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# Synthesis and mannosidase inhibitory activity of 6- and 7-substituted analogs of swainsonine

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**Abstract**—Swainsonine (**1**), an inhibitor of the important glycoprotein-processing enzyme Golgi α-mannosidase II, is a clinical candidate for cancer treatment. Analogs bearing substituents at C-6 and C-7 have been prepared and evaluated as inhibitors of α-mannosidase (jack bean), a closely related enzyme. © 2001 Published by Elsevier Science Ltd.

## 1. Introduction

Swainsonine (**1**) is a potent inhibitor of certain α-mannosidases, and has proven useful as a biochemical tool for the study of glycoprotein processing, since it inhibits a key late-stage enzyme in the biosynthesis of glycoproteins.[1–6] That enzyme, Golgi α-mannosidase II (GMII), is necessary for the formation of so-called 'complex glycoproteins'. The altered distribution of such glycoproteins on the surface of cancer cells is associated with metastasis and disease progression, hence inhibitors of GMII are potentially useful for cancer treatment.[7–10] Unfortunately, human GMII has proven difficult to isolate and characterize.[3,11,12] More selective inhibition of GMII over other mannosidases[13] is a desirable goal for cancer drug development, and makes the synthesis of analogs of swainsonine a significant undertaking (see accompanying paper). Many analogs of swainsonine have been reported, e.g. those where the oxygenation pattern, ring size, or configuration has been modified,[14] but these changes usually result in a diminution of potency. We report herein the synthesis and initial biological evaluation of 6- and 7-substituted analogs of swainsonine (**2** and **3**, respectively) (Fig. 1). We hoped that such substituents would afford more selectivity and potency in the inhibition of GMII, and we were also interested in collecting SAR data for this poorly studied system. In addition, we hoped that the relative remoteness of the planned substituents from the key functionality of swainsonine would allow linking to an affinity matrix in order to facilitate the isolation of human GMII.[15]

## 2. Results and discussion

The major goal of our initial work was to develop a route to both 6- and 7-substituted analogs of swainsonine, hopefully from a common intermediate. We were also interested in obtaining both diastereomers of each of these types of analogs. The scarcity of swainsonine and difficulties associated with the manipulation of its functional groups made it a poor choice of starting materials; thus, a totally synthetic route was developed.

Our initial efforts were aimed at producing 6-substituted swainsonine analogs, and resulted in the synthesis of both epimers of 6-ethylswainsonine (**18** and **20**) and

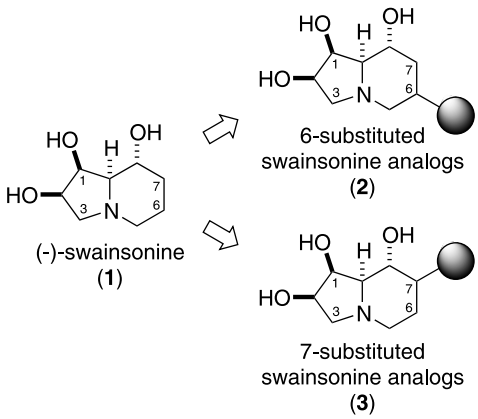


**Figure 1.** Swainsonine (**1**) and potential analogs (**2**) and (**3**).

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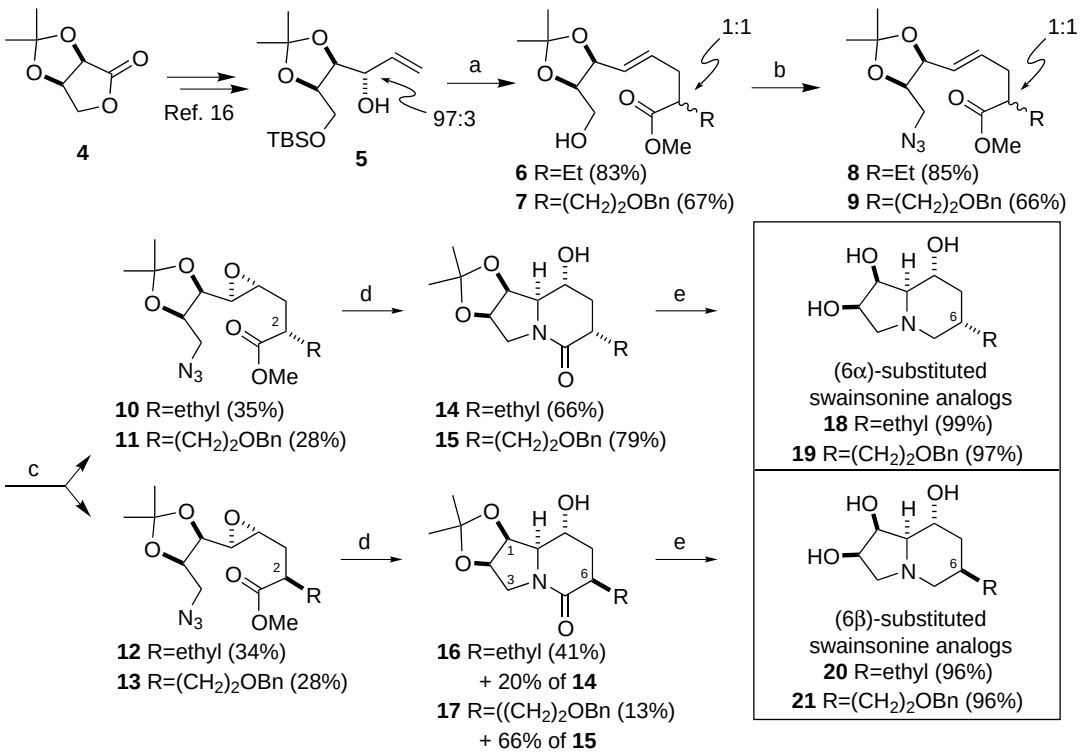
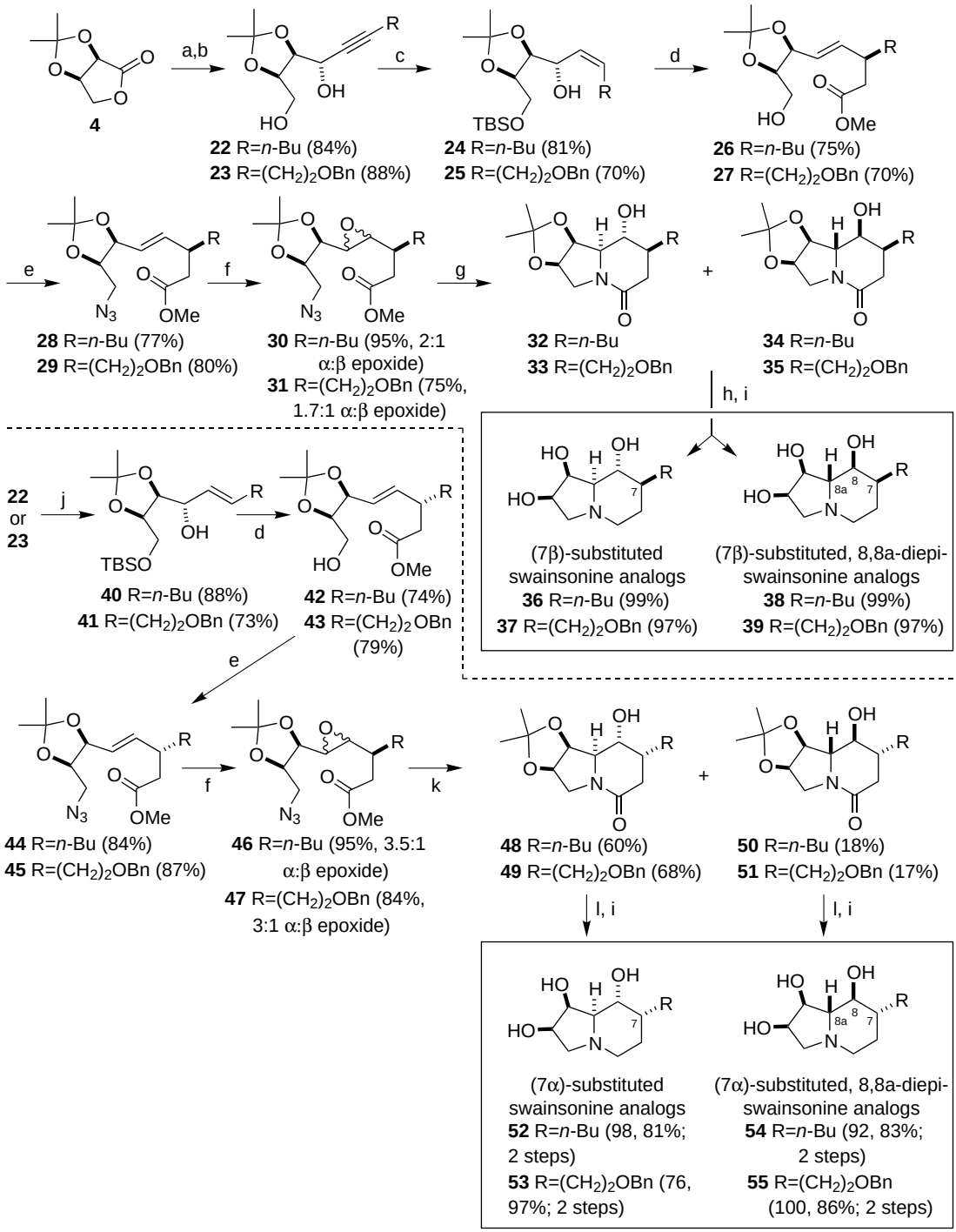
6-(2-benzyloxy)ethylswainsonine (**19** and **21**), as shown in Scheme 1. The allylic alcohol **5** was prepared from **4** as reported in our earlier work on the synthesis of swainsonine itself.[16] A Johnson orthoester Claisen rearrangement[17] using either trimethyl orthobutyrate or 4-benzyloxy-1,1,1-trimethoxybutane[18] gave the esters **6** and **7**, respectively, after desilylation, each as a 1:1 mixture of diastereomers. We chose this nonstereoselective method because we wished to prepare both epimers of the C-6 analogs. The Ireland version of the Claisen rearrangement[19] could presumably be used for the stereoselective formation of either of these diastereomers. Conversion of **6** and **7** to the azides **8** and **9** was followed by epoxidation and diastereomer separation to give **10**–**13**.[20] Reductive cyclization then afforded the indolizidin-5-ones **14**–**17**. Substantial epimerization was observed in the formation of **16** and **17**, presumably because of the axial orientation of the C-6 substituents in these compounds. Finally, lactam reduction and ketal hydrolysis afforded the desired 6-substituted swainsonine analogs **18**–**21**.

A similar route was chosen for the preparation of 7-substituted analogs of swainsonine (Scheme 2). Reduction of **4** followed by the addition of two different alkynyl Grignard reagents and primary alcohol protection gave the propargylic alcohols **22** and **23**, which were transformed in a stereoselective fashion into the *Z* or *E* allylic alcohols **24**/**25** and **40**/**41**, respectively, by selection of the appropriate reduction methods. Control of the allylic alcohol geometry, when combined with the Johnson orthoester Claisen rearrangement, should allow control of the configuration of the *R* substituent. Thus, Claisen rearrangements on **24**, **25**, **40**, and **41** followed by desilylation gave the esters **26**, **27**, **42**, and **43**, where the alkene geometry of the allylic alcohols had been completely translated into stereocontrol at the *R* substituents. Azide formation, epoxidation, and reductive cyclization was employed as described above to produce the indolizidinones **32**–**35** and **48**–**51**. In this case, the undesired epoxide diastereomers were carried on to the indolizidinones (i.e. **34**, **35**, **50**, and **51**), despite having the wrong configurations at C-8 and C-8a, since we were curious about the mannosidase inhibitory power of these bis-epimers. Lactam reduction and acetonide removal gave the 7-substituted swainsonine analogs **36**, **37**, **52**, and **53**, as well as the 7-substituted-8,8-diepiswainsonine analogs **38**, **39**, **54**, and **55**.

Screening of the new analogs **18**–**21**, **36**–**39**, and **52**–**55** against jack bean α-mannosidase were carried out using standard methods.[21] This enzyme is a commercially available enzyme that is a useful model for mammalian α-mannosidases such as GMII.[22] The $IC_{50}$s measured are reported in Table 1, where they are compared to those of swainsonine (**1**). Alkylation of the swainsonine backbone results in compounds with varying degrees of α-mannosidase inhibitory activity. The analogs **18** and **19** that have equatorially-disposed substituents at the C-6 position have the highest degree of inhibitory activ-




**Scheme 1.** Synthesis of 6-substituted swainsonine analogs. *Reagents and conditions:* (a) $PrC(OMe)_3$ or $BnO(CH_2)_3C(OMe)_3$, cat. $EtCO_2H$, toluene, reflux; *n*-$Bu_4NF$, THF; (b) $HN_3$, $PPh_3$, $EtO_2CN{=}NCO_2Et$, PhH; (c) *m*-CPBA, $CH_2Cl_2$; separate diastereomers. Each reaction also produced the other epoxide diastereomer (18% of 1:1 at C-2 for R = Et; 22% of 1:1 at C-2 for R = $(CH_2)_2OBn$); (d) $H_2$, $Pd(OH)_2$/C, MeOH, EtOAc; NaOMe, MeOH reflux; (e) $BH_3{\cdot}SMe_2$; 6N HCl/THF.




**Scheme 2.** *Reagents and conditions*: Synthesis of 7-substituted swainsonine analogs. (a) DIBAL-H; (b) RC CMgBr; (c) $H_2$, $Pd/BaSO_4$; $^tBuMe_2SiCl$, imidazole; (d) $MeC(OMe)_3$, cat. $EtCO_2H$, toluene, reflux; $n$-$Bu_4NF$, THF; (e) $HN_3$, $PPh_3$, $EtO_2CN{=}NCO_2Et$, PhH; (f) $m$-CPBA, $CH_2Cl_2$. Diastereomeric epoxides were not separated. (g) $H_2$, $Pd(OH)_2/C$, MeOH, EtOAc; NaOMe, MeOH reflux. Diastereomers at **8,8a** were not separated. Yields (ratio of swainsonine configuration:diepi-swainsonine configuration): R = $n$-Bu:60% (2.4:1); R = $(CH_2)_2OBn$:89% (1.7:1). (h) $BH_3{\cdot}SMe_2$; separate diastereomers; (i) 6N HCl/THF; (j) Red-Al®; $^tBuMe_2SiCl$, imidazole; (k) $H_2$, $Pd(OH)_2/C$, MeOH, EtOAc; NaOMe, MeOH reflux; separate diastereomers; (l) $BH_3{\cdot}SMe_2$.

ity. Increasing the substituent size from ethyl (**18**) to 2-(benzyloxy)ethyl (**19**) results in a slight decrease in inhibitory activity, while switching from equatorial to axial substitution at C-6 (**20** and **21**) leads to a significant decrease in activity. In contrast, most of the C-7 $n$-butyl and 2-(benzyloxy)ethyl-substituted compounds show complete loss of inhibitory activity, except for **53**, which is a weak inhibitor. As expected the 8,8a-diepiswainsonine analogs (**38**, **39**, **54, 55**) are devoid of activity.

**Table 1.** Concentration of swainsonine and analogs required to jack bean α-mannosidase by 50% ($IC_{50}$ inM)

| Compound | $IC_{50}$ (μM) |
|---|---|
| Swainsonine (**1**) | 0.4–0.1,[23,24] 0.1 (our labs)[25] |
| (6α)-Substituted analogs | |
| **18** (6α-Ethyl) | 30 |
| **19** (6α-$CH_2CH_2OBn$) | 230 |
| (6β)-Substituted analogs | |
| **20** (6β-Ethyl) | 70 |
| **21** (6β-$CH_2CH_2OBn$) | 275 |
| (7β)-Substituted analogs | |
| **36** (6β-*n*-Butyl) | NI |
| **37** (6β-$CH_2CH_2OBn$) | NI |
| (7α)-Substituted analogs | |
| **52** (6α-*n*-Butyl) | NI |
| **53** (6α-$CH_2CH_2OBn$) | 890 |
| 7-Substituted -8,8-diepi analogs | |
| **38** (6β-*n*-butyl) | NI |
| **39** (6β-$CH_2CH_2OBn$) | NI |
| **54** (6α-*n*-butyl) | NI |
| **55** (6α-$CH_2CH_2OBn$) | NI |

NI = no inhibition.

Our preliminary results indicate that there is a very low steric tolerance in the mannosidase binding pocket for substituents at C-7 of swainsonine, while substituents at C-6 are better accommodated. Further, the top (*beta*) face of swainsonine appears more sensitive to substitution than the bottom (*alpha*) face. This general pattern is consistent with the idea that the natural α-linked oligomannoside substrate has a sterically bulky α-face compared to its β-face. Note that the addition of the more bulky 2-(benzyloxy)ethylsubstituent in **19** has a relatively small effect on inhibitory activity compared to the ethyl-substituted **18**. It appears then that interactions between the β-face of swainsonine and the enzyme active site play the most important roles in binding ability. The α-face of swainsonine, on the other hand, is less essential for important binding interactions and thus synthetic modifications in this region may provide a means to differentiate between the various mannosidases while maintaining good inhibitory activity. Finally, the tolerance for relatively large substituents at C-6 bodes well for the construction of affinity labeling compounds and ligands for affinity chromatography. Results of these studies, as well as the results of inhibition studies with GMII, will be reported elsewhere.

## Acknowledgements

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