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Preliminary communication

Synthetic studies on the relationship between anti-HIV activities and micelle forming abilities of various alkylated glycyrrhetinate diglycoside sodium sulfates and related compounds

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Summary — Sodium sulfates **11–14**, **29–32**, **35** and **37** of various alkyl glycyrrhizin and related compounds were synthesized. In vitro anti-HIV activities of the sulfates were compared to the activities of glycyrrhizin **1** in the inhibition of replications of HTLV-III and GUN-4. The activities of the sulfates were increased 11.1, 15.2, 9.1 and 5.0 times for **11–14**, 100.0, 125.5, 83.3 and 11.6 times for **29–32**, and 11.6 and 50.0 times for **35** and **37**. From the relationship between CMC values and anti-HIV activities of the sulfates, it appeared that the sulfates exhibiting more potent antiviral activities had higher micelle forming abilities. Sodium sulfates having a triterpenoid or steroid ring in the molecule showed more potent activities than those of thioglycosides which had no such ring. From the investigation of syncytium formation, we suggest that the active sulfates inhibited HIV-1 infection early in the replication cycle of the virus.

glycyrrhizin / alkyl glycyrrhetinate diglycoside / sodium sulfate / anti-HIV activity / micelle formation

Introduction

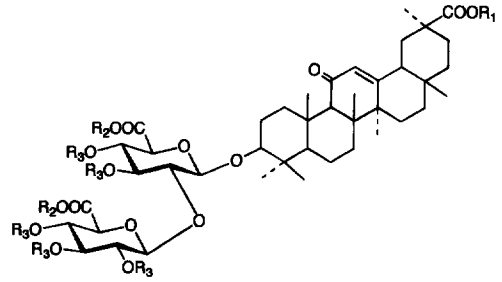
Glycyrrhizin **1** has been found to have antihepatotoxic [1, 2], antiulcerogenic [3, 4], antiallergenic [5, 6] and antiinflammatory activities [7, 8] as well as inhibitory activities in the replication of several DNA and RNA viruses in vitro [9]. Recently **1** and its sodium sulfate **2** have been proved to have in vitro anti-HIV (human immunodeficiency virus) activities [10, 11], though the activities are much lower than those of clinically used AZT (3'-azido-2',3'-dideoxythimidine) [12], DDI (2',3'-dideoxyinosine) [13] and DDC (2',3'-dideoxycytidine) [13]. Although the latter clinical drugs are known to have high antiviral activities for HIV and lower cell toxicity [14], the long term administration of them to AIDS (aquired immune deficiency syndrome) patients has caused critical side effects such as bone marrow suppression and carcinogenesis [15]. As it was not known that **1** and **2** have such side effects, derivatives having more potent anti-HIV activities obtained from **1** were sought. In this paper, we report syntheses of sodium sulfate derivatives of various alkyl glycyrrhizins and related compounds and compare the anti-HIV activities of the sulfate derivatives with that of **1** by the assay system using HTLV-IIIB specific antigens in MT-4 cells which are highly susceptible to HIV replication [16]. The activities of some of these synthetic compounds are also investigated by another assay system using GUN-4 specific antigens (see the *Experimental protocols*) in C8166 cells [17]. The relationships between the anti-HIV activities and the abilities of the sulfate derivatives to form micelles are reported. The effects on expression of the CD4 molecules on cell surface are also reported for the same potent active sulfates.

Chemistry

Various esterifications of the carboxyl group at the position of C-30 of **1** were carried out stepwise. Methylation of **1** with diazomethane, and treatment of **1** with 1-hexanol, 1-undecanol and 1-octadecanol in pyridine in the presence of 2-chloro-1,3-dimethylimidazolium chloride (DMC) gave corresponding trialkylated glycyrrhizins (**3–6**). Compounds **3–6** were

carefully hydrolyzed with 5% KOH in EtOH/H_2O (1:1) to obtain monoalkylated glycyrrhizins (**7–10**). The fact that the alkyl groups in **7–10** linked to the carboxyl groups at the position of C-30 on the aglycons was confirmed by acid hydrolysis of **7–10** to afford the corresponding alkyl glycyrrhetinates **16–19**. Reaction of **7–10** with anhydrous SO_3-pyridine complex [18, 19], followed by treatment with 1.0 M NaOH to adjust the pH to 7–8, gave sodium sulfate derivatives **11–14** respectively. Compounds **1–14** are shown in figure 1.

Reaction of glycyrrhetic acid **15** with 1-hexanol, 1-undecanol and 1-octadecanol in the presence of DMC in dry pyridine gave *n*-hexyl **17** (83.7%), *n*-undecyl **18** (71.2%) and *n*-octadecyl glycyrrhetinate **19** (74.4%) respectively (fig 2). Glycosylation of methyl glycyrrhetinate **16** [20] and the esters **17–19**



1 $R_1 = R_2 = R_3 = H$

2 $R_1 = R_2 = Na$, $R_3 = SO_3Na$

3 $R_1 = R_2 = CH_3$, $R_3 = H$

4 $R_1 = R_2 = CH_2(CH_2)_4CH_3$, $R_3 = H$

5 $R_1 = R_2 = CH_2(CH_2)_9CH_3$, $R_3 = H$

6 $R_1 = R_2 = CH_2(CH_2)_{16}CH_3$, $R_3 = H$

7 $R_1 = CH_3$, $R_2 = R_3 = H$

8 $R_1 = CH_2(CH_2)_4CH_3$, $R_2 = R_3 = H$

9 $R_1 = CH_2(CH_2)_9CH_3$, $R_2 = R_3 = H$

10 $R_1 = CH_2(CH_2)_{16}CH_3$, $R_2 = = R_3 H$

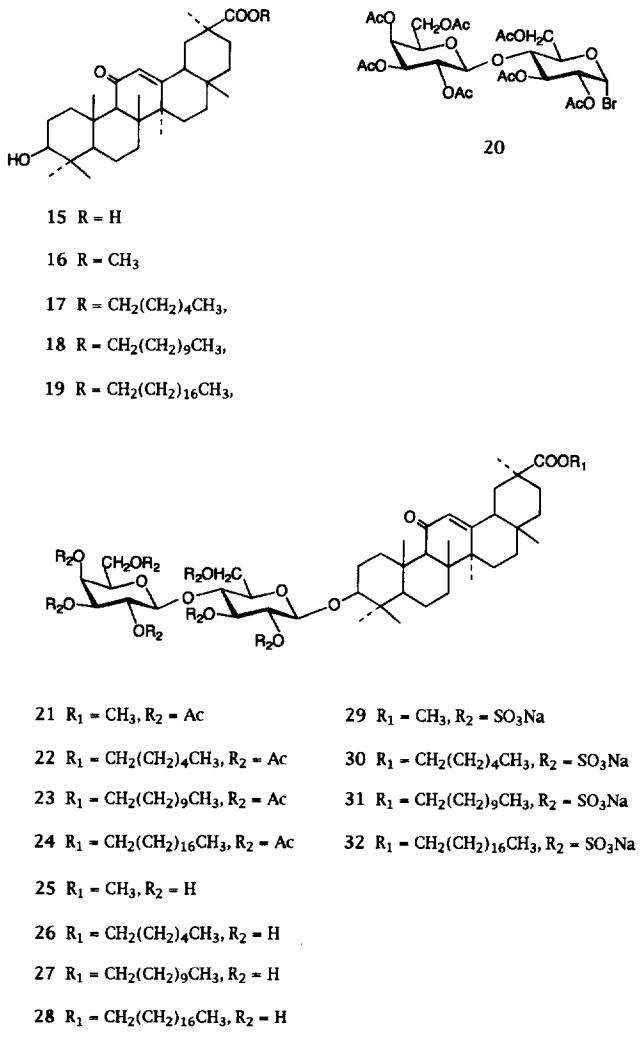
11 $R_1 = CH_3$, $R_2 = Na$, $R_3 = SO_3Na$

12 $R_1 = CH_2(CH_2)_4CH_3$, $R_2 = Na$, $R_3 = SO_3Na$

13 $R_1 = CH_2(CH_2)_9CH_3$, $R_2 = Na$, $R_3 = SO_3Na$

14 $R_1 = CH_2(CH_2)_{16}CH_3$, $R_2 = Na$, $R_3 = SO_3Na$

Fig 1. Structures of compounds **1–14**.



15 R = H

16 R = CH_3

17 R = $CH_2(CH_2)_4CH_3$,

18 R = $CH_2(CH_2)_9CH_3$,

19 R = $CH_2(CH_2)_{16}CH_3$,

20

21 $R_1 = CH_3$, R_2 = Ac

22 $R_1 = CH_2(CH_2)_4CH_3$, R_2 = Ac

23 $R_1 = CH_2(CH_2)_9CH_3$, R_2 = Ac

24 $R_1 = CH_2(CH_2)_{16}CH_3$, R_2 = Ac

25 $R_1 = CH_3$, $R_2 = H$

26 $R_1 = CH_2(CH_2)_4CH_3$, $R_2 = H$

27 $R_1 = CH_2(CH_2)_9CH_3$, $R_2 = H$

28 $R_1 = CH_2(CH_2)_{16}CH_3$, $R_2 = H$

29 $R_1 = CH_3$, $R_2 = SO_3Na$

30 $R_1 = CH_2(CH_2)_4CH_3$, $R_2 = SO_3Na$

31 $R_1 = CH_2(CH_2)_9CH_3$, $R_2 = SO_3Na$

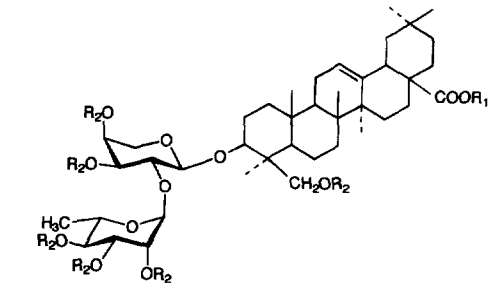
32 $R_1 = CH_2(CH_2)_{16}CH_3$, $R_2 = SO_3Na$

Fig 2. Structures of compounds **15–32**.

with 4-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **20** [21] in the presence of Ag_2CO_3 in dry CH_2Cl_2 gave corresponding diglycosides **21–24** in yields of 57.0, 52.0, 50.3 and 40.4% respectively. Compounds **21–24** exhibited pairs of doublets assigned to anomeric protons of two β-arranged pyranose rings at δ 4.52 (J = 8.1 Hz) and 4.52 (J = 8.1 Hz), 4.48 (J = 7.7 Hz) and 4.51 (J = 8.1 Hz), 4.47 (J = 8.1 Hz) and 4.50 (J = 7.7 Hz), and 4.48 (J = 7.7 Hz) and 4.50 (J = 7.3 Hz), respectively, in the ^{1}H-NMR spectra (table III). Deacetylation of the derivatives **21–24** by treatment with 5% KOH in EtOH/H_2O (1:1) gave compounds **25–28** (fig 2) in yields of 82.7, 80.5, 70.9 and 81.0% respectively. Reactions of **25–28** with anhydrous SO_3-

pyridine complex in dry pyridine gave, after purification by column chromatography using Diaion HP-20, compounds **29–32** (fig 2) in yields of 84.6, 73.3, 75.9 and 80.4% respectively. Sodium sulfate derivatives **35** and **37** (fig 3) were obtained from naturally occurring compounds, 3-*O*-[2'-*O*-(α-L-arabinopyranosyl)-α-L-rhamnopyranosyl)hederagenin **33** [22] and timosaponin A-III **36** [23] respectively. Methyl ester **34**, obtained by methylation of **33** with diazomethane, was treated with an SO_3–pyridine complex and worked up as described for the preparation of **11–14** to give **35** (85.2% yield). Sulfation of **36** was carried out by the same reaction as **35** to give **37** (87.5% yield).

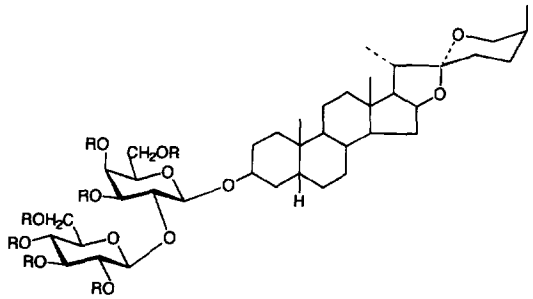
For the investigation of the role of triterpenoid and steroid rings in anti-HIV activity, alkyl thioglycosides without such rings were also prepared. *n*-Octadecyl β-D-thioglucopyranose **40** was synthesized by the same preparative methods as **38**, which has been previously synthesized [24]. Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **49** with thio-



33 $R_1 = R_2 = H$

34 $R_1 = CH_3, R_2 = H$

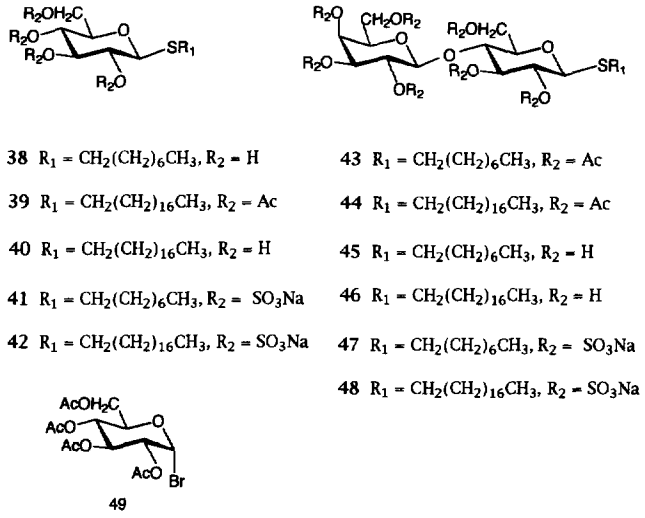
35 $R_1 = CH_3, R_2 = SO_3Na$



36 R = H

37 R = SO_3Na

Fig 3. Structures of compounds **33–37**.



38 $R_1 = CH_2(CH_2)_6CH_3, R_2 = H$

39 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = Ac$

40 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = H$

41 $R_1 = CH_2(CH_2)_6CH_3, R_2 = SO_3Na$

42 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = SO_3Na$

43 $R_1 = CH_2(CH_2)_6CH_3, R_2 = Ac$

44 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = Ac$

45 $R_1 = CH_2(CH_2)_6CH_3, R_2 = H$

46 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = H$

47 $R_1 = CH_2(CH_2)_6CH_3, R_2 = SO_3Na$

48 $R_1 = CH_2(CH_2)_{16}CH_3, R_2 = SO_3Na$

49

Fig 4. Structures of compounds **38–49**.

urea afforded 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl thiopseudourea hydrobromide which was, without purification, treated with 1-octadecylbromide in acetone/H_2O (1:1) in the presence of K_2CO_3 and $NaHSO_3$ to give **39**. **39** showed an anomeric proton signal at δ 4.48 with coupling constant J = 9.9 Hz, which suggested that the alkyl-thio group has the β-configuration. Deacetylation of **39** gave **40**. Reaction of **20**, instead of **49**, with thiourea afforded 4-*O*-(2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl thiopseudourea hydrobromide, which was reacted with 1-octyl- and 1-octadecylbromide as in the preparation of **39** to give compounds **43** and **44**, in yields of 89.0 and 72.3% respectively. **43** and **44** showed pairs of anomeric proton signals observed at δ 4.47 (J = 10.3 Hz) and 4.50 (J = 7.7 Hz), and δ 4.46 (J = 9.9 Hz) and 4.48 (J = 7.7 Hz) respectively, which suggested that the alkyl-thio groups in **43** and **44** have the same β-configuration as **39**. Deacetylation of **43** and **44** gave compounds **45** and **46** in yields of 83.3 and 79.7% respectively. Sulfation of **38**, **40**, **45** and **46** gave compounds **41**, **42**, **47** and **48** in yields of 61.9, 59.9, 35.1 and 64.5% respectively. The structures of all products obtained in this study were confirmed by their fast atom bombardment mass spectra (FAB-MA) (table XIV) and ^{1}H-NMR (tables III and VII) and ^{13}C-NMR spectra (tables I, II, IV–VI and VIII).

Discussion of anti-HIV activity

It has been reported that glycyrrhizin **1** and its sodium sulfate **2** completely inhibit HIV-induced syncytia

Table I. ^{13}C-NMR chemical shifts for compounds **7–10**[a].

	7	*8*	*9*	*10*
Aglycon				
C-1	39.8[b]	39.4[b]	39.8[b]	39.4[b]
C-2	26.5[c]	26.6[c]	26.3[c]	26.2
C-3	89.1	89.1	89.4	89.3
C-4	39.9[b]	39.9[b]	39.9[b]	39.7[b]
C-5	55.3	55.4	55.6	55.5
C-6	17.9	17.6	17.8	17.7
C-7	33.0	32.9	33.1	32.9
C-8	43.4	43.4	43.1	43.3
C-9	62.0	62.1	62.1	62.0
C-10	37.1	37.2	37.6	37.3
C-11	199.6	199.3	199.5	198.1
C-12	128.6	128.6	128.7	128.7
C-13	168.1	169.0	168.9	168.7
C-14	45.5	45.5	45.8	45.3
C-15	28.1	26.7[c]	26.8[c]	26.5[c]
C-16	26.7[c]	26.6[c]	26.7[c]	26.3[c]
C-17	32.0	32.8	32.5	32.2
C-18	48.6	48.7	48.9	48.8
C-19	41.3	41.2	41.3	41.2
C-20	44.2	44.2	44.2	44.2
C-21	31.2	31.5	31.4	31.3
C-22	38.1	38.1	38.0	38.1
C-23	28.2	27.8	28.4	27.8
C-24	16.7[d]	16.5[d]	16.7	16.5[d]
C-25	16.8[d]	16.7[d]	17.2	16.8[d]
C-26	18.8	19.1	19.5	19.2
C-27	23.4	24.3	23.5	24.0
C-28	28.6	28.6	29.5	28.4
C-29	30.0	29.6	30.0	29.7
C-30	176.9	176.3	176.4	176.0
$-OCH_2-$	51.7 (OCH_3)	64.9	64.6	65.1
$-(CH_2)_n-$	–	22.7, 30.7 30.9, 31.5 32.0	22.9, 28.3, 29.8 x 5, 32.1 x 2	22.3, 29.6 x 2, 30.0 x 10, 31.9 x 2, 32.0
$-CH_3$	–	14.1	14.3	14.2
Inner Sugar				
C-1	106.8	106.7	106.9	106.5
C-2	84.4	84.3	85.5	85.2
C-3	77.2	76.5	76.8	76.3
C-4	73.2	73.0	73.7	73.1
C-5	77.5	77.4	77.6	77.5
C-6	169.2	169.6	169.3	169.4
Outer Sugar				
C-1	105.0	104.9	104.6	104.5
C-2	76.7	76.7	76.9	76.5
C-3	77.6	77.3	77.4	77.5
C-4	73.2	73.4	73.3	73.1
C-5	77.9	77.8	77.8	77.9
C-6	172.1	169.7	169.8	169.9

[a]Spectra were obtained in d_5-pyridine. The signal assignments were based on the H-C COSY method and reported spectral data [33–35]. [b,c,d]These values may be interchangeable in each column.

Table II. ^{13}C-NMR chemical shifts for compounds **11–14**[a].

	11	*12*	*13*	*14*
Aglycon				
C-1	39.9[b]	39.6[b]	39.7[b]	39.5[b]
C-2	27.0	26.8[c]	26.5[c]	26.9[c]
C-3	91.0	90.7	90.5	90.9
C-4	40.0[b]	39.8[b]	39.9[b]	40.1[b]
C-5	55.4	55.5	55.6	55.3
C-6	17.7	17.1	17.5	17.4
C-7	34.3	32.4	33.9	32.9
C-8	43.1	43.9	43.7	43.8
C-9	62.3	62.4	62.5	62.7
C-10	37.2	37.2	37.3	37.4
C-11	204.1	200.8	201.0	200.7
C-12	128.3	128.3	128.5	128.6
C-13	173.0	171.0	172.6	172.9
C-14	46.5	46.1	46.3	46.4
C-15	27.0	26.4[c]	26.7[c]	26.6[c]
C-16	27.0	26.6[c]	26.6[c]	26.7[c]
C-17	32.5	32.1	32.4	32.2
C-18	49.2	48.8	48.7	48.8
C-19	41.6	41.8	41.7	41.5
C-20	45.0	44.5	44.8	44.9
C-21	31.4	31.2	31.3	31.3
C-22	39.7	38.6	39.0	38.9
C-23	28.0	27.8	28.1	27.9
C-24	16.8[d]	16.6[d]	16.7[d]	16.5[d]
C-25	16.9[d]	16.9[d]	16.9[d]	16.8[d]
C-26	19.3	19.0	19.2	19.1
C-27	23.5	23.2	23.5	23.9
C-28	28.4	28.6	28.9	28.5
C-29	29.3	29.6	29.5	29.0
C-30	180.0	177.0	177.5	177.6
$-OCH_2-$	51.7 (OCH_3)	66.4	65.5	65.9
$-(CH_2)_n-$	–	25.8 29.4 x 2, 31.9	23.0, 28.2, 29.9 x 5, 31.5 x 2	22.5, 29.8 x 2, 30.1 x 10, 32.0 x 2, 32.1
$-CH_3$	–	14.7	14.3	14.2
Inner Sugar				
C-1	103.5	102.3	104.6	104.5
C-2	84.2	84.5	85.1	84.2
C-3	76.1[d]	75.5	76.4	76.6
C-4	74.2	74.3	74.8	74.5
C-5	76.7	77.6	77.6	77.2
C-6	173.4	171.7	172.3	171.9[d]
Outer Sugar				
C-1	102.7	101.7	102.9	103.1
C-2	75.8	76.1	75.9	75.6
C-3	77.5	77.3	77.0	77.1
C-4	73.6	74.2	73.3	73.1
C-5	78.2	78.2	78.1	78.3
C-6	173.7	172.3	170.0	169.9[d]

[a]Spectra were obtained in d_5-pyridine. The signal assignments were based on the H-C COSY method, the reported spectral data [33–35] and the data listed in table I. [b,c,d]These values may be interchangeable in each column.

Table III. ^{1}H-NMR chemical shifts and coupling constants for compounds **21–24**[a].

	21	*22*	*23*	*24*
Aglycon[b]				
CH_3	0.76, 0.81, 0.93, 1.12, 1.12, 1.14, 1.36	0.75, 0.80, 0.92, 1.12, 1.13, 1.13, 1.35	0.76, 0.80, 0.92, 1.11, 1.12, 1.14, 1.35	0.76, 0.80, 0.92, 1.12, 1.12, 1.14, 1.35
$COOCH_3$	3.96 (s)	–	–	–
$COOCH_2$-	–	4.04–4.16	4.08 (dd, 6.2, 6.2)	4.08 (dd, 6.2, 6.2)
-$(CH_2)_n$-	–	1.32 (s, 8H)	1.26 (s, 18H)	1.25 (s, 32H)
CH_2CH_3	–	0.89 (dd, 6.6, 6.6)	0.88 (dd, 6.2, 6.2)	0.88 (dd, 6.2, 6.2)
H-3	3.09 (dd, 7.9, 7.9)	3.08 (dd, 8.1, 8.1)	3.08 (dd, 8.4, 8.4)	3.08 (dd, 8.1, 8.1)
H-9	2.31 (s)	2.31 (s)	2.31 (s)	2.31 (s)
H-12	5.65 (s)	5.65 (s)	5.65 (s)	5.64 (s)
H-18	2.78 (broad d, 11.4)	2.79 (broad d, 13.6)	2.79 (broad d, 13.6)	2.79 (broad d, 13.2)
Inner Sugar				
H-1'	4.52 (d, 8.1)	4.48 (d, 7.7)	4.47 (d, 8.1)	4.48 (d, 7.7)
H-2'	4.93 (dd, 9.5, 8.1)	4.93 (d, 9.5, 7.7)	4.94 (dd, 9.2, 8.1)	4.93 (dd, 9.5, 7.7)
H-3'	5.19 (dd, 9.5, 9.5)	5.19 (dd, 9.5, 9.5)	5.19 (dd, 9.2, 9.2)	5.19 (dd, 9.5, 9.5)
H-4'	3.74 (dd, 9.5, 9.5)	3.72 (dd, 9.5, 9.5)	3.72 (dd, 9.2, 9.2)	3.72 (dd, 9.5, 9.5)
H-5'	3.65 (ddd, 9.5, 6.6, 1.8)	3.61 (ddd, 9.5, 5.9, 1.8)	3.60 (ddd, 9.2, 6.2, 1.8)	3.60 (ddd, 9.5, 6.2, 1.8)
H-6'a	4.42 (dd, 11.0, 1.8)	4.42 (dd, 11.7, 1.8)	4.41 (dd, 11.7, 1.8)	4.41 (dd, 11.7, 1.8)
H-6'b	4.10 (dd, 11.0, 6.6)	4.09 (dd, 11.7, 5.9)	4.08 (dd, 11.7, 6.2)	4.07 (dd, 11.7, 6.2)
Outer Sugar				
H-1"	4.52 (d, 8.1)	4.51 (d, 8.1)	4.50 (d, 7.7)	4.50 (d, 7.3)
H-2"	5.10 (dd, 10.3, 8.1)	5.11 (d, 10.6, 8.1)	5.11 (d, 10.3, 7.7)	5.10 (dd, 10.3, 7.3)
H-3"	4.97 (dd, 10.3, 3.3)	4.95 (dd, 10.6, 3.3)	4.95 (dd, 10.3, 2.6)	4.95 (dd, 10.3, 2.9)
H-4"	5.34 (d, 3.3)	5.35 (d, 3.3)	5.34 (d, 2.6)	5.34 (d, 2.9)
H-5"	3.92 (dd, 6.6, 6.6)	3.88 (dd, 6.6, 6.6)	3.88 (dd, 6.6)	3.88 (dd, 6.3, 6.3)
H-6"a and b	4.05–4.16	4.04–4.16	4.06–4.16	4.04–4.16
Acetyl protons	1.98, 2.02, 2.04, 2.05, 2.06, 2.13, 2.15	1.96, 2.02, 2.04, 2.05, 2.06, 2.09, 2.15	1.96, 2.02, 2.04, 2.05, 2.06, 2.09, 2.15	1.96, 2.02, 2.04, 2.05, 2.06, 2.09, 2.15

[a]Spectra were obtained in $CDCl_3$. Coupling constants (J in Hz) are given in parentheses. The signal assignments were based on the H-H COSY method. [b]Only assignable protons on the aglycons are listed.

formation in MT-4 cells at drug concentrations of 1.0 mg/mL and 0.25 mg/mL respectively [10, 11]. We first examined anti-HIV activities and cell toxicities of compounds **11–14** against HTLV-IIIB [25] using MT-4 cells [26] at drug concentrations of 1000, 100, 10 and 1 μg/mL, and compared them with the corresponding activities of **1** (table IX). At concentrations of less than 10 μg/mL, **11–14** exhibited no growth inhibition of the virus. At a concentration of 100 μg/mL, **11–14** showed replications of 20, 10, 15 and 50% respectively. From the relative activities (RAs) (table XII) estimated by comparisons of effective concentrations (EC_{50}) of **11–14** for 50% inibition of replication with that of **1**, the anti-HIV activities of **11–14** against HTLV-IIIB were 11.1, 10.0, 9.1 and 5.0 times higher than that of **1**. However, at the much higher concentration of 1 mg/mL, **11–14** induced cell toxicities, in contrast to **1**. The effects of **11–14** on the inhibition of the replication of GUN-4 [27] using C8166 cells (Hoshino, unpublished data) were further investigated. It is known that sulfate polysaccharides have weak inhibitory activity against replication [29, 30]. Sulfates **11–14** showed no growth inhibition of GUN-4 at drug concentrations less than 100 μg/mL (table X), in contrast to the cases against HTLV-IIIB (table IX).

Next, compounds **29–32**, in which the kinds and linking positions of the two pyranoses were different from those of **11–14**, were assessed for their effects on the inhibition of replication of both HTLV-IIIB (table IX) and GUN-4 (table X). Although no effect was observed at a concentration of 1 μg/mL, **29–32** completely inhibited the replication of HTLV-IIIB in MT-4 cells at a concentration of 100 μg/mL. The RAs of **29–32** (table XII) indicated that the anti-HIV activities of **29–32** were respectively 100, 125.5, 83.3 and 16.7 times higher than that of **1**. In these cases, cell toxicities were also induced at the much higher

Table IV. ^{13}C-NMR chemical shifts for compounds **25–28**[a].

	25	*26*	*27*	*28*
Aglycon				
C-1	39.5	39.5	39.5	39.3
C-2	26.6[b]	26.5[b]	26.4[b]	26.5[b]
C-3	88.8	88.7	88.7	88.7
C-4	39.9	39.8	39.8	39.7
C-5	54.8	55.3	55.4	55.3
C-6	17.7	17.6	17.6	17.5
C-7	32.9	32.9	32.9	32.8
C-8	44.2	44.2	44.2	44.0
C-9	62.0	61.9	61.9	61.7
C-10	37.3	37.2	37.3	37.1
C-11	199.6	199.4	199.3	199.2
C-12	128.7	128.6	128.7	128.6
C-13	169.2	169.2	169.0	168.8
C-14	45.6	45.5	45.5	45.5
C-15	26.8[b]	26.7[b]	26.6[b]	26.6[b]
C-16	25.5	25.9	26.5[b]	26.2
C-17	32.1	32.0	32.1	32.0
C-18	48.7	48.7	48.7	48.5
C-19	41.3	41.3	41.3	41.2
C-20	43.4	43.4	43.4	43.3
C-21	31.3	31.3	31.4	31.2
C-22	38.2	38.1	38.2	38.0
C-23	28.2	28.3[c]	28.2	28.3
C-24	16.8	16.7	16.7	16.6[c]
C-25	17.1	17.0	17.0	16.8[c]
C-26	18.8	18.8	18.8	18.7
C-27	23.5	23.4	23.5	23.4
C-28	28.2	28.1[c]	28.7	28.6
C-29	28.6	28.7	29.1	28.9
C-30	176.9	176.4	176.4	176.1
-OCH_2-	51.2 (OCH_3)	64.5	64.6	64.4
-$(CH_2)_n$-	–	22.7 x 2, 29.0, 31.5	22.9, 26.7, 28.3, 29.4, 29.5, 29.7, 29.8 x 2, 32.1	22.8, 26.3, 28.9, 29.3, 29.5, 29.6, 29.7 x 3, 29.8 x 4, 31.9
-CH_3	–	14.1	14.3	14.2
Inner Sugar				
C-1	105.8	105.7	105.7	105.4
C-2	75.3[c]	75.1	75.2	74.7
C-3	77.1	77.1	77.1	76.7
C-4	82.5	82.3	82.5	82.1
C-5	76.9	76.8	76.8	76.4
C-6	62.5	62.3	62.5	62.2
Outer Sugar				
C-1	106.5	106.4	106.4	106.1
C-2	75.2[c]	75.1	75.1	74.7
C-3	72.5	72.4	72.5	72.0
C-4	70.0	69.9	69.9	69.7
C-5	76.2	76.1	76.1	75.7
C-6	62.1	62.1	62.1	61.9

[a]Spectra were obtained in d_5-pyridine. The signal assignments were based on H-C COSY and the reported spectral data [33–35]. [b,c]These values may be interchangeable in each column.

Table V. ^{13}C-NMR chemical shifts for compounds **29–32**[a].

	29	*30*	*31*	*32*
Aglycon				
C-1	39.6	39.6	40.1	39.5
C-2	27.2	27.2	27.4	27.3
C-3	91.5	91.5	91.6	91.4
C-4	39.9	39.9	39.7	40.0
C-5	55.4	55.3	55.5	55.1
C-6	18.0	17.7	17.8	18.1
C-7	33.3	33.3	33.5	33.3
C-8	44.9	44.8	44.6	44.9
C-9	58.3	58.2	58.9	58.3
C-10	37.3	37.4	37.1	37.4
C-11	203.5	202.9	202.9	202.9
C-12	128.5	128.7	128.4	128.8
C-13	172.7	171.7	171.6	171.4
C-14	46.3	46.3	46.0	46.2
C-15	27.2	27.2	27.4	27.3[b]
C-16	26.3	26.4	26.7	27.0[b]
C-17	32.5	32.5	32.2	32.5
C-18	49.1	48.9	48.5	48.8
C-19	41.4	41.4	42.0	41.2
C-20	44.1	44.1	44.2	44.1
C-21	31.5	31.7	31.5	31.7
C-22	38.9	39.1	38.9	39.2
C-23	27.8	28.4	28.1	28.5
C-24	16.6[b]	16.6	17.6	17.1
C-25	17.0[b]	17.0	18.3	17.1
C-26	19.6	19.6	19.8	19.6
C-27	23.6	23.5	23.3	23.6
C-28	28.4	27.7	27.6	27.7[b]
C-29	29.5	29.3	29.4	29.5
C-30	179.8	178.2	179.2	178.2
$-OCH_2-$	51.2 (OCH_3)	65.5	65.4	65.3
$-(CH_2)_n-$	–	23.2 x 2, 29.6, 32.0	21.6, 25.1 x 2, 31.3, 32.3 x 3, 34.4 x 2	23.4 x 2, 29.6, 30.2 x 2, 30.5 x 5, 30.6 x 4, 32.7 x 2
$-CH_3$	–	14.7	16.5	14.7
Inner Sugar				
C-1	101.2	101.3	101.5	101.3
C-2	74.1	74.1	74.3	74.1
C-3	78.3	78.3	78.6	78.5
C-4	80.5	80.5	80.6	80.4
C-5	76.2[c]	76.3	76.5	76.3
C-6	62.4	62.4	64.0	62.3[c]
Outer Sugar				
C-1	103.8	103.7	103.8	103.7
C-2	72.9	72.9	73.0	72.9
C-3	68.1	68.0	68.2	68.0
C-4	66.7	66.7	68.2	66.6
C-5	76.0[c]	76.0	76.2	76.3
C-6	62.4	62.4	62.5	62.2[c]

[a]Spectra were obtained in d_5-pyridine. The signal assignments were based on the reported spectral data [33–35] and the data listed in table IV. [b,c]These values may be interchangeable in each column.

Table VI. ^{13}C-NMR chemical shifts for compounds **35** and **37**[a].

	35	*37*
Aglycon		
C-1	37.5	30.9[b]
C-2	28.3	28.0
C-3	81.3	75.2
C-4	42.4	30.8[b]
C-5	48.3[b]	36.3
C-6	19.1	26.4
C-7	33.3	26.4
C-8	39.9	36.0
C-9	48.2[b]	40.0
C-10	34.4	35.6
C-11	24.7	21.1
C-12	122.9	40.8
C-13	142.1	41.7
C-14	42.3	58.6
C-15	26.7[c]	32.3
C-16	18.1	83.0
C-17	47.6	62.1
C-18	41.9	19.1[c]
C-19	46.8	24.1
C-20	31.3	19.7
C-21	34.1	18.7[c]
C-22	33.9	116.9
C-23	62.4	24.2[d]
C-24	17.2	24.1[d]
C-25	13.7	27.8
C-26	17.9	66.6
C-27	26.8[c]	19.0
C-28	181.0	–
C-29	33.7	–
C-30	23.9	–
-OCH_3	51.2	–
Inner Sugar		
C-1	104.7	100.5
C-2	83.4	84.1
C-3	74.1	74.7
C-4	70.3	69.8
C-5	77.4	64.1
C-6	61.9	–
Outer Sugar		
C-1	105.3	100.2
C-2	77.6	73.8
C-3	78.6	72.5
C-4	71.3	74.0
C-5	80.0	69.6
C-6	62.0	19.1

[a]Spectra were obtained in d_5-pyridine. The signal assignments were based on the reported spectral data [36, 37]. [b,c,d]These values may be interchangeable in each column.

concentration of 1 mg/mL. While **29–32** showed weaker effects against GUN-4 in C8166 cells than against HTLV-IIIB in MT-4 cells, they had stronger growth inhibitory activities against the former virus than did **11–14**. At a concentration of 100 μg/mL, sulfates **29–32** suppressed the replication of GUN-4 by 20, 30, 25 and 50% respectively, though no effect was observed at concentrations of less than 10 μg/mL.

Finally the effects of sulfates **35** and **37**, which differed both in kinds of pyranoses and structures of aglycons from **11–14**, and alkyl thioglycoside sodium sulfates **41**, **42**, **47** and **48**, with no triterpenoid or steroid rings in the molecules, were investigated against HTLV-IIIB using MT-4 cells (table XI). Sulfates **35** and **37** suppressed the replication by 75 and 50% respectively at a concentration of 10 μg/mL, and completely inhibited the growth of the virus at a concentration of 100 μg/mL. The RAs of **35** and **37** indicated that the anti-HIV activities of **35** and **37** were respectively 11.6 and 50.0 times higher than that of **1** (table XII). Although thioglycoside and thiolactoside sodium sulfates **41** and **47**, with C_8-alkyl chains, showed no effect at concentrations less than 100 μg/mL, sulfates **42** and **48**, with longer alkyl chains (C_{18}), suppressed the replications by 80 and 60% respectively at a concentration of 10 μg/mL and **42** by 30% at a concentration of 100 μg/mL. At the latter concentration, however, **48** showed cell toxicity.

As sodium sulfates **11–14**, **29–32**, **35**, **37**, **41**, **42**, **47** and **48** as well as **1** and **2** seemed to be surfactants because of the presence of both hydrophilic groups (sugar moieties) and hydrophobic ones (aglycons and alkyl groups) in the molecules, the relationship between the anti-HIV activity and micelle forming ability of each sodium sulfate were investigated. Anti-HIV activity was assessed with HTLV-IIIB, and micelle formation was estimated from critical micelle concentrations (CMCs) of the sulfates, obtained by colorimetric determinations using pynacyanol chloride [28]; it is generally accepted that the CMC value is inversely related to micelle forming ability. The CMC values of the sodium sulfates, together with the relative anti-HIV activities, are listed in table XII.

From the results shown in table XII, sodium sulfates exhibiting lower CMC values (ie, higher abilities to form micelles), seemed to have more potent anti-HIV activities. When the CMC values of sodium sulfates of alkylated glycosides, **11–14**, were compared with that of **2**, the value of **13** (7.5×10^{-3} M) was found to be near to that of **2** (8.0×10^{-3} M), the values of **11** (4.8×10^{-3} M) and **12** (2.6×10^{-3} M) were lower, and in contrast the value of **14** (5.2×10^{-2} M) was higher than that of **2**. These results were consistent with the trends in the anti-HIV activities: those of **11** and **12** were higher than that of **2**, the activity of **14** was lower than that of **2**, and the

Table VII. ^{1}H-NMR data of compounds **39**, **43** and **44**[a].

	39	*43*	*44*
Glc			
H-1	4.48 (d, 9.9)[b]	4.47 (d, 10.3)	4.46 (d, 9.9)
H-2	5.05 (dd, 9.9)	4.93 (dd, 10.3, 9.5)	4.89–4.98
H-3	5.22 (dd, 9.9, 9.9)	5.21 (dd, 9.5, 9.5)	5.21 (dd, 9.5, 9.5)
H-4	5.08 (dd, 9.9, 9.9)	3.78 (dd, 9.5, 9.5)	3.78 (dd, 9.5, 9.5)
H-5	3.70 (ddd, 9.9, 4.8, 2.2)	3.62 (ddd, 9.5, 5.1, 1.6)	3.62 (ddd, 9.5, 6.9, 1.8)
H-6a	4.25 (dd, 12.5, 5.1)	4.06–4.11	4.05–4.15
H-6b	4.15 (dd, 12.5, 2.2)	4.06–4.11	4.05–4.15
Gal			
H-1'	–	4.50 (d, 7.7)	4.48 (d, 7.7)
H-2'	–	5.10 (dd, 10.3, 7.7)	5.11 (dd, 10.3, 7.7)
H-3'	–	4.96 (dd, 10.3, 3.7)	4.89–4.98
H-4'	–	5.34 (d, 3.7)	5.35 (d, 3.7)
H-5	–	3.88 (dd, 6.6, 6.6)	3.88 (dd, 6.9, 6.9)
H-6'a	–	4.06–4.11	4.05–4.15
H-6'b	–	4.44–4.49	4.40–4.50
$-SCH_2-$	2.65 (s)	2.62 (s)	2.62 (s)
$-CH_2-$	1.28–1.50 (CH_2 x 16)	1.26–1.59 (CH_2 x 6)	1.25–1.58 (CH_2 x 16)
$-CH_3-$	0.89 (t, 6.6)	0.88 (t, 6.9)	0.88 (t, 6.2)
OAc	2.00, 2.03, 2.06, 2.08, 2.17	1.96, 2.05 x 2, 2.06, 2.11, 2.12, 2.15	1.97, 2.04, 2.05 x 2, 2.07, 2.11, 2.15

[a]Spectra were obtained in $CDCl_3$. The signal assignments were based on the H-H COSY method. [b]Coupling constants (J in Hz) are given in parentheses.

Table VIII. ^{13}CMR chemical shifts for compounds **40–42** and **45–48**.

	40[a]	*45*[b]	*46*[b]	*41*[a]	*42*[a]	*47*[b]	*48*[b]
Glc							
C-1	87.7	86.8	86.8	87.1	87.7	86.9	86.7
C-2	71.1	74.2	74.2	70.9	71.0	74.6	74.5
C-3	77.6	78.2	78.3	77.5	77.6	78.4	78.3
C-4	70.2	70.1	70.1	70.2	70.2	70.0	70.3
C-5	79.5	81.8	81.4	80.5	79.5	81.5	81.4
C-6	62.2	62.0	62.0	62.3	62.2	62.4	62.0
Gal							
C-1'	–	105.7	105.8	–	–	101.7	101.3
C-2'	–	72.5	72.2	–	–	72.6	72.6
C-3'	–	77.3	77.2	–	–	77.4	77.3
C-4'	–	75.2	75.2	–	–	75.8	75.7
C-5'	–	80.6	80.6	–	–	80.6	80.3
C-6'	–	62.2	62.2	–	–	62.3	62.2
$-SCH_2-$	23.3	22.9	22.9	23.4	23.3	22.9	22.9
$-CH_2-$	30.1 x 3, 30.3 x 2, 30.4 x 3, 30.6 x 6, 32.6 x 2	29.2, 29.4 x 2, 30.1, 30.3, 32.0	29.5, 29.6, 29.8 x 2, 29.9 x 2, 30.0 x 7	29.7, 29.8 x 2, 30.6, 30.7, 32.6	30.1 x 2, 30.3 x 2, 30.4 x 2, 30.6 x 8, 32.6 x 2	28.6, 28.7, 28.9 x 2, 29.1 x 2	29.1 x 2, 29.4 x 2, 32.1 x 2, 30.0 x 10
$-CH_3-$	14.1	14.3	14.3	14.6	14.8	14.3	14.3

[a]These spectra were obtained in C_5H_5N. [b]These spectra were obtained in D_2O, and dioxane was used as an internal standard.

Table IX. Replications (%) and cell toxicities on the expression of HTLV-IIIB-specific antigens in MT-4 cells after administration of compounds **1**, **2**, **11–14** and **29–32**.

Compound	*Drug concentration (μg/mL)*	*Percentage of IF-positive cells (Cell toxicity; –, ++)*[a]				*EC_{50}*[b] *(μg/mL)*
		1000	*100*	*10*	*1*	
1		0 (–)	100 (–)	100 (–)	100 (–)	500
2		NT[c] (++)	24 (–)	100 (–)	100 (–)	60[d]
11		NT (++)	20 (–)	100 (–)	100 (–)	50
12		NT (++)	10 (–)	100 (–)	100 (–)	40
13		NT (++)	15 (–)	100 (–)	100 (–)	55
14		NT (++)	50 (–)	100 (–)	100 (–)	100
29		NT (++)	0 (–)	10 (–)	100 (–)	5
30		NT (++)	0 (–)	5 (–)	100 (–)	4
31		NT (++)	0 (–)	15 (–)	100 (–)	6
32		NT (++)	0 (–)	80 (–)	100 (–)	30

[a]Cell toxicity: –, none; ++, strong. [b]Half value of effective drug concentration for inhibition of replication. [c]Not tested because of cell toxicity. [d]Nakashima et al [11] gave the EC_{50} of 55 μg/mL for this compound.

activities of **13** and **2** were similar. Sulfates **29–32** included the same alkylated aglycons as **11–14**, respectively, but a different sugar moiety, β-lactopyranose (4-*O*-β-D-galactopyranosyl-β-D-glucopyranose), from that of the latters. The CMC values of **29–31** were lower by the order of about 10^{-1} M than those of the corresponding sulfates **11–13**. The anti-HIV activities of **29–31** increased remarkably: the RAs of **29**, **30** and **31** were measured as 100.0, 125.5 and 83.3 respectively. The CMC value (2.0×10^{-3} M) of **32**, which bears an *n*-octadecyl group, was higher than the CMC values (3.3×10^{-4}, 2.5×10^{-4} and 5.4×10^{-4} M respectively) of **29–31**, which bear shorter alkyl groups (methyl, *n*-hexyl and *n*-undecyl groups respectively); and the relative activity (RA = 16.7) of **32** was lower than those of **29–31**. These results indicated that the sulfates with proper length alkyl chains (methyl, hexyl and undecyl groups) on the aglycons exhibited lower CMC values and higher anti-HIV activities than **2**, with no alkyl chain. Sodium sulfates **35** and **37**, with different aglycons from **11–14** and **29–32**, showed CMC values of 3.5×10^{-3} and 1.7×10^{-4} M, and RAs of 11.6 and 50.0, respectively, which suggests that the anti-HIV activity of glycoside sulfates does not depend on the structure of the aglycon but on the ability to form micelles.

The relationship of anti-HIV activity to micelle forming ability of sodium sulfates without triterpenoid or steroid rings in the molecules was also investigated. Octyl thioglycoside and octyl thiolactoside sulfates **41** and **47** showed higher CMC values, of 4.0×10^{-2} and 1.5×10^{-2} M respectively, and they had no activities at concentrations lower than 100 μg/mL. On the other hand, replication was considerably inhibited by octadecyl thioglucoside sulfate **42** with a CMC of 5.2×10^{-4} M: by 80 and 30% at concentrations of 10 and

Table X. Replications (%) and cell toxicities on the expression of GUN-4-specific antigens in C8166 cells after administration of compounds **11–14** and **29–32**.

Compound	*Drug concentration (μg/mL)*	*Percentage of IF-positive cells (Cell toxicity; –, ++)*[a]			EC_{50}[b] *(μg/mL)*
		100	*10*	*1*	
11		100 (–)	100 (–)	100 (–)	> 100[c]
12		100 (–)	100 (–)	100 (–)	> 100[c]
13		100 (–)	100 (–)	100 (–)	> 100[c]
14		100 (–)	100 (–)	100 (–)	> 100[c]
29		20 (–)	100 (–)	100 (–)	70
30		30 (–)	100 (–)	100 (–)	80
31		25 (–)	100 (–)	100 (–)	70
32		50 (–)	100 (–)	100 (–)	100

[a]Cell toxicity: –, none; ++, strong. [b]Half value of effective drug concentration for inhibition of replication. [c]Not obtained because of cell toxicity.

Table XI. Replications (%) and cell toxicities on the expression of HTLV-IIIB-specific antigens in C8166 cells after administration of compounds **35**, **37**, **40**, **41**, **47** and **48**.

Compound	*Drug concentration (μg/mL)*	*Percentage of IF-positive cells (Cell toxicity; –, ++)*[a]			EC_{50}[b] *(μg/mL)*
		100	*10*	*1*	
35		0 (–)	75 (–)	100 (–)	45
37		0 (–)	50 (–)	100 (–)	10
41		100 (–)	100 (–)	100 (–)	> 100[c]
42		30 (–)	80 (–)	100 (–)	60
47		100 (–)	100 (–)	100 (–)	> 100[c]
48		NT (–)	60 (–)	100 (–)	> 10[c]

[a]Cell toxicity: –, none; ++, strong. [b]Half value of effective drug concentration for inhibition of replication. [c]Not obtained because of cell toxicity.

Table XII. Comparison of anti-HIV activities against HTLV-IIIB with abilities of micelle formations of sodium sulfates of alkylated glycosides **11–14**, **29–32**, **35** and **37** and thioglycosides **40**, **41**, **47** and **48**.

Compound	*Alkyl group*	*CMC (M)*[a]	*Relative activity (RA)*[b]
1[e]	–	**[c]	
2[e]	–	8.0×10^{-3}	8.3
11[e]	Methyl	4.8×10^{-3}	11.1
12[e]	Hexyl	2.6×10^{-3}	15.2
13[e]	Undecyl	7.5×10^{-3}	9.1
14[e]	Octadecyl	5.2×10^{-2}	5.0
29[e]	Methyl	3.3×10^{-4}	100.0
30[e]	Hexyl	2.5×10^{-4}	125.5
31[e]	Undecyl	5.4×10^{-4}	83.3
32[e]	Octadecyl	2.0×10^{-3}	16.7
35[e]		3.5×10^{-3}	11.6
37[e]		1.7×10^{-4}	50.0
41	Octyl	4.0×10^{-2}	NT[d]
42[e]	Octadecyl	5.2×10^{-4}	8.3
47	Octyl	1.5×10^{-2}	NT[d]
48	Octadecyl	1.0×10^{-4}	NT[d]

[a]These values were obtained by the colorimetric method reported by Corrin et al [28]. [b]These values were obtained by comparisons of the EC_{50}s of compounds with that of **1**. [c]Not obtained because of the low solubility of **1**. [d]Not tested because of the cell toxicity. [e]A statistically significant correlation: $p < 0.01$, which was obtained by calculating the Spearman's rank-order correlation coefficient (Is = –0.945) for the correlation of CMC values and relative anti-HIV activities of these compounds.

100 μg/mL respectively. *n*-Octadecyl thiolactoside sulfate **48**, with a CMC value of 1.0×10^{-4} M, inhibited the replication to the extent of 60% at a concentration of 10 μg/mL. However, the inhibition was not determined at concentrations greater than 100 μg/mL because of the resulting cell toxicity. Thus, it was revealed that even sulfates with no triterpenoid or steroid ring in the molecule had anti-HIV activities. However, in spite of the lower CMC of **42** (5.2×10^{-4} M) compared with those of **11–13** (2.6×10^{-3} – 7.5×10^{-3} M) and **35** (3.5×10^{-3} M), the relative activity of **42** (RA = 8.3) was still weaker than **11**, **12**, **13** and **35** (RA = 11.1, 15.2, 9.1 and 11.6 respectively). These results suggest that the presence of triterpenoid or steroid rings in the glycoside molecules increases the activity, though the role of the rings in the appearance of the activity has not been elucidated yet. In order to examine the effects of sulfates **29**, **30** and **31** on the early stage of HIV-1 infection, the syncytium formation assay was performed with a slight modification to a published procedure [29]. From table XIII, it appears that these sulfates markedly inhibited syncytium formation at concentrations of 100 μg/mL or more, although these sulfate were slightly cytotoxic at 1000 μg/mL. This finding indicates that the sulfates inhibited HIV-1 infection at early steps of the replication cycle of HIV-1.

Conclusions

In vitro anti-HIV activities of synthesized sodium sulfates, **11–14**, **29–32**, **35** and **37**, of various alkyl glycyrrhizins and related compounds were compared with that of glycyrrhizin **1**. The activities were evaluated by the inhibition in replication of HTLV-IIIB using MT-4 cells and of GUN-4 using C8166 cells. The sulfates inhibited replication of HTLV-IIIB much more than the replication of GUN-4. Sulfates **29–32** inhibited completely the replication of HTLV-IIIB at drug concentrations less than 100 μg/mL, and showed EC_{50}s of 5, 4, 6 and 30 μg/mL respectively. On the

Table XIII. Inhibition of syncytium formation induced by HIV-1 by sulfates **29**, **30** and **31**[a].

Compound	*Drug concentration (μg/mL)*	*Number of syncytia (Cell toxicity; –, +)*[b]				
		1000	*100*	*10*	*1*	*0*
29		0[c] (±)	9 (–)	54 (–)	52 (–)	64 (–)
30		0 (+)	5 (–)	59 (–)	69 (–)	64 (–)
31		0 (+)	7 (–)	39 (–)	72 (–)	64 (–)

[a]MOLT-4 cells were cocultivated with HIV-1 with HIV-1-producing MOLT-4/HTLV-IIIB cells for 24 h in the presence of sulfates **29**, **30** and **31**. Syncytia in wells were counted under an inverted microscope. [b]Cell toxicity: –, none; +, weak. [c]Number of syncytia.

other hand, sulfates **11–14** still showed replications of 10–50% at a concentration of 100 μg/mL, and showed EC_{50}s of 50, 40, 55 and 100 μg/mL respectively. Among these sulfates, **11–14**, **29–32**, **35** and **37**, the anti-HIV activities of **29**, **30** and **31** were increased remarkably, by 100.0, 125.5 and 83.3 times respectively, from that of **1**. These potent active compounds had CMC values of 2.5–5.4 x 10^{-4} M, lower than compounds of lower activity such as **2**, **11–14** and **32** (2.6 x 10^{-3} – 5.2 x 10^{-2} M). Thus, the higher the ability to form micelles, the greater the anti-HIV activity. However, in spite of the lower CMC values of **41** (5.2 x 10^{-4} M) and **48** (1.0 x 10^{-4} M), their activities were lower than expected, which suggested that the presence of a triterpenoid or steroid ring in the molecule was required for enhancement of the activity. From the syncytium formation assay, it appeared that the sulfates with anti-HIV activities inhibited HIV-1 infection at early stages of the replication cycle of the virus.

Experimental protocols

General procedures

Dry dichloromethane (CH_2Cl_2) was obtained by refluxing with NaH followed by distillation. DMC was kindly donated by Shiratori Pharmaceutical Co Ltd (Tsudanuma 6-11-24, Narashino Chiba 275, Japan). Other chemicals and solvents were of reagent grade, and were obtained from commercial sources. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The thin-layer chromatography (TLC) utilized Kieselgel 60 F_{254} (Merck), and spots were detected by spraying the plates with $Ce(SO_4)_2$/10% H_2SO_4 (1:9) reagent, followed by heating at 100 °C for 10 min. Column chromatography was carried out on a Wakogel C-200 and the eluates were monitored by TLC. An SSC-6300 (Senshu Scientific Co Ltd) equipped with an SSC-3000A was employed for analytical HPLC using ODS-1251-D (4.6 x 250 mm), and was further equipped with an SSC autoinjector 6310 and an SSC fraction collector 6320 for preparative HPLC using ODS-4521-D (10 x 250 mm; flow rate, 1.0 mL/min; column temp, 40 °C). ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-GX NMR spectrometer at 270 and 67.8 MHz respectively. Tetramethylsilane was used as an internal standard, unless otherwise stated, and chemical shifts are given in ppm. Multiplicities of ^{1}H-NMR signals are indicated as s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-DX 300 mass spectrometer. Visible spectra were recorded on a Hitachi-U-3200 spectrophotometer.

Chemistry

*Glycyrrhizin trimethylester **3***
To a solution of glycyrrhizin **1** (10.0 g, 12.4 mmol) in MeOH (100 mL) was added freshly prepared diazomethane ether solution at 0 °C until nitrogen gas was no longer evolved. The reaction mixture was evaporated to yield a residue that was subjected to column chromatography (a gradient of 0–5% MeOH in CH_2Cl_2), followed by recrystallization from 95% EtOH, to give **3** (8.5 g). Physical data for **3** are listed in table XIV.

*Preparation of trialkylated glycyrrhizins **4–6**. Standard procedure*
To a solution of **1** (5.0 g, 6.2 mmol) and 1-hexanol (32 mL, 25.5 mmol) in pyridine (80 mL) was added 2-chloro-1,3-dimethylimidazolium chloride (DMC) (2.0 g, 11.8 mmol), which was then stirred for 24 h at room temperature. The reaction mixture was worked up as described for **3** to give **4** (3.7 g). Table XIV gives the physical data for products **4–6**.

*Preparation of monoalkylated glycyrrhizins **7–10**. Standard procedure*
A solution of **4** (2.4 g, 2.8 mmol) in 5% KOH in EtOH/H_2O (1:1) (10 mL) was allowed to stand for 5 h at room temperature. After neutralization with AcOH, the mixture was passed through Amberlite IR-120B (H^+ form) column chromatography and eluted with distilled water. The eluent was evaporated to yield a residue that was subjected to column chromatography ($CHCl_3$/MeOH/H_2O = 65:35:10, lower layer) to give **7** (1.7 g). Physical data for products **7–10** are listed in table XIV.

*Acid hydrolysis of **7–10**. Standard procedure*
Compound **7** (5 mg, 5.3 μmol) was dissolved in 1 N H_2SO_4 and heated at 80 °C for 2 h. After cooling, the mixture was neutralized with $BaCO_3$-saturated water, then centrifuged to give a supernatant solution. The solution was passed through Amberlite IR-120B (H^+ form) column chromatography and eluted with distilled water. The eluent was evaporated to compound **16** which was confirmed by TLC with the authentic sample. Similarly acid hydrolysis of **8**, **9** and **10** gave **17**, **18** and **19** respectively.

*Preparation of sodium sulfates of monoalkylated glycyrrhizins, **11–14**. Standard procedure*
A solution of **7** (470 mg, 0.5 mmol) and SO_3-pyridine complex (8.0 g, 5.0 mmol) in dry pyridine (20 mL) was stirred under shielding from light for 24 h at room temperature. After adjusting the pH to 8–9, the mixture was evaporated to obtain a residue. An aqueous solution (2 mL) of the residue was passed through Diaion HP-20 column chromatography and eluted first with distilled water, then with 50% H_2O/MeOH. The 50% MeOH eluent was evaporated to give **11** (570 mg). Physical data for products **11–14** are listed in table XIV.

*Alkylations of glycyrrhetic acid **17–19**. Standard procedure*
1-Undecyl alcohol (5 mL, 23.9 mmol) and DMC (3.6 g, 21.3 mmol) were added to a solution of glycyrrhetic acid **15** (5 g, 10.6 mmol) in dry pyridine (20 mL). After stirring for 24 h at room temperature, the mixture was poured into ice-water (100 mL), then extracted with CH_2Cl_2 (80 mL x 3). The combined organic extracts were successively washed with $NaHCO_3$-saturated water and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to afford a residue. The residue was subjected to column chromatography (a gradient of 0–2% acetone in benzene) to give *n*-undecyl glycyrrhetinate **18** (4.7 g) as a colorless amorphous powder. Physical data for products **17–19** are listed in table XIV. ^{1}H-NMR spectrum of **17** (only assignable signals are listed) ($CDCl_3$): δ 0.81, 0.81, 1.01, 1.13, 1.14, 1.15, 1.37 (each s, 3H, CH_3), 0.89 (dd, J = 6.6, 6.6 Hz, 3H, -CH_2CH_3), 1.33 (s, 8H, -CH_2- x 4), 2.34 (s, 1H, H-9), 2.79 (broad d, J = 13.6 Hz, 1H, H-18), 3.22 (dd, J = 10.3, 5.9 Hz, 1H, H-3), 4.09 (dd, J = 6.6, 6.6 Hz, 2H, -$COOCH_2$-), 5.65 (s, 1H, H-12). ^{1}H-NMR spectrum of **18** (only assignable

Table XIV. Physical data for the compounds synthesized in this study.

Starting material	*Product yield (%)*	*FABMS*[a]	*Formula*	*Calc*		*Found*	
				C	*H*	*C*	*H*
1	**3** (77.7) mp 194–196[b]	887	$C_{45}H_{68}O_{16}\cdot H_2O$	61.21	7.99	61.41	8.21
1	**4** (55.9)	1097	$C_{60}H_{98}O_{16}\cdot H_2O$	65.91	9.22	65.68	9.30
1	**5** (40.6)	1307	$C_{75}H_{128}O_{16}\cdot 1/2H_2O$	69.57	10.00	69.43	10.05
1	**6** (54.5)	1601	$C_{96}H_{170}O_{16}\cdot H_2O$	72.14	10.85	72.08	10.93
3	**7** (72.1)	859	$C_{43}H_{64}O_{16}\cdot H_2O$	60.41	7.78	60.55	7.81
4	**8** (85.9)	897	$C_{48}H_{74}O_{16}\cdot H_2O$	62.32	8.28	62.22	8.40
5	**9** (70.8)	999	$C_{53}H_{84}O_{16}\cdot 1/2H_2O$	64.54	8.69	64.33	8.84
6	**10** (88.1)	1065	$C_{60}H_{98}O_{16}\cdot H_2O$	65.91	9.22	65.77	9.35
7	**11** (73.1)	1413	$C_{43}H_{57}O_{31}S_5Na_7\cdot 2H_2O$	36.19	4.31	35.98	4.13
8	**12** (62.2)	1483	$C_{48}H_{67}O_{31}S_5Na_7\cdot 3/2H_2O$	38.74	4.74	38.48	4.80
9	**13** (73.5)	1553	$C_{53}H_{77}O_{31}S_5Na_7\cdot 3H_2O$	40.15	5.28	39.97	5.40
10	**14** (33.0)	1651	$C_{60}H_{91}O_{31}S_5Na_7\cdot 5/2H_2O$	43.03	5.78	42.85	5.94
15	**17** (83.7)	577	$C_{36}H_{58}O_4$	77.92	10.54	77.81	10.35
15	**18** (71.2)	647	$C_{41}H_{68}O_4$	78.79	10.97	78.83	10.77
15	**19** (74.4)	745	$C_{48}H_{82}O_4$	79.72	11.43	79.58	11.49
16	**21** (57.0) mp 214–215[c]	1125	$C_{57}H_{82}O_{21}$	62.05	7.49	62.23	7.43
17	**22** (52.0)	1195	$C_{62}H_{92}O_{21}$	63.46	7.90	63.29	8.01
18	**23** (50.3)	1265	$C_{67}H_{102}O_{21}$	64.71	8.27	64.36	8.40
19	**24** (40.4)	1363	$C_{74}H_{116}O_{21}$	66.24	8.71	66.36	8.65
21	**25** (82.7)	831	$C_{43}H_{68}O_{14}\cdot 1/2H_2O$	63.14	8.50	63.24	8.38
22	**26** (80.5)	901	$C_{48}H_{78}O_{14}\cdot 1/2H_2O$	64.91	8.97	64.94	8.86
23	**27** (70.9)	971	$C_{53}H_{88}O_{14}\cdot 1/2H_2O$	66.29	9.55	66.38	9.36
24	**28** (81.0) mp 246–247[b]	1069	$C_{60}H_{102}O_{14}\cdot 1/2H_2O$	68.21	9.84	67.97	9.72
25	**29** (84.6)	1545	$C_{43}H_{61}O_{35}S_7Na_7\cdot H_2O$	33.51	3.97	33.37	4.13
26	**30** (73.3)	1615	$C_{48}H_{71}O_{35}S_7Na_7\cdot 1/2H_2O$	35.98	4.47	35.67	4.51
27	**31** (75.9)	1685	$C_{53}H_{81}O_{35}S_5Na_7\cdot H_2O$	37.86	4.86	37.81	4.90
28	**32** (80.4)	1783	$C_{60}H_{102}O_{31}S_5Na_7\cdot H_2O$	40.49	5.38	40.23	5.44
33	**34** (99.2)	787	$C_{42}H_{68}O_{12}$	65.94	8.96	65.74	9.23
34	**35** (85.2)	1399	$C_{42}H_{62}O_{30}S_6Na_6\cdot 2H_2O$	35.69	4.71	35.51	4.86
36	**37** (87.5)	1477	$C_{39}H_{57}O_{34}S_7Na_7\cdot 3/2H_2O$	26.31	4.08	26.15	4.29
49	**39** (90.0)	639	$C_{32}H_{56}O_9S$	62.31	9.15	62.12	9.30
39	**40** (79.2)	471	$C_{24}H_{48}O_5S\cdot 1/2H_2O$	63.00	10.79	62.82	11.03
38	**41** (61.9)	739	$C_{14}H_{24}O_{17}S_5Na_4\cdot 5/2H_2O$	21.96	3.82	21.79	3.55
40	**42** (59.9)	879	$C_{24}H_{44}O_{17}S_5Na_4\cdot 2H_2O$	32.28	5.42	32.15	5.48
19	**43** (89.0)	787	$C_{34}H_{52}O_{17}S$	53.39	6.85	53.14	6.97
19	**44** (72.3)	927	$C_{44}H_{72}O_{17}S$	58.39	8.02	58.19	8.21
43	**45** (81.4)	493	$C_{20}H_{38}O_{10}S\cdot 1/3H_2O$	50.40	8.11	50.26	8.32
44	**46** (83.3)	633	$C_{30}H_{58}O_{10}S\cdot 2/3H_2O$	57.85	9.38	57.97	9.34
45	**47** (35.1)	1207	$C_{20}H_{31}O_{31}S_8Na_7\cdot 3/2H_2O$	19.82	2.83	19.62	3.01
46	**48** (64.5)	1347	$C_{30}H_{51}O_{31}S_8Na_7\cdot 2H_2O$	26.47	4.07	26.40	4.29

[a]Peaks were observed as the quasimolecular ion $[M + Na]^+$. [b]After recrystallization from 50% EtOH. [c]After recrystallization from Et_2O/petroleum ether.

signals are listed) ($CDCl_3$): δ 0.81, 0.81, 1.01, 1.13, 1.14, 1.15, 1.37 (each s, 3H, CH_3), 0.88 (dd, J = 7.0, 7.0 Hz, 3H, -CH_2CH_3), 1.26 (s, 18H, -CH_2- x 9), 2.34 (s, 1H, H-9), 2.79 (broad d, J = 13.6 Hz, 1H, H-18), 3.23 (dd, J = 10.3, 5.9 Hz, 1H, H-3), 4.09 (dd, J = 7.0, 7.0 Hz, 2H, -$COOCH_2$-), 5.65 (s, 1H, H-12). ^{1}H-NMR spectrum of **19** (only assignable signals are listed) ($CDCl_3$): δ 0.81, 0.81, 1.01, 1.13, 1.14, 1.15, 1.37 (each s, 3H, CH_3), 0.88 (dd, J = 6.6, 6.6 Hz, 3H, -CH_2CH_3), 1.25 (s, 32H, -CH_2- x 16), 2.34 (s, 1H, H-9), 2.80 (broad d, J = 13.6 Hz, 1H, H-18), 3.23 (dd, J = 9.9, 5.9 Hz, 1H, H-3), 4.09 (dd, J = 6.6, 6.6 Hz, 2H, -$COOCH_2$-), 5.65 (s, 1H, H-12).

*Glycosylations of alkyl glycyrrhetinates **16–19** with 6-O-(2',3',4',6'-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,4-tri-O-acetyl-α-D-glucopyranosyl bromide **20**. Standard procedure*
Ag_2CO_3 (3 g, 10.9 mmol) and Drierite (1 g, 7.3 mmol) were added to a solution of **18** (1.8 g, 2.9 mmol) in dry CH_2Cl_2 (10 mL). After stirring under shielding from light at room temperature for 1 h, **20** (6.5 g, 9.3 mmol) was added and the mixture further stirred at room temperature for 2 days. The reaction mixture was filtered, and the filtrate was poured into ice-water (100 mL), then extracted with CH_2Cl_2 (80 mL x 3). The combined organic extracts were successively washed with $NaHCO_3$-saturated water and water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to afford a residue. The residue was subjected to column chromatography (a gradient of 0–4% AcOEt in benzene), followed by application of preparative HPLC (MeOH), to give *n*-undecyl 3-*O*-[4'-*O*-(2",3",4",6"-tetra-*O*-acetyl-β-D-galactopyranosyl)-2',3',6'-tri-*O*-acetyl-β-D-glucopyanosyl]-glycyrrhetinate **23** (1.8 g) as a colorless amorphous powder. Physical data for **21–24** are listed in table XIV.

*Deacetylation of compounds **21–24**. Standard procedure*
A solution of **23** (1.5 g, 1.2 mmol) in 5% KOH in EtOH/H_2O (1:1) (25 mL) was allowed to stand overnight at room temperature. After neutralization with AcOH, the mixture was evaporated to afford a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O = 65:35:10, lower layer) to give **27** (820 mg) as a colorless amorphous powder. Physical data are listed in table XIV.

*Sulfation of compounds **25–28**. Standard procedure*
A solution of **27** (500 mg, 0.52 mmol) and anhydrous SO_3-pyridine complex (1.8 g, 11.3 mmol) in dry pyridine (8 mL) was allowed to stand under shielding from light at room temperature for 24 h. NaOH (1.0 M) was added to the reaction mixture to adjust the pH to 8–9, then evaporated to obtain a residue. The residue was dissolved in distilled water (5 mL), applied on a Diaion (HP-20) column, then successively eluted with distilled water (150 mL) and 50% MeOH in H_2O (150 mL). The 50% MeOH eluent was evaporated to give **31** (673 mg) as a colorless amorphous powder. Physical data for **29–32** are listed in table XIV.

Pharmacology

Drugs and cells
Stock solutions of drugs were prepared in DMSO at 10 mg/mL and stored at 4 °C. MT-4 and C8166 cells were maintained in RPMI1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum.

Anti-HIV assay. Standard procedure
Five hundred microliters of MT-4 cells (2 x 10^5/mL) were seeded into 48-well Costar plates. Serially-diluted drug solution (50 μL) in PBS was then added to each well. After incubation for a few hours, HIV-1 [HTLV-IIIB] was inoculated into the wells at a multiplicity of infection of 0.05. Four days later the cells were smeared onto glass slides and fixed with acetone. Expression of HIV-1 antigens in MT-4 cells was detected by indirect immunofluorescence assay [31]. Serum from an HIV-1-infected subject and FITC-conjugated anti-human IgG were used as the first and second antibodies respectively. Fluorescent cells were counted under a fluorescence microscope. Assay of GUN-4 using C8166 cells was performed using the same procedure as for HTLV-IIIB. The GUN-4 strain of HIV-1, derived from a Japanese hemophiliac with AIDS, is a syncytium-inducing type of clinical isolate, which has been passaged a few times in established T cell lines [27, 32]. This strain was used to examine anti-HIV-1 activities of drugs against a clinical isolate.

Syncytium assay
A suspension of MOLT-4 (2 x 10^5 cells/mL) in RPMI1640 medium (total 100 μL) was added to each drug. After incubation at 37 °C for 1 h, the suspension was cocultured with MOLT-IIIB (5 x 10^4 cells/mL) (100 μL) at the same temperature for 1 day. The cells were seeded into a 96-well microplate (the total amount of culture medium in each well was 200 μL). Numbers of syncytia were counted under an inverted microscope.

References

1 Suzuki H, Ohta Y, Takino Y, Fujisawa K, Hiroshima C, Shimizu N, Aso Y (1977) *Igaku no Ayumi* 102, 562–577
2 Kiso Y, Tohkin M, Hikino H, Hattori M, Sakamoto T, Namba T (1984) *Planta Medica* 298–302
3 Okabe S, Takata Y, Takeuchi K, Naganuma T, Takagi K (1976) *Dig Dis Sci* 21, 618–625
4 Yano S, Harada M, Watanabe K et al (1989) *Chem Pharm Bull* 37, 2500–2504
5 Sotomatsu S, Takaishi Y, Hiroi J, Namikata A, Okano N (1959) *Skin Urol* 21, 138–144
6 Kuroyanagi T, Kurusu A, Sugiyama H, Saito M (1962) *Jpn J Med Prog* 49, 458–465
7 Finney RSH, Tarnoky AL (1960) *J Pharm Pharmacol* 12, 49–58
8 Yamamoto S, Miyamoto A, Awachi Y, Hiroshima Y (1963) *J Ther* 45, 157–168
9 Pompei R, Flore O, Marccialis MA, Pani A, Loddo B (1979) *Nature* 218, 689–690
10 Ito M, Nakashima H, Baba M et al (1987) *Anviral Res* 7, 127–137
11 Nakashima H, Matsui T, Yoshida O et al (1987) *Jpn J Cancer Res* 78, 767–771
12 Mitsuya H, Weinhold KJ, Furman PA et al (1985) *Proc Natl Acad Sci USA* 82, 7096–7100
13 Mitsuya H, Broder S (1986) *Proc Natl Acad Sci USA* 83, 1911–1915
14 Ostertag W, Roesler G, Krieg CJ et al (1974) *Proc Natl Acad Sci USA* 71, 4980–4985
15 Aldhous P (1991) *Nature* 352, 269
16 Harada S, Koyanagi Y, Yamamoto N (1985) *Virology* 146, 272–281
17 Salahudin SZ, Markham PD, Wong-Staal F, Francini G, Kalyanaraman VS, Gallo RC (1983) *Virology* 129, 51–64
18 Ichikawa Y, Monden R, Kuzuhara H (1983) *Carbohydr Res* 172, 37–64
19 Broker T, Lindhorst TK, Thiem J, Vill V (1992) *Carbohydr Res* 230, 245–256
20 Takeda K (1938) *Yakugaku Zasshi* 58, 731–738
21 Haynes LJ, Newth FH (1995) *Advances in Carbohydrate Chemistry* (Wolfrom ML, ed) Academic, New York, 10, 207–256

22 Saito S, Sumita S, Tamura N et al (1990) *Chem Pharm Bull* 38, 411–414
23 Saito S, Nagase S, Ichinose K (1994) *Chem Pharm Bull* 42, 2342–2345
24 Saito S, Tsuchiya T (1985) *Chem Pharm Bull* 33, 503–508
25 Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) *Science* 224, 497–500
26 Miyoshi I, Kubonishi I, Yoshimoto S et al (1981) *Nature* 297, 770–771
27 Shimizu NS, Shimizu NG, Takeuchi Y, Hoshino H (1994) *J Virology* 68, 6130–6135
28 Corrin ML, Klevens HB, Harkins WD (1946) *J Chem Phys* 14, 480–486
29 Honda A, Hoshino H, Nakajima K et al (1991) *Biochem Biophys Res Commun* 175, 1–9
30 Nagumo T, Hoshino H (1988) *Jpn J Cancer Res (Gann)* 79, 9–11
31 Seki J, Shimada N, Takahashi K, Takita T, Takeuchi T, Hoshino H (1989) *Antimicrob Agents Chemother* 33, 773–775
32 Shimizu N, Takeuchi Y, Naruse T et al (1992) *J Mol Evol* 35, 329–336
33 Khalilov LM, Baltina LA, Spirikhin LB et al (1989) *Khim Prir Soedin*, 500–505
34 Saito S, Kuroda K, Hayashi Y et al (1991) *Chem Pharm Bull* 39, 2333–2339
35 Saito S, Sasaki Y, Kuroda K et al (1993) *Chem Pharm Bull* 41, 539–543
36 Saito S, Sumita S, Furumoto T, Ebashi J, Nagamura Y, Ishiguro I (1994) *Eur J Med Chem* 29, 455–470
37 Saito S, Nagase S, Ichinose K (1994) *Chem Pharm Bull* 42, 2342–2345