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Synthesis of the New Mannosidase Inhibitors, Diversity-Oriented 5-Substituted Swainsonine Analogues, via Stereoselective Mannich Reaction

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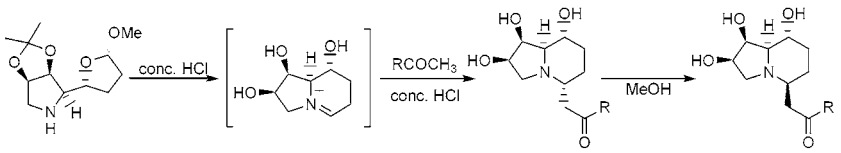
ABSTRACT



5α-Substituted swainsonine analogues were synthesized by Mannich reaction of an in situ generated (−)-swainsonine iminium ion intermediate. 5α-Substituted swainsonine analogues were epimerized to their 5*β*-isomers in protic solvent.

Golgi α-mannosidase II (GMII), a key enzyme in the biosynthesis of *N*-linked glycoproteins, is a molecular target for anticancer agents.[1] The distribution of the *N*-linked sugars on the cell surface is altered in various tumor cell lines, and this unusual protein glycosylation correlates with the progression of tumor metastasis.[2] In preliminary clinical trials, (−)-swainsonine (**1**), a potent inhibitor of GMII, was found to reduce tumor growth and metastasis.[3] It has also been shown to exhibit pleiotropic effects as an immunomodulatory agent. These effects include augmentation of tumoricidal activities of natural killer cells[4] and macrophages,[5] as well as stimulation of bone marrow cells.[6]

Owing to such biological properties, many molecular modifications of swainsonine (**1**) (summarized in Figure 1) have been made and studied in efforts to gain insight on the molecular bases of these activities as well as to develop more potent immunomodulatory anticancer agents.[7] For example, Pearson synthesized the first analogues of swainsonine bearing carbohydrate-like α-substituents at C(3).[7e] These were shown to be more potent inhibitors of jack bean α-mannosi-

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(1) (a) Goss, P. E.; Baker, M. A.; Carver, J. P.; Dennis, J. W. *Clin. Cancer Res.* **1995**, *1*, 935. (b) Roberts, J. D.; Klein, J. L.; Palmantier, R.; Dhume, S. T.; George, M. D.; Olden, K. *Cancer Detect. Prev.* **1998**, *22*, 455.
(2) (a) Dennis, J. W.; Granovsky, M.; Warren, C. E. *Bioessays* **1999**, *21*, 412. (b) Dennis, J. W.; Granovsky, M.; Warren, C. E. *Biochim. Biophys. Acta* **1999**, *1473*, 21.
(3) (a) Goss, P. E.; Reid, C. L.; Bailey, D.; Dennis, J. W. *Clin. Cancer Res.* **1997**, *3*, 1077. (b) Goss, P. E.; Baptiste, J.; Fernandes, B.; Baker, M.; Dennis, J. W. *Cancer Res.* **1994**, *54*, 145.
(4) (a) Galustian, C.; Foulds, S.; Dye, J. F.; Guillou, P. J. *Immunopharmacol.* **1994**, *27*, 165. (b) Fujieda, S.; Noda, I.; Saito, H.; Hoshino, T.; Yagita, M. *Arch. Otolaryngol. Head Neck Surg.* **1994**, *120*, 389.
(5) (a) Das, P. C.; Roberts, J. D.; White, S. L.; Olden, K. *Oncol. Res.* **1995**, *7*, 425. (b) Grzegorzewski, K.; Newton, S. A.; Akiyama, S. K.; Sharrow, S.; Olden, K.; White, S. L. *Cancer Commun.* **1989**, *1*, 373.
(6) Oredipe, O. A.; Furbert-Harris, P. M.; Green, W. R.; White, S. L.; Olden, K.; Laniyan, I.; Parish-Gause, D.; Vaughn, T.; Griffin, W. M.; Sridhar, R. *Pharmacol. Res.* **2003**, *47*, 69.
(7) (a) Michael, J. P. *Nat. Prod. Rep.* **2003**, *20*, 458. (b) Asano, N.; Kato, A.; Watson, A. A. *Mini Rev. Med. Chem.* **2001**, *1*, 145. (c) El Nemr, A. *Tetrahedron* **2000**, *56*, 8579. (d) Pearson, W. H.; Hembre, E. J. Book of Abstracts. In *213th ACS National Meeting*; San Francisco, April 13−17, 1997; American Chemical Society: Washington, DC, 1997; ORGN. (e) Pearson, W. H.; Guo, L. *Tetrahedron Lett.* **2001**, *42*, 8267. (f) Carmona, A. T.; Fuentes, J.; Robina, I.; Rodriguez Garcia, E.; Demange, R.; Vogel, P.; Winters, A. L. *J. Org. Chem.* **2003**, *68*, 3874.

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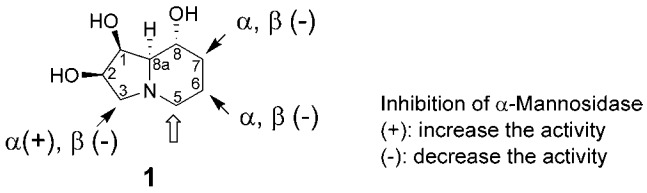

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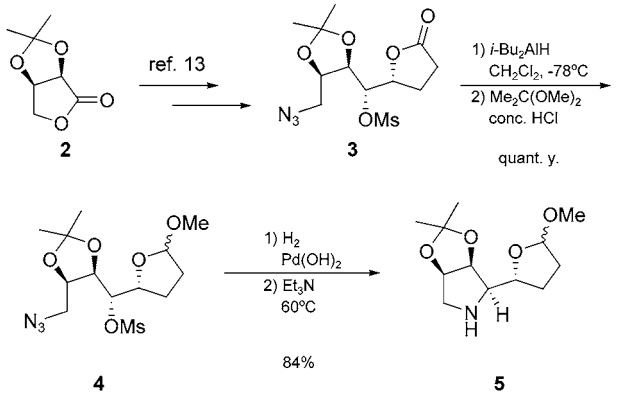
Figure 1. Molecular modifications of (−)-swainsonine **(1)**.

dase than swainsonine itself. The X-ray crystallographic structure of GMII in the presence of swainsonine supported his proposal that the 3-substituent is an effect mimic of a disaccharide.[8,9] Introduction of 6- or 7-substituents in either axial and equatorial orientations resulted in loss of activity.[10] These molecular modifications were viewed as a key to a diversity-oriented synthetic strategy.[11] The goals were to optimize the binding interactions between drug and target macromolecule and to delineate the disease-related biological pathways.

As part of our contributions to the development of new swainsonine analogues to help achieve these goals, we describe herein the stereoselective synthesis of 5-substituted swainsonine analogues and the structure−activity relationship (SAR) of their α-mannosidase inhibitory activities. To introduce substituents to (−)-swainsonine (**1**) in the 5-position, a Mannich reaction was performed with a swainsonine iminium ion generated in situ from amine acetal **5**. As shown in Scheme 1, the key iminium salt precursor **5** was

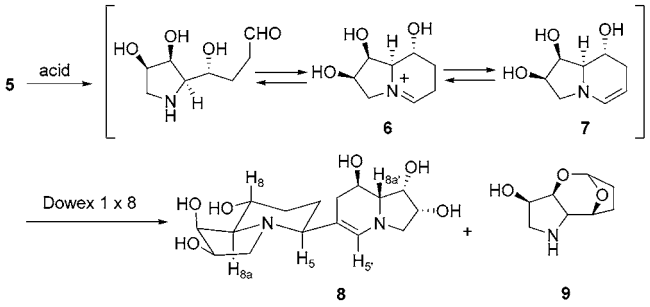
Scheme 1



2 3 4 5

synthesized from azido lactone **3**,[12] which was prepared from commercially available 2,3-*O*-isopropylidene-D-erythronolactone (**2**) as described in the literature.[13] The azido lactone **3** was subjected to reduction with diisobutylalumium hydride,

Scheme 2



methylation, and then palladium-catalyzed hydrogenolysis to give the cyclic amine acetal **5** in good yields.

Acid-catalyzed cleavage of protective groups present in amine acetal **5** in protic solvent followed by treatment with anion-exchange resin (Dowex 1X8) did not produce the desired iminium salt. Instead, a swainsonine dimer **8** was formed by reaction of iminium ion **6** with the corresponding enamine **7** (Scheme 2) (Table 1, entries 1 and 2).

Table 1. Solvent Effect on Dimerization Reaction

				yield[a] (%)	
entry	acid	solvent	time (h)	**8**	**9**
1	HCl	H_2O	21.5	quant	0
2	HCl	MeOH	24	68[b]	33[b]
3	HCl	CH_3CN	1.5	7[b]	92[b]
4	$BF_3{\cdot}Et_2O$	CH_3CN	24	0	81

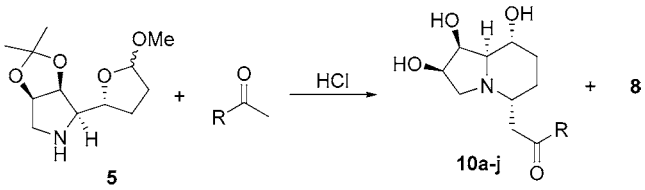
[a] Isolated yields. [b] Determined by ^{1}H NMR.

In contrast, the intramolecular acetal **9** was obtained as a major product if deprotection were carried out in acetonitrile as a solvent (Table 1, entries 3 and 4). The iminium salt **6** apparently is not so readily formed in acetonitrile because the nitrogen lone pair is less available under the aprotic acidic conditions that stabilize cationic species, than in protic solvents. However, intramolecular acetal **9** could be converted into the desired iminium salt **6** under these conditions since the ratio of **8** to **9** increased from 68/33 (Table 1, entry 2) to 87/4 after and additional 66 h reaction time.

With respect to dimer formation, ^{1}H NMR analysis of **8** revealed that enamine **7** attacked the iminium salt **6** from an equatorial direction to afford the more stable adduct **8** selectively. The chemical shift for H-5 was observed at δ 2.24 (dd, J = 2.1, 11.3 Hz) in swainsonine dimer **8**. The large J value (11.3 Hz) between H-5 and H-6 was also indicative of trans diaxial coupling and equatorial orientation (β-position) of the swainsonine enamine moiety, the vinyl

(8) van den Elsen, J. M.; Kuntz, D. A.; Rose, D. R. *EMBO J.* **2001**, *20*, 3008.
(9) Numao, S.; Kuntz, D. A.; Withers, S. G.; Rose, D. R. *J. Biol. Chem.* **2003**, *278*, 48074.
(10) Pearson, W. H.; Hembre, E. J. *Tetrahedron Lett.* **2001**, *42*, 8273.
(11) Schreiber, S. L. *Science* **2000**, *287*, 1964.
(12) Selected data for **3**: $[\alpha]^{22}_D$ +78.6 (*c* 0.51, $CHCl_3$); mp 140 °C (from $CHCl_3$/Et_2O) [lit.[13] $[\alpha]^{23}_D$ +75.0 (*c* 0.52, $CHCl_3$); mp 136 °C (from $CHCl_3$/Et_2O)]. **3** was converted to (−)-swainsonine ($[\alpha]^{24}_D$ −86.9 (*c* 0.13, MeOH) [lit.[13] $[\alpha]^{23}_D$ −74.0 (*c* 0.98, MeOH), lit.[17] $[\alpha]^{20}_D$ −87.2 (*c* 1, MeOH)] with an overall yield of 46% according to ref 13.
(13) Pearson, W. H.; Hembre, E. J. *J. Org. Chem.* **1996**, *61*, 7217.

Table 2. Mannich Reaction of Amine Acetal **5** and Various Ketones



						yield (%)	
entry	R	solvent	T (°C)	time h		**10**	**8**
1[a]	Ph[d]	EtOH	rt	28	**a**	46	53
2[b]	Ph	EtOH	reflux	1	**a**	38	42
3[c]	Ph[d]	80% CH_3CN/EtOH	70	3	**a**	72	30
4[a]	*p*-MePh[d]	EtOH	70	2	**b**	49	32
5[b]	*p*-*t*-BuPh	EtOH	reflux	2	**c**	34	36
6[b]	*p*-BrPh	EtOH	reflux	3	**d**	29	55
7[b]	*p*-IPh	EtOH	reflux	0.5	**e**	18	61
8[b]	Et	MeOH	reflux	4	**f**	28	50
9[b]	Pr	MeOH	reflux	11.5	**g**	32	23
10[b]	*n*-Bu	MeOH	reflux	32	**h**	31	4
11[b]	Cy	EtOH	reflux	32	**i**	29	36
12[b]	*t*-Bu	MeOH	reflux	19	**j**	22	58

[a] HCl (3 equiv). [b] 11 equiv. [c] 1.5 equiv. [d] When the SM spot disappeared on TLC, 6 N HCl was added and the solution was stirred for 30 min at 70 °C.

proton H-5′ of which appeared at the lowest field (δ 5.97). The large J value (9.2 Hz) between H-8a (δ 1.75, dd, J = 3.8, 9.2 Hz) and H-8 (δ 3.76, ddd, J = 4.8, 9.2, 10.9 Hz) also indicated that they were in a trans diaxial relationship. The axial orientation of the bridgehead proton H-8a and a Bohlmann band (2854 cm^{-1}) in the IR spectrum were consistent with the trans-fused indolizidine structure.[14]

Formation of this trans-fused indolizidine dimer indicated that stereoselective acid-catalyzed Mannich reactions of iminium salt **6** derived from **5** would occur in protic solvent to produce 5-substituted swainsonine analogues. We next examined reactions of amine acetal **5** and various ketones (Table 2) in order to introduce various alkyl groups at C(5) of swainsonine. [15] In all reactions, two major fractions were obtained, as shown by reversed-phase HPLC. The less polar product proved to be the Mannich adduct. The more polar product was not identified, but this same byproduct was obtained in every reaction with various ketones. Upon being passed through a basic ion-exchange column (Dowex 1X8), this byproduct was converted to swainsonine dimer **8**. Whereas the addition of acetonitrile to protic solvent

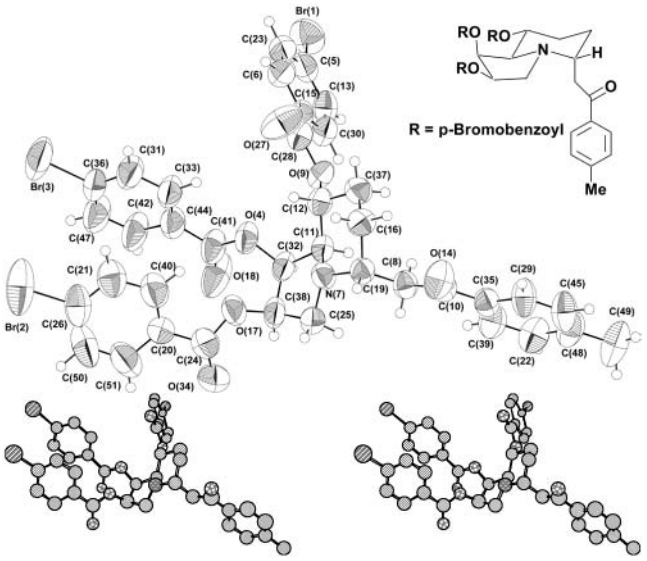


Figure 2. Perspective view and stereoview of tri-4-bromobenzoate of **10b**.

improved the yield of Mannich product (Table 2, entry 3), attempted reaction in pure acetonitrile led only to decomposition of starting material. Aromatic ketones were more reactive than aliphatic ones presumably due to greater stabilization of their enol form (Table 2, entries 1−5). Unsymmetrical dialkyl ketones gave a single regioisomer by reaction at the less substituted α carbon (Table 2, entries 8−11).[16] It was interesting to note that the less stable diastereomers resulting from axial attack (α-position) of enols were formed selectively. This result is in contrast to the formation of the swainsonine dimer byproduct **8** that occurs through equatorial attack. The stereochemistries of Mannich adducts were determined by their NMR analyses and X-ray diffraction. The Mannich adduct **10b** [(5*R*)-5-[2′-oxo-2′-(4-methylphenyl)ethyl]swainsonine] was converted to the 4-bromobenzoylate to give a single crystal for X-ray determination of the absolute stereochemistry (Figure 2). Its X-ray structure confirmed that the 5-substituent occupied the axial position and that the ring junction was trans-fused. In all adducts, the chemical shift for H-5 appeared in the 3.62−3.35 ppm range (Table 3) and a Bohlmann band (2800−2850 cm^{-1}) was present in the IR spectrum. These data indicated that products were formed exclusively by axial attack. Axial attack on the cyclic iminium salt in a half-chair conformation through a stable chairlike transition state would be favored under conditions of kinetic control.

We also found that it is possible to epimerize the stereocenter at C(5) to afford the (5β)-diastereomers of the Mannich adducts. This can be effected in reasonable yield by incubation in MeOH for several days or by the treatment with basic ion-exchange resin (Dowex 1X8) in 50% MeOH/water for several hours (Scheme 3). After this inversion to

(14) (a) Bohlmann, F.; Mueller, H. J.; Schumann, D. *Chem. Ber.* **1973**, *106*, 3026. (b) Bohlmann, F.; Schumann, D.; Arndt, C. *Tetrahedron Lett.* **1965**, 2705.

(15) **General Procedure.** A solution of **5** (10 mg, 0.04 mmol) in a solvent (500 μL) was added to a solution of 40 to 100% excess of various ketones (250−500 μL) and concentrated HCl in a solvent (500 μL) under reflux. After the reaction for the period of time indicated in Table 2, the mixture was evaporated, dissolved in water and extracted with Et_2O. The aqueous layer was lyophilized and purified by preparative reversed-phase HPLC (C18, acetonitrile/water gradient elution system from 0 to 100% over the period of 60 min).

(16) Jasor, Y.; Gaudry, M.; Luche, M. J.; Marquet, A. *Tetrahedron* **1977**, *33*, 295.

Table 3. NMR Chemical Shifts[a] (H-5) and α-Mannosidase Inhibitory Activity[b] of 5-Substiutued Swainsonine Analogues

	5α analogue			5β analogue	
R	H-5eq (ppm)	IC_{50} (μM)		H-5ax (ppm)	IC_{50} (μM)
Ph **10a**	3.62	0.51	**11a**	2.54	1.55
p-MePh **10b**	3.59	0.25	**11b**	2.62	0.91
p-*t*-BuPh **10c**	3.51	0.22	**11c**	2.52	2.54
p-BrPh **10d**	3.50	0.42	**11d**	2.67	ND[c]
p-IPh **10e**	3.51	0.33	**11e**	2.54	ND[c]
n-Bu **10h**	3.45	1.27	**11h**	2.40	2.70
Cy **10i**	3.35	0.82	**11i**	2.31	3.29
t-Bu **10j**	3.48	2.45	**11j**	ND[c]	ND[c]
			Dimer **8**	2.24	3.80

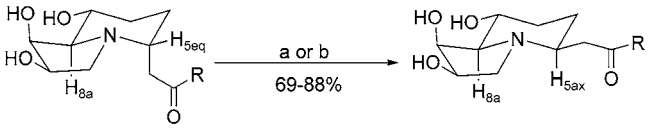
[a] δ values in ppm from TMS. [b] The IC_{50} for inhibition of jack bean α-mannosidase, IC_{50} of (−)-swainsonine; 0.25 μM, (lit.[18] 0.4 μM). [c] ND: not determined.

the β configuration, H-5 protons exhibited a upfield shift to 2.67−2.31 ppm in the ^{1}H NMR spectra. These relationships are similar to that between axial proton H-5ax (δ 1.85) and equatorial proton H-5eq (δ 2.90) of swainsonine.[17]

Mannosidase inhibitory activities of the new 5-substituted swainsonine analogues were evaluated against jack bean α-mannosidase.[18] All of the (5α)-substituted swainsonine analogues have higher inhibitory activity than the corresponding (5β) epimer (Table 3). The axial-oriented (α-face) aromatic substituents at C(5) position proved to be more effective for inhibitory activity than aliphatic groups. Increasing the size of a para-subsutituent of the phenyl ketone group on the α-face of swainsonine also resulted in an increase in the activity. Analogue **10c**, with an α-oriented 2-oxo-2-(4-*tert*-butylphenyl)ethyl substituent, and **10b**, with an α-oriented 2-oxo-2-(4-methylphenyl)ethyl substituent, compared favorably with swainsonine. On the other hand, bulky groups on the β-face tended to diminish potency. These data suggest that the substrate or inhibitor binding site of α-mannosidase afforded enough space for an aromatic

(17) Kardono, L. B. S.; Kinghorn, A. D.; Molyneux, R. J. *Phytochem. Anal.* **1991**, *2*, 120.
(18) Dennis, J. W.; White, S. L.; Freer, A. M.; Dime, D. *Biochem. Pharmacol.* **1993**, *46*, 1459.

Scheme 3[a]



10a-i **11a-i**

[a] Key: (a) Dowex 1X8 OH^- form, 50% MeOH/H_2O; (b) MeOH.

subsutituent on the (5α)-face of swainsonine. This is supported by the crystal structure of GMII in the presence of swainsonine that shows C(3) and C(5) of swainsonine located at the entrance of the binding pocket.[8]

In summary, we have developed amine acetal **5** as a versatile intermediate for the preparation of 5α- and 5β-substituted swainsonine analogues by stereoselective Mannich reaction and epimerization. The new 5α-substituted swainsonine analogue **10c** prepared by this route was found to be a more potent α-mannosidase inhibitor than swainsonine itself. Using the present procedure and other approaches, we are exploring greater diversity of C(5) substituted swainsonine analogues to study the SAR of swainsonine with respect to GMII inhibitor and immunomodulatory anticancer activity.

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Note Added after ASAP Posting. The IC_{50} values in Table 3 were incorrect in the version posted February 10, 2004; the corrected version was posted February 12, 2004.

Supporting Information Available: Spectral data for all new compounds and X-ray crystallographic data of tri-4-bromobenzoate of **10b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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