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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 vineomycin[1] and urdamycin[2] families and PI-080[3] (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 B_2 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 B_2,[5] we became interested in structure-activity relationships (SAR) of vineomycin B_2, 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 B_2 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 B_2 (**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**[5] and **7**, which was prepared from methyl α-D-olivoside, using NIS and TfOH proceeded smoothly to provide the protected trisaccharide **8** in high yield with high α-stereoselectivity. Subsequent removal of the ClAc group[6] in **8** using thiourea and 2,6-lutidine gave **1**. Analogues **2** and **3** were

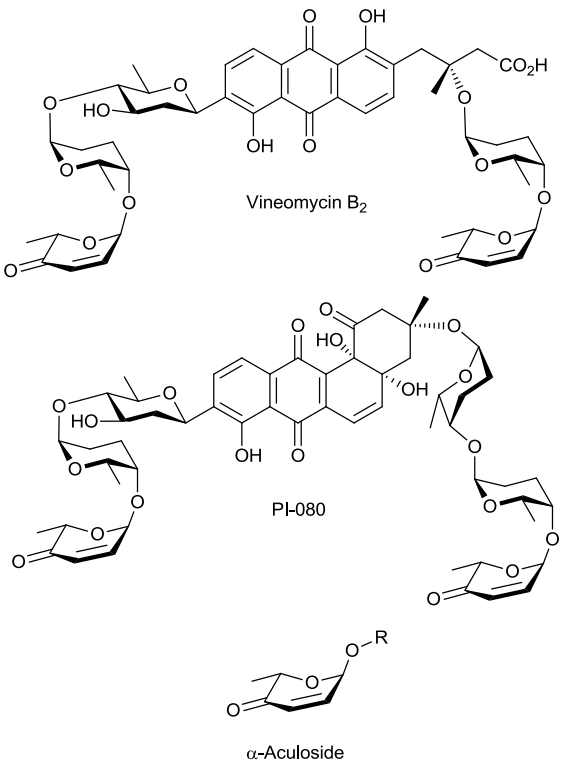

Fig. 1 Chemical structures of vineomycin B_2, PI-080, and α-aculoside.

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**,[5] followed by deprotection of the naphthyl group in the resulting disaccharide **10** and the ClAc group in **11**.

With the trisaccharide analogues of vineomycin B_2 (**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 (IC_{50}: 16.8 μM) and sarcoma 180 (IC_{50}: 6.3 μM).[7] In contrast, **2**–**4** did not show cytotoxic activity against MCF-7 (IC_{50}: > 100 μM) or sarcoma 180 (IC_{50}: > 100 μM). These results clearly indicated that α-aculoside was an indispensable unit in the trisaccharide's

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cytotoxicity against cancer cells.

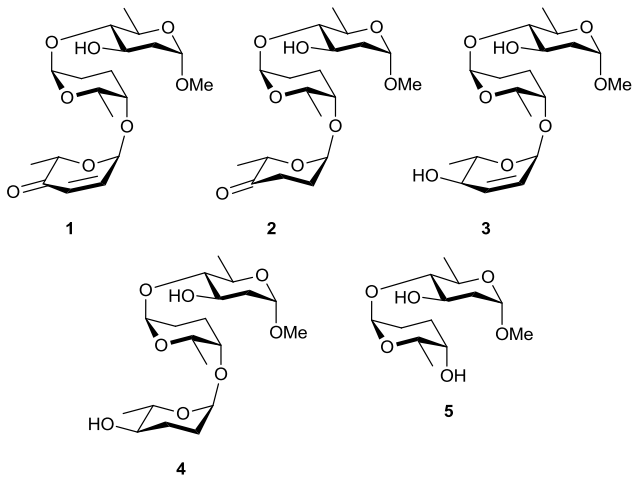
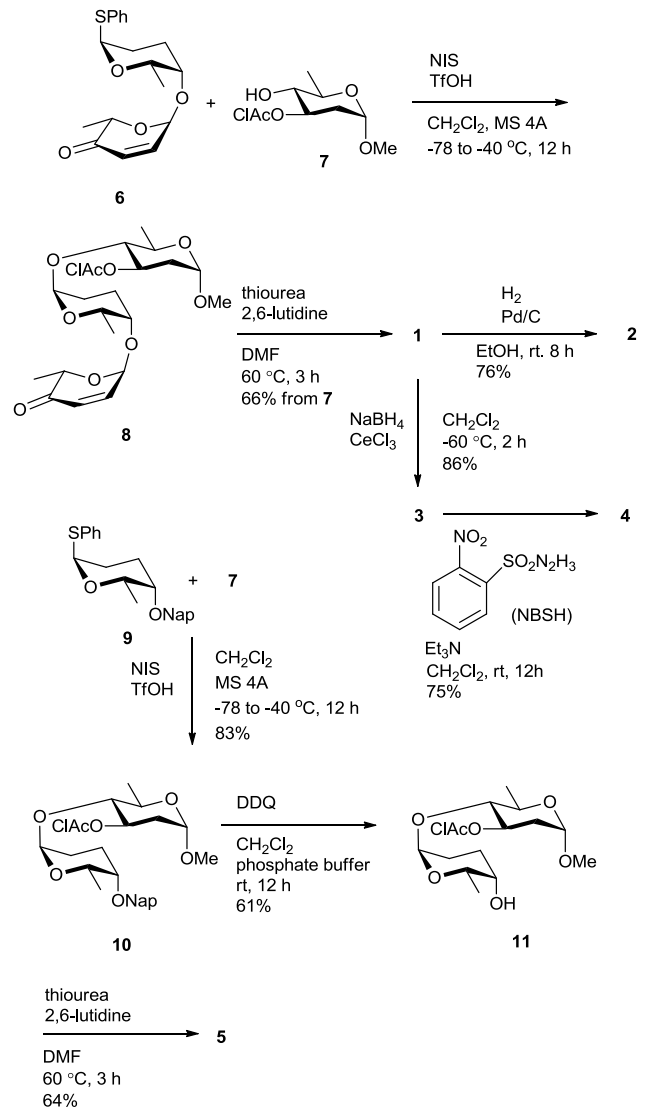


Fig. 2 Chemical structures of vineomycin B_2 trisaccharide analogues **1**–**5**.



Scheme 1. Synthesis of vineomycin B_2 trisaccharide analogues **1**–**5**.

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

Compound / Cancer cells	1	2	3	4	5
MCF-7 (IC50: μM)	16.0	>100	>100	>100	>100
Sarcoma 180 (IC50: μM)	6.3	>100	>100	>100	>100

Based on these results, we expected that α-aculoside would be a Michael acceptor,[8] 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 acceptor[9] 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 anomeric 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**[10] with 2-bromoethanol by CSA afforded the glycoside **31**, whose bromo group was converted to an azide group using NaN_3 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**[11] 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 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).

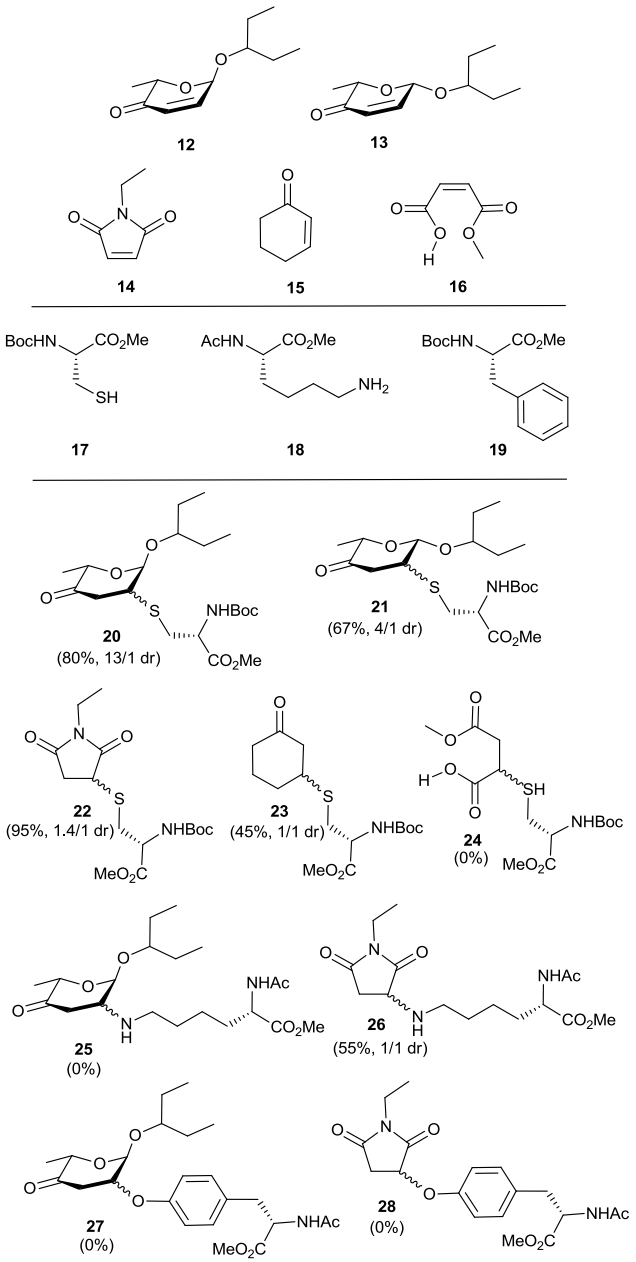
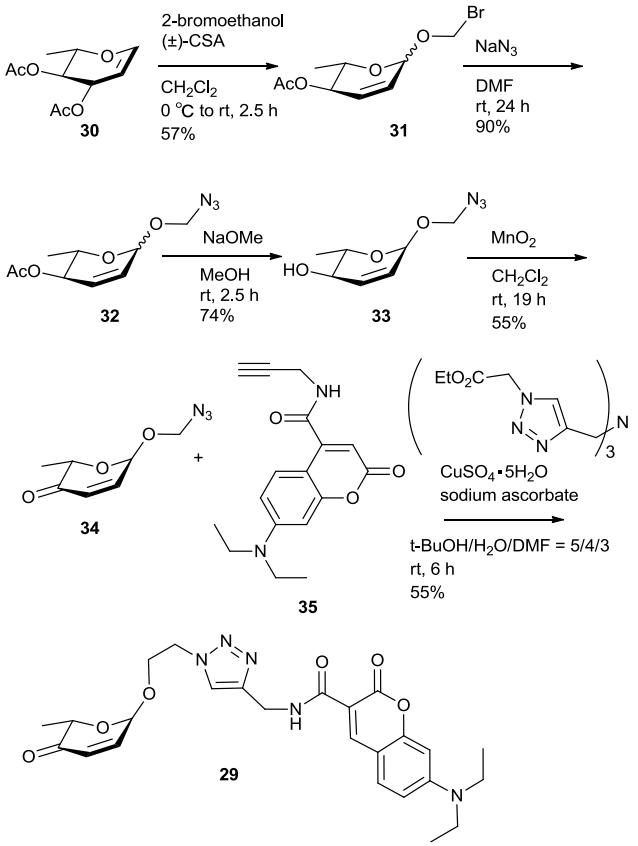


Fig. 3 Chemical structures of Michael acceptors **12**–**16**, donors **17**–**19**, and adducts **20**–**28**.

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.[12] These results indicated that **29** showed marked fluorescence enhancement of the thiol function of cysteine in cells.



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

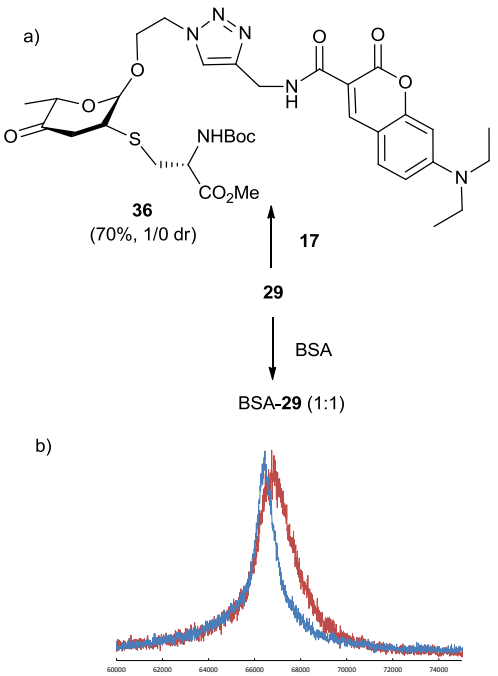


Fig. 4 a) Reactivity of **29** toward **17** and BSA; b) MALDI-TOF MS profiles of BSA (blue line) and BSA-**29** (1:1) adduct (red line).

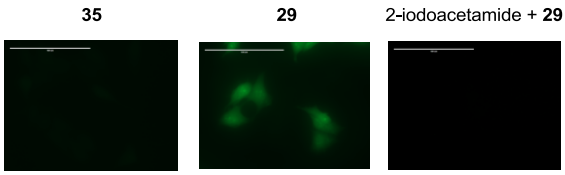


Fig. 5 Fluorescence microscopic analysis of MFC-7 cells treated with **29** (33 μM) and **35** (33 μM). The images of the cells were obtained using excitation at 470 nm and filters for emission at 525 nm. For the 2-iodoacetamide-treated sample, before the media were finally replaced with PBS containing **29**, the cells were incubated with media containing 2-iodoacetamide (10 mM).

In conclusion, we have demonstrated for the first time that α-aculosides are novel and selective Michael acceptors for the thiol function of cysteine residues. In addition, 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 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-α-aculoside hybrid to control the specific functions of certain proteins is now under way in our laboratories.

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