Synthesis of 3-*C*-hydroxymethyl- and 3-deoxyisofagomine and investigation of their binding to β-glucosidase

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The syntheses of two isofagomine analogues, 3-*C*-hydroxymethylisofagomine and 3-deoxyisofagomine, are reported.

Introduction

Inhibitors of glycoside processing enzymes are of increasing interest due to their potential application as drugs or as biochemical tools for investigating glycobiology.[1] A new type of potent and selective inhibitor of carbohydrate processing enzymes are the 1-azasugars, with isofagomine† (**1**) a strong β-glucosidase inhibitor being a typical example.[2] Structural modifications of such inhibitors may lead to insight into their binding mode or perhaps even stronger inhibitors.

Studies of isofagomine have established that the 3-hydroxy group is important for binding to β-glucosidase.[3] Recently it was found that substitution of this hydroxy group with a hydroxymethyl group as in **2** led to a large decrease in inhibition of β-glucosidase.[4] Surprisingly the 3-epimer of **2**, **3**, was a much better inhibitor than **2**. This result might suggest that the 3-hydroxymethyl group of **3** had a previously unexploited binding interaction with the enzyme, provided that **3** was a stronger inhibitor than the corresponding 3-deoxy-compound **4**. If so, a hybrid compound **5** containing both the 3-hydroxymethyl group of **3** and the 3-hydroxy group of **1** might be expected to be an even stronger inhibitor than **1** itself (Fig. 1).

In the present study we have synthesised the new compounds **4** and **5** and tested this hypothesis.

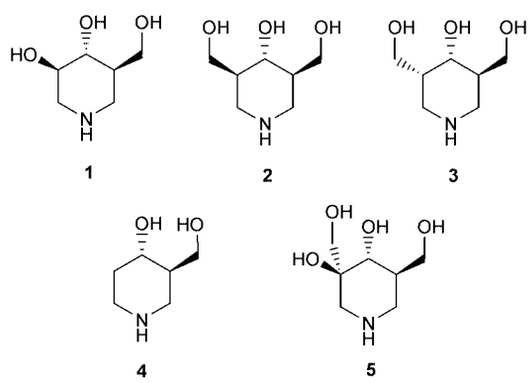
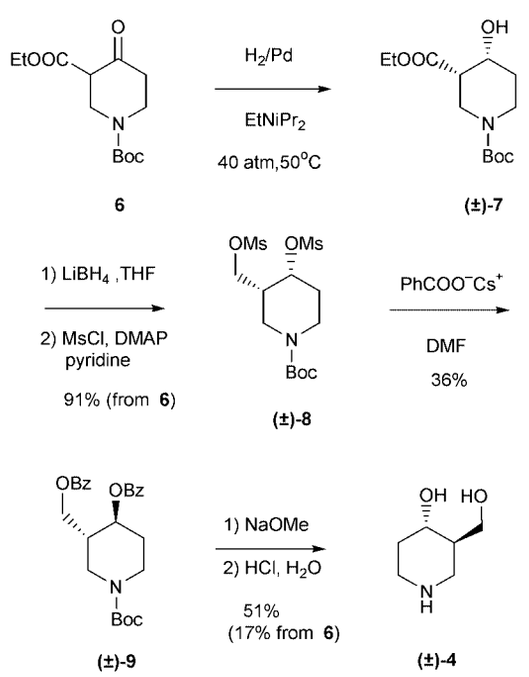


Fig. 1 Isofagomine (**1**) and analogues.

Results and discussion

For the synthesis of **4** the known piperidone **6**[5] was chosen as starting material (Scheme 1). It could be prepared in one step from commercial material and previous experiments in our group have shown that the ketone could be reduced stereoselectively by hydrogenation. Thus hydrogenation of **6** at 40 atm of H_2-pressure and at 50 °C in EtOH in the presence of diisopropylethylamine (DIEA) and Pd/C gave **7** as a single product. The ester group of **7** was reduced with $LiBH_4$ in THF to give a diol, which was reacted with methanesulfonyl chloride in pyridine in the presence of a catalytic amount of DMAP to give the dimesylate **8**. The yield from **6** was 91%.

The dimesylate was now subject to nucleophilic substitution with caesium benzoate in DMF at 50 °C. This was not expected to be a high yielding reaction, because secondary mesylates are often unreactive and prone to elimination. However after 3 days of reaction time the dibenzoate **9** was isolated in 36% yield, while 50% of elimination products were removed by chromatography. Even though the yield of **9** was modest, the product has a stereochemistry that is difficult to obtain by other means.



Scheme 1 Synthesis of 3-deoxyisofagomine (**4**).

Finally the dibenzoate **9** was deprotected by Zemplén deacylation followed by treatment with hydrochloric acid. This gave the target compound **4** in 17% overall yield.

Compound **4** was tested for inhibition of β-glucosidase. It was found to be an extremely weak inhibitor with a K_i of 5 mM. In contrast the K_i of **3** against β-glucosidase was 47 μM and thus 100 times more potent. This established that the

† The IUPAC name for isofagomine is (3*R*,4*R*,5*R*)-5-hydroxymethyl-3,4-dihydroxypiperidine.

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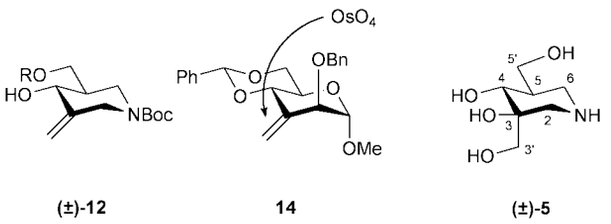
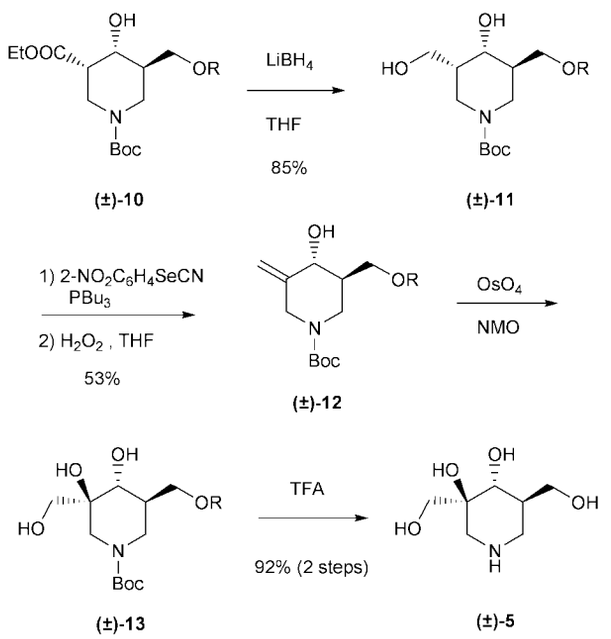


(±)-12 14 (±)-5

Fig. 2 Comparison of the dihydroxylation of **12** with the literature precedent: the dihydroxylation of **14**.



(±)-10 (±)-11 (±)-12 (±)-13 (±)-5

Scheme 2 Synthesis of 3-*C*-hydroxymethylisofagomine (**5**). R = CH_2-$CH_2Si(CH_3)_3$.

3-hydroxymethyl group of **3** definitely contributed to binding of the inhibitor. Incorporating this group into **1** could thus increase the strength of its binding.

The synthesis of **5** (Scheme 2) started from the known ester **10**, which is readily available because it is the main product when the 5-alkylate of **6** is hydrogenated (Scheme 1).[6] Reduction of **10** with $LiBH_4$ in THF gave a 85% yield of the diol **11**. This compound was converted to an alkene by the following sequence of reactions. The primary alcohol was selectively substituted with a nitrophenylselenium group using a Mitsunobu type reaction employing $NO_2C_6H_4SeCN$ and PBu_3 in THF.[7] The crude selenide was subsequently oxidised with hydrogen peroxide to give the alkene **12** in 53% overall yield. Now the alkene **12** was subjected to osmium catalysed dihydroxylation using OsO_4 and *N*-methylmorpholine *N*-oxide (NMO) to give a single diol **13**. The closest known example to this reaction was the OsO_4 dihydroxylation of compound **14** (Fig. 2).[8] In that case also only one stereoisomer with the indicated stereochemistry was obtained. Both **12** and **14** have identical stereochemistry at C-4 and C-5, which suggests a similar configuration of **13**. One might argue that the axial 2-benzyloxy group of **14**, which is absent in **12**, might affect the stereochemical outcome by giving sterical hindrance. However, if so it should affect the reaction of **14** by promoting addition from the opposite face to the one observed.

Finally **13** was deprotected with TFA to give **5** in 92% yield from alkene **12**. Conclusive evidence for the stereochemistry at C-3 was obtained from NMR spectroscopy of **5**. Firstly all ^1H and ^{13}C chemical shifts, except the tertiary C-3 were assigned based on the COSY and HSQC spectra (Table 1). Secondly the predominant conformation of **5** with an axial orientation of protons H-4, H-5, H-6ax and H-2ax was determined based on relative chemical shifts, coupling constants and NOEs, *e.g.*,

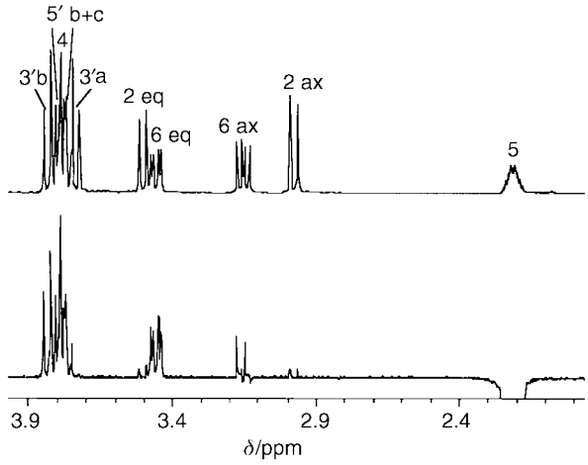


Fig. 3 One dimensional ^1H NMR spectrum of compound **5** (top) and one dimensional selective NOESY spectrum with selection of H-5.

medium strength NOEs between H-2ax and H-4, H-4 and H-6ax, and H-2ax and H-6ax in 2D NOESY or 2D HSQC-NOESY (Fig. 2).

The axial orientation of the hydroxymethyl group at the 3 position and equatorial orientation of the hydroxy group could be proved by NOE contacts between H-5 and H-3′b as seen in the 1D-NOESY in Fig. 3. Likewise, a NOE could be observed between H-2eq and H-3′b. Furthermore, the NOE's could be confirmed in the 2D HSQC-NOESY, where the overlap between H-3′b, H-4 and H-5′c could be resolved by the corresponding ^{13}C chemical shifts.

Compound (±)-**5** inhibited almond β-glucosidase with a K_i of 7 μM. This means that **5** is quite a strong inhibitor of the enzyme yet 64 times weaker than **1**, which has a K_i value of 0.11 μM.[2a] This value is however for enantiopure **1**, while **5** is racemic. Assuming that the L-*gluco* enantiomer of **5** does not inhibit the enzyme, which is a reasonable assumption since it has been found that the L-*gluco* enantiomer of 1-azafagomine is a very poor glucosidase inhibitor,[9] the active enantiomer of **5** is twice as strong and the difference in K_i value between **1** and **5** is only times 32.

The results show that although the binding of **4** is increased by introducing a hydroxymethyl group (in **3**) or a hydroxy group (in **1**), the effects are not additive. This suggests that both the 3-hydroxymethyl group and the 3-OH are competing for binding to the same functionality in the enzyme. Thus a kind of induced fit of the enzyme to the inhibitor seems to be in operation.

In the present work we have synthesised two new isofagomine analogues, and found that the 3-OH group of **1** is essential for its inhibition of β-glucosidase. Other polar groups at the 3-position also increase binding compared to deoxy, but a combination of two functionalities as in **5** does not further increase, rather decreases binding. This may be due to the possibility that the two groups are competing for the same binding site.

Experimental

General

All reactions were carried out under an inert atmosphere in preheated glass equipment. Solvents were distilled under anhydrous conditions. Thus THF was distilled from sodium–benzophenone and used directly. All reagents were used as purchased without further purification. Columns were packed with silica gel 60 (230–400 mesh) as the stationary phase. TLC-plates (Merck, 60, F_{254}) were visualised by spraying with ninhydrin (2% in butanol) and heating until coloured spots appeared. All enzymes and substrates for the enzyme assays were purchased from Sigma.

Table 1 ^{1}H and ^{13}C chemical shifts of compound **5** and assigned NOEs

	^{1}H	*J*/Hz		^{13}C	NOE[a]				
2ax	2.976	13.0		48.8	2eq (s)	4 (m)	6ax (m)		
2eq	3.507	13.0			2ax (s)	3′b (m)			
3′a	3.739	12.0		63.8					
3′b	3.837	12.0			2eq (m)	5 (m)			
4	3.783			71.6	2ax (m)	5 (m)	6ax (m)		
5	2.213			42.1	3′b (m)	4 (m)	5′b (m)	5′c (m)	6ax (w)	6eq (m)
5′b	3.770			61.3	5 (m)				
5′c	3.813	11.8	4.2		5 (m)				
6ax	3.155	13.1	8.8	45.1	2ax (m)	4 (m)	5 (w)	6eq (s)	
6eq	3.460	13.1	4.6		5 (m)	6ax (s)			

[a] Relative NOE intensities from 2D NOESY; w: weak, m: medium, s: strong.

(3,4-*trans*)-3-Hydroxymethyl-4-hydroxypiperidine (4)

The Boc-protected 3-ethoxycarbonyl-4-piperidone **6**[5] (300 mg, 1.1 mmol) was dissolved in absolute EtOH (30 mL), and DIEA (140 mg, 1.1 mmol) was added. N_2 was bubbled through the solution for 5 min before 10% Pd/C (200 mg) was added. The mixture was hydrogenated for 24 h (40 atm, 50 °C) before filtering through Celite® and evaporating to dryness. To remove the DIEA the remaining oil was dissolved in Et_2O (20 mL) and a saturated solution of $KHSO_4$ (20 mL) was added. The aqueous phase was extracted 3 times with Et_2O (20 mL) before the combined organic phases were dried ($MgSO_4$) and evaporated to crude **7**.

Without further purification the resulting alcohol **7** (300 mg, 1.1 mmol) was dissolved in dry THF (50 mL) and treated with $LiBH_4$ (24 mg, 1.1 mmol). The solution was refluxed for a 10 min period and cooled to 0 °C before a solution of saturated $KHSO_4$ (40 mL) was slowly added. The two layers were separated, and the aqueous phase was extracted with EtOAc (2 × 30 mL) before the combined organic phases were washed using 40 mL of brine, dried over $MgSO_4$ and evaporated to dryness.

The resulting diol (230 mg, 1.0 mmol) was dissolved in pyridine (10 mL), and a catalytic amount of DMAP was added. Methanesulfonyl chloride (242 mg, 2.1 mmol) was slowly added at ambient temperature, and the reaction was monitored by TLC (eluent: EtOAc). After *ca.* 90 min the starting material was no longer observed, and water was added (20 mL). The phases were separated, and the water phase was extracted with EtOAc (2 × 15 mL). The organic phases were combined and dried over $MgSO_4$ before evaporating. The resulting crystalline compound **8** (380 mg, 91% over 3 steps) was sufficiently pure for further reaction.

To a solution of the mesylated diol **8** (380 mg, 1.0 mmol) in 20 mL dry DMF, was added caesium benzoate (500 mg, 2.0 mmol). The reaction mixture was stirred at 50 °C for 3 days before the DMF was removed at reduced pressure. Water and EtOAc (15 mL of each) were added, and the water phase extracted with EtOAc (2 × 15 mL). The organic phases were combined and dried ($MgSO_4$) before evaporating to dryness. The resulting oil underwent flash chromatography (eluent: pentane–EtOAc, 8 : 1, R_f: 0.2) and gave 158 mg (36%) of the dibenzoylated compound **9**. ^{1}H-NMR ($CDCl_3$): δ 7.30–8.10 (m, 10H, Ar-*H*), 5.09 (dt, 1H, $J_{4,3eq}$ 4.4 Hz, $J_{4,3ax}$ 9.2 Hz, $J_{4,5}$ 9.2 Hz, H-4), 4.42 (dd, 1H, $J_{5'a,5}$ 4.4 Hz, $J_{5'a,5'b}$ 11.4 Hz, H-5′a), 4.20 (dd, 1H, $J_{5'b,5}$ 7.0 Hz, H-5′b), 4.00–4.20 (m, 1H, H-2eq), 3.95 (ddt, 1H, $J_{6eq,2eq}$ 1.6 Hz, $J_{6eq,5}$ 4.4 Hz, $J_{6eq,6ax}$ 14 Hz, H-6eq), 2.90–3.14 (m, 2H, H-2ax, H-6ax), 2.22–2.33 (m, 1H, H-5), 2.05–2.20 (m, 1H, H-3eq), 1.56–1.70 (m, 1H, H-3ax), 1.39 (s, 9H, C(CH_3)$_3$); ^{13}C-NMR ($CDCl_3$): δ 164.7, 165.2 (*C*O_2Ph), 153.6 (N*C*O_2C(CH_3)$_3$), 132.1, 132.0, 128.9, 128.7, 128.6, 127.4, 127.3 (Ar), 79.0 (O*C*(CH_3)$_3$), 70.5 (C-4), 62.3 (C-7), 43.5, 40.4, 39.4 (C-2, C-5, C-6), 29.1 (C-3), 27.3 (C(*C*H_3)$_3$).

The dibenzoylated compound **9** (158 mg, 0.36 mmol) was debenzoylated by dissolving it in MeOH (25 mL) and adding a catalytic amount of sodium. The solution was stirred at room temp. for 20 h and then evaporated and purified using flash chromatography (EtOAc, R_f 0.34). The resulting diol was *N*-deprotected by stirring it in HCl (4 M, 10 mL) overnight and concentrating to dryness to give pure **4** as the hydrochloride. Yield: 31 mg (51% over 2 steps, 17% from **6**). ^{1}H-NMR (D_2O): δ 3.57 (dd, 1H, $J_{5'a,5}$ 3.6 Hz, $J_{5'a,5'b}$ 11.6 Hz, H-5′a), 3.43–3.57 (m, 1H, H-4), 3.45 (dd, 1H, $J_{5'b,5}$ 6.2 Hz, H-5′b), 3.17–3.32 (m, 2H, H-2eq, H-5eq), 2.80 (dt, 1H, $J_{2ax,3eq}$ 3.2 Hz, $J_{2ax,2eq}$ 13.2 Hz, $J_{2ax,3ax}$ 13.2 Hz, H-2ax), 2.67 (t, 1H, $J_{6ax,5}$ 12.4 Hz, $J_{6ax,6eq}$ 12.4 Hz, H-6ax), 1.90–2.0 (m, 1H, H-5), 1.37–1.74 (m, 2H, H-3ax, H-3eq); ^{13}C-NMR (D_2O): δ 64.5 (C-4), 58.3 (C-7), 43.5, 41.5, 41.4 (C-2, C-5, C-6), 29.1 (C-3). HRMS(ES) calcd. for $C_6H_{13}O_2N$ + Na: 154.0844, found: 154.0819.

(4*RS*,5*RS*)-1-*tert*-Butoxycarbonyl-4-hydroxy-3-methylene-5-(trimethylsilylethoxymethyl)piperidine

The ester **10**[4,6] (0.26 g, 0.65 mmol) was dissolved in dry THF (10 mL), and $LiBH_4$ (10 mg, 0.45 mmol) was added. The mixture was heated to reflux for a 10 min period, and cooled to 0 °C before a solution of $KHSO_4$ (sat., 15 mL) was added slowly. The two layers were separated, and the water phase was extracted with EtOAc (2 × 15 mL). The combined organic phases were washed with NaCl (30 mL), dried over $MgSO_4$ and evaporated to dryness. To the resulting crude diol **11** (200 mg, 0.55 mmol, 85%) and 2-$NO_2C_6H_4SeCN$ (125 mg, 0.55 mmol) in dry THF (5 mL) was added PBu_3 (112 mg, 0.55 mmol) over a period of 5 min. The mixture was stirred for 5 h and $NaHCO_3$ (5% 10 mL) was added followed by extraction with EtOAc (2 × 10 mL). The combined organic phases were washed with NaCl (sat.) and evaporated to dryness. The resulting yellow oil was redissolved in THF (10 mL) and treated with a large excess of 35% aq. H_2O_2 (10 equiv.). After stirring overnight, a solution of $NaHSO_3$ (10%, 10 mL) was added to destroy the excess of H_2O_2, and the mixture was extracted with EtOAc (2 × 10 mL). The organic phases were washed with NaCl (sat., 15 mL) and evaporated to dryness. The crude product was purified by column chromatography on silica gel using a mixture EtOAc–pentane 1 : 4 as eluent. Yield of **12**: 100 mg (53%, 2 steps). ^{1}H-NMR ($CDCl_3$): δ 5.16 (s, 1H, H-3′a), 4.98 (s, 1H, H-3′b), 4.38 (d, 1H, $J_{2ax,2eq}$ 14.2 Hz, H-2eq), 4.10 (d, 1H, $J_{4,5}$ 8.8 Hz, H-4), 3.94 (br d, 1H, $J_{6ax,6eq}$ 11.8 Hz, H-6eq), 3.36–3.56 (m, 6H, H-2ax, H-5′a, H-5′b, H-6ax, OCH_2CH_2TMS), 2.73 (br s, 1H, O*H*), 1.84 (m, 1H, H-5), 1.43 (s, 9H, OC(CH_3)$_3$), 0.93 (t, 2H, *J* 8.8 Hz, CH_2CH_2TMS), 0.00 (s, 9H, Si(CH_3)$_3$); ^{13}C-NMR ($CDCl_3$): δ 154.5 (*C*=O), 143.5 (C-3), 108.6 (C=*C*H_2), 79.8 (O*C*(CH_3)$_3$), 74.1, 71.7 (C-4, C-5′), 68.9 (O*C*H_2CH_2TMS), 49.5, 48.5, 44.1 (C-2, C-5, C-6), 28.3 (C(*C*H_3)$_3$), 18.1 (OCH_2*C*H_2-TMS), −1.5 (Si(*C*H_3)$_3$). HRMS(ES) calcd. for $C_{17}H_{33}NO_4Si$ + Na: 366.2077, found: 366.2073.

(3*SR*,4*RS*,5*RS*)-3,5-Bis(hydroxymethyl)-3,4-dihydroxypiperidine (5)

The alkene **12** (100 mg, 0.29 mmol) was dissolved in a mixture

of water and acetone (1 : 1, 5 mL), and *N*-methylmorpholine *N*-oxide (43 mg, 0.32 mmol) and OsO_4 (0.74 mL of a 1% solution in *tert*-butyl alcohol, 0.029 mmol) were added. The mixture was stirred for 5 h, and an excess of $NaHSO_3$ was added, and stirring was continued for another 10 min. The mixture was extracted with EtOAc (2 × 5 mL), and the combined organic phases were washed with a solution of $NaHCO_3$ (5%, 1 × 10 mL), NaCl (sat., 1 × 10 mL), dried over $MgSO_4$ and evaporated to dryness to give **13**. The resulting oil was treated with a mixture of TFA and CH_2Cl_2 (50%, 5 mL) for 1 hour at 0 °C before evaporating to dryness. The residue was dissolved in 1 M hydrochloric acid (5 mL), filtered and evaporated to dryness to give **5** as a white solid. Yield: 57 mg (92%, for 2 steps). HRMS(ES) calcd. for $C_7H_{15}O_4N + H^+$: 178.1079, found: 178.1071.

NMR spectroscopy of 5. Data were obtained with a sample of 10 mg of **5** in 0.6 mL D_2O at 27 °C. Spectra were recorded in a 5-mm tube at 500.13 MHz or 600.13 MHz for ^1H and 125.77 MHz or 150.90 MHz for ^{13}C on a Varian Unity INOVA 500 or a Bruker Avance 600 spectrometer. Chemical shifts are reported relative to internal acetone (2.225 ppm for ^1H and 31.5 ppm for ^{13}C) and coupling constants (±0.5 Hz) were determined on a first-order basis. The phase-sensitive correlated spectroscopy (COSY) experiment were performed using double-quantum filtering[10,11] with the standard Bruker microprogram COSY-PHDQ. In the F_2 dimension, 4096 data points were collected giving an acquisition time of 0.37 s. The data matrix was zero filled in F_1 to give a matrix of 4096 × 1024 points and was resolution-enhanced in both dimensions by a shifted sine-bell function before Fourier transformation. The nuclear Overhauser effect spectroscopy, NOESY[12] was carried out in phase-sensitive mode, and was performed with a mixing time of 800 ms. The intensities of the NOESY cross-peaks were classified as either strong, medium or weak. The ^1H,^{13}C correlation experiments were performed in the inverse mode, as a heteronuclear single quantum coherence (HSQC) experiment[13] using pulse field gradients in the standard Varian program GHSQC with gradient strength of 4, 4 and 2 G cm^{-1}, respectively, and a HSQC-NOESY using 800 ms mixing time. Selective 1D NOESY were recorded using 800 ms mixing time.[14] All spectra were assigned using the Bruker software XWINNMR, spectra recorded on Varian system were converted into Bruker format.

Testing for inhibition of β-glucosidase

The assay for testing for inhibition of almond β-glucosidase was carried out as previously described.[3]

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