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Chemical and biological evaluation of unusual sugars, α-aculosides, as novel Michael acceptors

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The unusual sugars α-aculosides, which appear in certain antibiotics and have an α,β-unsaturated ketone structure, were found to be novel and selective Michael acceptors for the thiol function of cysteine residues. A coumarin derivative possessing α-aculoside as a Michael acceptor effectively and irreversibly operated as a fluorescent probe in cells. Furthermore, α-aculosides exhibited cytotoxic activity against several cancer cell lines.

The unusual sugars α-aculosides, which contain an α,β-unsaturated ketone structure, appear in certain antibiotics such as the vineomycin1 and uredamycin2 families and PI-0803 (Figure 1). While the biological activity of these antibiotics has been attributed to the aglycon portion, the anticoagulant activity of PI-080 and the cytotoxic activity of vineomycin B2 against cancer cells appeared to be associated with the trisaccharide moieties of the natural products.3,4 As a part of our recent study on the total synthesis of vineomycin B2,5 we became interested in structure-activity relationships (SAR) of vineomycin B2, particularly the detailed SAR and mode of action of the trisaccharide moiety. Herein we describe the importance of α-aculoside in the trisaccharide moiety of vineomycin B2 in the development of cytotoxicity against cancer cells. In addition, we report the attractive and unique nature of α-aculoside as a selective Michael acceptor and its utility for protein labeling.

First, as illustrated in Figure 2, we designed and synthesized trisaccharide analogues of vineomycin B2 (1–5). Analogue 1 possessed a methyl group as the aglycon. Analogues 2 and 3 lacked a double bond and a ketone function, respectively, in the α-aculoside moiety, while 4 contained neither functional group, and 5 was missing the entire α-aculoside moiety.

The synthesis of 1–5 is described in Scheme 1. Glycosylation of 6 and 7, which was prepared from methyl α-d-olivoside, using NIS and TIOH proceeded smoothly to provide the protected trisaccharide 8 in high yield with high α-stereoselectivity. Subsequent removal of the ClAc group6 in 8 using thiourea and 2,6-lutidine gave 1. Analogues 2 and 3 were obtained by hydrogenation of the double bond and reduction of the ketone in 1, respectively. Further reduction of the double bond in 3 using NBSH afforded 4, while the disaccharide analogue 5 was prepared by glycosylation of 7 and 9,7 followed by deprotection of the naphthyl group in the resulting disaccharide 10 and the ClAc group in 11.

With the trisaccharide analogues of vineomycin B2 (1–5) in hand, the cytotoxicity of 1–5 against MCF-7 human breast cancer cells and sarcoma 180 solid tumor cells in mice was examined by treating the cells with different doses for 24 h. The results are summarized in Table 1. It was found that only 1, containing α-aculoside, showed cytotoxic activity against MCF-7 (IC50: 16.8 μM) and sarcoma 180 (IC50: 6.3 μM). In contrast, 2–4 did not show cytotoxic activity against MCF-7 (IC50 > 100 μM) or sarcoma 180 (IC50 > 100 μM). These results clearly indicated that α-aculoside was an indispensible unit in the trisaccharide’s
cytotoxicity against cancer cells.

Fig. 2 Chemical structures of vineomycin B₂ trisaccharide analogues 1–5.


Table 1. Cytotoxic activities of 1–5 against MCF-7 and sarcoma 180 cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MCF-7 (IC50; μM)</th>
<th>Sarcoma 180 (IC50; μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>16.0</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td>6.3</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Based on these results, we expected that α-aculoside would be a Michael acceptor, exhibiting a unique nature due to its unusual structure containing an α,β-unsaturated ketone function in the strained pyranose ring system. Therefore, we next examined the chemical nature of α-aculoside 12 as a Michael acceptor by comparison with β-aculoside 13, N-ethylmaleimide (14), cyclohexenone (15), and monomethyl maleate (16), all of which contain α,β-unsaturated carbonyl functions in different scaffolds (Figure 3). After chemical synthesis of 12 and 13 (see ESI: Scheme S1), we examined the reactivity of 12–16 with a cysteine derivative 17 at pH 7.4 and 37 °C for 1 min. It was found that α-aculoside 12 reacted smoothly with 17 to give the corresponding Michael adduct 20 in 80% yield. The result was comparable to that of 14, which is known and used as a Michael acceptor⁹ and produced the corresponding Michael adduct 22 in 95% yield in the reaction with 17. In contrast, β-aculoside 13 gave the adduct 21 in lower yield (67%). Furthermore, the reaction of 15 with 17 afforded the adduct 23 in much lower yield (45%), and 16 did not provide the adduct 24 at all under the same reaction conditions. These results indicated that α-aculoside 12, as well as 14, exhibited high reactivity against the thiol function of cysteine derivative 17. Furthermore, it was revealed that the α-configuration of the anomic center and the strained α,β-unsaturated ketone structure of α-aculoside 12 significantly influenced its high reactivity with 17. We therefore selected 12 and 14 for further study, examining their reactivity toward lysine and tyrosine derivatives 18 and 19 at pH 7.4 and 37 °C for 4 h. It was found that 14 reacted with 18 to provide the adduct 26 in 55% yield, but did not react with 19 to produce adduct 28. In contrast, 12 reacted with neither 18 nor 19, and adducts 25 and 27 were not produced. These results indicated that α-aculoside 12 operated as a selective Michael acceptor for the thiol function of cysteine.

With these favourable results, we next examined whether a coumarin derivative possessing α-aculoside as a Michael acceptor worked as a fluorescent probe in living cells. For this purpose, we designed and synthesized the coumarin-α-aculoside hybrid 29. The synthesis of 29 is summarized in Scheme 2. Glycosylation of the known glycal 30 with 2-bromomethanol by CSA afforded the glycoside 31, whose bromo group was converted to an azide group using NaN₃ to give 32. Deprotection of the acetyl group in 32 followed by oxidation of the resulting 33 furnished 34. Finally, a click reaction of 34 with coumarin derivative 35 proceeded smoothly to furnish the desired coumarin-α-aculoside hybrid 29.

With the α-aculoside-containing coumarin derivative 29 in hand, we next examined its reactivity toward cysteine derivative 17 and the protein BSA (Figure 4). It was found that 29 reacted with 17 at pH 7.4 and 37 °C for 1 min to afford the Michael adduct 36 in 70% yield. In addition, it was confirmed by MALDI-TOF MS analysis that reaction of 10 equiv. of 29 with
BSA, which has one free cysteine thiol, fifty-nine lysine amino acid and twenty tyrosine hydroxyl groups, gave the 29-BSA adduct 37, containing only one unit of 29. These results indicated that the coumarin derivative 29, possessing α-aculoside as a Michael acceptor, selectively and effectively reacted with the thiol function of cysteine residues even in a protein (BSA).

In order to further demonstrate that the coumarin derivative 29, possessing α-aculoside as a Michael acceptor, could operate as a thiol-selective fluorescent probe in cells, fluorescence microscopy experiments were conducted. Figure 5 shows fluorescence images of MCF-7. It was found that when MCF-7 cells were treated with coumarin derivative 35, containing no α-aculoside, no fluorescence image was detected. In contrast, treatment of MCF-7 cells with 29 clearly gave a fluorescence image, and the fluorescence intensity decreased upon addition of a thiol trapping agent, 2-iodoacetamide. These results indicated that 29 showed marked fluorescence enhancement of the thiol function of cysteine in cells.

![Chemical structures of Michael acceptors 12-16, donors 17-19, and adducts 20-28.](image)

**Fig. 3** Chemical structures of Michael acceptors 12–16, donors 17–19, and adducts 20–28.

![Scheme 2. Synthesis of coumarin-α-aculoside hybrid 29.](image)

**Scheme 2.** Synthesis of coumarin-α-aculoside hybrid 29.

![Fig. 4 a) Reactivity of 29 toward 17 and BSA; b) MALDI-TOF MS profiles of BSA (blue line) and BSA-29 (1:1) adduct (red line).](image)

**Fig. 4** a) Reactivity of 29 toward 17 and BSA; b) MALDI-TOF MS profiles of BSA (blue line) and BSA-29 (1:1) adduct (red line).
In conclusion, we have demonstrated for the first time that \( \alpha \)-aculosides are novel and selective Michael acceptors for the thiol function of cysteine residues. In addition, a coumarin derivative possessing \( \alpha \)-aculoside as a Michael acceptor effectively and irreversibly operated as a fluorescent probe in cells. Furthermore, \( \alpha \)-aculosides exhibited cytotoxic activity against several cancer cell lines. The results presented here will assist in the molecular design of novel and selective protein-labeling or alkylating agents which will, in turn, help provide a means of visualizing or controlling the target proteins. A study using a ligand-\( \alpha \)-aculoside hybrid to control the specific functions of certain proteins is now under way in our laboratories.

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Notes and references