Organic & Biomolecular Chemistry

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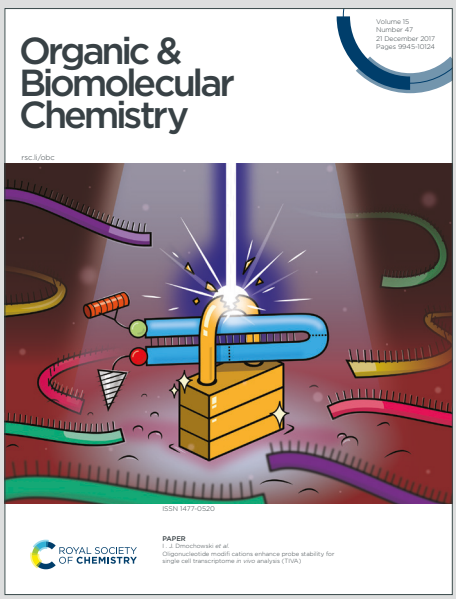

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DOI: 10.1039/C9OB02029B

ARTICLE

Synthesis and glycosidase inhibition of *N*-substituted derivatives of 1,4-dideoxy-1,4-imino-D-mannitol (DIM)

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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N-Substituted derivatives of 1,4-dideoxy-1,4-imino-D-mannitol (DIM), the pyrrolidine core of swainsonine, have been synthesized efficiently and stereoselectively from D-mannose with 2,3:5,6-di-*O*-isopropylidene DIM (**10**) as key intermediate. These *N*-substituted derivatives include *N*-alkylated, *N*-alkenylated, *N*-hydroxyalkylated and *N*-aralkylated DIMs with the carbon number of the alkyl chain ranging from one to nine. The obtained 33 *N*-substituted DIM derivatives were assayed against various glycosidases, which allowed a systematic evaluation of their glycosidase inhibition profiles. Though *N*-substitution of DIM decreased their α-mannosidase inhibitory activities, some of the derivatives showed significant inhibiton of other glycosidases.

Introduction

Golgi α-mannosidase II plays an important role in the processing of *N*-linked carbohydrates of newly synthesized glycoproteins, and is a pharmaceutical target for the design of inhibitors with anti-cancer activity.[1,2] Inhibition of the enzyme would affect glycoprotein processing which mediates a series of cancer-related biological behaviours such as cell proliferation, migration, adhesion and metastasis.[1,3,4] Swainsonine (**1**)[5] is a potent and competitive inhibitor of lysosomal α-mannosidase[6] and Golgi α-mannosidase II,[7] and has been selected for clinical trial based on its anticancer, antimetastatic, antitumor-proliferative and immunoregulating activities.[4,8] However, a phase II study of efficacy and safety of oral GD0039 (hydrochloride of swainsonine) in patients with renal carcinoma was discouraging due to severe side effects such as fatigue, anorexia, nausea and diarrhea.[9] Unexpectedly, recent research showed that swainsonine (**1**) promoted tumour progression and growth by increase in myeloid derived suppressor cells population for patients with HPV associated tumors.[10]

In order to elucidate the biological activities and structure-activity relationship (SAR) of swainsonine (**1**) and its derivatives,[11,12], the many studies on the synthesis and biological activity of the pyrrolidine core, 1,4-dideoxy-1,4-imino-D-mannitol (DIM, **2**)[13,14] have attracted our interest.[12] Structurally, the configurations of all chiral centers in DIM (**2**) are identical to those of swainsonine (**1**). Biological studies also showed that DIM (**2**) exhibited effective and similar inhibition of α-mannosidase to swainsonine (**1**). However, DIM (**2**) is much less effective than swainsonine (**1**) in inhibiting the formation of the complex types of oligosaccharides in cell culture,[15] which may be attributed to its high hydrophilicity and low permeability that preventing it from entering the cells.

Structure modification has been proved one of the most effective strategies to balance the contradiction between biological activities and toxicities of compounds.[16] Most of the listed iminosugar drugs or those under clinical trials are this class of compounds.[17] In the case of iminosugars, numerous *N*-substituted derivatives have been synthesized and proved to be highly bioactive drug candidates;[18-20] among them the most successful are the *N*-substituted derivatives of DNJ (**3**), Miglitol (**4**) and Miglustat (**5**) (Fig. 1), which are drugs for type II diabetes and Gaucher's disease, respectively. Substitution of the proton on the the ring nitrogen of iminosugars affects lipophilicity, hydrogen-bonding, basicity and the conformation of the heterocycle, and therefore influences the interaction with enzymes.

If DIM (**2**) can be regarded as the pharmacophore of swainsonine (**1**), modification of the structurally simpler single-ring iminosugar would be more efficient in the pursuit of potent glycosidase inhibitory activities and adjustment of inhibitory spectrum. Though previous reports showed that *NH*-substitution decreased mannosidase inhibition of the corresponding DIM derivatives,[21] the substitution groups have undoubted influence on their inhibition profile. Based on the previous results,[18,21-23] it is clear that both length and chain-

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Electronic Supplementary Information (ESI) available: Copies of ^{1}H NMR, ^{13}C NMR spectra and data of crystal structure. See DOI: 10.1039/x0xx00000x

end functional group of the alkyl chain affected the potency and selectivity. Herein, we report the synthesis and glycosidase inhibition profile of four series of *N*-substituted DIM derivatives with alkyl chain length ranging from one to nine carbons.

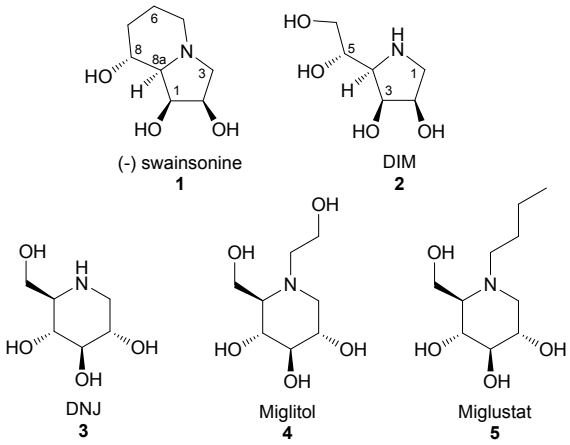


Fig. 1 Swainsonine, DIM and DNJ related compounds

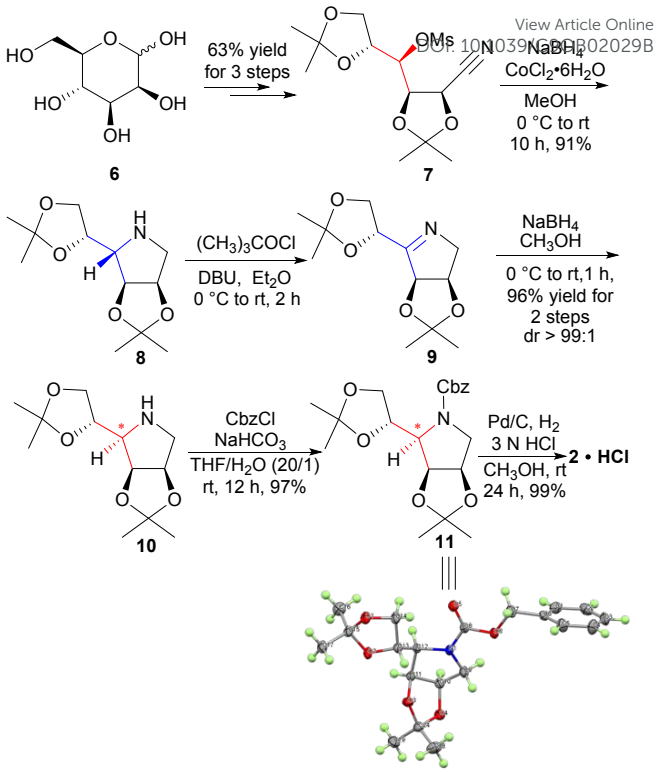
Results and discussion

Synthesis of 1,4-dideoxy-2,3:5,6-di-*O*-isopropylidene-1,4-imino-D-mannitol (10)

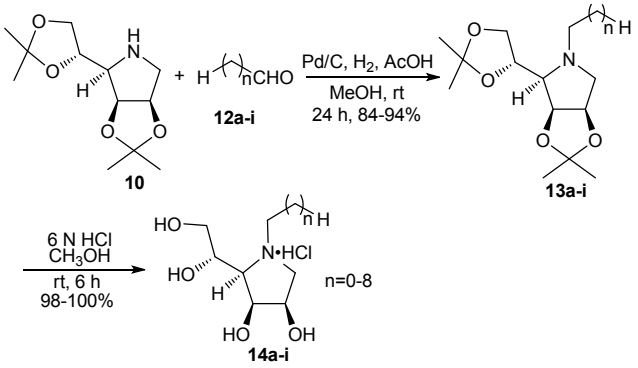
The mesylate (**7**) can be easily prepared on a large scale from D-mannose in 63% total yield according to the reported method with slight modifications (Scheme 1).[24] Reductive cyclization of **7** by sodium borohydride and cobalt (II) chloride gave pyrrolidine **8** in 91% yield. Other reductants including lithium aluminum hydride, borane etc. gave lower yields. Oxidation of the C4 *S*-configuration **8** by *tert*-butylhypochlorite and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) afforded the unstable imine **9**, which was sensitive to water or acid; the crude **9** was therefore directly reduced by sodium borohydride to give the key intermediate **10** with C4 configuration as *R* in high stereoselectivity (dr > 99:1). Reaction of the amine with CbzCl provided the fully protected compound **11** allowing purification; and finally deprotected to give the target product DIM (**2**) by routine hydrogenation. X-ray crystallographic analysis of **11** unambiguously confirmed the configuration at C4 (Scheme 1).

Synthesis of *N*-substituted DIM derivatives

With the key intermediate **10** in hand, the *N*-alkylated DIM derivatives were efficiently synthesized by reductive amination. Compound **10** was hydrogenated in the existence of a series of aldehydes (**12a-i**) ranging from formaldehyde to nonaldehyde; the resulting tertiary amines (**13a-i**) were then deprotected in 6 N hydrochloric acid to afford the target *N*-alkylated DIM derivatives (**14a-i**) in quantitative yield (Scheme 2).

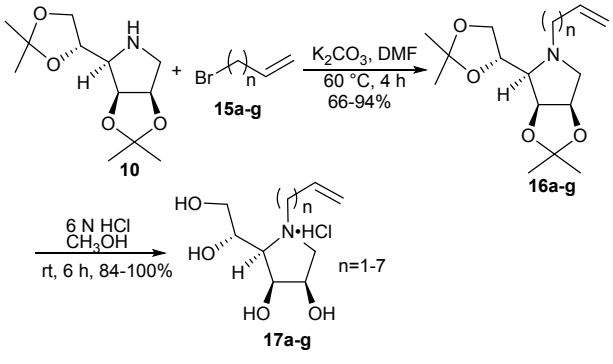


Scheme 1 The synthesis of key intermediate **10** and DIM·HCl (**2·HCl**).



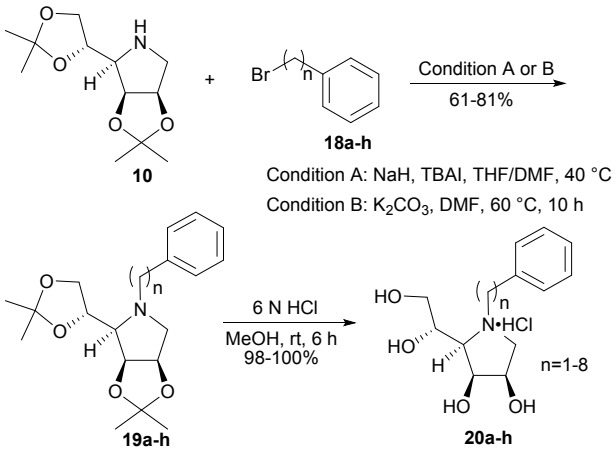
Scheme 2 Synthesis of *N*-alkylated DIM derivatives

DIM derivatives (**17a-g**) with alkenyl groups on the ring nitrogen were prepared by the nucleophilic substitution of intermediate **10** with alkenyl bromides (**15a-g**) followed by acidic deprotection in high yields (Scheme 3).

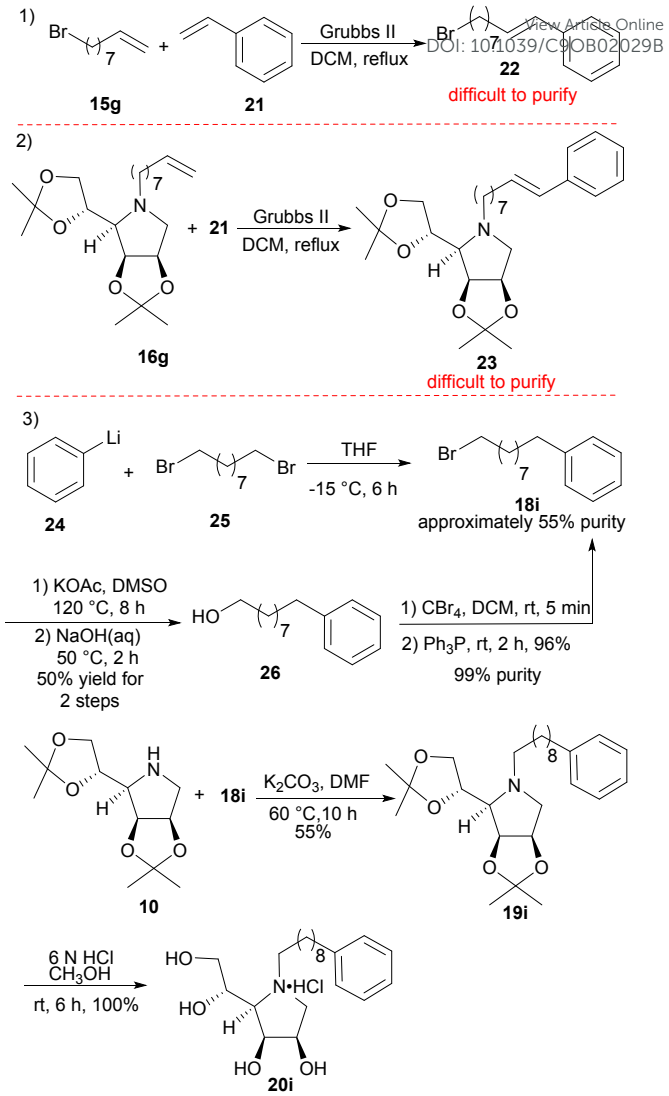


Scheme 3 Synthesis of *N*-alkenylated DIM derivatives

N-Aralkylated DIM derivatives (**20a-h**) with alkyl chain length ranging from one to eight carbons can be prepared by reaction of **10** with the corresponding commercially available aralkyl bromides (**18a-h**) in the presence of sodium hydride or potassium carbonate as base (Scheme 4). The synthesis of *N*-phenylnonyl-DIM (**20i**) started from the preparation of (9-bromonon-1-en-1-yl)benzene (**22**). Olefin metathesis reaction of 9-bromo-1-nonene (**15g**) and styrene (**21**) afforded a mixture of the target product **22** and self-coupling product of **15g**, which was difficult to purify by column chromatography. In the second trial, the previously obtained *N*-alkenylated DIM derivative **16g** was used as the substrate of olefin metathesis reaction, which unfortunately underwent similar separation problem as in the synthesis of **23**. Finally, the nucleophilic substitution of phenyl lithium and 1,9-dibromo-nonane was used in the preparation of (9-bromononyl)benzene (**18i**) with approximately 55% ^{1}H NMR purity. The crude **18i** was transformed to (9-hydroxylnonyl)benzene (**26**) by a substitution-hydrolysis process to facilitate separation with impurities accompanying **18i**. Subsequent bromination of **26** by Appel's condition[25] afforded bromide **18i** with 99% ^{1}H NMR purity. *N*-aralkylated compound **20i** was then obtained by nucleophilic substitution of **18i** with intermediate **10** and subsequent acidic deprotection (Scheme 5).

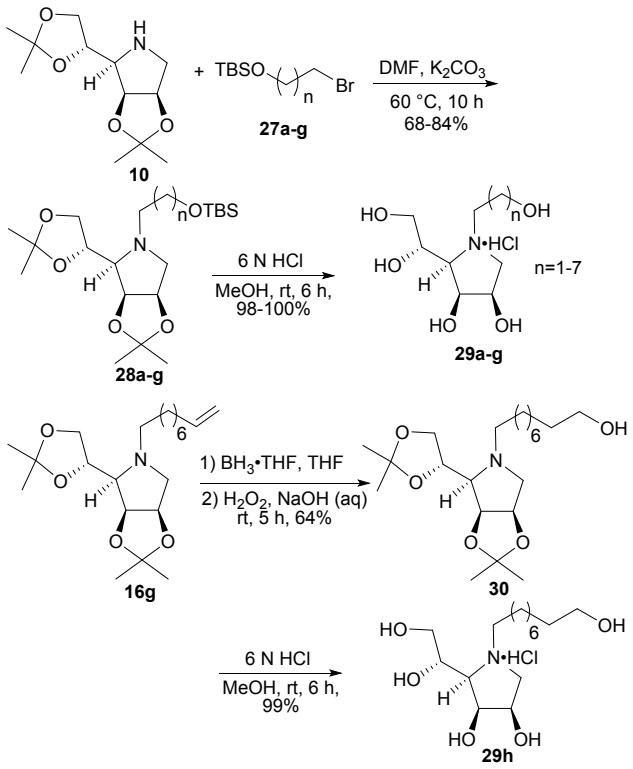


Scheme 4 Synthesis of *N*-aralkylated DIM derivatives



Scheme 5 Synthesis of *N*-aralkylated DIM derivatives

N-Hydroxyalkylated DIM derivatives (**29a-h**) were synthesized with intermediate **10** and bromides **27** according to similar procedures to those of *N*-alkenylated DIM derivatives with **29h** as an exception. Preparation of **29h** started from intermediate **16g**, which was treated with BH_3·THF and H_2O_2 successively to afford alcohol **30**. Final acidic deprotection provided the target **29h** in quantitative yield (Scheme 6).



Scheme 6 Synthesis of *N*-hydroxyalkylated DIM derivatives

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DOI: 10.1039/C9OB02029B

Evaluation against various glycosidases of *N*-substituted DIM derivatives

All synthesized DIM derivatives (**2·HCl**, **14a-i**, **17a-g**, **20a-i** and **29a-h**) were assayed according to our reported method[23] as potential inhibitors of a range of glycosidases; the results are summarized in Tables 1-4.

Table 1 Concentration of DIM (2·HCl) and its *N*-alkylated derivatives (14a-i) giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)									
	2·HCl	**14a**	**14b**	**14c**	**14d**	**14e**	**14f**	**14g**	**14h**	**14i**
α-Glucosidase										
Rice	NI[a] (39.0%)[b]	NI (4.9%)	NI (2.1%)	NI (6.2%)	NI (5.3%)	NI (6.7%)	NI (5.9%)	NI (0.5%)	NI (0%)	NI (4.7%)
Rat intestinal maltase	NI (26.7%)	NI (12.7%)	NI (13.1%)	NI (13.1%)	NI (23.9%)	NI (27.9%)	NI (7.4%)	NI (8.6%)	NI (9.4%)	NI (11.4%)
Yeast	NI (8.8%)	NI (7.4%)	NI (10.2%)	NI (2.9%)	NI (5.4%)	NI (16.9%)	NI (18.5%)	NI 14.0%)	NI (20.5%)	NI (44.6%)
β-Glucosidase										
Almond	NI (26.8%)	NI (41.6%)	NI (0%)	NI (1.0%)	NI (20.6%)	**270**	NI (34.6%)	**279**	NI (6.7%)	NI (19.1%)
Bovine liver	**888**	NI (12.2%)	NI (5.5%)	NI (0%)	NI (11.7%)	NI (25.7%)	NI (12.5%)	**1223**	**1985**	NI (39.1%)
Human lysosome	NI (21.1%)	NI (0%)	NI (0%)	NI (0.8%)	NI (15.5%)	NI (0%)	NI (5.8%)	NI (0%)	NI (30.6%)	**673**
α-Galactosidase										
Coffee beans	NI (12.6%)	NI (13.0%)	NI (5.5%)	NI (13.0%)	NI (8.4%)	NI (10.1%)	NI (18.9%)	NI (16.4%)	NI (21.6%)	NI (22.3%)
β-Galactosidase										
Bovine liver	**508**	NI (21.1%)	NI (13.7%)	NI (0%)	NI (9.2%)	629	NI (41.7%)	**292**	**380**	**182**
α-Mannosidase										
Jack bean	**3.2**	**492**	**1165**	**513**	NI (36.8%)	NI (39.2%)	**1231**	**285**	NI (27.0%)	**958**
β-Mannosidase										
Snail	NI (8.2%)	NI (0%)	NI (0%)	NI (0%)	NI (3.0%)	NI (3.0%)	NI (0.8%)	NI (0.5%)	NI (0.5%)	NI (0%)
α-L-fucosidase										
Bovine kidney	NI (5.2%)	NI (0.3%)	NI (0%)	NI (1.8%)	NI (0.6%)	NI (0%)	NI (6.5%)	NI (3.8%)	NI (0%)	NI (2.9%)
Trehalase										
Porcine kidney	NI (4.6%)	NI (0%)	NI (14.9%)	NI (3.0%)	NI (0%)	NI (13.2%)	NI (4.8%)	NI (7.4%)	NI (7.4%)	NI (5.7%)
Amyloglucosidase										
A. niger	NI (5.4%)	NI (2.2%)	NI (0%)	NI (1.2%)	NI (6.4%)	NI (0%)	NI (5.2%)	NI (2.9%)	NI (0%)	NI (14.7%)
α-L-rhamnosidase										
Penicillium decumbens	NI (21.9%)	**786**	NI (27.1%)	NI (7.2%)	NI (7.6%)	NI (11.5%)	NI (11.8%)	NI (17.7%)	NI (32.6%)	**986**
β-Glucuronidase										
E.coli	NI (14.2%)	NI (0.9%)	NI (0%)	NI (0%)	NI (4.7%)	NI (11.8%)	NI (12.0%)	NI (27.2%)	NI (32.6%)	**659**
Bovine liver	NI (0%)	NI (0%)	NI (0%)	NI (4.2%)	NI (0%)	NI (26.0%)	NI (1.6%)	NI (6.5%)	NI (7.6%)	NI (7.3%)

[a]NI: No inhibition (less than 50% inhibition at 1000 μM); [b]( ): inhibition % at 1000 μM.

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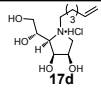
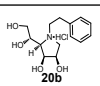
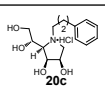
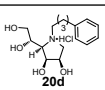
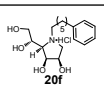
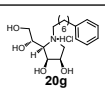
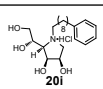
Table 2 Concentration of *N*-alkenylated DIM derivatives (17a-g) giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)							
	 1	 17a	 17b	 17c	 17d	 17e	 17f	 17g
α-Glucosidase								
Rice	NI[a] (45.3%)[b]	NI (3.9%)	NI (4.6%)	NI (5.0%)	NI (34.3%)	NI (7.1%)	NI (16.1%)	NI (1.8%)
Rat intestinal maltase	NI (42.9%)	NI (0%)	NI (0%)	NI (3.2%)	NI (0.3%)	NI (9.1%)	NI (6.4%)	NI (0%)
Yeast	NI (0.3%)	NI (4.8%)	NI (0%)	NI (0%)	NI (17.3%)	NI (21.3%)	NI (4.7%)	NI (17.3%)
β-Glucosidase								
Almond	NI (1.2%)	NI (23.1%)	NI (8.2%)	**887**	NI (10.8%)	NI (14.8%)	NI (22.8%)	NI (17.6%)
Bovine liver	NI (30.3%)	NI (14.7%)	NI (21.9%)	NI (19.8%)	NI (16.7%)	NI (24.9%)	NI (42.9%)	NI (41.8%)
Human lysosome	ND	NI (15.9%)	NI (36.0%)	NI (0%)	NI (0%)	NI (42.4%)	NI (5.1%)	NI (37.4%)
α-Galactosidase								
Coffee beans	NI (1.8%)	NI (6.9%)	NI (13.2%)	NI (110%)	NI (13.5%)	NI (17.3%)	NI (22.3%)	NI (20.5%)
β-Galactosidase								
Bovine liver	**546**	NI (16.5%)	NI (28.2%)	NI (37.2%)	NI (25.3%)	NI (36.1%)	**330**	**361**
α-Mannosidase								
Jack bean	**0.73**	**159**	NI (27.5%)	**350**	NI (18.5%)	NI (43.6%)	NI (29.3%)	NI (23.3%)
β-Mannosidase								
Snail	NI (0%)	NI (2.5%)	NI (0.7%)	NI (0%)	NI (0%)	NI (2.7%)	NI (0%)	NI (0%)
α-L-fucosidase								
Bovine kidney	NI (0%)	NI (2.5%)	NI (0%)	NI (0%)	NI (3.2%)	NI (0%)	NI (0%)	NI (0%)
Trehalase								
Porcine kidney	NI (0%)	NI (0.3%)	NI (0%)	NI (0%)	NI (0%)	NI (1.3%)	NI (8.8%)	NI (0.8%)
Amyloglucosidase								
A. niger	NI (9.3%)	NI (5.7%)	NI (2.1%)	NI (0%)	NI (22.4%)	NI (5.9%)	NI (1.9%)	NI (4.6%)
α-L-rhamnosidase								
Penicillium decumbens	NI (4.9%)	NI (28.3%)	NI (10.2%)	NI (3.9%)	NI (5.9%)	NI (14.5%)	NI (26.0%)	NI (32.2%)
β-Glucuronidase								
E.coli	NI (0.7%)	NI (11.9%)	NI (2.8%)	NI (13.8%)	NI (10.9%)	NI (19.8%)	NI (30.4%)	NI (44.5%)
Bovine liver	NI (6.5%)	NI (0%)	NI (0.6%)	NI (3.8%)	NI (0%)	NI (4.3%)	NI (31.1%)	NI (14.1%)

[a]NI: No inhibition (less than 50% inhibition at 1000 μM); [b]( ): inhibition % at 1000 μM.

Table 3 Concentration of *N*-aralkylated DIM derivatives (20a-i) giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)								
	 20a	 20b	 20c	 20d	 20e	 20f	 20g	 20h	 20i
α-Glucosidase									
Rice	NI[a] (34.2%)[b]	NI (0.5%)	NI (3.7%)	NI (8.1%)	NI (4.4%)	NI (9.7%)	100	NI (17.7%)	NI (4.6%)
Rat intestinal maltase	NI (16.6%)	NI (15.6%)	NI (14.9%)	NI (24.8%)	NI (25.1%)	NI (6.2%)	261	NI (10.6%)	NI (8.0%)
Yeast	NI (6.5%)	NI (7.1%)	NI (12.6%)	NI (7.7%)	NI (8.4%)	NI (14.6%)	NI (17.4%)	**1390**	**335**
β-Glucosidase									
Almond	NI (6.1%)	NI (43.7%)	**459**	**499**	**461**	**459**	**526**	**338**	**201**
Bovine liver	NI (9.5%)	NI (23.9%)	NI (31.6%)	**811**	**623**	**204**	**154**	**79.8**	**56.6**
Human lysosome	NI (4.7%)	NI (44.3%)	NI (15.3%)	NI (36.0%)	**428**	**1108**	**208**	**80.1**	**51.7**
α-Galactosidase									
Coffee beans	NI (8.8%)	NI (17.1%)	NI (15.9%)	NI (23.9%)	NI (34.9%)	NI (33.2%)	**1264**	**601**	**375**
β-Galactosidase									
Bovine liver	NI (7.9%)	**1129**	**1082**	**284**	**178**	**58**	**46.1**	**26.7**	**26.6**
α-Mannosidase									
Jack bean	NI (27.1%)	NI (37.1%)	**203**	**281**	**531**	**571**	NI (30.0%)	**413**	**334**
β-Mannosidase									
Snail	NI (4.4%)	NI (0.6%)	NI (3.7%)	NI (5.7%)	NI (6.8%)	NI (16.1%)	NI (5.1%)	**1267**	**556**
α-L-fucosidase									
Bovine kidney	NI (8.4%)	NI (0.2%)	NI (0%)	NI (0%)	NI (4.0%)	NI (10.2%)	NI (13.4%)	**718**	**623**
Trehalase									
Porcine kidney	NI (0%)	NI (0%)	NI (0%)	NI (0%)	NI (0%)	NI (0%)	NI (3.0%)	NI (0%)	NI (0%)
Amyloglucosidase									
A. niger	NI (11.4%)	NI (0%)	NI (0.5%)	NI (0.3%)	NI (5.1%)	NI (5.1%)	NI (4.9%)	NI (6.6%)	NI (16.9%)
α-L-rhamnosidase									
Penicillium decumbens	NI (11.5%)	NI (28.5%)	NI (17.8%)	**2581**	**1263**	**984**	**730**	**409**	**258**
β-Glucuronidase									
E.coli	NI (9.8%)	NI (24.1%)	NI (36.0%)	**1145**	**1471**	**429**	**255**	**253**	**95.5**

Bovine liver	NI (0%)	NI (3.7%)	NI (10.3%)	NI (31.9%)	NI (29.2%)	NI (0%)	NI (27.7%)	**643**	**500**

[a]NI: No inhibition (less than 50% inhibition at 1000 μM); [b]( ): inhibition % at 1000 μM.

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DOI: 10.1039/C9OB02029B

Table 4 Concentration of *N*-hydroxyalkylated DIM derivatives (29a-i) giving 50% inhibition of various glycosidases

Enzyme	IC_{50} (μM)							
	 **29a**	 **29b**	 **29c**	 **29d**	 **29e**	 **29f**	 **29g**	 **29h**
α-Glucosidase								
Rice	NI[a] (6.5%)[b]	NI (1.0	NI (0.1%)	NI (5.9%)	NI (4.1%)	NI (4.7%)	NI (6.4%)	NI (6.4%)
Rat intestinal maltase	NI (0%)	NI (8.2%)	NI (2.0%)	NI (7.5%)	NI (4.9%)	NI (7.1%)	NI (9.3%)	NI (14.4%)
Yeast	NI (0.3%)	NI (1.9%)	755	NI (23.5%)	NI (8.5%)	NI (1.7%)	NI (11.0%)	NI (12.7%)
β-Glucosidase								
Almond	NI (20.4%)	NI (3.8%)	NI (4.3%)	NI (19.4%)	NI (8.3%)	NI (31.9%)	NI (23.9%)	NI (12.7%)
Bovine liver	NI (19.5%)	NI (15.4%)	NI (5%)	NI (27.8%)	NI (27.2%)	NI (33.3%)	**800**	NI (20.4%)
Human lysosome	NI (0%)	NI (16.2%)	**149**	NI (34.5%)	NI (0%)	NI (0%)	NI (14.9%)	NI (0%)
α-Galactosidase								
Coffee beans	NI (11.8%)	NI (2.0%)	NI (11.8%)	NI (15.3%)	NI (26.9%)	NI (19.7%)	NI (13.7%)	NI (36.1%)
β-Galactosidase								
Bovine liver	NI (34.9%)	NI (32.6%)	NI (12.9%)	NI (38.4%)	NI (34.3%)	**510**	**256**	NI (42.2%)
α-Mannosidase								
Jack bean	**254**	**745**	NI (19.2%)	**712**	NI (34.3%)	**483**	**818**	NI (31.8%)
β-Mannosidase								
Snail	NI (0%)	NI (0%)	NI (0%)	NI (0%)	NI (1.9%)	NI (0%)	NI (0 %)	NI (0%)
α-L-fucosidase								
Bovine kidney	NI (0%)	NI (0%)	NI (0%)	NI (1.5%)	NI (1.0%)	NI (4.4%)	NI (0 %)	NI (4.1%)
Trehalase								
Porcine kidney	NI (2.2%)	NI (0%)	NI (0.3%)	NI (4.5%)	NI (2.5%)	NI (0%)	NI (1.2%)	NI (1.5%)
Amyloglucosidase								
A. niger	NI (3.5%)	NI (4.3%)	NI (0.5%)	NI (8.8%)	NI (7.8%)	NI (2.5%)	NI (12.1%)	NI (10.2%)
α-L-rhamnosidase								
Penicillium decumbens	**659**	**340**	NI (8.5%)	NI (6.0%)	NI (6.7%)	NI (7.1%)	NI (9.9%)	NI (20.0%)
β-Glucuronidase								
E.coli	NI (1.2%)	NI (0.4%)	NI (17.6%)	NI (9.4%)	NI (12.0%)	**251**	**58.5**	**182**
Bovine liver	NI (0%)	NI (0%)	NI (0%)	NI (7.2%)	NI (4.4%)	NI (32.5%)	NI (41.2%)	NI (17.8%)

[a]NI: No inhibition (less than 50% inhibition at 1000 μM); [b]( ): inhibition % at 1000 μM.

Though the synthesized *N*-substituted DIM derivatives are indeed open-chain analogues of swainsonine, all these compounds show reduced inhibition potency. In contrast to the potent and selective α-mannosidase inhibitory activities of swainsonine (**1**) and DIM·HCl (**2·HCl**), the *N*-substituted derivatives are only weak inhibitors of α-mannosidase or have no inhibition at all. Besides, increasing of *N*-substitution chain length led to broadening of inhibitory spectrum. With compound **20h** and **20i** as examples, they show moderate or weak inhibition towards almost all the tested glycosidases.

For DIM derivatives **14e**, **14g** and **14i** with relatively long alkyl chains, *N*-alkylation slightly improved their inhibition of β-glucosidase. *N*-methyl DIM (**14a**) and *N*-nonyl DIM (**14i**) are also weak inhibitors of α-L-rhamnosidase.

Comparing to the parent compound DIM, *N*-alkenylation mainly resulted in sharp decrease or complete loss of α-mannosidase inhibition. In the case of β-glucosidase and β-galactosidase, inhibitory activities of the *N*-alkenylated derivatives had no substantial changes or disappeared completely.

N-Aralkylated DIM derivatives with short alkyl chains (**20a** and **20b**) are inactive towards all the glycosidases tested. However, their inhibition towards β-glucosidase, β-galactosidase and β-glucuronidase exhibit an increasing tendency as the *N*-substitution chain lengthened. Both **20h** and **20i** were found to be moderate bovine liver β-galactosidase inhibitor (IC_{50} = 26.7 μM, 26.6 μM, respectively), compound **20i** also showed moderate *E. coli* β-glucuronidase inhibitory activity (IC_{50} = 95.5 μM).

As for DIM derivatives **29a-h**, *N*-hydroxyalkylation endows them weak *P. decumbens* α-L-rhamnosidase and moderate *E. coli* β-glucuronidase inhibitory activities in addition to sharp decrease of α-mannosidase inhibition. For example, compound **29g** is a moderate inhibitor of *E. coli* β-glucuronidase (IC_{50} = 58.5 μM).

In this work, the *N*-substitution derivatives were originally designed to be good α-mannosidase inhibitors since all of them can be regarded as open-chain analogues of swainsonine. However, the assay results suggested that open-chain structures are not suitable for the α-mannosidase inhibition. All these compounds showed reduced α-mannosidase inhibition potency, indicating that the piperidine of swainsonine or the free secondary amine structure of DIM may be important factors for the inhibition of α-mannosidase.

Among these *N*-substitution derivatives, *N*-aralkylated DIM derivatives exhibited broad inhibitory activities against almost all the glycosidases assayed. The adjustment of inhibitory spectra of these DIM derivatives might be related with the existence of the terminal aromatic-ring and flexible long alkyl

View Article Online DOI: 10.1039/C9OB02939B

chains, which facilitate their insertion and interaction with various glycosidases.

The assay results and structure-activity relationship (SAR) discovered in this work may be valuable for further studies of swainsonine and DIM-related iminosugars.

Conclusion

In summary, with D-mannose as starting material, we have developed an efficient and scalable synthetic route for DIM (**2**) and its *N*-substituted derivatives, which is featured by a highly stereoselective conversion of the C-4 configuration. Though assay results suggested that all *N*-substituted DIM derivatives exhibited reduced α-mannosidase inhibition, systematic syntheses of the *N*-substituted derivatives with their alkyl chain ranging from one to nine carbons provided a series of reliable data to understand their SAR. Besides, the changes of inhibitory spectra discovered in this work is very interesting and may be positive for the design of new glycosidase inhibitors.

Experimental

General methods

All chemicals and solvents were purchased from commercial suppliers without further purification or prepared as described in the literature. Ethyl ether, tetrahydrofuran, dichloromethane, triethylamine, pyridine and *N*,*N*-dimethylformamide were dried over activated 4Å molecular sieves before use. NMR spectra were measured in D_2O or $CDCl_3$ (with TMS as internal) on a Bruker AV400 (^{1}H at 400MHz, ^{13}C at 100 MHz) or a Bruker AV500 (^{1}H at 500 MHz, ^{13}C at 125 MHz) magnetic resonance spectrometer. Chemical Shifts (δ) were reported in parts per million (ppm), coupling constants (*J*) were reported in hertz (Hz). Multiplicity was indicated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiple dd = doublet of doublet, bs = broad singlet, ddd = doublet doublet of doublet, dt = doublet of triplet. High-resolution mass spectra (HRMS) were recorded on Thermo Fisher Exactive Spectrometer. Polarimetry were measured with Rudolph Autopl VI polarimeter. Infrared spectra were recorded on Nicolet-6700 FT-IR spectrometer. Melting points were determined using an electrothermal melting point apparatus. TLC plates were visualized by a spray of Pancaldi reagent ($(NH_4)_6MoO_4$, $Ce(SO_4)_2$,H_2SO_4 ,H_2O) or ultraviolet light. Chromatographic purification of products was carried out by flash column chromatography on silica gel (200–300 mesh).

Material and methods for the enzyme inhibition assay

With rat intestinal maltase as an exception, other enzymes were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo. USA). Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[26] were assayed at pH 6.8 for rat intestinal maltase using maltose. The released D-glucose was determined colorimetrically using the Glucose CII-test Wako (Wako Pure Chemical Ind.; Osaka, Japan). Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate in a buffer solution at the optimal pH value of each enzyme. The reaction was stopped by adding 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

2,3:5,6-Di-*O*-isopropylidene-4-*O*-methylsulphonyl-D-mannononitrile (7)

Compound **7** was synthesized in three steps from D-mannose as described in the literature.[24,27] White crystalline, 58.6 g, 0.18 mol, 63% yield from D-mannose (**6**) (50.0 g, 0.28 mol). M.p. 80-81 °C; [α]26 D+48.5 (*c* 2.24 in CH_2Cl_2); ν_{max}/cm^{-1} 2991 s, 2941 m, 2904 w, 1457 w, 1412 w, 1362 vs , 1231 s, 1178 m, 1086 vs, 1055 s, 921 w, 872 s, 830 s, 785 m, 744 w, 601 w, 525 s; δ_H (500 MHz; $CDCl_3$) 4.91 (1H, d, *J* = 4.7 Hz), 4.82 (1H, t, *J* = 9.0 Hz), 4.30-4.27 (2H, m), 4.14 (1H, dd, *J* = 9.0 Hz, 6.7 Hz), 4.11-4.06 (1H, m), 3.15 (3H, s), 1.62 (3H, s), 1.49 (3H, s), 1.42 (3H, s), 1.36 (3H, s); δ_C (125 MHz; $CDCl_3$) 116.6, 112.0, 111.6, 80.8, 77.8, 74.1, 67.7, 66.6, 38.9, 26.9, 26.2, 25.6, 25.4; HRMS(ESI) calcd for $C_{13}H_{22}NO_7S^+$ $[M+H]^+$ 336.11115, found 336.11087.

1,4-Dideoxy-2,3:5,6-di-*O*-isopropylidene-1,4-imino-D-talitol (8)

Cobalt (II) chloride hexahydrate (4.76 g, 20 mmol) was added to a solution of nitrile **7** (3.35 g, 10 mmol) in methanol (100 mL) at 0 °C. The mixture was stirred for 0.5 h, and then sodium borohydride (3.80 g, 0.10 mol) was added. The suspension was gradually raised to room temperature and stirred for 12 h. The reaction was then quenched with sat. aq. NH_4Cl (20 mL), and methanol was removed in vacuo. Water (100 mL) was added, and then extracted with EtOAc (3 × 30 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo again. The crude product was purified by flash column chromatography (silica gel, EtOAc/Et_3N 200:1) to give pyrrolidine **8** (2.23 g, 91% yield) as a white solid. M.p. 58-59°C ; [α]23 D-31.5 (*c* 1.14 in CH_2Cl_2); ν_{max}/cm^{-1} 2985 s, 2935 s, 2884 w, 1460 w, 1370 s, 1255 m, 1209 vs, 1160 s, 1098 m, 1053 vs, 983 w, 889 m, 867 m, 792 w, 515 w; δ_H (500 MHz; $CDCl_3$) 4.73-4.71 (1H, m), 4.47 (1H, dd, *J* = 5.8 Hz, 1.4 Hz), 4.07 (1H, dd, *J* = 11.7 Hz, 6.3 Hz), 4.02 (1H, dd, *J* = 7.7 Hz, 6.4 Hz), 3.84 (1H, t, *J* = 7.5 Hz), 3.15 (1H, dd, *J* = 5.7 Hz, 1.1 Hz), 3.09-3.02 (2H, m), 2.32 (1H, br), 1.47 (3H, s), 1.41 (3H, s), 1.33 (3H, s), 1.31 (3H, s); δ_C (125 MHz; $CDCl_3$) 111.5, 109.4, 84.0, 82.2, 76.0, 66.83, 66.81, 53.2, 26.48, 26.46, 25.3, 24.2;[24] HRMS(ESI) calcd for $C_{12}H_{22}NO_4^+$ $[M+H]^+$ 244.15433, found 244.15414.

(2*R*,3*S*)-5-((*S*)-2,2-Dimethyl-1,3-dioxolan-4-yl)-2,3-*O*-isopropylidene-2,3-dihydroxy-2,3-dihydro-2*H*-pyrrole (9)

tert-Butylhypochlorite (1.23 mL, 10.9 mmol) was added to a solution of pyrrolidine **8** (2.20 g, 9.1 mmol) in dry ether (15 mL) at 0°C. After stirring for 1 h, 1,8-diazabicyclo[5.4.0]-undec-7-ene (1.77 mL, 11.8 mmol) was added dropwise. The mixture was stirred at room temperature for another 1 h, and then filtered. The filtrate was concentrated in vacuo to give the crude imine **9** which was used directly in the next step. Part of the crude product was purified by flash column chromatography (silica gel, dry dichloromethane/Et_3N 100:1) to afford the target imine **9** as a light yellow syrup. [α]26 D-66.3 (*c* 0.18 in CH_2Cl_2); ν_{max}/cm^{-1} 2987 m, 2935 s, 2738 w, 2676 m, 2491 w, 1647 w, 1457 w, 1372 s, 1247 m, 1211 s,1154 s,

1065 vs, 985 w, 845 m, 512 w; δ_H (400 MHz; $CDCl_3$) 5.15 (1H, d, *J* = 5.8 Hz), 4.87 (1H, t, *J* = 6.2 Hz), 4.76 (1H, t, *J* = 5.1 Hz), 4.30-4.24 (2H, m), 4.07-3.94 (2H, m), 1.43 (3H, s), 1.42 (3H, s), 1.38 (3H, s), 1.36 (3H, s); δ_C (100 MHz; $CDCl_3$) 174.9, 112.0, 110.0, 85.5, 77.7, 73.4, 67.3, 65.4, 26.8, 26.3, 25.39, 25.36; HRMS(ESI) calcd for $C_{12}H_{20}NO_4^+$ $[M+H]^+$ 224.13868, found 242.13872.

1,4-Dideoxy-2,3:5,6-di-*O*-isopropylidene-1,4-imino-D-mannitol (10)

Sodium borohydride (1.60 g, 42.0 mmol) was added in portions to a solution of crude **9** (3.37 g, 11.9 mmol) in methanol (30 mL) at 0 °C. The mixture was stirred for 1 hours at room temperature, and then quenched with sat. aq. NH_4Cl (10 mL). Methanol was removed in vacuo. Water (50 mL) was added to the residue, and then extracted with EtOAc (3 × 20 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo again. The crude product was purified by flash column chromatography (silica gel, EtOAc/petroleum ether /Et_3N 200:200:1) to give the pyrrolidine **10** (2.10 g, 96% yield for two steps, dr > 99:1) as a colorless syrup. [α]26 D -39.9 (*c* 0.94 in CH_2Cl_2); ν_{max}/cm^{-1} 2985 s, 2935 s, 1457 w, 1371 s, 1258 m, 1208 vs, 1153 m, 1070 vs, 1031 s, 979 w, 927 w, 901 w, 851 s, 517 m; δ_H (400 MHz; $CDCl_3$) 4.70-4.64 (2H, m), 4.26-4.21 (1H, m), 4.09 (1H, dd, *J* = 8.4 Hz, 6.2 Hz), 3.99 (1H, dd, *J* = 8.4 Hz, 5.5 Hz), 3.09 (1H, d, *J* = 13.0 Hz), 2.69 (1H, dd, *J* = 8.2 Hz, 3.7 Hz), 2.62 (1H, dd, *J* = 13.0 Hz, 3.7 Hz), 1.45 (3H, s), 1.42 (3H, s), 1.38 (3H, s), 1.32 (3H, s); δ_C (100 MHz; $CDCl_3$) 110.7, 108.9, 81.6, 80.7, 73.9, 67.7, 66.4, 53.3, 26.9, 25.7, 25.4, 23.8;[12] HRMS(ESI) calcd for $C_{12}H_{22}NO_4^+$ $[M+H]^+$244.15433, found 244.15452.

***N*-Benzyloxycarbonyl-2,3:5,6-di-*O*-isopropylidene-1,4-dideoxy-1,4-imino-D-mannitol (11)**

To a stirred solution of pyrrolidine **10** (11.0 g, 45.2 mmol) and sodium bicarbonate (5.7 g, 67.8 mmol) in THF (40 mL) and water (2 mL) was added benzyl chloroformate (7.7 mL, 54.3 mmol) dropwise at room temperature. After TLC showed completion of the reaction, water (100 mL) was added, and extracted with EtOAc (3 × 50 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, EtOAc/petroleum ether 1:10) to afford the target product **11** (16.5 g, 97%) as a white crystalline. M.p. 81-82 °C; [α]24 D-25.4 (*c* 1.75 in CH_2Cl_2); ν_{max}/cm^{-1} 2985 s, 2937 m, 2893 w, 1708 vs, 1456 m, 1412 m, 1370 m, 1338 w, 1241 w, 1211 vs, 1160 m, 1087 m, 1062 s, 1006 w, 856 m, 756 w, 699 m, 514 w; δ_H (400 MHz; $CDCl_3$) 7.36-7.28 (5H, m), 5.10 (2H, ABq, *J*= 12.3 Hz), 4.77 (1H, t, *J* = 6.0 Hz), 4.66 (1H, m), 4.55 (1H, dd, *J* = 13.8 Hz, 6.1 Hz), 4.10-4.07 (1H, m), 4.01-3.94 (2H, m), 3.76-3.75 (1H, m), 3.50 (1H, dd, *J* = 13.4 Hz, 2.7 Hz), 1.47 (3H, s), 1.40 (3H, s), 1.36 (3H, s), 1.34 (3H, s); δ_C (100 MHz; $CDCl_3$) 155.2, 136.3, 128.5, 128.2, 128.0, 113.1, 109.0, 80.0, 77.9, 74.5, 67.8, 67.3, 62.7, 52.1, 26.8, 26.5, 25.4, 24.9;[12] HRMS(ESI) calcd for $C_{20}H_{27}NO_6Na^+$ $[M+Na]^+$ 400.17306, found 400.17224.

General reductive amination procedure

To a solution of pyrrolidine **10** (1 eq) and aldehyde **12a-i** (3 eq) in methanol was added 10% Pd/C (15 wt%) and several drops of AcOH. The suspension was stirred under hydrogen atmosphere for 24-48 h when TLC showed completion of the reaction. Hydrogen was replaced by nitrogen, catalyst was removed from the reaction mixture by filtration, and then washed with EtOAc for three times. The filtrate was concentrated in vacuo and purified by silica gel flash column chromatography to give the protected *N*-alkylated DIM derivatives (**13a-i**).

General nucleophilic substitution procedure

To a solution of pyrrolidine **10** (1 eq) and bromides (**15a-g**, **18b-h or 27a-g**)(1.5 eq) in *N*,*N*-dimethylformamide was added potassium carbonate (3 eq), the suspension was stirred at 65 ℃ for 4–24 h when TLC showed completion of the reaction. Water was added, and extracted with EtOAc. The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by silica gel flash column chromatography to afford the target *N*-substituted DIM derivatives (**16a-g**, **19b-h** and **28a-g**).

General deprotection procedure

To a solution of pyrrolidine **10** or protected *N*-substituted DIM derivatives (**13a-i**, **16a-g**, **19a-h**, **28a-g** and **30**) in methanol (15 mL) was added 6 N HCl (1 mL), and stirred for 6 h at room temperature when TLC showed completion of the reaction. The mixture was then concentrated in vacuo to give the target DIM derivatives as hydrochloride salt (**2·HCl**, **14a-i**, **17a-g**, **20a-i** and **29a-h**).

For the experimental data of compound 13b-i, 14b-i, 16b-g, 17b-g, 19b-h, 20b-h, 28b-g and 29b-g, please see the Electronic Supplementary Information (part I).

1,4-Dideoxy-1,4-imino-D-mannitol hydrochloride (2·HCl)

According to the general deprotection procedures, product **2·HCl** (40.9 mg, 99% yield) was obtained from **10** (50.0 mg, 0.21 mmol) as a white crystalline. M.p. 147-148 °C; [α]29 D-25.7 (*c* 0.94 in CH_3OH); ν_{max}/cm^{-1} 3502 w, 3336 s, 2942 m, 1125 m, 1084 w, 1035 w; δ_H (400 MHz; D_2O) 4.55-4.50 (1H, m), 4.41 (1H, t, *J* = 3.4 Hz), 4.14 (1H, dt, *J* = 8.5 Hz, 4.9 Hz), 3.78-3.68 (2H, m), 3.66-3.60 (2H, m), 3.21 (1H, dd, *J* = 11.9 Hz, 8.6 Hz); δ_C (100 MHz; D_2O) 69.9, 69.8, 67.1, 62.8, 62.2, 47.2; HRMS(ESI) calcd for $C_6H_{14}NO_4^+$ $[M+H]^+$ 164.09173, found 164.09154.

***N*-Methyl-1,4-dideoxy-2,3:5,6-di-*O*-isopropylidene-1,4-imino-D-mannitol (13a)**

According to the general reductive amination procedures, product **13a** (47.5 mg, 71% yield) was obtained from **10** (63.5 mg, 0.26 mmol) and 37% aq. formaldehyde (58.6 µL, 0.78 mmol) as a colorless syrup. [α]26 D-36.8 (*c* 1.11 in CH_2Cl_2); ν_{max}/cm^{-1} 2986 m, 2937 m, 2783 w, 2868 m, 1706 w, 1458 w, 1380 s, 1268 m, 1208 vs, 1157 m, 1106 m, 1052 s, 929 w, 859 m, 514 w; δ_H (400 MHz; $CDCl_3$) 4.61-4.55 (2H, m), 4.44 (1H, td, *J* = 7.3 Hz, 2.0 Hz), 4.15 (1H, t, *J* = 7.7 Hz), 4.02 (1H, t, *J* = 7.4 Hz), 3.16 (1H, d, *J* = 11.2 Hz), 2.39 (1H, s), 2.33 (3H, s), 2.17 (1H, dd, *J* = 11.2 Hz, 4.2 Hz), 1.46 (3H, s), 1.45 (3H, s), 1.34 (3H, s), 1.28 (3H, s); δ_C (100 MHz; $CDCl_3$) 110.9, 107.2, 81.3, 77.7, 75.0, 70.0, 65.9, 62.5, 41.6, 26.3, 25.7, 24.4, 24.2; HRMS(ESI) calcd for $C_{13}H_{24}NO_4^+$ $[M+H]^+$ 258.16998, found 258.16987.

***N*-Methyl-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (14a)**

According to the general deprotection procedures, product **14a** (13.3 mg, 100% yield) was obtained from **13a** (16.0 mg,

0.06 mmol) as a light yellow syrup. [α]25 D-31.3 (*c* 0.39 in CH_3OH); ν_{max}/cm^{-1} 3347 vs, 2924 w, 1633 w, 1455 w, 1138 m, 1096 m, 1138 m; δ_H (400 MHz; $CDCl_3$) 4.62-4.55 (2H, m), 4.24 (1H, dd, *J* = 10.4 Hz, 4.8 Hz), 3.90-3.81 (2H, m), 3.66-3.61 (2H, m), 3.50 (1H, dd, *J* = 12.4 Hz, 8.4 Hz), 3.00 (3H, s); δ_C (100 MHz; $CDCl_3$) 70.9, 69.7, 68.5, 67.0, 62.5, 58.4, 39.9;[28] HRMS(ESI) calcd for $C_7H_{16}NO_4^+$ $[M+H]^+$ 178.10738, found 178.10702.

N-Allyl-1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-mannitol (16a)

According to the general nucleophilic substitution procedures, product **16a** (3.0 g, 87% yield) was obtained from **10** (3.0 g, 12.3 mmol) and allyl bromide (1.59 mL, 18.45 mmol) as a light yellow syrup. [α]26 D -52.3 (*c* 1.60 in CH_2Cl_2); ν_{max}/cm^{-1} 2985 s, 2935 m, 2795 w, 1457 w, 1380 s, 1266 m, 1208 vs, 1152 m, 1097 m, 1039 s, 991 w, 926 w, 859 m, 842 m, 802 w, 513 w; δ_H (500 MHz; $CDCl_3$) 5.92-5.84 (1H, m), 5.17 (1H, dd, *J* = 17.5 Hz, 1.5 Hz), 5.08 (1H, d, *J* = 10.5 Hz), 4.58-4.54 (2H, m), 4.47 (1H, td, *J*= 7.5 Hz, 2.0 Hz), 4.22 (1H, t, *J* = 8.0 Hz), 4.00 (1H, t, *J* = 7.5Hz), 3.78-3.74 (1H, m), 3.19 (1H, d, *J* = 11.3 Hz), 2.75-2.70 (2H, m), 2.13 (1H, dd, *J* = 11.3 Hz, 4.2 Hz), 1.44 (3H, s), 1.43 (3H, s), 1.32 (3H, s), 1.26 (3H, s); δ_C (125 MHz; $CDCl_3$) 135.2, 116.8, 111.0, 107.1, 81.0, 77.5, 75.2, 66.8, 65.7, 58.6, 56.5, 26.2, 25.8, 24.6, 23.9; HRMS(ESI) calcd for$C_{15}H_{26}NO_4^+$ $[M+H]^+$ 284.18454, found 284.18510.

N-Allyl-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (17a)

According to the general deprotection procedures, product **17a** (11.4 mg, 100% yield) was obtained from **16a** (13.5 mg, 0.05 mmol) as a light yellow syrup. [α]26 D-36.7 (*c* 1.06 in CH_3OH); ν_{max}/cm^{-1} 3355 vs, 2940 m, 1645 w, 1428 m, 1122 m, 1027 m, 951 m, 548 w; δ_H (400 MHz; D_2O) 6.02-5.91 (1H, m), 5.66-5.60 (2H, m), 4.53-4.48 (2H, m), 4.22 (1H, q, *J* = 5.2 Hz), 4.01 (1H, dd, *J* = 13.2 Hz, 6.4 Hz), 3.87-3.68 (4H, m), 3.60-3.55 (1H, m), 3.46 (1H, dd, *J* = 12.1 Hz, 6.4 Hz); δ_C(100 MHz; D_2O) 126.7, 125.6, 70.9, 68.5, 67.6, 67.3, 62.5, 55.8, 54.6; HRMS(ESI) calcd for $C_9H_{18}NO_4^+$ $[M+H]^+$ 204.12303, found 204.12256.

N-Benzyl-1,4-dideoxy-2,3:5,6-di-O-isopropylidene-1,4-imino-D-mannitol (19a)

To a suspension of NaH (60%, 7.3 mg, 0.3 mmol) and TBAI (5.5 mg, 0.015 mmol) in dry THF (10 mL) was added the solution of **10** (17.0 mg, 0.15 mmol) in dry DMF (2 mL) dropwise at room temperature. After stirring for 10 min, benzyl bromide (39.3 mg, 0.23 mmol) was added slowly. The mixture was stirred at 40 °C for 1 h when TLC showed completion of the reaction. The reaction was quenched with sat. aq. NH_4Cl (10 mL), water (50 mL) was added, and then extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo again. The crude product was purified by flash column chromatography (silica gel, petroleum ether/EtOAc/NH_4OH 200:10:1) to give pyrrolidine **19a** (40.0 mg, 79%) as a colorless syrup. [α]26 D-30.6 (*c* 0.62 in CH_2Cl_2); ν_{max}/cm^{-1} 2986 m, 2934 m, 2796 w, 1454 w, 1380 s, 1268 w, 1207 vs, 1149 m, 1096 s, 1037 s, 991 w, 930 w, 859 m, 843 m, 757 s, 701 m, 514 w; δ_H (500 MHz; $CDCl_3$) 7.35 (2H, d, *J* = 7.2 Hz), 7.32-7.29 (2H, m), 7.22 (1H, t, *J* = 7.2 Hz), 4.62-4.53 (3H, m), 4.46-4.42 (2H, m), 4.07 (1H, t, *J* = 7.6 Hz), 3.14 (1H, d, *J* = 13.6 Hz), 3.04 (1H, d, *J* = 11.3 Hz), 2.83 (1H, d, *J* =4.65 Hz), 2.05 (1H, dd, *J* = 11.3 Hz, 4.6 Hz), 1.46 (3H, s), 1.43 (3H, s), 1.36 (3H, s), 1.26 (3H, s); δ_C (125 MHz; $CDCl_3$) 138.8, 128.8, 128.2, 126.8, 111.1, 107.3, 81.0, 77.6, 75.4, 67.0, 65.8, 59.1, 58.2, 26.3, 25.9, 24.7, 23.8;[14] HRMS(ESI) calcd for $C_{19}H_{28}NO_4^+$ $[M+H]^+$ 334.20128, found 334.20082.

N-Benzyl-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (20a)

According to the general deprotection procedures, product **20a** (11.3 mg, 100% yield) was obtained from **19a** (13.0 mg, 0.04 mmol) as a yellow-green syrup. [α]26 D-25.2 (*c* 0.27 in CH_3OH); ν_{max}/cm^{-1} 3344 vs, 2925 m, 2853 w, 2868 m, 1456 w, 1417 w, 1213 w, 1126 m, 1046 w, 701 w; δ_H (400 MHz; D_2O) 7.58-7.51 (5H, m), 4.61 (1H, d, *J* = 13.0 Hz), 4.54-4.47 (2H, m), 4.36 (1H, d, *J* = 13.0 Hz), 3.96 (1H, q, *J* = 5.0 Hz), 3.89-3.78 (3H, m), 3.64 (1H, dd, *J* = 12 Hz, 7.2 Hz), 3.38 (1H, dd, *J* = 12 Hz, 7.2 Hz); δ_C (100 MHz; D_2O) 131.0, 130.4, 129.4, 129.0, 70.9, 68.5, 68.4, 67.7, 62.6, 58.3, 55.2;[20] HRMS(ESI) calcd for $C_{13}H_{20}NO_4^+$ $[M+H]^+$ 254.13868, found 254.13842.

9-Phenylnonan-1-ol (26)

To a solution of 1,9-dibromononane (18) (3.0 g, 10.5 mmol) in dry THF (30 mL) was added dropwise phenyllithium (1.0 M solution in ethyl ether, 10.5 mL, 10.5 mmol) at -15 °C under Ar atmosphere. The solution was allowed to stir at -15 °C for 6 h, and then moved to room temperature to stir for 1 h. The reaction was quenched by dropwise addition of sat. aq. NH_4Cl (10 mL). Water (50 mL) was added, and extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in DMSO (25 mL) and potassium acetate (5.5 g, 55.5 mmol) was added. The solution was heated at 120 °C for 8 h when TLC showed completion of the reaction. Aq. NaOH solution (2 M, 20 mL) was added to the above solution, and stirred at 50 °C for another 2 h. Water (100 mL) was added, and extracted with EtOAc (3 × 20 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo again. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc 3:1) to give 9-phenylnonan-1-ol (26) (1.15 g, 50% yield) as a colorless oil. δ_H (400 MHz; $CDCl_3$) 7.28-7.24 (2H, m), 7.18-7.14 (3H, m), 3.61 (2H, t, *J* = 6.6 Hz), 2.59 (2H, t, *J* = 7.6 Hz), 1.62-1.51 (4H, m), 1.39-1.23 (10H, m); δ_C (100 MHz; $CDCl_3$) 142.9, 128.4, 128.2, 125.6, ,63.0, 36.0, 32.8, 31.5, 29.55, 29.45, 29.4, 29.3, 25.8.[30]

9-Phenyl-1-bromononane (18i)

To a solution of 9-phenylnonan-1-ol (26) (880 mg, 4.0 mmol) in dry DCM (20 mL) was added CBr_4 (1.45 g, 4.4 mmol), and stirred at room temperature for 5 min. PPh_3 (1.2 g, 4.65 mmol) in dry DCM (2 mL) was added dropwise, and then reacted at room temperature for 2 h when TLC indicated completion of the reaction. Aq. $NaHCO_3$ (50 Ml) was used to quench the reaction, then extracted with DCM (3 × 20 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel, petroleum ether/EtOAc 15:1) to afford the target bromide 18i (1.08 g, 96% yield) as a pale yellow oil. δ_H (400 MHz; $CDCl_3$) 7.28-7.23 (2H, m), 7.18-7.14 (3H, m), 3.39 (2H, t, *J* = 6.8 Hz), 2.59 (2H, t, *J* = 7.6 Hz), 1.87-

1.80 (2H, m), 1.62-1.57 (2H, m), 1.42-1.37 (2H, m), 1.30-1.29 (8H, m); δ_C (100 MHz; $CDCl_3$) 142.9, 128.4, 128.2, 125.6 , 36.0, 34.0, 32.9, 31.5, 29.41, 29.38, 29.3, 28.8, 28.2.[30]

_N_-(Phenylnonyl)-1,4-dideoxy-2,3:5,6-di-_O_-isopropylidene-1,4-imino-D-mannitol (19i)

According to the general nucleophilic substitution procedures, product **19i** (201.5 mg, 55% yield) was obtained from **10** (200.0 mg, 0.82 mmol) and 9-phenyl-1-bromononane (348.4 mg, 1.23 mmol) as a colorless syrup. [α]26 D-32.2 (*c* 1.74 in CH_2Cl_2); ν_{max}/cm^{-1} 3026 vw, 2985 m, 2929 vs, 2856 m, 2783 w, 1706 w, 1454 w, 1379 m, 1267 s, 1208 s, 1157 m, 1103 w, 1050 m, 860 m, 699 w, 514 vw; δ_H (500 MHz; $CDCl_3$) 7.29-7.26 (2H, m), 7.18-7.15 (3H, m), 4.59-4.55 (2H, m), 4.46 (1H, td, *J* = 7.3 Hz, 1.5 Hz), 4.20 (1H, t, *J* = 7.7 Hz), 3.99 (1H, t, *J* = 7.5 Hz), 3.25 (1H, d, *J* = 11.1 Hz), 3.06 (1H, dt, *J* = 12.2 Hz, 8.3 Hz), 2.61-2.58 (3H, m), 2.06-1.98 (2H, m), 1.63-1.56 (2H, m), 1.44-1.28 (24H, m); δ_C (125 MHz; $CDCl_3$) 143.0, 128.4, 128.2, 125.5, 111.0, 107.1, 81.1, 77.7, 75.3, 68.0, 65.8, 59.1, 54.3, 36.0, 31.5, 29.6, 29.6, 29.5, 27.7, 27.4, 26.3, 25.9, 24.9, 24.0; HRMS(ESI) calcd for $C_{27}H_{44}NO_4^+$ $[M+H]^+$ 446.32649, found 446.32602.

_N_-(Phenylnonyl)-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (20i)

According to the general deprotection procedures, product **20i** (55.9 mg, 100% yield) was obtained from **19i** (62.0 mg, 0.14 mmol) as a light yellow syrup. [α]26 D-41.4 (*c* 0.35 in CH_3OH); ν_{max}/cm^{-1} 3447 vs, 2929 w, 2854 w, 1649 m, 1460 w, 1124 w, 1033 w, 697 w; δ_H (500 MHz; D_2O) 7.13-7.10 (2H, m), 7.05-7.00 (3H, m) 4.55-4.47 (2H, m), 4.18 (1H, d, *J* = 4.6 Hz), 3.88-3.81 (2H, m), 3.63-3.55 (1H, m), 3.51-3.48 (1H, m), 3.34-3.26 (2H, m), 2.95-2.93 (1H, m), 2.45 (2H, t, *J* = 7.5 Hz), 1.64-1.58 (2H, m), 1.48 (2H, m), 1.29-1.11 (10H, m); δ_C(125 MHz; D_2O) 142.7, 128.3, 128.2, 125.6, 70.6, 69.1, 68.6, 67.3, 62.6, 55.4, 54.4, 35.7, 31.3, 29.2, 29.1, 28.8, 26.2, 24.7; HRMS(ESI) calcd for $C_{21}H_{36}NO_4^+$ $[M+H]^+$ 366.26389, found 366.26303.

7-((_tert_-Butyldimethylsilyl)oxy)-1-bromoheptane (27f)

Product 27f (2.87 g, 93% yield) was obtained from 7-bromo-1-heptanol (1.95 g, 10.00 mmol) and *tert*-Butyldimethylsilyl chloride (2.26 g, 15.00 mmol) as a colorless syrup.[31] δ_H (400 MHz; $CDCl_3$) 3.60 (2H, t, *J* = 6.5 Hz), 3.40 (1H, t, *J* = 6.9 Hz), 1.86 (2H, quint , *J* = 6.9 Hz), 1.53-1.48 (2H, m), 1.48-1.40 (2H, m), 1.35-1.31 (2H, m), 1.35-13.1 (4H, m) , 0.89 (9H, s) , 0.05 (6H, s); δ_C (100 MHz; $CDCl_3$) 63.2, 34.0, 32.8, 32.7, 28.6, 28.2, 26.0, 25.6, 18.4, -5.3.[32]

8-((_tert_-Butyldimethylsilyl)oxy)-1-bromooctane (27g)

Product 27g (2.9 g, 94% yield) was obtained from 8-bromo-1-octanol (2.0 g, 9.56 mmol) and *tert*-butyldimethylsilyl chloride (2.16 g, 14.34 mmol) as a colorless syrup.[31] δ_H (400 MHz; $CDCl_3$) 3.58 (2H, t, *J* = 6.5 Hz), 3.38 (1H, t, *J* = 6.9 Hz), 1.84 (2H, quint , *J* = 6.9 Hz), 1.51-1.45 (2H, m), 1.43-1.38 (2H, m), 1.30 (6H, s) , 0.88 (9H, s) , 0.03 (6H, s); δ_C (100 MHz; $CDCl_3$) 63.2, 33.7, 32.82, 32.81, 29.2, 28.7, 28.1, 26.0, 25.7, 18.3, -5.3.[31]

_N_-(2-((_tert_-Butyldimethylsilyl)oxy)ethyl)-1,4-dideoxy-2,3:5,6-di-_O_-isopropylidene-1,4-imino-D-mannitol (28a)

According to the general nucleophilic substitution procedures, product 28a (52.4 mg, 55% yield) was obtained from 10 (60.0 mg, 0.25 mmol) and 2-((*tert*-butyldimethylsilyl)oxy)-1-bromoethane (89.7 mg, 0.38 mmol) as a light yellow syrup. [α]29 D-23.7 (*c* 1.07 in CH_2Cl_2); ν_{max}/cm^{-1} 2986 m, 2931 s, 2858 m, 2798 vw, 1705 m, 1472 w, 1380 m, 1256 m, 1209 s, 1157 m, 1100 vs, 1061 m, 837 vs, 777 m; δ_H (400 MHz; $CDCl_3$) 4.59-4.52 (2H, m), 4.46 (1H, td, *J* = 7.2 Hz, 1.1 Hz), 4.22 (1H, t, *J* = 7.8 Hz), 3.97 (1H, t, *J* = 7.5 Hz), 3.75-3.66 (2H, m), 3.34 (1H, d, *J* = 11.3 Hz), 3.28 (1H, dt, *J* = 12.8 Hz, 6.2 Hz), 2.71 (1H, d, *J* = 1.7 Hz), 2.25-2.19 (2H, m), 1.43 (6H, s), 1.32 (3H, s), 1.26 (3H, s), 0.88 (9H, s), 0.04 (6H, s); δ_C (100 MHz; $CDCl_3$) 111.0, 107.1, 80.7, 78.0, 75.3, 67.7, 65.7, 62.1, 60.5, 56.1, 26.2, 25.9, 25.8, 24.7, 24.0, 18.2, -5.5; HRMS(ESI) calcd for $C_{20}H_{40}NO_5Si^+$ $[M+H]^+$ 402.26703, found 402.26706.

_N_-(2-Hydroxyethyl)-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (29a)

According to the general deprotection procedures, product **29a** (18.2 mg, 99% yield) was obtained from **28a** (30.0 mg, 0.07 mmol) as a light yellow syrup. [α]26 D-24.9 (*c* 0.33 in CH_3OH); ν_{max}/cm^{-1} 3362 vs, 2942 w, 1456 w, 1131 m, 1041 w, 1000 w, 517 w; δ_H (400 MHz; D_2O) 4.59-4.55 (2H, m), 4.26 (1H, d, *J* = 4.4 Hz), 3.96-3.81 (5H, m), 3.66-3.59 (3H, m), 3.35-3.32 (1H, m); δ_C (100 MHz; D_2O) 70.4, 69.4, 68.7, 67.1, 62.5, 56.3, 56.1, 55.7; HRMS(ESI) calcd for $C_8H_{18}NO_5^+$ $[M+H]^+$ 208.11795, found 208.11795.

_N_-(9-Hydroxynonyl)-1,4-dideoxy-2,3:5,6-di-_O_-isopropylidene-1,4-imino-D-mannitol (30)

To a solution of **16g** (130 mg, 0.35 mmol) in dry THF (20 mL) was added BH_3·THF (1 M, 0.39 mL, 0.39 mmol), and the mixture was allowed to stir at room temperature for 4 h. Aq. NaOH (1 M, 0.53 mL) and H_2O_2 (30%, 0.56 mL) were carefully added successively. The mixture was stirred at room temperature for 1 h, and then quenched by sat. aq. $Na_2S_2O_3$ (10 mL). Water (30 mL) was added, and extracted with EtOAc (3 × 10 mL). The organic phases were combined, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography (silica gel, petroleum ether/EtOAc/Et_3N 200:50:1) to give compound **30** (87.3 mg, 64% yield) as a colorless syrup. [α]25 D -44.3 (*c* 0.77 in CH_2Cl_2); ν_{max}/cm^{-1} 3446 w, 2985 w, 2928 vs, 2856 m, 2784 w, 1456 w, 1370 m, 1267 w, 1208 s, 1157 m, 1092 w, 1051 s, 990 w, 860 m, 800 w, 514 w; δ_H (400 MHz; $CDCl_3$) 4.59-4.54 (2H, m), 4.46 (1H, td, *J* = 7.3 Hz, 1.6 Hz), 4.19 (1H, t, *J* = 7.7 Hz), 3.97 (1H, t, *J* = 7.6 Hz), 3.62 (2H, t, *J* = 6.6 Hz), 3.24 (1H, d, *J* = 11.0 Hz), 3.05 (1H, dt, *J* = 11.9 Hz, 8.1 Hz), 2.58 (1H, d, *J* = 2.7 Hz), 2.06-1.97 (2H, m), 1.58-1.50 (2H, m), 1.43-1.27 (24H, m); δ_C (100 MHz; $CDCl_3$), 111.0, 107.1, 81.1, 77.7, 75.3, 68.0, 65.7, 63.0, 59.1, 54.2, 32.8, 29.5, 29.4, 29.3, 27.7, 27.7, 26.3, 25.9, 25.7, 24.8, 24.0; HRMS(ESI) calcd for $C_{21}H_{40}NO_5^+$ $[M+H]^+$ 386.29010, found 386.28946.

_N_-(9-Hydroxynonyl)-1,4-dideoxy-1,4-imino-D-mannitol hydrochloride (29h)

According to the general deprotection procedures, product **29h** (30.7 mg, 99% yield) was obtained from 3**0** (35.0 mg, 0.09 mmol) as a white solid. M.p. 75-76 °C; [α]28 D-37.3 (*c* 1.28 in CH_3OH); ν_{max}/cm^{-1} 3347 vs, 2928 vs, 2855 s, 1647 w, 1458 m, 1128 m,1046 m, 557 w; δ_H (500 MHz; $CDCl_3$) 4.57-4.51 (2H, m), 4.22 (1H, q, *J* = 5.0 Hz), 3.88-3.81 (2H, m), 3.67 (1H, t, *J* = 4.2

Hz), 3.60 (2H, t, *J* = 6.5 Hz), 3.55 (2H, d, *J* = 7.5 Hz), 3.43-3.37 (1H, m), 3.13-3.07 (1H, m), 1.79-1.69 (2H, m), 1.56-1.53 (2H, m), 1.41-1.33 (10H, m); δ_C (125 MHz; $CDCl_3$) 77.7, 68.7, 68.6, 67.3, 62.5, 61.8, 55.3, 54.5, 31.2, 28.3, 28.3, 28.0, 25.6, 24.9, 24.3; HRMS(ESI) calcd for $C_{15}H_{32}NO_5^+$ $[M+H]^+$ 306.22750, found 306.22690.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Financial support from the National Natural Science Foundation of China (No. 21772206) and National Engineering Research Center for Carbohydrate Synthesis of Jiangxi Normal University is gratefully acknowledged. This work was supported in part by an Emeritus Leverhulme Research Fellowship (GWJF) and a Grant-in-Aid for Scientific Research (C) from the Japanese Society for the Promotion of Science (JSPS KAKENHI Grant Number JP17K08362) (AK).

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DOI: 10.1039/C9OB02029B

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