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Diacylglycerols with Lipophilically Equivalent Branched Acyl Chains Display High Affinity for Protein Kinase C (PK-C). A Direct Measure of the Effect of Constraining the Glycerol Backbone in DAG Lactones

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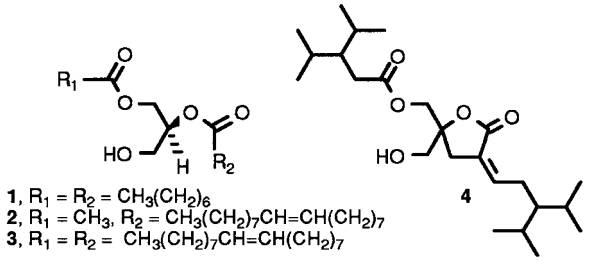
Abstract—New synthetic diacylglycerols (DAGs) with equivalent branched acyl chains were compared with commercially available DAGs as PK-C ligands. The results support the view that there is a minimal lipophilic requirement provided by the equivalent acyl groups that results in high binding affinity. Locking the glycerol backbone of the most potent DAG into a five-member lactone resulted in a 10-fold increase in potency. Published by Elsevier Science Ltd.

Classical (α, β, and γ) as well as novel (δ, ϵ, η, and θ) PK-C isozymes become activated as a result of the association of the inactive cytosolic enzyme with membranes containing acid phospholipids.[1,2] This association is strongly facilitated by the lipophilic second messenger *sn*-1,2-diacylglycerol (DAG) which is generated as a result of stimulus-generated activation of phospholipase C.[3–5] Pharmacologically, the high affinity phorbol esters can bypass this process and directly activate PK-C.[6] DAG and the phorbol esters bind to the C1 domains of the classic and novel members of the protein kinase C (PK-C) family,[3–5] as well as to C1 domain(s) in four other families of proteins involved in signal transduction, namely PK-D,[7] the chimaerins,[8] RasGRP,[9] and Unc-13.[10]

Among the most commonly used DAG analogues in PK-C studies are the commercially available 1,2-dioctanoyl-*sn*-glycerol (**1**, diC8), 1-oleoyl-2-acetyl-*sn*-glycerol (**2**, OAG) and 1,2-dioleoyl-*sn*-glycerol (**3**, diolein). These compounds, however, have reduced PK-C binding affinity and lower metabolic stability when compared to the phorbol esters.



1, $R_1 = R_2 = CH_3(CH_2)_6$
2, $R_1 = CH_3$, $R_2 = CH_3(CH_2)_7CH{=}CH(CH_2)_7$
3, $R_1 = R_2 = CH_3(CH_2)_7CH{=}CH(CH_2)_7$

We have recently developed highly potent PK-C ligands based on the DAG structure by constraining the glycerol backbone into a five-member lactone ring (DAG-lactones) and by incorporating branched alkyl chains designed to optimize hydrophobic interactions with a group of highly conserved hydrophobic amino acids along the rim of the C1 domain.[11,12] Since the effect of branched chains in increasing the binding affinity of DAG-lactones was significant, as exemplified by compound **4** ($K_i = 2.9$ nM),[12] we wished to investigate if analogous structural changes on the endogenous DAG ligand would have a similar beneficial outcome, beyond the passive role of facilitating partitioning or transport between biological phases. To quickly scan a possible domain of branched structures with different levels of lipophilicity, the 2,2-dimethylpropanoyl- (pivaloyl),

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4-methyl-3-(methylethyl)pentanoyl- and the 1-adamantanecarbonyl-moieties were selected as examples of small (a), medium (b) and large size acyl groups (c). The target structures (**9a–c**) are shown in Scheme 1.

Results and Discussion

The target compounds (**9a–c**) were prepared in four simple steps from commercially available (*S*)-di-*O*-isopropylideneglycerol (**5**, Scheme 1). Protection of the primary alcohol as a benzyl ether and removal of the acetonide group was followed by acylation with the corresponding acid chloride. The diacylation reaction was complete only for the pivaloyl analogue **8a**. In the other two cases (**8b**,**c**), the diacylated product was accompanied by a small amount of the monoacyl analogue at the *sn*-1 position. After chromatographic separation, catalytic hydrogenation of **8a–c** afforded the target compounds (**9a–c**) which were fully characterized.[13–15]

The measured binding affinities (K_is) of compounds **9a–c** for PK-Cα were compared with those of commercially available DAGs (**1–3**), and the values appear listed in Table 1 in order of decreasing potency. Binding affinity was assessed in terms of the ability of the ligand to displace [20-^{3}H]phorbol 12,13-dibutyrate (PDBU) as already described.[16–18] The octanol/water partition coefficients (log *P*) were calculated according to the fragment-based program KOWWIN 1.63.[19] Despite the additional number of aliphatic carbons in compounds **9c** (20 carbons) and **9b** (16 carbons) relative to **1** (14 carbons), the calculated partition coefficients reflect the effect of branching in lowering the log *P* value.[20] Since these compounds (**9b**, **1** and **9c**) have comparable potencies (K_is) and log *P* values, it is likely that having

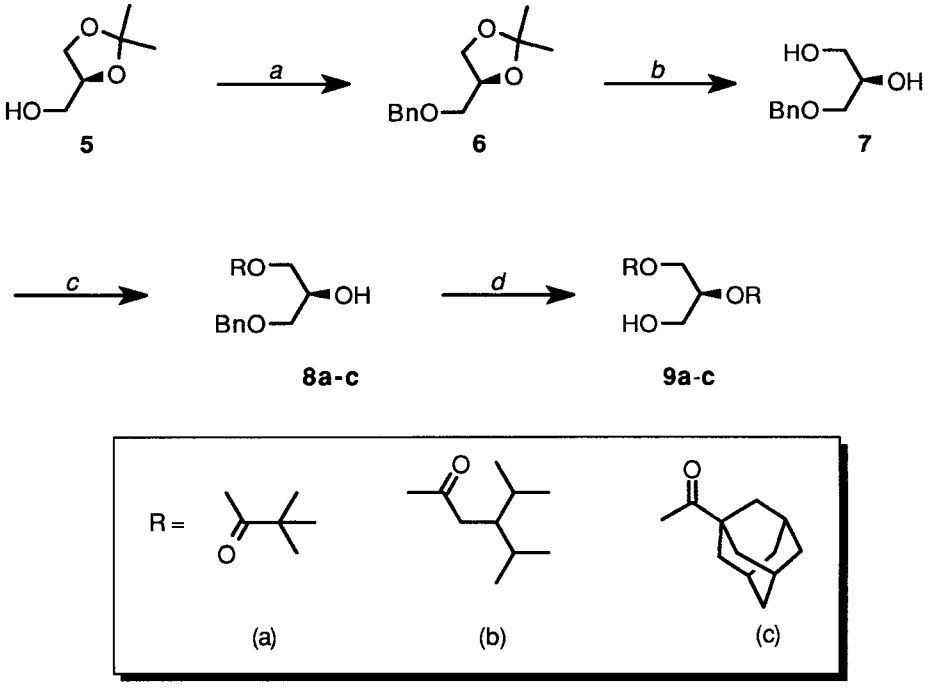
Table 1. PK-Cα affinities and Calculated log *P* values

Compound	$K_i \pm$ SEM (nM)[a]	Log *P*[b]
4	2.9 ± 0.2	5.9
9b	28.6 ± 3.2	5.8
1 (diC8)	33.0 ± 1.5	5.3
9c	38.7 ± 6.3	5.5
2 (OAG)	50.4 ± 4.2	7.0
3 (diolein)	87.2 ± 1.6	14.6
9a	3430 ± 580	2.1

[a]Values represent the mean ± standard error (triplicate).
[b]Octanol/water partition coefficients (log *P*).

two equivalent lipophilic chains (lipophilic balance) results in high binding affinities. Due to their random motion, the short and flexible chains of diC8 (**1**) are probably able to occupy the same conformational space as the branched chains in **9b** and **9c**, and thus no exceptional advantage in binding is derived from branching. However, the lipophilically unbalanced OAG and the very lipophilic diolein displayed lower binding affinities despite their high log *P* values. These results suggest that for efficient PK-C binding it would be desirable to diminish lipophilicity to a point that it does not compromise binding, since excess lipophilicity would result in higher K_i's (lower affinity) through nonspecific interactions. Hence, the results in Table 1 suggest that a log *P* value between 5 and 6 is close to ideal for DAGs. On the other hand, reduced lipophilicity beyond a certain threshold is detrimental as shown for compound **9a** whose log *P* of 2.1 may be too low for efficient membrane partitioning and binding.

Although we have provided numerous examples whereby locking the conformation of DAG into a lactone template results in an increase binding affinity



Scheme 1. Reagents. (a) BnBr/NaH/n-Bu_4NI/DMF, rt, 18 h. (b) 0.5 N HCl/THF, reflux, 2 h. (c) Acyl chloride/pyridine/CH_2Cl_2, 18 h. (d) H_2/Pd, EtOH, rt, 2 h.

for PK-C,[16–18] the results in Table 1 show, for the first time, a direct measure of the effect that constraining the glycerol backbone has on binding affinity. The 10-fold difference in binding affinity between compounds **4** and **9b**, which have almost identical log *P* values, is the direct result of the entropic advantage of constraining the glycerol backbone. This difference could even be larger if one considers that compound **4** is racemic and **9b** is chiral.

In conclusion, efficient DAG and DAG-lactone ligands can be constructed provided that they have equivalent, short acyl chains (branched or unbranched) with adequate lipophilicity. The optimal acyl chain size appears to be 7 or 8. An additional advantage of having branched acyl chains may be derived from an increase in stability toward hydrolysis by esterases, a factor that is of considerable importance for displaying activity in whole cells. This was recently shown in the antitumor screening of comparable DAG-lactones bearing branched versus linear acyl chains.[12]

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13. Compound **9a**: oil; IR (neat) 3508 (OH), 2972 (CH), 1734 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 5.15 (m, 1 H, CHOCO), 4.40 (dd, $J=11.9$, 3.9 Hz, 1 H, CHHOCO), 4.29 (dd, $J=11.9$, 5.6 Hz, 1 H, CHHOCO), 3.79 (d, $J=5.3$ Hz, 2 H, CH_2OH), 2.30 (br s, 1 H, OH), 1.28–1.27 (2 s, 18 H, $2\times C(CH_3)_3$); FAB MS (*m/z*, relative intensity) 261 (MH^+, 21). Anal. calcd for $C_{13}H_{24}O_5$: C, 59.98; H, 9.29. Found: C, 59.98; H, 9.38.
14. Compound **9b**: oil; IR (neat) 3472 (OH), 2960 (CH), 1739 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 5.15 (m, 1 H, CHOCO), 4.39 (dd, $J=11.9$, 4.2 Hz, 1 H, CHHOCO), 4.30 (dd, $J=11.9$, 5.6 Hz, 1 H, CHHOCO), 3.81 (d, $J=5.1$ Hz, 2 H, CH_2OH), 2.28 (m, 4 H, $2\times CH_2CH(i\text{-}Pr)_2$), 1.81 (m, 4 H, $4\times CH(CH_3)_2$), 1.70 (m, 2 H, $2\times CH(i\text{-}Pro)_2$), 0.99–0.97 (2 d, $J=1.9$ and 2.2 Hz, 12 H, $2\times CH(CH_3)_2$), 0.90–0.88 (2 d, $J=1.7$ Hz, 12 H, $2\times CH(CH_3)_2$); FAB MS (*m/z*, relative intensity) 373 (MH^+, 15). Anal. calcd for $C_{21}H_{40}O_5$: C, 67.70; H, 10.82. Found: C, 67.73; H, 10.75.
15. Compound **9c**: oil; IR (neat) 3485 (OH), 2912 (CH), 1721 (CO) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 5.15 (m, 1 H, CHOCO), 4.37 (dd, $J=11.9$, 4.3 Hz, 1 H, CHHOCO), 4.24 (dd, $J=11.9$, 5.9 Hz, 1 H, CHHOCO), 3.76 (d, $J=5.1$ Hz, 2 H, CH_2OH), 2.47 (br s, 1 H, OH), 2.07 (br s, 6 H, CH-adamantyl), 1.95 (br s, 12 H, CH_2-adamantyl), 1.75 (m, 12 H, CH_2-adamantyl); FAB MS (*m/z*, relative intensity) 417 (MH^+, 8). Anal. calcd for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.68; H, 8.84.
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