Clarification of the Binding Mode of Teleocidin and Benzolactams to the Cys2 Domain of Protein Kinase Cδ by Synthesis of Hydrophobically Modified, Teleocidin-Mimicking Benzolactams and Computational Docking Simulation

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Phorbol esters (12-*O*-tetradecanoylphorbol 13-acetate; TPA) and teleocidins are known to be potent tumor promoters and to activate protein kinase C (PKC) by binding competitively to the enzyme. The relationship between the chemical structures and the activities of these compounds has attracted much attention because of the marked structural dissimilarities. The benzolactam **5**, with an eight-membered lactam ring and benzene ring instead of the nine-membered lactam ring and indole ring of teleocidins, reproduces the active ring conformation and biological activities of teleocidins. Herein we describe the synthesis of benzolactams with hydrophobic substituents at various positions. Structure−activity data indicate that the existence of a hydrophobic region between C-2 and C-9 and the steric factor at C-8 play critical roles in the appearance of biological activities. We also computationally simulated the docking of teleocidin and the modified benzolactam molecules to the Cys2 domain structure observed in the crystalline complex of PKCδ with phorbol 13-acetate. Teleocidin and benzolactams fitted well into the same cavity as phorbol 13-acetate. Of the three functional groups hydrogen-bonding to the protein, two hydrogen-bonded with protein atoms in common with phorbol 13-acetate, but the third one hydrogen-bonded with a different protein atom from that in the case of phorbol 13-acetate. The model explains well the remarkable difference in activity between **5** and its analogue having a bulky substituent at C-8.

Introduction

The teleocidins (e.g., teleocidin B-4, **1**) (Figure 1) are a family of TPA-type tumor promoters[1] which include diterpene esters represented by 12-*O*-tetradecanoylphorbol 13-acetate (TPA, **2**) and aplysiatoxins. (−)-Indolactam-V ((−)-IL-V, **3**), which lacks the hydrophobic group at the 6,7-position of the indole ring of teleocidins, was synthesized by us[2] and was later isolated from *Streptoverticillium blastmyceticum*.[3] (−)-IL-V is considered to be the minimum-sized structure exhibiting tumor-promoter activity,[4] and the synthesis of (−)-IL-V has become of interest in recent years.[5] All teleocidins including (−)-IL-V exist in an equilibrium of two conformational states, twist and sofa forms,[6] as shown in Figure 2. The twist form is characterized by the *cis*-amide bond, whereas the sofa form is characterized by the *trans*-amide bond. The twist−sofa equilibrium is considered to be due to cis−trans isomerization of the amide bond and the steric effects of substituents on the nine-membered lactam ring. However, the low-energy barrier between the two conformers found in kinetic studies[6b] (the observed free energy of activation of (−)-IL-V acetate was $\Delta G^{\ddagger} = 19.2$ kcal/mol at −10 °C) and the short half-life of interconversion (estimated to be 1.2 s at 37 °C for (−)-IL-V acetate) made it difficult to interpret the mode of interaction of these promoters with common macromolecular targets (i.e., protein kinase C (PKC)). It is particularly important to determine the active ring conformation of teleocidins in order to explain the relationships between the structures and activities of several classes of TPA-type tumor promoters with various skeletal structures. Several molecular modeling studies based upon ligand structures and the structure−activity relationships of TPA-type tumor promoters with different skeletons have failed to provide conclusive evidence to identify the common structural requirements.[7]

After previous synthetic efforts[8] and computational prediction[9] of the conformational status of the indolactams, we had designed and synthesized benzolactam-Vs, in which the indole ring of indolactams is replaced with a benzene ring, in an attempt to reproduce separately the two conformations of teleocidins. Among these benzolactams, eight-membered lactams (benzolactam-V8, (−)-BL-V8 (**4**)) can only exist in the twist form and nine-membered lactams (benzolactam-V9) exist exclusively in the sofa form in solution.[10] Eight-membered derivatives having a sufficiently hydrophobic moiety, (e.g., (−)-benzolactam-V8-310, (−)-BL-V8-310, **5**) exhibited biological activities similar to those of teleocidin B-4, i.e., differentiation-inducing activity

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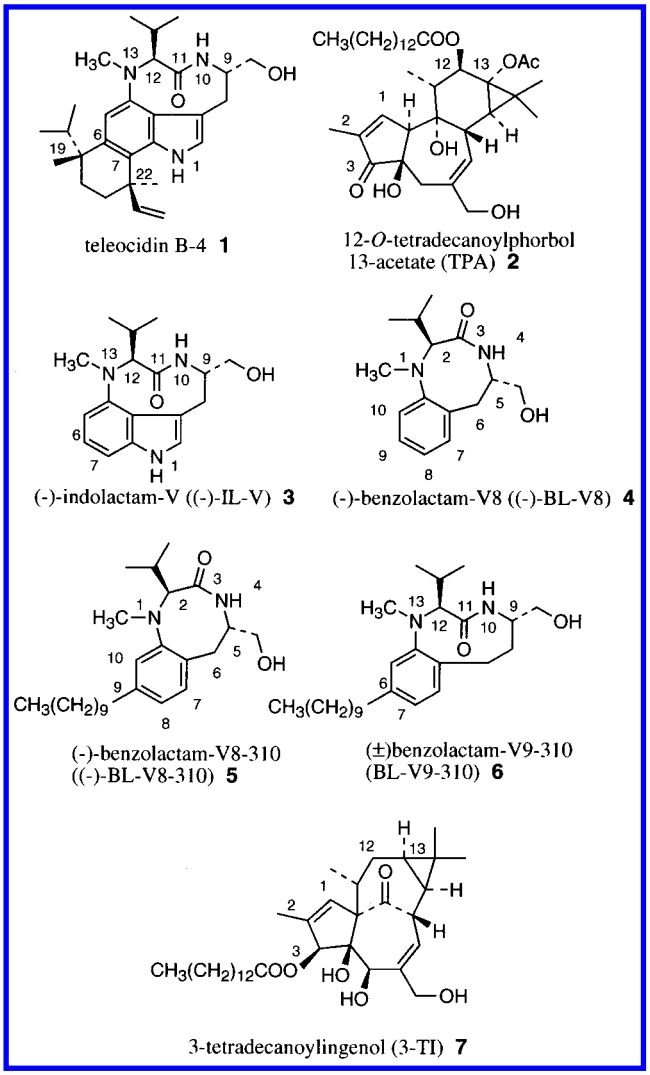


Figure 1. Structures of TPA-type tumor promoters and related compounds.

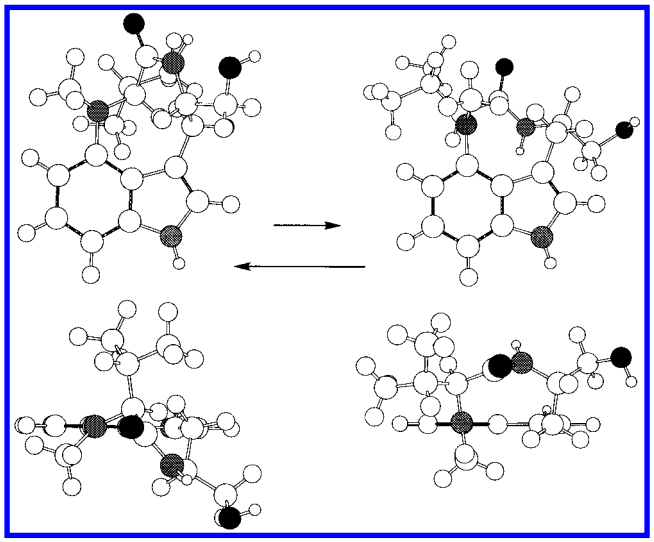


Figure 2. Equilibrium between the twist (left) and the sofa (right) forms in (−)-IL-V. Top: Views from the face of the indole ring. Bottom: Views from the side of the indole ring.

toward human promyelocytic leukemia (HL-60) cells and binding activity to PKC.[10] (−)-BL-V8-310 showed tumor-promoting activity in two-stage carcinogenesis experiments on mouse skin.[11] On the other hand, nine-membered derivatives, such as benzolactam-V9-310 ((±)-BL-V9-310, **6**), were inactive in these biological assays. These results clearly indicated that the twist form is close to the biologically active conformation of teleocidins. Irie et al. recently established the importance of the twist form of teleocidin by a different approach based on the synthesis of conformationally restricted analogues of (−)-IL-V.[12]

Recently, a crystallographic study revealed the direct interaction of phorbol 13-acetate with the PKCδ Cys2 domain.[13] The binding site thus identified can only bind with the twist form, but not the sofa form, of teleocidins in computer modeling analyses.[14] This is in good agreement with our conclusion based on synthesis and biological activity assay of benzolactams. Derivatization of (−)-BL-V8-310, a PKC modulator with a new skeletal systems, would provide further opportunities for examination of the interaction of ligands with PKC.

We report herein the synthesis of benzolactams with hydrophobic substituents at various positions for investigation of specific hydrophobic contacts in the PKC−ligand interaction.[15] We also report a computational simulation of the docking of these molecules and teleocidin B-4 to the Cys2 domain of PKCδ.[14] This modeling study explains well the remarkable differences in biological activity for benzolactams with hydrophobic substituents at specified positions.

Chemistry

The relative position of the hydrogen-bonding functional groups of teleocidins required for the appearance of biological activity has been established by the synthesis and biological evaluation of two benzolactams ((−)-BL-V8-310 (**5**) and (±)-BL-V9-310 (**6**)) reproducing respectively the two conformational states of teleocidins.[10] Although hydrogen-bonding and other polar interactions are important factors for *recognition* of a biologically active molecule at a receptor, hydrophobic interaction is also important for *stability* of binding to many receptors. In fact, the hydrophobic moiety on the indole ring of teleocidins plays a critical role in increasing the biological potency. The activity of teleocidin B-4 (**1**) in terms of the binding affinity to PKC and growth inhibition of HL-60 cells is 100−500 times higher than that of (−)-IL-V (**3**), which lacks the hydrophobic moiety.[16] Various structure−activity studies have reported that teleocidins B-1−B-4, which are stereochemical isomers of the 19- and 22-positions of the terpenoid side chain, and their open-chain analogues, teleocidins A-1 and A-2, exhibit similar biological activities.[17] The terpenoid substituents of teleocidins can be replaced by a simple linear alkyl group without loss of activity.[18] The active PKC−ligand complex is thought to contain from 3 to as many as 12 molecules of phospholipid bound to PKC.[19] At present, studies on the role of the hydrophobic moieties of TPA-type tumor promoters are focused on distinguishing between the direct interaction of their hydrophobic regions with PKC versus external hydrophobic interaction with phospholipid. The benzolactams are useful compounds for analyzing these points. In the case of benzolactams, the activity of (−)-BL-V8 (**4**), which lacks the hydrophobic moiety, is 1000 times weaker than that of (−)-BL-V8-310 (**5**). Recently, we have reported the synthesis and biological activity

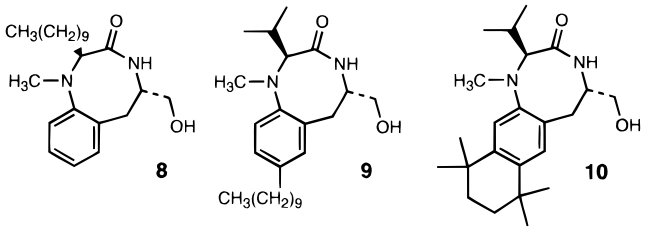


Figure 3. Structures of designed benzolactams ((−)-BL-8-C10 (**8**), (−)-BL-VA-210 (**9**), and (−)-BL-V8-23TM (**10**)).

of 9-alkylated (−)-BL-V8s.[20] Among the (−)-BL-V8s, substitution of a C10−C14 linear alkyl chain at the 9-position of the aromatic nucleus is optimum for maximal biological activity, though substitution of a C8−C16 cyclic alkyl group (cyclooctyl, cylcododecyl, cyclohexadecyl) or even a bulky adamantanemethyl group at the 9-position retains almost the same activity. This suggests that the hydrophobic alkyl group on (−)-BL-V8s is folded when the molecule binds to a receptor. Diterpene ester tumor promoters, such as TPA (**2**) and 3-tetradecanoylingenol (3-TI, **7**), which are essentially identical pharmacologically, have different skeletons with hydrophobic esters at different positions on the molecules. Thus, it seems likely that a large, appropriately oriented hydrophobic region on the molecule plays a critical role in the appearance of biological activities.

For the purpose of characterizing the hydrophobic region, (−)-BL-8-C10 (**8**) with a decyl group at the 2-position and (−)-BL-V8-210 (**9**) with a decyl group at the 8-position of the (−)-BL-V8 skeleton were designed. We supposed that location of the hydrophobic group in the former corresponds to that in 3TI, and the latter to that in TPA, in analogy to (−)-BL-V8-310 (**5**). In addition to these (−)-BL-V8s with a linear alkyl substituent, (−)-BL-V8-23TM (**10**) with an 8,9-cyclized alkyl group, which corresponds to that in teleocidin Bs, was designed (Figure 3).

Syntheses of the benzolactams (BLs) were carried out in a manner similar to that used for (−)-BL-V8-310 (**5**)[10c] (Scheme 1). In the synthesis of (−)-BL-8-C10 (**8**), the *n*-decylglycine unit was introduced as the triflate of (*R*)-benzyl α-hydroxy-*n*-dodecanoate (**11**), which was prepared as follows. Racemic *n*-decylglycine (**12**) was optically resolved by stereoselective hydrolysis of the *N*-chloroacetate **13** using aminoacylase (yield: 35%) according to Mori and Otsuka.[21] After hydrolysis of (*R*)-**13**, conversion of the amino group of (*R*)-**12** to a hydroxy group via the diazotization followed by esterification with benzyl alcohol−$SOCl_2$ gave (*R*)-benzyl α-hydroxy-*n*-dodecanoate (**14**; 53%). Treatment of **14** with triflic anhydride gave the triflate **11** (74%). Substitution of *N*-Boc-2-(methylamino)phenylalaninol (**15**)[10c] with the triflate **11** gave **16** (80%). Deprotection of the benzyl ester, formation of an activated ester, deprotection of the *N*-Boc group, and cyclization afforded **8** (27%) and its epimer **17** (24%).

(−)-BL-V8-210 (**9**) was prepared starting from 3-carbomethoxy-4-nitrobenzoic acid (**18**). Reduction of **18** with borane−dimethyl sulfide complex followed by oxidation with PCC gave 3-carbomethoxy-4-nitrobenzaldehyde (**19**) (86%). Reaction of the aldehyde with nonylphosphoniumylide gave **20** (84%), which was converted to the tosylate **21** by hydride reduction and tosylation (63%). Condensation of **21** with diethyl acetamidomalonate gave **22** (77%). Deprotection and decarboxylation gave the amino acid, which was protected as the ethyl ester for the carboxylic acid group and with Boc for the amino group, and the ester group was reduced with $LiBH_4$ to afford **23** (49%). The nitro alcohol **23** was converted into the methylamino alcohol **24** (99%) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **24** with the triflate of (*R*)-benzyl α-hydroxyisovalerate[5d] gave diastereomeric esters **25** (88%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters (96%). After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **9** (48%) and the epimer **26** (43%), which were isolated at this stage.

Synthesis of (−)-BL-V8-23TM (**10**) was performed from 1,2,3,4-tetrahydro-1,1,4,4,7-pentamethylnaphthalene (**27**), prepared by Friedel−Crafts alkylation of toluene with 2,5-dichloro-2,5-dimethylhexane. Nitration of **27** followed by benzylic bromination gave the nitro bromide **28** (51%). Condensation of **28** with diethyl acetamidomalonate gave **29** (90%). Deprotection and decarboxylation gave the amino acid, which was protected as the ethyl ester for the carboxylic acid group and with Boc for the amino group, and the ester group was reduced with $LiBH_4$ to afford **30** (94%). The nitro alcohol **30** was converted into the methylamino alcohol **31**, (83%) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **31** with the triflate of (*R*)-benzyl α-hydroxyisovalerate gave diastereomeric esters **32** (88%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters (96%). After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **10** (48%) and the epimer **33** (43%).

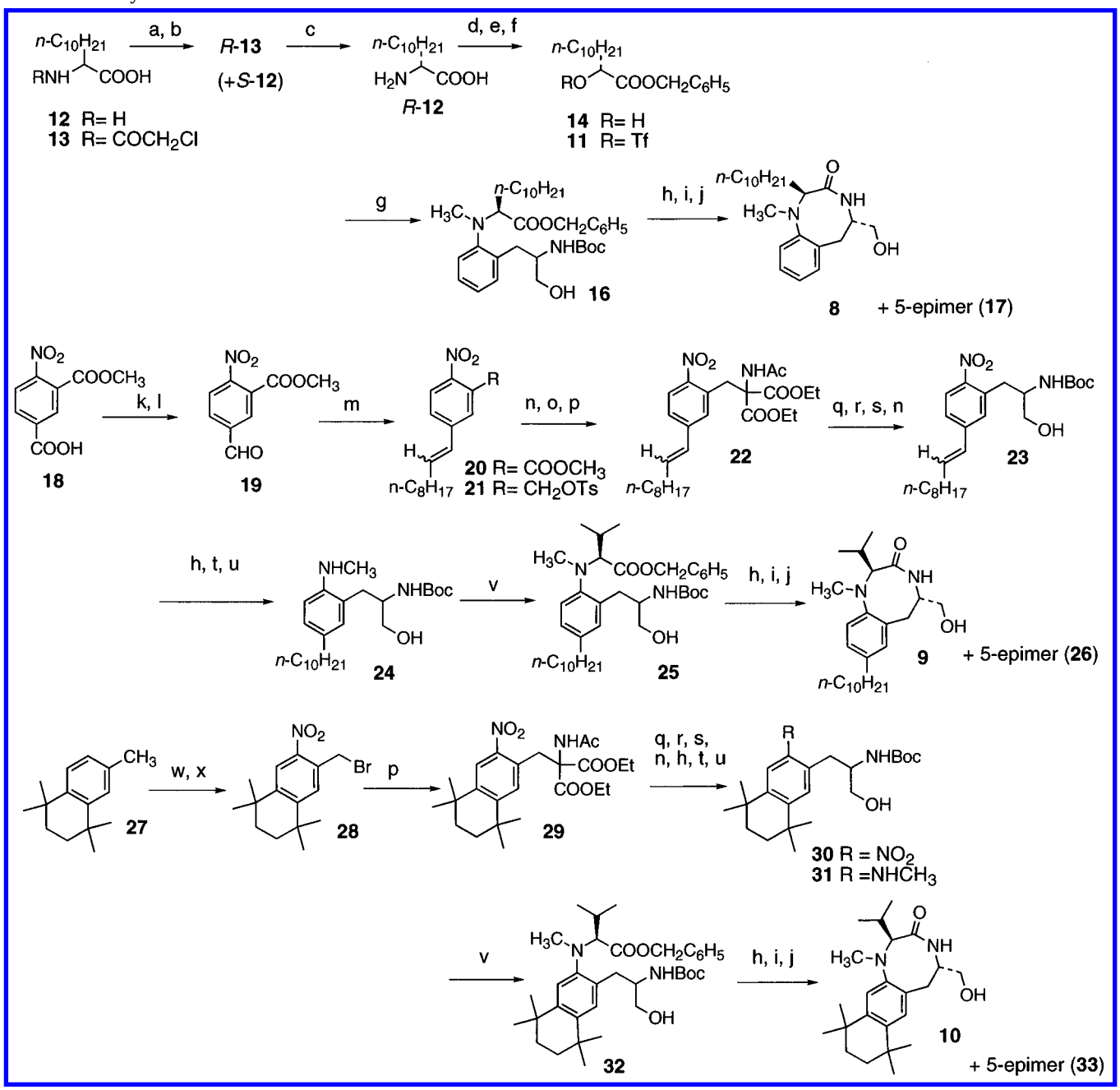
The benzolactams **8**−**10** were confirmed to take the twist conformational form, which has been established in the case of **4**, on the basis of the similarity of the ^1H NMR spectral data and nuclear Overhauser effect (NOE) experiments. Optical purity of the target compounds was determined by ^1H NMR measurements with (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid ((−)-MTPA) to be >99.8% ee.

Biological Evaluation

The biological activities of the benzolactams **8**−**10** were examined by means of two bioassays related to in vivo tumor promotion. One of the most sensitive and specific biological activities of the TPA-type tumor promoters is induction of growth inhibition, cell adhesion, and differentiation to monocytes of HL-60 cells.[22] The effective concentration (EC_{50}) of teleocidin B-4 for growth inhibition is 5×10^{-9} M, whereas that of (−)-IL-V is 5×10^{-7} M. Results are presented in Figure 4 along with comparative data for (−)-BL-V8-310 (**5**) and (−)-BL-V8 itself (**4**), which lacks a hydrophobic group at C-9.

Despite the switching of its decyl group from C-9 to C-2, (−)-BL-8-C10 (**8**) showed distinct, though decreased, activity. Its activity is 50 times stronger than that of (−)-BL-V8 (**4**). This suggests that the hydro-

Scheme 1. Synthesis of Benzolactams **7**–**9**[a]



[a] Key: (a) $ClCH_2COCl$, $NaOH/H_2O$; (b) *Aspergillus* aminoacylase, $CoCl_2$, pH 7.26; (c) HCl/H_2O; (d) $NaNO_2$, H_2SO_4/H_2O; (e) $C_6H_5CH_2OH$, $SOCl_2$; (f) Tf_2O, 2,6-lutidine/CH_2Cl_2; (g) (*S*)-2-(methylamino)phenylalaninol (**15**), 2,6-lutidine/CH_2ClCH_2Cl; (h) H_2, Pd–C/EtOH; (i) *N*-hydroxysuccinimide, DCC/CH_3CN; (j) CF_3COOH/CH_2Cl_2 then aq $NaHCO_3/CH_3COOEt$; (k) BH_3–$S(CH_3)_2$/THF; (l) PCC/CH_2Cl_2; (m) $C_9H_{19}P^+Br^-$, *n*-BuLi/THF; (n) $LiBH_4$/THF; (o) NaH, TsCl/toluene; (p) $CH_3CONHCH(COOC_2H_5)_2$, NaH/DMF; (q) HCl, AcOH; (r) $SOCl_2$/EtOH; (s) Boc_2O/CH_2Cl_2; (t) HCOOH, Ac_2O; (u) BH_3/THF; (v) triflate of benzyl (*R*)-α-hydroxyisovalerate, 2,6-lutidine/CH_2ClCH_2Cl; (w) HNO_3, H_2SO_4; (x) NBS/CCl_4, *hν*.

phobic region at C-2 and C-9 has a common function in determining biological activity. Unexpectedly, switching of the decyl group from C-9 to the neighboring C-8 also caused a distinct decrease of the activity; i.e., the activity of (−)-BL-V8-210 (**9**) is 30 times weaker than that of **5**. Further introduction of a bulky substituent at C-8 caused a significant decrease in activity; i.e., the activity of (−)-BL-V8-23TM (**10**) is 1000 times weaker than that of **5** and almost the same as that of **4**.

Binding affinity of the BLs to PKCδ is directly related to ligand–PKC interaction and is useful for interpreting modeling studies. Assay of the inhibition of [^{3}H]PDBu binding (K_d = 2.9 nM) to recombinant PKCδ and the determination of inhibition constants from binding curve IC_{50} values were done as previously described.[23] The difference in PKCδ binding potency between **5** (K_i = 5.9 ± 1.7 nM) and **10** (K_i = 5100 ± 730 nM) is particularly striking. These BLs (**4**, **5**, **8**–**10**) and teleocidins exist in a similar conformational state, which means that the hydrophilic functional groups are arranged in appropriate positions for binding to the receptor. Therefore, the significant difference in the activity caused by the substituents indicates that a steric factor that disfavors binding to the receptor exists around the 8-position of BLs. This is apparently the first observation of an effect on PKC binding attribut-

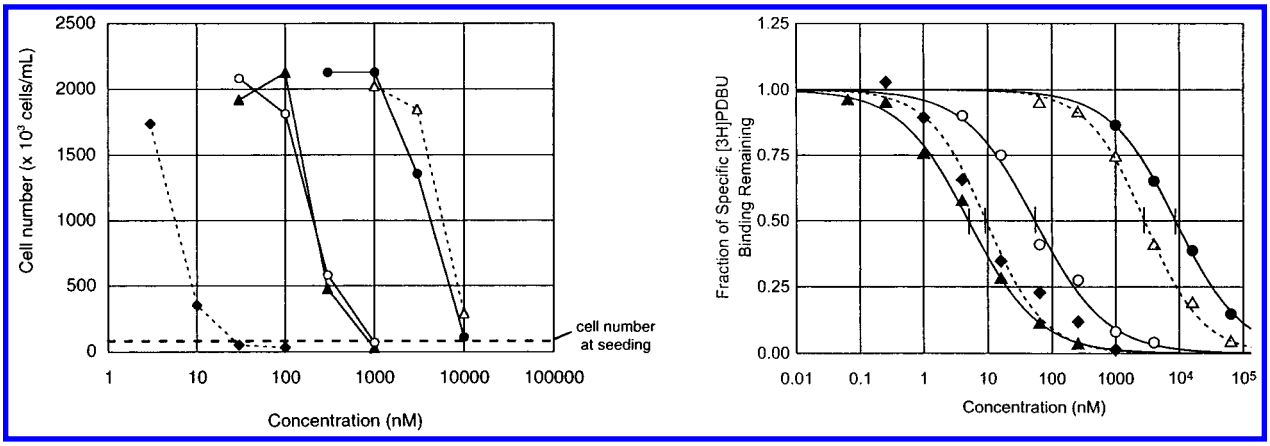


Figure 4. Biological activities of benzolactams **8**−**10**. Left: Growth inhibition of HL-60 cells. Right: Inhibition of specific [^{3}H]-PDBu binding with PKCδ. Legend: ◆, BL-V8-310 (**5**); ▲, BL-8-C10 (**8**); ○, BL-V8-210 (**9**); ●, BL-V8-23TM (**10**); △, BL-V8 (**4**).

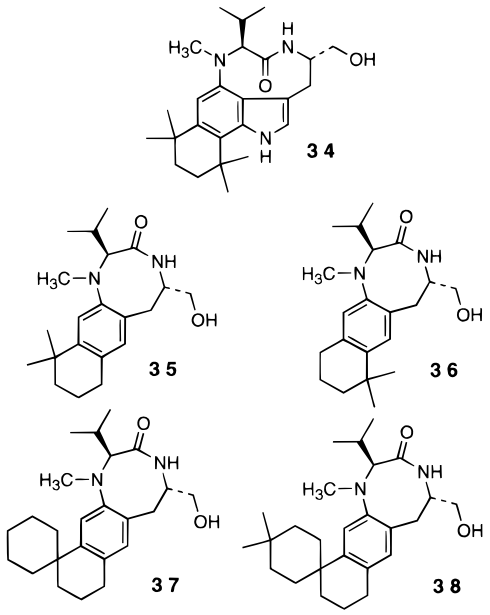


Figure 5. Structures of 6,7-cyclic (−)-IL-V **34** and 8,9-cyclic (−)-BL-Vs **35**−**38**.

able to essentially steric changes in the hydrophobic alkyl portion of a PKC ligand. Also, the difference between the K_i value of 3.0 ± 0.45 nM of **8** and its EC_{50} of 200 nM for HL-60 differentiation suggests that **8** will be useful as a probe for examination of the mechanism of the biological effects of binding of TPA-type tumor promoters to PKC.

Further Evaluation of Specific Hydrophobic Contacts in the PKC−Ligand Interaction. The activity of the BLs is markedly affected by the nature of the substituent at the 8-position. However, the tetramethyltetramethylene analogue of (−)-IL-V (Figure 5, **34**) has been shown to possess activity comparable to that of TPA in the PKC binding assay.[24] Superposition of the active twist form of teleocidin B-4 (**1**) and (−)-BL-V8-23TM (**10**) as determined by MM-2 calculations is illustrated in Figure 6. The significant difference in the activity seems to be caused by an extremely specific steric effect of the substituent on the 8-position of the BL molecule, which would hinder fitting into the cavity through van der Waals contact with PKCδ. For the purpose of investigation of the steric hindrance and the appropriate direction of the hydrophobic moiety for strong binding, four (−)-BL-V8s with 8,9-cyclized alkyl groups (Figure 5, **35**−**38**) were designed and synthesized. The syntheses were carried out in a manner similar to that used for (−)-BL-V8-23TM (**10**) as shown in Schemes 2 and 3. (−)-BL-V8-DM1 (**35**), which has two methyl groups on the C-8 side, was prepared starting from 4,4-dimethyl-γ-butyrolactone. Friedel−Crafts reaction of benzene with the lactone followed by bromination on the aromatic nucleus gave 7-bromo-4,4-dimethyl-1,2,3,4-tetrahydronaphthalen-1-one (**39**) (47%). Reduction of the ketone to a methylene group with triethylsilane gave 6-bromo-1,1-dimethyl-1,2,3,4-tetrahydronaphthalene (**40**) (89%). Conversion of the bromide **40** to an aldehyde via lithiation/DMF followed by hydride reduction gave the alcohol **41** (95%). This was converted to the benzyl bromide **42** (82%), which was nitrated to give the 7-nitro bromide **43** (32%) and its 5-nitro isomer. Condensation of **43** with diethyl acetamidomalonate gave **44** (97%). Deprotection and decarboxylation gave the amino acid, which was protected as the ethyl ester for the carboxylic acid group and with Boc for the amino group, and the ester group was reduced with $LiBH_4$ to afford **45** (71%). The nitro alcohol **45** was converted into the methylamino alcohol **46** (75%) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **46** with the triflate of (*R*)-benzyl α-hydroxyisovalerate gave diastereomeric esters **47** (82%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters. After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **35** (33%) and the epimer **48** (30%).

(−)-BL-V8-DM3 (**36**), which has two methyl groups on the C-9 side, was prepared starting from 4-bromobenzaldehyde (Scheme 2). Aldol condensation of the aldehyde with 3-methylbutan-2-one afforded the α,β-unsaturated ketone **49** (45%). Hydride reduction followed by intramolecular Friedel−Crafts reaction employing polyphosphoric acid gave 7-bromo-1,1-dimethyl-1,2,3,4-tetrahydronaphthalene (**50**) (64%). Conversion of the bromide **50** to an aldehyde via lithiation/DMF followed by hydride reduction gave the alcohol **51** (93%), which was converted to the benzyl bromide **52** (63%). Nitration, followed by condensation with diethyl acetamidomalonate, gave **53** (22%). Deprotection and decarboxylation gave the amino acid, which was protected as the ethyl ester for the carboxylic acid group and with

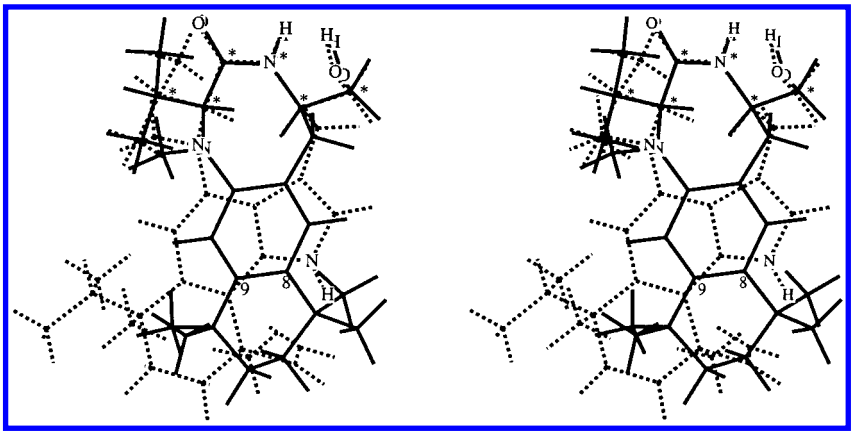
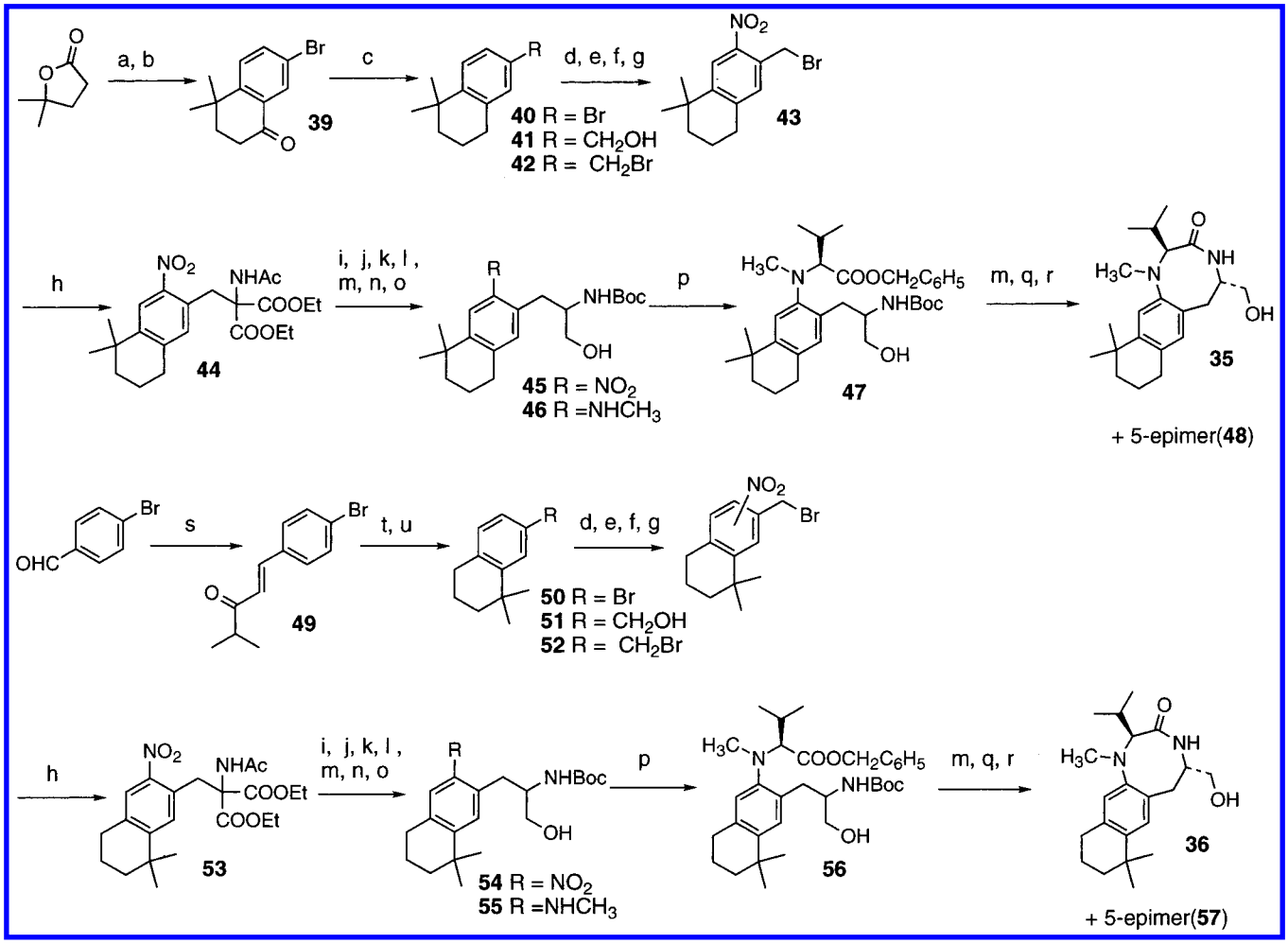


Figure 6. Molecular superposition of teleocidin B-4 (**1**) (dotted line) and (−)-BL-V8-23TM (**10**) (solid line). The two molecules were superposed on the basis of root-mean-square fit of the pairs of atoms marked with asterisks.

Scheme 2. Synthesis of Benzolactams **35** and **36**[a]

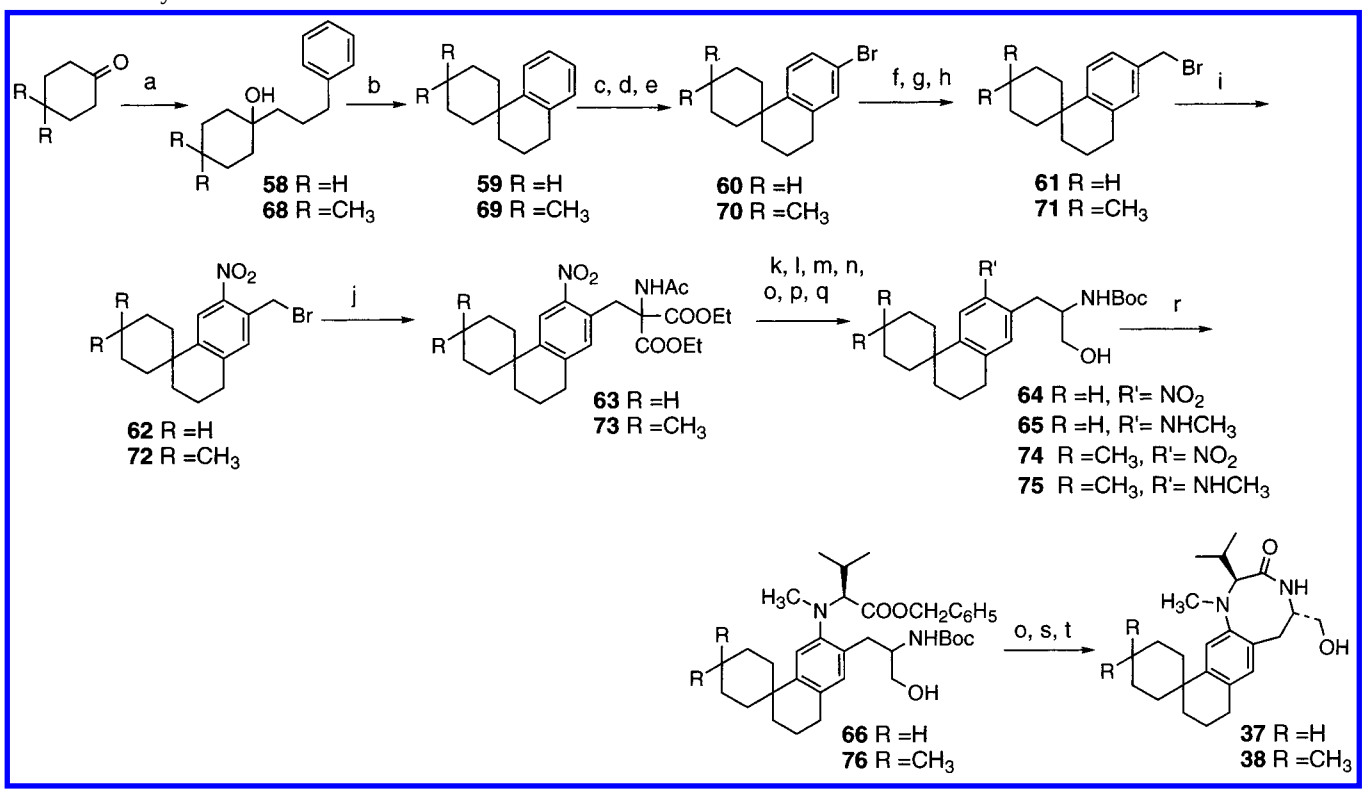


[a] Key: (a) benzene, $AlCl_3$; (b) Br_2, $AlCl_3/CH_2Cl_2$; (c) $(C_2H_5)_3SiH$, CF_3COOH; (d) *n*-BuLi/Et_2O then DMF; (e) $NaBH_4$/MeOH; (f) $CHBr_3$, Ph_3P/CH_2Cl_2; (g) fuming HNO_3/Ac_2O; (h) $CH_3CONHCH(COOC_2H_5)_2$, NaH/DMF; (i) HCl, AcOH; (j) $SOCl_2$/EtOH; (k) Boc_2O/CH_2Cl_2; (l) $LiBH_4$/THF; (m) H_2, Pd−Cl/EtOH; (n) HCOOH, Ac_2O; (o) BH_3/THF; (p) triflate of benzyl (*R*)-α-hydroxyisovalerate, 2,6-lutidine/CH_2ClCH_2Cl; (q) *N*-hydroxysuccinimide, DCC/CH_3CN; (r) CF_3COOH/CH_2Cl_2 then aq $NaHCO_3/CH_3COOEt$; (s) 3-methylbutan-2-one, aq NaOH/EtOH; (t) $LiAlH_4/Et_2O$; (u) polyphosphoric acid.

Boc for the amino group, and the ester group was reduced with $LiBH_4$ to afford **54** (89%). The nitro alcohol **54** was converted into the methylamino alcohol **55** (75%) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **55** with the triflate of (*R*)-benzyl α-hydroxyisovalerate gave diastereomeric esters **56** (84%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters. After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **36** (38%) and the epimer **57** (38%).

(−)-BL-V8-SP1 (**37**), which has a spiro[cyclohexane] group on the C-9 side as a hydrophobic moiety, was formed as follows (Scheme 3). Reaction of cyclohexanone with (3-phenylpropyl)magnesium bromide gave

Scheme 3. Synthesis of Benzolactams **37** and **38**[a]



[a] Key: (a) (3-phenylpropyl)magnesium bromide/EtO; (b) 90% H_2SO_4; (c) CrO_3/AcOH; (d) Br_2, $AlCl_3/CH_2Cl_2$; (e) $(C_2H_5)_3SiH$, CF_3COOH; (f) *n*-BuLi/Et_2O then DMF; (g) $NaBH_4$/MeOH; (h) $CHBr_3$, Ph_3P/CH_2Cl_2; (i) fuming HNO_3/Ac_2O; (j) $CH_3CONHCH(COOC_2H_5)_2$, NaH/DMF; (k) HCl, AcOH; (l) $SOCl_2$/EtOH; (m) Boc_2O/CH_2Cl_2; (n) $LiBH_4$/THF; (o) H_2, Pd−C/EtOH; (p) HCOOH, Ac_2O; (q) BH_3/THF; (r) triflate of benzyl (*R*)-α-hydroxyisovalerate, 2,6-lutidine/CH_2ClCH_2Cl; (s) *N*-hydroxysuccinimide, DCC/CH_3CN; (t) CF_3COOH/CH_2Cl_2 then aq $NaHCO_3$/CH_3COOEt.

1-(3-phenylpropyl)cyclohexanol (**58**) (70%). Intramolecular Friedel−Crafts reaction of **58** afforded 3′,4′-dihydrospiro[cyclohexane-1,1′(2′*H*)-naphthalene] (**59**) (88%). After benzylic oxidation with CrO_3, introduction of a bromo group at the 6′-position followed by reduction with triethylsilane gave the bromide **60** (35%). This was converted to the benzyl bromide **61** in a manner similar to that used for **43** (69%). Nitration of **61** afforded the nitro bromide **62** (40%) and its isomer. Condensation of **62** with diethyl acetamidomalonate gave **63** (94%). Deprotection and decarboxylation gave the amino acid, which was protected as the ethyl ester for the carboxylic acid group and with Boc for the amino group, and the ester group was reduced with $LiBH_4$ to afford **64** (86%). The nitro alcohol **64** was converted into the methylamino alcohol **65** (75%) by catalytic hydrogenation and formylation, followed by reduction with BH_3. Reaction of **65** with the triflate of (*R*)-benzyl α-hydroxyisovalerate gave diastereomeric esters **66** (64%). After hydrogenolysis of the benzyl ester, condensation with *N*-hydroxysuccinimide using DCC gave the activated esters. After removal of the Boc group using CF_3COOH, cyclization was carried out under dilute conditions to give **37** (38%) and the epimer **67** (28%).

BL-V8-SP3 (**38**), which has a dimethylspiro[cyclohexane] group on the C-9 side as a hydrophobic moiety, was prepared in the same manner as that used for **37**, starting from 4,4-dimethylcyclohexanone. The intermediates and yields are described in the Experimental Section. Optical purity of the target compounds (**35**−**38**) was determined by ^{1}H NMR measurements with (−)-MTPA to be >99.8% ee.

The biological activities of the benzolactams **35**−**38** were examined by assay of growth inhibition of HL-60 cells and inhibition of specific binding of [^{3}H]PDBu to recombinant PKCδ. The results are presented in Figure 7 along with comparative data for (−)-BL-V8-310 (**5**) and (−)-BL-V8-23TM (**10**). As is apparent, (−)-BL-V8-DM3 (**36**) with two methyl groups on the C-8 side showed weak activity (EC_{50} of 7 μM for HL-60 growth inhibition and $K_i = 1700 \pm 220$ nM for PKC affinity). On the other hand, (−)-BL-V8-DM1 (**35**), with two methyl groups on the C-9 side, showed about 30 times stronger activity than **36**. The difference in PKCδ binding potency between **36** and **35** ($K_i = 65 \pm 0.6$ nM) clearly indicates that a steric factor that disfavors binding to the receptor exists around the 8-position of BLs. The relatively weak activities of **35** compared to **5** can be explained by a lack of some favorably directed hydrophobic group. Introduction of the hydrophobic spiro[cyclohexane] group on the C-9 side enhanced the biological activity. (−)-BL-V8-SP1 (**37**) and (−)-BL-V8-SP3 (**38**) potently inhibited the growth of HL-60 cells (EC_{50} = 70 and 20 nM, respectively) and bound strongly to PKCδ ($K_i = 19 \pm 1.1$ and 14 ± 0.94 nM, respectively). The difference in PKCδ binding potency between **38** and **9**, with a linear decyl group at C-8 ($K_i = 32 \pm 8.7$ nM) indicates that the hydrophobic region of **38** is in a favorable location for biological activity.

Docking Study. In 1995, the crystal structure of the PKCδ Cys2 domain was elucidated in the complex with phorbol 13-acetate.[13] Docking simulations to this structure would be useful to clarify the relationship between the three-dimensional structures and activities of other

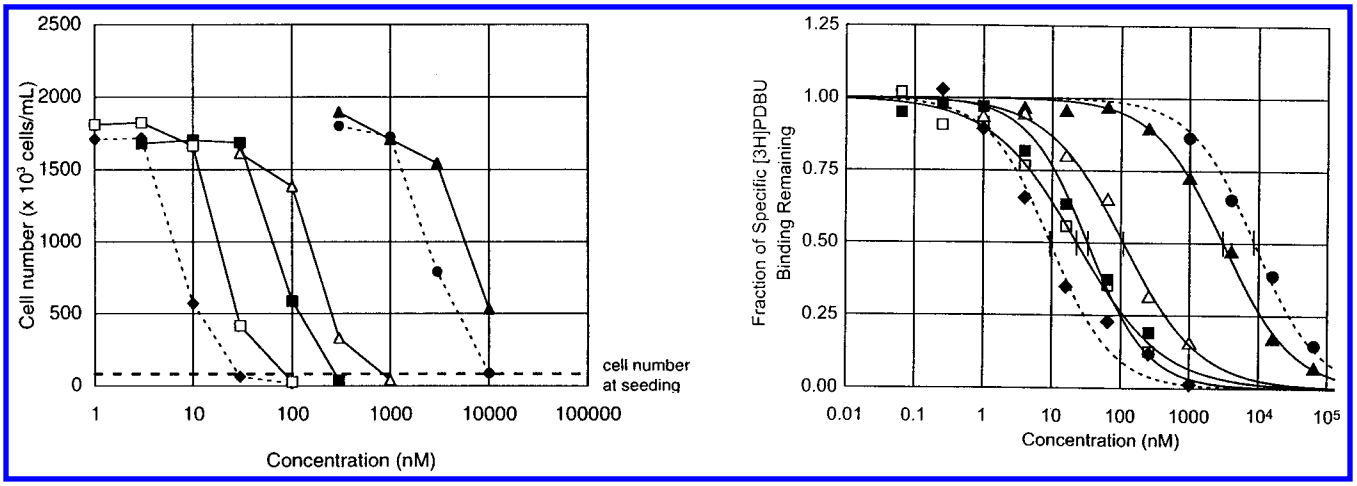


Figure 7. Biological activities of hydrophobically modified benzolactams **35**−**38**. Left: Growth inhibition of HL-60 cells. Right: Inhibition of specific [^{3}H]PDBu binding with PKCδ. Legend: ◆, BL-V8-310 (**5**); △, BL-V8-DM1 (**35**); ▲, BL-V8-DM3 (**36**); ■, BL-V8-SP1 (**37**); □, BL-V8-SP3 (**38**); ●, BL-V8-23TM (**10**).

PKC activators. Such simulation would also be useful to distinguish direct hydrophobic interaction of the hydrophobic moiety of teleocidins and indolactams with PKC from external hydrophobic interaction of the moiety with external phospholipids. Thus, we performed a docking study to obtain stable docking models[14] for the twist form of (−)-IL-V (**3**), (−)-BL-V8 (**4**), teleocidin B-4 (**1**), and two (−)-BL-V8s with 8,9-cyclic alkyl groups (**10** and **35**) bound to the PKCδ Cys2 domain, based upon the coordinates of the X-ray structure.[13]

Docking simulations in this study were performed using an automatic docking program (ADAM) developed by us previously.[25] The most stable docking model between a given protein and a small molecule is obtained by this method without presumptions, covering all possible docking combinations of ligand conformations and binding modes. All the flexibilities in rotatable bonds of the ligand molecule are automatically considered in the docking procedure, whereas the protein structure is treated as fixed in the step of obtaining initial docking models. As regards ring conformations, it is necessary to for possible conformers to be input in advance. Force-field energy values are used to select energetically favorable conformations together with favorable binding modes at the same time throughout the docking process. A noteworthy feature of the method is that torsion angles in the ligand are repeatedly optimized in continuous space together with relative positions and orientations of the two molecules during the process of docking. The efficiency of the method was confirmed by the fact that crystal structures of more than 10 enzyme−inhibitor complexes were well-reproduced by the program ADAM. In every case, not only was the docking mode in the crystal reproduced by the most stable docking model output from ADAM, but also the predicted structure coincided with the crystal structure very accurately with rms values of less than 2 Å. If torsion angles were not optimized during the docking process, the correct model would not be predicted or reproduced by the highest ranked model (most stable docking model) with such accuracy, even if conformational flexibilities were considered by systematic rotation of rotatable bonds in increments of 30° or 60°.

In advance of the ADAM docking, the allowed binding region for the compounds to be docked was roughly indicated on the protein structure interactively by the program GREEN,[26] so that the region fully included the phorbol 13-acetate molecule complexed with the enzyme in the crystal. A three-dimensional grid is generated inside the region, and various potentials are calculated in order to estimate intermolecular interaction energy very rapidly. van der Waals potentials for several probe atoms and electrostatic potentials at each grid point are calculated, using all protein atoms. Then, several dummy atoms, used as probes to cover likely binding modes and ligand conformations at the initial step of ADAM, were located at the estimated positions of partner atoms of hydrogen-bonding groups of the enzyme inside the allowed region.

Up to several dozen stable docking models initially output from the ADAM program for each molecule were reranked after energy minimization using the SANDER program of the AMBER package[27] in order to take into account conformational changes in the protein structure upon ligand binding. In this study, the most stable docking model after minimization was taken as a candidate structure for each compound and used as the basis for later discussion.

First, the redocking of phorbol 13-acetate was attempted in order to confirm the reliability of the docking method and the validity of all assumptions concerning the protein structure, using the modeled structure of phorbol 13-acetate itself. The most stable docking model selected by the docking method closely resembled the structure of the complex in the crystal, fully reproducing the ligand conformation and relative position and orientation, as shown in Figure 8, top. The hydrogen-bond network found in the crystal was also reproduced accurately in the predicted model. Three functional groups hydrogen-bonded to main-chain amide groups at the bottom of the cavity: the C3-carbonyl to the NH of Gly253, C-4 OH to the carbonyl of Gly253, and C-20 OH to both the carbonyl of Leu251 and the NH of Thr242, as found in the crystal (Table 1). Then, the twist forms of (−)-IL-V (**3**) and (−)-BL-V8 (**4**) were docked into the cavity by the same procedure. In the most stable docking structure for **3**, the molecule was well-fitted to the cavity, to the same extent as phorbol

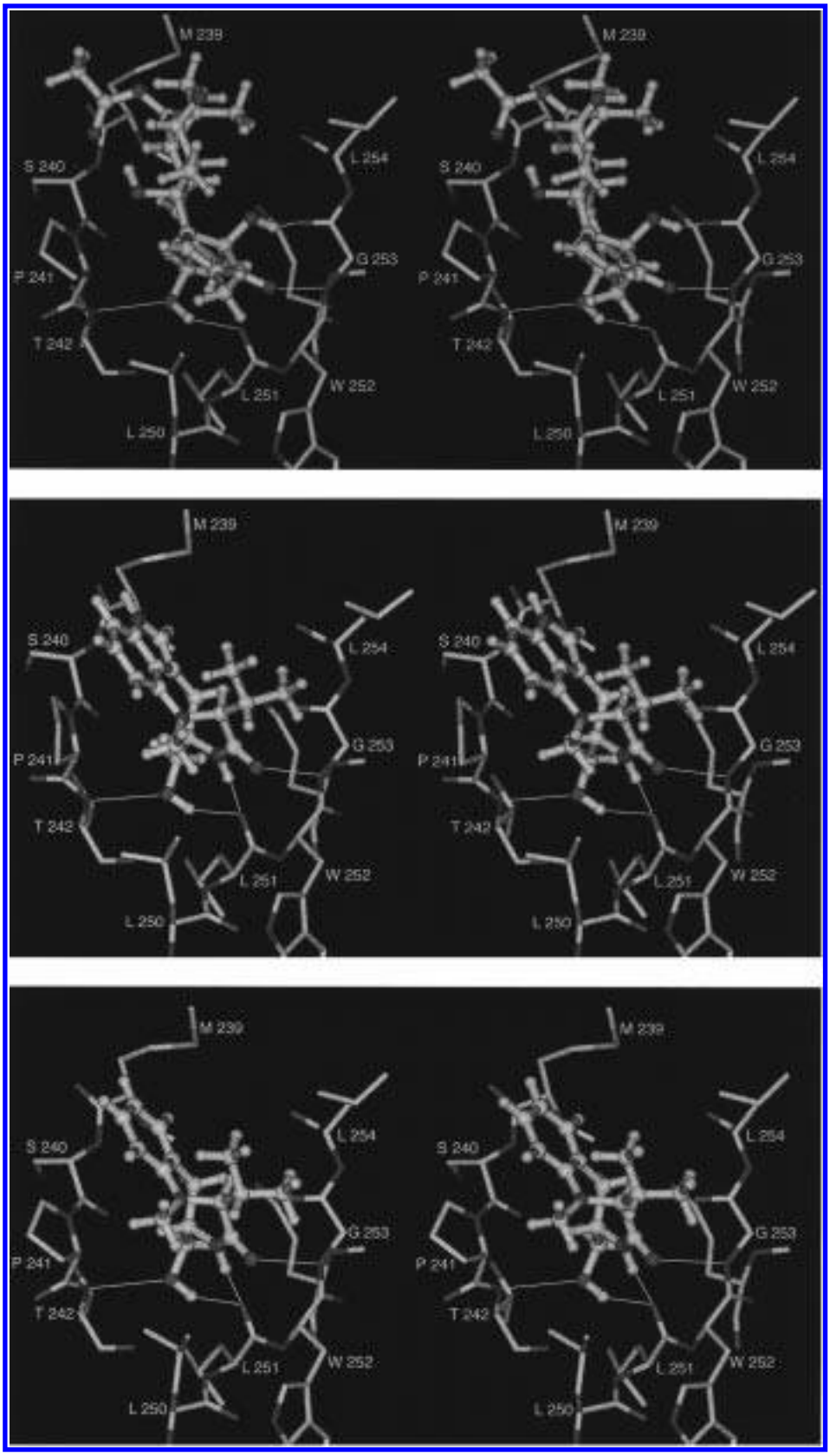


Figure 8. Stereoviews of the most stable docking models for phorbol 13-acetate (top), (−)-IL-V (**3**) (middle), and (−)-BL-V8 (**4**) (bottom). Bound ligands are shown with broad lines, and intermolecular hydrogen bonds (distances less than 3.2 Å) are shown with yellow lines.

13-acetate, forming four hydrogen bonds with the enzyme, as shown in Figure 8, middle. Three functional groups, the amide carbonyl, amide NH, and C-14 OH, were involved in the hydrogen-bond network. The amide carbonyl group hydrogen-bonded to the NH of Gly253 and the C-14 OH to both the carbonyl of Leu251 and the NH of Thr242, corresponding to the C3-carbonyl and C-20 OH groups in phorbol 13-acetate, respectively.

Table 1. Hydrogen-Bond Distances (Heteroatom−Heteroatom Distance in Å) in the Most Stable Docking Models and Those in the Crystal Structure

ligand	T242 N···HO[a]	L251 O···HO[a]	L251 O···HN[b]	G253 NH···O=C[c]	G253 O···HO[d]
phorbol 13-acetate (crystal)	2.95	2.81	none	3.10	2.64
phorbol 13-acetate (docked)	2.94	2.81	none	3.10	2.77
(−)-IL-V (**3**)	3.00	2.80	2.96	2.99	none
(−)-BL-V8 (**4**)	3.03	2.78	2.97	3.03	none
teleocidin B-4 (**1**)	3.01	2.78	2.97	2.97	none
(−)-BL-V8-23TM (**10**)	3.07	2.88	2.97	3.09	none
(−)-Bl-V8-DM1 (**35**)	3.04	2.78	2.93	2.98	none

[a] Phorbol 20-OH; (−)-IL-V and teleocidin B-4 14-OH; (−)-BL-Vs 5-OH. [b] (−)-IL-V and teleocidin B-4 10-NH; (−)-BL-Vs 4-NH. [c] Phorbol 3-C=O; (−)-IL-V and teleocidin B-4 11-C=O; (−)-BL-Vs 3-C=O. [d] Phorbol 4-OH.

Most notably, however, the partner of the amide NH in **3** was the carbonyl of Leu251, different from the partner of the third hydrogen-bonding group (C-4 OH) in phorbol 13-acetate, i.e.; the carbonyl of Gly253. The (−)-BL-V8 (**4**) molecule also formed a stable complex with the same hydrogen-bond network as IL-V, as expected from the superposition study of (−)-BL-V8 skeleton and teleocidin B-4 (**1**) (see Figure 6). The amide carbonyl hydrogen-bonded to the NH of Gly253, the C-5 CH_2OH hydrogen-bonded to both the carbonyl of Leu251 and the NH of Thr242, and the amide NH hydrogen-bonded to the carbonyl of Leu251, as shown in Figure 8, bottom.

Then, docking of teleocidin B-4 (**1**) was attempted in order to investigate the influence of the large hydrophobic group. The molecule **1** fitted well into the enzyme cavity without any unfavorable contact with protein atoms, as shown in Figure 9, top. The hydrophobic moiety of **1** points toward the outside of the protein, which is supposed to be important for the interaction with the lipid membrane. The docking of the two congeners of (−)-BL-V8, (−)-BL-V8-23TM (**10**) and (−)-BL-V8-DM1 (**35**), was also attempted in order to assess the effect of hydrophobic substituent groups on binding affinity. In the initial docking models, one of the methyl groups near C-8 of **10** clashed slightly with protein atoms at the entrance of the cavity, whereas **35** fitted well in the cavity. The clash was removed by energy minimization without causing marked changes in the hydrogen-bond networks with the proteins, as shown in Figure 9, middle and bottom.

Discussion

Recently, molecular modeling of the (−)-BL-V8 (**4**) molecule complexed with the PKCδ Cys2 domain using another calculation method was reported by Kozikowski et al.[28] The hydrogen-bonding pattern that they obtained was the same as the present result. They also described the importance of the hydrophobic interaction between the hydrophobic moiety of **4** and hydrophobic residues in the protein; the phenyl ring resides on top of Pro241, parallel to the proline ring, and the N-1 methyl group and the isopropyl group of **4** all interact with the hydrophobic side chains of Leu251 and Leu 254, respectively.

Although the hydrogen-bonding patterns of (−)-IL-V (**3**) and (−)-BL-V8 (**4**) are very similar, the structure−activity results for these two types of compounds are somewhat different. For example, there is a difference in PKCδ binding potency between **3** (80 nM) and **4** (1700 nM) and a difference in HL-60 growth-inhibitory activity between **3** (EC_{50}: 10^{-7} M) and **4** ($EC_{50} > 10^{-6}$ M). The difference in activity may be explained in terms of the difference between the hydrophobic interaction of the hydrophobic proline ring with the indole ring versus the benzene ring. The indole ring of **3** seems to have a more effective hydrophobic interaction compared to the benzene ring of **4** as judged from the present docking of **3** to the PKCδ Cys2 domain. The difference of electrostatic interaction between the 1-NH of **3** and an amide group of the peptide backbone may be also affected. 1-NH of **3** is close to the carbonyl of Met239 (N···O distance: 2.8 Å), but relative orientation of the two groups is not ideal for forming a stable hydrogen bond. Nevertheless, some electrostatic interaction is expected between them.[29] A more important difference is the difference in substitution effects between **3** and **4**. The 6,7-tetramethyltetramethylene analogue of (−)-IL-V (**34**) has a greater PKC binding activity than (−)-IL-V (**3**); its potency is comparable to that of TPA.[24] On the other hand, (−)-BL-V8-23TM (**10**), which has an 8,9-tetramethyltetramethylene group, showed very low activity. When we designed (−)-BL-V8-310,[10] which is still the most potent benzolactam known, such a subtle substituent effect was not expected. The present docking model explains well the great difference in activity. Teleocidin B-4 formed a stable complex with the same hydrogen-bonding pattern as the twist structure of **3** (Figure 9, top). Substituent groups at the 7-position of teleocidin B-4 do not hinder the fitting. Methyl and vinyl groups at the 22-position of teleocidin B-4 also satisfactorily evade van der Waals contacts with the protein. In contrast, bulky substituent groups at the 8-position of **10** would hinder the fitting of the molecule into the cavity through van der Waals contacts with Ser240 and Met239 (Figure 9, middle). Compound **35**, which lacks the bulky dimethyl group at the 8-position, formed a complex with the same hydrogen-bonding pattern as the twist structure of **3** (Figure 9, bottom).

To interpret the structure−activity relations among these compounds, force-field energies of all the most stable docking models were calculated by using the ANAL program of the AMBER package. Intramolecular, intermolecular, and total interaction energy values are summarized in Table 2. Total energy is defined as the sum of difference-intramolecular energy and intermolecular interaction energy. Here, the difference-intramolecular energy is defined as the difference between the conventional intramolecular energy for the ligand structure in the most stable docking model and that for the energy-minimized structure of the ligand alone extracted from the complex. For considering intramolecular relative stabilities among compounds of different structural classes, the raw energy values obtained by force-field calculation are less reliable than

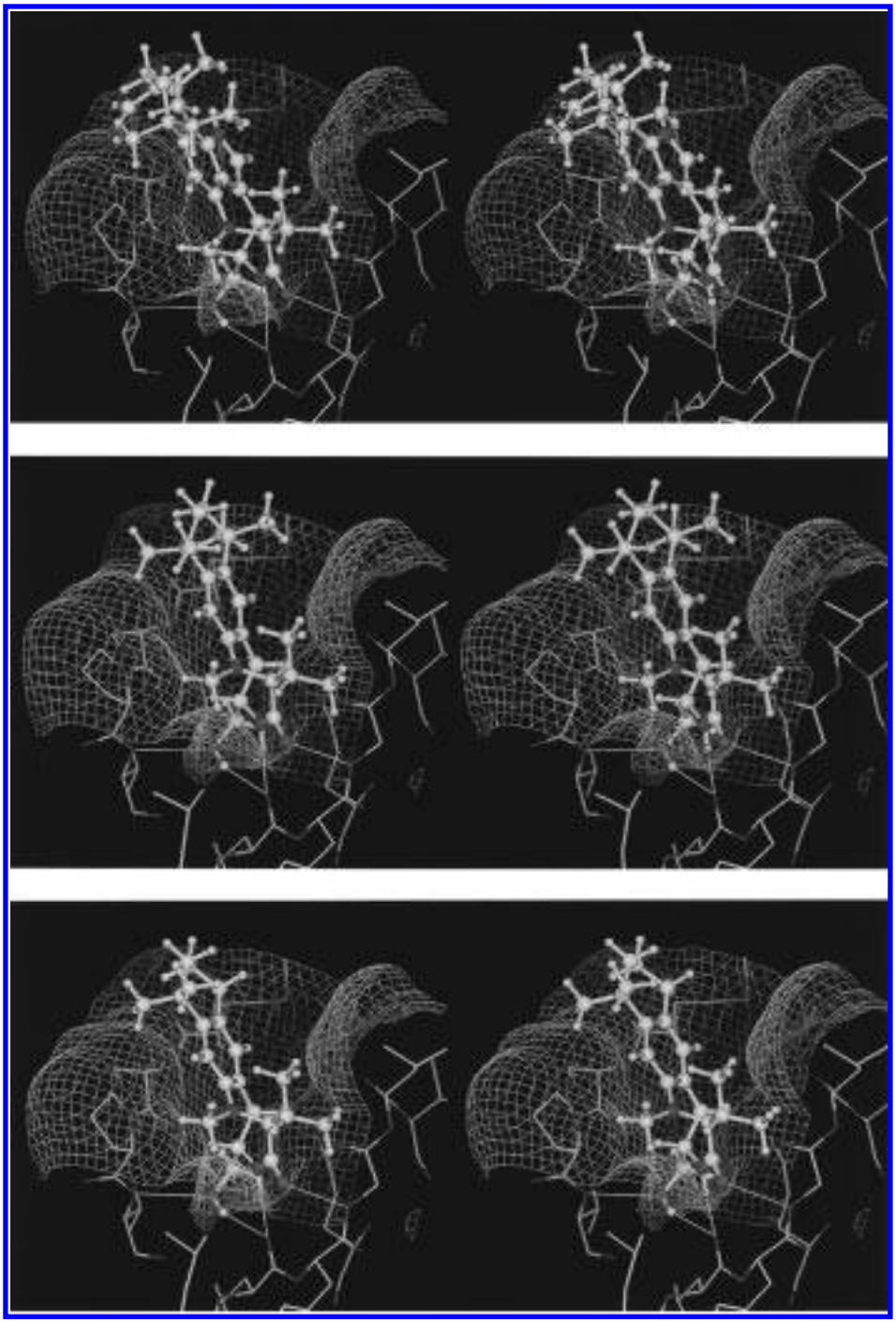


Figure 9. Stereoviews of the most stable docking models for teleocidin B-4 (**1**) (top), (−)-BL-V8-23TM (**10**) (middle), and (−)-BL-V8-DM1 (**35**) (bottom). The colored cage represents the allowed region for carbon atoms in ligands, calculated from the protein structure. Colors represent the electrostatic potential levels (positive, red; neutral, green; negative, blue).

Table 2. Energy Decomposition Analysis of the Most Stable Docking Models of (−)-IL-V and (−)-BLs[a]

ligand	$E_{ligand,bound}$	$E_{ligand,free}$	$E_{ligand,\Delta}$	E_{inter}	E_{total}	K_i for PKCδ (nM)
(−)-IL-V (**3**)	22.15	21.43	0.72	−30.31	−29.59	80 ± 29
(−)-BL-V8 (**4**)	24.31	23.54	0.77	−27.06	−26.29	1700 ± 30
teleocidin B-4 (**1**)	32.58	31.56	1.02	−31.25	−30.23	0.32 ± 0.043
(−)-BL-V8-23TM (**10**)	31.53	29.91	1.62	−27.97	−26.35	5100 ± 730
(−)-BL-V8-DM1 (**35**)	27.65	26.87	0.78	−29.16	−28.38	65 ± 0.6

[a] Energies are in kcal/mol. $E_{ligand,bound}$: energy of the ligand in the complexed structure. $E_{ligand,free}$: energy of the ligand minimized alone. $E_{ligand,\Delta}$: $E_{ligand,bound} - E_{ligand,free}$. E_{inter}: interaction energy between protein and ligand. E_{total}: $E_{ligand,\Delta} + E_{inter}$.

the difference energies used here, because of the high dependency on the force-field parameters.

The order of stability, based on the total energy for related teleocidin-type benzolactams: teleocidin B-4 (**1**) (−30.23), (−)-BL-V8-DM1 (**35**) (−28.38), (−)-BL-V8-23TM (**10**) (−26.35), and (−)-BL-V8 (**4**) (−26.29), can be well-correlated with the order of PKCδ binding potency, as seen from Table 2. The reason why docking models

of **10** and **4** exhibit almost the same stability seems to be as follows: the lower stability of **10** compared to **35**, by 2.03 kcal/mol, can be ascribed to the steric hindrance of the additional dimethyl groups, whereas the higher stability of **35** compared to **4**, by 2.09 kcal/mol, can be ascribed to favorable van der Waals interaction of the additional dimethylcyclohexyl group with protein atoms.

The hydrophobic regions of ligands bound to PKC are known to interact with the hydrophobic regions of phospholipid molecules presumably specifically associated with PKC.[30] Moreover, increasing the size of the hydrophobic regions of PKC ligands does increase their affinity for PKC. Thus it might be expected that the greater potency of more hydrophobic ligands for PKC in comparison to less hydrophobic ligands due to hydrophobic interactions with phospholipid would not be fully reflected in calculations of binding interactions of PKC ligand with PKC. However, this calculation of hydrophobically modified benzolactams reveals that the direct hydrophobic interaction of the ligand with PKC plays an important role in the affinity.

Conclusion

Synthesis and testing of benzolactams as conformationally restricted analogues of the biologically active conformation of teleocidins have clarified the conformational flexibility problem in structure−activity studies of teleocidins. However, the roles of the hydrophobic moieties of teleocidins and benzolactams and the flexible hydrophobic ester chains of phorbol esters are still not clear. Benzolactams are useful medicinal leads for elucidation of the role of PKC and the mechanism of tumor promotion, especially in conjunction with the structural determination of the PKCδ Cys2 domain−phorbol 13-acetate complex.[13] The present synthesis and biological evaluation of hydrophobically modified benzolactams have demonstrated the importance of a continuous substituent in hydrophobic region between C2 and C9 of BLs for binding to PKCδ Cys2 domain. It is noteworthy that the significant difference in the activity between (−)-BL-V8-310 (**5**) and (−)-BL-V8-23TM (**10**), as well as the difference between **5** and (−)-BL-V8-210 (**8**), can be accounted for by steric hindrance in binding of the ligand with PKCδ: docking simulation of teleocidin, (−)-IL-V, and benzolactams to the PKCδ Cys2 domain based upon the coordinates of the X-ray structure of the complex with phorbol 13-acetate, using an advanced docking program, explains well the above experimental results. These findings should be helpful in the design of compounds as biological tools for analyzing the mechanism of tumor promotion, or in designing potent antagonists.

Experimental Section

General Remarks. Melting points were obtained on a Yanagimoto micro-hot-stage apparatus without correction. Spectra were recorded with the following instruments: ^{1}H NMR spectra, JEOL JMN-GX-400 (400 MHz); mass spectra, JEOL JMN-DX 303; IR spectra, JASCO DS-402G; optical rotational spectra, JASCO DIP-181. NMR spectra were recorded with tetramethylsilane as an internal standard, and the chemical shifts are given as δ values from TMS. Column chromatography was performed on silica gel (Merck 9385).

(±)-2-[(Chloroacetyl)amino]dodecanoic Acid (13). A solution of *n*-decylglycine ((±)-**12**; 51.1 g, 237.6 mmol) in 2 N NaOH (120 mL) was stirred and cooled at 0 °C. To this was added a solution of chloroacetyl chloride (62 g, 548.6 mmol) in 2 N NaOH (200 mL) dropwise over 2 h with stirring. After the addition, the solution was acidified to pH 2 with concentrated HCl. The precipitate was collected and dissolved in AcOEt. The solution was washed with brine, dried (Na_2SO_4), and concentrated in vacuo to give **13** as a solid. Recrystallization from acetone−*n*-hexane gave 20.5 g (63%) of pure **13**. Mp: 91 °C. Anal. ($C_{14}H_{26}NO_3Cl$) C, H, N.

(*R*)-2-Aminododecanoic Acid ((*R*)-12). A solution of **13** (22.56 g, 97.4 mmol) in NaOH(aq) (3.44 g in 800 mL of distilled water) was warmed to 37 °C, adjusted to pH 7.27 with 3 N HCl, and diluted with 2 L of distilled water. To this were added *Aspergillus* aminoacylase (Tokyo Kasei Co.; 3.58 g) and $CoCl_2$ (15 mg), and the mixture was left to stand for 19 h at 37 °C. The separated (*S*)-**12** was collected, washed with water, methanol, and Et_2O, and dried to give 8.22 g of (*S*)-**12** (49%, mp 220 °C). The aqueous filtrate was left to stand for 22 h at 37 °C, and the precipitate was removed again by filtration. The filtrate was acidified with 3 N HCl, and the separated *(R)*-**13** was collected. It was dissolved in AcOEt. The solution was washed with 2 N HCl, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from acetone−*n*-hexane to give 7.92 g (35%) of (*R*)-**13**. Mp: 79−80.5 °C. (*R*)-**13** (5.60 g, 24.2 mmol) and 3 N HCl (100 mL) were stirred and heated under reflux for 4.5 h. After cooling, the mixture was neutralized with 15% aqueous NH_3. The precipitate was collected, washed with water, methanol, and AcOEt, and dried in vacuo to give 4.74 g (99%) of (*R*)-**12**. Mp: 205 °C dec. $[\alpha]^{22}_D$ −20.5° (*c* = 1.02, AcOH). Anal. ($C_{12}H_{25}NO_2$) C, H, N.

(*R*)-Benzyl 2-Hydroxydecanoate (14). A solution of sodium nitrite (2.0 g, 29.0 mmol) in water (23 mL) was added dropwise to a stirred solution of (*R*)-**12** (2.69 g, 12.5 mmol) in 2 N sulfuric acid over 1 h at 95 °C. After stirring for 2.5 h at 95 °C and cooling, the mixture was extracted with AcOEt, washed with brine, and dried over $MgSO_4$. Concentration gave the crude (*R*)-2-hydroxydecanoic acid as a colorless solid, which was used without purification. The acid was dissolved in dry benzene (50 mL); then benzyl alcohol (8.5 mL, 81.8 mmol) and thionyl chloride (1.0 mL, 13.7 mmol) were added. The solution was heated at reflux with a Dean−Stark trap for 10 h. An additional portion of thionyl chloride (1.0 mL) was added, and the reaction was continued for 16 h. After cooling, the solution was diluted with saturated $NaHCO_3$(aq) and extracted with AcOEt. The organic phase was washed with water, 10% citric acid aqueous solution, water, and brine. After drying ($MgSO_4$) and concentration in vacuo, the resultant residue was chromatographed on silica gel (hexane/AcOEt, 5:2) to give (*R*)-benzyl 2-hydroxydecanoate (**14**) as a viscous liquid (2.04 g, 53%). $[\alpha]^{22}_D$ +10.46° (*c* = 1.09, $CHCl_3$).

(*R*)-Benzyl 2-[[(Trifluoromethyl)sulfonyl]oxy]decanoate (11). A solution of **14** (1.17 g, 4.12 mmol) in dry dichloromethane (20 mL) was cooled to −40 °C, and 2,6-lutidine (0.95 g, 8.88 mmol) was added, followed by triflic anhydride (1.92 g, 6.8 mmol). After 1 h, the mixture was warmed to room temperature, and ice−water was added. The organic phase was separated, washed with water and brine, dried ($MgSO_4$), and concentrated to give an oil. Purification by column chromatography on silica gel (hexane/AcOEt, 5:1) gave (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]decanoate (**11**) as a colorless oil. $[\alpha]^{22}_D$ +27.5° (*c* = 1.16, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, *J* = 7 Hz, $(CH_2)_8CH_3$), 1.24 (br, 12H, $-(CH_2)_6CH_3$), 1.38 (br, 2H, $-CH_2(CH_2)_6CH_3$), 1.48 (br, 2H, $-CH_2(CH_2)_7CH_3$), 1.65 (m, 1H, $CHCH_2-$), 1.78 (m, 1H, $CHCH_2-$), 5.14 (t, *J* = 6 Hz, 1H, C*H*OTf), 5.26 (m, 2H, $CH_2C_6H_5$), 7.38 (m, 5H, C_6H_5).

Diastereomeric Esters 16. A mixture of 640 mg (2.29 mmol) of (±)-2-(methylamino)-*N*-Boc-phenylalaninol (**15**),[10c] the (*R*)-triflate **11** (978 mg, 2.30 mmol), and 2,6-lutidine (294 mg, 2.75 mmol) in 10 mL of CH_2ClCH_2Cl was refluxed for 2 days, and then the solvent was removed by evaporation. The residue was chromatographed on silica gel to give 1.01 g of a diastereomeric mixture of **16** as a colorless oil (80%). ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, *J* = 7 Hz, $(CH_2)_8CH_3$), 1.24 (br, 16H, $-(CH_2)_8CH_3$), 1.44 (s, 9H, Boc-CH_3), 1.49 (m, 0.5H, $-CH_2(CH_2)_8-$

CH_3), 1.66 (m, 0.5H, $-CH_2(CH_2)_8CH_3$), 1.90 (m, 1H, $-CH_2(CH_2)_8CH_3$), 2.72 (s, 1.5H, N-CH_3), 2.77 (s, 1.5H, N-CH_3), 2.7−3.0 (m, 1H, $ArCH_2CH-$), 3.27 (m, 0.5H, CH_2OH), 3.38 (m, 1H, CH_2OH), 3.61 (m, 1H, CH_3-N-CH-CO), 3.70 (m, 0.5H, CH_2OH), 4.00 (m, 1H, $-CHCH_2OH$), 5.06 (s, 1H, $CH_2C_6H_5$), 5.14 (d, 0.5H, J = 12 Hz, $CH_2C_6H_5$), 5.18 (d, 0.5H, J = 12 Hz, $CH_2C_6H_5$), 5.42 (bs, 0.5H, NHBoc), 5.49 (d, 0.5H, J = 8 Hz, NHBoc), 7.05−7.25 (m, 4H, Ar*H*), 7.3−7.4 (m, 5H, $CH_2C_6H_5$).

(−)-BL-8-C10 (8) and (−)-Epi-BL-8-C10 (17). A mixture of the diastereomeric esters **16** (1.01 g, 1.78 mmol) and 150 mg of 10% Pd−C in 120 mL of ethanol was vigorously stirred under 1 atm of H_2 at room temperature for 2 h and then filtered. The filtrate was concentrated to afford the crude carboxylic acid. The acid and 818 mg (7.1 mmol) of *N*-hydroxysuccinimide were dissolved in 18 mL of CH_3CN, and then a solution of 733 mg (3.6 mmol) of DCC in 2 mL of CH_3CN was added at 0 °C with stirring. Stirring was continued for 2 h at room temperature; then the solvent was removed under reduced pressure. The residue was suspended in a small amount of AcOEt, and insoluble urea was filtered off. The filtrate was concentrated and purified by column chromatography on silica gel to give an activated ester as a colorless liquid. This product was unstable and was used without further purification. Trifluoroacetic acid (5 mL) was added to a solution of the activated ester in 5 mL of CH_2Cl_2 at 0 °C with stirring. The mixture was stirred for 4 h at room temperature; then the solvent was removed under reduced pressure at below 30 °C. The residue was dissolved in 700 mL of AcOEt, and 50 mL of saturated $NaHCO_3$(aq) was added. The mixture was refluxed for 6 h with vigorous stirring. The organic phase was separated, and the aqueous layer was extracted with AcOEt. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was separated by column chromatography to give 171 mg (27%) of (−)-BL-8-C10 (**8**) and 151 mg (24%) (−)-epi-BL-8-C10 (**17**).

The optical purity of the BL-8-C10s was 80% ee, because the hydroxydediazoniation in the preparation of (*R*)-benzyl 2-hydroxydecanoate (**14**) required high temperature. Final optical purification was conducted as follows: the optically crude **8** was converted to *N*-tosylvaline ester,[6b] which was purified by silica gel column chromatography and recrystallization. Hydrolysis of the purified ester afforded optically pure **8**, colorless viscous oil. $[\alpha]^{22}_D$ −237.9° (c = 0.66, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, J = 7 Hz, $(CH_2)_8CH_3$), 1.22 (m, 16H, $-(CH_2)_8CH_3$), 1.90 (m, 2H, $-CH_2(CH_2)_8CH_3$), 2.77 (s, 3H, N-CH_3), 2.93 (m, 2H, $ArCH_2CH$-), 3.57 (m, 1H, CH_2OH), 3.67 (t, 1H, CH_3-N-CH-CO), 3.75 (m, 1H, CH_2OH), 4.31 (m, 2H, $-CHCH_2OH$), 6.28 (bs, 1H, $-NHCO-$), 6.93 (m, 1H, Ar*H*), 7.05 (m, 2H, Ar*H*), 7.20 (m, 1H, Ar*H*). HRMS: calcd for $C_{22}H_{36}N_2O_2$, 360.2777; found, 360.2834.

3-Carbomethoxy-4-nitrobenzaldehyde (19). A solution of 3-carbomethoxy-4-nitrobenzoic acid (**18**; 22.6 g, 100.4 mmol) in THF (450 mL) was cooled to 0 °C, and BH_3−methyl sulfide complex (16 mL, 168.8 mmol) was added. The mixture was stirred for 30 min at room temperature, refluxed for 5 h, and then cooled. The reaction was quenched by the addition of methanol, and the whole was concentrated. The residue was diluted with saturated $NaHCO_3$(aq) and extracted with AcOEt. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was crystallized from hexane−AcOEt to give 3-carbomethoxy-4-nitrobenzyl alcohol (19.2 g, 91%). A solution of the benzyl alcohol (15.5 g, 73.5 mmol) in CH_2Cl_2 (100 mL) was added to a suspension of pyridinium chlorochromate (PCC; 16.8 g, 78 mmol) and neutral activity I Al_2O_3 (21.1 g; Merck 70−230 mesh) in CH_2Cl_2 (150 mL) under Ar at 0 °C with vigorous stirring. The stirring was continued for 18 h; then the mixture was concentrated, charged on a silica gel column, and chromatographed to give 3-carbomethoxy-4-nitrobenzaldehyde (**19**) (14.4 g, 94%). Mp: 74.5−75.0 °C. Anal. ($C_9H_7NO_5$) C, H, N.

Nitro Ester 20. A solution of nonyltriphenylphosphonium bromide (45.8 g, 97.7 mmol) in 800 mL of THF was treated with 30 mL of 1.6 M *n*-BuLi in hexane at 0 °C, and the mixture was stirred for 40 min under an Ar atmosphere. To the ylide solution was added a solution of the aldehyde **19** (8.83 g, 42.2 mmol) in 100 mL of THF at 0 °C. The mixture was stirred for 3.5 h at 0 °C. The reaction was quenched by the addition of 5 mL of 2 N HCl, and then the solvent was evaporated. The residue was poured into 2 N HCl and extracted with CH_2Cl_2. The extract was washed with water and brine and dried over $MgSO_4$. Concentration and purification by column chromatography on silica gel with CH_2Cl_2/*n*-hexane = 1:1 afforded 11.3 g of **20** as a yellow viscous liquid (84%), *E*,*Z*-mixture of the olefin. ^{1}H NMR ($CDCl_3$): 0.87 (m, 3H, $CH_2(CH_2)_5CH_3$), 1.27 (br, 10H, $CH_2(CH_2)_5CH_3$), 1.46 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 2.23−2.33 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 3.93 (s, 3H, $COOCH_3$), 5.90 (m, 0.6H, $ArCH=CH(CH_2)_7CH_3$), 6.44 (m, 1.4H, $ArCH=CH(CH_2)_7CH_3$), 7.48 (m, 1H, Ar*H*), 7.54−7.59 (m, 1H, Ar*H*), 7.93 (m, 1H, Ar*H*).

Nitro Tosylate 21. To a solution of 2.7 g (126.0 mmol) of $LiBH_4$ in 500 mL of THF was added 11.2 g (35.1 mmol) of **20** at 0 °C with stirring. The solution was stirred for 1 h at 0 °C and then for 16 h at room temperature and concentrated. The residue was poured carefully into ice−water and extracted with AcOEt. The extract was washed with 10% citric acid(aq), water, and brine and dried over $MgSO_4$. Evaporation and purification by column chromatography on silica gel (CH_2Cl_2/AcOEt, 10:1) afforded 7.64 g of the benzyl alcohol (82%) as a pale-yellow oil. Sodium hydride (60% in oil; 1.20 g, 30 mmol) was washed with *n*-hexane, dried under reduced pressure, and suspended in 40 mL of toluene under an Ar atmosphere. To this suspension was added a solution of the above benzyl alcohol (6.4 g, 22.0 mmol) in toluene (80 mL) followed by *p*-toluenesulfonyl chloride (5.75 g, 30.2 mmol). After stirring for 2 h at room temperature, the mixture was poured into 10% citric acid(aq) and extracted with AcOEt. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was chromatographed on silica gel (hexane/CH_2Cl_2, 1:1) to give the tosylate **21** as a pale-red, viscous oil, *E*,*Z*-mixture of the olefin. ^{1}H NMR ($CDCl_3$): 0.87 (m, 3H, $CH_2(CH_2)_5CH_3$), 1.27 (br, 10H, $CH_2(CH_2)_5CH_3$), 1.46 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 2.30 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 2.46 (s, 3H, $ArCH_3$), 5.48 (m, 2H, $ArCH_2OH$), 5.90 (m, 0.6H, $ArCH=CH(CH_2)_7CH_3$), 6.43 (m, 1.4H, $ArCH=CH(CH_2)_7CH_3$), 7.36 (m, 3H, Ts, Ar*H*), 7.61 (s, 0.7H, Ar*H*), 7.63 (s, 0.3H, Ar*H*), 7.61 (s, 0.3H, Ar*H*), 7.84 (d, 2H, J = 9 Hz, Ts), 8.07 (d, 0.3H, J = 8 Hz, Ar*H*), 8.10 (d, 0.7H, J = 8 Hz, Ar*H*).

Diester 22. Sodium hydride (60% in oil; 1.15 g, 28.8 mmol) was washed with *n*-hexane, dried under reduced pressure, and suspended in 50 mL of DMF under an Ar atmosphere. A solution of 6.67 g (30.9 mmol) of diethyl acetamidomalonate in 10 mL of DMF was added to it, followed by a solution of tosylate **21** (10.77 g, 24.2 mol) in 50 mL of DMF, and the reaction mixture was stirred for 16 h at room temperature. The solvent was removed under reduced pressure. The residue was poured into 2 N HCl and the mixture was extracted with AcOEt. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated. Purification by column chromatography on silica gel (CH_2Cl_2/AcOEt, 10:1) afforded 9.12 g (77%) of the diester **22** as pale-yellow columns. Mp: 58−61 °C. ^{1}H NMR ($CDCl_3$): 0.87 (t, 3H, J = 7 Hz, $CH_2(CH_2)_5CH_3$), 1.27 (m, 16H, $CH_2(CH_2)_5CH_3$, 2 × $-OCH_2CH_3$), 1.45 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 1.95 (s, 3H, $NHCOCH_3$), 2.28 (m, 2H, $CH=CHCH_2CH_2(CH_2)_5CH_3$), 4.08 (m, 2H, $ArCH_2CH$), 4.25 (m, 4H, 2 × $-OCH_2CH_3$), 5.84 (m, 0.6H, $ArCH=CH(CH_2)_7CH_3$), 6.34 (m, 1.4H, $ArCH=CH(CH_2)_7CH_3$), 6.46 (bs, 1H, $-NHCO-$), 7.10 (d, 0.7H, J = 1 Hz, Ar*H*), 7.17 (d, 0.3H, J = 1 Hz, Ar*H*), 7.29 (m, 1H, Ar*H*), 7.83 (d, 0.3H, J = 8 Hz, Ar*H*), 7.84 (d, 0.7H, J = 8 Hz, Ar*H*). Anal. ($C_{26}H_{38}N_2O_7$) C, H, N.

Nitro Alcohol 23. A mixture of 9.12 g (18.6 mmol) of **22**, 20 mL of acetic acid, and 50 mL of concentrated HCl was refluxed gently for 24 h. The mixture was cooled to room temperature and added to 200 mL of ice water. The aqueous layer was washed twice with 50 mL of CH_2Cl_2 and then concentrated to dryness. The residue was dried under reduced pressure over P_2O_5 to give the amino acid as a white solid. A

solution of the crude amino acid in dry ethanol (40 mL) was added to a solution of 20.86 g of $SOCl_2$ in 100 mL of dry ethanol (prepared at −10 °C) with stirring at −40 °C. The mixture was stirred for 1 h at −40 °C and for 30 h at room temperature and then concentrated under reduced pressure to remove most of the ethanol. The residue was poured into saturated $NaHCO_3$(aq) and extracted with CH_2Cl_2. The organic phase was washed with saturated $NaHCO_3$(aq), water, and brine. The solution was dried over $MgSO_4$ and then concentrated, and the residue was redissolved in 200 mL of CH_2Cl_2. To this solution was added 5.04 g (23.1 mmol) of Boc_2O at 0 °C. The mixture was stirred for 18 h at room temperature and concentrated. Purification of the residue by column chromatography on silica gel (CH_2Cl_2) afforded 8.10 g of the *N*-Boc-amino acid ethyl ester (91%) as a pale-yellow oil. A solution of the *N*-Boc-amino acid ethyl ester (8.10 g, 17.0 mmol) in THF (50 mL) was added to a stirred solution of 1.28 g (59.7 mmol) of $LiBH_4$ in THF (150 mL). The solution was stirred for 1 h at 0 °C and for 17.5 h at room temperature and then concentrated. The residue was poured carefully into ice water and extracted with CH_2Cl_2. The extract was washed with water and brine and dried over $MgSO_4$. Evaporation and purification by column chromatography on silica gel afforded 3.93 g of the nitro alcohol **23** (49%) as colorless needles. Mp: 91−93 °C. ^{1}H NMR ($CDCl_3$): 0.88 (m, 3H, $CH_2(CH_2)_5CH_3$), 1.27 (m, 10H, $CH_2(CH_2)_5CH_3$), 1.37 (s, 9H, Boc-CH_3), 1.46 (m, 2H, $CH{=}CHCH_2CH_2(CH_2)_5CH_3$), 2.24 (dd, 0.6H, J = 7, 13 Hz, $CH{=}CHCH_2CH_2(CH_2)_5CH_3$), 2.31 (m, 1.4H, $CH{=}CHCH_2CH_2(CH_2)_5CH_3$), 2.41 (bs, 1H, O*H*), 3.06 (m, 2H, ArCH_2CH), 3.70 (m, 2H, CH_2OH), 4.00 (m, 1H, C*H*CH_2OH), 5.04 (bs, 1H, −N*H*CO−), 5.85 (m, 0.6H, Ar$CH{=}CH(CH_2)_7CH_3$), 6.39 (m, 1.4H, Ar$CH{=}CH(CH_2)_7CH_3$), 7.31 (m, 2H, Ar*H*), 7.95 (m, 1H, Ar*H*). Anal. ($C_{24}H_{38}N_2O_5$) C, H, N.

Methylamino Alcohol 24. A mixture of 2.44 g (5.62 mmol) of **23** and 240 mg of 10% Pd−C in 200 mL of ethanol was vigorously stirred under 1 atm of H_2 at room temperature for 10 h and then filtered. The filtrate was concentrated, and the residue was crystallized from benzene to afford 2.09 g of the amine with a saturated alkyl substituent as colorless needles (92%). Mp: 115 °C. Anal. ($C_{24}H_{42}N_2O_3$) C, H, N. A solution of the amine (1.99 g, 4.90 mmol) in 30 mL of THF was added at 0 °C to a mixed anhydride solution (prepared by addition of 1.22 g of formic acid to 2.66 g of acetic anhydride and THF (5 mL) at 0 °C, followed by heating for 2 h at 60−70 °C). After having been stirred for 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was poured into saturated $NaHCO_3$(aq), and the whole was extracted with AcOEt. The extract was washed with water and brine and dried over $MgSO_4$. Concentration and purification by column chromatography on silica gel with AcOEt/*n*-hexane = 1:1 afforded 1.97 g of the *N*-formate as a pale-yellow oil (93%). Mp: 93 °C. Anal. ($C_{25}H_{42}N_2O_4$) C, H, N. To a solution of the formate (1.89 g, 4.35 mmol) in 300 mL of THF was added dropwise 19 mL of 1.0 M BH_3 in THF solution at 0 °C, and the mixture was stirred for 2.5 h at 0 °C. The reaction was quenched by the addition of 10 mL of 10% citric acid, and the whole was extracted with AcOEt. After concentration, the residue was poured into saturated $NaHCO_3$(aq) and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. Concentration and purification by column chromatography on silica gel with AcOEt/*n*-hexane = 1:1 gave 1.82 g of the methylamino alcohol **24** as colorless prisms (99%). Mp: 53−55 °C. ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, J = 7 Hz, $CH_2(CH_2)_5CH_3$), 1.26 (m, 14H, $CH_2(CH_2)_7CH_3$), 1.47 (s, 9H, Boc-CH_3), 1.54 (m, 2H, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.48 (t, 2H, J = 8 Hz, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.83 (m, 2H, ArCH_2CH), 2.85 (s, 3H, N-C*H*$_3$), 3.50 (dd, 1H, J = 2, 8 Hz, C*H*$_2$OH), 3.56 (d, 1H, J = 8 Hz, C*H*$_2$OH), 3.70 (m, 1H, C*H*CH_2OH), 5.12 (bd, 1H, −N*H*CO−), 6.61 (d, 1H, J = 8 Hz, Ar*H*), 6.85 (d, 1H, J = 2 Hz, Ar*H*), 6.99 (dd, 1H, J = 2, 8 Hz, Ar*H*). Anal. ($C_{25}H_{44}N_2O_3$) C, H, N.

Diastereomeric Ester 25. A mixture of 870 mg (2.07 mmol) of **24**, (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]-isovalerate[5d] (808 mg, 2.38 mmol), and 2,6-lutidine (510 mg, 4.77 mmol) in 15 mL of CH_2ClCH_2Cl was refluxed for 24 h, and then the solvent was removed by evaporation. The residue was chromatographed on silica gel to give 1.095 g of a diastereomeric mixture of **25** as a colorless oil (86%). ^{1}H NMR ($CDCl_3$): 0.88 (m, 6H, $CH_2(CH_2)_5CH_3$, $CH(CH_3)_2$), 1.16 (m, 3H, $CH(CH_3)_2$), 1.26 (m, 14H, $CH_2(CH_2)_7CH_3$), 1.37 (s, 4.5H, Boc-CH_3), 1.45 (s, 4.5H, Boc-CH_3), 1.55 (m, 2H, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.26 (m, 1H, C*H*$(CH_3)_2$), 2.50 (m, 2H, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.82 (s, 1.5H, N-C*H*$_3$), 2.84 (s, 1.5H, N-C*H*$_3$), 2.95 (m, 2H, ArCH_2CH), 3.20 (d, 0.5H, J = 10 Hz, $CH_3NCHCO-$), 3.26 (d, 0.5H, J = 10 Hz, $CH_3NCHCO-$), 3.41 (m, 0.5H, C*H*$_2$OH), 3.60 (m, 1H, C*H*$_2$OH), 3.73 (m, 1H, C*H*$_2$OH, C*H*CH_2OH), 3.95 (m, 0.5H, C*H*CH_2OH), 4.87 (d, 0.5H, J = 11 Hz, $COOCH_2C_6H_5$), 4.91 (d, 0.5H, J = 11 Hz, $COOCH_2C_6H_5$), 4.94 (m, 1H, $COOCH_2C_6H_5$), 5.41 (bd, 0.5H, J = 5 Hz, −N*H*CO−), 6.06 (bd, 0.5H, J = 2 Hz, −N*H*CO−), 6.85−7.07 (m, 5H, Ar*H*), 7.26 (m, 3H, $COOCH_2C_6H_5$).

(−)-BL-V8-210 (9) and (−)-Epi-BL-V8-210 (26). A mixture of diastereomeric esters **25** (1.09 g, 1.78 mmol) and 150 mg of 10% Pd−C in 120 mL of ethanol was vigorously stirred under 1 atm of H_2 at room temperature for 2 h and then filtered. The filtrate was concentrated to afford the crude carboxylic acid. The acid and 818 mg (7.1 mmol) of *N*-hydroxysuccinimide were dissolved in 18 mL of CH_3CN, and then a solution of 733 mg (3.6 mmol) of DCC in 2 mL of CH_3CN was added at 0 °C with stirring. Stirring was continued for 2 h at room temperature; then the solvent was removed under reduced pressure. The residue was suspended in a small amount of AcOEt, and insoluble urea was filtered off. The filtrate was concentrated and purified by column chromatography on silica gel to give an activated ester as a colorless liquid. This product was unstable and was used without further purification. Trifluoroacetic acid (5 mL) was added to a solution of the activated ester in 5 mL of CH_2Cl_2 at 0 °C with stirring. The mixture was stirred for 4 h at room temperature; then the solvent was removed under reduced pressure at below 30 °C. The residue was dissolved in 1.2 L of AcOEt, and 100 mL of saturated $NaHCO_3$(aq) was added. The mixture was refluxed for 6 h with vigorous stirring. The organic phase was separated, and the aqueous layer was extracted with AcOEt. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was separated by column chromatography to give 240 mg (40%) of (−)-BL-V8-210 (**9**) and 151 mg (38%) of (−)-epi-BL-V8-210 (**26**). **9**: colorless viscous oil. $[\alpha]^{22}_D$ −231.9° (c = 1.16, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.88 (t, 3H, J = 7 Hz, $(CH_2)_2CH_3$), 0.93 (d, 3H, J = 7 Hz, $CH(CH_3)_2$), 1.07 (d, 3H, J = 7 Hz, $CH(CH_3)_2$), 1.26 (m, 14H, $-CH_2(CH_2)_7CH_3$), 1.58 (m, 2H, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.48 (m, 1H, C*H*$(CH_3)_2$), 2.49 (t, 2H, J = 8 Hz, Ar$CH_2CH_2(CH_2)_7CH_3$), 2.78 (s, 3H, N-C*H*$_3$), 2.85 (d, 1H, J = 17 Hz, ArCH_2CH−), 3.00 (dd, 1H, J = 8, 17 Hz, ArCH_2CH−), 3.42 (d, 1H, J = 8 Hz, CH_3-N-C*H*-CO), 3.55 (dd, 1H, J = 8, 11 Hz, C*H*$_2$OH), 3.74 (dd, 1H, J = 4, 11 Hz, C*H*$_2$OH), 4.27 (m, 1H, −C*H*CH_2OH), 6.21 (bd, 1H, J = 2 Hz, −N*H*CO−), 6.85 (s, 1H, Ar*H*), 6.96 (d, 1H, J = 8 Hz, Ar*H*), 7.00 (dd, 1H, J = 1, 8 Hz, Ar*H*). HRMS: calcd for $C_{25}H_{42}N_2O_2$, 402.3248; found, 402.3237. **26**: colorless viscous oil. $[\alpha]^{22}_D$ −145.9° (c = 0.92, $CHCl_3$). HRMS: calcd for $C_{25}H_{42}N_2O_2$, 402.3248; found, 402.3219.

7-Nitro-6-(bromomethyl)-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene (28). To a solution of 1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene (**27**; 16.6 g, 82.2 mmol) in 65 mL of AcOH was added dropwise a mixture of concentrated HNO_3 (8.2 mL) and concentrated H_2SO_4 (18 mL) at below 20 °C (required ice bath) with vigorous stirring. After stirring for 4 h, the mixture was poured into ice−water (200 mL), and the precipitate was collected. The precipitate was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$(aq) and water. Drying ($MgSO_4$) and concentration gave a pale-yellow solid. The crude product was recrystallized from ethanol to give 12.6 g (62.1%) of 7-nitro-1,1,4,4,6-pentamethyl-1,2,3,4-tetrahydronaphthalene. Mp: 152−153 °C. Anal. ($C_{15}H_{21}NO_2$) C, H, N. To a solution of the nitrotoluene (11.3 g, 45.7 mmol) and *N*-bromosuccinimide (7.65 g, 45.7 mmol) in

CCl_4 (100 mL) was added AIBN (150 mg, 0.91 mmol), followed by a catalytic amount of benzyltrimethylammonium tribromide at room temperature with stirring. The mixture was heated to reflux for 3 h. After removal of the precipitate by filtration, the filtrate was concentrated and chromatographed on silica gel (hexane/AcOEt, 10:1) to afford 7.37 g (49%) of the benzyl bromide **28**. Mp: 87.5−89 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.31 (m, 12H, C*H*$_3$), 1.71 (s, 4H, −(C*H*$_2$)$_2$−), 4.83 (s, 2H, ArC*H*$_2$-Br), 7.43 (s, 1H, Ar*H*), 8.02 (s, 1H, Ar*H*). Anal. ($C_{15}H_{20}NO_2$-Br) C, H, N.

Diester 29. The nitrobenzyl bromide **28** (7.37 g, 22.6 mmol) was converted into the diester **29** by the same method as that used for the preparation of **22** employing 1.08 g (27.0 mmol) of NaH, 4.90 g (22.6 mmol) of diethyl acetamidomalonate, and 50 mL of DMF for 2.5 h at room temperature under an Ar atmosphere. Purification by column chromatography on silica gel (hexane/AcOEt, 4:1) gave 9.44 g (90%) of the diester **29** as pale-yellow prisms. Mp: 131−132 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$): 1.28 (m, 18H, C*H*$_3$, 2 × −OCH$_2$C*H*$_3$), 1.69 (s, 4H, −(C*H*$_2$)$_2$−), 1.95 (s, 3H, NHCOC*H*$_3$), 4.03 (s, 2H, ArC*H*$_2$-CH), 4.2 (m, 4H, 2 × −OC*H*$_2$CH$_3$), 6.46 (bs, 1H, −N*H*CO−), 7.13 (s, 1H, Ar*H*), 7.79 (s, 1H, Ar*H*). Anal. ($C_{24}H_{34}N_2O_7$) C, H, N.

Nitro Alcohol 30. A mixture of 9.24 g (20.0 mmol) of **29**, 40 mL of acetic acid, and 20 mL of concentrated HCl was refluxed gently for 14 h. The mixture was concentrated, and water was removed azeotropically by the addition of ethanol three times. The residue was dissolved in dry ethanol (40 mL), and the solution was added to a solution of 20.0 g of $SOCl_2$ in 60 mL of dry ethanol (prepared at −10 °C) with stirring at −40 °C. The mixture was stirred for 1 h at −40 °C and then for 20 h at room temperature and then concentrated under reduced pressure to remove most of the ethanol. The residue was poured into saturated $NaHCO_3$(aq) and extracted with AcOEt. The organic phase was washed with saturated $NaHCO_3$(aq), water, and brine. The solution was dried over $MgSO_4$ and then concentrated, and the residue was redissolved in 20 mL of CH_2Cl_2. To this solution was added 5.45 g (25.0 mmol) of Boc_2O at 0 °C. The mixture was stirred for 18 h at room temperature and concentrated. Purification by column chromatography on silica gel (hexane/AcOEt, 8:1) afforded 8.41 g of the *N*-Boc-amino acid ethyl ester (94%) as colorless plates. Mp: 134−135.5 °C (hexane). Anal. ($C_{24}H_{36}N_2O_6$) C, H, N. A solution of the *N*-Boc-amino acid ethyl ester (8.41 g, 18.8 mmol) in THF (80 mL) was added to a stirred solution of 1.23 g (56.4 mmol) of $LiBH_4$ in THF (80 mL). The solution was stirred for 1 h at 0 °C and for 18 h at room temperature and concentrated. The residue was poured carefully into ice water and extracted with AcOEt. The extract was washed with water and brine and dried over $MgSO_4$. Evaporation afforded 7.66 g of the nitro alcohol **30** (100%) as a pale-yellow gum. ^{1}H NMR ($CDCl_3$): 1.30 (m, 12H, C*H*$_3$), 1.36 (s, 9H, Boc-C*H*$_3$), 1.70 (s, 4H, −(C*H*$_2$)$_2$−), 2.96 (m, 1H, ArC*H*$_2$CH), 3.20 (m, 1H, ArC*H*$_2$CH), 3.78 (m, 2H, C*H*$_2$OH), 3.98 (m, 1H, C*H*CH$_2$OH), 4.95 (bd, 1H, J = 8 Hz, −N*H*CO−), 7.24 (s, 1H, Ar*H*), 7.89 (s, 1H, Ar*H*). HRMS: calcd for $C_{22}H_{34}N_2O_5$, 406.2468; found, 406.2467.

Methylamino Alcohol 31. A mixture of 5.66 g (13.9 mmol) of **30** and 500 mg of 10% Pd−C in 500 mL of ethanol was vigorously stirred under 1 atm of H_2 at room temperature for 6 h and then filtered. The filtrate was concentrated, and the residue was crystallized from *n*-hexane to afford 4.84 g of the amine as a colorless powder (92%). Mp: 114−115 °C. Anal. ($C_{22}H_{36}N_2O_3$) C, H, N. A solution of the amine (3.0 g, 7.98 mmol) in 30 mL of THF was added at 0 °C to a mixed anhydride solution (prepared by addition of 2.0 g of formic acid to 4.0 g of acetic anhydride and THF (5 mL) at 0 °C, followed by heating at 60 °C for 2 h at 60−70 °C). After having been stirred for 2.5 h at room temperature, the mixture was concentrated under reduced pressure. The residue was poured into saturated $NaHCO_3$(aq), and the whole was extracted with AcOEt. The extract was washed with water and brine and dried over $MgSO_4$. Concentration and purification by column chromatography on silica gel (hexane/AcOEt, 5:6) afforded 2.90 g of the *N*-formate as a colorless amorphous gum (90%). To a solution of the formate (2.90 g, 7.18 mmol) in 80 mL of THF was added dropwise 21 mL of 1.0 M BH_3 in THF solution at 0 °C, and the mixture was stirred for 3 h at 0 °C. The reaction was quenched by the addition of 10 mL of 10% citric acid, and the whole was extracted with AcOEt. After concentration, the residue was poured into saturated $NaHCO_3$(aq) and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. Concentration and purification by column chromatography on silica gel (hexane/AcOEt, 2:1) gave 2.72 g of the methylamino alcohol **31** as a colorless amorphous gum (97%). ^{1}H NMR ($CDCl_3$): 1.25 (m, 12H, C*H*$_3$), 1.46 (s, 9H, Boc-C*H*$_3$), 1.65 (s, 4H, −(C*H*$_2$)$_2$−), 2.67 (m, 1H, ArC*H*$_2$CH), 2.83 (m, 1H, ArC*H*$_2$CH), 2.87 (s, 3H, N-C*H*$_3$), 3.52 (m, 2H, C*H*$_2$OH), 3.79 (m, 1H, C*H*CH$_2$OH), 5.13 (bs, 1H, −N*H*CO−), 6.70 (s, 1H, Ar*H*), 6.97 (s, 1H, Ar*H*). HRMS: calcd for $C_{23}H_{38}N_2O_3$, 390.2883; found, 390.2896.

Diastereomeric Ester 32. A mixture of 2.0 g (5.12 mmol) of **31**, (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]isovalerate[5d] (4.36 g, 12.8 mmol), and 2,6-lutidine (1.65 g, 15.4 mmol) in 80 mL of CH_2ClCH_2Cl was refluxed for 48 h, and then the solvent was removed by evaporation. The residue was chromatographed on silica gel to give 2.42 g of a diastereomeric mixture of **32** as a colorless amorphous gum (82%).

(−)-BL-V8-23TM (10) and (−)-Epi-BL-V8-23TM (33). A mixture of diastereomeric esters **32** (2.40 g, 4.14 mmol) and 300 mg of 10% Pd−C in 200 mL of ethanol was vigorously stirred under 1 atm of H_2 at room temperature for 2 h and then filtered. The filtrate was concentrated to afford the crude carboxylic acid. The acid and 952 mg (8.28 mmol) of *N*-hydroxysuccinimide were dissolved in 18 mL of CH_3CN, and then a solution of 1.27 g (6.17 mmol) of DCC in 10 mL of CH_3-CN was added at 0 °C with stirring. Stirring was continued for 2 h at room temperature; then the solvent was removed under reduced pressure. The residue was suspended in a small amount of AcOEt, and insoluble urea was filtered off. The filtrate was concentrated and purified by column chromatography on silica gel (hexane/AcOEt, 5:3) to give an activated ester as a colorless amorphous gum. This product was unstable and was used without further purification. Trifluoroacetic acid (20 mL) was added to a solution of the activated ester in 20 mL of CH_2Cl_2 at 0 °C with stirring. The mixture was stirred for 2 h at room temperature; then the solvent was removed under reduced pressure at below 30 °C. The residue was dissolved in 800 mL of AcOEt, and 60 mL of saturated $NaHCO_3$(aq) was added. The mixture was refluxed for 4 h with vigorous stirring. The organic phase was separated, and the aqueous layer was extracted with AcOEt. The combined extract was washed with brine, dried over $MgSO_4$, and concentrated. The crude product was separated by column chromatography to give 532 mg (37%) of (−)-BL-V8-23TM (**10**) and 485 mg (33%) of (−)-epi-BL-V8-23TM (**33**). **10**: colorless needles. Mp: 210−212 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −287.0° (c = 0.46, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.93 (d, 3H, J = 7 Hz, CH(C*H*$_3$)$_2$), 1.07 (d, 3H, J = 7 Hz, CH(C*H*$_3$)$_2$), 1.24 (m, 12H, C*H*$_3$), 1.65 (s, 4H, −(C*H*$_2$)$_2$−), 2.42 (m, 1H, C*H*(CH$_3$)$_2$), 2.78 (m, 1H, ArC*H*$_2$CH−), 2.79 (s, 3H, N-C*H*$_3$), 3.01 (m, 1H, ArC*H*$_2$CH−), 3.50−3.78 (m, 3H, C*H*$_2$OH, CH$_3$-N-C*H*-CO), 4.10 (m, 1H, −C*H*CH$_2$OH), 6.21 (bs, 1H, −N*H*CO−), 6.90 (s, 1H, Ar*H*), 6.98 (s, 1H, Ar*H*). Anal. ($C_{23}H_{36}N_2O_2$) C, H, N. **33**: colorless needles. Mp: 209.5−211 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −153.5° (c = 0.34, $CHCl_3$). HRMS: calcd for $C_{22}H_{36}N_2O_2$, 372.2787; found, 372.2763.

7-Bromo-3,4-dihydro-4,4-dimethyl-1(2*H*)-naphthalenone (39). A solution of 4,4-dimethyl-γ-butyrolactone (20.45 g, 0.179 mol) in dry benzene (30 mL) was added over 45 min to a stirred mixture of $AlCl_3$ (71.5 g, 0.536 mol) and dry benzene (120 mL) held at 5 °C. The mixture was warmed slowly to 90−100 °C and held at that temperature for 2.5 h. Decomposition with ice and HCl gave a benzene layer, which was washed successively with 2 N HCl, water, and saturated $NaHCO_3$(aq), then dried ($MgSO_4$), and concentrated. Distillation of the residue through a Vigreux column gave 22.32 g (72%) of 3,4-dihydro-4,4-dimethyl-1(2*H*)-naphthalenone[31] as a colorless liquid. Bp: 120 °C (7 mmHg). This ketone (22.32 g)

was added dropwise to a stirred mixture of $AlCl_3$ (42.47 g, 0.318 mol) and CH_2Cl_2 (10 mL), followed by similar addition of bromine (8 mL).[32] The mixture was vigorously stirred for 30 min at 80 °C. After cooling, the mixture was acidified with 6 N HCl at 0 °C and extracted with ether. The organic phase was washed with water and saturated $NaHCO_3$(aq), dried (Na_2SO_4), and concentrated. Distillation afforded 21.17 g (65%) of 7-bromo-3,4-dihydro-4,4-dimethyl-1(2*H*)-naphthalenone (**39**) as colorless cubes. Bp: 110 °C (0.3 mmHg). Mp: 56−57 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.38 (s, 6H, C*H*$_3$), 2.01 (t, 2H, *J* = 8.2 Hz, −COCH$_2$C*H*$_2$−), 2.73 (t, 2H, *J* = 8.2 Hz, −COC*H*$_2$CH$_2$−), 7.31 (d, 1H, *J* = 8.4 Hz, Ar*H*), 7.62 (dd, 1H, *J* = 2.2, 8.4 Hz, Ar*H*), 8.13 (dd, 1H, *J* = 1.5, 2.2 Hz, Ar*H*). Anal. ($C_{12}H_{13}OBr$) C, H, N.

6-Bromo-1,1-dimethyl-1,2,3,4-tetahydronaphthalene (40). A solution of **39** (11.52 g, 45.5 mmol) in CF_3COOH (50 mL) was added to triethylsilane (12.77 g, 0.11 mol) at 0 °C with stirring.[33] The mixture was stirred for 2 h at 0 °C and then for 6 h at room temperature. It was poured into saturated $NaHCO_3$(aq) and extracted with hexane. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated. Distillation afforded 21.17 g (65%) of 6-bromo-1,1-dimethyl-1,2,3,4-tetrahydronaphthalene (**40**) as a colorless liquid. Bp: 88−89 °C (1 mmHg). ^{1}H NMR ($CDCl_3$): 1.38 (s, 6H, C*H*$_3$), 1.64 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.78 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 2.73 (t, 2H, *J* = 6.4 Hz, ArC*H*$_2$CH$_2$CH$_2$), 7.18 (m, 2H, Ar*H*), 7.24 (dd, 1H, *J* = 2.2, 8.4 Hz, Ar*H*). HRMS: calcd for $C_{12}H_{15}{}^{79}Br$, 238.0358; found, 238.0347.

1,1-Dimethyl-6-(hydroxymethyl)-1,2,3,4-tetrahydronaphthalene (41). A stirred solution of **40** (7.98 g, 33.4 mmol) in dry ether (80 mL) was treated with 1.6 M *n*-BuLi in hexanes (25 mL, 40 mmol) at room temperature. The mixture was stirred for 20 min at room temperature; then 2.6 mL of dimethylformamide was added at −20 °C. Stirring was continued at 0 °C for 20 min; then the reaction was quenched by the addition of a small amount of water, and the mixture was poured into 2 N HCl. The mixture was extracted with ether and washed with water and brine. After drying ($MgSO_4$), the solution was concentrated to give 1,1-dimethyl-1,2,3,4-tetrahydro-6-naphthalenaldehyde. The aldehyde was dissolved in methanol (180 mL), and $NaBH_4$ (2.28 g) was added at 0 °C, with stirring. After stirring for 30 min at 0 °C, the mixture was extracted with ether. The organic phase was washed with brine and dried ($MgSO_4$). Concentration and purification by column chromatography on silica gel (hexane/CH_2Cl_2, 1:0 then 1:3) afforded 6.03 g (95%) of 1,1-dimethyl-6-(hydroxymethyl)-1,2,3,4-tetrahydronaphthalene (**41**) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.28 (s, 6H, C*H*$_3$), 1.66 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.81 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 2.77 (t, 2H, *J* = 6.4 Hz, ArC*H*$_2$CH$_2$CH$_2$), 4.62 (s, 2H, ArC*H*$_2$OH), 7.06 (s, 1H, Ar*H*), 7.15 (d, 1H, *J* = 8.1 Hz, Ar*H*), 7.33 (d, 1H, *J* = 8.1 Hz, Ar*H*). HRMS: calcd for $C_{13}H_{18}O$, 190.1358; found, 190.1360.

1,1-Dimethyl-6-(bromomethyl)-1,2,3,4-tetrahydronaphthalene (42). Triphenylphosphine (13.22 g, 50.4 mmol) was added as a bolus to a stirred solution of **41** (6.40 g, 33.6 mmol) and CCl_4 (13.95 g, 42.1 mmol) in CH_2Cl_2 (50 mL) at 0 °C. After stirring for 15 min at 0 °C, the mixture was concentrated, and ether was added. The precipitate was filtered off, and the filtrate was chromatographed on silica gel (*n*-hexane) to give 1,1-dimethyl-6-(bromomethyl)-1,2,3,4-tetrahydronaphthalene (**42**) as a pale-yellow oil. HRMS: calcd for $C_{13}H_{17}{}^{79}Br$, 252.0513; found, 252.0517.

1,1-Dimethyl-6-(bromomethyl)-7-nitro-1,2,3,4-tetrahydronaphthalene (43). To a solution of **42** (5.6 g, 22.2 mmol) in 12 mL of Ac_2O was added dropwise a mixture of fuming HNO_3 (2 mL), AcOH (2 mL), and Ac_2O (2 mL) at 0 °C with vigorous stirring. After stirring for 20 min at 0 °C, the mixture was poured into ice−water (100 mL) and extracted with AcOEt. The organic phase was washed with saturated water and brine. Drying (Na_2SO_4) and concentration gave a pale-yellow solid. The crude product was chromatographed on silica gel (hexane/CH_2Cl_2, 1:0 then 1:8) to give 2.09 g (32%) of 1,1-dimethyl-6-(bromomethyl)-7-nitro-1,2,3,4-tetrahydronaphthalene (**43**) and the 5-nitro isomer (1.31 g, 19%). ^{1}H NMR ($CDCl_3$): 1.31 (s, 6H, C*H*$_3$), 1.69 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.84 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 2.81 (t, 2H, *J* = 6.4 Hz, ArC*H*$_2$-CH$_2$CH$_2$), 4.80 (s, 2H, ArC*H*$_2$Br), 7.20 (s, 1H, Ar*H*), 8.05 (s, 1H, Ar*H*). HRMS: calcd for $C_{13}H_{16}NO_2{}^{79}Br$, 299.0345; found, 299.0335.

Diester 44. The nitrobenzyl bromide **43** (2.86 g, 9.59 mmol) was converted into the diester **44** by the same method as that used for the preparation of **29**, employing 387 mg (9.68 mmol) of NaH, 2.09 g (9.62 mmol) of diethyl acetamidomalonate, and 30 mL of DMF for 2.5 h at room temperature under an Ar atmosphere. Purification by column chromatography on silica gel (hexane/AcOEt, 4:1) gave 4.04 g of the diester **44** as a colorless powder (97%). Mp: 131−132 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$): 1.28 (m, 12H, C*H*$_3$, 2 × −OCH$_2$C*H*$_3$), 1.66 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.81 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 1.97 (s, 3H, NHCOC*H*$_3$), 2.72 (t, 2H, *J* = 6.4 Hz, ArC*H*$_2$CH$_2$CH$_2$), 3.99 (s, 2H, ArC*H*$_2$CH), 4.24 (m, 4H, 2 × −OC*H*$_2$CH$_3$), 6.45 (bs, 1H, −N*H*CO−), 6.86 (s, 1H, Ar*H*), 7.81 (s, 1H, Ar*H*). Anal. ($C_{22}H_{30}N_2O_7$) C, H, N.

Nitro Alcohol 45. The diester **44** (3.97 g, 9.14 mmol) was converted into the nitro alcohol **45** by the same method as that used for the preparation of **30** from **29**. Purification of the intermediate *N*-Boc nitro monoester by column chromatography on silica gel (*n*-hexane/AcOEt, 2:1) and recrystallization of the nitro alcohol afforded 2.76 g (71%) of **45** as colorless prisms. Mp: 138.5−140 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$): 1.27 (s, 6H, C*H*$_3$), 1.37 (s, 9H, Boc-C*H*$_3$), 1.68 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.82 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 2.78 (t, 2H, *J* = 6.4 Hz, ArC*H*$_2$CH$_2$CH$_2$), 3.00 (m, 1H, ArC*H*$_2$CH), 3.15 (m, 1H, ArC*H*$_2$CH), 3.70 (m, 2H, C*H*$_2$OH), 3.95 (m, 1H, C*H*CH$_2$-OH), 5.03 (bs, 1H, −N*H*CO−), 7.08 (s, 1H, Ar*H*), 7.93 (s, 1H, Ar*H*). Anal. ($C_{20}H_{30}N_2O_5$) C, H, N.

Methylamino Alcohol 46. The procedure was the same as that used for the preparation of **31**, employing 2.76 g (7.30 mmol) of **45**. Purification by column chromatography on silica gel (*n*-hexane/AcOEt, 3:1) afforded 1.97 g (75%) of the methylamino alcohol **46** as a colorless amorphous gum. ^{1}H NMR ($CDCl_3$): 1.28 (s, 3H, C*H*$_3$), 1.29 (s, 3H, C*H*$_3$), 1.46 (s, 9H, Boc-C*H*$_3$), 1.63 (m, 2H, −CH$_2$C*H*$_2$C(CH$_3$)$_2$), 1.76 (m, 2H, ArCH$_2$C*H*$_2$-CH$_2$), 2.64 (m, 3H, ArC*H*$_2$CH$_2$CH$_2$, ArC*H*$_2$CH), 2.79 (m, 1H, ArC*H*$_2$CH), 2.87 (s, 3H, NC*H*$_3$), 3.53 (m, 2H, C*H*$_2$OH), 3.71 (m, 1H, C*H*CH$_2$OH), 5.16 (bs, 1H, −N*H*CO−), 6.71 (s, 1H, Ar*H*), 6.75 (s, 1H, Ar*H*). HRMS: calcd for $C_{21}H_{34}N_2O_3$, 362.2569; found, 362.2581.

Diastereomeric Ester 47. The methylamino alcohol **46** (1.61 g, 4.44 mmol) was converted into the diastereomeric ester **47** by the same method as that used for the preparation of **32**, employing 6.64 g (19.5 mmol) of (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]isovalerate, 1.62 g (15.1 mmol) of 2,6-lutidine, and 50 mL of CH_2ClCH_2Cl for 40 h at refluxing temperature. Purification by column chromatography on silica gel (hexane/AcOEt, 20:1) gave 2.01 g (82%) of the diastereomeric ester **47** as a colorless amorphous gum.

(−)-BL-V8-DM1 (35) and (−)-Epi-BL-V8-DM1 (48). The procedure was the same as that used for the preparation of **10** and **33** employing 1.68 g (3.33 mmol) of **47** via the activated ester. Purification of the activated ester by column chromatography on silica gel (*n*-hexane/AcOEt, 7:1) followed by condensation and separation of **35** and **48** by column chromatography on silica gel (*n*-hexane/AcOEt, 1:2) afforded 343 mg (33%) of (−)-BL-V8-DM1 (**35**) and 310 mg (30%) of (−)-epi-BL-V8-DM1 (**48**). **35**: colorless needles. Mp: 179−181 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −294.0° (*c* = 0.94, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.94 (d, 3H, *J* = 6.6 Hz, CH(C*H*$_3$)$_2$), 1.06 (d, 3H, *J* = 6.6 Hz, CH(C*H*$_3$)$_2$), 1.25 (s, 6H, C*H*$_3$), 1.62 (m, 2H, −CH$_2$C*H*$_2$C-(CH$_3$)$_2$), 1.76 (m, 2H, ArCH$_2$C*H*$_2$CH$_2$), 2.42 (m, 1H, C*H*(CH$_3$)$_2$), 2.64 (t, 2H, *J* = 6.3 Hz, ArC*H*$_2$CH$_2$CH$_2$), 2.79 (s, 3H, N-C*H*$_3$), 2.80 (m, 1H, ArC*H*$_2$CH−), 2.94 (dd, 1H, *J* = 8.4, 16.4 Hz, ArC*H*$_2$CH−), 3.42 (d, 1H, CH$_3$-N-C*H*-CO), 3.52 (dd, 1H, *J* = 8.0, 10.6 Hz, −CHC*H*$_2$OH), 3.70 (dd, 1H, *J* = 4.0, 10.6 Hz, −CHC*H*$_2$OH), 4.26 (m, 1H, −C*H*CH$_2$OH), 6.33 (bs, 1H, −N*H*-CO−), 6.72 (s, 1H, Ar*H*), 7.00 (s, 1H, Ar*H*). Anal. ($C_{21}H_{32}N_2O_2$)

C, H, N. **48**: colorless needles. Mp: 215.5−217 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −154.4° (c = 1.08, $CHCl_3$). Anal. ($C_{21}H_{32}N_2O_2$) C, H, N.

1-(4′-Bromophenyl)-4-methyl-1-penten-3-one (49). To a stirred solution of 4-bromobenzaldehyde (29.45 g, 0.16 mol) and 3-methylbutan-2-one (13.71 g, 0.16 mol) in ethanol was added 10% NaOH(aq) (6 mL). The mixture was stirred at room temperature for 3 h; then water was added, and the whole was extracted with benzene. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated. Distillation gave 1-(4′-bromophenyl)-4-methyl-1-penten-3-one (**49**) as a yellow oil. Bp: 120 °C (1.5 mmHg). HRMS: calcd for $C_{12}H_{13}O^{79}Br$, 252.0150; found, 252.0134.

7-Bromo-1,1-dimethyl-1,2,3,4-tetrahydronaphthalene (50). A solution of **49** (15.99 g, 63.1 mmol) in dry ether (100 mL) was added to a stirred suspension of $LiAlH_4$ (1.55 g) in dry ether (60 mL) at 0 °C under an Ar atmosphere. The mixture was stirred at room temperature for 15 min; then the reaction was quenched by the addition of a small amount of AcOEt, and the mixture was poured into 2 N HCl. The mixture was extracted with AcOEt, and the extract was washed with water and saturated $NaHCO_3$(aq). Drying ($MgSO_4$) and concentration gave the crude alcohol. To the alcohol was added polyphosphoric acid (96 g), and the mixture was heated at 150 °C for 15 min with stirring and then cooled. Water was added, and the whole was extracted with ether. The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated. Distillation gave 7-bromo-1,1-dimethyl-1,2,3,4-tetrahydronaphthalene (**50**) as a colorless oil. Bp: 105 °C (2 mmHg). ^{1}H NMR ($CDCl_3$): 1.26 (s, 6H, CH_3), 1.64 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.80 (m, 2H, $ArCH_2CH_2CH_2$), 2.69 (t, 2H, J = 6.4 Hz, $ArCH_2CH_2CH_2$), 6.90 (d, 1H, J = 8.1 Hz, ArH), 7.16 (dd, 1H, J = 2.2, 8.1 Hz, ArH), 7.42 (d, 1H, J = 8.1 Hz, ArH). HRMS: calcd for $C_{12}H_{15}{}^{79}Br$, 238.0358; found, 238.0368.

1,1-Dimethyl-7-(hydroxymethyl)-1,2,3,4-tetrahydronaphthalene (51). The procedure was the same as that used for the preparation of **41**, employing 9.35 g (39.1 mmol) of **50**. Purification by column chromatography on silica gel (*n*-hexane/CH_2HCl_2, 1:0 then 1:3) afforded 6.90 g (93%) of 1,1-dimethyl-7-(hydroxymethyl)-1,2,3,4-tetrahydronaphthalene (**51**) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.29 (s, 6H, CH_3), 1.67 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.80 (m, 2H, $ArCH_2CH_2CH_2$), 2.76 (t, 2H, J = 6.4 Hz, $ArCH_2CH_2CH_2$), 4.64 (s, 2H, $ArCH_2OH$), 7.04 (d, 1H, J = 7.7 Hz, ArH), 7.07 (dd, 1H, J = 1.8, 7.7 Hz, ArH), 7.32 (s, 1H, ArH). HRMS: calcd for $C_{13}H_{18}O$, 190.1358; found, 190.1356.

1,1-Dimethyl-7-(bromomethyl)-1,2,3,4-tetrahydronaphthalene (52). The procedure was the same as that used for the preparation of **42**, employing 6.85 g (36.0 mmol) of **51**. Purification by column chromatography on silica gel (*n*-hexane) afforded 5.76 g (63%) of 1,1-dimethyl-7-(bromomethyl)-1,2,3,4-tetrahydronaphthalene (**52**) as a colorless oil. HRMS: calcd for $C_{13}H_{17}{}^{79}Br$, 252.0513; found, 252.0507.

Diester 53. A mixture of fuming HNO_3 (2 mL), AcOH (2 mL) and Ac_2O (2 mL) was added dropwise to a solution of **52** (5.75 g, 22.7 mmol) in 12 mL of Ac_2O at 0 °C with vigorous stirring. The mixture was stirred for 20 min at 0 °C, poured into ice−water (100 mL), and extracted with AcOEt. The organic phase was washed with saturated water and brine. Drying (Na_2SO_4) and concentration gave a pale-yellow solid. The crude product was chromatographed on silica gel (hexane/CH_2Cl_2, 1:30) to give a mixture of 1,1-dimethyl-7-(bromomethyl)-6-nitro-1,2,3,4-tetrahydronaphthalene and the 5-nitro isomer (4.97 g, 73%). The mixture of nitrobenzyl bromides (4.96 g, 16.6 mmol) was converted into the diester **53** by the same method as that used for the preparation of **44**, employing 669 mg (16.75 mmol) of NaH, 3.61 g (16.6 mmol) of diethyl acetamidomalonate, and 30 mL of DMF for 2.5 h at room temperature under an Ar atmosphere. Purification by column chromatography on silica gel (hexane/AcOEt, 4:1) gave 2.19 g of the diester **53** as a yellow amorphous gum (30%). ^{1}H NMR ($CDCl_3$): 1.29 (m, 12H, CH_3, 2 × $-OCH_2CH_3$), 1.67 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.82 (m, 2H, $ArCH_2CH_2CH_2$), 1.96 (s, 3H, NHCOCH_3), 2.77 (t, 2H, J = 6.4 Hz, $ArCH_2CH_2CH_2$), 4.04 (s, 2H, $ArCH_2CH$), 4.24 (m, 4H, 2 × $-OCH_2CH_3$), 6.44 (bs, 1H, $-NHCO-$), 7.15 (s, 1H, ArH), 7.55 (s, 1H, ArH). HRMS: calcd for $C_{22}H_{30}N_2O_7$, 434.2053; found, 434.2069.

Nitro Alcohol 54. The diester **53** (2.19 g, 5.04 mmol) was converted into the nitro alcohol **54** by the same method as that used for the preparation of **30** from **29**. Purification of the intermediate *N*-Boc nitro monoester by column chromatography on silica gel (*n*-hexane/AcOEt, 2:1) and column chromatography of the nitro alcohol on silica gel (*n*-hexane/AcOEt, 2:1) afforded 1.12 g (61%) of **54** as a yellow amorphous gum. ^{1}H NMR ($CDCl_3$): 1.29 (s, 6H, CH_3), 1.38 (s, 9H, Boc-CH_3), 1.68 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.82 (m, 2H, $ArCH_2CH_2CH_2$), 2.79 (t, 2H, J = 6.2 Hz, $ArCH_2CH_2CH_2$), 3.01 (m, 1H, $ArCH_2CH$), 3.18 (m, 1H, $ArCH_2CH$), 3.70 (m, 2H, CH_2OH), 3.96 (m, 1H, $CHCH_2OH$), 4.97 (bs, 1H, $-NHCO-$), 7.30 (s, 1H, ArH), 7.67 (s, 1H, ArH). HRMS: calcd for $C_{20}H_{30}N_2O_5$, 378.2155; found, 378.2142.

Methylamino Alcohol 55. The procedure was the same as that used for the preparation of **31**, employing 1.10 g (2.89 mmol) of **54**. Purification by column chromatography on silica gel (*n*-hexane/AcOEt, 2:1) afforded 621 mg (63%) of the methylamino alcohol **55** as a colorless amorphous gum. ^{1}H NMR ($CDCl_3$): 1.26 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.46 (s, 9H, Boc-CH_3), 1.63 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.76 (m, 2H, $ArCH_2CH_2CH_2$), 2.64 (m, 3H,$ArCH_2CH_2CH_2$, $ArCH_2CH$), 2.72 (t, 2H, J = 6.2 Hz, $ArCH_2CH$), 2.82 (m, 1H, $ArCH_2CH$), 2.83 (s, 3H, NCH_3), 3.53 (m, 2H, CH_2OH), 3.71 (m, 1H, $CHCH_2OH$), 5.18 (bs, 1H, $-NHCO-$), 6.36 (s, 1H, ArH), 6.96 (s, 1H, ArH). HRMS: calcd for $C_{21}H_{34}N_2O_3$, 362.2569; found, 362.2567.

Diastereomeric Ester 56. The methylamino alcohol **55** (511 mg, 1.41 mmol) was converted into the diastereomeric ester **56** by the same method as that used for the preparation of **32**, employing 1.08 g (3.18 mmol) of (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]isovalerate, 509 mg (4.75 mmol) of 2,6-lutidine, and 15 mL of CH_2ClCH_2Cl for 40 h at refluxing temperature. Purification by column chromatography on silica gel (hexane/AcOEt, 20:1) gave 654 mg (84%) of the diastereomeric ester **56** as a colorless amorphous gum.

(−)-BL-V8-DM3 (36) and (−)-Epi-BL-V8-DM3 (57). The procedure was the same as that used for the preparation of **10** and **33** employing 612 mg (1.11 mmol) of **56** via the activated ester. Purification of the activated ester by column chromatography on silica gel (*n*-hexane/AcOEt, 7:1), followed by condensation and separation of **36** and **57** by column chromatography on silica gel (*n*-hexane/AcOEt, 1:2), afforded 148 mg (38%) of (−)-BL-V8-DM3 (**36**) and 148 mg (38%) of (−)-epi-BL-V8-DM3 (**57**). **36**: colorless needles. Mp: 186−187.5 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −299.7° (c = 0.86, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.93 (d, 3H, J = 6.6 Hz, $CH(CH_3)_2$), 1.07 (d, 3H, J = 6.6 Hz, $CH(CH_3)_2$), 1.23 (s, 6H, CH_3), 1.62 (m, 2H, $-CH_2CH_2C(CH_3)_2$), 1.76 (m, 2H, $ArCH_2CH_2CH_2$), 2.43 (m, 1H, $CH(CH_3)_2$), 2.68 (t, 2H, J = 6.3 Hz, $ArCH_2CH_2CH_2$), 2.76 (s, 3H, N-CH_3) 2.84 (m, 1H, $ArCH_2CH-$), 2.99 (dd, 1H, J = 8.4, 16.5 Hz, $ArCH_2CH-$), 3.43 (d, 1H, CH_3-N-CH-CO), 3.55 (dd, 1H, J = 8.0, 10.6 Hz, $-CHCH_2OH$), 3.74 (dd, 1H, J = 4.0, 10.6 Hz, $-CHCH_2OH$), 4.27 (m, 1H, $-CHCH_2OH$), 6.24 (bs, 1H, $-NHCO-$), 6.69 (s, 1H, ArH), 6.94 (s, 1H, ArH). Anal. Calcd for $C_{21}H_{32}N_2O_2$: C, 73.22; H, 9.36; N, 8.13. Found: C, 72.94; H, 9.41; N, 8.04. **57**: colorless amorphous gum. $[\alpha]^{22}_D$ −142.4° (c = 1.08, $CHCl_3$). HRMS: calcd for $C_{21}H_{32}N_2O_2$, 344.2464; found, 344.2468.

1-(3-Phenylpropyl)cyclohexanol (58). A solution of 1-bromo-3-phenylpropane (19.9 g, 99.9 mml) in dry ether (30 mL) was added to a stirred mixture of Mg turnings (2.39 g) and dry ether (5 mL) under gentle reflux, under an Ar atmosphere. The mixture was cooled to 0 °C, and a solution of cyclohexanone (9.80 g, 132.4 mmol) in dry ether (20 mL) was added dropwise with stirring. Stirring was continued at room temperature for 1 h; then the mixture was poured into 2 N HCl and extracted with ether. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Distillation gave 15.17 g (70%) of 1-(3-phenylpropyl)cyclohexanol (**58**) as a colorless oil. Bp: 152 °C (3 mmHg). ^{1}H NMR ($CDCl_3$): 1.22−

1.60 (m, 12H, aliphatic), 1.69 (m, 2H, $ArCH_2CH_2CH_2$), 2.61 (m, 2H, $ArCH_2CH_2CH_2$), 7.15−7.19 (m, 3H, Ar*H*), 7.25−7.29 (m, 2H, Ar*H*). HRMS: calcd for $C_{15}H_{22}O$-H_2O, 200.1565; found, 200.1589.

3′,4′-Dihydrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (59). The alcohol **58** (15.0 g, 68.8 mmol) was added to 50 mL of 85% H_2SO_4 at 0 °C over a period of 20 min with stirring.[34] After stirring at room temperature for 30 min, the mixture was poured into ice−water and extracted with hexane. The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Distillation gave 9.36 g (68%) of the spiro-[cyclohexane-1,1′-tetralin] **59** as a colorless oil. Bp: 111 °C (2 mmHg). ^{1}H NMR ($CDCl_3$): 1.22−1.76 (m, 12H, aliphatic), 1.82 (m, 2H, $ArCH_2CH_2CH_2$), 2.75 (t, 2H, J = 6.2 Hz, $ArCH_2CH_2$-CH_2), 7.05 (m, 2H, Ar*H*), 7.16 (dt, 1H, J = 2.2, 6.2 Hz, Ar*H*), 7.41 (d, 1H, J = 8.1 Hz, Ar*H*). HRMS: calcd for $C_{15}H_{20}$, 200.1565; found, 200.1561.

6′-Bromo-3′,4′-dihydrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (60). A solution of CrO_3 (39.59 g) in AcOH−H_2O (220 mL, 10:1) was added to a stirred solution of **59** (21.5 g, 107.3 mol) in AcOH (1000 mL) over a period of 1.5 h.[35] The mixture was stirred for 1.5 h, 2-propanol (2 mL) was added, and the whole was concentrated in vacuo. The residue was taken up in water and extracted with hexane. The organic phase was washed with water and brine, dried ($MgSO_4$), and concentrated. Purification by column chromatography (hexane/CH_2Cl_2, 2:1) gave 14.3 g (62%) of the 4′-ketone. The ketone (19.14 g, 89.3 mmol) was added dropwise to a mixture of $AlCl_3$ (29.42 g, 220.6 mmol) and CH_2Cl_2 (10 mL) at room temperature with vigorous stirring. Then 5.5 mL of bromine was added. The mixture was stirred for 1 h, poured into 6 N HCl, and extracted with ether. The organic phase was washed with water and saturated $NaHCO_3$(aq), dried (Na_2SO_4), and concentrated. Purification by column chromatography (hexane/CH_2Cl_2, 3:1) gave 14.95 g (57%) of the 6′-bromo 4′-ketone as colorless needles. Mp: 115.5−116.5 °C (*n*-hexane) Anal. ($C_{15}H_{17}OBr$) C, H. A solution of the bromo ketone (14.68 g, 50.1 mmol) in CF_3COOH (50 mL) was added to triethylsilane (17.64 g, 0.152 mol) at 0 °C with stirring.[31] The mixture was stirred for 1 h at 0 °C and for 18 h at room temperature, then poured into saturated $NaHCO_3$(aq) and extracted with hexane. The organic phase was washed with water and brine, dried ($MgSO_4$) and concentrated. Purification by column chromatography (*n*-hexane) gave 13.92 g (100%) of 6′-bromo-3′,4′-dihydrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (**60**) as a colorless oil. ^{1}H NMR ($CDCl_3$): 1.26−1.82 (m, 14H, aliphatic), 2.72 (t, 2H, J = 6.8 Hz, $ArCH_2CH_2$), 7.18−7.29 (m, 3H, Ar*H*). HRMS: calcd for $C_{15}H_{19}{}^{79}Br$, 278.0671, found, 278.0673.

6′-(Bromomethyl)-3′,4′-dihydrospiro[cyclohexane-1,1′-(2*H*)-naphthalene] (61). The procedure was the same as that used for the preparation of **42** from **40**, employing 11.82 g (42.3 mmol) of **60**. Purification by column chromatography on silica gel (*n*-hexane) afforded 8.70 g (70%) of 6′-(bromomethyl)-3′,4′-dihydrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (**61**) as colorless prisms. Mp: 55−57 °C (methanol). ^{1}H NMR ($CDCl_3$): 1.27−1.82 (m, 14H, aliphatic), 2.73 (t, 2H, J = 6.2 Hz, $ArCH_2CH_2$), 4.45 (s, 2H, $ArCH_2Br$), 7.07 (s, 1H, Ar*H*), 7.18 (dd, 1H, J = 2.0, 8.2 Hz, Ar*H*), 7.38 (d, 1H, J = 8.2 Hz, Ar*H*). Anal. ($C_{16}H_{21}Br$) C, H.

6′-(Bromomethyl)-3′,4′-dihydro-7′-nitrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (62). The procedure was the same as that used for the preparation of **43** from **42**, employing 8.61 g (29.4 mmol) of **61**. Purification by column chromatography on silica gel (*n*-hexane) afforded 4.02 g (40%) of 6′-(bromomethyl)-3′,4′-dihydro-7′-nitrospiro[cyclohexane-1,1′-(2*H*)-naphthalene] (**62**) as pale-yellow prisms and its 5′-nitro isomer (3.08 g, 31%). Mp: 90.5−91.5 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$): 1.26−1.85 (m, 14H, aliphatic), 2.79 (t, 2H, J = 6.2 Hz, $ArCH_2CH_2$), 4.80 (s, 2H, $ArCH_2Br$), 7.20 (s, 1H, Ar*H*), 8.15 (s, 1H, Ar*H*). Anal. ($C_{16}H_{20}NO_2Br$) C, H, N.

Diester 63. The nitrobenzyl bromide **62** (3.94 g, 11.65 mmol) was converted into the diester **63** by the same method as that used for the preparation of **29**, employing 466 mg (11.65 mmol) of NaH, 2.54 g (11.69 mmol) of diethyl acetamidomalonate, and 30 mL of DMF for 2.5 h at room temperature under an Ar atmosphere. Purification by column chromatography on silica gel (hexane/AcOEt, 4:1) gave 5.18 g of the diester **63** as colorless prisms (94%). Mp: 169−170 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$): 1.25−1.83 (m, 20H, aliphatic, 2 × $-OCH_2CH_3$), 1.97 (s, 3H, $NHCOCH_3$), 2.70 (t, 2H, J = 6.4 Hz, $ArCH_2CH_2CH_2$), 3.99 (s, 2H, $ArCH_2CH$), 4.23 (m, 4H, 2 × $-OCH_2CH_3$), 6.45 (bs, 1H, −N*H*CO−), 6.86 (s, 1H, Ar*H*), 7.91 (s, 1H, Ar*H*). Anal. ($C_{25}H_{34}N_2O_7$) C, H, N.

Nitro Alcohol 64. The diester **63** (5.05 g, 10.64 mmol) was converted into the nitro alcohol **64** by the same method as that used for the preparation of **30** from **29**. Purification of the intermediate *N*-Boc nitro monoester by column chromatography on silica gel (*n*-hexane/AcOEt, 8:1) and column chromatography of the nitro alcohol on silica gel (*n*-hexane/AcOEt, 2:1) afforded 3.75 g (86%) of **64** as pale-yellow plates. Mp: 152−153 °C (hexane/AcOEt). ^{1}H NMR ($CDCl_3$): 1.24−1.84 (m, 23H, aliphatic, Boc-CH_3), 2.76 (t, 2H, J = 6.4 Hz, $ArCH_2CH_2$-CH_2), 3.00 (m, 1H, $ArCH_2CH$), 3.15 (m, 1H, $ArCH_2CH$), 3.69 (m, 2H, CH_2OH), 3.95 (m, 1H, $CHCH_2OH$), 5.03 (bs, 1H, −N*H*CO−), 7.07 (s, 1H, Ar*H*), 8.03 (s, 1H, Ar*H*). Anal. ($C_{23}H_{34}N_2O_5$) C, H, N.

Methylamino Alcohol 65. The procedure was the same as that used for the preparation of **31**, employing 2.67 g (6.38 mmol) of **64**. Purification of the methylamino alcohol by column chromatography on silica gel (*n*-hexane/AcOEt, 3:1) afforded 1.77 g (64%) of the methylamino alcohol **65** as a colorless amorphous gum. ^{1}H NMR ($CDCl_3$): 1.24−1.77 (m, 23H, aliphatic, Boc-CH_3), 2.62 (m, 3H, $ArCH_2CH_2CH_2$, $ArCH_2$-CH), 2.78 (m, 1H, $ArCH_2CH$), 2.88 (s, 3H, NCH_3), 3.53 (m, 2H, CH_2OH), 3.71 (m, 1H, $CHCH_2OH$), 5.13 (bs, 1H, −N*H*CO−), 6.37 (s, 1H, Ar*H*), 6.74 (s, 1H, Ar*H*). HRMS: calcd for $C_{24}H_{38}N_2O_3$, 402.2883; found, 402.2883.

Diastereomeric Ester 66. The methylamino alcohol **65** (1.66 g, 4.12 mmol) was converted into the diastereomeric ester **66** by the same method as that used for the preparation of **32**, employing 3.76 g (11.1 mmol) of (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]isovalerate, 1.54 g (14.4 mmol) of 2,6-lutidine, and 50 mL of CH_2ClCH_2Cl for 40 h at refluxing temperature. Purification by column chromatography on silica gel (hexane/AcOEt, 20:1) gave 2.02 g (83%) of the diastereomeric ester **66** as a colorless amorphous gum.

(−)-BL-V8-SP1 (37) and (−)-Epi-BL-V8−SP1 (67). The procedure was the same as that used for the preparation of **10** and **33**, employing 1.94 g (3.28 mmol) of **66** via the activated ester. Purification of the activated ester by column chromatography on silica gel (*n*-hexane/AcOEt, 7:1), followed by condensation and separation of **37** and **67** by column chromatography on silica gel (*n*-hexane/AcOEt, 1:2), afforded 477 mg (38%) of (−)-BL-V8-SP1 (**37**) and 357 mg (28%) of (−)-epi-BL-V8-SP1 (**67**). **37**: colorless needles. Mp: 198−199 °C (CH_2Cl_2/hexane). $[\alpha]^{22}{}_D$ −297.9° (c = 0.98, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 0.95 (d, 3H, J = 6.6 Hz, $CH(CH_3)_2$), 1.07 (d, 3H, J = 6.6 Hz, $CH(CH_3)_2$), 1.26−1.80 (m, 14H, aliphatic), 2.44 (m, 1H, $CH(CH_3)_2$), 2.64 (t, 2H, J = 6.2 Hz, $ArCH_2CH_2CH_2$), 2.84 (s, 3H, N-CH_3), 2.84 (m, 1H, $ArCH_2CH$−), 2.93 (dd, 1H, J = 8.4, 16.5 Hz, $ArCH_2CH$−), 3.43 (d, 1H, J = 8.1 Hz, CH_3-N-CH-CO), 3.51 (dd, 1H, J = 7.9, 10.8 Hz, $-CHCH_2OH$), 3.70 (dd, 1H, J = 3.9, 10.8 Hz, $-CHCH_2OH$), 4.29 (m, 1H, $-CHCH_2$-OH), 6.42 (bs, 1H, −N*H*CO−), 6.72 (s, 1H, Ar*H*), 7.09 (s, 1H, Ar*H*). Anal. ($C_{24}H_{36}N_2O_2$) C, H, N. **67**: colorless needles. $[\alpha]^{22}{}_D$ −133.7° (c = 1.43, $CHCl_3$). Anal. ($C_{24}H_{36}N_2O_2$) C, H, N.

1-(3-Phenylpropyl)-4,4-dimethylcyclohexanol (68). The procedure was the same as that used for the preparation of **58**, employing 9.80 g (77.7 mmol) of 4,4-dimethylcyclohexanone. Purification by distillation gave 34.44 g (66%) of 1-(3-phenylpropyl)-4,4-dimethylcyclohexanol (**68**) as a colorless oil. Bp: 146 °C (1 mmHg). HRMS: calcd for $C_{17}H_{26}O{\cdot}H_2O$, 228.1878; found, 228.1876.

3′,4′-Dihydro-4,4-dimethylspiro[cyclohexane-1,1′(2*H*)-naphthalene] (69). The procedure was the same as that used for the preparation of **59**, employing 34.43 g (0.14 mol) of **68**. Purification by distillation gave 23.78 g (74%) of the spiro-

[cyclohexane-1,1′-tetralin] **69** as a colorless oil. Bp: 148 °C (4 mmHg). HRMS: calcd for $C_{17}H_{24}$, 228.1878; found, 228.1875.

6′-Bromo-3′,4′-dihydro-4,4-dimethylspiro[cyclohexane-1,1′(2*H*)-naphthalene] (70). The spiro[cyclohexane-1,1′-tetralin] **69** (23.75 g, 0.104 mol) was converted into the bromide **70** by the same method as that used for the preparation of **60** from **59**. Purification of the final product by column chromatography (hexane) gave 17.84 g (57%) of 6′-bromo-3′,4′-dihydro-4,4-dimethylspiro[cyclohexane-1,1′(2*H*)-naphthalene] (**70**) as colorless prisms. Mp: 47−48 °C (*n*-hexane). Anal. ($C_{17}H_{23}$Br) C, H.

6′-(Bromomethyl)-3′,4′-dihydro-4,4-dimethylspiro[cyclohexane-1,1′(2H)-naphthalene] (71). The procedure was the same as that used for the preparation of **42** from **40**, employing 12.15 g (39.5 mmol) of **70**. Purification by column chromatography on silica gel (*n*-hexane) afforded 8.90 g (70%) of 6′-(bromomethyl)-3′,4′-dihydro-4,4-dimethylspiro[cyclohexane-1,1′(2*H*)-naphthalene] (**71**) as colorless needles. Mp: 68−69 °C (methanol). Anal. Calcd for $C_{18}H_{25}Br$: C, 67.29; H, 7.84. Found: C, 67.05; H, 7.81.

6′-(Bromomethyl)-3′,4′-dihydro-4,4-dimethyl-7′-nitrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (72). The procedure was the same as that used for the preparation of **43** from **42**, employing 8.67 g (27.0 mmol) of **71**. Purification by column chromatography on silica gel (*n*-hexane/CH_2Cl_2) afforded 3.61 g (37%) of 6′-(bromomethyl)-3′,4′-dihydro-4,4-dimethyl-7′-nitrospiro[cyclohexane-1,1′(2*H*)-naphthalene] (**72**) as pale-yellow prisms and its 5′-nitro isomer (2.68 g, 27%). Mp: 82−83 °C (*n*-hexane). Anal. ($C_{18}H_{24}NO_2Br$) C, H, N.

Diester 73. The nitrobenzyl bromide **72** (3.38 g, 9.23 mmol) was converted into the diester **73** by the same method as that used for the preparation of **29**, employing 371 mg (9.27 mmol) of NaH, 2.0 g (9.23 mmol) of diethyl acetamidomalonate, and 25 mL of DMF for 2 h at room temperature under an Ar atmosphere. Purification by column chromatography on silica gel (hexane/AcOEt, 4:1) gave 4.66 g of the diester **73** as colorless prisms (100%). Mp: 131−132 °C (hexane/AcOEt). Anal. ($C_{27}H_{38}N_2O_7$) C, H, N.

Nitro Alcohol 74. The diester **73** (4.05 g, 8.07 mmol) was converted into the nitro alcohol **74** by the same method as that used for the preparation of **30** from **29**. Purification of the intermediate *N*-Boc nitro monoester by column chromatography on silica gel (*n*-hexane/AcOEt, 8:1) and column chromatography of the nitro alcohol on silica gel (*n*-hexane/AcOEt, 2:1) afforded 2.82 g (84%) of the nitro alcohol **74** as a pale-yellow powder. Mp: 116−117 °C (*n*-hexane). HRMS: calcd for $C_{25}H_{38}N_2O_5$, 446.2781; found, 446.2768.

Methylamino Alcohol 75. The procedure was the same as that used for the preparation of **31**, employing 1.97 g (4.39 mmol) of **74**. Purification of the methylamino alcohol by column chromatography on silica gel (*n*-hexane/AcOEt, 3:1) afforded 980 mg (55%) of the methylamino alcohol **75** as a colorless amorphous gum. HRMS: calcd for $C_{26}H_{42}N_2O_3$, 430.3195; found, 430.3198.

Diastereomeric Ester 76. The methylamino alcohol **75** (920 mg, 2.14 mmol) was converted into the diastereomeric ester **76** by the same method as that used for the preparation of **32**, employing 1.94 g (5.70 mmol) of (*R*)-benzyl 2-[[(trifluoromethyl)sulfonyl]oxy]isovalerate, 798 mg (7.46 mmol) of 2,6-lutidine, and 30 mL of CH_2ClCH_2Cl for 40 h at refluxing temperature. Purification by column chromatography on silica gel (hexane/AcOEt, 20:1) gave 826 mg (62%) of the diastereomeric ester **76** as a colorless amorphous gum.

BL-V8-SP3 (38) and Epi-BL-V8-SP3 (77). The procedure was the same as that used for the preparation of **10** and **33**, employing 797 mg (1.28 mmol) of **76** via the activated ester. Purification of the activated ester by column chromatography on silica gel (*n*-hexane/AcOEt, 7:1), followed by condensation and separation of **38** and **77** by column chromatography on silica gel (*n*-hexane/AcOEt, 1:2), afforded 211 mg (40%) of (−)-BL-V8-SP3 (**38**) and 145 mg (27%) of (−)-epi-BL-V8-SP3 (**77**). **38**: colorless needles. Mp: 207−209 °C (CH_2Cl_2/hexane). $[\alpha]^{22}_D$ −252.9° (c = 1.13, $CHCl_3$). ^{1}H NMR ($CDCl_3$):: 0.95 (d, 3H, J = 6.6 Hz, CH(CH_3)$_2$), 0.96 (s, 3H, −CH_3), 1.05 (s, 3H, −CH_3), 1.07 (d, 3H, J = 6.6 Hz, CH(CH_3)$_2$), 1.05 (s, 3H, −CH_3), 1.15−1.93 (m, 12H, aliphatic), 2.41 (m, 1H, CH(CH$_3$)$_2$), 2.63 (t, 2H, J = 6.0 Hz, ArCH_2CH$_2$CH$_2$), 2.79 (m, 1H, ArCH_2CH−), 2.80 (s, 3H, N-CH_3), 2.92 (dd, 1H, J = 8.4, 16.5 Hz, ArCH_2-CH−), 3.42 (d, 1H, J = 8.1 Hz, CH$_3$-N-CH-CO), 3.51 (dd, 1H, J = 7.9, 10.8 Hz, −CHCH_2OH), 3.69 (dd, 1H, J = 3.7, 10.8 Hz, −CHCH_2OH), 4.31 (m, 1H, −CHCH$_2$OH), 6.63 (bs, 1H, −NHCO−), 6.72 (s, 1H, ArH), 7.11 (s, 1H, ArH). Anal. Calcd for $C_{26}H_{40}N_2O_2$: C, 75.68; H, 9.77; N, 6.79. Found: C, 75.66; H, 9.81; N, 6.89. **77**: colorless amorphous gum. $[\alpha]^{22}_D$ −113.7° (c = 1.11, $CHCl_3$). HRMS: calcd for $C_{26}H_{40}N_2O_2$, 412.3090; found, 412.3087.

HL-60 Growth-Inhibitory Test. The HL-60 cells were provided by Prof. F. Takaku (Faculty of Medicine, University of Tokyo) and were maintained in continuous suspension culture. The cells were cultured in plastic flasks in RPMI1640 medium, supplemented with 5% fetal bovine serum (FBS, not delipidized) and antibiotics (penicillin G and streptomycin), in a humidified atmosphere of 5% CO_2 in air at 37 °C. Test compounds were dissolved in ethanol at 2 mM and added to the cells, which were seeded at about 8×10^4 cells/mL. The final ethanol concentration was kept below 0.5%. The concentration of ethanol did not affect the HL-60 cell growth by comparison with control (ethanol-free) experiment. Cell growth was determined from a count of the cell number after 4 days.

Inhibition of Specific [^{3}H]PDBu Binding to PKCδ. Assay of the inhibition of [^{3}H]PDBu binding (K_d = 2.9 nM) to recombinant PKCδ and the determination of inhibition constants from binding curve IC_{50} values were done as previously described.[23] K_i values of the testing compounds are described below. The number of repetitions for each experiment is noted in parentheses: **1**, 0.32 ± 0.043 nM (2); **3**, 80 ± 29 nM (2); **4**, 1700 ± 30 nM (2); **5**, 5.9 ± 1.7 nM (4); **8**, 3.0 ± 0.45 nM (3); **9**, 32 ± 8.7 nM (2); **10**, 5100 ± 730 nM (3); **35**, 65 ± 0.6 nM (2); **36**, 1700 ± 220 nM (2); **37**, 19 ± 1.1 nM (2); **38**, 14 ± 0.94 nM (3).

Modeling and Docking Simulation. The most stable docking models for all compounds were estimated by using the automatic docking program ADAM version 3.4. Energy minimization of the docking models was done by the SANDER module of the AMBER 4.1 program package. Energy decomposition analyses of the final docking models were done by the ANAL module of AMBER 4.1. The structural data used for docking were prepared as follows. The atomic coordinates of phorbol 13-acetate were constructed by adding an acetyl group to 13-OH of the crystal structure of phorbol.[36] The structure of the twist form of teleocidin B-4 (**1**) was taken from that in the crystal.[37] The structure of the twist form of (−)-IL-V (**3**) was modeled by removing the alkyl moieties on the indole ring of the teleocidin B-4 structure. The structure of (−)-BL-V8 (**4**) was obtained computationally, because neither the molecule nor its congeners could be crystallized. Starting from a structure constructed by conventional modeling procedures, stable structures were searched by high-temperature molecular dynamics calculation using the AMBER program.[27] The stable conformers were selected from 2000 snapshot structures sampled from a 100-ps MD calculations (dielectric constant: $\epsilon = 4r$) at 3000 K, followed by energy minimization. The force-field parameters used for both MD and energy-minimization calculations were those which we previously employed to reproduce the conformational equilibrium between twist and sofa forms observed in (−)-IL-V.[9a] As the most stable conformer was very similar to the twist form of (−)-IL-V having the *cis*-amide structure, this was used for docking as the putative active conformation. The structures of (−)-BL-V8-23TM (**10**) and BL-V8-DM1 (**35**) were modeled by adding the corresponding alkyl moieties to the (−)-BL-V8 structure. In all compounds, hydrogen atoms were computationally located or relocated at appropriate positions. The atomic charges on atoms of all molecules were calculated by the Mulliken analysis method based on molecular orbital calculation using the MNDO method in the MOPAC program.[38] The structure of the PKCδ Cys2 domain was obtained from the complexed crystal structure of the PKCδ Cys2 domain and phorbol 13-

acetate (Protein Data Bank code 1PTR)[13] by removing the phorbol 13-acetate molecule. All hydrogen atoms that are missing in the crystal structure but should exist in the protein structure were computationally located at appropriate positions and optimized further by the AMBER program.

Supporting Information Available: ^{1}H NMR data for the compounds including synthetic intermediates and the epimers of the target molecules (9 pages). Ordering information is given on any current masthead page.

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