New Analogues of the Potent Cytotoxic Saponin OSW-1

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Saponin OSW-1 (5e-G2; 3β,16β,17α-trihydroxycholesterol-5-en-22-one 16-O-{O-[2-O-(4-methoxybenzoyl)-β-D-xlyopyranosyl]-[1→3]-2-O-acetyl-α-arabinopyranoside}) analogues: with modified side chain (5a-d-G2), 22-deoxo-23,24,25,26,27-pentanor- (14), 22-deoxo-23-oxa- (17), glycosylated with various monosaccharides (5e-G4/G6/G8), and OSW-1 structural isomer (10) were obtained. The analogues were synthesized using a previously published method for the synthesis of OSW-1. The structures of analogues were fully confirmed by spectroscopic methods, and the S-chirality at C-22 of the structural isomer was established by conformational analysis combined with the NMR spectrometry. The cytotoxicity of the analogues toward several types of malignant tumor cells was examined and compared with that of OSW-1. The results suggest that modification of the steroidal aglycone may lead to compounds with high cytotoxicity.

1. Introduction

The value of screening extracts of natural product in the search for novel, complex lead structures for drug discovery has been demonstrated in many studies.1–5 Indeed, most currently used cancer drugs are synthesized by simple modifications of natural products.6 Recently, a new group of saponins was isolated from the bulbs of Ornithogalum saundersiae, a perennial grown in southern Africa, where it is cultivated as a cut flower and garden plant.7 These saponins, each of which contains a novel 16β,17α-dihydroxycholesterol-22-one aglycone unit glycosylated at the 16-0H group with an acylated disaccharide, proved to be strongly cytotoxic, with very similar cytotoxicity profiles to those of cephalostatins.8–10 The most abundant saponin in the plant, OSW-1, is weakly toxic toward normal cells but inhibits the growth of various types of malignant tumor cells and is 10–100 times more potent than clinically applied anticancer agents, such as Adriamycin, cisplatin, camptothecin, and taxol.

The action mechanism of OSW-1 has been recently shown to damage the mitochondrial membrane and cristae in human leukemia and pancreatic cancer cells, leading to losses of transmembrane potential, increases in cytosolic calcium contents, and activation of calcium-dependent apoptotic pathways.11 This mechanism differs from those of all other anticancer compounds examined to date. Thus, OSW-1 has high apparent potential for effectively treating some cancers that are strongly resistant to currently available drugs, and it clearly warrants detailed further investigation.

Several research groups have described the synthesis of OSW-1 aglycone.12–15 Numerous methods of saponin OSW-1 semisynthesis are also known, including coupling of the aglycone with the sugar moiety.14,16–18 Various analogues of OSW-1 have also been prepared and tested for cytotoxicity recently.19–25 It was found that the C17 side chain of OSW-1 could tolerate certain modifications without significant loss of its antiproliferative potency. This includes replacement of the ketone group in the side chain by an ester group. Attempts to synthesize structurally simpler OSW-1 analogues with full antitumor activity led to the discovery of a potent analogue containing a monosaccharide sugar part.26 However, much remains to be discovered about the structural elements of saponin OSW-1 that are associated with its high activity, so we synthesized two series of novel analogues of the compound with modified side chains and various sugar moieties, and evaluated their toxicity toward a battery of cancer cell lines.

2. Results and Discussion

We have already published preliminary reports on the synthesis of OSW-1 analogues,27,28 using a procedure based on the previously described synthesis of saponin OSW-1.17 One of the intermediates in the OSW-1 synthesis route, 17α-hydroxyxactone 1, was successfully used to synthesize analogues with modified side chains. Various aglycones with side chains of various sizes and shapes (linear or branched) in hemiketals were obtained. By reacting 1 with corresponding alkyllithium reagents in high yields (Scheme 1). All aglycones were coupled with the OSW-1 disaccharide trichloroacetimidate (CCl3C(NH)O~G1) prepared according to the procedure described in the literature.16 The reactions were catalyzed by trimethylsilyl triflate. Apart from the desired 16β-glycosides 4a–f, variable amounts of isomeric hemiketal glycosides 3a–f were formed (Scheme 2). In the case of 2a, the glycosylation product 3a was not found in the reaction mixture, in contrast to the reaction with 2f, which did not afford the desired 16β-glycoside 4f. The observed difference in the results of the attempted glycosylation may be attributable to steric hindrance present in the cyclic aglycone. During glycosylation, the glycosyl donor may be attacked by either the oxygen atom at C16 (for 16β-O-glycosides) or that at C22 (for 22-O-glycosides). Because there is no spectral evidence for the presence of an open-chain form in equilibrium with the cyclic aglycone in any cases studied, the desired 16β-O-glycosides were probably formed by the direct attack of the ring oxygen on the sugar donor. The relative rates of the competing reactions determine the proportions of the products. However, there is no simple explanation...
for the obtained results because both the structure and the conformation of the side chain affect the course of reactions.

Products 3 and 4 were readily distinguished by analysis of their $^1$H and $^{13}$C NMR spectra. The most characteristic $^1$H signals of the 16β-glycosides 4 are a quartet at about 3.1 ppm deriving from the proton at C-20, a doublet ($\delta \sim 1.1$ ppm) arising from the 21-methyl protons, and a peak at around 4.8 ppm corresponding to the anemic proton. For compounds 3, the anemic proton signal was shifted downfield to $\sim 5.4$ ppm, the 20-H quartet appeared at $\sim 2.4$ ppm, and there was a characteristic upfield shift of acetate protons from their normal resonance at $\sim 2$ ppm to $\sim 1.7$ ppm. In the $^{13}$C NMR spectra, a quaternary carbon C-22 signal appeared at about 115 ppm for compounds 3, while 16β-glycosides 4 showed a characteristic signal for ketones at $\sim 218$ ppm. The last step of the synthesis of the saponin OSW-1 analogues 5a–e consisted of the simultaneous removal of the protective groups from both the steroid and the sugar moieties by hydrolysis in the presence of $p$-TsOH under controlled conditions (2 h at 80 °C). The best overall yield from 17α-hydroxy lactone 1 was obtained for saponin 5c (9%).

To obtain an isomer of OSW-1 with a carbonyl group at C-16 and a sugar moiety attached at C-22 (compound 10), the 16β, 17α,22-triol 6 was glycosylated (Scheme 3). Compound 6 was obtained by a LiAlH$_4$ reduction of OSW-1 aglycone in its hemiketal form (compound 2e). The stereoselectivity of the reaction was not satisfactory — a mixture of epimers at C-22 was formed although the mixture was homogeneous according to TLC analysis. Previous studies on the benzylolation$^{13}$ of this compound indicated that the hydroxyl group at C-22 is the most reactive one. However, the reaction of the 16β,17α,22-triol 6 with glycosyl trichloroacetimidate (CCl$_3$C(NH)O~G1) was not regioselective. Both products, glycosylated at C-16 (7) and at C-22 (8), were formed. They were quite easy to separate because compound 7 proved to be significantly less polar than its regioisomer 8. Both TLC and NMR analysis indicated that compound 8 was homogeneous. One of the epimers at C-22 appeared to react more quickly with a glycosyl donor than the other. The regioisomers (7 and 8) were subjected to oxidation with pyridinium dichromate. The isomeric ketones showed very similar polarity. One of them proved to be identical to the protected OSW-1 (4c-G1) previously obtained. The isomeric product 9 was subjected to simultaneous desilylation and cycloreversion with $p$-TsOH to afford the saponin OSW-1 isomer 10. The structure of compound 10 was fully confirmed by spectral analysis (IR, NMR, MS).

We also took effort to assign the configuration at the C-22 stereogenic center. The combination of NMR data with the exhaustive conformational analysis for the analogue in question allowed us to solve the problem. Initially, almost all proton and carbon signals were assigned by different types of NMR measurements. The chemical shifts observed in two solvents (Table 1) were in good agreement with those measured earlier for similar compounds.$^{7,29,30}$ The correlation signals observed in the 2D homonuclear spectra of compound 10 as well as those observed in their 2D heteronuclear spectra revealed long range connectivities allowed us to fully confirm the structure. Thus, numerous correlations between sugar units, between sugar and OMBz moieties, and from the aliphatic chain to either ring D or sugar were observed. This in turn allowed us to determine the positions of all groups and residues. However, the analysis provided no clear, unambiguous evidence for the chirality at C-22. Therefore, conformational analysis of both possible isomers was carried out.

Because the signal assignment obtained for 10 in chloroform was more complete than that obtained in the mixed solvent (chloroform/pyridine), we performed the conformational analysis using the data acquired from the former. The number of papers concerning full conformational analysis of saponins is rather limited.$^{31,32}$ In most other cases, structural analyses have been restricted to evaluations of the isolated conformations.$^{30}$ Here we attempted to analyze the conformational properties of saponin 10 by means of an exhaustive conformational search. The final set of 500 structures obtained for each isomer (R and S) by the procedure described in detail in the Experimental Section consists of molecular structures corresponding to
possible minima in the conformational space of the studied molecules. As some of the conformers were very similar to each other, to simplify analysis, the values of dihedral angles measured in generated structures were chosen as a criterion describing their similarity. Two conformers were assumed identical if the largest difference observed between the values describing their similarity. Two conformers were assumed measured in generated structures were chosen as a criterion.

For **Proton and Carbon NMR Chemical Shifts (in ppm) Measured for 10 in Solution**, see Table 1. Table 1. Proton and Carbon NMR Chemical Shifts (in ppm) Measured for 10 in Solution

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* Numbers followed by ′, ″, and ′′ refer to carbon atoms in arabinose, xylose, and the MBz group, respectively. * NA = not assigned.

In a recent study 21 it was claimed that the size of the side chain and even the presence of a carbonyl group were not very important for cytotoxicity of saponins. To check this hypothesis, an analogue 14 lacking the carbonyl group with a short side observed for meta protons. These findings indicate that one might be able to establish the chirality at C-22 by direct quantitative comparison of the conformationally averaged distances measured by NMR with those obtained from conformational analysis. However, this approach did not work because all distances obtained from NOE for the saponin were systematically shorter than those obtained from generated structures. It seems that the main source of this systematic discrepancy comes from the indirect effect,35 which clearly disturbs not only the intensity of NOE of methylene protons usually used for calibration but also the intensity of all peaks measured for the multispin system of saponins. In this situation, only qualitative analysis was possible. Figure 2 shows the distances between ortho protons of the OMBz ring and 7α, H, 9α, H, 14α, H, and 15α H observed in the conformers of both isomers of 10. The figure shows that quite substantial proportions of generated conformers of the isomer S have distances of about 5 Å, which is not the case for the isomer R. Furthermore, only one conformer of the S isomer was found in which the MBz ring is positioned over the β surface of the steroid; in all other conformers of this isomer, the MBz ring is over the α surface. The opposite situation obtains with the R isomer, where only two conformers were identified in which the MBz ring lies over the α surface. It is important to note that the relative population of the identified conformers cannot be accurately predicted, but these findings strongly suggest that the conformational preferences of the C-22 epimers are distinct and support the assignment of compound 10 as the C-22 S epimer.

Figure 1. Sets of conformers of compound 10 obtained by the procedure described in the text. Molecules are superposed with regard to heavy atoms of the steroid moiety. The lowest energy conformer is shown in bold: (A) 58 conformers of the 22S isomer; (B) 29 conformers of the 22R isomer. Lowest energy conformers with a cutoff of 5 kcal/mol: (C) 30 conformers of the 22S isomer; (D) 15 conformers of the 22R isomer.

Abbreviations: NA, not assigned; NT, not tested; DEPT, distortionless enhancement by polarization transfer; DQ COSY, double quantum filtered correlated spectroscopy; GHSQC, gradient heteronuclear single quantum coherence; HMQC, heteronuclear multiple-bond correlation; ROESY, rotating-frame Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy.
of OSW-1, but different glycosyl donors were used (G3, G5, G7, or G9). Generally, yields for coupling aglycone 2e with monosaccharide trichloroacetimidates (D-xylopyranose and L-arabinopyranose derivatives) were higher than those obtained with CCl3C(NH)O~G1. However, the reaction with the glucose derivative was rather sluggish, and the desired 16β-glucoside was not obtained (the hemiketal glucoside 3e-G9 was the major product). In some reactions, orthoacetates (e.g., 4e-G10), which showed a characteristic signal in the 13C NMR spectrum at about 122 ppm, were isolated as byproducts. Both hemiketal glycosides and orthoacetates proved to be acid-sensitive. During attempts to deprotect them under acidic conditions (p-TsOH), these products underwent fragmentation to the starting sugar and furan derivative of the steroid aglycone. The formation of this compound with a heteroaromatic ring E under acidic conditions from OSW-1 aglycone via a Ferrier type of rearrangement was reported several times in the literature.12,34,35

Figure 2. Comparison of distances between ortho protons of the OMBz ring and 7α H, 9α H, 14α H, and 15α H observed in the conformers of the two isomers of 10 shown in Figure 1: (A) isomer 22S; (B) isomer 22R.

Scheme 4

Further studies22,23 have shown that compounds containing an oxygen atom instead of a carbon atom at position 23 are equally or even more potent than saponin OSW-1. Combining this finding with the previous mentioned claim that the carbonyl group is not essential for biological activity,21 the simplified OSW-1 analogue 17 lacking the 22-carbonyl group and with oxygen in place of C-23 was designed, as a good candidate for a cytotoxic agent. The required ether aglycone 15 (which we previously synthesized from lactol 11 via reduction followed by a selective Williamson reaction28) was subjected to glycosylation with the OSW-1 disaccharide. After routine removal of the protective groups, the desired 23-oxa-22-deoxy-OSW-1 analogue (17) was obtained in 20% overall yield (four steps from lactol 11: Scheme 5).

A number of OSW-1 analogues with different sugar moieties were obtained. We reasoned that truncation of the disaccharide residue in OSW-1 into a monosaccharide derivative with the acetyl and p-methoxybenzoyl groups retained at their positions would not significantly affect its antiproliferative activity. The synthetic procedure was essentially the same as for synthesis of OSW-1, but different glycosyl donors were used (G3, G5, G7, or G9). Generally, yields for coupling aglycone 2e with monosaccharide trichloroacetimidates (D-xylopyranose and L-arabinopyranose derivatives) were higher than those obtained with CCl3C(NH)O~G1. However, the reaction with the glucose derivative was rather sluggish, and the desired 16β-glucoside was not obtained (the hemiketal glucoside 3e-G9 was the major product). In some reactions, orthoacetates (e.g., 4e-G10), which showed a characteristic signal in the 13C NMR spectrum at about 122 ppm, were isolated as byproducts. Both hemiketal glycosides and orthoacetates proved to be acid-sensitive. During attempts to deprotect them under acidic conditions (p-TsOH), these products underwent fragmentation to the starting sugar and furan derivative of the steroid aglycone. The formation of this compound with a heteroaromatic ring E under acidic conditions from OSW-1 aglycone via a Ferrier type of rearrangement was reported several times in the literature.12,34,35

The anticancer activity of the new OSW-1 analogues was evaluated in vitro using eight cancer cell lines of different histopathological origins and normal mouse fibroblast NIH 3T3 cells. The results are summarized in Table 2. The cancer cell lines exhibited distinct sensitivity to OSW-1 (5e-G2) and its analogues, with CEM, K 562, and A 549 cell lines being the most sensitive and G 361 human melanoma cells being the least sensitive. In contrast, no compounds tested showed cytotoxicity to the normal mouse NIH 3T3 fibroblasts. Among the tested analogues, the most active appeared to be compound 5d-G2, which showed similar antitumor potency to that of OSW-1. Against the ARN 8 cell line, it was even 10 times more active than natural saponin. However, shortening of the alkyl side chain (5a-G2) led to a slight loss of activity. The results demonstrate that small variations in the structure, for example, in the size of the cholestane side chain, do not affect antitumor activity significantly.21–23

The monosaccharide analogues of OSW-1 (5e-G4, 5e-G8) appeared to be lethal to 50% of the tumor cells at concentrations of 0.2–7.7 μM, that is, they are about 1000 times less active than OSW-1 (5e-G2). These findings clearly indicate that the disaccharide moiety is essential for the antitumor activities of OSW-1.

Compound 10, the structural isomer of saponin OSW-1, was also tested using the panel of cancer and normal cells. The cytotoxicity (TSC50) values of 10 varied between 0.28–14.4 μM and were about 1000 times lower than that of OSW-1, proving that the position of the disaccharide moiety is also important.

Contrary to previously published assertions,21 the presence of a carbonyl group at C22 also seems to be a pharmacophore requirement. Compound 14 showed much lower activity in anticancer tests than OSW-1. Similarly, the OSW-1 ether analogue 17 appeared to be much less active than the very potent ester analogue (23-oxa analogue of OSW-1) described recently.22

3. Experimental Section

3.1. General Remarks. Melting points were determined using a Kofler apparatus of the Boetius type. NMR spectra were recorded with a Bruker AC 200F or Varian UNITY 500plus (equipped with
a Performa II gradient generator unit, WFG, Ultrashims, high stability temperature unit and a 5 mm H/3/C/3/N-FPP- triple resonance inverse probe head) spectrometer using CDCl3/C6D6 solutions with TMS as the internal standard. Only selected signals in the 1H NMR spectra are reported. Infrared spectra were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer from chloroform solutions. Mass spectra were obtained at 70 eV with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J. T. Baker). TLC was carried out using commercially available plates (Ehler, silica gel 60 F254).

Compound 1, glycosyl trichloracetimides (Cl2C(NH2)O–G), lactol 11, and ether 15 were obtained according to procedures described, respectively, in refs 34, 16, 35, and 28.

### 3.2. Chemical Synthesis. Representative Procedure for Synthesizing Aglycones 2a–2f.

A solution of n-butyl lithium in anhydrous ether was prepared from lithium (13 mmol) and n-butyl bromide (13 mmol). The reagent was added, dropwise, over 1 h to a stirred solution of lactone 1 (1.3 mmol) in 100 mL of anhydrous ether at room temperature under argon. The reaction mixture was stirred for an additional hour, then quenched with saturated aqueous NH4Cl, and the reaction product was extracted with ether. Evaporation of the solvent from dried (anhydrous MgSO4) extract afforded crude product 2c, which was purified by silica gel column chromatography.

### 6β-Methoxy-3α,5-cyclo-27-nor-5α-furostane-17α,22α,20-diol 2c.

Yield 73%; an oil product eluted with hexane–ethyl acetate (77.5: 22.5). IR (CHCl3) 3549, 3469, 1091 cm−1. 1H NMR (200 MHz, CDCl3) δ 4.17 (1 H, J = 7.5 Hz), 3.32 (3 H, s), 1.35 (15 H, br s), 2.78 (1 H, m), 2.29 (1 H, q, J = 7.4 Hz), 1.04 (3 H, s), 0.94 (3 H, d, J = 7.4 Hz), 0.92 (2 × 3 H, m), 0.66 (1 H, m), 0.45 (1 H, dd, J = 7.8, 5.2 Hz). 13C NMR (50 MHz, CDCl3) δ 111.2 (C), 90.5 (C), 120.8 (CH), 56.4 (CH2), 52.7 (CH2), 47.5 (CH3), 44.5 (C), 43.3 (C), 42.2 (CH2), 37.4 (CH2), 35.01 (C), 34.97 (CH3), 33.2 (CH2), 32.0 (CH2), 30.8 (CH2), 29.9 (CH), 25.5 (CH2), 24.8 (CH2), 22.8 (CH2), 22.2 (CH3), 21.3 (CH2), 19.2 (CH), 17.6 (CH3), 13.9 (CH3), 13.0 (CH2), 8.4 (CH3). EI-MS m/z (%) 414 (M–H–O2, 21), 385 (47), 382 (9), 269 (100).

**Representative Procedure for Glycosylation of Aglycones 2a–2f, 6, 12, and 15 with CCl3C(NH2)O–G (G1, G3, G5, G7, or G9).**

A solution of the glycosyl trichloroacetimidate (CCl3C(NH2)O–G1, 0.64 mmol) and steroid aglycone 2c (0.55 mmol) in dry dichloromethane (15 mL) was stirred with 4 A molecular sieves (1.5 g) at room temperature for 15 min, and then the reaction mixture was cooled to −68 °C (ethanol−dry ice bath) and a 0.14 M solution of TMSOTf in CH2Cl2 (1.3 mL) was slowly added. The reaction mixture was stirred for an additional 30 min, quenched with triethylamine, and the molecular sieves were filtered out. The filtrate was evaporated in vacuo, and the products, protected saponin 4c and its cyclic isomer 3c, were separated by silica gel column chromatography.

The above method was also used for the preparation of the glycosides 4a–4e. The compounds 4a–4e were subjected to deprotection with full characterization. The crude products of glycosylation of diol 6 (compounds 7 and 8) were separated and oxidized to the corresponding ketones (vide infra).

**Table 2. Antitumor Activities of the New OSW-I Analogues (TC50 in nM) in the Calcin AM Cytotoxicity Assays**

<table>
<thead>
<tr>
<th>compound</th>
<th>CEM</th>
<th>MCF7</th>
<th>K 562</th>
<th>ARN 8</th>
<th>G 361</th>
<th>HeLa</th>
<th>HOS</th>
<th>A 549</th>
<th>NIH 3T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>5c-G2</td>
<td>0.3 ± 0.03</td>
<td>50 ± 2</td>
<td>0.8 ± 0.4</td>
<td>4 ± 0.4</td>
<td>1000 ± 31</td>
<td>8 ± 0.4</td>
<td>40 ± 2</td>
<td>0.5 ± 0.01</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>5a-G2</td>
<td>0.5 ± 0.02</td>
<td>90 ± 4</td>
<td>1.4 ± 0.4</td>
<td>3 ± 0.2</td>
<td>3100 ± 26</td>
<td>12 ± 0.5</td>
<td>140 ± 11</td>
<td>0.9 ± 0.03</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>5d-G2</td>
<td>0.2 ± 0.01</td>
<td>40 ± 1.5</td>
<td>0.7 ± 0.05</td>
<td>0.6 ± 0.05</td>
<td>1400 ± 52</td>
<td>8 ± 0.15</td>
<td>42 ± 6</td>
<td>0.5 ± 0.025</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>14</td>
<td>1300 ± 90</td>
<td>18200 ± 130</td>
<td>5100 ± 73</td>
<td>6800 ± 66</td>
<td>9300 ± 101</td>
<td>6400 ± 87</td>
<td>17400 ± 202</td>
<td>3200 ± 85</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>10</td>
<td>280 ± 10</td>
<td>3400 ± 66</td>
<td>430 ± 17</td>
<td>500 ± 7.5</td>
<td>14400 ± 99</td>
<td>1000 ± 54</td>
<td>11700 ± 150</td>
<td>480 ± 39</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>17</td>
<td>&lt;200</td>
<td>860 ± 32</td>
<td>&lt;400</td>
<td>&lt;400</td>
<td>1900 ± 78</td>
<td>350 ± 25</td>
<td>1800 ± 63</td>
<td>&lt;200</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>5e-G4</td>
<td>200 ± 20</td>
<td>1300 ± 150</td>
<td>430 ± 17</td>
<td>290 ± 61</td>
<td>2200 ± 91</td>
<td>570 ± 31</td>
<td>4200 ± 92</td>
<td>4200 ± 96</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>5e-G8</td>
<td>1000 ± 80</td>
<td>3500 ± 63</td>
<td>1800 ± 35</td>
<td>1500 ± 27</td>
<td>3500 ± 85</td>
<td>2300 ± 100</td>
<td>7200 ± 103</td>
<td>7200 ± 120</td>
<td>&gt;50 000</td>
</tr>
<tr>
<td>daunorubicin</td>
<td>62 ± 10</td>
<td>140 ± 12</td>
<td>230 ± 9T</td>
<td>11NTb</td>
<td>670 ± 20</td>
<td>670 ± 31</td>
<td>330 ± 49</td>
<td>NTb</td>
<td>NTb</td>
</tr>
</tbody>
</table>

* The values shown are the mean ± SDs obtained in three experiments. Daunorubicin was used as a positive control. t NT: not tested.
The oxidation product of compound 7 (eluted with hexane–ethyl acetate (92:8)) was shown to be identical in all respects to the compound 4e-G1 obtained by direct glycosylation of the aglycone 2e, as described in ref 17.

(22S)-6β-Methoxy-17α,22-dihydroxy-3e,5c-cyclo-5c-cholestan-16-one 22-O-([O-2-0-(4-methoxybenzoyl)-3,4-di-O-tritylthiophenyl]-β-D-xlyopyranosyl-1–3)-2-O-acetyl-4-O-tritylthiophenyl-α-L-arabinopyranoside) (9-G1). Product 9-G1 was eluted with hexane–ethyl acetate (87:5:12.5). IR (CHCl3) 3480, 1753, 1735, 1607, 1511, 1169, 1255. 1094 cm⁻¹. 1H NMR (200 MHz, CDCl3) δ 6.97 (2 H, d, J = 8.7 Hz), 6.91 (2 H, d, J = 8.7 Hz), 5.05 (1 H, dd, J = 8.9, 7.0 Hz), 4.94 (2 H, m), 4.64 (1 H, d, J = 6.5 Hz), 4.44 (1 H, d, J = 6.9 Hz), 4.00 (2 H, m), 3.91 (1 H, s), 3.86 (3 H, s), 3.77 (1 H, m), 3.71 (3 H, m), 3.50 (1 H, m), 2.30 (1 H, m), 2.18 (1 H, m), 1.02 (s, 3 H), 0.98 (3 H, d, J = 6.9 Hz). 13C NMR (50 MHz, CDCl3) δ 218.9 (C), 168.9 (C), 164.5 (C), 163.1 (C), 131.9 (2 CH), 122.9 (C), 113.3 (3 × CH), 102.1 (CH), 101.3 (CH), 83.1 (C), 82.8 (CH), 82.1 (CH), ESI-MS m/z (%): 1252 (MNa+), 1149 (MMeNa+).

Wolff–Kishner Reduction of Lactol 11. To a stirred solution of lactol 11 (0.276 g, 0.73 mmol) in ethanol (15 mL), an 89% solution of hydrazine hydrate (0.05 mL, 1.1 equiv) and triethylamine (0.76 mL, 5.5 equiv) was added. The reaction mixture was stirred for 16 h. Evaporation of the solvent from the reaction mixture afforded crude hydrazine. The product, without further purification, was dissolved in DMSO, and a solution of potassium tert-butoxide in DMSO was added dropwise. The reaction mixture was stirred at room temperature under argon for 4 h, then it was poured into water, and the product was extracted with ether. Evaporation of the solvent from dried (anhydrous MgSO4) extract afforded crude product, which was purified by silica gel column chromatography. Elution with hexane–ethyl acetate (85:15) yielded compound 12 (0.133 g; 50%).

6b-Methoxy-3e,5c-cyclo-20a-homo-5c-pregnan-16β,17β-diol (12). IR (CHCl3) 3510, 3468, 1725, 1092 cm⁻¹. 1H NMR (200 MHz, CDCl3) δ 3.87 (1 H, dd, J = 4.7, 7.9 Hz), 3.31 (3 H, s), 2.77 (1 H, m), 2.30 (2 H, m), 1.02 (s, 3 H), 0.98 (3 H, d, J = 6.7 Hz), 0.96 (3 H, s), 0.93 (3 H, d, J = 6.9 Hz), 0.64 (1 H, m), 0.42 (1 H, dd, J = 8.0, 5.1 Hz). 13C NMR (50 MHz, CDCl3) δ 86.3 (C), 82.2 (CH), 80.8 (CH), 56.4 (CH), 48.3 (CH), 47.5 (CH), 46.9 (C), 43.2 (C), 35.9 (CH2), 35.1 (C), 34.9 (CH2), 33.2 (CH2), 33.1 (CH2), 30.3 (CH), 28.9 (CH), 24.8 (CH2), 22.0 (CH2), 21.4 (CH), 19.2 (CH), 18.7 (CH2), 17.0 (CH2), 13.3 (CH2), 13.00 (CH2), ESI-MS m/z (%): 747.6 (MNa+), 385.3 (MMeNa+). 

3.3. NMR and Conformational Analysis of Compound 10. For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm). For NMR measurements, compound 10 of pyridine-d5 (D 99.5%, Aldrich, St. Louis, MO) and CDCl3 (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA) were used, and 101.8 ppm) and chemical shift of chloroform in 13C spectra (77.0 ppm).


