Branched Diacylglycerol-Lactones as Potent Protein Kinase C Ligands and α-Secretase Activators

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Using as our lead structure a potent PKC ligand (**1**) that we had previously described, we investigated a series of branched DAG-lactones to optimize the scaffold for PKC binding affinity and reduced lipophilicity, and we examined the potential utility of select compounds as α-secretase activators. Activation of α-secretase upon PKC stimulation by ligands causes increased degradation of the amyloid precursor protein (APP), resulting in enhanced secretion of sAPPα and reduced deposition of *β*-amyloid peptide (A*β*), which is implicated in the pathogenesis of Alzheimer's disease. We modified in a systematic manner the C_5-acyl group, the 3-alkylidene, and the lactone ring in **1** and established structure−activity relationships for this series of potent PKC ligands. Select DAG-lactones with high binding affinities for PKC were evaluated for their abilities to lead to increased sAPPα secretion as a result of α-secretase activation. The DAG-lactones potently induced α-secretase activation, and their potencies correlated with the corresponding PKC binding affinities and lipophilicities. Further investigation indicated that **2** exhibited a modestly higher level of sAPPα secretion than did phorbol 12,13-dibutyrate (PDBu).

Introduction

The protein kinase C (PKC)[1,2] family of serine/threonine kinases are key enzymes in cellular signal transduction, being activated by diacylglycerol (DAG) generated either by phospholipase C (PLC) mediated hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP_2) or indirectly by the action of phospholipase D and phosphatidic acid hydrolase.[3] DAG induces the translocation of cytosolic PKC to the inner leaflet of the cellular membrane and activates both the calcium-dependent classical PKC isoforms (PKC-α, *β*, and *γ*) and the novel or calcium-independent PKC isoforms (PKC-*δ*, *ϵ*, *η*, and *θ*) by binding to the C1 domains of the enzymes and promoting association with the membrane phospholipids.[4]

Whereas the transiently generated DAG binds only weakly to the C1 domains of the enzyme, phorbol esters bind to the same DAG-binding site in a competitive manner with affinities several orders of magnitude greater than those of DAGs and have provided powerful pharmacological tools for studying PKC function.[5,6] Phorbol esters function as potent and metabolically stable DAG surrogates because their conformationally rigid scaffold, unlike the flexible glycerol backbone of DAG, is able to specifically direct the hydrophilic pharmacophores.

Over the past several years, we have attempted to bridge the affinity gap between phorbol esters and DAGs by two independent but mutually complementary approaches.[7,8] The first approach, the pharmacophore-guided approach, seeks to reduce the entropic penalty associated with DAG binding by constraining the glycerol backbone into a five-member DAG-lactone. The second approach, the receptor-guided approach, involves the use of highly branched alkyl chains to improve the interaction of the DAG-lactone ligand with a cluster of conserved hydrophobic amino acids in the space between the two *β*-sheets of the C1 domain. We have found that the alkyl chains in the DAG-lactones are of importance in controlling binding affinity as a function of size, position on the glycerol scaffold, and degree of branching.

Using these approaches, we have obtained derivatives of a DAG-lactone scaffold bearing branched alkyl groups with reduced lipophilicity and with high affinities as PKC ligands.[9,10] For example, DAG-lactone **1a** (log $P = 5.03$) displayed a high affinity with a $K_i = 2.9$ nM, which was several hundred-fold more potent than the corresponding straight-chain-substituted DAG with similar lipophilicity (K_i = ca. 1 μM).[9] Furthermore, recent enantioselective synthesis confirmed that DAG-lactone **2**, the (*R*)-enantiomer of **1a**, proved to be the "active" isomer with exactly one-half ($K_i = 1.45$ nM) of the K_i value found for the racemate.[11]

The *β*-amyloid peptide (A*β*), a 39−43 amino acid peptide, has been implicated in the pathogenesis of Alzheimer's disease (AD) and is generated from amyloid precursor protein (APP) by stepwise proteolytic processing by the *β*- and *γ*-secretases. Thus, inhibition of the *β*- and *γ*-secretases has been regarded as a promising approach for the development of novel anti-Alzheimer agents.[12−15]

APP is also cleaved by the α-secretase within the A*β* sequence at Lys686−Leu689 to release a large secreted fragment termed sAPPα and a second fragment C83, which is further processed to the *N*-terminally truncated A*β* variant called p3.[16] These fragments are of no pathological significance and rather have shown neuroprotection and synaptotrophic effects.[17] Thus, enhancement of α-secretase activity leads to a reduction of the

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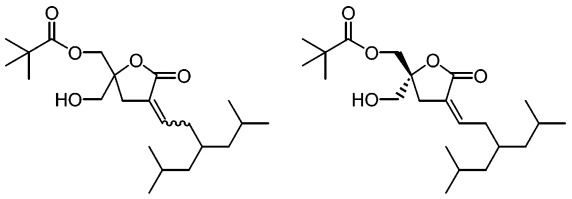
Chart 1



1a Z-isomer
1b E-isomer

2

APP substrate that is available for Aβ formation. Conversely, since the secretases seem to compete for a single pool of APP, decreased α-secretase processing causes elevated Aβ production by shunting more APP into the β-secretase pathway, resulting in increased Aβ deposition.[18,19] Thus, the activation of α-secretase is an attractive pathway to lower Aβ deposition, complementing strategies for inhibition of β- and γ-secretases.

PKC is known to participate in the processing of APP. Although cells contain a certain level of basal α-secretase activity, PKC activators, such as phorbol esters and benzolactams, substantially enhance this proteolysis through α-secretase activation, leading to enhanced production of sAPPα.[15,20] Moreover, activation of receptors that work through protein kinase C can augment α-secretase cleavage of APP with concomitant reduction in β-secretase processing. Thus, these ligands are potential anti-AD drug candidates along with β- and γ-secretase inhibitors.

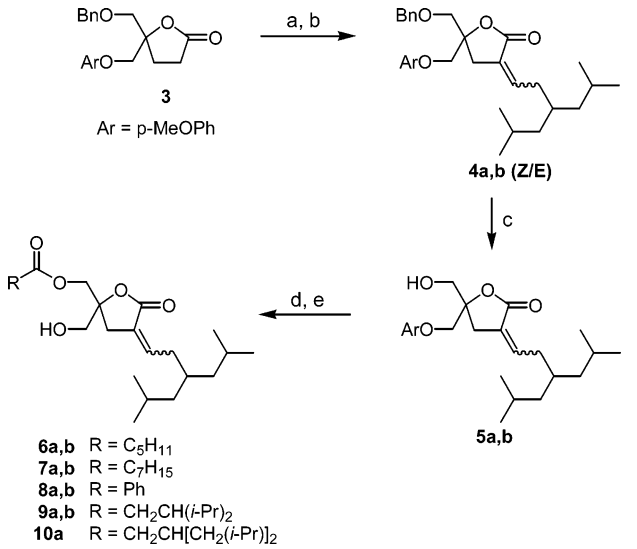
In this work, we investigated the structure−activity relationships of the highly potent DAG-lactone scaffold (**1**) and evaluated the abilities of potent DAG-lactones to inhibit [^{3}H]PDBu binding to PKC-α. We then evaluated a subset of these DAG-lactones as potential anti-amyloidic agents, determining their ability to lead to activation of the α-secretase by measuring the generation of sAPPα, a hydrolyzed product from APP.

Design and Synthesis

The lead branched DAG-lactone (**1**) possesses high binding affinity for PKCα. We used three approaches to optimize its activity. First, the 5-pivaloyl group of **1** was substituted with diverse acyl groups and with *tert*-butyl acrylate as an isostere, while the 3-alkylidene group was fixed as the 3,3-diisobutylpropylidene group. Second, the 3-(3,3-diisobutylpropylidene) chain was replaced by 3,3-diisopentyl or 3,3-diisopentenyl groups to change the disposition of the side chain. Finally, the γ-lactone moiety was replaced with a one-carbon-enlarged δ-lactone scaffold.

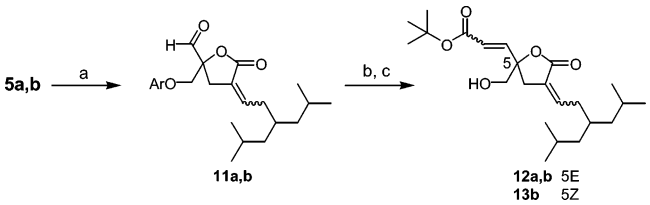
The syntheses of 3-(3,3-diisobutylpropylidene) DAG-lactone analogues (**6**−**10**) were completed from lactone **3**[18] employing a well-established methodology developed in our laboratory involving aldol condensation with 3,3-diisobutyl-1-propionaldehyde followed by elimination of the β-hydroxy lactone intermediate. Consistent with previously synthesized DAG-lactones, the *E*/*Z* geometry was assigned based on the relative chemical shift of vinyl protons in which the vinyl proton of the *E* isomer was farther downfield than that of the *Z* isomer by $\delta = 0.6-0.8$. After separation of the geometric isomers **4a** and **4b**, the isomers individually were converted to the corresponding DAG-lactones with different 5-acyl groups by conventional methods (Scheme 1). The syntheses of the *tert*-butyl acrylate analogues (**12**, **13**) were accomplished using Wittig olefination from aldehyde intermediate **11**, which was prepared from **5** by Swern oxidation (Scheme 2). 3,3-Bisalkylated lactones (**15**, **16**) were synthesized from lactone **3** by the enolate alkylation using

Scheme 1[a]



[a] Reagents and Conditions: (a) LiHMDS, $[(CH_3)_2CHCH_2]_2CHCH_2CHO$, −78 °C, THF; (b) (1) MsCl, NEt_3, CH_2Cl_2, (2) DBU; (c) BCl_3, CH_2Cl_2, −78 °C; (d) RCOCl, NEt_3, CH_2Cl_2; (e) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O, 0 °C.

Scheme 2[a]



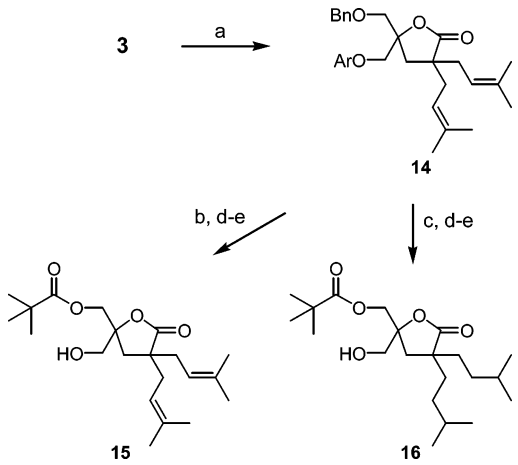
[a] Reagents and Conditions: (a) $(COCl)_2$, DMSO, NEt_3, CH_2Cl_2, −78 °C to rt; (b) Ph_3PCHCO_2tBu, CH_2Cl_2, rt; (c) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O, 0 °C.

2 equiv of isopentenyl bromide (Scheme 3). The syntheses of δ-lactone surrogates (**24**−**26**) were initially attempted employing the protocol of direct aldol condensation to the δ-lactone moiety with branched aldehydes as described in Scheme 1. Unfortunately, the condensation resulted in very low yield, and the strategy was therefore revised to the method described in Scheme 4. The intermediate **18**, previously reported,[21] was converted to the corresponding aldehyde and then elongated by two carbons in two steps. Aldol condensation of **21** with aldehyde and subsequent elimination produced **22** as an intractable mixture of *E*/*Z* isomers. The mixture underwent cyclization under acidic conditions to provide lactone **23**, whose geometric isomers, **23a** and **23b**, could be separated at this stage by column chromatography. With each isomer, the complete syntheses of **24**−**26** were performed individually.

Results and Discussion

Binding Studies. The interaction of the target DAG-lactones with PKC was assessed in terms of the ability of the ligands to displace bound [20-^{3}H]phorbol 12,13-dibutyrate (PDBu) from recombinant PKC-α in the presence of phosphatidylserine as previously described.[10] The IC_{50} values were determined by fitting the data points to the theoretical competition curve. The K_i values for inhibition of binding were calculated from the corresponding IC_{50} values (Tables 1−3). On the basis of the lead branched DAG-lactones (**1a**, $K_i = 2.90$ nM; **1b**, $K_i = 4.51$ nM) previously communicated, we fully explored the structure−activity relationships of the pharmacophoric regions. The cLog

Scheme 3[a]



[a] Reagents and Conditions: (a) LiHMDS, $(CH_3)_2C{=}CHCH_2Br$, −78 °C, HMPA, THF; (b) BCl_3, CH_2Cl_2, −78 °C; (c) H_2, Pd−C, EtOH; (d) $(CH_3)_3CCOCl$, NEt_3, CH_2Cl_2; (e) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O, 0 °C.

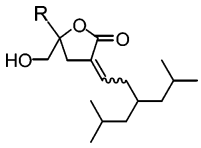
P values were calculated according to the fragment-based program KOWWIN.[22]

We modified the C_5-acyl moiety (pivaloyl group in **1**) by substituting with linear acyl groups (**6**, R = C_5H_{11}; **7**, R = C_7H_{15}), an aryl group (**8**, R = Ph), and bulkier branched acyl groups (**9**, R = $CH_2CH(i\text{-Pr})_2$; **10**, R = $CH_2CH[CH_2(i\text{-Pr})]_2$). Although they still retained high binding affinities for PKC-α (K_i = 3.78−16.1 nM), the potencies did not exceed that of the lead compound despite their higher log *P* values.

Previously, we demonstrated that the isosteric substitutions of the C_5-ester moiety in DAG-lactones with amide or *N*-hydroxyamide led to a substantial loss of activity.[9,23] Thus, as a second approach we replaced the C_5-acyloxy moiety with *tert*-butyl *E*/*Z*-acrylate (**12**, *E*; **13**, *Z*), a transposed isostere of 5-pivaloyloxymethyl. The result revealed that the activities were very sensitive to the geometry of the acrylate. Whereas the replacement with *tert*-butyl *E*-acrylate led to moderate reductions in binding affinities (6-fold in **12a**, 38-fold in **12b**) as compared to **1a** and **1b**, respectively, *tert*-butyl *Z*-acrylate (**13**) was observed to have very low affinity. To date, the SAR analysis of the C_5-acyl group in a series of 3-(3,3-diisobutylpropylidene) DAG-lactones has indicated that the pivaloyl group is optimal as the C_5-acyl group.

After identifying the optimizing group for the C_5-ester moiety, we next turned to the SAR of the 3-alkylidene group in lead **1**. We previously found that, in a series of 3-alkylidenes, the branched alkyl chains conferred higher binding affinities and lower lipophilicities than did the corresponding straight chains.[10]

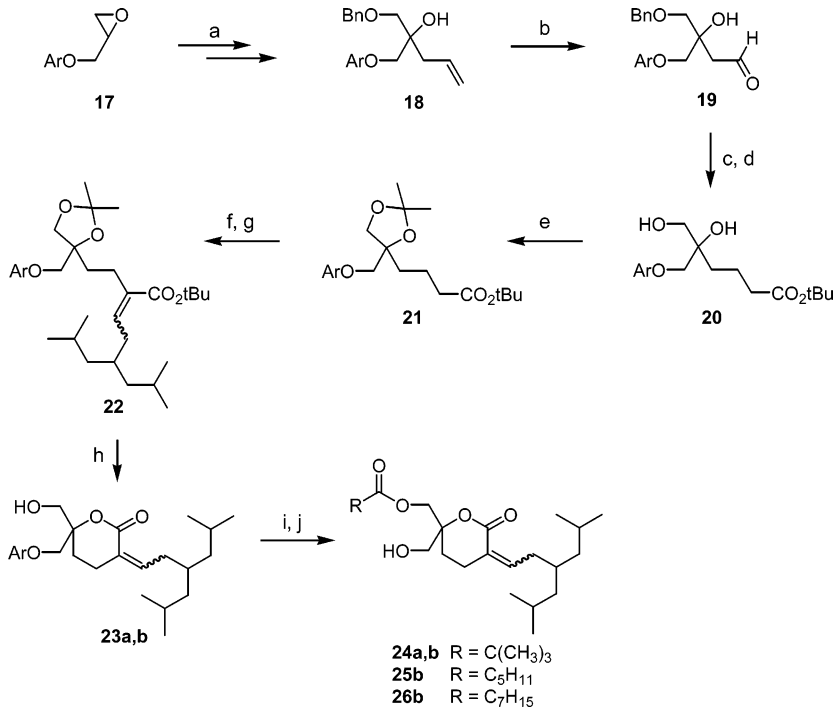
Table 1. Binding Affinities of 3-Alkylidene DAG-γ-Lactones to PKC-α



no.	R	*E*/*Z*	CLog *P*	K_i (nM)
1a	$CH_2OCOC(CH_3)_3$	*Z*	5.03	2.90 (±0.35)
1b	$CH_2OCOC(CH_3)_3$	*E*	5.03	4.51 (±0.49)
2[a]	$CH_2OCOC(CH_3)_3$	*Z*	5.03	1.45 (±0.20)
6a	$CH_2OCOC_5H_{11}$	*Z*	5.63	3.78 (±0.18)
6b	$CH_2OCOC_5H_{11}$	*E*	5.63	6.18 (±0.77)
7a	$CH_2OCOC_7H_{15}$	*Z*	6.61	4.97 (±0.17)
7b	$CH_2OCOC7H_{15}$	*E*	6.61	6.04 (±0.55)
8a	CH_2OCOPh	*Z*	5.12	6.66 (±0.90)
8b	CH_2OCOPh	*E*	5.12	16.1 (±0.4)
9a[b]	$CH_2OCOCH_2CH(i\text{-Pr})_2$	*Z*	6.88	6.87 (±0.56)
9b[b]	$CH_2OCOCH_2CH(i\text{-Pr})_2$	*E*	6.88	4.46 (±0.37)
10a	$CH_2OCOCH_2CH[CH_2(i\text{-Pr})]_2$	*Z*	7.86	6.88 (±0.67)
12a	(*E*)-CH=$CHCO_2C(CH_3)_3$	*Z*	5.30	18.0 (±1.6)
12b	(*E*)-CH=$CHCO_2C(CH_3)_3$	*E*	5.30	173 (±16)
13b	(*Z*)-CH=$CHCO_2C(CH_3)_3$	*E*	5.30	21700 (±1600)

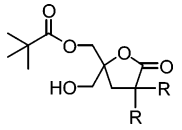
[a] Chiral *R* isomer.[11] [b] Reference 9.

Scheme 4[a]



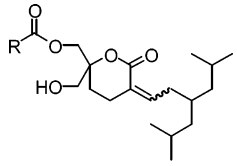
[a] Reagents and Conditions: (a) ref 21; (b) 4-NMO, OsO_4, $NaIO_4$, acetone−H_2O; (c) Ph_3PCHCO_2tBu, toluene; (d) H_2, Pd−C, EtOAc; (e) *p*-TsOH, acetone; (f) LiHMDS, $[(CH_3)_2CHCH_2]_2CHCH_2CHO$, −78 °C, THF; (g) (1) MsCl, NEt_3, CH_2Cl_2, (2) DBU; (h) CF_3CO_2H, CH_2Cl_2; (i) RCOCl, NEt_3, DMAP, CH_2Cl_2; (j) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O, 0 °C.

Table 2. Binding Affinities of 3,3-Bisalkyl DAG-γ-Lactones to PKC-α



no.	R	CLog *P*	K_i (nM)
15	$CH_2CH{=}C(CH_3)_2$	4.49	2100 (±260)
16	$CH_2CH_2CH(CH_3)_2$	4.66	18300 (±1600)

Table 3. Binding Affinities of Branched DAG-δ-Lactones to PKC-α



no.	R	*E*/*Z*	CLog *P*	K_i (nM)
24a	$C(CH_3)_3$	*Z*	5.52	28 (±1)
24b	$C(CH_3)_3$	*E*	5.52	48 (±2)
25b	C_5H_{11}	*E*	6.12	147 (±4)
26b	C_7H_{15}	*E*	7.10	129 (±8)

This time we decided to replace the 3-alkylidene group with a 3,3-bis-alkyl group. In this study, we introduced isopentenyl (**15**) and isopentyl (**16**) groups at the 3-position because they have similar numbers of carbons as does the 3,3-diisobutylpropylidene group in **1**. Unfortunately, incorporation of two branched side chains led to a dramatic (more than 1000-fold) reduction in binding affinity.

Finally, we investigated the SAR of the lactone ring in the lead compound. It was previously observed that the substitutions with five-membered ring isosteres, such as lactam, *N*-hydroxylactam, and cyclopentanone, did not further optimize the potent lead PKC ligand.[9,24] Thus, we explored six-membered lactone surrogates (**24**−**26**) as an approach to ring expansion. δ-Lactone surrogates (**24a**, **24b, 25**, and **26**) retained good binding affinities but with 10−20-fold lower potencies as compared to the corresponding parent compounds (**1a**, **1b, 6b**, and **7b**), respectively. Interestingly, the reduction in potencies upon ring expansion was very consistent, implying that a series of δ-lactone surrogates has a SAR pattern very similar to that found in the γ-lactone series.

α-Secretase Activation in Vitro. The abilities of the synthesized DAG-lactones to activate α-secretase were evaluated by measuring the amount of secreted sAPPα in W4 cells,[25] which are a human APP695 transfected rat neuroblastoma cell line.[26] The amount of secreted sAPPα, the hydrolysis product of APP upon cleavage by the α-secretase, was measured by gel electrophoresis and immunoblot analysis with monoclonal antibody 6E10 that recognizes the *N*-terminus of the Aβ peptide. The increased amount of sAPPα reflects increased α-secretase activity.[25,27] The intensity of the sAPPα band in the experimental groups was analyzed by densitometry and compared to that of the control group. Three independent experiments were conducted for each compound at a concentration of 1 μM. The results are shown in Table 4 and Figure 1, and phorbol 12,13-dibutyrate (PBDu) was used as a reference compound.

Nine selected DAG-lactones with high binding affinities were initially screened for induction of sAPPα secretion at a concentration of 1 μM (Table 4). As anticipated, all tested compounds showed a substantial effect (130−225%) as compared to the vehicle control (100%). The amount of secreted

Table 4. Activation of α-Secretase by Branched DAG-Lactones

compound	sAPPα secretion (% of control)[a]
control	100
PDBu	185 (±19)
1a	177 (±16)
1b	133 (±7)
2	209 (±22)
7a	158 (±8)
7b	141 (±12)
8a	157 (±9)
8b	131 (±10)
9b	225 (±7)
12a	200 (±8)

[a] DAG-lactones increase production of sAPPα after challenge to W4 cells. The activation represented % of control. Values represent the mean ± SEM of three experiments.

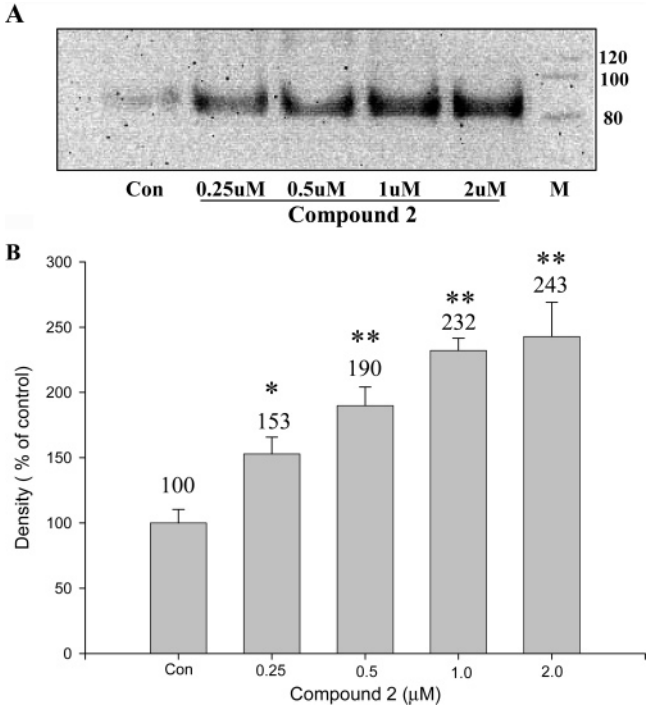


Figure 1. DAG-lactone **2** activated α-secretase activity in a dose-dependent manner. (A) Western blot of the amount of secreted sAPPα as a function of the concentration of DAG-lactone **2**. Results shown are representative of three independent experiments. (B) Densitometric analysis of the amount of secreted sAPPα as a function of the concentration of DAG-lactone **2**. Values indicate the mean ± SEM of three experiments (*, $P < 0.05$; **, $P < 0.01$, paired *t*-test).

sAPPα correlated well with the binding affinities to PKC-α for a series of DAG-lactones with the same log *P* values (**1a** vs **1b**, **7a** vs **7b**, and **8a** vs **8b**). In addition, for pairs of compounds with similar K_i values for PKCα binding but with different lipophilicities, the secretion was enhanced for the compound with the greater lipophilicity (**7b** vs **9b** and **8b** vs **12a**). These results imply that both PKC binding affinity and lipophilicity contribute to α-secretase activation leading to sAPPα secretion. To further explore quantitatively the effect of DAG-lactones on sAPPα secretion, two potent PKC ligands, **1a** ($K_i = 2.9$ nM) and its active enantiomer **2** ($K_i = 1.45$ nM),[11] were assayed and compared with PDBu (1 μM). As shown in Table 4, the two ligands exhibited significantly enhanced secretion of sAPPα. The relative levels of secreted sAPPα induced by **1a** and **2** were 177 ± 16% and 209 ± 22% (mean ± SEM), respectively, compared to control ($p = 0.068$ and 0.002, respectively, paired *t*-test). These values are similar to the level of secretion induced by PDBu (185 ± 19%). Furthermore, sAPPα secretion by **2**

increased in a dose-dependent manner as illustrated in Figure 1, in which the level of secreted sAPPα increased as the concentration of **2** increased up to 2 μM. These results demonstrate that the DAG-lactones enhanced sAPPα secretion through α-secretase activation in a fashion similar to the phorbol esters.

In conclusion, we investigated a series of branched DAG-lactones as PKC ligands and α-secretase activators based on a potent lead PKC ligand (**1**). The C_5-acyl group, 3-alkylidene, and lactone ring in **1** were systematically modified, and structure−activity relationships within the series have been analyzed. The measurement of the amount of secreted sAPPα generated from α-secretase activation by ligands indicated that they were potent α-secretase activators with potencies which correlated with their PKC binding affinities and lipophilicities. Further investigation with **1a** and its active enantiomer **2** demonstrated that they induce as high or higher a level of secreted sAPPα as does PDBu, and the activity was dose dependent. Our results support the approach of using PKC ligands to enhance sAPPα secretion by α-secretase activation and thus reduce plaque burden and suggest that DAG-lactones are potential anti-Alzheimer's drug candidate.

Experimental Section

All chemical reagents were commercially available. Melting points were determined on a Melting Point Büchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230−400 mesh, Merck. Proton NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Infrared spectra were recorded on a Perkin-Elmer 1710 Series FTIR. Mass spectra were recorded on a VG Trio-2 GC-MS. Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments.

5-[(Benzyloxy)methyl]-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furanone (4a,b). A cooled solution of **3** (2 g, 5.85 mmol) in THF (40 mL) at −78 °C was treated dropwise with lithium bis(trimethylsilyl)amide (8.2 mL, 1.0 M in THF) and stirred for 20 min. A solution of 3-isobutyl-5-methylhexanal (1.39 g, 8.2 mmol) was added dropwise to the lithium enolate at the same temperature. After being stirred at −78 °C for 3 h, the reaction was quenched by the slow addition of a saturated aqueous solution of ammonium chloride and then warmed to room temperature. The aqueous layer was extracted three times with diethyl ether, and the combined organic extracts were washed with water followed by brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:3) as eluent to afford *β*-hydroxylactone as an oil (2.85 g, 95%)

A cooled solution of *β*-hydroxylactone (2.85 g, 5.56 mmol) in CH_2Cl_2 (40 mL) at 0 °C was treated with triethylamine (1.55 mL, 11.12 mmol) followed by methanesulfonyl chloride (0.65 mL, 8.34 mmol). The reaction mixture was warmed to room temperature and stirred for 3 h. 1,8-Diazabicyclo[5.4.0]undec-7-ene (3.4 mL, 22.24 mmol) was added, and the resulting solution was stirred for 30 min at ambient temperature. The reaction mixture was neutralized with acetic acid and diluted with CH_2Cl_2. The organic layer was washed with water followed by brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc:hexane (1:3) as eluent to afford **4a** (*Z* isomer) and **4b** (*E* isomer) as an oil, respectively (ratio = 5:4, 2.09 g, 76%).

4a: R_f = 0.62 (EtOAc:hexanes = 1:3). ^{1}H NMR ($CDCl_3$) *δ* 7.30 (m, 5 H, phenyl), 6.81 (s, 4 H, 4-methoxyphenyl), 6.21 (m, 1 H, >C═CH), 4.59 (AB dd, 2 H, *J* = 12.4 and 14.4 Hz, $PhCH_2O$), 4.02 (AB dd, 2 H, *J* = 9.7 and 22.7 Hz, CH_2Ar), 3.76 (s, 3 H, OCH_3), 3.66 (AB dd, 2 H, *J* = 10.2 and 18.0 Hz, $BnCH_2O$), 2.92 (m, 2 H, H-4), 2.70 (m, 2 H, >C═CH−CH_2), 1.55−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.10 (t, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (d, 12 H, 4 × CH_3); IR (neat) 1760 (C═O), 1680 cm^{-1}.

4b: R_f=0.60 (EtOAc:hexanes=1:3). ^{1}H NMR ($CDCl_3$) *δ* 7.25 (m, 5 H, phenyl), 6.75 (m, 5 H, >C═CH and 4-methoxyphenyl), 4.53 (s, 2 H, $PhCH_2O$), 3.98 (AB dd, 2 H, *J* = 9.7 and 19.5 Hz, CH_2Ar), 3.69 (s, 3 H, OCH_3), 3.57 (AB dd, 2 H, *J* = 10.2 and 18.0 Hz, $BnCH_2O$), 2.78 (m, 2 H, H-4), 2.08 (m, 2 H, >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.04 (t, 4 H, 2 × $CHCH_2CHMe_2$), 0.80 (d, 12 H, 4 × CH_3); IR (neat) 1759 (C═O), 1680 cm^{-1}.

5-Hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furanone (5a,b). A cooled solution of **4a** (or **4b**) (1.16 g, 2.35 mmol) in CH_2Cl_2 (20 mL) at −78 °C was treated with boron trichloride (4.70 mL, 1 M in CH_2Cl_2, 4.7 mmol) and stirred for 2 h. The reaction mixture was quenched with saturated $NaHCO_3$ solution at −78 °C and warmed to room temperature. The resulting solution was extracted with diethyl ether several times. The combined organic layer was washed with water followed by brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:1) as eluent to afford **5a** (or **5b**) as an oil (0.94 g, 99%).

5a (*Z* isomer): ^{1}H NMR ($CDCl_3$) *δ* 6.82 (s, 4 H, Ar), 6.26 (m, 1 H, >C═CH), 4.02 (AB dd, 2 H, *J* = 9.5 and 25.6 Hz, CH_2Ar), 3.82 (AB ddd, 2 H, CH_2OH), 3.77 (s, 3 H, OCH_3), 2.98 (m, 1 H, H-4a), 2.88 (m, 1 H, H-4b), 2.70 (m, 2 H, > C═CH−CH_2), 1.91 (t, 1 H, OH), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.10 (t, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 12 H, 4 × CH_3).

5b (*E* isomer): ^{1}H NMR ($CDCl_3$) *δ* 6.82 (m, 5 H, >C═CH and Ar), 4.02 (AB dd, 2 H, *J* = 9.5 and 26.3 Hz, CH_2Ar), 3.83 (AB ddd, 2 H, CH_2OH), 3.76 (s, 3 H, OCH_3), 2.92 (m, 1 H, H-4a), 2.77 (m, 1 H, H-4b), 2.15 (m, 2 H, >C═CH−CH_2), 1.93 (t, 1 H, OH), 1.55−1.75 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 12 H, 4 × CH_3).

General Procedure for Acylation and Deprotection. A stirred solution of **5a** (or **5b**) (404 mg, 1 mmol), (dimethylamino)pyridine (12 mg, 0.1 mmol), and triethylamine (0.28 mL, 2 mmol) in CH_2Cl_2 (20 mL) was cooled to −10 °C and treated slowly with acyl chloride (1.4 mmol). The reaction mixture was warmed to room temperature and stirred for 3 h. The mixture was quenched with water and extracted with diethyl ether several times. The combined organic layer was washed with water followed by brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with an appropriate eluent.

A stirred solution of the above ester in acetonitrile−water (4:1, 25 mL) was cooled to −0 °C and treated with ammonium cerium nitrate (1.1 g, 2 mmol). Stirring in an ice bath continued for 40 min, and the reaction mixture was diluted with CH_2Cl_2. The organic layer was washed with water followed by brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with an appropriate eluent to give **6**−**10**.

5-Hexanoyloxymethyl-5-hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]tetrahydro-2-furanone (6a,b). 6a (*Z* isomer): 63% yield, oil; ^{1}H NMR ($CDCl_3$) *δ* 6.26 (m, 1H, >C═CH), 4.29 (AB d, 1 H, *J* = 11.9 Hz, CH_2OCOR), 4.16 (AB d, 1 H, *J* = 11.9 Hz, CH_2OCOR), 3.66 (ddd, 2 H, CH_2OH), 2.91 (m, 1 H, H-4a), 2.6−2.75 (m, 3 H, H-4b and >C═CH−CH_2), 2.34 (t, 2 H, *J* = 7.3 Hz, $COCH_2$), 2.10 (t, 1 H, OH), 1.55−1.7 (m, 5 H, $COCH_2CH_2$, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.30 (m, 4 H, $CO(CH_2)_2(CH_2)_2CH_3$), 1.09 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.85−0.92 (m, 15 H, 5 × CH_3); IR (neat) 3456 (OH), 1755 (C═O) cm^{-1}; MS (FAB) *m*/*z* 397 (MH^+). Anal. ($C_{23}H_{40}O_5$) C, H.

6b (*E* isomer): 65% yield, oil; ^{1}H NMR ($CDCl_3$) *δ* 6.80 (m, 1 H, >C═CH), 4.30 (AB d, 1 H, *J* = 11.9 Hz, CH_2OCOR), 4.16 (AB d, 1 H, *J* = 11.9 Hz, CH_2OCOR), 3.68 (AB ddd, 2 H, *J* = 7.1, 11.9 and 24.6 Hz, CH_2OH), 2.81 (m, 1 H, H-4a), 2.64 (m, 1 H, H-4b), 2.33 (t, 2 H, *J* = 7.3 Hz, $COCH_2$), 2.1−2.2 (m, 3 H, >C═CH−CH_2 and OH), 1.55−1.75 (m, 5 H, $COCH_2CH_2$, 2 ×

$CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.30 (m, 4 H, $CO(CH_2)_2(CH_2)_2CH_3$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.88 (m, 15 H, 5 × CH_3); IR (neat) 3444 (OH), 1747 (C═O), 1681 cm^{-1}; MS (FAB) *m*/*z* 397 (MH^+). Anal. ($C_{23}H_{40}O_5$) C, H.

5-Hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-5-octanoyloxymethyltetrahydro-2-furanone (7a,b). 7a (*Z* isomer): 64% yield, oil; ^1H NMR ($CDCl_3$) δ 6.25 (m, 1H, >C═CH), 4.28 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 4.16 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 3.65 (AB dd, 2 H, J = 27.9 and 12.1 Hz, CH_2OH), 2.90 (m, 1 H, H-4a), 2.6−2.75 (m, 3 H, H-4b and >C═CH−CH_2), 2.33 (t, 2 H, J = 7.3 Hz, $COCH_2$), 1.55−1.7 (m, 5 H, $COCH_2CH_2$, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.30 (m, 8 H, $CO(CH_2)_2(CH_2)_4CH_3$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.88 (m, 15 H, 5 × CH_3); IR (neat) 3447 (OH), 1745 (C═O), 1670 cm^{-1}; MS (FAB) *m*/*z* 425 (MH^+). Anal. ($C_{25}H_{44}O_5$) C, H.

7b (*E* isomer): 96% yield, oil; ^1H NMR ($CDCl_3$) δ 6.80 (m, 1 H, >C═CH), 4.30 (AB d, 1 H, J = 11.7 Hz, CH_2OCOR), 4.16 (AB d, 1 H, J = 11.7 Hz, CH_2OCOR), 3.68 (AB ddd, 2 H, CH_2OH), 2.81 (m, 1 H, H-4a), 2.63 (m, 1 H, H-4b), 2.33 (t, 2 H, J = 7.6 Hz, $COCH_2$), 2.13 (t, 2 H, >C═CH−CH_2), 2.06 (t, 1 H, OH), 1.5−1.75 (m, 5 H, $COCH_2CH_2$, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.28 (m, 8 H, $CO(CH_2)_2(CH_2)_4CH_3$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.88 (m, 15 H, 5 × CH_3); IR (neat) 3447 (OH), 1745 (C═O), 1670 cm^{-1}; MS (FAB) *m*/*z* 425 (MH^+). Anal. ($C_{25}H_{44}O_5$) C, H.

5-Benzoyloxymethyl-5-hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]tetrahydro-2-furanone (8a,b). 8a (*Z* isomer): 65% yield, oil; ^1H NMR ($CDCl_3$) δ 7.92 (d, 2 H, Ph), 7.52 (t, 1 H, Ph), 7.37 (t, 2 H, Ph), 6.19 (m, 1H, >C═CH), 4.40 (AB dd, 2 H, J = 21 and 11.9 Hz, CH_2OCOR), 3.64 (ddd, 2H, CH_2OH), 2.90 (m, 1 H, H-4a), 2.6−2.75 (m, 3 H, H-4b and >C═CH−CH_2), 2.34 (t, 2 H, J = 7.3 Hz, $COCH_2$), 1.60 (m, 3 H, 2 × $CHMe_2$, $CH(i\text{-}Bu)_2$), 1.18 (d, 2 H, $CHCH_2CHMe_2$), 1.01 (t, 2 H, $CHCH_2CHMe_2$), 0.78 (d, 12 H, 4 × CH_3); IR (neat) 3447 (OH), 1745 (C═O), 1670 cm^{-1}; MS (FAB) *m*/*z* 403 (MH^+). Anal. ($C_{24}H_{34}O_5$) C, H.

8b (*E* isomer): 98% yield, oil; ^1H NMR ($CDCl_3$) δ 8.10 (d, 2 H, Ph), 7.60 (t, 1 H, Ph), 7.44 (t, 2 H, Ph), 6.80 (m, 1 H, >C═CH), 4.47 (AB dd, 2 H, J = 11.9 and 21.2 Hz, CH_2OCOR), 3.76 (AB dd, 2 H, J = 12.2 and 24.1 Hz, CH_2OH), 2.88 (m, 1 H, H-4a), 2.73 (m, 1 H, H-4b), 2.10 (m, 2 H, >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.06 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.83 (d, 12 H, 4 × CH_3); IR (neat) 3441 (OH), 1686 (C═O) cm^{-1}; MS (FAB) *m*/*z* 403 (MH^+). Anal. ($C_{24}H_{34}O_5$) C, H.

5-Hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-5-(3-isopropyl-4-methyl-pentanoyloxymethyl)tetrahydro-2-furanone (9a,b). 9a (*Z* isomer): 28% yield, oil; ^1H NMR ($CDCl_3$) δ 6.26 (m, 1 H, >C═CH), 4.27 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 4.14 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 3.66 (AB dd, 2 H, J = 11.9 and 24.6 Hz, CH_2OH), 2.85−2.95 (m, 1 H, H-4a), 2.6−2.75 (m, 1 H, H-4b and >C═CH−CH_2), 2.21 (d, 2 H, J = 5.8 Hz, $COCH_2$), 2.04 (bs, 1 H, OH), 1.55−1.8 (m, 6 H, 4 × $CHMe_2$, $CH(i\text{-}Pr)_2$ and $CH(i\text{-}Bu)_2$), 1.09 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.75−0.95 (m, 24 H, 8 × CH_3); IR (neat) 3440 (OH), 1685 (C═O) cm^{-1}; MS (FAB) *m*/*z* 439 (MH^+). Anal. ($C_{26}H_{46}O_5$) C, H.

9b (*E* isomer): 32% yield, oil;^1H NMR ($CDCl_3$) δ 6.80 (m, 1 H, >C═CH), 4.29 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 4.14 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 3.68 (AB dd, 2 H, J = 11.9 and 24.6 Hz, CH_2OH), 2.81 (m, 1 H, H-4a), 2.64 (m, 1 H, H-4b), 2.20 (d, 2 H, J = 5.8 Hz, $COCH_2$), 2.13 (m, 2 H, >C═CH−CH_2), 2.04 (bs, 1 H, OH), 1.55−1.8 (m, 6 H, 4 × $CHMe_2$, $CH(i\text{-}Pr)_2$ and $CH(i\text{-}Bu)_2$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.75−0.9 (m, 24 H, 8 × CH_3); IR (neat) 3440 (OH), 1685 (C═O) cm^{-1}; MS (FAB) *m*/*z* 439 (MH^+). Anal. ($C_{26}H_{46}O_5$) C, H.

5-Hydroxymethyl-3-[(*Z*)-3-isobutyl-5-methylhexylidene]-5-(3-isobutyl-5-methyl-hexanoyloxymethyl)tetrahydro-2-furanone (10a). 10a (*Z* isomer): 22% yield, oil; ^1H NMR ($CDCl_3$) δ 6.25 (m, 1 H, >C═CH), 4.27 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 4.16 (AB d, 1 H, J = 11.9 Hz, CH_2OCOR), 3.66 (AB dd, 2 H, J = 11.9 and 24.6 Hz, CH_2OH), 2.85−2.95 (m, 1 H, H-4a), 2.6−2.75 (m, 1 H, H-4b and >C═CH−CH_2), 2.25 (d, 2 H, J = 6.6 Hz, $COCH_2$), 2.05 (bs, 1 H, OH), 1.55−1.75 (m, 6 H, 4 × $CHMe_2$ and 2 × $CH(i\text{-}Bu)_2$), 1.0−1.15 (m, 8 H, 4 × $CHCH_2CHMe_2$), 0.75−0.95 (m, 24 H, 8 × CH_3); IR (neat) 3440 (OH), 1685 (C═O) cm^{-1}; MS (FAB) *m*/*z* 467 (MH^+). Anal. ($C_{28}H_{50}O_5$) C, H.

5-Formyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-5-[(4-methoxyphenoxy)methyl]tetrahydro-2-furanone (11a,b). A cooled solution of oxalyl chloride (0.14 mL, 1.61 mmol) in CH_2Cl_2 (13 mL) at −78 °C was treated with DMSO (0.17 mL, 2.48 mmol) followed by a solution of **5a** (or **5b**) (0.5 g, 1.24 mmol) in CH_2Cl_2 (7 mL). After being stirred at −78 °C for 1 h, the reaction mixture was treated with triethylamine (0.43 mL, 3.1 mmol) and allowed to warm to room temperature. The mixture was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:1) as eluent to afford **11a** (or **11b**) as an oil (0.493 g, 99%).

11a (*Z* isomer): ^1H NMR ($CDCl_3$) δ 9.81 (s, 1 H, CHO), 6.82 (s, 4 H, 4-methoxyphenyl), 6.32 (m, 1 H, >C═CH), 4.21 (s, 2 H, CH_2Ar), 3.76 (s, 3 H, OCH_3), 3.06 (m, 2 H, H-4), 2.70 (m, 2 H, >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.10 (t, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 12 H, 4 × CH_3).

11b (*E* isomer): ^1H NMR ($CDCl_3$) δ 9.75 (s, 1 H, CHO), 6.74 (m, 5 H, 4-methoxyphenyl and >C═CH), 4.0 (m, 2 H, CH_2Ar), 3.69 (s, 3 H, OCH_3), 2.95 (m, 2 H, H-4), 2.10 (m, 2 H, >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.10 (t, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 12 H, 4 × CH_3).

5-[(*E*)-3-(*tert*-Butoxy)-3-oxo-1-propenyl]-5-hydroxymethyl-3-[(*Z*)-3-isobutyl-5 -methylhexylidene]tetrahydro-2-furanone (12a). A solution of **11a** (0.13 g, 0.323 mmol) in CH_2Cl_2 (40 mL) was treated with (*tert*-butoxycarbonylmethylene)triphenylphosphorane (0.186 g, 0.485 mmol) and stirred at room temperature for 24 h. The reaction mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:3) as eluent to afford only *E*-unsaturated ester as an oil (0.16 g, 99%).

A cooled solution of the above unsaturated ester (0.16 g, 0.32 mmol) in acetonitrile (10 mL) and water (4 mL) in an ice bath was treated with CAN (ammonium cerium nitrate, 0.526 g, 0.96 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h. The resulting mixture was filtered through Celite with additional CH_2Cl_2, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc:hexanes (1:3) as eluent to afford **12a** as a white solid (0.066 g, 52%).

12a: mp 135 °C; ^1H NMR ($CDCl_3$) δ 6.74 (d, 1 H, J = 15.6 Hz, CH═$CHCO_2(CH_3)_3$), 6.22 (m, 1 H, >C═CH), 6.04 (d, 1 H, J = 15.6 Hz, CH═$CHCO_2(CH_3)_3$), 3.74 (AB dd, 1 H, J = 6.1 and 12.2 Hz, CH_2OH), 3.60 (AB dd, 1 H, J = 6.1 and 12.2 Hz, CH_2OH), 3.12 (m, 1 H, H-4a), 2.58−2.76 (m, 3 H, H-4b and >C═CH−CH_2), 1.87 (t, 1 H, J = 6.1 Hz, OH), 1.55−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.45 (s, 9 H, $CO_2C(CH_3)_3$), 1.05 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.84 (m, 12 H, 4 × CH_3); IR (neat) 3433, 1715 cm^{-1}; MS (FAB) *m*/*z* 395 (MH^+). Anal. ($C_{23}H_{38}O_5$) C, H.

5-[(*E*/*Z*)-3-(*tert*-Butoxy)-3-oxo-1-propenyl]-5-hydroxymethyl-3-[(*E*)-3-isobutyl-5-methylhexylidene]tetrahydro-2-furanone (12b and 13b). By following the procedure described for the synthesis of **12a**, **12b** (5*E* isomer) and **13b** (5*Z* isomer) with a ratio of 1:1 were obtained with 64% yield from **11b**.

12b: R_f = 0.5 (EtOAc:hexanes = 1:2); ^1H NMR ($CDCl_3$) δ 6.80 (m, 1 H, >C═CH), 6.76 (d, 1 H, J = 15.6 Hz, CH═$CHCO_2(CH_3)_3$), 6.05 (d, 1 H, J = 15.6 Hz, CH═$CHCO_2(CH_3)_3$), 3.76 (AB dd, 1 H, J = 6.1 and 12.2 Hz, CH_2OH), 3.62 (AB dd, 1 H, J = 6.1 and 12.2 Hz, CH_2OH), 3.02 (m, 1 H, H-4a), 2.68 (m, 1 H, H-4b), 2.10 (m, 2 H, >C═CH−CH_2), 1.85 (t, 1 H, J = 6.1 Hz, OH), 1.55−1.7 (m, 3 H, 2 × $CHMe_2$ and $CH(i\text{-}Bu)_2$), 1.46 (s, 9 H, $CO_2C(CH_3)_3$), 1.04 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.84 (d, 12 H, 4 × CH_3); IR (neat) 3435, 1718 cm^{-1}; MS (FAB) *m*/*z* 395 (MH^+). Anal. ($C_{23}H_{38}O_5$) C, H.

13b: R_f = 0.45 (EtOAc:hexanes = 1:2); ^1H NMR ($CDCl_3$) δ 6.72 (m, 1 H, >C═CH), 6.25 (d, 1 H, J = 12.7 Hz, CH═$CHCO_2(CH_3)_3$), 5.83 (d, 1 H, J = 12.7 Hz, CH═$CHCO_2(CH_3)_3$), 3.88 (d, 1 H, J = 6.8 Hz, CH_2OH), 3.24 (m, 1 H, H-4a), 2.82 (m, 1 H,

H-4b), 2.16 (t, 1 H, J = 6.8 Hz, OH), 2.10 (m, 2 H, >C=CH−CH_2), 1.55−1.7 (m, 3 H, 2 × CHMe$_2$ and CH(*i*-Bu)$_2$), 1.48 (s, 9 H, $CO_2C(CH_3)_3$), 1.07 (m, 4 H, 2 × CHCH_2CHMe$_2$), 0.84 (d, 12 H, 4 × CH_3); IR (neat) 3434, 1715 cm^{-1}; MS (FAB) *m*/*z* 395 (MH^+). Anal. ($C_{23}H_{38}O_5$) C, H.

5-[(Benzyloxy)methyl]-5-[(4-methoxyphenoxy)methyl]-3,3-bis-(3-methyl-2-butenyl)dihydro-2(3*H*)-furanone (14). A solution of **3** (342 mg, 1 mmol) in THF (10 mL) was cooled to −78 °C and treated slowly with lithium bis(trimethylsilyl)amide (1.06 M in THF, 2.36 mL, 2.5 mmol). After stirring for 30 min, a solution of 4-bromo-2-methyl-2-butene (0.576 mL, 5 mmol) and hexamethylphosphoramide (4 mL) was added and stirred for 1 h at −78 °C. The reaction mixture was quenched with a solution of ammonium chloride and extracted with diethyl ether several times. The organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexane (1:6) as eluent to afford **14** (392 mg, 82%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.25−7.35 (m, 5 H, Ph), 6.81 (s, 4 H, Ar), 5.05−5.18 (m, 2 H, 2 × CH=C<), 4.56 (dd of AB, 2 H, J = 12 Hz, OCH_2Bn), 4.03 (d, 1 H, J = 9.5 Hz, OCH_2Ar), 3.91 (d, 1 H, J = 9.5 Hz, OCH_2Ar), 3.76 (s, 3 H, OCH_3), 3.59 (dd of AB, 2 H, J = 10 Hz, CH_2OBn), 2.3−2.45 (m, 4 H, 2 × >CCH_2CH=), 2.14 (d, 1 H, J = 13.7 Hz, H-4a), 2.04 (d, 1 H, J = 13.7 Hz, H-4b), 1.66 (s, 6 H, =C(CH$_3$)$_2$), 1.59 (s, 6 H, =C(CH$_3$)$_2$).

5-(Hydroxymethyl)-5-(pivaloyloxymethyl)-3,3-bis(3-methyl-2-butenyl)dihydro-2(3*H*)-furanone (15). By following the procedure described for the synthesis of **6**−**10**, the title compound was obtained as an oil in 65% yield starting from **14**. ^{1}H NMR ($CDCl_3$) δ 5.11 (bt, 2 H, 2 × CH=C<), 4.14 (dd of AB, 2 H, J = 11.7 Hz, CH_2OCO), 3.57 (bs, 2 H, CH_2OH), 2.3−2.45 (m, 4 H, 2 × >CCH_2CH=), 2.17 (s, 1 H, OH), 2.08 (d, 1 H, J = 13.9 Hz, H-4a), 1.85 (d, 1 H, J = 13.9 Hz, H-4b), 1.75 (s, 6 H, =C(CH$_3$)$_2$), 1.64 (s, 6 H, =C(CH$_3$)$_2$), 1.22 (s, 9 H, C(CH$_3$)$_3$); IR (neat) 3479, 2971, 1770, 1737, 1636, 1451 cm^{-1}; MS (EI) *m*/*z* 366 (M^+). Anal. ($C_{21}H_{34}O_5$) C, H.

5-(Hydroxymethyl)-5-(pivaloyloxymethyl)-3,3-bis(3-methylbutyl)dihydro-2(3*H*)-furanone (16). By following the procedure described for the synthesis of **6**−**10**, the title compound was obtained as an oil in 68% yield starting from **14**. ^{1}H NMR ($CDCl_3$) δ 4.29 (d, 1 H, J = 11.9 Hz, CH_2OCO), 4.12 (d, 1 H, J = 11.9 Hz, CH_2OCO), 3.63 (dd of AB, 2 H, J = 12 Hz, CH_2OH), 2.45 (bs, 1 H, OH), 2.15 (d, 1 H, J = 13.3 Hz, H-4a), 1.90 (d, 1 H, J = 13.3 Hz, H-4b), 1.45−1.73 (m, 6 H), 1.25−1.35 (m, 2 H), 1.05−1.2 (m, 2 H), 1.23 (s, 9 H, C(CH$_3$)$_3$), 0.85−0.93 (m, 12 H, 4 × CH_3); IR (neat) 3502, 2956, 1769, 1736, 1538, 1463 cm^{-1}; MS (EI) *m*/*z* 370 (M^+). Anal. ($C_{21}H_{38}O_5$) C, H.

4-Benzyloxy-3-hydroxy-3-[(4-methoxyphenoxy)methyl]-1-butanal (19). A solution of **18**[21] (6.54 g, 20 mmol) in acetone (20 mL) and H_2O (20 mL) was treated with 4-methylmorpholine *N*-oxide (2.34 g, 20 mmol), sodium periodate (4.28 g, 20 mmol), and osmium tetraoxide (2.5 mL, 2.5 wt % in 2-methyl-2-propanol, 0.2 mmol) and stirred for 20 h at room temperature. The reaction mixture was diluted with EtOAc and filtered. The filtrate was sequentially washed with sodium thiosulfate solution, H_2O, and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:3) as eluent to afford **19** as an oil (3.94 g, 58%). ^{1}H NMR ($CDCl_3$) δ 9.90 (t, 1 H, J = 2.4 Hz, CHO), 7.25−7.4 (m, 5 H, phenyl), 6.82 (s, 4 H, Ar), 4.56 (s, 2 H, OCH_2Ph), 3.92 (s, 2 H, CH_2OAr), 3.77 (s, 3 H, OCH_3), 3.60 (AB q, 2 H, J = 9.3 Hz, CH_2OBn), 3.12 (bs, 1 H, OH), 2.77 (d, 2 H, J = 2.4 Hz, CH_2CHO).

***tert*-Butyl 5,6-Dihydroxy-5-[(4-methoxyphenoxy)methyl]hexanoate (20).** A mixture of **19** (1.725 g, 5.2 mmol) and (*tert*-butoxycarbonylmethylene)triphenylphosphorane (3.915 g, 10.4 mmol) in toluene (10 mL) was refluxed for 2 h and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:2) as eluent to afford unsaturated ester as an oil (1.465 g, 73%). A suspension of above ester (1.465 g, 3.8 mmol) and 10% palladium on carbon (0.73 g) in EtOAc (8 mL) was hydrogenated under a balloon of hydrogen for 2 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:1) as eluent to afford **20** as an oil (1.112 g, 97%). ^{1}H NMR ($CDCl_3$) δ 6.84 (s, 4 H, Ar), 3.89 (AB q, 2 H, J = 9.2 Hz, CH_2OAr), 3.77 (s, 3 H, OCH_3), 3.65 (AB q, 2 H, J = 11.2 Hz, CH_2OH), 2.76 (bs, 1 H, OH), 2.36 (t, 2 H, J = 6.8 Hz, CH_2CO_2), 1.6−1.8 (m, 4 H, >CCH_2CH_2), 1.43 (s, 9 H, $CO_2C(CH_3)_3$).

***tert*-Butyl 4-{[(4-Methoxyphenoxy)methyl]-2,2-dimethyl-1,3-dioxolan-4-yl}butanoate (21).** A mixture of **20** (2.04 g, 6 mmol) and *p*-toluenesulfonic acid (0.114 g, 6 mmol) in acetone (10 mL) was stirred for 1 h at room temperature. The mixture was neutralized with solid $NaHCO_3$ and filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:4) as eluent to give **21** as an oil (1.96 g, 85%). ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 4 H, Ar), 4.08 (d, 1 H, J = 8.8 Hz, CH_2OAr), 3.83 (m, 3 H, CH_2OAr and >CCH_2O), 3.77 (s, 3 H, OCH_3), 2.25 (t, 2 H, J = 6.6 Hz, CH_2CO_2), 1.6−1.8 (m, 4 H, >CCH_2CH_2), 1.44 (m, 15 H, $CO_2C(CH_3)_3$ and >C(CH$_3$)$_2$).

6-Hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-6-[(4-methoxyphenoxy)methyl]tetrahydro-2-pyranone (23a,b). A stirred solution of **21** (1.14 g, 3 mmol) in THF (6 mL) was cooled to −78 °C and treated dropwise with lithium bis(trimethylsilyl)-amide (1 M in THF, 4 mL, 4 mmol). After 30 min, 3-isobutyl-5-methylhexanal (0.47 g, 3 mmol) in THF (3 mL) was added and stirring was continued for 2 h at −78 °C. The reaction was quenched by the slow addition of a saturated aqueous solution of ammonium chloride and extracted with ether several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:10) as eluent to give β-hydroxylactone as an oil (660 mg, 40%). A solution of above compound (660 mg, 1.2 mmol) in CH_2Cl_2 (6 mL) was cooled to 0 °C and treated with triethylamine (0.668 mL, 4.8 mmol) and methanesulfonyl chloride (274 mg, 2.4 mmol). After stirring for 4 h at room temperature, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 730 mg, 4.8 mmol) was added and the resulting solution was refluxed for 24 h. The reaction mixture was concentrated in vacuo, and the residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:10) as eluent to give **22** as an inseparable mixture of *E* and *Z* isomers as oils (290 mg, 45%). **22** (290 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (5 mL), cooled to 0 °C, and treated with trifluoroacetic acid (0.5 mL). After being stirred for 24 h at room temperature, the mixture was concentrated in vacuo and the residue purified by flash column chromatography on silica gel with EtOAc:hexanes (1:4) as eluent to give **23a** (*Z* isomer, 18 mg, 8%) and **23b** (*E* isomer, 75 mg, 36%) as oils, respectively.

23a: ^{1}H NMR ($CDCl_3$) δ 6.15 (m, 1 H, >C=CH), 4.08 (AB d, 1 H, J = 9.2 Hz, CH_2OAr), 3.96 (AB d, 1 H, J = 9.3 Hz, CH_2OAr), 3.77 (AB q, 2 H, J = 7.0 Hz, CH_2OH), 3.77 (s, 3 H, OCH_3), 2.5−2.7 (m, 2 H, H-4 and >C=CH−CH_2), 1.9−2.2 (m, 2 H, H-5), 1.5−1.75 (m, 3 H, 2 × CHMe$_2$, CH(*i*-Bu)$_2$), 1.10 (m, 4 H, 2 × CHCH_2CHMe$_2$), 0.86 (d, 12 H, 4 × CH_3).

23b: ^{1}H NMR ($CDCl_3$) δ 7.15 (m, 1 H, >C=CH), 4.11 (AB d, 1 H, J = 9.3 Hz, CH_2OAr), 3.94 (AB d, 1 H, J = 9.3 Hz, CH_2OAr), 3.81 (s, 2 H, CH_2OH), 3.77 (s, 3 H, OCH_3), 2.58 (m, 2 H, H-4), 1.95−2.2 (m, 4 H, H-5 and >C=CH−CH_2), 1.5−1.75 (m, 3 H, 2 × CHMe$_2$, CH(*i*-Bu)$_2$), 1.10 (m, 4 H, 2 × CHCH_2CHMe$_2$), 0.86 (d, 12 H, 4 × CH_3).

6-Hydroxymethyl-3-[(*Z*/*E*)-3-isobutyl-5-methylhexylidene]-6-pivaloyloxymethyl-tetrahydro-2-pyranone (24a,b). A cooled solution of **23a** (or **23b**) (30 mg, 0.07 mmol) in CH_2Cl_2 (2 mL) at 0 °C was treated with triethylamine (28 mg, 0.28 mmol), pivaloyl chloride (17 mg, 0.14 mmol), and a catalytic amount of 4-dimethylaminepyridine and stirred for 3 h at room temperature. The mixture was concentrated in vacuo, and the residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:10) as eluent to give the acylated product (32 mg, 90%).

Acylated Product. A solution of the acylated product (32 mg, 0.064 mmol) in CH_3CN-H_2O (4:1, 2 mL) was cooled to 0 °C and treated with ammonium cerium(IV) nitrate (70 mg, 0.128 mmol). After being stirred for 30 min at 0 °C, the mixture was diluted

with CH_2Cl_2. The organic layer was washed with aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc:hexanes (1:2) as eluent to give **24a** (or 24b) as an oil (20 mg, 76%).

24a (*Z* isomer): ^{1}H NMR ($CDCl_3$) δ 6.15 (m, 1 H, >C═CH), 4.30 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 4.13 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 3.64 (AB q, 2 H, J = 11.9 Hz, CH_2OH), 2.5−2.7 (m, 4 H, H-4 and >C═CH−CH_2), 1.75−2.05 (m, 2 H, H-5), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$, CH(*i*-Bu)$_2$), 1.22 (s, 9 H, $C(CH_3)_3$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (d, 12 H, 4 × CH_3); IR (neat) 3443, 1730, 1715, 1634 cm^{-1}; MS (FAB) *m*/*z* 397 (MH^+). Anal. ($C_{23}H_{40}O_5$) C, H.

24b (*E* isomer): ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 1 H, >C═CH), 4.28 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 4.15 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 3.63 (AB q, 2 H, J = 12.2 Hz, CH_2OH), 2.55 (m, 2 H, H-4), 1.8−2.15 (m, 4 H, H-5 and >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$, CH(*i*-Bu)$_2$), 1.22 (s, 9 H, $C(CH_3)_3$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (d, 12 H, 4 × CH_3); IR (neat) 3442, 1732, 1718, 1633 cm^{-1}; MS (FAB) *m*/*z* 397 (MH^+). Anal. ($C_{23}H_{40}O_5$) C, H.

6-Hydroxymethyl-3-[(*E*)-3-isobutyl-5-methylhexylidene]-6-hexanoyloxymethyl-tetrahydro-2-pyranone (25b). The compound was prepared by following the procedure described for the synthesis of **24** using hexanoyl chloride from **23b** in 82% yield. ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 1 H, >C═CH), 4.31 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 4.16 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 3.66 (AB q, 2 H, J = 12.2 Hz, CH_2OH), 2.55 (m, 2 H, H-4), 2.35 (t, 2 H, $CH_2CH_2CO_2$), 1.8−2.15 (m, 4 H, H-5 and >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$, CH(*i*-Bu)$_2$), 1.25−1.4 (m, 6 H, $CH_3(CH_2)_3CH_2CO_2$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 15 H, 5 × CH_3); IR (neat) 3442, 1732, 1718, 1633 cm^{-1}; MS (FAB) *m*/*z* 411 (MH^+). Anal. ($C_{24}H_{42}O_5$) C, H.

6-Hydroxymethyl-3-[(*E*)-3-isobutyl-5-methylhexylidene]-6-octanoyloxymethyl-tetrahydro-2-pyranone (26b). The compound was prepared by following the procedure described for the synthesis of **24** using octanoyl chloride from **23b** in 80% yield. ^{1}H NMR ($CDCl_3$) δ 7.14 (m, 1 H, >C═CH), 4.31 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 4.16 (AB d, 1 H, J = 11.7 Hz, CH_2OCO), 3.66 (AB q, 2 H, J = 12.2 Hz, CH_2OH), 2.55 (m, 2 H, H-4), 2.35 (t, 2 H, $CH_2CH_2CO_2$), 1.8−2.15 (m, 4 H, H-5 and >C═CH−CH_2), 1.5−1.7 (m, 3 H, 2 × $CHMe_2$, CH(*i*-Bu)$_2$), 1.25−1.4 (m, 10 H, $CH_3(CH_2)_5CH_2CO_2$), 1.10 (m, 4 H, 2 × $CHCH_2CHMe_2$), 0.86 (m, 15 H, 5 × CH_3); IR (neat) 3442, 1732, 1718, 1633 cm^{-1}; MS (FAB) *m*/*z* 439 (MH^+). Anal. ($C_{26}H_{46}O_5$) C, H.

Protocol for α-Secretase Activation Assay. *Cell Culture and Compounds Treatment.* B103 rat neuroblastoma cells[23] transfected with human APP695 (W4 cells) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Grand island, NY) containing 10% fetal bovine serum (FBS) (Hyclone, Irvine, CA) and 5% penicillin/streptomycin (Sigma, St. Louis, MO) at 37 °C in 8% CO_2. At the outset 90% confluent cells were dissociated and plated at 2 × 10^6 cells in a 100 mm dish. Cells were starved in plain DMEM for 1 h and then incubated for 18 h in the presence of each drug. Three independent experiments were conducted for each compound at a concentration of 1 μM.

*SDS−PAGE and Western Blot Analysis of sAPP*α. For sAPPα measurement, cultured supernatants were collected and centrifuged briefly to remove cell debris. Proteins were precipitated by incubation with trichloroacetic acid (TCA) (final concentration, 10%) for 2 h at 4 °C and centrifuged at maximum speed for 30 min. The supernatant fluid was removed, and the precipitate was washed with 500 μL of cold acetone and, after evaporation of the acetone, dissolved in sample buffer. Samples were boiled for 10 min and resolved by 10% SDS−polyacrylamide gel (Invitrogen, Carlsbad, CA) electrophoresis.

After blotting to poly(vinylidene difluoride) (PVDF) membranes, nonspecific binding was blocked by incubating the membrane for 1 h in 20 mM Tris HCl, pH 7.8, 137 mM NaCl, 0.05% Tween 20 (TBST) with 5% nonfat dried milk. Membranes were washed in TBST and incubated overnight at 4 °C with sAPPα-specific monoclonal antibody (6E10-Chemicon, Temecula, CA) After further washes, membranes were incubated for 1 h with horseradish peroxidase-linked anti-mouse IgG antibody, and the immunoreactive bands were detected by enhanced chemiluminescence (ECL) (Amersham Pharmacia, Piscataway, NJ).

Densitometric analysis of the western blot data was performed using a Bio-Imaging analyzer (ChemiDoc XRS, Bio-Rad, Hercules, CA & Image Gauge, Fuji, Tokyo).

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Supporting Information Available: Elemental analysis data for final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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