Conformationally Constrained Analogues of Diacylglycerol. 11.[1] Ultrapotent Protein Kinase C Ligands Based on a Chiral 5-Disubstituted Tetrahydro-2-furanone Template

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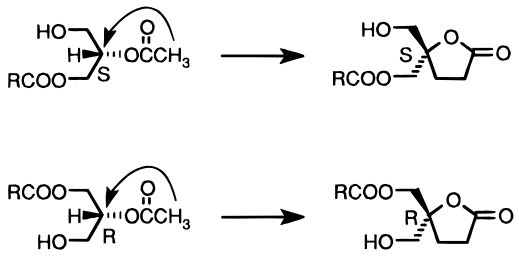
Conformationally constrained analogues of diacylglycerol (DAG) built on a racemic 5-[(acyloxy)-methyl]-5-(hydroxymethyl)tetrahydro-2-furanone template were shown previously to have excellent binding affinities for protein kinase C (PK-C). Since the interaction of PK-C with DAG is stereospecific, it was anticipated that PK-C would bind tightly to only one enantiomeric form of the compounds constructed with this new lactone template. Separation of enantiomers by chiral HPLC was discarded due to the ease with which acyl migration occurs in these class of compounds, and a total chiral synthesis was undertaken. Prior to chemical synthesis, the selection of the "correct" enantiomeric template was predicted by a molecular conformational analysis that compared the two enantiomers of DAG in their presumed "active" conformation with the two enantiomeric lactone templates. This presumed "active" conformation for DAG was derived from a previously developed pharmacophore model that uses the molecule of a potent phorbol diester as the ideal rigid template. The results from this analysis indicated that the "correct" lactone template corresponded to the inactive (*R*)-isomer of DAG. This analysis also predicted that the lactone template corresponding to the active (*S*)-DAG enantiomer would not fit adequately into the pharmacophore. The chiral syntheses of target compounds **2**, **4**, and **6**, constructed on the selected, and presumably "correct" lactone template, were achieved from a common bicyclic intermediate (5*R*,8*R*,9*R*)-8,9-*O*-isopropylidene-2-keto-1,7-dioxaspiro[4.4]nonane (**10**) that was synthesized from commercially available 1,2:3,5-di-*O*-isopropylidene-α-D-*threo*-apiofuranose (**7**) by a very effective spirolactonization approach. On the basis of their ability to inhibit the binding of [^{3}H-20]phorbol 12,13-dibutyrate (PDBU) to PK-Cα, the enantiomeric ligands **2**, **4**, and **6** were twice as potent as the corresponding racemates. These results confirm that binding of these lactones is stereospecific and consistent with a binding mechanism similar to that of DAG.

Introduction

The high binding affinity of ligands to either catalytic or allosteric sites in enzymes is normally stereospecific. Protein kinase C (PK-C) is no exception, and activation of this enzyme ensues only after the binding of the (S)-DAG enantiomer to the regulatory site of PK-C.[2−4] In our previous paper,[1] we have disclosed the properties of a new lactone, 5-[(acyloxy)methyl]-5-(hydroxymethyl)-tetrahydro-2-furanone (Chart 1), that serves as a template for the construction of compounds that function as semirigid DAG mimetics.[1] The increased binding affinities for PK-C shown by compounds constructed on this template appear to be derived principally from a reduction in the entropic penalty paid during the binding of the more rigid analogues, relative to the binding of the more flexible DAG molecule. In addition, the lactone template provides for an excellent spatial disposition of the DAG pharmacophores (two ester carbonyls and a primary alcohol function) that is possibly very similar to that found in bound DAG. Since our previous investigations with this template were performed with racemic compounds, it was reasonable to expect that PK-C would bind tightly to only one enantiomeric form of the compounds built with this new lactone template.

Chart 1



If the same idealized intramolecular cyclization that led to the racemic 5-[(acyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone template is now executed with either (*S*)-DAG or (*R*)-DAG (Chart 1), the resulting templates would be chiral also. In such a process, the relative chiralities of the *sn*-2 carbon in the starting DAG and the corresponding C-5 carbon in the lactone remain unchanged, and a first educated guess would perhaps predict that the lactone derived from the active (*S*)-DAG enantiomer should provide the "active" template. However, when the resulting (*S*)- and (*R*)-

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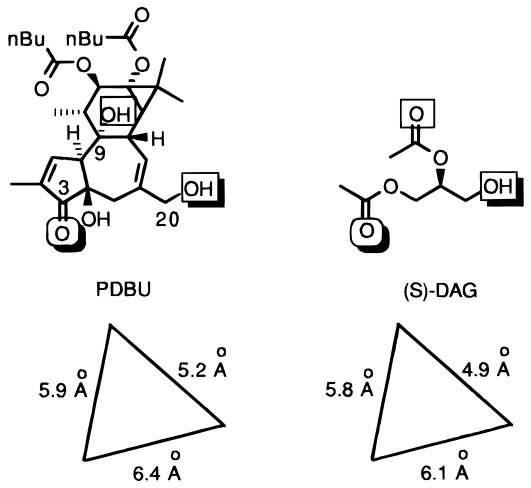
Chart 2



lactones were modeled to fit the phorbol ester pharmacophore which was derived from phorbol 12,13-dibutyrate (PDBU),[5] only the (*R*)-lactone provided an excellent fit to phorbol (*vide infra*).

Selection of the Correct Enantiomeric Template. The selection of the correct enantiomeric template was performed before embarking on a total enantioselective synthesis. As a first step, simple (*S*)- and (*R*)-DAG diacetates were modeled to fit the phorbol ester pharmacophore. The critical functional pharmacophores in the phorbol esters that are responsible for PK-C recognition are thought to be the C-20 hydroxyl, the C-3 carbonyl, and the C-9 hydroxyl (PDBU, Chart 2).[5] The latter two appear to function as hydrogen bond acceptors, while the primary alcohol at C-20 functions as a hydrogen bond donor. Correspondingly in DAG, the two ester carbonyls behave as hydrogen bond acceptors and the primary alcohol as a hydrogen bond donor.[5] The conformation of (*S*)-DAG demanded by the phorbol ester pharmacophore (Figure 1a) was found to be a stable and low-energy conformation only 4 kcal/mol above the global energy minimum. On the other hand, the conformation of (*R*)-DAG demanded by the phorbol ester pharmacophore (Figure 1b) was a high-energy conformation 10 kcal/mol above the global energy minimum. Although (*R*)-DAG could in principle interact with PK-C, the resulting system would have little or no free energy gain since it would have to pay a larger conformational energy penalty. This would explain why PK-C binds exclusively to (*S*)-DAG and why (*R*)-DAG is not an effective ligand. For the lactones, which are conceptually generated as indicated in Chart 1, the situation is reversed. Cyclization of (*S*)-DAG into the (*S*)-lactone changes the desired orientation of pharmacophores as a result of the C−O bond rotation required to achieve ring closure (Figure 2a). Conversely, when (*R*)-DAG is cyclized into the (*R*)-lactone, the unstable but desired conformation of (*R*)-DAG becomes a stable conformation in the (*R*)-lactone with the pharmacophores maintained in their correct disposition (Figure 2b). On the basis of this analysis, the (*R*)-lactone was selected as the potentially "active" template for the chemical synthesis of chiral ligands. This conclusion was validated by our results comparing the PK-C binding data of compounds containing the (*R*)-lactone template versus the corresponding racemates (*vide infra*).

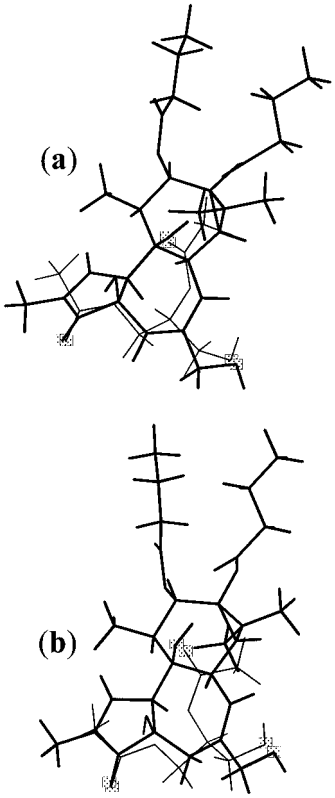


Figure 1. (a) Molecular superposition of a conformation of (*S*)-DAG (4.07 kcal/mol above the global minimum) that provides the best fit (rms = 0.170 Å) to phorbol 12,13-dibutyrate (PDBU). (b) Molecular superposition of a conformation of (*R*)-DAG (10.35 kcal/mol above the global minimum) that provides the best fit (rms = 0.330 Å) to phorbol 12,13-dibutyrate (PDBU).

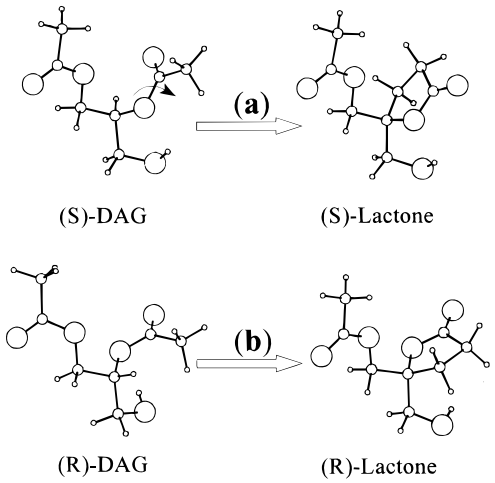
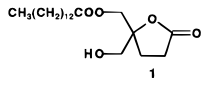
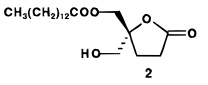
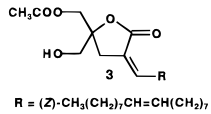
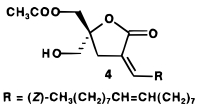
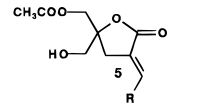
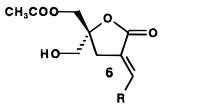


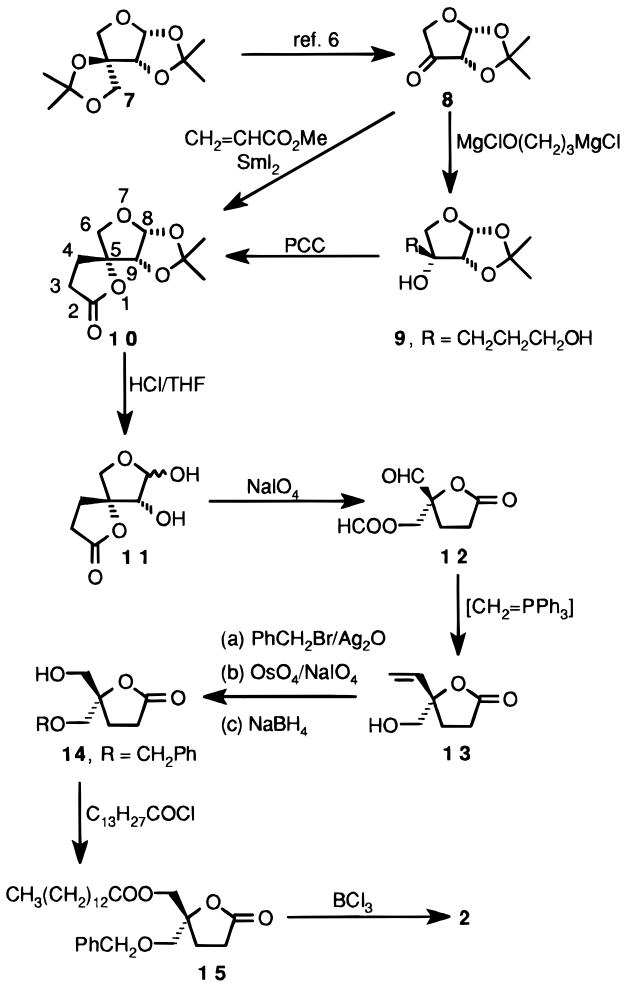
Figure 2. (a) Idealized cyclization of diacetyl (*S*)-DAG from Figure 1a into a lactone template. The C−O bond indicated by the arrow must rotate as shown to achieve ring closure. (b) Idealized cyclization of diacetyl (*R*)-DAG from Figure 1b into a lactone template. No bond rotations are required and the correct orientation of pharmacophores is maintained.

The decision to proceed with the stereospecific synthesis of the presumed active (*R*)-lactones (**2**, **4**, and **6**, Table 1), as opposed to the alternative of separating each of the enantiomers by chiral HPLC from the racemates (**1**, **3**, and **5**, Table 1), was based on the knowledge that a rapid acyl migration anticipated to occur during chromatography would inevitably cause racemization.

Table 1. Apparent K_i (nM) Values for Ligands as Inhibitors of PDBU Binding to PK-C

Racemate	K_i	(R)-Enantiomer	K_i
 1	138±24	 2	96±3.1
 3 R = (*Z*)-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	24±2.87	 4 R = (*Z*)-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	12±0.40
 5 R = (*Z*)-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	28±2.19	 6 R = (*Z*)-$CH_3(CH_2)_7CH{=}CH(CH_2)_7$	13±1.3

Scheme 1



Chemistry

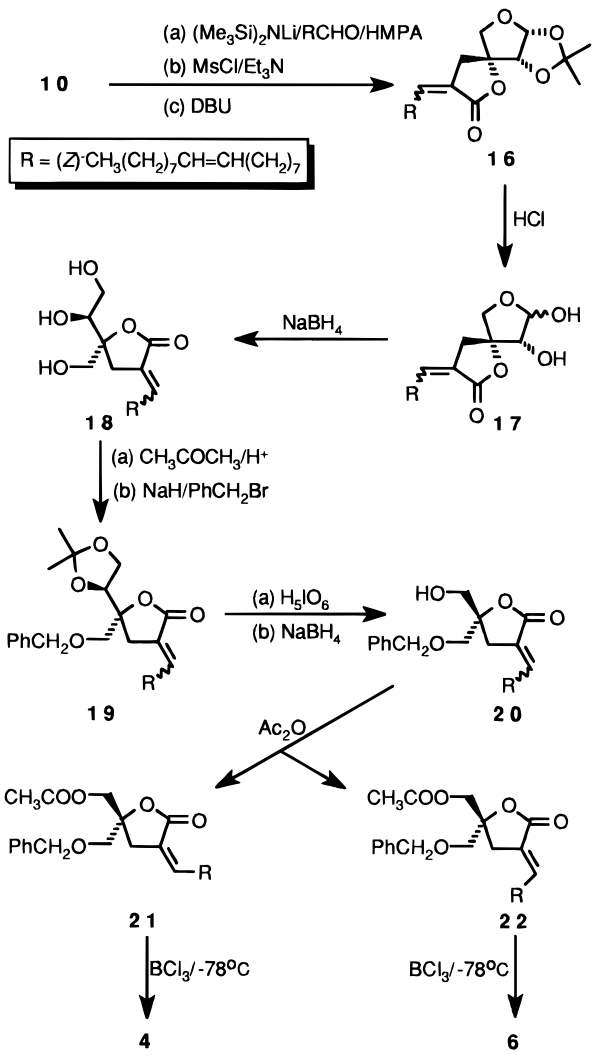
The simplest compound with the (*R*)-lactone template and with the myristate acyl chain (compound **2**) was constructed as shown in Scheme 1. Commercially available 1,2:3,5-di-*O*-isopropylidene-α-D-*threo*-apiofuranose (**7**) was hydrolyzed under mild conditions to selectively remove the 3,5-*O*-isopropylidene group, and the resulting free diol was oxidized to the known keto intermediate **8**.[6] Two spirolactonization approaches were attempted.[7] The first one began with the addition of the Grignard reagent, $MgClO(CH_2)_3MgCl$,[8] to give a new diol intermediate **9**. This diol was oxidized with pyridinium chlorochromate (PCC) which after the ensuing cyclization to the lactol was further oxidized *in situ* to the spirolactone **10**. Alternatively, and with the identical stereochemical outcome, a one-pot SmI_2-catalyzed reductive coupling of **8** with methyl acrylate[9] produced **10** directly but in lower yield. Since only the convex face of **8** is accessible, the attacking reagents added stereospecifically from the less hindered β-side ensuring that the resulting spirolactone **10** contained the required stereochemistry for the construction of the target chiral template. Indeed, after removal of the acetonide group, metaperiodate cleavage of the vicinal diol group in **11** provided the required lactone **12**. Wittig olefination of **12** with methyltriphenylphosphonium bromide proceeded with the simultaneous cleavage of the formate ester to give **13**. Protection of the primary alcohol function as the benzyl ether was followed by the simultaneous treatment with OsO_4 and sodium metaperiodate to cleave the *cis*-hydroxylated intermediate to the aldehyde stage. Sodium borohydride reduction of the aldehyde produced the chiral, singly protected lactone **14**. This indirect approach was necessary since reduction of aldehyde **12** would have given an achiral diol. Esterification with myristoyl chloride to the penultimate intermediate **15** and cleavage of the benzyl ether with BCl_3 at −78 °C was followed by a careful, low-temperature workup that involved quenching the reaction with a neutral buffer and extraction of the desired chiral lactone with ethyl ether. As anticipated, exposure of compound **2** to either chromatographic conditions, silica or neutral alumina, caused rapid racemization.

The more complex α-alkylidene lactones **4** and **6** were prepared from the pivotal intermediate spirolactone **10** (Scheme 2). In this instance, only the oleoyl aldehyde was used which after dehydration gave compound **16** as a mixture of *Z*- and *E*-isomers. Removal of the isopropylidene function from **16** gave **17**, and sodium borohydride reduction of the resulting lactol afforded the α-alkylidene lactone **18** with the desired chirality at C-4 (C-5 if named as a tetrahydro-2-furanone). The vicinal diol function on the side chain was protected as the acetonide, while the remaining primary alcohol function was protected as a benzyl ether to give **19**. Metaperiodic acid cleavage of **19**, followed by reduction with sodium borohydride, gave the antepenultimate intermediate **20**. After the conversion of **20** to a mixture of acetates, compounds **21** and **22** were separated by column chromatography. As before, cleavage of the benzyl ether with BCl_3 at −78 °C was followed by a similar workup as described before. This approach produced the individual target *Z*- and *E*-isomers **4** and **6**.

Biological Results

The affinity of the enantiomeric ligands **2**, **4**, and **6** for PK-Cα was expressed in terms of their ability to displace bound [^{3}H-20]phorbol 12,13-dibutyrate (PDBU) and was measured in the same manner as for the racemates described in the preceding paper.[1] Comparing the inhibition constants (K_i) obtained for each enantiomer with those of the corresponding racemate (Table 1), one finds, as expected, that the K_i values for the active enantiomers are very close to half the values for the racemates. These results not only substantiate the

Scheme 2



stereospecificity of PK-C for these lactones but also indirectly confirm the validity of our pharmacophore model[5] used to predict the correct enantiomeric lactone. In addition, these results support the concept that the new ligands are interacting at the binding site of the enzyme with their key pharmacophores spatially arranged in a manner similar to that encountered in the natural bound ligand DAG, but with a definitive entropic advantage.[1] To date, compounds **4** and **6** represent the most potent PK-C agonists possessing a diacylglycerol-like structure. These results contradict an earlier notion that the DAG molecule was considered too simple to give rise to a high-affinity ligand.[10]

Experimental Section

General Experimental. All chemical reagents were commercially available. Melting points were determined on a Mel-Temp II apparatus, Laboratory Devices, USA, and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230−400 mesh (E. Merck). Proton and ^{13}C NMR spectra were recorded on a Bruker AC-250 instrument at 250 and 62.9 MHz, respectively. Spectra were referenced to the solvent in which they were run (7.24 ppm for $CDCl_3$). Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR, and specific rotations were measured in a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

Analysis of Inhibition of [^{3}H]PDBU Binding by Non-radioactive Ligands. Enzyme−ligand interactions were analyzed by competition with [^{3}H]PDBU binding essentially as described in our previous work,[11] except that the PK-C preparation used here was the single isozyme PK-Cα. This recombinant PK-Cα was expressed in the baculovirus system and was isolated as described in ref 12. The ID_{50} values were determined from the competition curves, and the corresponding K_i values for the ligands were calculated from the ID_{50} values as described before.[11,12] Values represent the mean ± standard error (three determinations).

Molecular Modeling. All molecular mechanics calculations were carried out with QUANTA molecular modeling package (version 3.1) with CHARMm 2.2 parameter set running on a Silicon Graphics IRIS Indigo workstation. Since the molecular mechanics calculation depended upon the atom types, the atom type for every atom was carefully examined to ensure it was correct before any molecular calculations were conducted. All the conformational analysis was performed with the conformational search module within QUANTA. Monte Carlo random sampling algorithm was used to generate 5000 conformations for each structure. For each generated conformation, 5000 steps of adopted-basis Newton Raphson (ABNR) minimization was carried out or until convergence (with a convergence criterion equal to 0.01 kcal $Å^{-1}$). For both (*S*)-DAG and (*R*)-DAG diacetates (Figure 1a), the seven sp^3-sp^3 bonds between the hydroxyl oxygen and the two carbonyl carbons, which are relevant to the pharmacophore, were sampled in the Monte Carlo conformational analysis. Similarly, for both (*S*)- and (*R*)-lactones (Figure 1b), the five sp^3-sp^3 bonds between the hydroxyl oxygen and the two carbonyl carbons were sampled. An angle increment of 60° was used for each defined bond in the Monte Carlo conformational sampling, and a dielectric constant of 1 was used (CDIE = 1) in all the calculations. For each minimized conformer, the relevant distance information, as well as the conformational energy, was downloaded into an ASCII file. A program was then developed to compare automatically these conformations to the phorbol pharmacophore template to identify the best conformations according to considerations of both the root mean square (rms) value and the conformational energy. The phorbol 12,13-dibutyrate (PDBU) structure (Figure 1) was constructed starting from the crystal structure of phorbol[13] obtained from the Cambridge Structural Database,[14] using the molecular editor within QUANTA. Due to the lack of some CHARMm parameters for PBDU, a semiempirical quantum mechanics method, AM1 in the MOPAC 6.0 package running on a host mainframe Convex C240, was employed to minimize the PDBU structure which was used in this study.

1,2-*O*-Isopropylidene-α-D-*glycero*-tetrose-3-ulose (8). This compound was prepared from di-*O*-isopropylidene-α-D-apiose **7** in two steps according to the method of Carey et al.[6]

1,2-*O*-Isopropylidene-3-C-(hydroxypropyl)-α-D-erythrofuranose (9). A solution of 3-chloropropanol (2.84 g, 30 mmol) in THF (10 mL) was cooled to −20 °C, treated dropwise with methylmagnesium chloride (3 M in tetrahydrofuran, 10 mL, 30 mmol), and stirred for 20 min. The reaction mixture was warmed to room temperature, and magnesium (1.10 g, 45 mmol) was added. The suspension was refluxed for 3 h with intermittent addition of dibromoethane (0.02 mL each at 0, 1 and 2 h) and cooled to room temperature. A solution of **8** (1.58 g, 10 mmol) in THF (10 mL) was added dropwise to this mixture, and after stirring for 1 h, the reaction mixture was cooled over an ice bath and the reaction quenched by the slow addition of a saturated NH_4Cl solution (20 mL). The reaction mixture was filtered, and the filtrate was extracted several times with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (5:1) as eluant to give the title compound **9** (1.702 g, 78%) as a white solid: mp 93−95 °C; $[\alpha]^{22}_D$ +28.46° (*c* 1.3, $CHCl_3$); IR ($CHCl_3$) 3433 cm^{-1} (OH); ^1H NMR ($CDCl_3$) δ 1.34 and 1.56 (singlets, 3 H, CH_3), 1.60−1.80 (m, 4 H, CH_2CH_2-CH_2OH), 2.48 (br s, 2 H, OH), 3.60−3.76 (m, 4 H, CH_2OH, H-$4_{a,b}$), 4.14 (d, J = 3.8 Hz, 1 H, H-2), 5.79 (d, J = 3.8 Hz, 1 H, H-1); ^{13}C NMR ($CDCl_3$) δ 26.32, 26.49, 26.53, 32.03, 62.44, 72.49, 78.20, 81.83, 105.10, 112.43. Anal. ($C_{10}H_{18}O_5$) C, H.

(5*R*,8*R*,9*R*)-8,9-*O*-Isopropylidene-2-keto-1,7-dioxaspiro-[4.4]nonane (10): Method A. A solution of **9** (1.702 g, 7.8 mmol) in CH_2Cl_2 (100 mL) was treated with pyridinium chlorochromate (6.725 g, 31.2 mmol) and 4 Å molecular sieves (7.8 g). After the reaction mixture was stirred for 1 h at room temperature, the reaction was quenched with a slurry of Celite in ether and the mixture stirred for 30 min more. The suspension was filtered through a pad of silica gel and further washed with EtOAc. The filtrate was concentrated, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (2:1) as eluant to give the title compound **10** (1.654 g, 99%) as a white solid: mp 101−102 °C; $[\alpha]^{22}_D$ +74.0° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) 1785 cm^{-1}(C═O); ^1H NMR ($CDCl_3$) δ 1.34 and 1.59 (singlets, 3 H, CH_3), 2.07−2.33 (m, 2 H, H-$4_{a,b}$), 2.64 (t, J = 8.3 Hz, 2 H, H-$3_{a,b}$), 3.70 (d of AB, J = 8.5 Hz, 1 H, H-6_a), 4.16 (d of AB, J = 8.5 Hz, 1H, H-6_b), 4.33 (d, J = 3.5 Hz, 1 H, H-9), 5.82 (d, J = 3.5 Hz, 1 H, H-8); ^{13}C NMR ($CDCl_3$) δ 26.51, 26.68, 27.47, 30.05, 70.49, 82.31, 86.71, 104.87, 114.23, 174.81. Anal. ($C_{10}H_{14}O_5$) C, H.

Method B. A solution of **8** (0.316 g, 2 mmol) in THF (5 mL) was cooled to 0 °C and treated with a solution of methyl acrylate (0.36 mL, 4 mmol) in a mixture of 2-propanol (0.23 mL, 3 mmol) and hexamethylphosphoramide (2 mL). Samarium iodide (0.1 M in THF, 60 mL, 6 mmol) was added dropwise to the reaction mixture which was then allowed to warm to room temperature. After stirring for 30 min, the mixture was quenched with ether (50 mL) and filtered through a pad of silica gel. The filtrate was concentrated, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (2:1) as eluant to give the identical compound **10** (0.124 g, 30%) as a white solid.

(*S*)-5-Vinyl-5-(hydroxymethyl)tetrahydro-2-furanone (13). A solution of **10** (0.857 g, 4 mmol) in THF (20 mL) was treated with 1 N HCl solution (20 mL) and stirred for 20 h at room temperature. The reaction mixture was neutralized with solid $NaHCO_3$ and diluted with EtOAc. The mixture was dried and concentrated to give hemiacetal **11**, which was used in the next step without further purification. This compound was dissolved in a mixture of MeOH (40 mL) and water (20 mL), and the solution was stirred with sodium metaperiodate (1.71 g, 8 mmol) for 2 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated to dryness. The residue was dissolved in EtOAc, dried (Na_2SO_4), and concentrated to give aldehyde **12**, which was also used without further purification. Aldehyde **12** was dissolved in THF (10 mL), and the solution was slowly added to a suspension of methylphosphonium ylide [this ylide was prepared from methyltriphenylphosphonium bromide (2.858 g, 8 mmol) and potassium *tert*-butoxide (1.0 M in THF, 8 mL, 8 mmol) in THF (10 mL) after stirring for 30 min at room temperature]. The reaction mixture was stirred for 1 h at room temperature and for 1 h at 60 °C before it was cooled to 0 °C. The reaction mixture was then quenched with AcOH (0.5 mL) and filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (4:1) as eluant to give the title compound **13** (0.387 g, 68% from **10**) as an oil: IR (neat) 3440 (OH), 1770 (C═O), and 1644 cm^{-1} (C═C); ^1H NMR ($CDCl_3$) δ 2.05 (m, 1 H, H-4_a), 2.25 (s, 1 H, OH), 2.35−2.70 (m, 3 H, H-4_b, H-$3_{a,b}$), 3.54 (d of AB, J = 12.3 Hz, 1 H, C*H*OH), 3.75 (d of AB, J = 12.3 Hz, 1 H, CH*H*OH), 5.26 (d, J = 10.9 Hz, 1 H, CH═C*H*H), 5.37 (d, J = 17.2 Hz, 1 H, CH═CH*H*), 5.83 (dd, J = 17.2, 10.9 Hz, 1 H, C*H*═CH_2); ^{13}C NMR ($CDCl_3$) δ 28.05, 28.79, 66.70, 88.37, 116.10, 136.46, 177.41. Anal. ($C_7H_{10}O_3$) C, H.

(*S*)-5-[(Benzyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone (14). A solution of **13** (0.140 g, 1.0 mmol) in DMF (5 mL) was stirred with silver(I) oxide (0.232 g, 1.0 mmol) and benzyl bromide (0.30 mL, 2.5 mmol) for 5 days at room temperature. The reaction mixture was filtered, diluted with water, and extracted three times with EtOAc. The organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography over silica gel with hexanes/EtOAc (2:1) as eluant to give the intermediate 5-[(benzyloxy)methyl]-5-vinyltetrahydro-2-furanone (0.186 g, 80%) as an oil: $[\alpha]^{22}_D$ −29.68° (*c* 3.08, $CHCl_3$); IR (neat) 1772 (C═O) and 1653 cm^{-1} (C═C); ^1H NMR ($CDCl_3$) δ 2.05 (m, 1 H, H-4_a), 2.35−2.74 (m, 3 H, H-4_b, H-$3_{a,b}$), 3.55 (AB q, J = 10.4 Hz, 2 H, CH_2OH), 4.60 (AB q, J = 12.0 Hz, 2 H, CH_2OCH_2Ph), 5.22 (dd, J = 10.9, 0.5 Hz, 1 H, CH═C*H*H), 5.37 (dd, J = 17.3, 0.6 Hz, 1 H, CH═CH*H*), 5.88 (dd, J = 17.3, 10.9 Hz, 1 H, C*H*═CH_2); ^{13}C NMR ($CDCl_3$) δ 28.88, 29.37, 73.66, 74.50, 86.81, 115.73, 127.58, 127.80, 128.46, 136.72, 137.65, 176.82. Anal. ($C_{14}H_{16}O_3$) C, H. The above compound (0.162 g, 0.7 mmol) was dissolved in aqueous acetone (1:1, 10 mL) and the solution was stirred with 4-methylmorpholine *N*-oxide (0.164 g, 1.4 mmol), sodium metaperiodate (0.300 g, 1.4 mmol), and osmium tetroxide (2.5 wt % in *tert*-butyl alcohol, 0.88 mL, 0.07 mmol) for 20 h at room temperature. The reaction mixture was then quenched with saturated sodium thiosulfate solution (5 mL), stirred for 10 min, and extracted three times with EtOAc. The combined organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (4:1) as eluant to give the corresponding aldehyde (0.156 g, 96%) as an oil. This compound was immediately dissolved in MeOH (10 mL), cooled to −10 °C, and treated with sodium borohydride (0.090 g, 2.4 mmol). After stirring for 30 min, the reaction mixture was quenched with 1 N HCl and diluted with EtOAc. The organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (from 3:1 to 6:1) as eluant to give the title compound **14** (0.110 g, 70%) as a white solid: mp 76−78 °C; $[\alpha]^{22}_D$ +7.73° (*c* 4.4, $CHCl_3$); IR ($CHCl_3$) 3446 (OH) and 1771 cm^{-1} (C═O); ^1H NMR ($CDCl_3$) δ 2.10−2.20 (m, 2 H, H-$4_{a,b}$), 2.50−2.75 (m, 2 H, H-$3_{a,b}$), 3.60 (AB q, J = 10.1 Hz, 2 H, CH_2OH), 3.62 (d of AB, J = 12.1 Hz, 1 H, C*H*HOCH_2Ph), 3.75 (d of AB, J = 12.1 Hz, 1 H, CH*H*OCH_2Ph), 4.54 (br s, 2 H, CH_2OCH_2Ph), 7.20−7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 25.70, 29.15, 65.53, 72.43, 73.72, 87.46, 127.62, 127.90, 128.50, 137.49, 177.24. Anal. ($C_{13}H_{16}O_4$) C, H.

(*R*)-5-[(Benzyloxy)methyl]-5-[(tetradecanoyloxy)methyl]tetrahydro-2-furanone (15). A solution of **14** (0.024 g, 0.1 mmol) in CH_2Cl_2 (5 mL) was stirred with a drop of pyridine and a catalytic amount of (dimethylamino)pyridine (0.002 g, 0.016 mmol) and treated with tetradecanoyl chloride (0.054 mL, 0.2 mmol) for 2 h at room temperature. The solution was concentrated, and the residue was purified by flash column chromatography over silica gel with hexane/EtOAc (2:1) as eluant to give the title compound **15** (0.041 g, 92%) as an oil: $[\alpha]^{22}_D$ +1.43° (*c* 4.2, $CHCl_3$); IR (neat) 1783 and 1743 cm^{-1} (C═O); ^1H NMR ($CDCl_3$) δ 0.85 (distorted triplet, 3 H, CH_3), 1.10−1.40 (m, 20 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 1.60 (br m, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.00−2.30 (m, 2 H, H-$4_{a,b}$), 2.30 (t, J = 7.5 Hz, 2 H, $CH_3(CH_2)_{10}CH_2CH_2CO$), 2.50−2.75 (m, 2 H, H-$3_{a,b}$), 3.60 (br s, 2 H, CH_2OCH_2Ph), 4.20 (AB q, J = 11.9 Hz, 2 H, CH_2OCO), 4.55 (br s, 2 H, CH_2OCH_2Ph), 7.20−7.40 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.65, 24.81, 26.40, 28.78, 29.07, 29.20, 29.32, 29.42, 29.57, 29.61, 29.64, 31.89, 34.03, 65.99, 72.21, 73.76, 84.94, 127.63, 127.93, 128.49, 137.33, 173.14, 176.30. Anal. ($C_{27}H_{42}O_5$) C, H.

(*R*)-5-[(Tetradecanoyloxy)methyl]-5-(hydroxymethyl)-tetrahydro-2-furanone (2). A solution of **15** (0.036 g, 0.08 mmol) in CH_2Cl_2 (4 mL) was cooled to −78 °C, treated with boron trichloride (1.0 M in dichloromethane, 0.24 mL, 0.24 mmol), and stirred at that temperature for 1.5 h. The reaction was quenched by the slow addition of a saturated $NaHCO_3$ solution (0.3 mL) at −78 °C, and the mixture was immediately partitioned between ice-cold ether and a pH 7 phosphate buffer solution. The organic layer was washed five times with the pH 7 buffer solution, dried (Na_2SO_4), and concentrated to give a white solid. This solid was washed with cold hexane several times to give a pure sample of the title compound **2** (24 mg, 84%) as a solid: mp 65−66 °C; $[\alpha]^{22}_D$ +1.43° (*c* 4.2, $CHCl_3$). The IR, ^1H NMR, and ^{13}C NMR were identical to those reported for the racemate in the preceding paper (compound number **3e**).

(*R*)-(*Z*)-5-[(Acetyloxy)methyl]-5-(hydroxymethyl)-3-[(*Z*)-9-octadecaenylidene]tetrahydro-2-furanone (4) and (*R*)-(*E*)-5-[(Acetyloxy)methyl]-5-(hydroxymethyl)-3-[(*Z*)-9-octadecaenylidene]tetrahydro-2-furanone (6). A stirred

solution of **10** (0.428 g, 2.0 mmol) in THF (4 mL) was cooled to −78 °C and treated slowly with lithium bis(trimethylsilyl) amide (1.0 M in tetrahydrofuran, 2.4 mL, 2.4 mmol) for 1 h. A mixture of oleyl aldehyde (0.640 g, 2.4 mmol) and hexamethylphosphoramide (0.430 g, 5.35 mmol) was added, and stirring was continued for 1 h at −78 °C and for 1 h at −40 °C. The mixture was quenched with a solution of saturated ammonium chloride and diluted with ether. The organic layer was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography over silica gel with hexanes/EtOAc (3:1) as eluant to give the intermediate β-hydroxy lactone (0.865 g, 90%). This compound was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. The solution was then stirred with triethylamine (1.12 mL, 8 mmol) and methanesulfonyl chloride (0.31 mL, 4 mmol) for 30 min. The reaction mixture was warmed to room temperature and stirred for 1 h before the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (1.5 mL, 10 mmol). After further stirring for 14 h at room temperature, the mixture was concentrated and diluted with ether. The ethereal solution was washed with diluted HCl, water, and brine. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography using silica gel with hexanes/EtOAc (4:1) as eluant to give an inseparable oily mixture of *E*- and *Z*-isomers (**16**, 0.800 g, 96%) with a *E*/*Z* ratio of 2:1 according to ^{1}H NMR. The 0.50 ppm difference between the *β-cis* (δ = 6.82 ppm, distortet triplet) and *β-trans* (δ = 6.32 ppm, distorted triplet) protons of the α,*β*-enone system in **16** allows one to differentiate the *E*-isomer from the *Z*-isomer. The above mixture of **16** (0.463 g, 1 mmol) was dissolved in THF (15 mL) and stirred in the presence of 2 N HCl (15 mL) for 5 days at room temperature. The solution was then cooled over an ice bath and neutralized with solid $NaHCO_3$. The reaction mixture was filtered, and the filtrate was concentrated. The residue was diluted with EtOAc, dried (Na_2SO_4), and concentrated to give hemiacetal **17** as an oil, which was used in the next step without further purification. Hemiacetal **17** was dissolved in MeOH (10 mL), cooled to −10 °C, and treated with small portions of sodium borohydride until all the starting material was consumed. The reaction mixture was slowly acidified with acetic acid and concentrated. The residue was diluted with EtOAc and was washed with 1 N HCl and water. The organic layer was dried (Na_2SO_4) and concentrated to give triol **18** as an oil, which also was used for the next step without further purification. The above triol **18** was dissolved in acetone (20 mL) and cooled to 0 °C. The solution was treated with a catalytic amount of *p*-toluenesulfonic acid and stirred for 1 h. It was then neutralized with solid $NaHCO_3$, filtered, and concentrated. The residue was purified by flash column chromatography over silica gel with hexanes/EtOAc (2:1) as eluant to give the corresponding mixture of *E*- and *Z*-acetonides (0.232 g, 50%) as an oil. The above mixture of acetonides (0.232 g, 0.5 mmol) was dissolved in THF (10 mL) and treated with sodium hydride (60% dispersion, 40 mg, 1 mmol) and a mixture of benzyl bromide (0.12 mL, 1 mmol) and tetrabutylammonium iodide (0.037 g, 0.1 mmol). The reaction mixture was stirred for 12 h at room temperature and quenched by the addition of acetic acid (0.1 mL) and ether. The suspension was filtered through a short pad of silica gel which washed with ether. The filtrate was concentrated, and the residue was purified by flash column chromatography over silica gel with hexanes/EtOAc (4:1) as eluant to give **19** (0.240 g, 86%) as oil. Despite the very similar R_f values for the *E*- and *Z*-isomers of **19**, small amounts of each isomer were separated in some fractions, as semisolid materials, in the above chromatographic experiment.

Z-isomer **19**: ^{1}H NMR ($CDCl_3$) δ 0.86 (distorted t, 3 H, CH_3), 1.20−1.50 (m, 28 H, isopropylidene: 2 × CH_3, $CH_2(CH_2)_5CH_2$-$CH{=}CHCH_2(CH_2)_6CH_3$), 2.00 (m, 4 H, $CH_2CH{=}CHCH_2$), 2.65 (m, 2 H, >C=CHCH_2), 2.85 (AB q, H-$4_{a,b}$), 3.50 (s, 2 H, CH_2-OCH_2Ph), 4.00 (m, 2 H, OCH_2CHO), 4.23 (t, 1 H, OCH_2CHO), 4.55 (s, 2 H, OCH_2Ph), 5.35 (m, 2 H, $CH_2CH{=}CHCH_2$), 6.15 (m, 1 H, >C=CH), 7.20−7.50 (m, 5 H, Ph).

E-isomer **19**: ^{1}H NMR ($CDCl_3$) δ 0.86 (distorted t, 3 H, CH_3), 1.20−1.50 (m, 28 H, isopropylidene: 2 × CH_3, $CH_2(CH_2)_5CH_2$-$CH{=}CHCH_2(CH_2)_6CH_3$), 2.00 (m, 4 H, $CH_2CH{=}CHCH_2$), 2.15 (m, 2 H, >C=CHCH_2), 2.75 (AB q, H-$4_{a,b}$), 3.50 (s, 2 H, CH_2-OCH_2Ph), 4.00 (m, 2 H, OCH_2CHO), 4.25 (t, 1 H, OCH_2CHO), 4.55 (s, 2 H, OCH_2Ph), 5.33 (m, 2 H, $CH_2CH{=}CHCH_2$), 6.70 (m, 1 H, >C=CH), 7.20−7.50 (m, 5 H, Ph).

The mixture of *E*- and *Z*-isomers (compound **19**, 0.239 g, 0.43 mmol) was dissolved in ether (30 mL) and treated with periodic acid (0.490 g, 2.15 mmol). The reaction mixture was stirred for 20 h at room temperature, filtered, and concentrated. The residue was purified by flash column chromatography over silica gel with hexanes:EtOAc (3:2) as eluant to give the corresponding mixture of *E*- and *Z*-aldehydes (0.200 g, 96%) as an oil. This mixture was immediately dissolved in THF (10 mL) and H_2O (1 mL), cooled to −10 °C, and treated with small portions of sodium borohydride (total amount: 0.030 g) until all starting material was consumed. The reaction mixture was acidified by the slow addition of acetic acid and concentrated. The residue was diluted with ether and washed with 1 N HCl solution and water. The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography using silica gel with hexanes/EtOAc (3:2) as eluant to give **20** (0.145 g, 72%) as an oil. The above alcohol **20** (0.145 g, 0.3 mmol) was dissolved in CH_2Cl_2 (20 mL), cooled to −10 °C, and treated with pyridine (0.15 mL, 1.85 mmol), acetic anhydride (0.15 mL, 1.59 mmol), and a catalytic amount of (dimethylamino)pyridine (0.015 g, 0.13 mmol). After stirring for 30 min, the reaction mixture was concentrated at 0 °C. The residue was purified by flash column chromatography over silica gel with ether/hexanes (1:1) as eluant to give the *Z*-isomer **21** (0.052 g, 33%) and the *E*-isomer **22** (0.103 g, 65%) as semisolid materials.

Z-Isomer **21**: $[\alpha]^{22}_D$ +1.43° (*c* 0.28, $CHCl_3$); IR (neat) 1752 and 1670 (C=O) and 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (distorted t, 3 H, CH_3), 1.00−1.50 (m, 22 H, $CH_2(CH_2)_5CH_2$-$CH{=}CHCH_2(CH_2)_6CH_3$), 2.00 (m, 4 H, $CH_2CH{=}CHCH_2$), 2.02 (s, 3 H, CH_3CO), 2.60−2.90 (m, 4 H, >C=CHCH_2, H-$4_{a,b}$), 3.49 (d of AB, J = 9.9 Hz, 1 H, CHHOCH_2Ph), 3.57 (d of AB, J = 9.9 Hz, CHHOCH_2Ph), 4.19 (s, 2 H, CH_2COCH_3), 4.54 (s, 2 H, OCH_2Ph), 5.33 (m, 2 H, $CH_2CH{=}CHCH_2$), 6.17 (m, 1 H, >C=CH), 7.20−7.40 (m, 5 H, Ph).

E-Isomer **22**: $[\alpha]^{22}_D$ −2.58° (*c* 0.62, $CHCl_3$); IR (neat) 1752 and 1684 (C=O) and 1456 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.86 (distorted t, 3 H, CH_3), 1.10−1.50 (m, 22 H, $CH_2(CH_2)_5CH_2$-$CH{=}CHCH_2(CH_2)_6CH_3$), 2.00 (m, 4 H, $CH_2CH{=}CHCH_2$), 2.01 (s, 3 H, CH_3CO), 2.15 (m, 2 H, >C=CHCH_2), 2.62 (d of AB, J = 17.0 Hz, 1 H, H-4_a), 2.82 (d of AB, J = 17.0 Hz, 1 H, H-4_b), 3.50 (d of AB, J = 9.9 Hz, 1 H, CHHOCH_2Ph), 3.58 (d of AB, J = 9.9 Hz, CHHOCH_2Ph), 4.21 (s, 2 H, CH_2COCH_3), 4.55 (s, 2 H, OCH_2Ph), 5.33 (m, 2 H, $CH_2CH{=}CHCH_2$), 6.72 (m, 1 H, >C=CH), 7.20−7.40 (m, 5 H, Ph).

A solution of *Z*-isomer **21** (0.103 g, 0.196 mmol) in CH_2Cl_2 (15 mL) was cooled to −78 °C, treated with boron trichloride (1.0 M in dichloromethane, 0.8 mL, 0.8 mmol), and stirred for 1.5 h. The reaction mixture was quenched by the slow addition of a saturated solution of $NaHCO_3$ (0.8 mL) and immediately partitioned between ice-cold ether and a pH 7 phosphate buffer solution. The organic layer was washed five times with the pH 7 buffer solution, dried (Na_2SO_4), and concentrated to give the title compound **4**, which precipitated in cold hexane. The solid was filtered off and washed with cold hexane several times to give optically active **4** (0.068 g, 80%) as a semisolid gum; $[\alpha]^{22}_D$ +3.00° (*c* 0.3, $CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR were identical to the spectra reported for the racemate in the preceding paper (compound number **10f**). The corresponding *E*-isomer **6** was also prepared by the same procedure in 80% yield: mp 46 °C; $[\alpha]^{22}_D$ +12.5° (*c* 0.24, $CHCl_3$). The IR, ^{1}H NMR, and ^{13}C NMR were identical to the spectra reported for the racemate in the preceding paper (compound number **11f**).

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