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# Preparation of immobilized swainsonine analogs on solid support

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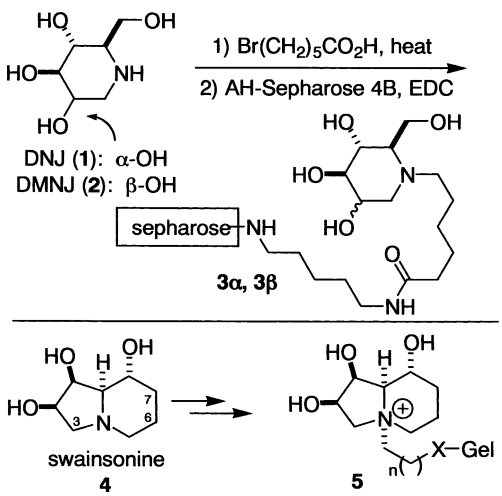
**Abstract**—Golgi α-mannosidase II and lysosomal mannosidase are examples of biologically important α-mannosidases that are difficult to purify. Swainsonine is an effective inhibitor of these α-mannosidases. In this report, analogs of swainsonine bearing an aminoalkyl group at C3 or C6 are synthesized and coupled with Affi-Gel 10 to prepare affinity matrices that may be useful for the preparation of α-mannosidases. Model ligands, where the aminoalkyl group is capped with an acyl group, are evaluated against a commercial α-mannosidase. 

## 1. Introduction

The purification of the various enzymes encountered in glycoprotein processing, particularly in mammalian systems, continues to be a challenge.[1–6] For example, the purification of mannosidase II from mung bean seedlings is laborious, involving many steps.[3] The isolation of Golgi α-mannosidase II from rat liver Golgi membranes is also tedious.[7–9] The difficult isolation of α-mannosidase II from any source, even with the advent of the cloning and expression work of Moremen and others,[5,7,10] has hampered the study of these important enzymes. Golgi α-mannosidase II, for example, is involved in the late-stage trimming of glycoproteins that are ultimately expressed as complex-type glycoproteins on the cell surface. The altered glycosylation patterns of such glycoproteins on the surface of cancer cells is associated with metastasis and disease progression, hence inhibitors of this enzyme are useful for cancer treatment.[11] Difficulties are also encountered in the isolation of human lysosomal mannosidase, an enzyme that is important in assaying the selectivity of potential cancer drugs.[12] The human lysosomal enzyme, although cloned and expressed by Moremen, is not yet available in sufficient quantities and purity to use in in vitro assays.

Affinity chromatography is a powerful tool for enzyme purification, assuming an appropriate ligand can be anchored to the polymer matrix.[13] Deoxynojirimycin (DNJ, **1**), an inhibitor of glucosidase I, and deoxymannojirimycin (DMNJ, **2**), an inhibitor of mannosidase I, have been used successfully as affinity ligands for the purification of these enzymes.[6] Scheme 1 shows the strategy that was used. *N*-Alkylation of the azasugars produced derivatives bearing a carboxyl group in the side-chain, which was used to anchor the azasugar to an agarose gel by amide bond formation, resulting in the affinity chromatography matrices **3**. Note that (**1**) and (**2**) are *secondary* amines. To prepare an affinity column for the purification of Golgi or lysosomal α-mannosidases, swainsonine (**4**) is an attractive ligand because of its strong inhibition of these enzymes.[14]




**Scheme 1.** Literature examples of azasugars as affinity ligands.[6,15] EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride.

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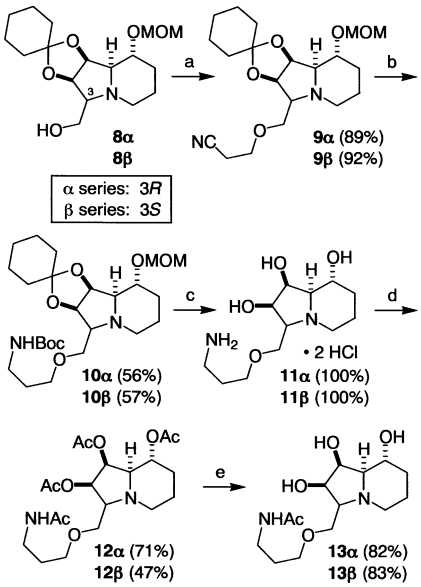
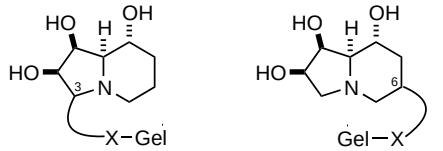



**Scheme 2.** *Reagents and conditions:* (a) NaOMe (2.2–2.8 equiv.), $CH_2$=CHCN (solvent), 0°C to rt. (b) $LiAlH_4$ (8–10 equiv.), $Et_2O$, rt; $NEt_3$ (1.1 equiv.), $Boc_2O$ (1.5–3 equiv.), MeOH, rt. (c) 6N HCl, THF, reflux, 1.5–2 days, concentrate, not further purified. (d) Dowex 1×8–200 (hydroxide form); aq. MeOH, $NEt_3$ (excess), $Ac_2O$ (excess), rt. (e) NaOMe (3.8 equiv.), MeOH, rt.

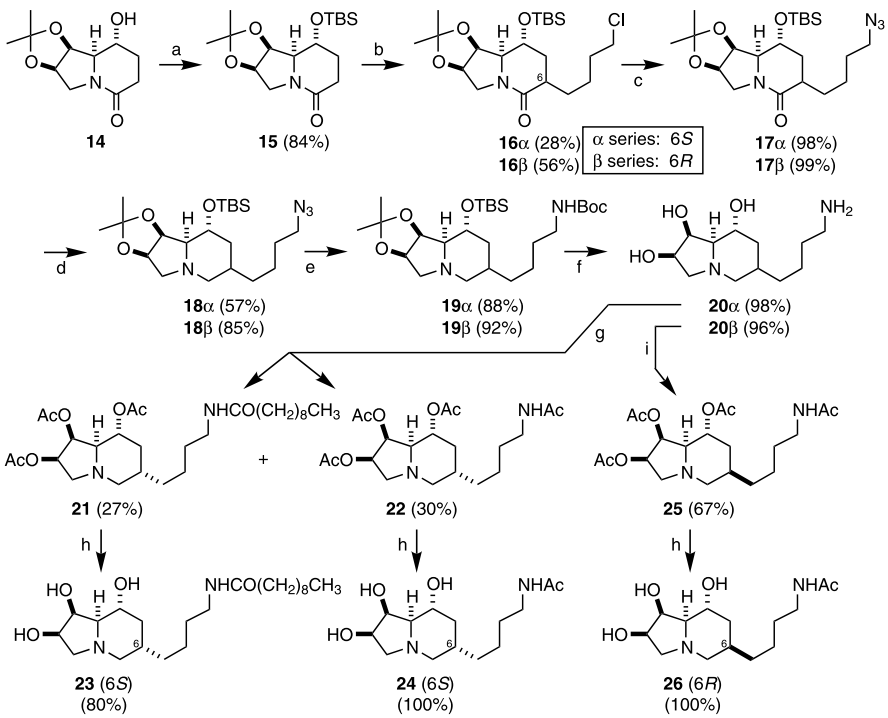
However, as a tertiary amine, functionalization by *N*-alkylation as was carried out for **1** and **2** results in a quaternary ammonium salt (e.g. **5**). This approach has been attempted, but has failed to produce a viable affinity matrix.[15] Attachment of the linker via one of the hydroxyl groups of swainsonine is an unattractive strategy, since they are required for recognition by the enzyme.

Our recent work on the synthesis of swainsonine analogs bearing side-chains at C3, C6, and C7 should allow us to build in a suitably tethered functional group for attachment to a gel without sacrificing the micromolar binding affinity necessary for affinity purification of α-mannosidases.[16–19] We now report the synthesis of such compounds, their evaluation as inhibitors of a suitable commercially available α-mannosidase, and the attachment of these affinity ligands to Affi-Gel 10 (Bio-Rad), e.g. **6** and **7** below. Affinity chromatography using these supports may prove useful in obtaining useful quantities of Golgi α-mannosidase II and lysosomal mannosidase enzymes that are important for assaying potential anticancer drugs.[11,12]




C3-tethered swainsonine ligand **6**

C6-tethered swainsonine ligand **7**




**Scheme 3.** *Reagents and conditions:* (a) *t*-$BuMe_2SiCl$ (TBSCl), imidazole, DMF, 0°C to rt. (b) *s*-BuLi (1.3 equiv.), THF, DMPU, −78°C, 1 h; $Br(CH_2)_4Cl$ (3 equiv.), 1 h. Yields shown are of the separated diastereomers **16α** and **16β**. (c) $NaN_3$, DMSO, 80°C, 3 days. Products were not purified further. (d) $BH_3{\cdot}SMe_2$ (4–8 equiv.), THF, 0°C to rt, 2 h; quench with ethanol, evaporate, then reflux with ethanol overnight. (e) $Boc_2O$ (2 equiv.), 20 weight% of 10% Pd–C, $H_2$ (1 atm), rt, 4 h. (f) 6N HCl, THF, rt, 20 h; Dowex 1×8–200 (hydroxide form), aqueous MeOH, overnight. (g) DCC (1.2 equiv.), decanoic acid (1 equiv.), MeCN, rt, overnight; excess $NEt_3$, excess $Ac_2O$, rt, overnight, separate **21** and **22**. (h) NaOMe (1.5–2 equiv.), MeOH, rt, overnight. (i) Excess $NEt_3$, excess $Ac_2O$, rt, overnight.

## 2. Results and discussion

Installation of an amine-bearing side-chain at C3 of swainsonine was accomplished as shown in Scheme 2. We have reported the synthesis of both C3 epimers of **8** from D-ribose.[16] Since we were uncertain of the relative effectiveness of the two epimers at C3 for affinity purification, we pursued both series. Conjugate addition of either **8α** or **8β** to acrylonitrile proceeded smoothly to produce the β-alkoxy nitriles **9α** and **9β**, which were reduced to the primary amines and protected as their Boc derivatives **10α** and **10β** to facilitate purification. Acidic hydrolysis of these compounds then gave the deprotected azasugars **11α** and **11β**, which were used to prepare the desired affinity matrices (vide infra). In order to verify that an amide-containing side-chain would be tolerated by α-mannosidases, the model compounds **13α** and **13β** were also prepared.

The synthesis of swainsonine analogs bearing an aminoalkyl group at C6 is shown in Scheme 3. We have already described a practical synthesis of the lactam **14**.[20] Protection of the alcohol of **14** followed by enolate alkylation gave the epimeric 6-(4-chlorobutyl)indolizidin-5-ones **16α** and **16β** in 28 and 56% yields, respectively, after separation. Again, we pursued the synthesis of both diastereomeric series. Azide displacement, amide reduction, azide reduction, and protection of the amines gave **19α** and **19β**. Acidic hydrolysis followed by ion-exchange chromatography gave the desired analogs **20α** and **20β**, ready for coupling to the affinity gel (vide infra). As in Scheme 2, simple amide analogs were also prepared in order to test the inhibitory power of these swainsonine analogs against a readily available α-mannosidase. Thus, further functional group manipulation afforded the amides **23**, **24**, and **26**.

Before proceeding with the coupling of **11** and **20** to an affinity gel, we screened the simple amide analogs **13α**, **13β**, **24**, **25**, and **26** against commercial jack bean α-mannosidase using standard methods.[21,22] This enzyme has been shown to be a useful model for mammalian α-mannosidases.[23] The free amines **11α** and **11β** were also tested. The $IC_{50}$s measured are reported in Fig. 1, where they are compared to those of swainsonine (**4**).[16,24,25] The C3-α analogs **11α** and **13α** were more potent inhibitors than the β-analogs **11β** and **13β**, as expected from our earlier work[16,17] and the presumed tolerance of α-mannosidases for an α-oriented group at this position, which correlates with C1 of mannose, the

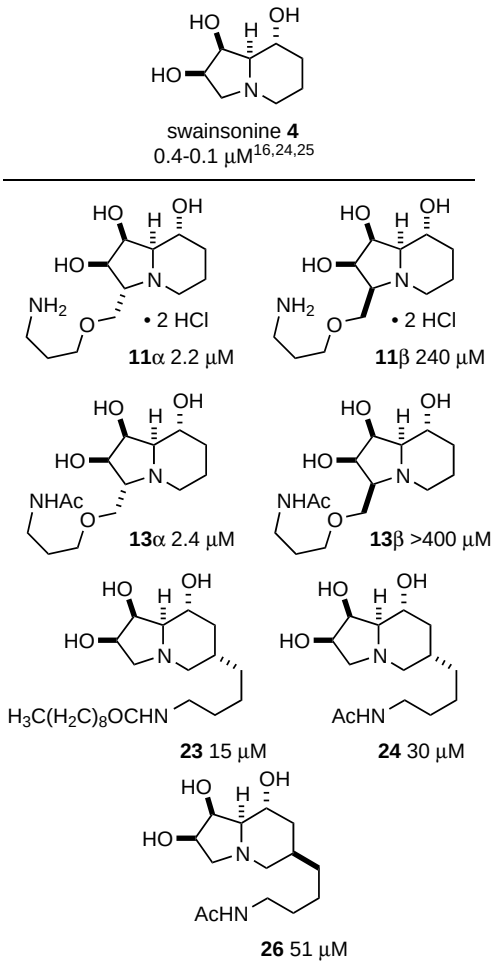
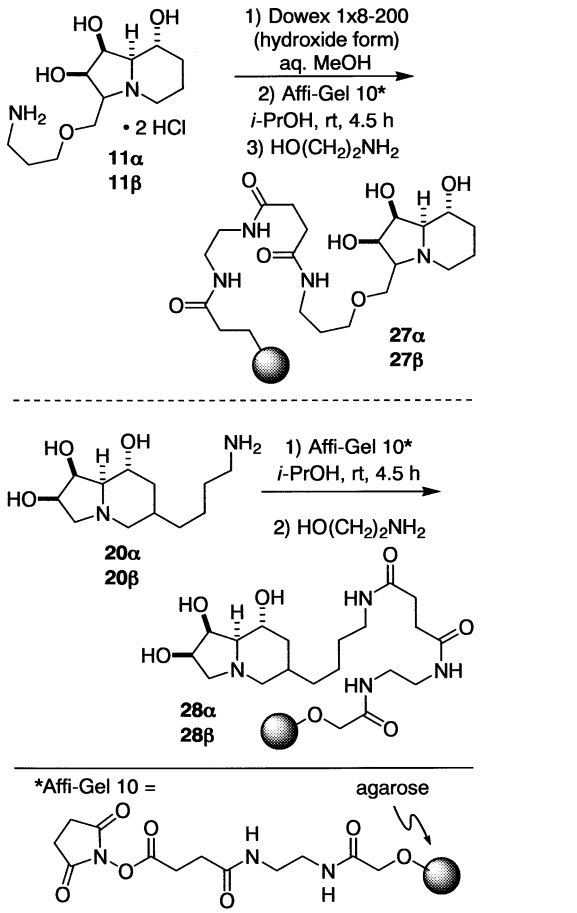



**Figure 1.** Concentration of swainsonine and analogs required to inhibit jack bean α-mannosidase by 50% ($IC_{50}$). The hydrochloride salts **11α** and **11β** were neutralized with Dowex 1×8–200 (hydroxide form) before testing. Concentration of substrate (*p*-nitrophenyl mannoside) = 0.8 mM; $K_m$ at 25°C = 1.52 mM; $K_m$ at 37°C = 0.68 mM.




**Scheme 4.** Coupling of swainsonine ligands to Affi-Gel 10 (Bio-Rad).

site of glycosidic cleavage. The C6 analogs **23**, **24**, and **26** showed less of a preference for a given configuration at this position. Based on these results, we hypothesized that affinity matrices derived from **11α**, **20α**, and **20β** would be the most useful.

The amines **11α**, **11β**, **20α**, and **20β** were coupled with Affi-Gel 10 (Bio-Rad) using the standard anhydrous coupling technique,[26,27] as shown in Scheme 4, producing the affinity matrices **27α**, **27β**, **28α**, and **28β**, respectively.

In summary, several affinity matrices have been prepared bearing tethered swainsonine ligands. Gel-free model compounds were also synthesized and found to be micromolar inhibitors of jack bean α-mannosidase, providing evidence that the immobilized ligands will be useful for affinity purification of more significant α-mannosidases. The use of these affinity matrices will be described elsewhere.

## Acknowledgements

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22. Enzyme inhibition was assayed colorimetrically by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl α-mannoside (Sigma) at pH 4.5 in 0.1 M sodium acetate buffer. The enzyme and inhibitor were preincubated for 20 min at 37°C in a total volume of 450 μL. The *p*-nitrophenyl α-mannoside substrate (50 μL of a 8.0 mM solution) was then added. After 25 min, 3 mL of a 2% sodium carbonate solution was added, and the concentration of *p*-nitrophenolate was measured at 410 nm. The assays were performed under conditions where the amount of *p*-nitrophenol released was linear with respect to both time and enzyme concentration.
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26. Coupling procedures may be found in the booklet 'Activated Immunoaffinity Supports' and Bulletin 1085, available from Bio-Rad Laboratories, Inc., Life Sciences Group, 2000 Alfred Nobel Drive, Hercules, CA 94547, USA.
27. For example, 27 mL of Affi-Gel 10 (0.41 mmol of activated ester) was washed with 10 volumes of cold isopropanol, then combined with 0.14 mmol of **20β** at rt. After 4.5 h, the unreacted acyloxysuccinates were blocked by the addition of 2.7 mL of a 1 M aqueous solution of ethanolamine (adjusted to pH 8 with HCl). The resulting support was washed with isopropanol and water, then stored in aqueous 0.2% sodium azide at 4°C.