Conformationally constrained analogues of diacylglycerol. 5. 2,5-Dideoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone: A superior homologue of 3-O-tetradecanoyl-2-deoxy-L-ribonolacton...

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · OCTOBER 1993

Impact Factor: 2.42 · DOI: 10.1016/S0960-894X(01)81002-7

Bioorganic & Medicinal Chemistry Letters, Vol.3, No.10, pp. 1993-1998, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

Conformationally Constrained Analogues of Diacylglycerol. 5[1]. 2,5-Dideoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone: A Superior Homologue of 3-O-Tetradecanoyl-2-deoxy-L-ribonolactone with PK-C Binding Affinity.

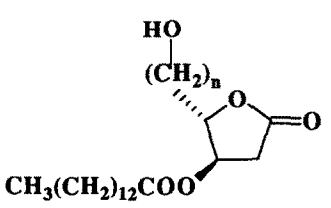
Rajiv Sharma†, Victor E. Marquez†*, George W. A. Milne†, Nancy E. Lewin‡, and Peter M. Blumberg‡

†*Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment and* ‡*Molecular Mechanisms of Tumor Promotion Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892 (USA)*

(*Received in USA* 15 *June* 1993; *accepted* 2 *July* 1993)

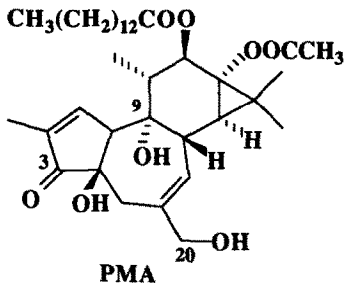
Abstract. A computer-generated molecular superposition of the key pharmacophores in 3-O-tetradecanoyl-2-deoxy-L-ribonolactone (**1**) and phorbol-12-myristate-13-acetate (PMA) indicated that the corresponding one carbon homologue, 2,5-dideoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone (**2**) would have an improved fit to PMA. Compound **2** was synthesized, and, consistent with the model's prediction, demonstrated superior binding affinity for PK-C relative to **1**.

Protein kinase C (PK-C) signal transduction, which is activated by growth factors, neurotransmitters, and hormones, is one of the most studied signalling pathways.[2] PK-C is cytoplasmic in its inactive state and becomes activated when diacylglycerol (DAG) is generated in the cell membrane by the phospholipase C-mediated cleavage of phosphatidylinositol-4,5-biphosphate.[2] DAG is an endogenous activator of PK-C which mimics many of the effects of the potent phorbol ester tumor promoters.[2] The DAG-mediated activation of PK-C is stereospecific, with only the S-enantiomer being an effective activator of the enzyme.[3] Consistent with a stereospecific mode of binding to PK-C, we found that 3-O-tetradecanoyl-2-deoxy-L-ribonolactone (**1**) possessed the highest binding affinity for PK-C among a group of isomeric γ-lactones.[4] This compound functioned as a conformationally rigid DAG analogue and inhibited the binding of [^{3}H]phorbol-12,13-dibutyrate to PK-C with a K_i of 2.5 μM.[4] This value, however, indicated a five-fold lower binding affinity than the value measured for the equivalent, open chain glycerol-1-myristate-2-acetate (K_i = 0.5 μM) used as a control.[4]



1, n = 1

2, n = 2



PMA

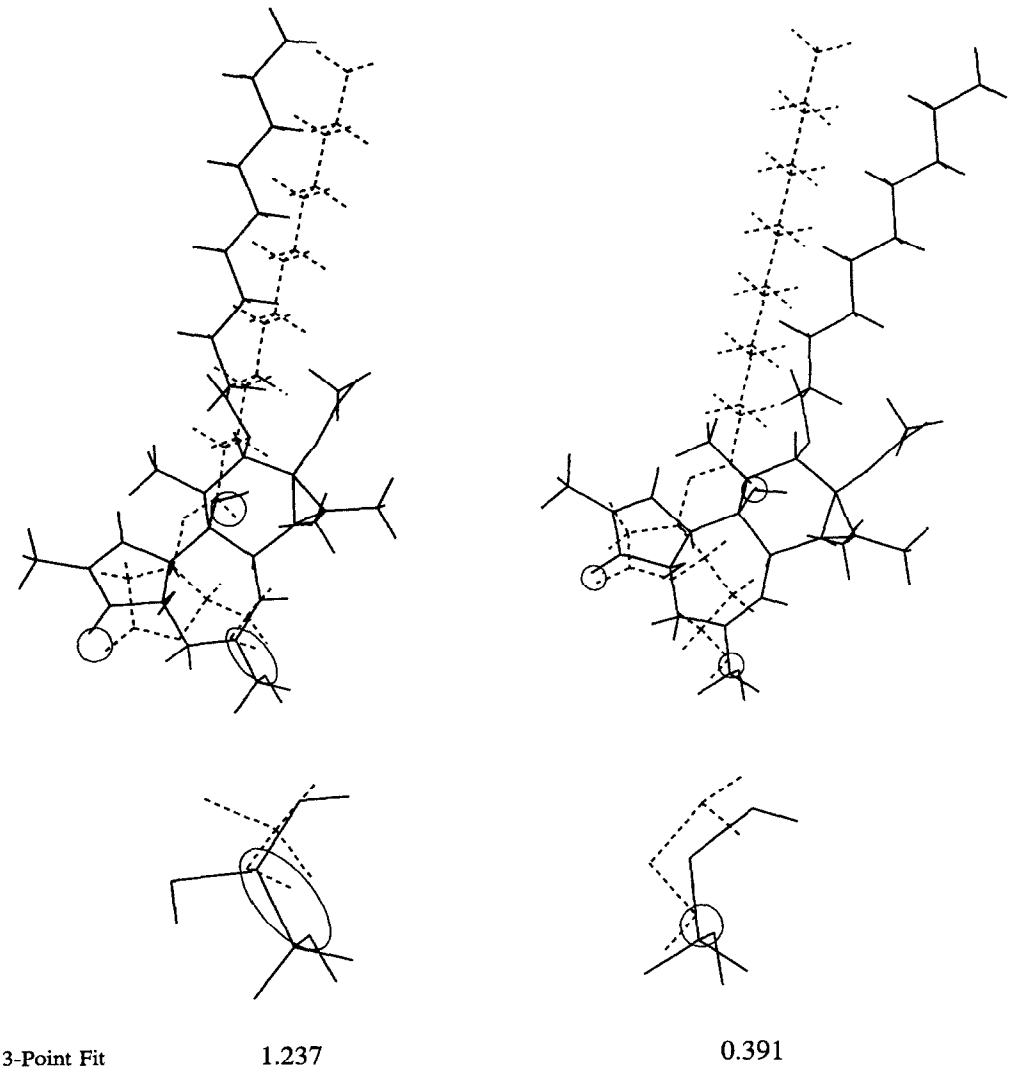


Figure 1. Superposition of Phorbol (solid line) on the γ-lactones 1 (left) and 2. The inset shows detail of the C_{20} hydroxyl of the phorbol and the side chain hydroxyl of the lactones.

A computer-generated molecular superposition of 3-O-tetradecanoyl-2-deoxy-L-ribonolactone (**1**) and phorbol-12-myristate-13-acetate (PMA) showed that any advantage accrued by the excellent overlap between the carbonyl oxygens of **1** and the C-3 and C-9 oxygens of PMA was opposed by a poorer overlap between the primary hydroxyl group of **1** and the C-20 hydroxyl of PMA.[4] In principle, formation of the lactone ring had reduced the "effective reach" of this important primary alcohol function (Figure 1). In order to compensate for this shortening effect, the homologue, 2,5-dideoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone (**2**) was synthesized and evaluated. As seen in Figure 1, for an equivalent side-chain orientation, the three-point fit for the proposed homologue **2** to PMA was significantly better (rms 0.391 vs. 1.237) than that of **1** to PMA. The actual comparative test between compounds **1** and **2** took special significance in view of the reduced potency, relative to DAG, that was reported for the corresponding butanetriol homologue.[5-7]

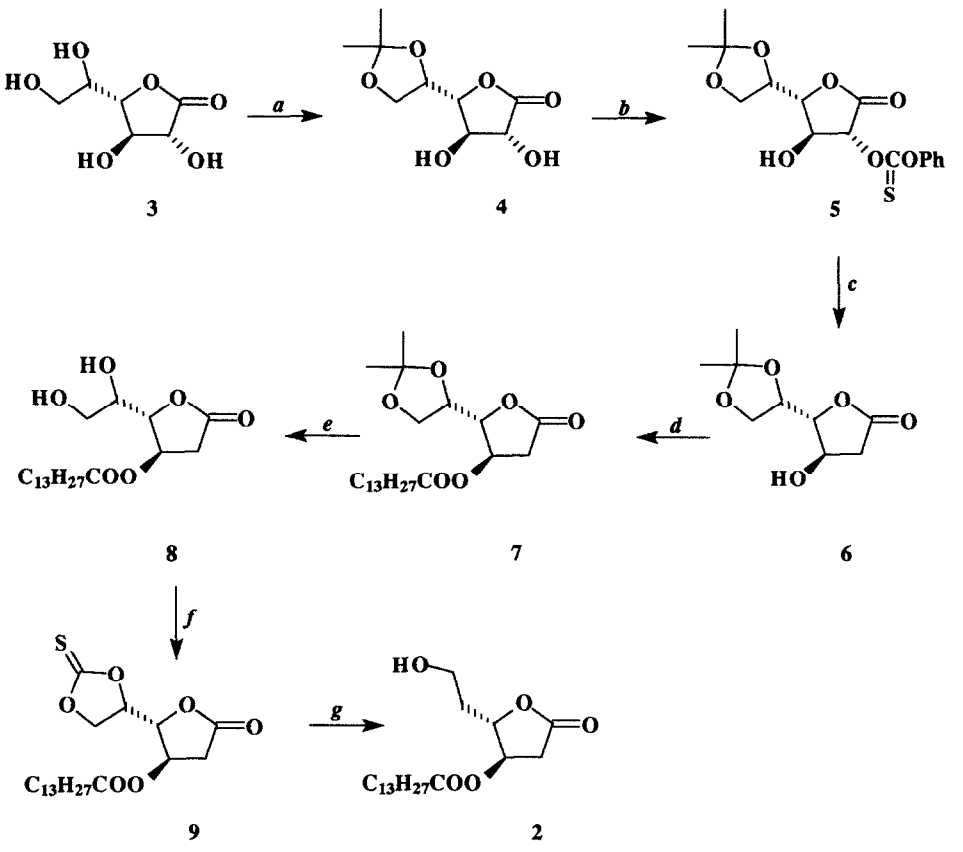
The synthesis of **2** commenced with commercially available D-galactono-1,4-lactone (**3**) which was protected as the 5,6-O-isopropylidene derivative **4** by a published method.[8] Selective acylation with phenyl chlorothionoformate produced intermediate **5** which was readily reduced to the 2-deoxygalactonolactone **6** (Scheme 1). At this stage, the myristate side chain was incorporated to give the corresponding ester **7**. Removal of the isopropylidene protection, followed by reaction with thiophosgene afforded the cyclic thionocarbonate **9** which underwent radical deoxygenation with the expected regioselectivity[9] to give the desired compound **2**.

Compounds **1** and **2** were evaluated for their ability to inhibit [20-^{3}H]phorbol-12,13-dibutyrate (PDBU) binding to purified PK-Cα[10] using the same experimental protocol described before.[4] The inhibition curves were of the type expected for competitive inhibition, and the data points were fitted to a theoretical non-cooperative competition curve to yield the ID_{50} values. The calculated K_i values from the ID_{50} were 1.5 μM $\pm$ 0.10 (n = 3) for compound **1** and 0.88 μM $\pm$ 0.13 (n = 3) for compound **2**. This approximately two-fold increase in binding affinity parallels the three-fold improvement in the fit determined by comparing the rms values calculated from molecular modeling. These results further validate our model pharmacophore and support its use as a guide for the synthesis of other constrained DAG analogues with improved binding affinity for PK-C. The fact that the binding affinity of **2** increased despite an increment in the number of rotamers for the extended hydroxyethyl chain in **2** is significant. This observation suggests that a specific orientation for the hydroxyalkyl chain bearing the primary alcohol function may not be as critical as the orientation of the two other oxygen (carbonyl) pharmacophores, provided that the chain is long enough to allow the hydroxyl group to reach effectively at the site where the requisite hydrogen bond is formed with the receptor.

Experimental Section

2-O-Phenoxythiocarbonyl-5,6-O-isopropylidene-D-galactono-1,4-lactone (**5**). A solution of 5,6-O-isopropylidene-D-galactono-1,4-lactone (**4**,[8] 1.485 g, 6.81 mmol) in anhydrous CH_3CN (35 mL) was treated with phenyl chlorothionoformate (1.418 g, 8.17 mmol) and dimethylaminopyridine (DMAP, 1.25 g, 10.21 mmol) under a blanket of argon at 0 °C. The reaction was quenched with water (1 mL) after 75 min, while the mixture was still cold, and the mixture was then concentrated under vacuum near 0 °C. The

Scheme 1



Reagents: (*a*) ref. 8 (*b*) PhOC(S)Cl, DMAP, CH_3CN (*c*) Bu_3SnH/azobis(isobutyronitrile), benzene (*d*) $CH_3(CH_2)_{12}COCl$, DMAP, pyridine, CH_2Cl_2 (*e*) 1M HCl, THF (*f*) thiophosgene, DMAP, pyridine, THF (*g*) Bu_3SnH/azobis(isobutyronitrile), toluene.

resulting solution was extracted with EtOAc (50 mL) and washed successively with 1 M HCl (2 x 20 mL), water (20 mL), satd $NaHCO_3$ soln (20 mL) and water (20 mL). The organic layer was dried (Na_2SO_4) and concentrated to give a yellow solid which was purified by flash column chromatography over silica gel. Elution with hexane:EtOAc (4:1), followed by hexane:EtOAc (3:1), gave **4** as an off-white solid (1.262 g, 52%), mp 120-121 °C (EtOAc/hexane); $[\alpha]_D^{25}$ = -88.17° (c 1.26, $CHCl_3$); IR (KBr) 3465, 1784, 1492, 1458, 1372, 1287 and 1248 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.38 (s, 3 H, $C\underline{H}_3$), 1.41 (s, 3 H, $C\underline{H}_3$), 3.39 (d, J = 2.9 Hz, 1 H, OH, D_2O exchanged), 4.02 (dd, 1 H, J = 8.5, 6.6 Hz, $H6_a$), 4.14 (dd, J = 8.5, 6.7 Hz, 1 H, $H6_b$), 4.32 (dd, J = 6.9, 2.9 Hz, 1 H, H4), 4.38 (dt, J = 6.6, 2.9 Hz, 1 H, H5), 4.76 (dt , J = 7.0, 2.8 Hz, 1 H, H3, converted to a triplet after D_2O exchanged), 6.05 (d, J = 7.2 Hz, 1 H, H2), 7.10-7.50 (m, 5 H, Ph); ^{13}C NMR ($CDCl_3$) δ 25.43, 25.92, 64.97, 73.09, 73.76, 79.95, 82.48, 110.51, 121.48, 127.12, 129.76, 153.50, 167.73, 195.38. Anal. Calcd for $C_{16}H_{18}O_7S$: C, 54.23; H, 5.12; S, 9.03. Found: C, 54.33; H, 5.12; S, 9.13.

2-Deoxy-5,6-O-isopropylidene-D-galactono-1,4-lactone (6). A solution of **5** (1.177 g, 3.32 mmol), tri-*n*-butyltin hydride (1.16 g, 3.98 mmol) and azobis(isobutyronitrile) (AIBN, 0.050 g) in anhydrous benzene (30 mL) was refluxed with vigorous stirring under argon for 75 min. The reaction mixture was concentrated and purified by flash chromatography over silica gel using hexane:EtOAc (3:1) as eluant. Compound **6** was isolated as a white solid (0.437 g, 65%), mp 71-73 °C (EtOAc/hexane); $[\alpha]_D^{25}$ = -12.83° (c 1.06, $CHCl_3$); IR (KBr) 3482, 1764, 1382, and 1344 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (s, 6 H, $C\underline{H}_3$), 2.40 (dd, J = 18, 1.9 Hz, 1 H, $H2_a$), 2.86 (br s, 1 H, OH), 2.95 (dd, 1 H, J =18.0, 6.6 Hz, $H2_b$), 3.92 (dd, J = 8.3, 7.4 Hz, 1 H, $H6_a$), 4.02 (dd, J = 8.3, 6.8 Hz, 1 H, $H6_b$), 4.26 (dt, J = 7.0, 1.7 Hz, 1 H, H5), 4.37 (br t , J = 1.5 Hz, 1 H, H4), 4.55 (m, 1 H, H3); ^{13}C NMR ($CDCl_3$) δ 25.48, 25.60, 38.24, 65.29, 70.10, 75.31, 85.47, 110.28, 176.29. Anal. Calcd for $C_9H_{14}O_5 \cdot 0.25\ H_2O$: C, 52.29; H, 7.07. Found: C, 52.43; H, 6.90.

2-Deoxy-3-O-tetradecanoyl-5,6-O-isopropylidene-D-galactono-1,4-lactone (7). A stirred solution of **6** (0.236 g, 1.16 mmol) in anhydrous CH_2Cl_2 (20 mL) at 0 °C containing anhydrous pyridine (0.369 g, 4.67 mmol) and DMAP (0.036 g, 0.29 mmol) was treated with myristoyl chloride (0.576 g, 2.33 mmol) and stirred at rt for 24 h. Water (1 mL) was added and the reaction mixture was concentrated under vacuum and extracted with EtOAc (50 mL). The organic extract was washed successively with 1 M HCl (2 x 15 mL), water (15 mL), satd $NaHCO_3$ soln (10 mL) and water (15 mL), and dried over Na_2SO_4. After removal of the solvent, the residue was purified by flash column chromatography over silica gel using hexane:EtOAc (9:1) as eluant. Compound **7** was isolated as a white solid (0.392 g, 81%), mp 49.0-50.0 °C (EtOAc/hexane); $[\alpha]_D^{25}$ = +21.50° (c 1.00, $CHCl_3$); IR (KBr) 1764, 1731, 1382, 1256, 1208 and 1179 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, $C\underline{H}_3(CH_2)_{12}CO$), 1.20-1.40 (m, 26 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$, $C(C\underline{H}_3)_2$), 1.60 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CO$), 2.30 (t, $J \approx$ 7.5 Hz, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CO$), 2.48 (d, J = 18.5 Hz, 1 H, $H2_a$), 3.01 (dd, J = 18.5, 7.2 Hz, 1 H, $H2_b$), 3.94 (distorted t, 1 H, $H6_a$), 4.08 (dd, J = 8.3, 7.0 Hz, 1 H, $H6_b$), 4.40 (m, 2 H, H4, H5), 5.31 (d, J = 7.1 Hz, 1 H, H3); ^{13}C NMR ($CDCl_3$) δ 14.10, 22.66, 24.70, 25.53, 29.04, 29.18, 29.32, 29.38, 29.55, 29.61, 29.63, 31.90, 34.03, 34.66, 65.20, 72.40, 75.27, 82.84, 110.41, 173.47, 174.93. Anal. Calcd for $C_{23}H_{40}O_6$: C, 66.94; H, 9.78. Found: C, 66.78; H, 9.85.

2-Deoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone (8). A solution of **7** (2.366 g, 5.73 mmol) in THF (160 mL) was treated with aqueous 1 M HCl (100 mL) and stirred at rt for 72 h. The reaction mixture was concentrated and extracted with EtOAc (3 x 25 mL), washed with water (2 x 20 mL) and dried (Na_2SO_4). Evaporation of the organic layer gave a white solid (1.796 g, 84%), mp 79.0-81.5 °C (EtOAc/hexane); $[\alpha]_D^{25}$ = +18.09° (c 1.10, $CHCl_3$); IR (KBr) 3547, 3404, 1774, 1731, 1354 and 1165 cm^{-1}; ^1H NMR $CDCl_3$) δ 0.85 (distorted t, 3 H, $C\underline{H}_3(CH_2)_{12}CO$), 1.20-1.35 (m, 20 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$), 1.60 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CO$), 2.30 (t, $J \approx$ 7.5 Hz, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CO$), 2.50 (dd, J = 18.6, 1.3 Hz, 1 H, $H2_a$), 3.08 (dd, J = 18.6, 7.5 Hz, 1 H, $H2_b$), 3.73 (d, J = 5.8 Hz, 2 H, $H6_{ab}$), 4.01 (m, 1 H, H5), (4.47 (br s, 1 H, H4), 5.38 (d, J = 7.4 Hz, 1 H, H3); ^{13}C NMR ($CDCl_3$) δ 14.10, 22.66, 24.67, 29.10, 29.20, 29.32, 29.41, 29.57, 29.61, 31.89, 34.04, 34.83, 63.12, 71.36, 72.33, 85.40, 173.63, 175.66. Anal. Calcd for $C_{20}H_{36}O_6$: C, 64.49; H, 9.74. Found: C, 64.44; H, 9.76.

2-Deoxy-3-O-tetradecanoyl- 5,6-O-thionocarbonate-D-galactono-1,4-lactone (9). A stirred solution of **8** (0.332 g, 0.891 mmol) in dry THF (9 mL) at rt was added DMAP (0.109 g, 0.891 mmol) and pyridine (0.352 g, 4.45 mmol). Thiophosgene (0.128 g, 1.114 mmol) was added to the solution and after 25 min the reaction was quenched with 1 M HCl (ca 10 mL). The two layers were separated and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic extract was washed successively with 1 M HCl (2 x 10 mL) and water (10 mL) and dried (Na_2SO_4). The solvent was evaporated and the residue was purified by flash column chromatography over silica gel using hexane/EtOAc (7:3) as eluant to give **9** (0.288 g, 78%). The compound solidified on standing, mp 48-49 °C; $[\alpha]_D^{25}$ = +26.92° (c 1.04, $CHCl_3$); IR (KBr) 1794, 1780, 1742, 1387, 1286 and 1155 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, $C\underline{H}_3(CH_2)_{12}CO$), 1.20-1.35 (m, 20 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$), 1.60 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CO$), 2.33 (t, $J \approx 7.5$ Hz, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CO$), 2.67 (dd, J = 18.9, 1.8 Hz, 1 H, $H2_a$), 3.10 (dd, J = 18.9, 7.9 Hz, 1 H, $H2_b$), 4.49 (distorted t, 1 H, H4), 4.65 (dd, J = 8.9, 7.2 Hz, 1 H, $H6_a$), 4.75 (t, $J \approx 8.7$ Hz, 1 H, $H6_b$), 5.33 (m, 2 H, H3, H5); ^{13}C NMR ($CDCl_3$) δ 14.10, 22.66, 24.60, 29.02, 29.15, 29.31, 29.37, 29.55, 29.60, 29.63, 31.88, 33.69, 33.81, 69.97, 70.83, 79.71, 82.42, 172.46, 173.77, 189.83. Anal. Calcd for $C_{21}H_{34}O_6S$: C, 60.84; H, 8.27; S, 7.73. Found: C, 60.86; H, 8.32; S, 7.80.

2,5-Dideoxy-3-O-tetradecanoyl-D-galactono-1,4-lactone (2). A mixture of **9** (0.195 g, 0.47 mmol), tri-*n*-butyltin hydride (0.151 g, 0.517 mmol) and azobis(isobutyronitrile) (AIBN, 0.050 g) in anhydrous toluene (8 mL) was added dropwise to a flask containing refluxing toluene (5 mL) under an argon atmosphere. After 1 h of reflux, the reaction mixture was allowed to cool to rt, concentrated, and purified by flash column chromatography over silica gel using hexane/EtOAc (4:1) followed by hexane:EtOAc (7:3) as eluant mixtures. Compound **2** (0.064 g, 38%) was isolated as a white solid, mp 65.5-66.5 °C (EtOAc/hexane); $[\alpha]_D^{25}$ = +20.29° (c 1.02, $CHCl_3$); IR (KBr) 3456, 1740, 1730, 1227, and 1179 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, $C\underline{H}_3(CH_2)_{12}CO$), 1.20-1.30 (m, 20 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$), 1.60 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CO$), 1.90 (m, 2 H, $H5_{a,b}$), 2.32 (t, $J \approx 7.5$ Hz, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CO$), 2.58 (dd, J = 18.6, 1.9 Hz, 1 H, $H2_a$), 2.95 (dd, J = 18.6, 7.0 Hz, 1 H, $H2_b$), 3.84 (m, 2 H, $H6_{a,b}$), 4.64 (distorted t, 1 H, H4), 5.22 (dm, J = 7.0 Hz, 1 H, H3); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.67, 24.69, 29.04, 29.18, 29.32, 29.39, 29.56, 29.61, 31.89, 33.80, 34.06, 35.33, 58.56, 73.17, 83.52, 173.73, 174.00; FAB MS *m/z* (rel intensity) 357 (MH^+, 86), 297 (MH-HOAc, 9), 211 ($C_{13}H_{27}CO^+$, 23), 129 (MH-$C_{13}H_{27}COOH$, 100). Anal. Calcd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.30; H, 10.15.

References and Notes

1. Paper #4 in the series, Lee, J.; Marquez, V. E.; Lewin, N. E.; Bahador, A.; Kazanietz, M.; Blumberg, P. M. *BioMed Chem. Lett.* **1993**, *3*, 1107.
2. For a Review, see *Protein Kinase C. Current Concepts and Future Perspectives*; Lester, D. S.; Epand, R. M., Ed.; Ellis Horwood: New York, 1992.
3. Rando, R. R.; Young, N. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 818.
4. Teng, K.; Marquez, V. E.; Milne, G. W. A.; Barchi, Jr., J. J.; Kazanietz, M. G.; Lewin, N. E.; Blumberg, P. M.; Abushanab, E. *J. Am. Chem. Soc.* **1992**, *114*, 1059.
5. Ganong, B. R.; Loomis, C. R.; Hannun, Y. A.; Bell, R. M. *Proc. Natl. Acad. Sci. USA*, **1986**, 1184.
6. Molleyres, L. P.; Rando, R. R. *J. Biol. Chem.*, **1988**, *263*, 14832.
7. Wender, P. A.; Cribbs, C. M. *Advances in Medicinal Chemistry*; Maryanoff, B. E.; Maryanoff, C. A., Ed.; Jai Press, Inc.: Greenwich, Connecticut; London, England, **1992**; Vol. 1, pp 1-53.
8. Morgenlie, S. *Acta Chem. Scand.* **1975**, *B29*, 367.
9. Barton, D. H. R.; Subramanian, R. *J. Chem. Soc. Perkin I*, **1977**, 1718.
10. Kazanietz, M. G.; Krausz, K. W.; Blumberg, P. M.; *J. Biol. Chem.* **1992**, *267*, 20878.