Conformationally Constrained Analogues of Diacylglycerol (DAG). 27. Modulation of Membrane Translocation of Protein Kinase C (PKC) Isozymes α and δ by Diacylglycerol Lactones (DAG-Lactones) Containing Rigid-Rod Acyl Groups

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Received November 3, 2006

Highly rigid and geometrically well-defined rods composed of ethynylene-substituted aromatic spacers [oligo(*p*-phenyleneethynylene), OPE] were incorporated as acyl moieties on diacylglycerol lactones (DAG-lactones) and investigated for their ability to bind to protein kinase C (PKC) and translocate PKC α and δ isoforms to plasma and internal membranes. The kinetics of PKC translocation were correlated with biological responses, viz. ERK phosphorylation, induction of IL-6 secretion, inhibition of cell proliferation, and induction of cellular attachment, that display very different time courses. Because OPE rods assemble through noncovalent forces and form stable films, they may influence the microdomain environment around the DAG-lactone membrane-binding site. A comparison of two DAG-lactones (**1** and **10**), one with two PE units (**1**) and the other with an equivalent flexible acyl chain (**10**) of matching lipophilicity, clearly demonstrated the effect of the rigid OPE chain in substantially prolonging the translocated state of both PKC α and δ.

Introduction

Natural membranes possess a lamellar bilayer organization as revealed by X-ray crystallography.[1] The lipid composition of the membrane controls whether such a lamellar bilayer exists in a liquid disordered state (I_d)[a] or in a liquid ordered state (I_o).[2] In general, lipids that contain unsaturated fatty acid chains with kinks drive the bilayer toward an I_d state, whereas saturated acyl chains tend to stabilize an ordered I_o state. The equilibrium between the I_d and I_o states is dynamic and can be affected by several factors, most notably by the concentration of cholesterol, which is constitutively present in membranes.[3] Cholesterol's rigid ring system and its ability to fill interstitial spaces facilitate a more tightly packed I_d state for the acyl chains of the bilayer.[4] Hence, cholesterol is an important determinant of membrane organization. The cholesterol concentration in different cellular organelles varies; for example, the plasma membrane and the Golgi apparatus have a high concentration of cholesterol and highly ordered membranes.[3] The endoplasmic reticulum (ER), however, has a low concentration of cholesterol and a more disordered membrane organization.[3] These different biophysical properties in membranes can have an important effect on the regulation of signal transduction by aiding or preventing the formation of microdomains that bring together both lipids and proteins involved in cell signaling[5,6]

During the course of our work with diacylglycerol lactones (DAG-lactones), which function as potent surrogates of diacylglycerol to strongly activate protein kinase C (PKC), the nature of the alkyl groups at both the acyl (R_1) and α-alkylidene (R_2) positions emerged as one of the principal determinants in controlling biological activity (Figure 1).[7] For example, switching from simple *n*-alkyl chains to branched alkyl chains[8] or incorporating aromatic moieties at R_1 and/or R_2 produced compounds that in some cases displayed significant degrees of specificity for PKC isozymes and other proteins containing DAG-responsive C1 domains.[9]

Because of its unique role, we initially considered modifying the acyl chain of DAG-lactones with the rigid cholesterol ring system. However, because the resulting DAG-lactones would be extremely lipophilic, we decided instead to replace the acyl chain with an ensemble of simpler, repetitive rigid elements that could be fine-tuned to a desired length and lipophilicity capable of mimicking the effects of cholesterol on the biophysical properties of the membrane. To that effect, we selected

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[a] Abbreviations: BOP−Cl, bis(2-oxo-3-oxazolidinyl)phosphinic acid chloride; CAN, ceric ammonium nitrate; CHO, Chinese hamster ovary; DAG, diacylglycerol; DMAP, dimetheylaminopyridine; DMEM, Dulbecco's modified Eagle's medium; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FRET-responsive, fluorescence resonance energy transfer-responsive; GFP-tagged, green fluorescent protein-tagged; I_d, liquid disordered state; IL, interleukin; I_o, liquid ordered state; LDA, lithium diisopropylamide; LNCaP, androgen-sensitive human prostate carcinoma cells; MRCK, myotonic dystrophy kinase-related cdc42-binding kinase; MsCl, methanesulfonyl chloride; Munc-13, unc-13 homolog; NBA, 3-nitrobenzyl alcohol; OPE, oligo(*p*-phenyleneethynylene); PDBu, [20-^{3}H]phorbol 12,13-dibutyrate; PE, *p*-phenyleneethynylene; PEG, polyethyleneglycol; PKC, protein kinase C; PKD, protein kinase D; PMA, phorbol 12-myristate 13-acetate; RasGRP, ras guanyl releasing protein; RPMI-1640, medium culture developed at Roswell Park Memorial Institute; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM, standard error of the mean; TBAF, tetra-*n*-butylammonium fluoride; TFA, trifluoroacetic acid; THF, tetrahydrofuran; WEHI-231, immature B-cell line.

10.1021/jm061289j
Published on Web 02/07/2007

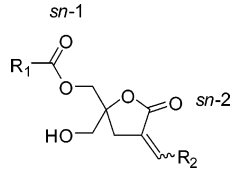
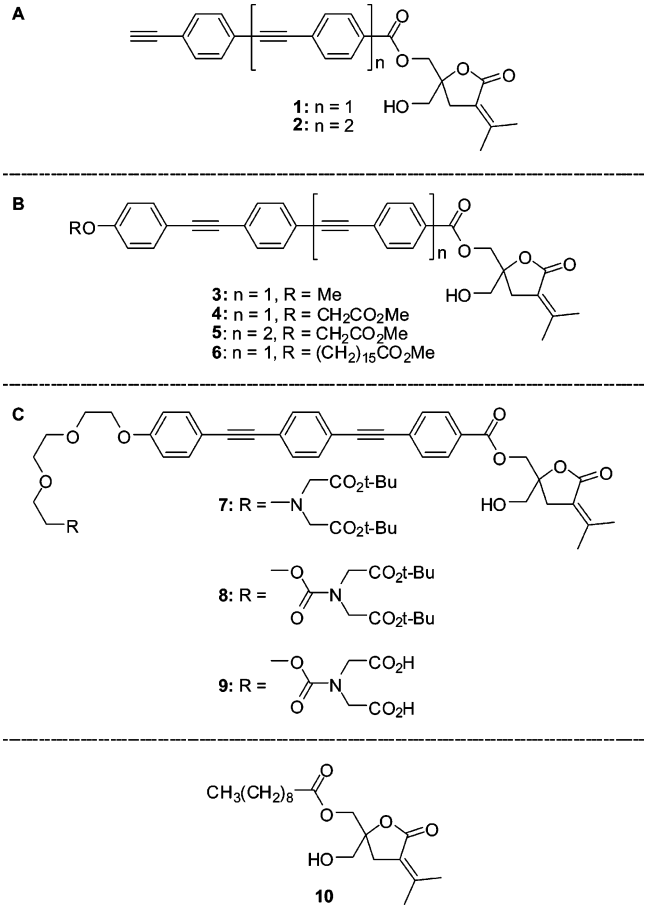


Figure 1. General structure of a DAG-lactone with acyl (R_1) and α-alkylidene (R_2) substitutions.

Scheme 1

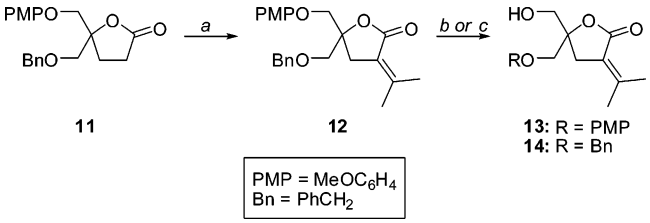


highly rigid and geometrically well-defined oligo(*p*-phenyleneethynylene) (OPE) acyl units of different lengths as membrane phase-modulators designed to achieve various levels of membrane organization and penetration. It is well known that DAGs containing different acyl chain lengths and degrees of unsaturation differentially perturb membranes and PKC activation.[10] Therefore, we expected that rigid OPE rods on DAG-lactones would stabilize a flat lamellar phase[11] and perhaps counter the nonbilayer-prone effect of typical DAGs that tend to induce a localized negative curvature on the membrane.[10] These OPE rigid rods with a conjugated π-electron system have gained considerable attention as polymeric structures in material science because they tend to form main-chain liquid crystals;[12] thus, we expected that their shape-persistent architecture would induce a more ordered I_o state microdomain around the DAG-lactone membrane-binding environment. Because these highly conjugated systems tend to form molecular aggregates, it was also important to explore several rigid-rod sizes to match the optimal length with maximal biological activity. In addition to the expected effect on membrane organization, the OPE backbone could also complex with hydrophobic patches on the surface of PKC as has been reported with other proteins.[13] Taken together, these observations made OPEs attractive candidates to explore as acyl chains in a novel set of DAG-lactones that we report herein.

Table 1. PKC Binding Affinities (K_i) for the α Isozyme and log *P*[18] Values of Target Compounds

compound	log *P*	K_i (nM)
1	3.24	5.23 ± 0.5
2	4.64	11.6 ± 0.3
3	4.67	5.88 ± 0.91
4	4.21	15.8 ± 5.9
5	5.61	126.9 ± 7.3
6	9.28	52.7 ± 5.4
7	6.05	111.1 ± 6.9
8	6.19	18.3 ± 2.6
9	3.68	1391 ± 57
10	3.68	15.9 ± 1.1

Scheme 2[a]



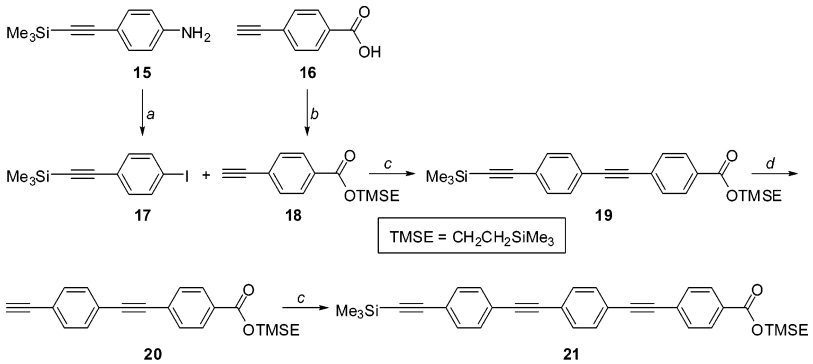
[a] Conditions and reagents: (*a*) i. LDA Acetone, THF, −78 °C. ii. MsCl, DMAP, CH_2Cl_2. (*b*) BCl_3, CH_2Cl_2, −78 °C; (*c*) CAN, CH_3CN/H_2O.

Design of DAG-Lactones with OPE Acyl Chains. The simplest OPE-containing DAG-lactones targeted for synthesis were those with 2 and 3 phenyleneethynylene (PE) units (**1** and **2**, Scheme 1A, Table 1). Compounds with more than three PE units proved to be poorly soluble and difficult to purify, possibly as a result of the well-known propensity of OPEs to form aggregates in solution.[14] This limitation on solubility prevented us from attempting to build DAG-lactones with OPE tethers matching the ca. 30 Å hydrophobic portion of the membrane, so as to span the entire width of the membrane with the rigid rod. Nevertheless, it is possible that the required length of the OPE tether to reach the outer surface of the membrane might be significantly shorter on the basis of the fact that the activated PKC C1 domain penetrates the membrane to a depth of at least 10 Å.[15,16]

1 and **2** were soluble and appeared to be optimal in terms of the log *P* value (Table 1). Log *P*, which measures a compound's ability to distribute between aqueous and lipid milieus, was between 4 and 5, well within the desirable range of other active DAG-lactones (Table 1), while an extra PE unit would increase the log *P* to 6.04. For the sake of simplicity and to avoid the separation of geometrical E and Z isomers, the α-alkylidene fragment was maintained constant as a 3-(methylethylidene) group in all of the compounds.

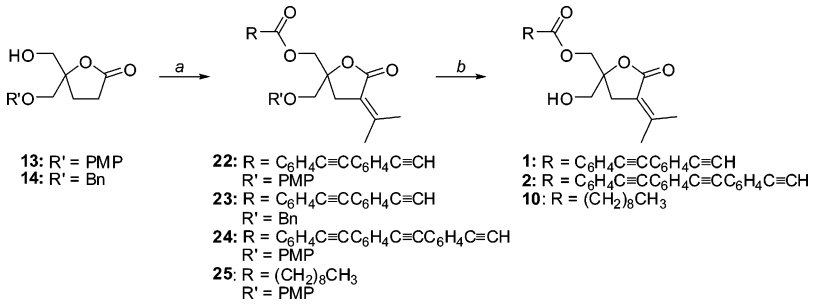
We also investigated the effect of non-ionizable and ionizable polar substituents located at various distances from the terminus of the OPE (Scheme 1). One objective was to modulate interactions with the inner core of the lipid bilayer; a second objective was to explore the possibility of spanning the width of the membrane to interact with the polar outer surface. For a polar, non-ionizable group on the shorter rod, we chose the OMe group (**3**, $n = 1$, Scheme 1B). For a polar ionizable group, we chose the CH_2CO_2Me group (**4**, $n = 1$, Scheme 1B), which might be expected to undergo hydrolysis by esterases in the cellular medium. The CH_2CO_2Me was also placed at the end of the longer OPE rod (**5**, $n = 2$, Scheme 1B) and at the end of a 14-carbon alkyl chain attached to the OPE that was long enough to span the width of the membrane (**6**, $n = 1$, Scheme

Scheme 3[a]



[a] Conditions and reagents: (*a*) $NaNO_2$, HCl, KI. (*b*) $TMSCH_2CH_2OH$, BOP−Cl, CH_2Cl_2. (*c*) **17**, $PdCl_2(PPh_3)_2$, piperidine, CuI, THF. (*d*) TFA, CH_2Cl_2.

Scheme 4[a]



[a] Conditions and reagents: (*a*) for **22**: i. **19**, TBAF, THF. ii. BOP−Cl, DMAP, **13**, CH_2Cl_2. For **23**: i. **19**, TBAF, THF. ii. BOP−Cl, DMAP, **14**, CH_2Cl_2. For **24**: i. **21**, TBAF, THF. ii. BOP−Cl, DMAP, **13**, CH_2Cl_2. For **25**: decanoic anhydride, DMAP, **13**, CH_2Cl_2. (*b*) CAN, CH_3CN/H_2O, CH_2Cl_2.

1B). As mentioned before, compounds with longer rods ($n > 2$) were not soluble and formed molecular aggregates. Inspired by the work of Matile et al.,[17] who used plain oligo(1,4-phenylene) amphiphiles in cell membrane recognition studies, we also chose to attach at the terminus of the OPE a similar polyethylene glycol tether capped with an ion-chelating iminodiacetate moiety or oxocarbonyliminodiacetate, both of which were synthesized as bis-*t*-butyl esters **7** and **8** ($n = 1$, Scheme 1C). The hydrolysis of **8** under special conditions afforded **9** ($n = 1$) as a free dicarboxylic acid. Finally, to discriminate between the effect of a rigid OPE chain on membrane organization versus an effect on lipophilicity (log *P*), which controls the partition of the molecule into a lipid environment, for direct comparison we synthesized DAG-lactone **10** (log $P = 3.68$) with a flexible decanoic acid chain closely matching the lipophilicity of the simplest OPE-containing DAG-lactone **1** (log $P = 3.24$).

Chemistry

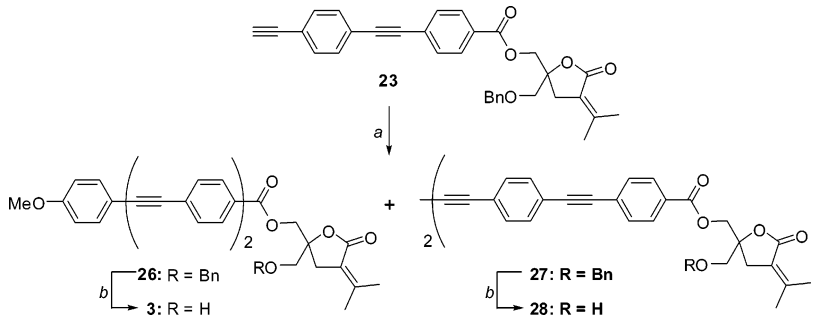
Starting from racemic lactone **11**,[19,20] aldol condensation with acetone followed by mesylation and elimination of the aldol product gave **12** in 84% yield (Scheme 2). Parallel deprotection of **12** with BCl_3 or ceric ammonium nitrate (CAN) afforded either **13** or **14** in 94 and 90% yields, respectively.

DAG-Lactones with Plain OPE Acyl Rods. For the synthesis of DAG-lactones with OPE acyl rods that are two to three PE units long (Scheme 1A), the corresponding OPE acids were prepared first. The shorter ester precursor **19** was obtained in 65% yield by coupling [2-(trimethylsilyl)ethyl]-4-ethynylbenzoate (**18**) with 4-[(trimethylsilyl)ethynyl]iodobenzene **17**,[21] under Sonogashira conditions[22] (Scheme 3). **17** was prepared from commercially available 2-[(trimethylsilyl)ethynyl]aniline (**15**) according to the literature,[21] and **18** was obtained after BOP−Cl [bis(2-oxo-3-oxazolidinyl)phosphinic acid chloride] mediated coupling of 4-ethynylbenzoic acid (**16**) and 2-(trimethylsilyl)ethanol. Acid **16** was obtained from the commercially available sodium salt. Following the selective removal of the terminal TMS group of **19** with trifluoroacetic acid (TFA), further coupling with **17**, also under Sonogashira conditions, gave the elongated trimeric OPE acyl rod precursor **21**. Alternatively, the trimethylsilylethyl group and the terminal TMS group in **19** were easily removed under neutral conditions with tetra-*n*-butylammonium fluoride (TBAF), and the fully deprotected acid underwent BOP−Cl mediated coupling with lactones **13** and **14**, respectively, to give **22** (78%) and **23** (62%) after two steps (Scheme 4). The longer, trimeric OPE acid rod was obtained from **21** in a similar manner with TBAF and coupling with **13** gave **24** in 68% yield. To compare the role of the rigid OPE acyl chain in **22** to that of an equivalent flexible chain, acylation of **13** with decanoic anhydride in the presence of DMAP was carried out to give **25**. CAN-mediated deprotection of the PMP groups in **22**, **24**, and **25** provided the corresponding targets **1** (75%), **2** (64%), and **10** (71%).

DAG-Lactones with Terminally Functionalized Ope Acyl Rods. With the idea of exploring the effect of a polar cap at the end of the rigid rod, some simple compounds ending with phenylmethoxy (**3**) or methyl phenoxyacetate (**4**) moieties were synthesized (Scheme 1B). For the phenylmethoxy compound, coupling of **22** with *p*-iodoanisole was to be avoided because of the susceptibility of the anisyl group to oxidation with CAN during the final deprotection step. Therefore, extension of the already assembled benzyl-protected DAG-lactone **23** with *p*-iodoanisole was attempted under Sonogashira's conditions in the presence of Cs_2CO_3. The desired DAG-lactone **26** was obtained in 50% yield accompanied by a small amount of the dimer **27** (12%) (Scheme 5). However, when the coupling was performed without Cs_2CO_3, formation of the dimer predominated. Deprotection of **26** with BCl_3 afforded **3** in 60% yield. Deprotection of **27** gave dimer **28**, which was not purified.

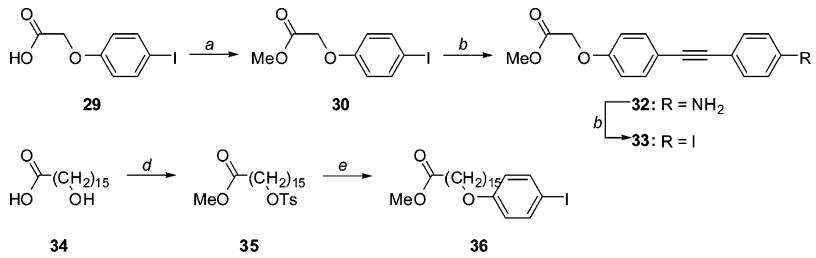
To synthesize the compound capped with the methyl phenoxyacetate moiety, commercially available 4-iodophenoxy-

Scheme 5[a]



[a] Conditions and reagents: (*a*) 4-iodoanisole, $PdCl_2(PPh_3)_2$, Et_3N, CuI, THF or 4-iodoanisole, $PdCl_2(PPh_3)_2$, $CsCO_3$, CuI, THF. (*b*) BCl_3, −78 °C, CH_2Cl_2.

Scheme 6[a]



[a] Conditions and reagents: (*a*) MeI, $NaHCO_3$, DMF. (*b*) 4-ethynylaniline (**31**), $PdCl_2(PPh_3)_2$, piperidine, CuI, THF. (*c*) $NaNO_2$, 2 N HCl, KI. (*d*) i. MeI, $NaHCO_3$, DMF, 40 °C. ii. TsCl, DMAP, CH_2Cl_2. (*e*) 4-iodophenol, $CsCO_3$, DMF.

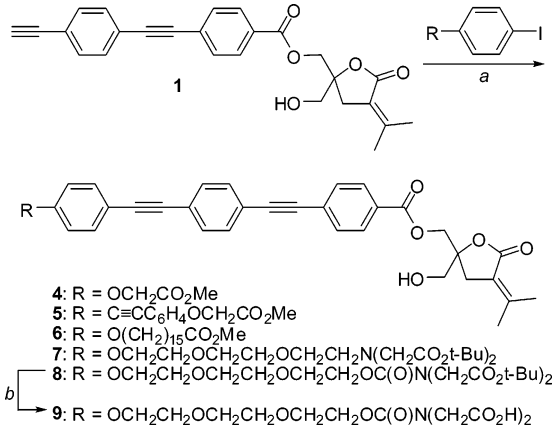
acetic acid (**29**) was converted to its methyl ester **30**, which was then coupled under Sonogashira conditions with 4-ethynylaniline (**31**) to give compound **32** in 74% yield (Scheme 6). Diazotization of the amine in the presence of KI afforded the corresponding iodide **33**. For reasons discussed earlier in this article, we wanted to move the polar ester moiety away from the end of the rigid rod, and for that purpose, commercially available 16-hydroxyhexadecanoic acid (**34**) was esterified, and the terminal hydroxyl group was converted to the tosyl ester to give **35**. The tosyl group in **35** was then displaced with *p*-iodophenol to give **36**, which, relative to **30**, separated the polar ester moiety 14-carbon atoms away from the end of the OPE rod (Scheme 6).

In addition to being a final DAG-lactone target, **1** also served as a useful partner for palladium-catalyzed couplings with the various substituted aryl iodides **30**, **33**, and **36** (Scheme 6), bypassing the final deprotection step. As explained later, the reaction conditions developed by Mori and co-workers[23] using $Pd(PPh_3)_4$ and Ag_2O as an activator and the inclusion of Cs_2CO_3 to reduce the formation of dimeric products instead of the standard Sonogashira conditions. Coupling of **1** with **30**, **33**, and **36** under these conditions gave **4**, **5**, and **6** in yields ranging from 58 to 63% (Scheme 7).

Another manner of capping the rigid acyl rod that was explored employed a more complex multifunctional unit consisting of a short polyethyleneglycol (PEG) spacer connected to an iminodiacetate moiety (Scheme 1C) in a fashion similar to that done by Matile et al. with some amphiphilic poly(phenylene) rods.[17] The functionalization of the PEG spacer was accomplished by reacting commercially available **37** and 4-iodophenol at 50 °C to give **38** (Scheme 8). The reaction of this intermediate with di-*t*-butyliminodiacetate gave the ester-protected tertiary amino derivative **39**, whereas a similar reaction in the presence of equimolar amounts of $CsCO_3$ acting as a carboxylating reagent[24] gave the neutral carbamate **40** (Scheme 8).

The syntheses of elongated DAG-lactones containing the terminal PEG spacer connected to the iminodiacetate or oxycarbonyliminodiacetate "caps" (**7** and **8**, Scheme 1C) did not

Scheme 7[a]



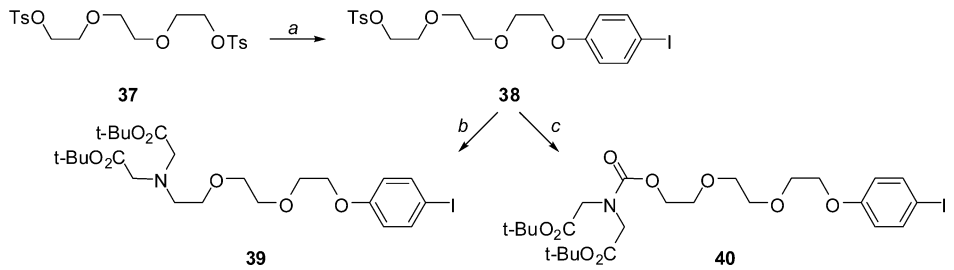
[a] Conditions and reagents: (*a*) $Pd(PPh_3)_4$, $CsCO_3$, Ag_2O, THF, 60 °C. (*b*) Monmorillonite KSF, CH_3CN, microwave.

proceed well under the standard Sonogashira conditions possibly as a result of the apparent chelation of Pd^{2+} with the PEG component of **39** and **40**. The reaction was then conducted under the conditions developed by Mori et al.[23] as mentioned previously. Coupling of **1** with **39** or **40** under these conditions gave **7** and **8** in 45 and 56% yields, respectively. Of the two PEG-tethered products, the synthesis and purification of **7** was difficult, whereas that of **8** was more reproducible, and hence this compound was hydrolyzed under microwave conditions using a clay mineral, montmorillonite ($Na_{0.2}Ca_{0.1}Al_2Si_4O_{10}(OH)_2(H_2O)_{10}$) KSF[25,26] in acetonitrile to give **9** in 27% yield (Scheme 7).

PKC Binding Studies. Enzyme−ligand interactions were assessed in vitro in terms of the ability of the ligand to displace bound [20-^{3}H]phorbol 12,13-dibutyrate (PDBu) from a recombinant single isozyme (PKC α) in the presence of phosphatidylserine.[27] The partition coefficients (log *P*) were calculated according to the atom-based program MOE Slog *P*[18] (Table 1).

DAG-Lactones with Plain OPE Acyl Rods (Scheme 1A). **1** and **2** are the simplest OPE-containing DAG-lactones with

Scheme 8[a]



[a] Conditions and reagents: (*a*) 4-iodophenol, $CsCO_3$, DMF, 50 °C. (*b*) Di-*t*-butyliminodiacetate, DMF, 50 °C. (*c*) Di-*t*-butyliminodiacetate, $CsCO_3$, DMF, 50 °C.

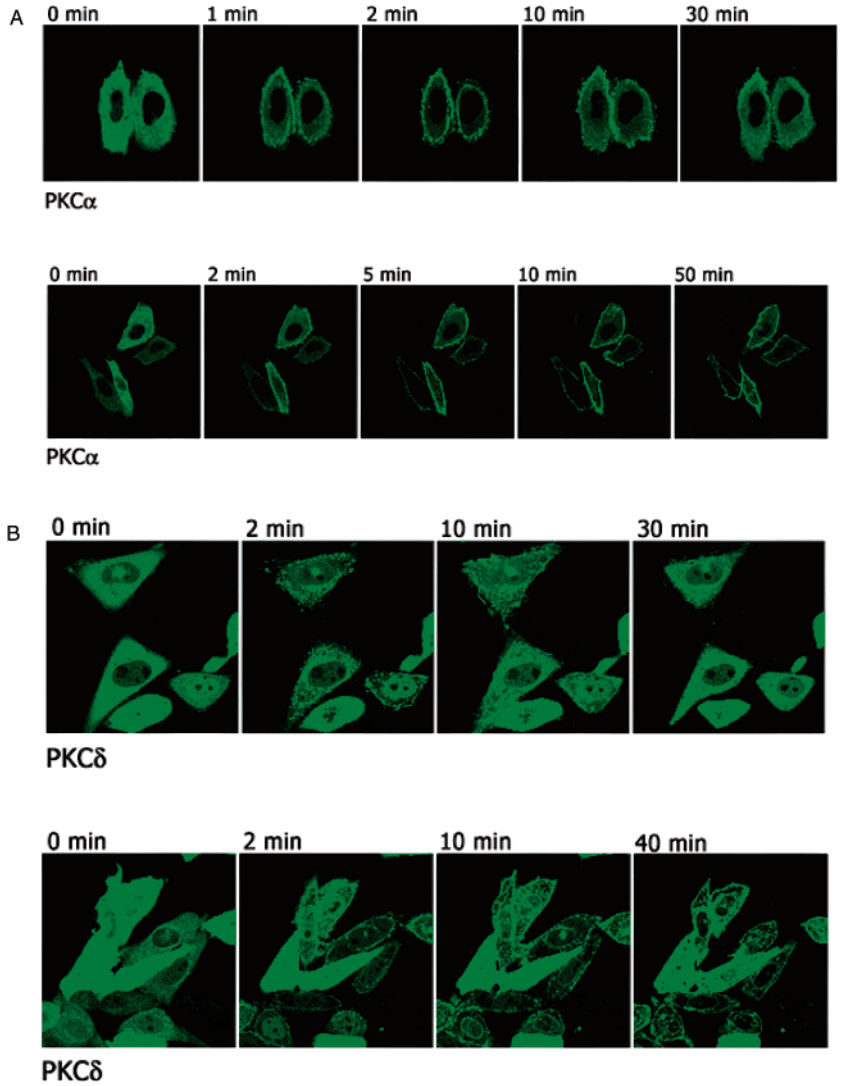


Figure 2. (A) Translocation of PKC α in CHO cells by **10** (top) and **1** (bottom) as a function of time. Cells were treated at 10 μM. Results are representative of three independent experiments. (B) Translocation of PKC δ in CHO cells by **10** (top) and **1** (bottom) as a function of time. Cells were treated at 10 μM. Results are representative of three independent experiments.

two and three PE units, respectively (Scheme 1A). **1** showed excellent affinity for PKC in the low nanomolar range (Table 1). Surprisingly, increasing the length of the acyl rod by one additional PE unit (**2**) reduced binding affinity by more than $^1/_2$ despite an increase in log *P* of 1.4. As with previous DAG-lactones synthesized in our laboratory, we have sought to develop potent ligands with the lowest possible log *P* value in order to minimize nonselective lipid binding.[28,29] Therefore, the same principle of achieving the lowest possible log *P* value compatible with a high binding affinity was also pursued in this investigation. A comparison of the binding affinities of the flexible DAG-lactone **10** and of the OPE-containing DAG-lactone **1**, both of which have comparable log *P* values, illustrates this approach. **1**, with the rigid acyl rod, was 3-fold more potent than **10** even though it was 0.45 log *P* units more hydrophilic than DAG-lactone **10**. Therefore, **1** looked like an excellent template for further exploration in our quest to construct target-specific PKC agonists (vide infra).

DAG-Lactones with Terminally Functionalized OPE Acyl Rods (Scheme 1, B and C). Lengthening the two-unit PE acyl rod in **1** by replacing the terminal hydrogen with a phenylmethoxy moiety (**3**, Scheme 1B) restored most of the binding

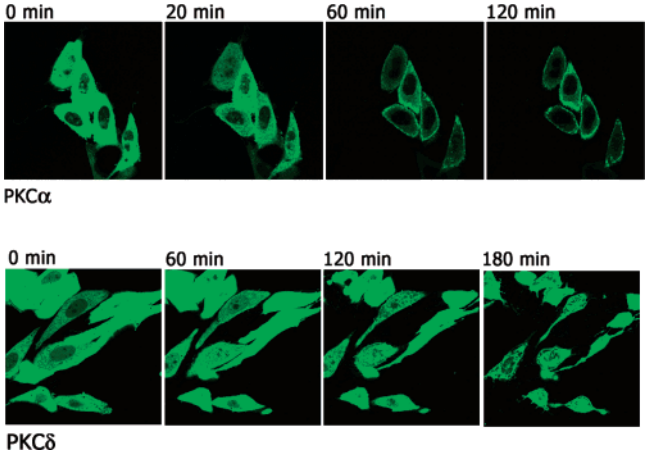


Figure 3. Translocation of PKC α (top) and PKC δ (bottom) in CHO cells by **2** (20 μM). Results are representative of three independent experiments.

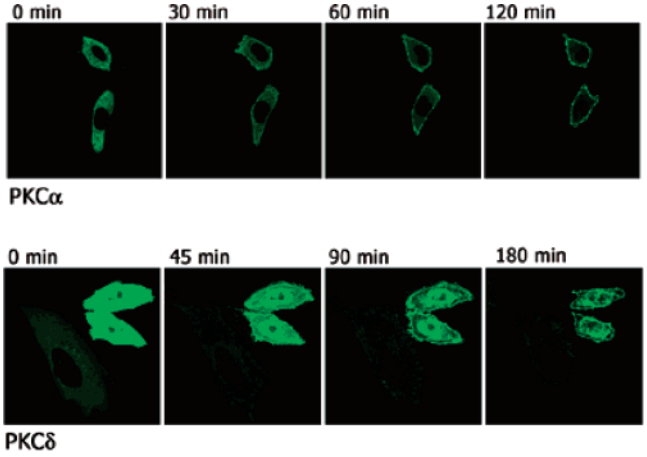


Figure 4. Translocation of PKC α (top) and PKC δ (bottom) in CHO cells by **6** (20 μM). Results are representative of three independent experiments.

lost in **2** with little change in log *P* (Table 1). However, a similar enlargement of **1** with a methyl phenoxyacetate moiety (**4**) was not as effective as the binding affinity decreased by ca. 3-fold, whereas the log *P* decreased only slightly. Surprisingly, the same methyl phenoxyacetate moiety positioned at the terminus of the longer OPE DAG-lactone **2** caused a 24-fold decrease in binding affinity (**5**), suggesting that increasing the OPE rigid rod beyond three PE units might be unproductive. The loss of binding in **5** appears to be associated more with the number of PE units rather than with the absolute length of the acyl chain because spacing the polar methyl acetate moiety in **4** by 15 carbons from the OPE terminus gave **6**, which registered a lower 10-fold decrease in binding affinity relative to that of **1** and only a ca. 3-fold decrease in binding relative to that of **4**. From the binding data (Table 1), the 2-PE acyl rod unit in **1** appears to be optimal in combining binding affinity and log *P* value.

When the terminus of the rigid acyl rod of **1** was capped with the short polyethyleneglycol (PEG) spacer used by Matile et al.[17] bearing the esterified iminodiacetate moiety (**7**) or the esterified oxocarbonyliminodiacetate moiety (**8**), the results showed that **8** with the oxocarbonyliminodiacetate terminus was 6-fold more potent than **7** and only 3.5-fold less potent than **1** (Table 1). This striking difference between **7** and **8**, which have comparable log *P* values, could be related to a change of polarity in going from a basic amine to a neutral carbamate. Unfortunately, the binding affinity of **8** decreased 76-fold when it was hydrolyzed to **9**, which displays a free oxocarbonyliminodiacetic acid moiety. It is likely that the neutral di-*t*-butyl esters in **8** can be better accommodated inside the lipid bilayer as opposed to the ionized oxocarbonyliminodiacetic acid moiety in **9**. We believe that the acyl moieties in **7**−**9** are perhaps too short to span the lipid bilayer; consequently, the polar termini of these molecules have difficulty settling inside the hydrophobic core. These observations were confirmed in cellular translocation studies in which **6** appears to be the only compound with measurable biological activity that is capable of spanning the 30 Å width of the cellular membrane (vide infra).

Cellular Translocation Studies of PKC α and PKC δ in CHO Cells. Translocation of PKCs from cytosol to membrane compartments provides one measure of their activation. This process can be detected in real time by overexpressing different green fluorescent protein-tagged (GFP-tagged) PKC isoforms in Chinese hamster ovary (CHO) cells and following their localization before and after ligand treatment using confocal microscopy. DAGs and phorbol esters translocate PKCs from the cytosol to the plasma membrane with kinetics dependent on their potencies and on their applied concentrations relative to their potencies. Higher relative concentrations of ligands usually result in faster kinetics.[30] PKC δ is unusual among PKC isoforms in that it translocates not only to the plasma membrane but also to other internal membrane compartments (nuclear membrane, mitochondria, other cellular organelles).[31] The pattern of PKC δ localization depends on the structure and lipophilicity of the activating drug.[30] The localization the of activated PKC isoform is very important in determining the downstream biological response, because PKC will only phosphorylate those substrates to which it has access through co-localization.

Addressing the biological activity of these DAG-lactones, we first tested the pattern and kinetics of the translocation of PKC α and δ in CHO cells. Both DAG-lactone **1** with a rigid OPE acyl rod and the hydrophobically equivalent DAG-lactone **10** with a flexible acyl chain translocated PKC α (Figure 2A) and PKC δ (Figure 2B) very quickly, within minutes. PKC δ translocated simultaneously to the plasma membrane and internal membranes (Figure 2B). Interestingly, the translocation of PKC α and PKC δ caused by **10** was transient, unlike that of **1**. This transient translocation was not due to desensitization of the PKCs because both PKC isoforms translocated again after the addition of new compound (data not shown), suggesting that the instability of **10** in the presence of the cells is the probable explanation.

An increase in the OPE acyl rod length by just one PE segment (**2**) had a dramatic effect (Figure 3, top). PKC-induced translocation by **2** even at high concentrations (20 μM) was much slower than that induced by **1** and **10**; PKC α translocation started in 20 min and reached completion after 120 min. Translocation of PKC δ was even slower; it began to become evident after 30−40 min and had not reached completion within 120 min (Figure 3, bottom).

Behavior similar to that of **2**, but with even slower kinetics of translocation, was observed for **3** at 20 μM where translocation of PKC α started after ca. 120 min. Translocation of PKC δ was even slower and was incomplete after 120 min (data not shown). Remarkably, there was no observable translocation over this time span with **4** and **5** even at 20 μM, possibly because the polar carboxylate moiety resulting from hydrolysis by esterases in the medium prevented the insertion of the acyl rod into the membrane.

6 was of particular interest in terms of translocation. It translocated PKC α and PKC δ slowly, beginning after ca. 30

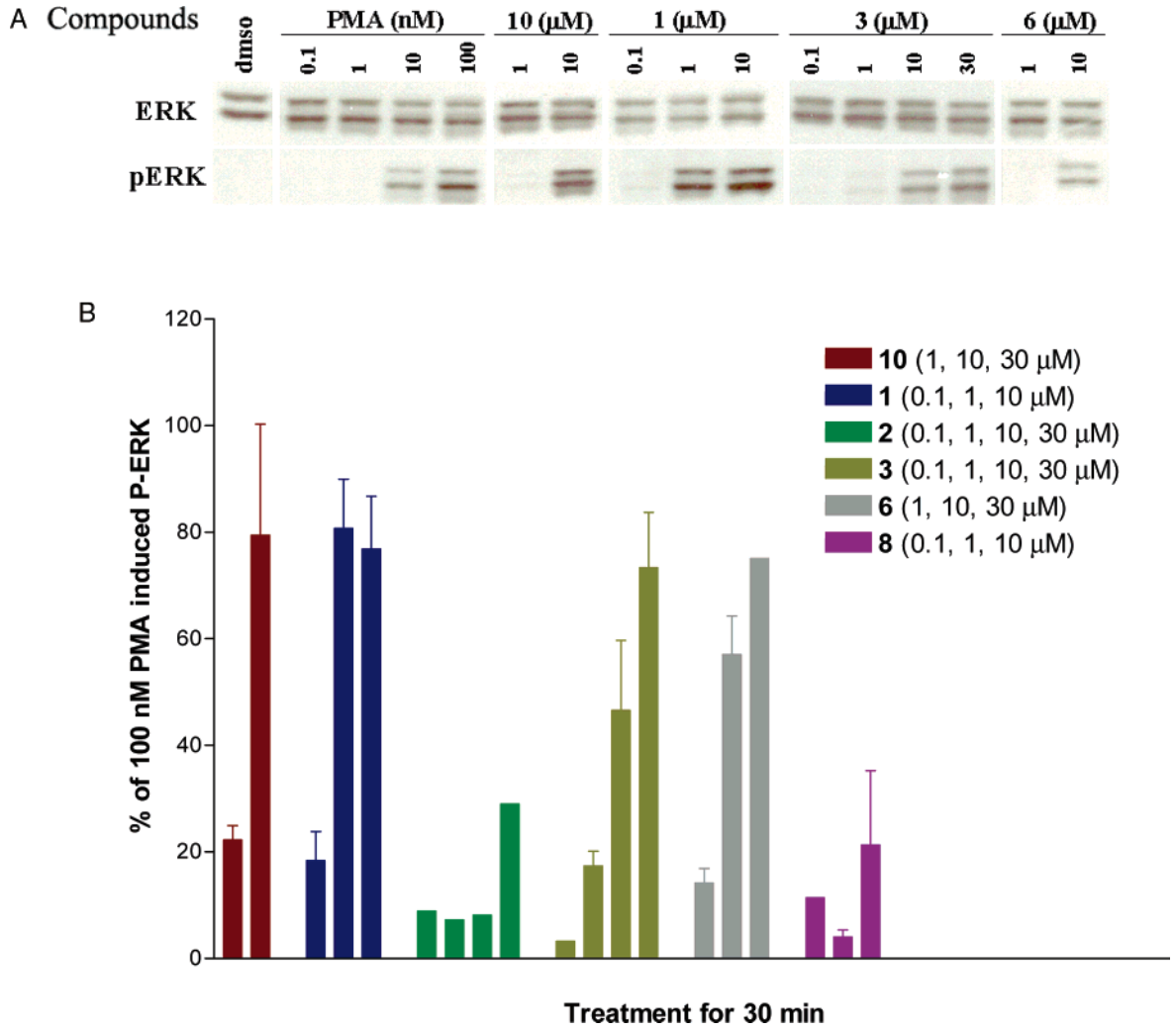


Figure 5. (A) ERK phosphorylation induced by the short rigid-rod DAG-lactones (**10**, **1**, **3**, and **6**) and PMA after 30 min in LNCaP cells. Western blots were probed with anti-ERK and anti-phospho-ERK antibodies. (B) Relative intensities of phospho-ERK from Western blots (PMA at 100 nM = 100%) for the compounds in A plus DAG-lactones **2** and **8**). Results are representative of three independent experiments except for **2**, which represents a single experiment. (B) Phosphorylation of ERK after 30 min of treatment of LNCaP cells with rigid-rod compounds.

min (Figure 4). PKC δ translocated to the plasma membrane first, followed later by translocation to the internal membranes and the nuclear membrane. The delay in PKC δ translocation to the plasma membrane and the internal membranes was longer than that caused by phorbol 12-myristate 13-acetate (PMA) (data not shown). This longer interval between these two destinations for PKC δ translocation could potentially result in different biology.

Not surprisingly, PKC-induced translocation with the more complex DAG-lactone **8** was even slower (starting after ca. 90−120 min) than that induced by **6** (data not shown). **8** had solubility problems reflected by the appearance in the medium of drug aggregates. No PKC translocation was achieved with **9**.

In summary, we conclude that none of the rigid-rod compounds were able to change the pattern of PKC δ translocation significantly, in particular by translocating PKC δ to the plasma membrane but not to internal membranes. There was a large difference in the kinetics of translocation caused by the different DAG-lactones because the one with the shortest OPE rod (two PE units) induced a quick translocation, and the ones with three or more PE units caused progressively slower translocation. The kinetics of translocation varied markedly, and **6** was noteworthy because of the long delay between PKC δ translocation to the plasma membrane and to the internal membranes. The potential consequences of these kinetic variations in translocation were investigated using a series of biological end points that differed in their time courses for response.

Other Biological Assays. In response to PMA treatment, stimulation of ERK phosphorylation in LNCaP cells occurs in minutes, secretion of IL-6 from WEHI-231 cells occurs over hours, and inhibition of WEHI-231 cell proliferation or differentiation of U937 cells occurs over several days. We evaluated these responses upon treatment with the rigid-rod DAG-lactones and compared them with the responses of PMA.

ERK Phosphorylation in LNCaP Cells. DAGs and phorbol ester induce ERK phosphorylation in many cellular systems.[9] Phosphorylation of ERK occurs shortly (10−30 min) after agonist treatment, as revealed by the appearance of the bands detected with anti-phosphoERK antibodies. The quickly acting **1** and **10** activated ERK phosphorylation very effectively with approximate EC_{50} values (as assessed from the graph) of 0.4 and 3 μM, respectively (Figure 5). Slowly acting **2** was less efficient and less potent in this short term assay, unlike its methoxy derivative **3**. The rigid-rod-containing DAG-lactone **6** with the 15-carbon chain linked to the rigid rod was also able to induce a strong response of ERK phosphorylation at 10 μM. These results agree with the previously shown results for translocation and indicate that **6** can translocate and probably span the width of the membrane. There is some variability in this assay caused by the short-term treatment (variations in the

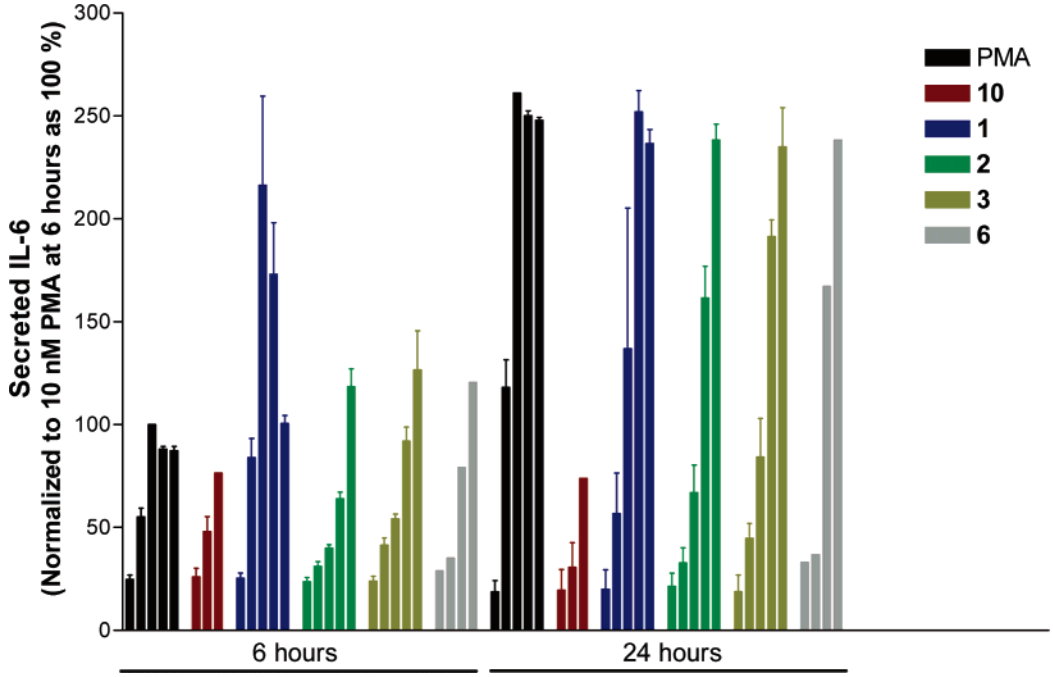


Figure 6. Induction of IL-6 secretion in WEHI-231 cells after 6 and 24 h. The level of secretion was normalized to that induced by 10 nM PMA after 6 h. Values represent the mean ± SEM of three independent experiments, except for **6**, which represents the results of a single experiment. PMA (0.1, 1, 10, 100, and 300 nM), **10** (1, 10, and30 μM), **1** (0.1, 1, 3, 10, and30 μM), **2** (0.1, 1, 3, 10, and 30 μM), **3** (0.1, 1, 3, 10, and 30 μM), and **6** (0.1, 1, 10, and 30 μM).

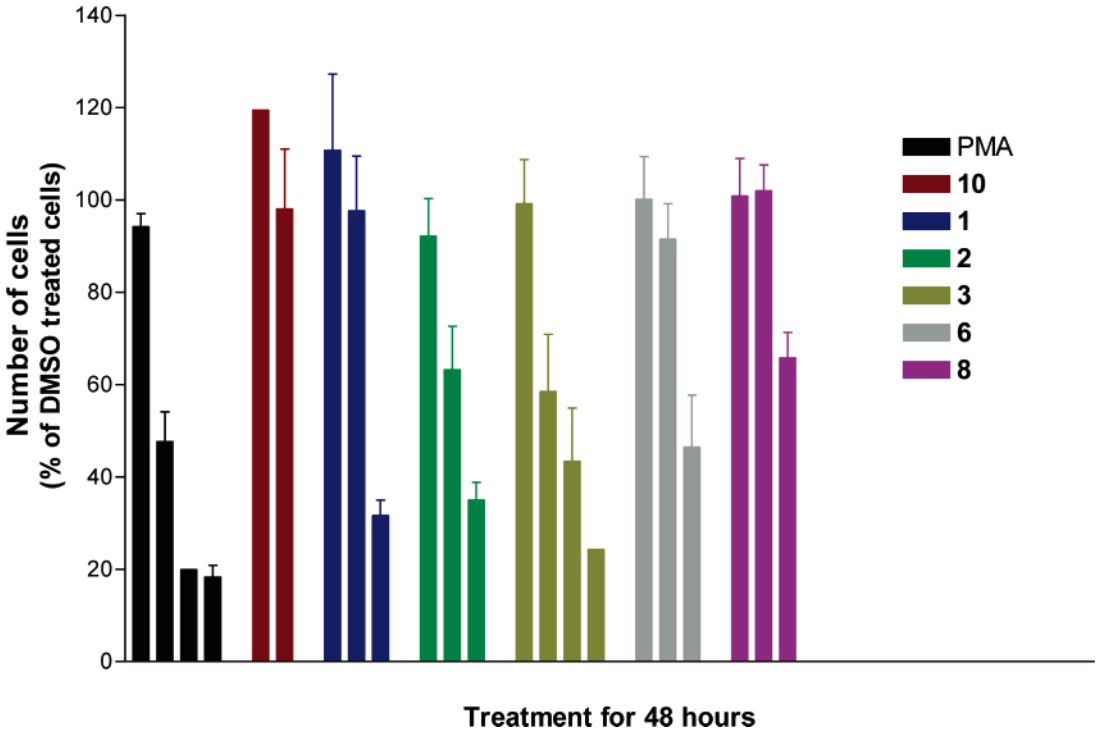


Figure 7. Inhibition of WEHI-231 cell proliferation. PMA (0.1, 1, 10, and 100 nM), **10** (1 and 10 μM), **1** (0.1, 1, and 10 μM), **2** (0.1, 1, and 10 μM), **3** (0.1, 1, 10, and 30 μM), **6** (0.1, 1, and 10 μM), and **8** (0.1, 1, and 10 μM). Values represent the mean ± SEM of three independent experiments, except for treatment with 1 μM of **10**, which was performed once.

drug absorption at 10 and 30 min) and the semiquantitative nature of western blotting.

Secretion of IL-6 from WEHI-231 Cells. We showed previously that PMA and other phorbol esters induce secretion of IL-6 from WEHI-231 pre-B cells starting about 3 to 4 h after treatment.[32] Ingenol 3-angelate induces a biphasic IL-6 response at early time points (6 h), whereas the response to PMA is monophasic and fails to reach the level induced by the optimal dose of ingenol 3-angelate; at 24 h the response to both compounds is similar.[32] We measured secreted IL-6 after 6 and 24 h of treatment with the rigid-rod compounds and compared the responses with those of PMA.

All of the the compounds that were active in ERK phosphorylation could induce IL-6 secretion. The quickly acting **1** was special in the IL-6 secretion assay as it caused a biphasic dose−response curve after 6 h of treatment, unlike PMA but similar

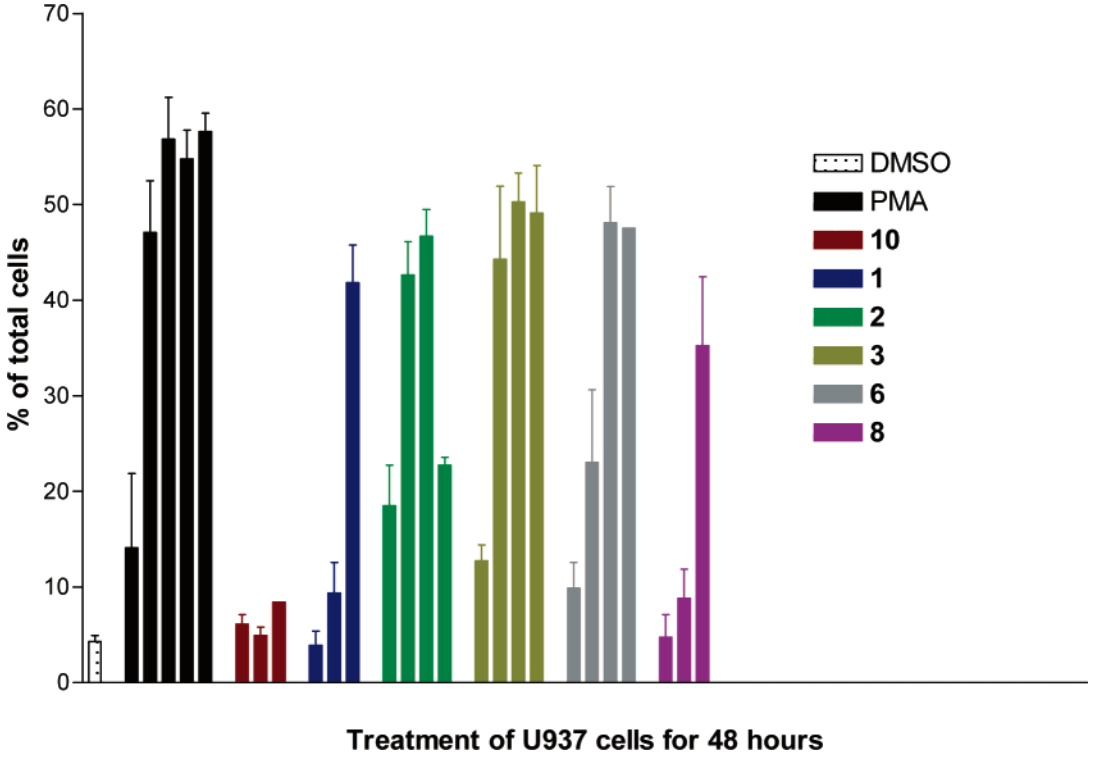


Figure 8. Attachment of U937 cells to the bottom of the dish after 48 h treatment. PMA (0.1, 1, 10, and 100 nM), **10** (1, 10, and 30 μM), **1** (0.1, 1, and 10 μM), **2** (0.1, 1, 10, and 30 μM), **3** (0.1, 1, 10, and 30 μM), **6** (0.1, 1, 10, and 30 μM), and **8** (0.1, 1, and 10 μM). Values are the mean $\pm$ SEM of three independent experiments, except for treatment with 30 μM of **10** and 30 μM of **6** that were performed once.

to the behavior we described earlier for ingenol 3-angelate. The other rigid-rod compounds tested did not show a biphasic curve, but interpretation is clouded by relatively low potencies, which might mean that sufficient doses were not achieved to reveal the biphasic response. At higher concentrations (3 μM and above), there is a solubility problem with many of the compounds (**2**, **3**, and **8**); drug aggregates in the aqueous phase were visible by light microscopy. **6** did not show unique behavior in this assay. Interestingly, **10**, which caused only transient translocation of PKCs, was much less effective in these assays at 6 and 24 h: **10** (30 μM) induced about 80% of the level of IL-6 secreted upon 10 nM PMA treatment for 6 h and only 25% of the level induced by PMA at 24 h. This result highlights the role of the rigid rod, in that **10** contains a flexible side chain lipophilically equivalent to that of **1**.

Inhibition of WEHI-231 Cell Proliferation. Phorbol esters inhibit the proliferation of many cell types. We checked the effect of the rigid-rod compounds on the proliferation of WEHI-231 pre-B cells after 48 h of treatment with different concentrations of compound. PMA inhibited proliferation of WEHI-231 cells with an EC_{50} of about 0.85 nM. In this assay, **2** and **3** were the most potent of the rigid-rod compounds with EC_{50} values of 0.4 ± 0.14 and 0.43 ± 0.03 μM, respectively, followed by **1** and **6** with an EC_{50} about 3 μM (Figure 7). The detailed dose−response curves of **2** and **3** showed no difference between the two compounds (data not shown). **8**, which caused very slow PKC translocation and had little activity in short term assays, showed 35% inhibition of cell proliferation at 10 μM. **10**, which caused transient translocation of PKCs, had hardly any effect on WEHI-231 cell proliferation.

Attachment of U937 Cells. Treatment of the U937 monocytic cell line with PMA and other phorbol esters results in their differentiation and attachment to the bottom of the cell culture dish. Bryostatin 1 is special in this aspect as it does not cause differentiation and attachment by itself, and it inhibits the differentiation and attachment induced by PMA.[33] We tested the rigid-rod compounds for differentiation of U937 monocytes after 48 h of treatment to check the long-term potency of the compounds and to see if any of them show bryostatin-1-like unique effects. In this assay, the most potent DAG-lactones were those with longer rigid rods, which were the same ones that showed slow kinetics of translocation. PMA induced differentiation and attachment of U937 monocytes with an EC_{50} of 0.45 $\pm$ 0.08 nM, and **2** and **3** had EC_{50} values of 0.25 ± 0.046 and 0.26 ± 0.065 μM, respectively. **2** (30 μM) reproducibly reduced U937 cell attachment by reducing the number of total cells. **6** was potent in this assay with an EC_{50} of 1 ± 0.43 μM. **1** and **8** were less potent, although **8** was more potent in this assay than in the short-term assays, possibly reflecting the slow kinetics of the compound. **10** did not have any effect in this assay, similar to its lack of inhibition of WEHI-231 cell proliferation.

Conclusions

The lipophilic second messenger *sn*-1,2-diacylglycerol is a central element in cellular signaling, controlling many downstream biological responses including cell proliferation, differentiation, apoptosis, and secretion.[34] Functioning as the DAG recognition motif, DAG-responsive C1 domains are present in multiple families of signal-transducing proteins, including PKC, PKD, RasGRP, the chimaerins, MRCK, and Munc-13, as well as in DAG kinases.[35] Additional complexity to the signaling is contributed by the different specificities of the individual members within a signaling family, with individual members even being antagonistic in their functions. For example, PKC δ is often pro-apoptotic, in contrast to PKC ϵ, which is anti-apoptotic.[36] Moreover, there can be cross talk between these families of DAG-responsive signaling proteins. PKC phosphorylation of PKD and RasGRP3 contributes to their activation; DAG kinase metabolizes DAG, terminating response from PKC

and the other families of DAG-responsive signaling proteins.[37,38] A central question in biology is how the cell controls the flow of information through these signaling pathways in a selective fashion.

From the perspective of therapeutics, the corresponding question is how one can exploit such mechanisms of selectivity to obtain novel and selective drug action for this class of drug targets. The compounds described here represent one potential strategy, drawing together several conceptual themes that have emerged from studies on PKC regulation and pharmacology.

An early observation has been that much of the diversity of the effect of natural products acting through C1 domains is driven by their patterns of lipophilic substitution on a conserved binding motif. A powerful example is the inhibition of tumor promotion exerted by the short-chain substituted 12-deoxyphorbol esters such as prostratin in contrast to the tumor promoting activity of its more lipophilic congener 12-deoxyphorbol 13-tetradecanoate.[39] Likewise, phorbol diesters with unsaturated side chains have long been characterized as being inflammatory but only weakly tumor promoting, unlike their corresponding analogs with saturated aliphatic chains.[40]

PKC has relatively broad substrate requirements. An important contributor to selectivity is therefore its selective proximity to appropriate substrates. We have described how PKC ligands with different patterns of biological response induce different patterns of localization of PKC δ, visualized using GFP-tagged PKC δ. The tumor promoting derivatives PMA and 12-deoxyphorbol 13-tetradecanoate first cause the accumulation of GFP-tagged PKC δ at the plasma membrane, followed by a shift to the internal membranes and the nuclear membrane. In contrast, bryostatin 1 and ingenol 3-angelate cause little plasma membrane translocation of PKC δ.[30,32] In elegant complementary studies, Newton and co-workers have employed a FRET-responsive PKC substrate, positioned in different cellular compartments, to show that different PKC isoforms have different kinetics of activation and different patterns of substrate phosphorylation.[41]

The relationship of the ligand relative to the surface of the membrane represents a third potential opportunity for selectivity. Cho and co-workers[42] have shown that different C1 domains penetrate the lipid surface to different extents. Such penetration will be controlled both by residues on the outer surface of the C1 domain, less conserved than the binding cleft itself, or indeed by other portions of the protein. The influence of structural elements other than the C1 domains on the behavior of PKC is reflected in the large differences observed between the behavior of isolated C1 domains and that of the intact protein. A mechanism driving such differences is the need to disrupt intramolecular contacts to render the C1 domains accessible.[42] This mechanism is strongly supported by the effects of peptides mimicking such postulated sites of interaction[43] and, in the case of β2-chimaerin, is shown directly from the X-ray structure, in which the C1 domain is shielded in the inactive conformation of the protein by interdomain linkers.[44]

We believe that the approach described here with the rigid-rod-substituted DAG-lactones has the potential to probe these different opportunities for selectivity. Aggregation of ligand within the surface of the membrane may lead to a distinct pattern of distribution of PKC. Selectivity of interaction with lipids may yield preferential localization in membrane compartments. Different kinetics of penetration will yield different kinetics of response. Rigid rods capped with appropriately hydrophilic substituents may insert into the membrane but penetrate no further, sequestering the PKC at the inner face of the membrane. Our initial structural exploration of these rigid-rod-substituted DAG-lactones has helped to delineate the chemical and pharmacological feasibility of this approach.

Although the described structures represent only the first generation of such compounds, we have defined structures that show a much longer persistence of action compared to the corresponding aliphatic chain substituted analog. We have likewise shown that we can achieve marked control over the rate of induction of translocation, presumably reflecting variable rates of penetration of the ligands, as shown directly elsewhere with fluorescent phorbol esters.[45] Using a battery of assays of biological responses chosen to reflect responses possessing different intrinsic time courses for responses, we have demonstrated that the patterns of biological response correlate with the persistence and time course of translocation for the different ligands. In addition, we have identified at least one ligand that displays substantial separation of the time courses for plasma membrane and internal translocation of PKC δ, providing a tool with which to identify potential consequences of this temporal separation of PKC δ activation at these different sites. Finally, we have demonstrated that at least one of these ligands displays the profile of induction of the cytokine IL-6 characteristic of the PKC activator ingenol 3-angelate (PEP005) rather than that characteristic of PMA, in which ingenol 3-angelate is under evaluation as a cancer chemotherapeutic agent and PMA is a tumor promoter.[32]

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 PKC α essentially as described previously.[27] The ID_{50} values were determined by least-squares fitting of the theoretical sigmoidal competition curve to the binding data. The K_i values were calculated from the ID_{50} values according to the relationship $K_i = ID_{50}/(1 + L/K_d)$, where L is the concentration of free [^{3}H]PDBu at the ID_{50} and K_d is the dissociation constant for [^{3}H]PDBu under the assay conditions.[27] Values represent the mean $\pm$ standard error (three determinations). The octanol/water partition coefficients (log P) were calculated according to the atom-based program MOE Slog P.[18]

Biological Experiments. Translocation of GFP-Tagged PKC Isoforms in CHO Cells. CHO cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in F12-K medium supplemented with 10% fetal bovine serum and antibiotics (penicillin 50 units/mL and streptomycin 0.05 mg/mL). CHO cells plated onto T delta dishes (Bioptechs Inc, Butler, PA) were transfected with GFP-tagged PKC α or PKC δ using Lipofectamine reagent (Invitrogen, Carlsbad, CA).[32] For confocal images, cells were examined with a Zeiss LSM 510 confocal microscope (Carl Zeiss Inc, Thornwood, NY) with an Axiovert 100M inverted microscope operating with a 25 mW argon laser tuned to 488 nm. Cells were imaged with a 63 $\times$ 1.4 NA Zeiss Plan-Apochromat oil immersion objective and with varying zooms (1.4 to 2). Time-lapse images were collected every 30 s using the Zeiss AIM software, in which the green emission was collected in a PMT with a LP 505 filter.

ERK Phosphorylation in LNCaP Cells. Androgen-sensitive LNCaP prostate cancer cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (penicillin 50 units/mL and streptomycin 0.05 mg/mL). Cells were plated in 6 cm dishes at about 60% confluency and 24 h later were treated with different concentrations of the test compound for 15 or 30 min. After treatment, the cells were washed with phosphate buffered saline, total cell lysates were prepared, and the lysates were analyzed by 10% SDS-PAGE followed by electrotransfer and immunostaining. The primary antibodies used for immunostaining against phospho-ERK (9101) and total ERK

(9102) were from Cell Signaling (Danvers, MA). The immunostaining was visualized by ECL (Amersham Biosciences). Films were scanned, densitometry was performed using Image J (developed at National Institute of Mental Health, NIH), and the results were expressed relative to the intensity of the phosphorylated ERK band induced by 100 nM PMA. Values for the mean ± SEM of three independent experiments (except for **2,** which was a single experiment) were graphed using Prism2 (GraphPad Software Inc, San Diego, CA).

Induction of IL-6 Secretion in WEHI-231 Cells. WEHI-231 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in DMEM supplemented with 10% fetal bovine serum, antibiotics (penicillin 50 units/mL and streptomycin 0.05 mg/mL), and 50 μM β-mercaptoethanol. The concentration of IL-6 secreted from WEHI-231 cells was determined as described previously[32] using an IL-6 ELISA kit (Biosource International Inc, Camarillo, CA). One × 10^5 viable cells/mL were plated in six-well plates (2 mL) treated after 24 h with the specified concentrations of the compounds (final DMSO concentration 0.1%) for 6 and 24 h, after which aliquots of supernatant were removed for IL-6 determination. Cells were pelleted from supernatants by centrifugation at 1000*g* for 10 min. The IL-6 concentration in the supernatants was measured following the manufacturer's protocol. Secreted IL-6 was determined in a single measurement per experiment and expressed as a percentage of IL-6 induced by 10 nM PMA at 6 h. The values for the mean ± SEM from three experiments (except for **6,** a single measurement) were graphed using Prism2 (GraphPad Software Inc, San Diego, CA).

Inhibition of WEHI-231 Cell Proliferation. One × 10^5 viable cells/mL were plated in six-well plates (2 mL) and treated after 24 h with the specified concentrations of the compounds (final DMSO concentration 0.1%) for 48 h. After trituration to form a single cell suspension, cells were counted using a Coulter particle counter. The numbers of cells were presented as a percentage of that of the DMSO treated control. The values of the mean ± SEM from three independent experiments were graphed using Prism2 (GraphPad Software Inc, San Diego, CA).

Attachment of U937 Cells. U937 cells, purchased from the American Type Culture Collection (Manassas, VA), were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (penicillin 50 units/mL and streptomycin 0.05 mg/mL). Attachment of U937 cells to the bottom of the culture dishes was measured as described previously with some modifications.[33] Cells plated at 1 × 10^5 cells/mL in 2 cm dishes were treated with different concentrations of compounds (final DMSO concentration 0.1%) for 48 h. Non-attached and attached cells (the latter measured after trypsinization) were counted using a Coulter particle counter. The numbers of attached cells were expressed as a percentage of total cells. The values of the mean ± SEM from three independent experiments were graphed using Prism2 (GraphPad Software Inc, San Diego, CA).

General Chemical Procedures. All of the chemical reagents were commercially available. Column chromatography was performed on silica gel 60, 230−400 mesh (E. Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova instrument at 400 and 100 MHz, respectively. Spectra are referenced to the solvent in which they were run (7.26 ppm for $CDCl_3$). Infrared spectra were recorded on a Jasco model 615 FTIR instrument. Fast-atom bombardment (FAB) mass spectra were run on a VG 7070E-HF double-focusing mass spectrometer and were obtained in positive ion mode except where noted. A sample matrix of 3-nitrobenzyl alcohol (NBA) was employed in all cases, and ionization was effected by a beam of xenon atoms generated in a saddle-field ion gun at 8.0 ± 0.5 kV. The FAB mass spectra of all of the compounds indicated a molecular species (MH^+, $M^{+\bullet}$, M + Na^+, or M − H) and fragment ions that are highly indicative of structure. In some cases, molecular identity was additionally confirmed in the positive ion mode by alkali metal cationization to form a M + K^+ species by adding 1.0 μL 1.0 N KCl in 90% methanol/H_2O to the sample before mixing with matrix and subsequent FABMS analysis. Nominal mass MS were obtained at a resolution of 1200, whereas accurate mass analysis (HRFABMS) was carried out at a resolution of 3500−4500. For the latter, a limited-range V/E scan was employed under control of a MASPEC-II[32] data system for Windows (MasCom GmbH, Bremen, Germany). Matrix-derived ions, including K^+ adducts, were utilized as the internal mass references for accurate mass determinations. Both ^{1}H and ^{13}C NMR data were used to set constraints for the calculation of all of the possible elemental compositions within 10 ppm of the measured accurate mass. In each case where an accurate mass was reported, a unique molecular formula could be determined by consideration of the molecular ion species and appropriate fragment ions. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

5-[(4-Methoxyphenoxy)methyl]-3-(methylethylidene)-5-[(phenylmethoxy)methyl]-4,5-dihydrofuran-2-one (12). A stirred solution of lactone **11**[19,20] (1.16 g, 3.39 mmol) in THF (8 mL) was treated with LDA (2.0 M, 3.4 mL, 6.8 mmol) at −78 °C. After 50 min, acetone (1.25 mL, 17.0 mmol) was added and stirred for 1.5 h at the same temperature. The reaction was quenched with saturated aqueous NH_4Cl and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated aqueous NH_4Cl solution (2 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude product was then dissolved in CH_2Cl_2 (20 mL) and treated with MsCl (0.52 mL, 6.8 mmol) and DMAP (1.66 g, 13.6 mmol) at room temperature overnight. The reaction mixture was then diluted with CH_2Cl_2 (50 mL), washed with saturated aqueous NH_4Cl (2 × 25 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (EtOAc/hexanes, 1/3) afforded **12** (1.2 g, 88%); mp 78−79 °C; FTIR (neat) 2360 (CH), 2840 (CH), 1729 (C═O), 1666 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.23−7.32 (m, 5H, C_6H_5-CH_2OCH_2C), 6.79 (s, 4H, $CH_3OC_6H_4OCH_2C$), 4.56 (AB q, J = 12.2 Hz, 2H $C_6H_5CH_2OCH_2C$), 3.99 (AB q, J = 9.7 Hz, 2 H, CH_3-$OC_6H_4OCH_2C$), 3.73 (s, 3H, $CH_3OC_6H_4OCH_2C$), 3.63 (AB q, J = 10.2 Hz, 2H, $C_6H_5CH_2OCH_2C$), 2.76−2.88 (m, 2H, H-$4_{a,b}$), 2.25 (t, J = 2.1 Hz, 3H, C═C(CH_3)$_2$), 1.83 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.11, 154.16, 152.55, 150.61, 137.65, 128.31, 127.64, 137.50, 119.19, 115.65, 114.53, 80.92, 73.56, 71.99, 70.57, 55.60, 32.63, 24.45, 19.80. FAB MS (*m*/*z*, relative intensity) 382 ($M^{\bullet+}$, 100); Anal. ($C_{23}H_{26}O_5$) C, H.

5-(Hydroxymethyl)-5-[(4-methoxyphenoxy)methyl]-3-(methylethylidene)-4,5-dihydrofuran-2-one (13). A solution of BCl_3 in CH_2Cl_2 (1.0 M, 31.4 mL) was added to a solution of **12** (175 mg, 0.46 mmol) in CH_2Cl_2 (15 mL) and stirred at −78 °C for 2 h. The reaction was quenched with saturated $NaHCO_3$ solution (30 mL), warmed to room temperature, and extracted with CH_2Cl_2 (2 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated. Purification by column chromatography on silica gel (EtOAc/hexanes, 1/1) afforded **13** (118 mg, 88%) as a white solid, mp 78−79 °C; FTIR (neat) 3442 (OH), 2934 (CH), 1744 (C═O), 1665 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.81 (s, 4H, CH_3-$OC_6H_4OCH_2C$), 3.99 (AB q, J = 9.5 Hz, 2H, $CH_3C_6H_4CH_2$-OCH_2C), 3.79 (AB q, J = 12.1 Hz, 2H, $HOCH_2C$), 3.75 (s, 3H, $CH_3C_6H_4CH_2OCH_2C$), 2.77−2.93 (m, 2H, H-$4_{a,b}$), 2.53 (br s, 1H, $HOCH_2C$), 2.26(t, J = 2.2 Hz, 3H, C═C(CH_3)$_2$), 1.88 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.30, 154.26, 152.48, 151.58, 119.14, 115.61, 114.62, 81.74, 70.29, 65.39, 55.67, 32.08, 24.56, 19.93; FAB MS (*m*/*z*, relative intensity) 292 ($M^{\bullet+}$,100); Anal. ($C_{16}H_{20}O_5$) C, H.

5-(Hydroxymethyl)-3-(methylethylidene)-5-[(phenylmethoxy)-methyl]-4,5-dihydrofuran-2-onephenylmethoxy)methyl]-4,5-dihydrofuran-2-one (14). Cerium(IV) ammonium nitrate (300 mg, 0.55 mmol) was added to a solution of **12** (69 mg, 0.18 mmol) in CH_3CN (2 mL) and H_2O (0.5 mL) and stirred for 10 min. The reaction was then quenched with sodium thiosulfate (60 mg) and concentrated to a solid under reduced pressure. The residue was dissolved in EtOAc (25 mL), washed with H_2O (25 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (EtOAc/hexanes, 1/3) afforded **14** (45 mg, 91%) as an oil; FTIR (neat) 3464 (OH), 2872 (CH), 2836 (CH), 1744 (C═O),1663 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.27−7.37 (m, 5H, C_6H_5),

4.57 (AB q, J = 12.1 Hz, 2H $C_6H_5CH_2OCH_2C$), 3.70 (AB q, J = 11.9 Hz, 2H, $HOCH_2C$), 3.56 (AB q, J = 9.9 Hz, 2H, $C_6H_5CH_2OCH_2C$), 2.70−2.81 (m, 2H, H-4$_{a,b}$), 2.25 (t, J = 2.2 Hz, 3H, C═C(CH_3)$_2$), 2.11 (br s, 1H, CH_2OH), 1.86 (br s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.51, 150.92, 137.58, 128.34, 127.71, 127.52, 119.45, 82.24, 73.57, 72.04, 65.45, 32.08, 24.46, 19.82. FAB MS (*m/z*, relative intensity) 277 (MH^+, 93). Anal. ($C_{16}H_{20}O_4$ 0.25 H_2O) C, H.

3,3-Dimethyl-3-silabutyl 4-ethynylbenzoate (18). A solution of 4-ethynylbenzoic acid (869 mg, 5.95 mmol), trimethylsilyl ethanol (808 mg, 6.85 mmol), and DMAP (2.5 g, 20.5 mmol) in CH_2Cl_2 was treated with BOP−Cl (3.46 g, 13.6 mmol). After 30 min of stirring, the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with 2 N HCl (2 × 25 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (EtOAc/hexanes, 1/19) afforded ester **18** (1.35 g) as a yellow oil in 92% yield; FTIR (neat) 3297, 1717 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90 (d, J = 8.6 Hz, 2H), 7.45 (d, J = 8.5 Hz, 2H), 4.36−4.31 (m, 2 H), 3.16 (s, 1H), 1.07−1.03 (m, 2H), 0.0 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 165.9, 131.9. 130.6, 129.3, 126.5, 82.8, 79.9, 63.4, 17.3, −1.5; FAB MS (*m/z*, relative intensity) 219 ($MH^+-C_2H_4$). Anal. ($C_{14}H_{18}O_2Si$), C, H.

3,3-Dimethyl-3-silabutyl 4-{2-[4-(3,3-dimethyl-3-silabut-1-ynyl)phenyl]ethynyl} benzoate (19). A solution of **18** (849 mg, 3.44 mmol), **17** (1.04 g, 3.44 mmol), $PdCl_2(PPh_3)_2$ (48 mg, 68.8 μmol, 2 mol %), and piperidine (10 mL) in THF (50 mL) was degassed and treated with CuI (26 mg, 136.8 μmol, 4 mol %). After stirring for 3 h, the reaction was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (50 mL), washed with saturated aqueous NH_4Cl (2 × 25 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/CH_2Cl_2, 3/1) afforded **19** (930 mg, 65%) as a white solid, mp 97−98 °C; FTIR (neat) 2953 (CH), 1705(C═O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 and 8.02 (doublets, $J \approx$ 9 Hz, 4H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 7.45 (m, 4H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 4.43 (m, 2H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 1.14 (m, 2H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 0.26 (s, 9H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 0.09 (s, 9H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.13, 131.93, 131.49, 131.45, 130.19, 129.44, 127.47, 123.41, 122.73, 104.47, 96.61, 91.77, 90.50, 63.45, 17.43, −0.11, −1.45. FAB MS (*m/z*, relative intensity) 419 (MH^+, 16), 418 ($M^{\bullet+}$, 26). Anal. ($C_{25}H_{30}O_2Si_2$) C, H.

3,3-Dimethyl-3-silabutyl 4-[2-(4-ethynylphenyl)ethynyl]benzoate (20). Trifluroacetic acid (0.5 mL) was added to a solution of **19** (220 mg, 0.53 mmol) in CH_2Cl_2 (2 mL). After stirring for 1.5 h, the reaction was quenched with $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 25 mL). The combined organics were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/CH_2Cl_2, 3/1) afforded **20** (150 mg, 82%) as a white solid, mp 91−92 °C; FTIR (neat) 2954 (CH), 1706(C═O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 and 7.58 (doublets, J = 8.6 Hz, 4H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 7.49 (s, 4H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 4.41−4.45 (m, 2H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 3.19 (s, 1H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 1.14 (m, 2H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 0.09 (s, 9H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.15, 132.12, 131.58, 131.49, 130.27, 129.46, 127.39, 123.19, 122.38, 91.54, 90.57, 83.14, 79.16, 63.48, 17.44, −1.44. FAB MS (*m/z*, relative intensity) 347 (MH^+, 17). Anal. ($C_{22}H_{22}O_2Si$·0.5 H_2O) C, H.

3,3-Dimethyl-3-silabutyl 4-[2-(4-{2-[4-(3,3-dimethyl-3-silabut-1-ynyl)phenyl]ethynyl} phenyl)ethynyl]benzoate (21). A solution of **20** (125 mg, 0.30 mmol), **17** (108 mg, 0.36 mmol), $PdCl_2(PPh_3)_2$ (14 mg, 19.9 μmol, 6.7 mol %), and piperidine (1 mL) in THF (8 mL) was degassed and treated with CuI (4.5 mg, 23.6 μmol, 8 mol %). After 3 h, the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (25 mL), washed with saturated aqueous NH_4Cl (2 × 15 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (CH_2Cl_2/hexanes, 1/1) afforded **21** (120 mg, 68%) as a white solid, mp 176−178 °C; FTIR (neat) 2157 (CH), 1709 (C═O) cm^{-1}; H NMR (400 MHz, $CDCl_3$): δ 7.58 and 8.02 (doublets, J = 8.2 Hz, 4H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 7.52 (s, 4H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 7.45 (s, 4H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 4.41−4.45 (m, 2H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 1.12−1.17 (m, 2H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 0.26 (s, 9H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$), 0.09 (s, 9H, $(CH_3)_3SiC{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2CH_2Si(CH_3)_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.17, 131.93, 131.68, 131.59, 131.49, 131.41, 130.22, 129.46, 127.48, 123.32 123.21, 122.97, 122.73, 104.54, 96.51, 91.80, 91.15, 90.85, 90.62, 63.48, 17.44, −0.10, −1.44. FAB MS (*m/z*, relative intensity) 519 (MH^+, 30), 518 ($M^{\bullet+}$, 47); HRMS (FAB): (MH^+) calcd for $C_{33}H_{35}O_2Si_2$, 519.2176; found, 519.2182. HRMS (FAB): ($M^{\bullet+}$) calcd for $C_{33}H_{35}O_2Si_2$, 518.2097; found, 518.2111.

{2-[(4-Methoxyphenoxy)methyl]-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl}methyl 4-[2-(4-ethynylphenyl)ethynyl]benzoate (22). Tetrabutylammonium fluoride (TBAF, 5 mL, 5 mmol, 1M in THF) was added to **19** (450 mg, 1.08 mmol). After stirring for 15 min, the reaction was quenched with dilute HCl, and the product was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was azeotroped with toluene, then combined with **13** (314 mg, 1.08 mmol) and DMAP (788 mg, 6.46 mmol) in CH_2Cl_2 (10 mL), and treated with BOP−Cl (1.09 g, 4.3 mmol). After stirring for 1 h, the reaction mixture was concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc, 6/1) afforded **22** (440 mg, 78%), mp 144−146 °C; FTIR (neat) 2926 (CH), 2854 (CH), 1752 (C═O), 1724 (C═O), 1666 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 and 7.96 (doublets, 4H, J = 8.1 Hz, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.49 (br s, 4H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.81−6.86 (m, 4H, $CH_3OC_6H_4CH_2OCH_2C$), 4.58 (AB q, J = 11.8 Hz, 2H $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.09 (AB q, J = 9.5 Hz, 2H $CH_3OC_6H_4CH_2OCH_2C$), 3.76 (s, 3H, $CH_3OC_6H_4CH_2OCH_2C$), 3.20 (s, 1H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 2.95 (br AB q, J = 16.5 Hz, 2H, H-4$_{a,b}$), 2.27 (s, 3 H, C═C(CH_3)$_2$), 1.87 (s, 3 H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.73, 165.37, 154.48, 152.29, 151.53, 132.11, 131.59, 129.65, 128.86, 128.08, 123.01, 122.49, 118.70, 115.69, 114.71, 92.03, 90.34, 83.09, 79.58, 79.25, 70.57, 66.78, 55.70, 33.16, 24.58, 19.94. FAB MS (*m/z*, relative intensity) 521 (MH^+, 28). Anal. ($C_{33}H_{28}O_6$) C, H.

{4-(Methylethylidene)-5-oxo-2-[(phenylmethoxy)methyl]-2-2,3-dihydrofuryl}methyl 4-[2-(4-ethynylphenyl)ethynyl]benzoate (23). TBAF (162 μL, 1 M in THF) was added to **19** (34 mg, 81 μmol) and stirred for 10 min. The reaction was then quenched with diluted HCl and extracted with EtOAc (2 × 10 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was azeotroped with toluene, then combined with **14** (22 mg, 79.7 μmol) and DMAP (59 mg, 0.49 mmol) in CH_2Cl_2 (2 mL), and treated with BOP−Cl (82 mg, 0.32 mmol). After stirring for 1 h, the reaction mixture was concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc, 6/1) afforded **23** (25 mg, 62%), mp 98−100 °C; FTIR (neat) 2867 (CH), 1749 (C═O), 1724 (C═O), 1666 (C═C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 and 7.93 (doublets, J = 8.1 Hz, 4H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2$), 7.50 (br s, 4H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.30−7.33 (m, 5H, $C_6H_5CH_2OCH_2C$), 4.60 (s, 2H, $C_6H_5CH_2OCH_2C$), 4.48 (AB q, J = 11.7 Hz, 2H $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.64 (AB q, J = 9.9 Hz, 2H, $C_6H_5CH_2OCH_2C$), 3.20 (s, 1H, $HC{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 2.75 (br AB q, J = 16.5 Hz, 2 H, H-4$_{a,b}$), 2.24 (t, J = 2.0 Hz, 3H, C═C(CH_3)$_2$), 1.83 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.91, 165.32, 150.87, 137.34, 132.09, 131.56, 131.51, 129.61, 128.93, 128.42, 127.94, 127.86, 127.68, 122.99, 122.46,

119.01, 91.95, 90.35, 83.06, 80.32, 79.26, 73.67, 71.71, 66.81, 33.11, 24.47, 19.83. FAB MS (*m/z*, relative intensity) 505 (MH^+, 65). Anal. ($C_{33}H_{28}O_5 \cdot 0.5\ H_2O$) C, H.

{2-[(4-Methoxyphenoxy)methyl]-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl}methyl 4-(2-{4-[2-(4-Ethynylphenyl)ethynyl]-phenyl}ethynyl)benzoate (24). TBAF (0.3 mL, 0.3 mmol) was added to **21** (66 mg, 0.13 mmol, 1M in THF). After stirring for 15 min, the reaction was quenched with diluted HCl, and the product was extracted with EtOAc (2 × 20 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was azeotroped with toluene, then combined with **13** (37 mg, 0.13 mmol) and DMAP (140 mg, 1.14 mmol) in CH_2Cl_2 (2 mL), and treated with BOP−Cl (193 mg, 0.75 mmol). After stirring for 1 h, the reaction mixture was then directly chromatographed on silica gel (hexanes/EtOAc, 6/1) to afford **24** (53 mg, 68%), mp 173−175 °C; FTIR (neat) 2925 (CH), 1724 (C═O), 1666 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.57 and 7.96 (doublets, J = 8.3 Hz, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2$-CH_2C), 7.52 (s, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 7.48 (s, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 6.81−6.87 (m, 4H, $CH_3OC_6H_4OCH_2$C), 4.58 (AB q, J = 11.8 Hz, 2H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 4.09 (AB q, J = 9.6 Hz, $CH_3OC_6H_4OCH_2$C), 3.76 (s, 3H, C$H_3OC_6H_4OCH_2$C), 3.19 (s, 1H, *H*C≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 3.03 (br AB q, J = 16 Hz, 2H, H-4_{ab}),2.28 (br s, 3H, C═C(CH_3)$_2$), 1.87 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.72, 165.36, 154.46, 152.28, 151.49, 132.07, 131.69, 131.60, 131.56, 131.46, 129.64, 128.82, 128.12, 123.33, 122.61, 122.16, 118.70, 115.67, 114.69, 92.26, 90398, 90.88, 90.41, 83.16, 79.75, 79.10, 70.56, 66.77, 55.68, 33.14, 24.57, 19.92. FAB MS (*m/z*, relative intensity) 621 (MH^+, 23). Anal. ($C_{41}H_{32}O_6 \cdot 0.5H_2O$) C, H.

{2-[(4-Methoxyphenoxy)methyl]-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl} Methyl Decanoate (25). Decanoic anhydride (44 μL, 119.5 μmol) was added to a stirring solution of **13** (23 mg, 78.7 μmol) and DMAP (29 mg, 237.7 μmol) in CH_2Cl_2 (1 mL). After 30 min, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (2 × 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (EtOAc/hexanes 15/85) afforded **25** (34 mg, 97%) as an oil; FTIR (neat) 2926 (CH), 2855 (CH), 1746 (C═O), 1668 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.82 (s, 4H, $CH_3OC_6H_4OCH_2$C), 4.31 (AB q, J = 11.6 Hz, 2H, $CH_3C_6H_{12}CH_2CH_2C(O)OCH_2$C), 3.98 (AB q, J = 9.2 Hz, 2H $CH_3C_6H_4CH_2OCH_2$C), 3.76 (s, 3H, C$H_3C_6H_4CH_2$-OCH_2C), 2.96 (dm, J = 16.8 Hz, 1H, H-4_a), 2.75 (dm, J = 16.8 Hz, 1H, H-4_b), 2.23−2.33 (m, 5H, $CH_3C_6H_{12}CH_2CH_2C(O)OCH_2$C and C═C(C$H_3$)$_2$), 1.88 (s, 3H, C═C(C$H_3$)$_2$), 1.58 (m, 1H, $CH_3C_6H_{12}CH_2CH_2C(O)OCH_2$C), 1.24 (br s, 12H, $CH_3C_6H_{12}CH_2$-$CH_2C(O)OCH_2$C), 0.88 (irregular t, 3H, C$H_3C_6H_{12}CH_2CH_2C(O)$-OCH_2C); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.27, 168.69, 154.41, 152.34, 151.54, 118.59, 115.64, 114.66, 79.52, 70.32, 65.67, 55.68, 34.07, 32.87, 31.82, 29.36, 29.23, 29.17, 29.08, 24.80, 24.59, 22.64, 19.95, 14.07; FAB MS (*m/z*, relative intensity) 446 ($M^{\bullet+}$,100). Anal. ($C_{26}H_{38}O_6$) C, H.

[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl 4-[2-(4-Ethynylphenyl)ethynyl]benzoate (1). CAN **(**2.4 g, 4.4 mmol) was added to a stirred solution of **22** (800 mg, 1.54 mmol) in CH_3CN (8 mL) and H_2O (2 mL). After 10 min, the reaction was quenched with 5% sodium thiosulfate and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (25 mL), washed with water (15 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (hexanes/EtOAc 2.5/1) afforded **1** (476 mg, 75%), mp 138−140 °C; FTIR (neat) 3413 (OH), 3288 (OH), 2925 (CH), 1717 (C═O), 1667 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 and 7.90 (doublets, J = 8.0 Hz, 4H, HC≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 7.43 (s, 4H, HC≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 4.41 (AB q, J = 12.0 Hz, 2H, HC≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 3.70 (AB q, J = 12 Hz, 2H, HOCH_2C), 3.19 (s, 1H, *H*C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 2.76 (AB q, J = 16.4 Hz, 2H, H-4_{ab}), 2.25 (t, J = 2.1 Hz, 3H, C═C(CH_3)$_2$), 1.86 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.96, 165372, 151.80, 132.14, 131.62, 129.71, 128.71, 128.25, 122.99, 122.54, 118.91, 92.15, 90.30, 83.10, 81.33, 79.27, 66.35 64.85, 32.32, 24.59, 19.96; FAB MS (*m/z*, relative intensity) 415 (MH^+, 58). Anal. ($C_{26}H_{22}O_5 \cdot 0.25H_2O$) C, H.

[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl 4-(2-{4-[2-(4-Ethynylphenyl)ethynyl]phenyl}-ethynyl)benzoate (2). CAN (80 mg, 0.14 mmol) was added to a suspension solution of **24** (30 mg, 0.048 mmol) in CH_3CN (1 mL), CH_2Cl_2 (0.5 mL), and H_2O (0.25 mL). After stirring 10 min, the reaction was quenched with solid sodium thiosulfate, and the mixture was concentrated under reduced pressure. The residue was taken up in EtOAc (20 mL), washed with water (10 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (hexanes/EtOAc 11/9) afforded **2** (16 mg, 64%) as a white solid, mp 205−207 °C; FTIR (neat) 2968 (CH), 1707 (C═O), 1666 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CD_3OD/CDCl_3$): δ 7.56 and 7.95 (doublets, J = 8.2 Hz, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2$-CH_2C), 7.50 (s, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 7.45 (s, 4H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 4.45 (AB q, J = 11.6 Hz, 2H, HC≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 3.73 (AB q, J = 12 Hz, 2H, HOCH_2C), 3.15 (s, 1H, *H*C≡CC_6H_4C≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 2.80 (br AB q, J = 16.4 Hz, 2H, H-$4_{a,b}$), 2.23 (br s, 3H, C═C(CH_3)$_2$), 1.83 (s, 3H, C═C(CH_3)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.91, 165.74, 151.81,132.11, 131.72, 131.63, 131.62, 131.50, 129.73, 128.67, 128.32, 123.41, 123.38, 122.61, 122.19, 118.89, 92.40, 91.02, 90.88, 90.37, 83.18, 81.30, 79.00, 66.33, 64.87, 32.33, 24.60, 19.97; FAB MS (*m/z*, relative intensity) 515 (MH^+, 22). Anal. ($C_{34}H_{26}O_5 \cdot 1.25H_2O$) C, H.

[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl Decanoate (10). CAN (89 mg, 0.162 mmol) was added to a solution of **25** (24 mg, 0.059 mmol) in CH_3CN (1 mL) and H_2O (0.25 mL), and stirred for 10 min. The reaction was then quenched with sodium thiosulfate (60 mg) and concentrated under reduced pressure. The solid residue was rinsed with EtOAc (25 mL); the filtrate was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (EtOAc/hexanes 1/3) afforded **10** (13 mg, 71%) as an oil; FTIR (neat) 3459 (OH), 2927 (CH), 2856 (CH), 1738 (C═O), 1667 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 4.21 (AB q, J = 11.8 Hz, 2H, $CH_3C_6H_{12}CH_2CH_2C(O)OCH_2$C), 3.65 (AB q, J = 12.1 Hz, 2H HOCH_2C), 2.76 (dm, J = 16.5 Hz, 1H, H-4_a), 2.58 (dm, J = 16.5 Hz, 1H, H-4_b), 2.33 (t, J = 7.6 Hz, 2H, $CH_3C_6H_{12}CH_2CH_2C$-$(O)OCH_2$C), 2.26 (irregular t, 2H, C═C(CH_3)$_2$), 1.88 (s, 3H, C═C(CH_3)$_2$), 1.60 (irregular pentet, 1H, $CH_3C_6H_{12}CH_2CH_2C(O)$-$OCH_2$C), 1.26 (br s, 12H, $CH_3C_6H_{12}CH_2CH_2C(O)OCH_2$C), 0.88 (irregular t, 3H, C$H_3C_6H_{12}CH_2CH_2C(O)OCH_2$C); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.72, 168.91, 151.80, 118.79, 81.05, 65.27, 64.82, 34.08, 32.14, 31.83, 29.38, 29.32, 29.23, 29.10, 24.82, 24.60, 22.65, 19.98, 14.09; FAB MS (*m/z*, relative intensity) 341 (MH^+,-100). Anal. ($C_{19}H_{32}O_5$) C, H.

{4-(Methylethylidene)-5-oxo-2-[(phenylmethoxy)methyl]-2-2,3-dihydrofuryl}methyl 4-(2-{4-[2-(4-Methoxyphenyl)ethynyl]-phenyl}ethynyl)benzoate (26). A solution of **23** (264 mg, 0.53 mmol), 4-methoxyiodobenzene (135 mg, 0.58 mmol), $PdCl_2(PPh_3)_2$ (15 mg, 0.02 mmol, 2 mol %), and Cs_2CO_3 (205 mg, 0.64mmol) in THF (5 mL) was degassed and treated with CuI (2.5 mg, 0.01 mmol, 2.5 mol %). After stirring for 5 h, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (25 mL), washed with water (2 × 15 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (EtOAc/hexanes 1/6) afforded **26** (162 mg, 50%) and dimeric byproduct **27** (40 mg, 12%).

26: mp 104−106 °C; FTIR (neat) 2913 (CH), 1750 (C═O), 1722 (C═O), 1666 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 and 7.92 (doublets, J = 8.7 Hz, 4H, $CH_3OC_6H_4C$≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 7.51 (s, 4H, $CH_3OC_6H_4C$≡CC_6H_4C≡$CC_6H_4CO_2$-CH_2C), 7.48 (d, J = 8.9 Hz, 2H, $CH_3OC_6H_4C$≡CC_6H_4C≡CC_6H_4-CO_2CH_2C), 7.28−7.33 (m, 5H, $C_6H_5CH_2OCH_2$C), 6.89 (d, J = 8.9 Hz, 2H, $CH_3OC_6H_4C$≡CC_6H_4C≡$CC_6H_4CO_2CH_2$C), 4.60 (s, 2H, $C_6H_5CH_2OCH_2$C), 4.48 (AB q, J = 11.7 Hz, 2H, $CH_3OC_6H_4C$≡

$CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.83 (s, 3H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.64 (AB q, $J = 9.9$ Hz, 2H, $C_6H_5CH_2OCH_2C$), 2.86 (br AB q, $J = 16.3$ Hz, 2H, H-4_{ab}), 2.25 (br s, 3H, $C{=}C(CH_3)_2$), 1.83 (s, 3H, $C{=}C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.93, 165.37, 159.81, 150.89, 137.36, 133.09, 131.62, 131.48, 131.37, 129.61, 128.80, 128.43, 128.14, 127.87, 127.69, 124.10, 121.90, 119.02, 114.95, 114.03, 92.41, 91.74, 90.11, 87.73, 80.33, 73.68, 71.73, 66.80, 55.28, 33.12, 24.48, 19.84. FAB MS (*m/z*, relative intensity) 611 (MH^+, 40). Anal. ($C_{40}H_{34}O_6{\cdot}1.3H_2O$) C, H.

27: FTIR (neat) 2920 (CH), 2867 (CH), 1748 (C=O), 1725 (C=O), 1666 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 and 7.93 (doublets, $J = 8.4$ Hz, 8H, $CCH_2O_2CC_6H_4C{\equiv}CC_6H_4C{\equiv}C{-}C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.53 (s, 8H, $CCH_2O_2CC_6H_4C{\equiv}CC_6H_4C{\equiv}C{-}C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.30−7.33 (m, 10H, 2 × $C_6H_5CH_2OCH_2C$), 4.60 (s, 4H, 2 × $C_6H_5CH_2OCH_2C$), 4.48 (AB q, $J = 11.7$ Hz, 4H, $CCH_2O_2CC_6H_4C{\equiv}CC_6H_4C{\equiv}C{-}C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.64 (AB q, $J = 9.9$ Hz, 4H, 2 × $C_6H_5CH_2OCH_2C$), 2.83 (br AB q, $J = 16.5$ Hz, 4H, 2 × H-4_{ab}), 2.25 (br s, 6H, $C{=}C(CH_3)_2$), 1.84 (s, 6H, $C{=}C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.95, 165.36, 150.95, 137.37, 132.49, 131.73, 131.57, 129.66, 129.04, 128.46 127.90, 127.72, 123.56,-121.94 119.02, 114.05, 91.96, 91.15, 82.09, 80.34, 75.85, 73.72, 71.74, 66.86, 33.16, 24.52, 19.87. FAB MS (*m/z*, relative intensity) 1007 (MH^+, 2).

[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl 4-(2-{4-[2-(4-Methoxyphenyl)ethynyl]phenyl}-ethynyl)benzoate (3). A solution of BCl_3 in CH_2Cl_2 (380 μL, 0.38 mmol) was added to a solution of **26** (48 g, 0.95 mmol) in CH_2Cl_2 (15 mL) and stirred at −78 °C for 1.5 h. The reaction was quenched with a saturated $NaHCO_3$ solution (20 mL), warmed to room temperature, and extracted with CH_2Cl_2 (2 × 30 mL). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (CH_2Cl_2/MeOH, 50/1) afforded **3** (24 mg, 60%) as a white solid, mp 187−189 °C. FTIR (neat) 2923 (CH), 17470 (C=O), 1716 (C=O), 1668 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 and 7.97 (doublets, $J = 8.4$ Hz, 4H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.51 (s, 4H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.89 and 7.47 (doublets, $J = 8.8$ Hz, 4H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.48 (AB q, $J = 11.8$ Hz, 2H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.83 (s, 3H, $CH_3OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.73 (dd, $J = 12.0$, 6.0 Hz, 1H, HOC*H*HC), 3.80 (dd, $J = 12.0$, 6.0 Hz, 1H, HOCH*H*C), 2.83 (br AB q, $J = 16.4$ Hz, 2H, *H*-4_{ab}), 2.29 (app t, 1H, *H*OCH_2C), 2.25 (s, 3H, $C{=}C(CH_3)_2$), 1.86 (s, 3H, $C{=}C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.97, 165.75, 159.85, 151.79, 133.12, 131.65, 131.58, 131.41, 129.70, 128.56, 128.43, 124.16, 121.88, 118.92, 114.99, 114.06, 92.61, 91.77, 90.05, 87.75, 81.35, 66.33, 64.85, 55.31, 32.31, 24.59, 19.95. FAB MS (*m/z*, relative intensity) 521 (MH^+, 63), 520 ($M^{\bullet+}$, 71). Anal. ($C_{33}H_{28}O_6{\cdot}2H_2O$) C, H.

Methyl 2-(4-iodophenyl)acetate (30). Methyl iodide (4.67 mL, 74.9 mmol) was added to a suspension of **29** (4.17 g, 15 mmol) and $NaHCO_3$ (1.37 g, 16.5 mmol) in DMF. After stirring for 2 days, the reaction mixture was diluted with EtOAc (100 mL) and washed with water (3 × 25 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/ EtOAc 9/1) afforded **30** (3.9 g, 89%), mp 104−106 °C. FTIR (neat) 2970 (CH), 1768 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.54 and 7.57 (doublets, $J \approx 8$ Hz, 4H, $CH_3O_2CCH_2OC_6H_4I$), 4.61 (s, 2H, $CH_3O_2CCH_2OC_6H_4I$), 3.80 (s, 3H, $CH_3O_2CCH_2OC_6H_4I$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.90, 157.54, 138.27, 116.90, 84.07, 65.13, 52.25; FAB MS (*m/z*, relative intensity) 293 (MH^+, 22), 292 ($M^{\bullet+}$, 71). Anal. ($C_9H_9IO_3$) C, H, I.

Methyl 2-{4-[2-(4-Aminophenyl)ethynyl]phenoxy}acetate (32). A solution of 4-ethynylaniline (**31**, 110 mg, 0.94 mmol), **30** (270 mg, 0.92 mmol), $PdCl_2(PPh_3)_2$ (13 mg, 18.5 μmol, 2 mol %), and piperidine (2 mL) in THF (10 mL) was degassed and treated with CuI (7 mg, 36.8 μmol, 4 mol %). After stirring for 3 h, the reaction mixture was concentrated under reduced pressure, dissolved in CH_2Cl_2 (25 mL), washed with saturated aqueous NH_4Cl (2 × 25 mL), and dried ($MgSO_4$). Purification by column chromatography on silica gel (hexanes/EtOAc 3/1) afforded **32** (193 mg, 74%), mp 144−146 °C; FTIR (neat) 3461 (NH_2), 3357 (NH_2), 2359 (CH), 2341 (CH), 1741 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 and 7.45 (app doublets, $J \approx 8.3$ Hz, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4NH_2$), 6.62 and 6.88 (app doublets, $J \approx 9.2$ Hz, 4H, $CH_3O_2CH_2OC_6H_4C{\equiv}CC_6H_4NH_2$), 4.64 (s, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4NH_2$), 3.81 (s, 3H, $CH_3O_2CH_2OC_6H_4C{\equiv}CC_6H_4NH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.11, 157.25, 146.43, 132.81, 132.79, 117.33, 114.74, 114.59, 112.79, 89.03, 86.78, 65.26, 52.29; FAB MS (*m/z*, relative intensity) 282 (MH^+, 55), 281 ($M^{\bullet+}$, 100). Anal. ($C_{17}H_{15}NO_3{\cdot}0.25H_2O$) C, H.

Methyl 2-{4-[2-(4-Iodophenyl)ethynyl]phenoxy}acetate (33). Aqueous $NaNO_2$ (278 mL) was added to **32** (1.06 g) in 2 N HCl (25 mL) at 0 °C and stirred for 15 min. Solid KI (913 mg) was added, and the reaction mixture was stirred for an additional 20 min. The reaction was then quenched with sodium thiosulfate, and the product was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc, 9/1) afforded **33** (371 mg, 44%), mp 174−175 °C; FTIR (neat) 2960 (CH), 2360 (CH), 2341 (CH), 1726 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 and 7.68 (app doublets, $J \approx 8.0$ Hz, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4I$), 6.88 and 7.22 (app doublets, $J \approx 8.4$ Hz, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4I$), 4.66 (s, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4I$), 3.81 (s, 3H, $CH_3OCCH_2OC_6H_4C{\equiv}CC_6H_4I$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.97, 157.87, 137.46 ,133.14, 132.95, 122.95, 116.26, 114.70, 93.77, 90.43, 87.56, 65.21, 52.33; FAB MS (*m/z*, relative intensity) 393 (MH^+, 43), 392 ($M^{\bullet+}$, 100); HRMS (FAB): (M^+) calcd for $C_{17}H_{13}O_3I$, 391.991; found, 391.990.

Methyl 16-[(4-Methylphenyl)sulfonyloxy]hexadecanoate (35). A suspension of commercially available juniperic acid (**34**, 1 g, 3.68 mmol), MeI (2.28 mL, 36.6 mmol), and $NaHCO_3$ (615 mg, 7.32 mmol) in DMF (10 mL) was stirred at 40 °C for 2 days. The reaction mixture was then diluted with EtOAc (25 mL) and washed with water (2 × 20 mL). The organic layer was dried ($MgSO_4$), concentrated under reduced pressure, and then added to toluene, forming an azeotrope. The residue was stirred with TsCl (1.05 g, 5.52 mmol) and DMAP (1.35 g, 11.03 mmol) in CH_2Cl_2 (20 mL) for 1 h. Purification by column chromatography on silica gel (hexanes/EtOAc 10/1) afforded **35** (900 mg, 55%) as a white solid, mp 54−55 °C; FTIR (neat) 2915 (CH), 2849 (CH), 1733 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 and 7.75 (doublets, $J = 8.4$ Hz, 4H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 4.01 (t, $J = 6.6$ Hz, 2H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 3.66 (s, 3H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 2.44 (s, 3H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 2.30 (t, $J = 7.6$ Hz, 2H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 1.57−1.66 (m, 4H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$), 1.21−1.31 (m, 22H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OSO_2C_6H_4CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.30, 144.56, 133.26, 129.75, 127.85, 70.68, 51.40, 34.10, 29.59, 29.56, 29.46, 29.42, 29.36, 29.23, 29.13, 28.90, 28.79, 25.30, 24.94, 21.61; FAB MS (*m/z*, relative intensity) 441 (MH^+, 91); HRMS (FAB): (MH^+) calcd for $C_{24}H_{41}O_5S$, 441.267; found, 441.270.

Methyl 16-(4-Iodophenoxy)hexadecanoate (36). A suspension of **35** (470 mg, 1.07 mmol), 4-iodophenol (235 mg, 1.07 mmol), Cs_2CO_3 (348 mg, 1.07 mmol) in DMF (5 mL) was stirred at 50 °C overnight. The reaction mixture was then diluted with EtOAc (20 mL) and washed with water (2 × 15 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc 10/1) afforded **36** (500 mg, 96%) as a white solid, mp 154−155 °C; FTIR (neat) 2918 (CH), 2849 (CH), 1736 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 and 7.52 (doublets, $J = 8.0$ Hz, 4H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OC_6H_4I$), 3.90 (t, $J = 6.6$ Hz, 2H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OC_6H_4I$), 3.66 (s, 3H, $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OC_6H_4I$), 2.29 (t, $J = 7.6$ Hz, 2H $CH_3O_2CCH_2CH_2(CH_2)_{11}CH_2CH_2OC_6H_4I$), 1.72−1.79 (m, 2H, CH_3O_2-

$CCH_2CH_2(CH_2)_{11}CH_2CH_2OC_6H_4I$), 1.58−1.65 (m, 2H, $CH_3O_2CCH_2CH_2$ $(CH_2)_{11}CH_2CH_2OC_6H_4I$), 1.25 (br s, 22H, $CH_3O_2CCH_2CH_2(CH_2)_{11}$-$CH_2CH_2OC_6H_4I$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.30, 158.98, 138.10, 116.91, 82.34, 68.10, 51.41, 34.10, 29.61, 29.57, 29.43, 29.34, 29.24, 29.12, 25.96, 24.94; FAB MS (*m/z*, relative intensity) 489 (MH^+, 65), 488 ($M^{\bullet+}$, 100); HRMS (FAB): (MH^+) calcd for $C_{23}H_{37}O_3I$, 489.187; found: 489.185; HRMS (FAB): Calcd for $C_{23}H_{37}O_3I$ ($M^{\bullet+}$); 488.179; found, 488.176.

Methyl 2-{4-[2-(4-{2-[4-({[2-(hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl}oxycarbonyl)phenyl]-ethynyl}phenyl)ethynyl]phenoxy}acetate (4). A suspension of **1** (2.9 mg, 7 μmol), **30** (2.3 mg, 8.2 mmol), $Pd(PPh_3)_4$ (1.6 mg, 1.4 μmol) Cs_2CO_3 (1.2 mg, 3.5 μmol), and Ag_2O (0.9 mg, 3.9 μmol) in THF (3 mL) was degassed and heated to 60 °C. After stirring at 60 °C for 90 min, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc 2/1) afforded **4** (2.5 mg, 63%) as a white solid, mp 188−190 °C; FTIR (neat) 2958 (CH), 2926 (CH), 1721 (C═O), 1669 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.58 and 7.97 (doublets, J = 8.1 Hz, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.49 (s, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.89 and 7.48 (doublets, J = 8.5 Hz, 4H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.67 (s, 3H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.48 (AB q, J = 11.6 Hz, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.82, (s, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.77 (AB q, J = 12.0 Hz, 2H, $HOCH_2C$), 2.84 (br AB q, J = 16.5 Hz, 2 H, H-$4_{a,b}$), 2.25 (s, 3H, $C{=}C(CH_3)_2$), 1.88 (s, 3 H, $C{=}C(CH_3)_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.99, 168.95, 165.74, 157.92, 151.79, 133.21, 131.66, 131.59, 131.46,129.71, 128.59, 128.40, 123.98, 122.04, 118.91, 116.29, 114.73, 92.55, 91.34, 90.11, 88.11, 81.33, 66.33, 65.22, 64.86, 52.35, 32.32, 24.60, 19.96; FAB MS (*m/z*, relative intensity) 579 (MH^+, 36), 578 ($M^{\bullet+}$, 44). HRMS (FAB): (MH^+) calcd for $C_{35}H_{30}O_8$, 579.202; found, 579.201; ($M^{\bullet+}$) calcd for $C_{35}H_{30}O_8$, 578.194; found 578.195.

Methyl 2-[4-(2-{4-[2-(4-{2-[4-({[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl}oxycarbonyl)-phenyl]ethynyl}phenyl)ethynyl]phenyl} ethynyl)phenoxy]acetate (5). A suspension of **1** (9 mg, 21.7 μmol), **33 (**9 mg, 22.9 μmol), $Pd(PPh_3)_4$ (5.3 mg, 4.6 μmol), Cs_2CO_3 (39 mg, 0.12 mmol) and Ag_2O (3.0 mg, 12.9 μmol) in THF (5 mL) was degassed and stirred at 60 °C for 50 min. The reaction mixture was concentrated under reduced pressure and directly purified by preparative TLC (SiO_2, MeOH/CH_2Cl_2 1/49) to afford **5** (8.5 mg, 58%) as a white solid, mp 180−184 °C; FTIR (neat) 2956 (CH), 2925 (CH), 1721 (C═O), 1666 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CH_3OD/CDCl_3$): δ 7.88 (d, J = 8.6 Hz, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.40−7.53 (m, 12H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.83 (d, J = 9.0 Hz, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.60 (s, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.39 (AB q, J = 11.8 Hz, 2H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.75, (s, 3H, $CH_3O_2CCH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.66 (AB q, J = 12.2 Hz, 2H, $HOCH_2C$), 2.78 (br AB q, J = 16.7 Hz, 2H, H-$4_{a,b}$), 2.17 (s, 3H, $C{=}C(CH_3)_2$), 1.79 (s, 3 H, $C{=}C(CH_3)_2$); ^{13}C NMR (100 MHz, $CH_3OD/CDCl_3$): δ 169.89, 165.61, 157.73 ,151.61, 133.05, 131.57, 131.46, 131.40, 131.27, 129.47, 128.60, 123.46, 123.39, 122.35, 122.31, 119.20, 116.21, 116.07, 114.58, 92.26, 91.19, 90.97, 90.51, 90.14, 88.61, 88.01, 81.92, 66.49, 65.03, 64.35, 32.09, 19.72; FAB MS (*m/z*, relative intensity) 679 (MH^+, 5), 678 ($M^{\bullet+}$, 5); HRMS was not possible because of the formation of aggregates.

Methyl 16-{4-[2-(4-{2-[4-({[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo-2-2,3-dihydrofuryl]methyl}oxycarbonyl)phenyl]-ethynyl}phenyl)ethynyl]phenoxy}hexadecanoate (6). A suspension of **1** (8 mg, 19.3 μmol), **36** (9.4 mg, 19.3 μmol), $Pd(PPh_3)_4$ (4.5 mg, 3.9 μmol), Cs_2CO_3 (3.2 mg, 9.5 μmol), and Ag_2O (2.3 mg, 1.95 μmol) in THF (6 mL) was degassed and stirred at 60 °C for 90 min. The reaction mixture was then concentrated under reduced pressure and directly purified by column chromatography on silica gel (hexanes/EtOAc 2/1) to give **6** (9.1 mg, 61%) as a white solid, mp 174−176 °C; FTIR (neat) 3406 (OH), 2918 (CH), 2915 (CH), 1716 (C═O), 1668 (C═C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.59 and 7.98 (doublets, J = 8.3 Hz, 4H, $CH_3O_2C(CH_2)_{15}$-$OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.51−7.54 (m, 4H, CH_3O_2C-$(CH_2)_{15}OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.88 and 7.46 (doublets, J = 8.8 Hz, 4H, CH_3O_2C $(CH_2)_{15}OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4$-$CO_2CH_2C$), 4.49 (AB q, J = 11.8 Hz, 2H, $CH_3O_2C(CH_2)_{15}OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.98 (t, J = 6.6 Hz, 2H, CH_3O_2C-$(CH_2)_{14}CH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 3.82 (br AB q, J = 12.1 Hz, 2H, $HOCH_2C$), 3.67 (s, 3H, $CH_3O_2C(CH_2)_{15}OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 2.84 (br AB q, J = 16.0 Hz, 2H, H-$4_{a,b}$), 2.30 (t, J = 7.6 Hz, 2H, $CH_3O_2CCH_2(CH_2)_{14}OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 2.26 (t, J = 2.0 Hz, 3H, $C{=}C(CH_3)_2$), 1.87 (s, 3 H, $C{=}C(CH_3)_2$), 1.75−1.83, 1.60−1.64 and 1.26−1.54 (multiplets, 26H, $CH_3O_2CCH_2(CH_2)_{13}CH_2OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.90, 165.77, 159.48, 151.83, 133.10, 131.69, 131.65, 131.60, 131.41, 129.72, 128.56, 128.47, 124.25, 121.84, 118.88, 114.70, 114.60, 92.65, 91.93, 90.04, 87.66, 81.29, 68.12, 66.31, 64.88, 51.43, 34.12, 32.34, 29.64, 29.59, 29.45, 29.38, 29.26, 29.16, 26.02, 24.97, 24.60, 24.58, 24.57, 19.97; FAB MS (*m/z*, relative intensity) 775 (MH^+, 32), 774 ($M^{\bullet+}$, 43); HRMS (FAB): (MH^+) calcd for $C_{49}H_{59}O_8$, 775.424; found, 775.422; ($M^{\bullet+}$) 774.418; found, 774.413.

2-{2-[2-(4-Iodophenoxy)ethoxy]ethoxy}ethyl 4-methylbenzenesulfonate (38). Commerically available tri(ethylene glycol) di-*p*-tosylate (**37**, 458 mg, 1 mmol) was added to a mixture of 4-iodophenol (220 mg, 1 mmol) and Cs_2CO_3 (326 mg, 2 mmol) in DMF (5 mL) and stirred at 50 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with EtOAc (40 mL), and washed with saturated NH_4Cl (2 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc 9/1) afforded **38** (216 mg, 42%) and the double displacement product (133 mg, 21%), which was not fully characterized. FTIR (neat) 2873 (CH) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.20 and 7.70 (doublets, J = 8.0 Hz, 4H, $CH_3C_6H_4SO_3CH_2CH_2OCH_2CH_2OCH_2$-$CH_2OC_6H_4I$), 6.60 and 7.45 (app doublets, $J \approx$ 8.8 Hz, 4H, $CH_3C_6H_4SO_3CH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 3.48−4.10 (multiplets, 12H, $CH_3C_6H_4SO_3CH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 2.33 (s, 3H, $CH_3C_6H_4SO_3CH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.33, 144.51, 137.78, 132.57, 129.54, 116.76, 82.60, 70.34, 69.24, 69.02, 68.31, 37.18, 21.33. FAB MS (*m/z*, relative intensity) 507 (MH^+, 100). Anal. ($C_{19}H_{23}$-IO_6S) C, H, I, S.

***tert*-Butyl 2-[{[(*tert*-Butyl)oxycarbonyl]methyl}(2-{2-[2-(4-iodophenoxy)ethoxy] ethoxy}ethyl)amino]acetate (39).** A solution of **38** (718 mg, 1.35 mmol), and di-*tert*-butyliminodiacetate (661 mg, 2.7mmol) in DMF (5 mL) was stirred at 50 °C for 3 days. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was then dissolved in EtOAc (40 mL) and washed with water (2 × 20 mL). The organic layer was dried ($MgSO_4$) and concentrated under reduced pressure. Purification by column chromatography on silica gel (hexanes/EtOAc 9/1) afforded **39** (550 mg, 70%); FTIR (neat) 2975 (CH), 2929 (CH), 1738 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.70 and 7.54 (app doublets, J = 7.2 Hz, 4H, $NCH_2CH_2OCH_2$-$CH_2OCH_2CH_2OC_6H_4I$), 4.07 (m, 2H, $NCH_2CH_2OCH_2CH_2OCH_2CH_2$-$OC_6H_4I$), 3.84 (m, 2H, $NCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 3.70 (m, 2H, $NCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 3.63 (m, 4H, $NCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 3.50 (s, 4H, $((CH_3)_3CO_2$-$CCH_2)_2N$), 2.95 (m, 2H, $NCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 1.44 (s, 18H, $((CH_3)_3CO_2CCH_2)_2N$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.75, 158.66, 138.12, 117.03, 82.87, 80.83, 70.79, 70.47, 70.33, 69.56, 67.51, 56.65, 53.35, 28.15; FAB MS (*m/z*, relative intensity) 580 (MH^+, 59). HRMS (FAB): (MH^+) calcd for $C_{24}H_{39}O_7N$, 580.177; found, 580.175.

***tert*-Butyl 2-[*N*-{[(*tert*-Butyl)oxycarbonyl]methyl}(2-{2-[2-(4-iodophenoxy)ethoxy] ethoxy}ethoxy)carbonylamino]acetate (40).** A suspension of **38** (2.1 g, 4.15 mmol), di-*tert*-butyliminodiacetate

(1.0 g, 4.15 mmol), and Cs_2CO_3 (1.4 g, 4.15 mmol) in DMF (20 mL) was stirred at 70 °C for 30 h. The reaction mixture was then cooled to room temperature, diluted with EtOAc (50 mL), washed with saturated aqueous NH_4Cl solution (2 × 25 mL), dried ($MgSO_4$), and concentrated. Purification by column chromatography on silica gel (hexanes/EtOAc/AcOH 13/5/2) afforded small amounts of **39** (1.32 mg) and **40** (600 mg, 51%).

40: FTIR (neat) 2979 (CH), 2881 (CH), 1742 (C═O), 1712 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.68 and 7.52 (app doublets, $J \approx 9.2$ Hz, 4H, $OCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 4.24 (m, 2H, $OCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 4.07 (m, 2H, $OCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 4.00 (s, 2H, $(CH_3)_3CO_2CCH_2N$), 3.95 (s, 2H, $(CH_3)_3CO_2CCH_2N$), 3.82 (m, 2H, $OCH_2CH_2OCH_2CH_2OCH_2CH_2OC_6H_4I$), 3.60−3.69 (m, 6H, $OCH_2CH_2OCH_2CH_2OC_6H_4I$), 1.44 (s, 3H, $((CH_3)_3CO_2CCH_2)_2N$), 1.45 (s, 3H, $((CH_3)_3CO_2CCH_2)_2N$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.60, 168.47, 158.62, 155.98, 138.09, 117.00, 82.83, 81.83, 81.80, 70.75, 70.58, 69.58, 69.34, 67.47, 65.11, 56.62, 49.90, 49.75, 28.13, 28.03. FAB MS (*m/z*, relative intensity) 624 (MH^+, 13), 623 ($M^{\bullet+}$, 16); HRMS (FAB): (M^+) calcd for $C_{25}H_{38}O_9NI$, 623.159; found, 623.158. Anal. ($C_{25}H_{38}INO_9$) C, H, I, N.

***tert*-Butyl 2-({[(*tert*-Butyl)oxycarbonyl]methyl}{2-[2-(2-{4-[2-(4-{2-[4-({[2-(hydroxymethyl)-4-(methylethylidene)-5-oxo(2-2,3-dihydrofuryl)]methyl}oxycarbonyl) phenyl]ethynyl}phenyl)-ethynyl]phenoxy}ethoxy)ethoxy]ethyl}amino)acetate (7).** A suspension of **1** (2 mg, 4.6 μmol), **39** (3 mg, 5.18 μmol), $Pd(PPh_3)_4$ (0.4 mg, 0.3 μmol, 8 mol %), Cs_2CO_3 (2 mg, 12.3 μmol), and Ag_2O (1 mg, 4.3 μmol) in THF (10 mL) was degassed and stirred at 60 °C for 50 min. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure and purified by preparative TLC (SiO_2, CH_2Cl_2/MeOH 100/1) to give **7** (2 mg, 45%) as a semisolid product; ^1H NMR (400 MHz, $CDCl_3$): δ 7.52 and 7.98 (app doublets, $J \approx 8.4$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.50 (s, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.80 and 7.45 (app doublets, $J \approx 8.8$ Hz, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.48 (AB q, $J = 11.8$ Hz, 2H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.15 (irregular t, 2H, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$), 3.87 (irregular t, 2H, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$), 3.59−3.75 (m, 6H, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$), 3.49 (s, 4H, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$), 2.95 (t, 2H, $J = 6.0$ Hz, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$), 2.84 (br AB q, $J = 16.0$ Hz, 2H, $H\text{-}4_{a,b}$), 2.26 (t, $J = 2.0$ Hz, 3H, $C{=}C(CH_3)_2$), 1.86 (s, 3H, $C{=}C(CH_3)_2$), 1.45 (s, 18H, $((CH_3)_3CO_2CCH_2)_2NCH_2CH_2O(CH_2)_2OCH_2CH_2O$). FAB MS (*m/z*, relative intensity) 904 ($M + K^+$, 34), 888 ($M + Na^+$, 5), 866 (MH^+, 21). HRMS (FAB): ($M + K^+$) calcd for $C_{50}H_{59}NO_2K$, 904.37; found, 904.34; ($M + Na^+$) 866.38; found, 866.39. Isotopic distributions are the same as those calculated for expected elemental analysis.

{2-[2-(2-{4-[2-(4-{2-[4-({[2-(Hydroxymethyl)-4-(methylethylidene)-5-oxo(2-2,3-dihydrofuryl)]methyl}oxycarbonyl)phenyl]-ethynyl}phenyl)ethynyl]phenoxy}ethoxy)ethoxy]ethoxy}-carbonylamino)acetate (8). A suspension of **1** (40 mg, 0.096 mmol), **40** (44 mg, 0.070 mmol), $Pd(PPh_3)_4$ (20 mg, 17.3 μmol), Cs_2CO_3 (40 mg, 3.5 μmol), and Ag_2O (24 mg, 0.1 mmol) in THF (15 mL) was degassed and stirred at 60 °C for 30 min. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was then taken up in water (20 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were then dried ($MgSO_4$) and concentrated. Purification by column chromatography on silica gel (ether/EtOAc 9/1) afforded **8** (36 mg, 56%), mp 82−83 °C; FTIR (neat) 2981 (CH), 2881 (CH), 1743 (C═O), 1699 (C═O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.57 and 7.96 (app doublets, $J \approx 8.8$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 7.50 (d, $J = 0.8$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 6.90 and 7.95 (app doublets, $J \approx 8.8$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.47 (AB q, $J = 11.8$ Hz, 2H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.26 (m, 2H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 4.15 (m, 2H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 4.01 (s, 2H, $(CH_3)_3CO_2CCH_2N$), 3.96 (s, 2H, $(CH_3)_3CO_2CCH_2N$), 3.86 (m, 2H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 3.64−3.81 (m, 8H, $NCO_2CH_2CH_2OCH_2CH_2OCH_2CH_2O$ and $HOCH_2C$), 2.90 (dm, $J = 16.8$ Hz, 1H, $H\text{-}4_a$), 2.75 (dm, $J = 16.8$ Hz, 1H, $H\text{-}4_b$), 2.40 (br s, 1H, $HOCH_2C$), 2.25 (t, $J = 2.0$ Hz, 3H, $C{=}C(CH_3)_2$), 1.85 (s, 3H, $C{=}C(CH_3)_2$), 1.45 and 1.46 (s, 6H, $(CH_3)_3CO_2CCH_2N$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.94, 168.68, 168.55, 165.75, 159.09, 156.07, 151.78, 133.09, 131.65, 131.58, 131.41, 129.71, 128.57, 128.44, 124.18, 121.88, 118.91, 115.14, 114.72, 92.62, 91.78, 90.05, 87.77, 81.91, 81.88, 81.32, 70.83, 70.64, 69.67, 69.41, 67.49, 66.32, 65.18, 64.86, 49.97, 49.82, 32.32, 28.08, 28.06, 24.59, 19.96. FAB MS (*m/z*, relative intensity) 909 ($M^{\bullet+}$, 9). Anal. ($C_{51}H_{59}NO_{14}\cdot H_2O$) C, H, N.

2-(*N*-(Carboxymethyl){2-[2-(2-{4-[2-(4-{2-[4-({[2-(hydroxymethyl)-4-(methyl ethylidene)-5-oxo(2-2,3-dihydrofuryl)]methyl}-oxycarbonyl)phenyl]ethynyl}phenyl)ethynyl]phenoxy}ethoxy)-ethoxy]ethoxy}carbonylamino)acetic acid (9). A suspension of **8** (6.1 mg, 6.6 μmol) and montmorillonite KSF (17 mg) in MeCN (1 mL) was microwaved for 7 min (ramp 2 min, reaction time 5 min) at 145 °C. The reaction mixture was filtered, and the filtrate was directly purified by semipreparative reverse-phase HPLC using a 250 mm × 20 mm YMC ODS-H80 column eluted with a gradient of H_2O/MeCN (9/1) to 100% MeCN over 40 min. At 25 min, the collected fraction afforded **9** (1.5 mg, 27%) as a solid, mp 194−195 °C; FTIR (neat) 2954 (CH), 2899 (CH), 1709 (C═O), 1606 (C═O) cm^{-1}; ^1H NMR (400 MHz, CD_3OD δ): 7.60 and 7.95 (app doublets, $J \approx 8.4$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2$), 7.50 (app doublet, $J \approx 4.8$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2$), 6.94 and 7.44 (app doublets, $J \approx 8.8$ Hz, 4H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2$), 4.48 (AB q, $J = 11.8$ Hz, 2H, $OC_6H_4C{\equiv}CC_6H_4C{\equiv}CC_6H_4CO_2CH_2C$), 4.23 (m, 2H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 4.16 ($NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 4.07 and 4.08 (singlets, 4H, $((CH_3)_3CO_2CCH_2)_2N$), 3.87 (m, 2H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$), 3.66−3.74 (m, 8H, $NOCOCH_2CH_2OCH_2CH_2OCH_2CH_2O$ and $HOCH_2C$), 2.88 (br AB q, $J = 18$ Hz, 2H, $H\text{-}4_{a,b}$), 2.21 (t, $J = 1.9$ Hz, 3H, $C{=}C(CH_3)_2$), 1.88 (s, 3H, $C{=}C(CH_3)_2$); FAB MS (*m/z*, relative intensity) 796 (M−H, 50); HRMS was not possible because of the formation of aggregates.

Acknowledgment. This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

Supporting Information Available: ^1H NMR spectra, ^{13}C NMR spectra, and combustion analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jain, M. K. In *Introduction to Biological Membranes*, 2nd ed. Wiley & Sons: New York, 1988; pp 51−85.
(2) Brown, D. A.; London, E. Structure and origin of ordered lipid domains in biological membranes. *J. Membr. Biol.* **1998**, *164*, 103−114.
(3) Maxfield, F. R.; Tabas, I. Role of cholesterol and lipid organization in disease. *Nature* **2005**, *438*, 612−621.
(4) Huang, J. Y.; Feigenson, G. W. A microscopic interaction model of maximum solubility of cholesterol in lipid bilayers. *Biophys. J.* **1999**, *76*, 2142−2157.
(5) Simons, K.; Ikonen, E. Functional rafts in cell membranes. *Nature* **1997**, *387*, 569−572.
(6) Cho, W. Building signaling complexes at the membrane. *Sci. STKE* **2006**, 7.
(7) Marquez, V. E.; Blumberg, P. M. Synthetic diacylglycerols (DAG) and DAG-lactones as activators of protein kinase C (PK-C). *Acc. Chem. Res.* **2003**, *36*, 434−443.
(8) Garcia-Bermejo, M. L.; Leskow, F. C.; Fujii, T.; Wang, Q.; Blumberg, P. M.; Ohba, M.; Kuroki, T.; Han, K. C.; Lee, J.; Marquez, V. E.; Kazanietz, M. G. Diacylglycerol (DAG)-lactones, a new class of protein kinase C (PKC) agonists, induce apoptosis in LNCaP prostate cancer cells by selective activation of PKCalpha. *J. Biol. Chem.* **2002**, *277*, 645−655. (Published erratum appears in *J. Biol. Chem.* **2004**, *279*, 23846.)

(9) Pu, Y.; Perry, N. A.; Yang, D.; Lewin, N. E.; Kedei, N.; Braun, D. C.; Choi, S. H.; Blumberg, P. M.; Garfield, S. H.; Stone, J. C.; Duan, D.; Marquez, V. E. A novel diacylglycerol-lactone shows marked selectivity in vitro among C1 domains of protein kinase C (PKC) isoforms {alpha} and {delta} as well as selectivity for RasGRP compared with PKC{alpha}. *J. Biol. Chem.* **2005**, *280*, 27329−27338.

(10) Szule, J. A.; Fuller, N. L.; Rand, R. P. The effects of acyl chain length and saturation of diacylglycerols and phosphatidylcholines on membrane monolayer curvature. *Biophys. J.* **2002**, *83*, 977−984.

(11) Muller, P. U.; Weber, E.; Rheinwald, G.; Seichter, W. Oligofunctional amphiphiles featuring geometric core group preorganization: Synthesis and study of Langmuir and Langmuir−Blodgett films. *Org. Biomol. Chem.* **2005**, *3*, 3757−3766.

(12) Schnablegger, H.; Antonietti, M.; Goltner, C.; Hartmann, J.; Colfen, H.; Samori, P.; Rabe, J. P.; Hager, H.; Heitz, W. Morphological characterization of the molecular superstructure of polyphenylene ethynylene derivatives. *J. Colloid Interface Sci.* **1999**, *212*, 24−32.

(13) Kim, I. B.; Dunkhorst, A.; Bunz, U. H. F. Nonspecific interactions of a carboxylate-substituted PPE with proteins. A cautionary tale for biosensor applications. *Langmuir* **2005**, *21*, 7985−7989.

(14) James, D. K.; Tour, J. M. Organic synthesis and device testing for molecular electronics. *Aldrichimica Acta* **2006**, *39*, 47−56.

(15) Xu, R. X.; Pawelczyk, T.; Xia, T. H.; Brown, S. C. NMR structure of a protein kinase C-gamma phorbol-binding domain and study of protein-lipid micelle interactions. *Biochemistry* **1997**, *36*, 10709−10717.

(16) Hurley, J. H.; Meyer, T. Subcellular targeting by membrane lipids. *Curr. Opin. Cell Biol.* **2001**, *13*, 146−152.

(17) Ghebremariam, B.; Sidorov, V.; Matile, S. Direct evidence for the importance of hydrophobic mismatch for cell membrane recognition. *Tetrahedron Lett.* **1999**, *40*, 1445−1448.

(18) Wildman, S. A.; Crippen, G. M. Prediction of physicochemical parameters by atomic contributions. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868−873.

(19) Lee, J. Design and synthesis of bioisosteres of ultrapotent protein kinase C (PKC) ligand, 5-acetoxymethyl-5-hydroxymethyl-3-alkylidene tetrahydro-2-furanone. *Arch. Pharm. Res.* **1998**, *21*, 452−457.

(20) Choi, Y.; Kang, J. H.; Lewin, N. E.; Blumberg, P. M.; Lee, J.; Marquez, V. E. Conformationally constrained analogues of diacylglycerol. 19. Synthesis and protein kinase C binding affinity of diacylglycerol lactones bearing an N-hydroxylamide side chain. *J. Med. Chem.* **2003**, *46*, 2790−2793.

(21) Aujard, I.; Baltaze, J. P.; Baudin, J. B.; Cogne, E.; Ferrage, F.; Jullien, L.; Perez, E.; Prevost, V.; Qian, L. M.; Ruel, O. Tetrahedral onsager crosses for solubility improvement and crystallization bypass. *J. Am. Chem. Soc.* **2001**, *123*, 8177−8188.

(22) Sonogashira, K.; Tohda, Y.; Hagihara, N. Convenient synthesis of acetylenes - catalytic substitutions of acetylenic hydrogen with bromoalkenes, iodoarenes, and bromopyridines. *Tetrahedron Lett.* **1975**, 4467−4470.

(23) Mori, A.; Kawashima, J.; Shimada, T.; Suguro, M.; Hirabayashi, K.; Nishihara, Y. Non-Sonogashira-type palladium-catalyzed coupling reactions of terminal alkynes assisted by silver(I) oxide or tetrabutylammonium fluoride. *Org. Lett.* **2000**, *2*, 2935−2937.

(24) Inesi, A.; Mucciante, V.; Rossi, L. A convenient method for the synthesis of carbamate esters from amines and tetraethylammonium hydrogen carbonate. *J. Org. Chem.* **1998**, *63*, 1337−1338.

(25) Yadav, Y. S.; Reddy, B. V. S.; Rao, K. S.; Harikishan, K. Montmorillonite clay: A novel reagent for the chemoselective hydrolysis of t-butyl esters. *Synlett* **2002**, 826−828.

(26) Lee, J. C.; Yoo, E. S.; Lee, J. S. Facile deprotection of aromatic tert-butyl and allylic esters under microwave irradiation conditions. *Synth. Commun.* **2004**, *34*, 3017−3020.

(27) Lewin, N. E.; Blumberg, P. M. [3H]-Phorbol 12,13-dibutyrate binding assay for protein kinase C and related proteins. *Methods Mol. Biol.* **2003**, *233*, 129−156.

(28) Nacro, K.; Sigano, D. M.; Yan, S. Q.; Nicklaus, M. C.; Pearce, L. L.; Lewin, N. E.; Garfield, S. H.; Blumberg, P. M.; Marquez, V. E. An optimized protein kinase C activating diacylglycerol combining high binding affinity (K_i) with reduced lipophilicity (log *P*). *J. Med. Chem.* **2001**, *44*, 1892−1904.

(29) Sigano, D. M.; Peach, M. L.; Nacro, K.; Choi, Y.; Lewin, N. E.; Nicklaus, M. C.; Blumberg, P. M.; Marquez, V. E. Differential binding modes of diacylglycerol (DAG) and DAG-lactones to protein kinase C (PK-C). *J. Med. Chem.* **2003**, *46*, 1571−1579.

(30) Wang, Q. J.; Fang, T. W.; Fenick, D.; Garfield, S.; Bienfait, B.; Marquez, V. E.; Blumberg, P. M. The lipophilicity of phorbol esters as a critical factor in determining the pattern of translocation of protein kinase C delta fused to green fluorescent protein. *J. Biol. Chem.* **2000**, *275*, 12136−12146.

(31) Li, L.; Lorenzo, P. S.; Bogi, K.; Blumberg, P. M.; Yuspa, S. H. Protein kinase C delta targets mitochondria, alters mitochondrial membrane potential, and induces apoptosis in normal and neoplastic keratinocytes when overexpressed by an adenoviral vector. *Mol. Cell. Biol.* **1999**, *19*, 8547−8558.

(32) Kedei, N.; Lundberg, D. J.; Toth, A.; Welburn, P.; Garfield, S. H.; Blumberg, P. M. Characterization of the interaction of ingenol 3-angelate with protein kinase C. *Cancer Res.* **2004**, *64*, 3243−3255.

(33) Vrana, J. A.; Saunders, A. M.; Chellappan, S. P.; Grant, S. Divergent effects of bryostatin 1 and phorbol myristate acetate on cell cycle arrest and maturation in human myelomonocytic leukemia cells (U937). *Differentiation* **1998**, *63*, 33−42.

(34) Dempsey, E. C.; Newton, A. C.; Mochly-Rosen, D.; Fields, A. P.; Reyland, M. E.; Insel, P. A.; Messing, R. O. Protein kinase C isozymes and the regulation of diverse cell responses. *Am. J. Physiol.: Lung Cell Mol. Physiol.* **2000**, *279*, L429−L438.

(35) Yang, C. F.; Kazanietz, M. G. Divergence and complexities in DAG signaling: Looking beyond PKC. *Trends Pharmacol. Sci.* **2003**, *24*, 602−608.

(36) Mischak, H.; Goodnight, J.; Kolch, W.; Martinybaron, G.; Schaechtle, C.; Kazanietz, M. G.; Blumberg, P. M.; Pierce, J. H.; Mushinski, J. F. Overexpression of protein kinase-C-delta and kinase-epsilon in Nih 3t3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. *J. Biol. Chem.* **1993**, *268*, 6090−6096.

(37) Brodie, C.; Steinhart, R.; Kazimirsky, G.; Rubinfeld, H.; Hyman, T.; Ayres, J. N.; Hur, G. M.; Toth, A.; Yang, D. Z.; Garfield, S. H.; Stone, J. C.; Blumberg, P. M. PKC delta associates with and is involved in the phosphorylation of RasGRP3 in response to phorbol esters. *Mol. Pharmacol.* **2004**, *66*, 76−84.

(38) Wang, Q. J. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol. Sci.* **2006**, *27*, 317−323.

(39) Szallasi, Z.; Blumberg, P. M. Prostratin, a nonpromoting phorbol ester, inhibits induction by phorbol 12-myristate 13-acetate of ornithine decarboxylase, edema, and hyperplasia in Cd-1 mouse skin. *Cancer Res.* **1991**, *51*, 5355−5360.

(40) Szallasi, Z.; Krsmanovic, L.; Blumberg, P. M. Nonpromoting 12-deoxyphorbol 13-esters inhibit phorbol 12-myristate 13-acetate induced tumor promotion in Cd-1 mouse skin. *Cancer Res.* **1993**, *53*, 2507−2512.

(41) Gallegos, L. L.; Kunkel, M. T.; Newton, A. C. Targeting protein kinase C activity reporter to discrete intracellular regions reveals spatiotemporal differences in agonist-dependent signaling. *J. Biol. Chem.* **2006**, *281*, 30947−30956.

(42) Bittova, L.; Stahelin, R. V.; Cho, W. Roles of ionic residues of the C1 domain in protein kinase C-alpha activation and the origin of phosphatidylserine specificity. *J. Biol. Chem.* **2001**, *276*, 4218−4226.

(43) Mochly-Rosen, D.; Gordon, A. S. Anchoring proteins for protein kinase C: A means for isozyme selectivity. *FASEB J.* **1998**, *12*, 35−42.

(44) Canagarajah, B.; Leskow, F. C.; Ho, J. Y. S.; Mischak, H.; Saidi, L. F.; Kazanietz, M. G.; Hurley, J. H. Structural mechanism for lipid activation of the Rac-specific GAP, beta 2-chimaerin. *Cell* **2004**, *119*, 407−418.

(45) Braun, D. C.; Cao, Y. Y.; Wang, S. M.; Garfield, S. H.; Hur, G. M.; Blumberg, P. M. Role of phorbol ester localization in determining protein kinase C or RasGRP3 translocation: Real-time analysis using fluorescent ligands and proteins. *Mol. Cancer Ther.* **2005**, *4*, 141−150.

JM061289J