of **15a** (0.7 g, 1 mmol) in THF (10 mL) was added, and the mixture was stirred for an additional 18 h. The mixture was poured into water, and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were dried and evaporated and the residue chromatographed (hexane/EtOAc 3/1) to give the product (0.3 g, 37%) as an oil: IR (film) 2980, 2940, 1700, 1415, 1390, 1370, 1250, and 1155 cm^{-1}; NMR ($CDCl_3$) 5.95 (bs, 2 H), 4.1 (s, 4 H), 3.8 (s, 4 H), 3.1 (bs, 4 H), 2.8 and 2.87 (s, 6 H total), 1.4 (s, 40 H), and 1.35 (s, 6 H); MS (CI/CH_4) 779 (M + H). Anal. ($C_{36}H_{65}Cl_2N_4O_8$) C, H, N.

Similarly prepared was **19b** (oil): IR (film) 2976, 2932, 1698, 1479, 1457, 1414, 1392, 1367, 1250, and 1152 cm^{-1}; NMR ($CDCl_3$) 5.95 (s, 2 H), 4.12 (s, 4 H), 3.82 (s, 4 H), 3.1 (bs, 4 H), 2.8 and 2.85 (s, 6 H), 1.48 (s, 40 H), and 1.35 (s, 6 H); MS (CI/CH_4) 765 (M + H). Anal. ($C_{37}H_{66}Cl_2N_4O_5$) C, H, N.

Deprotection of **19a** and **19b** (methanolic HCl) as previously described gave compounds **20a** and **20b**, respectively. Compound **20a**: mp 210-212 °C; IR (KBr) 2920, 2780, 2680, 2430, and 1460 cm^{-1}; NMR (D_2O) 7.05 (s, 2 H), 4.0 (s, 4 H), 3.9 (s, 4 H), 3.2-3.05 (m, 4 H), 2.8 and 2.73 (s, 6 H), 1.7 (bs, 4 H), and 1.4 (s, 8 H); MS (CI/CH_4) 378 (M + H). Anal. ($C_{18}H_{36}Cl_2N_4 \cdot 4HCl \cdot H_2O$) C, H, N; Cl: calcd, 39.16; found, 38.53. Compound **20b**: mp 228-230 °C; IR (KBr) 2940, 2854, 2754, 2690, 2510, 2456, 2412, 1608, 1468, and 824 cm^{-1}; NMR (D_2O) 7.05 (s, 2 H), 4.0 (s, 4 H), 3.9 (s, 4 H), 3.15-3.1 9 (m, 4 H), 2.8 and 2.75 (s, 6 H), 1.7 (bs, 4 H), and 1.4 (s, 6 H): MS (CI/CH_4) 365 (M + H). Anal. ($C_{17}H_{34}Cl_2N_4 \cdot 4HCl$) C, H, Cl, N.

By utilizing conditions similar to those used in the synthesis of **19**, compound **15b** was reacted with ethyltriphenylphosphonium bromide to give compound **21b** as an oil: IR (film) 1696, 1456, 1416, 1392, 1366, 1250, 1172, 1148, 936, 880, and 758 cm^{-1}; NMR ($CDCl_3$) 5.4 (bs, 2 H), 3.92 (s, 4 H), 3.7 (s, 4 H), 3.08 (s, 4 H), 2.82 and 2.72 (s, 6 H), 1.7 (d, J = 7.5 Hz, 6 H), 1.45 (s, 40 H), and 1.25 (s, 6 H); MS (CI/CH_4) 725 (M + H). Anal. ($C_{39}H_{72}N_4O_6 \cdot {}^1/_2PhCH_3$) C, H, N.

Deprotection (methanolic HCl) gave **22b**: mp 224-226 °C; IR (KBr) 2982, 2940, 2852, 2786, 2688, 2582, 2434, 1598, 1468, and 1456 cm^{-1}; NMR (D_2O) 6.4-6.3 (m, 2 H), 3.85 (s, 4 H), 3.75 (s, 4 H), 3.1-3.0 (m, 4 H), 2.75 and 2.73 (s, 6 H), 1.85 (d, J = 7.5 Hz, 6 H), 1.7 (s, 4 H), and 1.4 (s, 6 H); MS (CI/CH_4) 325 (M + H). Anal. ($C_{15}H_{40}N_4 \cdot 4HCl$) C, H, N, Cl.

Materials and Methods. HeLa Growth Inhibition Studies. HeLa cells were seeded in 60-mm tissue culture dishes at an initial density of 1×10^5 cells/dish. After 2 days growth, the average number of cells per dish was determined and this number was used as the baseline from which growth was calculated. Polyamine analogues were added to cells in complete medium and incubated for 2 days. At the end of this time, cells were counted, and the increase in cell number in drug-treated cultures was compared to that in untreated control cultures. The IC_{50} was that drug concentration that resulted in a 50% decrease in cell growth relative to controls.

Ethidium Bromide Displacement Assays. Measurement of polyamine analogue binding to calf thymus (CT) DNA by displacement of bound ethidium bromide was conducted as previously described.[7] Briefly, ethidium bromide (1.6 μM final concentration) (Sigma Chemical Co., St. Louis, MO) was added to 3 mL of buffer (2 mM HEPES, 10 μM EDTA, 9.4 mM NaCl, pH 7.0), and the fluorescence was recorded on an SLM-Aminco SPF-500C spectrofluorometer. Emission and excitation wavelengths were 598 and 546 nm, respectively. Upon the addition of calf thymus DNA, poly(dG-dC)·poly(dG-dC), or poly(dA-dT)·poly(dA-dT) (final concentration 2 μM, DNA phosphate), fluorescence increased on average 8-fold. Polyamine analogue was added in 10-μL aliquots and the decrease in fluorescence recorded. The IC_{50} value was defined as the concentration of polyamine required to decrease the fluorescence of the ethidium-DNA complex to 50%. None of the compounds tested absorbed or fluoresced at the critical wavelengths. Variability of multiple trials was generally less than 5%.

Aggregation of HeLa DNA. HeLa cell DNA was radioactively labeled by treatment of cultures with 4 μCi [^{3}H]thymidine (ICN, 60 Ci/mmol) for 3 days. DNA was then isolated by phenol/chloroform extraction and washed and the labeling quantitated. To 100 μL of assay buffer (25 mM Tris, 4.5 mM KCl, 3 mM $MgCl_2$, pH 7.6) were added 1.5 μg (10 μL) of HeLa DNA (approximately 20 000 cpm) and 10 μL of polyamine analogue at various concentrations. The mixture was incubated at 37 °C for 10 min, spun in an Eppendorf centrifuge for 2.5 min, and 50 μL of the resulting supernatant was counted for radioactivity. The IC_{50} value was defined as that concentration of polyamine analogue required to cause 50% of the radioactivity to aggregate and thus sediment out of the mixture. Due to cooperative binding of polyamines, aggregation profiles were very steep, the concentrations showing no aggregation and complete aggregation usually spanning only about 100 μM. For this reason, the error associated with IC_{50} determinations is estimated to be ±25%.

Supplementary Material Available: Preparation and analytical data for compounds **3**, **4**, **12a,b**, **13a,b**, **14a**, and **23-40** (6 pages). Ordering information is given on any current masthead page.

Synthesis of Structural Analogues of Lyngbyatoxin A and Their Evaluation as Activators of Protein Kinase C

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Syntheses of several new analogues of lyngbyatoxin A from a single common intermediate are described. These compounds bear a carbon chain at the 7-position of the indolactam V (ILV) nucleus which contains either a hydrophilic or a lipophilic group. The effect of these minor structural alterations on the ability of the ILV analogues to activate the enzyme protein kinase C (PKC) was determined by measuring the extent of phosphorylation of calf thymus histone (III-S). Introduction of a hydroxyl group on the C-7 appendage was found to dramatically decrease compound **3**'s ability to activate PKC. This result is interpreted in terms of the decreased ability of **3** to associate with the membrane bilayer.

The enzyme protein kinase C (PKC) was discovered in 1977.[1] Over the intervening years, PKC was found to be a Ca^{2+}-activated, phospholipid-dependent enzyme[2] and was shown to play an important role in signal transduction. When a ligand binds to certain receptors on the cell surface thereby stimulating the cell, inositol phospholipids are

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hydrolyzed to diacylglycerol and an inositol phosphate, both of which are known to act as second messengers in signal transduction. Inositol 1,4,5-trisphosphate plays an important role in mobilizing intracellular Ca^{2+}; these Ca^{2+} changes trigger in turn a series of intracellular events. On the other hand, the primary effect of diacylglycerol is to activate PKC thereby increasing the affinity of PKC for Ca^{2+}.[3] Depending upon the precise nature of the tissue, fully activated PKC plays an important role in secretion and exocytosis, ion conductance modulation, signal transduction apparatus regulation, gene expression, and cell proliferation. Additionally, the activation of PKC may underlie the phenomenon of synaptic plasticity and the expression of long term potentiation, processes fundamental to memory function.[4]

More specifically, PKC is activated when an extracellular signal binds to its receptor and causes the activation of phospholipase C (PLC), an enzyme that catalyzes the degradation of phosphatidylinositol 4,5-bisphosphate to *myo*-inositol 1,4,5-trisphosphate 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. Tumor promoters, for instance, the phorbol esters[5] and indole alkaloids such as lyngbyatoxin A and the teleocidins[6] can also activate PKC. These substances are able to mimic the effect of diacylglycerol in activating PKC, but unlike diacylglycerol, they cause a persistent activation of the enzyme. The persistent activation of PKC by these tumor promoters appears to be linked to the down regulation of the enzyme, a process which may be related to the tumor-promoting action of these substances.[7]

As part of an effort to turn one of Nature's toxins, lyngbyatoxin A, into selective antagonists of PKC, agents which may offer new approaches to cancer chemotherapy, we have recently developed an efficient synthetic pathway to the simplified lyngbyatoxin analogues 1 and 2.[8] These analogues were found to activate PKC in the same way as lyngbytoxin A, and most interestingly, the *n*-hexyl analogue 2 has recently been shown to increase the mean survival time of tumor-bearing mice by at least 2-fold.[9]

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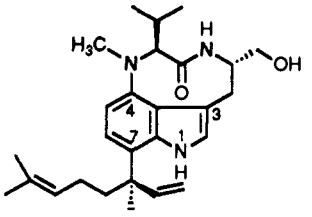
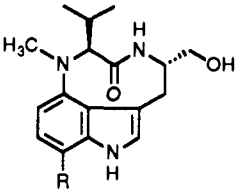


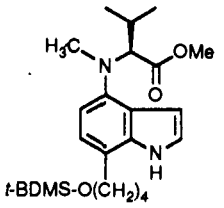
lyngbyatoxin A



R = *t*-Bu, 7-*tert*-butylindolactam V (1)
R = *n*-Hex, 7-*n*-hexylindolactam V (2)
R = $(CH_2)_4OH$, 7-(4-hydroxybutyl)indolactam V (3)
R = $(CH_2)_3CH{=}C(CH_3)_2$, 7-(5-methylhex-4-enyl)indolactam V (4)

As the difference in potency among the simplified analogues of lyngbyatoxin A and lyngbyatoxin A itself relates to the precise nature of the hydrophobic moiety at the 7-position of the indole ring, we believe that this hydrophobic moiety plays a crucial role in the activation as well as, perhaps, the down regulation of PKC. Structure–activity studies by various workers have linked the presence of a larger hydrophobic moiety to enhanced activity, a fact that may be related to the interaction of this hydrophobic moiety with the membrane lipid bilayer.[10] By the same token, the down-regulation of PKC may also be related to the interaction of the same lipophilic moiety with the membrane lipid bilayer. As PKC is known to exert negative-feedback control on some membrane receptors and, consequently, down regulation of the enzyme may be deleterious to this process, the investigation of lyngbyatoxin analogues bearing altered appendages at the 7-position may lead to new research tools for probing the biological significance of the negative-feedback loop.

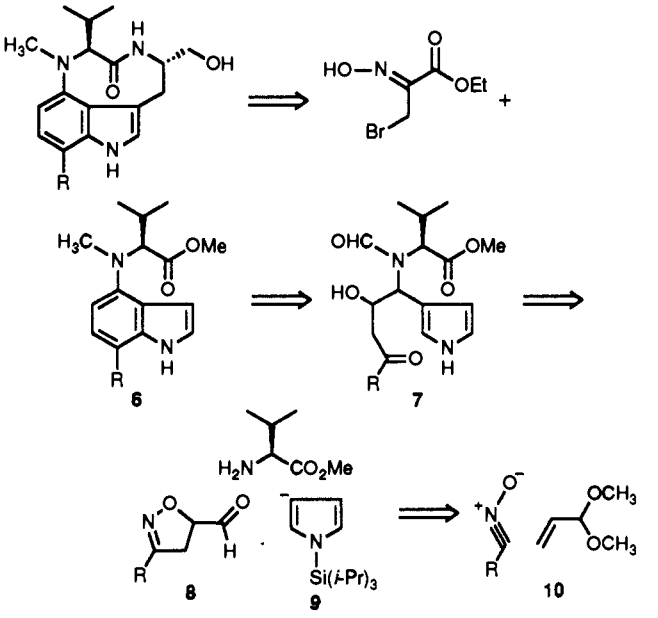
Herein, we report the synthesis and PKC activating abilities of several analogues of lyngbyatoxin A (compounds **3**, **4**, and **32**).[11] Compound **3** contains a four-carbon chain bearing a polar hydroxyl group which is attached to the 7-position of the indole ring, while compounds **4** and **32** contain modified lipophilic moieties. These analogues were synthesized from one common intermediate **5**, which can be prepared efficiently starting from 1,5-pentanediol. The presence of the hydroxy group



5

in the C-7 appendage of **3** makes it an attractive intermediate for the synthesis of other analogues and homologues. Furthermore, this hydroxyl function can serve as an important linker for the incorporation of various reporter groups.

Scheme I. Retrosynthetic Analysis of 7-Substituted ILV Analogues



The examination of the PKC activity of indolactam V (ILV) analogues containing a polar group located in the C-7 appendage at some distance from the indole ring has not been studied in any detail. The only other compounds to be tested are those containing an aminoethyl group or a 3-(carbomethoxy)propanoyl group.[12]

Our synthetic 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.[13] A retrosynthetic analysis of these molecules is presented in Scheme I. Although other routes to ILV analogues have been developed including the direct functionalization of natural ILV, these routes are not any more efficient than the methods detailed herein.[14]

Synthetic Chemistry

1,5-Pentanediol was monosilylated and oxidized by the Swern procedure to give the silyl ether of 5-hydroxypentanal **11**[15] (Scheme II). Aldehyde **11** was converted to oxime **12** by hydroxylamine hydrochloride in the

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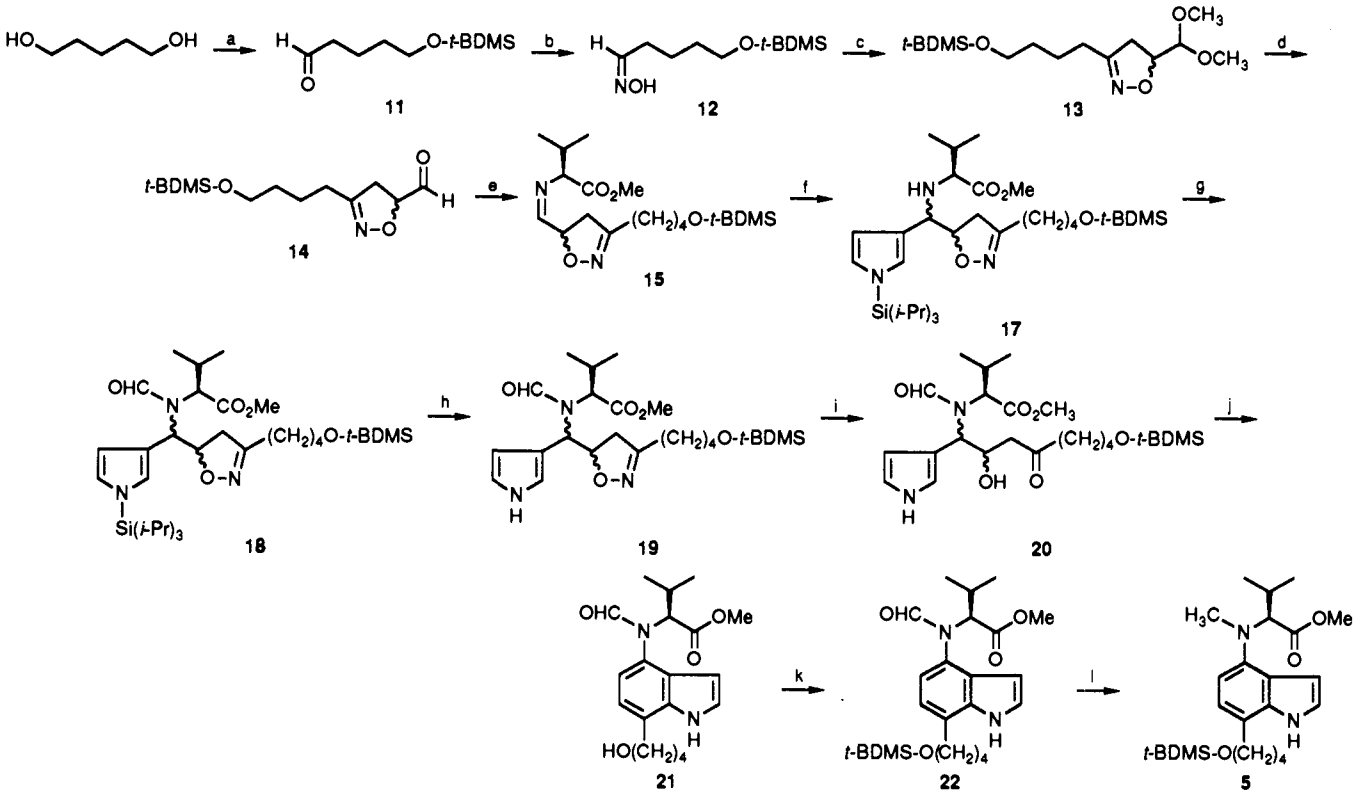
Scheme II. Synthesis of Key Intermediate 5[a]

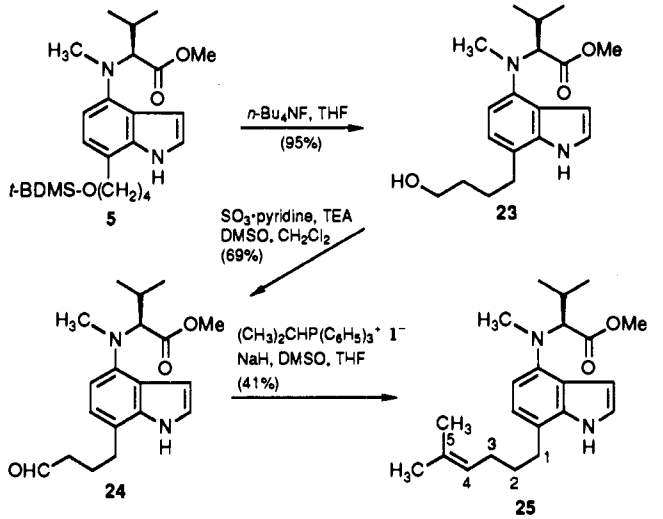


[a] Reagents and Conditions: (a) i, *t*-BDMSCl, imidazole, DMF (88%); ii, Swern oxidation (87%); (b) $NH_2OH \cdot HCl$, Na_2CO_3, H_2O (96%); (c) i, NCS, DMF; ii, $H_2C{=}CHCH(OMe)_2$, TEA, CH_2Cl_2 (78%); (d) i, 10% H_2SO_4/H_2O, DME (90%); ii, *t*-BDMSCl, imidazole, DMF (66%); (e) L-valine methyl ester, $MgSO_4$, PhH; (f) 3-bromo-1-(triisopropylsilyl)pyrrole (16), *t*-BuLi, THF (40%); (g) AcOCHO (neat) (76%); (h) *n*-Bu_4NF, THF (88%); (i) H_2, Raney Ni, $MeOH/H_2O/AcOH$ (50%); (j) *t*-BDMSOTf, CH_2Cl_2 (54%); (k) *t*-BDMSCl, imidazole, DMF (91%); (l) $BH_3 \cdot Me_2S$, THF (82%).

presence of sodium carbonate, and this oxime was treated in turn with *N*-chlorosuccinimide to give the corresponding hydroximic acid chloride.[16] In the presence of triethylamine the nitrile oxide derived from the hydroximic acid chloride underwent [3 + 2] cycloaddition with acrolein dimethyl acetal to give the Δ^2-isoxazoline **13**. Attempts to cleave the dimethyl acetal of **13** with trimethylsilyl iodide[17] to the corresponding aldehyde **14** without removing the silyl ether protecting group were not successful; thus, isoxazoline **13** was treated with 10% sulfuric acid in dimethoxyethane to hydrolyze the dimethyl acetal and to remove the *t*-BDMS protecting group. The hydroxyl group was then reprotected as the corresponding silyl ether **14**. While some attempts were made to synthesize compound **14** in a more direct manner by reacting the nitrile oxide derived from **12** with acrolein, the yields obtained for this reaction were disappointing (10–20%).

Next, aldehyde **14** was condensed with L-valine methyl ester to give the crude imine **15**, which was immediately condensed with *N*-(triisopropylsilyl)-3-lithiopyrrole[18] to give amine **17**. Amine **17** was formylated with acetic formic anhydride[19] to afford the corresponding formamide **18**, and the triisopropylsilyl protecting group was selectively removed with tetra-*n*-butylammonium fluoride to provide pyrrole **19**. Treatment of the (isoxazolylmethyl)pyrrole **19**

Scheme III. Introduction of the Unsaturated C-7 Hydrocarbon Tail



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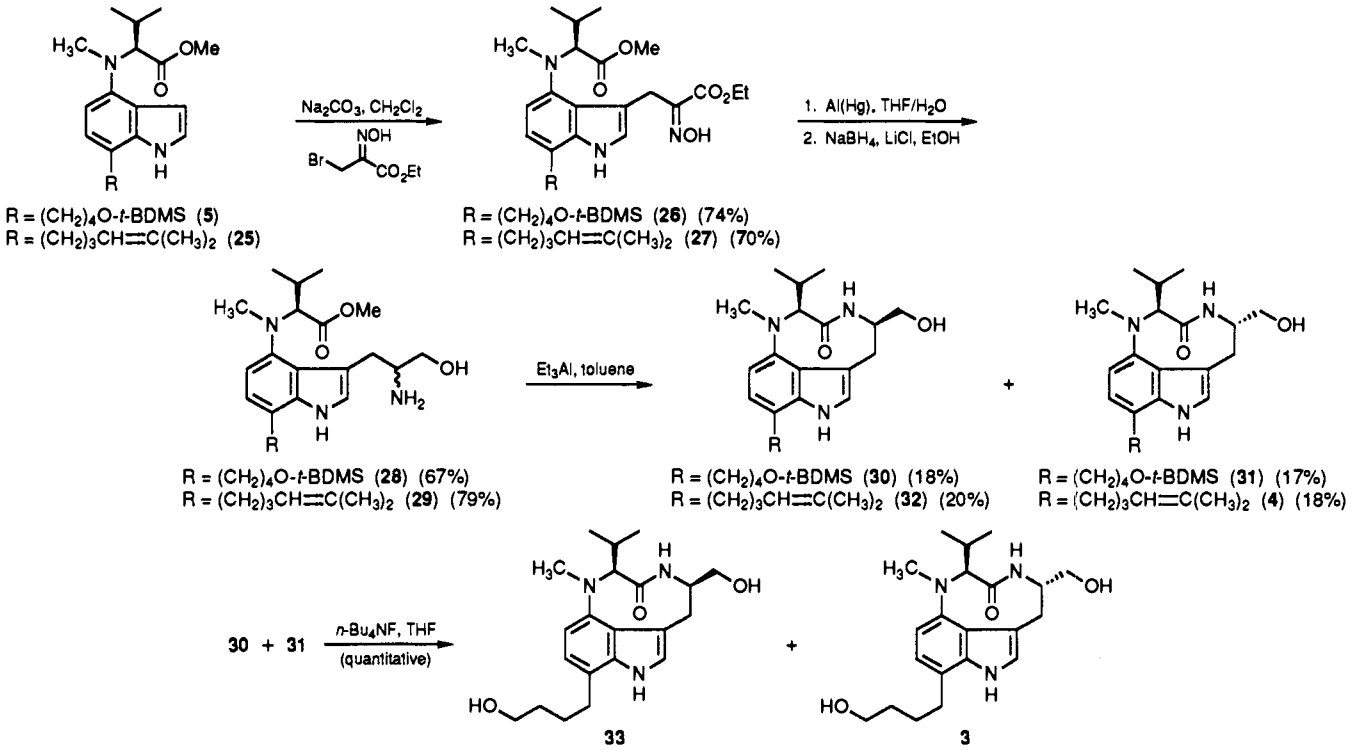
(18) (a) Kozikowski, A. P.; Cheng, X.-M. A Synthesis of 3-Substituted Pyrroles through the Halogen-Metal Exchange Reaction of 3-Bromo-1-(triisopropylsilyl)pyrrole. *J. Org. Chem.* **1984**, *49*, 3239–3240. (b) Muchowski, J. M.; Solas, D. R. β-Substituted Pyrrole via Electrophilic Substitution of N-Triisopropylsilylpyrrole. *Tetrahedron Lett.* **1983**, *24*, 3455–3456.

(19) (a) Krimen, L. I. Acetic Formic Anhydride. *Org. Synth.* **1970**, *50*, 1–3. (b) Krishnamurthy, S. A Highly Efficient and General N-Monomethylation of Functionalized Primary Amines via Formylation—Borane:Methyl Sulfide Reduction. *Tetrahedron Lett.* **1982**, *23*, 3315–3318.

Scheme IV. Completion of the ILV Analogues



with Raney nickel and hydrogen transformed the isoxazoline ring to the β-hydroxy ketone **20**. Intermediate **20** was cyclized in turn to the corresponding indole **21** by using *tert*-butyldimethylsilyl triflate as the Friedel-Crafts catalyst. Since the *t*-BDMS protecting group was lost in the cyclization step, a consequence presumably of the generation of triflic acid during the reaction, reprotection of the hydroxyl group was required. The *N*-formyl group of **22** was now reduced to give the corresponding methylamine **5** by use of the borane dimethylsulfide complex.[20] Some deformylated indole (approximately 10% based upon ^{1}H NMR analysis) was also isolated from this reaction. The optical rotation of indole **5** was found to be similar in sign and magnitude to the rotation displayed by related analogues which have been synthesized in our laboratories.

Scheme III shows the method of conversion of the 4-hydroxybutyl group at the 7-position of indole **5** to the 5-methylhex-4-enyl group. Indole **5** was firstly desilylated by treatment with tetra-*n*-butylammonium fluoride to give indole alcohol **23**. Attempts to oxidize alcohol **23** by Swern oxidation or DMSO/acetic anhydride gave low yields of the desired indole aldehyde **24**. PDC and PCC, on the other hand, caused decomposition of indole alcohol **23**. Fortunately, oxidation by SO_3·pyridine–DMSO–triethylamine gave the desired product **24** in good yield.[21] Aldehyde **24** was then converted to the corresponding dimethyl olefin **25** by Wittig reaction.[22]

Gilchrist's reagent, ethyl 3-bromo-2-ketopropionate oxime,[23] was employed to introduce the three-carbon fragment at the C-3 position of the indole nucleus. Indole **5** reacted with Gilchrist's reagent in the presence of sodium carbonate to give α-oximino ester **26** (Scheme IV). Small amounts of the undesired 5-substituted and 3,5-disubstituted indoles were also obtained. The oxime group of **26** was reduced to amine by aluminum amalgam,[24] and the less hindered ester group was selectively reduced to the amino alcohol **28** by sodium borohydride and lithium chloride. The final cyclization was achieved by refluxing amino alcohol **28** with triethylaluminum[25] in toluene under an anhydrous oxygen-free atmosphere for 15–16 h to give the diastereomeric indolactams **30** and **31**. The pair of diastereomeric indolactams could be readily separated by silica gel flash chromatography. Indolactam **4** and *epi*-indolactam **32** were obtained from indole **25** in the same manner as described above. Indolactam **3** and *epi*-indolactam **33** were obtained in quantitative yields by simply treating **30** and **31** with tetra-*n*-butylammonium fluoride.

^{1}H NMR spectra of *epi*-indolactams **30**, **32**, and **33** reveal that these compounds exist in a single conformation, as

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

addition, 1 μM	protein kinase C activity,[a] pmol min^{-1} (mg of protein)$^{-1}$
none	107 ± 5.0
TPA	899 ± 104
(−)-ILV	454 ± 32
(−)-*n*-hexylindolactam V	606 ± 80
4	763 ± 68
32	415 ± 15
31	703 ± 12
30	403 ± 7
3	256 ± 48
33	163 ± 14

[a] Protein kinase C activity was determined in the presence of 1 μM TPA or lyngbyatoxin A analogues as described in the Experimental Section.

only one *N*-methyl signal is observed. On the other hand, indolactams **2**, **3**, **4**, and **31** exist in two conformations, twist and sofa. Two *N*-methyl signals in an approximately 1:1 ratio are observed in the ^{1}H NMR (CD_3OD) spectra of these compounds. The nature of the substituents at the (6),7-positions of the indole nucleus may possibly affect the conformer ratio. For example, the ratio of twist to sofa conformations of oliveroretin A, oliveroretin D, blastmycetin D, blastmycetin E, and lyngbyatoxin A are 12:1 ($CDCl_3$), 7:1 ($CDCl_3$), 5:1 ($CDCl_3$), 1:2.4 ($CDCl_3$), and 5:1,[26] respectively. The ratio of conformations of these compounds in solution is solvent dependent. Although a receptor-model study based upon the structural superposition of the teleocidins and the phorbol esters favors the sofa form as the biologically active conformation of the former,[27] more stringent experiments are needed to determine which of the two conformations of the teleocidins is important to their biological activity.

Biological Results

The abilities of these newly synthesized analogues to activate PKC were assayed by using enzyme partially purified from cultured HeLa cells. The enzyme activity was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into calf thymus histone (III-S). The abilities of these six compounds together with those of 12-*O*-tetradecanoylphorbol 13-acetate (TPA), (−)-ILV, and (−)-*n*-hexylindolactam V to activate PKC are displayed in Table I. As expected based upon hydrophobicity parameters as well as stereochemical considerations, the 5-methyl-4-hexenyl analogue **4** and the [(*tert*-butyldimethylsilyl)oxy]butyl analogue **31** are somewhat more potent than *n*-hexyl-ILV in activating PKC. The olefin **32** and silyl ether **30** of "unnatural" stereochemistry at the hydroxymethyl bearing center are both less effective than their "natural" stereoisomers when tested at 1.0 μM concentrations. When tested at higher concentrations (10.0 μM) the differences among these five compounds become less apparent as seen in the complete concentration–response curve shown in Figure 1.

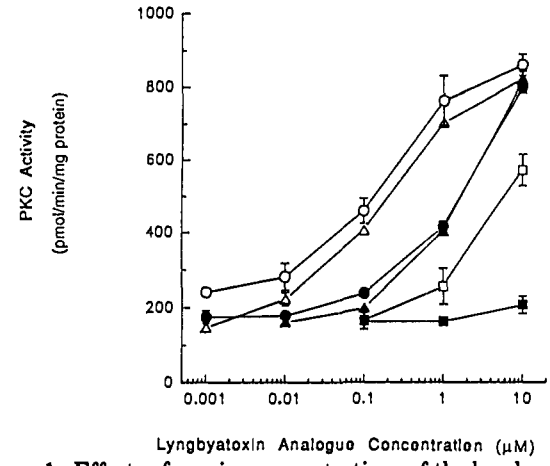

Figure 1. Effects of varying concentrations of the lyngbyatoxin A analogues on the activation of PKC. The PKC activity was determined in vitro in the presence of compounds **4** (○), **32** (●), **31** (△), **30** (▲), **3** (□), or **33** (■) as described in the Experimental Section.

Rather interestingly, the 4-hydroxybutyl analogues **3** and **33** are only weak activators of PKC even at concentrations of 1.0 μM. However, at 10 μM, compound **3** is capable of activating PKC moderately. Compounds **3** and **33** were found to be incapable of antagonizing PKC stimulation caused by the physiological activator, diacylglycerol (1,2-diolein, Avanti Polar Lipids, Inc., Alabastar, AL).

Apparently, the simple introduction of a polar hydroxyl group on the C-7 appendage of ILV decreases the compound's ability to interact with the membrane bilayer. The decreased association of the analogue with the membrane could compromise, in turn, formation of the ternary complex with PKC, thus leading to only weak activation of the enzyme. *The failure of 3 to antagonize the action of diacylglycerol may relate to the lower relative affinity of 3 for PKC compared to DAG.*

The present results indicate that it will be important to test the action of ILV analogues bearing a polar (charged) group in the C-7 appendage which is located at a distance of greater than four carbon atoms from the indole nucleus. Such analogues may still permit efficient binding to the phorbol ester recognition site of the PKC molecule, but may lead to the disruption of the association of PKC with the membrane lipids.[28] Such structural modifications may possibly lead to the discovery of selective PKC antagonists.

Experimental Section

All reactions were carried out in oven- or flame-dried glassware under an anhydrous argon atmosphere unless otherwise stated. Distilled, reagent-grade solvents were used for chromatography and extraction. Tetrahydrofuran (THF) was distilled over sodium benzophenone ketyl. Benzene and toluene were distilled over calcium hydride. Methylene chloride was distilled over calcium hydride and stored over molecular sieves (4 Å). Dimethylformamide (DMF) was distilled over calcium hydride under reduced pressure and stored over molecular sieves (4 Å). Triethylamine

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(TEA) was distilled over calcium hydride and stored over potassium hydroxide. Dimethyl sulfoxide (DMSO) was distilled over calcium hydride and stored under argon. All other reagents were used as supplied unless otherwise stated.

Infrared spectra (neat film) were obtained on an IBM IR/32 FTIR spectrophotometer. ^1H and ^{13}C NMR were recorded at 300 MHz and 75 MHz (Bruker WH-300), respectively, in the solvent(s) noted. ^1H chemical shifts (δ) were reported with Me_4Si (δ = 0.00 ppm) or $CHCl_3$ (δ = 7.26 ppm) as internal standards. ^{13}C chemical shifts (δ) were reported with $CHCl_3$ (central peak, δ = 77.00 ppm) as internal standard. The following abbreviations are used: br = broad, d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet. Low- and high-resolution mass spectra were determined on a VG 70-SE double focusing magnetic sector spectrometer.

Silica gel 60 (Merck, 230-400 mesh for flash chromatography) was used for column chromatography. Thin-layer chromatography (TLC) was performed on Merck Silica Gel 60 F-254 (0.25 mm, precoated on glass). Visualization of compounds on TLC was accomplished by UV illumination, or by staining with a solution prepared from 25 g of ammonium molybdate and 1 g of ceric sulfate in 500 mL of 10% sulfuric acid, followed by heating.

5-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-1-pentanol. To a solution of 1,5-pentanediol (50 mL, 662.4 mmol) and imidazole 22.54 g, 331.2 mmol) in DMF (50 mL) was added dropwise *t*-BDMSCl (25.00 g, 165.6 mmol) in DMF (100 mL). The reaction mixture was stirred at room temperature overnight, then diluted with water, and extracted with ethyl acetate (3×). The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10% to 25%) to give 32.06 g (88%) of the desired product and 3.57 g (6%) of the disilylated product: IR (film) 3339, 2932, 2859, 1463, 1256, 1101, 836, 775 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.05 (s, 6 H), 0.90 (s, 9 H), 1.30–1.66 (m, 7 H), 3.51–3.71 (m, 4 H); ^{13}C NMR δ -5.36, 14.12, 18.31, 20.99, 21.97, 25.92, 32.42, 62.73, 63.10; MS (*m/z*) 161 (M^+ - 57), 143, 115, 105, 75, 69; HRMS calcd for $C_7H_{17}O_2Si$ (M^+ - C_4H_9) 161.0998, found 161.0998.

5-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pentanal (11). A solution of oxalyl chloride (6.7 mL, 76.8 mmol) in methylene chloride (120 mL) was placed in a three-necked round-bottomed flask equipped with two pressure-equalizing addition funnels containing DMSO (10 mL, 140.8 mmol) in methylene chloride (60 mL) in one of the funnels and the monosilylated pentanediol (14.0 g, 64 mmol) in methylene chloride (150 mL) in the other. After the solution was cooled to -78 °C, the DMSO solution was added (ca. 1 min). After bubbling ceased, the alcohol solution was added slowly (ca. 5 min). After being stirred for another 15 min, the reaction was quenched with TEA (45 mL, 320 mmol). The cooling bath was removed, and the reaction mixture was allowed to warm to room temperature. The mixture was diluted with water and extracted with methylene chloride (2×). The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The oil residue was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10%) to yield 12.07 g (87%) of the desired product: IR (film) 2929, 2858, 1728, 1256, 1102, 836, 776 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 (s, 6 H), 0.86 (s, 9 H), 1.47–1.61 (m, 2 H), 1.61–1.76 (m, 2 H), 2.44 (dt, 2 H, *J* = 1.5, 7.3 Hz), 3.60 (t, 2 H, *J* = 6.2 Hz), 9.74 (t, 1 H, *J* = 1.5 Hz); ^{13}C NMR δ -5.39, 18.26, 18.57, 25.89, 32.05, 43.57, 62.53, 202.61; MS (*m/z*) 201, 189, 159 (M^+ - 57), 141, 101, 75; HRMS calcd for $C_7H_{15}O_2Si$ (M^+ - C_4H_9) 159.0841, found 159.0841.

5-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]pentanal Oxime (12). The protected aldehyde 11 (8.35 g, 38.5 mmol) was suspended in water (30 mL). Hydroxylamine hydrochloride (3.21 g, 46.2 mmol) neutralized by sodium carbonate (2.65 g, 25 mmol) in water (50 mL) was added slowly to the above suspension. After being stirred at room temperature for 1 h, the reaction mixture was extracted with diethyl ether (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude oil was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10% to 25%) to yield 8.54 g (96%) of the desired product as a mixture of anti and syn isomers: IR (film) 3279, 2930, 2859, 1463, 1256, 1101, 814, 775 cm^{-1}; ^1H NMR ($CDCl_3$) syn oxime δ 0.03 (s, 6 H), 0.87 (s, 9 H), 1.54 (m, 4 H), 2.19 (m, 2 H), 3.60 (m, 2 H), 7.40 (t, 1 H, *J* = 6.1 Hz), 9.20 (br, 1 H); anti oxime δ 0.04 (s, 6 H), 0.88 (s, 9 H), 1.55 (m, 4 H), 2.39 (m, 2 H), 3.62 (m, 2 H), 6.71 (t, 1 H, *J* = 5.4 Hz), 9.44 (br, 1 H); ^{13}C NMR syn oxime δ -5.36, 18.29, 22.94, 25.91, 29.19, 32.03, 62.63, 151.92; anti oxime δ -5.36, 18.29, 22.39, 24.68, 25.92, 32.36, 62.61, 152.56; MS (*m/z*) 214, 174 (M^+ - 57), 156, 105; HRMS calcd for $C_7H_{16}NO_2Si$ (M^+ - C_4H_9) 174.0950, found 174.0950. Anal. ($C_{11}H_{25}NO_2Si$) C, H, N.

(±)-3-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-5-(dimethoxymethyl)-4,5-dihydroisoxazole (13). To a solution of the oxime 12 (0.20 g, 0.86 mmol) in DMF (5 mL) was added NCS (0.13 g, 1.00 mmol). The reaction mixture was stirred at 40–45 °C for 3 h, then diluted with water and extracted with diethyl ether (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield a greenish oil. The crude hydroximic acid chloride was used immediately in the next step without further purification.

To a solution of acrolein dimethyl acetal (1 mL, 8.4 mmol) and TEA (1 mL, 7.2 mmol) in methylene chloride (5 mL) was added the crude hydroximic acid chloride (from the above reaction) in methylene chloride (5 mL). After being stirred at room temperature overnight, the reaction mixture was diluted with water and extracted with methylene chloride (2×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The oily residue was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (20%) to yield 0.22 g (78%) of the desired product: IR (film) 2931, 2858, 1463, 1255, 1095, 837, 776 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 (s, 6 H), 0.86 (s, 9 H), 1.47–1.69 (m, 4 H), 2.33 (t, 1 H, *J* = 2.8 Hz), 3.42 (d, 6 H, *J* = 1.7 Hz), 3.60 (t, 2 H, *J* = 6.1 Hz), 4.27 (d, 1 H, *J* = 4.6 Hz), 5.45 (m, 1 H); ^{13}C NMR δ -5.40, 18.22, 22.69, 25.85, 27.13, 32.07, 55.12, 55.99, 62.42, 104.51, 158.97; MS (*m/z*) 316, 274 (M^+ - 57), 258, 242, 202, 156, 89, 75, 55; HRMS calcd for $C_{12}H_{24}NO_4Si$ (M^+ - C_4H_9) 274.1475, found 274.1475. Anal. ($C_{16}H_{33}NO_4Si$) C, H, N.

(±)-3-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-4,5-dihydro-5-isoxazolecarboxaldehyde (14). Dimethyl acetal 13 (14.4 g, 0.043 mol) was dissolved in 200 mL of 1,2-dimethoxyethane–water–concentrated sulfuric acid (7.0/2.7/0.3). The reaction mixture was refluxed at 90 °C for ca. 3–4 h. After cooling to room temperature, it was neutralized by the addition of solid sodium bicarbonate. The solid residue was removed by filtration, and the filtrate was concentrated in vacuo. The oily residue was purified by silica gel chromatography with 5% methanol-ethyl acetate used as eluent to yield 7.69 g (90%) of 3-(4-hydroxybutyl)-2-isoxazoline-5-carboxaldehyde.

To a solution of this compound (501.5 mg, 2.65 mmol) and imidazole (433.0 mg, 6.16 mmol) in DMF (25 mL) was added *t*-BDMSCl (479.3 mg, 3.18 mmol) under an anhydrous argon atmosphere. After being stirred at room temperature overnight, the reaction was quenched with water and extracted with ethyl acetate. The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The oily residue was purified by silica gel chromatography by using ethyl acetate–hexane (50% to 67%) to yield 531.6 mg (66%) of the desired product as a mixture of the aldehyde and its hydrate: IR (film) 3394, 2929, 2859, 1738, 1629, 1463, 1256, 1103, 837, 776 cm^{-1}; ^1H NMR (for aldehyde) ($CDCl_3$) δ 0.02 (s, 6 H), 0.87 (s, 9 H), 1.57 (m, 4 H), 2.34 (m, 2 H), 3.00 (m, 2 H), 3.59 (t, 2 H, *J* = 3.7 Hz), 4.52 (m, 1 H), 9.69 (s, 1 H); a good ^{13}C NMR spectrum could not be obtained for the mixture; MS (for aldehyde) (*m/z*) 285 (M^+), 270, 256, 228, 156, 128, 75; HRMS calcd for $C_{14}H_{27}NO_3Si$ (M^+) 285.1760, found 285.1760.

***N*-[[[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-4,5-dihydro-5-isoxazolyl][1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl]methyl]-L-valine Methyl Ester (17).** The aldehyde 14 (2.86 g, 9.41 mmol), L-valine methyl ester (1.23 g, 9.41 mmol), and anhydrous $MgSO_4$ (excess) were suspended in benzene (30 mL). After being stirred at room temperature for 1 h, the reaction mixture was filtered. The filtration cake was washed quickly with benzene, and the filtrate was concentrated in vacuo. The oily residue was redissolved in benzene and concentrated to dryness in vacuo (repeated 3×). The crude imine 15 was used immediately in the next reaction step.

To a solution of 3-bromo-1-(triisopropylsilyl)pyrrole (16) (4.28 g, 14.1 mmol) in THF (20 mL) at -78 °C was added *tert*-butyl-

lithium (1.7 M in pentane, 16.6 mL, 28.2 mmol). After being stirred at -78 °C for 20 min, the solution was added by cannula to a solution of the crude imine **15** (from above) in THF (30 mL) at -78 °C.

After 1 h, the reaction was quenched with saturated aqueous ammonium chloride, and the cooling bath was removed. The reaction mixture was diluted with water, extracted with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude oil was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10% to 25%) to yield 2.34 g (40%) of the title compound as a diastereomeric mixture. More polar isomer: IR (film) 3321, 2957, 2888, 1738, 1464, 1256, 1101, 836, 776 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 (s, 6 H), 0.82–0.97 (m, 15 H), 1.07 (d, 18 H, J = 7.3 Hz), 1.40 (m, 3 H), 1.48–1.63 (m, 4 H), 1.90 (m, 1 H), 2.20 (m, 1 H), 2.26 (t, 2 H, J = 6.6 Hz), 2.70 (m, 2 H), 3.11 (d, 1 H, J = 6.2 Hz), 3.48 (s, 3 H), 3.58 (m, 3 H), 4.70 (dt, 1 H, J = 7.2, 9.8 Hz), 6.21 (dd, 1 H, J = 1.4, 2.6 Hz), 6.62 (d, 1 H, J = 1.3 Hz), 6.65 (t, 1 H, J = 2.6 Hz); ^{13}C NMR δ -5.37, 11.56, 17.75, 18.26, 18.73, 18.87, 22.66, 25.89, 27.41, 31.34, 32.22, 40.18, 51.09, 59.46, 62.49, 65.74, 83.89, 109.99, 122.65, 123.40, 124.26, 158.86, 175.18; MS (m/z) 564 (M^+ - 57), 522, 491, 475, 433, 365, 305; HRMS calcd for $C_{29}H_{54}N_3O_4Si_2$ (M^+ - C_4H_9) 564.3653, found 564.3653.

***N*-[[[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-4,5-dihydro-5-isoxazolyl][1-[tris(1-methylethyl)silyl]-1*H*-pyrrol-3-yl]methyl]-*N*-formyl-L-valine Methyl Ester (18).** A diastereomeric mixture of amine **17** (4.41 g, 7.09 mmol) and acetic formic anhydride (5 mL) were stirred at room temperature for 30 min. The reaction was quenched with saturated aqueous sodium bicarbonate, and the solution was extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10% to 30%) to give 3.50 g (76%) of the formylated product. Less polar isomer: IR (film) 2952, 2867, 1742, 1667, 1101 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.03 (s, 6 H), 0.47 (d, 3 H, J = 6.7 Hz), 0.80 (d, 3 H, J = 6.5 Hz), 0.87 (s, 9 H), 1.07 (d, 18 H, J = 7.4 Hz), 1.47 (m, 3 H), 1.52–1.67 (m, 4 H), 1.95–2.12 (m, 1 H), 2.25–2.42 (m, 2 H), 2.69–2.94 (m, 2 H), 3.61 (m, 2 H), 3.72 (s, 3 H), 3.82 (d, 1 H, J = 10.2 Hz), 4.93 (m, 1 H), 5.77 (d, 1 H, J = 3.8 Hz), 6.31 (s, 1 H), 6.69 (s, 1 H), 6.92 (s, 1 H), 8.50 (s, 1 H); ^{13}C NMR δ -5.41, 11.44, 17.64, 18.22, 19.29, 19.77, 22.59, 25.85, 27.25, 31.16, 32.19, 40.61, 52.17, 52.38, 62.43, 81.25, 112.74, 116.96, 124.08, 125.14, 159.01, 163.23, 173.31; MS (m/z) 592 (M^+ - 57), 574, 491, 474, 433, 393, 365; HRMS calcd for $C_{30}H_{54}N_3O_5Si_2$ (M^+ - C_4H_9) 592.3602, found 592.3602. Anal. ($C_{34}H_{63}N_3O_5Si_2$) C, H, N.

***N*-[[[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-4,5-dihydro-5-isoxazolyl]-1*H*-pyrrol-3-ylmethyl]-*N*-formyl-L-valine Methyl Ester (19).** To a solution of the formylated amine **18** (416.7 mg, 0.64 mmol) in THF (30 mL) at 0 °C was added dropwise tetra-*n*-butylammonium fluoride hydrate (45 mg, 0.13 mmol) in THF. The reaction was carefully monitored by TLC to avoid the removal of the *t*-BDMS protecting group. As soon as all the starting material disappeared, the mixture was poured into water and extracted with diethyl ether (2×). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (30% to 50%) to yield 263.9 mg (83%) of the desired product. Less polar isomer: IR (film) 2931, 2858, 1740, 1661, 1253, 1105, 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.04 (s, 6 H), 0.52 (d, 3 H, J = 6.7 Hz), 0.82 (d, 3 H, J = 5.4 Hz), 0.89 (s, 9 H), 1.51–1.69 (m, 1 H), 2.05 (m, 1 H), 2.24–2.42 (m, 2 H), 2.82 (m, 2 H), 3.63 (t, 2 H, J = 6.0 Hz), 3.73 (s, 3 H), 3.82 (d, 1 H, J = 10.1 Hz), 4.98 (m, 1 H), 5.76 (d, 1 H, J = 4.4 Hz), 6.25 (m, 1 H), 7.03 (m, 1 H), 8.53 (s, 1 H); ^{13}C NMR δ -5.37, 18.26, 19.38, 19.86, 25.89, 27.54, 31.10, 32.26, 40.35, 50.94, 52.40, 62.54, 63.76, 81.27, 109.75, 117.91, 118.20, 118.96, 160.08, 163.92, 172.83; MS (m/z) 448, 436 (M^+ - 57), 403, 357, 334, 319; HRMS calcd for $C_{21}H_{34}N_3O_5Si$ (M^+ - C_4H_9) 436.2268, found 436.2268.

***N*-[8-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]-2-hydroxy-4-oxo-1-(1*H*-pyrrol-3-yl)octyl]-*N*-formyl-L-valine Methyl Ester (20).** A mixture of the desilylated pyrroles **19** (51.7 mg, 0.105 mmol) and W-2 Raney nickel (2 spatula tips) in MeOH (2.5 mL), water (0.7 mL), and glacial acetic acid (4 drops) were stirred under a hydrogen-filled balloon for 4 h. The nickel catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The oily residue was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (40% to 50%) to give 25.8 mg (50%) of the desired hydroxy ketone as a mixture of isomers: IR (film) 3328, 2954, 2930, 2858, 1742, 1716, 1653, 1252, 1100, 836, 776, 657 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.02 (s, 12 H), 0.26 (d, 3 H, J = 7.0 Hz), 0.47 (d, 3 H, J = 6.7 Hz), 0.81 (d, 3 H, J = 6.5 Hz), 0.86 (s, 21 H), 1.48–1.66 (m, 8 H), 2.00–2.20 (m, 2 H), 2.39 (t, 5 H, J = 7.1 Hz), 2.47–2.72 (m, 4 H), 3.31 (d, 1 H, J = 3.8 Hz), 3.56 (t, 4 H, J = 6.3 Hz), 3.72 (s, 3 H), 3.75 (s, 1 H), 3.79 (s, 3 H), 4.38–4.57 (m, 3 H), 4.72 (br, 1 H), 5.53 (d, 1 H, J = 6.6 Hz), 6.10 (br, 1 H), 6.18 (br, 1 H), 6.67–6.79 (m, 3 H), 6.92 (br, 1 H), 8.27 (s, 1 H), 8.50 (s, 2 H), 8.67 (br, 1 H); ^{13}C NMR δ -5.45, 18.19, 18.85, 18.99, 19.69, 19.77, 21.58, 25.82, 27.35, 30.76, 31.97, 43.22, 43.62, 46.30, 46.73, 52.09, 52.77, 53.64, 60.50, 60.97, 61.01, 62.63, 62.71, 63.33, 67.23, 68.82, 107.99, 108.96, 117.41, 117.79, 117.94, 118.02, 118.22, 118.83, 163.49, 163.80, 172.89, 173.04, 209.35, 212.05; MS (m/z) 478, 464, 448, 432. Anal. ($C_{25}H_{44}N_2O_6Si$) C, H, N.

***N*-Formyl-*N*-[7-(4-hydroxybutyl)-1*H*-indol-4-yl]-L-valine Methyl Ester (21).** To a solution of hydroxy ketone **20** (1.45 g, 2.98 mmol) in methylene chloride (150 mL) was added *tert*-butyldimethylsilyl triflate (1.18 mL, 4.47 mmol). The reaction mixture was stirred at room temperature for 5 min, then quenched with saturated aqueous sodium bicarbonate, and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude oil was purified by silica gel chromatography with ethyl acetate–hexane (40% to 90%) used as eluent to give 0.50 g (48%) of the desired product and 76 mg (6%) of **22**: IR (film) 3301, 2937, 2874, 1744, 1661, 1367, 1285, 1207, 735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.98 (d, 3 H, J = 7.0 Hz), 1.59 (br, 1 H), 1.69 (m, 2 H), 1.88 (m, 2 H), 2.45 (m, 1 H), 2.91 (t, 2 H, J = 7.4 Hz), 3.67 (s, 3 H), 3.80 (t, 2 H, J = 6.1 Hz), 4.74 (d, 1 H, J = 9.4 Hz), 6.54 (dd, 1 H, J = 2.0, 2.9 Hz), 7.07 (d, 1 H, J = 7.7 Hz), 7.23 (t, 1 H, J = 2.5 Hz), 8.45 (s, 1 H), 8.91 (br, 1 H); ^{13}C NMR δ 19.90, 20.03, 26.08, 28.24, 30.55, 31.58, 51.86, 62.12, 64.45, 99.53, 118.02, 121.17, 125.32, 125.60, 125.75, 129.33, 136.06, 164.31, 170.78; MS (m/z) 346 (M^+), 318, 287, 275, 259, 232, 215; HRMS calcd for $C_{19}H_{26}N_2O_4$ (M^+) 346.1892, found 346.1892. Anal. ($C_{19}H_{26}N_2O_4$) C, H, N.

***N*-[7-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-1*H*-indol-4-yl]-*N*-formyl-L-valine Methyl Ester (22).** To a solution of the indole alcohol **21** (0.62 g, 1.78 mmol) and imidazole (0.35 g, 5.08 mmol) in DMF (25 mL) was added *t*-BDMSCl (0.35 g, 2.31 mmol). After being stirred at room temperature overnight, the reaction mixture was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude oil was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (40% to 50%) to give 0.75 g (91%) of the desired product: $[\alpha]^{22}_D$ -72° (c = 2.62 mg/mL, CH_2Cl_2); IR (film) 2937, 2860, 1746, 1664, 1256, 1101, 836, 777 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.11 (s, 6 H), 0.93 (s, 9 H), 0.98 (d, 3 H, J = 6.7 Hz), 1.01 (d, 3 H, J = 6.7 Hz), 1.61 (m, 2 H), 1.85 (m, 2 H), 2.45 (m, 1 H), 2.91 (t, 2 H, J = 7.4 Hz), 3.67 (s, 3 H), 3.78 (t, 2 H, J = 5.9 Hz), 4.72 (d, 1 H, J = 9.4 Hz), 6.54 (dd, 1 H, J = 2.2, 3.2 Hz), 6.98 (d, 1 H, J = 7.7 Hz), 7.07 (d, 1 H, J = 7.7 Hz), 8.45 (s, 1 H), 8.90 (br, 1 H); ^{13}C NMR δ -5.26, 18.44, 20.00, 20.16, 25.98, 26.47, 28.31, 30.45, 31.43, 51.89, 63.37, 64.34, 100.20, 118.34, 121.51, 124.91, 125.59, 125.69, 129.70, 136.21, 164.18, 170.82; MS (m/z) 460 (M^+), 445, 432, 403, 389, 373, 329, 288; HRMS calcd for $C_{25}H_{40}N_2O_4Si$ (M^+) 460.2747, found 460.2747.

***N*-[7-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-1*H*-indol-4-yl]-*N*-methyl-L-valine Methyl Ester (5).** To a solution of the formamide **22** (0.39 g, 0.84 mmol) in THF (60 mL) at 0 °C was added borane–dimethyl sulfide complex (2.0 M, 1.05 mL, 2.1 mmol). After being stirred at 0 °C for 3–5 min, the reaction mixture was warmed to 45–50 °C for 2 h and then cooled to room temperature, and methanol (1 mL) was added. After 10 min, the mixture was diluted with saturated aqueous sodium bicarbonate and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane

(10% to 20%) to give 0.31 g (82%) of the desired product and 30.4 mg (8%) of the deformylated product: $[\alpha]^{22}_D$ −130° (c = 2.89 mg/mL, CH_2Cl_2); IR (film) 2932, 2858, 1718, 1504, 1255, 1101, 836 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.09 (s, 6 H), 0.92 (s, 9 H), 0.93 (d, 3 H, J = 8.7 Hz), 1.07 (d, 3 H, J = 6.6 Hz), 1.62 (m, 2 H), 1.81 (m, 2 H), 2.38 (m, 1 H), 2.81 (t, 2 H, J = 7.3 Hz), 3.00 (s, 3 H), 3.64 (s, 3 H), 3.74 (t, 2 H, J = 6.1 Hz), 4.07 (d, 1 H, J = 10.9 Hz), 6.43 (d, 1 H, J = 7.7 Hz), 6.73 (t, 1 H, J = 2.5 Hz), 6.86 (d, 1 H, J = 7.7 Hz), 7.13 (t, 1 H, J = 2.8 Hz), 8.68 (br, 1 H); ^{13}C NMR δ −5.24, 18.45, 19.45, 19.87, 26.02, 26.47, 28.07, 30.33, 31.78, 33.98, 50.96, 63.33, 70.59, 101.91, 107.72, 118.57, 120.25, 121.84, 122.00, 136.36, 143.90, 172.59; MS (m/z) 446 (M^+), 403, 387, 199; HRMS calcd for $C_{25}H_{42}N_2O_3Si$ (M^+) 446.2965, found 446.2965.

N-[7-(4-Hydroxybutyl)-1H-indol-4-yl]-N-methyl-L-valine Methyl Ester (23). To a solution of the silylated alcohol **5** (140.5 mg, 0.314 mmol) in THF (30 mL) was added $(n\text{-Bu})_4NF$ (1.0 M in THF, 1.57 mL, 1.57 mmol). After the reaction mixture had been stirred at room temperature for 2 h, it was diluted with water and extracted with ethyl acetate (2×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (50% to 60%) to give 99 mg (95%) of the desired product: $[\alpha]^{22}_D$ −147° (c = 2.1 mg/mL, CH_2Cl_2); IR (film) 3406, 2953, 2872, 1732, 1505, 1374, 1196, 1007, 735 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.95 (d, 3 H, J = 6.5 Hz), 1.09 (d, 3 H, J = 6.7 Hz), 1.62 (m, 2 H), 1.77 (m, 2 H), 2.37 (m, 1 H), 2.77 (t, 2 H, J = 7.4 Hz), 3.01 (s, 3 H), 3.62 (s, 3 H), 3.67 (t, 2 H, J = 6.2 Hz), 4.09 (d, 1 H, J = 10.8 Hz), 6.55 (d, 1 H, J = 7.7 Hz), 6.72 (t, 1 H, J = 2.3 Hz), 6.86 (d, 1 H, J = 7.7 Hz), 7.04 (t, 1 H, J = 2.8 Hz), 8.77 (br, 1 H); ^{13}C NMR δ 19.35, 19.77, 26.02, 27.98, 30.38, 31.80, 33.88, 50.92, 62.40, 70.53, 101.58, 107.54, 118.40, 120.22, 121.67, 122.28, 136.11, 143.77, 172.63; MS (m/z) 332 (M^+), 289, 273, 215; HRMS calcd for $C_{19}H_{28}N_2O_3$ (M^+) 332.2100, found 332.2100.

N-Methyl-N-[7-(4-oxobutyl)-1H-indol-4-yl]-L-valine Methyl Ester (24). To a solution of indole alcohol **23** (86.66 mg, 0.261 mmol) and TEA (1 mL) in DMSO (10 mL) and CH_2Cl_2 (10 mL) was added SO_3·pyridine (207 mg, 1.31 mmol). After being stirred at room temperature for 3–4 h, the reaction mixture was quenched with saturated sodium bicarbonate and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by silica gel chromatography with ethyl acetate–hexane (40%) and as eluent to give 59.9 mg (69%) of the desired aldehyde: $[\alpha]^{22}_D$ −181° (c = 11.6 mg/mL, CH_2Cl_2); IR (film) 3395, 2957, 1725, 1505, 1194 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.97 (d, 3 H, J = 6.5 Hz), 1.10 (d, 3 H, J = 6.7 Hz), 1.97 (m, 2 H), 2.41 (m, 2 H), 2.59 (t, 2 H, J = 6.3 Hz), 2.75 (t, 2 H, J = 7.4 Hz), 3.03 (s, 3 H), 3.66 (s, 3 H), 4.13 (d, 1 H, J = 10.8 Hz), 6.54 (d, 1 H, J = 7.7 Hz), 6.74 (t, 1 H, J = 2.6 Hz), 6.84 (d, 1 H, J = 7.7 Hz), 7.20 (t, 1 H, J = 2.8 Hz), 9.12 (br, 1 H), 9.83 (s, 1 H); ^{13}C NMR δ 19.38, 19.79, 21.87, 28.02, 30.34, 33.89, 43.17, 50.93, 70.47, 101.72, 107.28, 117.46, 120.22, 121.71, 122.42, 136.23, 144.18, 172.55, 203.53; MS (m/z) 330 (M^+), 312, 287, 269, 253; HRMS calcd for $C_{19}H_{26}N_2O_3$ (M^+) 330.1939, found 330.1939.

N-Methyl-N-[7-(5-methyl-4-hexenyl)-1H-indol-4-yl]-L-valine Methyl Ester (25). To washed NaH (72 mg, 3.00 mmol) was added DMSO (5 mL). The suspension was heated at 75–80 °C for ca. 45 min. After the mixture was cooled to 0 °C, 2-propyltriphenylphosphonium iodide (1.3 g, 3.00 mmol) in DMSO (5 mL) was added via cannula. After 1 h, the resulting ylide (4.8 mL, 1.44 mmol) was added to a solution of indole aldehyde **24** (94.76 mg, 0.287 mmol) in THF (10 mL). The reaction mixture was stirred at room temperature for 1 h, and then it was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (10% to 15%) to give 42.2 mg (41%) of the desired product: $[\alpha]^{22}_D$ −154° (c = 6.01 mg/mL, CH_2Cl_2); IR (film) 3408, 2962, 2929, 1734, 1504, 1375, 1195, 1008, 727 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.93 (d, 3 H, J = 6.5 Hz), 1.07 (d, 3 H, J = 6.6 Hz), 1.58 (s, 3 H), 1.72 (s, 3 H), 1.75 (m, 2 H), 2.05 (m, 2 H), 2.37 (m, 1 H), 2.74 (t, 2 H, J = 7.5 Hz), 2.99 (s, 3 H), 3.62 (s, 3 H), 4.06 (d, 1 H, J = 10.9 Hz), 5.18 (t, 1 H, J = 7.3 Hz), 6.54 (d, 1 H, J = 7.8 Hz), 6.72 (s, 1 H), 6.86 (d, 1 H, J = 7.7 Hz), 7.09 (t, 1 H, J = 2.8 Hz), 8.13 (br, 1 H); ^{13}C NMR δ 17.77, 19.41, 19.84, 25.74, 27.76, 28.02, 29.70, 30.13, 33.93, 50.93, 70.62, 102.01, 107.83, 118.31, 120.18, 121.77, 121.96, 124.34, 132.08, 143.80, 172.56; MS (m/z) 356 (M^+), 313, 297, 240; HRMS calcd for $C_{22}H_{32}N_2O_2$ (M^+) 356.2438, found 356.2438.

(S)-7-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-α-(hydroxyimino)-4-[[1-(methoxycarbonyl)-2-methylpropyl]methylamino]-1H-indole-3-propanoic Acid Ethyl Ester (26). To a mixture of indole **5** (297.5 mg, 0.666 mmol) and sodium carbonate (282 mg, 2.66 mmol) in methylene chloride (60 mL) was added ethyl 3-bromo-2-ketopropionate oxime (143 mg, 0.679 mmol). The resulting mixture was stirred at room temperature overnight. The reaction was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with ethyl acetate–hexane (25% to 60%) as eluent to give 284.7 mg (75%) of the desired product: IR (film) 3376, 2954, 2859, 1728, 1257, 1201, 1098, 1021, 837 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.07 (s, 6 H), 0.90 (s, 9 H), 0.94 (d, 3 H, J = 6.7 Hz), 1.12 (d, 3 H, J = 6.7 Hz), 1.25 (t, 3 H, J = 7.1 Hz), 1.59 (m, 3 H), 1.77 (m, 2 H), 2.30 (m, 1 H), 2.77 (m, 2 H), 2.91 (s, 3 H), 3.57 (s, 3 H), 3.69 (m, 3 H), 4.25 (q, 2 H, J = 7.2 Hz), 4.41 (d, 1 H, J = 17.4 Hz), 4.52 (d, 1 H, J = 17.4 Hz), 6.75 (d, 1 H, J = 2.0 Hz), 6.84 (s, 2 H), 8.34 (br, 1 H), 9.39 (br, 1 H); MS (m/z) 575 (M^+), 558, 532, 516, 502, 460; HRMS calcd for $C_{30}H_{49}N_3O_6Si$ (M^+) 575.3391, found 575.3391.

(S)-α-(Hydroxyimino)-4-[[1-(methoxycarbonyl)-2-methylpropyl]methylamino]-7-(5-methyl-4-hexenyl)-1H-indole-3-propanoic Acid Ethyl Ester (27). In a similar manner as described for the synthesis of **26**, 36.5 mg (70%) of oxime ester **27** was obtained from indole **25** (38 mg, 0.107 mmol), sodium carbonate (50 mg, 0.476 mmol), and ethyl 3-bromo-2-ketopropionate oxime (27.5 mg, 0.131 mmol): IR (film) 3389, 2964, 2933, 1727, 1434, 1201, 1021, 771 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.94 (d, 3 H, J = 6.6 Hz), 1.12 (d, 3 H, J = 6.6 Hz), 1.25 (t, 3 H, J = 7.1 Hz), 1.58 (s, 3 H), 1.70 (s, 3 H), 1.75 (m, 2 H), 2.05 (m, 2 H), 2.31 (m, 1 H), 2.71 (t, 2 H, J = 7.4 Hz), 2.90 (s, 3 H), 3.57 (s, 3 H), 3.67 (d, 1 H, J = 8.5 Hz), 4.25 (q, 2 H, J = 7.1 Hz), 4.40 (d, 1 H, J = 15.1 Hz), 4.52 (d, 1 H, J = 15.1 Hz), 5.15 (t, 1 H, J = 7.0 Hz), 6.74 (s, 1 H), 6.85 (s, 2 H), 7.97 (br, 1 H), 9.72 (br, 1 H); MS (m/z) 485 (M^+), 469, 442, 426, 410.

(RS)-N-[3-(2-Amino-3-hydroxypropyl)-7-[4-[[(1,1-dimethylethyl)dimethylsilyl]oxy]butyl]-1H-indol-4-yl]-N-methyl-L-valine Methyl Ester (28). To a solution of oxime ester **26** (36.5 mg, 0.063 mmol) in THF–water (9:1, 10 mL) was added aluminum amalgam (in excess). The reaction mixture was stirred at room temperature for 3–4 h and then filtered through Celite, and the filter cake was washed thoroughly with ethyl acetate. The filtrate was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give 29.2 mg (82%) of the crude product, which was used in the next step without further purification.

To a suspension of the amino ester from above (73 mg, 0.125 mmol) and lithium chloride (42.2 mg, 1.00 mmol) in THF–ethanol (2:1, 15 mL) was added sodium borohydride (37.8 mg, 1.00 mmol). After the suspension had been stirred at room temperature for ca. 5–6 h, it was filtered through a medium-pore sintered-glass funnel. The filtrate was concentrated, and the oily residue was chromatographed on silica gel with methanol–methylene chloride (10% to 30%) as eluent to give 53 mg (82%) of the desired product: IR (film) 3366, 3255, 2953, 2858, 1732, 1511, 1463, 1255, 1099, 836, 775 cm^{-1}; ^1H NMR ($CDCl_3$–CD_3OD, 4:1) δ −0.09 (s, 6 H), 0.75 (s, 9 H), 0.91 (d, 3 H, J = 7.0 Hz), 0.97 (d, 3 H, J = 7.0 Hz), 1.49 (m, 2 H), 1.61 (m, 2 H), 2.28 (br, 1 H), 2.58 (s, 3 H), 2.68 (t, 2 H, J = 8.3 Hz), 2.89 (dd, J = 6.0, 12.0 Hz) and 3.02 (dd, J = 6.0, 12.0 Hz, total 1 H), 3.20 (m, 2 H), 3.39 (s) and 3.44 (s, total 4 H), 3.54 (t, 2 H, J = 6.2 Hz), 3.64 (dd, J = 3.0, 12.0 Hz) and 3.75 (dd, J = 3.0, 12.0 Hz, total 2 H), 3.64 (m, 1 H), 6.72 (d, 1 H, J = 7.6 Hz), 7.03 (d, 1 H, J = 5.5 Hz); MS (m/z) 519 (M^+), 502, 486, 460, 417, 401.

(RS)-N-[3-(2-Amino-3-hydroxypropyl)-7-(5-methyl-4-hexenyl)-1H-indol-4-yl]-N-methyl-L-valine Methyl Ester (29). In the same manner as described above, 21 mg (79%) of **29** was obtained from the corresponding oxime ester **27** (30 mg, 0.06 mmol): IR (film) 3395, 2931, 1733, 1471, 1447, 1171 cm^{-1}; ^1H NMR (CD_3OD) δ 1.07 (t, 6 H, J = 6.0 Hz), 1.57 (s, 3 H), 1.67

(s, 3 H), 1.71 (m, 2 H), 2.02 (m, 2 H), 2.41 (m, 1 H), 2.72 (s, 3 H), 2.79 (t, 2 H, J = 7.7 Hz), 3.05 (dd, J = 6.2, 14.6 Hz) and 3.18 (dd, J = 4.7, 14.4 Hz, total 1 H), 3.37 (m, 2 H), 3.52 (s) and 3.55 (s, total 3 H), 3.64 (d, 1 H, J = 7.2 Hz), 3.82 (m, 2 H), 5.14 (t, 1 H, J very small), 6.81 (s, 2 H), 7.18 (d, J = 10.0 Hz, 1 H); MS (m/z) 429 (M^+), 412, 386, 370, 353, 327.

[2*S*-(2*R,5*R**)]-9-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-1,2,4,5,6,8-hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (30) and [2*S*-(2*R**,5*S**)]-9-[4-[[(1,1-Dimethylethyl)dimethylsilyl]oxy]butyl]-1,2,4,5,6,8-hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (31).** To a solution of amino alcohol **28** (22.32 mg, 0.043 mmol) in toluene (50 mL) under an anhydrous argon atmosphere was added triethylaluminum (1.9 M, in toluene, 0.249 mL, 0.47 mmol). After being stirred at room temperature for 45 min, the reaction mixture was refluxed at 125 °C (oil bath temperature) for 14–16 h. The reaction mixture was cooled to room temperature, and methanol (1 mL) was added. After being stirred for an additional 10 min, the reaction mixture was diluted with saturated sodium bicarbonate and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude oil was loaded onto a silica gel column, and the column was eluted with ethyl acetate–hexane (50% to 100% in 10% increments) to give 3.65 mg (17%) of the more polar indolactam **31** and 3.72 mg (18%) of the less polar *epi*-indolactam **30**.

Indolactam **31**: $[\alpha]^{22}_D$ −93° (c = 3.17 mg/mL, methanol); IR (film) 3310, 2930, 2857, 1653, 1558, 1389, 1257, 1100, 837 cm^{-1}; ^1H NMR (CD_3OD) twist form δ 0.08 (s, 6 H), 0.68 (d, 3 H, J = 6.5 Hz), 0.94 (s, 12 H), 1.69 (m, 2 H), 1.78 (m, 2 H), 2.61 (m, 1 H), 2.92 (m, 2 H), 2.93 (s, 3 H), 3.13 (m, 2 H), 3.49 (d, 1 H, J = 9.3 Hz), 3.71 (m, 3 H), 4.30 (m, 1 H), 4.48 (d, 1 H, J = 10.1 Hz), 6.45 (d, 1 H, J = 7.7 Hz), 6.94 (s, 1 H), 7.00 (s, 1 H); sofa form δ 0.08 (s, 6 H), 0.94 (s, 12 H), 1.29 (d, 3 H, J = 6.6 Hz), 1.69 (m, 2 H), 1.78 (m, 2 H), 2.35 (m, 1 H), 2.75 (s, 3 H), 2.83 (t, 2 H, J = 7.5 Hz), 2.91 (m, 1 H), 3.04 (m, 1 H), 3.13 (m, 1 H), 3.29 (d, 1 H, J = 4.1 Hz), 3.36 (m, 1 H), 3.71 (m, 1 H), 4.30 (m, 1 H), 6.81 (d, 1 H, J = 6.2 Hz), 6.94 (s, 1 H), 7.17 (d, 1 H, J = 1.8 Hz); MS (m/z) 487 (M^+), 444, 401, 357; HRMS calcd for $C_{27}H_{45}N_3O_3Si$ (M^+) 487.3230, found 487.3230.

***epi*-Indolactam 30**: $[\alpha]^{22}_D$ −100° (c = 1.11 mg/mL, methanol); IR (film) 3310, 2930, 2857, 1653, 1509, 1453, 1258, 1100, 837 cm^{-1}; ^1H NMR (CD_3OD) δ 0.07 (s, 6 H), 0.76 (d, 3 H, J = 7.0 Hz), 0.79 (d, 3 H, J = 6.7 Hz), 0.93 (s, 9 H), 1.62 (m, 2 H), 1.79 (m, 2 H), 2.63 (m, 1 H), 2.83 (m, 2 H), 3.11 (s, 3 H), 3.37 (m, 2 H), 3.69 (t, 2 H, J = 6.5 Hz), 3.80 (m, 3 H), 4.02 (d, 1 H, J = 15.0 Hz), 6.70 (d, 1 H, J = 7.7 Hz), 6.80 (d, 1 H, J = 7.7 Hz), 6.99 (s, 1 H); ^{13}C NMR δ −5.13, 19.17, 20.71, 20.89, 26.43, 27.20, 29.17, 31.46, 33.05, 33.57, 33.96, 58.31, 64.36, 65.48, 70.19, 110.14, 114.64, 120.79, 121.81, 121.98, 123.83, 138.84, 146.93, 176.53; MS (m/z) 487 (M^+), 486, 444, 401, 357; HRMS calcd for $C_{27}H_{45}N_3O_3Si$ (M^+) 487.3230, found 487.3230.

[2*S*-(2*R,5*R**)]-1,2,4,5,6,8-Hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-9-(5-methyl-4-hexenyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (32) and [2*S*-(2*R**,5*S**)]-1,2,4,5,6,8-Hexahydro-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-9-(5-methyl-4-hexenyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (4).** In the same manner as described above, 2.39 mg (18%) of the more polar indolactam **4** and 2.73 mg (20%) of the less polar *epi*-indolactam **32** were obtained from the corresponding amino alcohol (14.77 mg, 0.034 mmol).

Indolactam **4**: $[\alpha]^{22}_D$ −93° (c = 1.01 mg/mL, methanol); IR (film) 3306, 2922, 2857, 1649, 1508, 1445, 1036, 737 cm^{-1}; ^1H NMR (CD_3OD) twist form δ 0.64 (d, 3 H, J = 6.7 Hz), 0.90 (d, 3 H, J = 6.1 Hz), 1.61 (s, 3 H), 1.71 (s, 3 H), 1.73 (m, 2 H), 2.07 (m, 2 H), 2.57 (m, 1 H), 2.84 (m, 2 H), 2.89 (s, 3 H), 3.10 (s, 2 H), 3.59 (d, 1 H, J = 9.3 Hz), 3.64 (dd, 1 H, J = 4.7, 10.8 Hz), 4.28 (m, 1 H), 4.44 (d, 1 H, J = 10.1 Hz), 5.19 (m, 1 H), 6.42 (d, 1 H, J = 7.9 Hz), 6.90 (s, 1 H), 6.97 (s, 1 H); sofa form δ 0.90 (d, 3 H, J = 6.1 Hz), 1.25 (d, 3 H, J = 6.6 Hz), 1.57 (s, 3 H), 1.71 (s, 3 H), 1.73 (m, 2 H), 2.07 (m, 2 H), 2.31 (m, 1 H), 2.72 (s, 3 H), 2.76 (m, 2 H), 2.84 (m, 1 H), 3.01 (m, 1 H), 3.10 (s, 1 H), 3.27 (m, 1 H), 3.33 (m, 1 H), 4.28 (m, 1 H), 5.19 (m, 1 H), 6.77 (d, 1 H, J = 7.7 Hz), 6.90 (s, 1 H), 7.13 (s, 1 H).

***epi*-Indolactam 32**: $[\alpha]^{22}_D$ −104° (c = 1.26 mg/mL, methanol); IR (film) 3308, 2926, 2855, 1553, 1508, 1456, 1373, 1030, 737 cm^{-1}; ^1H NMR (CD_3OD) δ 0.73 (d, 3 H, J = 6.6 Hz), 0.76 (d, 3 H, J = 6.6 Hz), 1.56 (s, 3 H), 1.69 (s, 3 H), 1.71 (m, 2 H), 2.04 (m, 2 H), 2.58 (m, 1 H), 2.75 (m, 2 H), 2.95 (d, 1 H, J = 13.1 Hz), 3.06 (s, 3 H), 3.14 (d, 1 H, J = 15.1 Hz), 3.77 (m, 3 H), 4.00 (d, 1 H, J = 10.5 Hz), 5.18 (t, 1 H, J = 7.1 Hz), 6.67 (d, 1 H, J = 7.7 Hz), 6.75 (d, 1 H, J = 7.6 Hz), 6.96 (s, 1 H).

[2*S*-(2*R,5*S**)]-1,2,4,5,6,8-Hexahydro-9-(4-hydroxybutyl)-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (3).** To a solution of indolactam **31** (5.16 mg, 0.011 mmol) in THF (2 mL) at 0 °C was added tetra-*n*-butylammonium fluoride (1.0 M, 4–5 drops). After 30 min, the reaction mixture was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was dissolved in a minimal amount of methanol. The solution was loaded onto a short silica gel column, and the column was eluted with methanol–ethyl acetate (0% to 5%) to give 4.17 mg (quantitative) of the desired product: $[\alpha]^{22}_D$ −86° (c = 2.09 mg/mL, methanol); IR (film) 3307, 2920, 2865, 1650, 1490, 1025 cm^{-1}; ^1H NMR (CD_3OD) twist form δ 0.63 (d, 3 H, J = 6.7 Hz), 0.90 (d, 3 H, J = 6.3 Hz), 1.67 (m, 2 H), 1.75 (m, 2 H), 2.55 (m, 1 H), 2.89 (s, 3 H), 2.90 (m, 2 H), 3.09 (s, 2 H), 3.49 (d, 1 H, J = 10.0 Hz), 3.61 (m, 3 H), 4.26 (m, 1 H), 4.44 (d, 1 H, J = 10.1 Hz), 6.41 (d, 1 H, J = 7.7 Hz), 6.91 (s, 1 H), 6.97 (s, 1 H); sofa form δ 0.90 (d, 3 H, J = 6.3 Hz), 1.28 (m, 3 H), 1.67 (m, 2 H), 1.75 (m, 2 H), 2.31 (m, 1 H), 2.71 (s, 3 H), 2.79 (t, 2 H, J = 7.2 Hz), 2.87 (m, 1 H), 3.03 (m, 1 H), 3.09 (s, 1 H), 3.26 (d, 1 H, J = 6.7 Hz), 3.32 (m, 1 H), 3.61 (m, 2 H), 4.26 (m, 1 H), 6.79 (d, 1 H, J = 7.7 Hz), 6.91 (s, 1 H), 7.13 (s, 1 H); MS (m/z) 373 (M^+), 330, 287, 256, 243; HRMS calcd for $C_{21}H_{31}N_3O_2$ (M^+) 373.2365, found 373.2365.

[2*S*-(2*R,5*R**)]-1,2,4,5,6,8-Hexahydro-9-(4-hydroxybutyl)-5-(hydroxymethyl)-1-methyl-2-(1-methylethyl)-3*H*-pyrrolo[4,3,2-*gh*]-1,4-benzodiazonin-3-one (33).** In the same manner as described above, 6.29 mg (99%) of the indolactam alcohol **30** was obtained from the corresponding indolactam silyl ether **30** (8.22 mg, 0.017 mmol): $[\alpha]^{22}_D$ −102° (c = 1.15 mg/mL, methanol); IR (film) 3307, 2930, 2868, 2806, 1650, 1498, 1033 cm^{-1}; ^1H NMR (CD_3OD) δ 0.72 (d, 3 H, J = 6.6 Hz), 0.76 (d, 3 H, J = 6.5 Hz), 1.61 (m, 2 H), 1.75 (m, 2 H), 2.59 (m, 1 H), 2.79 (m, 2 H), 2.95 (d, 1 H, J = 15.3 Hz), 3.07 (s, 3 H), 3.13 (d, 1 H, J = 15.3 Hz), 3.59 (t, 2 H, J = 6.4 Hz), 3.76 (m, 3 H), 4.00 (d, 1 H, J = 10.4 Hz), 6.67 (d, 1 H, J = 7.7 Hz), 6.77 (d, 1 H, J = 7.6 Hz), 6.96 (s, 1 H); ^{13}C NMR δ 20.87, 21.05, 27.34, 29.25, 31.73, 33.14, 33.57, 34.07, 59.33, 62.92, 65.46, 70.18, 110.12, 114.61, 120.83, 121.77, 121.96, 123.95, 138.81, 146.93, 176.55; MS (m/z) 373 (M^+), 353, 343, 330, 287, 243; HRMS calcd for $C_{21}H_{31}N_3O_2$ (M^+) 373.2365, found 373.2365.

Isolation of Protein Kinase C. Protein kinase C was partially purified from HeLa cells by using DE52-cellulose chromatography.[29] Briefly, cells were harvested and cell pellets were washed with ice-cold phosphate-buffered saline without any divalent cations. All subsequent procedures were carried out at 4 °C. Cells were homogenized in 20 mM Tris-HCl (pH 7.5), 0.25 M sucrose, 2 mM EDTA, 5 mM EGTA, 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 0.01% leupeptin (buffer A). The total PKC was extracted by stirring for 30 min with 0.5% Triton X-100 and centrifugated at 100000g for 1 h. The resulting supernatant was subjected to DE-52 chromatography and eluted with 0.1 M NaCl in 20 mM Tris-HCl (pH 7.5), 0.25 M sucrose, 0.5 mM EDTA, 0.5 mM EGTA, and 10 mM β-mercaptoethanol.

Protein Kinase C Assay. The enzyme activity was determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into calf thymus histone (III-S) (Sigma).[30] The reaction mixture

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contained 20 mM Tris-HCl (pH 7.5), 20 mM ATP, 7.5 mM magnesium acetate, 25 mg/mL phosphatidylserine, 0.1 mM EGTA, 0.1 mM $CaCl_2$, 1 nM to 10 μM lyngbyatoxin A analogues, and 1 to 3 mg of enzyme preparation in a final volume of 0.1 mL. The reaction mixture was incubated at 28 °C for 5 min and stopped by spotting onto Whatman p81 filter papers. Under these conditions the assay was linear with time and amount of protein. The filters were washed four times in 75 mM phosphoric acid, dried, and counted in a liquid scintillation counter. Calcium and phospholipid-dependent protein kinase C activity was determined by subtracting the activity determined in the absence of phosphatidylserine and DAG from that in the presence of phosphatidylserine and DAG. In the presence of either Ca^{2+} or phospholipid alone the enzyme activity was less than 5% of the activity when both were present. Protein was determined by the method of Bradford[31] with bovine serum albumin used as a standard.

One unit of protein kinase C activity is defined as that amount of enzyme which catalyzes the transfer of 1 pmol of phosphate from ATP to histone per minute at 28 °C.

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Pseudopeptide Analogues of Substance P and Leucine Enkephalinamide Containing the Ψ(CH_2O) Modification: Synthesis and Biological Activity

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The isosteric methyleneoxy Ψ(CH_2O) function was employed as a novel peptide-bond surrogate and incorporated into sequences of two neuropeptides, substance P (SP) and enkephalin. A pseudopeptide analogue [pGlu^6,Phe^{8}Ψ(CH_2O)Gly^9]SP$_{6-11}$ (7) of SP related C-terminal hexapeptide [pGlu^6]SP$_{6-11}$ and two pseudopeptide analogues of [Leu^5]enkephalinamide, [Tyr^{1}Ψ(CH_2O)Gly^2,Leu^5]enkephalinamide (11) and [Gly^{2}Ψ(CH_2O)-Gly^3,Leu^5]enkephalinamide (17), were synthesized. The N$^\alpha$-protected pseudodipeptidic units were incorporated in the appropriate peptide sequences by using conventional coupling methods in solution. Compound 7 was a potent agonist (EC_{50} = 4.8 nM) of substance P as compared to the parent peptide [pGlu^6]SP$_{6-11}$ (EC_{50} = 1.2 nM), in stimulating contraction of the isolated guinea pig ileum (GPI). Analogue 7 was more potent on the neuronal (NK-3) than on the muscular (NK-1) tachykinin receptors in the GPI as shown by the ratio of activities, EC_{50}(NK-1)/EC_{50}(NK-3) = 3.16, thus displaying an improved selectivity for the NK-3 tachykinin receptor subtype as compared to that of [pGlu^6]SP$_{6-11}$, EC_{50}(NK-1)/EC_{50}(NK-3) = 0.44. In the rat vas deferens (RVD) assay, a typical NK-2 system, the pseudopeptide analogue 7 was (EC_{50} = 2 μM) 10-fold more potent than the parent peptide and 20-fold less potent than eledoisin, an NK-2 selective tachykinin. The pseudopeptide enkephalin analogue 17 had low biological activity when tested in the electrically induced GPI (EC_{50} = 2.3 μM) and was inactive in the mouse vas deferens (MVD) assay. In the rat brain membrane (RBM) binding assay analogue 17 had low affinity (in the micromolar range) for both the μ and δ binding sites. In contrast, analogue 11 was a potent enkephalin agonist (EC_{50} = 30 nM), being equipotent to [D-Ala^2,Leu^5]enkephalinamide (DALE) in the GPI assay. In the MVD, analogue 11 showed a substantially reduced activity (EC_{50} = 92 nM), being about 10-fold less potent than DALE. In the RBM binding assay analogue 11 showed high affinity (in the nanomolar range) for both the μ and δ binding sites with increased selectivity for the δ sites as shown by the ratio of the apparent affinities for both receptors, $K_i(\delta)/K_i(\mu)$ = 2.1. The contribution of the modified peptide bonds in the mode of interaction of SP and enkephalin at their corresponding receptors is discussed.

Backbone modifications of peptide hormones and neuropeptides play an important role in structure-activity relationship studies and were found to affect potency, enzymatic stability, solubility, and conformational properties. Peptide backbone modifications have been introduced in an increasing number of biologically active peptides.[1] Modifications such as Ψ(NHCO)[2-4] Ψ(CONMe),[5] Ψ($COCH_2$),[6,7] Ψ(CSNH),[8] Ψ(CH_2S),[9] Ψ(CH_2NH), Ψ-(CH_2CH_2),[10] and *E* or *Z* Ψ(CH=CH)[11,12] have resulted in several analogues with increased biological activity and

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