

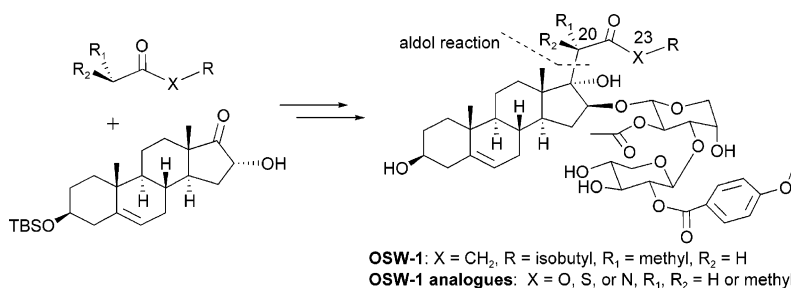
OSW Saponins: Facile Synthesis toward a New Type of Structures with Potent Antitumor Activities

Bingfeng Shi,[†] Pingping Tang,[†] Xiaoyi Hu,[‡] Jun O. Liu,[‡] and Biao Yu^{*,†}

State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China, and Departments of Pharmacology and Neuroscience, Johns Hopkins School of Medicine, 725 North Wolfe Street, Baltimore, Maryland 21205

byu@mail.sioc.ac.cn

Received July 23, 2005



OSW saponins, featuring a 16 β ,17 α -dihydroxycholest-22-one aglycon and an acylated β -D-xylopyranosyl-(1 \rightarrow 3)- α -L-arabinopyranosyl residue attached to the 16-hydroxyl group, have recently been discovered from a group of lily plants, which show potent antitumor activities with a novel mechanism of action. This paper describes an aldol approach to the stereoselective construction of the 16 α ,17 α -dihydroxycholest-22-one structure from 16 α -hydroxy-5-androsten-17-ones and propionates. Elaboration of the aldol adducts toward OSW-1, involving installation of the isoamyl ketone side chain, inversion of the 16-hydroxyl configuration, and selective protection of the C22-oxy function, has been explored and accomplished. In particular, the present route was found convenient for the synthesis of OSW saponin analogues with a C22-ester side chain. Thus, the 23-oxa-analogue of OSW-1 (**40**) was prepared starting from the industrial dehydroisoandrosterone (**1**) in a linear eight-step sequence and in 26% overall yield. Analogues with a variety of modified side chains were prepared, via aldol condensation with propionates of varying length, thiopropionate, and acetate (for preparation of **68–75**) or via aminolysis of the 22,16-lactone **26** (for preparation of the 23-*N*-analogues). Cross metathesis (CM) reaction was also found feasible for modification at the final stage from C22-allyl ester **70**. Valuable structure–activity relationships (SAR), together with the practical synthetic approach, have thus been provided to set a new stage for further studies on this new type of antitumor structures.

Introduction

In 1992, Sashida, Mimaki, and co-workers¹ reported a new type of cholestane glycosides from the bulbs of *Ornithogalum saundersiae*, a perennial garden plant of the lily family cultivated in southern Africa. These saponins, featuring a novel 16 β ,17 α -dihydroxycholest-22-one agly-

con and an acylated β -D-xylopyranosyl-(1 \rightarrow 3)- α -L-arabinopyranosyl residue attached to the 16-hydroxyl group (Figure 1), were found, in 1995, to possess extremely potent cytotoxicity toward leukemia HL-60 cells with IC₅₀ values ranging between 0.1 and 0.3 nM.² The major constituent, namely, OSW-1, was then tested against the NCI (U.S. National Cancer Institute) 60 cell lines,

* Corresponding author: fax (0086)-21-64166128.

[†] Shanghai Institute of Organic Chemistry.

[‡] Johns Hopkins School of Medicine.

(1) Kubo, S.; Mimaki, Y.; Terao, M.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1992**, *31*, 3969.

(2) (a) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Sashida, Y.; Hirano, T.; Oka, K.; Maekawa, R.; Wada, T.; Sugita, K.; Beutler, J. A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 633. (b) Rouhi, A. M. *Chem. Eng. News* **1995**, September 11, 28.

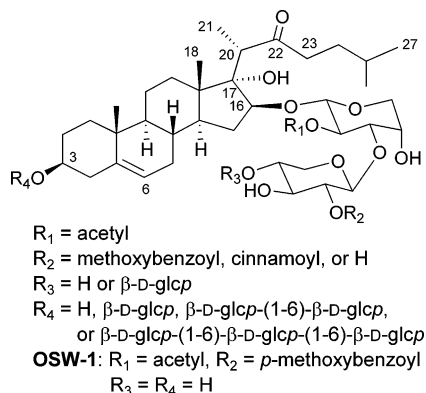


FIGURE 1. Naturally occurring OSW saponins.

showing a mean IC_{50} of 0.78 nM, which is 10–100 times more potent than those of the clinically applied anticancer agents such as mitomycin C, adriamycin, cisplatin, camptothecin, and taxol. Moreover, OSW-1 demonstrated little toxicity toward normal human pulmonary cells in vitro and prolonged the life span of mice injected with the P388 leukemia cell line by 59% via a single administration of 10 $\mu\text{g}/\text{kg}$.^{2a} Also intriguing is that OSW-1 showed equal potency against tumor cells resistant to other anticancer agents and displayed a characteristic antitumor profile that is only comparable to those of the cephalostatins.^{2a,3} These results imply a new anticancer mechanism underlying the action of this new type of natural products.

Continuous search in *O. saundersiae* and its taxonomically related plants, for example, *Ornithogalum thyrsoides* and *Galtonia candicans*, has led to the identification of some 20 more OSW saponins (Figure 1).⁴ Thus a preliminary set of SAR (structure–activity relationship) information has been obtained: (1) The acyl groups on the disaccharide segment (R_1 and R_2) are crucial to the antitumor activity of the molecules; lack of the R_2 methoxybenzoyl or (*E*)-cinnamoyl substitution reduces the activity by ~ 100 fold, while removal of both R_1 (acetyl) and R_2 reduces the activity by ~ 1000 -fold. (2) An additional β -D-glucopyranosyl residue (R_3) on the disaccharide reduces the antitumor activity by ~ 100 fold. (3) Presence of a β -D-glucopyranosyl residue at the 3-OH (R_4) does not affect the activity; but a di- or trisaccharide R_4 substitution diminishes the activity by more than 1000-fold.

Synthetic efforts have been made toward OSW saponins since the discovery of their potent antitumor activities.^{5–9} Notably, Guo and Fuchs⁵ achieved the first synthesis of the $16\beta,17\alpha$ -dihydroxycholest-22-one aglycon in 1998. They assembled the side chain onto 5-androsten-

3β -ol-17-one via a sequence of Wittig olefination and ene reaction and then introduced the $16\beta,17\alpha$ -diol by dihydroxylation (of the $16,17$ -ene with 1 equiv of OsO_4) followed by inversion of the resulting 16α -OH. Shortly thereafter, we succeeded in coupling of a suitably protected aglycon with a disaccharide trichloroacetimidate to complete the first total synthesis of OSW-1.⁶ Yu and Jin⁷ have then improved the synthesis by introducing the side chain via a 1,4-addition of a $17(20)$ -en-1-one with an α -alkoxy vinyl cuprate, followed by the elaboration of the 17α -OH by oxidation of the resulting $16,17$ -enolate with Davis reagent. Morzycki et al.⁸ realized an intramolecular ring opening of the $16\alpha,17\alpha$ -epoxide by the C22-carbonyl function to start further elaboration.

Although these developments have allowed access to the natural OSW-1 saponin and its analogues, the existing syntheses are time-consuming and require subtle control of the reaction conditions for some of the transformations. As a result, only a limited number of OSW saponin analogues have been obtained by means of chemical synthesis for SAR studies. Nevertheless, several key structural elements in OSW saponins essential for their activity have been identified. It was shown that either the aglycon analogues¹⁰ or the disaccharide derivatives (bearing disparate steroidal aglycons)¹¹ were nearly inactive toward tumor cells. The C16-epimer of OSW-1 was also inactive.¹² In contrast, saturation of the C5–C6 double bond¹³ and dimerization with a terephthalic acid at the 3-OH did not affect significantly the antitumor activity.¹² And the C17-side chain can tolerate modification, for example, reduction of the C22-carbonyl function into CH_2 or removal of the terminal CH_3 , without apparently changing the antitumor potency.¹⁴

Thus, a stage has been set for altering the nature of the synthetic challenge from total synthesis of the natural compounds or random preparation of their analogues to the preparation of a variety of targeted analogues and derivatives to extend the SAR profile and to study the mechanism of action of OSW saponins. Another goal is to find a lead compound that could be prepared in multigram quantities for toxicological and pharmacokinetic studies. In fact, we have recently developed an efficient aldol approach to the construction of the $16\alpha,17\alpha$ -dihydroxycholest-22-one structure, which can be converted to the 23-oxa analogues of OSW saponins conveniently. And importantly, these C17 side-chain-modified 23-oxa analogues were found to be as potent as the parent natural products against the growth of tumor cells.¹⁵ Herein, we report a comprehensive account on this matter.

(3) For the GI_{50} , TGI, and LC_{50} values of OSW-1 against the NCI 60 cell-line tumor panel, see the Supporting Information in ref 4c.

(4) (a) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Hasegawa, F.; Sashida, Y. *J. Nat. Prod.* **2002**, *65*, 1417. (b) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y. *Chem. Pharm. Bull.* **2001**, *49*, 1042. (c) Kuroda, M.; Mimaki, Y.; Yokosuka, A.; Sashida, Y.; Beutler, J. A. *J. Nat. Prod.* **2001**, *64*, 88.

(5) Guo, C.; Fuchs, P. L. *Tetrahedron Lett.* **1998**, *39*, 1099.

(6) Deng, S.; Yu, B.; Lou, Y.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 202.

(7) (a) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2001**, *123*, 3369. (b) Yu, W.; Jin, Z. *J. Am. Chem. Soc.* **2002**, *124*, 6576.

(8) (a) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzebska, I. *Tetrahedron Lett.* **2000**, *41*, 3751. (b) Morzycki, J. W.; Wojtkielewicz, A. *Carbohydr. Res.* **2002**, *337*, 1269.

(9) For other efforts toward the synthesis of OSW saponins, see (a) Morzycki, J. W.; Gryszkiewicz, A. *Pol. J. Chem.* **2001**, *75*, 983. (b) Morzycki, J. W.; Gryszkiewicz, A.; Jastrzebska, I. *Tetrahedron* **2001**, *57*, 2185. (c) Morzycki, J. W.; Wojtkielewicz, A.; Gryszkiewicz, A.; Wolczynski, S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3323. (d) Xu, Q.; Peng, X.; Tian, W. *Tetrahedron Lett.* **2003**, *44*, 9375.

(10) Guo, C.; LaCour, T. G.; Fuchs, P. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 419.

(11) (a) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Carbohydr. Res.* **2000**, *329*, 495. (b) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Carbohydr. Res.* **2001**, *334*, 159.

(12) Ma, X.; Yu, B.; Hui, Y.; Miao, Z.; Ding, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2153.

(13) Deng, L.; Wu, H.; Yu, B.; Jiang, M.; Wu, J. *Chin. J. Chem.* **2004**, *22*, 994.

(14) Deng, L.; Wu, H.; Yu, B.; Jiang, M.; Wu, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2781.

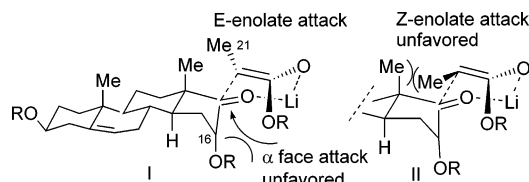


FIGURE 2. Proposed stereochemistry in the aldol approach to the construction of the cholestane 20(*S*)-16 α ,17 α -diol structure.

Results and Discussion

Aldol Approach to the Cholestane 16 α ,17 α -Dihydroxy-22-one Structure. A major challenge in the synthesis of OSW saponins has been the construction of the 16 β ,17 α -dihydroxycholest-22-one structure.^{5–9} When this synthetic target is envisioned to be a α -methyl- β , γ -diol-one, an aldol condensation between an α -hydroxyketone and a propionate could be the direct approach.¹⁶ Then the challenge lies in the stereo control of the aldol reaction to place the 21-methyl and 17-hydroxyl groups in *S*(C20) and α orientations, respectively. We anticipated that condensation with a 16 α -hydroxy-17-ketone steroidal substrate could lead to the formation of the desired stereochemistry at C-17 and C-20, considering the 16 α -OR group could force the approach of the enolate from the β face via intermediate I (Figure 2) in a nonchelation model,¹⁷ and the chair transition state (in a Zimmerman–Traxler model)^{17a} could prefer the *E*- to the *Z*-enolate to avoid the interaction of the 18-methyl group and the methyl of the propionate enolate (intermediate II). Although it has long been known that zinc enolates of propionates under the Reformatsky conditions approached the 16 α -acetoxy-17-oxo-androstanes from the α face,¹⁸ encouraging results have been provided by Doller and Gros¹⁹ that lithium enolate of *tert*-butyl propionate proceeded by a β -face attack.

The required 16 α -hydroxy-5-androsten-17-one derivatives **5** and **6**, with their 3-OH being protected with a robust TBDPS (*tert*-butyldiphenylsilyl) and a TBS (*tert*-butyldimethylsilyl) ether, respectively, were readily prepared from the industrial acetate **1** following modification of a literature procedure (Figure 3).²⁰ Thus, bromination of ketone **1** with 3 equiv of CuBr₂ in methanol under reflux provided 16 α -bromide **2** in 91% yield, with the 3-*O*-acetyl group being fully cleaved. Protection of the 3-OH with TBDPSCl or TBSCl in the presence of imidazole in *N,N*-dimethylformamide (DMF) gave **3** and **4**. Epimerization between the 16 α -bromide and its 16 β isomer took place readily under alkaline conditions, where they were subjected to S_N2 displacement with hydroxy ion, leading stereospecifically (from the 16 β -bromides only) to the 16 α -ols **5** and **6** in 95% yields. The reported ketol rearrange-

ment (leading to the corresponding 17 β -hydroxy-16-oxo steroids) was not detected.²⁰ Blocking the 16 α -OH of **5** with TBS ether provided **7**.

We first examined the aldol condensation of ketones **5–7** with lithium *E*-enolate of ethyl propionate, which was generated with solid lithium diisopropylamide (LDA) in 23% hexamethylphosphoric triamide (HMPA)–tetrahydrofuran (THF) via the method of Ireland (conditions A, Figure 4),²¹ where the highest percentage of the *E* isomer resulted from rapid addition of a slight excess (1.05–1.10 equiv) of the ester neat to a solution of the base.^{21b,c,22} As expected, only the 17 α -hydroxy(silyloxy)-20*S*- products **8a**, **9a**, and **10a** were isolated (in 41%, 63%, and 43% yield, respectively) (entries 1–3). Blocking the neighboring 16 α -OH was proven unnecessary for the present aldol condensation. For steric reasons the trans-fused five-membered 20,16-lactone could not be produced.^{18,23} Without control of the exclusive generation of the *E*-enolate of ethyl propionate (conditions B, without use of hexane-free LDA), reaction of ketone **5** provided the desired 20*S* product **8a** in a comparable 41% yield, but its 20*R* isomer **8b** was also isolated in 29% yield (entry 4). However, reaction of the 3-*O*-TBS-17-ketone **6** under similar conditions in the absence of HMPA (conditions C) afforded the desired **9a** in a satisfactory 75% yield, with its 20*R* isomer **9b** being isolated in only 12% yield (entry 5). Interestingly, under the relatively convenient conditions C, condensation of **6** with isobutyl and dodecyl propionate provided the desired 17 α -hydroxy-20*S* adducts **11a** and **12a** in good yields (78% and 81% yield, respectively); their 20*R* products were not isolated (entries 6 and 7). The predominant formation of the 20*S* isomers under conditions B and C could be explained by the following rationale: upon addition of a trapping agent such as excess esters, a kinetic resolution process led through preferential destruction of the (*Z*)-lithium enolate to a relative increase of the amount of the (*E*)-lithium enolate formed;^{21b,24} and with the *E*-enolate preferred in the aldol reaction, the thermodynamic equilibrium for (*E*)- to (*Z*)-enolate was expedited. The increased dominance of the *E*-enolates in the reaction conditions due to the increased size of the isobutyl and dodecyl group (compared to that of the ethyl group) was somewhat responsible for the latter results.²⁵

The C17 ester chain was assigned as β -oriented in the aldol adducts **8a–12a**, that is, trans to the 16 α -OH, because otherwise the corresponding 16,22- γ -lactone would be readily formed in the presence of mineral acids.¹⁸ Direct evidence was provided by the ready formation of the 16,17-*O*-acetonide **13** from diol **8a** (Figure 5).

At this stage, however, the stereochemistry at C20 in the aldol adducts could not be established definitely. Analysis of the chair transition state (Figure 2) and

(15) Shi, B.; Wu, H.; Yu, B.; Wu, J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4324.

(16) Reetz, M. T. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 556.

(17) (a) Zimmerman, H. E.; Traxler, M. D. *J. Am. Chem. Soc.* **1957**, *79*, 1920. (b) Kleschick, W. A.; Buse, C. T.; Heathcock, C. H. *J. Am. Chem. Soc.* **1977**, *99*, 247. (c) Evans, D. A. *Top. Stereochem.* **1982**, *13*, 1.

(18) Mazur, Y.; Danieli, N.; Sondheimer, F. *J. Am. Chem. Soc.* **1960**, *82*, 5889.

(19) Doller, D.; Gros, E. G. *Synth. Commun.* **1990**, *20*, 3115.

(20) Numazawa, M.; Nagaoka, M.; Osawa, Y. *J. Org. Chem.* **1982**, *47*, 4024.

(21) (a) Ireland, R. E.; Mueller, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 2891. (b) Ireland, R. E.; Wipf, P.; Armstrong, J. D., III *J. Org. Chem.* **1991**, *56*, 650. (c) Oare, D. A.; Heathcock, C. H. *J. Org. Chem.* **1990**, *55*, 157.

(22) Corey, E. J.; Gross, A. W. *Tetrahedron Lett.* **1984**, *25*, 495.

(23) Grigsby, W. E.; Hind, J.; Chanley, J.; Westheimer, F. H. *J. Am. Chem. Soc.* **1942**, *64*, 2606.

(24) Heathcock, C. H. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: Orlando, FL, 1984; Vol. 3, p 111 and references therein.

(25) Otera, J.; Fujita, Y.; Fukuzumi, S. *Synlett* **1994**, 213.

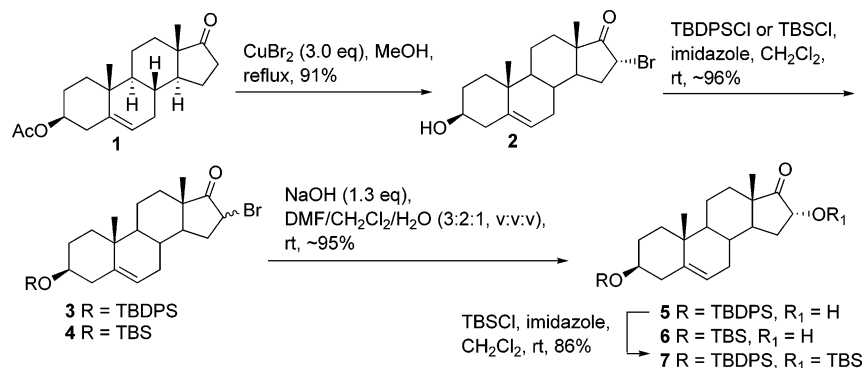
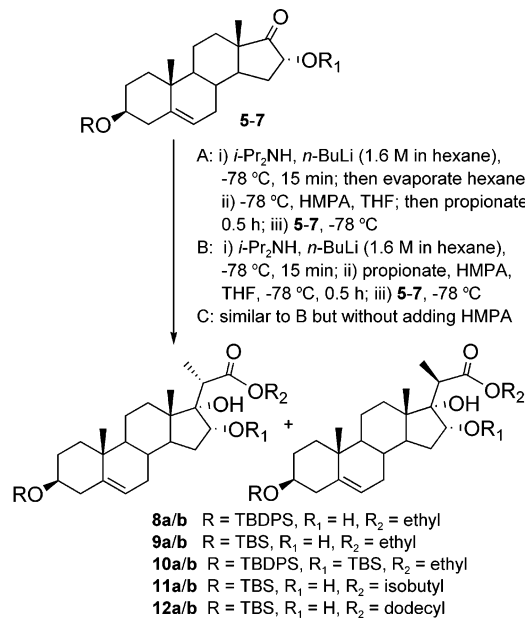


FIGURE 3. Introduction of the 16 α -OH.



Entry	Ketone	Propionate	Conditions	Product (yield)
1	5	ethyl	A	8a (41%), 8b (0)
2	6	ethyl	A	9a (63%), 9b (0)
3	7	ethyl	A	10a (43%), 10b (0)
4	5	ethyl	B	8a (41%), 8b (29%)
5	6	ethyl	C	9a (75%), 9b (12%)
6	6	isobutyl	C	11a (78%), 11b (0)
7	6	dodecyl	C	12a (81%), 12b (0)

FIGURE 4. Aldol condensation with propionate enolates.

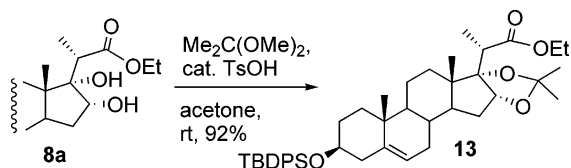


FIGURE 5. Formation of the 16,17-O-acetonide 13.

inspection of the literature results on addition of steroidal 17-ones with ethyl α -bromo(iodo)-propionates under Reformatsky conditions concluded favorably to the forma-

tion of the natural 20S isomers as the major products.^{18,26} These assignments were later confirmed unambiguously by NOE correlation and X-ray diffraction analysis of the transformed compounds. Upon comparison of the ¹H NMR spectra of the 20S and 20R isomers, the 21-methyl signal of the 20S isomers was observed at a relatively lower field, that is, 1.26 and 1.29 ppm, respectively, for the 21-methyl protons in the 20S isomers 8a and 9a versus 1.30 and 1.32 ppm in the 20R isomers 8b and 9b. A similar chemical shift differentiation has been found in steroids unsaturated at the 16(17) position,²⁷ while an opposite trend was found in steroids saturated at the 16(17) position.²⁸

Elaboration of the Cholestane Isoamyl Ketone Side Chain. Starting from the 22-esters 8a and 10a, we first tried to install the isoamyl residue on the cholestane side chain via addition to the corresponding Weinreb amides.²⁹ Unexpectedly, preparation of the desired Weinreb amides from the 22-esters was found to be difficult (Figure 6). Under the action of Et₂AlCl (conditions A; AlMe₃³⁰ or Me₂AlCl³¹ is usually used, but these reagents were not accessible to us), reaction of ester 10a with Me-(MeO)NH \cdot HCl did not produce the desired amide; instead, the 21-methyl epimerized compound 10b was isolated as the only product (53%, entry 1). Ester 8a remained intact under similar conditions. We then examined Merck's conditions with $i\text{-PrMgCl}$ as a promoter (conditions B) for the amide formation.³² Modified conditions with an excess amount of isoamylmagnesium chloride instead (conditions C) were also examined; thus an in situ addition to the newly formed Weinreb amide might take place to provide the desired cholestane directly.³² Ester 10a with its 16 α -OH being blocked was inert toward these conditions. Fortunately, the 16 α -OH unprotected 8a was readily converted into amide 14 in 75% yield in the presence of $i\text{-PrMgCl}$ (entry 4).

(26) (a) Oka, K.; Hara, S. *J. Org. Chem.* **1978**, *43*, 4408. (b) Lardon, A.; Reichstein, T. *Helv. Chim. Acta* **1941**, *24*, 1127. (c) Hey, D. H.; Honeyman, J.; Peal, W. *J. Chem. Soc.* **1954**, 185, 2648.

(27) (a) Marino, J. P.; Abe, H. *J. Am. Chem. Soc.* **1981**, *103*, 2907. (b) Schmuff, N. R.; Trost, B. M. *J. Org. Chem.* **1983**, *48*, 1404.

(28) (a) Piatak, D. M.; Wicha, J. *Chem. Rev.* **1978**, *78*, 199. (b) Ibuka, T.; Taga, T. *J. Org. Chem.* **1988**, *53*, 3947. (c) Nes, W. R.; Varkey, T. E.; Krevitz, K. *J. Am. Chem. Soc.* **1977**, *99*, 260.

(29) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.

(30) (a) Basha, A.; Lipton, M.; Weinreb, S. M. *Tetrahedron Lett.* **1977**, *18*, 4171. (b) Levin, J. I.; Turos, E.; Weinreb, S. M. *Synth. Commun.* **1982**, *12*, 989.

(31) Shimizu, T.; Osako, K.; Nakata, T. *Tetrahedron Lett.* **1997**, *38*, 2685.

(32) Williams, J. M.; Jobson, R. B.; Yasuda, N.; Marchesini, G. Dolling, U.-.; Grabowski, E. J. *J. Tetrahedron Lett.* **1995**, *36*, 5461.

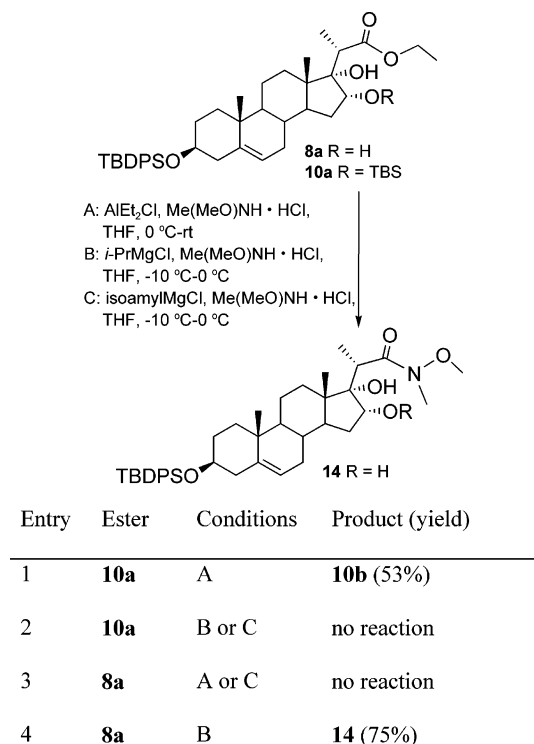


FIGURE 6. Preparation of the Weinreb amides.

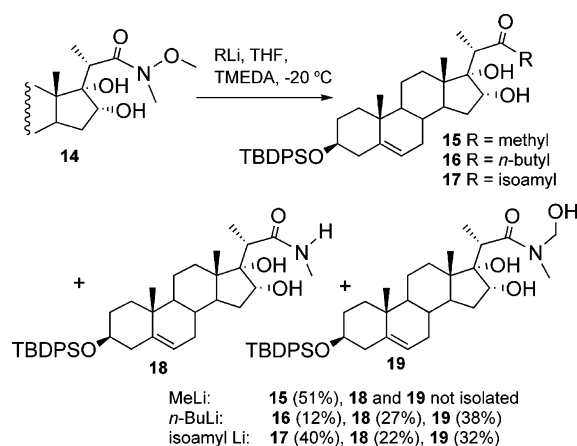


FIGURE 7. Alkylation of Weinreb amide **14**.

These experimental results for the preparation of Weinreb amides implied that the C22-carbonyl carbon in the 16 α ,17 α -dihydroxy steroidal structure was sterically very hindered. Therefore, it was not surprising that alkylation of 22-amide **14** with Grignard reagents, that is, isoamylmagnesium chloride, did not proceed at all. Then we tried substitution with alkyl lithium reagents (Figure 7). Alkylation of amide **14** with MeLi in the presence of TMEDA in THF at $-10\text{ }^{\circ}\text{C}$ afforded the desired methyl ketone **15** as the major product (51%). When the bulkier *n*-BuLi was employed, the corresponding butyl ketone **16** was obtained in only 12% yield, while considerable amounts of the amide derivatives **18** and **19** were produced (in 27% and 38% yield, respectively). The reaction with isoamyl lithium afforded the ketone product **17** in a decent 40% yield, although **18** and **19** were also isolated in comparable amounts (22% and 32% yield, respectively). These results proved the steric

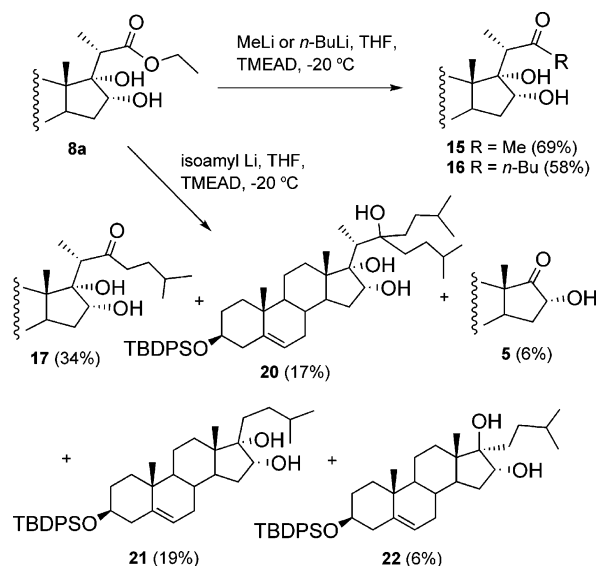


FIGURE 8. Alkylation of 22-ethyl ester **8a**.

hindrance at the C22-carbonyl carbon of Weinreb amide **14**. The production of the demethoxylation derivatives (e.g., **18** and **19**) from Weinreb amides in the presence of a strong base has been previously observed.^{33,34} An E₂ pathway was postulated; thus the basicity of the alkylation agents prevailed to effect abstraction of a proton from the *N*-methoxy group, leading to amide derivatives such as **18** and **19**. It should also be noted that the above alkylation reactions had to be quenched by pouring into a cold 5% HCl solution, otherwise the corresponding retro-aldol product **5** was produced.

In view of the hindrance of the C22-carbonyl function in the above experiments, we rationalized that alkylation of the 22-esters (instead of the bulkier Weinreb amides) would proceed much more easily, and overaddition to the ketone products could not take place at all. Thus similar conditions as described above for alkylation of the Weinreb amide **14** were then applied to ethyl ester **8a** (Figure 8). Encouragingly, reaction of ester **8a** with MeLi and *n*-BuLi gave the corresponding ketones **15** and **16** in good yields (69% and 58%, respectively). However, alkylation with isoamyl lithium led to the isoamyl ketone **17** in only moderate yield (34%). Byproducts were then carefully isolated and identified to explain the alkylation processes. Overalkylation on ketone **17** took place, providing 22-ol **20** (17%). Retro-aldol reaction of the substrate ester **8a** also proceeded, leading to ketone **5**. Alkylation of **5** afforded a pair of the 17 α / β -ol isomers **21** (19%) and **22** (6%). The presence of **21** and **22** demonstrated that the retro-aldol reaction took place before the reaction was quenched. It was also noticed that the β -face addition of the lithium reagent onto the 16 α -hydroxy-17-one **5** was favored over the α -face addition, leading to a **21**:**22** ratio of \sim 3:1; while the previous aldol addition with lithium enolate of ethyl propionates effected exclusive β -face addition.

We have also attempted to attach the isoamyl residue via umpolung of the C22-carbonyl carbon (Figure 9).

(33) Graham, S. L.; Scholz, T. H. *Tetrahedron Lett.* **1990**, *31*, 6269.
 (34) (a) Sibi, M. P.; Sharma, R.; Paulson, K. L. *Tetrahedron Lett.* **1992**, *33*, 1941. (b) Sibi, M. P.; Marvin, M.; Sharma, R. *J. Org. Chem.* **1995**, *60*, 5016.

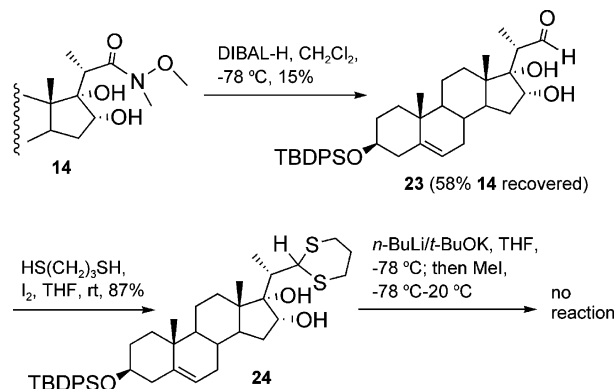


FIGURE 9. Attempt to extend the steroidal side chain via an unpolung approach.

Thus, the Weinreb amide **14** at hand was subjected to reduction with 4 equiv of diisobutylaluminum hydride (DIBAL-H) at $-78\text{ }^{\circ}\text{C}$ to produce the 22-aldehyde **23**. Again, the C22-carbonyl was resistant to reaction, leading to **23** in no more than 15% yield, with $\sim 58\%$ of the starting amide being recovered. Aldehyde **23** was then readily transformed into 1,3-dithiane **24** in 87% yield. Unfortunately, generation of the α -thioacetal anion with super base $n\text{-BuLi}/t\text{-BuOK}$,³⁵ followed by quenching with methyl iodide, did not give any product. Yu and Jin^{7b} have previously demonstrated that generation of the α -anion of a $16\beta,17\alpha$ -hydroxy-22-*S,S'*-diphenyl acetal was effected by $n\text{-BuLi}/t\text{-BuOK}$, but substitution of the anion onto methyl iodide was not successful.

Completion of the Total Synthesis of OSW-1. Reversion of the configuration of the 16α -hydroxyl group on the cholestane $16\alpha,17\alpha$ -dihydroxy-22-one structure required a sequence of oxidation (into the 16-ketone) and reduction,^{5,6} thus the C22-carbonyl group needed to be blocked first. In fact, protection of the C22-carbonyl on **17** with an ethylene glycol would furnish a formal total synthesis of OSW-1.⁶ Again, such an effort on the C22-carbonyl was proven futile. In comparison to the previous synthesis, where a $16,17$ -ene-22-one was readily converted into its C22-ketal, the presence of the $16\alpha,17\alpha$ -dihydroxyl groups (in **17**) should be the reason causing the inertness of the C22-carbonyl group.

We noticed that preferential benzoylation^{5b} and silylation^{9a} on the 22-OH could be achieved on a cholestane $16\beta,17\alpha,22$ -triol. Thus, starting from the 3-*O*-TBS-22-ester **9a**, we attempted to reverse the configuration of the 16α -hydroxyl group before introducing the isoamyl residue on the side chain (Figure 10). Treatment of diol **9a** with TPAP (tetrapropylammonium perruthenate) and NMO (*N*-methylmorpholine *N*-oxide) in the presence of 4 Å molecular sieve gave C16-ketone **25** in 93% yield.³⁶ The present protocol has recently been proven superior to Swern oxidation for oxidation of the steroidal 16α -OH.¹⁴ Reduction of ketone **25** with $\text{NaBH}_4/\text{CeCl}_3$ led stereoselectively to $16\beta,17\alpha$ -diol **27**. However, when this reaction was quenched at room temperature with addition of methanol and water, only the intramolecular lactonization product **26** could be isolated (92%). This

required us to quench the reaction at low temperature (-40 to $-78\text{ }^{\circ}\text{C}$) to obtain the desired diol **27** (76%). Both lactone **26** and ester **27** could be converted into hemiketal **28** quantitatively upon addition of isoamyllithium. However, an attempt to reduce hemiketal **28** into $16,17,22$ -triol **30** with LiAlH_4 (or HLiBEt_3) in THF led only to the furan derivative **29** formed via elimination of two molecules of water.^{5,9b} Fortunately, this problem was solved by replacement of the THF solvent with Et_2O .^{9a} Thus, reduction of **28** with LiAlH_4 in Et_2O afforded triol **30** in a good 87% yield.

In line with our expectation, treatment of triol **30** with 3 equiv of AZMBCl [2-(azidomethyl)benzoyl chloride] in the presence of 4-(*N,N*-dimethylamino)pyridine (DMAP) in THF afforded the 22-*O*-AZMB product **31** mainly in 48% yield.³⁷ The 16β -*O*-AZMB product **32** was isolated in 7% yield, and the corresponding $16,22$ -di-*O*-AZMB product was not isolated (Figure 11). Glycosylation of $16\beta,17\alpha$ -diol **31** with the disaccharide trichloroacetimidate **33** by use of TMSOTf as a promoter in the presence of 4 Å molecular sieve at $-20\text{ }^{\circ}\text{C}$ afforded the desired glycoside **34** in 61% yield.⁶ Then, the 22-*O*-AZMB group was selectively removed with PBU_3 , providing 22-ol **35** in a good 82% yield. Subsequent oxidation of the resulting 22-OH with PDC afforded ketone **36** in 97% yield. Finally, the silyl groups on **36** were removed with $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ in acetone–water, furnishing OSW-1 in 82% yield.

Synthesis of OSW Saponin Analogues with Modified Side Chains. We have recently found that the C17-side chain of OSW saponins was tolerant toward certain modifications without a significantly effect on the anti-tumor potency of the compounds.¹⁴ The present approach to the synthesis of the steroidal $16\beta,17\alpha$ -hydroxyl-22-ester structure, for example, **27**, could allow us to prepare very easily those OSW saponin analogues with a variety of modifications on the side chain. Our first target was the 23-oxa analogue of OSW-1 **40** (Figure 12), which has a C22-isobutyl ester in place of the isoamyl ketone in OSW-1. This minimal modification, that is, replacement of the CH_2 with an oxygen at the 23 position, would lead to a clear implication on SAR. Thus, a new stage would be set for further SAR study via analogue synthesis. Starting from the aldol condensation product **11a**, preparation of the desired 23-oxa-OSW-1 **40** was straightforward, involving only four steps of reaction that have already been examined in the synthesis of OSW-1 (Figure 11). Thus, the 16α -OH on **11a** was reversed following the sequence of oxidation with TPAP/NMO and reduction with $\text{NaBH}_4/\text{CeCl}_3$, giving 16β -ol **38**. Glycosylation of $16\beta,17\alpha$ -diol **38** with disaccharide trichloroacetimidate **33** under the action of TMSOTf provided glycoside **39**. Final removal of the silyl groups with $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ led to the desired **40**. Remarkably, such a synthetic approach to the 23-oxa-analogue of OSW-1 **40** requires only eight linear steps (in 26% overall yield) starting from the industrial isoandrosterone **1**.

As expected, the readily synthesized 23-oxa analogue **40** showed strong activity against the growth of tumor cells (Table 1). Therefore, we set about our synthesis of

(35) Schlosser, M.; Strunk, S. *Tetrahedron Lett.* **1984**, *25*, 741.

(36) Paquette, L. A.; Wang, H.; Zhao, M. *J. Am. Chem. Soc.* **1998**, *120*, 5213.

(37) (a) Wada, T.; Ohkubo, A.; Mochizuki, A.; Sekine, M. *Tetrahedron Lett.* **2001**, *42*, 1069. (b) Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 8165.

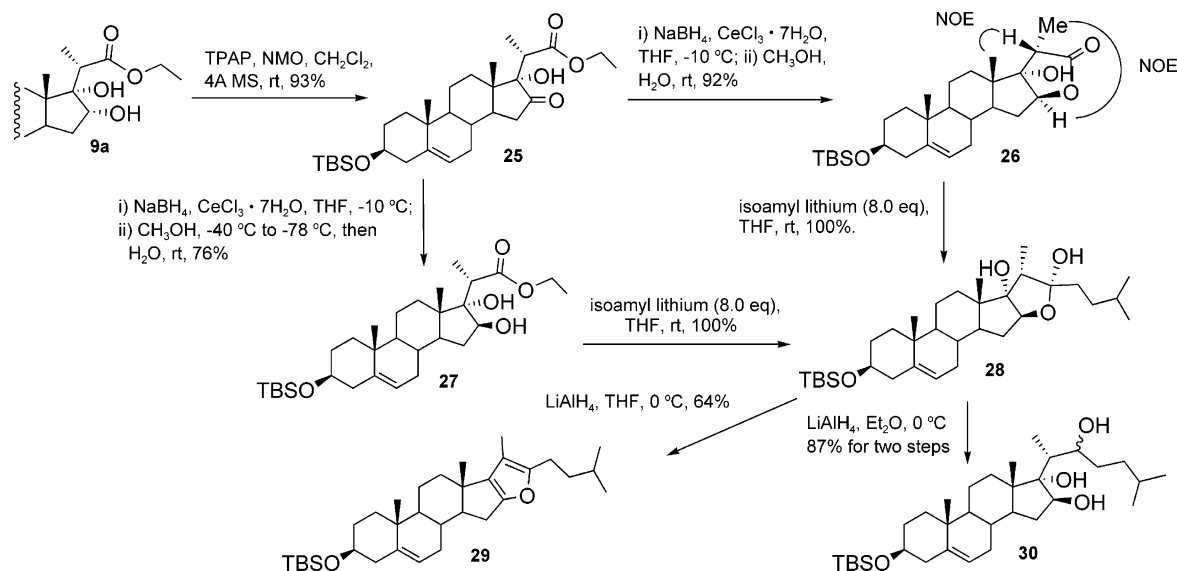


FIGURE 10. Conversion of the 16 α -hydroxyl configuration.

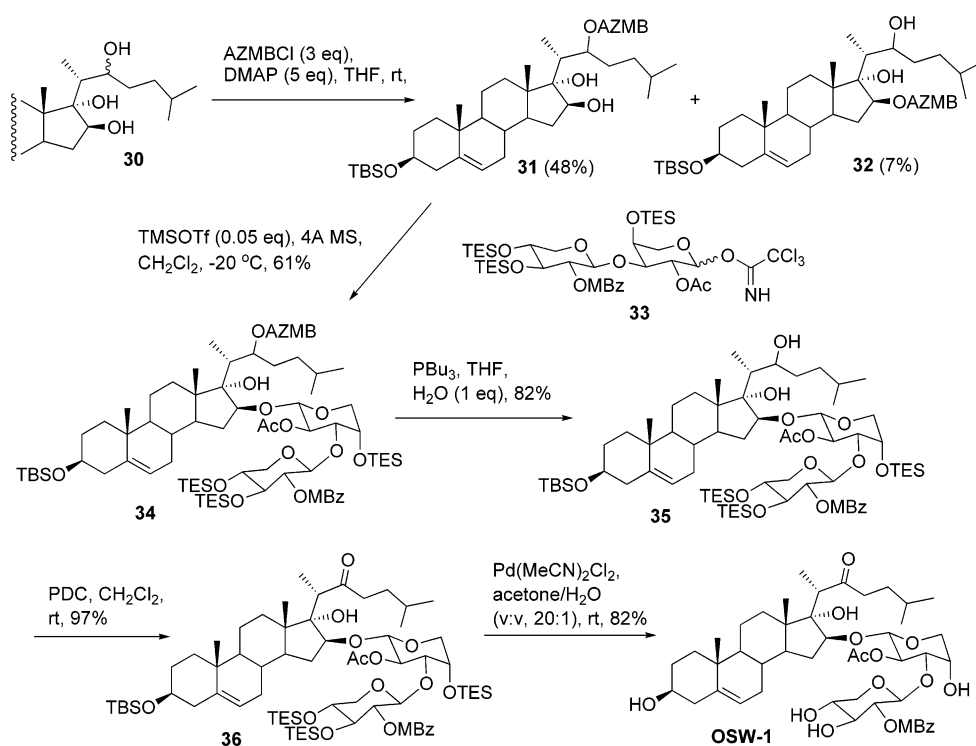


FIGURE 11. Completion of the total synthesis of OSW-1.

a variety of the analogues based on the structure of **40** to decipher the influence of the side chain on the antitumor activity of the OSW saponins (Figure 13).

Employing the aldol condensation of 3-*O*-TBS-16 α -hydroxy-5-androsten-17-one **6** with ethyl, isobutyl, and dodecyl propionates, we have already obtained the C22-ethyl ester **9a** and its 20*R* epimer **9b**, isobutyl ester **11a**, and dodecyl ester **12a** (Figure 4). Under similar conditions, aldol condensation of **6** with allyl, heptyl, and octadecyl propionates provided the corresponding esters **41–43**, which were applied directly to oxidation with TPAP/NMO, affording ketones **47**, **48**, and **50**, respectively (Figure 13). At this stage, the corresponding 20*R* epimers resulting from the aldol condensation could be

easily separated by silica gel column chromatograph. Allyl and heptyl esters **47** and **48** were so obtained in good yields (55% and 60% yield, respectively, for two steps); their 20*R* epimers were isolated in 8% and 5% yields, respectively. However, the longer octadecyl ester **50** was obtained in only a moderate yield of 32%; while its 20*R* epimer was obtained in 22% yield. This might be due to the poor solubility of the octadecyl propionate in the aldol reaction at low temperature. Detection of octadecyl alcohol in the reaction mixture indicated that esterolysis might be another reason. It should also be mentioned that once we scaled up the aldol condensation of **6** with dodecyl propionate at a 2.0 g scale, after oxidation, the desired 20*S* ester **49** was obtained in 51%

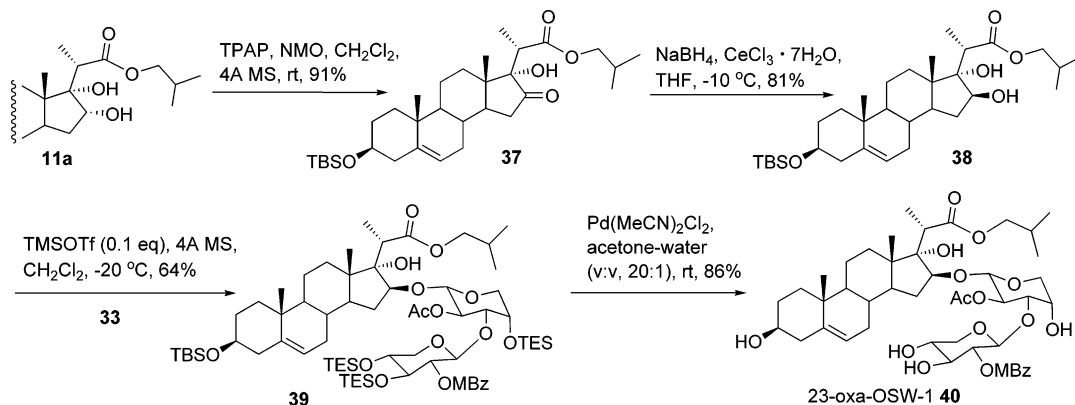


FIGURE 12. Synthesis of 23-oxa-OSW-1 40.

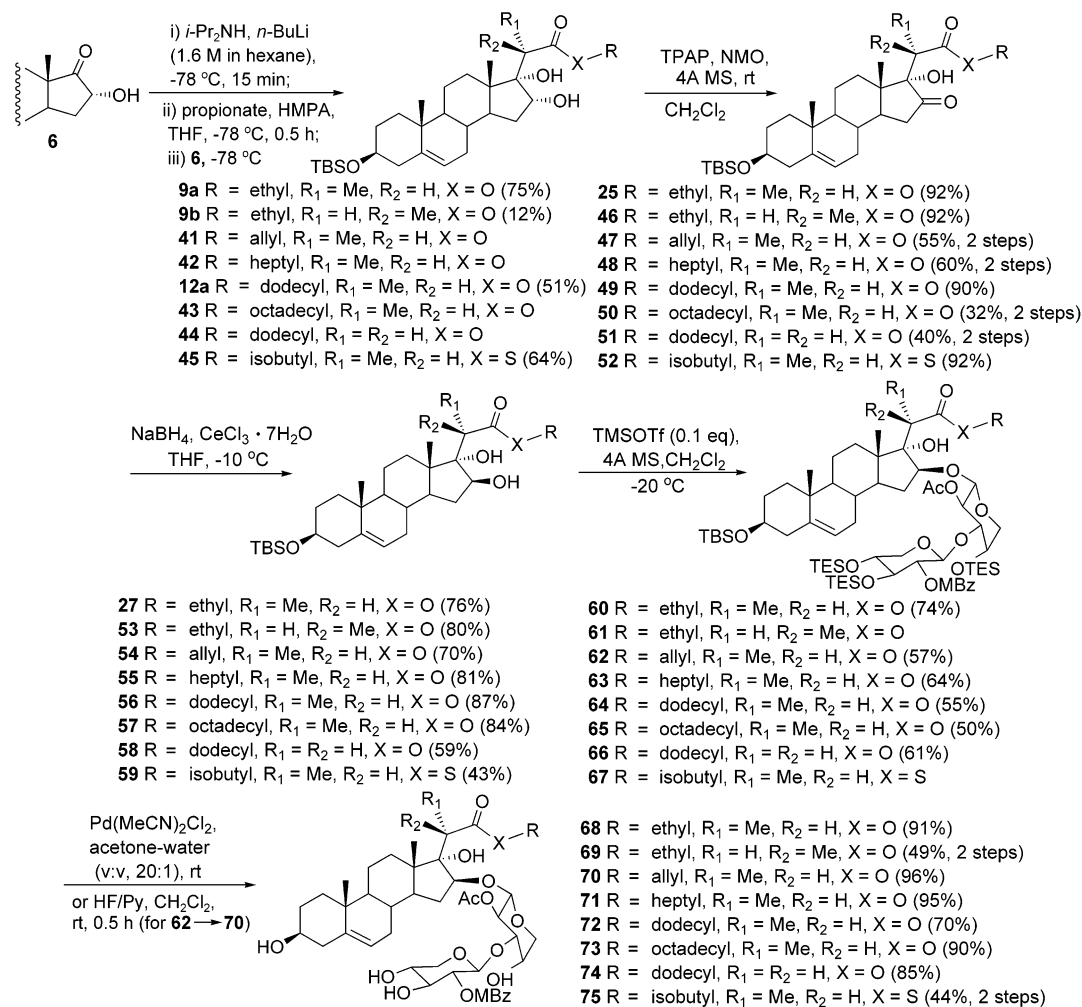


FIGURE 13. Synthesis of OSW saponin analogues with modified side chains.

yield, while its 20R isomer was also isolated in 10% yield; although a previous small-scale reaction (~50 mg) afforded the 20S isomer **12a** in 81% yield, without detection of the 20R isomer **12b** (Figure 4, entry 7). The oxidation step with TPAP/NMO converted the 16 α -OH into 16-ketone in >90% yields, which did not affect the stereochemistry of the 21-methyl group (i.e., **9a/b** \rightarrow **25/46**, 92%; **12a** \rightarrow **49**, 90%; **45** \rightarrow **52**, 92%). To obtain an analogue in absence of the 21-methyl group (i.e., **74**), aldol condensation of **6** with dodecyl acetate was carried out, after

oxidation, to lead to ketone **51** (40%). In addition, aldol condensation of **6** with isobutyl thiopropionate was also performed to give thioester **45** (64%), which would be converted into the 23-S-analogue of OSW-1 (i.e., **75**).

By transformations similar to those already described in the synthesis of the 23-O-analogue of OSW-1 **40** (Figure 12), that is, reduction with NaBH₄/CeCl₃ (**25** \rightarrow **27**, **46**–**52** \rightarrow **53**–**59**), glycosylation with disaccharide imidate **33** (**27** and **53**–**59** \rightarrow **60**–**67**), and removal of silyl protection (**60**–**67** \rightarrow **68**–**75**), the desired analogues **68**–

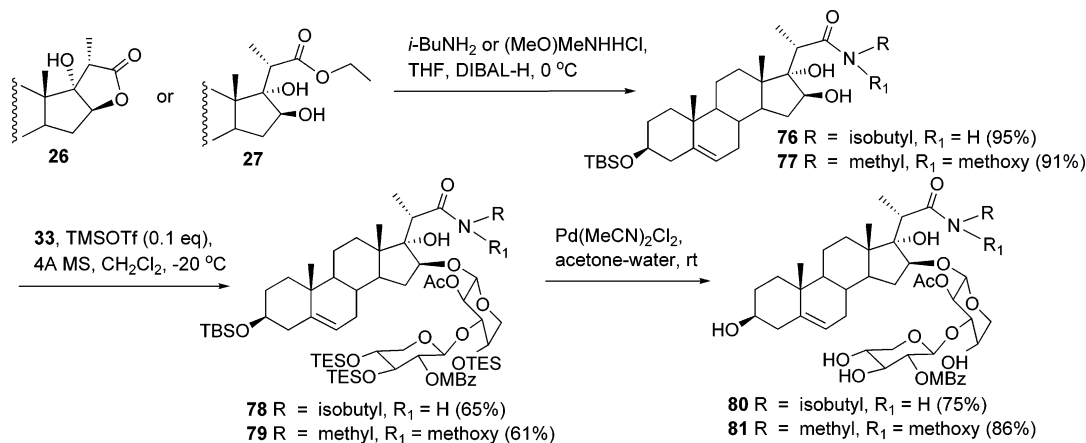


FIGURE 14. Synthesis of 23-*N*-analogues of OSW saponins.

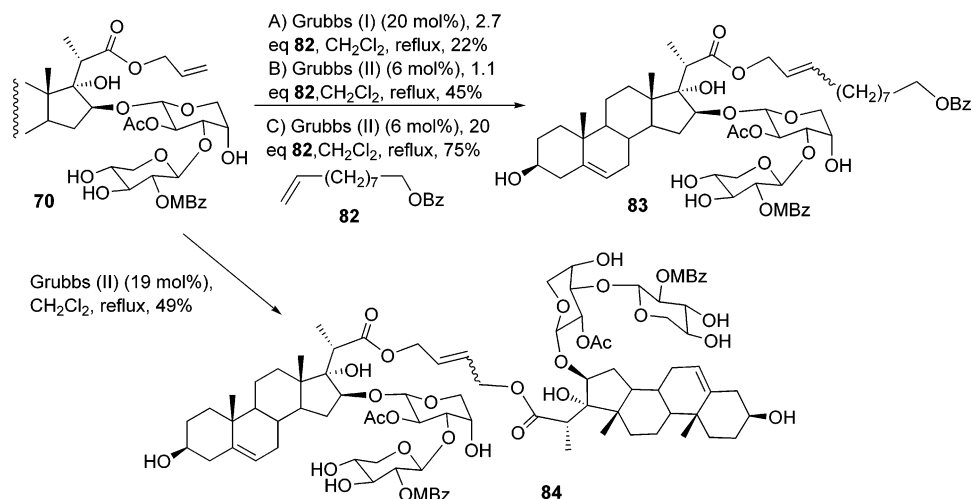


FIGURE 15. CM approach to the modification of the side chain of OSW saponin analogues.

75 were obtained conveniently (Figure 13). Among these transformations, it is worth noting that reduction of the C16-carbonyl group on C22-thioester **52** led to a considerable amount of the lactonization product **26** even upon careful quenching at low temperature. For reduction of ketone **51**, which lacks the 21-methyl group as compared to the other substrates, the desired 16 β -ol **58** was obtained in only 59% yield, while the corresponding 16 α -ol **44** was isolated in 21% yield.

Utilizing the lactone byproduct **26**, we also prepared the 23-*N*-analogues **80** and **81** of OSW saponins (Figure 14). A recently developed aminolysis protocol was successfully applied;³⁸ thus, treatment of lactone **26** with *i*-BuNH₂ or (MeO)MeNH in the presence of DIBAL-H at 0 °C provided isobutyl amide **76** and Weinreb amide **77** in excellent yields. Under similar conditions, ethyl ester **27** could also be converted into amides **76** and **77** in excellent yields.

Recalling the previous difficulty encountered in transferring 16 α ,17 α -dihydroxyl-22-ethyl ester **8a** into the corresponding Weinreb amide **14** (Figure 5), we reexamined this transformation under the present aminolysis conditions. In contrast to 16 β -22-ethyl ester **27**, 16 α -22-ethyl ester **8a** did not react at all. This result showed

clearly that the 16-hydroxyl configuration has a dramatic impact, steric in origin, on the reactivity of the C22-carbonyl function.

16 β ,17 α -Diols **76** and **77** were then subjected to glycosylation with disaccharide imidate **33** to provide glycosides **78** and **79**. Final removal of the silyl groups afforded the desired 23-*N*-analogues **80** and **81**.

The above synthetic approach to OSW saponin analogues is very convenient. However, preparation of each analogue with a different side chain requires starting from the aldol condensation of the 16 α -hydroxy-5-androsten-17-one **6** with different esters; the subsequent four steps of transformation are repetitive. It would be ideal to modify the side chain at the final step. Such a task could be realized by cross metathesis (CM) reaction on a multifunctional molecule such as OSW-1.³⁹ Thus, we tested the CM reaction with 22-allyl ester **70** (Figure 15). Gratifyingly, treatment of allyl ester **70** with 9-decen-1-yl benzoate **82**⁴⁰ (2.7 equiv) in the presence of the Grubbs(I) catalyst (20 mol %) in refluxing CH₂Cl₂ provided the corresponding coupling product **83** in 22% yield.

(39) For a recent review on cross metathesis, see Connon, S. J.; Blechert, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 1900.

(40) Blackwell, H. E.; O'Leary, D. J.; Chatterjee, A. K.; Washenfelder, R. A.; Bussmann, D. A.; Grubbs, R. H. *J. Am. Chem. Soc.* **2000**, *122*, 58.

(38) Huang, P.; Zheng, X.; Deng, X. *Tetrahedron Lett.* **2001**, *42*, 9039.

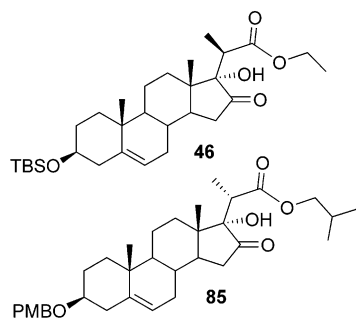


FIGURE 16. Compounds **46** and **85** for X-ray diffraction analysis.

This yield was increased to 45% when Grubbs(II) catalyst (6 mol %) was used with the amount of 9-decen-1-yl benzoate **82** was lowered to 1.1 equiv. When the amount of 9-decen-1-yl benzoate **82** was increased to 20 equiv,⁴¹ the yield of **83** was increased to 75% with Grubbs(II) catalyst (6 mol %). In the absence of the external olefin reagent, refluxing of **70** in CH_2Cl_2 in the presence of the Grubbs(II) catalyst (19 mol %) provided the dimeric compound **84** in 49% yield.

Confirmation of the Stereochemistry in the Steroidal Aglycon Synthesis. The ready formation of the 16,22- γ -lactone **26** (Figure 10) after inversion of the configuration of 16 α -OH (of **25**) implied the β orientation of the C17 side chain, which was installed by the previous aldol condensation. In addition, NOE interactions between the α -oriented 16-H and the 21-methyl protons and between the β -oriented 20-H and the 18-methyl protons were observed on this conformation-fixed lactone (Figure 10), confirming the *S* configuration at C-20 generated by the same aldol reaction.

The previously assigned stereochemistry on the aldol adducts was confirmed unambiguously by X-ray diffraction analysis of the 16-ketone derivatives **46**¹⁵ and **85** (Figure 16).⁴² The 20*R* ketone **46** was obtained from the minor aldol product **9b** by TPAP/NMO oxidation (Figure 13). The 20*S* ketone **85** was readily synthesized from 16-bromo-5-androsten-17-one **2** via blocking of the 3-OH with PMB group followed by a similar transformation as described for **4** \rightarrow **6** \rightarrow **11** \rightarrow **37**. Interestingly, it is revealed that the C17 side chains on the 20*R* and 20*S* steroids (e.g., **46** and **85**) adopt dramatically different trajectories in crystals. This might help to explain the good stereo differentiation in the present aldol condensation and previous Reformatsky addition with steroidal 17-ketones.

Antitumor Activities of the OSW Saponin Analogues with Modified Side Chains. The *in vitro* activities of the synthetic OSW-1 and its side-chain-modified analogues (**40**, **68–75**, **80**, **81**, **83**, and **84**) against the proliferation of several human cancer cell lines including HeLa, Jurkat T cells, and human MCF-7 breast cancer cell line were determined by following the incorporation of [³H]thymidine.⁴³ The results are summarized in Table 1.

(41) Chatterjee, A. K.; Choi, T. L.; Sanders, D. P.; Grubbs, R. H. *J. Am. Chem. Soc.* **2003**, *125*, 11360.

(42) For the X-ray diffraction analysis data, see Supporting Information.

(43) Zhang, Y.; Griffith, E. C.; Sage, J.; Jacks, T.; Liu, J. O. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6427.

The three cell lines tested exhibited distinct sensitivity to OSW-1, with Jurkat T cells being most sensitive and MCF-7 least sensitive (Table 1). Moreover, the three cell lines have different IC_{50} profiles for the various side-chain-modified OSW-1 analogues. The isosteres of OSW-1 with its 23- CH_2 group replaced by S or NH group (compounds **75** and **80**) showed similar antiproliferative potency toward HeLa and Jurkat T cells. However, the same analogues suffered from an over 20-fold decrease in potency in MCF-7 cells. For the C23 oxygen isostere (**40**), it is slightly more potent in Jurkat T cells but is over 10-fold less potent in either HeLa or MCF-7 cells. A variety of the C22-ester changes, that is, from ethyl (in **68**), allyl (in **70**), heptyl (in **71**), dodecyl (in **72**), to the 9-decen-1-yl benzoate (in **83**), did not affect dramatically their antitumor activities, except for **68** and **71**, toward MCF-7 cells with a significant decrease in activity. A longer C-17 side chain, that is, octadecyl residue (in **73**), abolished the cellular activity; and branching at the 23 position (in **81**) significantly lowered the potency for Jurkat and MCF-7 cell lines without much change in its activity for HeLa cells. The 21-methyl group has a significant yet intriguing impact on the cellular activities of the compounds. The pair of 20*S* and 20*R* isomers **68** and **69** showed opposite selectivity for the inhibition of Jurkat T in comparison to HeLa and MCF-7 cells. Whereas the 20*S* isomer **68** is more potent than its epimeric counterpart **69** in Jurkat T cells, it is significantly less active than **69** in both HeLa and MCF-7 cells. The importance of the 21-methyl group was underscored by the significant reduction in potency of **74** in comparison with its congener **72** against all three cell lines. The activity of the dimer **84** was significantly lower than that of the corresponding monomer **70** in Jurkat and MCF-7 cells but was comparable to **70** in HeLa cells.

Summary

OSW saponins, featuring a 16 β ,17 α -dihydroxycholesterol-22-one aglycon and an acylated β -D-xylopyranosyl-(1 \rightarrow 3)- α -L-arabinopyranosyl residue attached to the 16-hydroxyl group (Figure 1), represent a new structural class of natural products with extremely potent antitumor activities and a new type of mechanism of action. We found that aldol condensation of the readily available 16 α -hydroxy-5-androsten-17-ones (Figure 3) and propionates provided the desired 20(*S*)-16 α ,17 α -dihydroxy steroidal structure (Figures 4 and 13) in a stereocontrolled manner in accordance with the proposed mechanism (Figure 2). The X-ray diffraction analysis of a pair of the 20*R*-16-ketone derivatives **46** and 20*S*-**85** confirmed unambiguously the stereochemistry in the present synthesis and revealed clearly a dramatic conformational difference adopted by the steroidal 20*S* and 20*R* isomers.

Elaboration of the aldol adducts toward OSW-1 has been extensively explored. Unexpectedly, the steroidal C22 carbonyl or hydroxyl function in the presence of the 16 α ,17 α -dihydroxy groups was found to react sluggishly; for example, aminolysis of the 22-esters (Figure 6), alkylation (Figure 7) and reduction (Figure 9) of 22-Weinreb amide **14**, alkylation of 22-ester **8a** (Figure 8), and umpolung alkylation of the 22-dithiane **24** (Figure 9). In sharp contrast, after inversion of the 16 α -hydroxyl configuration, the corresponding steroidal C22-carbonyl

TABLE 1. Antitumor Activities of the Synthetic OSW-1 and Its Side Chain Modified Analogues

Compounds	C17 side chain	IC ₅₀ (μM)		
		HeLa	JurKat T	MCF-7
OSW-1		0.012	0.0022	0.094
40		0.23	0.00068	0.9
68		0.24	0.0053	>10
69		0.034	0.042	0.13
70		0.065	0.0027	0.1
71		0.002	0.0014	2.6
72		0.0033	0.0073	0.14
73		>10	>10	>10
74		10	0.073	1.4
75		0.0013	0.0009	5.2
80		0.0084	0.0053	1.8
81		0.012	0.035	16.5
83		0.04	0.003	0.19
84		0.07	0.066	2.8

or hydroxyl functions were no longer resistant to reaction; for example, selective acylation on the 22-OH in 16 β -17 α ,22-triol **30** (Figure 11) or alkylation (Figure 10) and aminolysis (Figure 14) of 22-ester **27** and 22,16-lactone **26**.

By the new synthetic route, the synthesis of OSW-1 was finally achieved in a linear sequence of nine steps and 14% overall yield from the aldol adduct **9a** (Figures 10 and 11) without much optimization. We found that the present chemistry was extremely convenient for the synthesis of the OSW saponin analogues with a C22-ester side chain. Remarkably, a 23-oxa analogue of OSW-1, **40**, was prepared starting from the industrial dehydroisoandrosterone **1** in eight linear steps and in 26% overall yield (Figure 12). And this 23-oxa analogue was found to be as active as the parent OSW-1. Thus, a new stage was set for further synthesis to decipher SAR of OSW saponins.

With the ready chemistry in hand, analogues with a variety of modified side chains were easily prepared, via aldol condensation with propionates of varying length, with thiopropionate and acetate (for preparation of **68–75**; Figure 13), or via aminolysis of the 16,22-lactone **26** (for preparation of the 23-*N*-analogues; Figure 14). CM reaction was also proven feasible for modification at the final stage from C22-allyl ester **70** (Figure 15). Examination of these new analogues revealed a valuable SAR for the side chain of OSW saponins (Table 1). The new synthetic routes to OSW-1 and its side-chain analogues also pave the way to synthesis of new probes for mechanistic studies of OSW-1 and of selected active analogues in gram quantities for animal studies.

Experimental Section⁴⁴

Isoamyl Ketone 17 (and Byproducts 20–22). A solution of **8a** (400 mg, 0.62 mmol) in dry THF (6 mL) was cooled to $-20\text{ }^{\circ}\text{C}$ under Ar. Isoamyllithium (8 mL, 0.78 M in Et₂O) was added dropwise with stirring over 15 min. After being stirred at $-20\text{ }^{\circ}\text{C}$ for an additional 1.5 h, the resulting reaction mixture was pooled into 5% HCl that has been cooled to $-40\text{ }^{\circ}\text{C}$. The product was extracted with CH₂Cl₂, and the combined organic layers were dried over Na₂SO₄. After evaporation of the solvent, the residue was purified by flash column chromatography (petroleum ether/EtOAc 12:1) to afford **17** (121 mg, 34%), **20** (69 mg, 17%), **21** (80 mg, 19%), **22** (24 mg, 6%), and **5** (6%) as white solids.

Compound 17: $[\alpha]_{\text{D}}^{24} = -52.5$ (*c* 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 7.69–7.34 (m, 10 H), 5.10 (m, 1 H), 4.04 (br s, 1 H), 4.05–3.96 (m, 1 H), 3.54 (m, 1 H), 2.88 (q, *J* = 7.1 Hz, 1 H), 2.54 (t, *J* = 7.4 Hz, 2 H), 2.54 (br s, 1 H), 1.16 (d, *J* = 7.1 Hz, 3 H), 1.06 (s, 9 H), 0.97 (s, 3 H), 0.9 (s, 3 H), 0.88 (s, 3 H), 0.78 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 218.4, 141.2, 135.7, 134.8, 129.4, 127.4, 120.8, 82.6, 76.9, 73.1, 79.4, 49.3, 48.2, 48.2, 42.4, 41.6, 37.0, 36.4, 35.2, 32.3, 32.1, 31.8, 31.7, 27.5, 27.0, 22.3, 20.2, 19.4, 19.1, 14.5, 12.7; ESIMS *m/z* 693.4 (M + Na⁺), HRMS (ESI) *m/z* 693.4288 (M + Na⁺), calcd for C₄₃H₆₂O₄SiNa 693.4310.

Compound 20: ¹H NMR (500 MHz, CDCl₃) δ 7.67–7.35 (m, 10 H), 5.10 (m, 1 H), 4.52 (br s, 1 H), 4.39 (m, 1 H), 3.54 (m, 1 H), 2.99 (br s, 1 H), 1.06 (s, 9 H), 0.97 (s, 3 H), 0.9 (s, 3 H), 0.89–0.86 (16 H), 0.78 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 141.2, 135.7, 134.8, 129.4, 127.4, 121.0, 84.6, 79.7, 75.8, 73.2, 49.4, 48.1, 43.0, 42.5, 37.0, 33.33, 33.28, 42.25, 31.93, 31.86, 31.80, 28.6, 27.0, 22.97, 22.82, 22.66, 22.39, 19.4, 14.3, 13.0;

ESIMS *m/z* 765.5 (M + Na⁺), HRMS (ESI) *m/z* 765.5226 (M + Na⁺), calcd for C₄₈H₇₄O₄SiNa 765.5249.

Compound 21: $[\alpha]_{\text{D}}^{24} = -56.7$ (*c* 1.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.69–7.34 (m, 10 H), 5.11 (m, 1 H), 4.06 (m, 1 H), 3.53 (m, 1 H), 1.06 (s, 9 H), 0.98 (s, 3 H), 0.89–0.87 (8 H), 0.70 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 141.3, 135.8, 134.9, 129.5, 127.5, 121.0, 81.9, 77.1, 73.3, 49.8, 48.6, 47.1, 42.5, 37.2, 36.6, 33.2, 32.7, 32.0, 31.9, 31.8, 31.2, 28.9, 27.1, 22.8, 22.7, 20.2, 19.5, 19.2, 15.4; ESIMS *m/z* 637.4 (M + Na⁺), 1251.8 (2M + Na⁺).

Compound 22: $[\alpha]_{\text{D}}^{24} = -57.4$ (*c* 1.5, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.69–7.34 (m, 10 H), 5.11 (m, 1 H), 4.32 (m, 1 H), 3.56–3.48 (m, 1 H), 1.05 (s, 9 H), 0.99 (s, 3 H), 0.91 (s, 3 H), 0.89 (s, 3 H), 0.86 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 141.7, 136.2, 135.2, 129.8, 127.8, 121.1, 85.5, 81.8, 73.5, 50.2, 48.9, 47.6, 42.8, 37.5, 36.9, 34.1, 33.8, 33.3, 32.8, 32.2, 31.9, 29.8, 28.5, 27.4, 23.1, 23.0, 20.7, 19.8, 19.5, 15.1; ESIMS *m/z* 637.4 (M + Na⁺), HRMS (ESI) *m/z* 637.4031 (M + Na⁺), calcd for C₄₀H₅₈O₃SiNa 637.4047.

16,22- γ -Lactone 26. A suspension of **25**¹⁵ (318 mg, 0.61 mmol), CeCl₃·7H₂O (320 mg, 0.86 mmol), and NaBH₄ (160 mg, 4.21 mmol) in dry THF was stirred at 0 °C for 1 h and then quenched with methanol. After the mixture was stirred for about 0.5 h, water was added. The resulting mixture was diluted with CH₂Cl₂. The organic layer was washed with 5% HCl and brine, respectively, and then dried over Na₂SO₄ and filtered. The filtrate was concentrated in vacuo to give a residue, which was purified by flash column chromatography (petroleum ether/EtOAc/CH₂Cl₂ 9:1:2) to afford **26** (267 mg, 92%) as a white solid. $[\alpha]_{\text{D}}^{26} = -85.2$ (*c* 0.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 5.26 (m, 1 H), 4.43 (dd, *J* = 4.7 and 3.2 Hz, 1 H), 3.46–3.39 (m, 1 H), 2.67 (q, *J* = 7.7 Hz, 1 H), 1.29 (d, *J* = 7.7 Hz, 3 H), 1.03 (s, 3 H), 0.89 (s, 9 H), 0.81 (s, 3 H), 0.06 (s, 6 H); ESIMS *m/z* 492.4 (M + Na⁺), 971.6 (2M + Na⁺). Anal. Calcd for C₂₈H₄₆O₄Si: C, 70.84; H, 9.77. Found: C, 71.03; H, 9.78.

16 β ,17 α ,22-Triol 30. To a solution of lactone **26** (147 mg, 0.31 mmol) in THF (5 mL) was added dropwise a solution of isoamyllithium in Et₂O (1.35 M, 1.83 mL, 2.48 mmol) during 0.5 h at room temperature under argon. The reaction mixture was quenched with saturated aqueous NH₄Cl. The product was extracted with CH₂Cl₂. The combined organic layer was washed with brine and dried over anhydrous Na₂SO₄. The solvent was removed to afford the crude hemiketal **28**, which was used immediately without further purification and identification. Hemiketal **28** was dissolved in anhydrous ether (20 mL). The solution was cooled to 0 °C and treated with a suspension of LiAlH₄ (59 mg, 1.55 mmol) in ether (2 mL). The reaction mixture was stirred overnight at room temperature. After completion of the reaction, excess LiAlH₄ was quenched carefully with water. The product was extracted with ether. The organic extract was washed with 5% HCl and brine, respectively, and was then dried over Na₂SO₄ and filtered. The filtrate was concentrated in vacuo to give a residue, which was purified by silica gel flash column chromatography (petroleum ether/EtOAc/CH₂Cl₂ 6:1:1) to afford **30** (147 mg, 87%) as a white solid. Compound **30** is a mixture of the epimers at C22.

22-O-AZMB Ester 31 (and 16-O-AZMB Ester 32). To a stirring solution of triol **30** (140 mg, 0.26 mmol) in anhydrous THF (6 mL) at room temperature was added DMAP (120 mg, 0.98 mmol), followed by addition of a solution of 2-(azidomethyl)benzoyl chloride³⁷ (3.9 mL, 0.33 M) in THF. After completion of the reaction, the reaction mixture was diluted with CH₂Cl₂ and washed twice with saturated aqueous NaHCO₃ and once with water. The organic layer was dried over Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography (petroleum ether/EtOAc 30:1 to petroleum ether/EtOAc 15:1) to afford **31** (87 mg, 48%) and the 16-O-AZMB ester **32** (7%) as white solids.

Compound 31: $[\alpha]_{\text{D}}^{14} = -11.1$ (*c* 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 8.05 (d, *J* = 8.1 Hz, 1 H), 7.58 (d, *J* = 7.2 Hz, 1 H), 7.55 (t, *J* = 7.2 Hz, 1 H), 7.43 (t, *J* = 7.2 Hz, 1 H), 5.31

(44) For compounds not been described in this section, see Supporting Information.

(m, 1 H), 5.16 (d, $J = 10.2$ Hz, 1 H), 4.91–4.77 (m, 3 H), 4.15 (m, 1 H), 3.56–3.40 (m, 1 H), 2.46 (q, $J = 6.4$ Hz, 1 H), 1.12 (d, $J = 7.1$ Hz, 3 H), 1.01 (s, 3 H), 0.97 (s, 3 H), 0.89 (s, 9 H), 0.88 (d, $J = 7.2$ Hz, 6 H), 0.06 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 168.0, 141.4, 137.9, 133.1, 131.1, 129.7, 128.3, 128.1, 120.9, 86.4, 79.5, 78.9, 72.5, 53.1, 49.8, 48.5, 47.1, 42.7, 38.9, 37.3, 36.5, 35.8, 34.5, 32.7, 32.0, 31.9, 31.8, 28.5, 27.8, 25.9, 22.9, 22.3, 20.4, 19.4, 18.3, 12.8, 9.9, –4.6; ESIMS m/z 730.5 (M + Na⁺), HRMS (ESI) m/z 730.4596 (M + Na⁺), calcd for $\text{C}_{41}\text{H}_{65}\text{O}_5\text{N}_3\text{SiNa}$ 730.4586.

Compound 32: ^1H NMR (300 MHz, CDCl_3) δ 8.04 (d, $J = 8.1$ Hz, 1 H), 7.56 (d, $J = 7.2$ Hz, 1 H), 7.52 (t, $J = 7.2$ Hz, 1 H), 7.42 (t, $J = 7.2$ Hz, 1 H), 5.64–5.59 (dd, $J = 7.8$ and 9.0 Hz, 1 H), 5.32 (m, 1 H), 4.97–4.74 (AB, 2 H), 4.83 (m, 1 H), 4.23 (m, 1 H), 3.48 (m, 1 H), 1.13 (d, $J = 7.2$ Hz, 3 H), 1.01 (s, 3 H), 0.94 (s, 3 H), 0.89 (s, 9 H), 0.88 (d, $J = 7.2$ Hz, 6 H), 0.06 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 167.7, 141.5, 137.5, 132.7, 131.0, 129.6, 128.1, 120.9, 86.8, 80.6, 77.2, 72.5, 53.2, 49.7, 48.4, 46.6, 42.8, 38.6, 37.3, 37.1, 36.5, 34.8, 32.6, 32.4, 32.0, 31.9, 31.9, 27.9, 26.0, 22.8, 22.4, 20.5, 19.4, 18.3, 13.4, 9.0, –4.6.

Glycoside 34. A solution of the disaccharide imidate **33**⁶ (160 mg, 0.13 mmol), aglycon **31** (81 mg, 0.11 mmol), and 4 Å molecular sieve in dry CH_2Cl_2 was stirred at room temperature for 15 min and then cooled to –20 °C. A solution of TMSOTf (0.05 equiv) in CH_2Cl_2 was slowly added to the reaction. After being stirred for 1 h, the reaction was quenched with Et_3N and filtered. The filtrates were concentrated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether/EtOAc 20:1) to afford **34** (104 mg, 61%) as a white foam. $[\alpha]_{\text{D}}^{20} = -25.5$ (c 1.7, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 7.86 (d, $J = 8.1$ Hz, 3 H), 7.49 (m, 3 H), 6.76 (d, $J = 8.1$ Hz, 2 H), 5.31 (m, 1 H), 5.20 (m, 2 H), 4.90 (m, 2 H), 4.87 and 4.69 (AB, 2 H), 4.52 (d, $J = 3.6$ Hz, 1 H), 4.24 (br s, 1 H), 4.02 (m, 1 H), 3.84 (s, 3 H), 1.68 (s, 3 H), 1.07 (d, $J = 6.9$ Hz, 3 H), 0.07 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 168.0, 163.9, 162.5, 140.8, 136.8, 131.6, 131.2, 130.3, 129.2, 128.6, 127.1, 122.1, 120.5, 112.7, 100.5, 86.4, 76.7, 76.1, 72.0, 54.8, 52.4, 49.3, 48.3, 47.1, 42.2, 38.0, 36.8, 35.9, 35.0, 32.1, 31.5, 31.4, 31.2, 30.0, 29.2, 27.4, 25.4, 24.2, 22.3, 20.1, 20.0, 18.7, 17.8, 17.7, 12.6, 10.5, 6.4, 6.38, 6.3, 4.6, 4.5, 4.4, 4.4, 4.3, 4.2, –5.1; ESIMS m/z 1513.1 (M + Na⁺), HRMS (ESI) m/z 1512.8509 (M + Na⁺), calcd for $\text{C}_{75}\text{H}_{131}\text{O}_{16}\text{N}_3\text{Si}_4\text{Na}$ 1512.8499.

22-Alcohol 35. To a solution of **34** (30 mg, 0.02 mmol) in THF (1 mL) was added water (5 equiv), followed by tributylphosphine (15 μL , 0.06 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was diluted with CH_2Cl_2 and washed once with saturated aqueous NaHCO_3 and twice with water. The organic layer was dried over Na_2SO_4 , filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (petroleum ether/EtOAc 15:1) to afford **35** (22 mg, 82%) as a white solid. $[\alpha]_{\text{D}}^{21} = -36.8$ (c 1.4, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.00 (d, $J = 9.6$ Hz, 2 H), 6.88 (d, $J = 8.7$ Hz, 2 H), 5.30 (m, 1 H), 4.94 (dd, $J = 2.4$ and 5.4 Hz, 1 H), 4.87 (m, 2 H), 4.49 (d, $J = 3.0$ Hz, 1 H), 4.26 (m, 1 H), 4.22 (m, 1 H), 3.86 (s, 3 H), 2.61 (m, 1 H), 1.95 (s, 3 H), 0.07 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3): δ 170.0, 164.7, 163.3, 141.5, 131.9, 122.6, 121.1, 113.4, 113.1, 100.5, 90.4, 87.1, 82.4, 77.2, 75.6, 72.9, 72.6, 70.7, 70.1, 62.9, 56.6, 56.3, 55.4, 49.6, 48.4, 48.1, 47.0, 44.6, 43.4, 42.8, 41.0, 37.5, 37.3, 36.5, 35.7, 35.0, 34.7, 33.3, 32.2, 32.1, 31.9, 31.5, 30.1, 29.7, 27.8, 25.9, 25.0, 24.4, 22.9, 22.2, 21.4, 20.9, 20.6, 19.2, 18.2, 17.1, 13.3, 13.1, 6.9, 6.8, 4.9, 4.8, –4.6; ESIMS m/z 1354.5 (M + Na⁺), HRMS (ESI) m/z 1353.8064 (M + Na⁺), calcd for $\text{C}_{71}\text{H}_{126}\text{O}_{15}\text{Si}_4\text{Na}$ 1353.8066.

22-Ketone 36. To a solution of **35** (21 mg, 0.016 mmol) and 3 Å molecular sieve (20 mg) in CH_2Cl_2 was added pyridinium dichromate (12 mg, 0.032 mmol). After being stirred for 1 h at room temperature, the reaction mixture was filtered. The filtrates were evaporated in vacuo. The crude product was purified by silica gel column chromatography (petroleum ether/EtOAc 15:1) to afford **36** (20.3 mg, 97%) as a white solid. $[\alpha]_{\text{D}}^{20} = -43.5$ (c 0.7, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.00 (d,

$J = 9.3$ Hz, 2 H), 6.85 (d, $J = 8.7$ Hz, 2 H), 5.24 (m, 1 H), 4.87 (m, 1 H), 4.76 (m, 1 H), 4.69 (m, 1 H), 4.17 (m, 1 H), 3.81 (s, 3 H), 3.06 (q, $J = 7.2$ Hz, 1 H), 2.20–2.13 (m, 1 H), 1.90 (s, 3 H), 1.09 (d, $J = 7.2$ Hz, 3 H), 0.07 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 219.5, 168.7, 164.7, 163.5, 141.4, 132.0, 122.6, 121.1, 113.5, 100.0, 85.9, 72.6, 69.8, 55.4, 49.6, 48.1, 46.1, 45.9, 42.8, 39.0, 37.3, 36.5, 34.6, 32.2, 31.9, 29.7, 27.4, 25.9, 22.4, 22.1, 20.7, 19.3, 18.3, 13.9, 11.8, 6.9, 6.8, 4.9, 4.8, –4.6; ESIMS m/z 1351.8 (M + Na⁺), HRMS (ESI) m/z 1351.7890 (M + Na⁺), calcd for $\text{C}_{71}\text{H}_{124}\text{O}_{15}\text{Si}_4\text{Na}$ 1351.7910.

OSW-1. A solution of **36** (14.1 mg, 0.011 mmol) and $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ (cat. 1.5 mg) in acetone and water (20:1 v/v, 1 mL) was stirred at room temperature until TLC indicated the reaction has finished. Then the solution was directly concentrated in vacuo to give a residue, which was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 15:1) to give OSW-1⁶ (7.6 mg, 82%) as a white solid. $[\alpha]_{\text{D}}^{16} = -42.9$ (c 0.14, CH_3OH).

(20S)-Allyl-23-oxa-OSW-1 70. A solution of **62** (188 mg, 0.143 mmol) in dry CH_2Cl_2 (10 mL) was treated with HF-pyridine (2 drops) at room temperature. After being stirred for 0.5 h, the reaction mixture was poured into a saturated NaHCO_3 solution. The organic layer was washed with brine and dried over Na_2SO_4 . After evaporation of the solvent, the residue was purified by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 30:1) to afford **70** (118 mg, 96%) as a white foam. $[\alpha]_{\text{D}}^{26} = -31.3$ (c 0.59, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.03 and 6.93 (AB, 4 H), 5.71–5.59 (m, 1 H), 5.32 (m, 1 H), 5.16 (d, $J = 7.8$ Hz, 1 H), 5.11 (br s, 1 H), 4.93 (dd, $J = 6.6$ and 6.3 Hz, 1 H), 4.83–4.76 (m, 2 H), 4.55 (dd, $J = 12.6$ and 6.0 Hz, 1 H), 3.91 (s, 3 H), 2.61 (q, $J = 7.2$ Hz, 1 H), 1.94 (s, 3 H), 1.05–0.90 (m, 7 H), 0.78 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 181.2, 172.2, 168.6, 166.6, 143.2, 134.8, 134.4, 124.1, 124.0, 121.3, 116.5, 103.2, 102.6, 92.2, 87.3, 76.4, 75.7, 74.4, 72.7, 72.0, 67.7, 67.4, 66.5, 64.1, 58.1, 56.0, 52.2, 50.9, 48.5, 44.9, 43.2, 39.8, 39.1, 37.4, 34.4, 34.2, 23.4, 23.2, 22.1, 15.9, 15.3; ESIMS m/z 881.5 (M + Na⁺), HRMS (ESI) m/z 881.3949 (M + Na⁺), calcd for $\text{C}_{45}\text{H}_{62}\text{O}_{16}\text{Na}$ 881.3930.

22-Amide 76. A solution of DIBAL-H (1 M in toluene, 4.9 mL, 4.9 mmol) was added to a cool (0~5 °C) solution of isobutylamine (0.5 mL, 0.5 mmol) in THF (2 mL) under argon. The mixture was allowed to warm and was stirred at room temperature for 2 h. The concentration of the prepared DIBAL-H/*i*-BuNH₂ complex was about 0.72 mol/L.

To a solution of **26** (30 mg, 0.063 mmol) in THF (2 mL) were added, under Ar at room temperature, the DIBAL-H/*i*-BuNH₂ complexes (0.44 mL, 0.72 mol/L). After being stirred at room temperature for 2 h, the reaction was cooled to 0 °C and then quenched with H₂O (0.1 mL) and a 1 N aqueous solution of KHSO_4 (5 mL). The resulting mixture was extracted with CH_2Cl_2 . The combined organic layers were washed with brine, dried over Na_2SO_4 , and concentrated. The residue was purified by flash chromatography (petroleum ether/EtOAc 5:1) to afford **76** (33 mg, 95%) as a white solid. $[\alpha]_{\text{D}}^{20} = -39.2$ (c 0.5, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 5.77 (br s, 1 H), 5.29 (m, 1 H), 4.21 (s, 1 H), 3.92 (m, 1 H), 3.50–3.43 (m, 1 H), 3.11 (m, 2 H), 2.86 (q, $J = 7.2$ Hz, 1 H), 2.66 (m, 1 H), 1.24 (d, $J = 7.2$ Hz, 3 H), 1.13 (s, 3 H), 0.98 (s, 3 H), 0.91 (s, 3 H), 0.88 (d, 9 H); ^{13}C NMR (75 MHz, CDCl_3) δ 178.4, 141.5, 120.8, 85.1, 81.8, 72.5, 49.6, 48.4, 46.7, 46.3, 42.7, 41.8, 37.3, 36.5, 35.8, 32.6, 32.0, 31.9, 31.8, 28.5, 25.9, 20.4, 20.1, 19.4, 18.2, 13.4, –4.6; ESIMS m/z 570.4 (M + Na⁺), HRMS (ESI) m/z 570.3963 (M + Na⁺), calcd for $\text{C}_{32}\text{H}_{57}\text{NO}_4\text{SiNa}$ 570.3949.

Protected 23-Aza-OSW-1 78. A procedure similar to that described for the preparation of **34** was employed. Thus treatment of **76** (33 mg, 0.06 mmol) and disaccharide imidate **33** (70 mg, 0.072 mmol) in the presence of TMSOTf (30 μL , 0.1 M solution in CH_2Cl_2) provided **78** (52 mg, 65%) as a white foam. $[\alpha]_{\text{D}}^{20} = -24.3$ (c 1.6, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.03 and 6.93 (AB, 4 H), 7.20 (m, 1 H), 5.62 (s, 1 H), 5.30 (m, 1 H), 4.89 (s, 1 H), 4.82 (m, 2 H), 4.45 (2 H), 3.89 (s, 3 H), 3.49 (m, 1 H), 3.12 (q, $J = 7.4$ Hz, 1 H), 1.99 (s, 3 H), 1.32 (d, $J =$

7.4 Hz, 3 H), 0.06 (s, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 180.1, 169.1, 166.7, 163.4, 141.3, 132.0, 122.0, 121.1, 113.6, 100.5, 98.1, 91.2, 85.5, 76.1, 73.9, 72.5, 71.9, 70.6, 68.4, 65.3, 63.2, 59.7, 55.5, 49.6, 48.2, 46.1, 45.7, 42.9, 40.4, 37.2, 36.5, 35.0, 32.3, 32.1, 31.9, 28.2, 25.9, 20.7, 20.6, 20.2, 20.1, 19.2, 18.2, 14.5, 14.4, 6.9, 6.7, 5.1, 5.0, 4.7, -4.6; MALDI-MS m/z 1352.7 (M + Na⁺), HRMS (MALDI) m/z 1352.7905 (M + Na⁺), calcd for $\text{C}_{70}\text{H}_{123}\text{NaNO}_{15}\text{Si}_4$ 1352.7862.

23-Aza-OSW-1 analogue 80. A procedure similar to that described for the preparation of OSW-1 was employed. Thus treatment of **78** (40 mg, 0.030 mmol) with $\text{Pd}(\text{MeCN})_2\text{Cl}_2$ (1.8 mg) provided **80** (19.6 mg, 75%) as a white foam. $[\alpha]_D^{20} = -45.3$ (c 0.9, CHCl_3); ^1H NMR (300 MHz, CDCl_3) δ 8.07 and 6.99 (AB, 4 H), 5.64 (m, 1 H), 5.32 (m, 1 H), 5.27 (s, 1 H), 4.90 (br s, 1 H), 4.89 (m, 1 H), 4.71 (m, 1 H), 4.39 (br s, 1 H), 4.32 (m, 1 H), 4.28 (m, 1 H), 3.96 (br s, 1 H), 3.88 (s, 3 H), 2.58 (q, $J = 7.4$ Hz, 1 H), 2.02 (s, 3 H), 1.19 (d, $J = 7.4$ Hz, 3 H), 1.00 (s, 3 H), 0.84 (s, 3 H), 0.79 (d, $J = 6.6$ Hz, 6 H); ^{13}C NMR (75 MHz, CDCl_3) δ 179.4, 169.6, 166.0, 164.3, 140.5, 132.2, 121.6, 121.0, 114.2, 99.5, 99.4, 90.4, 85.3, 76.1, 72.6, 72.4, 71.7, 70.3, 69.1, 63.7, 63.0, 59.6, 55.6, 49.5, 48.0, 46.2, 45.7, 42.3, 40.6, 37.1, 36.4, 34.7, 32.2, 31.8, 31.6, 28.2, 20.8, 20.6, 20.0, 19.9, 19.4, 14.5, 13.5; MALDI-MS m/z 896.5 (M + Na⁺), HRMS (MALDI) m/z 896.4446 (M + Na⁺), calcd for $\text{C}_{46}\text{H}_{67}\text{O}_{15}\text{NaN}$ 896.4403.

Compound 83. Allyl ester **70** (21 mg, 0.025 mol) and 9-decen-1-yl benzoate **82** (125 mg, 0.48 mmol) were added via syringe to a stirring solution of Grubbs second-generation catalyst (1.5 mg, 0.0018 mmol) in CH_2Cl_2 (2 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 h. The reaction mixture was then concentrated and purified directly by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 30:1) to afford **83** (20 mg, 75%) as a white solid. $[\alpha]_D^{26} = -21.7$ (c 0.76, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ 8.03 (m, 4 H), 7.55 (m, 1 H), 7.44 (m, 2 H), 6.93 (d, $J = 5.0$ Hz, 2 H), 5.64 (m, 1 H), 5.36–5.29 (m, 2 H), 4.94 (br s, 1 H), 4.84 (m, 2 H), 4.57 (m, 1 H), 4.31 (t, $J = 10.0$ Hz, 2 H), 4.23 (br s, 1 H), 4.16 (m, 1 H), 4.02 (m, 1 H), 3.86 (br s, 1 H), 3.84–3.78 (m, 9 H), 3.67 (m, 1 H), 3.51–3.37 (m, 4 H), 1.96 (s, 3 H), 0.78 (s, 3 H); ^{13}C NMR (125 MHz, CDCl_3) δ 178.1, 169.1, 166.2, 165.4, 163.4, 140.1, 136.5, 132.3, 131.6, 130.0, 129.0, 127.8, 122.8, 121.0, 120.9, 113.4, 99.9, 99.5, 89.1, 84.2, 76.7, 76.5, 76.2, 72.5, 71.3,

69.5, 64.6, 64.5, 54.9, 49.0, 47.8, 45.3, 40.0, 36.7, 35.9, 31.7, 31.6, 31.3, 31.2, 31.1, 29.2, 28.8, 28.7, 28.6, 28.3, 28.2, 25.5, 20.2, 18.9, 12.8, 12.1; ESIMS m/z 1113.65 (M + Na⁺), HRMS (MALDI) m/z 1113.5371 (M + Na⁺), calcd for $\text{C}_{60}\text{H}_{82}\text{O}_{18}\text{Na}$ 1113.5393.

Dimer 84. Allyl ester **70** (25 mg, 0.029 mmol) was added via syringe to a stirring solution of Grubbs second-generation catalyst (4.7 mg, 0.0055 mmol) in CH_2Cl_2 (5 mL). The flask was fitted with a condenser and refluxed under nitrogen for 16 h. The reaction mixture was then concentrated and purified directly by silica gel column chromatography ($\text{CH}_2\text{Cl}_2/\text{MeOH}$ 30:1) to afford **84** as a white solid (12 mg, 49%). $[\alpha]_D^{21} = -10.1$ (c 0.6, CHCl_3); ^1H NMR (500 MHz, $\text{C}_6\text{D}_5\text{N}$) δ 8.42 (d, $J = 10.0$ Hz, 1 H), 7.18 (d, $J = 10.0$ Hz, 1 H), 6.09 (m, 1 H), 5.77 (m, 2 H), 5.45 (br s, 1 H), 5.34 (d, $J = 5.0$ Hz, 1 H), 4.70 (d, $J = 5.0$ Hz, 1 H), 4.58–4.28 (m, 10 H), 3.87 (m, 6 H), 3.08 (m, 1 H), 2.77 (br s, 1 H), 2.68–2.67 (d, $J = 5.0$ Hz, 1 H), 2.10 (m, 6 H), 1.13 (s, 3 H); ^{13}C NMR (125 MHz, $\text{C}_6\text{D}_5\text{N}$) δ 178.1, 169.8, 165.7, 164.0, 142.0, 132.5, 129.1, 121.2, 114.2, 103.2, 85.1, 75.9, 75.2, 71.6, 71.4, 70.8, 55.7, 50.3, 49.0, 46.7, 43.6, 41.4, 37.9, 36.9, 32.7, 32.3, 32.2, 30.8, 30.1, 30.0, 29.7, 29.3, 23.0, 21.0, 21.0, 19.7, 17.8, 14.3, 13.6, 13.4; ESIMS m/z 1711.7 (M + Na⁺), HRMS (ESI) m/z 1711.7637 (M + Na⁺), calcd for $\text{C}_{88}\text{H}_{120}\text{O}_{32}\text{Na}$ 1711.7654.

Acknowledgment. This work is supported by the National Natural Science Foundation of China (20372070, 20321202, and 29925203), the Chinese Academy of Sciences (KGCX2-SW-209), and the Committee of Science and Technology of Shanghai (04DZ14901 and 04DZ05603).

Supporting Information Available: Experimental procedures for synthesis and cell proliferation assay, spectroscopic data for other new compounds, reproductions of ^1H and ^{13}C NMR spectra for selected compounds (in total 159 spectra), and X-ray diffraction data for compounds **46** and **85**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JO051536B