



# Isolation from *Stevia rebaudiana* of DMDP acetic acid, a novel iminosugar amino acid: synthesis and glycosidase inhibition profile of glycine and $\beta$ -alanine pyrrolidine amino acids

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## Abstract

DMDP acetic acid [*N*-carboxymethyl-2,5-dideoxy-2,5-imino-D-mannitol] **5** from *Stevia rebaudiana* is the first isolated natural amino acid derived from iminosugars bearing an *N*-alkyl acid side chain; it is clear from GCMS studies that such derivatives with acetic and propionic acids are common in a broad range of plants including mulberry, *Baphia*, and English bluebells, but that they are very difficult to purify. Reaction of unprotected pyrrolidine iminosugars with aqueous glyoxal gives the corresponding *N*-acetic acids in very high yield; Michael addition of both pyrrolidine and piperidine iminosugars and that of polyhydroxylated prolines to *tert*-butyl acrylate give the corresponding *N*-propionic acids in which the amino group of  $\beta$ -alanine is incorporated into the heterocyclic ring. These easy syntheses allow the identification of this new class of amino acid in plant extracts and provide pure samples for biological evaluation. DMDP *N*-acetic and propionic acids are potent  $\alpha$ -galactosidase inhibitors in contrast to potent  $\beta$ -galactosidase inhibition by DMDP.

**Keywords** Iminosugars · Amino acid · *Stevia* ·  $\beta$ -Galactosidase ·  $\alpha$ -Galactosidase · Hydroxy proline · Iminosugar acid · Sugar amino acids

## Introduction

Many iminosugars, sugar mimics in which the ring oxygen of a pyranose or furanose is replaced by nitrogen to form polyhydroxylated pyrrolidines [e.g. DMDP

(2,5-dideoxy-2,5-imino-D-mannitol) **1**] and piperidines [e.g., DNJ (deoxynojirimycin) **2**], have been isolated from plants (Fig. 1) (Asano et al. 2000; Watson et al. 2001). In contrast, 7*a*-epialexaflorine **3** from *Alexa grandiflora* (Pereira et al. 1991) and BR1 **4** from *Baphia racemosa* (di Bello et al. 1984; Booth et al. 2007; Fleet et al. 1986, 1987; Manning et al. 1985) are among the very few corresponding iminosugar amino acids (Risseeuw et al. 2007, 2013) that have been identified as natural products. Sugar amino acids are much more difficult to isolate and identify

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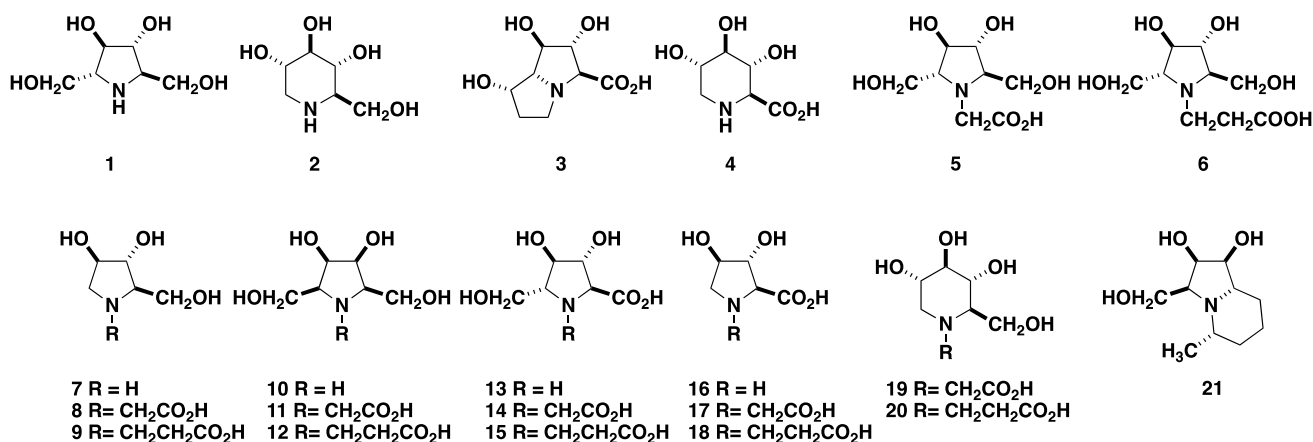


Fig. 1 Structure of iminosugars and sugar amino acids

than their iminosugar analogues due to the high content of acidic and neutral amino acids in plants. DMDP **1** is the most widely occurring iminosugar; GCMS studies on a number of diverse plant extracts from *Stevia rebaudiana*, mulberry, and bluebell (*Hyacinthoides non scripta*) show that *N*-alkylated amino acids such as DMDP acetic **5** and DMDP propionic **6** acids are present (Gallagher and Yu 2014; Nash et al. 2018; Wilson et al. 2010). This paper describes (a) the isolation of DMDP acetic acid **5** from *Stevia* in which the amino group of glycine is incorporated into the hydroxylated pyrrolidine ring, (b) the efficient conversion of the unprotected pyrrolidine iminosugars DMDP **1**, DAB [1,5-dideoxy-1,5-imino-D-arabinitol] **7** and *galacto*-DMDP [2,5-dideoxy-2,5-iminogalactitol] **10** to pyrrolidine acetic acids **5**, **8**, and **11** by treatment with aqueous glyoxal, (c) Michael addition of unprotected pyrrolidine **5**, **7**, **10** and piperidine DNJ **2** iminosugars and the sugar amino acids **13** and **16** to *tert*-butyl acrylate to give *N*-propionic acid derivatives in which the amine of  $\beta$ -alanine is incorporated into the heterocyclic ring, and (d) the effect of the *N*-glycine and *N*- $\beta$ -alanine sidechains on glycosidase inhibition, in particular on the change in  $\alpha$ - and  $\beta$ -galactosidase activity.

## Results and discussion

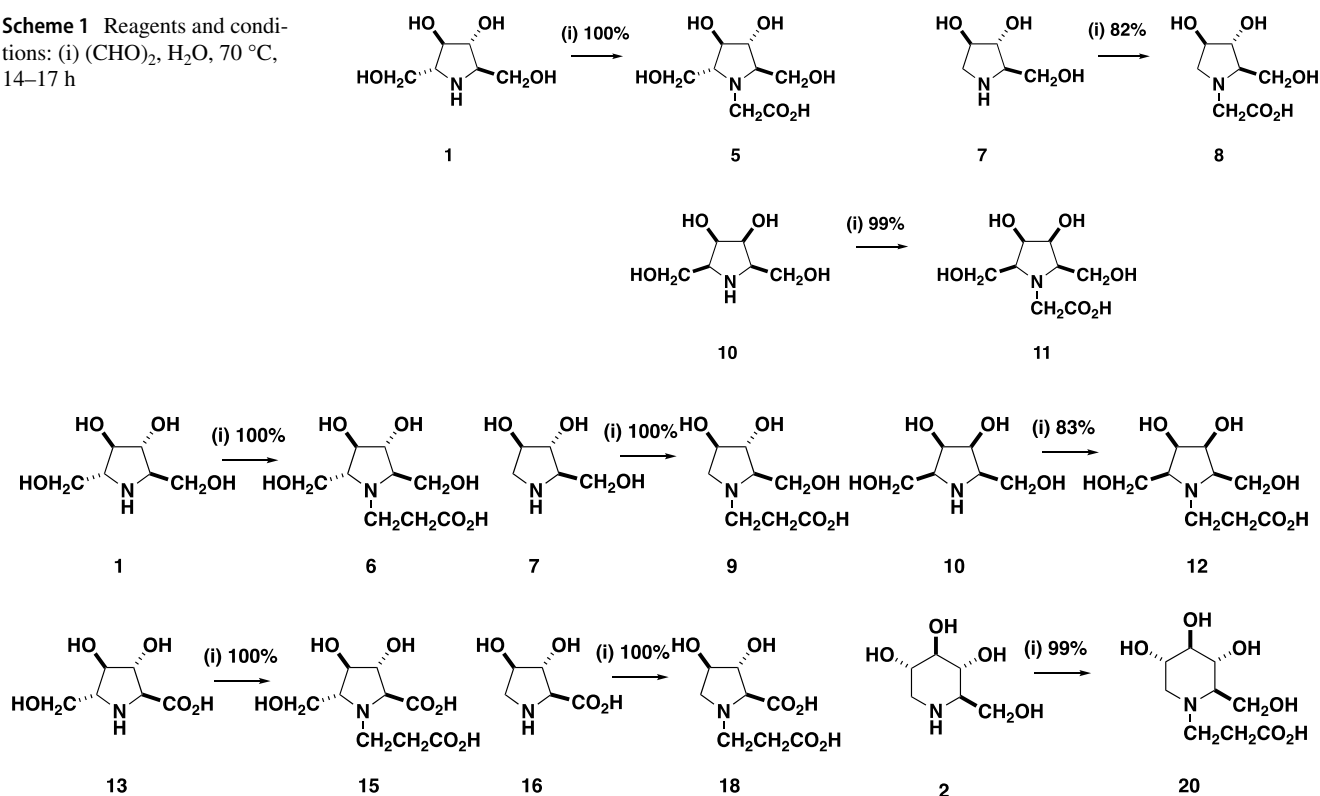
### Isolation of DMDP acetic acid **5** from *Stevia rebaudiana*

Steviamine **21**, [with the same relative pyrrolidine ring stereochemistry as *galacto*-DMDP **10**] was the first alkaloid isolated from *Stevia* (Michalik et al. 2010; Thompson et al. 2009) and has shown potential anti-metastatic activity by inhibiting the hexosaminidase released by a breast cancer cell line (Ramessur et al. 2010). A number of intensely sweet glycosides of steviol are widely available commercially; yet,

the iminosugars of *Stevia* are hitherto unreported despite their strong biological activity. The iminosugar content can be as high as 0.5% of stevia leaves and comprises mainly DMDP **1**; some 2 g of DMDP along with 50 mg of DMDP acetic acid **5** were isolated from 2.2 kg of leaves from *Stevia rebaudiana* Betoni (Asteraceae), a herb native to South America. Many plants containing iminosugars also contain their *N*-acetic and *N*-propionic acids which have so far evaded identification and isolation due to the complex free amino acid profile of most plants (Bell 1976).

### Synthesis of pyrrolidine *N*-acetic acids from unprotected iminosugars

Easy syntheses of *N*-alkylated amino acids allow the identification of natural products in crude extracts from plants where isolation of the pure natural product is difficult. Although the mechanism is unclear (Amadori or intramolecular Cannizzaro rearrangements), the reaction of secondary amines with aqueous glyoxal gives *N,N*-dialkyl glycines in good yield (Scheme 1) (Farfán et al. 1987). Treatment of the iminosugars DMDP **1**, DAB **7**, and *galacto*-DMDP **10** with aqueous glyoxal at 70 °C for 14–17 h gave the corresponding acetic acids **5**, **8**, and **11** in yields of 100%, 82%, and 99%, respectively, and were readily isolated with a minimum of purification. This transformation was unsuccessful when applied to hydroxylated prolines [the monocyclic equivalent of epialexaflorine **13** and the dihydroxyproline **16**]; the acetic acids **14** and **17** were not isolated. Surprisingly, the piperidine iminosugar DNJ **2** with glyoxal under the same conditions gave a complex reaction mixture and did not allow the isolation of DNJ acetic acid **19**.

**Scheme 1** Reagents and conditions: (i) (CHO)<sub>2</sub>, H<sub>2</sub>O, 70 °C, 14–17 h**Scheme 2** Reagents and conditions: (i) CH<sub>2</sub>=CHCOO<sup>t</sup>Bu, MeOH, 50 °C, 48 h; CF<sub>3</sub>CO<sub>2</sub>H, H<sub>2</sub>O, rt, 48 h

### Synthesis of pyrrolidine and piperidine *N*-propionic acids from unprotected iminosugars and polyhydroxylated amino acids

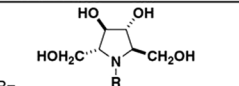
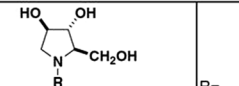
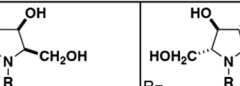
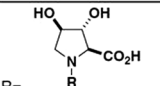
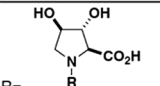
The Michael addition of unprotected polyhydroxylated prolines, and pyrrolidine and piperidine iminosugars, with *tert*-butyl acrylate in the presence of methanol (to avoid polymerization of the acrylate) at 50 °C, followed by hydrolysis with aqueous trifluoroacetic acid, gave consistent excellent yields of β-alanine analogues in which the amine formed part of a pyrrolidine or piperidine ring (Scheme 2). Minimal purification was necessary. The pyrrolidine iminosugars DMDP **1**, DAB **7** and *galacto*-DMDP **10** gave the *N*-propionic acids **6** (100%), **9** (100%), and **12** (83%). The hydroxyprolines **13** and **16** formed **15** and **18** in quantitative yield; the structure of **15** was unequivocally confirmed by X-ray diffraction (Edgeley et al. 2012). DNJ **2** produced DNJ-propionic acid **20** in 99% yield.

### Glycosidase inhibition

The glycosidase inhibition profiles of a number of *N*-acetic and *N*-propionic acids were compared with their parent iminosugars and prolines (Table 1). DMDP **1** showed potent inhibitions of bovine liver β-glucosidase and β-galactosidase with IC<sub>50</sub> values of 9.7 and 3.3 μM, respectively (Best et al.

2010); **1** also showed weak and broad inhibition against α-glucosidase, β-mannosidase, trehalase, and amyloglucosidase. Both DMDP acetic **5** and DMDP propionic **6** acids showed significantly weaker β-glucosidase and β-galactosidase inhibition, but both were good inhibitors of α-galactosidase with IC<sub>50</sub> value of 35 μM and 67 μM, respectively; this is the first report of good α-galactosidase inhibition by DMDP derivatives. *N*-Ethyl and *N*-propyl derivatives of DMDP did not show such changes in their glycosidase inhibition profile (Asano et al. 1995). *galacto*-DMDP **10** is a potent and specific inhibitor of coffee bean α-galactosidase (IC<sub>50</sub> 0.19 μM) (Ayers et al. 2012), but had no inhibition of β-galactosidase. The corresponding acetic **11** and propionic **12** acids remain potent inhibitors of α-galactosidase, but showed no significant inhibition of β-galactosidase. *galacto*-DMDP propionic acid **12** was a highly selective α-galactosidase inhibitor. These results indicated that COOH group of the *N*-terminal chain caused selective and potent α-galactosidase inhibitions. DAB **7**, related to DMDP **1** by the loss of a hydroxymethyl group, is a good inhibitor of some α-glucosidases, but does not have any effect on galactosidases; both DAB acetic **8** and propionic **9** acids were significantly weaker glycosidase inhibitors and did not show any α-galactosidase inhibition. Neither of the propionic acids **15** or **18** derived from the

**Table 1** Concentration of iminosugar pyrrolidine carboxylic acids giving 50% inhibition of various glycosidases

Enzyme	IC <sub>50</sub> (μM)												
	 R=			 R=			 R=			 R=		 R=	
	H	CH <sub>2</sub> CO <sub>2</sub> H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	H	CH <sub>2</sub> CO <sub>2</sub> H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	H	CH <sub>2</sub> CO <sub>2</sub> H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H	H	(CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> H
1	5	6	7	8	9	10	11	12	13	15	16	18	
<b>α-Glucosidase</b>													
Rice	214	787	NI (17.8%)	250	NI (16.7%)	NI (38.3%)	NI (38.9%)	72	NI (12.2%)	290	NI (33.9%)	NI (16.0%)	NI (0%)
Yeast	<sup>a</sup> NI (15.6%)	100	89	0.15	NI (11.3%)	37	NI (0%)	NI (0%)	NI (0%)	144	NI (17.8%)	NI (23.0%)	NI (6.1%)
Rat intestinal maltase	290	1000	NI (30.1%)	55	834	922	NI (44.2%)	302	NI (28.3%)	204	NI (28.0%)	NI (43.8%)	NI (5.4%)
<i>A. niger</i>	24	113	77	66	NI (7.0%)	381	NI (0%)	NI (1.1%)	NI (0%)	<sup>c</sup> ND	NI (11.5%)	NI (0.8%)	NI (0%)
<b>β-Glucosidase</b>													
Almond	10	193	256	250	NI (8.0%)	NI (15.1%)	597	NI (17.3%)	NI (41.3%)	NI (48.6%)	654	NI (0%)	NI (3.1%)
Bovine liver	9.7	430	424	<sup>b</sup> NI (27.3%)	NI (3.2%)	NI (16.0%)	NI (44.5%)	NI (0%)	NI (5.0%)	NI (36.7%)	NI (22.7%)	NI (19.9%)	NI (1.5%)
<i>Aspergillus niger</i>	123	NI (25.1%)	NI (15.8%)	NI (38.6%)	NI (0%)	NI (4.5%)	NI (0%)	NI (0%)	NI (0%)	NI (23.6%)	NI (0%)	NI (0%)	NI (0%)
<b>α-Galactosidase</b>													
Coffee beans	NI (10.5%)	<b>35</b>	<b>67</b>	NI (6.6%)	NI (0%)	NI (17.9%)	<b>0.19</b>	<b>4.3</b>	<b>0.50</b>	NI (4.2%)	NI (0%)	NI (0%)	NI (0%)
<b>β-Galactosidase</b>													
Bovine liver	<b>3.3</b>	<b>246</b>	<b>203</b>	NI (37.4%)	NI (0%)	NI (11.7%)	NI (48.2%)	NI (20.2%)	NI (12.6%)	NI (46.2%)	NI (10.7%)	NI (0%)	NI (18.3%)
<b>α-Mannosidase</b>													
Jack bean	NI (31.5%)	NI (0.7%)	NI (4.7%)	320	NI (0%)	NI (11.3%)	NI (10.2%)	NI (0%)	NI (5.7%)	NI (1.1%)	NI (0.7%)	NI (0%)	NI (0%)
<b>β-Mannosidase</b>													
Snail	721	NI (5.2%)	NI (5.2%)	NI (10.0%)	NI (0%)	NI (0%)	NI (0%)	NI (0%)	NI (1.2%)	NI (6.4%)	NI (4.4%)	NI (0%)	NI (0%)
<b>α-L-Rhamnosidase</b>													
<i>P. decumbens</i>	NI (24.1%)	NI (1.8%)	NI (3.2%)	NI (5.4%)	NI (5.3%)	NI (1.6%)	550	NI (11.3%)	NI (46.1%)	NI (0%)	NI (1.2%)	NI (1.9%)	NI (0%)
<b>α-L-Fucosidase</b>													
Bovine kidney	NI (37.2%)	NI (0%)	NI (0.3%)	NI (11.2%)	NI (0%)	NI (0%)	41	NI (45.6%)	151	NI (4.8%)	NI (0%)	NI (0%)	NI (0%)
<b>β-Glucuronidase</b>													
<i>E.coli</i>	NI (0.4%)	NI (0%)	NI (1.0%)	NI (2.8%)	NI (1.0%)	NI (1.5%)	NI (21.3%)	NI (0%)	NI (0%)	NI (12.9%)	NI (2.1%)	NI (17.6%)	NI (0.4%)
Bovine liver	NI (0%)	NI (0%)	NI (0%)	NI (2.6%)	NI (3.1%)	NI (1.7%)	NI (11.4%)	NI (19.0%)	NI (19.0%)	NI (4.0%)	NI (0%)	NI (29.3%)	NI (0%)
<b>Trehalase</b>													
Porcine kidney	200	NI (29.0%)	NI (28.1%)	61	NI (0%)	502	NI (8.4%)	NI (0%)	NI (1.2%)	NI (5.2%)	NI (1.5%)	NI (0%)	NI (0%)
<b>Amyloglucosidase</b>													
<i>A. niger</i>	113	340	358	362	NI (3.3%)	NI (27.4%)	NI (3.4%)	NI (3.0%)	NI (0%)	ND	NI (4.2%)	NI (6.1%)	NI (0.2%)
<i>Rhizopus sp</i>	54	228	226	160	NI (5.8%)	1000	NI (3.7%)	NI (1.6%)	NI (7.6%)	ND	NI (5.9%)	NI (3.1%)	NI (2.6%)

<sup>a</sup>NI no inhibition (less than 50% inhibition at 1000 μM)

<sup>b</sup>( ): inhibition % at 1000 μM

<sup>c</sup>ND not determined

polyhydroxylated prolines **13** and **16** showed inhibition of any glycosidases.

## Experimental

All commercial reagents were used as supplied. Methanol was purchased dry from Alfa Aesar in sure-seal bottles. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Melting points were recorded on a Kofler hot block and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations are quoted in g 100 mL<sup>-1</sup>. Infrared spectra were recorded on a Perkin-Elmer 1750 IR Fourier Transform spectrophotometer using thin films on a diamond ATR surface (thin film). Only the characteristic peaks are quoted. Low-resolution mass spectra (*m/z*) were recorded on an Agilent 6120 spectrometer and high-resolution mass spectra (HRMS *m/z*) on a Bruker microTOF mass analyzer using electrospray ionization (ESI). Nuclear magnetic resonance (NMR) spectra were recorded on Bruker

AVIII 400 HD nanobay and Bruker DQX 400 spectrometers (<sup>1</sup>H: 400 MHz and <sup>13</sup>C: 100.6 MHz) in the deuterated solvent stated. All chemical shifts ( $\delta$ ) are quoted in ppm and coupling constants (*J*) in Hz. Residual signals from the solvents were used as an internal reference, except in the case of deuterium oxide, where acetonitrile was used as the reference. The syntheses of all *N*-alkyl derivatives were carried out directly from their unprotected parent iminosugars and amino acids as previously prepared: 2,5-dideoxy-2,5-imino-D-mannitol (DMDP) **1** (Best et al. 2010), 2,5-dideoxy-2,5-imino-D-mannonic acid [(3*S*)-3-hydroxy-bulgecinine] **13** (Best et al. 2010), and deoxyojirimycin (DNJ) **2** (Best et al. 2010) and 2,5-dideoxy-2,5-iminogalactitol (*galacto*-DMDP) **10** from glucuronolactone (Ayers et al. 2012), 1,4-dideoxy-1,4-imino-D-arabinitol (DAB) **7** and *trans,trans*-dihydroxyproline **16** from diacetone glucose (Fleet and Witty 1990).

## Isolation of DMDP acetic acid **5** and DMDP **1** from *Stevia*

Leaves of commercially supplied *S. rebaudiana* Betoni (Asteraceae), a herb native to South America, were used for the isolation of DMDP acetic acid **5**. *Stevia* leaf (2.2 kg) was obtained from Cotswold Health Products UK (batch 060621) and extracted in water for 15 h. The filtrate was applied to a cation exchange column (Amberlite IR120) in the H<sup>+</sup> form and washed with water, and then the amino acids and SAA displaced with 2 M ammonia solution. After removal of the ammonia under reduced pressure at 40 °C, the material was applied to anion exchange resin (Amberlite CG400 OH<sup>-</sup> form) and washed with water to give an unretained fraction containing DMDP **1** and the neutral and acidic compounds displaced with 1 M acetic acid. Both fractions were reduced in volume at reduced pressure and the DMDP purified using Amberlite CG50 resin in the ammonia form with 20 water fractions collected. The DMDP acetic acid **5** was purified from other amino acids by applying the CG400 retained fraction back on the CG400 in the acetate form and 20 fractions collected with water. Analysis of fractions was by GCMS of the trimethylsilylation of aliquots of the fractions. DMDP **1** gives a distinctive EI fragmentation with major ions 217 (100%), 258 (50%), and 348 (80%) 436 M<sup>+</sup> - 15 (methyl) (2%) amu (retention time 8.7 min) and the DMDP acetic acid **5** with retention time 10.8 min has ions 147 (100%), 298 (50%), and 478 (80%), 566 M<sup>+</sup> - 15 (methyl) (2%) amu. Mannitol as reference on the same mid-polarity column has a retention time of 10.5 min. Yields were: DMDP **1** (2 g) and DMDP acetic acid **5** (50 mg). The GCMS and NMR spectra of DMDP **1** and DMDP acetic acid **5** were identical to those of the synthetic compounds in the paper.

### General procedure for pyrrolidine *N*-acetic acids

Glyoxal solution (40% w/w) in water (1.1 eq) was added to a stirred solution of the corresponding iminosugar (1.0 eq) in water (15 mg/mL). The mixture was stirred at 70 °C for 14–17 h, after which the reaction mixture was concentrated in vacuo. The remaining residue was loaded onto a Dowex ion-exchange resin (50WX8, H<sup>+</sup>). The column was flushed with water and then eluted with 2 M aqueous ammonia. The ammoniacal fraction was concentrated in vacuo to afford the corresponding compounds.

### *N*-Carboxymethyl-2,5-dideoxy-2,5-imino-*D*-mannitol **5**

DMDP **1** (20 mg, 0.12 mmol) gave DMDP acetic acid **5** (27 mg, 100%), [ $\alpha$ ]<sub>D</sub><sup>25</sup> +5.9 (*c* 0.15, H<sub>2</sub>O);  $\nu_{\max}$  (thin film) 3235 (OH), 1614, 1393 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 3.77

(2H, m, H-2 and H-5), 4.00 (6H, m, CH<sub>2</sub>N, H1 and H6), 4.23 (2H, d, *J*<sub>3,2</sub>/*J*<sub>4,5</sub> 5.6, H3 and H4);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 52.8 (CH<sub>2</sub>N), 57.2 (C1 and C6), 70.5 (C2 and C5), 74.8 (C3 and C4), 171.3 (C=O); *m/z* (ESI<sup>+</sup>): 222 ([M+H]<sup>+</sup>, 21%), 244 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>): found 244.0793 ([M+Na]<sup>+</sup>); C<sub>8</sub>H<sub>15</sub>NNaO<sub>6</sub> requires 244.0792.

### *N*-Carboxymethyl-1,4-dideoxy-1,4-imino-*D*-arabinitol **8**

DAB **7** (11 mg, 0.08 mmol) gave **8** (13 mg, 82%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> +20.7 (*c* 0.47, H<sub>2</sub>O);  $\nu_{\max}$  (thin film) 3234 (OH), 1614, 1396 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 3.63 (1H, m, H2), 3.66 (1H, dd, *J*<sub>5,4</sub> 5.4, *J*<sub>5,5'</sub> 7.0, H5), 3.83 (1H, m, H5'), 3.87 (1H, d, *J*<sub>1,1'</sub> 16.0, H1), 4.01 (2H, m, CH<sub>2</sub>N), 4.07 (1H, d, *J*<sub>1',1</sub> 16.0, H1'), 4.15 (1H, t, *J*<sub>3,4</sub>/*J*<sub>3,2</sub> 3.6, H3), 4.38 (1H, m, H4);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 58.2, 58.4 (CH<sub>2</sub>N and C1), 60.4 (C5), 74.2, 74.4 (C2 and C4), 76.6 (C3), 170.8 (C=O); *m/z* (ESI<sup>+</sup>): 192 ([M+H]<sup>+</sup>, 15%), 214 ([M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>): found 214.0687 ([M+Na]<sup>+</sup>); C<sub>7</sub>H<sub>13</sub>NNaO<sub>5</sub> requires 214.0686.

### *N*-Carboxymethyl-2,5-dideoxy-2,5-iminogalactitol **11**

galacto-DMDP **10** (15 mg, 0.09 mmol) gave the *N*-acetic acid **11** (20 mg, 99%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> 0.0 (*c* 0.30, H<sub>2</sub>O);  $\nu_{\max}$  (thin film): 3360 (OH), 3250 (OH), 1741 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz): 3.06 (2H, ddd, *J*<sub>2,1</sub> 4.3, *J*<sub>2,3</sub> 5.3, *J*<sub>2,1'</sub> 6.1, H2), 3.24 (2H, s, CH<sub>2</sub>N), 3.54 (2H, dd, *J*<sub>1,2</sub> 4.3, *J*<sub>1,1'</sub> 11.3, H1), 3.61 (2H, dd, *J*<sub>1',2</sub> 6.1, *J*<sub>1',1</sub> 11.3, H1'), 4.25 (2H, d, *J*<sub>3,2</sub> 5.3, H3);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz): 57.0 (CH<sub>2</sub>N), 60.2 (C1), 66.4 (C2), 72.3 (C3), 180.4 (C=O); *m/z* (ESI<sup>+</sup>): 222 ([M+H]<sup>+</sup>, 100%), 244 ([M+Na]<sup>+</sup>, 35%); (ESI<sup>-</sup>): 220 ([M-H]<sup>-</sup>, 100%). HRMS (ESI<sup>+</sup>): found 220.0830 ([M-H]<sup>-</sup>); C<sub>8</sub>H<sub>14</sub>NO<sub>6</sub> requires 220.0827.

### General procedure for *N*-propionic acids

*tert*-Butylacrylate (10 eq) was added to a stirred solution of the corresponding iminosugar or hydroxylated proline (1 eq) in methanol (10 mg/mL). The mixture was stirred at 50 °C for 48 h, after which the reaction was shown to be complete by mass spectrometry. The mixture was concentrated in vacuo to afford the crude ester, which was dissolved in a mixture of trifluoroacetic acid (0.5 mL) and water (0.5 mL) and stirred at room temperature. After 48 h, the solvents were removed in vacuo. The remaining residue was loaded onto a Dowex ion-exchange resin (50WX8, H<sup>+</sup>). The column was flushed with water and then eluted with 2 M aqueous

ammonia. The ammoniacal fraction was concentrated in vacuo to afford the title compounds.

### ***N*-(2-Carboxyethyl)-2,5-dideoxy-2,5-imino-D-mannitol 6**

DMDP **1** (33 mg, 0.20 mmol) gave DMDP propionic acid **6** (48 mg, 100%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 25.2 (*c* 0.1, methanol);  $\nu_{\max}$  (thin film) 3229 (OH), 1577, 1390 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 2.56 (2H, m, CH<sub>2</sub>COO), 3.45 (2H, t, *J* 6.4, CH<sub>2</sub>N), 3.59 (2H, bs, H2 and H5), 3.93 (4H, d, *J*<sub>1,2</sub>/*J*<sub>6,5</sub> 3.2, H1 and H6), 4.13 (2H, d, *J*<sub>3,2</sub>/*J*<sub>4,5</sub> 3.2, H3 and H4);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 31.7 (CH<sub>2</sub>COO), 46.7 (CH<sub>2</sub>N), 57.5 (C1 and C6), 70.4 (C2 and C5), 76.3 (C3 and C4), 179.1 (C=O); *m/z* (ESI<sup>+</sup>): 236 ([M+H]<sup>+</sup>, 100%), 258 ([M+Na]<sup>+</sup>, 89%), 493 ([2M+Na]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>): found 236.1131 ([M+H]<sup>+</sup>); C<sub>9</sub>H<sub>18</sub>NO<sub>6</sub> requires 236.1129.

### ***N*-(2-Carboxyethyl)-1,4-dideoxy-1,4-imino-D-arabitol 9**

DAB **1** (9 mg, 0.07 mmol) gave DAB propionic acid **9** (14 mg, 100%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> + 12.7 (*c* 0.43, water);  $\nu_{\max}$  (thin film) 3239 (OH), 1579, 1388 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 2.70 (2H, m, CH<sub>2</sub>COO), 3.40 (1H, m, CH<sub>2</sub>N), 3.54 (2H, m, H2 and H5), 3.72 (2H, m, H5' and CH<sub>2</sub>N), 3.99 (2H, m, H1 and H1'), 4.16 (1H, m, H3), 4.37 (1H, m, H4);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 31.9 (CH<sub>2</sub>COO), 53.3 (CH<sub>2</sub>N), 58.7 (C1 and C5), 74.2, 75.5, 76.6 (C2, C3, and C4), 178.0 (C=O); *m/z* (ESI<sup>+</sup>): 206 ([M+H]<sup>+</sup>, 92%), 228 ([M+Na]<sup>+</sup>, 100%), 433 ([2M+Na]<sup>+</sup>, 46%); HRMS (ESI<sup>+</sup>): found 228.0847 ([M+Na]<sup>+</sup>); C<sub>8</sub>H<sub>15</sub>NNaO<sub>5</sub> requires 228.0842.

### ***N*-(2-Carboxyethyl)-2,5-dideoxy-2,5-iminogalactitol 12**

galacto-DMDP **10** (21 mg, 0.13 mmol) gave the propionic acid **12** (25 mg, 83%) [ $\alpha$ ]<sub>D</sub><sup>25</sup> 0.0 (*c* 1.1, H<sub>2</sub>O);  $\nu_{\max}$  (thin film): 3451 (OH), 3343 (OH), 1738 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz): 2.51–2.63 (2H, m, CH<sub>2</sub>COO), 3.00 (2H, a–q, *J*<sub>2,1</sub>/*J*<sub>2,1</sub>/*J*<sub>2,3</sub> 5.1, H2), 3.25 (2H, a–t, *J* 6.0, CH<sub>2</sub>N), 3.48 (2H, dd, *J*<sub>1,2</sub> 4.5, *J*<sub>1,1'</sub> 11.2, H1), 3.55 (2H, dd, *J*<sub>1,2</sub> 5.8, *J*<sub>1,1'</sub> 11.2, H1'), 4.20 (2H, d, *J*<sub>3,2</sub> 5.0, H3);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz): 33.2 (CH<sub>2</sub>COO), 48.5 (CH<sub>2</sub>N), 60.1 (C1), 66.3 (C2), 72.2 (C5), 180.4 (C=O); *m/z* (ESI<sup>+</sup>): 236 ([M+H]<sup>+</sup>, 100%), 258 ([M+Na]<sup>+</sup>, 85%); (ESI<sup>–</sup>): 234 ([M–H]<sup>–</sup>, 100%). HRMS (ESI<sup>–</sup>): found 234.0990 [M–H]<sup>–</sup>; C<sub>9</sub>H<sub>16</sub>NO<sub>6</sub> requires 234.0983.

### ***N*-(2-Carboxyethyl)-2,5-dideoxy-2,5-imino-D-mannonic acid 15**

*tert*-Butylacrylate (0.25 mL, 1.7 mmol) and triethylamine (0.04 mL, 0.29 mmol) were added to a stirred solution of the iminomannonic acid **13** (30 mg, 0.17 mmol) in methanol (1.5 mL). The mixture was stirred at 50 °C for 48 h and then concentrated in vacuo. The crude was redissolved in a mixture of trifluoroacetic acid (1.5 mL) and water (1.5 mL), and the resulting solution was stirred at room temperature. After 48 h, the reaction was judged to be complete by mass spectrometry. The solvents were removed in vacuo and the remaining residue was loaded onto an Amberlite CG-400 strongly basic ion-exchange resin. The column was flushed with water and then eluted with 2 M acetic acid. The acidic fraction was concentrated in vacuo to afford the propionic acid **15** (42 mg, 100%). [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 6.7 (*c* 0.75, water);  $\nu_{\max}$  (thin film) 3262 (OH), 1711, 1621 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 2.90 (2H, m, CH<sub>2</sub>COO), 3.64 (1H, m, CH<sub>2</sub>N), 3.86 (1H, m, CH<sub>2</sub>N), 4.02 (1H, m, H5), 4.09 (2H, m, H6), 4.13 (1H, d, *J*<sub>2,3</sub> 2.8, H2), 4.28 (1H, t, *J*<sub>4,5</sub>/*J*<sub>4,3</sub> 2.8, H4), 4.33 (1H, d, *J*<sub>3,2</sub>/*J*<sub>3,4</sub> 2.8, H3);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 30.3 (CH<sub>2</sub>COO), 47.3 (CH<sub>2</sub>N), 57.2 (C6), 72.3 (C5), 74.2 (C2), 76.8 (C4), 79.2 (C3), 170.8, 174.9 (C=O); *m/z* (ESI<sup>–</sup>): 248 ([M–H]<sup>–</sup>, 100%); HRMS (ESI<sup>+</sup>): found 272.0738 ([M+Na]<sup>+</sup>); C<sub>9</sub>H<sub>15</sub>NNaO<sub>7</sub> requires 272.0741.

### ***N*-(2-Carboxyethyl)-2,5-dideoxy-2,5-imino-D-lyxonic acid 18**

The *trans,trans*-dihydroxyproline **16** (33 mg, 0.22 mmol) gave the propionic acid **18** (47 mg, 100%) by the same procedure used for the synthesis of **15**. [ $\alpha$ ]<sub>D</sub><sup>25</sup> – 2.3 (*c* 0.48, water);  $\nu_{\max}$  (thin film): 3367 (OH), 1623, 1707 (COO);  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz, HCl Salt): 2.77–2.85 (2H, m, CH<sub>2</sub>COO), 3.51–3.54 (3H, m, CH<sub>2</sub>N and H5), 3.77 (1H, d, H5', *J*<sub>5',5</sub> 12.1), 4.04 (1H, s, H2), 4.24 (1H, s, H4), 4.43 (1H, s, H3);  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz, HCl salt): 30.3 (CH<sub>2</sub>COO), 52.7 (CH<sub>2</sub>N), 60.4 (C5), 74.4 (C4), 76.2 (C2), 79.1 (C3), 174.7, 177.3 (C=O); *m/z* (ESI<sup>+</sup>): 220 ([M+H]<sup>+</sup>, 100%); HRMS (ESI<sup>+</sup>): found 220.0806 [M+H]<sup>+</sup>; C<sub>8</sub>H<sub>14</sub>NO<sub>6</sub> requires 220.0816.

### ***N*-(2-carboxyethyl)-1,5-dideoxy-1,5-imino-D-glucitol (DNJ-propionic acid) 20**

*tert*-Butyl acrylate (0.15 mL, 1.04 mmol) was added to a stirred solution of DNJ **2** (17 mg, 0.1 mmol) in methanol (1 mL). The mixture was stirred at 50 °C for 41 h after which more *tert*-butyl acrylate (0.15 mL) was added. After

a further 24 h, the reaction mixture was concentrated in vacuo. The crude residue was re-dissolved in a mixture of trifluoroacetic acid (1 mL) and water (1 mL), and the resulting solution was stirred at rt. After 48 h, the solvents were removed in vacuo and the remaining residue was loaded onto a Dowex ion-exchange resin (50WX8 H<sup>+</sup>). The column was flushed with water and then eluted with 2 M aqueous ammonia. The ammoniacal fraction was reduced in vacuo to afford the *N*-propionic acid **20** as white solid (25 mg, 99%). [ $\alpha$ ]<sub>D</sub><sup>20</sup> – 8.0 (*c*, 0.95 in water);  $\nu_{\max}$  (thin film) 3206 (OH), 1563 (COO); m.p. 196–198 °C;  $\delta_{\text{H}}$  (D<sub>2</sub>O, 400 MHz) 2.52 (m, 2H, CH<sub>2</sub>COO), 2.65 (t,  $J_{\text{gem}}=J_{1,2}=11.6$ , 1H, H-1), 2.71 (m, 1H, H-5), 3.12 (m, 1H, CH<sub>2</sub>N), 3.27 (d,  $J_{\text{gem}}=11.6$ ,  $J_{1',2}=4.8$ , 1H, H-1'), 3.33 (m, 1H, CH<sub>2</sub>N), 3.38 (t,  $J_{3,2}=J_{3,4}=9.4$ , 1H, H-3), 3.53 (t,  $J_{4,5}=J_{4,3}=9.4$ , 1H, H-4), 3.66 (ddd,  $J_{2,1'}=4.8$ ,  $J_{2,3}=9.4$ ,  $J_{2,1}=11.6$ , 1H, H-2), 3.95 (dd,  $J_{6,5}=2.4$ ,  $J_{6,6'}=13.5$ , 1H, H-6), 4.00 (dd,  $J_{6',5}=2.7$ ,  $J_{6',6}=13.5$ , 1H, H-6');  $\delta_{\text{C}}$  (D<sub>2</sub>O, 100.6 MHz) 31.9 (CH<sub>2</sub>COO), 49.6 (CH<sub>2</sub>N), 54.6 (C-1), 56.2 (C-6), 65.6 (C-5), 68.1 (C-2), 69.3 (C-4), 77.7 (C-3), 180.2 (C=O); *m/z* (ESI<sup>+</sup>): 236 ([M+H]<sup>+</sup>, 100%), 258 ([M+Na]<sup>+</sup>, 42%), 493 ([2M+Na]<sup>+</sup>, 25%); HRMS (ESI<sup>+</sup>): found 236.1137 ([M+H]<sup>+</sup>); C<sub>9</sub>H<sub>18</sub>NO<sub>6</sub><sup>+</sup> requires 236.1129.

### Glycosidase inhibition (Kato et al. 2015)

The enzymes  $\alpha$ -glucosidase (from rice, yeast, and *A. niger*),  $\beta$ -glucosidase (from almond, bovine liver, and *A. niger*),  $\alpha$ -galactosidase (from coffee beans),  $\beta$ -galactosidase (from bovine liver),  $\alpha$ -mannosidase (from jack bean),  $\beta$ -mannosidase (from snail),  $\alpha$ -L-rhamnosidase (from *Penicillium decumbens*),  $\alpha$ -L-fucosidase (from bovine kidney),  $\beta$ -glucuronidases (from *E. coli* and bovine liver), amyloglucosidase (*A. niger*, and *Rhizopus* sp.), *p*-nitrophenyl glycosides, and various disaccharides were purchased from Sigma-Aldrich Co. Brush border membranes were prepared from the rat small intestine (Kessler et al. 1978), and were assayed at pH 6.8 for rat intestinal maltase using maltose. For rat intestinal maltase activities, the reaction mixture contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10 min at 37 °C. The reaction was stopped by heating at 100 °C for 3 min. After centrifugation (600g; 10 min), the resulting reaction mixture were added to the Glucose CII-test Wako (Wako Pure Chemical Ind., Osaka, Japan). The absorbance at 505 nm was measured to determine the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 400 mM Na<sub>2</sub>CO<sub>3</sub>. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

## Conclusions

Glycosides from leaves of *S. rebaudiana* are widely used as low calorie sweeteners (Samuel et al. 2018). Other beneficial properties from *Stevia* extracts have been noted (Chat-sudthipong and Muanprasat 2009; Zangeneh et al. 2017); DMDP **1** was shown to be the major iminosugar in *Stevia* together with smaller quantities of the DMDP acetic acid **5**. Although such polyhydroxylated amino acids are difficult to isolate, they may readily be obtained from unprotected pyrrolidine iminosugars with aqueous glyoxal as the corresponding *N*-acetic acids and from both pyrrolidine and piperidine iminosugars and of polyhydroxylated prolines with *tert*-butyl acrylate as the *N*-propionic acids. The syntheses will allow the easy identification of this new class of amino acid in plant extracts and provide pure sample for biological evaluation. The potent  $\beta$ -galactosidase inhibition by DMDP **1** was replaced by potent  $\alpha$ -galactosidase inhibition by DMDP *N*-acetic **5** and propionic **6** acids; in contrast, *galacto*-DMDP **10** and both its acetic **11** and propionic **12** acids are  $\alpha$ -galactosidase inhibitors.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Research involving human participants and/or animals** This article does not contain any studies with human participants or animals performed by any of the authors.

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