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Synthesis and Hemolytic Properties of Lactosides of Glycyrrhetic Acid Derivatives

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Summary. The partial synthesis of lactosides of glycyrrhetic acid, its 11-deoxo and 18α-derivatives, and their methyl esters is described. The influence of the aglycon structure on the hemolytic properties of the title compounds is discussed and compared with those of oleanolic acid derivatives. Furthermore, we describe an optimized preparation of 18α-glycyrrhetic acid.

Keywords. Hemolytic activity; Glycosidation; Glycyrrhetic acid; Lactosides.

Introduction

Recently, we have reported on the hemolytic properties of oleanolic acid glycosides containing galactose units [1]. Amongst these compounds the glycoside with a lactosyl residue, calenduloside A, was the most active substance, exhibiting a remarkable hemolytic index of 150700.

To examine the influence of variations of the aglycon structure on the hemolytic activity we have prepared lactosides of structurally related glycyrrhetic acid derivatives. During these investigations the influence of the position of the carboxyl group, of an additional oxo group, and of the linkage between rings D and E has been studied. Besides, we synthesized the methyl esters of the glycosides in order to verify if the hemolytic activity is in general slightly increased by esterification of acidic triterpene saponins as presumed by some authors [2, 3].

Results and Discussion

As starting material for the synthesis of the aglyca we used glycyrrhetic acid (**1**). The 18α-isomer of **1** (**2**) was synthesized in order to reveal the influence of the linkage between rings D and E. Furthermore, we removed the oxo group in position 11 of **1** to obtain the 11-deoxo derivative **3** which differs from oleanolic acid (**4**) only by the interchanged positions of the carboxyl group and a methyl group.

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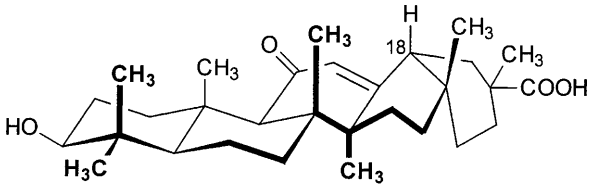
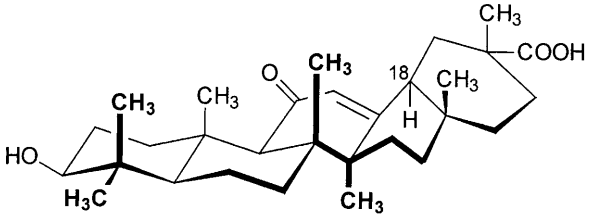


1



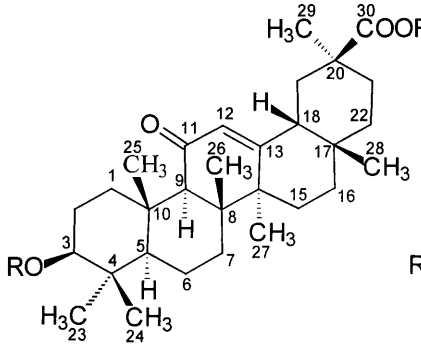
2

The benzhydrylic esters **5**–**7** of aglyca **1**–**3** were glycosylated by *Königs-Knorr* procedures [4, 5] to yield the protected saponins **8**–**10**. Deacetylation and hydrogenation of **8**–**10** afforded the glycosides **11**–**13**. The methyl esters **14** and **15** were prepared from the saponins **11** and **12**. Likewise, the methyl ester **16** was obtained from the readily synthesized calenduloside A (**17**) [1]. The hemolytic properties of all synthesized saponins were detected as reported [6].

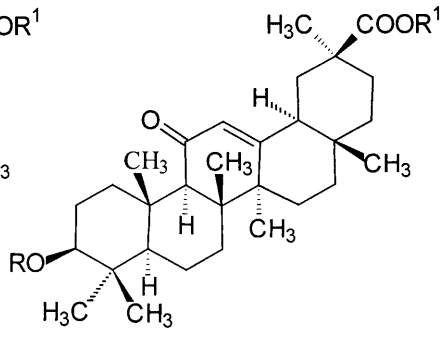
Syntheses

Beaton and *Spring* have reported the syntheses of 18α-glycyrrhetic acid (**2**) from 18β-glycyrrhetic acid (**1**) and its methyl ester under acidic or alkaline conditions [7]. Refluxing for 120 h with aqueous KOH gave 38% of **2**. However, we obtained 45% of **2** within 2 h by heating of **1** in diethylene glycol at 220°C. 11-Deoxo-18β-glycyrrhetic acid (**3**) was synthesized by hydrogenation of **1** using platinum as catalyst. The diphenylmethyl esters of triterpene acids **1**–**3** were prepared using diphenyldiazomethane [8, 9]. As glycosyl donor, 4β-*D*-galactopyranosyl-α-*D*-glucopyranosylbromide heptaacetate (acetobromolactose) was used [1].

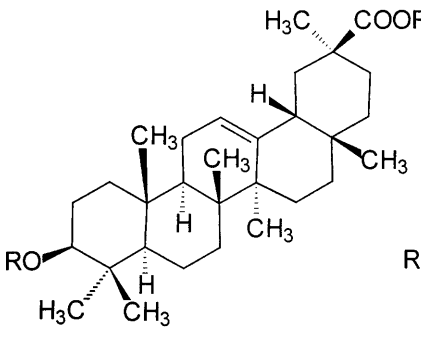
Glycosidations were performed by application of *Königs-Knorr* procedures with mercury (II) cyanide [10] and silver oxide [11] as catalysts. Recently, we have reported that silver oxide catalyzed glycosidations of oleanolic acid with $1 \rightarrow 4$ linked dissacharide donors affords orthoesters [12]. However, under the same conditions no formation of orthoesters was observed during the reactions of **5** and **6** with acetobromolactose. Deacetylation of protected glycosides **8**–**10** were carried out by treatment with sodium methylate according to a procedure given in Ref. [13]. The benzhydrylic esters were cleaved by hydrogenation with Pd on charcoal (10%), affording glycosides **11**–**13**. Those and calenduloside A (**17**) [1] were methylated using an etheral solution of diazomethane giving compounds **14**–**16**.



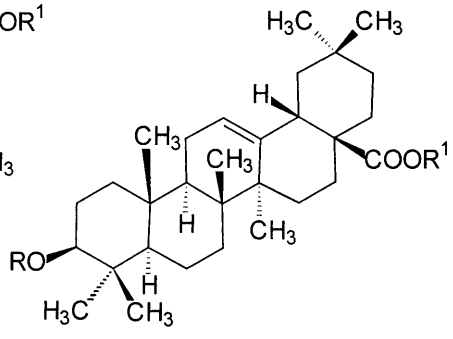
1: R = H, R^1 = H
5: R = H, R^1 = $CH(Ph)_2$
8: R = *A*, R^1 = $CH(Ph)_2$
11: R = *B*, R^1 = H
14: R = *B*, R^1 = CH_3



2: R = H, R^1 = H
6: R = H, R^1 = $CH(Ph)_2$
9: R = *A*, R^1 = $CH(Ph)_2$
12: R = *B*, R^1 = H
15: R = *B*, R^1 = CH_3



3: R = H, R^1 = H
7: R = H, R^1 = $CH(Ph)_2$
10: R = *A*, R^1 = $CH(Ph)_2$
13: R = *B*, R^1 = H



4: R = H, R^1 = H
16: R = *B*, R^1 = CH_3
17: R = *B*, R^1 = H

A: *β-D*-(2'',3'',4'',6''-Tetraacetyl-*galp*)-(1''→4')-*β-D*-(2',3',6'-triacetyl-*glcp*)-(1'→
B: *β-D*-(*galp*)-(1''→4')-*β-D*-(*glcp*)-(1'→

Structure determination

The resonances in the NMR spectra were assigned with the aid of $^1H,^1H$ – COSY, HSQC, and HMBC spectra (optimized for a long-range coupling constant of 8 Hz). The crosspeaks in the latter from H-1′ of compounds **8**–**17** to C-3 established the bond between the carbohydrate moiety and the aglycon. Within the carbohydrate units, the ^1H signals were assigned by means of 1D TOCSY experiments. All

Table 1. Hemolytic activity of compounds **11**–**17**

Saponin	*HI*
11	<10000
12	<10000
13	<10000
14	135000
15	19500
16	10000
17	150700

saponins have β-configuration at the inner anomeric position as indicated by large coupling constants ($J \cong 8$ Hz) of their H-1′ in the ^{1}H NMR spectra. The formation of 18α-glycyrrhetic acid was verified from its ^{13}C NMR spectrum. We observed a typical low-field shift for the signal of C-16 (10 ppm), whereas the resonances of C-18 (9 ppm), C-19 (9 ppm), and C-28 (12 ppm) were observed at higher field. This is consistent with data reported for 18α- and 18β-methylglycyrrhetinate [14].

Determination of hemolytic activity

The lactosides **11**–**13** possess no detectable hemolytic properties. The 11-deoxo compound **13** differs from the highly active oleanolic acid lactoside **17** only by interchanged positions of a methyl and the carboxyl group. Therefore, a carboxy group in position 17 may be assumed as a basic requirement for high hemolytic activity of lactosides of pentacyclic triterpene acids. However, the corresponding methyl esters show a reverse activity order. The above mentioned presumption that esterification increases the hemolytic activity of triterpene saponins seems to be true in the case of glycosides derived from glycyrrhetic acid. Compounds **14** and **15** possess increased activities, whereas esterification of the most active oleanolic acid lactoside **17** led to the almost inactive compound **16**. The linkage between rings D and E has remarkable influence on the activity of esters of glycyrrhetic acid lactosides. The 18β-isomer **14** exhibits far higher hemolytic potency than its 18α-analogue **15**.

Experimental

General

Optical rotation: polarimeter 241 MC (Perkin Elmer). MS: Varian MAT 711 spectrometer (70 eV electron impact and field desorption). IR spectra: infrared spectrometer system 2000 FT (Perkin Elmer). UV/Vis: Lambda 17 UV/Vis-spectrometer (Perkin Elmer). NMR spectra: Varian Inova 400 (300 K), 5 mm tubes, solvent resonance as internal standard. ^{1}H and ^{13}C resonances were assigned using ^{1}H,^{1}H- and ^{1}H,^{13}C-correlation techniques; the atom numbering corresponds to that in the formulae. Assignments marked with an asterisk are interchangeable.

Materials: column chromatography (CC): silica gel 60 (Merck 70–230 mesh, pore-diameter 60 Å); thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F_{254}, 0.2 mm, 200 × 200 mm); preparative TLC: PLC plates (Merck, silica gel 60 F_{254}, 1 mm, 200 × 200 mm); the compounds were detected in UV light at 254 nm and by spraying with MeOH:H_2SO_4 = 9:1 and

subsequent heating with a hot gun. Glycyrrhetic acid is commercially available (Fluka). Acetobromolactose was synthesized as reported [1]. $CHCl_3$ was dried by distillation over P_2O_5 and deacidified with basic Al_2O_3. Benzene was dried with Na, nitromethane was deacidified with Al_2O_3. The hemolytic activity (expressed as Hemolytic Index, *HI*) was evaluated by the method of the Austrian Pharmacopoeia (OeAB 1994) using the *Austrian Saponinstandard* as reference ($HI =$ 30000).

18α-glycyrrhetic acid (**2**)

4 g KOH (0.071 mol) were added to 25 cm^3 of diethylene glycol and heated to 100°C giving a dark brown solution. 10 g (0.02 mol) of glycyrrhetic acid (**1**) were added, and the solution was stirred at 220°C for two h. The solidified mixture was cooled to room temperature, and H_2O and concentrated HCl were added. The brownish solid was filtered of, washed with H_2O to remove the HCl, and then extracted with hot acetone. The remaining white solid was filtered by suction and recrystallized from 800 cm^3 of EtOH giving 4.5 g (45%) of **2**.

M.p.: 330°C (Ref. [7]: 330–335°C); $[\alpha]_D^{20} = +74.5°$, $[\alpha]_{546}^{20} = +85.7°$ ($c = 0.081$, EtOH); IR (KBr): $\bar{\nu} =$ 3496 (w), 2969 (m), 2926 (m), 2867 (w), 1704 (s), 1661 (s), 1297 (w), 1200 (w), 1112 (w), 1028 (w), 989 (w), 658 (w) cm^{-1}; ^{1}H NMR (400 MHz, δ, *DMSO*-d_6): 0.64–0.68 (m, 7H, 5-H, 24-H, 28-H), 0.89 (s, 3H, 23-H), 0.93–0.99 (m, 1H, 1-H), 1.03 (s, 3H, 26-H), 1.08 (s, 3H, 25-H), 1.15 (s, 3H, 29-H), 1.19–1.66 (m, 17H, 2-H, 6-H, 7-H, 15-H, 16-H, 19-H, 21-H, 22-H, 27-H), 1.77 (dt, $J = 13.8$, 3.6 Hz, 1H, 21-H), 1.88 (dt, $J = 13.3$, 3.8 Hz, 1H, 15-H), 2.25–2.28 (m, 2H, 9-H, 18-H), 2.45 (d, b, $J = 13.3$ Hz, 1H, 1-H), 3.00 (dd, $J = 10.8$, 3.5 Hz, 1H, 3-H), 5.32 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, *DMSO*-d_6): 15.82 (C-28), 16.23 (C-24), 16.54 (C-25), 17.42 (C-6), 18.38 (C-26), 20.58 (C-27), 20.77 (C-29), 26.53 (C-15), 27.10 (C-2), 28.34 (C-23), 28.52 (C-21), 31.59 (C-19), 33.36 (C-7), 35.27 (C-17), 35.43 (C-22), 36.62 (C-10), 36.82 (C-16), 38.60 (C-1), 38.86 (C-4), 39.66 (C-18), 41.75 (C-20), 43.50 (C-8), 44.84 (C-14), 54.34 (C-5), 60.14 (C-9), 76.79 (C-3), 123.16 (C-12), 166.21 (C-13), 179.58 (C-30), 198.89 (C-11) ppm.

11-deoxo-18β-glycyrrhetic acid (**3**)

3 was obtained from glycyrrhetic acid by catalytic hydrogenation with Pt following a procedure given in the literature [15].

Diphenylmethyl 18β-glycyrrhetinate (**5**)

5 was prepared according to Ref. [16].

Diphenylmethyl 18α-glycyrrhetinate (**6**; $C_{43}H_{56}O_4$)

The carboxylic group of **2** was protected with diphenylmethyl diazomethane as described for oleanolic acid [17] to give **6** as a colourless resin.

$[\alpha]_D^{20} = +57.6°$, $[\alpha]_{546}^{20} = +60.85°$ ($c = 0.061$, CH_2Cl_2); IR (KBr): $\bar{\nu} =$ 2971 (s), 2934 (s), 2866 (m), 1730 (s), 1663 (s), 1455 (m), 1387 (m), 1277 (w), 1225 (s), 1192 (m), 1156 (w), 1100 (s), 1032 (m), 994 (w), 756 (m), 699 (s) cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} ($\log\epsilon$) = 241 (3.884) nm; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 0.64–0.68 (m, 4H, 5-H, 28-H), 0.79 (s, 3H, 24-H), 0.87–0.95 (m, 1H, 1-H), 0.98 (s, 3H, 23-H), 1.11 (s, 3H, 26-H), 1.18 (s, 3H, 25-H), 1.20–1.25 (m, 1H, 15-H), 1.26 (s, 3H, 29-H), 1.32 (s, 3H, 27-H), 1.33–1.71 (m, 13H, 2-H, 6-H, 7-H, 16-H, 19-H, 21-H, 22-H), 1.88–2.03 (m, 2H, 15-H, 21-H), 2.20–2.28 (m, 2H, 9-H, 18-H), 2.67 (dt, $J = 13.4$, 3.3 Hz, 1H, 1-H), 3.19 (dd, $J =$ 11.3, 4.9 Hz, 1H, 3-H), 5.51 (s, 1H, 12-H), 6.83 (s, 1H, $CHPh_2$), 7.24–7.32 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 15.62 (C-24), 15.96 (C-28), 16.49 (C-25), 17.52 (C-6), 18.47

(C-26), 20.63 (C-29), 20.72 (C-27), 26.61 (C-15), 27.20 (C-2), 28.06 (C-23), 28.36 (C-21), 31.62 (C-19), 33.74 (C-7), 35.47 (C-17), 35.86 (C-22), 36.80 (C-10), 37.57 (C-16), 39.00 (C-4), 39.09 (C-1), 40.27 (C-18), 42.64 (C-20), 43.75 (C-8), 44.87 (C-14), 54.94 (C-5), 60.62 (C-9), 76.74 ($CHPh_2$), 78.66 (C-3), 124.11 (C-12), 125.93, 126.79, 127.74, 127.79, 128.45, 128.49 (aromatic C), 140.35 (aromatic C_q), 165.38 (C-13), 176.85 (C-30), 199.65 (C-11) ppm; MS (ES +): m/z (%) = 637 (82.0) [M + H^+], 317 (4.2), 241 (4.3), 167 (5.1), 143 (5.1), 130 (30.2), 115 (100.0).

Diphenylmethyl 11-deoxo-18β-glycyrrhetinate (**7**; $C_{43}H_{58}O_3$)

The carboxylic group of **3** was protected with diphenylmethyl diazomethane as described for oleanolic acid [17] giving **7** as a colourless resin.

$[\alpha]_D^{20} = +94.9°$, $[\alpha]_{546}^{20} = +111.1°$ ($c = 0.222$, CH_2Cl_2); IR (KBr): $\bar{\nu} = 2929$ (s), 2867 (m), 1730 (s), 1453 (m), 1382 (w), 1213 (w), 1149 (s), 1082 (m), 1029 (m), 739 (w), 699 (s) cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (logϵ) = 250 (3.028), 236 (2.992) nm; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 0.67 (s, 3H, 28-H), 0.73 (d, $J = 11.5$ Hz, 1H, 5-H), 0.79 (s, 3H, 24-H), 0.84 (dt, $J = 13.4$, 2.1 Hz, 1H, 16-H), 0.93 (s, 6H, 25-H, 26-H), 0.96–0.98 (m, 2H, 1-H, 15-H), 1.00 (s, 3H, 23-H), 1.13 (s, 3H, 27-H), 1.14 (s, 3H, 29-H), 1.26–2.02 (m, 19H, 1-H, 2-H, 6-H, 7-H, 11-H, 15-H, 16-H, 18-H, 19-H, 21-H, 22-H), 3.22 (d, b, $J = 8.2$ Hz, 1H, 3-H), 5.12 (t, $J = 3.4$ Hz, 1H, 12-H), 6.90 (s, 1H, $CHPh_2$), 7.26–7.38 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 15.48 (C-25), 15.57 (C-24), 16.75 (C-26), 18.33 (C-6), 23.51 (C-11), 25.92 (C-27), 26.11 (C-15), 26.91 (C-16), 27.21 (C-2), 27.97 (C-29), 28.08 (C-23), 28.43 (C-28), 31.32 (C-21), 31.86 (C-17), 32.65 (C-7), 36.91 (C-10), 38.08 (C-22), 38.60 (C-1), 38.76 (C-4), 39.75 (C-8), 41.49 (C-14), 42.80 (C-19), 44.24 (C-20), 47.58 (C-9), 47.93 (C-18), 55.15 (C-5), 76.40 ($CHPh_2$), 78.97 (C-3), 122.52 (C-12), 126.97, 127.30, 127.69, 127.79, 128.37 (aromatic C), 140.39, 140.45 (aromatic C_q), 144.25 (C-13), 175.91 (C-30) ppm; MS (ES +): m/z (%) = 447 (14.7), 337 (4.2), 289 (7.7), 282 (18.6), 281 (100.0), 269 (7.1), 241 (16.0), 229 (22.4), 211 (4.5), 205 (10.9), 195 (20.8), 187 (11.9), 182 (6.4), 168 (13.5), 167 (80.8).

Diphenylmethyl 2′,3′,6′,2″,3″,4″,6″-heptaacetyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18β-glycyrrhetinate (**8**; $C_{69}H_{90}O_{21}$)

To a solution of 1.6 g (2.5 mmol) **5** in 20 cm^3 of dry $CHCl_3$, 3.9 g Ag_2O and 6.0 g drierite were added, and the mixture was stirred in the dark under Ar for 2 h to remove last traces of moisture. Then, 0.32 g I_2 were added, and a solution of 1.5 g (3.8 mmol) of acetobromolactose in 10 cm^3 of $CHCl_3$ was added dropwise within an hour. The reaction mixture was stirred in the dark under Ar for 48 h at room temperature. Solids were filtered off by suction, and the solvent was evaporated *in vacuo* at room temperature. The residue was purified by CC over silica gel eluting first with CH_2Cl_2: CH_3OH = 9:1 and subsequently changing to CH_2Cl_2:CH_3OH = 8:2 to obtain 1.05 g (34.1%) of **8** as a resin.

$[\alpha]_D^{20} = +58.5°$, $[\alpha]_{546}^{20} = +65.5°$ ($c = 0.099$, CH_2Cl_2); IR (KBr): $\bar{\nu} = 2950$ (m), 2871 (w), 1756 (s), 1661 (m), 1455 (m), 1368 (m), 1222 (s), 1165 (m), 1149 (m), 1132 (m), 1053 (s), 984 (w) cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (logϵ) = 248 (4.030) nm; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 0.63–0.67 (m, 4H, 5-H, 28-H), 0.73 (s, 3H, 24-H), 0.85–0.97 (m, 5H, 1-H, 15*-H, 23-H), 1.05 (s, 3H, 26-H), 1.09 (s, 3H, 25-H), 1.14 (s, 3H, 29-H), 1.16–1.18 (m, 1H, 16*-H), 1.21–1.39 (m, 8H, 6-H, 7-H, 21-H, 22-H, 27-H), 1.49–1.80 (m, 6H, 2-H, 6-H, 7-H, 15*-H, 19-H), 1.92–2.10 (m, 4H, 16*-H, 18-H, 19-H, 21-H), 1.94 (s, 3H, CH_3COO), 1.99 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.06 (s, 3H, CH_3COO), 2.12 (s, 3H, CH_3COO), 2.26 (s, 1H, 9-H), 2.76 (d, b, $J = 13.4$ Hz, 1H, 1-H), 3.06 (t, $J = 8.1$ Hz, 1H, 3-H), 3.56–3.60 (m, 1H, 5′-H), 3.71 (t, $J = 9.4$ Hz, 1H, 4′-H), 3.85 (t, $J = 6.8$ Hz, 1H, 5″-H), 4.03–4.12 (m, 3H, 6′-H, 6″-H), 4.40 (d, $J = 11.7$ Hz, 1H, 6′-H), 4.45 (d, $J = 7.9$ Hz, 1H, 1″-H), 4.48 (d, $J = 8.2$ Hz, 1H, 1′-H), 4.88–4.95 (m, 2H, 2′-H, 3″-H), 5.08 (dd, $J = 10.2$, 7.9 Hz, 1H, 2″-H), 5.17 (t, $J = 9.2$ Hz, 1H, 3′-H), 5.32 (d, $J = 3.0$ Hz, 1H, 4″-H), 5.48

(s, 1H, 12-H), 6.90 (s, 1H, $CHPh_2$), 7.24–7.37 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 16.25 (C-25), 16.33 (C-24), 17.33 (C-6), 18.63 (C-26), 20.46, 20.59, 20.69, 20.79, 20.80 (CH_3COO), 23.27 (C-27), 25.64 (C-2), 26.34, 26.41 (C-15, C-16), 27.66 (C-23), 28.21, 28.26 (C-28, C-29), 31.14 (C-21), 31.70 (C-17), 32.69 (C-7), 36.77 (C-10), 37.45 (C-22), 39.05 (C-1), 39.15 (C-4), 41.11 (C-19), 43.10 (C-14), 43.96 (C-20), 45.28 (C-8), 47.99 (C-18), 55.20 (C-5), 60.82 (C-6″), 61.67 (C-9), 62.27 (C-6′), 66.62 (C-4″), 69.11 (C-2″), 70.67 (C-5″), 70.95 (C-3″), 72.05 (C-2′), 72.33 (C-5′), 72.91 (C-3′), 76.60 ($CHPh_2$), 76.83 (C-4′), 90.40 (C-3), 101.06 (C-1″), 102.63 (C-1′), 126.96, 127.25, 127.81, 128.12, 128.42, 128.47, 128.60 (aromatic C, C-12), 140.02, 140.07 (aromatic C_q), 168.83, 169.09, 169.35, 169.82, 169.97, 170.08, 170.31 (CH_3COO, C-13), 175.14 (C-30), 199.88 (C-11) ppm; MS (ES +): m/z (%) = 1255 (100.0) [M + H^+], 1213 (6.6), 619 (5.9), 391 (4.9), 332 (5.6), 331 (34.2), 313 (4.3), 289 (3.9), 205 (3.3), 169 (3.9), 168 (12.2), 167 (80.3), 130 (14.5), 115 (42.1).

Diphenylmethyl 2′,3′,6′,2″,3″,4″,6″-heptaacetyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18α-glycyrrhetinate (**9**; $C_{69}H_{90}O_{21}$)

Under the same conditions as described for **8**, 1.6 g (2.5 mmol) of **6** gave 980 mg (31.1%) of **9** as a resin.

$[\alpha]_D^{20} = +33.2°$, $[\alpha]_{546}^{20} = +26.2°$ ($c = 0.057$, CH_2Cl_2); IR (KBr): $\bar{\nu}$ = 2971 (m), 2946 (w), 2871 (w), 1756 (s), 1665 (m), 1455 (w), 1369 (m), 1226 (s), 1172 (m), 1053 (s), 984 (w) cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (logϵ) = 241 (4.057) nm; ^1H NMR (400 MHz, δ, $CDCl_3$): 0.64–0.68 (m, 4H, 5-H, 28-H), 0.73 (s, 3H, 24-H), 0.76–0.79 (m, 1H, 1-H), 0.89 (s, 3H, 23-H), 1.10 (s, 3H, 26-H), 1.12–1.14 (m, 1H, 15-H), 1.16 (s, 3H, 25-H), 1.25 (s, 3H, 29-H), 1.31 (s, 3H, 27-H), 1.35–1.82 (m, 13H, 2-H, 6-H, 7-H, 16-H, 19-H, 21-H, 22-H), 1.90–2.07 (m, 2H, 15-H, 21-H), 1.94 (s, 3H, CH_3COO), 2.00 (s, 3H, CH_3COO), 2.01 (s, 3H, CH_3COO), 2.02 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.12 (s, 3H, CH_3COO), 2.20–2.26 (m, 2H, 9-H, 18-H), 2.66 (d, b, J = 13.5 Hz, 1H, 1-H), 3.04 (dd, J = 10.0, 6.4 Hz, 1H, 3-H), 3.56–3.59 (m, 1H, 5′-H), 3.69 (t, J = 9.5 Hz, 1H, 4′-H), 3.85 (t, J = 6.8 Hz, 1H, 5″-H), 4.03–4.12 (m, 3H, 6′-H, 6″-H), 4.39 (d, J = 10.0 Hz, 1H, 6′-H), 4.43 (d, J = 8.1 Hz, 1H, 1″-H), 4.47 (d, J = 8.3 Hz, 1H, 1′-H), 4.89–4.95 (m, 2H, 2′-H, 3″-H), 5.08 (dd, J = 10.3, 8.1 Hz, 1H, 2″-H), 5.14 (t, J = 9.4 Hz, 1H, 3′-H), 5.32 (d, J = 2.8 Hz, 1H, 4″-H), 5.50 (s, 1H, 12-H), 6.83 (s, 1H, $CHPh_2$), 7.24–7.32 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 16.02 (C-28), 16.47 (C-24, C-25), 17.46 (C-6), 18.52 (C-26), 20.48, 20.61, 20.71, 20.81 (C-27, C-29, CH_3COO), 25.64 (C-2), 26.65 (C-15), 27.71 (C-23), 28.41 (C-21), 31.66 (C-19), 33.77 (C-7), 35.53 (C-17), 35.93 (C-22), 36.58 (C-10), 37.62 (C-16), 39.11 (C-1, C-4), 40.35 (C-18), 42.68 (C-20), 43.81 (C-8), 44.91 (C-14), 55.30 (C-5), 60.62 (C-9), 60.84 (C-6″), 62.28 (C-6′), 66.64 (C-4″), 69.12 (C-2″), 70.70 (C-5″), 70.97 (C-3″), 72.05 (C-2′), 72.34 (C-5′), 72.92 (C-3′), 76.83 (C-4′, $CHPh_2$), 90.47 (C-3), 101.08 (C-1″), 102.67 (C-1′), 124.17 (C-12), 126.86, 127.80, 127.85, 128.50, 128.54 (aromatic C), 140.39 (aromatic C_q), 165.45 (C-13), 169.11, 169.38, 169.84, 169.99, 170.10, 170.33 (CH_3COO), 176.88 (C-30), 199.56 (C-11) ppm; MS (ES +): m/z (%) = 1255 (87.5) [M + H^+], 1213 (4.3), 619 (11.5), 469 (5.3), 391 (6.3), 331 (27.0), 183 (4.6), 168 (14.5), 167 (100.0), 130 (26.0), 115 (93.4).

Diphenylmethyl 2′,3′,6′,2″,3″,4″,6″-heptaacetyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-11-deoxo-18β-glycyrrhetinate (**10**; $C_{69}H_{92}O_{20}$)

2.3 g (3.7 mmol) of **7** were dissolved in 180 cm^3 of a 1:1 mixture of deacidified nitromethane and dry benzene. Half of the amount of the solvent was evaporated at 50°C to remove traces of moisture. 3.7 g (17.1 mmol) of mercury (II) cyanide and 3.0 g (4.3 mmol) of acetobromolactose were added. After refluxing for 4 h with vigorous stirring the solvent was removed *in vacuo* and the residue extracted with $CHCl_3$. The solution was shaken three times with KI solution (5%), twice with an aqueous $NaHCO_3$ solution (10%), and finally with H_2O. After drying over $CaCl_2$ the solvent was

removed *in vacuo* and the residue was purified by CC with CH_2Cl_2:ethyl acetate = 9:1 as eluent to afford 2.6 g (56.6%) of **10** as a resin.

$[\alpha]_D^{20} = +52.1°$, $[\alpha]_{546}^{20} = +57.5°$ ($c = 0.188$, CH_2Cl_2); IR (KBr): $\bar{\nu} = 2947$ (m), 2872 (w), 1757 (s), 1455 (m), 1432 (w), 1369 (s), 1227 (s), 1148 (m), 1051 (s), 981 (w) cm^{-1}; UV/Vis (CH_2Cl_2): λ_{max} (logϵ) = 250 (3.299), 235 (3.293) nm; ^{1}H NMR (400 MHz, δ, $CDCl_3$): 0.66 (s, 3H, 28-H), 0.71–0.74 (m, 4H, 5-H, 24-H), 0.80–0.86 (m, 1H, 16-H), 0.88–0.96 (m, 10H, 1-H, 23-H, 25-H, 26-H), 0.97–1.00 (m, 1H, 15-H), 1.12 (s, 3H, 27-H), 1.14 (s, 3H, 29-H), 1.27–1.85 (m, 16H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 18-H, 19-H, 21-H, 22-H), 1.89–2.10 (m, 3H, 16-H, 19-H, 21-H), 1.97 (s, 3H, CH_3COO), 2.03 (s, 3H, CH_3COO), 2.04 (s, 3H, CH_3COO), 2.05 (s, 3H, CH_3COO), 2.06 (s, 3H, CH_3COO), 2.11 (s, 3H, CH_3COO), 2.15 (s, 3H, CH_3COO), 3.08 (dd, $J = 11.5$, 4.6 Hz, 1H, 3-H), 3.58–3.62 (m, 1H, 5′-H), 3.75 (t, $J = 9.4$ Hz, 1H, 4′-H), 3.88 (t, $J = 6.9$ Hz, 1H, 5″-H), 4.06–4.16 (m, 3H, 6′-H, 6″-H), 4.44–4.52 (m, 3H, 1′-H, 1″-H, 6′-H), 4.91–4.98 (m, 2H, 2′-H, 3″-H), 5.09–5.14 (m, 2H, 2″-H, 12-H), 5.20 (t, $J = 9.3$ Hz, 1H, 3′-H), 5.35 (d, $J = 3.0$ Hz, 1H, 4″-H), 6.90 (s, 1H, $CHPh_2$), 7.27–7.38 (m, 10H, aromatic H) ppm; ^{13}C NMR (100 MHz, δ, $CDCl_3$): 15.37 (C-25), 16.32 (C-24), 16.69 (C-26), 18.15 (C-6), 20.45, 20.57, 20.68, 20.77, 20.82 (CH_3COO), 23.47 (C-11), 25.66 (C-2), 25.85 (C-27), 26.05 (C-15), 26.85 (C-16), 27.65 (C-23), 27.92 (C-28), 28.39 (C-29), 31.27 (C-21), 31.81 (C-17), 32.58 (C-7), 36.57 (C-10), 38.03 (C-22), 38.50 (C-1), 38.81 (C-4), 39.73 (C-8), 41.43 (C-14), 42.77 (C-19), 44.19 (C-20), 47.51 (C-9), 47.87 (C-18), 55.38 (C-5), 60.79 (C-6″), 62.07 (C-6′), 66.58 (C-4″), 69.09 (C-2″), 70.63 (C-5″), 70.92 (C-3″), 71.99 (C-2′), 72.31 (C-5′), 72.82 (C-3′), 76.36 ($CHPh_2$), 76.60 (C-4′), 90.51 (C-3), 100.99 (C-1″), 102.70 (C-1′), 122.45 (C-12), 126.94, 127.28, 127.66, 127.77, 128.34 (aromatic C), 140.33, 140.39 (aromatic C_q), 144.24 (C-13), 169.01, 169.38, 169.77, 169.98, 170.07, 170.26, 170.28 (CH_3COO), 175.84 (C-30) ppm; MS (ES+): m/z (%) = 1242 (20.1) [M + H$^+$], 1076 (4.4), 938 (12.5), 937 (32.0), 637 (5.6), 620 (22.6), 619 (100.0), 437 (3.8), 331 (14.7).

β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18β-glycyrrhetic acid (**11**; $C_{42}H_{66}O_{14}$)

1.05 g (0.83 mmol) of **8** were dissolved in 11 cm^3 of dry $CHCl_3$ and cooled to −20°C. 1 g of Na in 20 cm^3 MeOH cooled to −20°C was added, and the mixture was allowed to stand for 1.5 h at this temperature. Then, ice water and brine were added. The mixture was extracted five times with $CHCl_3$, and the combined organic layers were washed with a saturated aqueous solution of NH_4Cl and H_2O, dried over Na_2SO_4, and filtered. The solvent was evaporated *in vacuo*, and the residue (550 mg) was dissolved in 36 cm^3 of dry MeOH. 500 mg of Pd/C (10%) were added, and the mixture was allowed to shake with H_2 overnight at a pressure of 3450 hPa at room temperature. The solids were filtered off, and the solvent was evaporated *in vacuo* at room temperature. The residue was purified in portions of 200 mg by preparative TLC (CH_2Cl_2:MeOH = 8:2) yielding 180 mg (27.2%) of **11** as an amorphous solid.

M.p.: 283–285°C (decomp.); $[\alpha]_D^{20} = +66.4°$, $[\alpha]_{546}^{20} = +69.1°$ ($c = 0.219$, CH_3OH); IR (KBr): $\bar{\nu} = 3406$ (s), 2949 (m), 2875 (m), 1648 (m), 1560 (m), 1465 (w), 1389 (m), 1362 (w), 1288 (w), 1215 (w), 1075 (s), 1049 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.79 (d, $J = 12.1$ Hz, 1H, 5-H), 0.82 (s, 3H, 28-H), 0.87 (s, 3H, 24-H), 0.90–1.03 (m, 2H, 1-H, 15*-H), 1.08 (s, 3H, 23-H), 1.09 (s, 3H, 29-H), 1.14 (s, 3H, 26-H), 1.15 (s, 3H, 25-H), 1.22–1.35 (m, 3H, 16*-H, 21-H, 22-H), 1.42 (s, 3H, 27-H), 1.46–1.97 (m, 11H, 2-H, 6-H, 7-H, 15*-H, 19-H, 21-H, 22-H), 2.15 (dt, $J = 13.0$, 3.8 Hz, 1H, 16*-H), 2.31 (d, b, $J = 11.5$ Hz, 1H, 18-H), 2.44 (s, 1H, 9-H), 2.71 (dt, $J = 13.0$, 3.0 Hz, 1H, 1-H), 3.19 (dd, $J = 11.9$, 4.4 Hz, 1H, 3-H), 3.30 (t, $J = 8.4$ Hz, 1-H, 2′-H), 3.40–3.43 (m, 1H, 5′-H), 3.51–3.64 (m, 5H, 2″-H, 3′-H, 3″-H, 4′-H, 5″-H), 3.73 (dd, $J = 11.6$, 4.1 Hz, 1H, 6″-H), 3.77–3.87 (m, 4H, 4″-H, 6′-H, 6″-H), 4.38 (d, $J = 8.4$ Hz, 1H, 1′-H), 4.40 (d, $J = 9.1$ Hz, 1H, 1″-H), 5.70 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 17.30 (C-24, C-25), 18.74 (C-6), 19.65 (C-26), 24.16 (C-27), 27.29 (C-2), 27.90, 27.99 (C-15, C-16), 28.82 (C-23), 29.65 (C-28), 29.86 (C-29), 33.27 (C-17, C-21), 34.16 (C-7), 38.33 (C-10), 39.78 (C-22), 40.62 (C-1), 40.79 (C-4), 44.01 (C-19), 44.91 (C-8*), 46.47 (C-20), 47.01 (C-14*), 50.15 (C-18), 56.83 (C-5), 62.34 (C-6′), 62.80 (C-6″), 63.38 (C-9),

70.62 (C-4″), 72.79 (C-2″), 75.00 (C-3″), 75.58 (C-2′), 76.46 (C-5′), 76.70 (C-3′), 77.30 (C-5″), 81.10 (C-4′), 90.91 (C-3), 105.38 (C-1″), 106.76 (C-1′), 129.17 (C-12), 174.06 (C-13), 184.66 (C-30), 202.93 (C-11) ppm; MS (ES +): m/z (%) = 795 (100.0) [M + H$^+$], 633 (3.3), 471 (6.3), 241 (13.8), 200 (6.6), 159 (6.9), 130 (29.3), 115 (97.4).

β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18α-glycyrrhetic acid (**12**; $C_{42}H_{66}O_{14}$)

980 mg (0.78 mmol) of **9** were dissolved in 10 cm^3 of dry $CHCl_3$ and cooled to −20°C. 980 mg of Na in 20 cm^3 MeOH cooled to −20°C were added, and the mixture was allowed to stand for 1.5 h at this temperature. Then it was worked up as described for **11**. The residue (405 mg) was dissolved in 27 cm^3 of dry MeOH. 400 mg of Pd/C (10%) were added, and the mixture was treated as described for **11** giving a residue (314 mg) which was purified by preparative TLC (CH_2Cl_2:MeOH = 8:2) yielding 85 mg (13.7%) of **12** as an amorphous solid.

M.p.: 270–272°C (decomp.); $[\alpha]_D^{20} = +29.6°$, $[\alpha]_{546}^{20} = +31.4°$ ($c = 0.112$, CH_3OH); IR (KBr): $\bar{\nu} = 3406$ (s), 2932 (m), 1655 (m), 1561 (m), 1459 (w), 1390 (m), 1218 (w), 1074 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.74 (s, 3H, 28-H), 0.78 (d, $J = 11.7$ Hz, 1H, 5-H), 0.87 (s, 3H, 24-H), 0.97–1.03 (m, 1H, 1-H), 1.07 (s, 3H, 23-H), 1.14 (s, 3H, 26-H), 1.18 (s, 3H, 29-H), 1.20 (s, 3H, 25-H), 1.28–1.34 (m, 2H, 15-H, 22-H), 1.38 (s, 3H, 27-H), 1.41–2.25 (m, 14H, 2-H, 6-H, 7-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.31 (d, $J = 12.9$ Hz, 1H, 18-H), 2.35 (s, 1H, 9-H), 2.61 (d, b, $J = 13.7$ Hz, 1H, 1-H), 3.19 (dd, $J = 11.5$, 4.4 Hz, 1H, 3-H), 3.29 (t, $J = 8.6$ Hz, 1H, 2′-H), 3.40 (dt, $J = 9.4$, 3.1 Hz, 1H, 5′-H), 3.47–3.62 (m, 5H, 2″-H, 3′-H, 3″-H, 4′-H, 5″-H), 3.72 (dd, $J = 11.5$, 4.5 Hz, 1H, 6″-H), 3.77–3.86 (m, 4H, 4″-H, 6′-H, 6″-H), 4.36 (d, $J = 7.8$ Hz, 1H, 1′-H), 4.38 (d, $J = 7.4$ Hz, 1H, 1″-H), 5.57 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.88 (C-28), 17.36 (C-24), 17.50 (C-25), 18.83 (C-6), 19.42 (C-26), 21.50 (C-27), 22.17 (C-29), 27.28 (C-2), 28.19 (C-15), 28.77 (C-23), 30.84 (C-21), 34.27 (C-19), 35.15 (C-7), 37.04 (C-17), 37.98 (C-22), 38.13 (C-10), 39.07 (C-16), 40.58 (C-1), 40.74 (C-4), 42.48 (C-18), 44.83 (C-20), 45.42 (C-8), 46.60 (C-14), 56.86 (C-5), 62.28 (C-6′, C-9), 62.80 (C-6″), 70.62 (C-4″), 72.82 (C-2″), 75.06 (C-3″), 75.56 (C-2′), 76.49 (C-5′), 76.81 (C-3′), 77.34 (C-5″), 81.00 (C-4′), 90.92 (C-3), 105.37 (C-1″), 106.84 (C-1′), 124.91 (C-12), 170.09 (C-13), 187.00 (C-30), 202.50 (C-11) ppm; MS (ES +): m/z (%) = 795 (49.3) [M + H$^+$], 633 (2.0), 471 (2.8), 317 (2.5), 241 (12.5), 200 (3.9), 167 (6.6), 143 (5.3), 130 (30.9), 115 (100.0).

β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-11-deoxo-18β-glycyrrhetic acid (**13**; $C_{42}H_{68}O_{13}$)

2.6 g (2.1 mmol) of **10** were dissolved in 80 cm^3 of dry $CHCl_3$ and cooled to −20°C. 2.4 g of Na in 200 cm^3 MeOH cooled to −20°C were added, and the mixture was allowed to stand for 1.5 h at this temperature. Then it was worked up as described for **11**. The resulting residue (2.1 g) was dissolved in 100 cm^3 of dry MeOH. 2 g of Pd/C (10%) were added, and the mixture was treated as described for **11**. The residue was purified by CC (CH_2Cl_2:MeOH = 8:2) yielding 325 mg (19.8%) of **13** as an amorphous solid.

M.p.: 235–238°C (decomp.); $[\alpha]_D^{20} = +36.0°$, $[\alpha]_{546}^{20} = +39.6°$ ($c = 0.222$, CH_3OH); IR (KBr): $\bar{\nu} = 3419$ (s), 2946 (m), 1652 (s), 1634 (s), 1538 (w), 1464 (m), 1455 (m), 1390 (m), 1310 (w), 1229 (m), 1163 (m), 1075 (s), 1047 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, CD_3OD): 0.82 (s, 3H, 28-H), 0.83–0.85 (m, 1H, 5-H), 0.90 (s, 3H, 24-H), 0.92–0.94 (m, 1H, 16-H), 1.01–1.03 (m, 7H, 1-H, 25-H, 26-H), 1.06–1.08 (m, 1H, 15-H), 1.10 (s, 3H, 23-H), 1.15 (s, 3H, 29-H), 1.21 (s, 3H, 27-H), 1.32–2.12 (m, 19H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 18-H, 19-H, 21-H, 22-H), 3.24 (dd, $J = 11.6$, 4.1 Hz, 1H, 3-H), 3.33 (t, $J = 8.4$ Hz, 1-H, 2′-H), 3.45–3.47 (m, 1H, 5′-H), 3.57–3.71 (m, 5H, 2″-H, 3′-H, 3″-H, 4′-H, 5″-H), 3.79–3.90 (m, 4H, 6′-H, 6″-H), 3.95 (s, b, 1H, 4″-H), 4.44 (d, $J = 7.8$ Hz, 1H, 1′-H), 4.45 (d, $J = 7.3$ Hz, 1H, 1″-H), 5.31 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, CD_3OD): 16.36 (C-25), 17.26 (C-24), 17.75 (C-26), 19.60 (C-6), 24.88 (C-11), 26.75 (C-27), 27.29

(C-2), 27.59 (C-15), 28.36 (C-16), 28.84 (C-23), 29.17 (C-28), 29.65 (C-29), 32.70 (C-21), 33.30 (C-17), 34.16 (C-7), 38.06 (C-10), 40.03 (C-22), 40.20 (C-1), 40.41 (C-4), 41.32 (C-8*), 42.99 (C-14*), 44.68 (C-19), 45.62 (C-20), 49.30 (C-9), 49.87 (C-18), 57.26 (C-5), 62.32 (C-6′), 63.00 (C-6″), 71.00 (C-4″), 72.72 (C-2″), 74.98 (C-3″), 75.62 (C-2′), 76.45 (C-5′), 76.76 (C-3′), 76.86 (C-5″), 80.99 (C-4′), 91.14 (C-3), 105.25 (C-1″), 106.45 (C-1′), 123.82 (C-12), 146.38 (C-13), 184.29 (C-30) ppm; MS (ES +): m/z (%) = 781 (12.7) [M + H$^+$], 412 (4.1), 318 (4.7), 317 (36.2), 277 (4.4), 276 (33.6), 241 (28.3), 200 (13.8), 159 (8.8), 146 (30.8), 143 (100.0).

β-D-Galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-oleanolic acid
(**17**; calenduloside A; $C_{43}H_{70}O_{13}$)

17 was prepared as reported in Ref. [1].

Methyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18β-glycyrrhetinate
(**14**; $C_{43}H_{68}O_{14}$)

Diazomethane in diethyl ether was added to a suspension of 60 mg (0.075 mmol) of **11** in 15 cm^3 of MeOH until the yellow colour persisted. The solution was allowed to stand for 1 h; then acetic acid was added, and the mixture was evaporated *in vacuo* to afford 75 mg of a residue which was purified by preparative TLC (CH_2Cl_2:MeOH = 8:2) yielding 39 mg (63.9%) of **14** as an amorphous solid.

M.p.: 229–231°C (decomp.); $[\alpha]_D^{20} = +53.9°$, $[\alpha]_{546}^{20} = +60.4°$ ($c = 0.182$, CH_3OH); IR (KBr): $\bar{\nu} = 3387$ (s), 2948 (s), 2873 (m), 1732 (m), 1659 (s), 1577 (s), 1453 (w), 1412 (m), 1387 (m), 1217 (w), 1156 (s), 1075 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.76 (s, 3H, 28-H), 0.76–0.80 (m, 1H, 5-H), 0.89–0.93 (m, 1H, 15*-H), 1.05 (s, 3H, 24-H), 1.05–1.10 (m, 2H, 1-H, 16*-H), 1.09 (s, 3H, 26-H), 1.17 (s, 3H, 29-H), 1.22–1.28 (m, 1H, 7-H), 1.26 (s, 3H, 25-H), 1.30–1.44 (m, 3H, 21-H, 22-H), 1.33 (s, 3H, 23-H), 1.38 (s, 3H, 27-H), 1.53–2.40 (m, 11H, 2-H, 6-H, 7-H, 15*-H, 16*-H, 18-H, 19-H, 21-H), 2.47 (s, 1H, 9-H), 3.07 (d, b, $J = 13.3$ Hz, 1H, 1-H), 3.36 (dd, $J = 11.6$, 4.3 Hz, 1H, 3-H), 3.67 (s, 3H, OCH_3), 3.88–3.94 (m, 1H, 5′-H), 4.02–4.06 (m, 1H, 2′-H), 4.12–4.16 (m, 2H, 3″-H, 5″-H), 4.24–4.28 (m, 2H, 3′-H, 4′-H), 4.37 (dd, $J = 10.6$, 5.3 Hz, 1H, 6″-H), 4.42–4.55 (m, 5H, 2″-H, 4″-H, 6′-H, 6″-H), 4.86 (d, $J = 8.0$ Hz, 1H, 1′-H), 5.10 (d, $J = 8.0$ Hz, 1H, 1″-H), 5.86 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 16.40 (C-25), 16.70 (C-24), 17.28 (C-6), 18.42 (C-26), 23.08 (C-27), 26.15, 26.22, 26.38 (C-2, C-15, C-16), 27.81 (C-23, C-29), 28.20 (C-28), 30.93 (C-21), 31.67 (C-17), 32.53 (C-7), 36.90 (C-10), 37.79 (C-22), 39.15 (C-1), 39.52 (C-4), 40.92 (C-19), 43.06 (C-14), 43.85 (C-20), 45.17 (C-8), 48.28 (C-18), 51.30 (OCH_3), 55.00 (C-5), 61.63 (C-6″), 61.75 (C-9), 62.13 (C-6′), 69.69 (C-4″), 72.16 (C-2″), 74.82 (C-3″), 74.92 (C-2′), 75.87 (C-5′), 76.55 (C-3′), 76.82 (C-5″), 82.13 (C-4′), 88.36 (C-3), 105.48 (C-1″), 106.11 (C-1′), 128.35 (C-12), 168.67 (C-13), 176.46 (C-30), 199.12 (C-11) ppm; MS (ES +): m/z (%) = 810 (100.0) [M + H$^+$], 729 (4.3), 647 (3.2), 486 (3.3), 485 (9.6), 241 (11.2), 200 (5.3), 173 (22.1).

Methyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-18α-glycyrrhetinate
(**15**; $C_{43}H_{68}O_{14}$)

60 mg (0.075 mmol) of **12** were treated with diazomethane as described for **14**. The residue was purified by preparative TLC (CH_2Cl_2:MeOH = 8:2) yielding 40 mg (65.5%) of **15** as an amorphous solid.

M.p.: 190–192°C (decomp.); $[\alpha]_D^{20} = +87.1°$, $[\alpha]_{546}^{20} = +70.7°$ ($c = 0.074$, *DMSO*); IR (KBr): $\bar{\nu} = 3386$ (s), 2944 (s), 1726 (m), 1662 (s), 1570 (m), 1458 (m), 1411 (m), 1387 (m), 1280 (w), 1230 (m), 1194 (w), 1159 (m), 1116 (s), 1079 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.66 (s, 3H, 28-H), 0.80 (d, $J = 11.5$ Hz, 1H, 5-H), 1.06 (s, 3H, 24-H), 1.08–1.15 (m, 1H, 1-H), 1.12 (s, 3H, 26-H), 1.17–1.20 (m, 1H, 15-H), 1.24–1.37 (m, 2H, 16-H, 22-H), 1.29 (s, 3H, 29-H), 1.31 (s, 3H, 27-H),

1.34 (s, 6H, 23-H, 25-H), 1.40–2.22 (m, 13H, 2-H, 6-H, 7-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.27 (d, $J = 11.5$ Hz, 1H, 18-H), 2.37 (s, 1H, 9-H), 2.97 (d, b, $J = 13.4$ Hz, 1H, 1-H), 3.39 (dd, $J = 11.6$, 4.2 Hz, 1H, 3-H), 3.69 (s, 3H, OCH_3), 3.86–3.94 (m, 1H, 5′-H), 4.00–4.09 (m, 1H, 2′-H), 4.10–4.14 (m, 2H, 3″-H, 5″-H), 4.22–4.27 (m, 2H, 3′-H, 4′-H), 4.36 (dd, $J = 10.7$, 5.3 Hz, 1H, 6″-H), 4.40–4.52 (m, 5H, 2″-H, 4″-H, 6′-H, 6″-H), 4.85 (d, $J = 7.9$ Hz, 1H, 1′-H), 5.07 (d, $J = 7.9$ Hz, 1H, 1″-H), 5.70 (s, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 15.65 (C-28), 15.58 (C-25), 16.77 (C-24), 17.43 (C-6), 18.34 (C-26), 20.32, 20.43 (C-27, C-29), 26.22 (C-2), 26.60 (C-15), 27.89 (C-23), 28.67 (C-21), 31.79 (C-19), 33.64 (C-7), 35.22 (C-17), 35.74 (C-22), 36.76 (C-10), 37.28 (C-16), 39.22 (C-1), 39.49 (C-4), 40.06 (C-18), 42.53 (C-20), 43.69 (C-8), 44.81 (C-14), 51.49 (OCH_3), 55.16 (C-5), 60.66 (C-9), 61.68 (C-6″), 62.23 (C-6′), 69.73 (C-4″), 72.16 (C-2″), 74.84 (C-3″), 74.96 (C-2′), 75.88 (C-5′), 76.56 (C-3′), 76.82 (C-5″), 82.13 (C-4′), 88.45 (C-3), 105.44 (C-1″), 106.09 (C-1′), 123.97 (C-12), 165.00 (C-13), 178.19 (C-30), 198.69 (C-11) ppm; MS (ES+): m/z (%) = 810 (100.0) [M + H$^+$], 729 (5.3), 647 (4.2), 526 (3.4), 486 (3.2), 485 (8.9), 241 (5.8), 205 (6.3), 173 (7.2).

Methyl-β-D-galactopyranosyl-(1 → 4)-β-D-glucopyranosyl-3-O-oleanolate (**16**; $C_{43}H_{70}O_{13}$)

150 mg (0.19 mmol) of **17** were treated with diazomethane as described for **14**. The residue was crystallized from MeOH giving 75 mg (49.1%) of **16** as white needles.

M.p.: 260–265°C (decomp.); $[\alpha]_D^{20} = +35.8°$, $[\alpha]_{546}^{20} = +33.0°$ ($c = 0.140$, CH_3OH); IR (KBr): $\bar{\nu} = 3433$ (s), 2947 (s), 1723 (m), 1463 (m), 1388 (m), 1364 (m), 1304 (w), 1264 (m), 1231 (w), 1206 (m), 1163 (s), 1074 (s), 1033 (s) cm^{-1}; ^{1}H NMR (400 MHz, δ, C_5D_5N): 0.77 (d, $J = 11.0$ Hz, 1H, 5-H), 0.81 (s, 3H, 26-H), 0.84 (s, 3H, 25-H), 0.86–0.89 (m, 1H, 1-H), 0.91 (s, 3H, 29-H), 0.92 (s, 3H, 30-H), 1.00 (s, 3H, 24-H), 1.06–1.19 (m, 4H, 7-H, 15-H, 19-H, 21-H), 1.23 (s, 3H, 27-H), 1.31 (s, 3H, 23-H), 1.35–1.98 (m, 14H, 1-H, 2-H, 6-H, 7-H, 9-H, 11-H, 15-H, 16-H, 19-H, 21-H, 22-H), 2.01 (dt, $J = 12.8$, 3.3 Hz, 1H, 16-H), 2.13 (dd, $J = 13.7$, 3.5 Hz, 1H, 2-H), 3.08 (dd, $J = 13.4$, 3.7 Hz, 1H, 18-H), 3.32 (dd, $J = 12.0$, 4.4 Hz, 1H, 3-H), 3.69 (s, 3H, OCH_3), 3.94–3.96 (m, 1H, 5′-H), 4.03–4.07 (m, 1H, 2′-H), 4.10–4.17 (m, 2H, 3′-H, 4′-H), 4.25–4.29 (m, 2H, 3″-H, 5″-H), 4.37–4.56 (m, 6H, 2″-H, 4″-H, 6′-H, 6″-H), 4.87 (d, $J = 7.7$ Hz, 1H, 1′-H), 5.10 (d, $J = 7.9$ Hz, 1H, 1″-H), 5.37 (s, b, 1H, 12-H) ppm; ^{13}C NMR (100 MHz, δ, C_5D_5N): 15.10 (C-25), 16.67 (C-24), 16.78 (C-26), 18.09 (C-6), 23.02 (C-16), 23.29 (C-30), 23.36 (C-11), 25.77 (C-27), 26.14 (C-2), 27.70 (C-15), 27.84 (C-23), 30.45 (C-20), 32.42 (C-22), 32.65 (C-7), 32.78 (C-29), 33.57 (C-21), 36.59 (C-10), 38.29 (C-1), 39.13 (C-4), 39.28 (C-8), 41.43 (C-18), 41.57 (C-14), 45.69 (C-19), 46.56 (C-17), 47.52 (C-9), 51.23 (OCH_3), 55.42 (C-5), 61.64 (C-6″), 62.22 (C-6′), 69.70 (C-4″), 72.14 (C-2″), 74.85 (C-3″), 74.93 (C-2′), 75.90 (C-5′), 76.56 (C-3′), 76.85 (C-5″), 81.18 (C-4′), 88.56 (C-3), 105.51 (C-1″), 106.19 (C-1′), 122.46 (C-12), 143.79 (C-13), 177.61 (C-28) ppm; MS (ES+): m/z (%) = 795 (78.0) [M + H$^+$], 471 (5.6), 453 (16.4), 377 (7.9), 343 (7.9), 317 (29.2), 280 (8.2), 279 (42.5), 276 (29.6), 271 (20.8), 260 (11.9), 241 (100.0), 223 (9.1), 200 (50.9), 171 (7.9), 167 (11.6), 163 (13.8), 159 (41.5), 146 (71.1), 126 (73.0), 120 (86.8).

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References

[1] Seebacher W, Weis R, Jurenitsch J, Rauchensteiner K, Haslinger E (2000) Monatsh Chem **131**: 985

[2] Wulff G (1968) Dtsch Apothek Ztg **108**: 797

[3] Winkler W (1961) Kolloid Z **177**: 63
[4] Königs W, Knorr E (1900) Sitzber Math Naturw Kl Bayer Akad Wiss München **30**: 108
[5] Königs W, Knorr E (1901) Ber **34**: 957
[6] Seebacher W, Haslinger E, Rauchensteiner K, Jurenitsch J, Presser A, Weis R (1999) Monatsh Chem **130**: 887
[7] Beaton JM, Spring FS (1955) J Chem Soc 3126
[8] Staudinger H, Anthes E, Pfenninger F (1917) Ber Chem Ges **49**: 1928
[9] Coleman GH, Gilman H, Adams CE, Pratt PE (1939) J Org Chem **3**: 99
[10] Janiszowska W, Wilkomirski B, Kasprzyk Z (1980) Pol J Chem **54**: 2147
[11] Helferich B, Klein W (1926) Liebigs Ann Chem **450**: 219
[12] Seebacher W, Haslinger E, Weis R (2001) Monatsh Chem **132**: 839
[13] Helferich B, Schäfer W (1926) Liebigs Ann Chem **450**: 229
[14] Duddeck H, Elgamal MHA, Ricca GS, Danieli B, Palmisano G (1978) Org Magn Reson **11**: 130
[15] Ruzicka L, Leuenberger H, Schellenberg H (1937) Helv Chim Acta **20**: 1271
[16] Ullah N, Seebacher W, Weis R, Jurenitsch J, Rauchensteiner K, Haslinger E (2000) Monatsh Chem **131**: 787
[17] Hardegger E, El Heweihi Z, Robinet FG (1948) Helv Chim Acta **31**: 439

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