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CONFORMATIONALLY CONSTRAINED ANALOGUES OF DIACYLGLYCEROL (DAG). 14.[1] DISSECTION OF THE ROLES OF THE *sn*-1 AND *sn*-2 CARBONYLS IN DAG MIMETICS BY ISOPHARMACOPHORE REPLACEMENT

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Abstract: The replacement of the *sn*-1 and *sn*-2 carbonyl esters in DAG-surrogate lactones by sulfonate esters showed that their isosteric properties in protein kinase C binding are controlled by the location of the hydrophobic alkyl chain on the molecule. The CO and SO_2 groups appear to be true isosteres only when they are adjacent to the alkyl chain, which is presumed to insert normal to the lipid bilayer. Published by Elsevier Science Ltd.

sn-1,2-Diacylglycerol (DAG) is the endogenous lipid activator of protein kinase C (PK-C).[2] PK-C is a multifunctional serine/threonine-specific protein kinase that requires translocation to the membrane for full activation.[3] As a result of a receptor-mediated process, the high concentration of DAG released locally at the membrane decreases chain ordering in the neighboring bilayer causing a phase transition that facilitates translocation of PK-C.[4] Additionally, DAG binds stereospecifically to one of two highly conserved cysteine-rich regions (C1a or C1b), which are part of the regulatory domain of PK-C.[3,5] The phorbol esters are also known to bind tightly to the same domains and can effectively compete with DAG.[6]

The bilayer-associated conformation of the glycerol backbone and acyl chains in membrane-bound DAG appears to be responsible for maintaining the orientational inequivalence of its two acyl chains.[7,8] Although the exact molecular interaction of DAG with PK-C remains to be defined, it is possible that the bilayer-associated conformation of DAG is able to determine the role of the *sn*-1 and *sn*-2 carbonyls in binding to PK-C. Based on the X-ray structure of the PK-Cδ C1b–phorbol-13-acetate complex,[9] only two of the three important pharmacophores in the phorbol esters (the C-20 hydroxyl and the C-3 carbonyl) are directly involved in binding to the C1b domain. The third important pharmacophore (the C-9 hydroxyl) is not involved at all, which suggests that this group could reside near the lipid bilayer interface after the translocation of PK-C to the membrane. Since there is a direct correspondence of pharmacophores between phorbol esters and DAG,[10–12] it is reasonable to speculate that one of the carbonyl groups of DAG would also stay uninvolved with the CRD2 domain and thus remain free to bind at the lipid bilayer interface.

The conformation of DAG in the membrane appears to be controlled by the lipid lattice;[7,13,14] therefore, the constrained DAG lactones **1** and **2** (Figure 1),[10] which contain a single hydrophobic alkyl chain, may help discern the role of the two carbonyl moieties in DAG. If one assumes that in compound **1** the long alkylacyl chain becomes normal to the bilayer, the glycerol backbone (C1-C3) attached to the lactone will also be normal to the bilayer, and the *sn*-1 carbonyl will be closer to the bilayer interface, akin to what has been shown for DAG in a lipid membrane[7] (Figure 1a). Alternatively, in α-alkylidenes **2a** and **2b** the role of the carbonyl

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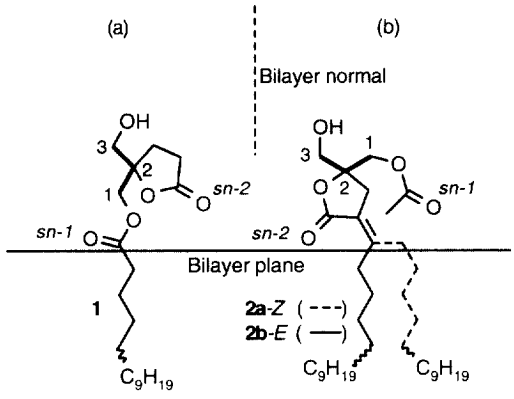


Figure 1. Schematic orientation of lactones **1** and **2** in a lipid bilayer.

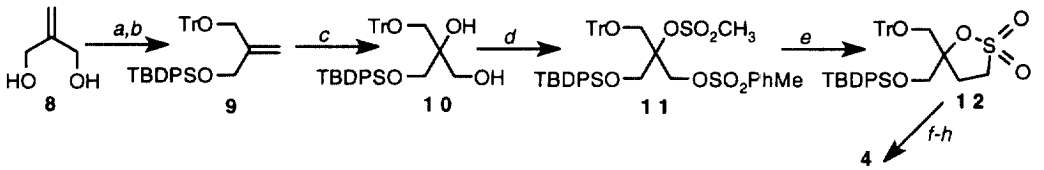
groups could be essentially reversed, since the long alkyl chain attached to the lactone would become normal to the bilayer (Figure 1b). In this case, the lactone carbonyl (equivalent to the *sn*-2 carbonyl of DAG) would be closer to the bilayer interface. Therefore, the location of the alkyl chain could determine which of carbonyl moieties in **1** and **2** will be accessible for the highly stereospecific binding to the C1 domain of PK-C. In the case of **1**, it would be the *sn*-3 hydroxyl and the *sn*-2 carbonyl (lactone carbonyl), whereas in the case of **2**, it would be the *sn*-3 hydroxyl and the *sn*-1 carbonyl ester. It is also known that the affinity of PK-C for the membrane increases with the mol % of the anionic lipid phosphatidylserine (PS), which produces nonspecific accumulation of cations (i.e., Ca^{+2}) at the membrane interface.[15] Since both PS and DAG binding sites are located in close proximity on the C1 region of the regulatory domain of the enzyme,[16] it is possible that the carbonyl function closest to the bilayer plane will interact with this hydrated milieu[17] through hydrogen bonding. Clearly, hydrogen bonding interaction in this heterogeneous medium is not expected to be as stereoselective as hydrogen bonding with the C1 domain of PK-C.

Despite the fact we have generally detected higher binding affinities with α-alkylidene branched lactones relative to their acyl-branched counterparts,[10–12] it is not known whether these differences truly reflect a divergence in the role of the carbonyl moieties. In order to better understand the role of the *sn*-1 and *sn*-2 carbonyls in situations where the alkyl chain might reverse their location in the lipid bilayer, we decided to systematically exchange each -O-C=O group (ester or lactone) in compounds **1** and **2** with a different isosteric group, such as the $-O-SO_2$ (sulfonate ester or sultone) (Table 1). The hypothesis was that this change would have a more significant effect, positive or negative, on the more stereochemically demanding C1 binding site than on the less stereochemically demanding binding site at the bilayer interface. The results presented below support this hypothesis.

Chemistry

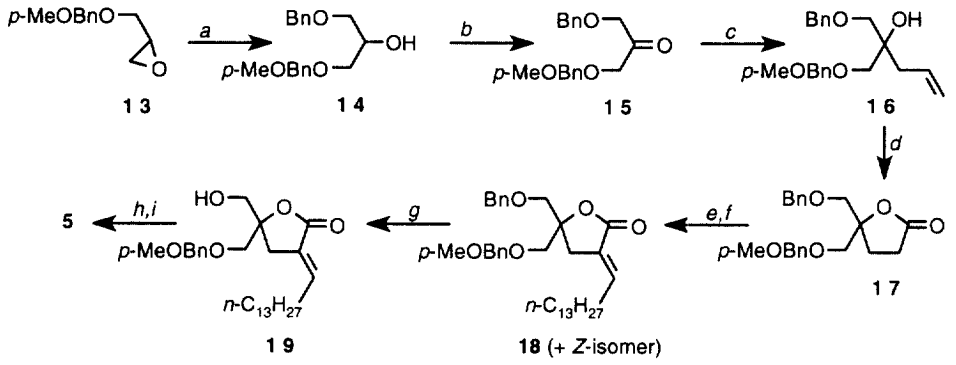
Since even as racemates most constrained DAG lactones display significant potency as PK-C ligands,[10–12] all the new compounds designed for this study were prepared in racemic form. Also, in the case of targets **2**, **5**, and **6**, only the more abundant *E*-isomers were isolated. The first target compound **3**[18] (Table 1) was easily synthesized from 5,5-bis(hydroxymethyl)oxolan-2-one[10] after treatment with one equivalent of chlorotetradecyl sulfone in pyridine/DMAP. The syntheses of the remaining three targets **4-6** (Table 1) were more elaborate as illustrated in Schemes 1–3. The critical assembly of sultones **4**[19] and **6**[20] relied on the selective tosylation and mesylation of the primary and secondary alcohol moieties of **10** and **21** to give intermediates **11** (Scheme 1) and **22** (Scheme 3), respectively. The ensuing intramolecular cyclization to the protected sultones **12** (Scheme 1) and **23** (Scheme 3) was achieved by a base-catalyzed intramolecular displace-

Scheme 1



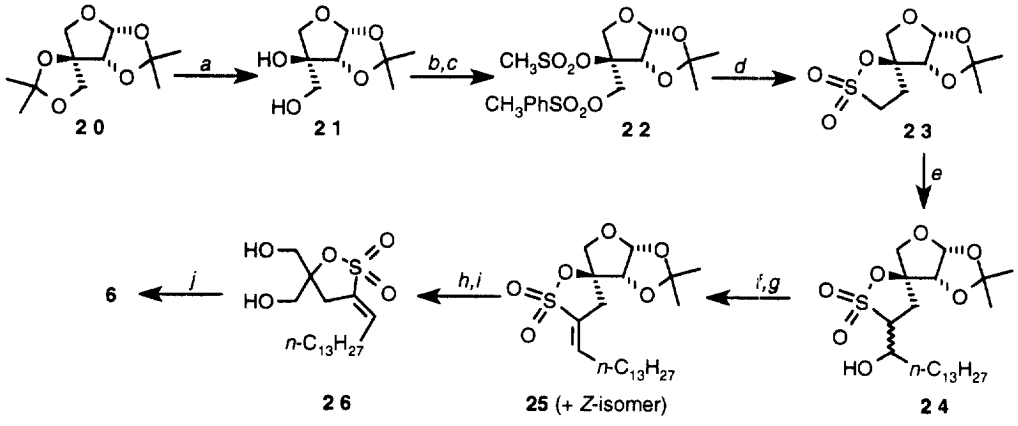
Scheme 1. (*a*) Ph_3CCl (TrCl), DMAP/Et_3N, DMF, rt (67%); (*b*) Me_3CPh_2SiCl (TBDPSCl), Imidazole, DMF, rt (91%); (*c*) OsO_4, NMMO, acetone/H_2O, rt (100%); (*d*) i. $MePhSO_2Cl$, pyridine, 0 °C (90%), ii. CH_3SO_2Cl, DMAP/pyridine, 50 °C (36%); (*e*) $HC{\equiv}CLi{\bullet}H_2NCH_2CH_2NH_2$, DMSO, rt (76%); (*f*) HCOOH, Et_2O, rt (71%); (*g*) n-$C_{13}H_{27}COCl$, DMAP/pyridine, rt (95%); (*h*) t-Bu_4NF, THF, rt (74%).

Scheme 2



Scheme 2. (*a*) BnOH, NaH, DMF, 0 °C → 80 °C (90%); (*b*) PCC, 4 Å mol. sieves, CH_2Cl_2, rt (92%); (*c*) $CH_2{=}CH_2CH_2MgCl$, THF, 0 °C → rt (88%); (*d*) i. $BH_3{\bullet}SMe_2$, THF, -78 °C → rt, ii. PCC, CH_2Cl_2, rt, 3 days (64%); (*e*) $[(CH_3)_3Si]_2NLi$, $CH_3(CH_2)_{12}CHO$, THF, -78 °C; (*f*) i. CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C → rt, ii. DBU, CH_2Cl_2, rt (93%); (*g*) BCl_3, CH_2Cl_2, -78 °C (74%); (*h*) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C (94%); (*i*) $(NH_4)_2Ce(NO_3)_6$, CH_3CN-H_2O, 0 °C, (72%).

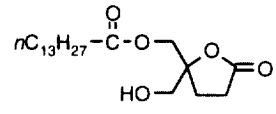
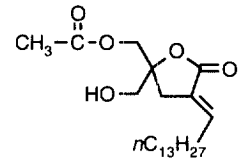
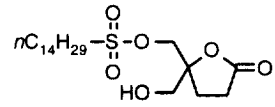
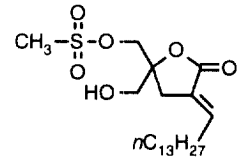
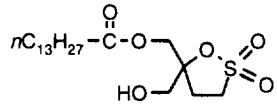
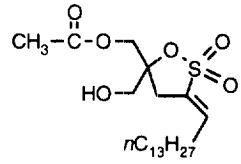
Scheme 3



Scheme 3. (*a*) AcOH-H_2O, rt (100%); (*b*) $MePhSO_2Cl$, pyridine, 0 °C → rt (85%); (*c*) CH_3SO_2Cl, DMAP/Et_3N, rt (98%); (*d*) $HC{\equiv}CLi{\bullet}H_2NCH_2CH_2NH_2$, DMSO, rt (65%); (*e*) n-BuLi, $CH_3(CH_2)_{12}CHO$, THF, -78 °C (83%); (*f*) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C → rt (98%); (*g*) DBU, benzene, 0 °C → rt (82%); (*h*) 10% H_2SO_4, dioxane, 90 °C (75%); (*i*) i. $NaIO_4$, MeOH-H_2O, rt, ii. $NaBH_4$, MeOH, 0 °C (69%); (*j*) Ac_2O, DMAP/pyridine, 0 °C (53%).

ment of the tosylate. Unfortunately, the synthesis of **6** could not be achieved directly from intermediate **12** in Scheme 1. Synthesis of **5** started from commercially available glycidyl 4-methoxyphenyl ether **13** (Scheme 2) to give the key 1,3-dihydroxyketone intermediate **15**. Assembly of the lactone from **15** and introduction of the α-alkylidene chain was performed essentially as reported previously[9–11] to give the major *E*-isomer **5**.[21]

Table 1. Selected target compounds. K_i values represent the mean ± standard error (triplicate)

Cpd #	Structure	K_i (nM)	Cpd #	Structure	K_i (nM)
1		138 ±24	**2a**		77.9 ±4.7
3		90.7 ±5.0	**5**		823 ±74
4		1,040 ±310	**6**		320 ±20

Results and Discussion

The affinity of these ligands for PK-C was assessed in terms of their ability to displace [20-^{3}H]phorbol 12,13-dibutyrate (PDBU) from a recombinant single isozyme, PK-Cα, as already described (Table 1).[10–12] With compounds **1** and **3** there appears to be complete isosteric bioequivalence between carboxylate and sulfonate esters. If we assume that the lipid lattice forces the orientation of the long alkyl chain to be normal to the bilayer as in Figure 1a, then the *sn*-1 CO in **1** and the SO_2 in **3** could effectively form hydrogen bonds with the hydrated milieu at the bilayer interface. In this conformation the glycerol backbone would be normal to the bilayer and the lactone carbonyl (*sn*-2 CO) would be poised to interact effectively with PK-C. As we have demonstrated earlier, this type of conformationally constrained lactones has a significant entropic advantage in binding relative to DAG.[10–12] If the CO and SO_2 groups in compound **3** are interchanged to give **4**, the lipid

lattice would maintain the *sn*-1 CO at the bilayer interface and leave the SO_2 of the sultone to interact with PK-C. The >10-fold loss in affinity relative to **3** would indicate that the SO_2 group in **4** is a disfavored bioisostere of the lactone carbonyl (*sn*-2 CO) when binding to the C1 domain of PK-C.

With the α-alkylidene lactones **5-6** it is possible that the mode of binding is reversed. In the parent **2a**, the α-alkylidene would be normal to the bilayer (Figure 1b) forcing the lactone *sn*-2 CO to lie closer to the bilayer interface. In this conformation, the glycerol backbone is parallel to the bilayer and the *sn*-1 CO of the short acyl chain becomes available for binding to PK-C. This appears to be a preferred arrangement since we have consistently detected higher affinities for the α-alkylidene branched lactones relative to their acyl-branched counterparts.[10–12] We have also found that the directionality of the α-alkylidene chain is important and the *Z*-isomers, like **2b** (not included in this study), are ca. 2-fold better ligands than the corresponding *E*-isomers. This probably reflects a small penalty in bending that would be required for the *E*-isomer (**2a**) to adjust its alkyl chain parallel to the lipid lattice (Figure 1b, dotted line). The very efficient mode of binding of **2a** deteriorated by >10-fold when the acetate function was replaced by the methyl sulfonate ester (compound **5**). This loss of binding reflects the inability of the SO_2 (in this case an isostere of the *sn*-1 CO) to bind effectively to the more stereospecifically demanding site on PK-C. If this is true, a reversal of the CO and SO_2 groups relative to **5** (compound **6**) should produce a better ligand, since the SO_2 of the sultone would be forced by the adjacent α-alkylidene chain to engage in hydrogen bonding at the less stereospecifically demanding lipid interface. Indeed, compound **6** was 2.5 times more potent than **5**. The reason why the swapping of isosteric functionalities between **1** and **3** proved to be better than that between **2** and **6** probably reflects a level of organization at the lipid interface that allows the freer SO_2 group in **3** to adjust better than the more encumbered SO_2 of the sultone.

The data presented here support the hypothesis that the binding environment of the *sn*-1 and *sn*-2 carbonyls in these DAG lactones is probably different and it is controlled by the preference of the lipid alkyl chain to lie normal to the bilayer. Furthermore, the replacement of the *sn*-1 and *sn*-2 carbonyl esters with sulfonate esters showed that their isosteric properties are structurally dependent. The CO and SO_2 groups appear to be true isosteres when forced to reside near the lipid interface by the adjacent hydrophobic alkyl chain. The same groups do not show equivalent isosteric properties when they reside away from the lipid bilayer, at a site where they are expected to bind to the C1 domain of PK-C.

References and Notes

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18. *rac*-5-(Hydroxymethyl)-5-[(tetradecylsulfonyloxy)methyl]oxolan-2-one (**3**): oil; ^{1}H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, CH_3), 1.20–1.50 (m, 22 H, $CH_3(C\underline{H}_2)_{11}CH_2CH_2SO_3$), 1.65 (br s, 1 H, OH), 1.82 (m, 2 H, $CH_3(CH_2)_{11}C\underline{H}_2CH_2SO_3$), 2.20 (m, 2 H, H-4), 2.68 (m, 2 H, H-3), 3.15 (m, 2 H, $CH_3(CH_2)_{11}CH_2C\underline{H}_2SO_3$), 3.72 (s, 2 H, $C\underline{H}_2OH$), 4.30 (AB d, J = 12.5 Hz, 1 H, $C\underline{H}_2OSO_2$); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.67, 23.36, 25.27, 28.10, 28.46, 28.93, 29.22, 29.32, 29.46, 29.56, 29.62, 29.64, 31.89, 50.71, 64.72, 69.76, 85.43, 175.96; FABMS (*m/z*, relative intensity) 407 (MH^+, 54), 129 (MH^+-CH_3SO_3H, 100). Anal calcd for $C_{20}H_{38}O_6S$: C, 59.08, H, 9.42. Found: C, 58.85; H , 9.34.
19. *rac*-[1-(Hydroxymethyl)-3,3-dioxo-2,3-oxathiolanyl]methyltetradecanoate (**4**): oil, ^{1}H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, CH_3), 1.25 (m, 20 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CO$), 1.60 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CO$), 2.35 (t, J = 7.6 Hz, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CO$), 2.45 (t, J = 7.9 Hz, 1 H, OH), 2.65 (m, 2 H, H-5), 3.35 (m, 2 H, H-4), 3.72 (AB q, J = 12.5 Hz, 2 H, $C\underline{H}_2OH$), 4.28 (AB q, J = 12.3 Hz, 2 H, $C\underline{H}_2OCO$); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.66, 24.75, 27.19, 29.06, 29.19, 29.32, 29.40, 29.56, 29.61, 29.64, 31.89, 33.95, 45.64, 64.49, 64.74, 88.25, 173.44; FABMS (*m/z*, relative intensity) 393 (MH^+, 65), 211 ($C_{13}H_{27}CO^+$, 100). Anal calcd for $C_{19}H_{36}O_6S$: C, 58.13; H, 9.25; S, 8.15. Found: C, 58.08; H, 9.30; N, 8.22.
20. *rac*-(*E*)-5-Hydroxymethyl-5-[(methylsulfonyloxy)methyl]-3-tetradecylideneoxolan-2-one (**5**): oil; ^{1}H NMR ($CDCl_3$) δ 0.88 (distorted t, 3 H, CH_3), 1.20–1.60 (m, 22 H, $CH_3(C\underline{H}_2)_{11}CH_2CH$=), 2.11 (t, 1 H, OH), 2.14-2.23 (m, 2 H, $CH_3(CH_2)_{11}C\underline{H}_2CH$=), 2.78 (m, 2 H, H-4), 3.07 (s, 3 H, CH_3SO_3), 3.74 (AB d, J = 6.0 Hz, 2 H, CH_2OH), 4.35 (AB q, J = 11.0 Hz, 2 H, $C\underline{H}_2OSO_2CH_3$), 6.81 (m, 1 H, >C=CH); ^{13}C NMR ($CDCl_3$) δ 14.09, 22.66, 27.99, 29.33, 29.36, 29.49, 29.53, 29.60, 29.62, 29.65, 30.40, 31.90, 37.62, 64.63, 69.66, 82.28, 124.75, 143.40, 169.43; EIMS (*m/z*) 418 (M^+). Anal calcd for $C_{21}H_{38}O_6S$: C, 60.26; H, 9.15. Found: C, 60.17; H, 9.13.
21. *rac*-(*E*)-[1-(Hydroxymethyl)-3,3-dioxo-4-tetradecylidene-2,3-oxathiolanyl]methyl acetate (**6**): oil; ^{1}H NMR ($CDCl_3$) δ 0.85 (distorted t, 3 H, CH_3), 1.25 (m, 20 H, $CH_3(C\underline{H}_2)_{10}CH_2CH_2CH$=), 1.50 (m, 2 H, $CH_3(CH_2)_{10}C\underline{H}_2CH_2CH$=), 2.10 (s, 3 H, CH_3CO), 2.15 (m, 2 H, $CH_3(CH_2)_{10}CH_2C\underline{H}_2CH$=), 3.00 (AB q, J = 15 Hz, 2 H, H-5), 3.70 (AB q, J = 12.5 Hz, 2 H, $C\underline{H}_2OH$), 4.30 (AB q, J = 12.3 Hz, 2 H, $C\underline{H}_2OCO$), 6.50 (m, 1 H, >C=CH); ^{13}C NMR ($CDCl_3$) δ 14.09, 20.60, 22.66, 27.99, 28.77, 29.19, 29.29, 29.32, 29.44, 29.58, 29.61, 31.89, 63.97, 64.37, 86.26, 132.73, 136.37, 170.71; FABMS (*m/z*, relative intensity) 419 (MH^+, 20). Anal calcd for $C_{21}H_{38}O_6S$: C, 60.25; H, 9.16. Found: C, 60.30; H, 9.21.