Pergamon

Tetrahedron 58 (2002) 2101–2115

TETRAHEDRON

Synthesis, conformation and PKC isozyme surrogate binding of new lactone analogues of benzolactam-V8s

Yu Nakagawa, Kazuhiro Irie,* Akiko Masuda and Hajime Ohigashi

Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

Received 14 December 2001; accepted 23 January 2002

Abstract—To investigate the role of the amide hydrogen of benzolactam-V8s (**1**–**3**) on protein kinase C (PKC) isozyme binding, new lactone analogues of benzolactam-V8s with hydrophobic side chains at positions 8 and/or 9 (**5**–**8**) were synthesized. The PKC binding affinities of 8- and 9-decylbenzolactone-V8 (**5**,**6**) were much lower than those of 8- and 9-decylbenzolactam-V8 (**2**,**3**), respectively, indicating that the amide hydrogen of benzolactam-V8s plays a critical role in PKC binding. 8-Decylbenzolactam-V8 (**2**) showed lower binding affinities to all PKC isozymes compared with those of 9-decylbenzolactam-V8 (**3**). The binding affinities of 8-substituted benzolactones (**5**,**7**,**8**) were also lower than those of 9-decylbenzolactone-V8 (**6**), but their PKC isozyme selectivity was higher than those of **2**, **3** and **6**. 8-Decybenzolactone-V8 (**5**) exhibited the most significant η-C1B selectivity among the four benzolactones (**5**–**8**) synthesized in this study.

1. Introduction

Tumor promoters such as teleocidins and phorbol esters activate protein kinase C (PKC) isozymes, which mediate cellular signal transduction by binding to the cysteine-rich C1 domains (C1A, C1B).[1,2] PKCs consisting of more than 11 isozymes are subdivided into three groups: conventional PKCs (α, βI, βII, γ), which are calcium-dependent, novel PKCs (δ, ε, η, θ), which are calcium-independent, and atypical PKCs (ζ, λ/ι), which lack the ability to bind tumor promoters. Recent studies[3,4] using transgenic or knockout mice revealed that novel PKCs play an important role in tumor promotion. However, the precise mechanism of tumor promotion and the role of each PKC isozyme remain unclear because naturally occurring tumor promoters show little PKC isozyme selectivity.[5,6] Design of analogues with high PKC isozyme selectivity is also quite difficult due to their complex structures.

As artificial tumor promoters benzolactam-V8s,[7,8] recently developed as simplified analogues of teleocidins,[9] bind to PKC with high affinities, they are promising lead compounds that selectively modulate the individual PKC isozymes (Fig. 1). Kozikowski et al.[8] and Endo et al.[10] independently reported the binding mode of benzolactam-V8s to PKCδ-C1B including the hydrogen bond network and the hydrophobic interaction by computational docking studies based on the X-ray structure analysis of PKCδ-C1B in complex with phorbol 13-acetate.[11] However, there have been no reports of experimental results or of computational

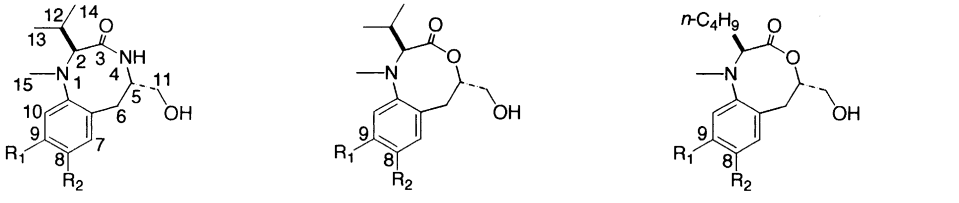
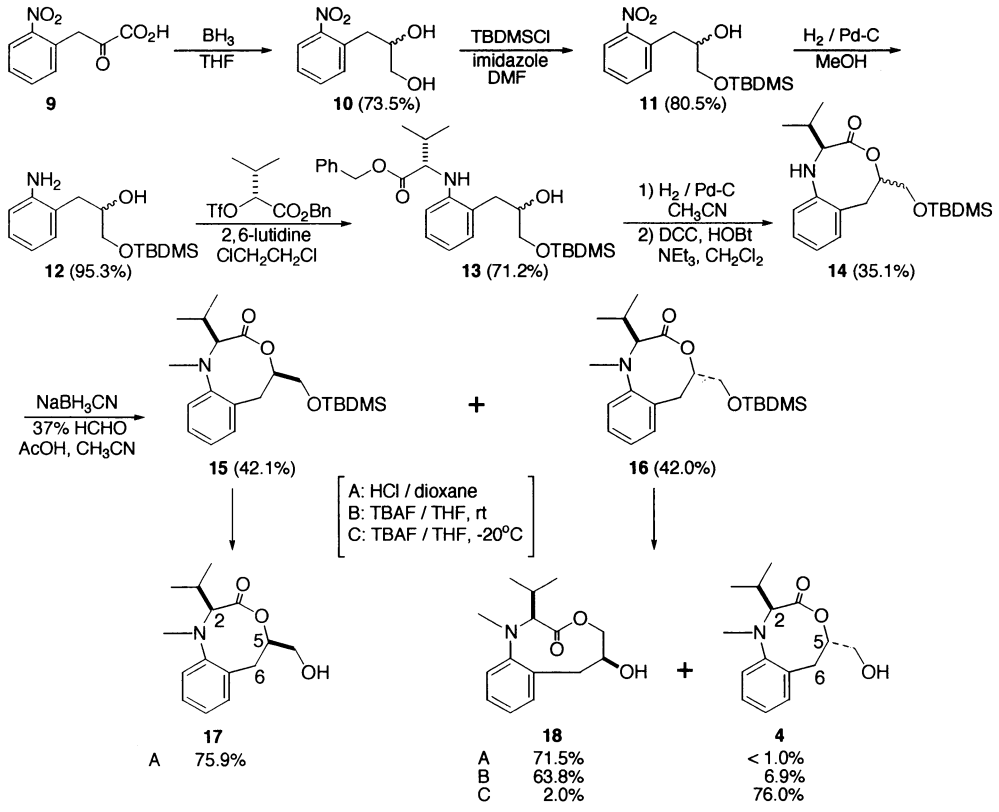


Figure 1. Structures of benzolactam-V8s and benzolactones.

Keywords: benzolactam-V8; benzolactone; phorbol ester; protein kinase C; tumor promoter.
* Corresponding author. Tel.: +81-75-753-6282; fax: +81-75-753-6284; e-mail: irie@kais.kyoto-u.ac.jp

0040–4020/02/$ - see front matter
PII: S0040-4020(02)00099-6



Scheme 1. Synthesis of benzolactone-V8 (**4**).

analyses on the interaction between benzolactam-V8s and other PKC C1 domains, interrupting the rational design of PKC isozyme-selective modulators.

The computational docking studies[8,10] indicated that three functional groups of both benzolactam-V8s and phorbol esters form hydrogen bonds with PKCδ-C1B. The hydrogen-binding sites in PKCδ-C1B of the hydroxyl group and the amide carbonyl group of benzolactam-V8s were the same to those of the C-20 hydroxyl group and the C-3 carbonyl group of phorbol esters, respectively. However, the hydrogen-bonding site of amide hydrogen of benzolactam-V8s differs from that of the C-4 hydroxyl group of phorbol esters. Moreover, we recently clarified that the C-4 hydroxyl group of phorbol esters is not necessary for PKC binding.[12] As the involvement of the amide hydrogen of benzolactam-V8s in the PKC isozyme binding remains unknown, we modified the amide function of benzolactam-V8s to clarify its role in PKC isozyme binding and to obtain insight toward PKC isozyme selective-modulators.

We have recently reported the synthesis and PKC binding of 8-decylbenzolactone-V8 (**5**), a new lactone analogue of 8-decylbenzolactam-V8 (**2**), as a preliminary communication.[13] Endo et al. reported that benzolactam-V8s with hydrophobic side chains at position 8 showed the weaker binding to PKCδ than 9-decylbenzolactam-V8 (**3**).[10] This difference in activities between 8- and 9-substituted benzolactam-V8s is explained well by the computational docking study; the side chain at position 8 of benzolactam-V8s crushes with the entrance of the tumor-promoter-binding cavity of PKCδ-C1B.[10] A quite similar substituent effect would be observed in benzolactone-V8s. We report here the synthesis of 9-decylbenzolactone-V8 (**6**) and 8,9-disubstituted benzolactones (**7**,**8**), and their conformation and binding affinities to the C1 domains of all PKC isozymes along with those of **5**.

2. Results and discussion

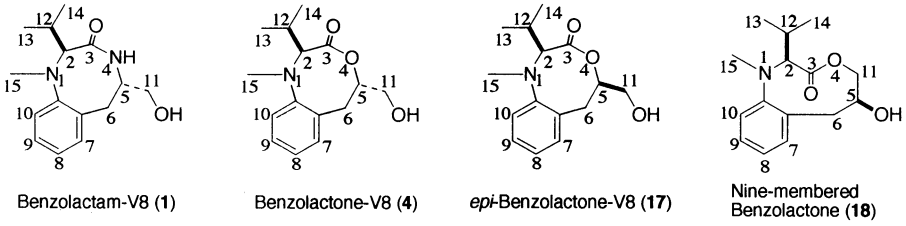
2.1. Synthesis and conformational analysis of benzolactone-V8 (4)

Benzolactone-V8 (**4**), the core structure of benzolactone-V8s (**5**,**6**), was synthesized from 2-nitrophenylpyruvic acid (**9**) as shown in Scheme 1. Reduction of both the ketone and the carboxyl group of **9** with borane gave the diol (**10**, 73.5%). Selective protection of the primary hydroxyl group of **10** with a *tert*-butyldimethylsilyl (TBDMS) group was achieved with TBDMSCl and imidazole in *N*,*N*-dimethylformamide (DMF) at 0°C (80.5%). The nitro group of **11** was reduced by catalytic hydrogenation to give the aniline derivative **12** (95.3%). The valine subunit was introduced by substitution of **12** with D-valine-derived triflate[14] to give two diastereomeric esters (**13**, 71.2%), the separation of which was quite difficult at this point. After deprotection of the benzyl group of the diastereomeric mixture (**13**) by hydrogenation in CH_3CN, intramolecular esterification was accomplished with 1,3-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt) and triethylamine in dichloromethane to give **14** (35.1%). This slightly lower cyclization yield was expected as the synthesis of an eight- or a nine-membered lactone is generally

Table 1. ^{1}H NMR chemical shifts of benzolactam-V8 (**1**), benzolactone-V8 (**4**), *epi*-benzolactone-V8 (**17**) and nine-membered benzolactone (**18**) in deuteriochloroform (500 MHz, 300 K)

No.	δ (Multiplicity, *J* in Hz)			
	Benzolactam-V8 (**1**)[a]	Benzolactone-V8 (**4**)[b]	*epi*-Benzolactone-V8 (**17**)[c]	Nine-membered Benzolactone-V8 (**18**)[d]
2	3.46 (d, *J*=8.6)	3.34 (d, *J*=10.2)	3.28 (d, *J*=10.8)	3.14 (d, *J*=9.7)
5	4.05 (m)	4.82 (m)	4.65 (m)	4.17 (m)
6α	3.08 (d, *J*=16.9, 2.2)	3.05 (dd, *J*=16.3, 3.7)	2.94 (dd, *J*=15.5, 5.8)	3.04 (dd, *J*=12.7, 10.4)
6β	2.81 (d, *J*=16.9, 8.0)	2.98 (dd, *J*=16.3, 3.7)	2.82 (dd, *J*=15.5, 2.4)	2.79 (dd, *J*=12.7, 2.7)
7	7.02 (d, *J*=7.6)	7.08 (d, *J*=7.6)	7.11 (d, *J*=7.4)	7.15–7.24 (m)
8	7.18 (t, *J*=7.6)	7.22 (t, *J*=7.6)	7.22 (t, *J*=7.4)	7.15–7.24 (m)
9	6.88 (t, *J*=7.6)	7.04 (t, *J*=7.6)	7.06 (t, *J*=7.4)	7.15–7.24 (m)
10	7.04 (d, *J*=7.6)	7.09 (d, *J*=7.6)	7.19 (d, *J*=7.4)	7.15–7.24 (m)
11	3.52 (m)	3.69 (m)	3.77 (dd, *J*=11.9, 4.2)	4.00 (m)
	3.70 (m)		3.82 (dd, *J*=11.9, 7.2)	4.57 (m)
12	2.43 (m)	2.23 (m)	2.32 (m)	2.23 (m)
13	1.06 (d, *J*=6.5)	1.02 (d, *J*=6.6)	1.06 (d, *J*=6.6)	1.13 (d, *J*=6.7)
14	0.89 (d, *J*=6.8)	0.99 (d, *J*=6.5)	0.88 (d, *J*=6.5)	1.00 (d, *J*=6.6)
15	2.79 (s)	2.80 (s)	2.91 (s)	2.71 (s)

[a] 0.082 M.
[b] 0.102 M.
[c] 0.061 M.
[d] 0.061 M. Numbering of **18** is assigned as shown for convenience of comparison with **1**, **4**, and **17**.



quite difficult.[15] Methylation of the diastereomeric lactones (**14**) by the method of Kozikowski et al.[8] gave two diastereomers, **15** (42.1%) and **16** (42.0%), which were easily separated by silica gel column chromatography.

Deprotection of each TBDMS group of **15** and **16** with 1N HCl in dioxane gave a single product, **17** and **18**, respectively, whose ^{1}H NMR spectra in deuteriochloroform showed that each compound existed as a single conformer at room temperature (Table 1). The free hydroxymethylene signal (δ 3.77 and 3.82) and significant NOE between H-2 (δ 3.28) and H-5 (δ 4.65) protons were observed in **17**, indicating that **17** was *epi*-benzolactone-V8 with the *R* configuration at position 5. On the other hand, the hydroxylmethylene signals of **18** were shifted downfield (δ 4.00 and 4.57), and the cross-peak between the hydroxyl proton (δ 2.01) and the hydroxymethyne proton (δ 4.17) was observed in the ^{1}H–^{1}H COSY spectrum. These results suggested that **18** is not the expected benzolactone-V8 (**4**) but the nine-membered lactone deduced to be formed by intramolecular transesterification under acidic conditions in the TBDMS deprotection step (1N HCl–dioxane). We performed deprotection of the TBDMS group of **16** with tetrabutylammonium fluoride (TBAF) in tetrahydrofuran (THF) at room temperature to obtain a major product identified as **18** (63.8%) along with a minor product **4** (6.9 %), which was thought to be the desired eight-membered lactone. To suppress transesterification, we conducted this reaction at −20°C and succeeded in obtaining mainly **4** (76.0%). The ^{1}H NMR spectrum showed that **4** existed as a single conformer in deuteriochloroform at room temperature. The free hydroxymethylene signal (δ 3.69) and cross-peak between the hydroxyl proton and the methylene proton were observed, suggesting that **4** is an eight-membered lactone. The lack of NOE enhancement between H-2 (δ 3.34) and H-5 (δ 4.82) protons in the NOESY spectrum strongly indicated that **4** is benzolactone-V8 with the *S* configuration at position 5. The significant NOE enhancement between H-2 (δ 3.34) and H-6α (δ 3.05), which is characteristic of benzolactam-V8 (**1**),[7] was observed in **4**.

Conformational analysis of **4** was performed by molecular mechanics and semiempirical quantum mechanics calculations. The initial structure was calculated by MM2 on the condition that the distance between H-2 and H-6α was fixed to 2 Å because of the significant NOE enhancement between these protons. Optimization of this structure was carried out by PM3. The calculated structure of **4** along with that of **1** reported by Endo et al.[7] are shown in Fig. 2 (left and center). The conformation of the ester group was *syn* geometry corresponding to *cis* geometry of the amide group, and the ring conformation of **4** was quite similar to that of **1**. These results indicate that benzolactone-V8 (**4**) is a three-dimensional mimic of benzolactam-V8 (**1**).

2.2. Synthesis of 8-decylbenzolactone-V8 (5) and 9-decylbenzolactone-V8 (6)

Although the binding affinity to PKC of benzolactam-V8 (**1**) itself is quite weak, 8- and 9-decylbenzolactam-V8 (**2**,**3**) are strong PKC binders.[10] We tried to synthesize 8-decylbenzolactone-V8 (**5**) from benzolactone-V8 (**4**). Introduction of a

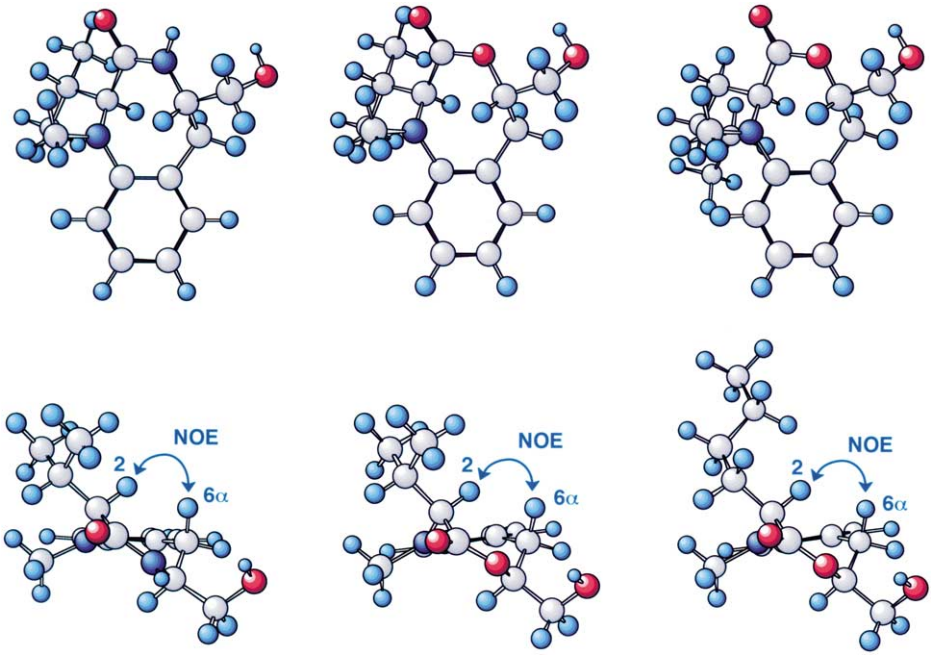
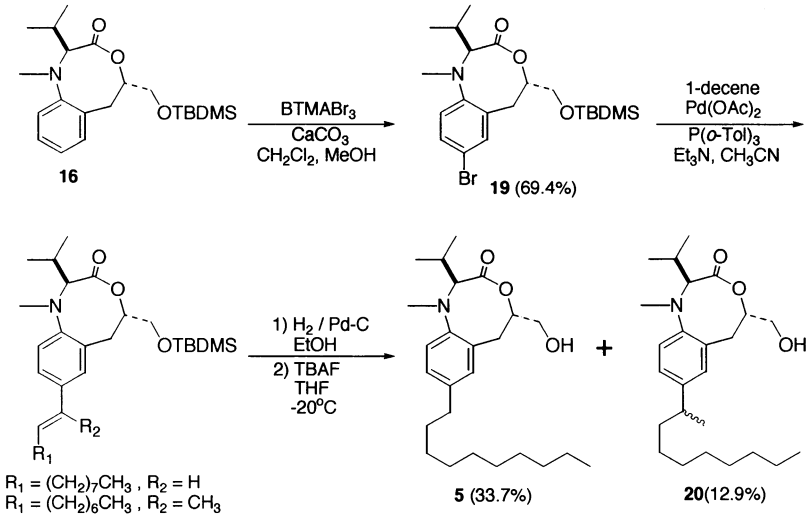


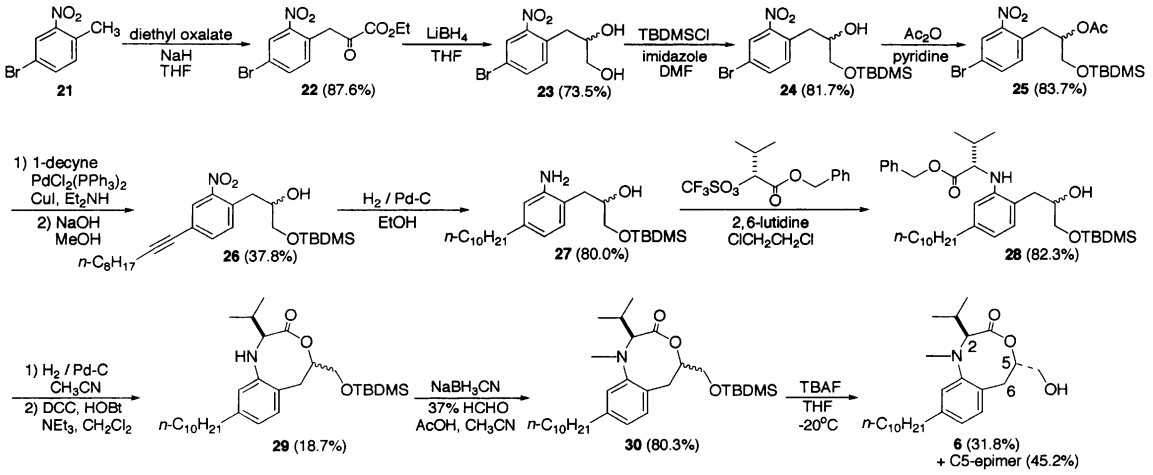
Figure 2. Stable conformation of benzolactam-V8 (**1**, left), benzolactone-V8 (**4**, center) and benzolactone-Nle8 (right).

decyl group at position 8 of **4** was accomplished as shown in Scheme 2. Unexpectedly, electrophilic substitution of **16** with iodine in pyridine did not proceed at all although 11-*O*-acetylbenzolactam-V8 was iodinated under the same reaction conditions.[8] As transesterification might occur in conventional iodination and bromination under strong acidic conditions, we used a strong bromination reagent, benzyltrimethylammonium tribromide ($BTMABr_3$),[16] under neutral conditions. Bromination of **16** using $BTMABr_3$ proceeded at room temperature to give the 8-bromo derivative (**19**, 69.4%). Heck reaction[17] of **19** with 1-decene gave two coupling products, followed by hydrogenation and deprotection of the TBDMS group with TBAF at −20°C, gave 8-decylbenzolactone-V8 (**5**, 33.7%) and its regio isomer (**20**, 12.9%).

9-Decylbenzolactone-V8 (**6**) is a new lactone analogue of 9-decylbenzolactam-V8 (**3**), which is the most potent PKC binder among benzolactam-V8s[10] synthesized to date. Compound **6** was synthesized from 4-bromo-2-nitrotoluene (**21**) as shown in Scheme 3. S_N2 substitution of **21** with diethyl oxalate gave the ethyl pyruvate derivative **22** (87.6%). Reduction of both the ketone and the ester groups of **22** (73.5%) followed by selective protection of primary hydroxyl group with a TBDMS group gave **24** (81.7%), the second hydroxyl group of which was protected with an acetyl group (83.7%). Modified Sonogashira coupling[18] of **25** with 1-decyne followed by alkaline hydrolysis gave the alkyne (**26**, 37.8%). Saturation of the triple bond and reduction of the nitro group of **26** was accomplished in one step by hydrogenation to give the 3-decylaniline derivative



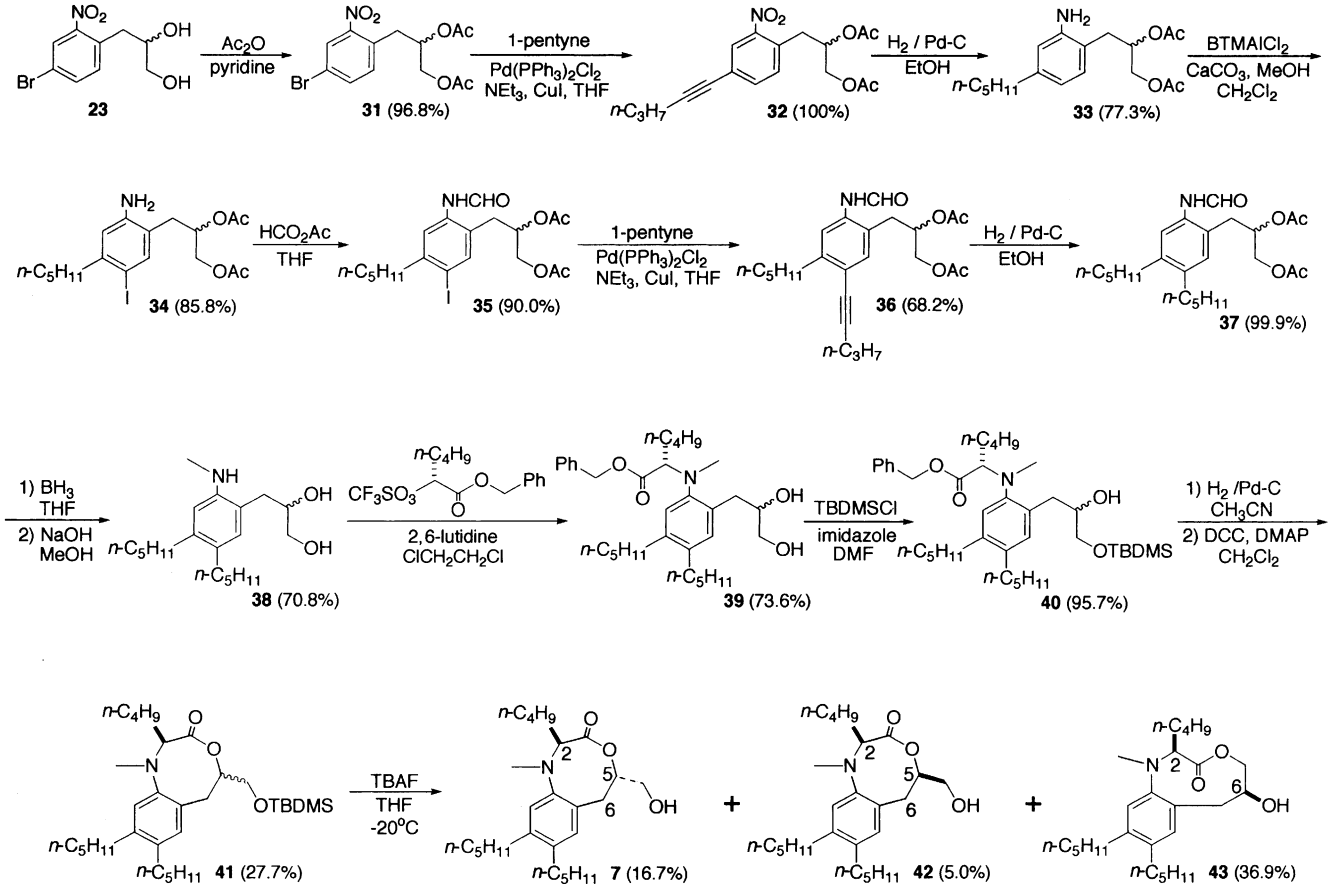
Scheme 2. Synthesis of 8-decylbenzolactone-V8 (**5**).



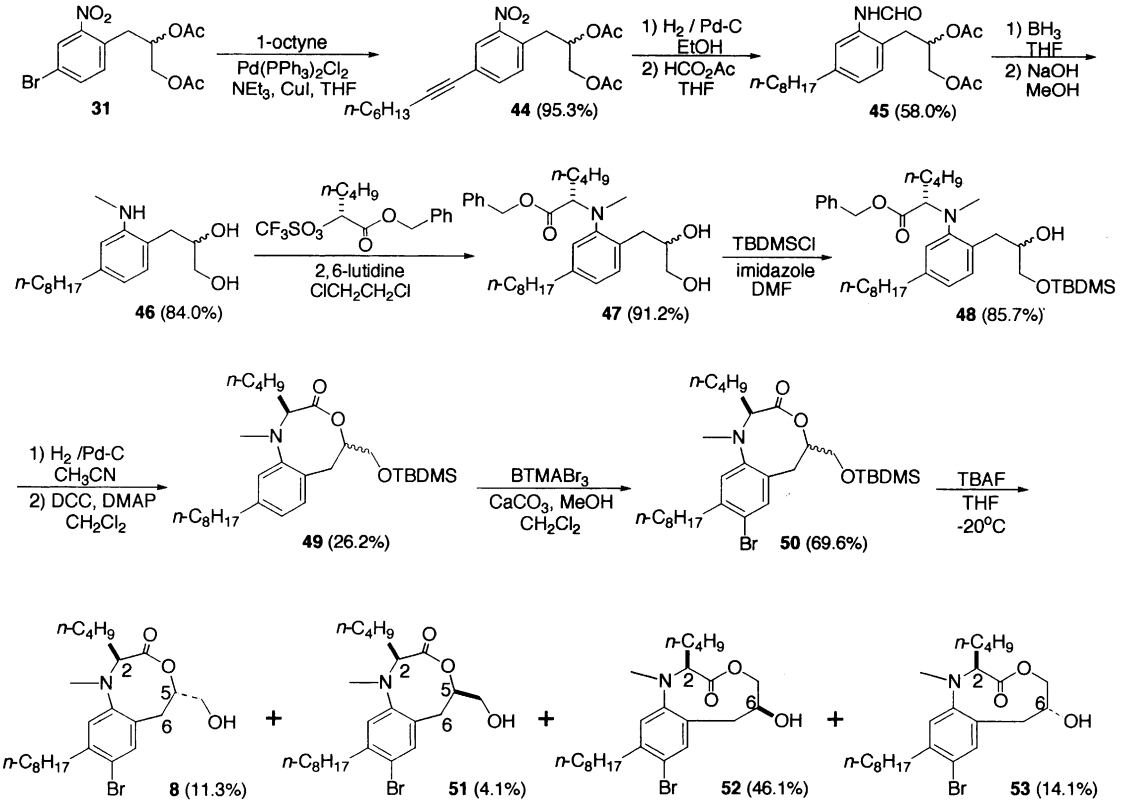
Scheme 3. Synthesis of 9-decylbenzolactone-V8 (**6**).

(**27**, 80.0%). Introduction of the valine subunit gave the diastereomeric esters (**28**, 82.3%), the benzyl ester of which was removed by hydrogenation followed by intramolecular esterification to yield diastereomeric lactones (**29**, 18.7%). *N*-Methylation of **29** gave 9-decyl-11-*O*-TBDMS-benzolactone-V8 (**30**, 80.3%). Deprotection of the TBDMS group of **30** with TBAF at −20°C gave 9-decylbenzolactone-V8 (**6**, 31.8%) and its C-5 epimer (45.2%), which were easily separated by column chromatography and identified by the NOESY spectra, respectively. Significant NOE enhancement between H-2 (δ 3.27) and H-5 (δ 4.63) was observed in the C-5 epimer but not in **6**.

^{1}H NMR spectrometry showed that both **5** and **6** existed as single conformers in deuteriochloroform at room temperature. Computational calculations similar to those mentioned above indicated that the conformations of **5** and **6** were almost the same as that of benzolactone-V8 (**4**). These results suggest that the conformations of 8-decylbenzolactone-V8 (**5**) and 9-decylbenzolactone-V8 (**6**) are very



Scheme 4. Synthesis of 8,9-dipentylbenzolactone-Nle8 (**7**).



Scheme 5. Synthesis of 8-bromo-9-octylbenzolactone-Nle8 (**8**).

similar to those of 8-decylbenzolactam-V8 (**2**) and 9-decylbenzolactam-V8 (**3**), respectively.

2.3. Synthesis of 8,9-dipentylbenzolactone-Nle8 (7) and 8-bromo-9-octylbenzolactone-V8 (8)

Endo et al.[19] reported that PKC binding affinities of benzolactam-V8s with an 8,9-cyclized alkyl group are far weaker than those of the monosubstituted benzolactam-V8s. On the other hand, the hydrophobic alkyl chain at position 2 of benzolactams increases the binding affinity to PKCδ.[20] Based on these observations, we designed two acyclic 8,9-disubstituted benzolactones with a norleucine subunit instead of valine, 8,9-dipentylbenzolactone-Nle8 (**7**) and 8-bromo-9-octylbenzolactone-Nle8 (**8**).

Synthesis of **7** is shown in Scheme 4. After protection of the two hydroxyl groups of **23** with acetyl groups (96.8%), modified Sonogashira coupling[18] with 1-pentyne gave **32** (100%). Hydrogenation of **32** accomplished saturation of the triple bond and reduction of the nitro group (77.3%). Iodination using benzyltrimethylammonium dichloroiodate ($BTMAICl_2$)[21] selectively gave the *p*-iodinated aniline derivative (**34**, 85.8%). As aromatic halides with electron-donating groups are generally poor substrates for palladium-catalyzed coupling reactions, we formylated the amine group of **34** (90.0%) by the method of Endo et al.[7] before Sonogashira reaction. Coupling of the *p*-iodoformanilide derivative **35** with 1-pentyne proceeded readily at room temperature to yield the alkyne (**36**, 68.2%). After saturation of the triple bond of **36** by hydrogenation (99.9%), reduction of the formyl group followed by alkaline hydrolysis gave the 3,4-dipentyl-*N*-methylaniline derivative (**38**, 70.8%). The norleucine subunit was introduced by substitution of **38** with the D-norleucine-derived triflate, which was synthesized by a method similar to D-valine-derived triflate synthesis[14] to give **39** (73.6%), the primary alcohol of which was selectively protected with a TBDMS group at 0°C (95.7%). After hydrogenolysis of the benzyl ester of **40**, intramolecular esterification was carried out with DCC and 4-(dimethylamino)pyridine (DMAP) in dichloromethane to give the major and minor diastereomeric lactones (**41**, 27.7%) in a ratio of 3:1. As separation of these diastereomers was difficult at this point, the TBDMS groups were deprotected. Treatment of **41** with TBAF at −20°C gave mainly three products, **7**, **42** and **43**, in yields of 16.7, 5.0 and 36.9%, respectively.

^{1}H NMR spectra of **7**, **42** and **43** showed that each compound existed as a single conformer in deuteriochloroform at room temperature. The free hydroxymethylene signals were observed in **7** and **42** (δ 3.77 for **7**, δ 3.75 for **42**) but the hydroxymethylene proton signal was shifted downfield (δ 4.17) in **43**, indicating that **7** and **42** are eight-membered lactones, but that **43** is a nine-membered lactone formed by intramolecular transesterification. As the side chain of norleucine is less hindered compared with that of valine, transesterification might have occurred more easily in this case. Deprotection of the TBDMS group at temperature lower than −20°C did not proceed at all or required long reaction time resulting in a poor yield. The absolute configuration of **43** at position 6 was determined to be *S* because of the significant NOE between H-2 (δ 3.40) and H-6 (δ 4.18) protons of **43**. On the other hand, NOE between

H-2 (δ 3.63) and H-5 (δ 4.74) protons was observed in **42** but not in **7**, suggesting that **7** was the desired 8,9-dipentyl-benzolactone-Nle8 with *S* configuration at position 5, and that **42** was its C-5 epimer. Significant NOE was also detected between H-2 (δ 3.69) and H-6α (δ 3.01) protons of **7**, which is characteristic of benzolactone-V8 (**4**).

8-Bromo-9-octylbenzolactone-Nle8 (**8**) was synthesized from **31** as shown in Scheme 5. After modified Sonogashira coupling[17] of **31** with 1-octyne (95.3%), hydrogenation followed by N-formylation gave the 3-octylformanilide derivative (**45**, 58.0%). Reduction of the formyl group and deprotection of the two acetyl groups yielded **46** (84.0%). Substitution of **46** with the D-norleucine-derived triflate (91.2%), selective protection of the primary hydroxyl group with a TBDMS group (85.7%), and hydrogenolysis of the benzyl ester followed by intramolecular esterification gave the major and minor diastereomeric lactones (**49**, 26.2%) in a ratio of 3: 1. Bromination of **49** using $BTMABr_3$ selectively gave the 8-bromo derivative (**50**, 69.6%). Deprotection of the TBDMS group of **50** with TBAF at -20°C gave four products, **8**, **51**–**53**, in yields of 11.3, 4.1, 46.1 and 14.1%, respectively. ^{1}H NMR spectra showed that each lactone also existed as a single conformer in deuteriochloroform at room temperature. The hydroxymethylene signals (δ 3.72 for **8**, δ 3.76 for **51**, δ 4.20 for **52**, δ 4.29 for **53**) indicated that **8** and **51** are eight-membered lactones, while **52** and **53** are nine-membered lactones. Significant NOE between H-2 (δ 3.62) and H-5 (δ 4.72) protons was observed in **51** but not in **8**, suggesting that the absolute configurations of **8** and **51** at position 5 are *S* and *R*, respectively. Characteristic NOE between H-2 (δ 3.71) and H-6α (δ 2.88) protons of **8** also supported this assignment. The absolute configurations of **52** and **53** at position 6 were determined to be *S* and *R*, respectively, on the basis of the NOE between H-2 (δ 3.39) and H-6 (δ 4.18) protons of **52**.

Conformational analyses of **7** and **8** by the same method as described for benzolactone-V8 (**4**) indicated that the lactone ring conformations of **7** and **8** were similar to each other. The optimized conformation of benzolactone-Nle8 subunit, the core structure of **7** and **8**, is shown in Fig. 2 (right). The conformation of the ester group is *syn* geometry, and the ring conformation is almost the same as that of benzolactone-V8 (**4**). Neither benzolactone-V8s (**5**,**6**) nor benzolactone-Nle8s (**7**,**8**) were converted to nine-membered lactones in Tris-maleate buffer (pH 7.4) at 4°C, which was used in the PKC surrogate peptide binding assay described below.

2.4. PKC surrogate binding of benzolactam-V8s (2,3) and benzolactones (5–8)

Binding affinity to the PKC isozymes of benzolactones (**5**–**8**) along with benzolactam-V8s (**2**,**3**), which were synthesized by the method of Endo et al.[10] with slight modifications, was evaluated by inhibition of the specific binding of [^{3}H]phorbol-12,13-dibutyrate (PDBu) (Fig. 3) to the PKC isozyme C1 domains as reported previously.[22,23] We have synthesized individual C1A and C1B domains of all PKC isozymes consisting of about 50 amino acids by solid-phase synthesis and measured the dissociation constants (K_d) of [^{3}H]PDBu.[23] Using these PKC C1 peptides, the concentration required to cause 50% inhibition, IC_{50}, of [^{3}H]PDBu binding was measured. The binding affinities of the benzolactones and the benzolactam-V8s to each PKC C1 peptide were expressed as K_i values calculated from the IC_{50} and the K_d of [^{3}H]PDBu as reported by Sharkey and Blumberg.[22] Table 2 summarizes the K_i values of **2**, **3**, **5** and **6**.

Corresponding to the results of Endo et al.[10] using native PKCδ, **3** showed much higher binding affinities for all PKC C1 peptides than **2**. A similar tendency was observed in benzolactone-V8s (**5**,**6**). 9-Decylbenzolactone-V8 (**6**) bound to PKC C1 peptides 3-30-fold more strongly than 8-decylbenzolactone-V8 (**5**). However, the binding affinities of **5** and **6** to all PKC C1 peptides were significantly lower than those of **2** and **3**, respectively. These results indicate

Table 2. K_i values for inhibition of the specific binding of [^{3}H]PDBu by 8-decylbenzolactam-V8 (**2**), 9-decylbenzolactam-V8 (**3**), 8-decylbenzolactone-V8 (**5**) and 9-decylbenzolactone-V8 (**6**)

PKC C1 peptide	K_i (nM)			
	8-Decyl-benzolactam-V8 (**2**)	9-Decyl-benzolactam-V8 (**3**)	8-Decyl-benzolactone-V8 (**5**)	9-Decyl-benzolactone-V8 (**6**)
α-C1A (72-mer)[a]	322.5 (36.1)[b]	17.5 (5.0)	>10,000	413.6 (6.4)
α-C1B	4690 (201)	119.1 (10.7)	>10,000	4052 (153)
β-C1A (72-mer)	442.4 (34.7)	20.0 (2.0)	>10,000	1135 (216)
β-C1B	260.8 (16.7)	12.4 (0.7)	22,010 (1896)	610.2 (23.8)
γ-C1A	1664 (99.7)	16.1 (2.6)	6321 (536)	2358 (187)
γ-C1B	150.6 (6.8)	21.3 (3.7)	17,574 (1648)	674.6 (23.1)
δ-C1A	2771 (240)	119.3 (28.8)	70,641 (3322)	6146 (304)
δ-C1B	14.6 (1.6)	1.6 (0.2)	1155 (46)	149.4 (0.9)
ε-C1A	8361 (385)	87.4 (15.3)	>10,000	4831 (112)
ε-C1B	13.1 (0.2)	3.5 (0.6)	262.3 (3.5)	82.7 (3.9)
η-C1A	2489 (74)	43.5 (17.4)	>10,000	1591 (95)
η-C1B	6.4 (1.2)	1.1 (0.2)	119.9 (6.7)	37.0 (0.9)
θ-C1A	NT[c]	NT	NT	NT
θ-C1B	13.2 (2.6)	1.7 (0.4)	1097 (53)	62.0 (5.2)

[a] Ten residues from both N and C-termini of the previous α-C1A and β-C1A were elongated as the solubility of the original 52-mer peptides was extremely low.
[b] Standard deviation of at least two separate experiments.
[c] Not tested. The K_d value of [^{3}H]PDBu to θ-C1A could not be measured because of its very weak binding affinity.

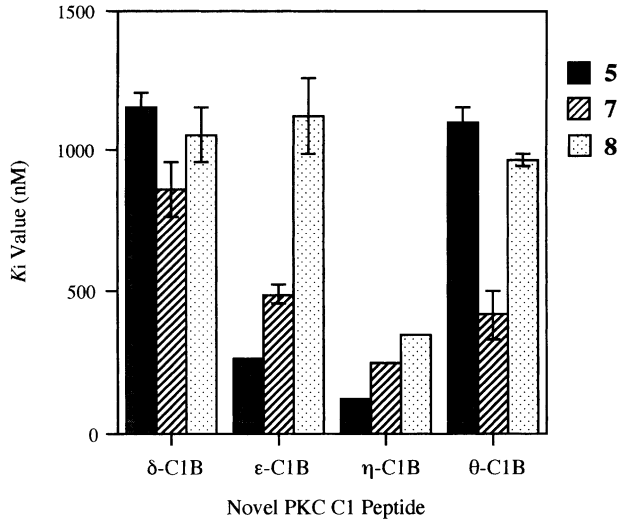


Figure 3. K_i values for inhibition of the specific binding of [^{3}H]PDBu by 8-decylbenzolactone-V8 (**5**), 8,9-dipentylbenzolactone-Nle8 (**7**) and 9-bromo-8-decylbenzolactone-Nle8 (**8**).

that the amide hydrogen of benzolactam-V8s is necessary to amplify the binding affinities for all PKC isozymes.

Although 8- and 9-decylbenzolactam-V8s (**2**,**3**) bound to the C1B peptides of novel PKCs more strongly than the other PKC C1 peptides, the selectivity among these C1B peptides was low. 9-Decylbenzolactone-V8 (**6**) exhibited a PKC isozyme- and C1 domain-selectivity similar to **2** and **3**. However, it is noteworthy that 8-decylbenzolactone-V8 (**5**) bound to η-C1B more selectively than **2**, **3** and **6**; the binding affinity of **5** for η-C1B was about 2-fold, 10-fold, and more than 50-fold higher than those for ε-C1B, δ-C1B and θ-C1B, and the other PKC C1 peptides, respectively. These results suggest that the 8-substitution of benzolactones might increase the PKC isozyme selectivity especially among novel PKC isozymes, but that of benzolactam-V8s has no effect on the PKC isozyme selectivity.

To investigate the effects of 8,9-disubstitution of benzolactones on the novel PKC C1B domain selectivity, we examined the binding affinities of 8,9-dipentylbenzolactone-Nle8 (**7**) and 8-bromo-9-octylbenzolactone-Nle8 (**8**) to the novel PKC C1B peptides (Fig. 3). Both compounds bound to η-C1B most strongly among these C1B peptides. However, the η-C1B selectivity of these compounds was relatively poor compared with that of **5**; the K_i values of **7** and **8** were 861±98 and 1050±100 nM for δ-C1B, 488±33 and 1120±134 nM for ε-C1B, 248±15 and 343±11 nM for η-C1B and 414±86 and 964±25 nM for θ-C1B, respectively. These results suggest that introduction of the substituent at position 8 of the benzolactones increases the η-C1B selectivity, but that the side chain at position 9 lowers the selectivity even in the presence of the subsitituent at position 8.

3. Conclusions

We have synthesized new lactone analogues of benzolactam-V8s (**5**–**8**) to investigate the role of the amide hydrogen of benzolactam-V8s in the PKC isozyme binding and to find new lead compounds with high PKC isozyme selectivity. The binding affinities for PKC C1 peptides of 8-decylbenzolactone-V8 (**5**) and 9-decylbenzolactone-V8 (**6**) were significantly lower than those of the corresponding lactams (**2**,**3**), respectively, indicating that the amide hydrogen of benzolactam-V8s plays a critical role in the PKC binding. Compound **5** also showed weaker activities than **2** in two in vitro bioassays (Epstein–Barr virus early antigen-inducing ability and superoxide generation-inducing ability) related to in vivo tumor promotion as reported previously.[13]

9-Decyl derivatives of benzolactam-V8 and benzolactone-V8 (**3**,**6**) were stronger PKC binders compared with the corresponding 8-decyl derivatives (**2**,**5**), respectively. This was probably due to steric hindrance between the 8-substituent and the entrance of the tumor promoter-binding cavity of PKC C1 domains as observed in the computational docking study of 8-decylbenzolactam-V8 (**2**) with PKCδ-C1B.[10] Although 9-decylbenzolactone-V8 (**6**) showed a PKC isozyme- and C1 domain-selectivity similar to **2** and **3**, a significant increase in the η-C1B selectivity was observed in 8-decylbenzolactone-V8 (**5**). The present results provided a basis for the rational design of new medicinal agents with PKC isozyme selectivity. As a recent investigation revealed that PKCη plays an important role in mouse skin carcinogenesis,[24] **5** might be useful for further studies of the mechanism of tumor promotion.

4. Experimental

4.1. General

The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-2200A, Digital Polarimeter, Jasco DIP-1000; ^{1}H NMR, Bruker ARX500 and AC300 (ref. TMS); HPLC, Waters Model 600E with Model 2487 UV detector; (HR) EI-MS and FAB-MS, JOEL JMS-600H. HPLC was carried out on a YMC packed SH-342-5 (ODS, 20 mm i.d.×250 mm) column (Yamamura Chemical Laboratory). Wako C-200 gel (silica gel, Wako Pure Chemical Industries) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography. [^{3}H]PDBu (17.0 Ci/mol) was purchased from NEN Research Products. All other chemicals and reagents were purchased from chemical companies and used without further purification.

4.1.1. Synthesis of benzolactone-V8 (4). To a solution of **9** (2.10 g, 10.2 mmol) in anhydrous THF (13 ml) was added dropwise 1.0 M BH_3 in THF solution (40 ml) at 0°C, and the mixture was stirred for 24 h at 0°C. The reaction was quenched with distilled water (20 ml), and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **10** (1.45 g, 7.50 mmol, 73.5%). Compound **10**: ^{1}H NMR δ ($CDCl_3$, 0.084 M, 500 MHz, 300 K) ppm: 2.53 (2H, br.s), 3.00 (1H, dd, J=13.7, 8.4 Hz), 3.14 (1H, dd, J=13.7, 4.4 Hz), 3.56 (1H, dd, J=11.2, 6.6 Hz), 3.75 (1H, dd, J=11.2, 3.3 Hz), 4.03 (1H, m), 7.40 (1H, dt, J=8.0,

1.2 Hz), 7.44 (1H, dd, *J*=8.0, 1.1 Hz), 7.56 (1H, dt, *J*=8.0, 1.1 Hz), 7.93 (1H, dd, *J*=8.0, 1.2 Hz); HR-FAB-MS *m/z*: 198.0772 (MH^+, calcd for $C_9H_{12}NO_4$, 198.0766).

To a solution of **10** (1.22 g, 6.19 mmol) in anhydrous DMF (6.5 ml) was added imidazole (842 mg, 12.4 mmol) and TBDMSCl (929 mg, 6.19 mmol) at 0°C, and the mixture was stirred for 45 min at 0°C. The reaction was quenched with distilled water (50 ml), and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **11** (1.55 g, 4.98 mmol, 80.5%). Compound **11**: ^{1}H NMR δ ($CDCl_3$, 0.064 M, 500 MHz, 300 K) ppm: 0.09 (6H, s), 0.91 (9H, s), 2.52 (1H, d, *J*=4.7 Hz), 2.98 (1H, dd, *J*=13.8, 8.8 Hz), 3.12 (1H, dd, *J*=13.8, 3.6 Hz), 3.54 (1H, dd, *J*=10.0, 6.5 Hz), 3.73 (1H, dd, *J*=10.0, 3.7 Hz), 3.95 (1H, m), 7.38 (1H, dt, *J*=7.8, 1.1 Hz), 7.46 (1H, d, *J*=7.1 Hz), 7.54 (1H, dt, *J*=7.5, 0.7 Hz), 7.93 (1H, dd, *J*=8.3, 0.6 Hz); HR-FAB-MS *m/z*: 312.1621 (MH^+, calcd for $C_{15}H_{26}NO_4Si$, 312.1631).

A mixture of **11** (1.99 g, 6.40 mmol) and 10% Pd–C (298 mg) in MeOH (13 ml) was vigorously stirred under 1 atm of H_2 at room temperature for 50 min. The reaction mixture was filtered and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **12** (1.72 g, 6.10 mmol, 95.3%). Compound **12**: ^{1}H NMR δ ($CDCl_3$, 0.045 M, 500 MHz, 300 K) ppm: 0.08 (6H, s), 0.91 (9H, s), 2.60 (1H, br.s), 2.71 (2H, d, *J*=6.0 Hz), 3.51 (1H, dd, *J*=10.0, 6.8 Hz), 3.63 (1H, dd, *J*=10.0, 4.3 Hz), 3.90 (1H, m), 4.04 (2H, br.s), 6.68 (1H, dd, *J*=7.9, 0.8 Hz), 6.72 (1H, dt, *J*=7.4, 1.0 Hz), 7.01 (1H, dd, *J*=7.5, 1.4 Hz), 7.05 (1H, dt, *J*=7.6, 1.5 Hz); HR-EI-MS *m/z*: 281.1778 (M^+, calcd for $C_{15}H_{27}NO_2Si$, 281.1811).

A solution of **12** (7.62 g, 27.1 mmol), 2,6-lutidine (4.50 ml, 38.7 mmol), D-valine-derived triflate[14] (10.5 g, 30.9 mmol) in dichloroethane (44.5 ml) was refluxed gently for 4.5 h. The reaction mixture was poured into water and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **13** (9.11 g, 19.3 mmol, 71.2%). A mixture of **13** (1.34 g, 2.85 mmol) and 10% Pd–C (147 mg) in CH_3CN (12 ml) was vigorously stirred under 1 atm of H_2 at room temperature for 20 min. The reaction mixture was filtered and concentrated to give the crude carboxylic acid. To a solution of the acid in CH_2Cl_2 (8.4 ml) was added triethylamine (428 μl, 3.10 mmol) and HOBt (383 mg, 2.85 mmol). After stirring for 15 min, DCC (589 mg, 2.85 mmol) in CH_2Cl_2 (4.0 ml) was added dropwise at 0°C to the solution. The reaction mixture was stirred for 40 h at room temperature and then filtered. The filtrate was concentrated and purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **14** (364 mg, 1.00 mmol, 35.1% in two steps), in which two diastereomers existed in a ratio of 1:1. Compound **14**: ^{1}H NMR δ ($CDCl_3$, 0.058 M, 500 MHz, 300 K) ppm: 0.08 (3H, s), 0.10 (3H, s), 0.10 (3H, s), 0.11 (3H, s), 0.92 (9H, s), 0.93 (9H, s), 0.99 (3H, d, *J*=6.7 Hz), 1.03 (3H, d, *J*=6.7 Hz), 1.20 (3H, d, *J*=6.7 Hz), 1.21 (3H, d, *J*=6.6 Hz), 2.03 (1H, m), 2.22 (1H, m), 2.90 (1H, dd, *J*=14.8, 2.4 Hz), 2.94 (1H, dd, *J*=15.8, 6.5 Hz), 3.16 (1H, dd, *J*=14.8, 8.4 Hz), 3.20 (1H, dd, *J*=15.8, 4.5 Hz), 3.30 (1H, d, *J*=9.5 Hz), 3.41 (1H, d, *J*=8.8 Hz), 3.62 (1H, dd, *J*=10.5, 6.4 Hz), 3.72 (1H, dd, *J*=10.5, 5.8 Hz), 3.75 (1H, dd, *J*=10.5, 6.4 Hz), 3.88 (1H, dd, *J*=10.5, 4.9 Hz), 4.62 (1H, m), 5.20 (1H, m), 6.99 (2H, m), 7.06 (4H, m), 7.17 (2H, m); HR-FAB-MS *m/z*: 363.2241 (M^+, calcd for $C_{20}H_{33}NO_3Si$, 363.2230).

AcOH (167 μl) was added to the mixture of **14** (182 mg, 0.500 mmol), 37% HCHO (800 μl, 9.00 mmol), and $NaBH_3CN$ (240 mg, 3.81 mmol) in CH_3CN (2 ml) at 0°C. The reaction mixture was stirred for 1 h at room temperature and then concentrated. Water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **15** (79.2 mg, 0.210 mmol, 42.0%) and **16** (79.4 mg, 0.211 mmol, 42.1%). Compound **15**: ^{1}H NMR δ ($CDCl_3$, 0.069 M, 500 MHz, 300 K) ppm: 0.09 (3H, s), 0.11 (3H, s), 0.86 (3H, d, *J*=6.5 Hz), 0.93 (9H, s), 1.06 (3H, d, *J*=6.5 Hz), 2.21 (1H, m), 2.83 (1H, dd, *J*=15.0, 5.0 Hz), 2.92 (3H, s), 3.07 (1H, dd, *J*=15.0, 2.6 Hz), 3.25 (1H, d, *J*=10.5 Hz), 3.60 (1H, dd, *J*=9.9, 7.9 Hz), 3.81 (1H, dd, *J*=9.9, 5.7 Hz), 4.62 (1H, m), 7.05 (1H, dt, *J*=7.1, 1.7 Hz), 7.12 (1H, dd, *J*=7.1, 0.8 Hz), 7.20 (2H, m); HR-FAB-MS *m/z*: 378.2483 (MH^+, calcd for $C_{21}H_{36}NO_3Si$, 378.2464). Compound **16**: ^{1}H NMR δ ($CDCl_3$, 0.085 M, 500 MHz, 300 K) ppm: 0.06 (3H, s), 0.08 (3H, s), 0.91 (9H, s), 0.97 (3H, d, *J*=6.5 Hz), 1.05 (3H, d, *J*=6.6 Hz), 2.21 (1H, m), 2.74 (3H, s), 2.89 (1H, dd, *J*=15.6, 3.7 Hz), 3.15 (1H, dd, *J*=15.6, 3.7 Hz), 3.29 (1H, d, *J*=10.3 Hz), 3.50 (1H, dd, *J*=10.1, 7.7 Hz), 3.65 (1H, dd, *J*=10.1, 5.6 Hz), 4.83 (1H, m), 7.08 (3H, m), 7.21 (1H, m); HR-FAB-MS *m/z*: 377.2396 (M^+, calcd for $C_{21}H_{35}NO_3Si$, 377.2386).

Deprotection of the TBDMS group of **15** and **16** was performed by the following three methods.

Method A: a solution of **15** (37.2 mg, 98.7 μmol) in 1N HCl–dioxane (1.2 ml) was stirred for 20 min and then concentrated. 5% K_2CO_3 aq. (5 ml) was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc followed by HPLC on YMC-SH-342-5 using 63% MeOH to give **17** (15.9 mg, 60.5 μmol, 75.9%). Compound **17**: $[\alpha]_D=-174.0°$ (*c*=0.57, MeOH, 27.8°C); UV λ_{max} (MeOH) nm (ε) 256 (5000), 207 (13,800); ^{13}C NMR δ ($CDCl_3$, 0.061 M, 125 MHz, 300 K) ppm: 19.58, 19.88, 27.43, 35.71, 38.05, 65.12, 72.21, 79.65, 124.77, 125.43, 127.89, 132.02, 135.94, 152.25, 171.21; HR-EI-MS *m/z*: 263.1500 (M^+, calcd for $C_{15}H_{21}NO_3$, 263.1521).

Compound **16** (31.9 mg, 84.6 μmol) was treated in a manner similar to that described for the synthesis of **17** to give **18** (15.9 mg, 60.5 μmol, 71.5%). Compound **18**: $[\alpha]_D=-75.0°$ (*c*=0.40, MeOH, 27.8°C); UV λ_{max}

(MeOH) nm (ε) 270 (2900), 235 (2500), 206 (11,500); HR-EI-MS *m/z*: 263.1497 (M^+, calcd for $C_{15}H_{21}NO_3$, 263.1521).

Method B: TBAF·$5H_2O$ (157 mg, 500 μmol) was added to **16** (22.0 mg, 58.4 μmol) in THF (1 ml) and the mixture was stirred for 15 min at room temperature. The solution was poured into water and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc followed by HPLC on YMC-SH-342-5 using 68% MeOH to give **18** (9.70 mg, 37.0 μmol, 63.8%) and **4** (1.10 mg, 4.18 μmol, 6.9%). Compound **4**: $[\alpha]_D=-124.0°$ (c=0.68, MeOH, 27.8°C); UV λ_{max} (MeOH) nm (ε) 254 (4300), 207 (14,000); ^{13}C NMR δ ($CDCl_3$, 0.061 M, 125 MHz, 300 K) ppm: 19.19, 20.00, 26.98, 35.52, 35.73, 64.77, 73.42, 77.94, 124.23, 125.46, 128.05, 132.14, 132.80, 150.66, 170.46; HR-EI-MS *m/z*: 263.1496 (M^+, calcd for $C_{15}H_{21}NO_3$, 263.1521).

Method C: TBAF·$5H_2O$ (127 mg, 403 μmol) was added to **16** (25.3 mg, 67.1 μmol) in THF (1 ml) at −20°C and the mixture was stirred for 1 h at −20°C. The solution was poured into ice-water and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc followed by HPLC on YMC-SH-342-5 using 68% MeOH to give **4** (13.4 mg, 51.0 μmol, 76.0%).

4.1.2. Synthesis of 8-decylbenzolactone-V8 (5). To a solution of **16** (100 mg, 0.265 mmol) in CH_2Cl_2 (2.5 ml) and MeOH (1.0 ml) was added $BTMABr_3$ (114 mg, 0.292 mmol) and $CaCO_3$ (98.4 mg, 0.981 mmol) at room temperature. The reaction mixture was stirred for 30 min and then filtered. After concentration of the filtrate, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on YMC A60-350/250 gel using water and increasing amounts of MeOH to give **19** (84.1 mg, 0.184 mmol, 69.4%). Compound **19**: ^1H NMR δ ($CDCl_3$, 0.013 M, 500 MHz, 300 K) ppm: 0.07 (3H, s), 0.10 (3H, s), 0.92 (9H, s), 0.94 (3H, d, J=6.5 Hz), 1.04 (3H, d, J=6.7 Hz), 2.18 (1H, m), 2.70 (3H, s), 2.86 (1H, dd, J=15.5, 3.6 Hz), 3.08 (1H, dd, J=15.5, 4.5 Hz), 3.21 (1H, d, J=10.5 Hz), 3.47 (1H, dd, J=10.1, 8.4 Hz), 3.66 (1H, dd, J=10.1, 5.4 Hz), 4.79 (1H, m), 6.96 (1H, d, J=8.3 Hz), 7.23 (1H, d, J=2.3 Hz), 7.31 (1H, dd, J=8.3, 2.3 Hz); HR-EI-MS *m/z*: 455.1502 (M^+, calcd for $C_{21}H_{34}NO_3SiBr$, 455.1491).

A mixture of **19** (78.0 mg, 0.171 mmol), 1-decene (64.6 μl, 0.342 mmol), palladium acetate (42.3 mg, 0.188 mmol), tri-*o*-tolylphosphine (78.0 mg, 0.257 mmol) and triethylamine (1.00 ml, 7.19 mmol) in CH_3CN (1.3 ml) was heated at 120°C in a sealed tube for 1.5 h and then filtered. After concentration of the filtrate, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give a mixture of alkenes (69.4 mg, 0.138 mmol, 78.9%). The alkenes and 5% Pd–C (17.4 mg) in EtOH (10 ml) were vigorously stirred under 1 atm of H_2 at room temperature for 3.5 h. The reaction mixture was filtered and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give a mixture of 8-decyl-11-*O*-TBDMS-benzolactone-V8 and its regio isomer (60.7 mg, 0.117 mmol, 87.0%). This mixture (44.5 mg, 86.0 μmol) was treated in a manner similar to that described for the synthesis of **4** to give **5** (17.8 mg, 44.2 μmol, 49.1%) and **20** (6.50 mg, 16.1 μmol, 18.7%). Compound **5**: $[\alpha]_D=-84.0°$ (c=0.42, MeOH, 24.3°C); UV λ_{max} (MeOH) nm (ε) 260 (2500); ^1H NMR δ ($CDCl_3$, 0.088 M, 500 MHz, 300 K) ppm: 0.88 (3H, t, J=6.9 Hz), 0.97 (3H, d, J=6.6 Hz), 1.05 (3H, d, J=6.6 Hz), 1.23–1.31 (14H, m), 1.58 (2H, m), 2.17 (2H, m), 2.53 (2H, t, J=7.8 Hz), 2.76 (3H, s), 2.87 (1H, dd, J=15.8, 5.1 Hz), 3.01 (1H, dd, J=15.8, 4.0 Hz), 3.28 (1H, d, J=10.2 Hz), 3.69 (2H, m), 4.87 (1H, m), 6.86 (1H, s), 7.02 (2H, s); ^{13}C NMR δ ($CDCl_3$, 0.088 M, 125 MHz, 300 K) ppm: 14.12, 19.28, 19.87, 22.70, 26.68, 29.34, 29.41, 29.52, 29.62, 29.64, 31.52, 31.93, 35.27, 35.31, 36.39, 64.49, 74.66, 77.54, 126.74, 127.93, 132.17, 133.41, 139.68, 148.30, 170.68; HR-EI-MS *m/z*: 403.3064 (M^+, calcd for $C_{25}H_{41}NO_3$, 403.3086). Compound **20**: ^1H NMR δ ($CDCl_3$, 0.032 M, 500 MHz, 300 K) ppm: 0.87 (3H, t, J=6.9 Hz), 0.98 (3H, d, J=6.6 Hz), 1.05 (3H, d, J=6.6 Hz), 1.20 (3H, d, J=6.9 Hz), 1.14–1.30 (12H, m), 1.52 (2H, m), 2.09 (1H, br.s), 2.16 (1H, m), 2.61 (1H, m), 2.76 (3H, s), 2.86 (1H, dd, J=15.8, 4.8 Hz), 3.02 (1H, dd, J=15.8, 4.0 Hz), 3.29 (1H, d, J=10.2 Hz), 3.69 (2H, m), 4.87 (1H, m), 6.84 (1H, s), 7.02 (2H, s); HR-EI-MS *m/z*: 403.3038 (M^+, calcd for $C_{25}H_{41}NO_3$, 403.3086).

4.1.3. Synthesis of 9-decylbenzolactone-V8 (6). 60% Sodium hydride in oil (1.93 g, 48.1 mmol) was washed with hexane and suspended in THF (10 ml) under an Ar atmosphere. To this suspension was added **21** (5.20 g, 24.1 mmol) in THF (10 ml) at 0°C. After stirring for 30 min, diethyl oxalate (26.0 ml, 193 mmol) was added dropwise at 0°C and the reaction mixture was stirred for 24 h at 40°C. The reaction was quenched by 1N HCl (50 ml), and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **22** (6.66 g, 21.1 mmol, 87.6%). Compound **22**: ^1H NMR δ ($CDCl_3$, 0.106 M, 500 MHz, 300 K) ppm: 1.40 (3H, t, J=7.2 Hz), 4.39 (2H, q, J=7.2 Hz), 4.50 (2H, s), 7.21 (1H, d, J=8.2 Hz), 7.76 (1H, dd, J=8.2, 2.1 Hz), 8.32 (1H, d, J=2.1 Hz); HR-FAB-MS *m/z*: 315.9817 (MH^+, calcd for $C_{11}H_{11}NO_5Br$, 315.9820).

Lithium borohydride (15.0 mg, 0.831 mmol) was added to **22** (87.1 mg, 0.277 mmol) in THF (1 ml), and the mixture was stirred for 1 h at room temperature. The reaction was quenched with distilled water (5 ml) and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel

C-200 using hexane and increasing amounts of EtOAc to give **23** (79.6 mg, 0.204 mmol, 73.5%). Compound **23** (2.98 g, 10.8 mmol) was treated in a manner similar to that described for the synthesis of **11** to give **24** (3.44 g, 8.82 mmol, 81.7%). Compound **24**: ^{1}H NMR δ ($CDCl_3$, 0.056 M, 500 MHz, 300 K) ppm: 0.09 (6H, s), 0.91 (9H, s), 2.48 (1H, d, J=4.8 Hz), 2.93 (1H, dd, J=13.9, 8.9 Hz), 3.07 (1H, dd, J=13.9, 3.5 Hz), 3.52 (1H, dd, J=10.0, 6.4 Hz), 3.72 (1H, dd, J=10.0, 3.7 Hz), 3.91 (1H, m), 7.36 (1H, d, J=8.3 Hz), 7.65 (1H, dd, J=8.3, 2.1 Hz), 8.06 (1H, d, J=2.1 Hz); HR-FAB-MS *m/z*: 390.0751 (MH^+, calcd for $C_{15}H_{25}NO_4SiBr$, 390.0736).

A mixture of **24** (1.65 g, 4.23 mmol) and acetic anhydride (2.00 ml, 21.2 mmol) in pyridine (5 ml) was stirred for 1 h at room temperature and then concentrated. The residue was poured into water and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **25** (1.53 g, 3.54 mmol, 83.7%). Compound **25**: ^{1}H NMR δ ($CDCl_3$, 0.051 M, 500 MHz, 300 K) ppm: 0.07 (6H, s), 0.91 (9H, s), 1.95 (3H, s), 3.12 (1H, dd, J=14.1, 8.7 Hz), 3.34 (1H, dd, J=14.1, 4.0 Hz), 3.64 (1H, dd, J=10.9, 5.1 Hz), 3.69 (1H, dd, J=10.9, 4.7 Hz), 5.11 (1H, m), 7.27 (1H, d, J=8.5 Hz), 7.64 (1H, dd, J=8.3, 2.0 Hz), 8.05 (1H, d, J=2.0 Hz); HR-FAB-MS *m/z*: 432.0838 (MH^+, calcd for $C_{17}H_{27}NO_5SiBr$, 432.0841).

A mixture of **25** (1.53 g, 3.54 mmol), $PdCl_2(PPh_3)_2$ (200 mg, 0.280 mmol), 1-decene (1.30 ml, 7.08 mmol) and CuI (27.0 mg, 0.140 mmol) in diethylamine (15 ml) was stirred for 20 h at room temperature under an Ar atmosphere and then filtered. After concentration of the filtrate, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give the alkyne, which was treated with 2N NaOH (1 ml) in MeOH (9 ml) for 1 h at room temperature and then concentrated. Water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **26** (599 mg, 1.34 mmol, 37.8% in two steps). Compound **26**: ^{1}H NMR δ ($CDCl_3$, 0.058 M, 500 MHz, 300 K) ppm: 0.08 (6H, s), 0.89 (3H, t, J=7.0 Hz), 0.91 (9H, s), 1.29–1.31 (8H, m), 1.44 (2H, m), 1.59 (2H, m), 2.40 (2H, t, J=7.1 Hz), 2.48 (1H, d, J=4.7 Hz), 2.95 (1H, dd, J=13.8, 8.7 Hz), 3.08 (1H, dd, J=13.8, 3.6 Hz), 3.52 (1H, dd, J=9.9, 6.4 Hz), 3.71 (1H, dd, J=9.9, 3.6 Hz), 3.91 (1H, m), 7.36 (1H, d, J=8.0 Hz), 7.51 (1H, dd, J=7.9, 1.0 Hz), 7.91 (1H, s); HR-FAB-MS *m/z*: 448.2912 (MH^+, calcd for $C_{25}H_{42}NO_4Si$, 448.2883).

A mixture of **26** (738 mg, 1.65 mmol) and 10% Pd–C (148 mg) in EtOH (15 ml) was stirred vigorously under 1 atm of H_2 at room temperature for 1 h. The reaction mixture was filtered and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **27** (554.2 mg, 1.32 mmol, 80.0%). Compound **27**: ^{1}H NMR δ ($CDCl_3$, 0.040 M, 500 MHz, 300 K) ppm: 0.08 (6H, s), 0.88 (3H, d, J=6.9 Hz), 0.91 (9H, s), 1.26–1.32 (14H, m), 1.56 (2H, m), 2.48 (2H, t, J=7.8 Hz), 2.64 (1H, br.s), 2.67 (2H, m), 3.50 (1H, dd, J=10.0, 6.4 Hz), 3.62 (1H, dd, J=10.0, 4.8 Hz), 3.88 (1H, m), 3.97, (2H, br.s), 6.51 (1H, d, J=1.4 Hz), 6.54 (1H, dd, J=7.6, 1.4 Hz), 6.91 (1H, d, J=7.6 Hz); HR-EI-MS *m/z*: 421.3350 (M^+, calcd for $C_{25}H_{47}NO_2Si$, 421.3376).

Compound **27** was treated in a manner similar to that described for the synthesis of **4** to give **6** and its C-5 epimer. Compound **6**: $[\alpha]_D$=−91.0° (c=0.61, MeOH, 28.6°C); UV λ_{max} (MeOH) nm (ε) 256 (1470), 213 (3770), 202 (2700); ^{1}H NMR δ ($CDCl_3$, 0.088 M, 500 MHz, 300 K) ppm: 0.88 (3H, t, J=7.0 Hz), 0.99 (3H, d, J=6.5 Hz), 1.03 (3H, d, J=6.6 Hz), 1.23–1.31 (14H, m), 1.59 (2H, m), 2.23 (2H, m), 2.55 (2H, t, J=7.8 Hz), 2.78 (3H, s), 2.92 (1H, dd, J=16.2, 5.1 Hz), 3.01 (1H, dd, J=16.2, 3.8 Hz), 3.33 (1H, d, J=10.2 Hz), 3.69 (2H, m), 4.81 (1H, m), 6.86 (1H, dd, J=7.7, 1.4 Hz), 6.89 (1H, d, J=1.4 Hz), 6.96 (1H, J=7.7 Hz); ^{13}C NMR δ ($CDCl_3$, 0.088 M, 125 MHz, 300 K) ppm: 14.11, 19.23, 20.04, 22.69, 26.99, 29.34, 29.36, 29.51, 29.62, 31.41, 31.92, 35.18, 35.52, 35.83, 64.82, 73.55, 78.12, 124.31, 125.59, 129.86, 131.94, 143.07, 150.46, 170.59; HR-EI-MS *m/z*: 403.3055 (M^+, calcd for $C_{25}H_{41}NO_3$, 403.3086). C-5 epimer of **6**; $[\alpha]_D$=−102.0° (c=0.82, MeOH, 28.6°C); UV λ_{max} (MeOH) nm (ε) 256 (1600), 215 (7000); ^{1}H NMR δ ($CDCl_3$, 0.075 M, 500 MHz, 300 K) ppm: 0.88 (3H, t, J=7.0 Hz), 0.88 (3H, d, J=6.5 Hz), 1.06 (3H, d, J=6.6 Hz), 1.26–1.31 (14H, m), 1.58 (2H, m), 2.28 (2H, m), 2.55 (2H, m), 2.78 (1H, dd, J=15.4, 2.4 Hz), 2.89 (3H, s), 2.89 (1H, dd, J=15.4, 6.0 Hz), 3.27 (1H, d, J=10.5 Hz), 3.78 (2H, m), 4.63 (1H, m), 6.87 (1H, dd, J=7.7, 1.5 Hz), 6.98 (1H, d, J=1.5 Hz), 7.00 (1H, J=7.7 Hz); ^{13}C NMR δ ($CDCl_3$, 0.075 M, 125 MHz, 300 K) ppm: 14.12, 19.56, 19.90, 22.69, 27.43, 29.28, 29.34, 29.50, 29.62, 31.46, 31.91, 35.51, 35.60, 37.71, 65.17, 72.12, 79.87, 124.72, 125.37, 131.79, 132.80, 142.90, 152.11, 171.28; HR-EI-MS *m/z*: 403.3013 (M^+, calcd for $C_{25}H_{41}NO_3$, 403.3086).

4.1.4. Synthesis of 8,9-dipentylbenzolactone-Nle8 (7). A mixture of **23** (10.3 g, 37.3 mmol) and acetic anhydride (14.0 ml, 149 mmol) in pyridine (9 ml) was stirred for 30 min at room temperature and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **31** (13.0 g, 36.1 mmol, 96.8%). To a mixture of **31** (3.60 g, 10.0 mmol), $PdCl_2(PPh_3)_2$ (527 mg, 0.751 mmol), 1-pentyne (2.20 ml, 22.0 mmol), triethylamine (2.80 ml, 20.0 mmol) in THF (30 ml) was added CuI (57.0 mg, 0.300 mmol) at room temperature under an Ar atmosphere. The reaction mixture was stirred for 18 h at room temperature and then filtered. After concentration of the filtrate, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **32** (3.47 g, 10.0 mmol, 100%). Compound **32**: ^{1}H NMR δ ($CDCl_3$, 0.140 M, 500 MHz, 300 K) ppm: 1.05 (3H, t, J=7.4 Hz), 1.63 (2H, m), 1.96 (3H, s), 2.08 (3H,

s), 2.39 (2H, t, J=7.0 Hz), 3.14 (1H, dd, J=14.1, 8.9 Hz), 3.32 (1H, dd, J=14.1, 4.3 Hz), 4.09 (1H, dd, J=11.9, 5.6 Hz), 4.28 (1H, dd, J=11.9, 3.9 Hz), 5.34 (1H, m), 7.27 (1H, d, J=8.0 Hz), 7.51 (1H, dd, J=8.0, 1.6 Hz), 7.94 (1H, d, J=1.3 Hz); HR-FAB-MS m/z: 348.1452 (MH^+, calcd for $C_{18}H_{22}NO_6$, 348.1447).

Compound **32** (3.54 g, 10.0 μmol) was treated in a manner similar to that described for the synthesis of **27** to give **33** (2.48 g, 7.73 μmol, 77.3%). Compound **33**: ^{1}H NMR δ ($CDCl_3$, 0.035 M, 500 MHz, 300 K) ppm: 0.89 (3H, t, J=7.0 Hz), 1.32 (4H, m), 1.57 (2H, m), 2.07 (3H, s), 2.10 (3H, s), 2.47 (2H, t, J=7.8 Hz), 2.69 (1H, dd, J=14.1, 9.8 Hz), 2.89 (1H, dd, J=14.1, 4.3 Hz), 4.09 (2H, br.s), 4.12 (1H, dd, J=12.1, 6.4 Hz), 4.22 (1H, dd, J=12.1, 2.7 Hz), 5.18 (1H, m), 6.50 (1H, s), 6.50 (1H, d, J=8.0 Hz), 6.84 (1H, d, J=8.1 Hz); HR-EI-MS m/z: 321.1944 (M^+, calcd for $C_{18}H_{27}NO_4$, 321.1940).

To a solution of **33** (2.47 g, 7.69 mmol) in CH_2Cl_2 (50 ml) and MeOH (20 ml) was added $BTMAICl_2$ (2.81 g, 8.07 mmol) and $CaCO_3$ (1.00 g, 10.0 mmol) at room temperature. The reaction mixture was stirred for 2.5 h and then filtered. After concentration of the residue, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **34** (2.95 g, 6.60 mmol, 85.8%). Compound **34**: ^{1}H NMR δ ($CDCl_3$, 0.032 M, 500 MHz, 300 K) ppm: 0.91 (3H, t, J=6.9 Hz), 1.36 (4H, m), 1.54 (2H, m), 2.08 (3H, s), 2.10 (3H, s), 2.54 (2H, t, J=8.0 Hz), 2.64 (1H, dd, J=14.1, 9.5 Hz), 2.83 (1H, dd, J=14.1, 4.4 Hz), 4.12 (1H, dd, J=12.1, 6.2 Hz), 4.16 (2H, br.s), 4.22 (1H, dd, J=12.1, 2.9 Hz), 5.04 (1H, m), 6.55 (1H, s), 7.33 (1H, s); HR-EI-MS m/z: 447.0902 (M^+, calcd for $C_{18}H_{26}NO_4I$, 447.0907).

Formic acid (1.10 ml, 29.2 mmol) was added to acetic anhydride (1.90 ml, 20.2 mmol) at 0°C, and the mixture was stirred for 2 h at 50°C to give the mixed anhydride. To the mixed anhydride was added dropwise a solution of **34** (2.95 g, 6.60 mmol) in THF (6.4 ml) at 0°C. The mixture was stirred for 30 min at 0°C and then concentrated. Saturated aqueous $NaHCO_3$ was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **35** (2.82 g, 5.94 mmol, 90.0%), in which two conformers existed in a ratio of 1:0.5. Compound **35**: ^{1}H NMR δ ($CDCl_3$, 0.043 M, 500 MHz, 300 K) ppm for the major conformer: 0.91 (3H, t, J=6.7 Hz), 1.37 (4H, m), 1.57 (2H, m), 2.08 (3H, s), 2.16 (3H, s), 2.66 (2H, t, J=7.9 Hz), 2.70 (1H, dd, J=14.4, 10.3 Hz), 2.92 (1H, dd, J=14.4, 3.0 Hz), 4.18 (1H, dd, J=12.3, 6.1 Hz), 4.21 (1H, dd, J=12.3, 3.1 Hz), 4.81 (1H, m), 7.51 (1H, s), 8.18 (1H, s), 8.51, (1H, s), 8.74 (1H, br.s), for the minor conformer: 0.91 (3H, t, J=6.7 Hz), 1.37 (4H, m), 1.57 (2H, m), 2.09 (3H, s), 2.11 (3H, s), 2.66 (2H, t, J=7.9 Hz), 2.77 (1H, dd, J=14.4, 7.2 Hz), 2.90 (1H, dd, J=14.4, 6.4 Hz), 4.09 (1H, dd, J=12.1, 5.6 Hz), 4.27 (1H, dd, J=12.1, 3.4 Hz), 4.98 (1H, m), 7.02 (1H, s), 7.62 (1H, s), 8.31 (1H, br.d, J=9.8 Hz), 8.56 (1H, d, J=10.9 Hz); HR-EI-MS m/z: 475.0807 (M^+, calcd for $C_{19}H_{26}NO_5I$, 475.0856).

To a mixture of **35** (2.82 g, 5.94 mmol), $PdCl_2(PPh_3)_2$ (313 mg, 0.446 mmol), 1-pentyne (1.30 ml, 13.1 mmol), triethylamine (1.70 ml, 11.9 mmol) in THF (18 ml) was added CuI (33.9 mg, 0.178 mmol) at room temperature under an Ar atmosphere. The reaction mixture was stirred for 44 h at room temperature and then filtered. After concentration of the filtrate, water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **36** (1.68 g, 4.05 mmol, 68.2%), in which two conformers existed in a ratio of 1:0.6. Compound **36**: ^{1}H NMR δ ($CDCl_3$, 0.074 M, 500 MHz, 300 K) ppm for the major conformer: 0.90 (3H, t, J=6.7 Hz), 1.06 (3H, t, J=7.4 Hz), 1.34 (4H, m), 1.63 (4H, m), 2.08 (3H, s), 2.16 (3H, s), 2.41 (2H, t, J=6.9 Hz), 2.71 (1H, dd, J=14.5, 10.4 Hz), 2.72 (2H, t, J=7.8 Hz), 2.94 (1H, dd, J=14.5, 2.9 Hz), 4.17 (1H, dd, J=12.3, 6.4 Hz), 4.22 (1H, dd, J=12.3, 2.7 Hz), 4.81 (1H, m), 7.08 (1H, s), 8.15 (1H, s), 8.50, (1H, d, J=1.3 Hz), 8.78 (1H, br.s), for the minor conformer: 0.91 (3H, t, J=6.8 Hz), 1.06 (3H, t, J=7.4 Hz), 1.34 (4H, m), 1.63 (4H, m), 2.09 (3H, s), 2.10 (3H, s), 2.42 (2H, t, J=6.9 Hz), 2.71 (2H, t, J=7.8 Hz), 2.77 (1H, dd, J=14.4, 3.8 Hz), 2.92 (1H, dd, J=14.4, 5.9 Hz), 4.08 (1H, dd, J=12.1, 5.8 Hz), 4.27 (1H, dd, J=12.1, 3.2 Hz), 4.96 (1H, m), 6.99 (1H, s), 7.19 (1H, s), 8.41, (1H, br.d, J=10.8 Hz), 8.59 (1H, d, J=10.8 Hz); HR-EI-MS m/z: 415.2336 (M^+, calcd for $C_{24}H_{33}NO_5$, 415.2358).

A mixture of **36** (1.66 g, 4.00 mmol) and 10% Pd–C (116 mg) in EtOH (17 ml) was stirred vigorously under 1 atm of H_2 at room temperature for 1 h. The reaction mixture was filtered and then concentrated to give crude **37**. To a solution of **37** in THF (40 ml) was added dropwise 1.0 M BH_3 in THF solution (12 ml) at 0°C, and the mixture was stirred for 1 h at 0°C. The reaction was quenched with 10% citric acid (6 ml) and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine and dried over Na_2SO_4, and then concentrated to give the crude *N*-methylaniline derivative. To a solution of the *N*-methylaniline derivative in MeOH (10 ml) was added 1N NaOH (4 ml). The reaction mixture was stirred for 20 min at room temperature and then concentrated. Water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **38** (910 mg, 2.83 mmol, 70.8% in three steps). Compound **38**: ^{1}H NMR δ ($CDCl_3$, 0.071 M, 500 MHz, 300 K) ppm: 0.90 (3H, t, J=7.0 Hz), 0.91 (3H, t, J=6.9 Hz), 1.36 (8H, m), 1.52 (2H, m), 1.58 (2H, m), 2.48 (2H, t, J=8.0 Hz), 2.55 (2H, t, J=8.0 Hz), 2.67 (1H, dd, J=14.2, 5.4 Hz), 2.70 (1H, dd, J=14.2, 7.1 Hz), 2.82 (3H, s), 3.49 (1H, dd, J=11.2, 6.1 Hz), 3.65 (1H, dd, J=11.2, 3.5 Hz), 3.92 (1H, m), 6.49 (1H, s), 6.81 (1H, s); HR-EI-MS m/z: 321.2662 (M^+, calcd for $C_{20}H_{35}NO_2$, 321.2668).

To a solution of D-norleucine (20.0 g, 153 mmol) in 1N

H_2SO_4 (236 ml) was added dropwise $NaNO_2$ (15.8 g, 229 mmol) in distilled water (60 ml) at 0°C and the mixture was stirred for 2 h at 0°C. After stirring for 20 h at room temperature, the reaction mixture was neutralized with $NaHCO_3$ and then concentrated. The residue was acidified with 1N HCl and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine and dried over Na_2SO_4, and concentrated to give the crude α-hydroxycarboxylic acid (17.6 g, 133 mmol, 87.3%). To a solution of the α-hydroxycarboxylic acid (17.6 g, 133 mmol) in MeOH (50 ml) was added NaOH (5.87 g, 146.6 mmol) in distilled water (10 ml). The mixture was stirred for 30 min at room temperature and then concentrated. To a suspension of the residue in DMF (130 ml) was added benzyl bromide (17.4 ml, 147 mmol) at room temperature. After stirring for 20 h, the reaction mixture was poured into water and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give the α-hydroxylester (21.8 g, 98.2 mmol, 73.7%). To a solution of the α-hydroxylester (12.3 g, 55.6 mmol) and 2,6-lutidine (8.40 ml, 72.3 mmol) in CH_2Cl_2 (70 ml) was added dropwise trifluoromethane sulfonyl anhydride (16.0 g, 56.7 mmol) at −78°C, and the mixture was stirred for 2 h at −78°C. The reaction was quenched with water (500 ml) and the mixture was extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over Na_2SO_4 and then concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give the triflate (18.9 g, 53.4 mmol, 96.0%). (*R*)-Benzyl 2-[(trifluoromethylsulfonyl)oxy]hexanoate; $[\alpha]_D$=+13.0° (*c*=1.01, MeOH, 26.0°C); ^{1}H NMR δ ($CDCl_3$, 0.086 M, 500 MHz, 300 K) ppm: 0.89 (3H, t, *J*=7.1 Hz), 1.36 (4H, m), 1.99 (2H, dt, *J*=7.5, 6.2 Hz), 5.14 (2H, t, *J*=6.1 Hz), 5.26 (2H, m), 7.37 (5H, m); HR-FAB-MS *m/z*: 354.0764 (M^+, calcd for $C_{14}H_{17}O_5SF_3$, 354.0748).

A mixture of **38** (808 mg, 2.52 mmol), (*R*)-benzyl 2-[(trifluoromethylsulfonyl)oxy]hexanoate (1.20 g, 3.02 mmol), and 2,6-lutidine (0.88 ml, 7.56 mmol) in dichloroethane (10 ml) was refluxed for 45 min. The reaction was quenched with water (50 ml) and the mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated and purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **39** (973 mg, 1.85 mmol, 73.6%). Compound **39** (973 mg, 1.85 μmol) was treated in a manner similar to that described for the synthesis of **11** to give **40** (1.13 g, 1.77 μmol, 95.7%). A mixture of **40** (1.12 g, 1.75 mmol) and 10% Pd–C (112 mg) in CH_3CN (10 ml) was stirred vigorously under 1 atm of H_2 at room temperature for 15 min. The reaction mixture was filtered and concentrated to give the crude carboxylic acid. To a solution of this acid and DMAP (427 mg, 3.50 mmol) in CH_2Cl_2 (7 ml) was added dropwise DCC (541 mg, 2.63 mmol) in CH_2Cl_2 (3.5 ml) at 0°C, and the mixture was stirred for 11 h at room temperature. The reaction mixture was filtered and then concentrated. Water was added to the residue and the mixture was extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on Wakogel C-200 using hexane and increasing amounts of EtOAc to give **41** (257 mg, 0.484 mmol, 27.7% in two steps), in which two diastereomers existed in a ratio of 1:0.3. Compound **41**: ^{1}H NMR δ ($CDCl_3$, 0.054 M, 500 MHz, 300 K) ppm for the major diastereomer: 0.07 (3H, s), 0.09 (3H, s), 0.91 (9H, s), 0.92 (9H, m), 1.25–1.36 (12H, m), 1.55 (4H, m), 1.89 (2H, m), 2.51 (4H, m), 2.73 (1H, dd, *J*=15.9, 8.6 Hz), 2.75 (3H, s), 3.16 (1H, dd, *J*=15.9, 3.8 Hz), 3.63 (1H, t, *J*=7.9 Hz), 3.65 (1H, dd, *J*=10.6, 5.1 Hz), 3.74 (1H, dd, *J*=10.6, 6.3 Hz), 5.44 (1H, m), 6.80 (1H, s), 6.88 (1H, s), for the minor diastereomer: 0.07 (3H, s), 0.10 (3H, s), 0.91 (9H, s), 0.92 (9H, m), 1.25–1.36 (12H, m), 1.55 (4H, m), 1.89 (2H, m), 2.51 (4H, m), 2.81 (1H, dd, *J*=14.8, 5.2 Hz), 2.84 (3H, s), 3.02 (1H, dd, *J*=14.8, 2.8 Hz), 3.58 (2H, m), 3.76 (1H, m), 4.67 (1H, m), 6.86 (1H, s), 6.89 (1H, s); HR-EI-MS *m/z*: 531.4118 (M^+, calcd for $C_{32}H_{57}NO_3Si$, 531.4107).

Compound **41**: (118 mg, 0.222 μmol) was treated in a manner similar to that described for the synthesis of **4** to give **7** (15.3 mg, 36.7 μmol, 16.7%), **42** (4.5 mg, 10.8 μmol, 5.0%) and **43** (34.2 mg, 82.0 μmol, 36.9%). Compound **7**: $[\alpha]_D$=−93.0° (*c*=0.68, MeOH, 30.7°C); UV λ_{max} (MeOH) nm (ε) 252 (892), 203 (11,700); ^{1}H NMR δ ($CDCl_3$, 0.063 M, 500 MHz, 300 K) ppm: 0.89 (3H, t, *J*=7.2 Hz), 0.90 (6H, t, *J*=6.9 Hz), 1.26–1.36 (12H, m), 1.52 (4H, m), 1.89 (2H, m), 2.24 (1H, br.s), 2.49 (2H, t, *J*=8.1 Hz), 2.53 (2H, t, *J*=8.0 Hz), 2.78 (3H, s), 2.92 (1H, dd, *J*=16.2, 8.3 Hz), 3.01 (1H, dd, *J*=16.2, 3.4 Hz), 3.69 (1H, t, *J*=7.4 Hz), 3.70 (2H, m), 5.33 (1H, m), 6.83 (1H, s), 6.87 (1H, s); ^{13}C NMR δ ($CDCl_3$, 0.063 M, 125 MHz, 300 K) ppm: 13.92, 14.06, 22.58, 28.42, 28.64, 30.94, 31.00, 31.92, 32.05, 32.09, 32.52, 36.26, 37.24, 65.71, 69.77, 79.77, 123.01, 129.00, 132.23, 136.74, 140.35, 148.13, 172.49; HR-EI-MS *m/z*: 417.3223 (M^+, calcd for $C_{26}H_{43}NO_3$, 417.3243). Compound **42**: ^{1}H NMR δ ($CDCl_3$, 0.022 M, 500 MHz, 300 K) ppm: 0.88 (3H, t, *J*=7.2 Hz), 0.91 (6H, t, *J*=6.9 Hz), 1.24–1.37 (12H, m), 1.54 (4H, m), 1.75 (1H, m), 1.82 (1H, m), 2.15 (1H, dd, *J*=7.5, 5.0 Hz), 2.51 (2H, t, *J*=7.9 Hz), 2.54 (2H, t, *J*=7.9 Hz), 2.79 (1H, dd, *J*=15.3, 2.3 Hz), 2.83 (3H, s), 2.90 (1H, dd, *J*=15.3, 6.1 Hz), 3.63 (1H, dd, *J*=7.9, 6.8 Hz), 3.75 (2H, m), 4.74 (1H, m), 6.84 (1H, s), 6.91 (1H, s); ^{13}C NMR δ ($CDCl_3$, 0.022 M, 125 MHz, 300 K) ppm: 13.92, 14.06, 22.58, 22.64, 27.58, 28.62, 30.96, 31.09, 31.91, 32.05, 32.15, 32.44, 36.89, 37.17, 65.18, 66.76, 80.16, 125.26, 132.25, 132.47, 137.31, 140.12, 148.92, 172.76; HR-EI-MS *m/z*: 417.3210 (M^+, calcd for $C_{26}H_{43}NO_3$, 417.3243). Compound **43**: ^{1}H NMR δ ($CDCl_3$, 0.063 M, 300 MHz, 313 K) ppm: 0.91 (6H, t, *J*=7.1 Hz), 0.93 (3H, t, *J*=6.8 Hz), 1.33–1.37 (12H, m), 1.54 (4H, m), 1.86 (2H, m), 1.92 (1H, d, *J*=6.4 Hz), 2.53 (4H, m), 2.73 (3H, s), 2.78 (1H, dd, *J*=9.0, 4.3 Hz), 3.19 (1H, br.t, *J*=9.0 Hz), 3.40 (1H, dd, *J*=9.2, 5.9 Hz), 4.18 (3H, m), 6.94 (1H, s), 6.97 (1H, s); HR-EI-MS *m/z*: 417.3209 (M^+, calcd for $C_{26}H_{43}NO_3$, 417.3243).

4.1.5. Synthesis of 8-bromo-9-octylbenzolactone-Nle8 (8). To a mixture of **31** (3.40 g, 9.44 mmol), 1-octyne (2.80 ml, 18.9 mmol), $PdCl_2(PPh_3)_2$ (497 mg, 0.708 mmol), and triethylamine (2.60 ml, 18.9 mmol) in THF (25 ml) was added CuI (58.3 mg, 0.301 mmol) under an Ar atmosphere at room temperature. The reaction mixture was stirred for 7 h and then filtered. The filtrate was concentrated and purified by column chromatography on Wakogel

C-200 using hexane and increasing amounts of EtOAc to give **44** (3.50 g, 9.00 mmol, 95.3%). Compound **44**: ^{1}H NMR δ ($CDCl_3$, 0.081 M, 500 MHz, 300 K) ppm: 0.91 (3H, t, J=6.9 Hz), 1.28–1.49 (6H, m), 1.60 (2H, m), 1.96 (3H, s), 2.08 (3H, s), 2.41 (2H, d, J=7.0 Hz), 3.14 (1H, dd, J=14.0, 8.8 Hz), 3.32 (1H, dd, J=14.0, 4.3 Hz), 4.09 (1H, dd, J=11.9, 5.6 Hz), 4.28 (1H, dd, J=11.9, 3.9 Hz), 5.34 (1H, m), 7.26 (1H, d, J=8.0 Hz), 7.51 (1H, dd, J=8.0, 1.7 Hz), 7.93 (1H, d, J=1.7 Hz); HR-FAB-MS m/z: 390.1934 (MH^+, calcd for $C_{21}H_{28}NO_6$, 390.1916).

Compound **44** (3.50 g, 9.00 μmol) was treated in a manner similar to that described for the synthesis of **27** to give the crude aniline. This aniline was treated in a manner similar to that described for the synthesis of **35** to give **45** (2.04 g, 5.22 μmol, 58.0% in two steps), in which two conformers existed in a ratio of 1:0.7. Compound **45**: ^{1}H NMR δ ($CDCl_3$, 0.101 M, 500 MHz, 300 K) ppm for the major conformer: 0.87 (3H, t, J=7.0 Hz), 1.26–1.30 (10H, m), 1.63 (2H, m), 2.07 (3H, s), 2.15 (3H, s), 2.58 (2H, t, J=7.8 Hz), 2.75 (1H, dd, J=14.3, 10.4 Hz), 2.97 (1H, dd, J=14.3, 3.2 Hz), 4.16 (1H, dd, J=12.3, 6.2 Hz), 4.20 (1H, dd, J=12.3, 2.9 Hz), 4.83 (1H, m), 6.89 (1H, dd, J=7.8, 1.4 Hz), 6.98 (1H, d, J=7.8 Hz), 8.08 (1H, d, J=1.0 Hz), 8.50 (1H, d, J=1.6 Hz), 8.67 (1H, br.s), for the minor conformer: 0.87 (3H, t, J=6.9 Hz), 1.26–1.30 (10H, m), 1.63 (2H, m), 2.08 (3H, s), 2.10 (3H, s), 2.58 (2H, t, J=7.8 Hz), 2.81 (1H, dd, J=14.3, 7.5 Hz), 2.95 (1H, dd, J=14.3, 6.2 Hz), 4.08 (1H, dd, J=12.2, 5.7 Hz), 4.27 (1H, dd, J=12.2, 3.2 Hz), 4.99 (1H, m), 6.98 (1H, d, J=7.5 Hz), 6.99 (1H, s), 7.09 (1H, d, J=7.5 Hz), 8.38, (1H, br.d, J=10.8 Hz), 8.57 (1H, d, J=11.0 Hz); HR-EI-MS m/z: 391.2357 (M^+, calcd for $C_{22}H_{33}NO_5$, 391.2358).

Compound **45** (2.03 g, 5.20 mmol) was treated in a manner similar to that described for the synthesis of **38** to give **46** (1.28 g, 4.37 μmol, 84.0% in two steps). Compound **46**: ^{1}H NMR δ ($CDCl_3$, 0.071 M, 500 MHz, 300 K) ppm: 0.88 (3H, t, J=7.0 Hz), 1.27–1.35 (10H, m), 1.60 (2H, m), 2.55 (2H, t, J=7.9 Hz), 2.68 (2H, d, J=6.4 Hz), 2.83 (3H, s), 3.48 (1H, dd, J=11.3, 6.2 Hz), 3.65 (1H, dd, J=11.3, 3.4 Hz), 3.91 (1H, m), 6.50 (1H, d, J=1.4 Hz), 6.56 (1H, dd, J=7.5, 1.4 Hz), 6.93 (1H, d, J=7.5 Hz); HR-EI-MS m/z: 293.2343 (M^+, calcd for $C_{18}H_{31}NO_2$, 293.2355).

Compound **46** (1.26 g, 4.30 mmol) was treated in a manner similar to that described for the synthesis of **40** to give **48** (2.05 g, 3.36 μmol, 78.2% in two steps), in which two diastereomers existed in a ratio of 1:1. Compound **48**: ^{1}H NMR δ ($CDCl_3$, 0.085 M, 500 MHz, 300 K) ppm: 0.04 (3H, s), 0.05 (3H, s), 0.06 (6H, s), 0.85 (3H, t, J=7.1 Hz), 0.86 (3H, t, J=7.1 Hz), 0.87 (6H, t, J=6.7 Hz), 0.90 (9H, s), 0.91 (9H, s), 1.25–1.34 (28H, m), 1.50 (4H, m), 1.74 (2H, m), 1.88 (2H, m), 2.47 (2H, t, J=8.1 Hz), 2.48 (2H, t, J=7.7 Hz), 2.73 (3H, s), 2.77 (3H, s), 2.77 (1H, dd, J=14.1, 7.3 Hz), 2.83 (2H, dd, J=6.1 Hz), 2.92 (1H, dd, J=14.1, 4.2 Hz), 3.41 (1H, dd, J=9.9, 6.0 Hz), 3.47 (1H, d, J=10.0, 6.1 Hz), 3.51 (1H, dd, J=9.9, 6.2 Hz), 3.57 (1H, dd, J=10.0, 5.5 Hz), 3.69 (2H, dd, J=9.1, 5.7 Hz), 3.79 (1H, m), 3.86 (1H, m), 3.98 (1H, d, J=3.4 Hz), 4.37 (1H, d, J=3.0 Hz), 5.05 (2H, m), 5.08 (2H, m), 6.88 (1H, dd, J=7.7, 1.5 Hz), 6.89 (1H, dd, J=7.6, 1.5 Hz), 6.91 (2H, d, J=1.7 Hz), 7.10 (1H, d, J=7.7 Hz), 7.12 (1H, d, J=7.7 Hz), 7.18 (2H, m), 7.22 (2H, m), 7.31 (6H, m); HR-EI-MS m/z: 611.4373 (M^+, calcd for $C_{37}H_{61}NO_4Si$, 611.4370).

Compound **48** (2.03 g, 3.32 mmol) was treated in a manner similar to that described for the synthesis of **41** to give **49** (437 mg, 0.869 μmol, 26.2% in two steps), in which two diastereomers existed in a ratio of 1:0.3. Compound **49**: ^{1}H NMR δ ($CDCl_3$, 0.062 M, 500 MHz, 300 K) ppm for the major diastereomer: 0.07 (3H, s), 0.09 (3H, s), 0.88 (3H, d, J=7.1 Hz), 0.89 (3H, d, J=6.7 Hz), 0.90 (9H, s), 1.27–1.38 (14H, m), 1.58 (2H, m), 1.91 (2H, m), 2.54 (2H, t, J=8.2 Hz), 2.77 (3H, s), 2.80 (1H, dd, J=16.0, 8.3 Hz), 3.20 (1H, dd, J=16.0, 3.5 Hz), 3.65 (1H, dd, J=10.6, 5.2 Hz), 3.68 (1H, t, J=7.8 Hz), 3.75 (1H, dd, J=10.6, 6.3 Hz), 5.34 (1H, m), 6.83 (1H, dd, J=7.7, 1.4 Hz), 6.91 (1H, d, J=1.2 Hz), 6.96 (1H, d, J=7.7 Hz), for the minor diastereomer: 0.08 (3H, s), 0.10 (3H, s), 0.87 (3H, d, J=7.3 Hz), 0.90 (3H, d, J=7.6 Hz), 0.92 (9H, s), 1.27–1.38 (14H, m), 1.58 (2H, m), 1.74 (2H, m), 2.56 (2H, t, J=8.9 Hz), 2.85 (1H, dd, J=15.0, 6.1 Hz), 2.85 (3H, s), 3.05 (1H, dd, J=15.0, 2.6 Hz), 3.60 (1H, dd, J=10.2, 5.9 Hz), 3.61 (1H, t, J=7.3 Hz), 3.79 (1H, dd, J=10.2, 5.8 Hz), 4.64 (1H, m), 6.87 (1H, dd, J=7.7, 1.5 Hz), 6.94 (1H, d, J=1.4 Hz), 7.01 (1H, d, J=7.7 Hz); HR-EI-MS m/z: 503.3797 (M^+, calcd for $C_{30}H_{53}NO_3Si$, 503.3794).

Compound **49** (420 mg, 0.835 mmol) was treated in a manner similar to that described for the synthesis of **19** to give **50** (339 mg, 0.581 μmol, 69.6%), in which two diastereomers existed in a ratio of 1:0.3. Compound **50**: ^{1}H NMR δ ($CDCl_3$, 0.047 M, 500 MHz, 300 K) ppm for the major diastereomer: 0.07 (3H, s), 0.10 (3H, s), 0.89 (6H, m), 0.90 (9H, s), 1.20–1.35 (14H, m), 1.57 (2H, m), 1.90 (2H, m), 2.65 (2H, m), 2.76 (3H, s), 2.79 (1H, dd, J=16.2, 8.3 Hz), 3.14 (1H, dd, J=16.2, 3.4 Hz), 3.65 (1H, dd, J=10.5, 5.4 Hz), 3.66 (1H, t, J=7.4 Hz), 3.75 (1H, dd, J=10.5, 6.1 Hz), 5.23 (1H, m), 6.92 (1H, s), 7.22 (1H, s), for the minor diastereomer: 0.09 (3H, s), 0.11 (3H, s), 0.89 (6H, m), 0.92 (9H, s), 1.20–1.35 (14H, m), 1.57 (2H, m), 1.68 (2H, m), 2.65 (2H, m), 2.79 (1H, dd, J=14.8, 3.3 Hz), 2.84 (3H, s), 3.02 (1H, dd, J=14.8, 3.1 Hz), 3.57 (1H, t, J=7.2 Hz), 3.57 (1H, dd, J=15.3, 6.0 Hz), 3.77 (1H, dd, J=15.3, 5.7 Hz), 4.67 (1H, m), 6.95 (1H, s), 7.26 (1H, s); HR-EI-MS m/z: 581.2882 (M^+, calcd for $C_{30}H_{52}NO_3SiBr$, 581.2899).

Compound **50**: (58.7 mg, 0.101 mmol) was treated in a manner similar to that described for the synthesis of **4** to give **8** (5.30 mg, 11.3 μmol, 11.3%), **51** (1.90 mg, 4.05 μmol, 4.1%), **52** (21.6 mg, 46.1 μmol, 46.1%), and **53** (6.60 mg, 14.1 μmol, 14.1%). Compound **8**: $[\alpha]_D$= −120.0° (c=0.62, MeOH, 30.7°C); UV λ_{max} (MeOH) nm (ε) 262 (3250), 203 (21,500); ^{1}H NMR δ ($CDCl_3$, 0.039 M, 500 MHz, 300 K) ppm: 0.88 (6H, t, J=7.0 Hz), 1.22–1.34 (14H, m), 1.57 (2H, m), 1.90 (2H, m), 2.25 (1H, t, J=6.5 Hz), 2.65 (2H, t, J=7.9 Hz), 2.79 (3H, s), 2.99 (2H, d, J=5.4 Hz), 3.71 (1H, t, J=7.4 Hz), 3.72 (2H, m), 5.12 (1H, m), 6.90 (1H, s), 7.24 (1H, s); ^{13}C NMR δ ($CDCl_3$, 0.039 M, 125 MHz, 300 K) ppm: 13.92, 14.12, 22.49, 22.68, 28.28, 28.40, 29.28, 29.36, 29.42, 29.93, 31.90, 35.93, 36.02, 36.60, 65.75, 68.08, 79.62, 118.49, 123.46, 130.81, 135.26, 141.77, 149.70, 172.03; HR-FAB-MS m/z: 467.2019 (M^+, calcd for $C_{24}H_{38}NO_3Br$, 467.2034).

Compound **51**: ^{1}H NMR δ ($CDCl_3$, 0.018 M, 500 MHz, 300 K) ppm: 0.88 (6H, t, J=7.1 Hz), 1.23–1.34 (14H, m), 1.58 (2H, m), 1.74 (1H, m), 1.82 (1H, m), 2.11 (1H, dd, J=7.8, 4.8 Hz), 2.66 (2H, t, J=7.9 Hz), 2.81 (1H, dd, J=15.4, 2.7 Hz), 2.83 (3H, s), 2.89 (1H, dd, J=15.4, 6.0 Hz), 3.62 (1H, t, J=7.4 Hz), 3.76 (2H, m), 4.72 (1H, m), 6.97 (1H, s), 7.27 (1H, s); HR-FAB-MS *m/z*: 467.2074 (M^+, calcd for $C_{24}H_{38}NO_3Br$, 467.2034). Compound **52**: ^{1}H NMR δ ($CDCl_3$, 0.092 M, 300 MHz, 313 K) ppm: 0.88 (3H, t, J=7.0 Hz), 0.93 (3H, t, J=6.6 Hz), 1.29–1.45 (14H, m), 1.57 (2H, m), 1.83 (2H, m), 1.90 (1H, d, J=6.6 Hz), 2.64 (2H, m), 2.72 (3H, s), 2.74 (1H, dd, J=12.7, 3.4 Hz), 3.20 (1H, dd, J=12.7, 9.3 Hz), 3.39 (1H, dd, J=9.2, 6.0 Hz), 4.18 (3H, m), 7.03 (1H, s), 7.37 (1H, s); HR-FAB-MS *m/z*: 467.2045 (M^+, calcd for $C_{24}H_{38}NO_3Br$, 467.2034). Compound **53**: ^{1}H NMR δ ($CDCl_3$, 0.028 M, 300 MHz, 313 K) ppm: 0.89 (3H, t, J=7.0 Hz), 0.91 (3H, t, J=6.8 Hz), 1.29–1.35 (14H, m), 1.58 (2H, m), 1.77 (2H, m), 2.65 (2H, m), 2.80 (3H, s), 2.86 (2H, m), 3.51 (1H, dd, J=9.6, 5.6 Hz), 4.27 (3H, m), 7.00 (1H, s), 7.42 (1H, s); HR-FAB-MS *m/z*: 467.2078 (M^+, calcd for $C_{24}H_{38}NO_3Br$, 467.2034).

4.2. Conformation analyses of benzolactone-V8 (4) and benzolactone-Nle8

The most stable conformations of benzolactone-V8 (**4**) and benzolactone-Nle8 were estimated by Chem 3D program version 5.0 (CambridgeSoft) based on their NMR spectra. As significant NOE between H-2 and H-6α was observed in both compounds, the initial structures were calculated by MM2 calculation using default parameters with the distance between H-2 and H-6α fixed to 2 Å. The calculated structures were optimized by semiempirical quantum mechanics calculation using PM3 theory to give the most stable conformers. Conformation analysis of benzolactam-V8 (**1**) by the same method fully reproduced the same conformer reported by Endo et al.[7]

4.3. Inhibition of specific [^{3}H]PDBu binding to PKC isozyme C1 peptides

The [^{3}H]PDBu binding to the PKC isozyme C1 peptides was evaluated using the procedure of Sharkey and Blumberg[21] with modifications as reported previously[22] under the following conditions: 50 mM Tris-maleate buffer (pH 7.4 at 4°C), 5–20 nM PKC isozyme C1 peptide, 20–40 nM [^{3}H]PDBu (20.0 Ci/mmol), 50 μg/ml 1,2-di(*cis*-9-octadecenoyl)-*syn*-glycero-3-phospho-L-serine, 3 mg/ml bovine γ-globulin, and various concentrations of an inhibitor. Binding affinity was evaluated by the concentration required to cause 50% inhibition of the specific [^{3}H]PDBu binding, IC_{50}, which was calculated by a computer program (SAS) with a probit procedure. The binding constant, K_i, was calculated by the method of Sharkey and Blumberg.[22]

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (A) (2) (No. 13024245) from the Ministry of Education, Science, Culture, and Sports of Japan (for K. I.), and from Japan Society for the Promotion of Science for Young Scientists (for Y. N.).

References

1. Ron, D.; Kazanietz, M. G. *FASEB J.* **1999**, *13*, 1658–1676.
2. Kazanietz, M. G.; Caloca, M. J.; Fujii, T.; Eroles, P.; Garcia-Bermejo, M. L.; Reilly, M.; Wang, H. *Biochem. Pharmacol.* **2000**, *60*, 1417–1424.
3. Redding, P. J.; Dreckschimdt, N. E.; Ahrens, H.; Simsiman, R.; Tseng, C.-P.; Zou, J.; Oberley, T. D.; Verma, A. K. *Cancer Res.* **1999**, *59*, 5710–5718.
4. Redding, P. J.; Dreckschimdt, N. E.; Zou, J.; Bourguingnon, T. D.; Oberley, T. D.; Verma, A. K. *Cancer Res.* **2000**, *60*, 595–602.
5. Kazanietz, M. G.; Areces, L. B.; Bahador, A.; Mischak, H.; Goodnight, J.; Mushinski, J. F.; Blumberg, P. M. *Mol. Pharmacol.* **1993**, *44*, 298–307.
6. Irie, K.; Nakahara, A.; Nakagawa, Y.; Ohigashi, H.; Shindo, M.; Fukuda, H.; Konishi, H.; Kikkawa, U.; Kashiwagi, K.; Saito, N. *Pharmacol. Ther.* **2002** in press.
7. Endo, Y.; Ohno, M.; Hirai, M.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1996**, *118*, 1841–1855.
8. Kozikowski, A. P.; Wang, S.; Ma, D.; Yao, J.; Ahmad, S.; Glazer, R. I.; Bogi, K.; Acs, P.; Modarres, S.; Lewin, N. E.; Blumberg, P. M. *J. Med. Chem.* **1997**, *40*, 1316–1326.
9. Fujiki, H.; Sugiyama, T. *Adv. Cancer Res.* **1987**, *49*, 223–264.
10. Endo, Y.; Takehara, S.; Ohno, M.; Driedger, P. E.; Stabel, S.; Mizutani, M. Y.; Tomioka, N.; Itai, A.; Shudo, K. *J. Med. Chem.* **1998**, *41*, 1476–1496.
11. Zhang, G.; Kazanietz, M. G.; Blumberg, P. M.; Hurley, J. H. *Cell* **1995**, *81*, 917–924.
12. Tanaka, M.; Irie, K.; Nakagawa, Y.; Nakamura, Y.; Ohigashi, H.; Wender, P. A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 719–722.
13. Nakagawa, Y.; Irie, K.; Nakamura, Y.; Ohigashi, H. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 723–728.
14. Kogan, T. P.; Somers, T. C.; Venuti, M. C. *Tetrahedron* **1990**, *46*, 6623–6632.
15. Rousseau, G. *Tetrahedron* **1995**, *51*, 2777–2849.
16. Kajigaeshi, S.; Kakinami, T.; Inoue, K.; Kondo, M.; Nakamura, H.; Fujikawa, M.; Okamoto, T. *Bull. Chem. Soc. Jpn* **1988**, *61*, 597–599.
17. Plevyak, J. E.; Heck, R. F. *J. Org. Chem.* **1978**, *43*, 2454–2456.
18. Thorand, S.; Krause, N. *J. Org. Chem.* **1998**, *63*, 8551–8553.
19. Endo, Y.; Ohno, M.; Takehara, S.; Driedger, P. E.; Stabel, S.; Shudo, K. *Chem. Pharm. Bull.* **1997**, *45*, 424–426.
20. Endo, Y.; Yokoyama, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 63–66.
21. Kajigaeshi, S.; Kakinami, T.; Yamasaki, H.; Fujisaki, S.; Okamoto, T. *Bull. Chem. Soc. Jpn* **1988**, *61*, 600–602.
22. Sharkey, N. A.; Blumberg, P. M. *Cancer Res.* **1985**, *45*, 19–24.
23. Shindo, M.; Irie, K.; Nakahara, A.; Ohigashi, H.; Konishi, H.; Kikkawa, U.; Fukuda, H.; Wender, P. A. *Bioorg. Med. Chem.* **2001**, *9*, 2073–2081.
24. Chida, K.; Hara, T.; Hirai, T.; Konishi, C.; Nakamura, K.; Nakao, K.; Katsumi, M.; Kuroki, T. *SEIKAGAKU* **2000**, *72*, 674.