chloromethane was added, and the organic phase was worked up in the usual manner. Purification by flash chromatography (70% ethyl acetate in hexanes) gave 50 mg (99%) of the title compound **24**. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.31 (t, 6 H, J = 7.5 Hz, 2 CH_2CH_3), 1.84 (quint, 2 H, J = 7.5 Hz, $CH_2CH_2CH_2$), 2.32–2.47 (m, 4 H, $CH_2C{\equiv}C$, CH_2CO_2), 2.62 (dd, 2 H, J = 7.5 Hz, 22.5 Hz, $CH_2P{=}O$), 3.67 (s, 3 H, CO_2CH_3), 4.10 (quint, 4 H, J = 7.5 Hz, 2 CH_2O), 5.60 (br d, 1 H, $CHC{\equiv}C$), 5.93 (hept, 1 H, J = 7.5 Hz, $CHCH_2PO$). IR (neat): 2980, 1740 cm^{-1}. High-resolution mass spectrum: m/e calcd for $C_{14}H_{24}O_5P$ (M + H) 303.1362, found 303.1360.

Ethyl (5*S*,6*R*)-5-Hydroxy-6-[(cysteine methyl ester)-*S*-yl]-15-carbomethoxypentadeca-7,9(*E*)-dien-11-ynoate (16-Carbomethoxy-17,18,19,20-tetranor-14,15-dihydro-11,12-didehydro-LTE$_4$ Triester, 26). To a stirred solution of the phosphonate **24** (50 mg, 0.17 mmol) in THF (1 mL) was added at −70 °C a solution of LiHMDSi (0.34 M in THF, 0.54 mL, 0.18 mmol), and the resulting solution was stirred for 15 min under argon. Then a solution of the aldehyde **25** (34 mg, 0.18 mmol) in THF was added, and the temperature was raised slowly to room temperature. After 2.5 h the reaction was quenched with saturated ammonium chloride solution (0.5 mL) diluted with CH_2Cl_2, washed successively with a saturated solution of sodium bicarbonate and brine, and dried over anhydrous Na_2SO_4. Flash chromatography of the residue (20% ethyl acetate in hexanes containing 1% triethylamine) gave 12 mg (22%) of the corresponding epoxide. The epoxide (0.036 mmol) was dissolved in a mixture of methanol and triethylamine (3:1, 1 mL) containing L-cysteine methyl ester (15 mg, 0.089 mmol) and 4-hydroxy-TEMPO (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy free radical) (1 mg) at room temperature. After being stirred overnight evaporation of the solvent and flash chromatography (hexanes–AcoEt–MeOH, 8:5:1) gave 11 mg (65%) of the title compound (**26**). UV (MeOH) λ_{max}: 273 nm. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1..25 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.44–1.53 (m, 2 H), 1.60–1.91 (m, 6 H), 2.32 (t, 2 H, J = 7.2 Hz, CH_2CO_2), 2.39–2.48 (m, 2 H), 2.75 (dd, 1 H, J = 7.5, 13.7 Hz, SCH_2), 2.86 (dd, 1 H, J = 4.5, 13.8 Hz, SCH_2), 3.42 (dd, 1 H, J = 3.6, 9.7 Hz, H-6), 3.66 (m, 1 H), 3.68 (s, 3 H, CO_2CH_3), 3.74 (s, 3 H, CO_2CH_3), 4.12 (q, 2 H, J = 7.2 Hz, OCH_2), 5.59 (d, 1 H, $J_{9,10}$ = 15.7 Hz, H-10), 5.69 (dd, 1 H, $J_{6,7}$ = 10 Hz, $J_{7,8}$ = 15.6 Hz, H-7), 6.19 (dd, 1 H, $J_{7,8}$ = 15.1 Hz, $J_{8,9}$ = 10.7 Hz, H-8), 6.50 (dd, 1 H, $J_{8,9}$ = 10.7 Hz, $J_{9,10}$ = 15.4 Hz, H-9). High-resolution mass spectrum (glycerol) calcd for $C_{23}H_{36}O_7NS$ (M + H), 470.2212, found 470.2213.

Boc-L-Dmt-OH as a Fully N,S-Blocked Cysteine Derivative for Peptide Synthesis by Prior Thiol Capture. Facile Conversion of N-Terminal Boc-L-Dmt-Peptides to H-Cys(Scm)-Peptides

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An efficient, convenient preparation of *N*-L-(*tert*-butyloxycarbonyl)-2,2-dimethylthiazolidine-4-carboxylic acid (Boc-Dmt-OH) is reported. The following rate constants assess the coupling efficiency and racemization risk of Boc-L-Dmt-OC_6F_5 in THF at 22 °C. With H-Val-OMe, k_{couple} = 3.2 × 10^{-2} M^{-1} s^{-1}, and with Et_3N, k_{rac} < 10^{-8} M^{-1} s^{-1}. Conversions of derivatives of the sequence Cys-Gly-Gly-Ala to the corresponding Scm-functionalized (Scm = methoxycarbonylsulfenyl, MeOCOS) cysteine peptides illustrated transformation of –Dmt– to –Cys(Scm)– with or without prior Boc cleavage. A palladium black catalyzed hydrogenolysis of a benzyl ester in the presence of the thiazolidine is reported in the solution-phase synthesis of the tripeptide Boc-Dmt-Leu-Ala-OBzl. The functionalized octapeptide 58–51 of basic pancreatic trypsin inhibitor, H-Cys(Scm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH, was prepared by a 4 + 4 thiol capture and acyl-transfer strategy to demonstrate the applicability of the thiazolidine to this method.

In this paper we report recent findings on the particular utility of cysteine N,S-blocking through thiazolidine formation for amide ligation by means of thiol capture. Previous reports[1] from this laboratory have described in detail the strategy of peptide synthesis by prior thiol capture and its application to the synthesis of a 29 amino acid fragment of Basic pancreatic trypsin inhibitor, BPTI (58–30).[2] As shown in Scheme I, the prior thiol capture strategy requires that an unsymmetrical disulfide bond be formed between the sulfur of a thiol functionalized rigid dibenzofuran-derived template containing one peptide fragment **1** and the sulfur of a cysteine residue residing at the amino terminus of a second peptide fragment **2**. The resulting aryl cysteinyl disulfide **3** then undergoes intramolecular *O,N*-acyl transfer, forming the new peptide amide **4**. Reaction of **4** with phosphine cleaves the disulfide bond, and protection of the so generated cysteine thiol yields the desired polypeptide **5**, completing one thiol capture cycle. Subsequent activation of **5** to **6** prepares the peptide for another thiol capture cycle. This multistep procedure is designed for the specific purpose of coupling large peptide fragments prepared by the solid-phase method. Synthesis proceeds in a linear fashion beginning at the C-terminus, and fragment couplings are made at each cysteine.

Synthesis of a complex peptide by prior thiol capture thus requires very careful selection of the functions used to protect the cysteine sulfhydryl. Although formation of a disulfide selectively from one sulfur in the presence of others has been accomplished previously in multifunctionalized molecules,[3] there is no widely acceptable method for accomplishing this transformation in either small or large peptides or in proteins.

The linear tactical sequence of ligations abbreviated in Scheme II emphasizes the three types of cysteine S-

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(2) Fotouhi, N.; Galakatos, N.; Kemp, D. *J. Org. Chem.* **1989**, *54*, 2803–2817.

(3) Kamber, B. *Z. Naturforsch.* **1981**, *36b*, 508–514.

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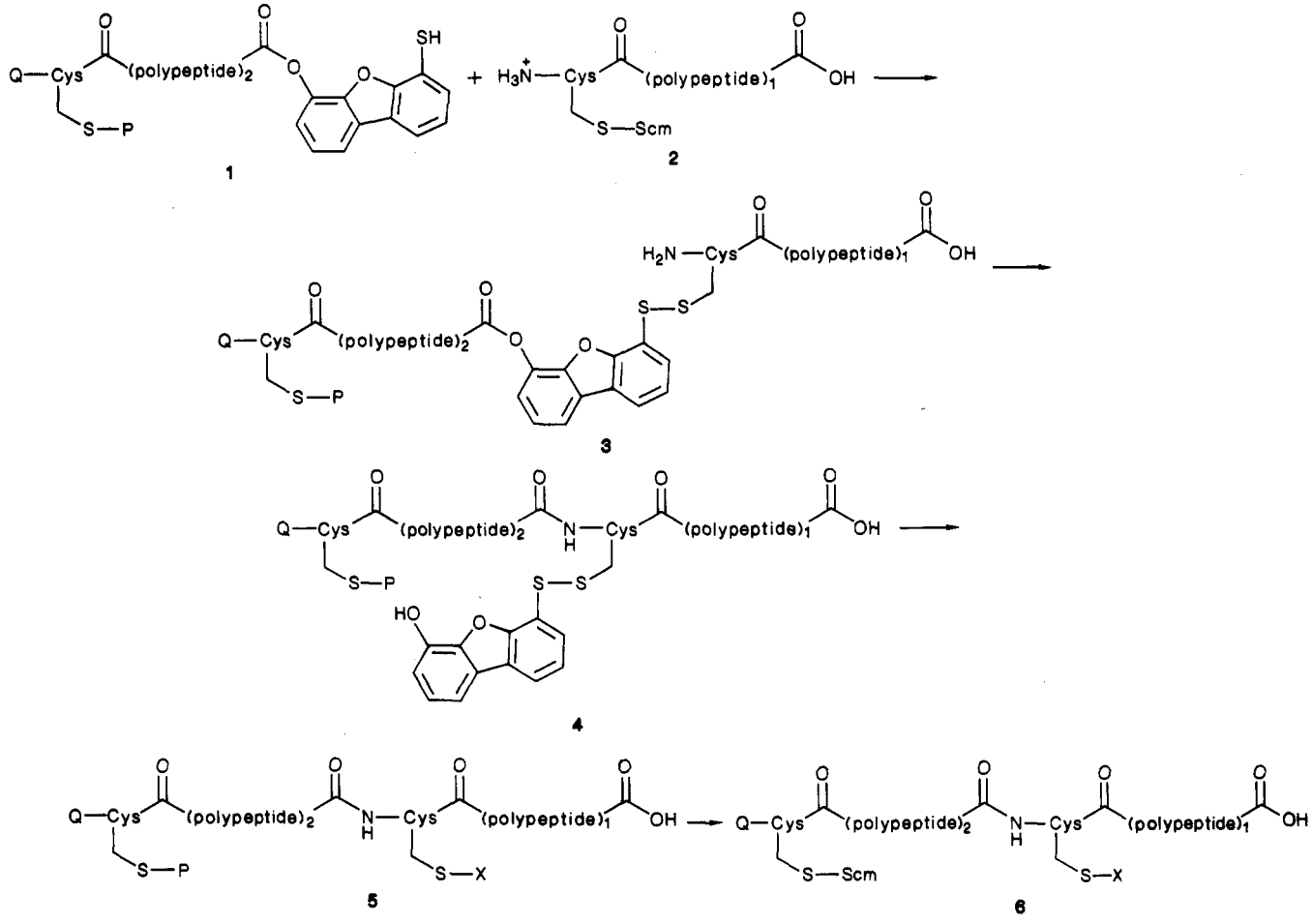
Scheme I[a]

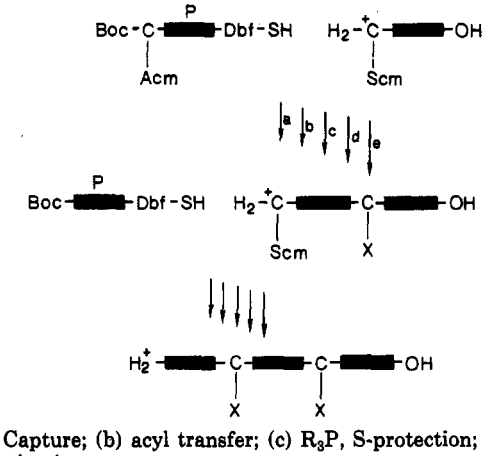


[a] Q = *tert*-butyloxycarbonyl (Boc) or 2-*p*-(biphenylyl)-2-propyloxycarbonyl (Bpoc); P = acetamidomethyl (Acm) or 2,2-dimethylthiazolidinyl; Scm = methoxycarbonylsulfenyl; X = dinitrophenyl.

functionalization that are needed for thiol capture: temporary protection, S-activation, and permanent protection. The thiol function used for S-activation must permit clean, facile formation of the S–S bond of Scheme I. The thiol function used for temporary protection must be stable to the conditions of the thiol capture reaction sequence, yet must be cleanly converted into the function used for S-activation. The thiol function used for permanent protection must resist all of the above manipulations including acidolytic side chain deblocking but must also be cleanly removable under mild conditions at the last step of the synthesis.

As previously discussed,[2] the function thus far used for S-activation is the methoxycarbonylsulfenyl (Scm).[4] The reagent used to generate the S-Scm function is Scm-Cl, which was introduced by Brois[4a] and has been used successfully by Hiskey[4b] and Kamber[4c] for converting free cysteine thiols as well as cysteine protected as acetamidomethyl (Acm) or trityl to the activated Scm species. There are drawbacks to the use of Scm-Cl, such as its short storage life (1–2 months at −20 °C in sealed ampules) and its relatively rapid hydrolysis in the protic media that are also required for the Cys to Cys(Scm) conversion to take place. Nevertheless, it has the unique feature both in our hands and in literature reports of forming stable crystalline Scm derivatives with cysteine peptides. Further, it is the only disulfide-forming reagent that has been shown to meet the stringent requirements of low concentration and protic solvent employed in the first step of the thiol capture method.

Scheme II[a]



[a] (a) Capture; (b) acyl transfer; (c) R_3P, S-protection; (d) TFA; (e) S-activation.

The permanent protection has optimally involved reaction of the thiol liberated in step 3 of Scheme I with 2,4-dinitrofluorobenzene (Dnp-F). The resulting Dnp-blocked internal cysteine residues are inert to reaction conditions required for selective conversion of the N-terminal cysteine to a Cys-Scm function, and the dinitro-

(4) (a) Brois, J.; Pilot, J.; Barnum, H. *J. Am. Chem. Soc.* **1970**, *92*, 7629. (b) Hiskey, R.; Li, C.; Vunnam, R. *J. Org. Chem.* **1975**, *40*, 3697. (c) Kamber, B. *Helv. Chim. Acta* **1973**, *56*, 1370.

phenyl group is removed at the end of the synthesis under mild thiolysis conditions.

Of the many methods of sulfhydyl protection in the literature of solid-phase synthesis,[5] only the Acm and trityl groups are compatible with all the requirements of the temporary protection function of the prior thiol capture strategy. Although the trityl group can be removed cleanly under mild acidolytic conditions, it was abandoned early since its steric bulk can lead to slow peptide coupling reactions and to sluggish reaction with Scm-Cl. Moreover, its high molecular weight and lipophilic character insolubilize trityl-functionalized peptides. The Cys-Acm group offers the advantage that it is easily made and purified and is commercially available as its N-Boc derivative in highly pure form. It is coupled very reliably in solid-phase synthesis, provides an NMR marker in the product peptide, and has been found to increase the solubility of large protected peptide fragments.[6]

Given the instability of Scm-Cl to the protic conditions of the S-activation reaction, the sluggish reactivity of the inductively deactivated thioether function of Cys(Acm) can result in competitive loss of Scm-Cl by reaction with solvent and is therefore a problematic feature. With larger Cys-containing peptides choices of solvents are frequently dictated by solubility and are often not compatible with the strict solvation requirements of the reaction with Scm-Cl. Often very large molar excesses of Scm-Cl must be used to achieve complete Acm to Scm conversion. Furthermore, in previous work with the prior thiol capture method, methionine-containing peptides can react with Scm-Cl to form intermediates that are converted to the desired cysteine-Scm peptide only upon concentration of the reaction medium. These intermediates are probably formed by reaction of Scm-Cl initially with the methionine thioether, which is a stronger nucleophile than the Acm thioether. A similar problematic feature should be expected with peptides containing a tryptophan in which the strongly nucleophilic indole is not protected. In these cases it would be desirable to have the option of running the reaction of Scm-Cl with the free thiol of the same peptide, but this option is not feasible with Acm since its deprotection to a free thiol requires the use of divalent mercury salts which would interfere with the blocking scheme of the internal cysteines.

An alternative solution to this problem is the development of a more versatile temporary cysteine protection which presents the sulfhydryl in a more nucleophilic form. A special feature of thiol capture simplifies the search for an alternative. All the peptide fragments for which this temporary protection applies bear an N-terminal cysteine. There is no solid-phase chain elongation past this cysteine, and, therefore, it is possible to employ a joint amine–sulfhydryl masking group, removable by mild acidolysis to yield the aminoethanethiol moiety of cysteine directly.

An ideal candidate for this purpose is 2,2-dimethylthiazolidine-4-carboxylic acid (H-Dmt-OH) (8), which was first identified as an undesired product and a source of error in Linderstrom–Lang (acetone–HCl) amino acid titrations of cysteine-containing peptides. Subsequently, Dmt was shown to be easily interconvertable with cysteine and acetone in aqueous solutions,[7] and more recent studies showed 2,2-bis(alkyl-substituted) thiazolidines to undergo reliable hydrolyses to aminosulfhydryl compounds under either basic or acidic conditions.[10] Dmt has been used as a protein modification reagent for introducing heavy atom binding sites used in X-ray crystal structure determinations,[9] and protected at the α-amine with a formyl group, Dmt has been successfully employed as a sulfhydryl masking group for cysteine in the synthesis of the tripeptide glutathione.[8]

Therefore, we proposed to investigate Dmt for the prior thiol capture method and in doing so to establish the following four points. The N-*tert*-butyloxycarbonyl derivative (Boc-Dmt-OH) had to be synthesized in suitable purity (>99.8%) for application to solid-phase synthesis. This degree of purity is mandatory since the effect of errors over the numerous steps in the synthesis of a peptide chain on the solid support is cumulative, and impure starting materials can make yields unbearably low and purification of the final product excessively difficult. It was necessary to establish that the potentially sterically hindered Boc-Dmt-OH could be coupled rapidly and quantitatively under standard conditions of solid- and solution-phase peptide synthesis. Since cysteine derivatives are very prone to racemization,[11] the extent of this problem had to be identified and quantitated for Dmt. Finally, it was necessary to show that a peptide containing an amino terminal Dmt residue could be converted to the corresponding amino-terminal Cys(Scm)-peptide under conditions compatible with thiol capture strategy.

Preparation of Boc-Dmt-OH (9). Boc-Dmt-OH (9) was previously prepared by Woodward et al.[12] as the starting material for the synthesis of Cephalosporin C by reaction of H-Dmt-OH (8) with highly unstable in situ generated *tert*-butyloxycarbonyl chloride. Designing a more facile preparation using the commercially available di-*tert*-butyl dicarbonate presents two difficulties. First, the nitrogen of the starting H-Dmt-OH is very hindered, being bound to a tertiary carbon and a secondary carbon with a bulky electron-withdrawing carboxylate. Second, thiazolidines substituted at the 2-position, such H-Dmt-OH (8), are very susceptible to alkaline hydrolysis,[8] and methods of Boc introduction which used strong base or even excesses of nonnuclephilic tertiary amine bases led in our hands to low yields (<10%) of intractable mixtures containing both Boc-cysteine and the desired product, Boc-Dmt-OH (9). Although elsewhere it has been noted that *tert*-butyloxycarbonylation is catalyzed by (dimethylamino)pyridine[13] or excess tertiary base, in this particular case the addition of these reagents always led to the aforementioned product mixtures and to a dramatic reduction in yield. A procedure that consistently works well combines the hydrochloride salt of H-Dmt-OH (8) with di-*tert*-butyl dicarbonate in acetonitrile containing 1.05 equiv of triethylamine at 22 °C for 2 days. Yields on a multigram scale were reproduced regularly above 75%.

Aminolysis and Racemization Rate Studies. Active esters of *N*-α-urethane-protected, *S*-alkyl-cysteine derivatives have been shown to racemize at unusually high rates

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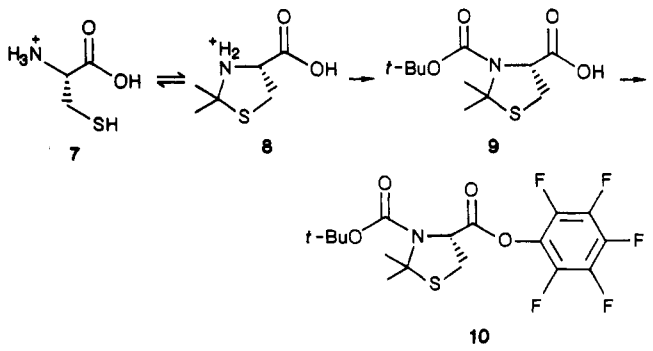

in the presence of tertiary bases. Extensive studies by Kovacs[14] confirmed this problem, found the racemization to proceed through a solvent-caged enolization mechanism,[15] and found that the coupling efficiency, a factor that takes into account both rates of coupling and racemization, can be optimized by using pentafluorophenyl esters. Further studies by Jones[16] identified thiazolidine-4-carboxylic acid as a structural anomaly that exhibits a marked resistance to racemization of at least 10^3 compared to the analogous active ester of an *S*-benzylcysteine. The pentafluorophenyl ester, Boc-Dmt-OPfp (**10**), was chosen for this study because not only have Pfp esters been shown to be effective acylating agents in solid-phase peptide synthesis,[17] but also because the Pfp ester provides a convenient handle for comparison to the previous literature reports.

Boc-Dmt-OPfp (**10**) was prepared in high yield and purity from 1-cysteine hydrochloride (**7**) in three steps. This active ester has been shown to couple (k_{couple} = 3.2 × 10^{-2} M^{-1} s^{-1}, *t* for 90% reaction is 36 min, 0.13 M) in THF at 22 °C with valine methyl ester in 86% isolated yield. Boc-Dmt-OPfp (**10**) was found to be stable to racemization in THF in the presence of 7× excess triethylamine (k_{rac} < 10^{-8} M^{-1} s^{-1}, no change in optical rotation in 144 h). For comparison Cbz-Cys-(*S*-benzyl)-OPfp[15] has been found to have k_{couple} = 4.0 × 10^{-2} M^{-1} s^{-1} (*t* for 90% reaction is 2.9 min, 0.13 M, 10× faster than Dmt) and k_{rac} = 33 × 10^{-4} M^{-1} s^{-1} (at least 10^4 more susceptible to racemization than Dmt). This demonstrated high coupling efficiency for Boc-Dmt-OPfp (**10**) confirms its usefullness as a reagent for introducing N-terminal cysteine in solid-phase peptide synthesis.

A solution-phase synthesis of the tripeptide Boc-Dmt-Leu-Ala-OBzl was accomplished by a DCC/HOBt coupling of Boc-Dmt-Leu-OH and H-Ala-OBzl in dichloromethane in 90% isolated yield. The dipeptide Boc-Dmt-Leu-OH was prepared from the precursor dipeptide benzyl ester by catalytic (Pd black) hydrogenolysis in methanol. Since hydrogenolysis of benzyl esters in sulfur-containing peptides is often problematic due to poisoning of the palladium catalyst by sulfur, the cleavage of the benzyl ester was monitored carefully by TLC and ^{1}H NMR analyses. After 2 h the cleavage was greater than 99% complete. Complete removal of the benzyl ester of the tripeptide was effected under the same conditions.

Conversion of Dmt-Peptides with Cys(Scm)-Peptides. As shown in Scheme III, peptides containing the dimethylthiazolidine moiety at the amino terminus provide the option of being converted to Cys-(Scm)-peptides by any of three different pathways. The first pathway is a direct conversion of a Boc-Dmt-peptide to a Boc-Cys(Scm)-peptide. The second involves Boc group cleavage and subsequent conversion of the resulting H-Dmt-peptide to an H-Cys(Scm)-peptide. The third route proceeds through cleavage of the Boc group and hydrolysis of the thiazolidine to yield an H-Cys-peptide, which is then converted to the H-Cys(Scm)-peptide. Boc-Dmt-Gly-Gly-Ala-OtBu (**11**), the model peptide that was used to study all of the above transformations, was prepared by a DCC/HOBt coupling of Boc-Dmt-OH and H-Gly-Gly-Ala-OtBu in dichloromethane in 84% isolated yield.

Although a careful examination of the equilibrium between acetone and cysteine had been reported,[6] it was still necessary to establish a reproducible protocol in varying peptide solvent mixtures for interconverting cysteine and Dmt in large peptides and for monitoring this interconversion. The *tert*-butyl protecting groups of **11** were removed in TFA, and the hydrolysis of the thiazolidine **12** was affected by dilution to 10^{-4} M in 1:1 (v/v) aqueous alcoholic (ethanol, methanol, or hexafluoroisopropyl alcohol) or acetonitrile solutions. The equilibrium between **12** and **13** could be forced in the direction of the cysteine by removal of the acetone in vacuo. Distinctive ^{1}H NMR markers made it an easy task to monitor the amount of cysteine or Dmt that was present by following the appearance and disappearance of the methine and methylene proton resonances of the Dmt (5.02, t; 3.86, dd; and 3.65, dd) and cysteine (4.36, t; 3.20, d), respectively, and by following the disappearance of the isopropylidine resonances (1.91, s). The disappearance of the Dmt methine resonance at 5.02 ppm is an especially good marker since its unusual downfield shift will almost always be clearly visible even in the complicated spectra of very large peptides. Thus Dmt-peptides can indeed be converted to H-Cys-peptides, and this conversion can be conveniently monitored and the products fully characterized.

Since the reaction of Cys(SH)-peptides with Scm-Cl to form Cys(Scm) peptides has a strong literature precedent,[3,4] the conversion of **13** to **15** was the first transformation to be examined. It was observed, however, that small amounts (5–10%) of the undesired symmetrical disulfide were produced when the reaction was carried out in chloroform/alcohol (methanol or HFIP) solvent mixtures. Addition of catalytic amounts of triethylamine to the reaction mixture caused the yield of symmetrical disulfide to become as great as 30–40%. This side reaction is probably the result of trace amounts of thiolate ion in the reaction mixture that react rapidly with the Cys-(Scm)-peptide as it forms. Addition of acetic acid as a cosolvent in the reaction mixture suppressed formation of thiolate ions and avoided this side reaction. The solvent mixture of acetic acid/DMF/water (80:10:5) consistently led to clean conversions of **13** to **15** with reproducibly high yields (>90%), and this solvent mixture was subsequently used for all Scm-Cl reactions. The presence of HFIP, methanol, or chloroform in the solvent mixture did not lead to any symmetrical disulfide formation as long as acetic acid was also present.

The conversion of a Dmt-peptide to a Cys-peptide directly by reaction of Scm-Cl with intact thiazolidine, as in **11** to **14** and **12** to **15**, proceeded in high yield (>90%) in the acetic acid/DMF/water solvent mixtures. A sample of **14** prepared by this direct route from Dmt was shown to have the same physical properties and to be chromatographically indistinguishable from a sample of **14** pre-

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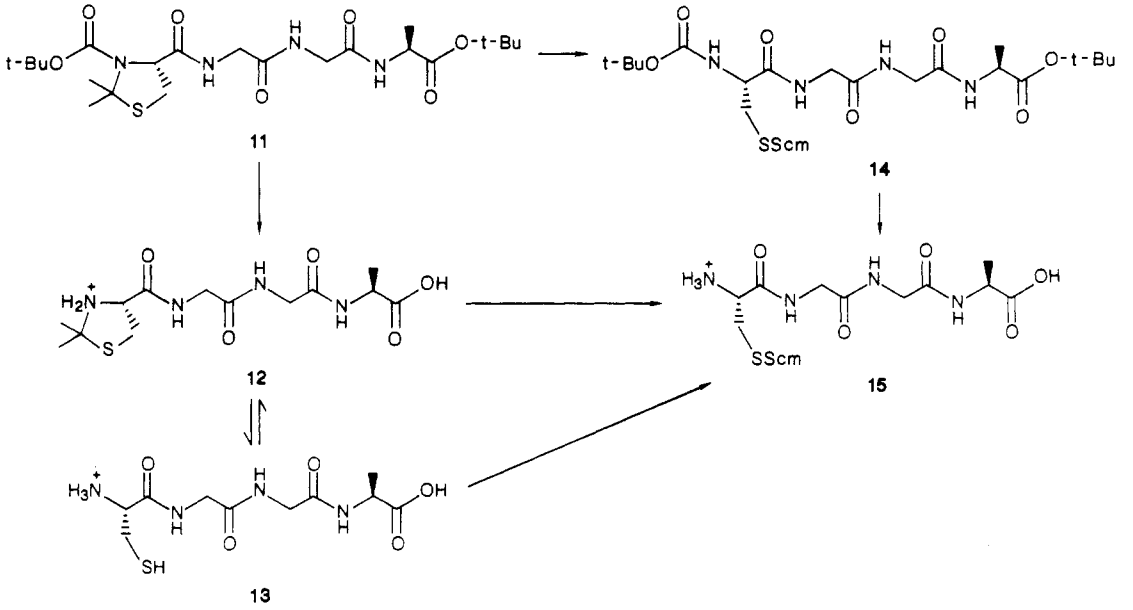
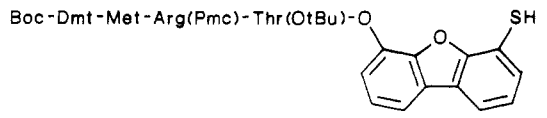
Scheme III



pared independently from Boc-Cys(Acm)-Gly-Gly-Ala-OtBu. Samples of **15** prepared from **12**, **13** or a precursor H-Cys-(Acm)-peptide were all similarly indistinguishable from one another.

In a realistic test of the feasibility of Dmt as an amino terminal cysteine blocking group for the prior thiol capture strategy, an entire synthetic cycle was completed in the synthesis of the Scm-activated fragment 58–51 of BPTI **17**. The template-charged tetrapeptide **16** was synthesized by standard protocols on a solid-phase resin functionalized with a 4,6-dibenzofuran template.[2] The coupling of Boc-Dmt-OH (**9**) as either the symmetrical anhydride (3 equiv) or as Boc-Dmt-OPfp (**10**) (6 equiv) in dichloromethane was greater than 99% by HPLC analysis. After cleavage from the resin by phosphine reduction of the disulfide, crude **16** was purified by preparative HPLC (70% isolated yield) and treated immediately with **15** to begin a thiol capture cycle. Following the thiol capture, acyl transferase, and cleavage from the template, the crude octapeptide was treated with dinitrofluorobenzene to protect the internal cysteine. At this stage of the cycle, it was possible to effect the acidolytic deprotection of both the arginine and threonine side chains under the mild conditions for removing *tert*-butyl groups (TFA, 1 h) since the choice for arginine protection was the very acid labile pentamethylchromansulfonyl (Pmc).[18] The crude peptide mixture from the TFA deprotection was isolated and without further purification was treated with Scm-Cl (2 equiv) under the same conditions used to convert **12** to **15**. The Scm-activated octapeptide **17** was purified by preparative HPLC in 48% isolated yield and found to be identical in all physical and chromatographic characteristics with a fully characterized, authentic sample of **17**, which had been prepared previously by using Boc-Cys-(Acm) precursor peptides.



16

$H_3\overset{+}{N}$-Cys(Scm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH

17

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In summary, the preparation and properties of Boc-Dmt-OH (**9**) have been described, and the conversion of peptides functionalized at the N-terminus with Boc-Dmt to the corresponding H-Cys(Scm)-peptides has been demonstrated with a unique application to the thiol capture method of peptide synthesis.

Experimental Section

IR spectra and kinetic studies were recorded on a Mattson Instruments Cygnus 100 FT spectrometer. High-resolution ^1H NMR spectra were obtained on either a Bruker WM-250 (FT) or a Varian XL-300 instrument. Chemical shifts are reported in ppm downfield from tetramethylsilane and splitting patterns are designated as s, singlet; d, doublet, t, triplet; q, quartet; p, pentaplet; dd, doublet of doublets; m, multiplet; b, broad. Low-resolution, high-resolution, and fast atom bombardment (FAB) mass spectra were recorded on a Varian MAT-44, CEC-110, or a Finnigan MAT-731 mass spectrometer. Microanalyses were performed by Multichem Laboratories, Lowell, MA. Melting points were determined on a Thomas-Hoover melting point apparatus. Optical rotations were recorded on a Rudolph Research Autopol III automatic polarimeter.

Analytical high-pressure liquid chromatography (HPLC) was performed on a Waters system consisting of two 6000A pumps, a Waters 490 programmable multiwavelength detector, a Model 730 data module, a Model U6-K injector, a Model 680 automated gradient controller, and a Vydac 218TP54 reverse-phase C-18 column. Preparative HPLC was performed on a system including a Waters Model 590 pump fitted with preparative heads, a Rheodyne injector, an Autochrome OPG/S prepump solvent mixer, a Waters Model 450 variable UV–vis detector, and a Vydac 218TP1022 reverse-phase, C-18 column. Amino acid analysis was obtained by using the Waters Picotag system in connection with the analytical HPLC system described above. Samples for amino acid analysis were prepared in the gas phase by propionic acid/HCl at 150 °C for 24 h. Analytical thin-layer chromatography was performed on aluminum precoated silica gel 60 plates (Merck F-254).

Unless otherwise specified all amino acids are of the L configuration. Scm-Cl was prepared from $COCl_2S$ (Aldrich) by a literature procedure,[4] purified by fractional distillation at reduced pressure, and stored at −20 °C in sealed ampules (stable for 1–2 months). The following abbreviations are used for dichloromethane (DCM), tetrahydrofuran (THF), acetonitrile (MeCN), dicyclohexylcarbodiimide (DCC), dicyclohexylurea (DCU), diisopropylethylamine (DIEA), and dimethylformamide (DMF).

L-(−)-2,2-Dimethylthiazolidine-4-carboxylic Acid Hydrochloride Salt [(H-Dmt-OH) HCl, 8]. L-Cysteine hydrochloride monohydrate (20 g, 0.113 mol) was refluxed in dry acetone (800

mL) under dry nitrogen for 1.5 h. Large white crystalline plates of the thiazolidine (22.4 g, 100%, mp 165–168 °C) were collected by filtration and shown to be 95% pure by ^{1}H NMR analysis. Refluxing a second time in dry acetone yielded 99+% pure thiazolidine (21 g, 94%): mp 169–170 °C (lit.[13], mp 165–168 °C); ^{1}H NMR (300 MHz, D_2O), δ 4.94 (1 H, t, J = 9 Hz, methine), 3.65 and 3.79 (2 H, dd, J = 9 Hz and J = 18 Hz, methylene), 2.02 (3 H, s) and 1.95 (3 H, s) (2,2-dimethyl).

L-(−)-(*tert*-Butyloxycarbonyl)-2,2-dimethylthiazolidinecarboxylic Acid (Boc-L-Dmt-OH) (9). To a suspension of (H-Dmt-OH) HCl (13.6 g, 0.069 mol) and di-*tert*-butyl dicarbonate (20 g, 0.092 mol) in dry MeCN was added DIEA (1.1 equiv, 13.2 mL, 0.076 mol). The suspension was allowed to stir for 2 days. The MeCN was removed in vacuo, and the remaining oil was taken up in ether and concentrated in vacuo to an oily solid. The oily solid was again taken up in ether, and the amine salt was removed from the ether solution by filtration through Celite. The ethereal filtrate was washed with 0.1 N HCl (2×), water (2×), and brine (1×), dried, and concentrated to a clear oil that was dissolved in hexanes and concentrated in vacuo to a white solid. Crystallization from hexanes yielded white needles (12.8 g, 75%): mp 113–114 °C (lit.[12] mp 114–114.5 °C); $[\alpha]^{20}_{589}$ (c = 1.34, chloroform) (lit. −85°); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.09 (m) and 4.90 (m) (1 H, C_α-H), 3.30 (m, 2 H, C_β-H_2), 1.95 (s) and 1.90 (s) (6 H, isopropylidine methyls), 1.50 (s) and 1.60 (s) (9 H, *tert*-butyl).

Boc-Dmt-Leu-OBzl. To a solution of Boc-Dmt-OH (1.27 g, 0.0051 mol), H-Leu-OBzl (1.13 g, 0.0051 mol), 1-hydroxybenzotriazole hydrate (0.78 g, 0.0051 mol) in DCM (10 mL), and DMF (1 mL) at 0 °C under nitrogen was added DCC (1.05 g, 0.0051 mol), and the reaction was stirred at 20 °C for 4 h. The DCU was removed by filtration through Celite, and the solution was concentrated in vacuo to an oil that was taken up in ethyl acetate. At 0 °C more DCU precipitated and was removed by filtration. The filtrate was then washed with 0.1 N HCl (2×), water (2×), and brine (2×), dried, and concentrated to an oil that was dissolved in ether and concentrated in vacuo to a white solid (2×). Crystallization from ether/hexanes yielded long white needles (1.5 g, 63%): mp 79–80 °C; TLC (CH_2Cl_2) R_f 0.05; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5 H), 5.17 (m, 2 H), 4.78 (m) and 4.68 (m) (1 H, Dmt methine), 4.65 (m, 1 H, leucine methine), 3.25 (m, 2 H, Dmt methylene), 1.87 (s) and 1.80 (s) (6 H, 2,2-dimethyl), 1.65 (m, 2 H, Leu), 1.55 (m, 1 H, Leu), 1.42 (s, 9 H, Boc), 0.92 (dd, 6 H, Leu); FABMS (glycerol) 465 (M^+), 409 ($M^+ - C_4H_9$).

Boc-Dmt-Leu-Ala-OBzl. To a solution of Boc-Dmt-Leu-OBzl (390 mg, 0.84 mmol) in methanol was added palladium black (25 mg), and the suspension shaken under hydrogen (40 psi) for 2 h. The reaction was filtered through Celite, and the methanol was removed in vacuo to yield a clear oil that crystallized from ether/hexanes to yield long white needles (300 mg, 95%).

A solution of the Boc-Dmt-Leu-OH (140 mg, 0.374 mmol), H-Ala-OBzl (67 mg, 0.374 mmol), and 1-hydroxybenzotriazole hydrate (57.3 mg, 1 equiv) in DCM was cooled to 0 °C in an ice bath. To this was added DCC (77.2 mg, 1 equiv), and the reaction was stirred for 1 h at 0 °C and for 4 h at 20 °C. The reaction was filtered and reduced in vacuo to a clear oil that was taken up in ethyl acetate and washed with 0.1 N HCl (2×), 5% $NaHCO_3$ (2×), water (2×), and brine. Drying over magnesium sulfate and filtration yielded a clear solution that was concentrated in vacuo to a white solid that crystallized from ethyl acetate/hexanes in long white needles (180 mg, 90%): mp 137–138 °C; HPLC t_R 10.7 min, 98% (60% CH_3CN/40% 0.1% TFA); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (m, 2 H), 6.70 (m, 2 H), 4.77 (m, 1 H, Dmt methine), 4.58 (t, 1 H, J = 8 Hz, Leu methine), 4.45 (q, 1 H, J = 8 Hz, Ala methine), 3.51 and 3.25 (dd, 2 H, J = 8 Hz and J = 16 Hz, Dmt methylene), 1.84 and 1.76 (s, 6 H, Dmt-2,2-dimethyl), 1.65 (m, 2 H, Leu), 1.55 (m, 1 H, Leu), 1.45 (s, 9 H, Boc), 1.40, (d, 3 H, Ala), 0.90 (m, 6 H, Leu); FABMS (3-nitrobenzyl alcohol) 536 (M^+), 480 (M^+ − tBu), 4.36 (M^+ − tBu, $-CO_2$), 301 (M^+ − tBu, $-CO_2$, −benzyl, −isopropylidine). Anal. Calcd for $C_{27}H_{41}N_3O_6S$: C, 60.54; H, 7.71; N, 7.84. Found: C, 60.61; H, 7.62; N, 7.83.

Boc-Dmt-Gly-Gly-Ala-OtBu (11). To a solution of Boc-Dmt-OH (200 mg, 0.762 mol) and H-Gly-Gly-Ala-OtBu (0.762 mmol) in DCM at 0 °C under nitrogen was added DCC (157 mg, 0.762 mmol). The reaction was stirred at 20 °C for 4 h. Filtration through Celite and removal of the DCM in vacuo left a clear oil that was taken up in ethyl acetate. At −20 °C more DCU precipitated and was separated by filtration. The filtrate was washed with 0.1 N HCl (2×), 5% $NaHCO_3$ (2×), water (2×), and brine, dried over magnesium sulfate, and concentrated in vacuo to a clear oil that crystallized as long white needles from EtOAc/hexanes: yield (320 mg, 84%); mp 151–152 °C; HPLC t_R 12.6 min 100% (40% CH_3CN/60% 0.1% TFA); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (t, 2 H), 6.61 (d, 1 H), 4.76 (t, 1 H, J = 6 Hz), 4.44 (m, 1 H, J = 8 Hz), 4.05 (dd, 2 H), 3.95 (d, 2 H, J = 6 Hz), 3.38 (d, 2 H, J = 6 Hz), 1.92 and 1.80 (s, 6 H), 1.47 (s, 18 H), 1.40 (d, 3 H, J = 8 Hz); FAB mass spectrum (glycerol) 503 (M^+), 446 ($M^+ - C_4H_9$), 403 ($M^+ - C_4H_9$ and CO_2), 347 ($M^+ - 2C_4H_9$ and CO_2); high-resolution FAB mass spectrum calcd for $C_{22}H_{38}N_4O_7S$ 502.2461, found 502.2476.

H-Cys-Gly-Gly-Ala-OH (13). Sequential Removal of Boc and Conversion of Dmt to Cys with a Boc-Dmt-Peptide. To a solution of trifluoroacetic acid (1.5 mL) in DCM (0.5 mL) was added Boc-Dmt-Gly-Gly-Ala-OtBu (13 mg, 0.026 mmol). This solution was stirred under nitrogen for 2 h and then concentrated in vacuo to a white powder, which was triturated with 1:1 ether/petroleum ether and identified by ^{1}H NMR spectroscopy as the trifluoroacetate salt of H-Dmt-Gly-Gly-Ala-OH (12): ^{1}H NMR (300 MHz, D_2O) δ 5.02 (t, 1 H, J = 8.6 Hz, Dmt methine), 4.53 (q, 1 H, J = 8.0 Hz, Ala methine), 4.20 (s, 2 H, Gly), 4.10 (s, 2 H, Gly), 3.86 and 3.65 (dd, 2 H, J = 9 Hz and J = 18 Hz, Dmt methylene), 1.91 (s, 6 H, Dmt-2,2-dimethyl), 1.53 (d, 3 H, J = 8.0 Hz, Ala).

The above-prepared white powder was dissolved in distilled water–ethanol (1:1, 50 mL, 0.5 mM) and concentrated in vacuo again to a white powder. This dilution process was repeated (3×) to push the equilibrium to a quantitative conversion to the free cysteine thiol and acetone. Concentration in vacuo yielded a white powder (11 mg, 100%): HPLC: t_R 6.57 min (100%) 100%, 0.1% TFA; ^{1}H NMR (300 MHz, D_2O) δ 4.50 (q, 1 H, J = 8.0 Hz, Ala methine), 4.36 (t, 1 H, J = 5 Hz, Cys methine), 4.15 (s, 2 H, Gly), 4.06 (s, 2 H, Gly), 3.20 (d, 2 H, J = 5.0 Hz, Cys methylene), 1.53 (d, 3 H, J = 8.0 Hz, Ala).

H-Cys(Scm)-Gly-Gly-Ala-OH Trifluoroacetate Salt (15). Conversion of a H-Dmt-Peptide to a H-Cys(Scm)-Peptide. The trifluoroacetate salt of H-Dmt-Gly-Gly-Ala-OH was prepared from Boc-Dmt-Gly-Gly-Ala-OtBu (13 mg, 0.026 mmol) as described above and dissolved in a 0 °C premixed sovent consisting of glacial acetic acid (8 parts), DMF (1 part), and water (0.5 parts). To this solution was added Scm-Cl (2.8 μL, 1.2 equiv), and the reaction was stirred at 0 °C under nitrogen for 30 min. The solvent was removed in vacuo to leave a clear oil that was dissolved in dioxane and reduced immediately in vacuo to leave a white solid. Trituration with ether/petroleum ether (2×) yielded a white powder (12 mg, 90%), mp 85–90 °C dec, identical by HPLC and ^{1}H NMR with an authentic sample: ^{1}H NMR (300 MHz, D_2O) δ 4.43 (m, 1 H), 4.41 (m, 1 H), 4.17 (d, 2 H, J = 5.0 Hz, Gly), 4.09 (s, 2 H, Gly), 4.05 (s, 3 H, OMe), 3.56 (dd, 1 H, J = 7 Hz and J = 20 Hz) and 3.39 (dd, 2 H, J = 10 Hz and J = 20 Hz) (Cys methylene), 1.53 (d, 3 H, J = 7.8 Hz, Ala).

Boc-Cys(Scm)-Gly-Gly-Ala-OtBu (14). Conversion of a Boc-Dmt-Peptide to a Boc-Cys(Scm)-Peptide. To a solution of Boc-Dmt-Gly-Gly-AlaOtBu (13.6 mg, 0.026 mmol) in glacial acetic acid/DMF/Water (8:1:0.5) at 0 °C under nitrogen was added with stirring Scm-Cl (2.8 μL, 1.2 equiv). After 1 h the solvent was removed in vacuo to a clear oil that was suspended in hexanes and immediately concentrated in vacuo (3×) and triturated with hexanes (3×). Lyophilization from MeCN/water (1:2) yielded a white powder (14 mg, 94%): mp 142–143 °C (lit.[2] mp 143–143 °C); HPLC t_R 8 min (45% CH_3CN/55% 0.1% TFA), identical with an authentic sample;[2] ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.64 (b s, 1 H), 7.19 (b s, 1 H), 6.66 (b d, 1 H, J = 7.8 Hz), 5.86 (b d, 1 H, J = 8.2 Hz), 4.45 (p, 1 H, J = 7 Hz, Ala methine), 4.27 (q, 1 H, J = 8 Hz, Cys methine), 4.05 (d, 2 H, J = 6 Hz), 4.02 (dd, 1 H, J = 6 Hz and J 15 Hz, Gly), 3.94 (s, 3 H, OMe), 3.90 (dd, 1 H, J = 6 Hz and J = 15 Hz), 3.26 (dd, 1 H, J = 5.8 Hz and 14 Hz, Cys methylene), 3.12 (dd, 1 H, J = 7.3 Hz and J = 14 Hz, Cys methylene), 1.46 (s, 9 H), 1.44 (s, 9 H), 1.40 (d, 3 H, J = 7 Hz); FAB mass spectrum (glycerol) 553 (M^+). Anal. Calcd for $C_{21}H_{38}N_4O_{10}S_2$: C, 44.75; H, 6.90; N, 9.94. Found: C, 44.84; H, 6.57; N, 9.95.

L-(−)-*N*-(*tert*-Butyloxycarbonyl)-2,2-dimethylthiazolidinecarboxylic Acid Pentafluorophenyl Ester

(Boc-Dmt-OPfp) (10). To a solution of Boc-Dmt-OH (1.3 g, 5 mmol) and pentafluorophenol (1.0 g, 5.5 mmol) in ethyl acetate at 0 °C under nitrogen was added DCC (1.13 g, 5.5 mmol). After 1.5 h the reaction was filtered through paper, the collected DCU was washed with cold ethyl acetate, and the combined filtrate was concentrated in vacuo to a white solid, which was triturated with 4 °C hexanes. Recrystallization from boiling hexanes yielded long white needles (1.6 g, 75%): mp 104–105 °C; TLC R_f 0.51 (DCM), R_f 0.30 (3:2, DCM–hexanes); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.28 (dd, J = 3.8 Hz and J = 8.6 Hz) and 5.1. (dd, J = 1.9 Hz and J = 8.6 Hz) (1 H, methine), 3.48 (dd, J = 7.2 Hz and 15 Hz) and 3.28 (dd, J = 2.4 Hz and 15 Hz) (2 H, methylene), 1.92, 1.85, and 1.81 (s, 6 H, 2,2-dimethyl), 1.53 and 1.48 (s, 9 H, Boc). Anal. Calcd for $C_{17}H_{18}NO_4SF_5$: C, 47.78; H, 4.24; N, 3.27. Found: C, 47.81; H, 4.34; N, 3.27.

Boc-Dmt-Val-OMe. To a solution of H-Val-OMe (66 mg, 0.50 mmol) in THF (3 mL) was added Boc-Dmt-OPfp (214 mg, 0.50 mmol), and the reaction was stirred under nitrogen at 22 °C for 30 min. The THF was removed in vacuo to leave a clear oil that was taken up in EtOAc and washed with 0.1 N HCl, 5% $NaHCO_3$, water, and brine, dried over magnesium sulfate, and concentrated in vacuo to an oil that solidified in ether. Trituration with ether/petroleum ether yielded a white oily solid (160 mg, 86%): TLC R_f 0.64 (98% EtOAc/2% acetic acid); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.81 (b, 1 H, Dmt methine), 4.59 (dd, 1 H, Val methine), 3.74 (s, 3 H, OMe), 3.25 (b, 2 H, Dmt methylene), 2.23 (m, 1 H, J = 6 Hz and J = 10 Hz, Val), 1.90 and 1.83 (s, 6 H, 2,2-dimethyl), 1.48 (s, 9 H, Boc), 0.94 (dd, 6 H, J = 6 Hz and J = 10 Hz, Val).

Racemization and Aminolysis Rate Studies of L-(−)-(*tert*-Butyloxycarbonyl)-2,2-dimethylthiazolidinecarboxylic Acid Pentafluorophenyl Ester (Boc-Dmt-OPfp) (9). Both the racemization and the aminolysis rate studies were modeled after previous comprehensive literature studies of protected cysteine derivatives by Kovacs[15] and Jones.[16]

For the racemization rate study the preparation of all the solutions was carried out under dry nitrogen to prevent hydrolysis of the pentafluorophenyl ester. The concentration of Boc-Dmt-OPfp was 0.025 M in THF, and racemization was initiated by adding 7 equiv of TEA per mole of active ester used. The kinetics were followed by observing optical rotation at 589 nm. The initial reading was taken within 2 min of mixing. This experiment was repeated with 35 equiv of TEA. No change in optical rotation was observed in either case over a time period of 144 h, placing an upper limit on k_{rac} of $<10^{-8}$ M^{-1} s^{-1}. Under these same conditions the k_{rac} for Boc-Cys(Bzl)-OPfp was 3.3×10^{-3} M^{-1} s^{-1}.[15]

For the aminolysis rate study, the concentrations of Boc-Dmt-OPfp and valine methyl ester were each 0.13 M in THF. The concentration of active ester was followed by IR spectroscopy, measuring the disappearance of the band in the 5.6 μm region. The initial reading was taken 3 min after mixing the solutions, and the second-order rate constant (k_{couple}) for coupling was found to be 3.2×10^{-2} M^{-1} s^{-1}. Under the same conditions, k_{couple} for Boc-Cys-(Bzl)-OPfp was 40.4×10^{-2} M^{-1} s^{-1}.[15]

Preparation of 4-[*N*-(*tert*-Butyloxycarbonyl)-L-2,2-dimethylthiazolidinyl-L-methionyl-L-arginyl(N^G-2,2,6,7,8-pentachromansulfonyl)-L-threonyloxy(*O*-*tert*-butyl)]-6-mercaptodibenzofuran (16). The above tetrapeptide was prepared on a solid-phase resin (100 mg, 0.49 mmol/gram) functionalized with a polystyrene-linked benzyl ester of *S*-((4-Hydroxy-6-dibenzofuran)thio)-*N*-(benzyloxycarbonyl)-L-cysteine. The solid-phase synthesis protocols and reagents were the same as those previously reported in the synthesis of the analogous Cys(Acm) tetrapeptide. Boc-Dmt was coupled quantitatively by conversion either to the symmetrical anhydride (3 equiv in DCM) or to the pentafluorophenyl ester (6 equiv in DCM). Typically these couplings were complete in 30 min, which is normal for solid-phase synthesis couplings. The extent of coupling was monitored at each step by HPLC analysis of the phosphine cleavage product of a small sample (<1 mg) of the protected peptide from the resin. The peptide was cleaved from the resin in hexafluoroisopropyl alcohol/DCM (1:4) with tributylphosphine (1.2 equiv) and purified immediately by preparative HPLC (eluent 75%/25%, MeCN/0.1% TFA(aq)]. The appropriate fractions were collected and lyophilized to a white powder (40 mg, 70.1% based on the initial loading of the resin). FABMS [$C_{56}H_{79}N_7O_{12}S_4$ (3-nitrobenzyl alcohol matrix)]: 1171.5 (M^+, 100%).

Preparation of L-Cysteinyl(*S*-methoxycarbonylthio)-L-methionyl-L-arginyl-L-threonyl-L-cysteinyl(*S*-dinitrophenyl)-glycylglycyl-L-alanine [H-Cys-(Scm)-Met-Arg-Thr-Cys(Dnp)-Gly-Gly-Ala-OH] (17). Treatment of **16** (8.0 mg, 0.0068 mmol) with **15** (1 equiv) under the standard conditions[2] for thiol capture, acyl transfer, disulfide cleavage, and S-protection of the internal cysteine with dinitrofluorobenzene yielded a crude mixture containing the desired octapeptide Boc-Dmt-Met-Arg-(Pmc)-Thr(OtBu)-Cys(Dnp)-Gly-Gly-Ala-OH. Following acidolytic removal of the *tert*-butyl and Pmc protecting groups with trifluoroacetic acid containing 0.5% water, the crude octapeptide product mixture was triturated with ether (3×), treated with Scm-Cl under the same conditions used for the preparation of **15**, and purified immediately by preparative HPLC (eluent 20%/80%, MeCN/0.1% TFA for 6 min, 28% MeCN for 2 min, and 34% MeCN for 2 min; t_R = 9 min for **17**). Lyophylization left a yellow powder (4.1 mg, 48% based on **16**). As seen by analytical HPLC (t_R) and ^{1}H NMR there no difference between **17** prepared in this way and an authentic sample of **17** previously prepared and characterized:[2] FABMS [$C_{36}H_{58}N_{13}O_{16}S_4$ (3-nitrobenzylalcohol matrix)] 1055 (M^+, 100%). Amino Acid Analysis: $Gly_{(2.0)}$, 2.02; $Arg_{(1.0)}$, 1.03; $Thr_{(1.0)}$, 0.87; $Met_{(1.0)}$, 0.82; $Ala_{(1.0)}$, 1.00; $Cys_{(1.0)}$, 0.90. Cys(Dnp) is stable to hydrolysis conditions.

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Registry No. 7·HCl, 52-89-1; 8·HCl, 67089-92-3; **9**, 5317-15-7; **10**, 120711-54-8; **11**, 120711-55-9; **12**·TFA, 120711-57-1; **13**·TFA, 120711-59-3; **14**, 120411-21-4; **15**·TFA, 120386-61-0; **16**, 120711-60-6; **17**, 120386-69-8; Scm-Cl, 26555-40-8; H-Leu-OBzl, 1738-69-8; H-Gly-Gly-Ala-OBu-*t*, 120386-72-3; BOC-Dmt-Leu-OBzl, 120711-51-5; BOC-Dmt-Leu-OH, 120711-52-6; H-Ala-OBzl, 17831-01-5; BOC-Dmt-Leu-Ala-OBzl, 120711-53-7; BOC-Dmt-Val-OMe, 120711-61-7; H-Val-OMe, 4070-48-8; BOC-Dmt-Met-Arg(Pmc)-Thr(*t*-Bu)-Cys(Dnp)-Gly-Gly-Ala-OH, 120711-62-8.