Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 20, pp. 2405-2410, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00348-3

CONFORMATIONALLY CONSTRAINED ANALOGUES OF DIACYLGLYCEROL. 9.[1] THE EFFECT OF SIDE-CHAIN ORIENTATION ON THE PROTEIN KINASE C (PK-C) BINDING AFFINITY OF δ-LACTONES

Jeewoo Lee,[1] Nancy E. Lewin,[2] Peter M. Blumberg,[2] and Victor E. Marquez,[1]*

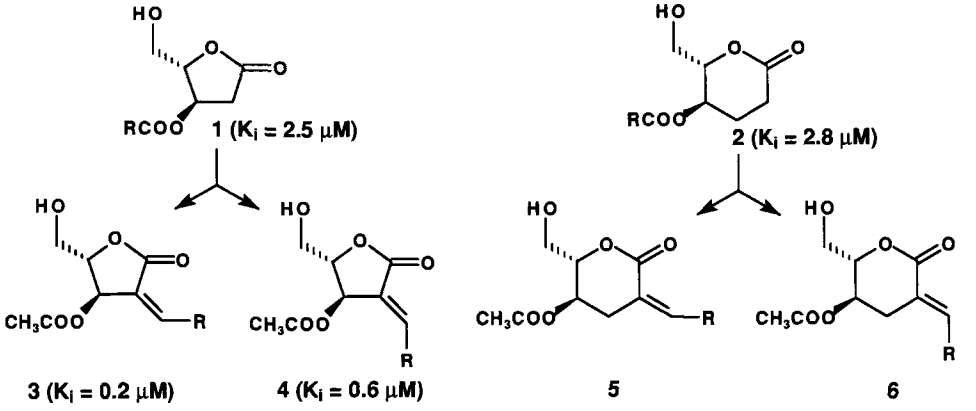
[1]*Laboratory of Medicinal Chemistry, Developmental Therapeutics Program, Division of Cancer Treatment and* [2]*Molecular Mechanisms of Tumor Promotion Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, NIH, Bethesda, Maryland 20892 (USA)*

Abstract. The construction of conformationally constrained diacylglycerol analogues on an α-alkylidene-δ-lactone template was achieved stereospecifically from L-xylose. The increased PK-C binding affinity of the *E*-isomer (**6**) over the *Z*-isomer (**5**) contrasts with the more modest difference observed for the same geometrical isomers built on an α-alkylidene-γ-lactone template. The more effective discrimination between isomeric α-alkylidene-δ-lactones by PK-C forcefully argues that orientation of the side-chain is a key determinant for a strong interaction with the enzyme.

Protein kinase C (PK-C) plays a pivotal role in cell signal transduction and as such it is intimately involved in the regulation of cell growth and differentiation.[2,3] The molecular heterogeneity of the PK-C family of enzymes,[4,5] their different tissue distribution,[6] and the possibility that they might be separately implicated in arresting or stimulating cell growth[2,3] make them attractive targets for antitumor drug development.[7] In most PK-C isozymes, activation is rapid after the enzyme binds to diacylglycerol (DAG), one of the products released from the receptor-activated hydrolysis of phosphatidylinositol-4,5-bisphosphate catalyzed by phospholipase C.[8] PK-C is also the intracellular receptor for the tumor-promoting phorbol esters which have the capacity to bind to PK-C with much greater affinity than DAG.[9]

Over the past few years, we have synthesized a series of conformationally rigid DAG analogues designed to minimize the entropic penalty associated with the binding of the linear and flexible molecule of DAG.[1] We have been able to establish that 5- and 6-member lactones corresponding, respectively, to 3-*O*-tetradecanoyl-2-deoxy-L-ribonolactone (**1**)[10] and the stereochemically equivalent 4-*O*-tetradecanoyl-2,3-dideoxy-L-glucono-1,5-lactone (**2**)[11] can bind to PK-C with nearly equal affinity. We have also determined that in a manner equivalent to the transposition of the long acyl chain from *sn*-1 to *sn*-2 in DAG —which does not change biological properties in DAG[12]— a comparable modification of the parent lactone **1**, consisting in abbreviating the acyl function as the acetate and extending the lipophilic chain from the α-carbon of the ring, resulted in compounds with improved binding affinities for PK-C relative **1**.[13] More importantly, the enantiomeric forms of these lactones were strictly differentiated by PK-C, which contrasts with the reduced specificity shown by the optical antipodes of **1**.[10] Taken together these findings support the concept that a specific orientation of the lipophilic component of the molecule (the alkyl chain) improves affinity and specificity. An additional improvement in binding was also evidenced when the side chain was steered in a direction coplanar to the five-member ring from its point of insertion. Indeed, the

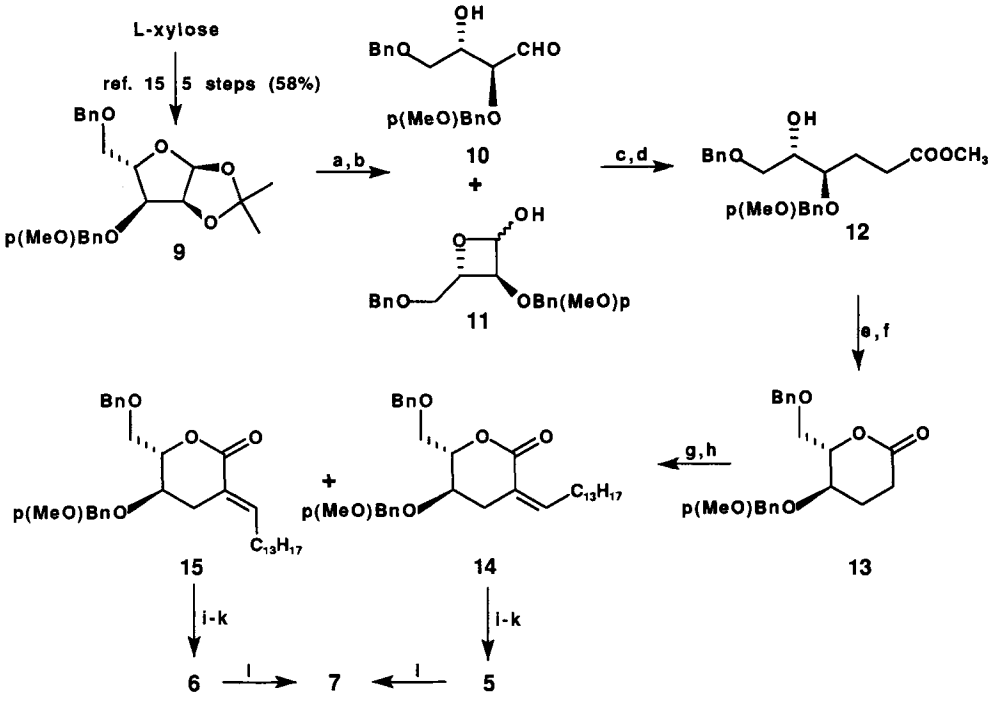
corresponding *Z*- and *E*-α-alkylidene lactones **3** and **4** had higher binding affinities for PK-C with the *Z*-isomer (**3**) being slightly preferred.[14] In this manuscript, we wish to report the results of a similar study



with the 6-member lactones (δ-lactones) represented by the target compounds **5** and **6**. Additionally, we describe the synthesis and biological evaluation of the alkyl analogue **7**, as well as the new lactone analogue **8**, where the alkylacyl chain appears displaced one position away from its location in **2**.

Synthesis of the suitably protected 2,3-dideoxy-L-glucono-1,5-lactone **13** was achieved from L-xylose (Scheme 1). Removal of the isopropylidene group from compound **9** —which was synthesized earlier in our laboratory[15]— was followed by periodate cleavage of the resulting diol to give 82% of a mixture of **10/11** (ca. 1:5). This mixture was used in a Wittig reaction with methyl(triphenylphosphoranylidene)acetate to give the corresponding unsaturated ester which was immediately reduced to the saturated methyl hexanoate ester **12** with $NaBH_4$ in the presence of $NiCl_2$. The ester in **12** was hydrolyzed and the ensuing lactonization in the presence of dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) as condensing agents produced the desired six-member lactone **13** in good yield. Reaction of the enolate anion of **13** with myristyl aldehyde gave a mixture of diastereomeric alcohols which after treatment with methanosulfonyl chloride in the presence of triethylamine afforded the corresponding *Z*- and *E*-isomers, **14** and **15**. Separation of these isomers (**15/14** ≈ 20) was achieved by silica gel column chromatography using a mixture of ethyl acetate:hexanes (1:3) as eluant. As in

Scheme 1

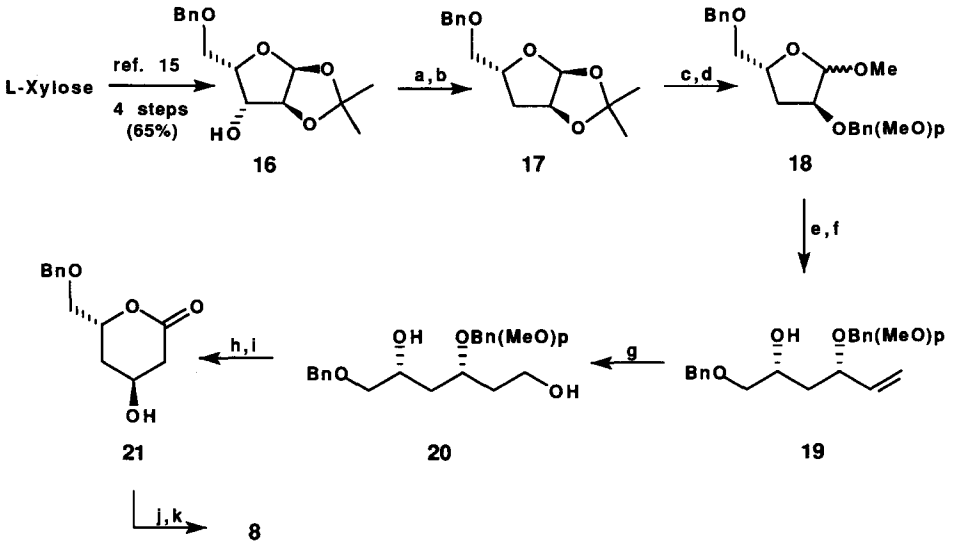


Reagents: (**a**) HCl, dioxane-H_2O (**b**) $NaIO_4$, $NaHCO_3$, CH_2Cl_2-H_2O (82%, two steps) (**c**) $Ph_3PCHCOOMe$, PhCOOH (cat.), benzene (98%) (**d**) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH (93%) (**e**) NaOH, THF-H_2O (100%) (**f**) DCC, DMAP, CH_2Cl_2 (82%) (**g**) LDA, THF, −78 °C/$C_{13}H_{27}CHO$, $ZnCl_2$ (**h**) CH_3SO_2Cl, Et_3N, CH_2Cl_2 (75%, two steps) (**i**) DDQ, CH_2Cl_2-H_2O (83-84%) (**j**) Ac_2O, pyridine, CH_2Cl_2 (98-99%) (**k**) BCl_3, CH_2Cl_2, −78 °C (97-98%) (**l**) H_2, Pd/C, MeOH (99%)

the case of the five-member lactones, identification of each isomer was readily done by examining the chemical shift of the vinyl protons of this cisoid enone system. For the *E*-isomer (**15**), the β-cis vinyl proton centered at δ 7.03 (tt, J = 7.5, 2.2 Hz) showed the expected downfield shift relative to the equivalent signal of the β-trans proton of the *Z*-isomer **14** (δ 6.03, t, J = 7.2 Hz). Selective oxidative removal of the p-methoxybenzyl ether by 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) performed on each individual isomer enabled the selective acylation of the secondary alcohol with acetic anhydride. Removal of the benzyl ether with BCl_3 afforded the target α-alkylidene-δ-lactones **5** and **6**.[16] Unfortunately, the catalytic hydrogenation of either olefin afforded an inseparable mixture of the α-alkyl-δ-lactone **7**.

The final target, (4S,6R)-4-tetradecanoyloxy-6-hydroxymethyl-3,4,5,6-tetrahydro-2*H*-pyran-2-one (**8**), which is isomeric to lactone **2**, was synthesized according to Scheme 2. The free hydroxyl group of intermediate **16**, previously synthesized as a precursor of **9**[15] (Scheme 1), was removed via the Barton-McCombie reduction to give the deoxygenated compound **17** after treatment with tributyltin hydride/AIBN. Acid-catalyzed methanolysis of the isopropylidene group in **17** and protection of the remaining free

Scheme 2



Reagents: (**a**) NaH, CS_2, MeI, THF (**b**) *n*-Bu_3SnH, AIBN, Toluene, Δ (66%, two steps) (**c**) Dowex 50W-X4 (H^+) resin, MeOH (100%) (**d**) *p*-$MeOPhCH_2Br$, NaH, THF (86%) (**e**) HCl, THF-H_2O (82%) (**f**) Ph_3PCH_3Br, *t*-BuOK, benzene, Δ (82%) (**g**) BH_3-SMe_2, THF; $NaBO_3$, H_2O (67%) (**h**) PCC, 4 Å Molecular sieves, CH_2Cl_2 (96%) (**i**) DDQ, CH_2Cl_2-H_2O (73%) (**j**) $CH_3H_{27}COCl$, DMAP, pyridine, CH_2Cl_2 (97%) (**k**) H_2, Pd/C, MeOH (94%).

hydroxyl group as the p-methoxybenzyl ether gave **18**. Conversion of **18** to the lactol was then followed by reaction with methyltriphenylphosphonium bromide/t-BuOK to give the expected olefin **19**, which was converted to alcohol **20** by the usual hydroboration/oxidation protocol. Pyridinium chlorochromate (PCC) oxidation of the primary alcohol in **20** proceeded via the *in situ* cyclization to the lactol which underwent further oxidation to the desired δ-lactone. As before, oxidative removal of the p-methoxybenzyl ether with DDQ enabled the selective acylation of **21** with myristoyl chloride, and removal of the benzyl ether by catalytic hydrogenation afforded the required target lactone **8**.[17]

The potencies of the test compounds to function as competitive binding inhibitors of [^{3}H-20]-phorbol-12,13-dibutyrate (PDBU) to PK-C are expressed in terms their inhibition constants K_i (Table 1). During the course of our synthetic work, the PK-C preparation used for the bioassay was changed from a mixture of the PK-C isozymes α, β, and γ purified from mouse brain to a single recombinant isozyme, PK-Cα from bovine brain. As reported before, we have observed little variance between results obtained with the two types of enzyme preparation.[8] As discussed in our previous communication, the results obtained with the 5-member lactones showed similar affinities for the *Z*- and *E*-isomers (compounds **3** and **4**), with a slight advantage in favor of the *Z*-isomer. A discreet advantage of one isomer relative to the other in the 5-member lactones was magnified to ca. 7-fold in the case of the 6-member lactones, except that the advantage shifted in favor of the *E*-isomer (compare compounds **5** and **6**). This difference suggests that,

with other factors being constant, the distinct orientation of the hydrophobic α-alkylidene moiety in the more puckered 6-member lactone affects the binding of the ligand. Since a change in orientation of the alkyl chain is expected to be strictly related to hydrophobic binding interactions at constant hydrophobicity, the effect on binding cannot be as dramatic as with changes in hydrogen bonding or electrostatic interactions. This concept is entirely consistent with the more moderate differences in K_i observed between these compounds (Table 1).

The results on Table 1 also indicate that a completely saturated chain as found in compound **7** (mixture of isomers) was not as effective as the α-alkylidene side chains. In this case, however, since the mixture of isomers could not be separated the question of orientation cannot be fully addressed. Finally, the relocation of the alkylacyl chain from C-3 (compound **2**) to C-4 (compound **8**) produced an isomeric 6-member lactone with a significant reduction in binding affinity.

Is summary, the results presented here strengthen the concept that δ-lactones are as effective as γ-lactones as templates to support the construction of rigid DAG analogues. However, due to the more discriminating nature of the δ-lactone, it could have a definitive advantage over the γ-lactone as a template for the unraveling of structure-activity relationship problems, particularly those related to orientation of the side chain.

Table 1. Apparent K_i (μM) Values (n = 3) for Ligands **2-8** as Inhibitors of PDBU Binding to PK-C

	2	**3**	**4**	**5**	**6**	**7**	**8**
PK-C mix	2.8^{11} ±0.4	0.2^{14} ±0.0	0.6^{14} ±0.0	—	—	—	—
PK-C α	—	0.2^{14} ±0.0	0.3^{14} ±0.0	1.3 ±0.0	0.2 ±0.0	1.9 ±0.1	20.5 ±0.5

References and Notes

1. Paper #8 in series: Lee, J.; Sharma, R.; Teng, K.; Lewin, N. E.; Blumberg, P. M.; Marquez, V. E. *BioMed. Chem. Lett.* **1994**, *4*, 1369.

2. Clemens, M. J.; Trayner, I,; Menaya, J. *J. Cell Science* **1992**, *103*, 881.

3. Macfarlane, D. E.; Manzel, L. *J. Biol. Chem.* **1994**, *269*, 4327.

4. Nishizuka, Y. *Nature* **1988**, *334*, 661.

5. Nishizuka, Y. *J. Amer. Med. Assoc.* **1989**, *262*, 1826.

6. Wetsel, W. C; Khan, W. A.; Merchenthaler, I.; Rivera, H.; Halpern, A. E.; Phung, H. M.; Negro-Vilar, A.; Hannun, Y. A. *J. Cell. Biol.* **1992**, *117*, 121.

7. Gescher, A. *Br. J. Cancer* **1992**, *66*, 10.

8. For a review, see *Protein Kinase C. Current Concepts and Future Perspectives*; Lester, D. S.; Epand, R. M. (Eds.), Ellis Horwood: New York, 1992.

9. Sharkey, N. E.; Leach, K. L.; Blumberg, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 607.

10. 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.

11. Lee, J.; Marquez, V. E.; Blumberg, P. M.; Krausz, K. W.; Kazanietz, M. G. *Bioorg. Med. Chem.* **1993**, *1*, 119.

12. Mori, T.; Takai, Y.; Binzu, Y.; Takahashi, J.; Nishizuka, Y.; Fujikura, T. *J. Biochem.* **1982**, *91*, 427.

13. Lee, J.; Marquez, V. E.; Lewin, N. E.; Kazanietz, M. G.; Bahador, A.; Blumberg, P. M. *BioMed. Chem. Lett.* **1993**, *3*, 1101.

14. Lee, J.; Marquez, V. E.; Lewin, N. E.; Bahador, A.; Kazanietz, M. G.; Blumberg, P. M. *BioMed. Chem. Lett.* **1993**, *3*, 1107.

15. Lee, J.; Marquez, V. E.; Lewin, N. E.; Blumberg, P. M. *BioMed. Chem. Lett.* **1994**, *4*, 543.

16. All intermediates leading to **5** and **6** were fully characterized. Compound **5**: mp 60 °C, $[\alpha]^{25}_D$ = –61.43° (c 0.28, $CHCl_3$); IR (KBr) 3456 (OH), 1735, 1713 (C=O) 1636 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.12 (t, *J* = 7.3 Hz, 1 H, C=C<u>H</u>), 5.09 (m, 1 H, H-4), 4.29 (m, 1 H, H-5), 3.86 (dd, *J* = 12.4, 3.3 Hz, 1 H, CH<u>H</u>OH), 3.73 (dd, *J* = 12.4, 4.4 Hz, 1 H, C<u>H</u>HOH), 2.94 (m, 1 H, H-3a), 2.58 (m, 3 H, H-3b, C<u>H</u>$_2$-CH=C), 2.07 (s, 3 H, C<u>H</u>$_3$CO), 1.70 (br s, 1 H, OH), 1.00-1.50 (m, 22 H), 0.86 (distorted t, 3 H, C<u>H</u>$_3$); ^{13}C NMR δ 170.10, 164.02, 151.12, 120.61, 80.51, 66.53, 62.05, 34.54, 31.91, 29.73, 29.66, 29.64, 29.57, 29.43, 29.34, 29.08, 22.68, 20.94, 14.10; FAB MS *m/e* (relative intensity) 383 (MH^+, 100). Anal. Calc'd for $C_{22}H_{38}O_5$: C, 69.07; H, 10.01. Found: C, 68.90; H, 9.98. Compound **6**: mp 58 °C, $[\alpha]^{25}_D$ = –6.58 ° (c 1.00, $CHCl_3$); IR (KBr) 3455 (OH), 1735, 1713 (C=O) 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.12 (tt, *J* = 7.5, 2.1 Hz, 1 H, C=C<u>H</u>), 5.11 (m, 1 H, H-4), 4.35 (m, 1 H, H-5), 3.88 (dd, *J* = 12.6, 3.2 Hz, 1 H, CH<u>H</u>OH), 3.76 (dd, *J* = 12.6, 4.2 Hz, 1 H, C<u>H</u>HOH), 2.99 (m, 1 H, H-3a), 2.52 (m,1 H, H-3b), 2,13 (m, 2 H, C<u>H</u>$_2$-CH=C), 2.09 (s, 3 H, C<u>H</u>$_3$CO),1.15-1.60 (m, 22 H), 0.86 (distorted t, 3 H, C<u>H</u>$_3$); ^{13}C NMR δ 170.02, 165.05, 149.37, 121.43, 79.85, 65.53, 61.61, 31.89, 29.62, 29.49, 29.37, 29.32, 28.56, 28.21, 28.05, 22.65, 20.95, 14.09; FAB MS *m/e* (relative intensity) 383 (MH^+, 100). Anal. Calc'd for $C_{22}H_{38}O_5$: C, 69.07; H, 10.01. Found: C, 69.12; H, 10.00.

17. All intermediates leading to **8** were fully characterized. Compound **8**: mp 69 °C; $[\alpha]^{25}_D$ = –1.20° (c 1.00 $CHCl_3$); IR (KBr) 3504 (OH), 1734, 1707 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.32 (m, 1 H, H-3), 4.66 (m, 1 H, H-5), 3.89 (dd, *J* = 12.4, 2.9 Hz, 1 H, CH<u>H</u>OH), 3.65 (dd, *J* = 12.4, 4.6 z, 1 H, C<u>H</u>HOH), 2.74 (d, *J* = 3.9 Hz, 2 H, H-2), 2.30 (t, *J* = 7.5 Hz, 2 H, OCC<u>H</u>$_2$C$_{12}$H$_{25}$), 2.02 (dd, *J* = 7.7, 3.3 Hz, 2 H, H-4), 1.60 (m, 2 H, OCCH$_2$C<u>H</u>$_2$C$_{11}$H$_{23}$), 0.86 (distorted t, 3 H, C<u>H</u>$_3$); ^{13} C NMR δ 172.80, 168.67, 76.79, 65.22, 64.32, 35.34, 34.26, 31.88, 29.63, 29.60, 29.55, 29.40, 29.31, 29.19, 29.07, 28.47, 24.81, 22.65, 14.08; FAB MS *m/e* (relative intensity) 357 (MH^+, 1.53). Anal. Calc'd for $C_{20}H_{36}O_5$: C, 67.38; H, 10.18. Found: C, 67.30; H, 10.19.

(*Received in USA* 1 *August* 1994; *accepted* 8 *September* 1994)