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Conformationally Constrained Analogues of Diacylglycerol. 24. Asymmetric Synthesis of a Chiral (*R*)-DAG-Lactone Template as a Versatile Precursor for Highly Functionalized DAG-Lactones

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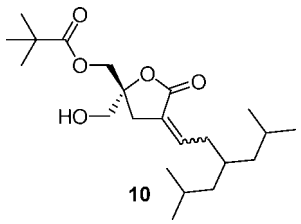
ABSTRACT



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Commercially available 2-methylenepropane-1,3-diol was converted to chiral epoxide (*R*)-2 via Sharpless asymmetric epoxidation in >96% ee. Regiospecific epoxide ring opening and reduction of the intermediate alkyne set the stage for a one-pot lactonization to give (*R*)-6, a convenient precursor for all functionalized chiral DAG-lactones used as potent PK-C ligands. The synthesis of the most potent DAG-lactones known to date, (*Z*)-10 and (*E*)-10, served to confirm PK-C's exclusive preference for the (*R*)-stereochemistry in this class of compounds.

The central role of protein kinase C in cell signal transduction has been well established since its discovery more than 2 decades ago.[1] The classic (α, β1, β2, and γ) and novel (δ, ϵ, η, and θ) PK-C isozymes contain in their regulatory domain two copies of a cysteine-rich motif (C1 domains) about 50 amino acids long, which are the receptors for the phorbol ester tumor promoters and the second messenger diacylglyerol (DAG). Over the past several years, we have synthesized a number of potent PK-C ligands based on a constrained glycerol scaffold (DAG-lactone) that bind to these C1 domains with high affinity.[2,3] During our investigations, we have demonstrated the importance of the alkyl chains in controlling binding affinity as a function of size,

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position on the glycerol scaffold, and degree of branching.[4] The evolution of this process can be visualized in Figure 1,

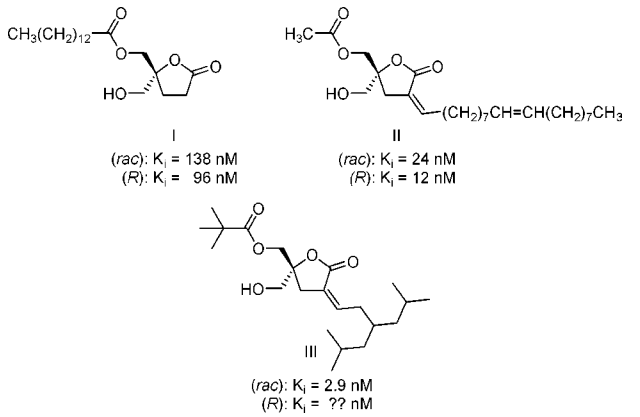


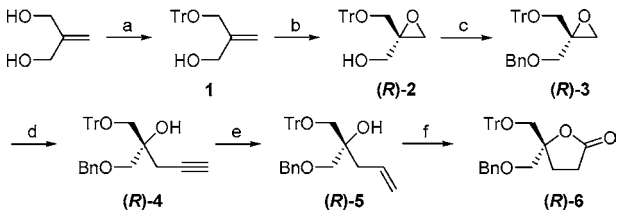
Figure 1. Structures and PK-Cα binding affinity of racemic and (*R*)-enantiomeric DAG-lactones with templates of increasing complexity (I, II, and III).

where it can also be seen that for scaffolds I and II the active enantiomer has the (*R*)-stereochemistry. In the few cases where both (*R*)- and (*S*)-enantiomers were synthesized, binding affinity differences greater than two orders of magnitude were observed with activity residing exclusively with the (*R*)-enantiomer.[5] An important improvement in potency occurred when transferring the bulk of the alkyl group from the acyl position in I to the α-alkylidene position in II, which reduced the acyl group to the simplest acetyl moiety and resulted in a 5- to 8-fold increase in binding affinity.[6]

Because our most recent potent compounds belong to scaffold type III, where the alkyl chains are distributed between both acyl and α-alkylidene positions, we wanted to confirm that, in agreement with scaffolds I and II, the required stereochemistry for III was also (*R*). This confirmation was considered important because normally for the sake of convenience the search for novel compounds is initially conducted with racemic DAG-lactones, with the synthesis of the pure enantiomer postponed until after the initial screen. In the present manuscript, we wish to present a general approach to a simple, chiral DAG-lactone [(*R*)-**6**] that serves as chiral precursor for all three scaffolds (I−III). This new process appears to be vastly superior in efficiency and simplicity when compared to previous methods of syntheses of (*R*)-DAG-lactones (scaffolds I and II) from chiral carbohydrate precursors, such as 1,2:3,5-di-*O*-isopropylidene-α-D-threo-apiofuranose[5] and D-arabinose.[6] Furthermore, because compounds having a type III scaffold have not yet been synthesized in pure enantiomeric form, we chose to demonstrate the utility of our approach by synthesizing type III molecules, such as compounds (*E*)-**10** and (*Z*)-**10**, as (*R*)-enantiomers. As anticipated, the binding affinities of both geometric isomers confirmed the preference of PK-C for the (*R*)-enantiomers.

The synthesis began with protection of commercially available 2-methylenepropane-1,3-diol as its monotrityl ether **1** (Scheme 1). Use of Sharpless mnemonic rules led to the

Scheme 1[a]



[a] Reagents and conditions: (a) Et_3N, TrCl, CH_2Cl_2 (52%); (b) (+)-DET (2% mol), Ti(O*i*-Pr)$_4$ (10% mol), *t*-BuOOH, CH_2Cl_2, −20 °C (80%); (c) NaH, BnBr, DMF (86%); (d) LiC≡CH·EDA, DMSO (79%); (e) Lindlar cat. (50% w), H_2, quinoline (50% w), hexane (97%); (f) BH_3·SMe_2, THF, −78 °C; then PCC, CH_2Cl_2 (48% for 2 steps).

selection of L-(+)-diethyl tartrate as the optically active reagent for the chiral epoxidation of alkene **1** to produce the desired DAG-lactone (*R*)-**6**. Epoxidation of **1** with *t*-BuOOH in the presence of catalytic amounts of titanium tetraisopropoxide and L-(+)-diethyl tartrate gave an 80% yield of the desired, chiral epoxide (*R*)-**2** with >96% ee as confirmed by its Mosher ester. Protection of the remaining free alcohol as a benzyl ether provided compound (*R*)-**3** and set the stage for the ensuing nucleophilic opening of the epoxide moiety with lithium acetylide (ethylenediamine complex) to give the key intermediate (*R*)-**4**. In the presence of Lindlar catalyst (Pd-$CaCO_3$-PbO), the alkyne group in (*R*)-**4** was successfully reduced to the alkene to give the tertiary homoallylic alcohol (*R*)-**5**. As was the case for the synthesis of racemic lactones, formation of lactone (*R*)-**6** was achieved in "one pot" after hydroboration of the olefin and immediate oxidation with pyridinium chlorochromate.[7]

From lactone (*R*)-**6**, the synthesis of chiral (*R*)-DAG-lactones (*E*)-**10** and (*Z*)-**10** was completed using a well-established methodology developed in our laboratory[2] involving aldol condensation with 5-methyl-3-(2-methylpropyl)-hexan-1-one followed by olefination (Scheme 2). Separation of geometric isomers (*E*)-**7** and (*Z*)-**7** was achieved at this stage by column chromatography, and completion of the synthesis was performed individually for each isomer. Removal of the trityl ether gave the free alcohols (*E*)-**8** and

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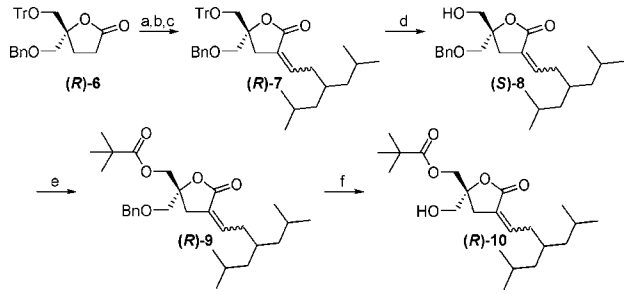
Scheme 2[a]



[a] Reagents and conditions: (a) LHMDS, $(i\text{-Bu})_2CHCH_2CHO$, THF, −78 °C; (b) Et_3N, MsCl, CH_2Cl_2; (c) DBU, CH_2Cl_2 (*Z* 43%, *E* 40%); (d) formic acid, CH_2Cl_2 (*Z* 60%, *E* 92%); (e) Et_3N, pivaloyl chloride, DMAP, CH_2Cl_2 (*Z* 100%, *E* 100%); (f) BCl_3, CH_2Cl_2, −78 °C (*Z* 79%, *E* 82%).

(*Z*)-**8** with the (*S*)-stereochemistry. Acylation of the free alcohol with pivalolyl chloride gave compounds (*E*)-**9** and (*Z*)-**9**, which after final removal of the benzyl group afforded the target compounds (*E*)-**10** and (*Z*)-**10** with the (*R*)-stereochemistry. Consistent with previously synthesized DAG-lactones, the vinyl proton of the (*Z*)-isomer displayed a characteristic signal at $\delta = 6.0$ in its ^{1}H NMR spectrum, while the corresponding signal of the (*E*)-isomer appeared more downfield at $\delta = 6.7$.[2]

Although the pivaloyl group is sufficiently hindered to prevent spontaneous racemization by acyl migration, as we have previously observed with linear chains,[5] we wanted to confirm this by authenticating the structural integrity of the targets. First, we used the chiral NMR shift reagent europium tris[3-(trifluoromethylhydroxymethylene)-(+)-camphorate] to determine enantiomeric purity.[8] In the presence of the Eu chiral reagent, both racemic samples of (*E*)-**10**[9] and (*Z*)-**10**[9] showed the largest pseudocontact-shift difference for one of the enantiotopic protons (underscored) on the C<u>H</u>HOCOC-

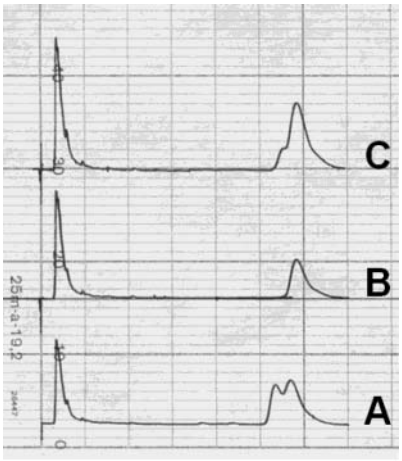


Figure 2. (A) Racemic (*E*)-**10** (100 μL, 1.8 mg/mL). (B) Enantiopure (*R*)-(*E*)-**10** (50 μL, 1.7 mg/mL). (C) Racemic (*E*)-**10** (50 μL) and enantiopure (*R*)-(*E*)-**10** (50 μL).

$(CH_3)_3$ side chain. This proton's signal, which in *rac*-(*Z*)-**10** resonates at $\delta = 4.08$ (d, $J_{gem} = 11.8$ Hz) shifted to $\delta = 4.20$ and was split into two doublets with identical geminal coupling constants. A similar shift to lower field occurred for *rac*-(*E*)-**10**, which showed the same splitting pattern. When the same experiment was performed with enantiopure (*E*)-**10** and (*Z*)-**10**, the Eu chiral reagent induced the expected downfield shift, and a second set of very minor signals was observed. Peak areas measured for the newly resolved signals for both (*E*)-**10** and (*Z*)-**10** enantiomers corresponded to optical purities >90%. Enantiomeric purity was also assessed by chiral HPLC analysis on a ChiraCel OD column (Figure 2). Whereas the racemate could be partially resolved into two distinct peaks, both (*E*)-**10** and (*Z*)-**10** enantiomers eluted essentially as single peaks. Although the poor resolution precluded accurate integration of peak areas, the lack of any "shoulders" present on the peaks by visual inspection allowed us to estimate an enantiomeric purity of >90%.

The PK-C binding affinities for (*E*)-**10** and (*Z*)-**10** are expressed in terms of the parameter K_i, which measures the ability of the ligand to displace PK-Cα-bound-[20-^{3}H]-phorbol-12,13-dibutyrate (PDBU) in the presence of phosphatidylserine. The inhibition curves obtained were of the type expected for competitive inhibition, and the K_i values were calculated from the ID_{50} values.[10] The K_i values for (*E*)-**10** and (*Z*)-**10** were 2.4 ± 0.34 and 1.45 ± 0.2 nM, respectively. These values are almost exactly half of the K_i values for the racemates (Figure 3),[9,11] thus confirming that

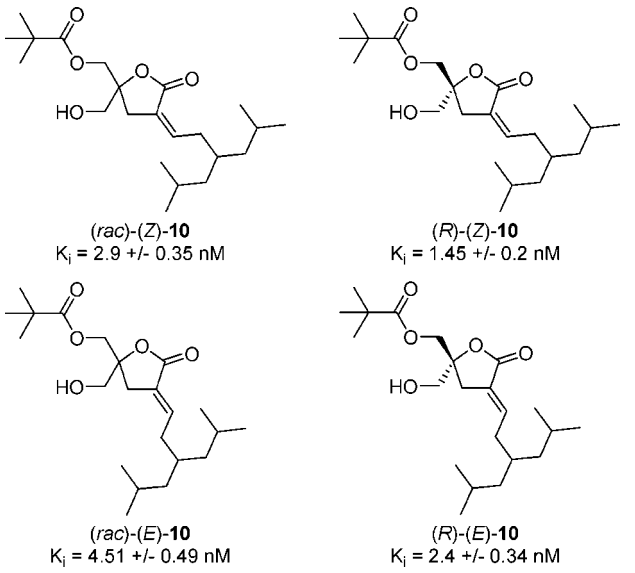


Figure 3. Comparison of PK-Cα binding affinities between racemic and chiral DAG-lactones with (*Z*)- and (*E*)-geometries.

the (*R*)-enantiomers uniformly represent the "active" template regardless of the pattern of substitution or balance of alkyl groups at either the acyl or α-alkylidene positions on the DAG-lactones. These results also confirm the typically observed trend of a ca. 2-fold difference in favor of the (*Z*)-isomers.

In conclusion, we have developed a general approach to a simple, chiral DAG-lactone [(*R*)-**6**] that serves as a convenient precursor for all three types of functionalized, chiral DAG-lactones used as potent PK-C ligands. In addition, the synthesis of (*Z*)-**10** and (*E*)-**10** confirm the exclusive preference for the (*R*)-stereochemistry in this class of compounds by the PK-C enzyme.

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Supporting Information Available: General experimental procedures and complete characterization data for all new compounds, plus ^{1}H NMR spectra of racemic and optically pure samples of (*E*)-**10** and (*Z*)-**10** with the chiral shift reagent. This material is available free of charge via the Internet at http://pubs.acs.org.

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