Synthesis of the Marine Sponge Cycloheptapeptide Stylopeptide 1[1]

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The solution-phase synthesis of the marine sponge stylopeptide 1 (**2**), cyclo-(Leu-Ile-Phe-Ser-Pro-Ile-Pro), was conveniently accomplished using *N*-terminal Fmoc- and *C*-terminal *tert*-butyl ester protection with *tert*-butyl ether side-chain blocking for serine. Peptide bond formation for each step except for the final cyclization was effected with diethyl phosphorocyanidate to give the linear heptapeptide in 19% yield. Both TBTU (**4**) or BOP-Cl (**5**) were used to cyclize the heptapeptide and resulted in 67% and 13% yields of stylopeptide 1 (**2**), respectively. Peptide **2** was obtained in 11% overall yield based on the TBTU cyclization procedure. The general approach represents a useful improvement in the synthesis of such cyclic peptides. The synthetic stylopeptide 1 (**2**) proved to be identical with the natural product.

In 1983, we located the orange Indo-Pacific sponge *Stylotella aurantium* northwest of New Ireland (PNG) in the Bismark Archipelago. Subsequently, we isolated the cycloheptapeptides designated stylostatin 1 (**1**)[2a] and stylopeptide 1 (**2**)[2b] and confirmed our initial structural assignments by X-ray crystal structure determinations. Stylopeptide 1 was also isolated from the Federated States of Micronesia (Chuuk) marine sponge *Phakellia costata*.[2c] In both sponges stylopeptide 1 was essentially a trace (~10^{-5}% yields) constituent. While the specimens of stylopeptide 1 from the two different sponge sources appeared quite pure by TLC, HPLC, mp, and high field (400 MHz) ^{1}H-NMR, their ability to inhibit growth of the P388 lymphocytic leukemia cell line differed by more than ten fold (ED_{50} ~10 *vs* 0.1 μg/mL, respectively) Such observations suggested the more cell growth inhibitory specimens of stylopeptide 1 from *P. costata* might be transporting (by complex or other means), or simply contaminated by one or more of the extraordinarily active halistatin-type[3ab] (*cf.*, **3**) antineoplastic agents in a trace amount only detectable by biological means. To resolve this cell growth inhibitory dilemma and to provide a larger supply of stylopeptide 1 (**2**) for additional biological evaluation, its total synthesis was achieved as described in the sequel.

A sequential amino acid addition and *N*-Fmoc/*tert*-butyl protection strategy[4] (Scheme 1) was employed to obtain stylopeptide 1 (**2**) that was similar to one we used recently to obtain axinastatins 2 and 3.[5] The *tert*-butyl esters[6] for *C*-terminal protection were selected because they do not readily undergo nucleophilic attack and are therefore useful to minimize diketopiperazine formation.[4b−d] For *N*-terminal blocking the 9-fluorenylmethoxycarbonyl (Fmoc) group[7abc] was chosen as cleavage[4b,c] is easily accomplished with diethylamine. Peptide bond formation was conducted in dichloromethane (DCM) employing diethyl phosphorocyanidate (DEPC)[8] with diisopropylethylamine (DIEA) as base. The solvent selection was based on studies that indicated less epimerization[9] than with DMF.[10] When DEPC was employed in the final peptide cyclization step, the yield of stylopeptide 1 (**2**) was barely detectable. Interestingly, the related azide DPPA has been used in peptide cyclization reactions with varying degrees of success.[11,12a] When TBTU[12ab,13] (**4**)/DIEA in DMF and BOP-Cl[14] (**5**)/DIEA in DCM were employed, moderate yields (21% and 13%, respectively) of stylopeptide 1 (**2**) were realized. Maximum yield (67%) of the cyclic peptide **2** was obtained using TBTU/DIEA in DCM. The initial low yields of stylopeptide 1 employing DEPC for the cyclization step

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(2) (a) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Erickson, K. L.; Doubek, D. L.; Schmidt, J. M.; Tackett, L. P.; Bakus, G. J. *J. Org. Chem.* **1992**, *57*, 7217. (b) Pettit, G. R.; Srirangam, J. K.; Herald, D. L.; Xu, J.-P.; Boyd, M. R.; Cichacz, Z.; Kamano, Y.; Schmidt, J. M.; Erickson, K. L. *J. Org. Chem.* **1995**, *60*, 8257. (c) Unpublished results.

(3) (a) Pettit, G. R.; Tan, R.; Gao, F.; Williams, M. D.; Doubek, D. L.; Boyd, M. R.; Schmidt, J. M.; Chapuis, J.-C.; Hamel, E.; Bai, R.; Hooper, J. N. A.; Tackett, L. P. *J. Org. Chem.* **1993**, *58*, 2538. (b) Pettit, G. R.; Gao, F.; Doubek, D. L.; Boyd, M. R.; Hamel, E.; Bai, R.; Schmidt, J. M.; Tackett, L. P.; Rützler, K. *Gazz. Chim. Ital.* **1993**, *123*, 371.

(4) (a) Perich, J. W.; Johns, R. B. *Aust. J. Chem.* **1990**, *43*, 1623; Riniker, B.; Flörsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307. (b) For an exception see Fischer, P. M.; Solbakken, M.; Undheim, K. *Tetrahedron*, **1994**, *50*, 2277. (c) Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350. (d) Høeg-Jensen, T.; Jakobsen, M. H.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 6387. (e) Paulsen, H.; Adermann, K. *Liebigs Ann. Chem.*, **1989**, 751 and 771.

(5) Pettit, G. R.; Holman, J. W.; Boland, G. M. *J. Chem. Soc., Perkin Trans. 1,* submitted.

(6) Adamson, J. G.; Blaskovich, M. A.; Groenevelt, H.; Lajoie, G. A. *J. Org. Chem.* **1991**, *56*, 3447. We obtained lower yields with this procedure using excess *p*-toluenesulfonic acid compared to a catalytic amount of sulfuric acid. Costopanagiotis, A. A.; Preston, J.; Weinstein, B. *J. Org. Chem.* **1966**, *31*, 3398. Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251. Anderson, G. W.; Callahan, F. M. *J. Am. Chem. Soc.* **1960**, *82*, 3359.

(7) (a) For a review see: Fields, G. B.; Noble, R. L. *Int. J. Pept. Protein Res.* **1990**, *35*, 161. (b) Chang, C.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E. O.; Haug, J. D. *Int. J. Pept. Protein Res.* **1980**, *15*, 59. (c) Bodanszky, M.; Deshmane, S. S.; Martinez, J. *J. Org. Chem.* **1979**, *44*, 1622.

(8) Hamada, Y.; Rishi, S.; Shioiri, T.; Yamada, S.-I. *Chem. Pharm. Bull.* **1977**, *25*, 224. DEPC is also called diethylphosphoryl cyanide or diethyl cyanophosphonate.

(9) Benoiton, N. L. *Int. J. Pept. Protein Res.* **1994**, *44*, 399.

(10) Haver, A. C.; Smith, D. D. *Tetrahedron Lett.* **1993**, *34*, 2239. Miyazawa, T.; Otomatsu, T.; Yamada, T.; Kuwata, S. *Int. J. Pept. Protein Res.* **1992**, *39*, 229. Benoiton, N. L.; Kuroda, K. *Int. J. Pept. Protein Res.* **1981**, *17*, 197.

(11) Schmidt, U.; Langner, J. *J. Chem. Soc., Chem. Commun.* **1994**, 2381.

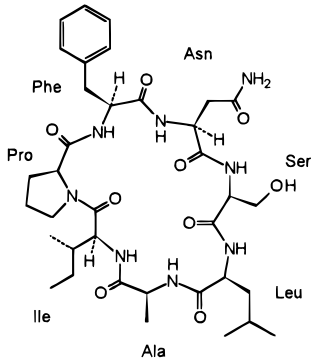
(12) (a) Zimmer, S.; Hoffmann, E.; Jung, G.; Kessler, H. *Liebigs Ann. Chem.* **1993**, 497. (b) Trzeciak, A.; Bannwarth, W. *Tetrahedron Lett.* **1992**, *33*, 4557.

(13) Ehrlich, A.; Rothemund, S.; Brudel, M.; Beyermann, M.; Carpino, L. A.; Bienert, M. *Tetrahedron Lett.* **1993**, *34*, 4781.

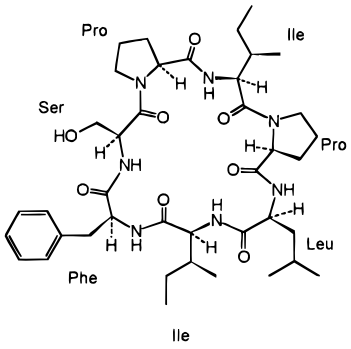
(14) Suggested by Prof. R. B. Bates, University of Arizona. Also designated *N,N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride.

0022-3263/96/1961-2322$12.00/0

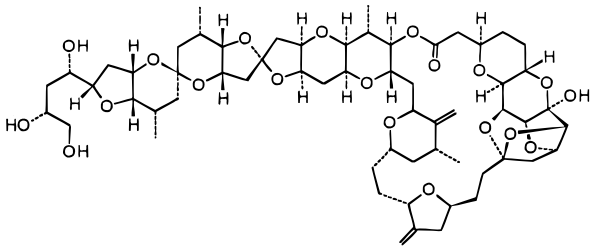
Chart 1



1, Stylostatin 1

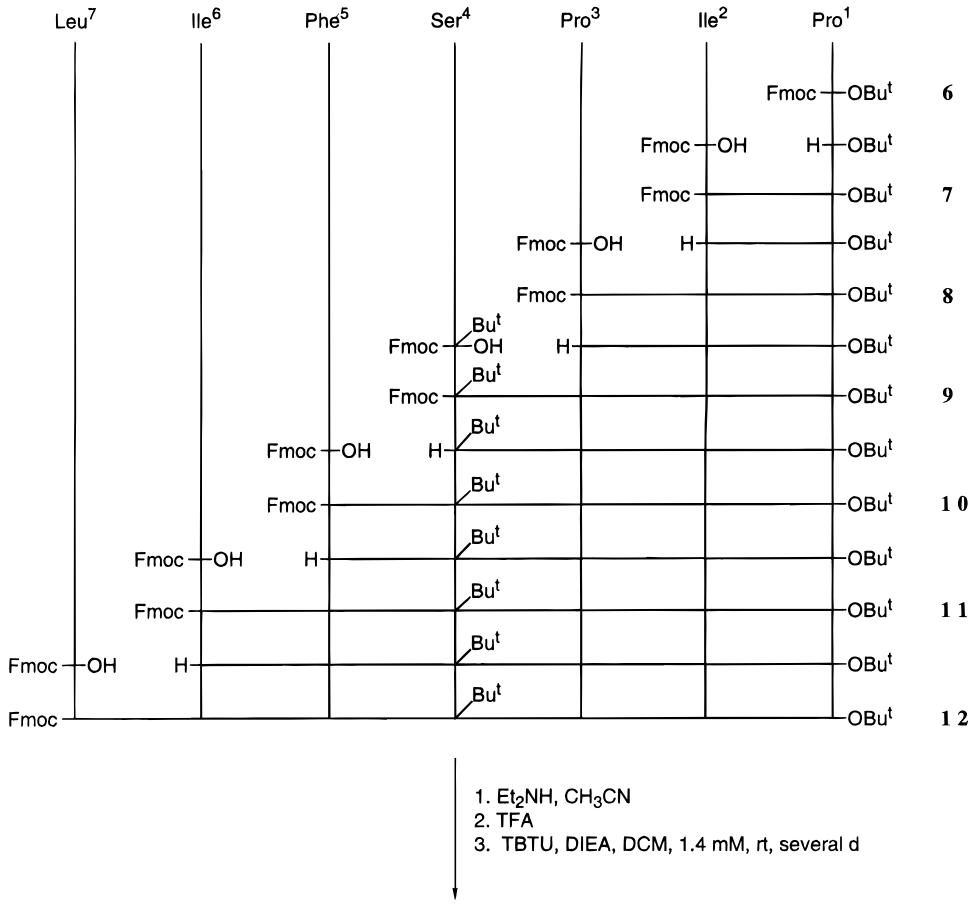


2, Stylopeptide 1



3, Halistatin 1

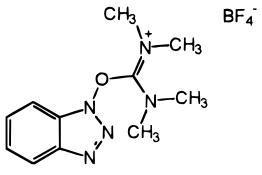
Scheme 1



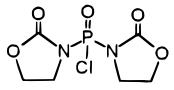
cyclo-(Leu^7-Ile^6-Phe^5-Ser^4-Pro^3-Ile^2-Pro^1)

2

may have been due in part to the unblocked serine hydroxyl group, though it is usually not necessary to protect this hydroxyl group.[15] Fortunately, the problem with DEPC was nicely avoided using TBTU (**4**).



4



5

The synthetic stylopeptide 1 (**2**) was found to be identical with the natural product by comparing mixture mp, TLC, $[\alpha]_D$, IR, and high field (400 MHz) ^{1}H NMR data. Our earlier concern about the possibility of the natural stylopeptide 1 serving as a carrier for an undetected (by physical and chemical means) but powerful halistatin-like (*cf.*, **3**) component was amply realized by results of careful evaluation of the synthetic cyclic peptide against the murine P388 lymphocytic leukemia cell line and a selection of human cancer cell lines. In each case

(15) Burke, T. R., Jr.; Knight, M.; Chandrasekhar, B.; Ferretti, J. A. *Tetrahedron Lett.* **1989**, *30*, 519. Fischer, P. M.; Retson, K. V.; Tyler, M. I.; Howden, M. E. H. *Int. J. Pept. Protein Res.* **1991**, *38*, 491. Barbato, G.; D'Auria, G.; Paolillo, L.; Zanotti, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 388.

the synthetic peptide (**2**) proved to be inactive while the natural specimens from *P. costata* maintained their activity suggesting the presence of this unknown active constituent. Since we have recently discovered an analogous situation among some of our other marine Porifera cyclic peptides,[5] it is now clear that such cyclic peptides are capable of associating with certain strongly active polyether-type antineoplastic agents that are only detectable by biological methods. In short, the results of these experiments should serve as a useful caution signal when evaluating such sponge peptides for cancer cell growth inhibitory properties.

Experimental Section

General. Except for acetonitrile (HPLC grade, EM Science) and DMF (anhyd, Aldrich), all solvents were redistilled. All the amino acids corresponded to the L-configuration, and coupling reactions were conducted under argon. The *N*-Fmoc-L-amino acids (Sigma-Aldrich), *N*,*N*-bis(2-oxo-3-oxazolidinyl)-phosphorodiamidic choride (BOP-Cl, **5**, TCI America Co.),[14] and *p*-toluenesulfonic acid monohydrate (TsOH·H_2O, Matheson, Coleman & Bell) were used as received. Diethyl phosphorocyanidate (DEPC, 93%),[8] diisopropylethylamine (DIEA), diethylamine (Et_2NH), and *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, **4**),[12,13] and trifluoroacetic acid (TFA) were used as received from Sigma-Aldrich Co. Thin layer chromatography was performed using silica gel GHLF Uniplates (Analtech), and the plates were visualized by UV light and/or ceric sulfate−sulfuric acid (by heating for 2−3 min). Column chromatography employed 230−400 mesh, 0.040−0.063 mm silica gel 60 (Merck). Melting points are uncorrected. Optical rotation data were collected using a 1 mL 1-dm cell at the sodium D line (589 nm and temperatures noted). The ^{1}H NMR δ values are relative to TMS in $CDCl_3$ (except for stylopeptide 1 which are relative to DMSO-d_6 at δ 2.49) and relative to $CDCl_3$ (δ 77.0) for ^{13}C NMR spectra. *J* values are in Hz. Analytical samples were dried *in vacuo* (Abderhalden over P_2O_5 at methanol or water reflux temperatures for several h). Elemental analyses were completed by Galbraith Laboratories, Inc. (Knoxville, TN).

General Deprotection Procedure.[4b,c,7b] A solution of the *N*-Fmoc *tert*-butyl ester in 1:1 CH_3CN/Et_2NH (∼0.2 M) was stirred at rt for 2 h. After several minutes, deprotection was observed to be complete (by TLC), but longer times were used to insure maximum deprotection. Reaction with ninhydrin by mild heating of the TLC plate indicated the free amino *tert*-butyl ester near the origin. Solvents were removed by rotary evaporation to yield viscous amber syrups. Further removal of trace solvent sometimes was done *in vacuo* (typically ∼0.3 torr, 30 min). The crude free amino *tert*-butyl ester was used immediately without further purification.

Amino Acid Coupling Procedure.[7b,8] A solution of the crude amino *tert*-butyl ester in DCM (∼0.5 M) containing DIEA (1 equiv) at rt was transferred slowly under positive argon pressure *via* cannula to a solution of the *N*-Fmoc-amino acid (1 equiv) and DEPC (1 equiv) in DCM (∼0.3 M) at −10 °C. (The calculated volume of 93% pure DEPC was added to give 1 equiv). Transfer was completed *via* cannula with small portions of DCM, so the final concentration with respect to the amino *tert*-butyl ester was ∼0.1−0.2 M. The reaction mixture was stirred under argon for 3−4 h between −10 and −5 °C. TLC monitoring showed dibenzofulvene (DBF) byproduct(s) and the desired *N*-Fmoc *tert*-butyl ester. If the order of addition was reversed (Fmoc-amino acid/DEPC added to the amino *tert*-butyl ester/DIEA mixture), the yields decreased. When the DEPC coupling (in DCM or DMF/DCM) was performed with anhydrous sodium carbonate[5] as base, the reverse order of addition was used. In general, the DEPC/DIEA method was judged to be more convenient.

Purification of the Peptides 7−12. After concentrating the reaction mixture, the crude product could be directly chromatographed quite effectively without an aqueous washing procedure.[8] The crude *N*-Fmoc *tert*-butyl ester-protected peptide was subjected to medium pressure silica gel chromatography.[16] To avoid clogging,[4c] filtration was sometimes required to remove insoluble byproducts (formed by allowing the oily residue to stand for several h) prior to column chromatography. Multiple precipitations/crystallizations of the product gave colorless powders (except for the di-, tri, and tetrapeptides which were foams) which were sufficiently pure for the next coupling reaction. During the process of multiple precipitations/crystallizations from hot solutions, filtration was sometimes required to remove insoluble material (polymer?) which probably formed from DBF.

***N*-Fmoc-Ile-Pro-OBu^t (7).** Using the above general procedures *N*-Fmoc-Pro-OBu^t (**6**, 3.00 g, 7.62 mmol)[5,6] was *N*-deprotected and coupled with *N*-Fmoc-Ile. The concentrated residue was subjected to silica gel chromatography (2:1 hexane/EtOAc, column: 50 mm x 6.5 in silica depth, flow: ∼1 in./90 s) to give 2.76 g (72% yield) of a foamy solid. Analytical purity was achieved by additional chromatography: R_f 0.53 (1:1 hexane/EtOAc); $[\alpha]^{28}_D$ −57.5° (*c* 1.275, $CHC1_3$); UV (nm, log ϵ): 235, 3.8; 267, 4.3; 289, 3.7; 300, 3.8; EIMS *m*/*z* calcd for $C_{30}H_{38}N_2O_5$ 506, found 506. Anal. Calcd for $C_{30}H_{38}N_2O_5$ (506.64): 71.12 C, 7.56 H, 5.53 N. Found: 71.26 C, 7.39 H, 5.20 N.

***N*-Fmoc-Pro-Ile-Pro-OBu^t (8).** Using the above general procedures *N*-Fmoc-Ile-Pro-OBu^t (**7**, 6.90 g, 13.62 mmol) was *N*-deprotected and coupled with *N*-Fmoc-Pro. The concentrated oil was purified by silica gel chromatography (4:1 EtOAc/hexane, column: 90 mm x 7.5 in silica depth, flow; ∼1 in./6 min = 23 mL/min to yield 7.6 g of a foam. Rechromatography gave 6.28 g (76% yield) of a colorless foam; mp 85−89 °C; R_f 0.45 (EtOAc); $[\alpha]^{23}_D$ −105° (*c* 0.11, $CHC1_3$); UV (nm, log ϵ) 235, 3.8; 267, 4.2; 289, 3.7; 300, 3.8; FABHRMS *m*/*z* calcd for $C_{35}H_{46}N_3O_6$ 604.3386, found 604.3372 $[M + H]^+$. Anal. Calcd for $C_{35}H_{45}N_3O_6 \cdot H_2O$ (621.77): 67.61 C, 7.62 H, 6.76 N. Found: 67.72 C, 7.72 H, 6.43 N.

***N*-Fmoc-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (9).** Using the above general procedures *N*-Fmoc-Pro-Ile-Pro-OBu^t (**8**, 6.66 g, 11.03 mmol) was *N*-deprotected and coupled with *N*-Fmoc-Ser(Bu^t). After concentration, silica gel chromatography (4:1 EtOAc/hexane, column: 90 mm x 8 in silica depth, flow: 23 mL/70 s) was used to afford 6.77 g (80% yield) of a foam. Analytical purity was achieved by additional chromatography: R_f 0.22−0.30 (4:1 EtOAc/hexane); $[\alpha]^{24.5}_D$ −72° (*c* 3.24, $CHC1_3$); FABHRMS *m*/*z* calcd for $C_{42}H_{59}N_4O_8$ 747.4333, found 747.4314 $[M + H]^+$. Anal. Calcd for $C_{42}H_{58}N_4O_8 \cdot H_2O$ (764.96): 65.95 C, 7.91 H, 7.32 N. Found: 66.10 C, 7.90 H, 7.29 N.

***N*-Fmoc-Phe-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (10).** Using the above general procedures *N*-Fmoc-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (**9**, 6.67 g, 8.93 mmol) was *N*-deprotected and coupled with *N*-Fmoc-Phe. The reaction became homogeneous as the reaction progressed. Silica gel chromatography (4:1 EtOAc/hexane, column: 90 mm x 6.5 in silica depth, flow: 23 mL/90 s) afforded 7.18 g (90% yield) of a powder. A pure sample was obtained by additional chromatography and precipitation: mp 114−119 °C; R_f 0.21−0.30 (4:1 EtOAc/hexane); $[\alpha]^{25}_D$ −78° (*c* 0.54, $CHC1_3$); FABHRMS *m*/*z* calcd for $C_{51}H_{68}N_5O_9$ 894.5017, found 894.5017 $[M + H]^+$. Anal. Calcd for $C_{51}H_{67}N_5O_9$ (894.12); 68.51 C, 7.55 H, 7.83 N. Found: 68.48 C, 7.68 H, 7.60 N.

***N*-Fmoc-Ile-Phe-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (11).** Using the above general procedures *N*-Fmoc-Phe-Ser-(Bu^t)-Pro-Ile-Pro-OBu^t (**10**, 7.25 g, 8.11 mmol) was *N* deprotected and coupled with *N*-Fmoc-Ile. Homogeneity of the mixture was observed as the reaction progressed. Silica gel chromatography (EtOAc, column: 90 mm x 6 in silica depth, flow: 23 mL/105 s) gave 5.34 g (65% yield) of a powder from EtOAc/hexane. Additional chromatography and precipitation provided an analytical sample: mp 154−159 °C; R_f 0.22 (10:1 EtOAc/hexane, 0.15 (4:1 EtOAc/hexane); $[\alpha]^{26}_D$ −68° (*c* 0.26, $CHC1_3$); IR (NaC1 thin film) ν_{max} (cm^{-1}): 3293 (s), 3065 (w), 2969 (s), 2936 (m), 2878 (m), 1724 (m), 1628 (s), 1535 (s), 1449 (s), 1391 (w), 1366 (m), 1234 (m), 1196 (w), 1155 (m), 1097 (w), 1034 (w), 758 (m), 743 (m); FABHRMS *m*/*z* calcd for $C_{57}H_{79}N_6O_{10}$

(16) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

1007.5857, found 1007.5871 $[M + H]^+$. Anal. Calcd for $C_{57}H_{78}N_6O_{10}$ (1007.28): 67.97 C, 7.81 H, 8.34 N. Found: 67.51 C, 7.83 H, 8.24 N.

***N*-Fmoc-Leu-Ile-Phe-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (12).** Using the above general procedures *N*-Fmoc-Ile-Phe-Ser(Bu^t)-Pro-Ile-Pro-OBu^t (**11**, 5.47 g, 5.43 mmol) was *N*-deprotected and coupled with *N*-Fmoc-Leu. Silica gel column chromatography (EtOAc, column: 90 mm x 6.5 in silica depth, flow: 23 mL/75 s) provided 4.59 g (76% yield) of a colorless powder. Additional chromatography and precipitation afforded a pure sample: R_f 0.32 (EtOAc); $[\alpha]^{26}_D$ −76° (*c* 0.55, $CHC1_3$); UV (nm, log ϵ): 236, 3.8; 266, 4.2; 289, 3.7; 300, 3.8; FABHRMS *m*/*z* calcd for $C_{63}H_{90}N_7O_{11}$ 1120.6698, found 1120.6721 $[M + H]^+$. Anal. Calcd for $C_{63}H_{89}N_7O_{11}$ (1120.44): 67.54 C, 8.01 H, 8.75 N. Found: 67.94 C, 8.36 H, 8.61 N.

Stylopeptide 1 (2). Method A. TBTU Coupling: *N*-Fmoc-Leu-Ile-Phe-Ser-(Bu^t)-Pro-Ile-Pro-OBu^t (**12**, 1.00 g, 0.897 mmol) was *N*-deprotected using the above general procedure in a 1 L round-bottom flask. After thorough removal of solvents, neat TFA (30 mL) was added to the crude Leu-Ile-Phe-Ser(Bu^t)-Pro-Ile-Pro-OBu^t at rt (precipitation was observed immediately), and the mixture was stirred for 2.5 h. Excess TFA was removed *in vacuo*, followed by evaporation of 2 × 5 mL portions of DCM. To the TFA salt in DCM (600 mL) was added TBTU (1.44 g, 4.48 mmol) dissolved in CH_3-CN (15 mL) at 0 °C under argon. DIEA (6 mL, 1% v/v) was added slowly (∼15 min) at rt while stirring. Dilution of the heptapeptide corresponded to 1.4 mM. The reaction mixture was stirred for 2 h at 0 °C and continued for 14 d at rt. After removal of solvent *in vacuo*, a solution of the crude product in DCM (200 mL) was washed with 10% aqueous citric acid (3 × 10 mL), saturated aqueous $NaHCO_3$ (3 × 10 mL), and water (10 mL). The aqueous layers were back-extracted with EtOAc (10 mL). The combined organic layers were dried ($MgSO_4$) and filtered. Solvents were removed *in vacuo* to yield 1.67 g of an oil which was chromatographed on silica gel using a 25 mm x 10 in (silica depth) column and elution with 1:1 hexane/*i*-PrOH under slight pressure to allow ∼23 mL/4 min. Stylopeptide 1 was followed by ceric sulfate−sulfuric acid-developed TLC (R_f 0.40, 4:1 EtOAc/CH_3OH). (Stylopeptide 1 was not visualized by UV or I_2). Straw-colored crystals were obtained from saturated solutions in CH_3OH. Recrystallization was continued until the mother liquor was completely colorless (at least three recrystallizations) to give 0.46 g of colorless needles (67% final step yield and 11% overall).

Comparison of the natural and synthetic specimens of stylopeptide 1 (**2**) by mixed mp, TLC, $[\alpha]_D$, IR, and ^{1}H NMR (400 MHz, CD_3OD and DMSO-d_6) showed that both were identical: mp 228.0−229.5 °C (synthetic), mp 228.0−229.0 °C (natural),[2b] mixed mp 227.0−228.0 °C; R_f 0.51 (*i*-PrOH); 0.40 (4:1 EtOAc/CH_3OH); 0.27−0.31 (2:1 *i*-PrOH/hexane; 0.18 (1:1 hexane/*i*-PrOH); $[\alpha]^{27}_D$ −129° (*c* 0.21, CH_3OH, synthetic) $[\alpha]^{25}_D$ −128° (*c* 0.20, CH_3OH, natural),[2b] synthetic FABHRMS *m*/*z* calcd for $C_{40}H_{62}N_7O_8$ 768.4660, found 768.4653 $[M + H]^+$; natural FABHRMS *m*/*z* found 768.4647 $[M + H]^+$.[2b] Anal. Calcd for $C_{40}H_{61}N_7O_8 \cdot 2H_2O$ (803.99): 59.76 C, 8.15 H, 12.19 N. Found: 59.54 C, 7.80 H, 12.23 N. Complete NMR (^{1}H, ^{13}C, HMBC, NOESY, ROESY in DMSO-d_6) and X-ray data for stylopeptide **1** have been previously reported.[2b]

Method B. BOP-C1 Coupling: The preceding Fmoc and *tert*-butyl deprotection reactions (method A) were repeated using **12** (54 mg, 0.0483 mmol). A solution of the TFA salt of Leu-Ile-Phe-Ser-Pro-Ile-Pro and DIEA (160 equiv) in DCM (35 mL) was transferred slowly *via* cannula (over 1 h) under argon to a stirred solution of BOP-C1 (22 equiv) in DCM (400 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and at rt for 3 weeks. The solvent was evaporated and the residue triturated and then dissolved in EtOAc (50 mL). The ethyl acetate solution was washed with water (3 × 2 mL), and the aqueous layer was back-extracted with EtOAc (3 × 2 mL). The combined EtOAc extract was washed with saturated aqueous $NaHCO_3$ (3 × 2 mL) and brine (2 mL), dried ($MgSO_4$), and filtered. Solvent was removed to yield an oil (0.1 g). The oil was subjected to silica gel chromatography using 2:1 *i*-PrOH/hexane as eluent. Stylopeptide 1 (**2**) was obtained as colorless needles from CH_3OH (4.9 mg, 13% yield). The synthetic peptide was identical by TLC with authentic natural stylopeptide 1.

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Supporting Information Available: List of ^{1}H (from ^{1}H-^{1}H COSY) and ^{13}C (APT and BB) NMR assignments for Fmoc *tert*-butyl ester intermediates, **7**−**12** and ^{1}H NMR spectra and IR spectra of stylopeptide 1 (**2**) (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal and can be ordered from the ACS; see any current masthead page for ordering information.

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