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Carbohydrate Research 337 (2002) 1269–1274

CARBOHYDRATE RESEARCH

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Synthesis of a cholestane glycoside OSW-1 with potent cytostatic activity

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Received 11 January 2002; received in revised form 7 May 2002; accepted 22 May 2002

Abstract

The potent antitumor agent OSW-1 was synthesized from the protected aglycone in different ways. It was proven that direct glycosylation of the aglycone in its hemiketal form could be achieved, affording the protected OSW-1 in a moderate yield. Alternatively, regioselective protection of the triol obtained by reduction of the aglycone, followed by glycosylation, deprotection and oxidation yielded the same OSW-1 derivative. The third approach to this compound consisted of glycosylation of the previously described lactol [Morzycki, J. W.; Gryszkiewicz, A. *Polish J. Chem.* **2001**, *75*, 983–989], reaction of the resulting aldehyde with a Grignard reagent, and oxidation. OSW-1 obtained on removal of the protective groups was identical with the natural product.

Keywords: OSW-1; Glycosylation; Saponins; Steroids; Antitumor activity

1. Introduction

The steroidal saponin OSW-1 (**5**) belongs to a family of similar compounds isolated from the bulbs of *Ornithogalum saundersiae*, a perennial grown in southern Africa.[1] The saponins feature common cholestane aglycone (3β,16β,17α-trihydroxycholest-5-en-22-one) and vary at the 2″ *O*-acyl group of the sugar moiety. In vitro assays showed that the saponins were extremely toxic against a broad spectrum of malignant tumor cells, including drug-resistant leukemia and various lung cancer cell lines. The anticancer activities (IC_{50} values) of OSW-1 are from 10 to 100 times more potent than those of the clinically used anticancer agents (e.g., mitomycin C, adriamycin, cisplatin, camptothecin, and taxol).[2]

We have recently reported a synthesis of the saponin OSW-1 aglycone.[3] The first synthesis of the aglycone was described by Fuchs et al.[4] The Chinese chemists first performed the coupling of the protected OSW-1 aglycone with a sugar imidate.[5] In a new synthetic route to the saponin OSW-1, a different approach to the synthesis of the steroid and the sugar parts of the molecule was recently reported, but the same coupling procedure was employed.[6]

The steroid aglycone and the sugar moiety are both important for biological activity of OSW-1. Removal of the acetyl and the 4-methoxybenzoyl groups from the sugar part diminished the cytotoxicity by 1000 times.[2] The steroidal glycosides bearing the disaccharide moiety of OSW-1 at different positions were proved at least 10,000 times less active than OSW-1.[7] The mechanism of action of OSW-1 is still unknown but its cytotoxicity profile is strikingly similar[2,8] to that of cephalostatins, a group of the dimeric steroid–pyrazine marine alkaloids.[9] It was hypothesized that these two groups of compounds might form a similar active intermediate, presumably a C-22 oxocarbenium ion.[4,9b] Therefore, the presence of both a 16β,17α-diol and a C-22 carbonyl group is essential for the biological activity of OSW-1. The recently performed structure–activity relationship studies confirmed this conclusion.[10] However, synthesis of further analogues is necessary for determination of the minimal pharmacophor requirements.

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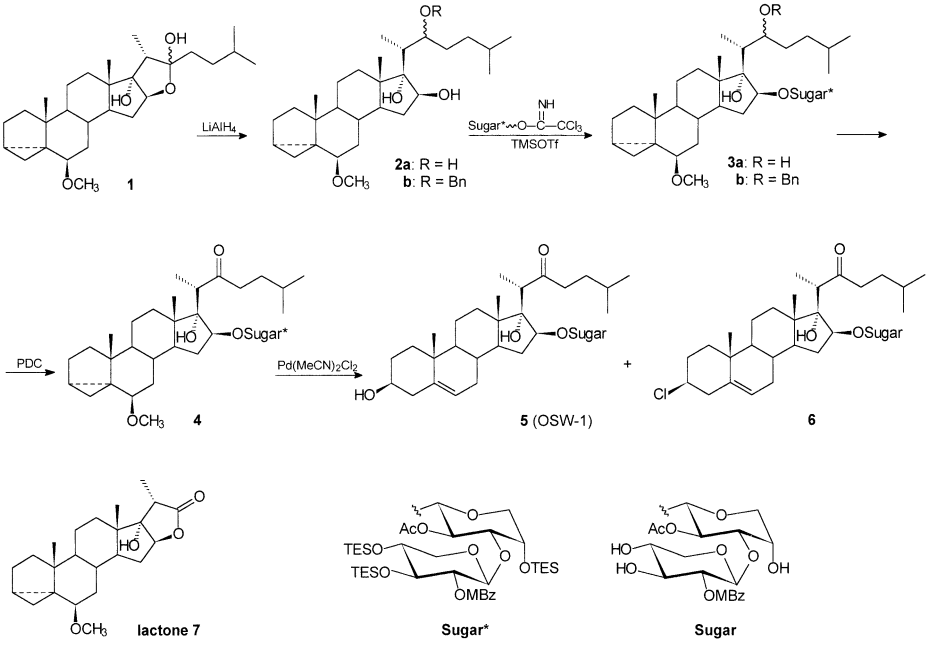
0008-6215/02/$ - see front matter
PII: S0008-6215(02)00126-X

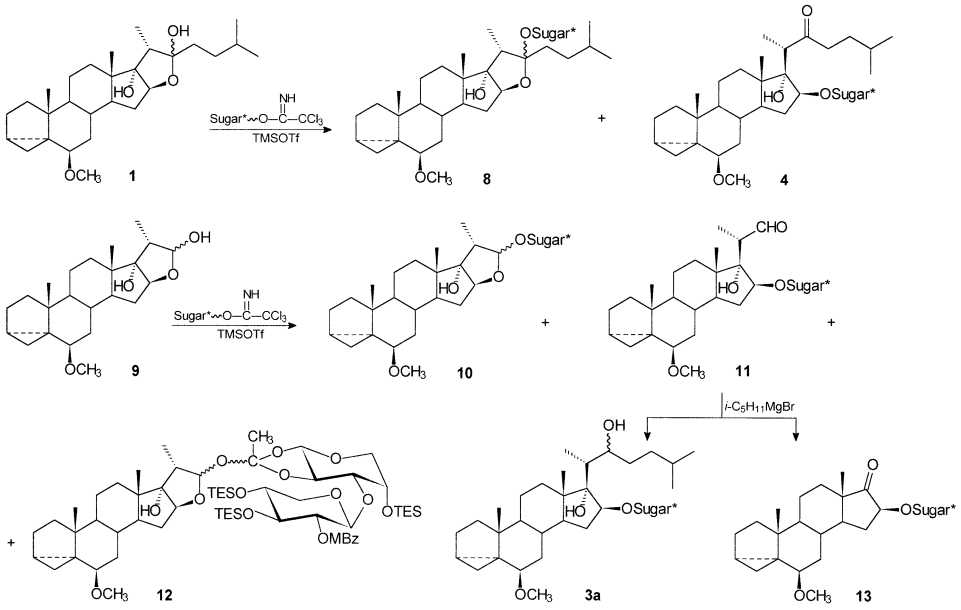
2. Results and discussion

In this paper the results of glycosylation of the protected OSW-1 aglycone (**1**) and related compounds are described. The disaccharide of OSW-1, namely 2-*O*-(4-methoxybenzoyl)-β-D-xylopyranosyl-(1 → 3)-2-*O*-acetyl-α-L-arabinopyranose, was obtained according to the known procedure.[5] The corresponding disaccharide trichloroacetimidate was also prepared by the method described in the literature[5] and used as a glycosyl donor. In the previous paper, an improved synthesis of the OSW-1 aglycone (**1**) in its hemiketal form, with the ring B double bond and the 3β-OH group protected as an *i*-steroid ether, was reported.[3] Compound **1** proved to be stereochemically pure, but its configuration at C-22 could not be elucidated directly from the spectra. Presumably, the configuration is 22*R* since this compound was calculated, using the MM^+ force field, to be over 4 kcal/mol more stable than its 22*S* epimer. $LiAlH_4$ reduction of **1** afforded the triol **2a** as a C-22 diastereoisomeric mixture with one epimer prevailing (Scheme 1). The triol **2a** was regioselectively protected at O-22 as the benzyl ether **2b**. Only one epimer of **2b** could be easily isolated in its pure state from the reaction mixture. This predominant epimer (its configuration was not established, but it was not important for further synthesis) was taken for the coupling reaction with a disaccharide donor. The glycosylation proceeded smoothly under the promotion of TMSOTf, yielding **3b**. The hydrogenolysis of the benzyl ether was followed by oxidation of the 22-OH group to the ketone **4** with pyridinium dichromate. Finally, all protective groups (3α,5α-*cyclo*-6β-methoxy protection of aglycone and TES groups on the sugar moiety) were simultaneously removed with a soft Lewis acid – $Pd(MeCN)_2Cl_2$ at room temperature. The yield of OSW-1 (**5**) was satisfactory, but the high cost of the reagent and the formation of a minor 3β-chloro product **6**, due to the presence of chlorides in the reaction mixture, encouraged the investigation of deprotection under different conditions. A high yield of OSW-1 (**5**) was also achieved using a catalytic amount of *p*-TsOH in dioxane/water at 75 °C (no glycoside hydrolysis was observed).

The direct glycosylation of the protected aglycone (**1**) with the disaccharide trichloroimidate was also studied. The conversion was 20% and two products were formed in the ratio of about 1:1. In addition to the desired protected OSW-1 (**4**), the glycoside **8** of the hemiketal was obtained (Scheme 2). Similar glycosylation of the hemiacetal **9** was also performed. Compounds **1** and **9** were obtained from the readily available hydroxy-lactone **7** by reaction with isoamyl lithium or diisobutyl aluminum hydride, respectively. In the case of the glycosylation of **9**, the conversion was slightly above 50% and three products were formed. The major one, aldehyde **11**, was accompanied by the hemiacetal glycoside **10** and the orthoester **12**. The latter compound was formed by the well known mechanism involving participation of acetate at the 2′ position.[11] The aldehyde **11** seems to be a versatile starting material for synthesis of OSW-1 analogues with different side chains. The reaction **11** with isoamyl magnesium bromide was carried out at low temperature (− 70 °C). The ester groups remained intact under these conditions, but an undesired retroaldol reaction accompanied the Grignard reaction. The alkylation product was



Scheme 1.



Scheme 2.

formed as a single stereoisomer, which occurred to be identical with compound **3a** obtained by the method described above. The 16-*O*-glycoside devoid side chain, 17-ketone **13**, was the retroaldol reaction product. The retroaldol reaction was promoted by a strongly basic Grignard reagent.

It can be concluded from this study that the previously described hydroxy-lactone **7** is a valuable intermediate in the synthesis of the highly potent cytostatic saponin OSW-1. Synthesis of the side chain analogues of OSW-1 from the hydroxy-lactone **7** and the aldehyde **11** using the reactions with less basic organometallic reagents, and evaluation of the antitumor activities of the analogues will be published in due course.

3. Experimental

General methods.—Melting points were determined on a Kofler apparatus of the Boëtius type. Optical rotations were measured on a Perkin–Elmer 141 polarimeter in $CHCl_3$ or in MeOH. NMR spectra were recorded with the Bruker AC 200F (200 MHz) and Avance 500 (500 MHz) spectrometers using $CDCl_3$ or C_5D_5N solutions with TMS as the internal standard (only selected signals in the NMR spectra are reported). Infrared spectra were recorded on a Nicolet Series II Magna-IR 550 FT-IR spectrometer as chloroform solutions unless otherwise stated. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. Compounds **1**, **2a** and **9** were obtained according to the earlier described procedures.[3a,3c] The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J. T. Baker). Thin-layer chromatograms were developed on aluminum TLC sheets precoated with silica gel 60 F_{254} (E. Merck) and visualized with 50% H_2SO_4 after heating. All solvents were dried and freshly distilled prior to use.

Selective benzylation of triol **2a**.—To the stirred solution of **2a** (100 mg, 0.22 mmol) in THF (6 mL), NaH (14 mg of 60% dispersion in mineral oil, 0.33 mmol) was added at 0 °C. The reaction mixture was stirred 15 min at 0 °C, then BnBr (0.04 mL, 0.33 mmol) and $Bu_4^+I^-$ (10 mg, 0.04 mmol) were added. The reaction mixture was stirred 1.5 h at reflux. The reaction was carefully quenched with water and extracted with ether. The extract was dried over $MgSO_4$ and solvent was evaporated in vacuo.

22-Benzyloxy-6β-methoxy-3α,5α-cyclocholestane-16β,17α-diol (**2b**). Pure **2b** (48 mg, 40%) was eluted from a silica gel column with EtOAc (12.5%)–hexane, mp 130–132 °C (rectangular plates); R_f 0.35 (7:3 hexane–EtOAc); $[\alpha]_D^{21}$ +11.1° (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): *ν* 3621, 3406, 1468, 1455, 1384, 1090 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): *δ* 7.33 (m, 5 H), 4.69 (s, 1 H), 4.68 (d, 1 H, *J* 11.2 Hz), 4.44 (d, 1 H, *J* 11.2 Hz), 4.11 (dd, 1 H, *J* 8.8, 4.2 Hz), 3.96 (m, 1 H), 3.34 (s, 3 H), 2.78 (m, 1 H), 1.05 (d, 3 H, *J* 7.5 Hz), 1.03 (s, 3 H), 0.94 (s, 3 H), 0.93 (d, 6 H, *J* 6.4 Hz), 0.65 (m, 1 H), 0.43 (dd, 1 H, *J* 8.0, 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): *δ* 137.9 (C), 128.5 (CH × 3), 127.8 (CH), 127.7 (CH), 87.2 (C), 82.5 (CH), 82.4 (CH), 81.3 (CH), 70.3 (CH_2), 56.6 (CH_3), 47.8 (C), 47.5 (CH), 47.1 (CH), 43.3 (C), 37.1 (CH_2), 35.11 (C), 35.06 (CH_2), 35.00 (CH_2 × 2), 34.97 (CH), 33.4 (CH_2), 30.5 (CH), 28.4 (CH), 28.3 (CH_2), 24.9 (CH_2), 22.8 (CH_3), 22.4 (CH_3), 22.3 (CH_2), 21.4 (CH), 19.2 (CH_3), 13.7 (CH_3), 13.1 (CH_2), 7.5 (CH_3).

Glycosylation of compound **2b**.—A solution of 2-*O*-acetyl-3-*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-D-xylopyranosyl]-4-*O*-(triethylsilyl)-β-L-arabinopyranosyl trichloroacetimidate (106 mg, 0.11 mmol) and compound **2b** (54 mg, 0.1 mmol) in dry CH_2Cl_2 (15 mL) was stirred with 4 Å molecular sieves (225 mg) at rt for 15 min, then the reaction mixture was cooled to −68 °C (EtOH–dry ice bath) and a 0.14 M solution of TMSOTf (0.24 mL) in CH_2Cl_2 was slowly added. The reaction mixture was stirred for further 30 min, quenched with Et_3N and filtered. The filtrate was evaporated in vacuo and the crude product **3b** was purified by silica gel column chromatography with EtOAc (2.5%)–hexane.

22-Benzyloxy-6β-methoxy-3α,5α-cyclocholestane-16β,17α-diol 16-O-{O-[3,4-di-O-triethylsilyl-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (**3b**). Compound **3b** was obtained in 59% (78 mg) as an amorphous white solid; R_f 0.39 (19:1 benzene–EtOAc). IR ($CHCl_3$): *v* 3399, 1721, 1607, 1458, 1255, 1103, 1019 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.04 (d, 2 H, *J* 8.9 Hz), 7.29 (m, 5 H), 6.89 (d, 2 H, *J* 8.9 Hz), 5.11 (s, 1 H), 4.90 (m, 3 H), 4.66 (d, 1 H, *J* 11.5 Hz), 4.47 (m, 1 H), 4.36 (d, 1 H, *J* 11.5 Hz), 4.31 (bs, 1 H), 3.90–4.10 (m, 3 H), 3.87 (s, 3 H), 3.70–3.85 (m, 2 H), 3.53 (m, 2 H), 3.34 (m, 2 H), 3.30 (s, 3 H), 2.75 (m, 1 H), 2.05 (s, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.0 (C), 164.5 (C), 164.4 (C), 163.3 (C), 137.8 (CH), 132.1 (CH), 129.0 (C), 128.4 (CH), 128.2 (CH), 128.1 (CH), 127.7 (CH), 122.7 (C), 113.0 (CH). ESIMS (*m*/*z*): 1344.8 ($M+Na^+$).

Removal of the benzyl protective group in **3b**.—A suspension of **3b** (20 mg, 0.015 mmol), 10% Pd/C (30 mg) and Et_3N (0.007 mL) in EtOAc (1 mL) and EtOH (1 mL) was stirred under H_2 atmosphere (50 atm) at 50 °C overnight. The catalyst was then filtered off and the solvent was evaporated in vacuo from the filtrate affording crude product, which was purified by silica gel column chromatography. Elution with EtOAc (25%)–hexane yielded **3a**.

6β-Methoxy-3α,5α-cyclocholestane-16β,17α,22-triol 16-O-{O-[3,4-di-O-triethylsilyl-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (**3a**). Compound **3a** (16 mg, 86%) was obtained as an oil; R_f 0.31 (7:3 hexane–EtOAc); $[\alpha]_D^{21}$ −21.4° (*c* 0.3, $CHCl_3$). IR ($CHCl_3$): *v* 3592, 3435, 1720, 1607, 1463, 1255, 1101, 1018 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.01 (d, 2 H, *J* 8.9 Hz), 6.88 (d, 2 H, *J* 8.9 Hz), 4.88 (m, 2 H), 4.84 (m, 1 H), 4.46 (bs, 1 H), 4.29 (m, 1 H), 3.92–4.07 (m, 3 H), 3.86 (s, 3 H), 3.78 (m, 2 H), 3.57 (m, 1 H), 3.30 (s, 3 H and m, 1 H), 2.75 (m, 1 H), 2.30 (m, 1 H), 1.96 (s, 3 H), 1.02 (s, 3 H), 0.80–0.99 (m, 36 H), 0.77 (d, 3 H, *J* 6.6 Hz), 0.73 (d, 3 H, *J* 6.5 Hz), 0.50–0.65 (m, 20 H), 0.40 (dd, 1 H, *J* 7.8, 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 168.8 (C), 164.5 (C), 163.3 (C), 131.9 (CH), 122.7 (C), 113.3 (CH), 100.8 (CH), 88.7 (C). ESIMS (*m*/*z*): 1254.8 ($M+Na^+$).

Oxidation of alcohol **3a**.—To a solution of **3a** (10 mg, 0.008 mmol) in CH_2Cl_2 (3 mL) pyridinium dichromate (6 mg, 0.016 mmol) was added, and the reaction mixture was stirred for 1 h. The solvent was evaporated in vacuo at rt. The crude product was purified by silica gel column chromatography to afford **4** in quantitative yield. The protected OSW-1 (**4**) proved identical in all respects with the compound **4** obtained by direct glycosylation of the aglycone **1** as described below.

Glycosylation of hemiketal **1**.—A solution of 2-*O*-acetyl-3-*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-D-xylopyranosyl]-4-*O*-(triethylsilyl)-β-L-arabinopyranosyl trichloroacetimidate (600 mg, 0.64 mmol) and aglycone **1** (250 mg, 0.5 mmol) in dry CH_2Cl_2 (15 mL) was stirred with 4 Å molecular sieves (1.5 g) at rt for 15 min, then the reaction mixture was cooled to −68 °C (EtOH–dry ice bath) and a 0.14 M solution of TMSOTf (1.3 mL) in CH_2Cl_2 was slowly added. The reaction mixture was stirred for further 30 min, quenched with Et_3N and filtered. The filtrate was evaporated in vacuo and the products were separated by silica gel column chromatography. Elution with EtOAc (2.5%)–hexane afforded compound **4** (68 mg, 10%), further elution with EtOAc (4%)–hexane yielded **8** (64 mg, 9%). 1:2 EtOAc–hexane eluted the unreacted aglycone **1** (200 mg, 80%).

Protected OSW-1 **4**. Compound **4** was obtained as an oil; R_f 0.33 (19:1 benzene–EtOAc). IR ($CHCl_3$): *v* 3476, 1738, 1716, 1690, 1607, 1461, 1256, 1101, 1018 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 8.05 (d, 2 H, *J* 8.9 Hz), 6.91 (d, 2 H, *J* 8.9 Hz), 4.93 (dd, 1 H, *J* 4.5, 3.6 Hz), 4.82 (brs, 1 H), 4.75 (dd, 1 H, *J* 5.0, 2.8 Hz), 4.35 (s, 1 H), 4.29 (m, 1 H), 4.23 (d, 1 H, *J* 2.7 Hz), 3.98–3.89 (m, 2 H), 3.86 (s, 3 H), 3.82 (brs, 1 H), 3.77 (t, 1 H, *J* 5.1 Hz), 3.70 (dd, 1 H, *J* 7.7, 5.4 Hz), 3.61 (dd, 1 H, *J* 8.3, 5.1 Hz), 3.31 (s, 3 H), 3.25–3.29 (m, 1 H), 3.15 (d, 1 H, *J* 7.4 Hz), 2.75 (m, 1 H), 2.49 (m, 1 H), 2.26 (m, 2 H), 1.95 (s, 3 H), 1.15 (d, 3 H, *J* 7.4 Hz, H-21), 1.02 (s, 3 H), 0.9–1.0 (m, 34 H), 0.73 (m, 6 H), 0.55–0.65 (m, 18 H), 0.41 (dd, 1 H *J* 8.0, 5.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 219.6 (C), 168.7 (C), 164.7 (C), 163.4 (C), 132.0 (CH), 128.3 (CH), 122 (C), 113.4 (CH). ESIMS (*m*/*z*): 1252.8 ($M+Na^+$).

(20S)-6β-Methoxy-3α,5α-cyclofurostane-17α,22-diol 22-O-{O-[3,4-di-O-triethylsilyl-2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (**8**). Compound **8** was obtained as an oil; R_f 0.12 (19:1 benzene–EtOAc); $[\alpha]_D^{21}$ +9.8° (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): *v* 3510, 1727, 1607, 1460, 1256, 1098, 1011 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 7.96 (d, 2 H, *J* 8.9 Hz), 6.89 (d, 2 H, *J* 8.9 Hz), 5.35 (d, 1 H, *J* 3.2 Hz), 5.01 (dd, 1 H, *J* 9.3, 3.3 Hz), 4.96 (t, 1 H, *J* 7.3 Hz), 4.69 (d, 1 H, *J* 6.6 Hz), 4.23 (t, 1 H, *J* 7.6), 4.08 (bs, 1 H), 4.01 (dd, 1 H, *J* 11.5, 4.4

Hz), 3.86 (s, 3 H), 3.75 (t, 1 H, *J* 7.7), 3.69 (m, 1 H), 3.55 (dd, 1 H, *J* 11.8, 3.6 Hz), 3.31 (s, 3 H), 3.24 (dd, 1 H, *J* 11.6, 8.6 Hz), 2.76 (m, 1 H), 2.36 (m, 1 H), 1.75 (s, 3 H), 0.81–1.05 (m, 43 H), 0.50–0.65 (m, 19 H), 0.43 (dd, 1 H, *J* 7.9, 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.8 (C), 164.5 (C), 163.2 (C), 131.7 (CH), 128.3 (CH), 122.8 (C), 115.1 (C), 113.4 (CH). ESIMS (*m*/*z*): 1252.8 ($M + Na^+$).

Glycosylation of hemiacetal **9**.—The coupling reaction of **9** with the disaccharide donor (Sugar* ~ O–C(=NH)CCl_3) was performed according to the procedure described above. The products obtained (**10**, **11**, and **12**) were separated and purified by silica gel column chromatography. Pure aldehyde **11** (yield 30%) was eluted with EtOAc (5%)–hexane, further elution with EtOAc (7.5%)–hexane yielded consecutively compounds **10** (39%) and **12** (9%).

*(20*S*)-6β-Methoxy-16β,17α-dihydroxy-3α,5α-cyclobisnorcholanaldehyde 16-*O*-{*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-*D*-xylopyranosyl]-(1→3)-2-*O*-acetyl-4-*O*-triethylsilyl-α-*L*-arabinopyranoside}* (**11**). Compound **11** was obtained as a white foam; R_f 0.34 (23:2 benzene–EtOAc); $[\alpha]_D^{21}$ −13.3° (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): *v* 3517, 2735, 1725, 1607, 1255, 1097, 1033 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.54 (d, 1 H, *J* 0.8 Hz), 8.00 (d, 2 H, *J* 8.9 Hz), 6.92 (d, 2 H, *J* 8.9 Hz), 4.92 (dd, 1 H, *J* 6.7, 5.3 Hz), 4.86 (m, 1 H), 4.67 (d, 1 H, *J* 5.2 Hz), 4.14 (d, 1 H, *J* 4.5 Hz), 4.11 (m, 1 H), 3.98 (m, 1 H), 3.87 (s, 3 H), 3.65–3.86 (m, 4 H), 3.31 (s, 3 H), 3.22 (dd, 1 H, *J* 11.5, 7.4 Hz), 3.12 (m, 1 H), 3.07 (s, 1 H), 2.74 (m, 1 H), 1.90 (s, 3 H), 0.85–1.11 (m, 36 H), 0.50–0.65 (m, 20 H), 0.41 (dd, 1 H, *J* 7.9, 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 207.6 (CH), 168.9 (C), 164.6 (C), 163.3 (C), 131.8 (CH), 128.3 (CH), 122.7 (C), 113.4 (CH). ESIMS (*m*/*z*): 1182.7 ($M + Na^+$).

*(20*S*)-6β-Methoxy-16β,17α-dihydroxy-3α,5α-cyclobisnorcholanaldehyde 22,16-hemiacetal 22-*O*-{*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-*D*-xylopyranosyl]-(1→3)-2-*O*-acetyl-4-*O*-triethylsilyl-α-*L*-arabinopyranoside}* (**10**). Compound **10**: mp 126–130 °C (prisms from hexane–CH_2Cl_2); R_f 0.22 (23:2 benzene–EtOAc); $[\alpha]_D^{21}$ −12.0° (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): *v* 3518, 1731, 1606, 1458, 1255, 1097, 1004 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.98 (d, 2 H, *J* 8.9 Hz), 6.90 (d, 2 H, *J* 8.9 Hz), 5.34 (d, 1 H, *J* 4.8 Hz), 4.99 (t, 1 H, *J* 7.5 Hz), 4.90 (m, 1 H), 4.63 (d, 1 H, *J* 7.0 Hz), 4.55 (d, 1 H, *J* 5.7 Hz), 4.00 (m, 1 H), 3.97 (m, 1 H), 3.87 (s, 3 H), 3.83 (m, 1 H), 3.65–3.75 (m, 3 H), 3.39 (dd, 1 H, *J* 11.8, 1.8 Hz), 3.33 (s, 3 H), 3.19 (dd, 1 H, *J* 11.5, 8.9 Hz), 2.76 (m, 1 H), 2.41 (m, 1 H), 1.86 (s, 3 H), 1.04 (s, 3 H), 0.92–1.00 (m, 20 H), 0.82–0.87 (m, 14 H), 0.45–0.67 (m, 20 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.6 (C), 164.8 (C), 163.6 (C), 163.3 (C), 131.8 (CH), 128.2 (CH), 122.4 (C), 113.4 (CH), 105.2 (CH). ESIMS (*m*/*z*): 1182.7 ($M + Na^+$).

*(20*S*)-6β-Methoxy-16β,17α-dihydroxy-3α,5α-cyclobisnorcholanaldehyde 22,16-hemiacetal 22-*O*-{*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-*D*-xylopyranosyl]-(1→3)-4-*O*-triethylsilyl-α-*L*-arabinopyranosyl 1,2-orthoacetate}* (**12**). Compound **12** was obtained as an amorphous solid; R_f 0.25 (23:2 benzene–EtOAc); $[\alpha]_D^{21}$ −4.0° (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): *v* 3566, 1723, 1606, 1458, 1255, 1099, 1010 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.01 (d, 2 H, *J* 8.9 Hz), 6.92 (d, 2 H, *J* 8.9 Hz), 5.34 (d, 1 H, *J* 3.9), 5.29 (d, 1 H, *J* 4.8), 5.01 (t, 1 H, *J* 7.7 Hz), 4.77 (d, 1 H, *J* 7.2 Hz), 4.15 (m, 2 H), 4.02 (m, 1 H), 3.97 (dd, 1 H, *J* 11.6, 4.4 Hz), 3.87 (s, 3 H), 3.82–3.86 (m, 2 H), 3.70–3.75 (m, 2 H), 3.63 (dd, 1 H, *J* 11.5, 3.4 Hz), 3.33 (s, 3 H), 3.23 (dd, 1 H, *J* 11.6, 8.9 Hz), 2.77 (m, 1 H), 2.32 (dd, 1 H, *J* 7.0, 5.0 Hz), 1.67 (s, 3 H), 1.05 (s, 3 H), 0.80–1.00 (m, 40 H), 0.50–0.67 (m, 20 H), 0.45 (dd, 1 H, *J* 8.0, 5.1 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 164.7 (C), 163.2 (C), 131.6 (CH), 128.3 (CH), 122.9 (C), 121.6 (C), 113.5 (CH), 103.9 (CH), 100.6 (CH), 97.4 (CH). ESIMS (*m*/*z*): 1182.6 ($M + Na^+$).

Alkylation of the aldehyde **11**.—A solution of isoamyl magnesium bromide in anhyd ether was prepared from magnesium (6 mg, 0.26 mmol) and isoamyl bromide (0.032 mL, 0.26 mmol). The Grignard reagent was added dropwise to a stirred solution of compound **11** (150 mg, 0.13 mmol) in anhyd ether (10 mL) under argon at −70 °C. The reaction mixture was stirred 4 h at gradually increasing temperature to 0 °C. The reaction mixture was quenched with satd aq NH_4Cl and the product was extracted with ether. The extract was dried over $MgSO_4$ and solvent was evaporated in vacuo. The products were separated by silica gel column chromatography. Elution with EtOAc (15%)–hexane yielded ketone **13** (71 mg, 50%). Further elution with EtOAc (25%)–hexane afforded **3a** (31 mg, 20%) identical (R_f, optical rotation, NMR spectra) with the compound **3a** described above.

*6β-Methoxy-3α,5α-cycloandrostan-16β-ol-17-one 16-*O*-{*O*-[3,4-di-*O*-triethylsilyl-2-*O*-(4-methoxybenzoyl)-β-*D*-xylopyranosyl]-(1→3)-2-*O*-acetyl-4-*O*-triethylsilyl-α-*L*-arabinopyranoside}* (**13**). Compound **12** was obtained as an oil; R_f 0.25 (4:1 hexane–EtOAc); $[\alpha]_D^{21}$ −0.1° (*c* 1.5, $CHCl_3$). IR ($CHCl_3$): *v* 1739, 1726, 1607, 1255, 1096 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.95 (d, 2 H, *J* 8.8 Hz), 6.89 (d, 2 H, *J* 8.8 Hz), 4.98 (m, 2 H), 4.78 (d, 1 H, *J* 6.5 Hz), 4.68 (d, 1 H, *J* 6.9 Hz), 3.95 (m, 2 H), 3.85 (s, 3 H), 3.68–3.84 (m, 4 H), 3.32 (s, 3 H), 2.78 (m, 1 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 217.8 (C), 168.8 (C), 164.5 (C), 163.2 (C), 131.8 (CH), 128.3 (CH), 122.9 (C), 113.3 (CH), 101.7 (CH), 100.4 (CH), 81.9 (CH). ESIMS (*m*/*z*): 1123.7 ($M + Na^+$).

Deprotection of the functional groups.—Compound **4** (29 mg, 0.024 mmol) was dissolved in the solution of $PdCl_2$ (1.6 mg) in MeCN (2 mL), acetone (1.6 mL) and

water (0.06 mL). The reaction mixture was stirred 60 h at rt, evaporated in vacuo and chromatographed on silica gel column. Elution with MeOH (5%)–CH_2Cl_2 afforded compound **6** (5 mg, 26%), further elution with MeOH (7%)–CH_2Cl_2 yielded OSW-1 (**5**, 15 mg, 74%) as an amorphous solid.

Saponin OSW-1 (**5**). Compound **5**: R_f 0.18 (93:7 CH_2Cl_2–MeOH); $[\alpha]_D^{21}$ −44.0° (*c* 0.5, MeOH); lit. −43.2°, −45.2°.[1,5] IR ($CHCl_3$): *v* 3594, 3458, 1728, 1692, 1606, 1512, 1259, 1170, 1034 cm^{-1}. ^1H NMR (500 MHz, C_5D_5N): δ 8.30 (d, 2 H, *J* 8.9 Hz), 7.07 (d, 2 H, *J* 8.9 Hz), 5.64 (m, 1 H), 5.51 (dd, 1 H, *J* 8.0, 6.1 Hz), 5.37 (d, 1 H, *J* 4.0 Hz), 5.09 (d, 1 H, *J* 7.6 Hz), 4.76 (s, 1 H), 4.56 (d, 1 H, *J* 6.0 Hz), 4.37 (m, 1 H), 4.31 (dd, 1 H, *J* 11.4, 5.1 Hz), 4.19–4.25 (m, 2 H), 4.10–4.18 (m, 3 H), 3.81 (m, 1 H), 3.76 (s, 3 H), 3.65–3.75 (m, 2 H), 3.18 (q, 1 H, *J* 7.4 Hz), 1.96 (s. 3 H), 1.28 (d, 3 H, *J* 7.4 Hz), 1.07 (s, 3 H), 0.99 (s, 3 H), 0.89 (d, 3 H, *J* 6.3 Hz), 0.86 (d, 3 H, *J* 6.3 Hz). ^{13}C NMR (125 MHz, C_5D_5N): δ 219.8 (C), 170.1 (C), 166.3 (C), 164.8 (C), 142.8 (C), 133.3 (CH), 122.0 (CH), 115.0 (CH), 104.5 (CH), 101.7 (CH), 97.3 (C), 89.2 (CH), 86.6 (C), 81.7 (CH), 77.1 (CH), 75.9 (CH), 72.9 (CH), 72.2 (CH), 71.6 (CH), 68.6 (CH), 67.8 (CH_2), 66.3 (CH_2), 56.4 (CH_3), 51.0 (CH), 49.4 (CH), 47.4 (C), 47.2 (CH), 44.3 (CH_2), 40.1 (CH_2), 38.7 (CH_2), 37.7 (C), 35.5 (CH_2), 34.5 (C), 33.6 (CH_2), 33.5 (CH_2), 33.4 (CH_2), 33.1 (CH_2), 32.9 (CH), 28.6 (CH), 23.7 (CH_3), 23.4 (CH_3), 21.8 (CH_2), 21.7 (CH_3), 26.5 (CH_3), 14.5 (CH_3), 12.8 (CH_3). ESIMS (*m*/*z*): 895.4 ($M + Na^+$). Mass calcd for $C_{47}H_{68}NaO_{15}$: 895.4450; found: 895.4472.

3β-Chlorocholest-5-ene-16β,17α-diol-22-one 16-O-{O-[2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-α-L-arabinopyranoside} (**6**). Compound **12** was obtained as an amorphous solid; R_f 0.36 (93:7 CH_2Cl_2–MeOH). IR ($CHCl_3$): *v* 3584, 3466, 1728, 1693, 1606, 1512, 1259, 1170, 1033 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 8.08 (d, 2 H, *J* 8.8 Hz), 6.98 (d, 2 H, *J* 8.8 Hz), 5.36 (d, 1 H, *J* 4.1 Hz), 4.95 (m, 1 H), 4.71 (m, 2 H), 4.15 (m, 3 H), 3.88 (s, 3 H), 3.69–3.87 (m, 5 H), 3.38–3.48 (m, 3 H), 2.74 (q, 1 H, *J* 7.4 Hz), 1.95 (s. 3 H), 1.05 (s, 3 H), 0.80 (s, 3 H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 218.9 (C), 169.3 (C), 165.9 (C), 164.1 (C), 140.7 (C), 132.2 (CH), 121.4 (CH), 114.0 (CH), 85.6 (C). ESIMS (*m*/*z*): 913.4 ($M + Na^+$). Mass calcd for $C_{47}H_{67}ClNaO_{14}$: 913.4112; found: 913.4104.

Acknowledgements

We thank Dr. L. Siergiejczyk for assistance in recording some NMR spectra and Mrs. J. Maj for help in preparation of the manuscript. Financial support from the State Committee for Scientific Research, Poland (Grant No. 7 T09A 045 20) is gratefully acknowledged.

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