M^+ – TFA).

Method B. To a solution of freshly prepared $Tfa^-H_2^+$-L-Cys(Scm)-OMe (15) (60.30 mol) in HFIP (2.20 mL) was added in one portion at 0 °C a solution of 4-(benzyloxycarbonyl-L-alaninyloxy)-6-dibenzofuranthiol (13) (prepared as described for 14) (24.3 mg, 57.65 mol) in the same solvent. The resulting pale solution was stirred first at 0 °C for 7 min and then at 25 °C for 25 min, and the clear mixture was evaporated to dryness. The residue was dried under high vacuum and triturated with petroleum ether to afford a pale powder (37.1 mg, 96%), mp 84–86 °C dec.

This compound was identical with authentic 16 (method A) by mixture melting point and ^1H NMR analyses.

Methyl *N*-(*tert*-Butoxycarbonyl)-*S*-(4-dibenzofuranylthio)-L-cysteinate (18). The title substances was prepared in 87% yield from 8 and 17 following the procedure described for 16, except that TEA was not added, and 3:2 MeCN–HFIP was used as solvent. Purification by preparative layer chromatography (1000 μm; 9:1 $CHCl_3$–EtOAc) gave 18 as a pale oil. ^1H NMR (270 MHz, $CDCl_3$): δ 1.43 (9 H, s, Cys:boc), 3.21–3.43 (2 H, m, Cys:methylene), 3.73 (3 H, s, Cys:OMe), 4.65–4.76 (1 H, m, Cys:methine), 5.43 (1 H, b d, J = 8 Hz, urethane), 7.30 (1 H, dd, J = 8, 1 Hz), 7.37 (1 H, td, J = 8, 1 Hz), 7.48 (1 H, td, J = 8, 1 Hz), 7.63 (1 H, dd, J = 8, 1 Hz), 7.93 (1 H, dd, J = 8, 1 Hz). High-resolution mass spectrum calcd from $C_{21}H_{23}O_5NS_2$ 433.1018, found 433.1020. UV^{HFIP}: λ_{max} 283 (ε 15 700), 238 (ε 24 000), 209 nm (ε 38 600).

Rate Determination for the Thiol Capture Reaction: (17 + 8) → 18. Within 2 days before a kinetic run, 4-mercaptodibenzofuran (17) was distilled and Boc-L-Cys(Scm)-OMe 8 was recrystallized (hexane) to constant melting point (78 °C). TEA and DIEA were distilled first from ninhydrin and then from sodium and stored in sealed ampules at −20 °C; 2,6-di-*tert*-butyl-4-methylpyridine was distilled prior to use. HFIP was fractionally distilled; reagent grade MeCN was dried over molecular sieves (Linde 4A) for at least 2 days prior to use; water was freshly distilled (Corning AG-1B constant flow apparatus).

(34) Janczewski, M.; Maziarzyk, H. *Roczniki Chem.* **1977**, *51*, 891.

The cysteine derivative 8 was dissolved in HFIP or aqueous HFIP; thiol 17 and the amine bases in MeCN. Reactions were conducted at 4.3×10^{-4} M to 8.6×10^{-4} M in thiol, and zero to 17.5×10^{-3} M in amine at 25 °C. In all but one case pseudo-first-order conditions were employed with 8 in 21-fold excess of the thiol 17.

To initiate a run, volumes of freshly prepared solutions of the reagents were transferred by syringe (Hamilton) into a borosilicate microvial (Wheaton; capacity 0.30 mL) equipped with a magnetic flea bar and a Teflon-coated screw cap in the following order: First 8 (200 μL), second 17 (20 μL), and last the appropriate base (if any). The reaction time was measured with a digital timer (Precision Scientific Co., cat. no. 69235) and zero time was taken when the last reagent was added.

The reaction was followed by HPLC (Whatman Partisil 5/25 ODS-3 column; 90% methanol:1% acetic acid, gradient elution, no. 6 curve, 10 min, 1.0 mL/min), monitored by injecting 10.0-μL aliquots of the reaction mixture at regular intervals). The retention times of the peaks of interest were predetermined by injection of authentic samples of the appropriate compounds under the same conditions are as follows: 8 (5.58 min), 17 (10.17 min), 18 (11.95 min), 4-dibenzofuranyl disulfide (17.05 min).

The peaks of interest were integrated, and the sum of the integrals for 17, 18, and disulfide gave the value for A_0 at any given time. The relative concentration of thiol 17 (A_t) was obtained in every case by dividing the integral for 17 by A_0 (obtained as described above). The reaction was followed for at least 3 half-lives, and the infinity point was taken at 6 half-lives (corrected for the relative concentration of disulfide in the reaction mixture). The slope of a plot of $\ln (A_0 - A_\infty) - \ln (A_t - A_\infty)$ vs time gave pseudo-first-order rate constants; division by concentration of **1m** gave second-order rate constants.

Preparation of CF_3CO_2H·H-L-Cys(Scm)-Gly-Gly-L-Ala-OH (22) BPTI Sequence H-C(Scm)-(56–58)-OH. 1. Z-Gly-Gly-L-Ala-OtBu. A solution of Z-Gly-Gly-OH (7.00 g, 26.3 mmol) and Et_3N (3.60 mL, 26 mmol) in THF–DMF (300 mL 2:1) at 3 °C was treated dropwise with stirring with isobutyl chloroformate (3.65 mL, 26.3 mmol) and after 20 min with a slurry of Et_3N (3.6 mL, 26.3 mmol) and HCl·H-L-Ala-OtBu (4.8 g, 26.3 mmol) in THF (100 mL). After overnight stirring, the mixture was filtered and the precipitate was washed with cold THF. The oil resulting from evaporation of the filtrate was dissolved in EtOAc (250 mL), and the resulting solution was extracted with 5% $NaHCO_3$, pH 3.5 citrate buffer, water, and brine, dried over $MgSO_4$, and evaporated to yield a white solid, recrystallized from ethanol to give 7.8 g, 78%, of white needles, mp 115–117 °C. TLC (EtOAc–MeOH, 95:5): R_f 0.6.

Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 57.67; H, 6.99; N, 10.59.

2. Boc-L-Cys(Acm)-Gly-Gly-L-Ala-OtBu. A solution of Z-Gly-Gly-L-Ala-OtBu (1.5 g, 3.8 mmol) in MeOH (40 mL) was hydrogenated at 50 psi in the presence of Pd black for 1 h. After filtration through Celite, the filtrate and washings were evaporated to yield a clear oil H-Gly-Gly-L-tBu, 0.98 g, that solidified under high vacuum and that was used directly in the next step.

To a solution of Boc-L-Cys(Acm)-OH (1.1 g, 3.8 mmol) in THF (20 mL) at −10 °C was added with stirring Et_3N (0.53 mL, 3.8 mmol) and then isobutyl chloroformate (0.53 mL, 3.8 mmol). After 20 min at −12 °C a solution of H-Gly-Gly-L-Ala-OtBu (0.98 g, 3.8 mmol) in THF (10 mL) was added. Stirring with warming to 23 °C was allowed to continue overnight, and the slurry was filtered and the salts were washed with THF. The combined filtrates were evaporated to yield an oil that was dissolved in EtOAc and washed (with pooled back extractions) with 5% $NaHCO_3$, pH 3.5 citrate buffer, water, and brine, dried over $MgSO_4$, and evaporated to yield an oil that was purified by flash chromatography (eluent DCM–MeOH, 9:1) to afford a white foam, 1.74 g, 90%. TLC (DCM–MeOH, 9:1): R_f = 0.35. FAB MS: $[MH^+]$ at 535.

Anal. Calcd for $C_{22}H_{39}N_5O_8S$: C, 4..52; H, 7.37; N, 13.12; S, 6.01. Found: C, 48.65; H, 7.66; N, 12.87; S, 6.01.

3. Boc-L-Cys(Scm)-Gly-Gly-L-Ala-OtBu. To a 0 °C solution of the above compound (4.00 g, 7.50 mmol) in $CHCl_3$–MeOH (54 mL, 2:1) was added Scm-Cl (1.29 mL, 14.3 mmol. After 50 min, 1 M aqueous DEA (16 mL) was added, the two-phase mixture was poured into $CHCl_3$ (300 mL), and the organic phase was washed with 1 M citric acid, water, and brine, dried over $MgSO_4$, and evaporated to yield a white solid that was purified by flash chromatography (eluent DCM–MeOH, 94:6) to give 3.9 g (94.5%) of a white powder, mp 143–145 °C. Purity (HPLC) >99.5%. HPLC (45% MeCN–55% 0.1% TFA, 1 mL/min, 214 nm): t_R 8.0 min. ^1H NMR (300 MHz, $CDCl_3$): δ 7.62 (1 H, b m), 7.19 (1 H, b m), 6.64 (1 H, b d, J = 8.1 Hz), 5.83 (1 H, b d, J = 8.1 Hz), 4.45 (1 H, p, J = 6.8 Hz), 4.27 (1 H, dt, J = 7.0, 8.7 Hz), 4.05 (2 H, d, J = 5.4 Hz), 4.02 (1 H, dd, J = 5.4, 14.5 Hz), 3.94 (3 H, s), 3.90 (1 H, dd, J = 5.4, 14.5 Hz), 3.26 (1 H, dd, J = 5.9, 13.5 Hz), 3.12 (1 H, dd, J = 7.03, 13.5 Hz), 1.45 (18 H, s), 1.40 (3 H, d, J = 6.8 Hz). FAB MS: $[MH^+]$ 553.

Anal. Calcd for $C_{21}H_{36}N_4O_9S_2 \cdot {}^1/_2H_2O$: C, 44.75; H, 6.90; N, 9.94. Found: C, 44.70; H, 6.57; N, 9.95.

4. TFA·H-L-Cys(Scm)-Gly-Gly-L-Ala-OH (22). A solution of the above compound (30 mg, 54 μmol) in TFA–DCM (2 mL, 3:1, with anisole, 60 μL) was stirred for 2 h at 25 °C and then evaporated to yield 30 mg, 100%, of **22** as a white powder, mp 85–90 °C dec, which was used without further purification in capture experiments. ^1H NMR (300 MHz, D_2O): δ 4.49 (1 H, q, J = 7.2 Hz), 4.48 (1 H, m), 4.20 (2 H, d, J = 6.0 Hz), 4.12 (2 H, s), 4.10 (3 H, s), 3.60 (1 H, dd, J = 5.7, 15.0 Hz), 3.42 (1 H, dd, J = 8.9, 15.0 Hz), 1.55 (3 H, d, J = 7.2 Hz).

Synthesis of Bpoc-L-Lys(Boc)-L-Arg(Pmc)-L-Asn-OH. 1. Z-L-Arg(Pmc)-L-Asn-OH. To a 0 °C solution in 20 mL of DME was added with stirring Z-L-Arg(Pmc)-OH[27] (1.22 g, 2.1 mmol), *N*-hydroxysuccinimide (0.29 g, 2.6 mmol), and DCCI (0.52 g, 2.6 mmol). After 3 h the mixture was filtered, and the urea was washed with DME (3 × 3 mL). Evaporation yielded the OSu ester as a foam, which was used directly by solution in dioxane (100 mL) at 10 °C followed by mixing with a solution of H-L-Asn-OH·H_2O (1.89 g, 6.3 mmol) and $NaHCO_3$ (1.05 g, 6.3 mmol) in water (60 mL). After 15 min of vigorous stirring, most of the dioxane was removed in vacuum, and the remaining aqueous phase was acidified to pH 3.5 and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with water and brine, dried over $MgSO_4$, and evaporated to yield a white foam that was purified by flash chromatography (eluent 80:18:5 DCM–MeOH–AcOH) to afford the title compound as a white foam, 1.21 g, 84%. HPLC (50% MeCN–50% 0.1% TFA, 1 mL/min 214 nm): t_R 5.5 min, 99.5% pure.

2. H-L-Arg(Pmc)-L-Asn-OH and Bpoc-L-Lys(Boc)-L-Arg(Pmc)-L-Asn-OH. A solution of Z-L-Arg(Pmc)-L-Asn-OH (1.0 g, 1.4 mmol) in MeOH (75 mL) was hydrogenated for 4 h at 50 psi in the presence of Pd black. The solution was filtered through Celite and evaporated to yield a white amorphous solid, which was dried in vacuum for 3 h and then used directly in the next step.

To a 0 °C solution of Bpoc-L-Lys(Boc)-OH (0.63 g, 1.3 mmol) and *N*-hydroxysuccinimide (0.15 g, 1.3 mmol) in EtOAc (12 mL) was added DCCI (0.27 g, 1.3 mmol) with stirring. After 1 h at 0 °C and 3 h at 25 °C the suspension was filtered, and the collected urea was washed with EtOAc (2 × 10 mL). The combined filtrates were evaporated to yield the crude OSu ester, which was used directly. (The ester could be purified by flash chromatography—Eluant: 4:1 DCM–EtOAc, with significant losses.)

To a stirred 10 °C solution of the above active ester in dioxane (50 mL) was added a solution of H-L-Arg(Pmc)-L-Asn-OH (0.78 g, 1.4 mmol) and $NaHCO_3$ (0.12 g, 1.4 mmol) in H_2O–dioxane (80 mL, 1:1). After 3 h most of the dioxane was removed at aspirator pressure, and the residue was partitioned between EtOAc and pH 3.5 0.5 M citrate buffer (150 mL, 1:1). The separated aqueous phase was extracted with EtOAc (2 × 75 mL), and the combined organic phases were washed with water and brine, dried over $MgSO_4$, and then evaporated to give a solid that was triturated with ether (6 × 10 mL). Flash chromatography (eluant DCM–MeOH–dicyclohexylamine, 86:14:0.5) gave 1.0 g, 73%, of the DCHA salt of the title tripeptide as an amorphous white solid. HPLC (60% MeCN–40% 0.1% TFA, 1 mL/min, 214 nm): t_R 9.65 min, >99% pure. ^1H NMR (300 MHz, DMSO-d_6): δ 8.17 (1 H, d, J = 5.6 Hz), 7.80 (1 H, d, J = 7.6 Hz), 7.7–7.3 (10 H, m), 7.24 (1 H, d, J = 7.6 Hz), 6.90 (1 H, s), 6.75 (1 H, b t, J = 6, 0 Hz), 6.7–6.3 (3 H, b m), 4.50 (1 H, q, J = 7.5 Hz), 4.30 (1 H, m), 3.82 (1 H, m), 2.90 (2 H, m), 2.88 (2 H, m), 2.55 (2 H, b t, J = 7.5 Hz), 2.47 (6 H, s), 2.03 (3 H, s), 1.73 (3 H, s), 1.48 (9 H, s), 1.25 (6 H, s), 1.8–1.3 (10 H, m). FAB MS (glycerol): $[MH^+]$ 1021.9,

[MH^+ - Bpoc] 784 (100), [MH^+ - Pmc] 755, [MH^+ - Bpoc - Pmc] 518.

Synthesis of Boc-L-Cys(Acm)-L-Arg(Pmc)-OH. 1. H-L-Arg(Pmc)-OH. A solution of Z-L-Arg(Pmc)-OH[27] (1.38 g, 2.3 mmol) in MeOH (50 mL) was hydrogenated at 50 psi for 3 h in the presence of Pd black. After filtration through Celite and evaporation a white solid was obtained that was used in the next step without purification.

2. Boc-L-Cys(Acm)-OSu and Boc-L-Cys(Acm)-L-Arg(Pmc)-OH. To a solution of Boc-L-Cys(Acm)-OH (0.61 g, 2.1 mmol) and *N*-hydroxysuccinimide (0.24 g, 2.1 mmol) in DME (20 mL) at 0 °C was added with stirring DCCI (0.43 g, 2.1 mmol). After 1 h at 0 °C and 4 h at 25 °C the solution was filtered, and the precipitate was washed with DME, the combined filtrates were evaporated, and the residue was dissolved in dioxane (20 mL). The solution was cooled to 10 °C, and a solution of H-L-Arg(Pmc)-OH (0.96 g, 2.2 mmol) and $NaHCO_3$ (0.18 g, 2.2 mmol) in dioxane–H_2O (20 mL, 1:1) was added. The mixture was stirred for 1 h at 0 °C and then evaporated. The residue was dissolved in EtOAc (30 mL), and the solution was washed with 0.5 M citrate buffer pH 3.5, water, and brine, dried over $MgSO_4$, and evaporated. Flash chromatography (eluent DCM–MeOH–AcOH, 85:13:2) yielded an oil that solidified after manipulation with DCM–hexane, 1.3 g, 91%. ^1H NMR (300 MHz, DMSO-d_6): δ 8.60 (1 H, t, J = 7.0 Hz), 7.55 (1 H, d, J = 7.2 Hz), 7.0 (1 H, d, J = 8.1 Hz), 4.26 (1 H, dd, J = 6.5, 13.5 Hz), 4.10 (2 H, m), 3.90 (1 H, m), 3.00 (2 H, m), 2.90 (1 H, dd, J = 4.5, 13.5 Hz), 2.60 (1 H, dd, J = 9.8, 13.5 Hz), 2.56 (2 H, t, J = 6.5 Hz), 2.42 (6 H, s), 2.00 (3 H, s), 1.85 (3 H, s), 1.75 (2 H, t, J = 6.5 Hz), 1.35 (9 H, s), 1.25 (6 H, s).

***N*-(*tert*-Butoxycarbonyl)-*O*-(dinitrophenyl)-L-tyrosine (Boc-L-Tyr(Dnp)-OH).** To a clear solution of Boc-L-Tyr-OH (1.4 g, 5.0 mmol) and $KHCO_3$ (10 g, 100 mmol) in 50 mL of water was added with stirring at 25 °C Dnp-F (0.63 mL, 5.0 mmol) in MeOH (30 mL). After 45 min at 25 °C most of the MeOH was evaporated, and the remaining aqueous phase was acidified to pH 2 with 0.5 M citric acid and extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and evaporated to yield a residue that was crystallized from ether at 0 °C. Recrystallization from EtOAc–hexane gave 1.3 g, 59%, of the title compound as yellow needles, mp 113–116 °C dec. HPLC (60% MeCN–40% 0.1% TFA, 1 mL/min) t_R 5.40 min. ^1H NMR (250 MHz, $CDCl_3$): δ 8.83 (1 H, d, J = 3.0 Hz), 8.30 (1 H, dd, J = 3.0, 8.7 Hz), 7.30 (2 H, d, J = 6.8 Hz), 7.1–7.0 (3 H, m), 5.05 (1 H, b d, J = 7.5 Hz), 4.62 (1 H, b m), 3.25 (1 H, b dd, J = 5.3, 13.2 Hz), 3.08 (1 H, b dd, J = 6.4, 13.2 Hz), 1.40 (9 H, s).

Preparation of Polystyrene Resin Functionalized with Disulfide-Linked 4-Hydroxy-6-thiadibenzofuran. 1. 4-Aminomethyl Polystyrene. A 99% styrene–1% *p*-divinylbenzene copolymer (BioRad SX-1) (20 g) was washed and sieved according to the procedure of Gisin and Merrifield.[29] The resin was then functionalized in a two-step literature process[28] to yield a white solid (20 g) after drying in vacuum. The amine content of the resin was found to be 0.21 ± 0.01 mmol/g by independent picric acid titration and quantitative ninhydrin assay.[35]

2. Z-L-Cys(Scm)-OH·CHA. To a 5 °C solution of Z-L-Cys(Trit)-OH·DEA (10 g, 17.5 mmol) in $CHCl_3$–MeOH (225 mL, 2:1) was added Scm-Cl (3.0 mL 33 mmol) in one portion. The solution was stirred 1 h at 5 °C and then poured into $CHCl_3$ (1.3 L) containing DEA (1.8 mL, 17.5 mmol). The organic solution was washed with 0.5 M citric acid, water, and brine, dried over $MgSO_4$, and concentrated to a yellow oil, which was dissolved in ether (60 mL) to which CHA (2.0 mL, 1 equiv) was added. The resulting solid was recrystallized from benzene–hexane to yield 6.5 g, 83%, of Z-L-Cys(Scm)-OH·CHA as white needles, mp 100–101 °C. TLC ($CHCl_3$–EtOAc–AcOH, 88:10:2): R_f 0.5. HPLC (50% MeCN–50% 0.1% TFA, 1 mL/min, 214 nm): t_R 5.8 min.

3. Z-L-Cys(4-acetoxydibenzofuranyl-6-thia)-OH (21). To ether and 0.5 M citrate buffer, pH 3.5 (200 mL 1:1), was added 4.8 g, 10.5 mmol, of Z-L-Cys(Scm)-OH·CHA. The layers were separated, and the organic phase was washed with citrate buffer, water, and brine, dried over $MgSO_4$, and evaporated to yield the acid, which was dissolved in HFIP–DCM–H_2O (45 mL, 5:3:1). To this was added with stirring at 25 °C 4-acetoxy-6-mercaptodibenzofuran (2.68 g, 10.5 mmol). After 2 h, the solvent was evaporated, and the resulting solid was recrystallized twice from hot EtOH with slow cooling to yield **21**, 4.7 g, 88% of tiny white needles, mp 194–195 °C. HPLC (60% MeCN–40% 0.1% TFA, 1 mL/min, 214 nm): t_R 7.6 min.

Anal. Calcd for $C_{25}H_{21}NO_7S_2$: C, 58.70; H, 4.14; N, 2.74; S, 12.53. Found: C, 58.28; H, 4.16; N, 2.73; S, 12.96.

4. Polystyrene Resin Functionalized with Disulfide-Linked 4-Acetoxydibenzofuran-6-thia Functions. A fresh solution of **21** (205 mg, 0.40 mmol) in DCM–DMF (11 mL, 10:1) was added to preswelled (DCM) aminomethyl polystyrene (1.0 g, 0.2 mmol amine function) contained in the solid-phase reaction vessel. After 5 min a solution of DCCI (82.4 mg, 0.40 mmol) in DCM (8 mL) was added, the vessel was rotated for 16 h at 25 °C, and the resin was washed with DCM (6 × 20 mL; 1 min each). By quantitative ninhydrin assay[35] the amount of free amine was found to be 0.005 mmol/g, 98% reaction. The resin was acetylated by the following protocol: acetic anhydride–DIEA–DCM (10 mL; 2:0.5:8; 1 × 10 min); DCM (20 mL; 1 × 1 min); DCM (20 mL; 1 × 2 min); DCM (20 mL; 6 × 1 min). A quantitative ninhydrin assay[33] was now negative for free amine.

5. Deblocking of the Acetoxy Ester. A mixture at 0 °C of DMAc (8 mL) and freshly prepared aqueous 2 M hydroxylamine (2 mL, prepared by adding 4 M NaOH to a solution of 1.4 g hydroxylamine hydrochloride in 5 mL water to bring the pH to 7.0) was allowed to stand for 5 min and then filtered of salt. The filtrate was added to the resin prepared in 4, contained in the solid-phase reaction vessel, and previously preswelled in DMAc. After rotation for 2 h the solvent was removed by positive pressure filtration under N_2, and the resin was washed as follows: DMAc (20 mL; 2 × 1 min); DMAc–H_2O (20 mL; 4:1; 2 × 1 min); DMAc (20 mL; 2 × 1 min); DCM (20 mL; 6 × 1 min). The resin was then dried in high vacuum overnight, wt 1.1 g. No free amine could be detected by ninhydrin assay.

General Procedure for Thiol Capture Synthesis of Polypeptides. A. Solid-Phase Synthesis. 1. Apparatus. Solid-phase reaction vessels were custom-made according to Merrifield's prototype.[29] Three different vessel sizes were used, as follows: (1) for analytical and exploratory work, a vessel was used that accommodated 50–100 mg of resin with a maximum solvent volume of 5 mL; (2) for routine preparative work, a vessel for 100–500 mg of resin with solvent capacity 15 mL; (3) for large-scale preparation, a vessel for 2 g of resin holding 40 mL of solvent. The vessel was clamped and rotated through 360° at ca. 12 rpm by a Con-Torque mechanical stirrer (Eberbach Co.). Filtration of the reaction mixture was performed by unscrewing the Teflon-coated screw cap, attaching a screw-on nitrogen inlet adaptor (Ace cat. no. 10924-200), and forcing the solvent through the glass frit (medium porosity) with a positive pressure of nitrogen at about 10 psi. The wash solvents were transferred into the vessel via cannula under nitrogen pressure. To minimize resin adhesion, attrition, and loss, the solid-phase vessels were silanized with Sigma-Coat (Sigma).

2. Solvents and Reagents. All α-Boc-blocked amino acids were purchased from The Peptides Institute. All *N*-α-Bpoc-blocked amino acids were prepared as previously reported[22] and found to be >99% pure by NMR and HPLC. Samples were stored at −20 °C, and bottles were warmed to 25 °C before opening. Samples were routinely checked for constancy of melting point. Bu_3P and Et_3P were purchased from Aldrich and stored and transferred by syringe under N_2. Samples were discarded when routine tests revealed the presence of phosphine oxide. HFIP, TFE, gold-label silver nitrate, DCCI, DNP-F, and phenylmercuric acetate were purchased from Aldrich. Acetic anhydride, DMAc, and DCM were purchased from Mallinkrodt, Fluka, and Fischer, respectively, and used without purification. Sequanal grade TFA was purchased from Pierce. DIEA was distilled from ninhydrin and redistilled from CaH_2 and then stored in brown bottles at −20 °C. *N*-Hydroxysuccinimide (HOSu) and 4-(dimethylamino)pyridine (DMAP) were recrystallized from EtOAc; 1-hydroxybenzotriazole (HOBt) and sodium nitrite were recrystallized from EtOH–H_2O and H_2O, respectively. THF and dioxane were obtained dry and peroxide-free by distillation through a 1-m helix-packed column from sodium benzophenone ketyl. Reagent

(35) Gisin, B. F. *Anal. Chim. Acta* **1972**, *58*, 248. Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147.

grade DMSO was fractionally distilled at 45 °C at 0.1 mm; the first and last 25% were discarded; the middle cut was collected in a flame-dried flask and used for acyl transfer within 1 day. Ether was freed of moisture and traces of peroxides by passage through activity grade 1 alumina immediately prior to use. All solvent mixtures are prepared with indicated ratios by volume.

3. Synthesis of Symmetrical Anhydrides of α-Urethane-Blocked Amino Acids. Note that for Bpoc derivatives of Met and Gly, and for Boc-Tyr(Dnp)-OH, DCM replaced ether in the extractions, as shown by brackets. The α-N-protected amino acid salt (8 equiv) was suspended in ether [DCM] (10 mL) and extracted with 0.5 M pH 3.5 citrate buffer (2 × 10 mL). The combined extracts were washed with ether ([DCM]), and the pooled organic phases were washed with brine to neutral pH, dried over $MgSO_4$, and evaporated under high vacuum to a foam, which was weighed to determine the stoichiometry of the next step. To a ca. 0.3 M solution of the foam in DCM at 0 °C was added DCCI (4 equiv) with stirring. After 15 min the precipitated urea was filtered on a frit and washed with minimal cold DCM to give a final concentration of 0.15 M anhydride. The resulting clear solution of $(X\text{-}Yyy)_2O$ at 0–5 °C was used immediately in the solid-phase acylation step.

4. Protocols for Solid-Phase Peptide Synthesis. Three different protocols are used to extend the polypeptide chain by one unit. Protocol A is used exclusively when attaching the first amino acid residue to the resin-bound dibenzofuran template. Protocol C is the standard protocol, used with trouble-free amino acid derivatives. Protocol B is used with Asn, Arg, Cys, Gln, and peptide fragment couplings.

a. Protocol A. A weighed sample of preswelled (DCM) 4-hydroxydibenzofuran-6-thia--functionalized polystyrene is placed in the reaction vessel and subjected to the following washes and chemical steps: (1) DCM (10 mL, 4 × 1 min); (2) 0.15 M $(X\text{-}Yyy)_2O$ prepared as described above (4 equiv, 1 × 15–30 min) and DIEA (3 equiv) in DCM; (3) DCM (10 mL, 6 × 1 min); Ac_2O (1 mL of 1:10 in DCM, 1 × 5 min); DCM (10 mL, 6 × 1 min). During step 2 the completeness of acylation is assayed by phosphine cleavage, monitoring presence of hydroxy template. The acylation step is repeated if more than 2% of hydroxy template is detected. At the end of the complete three-step sequence, the resin is assayed by phosphine cleavage as described below. The functionalization is repeated with fresh resin and longer reaction times if less than 98% aminoacylated template (>2% acetoxy template) or the presence of hydroxy template can be detected.

b. Protocol B. An aminoacylated resin from protocols A, B, or C is subjected to the following washes and chemical steps: (1) 0.5% TFA in DCM (10 mL, 1 × 1 min); (2) 0.5% TFA in DCM (10 mL, 1 × 20 min); (3) DCM (10 mL, 6 × 1 min); (4) 5% DIEA in DCM (10 mL, 2 × 1 min); (5) DCM (10 mL, 6 × 1 min); (6) acylating agent as described below for each case (3 equiv at 0.1–0.15 M in DCM, 1 × 20–60 min); (7) DCM (10 mL, 3 × 1 min); (8) 1:1 DCM–DMF (10 mL, 2 × 1 min); (9) DCM (10 mL, 4 × 1 min). During step 6 the completeness of acylation is assayed by phosphine cleavage and the acylation time extended as needed. An acetic anhydride step equivalent to step 3 of protocol A is used if a final phosphine assay shows detectible unreacted amine.

Asn and Gln. Both were activated by the procedure of Konig and Geiger.[36] To a 5 °C solution of Bpoc-Asn-OH (0.4 M) in 1:1 DCM–DMF was added HOBt (1 equiv) followed by DCCI (1 equiv). After 10 min the DCU was retained on a glass frit and the filtrate was added immediately to the resin (step 6), and acylation was allowed to proceed for 5 min.

Cys. Acylation was performed for 45 min, with a 0.2 M solution of the symmetrical anhydride of Boc-Cys(S-Acm)-OH in 4:1 DCM–DMF.

Arg: A 5 °C solution of Bpoc-Arg(PMc)-OH (0.1 M) in DCM was added to the resin followed by a solution of DCCI (1 equiv) in DCM to obtain a final concentration of 0.05 M. Acylation was then carried out for 60 min.

c. Protocol C. An aminoacylated resin from protocols A, B, or C is subjected to the following washes and chemical steps: (1) 0.5% TFA in DCM (10 mL, 1 × 1 min); (2) 0.5% TFA in DCM (10 mL, 1 × 20 min); (3) DCM (10 mL, 6 × 1 min); (4) $(X\text{-}Yyy)_2O$ and DIEA (4 equiv each, at 0.1–0.15 M in DCM, 1 × 5–10 min); (5) DCM (10 mL, 6 × 1 min).

5. Assay of Peptide Purity and Preparative Cleavage from the Resin. Phosphine cleavage of the disulfide linkage releases the fully blocked, template-functionalized peptide from the resin. It is used for final recovery of peptide and for quality control during the solid-phase synthesis.

a. Solvent Optimization and Assay. Resin (1–2 mg) in a 1 × 8-cm test tube is treated with swirling with 100–150 μL of a solution in DCM–HFIP, 4:1, of Et_3P or Bu_3P (1.0 μL/mL). After 1 min, a 10-μL aliquot of the supernatant is monitored by HPLC. For heavily loaded resins, the solvent ratio may be changed to 85:15 or 9:1 to allow greater swelling of the resin. If the peptide is not released under any of these conditions, a mixture of DCM–TFE, 4:1, is used. After 10–20 min the cleavage reaction is monitored a second time to insure that the solvent system provides maximum cleavage and to examine the stability of the template–peptide linkage toward excess phosphine. Frequently a trace of 4-acetoxy-6-mercaptodibenzofuran is present in the cleavage mixture. This easily leached substance can be used as an internal standard to estimate maximum cleavage and to optimize the composition of the cleavage solvent by maximizing the ratio of [product]/[standard].

b. Preparative Cleavage. The resin bearing the peptide–template conjugate is dried at 25 °C in high vacuum for several hours and then is placed in the solid-phase reaction vessel and swelled with the optimal solvent found above. The expected yield of cleaved peptide is found from the following formula:

$$y = x(\mathrm{MW} + 1000/L)$$

where y = wt of dried resin in milligrams, x = expected yield of peptide in millimoles, MW = molecular weight of template-peptide, and L = resin loading in millimoles/gram.

The swelling solvent is removed by positive nitrogen pressure and replaced by 5–6 mL/g resin of the same solvent containing Et_3P (1.0 equiv based on the expected peptide yield). The vessel is rotated for 15 min, and the solvent is expelled with nitrogen into a round-bottomed flask. The resin is washed (3 × 1 min) with the same amount of solvent, the combined filtrates are evaporated under reduced pressure, and the resulting residue is dried for 1 min under high vacuum. Trituration with ether (6×) under nitrogen removes phosphine oxide and 4-acetoxydibenzofuran-6-thiol, affording the released peptide–template conjugate as a powder, which is used immediately in the capture step without further purification.

With the unusually reactive Asp–template or Gly–template linkages the procedure is modified by use of the more hindered Bu_3P (Asp case) or by use of 0.95 equiv of Et_3P (Gly case). Owing to the intense long-wavelength absorption of the 4-(acyloxy)-DBF-SH group, a relatively accurate photometric assay of the purity of the peptide–template conjugate is available by UV monitoring at 280 or 254 nm.

6. The Thiol Capture Step. In a tared round-bottomed flask is placed a weighed amount of the peptide–template conjugate released as described above. A solution of 1.0–1.05 equiv of the second, Scm-activated peptide (ca. 1 mM) in 3:1 HFIP–H_2O at 25 °C is added. (In rare cases of less soluble Scm derivatives, 10:1 HFIP–H_2O can be used, but the reaction time must be increased to 45–60 min.) The progress of the reaction is checked by HPLC after 10 min, and if the reaction is not complete, the volume of the reaction mixture is reduced to $^2/_3$ in vacuum, and the reaction is checked again. All solvent is then evaporated, and the product is obtained as a granular or fluffy powder by the addition of a small amount of ether, followed by drying in a gentle stream of nitrogen, with precautions to avoid loss of dust. The capture product can be stored at −20 °C but is best carried into the acyl transfer step immediately. Purification of this material by preparative HPLC is optional but not essential.

7. The Acyl-Transfer Step. A weighed amount of capture product from the preceding step is completely dissolved in freshly distilled DMSO at 25 °C to give a solution with final concentration close to 1×10^{-4} M. If model studies indicate that the half time for the acyl transfer is longer than 1 h, about 5–10 mol %, based on the capture product, of $AgNO_3$ is added to the DMSO, followed by 1–2 equiv of DIEA, based on capture product. For reactions with shorter half times, $AgNO_3$ is omitted. The solution is stirred

(36) König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034.

at 25 °C in the dark under N_2 for a time period corresponding to 10 half-lives of the acyl transfer. The progress of the reaction can be monitored by HPLC. Upon completion of the reaction, the DMSO is removed under high vacuum at 40–45 °C using a rotary evaporator equipped with a liquid nitrogen-filled cold finger. All the ground joints of the evaporator are greased with Apiezon N, and the evaporation flask contains a magnetic stirbar to facilitate the evaporation process. After the solvent removal is complete, the resulting oil is further dried under high vacuum (0.01–0.1 mm) for at least 30 min and is then used directly without purification in the next step.

8. The Cleavage of S–S Linkage to Template and 2,4-Dinitrophenylation of Released Thiol. The product from the preceding step is dissolved in ca. 5–10 mL/100 mg DCM–HFIP (1:1) containing 1% water, and 1.5 equiv, based on acyl-transfer product, of Et_3P is added. (Some experimentation with solvent composition may be required to achieve solubilization alternatively, 4:1 or 3:1 dioxane–water mixtures can be used.) The progress of the reduction is followed by HPLC, but should be complete in 15 min. The solvent is removed in vacuum, and the resulting residue is triturated with a spatula with ether (6×) in order to remove the released 4-hydroxy-6-mercaptodibenzofuran and to convert the product to a free-flowing powder.

Depending upon the solubility characteristics of the peptide and its side chain blocking groups, one of three dinitrophenylation protocols is appropriate.

a. Peptides with complete side chain protection (except for the guanidine of Arg) are dissolved under nitrogen in 2:1 DCM–DMF containing 1 equiv of DIEA and 2 equiv of Dnp-F. The reaction is followed by HPLC and is usually complete after 5–20 min. The solvent is evaporated, and the residue is triturated with ether, then with an ether–DCM mixture to remove Dnp-F. The concentrations of the reagents are not critical, since concentration during workup increases the reaction rate.

b. For blocked peptides that are insoluble in the above solvent mixture, TFE can usually be substituted in an otherwise identical procedure.

c. Peptides containing unblocked side chain functions, particularly the ϵ-amino group of Lys are best treated by the following protocol. The peptide is dissolved in TFE, which is diluted with an equal volume of pH 7.5 0.1 M phosphate buffer, and then treated with 2 equiv of Dnp-F. The concentration of peptide should be greater than 1 mg/mL, and the reaction is monitored by HPLC. Usually, complete reaction is observed after 5–20 min, and the mixture is then worked up as in a.

The product of dinitrophenylation is purified by preparative HPLC or by Sephadex gel filtration followed by HPLC or partition chromatography, and is stable to storage.

9. Conversion of Acm to Scm Functions. This derivatization is carried out in two ways, depending on the side-chain protection of the peptide.

a. For fully blocked peptides, the peptide is added (ca. 1 mg/mL) to a 0–5 °C mixture of 1:1 DCM–MeOH and stirred while diethylamine (1 equiv) is added followed by Scm-Cl (2 equiv). After 50 min at 5 °C, the solvent is evaporated, and the residue is purified by preparative HPLC. The Scm function is relatively stable in protic, weakly acidic solvents. It decomposes relatively rapidly in the basic dipolar aprotic solvents such as DMF.

b. For unblocked peptides, the peptide is added (ca. 2 mg/mL) to a 5 °C mixture of 9:1 HOAc–DMF containing 3% water. (More water can be used to increase solubility, but the enhanced solvolysis rate of Scm-Cl will necessitate use of 10–50 equiv of this reagent, with HPLC monitoring to determine completeness of the reaction.) To this solution is added a freshly prepared solution of 2 equiv of Scm-Cl in 1:1 HOAc–DMF. The reaction is monitored by HPLC and is usually complete in 50 min, at which point the solvent is evaporated, the residue dissolved in 2 M aqueous HOAc, and the resulting solution lyophilized to yield a final powder that can be purified as indicated in a.

Synthesis of BPTI Sequence 51–58: H-C(Acm)-(52–54)-C(Dnp)-(56–58)-OH (28). 1. Solid-Phase Synthesis of Boc-C(Acm)-M-R(Pmc)-T(tBu)-Dbf-SH (24). Amino acid are listed in order of acylation, starting from the C-terminus. In parentheses are listed the method of acylation and the purity of the resulting peptide; the purity is reported relative to the combined amounts of the peptide and 4-acetoxy-6-mercaptodibenzofuran and can be obtained directly from the HPLC trace: (1) T (A, 97%), (2) R (B, 97%), (3) M (C, 97%), (4) C (B, 97%).

2. The resin-bound tetrapeptide Boc-C(Acm)-M-R(Pmc)-T-(tBu) (340 mg, 0.054 mmol) was placed in the solid-phase vessel, washed with DCM–HFIP (10 mL, 4:1, 2 × 1 min), and then swelled in DCM–HFIP (3 mL, 4:1) containing Et_3P (8.5 μL, 1.2 equiv). After 20 min the solvent was filtered under positive N_2 pressure and collected in a 25-mL flask. The resin was washed with DCM–HFIP (3 mL, 4:1, 3 × 1 min), and the collected filtrates were evaporated. The resulting oil was triturated under N_2 with hexanes and with hexanes–ether, 1:1 (6 × 10 mL). The resulting white powdery residue was dried under high vacuum, 59 mg, 99%.

To the resulting free thiol was added a solution of **22** (1.2 mL, 5:1), and after 1.5 h, the solvent was evaporated, and the resulting oil was triturated with ether and 1:1 ether/DCM to yield a white powder of the unsymmetrical disulfide that was dried for at least 2 h under high vacuum, 75.1 mg, 94% (based on the weight of the free thiol **24**).

To a stirred solution of the disulfide in frehsly distilled DMSO (100 mL) was added $AgNO_3$ (1 mL of an 0.041 M solution in DMSO) followed by DIEA (8.1 μL, 1.0 equiv). The resulting clear, colorless solution was degassed and purged with N_2 (2×) and stirred in the dark for 20 h. The solvent was evaporated under high vacuum at 40 °C using a rotary evaporator with liquid nitrogen cooled cold finger to afford a pale brown oil, which was dissolved in HFIP–DCM (3 mL, 1:3) and treated with H_2O (0.1 mL) and Et_3P (9 μL, ca. 1.0 equiv). After 15 min, the solvent was evaporated, and the residue was triturated with ether (6×) under N_2. The resulting free thiol was dissolved in DMF–DCM (3 mL, 3:2) containing Dnp-F (12 μL, 2 equiv) and DIEA (8 μL, 1 equiv). After 5 min, the solvent was evaporated under high vacuum, and the resulting oil was triturated with ether (4×) to yield the crude octapeptide as a yellow solid, 79.5 mg. Preparative HPLC on 70 mg of this solid (55% 0.1% TFA–45% MeCN) afforded pure protected octapeptide **27** as a yellow solid, 45 mg, 68%. HPLC (60% 0.1% TFA–40% MeCN): t_R 10.0 min. ^1H NMR (300 MHz, DMF-d_7): δ 8.93 (1 H, d, J = 2.8 Hz, Dnp), 8.55 (2 H, m, amides), 8.5 (1 H, dd, J = 2.8, 8.6 Hz, Dnp), 8.32 (1 H, d, J = 7.4 Hz, amide), 8.23 (1 H, d, J = 7.2 Hz, amide), 7.0 (1 H, d, J = 8.0 Hz, urethane), 6.7 (3 H, b m, R NHs), 4.85 (1 H, J = 3.0, 8.0 Hz, C CH), 4.2–4.6 (8 H, m, CHs + Acm CH), 4.05 (1 H, m, T CH), 3.90 (2 H, d, J = 5.5 Hz, G), 3.88 (2 H, d, J = 6.3 Hz, C), 3.82 (1 H, dd, J = 4.6, 14.3 Hz, C), 3.6 (1 H, dd, J = 8.6, 14.3 Hz, C), 3.18 (2 H, m, R), 3.02 (1 H, dd, J = 4.6, 14.3 Hz, C), 2.80 (1 H, dd, J = 8.6, 14.3 Hz, C), 2.62 (2 H, t, J = 6.9 Hz, M), 2.55 (6 H, s, Pmc), 2.05 (3 H, s, Pmc), 2.04 (3 H, s, M), 1.95 (3 H, s, Acm), 1.50–1.85 (6 H, R + M), 1.4 (9 H, s, Boc), 1.38 (3 H, d, J = 7.4 Hz, A), 1.30 (6 H, s, Pmc), 1.19 (9 H, s, T Bu), 1.05 (3 H, d, J = 6.3 Hz, T).

The pure protected octapeptide (31 mg, 0.021 mmol) was dissolved in TFA containing 0.5% H_2O (5 mL), and the resulting bright yellow solution was stirred for 3 h and then concentrated to a yellow oil that was triturated with ether (6 × 5 mL). The residue was purified by preparative HPLC (80% 0.1% TFA–20% MeCN) to yield **28** as a yellow powder, 23.5 mg, 87%. FAB MS: 1035.4 [MH^+]. ^1H NMR (300 MHz, D_2O): δ 9.18 (1 H, d, J = 2.4 Hz, Dnp), 8.60 (1 H, dd, J = 2.4, 9.0 Hz, Dnp), 8.0 (1 H, d, J = 9.0 Hz, Dnp), 4.65 (1 H, dd, J = 6.0, 7.8 Hz), 4.35–4.55 (6 H, m, α-CH), 4.3 (1 H, m, T CH), 4.1 (4 H, s, G), 3.90 (1 H, dd, J = 5.4, 15 Hz), C CH_2), 3.63 (1 H, dd, J = 9.0, 15.0 Hz, C CH_2), 3.3 (3 H, m, R, C CH_2), 3.15 (1 H, dd, J = 6.6, 15.0 Hz, C CH_2), 2.7 (2 H, dt, J = 3.0, 7.2 Hz, M CH_2), 2.20 (3 H, s, M CH_3), 2.12 (3 H, s, Acm), 1.7–2.1 (6 H, m, R + M CH_2), 1.53 (3 H, d, J = 7.2 Hz, A), 1.25 (3 H, d, J = 6.0 Hz, T). Amino acid analysis: $Gly_{(2.0)}$ 2.0; $Arg_{(1.0)}$ 1.04; $Thr_{(1.0)}$ 0.74; $Ala_{(1.0)}$ 0.96; $Met_{(1.0)}$ 0.94.

S-Activation of BPTI Sequence 51–58: TFA·H-C(Scm)-(52–54)-C(Dnp)-(56–58)-OH (29). To a solution of **28** (44 mg, 24.9 μmol), in HOAc–DMF (9:1, 5 mL) and H_2O (0.15 mL) was added at 0 °C with stirring Scm-Cl (0.63 mL of a 0.11 M solution in 2:1 HOAc–DMF). After 30 min the solvent was evaporated, and the residue was dissolved in H_2O–HOAc (0.5 mL, 2 M) and applied to a preparative HPLC column. Elution (80% 0.1% TFA–20% MeCN, 6 min; 28% MeCN 2 min; 32% MeCN; 18 mL/min, 295 nm; t_R 10 min) gave **29** as a yellow powder after lyophilization, 38 mg, 86%. HPLC (75 to 0% 0.1% TFA, 25–100% MeCN in 15 min): t_R 9 min. ^1H NMR (300 MHz, D_2O): identical with the Acm derivative except without δ 2.12 and 3.15

and with the following: δ 4.80 (1 H, dd, J = 4.8, 7.2 Hz, C CH), 3.91 (3 H, s, Scm), 3.37 (1 H, dd, J = 5.7, 13.8 Hz, C CH_2), 3.25 (1 H, dd, J = 6.3, 13.8 Hz C CH_2).

Synthesis of BPTI Sequence 38–58: TFA·H-C(Acm)-(39–50)-C(Dnp)-(52–54)-C(Dnp)-(56–58)-OH (31). 1. Solid-Phase Synthesis of Boc-C(Acm)-R(Pmc)-A-K(Boc)-R(Pmc)-N-N-F-K(Boc)-S(tBu)-A-E(tBu)-D(tBu)-Dbf-SH (30b). Amino acid are listed in order of acylation, starting from the C-terminus. In parentheses are listed the method of acylation and the purity of the resulting peptide; the purity is reported relative to the combined amounts of the peptide and 4-acetoxy-6-mercaptodibenzofuran, and can be obtained directly from the HPLC trace: (1) D (A, 98%), (2) E (C, 98%), (3) A (C, 98%), (4) S (C, 98%), (5) K (C, 98%), (6) F (C, 98%), (7a) N (B, 94%), (7b) acetic anhydride (C, 94%), (8a) 2× Bpoc-K(Boc)-R(Pmc)-N (B, 82%), (8b) acetic anhydride (C, 82%), (9) 2× A (C, 80%), (10) 2× Boc-C(Acm)-R(Pmc) (B, 75%).

2. The resin-bound 13-peptide **30b** (ca. 9 μmol) was placed in the reaction vessel and washed with 2 mL and suspended in 2 mL of 4:1 DCM–TFE and then treated with Bu_3P (4 μL, 1.3 equiv). After 15 min, the solvent was filtered, and the resin was washed with DCM–TFE (4:1 2 mL, 3 × 1 min). The combined washings were concentrated to a gum that was triturated with ether (6 × 10 mL) to yield the crude template-functionalized 13-peptide as a white powder, 25 mg, 100%, which was dissolved in a solution of **29** (12 mg, 9.5 μmol) in HFIP-H_2O (15 mL, 3:1) and stirred for 30 min. The solvent was evaporated to yield a yellow powder that was dried under high vacuum and then dissolved in DMSO (100 mL) containing DIEA (3.2 μL, 2.0 equiv). The solution was stirred for 14 h in the dark under N_2, and the solvent was evaporated. The residue was dissolved in DCM–TFE (5 mL, 3:2) and treated with Bu_3P (3.5 μL, 1.5 equiv); after 15 min, the solvent was evaporated, the residue was triturated with ether (5 × 5 mL) to remove template, and the residual 21-peptide was dissolved in TFE containing Dnp-F (100 μL, 0.4 M in Dnp-F and 0.22 M in DIEA). After 30 min the solvent was evaporated, and the excess Dnp-F was removed by trituration with ether, ether–DCM, and ether to yield 33 mg of crude side-chain-blocked Boc-C(Acm)-(38–50)-C(Dnp)-(51–54)-C(Dnp)-(55–58)-OH, a sample of which was purified by preparative HPLC (Vydac 218TP1002, 1 cm, 4 mL/min, eluent 20–0% 0.1% TFA, 80–100% MeOH; t_R 20 min). FAB MS (abundance) calcd for $C_{160}H_{246}N_{39}O_{51}S_6$ 3721.62, found 3721.6 (41), 3722.6 (84), 3723.63 (100), 3724.6 (88), 3725.64 (63), 3726.65 (38).

The remaining crude peptide was dissolved in TFA (5 mL, with 1% H_2O), and the solution was stirred for 2 h, and then concentrated to a residue that was dissolved in 5% HOAc and applied to a Sephadex G-25 column (fine, 2.5 × 95 cm, eluent 5% HOAc, 7 mL/h). The fractions containing the unprotected peptide were pooled and concentrated to give 30 mg of residue that was further purified by preparative HPLC (Vydac 218TP1002, 73% 0.1% TFA–27% MeCN, 18 mL/min, t_R 8 min) to afford a yellow powder, 13.5 mg, 55% overall from **29** consumed. HPLC (75–65% 0.1% TFA, 25–35% MeCN over 20 min): t_R 18.5 min. FAB MS calcd for $C_{105}H_{162}N_{39}O_{39}S_4$ 2722.913, found 2722.3 (MH^+, centroid). ^1H NMR (500 MHz, D_2O): δ 9.13 (1 H, d, J = 2.5 Hz, Dnp), 9.08 (1 H, d, J = 2.5 Hz, Dnp, 8.57 (2 H, 2 dd, J = 9.0 Hz, Dnp), 7.94 (1 H, dd, J = 9.0 Hz, Dnp), 7.90 (1 H, dd, J = 9.0 Hz, Dnp), 7.42 (2 H, t, J = 7.5 Hz, F), 7.39 (1 H, t, J = 7.2 Hz, F), 7.31 (2 H, d, J = 7.5 Hz, F), 4.3–4.55 (14 H, m, α-CH), 4.11 (2 H, s, G), 4.09 (2 H, s, G), 4.03 (1 H, dd, J = 5.5, 16.5 Hz, S), 3.95 (1 H, dd, J = 5.4, 16.5 Hz, s), 3.87 (1 H, dd, J = 4.6, 13.8 Hz, C Dnp), 3.78 (1 H, dd, J = 4.6, 13.8 Hz, C Dnp), 3.63 (2 H, m, c Dnp), 3.3 (8 H, m, C Acm + R), 3.05 (1 H, dd, J = 5.5, 17.4 Hz, D), 2.97 (1 H, dd, J = 7.3, 17.4 Hz, D), 2.7–2.9 (4 H, m, N), 2.97 (1 H, dd, J = 6.9, 13.8 Hz, E), 2.62 (1 H, dd, J = 6.9, 13.8 Hz, E), 2.55 (2 H, t, J = 7.6 Hz, M), 2.2 (3 H, s, Acm), 2.15 (3 H, s, M), 2.09 (1 H, d, J = 7.8, 14.2 Hz, F), 2.0–1.7 (22 H, m, R + K), 1.53 (3 H, d, J = 7.4 Hz, A), 1.5 (6 H, d, J = 7.4, A), 1.30 (3 H, d, J = 6.4 Hz, T).

S-Activation of BPTI Sequence 38–58: TFA·H-C(Scm)-(39–50)-C(Dnp)-(52–54)-C(Dnp)-(56–58)-OH (32). To a solution of **31** (5.0 mg, 1.59 μmol) in wet HOAc–DMF (0.5 mL, 9:1 + water, 0.015 mL) was added at 0 °C Scm-Cl (0.35 mL, 0.11 M in 9:1 HOAc–DMF, 38 μmol). The mixture was checked periodically by HPLC to follow the course of the reaction, and after 45 min the solvent was evaporated, the residue was dissolved in H_2O (0.5 mL, 0.1% TFA), and the sample was purified by preparative HPLC (73% 0.1% TFA, 27% MeCN, t_R 9 min) to yield after solvent removal a yellow powder, 4.3 mg, 86%. HPLC (75–65% 0.1% TFA, 25–35% MeCN over 20 min): t_R 19.3 min. FAB MS (abundance) calcd for $C_{104}H_{159}N_{38}O_{40}S_5$ 2740.02, found (MH^+) 2739.96 (75), 2740.94 (100), 2741.94 (82), 2742.98 (68), 2743.95 (40), 2744.97 (28). Amino acid analysis (gas-phase hydrolysis 24 h, 108 °C, 6 M HCl): $Asp_{(3.0)}$ 2.74, $Glu_{(1.0)}$ 1.1, $Ser_{(1.0)}$ 0.95, $Gly_{(2.0)}$ 2.13, $Arg_{(3.0)}$ 3.02, $Thr_{(1.0)}$ 0.95, $Ala_{(3.0)}$ 3.07, $Met_{(1.0)}$ 1.00, $Phe_{(1.0)}$ 1.19, $Lys_{(2.0)}$ 2.2. Amino acid sequence analysis of the first 15 N-terminus amino acids coincided with the corresponding residues of BPTI(38–58).

Synthesis of BPTI Sequence 30–58: TFA·H-C(Acm)-(31–34)-Y(Dnp)-(36–37)-C(Dnp)-(39–50)-C(Dnp)-(52–54)-C-(Dnp)-(56–58)-OH (34). 1. Solid-Phase Synthesis of Boc-C(Acm)-Q-T(tBu)-F-V-Y(Dnp)-G-G-Dbf-SH. Amino acid are listed in order of acylation, starting from the C-terminus. In parentheses are listed the method of acylation and the purity of the resulting peptide; the purity is reported relative to the combined amounts of the peptide and 4-acetoxy-6-mercaptodibenzofuran and can be obtained directly from the HPLC trace: (1) G-G (A, 99%), (2) Y (C, 98%), (3) V (C, 98%), (4) F (C, 98%), (5) T (C, 98%), (6) Q (B, 96%), (7) C (B, 93%).

2. To a suspension of the resin-bound 9-peptide (1.6 μmol) in DCM–TFE (0.3 mL, 85:15) was added Et_3P (0.95 equiv, 75 μL of a 20.7 mM solution in the same solvent), and after 10 min the product was isolated as described for the side-chain-blocked Boc-C(Acm)-(38–50)-O-DBF-SH, 2.2 mg, 95%, 80% pure by HPLC. To this material was added a solution of **32** (1.1 μmol) in HFIP-H_2O (0.4 mL, 3:1). After 45 min the solvent was evaporated, and the product was purified by preparative HPLC (63–57% 0.1% TFA, 37–43% MeCN in 12 min, 18 mL/min, t_R 11 min) to give a yellow powder, 4.2 mg, 81% (based on **32**). HPLC (Vydac 218TP54-C18, 60–0% 0.1% TFA, 40–100% MeCN over 20 min, 1 mL/min, 280 nm): t_R 10.4 min.

To a solution of this material (4.0 mg, 0.86 μmol) in freshly distilled DMSO (10 mL) was added DIEA (1.0 equiv, 30 μL of 28.7 mM in DMSO; i.e. 50 μL in 10 mL), and the pale yellow solution was stirred under N_2 in the dark for 4 h. The solvent was removed under high vacuum using a rotary evaporator with a liquid N_2 cooled cold finger, and the residue was dissolved in dioxane–H_2O (1 mL, 7:3) to which 20 μL of 0.1 M Et_3P in the same solvent was added. The solution was stirred for 10 min, the solvent was evaporated, and the residue was triturated with ether (6 × 3 mL). The resulting thiol was dissolved in TFE–pH 7.5 $NaHCO_3$ buffer (1.0 mL, 1:1) containing Dnp-F (15 μL/mL, 4 equiv). Evaporation and trituration with ether, DCM, and ether afforded a light yellow powder, 80% pure by HPLC. A sample of this material was purified by preparative HPLC (63–53% 0.1% TFA, 37–47% MeCN over 12 min; t_R 14 min). FAB MS calcd for $C_{165}H_{236}N_{52}O_{60}S_2$ 4068.29, found centroid at 4067.84.

The crude side-chain-blocked peptide was dissolved in TFA (2 mL), and the solution was stirred for 1.5 h, followed by evaporation. The resulting oil was converted to a solid by trituration with ether. Preparative HPLC (70–63% 0.1% TFA, 30–37% MeCN, 10 min; t_R 12 min) gave 3 mg, 70%, of **34**. HPLC (70–45% 0.1% TFA, 30–55% MeCN over 20 min): t_R 14.7 min. Amino acid analysis (after gas phase hydrolysis by 6 M HCl at 108 °C for 24 h): $Asp_{(3.0)}$ 2.4, $Glu_{(2.0)}$ 2.00, $Ser_{(1.0)}$ 0.75, $Gly_{(4.0)}$ 3.97, $Arg_{(3.0)}$ 3.24, $Thr_{(2.0)}$ 2.01, $Ala_{(3.0)}$ 3.17, $Val_{(1.0)}$ 1.06, $Met_{(1.0)}$ 0.82, $Phe_{(2.0)}$ 1.48, $Lys_{(2.0)}$ 2.04. Cys(Dnp) and Tyr(Dnp) were not analyzed. Amino acid sequence analysis of the first 15 N-terminus amino acids coincided with the corresponding amino acid sequence of BPTI(30–58).

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Registry No. **5**, 101762-34-9; **6**, 101762-35-0; **7**, 101762-14-5; **8**, 53907-23-6; **9**, 55757-46-5; **13**, 103478-10-0; **14**, 101774-01-0; **15**, 82392-72-1; **16**, 103477-95-8; **17**, 42137-80-4; **18**, 120411-15-6; **21**,

120386-82-5; **22**, 120386-61-0; **23**, 120386-62-1; **24**, 120386-63-2; **25**, 120386-64-3; **26**, 120386-65-4; **27**, 120386-66-5; **28**, 120386-68-7; **29**, 120386-70-1; **30b**, 120411-20-3; **31**, 120411-17-8; **32**, 120411-19-0; **34**, 120544-84-5; BPTI, 9087-70-1; Dbf, 132-64-9; Scm-Cl, 26555-40-8; Dnp-F, 70-34-8; Scm-S-Dbf-OH, 101697-58-9; Z-Ala-O-Dbf-S-Scm, 101697-56-7; Z-Gly-Gly-OH, 2566-19-0; H-Ala-OBu-*t*·HCl, 13404-22-3; Z-Gly-Gly-Ala-OBu-*t*, 120386-71-2; H-Gly-Gly-Ala-OBu-*t*, 120386-72-3; Boc-Cys(Acm)-OH, 19746-37-3; Boc-Cys(Acm)-Gly-Gly-Ala-OBu-*t*, 120386-73-4; Boc-Cys(Scm)-Gly-Gly-Ala-OBu-*t*, 120411-21-4; Z-Arg(Pmc)-OH, 112160-32-4; Z-Arg(Pmc)-OSu, 120386-74-5; H-Asn-OH, 70-47-3; Z-Arg(Pmc)-Asn-OH, 120386-75-6; H-Arg(Pmc)-Asn-OH, 120386-76-7; Bpoc-Lys(Boc)-OH, 47766-94-9; Bpoc-Lys(Boc)-OSu, 120386-77-8; Bpoc-Lys(Boc)-Arg(Pmc)-Asn-OH, 120386-78-9; Bpoc-Lys(Boc)-Arg(Pmc)-Asn-OH·DCHA, 120386-79-0; H-Arg(Pmc)-OH, 112160-37-9; Boc-Cys(Acm)-OSu, 19746-38-4; Boc-Cys(Acm)-Arg(Pmc)-OH, 120411-22-5; Boc-Tyr-OH, 3978-80-1; Boc-Tyr(Dnp)-OH, 120386-80-3; Z-Cys(Trit)-OH·DEA, 53308-88-6; Z-Cys(Scm)-OH, 53907-19-0; Z-Cys(Scm)-OH·CHA, 120386-81-4; H-Cys(Boc-Cys(Acm)-Met-Arg(Pmc)-Thr(*t*-Bu)-O-Dbf-S)-Gly-Gly-Ala-OH, 120386-83-6; Boc-Cys(Acm)-Met-Arg(Pmc)-Thr(*t*-Bu)-Cys(HO-Dbf-S)-Gly-Gly-Ala-OH, 120411-23-6; H-Cys(Boc-Cys(Acm)-Arg(Pmc)-Ala-Lys(Boc)-Arg(Pmc)-Asn-Asn-Phe-Lys(Boc)-Ser(*t*-Bu)-Ala-Blu(OBu-*t*)-Asp(OBu-*t*)-O-Dbf-S)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH, 120474-54-6; Boc-Cys(Acm)-Gln-Thr(*t*-Bu)-Phe-Val-Tyr(Dnp)-Gly-Gly-O-Dbf-SH, 120411-14-5; H-Cys(Boc-Cys(Acm)-Gln-Thr(*t*-Bu)-Phe-Val-Tyr(Dnp)-Gly-Gly-O-Dbf-S)-Arg-Ala-Lys-Arg-Asn-Asn-Phe-Lys-Ser-Ala-Glu-Asp-Cys(Dnp)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH, 120474-55-7; Boc-Cys(Acm)-Gln-Thr(*t*-Bu)-Phe-Val-Tyr(Dnp)-Gly-Gly-Cys(Dnp)-Arg-Ala-Lys-Arg-Asn-Asn-Phe-Lys-Ser-Ala-Glu-Asp-Cys(Dnp)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH, 120474-53-5; Boc-C(Acm)-(38-50)-C(Dnp)-(51-54)-C(Dnp)-(55-58)-OH, 120474-52-4.

Synthesis of Bryostatins. 1. Construction of the C(1)–C(16) Fragment†

Mary A. Blanchette, Michael S. Malamas, Michael H. Nantz, John C. Roberts, Peter Somfai, David C. Whritenour, and Satoru Masamune*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Masanori Kageyama and Tadashi Tamura

Kao Institute for Fundamental Research, 2606 Akabane, Ichikaimachi, Tochigi 321-34, Japan

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The synthesis of fragment AB [C(1)–C(16)] of bryostatin 1 is described. Two aldol coupling reactions involving (i) chiral fragment A [C(1)–C(10)] with achiral B [C(11)–C(16)] and (ii) chiral fragment A1 [C(7)–C(10)] with achiral A2 [C(6)–C(1)] constitute crucial steps in which an external chiral boron reagent is used to control stereoselectivity in the creation of a new stereogenic center. This type of double asymmetric synthesis, although rarely precedented, provides a powerful means of stereocontrol over the fragment assembly.

Two decades ago, Pettit et al. found that extracts from the invertebrate colonial filter-feeder *Bugula neritina* were active against the murine P388 lymphocytic leukemia.[1] Subsequent efforts directed toward the isolation of the bioactive constituents have yielded 13 bryostatins of known structure, all but one of which differ only in their C(7) and C(20) substituents.[2] Whereas bryostatin 1 (**1**),[2a] the most abundant bryostatin, contains C(7) acetate and C(20) octadienoate substituents, there are various other ester derivatives, as well as three C(20)-deoxy bryostatins. Because of their attractive stereostructural features, anticancer properties, and relative scarcity, we have chosen **1** as a synthetic target. As depicted in Scheme I, a logical (and straightforward) retrosynthesis of **1** begins with the dissection of the lactonic linkage and the C(16)–C(17) double bond to provide the two major fragments AB [C(1)–C(16)] and CD [C(17)–C(27)]. The AB fragment can be further disassembled into fragments A [C(1)–C(10)] and B [C(11)–C(16)] and finally into A1 [C(7)–C(10)] and A2 [C(1)–C(6)]. In the coupling of A1 and A2, a stereogenic center is created at C(7) of A, and in the coupling of A and B, at C(11) of AB. Thus, these reactions are concerned with a fundamental, general problem of convergent synthesis, which involves the stereoselective assembly of two fragments (at least one of which is chiral) with concomitant creation of a new stereogenic center or centers.[3]

Stereogenic centers embedded in fragments generally correspond directly to those of a target molecule. When a center (or centers) is/are created in the coupling of two fragments, e.g. an enolate and an aldehyde, the product ratio heavily depends on the diastereoselectivities[3a] of the two reactants. The stereoselection attained in such a coupling, once a choice of fragments has been made, is therefore predetermined and normally unpredictable in both magnitude and sense. Thus, the fragment-coupling step often constitutes the least stereoselective step in the total synthesis, and, traditionally, such a reaction is performed with anticipated resignation to the ensuing product mixture with subsequent efforts directed toward separation of the congeners.[4] One approach to this problem, however, is externally altering (or ideally overpowering) the diastereofacial selectivities of the reactants. The use of enolates

† This paper is dedicated to Professor Frederick D. Greene in appreciation of his 27 years of service as Editor of *The Journal of Organic Chemistry*.

(1) Pettit, G. R.; Day, J. F.; Hartwell, J. L.; Wood, H. B. *Nature (London)* **1970**, *227*, 962.

(2) (a) Pettit, G. T.; Herald, C. L.; Doubec, D. L.; Herald, D. L.; Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1982**, *104*, 6846. (b) Pettit, G. R.; Kamano, Y.; Herald, C. L. *J. Org. Chem.* **1987**, *52*, 2854 and references cited therein.

(3) (a) Masamune, S.; Choy, W.; Petersen, J. S.; Sita, L. R. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1. (b) Masamune, S. In *Stereochemistry of Organic and Bioorganic Transformations*; Bartmann, W., Sharpless, K. B., Eds.; VCH Verlagsgesellschaft mbH: Weinheim, 1987; pp 49–71. As discussed in these references, the process of fragment assembly should be distinguished from one whereby stereogenic centers are created on a chiral substrate by a homochiral reagent or catalyst, as has been executed on numerous occasions in recent years.

(4) For instance, see: (a) Toshima, K.; Tatsuta, K.; Kinoshita, M. *Tetrahedron Lett.* **1986**, *27*, 4741. (b) Evans, D. A.; Bender, S. L.; Morris, J. *J. Am. Chem. Soc.* **1988**, *110*, 2506.

0022-3263/89/1954-2817$01.50/0