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Synthesis of glycyrrhetic acid diglycosides and their cytoprotective activities against CCl_4-induced hepatic injury in vitro

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Summary — Glycyrrhetic acid diglycosides **16**, **24**, **25**, **42** and **46**, with respectively β-D-glucuronopyranosyl-(1→3)-β-D-glucopyranose, -(1→6)-α–D-glucopyranose, -(1→6)-β-D-glucopyranose, -(1→6)-β-D-galactopyranose, and β-D-galacturonopyranosyl-(→2)-β-D-glucopyranose as sugar components at the *O*-3 positions on the aglycons, were synthesized. In vitro cytoprotective activities, against CCl_4-induced hepatic injury, of the synthetic diglycosides, methyl β-D-glucuronopyranosyl-(1→4)-α-D-glucopyranosyl-D-glycyrrhetinate **33** and methyl esters **15** and **23** (the precursors of **16** and **24** respectively) were compared with those of glycyrrhizin **1** and β-D-glucuronopyranosyl-(1→2)-β-D-glucopyranosyl-glycyrrhetic acid **2**. Of the glycosides **16**, **24**, and **25**, with β-D-glucuronopyranosyl-glucopyranose as the sugar component, **16** and **24** were as cytoprotective as **1** and **2**, whereas **25** showed no remarkable activity. From stereomodels of the glycosides these differences in activity were inferred to be due to the stereochemistries of the terminal β-D-glucuronopyranoses in the molecules. Glycoside **46**, in which the terminal β-D-glucuronopyranose of **2** was replaced by β-D-galacturonopyranose, was as potent as **2**. Further, it was confirmed that a free COOH group on the E ring of aglycon was essential for the activity.

glycyrrhizin / glycosylation / glycyrrhetic acid diglycoside / cytoprotective activity / hepatic injury

Introduction

Naturally-occurring saponins (steroidal and triterpenoidal glycosides) isolated from plant sources have a range of pharmacological and biological activities [1–6]. The activities are presumed to depend not only on the structures of the aglycons but on the variety, number, conformation, and type of linking of sugars in the saponin molecules [5].

In previous papers [7–9], we reported the syntheses of diglycosides of glycyrrhetic acid, 11-deoxyglycyrrhetic acid and 11-deoxyglycyrrhetol, in which various β-(1→2)-linked disaccharides were β-linked to the *O*-3 position of the aglycons. We also reported a comparison of their cytoprotective effects against experimental cytotoxicity in primary cultured rat hepatocytes with that of glycyrrhizin **1** isolated from *Glycyrrhiza glabra* L and allied plants (Leguminosae), which consisted of glycyrrhetic acid as an aglycon and 2-*O*-(β-D-glucuronopyranosyl)-β-D-glucuronopyranose as a sugar component linked to the *O*-3 position on the aglycon. Comparison of the activities revealed that at least one acidic glucuronopyranose in the diglycosides was essential for the appearance of the cytoprotective activities. Furthermore, the diglycosides with only β-D-glucuronopyranose (β-glcUA) as a terminal sugar component on the aglycons were more effective than those with β-glcUA as an inner sugar component. It was also revealed that the free carboxyl group at the C-20 position on the aglycon was essential for the appearance of the cytoprotective activities, and that the diglycosides without a carbonyl group at the C-11 position on the aglycons were slightly more active than those bearing the group.

In this paper, we report the synthesis of glycyrrhetic acid diglycosides with β-D-glucuronopyranosyl-(1→3)-β-D-glucopyranose (β-glcUA-(1→3)-β-glc), -(1→4)-β-D-glucopyranose (β-glcUA-(1→4)-β-glc), -(1→6)-β-D-glucopyranose (β-glcUA-(1→6)-β-glc), -(1→6)-β-D-glucopyranose (β-glcUA-(1→6)-β-glc), -(1→6)-β-D-galactopyranose (β-glcUA-(1→6)-β-gal) and β-D-galacturonopyranosyl-(1→2)-β-D-glucopyranose (β-galUA-(1→2)-β-glc) at the *O*-3 positions of the aglycons. We also compare their in vitro cytoprotective activities against CCl_4-induced hepatic injury with those of **1** and 2-*O*-(β-D-glucuronopyranosyl)-β-D-glucopyranosylglycyrrhetic acid **2** [7].

Chemistry

The construction of the glycyrrhetic acid diglycoside with the β-glcUA-(1→3)-β-glc unit was performed by stepwise glycosylations from monoglycoside to diglycoside (scheme 2). 3-*O*-Benzyl-2,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide **7**, used for the first glycosylation, was synthesized according to the method illustrated in scheme 1.

Acid hydrolysis of 3-*O*-benzyl-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose **5**, obtained by benzylation of 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose **4** [10], followed by acetylation, gave 3-*O*-benzyl-1,2,4,6-tetra-*O*-acetyl-β-D-glucopyranose **6** in 55% overall yield. Bromination of **6** with 20% HBr/AcOH afforded a very unstable mixture (87%). As purification of the mixture by column chromatography gave α-bromide **7** in only 15% yield, the first glycosylation of methyl glycyrrhetinate **9** was performed with the mixture in the presence of a mixed catalyst $Hg(CN)_2$/$HgBr_2$ [11] to obtain monoglycosides **10** and **11** in yields of 50 and 7% respectively.

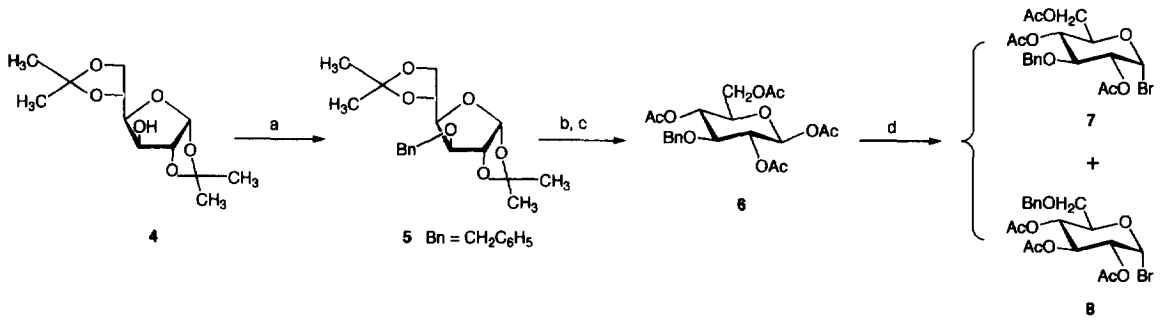
Both **10** and **11** showed a quasimolecular ion peak at *m/z* 885 $[M + Na]^+$ in the fast atom bombardment mass spectra (FAB-MS). Although **10** and **11** were thought to be anomeric isomers of each other, the ^{1}H-NMR spectra (table I) of **10** and **11** exhibited anomeric proton signals at δ 4.44 and 4.54, with coupling constants of J = 8.1 and 7.9 Hz respectively, as well as signals due to three acetyl groups and a benzyl group. The spectra suggest that both aglycons of **10** and **11** arrange in the β-configuration. Further, the signal of H-3' (δ 3.70) on the pyranose ring of **10** was observed at a higher field than that of **11** (δ 5.19), and the signals of H-6'a and 6'b (δ 4.07 and 4.20) of the pyranose ring of **10** were shifted to lower fields than those of **11** (δ 3.54 and 3.56). These results indicate that the benzyl group links to the *O*-3' position of the pyranose of **10**, but to the *O*-6' position of the pyranose of **11**.

Glycosylation of **9** with purified **7** under the same reaction conditions gave only **10** in 73% yield. Formation of a mixture of **10** and **11** in the former glycosylation was attributed to the presence of both **7** and **8** in the bromide mixture. Though the latter bromide could not be isolated, it was thought to be a rearrangement product in the process of bromination of **6**. Anomeric isomers of **10** and **11** were rarely detected by thin layer chromatography (TLC) in the glycosylation of **9** with **7**.

After hydrogenation of **10**, the resulting monoglycoside **12** was further glycosylated with methyl 2,3,4-tri-*O*-acetyl-α-D-glucuronatopyranosyl bromide **13** [12] to give the diglycoside derivative **14** in 55% yield (scheme 2). The FAB-MS of **14** showed a quasimolecular ion peak at *m/z* 1111 $[M + Na]^+$, and the ^{1}H-NMR spectrum of **14** exhibited a pair of anomeric proton signals at δ 4.38 (d, J = 8.1 Hz) and 4.64 (d, J = 8.1 Hz), which suggests that both pyranoses of **14** have the β-configuration. The α-anomeric isomer of **14** was not detected on the TLC.

Hydrolysis of **14** with 5% KOH in EtOH/H_2O (1:1) at room temperature afforded monomethyl ester **15** (72%). The position of the ester group was confirmed by acid hydrolysis of **15** to give **9**. Further hydrolysis of **15** with LiI in γ-collidine under reflux gave **16** (45%). Compound 16 showed a quasimolecular ion peak at *m/z* 831 $[M + Na]^+$ in the FAB-MS, and two anomeric carbon signals at δ 101.8 and 106.4 in its ^{13}C-NMR spectrum (table IV).

Preparation of glycyrrhetic acid diglycosides with β-glcUA(1→6)–α–glc and -β-glc was performed by glycosylation of **9** with 6-*O*-(methyl 2',3',4'-tri-*O*-acetyl-β-D-glucuronatopyranosyl)-2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide **19** (scheme 3). The bromide was obtained from 6-*O*-(methyl 2',3',4'-tri-*O*-acetyl-β-D-glucuronatopyranosyl)-1,2;3,5-di-*O*-isopropylidene-α-D-glucofuranose **17** [13] as follows: acid hydrolysis of **17**, followed by acetylation, gave **18**

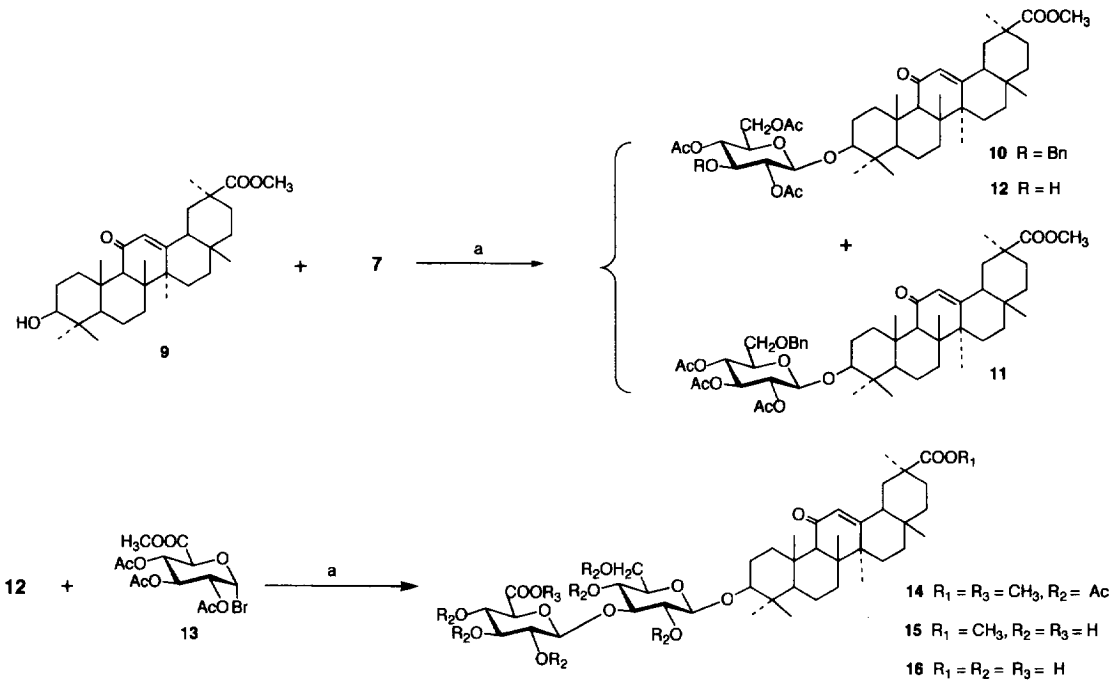


Scheme 1. Reagents: (a) Benzyl bromide, KOH, reflux, 4 h; (b) Amberlite IR-120 (H^+ form)/80% AcOH; (c) Ac_2O/pyridine (1:1); (d) 20% HBr/AcOH.

Table I. ^{1}H-NMR Spectral data of compounds **10**, **11**, **12**, **14** and **22**[a].

	10	*11*	*12*	*14*	*22*
Aglycon[b] and others					
CH_3	0.77, 0.80, 0.93, 1.12, 1.13, 1.14, 1.35	0.79, 0.80, 0.93, 1.12, 1.14, 1.15, 1.34	0.72, 0.81, 0.96, 1.12, 1.13, 1.14, 1.35	0.77, 0.80, 0.94, 1.12, 1.15, 1.15, 1.35	0.77, 0.80, 0.90, 1.12, 1.14, 1.25, 1.34
OCH_3	3.68	3.69	3.69	3.69	3.69, 3.73
H-3	3.09 (dd, 11.3, 4.8)[c]	3.13 (dd, 11.3, 5.2)	3.10 (dd, 11.3, 4.8)	3.12 (dd, 11.6, 4.4)	3.06 (dd, 11.3, 4.6)
H-9	2.31 (s)	2.30 (s)	2.31 (s)	2.30 (s)	2.35 (s)
H-12	5.66 (s)	5.67 (s)	5.66 (s)	5.67 (s)	5.66 (s)
H-18	2.80 (broad d, 13.3)	2.82 (broad d, 13.4)	2.80 (broad d, 13.9)	2.80 (broad d) 13.4)	2.79 (broad d, 13.6)
$COCH_3$	1.96, 2.00, 2.05	1.93, 2.00, 2.03	2.07, 2.11, 2.11	2.01, 2.03, 2.04	1.99, 2.01, 2.02, 2.05, 2.06, 2.12
$-CH_2-$	4.60 (s)	4.54 (ABq, 12.2)	–	–	–
C_6H_5	7.24–7.35	7.28–7.37	–	–	–
Inner sugar					
H-1'	4.44 (d, 8.1)	4.54 (d, 7.9)	4.47 (d, 8.1)	4.59 (d, 7.9)	4.38 (d, 8.1)
H-2'	5.10 (dd, 9.5, 8.1)	5.02 (dd, 9.5, 7.9)	4.88 (dd, 9.0, 8.1)	5.01 (dd, 9.8, 7.9)	5.04 (dd, 9.5, 8.1)
H-3'	3.70 (dd, 9.5, 9.5)	5.19 (dd, 9.5, 9.5)	3.59–3.74	5.24 (dd, 9.8, 9.8)	3.87 (dd, 9.5, 9.5)
H-4'	5.06 (dd, 9.5, 9.5)	5.00 (dd, 9,5, 9.5)	4.89 (dd, 10.1, 10.1)	5.01 (dd, 9.8, 9.8)	4.88 (dd, 9.5, 9.5)
H-5'	3.60 (ddd, 9.5, 5.5, 2.6)	3.60–3.68	3.59–3.74	3.53 (ddd, 9.8, 5.8, 2.4)	3.60–3.67
H-6'a	4.07 (dd, 12.1, 2.6)	3.54 (dd, 12.5, 2.2)	4.12 (dd, 12.1, 2.6)	3.60 (dd, 12.2, 5.80	4.13–4.20
H-6'b	4.20 (dd, 12.1, 5.5)	3.56 (dd, 12.5, 5.0)	4.25 (dd, 12.1, 5.9)	3.66-3.74	4.13–4.20
Terminal sugar					
H-1"					4.64 (d, 8.1)
H-2"					5.04 (dd, 9.5, 8.1)
H-3"					5.16 (dd, 9.5, 9.5)
H-4"					5.19 (dd, 9.5, 9.5)
H-5"					3.99 (d, 9.5)

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

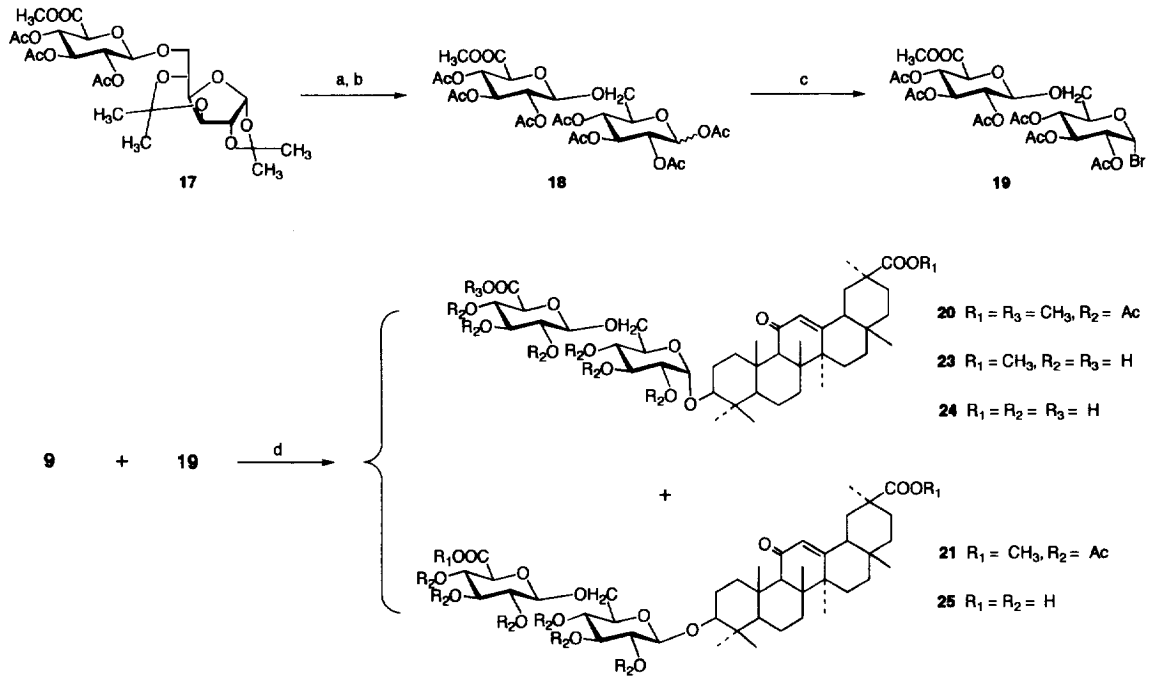


Scheme 2. (a) $Hg(CN)_2$, $HgBr_2$, Drierite, CH_2Cl_2.

(52%), which was treated with 20% HBr/AcOH to obtain bromide **19** in 86% yield. Glycosylation of **9** with **19** in the presence of $Hg(CN)_2/HgBr_2$ gave α-diglycoside **20** and β-diglycoside **21** in yields of 30 and 3% respectively. Both products showed the same quasimolecular ion peak at *m/z* 1111 $[M + Na]^+$ in the FAB-MS. In its ^{1}H-NMR spectra (table II), **20** exhibited two anomeric proton signals, at δ 5.20 and 4.56 with coupling constants of J = 4.0 and 7.9 Hz respectively, while **21** showed signals at δ 4.54 and 4.69 with coupling constants of J = 8.1 and 8.1 Hz respectively. Compound **21** was also obtained by the glycosylation of **22** derived from **11**. Hydrogenation of **11** gave **22** in 79% yield. Glycosylation of **22** with **13** gave **21** in 26% yield (scheme 4). Hydrolysis of major product **20** with 5% KOH in EtOH/H_2O (1:1) at room temperature gave **23** (79%), which was further hydrolyzed with LiI in γ-collidine under reflux to obtain **24** (51%). Compounds **23** and **24** showed quasimolecular ion peaks at *m/z* 845 and 831 $[M + Na]^+$ respectively. Direct hydrolysis of minor product **21** with LiI in γ-collidine under reflux gave **25** in 84% yield, which exhibited the same quasimolecular ion peak at *m/z* 831 $[M + Na]^+$ as **24**.

Preparation of the glycyrrhetic acid diglycoside with glcUA(1→4)-glc was also tried by stepwise glycosylation. The pyranose derivative **29** used for the first glycosylation was synthesized as follows (scheme 5): 4,6-*O*-benzylidene-D-glucose **26** [14] was acetylated in the presence of anhydrous AcONa in Ac_2O under refluxing to give β-acetate **27** (21%). Acid hydrolysis of **27** with 80% AcOH afforded **28** (93%), which was benzoylated to give the monobenzoate **29** (62%). Glycosylation of **9** with **29** in the presence of trimethylsilyl trifluoromethane sulfonate (Me_3Si-Triflate) [15] in dry CH_2Cl_2 gave β- (**30**) and α-monoglycoside (**31**) in yields of 34 and 12% respectively. Both **30** and **31** showed the same quasimolecular ion peak at *m/z* 875 $[M + Na]^+$ in the FAB-MS. In their ^{1}H-NMR spectra (table III), **30** exhibited an anomeric proton signal at δ 4.56 with a coupling constant of J = 7.7 Hz, whereas that of **31** was at δ 5.18 with a coupling constant of J = 4.0 Hz.

It is known that when all hydroxyl groups attached to C-2, C-3, and C-4 in an aldohexopyranoside have an equatorial orientation, the general order of reactivity in forming glycosidic linkages is 6-OH ≫ 3-OH > 2-OH > 4-OH [16]. An example of the slight reactivity of the 4-OH was proved by the reaction of methyl 2,3,6-tri-*O*-acetyl-β-D-glucopyranosylglycyrrhetinate **35** with bromide **13** in the presence of silver trifluoromethane sulfonate (Ag-Triflate) for over 12 h. This gave only product **36**, in which the 4-OH on the pyranose was intact and an enol α-glycosidic linkage



Scheme 3. (a) Amberlite IR-120 (H+ form)/80% AcOH, reflux, 5 h; (b) Ac_2O/pyridine (1:1); (c) 20% HBr/AcOH; (d) $Hg(CN)_2$, $HgBr_2$, Drierite, CH_2Cl_2.

was formed on the *C*-ring (scheme 6) [17, 18]. Although glycosylation of β-anomer **30** with **13** in the presence of Ag-Triflate was tried, no product was obtained within 5 h. In contrast, glycosylation of the α-anomer **31** with **13** under the same reaction conditions for 5 h gave product **32** in 31% yield, along with a small amount of another product, which was not purified. In the FAB-MS of **32**, a quasimolecular ion peak was observed at *m/z* 1173 $[M + Na]^+$. The ^{1}H-NMR spectrum (table III) of **32** exhibited a pair of anomeric proton signals at δ 5.95 (d, J = 4.0 Hz) and 4.45 (d, J = 8.1 Hz). Treatment of **32** with 5% KOH in EtOH/H_2O (1:1) at room temperature gave **33** (56%). However, **33** was refluxed in γ-collidine containing LiI to give monoglycoside **34** (64%), resulting from a breakdown of the terminal pyranose, in contrast to the reactions of **15** and **20**.

As mentioned above, while the glycosylation of monoglycoside **12** with bromide **13** gave only diglycoside **14**, in which both pyranoses are arranged in the more stable β-configuration, glycosylation of **9** with bromide **19** afforded diglycosides **20** having β-glcUA(1→6)-α-glc as a major product and **21** having β-glcUA(1→6)-β-glc as a minor product. These results were inferred from stereomodels obtained by molecular dynamic calculations using MM2 (Chem 3D plus, Cambridge Scientific Company, Inc) and are illustrated in figure 2. The most stable projection models of **16** (derived from **14**), **24** and **25** (derived from **20** and **21** respectively), **42** (derived from **41**), and **2** are depicted as models I–V (M-I–IV and V), respectively, shown in figure 2. In **16** (M-I), the terminal β-glcUA, represented by S2, was located sufficiently apart from the bulky aglycon that it was affected less by steric hindrance. When the stereomodels of **24** and **25** (M-II and M-III) were compared, the β-glcUA of **24** was also located apart from the bulky aglycon; on the other hand, the β-glcUA of **25** was located very close to the aglycon, so that the β-glcUA of **25** was influenced by steric hindrance more than that of **24**. **24** was therefore a more stable compound than **25**.

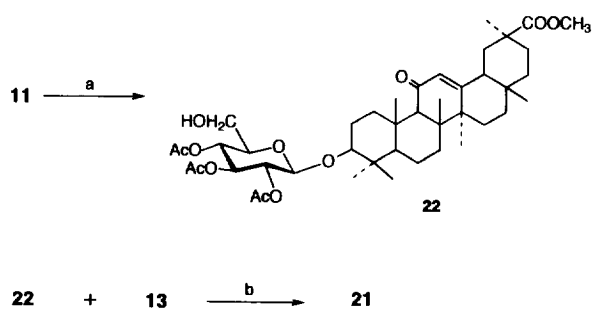
When the inner pyranose, β-glc, of **25** is replaced by β-gal, the projection model of the resulting diglycoside **42** can be depicted as M-IV in figure 2. In this model, the terminal β-glcUA residue is located apart from the aglycon in the absence of steric hindrance by the 4'-OH group on the β-gal. Therefore, it appeared that the diglycoside **42** could be readily synthesized (scheme 7). Glycosylation of 1,2;3,4-di-*O*-isopropylidene-α-D-galacto-pyranose **37** [19] with **13** gave

Table II. ^{1}H-NMR spectral data of compounds **20**, **21**, **41** and **45**[a].

	20	*21*	*41*	*45*
Aglycon[b] and others				
CH_3	0.81, 0.86, 1.01, 1.13, 1.14, 1.14, 1.37	0.71, 0.81, 0.94, 1.12, 1.13, 1.36, 1.45	0.78, 0.80, 0.94, 1.12, 1.13, 1.16, 1.45	0.81, 0.92, 1.10, 1.12, 1.15, 1.15, 1.36
OCH_3	3.69, 3.75	3.69, 3.75	3.69, 3.77	3.69, 3.72
H-3	3.16 (dd, 10.1, 5.5)[c]	3.19 (dd, 11.5, 5.1)	3.14 (dd, 10.6, 4.7)	3.15 (11.2, 4.9)
H-12	5.67 (s)	5.66 (s)	5.65 (s)	5.67 (s)
H-9	2.35 (s)	2.37	2.36	2.33
H-18	2.80 (broad d, 10.3)	2.83 (broad d, 10.3)	2.81 (broad d, 10.5)	2.80 (broad d, 13.6)
$COCH_3$	2.00, 2.02, 2.02, 2.03, 2.05, 2.06	1.98, 1.99, 2.02, 2.05, 2.06, 2.07	1.98, 2.00, 2.02, 2.04, 2.06, 2.14	1.98, 2.00, 2.04, 2.05, 2.05, 2.07
Inner sugar				
H-1'	5.20 (d, 4.0)	4.54 (d, 8.1)	4.50 (d, 8.1)	4.48 (d, 7.7)
H-2'	4.77 (dd, 10.1, 4.0)	4.99 (dd, 9.5, 8.1)	4.94 (dd, 10.1, 8.1)	3.90 (dd, 9.5, 7.7)
H-3'	5.41 (dd, 10.1, 9.5)	5.18 (dd, 9.9, 9.5)	4.95 (dd, 10.1, 3.7)	5.18 (dd, 9.5, 9.5)
H-4'	4.93 (dd, 9.5, 9.5)	4.88 (dd, 9,9, 9.9)	5.35 (d, 3.7)	4.91 (dd, 9.5, 9.5)
H-5'	4.13 (ddd, 9.5, 5.8, 2.4)	3.62-3.85	3.75-3.86	3.66
H-6a'	3.51 (dd, 10.7, 5.8)	3.62-3.85	3.75-3.86	4.05 (dd, 12.1, 2.6)
H-6b'	3.96 (dd, 10.7, 2.4)	3.62-3.85	3.75-3.86	4.26 (dd, 12.1, 5.9)
Terminal sugar				
H-1''	4.56 (d, 7.9)	4.69 (d, 8.1)*[d]	4.56 (d, 8.1)*	4.87 (d, 8.0)
H-2''	5.01 (dd, 8.6, 8.1)	4.96*	5.00*	5.13 (dd, 10.3, 8.0)
H-3''	5.21 (dd, 9.5, 8.6)	5.16-5.25*	5.17–5.25*	4.98 (dd, 10.3, 3.3)
H-4''	5.23 (dd, 9.5, 9.5)	5.16-5.25*	5.17–5.25*	5.65 (dd, 3.3, 1.1)
H-5''	3.97 (d, 9.5)	3.99*	4.01*	4.21 (d, 1.1)

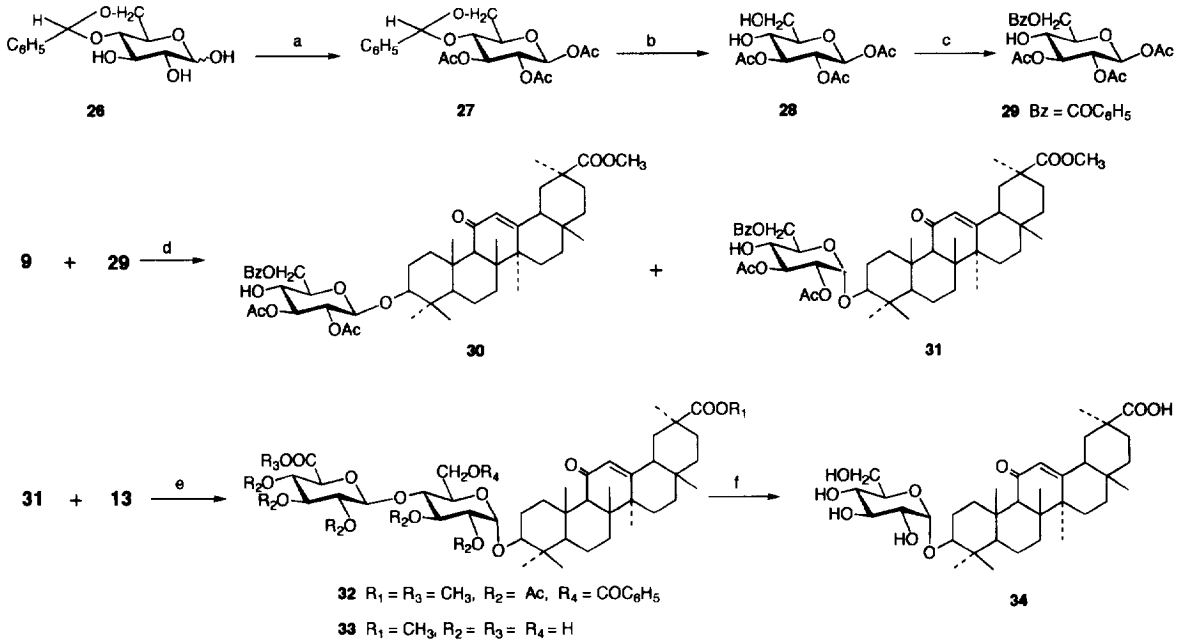
[a]Spectra were obtained in $CDCl_3$. The signal assignments were based on H-H COSY method. [b]Only assignable signals on the aglycons are listed. [c]Coupling constants (J in Hz) are given in parentheses. [d]Protons with asterisks (*) showed virtual long-range spin–spin coupling in five-spin system [13, 25, 26].

methyl 6-*O*-(2',3',4'-tri-*O*-acetyl-β-D-glucuronopyranosyl)-1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranose **38** (71%), from which **39** was obtained in 52% yield by acid hydrolysis. Bromination of **39** with 20% HBr/AcOH afforded bromide **40** (86%), which was reacted with **9** in the presence of $Hg(CN)_2/HgBr_2$ in dry CH_2Cl_2 to give diglycoside **41** in 43% yield. The FAB-MS of **41** showed a quasimolecular ion peak at m/z 1111 $[M + Na]^+$. In the ^{1}H-NMR spectrum (table II) of **41**, a pair of anomeric proton signals were observed at δ 4.50 and 4.56 (d, J = 8.1 Hz). Refluxing **41** in γ-collidine in the presence of LiI gave **42** in 44% yield. Compound **42** exhibited a quasimolecular ion peak at m/z 831 $[M + Na]^+$ in the FAB-MS, and a pair of anomeric carbon signals at δ 105.2 and 107.2 in the ^{13}C-NMR specrum (table IV).

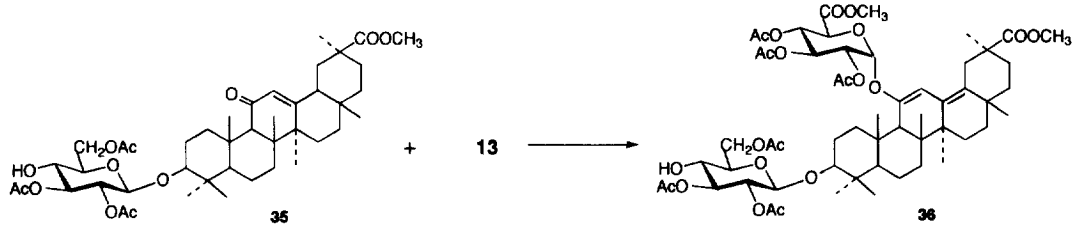


Scheme 4. Reagents: (a) H_2/Pd-C; (b) $Hg(CN)_2$, Drierite, CH_2Cl_2.

Glycyrrhetic acid diglycoside **46**, which had a terminal β-galUA in place of the β-glcUA in **2**, was synthesized to allow comparison of its cytoprotective effects with those of diglycosides with a terminal glcUA. Monoglycoside derivative **43** [8] was reacted with methyl 2,3,4-tri-*O*-acetyl-α-D-galacturonopyranosyl bromide **44** [20] to give diglycoside **45** in 58% yield (scheme 8). The FAB-MS of **45** showed a quasimolecular ion peak at m/z 1111 $[M + Na]^+$. The ^{1}H-NMR spectrum of **45** exhibited a pair of anomeric proton signals at δ 4.48 and 4.87 with coupling constants of J = 7.7 and 8.0 Hz respectively. Refluxing **45** with LiI in γ-collidine gave **46** in 43%



Scheme 5. (a) AcONa, Ac_2O, reflux, 1 h; (b) 80% AcOH, reflux, 1 h; (c) benzoyl chloride, pyridine; (d) Me_3Si-Triflate, CH_2Cl_2; (e) Ag-Triflate, TMU, CH_2Cl_2; (f) 5% KOH in $EtOH/H_2O$ (1:1).



Scheme 6.

yield. **46** showed a quasimolecular ion peak at *m/z* 831 $[M + Na]^+$ in the FAB-MS, and a pair of anomeric carbon signals at δ 104.9 and 106.2 in its ^{13}C-NMR spectrum (table IV).

Cytoprotective activity

In vitro cytoprotective activities of the synthetic diglycosides against CCl_4-induced hepatic injury were compared with that of glycyrrhizin **1**, which is known to have potent activity [21]. The activities were evaluated by assay of aspartate transaminase (AST) and alanine transaminase (ALT), which were released from the injured hepatocytes [22]. The lower the release of ALT and AST, the more potent the cytoprotective activity of the diglycoside. The reaction suspensions for the assay were composed of hepatocytes (2×10^6 cells) [23] and glycosides (0.05, 0.1 and 0.5 mg/mL) in Hanks solution (total volume 1.0 mL), and the suspension for the control was composed solely of hepatocytes (2×10^6 cells) in Hanks solution (total volume 1.0 mL). The suspensions were exposed to CCl_4 vapor at 37 °C for 1 h. After incubation of the cell suspensions, the supernants were collected by centrifugation at 1000 *g* for 30 s. The AST and ALT

Table III. ^{1}H-NMR spectral data of compounds **30**, **31** and **32**[a].

	30	*31*	*32*
Aglycon[b] and others			
CH_3	0.76, 0.81, 0.93, 1.01, 1.05, 1.16, 1.35	0.79, 0.87, 0.99, 1.03, 1.04, 1.14, 1.35	0.74, 0.80, 0.90, 1.07, 1.09, 1.16, 1.25
OCH_3	3.69	3.69	3.71, 3.77
H-3	3.10 (dd, 11.0, 5.2)[c]	3.16 (dd, 11.6, 4.6)	3.02 (dd, 10.3, 5.9)
H-12	5.68 (s)	5.66 (s)	5.67 (s)
H-9	2.27 (s)	2.27	2.20
H-18	2.75 (broad d, 13.2)	2.80 (broad d, 13.2)	2.64 (broad d, 13.6)
$COCH_3$	2.04, 2.08	2.05, 2.08	2.03, 2.05, 2.08, 2.08, 2.06
Aromatic proton	7.47 (dd, 8.1, 7.0) 7.59 (dd, 8.1, 7.9) 8.05 (d, 7.9)	7.42 (dd, 7.7, 7.0) 7.54 (7.7, 7.9) 8.05 (d, 7.0)	–
Inner sugar			
H-1'	4.56 (d, 7.7)	5.18 (d, 4.0)	5.95 (d, 4.0)
H-2'	4.98 (dd, 9.2, 7.7)	4.80 (dd, 9.9, 4.0)	5.11 (dd, 9.0, 4.0)
H-3'	5.09 (dd, 9.2, 9.2)	5.30 (dd, 9.9, 9.9)	5.15–5.21
H-4'	3.67–3.70	3.62 (dd, 9,9, 9.5)	3.65–3.69
H-5'	3.67–3.70	4.13 (ddd, 9.5, 4.4, 1.8)	3.65–3.69
H-6a'	4.12 (dd, 12.1, 2.6)	4.52 (dd, 12.1, 1.8)	4.39 (dd, 12.1, 5.0)
H-6b'	4.25 (dd, 12.1, 5.9)	4.71 (dd, 12.1, 4.4)	4.79 (dd, 12.1, 1.1)
Terminal sugar			
H-1"			4.45 (d, 8.1)
H-2"			4.99 (dd, 9.5, 8.1)
H-3"			5.15–5.21
H-4"			5.15–5.21
H-5"			4.22 (d, 9.5)

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

activities were assayed by the reported procedures [24], and the results are shown in table V. In this table, 'Non' describes the activities of AST and ALT in the supernatant obtained from non-injured cells.

Diglycoside methyl esters **15**, **23** and **33** showed no decrease in the release of AST or ALT from the CCl_4-injured hepatocytes at doses up to 0.5 mg/mL; thus these activities were nearly the same as that of the control. This observation confirms that the presence of the free carboxylic group at the C-20 position on the *E*-ring of the aglycons is essential for cytoprotective activity. Among the glycosides **2**, **16**, **24**, **25** and **42** with no ester group but with a free COOH group on the *E* ring of the aglycons, glycosides **2**, **16**, **24** and **42** at a dose of 0.5 mg/mL decreased the release of ALT and AST to nearly the same extent as did **1**, though no notable decrease in the release was observed at doses less than 0.1 mg/mL. In contrast, glycoside **25** at doses up to 0.5 mg/mL led to AST and ALT activities similar to control activities. Thus, the former compounds have cytoprotective activities as potent as that of glycyrrhizin **1**, while the latter has no remarkable activity.

Though the cause of this difference is still unclear, it may be explained as follows: in the stereomodels (fig 2), the terminal β-glcUA units of **2**, **16**, **24** and **42** are located efficiently apart from the bulky aglycon moieties. In addition, the 6"-COOH groups on the β-glcUAs of the glycosides are arranged in *exo* sites. Therefore, the glycosides may be fixed to the hepatic cell by linking the 6"-COOH group to the recognition site on the cell, and this binding is responsible for the cytoprotective activity. On the other hand, the proximity of the β-glcUA of **25** to the bulky aglycon, and the arrangement in the *endo* site of the 6"-COOH group of the β-glcUA, suggest that this inactive glycoside may not be fixed to the hepatic cell.

The cytoprotective activity of glycoside **46**, in which the β-glc of glycoside **2** was replaced by β-gal, was investigated. The decrease in the release of AST and ALT that resulted from this glycoside was similar to the decrease that resulted from glycoside **2**, which exhibited cytoprotective activity as potent as that of glycyrrhizin **1**. This result, together with the assay of diglycoside methyl esters **15**, **23** and **33**, which showed no cytoprotective activity, indicates that the presence of the COOH group on the terminal pyranose of diglycosides is essential for appearance of the cytoprotective activity even though the structure of the pyranose is changed, in accord with the result previously reported [9].

Conclusions

Glycosides **16** and **46** were synthesized by stepwise glycosylations from methyl glycyrrhetinate **9** via monoglycosides **12** and **43**. Glycosides **24**, **25** and **42** were synthesized by one-step glycosylation. Glycosylation of **9** with bromide **19** gave diglycoside derivatives **20** and **21**, from which **24** and **25** respectively were obtained by alkaline hydrolysis. Glycosylation of **9** with bromide **40** yielded the diglycoside derivative **41**, which was hydrolyzed to give **42**. An attempt to synthesize a diglycoside with a β-glcUA-(1→4)-α-glc unit was unsuccessful because the terminal β-glcUA residue of the precursor **33** was cleaved in alkaline hydrolysis to give the monoglycoside **34**.

Cytoprotective activities against CCl_4-induced hepatic injury of **16**, **24**, **25**, **42** and **46**, which had a free COOH group on the E rings of the aglycon, and methyl esters **15**, **23** and **33**, were compared with those of glycosides **1** and **2**. This comparison revealed that the terminal β-glcUA residues of glycosides **2**, **16**, **24** and **42**, which exhibited potent cytoprotective activities, were located away from bulky aglycons, and the 6"-COOH groups on the pyranoses were arranged in *exo* sites so that the glycosides might be fixed to the injured hepatic cells though binding of the groups to the cells. On the other hand, the proximity of the terminal β-glcUA of glycoside **25** to the bulky aglycon and the *endo* site of its 6"-COOH group may sterically hinder binding of the glycoside to the cells and result in the lack of remarkable activity. The lack of notable activity by glycosides **15**, **23** and **33** is consistent with the requirement for cytoprotective activity of a free COOH group on the E ring of the aglycon.

Experimental protocols

General procedures

Dry dichloromethane (CH_2Cl_2) was obtained by refluxing with NaH followed by distillation. Other chemicals and solvents were of reagent grade, and were obtained from commercial sources. The thin-layer chromatography (TLC) was run on 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 Wakogel C-200. An SSC-6300/SSC-3000 apparatus (Senshu Scientific Co Ltd) was employed for analytical HPLC, using an ODS-1251-D column (4.6 × 250 mm), with an SSC autoinjector 6310. An SSC fraction collector 6320 was used for preparative HPLC with an ODS-4251-D column (10 × 250 mm). ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL JNM-GX NMR spectrometer at 270 and 67.8 MHz respectively. Chemical shifts are given in δ with tetramethylsilane as an internal standard. Multiplicities of ^{1}H-NMR signals are indicated by s (singlet), d (doublet), dd (doublet of doublets) and m (multiplet). Only assignable signals for protons on aglycons in ^{1}H-NMR spectra are listed in the tables and *Experimental protocols*. FAB-MS were recorded on a JEOL JMS-DX 300 mass spectrometer.

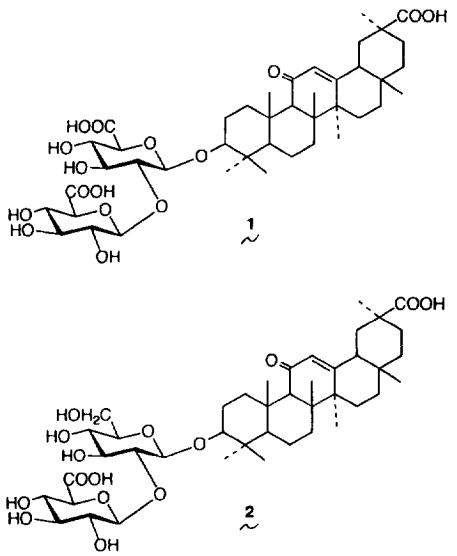
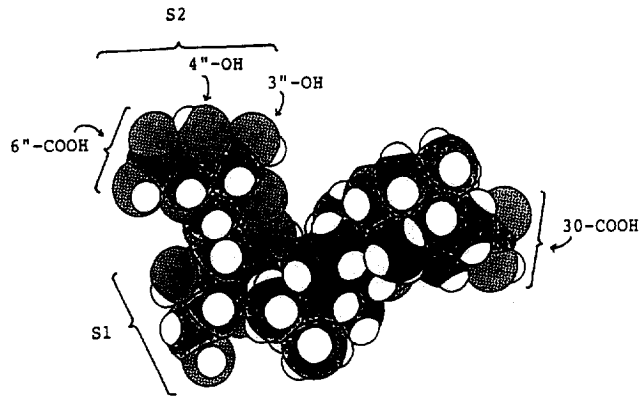
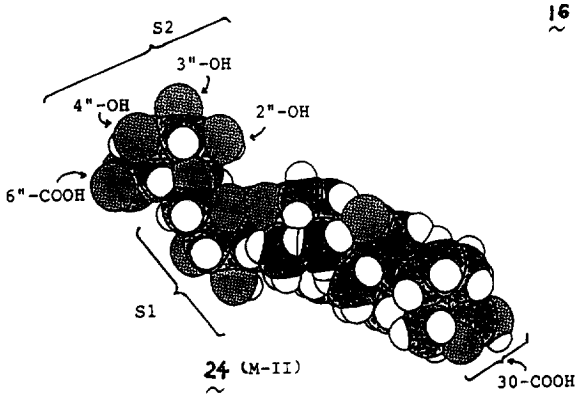


Fig 1. Compounds **1**, **2**.

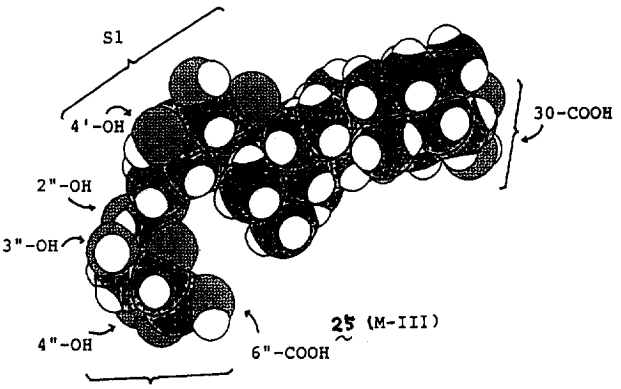
Fig 2. (Overleaf). Stereomodels of compounds **16** (M-I), **24** (M-II), **25** (M-III), **42** (M-IV) and **2** (M-V). Each projection medel is depicted in the direction that a steric relation between the aglycon and the terminal β-glcUA in the glycoside is well understandable. S1 and S2 reveal inner glc and terminal glcUA respectively.



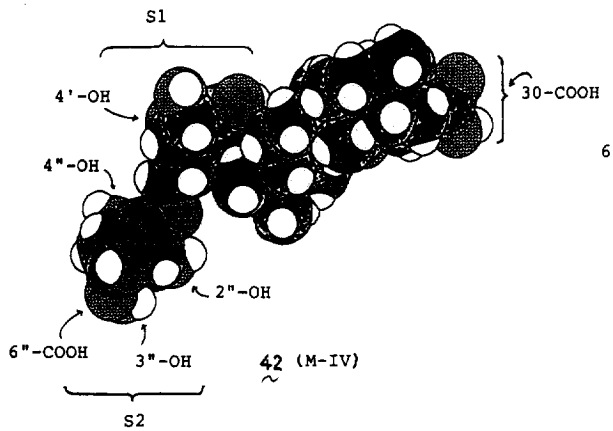
16 (M-I)



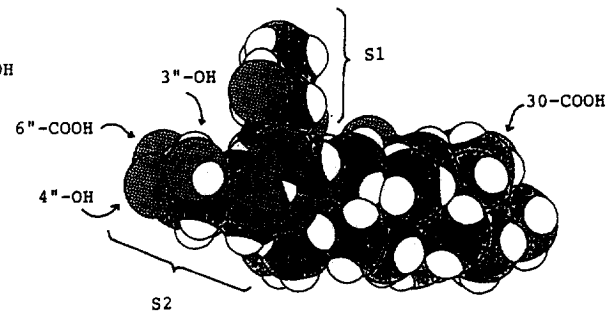
24 (M-II)



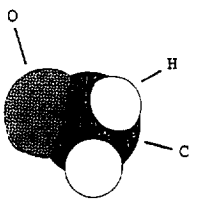
25 (M-III)

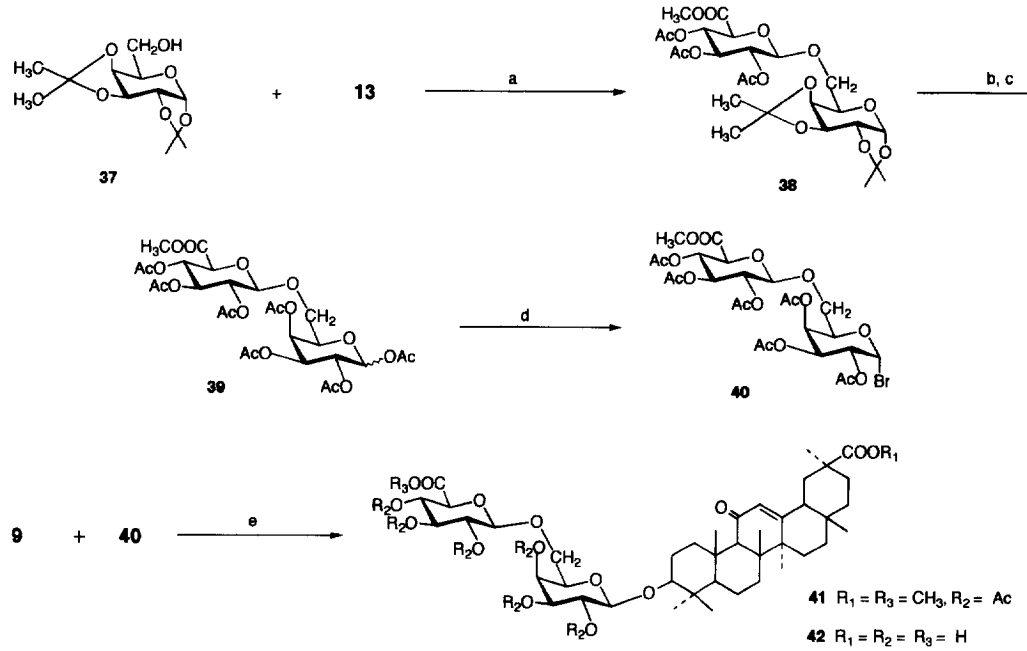


42 (M-IV)



2 (M-V)





Scheme 7. Reagents: (a) $Hg(NC)_2$, benzene/nitromethane (1:1), 40 °C, 12 h; (b) 80% AcOH, reflux, 36 h; (c) Ac_2O/pyridine (1:1); (d) 20% HBr/AcOH; (e) $HG(CN)_2$, $HgBr_2$, Drierite, CH_2Cl_2.

Chemistry

3-O-Benzyl-1,2,4,6-tetra-O-acetyl-β-D-glucopyranose **6**

A mixture of 1,2;3,5-di-*O*-isopropylidene-α-D-glucopyranose **4** (80 g, 307.3 mmol) and KOH (100 g, 1.78 mol) in benzyl bromide (320 mL, 2.69 mol) was stirred at 130 °C under reflux for 4 h. After cooling to room temperature, the reaction mixture was poured into ice-water (500 mL), then extracted with CH_2Cl_2 (3 × 300 mL). The combined CH_2Cl_2 extracts were washed with H_2O, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to give crude product **5**. Without purification, **5** was dissolved in 80% acetic acid (200 mL), added to Amberlite IR-120B (H^+ form), and then stirred at 80 °C for 3 h. The reaction mixture was neutralized with pyridine and evaporated to give a residue, which was dissolved in a solution of Ac_2O/pyridine (1:1, 200 mL) and allowed to stand at room temperature for 16 h. The mixture was coevaporated with toluene (5 × 200 mL) to give a residue, which was purified by column chromatography (a gradient of 0–5% acetone in benzene), to yield colorless needles of **6** (74 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 7.23–7.40 (5H, aromatic protons), 5.65 (1H, d, J = 8.2 Hz, H-1), 5.16 (1H, dd, J = 9.5, 8.2 Hz, H-2), 5.15 (1H, dd, J = 9.5, 9.5 Hz, H-4), 4.61 (2H, s, -$CH_2C_6H_5$), 4.22 (1H, dd, J = 12.5, 4.9 Hz, H-6a), 4.09 (1H, dd, J = 12.5, 2.4 Hz, H-6b), 3.76 (1H, dd, J = 9.5, 9.5 Hz, H-3), 3.73 (1H, ddd, J = 9.5, 4.9, 2.4 Hz, H-5), 1.98, 2.08 and 2.10 (each 3H, s, OAc).

3-O-Benzyl-1,2,4,6-tetra-O-acetyl-β-D-glucopyranosyl bromide **7**

A solution of compound **6** (40 g, 91.2 mmol) in CH_3COOH (40 mL) was added to 20% HBr/AcOH (80 mL) and stirred at 0 °C for 1 h. The reaction mixture was poured into ice-CH_2Cl_2 solution (500 mL) and washed successively with water, Na_2CO_3-saturated water and water. The CH_2Cl_2 solution was dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated to give a mixture of **7** and **8** (87% yield), which was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to afford compound **7** (6.3 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 7.24–7.37 (5H, aromatic protons), 6.64 (1H, d, J = 4.0 Hz, H-1), 4.78 (1H, dd, J = 9.5, 4.0 Hz, H-2), 4.66 (1H, dd, J = 9.5, 9.5 Hz, H-4), 4.60 (2H, s, -$CH_2C_6H_5$), 4.26 (1H, dd, J = 12.5, 4.4 Hz, H-6a), 4.17 (1H, dd, J = 12.5, 2.4 Hz, H-6b), 4.14–4.20 (1H, m, H-5), 4.06 (1H, dd, J = 9.5, 9.5 Hz, H-3), 1.95, 2.07 and 2.07 (each 3H, s, OAc).

Glycosylation of methyl glycyrrhetinate **9** *with* **7**

A mixture of methyl glycyrrhetinate **9** (3 g, 6.2 mmol), compound **7** (5.5 g, 8.2 mmol), $Hg(CN)_2$ (3.1 g, 12.3 mmol), $HgBr_2$ (4.5 g, 12.5 mmol) and Drierite (2 g, 14.7 mmol) in dry CH_2Cl_2 (40 mL) was stirred for 2 h at room temperature while shielded from light. The reaction mixture was filtered, and the filtrate was poured into ice-water (150 mL) and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were washed

Table IV. ^{13}C-NMR chemical shifts for compounds **15**, **16**, **23**, **24**, **25**, **33**, **34**, **42** and **46**[a].

	15	*16*	*23*	*24*	*25*	*33*	*34*	*42*	*46*
Aglycon									
C-1	39.2[b]	39.4[b]	39.1	38.7	39.3[b]	39.6[b]	39.2	39.5[b]	39.5[b]
C-2	26.4[c]	26.5[c]	26.7[b]	26.4[b]	26.3[c]	26.5[c]	26.5[b]	26.7[c]	26.6
C-3	86.7	84.9	83.9	84.7	85.5	89.1	89.7	84.7	88.5
C-4	39.4[b]	39.7[b]	39.1	38.7	39.1[b]	39.7[b]	39.4	39.4[b]	39.8[b]
C-5	54.9	55.3	55.2	54.8	55.8	55.3	55.3	55.3	55.5
C-6	17.2	17.5	17.7	17.3	17.3	17.6	17.4	17.6	17.6
C-7	31.8	32.8	32.9	32.4	32.7[b]	32.9	32.8	32.9	32.9
C-8	44.2	44.0	44.2	43.9	44.0	44.3	44.0	44.0	43.5
C-9	61.9	62.0	62.0	61.7	61.8	61.4	61.8	62.0	62.1
C-10	36.8	37.1	37.4	37.0	37.1	37.2	36.8	37.2	37.2
C-11	201.3	200.1	199.7	202.3	200.9	200.2	200.0	199.4	199.5
C-12	127.4	128.6	128.7	127.6	127.9	128.5	128.6	128.6	128.6
C-13	171.4	170.2	169.1	168.2	170.8	170.0	168.9	169.4	169.6
C-14	45.5	45.5	45.5	45.5	45.4	45.6	45.4	45.5	45.5
C-15	26.4[c]	26.7[c]	26.7[b]	26.4[b]	26.5[c]	26.7[c]	26.1[b]	26.8[c]	26.8
C-16	26.1[c]	26.4[c]	26.5[b]	26.2[b]	26.3[c]	26.5[c]	26.5[b]	26.6[c]	26.8
C-17	31.8	32.0	32.0	31.8[c]	31.9	32.0	31.8	32.1	32.1
C-18	48.6	48.6	48.7	48.8	48.8	48.7	48.3	48.7	48.7
C-19	40.9	41.5	41.3	42.1	41.7	41.3	39.5	41.6	41.0
C-20	43.3	43.4	43.6	43.3	43.3	43.5	43.2	43.4	44.0
C-21	30.8	31.4	31.3	31.7[c]	31.6	31.2	31.2	31.5	31.6
C-22	37.8	38.3	38.2[b]	38.2	38.1	38.1	37.8	38.4	38.2
C-23	27.8	28.1	28.3	28.6[d]	28.3[d]	28.2	28.0	28.1	28.2
C-24	16.4[d]	17.0[d]	16.7	16.3	16.6	16.7[e]	16.4	16.9[d]	16.9
C-25	16.7[d]	17.1[d]	17.1	17.8	17.7	17.0[e]	16.7	17.0[d]	16.9
C-26	18.5	18.7	18.8	18.7	18.8	18.8	18.7	18.8	18.8
C-27	23.1	23.5	23.5	22.9	23.3	23.4	23.5	23.5	23.6
C-28	27.9	28.5[e]	28.6[c]	28.7[d]	28.5[d]	28.2[e]	28.3[c]	28.6[e]	28.7
C-29	28.4	28.6[e]	28.8[c]	28.9[d]	29.0	28.6	28.5[c]	28.7[e]	28.7
C-30	178.2	173.8	176.9	176.1	176.8	177.3	176.8	173.3	179.1
OCH_3	52.2	–	51.7	–	–	51.9	—	—	
Inner sugar									
C-1	102.8	101.8	97.6	96.9	102.5	97.6	95.6	105.2	104.9
C-2	72.5	74.3	78.1	76.9	74.0	71.3	72.0	74.3	83.8
C-3	89.3	88.9	75.5	75.9	77.3	74.1	74.3	74.7	77.8
C-4	69.2	71.3	71.6	73.2[e]	70.1	80.6	70.5	75.0	71.6
C-5	74.7	73.5	78.1	77.5	76.1	73.2	72.8	77.7	76.9
C-6	61.9	62.1	69.9	68.6	68.9	62.1	62.1	69.6	62.7
Terminal sugar									
C-1	105.2	106.4	105.7	103.2	104.5	106.1		107.2	106.2
C-2	74.4	73.5	73.4[d]	72.7	74.8	74.8		73.9	76.8
C-3	76.5	77.1	77.9	75.9	77.3	77.3		77.7	76.9
C-4	74.2	73.5	73.5[d]	73.3[e]	72.0	74.3		73.2	71.7
C-5	76.5	77.1	75.1	76.5	75.5	76.3		75.1	77.8
C-6	176.1	179.3	172.6	173.2	176.2	171.5		172.8	173.3

[a]Spectra were obtained in pyridine-d_5. Chemical shifts of carbons on pyranoses were obtained by comparison with those previously reported [27–33]. [b,c,d,e]These values may be interchangeable in each column.

with $NaHCO_3$-saturated water then water, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to give compounds **10** (2.7 g) and **11** (340 mg). ^{1}H-NMR data for **10** and **11**: see table I.

*Methyl 3-O-(2', 4', 6'-tri-O-acetyl-β-D-glucopyranosyl)glycyrrhetinate **12***

A solution of compound **10** (2.8 g, 3.2 mmol) in AcOH (40 mL) was added to 10% palladium/charcoal catalyst (100 mg) and stirred at room temperature for 12 h under H_2. The reaction mixture was filtered, and the filtrate was evaporated to give a residue which was subjected to column chromatography (a gradient of 0–5% acetone in benzene) to give compound **12** (2.5 g). ^{1}H-NMR data: see table I.

*Methyl 3-O-[3'-O-(methyl 2",3",4"-tri-O-acetyl-β-D-glucuronatopyranosyl)-2',4',6'-tri-O-acetyl-β-D-glucopyranosyl]-glycyrrhetinate **14***

To a solution of compound **12** (1.0 g, 1.3 mmol) in CH_2Cl_2 (6 mL), $Hg(CN)_2$ (550 mg, 2.2 mmol), $HgBr_2$ (550 mg, 1.5 mmol) and Drierite (1 g, 7.3 mmol) were added, and the mixture was stirred for 1 h at room temperature with shielding from light. Methyl 2,3,4-tri-*O*-acetyl-α-D-glucuronatopyranosyl bromide **13** (1.1 g, 3.5 mmol) was added, and the mixture was stirred for an additional 4 h then worked up as described for the glycosylation of **9** with **7** to give a residue. The residue was subjected to column chromatography (a gradient of 0–6.2% acetone in benzene), followed by preparative HPLC (30% H_2O/acetone) to give compound **14** (780 mg). ^{1}H-NMR data: see table I.

*Methyl 3-O-[3'-O-(β-D-glucuronatopyranosyl)-β-D-glucopyranosyl]glycyrrhetinate **15***

A solution of compound **14** (620 mg, 0.57 mmol) in 5% KOH in EtOH/H_2O (1:1, 20 mL) was allowed to stand at room temperature for 12 h. The mixture was neutralized with acetic acid, then evaporated to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to obtain compound **15** (340 mg). ^{13}C-NMR data: see table II.

*Acid hydrolysis of **15***

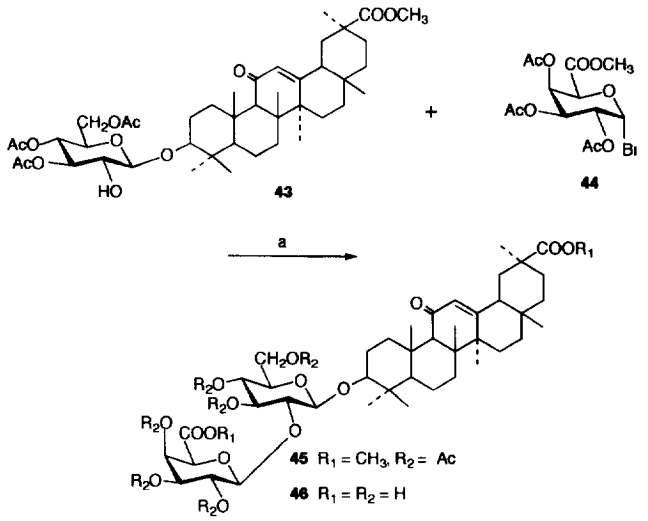
Compound **15** (5 mg, 5.9 μmol) was dissolved in 1N H_2SO_4 (1.0 mL) and heated at 80 °C for 2 h. After cooling, the mixture was neutralized with saturated aqueous $BaCO_3$, then centrifuged to give a supernatant solution. The solution was passed through Amberlite IR-120B (H^+ form) and eluted with distilled water. The eluent was evaporated to give methyl glycyrrhetinate **9**, which was identified by comparison with an authentic sample by TLC.

*3-O-[3'-O-(β-D-Glucuronatopyranosyl)-β-D-glucopyranosyl]-glycyrrhetinate **16***

A mixture of compound **15** (330 mg, 0.39 mmol) and LiI (500 mg, 2.9 mmol) in γ-collidine (4 mL) was stirred at 170 °C under argon. After cooling to room temperature, the mixture was neutralized with acetic acid and evaporated to afford a residue. The residue was subjected to column chromatograpy ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer), followed by preparative HPLC (35% H_2O/MeOH) to give compound **16** (146 mg). ^{13}C-NMR data: see table IV.

*6-O-(Methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-1,2,3,4-tetra-O-acetyl-D-glucopyranose **18***

A mixture of **17** (15.0 g, 24.2 mmol) and Amberlite IR-120 (H^+ form, 3 g) in 85% AcOH (350 mL) was stirred under reflux



Scheme 8. Reagents: (a) $Hg(CN)_2$, $HgBr_2$, Drierite, CH_2Cl_2.

for 5 h. After cooling to room temperature, the mixture was filtered. The filtrate was neutralized with pyridine and evaporated to obtain a residue. The residue was dissolved in pyridine/Ac_2O (1:1, 200 mL), and allowed to stand for 18 h at room temperature. The mixture was coevaporated with toluene (5 × 200 mL) to give a residue that was subjected to column chromatography (a gradient of 0–5.0% acetone in benzene) to give compound **18** (9 g). ^{13}C-NMR data: see table IV.

*6-O-(Methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl bromide **19***

A solution of compound **18** (8.0 g, 12.0 mmol) in AcOH (20 mL) to which was added 20% HBr/AcOH (50 mL) was stirred for 1 h at room temperature, and worked up as described for the preparation of **7** to give a residue. The residue was subjected to column chromatogaphy (a gradient of 0–5.0% acetone in benzene) to give compound **19** (8.0 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 6.61 (1H, d, J = 4.0 Hz, H-1), 5.52 (1H, dd, J = 9.5, 9.2 Hz, H-3), 5.25 (1H, dd, J = 9.5, 9.5 Hz, H-3'), 5.22 (1H, dd, J = 9.5, 9.5 Hz, H-4'), 5.08 (1H, d, J = 9.2 Hz, H-1'), 5.07 (1H, dd, J = 9.5, 9.5 Hz, H-4), 5.01 (1H, dd, J = 9.2, 9.2 Hz, H-2'), 4.78 (1H, dd, J = 9.5, 4.0 Hz, H-2), 4.20–4.32 (1H, m, H-5), 4.02 (1H, d, J = 9.5 Hz, H-5'), 3.97–4.04 (2H, H-6a and H-6b), 3.75 (3H, s, OCH_3), 2.01, 2.02, 2.02, 2.05, 2.08 and 2.09 (each 3H, s, OAc).

*Glycosylation of methyl glycyrrhecinate **9** with **19***

A mixture of **9** (1.0 g, 2.1 mmol), **19** (6.3 g, 9.2 mmol), $Hg(CN)_2$ (1.1 g, 4.4 mmol), $HgBr_2$ (1.0 g, 2.8 mmol), and Drierite (1.0 g, 7.3 mmol) in dry CH_2Cl_2 (10 mL) was stirred for 2 h at room temperature while shielded from light. The reaction mixture was worked up as described for the glycosylation of **9** with **7** to give a residue. The residue was purified by column chromatography (a gradient of 0–10% acetone in benzene), followed by preparative HPLC (30% H_2O/acetone) to give compound **20** (620 mg) and **21** (68.6 mg). ^{1}H-NMR data for **20** and **21**: see table II.

Table V. Cytoprotective effects of glycosides on CCl_4-induced hepatic injury[a].

Glycoside	*Dose*	*AST*		*ALT*	
	(mg/mL)	*IU*	*%*	*IU*	*%*
None	–	10.5 + 2	–	13.6 +1	–
Control	–	180.1 + 3	100	183.2 + 5	100
1	0.05	175.9 + 2	98	156.7 + 6	86
	0.1	168.5 + 3	96	149.1 + 5*	81
	0.5	75.6 + 4*	42	67.4 + 3*	37
2	0.05	170.1 + 9	94	168.9 + 7	92
	0.1	157.3 + 2*	87	154.6 + 3*	84
	0.5	82.8 + 7*	46	99.0 + 1*	54
15	0.05	177.3 + 1	98	180.8 + 5	99
	0.1	185.2 + 6	103	179.8 + 3	98
	0.5	189.4 + 5	105	185.2 + 3	101
16	0.05	183.5 + 3	102	179.2 + 2	98
	0.1	166.3 + 2**	92	168.1 + 4**	92
	0.5	73.6 + 8**	41	77.8 +5*	42
23	0.05	186.1 + 2	103	177.7 + 3	97
	0.1	179.6 + 7	100	180.3 + 4	98
	0.5	172.6 + 9	96	179.3 + 3	98
24	0.05	173.8 + 3	97	177.9 + 2	97
	0.1	161.2 + 5**	90	173.7 + 4**	95
	0.5	96.8 + 6*	54	99.0 + 5*	54
25	0.05	183.3 + 5	102	179.4 + 2	98
	0.1	178.5 + 3	99	176.5 + 6	95
	0.5	166.4 + 7**	92	172.6 + 3	94
33	0.05	177.3 + 4	98	181.5 + 2	99
	0.1	175.8 + 4	98	170.9 + 3	95
	0.5	193.6 + 7	107	166.4 + 10	91
42	0.05	178.5 + 3	99	181.1 + 2	99
	0.1	170.2 + 5	99	168.7 + 4**	92
	0.5	105.2 + 3**	58	86.4 + 5**	47
46	0.05	174.3 + 5	97	177.7 + 8	97
	0.1	143.2 + 12	76	154.3 + 5**	84
	0.5	70.2 + 5*	39	94.8 + 2*	52

[a]Glycosides were added at doses of 0.05, 0.1 and 0.5 mg in each suspension (1.0 mL). Both AST and ALT were assayed as described in the text. Activities of glycosides in doses of more than 1.0 mg/mL could not compared because of lower solubility of some glycosides. Significantly different from the control: ** < 0.05, * < 0.01

Methyl 3-O-(2',3'4'-tri-O-acetyl-β-D-glucopyranosyl)glycyrrhetinate **22**
A solution of compound **11** (300 mg, 0.35 mmol) in AcOH (5 mL) to which was added 15% palladium/charcoal catalyst (15 mg) was stirred at room temperature for 15 h under H_2. The reaction mixture was worked up as described for the preparation of **12** to give **22** (200 mg).

Glycosylation of **22** *with* **13**
A solution of **22** (200 mg, 0.26 mmol) in dry CH_2Cl_2 (5 mL) to which was added $Hg(CN)_2$ (250 mg, 0.99 mmol), $HgBr_2$ (250 mg, 0.69 mmol), and Drierite (200 mg, 1.5 mmol) was stirred for 1 h at room temperature while shielded from light. Bromide **13** (500 mg, 1.6 mmol) was added, and the mixture was stirred for a further 24 h at the same temperature. The reaction mixture was worked up as described for the glycosylation of **9** with **7** to obtain a residue. The residue was subjected to column chromatography (a gradient of 0–5% acetone in benzene), followed by preparative HPLC (30% H_2O/acetone), to give compound **21** (290 mg).

Methyl 3-O-[6'-O-(β-D-glucuronatopyranosyl)-α-D-glucopyranosyl]glycyrrhetinate **23**
A solution of compound **20** (500 mg, 0.46 mmol) in 5% KOH in EtOH/H_2O (1:1, 20 mL) was allowed to stand at room temperature for 8 h, then worked up as described for the preparation of **15** to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to obtain compound **23** (300 mg). ^{13}C-NMR data: see table IV.

3-O-[6'-O-(β-D-Glucuronatopyranosyl)-β-D-glucopyranosyl]-glycyrrhetinate **24**
A mixture of **23** (270 mg, 0.32 mmol) and LiI (250 mg, 1.5 mmol) was stirred at 170 °C for 2 h. The mixture was worked up as described for the preparation of **16** to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer), followed by preparative HPLC (35% H_2O/MeOH), to give **24** (135 mg). ^{13}C-NMR data: see table IV.

3-O-[6'-O-(β-D-Glucuronatopyranosyl)-β-D-glucopyranosyl]-glycyrrhetinate **25**
A solution of compound **21** (60 mg, 55.1 μmol) and LiI (100 mg, 0.59 mmol) in γ-collidine (2 mL) was stirred at 17 °C under argon, and worked up as described for the preparation of **15** to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to obtain compound **25** (38 mg). ^{13}C-NMR data: see table IV.

4,6-O-Benzylidene-1,2,3-tri-O-acetyl-β-D-glucopyranose **27**
To a refluxing suspension of anhydrous NaOAc (35 g, 0.43 mol) in acetic anhydride (700 mL) seventy 1 g portions of 4,6-*O*-benzylidene-D-glucose **26** (total: 70 g, 0.26 mol) were cautiously added. After addition of all of the **26**, the mixture was further refluxed for 1 h, then poured into ice-water (700 mL) and extracted with CH_2Cl_2 (3 × 700 mL). The combined CH_2Cl_2 extracts were washed with $NaHCO_3$-saturated water then water, dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to give a residue that was subjected to column chromatography (a gradient of 0–5% acetone in benzene), followed by preparative HPLC (30% H_2O in acetone), to give compound **27** (22.0 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 7.33–7.45 (5H, aromatic protons), 5.79 (1H, d, J = 8.1 Hz, H-1), 5.50 (1H, -C*H*C_6H_5), 5.37 (1H, dd, J = 9.2, 9.2 Hz, H-3), 5.13 (1H, dd, J = 9.2, 8.1 Hz, H-2), 4.39 (1H, dd, J = 10.3, 4.4 Hz, H-6a), 3.73 (1H, dd, J = 9.2, 9.2 Hz, H-4), 3.65–3.80 (2H, H-5 and 6a), 2.05, 2.06 and 2.11 (each 3H, s, OAc).

1,2,3-Tri-O-acetyl-β-D-glucopyranose **28**
A solution of compound **27** (20.0 g, 48.5 mmol) in 20% acetic acid (100 mL) was stirred at 80 °C for 10 min. The reaction mixture was evaporated to yield a residue that was purified by column chromatography (a gradient of 0–5% MeOH in CH_2Cl_2) to give compound **28** (14.4 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 5.72 (1H, d, J = 8.1 Hz, H-1), 5.14 (1H, dd, J = 9.5, 9.5 Hz, H-3), 5.00 (1H, dd, J = 9.5, 8.1 Hz, H-2), 4.58 (1H, dd, J = 12.5, 3.4 Hz, H-6a), 3.70–3.95 (2H, H-5 and 6a), 3.59 (1H, dd, J = 9.5, 9.5 Hz, H-4), 3.30–3.95 (2H, 2 × OH), 2.04, 2.09 and 2.11 (each 3H, s, OAc).

6-O-Benzoyl-1,2,3-tri-O-acetyl-β-D-glucopyranose **29**
Benzoyl chloride (6.4 mL, 55.0 mmol) was added to a solution of compound **28** (15 g, 49.0 mmol) in pyridine (100 mL), and the resulting solution was stirred at room temperature for 12 h. The reaction mixture was poured into ice-water (200 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The combined CH_2Cl_2 extracts were successively washed with 5% HCl, $NaHCO_3$-saturated water and water, then dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated to obtain a residue that was subjected to column choromatography (a gradient of 0–5% acetone in benzene) to give compound **29** (12.2 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 7.46–8.06 (5H, aromatic protons) 5.76 (1H, d, J = 8.4 Hz, H-1), 5.19 (1H, dd, J = 9.5, 9.5 Hz, H-3), 5.06 (1H, dd, J = 9.5, 8.4 Hz, H-2), 4.73 (1H, dd, J = 12.5, 4.0 Hz, H-6a), 4.55 (1H, dd, J = 12.5, 1.8 Hz, H-6b), 3.82 (1H, ddd, J = 9.5, 4.0, 1.8 Hz, H-5) 3.71 (1H, dd, J = 9.5, 9.5 Hz, H-4), 2.04, 2.09 and 2.10 (each 3H, s, OAc).

Glycosylation of **9** *with* **29**
A mixture of **9** (7.2 g, 14.9 mmol) in CH_2Cl_2 (50 mL) was stirred for 1 h at room temperature with shielding from light. Compound **29** (12 g, 30.4 mmol) and trimethylsilyl trifluoromethane sulfonate (Me_3Si-Triflate) (1.0 mL) were added, and the resulting mixture was stirred for 6 h. The mixture was worked up as described for glycosylation of **9** with **7** to give a residue. The residue was purified by column chromatography (a gradient of 0–10% acetone in benzene) to give compounds **30** (4.3 g) and **31** (1.5 g). ^{1}H-NMR data for **30** and **31**: see table III.

Methyl 3-O-[4'-O-(methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-6'-O-benzoyl-2',3'-di-O-acetyl-α-D-glucopyranosyl]glycyrrhetinate **32**
A mixture of compound **31** (1.4 g, 1.6 mmol), bromide **13** (3.0 g, 9.5 mmol), silver trifluoromethane sulfonate (Ag-Triflate) (540 mg, 2.1 mmol), and 1,1,3,3-tetramethyl urea (TMU) (0.72 mL, 6.2 mmol) in CH_2Cl_2 (50 mL) was stirred with shielding from light for 5 h at room temperature. The mixture was worked up as described for the glycosylation of **9** with **7** to give a residue. The residue was purified by column chromatography (a gradient of 0–20% AcOEt in benzene) to give compound **32** (610 mg). ^{1}H-NMR data: see table III.

Methyl 3-O-[4'-O-(β-D-glucuronatopyranosyl)-α-D-glucopyranosyl]glycyrrhetinate **33**
A solution of **32** (600 mg, 0.52 mmol) in 5% KOH in EtOH/H_2O (1:1, 5 mL) was allowed to stand at room temperature for 10 h, and worked up as described for the preparation of **15** to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to obtain compound **33** (240 mg). ^{13}C-NMR data: see table IV.

Table VI. Physical data for compounds synthesized in this study.

Starting material	*Product (yield, %)*	*FABMS*[a]	*Formula*	*Calc*		*Found*	
				C	*H*	*C*	*H*
4	**6** (55) mp 95–97[b]	461	$C_{21}H_{26}O_{10}$	57.53	5.38	57.51	5.40
6	**7** (15)	481	$C_{19}H_{23}O_8Br$	49.78	5.02	49.76	5.04
9	**10** (50) mp 212–214[c]	885	$C_{50}H_{70}O_{12}$	69.58	8.17	69.53	8.18
9	**11** (7)	885	$C_{50}H_{70}O_{12}$	69.58	8.17	69.55	8.21
10	**12** (99)	795	$C_{43}H_{64}O_{12}$	66.84	8.29	66.8	8.31
12	**14** (55)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.69	7.48
14	**15** (72)	845	$C_{43}H_{66}O_{15}\cdot H_2O$	61.41	8.15	61.18	8.21
15	**16** (45)	831	$C_{42}H_{64}O_{15}\cdot H_2O$	61.00	8.04	60.85	8.19
17	**18** (52)	687	$C_{27}H_{36}O_{19}$	48.80	5.46	48.72	5.53
18	**19** (86)	707	$C_{25}H_{33}O_{17}Br$	43.81	4.85	43.63	4.91
19	**20** (30)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.54	7.62
19	**21** (3)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.68	7.57
11	**22** (79)	795	$C_{43}H_{64}O_{12}$	66.84	8.29	66.79	8.33
22	**21** (26)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.68	7.57
20	**23** (79)	845	$C_{43}H_{66}O_{15}\cdot H_2O$	61.41	8.15	61.36	8.17
23	**24** (51)	831	$C_{42}H_{64}O_{15}\cdot H_2O$	61.00	8.04	60.93	8.09
21	**25** (84)	831	$C_{42}H_{64}O_{15}\cdot H_2O$	61.00	8.04	60.85	8.17
26	**27** (21)	417	$C_{19}H_{22}O_9\cdot H_2O$	55.34	5.87	55.29	5.92
27	**28** (93)	329	$C_{12}H_{18}O_9$	47.06	5.92	46.60	5.93
28	**29** (62)	433	$C_{19}H_{22}O_9$	55.61	5.40	55.34	5.33
29	**30** (30)	875	$C_{48}H_{66}O_{12}\cdot H_2O$	68.30	8.00	68.28	8.06
29	**31** (12)	875	$C_{48}H_{66}O_{12}\cdot H_2O$	68.30	8.00	68.12	8.23
31	**32** (31)	1173	$C_{61}H_{82}O_{21}$	63.64	7.18	63.38	7.24
32	**33** (56)	845	$C_{43}H_{66}O_{15}\cdot H_2O$	61.41	8.15	61.27	8.31
33	**34** (40)	655	$C_{36}H_{56}O_{19}\cdot H_2O$	66.43	8.98	66.15	9.12
37	**38** (71)	599	$C_{25}H_{36}O_{15}$	52.08	6.29	51.99	6.54
38	**39** (52)	687	$C_{27}H_{36}O_{19}$	48.80	5.46	48.83	5.51
39	**40** (86)	707	$C_{25}H_{33}O_{17}Br$	43.81	4.85	65.74	9.23
40	**41** (43)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.43	7.49
41	**42** (44)	831	$C_{42}H_{64}O_{15}\cdot 2H_2O$	59.70	8.11	59.68	8.25
43	**45** (58)	1111	$C_{56}H_{80}O_{21}$	61.75	7.40	61.56	7.39
45	**46** (43)	831	$C_{42}H_{64}O_{15}\cdot H_2O$	61.00	7.80	61.12	7.64

[a]Peaks were observed as the quasimolecular ion $[M + Na]^+$. [b]After recrystallization from 90% EtOH. [c]After recrystallization from Et_2O/petroleum ether.

3-O-(β-D-glucopyranosyl)glycyrrhetic acid ***34***
A solution of compound **33** (60 mg, 71.3 μmol) and LiI (150 mg, 0.88 mmol) in γ-collidine (2 mL) was stirred at 170 °C under argon, and worked up as described for the preparation of **15** to give a residue. The residue was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to obtain compound **34** (40 mg). ^{13}C-NMR data: see table IV.

6-O-(Methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-1,2;3,4-di-O-isopropylidene-α-D-galactopyranose ***38***
To a solution of 1,2;3,4-di-*O*-isopropylidene-α-D-galactopyranse **37** (15.0 g, 57.6 mmol) in benzene/nitromethane (1:1, 200 mL) was added pyranose bromide **13** (40.0 g, 126.1 mmol) and $Hg(CN)_2$ (20.0 g, 79.2 mmol); the mixture was stirred at 45 °C for 12 h with shielding from light. The reaction mixture was worked up as described for the glycosylation of **9** with **7** to give a residue. The residue was purified by column chromatography (a gradient of 0–5% acetone in benzene) to give compound **38** (23.7 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 2.02, 2.07, 2.07 (each s, Ac), 3.68 (1H, dd, *J* = 11.4, 8.1 Hz, H-6a), 3.72 (3H, s, OCH_3), 3.93 (1H, m, H-5), 4.03 (1H, d, *J* = 9.5 Hz, H-5'), 4.04 (1H, dd, *J* = 11.4, 2.6 Hz, H-6b), 4.17 (1H, d, *J* = 2.6 Hz, H-4), 4.29 (1H, dd, *J* = 5.1, 2.6 Hz, H-2), 4.58 (1H, dd, *J* = 7.7, 2.6 Hz, H-3), 4.66 (1H, d, *J* = 7.7 Hz, H-1'), 5.02 (1H, dd, *J* = 9.5, 7.7 Hz, H-2'), 5.20 (1H, dd, *J* = 9.5, 9.5 Hz, H-4'), 5.27 (1H, dd, *J* = 9.5, 9.5 Hz, H-3'), 5.49 (1H, d, *J* = 5.1 Hz, H-1).

6-O-(Methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-1,2,3,4-tetra-O-acetyl-D-galactopyranose ***39***
A solution of compound **38** (15.0 g, 26.0 mmol) in 80% AcOH (100 mL) was refluxed for 36 h. After cooling, the solution was neutralized with pyridine and evaporated to obtain a residue that was purified by column chromatography (a gradient of 0–5% acetone in benzene) to give compound **39** (9.0 g).

6-O-(Methyl 2',3',4'-tri-O-acetyl-β-D-glucuronatopyranosyl)-2,3,4-tri-O-acetyl-α-D-galactopyranosyl bromide ***40***
To a solution of compound **39** (9.0 g, 13.5 mmol) in acetic acid (10 mL) was added 20% HBr/AcOH (50 mL), and the mixture was stirred at 0 °C for 1 h. The reaction mixture was worked up as described for the preparation of **7** to give compound **40** (8.0 g). ^{1}H-NMR spectrum ($CDCl_3$) δ: 6.69 (1H, d, *J* = 4.0 Hz, H-1), 5.48 (1H, d, *J* = 3.3 Hz, H-4), 5.38 (1H, dd, *J* = 10.6, 3.3 Hz, H-3), 5.18–5.24 (2H, H-3' and H-4'), 5.02 (1H, dd, *J* = 10.6, 4.0 Hz, H-2), 4.99 (1H, dd, *J* = 8.1, 8.1 Hz, H-2'), 4.60 (1H, d, *J* = 8.1 Hz, H-1'), 4.03 (1H, d, *J* = 9.5 Hz, H-5'), 3.86 (1H, dd, *J* = 11.4, 5.5 Hz, H-6a), 3.76 (3H, s, OCH_3), 3.75 (1H, m, H-5), 3.72 (1H, dd, *J* = 11.4, 7.0 Hz, H-6b), 2.00, 2.02, 2.02, 2.07, 2.11 and 2.14 (each 3H, s, OAc).

Methyl 3-O-[6'-O-(methyl 2",3",4"-tri-O-acetyl-β-D-glucuronatopyranosyl)-2',4',6'-tri-O-acetyl-β-D-galactopyranosyl]-glycyrrhetinate ***41***
To a solution of **9** (1.6 g, 3.3 mmol) and bromide **40** (5.6 g, 8.2 mmol) in CH_2Cl_2 (30 mL) was added $Hg(CN)_2$ (550 mg, 2.2 mmol), $HgBr_2$ (550 mg, 1.5 mmol), and Drierite (1 g, 7.3 mmol), and the mixture was stirred for 3 h at room temperature while shielded from light. The mixture was worked up as described for the glycosylation of **9** with **7** to give compound **41** (1.6 g). ^{1}H-NMR data: see table II.

3-O-[6'-O-[(β-D-Glucuronatopyranosyl)-β-D-galactopyranosyl]glycryrrhetic acid ***42***
A solution of compound **41** (440 mg, 0.40 mmol) and LiI (120 mg, 0.71 mmol) in γ-collidine (5 mL) was refluxed for 2 h. After cooling, the mixture was neutralized with acetic acid and evaporated to obtain a residue that was subjected to column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer) to give compound **42** (150 mg). ^{13}C-NMR data: see table IV.

Methyl 3-O-[2'-O-(methyl 2",3",4"-tri-O-acetyl-β-D-galacturonatopyranosyl)-3',4',6'-tri-O-acetyl-β-D-glucopyranosyl]-glycyrrhetinate ***45***
A mixture of monoglycoside **43** (400 mg, 0.47 mmol), $Hg(CN)_2$ (260 mg, 1.0 mmol), $HgBr_2$ (370 mg, 1.0 mmol) and Drierite (300 mg, 2.2 mmol) in CH_2Cl_2 (20 mL) was stirred for 1 h at room temperature. Bromide **44** (800 mg, 2.5 mmol) was then added to the mixture, which was stirred for another 7 h at the same temperature. The mixture was worked up as described for the glycosylation of **9** to give a residue, which was subjected to column chromatography (a gradient of 0–3% acetone in benzene) to obtain **45** (328 mg). ^{1}H-NMR data: see table II.

3-O-[2'-O-(β-D-galacturonatopyranosyl)-β-D-glucopyranosyl]-glycyrrhetinate ***46***
A mixture of **45** (320 mg, 0.29 mmol) and LiI (300 mg, 1.8 mmol) in γ-collidine (5 mL) was heated at 170 °C for 2 h. The mixture was worked up as described for the preparation of **16** to afford a residue that was purified by column chromatography ($CHCl_3$/MeOH/H_2O, 65:35:10, lower layer), followed by preparative HPLC 30% H_2O/MeOH), to give **46** (105 mg). ^{13}C-NMR data: see table IV.

Cytoprotective activity

The cytoprotective activities of diglycosides were determined by measurement of the activities of AST and ALT which were assayed by autoanalyzer COBAS MRA (Roche). Commercial kits based on the principle of the AST and ALT assay method [24] were used. Statistical examination was carried out with the Student's *t* test.

References

1 Chandel RS, Rastogi RP (1980) *Phytochemistry* 19, 1889–1908
2 Kumagai A, Yano S, Otomo M, Takeuchi K (1957) *Endocrinol Jpn* 4, 17–27
3 Kumagai A, Nanaboshi M, Asanuma Y, Yagura T, Nishino K, Yamamura (1976) *Endocrinol Jpn* 14, 39–42
4 Takahashi K, Shibata S, Yano S et al (1980) *Chem Pharm Bull* 28, 3449–3452
5 Shoji J (1981) *Kagaku No Ryoiki* 35, 325–330
6 Shoji J (1981) *Kagaku No Ryoiki* 35, 414–423
7 Saito S, Kuroda K, Hayashi Y, Nagamura Y, Nishida K, Ishiguro I (1991) *Chem Pharm Bull* 39, 2333–2339
8 Saito S, Sasaki Y, Kuroda K et al (1993) *Chem Pharm Bull* 41, 539–543
9 Saito S, Sumita S, Furumoto T et al (1994) *Eur J Med Chem* 29, 455–470
10 Otto TS (1963) *Methods Carbohydr Chem* 2, 318–325
11 Paulsen H (1982) *Angew Chem* 21, 155–224
12 Fhelhaber H, Snatzke G, Vlahov I (1987) *Liebigs Ann Chem* 637–638
13 Saito S, Sasaki Y, Furumoto T, Sumita S, Hinomoto T (1994) *Carbohydr Res* 258, 59–75
14 Zervas L (1931) *Ber* 64, 2289–2296
15 Trumtel M, Veyrieres A, Sinay P (1989) *Tetrahedron Lett* 30, 2529–2532
16 Winkley WR (1988) *Mod Carb Chem* 297–322
17 Saito S, Sumita S, Kanda Y, Sasaki Y (1992) *Tetrahedron Lett* 33, 7381–7384
18 Saito S, Sumita S, Kanda Y, Sasaki Y (1994) *Chem Pharm Bull* 42, 1016–1027
19 Freudenberg K, Hixon RM (1923) *Chem Ber* 56, 2119–2127
20 Saito S, Sumita S, Ichinose K, Kanda Y (1993) *Chem Pharm Bull* 41, 90–96

21 Kiso Y, Tohkin M, Hikino H, Hattori M, Sakamoto T, Namba T (1984) *Planta Med* 50, 298–302
22 Fujisawa K, Kurihara N, Kojima M et al (1977) *Jpn J Med* 16, 14–23
23 Seglen PO (1976) *Methods Cell Biol* 13, 29–83
24 Heerspind W, Hafkensheidt JCM, Siepel H, van der Ven-Jongekryge J, Dijt CCM (1989) *Enzyme* 25, 333–341
25 Abraham RJ, Bernstein HJ (1961) *Can J Chem* 39, 216–230
26 Musher JI, Corey EJ (1962) *Tetrahedron* 18, 791–809
27 Dorman DE, Angyal SJ, Roberts JD (1970) *J Am Chem Soc* 90, 1351–1354
28 Dorman DE, Roberts JD (1970) *J Am Chem Soc* 90, 1355–1361
29 Choi JS, Woo WS (1987) *Planta Med* 53, 62–65
30 Kasai R, Matsumoto K, Nie OL, Zhou JL, Tanaka O (1988) *Chem Pharm Bull* 36, 234–243
31 Kitagawa I, Sakagami M, Hashiuchi F, Zhou JL, Yoshikawa M, Ren J (1989) *Chem Pharm Bull* 37, 551–553
32 Yahara S, Emura S, Feng H, Nohara T (1989) *Chem Pharm Bull* 37, 2136–2138
33 Konoshima T, Kozuka M, Haruna M, Ito K, Kimura T, Toduda H (1989) *Chem Pharm Bull* 37, 2731–2735