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Conformationally Constrained Analogues of Diacylglycerol (DAG)—II. Differential Interaction of δ-Lactones and γ-Lactones with Protein Kinase C (PK-C)

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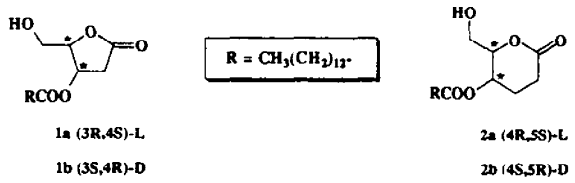
Abstract—Starting with L- or D-tri-*O*-acetylglucal, the corresponding L- and D-isomers of 4-*O*-tetradecanoyl-2,3-dideoxyglucono-1,5-lactone (**2a** and **2b**) were synthesized as rigid diacylglycerol (DAG) analogues. Consistent with results obtained previously with the equivalent L- and D-1,4-lactones (**1a** and **1b**), the L-isomer (**2a**) was more potent in activating protein kinase C (PK-C) and inhibiting the binding of [^{3}H]phorbol-12,13-dibutyrate to the enzyme's regulatory domain. In these experiments the difference in potency observed between the optical antipodes of the gluconolactones (**2a** and **2b**) was greatly increased relative to the corresponding ribonolactones (**1a** and **1b**). These results indicate that PK-C is more able to discriminate between optical antipodes, in favor of the L-isomer, as the lactone ring increases from five to six.

Introduction

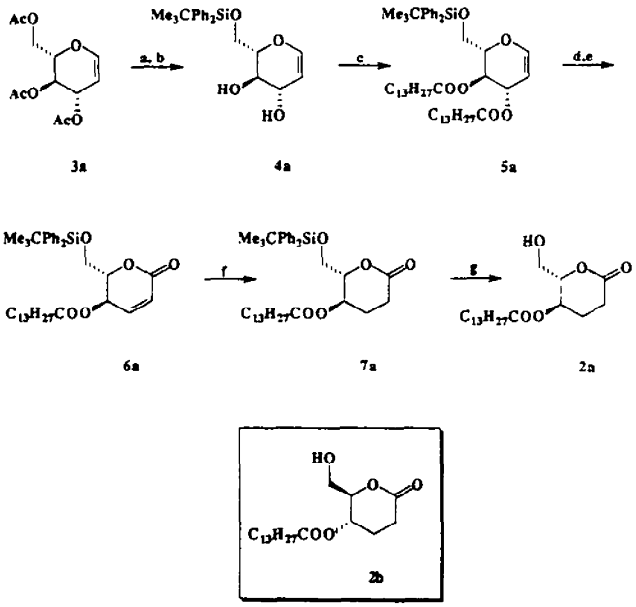
Since its discovery by Nishizuka in 1977,[1] protein kinase C (PK-C) has been extensively studied and recognized as a pivotal enzyme in the regulation of cell signal transduction.[2] The occupation of cell surface receptors by various agonists, such as hormones and growth factors, triggers the hydrolysis of phosphatidyl inositol, phosphatidyl choline, or phosphatidyl ethanolamine to generate diacyl glycerol (DAG).[3,4] The released DAG binds to the regulatory domain of PK-C and activates this cytoplasmic enzyme by causing its translocation to the membrane, while simultaneously increasing its affinity for calcium.[1,4] PK-C is a family of closely related protein kinases which are believed to have different activation properties.[5] Recent studies indicate that some signalling pathways might be specific for growth factors and oncogenes, thus offering the potential for chemotherapeutic intervention by specific PK-C inhibitors.[6] Phorbol esters and other chemically diverse tumor promoters also activate PK-C by acting as stable and highly potent DAG equivalents.[7] In the absence of detailed information from X-ray analysis or solution NMR studies, several groups have attempted to derive a receptor model for the regulatory domain of PK-C based on the disposition of equivalent pharmacophores in the three-dimensional structures of various PK-C activators.[8–13] Our attempt to understand the demands of this binding process is based on the progressive construction of increasingly complex and conformationally constrained DAG analogues in order to arrive at the optimal conformation of enzyme-bound DAG.[14,15] It is hoped that this approach will reveal important features of the "active" DAG conformation as a prelude to the development of structurally related PK-C inhibitors.

We have previously shown that among all the isomers of 2-deoxy-1,4-ribonolactone, 3-*O*-tetradecanoyl-2-deoxy-L-ribonolactone (**1a**) had the highest affinity for PK-C.[14] Mechanistically, this compound behaved as a competitive inhibitor of phorbol ester binding with a K_i of 2.5 μM.[14] In addition, the rate of phosphorylation of histone H1 by PK-C increased in the presence of the compound in a dose-dependent manner as would be expected for a PK-C activator.[14] These results suggested that compound **1a**, whose structure contains an embedded glycerol moiety, behaved as a surrogate of diacylglycerol (DAG), the physiologic activator of PK-C. However, despite the good activity of **1a**, the comparable acyclic DAG, glycerol-1-myristate-2-acetate, showed a five-fold greater affinity for the enzyme.[14] For this reason, the conformation of **1a** represents only an approximation of the "ideal" active rotamer of DAG. In addition, during these investigations only a small but reproducible difference in the binding affinity of **1a** and its optical antipode, 3-*O*-tetradecanoyl-2-deoxy-D-ribonolactone (**1b**, K_i = 5.3 μM), was detected.[14] Since PK-C activation by DAGs is known to be stereospecific (only the *S*-DAG enantiomer is active),[16] the possibility existed that the 1,4-lactones were binding to PK-C in a different manner than DAG. In order to investigate whether this phenomenon is observed with other cyclic lactones used as templates for DAG, we set out to synthesize the equivalent optical antipodes of the six-membered dideoxy-1,4-glucono-1,5-lactone (**2a** and **2b**) which in the same manner as **1a**,**b** contain an embedded glycerol molecule.



Synthesis

Starting with L- or D-tri-*O*-acetylglucal, the corresponding L- and D-isomers of 4-*O*-tetradecanoyl-2,3-dideoxygluconо-1,5-lactone (**2a,b**) were synthesized. Scheme I illustrates the synthesis for the L-isomer (**2a**). A critical step in this synthesis was the simultaneous oxidation β-elimination reaction of the glycal to give the corresponding α,β-unsaturated lactone.[17,18] Although the reaction proceeds well with acetate- or benzoate-protected glucals and the reduction to the saturated lactone is uneventful, removal of the protecting ester groups is accompanied by isomerization to the five-membered dideoxygluconolactone. For that reason, the starting tri-*O*-acetylglucal was deprotected first, and myristoyl groups were then selectively introduced at C3 and C4 prior to the oxidation–elimination reaction. The desired bis-myristoylation was easily accomplished after the primary alcohol function was protected with the bulky *tert*-butyldiphenylsilyl group. In this way, the ensuing PCC oxidation–elimination reaction afforded the compound with the desired 4-*O*-myristoyl group already in place. In practice, however, complete β-elimination was not achieved and further treatment with triethylamine was required to drive the reaction to completion. Reduction of the double bond and desilylation with HF–pyridine produced the desired target compounds.



Scheme I. Reagents: (a) K_2CO_3/MeOH, (b) Me_3CPh_2SiCl/DMF/-imidazole, (c) $C_{13}H_{27}COCl$/pyr, (d) PCC/1,2-dichloroethane, (e) Et_3N/-CH_2Cl_2, (f) H_2, Pd/C, (g) HF/pyr (0 °C).

Biological Results and Discussion

Both enantiomers **2a** and **2b** showed competitive inhibition kinetics in the phorbol ester binding assay with PK-C (K_i values of 2.8 and 30.5 μM, respectively). This difference in binding contrasts with the behavior displayed by the optical antipodes of the five-membered lactones (**1a** and **1b**)[14] and suggests that the optical antipodes of the six-membered 1,5-lactone appear to be better discriminated by PK-C (Table 1). These results are more consistent with a DAG binding mode for the gluconolactones. Unfortunately, an augmentation in potency was not accompanied by the increase in ring size. A similar higher level of enantiomer discrimination was also observed in the capacity of **2a** and **2b** to increase the PK-C-induced rate of phosphorylation of histone H1 (Figure 1). This again is more consistent with a DAG binding mode for the six- rather than the five-membered lactones.

Table 1. Apparent K_i values for lactones assayed as inhibitors of [^{3}H]-phorbol-12,13-dibutyrate binding to PK-C

compd	K_i (μM)
1a	2.5 ± 0.3 (n = 3)[14]
1b	5.3 ± 1.5 (n = 3)[14]
2a	2.8 ± 0.4 (n = 2)
2b	30.5 ± 7.4 (n = 3)

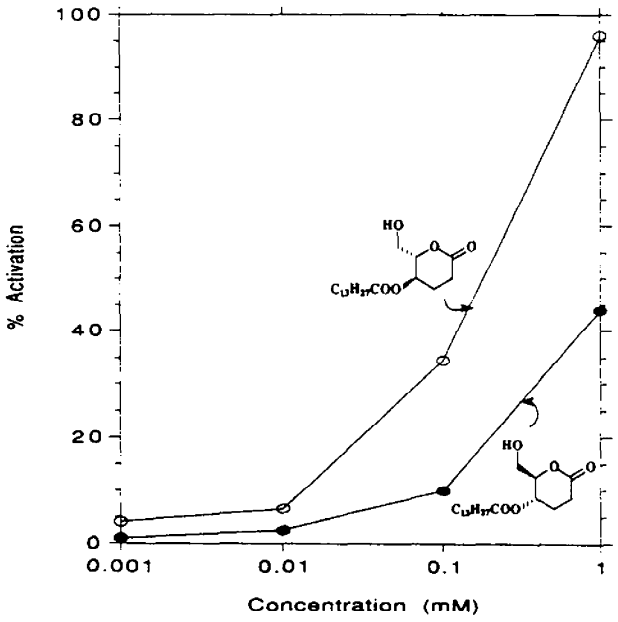


Figure 1. Activation of PK-C by lactones **2a** (○) and **2b** (●). Average of two experiments

We do not yet understand the reasons for the superior capacity of PK-C to better discriminate between the optical antipodes of the six-membered lactones. It is possible that the flatter five-membered ring allows these molecules to accommodate the receptor in both enantiomeric forms, which may be more difficult with the more puckered six-membered lactones. Since potency did not change between the most active enantiomers within each class of lactones, the relative orientation of the key pharmacophores may be

quite similar in both types of compounds, although still far from the ideal "active" DAG conformation. This work provides incentive for exploring larger lactones as PK-C agonists. Larger lactones might accommodate the demands of the receptor better, while still maintaining a certain degree of rigidity relative to the open-chain DAG. Such a progressive increase in lactone size will bring a decrease in strain energy. The smaller lactones (five- to eight-membered) have significantly higher strain energies than larger lactones as a result of the higher energy associated with an ester *E* conformation.[19] This energy decreases considerably as the larger lactones adopt a *Z* ester conformation.[19] Since some of the most potent PK-C activators found in nature are macrolactones,[13] this avenue of research might offer some interesting possibilities. In this connection, the high PK-C binding affinity displayed by some macrocyclic DAG analogues, constructed by connecting the two lipophilic groups of DAG, corroborates this assumption.[20]

Experimental Section

General experimental

All chemical reagents were commercially available. Melting points were determined on a Mel-Temp II apparatus, Laboratory Devices, U.S.A., and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh (E. Merck), and analytical TLC was performed on Analtech Uniplates silica gel GF with the solvents indicated for the individual experiments. Proton and ^{13}C NMR spectra were recorded in $CDCl_3$ unless otherwise indicated at 250 and 62.9 MHz, respectively, on a Bruker AC-250 instrument. Chemical shifts are expressed as δ values with reference to Me_4Si. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR and specific rotations were measured using a Perkin-Elmer Model 241 polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA.

Biological activity

Analysis of inhibition of [^{3}H]phorbol-12,13-dibutyrate by non-radioactive ligands and measurements of protein kinase activity were performed exactly as described in a previous manuscript.[14]

6-O-tert-Butyldiphenylsilyl-L-glucal (4a)

A solution of tri-*O*-acetyl-L-glucal (**3a**, 3 g, 11.02 mmol) in methanol was treated with potassium carbonate (0.1 g), stirred at room temperature for 3 h, and concentrated to dryness. The residue was purified through a short pad of silica gel with EtOAc/MeOH (8:1) as eluant to give L-glucal (1.6 g, 99%) as solid white flakes. This material was dissolved in DMF (25 mL) and treated with imidazole (2.0 g, 30 mmol) and *tert*-butyldiphenylsilyl chloride (3.3 g, 12 mmol). The reaction mixture was stirred at room temperature for 12h and concentrated to dryness. The residue was purified by flash column chromatography with hexanes/EtOAc (1:1) as eluant to give **4a** (3.62 g, 86%) as a colorless syrup: $[\alpha]^{25}_D$ = -18.72° (c 3.60, MeOH); ^1H NMR δ 7.64–7.71 (m, 4H), 7.34–7.47 (m, 6H), 6.30 (dd, 1H, *J* = 6.0, 1.5 Hz), 4.71 (dd, 1H, *J* = 6.0, 2.1 Hz), 4.26 (m, 1H), 3.97 (m, 2H), 3.75–3.95 (m, 2H), 2.85 (bs, 1H, OH), 2.25 (bs, 1H, OH), 1.05 (s, 9H); ^{13}C NMR δ 144.26, 135.60, 135.50, 132.87, 132.66, 129.89, 129.85, 127.80, 127.74, 102.32, 71.59, 69.60, 63.76, 26.77, 19.20; IR (neat) 3382 cm^{-1}. Anal. Calc'd for $C_{22}H_{28}O_4Si$: C, 68.71; H, 7.34. Found: C, 68.70; H, 7.36.

6-O-tert-Butyldiphenylsilyl-D-glucal (4b)

This compound was obtained from tri-*O*-acetyl-D-glucal in the same manner as **4a**: $[\alpha]^{25}_D$ = +18.82° (c 2.75, MeOH). The ^1H NMR, ^{13}C NMR and IR spectra were identical to those of its optical antipode. Anal. Calc'd for $C_{22}H_{28}O_4Si$: C, 68.71; H, 7.34. Found: C, 68.47; H, 7.41.

6-O-tert-Butyldiphenylsilyl-3,4-di-O-tetradecanoyl-L-glucal (5a)

A solution of **4a** (2.36 g, 9.0 mmol) in CH_2Cl_2 (150 mL) containing a mixture of pyridine (7.28 mL, 90.0 mmol) and DMAP (22 mg, 0.18 mmol) was treated with a single portion of myristoyl chloride (12.23 mL, 45 mmol) and kept at room temperature with stirring for 2 days. The reaction mixture was diluted with ether and the ethereal solution was washed with 10% aqueous HCl and water. The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was dissolved in hexane and the solution was cooled to -20°C. The solid formed was removed by filtration and the filtrate was reduced to dryness. This final residue was purified by flash column chromatography with pet. ether/CH_2Cl_2 (5:1) as eluant to give **5a** (6.81 g, 94%) as a colorless oil: $[\alpha]^{25}_D$= + 9.93° (c 4.17, $CHCl_3$); ^1H NMR δ 7.60–7.69 (m, 4H), 7.30–7.45 (m, 6H), 6.41 (dd, 1H, *J* = 6.1, 1.0 Hz), 5.22–5.38 (m, 2H), 4.74 (dd, 1H, *J* = 6.2, 3.3 Hz), 4.13 (m, 1H), 3.80 (m, 2H), 2.14–2.25 (m, 4H), 1.43–1.58 (m, 4H), 1.16–1.30 (m, 40H), 1.03 (s, 9H), 0.86 (t, 6H); ^{13}C NMR δ 173.21, 172.10, 145.83, 135.61, 133.18, 129.69, 129.66, 127.63, 93.36, 76.69, 67.29, 67.10, 61.77, 34.29, 34.13, 31.91, 29.66, 29.64, 29.60, 29.44, 29.34, 29.21, 29.09, 29.06, 26.70, 24.80, 24.76, 22.67, 19.19, 14.10; IR (neat) 1742 cm^{-1}. Anal. Calc'd for $C_{50}H_{80}O_6Si$: C, 74.58; H, 10.01. Found: C, 74.51; H, 9.99.

6-O-tert-Butyldiphenylsilyl-3,4-di-O-tetradecanoyl-D-glucal (5b)

This compound was obtained from **4b** in the same manner as **5a**: $[\alpha]^{25}_D$ = -9.60° (c 3.54, $CHCl_3$). The ^1H NMR, ^{13}C NMR and IR spectra were identical to those of its optical antipode. Anal. Calc'd for $C_{50}H_{80}O_6Si$: C, 74.58, H, 10.01. Found: C, 74.74; H, 10.15.

6-O-tert-Butyldiphenylsilyl-4-O-tetradecanoyl-2,3-dideoxy-didehydro-L-glucono-1,5-lactone (6a)

A mixture of **5a** (6.44 g, 8.0 mmol) and pyridinium chlorochromate (3.45 g, 16.0 mmol) in 1,2-dichloroethane

(150 mL) was refluxed for 18 h. After cooling to room temperature, the reaction mixture was passed through a short pad of silica gel previously impregnated with hexanes but eluted with ether. The filtrate was reduced to dryness to give a mixture of the desired unsaturated lactone (**6a**) plus the corresponding uneliminated precursor. This mixture was immediately dissolved in a solution of triethylamine (20 mL) and CH_2Cl_2 (40 mL) and stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography with pet. ether/CH_2Cl_2 (1:2) as eluant to give **6a** (4.03 g, 85%) as a colorless oil: $[\alpha]^{25}_D$ = -87.51° (c 3.65, $CHCl_3$); ^1H NMR δ 7.58–7.70 (m, 4H), 7.34–7.50 (m, 6H), 6.75 (dd, 1H, *J* = 9.9, 3.8 Hz), 6.11 (dd, 1H, *J* = 9.8, 1.0 Hz), 5.67 (m, 1H), 4.53 (dd, 1H, *J* = 9.5, 4.1 Hz), 3.83 (m, 2H), 2.30 (m, 2H), 1.60 (m, 2H), 1.23–1.34 (m, 20H), 1.04 (s, 9H), 0.88 (t, 3H); ^{13}C NMR δ 172.60, 161.92, 141.85, 135.61, 135.54, 132.59, 132.31, 129.94, 127.82, 123.01, 34.00, 31.89, 29.65, 29.61, 29.57, 29.39, 29.32, 29.18, 29.05, 26.68, 24.78, 22.66, 19.13, 14.10; IR (neat) 1743 cm^{-1}. Anal. Calc'd for $C_{36}H_{52}O_5Si$: C, 72.93; H, 8.84. Found: C, 72.99; H, 8.88.

6-O-tert-Butyldiphenylsilyl-4-O-tetradecanoyl-2,3-dideoxy-didehydro-D-glucono-1,5-lactone (6b)

This compound was obtained from **5b** in the same manner as **6a**: $[\alpha]^{25}_D$ = +90.40° (c 1.50, $CHCl_3$). The ^1H NMR, ^{13}C NMR and IR spectra were identical to those of its optical antipode. Anal. Calc'd for $C_{36}H_{52}O_5Si$: C, 72.93; H, 8.84. Found: C, 73.03; H, 8.85.

6-O-tert-Butyldiphenylsilyl-4-O-tetradecanoyl-2,3-dideoxy-L-glucono-1,5-lactone (7a)

A solution of **6a** (3.56 g, 6.0 mmol) in ethyl acetate (100 mL) was hydrogenated for 2 h in the presence of 10% Pd/C (4.0 g) under a hydrogen balloon. The reaction mixture was filtered and reduced to dryness. The residue was purified by flash column chromatography with hexanes/EtOAc (5:1) as eluant to give **7a** (3.5 g, 98%) as a colorless oil: $[\alpha]^{25}_D$ = -32.24° (c 3.65, $CHCl_3$); ^1H NMR δ 7.60–7.64 (m, 4H), 7.35–7.49 (m, 6H), 5.31 (m, 1H), 4.43 (m, 1H), 3.82 (m, 2H), 2.50–2.78 (m, 2H), 2.24–2.39 (m, 3H), 2.00 (m, 1H), 1.60 (m, 2H), 1.22–1.36 (m, 20H), 1.05 (s, 9H), 0.88 (t, 3H); ^{13}C NMR δ 172.74, 169.69, 135.64, 135.51, 132.19, 129.95, 127.86, 81.39, 65.98, 63.50, 31.29, 31.89, 29.61, 29.57, 29.42, 29.32, 29.20, 29.07, 26.71, 26.19, 24.87, 23.88, 22.66, 19.12, 14.10; IR (neat) 1742 cm^{-1}. Anal. Calc'd for $C_{36}H_{54}O_5Si$: C, 72.68; H, 9.15. Found: C, 72.56; H, 9.10.

6-O-tert-Butyldiphenylsilyl-4-O-tetradecanoyl-2,3-dideoxy-D-glucono-1,5-lactone (7b)

This compound was obtained from **6b** in the same manner as **7a**: $[\alpha]^{25}_D$ = +31.77° (c 3.10, $CHCl_3$). The ^1H NMR, ^{13}C NMR and IR spectra were identical to those of its optical antipode. Anal. Calc'd for $C_{36}H_{54}O_5Si$: C, 72.68; H, 9.15. Found: C, 72.65; H, 9.17.

4-O-Tetradecanoyl-2,3-dideoxy-L-glucono-1,5-lactone (2a)

A solution of **7a** (2.97 g, 5.0 mmol) in THF (50 mL) was cooled to 0 °C and treated with HF–pyridine (5 mL). The reaction mixture was stirred at that temperature for 24 h and immediately concentrated to dryness under high vacuum. The residue was dissolved in hot hexanes, filtered and concentrated *in vacuo*. The new residue was purified by flash column chromatography with pet. ether/EtOAc (2:1) as eluant to give **2a** (0.3 g, 17%) as a white solid which was recrystallized from hexanes: m.p. 46.5 °C; $[\alpha]^{25}_D$ = -40.00° (c 1.00, $CHCl_3$); ^1H NMR δ 5.15 (dd, 1H, *J* = 10.4, 5.7 Hz), 4.40 (m, 1H), 3.89 (dd, 1H, *J* = 12.6, 3.4 Hz), 3.77 (dd, 1H, *J* = 12.6, 4.1 Hz), 2.50–2.78 (m, 2H), 2.17–2.24 (m, 3H), 1.98 (m, 1H), 1.60 (m, 2H), 1.22–1.36 (m, 20H), 0.88 (t, 3H); ^{13}C NMR δ 173.01, 170.41, 81.61, 65.65, 62.19, 34.25, 31.88, 29.63, 29.60, 29.55, 29.40, 29.31, 29.18, 29.05, 26.57, 24.84, 24.09, 22.65, 14.08; IR (KBr) 3466, 1740 cm^{-1}. Anal. Calc'd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.28; H, 10.21.

4-O-Tetradecanoyl-2,3-dideoxy-D-glucono-1,5-lactone (2b)

This compound was obtained from **7b** in the same manner as **2a**: m.p. 46.5 °C; $[\alpha]^{25}_D$ = +39.80° (c 1.00, $CHCl_3$). The ^1H NMR, ^{13}C NMR and IR spectra were identical to those of its optical antipode. Anal. Calc'd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.12; H, 10.20.

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