New Amide-Bearing Benzolactam-Based Protein Kinase C Modulators Induce Enhanced Secretion of the Amyloid Precursor Protein Metabolite sAPPα

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Protein kinase C (PKC) is known to participate in the processing of the amyloid precursor protein (APP). Abnormal processing of APP through the action of the β- and γ-secretases leads to the production of the 39−43 amino acid Aβ fragment, which is neurotoxic and which is believed to play an important role in the etiology of Alzheimer's disease. PKC activation enhances α-secretase activity, which results in a decrease of the amyloidogenic products of β-secretase. In this article, we describe the synthesis of 10 new benzolactam V8 based PKC activators having side chains of varied saturation and lipophilicity linked to the aromatic ring through an amide group. The K_i values measured for the inhibition of phorbol ester binding to PKCα are in the nanomolar range and show some correlation with their lipophilicity. Compounds **5g** and **5h** show the best binding affinity among the 10 benzolactams that were synthesized. By use of a cell line derived from an AD patient, significant enhancement of sAPPα secretion was achieved at 1 μM concentration for most of the compounds studied and at 0.1 μM for compounds **5e** and **5f**. At 1 μM the enhancement of sAPPα secretion for compounds **5c**−**h** is higher than that observed for the control compound 8-(1-decynyl)benzolactam (BL). Of interest is the absence of activity found for the highly lipophilic ligand **5i**, which has a K_i of 11 nM. On the other hand, its saturated counterpart **5j**, which possesses a comparable K_i and ClogP, retains activity in the secretase assay. In the hyperplasia studies, **5f** showed a modest response at 100 μg and **5e** at 300 μg, suggesting that **5f** was approximately 30-fold less potent than the PKC activator mezerein and 100-fold less potent than TPA. **5e** was approximately 3-fold less active than **5f**. On the basis of the effect of unsaturation for other potent PKC ligands, we would predict that **5e** would retain biological activity in most assays but would show a marked loss of tumor-promoting activity. Compound **5e** thus becomes a viable candidate compound in the search for Alzheimer's therapeutics capable of modulating amyloid processing.

Introduction

Protein kinase C (PKC) represents a family of at least 12 serine/threonine kinases that are involved in signal transduction in response to a host of hormonal, neuronal, and growth factor stimuli.[1−4] Differences in structure as well as substrate requirements have led to the general classification of the isoforms into three groups, termed the classical, novel, and atypical PKCs. The N-terminal regulatory domain of the classical and novel PKCs contains a conserved C1 domain comprising two cysteine-rich zinc fingers that bind to the natural PKC activator diacylglycerol (DAG) as well as to certain natural products such as the phorbol esters and the indolactams. Normally, the classical and novel PKC isozymes are cytosolic and are translocated to the membrane upon interaction with Ca^{2+}, whereby they are activated through interaction with membrane phospholipids and DAG.[5,6] PKC has been shown to play a major role in a variety of disease states including diabetes, heart disease, cancer, and Alzheimer's disease, and thus, both isoform-selective activators and inhibitors may provide novel leads in the development of therapeutic agents. Bryostatin, a macrocyclic lactone from the bryozoan *Bugula neritina*, binds to the DAG regulatory site of PKC, yet it is not a tumor promoter but acts as an antineoplastic agent that has been used to treat murine melanoma.[7] In view of the fact that bryostatin-1 is in clinical evaluation as an anticancer agent, it is likely that other activators of PKC that are not tumor promoters will find use in treating neoplastic conditions.

There has been a growing interest in studying the relationship between Alzheimer's disease (AD) and PKC. Numerous researchers have found defective PKC in brains[8−13] and peripheral tissues of AD patients.[14−17] PKC is known to participate in the processing of the amyloid precursor protein (APP),[18] including the amyloidogenic fragments Aβ 1−40 and 1−42. There are three well-characterized proteolytic routes of amyloid processing, all of which seem to occur in normal and pathological states. α-Secretase,[19] a metalloprotease neither completely identified nor characterized, cleaves APP within the Aβ sequence (between residues K and L),

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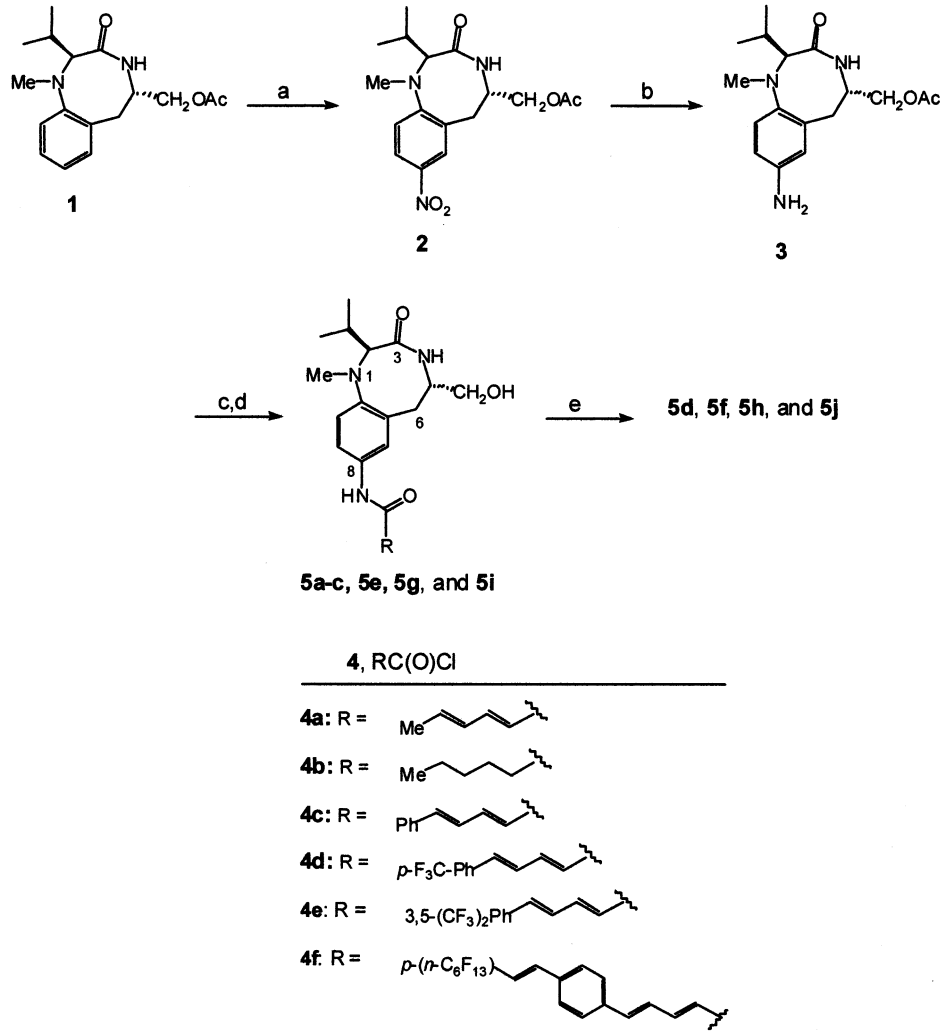
Scheme 1[a]



[a] Reagents and conditions: (a) HNO_3, Ac_2O, 5 min, 0 °C, 92%; (b) Pd/C, H_2, 3 h, room temp; (c) RCOCl, Et_3N, 4 h, room temp; (d) Na_2CO_3, MeOH, 1.5 h, room temp; (e) Pd/C, H_2, 3 h, room temp, 90%.

generating a large secreted fragment termed sAPPα and a smaller intracellular fragment P3. Both fragments are of no pathological significance. The process leading to Aβ formation or "amyloidogenic" processing and ultimately Aβ deposition and plaque formation in AD involves the participation of another enzyme, β-secretase.[20,21] This enzyme cleaves just outside the Aβ sequence, leaving a large membrane-bound fragment that is later cleaved by a yet to be identified γ-secretase[22] at position 711 or 713 and thereby generating Aβ 1−40 or 1−42, respectively. In-depth description and discussion of APP processing can be found elsewhere.[23−26] PKC activators belonging to the phorbol family have been shown to dramatically enhance α-secretase activity, thus leading to enhanced secretion of sAPPα.[18,27−32] Because the secretases seem to compete for a single pool of APP,[33,34] enhancing α-secretase activity would result in a decrease of amyloidogenic products of β-secretase. In fact, a direct decrease of Aβ 1−40 has been documented after PKC activation.[27,28] A few other studies fail to show the direct relationship between enhanced sAPPα and decreased Aβ 1−40. However, they still show that PKC activation has "positive" effects as reflected in an increase in the nonpathogenic sAPPα or a decrease in β-amyloid.[35−37] We have shown that 8-(1-decynyl)-benzolactam[38] restores an otherwise abnormal K channel activity in fibroblasts from AD patients, linked to enhanced translocation of PKCα.[39,40] Furthermore, the same compound was later shown to significantly enhance sAPPα secretion.[41]

In continuation of these studies, we have now examined the effect of altering the side chain appendage of the core benzolactam through introduction of both saturated and unsaturated amide residues. Interestingly, as described herein, we find that the majority of these ligands are more active than the 8-(1-decynyl)-benzolactam in promoting the non-amyloidogenic α-processing of APP.

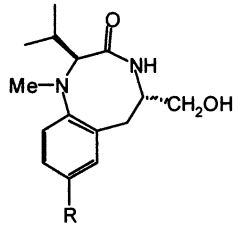
Design and Synthesis. An important role has been ascribed to the nature of the side chains present in certain PKC activators, since unsaturation in these side chains tends to increase inflammatory activity while decreasing tumor-promoting activity. For example, octahydromezerein is a tumor promoter while mezerein itself acts as a nonpromoting inflammatory agent.[42] Likewise, 12-*O*-acetylphorbol 13-(2,4-decadienoate) has been shown to have practically no effect as a tumor promoter in contrast to its saturated counterpart, 12-*O*-acetylphorbol 13-decanoate.[43] Guided by these observations, we chose to compare the properties of benzo-

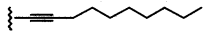
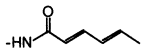
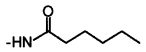
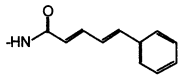
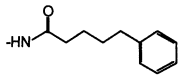
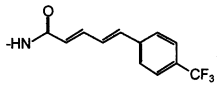
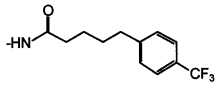
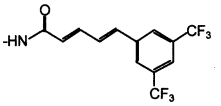
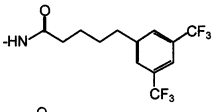
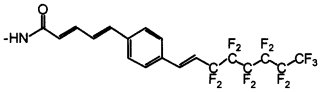
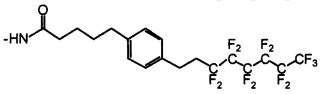
lactam derivatives having unsaturated and saturated side chains of the same length. We thus attached aliphatic side chains, both trans,trans dienic and saturated, linked to the benzolactam scaffold by means of an amide bond and terminating with a methyl, phenyl, *p*-trifluorophenyl, bis(3,5-trifluoromethyl)phenyl, and *p*-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooct-1-enyl)phenyl or *p*-(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)phenyl groups. The terminal aryl and fluoroalkylaryl groups will serve to increase the ClogP, as well as to modify the association of the protein−ligand complex with the membrane.[44] Linkage of the side chain to the benzolactam core by an amide bond was chosen for its synthetic convenience. It was also anticipated that this amido group might be able to form additional hydrogen bonds with proximal amino acid residues comprising the binding loops of PKCα. This possibility finds support in our molecular modeling studies as described below.

The synthesis of these 8-substituted benzolactam derivatives containing an amide moiety commences from the unsubstituted benzolactam, the synthesis of which has previously been published.[38] After O-acetylation, the benzolactam **1** can be nitrated under mild conditions with a solution of nitric acid in acetic anhydride (Scheme 1). The reaction produces exclusively the 8-nitro derivative **2** in an excellent yield of 92%, provided that the reaction is promptly terminated after completion. Prolonged exposure to the nitration reagent leads to a mixture of incompletely characterized products, with overnitration probably being the main process. Catalytic reduction of the nitro group over Pd/C afforded the desired amino derivative **3**, which was then reacted without purification with acyl chlorides **4a**−**f**. The resulting amides were subjected to deacetylation under basic conditions to obtain the final products **5a**−**c**, **5e**, **5g**, and **5i** in 31−42% yield over the three steps. Catalytic reduction of the unsaturated side chain appendages of **5c**, **5e**, **5g**, and **5i** over Pd/C provided the saturated analogues **5d**, **5f**, **5h**, and **5j**. The synthesis of the required trifluoromethylated acid chlorides was carried out using a modification of Huang's method,[45] involving the reaction between methoxycarbonylallylidenetriphenylarsorane generated in situ and the requisite benzaldehyde. Subsequent base hydrolysis of the ester and reaction with thionyl chloride provided the desired acylating agent. For the synthesis of **5i**, the required acid chloride was prepared by coupling of the *E*,*E*-isomer of methyl 5-(4-iodophenyl)-2,4-pentadienoate and 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-1-octene in the presence of $Pd(OAc)_2$, $NaHCO_3$, and n-Bu_4NHSO_4 in DMF under nitrogen,[46] followed by base hydrolysis and acid chloride formation.

Binding Studies. The interaction of the benzolactams **5a**−**j** with PKC was assessed by determining their ability to displace bound [20-^{3}H]phorbol 12,13-dibutyrate (PDBU) from recombinant PKCα in the presence of phosphatidylserine. The partition coefficients (ClogP) were calculated according to the fragment-based program KOWWIN 1.63.[47] The results are presented in Table 1. The K_i values of all compounds are in the nanomolar range, and the calculated log *P* values are in the range 2−11. Dienic ligands and their saturated counterparts generally display comparable binding affinities except in the cases of **5a** and **5b**.

Table 1. ClogP and K_i Values of BL and Amides **5a**−**j**



Compound	Appendage R =	ClogP	K_i (nM)
BL		6.0	14.7 ± 1.3
5a		2.0	1970 ± 8.0
5b		2.8	225 ± 15.7
5c		3.6	15.8 ± 0.3
5d		4.0	34.2 ± 1.0
5e		4.6	11.9 ± 1.1
5f		5.0	12.5 ± 0.7
5g		5.5	5.6 ± 0.2
5h		6.0	7.8 ± 0.7
5i		10.2	11.2 ± 1.6
5j		10.8	9.2 ± 0.9

sAPPα Secretion. The ability of our compounds to enhance sAPPα secretion using a cell line derived from an AD patient is summarized as bar graphs in Figure 1. All values are expressed relative to DMSO alone.

Four to six independent experiments were conducted for each compound at a concentration of 1 μM, and duplicate experiments for all the compounds were carried out at a concentration of 0.1 μM. A substantial effect was observed at 1 μM for all compounds tested with the exception of compound **5i**. The effect on sAPPα secretion correlates reasonably well with the K_i for inhibition of PDBU. Thus, compounds **5a** and **5b** exhibit only modest effects in this assay, since their K_i values are between 200 and 2000 nM. Of interest is the absence of activity found for the highly lipophilic ligand **5i**, which has a K_i of 11 nM. On the other hand, its saturated counterpart **5j**, which possesses a comparable K_i and ClogP, retains activity in the secretase activity. The

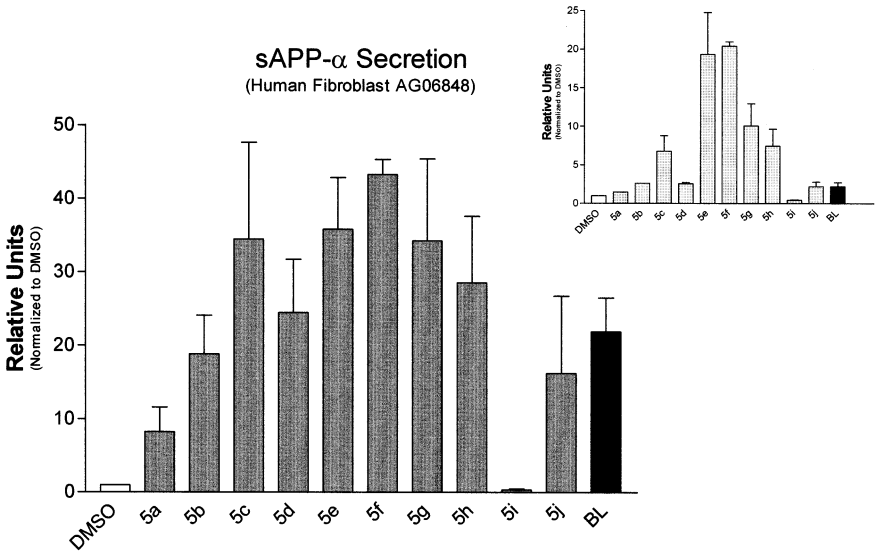


Figure 1. Secretion of sAPPα. Secretion was determined by densitometry analyses of immunoblots. Each bar represents a different treatment (all 1 μM). Results are from 4 to 6 independent experiments, except for **5f**,**i**,**j** (triplicate), and 12 repeats for BL (BL is the "+" reference or control). The units are relative to the signal obtained from cells treated with solvent (DMSO) alone, which reflects nonstimulated or basal secretion of sAPPα. The normalization was applied to each blot. Increased sAPPα was particularly marked after stimulation with **5e**−**g** (Kruskal−Wallis and Dunn's post-test). Noticeable increases were also achieved with **5c**,**d**,**h** and, to a lesser extent, BL. The inset shows that **5e** and **5f** also have a significant effect at 0.1 μM. Compounds **5c**,**g**,**h** also induce noticeable secretion. Most experiments for 0.1 μM are duplicates. Six repeats were carried out for **5g** and **5h** and single experiments for **5a** and **5b**.

enhancement of sAPPα secretion by compounds **5c**−**h** is higher than that observed for 8-(1-decynyl)benzolactam. Compounds **5c**,**e**−**h** enhanced sAPPα secretion even at 0.1 μM, although to a lesser degree than at the higher concentration. A noticeable difference is observed between most of the compounds and the ligands **5e** and **5f**, which are both lipophilic and bind potently to PKCα. Their activity in the sAPPα assay dropped by merely one-half when the concentration was decreased 10-fold. A representative Western blot illustrates the sAPPα signal analyzed for all of the compounds (Figure 2).

Hyperplasia Assays. Compounds **5e** and **5f** were evaluated for induction of hyperplasia after topical application to the shaved backs of outbred Sencar mice (NCI−Frederick, Frederick, MD). The extent of hyperplasia was estimated in terms of the number of cell layers in the epidermis (Table 2). The potencies of **5e** and **5f** were compared with those of TPA and of mezerein. Detectable hyperplasia was observed after a single application of 1 μg of TPA or after a single application of 3 μg of mezerein. **5f** showed a modest response at 100 μg and **5e** at 300 μg, suggesting that **5f** was approximately 30-fold less potent than mezerein and 100-fold less potent than TPA. **5e** was approximately 3-fold less active than **5f**. Similar relationships were observed after four applications, although the extent of hyperplasia was somewhat more marked.

Docking and MD Simulation. The truncated version of 8-(1-decynyl)benzolactam (BL) bearing only an acetylene group at position 8 and generic ligand **5** containing −NH(CO)−CH═CHMe (*E* double bond) as a side chain appendage were docked to the binding domain of PKCα C1b, and the region within an 8 Å sphere around the ligand was subjected to molecular dynamics simulations. Molecular modeling revealed that truncated BL forms four highly populated (close to 100%) hydrogen bonds with amino acid residues Ser 111, Thr113, Leu 122, and Gly124. It also forms a less

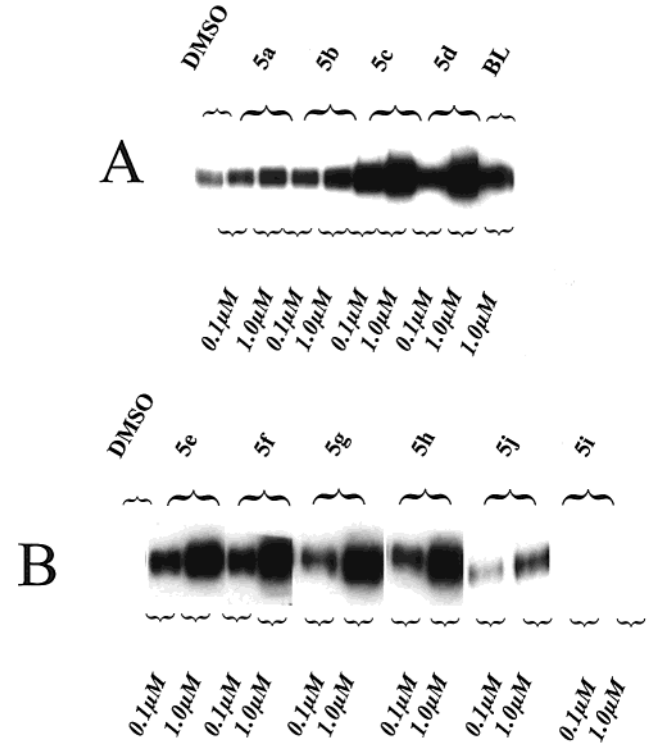


Figure 2. sAPPα detection. Conventional immunoblotting techniques were used to detect secreted sAPPα. Representative blots show the signal obtained with various compounds used at (A) 1 and (B) 0.1 μM. The two panels represent distinct experiments and blots.

populated hydrogen bond with Tyr 109 (21%) (Figure 3, Table 3). Generic ligand **5** also forms four hydrogen bonds with an occupancy close to 100% with Ser 111, Thr 113, Leu 122, and Gly 124. Like BL, ligand **5** forms a low occupancy bond with Tyr 109 (16%). However, in comparison to BL, the hydrogen atom from the amido

Table 2. Hyperplasia Induced by Compounds **5e** and **5f** in Comparison to TPA and Mezerein Estimated by the Number of Cell Layers in the Epidermis

compound	dose (μg/application)	epidermal cell layers: single application	epidermal cell layers: four applications
acetone control		1−2	1−2
	2	2−5	5−7
TPA	1	2−4	3−7
	0.3	1−3	1−4
	10	3−6	3−7
mezerein	3	2−6	2−6
	1	1−2	1−3
	300	1−4	2−4
	100	1−2	2−5
	30	1−2	1−4
5e	10	1−2	1−2
	3	1−2	1−2
	300	2−6	3−7
	100	1−5	2−4
	30	1−2	1−3
5f	10	1−2	1−3
	3	1−2	1−2
	1	1−2	1−2

group located at position 8 of **5** is able to form one additional hydrogen bond with Ser 110, which shows an occupancy of 67%.

Discussion and Conclusions

The nature of the benzolactam side chain has a substantial effect on the ability of the benzolactams to increase the formation of sAPPα. This is most clearly seen in comparing the decynyl-substituted benzolactam with the benzolactams **5c**,**e**−**g**. Of particular note is the improved activity of **5f** in comparison to 8-(1-decynyl)-benzolactam, although the ClogP of the former is one log unit lower while their K_i values are very similar. On the basis of our modeling studies, we find that the side chain amide group of these new benzolactams is able to form an additional H-bonding interaction with serine residue 110 present in loop A. We suggest that the effect of this extra H bond on the orientation of the side chain coupled with the possible interaction of the side chain amide carbonyl with complementary groups present in the membrane phospholipids may better anchor the PKC−ligand complex to the membrane, allowing it to be a more effective mediator of the events resulting in α-secretase activation. Compounds **5e** and **5f** are relatively potent PKC ligands[49] exhibiting binding affinities that are only 40 times poorer than that of PDBU (phorbol 12,13-dibutyrate). The in vitro assays show the most dramatic increase in the secretion of nonpathogenic sAPPα in the cases of **5e** and **5f**.

Concerning the hyperplasia results, our findings with **5e** and **5f** are consistent with the structure−activity relations that have been shown for other classes of DAG mimetics derived from phorbol and related diterpenes. Here, the concept that has emerged is that interaction with the binding site on protein kinase C is relatively independent of the pattern of side chain substitution. This independence can be understood from the X-ray crystallographic analysis of the complex between the C1b binding domain of protein kinase C and phorbol 13-acetate,[48] in which the primary hydroxyl group (C20) and several of the secondary hydroxy groups of the constrained diterpene ring system insert into a hydrophilic pocket in an otherwise hydrophobic face of the C1 domain, whereas the ester side chains project away from the C1 domain and presumably interact with the phospholipid bilayer. Computer modeling and site-directed mutagenesis have permitted extrapolation of this model to other classes of constrained ligands, including the resiniferonol derivative thymeleatoxin,[53] the ingenol 3-monoesters,[53] constrained diacylglycerol lactones,[54] and the indole alkaloids.[55] In all of these cases, the side chains project away from the C1 domain.

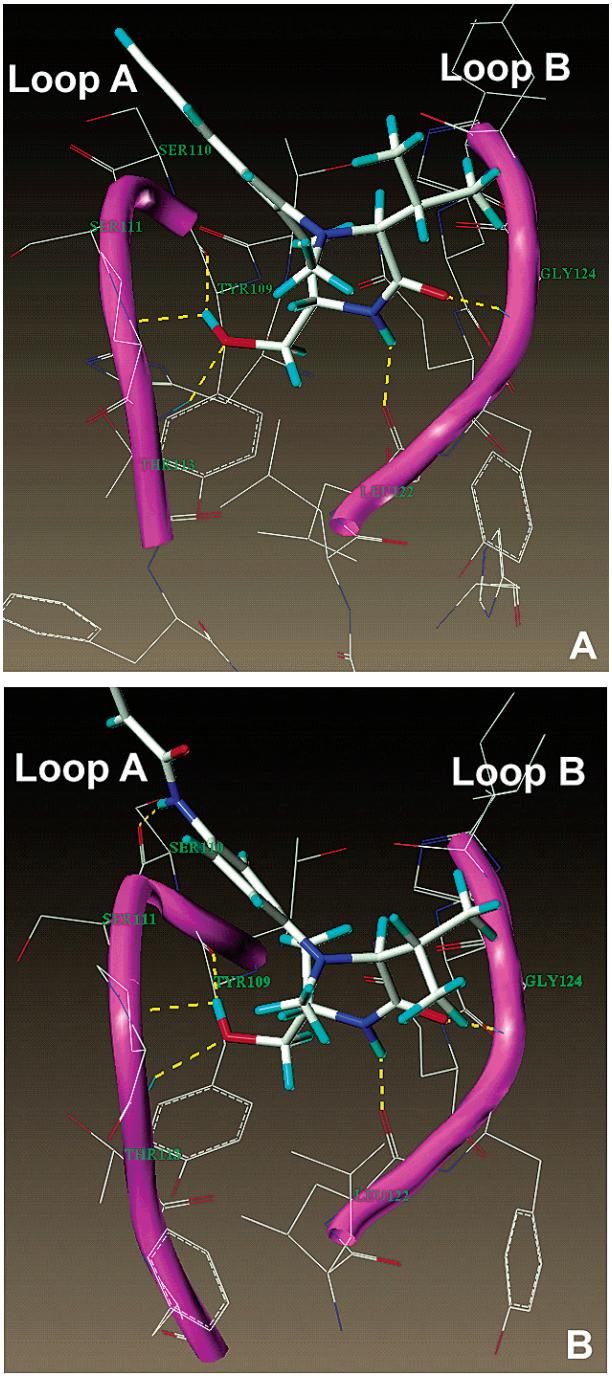


Figure 3. Hydrogen bonding interactions between PKCα C1b domain and ligands BL (A) and generic **5** (B).

Although the side chains do not directly interact with the C1 domain, studies at the cellular level clearly indicate that the side chains can influence into which membranes PKC can insert. This is most evident in the case of PKCδ.[56] PKCδ inserts into the plasma and

Table 3. Hydrogen Bond Interactions between PKCα C1b Domain and the Modeled Ligands

hydrogen bond	occupancy[a] (%)	average lifetime (ps)	parameters of hydrogen bonds			
			r_{DA} [b] (Å)	r_{AH} (Å)	α_{D-H-A} (deg)	ω_{H-A-AA} (deg)
			BL			
$O_{3-CO}\cdots HN_{Gly124}$	100	50.0	2.65 ± 0.17	1.76 ± 0.17	148.0 ± 13.6	35.4 ± 20.8[c]
$H_{4-NH}\cdots CO_{Leu122}$	85	42.4	3.06 ± 0.31	2.15 ± 0.34	152.7 ± 9.2	−57.0 ± 10.6[d]
$O_{11-OH}\cdots HN_{Thr113}$	90	44.8	2.85 ± 0.30	2.05 ± 0.30	138.7 ± 17.8	26.2 ± 29.1[e]
$H_{11-OH}\cdots CO_{Ser111}$	99	49.6	2.49 ± 0.12	1.77 ± 0.20	132.7 ± 16.7	−53.3 ± 57.7[f]
$H_{11-OH}\cdots CO_{Tyr109}$	21	10.3	3.67 ± 0.47	2.92 ± 0.49	137.7 ± 12.8	132.7 ± 28.1[g]
			Generic Ligand **5**			
$O_{3-CO}\cdots NH_{Gly124}$	100	50.0	2.57 ± 0.12	1.74 ± 0.13	138.9 ± 12.5	33.4 ± 23.0[c]
$H_{4-NH}\cdots CO_{Leu122}$	94	47.2	2.94 ± 0.23	2.03 ± 0.26	152.4 ± 8.4	−52.7 ± 11.1[d]
$O_{11-OH}\cdots NH_{Thr113}$	98	49.1	2.71 ± 0.18	1.93 ± 0.20	135.3 ± 16.0	13.7 ± 14.7[e]
$H_{11-OH}\cdots CO_{Ser111}$	96	47.9	2.59 ± 0.16	1.99 ± 0.26	121.7 ± 16.1	−78.2 ± 13.0[f]
$H_{11-OH}\cdots CO_{Tyr109}$	16	7.9	3.76 ± 0.46	3.03 ± 0.53	136.2 ± 12.9	115.3 ± 29.7[g]
$NH_{8-NHCO}\cdots CO_{Ser110}$	67	33.5	3.19 ± 0.59	2.45 ± 0.91	139.5 ± 31.5	79.8 ± 13.1[h]

[a] Resolution of 50 fs, counted as hydrogen bond only if $r_{AH} < 2.5$ Å. [b] Average distance during 50 ps production phase. "D" refers to a donor atom. "A" refers to an acceptor atom. [c] Torsion angle $H_{Gly124}-O-C-N_{ligand}$. [d] Torsion angle $H_{ligand}-O_{Leu122}-C-N$. [e] Torsion angle $H_{Thr113}-O-C-H_{ligand}$. [f] Torsion angle $H_{ligand}-O_{Ser111}-C-N$. [g] Torsion angle $H_{ligand}-O_{Tyr109}-C-N$. [h] Torsion angle $H_{ligand}-O_{Ser110}-C-N$.

nuclear membranes in response to the tumor-promoting derivative 12-deoxyphorbol 13-tetradecanoate, whereas it goes to the nuclear membrane and punctate aggregates, but not the plasma membrane, after treatment with 12-deoxyphorbol 13-acetate, a derivative that is inflammatory but that acts to inhibit tumor promotion. The important influence of hydrophobicity in the translocation of PKCδ was similarly shown for a series of homologous, symmetrically substituted phorbol 12,13-diesters, where both the kinetics and ultimate position of translocation of PKCδ depended on the hydrophobicity.[57] Since subcellular location should drive the availability of substrates for PKC, the ability of side chains on PKC ligands to control localization could plausibly contribute to differences in their biological activities.

In the case of derivatives that differ by being either unsaturated or saturated in their side chains, the biological effect has been consistent but the mechanism remains unresolved. In early studies, Hecker and coworkers described several series of diterpene esters in which unsaturation was associated with retention of inflammatory activity but loss of tumor-promoting activity. Examples include phorbol 12,13-dihexanoate versus phorbol 12,13-dihexa-2,4-dienoate,[43] phorbol 12-tetradecanoate 13-acetate versus phorbol 12-tetradeca-2,4,6,8-tetraenoate 13-acetate,[58] and 12-deoxyphorbol 13-decanoate versus 12-deoxyphorbol 13-deca-2,4-dienoate.[59] Some of us demonstrated that mezerein, a phorbol-related diterpene with a 5-phenylpenta-2,4-dienoate side chain at C12 that is only weak as a complete tumor promoter, regained tumor-promoting activity upon hydrogenation to generate octahydromezerein.[60] However, Mezerein did not induce any striking difference in the pattern of PKC translocation, suggesting that unsaturation may have a more subtle effect on PKC function, such as positioning PKC relative to cholesterol-rich rafts in the plasma membrane.

For **5e** and **5f**, we find similar affinities for PKCα and only a modest, 3-fold decrease in potency for skin hyperplasia for the unsaturated derivative. On the basis of the effect of unsaturation for other potent PKC ligands, we would predict that **5e** would retain biological activity in most assays but would show a marked loss of tumor-promoting activity.

Of considerable interest in understanding the complexity of these side chain interactions is the complete absence of activity observed in the sAPPα assay for the highly fluorinated analogue **5i**. Its binding affinity for PKCα is good, and its calculated lipophilicity is extremely high. It is unclear as to what is happening in this case, since its saturated counterpart is still effective, and thus solubility issues may be eliminated from consideration. However, we hypothesize that the somewhat more rigid side chain of the ligand **5i** is responsible for the lack of activity observed in the sAPPα assay. Particularly, since all double bonds in **5i** are trans and the orientation of the amido group in **5i** is fixed because of the presence of a hydrogen bond interaction with Ser 110, the overall rigidity of the long lipophilic side chain of **5i** is greater than that of **5j**. Although, the amido group in position 8 of **5j** also forms a hydrogen bond with Ser 110, unlike **5i**, the side chain of **5j** is much more flexible because of its saturated nature. These differences in side chain rigidity may accordingly alter the subcellular localization of PKC and contribute to the observed differences in their effects on secretase activity.

In light of the present findings, we believe that compounds such as **5e** that are capable of enhancing sAPPα secretion are worth exploring further as possible therapeutics in the treatment of Alzheimer's disease. Such compounds could be used alone or in combination with β-secretase inhibitors to decrease the formation of the neurotoxic β-amyloid peptide, thereby slowing the progression of the disease process.[72]

Experimental Section

Analysis of Inhibition of [^{3}H]PDBU Binding by Nonradioactive Ligands. Enzyme−ligand interactions were analyzed by competition with [^{3}H]PDBU binding to the single isozyme PKCα as described previously.[50]

Cells and Cell Culture. A well-characterized cell line from an AD patient (AG06848) was obtained from the Coriell Cell Repository (Camden, NJ), and then it was cultured and maintained as described[41] except that the plastic material was a 6 cm diameter Petri dish. Cells were typically grown to confluence, which took approximately 4−5 days. Cells were maintained in a complete medium until 2 h prior to treatment. At that point, the medium was replaced by DMEM without serum and left undisturbed for 2 h. Then, the cells were treated with 0.1 or 1 μM of the compounds to be tested. 8-(1-Decynyl)-benzolactam V (BL) and DMSO were used as positive and negative controls, respectively. The concentration of DMSO was maintained at less than 1% in all cases. The medium was collected after 3 h to measure the sAPPα secretion.

sAPPα Determinations. The concentration of secreted sAPPα was measured using conventional immunoblotting techniques,[51] following with minor modifications the protocol described elsewhere.[41] Precipitated protein extracts from each dish/treatment were loaded into freshly prepared 8% acrylamide Tris-HCl minigels and separated by SDS−PAGE. The volume of sample loaded was corrected for total cell protein per dish. Proteins were then electrophoretically transferred to PVDF membranes. Membranes were saturated with 5% nonfat dry milk to block nonspecific binding. Blocked membranes were incubated overnight at 4 °C with the commercially available antibody 6E10 (1:500), which recognizes sAPPα in the conditioned medium (SENETEK).[41,52] After being washed, the membranes were incubated at room temperature with horseradish peroxidase conjugated antimouse IgG secondary antibody (Jackson's Laboratories). The signal was then detected using enhanced chemiluminescence followed by exposure of Hyperfilm ECL (Amersham). The band intensities were quantified by densitometry using a BioRad GS-800 calibrated scanning densitometer and Multianalyst software (BioRad).

Hyperplasia Studies. Mice were 7 weeks old at the beginning of the treatments and were in the resting phase of the hair cycle. Compounds were dissolved in 0.2 mL of acetone and either were applied once or else were applied twice weekly for a total of four applications. Two animals were treated at each dose of compound, and 72 h after the last application, the animals were euthanized. Two portions of treated skin were removed from each animal, fixed in neutral buffered formalin, and stained with hematoxylin and eosin for histological analysis (staining of sections was performed by American Histolabs, Gaithersburg, MD). TPA (12-*O*-tetradecanoylphorbol 13-acetate) and mezerein were from LC Laboratories (Woburn, MA).

Chemistry. Analytical and preparative thin-layer chromatography (TLC) was performed in a solvent-vapor-saturated chamber on EM Science silica gel 60 F-254 plates. Spots were visualized by UV. Melting points (uncorrected) were determined in open capillaries on a Thomas-Hoover apparatus. Infrared spectra were obtained on an ATI Mattson Genesis series spectrometer in KBr pellets. NMR spectra were recorded on a Varian instrument (^{1}H frequency of 300 MHz) using TMS as an internal standard (^{1}H and ^{13}C) or $CFCl_3$ as an internal or external standard. Elemental analyses were performed by Micro-Analysis, Inc., and the results were within 0.4% of the theoretical value. Mass spectra were obtained on a Shimadzu QP-5000 mass spectrometer using a direct inlet probe and an electron beam energy of 70 eV. Determinations of purity by HPLC were performed with a Shimadzu LC-10 AD pump and a Waters 484 tunable absorbance detector using the following conditions: (A) Supelco Discovery RP amide C_{16} 250 mm × 3.0 mm; flow rate = 0.5 mL/min; detection at 280 nm; 0−30 min, 40−80% acetonitrile in water; 30−60 min, 80% acetonitrile in water; (B) Waters μBondapak C_{18} 300 mm × 7.8 mm; flow rate = 2.8 mL/min; detection at 280 nm; 0−30 min, 40−80% acetonitrile in water; 30−60 min, 80% acetonitrile in water; (C) Supelco Discovery RP amide C_{16} 250 mm × 3.0 mm; flow rate = 0.4 mL/min; detection at 280 nm; 0−15 min, 0−100% methanol in water; 15−50 min, methanol.

(2*S*,5*S*)-*O*-Acetyl-8-nitrobenzolactam V (2). A mixture of (2*S*,5*S*)-*O*-acetylbenzolactam V (**1**) (280 mg, 1.06 mmol) in Ac_2O (1.2 mL) was stirred at 0 °C. To this solution was added dropwise over 5 min a chilled mixture of Ac_2O (1.2 mL) and 70% HNO_3 (268 mg, 3.11 mmol). The reaction was allowed to proceed for 4−5 min at 0 °C and then was stopped by adding water (50 mL) in one portion to the vigorously stirred solution. Extraction with EtOAc (4 × 40 mL) followed by evaporation and column chromatography on silica gel (1:1 EtOAc/hexane as eluent) provided 340 mg (92%) of the product: mp 180−182 °C, yellow prisms (EtOH); $[\alpha]^{20}_D$ −1017 (*c* 1.44, $CHCl_3$); IR (KBr) 1743, 1670, 1505, 1326, 1231 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.77 (d, 3H, J = 6.8 Hz), 1.06 (d, 3H, J = 6.3 Hz), 2.13 (s, 3H), 2.50 (m, 1H), 2.93 (s, 3H), 2.96 (d, 1H, J = 17.5 Hz), 3.44 (dd, 1H, J = 7.2, 17.5 Hz), 3.63 (d, 1H, J = 10.3 Hz), 3.68 (m, 1H), 3.94 (dd, 1H, J = 9.4, 11.1 Hz), 4.29 (dd, 1H, J = 4.1, 11.2 Hz), 5.97 (s, 1H), 6.90 (d, 1H, J = 9.0 Hz), 8.01 (d, 1H, J = 2.4 Hz), 8.08 (dd, 1H, J = 2.7, 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 18.9, 20.5, 27.5, 33.5, 37.5, 51.5, 66.8, 66.9, 116.3, 123.8, 128.0, 139.2, 156.3, 170.2, 171.5; MS *m*/*z* 349 (M$^+$, 26%), 332, 306, 278, 203, 177, 133, 43 (100%). Anal. ($C_{17}H_{23}N_3O_5$) C, H, N.

General Procedure for the Synthesis of (2*S*,5*S*)-8-(Acylamino)benzolactams V (5a−d). A mixture of nitro compound **2** (100 mg, 287 μmol) and 10% Pd/C (45 mg) in ethanol (20 mL) was shaken in a Parr apparatus under 4.0 atm of hydrogen at room temperature for 3 h. The mixture was concentrated, and the residue was dried under vacuum for 1 h at 60 °C. To the crude amine **3**, dry THF (4 mL) was added under inert atmosphere, followed by dry Et_3N (0.5 mL) and the acyl chloride (1.6 equiv). The mixture was stirred at room temperature for 4 h and then was filtered and concentrated. Preparative TLC (ethyl acetate as developing solvent) provided an intermediate, which was dissolved in ethanol (30 mL). A solution of sodium carbonate (60 mg) in water (6 mL) was added with vigorous stirring. After 1.5−2 h at room temperature, the solution was concentrated and water (20 mL) was added. Extraction with ethyl acetate (4 × 30 mL) provided the crude amides **5a**−**d**, which were purified by preparative TLC as above.

(2*S*,5*S*)-(*E*,*E*)-8-(2,4-Hexadienoylamino)benzolactam V (5a). The title compound was obtained according to the general procedure in 38% yield as yellow oil: $[\alpha]^{20}_D$ −253 (*c* 0.80, MeOH); IR (KBr) 1646, 1539, 1506 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.06 (d, 3H, J = 6.8 Hz), 1.11 (d, 3H, J = 6.8 Hz), 1.85 (d, 3H, J = 6.1 Hz), 2.38 (m, 1H), 2.60 (s, 3H), 2.63, 2.85 (ABq, 2 H, J = 15.6 Hz, both parts d with J = 10.5 and 5.4 Hz, respectively), 3.32 (d, 1H, J = 5.6 Hz), 3.47−3.56 (m, 1H), 3.64−3.73 (m, 1H), 4.06 (br s, 1H), 4.94 (br s, 1H), 5.96 (d, 1 H, J = 15.1 Hz), 6.03−6.25 (m, 2H), 6.37 (s, 1H), 6.96 (d, 1H, J = 8.8 Hz), 7.23 (dd, 1H, J = 10.3, 14.9 Hz), 7.43 (br d, 1H, J = 7.3 Hz), 7.61 (dd, 1H, J = 2.0, 8.3 Hz), 8.02 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.2, 18.6, 20.9, 28.9, 37.0, 38.9, 52.5, 64.2, 78.1, 119.7, 122.4, 122.6, 123.0, 129.9, 134.3, 135.3, 137.9, 141.6, 147.7, 164.7, 174.6; MS *m*/*z* 371 (M$^+$, 13%), 340, 276, 255, 95, 44 (100%); HPLC retention time 9.5 min (96.1% purity) using conditions A, 29.6 min (96.9% purity) using conditions B.

(2*S*,5*S*)-8-(Hexanoylamino)benzolactam V (5b). The title compound was obtained according to the general procedure in 35% yield as colorless oil: $[\alpha]^{20}_D$ −166 (*c* 0.85, MeOH); IR (KBr) 3290 (br), 1650, 1540, 1506 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3H, J = 6.8 Hz), 1.02 (d, 3H, J = 6.8 Hz), 1.10 (d, 3H, J = 6.8 Hz), 1.27−1.42 (m, 4H), 1.68 (m, 2H), 2.27 (t, 2H, J = 7.4 Hz), 2.38 (m, 1H), 2.64 (s, 3H), 2.72, 2.88 (ABq, 2H, J = 15.6 Hz, both parts d with J = 9.8 and 4.8 Hz, respectively), 3.32 (d, 1H, J = 5.9 Hz), 3.46−3.56 (m, 1H), 3.64−3.73 (m, 1H), 4.01 (br s, 1H), 4.85 (br s, 1H), 6.50 (s, 1H), 7.00 (d, 1H, J = 8.8 Hz), 7.31 (d, 1H, J = 7.1 Hz), 7.48 (dd, 1H, J = 2.2, 8.8 Hz), 7.69 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.0, 18.4, 20.7, 22.5, 25.4, 28.8, 31.5, 37.1, 37.4, 38.6, 52.6, 64.3, 77.3, 119.7, 122.6, 134.2, 134.8, 147.8, 171.9, 174.4; MS *m*/*z* 375 (M$^+$, 14%), 344, 304, 259, 147, 44 (100%); HPLC retention time 11.6 min (98.5% purity) using conditions A, 29.6 min (98.9% purity) using conditions C.

(2*S*,5*S*)-(*E*,*E*)-8-(5-Phenyl-2,4-pentadienoylamino)benzolactam V (5c). The title compound was obtained according to the general procedure in 31% yield as a yellow oil: $[\alpha]^{20}_D$ −187 (*c* 0.56, MeOH); IR (KBr) 1653, 1540, 1507 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.07 (d, 3H, J = 6.8 Hz), 1.12 (d, 3H, J = 6.8 Hz), 2.40 (m, 1H), 2.63 (s, 3H), 2.70, 2.88 (ABq, 1H, J = 15.8 Hz, both parts d with J = 10.3 and 5.2 Hz, respectively), 3.36 (d, 1H, J = 5.6 Hz), 3.48−3.58 (m, 1H), 3.66−3.76 (m, 2H), 4.88 (br s, 1H), 6.18 (d, 1H, J = 14.9 Hz), 6.51 (s, 1H), 6.81−6.95 (m, 2H), 7.00 (d, 1H, J = 8.8 Hz), 7.25−7.49 (m, 7H), 7.58 (d, 1H, J = 7.6 Hz), 7.92 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.4, 20.9, 28.8, 37.1, 38.6, 52.7, 64.4, 77.5, 119.8, 122.6, 122.8, 125.0, 126.6, 127.0, 128.7, 128.8, 134.2, 134.9, 136.3, 139.3, 141.3, 147.9, 164.3, 174.6; MS *m*/*z* 433 (M$^+$, 46%), 402, 362, 317, 276, 157, 128, 44 (100%); HPLC retention time 20.3 min (98.3%

purity) using conditions A, 26.0 min (96.4% purity) using conditions B.

(2*S*,5*S*)-8-(5-Phenylpentanoylamino)benzolactam V (5d). A mixture of **5c** (40.0 mg, 92 μmol), Pd/C (10%) (20 mg), and methanol (20 mL) was shaken under 3.3 atm of H_2 at room temperature for 19 h. Filtration from the catalyst, evaporation, and thin-layer chromatography (EtOAc as eluent) provided **5d** (36.1 mg, 90%) as a colorless oil: $[\alpha]^{25}_D$ −129 (*c* 1.8, MeOH); IR (KBr) 1652, 1541, 1507 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.02 (d, 3H, *J* = 6.8 Hz), 1.09 (d, 3H, *J* = 6.6 Hz), 1.61−1.79 (m, 4H), 2.20−2.42 (m, 3H), 2.60 (s, 3H), 2.61−2.68 (m, 2H), 2.68, 2.85 (ABq, 2H, *J* = 16.0 Hz, both parts d with *J* = 10.0 and 5.0 Hz, respectively), 3.28 (d, 1H, *J* = 5.6 Hz), 3.45−3.54 (m, 1H), 3.62−3.70 (m, 1H), 4.06 (br s, 1H), 4.85 (br s, 1H), 6.46 (s, 1H), 6.93 (d, 1H, *J* = 8.5 Hz), 7.13−7.21 (m, 3 H), 7.24−7.34 (m, 3H), 7.45 (dd, 1H, *J* = 1.6, 8.7 Hz), 7.72 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.3, 20.8, 25.3, 28.8, 31.2, 35.7, 37.1, 37.2, 38.6, 52.6, 64.2, 77.5, 119.7, 122.5, 122.6, 125.7, 128.3, 128.4, 134.3, 134.8, 142.2, 147.8, 171.6, 174.5; MS *m*/*z* 437 (M^+, 12%), 406, 366, 321, 261, 147, 91, 44 (100%); HPLC retention time 19.9 min (97.9% purity) using conditions A, 30.4 min (97.3% purity) using conditions C.

(2*S*,5*S*)-(*E*,*E*)-8-[5-[4-(Trifluoromethyl)phenyl]-2,4-pentadienoylamino]benzolactam V (5e). A mixture of methyl (*E*,*E*)-5-[(4-trifluoromethyl)phenyl]-2,4-pentadienoate[45] (0.91 g, 3.55 mmol), KOH (0.60 g, 10.7 mmol), methanol (30 mL), and water (2 mL) was stirred at room temperature for 22 h. Solvents were evaporated, and the residue was dissolved in a minimum amount of water. The resulting solution was acidified with 3 M HCl. The precipitate that formed immediately was separated by filtration and dried under vacuum. This product (0.74 g, 3.06 mmol) and $SOCl_2$ (6.6 mL) were heated at 70 °C for 3 h. Evaporation of the excess of $SOCl_2$ provided the crude acid chloride, which was used in the synthesis of compound **5e** (see general procedure above) without purification. Compound **5e**: yield 42%; yellow oil; $[\alpha]^{20}_D$ −165 (*c* 0.52, MeOH); IR (KBr) 1653, 1541, 1507, 1324 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.11 (d, 3H, *J* = 7.1 Hz), 1.14 (d, 3H, *J* = 6.8 Hz), 2.41 (m, 1H), 2.62 (s, 3H), 2.67, 2.90 (ABq, 2H, *J* = 15.8 Hz, both parts d with *J* = 5.7 and 10.2 Hz, respectively), 3.35 (d, 1H, *J* = 5.4 Hz), 3.52−3.61 (m, 1H), 3.69−3.78 (m, 1H), 3.93 (br s, 1H), 5.00 (br s, 1H), 6.26 (d, 1H, *J* = 14.9 Hz), 6.33 (s, 1H), 6.80−7.02 (m, 3H), 7.38−7.68 (m, 7 H), 8.05 (br s, 1H); ^{13}C NMR ($CDCl_3$) 18.1, 21.0, 28.9, 37.0, 39.1, 52.5, 64.1, 78.6, 119.8, 122.4, 123.2, 124.0 (q, *J* = 272 Hz), 125.6 (q, *J* = 3.5 Hz), 126.7, 127.0, 128.9, 130.1 (q, *J* = 32.7 Hz), 134.7, 137.2, 139.7, 140.4, 147.9, 164.1, 174.8; MS *m*/*z* 501 (M^+, 94%), 470, 430, 415, 385, 276, 225 (100%), 177; HPLC retention time 26.0 min (98.9% purity) using conditions A, 20.2 min (97.3% purity) using conditions B.

(2*S*,5*S*)-8-[5-[4-(Trifluoromethyl)phenyl]pentanoylamino]benzolactam V (5f): $[\alpha]^{25}_D$ −127 (*c* 0.70, MeOH); IR (KBr) 3290 (br), 1649, 1504, 1326, 1121, 1068 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.02 (d, 3H, *J* = 6.8 Hz), 1.10 (d, 3H, *J* = 6.8 Hz), 1.63−1.82 (m, 4H), 2.21−2.43 (m, 3H), 2.60 (s, 3H), 2.65−2.78 (m, 3H), 2.87 (dd, 1H, *J* = 5.0, 15.7 Hz), 3.28 (d, 1H, *J* = 5.6 Hz), 3.47−3.59 (m, 1H), 3.65−3.75 (m, 1H), 3.90 (br s, 1H), 4.88 (br s, 1H), 6.38 (br s, 1H), 6.94 (d, 1H, *J* = 8.5 Hz), 7.29 (d, 2H, *J* = 8.1 Hz), 7.36 (br s, 1H), 7.47 (d, 1H, *J* = 8.8 Hz), 7.52 (d, 2H, *J* = 8.3 Hz), 7.59 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 18.2, 20.8, 25.2, 28.8, 30.8, 35.5, 37.0, 38.9, 52.5, 64.1, 78.1, 119.6, 122.5, 122.7, 124.3 (q, *J* = 272 Hz), 125.2 (q, *J* = 3.5 Hz), 128.1 (q, *J* = 32.2 Hz), 128.7, 134.5, 135.0, 146.3, 147.8, 171.4, 174.5; MS *m*/*z* 505 (M^+, 32%), 474, 462, 434, 419, 389, 261, 159, 44 (100%); HPLC retention time 24.9 min (99.7% purity) using conditions A, 19.0 min (99.5% purity) using conditions B.

(2*S*,5*S*)-(*E*,*E*)-8-[5-[3,5-Bis(trifluoromethyl)phenyl]-2,4-pentadienoylamino]benzolactam V (5g). The title compound was obtained according to the general procedure in 42% yield as a yellow oil: $[\alpha]^{20}_D$ −149 (*c* 0.25, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.11 (d, 3H, *J* = 6.8 Hz), 1.14 (d, 3H, *J* = 6.8 Hz), 2.43 (m, 1H), 2.63 (s, 3H), 2.70, 2.91 (ABq, 2H, *J* = 15.8 Hz, both parts d with *J* = 10.3 and 5.5 Hz, respectively), 3.36 (d, 1H, *J* = 5.4 Hz), 3.53−3.62 (m, 1H), 3.76 (br d, 1H, *J* = 10.5 Hz), 3.88 (br s, 1H), 5.01 (br s, 1H), 6.31 (d, 1H, *J* = 14.9 Hz), 6.35 (br s, 1H), 6.83−7.08 (m, 3H), 7.43 (dd, 1H, *J* = 10.5, 14.9 Hz), 7.55 (br s, 1H), 7.65 (d, 1H, *J* = 8.3 Hz), 7.76 (s, 1H), 7.83 (s, 2H), 8.04 (br s, 1H); ^{19}F NMR ($CDCl_3$) δ −63.5 (m); ^{13}C NMR ($CDCl_3$) δ 18.1, 21.0, 29.0, 37.0, 39.2, 52.4, 64.0, 78.9, 119.8, 121.7 (sept, *J* = 3.4 Hz), 122.4, 123.1 (q, *J* = 273 Hz), 123.3, 126.5 (narrow m), 127.8, 130.2, 132.1 (q, *J* = 33.4 Hz), 134.8, 135.2, 135.3, 138.4, 139.7, 148.1, 163.7, 174.9; MS *m*/*z* 569 (M^+, 3%), 453, 279, 245, 167, 149, 44 (100%), 177; HPLC retention time 31.3 min (98.4% purity) using conditions A, 32.1 min (96.7% purity) using conditions C.

(2*S*,5*S*)-8-[5-[3,5-Bis(trifluoromethyl)phenyl]pentanoylamino]benzolactam V (5h). A mixture of **5g** (40.0 mg, 70 μmol), Pd/C (10%) (20 mg), and methanol (20 mL) was stirred under 1 atm of H_2 at room temperature for 0.5 h. Filtration from the catalyst, evaporation, and thin-layer chromatography (EtOAc as eluent) provided **5h** (37.5 mg, 93%) as a colorless oil: $[\alpha]^{25}_D$ −101 (*c* 0.26, MeOH); IR (KBr) 3410 (br), 1642, 1540, 1505, 1380, 1280, 1128 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.01 (d, 3H, *J* = 6.8 Hz), 1.10 (d, 3H, *J* = 6.6 Hz), 1.69−1.82 (m, 4H), 2.28−2.45 (m, 3H), 2.64 (s, 3H), 2.71−2.84 (m, 3H), 2.88 (dd, 1H, *J* = 5.3, 16.0 Hz), 3.31 (d, 1H, *J* = 6.1 Hz), 3.48−3.60 (m, 2H), 3.66−3.78 (m, 1H), 4.81 (br s, 1H), 6.48 (br s, 1H), 6.97 (d, 1H, *J* = 8.8 Hz), 7.24 (br s, 1H), 7.39−7.47 (m, 2H), 7.64 (s, 2H), 7.70 (s, 1H); ^{19}F NMR ($CDCl_3$) δ −63.2 (m); ^{13}C NMR ($CDCl_3$) δ 18.5, 20.7, 25.1, 28.8, 30.7, 35.4, 36.9, 37.1, 38.4, 52.7, 64.4, 77.2, 119.7, 119.9 (sept, *J* = 3.8 Hz), 122.3, 122.7, 123.4 (q, *J* = 272 Hz), 128.5 (narrow m), 131.5 (q, *J* = 32.7 Hz), 134.2, 134.4, 144.6, 148.0, 171.0, 174.3; MS *m*/*z* 573 (M^+, 4%), 542, 502, 403, 261, 227, 44 (100%); HPLC retention time 29.3 min (99.0% purity) using conditions A, 31.7 min (98.3% purity) using conditions C.

(2*S*,5*S*)-(*E*,*E*,*E*)-8-[5-[4-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooct-1-enyl)phenyl]-2,4-pentadienoylamino]benzolactam V (5i). The title compound was obtained according to the general procedure in 41% yield as a yellow oil: $[\alpha]^{20}_D$ −87 (*c* 0.185, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.09 (d, 3H, *J* = 6.8 Hz), 1.13 (d, 3H, *J* = 6.8 Hz), 2.41 (m, 1H), 2.63 (s, 3H), 2.67−2.77 (m, 1H), 2.90 (dd, 1H, *J* = 5.3, 16.0 Hz), 3.36 (d, 1H, *J* = 5.6 Hz), 3.50−3.61 (m, 1H), 3.68−3.79 (m, 2H), 4.95 (br s, 1H), 6.20 (dt, 1H, *J* = 11.8 Hz (t), 16.1 Hz (d)), 6.22 (d, 1H, *J* = 14.6 Hz), 6.39 (br s, 1H), 6.83, 6.95 (ABq, 2H, *J* = 15.6 Hz, B part d with *J* = 10.7 Hz), 6.95 (dd, 1H, *J* = 10.6, 15.5 Hz), 7.00 (d, 1H, *J* = 8.8 Hz), 7.14 (dt, 1H, *J* = 16.1 Hz (d), 2.4 Hz (t)), 7.38−7.54 (m, 6H), 7.58−7.66 (m, 1H), 7.93 (br s, 1H); ^{19}F NMR ($CDCl_3$) δ −126.7 (m, 2F), −123.6 (m, 2F), −123.4 (br s, 2F), −122.1 (br s, 2F), −111.6 (m, 2F), −81.3 (m, 3F); ^{13}C NMR ($CDCl_3$) δ 18.3, 21.0, 28.9, 37.0, 38.9, 52.6, 64.3, 78.1, 114.4 (t, *J* = 22.9 Hz), 119.9, 122.5, 123.0, 126.1, 127.5, 128.0, 128.1, 133.6, 134.4, 135.1, 137.9, 138.2, 139.0 (t, *J* = 9.1 Hz), 140.8, 147.9, 164.1, 174.7; HPLC retention time 43.8 min (96.8% purity) using conditions A, 33.3 min (99.0% purity) using conditions C.

(2*S*,5*S*)-8-[5-[4-(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)phenyl]pentanoylamino]benzolactam V (5j). A mixture of **5i** (40.0 mg, 51 μmol), Pd/C (10%) (20 mg), and methanol (20 mL) was stirred under 1 atm of H_2 at room temperature for 0.5 h. Filtration from the catalyst, evaporation, and thin-layer chromatography (EtOAc as eluent) provided **5j** (38.6 mg, 96%) as a colorless oil: $[\alpha]^{20}_D$ −88 (*c* 1.24, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.02 (d, 3H, *J* = 6.8 Hz), 1.10 (d, 3H, *J* = 6.8 Hz), 1.60−1.80 (m, 4H), 2.23−2.44 (m, 5H), 2.62 (s, 3 H), 2.62−2.66 (m, 1 H), 2.73 (dd, 1 H, *J* = 9.9, 16.0 Hz), 2.81−2.92 (m, 4H), 3.30 (d, 1H, *J* = 6.1 Hz), 3.47−3.58 (m, 1H), 3.65−3.82 (m, 2H), 4.84 (br s, 1H), 6.45 (br s, 1H), 6.95 (d, 1H, *J* = 8.5 Hz), 7.07−7.17 (m, 4H), 7.28−7.36 (m, 1H), 7.44 (br d, 1H, *J* = 8.8 Hz), 7.54 (br s, 1H); ^{19}F NMR ($CDCl_3$) δ −126.7 (m, 2F), 124.1 (br s, 2F), −123.4 (br s, 2F), −122.4 (br s, 2F), −115.2 (m, 2F), −81.3 (m, 3F); ^{13}C NMR ($CDCl_3$, weak multiplets of fluorinated C omitted) δ 18.2, 20.8, 25.3, 25.9 (t, *J* = 4.0 Hz), 28.9, 31.2, 33.0 (t, *J* = 22.2 Hz), 35.3, 37.0, 37.2, 38.9, 52.5, 64.1, 78.2, 119.7, 122.4, 122.7, 128.2, 128.8, 134.5, 135.1, 136.4, 140.7, 147.7, 171.6, 174.6; HPLC

retention time 44.1 min (96.6% purity) using conditions A, 33.6 min (98.9% purity) using conditions C.

Docking and MD Simulations. The 3D model of PKCα[61] was obtained as described earlier.[62] The docking was performed using the FlexX module in Sybyl6.8.[63,64] The molecules were docked to the binding site of PKCα C1b domain formed by loop A comprising Thr 108, Tyr 109, Ser 110, Ser 111, Pro 112, Thr 113, and Phe 114 and by loop B comprising amino acids Leu 121, Leu 122, Tyr 123, Gly 124, Leu 125, Ile 126, His 127, and Gln 128. To simplify the analysis, the long alkyl tail in BL and ligand **5** was truncated, and the resulting two molecules were used for further studies. The 30 best docking solutions based on the FlexX docking functions were saved by FlexX. To produce a more robust evaluation of ligand−receptor interactions, a consensus of all five scoring functions (FlexX,[65] PMF,[66] GOLD,[67,68] Chemscore,[69] and DOCK[70]) available in CScore was generated using the CScore module in Sybyl6.8.[64] Among the 30 best solutions, only those that have a consensus of three or more functions and are ranked highest by a combination of GOLD, DOCK, and FlexX scores were taken into further consideration. The poses of BL and ligand **5** with the highest PMF score are shown in Figure 3 and were used for further MD simulations.

The molecules of BL and ligand **5** in the PKCα C1b domain obtained during the docking studies were minimized using the Tripos force field in Sybyl6.8[64] and the Powell method for 5000 steps or until the gradient is lower than 0.01 kcal mol^{-1} $Å^{-1}$. All simulations were performed with the molecular dynamics module (Sybyl6.8) in a vacuum with a constant dielectric constant $\epsilon = 1$ using the Tripos force field. The ligands were allowed to be fully flexible, while in PKCα, only residues within an 8 Å radius of the ligand were made fully flexible keeping the other residues fixed. The following parameters were used for MD: 0.5 fs time step, snapshot each 50 fs, Bolzmann initial velocities, constraint of all bonds to hydrogen atoms using the SHAKE algorithm.[71] The heating phase in the NTP ensemble of 50 ns was followed by a 200 ns equilibration phase and a 100 ns production phase. All molecular modeling studies were performed on a SGI Octane with two 300 MHz MIPS R12000 processors. The results of molecular modeling studies are summarized in Table 3.

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