New Analogues of the Potent Cytotoxic Saponin OSW-1

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Saponin OSW-1 (**5e-G2**; 3β,16β,17α-trihydroxycholest-5-en-22-one 16-*O*-{*O*-[2-*O*-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(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-dihydroxycholest-22-one aglycone unit glycosylated at the 16-OH 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-hydroxylactone **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 hemiketal forms (**2a−f**) 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
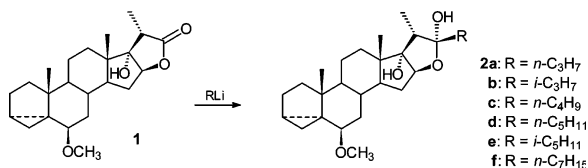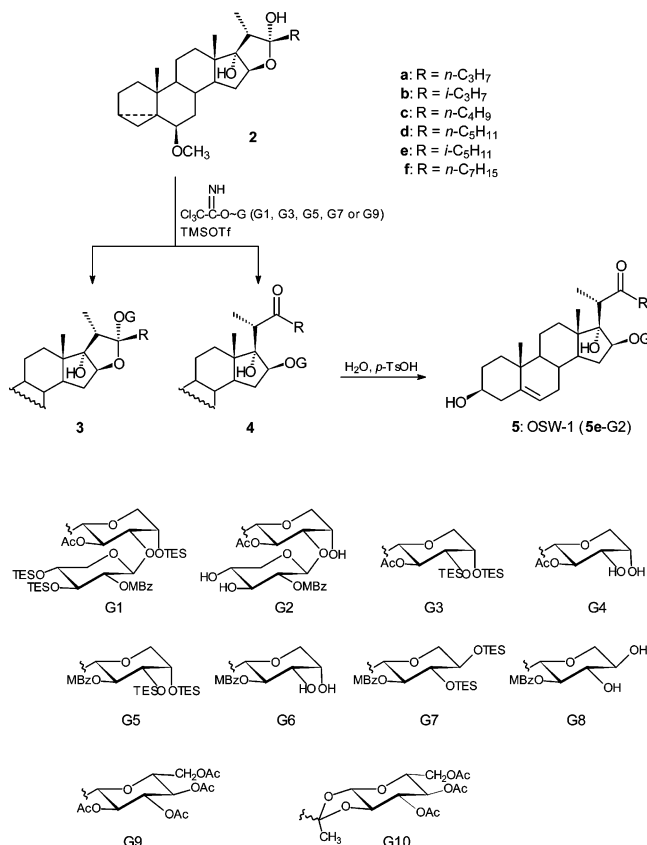
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Scheme 1

2a: R = *n*-C$_3$H$_7$
b: R = *i*-C$_3$H$_7$
c: R = *n*-C$_4$H$_9$
d: R = *n*-C$_5$H$_{11}$
e: R = *i*-C$_5$H$_{11}$
f: R = *n*-C$_7$H$_{15}$

Scheme 2[a]

a: R = *n*-C$_3$H$_7$
b: R = *i*-C$_3$H$_7$
c: R = *n*-C$_4$H$_9$
d: R = *n*-C$_5$H$_{11}$
e: R = *i*-C$_5$H$_{11}$
f: R = *n*-C$_7$H$_{15}$

5: OSW-1 (**5e**-G2)

G1   G2   G3   G4

G5   G6   G7   G8

G9   G10

[a] TMSOTf, trimethylsilyl triflate; MBz, 4-methoxybenzoyl; TES, triethylsilyl; *p*-TsOH, 4-toluenesulfonic acid.

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 ¹H and ¹³C NMR spectra. The most characteristic ¹H 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 anomeric proton. For compounds **3**, the anomeric 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 ¹³C NMR spectra, a

Scheme 3
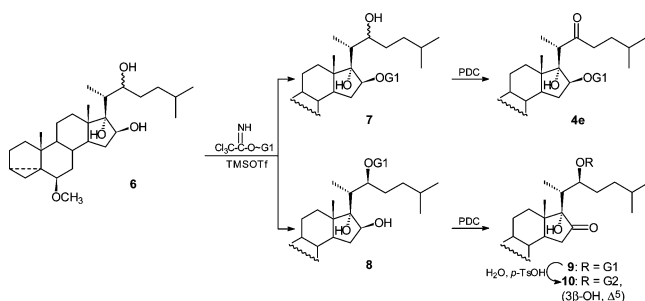
quaternary carbon C-22 signal appeared at about 115 ppm for compounds **3**, while 16β-glycosides **4** showed a characteristic signal for ketones at $\sim$218 ppm. The last step of the synthesis of the saponin OSW-1 analogues **5a**–**e** consisted of the simultaneous removal of the protective groups 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-hydroxylactone **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 benzylation[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$\sim$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 (**4e**-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 compond **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 revealing 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

Table 1. Proton and Carbon NMR Chemical Shifts (in ppm) Measured for **10** in Solution[a]

atom	in CDCl$_3$		in CDCl$_3$/C$_5$D$_5$N 1:1 (v/v)	
	^{1}H	^{13}C	^{1}H	^{13}C
1	1.120; 1.602	31.44	1.001; 1.745	NA[b]
2	1.514; 1.859	31.42	1.456; 1.790	35.02
3	3.543	71.62	3.412	74.52
4	2.234; 2.298	42.11	2.190; 2.226	NA[b]
5		NA[b]		144.80
6	5.340	121.08	5.229	124.00
7	1.581; 1.862	31.04	1.479; 1.782	NA[b]
8	NA[b]	NA[b]	NA[b]	45.74
9	1.058	49.17	NA[b]	52.59
10		NA[b]		39.95
11	1.491; 1.608	20.50	1.390; 1.443	NA[b]
12	1.259	32.03	1.533; 1.748	34.38
13		NA[b]		49.62
14	1.886	43.85	1.848	47.29
15	1.682; 2.109	37.84	1.628; 2.132	NA[b]
16		218.81		NA[b]
17		NA[b]		86.27
18	0.743	14.06	0.652	17.20
19	1.013	19.38	0.923	22.75
20	1.876	35.52	1.797	38.94
21	0.942	7.59	0.833	10.89
22	4.961	82.83	4.884	86.00
23	1.114; 1.856	37.05	1.177; 1.489	39.96
24	0.970; 1.123	35.11	0.886; 1.063	38.36
25	1.497	28.16	1.408	31.50
26	0.859	22.58	0.764	25.56
27	0.859	22.58	0.771	26.25
1′	4.571	100.58	4.439	104.70
2′	5.037	70.53	5.031	73.66
OAc	1.796	20.64	NA[b]	NA[b]
3′	3.800	80.84	3.676	83.34
4′	4.048	55.59	3.937	70.89
5′	3.523; 3.736	63.83	3.412; 3.925	68.06
1″	4.682	101.87	4.624	105.30
2″	4.934	74.29	4.971	77.16
3″	3.718	74.30	3.697	77.93
4″	3.823	69.67	3.722	73.01
5″	3.395; 4.136	64.59	3.310; 4.023	68.74
1‴		NA[b]		166.50
2‴; 6‴	7.972	132.22	7.870	135.30
3‴; 5‴	6.915	113.86	6.789	116.90
4‴		NA[b]		166.80
4‴OMe	3.864	55.67	3.741	58.79
7‴		NA[b]		168.60

[a] Numbers followed by ′, ″, and ‴ refer to carbon atoms in arabinose, xylose, and the MBz group, respectively. [b] NA = not assigned.

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 of all of their matching dihedral angles was less than 2 degrees and only one of them was further considered. This procedure led to a total number of 29 different conformers of *R* and 58 conformers of *S*; see Figure 1. For clarity, the numbers of lowest energy conformers of *R* and *S* after applying a cutoff of 5 kcal/mol were reduced to 15 and 30, respectively, see Figure 1.

Inspection of Figure 1 indicates that there is a distinctive difference in conformational freedom between the two isomers of **10**. In the *S* isomer, OMBz groups in the sugar units are generally positioned over the α-surface of the steroid moiety, whereas in the *R* isomer, these moieties are positioned over the β-surface. In ROESY[a] spectra, four cross-peaks have been observed between ortho protons of the OMBz group and four protons of the α-surface, namely, the 7α, 9α, 14α, and 15α protons. Clearly weaker cross-peaks of the same type were
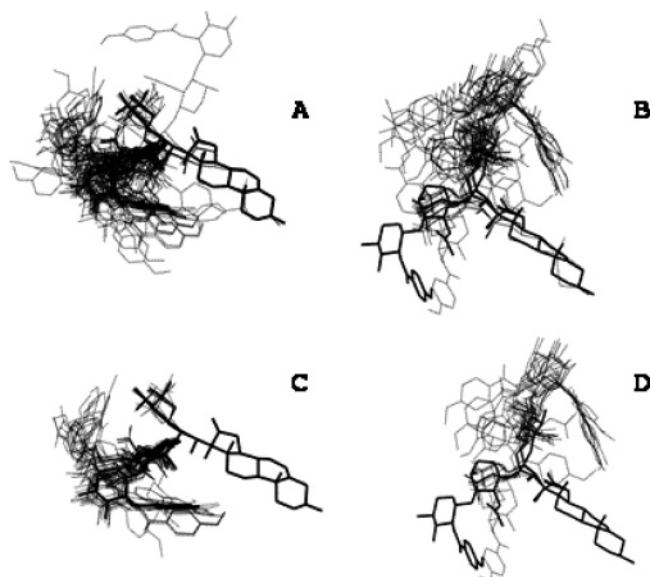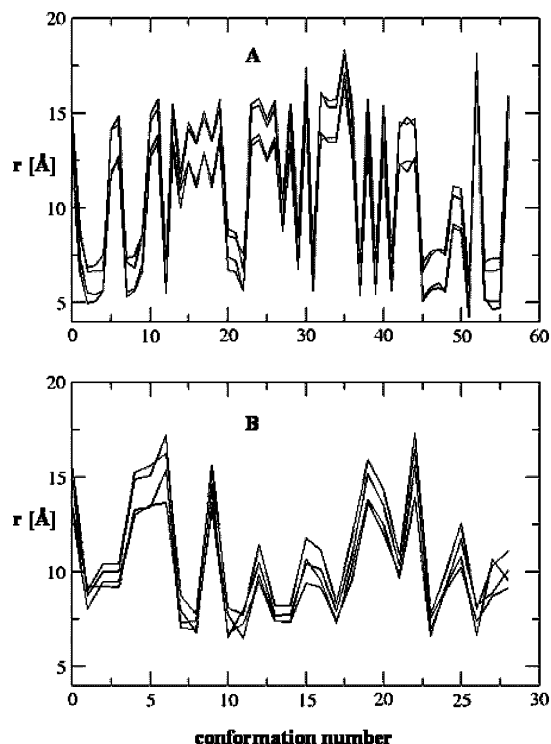

Figure 1. Sets of conformers of compound **10** obtained by the procedure described in the text. Molecules are superposed with regard to heavy atoms of the steroid moiety. The lowest energy conformer is shown in bold: (A) 58 conformers of the 22*S* isomer; (B) 29 conformers of the 22*R* isomer. Lowest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

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,[33] 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.
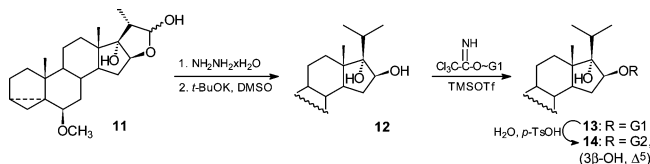
In a recent study[21] it was claimed that the size of the side chain and even the presence of a carbonyl group were not very important for cytotoxicity of saponins. To check this hypothesis, an analogue **14** lacking the carbonyl group with a short side

[a] Abbreviations: NA, not assigned; NT, not tested; DEPT, distortionless enhancement by polarization transfer; DQCOSY, double quantum filtered correlated spectroscopy; GHSQC, gradient heteronuclear single quantum coherence; HMBC, heteronuclear multiple-bond correlation; ROESY, rotating-frame Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy.

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 22*S*; (B) isomer 22*R*.

Scheme 4

Scheme 5

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 CCl$_3$C(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 ^{13}C 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 (TSC$_{50}$) 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]
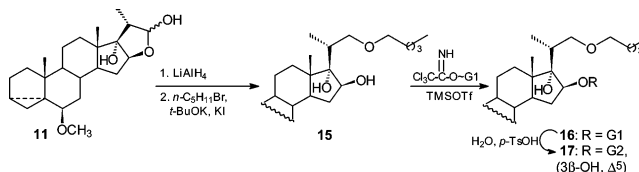
chain was designed. Lactol **11**, readily available from lactone **1** by DIBAL-H reduction, was treated with hydrazine and then with potassium *t*-butoxide in DMSO. The Wolff−Kishner reduction afforded 16β,17α-diol **12**, which was glycosylated with CCl$_3$C(NH)O~G1. Removal of protective groups in **13** with simultaneous cycloreversion resulted in the desired analogue **14** (Scheme 4).

Further studies[22,23] 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 reaction[28]) was subjected to glycosylation with the OSW-1 disaccharide. After routine removal of the protective groups, the desired 23-oxa-22-deoxo-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

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

Table 2. Antitumor Activities of the New OSW-1 Analogues (TCS_{50} in nM) in the Calcein AM Cytotoxicity Assay[a]

	cell line (TCS_{50} in nM)								
compound	CEM	MCF7	K 562	ARN 8	G 361	HeLa	HOS	A 549	NIH 3T3
5e-G2	0.3 ± 0.03	50 ± 2	0.8 ± 0.05	4 ± 0.4	1000 ± 31	8 ± 0.4	40 ± 2	0.5 ± 0.01	>50 000
5a-G2	0.5 ± 0.02	90 ± 4	1.4 ± 0.4	3 ± 0.2	3100 ± 26	12 ± 0.5	140 ± 11	0.9 ± 0.03	>50 000
5d-G2	0.2 ± 0.01	40 ± 1.5	0.7 ± 0.05	0.6 ± 0.05	1400 ± 52	8 ± 0.15	42 ± 6	0.5 ± 0.025	>50 000
14	1300 ± 90	18200 ± 130	5100 ± 73	6800 ± 42	9300 ± 101	6400 ± 87	17400 ± 202	3200 ± 85	>50 000
10	280 ± 10	3400 ± 66	430 ± 17	500 ± 7.5	14400 ± 99	1000 ± 54	11700 ± 150	480 ± 39	>50 000
17	<200	860 ± 32	<400	<400	1900 ± 78	350 ± 25	1800 ± 63	<200	>50 000
5e-G4	200 ± 20	1300 ± 15	430 ± 20	1950 ± 61	2200 ± 91	570 ± 31	4200 ± 92	4200 ± 96	>50 000
5e-G8	1000 ± 80	3500 ± 63	1800 ± 35	1500 ± 27	3500 ± 85	2300 ± 100	7200 ± 103	7200 ± 120	>50 000
daunorubicin	62 ± 10	140 ± 12	230 ± 9	NT[b]	670 ± 20	670 ± 31	330 ± 49	NT[b]	NT[b]

[a] The values shown are the mean $\pm$ SDs obtained in three experiments. Daunorubicin was used as a positive control. [b] NT = not tested.

a Performa II gradient generator unit, WFG, Ultrashims, high stability temperature unit and a 5 mm ^{1}H/^{13}C/^{15}N-PFG triple resonance inverse probe head) spectrometer using CDCl$_3$/C$_5$ND$_5$ solutions with TMS as the internal standard. Only selected signals in the ^{1}H 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 (Merck, Silica gel 60 F$_{254}$).

Compound **1**, glycosyl trichloroacetimidates (CCl$_3$C(NH)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*-butyllithium 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 NH$_4$Cl, and the reaction product was extracted with ether. Evaporation of the solvent from dried (anhydrous MgSO$_4$) extract afforded crude product **2c**, which was purified by silica gel column chromatography.

6β-Methoxy-3α,5-cyclo-27-nor-5α-furostane-17α,22α-diol (2c). Yield 73%; an oily product eluted with hexane−ethyl acetate (77.5: 22.5). IR (CHCl$_3$) 3594, 3469, 1091 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$) δ 4.17 (1 H, t, J = 7.5 Hz), 3.32 (3 H, s), 3.15 (1 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). ^{13}C NMR (50 MHz, CDCl$_3$) δ 111.2 (C), 90.5 (C), 90.3 (CH), 82.0 (CH), 56.4 (CH$_3$), 52.2 (CH), 47.5 (CH), 44.5 (C), 43.3 (C), 42.2 (CH), 37.4 (CH$_2$), 35.01 (C), 34.97 (CH$_2$), 33.2 (CH$_2$), 32.0 (CH$_2$), 30.8 (CH$_2$), 29.9 (CH), 25.5 (CH$_2$), 24.8 (CH$_2$), 22.8 (CH$_2$), 22.2 (CH$_2$), 21.3 (CH), 19.2 (CH$_3$), 17.6 (CH$_3$), 13.9 (CH$_3$), 13.0 (CH$_2$), 8.4 (CH$_3$). EI-MS *m/z* (%) 414 (M − H$_2$O, 21), 385 (47), 382 (9), 269 (100).

Representative Procedure for Glycosylation of Aglycones 2a−2f, 6, 12, and 15 with CCl$_3$C(NH)O-G (G1, G3, G5, G7, or G9). A solution of the glycosyl trichloroacetimidate (CCl$_3$C(NH)O-G1, 0.64 mmol) and steroid aglycone **2c** (0.5 mmol) in dry dichloromethane (15 mL) was stirred with 4 Å 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 CH$_2$Cl$_2$ (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 without full characterization. The crude products of glycosylation of diol **6** (compounds **7** and **8**) were separated and oxidized to the corresponding ketones (vide infra).

6β-Methoxy-16β,17α-dihydroxy-3α,5-cyclo-27-nor-5α-cholestan-22-one 16-O-{O-[2-O-(4-methoxybenzoyl)-3,4-di-O-triethylsilyl-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (4c-G1). Yield 9%; an oily product eluted with benzene−ethyl acetate (98:2). IR (CHCl$_3$) 3467, 1737, 1717, 1607, 1459, 1256, 1101, 1018 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$) δ 8.06 (2 H, d, J = 8.8 Hz), 6.92 (2 H, d, J = 8.8 Hz), 4.92 (1 H, t, J = 5.0 Hz), 4.81 (1 H, m), 4.75 (1 H, m), 4.37 (1 H, br s), 4.23 (1 H, m), 3.95 (1 H, m), 3.88 (3 H, s), 3.63−3.80 (3 H, m), 3.32 (3 H, s), 3.11 (1 H, q, J = 7.3 Hz), 2.76 (1 H, m), 1.95 (3 H, s), 1.13 (3 H, d, J = 7.3 Hz), 1.02 (3 H, s), 0.40 (1 H, dd, J = 8.0, 5.1 Hz).

6β-Methoxy-3α,5-cyclo-27-nor-5α-furostane-17α,22α-diol 22-O-{O-[2-O-(4-methoxybenzoyl)-3,4-di-O-triethylsilyl-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-4-O-triethylsilyl-α-L-arabinopyranoside} (3c-G1). Yield 9%; an oily product eluted with benzene−ethyl acetate (96:4). IR (CHCl$_3$) 3510, 1727, 1607, 1511, 1256, 1098, 1010 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$) δ 7.96 (2 H, d, J = 8.8 Hz), 6.89 (2 H, d, J = 8.8 Hz), 5.33 (1 H, d, J = 3.3 Hz), 4.97−5.03 (2 H, m), 4.68 (1 H, d, J = 6.7 Hz), 4.23 (1 H, t, J = 7.5 Hz), 3.93−4.19 (3 H, m), 3.86 (3 H, s), 3.60−3.82 (3 H, m), 3.55 (1 H, dd, J = 11.8, 3.5 Hz), 3.32 (3 H, s), 3.25 (1 H, m), 2.77 (1H, m,), 2.37 (1 H, q, J = 7.0 Hz), 1.74 (3 H, s). ^{13}C NMR (50 MHz, CDCl$_3$) δ 169.8 (C), 164.5 (C), 163.2 (C), 131.7 (2 × CH), 122.8 (C), 115.1 (C), 113.4 (2 × CH), 102.0 (CH), 91.8 (CH), 90.1 (CH), 89.8 (C), 82.1 (CH). ESI-MS *m/z* (%) 1238.2 (MNa$^+$).

Representative Procedure for the Deprotection of Glycosides. To the solution of a protected glycoside **4c** (0.012 mmol) in a mixture of dioxane (1.5 mL) and water (0.05 mL), *p*-TsOH × H$_2$O (0.002 mmol) was added. The reaction mixture was stirred for 2 h at 80 °C, the solvent was evaporated in vacuo at a temperature below 50 °C, and the residue was chromatographed on a silica gel column.

3β,16β,17α-Trihydroxy-27-norcholest-5-en-22-one 16-O-{O-[2-O-(4-methoxybenzoyl)-β-D-xylopyranosyl]-(1→3)-2-O-acetyl-α-L-arabinopyranoside} (5c-G2). Yield 98%; amorphous solid eluted with ethyl acetate. IR (CHCl$_3$) 3591, 3453, 1728, 1692, 1606, 1512, 1259, 1170, 1033 cm^{-1}. ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.10 (2 H, d, J = 8.7 Hz), 6.99 (2 H, d, J = 8.7 Hz), 5.34 (1 H, m), 4.94 (1 H, dd, J = 8.0, 7.2 Hz), 4.71 (2 H, m), 4.20 (1 H, br s), 4.17 (1 H, br s), 4.14 (1 H, dd, J = 11.6, 4.2 Hz), 3.89 (3 H, s), 3.77 (1 H, m), 3.66−3.73 (2 H, m), 3.38−3.58 (4 H, m), 3.23 (1 H, br s), 2.78 (1 H, br s), 2.67 (1 H, q, J = 7.4 Hz), 1.97 (3 H, s), 1.04 (3 H, s), 1.02 (3 H, d, J = 7.4 Hz), 0.82 (3 H, t, J = 7.0 Hz), 0.80 (3 H, s). ^{13}C NMR (125 MHz, CDCl$_3$) δ 218.9 (C), 170.1 (C), 168.3 (C), 166.4 (C), 142.8 (C), 132.5 (2 × CH), 121.7 (CH), 121.4 (C), 114.3 (2 × CH), 102.4 (CH), 99.4 (CH), 88.7 (CH), 85.8 (C), 80.2 (CH). ESI-MS *m/z* (%) 881.7 (MNa$^+$). Anal. (C$_{46}$H$_{66}$O$_{15}$) C, H.

Procedure for Oxidation of Compounds 7 and 8. To a solution of dihydroxy-compound **7** or **8** (0.008 mmol) in dichloromethane (3 mL), pyridinium dichromate (0.016 mmol) was added, and the reaction mixture was stirred for 2 h at room temperature. The solvent was evaporated in vacuo, and then the crude product was purified by silica gel column chromatography to afford the corresponding ketone (**4e**-G1 or **9**-G1) in a quantitative yield.

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.

(22*S*)-6*β*-Methoxy-17*α*,22-dihydroxy-3*α*,5-cyclo-5*α*-cholestan-16-one 22-*O*-{*O*-[2-*O*-(4-methoxybenzoyl)-3,4-di-*O*-triethylsilyl-*β*-D-xylopyranosyl]-(1→3)-2-*O*-acetyl-4-*O*-triethylsilyl-*α*-L-arabinopyranoside} (9-G1). Product **9**-G1 was eluted with hexane−ethyl acetate (87.5:12.5). IR (CHCl$_3$) 3480, 1753, 1735, 1607, 1511, 1169, 1255. 1094 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$) *δ* 7.97 (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), 3.61−3.71 (3 H, m), 3.40 (1 H, d, *J* = 11.8 Hz), 3.33 (3 H, s), 3.24 (1 H, m), 2.78 (1 H, m), 2.30 (1 H, dd, *J* = 17.2, 6.9 Hz), 1.87 (3 H, s). ^{13}C NMR (50 MHz, CDCl$_3$) *δ* 218.9 (C), 168.9 (C), 164.5 (C), 163.1 (C), 131.9 (2 × CH), 122.9 (C), 113.3 (2 × CH), 101.8 (CH), 101.3 (CH), 83.1 (C), 82.8 (CH), 82.1 (CH). ESI-MS *m/z* (%) 1252.5 (MNa$^+$).

Wolff−Kishner Reduction of Lactol 11.[13] 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 (0.05 mL) was added. The reaction mixture was stirred under reflux for 16 h. Evaporation of the solvent from the reaction mixture afforded crude hydrazone. The product, without further purification, was dissolved in DMSO, and a solution of potassium *t*-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 MgSO$_4$) 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%).

6*β*-Methoxy-3*α*,5-cyclo-20a-homo-5*α*-pregnan-16*β*,17*α*-diol (12). IR (CHCl$_3$) 3510, 3468, 1725, 1092 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$) *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). ^{13}C NMR (50 MHz, CDCl$_3$) *δ* 86.3 (C), 82.2 (CH), 80.8 (CH), 56.4 (CH$_3$), 48.3 (CH), 47.5 (CH), 46.9 (C), 43.2 (C), 35.9 (CH$_2$), 35.1 (C), 34.9 (CH$_2$), 33.2 (CH$_2$), 33.1 (CH$_2$), 30.3 (CH), 28.9 (CH), 24.8 (CH$_2$), 22.0 (CH$_2$), 21.4 (CH), 19.2 (CH$_3$), 18.7 (CH$_3$), 17.0 (CH$_3$), 13.3 (CH$_3$), 13.00 (CH$_2$). ESI-MS *m/z* (%) 747.6 (2MNa$^+$), 385.3 (MNa$^+$).

3.3. NMR and Conformational Analysis of Compound 10. For NMR measurements, compound **10** was dissolved in a mixture of pyridine-*d*$_5$ (D 99.5%, Aldrich, St. Louis, MO) and CDCl$_3$ (D 99.5%, Cambridge Isotope Laboratories, Inc., Andover, MA), 1:1 (v/v), or in CDCl$_3$ to a concentration of 5 mM. A total of 32 K data points were collected, and a spectral width of 6 kHz was used in 1D proton experiments. The 2D experiments were measured using a proton spectral width of 4.5 kHz collecting 2 K data points. TOCSY,[36] DQF-COSY,[37,38] and ROESY[39,40] spectra were measured with 256 increments. A mixing time of 80 ms was used in TOCSY, while 250 ms was used in ROESY measurements. The 2D {^{1}H, ^{13}C} GHSQC[41,42] and HMBC[43] experiments with gradients were performed in proton decoupled mode with a carbon spectral width of 25 kHz and 256 increments. The spectra were calibrated against the residual chemical shift of chloroform in proton spectra (7.26 ppm) and chemical shift of chloroform in ^{13}C spectra (77.0 ppm). Distances between protons were calculated from the volumes of the corresponding cross-peaks from ROESY spectra, and cross-peaks obtained for methylene protons were used for the calibration.

Conformational Analysis. Molecular structures of the 22*S* and 22*R* isomer of **10** were built using the TINKER[44,45] package (version 4.2).

Structure manipulations and analysis (computation of energy, rmsd calculations) were carried out with the set of tools provided with the TINKER package. Molecular dynamics simulations and the minimizations of structures over Cartesian coordinates employed the MM3[46] force field adopted for the TINKER suite, which was included in the standard program distribution. Obtained structures were visualized using the VMD program.[47]

Details of the Simulation Procedure. A single molecular dynamics trajectory at constant temperature was generated. Simulation started from an arbitrarily chosen conformer of the molecule of interest. The Berendsen bath coupling[48] method, with a time constant of 0.1 ps, was used to maintain constant temperature (1000 K) during the simulation.

The molecular dynamics trajectory was updated using a modified Beeman method[49] to integrate the Newtonian equations of motion. The time interval for the dynamics steps was set to 1 fs, and the total length of the simulated trajectory reached 1 ns. From the generated trajectory, 500 structures, equally spaced in time, were collected, with the interval between snapshots set to 2 ps. Each structure from the generated ensemble was then subjected to molecular dynamics simulated annealing computation. During a 2 ps simulation, the temperature changed between the starting value of 1000 K to the final value of 100 K. The linear scaling protocol was used.

Finally, all structures were minimized in Cartesian coordinate space using limited memory LM-BFGS[50,51] nonlinear optimization, with the value of the rms termination criterion set to 0.01 kcal/mol/Å. The value of the dielectric constant in all calculations was 1.5; the default value for the MM3 force field. Because all experimental measurements were conducted in media of low polarizability, this choice of dielectric constant seems to be justified. The final set of 500 minimized structures was then further analyzed.

3.4. Biological Tests. The ARN 8 cancer cell line was kindly provided by Dr. J. P. Blaydes (University of Dundee, Scotland). Other cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). The screening cell lines (T-lymphoblastic leukemia cell line CEM; breast carcinoma cell line MCF7, lung carcinoma cell line K 562, chronic myelogenous leukemia cell line K 562, epitheloid carcinoma cell line HeLa, melanoma cell line ARN 8, malignant melanoma cell line G 361, osteosarcoma cell line HOS, and mouse fibroblast NIH 3T3) were cultured in DMEM medium (Gibco BRL) supplemented with 10% fetal calf serum, 4 mM glutamine, 100 U/mL penicillin, and 100 *μ*g/mL streptomycin, at 37 °C in a fully humidified atmosphere containing 5% CO$_2$. Cell suspensions with approximately 1.25 × 10^5 cells/mL were distributed in 96-well microtiter plates, and after 3 h of stabilization, the tested OSW-1 analogues were added in 20 *μ*L aliquots in dimethylsulfoxide (DMSO) in serial (usually 6- and 4-fold) dilutions to the microtiter plate wells. Control cultures were treated with DMSO alone, and the final concentration of DMSO in the reaction mixture never exceeded 0.6%. In routine testing, the highest well concentration was 50 *μ*M, but for some analogues, this varied. After 72 h of culture, the cells were incubated with Calcein AM solution (Molecular Probes) for 1 h. The fluorescence of viable cells was quantified using a Fluoroscan Ascent fluorometer (Microsystems). The tumor cell survival (TCS) rate was calculated using the following equation: TCS = (OD$_{\text{drug−exposed well}}$/mean OD$_{\text{control wells}}$) × 100%. The TCS$_{50}$ value, the drug concentration lethal to 50% of the tumor cells, was calculated from the obtained dose−response curves.

Supporting Information Available: Synthetic procedures, spectral data for all new compounds (IR, ^{1}H NMR, ^{13}C NMR, MS), a set of spectra of compound **10** (^{1}H NMR, ^{13}C NMR, DEPT, DQCOSY, GHSQC, TOCSY, ROESY, IR, MS), and elemental analyses of all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Perry, M. C. *The chemotherapy sourcebook*; Williams & Wilkins: Baltimore, MD, 1992.

(2) Mukherjee, A. K.; Basu, S.; Sarkar, N.; Ghosh, A. C. Advances in cancer therapy with plant based natural products. *Curr. Med. Chem.* **2001**, *8*, 1467−1486.

(3) Petit, G. R.; Inoue, M.; Kamano, Y.; Herald, D. L.; Arm, C.; Dufresne, C.; Christie, N. D.; Schmidt, J. M.; Doubek, D. L.; Krupa, T. S. Isolation and structure of the powerful cell growth inhibitor cephalostatin 1. *J. Am. Chem. Soc.* **1988**, *110*, 2006−2007.

(4) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. Ritterazine A, a highly cytotoxic dimeric steroidal alkaloid, from the tunicate *Ritterella tokioka*. *J. Org. Chem.* **1994**, *59*, 6164−6166.

(5) Fan, W. Possible mechanisms of poclitaxel-induced apoptosis. *Biochem. Pharmacol.* **1999**, *57*, 1215−1221.

(6) Reddy, L.; Odhav, B.; Bhoola, K. D. Natural products for cancer prevention: A global perspective. *Pharmacol. Ther.* **2003**, *99*, 1−13.

(7) Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. Acylated cholestane glycosides from the bulbs of *Ornithogalum saundersiae*. *Phytochemistry* **1992**, *31*, 3969−3973.

(8) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Suita, K.; Beutler, J. A. Cholestane glycosides with potent cytostatic activities on various tumor cells from *Ornithogalum saundersiae* bulbs. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633−636.

(9) Mimaki, Y. Structures and biological activities of plant glycosides: cholestane glycosides from *Ornithogalum saundersiae, O. thyrsoides and Galtonia candicans*, and their cytotoxic and antitumor activities. *Nat. Prod. Commun.* **2006**, *1*, 247−253.

(10) Gryszkiewicz-Wojtkielewicz, A.; Jastrzębska, I.; Morzycki, J. W.; Romanowska, D. B. Approaches towards the synthesis of cephalostatins, ritterazines and saponins from *Ornithogalum saundersiae*— New natural products with cytostatic activity. *Curr. Org. Chem.* **2003**, *7*, 1257−1277.

(11) Zhou, Y.; Garcia-Prieto, C.; Carney, D. A.; Xu, R.; Pelicano, H.; Kang, Y.; Yu, W.; Lou, C.; Kondo, S.; Liu, J.; Harris, D. M.; Estrov, Z.; Keating, M. J.; Jin, Z.; Huang, P. OSW-1: A natural compound with potent anticancer activity and a novel mechanism of action. *J. Natl. Cancer Inst.* **2005**, *97*, 1781−1785.

(12) Guo, C.; Fuchs, P. L. The first synthesis of the aglycone of the potent anti-tumor steroidal saponin OSW-1. *Tetrahedron Lett.* **1998**, *39*, 1099−1102.

(13) Morzycki, J. W.; Gryszkiewicz, A. Synthesis of the potent antitumor saponin OSW-1 aglycone. *Pol. J. Chem.* **2001**, *75*, 983−989.

(14) Yu, W.; Jin, Z. A new strategy for the stereoselective introduction of steroid side chain via α-alkoxy vinyl cuprates: Total synthesis of highly potent antitumor natural product OSW-1. *J. Am. Chem. Soc.* **2001**, *123*, 3369−3370.

(15) Xu, Q.; Peng, X.; Tian, W. A new strategy for synthesizing the steroids with side chains from steroidal sapogenins: Synthesis of the aglycone of OSW-1 by using the intact skeleton of diosgenin. *Tetrahedron Lett.* **2003**, *44*, 9375−9377.

(16) Deng, S.; Yu, B.; Lou, Y.; Hui, Y. First total synthesis of an exceptionally potent antitumor saponin, OSW-1. *J. Org. Chem.* **1999**, *64*, 202−208.

(17) Morzycki, J. W.; Wojtkielewicz, A. Synthesis of a cholestane glycoside OSW-1 with potent cytostatic activity. *Carbohydr. Res.* **2002**, *337*, 1269−1274.

(18) Yu, W.; Jin, Z. The total synthesis of the natural product OSW-1. *J. Am. Chem. Soc.* **2002**, *124*, 6576−6583.

(19) Shi, B.; Wu, H.; Yu, B.; Wu, J. 23-Oxa-analogues of OSW-1: Efficient synthesis and extremely potent antitumor activity. *Angew. Chem., Int. Ed.* **2004**, *43*, 4324−4327.

(20) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. Synthesis of OSW-1 analogues and a dimer and their antitumor activities. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2153−2156.

(21) Deng, L.; Wu, H.; Yu, B.; Jiang, M.; Wu, J. Synthesis of OSW-1 analogs with modified side chains and their antitumor activities. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2781−2785.

(22) Shi, B.; Wu, H.; Yu, B.; Wu, J. 23-Oxa-analogues of OSW-1: Efficient synthesis and extremely potent antitumor activity. *Angew. Chem., Int. Ed.* **2004**, *43*, 4324−4327.

(23) Shi, B.; Tang, P.; Hu, X.; Liu, X. O.; Yu, B. OSW Saponins: Facile synthesis toward a new type of structures with potent antitumor activities. *J. Org. Chem.* **2005**, *70*, 10354−10367.

(24) Qin, J.; Tian, W.; Lin, W. A highly efficient synthesis of 22-deoxy-OSW-1 by utilizing the intact skeleton of diosgenin. *Tetrahedron Lett.* **2006**, *47*, 3217−3219.

(25) Morzycki, J. W.; Wojtkielewicz, A. Synthesis of a highly potent antitumor saponin OSW-1 and its analogues. *Phytochem. Rev.* **2005**, *4*, 259−277.

(26) Tang, P.; Mamdani, F.; Hu, X.; Liu, J. O.; Yu, B. Synthesis of OSW saponin analogs with modified sugar residues and their antiproliferative activities. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1003−1007.

(27) Morzycki, J. W.; Wojtkielewicz, A.; Wołczyński, S. Synthesis of analogues of a potent antitumor saponin OSW-1. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3323−3326.

(28) Kruszewska, A.; Wilczewska, A. Z.; Wojtkielewicz, A.; Morzycki, J. W. Synthesis of 23-oxa-22-deoxo analoues of OSW-1 aglycone. *Pol. J. Chem.* **2006**, *80*, 611−615.

(29) Morzycki, J. W.; Łotowski, Z.; Siergiejczyk, L.; Lipkowski, J.; Tabaszewska, A.; Wójcik, J. Structure of 3β-hydroxy-16-oxo-24-nor-17-azachol-5-eno-23-nitrile and its 20S epimer. *Steroids* **1995**, *60*, 195−203.

(30) Soliman, H. S. M.; Simon, A.; Tóth, G.; Duddeck, H. Identification and structure determination of four triterpene saponins from some middle-east plants. *Magn. Reson. Chem.* **2001**, *39*, 567−576.

(31) Akihisha, T.; Kimura, Y.; Tai, T. Arai, K. Astertarone B, a hydroxy-triterpenoid ketone from the roots of *Aster tataricus* L. *Chem. Pharm. Bull.* **1999**, *47*, 1161−1163.

(32) Kapou, A.; Fousteris, M. A.; Nikolaropoulos, S. S.; Zervou, M.; Grdadolnik, S. S.; Zoumpoulakis, P.; Kyrikou, I.; Mavromoustakos, T. 2D NMR and conformational analysis of a prototype antitumor steroidal ester. *J. Pharm. Biomed. Anal.* **2005**, *38*, 428−434.

(33) Neuhaus, D.; Williamson, M. P. The steady-state NOE in rigid multispin systems. *The nuclear Overhauser effect in structural and conformational analysis*, 2nd ed.; John Wiley & Sons: New York, 2000.

(34) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzębska, I. Some reactions of 16α,17α-oxido-steroids: A study related to the synthesis of the potent antitumor saponin OSW-1 aglycone. *Tetrahedron Lett.* **2000**, *41*, 3751−3754.

(35) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzębska, I. Neighboring group participation in epoxide ring cleavage in reactions of some 16α,-17α-oxidosteroids with lithium hydroperoxide. *Tetrahedron* **2001**, *57*, 2185−2193.

(36) Bax, A.; Davis, D. G. MLEV-17 based two-dimensional homonuclear magnetization transfer spectroscopy. *J. Magn. Reson.* **1985**, *65*, 355−360.

(37) Piantini, U.; Sørensen, O. W.; Ernst, R. R. Multiple quantum filters for elucidating NMR coupling networks. *J. Am. Chem. Soc.* **1982**, *104*, 6800−6801.

(38) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. Improved spectral resolution in COSY H-1-NMR spectra of proteins via double quantum filtering. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479−485.

(39) Bothner-By, A. A.; Stephens, R. L.; Lee, J.-M.; Warren, C. D.; Jeanloz, R. W. Structure determination of a tetrasaccharide: Transient nuclear Overhauser effects in the rotating frame. *J. Am. Chem. Soc.* **1984**, *106*, 811−813.

(40) Bax, A.; Davis, D. G. Practical aspects of two-dimensional transverse NOE spectroscopy. *J. Magn. Reson.* **1985**, *63*, 207−213.

(41) Palmer, A. G., III; Cavanagh, J.; Wright, P. E. Sensitivity improvement in proton-detected twodimensional heteronuclear correlation NMR spectroscopy. *J. Magn. Reson.* **1991**, *93*, 151−170.

(42) Kay, L. E.; Keifer, P.; Saarinen, T. Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity. *J. Am. Chem. Soc.* **1992**, *114*, 10663−10665.

(43) Bax, A.; Summers, M. F. ^{1}H and ^{13}C assignments from sensitivity-enhanced detection of heteronuclear multiple-bond connectivity by 2D multiple quantum NMR. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094.

(44) Ren, P.; Ponder, J. W. Polarizable atomic multipole water model for molecular mechanics simulation. *J. Phys. Chem. B* **2003**, *107*, 5933−5947.

(45) Ren, P.; Ponder, J. W. Consistent treatment of inter- and intramolecular polarization in molecular mechanics calculations. *J. Comput. Chem.* **2002**, *23*, 1497−1506.

(46) Allinger, N. L.; Yuh, Y. H.; Lii, J.-H. Molecular mechanics. The MM3 force field for hydrocarbons. *J. Am. Chem. Soc.* **1989**, *111*, 8551−8566.

(47) Humphrey, W.; Dalke, A.; Schulten, K. VMD−Visual molecular dynamics. *J. Mol. Graphics* **1996**, *14*, 33−38.

(48) Berendsen, H. J. C.; Postma, J. P. M.; van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* **1984**, *81*, 3684−3690.

(49) Beeman, D. Some multistep methods for use in molecular dynamics calculations. *J. Comput. Phys.* **1976**, *20*, 130−139.

(50) Nocedal, J.; Wright, S. J. *Numerical Optimization*; Springer-Verlag: New York, 1999.

(51) Nocedal, J. Updating quasi-Newton matrices with limited storage. *Math. Comp.* **1980**, *35*, 773−782.