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CONFORMATIONALLY CONSTRAINED ANALOGUES OF DAG.7.[1] INTERACTION OF A MEDIUM-SIZE ε-LACTONE WITH PROTEIN KINASE C (PK-C)

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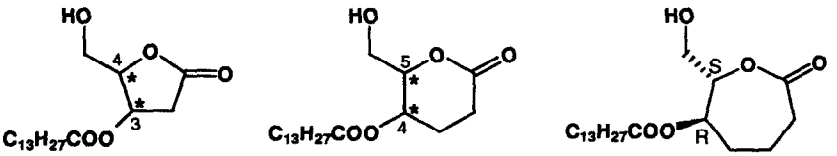
Abstract: Synthesis of (5R,6S)-5-O-tetradecanoyl-6-hydroxymethyl-6-heptanolide (**3**), designed as a rigid diacylglycerol (DAG) analogue, was achieved in 17 steps from L-xylose. Protein kinase (PK-C) binding affinity for this ε-lactone template was less than that observed for the stereochemically equivalent γ– and δ–lactones. A critical ring-size for PK-C binding affinity in larger lactones is proposed based on our data and results from the published literature.

The release of "second messenger" diacylglycerol (DAG) is part of a lipid-mediated signal transduction pathway that is initiated by the receptor-induced hydrolysis of membrane phosphatidylinositol 4,5-diphosphate.[2-4] In the presence of calcium and other phospholipid cofactors, DAG is capable of effectively activating protein kinase C (PK-C).[3,4] PK-C is in reality a family of closely related enzymes which have different modes of activation and tissue distribution.[4-6] The involvement of PK-Cs in numerous aspects of cell proliferation and differentiation, often times with opposite effects,[7] makes this family of enzymes attractive targets for chemotherapeutic intervention. PK-Cs are also the receptors for the plant diterpene phorbol esters which are known to be potent tumor promoters.[8-10] Molecular modeling comparisons between DAG, phorbol esters, and other natural PK-C agonists have led to the identification of equivalent pharmacophores in these molecules that define important structural requirements for PK-C activation.[11,12] Using this information, we have designed, with some success, various conformationally constrained analogues of DAG in which the essential pharmacophores of DAG are contained in a simple lactone structure.[13-16] In one of these investigations, we compared the effects of a pair of enantiomeric ribono- (**1a,b**) and gluconolactones (**2a,b**) on PK-C binding and activation, and showed that while for the five-membered ribonolactones PK-C demonstrated little discrimination between enantiomers, enantiomeric six-membered lactones were better discriminated by the enzyme.[16] An unexpected finding, however, was that despite the difference in ring size, the most potent enantiomer of the ribonolactones and the most potent enantiomer of the gluconolactones were virtually equivalent as inhibitors of binding of [^{3}H]-phorbol-12,13-dibutyrate to PK-C (K_i values 2.5 and 2.8 μM, respectively).[16] In an effort to explore further the issue of ring size versus PK-C affinity, and to investigate the limits of this equivalency, the corresponding seven-membered ε-lactone, having the same stereochemistry as the two most active enantiomeric γ– and δ–lactones, was synthesized and evaluated.



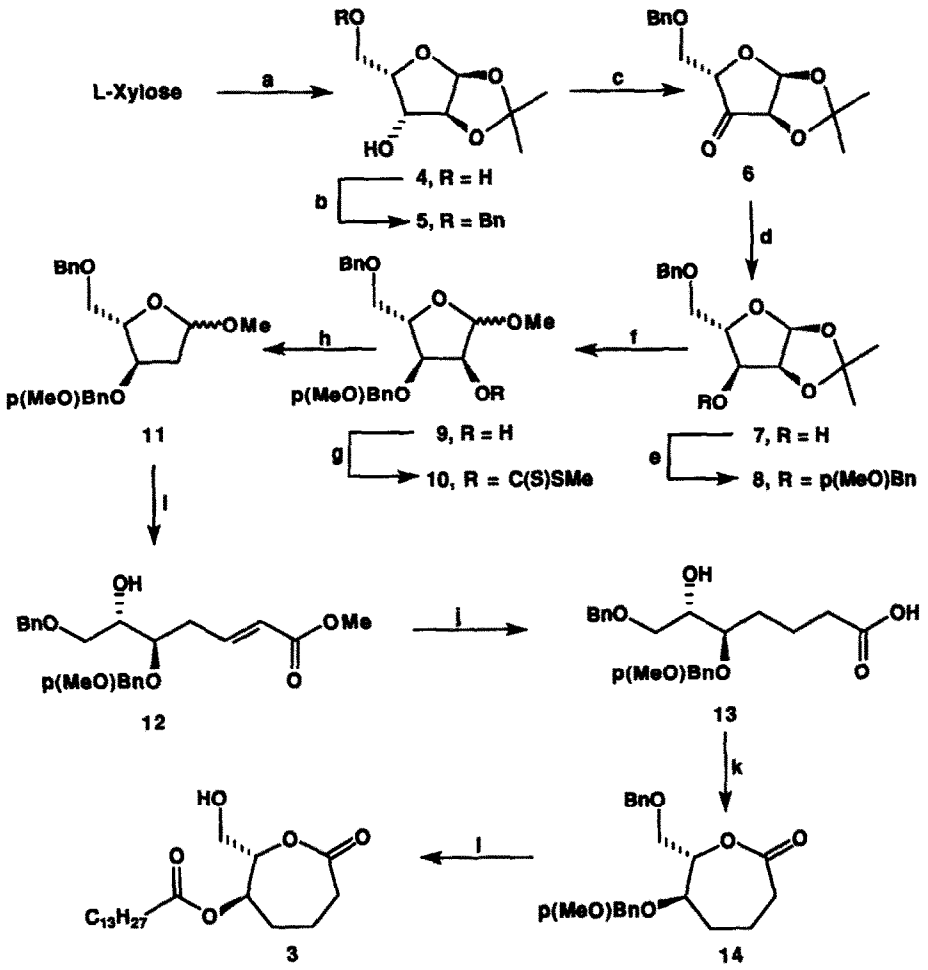
1a (3R,4S); K_i = 2.5 μM **2a (4R,5S); K_i = 2.8 μM** **3**

1b (3S,4R); K_i = 5.3 μM **2b (4S,5R); K_i = 30.5 μM**

The synthetic strategy (Scheme) was directed towards obtaining the requisite substituted heptanoic acid **13** with the appropriate protecting groups to lead to the exclusive formation of a seven-membered lactone. Using L-xylose as the starting chiral template, the 1,2-monoacetonide of L-xylose (**4**) was prepared and selectively protected as the monobenzyl ether **5**. Inversion of configuration of the hydroxyl group at C–3 was then accomplished via a common oxidation-reduction strategy that insured delivery of hydride from the less hindered α-side of keto intermediate **6**. The newly generated secondary alcohol function at C–3 was protected as the *p*-methoxybenzyl ether to insure its selective removal in the presence of the C–5 benzyl ether. In the following three steps, the acetonide group was removed by acid-catalyzed methanolysis and the C–2 hydroxyl group was deoxygenated by conversion to the xanthate ester and subsequent treatment with tributyltin hydride and AIBN in refluxing toluene to give compound **11**. Hydrolysis of the methylglycoside **11** to the lactol was followed by a Wittig reaction with methyl(triphenylphosphoranylidene)acetate that gave the open methyl 2-heptenoate ester as the trans-isomer **12**. Selective reduction of the conjugated double bond in **12** was performed with $NaBH_4$ in the presence of $NiCl_2$, according to the method of Kido et al.[17] At this point, the ensuing hydrolysis of the methyl ester and lactonization of the resulting acid in the presence of dicyclohexylcarbodiimide (DCC) as the condensing agent produced the desired seven-membered lactone as anticipated by the work of Keck and Boden.[18] The *p*-methoxybenzyl group was then selectively removed by oxidation with dichlorodicyanobenzoquinone (DDQ) which freed the secondary alcohol function at C-5 to allow formation of the corresponding myristate ester **14**. Removal of the remaining benzyl group by hydrogenolysis over Pd/C afforded the target compound **3**.[19]

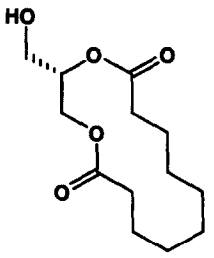
Biological evaluation of the seven-membered lactone **3** revealed that it had less binding affinity for PK-C than the stereochemically equivalent, lower homologs **1a** and **2a**. The K_i measured for inhibition of binding of [^{3}H]-phorbol-12,13-dibutyrate to PK-Cα under our standard conditions of assay[13-15] was 41 ± 11 μM (n = 3). This loss of affinity for the seven-membered template would suggest that other medium-size lactones, i.e., up to perhaps nine-membered lactones, might also give rise to poor PK-C ligands. A critical threshold, where good affinity could again reappear, lies possibly beyond the decalactone template where the lactone ester undergoes a change from the *E* to the more stable *Z* conformation.[20] Support for this idea comes independently from two laboratories that reported the synthesis of some macrolactones

Scheme



Reagents and conditions: a. i. H_2SO_4, $CuSO_4$, acetone. ii. 0.2% HCl, THF (82%, two steps). b. $(Bu_3Sn)_2O$, toluene; BnBr, Bu_4NBr (98%). c. PDC, AcOH, 4 Å molecular sieves, CH_2Cl_2 (96%). d. $NaBH_4$, MeOH (85%). e. p-(MeO)BnBr, NaH, THF (88%). f. HCl, MeOH (94%). g. CS_2, MeI, NaH, THF (83%). h. Bu_3SnH, AIBN, toluene, Δ (73%). i. i. HCl, aq. dioxane (95%). ii. $PPh_3CHCOOMe$, PhCOOH, benzene (95%). j. i. $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH (98%). ii. NaOH, THF-H_2O (100%). k. DCC, DMAP, DMAP·HCl, $CHCl_3$ (96%). l. i. DDQ, CH_2Cl_2, H_2O (92%). ii. $C_{13}H_{27}COCl$, pyridine, DMAP, CH_2Cl_2 (98%). iii. H_2, Pd/C, MeOH, $C_{13}H_{27}COOH$ (87%).

conceived by connecting the ends of the two lipophilic chains of DAG.[11,21] In the laboratory of Rando, a 14-membered cyclic DAG analogue (**15**) was found to have good affinity for PK-C, albeit significantly lower than that shown by the corresponding acyclic DAG analogue diolein.[21] In Wender's laboratory, however, a more successful result was obtained for a 20-membered macrocyclic lactone (**16**).[11] Although Wender's results have not yet been published in detail, K_i values for the inhibition of binding of [^{3}H]-phorbol-12,13-dibutyrate for this class of compounds are indicated to be in the 14 nM to 3.5 μM range.[11] Based on these data, it is possible to envision the effect that a large ring might have in influencing the orientation of the key DAG pharmacophores in these molecules. Moreover, it is also possible that with the larger rings, i.e. > 20-membered, the conformation adopted by the restricted side chain would be able to mimic almost perfectly the two independent chains of the acyclic DAG molecule as shown recently by Menger et al.[22] This would allow for a better interaction with the lipid bilayer which would explain the superior affinity shown by Wender's lactone.



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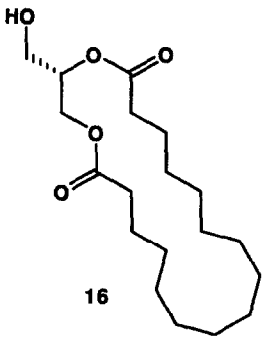
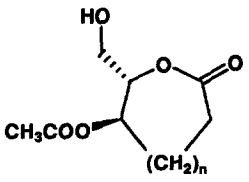


16



17, n = 13-15

In our more rigid 5-and 6-membered lactones (**1a** and **2a**) the orientation of the key pharmacophores, although far from perfect, might be close enough to the ideal that the overall binding process benefits from an entropy gain. For the medium-size lactones, such as **3**, the still rigid pharmacophores are probably thrown out of alignment and therefore binding affinity would be lost for enthalpic reasons. For the macrolactones, it would appear that the larger ring would permit a better alignment of the essential pharmacophores and facilitate hydrophobic binding to improve the overall fit at the active site. If these considerations are valid, macrolactone **17** with a similar stereochemistry to that present in compounds **1a** and **2b**, and macrolactones **15** and **16**, would be an attractive synthetic target.

In summary, PK-C affinity for conformationally restricted lactones that function as DAG surrogates peaks for 5- and 6-membered lactones and decreases beyond that point. Based on results from other laboratories, the limit for this decrease in activity appears to be in the 14-20-member range where affinity for PK-C again reappears. Notwithstanding the different modalities in which the DAG molecule appears embedded into the various lactones discussed here, we surmise that affinity for PK-C should be re-established again for a macrocyclic lactones such as **17**. This molecule should allow for efficient contacts

between the pharmacophores and the receptor, as well as for a better alignment of the rest of the hydrophobic ring with the lipid membrane.

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19. Compound **3**: mp 58 °C; $[\alpha]_D^{25}$ –14.86° (c 0.7, $CHCl_3$); IR (KBr) 3568 (OH), 1740 and 1730 cm^{-1} (CO); ^1H NMR ($CDCl_3$) δ 4.97 (m, 1 H, H-5), 4.54 (m, 1 H, H-6), 3.86 (AB m, 2 H, H-$7_{a,b}$), 2.40-2.70 (m, 2 H, H-$2_{a,b}$), 2.35 (t, 2 H, $CH_3(CH_2)_{11}C\underline{H}_2CO$), 1.14-2.02 (m, 26 H, H-$3_{a,b}$, H-$4_{a,b}$, $CH_3(C\underline{H}_2)_{11}$-), 0.86 (distorted t, 3 H, $C\underline{H}_3$); ^{13}C NMR δ 173.37, 170.44, 77.88, 74.83, 61.19, 34.28, 31.90, 29.66, 29.62, 29.59, 29.43, 29.33, 29.22, 29.09, 24.90, 23.95, 22.67, 18.20, 14.10; FAB MS *m/z* (rel intensity) 371 (MH^+, 86). Anal. Calcd for $C_{21}H_{38}O_5$: C, 68.07; H, 10.34. Found: C, 67.82; H,

10.40. All prior intermediates leading to this compound were fully characterized.

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