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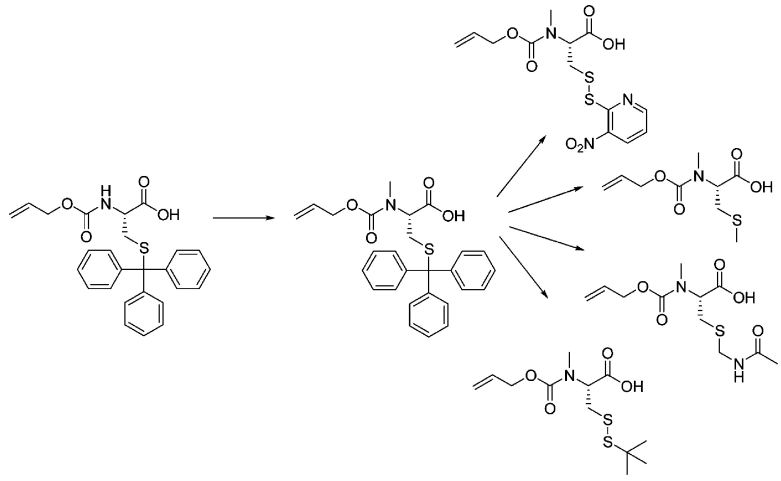

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Cysteine-*S*-trityl a Key Derivative to Prepare *N*-Methyl Cysteines

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S-Trt Cys are used as precursors for the synthesis of protected *N*Me−Cys. *N*-Methylation of Alloc−Cys(Trt)−OH and Boc−Cys(Trt)−OH gives the corresponding *N*-methylated derivatives in good yields and purities, which can be further derivatized in solution to obtain a myriad of *S*-protected derivatives. To further broaden the scope of this methodology, the N^{α}-amino protecting group of the *N*Me−*S*−protected Cys can be replaced easily either on the solid phase (from the Alloc precursor) or in solution (from the Boc precursor). Thus, this convenient route allows us to obtain many different protected *N*Me−Cys, which were of limited accessibility until now.

Introduction

N-Methylation is one of the most common post-translational modifications. *N*-methyl amino acids are found in linear or cyclic peptides, which present interesting biological activities. Cyclosporins, for example, which are produced by a fungus (Beauveria nivea), exhibit a myriad of antiinflammatory, antifungal, and immunosuppressive activities.[1–3]

The marine ecosystem offers many examples of *N*Me amino acid-containing peptides such as keramamide A, jaspamide, didemnins, halipeptin, and thiocoraline.[4–8] Peptides that contain *N*Me amino acids show better metabolic stability, higher hydrophobicity, and a more rigid conformational structure. This modification has been used in synthetic peptides to reduce flexibility and also to suppress intermolecular hydrogen bonds in order to avoid aggregation.[9,10] An interesting example is Cilengitide, a RGD-based cyclic peptide with *N*Me−Val, currently being evaluated in clinical trials for the treatment of recurrent glioma, a highly vascularized brain tumor.[11,12] *N*Me amino acids have also been taken into account in designing peptides that cross the hematoencephalic membrane.[13,14]

Due to their important role in pharmacology, many efforts have been directed at optimizing synthetic methods for the preparation of *N*Me amino acids and their use in peptide chemistry. A special and key case is the trifunctional amino acid Cys which is one of the most versatile natural amino acids, since it offers the possibility to form disulfide bonds and thioester linkages. In proteins, Cys is a key residue that maintains the protein in its folded and often active state.[15] Disulfide bridges provide the constraints that are necessary for the protein to fold, and in proteins with several cysteines, the folding pathway can be determined following the order in which disulfides form.[16] Misfolded analogues are usually inactive, but in some cases, they can lead to important human diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases.[17] In small peptides, which do not tend to fold naturally, constraints imposed by disulfide formation may improve its biological activity and its resistance to proteolysis.[18,19]

*N*Me−Cys is found in thiocoraline peptides,[20,21] playing a relevant ramification role. Thiocoraline is a cyclic octathiodepsipeptide where six of the residues are Cys derivatives. In thiocoraline, they form the disulfide bridge and the two thioester bonds which all together constitute the bicyclic core. Moreover, two Cys are also linked to key intercalator heterocycles.

To the best of our knowledge, no *N*Me−Cys are commercially available. Until now, the only synthetic method used harsh conditions (Na, NH_3)[22] and was not adequate for large-scale synthesis. In this sense, it is important to develop a versatile method that offers the possibility to obtain *N*Me−Cys with their amino and thiol functionalities conveniently protected.

Results and Discussion

***N*Me−Cys Using Conventional Methods.** Several efficient methods used to modify amino acids in solution have been described.[23–26] Methods to obtain *N*-methyl amino acids on solid supports from the corresponding α-*N*-nosyl protecting amino acid have also been recently described.[27–29] These general procedures have been tested in Cys derivatives. However, a robust method which would allow the synthesis in solution of a broad range of *N*Me−Cys derivatives that could respond to peptide synthetic requirements, has not yet been achieved.[30] Scheme 1 summarizes the results obtained in our laboratory when general methods described for *N*-methylating amino acids were applied to Cys.

The best results for Cys(Me) were obtained when using *N*-methylation with optimized conditions [MeI (2 equiv),

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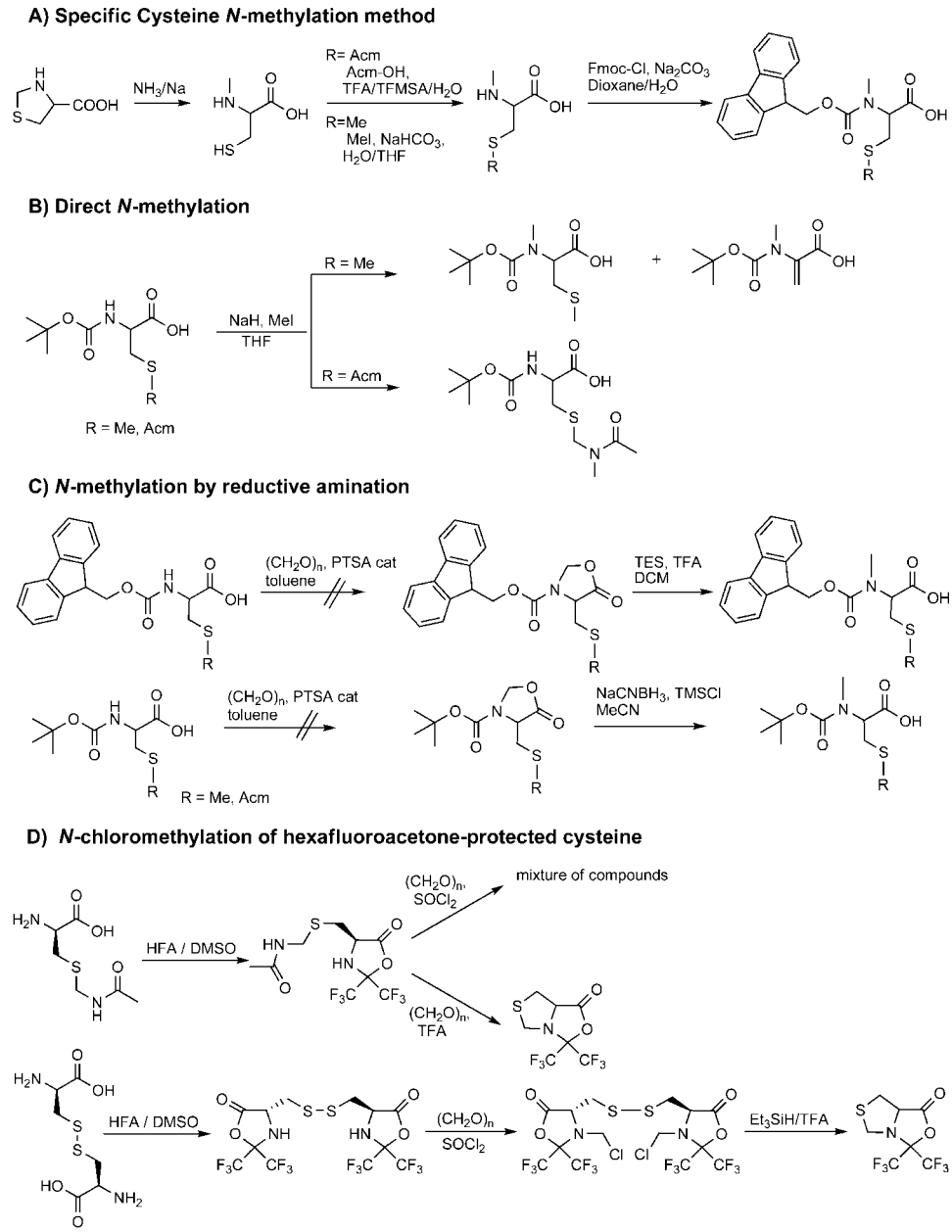
Scheme 1. *N*-Methylation Methods Applied to Cys[a]



[a] As the results corresponding to Scheme 1 were rather negative, the experimental procedures were not optimized and therefore have not been included in the experimental section.

NaH (3 equiv), 4 °C, overnight] (Scheme 1B). Although accompanied by β-elimination with the formation of the didehydroalanine derivative, this byproduct can be easily eliminated by removal of the Boc group and subsequent reprotection with Alloc, Fmoc, or *p*NZ. The application of the same method to the acetamidomethyl (Acm) protecting group derivative however, gave exclusively the Acm methylated compound (Scheme 1B).

The formation of oxazolidinones, which has been broadly explored as an *N*-Methylation method because of its efficiency and versatility with different protecting groups,[31–33] was also attempted on both Fmoc− and Boc−Cys derivatives (Me, Acm). But in any case, the formation of the cyclic compound was observed, and instead, a mixture of compounds was obtained (Scheme 1C). These results are consistent with those obtained in oxazolidinone formation of Z−Cys−OH (3% yield)[34] or Boc−Cys(Ac)−OH (51% yield, but where no further reduction was observed).[35] The oxazolidinone method appears to be also not straightforward with Ser and Thr derivatives. An additional nucleophilic group in the side-chain could compete with the correct cyclization between the amino group and the carboxylic moiety.

Hexafluoroacetone (HFA) protects both the amino function and the carboxylic group of α-amino acids to give highly acid-stable 2,2′-bistrifluoromethyl-1,3-oxazolidinones. *N*-Methylation of hexafluoroacetone-protected amino acids can be performed as a one-pot procedure by reaction with formaldehyde and thionyl chloride and subsequent reduction of the corresponding *N*-chloromethyl compounds with triethylsilane and trifluoroacetic acid.[36] By NMR, we observed *N*-chloromethyl intermediates of HFA−Cys(Acm) and also HFA−cystine, but upon addition of triisopropylsilane−trifluoroacetic acid (TIS−TFA), HFA−thioproline was formed and no methylated product was observed (Scheme 1D).

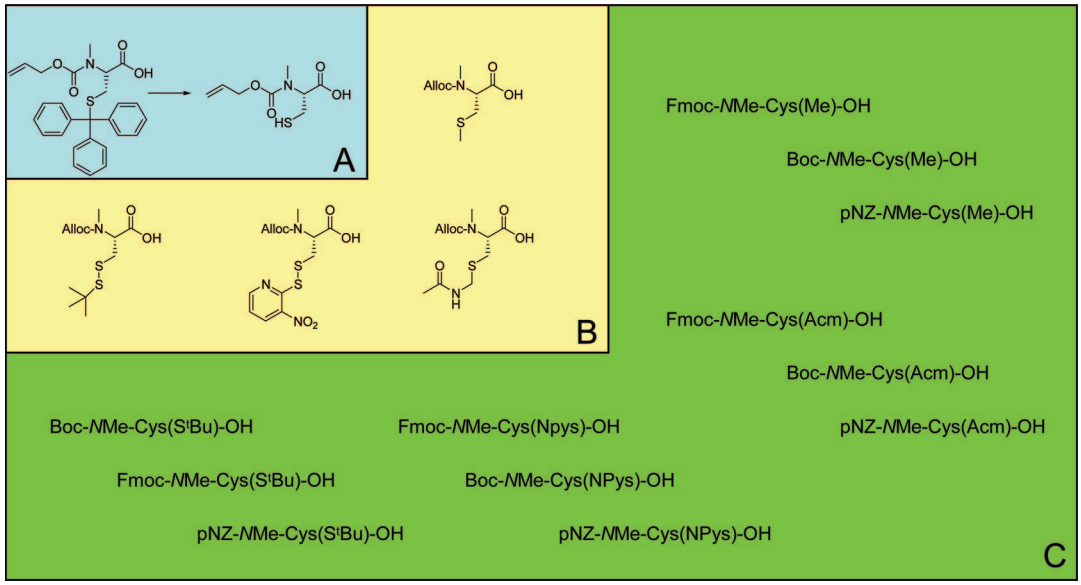
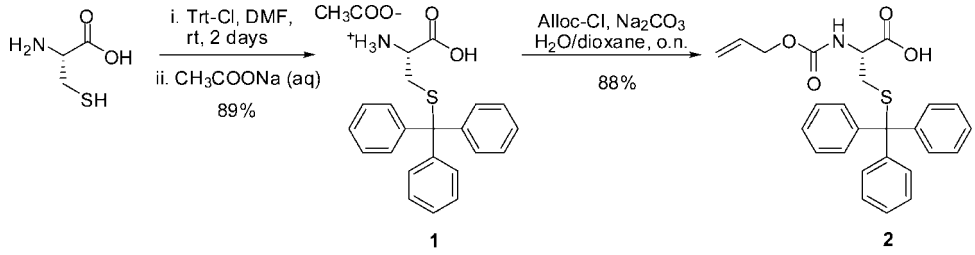


Figure 1. Alloc−*N*Me−Cys(Trt)−OH as a highly versatile starting material for *N*-Methylation.

Scheme 2. Obtention of the Starting Material Alloc−Cys(Trt)−OH (**2**) in Solution

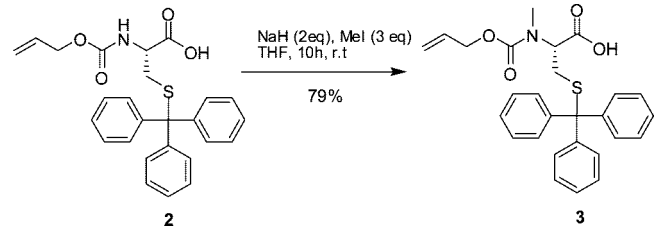


We propose an orthogonal strategy where side-chain protection needs to accomplish the following requirements: (a) being stable to *N*-Methylation conditions; (b) being easily removed on solution; (c) allowing interconversion to other side-chain protecting groups to obtain a robust method in order to get a broad range of *N*Me−Cys derivatives; and (d) being orthogonal to the α-amino protecting group. In this regard, of the most common amino protecting groups (Fmoc, Boc, Alloc), we choose the Alloc group mainly because it can be interconverted on the solid support and because it allows clean *N*-Methylation, as will be shown later. As for the key side-chain protecting group, the Trt group appears to be a good option because it is stable to basic media, orthogonal to the Alloc group, and easily removed using very mild acid conditions.

Alloc−Cys(Trt)−OH as a Starting Material to Diversify the Protection Scheme. As explained above, since the major byproduct was from a β-elimination reaction, Alloc−Cys(Trt)−OH, a more stable precursor to basic medium, was chosen as a starting material for *N*-Methylations. The Alloc group is suitable for several reasons: (i) it is compatible under basic conditions; (ii) it is orthogonal with the Trt group, and therefore, both groups can be converted independently to other suitable protecting groups (Figure 1A and B); and (iii) Alloc amino acids can be attached to 2-chlorotrityl chloride (CTC) resin to switch the α-amino protecting group (Figure 1C).

Alloc−Cys(Trt)−OH can be obtained on the solid phase by attaching commercially available Fmoc−Cys(Trt)−OH,[37]

Scheme 3. Key Step: *N*-Methylation of Compound Alloc−Cys(Trt)−OH (**2**)



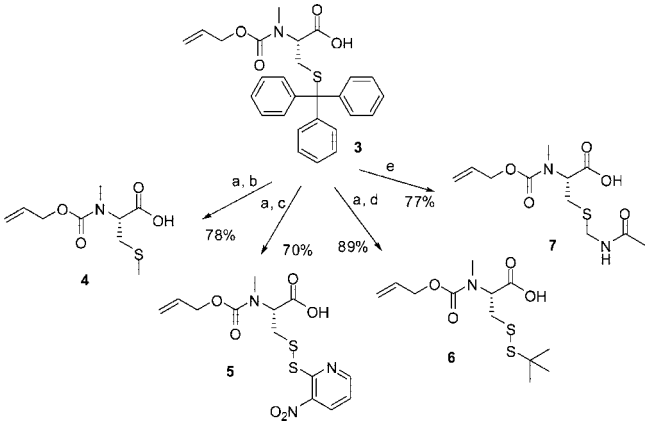
followed by removal and reprotection of the amino function with Alloc−Cl (73% yield,) or it can be obtained in solution in large scale from natural and commercially available H−Cys−OH hydrochloride in three reaction steps (78% overall yield without purification steps; Scheme 2).

Alloc−Cys(Trt)−OH (**2**) is subjected to standard procedures of *N*-Methylation of Boc amino acids which use more MeI than is used in Boc−Cys(Me)−OH. The Trt group, being much hindered, avoids the β-elimination reaction. The conversion is total, and no β-elimination side-product is observed (Scheme 3).

Interconversion of the Protecting Side-Chain in Solution. The Trt group may be easily removed under acidic conditions with the aim of synthesizing side-chain protected cysteines differently (Scheme 4).

In order to obtain the free thiol function, the amino acid (**3**) is treated with TFA in the presence of triisopropylsilane (TIS) in CH_2Cl_2 (4:1:35) for 1 h at room temperature to obtain Alloc−*N*Me−Cys−OH in quantitative yield. A strong

Scheme 4. *N*Me−Cys with Different Side-Chain Protection Obtained from Alloc−*N*Me−Cys(Trt)−OH (**3**)[a]



[a] Reaction conditions: (a) TFA−TIS−CH_2Cl_2 (4:1:35), 1 h; (b) MeI (1.4 equiv) in H_2O−THF (1:1), pH 7 (aq. 10% Na_2CO_3, 4 h); (c) Npys−Cl (1.2 equiv), CH_2Cl_2; (d) (i) Scm−Cl (2 equiv), CH_2Cl_2, pH 7 (DIEA), (ii) *t*Bu−SH (2 equiv), Et_3N (1 equiv), MeOH, 2 h, r.t.; (e) Acm–OH (3 equiv), TIS (4%), TFA−TFMSA (95:5), 5 h, r.t.

scavenger such as TIS is needed, because in the presence of milder scavengers such as H_2O and $PhSiH_3$, a low conversion of 10 and 40%, respectively, was obtained due to realkylation of the Trt group.

Different side-chain protections such as Me, 3-nitro-2-pyridyl (Npys), and S*t*Bu were introduced without the need for previous purification of the free thiol derivative because triphenylmethane and excess TIS remaining from deprotection reaction do not interfere in the subsequent protection reactions.

Cys derivative **4** was obtained in 78% yield under mild conditions with a slight excess of MeI in aqueous medium, maintaining neutral pH, and without purification steps.

Npys protection is useful for the formation of an unsymmetrical disulfide bond and suitable in Boc/Benzyl synthesis because it is stable under standard synthetic conditions, being also used to strengthen acids and tertiary amines. The amino acid **5** has been achieved starting from Alloc−*N*Me−Cys−OH by treatment with Npys−Cl in a mild basic medium (pH 8) for 4 h in 70% yield.

S*t*Bu protection requires the initial activation of the free thiol group with methoxycarbonylsulfenylchloride to yield the corresponding methoxycarbonylsulfenyl derivative under mild basic conditions. This is followed by a nucleophilic attack of 2-methylpropane-2-thiolate at the sulfur site.[38] The amino acid **6** was synthesized in 89% yield.

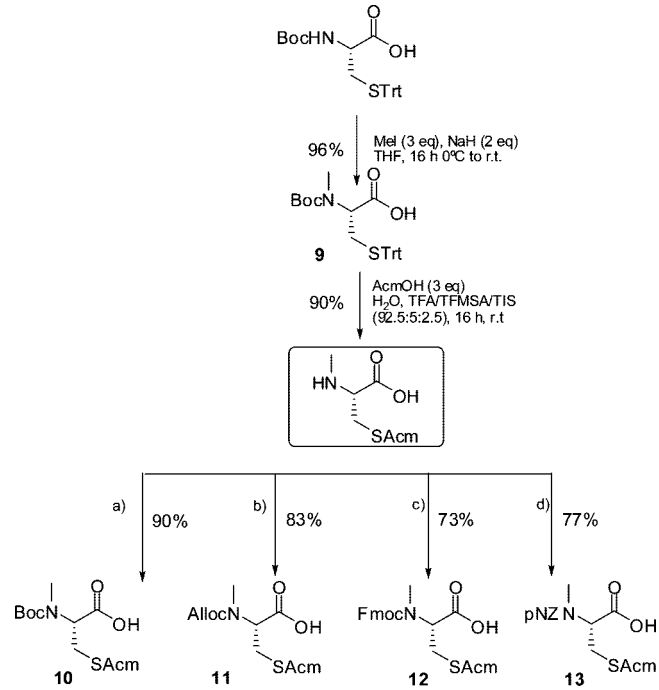
Synthesis of Cys derivative **7** starting from amino acid **3**, under acidic conditions and in the presence of TIS and acetamidomethanol (Acm−OH), is a useful reaction because it allows the removal of the Trt group and the introduction of Acm in a single step, without isolation of the free thiol intermediate. The *S*-Acm group has been reported to be stable under basic and acidic conditions. However, in the reaction medium, formation of Alloc−*N*Me−Cis−OH as a side-product was observed. Some undesired Acm removal and subsequent oxidation could form the undesired dimer.

In this case, some optimization in the reaction conditions was necessary. As shown in Table 1, the best result was observed in the presence of TIS, a large excess of Acm−OH,

Table 1. Conditions Tested in Deprotection−Reprotection of Cys Derivative **3** To Obtain **7** in One Pot

	Acms−OH (equiv)	TIS	*t* (h)	Ar atmosphere	dimer (%)
1	1.66		24		30
2	3		24		40
3	3	√	4	√	6

Scheme 5. Protected *N*Me−Cys(Acm) Derivatives Starting from Commercially Available Boc−Cys(Trt)−OH[a]



[a] Reaction conditions: (a) Boc_2O (2 equiv), H_2O−THF (1:1) pH 9 (aq. 50% NaOH), 24 h; (b) Alloc−Cl (2 equiv), aq. 2% Na_2CO_3−dioxane (1:1), 16 h; (c) Fmoc−Cl (1.2 equiv), aq. 2% Na_2CO_3−dioxane (1:1), 48 h; (d) *p*NZ−Cl (1 equiv), aq. 2% Na_2CO_3−dioxane (1:1), 72 h.

short reaction time, and under an inert atmosphere. Following these conditions, the formation of the dimer was minimized and the desired product was obtained in 80% yield with an excellent purity (>90%).

Dimer formation has already been described in a few cases during acidolytic cleavage of peptides containing Acm-protected cysteines. For example, dimer formation has resulted from Wang resin in the presence of scavengers such as TIS, phenol, or anisole[39] and from a polystyrene resin only under mild acidolysis conditions using TFA and 5% H_2O for 1 h at room temperature.[40] The cause of this reaction has not yet been elucidated, but it is thought to have been ascribed to an undefined sequence-specific effect, because the formation of the disulfide dimer does not occur during cleavage of all peptides containing Cys(Acm) residues. In our case, it has been observed that this kind of byproduct was obtained only when starting from **3**. When introducing the Acm moiety to H−*N*Me−Cys−OH, this side product was not observed.

In order to circumvent dimer formation, another route was envisioned to obtain Acm derivatives. Specifically, Boc−Cys(Trt)−OH was *N*-Methylated by using regular conditions (NaH, MeI) to obtain Boc−*N*Me−Cys(Trt)−OH (**9**) in good yields and purity.[41] Then, reaction with Acm−OH in acidic media [TFA−TFMSA−TIS (92.5:5:2.5)] simultaneously

removed the Trt and Boc groups and introduced the Acm moiety. In this case, no dimer formation was observed. Although this procedure seems a step back due to the cleavage of the Boc group, the intermediate obtained, H−*N*Me−Cys(Acm)−OH, is highly versatile and allows the introduction of many different protecting groups. For instance, the Fmoc−, *p*NZ−, and also Boc and Alloc derivatives can be easily obtained by reaction with Fmoc−Cl, *p*NZ−Cl, $(Boc)_2O$, and Alloc−Cl (Scheme 5).

Interchange of Amino Function on Solid Phase..[37] The development of a synthetic strategy for complex peptides such as thiocoraline analogues[42] or other peptides could require a rapid synthesis of other N^{α}-substituted *S*Trt derivatives. To satisfy this demand, amino acid **3** was loaded onto a CTC resin and the Alloc group was removed by reaction with $Pd(PPh_3)_4$ in the presence of $PhSiH_3$. The resultant H−*N*Me−Cys(Trt)−OCTC was reacted with *p*NZ−Cl or Fmoc−Cl with DIEA in CH_2Cl_2, to obtain the new *N*Me-protected derivatives *p*NZ and Fmoc. The compounds were released from the resin by treatment with 2% TFA in CH_2Cl_2 and dilution with water previous to solvent evaporation in order to avoid removal of the *S*Trt group. The compounds were obtained in good yields and purities (Scheme 6).

Conclusions

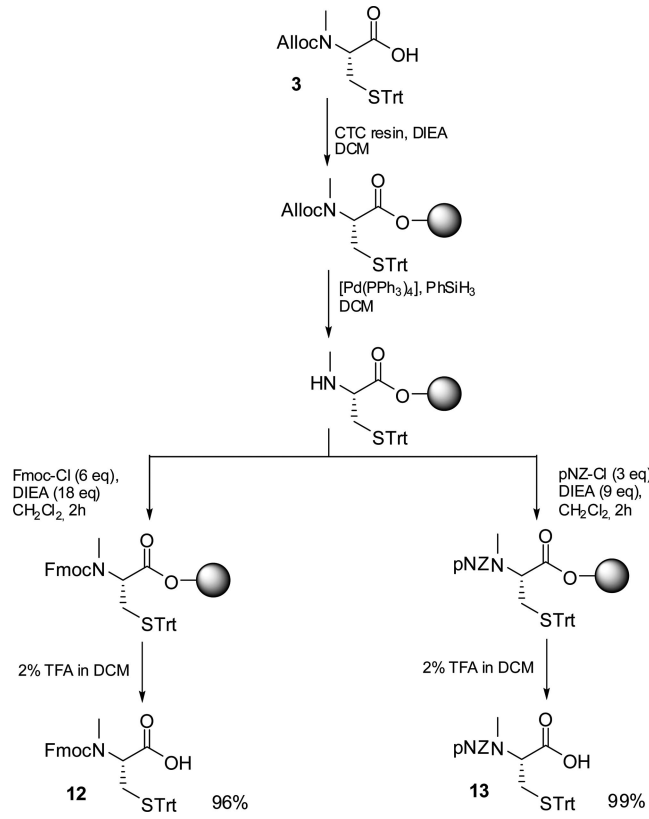
A number of N^{α}- and S-protected *N*Me−Cys can be accessed easily from *S*-Trt precursors by changing the protecting groups either in solution or on solid-phase. The good purities and yields obtained when using this strategy, combined with the milder reaction conditions, make this route attractive for large-scale production and could help fill the gap in the commercial availability of protected *N*Me−Cys.

Experimental Section

Abbreviations. Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC−IUB Commission of Biochemical Nomenclature in ref 43. The following additional abbreviations are used: Acm−OH, acetamidomethanol; ACN, acetonitrile; Alloc, allyloxycarbonyl; Boc, *t*-butyloxycarbonyl; CTC, chlorotrityl chloride (Barlos) resin; DCM, dichloromethane; DHB, 2,5-dihydroxybenzoic acid; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; ESMS, electrospray mass spectrometry; HPLC, high performance liquid chromatography; MS, mass spectrometry; Npys, 3-nitro-2-pyridinesulphenyl; *p*NZ, *p*-nitrobenzyloxycarbonyl; PTSA, *p*-toluenesulfonic acid; TBME, *tert*-butyl methyl ether; TFA, trifluoroacetic acid; TES, triethylsilane; TFMSA, trifluoromethanesulfonic acid; TIS, triisopropylsilane; Trt, trityl. Amino acid symbols denote an L-configuration unless indicated otherwise. All reported solvent ratios are expressed as v/v, unless otherwise stated.

General Methods. All commercial reagents and solvents were used as received with the exception of DMF and CH_2Cl_2, which were bubbled with nitrogen to remove volatile contaminants (DMF) and stored over activated 4 Å molecular sieves (Merck, Darmstadt, Germany), and THF which was distilled from sodium/benzophenone.

Scheme 6. Obtention of Different Protected N^{α} *N*Me−Cys(Trt)−OH Derivatives by Interchanging the N^{α} Protecting Group on a Solid Support Starting from **3**



Solution reactions were performed in round-bottomed flasks. Organic solvent extracts were dried over anhydrous $MgSO_4$, followed by solvent removal under reduced pressure at temperatures below 40 °C.

Solid-phase syntheses were performed in polypropylene syringes (2.5 mL) fitted with a polyethylene porous disk. Solvents and soluble reagents were removed by suction. Removal of the Fmoc group was carried out with piperidine−DMF (1:4, v/v) (1 × 1 min, 2 × 5 min). Washings between reaction steps were carried out with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) using 5 mL solvent·g^{-1} resin for each wash. Peptide synthesis transformations and washes were performed at 25 °C.

HPLC columns (a Symmetry C18 reversed-phase analytical column, 5.0 μm × 4.6 mm × 150 mm and a Symmetry C18 reversed-phase semipreparative column, 5.0 μm × 7.8 mm × 100 mm) were obtained from Waters (Ireland). Analytical HPLC was carried out on a Waters instrument comprising a separation module (Waters 2695), automatic injector, photodiode array detector (Waters 996), and system controller (Millenium[32] login). UV detection was at 220 and 254 nm, and linear gradients of ACN (+0.036% TFA) into H_2O (+0.045% TFA) were run at a 1.0 mL·min^{-1} flow rate over 15 min. Semipreparative HPLC was carried out on a Waters instrument comprising a separation module (Waters 600), automatic injector, and a dual absorbance detector (Waters 2487). UV detection was at 220 and 254 nm, and linear gradients of ACN (+0.05% TFA) into H_2O (+0.1% TFA) were run in the conditions specified for each case.

ES(+)-MS analyses of amino acids samples were performed on a Waters Micromass ZQ spectrometer and in an Agilent Ion Trap 1100 Series LC/MSD Trap. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopy was performed on a Varian Mercury 400. Chemical shifts (δ) are expressed in parts per million downfield from tetramethylsilyl chloride. Coupling constants are expressed in Hertz.

Alloc−Cys(Trt)−OH (2). $CH_3COOH\cdot H$−Cys(Trt)−OH[37] (4.69 g, 11.1 mmol) was dissolved in dioxane (55 mL) and Na_2CO_3 (2%) in H_2O (55 mL), and the solution was cooled to 4 °C. Alloc−Cl (1.5 equiv, 1.78 mL, 16.65 mmol) was dissolved in dioxane (5 mL) and added slowly to the amino acid solution. The mixture was stirred for 1 h at 4 °C and then for 2 h at 25 °C, the pH being maintained at 9–10. Dioxane was removed, and the aqueous solution was washed with TBME (3 × 50 mL). The aqueous layer was acidified with 1 N HCl to pH 6–7, and the product was extracted with EtOAc (3 × 50 mL). The solvent was evaporated to give 4.35 g of the title product as a white solid (88% yield; 85% purity). Analytical HPLC (linear gradient from 0 to 100% ACN over 15 min, 1 mL/min): t_R = 13.9 min. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (m, 6H), 7.21 (m, 6 H), 7.14 (m, 3H), 5.79 (m, 1 H), 5.27 (d, 1H), 5.18(m, 2 H), 4.41 (m, 2H), 3.94 (m, 1H), 2.59 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.5, 155.6, 144.1, 132.5, 129.4, 128.0, 126.8, 117.9, 67.0, 66.0, 52.7, 33.6. HPLC-ESMS: *m/z* calcd for $C_{26}H_{25}NO_4S$, 447.2; found, 445.5 $[M - H]^-$.

Alloc−*N*Me−Cys(Trt)−OH (3). In a round-bottomed flask, NaH (60% in mineral oil) (617 mg, 15.43 mmol) was suspended in THF anhydrous (20 mL) under a nitrogen atmosphere and the suspension was cooled at 4 °C. Alloc−Cys(Trt)−OH (2.87 g, 6.43 mmol) was dissolved in THF (4 mL), and the solution was added slowly. MeI (1.33 mL, 21.21 mmol) was dropped, and the mixture was stirred for 2 h at 4 °C and for 10 h at room temperature. MeOH and H_2O were added to quench the reaction, and the THF was removed under vacuum. The aqueous solution was washed with TBME (3 × 20 mL) and acidified with 1 N HCl to pH 6–7, and the product was extracted with EtOAc (3 × 30 mL). The solvent was dried with Na_2SO_4 and removed under vacuum. The product was redissolved in ACN and H_2O and lyophilized to give 2.34 g of the title compound as a white powder (79% yield; 97% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 14.5 min. ^{1}H NMR (400 MHz, $CDCl_3$): major conformer δ 7.42 (m, 6H), 7.28 (m, 6H), 7.20 (m, 3H), 5.85 (m, 1H), 5.21 (m, 2H), 4.54 (d, J = 5.2 Hz, 2H), 3.92 (m, 1H), 2.72 (m, 2H), 2.68 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): major conformer δ 174.7, 156.6, 144.4, 132.5, 129.5, 127.9, 126.8, 117.4, 67.0, 66.4, 60.4, 60.0, 32.9. HPLC-ESMS: *m/z* calcd for $C_{27}H_{27}NO_4S$, 461.2; found 459.4 $[M - H]^-$. HRMS: *m/z* calcd for $C_{27}H_{26}NO_4S$, 460.1577 $[M - H]^-$; found, 460.1572.

Boc−*N*Me−Cys(Me)−OH. Boc−Cys(Me)−OH (1 g, 4.24 mmol) was dissolved in anhydrous THF (3 mL). The amino acid solution was transferred to a NaH suspension in mineral oil (509 mg, 12.7 mmol) in THF (10 mL) cooled in an ice–water bath. MeI (528 mL, 8.48 mmol) was then added, and the reaction was stirred at 4 °C for 16 h. MeOH and H_2O were added to the reaction mixture to quench excess NaH, and the solution was then evaporated. The residue was dissolved in H_2O (50 mL) and the aqueous layer was washed with TBME (3 × 25 mL), acidified to pH 4.5, extracted with EtOAc (4 × 25 mL), dried ($MgSO_4$), and evaporated. The product was purified by preparative HPLC (linear gradient from 15 to 50% ACN over 30 min, C_8, 40 mL/min) to obtain 450 mg of the title compound as a white power (44% yield; 99% purity). Analytical HPLC (linear gradient from 0 to 100% ACN over 15 min, 1 mL/min): t_R = 10.3 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.18 (br s, 1H), 4.74 (m, 1H), 3.05 (m, 1H), 2.88 (m, 1H), 2.84 (s, 3H), 2.13 (s, 3H), 1.46 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.2, 156.5, 80.7, 58.0, 33.2, 32.1, 28.6, 15.6. HPLC-ESMS: *m/z* calcd for $C_{10}H_{19}NO_4S$, 249.1; found, 250.3 $[M + H]^+$, 272.3 $[M + Na]^+$.

Boc−*N*Me−Cys(Trt)−OH (9). Boc−Cys(Trt)−OH (5.0 g, 10.8 mmol) was dissolved in anhydrous THF (10 mL). The amino acid solution was transferred to a NaH suspension in mineral oil 60% (0.84 g, 25.9 mmol) in THF (35 mL) cooled in an ice–water bath. MeI (2.2 mL, 35.6 mmol) was then added dropwise, and the reaction was stirred overnight (0−25 °C). MeOH and H_2O were added to the reaction mixture to quench excess NaH, and the solution was then evaporated. The residue was dissolved in H_2O (200 mL), and the aqueous layer was washed with TBME (3 × 100 mL), acidified to pH 4.5, extracted with EtOAc (4 × 100 mL), dried ($MgSO_4$), and evaporated to afford 4.8 g of a white solid (96% yield; 97%purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 15.1 min. ^{1}H NMR ($CDCl_3$, 400 MHz) mixture of two rotamers δ 7.42 (m, 6H), 7.29 (m, 6H), 7.21 (m, 3H), 3.82 and 3.68 (dd, 1H), 2.84–2.72 (m, 2H), 2.69 and 2.67 (s, 3H), 1.45 and 1.38 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz) mixture of two rotamers δ 175.7 and 174.9, 156.4 and 154.9, 144.7, 129.8, 128.2, 127.0, 81.3 and 81.1, 67.2, 60.6 and 59.8, 34.3 and 33.8, 31.7 and 31.1, 28.5. HPLC-ESMS: *m/z* calcd for $C_{28}H_{31}NO_3S$, 477.20; found, 477.12 $[M - H]^-$.

Intercoversion of *S*-Protecting Groups in Solution. Alloc−*N*Me−Cys(Acm)−OH (11). Alloc−*N*Me−Cys(Trt)−OH (3.82 mmol, 1.76 g) was suspended in H_2O (8.5 mL), and the suspension was purged with argon. *N*-(Hydroxymethyl)acetamide (1.102 g, 11.47 mmol) was added, and the mixture was cooled to 4 °C under argon. TIS (1 mL) was added to a solution of TFA−TFMSA (95:5) (26 mL) and dropped into the suspension. The mixture was stirred at room temperature for 5 h, and the solvent was evaporated under vacuum and coevaporated with toluene. The aqueous layer was basified to pH 9 and washed with TBME (3 × 20 mL) then acidified to pH 3.5, and the product was extracted with EtOAc (3 × 50 mL) to obtain 0.85 g of the title product as a colorless oil (77% yield, 95% purity). Analytical HPLC (linear gradient from 10 to 100% for 15 min): t_R = 7.2 min. CCF (SiO_2,CH_2Cl_2−MeOH (9:1)): R_f = 0.21. ^{1}H NMR ($CDCl_3$, 400 MHz): mixture of rotamers δ 10.25 (br s, 1H), 7.34 (br s, 1H), 5.92 (m, 1H), 5.34 (dm, 1H, J = 17.2 Hz), 5.25 (dm, 1H, J = 10.4 Hz), 4.94 and 4.84 (m, 1H), 4.65 (m, 2H), 4.58 (dd, 1H, J = 14.4 Hz, J = 6.8 Hz), 4.24 (dd, 1H, J = 14.4 Hz, J = 5.2 Hz), 3.25 (dd, 1H, J = 16 Hz, J

=4.8 Hz), 2.92 (m, 4H), 2.11 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 174.0, 159.8, 158.2, 132.1, 118.2, 67.5, 59.1, 42.1, 31.7, 30.9, 22.4. HPLC-ESMS: *m/z*: calcd for $C_{11}H_{18}N_2O_5S$ 290.1; found, 288.2 $[M - H]^-$. HRMS: *m/z* calcd for $C_{11}H_{17}N_2O_5S$ 289.0853 $[M - H]^-$; found, 289.0849.

Alloc−*N*Me−Cys(Npys) (5). To a round-bottomed flask containing Alloc−*N*Me−Cys(Trt)−OH (0.44 mmol, 200 mg), a solution of TFA and TIS in CH_2Cl_2 (4:1:35, 4 mL) was added dropwise. The solution was stirred for 1 h at room temperature, and the solvent evaporated under reduced pressure and coevaporated with toluene. The product was dissolved in CH_2Cl_2 (2 mL) and DIEA was added to pH 7. Next, Npys−Cl (0.66 mmol, 126 mg), dissolved in CH_2Cl_2 (0.5 mL), was added to the amino acid solution. The reaction was controlled by the Ellman test, and the solvent evaporated under reduced pressure. H_2O was added, and the pH was basified to 9. The aqueous layer was washed with TBME (3 × 7 mL) and acidified to pH 3.5, and the product was extracted with EtOAc (3 × 10 mL) to obtain 116 mg of the title product as a yellow powder (70% yield, 92% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 11.0 min. ^{1}H NMR (400 MHz, $CDCl_3$): major conformer δ 8.86 (d, 1H, *J* = 3.2 Hz), 8.53 (d, 1H, *J* = 8 Hz), 4.39 (dd, 1H, *J* = 8 Hz, *J* = 4.4 Hz), 5.94 (m, 1H), 5.28 (m, 2H), 4.83 (m, 1H), 4.62 (m, 2H), 3.53 and 3.31 (m, 2 H), 3.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): major conformer δ 173.8, 156.8, 153.8, 142.8, 133.9, 132.3, 121.1, 117.8, 66.8, 59.9, 37.6, 33.5. HPLC-ESMS: *m/z* calcd for $C_{13}H_{15}N_3O_6S_2$, 373.0; found, 374.1 $[M + H]^+$, 396.1 $[M + Na]^+$. HRMS: *m/z* calcd for $C_{13}H_{16}N_3O_6S_2$, 373.0475 $[M + H]^+$; found, 374.0480.

Alloc−*N*Me−Cys(Me)−OH (4). To a round-bottomed flask containing Alloc−*N*Me−Cys(Trt)−OH (0.22 mmol, 100 mg) a solution of TFA and TIS in CH_2Cl_2 (4:1:35, 2 mL) was added dropwise. The solution was stirred for 1 h at room temperature, and the solvent evaporated under reduced pressure and coevaporated with toluene. The product was dissolved in H_2O−THF (1:1, 2.8 mL), and the solution was cooled to 4 °C. Maintaining the pH at 7, MeI (0.31 mmol, 20 μL) was added to the solution. The mixture was stirred for 4 h at room temperature and THF evaporated under vacuum. The aqueous layer was basified to pH 9, washed with TBME (3 × 5 mL), and acidified to pH 3.5. Then, the product was extracted with EtOAc (3 × 7 mL), dried ($MgSO_4$), and evaporated to obtain 40.0 mg of a colorless oil (78% yield, 80% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min): t_R = 9.2 min. ^{1}H NMR ($CDCl_3$, 400 MHz) major conformer: δ 5.93 (m, 1H), 5.35 (dd, 1H), 5.22 (dd, 1H), 4.88 (m, 1H), 4.65 (d, 2H), 3.12 (dd, 1H), 2.95 (s, 3H), 2.92 (dd, 1H), 2.15 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): major conformer δ 174.8, 157.2, 136.8, 117.9, 67.1, 58.8, 33.1, 32.0, 17.9. ESMS: *m/z* calcd for $C_9H_{15}NO_4S$, 233.07; found, 234.3 $[M + H]^+$.

Alloc−*N*Me−Cys(*S*^{t}Bu)−OH (6). To a round-bottomed flask containing Alloc−*N*Me−Cys(Trt)−OH (0.44 mmol, 200 mg) a solution of TFA and TIS in CH_2Cl_2 (4:1:35, 4 mL) was added dropwise. The solution was stirred for 1 h at room temperature, and the solvent evaporated under reduced pressure and coevaporated with toluene. The product was dissolved in CH_2Cl_2 anhydrous, and DIEA was added to reach pH 7. Scm−Cl (0.88 mmol, 80 μL) was added, and the reaction was controlled by Ellman test. The solvent was evaporated, and the Scm product was dissolved in MeOH (2 mL) and added to a round-bottomed flask containing *t*-BuSH (0.88 mmol, 100 μL) and Et_3N (0.4 mmol, 56 μL) in MeOH (2 mL). The mixture was stirred for 2 h at room temperature, and the solvent evaporated under reduced pressure. H_2O was added, and the pH was basified to 9. The aqueous layer was washed with TBME (3 × 7 mL) and then acidified to pH 3.5, and the product was extracted with EtOAc (3 × 10 mL), dried ($MgSO_4$), and evaporated to obtain 120 mg of a colorless oil. (89% yield, 60% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 12.4 min. The product was purified by semipreparative HPLC (linear gradient from 30 to 100% ACN in 30 min, 10 mL/min): t_R = 22.6 min. ^{1}H NMR (400 MHz, $CDCl_3$): major conformer δ 5.93 (m, 1H), 5.32 (m, 2H), 4.72 (m, 1H), 3.32 and 3.09 (m, 2H), 3.01 (s, 3H), 1.24 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): major conformer δ 175.2, 156.6, 132.5, 117.5, 66.6, 59.8, 48.2, 39.0, 33.8, 29.9. HPLC-ESMS: *m/z* calcd for $C_{12}H_{21}NO_4S_2$, 307.1; found, 308.1, 346.1 $[M + Na]^+$. HRMS: *m/z* calcd for $C_{12}H_{22}NO_4S_2$, 308.0985 $[M + H]^+$; found, 308.0980.

Interconversion of the N^{α}-Amino Group in Solution. Fmoc−*N*Me−Cys(Me)−OH. The amino acid Boc−*N*Me−Cys(Me)−OH was treated with TFA−H_2O (95:5) for 1 h. The solvent was then removed by vacuum evaporation followed by five coevaporations with dioxane. TFA·H*N*Me−Cys(Me)−OH (4.1 g, 23.8 mmol) was dissolved in 2% Na_2CO_3in dioxane–H_2O (1:1) (170 mL). Fmoc−Cl (10 g, 38.8 mmol) was dissolved in dioxane (8 mL) and added to the amino acid solution. The mixture was stirred for 2 h at 4 °C and for a further 3 days at 25 °C, with the pH maintained at 9–10. The dioxane was removed, and the resultant aqueous solution was washed with TBME (3 × 50 mL) and then acidified with HCl to pH 2. The crude product was extracted with EtOAc (3 × 50 mL), and the solvent removed under reduced pressure. Then, the product was purified by preparative HPLC (linear gradient from 30 to 70% ACN for 30 min, C_4 reversed-phase column, 20 mL/min) to give 1.23 g of a pale yellow power (99% purity, 7% yield). Analytical HPLC (linear gradient from 30 to 70% ACN for 15 min, 1 mL/min): t_R= 12.8 min. ^{1}H NMR ($CDCl_3$, 400 MHz): mixture of two rotamers δ 9.1 (bs, 1H), 7.75 (m, 2H), 7.60 (m, 2H), 7.55 (m, 2H), 7.34 (m, 2H), 4.87 and 4.67 (1:2) (m, 1H), 4.46 (m, 2H), 4.29 and 4.22 (1:2) (m, 1H), 3.11 and 2.82 (1:2) (m, 2H), 2.96 and 2.89 (1:2) (s, 3H), 2.12 and 1.88 (1:2) (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.0, 157.5, 143.9, 141.6, 128.0, 127.4, 125.3, 120.3, 68.0, 58.6, 47.3, 33.4, 32.1, 15.7. MALDI-TOF MS (DHB): calcd for $C_{20}H_{21}NO_4S$, 371.1; found, 372.0 $[M + H]^+$, 393.9 $[M + Na]^+$, 409.9 $[M + K]^+$.

Alloc−*N*Me−Cys(Me)−OH (4). In a 500 mL round-bottomed flask Boc−*N*Me−Cys(Me)−OH (3.0 g, 12.0 mmol) was reacted with TFA−CH_2Cl_2 (1:1) for 1 h. The mixture was evaporated to dryness, and coevaporations with dioxane were carried out in order to remove excess TFA. The solid mixture was dissolved in a 30% aqueous Na_2CO_3

solution (50 mL). Next, a solution of allyl chloroformate (1.5 mL, 14.4 mmol) in dioxane (50 mL) was added dropwise, and the pH maintained at 8–9 by the addition of 10% aqueous Na_2CO_3. The course of the reaction was followed by TLC [EtOAc−H_2O−AcOH−2-propanol (2:1:1:1)]. After completion of the reaction, the aqueous phase was washed with TBME (3 × 50 mL) and then acidified to pH 2 by adding 4 N HCl. Next, the title product was extracted with EtOAc (3 × 50 mL), dried ($MgSO_4$), and evaporated to obtain 2.5 g of a colorless oil (89.6% yield; 95% purity), which was characterized as described earlier in the experimental section.

Interconversion of N^{α}- and *S*-Protecting Groups in Solution. Boc−*N*Me−Cys(Acm)−OH (10). Boc−*N*Me−Cys(Trt)−OH (2.8 g, 5.36 mmol) was dissolved in H_2O (8.4 mL) and acetamidomethanol (0.62 g, 6.96 mmol) was added followed by a solution of TFA−TFMSA−TIS (92.5:5:2.5; 50 mL). The solution was stirred at 25 °C for 16 h, and then, it was evaporated. After three coevaporations with TBME (3 × 50 mL) to remove excess TFA, the residue, H−*N*Me−Cys(Acm)−OH, was dissolved in H_2O (25 mL) and THF (50 mL), and the pH was adjusted to 8–9 by adding 50% NaOH. Next, $(Boc)_2O$ (2.3 g, 10.72 mmol.) was added and the mixture kept to pH 8–9 by adding 50% NaOH. The reaction was stirred for 24 h at 25 °C. The solvent was evaporated, and the aqueous layer was washed with TBME (3 × 100 mL). The aqueous layer was brought to pH 4.0 by adding 1 N HCl and extracted with EtOAc (4 × 100 mL). The organic phase was dried ($MgSO_4$) and evaporated to afford 1.35 g of a white solid (90% yield, 93% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 7.9 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.72 (br s, 1H), 4.92 (m, 1H), 4.56 (m, 1H), 4.21 (m, 1H), 3.21 (m, 1H), 2.89 and 2.82 (m, 4H), 2.05 (m, 3H), 1.50 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.6, 159.1, 153.5, 80.9, 59.3, 40.6, 32.3, 31.4, 28.3, 22.9. HPLC-ESMS: *m/z* calcd for $C_{12}H_{22}N_2O_5S$, 306.12; found, 306.67 $[M + H]^+$.

Alloc−*N*Me−Cys(Acm)−OH (11) from Boc−*N*Me−Cys(Trt)−OH (9). The intermediate H−*N*Me−Cys(Acm)−OH obtained above (0.95 g, 4.6 mmol) was dissolved in 2% Na_2CO_3−dioxane (1:1, 40 mL). Alloc−Cl (0.98 mL, 9.2 mmol) was added, and the reaction was adjusted to pH 8 by adding 2 N NaOH. The reaction was stirred for 16 h at 25 °C, after which time it was adjusted again to pH 8–9 by adding 2 N NaOH. The dioxane was evaporated, and the aqueous phase extracted with TBME (3 × 100 mL). Next, the aqueous phase was acidified to pH 3.5 and extracted with EtOAc (3 × 100 mL). The organic layer was dried ($MgSO_4$) and evaporated, and the product was characterized as described earlier in the experimental section to obtain 1.10 g of powder (83% yield; 82% purity).

Fmoc−*N*Me−Cys(Acm)−OH (12) from Boc−*N*Me−Cys(Trt)−OH (9). The amino acid H−*N*Me−Cys(Acm)−OH obtained earlier (86.5 mg, 0.42 mmol) was dissolved in 2% Na_2CO_3−dioxane (1:1, 2 mL). Fmoc−Cl (0.16 g, 0.63 mmol) was added, and the reaction was adjusted to pH 8 by adding 1 N NaOH. The reaction was stirred for 48 h at 25 °C, after which time it was adjusted again to pH 8–9 by adding 1 N NaOH. The dioxane was evaporated, and the aqueous phase extracted with TBME (3 × 50 mL). Next, the aqueous phase was acidified to pH 2 and extracted with EtOAc (3 × 50 mL). The organic layer was dried ($MgSO_4$) and evaporated to obtain 0.13 g of product (73% yield; 99% purity). Analytical HPLC (linear gradient from 30 to 70% ACN for 15 min, 1 mL/min): t_R = 8.8 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76 (m, 2H), 7.58 (m, 2H), 7.39 (m, 2H), 7.29 (m, 2H), 7.01 and 6.55 (1:3.7) (m, 1H), 4.95 and 4.73 (m, 1H), 4.55, 4.25 (m, 4H), 4.21 (m, 1H), 3.25 and 2.9 (m, 2H), 2.91 and 2.90 (s, 3H), 2.02 and 1.99 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.6, 157.9, 156.0, 144.0, 141.6, 128.0, 127.4, 125.3, 120.2, 68.5, 58.8, 47.4, 42.1, 32.0, 31.3, 23.1. MALDI-TOF (DHB), *m/z* calcd for $C_{22}H_{24}N_2O_5S$, 428.1; found 429.2 $[M + H]^+$.

***p*NZ−*N*Me−Cys(Acm)−OH (13) from Boc−*N*Me−Cys(Trt)−OH (9).** H−*N*Me−Cys(Acm)−OH (2.15 g, 10.4 mmol) was dissolved in 2% Na_2CO_3−dioxane (1:1, 30 mL), and the solution was cooled at 4 °C. *p*-Nitrobenzyl chloroformate (2.23 g, 10.4 mmol) was dissolved in minimum dioxane and added to the amino acid solution. The reaction mixture was stirred at 4 °C for 2 h and then warmed at room temperature for 3 days. More *p*NZ−Cl was added during the second day of reaction. The pH was kept at 9–10 by an aqueous solution of 10% Na_2CO_3. Total conversion was controlled by CCF. The organic solvent was removed under vacuum, and the aqueous layer was washed with TBME (3 × 50 mL), acidified to pH = 3–4 with HCl aqueous solution, and extracted with EtOAc (3 × 50 mL). The organic layer was dried with Na_2SO_4, filtered, and then dried under vacuum to obtain 3.85 g of the title compound as a white powder (77% yield; 93% purity). Analytical HPLC (linear gradient from 0 to 100% ACN over 15 min, 1 mL/min): t_R = 9.1 min. ^{1}H NMR ($CDCl_3$, 400 MHz): mixture of rotamers δ 8.22 (d, 2H, J = 8.8 Hz), 7.52 (d, 2H, J = 8.4 Hz), 6.70 and 6.42 (br s, 1H), 5.28 (s, 2H), 4.98 and 4.94 (m, 1H), 4.68 and 4.58 (1: 2.5) (m, 1H), 4.22 and 3.99 (m, 1H), 3.25 and 2.96 (m, 2H), 2.96 and 2.94 (s, 3H), 2.07 and 2.05 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.6, 157.3, 156.0, 147.8, 144.1, 128.0, 124.0, 66.5, 58.8, 41.6, 31.6, 30.0, 23.2. HPLC-ESMS: *m/z*: calcd for $C_{15}H_{19}N_3O_7S$, 385.1; found, 386.1 $[M + H]^+$, 408.1 $[M + Na]^+$.

Interconversion of N^{α}-Amino Group on Solid Phase. *p*NZ−*N*Me−Cys(Trt)−OH (13). 2-Chlorotrityl resin (0.4 g, 1.5 mmol/g) was washed with DMF (3 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). Next, Alloc−*N*Me−Cys(Trt)−OH (0.18 g, 0.4 mmol) was dissolved in CH_2Cl_2 (0.5 mL), and DIEA (0.46 mL, 2.7 mmol) was added. Then, the mixture was added to the resin. After 5 min, more DIEA (0.23 mL, 1.3 mmol) was added, and the reaction was shaken for 1 h at 25 °C. MeOH (0.32 mL) was added in order to cap any unreacted sites and the syringe shaken for an additional 10 min. The resin was then washed with CH_2Cl_2 (3 × 0.5 min) and DMF (3 × 0.5 min). Cleavage of the Alloc group was achieved by treatment with $Pd(PPh_3)_4$ (46 mg, 0.04 mmol) and $PhSiH_3$ (0.49 mL, 4.0 mmol) in CH_2Cl_2 (1 mL) for 15 min The resin was washed with CH_2Cl_2, and the procedure repeated two more times. Lastly, the resin was washed extensively with CH_2Cl_2 (5 × 1 min). At this point, the resin was split into two syringes. In order to introduce the protecting group to the resin-bound H−*N*Me−Cys(Trt)−OH

(0.2 g, 0.2 mmol), *p*-nitrobenzyl chloroformate (0.26 g, 1.2 mmol) was previously dissolved in CH_2Cl_2 (0.5 mL) and DIEA (0.71 mL, 4.0 mmol) and then added to the resin. After 2 h, the De Clercq test was negative. The resin was washed with CH_2Cl_2 (3 × 0.5 min), MeOH (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min). The resin was cleaved with TFA−CH_2Cl_2 (1:49) (5 × 1 min), and the mixture was evaporated and lyophilized to obtain 110 mg of the title product as a white solid (99% yield; 96% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 14.9 min. ^{1}H NMR ($CDCl_3$, 400 MHz): mixture of rotamers δ 8.16 and 8.11 (2:1) (d, 2H, J = 8.8 Hz), 7.42 (m, 8H), 7.28 (m, 6H), 7.23 (m, 3H), 5.25 and 5.16 (m, 2H), 4.16 and 4.02 (1:2), 2.83 and 2.68 (2:1), 2.77 and 2.76 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 173.7, 147.7, 144.6, 144.5, 129.8, 129.7, 128.3, 128.2, 127.9, 127.2, 127.1, 123.9, 123.9, 67.3, 66.3, 59.7, 32.8, 31.3. HPLC-ESMS: *m/z* calcd for $C_{31}H_{28}N_2O_6S$, 556.17; found, 554.66 $[M - H]^-$. HRMS: *m/z* calcd for $C_{31}H_{27}N_2O_6S$, 555.1584; found, 555.1582.

Fmoc−*N*Me−Cys(Trt)−OH (12). Fmoc−Cl (0.31 g, 1.2 mmol) was dissolved in CH_2Cl_2 (0.5 mL), and DIEA (0.71 mL, 4.0 mmol) was added. The mixture was added to the resin-bound H−*N*Me−Cys(Trt)−OH (0.2 g, 0.2 mmol), as described above. After 2 h, the De Clercq test was negative. The resin was washed with CH_2Cl_2 (3 × 0.5 min), MeOH (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min). The resin was cleaved with TFA−CH_2Cl_2 (1:49) (5 × 1 min), and the mixture was evaporated and lyophilized to obtain 115 mg of the title product as a white solid (96% yield; 98% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 16.5 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.75 (m, 2H), 7.57 (m, 2H), 7.44 (m, 4H), 7.38 (m, 4H), 7.29 (d, 9H), 7.22 (m, 3H), 4.50 (m, 1H), 4.41 (m, 2H), 4.26 and 4.21 (2:1) (m, 1H), 2.85 and 2.79 (m, 2H), 2.74 and 2.70 (2:1) (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.4, 157.4, 144.6, 144.1, 141.5, 129.8, 129.7, 128.3, 127.9, 127.3, 127.1, 125.3, 125.3, 120.2, 67.2, 66.2, 60.5, 47.4, 33.1 and 32.3, 31.2. MALDI-TOF (DHB):*m/z* calcd for $C_{38}H_{33}NO_4S$, 599.21; found, 622.20 $[M + Na]^+$. HRMS: *m/z* calcd for $C_{38}H_{33}NNaO_4S$, 622.2023 $[M + Na]^+$; found, 622.2029.

***p*NZ−*N*Me−Cys(Me)−OH.** 2-Chlorotrityl resin (0.6 g, 1.5 mmol/g) was washed with DMF (3 × 0.5 min) and CH_2Cl_2 (3 × 0.5 min). Next, Alloc−*N*Me−Cys(Me)−OH (0.17 g, 0.6 mmol) was dissolved in CH_2Cl_2 (0.5 mL), and DIEA (0.70 mL, 4.0 mmol) was added. Then, the mixture was added to the resin. After 5 min, more DIEA (0.35 mL, 2.0 mmol) was added and the reaction was shaken for 1 h at 25 °C. MeOH (0.48 mL) was added in order to cap any unreacted sites, and the syringe was shaken for an additional 10 min. The resin was then washed with CH_2Cl_2 (3 × 0.5 min) and DMF (3 × 0.5 min).

Cleavage of the Alloc group was achieved by treatment with $Pd(PPh_3)_4$ (69 mg, 0.06 mmol) and $PhSiH_3$ (0.74 mL, 6 mmol) in CH_2Cl_2 (1 mL) for 15 min The resin was washed with CH_2Cl_2, and the procedure repeated two more times. Last, the resin was washed extensively with CH_2Cl_2 (5 × 1 min). At this point, the resin was split into three portions. In order to introduce the protecting group to the resin-bound H−*N*Me−Cys(Me)−OH (0.2 g, 0.2 mmol), *p*-nitrobenzyl chloroformate (0.13 g, 0.6 mmol) was previously dissolved in CH_2Cl_2 (0.5 mL) and DIEA (0.13 g, 0.6 mmol) and then added to the resin. After 2 h, the De Clercq test was negative. The resin was washed with CH_2Cl_2 (3 × 0.5 min), MeOH (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min). The resin was cleaved with TFA−CH_2Cl_2 (1:49) (5 × 1 min), and the product was evaporated and lyophilized to obtain 53 mg of product (80% yield; 98% purity). Analytical HPLC (linear gradient from 0 to 100% ACN for 15 min, 1 mL/min): t_R = 10.8 min. ^{1}H NMR ($CDCl_3$, 400 MHz) mixture of two conformers (80:20): major conformer δ 8.22 (d, 2H, J = 8.4 Hz), 7.52 (d, 2H, J = 8.4 Hz), 5.29 (d, 2H), 4.86 (dd, 2H, J = 10.8 Hz, J = 4.8 Hz), 3.12 (dd, 1H, J = 14.0 Hz, J = 4.8 Hz), 2.99 (s, 3H), 2.95 (dd, 1H, J = 14.0 Hz, J = 3.2 Hz), 2.14 (s, 3H); minor conformer δ 8.20 (d, 2H, J = 8.0 Hz), 7.50 (d, 2H, J = 8.0 Hz), 5.27 (d, 2H), 4.77 (dd, 2H, J = 10.8 Hz, J = 4.8 Hz), 3.09 (dd, 1H, J = 12.8 Hz, J = 4.8 Hz), 2.97 (s, 3H), 2.91 (dd, 1H, J = 14.0 Hz, J = 3.2 Hz), 2.11 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): major conformer δ 174.6, 156.8, 147.8, 144.0, 128.0, 124.0, 66.4, 58.5, 33.3, 32.0, 15.7; minor conformer δ 174.4 (CO), 155.8 (CO), 147.8 (C_{Ar}), 143.8 (C_{Ar}), 128.4 (CH_{Ar}), 124.0 (CH_{Ar}), 66.5 (CH_2*p*NZ), 58.8 (CH^{α}), 33.8 (CH_2^{β}), 32.2 (NCH_3), 16.1 (SCH_3). HRMS: *m/z* calcd for $C_{13}H_{17}N_2O_6S$, 329.0802 $[M + H]^+$; found, 329.0805.

Boc−*N*Me−Cys(Me)−OH via Change of Protecting Group in Solid Phase. *t*-Boc dicarbonate (0.22 g, 1.0 mmol) was dissolved in CH_2Cl_2 (0.5 mL), and DIEA (0.36 mL, 2.0 mmol) was added. The mixture was added to the resin-bound H−*N*Me−Cys(Me)−OH (0.2 g, 0.2 mmol). After 2 h, the De Clercq test was negative. The resin was washed with CH_2Cl_2 (3 × 0.5 min), MeOH (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min). The resin was cleaved with TFA−CH_2Cl_2 (1:49) (5 × 1 min), and the mixture was evaporated, lyophilized, and characterized as described earlier in the experimental section. A 46 mg portion of product was obtained (92% yield; 93% purity).

Fmoc−*N*Me−Cys(Me)−OH via Change of Protecting Group on Solid Phase. Fmoc−Cl (0.16 g, 0.6 mmol) was dissolved in CH_2Cl_2 (0.5 mL), and DIEA (0.36 mL, 2.0 mmol) was added. The mixture was added to the resin-bound H−*N*Me−Cys(Me)−OH (0.2 g, 0.2 mmol)). After 2 h, the De Clercq test was negative. The resin was washed with CH_2Cl_2 (3 × 0.5 min), MeOH (3 × 0.5 min), and CH_2Cl_2 (3 × 0.5 min). The resin was cleaved with TFA−CH_2Cl_2 (1:49) (5 × 1 min), and the mixture was evaporated, lyophilized, and characterized as described earlier in the experimental section. A 67 mg portion of product was obtained (90% yield; 95% purity).

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Supporting Information Available. General methods and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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