Structure of WS9326A, a Novel Tachykinin Antagonist from a *Streptomyces*

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A novel tachykinin antagonist WS9326A was isolated from *Streptomyces violaceoniger* no. 9326. It is an acylated macrocyclic heptapeptide lactone consisting of L-Thr, (*E*)-dehydro-*N*-methyltyrosine ((*E*)-ΔMeTyr), L-Leu, D-Phe, L-*allo*Thr, L-Asn, L-Ser, and 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid. Its structure, including absolute stereochemistry, was unequivocally determined as **1** on the basis of chemical and spectroscopic evidence. The spectroscopic investigations were carried out mainly with **2**, the triacetyl derivative of WS9326A. This is the first example of a tachykinin antagonist isolated from a natural source.

Introduction

Substance P[1] is a member of the family of structurally related peptides known as tachykinins[2] and is widely distributed in the body. There are three tachykinin receptors in human body designated as NK_1, NK_2, and NK_3. Several studies indicate that tachykinins play an important role in physiological activities, such as vasodilatation,[3] stimulation of salivary secretion,[4] and airway smooth muscle contraction.[5] In the course of our screening program for a tachykinin antagonist as a therapeutically useful antiasthmatic drug, WS9326A (**1**) was isolated from *Streptomyces violaceoniger* no. 9326. Compound **1** inhibits the binding of [^{3}H]substance P to a guinea pig lung membrane preparation with an IC_{50} value of 3.6×10^{-6} M and antagonizes the tracheal contractions induced by exogenously added substance P and neurokinin A with IC_{50} values of 9.7×10^{-6} M and 3.5×10^{-6} M, respectively.[6] Its discovery, isolation, characterization, biological, and pharmacological properties are described in a separate publication.[7] In this paper we report structure elucidation of this natural product on the basis of chemical and physical evidence.

Results and Discussion

From an ethyl acetate extract of mycelial cake of fermentation broth was obtained WS9326A (**1**) as a colorless powder: mp 187–190 °C, $[\alpha]^{23}_D$ −84° (*c* 1.0, MeOH). The molecular formula was established to be $C_{54}H_{68}N_8O_{13}$ by elementary analysis and high-resolution (HR) FABMS.[7] Compound **1** was negative to ninhydrin reagent and was positive to $FeCl_3$. Acetylation of **1** with Ac_2O/pyridine gave triacetyl derivative (**2**) (FABMS *m/z* 1163). Methylation with CH_2N_2 and subsequent acetylation gave diacetyl monomethyl derivative (**3**) (FABMS *m/z* 1135). These results indicated that **1** contains three hydroxyl groups, and one of them was phenolic in nature.

A strong absorption at 1650 cm^{-1}, together with a weak band at 1730 cm^{-1}, in the IR spectrum suggested the presence of a peptide linkage along with an ester or lactone functionality. Acid hydrolysis of **1** (6 N HCl, 110 °C, 18 h) gave products which were positive to ninhydrin on a TLC plate. This further substantiated the peptide-like nature of the molecule. Amino acid analysis of the hydrolysate revealed the presence of one residue each of Asp, Ser, Leu, Phe, methylamine, and NH_3 and two residues of Thr. One of the two Thr residues was observed to be *allo*Thr by chiral column GC–MS[8] (vide infra). Presumably, NH_3 was derived from either Asn or C-terminal carboxamide. The peak due to methylamine in the amino acid analysis suggested the presence of a dehydro *N*-methyl amino acid.[9] Indeed, the amino acid analysis of the hexahydro derivative **4**, obtained from **1** (Pd-black, 4 atm of H_2), showed absence of the peak due to methylamine. A new peak, which coeluted with authentic (DL)-*N*-methyltyrosine showed that the dehydroamino acid was dehydro-*N*-methyltyrosine (ΔMeTyr). The presence of ΔMeTyr residue was also confirmed by the extensive analysis of ^{13}C–^1H long-range coupling patterns in **2** (vide infra). Alkaline hydrolysis of **1** (6 N KOH, 24 h) gave an acid along with other byproducts. HRFABMS showed that the acid had a molecular formula $C_{14}H_{16}O_2$. The ^1H NMR spectrum of the acid showed the presence of a disubstituted benzene ring [δ 7.30 (3 H, m), 7.65 (1 H, d, *J* = 7.3 Hz)], an isolated trans ethylene [δ 6.41 (1 H, d, *J* = 16 Hz), 5.87 (1 H, d, *J* = 16 Hz)], and a *cis*-1-pentenyl group [δ 0.87 (3 H, t, *J* = 7.3 Hz), 1.42 (2 H, m), 2.03 (2 H, m), 5.87 (1 H, dt, *J* = 11.4, 7.4 Hz), 6.58 (1 H, d, *J* = 11.4 Hz)]. A UV spectrum of the hydrogenation product of the acid **6** compared well with that of *o*-xylene rather than *m*- or *p*-xylene (obsd 262, *o*-, 262.5, *m*-, 264.5, *p*-, 274 nm). On the basis of the above information structure **5**

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(1) The current nomenclature designates the receptors for substance P, neurokinin A, and neurokinin B as NK_1, NK_2, and NK_3.

(2) Chang, M. M.; Leeman, S. E. *J. Biol. Chem.* **1970**, *245*, 4784.

(3) Pernow, B.; Rosell, S. *Acta Physiol. Scand.* **1975**, *93*, 139.

(4) (a) Vogler, K. et al. *Ann. N. Y. Acad. Sci.* **1963**, *104*, 378. (b) Leeman, S. E.; Hammerschlag, R. *Endocrinology* **1967**, *81*, 803.

(5) Barnes, P. J. *Lancet* **1986**, 242–245.

(6) Both NK_1 and NK_2 receptors are present in the human airway, therefore dual NK_1/NK_2 receptor antagonist may have an added advantage in the treatment of asthma.

(7) (a) Hayashi, K.; Hashimoto, M.; Shigematsu, N.; Nishikawa, M.; Ezaki, M.; Yamashita, M.; Kiyoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H. *J. Antibiot.* **1992**, *45*, 1055–1063. (b) Hashimoto, M.; Hayashi, K.; Murai, M.; Fujii, T.; Nishikawa, M.; Kiyoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H. *J. Antibiot.* **1992**, *45*, 1064–1070.

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(9) Gross, E., Meienhofer, J., Eds. *The Peptides*; Academic Press: New York, 1983; Vol. 5, pp 310–311.

0022-3263/93/1958-0170$04.00/0

was assigned to the acid. This was further confirmed by comparison with the synthetic sample (see Experimental Section).

The even amino acids and the acyl component, identified above, account for all the elements present in 1. The molecular formula of 1 shows 24 degrees of unsaturation. However, the above fragments lead to 23 degrees of unsaturation. The remaining unsaturation is then due to the cyclic nature of the compound. The observation that compound 7, having molecular formula $C_{54}H_{70}N_8O_{14}$ (1 + H_2O), is obtained upon mild alkaline hydrolysis of 1 also indicates the presence of a lactone ring.

Careful examination of ^{13}C NMR spectra (100 MHz, CD_3OD) indicated that 1 exists as a mixture of conformers in solution (6:1).[10] This resulted in complicated ^1H and ^{13}C NMR spectra and also made it difficult to connect the eight fragments by application of modern 2D NMR techniques. Fortunately, however, ^1H and ^{13}C NMR spectra of the triacetyl derivative 2 in $CDCl_3$–CD_3OD (10:1)[11] showed the presence of a single conformer, and the signals were well-separated. Thus, it was suitable for extensive NMR analysis and was chosen for initial structure assignments.

NMR signals for individual amino acids ^{1}Thr, ^{3}Leu, ^{4}Phe, ^{5}Thr, ^{6}Asn (or Asp), ^{7}Ser,[12] and the acyl component attached to ^{1}Thr were readily assigned by 2D NMR experiments including ^1H–^1H COSY, ^{13}C–^1H COSY, and COLOC (Table I). The gross structure for 2 was readily assembled by connecting the individual amino acids on the basis of connectivities observed in the COLOC experiment (Figure 1). The ROESY spectrum further confirmed the amino acid sequence implied by the COLOC data. The results are shown in Figure 1. Long-range coupling between the carbonyl carbon of ^{7}Ser and the β-methine proton of ^{1}Thr showed the molecule formed a 22-membered lactone. The presence of Asn residue was ascertained by the observation of the C-terminal Ser carbonyl as a carbonyl contributor to the macrocyclic lactone. The observation of ROE between Ser NH and Asn CαH established the regiochemistry of the Asn linkage as the normal linkage. Thus, NMR spectral analysis of 2 well established the planar structure of 2 and thence of 1, as depicted in Figure 1.

Absolute Stereochemistry of WS9326A. To determine the stereochemistry of the standard amino acids, the acid hydrolysates described above were derivatized with 15% HCl/*n*-BuOH followed by trifluoroacetic anhydride (TFAA) to *N*,*O*-trifluoroacetyl *n*-butyl ester derivatives. Chiral column GC–MS analysis of the derivative allowed us to assign the L configuration for the Asp, Ser, Leu, Thr, and *allo*Thr and the D configuration for the Phe.

The ΔMeTyr unit of 2 showed a ROE between the β-olefin proton at δ 6.70 and the *N*-methyl proton at δ 3.56 indicating that ΔMeTyr had the *E* configuration as shown. It was also consistent with the coupling constant between the carbonyl carbon at δ 166.4 and the β-olefin proton at δ 6.70 in the ΔMeTyr unit of 2 (obsd $^3J_{C-H}$ = 9.5 Hz (lit.[13] *E*: $^3J_{C-H}$ = 8.6–10 Hz, *Z*: $^3J_{C-H}$ = 4.7–4.9 Hz)).

The configuration of the two double bonds in the acyl component of 2 was assigned on the basis of vicinal ^1H–^1H

Table I. ^1H and ^{13}C NMR Signal Assignments of Triacetyl-WS9326A (2) ($CDCl_3$–CD_3OH (10:1))

	^{13}C NMR		^1H NMR	
assignment		δ_C (m)	δ_H	(intn, mult, *J* (Hz))
acyl	1	167.03 (s)		
	2	121.29 (d)	6.85	(1 H, d, *J* = 16)
	3	140.74 (d)	7.88	(1 H, d, *J* = 16)
	1′	133.75 (s)		
	2′	138.74 (s)		
	3′	127.24 (d)	7.15–7.30	(1 H, m)
	4′	129.34 (d)	7.15–7.30	(1 H, m)
	5′	130.20 (d)	7.15–7.30	(1 H, m)
	6′	126.50 (d)	7.61	(1 H, d, *J* = 8)
	1″	126.95 (d)	6.49	(1 H, d, *J* = 12)
	2″	135.23 (d)	5.76	(1 H, dt, *J* = 12, 7.5)
	3″	30.65 (t)	1.93	(2 H, m)
	4″	22.79 (t)	1.28	(2 H, m)
	5″	13.82 (q)	0.71	(3 H, t, *J* = 7.5)
^{1}Thr	NH		8.02	(1 H, d, *J* = 8)
	α	53.22 (d)	5.46	(1 H, d, *J* = 8)
	β	70.99 (d)	5.48	(1 H, q, *J* = 6)
	γ	17.05 (q)	1.35	(3 H, d, *J* = 6)
	CO	169.79 (s)		
^{2}ΔMeTyr	NMe	39.39 (q)	3.56	(3 H, s)
	α	138.82 (s)		
	β	126.50 (d)	6.70	(1 H, s)
	1	131.31 (s)		
	2, 6	129.96 (d) × 2	7.28	(2 H, d, *J* = 8)
	3, 5	122.10 (d) × 2	7.03	(2 H, d, *J* = 8)
	4	151.02 (s)		
	CO	166.36 (s)		
	CH_3CO	21.21 (q)	2.26	(3 H, s)
	CH_3CO	169.55 (s)		
^{3}Leu	NH		7.45	(1 H, d, *J* = 7)
	α	52.66 (d)	4.22	(1 H, m)
	β	39.75 (t)	1.32–1.20	(1 H, m)
			1.58	(1 H, m)
	γ	24.26 (d)	0.84	(1 H, m)
	δ	21.42 (q)	0.72	(3 H, d, *J* = 6)
	δ	23.15 (q)	0.65	(3 H, d, *J* = 6)
	CO	173.23 (s)		
^{4}Phe	NH		8.25	(1 H, d, *J* = 8)
	α	56.10 (d)	4.65–4.56	(1 H, m)
	β	39.06 (t)	2.80–2.85	(2 H, m)
	1	137.12 (s)		
	2, 6	129.21 (d) × 2	6.98	(2 H, m)
	3, 5	128.56 (d) × 2	6.81	(2 H, m)
	4	126.74 (d)	6.96	(1 H, m)
	CO	173.03 (s)		
^{5}*allo*Thr	NH		7.70	(1 H, d, *J* = 6)
	α	58.13 (d)	4.31	(1 H, t, *J* = 6)
	β	69.22 (d)	4.93	(1 H, m)
	γ	16.18 (q)	1.07	(3 H, d, *J* = 6)
	CO	169.59 (s)		
	CH_3CO	20.99 (q)	1.85	(3 H, s)
	CH_3CO	170.84 (s)		
^{6}Asn	NH		7.86	(1 H, d, *J* = 8)
	α	49.93 (d)	4.75	(1 H, m)
	β	35.57 (t)	2.56	(1 H, dd, *J* = 4, 16)
			2.90	(1 H, dd, *J* = 6, 16)
	γCO	174.20 (s)		
	CO	171.02 (s)		
^{7}Ser	NH		7.30	(1 H, m)
	α	52.18 (d)	4.65–4.56	(1 H, m)
	β	63.73 (t)	4.18	(1 H, dd, *J* = 8, 11)
			4.46	(1 H, dd, *J* = 6, 11)
	CO	168.52 (s)		
	CH_3CO	20.83 (q)	2.00	(3 H, s)
	CH_3CO	171.32 (s)		

(10) Relative abundance of major and minor conformer changed with the choice of solvent: CD_3OD (6:1), DMF-d_7 (1:4), DMSO-d_6 (1:5). The solution conformation of WS9326A (1) and tetrahydro derivative 8 (code no. FK224) have been investigated by our analytical research laboratories, and the results will be reported in due course.

(11) $CDCl_3$–CD_3OH was employed instead of $CDCl_3$–CD_3OD in measurement of a series of 2D NMR of 2 in order to avoid D-exchange because an amide proton was useful in assigning the sequence analysis of a peptide.

(12) The left upper superscript implies the count number from the N-terminus.

(13) Vleggaar, R.; Wessels, P. L. *J. Chem. Soc., Chem. Commun.* **1980**, 160–162.

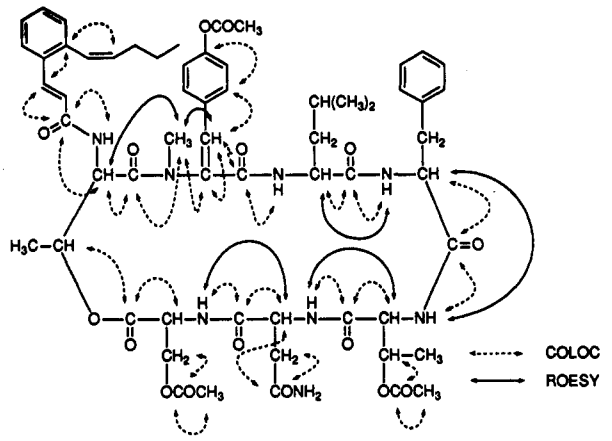


Figure 1. ^{13}C–^{1}H long-range coupling (COLOC) and ROESY of triacetyl-WS9326A (**2**).

coupling constants through the double bond. Thus, trans orientation of the isolated double bond, C(2)–C(3), was evident from the coupling constant of 16 Hz, whereas 12-Hz coupling indicated the cis geometry for 1-pentenyl substituent.

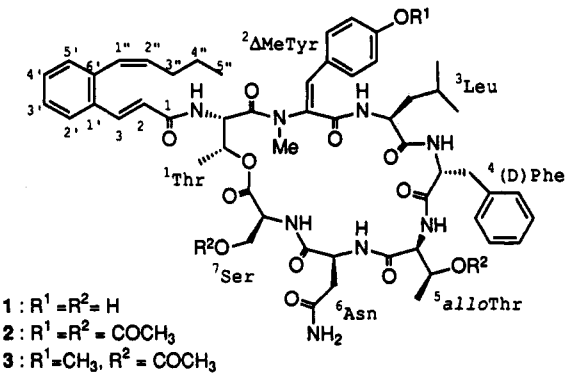
The only remaining structural problem, namely the positions of L-Thr and L-*allo*Thr, was solved by chemical methods as follows. Hydrogenation of **1** (Pd-black, 1 atm) gave tetrahydro derivative **8**.[14] Alkaline hydrolysis of **8** (1 N NaOH, 0 °C, 1 h) gave a linear peptide **9**, which upon mild acid hydrolysis (concd HCl, rt, 4 h) gave degradation products **10** and **11**. Compound **10** was negative to ninhydrin and had molecular formula $C_{18}H_{27}NO_4$ (HR-FABMS). ^1H NMR of **10** showed a disubstituted benzene ring [δ 7.05–7.20 (4 H, m)], an *n*-pentyl group [δ 0.92 (3 H, t, J = 7 Hz), 1.37 (4 H, m), 1.58 (2 H, m), 2.65 (2 H, m)], a 1,2-disubstituted ethyl group [δ 2.58 (2 H, m), 2.96 (2 H, m)], and a Thr unit [δ 1.11 (3 H, d, J = 6.5 Hz), 4.29 (1 H, dq, J = 3, 6.5 Hz), 4.44 (1 H, d, J = 3 Hz)]. Threonine in **10** had the L-configuration as was shown by chiral column GC–MS of the acid hydrolysis products of **10** (6 N HCl, 110 °C, 18 h). Structure of **10** was thus established to be 3-(2-pentylphenyl)propionyl-L-threonine. This result established in the cyclic peptide that the acylated ^{1}Thr was L-Thr, and hence the other threonine should be *allo*Thr. This was indeed the case. Compound **11** was positive to ninhydrin, and HRFABMS gave molecular formula $C_{26}H_{40}N_6O_9$. Acid hydrolysis (6 N HCl, 110 °C, 18 h) of **11** gave equimolar amounts of Asp, Thr, Ser, Leu, Phe, and NH_3 as shown by amino acid analyses. The amino acids were identified as L-Asp, L-*allo*Thr, L-Leu, L-Ser, and D-Phe by chiral column GC–MS. The sum of the molecular formulas for the five amino acid units and NH_3 satisfies the molecular formula of **11**. The structure of **11** was established by FABMS fragmentation analysis to be Leu-D-Phe-*allo*Thr-Asn-Ser (see Experimental Section).

The structure of **2** and consequently, the structure of WS9326A (**1**) was thus established (Chart I).

Conclusion

On the basis of extensive NMR analysis of **2** and chemical degradation of **1**, the structure of WS9326A (**1**) including absolute stereochemistry was established as being **1**. With

Chart I



the structure of **1** known, the assignment of ^1H and ^{13}C NMR spectra of the major conformer of **1** is shown in Table II. The compound has a novel amino acid, (*E*)-ΔMeTyr, and an acyl group 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid in the molecule. Compound **1** is the first example of a potent SP receptor antagonist isolated from a natural source.

Experimental Section

Triacetyl-WS9326A (2). To a solution of WS9326A (**1**) (300 mg) in pyridine (4.5 mL) were added acetic anhydride (1.5 mL) and 4-(dimethylamino)pyridine (DMAP) (1 mg), and the reaction mixture was allowed to stand at rt for overnight. The reaction mixture was evaporated to dryness to afford an oil which was purified by preparative TLC ($CHCl_3$–MeOH (10:1)). The product was triturated with diethyl ether to give triacetyl-WS9326A (**2**) as a colorless powder (332 mg): mp 141–143 °C; IR (KBr) 3350, 1730, 1650, 1520 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table I; $[\alpha]^{23}_D$ −122° (*c* 1.0, MeOH); FABMS *m/z* 1163 (M + H)$^+$; HRFABMS calcd for $C_{60}H_{75}N_8O_{16}$ 1163.5301 (M + H)$^+$, found 1163.5315.

Diacetylmonomethyl-WS9326A (3). To a solution of WS9326A (**1**) (200 mg) in MeOH (3 mL) was added a solution of CH_2N_2 in diethyl ether. After the solution was stirred for 5 min, the solvent was removed in vacuo. The residue was subjected to preparative TLC (Merck 5744) and developed with 20% MeOH in $CHCl_3$ to give monomethyl-WS9326A (180 mg): FABMS *m/z* 1051 (M + H)$^+$.

To a solution of monomethyl-WS9326A (100 mg) in pyridine (1 mL) were added acetic anhydride (0.5 mL) and DMAP (1 mg), and the reaction mixture was allowed to stand at rt for overnight. The reaction mixture was evaporated to dryness to afford an oil which was purified by preparative TLC ($CHCl_3$–MeOH (10:1)). The product obtained was triturated with diethyl ether to give diacetylmonomethyl-WS9326A (**3**) as a colorless powder (110 mg): mp 152–156 °C; IR (KBr) 3340, 1740, 1660, 1510 cm^{-1}; $[\alpha]^{20}_D$ −30.1° (*c* 0.11 MeOH); FABMS *m/z* 1135 (M + H)$^+$; HRFABMS calcd for $C_{59}H_{75}N_8O_{15}$ 1135.5351 (M + H)$^+$, found 1135.5340.

Acid Hydrolysis of WS9326A (1). For amino acid analysis, 2 mg of WS9326A (**1**) was dissolved in 0.8 mL of 6 N HCl in an evacuated glass tube and heated at 110 °C for 18 h. After evaporation, the residue was dissolved in 0.1 N HCl and subjected to amino acid analysis on a Hitachi 835 amino acid analyzer under the conditions for standard amino acids, in which Thr and *allo*Thr were not separated. The presence of *allo*Thr in the acid hydrolysate was verified by chiral column GC–MS analysis. Retention times in the amino acid analysis (min): Asp (13.21), Thr (14.06), Ser (14.85), Leu (36.52), Phe (39.09), NH_3 (42.96), $MeNH_2$ (46.04). The molar ratio of Thr was twice that of the other amino acids.

Hexahydro-WS9326A (4). To a solution of WS9326A (**1**) (85 mg) in MeOH (3 mL) was added Pd-black (200 mg, water wet) followed by hydrogenation (4 atm) at 40 °C for 4 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was triturated with diethyl ether to give hexahydro-WS9326A (**4**) as a colorless powder (78 mg): mp 158–160 °C; IR (KBr) 3325, 1735, 1650, 1515 cm^{-1};

(14) Compound **8** (code no. FK224) is 10 times more potent than **1** against NK_1 and NK_2 receptors and is currently in phase II clinical trials as an antiasthmatic agent.

Scheme I. Degradation products of WS9326A (1)

$[\alpha]^{21}_D$ = −29.4° (*c* 1.04, MeOH); FABMS *m/z* 1043 $(M + H)^+$; HRFABMS calcd for $C_{54}H_{75}N_8O_{13}$ 1043.5453 $(M + H)^+$, found 1043.5472.

Acid Hydrolysis of Hexahydro-WS9326A (4). Hexahydro-WS9326A (4) (2 mg) was hydrolyzed and subjected to amino acid analysis. Retention time (min): Asp (13.14), Thr (14.01), Ser (14.80), MeTyr (32.61), Leu (36.17), Phe (38.90), NH_3 (42.88). The molar ratio of Thr was twice that of the others.

Alkaline Hydrolysis of WS9326A (1). To a solution of WS9326A (1) (100 mg) in MeOH (2 mL) was added 6 N KOH (2 mL), and the mixture was refluxed for 24 h. The reaction mixture was then acidified with 6 N HCl, extracted with EtOAc, and dried ($MgSO_4$) and the solvent evaporated. The residue obtained was purified by preparative TLC ($CHCl_3$–MeOH (10:1)) to get the acyl component 5 as a white powder (12.5 mg): mp 94–96 °C; IR (Nujol) 1690, 1680, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (3 H, t, *J* = 7.3 Hz), 1.42 (2 H, m), 2.03 (2 H, m), 5.87 (1 H, dt, *J* = 11.4, 7.4 Hz), 6.41 (1 H, d, *J* = 16 Hz), 6.57 (1 H, d, *J* = 11.4 Hz), 7.3 (3 H, m), 7.65 (1 H, d, *J* = 7.3 Hz), 7.65 (1 H, d, *J* = 7.3 Hz), 8.02 (1 H, d, *J* = 16 Hz); FABMS *m/z* 217 $(M + H)^+$; HRFABMS calcd for $C_{14}H_{16}O_2$ 217.1128 $(M + H)^+$, found 217.1132.

Hydrogenesis of Acyl Component 5. To a solution of 5 (5 mg) in MeOH (1 mL) was added Pd-black (10 mg, water wet) and the mixture hydrogenated (1 atm) at rt for 1 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by preparative TLC ($CHCl_3$–MeOH (19:1)) to give the tetrahydroacyl component 6 as colorless prisms (5 mg): mp 43–45 °C; FABMS *m/z* 221 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 0.92 (3 H, t, *J* = 7 Hz), 1.38 (4 H, m), 1.61 (2 H, m), 2.63 (2 H, t, *J* = 8 Hz), 2.67 (2 H, t, *J* = 8 Hz), 3.00 (2 H, t, *J* = 8 Hz), 7.17 (4 H, m); UV λ_{max}^{MeOH} nm 255 (sh), 262 (ε 380), 270 (ε 320).

Chiral Column GC–MS Analysis. Chiral column GC–MS was carried out by using a Chirasil-L-Val capillary column (0.25 mm × 25 m) programmed from 80 to 210 °C at 3 °C/min and a ZAB-SE mass spectrometer operating in the positive EI mode (scan range between *m/z* 30 and 600 with repetition time of 1.5 s). Derivatization of amino acid residues was done as follows. The acid hydrolysate of WS9326A (1) was heated in 15% HCl in *n*-BuOH (0.25 mL) at 100 °C for 20 min in a screw-capped test tube. After removal of the *n*-butanolic HCl in vacuo, CH_2Cl_2 (0.3 mL) and trifluoroacetic anhydride (0.2 mL) were added, and the mixture was kept at 100 °C for 5 min. The product was evaporated, dissolved in EtOAc, and subjected to the analysis. Retention time (min): L-Thr (10:28), L-Ser (14:31), L-*allo*Thr (14:44), L-Leu (15:14), D-Phe (25:32), L-Asp (26:18).

WS9326A Acid (7). To a solution of 1 (300 mg) in MeOH (3.2 mL) was added aqueous 1 N NaOH solution (0.8 mL), and the reaction mixture was stirred at rt for 30 min. The mixture was applied directly to a column of DOWEX-50W X-2 (MeOH) and eluted with MeOH. The product fractions were collected and evaporated to dryness under reduced presser. The residue was triturated with diethyl ether to give WS9326 acid (7) as a colorless powder (274 mg): mp 150–154 °C; FABMS *m/z* 1055 $(MH)^+$; HRFABMS calcd for $C_{54}H_{70}N_8O_{14}$ 1055.5089 $(M + H)^+$, found 1055.5104; $[\alpha]^{22}_D$ = −56.5° (*c* 1.03, MeOH).

Tetrahydro-WS9326A (8). To a solution of WS9326A (1) (100 mg) in MeOH (3 mL) was added Pd-Black (25 mg, water wet) and the solution hydrogenated (1 atm) at rt for 3 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was triturated with diethyl ether to give tetrahydro-WS9326A (8) as a colorless powder (92 mg): mp 174–176 °C; IR (KBr) 3310, 1735, 1655, 1515 cm^{-1}; UV λ_{max}^{MeOH} 287 nm (ε 12 300); $[\alpha]^{22}_D$ = −93.9° (*c* 1.01, MeOH); FABMS *m/z* 1041 $(M + H)^+$; HRFABMS calcd for $C_{54}H_{73}N_8O_{13}$ 1041.5297 $(M + H)^+$, found 1041.5320.

Tetrahydro-WS9326A Acid (9). To a solution of tetrahydro-WS9326A (8) (600 mg) in MeOH (1 mL) was added 1 N NaOH (2 mL) at 0 °C. After the solution was stirred for 1 h, 1 N HCl (2 mL) was added to the solution. The solvent was removed in vacuo, and the residue was dissolved in EtOAc and dilute HCl. The organic layer was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the solid was filtered with EtOAc to give tetrahydro-WS9326A acid (9) (600 mg): mp 140–143 °C; IR (Nujol) 3300, 1710 (shoulder), 1645, 1510 cm^{-1}; $[\alpha]^{22}_D$ = −72.3° (*c* 1.00, MeOH); FABMS *m/z* 1059 $(M + H)^+$; HRFABMS calcd for $C_{54}H_{75}N_8O_{14}$ 1059.5402 $(M + H)^+$, found 1059.5423.

Mild Acid Hydrolysis of Tetrahydro-WS9326A Acid (9). Tetrahydro-WS9326A acid (9) (600 mg) was dissolved in concd HCl (6 mL) and allowed to stand at rt for 4 h. The reaction mixture was then neutralized with 5 N NaOH and extracted with EtOAc. The organic layer was evaporated in vacuo. The residue

Table II. ^1H and ^{13}C NMR Signal Assignments of WS9326A (1) (CD_3OH)[a]

		^{13}C NMR	^1H NMR	
assignment		δ_C (m)	δ_H	(intn, mult, J (Hz))
acyl	1	167.79 (s)		
	2	123.70 (d)	7.30	(1 H, d, J = 16)
	3	140.05 (d)	7.67	(1 H, d, J = 16)
	1′	134.85 (s)		
	2′	138.71 (s)		
	3′	130.70 (d)	7.25	(1 H, m)
	4′	129.90 (d)	7.36	(1 H, m)
	5′	128.55 (d)	7.43	(1 H, m)
	6′	128.04 (d)	7.79	(1 H, d, J = 7)
	1″	127.99 (d)	6.59	(1 H, d, J = 11.5)
	2″	135.27 (d)	5.87	(1 H, dt, J = 11.5, 7)
	3″	31.37 (t)	2.14	(2 H, m)
	4″	23.63 (t)	1.46	(2 H, m)
	5″	14.13 (q)	0.90	(3 H, t, J = 7)
[1]Thr	NH		8.28	(1 H, d, J = 9.5)
	α	53.64 (d)	5.35	(1 H, d, J = 9.5)
	β	73.46 (d)	5.55	(1 H, q, J = 6.5)
	γ	17.19 (q)	1.08	(3 H, d, J = 6.5)
	CO	171.04 (s)		
[2]ΔMeTyr	N-Me	34.58 (q)	2.89	(3 H, s)
	α	132.03 (s)		
	β	132.11 (d)	6.82	(1 H, s)
	1′	126.09 (s)		
	2′, 6′	131.69 (d) × 2	7.05	(2 H, d, J = 8.5)
	3′, 5′	115.63 (d) × 2	6.64	(2 H, d, J = 8.5)
	4′	159.20 (s)		
	CO	167.15 (s)		
[3]Leu	NH		8.15	(1 H, d, J = 3)
	α	55.55 (d)	3.70	(1 H, dt, J = 3, 7.5)
	β	39.85 (t)	0.95	(2 H, m)
	γ	24.56 (d)	0.60	(1 H, m)
	δ	22.71 (q)	0.53	(3 H, d, J = 6)
	δ	22.52 (q)	0.51	(3 H, d, J = 6)
	CO	175.69 (s)		
[4]Phe	NH		8.90	(1 H, d, J = 8)
	α	56.91 (d)	4.48	(1 H, ddd, J = 3, 8, 12)
	β	37.18 (t)	3.46	(1 H, dd, J = 3, 14)
			2.69	(1 H, dd, J = 12, 14)
	1′	139.12 (s)		
	2′, 6′	129.61 (d) × 2	7.10	
	3′, 5′	129.22 (d) × 2		(5 H, m)
	4′	127.38 (d)	7.45	
	CO	173.38 (s)		
[5]*allo*Thr	NH		8.69	(1 H, d, J = 10)
	α	59.53 (d)	4.68	(1 H, t, J = 10)
	β	71.34 (d)	3.62	(1 H, m)
	γ	21.17 (q)	1.21	(3 H, d, J = 6)
	CO	172.89 (s)		
[6]Asn	NH		8.83	(1 H, d, J = 8)
	α	52.10 (d)	5.10	(1 H, ddd, J = 3, 8, 9.5)
	β	37.09 (t)	2.94	(1 H, dd, J = 3, 16)
			2.74	(1 H, dd, J = 9.5, 16)
	γCO	174.70 (s)		
	CO	173.73 (s)		
[7]Ser	NH		8.84	(1 H, d, J = 8.5)
	α	56.76 (d)	4.55	(1 H, dt, J = 8.5, 6)
	β	62.80 (t)	3.92	(2 H, d, J = 6)
	CO	170.45 (s)		

[a] Major conformer exists in methanol.

was charged on a column of HP20SS (50 mL swollen with 50% MeOH) and eluted stepwise with 50% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, and 90% MeOH (each 100 mL). 80% MeOH eluate was evaporated in vacuo to give compound **10** as a colorless oil (50.4 mg): IR ($CHCl_3$) 3330, 1725, 1665, 1515 cm^{-1}; ^1H NMR (CD_3OD) δ 0.92 (3 H, t, J = 7 Hz), 1.11 (3 H, d, J = 6.5 Hz), 1.37 (4 H, m), 1.58 (2 H, m), 2.58 (2 H, m), 2.65 (2 H, m), 2.96 (2 H, m), 4.29 (1 H, dq, J = 3, 6.5 Hz), 4.44 (1 H, d, J = 3 Hz), 7.05–7.20 (4 H, m); $[\alpha]^{21}_D$ = −14.8° (c 1.0, $CHCl_3$); HRFABMS calcd for $C_{18}H_{27}NO_4Na$ 344.1838 $(M + Na)^+$, found 344.1833. Compound **10** was hydrolyzed and subjected to amino acid analysis and chiral column GC–MS analysis, t_R (min): Thr (11.66); L-Thr (10:29), respectively. The aqueous layer was concentrated under vacuum and charged on a column of HP20SS (50 mL swollen with H_2O). After the residue was washed with H_2O (150 mL), the column was eluted with 30% MeOH (100 mL). The eluate was evaporated in vacuo to give compound **11** as a white powder (31.2 mg): mp 206–209 °C; IR (KBr) 3280, 1720 (shoulder), 1620, 1530 cm^{-1}; ^1H NMR (D_2O) δ 0.77 (3 H, d, J = 6.5 Hz), 0.78 (3 H, d, J = 6.5 Hz), 1.16 (3 H, d, J = 6.5 Hz), 1.13–1.25 (3 H, m), 2.80 (1 H, dd, J = 8.5, 15.5 Hz), 2.92 (1 H, dd, J = 5, 15.5 Hz), 2.98 (1 H, dd, J = 10, 14 Hz), 3.24 (1 H, dd, J = 6, 14 Hz), 3.38 (1 H, m), 3.86 (2 H, m), 4.07 (1 H, dq, J = 6.5, 6.5 Hz), 4.30 (1 H, t, J = 5 Hz), 4.31 (1 H, d, J = 6.5 Hz), 4.78 (1 H, dd, J = 6, 10 Hz), 4.86 (1 H, dd, J = 5, 8.5 Hz), 7.29–7.43 (5 H, m); $[\alpha]^{20}_D$ = −12.2° (c 0.50, H_2O); FABMS m/z 603 $(M + Na)^+$, 581 $(M + H)^+$, 476 (M − Ser), 362 (M − Ser-Asn), 261 (M − Ser-Asn-Thr); HRFABMS calcd for $C_{26}H_{40}N_6O_9Na$ 603.2754 $(M + Na)^+$, found 603.2753. Compound **11** was hydrolyzed and subjected to amino acid analysis and chiral column GC–MS analysis, t_R (min): Asp (10.88), Thr (11.58), Ser (12.37), Leu (28.54), Phe (32.78), NH_3 (39.53); L-Asp (26:06), L-*allo*Thr (14:36), L-Ser (14:27), L-Leu (15:06), D-Phe (25:25), respectively.

Synthesis of Acyl Component 5. To a solution of *o*-phthalaldehyde (6.7 g) in CH_2Cl_2 (30 mL) was added carbethoxymethylenetriphenylphosphorane (17.42 g) and the mixture stirred for 30 min at rt. The reaction mixture was evaporated, and the residue was dissolved in diethyl ether. After the precipitate was filtered, the filtrate was evaporated. The residue was distilled under vacuum (125 °C/0.6 mmHg) to give 3-(2-formylphenyl)-2(*E*)-propenoic acid ethyl ester as a pale yellow oil (6 g): ^1H NMR ($CDCl_3$) δ 1.24 (3 H, t, J = 6.5 Hz), 4.19 (2 H, q, J = 6.5 Hz), 6.28 (1 H, d, J = 15 Hz), 7.5 (3 H, m), 7.77 (1 H, m), 8.43 (1 H, d, J = 15 Hz), 10.18 (1 H, s).

To a solution of butyltriphenylphosphonium bromide (3.2 g) in tetrahydrofuran (THF) (50 mL) was added potassium *tert*-butoxide (900 mg) under a N_2 atmosphere and the mixture stirred for 30 min at room temperature. The solution of 3-(2-formylphenyl)-2(*E*)-propenoic acid ethyl ester (2.0 g) in THF (30 mL) was added thereto over a period of 20 min and stirred for 1 h. After the solvent was evaporated, the residue was dissolved in diethyl ether and washed with brine and water. After the solution was dried ($MgSO_4$), the solvent was evaporated. The residue was subjected to a silica gel column chromatography (100 g) and eluted with hexane–EtOAc (3:1). The fractions containing the object compound were evaporated to give 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid ethyl ester as a colorless oil (2 g): ^1H NMR δ 0.88 (3 H, t, J = 7 Hz), 1.34 (3 H, t, J = 6.5 Hz), 1.42 (2 H, m), 2.05 (2 H, m), 4.27 (2 H, q, J = 6.5 Hz), 5.85 (1 H, dt, J = 7, 11 Hz), 6.39 (1 H, d, J = 16 Hz), 6.56 (1 H, d, J = 11 Hz), 7.3 (3 H, m), 7.61 (1 H, m), 7.92 (1 H, d, J = 16 Hz).

To a solution of 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid ethyl ester (2 g) in 20% aqueous MeOH was added KOH (2.3 g) and the mixture stirred for 2 h at 60 °C. The reaction mixture was acidified with 6 N HCl and extracted with EtOAc. After the solution was dried ($MgSO_4$), the solvent was evaporated. The residue was dissolved in hexane–EtOAc (4:1), and dicyclohexylamine (1.63 mL) was added to give crystals. The crystals were recrystallized from the same solvent. The adduct was dissolved in EtOAc and washed with 1 N H_2SO_4. After the solution was dried ($MgSO_4$), the solvent was evaporated to give 3-[2-(1(*Z*)-pentenyl)phenyl]-2(*E*)-propenoic acid (**5**) (1.8 g) as a white powder: FABMS m/z 217 $(M + H)^+$; HRFABMS calcd for $C_{14}H_{16}O_2$ 217.1128 $(M + H)^+$, found 217.1125. Its spectral data are identical with those of **5** derived from WS9326A (**1**) in all respects.

Synthesis of the Trans Isomer of the Acyl Component. To a solution of butyltriphenylphosphonium bromide (2.7 g) in THF (10 mL) was added 15% *n*-butyllithium in hexane (4.3 mL) under a N_2 atmosphere. The solution was cooled to −78 °C, and the solution of 3-(2-formylphenyl)-2(*E*)-propenoic acid ethyl ester (690 mg) in THF (3 mL) was added dropwise. After the solution was stirred for 1 h at −78 °C, EtOH (1 mL) was added and the mixture stirred for 1 h. After the solvent was evaporated, the residue was dissolved in diethyl ether and washed with brine and water. After the solution was dried (MgSO4), the solvent was evaporated. The residue was subjected to a silica gel column chromatography (30 g) and eluted with hexane–EtOAc (2:1). The fractions containing the object compound were evaporated to

give 3-[2-(1(*E*)-pentenyl)phenyl]-2(*E*)-propenoic acid ethyl ester as a colorless oil (400 mg): ^{1}H NMR δ 0.98 (3 H, t, J = 7 Hz), 1.34 (3 H, t, J = 6.5 Hz), 1.52 (2 H, m), 2.24 (2 H, m), 4.28 (2 H, q, J = 6.5 Hz), 6.08 (1 H, dt, J = 7, 15.5 Hz), 6.35 (1 H, d, J = 16 Hz), 6.69 (1 H, d, J = 15.5 Hz), 7.20–7.80 (4 H, m), 8.07 (1 H, d, J = 16 Hz).

To a solution of 3-[2-(1(*E*)-pentenyl)phenyl]-2(*E*)-propenoic acid ethyl ester (200 mg) in MeOH (5 mL) was added 1 N NaOH (5 mL) and the mixture stirred for 2 h at 60 °C. The reaction mixture was acidified with 6 N HCl and extracted with EtOAc. After the solution was dried ($MgSO_4$), the solvent was evaporated. The residue was dissolved in hexane–EtOAc (4:1), and dicyclohexylamine (140 mL) was added to give crystals. The crystals were recrystallized from the same solvent. The adduct was dissolved in EtOAc and washed with 1 N H_2SO_4. After the solution was dried ($MgSO_4$), the solvent was evaporated to give 3-[2-(1(*E*)-pentenyl)phenyl]-2(*E*)-propenoic acid (**13**) (140 mg) as a white powder: mp 70–72 °C; ^{1}H NMR ($CDCl_3$) δ 1.00 (3 H, t, J = 7.3 Hz), 1.54 (2 H, m), 2.27 (2 H, m), 6.10 (1 H, dt, J = 15.5, 7 Hz), 6.37 (1 H, d, J = 16 Hz), 6.71 (1 H, d, J = 15.5 Hz), 7.26 (1 H, m), 7.36 (1 H, m), 7.45 (1 H, d, J = 8 Hz), 7.56 (1 H, d, J = 8 Hz), 8.18 (1 H, d, J = 16 Hz); FABMS m/z 217 $(MH)^+$; HRFABMS calcd for $C_{14}H_{16}O_2$ 217.1128 $(M + H)^+$, found 217.1135.

Supplementary Material Available: NMR spectra of 1–11 and a chiral GC–MS chart (19 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.