Solid-Phase Total Synthesis of Oscillamide Y and Analogues

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We report an efficient solid phase synthesis of oscillamide Y and three analogues. The cyclic peptide was prepared using a combination of Fmoc and allyl chemistries and an acid labile Wang type linker. The urea functionality was smoothly incorporated using N^{α}-(4-nitrophenyloxycarbonyl)-N^{ϵ}-(9-fluorenylmethoxycarbonyl)-D-lysine allyl ester. Coupling to the *N*-methyl amino acid was readily achieved using HATU, monitoring the reaction using bromophenol blue. Allyl deprotection was accomplished using $Pd(PPh_3)_4$ and dimedone, and cyclization was smoothly accomplished using PyBroP. All reactions were monitored using mass spectrometry methodology. The cyclized materials were cleaved by acidolysis and purified by RP HPLC. In all cases the material isolated was the major product and gave the expected molecular ion information. HPLC comparison with an authentic sample of oscillamide Y showed that the isomer containing L-*N*-methylalanine and L-homotyrosine was the natural product. ^{1}H NMR and ^{1}H−^{1}H COSY NMR experiments further confirmed this identification. The four compounds were tested as competitive and slow-tight binding inhibitors of chymotrypsin but showed, contrary to literature expectations, no inhibitory activity.

Introduction

Oscillamide Y (Figure 1) is a cyclic ureido-containing hexapeptide produced by a "toxic" strain of the freshwater cyanobacterium *Oscillatoria agardhii* and whose structure was first reported in 1995 as a potent inhibitor of chymotrypsin.[1] It contains several unusual functionalities including a urea, *N*-methylalanine, and homotyrosine as well as a bridging D-lysine residue—a bridge found in other cyclic peptides such as bacitracin A[2] and glidobaktin[3] but usually with L-stereochemistry. It also bears a striking resemblance to the reported structure of konbamide,[4] a calmodulin antagonist isolated from the sea sponge *Theonella*. The stereochemistries of the homotyrosine and *N*-methylalaine residues were unknown[1] so we decided to synthesize four possible analogues of oscillamide Y to firstly determine the stereochemistry of the natural product and secondly, to determine the relative chymotrypsin inhibitory properties of the four compounds. The development of solid phase methodology to oscillamide Y was investigated because of our desire to use oscillamide Y as a template for library generation and the design of serine protease inhibitors.

Results and Discussion

Our synthetic approach is outlined in Scheme 1 and utilized Fmoc/Bu^t/allyl chemistries as have been reported previously by several other groups including our own.[2,5] The urea functionality was incorporated using a nitrophenyl carbamate activated lysine residue.

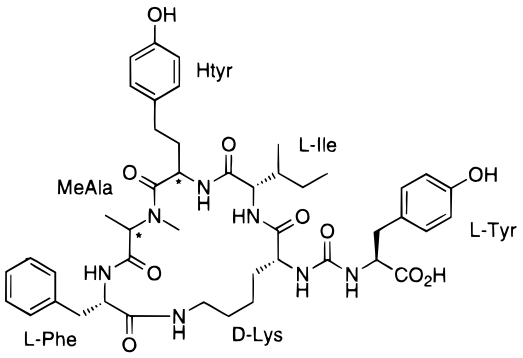

Figure 1. Oscillamide Y (* unknown stereochemistry).

Thus (hydroxymethyl)phenoxyacetic acid was coupled to aminomethyl polystyrene resin using diisopropylcarbodiimide (DIC)/hydroxybenzotriazole (HOBt) in dichloromethane/DMF. This, in our hands, provides a much more reliable resin-linker system than the traditional methods of Wang linker synthesis. Coupling of the first residue was carried out using FmocTyr(OBu^t)OH in THF/pyridine with DIC activation followed by capping with acetic anhydride and gave an initial loading of 0.4 mmol/g as determined by quantitative Fmoc and ninhydrin tests. The Fmoc group was removed with piperidine and the free amine treated with 4 equiv of N^{α}-(4-nitrophenyloxycarbonyl)-N^{ϵ}-(9-fluorenylmethoxycarbonyl)-D-lysine allyl ester **(2)** in DMF/diisopropylethylamine (DIPEA). Bases such as *N*-methylmorpholine and pyridine were found to be inapplicable in this step as was the use of dichloromethane. The N^{α}-activated lysine compound (**2**) was prepared as shown in Scheme 2 in good yield (59%) from D-Lys(Cbz)OH.

Successful urea formation was monitored by removal of a small quantity of resin (ca. 5 mg) followed by trifluoroacetic acid (TFA) treatment and subsequent HPLC and ES (electrospray) MS analysis.[6] Fmoc peptide synthesis continued on the side chain of the lysine residue using Fmoc-L-Phe and then either Fmoc-L-*N*-

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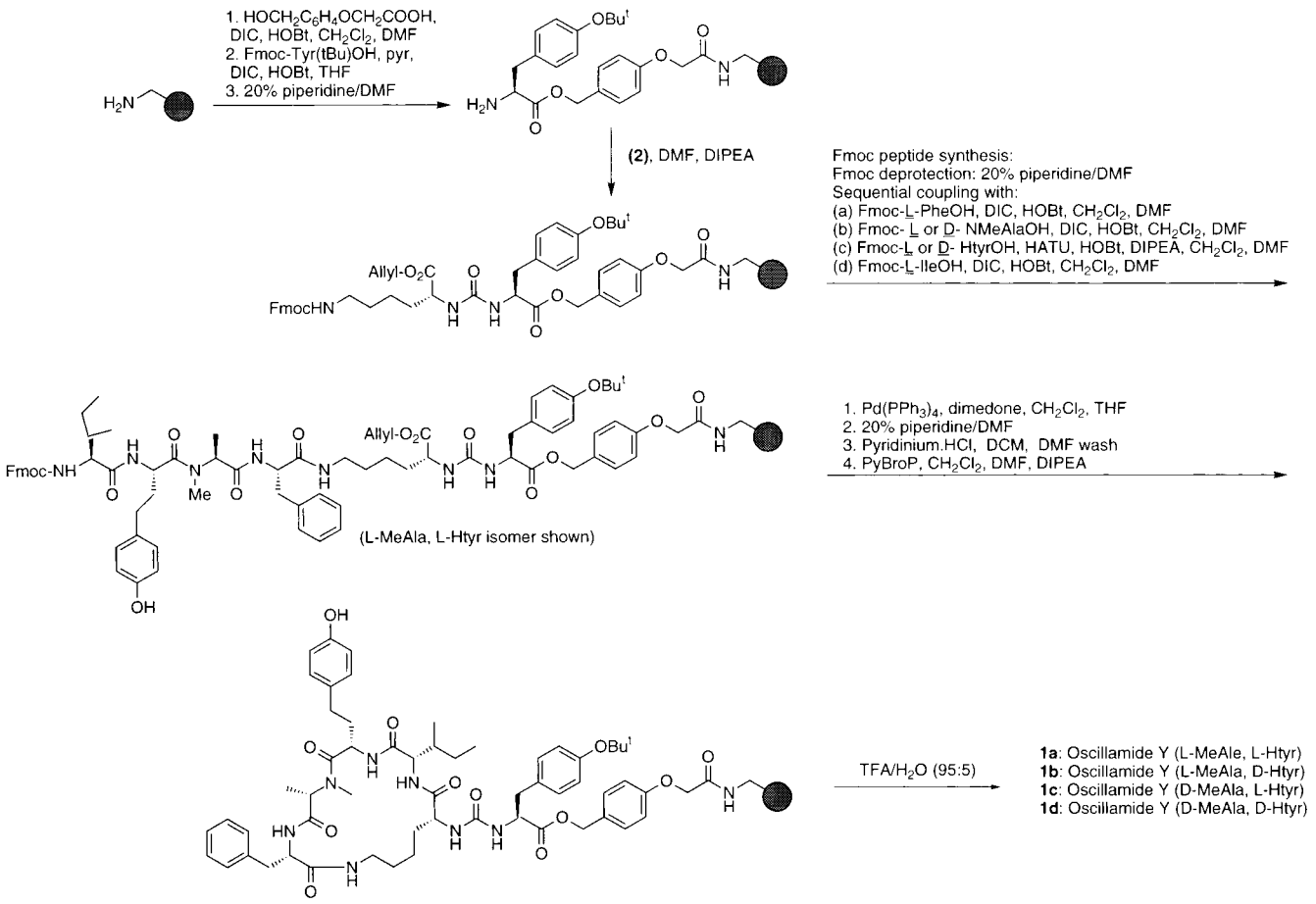
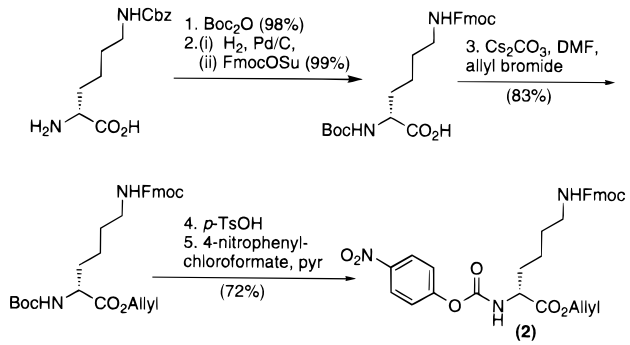
Scheme 1



Scheme 2



MeAla or Fmoc-D-*N*-MeAla. Coupling to the *N*-methylamino acid was attempted with three coupling mixtures. (i) DIC/HOBt, (ii) PyBroP,[7] (iii) HATU[8]/HOBt, monitoring the reaction in each case by the use of bromophenol blue.[9] HATU (*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) proved to be the most successful coupling agent giving complete coupling in 2−3 h.

The remaining residue (Fmoc-L-Ile) coupled without incident using DIC/HOBt followed by allyl ester removal using Pd(0) and dimedone as the scavenger.[10] The resin bound linear peptide was deprotected with piperidine and washed with pyridinium hydrochloride in DMF/dichloromethane (1:1) to ensure all traces of piperidine were removed. The success of this final allyl deprotection was monitored by mass spectrometry[5,6] and in all cases the full length, but deprotected peptide, was the only material observed. Cyclization proceeded without incident using PyBroP in DMF/dichloromethane with DIPEA and was again monitored by mass spectrometry. The compounds were cleaved from the support using TFA/H_2O (95:5) for 2 h, the solvent was evaporated, and the crude products were dissolved in water/acetonitrile and lyophilized before purification by reverse phase HPLC. The desired compound was the major component in all cases showing the high efficiency of the synthesis. HPLC comparison of the four synthetic materials and comparison with an authentic sample of oscillamide Y (kindly provided by Tomoharu Sano) showed that the isomer containing L-*N*-methylalanine and L-homotyrosine corresponded to the natural material (Figure 2).

The four oscillamide Y isomers were assayed as competitive inhibitors of chymotrypsin using the substrate succinyl-AlaAlaProPhe-*p*-nitroanilide,[11] monitoring the release of *p*-nitrophenol at 410 nm. At concentrations up to 100 μM, none of the four isomers showed any competitive inhibitory behavior with chymotrypsin. In a similar assay authentic oscillamide Y was also found not to inhibit chymotrypsin. The four isomers of oscil-

(6) Typical ESMS monitoring procedure: A 5−10 mg sample of resin is treated with 20% piperidine/DMF for 10 min and washed with DMF and dichloromethane before cleavage with TFA/H_2O (95:5, 1 mL) for 1 h. After filtration and evaporation of the solvents, the product is analyzed by ES MS.

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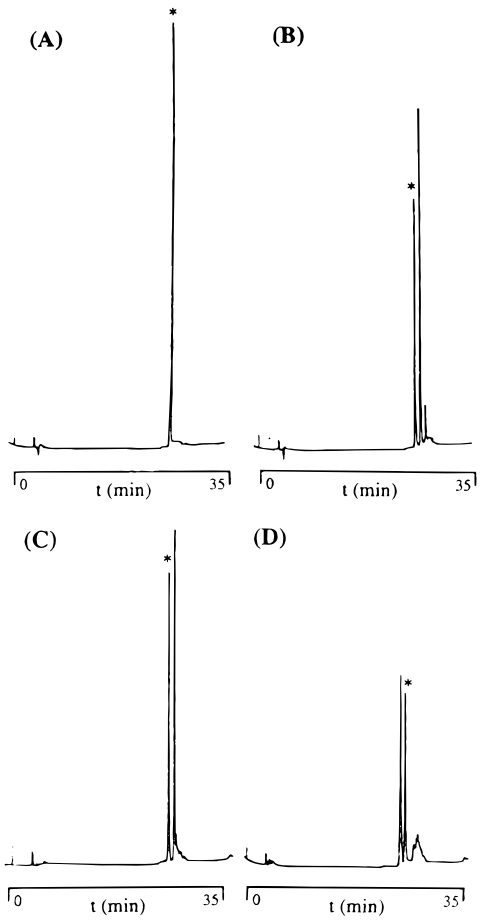

Figure 2. HPLC chromatograms showing coinjections of (A) oscillamide Y + synthetic oscillamide Y (L-MeAla, L-Htyr); (B) oscillamide Y + synthetic oscillamide Y (L-MeAla, D-Htyr); (C) oscillamide Y + synthetic oscillamide Y (D-MeAla, D-Htyr); (D) oscillamide Y + synthetic oscillamide Y (D-MeAla, L-Htyr) [gradient conditions: $t = 0$, 75% A, 25% B; $t = 10$, 75% A, 25% B; $t = 30$, 25% A, 75% B; $t = 35$, 100% B; A = water/0.1% TFA, B = acetonitrile/0.1% TFA]. Approximately equal amounts of synthetic and authentic oscillamide Y were coinjected in each run (as determined by UV absorbance). (* = Oscillamide Y).

lamide Y were tested for slow, tight-binding inhibitory activity of chymotrypsin, but again, no inhibition was observed, clearly at variance with other published results.[1]

We have therefore produced a very efficient solid phase synthesis of a urea-containing cyclic peptide and have set the stage for the use oscillamide Y as a template for solid phase chemistry. Unfortunately we could not repeat the work of previous workers who claimed that oscillamide Y was a potent inhibitor of chymotrypsin.

Experimental Section

General. An authentic sample of oscillamide Y was generously provided by Tomoharu Sano. Specific sources of reagents and solvents were as follows: Peptide synthesis grade *N*,*N*-dimethylformamide (DMF) and HPLC grade acetonitrile, Rathburn Chemical Co.; Fmoc N-Me-Ala (D and L), Htyr (D and L), HOBt and PyBroP, Advanced ChemTech; HATU, PerSeptive Biosystems; other Fmoc-protected amino acids, NovaBiochem.

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\epsilon}$-(benzyloxycarbonyl)-D-lysine.**[12] A solution of di-*tert*-butyl dicarbonate (3.82 g, 17.5 mmol) in dioxane (30 mL) was added to a solution of *N*$^{\epsilon}$-(benzyloxycarbonyl)-D-lysine (5.03 g, 18.0 mmol) in dioxane/water (1:1, 80 mL) and sodium hydroxide (2 M, 9 mL) at 4 °C and the mixture stirred for 1.5 h. The reaction mixture was concentrated under reduced pressure, poured into water (200 mL) and acidified with 2 M $KHSO_4$. The aqueous phase was extracted with ethyl acetate (60 mL, 5 × 30 mL), the extracts were combined, washed (brine, 60 mL), dried ($MgSO_4$), and filtered, and the solvent was removed *in vacuo* to give a sticky oil (6.55 g, 98%): ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.26−1.44 (s + m, 14 H), 1.50−1.64 (m, 1 H), 2.97 (dt, J = 5.9 and 6.6 Hz, 2 H), 3.82 (ddd, J = 8.1, 8.8 and 5.1 Hz, 1 H), 5.00 (s, 2 H), 7.02 (d, J = 8.1 Hz, 1 H), 7.24 (t, J = 5.9 Hz, 1 H), 7.29−7.38 (m, 5 H), 12.41 (broad s, 1H); ^{13}C NMR (75 MHz, d_6-DMSO) δ 22.8, 28.1, 28.9, 30.3, 39.9, 53.4, 65.0, 77.9, 127.6, 128.3, 137.2, 155.5, 156.0, 174.2.

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\epsilon}$-(9-fluorenylmethoxycarbonyl)-D-lysine.**[13] A solution of *N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-*N*$^{\epsilon}$-(benzyloxycarbonyl)-D-lysine (6.55 g, 17.2 mmol) in ethanol (250 mL) was purged with nitrogen. Palladium on activated carbon (10%, 0.65 g) was added, the solution degassed, the apparatus filled with hydrogen (×3), and the reaction mixture stirred overnight. The reaction was filtered through Celite and the solvent removed under reduced pressure. The product was redissolved in dioxane (100 mL) and $NaHCO_3$ (5% aq, 50 mL), the solution cooled in an ice bath, a solution of Fmoc-succinimide (5.50 g, 16.3 mmol) added dropwise over 40 min, and the mixture stirred for another 30 min. The reaction mixture was concentrated under reduced pressure, diluted to 250 mL by the addition of water, and acidified to pH 3 with 2 M $KHSO_4$. The aqueous phase was extracted with ethyl acetate (75 mL, 3 × 50 mL). The extracts were combined, washed (brine, 50 mL), dried ($MgSO_4$), and filtered, and the solvent was removed *in vacuo* to give the product as a colorless foam (7.57 g, 99%): ^{1}H NMR (300 MHz, d_6-DMSO) δ 1.25−1.45 (s + m, 14 H), 1.45−1.70 (m, 1 H), 2.97 (m, 2 H), 3.84 (ddd, J = 4.4, 5.2 and 7.4 Hz, 1 H), 4.20 (t, J = 6.6 Hz, 1H), 4.29 (d, J = 6.6 Hz, 2 H), 7.03 (d, J = 7.4 Hz, 1 H), 7.28 (t, J = 5.2 Hz, 1 H), 7.32 (t, J = 7.4 Hz, 2 H), 7.40 (t, J = 7.4 Hz, 2 H), 7.68 (d, J = 7.4 Hz, 2 H), 7.88 (d, J = 7.4 Hz, 2 H), 12.41 (broad s, 1 H); ^{13}C NMR (75 MHz, d_6-DMSO) δ 22.9, 27.9, 28.9, 30.4, 39.9, 46.7, 53.4, 66.3, 77.9, 120.0, 125.1, 127.0, 127.5, 140.7, 143.9, 155.6, 156.0, 174.2.

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\epsilon}$-(9-fluorenylmethoxycarbonyl)-D-lysine Allyl Ester.** *N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\epsilon}$-(9-fluorenylmethoxycarbonyl)-D-lysine (7.57 g, 16.2 mmol) was dissolved in DMF (40 mL) and cesium carbonate (3.98 g, 12.2 mmol) added. The mixture was stirred for 1 h. Allyl bromide (13.8 g, 9.9 mL, 0.114 mol) was added to the mixture and stirring continued for a further 1 h resulting in a milky solution. The mixture was diluted to 250 mL with water and acidified with 2 M $KHSO_4$. The aqueous phase was extracted with dichloromethane (80 mL, 3 × 40 mL). The extracts were combined, washed (brine, 80 mL), dried ($MgSO_4$), and filtered, and the solvent was removed *in vacuo* to give a white solid which was recrystallized from ethyl acetate/hexane to give white crystals (6.89 g, 83%): mp 119−121 °C; IR (Nujol) 3345, 1749, 1690, 1522; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40−1.50 (s + m, 11 H), 1.50−1.58 (m, 2 H), 1.62−1.71 (m, 1 H), 1.75−1.90 (m, 1 H), 3.19 (m, 2 H), 4.21 (t, J = 6.6 Hz, 1 H), 4.33 (m, 1 H), 4.40 (d, J = 6.6 Hz, 2 H), 4.63 (dt, J = 5.9 and 1.5 Hz, 2 H), 4.95 (broad s, 1 H), 5.15 (d, J = 7.4 Hz, 1 H), 5.25 (dd, J = 10.3 and 1.5 Hz, 1 H), 5.33 (dd, J = 16.9 and 1.5 Hz, 1 H), 5.84−6.00 (ddt, J = 16.9, 10.3 and 5.9 Hz, 1 H), 7.32 (dt, J = 1.5 and 7.4 Hz, 2 H), 7.41 (t, J = 7.4 Hz, 2 H), 7.60 (d, J = 7.4 Hz, 2 H), 7.77 (d, J = 7.4 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.4, 28.2, 29.3, 32.3, 40.5, 47.2, 53.2, 65.8, 66.4, 79.8, 118.8, 119.9, 125.0, 126.9, 127.6, 131.5, 141.2, 143.9, 155.4, 156.4, 172.4. HRMS: $C_{29}H_{37}O_6N_2$ requires 509.2539; found 509.2617.

***N*$^{\alpha}$-(4-Nitrophenyloxycarbonyl)-*N*$^{\epsilon}$-(9-fluorenylmethoxycarbonyl)-D-lysine Allyl Ester (2).** A solution of *N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-*N*$^{\epsilon}$-(9-fluorenylmethoxycarbonyl)-D-lysine allyl ester (0.97 g, 1.9 mmol) and *p*-toluenesulfonic acid (0.33 g,

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1.9 mmol) in dichloromethane/ethanol (1:1, 20 mL) was evaporated under reduced pressure at 45 °C and the evaporation repeated six times until no starting material remained. A solution of this product in dichloromethane (15 mL) was added to a suspension of 4-nitrophenyl chloroformate in dichloromethane (10 mL) and pyridine (1.2 mL, 15.2 mmol) with cooling in an ice/salt bath. After stirring and allowing the temperature to increase to 5 °C over 3 h, the reaction mixture was acidified by addition of 1 M $KHSO_4$ (50 mL). The organic layer was separated and the aqueous phase extracted with dichloromethane (3 × 15 mL). The extracts were combined, dried ($MgSO_4$), and filtered, and the solvent was removed *in vacuo*. The crude product was purified by column chromatography (silica gel eluting with 2:1 hexane/ethyl acetate to remove 4-nitrophenol and 2:3 hexane/ethyl acetate to elute the product) and gave the title compound as pale yellow crystals (0.78 g, 72%): mp 126−128 °C; IR (Nujol) 3327, 1724, 1683, 1530; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35−1.70 (m, 4 H), 1.70−2.05 (m, 2 H), 3.24 (m, 2 H), 4.21 (t, J = 6.6 Hz, 1 H), 4.41 (m, 3 H), 4.68 (d, J = 5.9 Hz, 2 H), 4.93 (t, J = 5.2 Hz, 1 H), 5.29 (d, J = 10.3 Hz, 1 H), 5.36 (d, J = 16.2 Hz, 1 H), 5.86−5.95 (ddt, J = 10.3, 16.2 and 5.9 Hz, 1 H), 6.02 (d, J = 8.1 Hz, 1 H), 7.22−7.32 (m, 4 H), 7.40 (t, J = 7.4 Hz, 2 H), 7.60 (d, J = 7.4 Hz, 2 H), 7.75 (d, J = 7.4 Hz, 2 H), 8.18 (d, J = 8.8 Hz, 2 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 22.2, 29.6, 31.6, 40.2, 47.3, 54.2, 66.3, 66.9, 119.4, 120.1, 122.0, 125.1, 125.2, 127.1, 127.9, 131.5, 141.4, 144.0, 144.9, 153.0, 155.8, 157.0, 171.7. HRMS: $C_{31}H_{32}O_8N_3$ requires 574.2189; found 574.2153.

(Hydroxymethyl)phenoxyacetamidomethyl Polystyrene Resin. A solution of diisopropylcarbodiimide (1.67 g, 2.0 mL, 13.2 mmol), HOBt (1.78 g, 13.2 mmol), and 4-(hydroxymethyl)phenoxyacetic acid (2.40 g, 13.2 mmol) in CH_2Cl_2/DMF (1:1, 30 mL) was added to aminomethyl polystyrene resin (8.26 g, 6.6 mmol NH_2) suspended in dichloromethane (130 mL) and the mixture shaken for 2.5 h. At this time a ninhydrin test was negative. The resin was filtered, washed with DMF (4 × 80 mL), dichloromethane (4 × 100 mL), and ether (2 × 80 mL) and dried *in vacuo*.

***N*$^\alpha$-(Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-tyrosinyl(hydroxymethyl)phenoxyacetamidomethyl Polystyrene Resin.** Diisopropylcarbodiimide (1.13 g, 1.39 mL, 8.0 mmol) was added to an ice cold solution of FmocTyr(tBu)OH (3.68 g, 8.0 mmol) and HOBt (1.19 g, 8.0 mmol) in THF (30 mL). The preactivated amino acid solution was added to (hydroxymethyl)phenoxyacetamidomethyl polystyrene resin (2.5 g, 2.0 mmol) suspended in pyridine (10 mL) and the mixture shaken for 3.5 h. The resin was filtered and washed with THF (2 × 30 mL), DMF (4 × 30 mL), and dichloromethane (4 × 30 mL) and dried *in vacuo*. Resin substitution was determined by quantitative Fmoc and ninhydrin analysis and found to be 0.4 mmol/g. Residual resin sites were capped by treatment with acetic anhydride (0.36 g, 0.34 mL, 3.6 mmol) and pyridine (0.28 g, 0.29 mL, 3.6 mmol) in dichloromethane (30 mL) for 1.25 h. The resin was filtered, washed with dichloromethane (3 × 30 mL), DMF (4 × 30 mL), and dichloromethane (5 × 30 mL), and dried *in vacuo*.

Oscillamide Y (L-MeAla, L-Htyr) (1a). *N*$^\alpha$-(Fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-tyrosinyl(hydroxymethyl)phenoxyacetamidomethyl polystyrene resin (0.27 g, 0.1 mmol) was treated with 20% piperidine in DMF (4 mL) for 30 min, filtered, and washed with DMF (4 × 4 mL) and dichloromethane (4 × 4 mL). The resin was suspended in DMF (2 mL), a solution of *N*$^\alpha$-(4-nitrophenyloxycarbonyl)-*N*$^\epsilon$-(9-fluorenylmethoxycarbonyl)-D-lysine allyl ester (229 mg, 0.4 mmol) in DMF (2 mL) and DIPEA (69 μL, 0.4 mmol) were added, and the mixture was shaken for 25 min. The resin was filtered and washed with DMF (6 × 4 mL) and dichloromethane (4 × 4 mL). A ninhydrin test showed complete coupling.

The resin was subjected to Fmoc-peptide synthesis using the following conditions:

(i) Fmoc deprotection: 20% piperidine in DMF (4 mL) for 30 min, followed by washing with DMF (4 × 4 mL) and dichloromethane (4 × 4 mL).

(ii) Coupling conditions: (a) Fmoc-PheOH (155 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol), and DIC (63 μL, 0.4 mmol) in dichloromethane/DMF (4 mL:10 drops), 1.5 h. (b) FmocN-Me-AlaOH (130 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol), and DIC (63 μL, 0.4 mmol) in dichloromethane/DMF (4 mL:10 drops), 1.5 h. (c) Fmoc-L-HtyrOH (168 mg, 0.4 mmol), HOBt (28 mg, 0.2 mmol), HATU (0.15 g, 0.4 mmol), and DIPEA (138 μL, 0.8 mmol) in dichloromethane/DMF (1:1), 2 h. A bromophenol blue test indicated complete coupling. (d) Fmoc-IleOH (141 mg, 0.4 mmol), HOBt (54 mg, 0.4 mmol), and DIC (63 μL, 0.4 mmol) in dichloromethane/DMF (4 mL:10 drops), 2 h. Following all couplings the resin was filtered and washed with DMF (6 × 4 mL) and dichloromethane (4 × 4 mL). All couplings (except c) were analyzed using the ninhydrin test.

A solution of palladium tetrakis (triphenylphosphine) (116 mg, 0.1 mmol) and dimedone (140 mg, 1.0 mmol) in dichloromethane/THF (1:1, 4 mL, sparged with nitrogen) was added to the resin and the mixture shaken overnight. The resin was filtered, washed with dichloromethane (2 × 4 mL), DMF (2 × 4 mL), 0.5% DIPEA + 0.5% diethyldithiocarbamic acid sodium salt in DMF (4 × 4 mL), DMF (3 × 4 mL), and dichloromethane (4 × 4 mL). The resin was treated with 20% piperidine in DMF (4 mL) for 30 min, filtered, and washed with DMF (4 × 4 mL), 10% pyridinium hydrochloride in dichloromethane/DMF (1:1, 3 × 4 mL), and dichloromethane (4 × 4 mL). A solution of PyBroP (280 mg, 0.6 mmol) and DIPEA (103 μL, 0.6 mmol) in dichloromethane/DMF (1:1, 4 mL) was added to the resin and the mixture shaken for 2.5 h. The resin was filtered and washed with dichloromethane (2 × 4 mL), DMF (4 × 4 mL), and dichloromethane (4 × 4 mL). A ninhydrin test was negative. The product was cleaved from the solid support by treatment with TFA/H_2O (95:5, 5 mL) for 2.5 h. The resin was filtered and washed with TFA (2 × 2 mL) and dichlorormethane (3 × 4 mL), and the filtrate and washes were combined. Removal of the solvent under reduced pressure gave a white solid which was dissolved in water/acetonitrile and lyophilized. Purification was achieved using semipreparative RP HPLC on a Phenomenex Prodigy ODS 5 μm 250 × 10

Table 1. ^{1}H NMR (360 MHz, d_7-DMF) Data for Oscillamide Y

	position	δ (ppm)	(m, Hz)
Phe	CH^α	4.67	(m)
	CH_2^β	3.52	(dd, J = 3.1, 13.3 Hz)
		2.98	(d, J = 13.3 Hz)
	aryl	7.37	(m)
		7.30	(m)
	NH	9.11	(d, J = 9.1 Hz)
MeAla	CH^α	5.09	(q, 6.8 Hz)
	CH_3^β	1.28	(d, J = 6.8 Hz)
	NMe	2.05	(s)
Htyr	CH^α	5.04	(m)
	CH_2^β	2.05	(m)
		1.98	(m)
	CH_2^γ	2.85	(obscured by solvent)
		2.62	(m)
	aryl	7.18	(overlapping with Tyr)
		6.91	(d, J = 8.5 Hz)
	NH	9.17	(d, J = 4.3 Hz)
Ile	CH^α	4.30	(t, J = 6.4 Hz)
	CH^β	2.05	(m)
	CH_2^γ	1.82	(m)
		1.37	(m)
	CH_3^γ	1.26	(d, J = 6.9 Hz)
	CH_3^δ	1.37	(t, J = 7.4 Hz)
	NH	7.54	(d, J = 6.4 Hz)
Lys	CH^α	4.26	(dd, J = 6.9, 9.0 Hz)
	CH_2^β	2.01	(m)
		1.85	(m)
	$CH_2^\gamma + CH_2^\delta$	1.60	(m)
	CH_2^δ	1.40	(m)
	CH_2^ϵ	3.72	(m)
		2.90	(obscured by solvent)
	NH^α	7.60	(broad)
	NH^ϵ	6.97	(d, J = 7.4 Hz)
Tyr	CH^α	4.63	(m)
	CH_2^β	3.14	(dd, J = 5.1, 14.0 Hz)
		3.00	(obscured by solvent)
	aryl	7.18	(overlapping with Htyr)
		6.87	(d, J = 8.5 Hz)
	NH	6.40	(d, J = 7.9 Hz)

mm column, eluting at 2.0 mL/min. The following gradient was used: $t = 0-10$ min, 75% A, 25% B; $t = 30$ min, 25% A, 75% B, $t = 35$ min, 100% B. (A = water, 0.1% TFA, B = MeCN, 0.1% TFA). Under these conditions (L-MeAla, L-Htyr) oscillamide Y eluted after 24.8 min. Yield 8 mg. λ_{max} (MeOH): 278 nm, $\epsilon = 2595$. [lit.[1] λ_{max} (H_2O): 278 nm, $\epsilon = 2595$]. ESMS: 858.3 $(M + H)^+$, 880.4 $(M + Na)^+$; HRMS: M + H $C_{45}H_{60}O_{10}N_7$ requires 858.4402, Found 858.4385. For ^1H NMR data see Table 1 and Supporting Information.

Oscillamide Y (L-MeAla, D-Htyr; D-MeAla, L-Htyr; and D-MeAla, D-Htyr Isomers) (1b, 1c, 1d). The above procedure was repeated using 0.88 g (0.4 mmol) of N^{α}-(fluorenylmethoxycarbonyl)-*O*-*tert*-butyl-L-tyrosinyl(hydroxymethyl)phenoxyacetamidomethyl polystyrene resin. After coupling of FmocPheOH, the resin was split into three equal portions (A, B, C). Fmoc-L-MeAlaOH was coupled to A, and Fmoc-D-MeAlaOH to B and C. At the next synthetic stage, Fmoc-D-HtyrOH was coupled to A, and B and C were treated with Fmoc-D-HtyrOH and Fmoc-L-HtyrOH, respectively. Subsequent synthetic steps were identical. Purification by semipreparative RP HPLC using the conditions above gave oscillamide Y (L-MeAla, D-Htyr), 25.5 min, 9 mg; oscillamide Y (D-MeAla, L-Htyr), 25.8 min, 3 mg; oscillamide Y (D-MeAla, D-Htyr), 25.3 min. ESMS: Oscillamide Y (L-MeAla, D-Htyr) 858.3 $(M + H)^+$, 880.4 $(M + Na)^+$. Oscillamide Y (D-MeAla, D-Htyr) 858.4 $(M + H)^+$, 880.4 $(M + Na)^+$. Oscillamide Y (D-MeAla, L-Htyr) 858.3 $(M + H)^+$, 880.2 $(M + Na)^+$.

Chymotrypsin Inhibition Assays. Bovine pancreatic α-chymotrypsin (from Calbiochem) was dissolved in 0.1 M Tris-HCl/0.01 M $CaCl_2$ buffer at a concentration of 20 μg/ mL. Kinetic analysis using the substrate Suc-Ala-Ala-Pro-Phe-pNA at pH 8 in 0.1 M Tris-HCl/0.01 M $CaCl_2$ and monitoring the release of 4-nitrophenol at 410 nm gave K_m and k_{cat} values of 48 μM and 350 s^{-1}, respectively, essentially identical to the literature.[11] Addition of any of the four oscillamide Y analogues, up to a concentration of 100 μM (using the substrate Suc-Ala-Ala-Pro-Phe-pNA at a concentration of 0.67 K_m), gave no observable inhibition. Preincubation of the oscillamide Y analogues (25 μM) with chymotrypsin likewise gave no observable inhibition.

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Supporting Information Available: ^1H and $^1H-^1H$ COSY NMR spectra for oscillamide Y (L-MeAla, L-Htyr) (4 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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