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Report

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Synthesis and antiproliferative activities of OSW-1 analogues bearing 2''-*O*-*p*-acylaminobenzoyl residues

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OSW-1 is a well-known natural saponin with potent antitumor activities. We have designed and prepared a small library of 22 OSW-1 analogues with a variety of *p*-acylamino-benzoyl groups installed at C2'' of the xylose residue, wherein a regioselective (1→3)-glycosylation of arabinoside 3,4-diol has been achieved by manipulation of the protecting groups on the imidate donors. Bioassays lead to new structure-activity relationships as well as two applicable fluorescent probes, which are found to localize to lysosomes in HeLa cells and could be used in further antitumor mechanism studies of OSW-1 in living cells.

Background and Originality Content

OSW-1 (**1**) is a disaccharide saponin isolated from the bulb of *Ornithogalum saundersiae* by Sashida and co-workers in the early 1990s (Figure 1).[1-2] Owing to its exceptionally strong antiproliferative activities against tumor cell lines,[3-9] OSW-1 (**1**) has attracted extensive researches on the synthesis, derivatization, and structure-activity relationships.[10-26] SBF-1 (**2**), a synthetic analogue bearing an ester chain at C22,[27] displays comparable antitumor activities as the natural product. Due to its easy accessibility via chemical synthesis, SBF-1 (**2**) has been used as a surrogate for OSW-1 (**1**) in biological studies.[28-34] Very recently, we revealed that analogue **3,** with the original ester at the xylose residue being replaced by amide, displayed even stronger antitumor activities with the IC_{50} value as low as 0.11 nM against Jurkat T cell lines.[35]

On the other hand, the antitumor mechanism of these molecules still remains elusive.[28-34, 36-41]. It is of great interest to identify the antitumor-associated binding proteins which could be exploited as drug targets for cancer treatment. Recently, Sakurai and co-workers synthesized several OSW-1-based probes with fluorescent tags installed at C3'' or C4'' on the xylose residue,[42-47] and found these probes could localize to the ER and Golgi apparatus in HeLa cells and induce Golgi stress.[41]

Based on our previous structure-activity relationship studies, we envisioned SBF-1-based analogues bearing a *p*-amino-benzoyl group at C2'' of the xylose residue and an azido-substituted aliphatic side chain on the aglycone moiety both highly active and readily derivatizable into various probes (Figure 1). Particularly, fluorescent probes were designed, in which 4-(*N*,*N*-dimethyl aminosulfonyl)-2,1,3-benzoxadiazole (DBD) was installed at either the 2''-(*p*-aminobenzoyl) group via acylation or the terminus of the side chain via click reaction. Herein, we report the synthesis of these new OSW-1 analogues and fluorescent probes bearing 2''-*O*-(*p*-acylamino-benzoyl)-xylose residues, their antiproliferative activities, and localization in HeLa cells.

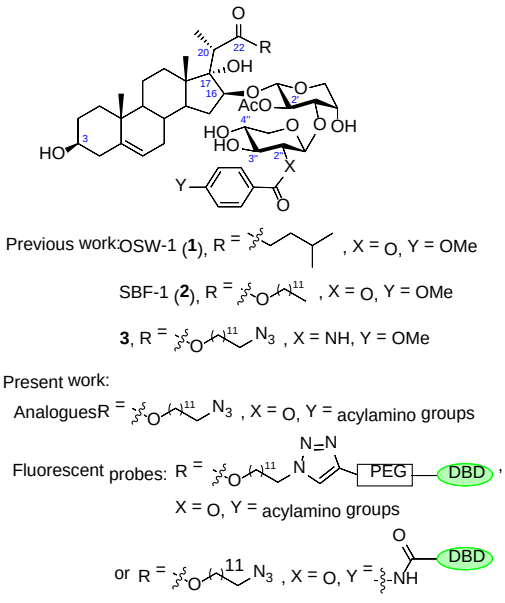
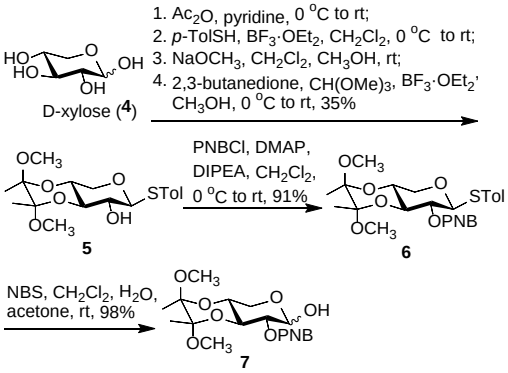


Figure 1 Structures of OSW-1 (**1**), its analogues, and fluorescent probes.

Results and Discussion

The synthesis commenced with the conversion of d-xylose into butane-bisacetal (BDA)-protected β-thioxyloside **5** (Scheme 1).[48-51] Thus, treatment of d-xylose (**4**) with Ac_2O in pyridine provided tetraacetyl xylose, which was subjected to condensation with *p*-TolSH in the presence of $BF_3{\cdot}OEt_2$. The resulting thioxyloside was treated with $NaOCH_3$ in a mixture solvent of CH_3OH and CH_2Cl_2 at rt to remove the acetyl groups;[48] subsequent protection under the conditions of 2,3-butanedione and $CH(OMe)_3$ in the presence of $BF_3{\cdot}OEt_2$ provided **5** in an overall yield of 35% (based on **4**).[49-51] As a precursor of the desired *p*-aminobenzoyl group for late-stage acylation, the relatively inert *p*-nitrobenzoyl (PNB) group was introduced into alcohol **5** to give **6** in 91% yield under the conditions of PNBCl, DIPEA, and DMAP in CH_2Cl_2. Treatment of thioglycoside **6** with NBS and H_2O resulted in hemiacetal **7** in 98% yield, which was a ready precursor for further preparation of imidate and *ortho*-alkynylbenzoate donors.

Scheme 1 Synthesis of BDA-protected hemiacetal **7**.



In our previous studies, it was found that xylosyl donor **8** bearing BDA protecting group at O3 and O4 led mainly to the undesired (1→4)-linked disaccharide product when coupled with l-arabinoside 3,4-diol **10**,[35] whereas the glycosylation with TES-protected donor **9** offered the desired (1→3)-linked disaccharide as the major product (Figure 2).[8, 11] In order to improve the regio-selectivity and verify our assumption that these regio-selectivities were majorly determined by the protecting groups at O3 and O4 of the donors instead of the substitutes at O2 or anomeric position,[50-53] we envisioned the TES-protected thioxyloside **11** as a counterpart of BDA-protected **6** in the present studies (Scheme 2). Thus, the BDA group on **6** was removed by a mixture of 95% CF_3COOH and CH_3CN (2:1), and the resulting diol was treated with TESOTf and 2,6-lutidine in CH_2Cl_2 at −20 °C to give 3,4-di-*O*-TES donor **11** in 89% yield (based on **6**). Subjection of thioglycoside **11** into hydrolysis under the conditions of NBS and H_2O in CH_2Cl_2 and acetone at rt gave the corresponding hemiacetal **12** in 51% yield.

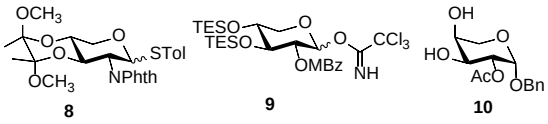
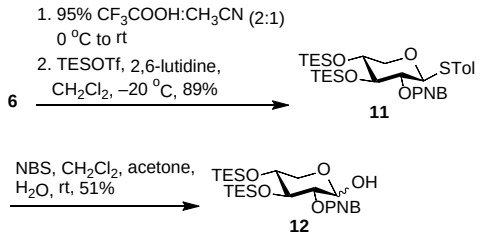


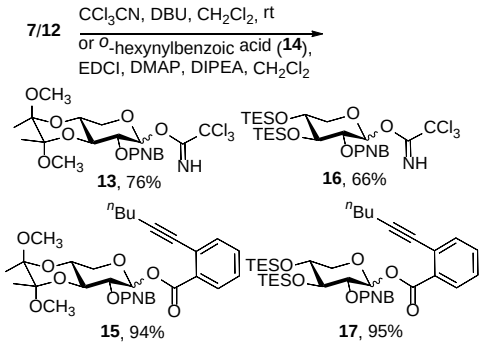
Figure 2 Structure of donors **8**, **9**, and acceptor **10** in previous studies.

Next, hemiacetal **7** was coupled with CCl_3CN in the presence of DBU at rt (Scheme 3), and the resulting trichloroacetimidate donor **13** was obtained in only 76% yield due to partial hydrolysis during column purification. On the other hand, the conversion of hemiacetal **7** into shelf-stable alkynylbenzoate donor **15** was highly efficient upon treatment with *o*-hexynylbenzoic acid (**14**) and EDCI in the presence of DIPEA and DMAP.[54-61] Similar results were obtained in the transformation of hemiacetal **12** into the corresponding trichloroacetimidate **16** and alkynylbenzoate **17**.

Scheme 2 Synthesis of TES-protected hemiacetal **12**.



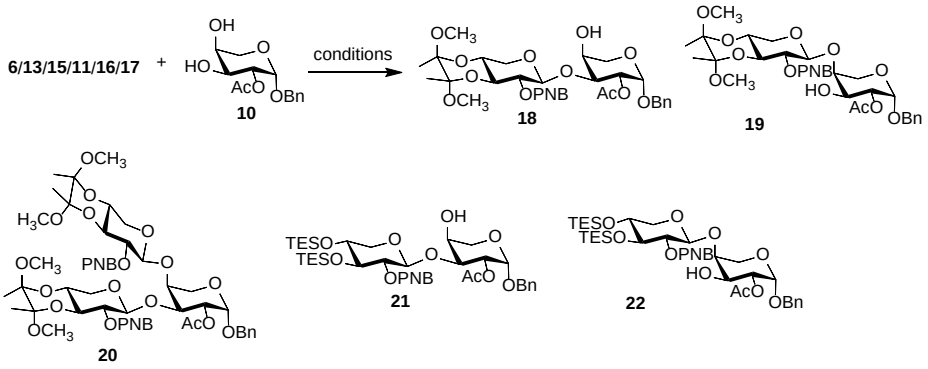
Scheme 3 Synthesis of xylosyl imidate and alkynylbenzoate donors.



With three BDA-protected donors (**6**, **13**, **15**) and their TES-protected counterparts (**11**, **16**, **17**) at hand, we set out to examine their performance in the glycosylation of l-arabinoside 3,4-diol **10**. The crude products were purified with a flash column chromatography to offer a mixture of the glycosylation products, which were then run on HPLC to determine the UV absorption of each product as a reference for their molar ratio. As depicted in Table 1, all three BDA-protected donors (**6**, **13**, **15**) led to the full consumption of diol **10** and provided three glycosylation products, i.e., (1→3)-linked disaccharide **18**, (1→4)-linked disaccharide **19**, and trisaccharide **20** (entries 1−10). The molar ratios of disaccharides **18**/**19** were approximately equal to the ratios of their UV absorption, whereas the UV absorption ratio of **18**/**20** could reflect the change of the molar ratio given the different UV absorptivity of the disaccharide and trisaccharide. All the three BDA-protected donors resulted in more (1→4)-linked disaccharide **19** than (1→3)-linked disaccharide **18** (entries 1−9). Lowering the reaction temperature from −20 °C to −60 °C gradually increased the proportion of **18** and decreased the proportions of **19** and trisaccharide **20** (entries 1 to 3, entries 5 to 7, and entries 8 to 10). The best proportion of **18** was obtained when alkynylbenzoate donors **15** was used at −60 °C under the conditions of $Ph_3PAuNTf_2$ (0.1 equiv) and 4Å MS in CH_2Cl_2 (entry 10), wherein **18** was found slightly more than **19**. The most effective suppression of the formation of trisaccharide **20** was observed in the pre-activation glycosylation with thioxyloside donor **6** under the conditions of BSP, TTBP, Tf_2O, and 3Å MS in CH_2Cl_2 at −70 °C (entry 4).[62-64] However, the resulting molar ratio of **18**/**19** (1.0:1.3) was not as good as that in entry 10 (1.0:0.90).

The results above revealed that low temperature was preferred for preparing the desired (1→3)-linked disaccharide, hence the following glycosylation with TES-protected donors (**11**, **16**, **17**) was conducted at −60 °C or −70 °C. No trisaccharide product was observed under these conditions, so that only the mixture of (1→3)-linked disaccharide **21** and (1→4)-linked disaccharide **22** was isolated and examined to give the ratios of **21**/**22** and the yields of **21** (Table 1, entries 11-15). As expected, TES-protected donors generally displayed better 3-OH selectivity than BDA-protected donors. Although treatment of thioglycoside donor **11** with NIS and TMSOTf at –70 °C led to a complex mixture (entry 11), the corresponding pre-activation glycosylation with BSP, Tf_2O, and TTBP gave **21**/ **22** in a ratio of 1.0:1.1 (entry 12), that was slightly better than the **18**/**19** ratio of 1.0:1.3 in entry 4. However, the yield of **21** was only 25% due to the formation of some unidentified by-products. Gratifyingly, alkynylbenzoate donor **17** under the catalysis of $Ph_3PAuNTf_2$ (0.2 equiv) was found to provide a satisfactory **21**/**22** ratio of 1.0:0.67 and much less by-products, giving the desired (1→3)-linked disaccharide **21** in 56% yield (entry 15). Notably this reaction was conducted at –60 °C that was uncommon for gold(I)-catalyzed glycosylation with alkynylbenzoates, implying the high reactivities of these xylosyl donors. For trichloroimidate donor **16**, the glycosylation underwent smoothly at –60 °C and the regio-selectivities were found to be greatly dependent on the promoters. When TMSOTf was employed (entry 13), a modest **21**/**22** ratio of 1.0:1.2 was obtained similar to the **18**/**19** ratio of 1.0:1.1 in entry 7, whereas the replacement with a catalytic amount of $BF_3{\cdot}OEt_2$ could significantly increase the ratio to 1.0:0.55. The best yield of **21** was obtained in 64% using TES-protected donor **16** under the catalysis of $BF_3{\cdot}OEt_2$ in CH_2Cl_2 at −60 °C.

Table 1 Glycosylation of arabinoside diol **10** with BDA-protected donors (**6**, **13**, **15**) and TES-protected donors (**11**, **16**, **17**).



Entry	donor	Conditions	Temperature	Ratio of the UV absorption of **18/19/20**	Ratio of the UV absorption of **21/22**	Yield of **21**
1	**6**	NIS, TMSOTf, 4Å MS, CH_2Cl_2	−20 °C	1.0:1.9:0.98	–	–
2			−40 °C	1.0:1.6:0.75	–	–
3			−60 °C	1.0:1.2:0.54	–	–
4		BSP, TTBP, Tf_2O, 3Å MS, CH_2Cl_2	−70 °C	1.0:1.3:0.23	–	–
5	**13**	TMSOTf, 4Å MS, CH_2Cl_2	−20 °C	1.0:1.6:0.52	–	–
6			−40 °C	1.0:1.4:0.60	–	–
7			−60 °C	1.0:1.1:0.40	–	–
8	**15**	$Ph_3PAuNTf_2$, 4Å MS, CH_2Cl_2	−20 °C	1.0:1.6:0.77	–	–
9			−40 °C	1.0:1.3:0.67	–	–
10			−60 °C	1.0:0.90:0.44	–	–
11	**11**	NIS, TMSOTf, 4Å MS, CH_2Cl_2	−70 °C	–	complex	
12		BSP, TTBP, Tf_2O, 3Å MS, CH_2Cl_2	−70 °C	–	1.0:1.1	25%
13	**16**	TMSOTf, 4Å MS, CH_2Cl_2	−60 °C	–	1.0:1.2	39%
14		$BF_3{\cdot}OEt_2$, 4Å MS, CH_2Cl_2	−60 °C	–	1.0:0.55	64%
15	**17**	$Ph_3PAuNTf_2$, 4Å MS, CH_2Cl_2	−60 °C	–	1.0:0.67	56%

Both (1→3)-linked disaccharides **18** and **21** could be used in the subsequent preparation of OSW-1 analogues. Thus, the BDA protecting group on **18** was removed in a mixture of 95% CF_3COOH and CH_3CN (Scheme 4), and the resulting triol was subjected to TES protection under the conditions of TESOTf and 2,6-lutidine at –20 °C to result in **23** in 62% yield. A highly efficient Pd/C-promoted reduction was conducted at 1 atm H_2 atmosphere for 3 h to convert the nitro group into amino group with the anomeric benzyl group being intact. The resulting **24** was then treated with Boc_2O in the presence of DMAP at rt to provide **25** in 43% yield and **26** in 35% yield, respectively. Next, the anomeric benzyl group on **26** was successfully removed by an enhanced 24-h hydrogenolysis with an excess amount of Pd/C, and the resulting hemiacetal was coupled with alkynylbenzoic acid **14**. A $Ph_3PAuNTf_2$-catalyzed glycosylation was then conducted with either azido-bearing aglycone **27** or alkyne-bearing aglycone **28** at 0 °C (See SI for the preparation of **28**),[35] and subsequent removal of the TES and Boc groups under the conditions of CF_3COOH and CH_2Cl_2 at rt furnished the desired OSW-1 analogues **29** and **30** which bear a 2-*O*-(*p*-amino-benzoyl)-xylose residue.

Alternatively, an enhanced hydrogenolysis of disaccharide **23** was employed to remove the anomeric benzyl group and simultaneously to convert the nitro group into amino group (Scheme 5). Subsequently selective condensation of the resulting acetal with alkynylbenzoic acid **14** gave a mixture of epimers **31** in 61% yield with the α/β ratio of 1:10. The intact amino group was then coupled with a variety of acyl chlorides, anhydrides, and

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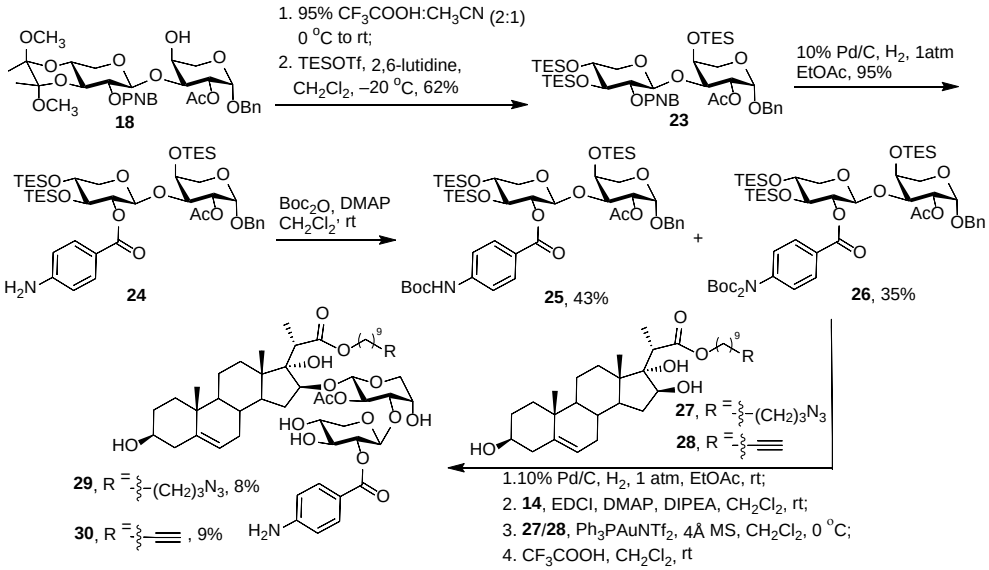
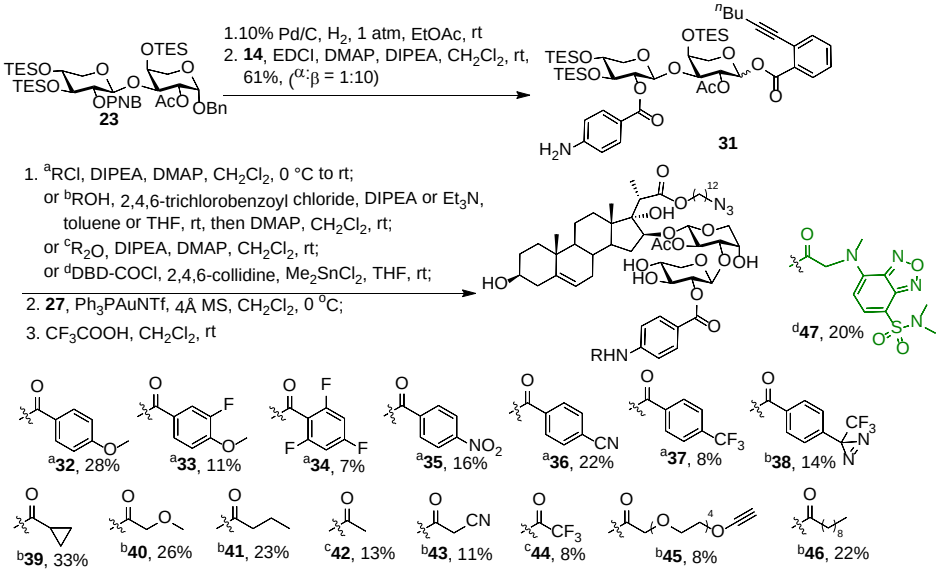
carboxylic acids. $Ph_3PAuNTf_2$-catalyzed glycosylation of aglycone **27** and final removal of the TES groups furnished sixteen OSW-1 analogues, including **32**–**38** with benzoyl-derived groups, **39**–**46** with aliphatic acyl groups, and **47** with the fluorescent DBD group.

Scheme 4 Synthesis of OSW-1 analogues **29** and **30** bearing 2-*O*-(*p*-amino-benzoyl)-xylose residue.



Scheme 5 Synthesis of OSW-1 analogues **32**–**47** bearing 2-*O*-(*p*-acylamino-benzoyl)-xylose residue.



Compound **38** was coupled with DBD-PEG2-alkyne **48** via a Cu-catalyzed click reaction in the presence of tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA) and sodium ascorbate in DMF and H_2O. The crude product was purified by HPLC to afford the desired probe **50** with the saponin residue connecting the fluorescent DBD tag via a flexible and hydrophilic PEG linker. Similar coupling of compounds **32**, **38**, and **47** with biotin-PEG4-alkyne **49** via click reaction provided three probes (i.e., **51**–**53**) with the biotin group extended from the aglycone side chain.

With these 22 OSW-1 analogues at hand, we evaluated their antiproliferative activities against Jurkat T tumor cell lines and CRL1999 normal cell lines (Table 2). Compounds **29** and **30** bearing *p*-aminobenzoyl group at C2′′ displayed similarly potent activities as OSW-1 (**1**) and SBF (**2**) bearing the *p*-methoxybenzoyl group. With an IC_{50} value of 1.0 nM, alkyne derivative **30** was found 10

time more potent than azide derivative **29** against Jurkat T, implying that the terminal group at the aglycone side chain could influence the antiproliferative activities. For those with an additional benzoyl-derived residue at the *p*-amino group, i.e., **32**–**33** and **35**–**36** with either an electron-donating methoxyl group or an electron-withdrawing nitro or nitrile group, were found to retain the potent activities with IC_{50} values against Jurkat T in the range of 2.5–15 nM, which was consistent with the previous presumption that a variation at this site could be tolerable.[3, 46, 65-66] The *p*-trifluoromethylbenzoyl derivative **37** was less potent, whereas compound **34** bearing 2,4,6-trifluorobenzoyl group was found to be inactive. Moreover, the activities of the diazirine-containing **38** also significantly descended, excluding it from further use as a photoaffinity probe.

On the other hand, compounds **39**–**42** bearing short aliphatic acyl residues showed better activities than the aforementioned benzoyl-derived analogues against Jurkat T cell lines with IC_{50} values in the range of 1.1–5.9 nM (Table 2). Increased IC_{50} values were observed on those bearing electron-withdrawing nitrile or trifluoromethyl groups (e.g. **43** and **44**) as well as the analogues with long acyl chains. Particularly, compound **46** bearing a very long hydrophobic side chain lost the antiproliferative activities against CRL1999 normal cell lines.

Scheme 6 Synthesis of OSW-1 probes bearing DBD or biotin residues.

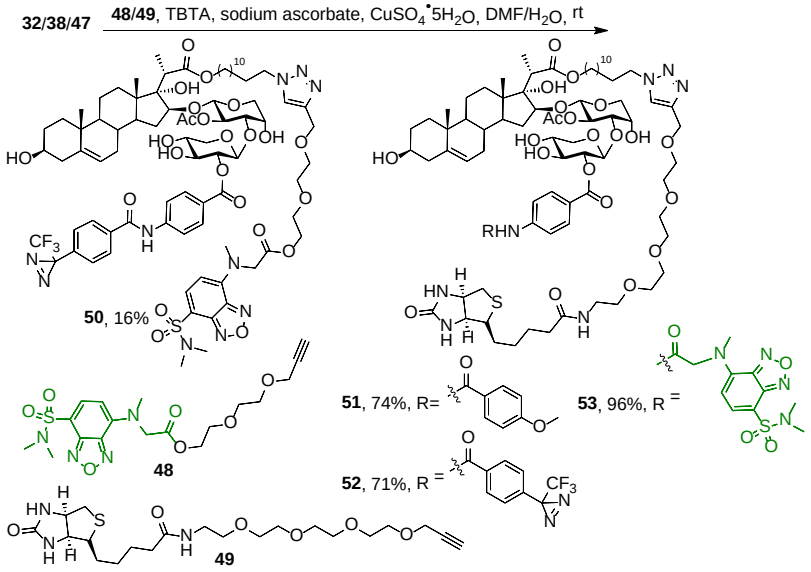


Table 2 Antiproliferative activities of the synthetic OSW-1 analogues and probes against Jurkat and CRL1999 cell lines.

Compounds	IC_{50} (nM)		Compounds	IC_{50} (nM)	
	Jurkat	CRL1999		Jurkat	CRL1999
29	12	17	**30**	1.0	7.0
32	5.1	27	**33**	15	19
34	>1000	>1000	**35**	7.4	14
36	2.5	9.0	**37**	89	96
38	>100	>100	**39**	1.1	3.8
40	2.7	13	**41**	2.7	8.1
42	5.9	9.1	**43**	58	49
44	33	78	**45**	51	78
46	>100	>1000	**47**	2.8	59
50	60	>100	**51**	21	82
52	18	>100	**53**	>100	>1000
Taxol	7.7	36			

It is interesting to find that compound **47** with a bulky DBD group at C2'' still retained excellent activities against Jurkat T cell lines (Table 2), making it a useful fluorescent probe for mechanism studies in living cells. The addition of a PEG-linked biotin group to the aglycone side chain on **47** decreased the activities of the resulting derivative **53**. And similar decrease of activity was observed in the transformation of **32** into its biotin-PEG-conjugated **51**. Nevertheless, similar transformations of the diazirine derivative **38** into its biotin-PEG-conjugated **52** and DBD-PEG-linked **50** were found to increase the activities against Jurkat cells.

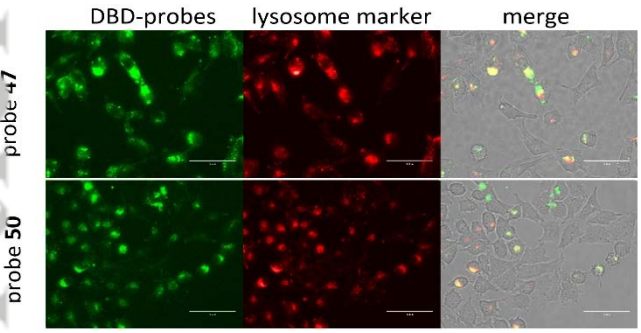


Figure 3 Intracellular colocalization analysis of DBD probes **47** (0.5 μM), **50** (0.5 μM), and Lysotracker® Red (lysosome marker) in HeLa cells. Cells were preincubated with 333 μM of Lysotracker® Red in DMEM at 37 °C for 1 h, washed with DMEM, then treated with **47** or **50** (0.5 μM) at 37 °C for 1h. Scale bars: 100 μm.

The fluorescent derivatives **47** and **50** were found active, therefore were applied to the intracellular localization studies. A preliminary examination was conducted and showed that **47** and **50** could be internalized into cells in 0.5 h, and presumably localized at lysosomes. Hence, colocalization experiments were performed with lysosome marker Lysotracker® Red in HeLa cells, which demonstrated that the green fluorescence from **47** and **50** could obviously overlay with lysosome marker. It is noteworthy that probe **50** with weaker antiproliferative activities gave higher fluorescence intensity. Further studies on the mechanism of OSW-1 compounds against cancer cells using these novel probes are yet to perform.

Conclusions

We have established an effective approach to the preparation of 2''-*O*-*p*-acylamino-benzoyl OSW-1 analogues, wherein the regioselective glycosylation of arabinoside 3,4-diol to construct the desired (1→3)-linked disaccharides was realized by employing 3,4-di-*O*-TES-xylosyl imidate donors under the catalysis of $BF_3{\cdot}OEt_2$ at low temperature. Thus, 22 analogues were synthesized and their antiproliferative activities against Jurkat T cell lines and CRL1999 cell lines were evaluated. Some of these new derivatives, such as **39**–**42** with short aliphatic acyl residues, show antiproliferative activities as potent as the parent natural product. In addition, two fluorescent probes are found to be able to localize at lysosomes of HeLa cells, which might be useful for further studies on the mechanisms of action of this important type of anticancer compounds.

Supporting Information

The supporting information for this article is available on the WWW under https://doi.org/10.1002/cjoc.2018xxxxx.

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