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Enzymatic desymmetrization of 5-bis(hydroxymethyl)tetrahydro-2-furanone: a template for protein kinase C ligands

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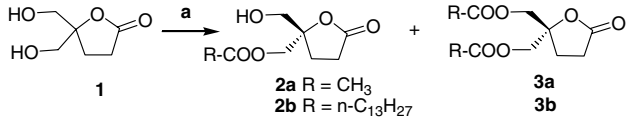
Abstract—The stereoselective acylation of *meso*-5-bis(hydroxymethyl)tetrahydro-2-funanone **1** by vinyl acetate or vinyl myristoate in the presence of *Pseudomonas cepacia* lipase in organic media gave the corresponding (*S*)-monoesters in high enantiomeric excess. The hydrolysis of the corresponding diacetate in the presence of the same enzyme provided the (*R*)-monoacetate.

1. Introduction

sn-1,2-Diacylglycerols (DAG) are intracellular second messengers and their main biological target is a family of protein kinase C (PK-C) isoenzymes.[1] These enzymes catalyze the O-phosphorylation of serine/threonine of proteins involved in signaling pathways (signal transduction)[2] that regulate cell growth, differentiation, apoptosis, and tumor promotion. PK-C enzymes contain a C-terminal catalytic domain and a N-terminal regulatory domain. The catalytic domain has binding sites for ATP and protein substrates. Bryostatins[3] (antineoplastic marine natural products), phorbol esters[4] (tumor promoters from plants), and DAG all compete for the same regulatory binding sites of PK-C as shown by displacement of binding assays. Protein phosphorylation by kinases regulates most aspects of cell function and selective protein kinase ligands are potential therapeutic agents for the treatment of a variety of diseases including cancer and diabetes.[5] The regulatory sites of PK-C are selective for the (*S*)-enantiomers of DAG ligands. Conformationally constrained analogues of DAG are more potent ligands than parent compounds.[6] Herein we report the enzymatic desymmetrization of achiral DAG-lactone **1**.

2. Results and discussion

DAG-lactone **1** was prepared according to the procedure reported by Marquez et al.[7] Next, we did some screening to find hydrolases, which were able to distinguish the enantiotopic hydroxymethyl groups of lactone **1**.[8] Of the enzymes and conditions studied, the esterification of lactone **1** with vinyl acetate in the presence of *Pseudomonas cepacia* lipase[9] (PCL) in acetonitrile (Scheme 1) gave chiral monoester (*S*)-**2a** (71% yield, ee = 96%) and the corresponding achiral diester **3a** (14%). Replacement of vinyl acetate by vinyl myristoate provided the enantiomerically pure monoester (*S*)-**2b** (62% yield, ee ⩾99%) and achiral diester **3b** (38%).



Scheme 1. Reagents and conditions: (a) vinyl acetate or vinyl myristoate, *P. cepacia* lipase, acetonitrile.

The enantiomeric composition of **2a** and **b** was determined by a reaction with (+)-α-methoxy-α-trifluoromethyl-α-phenylacetic acid in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl-carbodiimide hydrochloride (EDC) and dimethylaminopyridine (DMAP), followed by ^{19}F (376 MHz) and ^{1}H (400 MHz) NMR analysis of the resulting diastereomeric esters. For instance, the ^{19}F chemical shift difference for the α-CF_3

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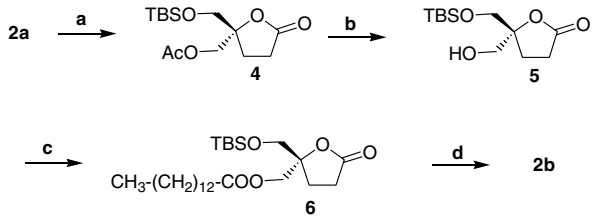
group of these diastereomeric derivatives in deuterated benzene is 0.15 ppm for both **2a** and **2b**.

Comparison of the specific rotation values $\{[\alpha]_D^{22} = -2.6$ (c 2.6, $CHCl_3$)$\}$ for sample **2b** obtained from the enzymatic desymmetrization with those reported in the lit.[10] $\{[\alpha]_D^{22} = +1.4$ (c 4.2, $CHCl_3$) for the (R)-enantiomer$\}$ confirmed the absolute configuration of (−)-**2b** to be *S*. The absolute configuration of monoester (*S*)-**2a** $\{[\alpha]_D^{22} = -27.7$ (c 0.96, C_6H_6)$\}$ was determined by correlation with compound (*S*)-**2b** of known absolute configuration (Scheme 2). Thus compound **2a** was protected as the TBS (*tert*-butyldimethylsilyl) ether and then the acetyl ester group of **4** hydrolyzed in the presence of *P. cepacia* lipase to give **5**. Acylation of **5** with myristoyl chloride followed by desilylation of **6** provided (*S*)-**2b** $\{[\alpha]_D^{22} = -2.2$ (c 1.0, $CHCl_3$); lit.[10] $[\alpha]_D^{22} = +1.4$ (c 4.2, $CHCl_3$) for the (R)-enantiomer$\}$.



Scheme 2. Reagents and conditions: (a) TBSCl, imidazole, DMAP, DMF, 75%; (b) *P. cepacia* lipase, H_2O ($CaCl_2$), Pyridine, Triton X, quantitative; (c) myristoyl chloride, pyridine, quantitative; (d) TBAF, CH_3COOH, THF, 88%.

As enzymes usually show the same enantioselectivity for acylation and deacylation reactions, the hydrolysis of **3a** and **b** appeared attractive since it would produce the monoesters of opposite configuration. While no conversion was observed with dimyristoyl ester **3b**, diacetate **3a** was hydrolyzed by PCL in aqueous/pyridine media to give (*R*)-(+)-**2a** $\{[\alpha]_D^{22} = +25.2$ (c 0.96, C_6H_6)$\}$ in high yield (85%) and good enantiomeric excess (88%). Empirical rules can predict some of the enantiopreferences of PCL but primary alcohols bearing an oxygen at the stereocenter (e.g., glycerol derivatives) do not fit these rules.[11]

Chiral non-racemic monoesters derived from *meso*-1,3-diols are susceptible to racemization via acid-catalyzed acyl migration.[11] This racemization is faster in aqueous solutions than in organic media. We did not observe any racemization during the enzyme-catalyzed esterification or purification of monoesters. However, this group migration may explain the slightly lower enantiomeric purity of monoester **2a** obtained by enzymatic hydrolysis in aqueous media.

Marquez et al. reported a multistep synthesis of monoester (*R*)-**2b** starting from the rare sugar D-*threo*-apiofuranose.[10] Herein we report an alternative route to the use of chiral pool precursors for the enantioselective preparation of conformationally constrained analogues of diacylglycerol.

3. Experimental section

3.1. General

Infrared spectra were recorded on a Bomem BM-100 spectrometer. ^1H, ^{19}F and ^{13}C NMR spectra were recorded at 400, 376 and 100 MHz, respectively, on a Varian Inova AS 400 spectrometer. Optical rotations were measured using a JASCO DIP-360 polarimeter (c as g of compound per 100 mL). Flash column chromatography was carried out using 40–63 μm (230–400 mesh) silica gel. *P. cepacia* lipase (formerly known as *Pseudomonas fluorescens* lipase) is available from Amano (lipase PS-30, containing diatomaceous earth, dextran and $CaCl_2$).

3.2. (*S*)-5-[(Acetyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2- furanone 2a from enzymatic esterification

Compound **1** (314.0 mg, 2.15 mmol) was dissolved in acetonitrile (31.4 mL) on powdered molecular sieves (3 Å, 315 mg). Lipase from *P. cepacia* (11,000 Units) and vinyl acetate (934 mg, 10.9 mmol) were then added and the mixture stirred at rt. The reaction was monitored by thin layer chromatography (2.5 h). The reaction was quenched by filtration of the enzyme and the volatiles evaporated. The crude product was purified by flash chromatography (hexane/ethyl acetate 1:1 to pure ethyl acetate) to give (*S*)-**2a** (287 mg, 71%) and **3a** (69.3 mg, 14%) as colorless oils. Compound (*S*)-**2a**: $[\alpha]_D^{22} = -27.7$ (c 0.96, C_6H_6). IR (neat) 3461, 1777, 1743, 1246 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (m, 1H), 2.11 (s, 3H), 2.27 (m, 1H), 2.45 (s, 1H), 2.66 (m, 2H), 3.64 (d, $J = 12.0$ Hz, 1H), 3.77 (d, $J = 12.0$ Hz, 1H), 4.15 (d, $J = 12.2$ Hz, 1H), 4.28 (d, $J = 12.2$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 20.9, 25.7, 29.1, 65.0, 65.9, 86.3, 170.9, 176.9; HRMS (CI, NH_3) calcd for $C_8H_{11}O_4$ $(M+H)^+$ 189.0763, found 189.0759.

Compound **3a**: IR (neat) 1784, 1745, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.09 (s, 6H), 2.17 (dd, $J = 9.2$ and 8.0 Hz, 2H), 2.66 (dd, $J = 9.2$ and 8.0 Hz, 2H), 4.18 (d, $J = 12.0$ Hz, 2H), 4.23 (d, $J = 12.0$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 20.9, 26.5, 28.6, 65.9, 83.5, 170.4, 175.8; HRMS calcd for $C_{10}H_{15}O_6$ $(M+H)^+$ 231.0869, found 231.0873.

3.3. (*S*)-5-[(Tetradecanoyloxy)methyl]-5-(hydroxymethyl)-tetrahydro-2-furanone 2b from enzymatic esterification

Compound **1** (228 mg, 1.56 mmol) was dissolved in acetonitrile (22.5 mL) on powdered molecular sieves (3 Å, 228 mg). PCL (8000 Units) and vinyl myristoate (1.98 g, 7.80 mmol) were then added and the mixture was stirred at rt. The reaction was monitored by TLC and stopped when all the starting material was consumed (2 h). The reaction was quenched by filtration of the enzyme and the solvent evaporated in vacuo. The crude product was purified by flash chromatography (hexane/AcOEt 9:1 to AcOEt 100% to AcOEt/MeOH (95:5) to

give (*S*)-**2b** (344 mg, 62%) and **3b** (336 mg, 38%) as white solids.

Compound **2b**: mp 64–66 °C, lit.[10] mp 65–66 °C; $[\alpha]_D^{22} = -2.6$ (*c* 2.6, $CHCl_3$), $[\alpha]_D^{22} = -16.0$ (*c* 1.32, C_6H_6); IR (KBr) 3415, 1747, 1728, 1161 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.88 (t, $J = 7.0$ Hz, 3H), 1.34 (m, 20H), 1.62 (m, 2H), 2.08 (m, 1H), 2.28 (m, 1H), 2.35 (t, $J = 7.6$ Hz, 2H), 2.67 (m, 2H), 3.64 (d, $J = 12.2$ Hz, 1H), 3.76 (d, $J = 12.2$ Hz, 1H), 4.14 (d, $J = 11.8$ Hz, 1H), 4.29 (d, $J = 11.8$ Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.8, 25.4, 29.0, 29.1, 29.2, 29.3, 29.4, 29.58, 29.62, 29.65, 31.9, 34.0, 64.7, 65.6, 86.5, 173.5, 177.2.

Compound **3b**: mp 63–65 °C; IR (KBr) 1747, 1730, 1160 cm^{-1}; ^1H NMR (C_6D_6) δ 0.85 (t, $J = 7.0$ Hz, 6H), 1.14 (m, 4H), 1.25 (m, 36H), 1.46 (m, 6H), 1.97 (m, 6H), 3.84 (d, $J = 11.6$ Hz, 2H), 3.94 (d, $J = 11.6$ Hz, 2H); ^{13}C NMR (C_6D_6) δ 14.4, 23.2, 25.2, 26.2, 28.2, 29.4, 29.71, 29.87, 29.95, 30.11, 30.16, 30.20, 32.4, 34.0, 65.6, 82.9, 172.5, 174.6; HRMS (CI, NH_3) calcd for $C_{34}H_{63}O_6$ $(M+H)^+$ 567.4624, found 567.4631.

3.4. (*R*)-5-[(Acetyloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone 2a from enzymatic hydrolysis

To a solution of **3a** (65.0 mg, 0,282 mmol), Triton X (1 drop, emulsifier) and pyridine (91.2 μL, 1.128 mmol) in aq 0.05 M $CaCl_2$ (3 mL) was added *P. cepacia* lipase (2300 Units). The mixture was stirred at rt for 2 h. The mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3 × 100 mL). The organic layers were dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash chromatography (hexane/ethyl acetate 1:1 to pure ethyl acetate) to give (*R*)-**2a** (45 mg, 85%). $[\alpha]_D^{22} = +25.4$ (*c* 0.96, C_6H_6).

3.5. (*R*)-5-[(Acetyloxy)methyl]-5-(*tert*-butyldimethylsilyloxy)methyl]tetrahydro-2-furanone 4

To a solution of (*S*)-(−)-**2a** (263.4 mg, 1.40 mmol), imidazole (142.2 mg, 2.80 mmol), and DMAP (1 mg, 0.012 mmol) in DMF (1.25 mL) cooled to 0 °C was added TBDMSCl (314.9 mg, 2.09 mmol). The solution was stirred at 0 °C for 12 h under dry N_2 atmosphere. The solution was diluted with ether (50 mL) and washed with 1 M HCl (3 × 50 mL), satd aq $NaHCO_3$ (2 × 50 mL) and water (2 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (pure hexane to hexane/ethyl acetate, 3:1) to give **4** (318 mg, 75%) as a colorless oil: $[\alpha]_D^{22} = -17.9$ (*c* 1.34, C_6H_6; IR (neat) 1784, 1748, 1251 cm^{-1}; ^1H NMR (C_6D_6) δ 0.00 (s, 3H), 0.01 (s, 3H), 0.89 (s, 9H), 1.53 (m, 1H), 1.65 (s, 3H), 1.75 (m, H), 2.24 (m, 2H), 3.42 (d, $J = 10.4$ Hz, 1H), 3.48 (d, $J = 10.4$ Hz, 1H), 3.99 (d, $J = 11.8$ Hz, 1H), 4.06 (d, $J = 11.8$ Hz, 1H); ^{13}C NMR (C_6D_6) δ −5.05, −4.97, 18.87, 20.73, 26.34, 26.45, 29.32, 66.36, 66.55, 85.51, 170.31, 176.02; HRMS (CI, NH_3) *m*/*z* calcd for $C_{14}H_{27}O_5Si$ $(M+H)^+$ 303.1628, found 303.1635.

3.6. (*R*)-5-[(*tert*-Butyldimethylsiloxy)methyl]-5-(hydroxymethyl)tetrahydro-2-furanone 5

To a solution of **4** (180 mg, 0.595 mmol), Triton X (1 drop, emulsifier) and pyridine (198 μL, 2.419 mmol) in aq 0.05 M $CaCl_2$ (27 mL) was added *P. cepacia* lipase (6500 Units). The mixture was stirred at rt for 12 h. The mixture was diluted with brine (50 mL) and extracted with ethyl acetate (3 × 300 mL). The organic layers were dried over $MgSO_4$ and evaporated in vacuo. The crude product was purified by flash chromatography (hexane/ethyl acetate, 7:3) to give **5** (154.8 mg, quantitative) as a colorless oil: $[\alpha]_D^{22} = -1.1$ (*c* 1.07, C_6H_6); IR (neat) 3439, 1776, 1253 cm^{-1}; ^1H NMR (C_6D_6) δ −0.02 (s, 3H), 0.01 (s, 3H), 0.89 (s, 9H), 1.60 (m, 2H), 2.20 (m, 2H), 3.20 (d, $J = 10.7$ Hz, 1H), 3.31 (d, $J = 10.7$ Hz, 1H), 3.35 (d, $J = 10.4$ Hz, H), 3.46 (d, $J = 10.4$ Hz, 1H); ^{13}C NMR (C_6D_6) δ −5.50, −5.42, 18.39, 25.24, 25.99, 29.66, 65.21, 66.20, 88.61, 177.60; HRMS (CI, NH_3) *m*/*z* calcd for $C_{12}H_{25}O_4Si$ $(M+H)^+$ 261.1522, found 261.1517.

3.7. (*R*)-5-[(*tert*-Butyldimethylsilyloxy)methyl]-5-[(tetradecanoyloxy)methyl]tetrahydro-2-furanone 6

To a solution of **5** (154.8 mg, 1.04 mmol) in anhydrous pyridine (1 mL) was added myristoyl chloride (186 μL, 1.04 mmol) and the solution stirred overnight at rt. The solution was transferred in a larger flask with ethyl acetate and co-evaporated three times with hexane (3 × 50 mL). The crude product was purified by flash chromatography (pure hexane to hexane/ethyl acetate 4:1) to give **6** (280 mg, quantitative) as a colorless oil: $[\alpha]_D^{22} = -11.6$ (*c* 1.18, C_6H_6), IR (neat) 1787, 1745, 1253 cm^{-1}; ^1H NMR (C_6D_6) δ −0.02 (s, 3H), −0.01 (s, 3H), 0.87 (s, 9 H), 0.90 (t, $J = 6.8$ Hz, 3H), 1.19 (m, 2H), 1.28 (m, 18H), 1.47 (m, 1H), 1.53 (m, 2H), 1.71 (m, 1H), 2.10 (t, $J = 7.4$ Hz, 2H), 2.19 (m, 2H), 3.39 (d, $J = 10.6$ Hz, 1H), 3.45 (d, $J = 10.6$ Hz, 1H), 3.98 (d, $J = 12.0$ Hz, 1H), 4.07 (d, $J = 12.0$ Hz, 1H); ^{13}C NMR (C_6D_6) δ −5.54, −5.46, 14.41, 18.37, 23.14, 25.25, 25.94, 25.89, 28.81, 29.45, 29.73, 29.85, 29.93, 30.09, 30.15, 30.18, 32.37, 34.09, 65.90, 65.92, 84.93, 172.57, 175.21; HRMS (CI, NH_3) *m*/*z* calcd for $C_{26}H_{51}O_5Si$ $(M+H)^+$ 471.3506, found 471.3512.

3.8. (*S*)-5-[(Tetradecanoyloxy)methyl]-5-(hydroxymethyl)-tetrahydro-2-furanone 2b

To a solution of **6** (126.2 mg, 0.297 mmol) in dry THF (5 mL) was added glacial acetic acid (51 μL, 0.885 mmol) and the solution stirred for 5 min at rt. Tetrabutylammonium fluoride (429 mg, 1.634 mmol) was then added and the solution stirred overnight at rt. The volatiles were evaporated and the crude product purified by flash chromatography (ethyl acetate/hexane 2:3) to give (*S*)-**2b** (84 mg, 88%). {$[\alpha]_D^{22} = -2.2$ (*c* 1.00, $CHCl_3$); lit.[10] $[\alpha]_D^{22} = +1.4$ (*c* 4.2, $CHCl_3$)} for the (*R*)-enantiomer.

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