Synthesis and Biological Studies of Simplified Analogues of Lyngbyatoxin A: Use of an Isoxazoline-Based Indole Synthesis. Quest for Protein Kinase C Modulators†

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Contribution from the Departments of Chemistry and Behavioral Neuroscience, Chevron Science Center, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, and Department of Pharmacology, School of Medicine, Scaife Hall, University of Pittsburgh, Pittsburgh, Pennsylvania 15261. *Received July 11, 1988. Revised Manuscript Received March 21, 1989*

Abstract: Efficient synthetic routes to optically active analogues of lyngbyatoxin A, a potent activator of protein kinase C, have been devised starting from L-valine methyl ester. Access to the indole nucleus of these molecules is gained through the nitrile oxide based annelation of an aromatic ring to a pyrrole ring. The biological action of several of the intermediates and analogues prepared during the course of these studies on protein kinase C activity is also presented.

Protein kinase C (PKC) serves as an important phosphorylating enzyme that is activated when an extracellular signal binds to its receptor and causes activation of a phospholipase C (PLC). This lipase degrades in turn phosphatidylinositol bis(phosphate) to *myo*-inositol 1,4,5-tris(phosphate) and diacylglycerol. Full activation of PKC requires its translocation to the inner membrane surface and the binding of Ca^{2+}, phosphatidylserine, and the diacylglycerol released by the action of PLC.[1] The phorbol esters[2] and indole alkaloids such as lyngbyatoxin A[3] and the teleocidins[4] are potent tumor promoters, which are able to mimic the effect of diacylglycerol in activating this kinase. PKC does furthermore appear to play a crucial role in the synaptic plasticity that underlies memory function.[1e]

As part of an effort to turn one of Nature's toxins, lyngbyatoxin A, into selective modulators of PKC, agents that may offer new approaches to cancer therapy, we have chosen to develop an efficient synthetic pathway to the simplified lyngbyatoxin analogues **1a** and **1b** (Scheme I). These compounds contain a *tert*-butyl or *n*-hexyl group in place of the more complex linalyl group present in the natural product.[5] Structure–activity studies by other workers have linked the presence of a hydrophobic group at the 7-position of the indole ring to enhanced biological activity, a fact that may be related to the interaction of this group with the cellular lipid bilayer.[6] Therefore, we required that our synthetic scheme be able to readily accommodate the introduction of the *tert*-butyl group or related alkyl groups at the 7-position.

Synthesis Scheme. Our synthesis scheme makes use of a new indole synthesis developed specifically for these molecules and is based upon annelation of an aromatic ring to a preformed pyrrole ring system.[7]

The synthesis begins by condensing *N*-(triisopropylsilyl)-β-lithiopyrrole[8] (**6**) with the crude imine **7** formed from the reaction of L-valine methyl ester (**5**) with the isoxazoline **4a** (Scheme II, illustrated for the *tert*-butyl analogue **1a**). The isoxazoline **4a** is readily prepared by dipolar cycloaddition reaction of acrolein dimethyl acetal with 2,2-dimethylpropanenitrile oxide (or heptanenitrile oxide for the synthesis of the *n*-hexyl derivative **4b**) followed by acetal hydrolysis using concentrated sulfuric acid in MeOH–H_2O at 90 °C. The resulting mixture of diastereomeric products **8a** is formylated by using formic acetic anhydride[9] and then the silyl group is cleaved with tetra-*n*-butylammonium fluoride in THF. Hydrogenolysis of the N–O bond of **9a** using Raney nickel as catalyst provides the corresponding β-hydroxy ketone **3a** (Scheme I), which is cyclized to the indole **10a**, $[\alpha]^{23}_D$ −55.9° (c = 0.788, CH_2Cl_2), using *tert*-butyldimethylsilyl triflate as catalyst.

While indole **10** will serve as a key starting material in the preparation of several conformationally constrained analogues of lyngbyatoxin A, it also provides a ready precursor to **1**. Accordingly, the *N*-formyl group of **10a** was reduced to an *N*-methyl group by treatment with borane–methyl sulfide complex.[10] In addition to the desired amine **2a**, a small amount (10%) of the N-deformylated product was also isolated. The optical purity of indoles **2a** and **2b** was checked at this stage by first reducing the ester group to alcohol ($LiAlH_4$) and then preparing both the (*R*)- and (*S*)-MTPA ester derivatives of each of these alcohols.[11] ^1H NMR (300 MHz) of the Mosher esters showed that **2b**, $[\alpha]^{25}_D$ −166.4° (c = 0.625, CH_2Cl_2), was a single enantiomer while **2a**, mp 139 °C, $[\alpha]^{25}_D$ −163.2° (c = 0.486, CH_2Cl_2), had undergone some racemization (~90% ee). The racemization observed in the latter case is a likely consequence of the prolonged time period (~24 h) required to achieve Raney nickel hydrogenolysis of the sterically encumbered *tert*-butyl-bearing isoxazoline **9a**.

Gilchrist's reagent **11**[12] was now employed to introduce an

† Dedicated to Professor W. G. Dauben on the occasion of his seventieth birthday.

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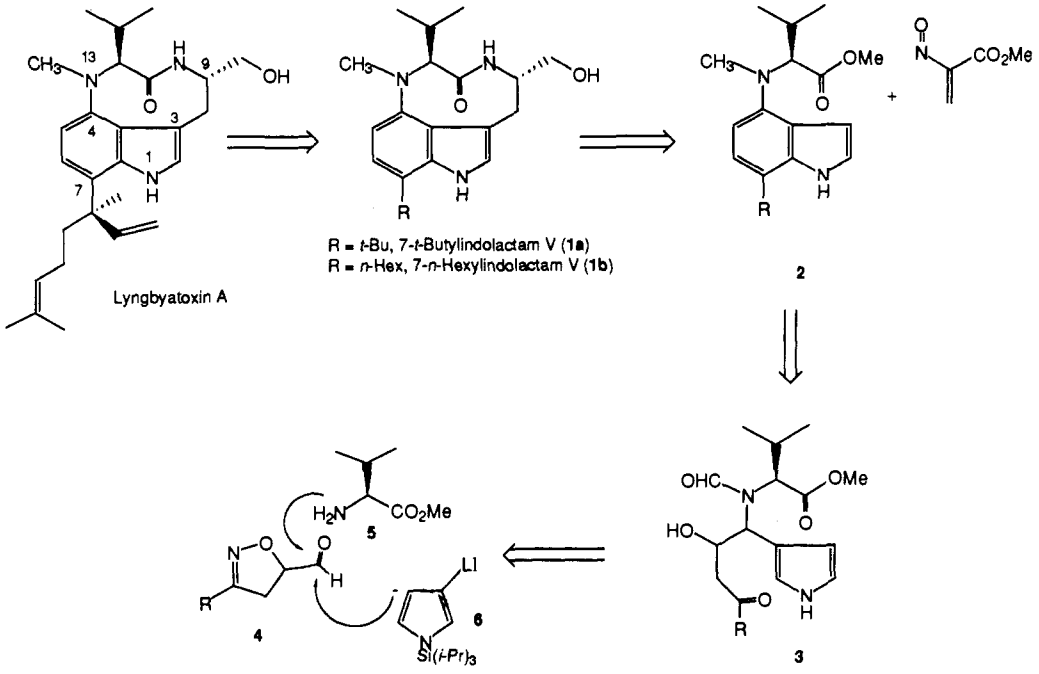
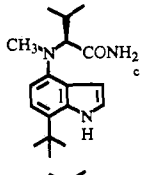
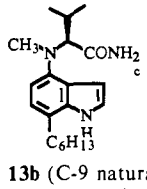
Scheme I. Simplification of the Lyngbyatoxin A Structure



α-oximino ester group at the C-3 position of the indole nucleus.[13] In this reaction, the undesired 5-substituted indole (3.5%) and the 3,5-disubstituted indole (5.7%) were also obtained as minor byproducts. The oxime group of **12a** was reduced to amine by the action of aluminum amalgam,[14] and the less hindered ester group was reduced in turn with sodium borohydride to the amino alcohol **13a**.[15] While numerous attempts were made to effect lactam ring formation through methods involving ester hydrolysis followed by carboxyl group activation, the overall process proved cumbersome as well as low yielding. On the other hand, by simply treating the amine **13a** with triethylaluminum[16] in toluene at reflux for 12 h, the desired lactamization reaction proceeded efficiently to provide a readily separable mixture of the diastereomeric lactams **1a**, $[\alpha]^{22}_D$ −120° (c = 0.060, MeOH), and **14a** $[\alpha]^{23}_D$ −85.4° (c = 0.130, MeOH), in 22% and 27% yields, respectively. Similarly, the treatment of **13b** with triethylaluminum gave the n-hexyl derivative **1b**, $[\alpha]^{22}_D$ −102° (c = 0.050, MeOH), and **14b**, $[\alpha]^{22}_D$ −87° (c = 0.045, MeOH), in 24% and 26% yields, respectively. Trimethylaluminum and triisobutylaluminum also promoted this cyclization reaction under the same conditions. The former reagent gave an 11% yield of **1b** and a 7% yield of **14b**, while the latter reagent gave 25% and 26% yields, respectively. The optical rotations of **1a** and **1b** are in line with that reported for natural lyngbyatoxin A (−108.4°).[3b]

The present synthetic work provides a novel and efficient solution to the procurement of lyngbyatoxin-related products in optically pure (or nearly so) form. The use of an isoxazoline-carboxaldehyde as the focal point for the joining of two other key building blocks, an amino acid and a pyrrole ring, in the elaboration of an indole is fundamental to the efficiency of the overall strategy. Given the relative simplicity (11 steps from **4**) of the process, the search for biologically promising "diacyl glycerol site specific" modulators of PKC can begin in earnest. The biological activity of several of the analogues and intermediates generated during the course of the present synthetic efforts are recorded below.

Table I. Effects of Various Synthetic Analogues and Intermediates of Lyngbyatoxin A on the Activation of Protein Kinase C

compound added	protein kinase C activity,[a] cpm/min per mg protein × 10^{-3}
none	722
TPA[b]	1940
lyngbyatoxin A	1825
 [c]	731
 [c]	797
13b (C-9 natural stereochemistry)	821
13b′ (C-9 epi stereochemistry)	699
1b	1713
14b	1209

[a] The results were representative of three experiments. [b] TPA, 12-*O*-tetradecanoylphorbol-13-acetate. [c] These amides were prepared from the corresponding esters by reaction with dimethylaluminum amide.

Effects of Intermediates and Analogues of Lyngbyatoxin A on Protein Kinase C Activity. We have examined the ability of several of our synthetic lyngbyatoxin analogues and intermediates to activate protein kinase C. Protein kinase C was partially purified from HeLa cells as described previously, and enzyme activity was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into H1 histone (Sigma).[17] As is apparent from Table

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(13) Attempts were made to introduce the indole C-3 appendage into **2** in enantiomerically correct form by use of the aziridine prepared from L-serine. Unfortunately, the Lewis acid promoted reactions of such aziridine derivatives with indole were low yielding.

(14) Drinkwater, D. J.; Smith, P. W. G. *J. Chem. Soc., Chem. Commun.* **1971**, 1305.

(15) We wish to thank Dr. M. Natsume for disclosing the experimental details of this ester reduction; see: Reference 5f.

(16) For the application of Et_3Al and $(i\text{-}Bu)_3Al$ in the construction of β-lactams, see: (a) Woodward, R. B.; Heusler, K.; Gosteli, J.; Naegeli, P.; Oppolzer, W.; Ramage, R.; Ranganathan, S.; Vorbrüggen, H. *J. Am. Chem. Soc.* **1966**, *88*, 852. (b) Cainelli, G.; Giacomini, D.; Panunzio, M.; Martelli, G.; Spunta, G. *Tetrahedron Lett.* **1987**, *28*, 3593.

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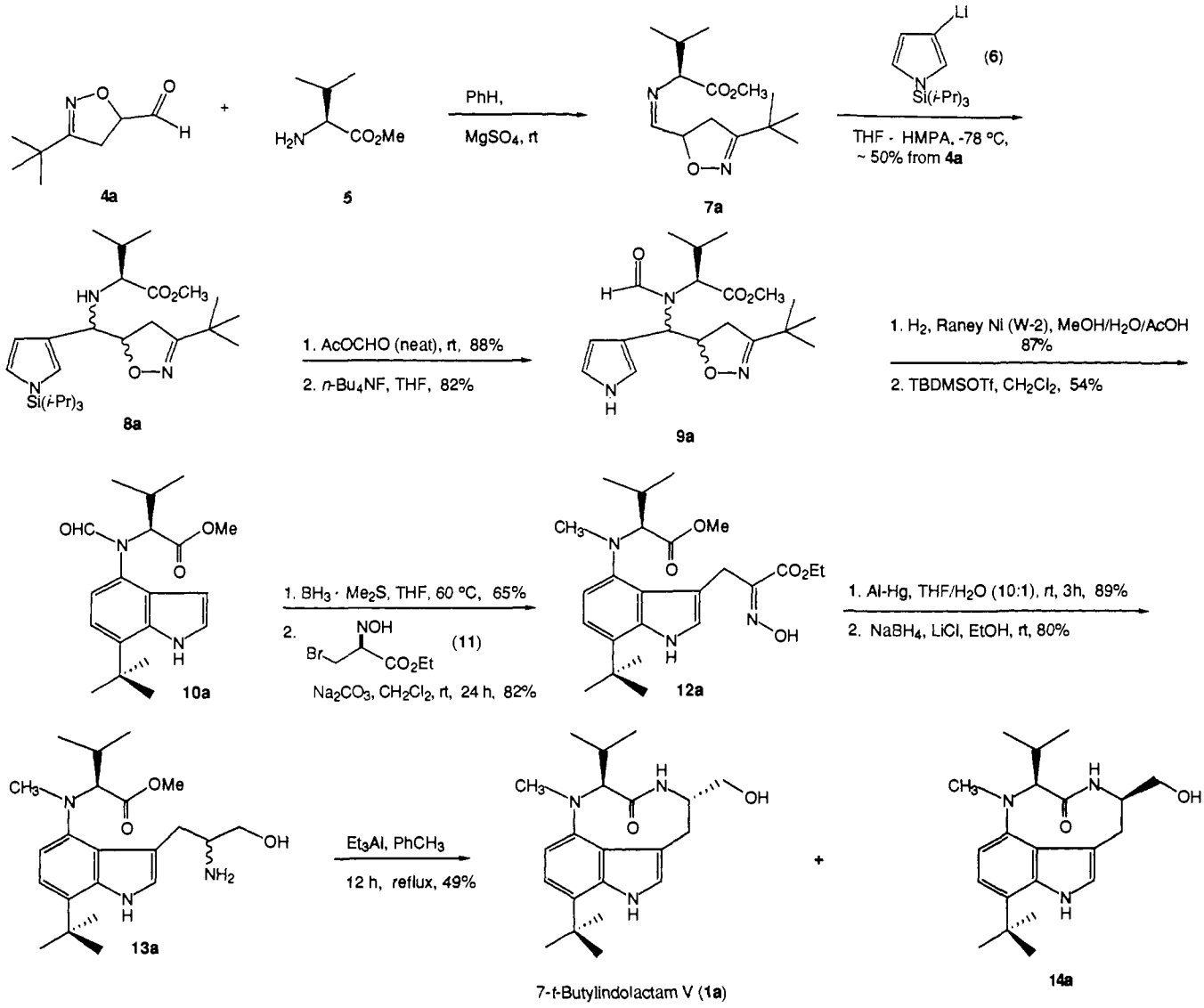
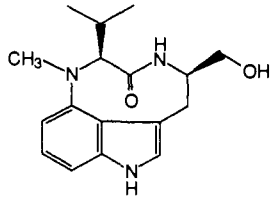
Scheme II. A Synthesis of 7-*tert*-Butylindolactam V



I, lyngbyatoxin A was as effective as phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), in stimulating protein kinase C activity. Compound **1b**, which retains the nine-membered lactam ring of lyngbyatoxin A but contains an *n*-hexyl group in place of the linalyl group at position 7 of the indole ring, also activated protein kinase C significantly. Interestingly, while (−)-epiindolactam V was found to be inactive in [^{3}H]TPA binding



(-) - epi-Indolactam V

experiments, human promyelocytic leukemia cell (HL-60) adhesion studies, and ornithine decarboxylase (ODC) induction assays, our epi analogue **14b** was found to be a good activator of protein kinase C (Table I) as well as ODC (data not shown).[18] In contrast to the PKC activity of **1b** and **14b**, all of the other compounds tested that lack the lactam ring structure failed to enhance the activity of protein kinase C.

From the present studies we conclude that the replacement of the more complex linalyl fragment of lyngbyatoxin A by the simpler *n*-hexyl fragment does not compromise protein kinase C stimulatory activity. The presence of the lactam ring structure appears essential for activity, while unnatural stereochemistry at C-9 (lyngbyatoxin A numbering system) does not lead to the extinction of activity. Further studies pertaining to the action of these compounds on protein kinase C are underway and will be described separately.

Experimental Section

3-Hexyl-2-isoxazoline-5-carbaldehyde (4b). To a solution of heptanaldoxime (8.50 g, 65.8 mmol) in DMF (30 mL) was slowly added *N*-chlorosuccinimide (9.66 g, 72.4 mmol) in DMF (30 mL), keeping the reaction temperature at 40–50 °C. After being stirred for an additional 30 min at 40–50 °C, the reaction solution was diluted with water and extracted with ether. The combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo to afford 13.02 g of 1-chloroheptanaldoxime as a crude oil.

Crude 1-chloroheptanaldoxime (13.02 g) was dissolved in methylene chloride (40 mL) and added to a solution of acrolein dimethyl acetal (40 mL, 0.336 mol) and triethylamine (27.5 mL, 0.198 mol) in methylene chloride (30 mL) at 10 °C. The resulting suspension was stirred for 3 h at room temperature and poured into water. The reaction mixture was

(18) Fujiki, H.; Suganuma, M.; Nakayasu, M.; Tahira, T.; Endo, Y.; Shudo, K.; Sugimura, T. *Gann* **1984**, *75*, 866. Compound **14b** was also found to be active in the ODC induction assay. We acknowledge Dr. Marcia Dawson of the Stanford Research Institute for carrying out this study.

extracted with methylene chloride, and the extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil (13.7 g) of crude 5-(dimethoxymethyl)-3-hexyl-2-isoxazoline was used in the next reaction without further purification.

A mixture of crude 5-(dimethoxymethyl)-3-hexyl-2-isoxazoline (13.77 g), methanol (450 mL), water (220 mL), and concentrated sulfuric acid (35 mL) was gently refluxed (the oil bath temperature was ~95 °C) for 12 h. After cooling, half of the volume of the reaction solution was removed in vacuo with a rotary evaporator, and the remainder was diluted with water and extracted with ether. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil was purified with silica gel chromatography using ethyl acetate–hexane in a gradient from 1:2 to 1:1 as eluent to yield 7.42 g (56.1%) of **4b** as a light yellow oil: IR (film) 3650–3100, 2970, 2930, 2860, 1628, 1460, 1450, 1435 cm^{-1}. The NMR spectrum showed that **4b** was a mixture of aldehyde and its hydrate (1:2). ^1H NMR ($CDCl_3$) δ 9.72 (s, 0.33 H), 4.84 (dd, 0.33 H, J = 10.1, 7.1 Hz), 4.4–4.6 (m, 0.67 H), 3.45 (d, 0.67 H, J = 2.4 Hz), 3.16 (dd, 0.33 H, J = 7.1, 17.9 Hz), 3.09 (dd, 0.33 H, J = 10.1, 17.9 Hz), 2.8–3.1 (m, 1.33 H), 2.34 (dd, 2 H, J = 7.6, 15.0 Hz), 1.29 (s, 8 H), 0.89 (t, 3 H, J = 6.7 Hz); mass spectrum, m/z 183 (M^+ for aldehyde), 154, 126, 133, 43; exact mass calcd for $C_{10}H_{17}NO_2$ 183.1259, found 183.1260.

3-*tert*-Butyl-2-isoxazoline-5-carbaldehyde (4a). In a similar manner as shown for the synthesis of **4b**, 4.53 g (57.5%) of **4a** was obtained from 4.60 g of *tert*-butanaldoxime as a light yellow oil: IR (film) 3100–3600, 2967, 2930, 2900, 2872, 1734, 1616, 1480, 1462, 1435, 1397, 1365 cm^{-1}. The NMR spectrum showed that **4a** was a mixture of aldehyde and its hydrate (1:1). ^1H NMR ($CDCl_3$) δ 9.71 (s, 0.5 H), 4.85 (dd, 0.5 H, J = 6.0, 11.0 Hz), 4.57 (m, 0.5 H), 3.47 (d, 0.5 H, J = 3.0 Hz), 2.9–3.3 (m, 2 H), 1.21 (s, 9 H); mass spectrum, m/z 155 (M^+ for aldehyde), 126, 84, 75; exact mass calcd for $C_8H_{13}NO_2$ 155.0946, found 155.0946.

3-Hexyl-5-[[[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino][1-(triisopropylsilyl)pyrrol-3-yl]methyl]-2-isoxazoline (8b). A mixture of aldehyde **4b** (2.36 g, 11.7 mmol), L-valine methyl ester (1.53 g, 11.7 mmol), and anhydrous $MgSO_4$ (1.4 g) in benzene (35 mL) was stirred for 1 h at room temperature. The reaction mixture was filtered, and the filter cake was washed with benzene. The combined benzene layers were concentrated in vacuo. In order to remove water as a benzene azeotrope, the residue was dissolved in benzene and concentrated in vacuo again to give the imine **7b** as a crude oil, which was used for further reaction without purification.

The NMR spectrum showed that **7b** was a mixture of two isomers (1:1): ^1H NMR ($CDCl_3$) δ 7.70 (d, J = 3.3 Hz) and 7.65 (d, J = 4.7 Hz) (1 H), 5.09–5.13 (m, 1 H), 3.73 (s) and 3.74 (s) (3 H), 3.55 (d, 1 H, J = 6.8 Hz), 3.10–3.22 (m, 2 H), 2.36 (t, 2 H, J = 7.7 Hz), 2.25 (octet, 1 H, J = 6.8 Hz), 1.56 (m, 2 H), 1.29 (br s, 6 H), 0.92 (d, 3 H, J = 6.8 Hz), 0.88 (d, 3 H, J = 6.8 Hz), 0.86 (t, 3 H, J = 6.7 Hz).

To a solution of 3-bromo-1-(triisopropylsilyl)pyrrole (6.03 g, 19.9 mmol) in THF (50 mL) at −23 °C was added under an argon atmosphere 12.8 mL (26.9 mmol) of *n*-butyllithium (2.1 M solution in hexane). After 30 min at −23 °C, the reaction solution was cooled to −78 °C, and then HMPA (10.0 mL, 58.5 mmol) was added. The mixture was stirred for 15 min at −78 °C and then added by syringe to a solution of crude **7b** in THF (150 mL) at −78 °C.

After 1 h at −78 °C, the mixture was diluted with aqueous ammonium chloride, and then the cooling bath was removed. The reaction mixture was warmed to 0 °C, poured into water, and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel with a stepwise gradient of ethyl acetate (10–40%) in hexane as eluent to give 2.91 g (47.9% from **4b**) of a diastereomeric mixture (ca. 1:1) of compound **8b** as an oil: R_f = 0.42 and 0.46 (hexane–ethyl acetate, 4:1). This mixture was used in the next reaction without further separation. For analytical use, the diastereomixture **8b** was partially separated by silica gel chromatography by using a gradient of hexane–ethyl acetate (8:1–4:1). Less polar isomer of **8b**: IR (film) 3100–3600 (m), 2940 (s), 2930 (s), 2870 (s), 1738 (s), 1464 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.66 (t, 1 H, J = 2.8 Hz), 6.61 (s, 1 H), 6.18 (t, 1 H, J = 1.2 Hz), 4.77 (td, 1 H, J_t = 10.8, J_d = 3.0 Hz), 3.85 (d, 1 H, J = 3.0 Hz), 3.57 (s, 3 H), 3.25 (d, 1 H, J = 6.3 Hz), 3.12 (dd, 1 H, J = 10.8, 16.9 Hz), 2.74 (dd, 1 H, J = 10.8, 16.9 Hz), 2.24 (t, 2 H, J = 7.7 Hz), 1.84 (octet, 1 H, J = 6.3 Hz), 1.3–1.55 (m, 5 H), 1.27 (br s, 6 H), 1.07 (d, 18 H, J = 7.4 Hz), 0.7–0.95 (m, 9 H); mass spectrum, m/z 460 (M^+ − 59), 389, 388, 365; exact mass calcd for $C_{22}H_{39}N_3OSi$ ($M^+ - C_7H_{14}O_2$) 389.2862, found 389.2862. More polar isomer of **8b**: IR (film) 3200–3600 (m), 2940 (s), 2930 (s), 2870 (s), 1738 (s), 1466 (s), 1435 (m); ^1H NMR ($CDCl_3$) δ 6.68 (t, 1 H, J = 2.3 Hz), 6.64 (s, 1 H), 6.23 (s, 1 H), 4.71 (ddd, 1 H, J v 6.9, 7.7, 10.0 Hz), 3.58 (d, 1 H, J = 6.9 Hz), 3.50 (s, 3 H), 3.14 (d, 1 H, J = 6.6 Hz), 2.76 (dd, 1 H, J = 10.0, 17.9 Hz), 2.66 (dd, 1 H, J = 7.7, 17.9 Hz), 2.25 (t, 2 H, J = 7.6 Hz), 1.91 (octet, 1 H, J = 6.6 Hz), 1.35–1.55 (m, 5 H), 1.30 (br s, 6 H), 1.08 (d, 18 H, J = 7.4 Hz), 0.94 (d, 3 H, J = 6.6 Hz), 0.91 (d, 3 H, J = 6.6 Hz), 0.88 (t, 3 H, J = 6.6 Hz); mass spectrum, m/z 389 (M^+ − 130), 388, 365.

3-*tert*-Butyl-5-[[[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino][1-(triisopropylsilyl)pyrrol-3-yl]methyl]-2-isoxazoline (8a). In a similar manner as shown for the synthesis of **8b**, 0.283 g (50.0%) of a diastereomeric mixture of **8a** was obtained from aldehyde **4a** (0.20 g, 1.15 mmol), L-valine methyl ester (0.159 g, 1.21 mmol), 3-bromo-1-(triisopropylsilyl)pyrrole (0.80 g, 2.65 mmol), and *n*-butyllithium (2.5 M solution in hexane, 1.11 mL, 2.78 mmol). This diastereomeric mixture was used for the next reaction without further separation: IR (film) 3335 (br w), 2957 (s), 2869 (s), 1738 (s), 1661 (w), 1530 (w), 1464 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.61–6.78 (m, 2 H), 6.19 (s) and 6.23 (s) (1 H), 4.60–4.95 (m, 1 H), 3.56–3.84 (m, 1 H), 3.50 (s) and 3.57 (s) (3 H), 3.15 (d, J = 6.0 Hz), and 3.25 (d, J = 6.0 Hz) (1 H), 2.76–3.04 (m, 2 H), 1.94 (m, 1 H), 1.35–1.50 (m, 3 H), 1.05–1.20 (m, 27 H), 0.89–0.95 (m, 6 H); mass spectrum m/z 445 (M^+ − 46), 443, 365.

Formylation of Amine 8b. A mixture of amine **8b** (1.50 g, 2.89 mmol) and acetic formic anhydride (2.0 mL) was stirred for 20 min at room temperature. The pH of the reaction mixture was adjusted to 7 with aqueous sodium bicarbonate, and the solution was poured into water and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil was purified with alumina column chromatography [activity III, a stepwise gradient of ethyl acetate (20–50%) in hexane as eluent] to give 1.50 g (98.7%) of N-formylated **8b** as an oil: IR (film) 2700–3600 (br m), 2950 (s), 2940 (s), 2870 (s), 1741 (s), 1668 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.53 (s), 8.51 (s) and 8.49 (s) (1 H), 7.18 (d, J = 2.2 Hz), 6.93 (br s), 6.88 (br s) and 6.18–6.33 (m) (2 H), 6.33 (t, J = 1.2 Hz) and 6.29 (t, J = 1.2 Hz) (1 H), 5.79 (d, J = 4.1 Hz) and 5.75 (d, J = 4.1 Hz) (1 H), 4.80–5.02 (m, 1 H), 3.95 (d, J = 10.2 Hz) and 3.83 (d, J = 10.2 Hz) (1 H), 3.80 (s), 3.74 (s) and 3.71 (s) (3 H), 2.52–2.95 (m, 2 H), 2.27 (t, J = 7.6 Hz) and 2.19 (t, J = 7.6 Hz) (2 H), 2.05 (m, 1 H), 1.48 (m, 5 H), 1.20–1.40 (m, 6 H), 1.05–1.13 (m, 18 H), 0.80–0.95 (m, 6 H), 0.56 (d, J = 6.7 Hz), 0.52 (d, J = 6.7 Hz) and 0.48 (d, J = 6.7 Hz) (3 H); mass spectrum, m/z 547 (M^+), 530, 516, 503, 473, 471, 445, 393, 365; exact mass calcd for $C_{30}H_{53}N_3O_4Si$ 547.3805, found 547.3808.

Formylation of Amine 8a. In a similar manner as described for the synthesis of N-formylated **8b**, 3.12 g (88.0%) of N-formylated **8a** was obtained from 3.36 g (6.83 mmol) of amine **8a** and acetic formic anhydride (1.5 mL): IR (film) 3100–3500 (br w), 2959 (s), 2868 (s), 1742 (s), 1669 (s), 1466 (m), 1200 (s), 884 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.47 (s) and 8.52 (s) (1 H), 7.18 (d, J = 2.0 Hz), 6.99 (d, J = 2.0 Hz), 6.96 (s) and 6.86 (s) (1 H), 6.69 (d, J = 2.2 Hz), 6.72 (d, J = 2.2 Hz) and 6.74 (d, J = 2.2 Hz) (1 H), 6.31 (s, 1 H), 5.74 (d, J = 4.0 Hz), 5.87 (d, J = 4.0 Hz) and 5.91 (d, J = 4.0 H) (1 H), 4.75–4.95 (m, 1 H), 3.86 (d, J = 10.1 Hz) and 4.01 (d, J = 10.1 Hz) (1 H), 3.73 (s), 3.74 (s) and 3.80 (s) (3 H), 2.63 (dd, J = 8.6, 16.9 Hz) and 2.70–2.98 (m) (2 H), 2.05 (m, 1 H), 1.42 (m, 3 H), 0.85–1.20 (m, 27 H), 0.79 (d, J = 6.6 Hz) and 0.81 (d, J = 6.6 Hz) (3 H), 0.49 (d, J = 6.6 Hz) and 0.50 (d, J = 6.6 Hz) (3 H); mass spectrum, m/z 473 (M^+ − 46), 471, 445, 443, 393, 365; exact mass calcd for $C_{21}H_{37}N_2O_3Si$ ($M^+ - C_7H_{12}NO$) 393.2573, found 393.2572.

3-Hexyl-5-[[*N*-formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]-amino](pyrrol-3-yl)methyl]-2-isoxazoline (9b). A mixture of N-formylated **8b** (1.56 g, 2.85 mmol) and tetrabutylammonium fluoride (1.1 M solution in THF, 10 mL, 11.0 mmol) in THF (10 mL) was stirred for 30 min at room temperature. The reaction solution was diluted with H_2O and extracted with ethyl acetate. The extract was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residual oil was purified with alumina column chromatography [activity III, a stepwise gradient of ethyl acetate (30–50%) in hexane as eluent] to afford 1.10 g (94.8%) of **9b** as an oil: IR (film) 3100–3500 (br w), 2960 (s), 2930 (s), 2872 (m), 2860 (m), 1740 (s), 1661 (s), 1553 (w), 1246 (m), 789 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.49 (s), 8.51 (s) and 8.53 (s) (1 H), 8.26 (br s, 1 H), 6.91 (s) and 7.03 (s) (1 H), 6.74 (br s) and 6.89 (br s) (1 H), 6.24 (s, 1 H), 5.76 (d, 1 H, J = 4.4 Hz), 4.85–5.01 (m, 1 H), 3.77–3.90 (m, 1 H), 3.72 (s), 3.74 (s) and 3.79 (s) (3 H), 2.60–2.93 (m, 2 H), 2.20–2.40 (m, 2 H), 2.05 (m, 1 H), 1.55 (m, 2 H), 1.2–1.4 (m, 6 H), 0.80–0.95 (m, 6 H), 0.52 (d, J = 6.6 Hz), 0.58 (d, J = 6.6 Hz) and 0.60 (d, J = 6.6 Hz) (3 H); mass spectrum, m/z 374 (M^+ − 17), 346, 332, 315, 287, 237, 209; exact mass calcd for $C_{12}H_{17}N_2O_3$ ($M^+ - C_9H_{16}NO$) 237.1239, found 237.1239. Anal. Calcd for $C_{21}H_{33}N_3O_4$: C, 64.42; H, 8.50; N, 10.74. Found: C, 64.19; H, 8.64; N, 10.86.

3-*tert*-Butyl-5-[[*N*-formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino](pyrrol-3-yl)methyl]-2-isoxazoline (9a). In a similar manner as shown in the synthesis of **9b**, 1.98 g (73.8%) of **9a** was obtained from 3.76 g (7.24 mmol) of N-formylated **8a** and tetrabutylammonium fluoride (1.1 M solution in THF, 19.7 mL, 21.7 mmol): IR (film)

3100–3500 (w), 2967 (s), 2930 (s), 2874 (s), 1740 (s), 1660 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.48 (s) and 8.53 (s) (1 H), 8.30 (br s) and 8.54 (br s) (1 H), 6.89 (d, J = 1.8 Hz) and 7.03 (d, J = 1.8 Hz) (1 H), 6.73 (m) and 6.80 (m) (1 H), 6.24 (m, 1 H), 5.73 (d, J = 4.8 Hz) and 5.83 (m) (1 H), 4.8–5.1 (m, 1 H), 3.82 (d, J = 10.1 Hz) and 3.95 (d, J = 10.0 Hz) (1 H), 3.75 (s) and 3.79 (s) (3 H), 2.6–3.0 (m, 2 H), 2.10 (m, 1 H), 1.09 (s), 1.10 (s), 1.18 (s) and 1.19 (s) (9 H), 0.80–0.84 (m, 3 H) and 0.51–0.60 (m, 3 H); mass spectrum, m/z 317 (M^+ – 46), 315, 289, 287, 237, 209; exact mass calcd for $C_{12}H_{17}N_2O_3$ (M^+ – $C_7H_{12}NO$) 237.1239, found 237.1239.

3-[1-[*N*-Formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino]-2-hydroxy-4-ketodecyl]pyrrole (3b) and 4-[*N*-Formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino]-7-hexylindole (10b). A mixture of **9b** (5.25 g, 11.7 mmol) and W-2 Raney nickel (three spatula scoops) in methanol (100 mL), water (25 mL), and acetic acid (2.5 mL) was stirred under a hydrogen-filled balloon for 8 h at room temperature. The nickel catalyst was filtered, and methanol was removed by evaporation in vacuo. The residue was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Silica gel chromatography [a stepwise gradient of ethyl acetate (25–75%) in hexane as eluent] of the crude product afforded 2.103 g (45.6%) of hydroxy ketone **3b** as an oil and 0.186 g (4.4%) of indole **10b** as an oil, which crystallized slowly.

3b: IR (film) 3100–3600 (m), 2959 (s), 2930 (s), 2874 (m), 1740 (s), 1651 (s), 1547 (w), 1242 (s), 1048 (m) cm^{-1}; mass spectrum, m/z 394 (M^+), 393, 376, 358, 347, 330, 316, 299, 287, 271, 259, 238, 209; exact mass calcd for $C_{21}H_{32}N_2O_4$ (M^+ – H_2O) 376.2362, found 376.2364.

10b: $[\alpha]^{24}_D$ –73.4° (c = 1.0055, CH_2Cl_2); mp 69–70 °C; IR (film) 3100–3600 (m), 2950 (s), 2940 (s), 2865 (s), 2860 (s), 1745 (s), 1662 (s), 1435 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.71 (br s, 1 H), 8.69 (s, 1 H), 7.22 (t, 1 H, J = 7.7 Hz), 7.08 (d, 1 H, J = 7.7 Hz), 6.99 (d, 1 H, J = 7.7 Hz), 6.53 (t, 1 H, J = 2.6 Hz), 4.75 (d, 1 H, J = 9.3 Hz), 3.67 (s, 3 H), 2.82 (t, 2 H, J = 7.7 Hz), 2.46 (heptet d, 1 H, J_h = 6.7 Hz, J_d = 9.3 Hz), 1.74 (quintet, 2 H, J = 7.5 Hz), 1.20–1.45 (m, 6 H), 1.02 (d, 3 H, J = 6.7 Hz), 0.98 (d, 3 H, J = 6.7 Hz), 0.88 (t, 3 H, J = 6.7 Hz); mass spectrum, m/z 358 (M^+), 330, 299, 287, 271, 259, 244, 227, 217, 199; exact mass calcd for $C_{21}H_{30}N_2O_3$ 358.2255, found 358.2256. Anal. Calcd for $C_{21}H_{30}N_2O_3$: C, 70.36; H, 8.44; N, 7.82. Found: C, 70.16; H, 8.47; N, 7.98.

TBDMSOTf-Promoted Indole Cyclization Reaction of 3b. To a solution of hydroxy ketone **3b** (247 mg, 0.626 mmol) in dichloromethane (150 mL) under an argon atmosphere was added *tert*-butyldimethylsilyl trifluoromethanesulfonate (201 μL, 0.876 mmol). The reaction turned orange-brown in color after 1 min. After 15 min, the reaction was quenched by adding saturated sodium bicarbonate (30 mL) and was extracted with dichloromethane. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified with silica gel chromatography (30% ethyl acetate in hexane as eluent) to afford 116 mg (51.8%) of indole **10b**.

3-[1-[*N*-Formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino]-5,5-dimethyl-2-hydroxy-4-ketohexyl]pyrrole (3a). In a similar manner (except that reaction time was 24 h) as shown in the synthesis of **3b**, 2.34 g (87.8%) of hydroxy ketone **3a** was obtained from **9a** (3.76 g, 7.24 mmol): IR (film) 3150–3600 (br m), 2969 (s), 2876 (s), 1740 (s), 1705 (s), 1651 (s) cm^{-1}; mass spectrum, m/z 348 (M^+ – 18), 334, 319, 281, 263, 238, 209; exact mass calcd for $C_{19}H_{28}N_2O_4$ (M^+ – H_2O) 348.2049, found 348.2048.

7-*tert*-Butyl-4-[*N*-formyl-*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]amino]indole (10a). In a similar manner as shown in the synthesis of **10b** by TBDMSOTf-promoted indole cyclization reaction, 169.2 mg (55.0%) of indole **10a** was obtained from hydroxy ketone **3a** (321.3 mg, 0.972 mmol): $[\alpha]^{23}_D$ –55.9° (c = 0.7875, CH_2Cl_2); IR (film) 3300–3500 (m), 2965 (s), 2874 (m), 1742 (s), 1665 (s), 1509 (m), 1280 (m), 1206 (m), 1102 (m), 756 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.43 (s, 2 H), 7.26 (br s, 1 H), 7.11 (d, 1 H, J = 7.0 Hz), 7.08 (d, 1 H, J = 7.0 Hz), 6.57 (br s, 1 H), 4.73 (d, 1 H, J = 7.4 Hz), 3.68 (s, 3 H), 2.50 (m, 1 H), 1.52 (s, 9 H), 1.02 (d, 3 H, J = 6.8 Hz), 0.99 (d, 3 H, J = 6.8 Hz); mass spectrum, m/z 330 (M^+), 302, 287, 271, 259, 243, 227, 216, 199; exact mass calcd for $C_{19}H_{26}N_2O_3$ 330.1943, found 330.1944.

7-Hexyl-4-[*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]-*N*-methylamino]indole (2b) and N-deformylated 10b. To a solution of formamide **10b** (645 mg, 1.80 mmol) in THF (100 mL) under an argon atmosphere was added borane–methyl sulfide complex (10 M solution in THF, 0.54 mL, 5.40 mmol) at 0 °C. The solution was stirred for 30 min at 50 °C. After cooling, methanol (2 mL) was added, and the reaction mixture was stirred for an additional 5 min.

Hydrochloric acid (10%) in methanol (1 mL) was added, followed by heating at 50 °C for 2 min. After cooling, the reaction solution was diluted with aqueous sodium bicarbonate and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel flash chromatography (a stepwise gradient of ethyl acetate (12–25%) in hexane as eluent) to give 438 mg (70.7%) of **2b** and 87 mg (14.7%) of N-deformylated **10b**.

2b: $[\alpha]^{24}_D$ –166.4° (c = 0.6250, CH_2Cl_2); IR (film) 3100–3700 (br s), 2960 (s), 2930 (s), 2860 (s), 2855 (s), 1716 (s), 1504 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.10 (br s, 1 H), 7.14 (t, 1 H, J = 2.6 Hz), 6.86 (d, 1 H, J = 7.7 Hz), 6.74 (t, 1 H, J = 2.6 Hz), 6.54 (d, 1 H, J = 7.7 Hz), 4.06 (d, 1 H, J = 10.9 Hz), 3.63 (s, 3 H), 2.99 (s, 3 H), 2.74 (t, 2 H, J = 7.7 Hz), 2.37 (heptet d, 1 H, J_h = 6.5 Hz, J_d = 10.9 Hz), 1.70 (quintet, 2 H, J = 7.5 Hz), 1.25–1.45 (m, 6 H), 1.07 (d, 3 H, J = 6.7 Hz), 0.93 (d, 3 H, J = 6.7 Hz), 0.88 (t, 3 H, J = 6.9 Hz); mass spectrum, m/z 344 (M^+), 330, 301, 285 (100); exact mass calcd for $C_{21}H_{32}N_2O_2$ 344.2464, found 344.2464.

N-Deformylated **10b**: IR (film) 3406 (m), 2958 (s), 2928 (s), 2856 (m), 1738 (s), 1728 (s), 1606 (m), 1530 (m), 1504 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.70 (br s, 1 H), 8.41 (s, 1 H), 7.13 (d, 2 H, J = 7.6 Hz), 7.08 (d, 2 H, J = 7.6 Hz), 4.72 (d, 1 H, J = 9.4 Hz), 3.68 (s, 3 H), 2.83 (t, 2 H, J = 7.7 Hz), 2.43 (m, 1 H), 1.73 (m, 2 H), 1.20–1.55 (m, 6 H), 1.01 (d, 3 H, J = 6.8 Hz), 0.98 (d, 3 H, J = 6.8 Hz), 0.90 (t, 3 H, J = 6.6 Hz); mass spectrum, m/z 330 (M^+), 301, 287, 271 (base), 259, 227, 215, 199.

7-*tert*-Butyl-4-[*N*-[(1*S*)-(methoxycarbonyl)-2-methylpropyl]-*N*-methylamino]indole (2a) and N-deformylated 10a. In a similar manner as shown in the synthesis of **2b**, 157 mg (65.7%) of **2a** and 23 mg (10.1%) of N-deformylated **10a** were synthesized from 250 mg (0.755 mmol) of **10a**.

2a: $[\alpha]^{25}_D$ –163.2° (c = 0.486, CH_2Cl_2); mp 139 °C (hexane); IR (Nujol) 3300–3500 (br m), 1713 (s), 1591 (m), 1506 (m), 1377 (m), 1365 (m), 1209 (m), 1099 (m), 725 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.25 (br s, 1 H), 7.15 (t, 1 H, J = 2.9 Hz), 6.97 (d, 1 H, J = 8.0 Hz), 6.74 (t, 1 H, J = 2.9 Hz), 6.57 (d, 1 H, J = 8.0 Hz), 4.02 (d, 1 H, J = 10.8 Hz), 3.65 (s, 3 H), 3.01 (s, 3 H), 2.38 (m, 1 H), 1.48 (s, 9 H), 1.09 (d, 3 H, J = 6.7 Hz), 0.94 (d, 3 H, J = 6.7 Hz); mass spectrum, m/z 316 (M^+), 301, 273, 257, 243, 227, 213, 201, 185; exact mass calcd for $C_{19}H_{28}N_2O_2$ 316.2151, found 316.2150.

N-Deformylated **10a**: ^1H NMR ($CDCl_3$) δ 8.27 (br s, 1 H), 7.14 (t, 1 H, J = 2.9 Hz), 6.94 (d, 1 H, J = 8.0 Hz), 6.54 (t, 1 H, J = 2.9 Hz), 6.19 (d, 1 H, J = 8.0 Hz), 4.50 (br s, 1 H), 4.01 (d, 1 H, J = 6.0 Hz), 3.71 (s, 3 H), 2.20 (octet, 1 H, J = 6.8 Hz), 1.45 (s, 9 H), 1.10 (d, 3 H, J = 6.8 Hz), 1.05 (d, 3 H, J = 6.8 Hz).

3-Substituted Indole 12b, 5-Substituted 2b, and 3,5-Disubstituted 2b. To a mixture of indole **2b** (126 mg, 0.365 mmol) and sodium carbonate (85 mg, 0.803 mmol) in dichloromethane (25 mL) was added ethyl 3-bromo-2-ketopropionate oxime (84.3 mg, 0.402 mol). The resulting mixture was stirred for 24 h at room temperature. The reaction solution was poured into water, neutralized with 1 N hydrochloric acid, and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified with silica gel flash chromatography [a stepwise gradient of ethyl acetate (25–100%) in hexane as eluent] to afford 111.1 mg (64.2%) of the desired 3-substituted indole **12b**, 19.0 mg (11.0%) of 5-substituted **2b**, and 10.4 mg (4.8%) of 3,5-disubstituted **2b**.

12b: IR (film) 3100–3700 (br s), 2930 (s), 2860 (s), 2700 (s), 2650 (s), 1726 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.5 (br s, 1 H), 7.90 (s, 1 H), 6.87 (d, 1 H, J = 8.5 Hz), 6.84 (d, 1 H, J = 8.5 Hz), 6.79 (s, 1 H), 4.51 (d, 1 H, J = 15.0 Hz), 4.41 (d, 1 H, J = 15.0 Hz), 4.28 (q, 2 H, J = 7.1 Hz), 3.67 (d, 1 H, J = 8.6 Hz), 3.57 (s, 3 H), 2.90 (s, 3 H), 2.75 (dd, 1 H, J = 13.0, 7.6 Hz), 2.63 (dd, 1 H, J = 13.0, 5.5 Hz), 2.20–2.41 (m, 1 H), 1.6–1.8 (m, 2 H), 1.30 (br s, 6 H), 1.28 (t, 3 H, J = 7.1 Hz), 1.25 (d, 3 H, J = 7.0 Hz), 1.12 (d, 3 H, J = 7.0 Hz), 0.88 (t, 3 H, J = 6.8 Hz); mass spectrum, m/z 473 (M^+), 457, 430, 414, 398, 366, 354, 340; exact mass calcd for $C_{26}H_{39}N_3O_5$ 473.2892, found 473.2890.

5-Substituted **2b**: IR (film) 3100–3550 (br s), 2950 (s), 2930 (s), 2870 (s), 2890 (s), 1732 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.6 (br s, 1 H), 8.01 (s, 1 H), 7.11 (s, 1 H), 6.70 (s, 1 H), 6.51 (s, 1 H), 4.38 (d, 1 H, J = 14.3 Hz), 4.25 (q, 2 H, J = 7.1 Hz), 4.09 (d, 1 H, J = 14.3 Hz), 3.44 (s, 3 H), 3.33 (d, 1 H, J = 10.1 Hz), 3.08 (s, 3 H), 2.70 (t, 2 H, J = 7.7 Hz), 2.30 (m, 1 H), 1.4–1.7 (m, 2 H), 1.30 (br s, 6 H), 1.29 (d, 3 H, J = 7.1 Hz), 1.28 (t, 3 H, J = 7.1 Hz), 1.23 (d, 3 H, J = 7.1 Hz), 0.88 (t, 3 H, J = 6.7 Hz); mass spectrum, m/z 473 (M^+), 457, 430, 414, 398.

3,5-Disubstituted **2b**: IR (film) 3100–3600 (br s), 2950 (s), 2930 (s), 2870 (s), 2860 (s), 1738 (s), 1732 (s), 1714 (s), 1699 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.65 (br s, 2 H), 7.81 (s, 1 H), 6.72 (s, 1 H), 6.47 (s, 1 H), 4.49 and 4.81 (AB q, 2 H, J = 15.8 Hz), 4.2–4.4 (m, 5 H), 3.86 and 4.08 (AB q, 2 H, J = 14.5 Hz), 3.77 (s, 3 H), 2.95 (s, 3 H), 2.65 (t, 2 H, J = 7.7 Hz), 1.95 (m, 1 H), 1.65 (br s, 2 H), 1.2–1.5 (m, 12 H), 0.98 (d, J = 6.8 Hz), 0.85 (br s) and 0.74 (d, J = 6.8 Hz) (9 H); mass spectrum, m/z 451 (M^+ – 151), 283, 253, 91.

3-Substituted Indole 12a, 5-Substituted 2a, and 3,5-Disubstituted Indole 2a. In a similar manner as described in the synthesis of **12b**, 121.2 mg (82.0%) of **12a**, 5.2 mg (3.5%) of 5-substituted **2a**, and 10.8 mg (5.7%) of 3,5-disubstituted **2a** were obtained from **2a** (105 mg), sodium carbonate (81 mg), and ethyl 3-bromo-2-ketopropionate oxime (80.2 mg).

12a: IR (film) 3100–3500 (br s), 2961 (s), 2905 (m), 2874 (m), 1726 (vs), 1512 (m), 1202 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.37 (br s, 1 H), 8.09 (br s, 1 H), 6.97 (d, 1 H, J = 8.0 Hz), 6.85 (d, 1 H, J = 8.0 Hz), 6.78 (d, 1 H, J = 2.4 Hz), 4.53 and 4.43 (AB q, 2 H, J = 15.1 Hz), 4.28 (q, 2 H, J = 7.0 Hz), 3.68 (d, 1 H, J = 8.4 Hz), 3.59 (s, 3 H), 2.89 (s, 3 H), 2.30 (octet, 1 H, J = 6.7 Hz), 1.45 (s, 9 H), 1.29 (3 H, t, J = 7.0 Hz), 1.12 (3 H, d, J = 6.7 Hz), 0.95 (3 H, d, J = 6.7 Hz); mass spectrum, m/z 445 (M^+), 429, 402, 386, 370, 312; exact mass calcd for $C_{24}H_{35}N_3O_5$ 445.2577, found 445.2577.

5-Substituted **2a**: IR (film) 3100–3600 (br s), 2963 (s), 2910 (m), 2870 (m), 1723 (vs), 1198 (s), 1020 (m), 736 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.04 (br s, 1 H), 8.20 (s, 1 H), 7.11 (t, 1 H, J = 2.6 Hz), 6.85 (s, 1 H), 6.50 (br s, 1 H), 4.41 and 4.08 (AB q, 2 H, J = 15.0 Hz), 4.24 (q, 2 H, J = 7.1 Hz), 3.46 (s, 3 H), 3.34 (d, 1 H, J = 9.9 Hz), 3.08 (s, 3 H), 2.31 (m, 1 H), 1.43 (s, 9 H), 1.26 (t, 3 H, J = 7.1 Hz), 1.24 (d, 3 H, J = 6.9 Hz), 0.89 (d, 3 H, J = 6.9 Hz); mass spectrum, m/z 445 (M^+), 425, 402, 386; exact mass calcd for $C_{24}H_{35}N_3O_5$ 445.2577, found 455.2575.

3,5-Disubstituted **2a**: IR (film) 3100–3600 (br s), 2965 (s), 1732 (s), 1206 (m), 1126 (m), 1024 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.6 (br s, 2 H), 7.97 (s, 1 H), 6.69 (s, 1 H), 6.58 (s, 1 H), 4.98 and 4.46 (AB q, 2 H, J = 16.0 Hz), 4.2–4.4 (m, 5 H), 4.08 and 3.87 (AB q, 2 H, J = 15.4 Hz), 3.77 (s, 3 H), 2.94 (s, 3 H), 1.9–2.1 (m, 1 H), 1.39 (s, 9 H), 1.25–1.35 (m, 6 H), 0.98 (d, 3 H, J = 6.9 Hz), 0.74 (d, 3 H, J = 6.9 Hz); mass spectrum, m/z 574 (M^+), 557, 541, 531, 515, 499; exact mass calcd for $C_{29}H_{42}N_4O_8$ 574.3003, found 574.3003.

Reduction of Oxime 12b. To a solution of oxime ester **12b** (24.6 mg, 0.521 mmol) in 10% H_2O–THF (30 mL) were added aluminum amalgam, which was prepared from 0.25 g of aluminum (granular) and 2% mercury(II) chloride (0.1 mL) in 10% H_2O–THF (0.5 mL). The reaction mixture was stirred for 3 h at room temperature. Sodium bicarbonate (3 mL) was added, and the reaction mixture was filtered. The filter cake was washed with ethyl acetate. The filtrate was diluted with water and extracted with ethyl acetate. The extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 203.4 mg (85.0%) of the desired reduced amine as a crude oil. This oil was used in the next step without further purification: IR (film) 3100–3500 (br w), 2957 (s), 2928 (s), 2860 (m), 1732 (s), 1456 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.16 (br s, 1 H), 7.03 (s, 1 H), 6.78–6.86 (m, 2 H), 4.12 (q, J = 7.1 Hz) and 4.18 (q, J = 7.1 Hz) (2 H), 3.6–3.75 (m, 2 H), 3.46 (s, 3 H), 3.35 (m, 1 H), 2.99 (dd, 1 H, J = 8.8, 14.3 Hz), 2.83 (s) and 2.85 (s) (3 H), 2.73 (t, 2 H, J = 7.7 Hz), 2.30 (m, 1 H), 1.70 (m, 2 H), 1.30 (br s, 6 H), 1.1–1.25 (m, 6 H), 0.9–1.05 (m, 6 H); mass spectrum, m/z 459 (M^+), 445, 429, 416, 400, 386, 357, 343, 315.

Reduction of Oxime 12a. In the same manner as described above, 64.8 mg (89.0%) of reduced amine was obtained as a crude product from **12a** (75.2 mg, 0.169 mmol): IR (film) 3300–3500 (br w), 2959 (s), 2874 (m), 1732 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.22 (br s, 1 H), 7.06 (s, 1 H), 6.97 (d, 1 H, J = 7.9 Hz), 6.81 (d, J = 7.9 Hz) and 6.82 (d, J = 7.9 Hz) (1 H), 4.12 (q, J = 7.1 Hz) and 4.18 (q, J = 7.1 Hz) (2 H), 3.6–3.75 (m, 2 H), 3.49 (s) and 3.50 (s) (3 H), 3.39 (m, 1 H), 3.03 (dd, 1 H, J = 8.6, 14.7 Hz), 2.83 (s) and 2.84 (s) (3 H), 2.30 (m, 1 H), 1.46 (s, 9 H), 1.21–1.38 (m, 3 H), 1.10–1.20 (m, 3 H), 0.95 (br s, 3 H); mass spectrum, m/z 431 (M^+), 417, 388, 372, 358, 329.

Amino Alcohol 13b. A mixture of the amino ester from oxime **12b** (318 mg, 0.692 mmol), lithium chloride (263 mg, 6.23 mmol) and sodium borohydride (236 mg, 6.23 mmol) in THF (12 mL), and ethanol (6 mL) was stirred under an argon atmosphere for 2 h at room temperature. Water (0.1 mL) was added to the reaction mixture, and the mixture was loaded directly on a silica gel flash column. Elution with 8% methanol in dichloromethane afforded 165.4 mg (57.3%) of **13b** as a gum: IR (film) 3000–3600 (br s), 2960 (s), 2926 (s), 2855 (s), 1733 (s), 1040 (s), 735 (m) cm^{-1}; ^1H NMR ($CDCl_3$ + MeOH-d_4, 8:1) δ 7.15 (d, 1 H, J = 11.8 Hz), 6.88 (d, 1 H, J = 7.8 Hz), 6.80 (d, 1 H, J = 7.8 Hz), 3.90 (dd, 1 H, J = 12.2 Hz, the other J is very small), 3.78 (dd, 1 H, J = 12.2, 4.4 Hz), 3.64 (d, J = 6.5 Hz) and 3.68 (d, J = 6.5 Hz) (1 H), 3.58 (s) and 3.61 (s) (3 H), 3.38 (m, 1 H), 3.16 (dd, 1 H, J = 14.5, 7.0 Hz), 3.04 (dd, 1 H, J = 14.5, 5.2 Hz), 2.80 (t, 2 H, J = 7.7 Hz), 2.73 (s, 3 H), 2.42 (br s, 1 H), 1.69 (m, 2 H), 1.3–1.45 (m, 6 H), 1.05–1.20 (m, 6 H), 0.89 (t, 3 H, J = 6.8 Hz); mass spectrum, m/z 417 (M^+), 400, 384, 374, 358, 315, 300; exact mass calcd for $C_{24}H_{39}N_3O_3$ 417.2991, found 417.2991.

Amino Alcohol 13a. In the same manner as described above, 20.9 mg (80.0%) of **13a** was obtained from the corresponding oxime ester (28.9 mg, 0.067 mmol) as a gum: IR (film) 3100–3600 (br s), 2963 (s), 1734 (s), 1508 (s), 1204 (m), 1044 (m), 814 (m), 737 (s) cm^{-1}; mass spectrum, m/z 389 (M^+), 358, 346, 330, 387, 271; exact mass calcd for $C_{22}H_{35}N_3O_3$ 389.2678, found 389.2678.

7-*n*-Hexylindolactam V (1b) and epi-7-*n*-hexylindolactam V (14b). To a solution of amino alcohol **13b** (8.0 mg, 0.019 mmol) in toluene (5 mL) under an argon atmosphere was added triethylaluminum (1.9 M solution in toluene, 90.9 μL, 0.173 mmol). After being stirred for 30 min at room temperature, the reaction solution was refluxed at 115 °C for 14 h and cooled. Methanol (0.1 mL) was added to the reaction, followed by stirring for 10 min. The solution was diluted with water and extracted with ethyl acetate. The extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified with silica gel column chromatography [a stepwise gradient of ethyl acetate (50–100%) in hexane as eluent] to give 1.8 mg (24%) of **1b** as a less polar compound and 1.9 mg (26%) of **14b** as a polar compound.

1b: $[\alpha]^{22}_D$ –102° (c = 0.050, MeOH); IR (film) 3100–3600 (br m), 2957 (m), 2927 (s), 2885 (m), 1646 (s), 1506 (m), 1447 (m), 1021 (m) cm^{-1}. ^1H NMR (MeOH-d_4) signals showed that **1b** was a mixture (1:1) of two conformers. Twist form: ^1H NMR (MeOH-d_4) δ 6.94 (s, 1 H), 6.75 (d, 1 H, J = 7.5 Hz), 6.39 (d, 1 H, J = 7.5 Hz), 4.42 (d, 1 H, J = 10.1 Hz), 4.25 (m, 1 H), 3.62 (dd, 1 H, J = 3.9, 11.2 Hz), 3.45 (m, 1 H), 3.06 and 3.10 (AB q, 2 H, J = 15.4 Hz), 2.87 (s, 3 H), 2.82 (t, 2 H, J = 7.4 Hz), 2.53 (m, 1 H), 1.67 (m, 2 H), 1.3–1.4 (m, 6 H), 0.89 (m, 6 H), 0.62 (d, 3 H, J = 6.6 Hz). Sofa form: ^1H NMR (MeOH-d_4) δ 7.11 (s, 1 H), 6.89 (d, 1 H, J = 6.8 Hz), 6.86 (d, 1 H, J = 6.8 Hz), 4.25 (m, 1 H), 3.35 (m, 1 H), 3.23 (dd, 1 H, J = 7.0, 10.4 Hz), 3.05 (d, 1 H, J = 11.0 Hz), 3.00 (dd, 1 H, J = 4.3, 14.4 Hz), 2.87 (m, 1 H), 2.74 (t, 2 H, J = 7.6 Hz), 2.70 (s, 3 H), 2.31 (m, 1 H), 1.67 (m, 2 H), 1.3–1.4 (m, 6 H), 1.24 (d, 3 H, J = 6.8 Hz), 0.89 (m, 6 H); mass spectrum, m/z 385 (M^+), 354, 342, 326, 314, 299, 255; exact mass calcd for $C_{23}H_{35}N_3O_2$ 385.2729, found 385.2728.

14b: $[\alpha]^{22}_D$ –87° (c = 0.045, MeOH); IR (film) 3100–3600 (br m), 2957 (s), 2927 (s), 2857 (m), 1653 (s), 1506 (m), 1451 (m), 1031 (m) cm^{-1}; ^1H NMR (300 MHz, MeOH-d_4) δ 6.93 (s, 1 H), 6.74 (d, 1 H, J = 7.7 Hz), 6.65 (d, 1 H, J = 7.7 Hz), 3.98 (d, 1 H, J = 10.5 Hz), 3.55–3.75 (m, 3 H), 2.93 and 3.12 (AB q, 2 H, J = 17.5 Hz), 3.06 (s, 3 H), 2.70 (m, 2 H), 2.56 (m, 1 H), 1.67 (m, 2 H), 1.25–1.4 (m, 6 H), 0.88 (m, 3 H), 0.74 (d, 3 H, J = 6.6 Hz), 0.71 (d, 3 H, J = 6.6 Hz); mass spectrum, m/z 385 (M^+), 360, 342, 315, 301, 257; exact mass calcd for $C_{23}H_{35}N_3O_2$ 385.2729, found 385.2728.

7-*tert*-Butylindolactam V (1a) and epi-7-*tert*-Butylindolactam V (14a). In a similar manner as described in the synthesis of **1b** and **14b**, 1.7 mg (22%) of **1a** and 2.0 mg (27%) of **14b** were obtained from amino alcohol **13a** (8.2 mg, 0.016 mmol) and triethylaluminum (1.9 M solution in toluene, 86.0 μL, 0.163 mmol).

1a: $[\alpha]^{23}_D$ –120° (c = 0.060, MeOH); IR (film) 3150–3600 (s), 2960 (s), 2928 (s), 2880 (s), 2715, 1653 (s), 1507 (s), 1256, 1202, 1174, 1120, 1115, 1095, 1065, 1044, 945, 868, 802, 739, 700 cm^{-1}; ^1H NMR (300 MHz, MeOH-d_4) signals showed that **1a** was a mixture (1:1) of two conformers. Twist form: ^1H NMR (300 MHz, MeOH-d_4) δ 10.26 (br s, 1 H), 7.12 (s, 1 H), 7.01 (d, 1 H, J = 8.0 Hz), 5.89 (d, 1 H, J = 8.0 Hz), 4.40 (d, 1 H, J = 10.0 Hz), 4.26 (m, 1 H), 3.5–3.75 (m, 2 H), 3.09 (br s, 2 H), 2.87 (s, 3 H), 2.52 (m, 1 H), 1.44 (s, 9 H), 0.89 (d, 3 H, J = 6.5 Hz), 0.64 (d, 3 H, J = 6.5 Hz). Sofa form: ^1H NMR (300 MHz, MeOH-d_4) δ 9.96 (br s, 1 H), 6.96 (s, 1 H), 6.89 (d, 1 H, J = 8.0 H), 6.42 (d, 1 H, J = 8.0 Hz), 4.26 (m, 1 H), 3.2–3.4 (m, 2 H), 3.06 (d, 1 H, J = 11.2 Hz), 3.00 (dd, 1 H, J = 14.3, 4.4 Hz), 2.87 (dd, 1 H, J = 14.3, 1.8 Hz), 2.71 (s, 3 H), 2.32 (m, 1 H), 1.47 (s, 9 H), 1.24 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.5 Hz); mass spectrum, m/z 357 (M^+, base), 342, 326, 314, 298, 286, 271, 255, 241, 227, 215; exact mass calcd for $C_{21}H_{31}N_3O_2$ 357.2416, found 357.2417.

14a: $[\alpha]^{23}_D$ –85.4° (c = 0.130, MeOH); IR (film) 3150–3600, 2957 (s), 2930 (s), 2872 (s), 1653 (s), 1508, 1254, 1196, 1173, 1095, 1073, 1036, 945, 806, 739, 704 cm^{-1}; ^1H NMR (300 MHz, MeOH-d_4) δ 9.95 (br s, 1 H), 6.96 (br s, 1 H), 6.87 (d, 1 H, J = 8.0 Hz), 6.67 (d, 1 H, J = 8.0 Hz), 3.95 (d, 1 H, J = 10.6 Hz), 3.71–3.82 (m, 3 H), 2.92 and 3.16 (AB q, 2 H, J = 15.1 Hz), 3.05 (s, 3 H), 2.57 (m, 1 H), 1.44 (s, 9 H), 0.74 (d, 3 H, J = 6.6 Hz), 0.70 (d, 3 H, J = 6.6 Hz); mass spectrum, m/z 357 (M^+, base), 342, 326, 314, 298, 286, 271, 255, 241, 227, 215; exact mass calcd for $C_{21}H_{31}N_3O_2$ 357.2416, found 357.2415.

Protein Kinase C Assay. Protein kinase C was partially purified from HeLa cells by DE-52 cellulose chromatography as described previously.[17]

The enzyme activity was determined[17] by measuring incorporation of ^{32}P from [γ-^{32}P]ATP into H1 histone (Sigma). The reaction mixture contained 20 mM Tris-HCl (pH 7.5), 15 μM ATP (1 μCi [γ-^{32}P]ATP), 10 mM magnesium acetate, 20 μg/mL phosphatidylserine, 0.5 mM EGTA, 100 nM of the lyngbyatoxin A analogue, and 2 μg of the enzyme preparation in a final volume of 200 μL. The reaction was stopped with 1 mL of 25% trichloroacetic acid after incubation at 28 °C for 10 min. The precipitated protein was washed four times and counted in a liquid scintillation counter.

Acknowledgment. We are indebted to the University of Pittsburgh Central Research Development Fund for the support of these studies. We acknowledge Dr. S. Sakai of Chiba University for the samples of natural lyngbyatoxin A and teleocidin A-2.

Biosynthetic Studies of Brevetoxins, Potent Neurotoxins Produced by the Dinoflagellate *Gymnodinium breve*

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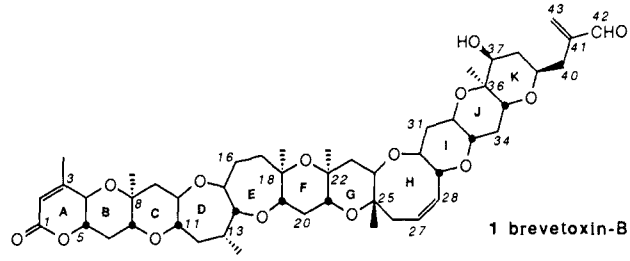
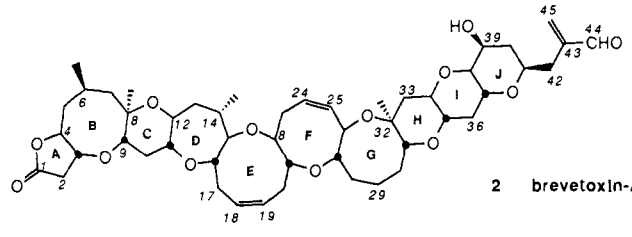
Received July 28, 1988. Revised Manuscript Received March 15, 1989

Abstract: Blooms of the dinoflagellate *Gymnodinium breve* (*Ptychodiscus brevis*) commonly known as "red tide" have led to massive fish kills, mollusk contamination, and human food intoxications along the Florida coast and the Gulf of Mexico. The toxins from *G. breve* responsible for these phenomena are the brevetoxins (BTX's), a group of potent neurotoxins with polycyclic trans-fused ether rings which presumably depolarize the sodium channels of the excitable membranes. BTX-B, $C_{50}H_{70}O_{14}$, the first of these neurotoxins whose structure was elucidated, has an unprecedented structure consisting of 6/6/6/7/7/6/6/8/6/6/6 ether rings trans-fused in a ladder-like manner. Another member of these toxins, BTX-A, $C_{49}H_{70}O_{13}$, has another remarkable structure consisting of trans-fused 5/8/6/7/9/6/6/6 ether rings. Although the carbon skeletons of BTX-B and BTX-A are different, both consist of a single carbon chain that is polyoxygenated with methyl substituent groups. This is consistent with polyketide biosynthesis, i.e., condensation of acetate units with the methyl groups originating from either *S*-adenosylmethionine or propionate. Labeling experiments using sodium [1-^{13}C]- and [2-^{13}C]acetate and [*methyl*-^{13}C]methionine demonstrate that the labeling patterns of BTX-B and BTX-A are similar and that the biosynthesis of brevetoxins is not of simple polyketide origin. These labeling studies suggest that the citric acid cycle is involved in the biosynthetis of BTX-B and BTX-A, the degree of its involvement being unusually high. Furthermore, CO_2 participates in a unique manner in the biosynthesis of C-1 of BTX-B and BTX-A.

Blooms of dinoflagellates commonly known as "red tide" have received much attention due to their toxic effects on the environment. One of the most toxic species, *Gymnodinium breve* (*Ptychodiscus brevis*), occurring along the Gulf of Mexico and Florida coast, has received much attention due to its devastating effects on fishing and tourist industries as well as its effects on the ecosystem.[1] Since 1968 numerous attempts had been made to isolate the lipid-soluble neurotoxins of *G. breve*[2] that depolarize the sodium channels of the excitable membranes.[3]

The structure of the first of these neurotoxins, brevetoxin-B (BTX-B) (**1**) $C_{50}H_{70}O_{14}$, was of unprecedented nature consisting of 11 ethereal 6/6/6/7/7/6/6/8/6/6/6 rings trans-fused in a ladder-like manner.[4] The structure of the major toxin BTX-B was determined by X-ray crystallography and the absolute configuration by the dibenzoate chirality method. The structures of five additional toxins in this series have been elucidated, i.e., BTX-C,[5] GB-3,[6] GB-5,[7] and GB-6.[7] This was followed by structure elucidation of BTX-A (**2**), $C_{49}H_{70}O_{13}$, the most potent and challenging of the brevetoxins which also had a remarkable structure consisting of trans-fused 5/8/6/7/9/8/8/6/6/6 ether rings. The structure of BTX-A (**2**) was elucidated by X-ray crystallography[8] and independently by extensive MS and NMR;[9] the spectroscopically derived structure was correct except for the C-6 configuration, which was assigned as 6α instead of 6β. The difficulty associated with NMR studies of BTX-A was due to the medium-sized rings which makes the molecule flexible, thus leading to broadening of signals; in contrast, BTX-B is quite stiff except around rings D/E where it can fold.





Recently, another class of toxins responsible for diarrheic shellfish poisoning (DSP) has received wide interest. Okadaic

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