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Synthesis and mannosidase inhibitory activity of 3-benzyloxymethyl analogs of swainsonine

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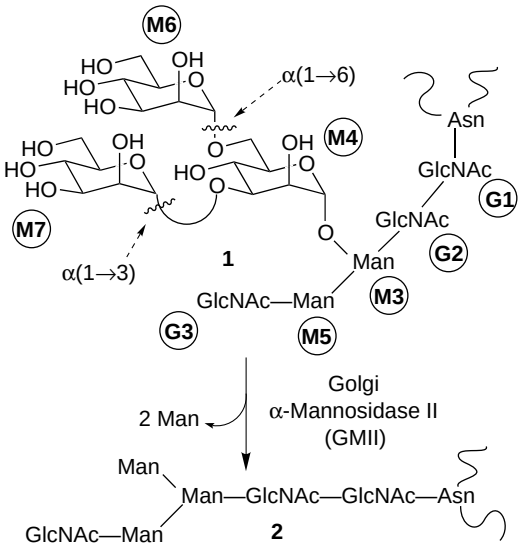
Abstract—Swainsonine (**3**), an inhibitor of Golgi α-mannosidase II, is a clinical candidate for cancer treatment. In order to avoid potential problems arising from its co-inhibition of lysosomal mannosidases, we have synthesized 3-benzyloxymethyl analogs of swainsonine (**17** and **18**). Initial screening of these new compounds is reported. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Golgi α-mannosidase II (GMII), a class II retaining[1,2] glycosyl hydrolase of family 38, is a key enzyme in the biosynthesis of *N*-linked glycoproteins, catalyzing the trimming of two mannose residues (M6 and M7) from the intermediate **1** ($GlcNAcMan_5GlcNAc_2AsnX$) to produce **2** ($GlcNAcMan_3GlcNAc_2AsnX$), the precursor of complex-type glycoproteins (Scheme 1).[3–9] The altered glycosylation patterns 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.[10–13]

The azasugars[14–17] swainsonine (**3**), deoxymannojirimycin (DMNJ, **4**), and 1,4-dideoxy-1,4-imino-D-mannitol (DIM, **5**) are examples of inhibitors of α-mannosidases, including GMII, and are thought to mimic the mannosyl cation **6** or the oxonium-like transition state encountered during hydrolysis (Fig. 1).[3,4,14,16,18–23] Swainsonine has been investigated as an



Scheme 1. Golgi α-mannosidase II (GMII) trims two mannose residues from the substrate **1** to produce **2**.

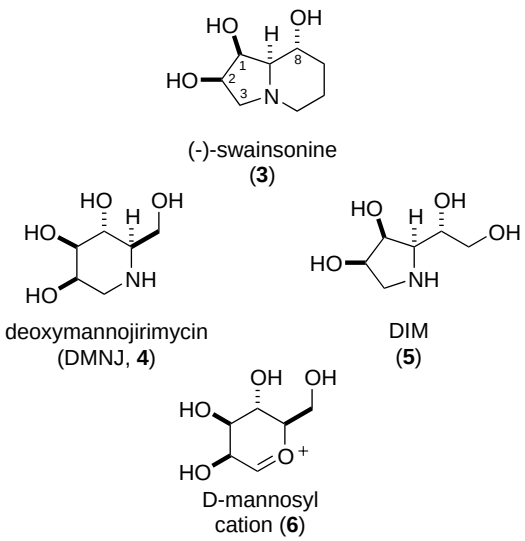


Figure 1. Inhibitors of α-mannosidases are proposed to mimic the mannosyl cation or the oxonium-like transition state encountered during hydrolysis.

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anti-cancer agent,[10,11,24–29] and in clinical trials, it has been found to reduce tumor growth and metastasis.[10,11] Although swainsonine shows low toxicity and good oral availability, it also inhibits lysosomal mannosidase,[1,4,30,31] a catabolic class II retaining α-mannosidase, causing the accumulation of high-mannose oligosaccharides in tissues (swainsonine-induced mannosidosis).[32–34] Synthetic analogs of swainsonine or other α-mannosidase inhibitors may allow selective inhibition of the Golgi enzyme over the lysosomal enzyme. Toward that end, we report herein the synthesis of 3-benzyloxymethyl analogs of swainsonine and their initial evaluation as inhibitors of jack bean α-mannosidase, a commercially available enzyme that is a useful model for mammalian α-mannosidases.[35]

2. Results and discussion

At the time these studies were initiated, there was no structural information available on GMII to guide inhibitor design, thus we began by considering the substrate for the enzyme, molecule **1**. Both M6 and M7 are α-linked to M4, thus we proposed that placing a sugar or sugar-like molecule on swainsonine at C(3), the position corresponding to the anomeric carbon of the mannosyl cation **6**, i.e. structure **7** in Fig. 2, would be tolerated by GMII, and may lead to the discovery of analogs with better selectivity for this enzyme over lysosomal α-mannosidase. It is perhaps unsurprising that swainsonine cannot discriminate between these two enzymes, since both are class II retaining α-mannosidases with high sequence homology.[1,31] More complex structures may be more selective; for example, a crucial requirement for the action of GMII is the presence of a particular *N*-acetylglucosamine residue (GlcNAc G3) (Scheme 1).[36]

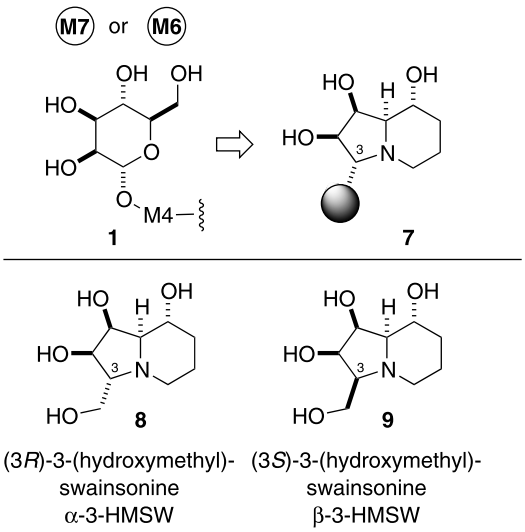


Figure 2. 3-Substituted swainsonine analogs **7** may mimic the oxonium-like transition state encountered in the hydrolysis of **1** by GMII. Compounds **8** and **9**, for example, have a hydroxymethyl group at the 'anomeric' carbon of swainsonine.

Our initial efforts resulted in the synthesis of (3*R*)- and (3*S*)-3-(hydroxymethyl)swainsonine, **8** and **9**, respectively.[37,38] Both were found to be inhibitors of α-mannosidases, although not as potent as swainsonine (Table 1). Regarding the goal of inhibition of GMII rather than lysosomal mannosidase, **8** was unselective (as is swainsonine **3**), but **9** was found to show promising selectivity, an unexpected result, since **9** has the β-configuration at C-3 (cf expectations based on structures **1** and **7**). Given this promising result, we were encouraged to study analogs of **8** and **9** wherein the 3-hydroxymethyl group had been modified. We chose to examine initially 3-benzyloxymethyl derivatives of swainsonine, not only for synthetic ease, but because the aryl substituents may serve as a second carbohydrate-like group, thus yielding disaccharide mimics of the M6–M4 or M7–M4 portion of **1** in Scheme 1, where the aryl group plays the role of M4.

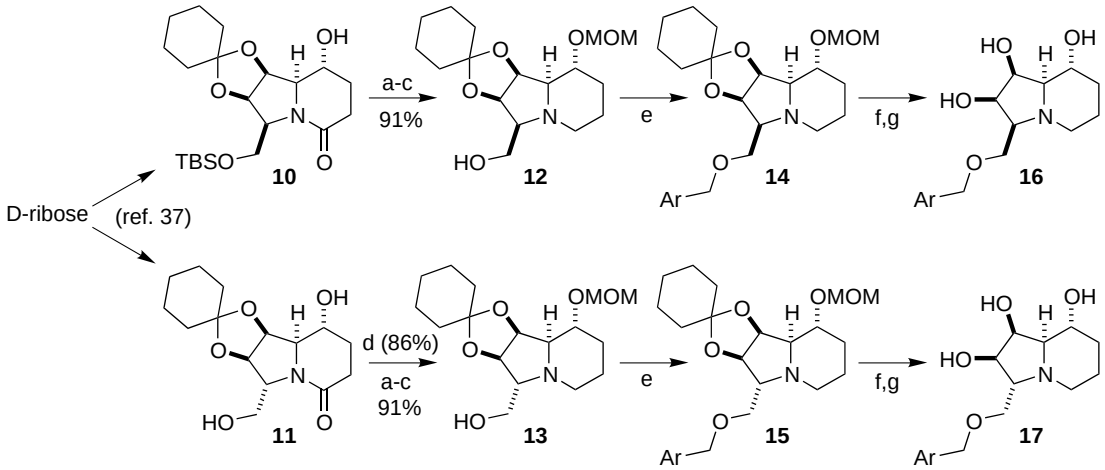
D-Ribose was transformed into the indolizidinones **10** and **11** using our previously reported procedures.[37] Protection of the C-8 hydroxyl group of **10** followed by desilylation and lactam reduction afforded **12**. *O*-Alkylation of **12** with several benzyl halides gave **14**, which could be converted to the desired analogs **16** of (3*S*)-3-(hydroxymethyl)swainsonine (**9**) by acid hydrolysis and subsequent ion-exchange chromatography. Selective monosilylation of **11** followed by a similar sequence provided the analogs **17** of (3*R*)-3-(hydroxymethyl)swainsonine (**8**) (Scheme 2).

Since GMII and lysosomal mannosidases are not readily available, as a first step toward evaluating these new analogs, we report their inhibitory action against jack bean α-mannosidase, a commercially available enzyme that has proven to be an excellent model for mammalian α-mannosidases. It is also a class II, family 38 retaining mannosidase, and shares high sequence homology with GMII.[1,35,42]

Screening of the new analogs **16a–d** and **17a–e** against jack bean α-mannosidase was carried out using stan-

Table 1. Concentration of swainsonine and analogs required to inhibit various α-mannosidase by 50% (IC_{50} in μM)

	Jack bean α-mannosidase	Lysosomal α-mannosidase (source)	Golgi α-mannosidase (source)
3	0.4–0.1[4,19]	0.07–0.20 (rat liver)[4,30]	0.08–0.20 (rat liver)[4,18,41]
	0.1 (our labs)[37]	0.08–0.5 (human)[34,39–41]	0.04 (human)[23]
8	1.2[37]	1.7 (human)[41]	1.3–2.2 (rat liver)[41]
9	45[37]	39 (human)[41]	1.8 (rat liver)[41]



Alkylating agent $ArCH_2X$	Benzylation product from step e (yield)	Deprotection product from steps f&g (yield)
$PhCH_2Br$	**14a** (84%)	**16a** (81%)
2-naphthyl-CH_2Br	**14b** (70%) + **12** (28%)	**16b** (70%)
4-Ph-$PhCH_2Br$	**14c** (72%) + **12** (21%)	**16c** (86%)
4-*t*-Bu-$PhCH_2Br$	**14d** (not purified)	**16d** (24%, 3 steps)
$PhCH_2Br$	**15a** (89%)	**17a** (82%)
2-naphthyl-CH_2Br	**15b** (65%) + **13** (23%)	**17b** (74%)
4-Ph-$PhCH_2Br$	**15c** (79%) + **13** (5%)	**17c** (100%)
4-*t*-Bu-$PhCH_2Br$	**15d** (85%)	**17d** (54%)
4-Me-$PhCH_2Cl$	**15e** (56%) + **13** (26%)	**17e** (93%)

Scheme 2. *Reagents and conditions*: (a) *i*-Pr_2NEt, CH_3OCH_2Cl (MOMCl), −10°C to rt; (b) *n*-Bu_4NF, THF, rt; (c) $BH_3{\cdot}SMe_2$, THF, rt. (d) *t*-$BuMe_2SiCl$ (TBDMSCl), 2,6-lutidine, CH_2Cl_2, −15°C; (e) NaH, $ArCH_2X$, *n*-Bu_4NI, THF, rt; (f) 6N HCl, THF, rt; (g) Dowex 1×8–200.

dard methods.[43] The IC_{50}s measured are reported in Table 2, where they are compared to those of swainsonine (**3**), (3*R*)-3-(hydroxymethyl)swainsonine (**8**, α-3-HMSW) and (3*S*)-3-(hydroxymethyl)swainsonine (**9**, β-3-HMSW), data obtained previously in our labs.[37,44] Analogs **17**, with an α-oriented 3-benzyloxymethyl substituent, are considerably more potent inhibitors than the β-3-benzyloxymethyl analogs **16**, which is consistent with the hypothesis that the C(3) substituent mimics the α-oriented carbohydrate that is cleaved by α-mannosidases, e.g. M4 in structure **1** (Scheme 1) and Fig. 2. Another important aspect of the data in Table 2 is that compounds **17** are as potent as or more potent than swainsonine in inhibiting this α-mannosidase. Swainsonine is considered to be one of the best α-mannosidase inhibitors known and, to date, attempts to make better inhibitors have met with difficulty. The importance of the α-3-substituent is further illustrated by the ability of **18** (8-epihomoaustraline)[45] to inhibit this α-mannosidase, despite the non-*manno* configuration. We had synthesized this compound earlier in studies aimed at inhibiting glucosidases, and were surprised to find that it was an inhibitor of a mannosidase, which led us to the design and synthesis of **8** and **9**. Although neither of these compounds proved to be as potent as swainsonine, it is gratifying to find that the α-3-benzyloxymethyl analogs reported herein are excellent α-mannosidase inhibitors.

In summary, we have synthesized the first analogs of swainsonine bearing carbohydrate-like substituent at C(3), the position that has been proposed to be analogous to the anomeric center of the mannose substrates of α-mannosidases. Some of the analogs were shown to exceed swainsonine in the ability to inhibit jack bean α-mannosidase, with IC_{50}s as low as 50 nM. We will report elsewhere the evaluation of these and other related analogs against Golgi α-mannosidase II and lysosomal mannosidases. Further, since the first X-ray crystallographic determination of the structure of an α-mannosidase II has appeared, including structures with swainsonine or deoxymannojirimycin bound in the active site,[46] we will hopefully be able to design more selective and potent inhibitors of GMII.

Acknowledgements

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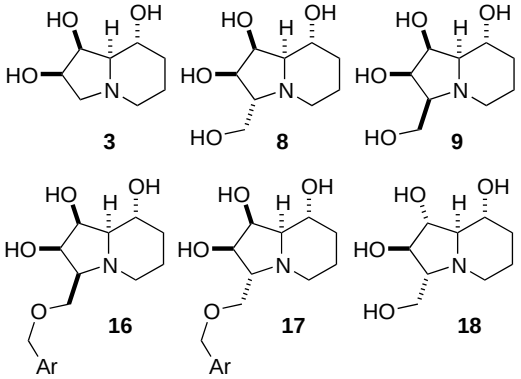
Table 2. Concentration of swainsonine and analogs required to inhibit jack bean α-mannosidase by 50%. Compound **18**,[45] having a non-*manno* configuration, is also shown (see text)



Compound	Ar	IC_{50} (μM)
3 (swainsonine)	–	0.40[4]
3 (swainsonine)	–	0.10[37,42]
8 (α-3-HMSW)	–	1.2[37]
9 (β-3-HMSW)	–	45[37]
16a	Ph	100
16b	2-Naphthyl	17
16c	4-Ph-Ph	>200
16d	4-*t*-Bu-Ph	>200
17a	Ph	0.39
17b	2-Naphthyl	0.11
17c	4-Ph-Ph	0.08
17d	4-*t*-Bu-Ph	0.05
17e	4-Me-Ph	0.07
18	–	150[45]

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263 [(3*S*)-3-(hydroxymethyl)swainsonine, **9**]. These data may be found in: Shah, R.; Carver, J.; Marino-Albernas, J.; Tvaroska, I.; Tropper, F.; Dennis, J., US Patent 6,048,870, 2000.

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