Efficient Synthesis of a Fluorescent Farnesylated Ras Peptide

Zuping Xia and Charles D. Smith*

Department of Pharmacology, Pennsylvania State University, Hershey, Pennsylvania 17033

cdsmith@psu.edu

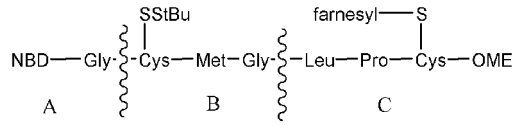
Received January 17, 2001

Introduction

The activities of several cellular proteins are dramatically altered by the covalent attachment of lipid, such as farnesyl, myristoyl, and palmitoyl moieties.[1] These lipidation modifications play crucial roles in signal transduction because they target specific proteins to the subcellular sites that are necessary for their activities. For example, newly translated Ras proteins are cytosolic, but rapidly undergo a series of modifications that result in their targeting to the inner surface of the plasma membrane. These modifications involve sequential farnesylation of a cysteine residue near the C-terminus, proteolytic removal of the terminal amino acid residues so that the farnesylated cysteine becomes the C-terminus, and carboxymethylation of the new C-terminus. Proteins encoded by the H-ras and N-ras genes, as well as several other proteins, are then modified through the covalent attachment of palmitate residues to cysteine residues. Unlike farnesylation and myristoylation, palmitoylation is a reversible reaction suggesting that it may be particularly important for regulating protein function.[2] In fact, this palmitoylation is required for transformation of cells by H-ras and so the enzyme that catalyzes this reaction is being explored as a target for the development of new anticancer drugs. We have recently described a series of alkyloxiranecarboxamides that inhibit protein palmitoylation;[3] however, further characterization of these compounds suffers from the lack of efficient in vitro and cellular assays for this palmitoyltransferase.

A fluorescently labeled farnesylated Ras peptide containing the proper unprotected cysteine residue may be a useful chemical tool for studying the palmitoylation of Ras proteins and to develop a high-throughput assay for palmitoyltransferase inhibitors. Although a significant amount of effort has been directed toward the preparation of prenylated peptides and peptide esters,[4] general methods for the synthesis of these compounds remain lacking. A labeled farnesylated peptide (**1**) has been previously demonstrated to undergo palmitoylation and redistribution of its subcellular localization in intact cells;[5] however, methods for the synthesis and the chemical characterization of this peptide were not complete. Therefore, we now report an efficient synthesis of 7-nitro-2,1,3-benzoxadiazolyl(NBD)-labeled farnesylated *N*-ras C-terminal heptapeptide [NBD-GCMGLPC(Far)-OMe (**1**)].

Scheme 1



Results and Discussion

It was noted that there are two chemically sensitive functional groups in the structure of the target peptide **1**. One is the farnesyl group that is readily removed under acidic conditions, requiring that all reactions after the farnesylated residue is generated be conducted in neutral or mildly basic conditions. The second sensitive group is the sulfhydryl group of the cysteine moiety that is easily oxidized by air or reacted with acylating agent. Hence, it must be masked during the synthesis and deprotected at the final stage to produce the site targeted by the palmitoyltransferase. Of the two common protecting groups for sulfhydryls, the acetamidomethyl group (Acm) and the *S*-*tert*-butylthio group (StBu), both are stable in acidic or basic conditions so that either can be used together with Fmoc protection or BOC protection during peptide synthesis. Additionally, each can be removed under neutral conditions. However, since Acm deprotection can be troublesome and incomplete,[6] protection by StBu appeared more attractive.

A segmental condensation strategy for the synthesis of cysteine-protected **1** is shown in Scheme 1. Because of the differential sensitivities of the necessary protecting groups and the thioether-linked farnesyl moiety, the peptide was synthesized as three separate segments and then coupled under conditions that did cause inappropriate cleavages. The individual reactions used for the formation of these segments and their linkage are described in Scheme 2.

The allyl ester strategy[7] was used to protect the C-terminal glycine in the synthesis of segment B. Glycine was reacted with allyl alcohol in the presence of toluenesulfonic acid to yield glycine allyl ester (**2**) in excellent yield (94%). Compound **2** was then condensed with BOC-methionine using DCC/HOBt to afford BOC-Met-Gly allyl ester (**3**) in excellent yield (98%). It is important to note that Fmoc-methionine could not be used at this point since the allyl group is not stable in basic conditions, so that it would be lost during the subsequent removal of Fmoc group. After deblocking the N-terminus of **3** using TFA, we could choose either Fmoc-(StBu)cysteine or BOC(Acm)cysteine for the next addition.

* To whom correspondence should be addressed. Phone: (717) 531-1672.

(1) (a) Dunphy, J. T.; Linder, M. E. *Biochim. Biophys. Acta* **1998**, *1436*, 245−261. 1 (b) Mundy, D. I. *Biochem. Soc. Trans.* **1995**, *23*, 572−576.

(2) (a) Milligan, G.; Parenti, M.; Magee, A. I. *Trends Biochem. Sci.* **1995**, *20*, 181−187. 2 (b) Mumby, S. M. *Curr. Opin. Cell Biol.* **1997**, *9*, 148−154. 2 (c) Veit, M.; Schmidt, M. F. G. *Methods Mol. Biol.* **1998**, *88*, 227−239.

(3) Lawrence, D. S.; Zilfou, J. T.; Smith, C. D. *J. Med. Chem.* **1999**, *42*, 4932−4941.

(4) (a) Naider, F. R.; Becker, J. M. *Biopolymers* **1997**, *43*, 3−14. 4 (b) Kadereit, D.; Kuhlmann, J.; Waldmann, H. *ChemBioChem* **2000**, *1*, 144−169.

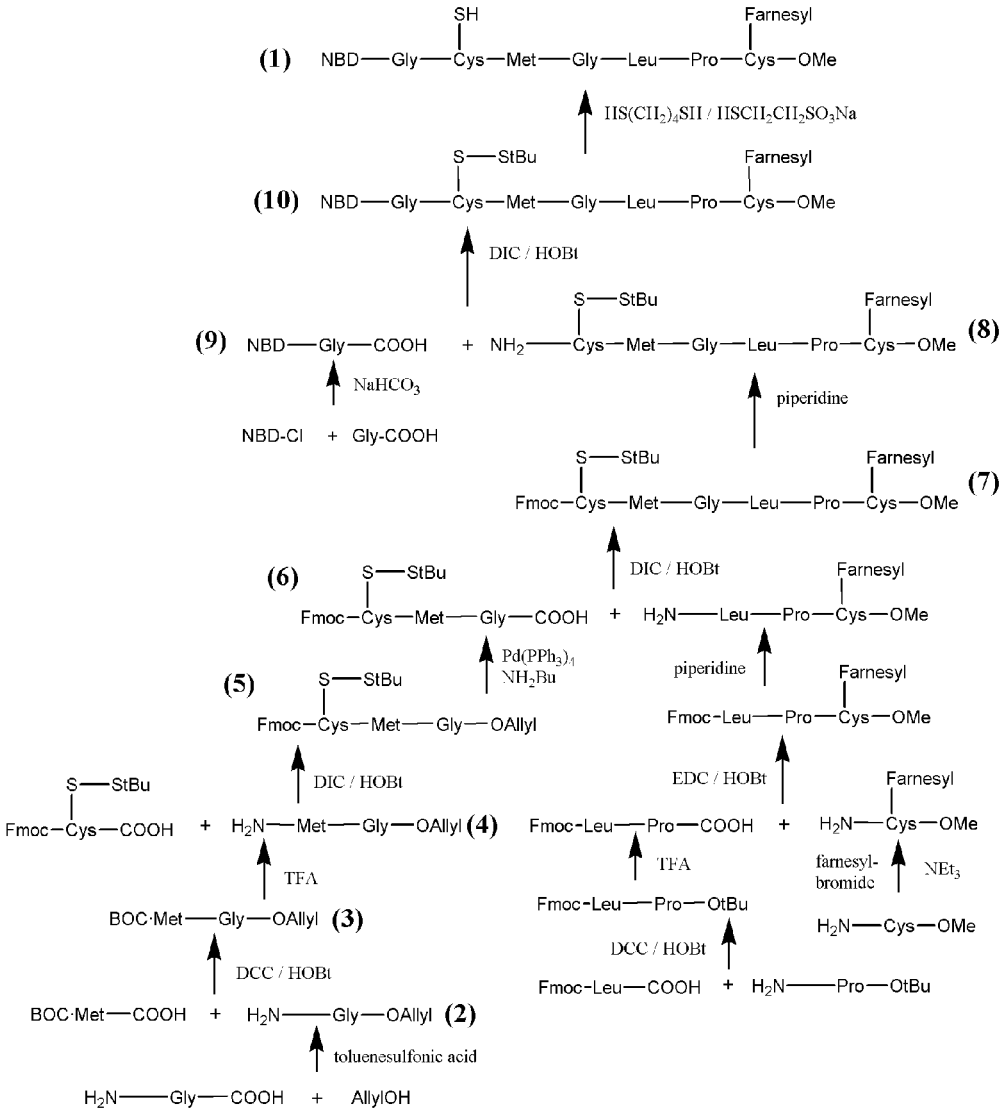
(5) Schroeder, H.; Leventis, R.; Rex, S.; Schelhaas, M.; Nägele, E.; Waldmann, H.; Silvius, J. R. *Biochemistry* **1997**, *36*, 13102−13109.

(6) Ramage, R.; Stewart, A. S. *J. Chem. Soc., Perkin. Trans. 1* **1993**, 1947−1993.

(7) (a) Kunz, H.; Waldmann, H.; Unverzagt, C. *J. Peptide Protein Res.* **1985**, *26*, 493−497. 7 (b) Schmittberger, T.; Waldmann, H. *Bioorg. Med. Chem.* **1999**, *7*, 749−762.

10.1021/jo015526w CCC: $20.00 © 2001 American Chemical Society
Published on Web 07/04/2001

Scheme 2



According to the design in Scheme 1, we would subsequently couple NBD-glycine to the peptide. Initially, we attempted to couple BOC-(Acm)cysteine to Met-Gly allyl ester so that the N-terminus of the tripeptide could be deblocked while the C-terminal allyl remained protected. However, this reaction was not satisfactory due to its difficult workup and low yield (19%). Consequently, an alternate strategy was pursued. By this route, we kept the N-terminus protected and deblocked the C-terminus of the tripeptide to couple it to segment C prior to the addition of segment A. This allowed the use of Fmoc-(StBu)cysteine instead of BOC-(Acm)cysteine since removal of the allyl group would not alter the N-terminal protection. Therefore, Fmoc-(StBu)cysteine was condensed with **4** using DIC/HOBt to obtain Fmoc-Cys-(StBu)-Met-Gly allyl ester (**5**) in good yield (65%). The allyl ester group was removed from the C-terminus using tetrakis(triphenylphosphine)palladium(0) in the presence of *n*-butylamine; however, cleavage under these conditions yielded only 50% of the expected product (**6**). This may be improved using other nucleophiles, such as *N*,*N*′-dimethylbarbituric acid as acceptors.[7b]

On the other hand, segment C (Leu-Pro-Cys(Far)-OMe) was smoothly synthesized as described in the literature.[8] Briefly, proline *tert*-butyl ester was condensed with Fmoc-leucine, followed by acid deprotection at the C-terminus to afford the dipeptide Fmoc-Leu-Pro-OH. Cysteine methyl ester was farnesylated using farnesyl bromide,[8] followed by coupling with Fmoc-Leu-Pro-OH to obtain a protected segment C. The Fmoc group at the N-terminus was then removed using piperidine to obtain segment C, which was then condensed with segment B using DIC/HOBt to yield Fmoc-C(StBu)MGLPC(Far)-OMe in excellent yield (97%). The N-terminus was then deblocked with piperidine and the peptide was coupled with NBD-glycine, which was synthesized using a modified literature method.[9] The final target peptide (**1**) was then obtained by removal of the StBu protecting group using 1,4-dithiobutane. The purity of the final product was at least 98% by HPLC analysis and its identity was confirmed by high-resolution mass spectroscopy.

Experimental Section

All reagents used were of reagent grade or better. Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were

(8) Strober, P.; Schelhaas, M.; Nagele, E.; Hagenbuch, P.; Retey, J.; Waldmann, H. *Bioorg. Med. Chem.* **1997**, *5*, 75−83.

(9) Ghosh, P. B.; Whitehouse, M. W. *J. Med. Chem.* **1968**, *11*, 305−311.

acquired using a Bruker AF-200 spectrometer. Chemical shifts were determined based on either an internal standard, tetramethylsilane, or deuterium solvent itself. High-resolution mass spectra were obtained from the Scripps Research Institute. The purity of the final product (**1**) was determined by HPLC [Beckman column (C18, 5 μm, 250 × 4.6 mm); UV 254 nm; 70% CH_3CN in water; 1 mL/min]. TLC analyses were conducted on Whatman PE SIL G/UV plates or Baker-flex silica gel IB-F plates. Anhydrous organic solvents were obtained from either Aldrich or by fresh distillation over the drying agents Na or CaH_2. Optical rotation was determined on a Polax-2L polarimeter. Silica gel chromatography was performed either on glass column or by radial chromatography on a Chromatotron. The synthesis of H-Leu-Pro-Cys(Far)-OMe was completed according to literature procedures (Strober, P. et al. *Bioorg. Med. Chem.* **1997**, *5*, 75−83).

Synthesis of NH_2-Gly-*O*-allyl Ester (2). A mixture of glycine (18.8 g, 0.25 mol), toluenesulfonic acid (48.5 g, 0.25 mol), and allyl alcohol (100 mL, 1.47 mol) in benzene (50 mL) was refluxed using a Dean−Stark trap. After ∼9 mL of water was collected, the reaction was stopped and the solvent was removed in vacuo. The residue was crystalized from ether (100 mL) to afford **2** (67.4 g, 94%) as a yellowish solid: ^{1}H NMR ($CDCl_3$) δ 8.02 (br s, 3 H), 7.69 (d, J = 8.0 Hz, 2 H), 7.08 (d, J = 8.0 Hz, 2 H), 5.75 (ddd, J = 22.4, 10.4, 5.7 Hz, 1 H), 5.22 (dd, J = 15.9, 1.2 Hz, 1 H), 5.15 (dd, J = 9.4, 1.2 Hz, 1 H), 4.48 (d, J = 5.7 Hz, 2 H), 3.71 (br d, J = 5.5 Hz, 2 H), 2.32 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 167.2, 140.7, 131.2, 129.0 (2 C), 126.1 (2 C), 119.0, 66.7, 40.6, 21.3.

Synthesis of BOC-Met-Gly-*O*-allyl Ester (3). *N*-Ethylmorpholine (1.28 mL, 0.010 mol) was added to a solution of BOC-Met (2.50 g, 0.010 mol), **2** (2.89 g, 0.010 mol), and HOBt (1.37 g, 0.010 mol) in anhydrous THF (35 mL). After the mixture was cooled to 0 °C, a solution of DCC (2.19 g, 0.011 mol) in anhydrous THF (5 mL) was added to it. The mixture was stirred at 0 °C for 1 h, followed by reaction at rt for 1 h. After filtration and removal of the solvent in vacuo, the residue was chromatographed on silica gel column with an elution gradient of 1−6% v/v MeOH in $CHCl_3$ to afford **3** (3.39 g, 98%) as a white solid: $[\alpha]_D$ = −37.8° (*c* 7.11, CH_3OH, 24 °C); R_f 0.56 (MeOH/$CHCl_3$ 3:97); ^{1}H NMR ($CDCl_3$) δ 5.91 (ddd, J = 22.8, 10.5, 5.8 Hz, 1 H), 5.34 (dd, J = 17.2, 1.2 Hz, 1 H), 5.27 (dd, J = 10.3, 1.2 Hz, 1 H), 4.65 (d, J = 5.8 Hz, 2 H), 4.38 (m, 1 H), 4.09 (d, J = 5.9 Hz, 1 H), 4.06 (d, J = 6.7 Hz, 1 H), 2.60 (t, J = 7.2 Hz, 2 H), 2.11 (s, 3 H), 1.98 (dt, J = 22, 7.2 Hz, 2 H), 1.44 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 172.0, 169.3, 155.6, 131.5, 119.0, 80.4, 66.0, 53.6, 41.3, 31.8, 30.2, 28.4 (3 C), 15.3.

Synthesis of NH_2-Met-Gly-O-allyl Ester (4). Trifluoroacitic acid (3.4 mL, 44.6 mmol) was dropwise added to a solution of **3** (1.05 g, 3.02 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The solution was then stirred at rt for 1 h, followed by addition of toluene (50 mL). After removal of the solvent in vacuo, **4** was obtained as a an oil (1.13 g, 104%), which was used immediately for the next reaction: $[\alpha]_D$ = 16.8° (*c* 2.17, $CHCl_3$, 23 °C); ^{1}H NMR ($CDCl_3$) δ 8.35 (br s, 2 H), 5.88 (ddd, J = 22.9 Hz, 10.4 Hz, 5.9 Hz, 1 H), 5.32 (dd, J = 17.1 Hz, 1.2 Hz, 1 H), 5.25 (dd, J = 10.6 Hz, 1.2 Hz, 1 H), 4.61 (d, J = 5.6 Hz, 2 H), 4.37 (m, 1 H), 3.90−4.20 (m, 2 H), 2.64 (t, J = 7.2 Hz, 2 H), 2.17 (m, 2 H), 2.06 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.5, 169.3, 131.4, 119.1, 66.4, 53.1, 41.5, 30.3, 29.1, 14.7.

Synthesis of Fmoc-Cys(StBu)-Met-Gly-*O*-allyl Ester (5). *N*-Ethylmorpholine (380 μL, 3.02 mmol) was added to a solution of **4** (1.05 g, 3.02 mmol) and HOBt (0.82 g, 6.04 mmol) in anhydrous THF (35 mL), followed by addition of a solution of Fmoc-Cys(StBu)-OH (1.3 g, 3.02 mmol) in anhydrous THF (5 mL). After the mixture was cooled to 0 °C, DIC (0.6 mL, 3.83 mmol) was added to the reaction mixture. The reaction was allowed to warm from 0 °C to rt and incubated for 24 h. After filtration and removal of the solvent in vacuo, the residue was dissolved in $CHCl_3$ (100 mL) and washed with 1 N HCl (40 mL), 5% $NaHCO_3$ (40 mL), and water (40 mL) sequentially. The $CHCl_3$ solution was dried over $MgSO_4$ and concentrated in vacuo, followed by chromatography on silica gel column with an elution gradient of 0−2% v/v MeOH in $CHCl_3$ to afford **5** (1.31 g, 65%) as a white solid: $[\alpha]_D$ = −35.1° (*c* 1.45, $CHCl_3$, 23 °C); R_f 0.38 (MeOH/$CHCl_3$ 2:98); ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 7.4 Hz, 2 H), 7.60 (d, J = 7.4 Hz, 2 H), 7.38 (dd, J = 7.4 Hz, 2 H), 7.28 (dd, J = 7.4 Hz, 2 H), 6.15 (d, J = 7.4 Hz, 1 H), 5.85 (ddd, J = 23.0, 10.4, 5.8 Hz, 1 H), 5.27 (dd, J = 17.2, 1.2 Hz, 1 H), 5.20 (dd, J = 9.8, 1.2 Hz, 1 H), 3.90−4.90 (m, 9 H), 3.12 (d,, J = 5.9 Hz, 2 H), 2.60 (t, J = 4.9 Hz, 2 H), 1.90−2.35 (m, 2 H), 2.06 (s, 3H), 1.32 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 171.1, 170.4, 169.2, 156.3, 143.9, 143.8, 141.4 (2 C), 131.6 C, 127.8 (2 C), 127.2 (2 C), 125.2 (2 C), 120.1 (2 C), 118.9, 67.8, 66.0, 55.1, 52.7, 48.6, 47.2, 42.3, 41.4, 31.0, 30.2, 29.9 (3 C), 15.3.

Synthesis of Fmoc-Cys(StBu)-Met-Gly-COOH (6). A solution of **5** (440 mg, 0.67 mmol) in THF (10 mL) was bubbled with argon for 5 min, and tetrakis(triphenylphosphine)palladium (0) (76 mg, 0.067 mmol) was added to the solution. After the mixture was stirred for 10 min, formic acid (40 μL, 1.06 mmol) and *n*-butylamine (66 μL, 0.67 mmol) were added, and the reaction was continued for 1 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (20 mL) and washed with 1 N HCl (6 mL) and saturated NaCl (6 mL). The EtOAc solution was dried over $MgSO_4$ and concentrated in vacuo. The residue was subjected to radial chromatography on silica with an elution gradient of 0−10% v/v MeOH in $CHCl_3$ to afford **6** (208 mg, 50%) as a yellow solid: $[\alpha]_D$ = −88.3° (*c* 1.28, CH_3OH, 24 °C); R_f 0.24 (MeOH/$CHCl_3$ 10:90); ^{1}H NMR (CD_3COCD_3) δ 7.85 (d, J = 7.5 Hz, 2 H), 7.72 (d, J = 7.2 Hz, 2 H), 7.41 (dd, J = 7.5 Hz, 7.2 Hz, 2 H), 7.32 (dd, J = 7.2 Hz, 2 H), 3.0−5.0 (m, 9 H), 2.58 (t, J = 7.0 Hz, 2 H), 2.02 (s, 3H), 1.90−2.20 (m, 2H), 1.32 (s, 9 H); ^{13}C NMR ($CDCl_3$) δ 172.0, 171.0, 170.9, 157.0, 144.8 (2 C), 142.0 (2 C), 128.4 (2 C), 127.9 (2 C), 126.0 (2 C), 120.6 (2 C), 67.6, 55.8, 53.2, 48.3, 47.9, 43.3, 41.3, 32.7, 30.6, 30.2 (3 C), 15.2.

Synthesis of Fmoc-Cys(StBu)-Met-Gly-Leu-Pro-Cys-(Far)-OMe (7). DIC (40 μL, 0.26 mmol) was added to a solution of **6** (128 mg, 0.21 mmol) and HOBt (57 mg, 0.42 mmol) in anhydrous THF (5 mL) at 0 °C. The solution was stirred at 0 °C for 1 h, followed by the addition of a solution of H-Leu-Pro-Cys-(Far)-OMe (113 mg, 0.21 mmol) in anhydrous THF (6 mL). The reaction was allowed to warm from 0 °C to rt and incubated for 24 h. After removal of the solvent in vacuo, the residue was subjected to radial chromatography on silica eluted with 3% MeOH in $CHCl_3$ to afford **7** (236 mg, 97%) as a yellowish foam: $[\alpha]_D$ = −41.2° (*c* 2.30, $CHCl_3$, 23 °C); R_f 0.30 (MeOH/$CHCl_3$ 5:95); ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 7.1 Hz, 2 H), 7.64 (d, J = 6.9 Hz, 2 H), 7.39 (dd, J = 7.5, 7.1 Hz, 2 H), 7.28 (dd, J = 7.5, 6.9 Hz, 2 H), 3.60−5.30 (m, 15 H), 3.59 (s, 3 H), 2.45−3.30 (m, 8 H), 2.06 (s, 3 H), 1.67 (s, 6H), 1.60 (s, 6 H), 1.24 (s, 9 H), 1.13 (d, J = 6.4 Hz, 6 H), 0.80−2.30 (m, 17 H); ^{13}C NMR ($CDCl_3$) δ 171.8, 171.6, 171.4, 171.3, 170.2, 168.2, 156.5, 144.0, 143.9, 141.4 (2 C), 139.8, 135.0, 131.3, 127.8 (2 C), 127.2 (2 C), 125.4 (2 C), 124.4, 123.9, 120.0 (2 C), 119.9, 67.6, 59.8, 54.6, 52.3, 52.2, 49.1, 48.1, 47.6, 47.2, 44.3, 43.5, 42.5, 42.2, 39.8 (2 C), 32.9, 31.9, 30.2, 29.9 (3 C), 29.7, 28.8, 26.8, 26.7, 25.7, 24.9, 24.7, 23.6, 22.1, 17.7, 16.2, 16.1, 15.3.

Synthesis of NH_2-Cys(StBu)-Met-Gly-Leu-Pro-Cys(Far)-OMe (8). Piperidine (1 mL, 10.1 mmol) was added to a solution of **7** (105 mg, 0.091 mmol) in THF (5 mL), and the solution was stirred at rt for 1 h. After removal of the solvent in vacuo, the residue was subjected to radial chromatography on silica with an elution gradient of 3−10% v/v MeOH in $CHCl_3$ to afford **8** (71 mg, 84%) as a semisolid: $[\alpha]_D$ = −83.0° (*c* 0.89, MeOH, 23 °C); R_f 0.38 (MeOH/$CHCl_3$ 10:90); ^{1}H NMR ($CDCl_3$) δ 4.50−5.30 (m, 8 H), 3.74 (s, 3 H), 3.50−3.90 (m, 4 H), 1.80−3.30 (m, 26 H), 1.68 (s, 6H), 1.60 (s, 6 H), 1.40−1.60 (m, 2 H), 1.34 (s, 9 H), 0.96 (d, J = 4.8 Hz, 3 H), 0.93 (d, J = 5.2 Hz, 3 H); ^{13}C NMR ($CDCl_3$) δ 173.2, 171.5, 170.5 (2 C), 170.3, 167.7, 139.1, 134.5, 130.4, 123.5, 122.9, 118.8, 58.9, 53.6, 51.5, 51.2, 51.1, 48.2, 47.5, 46.5, 44.3, 42.2, 40.9, 38.8 (2 C), 32.2, 30.3, 29.4, 29.0 (3 C), 28.8, 27.2, 25.9, 25.7, 24.8, 24.0, 23.8, 22.5, 21.0, 16.8, 15.3, 15.1, 14.5; HRMS (MALDI) m/z calcd for $C_{44}H_{76}N_6O_7S_4Na$ (M + Na) 951.4551, found 951.4588.

Synthesis of NBD-Gly-COOH (9). A solution of NBD-Cl (303 mg, 1.52 mmol) in MeOH (24 mL) was added dropwise to a solution of glycine (125 mg, 1.67 mmol) and $NaHCO_3$ (417 mg, 4.96 mmol) in water (6 mL) at 55 °C and incubated for 1 h. The reaction flask was covered with aluminum foil, and all subsequent experiments involving the fluorescent compound were performed under dim light. After removal of the MeOH in vacuo, the aqueous solution was applied to a Sephadex C25 column and eluted with water. The eluant was monitored by TLC, with fluorescence visualization, until **9** was collected. The solution

was acidified to pH 1.5 using 1 N HCl, and lyophilized. The dried residue was dissolved in acetone, filtered, and the solvent was removed in vacuo to afford crude **9** (367 mg, 101%) as an orange solid, which was immediately used for the next reaction: ^{1}H NMR (CD_3COCD_3) δ 8.58 (d, J = 8.6 Hz, 1 H), 6.51 (d, J = 8.6 Hz, 1 H), 4.53 (s, 2 H).

Synthesis of NBD-Gly-Cys(StBu)-Met-Gly-Leu-Pro-Cys-(Far)-OMe (10). DIC (28 μL, 0.18 mmol) was added to a solution of **9** (36 mg, 0.15 mmol) and HOBt (41 mg, 0.40 mmol) in anhydrous THF (5 mL) at 0 °C in a foil-covered reaction flask. The solution was stirred for 1 h at 0 °C, followed by the addition of a solution of **8** (70 mg, 0.075 mmol). The reaction was allowed to warm from 0 °C to rt and incubated for 24 h. After removal of the solvent in vacuo, the residue was dissolved in EtOAc (60 mL) and washed with 1 N HCl (20 mL), 5% $NaHCO_3$ (20 mL), and water (20 mL) sequentially. The EtOAc solution was dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to radial chromatography on silica with an elution gradient of 1−5% v/v MeOH in $CHCl_3$ to afford **10** (55 mg, 64%) as an orange solid: $[\alpha]_D = -100°$ (c 0.55, $CHCl_3$, 22 °C); R_f 0.51 (MeOH/$CHCl_3$ 10:90); ^{1}H NMR (CD_3OD) δ 8.48 (d, J = 8.6 Hz, 1 H), 6.31 (d, J = 8.6 Hz, 1 H), 4.20−5.25 (m, 10 H), 3.71 (s, 3 H), 3.50−4.10 (m, 4 H), 1.80−3.35 (m, 26 H), 1.65 (s, 6H), 1.59 (s, 6 H), 1.43 (m, 2 H), 1.30 (s, 9 H), 0.96 (d, J = 5.0 Hz, 6 H); ^{13}C NMR (CD_3OD) δ 174.0, 173.5 (2 C), 172.6, 172.4, 171.2, 170.5, 146.3, 140.7, 138.0 (2 C), 136.3, 132.1, 125.4, 125.1, 121.3 (2 C), 101.5, 61.5, 54.8, 54.5, 53.8, 52.9, 51.0, 48.6, 47.4, 43.5, 43.1, 41.4, 40.8, 40.6, 33.6, 31.4 (2 C), 30.6, 30.2 (3 C), 30.1, 28.7, 27.8, 27.4, 25.8 (2 C), 23.7, 22.0, 17.7, 16.3, 16.2, 15.2; HRMS (MALDI) m/z calcd for $C_{44}H_{76}N_6O_7S_4Na$ (M + Na) 951.4551, found 951.4588.

Synthesis of NBD-Gly-Cys-Met-Gly-Leu-Pro-Cys(Far)-OMe (1). Under a N_2 atmosphere in a glovebox, **10** (55 mg, 0.048 mmol) was dissolved in a mixed solution of DMF/0.5 M HEPES pH = 7.7 (3:1) (5 mL), followed by the addition of 1,4-dithiolbutane (50 μL, 0.43 mmol) and 2-mercaptoethanesulfonic acid sodium salt (MES, 91 mg, 0.56 mmol). The solution was stirred at 40 °C for 24 h, and then the solvent was removed by flushing with N_2 and the residue was dissolved in 0.1 M MES (50 mL). The MES solution was extracted with $CHCl_3$ (6 × 25 mL), and the $CHCl_3$ was collected and removed by flushing with N_2 to afford **1** (35 mg, 69%) as an orange solid: purity 98%; $[\alpha]_D = -93.5°$ (c 0.34, MeOH, 26 °C); R_f 0.47 (MeOH/$CHCl_3$ 10:90); ^{1}H NMR (CD_3OD) δ 8.51 (d, J = 8.6 Hz, 1 H), 6.32 (d, J = 8.6 Hz, 1 H), 4.20−5.30 (m, 10 H), 3.71 (s, 3 H), 3.45−4.10 (m, 4 H), 1.80−3.40 (m, 26 H), 1.65 (s, 6H), 1.58 (s, 6 H), 1.43 (m, 2 H), 0.95 (d, J = 4.8 Hz, 6 H); ^{13}C NMR (CD_3OD) δ 174.2, 173.8, 173.6, 172.7, 172.5, 171.3, 170.5, 140.8, 138.1 (2 C), 136.4, 132.2, 125.5, 125.2, 121.5, 121.4, 101.5, 61.6, 57.4, 54.6, 53.9, 53.0, 51.1, 48.6, 47.3, 43.6 (2 C), 41.5, 40.9, 40.8, 33.6, 31.6, 31.4, 30.6, 30.2, 27.9, 27.5, 26.9, 25.9 (2 C), 23.8, 22.1, 17.8, 16.4, 16.2, 15.3; HRMS (MALDI) m/z calcd for $C_{48}H_{72}N_{10}O_{11}S_3Na$ (M + Na) 1083.4436, found 1083.4410.

Acknowledgment. This work was supported by NIH Grant No. R01 CA 75248 to C.D.S.

JO015526W