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Structural determination of sulfoquinovosyldiacylglycerol by chiral syntheses

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Abstract

Chiral sulfoquinovosyldiacylglycerols (SQDGs) have been synthesized to determine the absolute stereochemistry and the biological activities. The ^{1}H NMR spectrum of a natural SQDG is comparable to that of synthetic (2*S*)-SQDG rather than that of the (2*R*) analogue. The biological activity of the respective isomers for DNA polymerase α and β inhibition was not distinguishable in the enzymatic assay.

Recently, DNA polymerase (pol) inhibitors, e.g. sulfolipids, fatty acids, sphingolipids, ceramides, tepenoids and aromatic compounds, have been isolated from natural resources.[1–12] The sulfolipids, sulfoquinovosyldiacylglycerol (SQDG) and sulfoquinovosylmonoacylglycerol (SQMG), are sulfonic analogs of D-glucose bound to glycerol bearing fatty acids. We reported that sulfolipids in the class of SQDG and SQMG from a fern,[1] an alga[2] and a marine invertebrate[3] are potent inhibitors of DNA pol α and β in vitro and human lung cancer in vivo. DNA polymerases are essential for DNA replication and repair, and hence, enzymatic inhibitors lead to cell death. SQDGs possess extensive biological activities such as anti-tumor effect,[3] P-selectin receptor inhibition,[13] HIV-RT inhibition,[2,14] AIDS-antiviral,[15] and so on.

The stereochemistry of cyanobacterial SQDG has been determined to be (2*S*) by the comparison of the optical rotation values.[16] Although (2*S*)-SQDG was synthesized,[16,17] the (2*R*) analogue has not been prepared so far. We modified our chiral synthetic method as follows to obtain both (2*S*)

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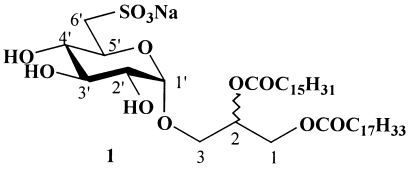
and (2*R*) analogues to determine the C-2 stereochemistry of natural SQDG (**1**) by comparison of the ^{1}H NMR data and the active isomer involved in in vitro DNA pol inhibition.



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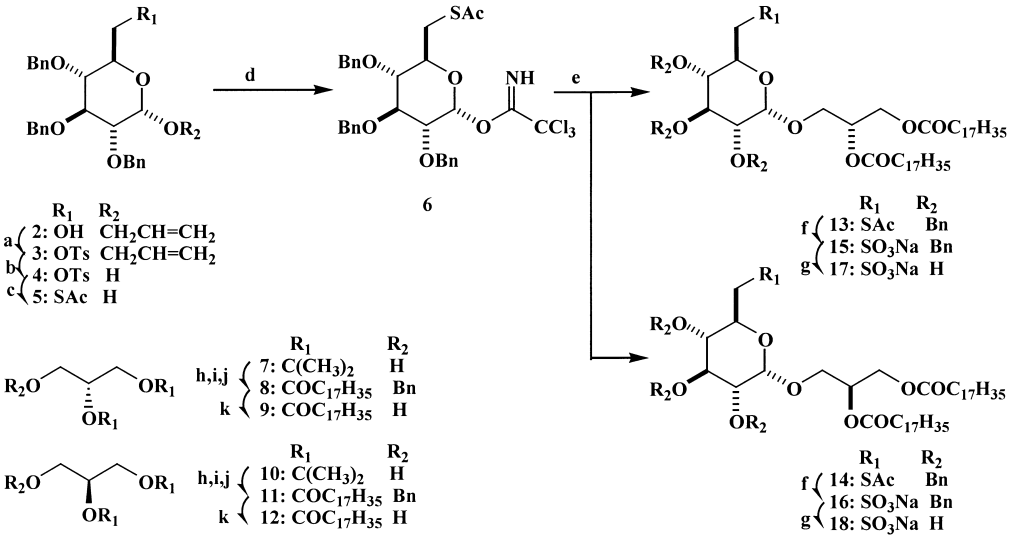
(1) D-Glucose was chosen as the glycosyl donor instead of D-glucal used in the syntheses of three anti-HIV-1 sulfolipids.[16] (2) As the glycosyl acceptor, enantiomerically pure (2*S*)-isopropylideneglycerol was used;[17–19] however, the fatty acid esters of the chiral glycerols were employed to avoid racemization under the acidic conditions of glycosylation.[16]

Compound **2** was prepared from D-glucose in several steps including the selective reduction of 4′,6′-*O*-benzylidene derivative with $AlCl_3$–$LiAlH_4$ in Et_2O–CH_2Cl_2.[20] Tosylation of **2** with TsCl afforded **3**, followed by deallylation with $PdCl_2$ in MeOH to give **4** (Scheme 1). The tosyloxy group was then converted into a thioacetyl group with potassium thioacetate in DMF to yield **5**. The reaction of **5** with CCl_3CN and DBU in CH_2Cl_2 selectively gave α-anomer **6** (H-1′, δ 6.41, $J = 3.48$ Hz),[21] while no β-anomer was found. The glycosyl acceptors (**9**, **12**) were prepared from (2*S*)- and (2*R*)-isopropylidene glycerols by the following reactions: benzylation, deprotection, esterification (**8**, **11**) and debenzylation (**9**, **12**). The imidates (**6**) were treated with (2*S*)- or (2*R*)-1,2-bis-*O*-stearylglycerol (**9** or **12**) under the presence of TMSOTf as Lewis acid. Two glycosylations (**6** and **9**, **6** and **12**) proceeded α-selectively and afforded only α-anomer **13** and **14**, respectively.[21] Oxydation of **13** and **14** with OXONE[16] gave sodium sulfonate derivatives (**15**, **16**), and then the



Scheme 1. (a) TsCl, DMAP, C_5H_5N, rt, 15hr, 92% (**3**). (b) $PdCl_2$, MeOH, rt, 18 h, 80% (**4**). (c) CH_3COSK, DMF, 80°C, 3 h, 86% (**5**). (d) CCl_3CN, DBU, CH_2Cl_2, rt, 5.5 h, 70% (**6**). (e) TMSOTf, CH_2Cl_2, MS4A, rt, 6 h, 62% (**13**), 67% (**14**). (f) OXONE, AcOH, AcOK, rt, 4 h, 68.8% (**15**), 55.1% (**16**). (g) Pd/C, H_2, rt, 18 h, 42.5% (**17**), 51.5% (**18**). (h) BnBr, NaH, DMF, rt, 3 h. (i) TsOH, MeOH, rt, 16 h. (j) $C_{17}H_{35}COOH$, EDCI, DMAP, C_5H_5N, rt, 6 h, 37.5% (three steps) (**8**), 84.8% (three steps) (**11**). (k) Pd/C, H_2, rt, 4 h, 94.5% (**9**), 80.7% (**12**).

catalytic hydrogenation with Pd–C afforded chiral SQDGs **17** and **18**, respectively.[21] The ^{1}H NMR spectra of (2*R*)-SQDG (**17**), (2*S*)-SQDG (**18**) and the natural product (**1**) in DMSO-d_6 indicated that the stereochemistry at C-2 of the natural product (**1**) (δ 4.33(H-1a)/4.11(H-1b)) was confirmed to be *S*-form, since the chemical shifts of glycerol H-1 were δ 4.30(H-1a)/4.22(H-1b) of **17** and 4.33(H-1a)/4.11(H-1b) of **18** (Table 1).

Table 1
^{1}H NMR data (600 MHz) of the SQDGs in DMSO-d_6

	δ_H (number of protons, multiplicity, J values in Hz)		
Position	Natural product	(2*S*)-SQDG (**18**)	(2*R*)-SQDG (**17**)
Glycerol			
1-a	4.33 (1H, dd, 11.7, 1.7)	4.33 (1H, dd, 12.1, 2.5)	4.30 (1H, dd, 11.8, 2.4)
1-b	4.11 (1H, dd, 11.7, 7.6)	4.11 (1H, dd, 12.1, 7.6)	4.22 (1H, dd, 11.8, 8.6)
2	5.08 (1H, m)	5.12 (1H, m)	5.12 (1H, m)
3-a	3.90 (1H, dd, 10.6, 5.9)	3.89 (1H, dd, 10.7, 5.6)	3.89 (1H, dd, 10.7, 6.1)
3-b	3.37 (1H, dd, 10.6, 5.9)	3.37 (1H, dd, 10.7, 5.6)	3.45 (1H, m)
Sulfoquinovose			
1'	4.55 (1H, d, 3.7)	4.54 (1H, d, 3.9)	4.55 (1H, d, 3.7)
2'	3.17 (1H, dd, 9.5, 3.7)	3.17 (1H, dd, 9.8, 3.9)	3.17 (1H, dd, 9.5, 3.7)
3'	3.34 (1H, t, 9.5)	3.33 (1H, t, 9.3)	3.33 (1H, t, 9.3)
4'	2.89 (1H, m)	2.89 (1H, m)	2.89 (1H, m)
5'	3.76 (1H, m)	3.76 (1H, m)	3.73 (1H, m)
6'-a	2.89 (1H, m)	2.89 (1H, m)	2.89 (1H, m)
6'-b	2.53 (1H, dd, 13.9, 7.1)	2.53 (1H, dd, 14.2, 7.3)	2.54 (1H, dd, 14.2, 6.9)

The synthetic compounds **17** and **18** inhibited eukaryotic DNA pol α and β[1] similarly as illustrated in Fig. 1. Since the IC_{50} values of the two compounds were not distinguishable, the chirality at C-2 of glycerol may not be essential for in vitro DNA pol inhibition. This observation may be useful for further research and development of SQDGs for clinical usage.

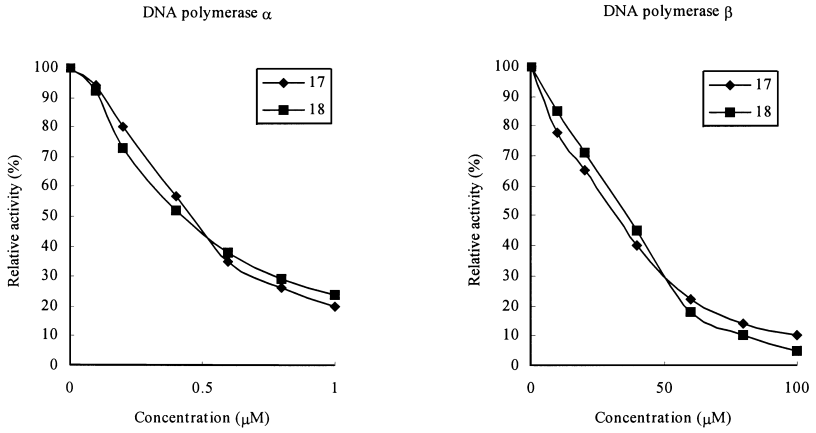


Figure 1. Inhibition of DNA polymerases α and β by synthetic SQDGs **17** and **18**[22]

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21. Physicochemical data: Compound **6**. $[\alpha]_D$ +56.0°(*c* 1.17, $CHCl_3$), IR (cm^{-1}): 3250, 3000, 2950, 2850, 1930, 1860, 1650, 1430, 1340, 1200, 1090–930, 880, 770, ^1H NMR (300 MHz, $CDCl_3$): δ 8.60 (1H, s, C=NH), 7.32 (15H, m, ArH), 6.40 (1H, d, $J=3.5$ Hz, anomeric H), 5.00 (1H, d, $J=13.6$ Hz, $ArCH_2O$), 4.90 (1H, d, $J=13.7$ Hz, $ArCH_2O$), 4.82 (1H, d, $J=10.7$ Hz, $ArCH_2O$), 4.74 (1H, d, $J=11.7$ Hz, $ArCH_2O$), 4.67 (1H, d, $J=11.7$ Hz, $ArCH_2O$), 4.62 (1H, d, $J=10.6$ Hz, $ArCH_2O$), 4.04 (2H, m), 3.71 (1H, dd, $J=9.6$ and 3.5 Hz), 3.42 (2H, m), 3.13 (1H, dd, $J=13.9$ and 6.7 Hz), 2.31 (3H, s, $SCOCH_3$), LRFABMS: *m/z* 654.1 $[M+H]^+$, HRFABMS: calcd *m/z* 654.1094 for $C_{31}H_{33}^{35}Cl_2^{37}ClNO_6S$ $[M+H]^+$, found *m/z* 654.1071. Compound **13**. $[\alpha]_D$ +23.4°(*c* 1.16, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): δ 7.24–7.34 (15H, m, ArH), 5.25 (1H, m), 4.59–4.98 (7H, m, anomeric H and $ArCH_2O$), 4.37 (1H, dd, $J=12.0$ and 3.8Hz), 4.21 (1H, dd, $J=11.9$ and 6.0 Hz), 3.93 (1H, t, $J=9.2$ Hz), 3.73–3.81 (2H, m), 3.48–3.56 (2H, m), 3.37 (1H, dd, $J=12.4$ and 2.9 Hz), 3.30 (1H, t, $J=9.2$ Hz), 3.03 (1H, dd, $J=13.7$ and 7.5 Hz), 2.33 (3H, s, $SCOCH_3$), 2.27 (4H, m, $OCOCH_2$), 1.59 (4H, m, $OCOCCH_2$), 1.25 (64H, br, CH_2), 0.86 (6H, t, $J=6.9$ Hz, CH_3), ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.7, 173.3, 172.9, 138.5, 138.1, 137.9, 127.5–128.5, 96.7, 81.5, 80.2, 79.9, 75.7, 75.1, 72.9, 69.7, 69.7, 65.7, 62.4, 34.2, 34.1, 29.0–30.7, 24.8, 22.6, 14.1. Compound **14**. $[\alpha]_D$ +32.5°(*c* 0.85, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (15H, m, ArH), 5.23 (1H, m), 4.96 (1H, d, $J=10.8$ Hz, $ArCH_2O$), 4.89 (d, 1H, $J=10.7$ Hz, $ArCH_2O$), 4.79 (d, 1H, $J=11.0$ Hz, $ArCH_2O$), 4.74 (d, 1H, $J=12.0$ Hz,

$ArCH_2O$), 4.69–4.58 (3H, m, $ArCH_2O$, anomeric H), 4.41 (1H, dd, $J = 11.9$ and 3.7 Hz), 4.21 (1H, dd, $J = 11.9$ and 6.3 Hz), 3.94(1H, t, $J = 9.2$ Hz), 3.77–3.75 (m, 2H), 3.53–3.46 (m, 2H), 3.40 (dd, 1H, $J = 13.7$ and 3.0 Hz), 3.31 (t, 1H, $J = 9.2$ Hz), 3.05 (dd, 1H, $J = 13.7$ and 7.5 Hz), 2.33 (3H, s, $SCOCH_3$), 2.31 (4H, m, $OCOCH_2$), 1.60 (4H, m, $OCOCCH_2$), 1.25 (64H, br, CH_2), 0.88 (6H, t, $J = 6.4$ Hz, CH_3), ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.7, 173.7, 173.0, 138.5, 138.1, 137.8, 128.4–127.6, 97.1, 81.5, 80.2, 80.1, 75.2, 73.0, 69.63, 69.59, 66.1, 62.4, 34.2, 34.1, 31.9, 30.7, 30.5, 29.7–29.1, 24.9, 22.6, 14.1. Compound **17**. $[\alpha]_D$ +23.6°(*c* 0.39, chloroform:methanol:water = 65:25:4), LRFABMS (negative mode) *m*/*z* 849.5 $[M–H]^-$, 583.3 $[M–C18:0]^-$, 565.5 $[M–C18:0\text{-}H_2O]^-$ HRFABMS calcd *m*/*z* 849.5762 for $C_{45}H_{85}O_{12}S$ $[M–H]^-$, found *m*/*z* 849.5764, ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.48, 172.36, 98.30, 74.46, 72.83, 71.59, 69.77, 68.55, 64.95, 62.36, 33.56, 33.43, 31.28, 29.04, 29.00, 28.95, 28.81, 28.78, 28.70, 28.44, 24.47, 22.08, 13.93. Compound **18**. $[\alpha]_D$ +38.8° (*c* 0.42, chloroform:methanol:water = 65:25:4), LRFABMS (negative mode) *m*/*z* 849.5 $[M–H]^-$, 583.3 $[M–C18:0]^-$, 565.5 $[M–C18:0–H_2O]^-$, HRFABMS calcd *m*/*z* 849.5762 for $C_{45}H_{85}O_{12}S$ $[M–H]^-$, found *m*/*z* 849.5694, ^{13}C NMR (125 MHz, DMSO-d_6): δ 172.50, 172.35, 98.28, 74.34, 72.88, 71.60, 69.70, 68.53, 64.60, 62.62, 33.55, 33.42, 31.29, 29.05, 29.01, 28.83, 28.79, 28.71, 28.46, 24.45, 22.09, 13.92.

22. The standard reaction mixture for pol α purified from calf thymus (or recombinant rat DNA pol β purified from *E. coli* JMpβ5) and Taq pol contained 50 mM tris–HCl, 1 mM dithiothreitol, 1 mM $MgCl_2$, 5 μM poly(dA)/oligo$(dT)_{12–18}$(2/1), 10 μM $[^3H]$dTTP (100 cpm/pmol), 15% glycerol and 8 μl of an enzyme inhibitor solution in DMSO. After incubation, the radioactive DNA product was collected on a DEAE-cellulose paper disc, and then the radioactivity was measured in a scintillation counter.