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Synthesis of OSW-1 Derivatives by Site-Selective Acylation and Their Biological Evaluation

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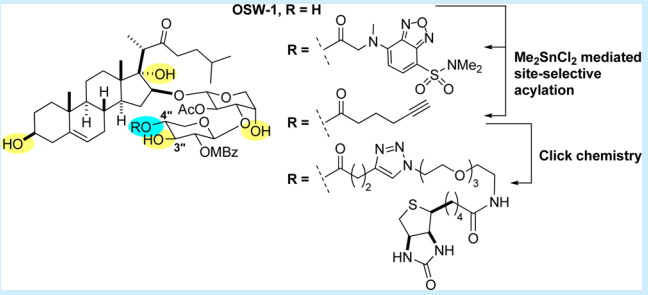
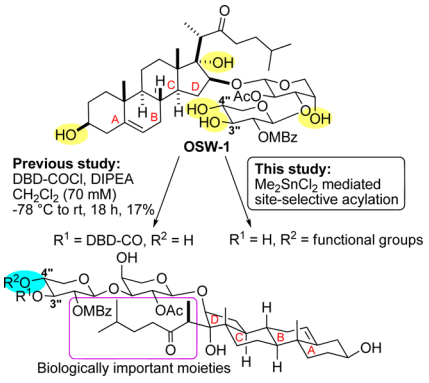
Supporting Information

ABSTRACT: A strategy to site-selectively monoacylate an antitumor saponin OSW-1 was developed using an organotin reagent to rapidly access its derivatives that are useful as chemical probes. 4″-*O*-Acylated OSW-1 derivatives bearing a fluorophore, an alkyne tag, or biotin were prepared in good yields and were shown to maintain highly cytotoxic activity.



OSW-1 belongs to a steroidal saponin family of natural products found in *Ornithogalum saundersiae*, which bears a distinctive acylated disaccharide moiety at the sterol D ring (Scheme 1).[1] OSW-1 has attracted a great deal of interest as a synthetic target, as a drug lead, as well as a biological tool due to its highly potent and selective anticancer activity shown by the National Cancer Institute 60-cell in vitro screen (mean IC_{50} = 0.78 nM).[2−5] However, the molecular basis underlying the cancer selective activity of OSW-1 is still unclear. Shair's group recently identified two cytoplasmic proteins, oxysterol binding protein (OSBP) and its paralog (ORP4L), as OSW-1 binding proteins.[6] More recently, Huang's group revealed that OSW-1 inhibits Na^+/Ca^{2+} exchanger 1, a membrane protein, which leads to apoptosis through increased calcium concentration in cytosol and mitochondria.[7] These studies therefore suggest that OSW-1 interacts with several proteins at different cellular locations and that further investigation is needed to fully understand its mechanism of action.

Scheme 1. One-Step Monoacylation of an Antitumor Steroidal Saponin OSW-1 by (a) Nondirected and (b) Catalyst-Directed Strategies



Synthesis of chemical probes based on OSW-1 with a judicious choice of functionalities such as affinity tags or fluorescent tags would provide useful tools to study how it selectively acts upon cancer cells.[8] To this end, chemical modification of OSW-1 offers an attractive approach for an expedient entry into a variety of its derivatives as chemical probes as well as drug leads. While a number of derivatives have been prepared through total synthesis and degradation of the natural product,[2,4] a few reports described the semisynthetic preparation of OSW-1 derivatives. For example, the 3″-*O*-carbamate derivative conjugated to sepharose resin has been prepared by Shair and coworkers for the affinity purification of OSW-1 binding proteins.[6] We have previously reported synthesis of a 3″-*O*-fluorescent derivative of OSW-1 by monoacylation, which was successfully used to show the cellular uptake and localization of OSW-1.[9] However, site-selective modification has not yet been developed for a facile access to appropriately functionalized derivatives of OSW-1. In our previous studies, synthesis of the 3″-*O*-monoacylated derivative relied on the intrinsic differential reactivity of the hydroxyl groups toward 1 equiv of an acylating reagent at low temperature in high dilution, which was prohibitively low yielding (17%, Scheme 1) and with low site-selectivity to be applied to synthesis of various other derivatives. Here we report a strategy to site-selectively acylate the C4″ hydroxyl group of OSW-1 using an organotin reagent to rapidly access its derivatives and the evaluation of their utility in biological studies. The 4″-*O*-fluorescent derivative was used to show the cellular localization, while the biotin-tagged derivative would be

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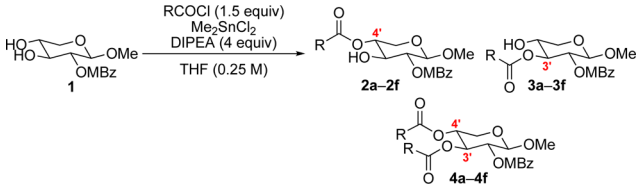
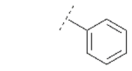
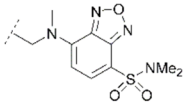
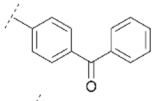
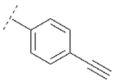
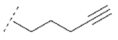
useful in the affinity purification of OSW-1 binding proteins. Our site-selective acylation method should be useful for a facile preparation of OSW-1 probes for biological studies as well as its potent derivatives.

Site-selective functionalization of polyhydroxylated natural products represents a significant challenge in synthetic chemistry. In recent years, site-selective acylation has emerged as a promising solution to this problem.[10] OSW-1 possesses five alcohols, among which are three isolated alcohol moieties and two in a 1,2-relationship (Scheme 1). Based on our previous structural studies, we anticipated that the functionalization at the C4″ hydroxyl group would be favorable in terms of bioactivity since it is distant from the putative pharmacophore of OSW-1.[11] Several strategies have been developed for site-selective monoacylation of free or partially protected carbohydrates, which involves catalysts to selectively activate 1,2-diols.[12] We thus envisaged that the 1,2-diol moiety of OSW-1 could be exploited for selective acylation of the C4″ hydroxyl group. Recently, the Onomura group reported use of catalytic dimethyltin dichloride (Me_2SnCl_2) to achieve highly efficient and site-selective benzoylation of various free monosaccharides, which possess consecutive arrays of 1,2-diols either in *cis* or *trans* arrangements.[13] It was proposed that Me_2SnCl_2 increases the acidities of 1,2-diols by weakly coordinating them, which allows acylation at the less sterically hindered hydroxyl group upon selective deprotonation by a hindered base such as DIPEA. Nevertheless, there has been no literature precedence which applied Me_2SnCl_2 as a catalyst for site-selective monoacylation of complex polyhydroxylated natural products. In this study, we explored the use of the organotin reagent to selectively acylate the C4″ hydroxyl group of OSW-1, which bears a bulky 4-methoxybenzoyl (MBz) group at the C2″−position (Scheme 1).

For the initial studies, we prepared a xyloside derivative **1** representing its substructure as a model substrate, since the isolated sample of OSW-1 was only available in limited quantities. Under the reported conditions using the catalytic amounts of Me_2SnCl_2,[12] the xyloside derivative **1** was selectively acylated to give 4′-*O*-monobenzoylated product **2a** in 94% (Table 1, entry 1). We next tested the organotin catalyzed site-selective acylation condition with acid chlorides bearing potentially useful functionalities for biological studies. A fluorophore-labeling reagent, 4-(*N*,*N*-dimethylamino sulfonyl)-7-*N*-methylamino)-2,1,3-benzoxadiazolyl chloride (DBD-COCl) was chosen since it is commercially available and it has proven useful in our previous fluorescent imaging studies of an OSW-1 derivative.[9] In the absence of the organotin catalyst, acylation with DBD-COCl provided 4′-*O*-acylated product (**2b**) in 17% yield, 3′-*O*-acylated (**3b**) in 12% and 3′,4′-*O*-bis-acylated product (**4b**) in 2% (entry 2). Although the reported conditions using Me_2SnCl_2 for acylation with DBD-COCl gave **2b** in 38% yield with slightly enhanced site-selectivity (entry 3), it is much less efficient in contrast to the case with benzoyl chloride. Screening of molar equivalents of the catalyst, the acylating reagent and the substrate concentration suggested that the excess amounts of the catalyst were needed to improve the reaction. Optimized conditions with 2 or 4 equiv of Me_2SnCl_2 and DBD-COCl at a diluted substrate concentration of 0.01 M led to generation of the desired 4′-*O*-acylated **2b** in 93% yield and 3′-*O*-acylated **3b** in 5% (entry 4). We also assessed the reactivity of **1** toward various acid chlorides bearing benzophenone group useful for photoaffinity labeling as well as azide group or alkyne group useful for click chemistry

Table 1. Selectivity of Me_2SnCl_2-Mediated Acylation of 1



Entry	R	Me_2SnCl_2 (equiv)	Yield (%)		
1[a]		0.05	**2a** (94)	**3a** (0)	**4a** (0)
2[b]		0	**2b** (17)	**3b** (12)	**4b** (2)
3		0.05	**2b** (38)	**3b** (1)	**4b** (2)
4[c]		4	**2b** (93)	**3b** (5)	**4b** (0)
5		0.05	**2c** (93)	**3c** (0)	**4c** (0)
6		0.05	**2d** (76)	**3d** (0)	**4d**(0)
7		0.05	**2e** (88)	**3e** (0)	**4e** (0)
8		0.05	**2f** (38)	**3f** (7)	**4f** (0)
9[d]		3	**2f** (64)	**3f** (8)	**4f** (0)

[a]2 equiv of DIPEA was used. [b]2 equiv of acylating agent was used. [c]4 equiv of acylating agent and 8 equiv of DIPEA was used with **1** at 0.01 M. [d]3 equiv of acylating agent and 6 equiv of DIPEA was used with **1** at 0.01 M.

(alkyne−azide cycloaddition) for introducing other functional groups of choice.[14] For the three acylating reagents which represent benzoyl derivatives (entries 5−7), **1** was selectively monoacylated at 4′-OH in high yields (76−93%), and no 3′-*O*-acylated nor bis-acylated products was isolated. However, in the case with hexynoyl chloride, the standard catalytic condition resulted in monoacylated products in poor yields (entry 8). Increasing the amounts of Me_2SnCl_2 and the acylating reagent to 4 equiv provided 4′-*O*-acylated **2f** at 64% with 3′-*O*-acylated **3f** at 8% (entry 9). We therefore demonstrated that the site-selective acylation of a 1,2-*trans*-diol system in the model xylose derivative could be implemented with various acid chlorides by using either catalytic or excess amounts of Me_2SnCl_2.

Having achieved site-selective acylation of the model system to install various functionalities, we next applied the optimized condition to selective functionalization of OSW-1. Using 4 equiv of the catalyst and DBD-COCl with the concentration of OSW-1 at 0.01 M, the desired 4″-*O*-acylated product (**5**) was obtained in 55% yield with the 3″-*O*-acylated product in 6%, while no bis-acylated product was observed (Scheme 2). The unreacted OSW-1 was recovered in 14% yield. The introduction of only one DBD group in **5** was confirmed by the ESI-MS spectrum (m/z = 1191.5005 $[M + Na]^+$) as well as by a set of ^1H NMR signals corresponding to a single DBD group (pyridine-d_5, δ = 7.86, 6.06, 3.17, 2.86 ppm). The

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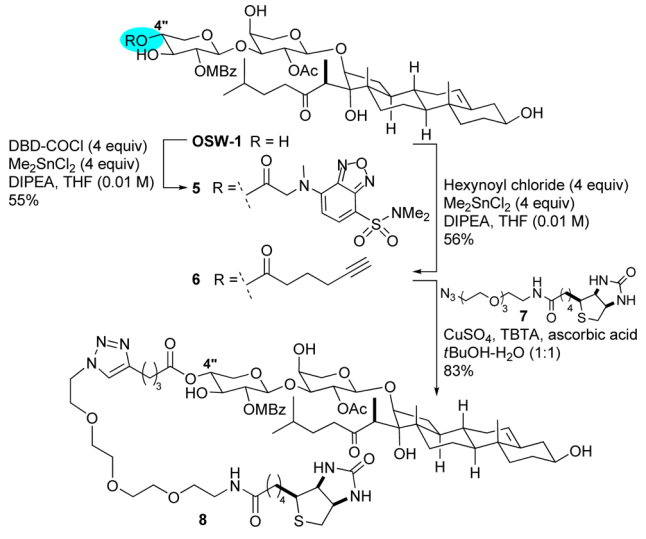
Scheme 2. Preparation of OSW-1 Derivatives by Me_2SnCl_2-Mediated Selective Monoacylation at the C4″-OH



derivatization at the C4″ position in **5** was confirmed by ^{1}H NMR, ^{13}C NMR, COSY, TOCSY, and HMQC spectra. In specific, the ^{1}H NMR spectrum of **5** in comparison with that of OSW-1 revealed a characteristic downfield shift of the H4″ peak (pyridine-d_5, δ = 5.37 ppm, $\Delta\delta$ = 1.18 ppm). A similar condition using hexynoyl chloride as an acylating reagent provided the 4″-*O*-acylated product **6** in 56%, the 3″-*O*-acylated product in 13%, and the recovered starting material (26%). Bis-acylated product was not isolated. Monoacylation of OSW-1 with hexynoyl group was verified by an ESI-MS peak at m/z = 989.4878 $[M + Na]^+$ and the position of the hexynoyl group at the C4″ by ^{1}H NMR of the H4″ peak at δ = 5.31 ppm (pyridine-d_5) with a distinctive downfield shift of $\Delta\delta$ = 1.12 ppm. It was further demonstrated that an alkyne derivative of OSW-1 is useful as a precursor to various chemical probes. The treatment of **6** with biotin-azide **7** under copper-promoted alkyne−azide cycloaddition condition[14] afforded the biotin-tagged affinity probe **8** in 83% yield (Scheme 2). Taken together, we successfully prepared a fluorescent DBD derivative and an alkyne derivative from OSW-1 in one step in good yields and in a site-selective fashion by using Me_2SnCl_2. To our knowledge, these are the first examples of Me_2SnCl_2-mediated site-selective acylation of a nonprotected polyol natural product. Our findings that excess amounts of Me_2SnCl_2 can efficiently promote monoacylation of diols at dilute substrate concentrations with little bisacylated product should be useful in cases where substrates are available only in minute quantities.

The antiproliferative activity of **5**, **6**, and **8** was tested using XTT assay with HeLa cells. All of the three OSW-1 derivatives were found to exhibit highly potent antiproliferative activities. The IC_{50} values of the DBD-tagged **5** and the alkyne-tagged **6** (**5**: 4.0 nM, **6**: 3.1 nM) were similar to that of OSW-1 (IC_{50} = 2.6 nM),[9] whereas that of the biotin-tagged **8** (IC_{50} = 46 nM) was approximately 20-fold less potent. On the other hand, the monosaccharide derivatives **2b** and **2f** as negative controls did not show any activity. We therefore demonstrated that functionalization at the C4″ position is suitable for accessing various OSW-1 derivatives.

To demonstrate its utility, the fluorescent probe **5** was subsequently applied in the cell-imaging studies. For an analysis of the cellular localization of OSW-1, HeLa cells were treated with **5** at 1 μM for 1 h, which were washed with buffer, fixed with 4% formaldehyde, and imaged by fluorescence microscopy. We found that the fluorescent probe **5** was rapidly internalized into cells and predominantly localized around the nuclei (Figure 1a). The localization pattern for **5** is similar to

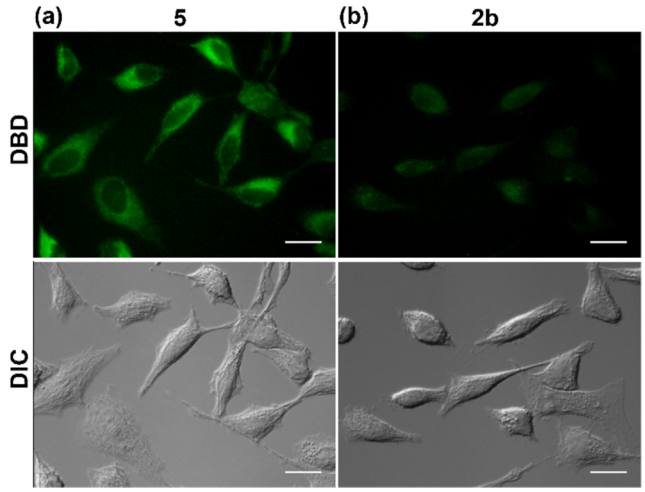


Figure 1. Cellular uptake and localization of DBD-tagged fluorescent probes. HeLa cells were treated with (a) **5** (1 μM) and (b) **2b** (1 μM) at 37 °C for 1 h and then fixed and visualized by fluorescence microscopy. Top panels: fluorescent images based on the fluorescence of DBD-tagged compounds. Bottom panels: differential interference contrast images. Scale bars: 20 μm.

that of 3″-*O*-DBD-tagged OSW-1, which was reported in our previous studies.[9,15] Since 3″-DBD-tagged OSW-1 has been shown previously to localize at the endoplasmic reticulum and the Golgi apparatus through more detailed colocalization analyses,[9] it indicated that **5** may also be distributed to these subcellular organelles. In contrast, the control experiment using the DBD-tagged compound **2b** showed no cellular uptake of the compound (Figure 1b). Therefore, these data strongly suggested that the cell internalization and a particular subcellular localization pattern of the fluorescent probe **5** were observed due to the activity of OSW-1 and not due to that of the DBD group. Our findings that both 4″-*O*-DBD-tagged **5** and the corresponding 3″-*O*-DBD-tagged OSW-1 are as potent as OSW-1 and that they displayed similar cellular localization are in support of our hypothesis that the C3″ and the C4″ hydroxyl groups are distant from the putative pharmacophore of OSW-1.[11] Taken together, our results in fluorescence imaging studies illustrated the utility of chemical probes, which was achieved through the site-selective acylation of OSW-1.

In summary, we have developed an effective strategy to selectively acylate the C4″ hydroxyl group of OSW-1 using Me_2SnCl_2 for a facile preparation of its derivatives that are useful for the biological studies. Our results present the first example of Me_2SnCl_2-mediated acylation of a nonprotected polyol natural product. The method used in this study is mild and can be performed at a low substrate concentration, which should be useful for functionalization of various other complex polyol compounds with a 1,2-diol system, especially in cases where compounds are available only in minute quantities. OSW-1 derivatives with a fluorescent tag, alkyne tag, as well as a biotin tag were found to maintain highly cytotoxic activity, demonstrating the C4″ hydroxyl group of OSW-1 as a suitable point of modification. The fluorescent DBD-tagged OSW-1 was

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used to show its cell uptake and cellular localization. Efforts to identify previously unknown OSW-1 binding proteins are currently ongoing using the biotin-tagged OSW-1.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, structural characterization data, and fluorescence spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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(15) For the cellular uptake and localization of 3″-DBD-tagged OSW-1 under the same experimental conditions as that for **5**, see Figure S3 (Supporting Information).