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Synthesis and evaluation of α-, β-glucosidase inhibition of 1-*N*-carboxamide-1-azafagomines and 5-*epi*-1-azafagomines

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ABSTRACT

1-*N*-Carboxamide 1-azafagomines and 5-*epi*-1-azafagomines were obtained from 1-azafagomine and 5-*epi*-1-azafagomine. The hydroxyl groups and the *N*-2 pyridazine position were protected prior to reaction with different isocyanates to form ureas. Protective groups were removed leading to the target compounds in 18–23% global yields. Final compounds were tested towards α- and β-glucosidases.

1. Introduction

1-Azafagomine and its derivatives are very good as α- and β-glucosidase inhibitors.[1,2] The introduction of a carboxamide group at *N*-1 was recently found to hugely increase the α-glucosidase inhibition so the compound became almost as potent as deoxynojirimycin (K_i = 0.44 μM).[3,4] The K_i value obtained for (−)-1-azafagomine *N*-phenyl carboxamide (**1**) was K_i = 3.36 μM, slightly better than the racemic 1-azafagomine (K_i = 3.9 μM).[2] It is also known that an alkyl group at the same *N*-position greatly enhanced the β-glucosidase inhibition, with the best result belonging to 3-phenylpropyl (K_i = 0.032 μM).[2] It was a surprising finding that the α-glucosidase inhibitory activity of the laevo- and the dextrorotatory enantiomers of 1-azafagomine *N*-phenyl carboxamide (**1**) were of the same order of magnitude;[4] In contrast Bols found the opposite trend with the two enantiomers of 1-azafagomine.[5] In this paper two new 1-*N*-carboxamide-1-azafagomines, and two 1-*N*-carboxamide-5-*epi*-1-azafagomines bearing different carboxamide moieties were synthetized, and its activity measured against α- and β-glucosidase. These compounds belong to *N*-sp^2-iminosugars classes known to be conformationally locked compounds displaying excellent selectivities.[6]

2. Results and discussion

The adopted synthetic methodology to these compounds relies on Diels–Alder cycloaddition of 2,4-pentadienol (**2**) to 4-phenyl-1,2,4-triazole-3,5-dione (PTAD)[1] and to diethyl azodicarboxylate (DEAD) leading to (±)-1-azafagomine[1] (**3**) and (±)-5-*epi*-1-azafagomine[1] (**6**). These compounds are precursors of the target carboxamide derivatives. (±)-1-Azafagomine (**3**) was obtained first by combination of 2,4-pentadienol (**2**) to PTAD.[1] DEAD was subsequently used due to its lower price compared to PTAD; the resulting cycloadduct **5** was obtained in 99% yield, a better yield than the equivalent cycloadduct **4** (88%). It was found that cycloadduct **5** could be *cis*-dihydroxylated with OsO_4 in good yield and total selectivity, but not *trans*-dihydroxylated; in this case the selectivity dropped to zero. Hydrazinolysis of the urethane group furnished **6** in 81% yield (Scheme 1).

^{1}H NMR spectrum of compound **5** showed two species in 1:1 ratio, found to be rotamers. In some regions of the ^{1}H NMR spectrum the duplication of peaks is clearly individualized signals. To assign the rotamers a 1D NOE difference experiment was performed in $CDCl_3$. Irradiation at δ_H = 3.38 ppm (due to H_a of CH_2OH group) in one rotamer inverts H_a of this rotamer, and H_a of the other rotamer at δ_H = 3.48 ppm. This fact was recently demonstrated by Dennis to occur when the protons are under significant chemical exchange on the saturation time scale.[7] Simultaneously a slight enhancement in intensity (8.2%) of H_b in both rotamers are observed due to nuclear Overhauser effect at δ_H = 3.69 ppm (H_b of both rotamers are coincident). ^{13}C NMR spectrum shows either broad peaks or duplication of signals for all carbons. The same trend was observed for triol intermediate **7**, either in ^{13}C or ^{1}H NMR spectra. These phenomena are probably related to the difficulty of nitrogen's inversion due to the two bulky groups attached to them.

1-Azafagomine (**3**) and 5-*epi*-1-azafagomine (**6**) were conveniently protected in order to keep *N*-1 as the only nucleophilic

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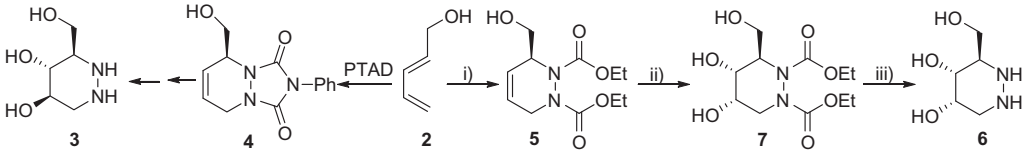
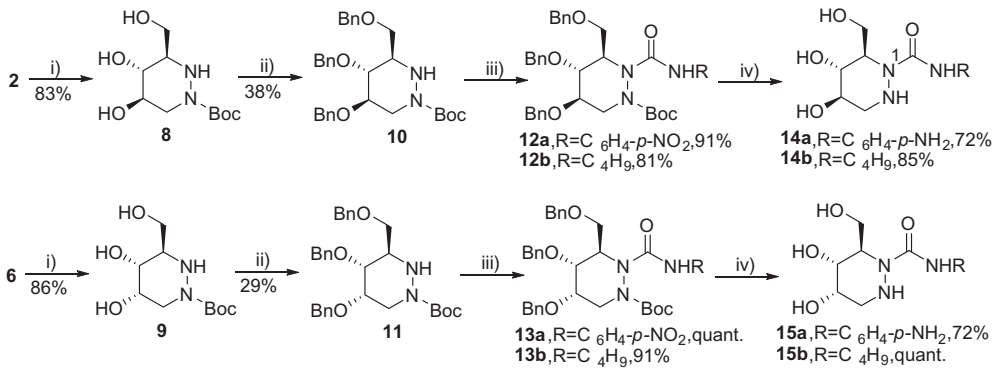
0008-6215/

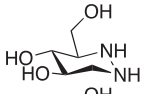
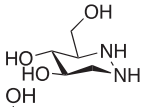
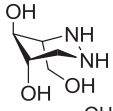
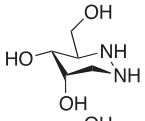
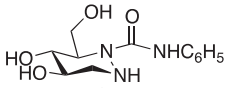
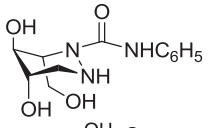
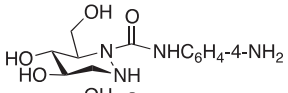
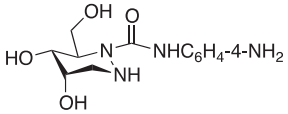
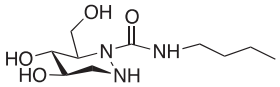
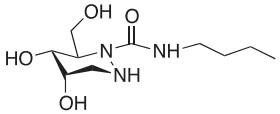


Scheme 1. Synthesis of 1-azafagomine (**3**) and 5-*epi*-1-azafagomine (**6**). Reagents and conditions: (i) DEAD, toluene, 24 h, rt, 99%; (ii) OsO_4, NMO, acetone/H_2O, 9:1, 24 h, rt, 96%; (iii) $NH_2NH_2{\cdot}H_2O$, 18 h, 100 °C, 81%.



Scheme 2. Synthesis of and *N*-1-carboxamide 1-azafagomines (**14a**, **b**) and *N*-1-carboxamide 5-*epi*-1-azafagomines (**15a**, **b**). Reagents and conditions: (i) Boc_2O, ethanol, $NaHCO_3$; (ii) BnBr (3.5 equiv), NaH (in paraffin), DMF (dry); (iii) isocyanate, toluene (dry), reflux; (iv) (a) Pd/C, H_2, HCl 3 M (1): ethanol (5), rt, 24 h, (b) Dowex 1×2–OH.

Table 1
K_i values (μM) for the Inhibition of α- and β-glucosidases by 1-azafagomine and 5-*epi*-azafagomine derivatives

Compound		α-Glucosidase (baker's yeast)	β-Glucosidase (almonds)
	(±)-**3**	3.9[a]	0.65[a]
	(−)-**3**	6.9[a]	0.3[a]
	(+)-**3**	2900[a]	>10,000[a]
	(±)-**6**	>1000[a]	137[a]
	(-)-**1**	3.4[b]	14.7[b]
	(+)-**1**	10.6[b]	25.2[b]
	(±)-**14a**	>100[b]	17.2[b]
	(±)-**15a**	12.7[b]	14.0[b]
	(±)-**14b**	n.d.[c]	20.8[b]
	(±)-**15b**	91.6[b]	9.0[b]

[a] pH 6.8.
[b] pH 7.0.
[c] n.d., not determined.

centre in the molecules, ready to react with isocyanates. *N*-2 was protected by reaction with Boc anhydride giving products in 83% (**8**) and 86% (**9**) yields. The hydroxyl groups were all protected by reaction with benzyl bromide in the presence of NaH, furnishing the hydroxyl-protected compounds in 38% yield (**10**) and 29% yield (**11**). Unfortunately simultaneous benzylation of *N*-1 occurred lowering the yields of target compounds **10** and **11**. The isolated compounds were reacted with *p*-nitrophenyl and butyl isocyanates; ureas **12a**, **b** and **13a**, **b** were formed in excellent yields (81%-quant.). Deprotection of these compounds occur by hydrogenation under H_2, Pd/C in HCl 3 M (1): ethanol (5) at rt to give the final compounds **14a**, **b** and **15a**, **b** in 72%-quant. yields (Scheme 2).

^{1}H NMR spectra of compounds **12a**, **b**, **13a**, **b** showed complex patterns due to the existence of rotamers, as referred to compounds **5** and **7**. The simpler ^{1}H NMR spectrum belongs to compound **13a** showing broad signals for all peaks which means that a slow interconversion between rotamers occurs in the spectrum acquisition time.

^{1}H and ^{13}C NMR spectra of compounds **14a**, **b** and **15a**, **b** showed of course simpler patterns. In these cases only the hydroxymethylene and the carbamate groups are neighbours. In general ^{1}H and ^{13}C NMR spectra of compounds **15a**, **b** showed a single set of peaks. There is an exception to the ^{13}C spectrum of compound **15a**: the aromatic region and carbonyl group show peak duplication. On the other hand, compound **14a** showed duplication of peaks in the aromatic region in both ^{1}H and ^{13}C NMR spectra, however **14b** showed two sets of peaks for all protons and carbons in the structure.

1-*N*-Phenyl carboxamide derivatives of 1-azafagomine, in either enantiomeric form was found to be potent α-glycosidase inhibitors in a previous work.[4] As an extension of this work we tested now four new compounds: two having the 1-azafagomine backbone **14a**, **b**, and two the 5-*epi*-1-azafagomine structure **15a**, **b**. We became interested in the 5-*epi*-1-azafagomine structure because according to our previous results with 1-azafagomine *N*-phenyl carboxamide derivative (**1**) the two enantiomers display comparable inhibition, either towards α- and β-glucosidases.[4] In fact we now acknowledge that the configuration change at C-5 of 1-azafagomine nucleus showed a small difference in inhibition activity towards α-glucosidase, baker's yeast source (**15a**, K_i = 12.7 μM) relatively to (+)-**1** and (−)-**1**, and interestingly one order of magnitude better than **14a**. However compound **15b** is one order of magnitude less potent (Table 1). The efficiency of the recognition mechanism between the inhibitor and the enzyme seems to be largely dependent on the group attached to the carboxamide functionality.

Bols have found that free *N*-1 is essential for the α-inhibition activity of 1-azafagomines. The K_i values of alkylated compounds towards α-glucosidases were found to be similar to that of isofagomine (K_i = 86 μM), a structure in which a carbon replaces *N*-1.[2] Possibly the good inhibition activity of **15a** as well as (−)-**1** and (+)-**1** can be explained by the existence of a carbonyl that is interacting with the enzyme in the same way as the free *N*-1 does.

A rapid inspection of Table 1 shows the same kind of K_i values to all carboxamide compounds (K_i = 9–25 μM) towards β-glucosidase, almond source. In this case the nature of the carboxamide group attached to *N*-1 is not expressed in the inhibition activity of 1-azafagomines. On the other hand free (±)-1-azafagomine (**3**) shows to be 1.5 order of magnitude more potent than carboxamide derivatives. The inhibitory activity is due exclusively to the laevorotatory enantiomer (K_i = 0.3 μM); the K_i of the dextrorotatory enantiomer is >10,000 μM. Although this work deals with racemic mixtures, considering that a small gap of activity exists between both enantiomers **1**, and that similar K_i values were obtained for all carboxamide derivatives, it is likely to suppose a similar inhibitory potential for either *dextro*- and *laevo*-enantiomers of carboxamide derivatives.

In conclusion *N*-carboxamide 5-*epi*-1-azafagomine derivatives display comparable α-inhibition potency to *N*-carboxamide 1-azafagomine derivatives in contrast to the free 5-*epi*-1-azafagomine which, unlike 1-azafagomine, is a very poor inhibitor. With respect to β-glucosidase inhibition the *N*-carboxamide derivatives of either 1-azafagomine or 5-*epi*-1-azafagomine exhibit the same potency. In general the carboxamide functionality is exerting a negative effect on the recognition by β-glucosidase of the compounds. The effect of the group attached to the carboxamide function is important in the α-glycosidase inhibition but not in β-glycosidase.

3. Experimental

3.1. General

Solvents were distilled under anhydrous conditions. 1-azafagomine (**3**) was obtained according to Bols's protocol;[5] 5-*epi*-1-azafagomine (**6**) was obtained by a modification of this protocol, by changing the dienophile/diethylazodicarboxylate (DEAD) instead of 4-phenyl-1,2,4-triazole-3,5-dione (PTAD). The synthetic sequence of 5-*epi*-1-azafagomine (**6**) followed the same steps: oxidation of the C=C double bond, and cleavage of the groups attached to the six-membered ring nitrogen atoms. 2,4-Pentadienol (**2**) was obtained by repeating the literature procedure.[8] All reagents were purchased and used without further purification. Glassware was dried prior to use. Compounds were purified by dry flash chromatography using silica 60, <0063 mm and water pump vacuum or by flash-chromatography using silica 60 Å 230–400 mesh as stationary phases. TLC plates (silica gel 60 F_{254}) were visualized either at a UV lamp or in I_2. ^{1}H NMR and ^{13}C NMR were run on a Varian Unity Plus 300, or Bruker Avance III 400 or Bruker BioSpin GmbH spectrometers. Infrared spectra were recorded on a Bomem MB 104. Samples were run as nujol mulls and oils as thin films. Melting points are uncorrected. MS spectra were recorded on a VG Autospec M. spectrometer.

3.2. Synthesis of 5-*epi*-1-azafagomine (6)

3.2.1. Diels–Alder cycloaddition of 2,4-pentadienol to diethyldiazodicarboxylate

To a solution of 2,4-pentadienol (1.33 g; 16.0 mmol) in dry toluene (10 mL) was added diethyldiazodicarboxylate (40% in toluene, 7.30 mL, 16.0 mmol) under magnetic stirring at rt. The stirring was continued for 24 h and the solvent removed to give cycloadduct **5** as an orange oil (4.32 g; 99%).

ν_{max} (neat) 3483, 1707 cm^{-1}. δ_H (400 MHz, $CDCl_3$)[†] 1.23–1.30 (12H, m, 4 × CH_3, A+B), 2.58 (1H, br s, OH), 3.35 (1H, dd, *J* 12.3, 9.5 Hz, H-3′, A), 3.45 (1H, dd, *J* 12.0, 9.8 Hz, H-3′, B), 3.56–3.69 (2H, m, 2 × H-3′, A+B), 3.77 (1H, dd, *J* 13.5, 4.3 Hz, H-6, A), 3.91 (1H, br s, H-6, B), 4.11–4.26 (8H, m, 4 × CH_2, A+B), 4.30 (1H, tdd, *J* 6.0, 3.9, 2.2 Hz, H-6, B), 4.34–4.44 (1H, m, H-6, A), 4.72 (2H, br s, H-3, A+B), 5.66–5.88 (4H, m, H-4+H-5, A+B) ppm. δ_C (100 MHz, $CDCl_3$)[‡] 14.3 (CH_3, A), 14.4 (CH_3, B), 42.2 (C-6, A), 43.6 (C-6, B), 55.9 (C-3, A), 56.9 (C-3, B), 61.9 (C-3′, A+B), 62.6, 62.7, 62.8, 62.9 (CH_2, A+B), 123.4, 124.2, 124,6, 125.2 (C-4 or C-5, A+B), 154.9, 155.7, 156.2, 156.3 (C=O, A+B) ppm. HRMS (ESI): calcd for $C_{11}H_{18}N_2NaO_5$: 281.1108; found: 281.1109.

3.2.2. *cis*-Dihydroxylation of compound 5

Cycloadduct **5** (1.85 g; 7.17 mmol) was dissolved in acetone (18.4 mL) and water (2.05 mL), *N*-methylmorpholine *N*-oxide

[†] ^{1}H NMR analysis showed a 1:1 mixture of rotamers A and B, due to inversion of the nitrogens lone pairs/rotation of the amide bond.

[‡] ^{13}C NMR also showed peak duplication.

(1.25 g; 9.30 mmol) was added followed by a solution of OsO_4 in water (4%; 0.76 mL; 0.12 mmol). The mixture was stirred for 1 d at rt. The reaction was quenched by addition of 5% aq soln $Na_2S_2O_3$ (10 mL). The acetone was removed by evaporation and the residue redissolved in AcOEt, filtered through a pad of silica, washed with AcOEt, and concentrated to give a yellow oil (**7**; 2.00 g; 96%).

ν_{max} (neat) 3419, 2983, 2937, 1698 cm^{-1}. δ_H (400 MHz, $CDCl_3$)[†] 1.23–1.32 (12H, m, 4 × CH_3, A+B), 3.12 (1H, br d, *J* 10.8 Hz, H-6, A), 3.21 (1H, br d, *J* 10.8 Hz, H-6, B), 3.50 (2H, t, *J* 11.6 Hz, H-3′, A), 3.62 (1H, d, *J* 8.0 Hz, H-3′, B), 3.65–3.95 (4H, br s, H-4+H-5, A+B), 4.01 (2H, dd, *J* 13.2, 5.6 Hz, H-6, A), 4.10–4.25 (9H, m, H-6, B+4 × CH_2, A+B), 4.52–4.64 (2H, br s, H-3, A+B) ppm. δ_C (100 MHz, $CDCl_3$)[‡] 14.3, 14.4 (CH_3, A,B), 44.0, 45.7 (C-6, A,B), 58.4, 59.0 (C-3, A,B), 61.9, 62.8 (C-3′, A,B), 63.0, 63.1 (2 × CH_2, A or B), 63.2, 63.3 (2 × CH_2, A or B), 64.0, 64.3 (C-4 or C-5, A,B), 66.4, 66.7 (C-4 or C-5, A,B), 155.2, 156.5 (C═O, A,B), 157.1, 157.3 (C═O, A,B) ppm. HRMS (ESI): calcd for $C_{11}H_{20}N_2NaO_7$: 315.1163; found: 315.1163.

3.2.3. Cleavage of ethyl carbamate groups in compound 7

Triol **7** (1.40 g; 4.80 mmol) was dissolved in hydrazine hydrate (82 mL) and heated at 100 °C for 18 h. The solution was concentrated and the crude product was subjected to dry-flash chromatography in EtOH/NH_4OH (25%) 99:1 giving pure **6** (0.57 g; 81%).

δ_H (300 MHz, D_2O) 3.00 (1H, d, *J* 14.4 Hz, H-6), 3.07 (1H, ddd, *J* 9.6, 6.4 e 3.2 Hz, H-3), 3.10 (1H, d, *J* 14.4 e 2.8 Hz, H-6), 3.64 (1H, dd, *J* 12.0 e 3.2 Hz, H-3′), 3.67 (1H, d, *J* 6.4 Hz, H-4), 3.84 (1H, dd, *J* 12.0 e 2.8 Hz, H-3′), 4.00 (1H, d, *J* 1.6 Hz, H-5) ppm.[1]

3.3. General procedure for *N*-1 protection of 1-azafagomine (3) and 5-*epi*-1-azafagomine (6)

To a stirred solution of **3** (0.43 mmol)/**6** (1.05 mmol) in ethanol, respectively 1.1 mL and 2.6 mL, was added sat. soln $NaHCO_3$ [1.08 mL (**3**); 1.29 mL (**6**)] and Boc_2O [0.43 mmol (**3**); 1.04 mmol (**6**)] at rt. The reaction mixture was stirred overnight, filtered through a pad of silica, and washed thoroughly with ethanol. The filtrate was concentrated to give *N*-Boc-1-azafagomine (**8**) and *N*-Boc-5-*epi*-1-azafagomine (**9**) as beige oils (**8**; 0.71 mmol; 83%) and (**9**, 0.91 mmol; 86%).

3.3.1. *N*-Boc-1-azafagomine (8)

ν_{max} (neat): 3300 (O–H), 1694 (C═O) cm^{-1}. δ_H (400 MHz, D_2O) 1.51 (9H, s, C(CH_3)$_3$), 2.73 (1H, ddd, *J* 9.2, 6.0, 2.8 Hz, H-3), 2.91 (1H, dd, *J* 13.2 e 10.8 Hz, H-6), 3.41 (1H, t, *J* 9,2 Hz, H-4), 3.60 (1H, ddd, *J* 10.8, 9.2 ,5.2 Hz, H-5), 3.71 (1H, dd, *J* 12.0, 6.0 Hz, H-3′), 3.90 (1H, dd, *J* 12.0, 2.4 Hz H-3′), 4.27 (1H, dd, *J* 13.2, 5.2 Hz, H-6) ppm. δ_C (100.6 MHz, D_2O) 27.6 (C(CH_3)$_3$), 49.3 (C-6), 59.2 (C-3′), 61.9 (C-3), 69.8 (C-5), 71.6 (C-4), 83.3 (*C*(CH_3)$_3$), 156.5 (C═O) ppm. HRMS (ESI): calcd for $C_{10}H_{20}N_2O_5$ $[M+Na]^+$ 271.1264; found: $[M+Na]^+$ 271.1267.

3.3.2. *N*-Boc-5-*epi*-1-azafagomine (9)

ν_{max} (neat): 3300 (O–H), 1637 (C═O) cm^{-1}. δ_H (400 MHz, D_2O) 1.49 (9H, s, C(CH_3)$_3$), 3.08 (1H, ddd, *J* 9.2, 6.4, 3.2 Hz H-3), 3.35 (1H, br d, *J* 13.2 Hz, H-6), 3.66–3.71 (1H, m, H-4), 3.68 (1H, dd, *J* 12.0, 6.4 Hz, H-3′), 3,85 (1H, dd, *J* 12.0, 3.2 Hz, H-3′), 3.98–4.03 (1H, m, H-5), 4.05–4.10 (1H, m, H-6) ppm. δ_C (100.6 MHz, D_2O) 29.6 (C(CH_3)$_3$), 49.7 (C-6), 59.7 (C-3′), 57.6 (C-3), 66.6 (C-5), 67.4 (C-4), 82.8 (*C*(CH_3)$_3$), 157.4 (C═O) ppm. HRMS (ESI) calcd for $C_{10}H_{20}N_2O_5$ $[M+Na]^+$ 271.1264; found: $[M+Na]^+$ 271.1263.

3.4. General procedure for protection of the hydroxyl groups in *N*-Boc-1-azafagomine (8) and *N*-Boc-5-*epi*-1-azafagomine (9)

To a solution of the *N*-Boc-1-azafagomine (**8**, 1.16 mmol)/*N*-Boc-5-*epi*-1-azafagomine (**9**, 1.08 mmol) in DMF (6.0 mL) was added portionwise NaH (80% in paraffin; 3.5 equiv) under magnetic stirring at 0 °C. The mixture was kept stirring for 30 min at 0 °C before addition of benzyl bromide (3.5 equiv), added dropwise at 0 °C. The reaction mixture was stirred further for 18 h at room temperature. The reaction was quenched, by pouring the reaction mixture onto ice, washed with water and brine. The organic layer was dried with $MgSO_4$ and concentrated under vacuum to give a crude product that was subjected to dry-flash chromatography (petroleum ether/ethyl acetate). The benzylated products were obtained as yellow oils: *N*-Boc-tri-*O*-benzyl-1-azafagomine (**10**; 0.41 mmol; 38%) and *N*-Boc-tri-*O*-benzyl-5-*epi*-1-azafagomine (**11**, 0.31 mmol; 29%).

3.4.1. *N*-Boc-tri-*O*-benzyl-1-azafagomine (10)

ν_{max} (neat): 3471 (N–H), 3087, 3063, 3029, 3005 (Ar), 1690 (C═O) cm^{-1}. δ_H (400 MHz, DMSO) 1.46 (9H, s, C(CH_3)$_3$) 2.90–2.96 (2H, m, H-3+H-6), 3.49 (1H, d, *J* 8.4 Hz, H-4), 3.54 (1H, dd, *J* 9.6, 3.2 Hz, H-3′), 3.56 (1H, d, *J* 6.0 Hz, H-5), 3.71 (1H, dd, *J* 9.6, 3.2 Hz, H-3′), 4.27–4.33 (1H, br s, H-6), 4.43 (1H, d, *J* 11.6 Hz, OCH_2-3′), 4.52 (1H, d, *J* 11.6 Hz, OCH_2-3′), 4.56 (1H, d, *J* 11.2 Hz, OCH_2-4), 4.67 (1H, d, *J* 12.0 Hz, OCH_2-5), 4.72 (1H, d, *J* 12.0 Hz, OCH_2-5), 4.88 (1H, d, *J* 11.2 Hz, OCH_2-4), 7.23–7.39 (15H, m, Ph) ppm. δ_C (100.6 MHz, DMSO) 28.2 (C(*C*H_3)$_3$), 47.5 (C-6), 60.1 (C-3), 68.2 (C-3′), 72.2 (OCH_2-3′), 73.2 (OCH_2-5), 74.9 (OCH_2-4), 78.7 (C-5), 78.9 (C-5), 80.9 (*C*(CH_3)$_3$), 127.6–128.7 (C-H, Ph), 137.9, 138.1, 138.2 (Cq, Ph), 154.9 (C═O) ppm. HRMS (ESI): calcd for $C_{31}H_{38}N_2O_5$ $[M+H]^+$ 519.2853; found: $[M+H]^+$ 519.2844.

3.4.2. *N*-Boc-tri-*O*-benzyl-5-epi-1-azafagomine (11)

ν_{max} (neat): 3322–3359 (N–H), 3088, 3063, 3030 (Ar), 1692 (C═O) cm^{-1}. δ_H (400 MHz, $CDCl_3$) 1.47 (9H, s, C(CH_3)$_3$), 3.03 (1H, br d, *J* 12.8 Hz, H-6), 3.46–3.52 (2H, m, H-3 and H-4), 3.60 (1H, dd, *J* 9.2, 6.0 Hz, H-3′), 3.75 (1H, s, H-5), 3.77 (1H, dd, *J* 9.2, 6.0 Hz, H-3′), 4.46 (1H, d, *J* 12.8 Hz, H-6), 4.32 (1H, d, *J* 12.0 Hz, OCH_2-4 or OCH_2-5), 4.51–4.58 (4H, m, OCH_2-4 or O*CH*$_2$-5 and OCH_2-3′), 7.25–7.40 (15H, m, Ph) ppm. δ_C (100.6 MHz, $CDCl_3$) 28.2 (C(*C*H_3)$_3$), 46.1 (C-6), 55.1 (C-3), 68.8 (C-3′), 69.6 (C-5), 70.4, 70.6 (OCH_2-4 and O*CH*$_2$-5), 73.2 (OCH_2-3′), 76.1 (C-4), 80.6 (*C*(CH_3)$_3$), 125.5–127.8 (C-H, Ph), 137.9, 138.0, 138.1 (Cq, Ph), 155.9 (C═O) ppm. HRMS (ESI): calcd for $C_{31}H_{38}N_2O_5$ $[M+Na]^+$ 519.2673; found: $[M+Na]^+$ 541.2653.

3.5. General procedure for reaction of compounds 10/11 with isocyanates to form compounds 12a, b and 13a, b

To a solution of protected 1-azafagomine (**10**)/5-epi-1-azafagomine (**11**) (0.11–0.16 mmol) in dry toluene (2 mL), kept under nitrogen and magnetic stirring, was added *p*-nitrophenyl/butyl isocyanate (1.0–3.0 equiv) dropwise at 0 °C. The solution mixture was transferred to an oil bath and refluxed for 4–17 h30 m, then evaporated till dryness, and re-dissolved in ether. The etherate was washed with 3 M aq HCl, the organic phase was dried over $MgSO_4$, the filtrate evaporated to give a brownish oil in 81–92% yield.

3.5.1. *N*-Boc-tri-*O*-benzyl-1-azafagomine-*N*-*p*-nitrophenylcarboxamide 12a

Compound **10** (0.06 g; 0.12 mmol); *p*-nitrophenyl isocyanate: 1.5 equiv; reflux for 6h30 min. Obtained **12a**, yellow oil (0.11 mmol; 91%). ν_{max} (neat): 3352 (N–H), 3030, 3064 (Ar), 1722 (C═O), 1690 (C═O), 1534, 1504, (NO_2) cm^{-1}. δ_H (400 MHz, C_3D_6O)[§] 1.35 (9H, s, C(CH_3)$_3$, M), 1.44 (9H, s, C(CH_3)$_3$, m), 3.42 (1H, dd, *J* 14.0, 0.8 Hz, H-3, M), 3.57 (1H, br d, *J* 14.8 Hz, H-3, m), 3.70 (1H, s, H-4, m), 3.76–3.84 (3H, m, 2 × H-6′, M, m+H-4, M), 3.89

[§] M, major rotamer; m, minor rotamer.

(1H, t, *J* 9.8 Hz, H-6′, M), 3.99 (1H, t, *J* 9.6 Hz H-6′, m), 4.02 (1H, s, H-5, M), 4.08 (1H, s, H-5, m), 4.34 (1H, d, *J* 14.8 Hz, H-3, m), 4.45–4.53 (1H, m, OCH_2-4), 4.46 (1H, d, *J* 12.0 Hz, OCH_2-6′), 4.50 (1H, d, *J* 14.0 Hz, H-3, M), 4.52 (1H, d, *J* 12.0 Hz, OCH_2-6′), 4.61 (1H, d, *J* 11.6 Hz, OCH_2-5), 4.75 (1H, d, *J* 11.6 Hz, OCH_2-5), 4.83 (1H, d, *J* 11.6 Hz, OCH_2-4), 5.13 (1H, t, *J* 7.2 Hz, H-6, M), 5.19 (1H, t, *J* 7.2 Hz, H-6, m), 7.28–7.39 (15H, m, 3xPh), 7.87 (2H, d, *J* 8.4 Hz, H_o, m), 7.95 (2H, d, *J* 8.8 Hz, H*o*, M), 8.19 (2H, d, *J* 8.8 Hz, H_m, m), 8.20 (2H, d, *J* 8.4 Hz, H_m, M) ppm. δ_C (100.6 MHz, C_3D_6O) 28.1 (C(CH_3)$_3$, m), 28.2 (C(CH_3)$_3$, M), 42.8 (C-3, M), 45.7 (C-3, m), 52.9 (C-6, M+m), 68.5 (C-6′, M), 68.8 (C-6′, m), 71.1 (C-5, m) 71.5 (OCH_2-4), 71.68 (OCH_2-5), 71.7 (C-5, M), 73.5 (OCH_2-6′), 73.6 (C-4, m), 73.8 (C-4, M), 81.2 (*C*(CH_3)$_3$, M), 81.3 (*C*(CH_3)$_3$, m), 119.4, 119.5 (C_o, M+m), 124.3 (C_m, M+m), 127.9–129.1 (C-H, Ph), 139.2 139.4, 139.6 (Cq, Ph), 147.0 (Cq, urea) 147.3 (Cq, C-NO_2) 154.9 (C=O, Boc) 156.2 (C=O, urea) ppm. HRMS (ESI): calcd for $C_{38}H_{42}N_4O_8$ $[M+Na]^+$ 705.2895; found: $[M+Na]^+$ 705.2881.

3.5.2. *N*-Boc-tri-*O*-benzyl-1-azafagomine-*N*-butylcarboxamide 12b

Compound **10** (0.09 g; 0.16 mmol); butyl isocyanate: 2.0 equiv; 14 h reflux. Obtained **12b**, brownish oil (0.13 mmol; 81%). ν_{max} (neat): 3365 (N–H), 3031, 3063, 3087 (Ar), 1712 (C=O), 1692 (C=O) cm^{-1}. δ_H (400 MHz, C_3D_6O)[§] 0.90–1.60 (16H, m, CH_3–CH_2–CH_2, C(CH_3), M+m), 3.15–5.15 (15H, m, NCH_2, 2 × H-3, H-4, H-5, H-6, 2 × H-6′, OCH_2-4, OCH_2-5, OCH_2-6′, M+m), 7.32–7.39 (15H, m, 3 × Ph) ppm. δ_C (100.6 MHz, C_3D_6O) 13.9, 14.0, 14.1, 14.2 (*C*H_3, M+m), 20.4, 20.5, 20.6, 20.7 (*C*H_2, M+m), 28.2, 28.7 (C(*C*H_3), M+m), 31.7, 32.6, 33.3, 33.4 (CH_2, M+m), 40.5, 40.6 (NCH_2, M+m), 41.7, 42.7 (H-3, M+m), 51.9 (C-6, M), 52.3 (C-6, m), 69.3, 71.5, 73.1, 73.2 (H-6′, OCH_2-4, OCH_2-5, OCH_2-6′), 71.7, 74.4 (C-4+C-5, M), 71.3, 74.3 (C-4+C-5, m), 81.3, 81.8 (*C*(CH_3)$_3$), 128.1–129.4 (C-H, Ph), 139.3, 139.4, 139.5, 139.6, 139.7, 139.9 (Cq, M+m), 155.7, 156.7, 157.0, 157.7 (C=O Boc, C=O urea) ppm. HRMS (ESI): calcd for $C_{36}H_{47}N_3O_6$ $[M+H]^+$ 618.3538; found: $[M+H]^+$ 618.3524.

3.5.3. *N*-Boc-tri-*O*-benzyl-5-*epi*-1-azafagomine-*N*-*p*-nitrophenylcarboxamide 13a

Compound **11** (0.057 g; 0.11 mmol); *p*-nitrophenyl isocyanate: 1.0 equiv; 4 h reflux. Obtained **13a**, yellow oil (0.11 mmol; 99%). ν_{max} (neat): 3351 (N–H), 3030 (CH, Ar), 1711 (C=O), 1691 (C=O) cm^{-1}. δ_H (400 MHz, C_3D_6O) 1.40 (9H, s, C(*C*H_3)$_3$), 3.25–3.28 (1H, br s, H-3), 3.50–3.59 (1H, br s, H-6a′), 3.70–3.78 (1H, br s, H-6b′), 3.91 (1H, br d, *J* 8.8 Hz, H-4), 4.22 (1H, s, H-5), 4.56–4.66 (4H, m, OCH_2-4, OCH_2-6′), 4.66 (1H, d, *J* 12.0 Hz, OCH_2-5), 4.80 (1H, d, *J* 12.0 Hz, OCH_2-5), 5.25–5.34 (1H, br s, H-6), 7.29 (2H, d, *J* 7.2 Hz, Ph), 7.30–7.40 (11H, m, Ph), 7.43 (2H, d, *J* 6.8 Hz, Ph), 7.93–8.00 (2H, br s, H_o), 8.19 (2H, d, *J* 8.8 Hz, H_m) ppm. δ_C (100.6 MHz, C_3D_6O) 28.8 (C(CH_3)$_3$), 43.5 (C-3), 54.9 (C-6), 68.5 (C-6′), 71.1 (OCH_2-4 or OCH_2-6′), 71.6 (C-4), 72.2 (OCH_2-5), 72.8 (C-5), 73.8 (OCH_2-4 or OCH_2-6′), 84.4 (*C*(CH_3)$_3$), 119.6 (C_o), 125.3 (C_m), 128.2–129.2 (C-H, Ph), 139.0, 139.5, 139.6 (Cq, Ph), 143.1 (Cq, urea), 147.2 (Cq, –NO_2), 155.1 (C=O), 156.2(C=O) ppm. HRMS (ESI): calcd for $C_{38}H_{42}N_4O_8$ $[M+Na]^+$ 705.2895; found: $[M+Na]^+$ 705.2882.

3.5.4. *N*-Boc-tri-*O*-benzyl-5-*epi*-1-azafagomine-*N*-butylcarboxamide 13b

Compound **11** (0.024 g; 0.046 mmol); butyl isocyanate: 3.0 equiv; 17h30 min, reflux. Obtained **13b** as a yellow oil, (0.04 mmol; 91%). ν_{max} (neat): 3365 (N–H), 3087, 3063, 3030 (Ar), 1711 (C=O), 1691 (C=O) cm^{-1}. δ_H (400 MHz, C_3D_6O)[§] 0.89–0.96 (3H, m, CH_3, M+m), 1.32–1.37 (2H, m, CH_2, M+m), 1.39, 1.40 (9H, s, C(CH_3)$_3$, M+m), 1.44–1.50 (2H, m, CH_2, M+m), 3.11–3.27 (5H, m, NCH_2 m, 2 × H-3m, H-3M), 3.25 (2H, td, *J* 7.2, 5.6 Hz, NCH_2, M), 3.47 (1H, br d, *J* 9.6 Hz, H-6′), 3.67 (1H, dd, *J* 9.6, 5.6 Hz, H-6′), 3.82 (1H, ddd, *J* 10.8, 4.8, 2.4 Hz, H-4), 4.08–4.11 (1H, m, H-3M), 4.14 (1H, br s, H-5), 4.52–4.61 (4H, m, OCH_2-4, OCH_2-6′), 4.81 (1H, d, *J* 12.0 Hz, OCH_2-5), 4.79 (1H, d, *J* 12.0 Hz, OCH_2-5), 5.24–5.29 (1H, br s, H-6), 7.28–7.37 (13H, m, Ph), 7.39 (2H, *J* 8.4 Hz, Ph) ppm. δ_C (100.6 MHz, C_3D_6O) 14.1 (CH_3, M), 14.0 (CH_3, m), 20.4 (CH_2 ,m), 20.7 (CH_2, M), 28.2, 28.3 (C(CH_3), M+m), 32.6, 33.2 (CH_2, M, m), 40.5 (NCH_2, M), 40.7 (NCH_2, m), 41.7 (H-3, m), 42.3 (H-3, M), 54.0 (C-6), 69.0 (C-6′), 70.7 (OCH_2-4), 71.7 (C-4), 71.8 (OCH_2-5), 72.7 (C-5), 73.7 (OCH_2-6′), 81.5 (*C*(CH_3)$_3$), 128.0–129.1 (C-H, Ph), 139.2, 139.6, 139.9 (Cq, Ph), 157.1 (C=O), 157.9 (C=O) ppm. HRMS (ESI): calcd for $C_{36}H_{47}N_3O_6$ $[M+H]^+$ 618.3538; found: $[M+H]^+$ 618.3527.

3.6. Cleavage of protective groups to give final products (14a, b/15a, b)

To a solution of *N*-carboxamido derivatives **12a**, **b**/**13a**, **b** (0.06–0.11 mmol) in EtOH (5–2.5 mL) was added aq HCl sol. (1 M, 1–0.5 mL), and Pd/C 10% (0.01–0.03 g). The reaction mixture was stirred under H_2 for 24 h at rt. After reaction completion the mixture was filtered through a pad of Celite, washed with ethyl acetate and the filtrate concentrated. The oil residue was re-dissolved in water (20 mL), and washed with ether (20 mL). The aqueous solution was neutralized with a Dowex (OH^-) basic resin and the aqueous solution concentrated to dryness to give the final products.

3.6.1. 1-Azafagomine-1*N*-(*p*-aminophenyl)carboxamide (14a)

Carboxamide **12a** (0.08 g; 0.11 mmol); EtOH (5 mL); HCl (1 M; 1 mL); Pd/C 10% (0.03 g). Obtained **14a**, yellow oil (0.08 mmol; 72%). ν_{max} (neat): 3583 (N–H), 3369 (O–H), 1713 (C=O) cm^{-1}. δ_H (400 MHz, D_2O)[§] 2.92 (1H, dd, *J* 14.2, 0.8 Hz, H-3), 3.32 (1H, dd, *J* 14.2, 1.6 Hz, H-3), 3.71 (1H, d, *J* 13.2 Hz, H-4), 3.81 (1H, dd, *J* 12.0, 4.8 Hz H-6′), 3.90 (1H, br s, H-5), 4.09 (1H, dd, *J* 12.0, 9.2 Hz, H-6′), 4.41–4.45 (1H, m, H-6), 6.88 (1H, t, *J* 9.2, 8.4 Hz H_o, M+m), 7.19 (2H, d, *J* 8.8 Hz, H_m, M+m), 7.25 (2H, d, *J* 8.8 Hz, H_m, M+m) ppm. δ_C (100.6 MHz, D_2O) 46.4 (C-3), 56.2 (C-6), 59.0 (C-6′), 64.9 (C-4), 66.2 (C-5), 116.2 (C_o, m), 116.8 (C_o, M), 124.4, 124.6 (C_m, M, m), 129.8, 129.9 (C_q, urea), 142.8, 144.6 (C_q, C–NO_2) 155.1, 156.2 (C=O, M, m) ppm. HRMS (ESI): calcd for $C_{10}H_{18}N_4O_4$ $[M+H]^+$ 283.1401; found: $[M+H]^+$ 283.1399.

3.6.2. 1-Azafagomine-1*N*-butylcarboxamide (14b)

Carboxamide **12b** (0.06 g; 0.10 mmol); EtOH (5 mL); HCl sol (1 mL); Pd/C 10% (0.02 g). Obtained compound **14b**, yellow oil (0.08 mmol; 85%). ν_{max} (neat): 3388 (O–H), 1645 (C=O) cm^{-1}. δ_H (400 MHz, D_2O)[§] 0.91 (3H, t, *J* 7.6 Hz, CH_3, m), 0.93 (3H, t, *J* 7.2 Hz, CH_3, M), 1.31–1.40 (2H, m, CH_2, M+m), 1.46–1.55 (2H, m, CH_2, M+m), 2.78 (1H, dd, *J* 13.2, 10.8 Hz, H-3, m), 2.81 (1H, dt, *J* 14.8, 1.2 Hz, H-3, M), 3.15–3.20 [3H(M)+2H(m), m, NCH_2, M+m,+H-3, M], 3.37 (1H, dd, *J* 10.0, 9.2 Hz, H-5, m), 3.55–3.59 (1H, m, H-4, m), 3.65–3.67 (1H, m, H-4, M), 3.71 (1H, dd, *J* 12.0, 6.4 Hz, H-6′, m), 3.76 (1H, dd, *J* 12.0, 4.2 Hz, H-6′, M), 3.88–3.86 (1H, m, H-5, M), 3.92 (1H, dd, *J* 12.0, 2.8 Hz, H-6′, m), 4.03 (1H, dd, *J* 12.0, 9.0 Hz, H-6′, M), 4.31–4.34 (1H, m, H-6, M), 4.37–4.40 (2H, m, H-3+H-6, m). δ_C (100.6 MHz, D_2O) 13.0 (CH_3, m), 13.0 (CH_3, M), 19.3 (CH_2, m), 19.4 (CH_2, M), 31.5 (CH_2, m), 31.6 (CH_2, M), 39.5 (CH_2, m), 39.7 (CH_2, M), 46.2 (C-3, M), 47.6 (C-3, m), 56.2 (C-6, M), 62.0 (C-6, m), 59.1 (C-6′, M), 59.4 (C-6′, m), 65.0 (C-5, M), 66.2 (C-4, M), 69.9 (C-5, m), 71.9 (C-4, m), 159.7 (C=O, m), 160.9 (C=O, M) ppm. HRMS (ESI): calcd for $C_{10}H_{21}N_3O_4$ $[M+Na]^+$ 270.1424; found: $[M+H]^+$ 270.1421.

3.6.3. 5-*epi*-1-Azafagomine-1*N*-(*p*-aminophenyl)carboxamide (15a)

Carboxamide **13a** (0.07 g; 0.09 mmol); EtOH (7 mL); HCl sol. (2 mL); Pd/C 10% (0.03 g). Obtained compound **15a** as a yellow

oil (0.07 mmol; 73%). ν_{max} (neat): 3446 (OH and NH), 1640 (C=O) cm^{-1}. δ_H (400 MHz, D_2O) 2.89–2.92 (1H, m, H-6), 2.96–2.99 (2H, m, H-3), 3.78–3.87 (1H, m, H-6′), 3.89–3.95 (1H, m, H-4), 4.08 (1H, t, *J* 2.6 Hz, H-5), 4.65 (1H, ddd, *J* 14.4, 5.6, 2.4 Hz, H-6′), 7.12 (1H, d, *J* 8.0 Hz, H_o), 7.35 (1H, d, *J* 8.0 Hz, H_m) ppm. δ_C (100.6 MHz, D_2O) 42.0 (C-6), 45.1 (C-3), 58.3 (C-6′), 64.4 (C-4), 66.5 (C-5), 117.2 (C_o), 123.4 (C_m), 123.39, 131.80 (C_q), 146.12 (C=O) ppm. HRMS (ESI): calcd for $C_{10}H_{18}N_4O_4$ $[M\text{-}C_7H_7N_2O]^+$ 148.0770 ; found: $[M\text{-}C_7H_7N_2O\cdot]^+$ 148.0788.

3.6.4. 5-*epi*-1-Azafagomine-1*N*-butylcarboxamide (15b)

Carboxamide **13b** (0.04 g; 0.06 mmol); EtOH (2.5 mL); HCl sol (1 M; 0.5 mL); Pd/C (0.01 g). Obtained compound **15b**, yellow oil (0.06 mmol; quant.). ν_{max} (neat): 3388 (O-H), 1645 (C=O) cm^{-1}. δ_H (400 MHz, D_2O) 0.94 (3H, t, *J* 7.4 Hz, CH_3) 1.34–1.36 (2H, m, CH_2), 1.48–1.52 (2H, m, CH_2), 2.86–2.89 (2H, m, H-3), 3.01–3.20 (2H, m, NCH_2), 3.75 (1H, dd, *J* 12.0, 6.4 Hz, H-6′), 3.81 (1H, dd, *J* 12.0, 9.6 Hz, H-6′), 3.86 (1H, ddd, *J* 9.6, 6.8, 2.8 Hz, H-4), 4.04 (1H, t, *J* 2.8 Hz, H-5), 4.57 (1H, ddd, *J* 8.4, 6.4, 2.4 Hz H-6) ppm. δ_C (100.6 MHz, D_2O) 13.1 (CH_3), 19.4 (CH_2), 31.6 (CH_2), 39.5 (NCH_2), 44.9 (C-3), 58.3 (C-6), 58.4 (C-6′), 64.5 (C-4), 66.5 (C-5), 161.0 (C=O) ppm. HRMS (ESI): calcd for $C_{10}H_{21}N_3O_4$ $[M+Na]^+$ 270.1424; found: $[M+Na]^+$ 270.1423.

3.7. Measurement of glycosidase inhibition

α-Glucosidase from bakers' yeast (EC 3.2.1.20, Sigma G-5003) and β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395) were used as model glucosidases. Enzyme assays were conducted in 96 wells Nunc plates using 4-nitrophenyl α-D-glucopyranoside or 4-nitrophenyl β-D-glucopyranoside as substrates, in phosphate buffer 100 mM, pH 7.0 at 25 °C. A range of substrate concentrations from 83.3×10^{-6} M to 4.17×10^{-3} M (10 different concentrations) for α-glucosidase and from 333.3×10^{-6} M to 15.3×10^{-3} M (8 different concentrations) for β-glucosidase, in a final volume of 300 μL, was tested using 1 unit/mL of β-glucosidase or 0.21 units/mL of α-glucosidase, in the absence and in the presence of inhibitor (**14a**, **b** and **15a**, **b**: 6.66×10^{-6} to 50×10^{-6} M). Blanks were set containing all reaction components but enzyme. All assays were performed in triplicate.

The formation of 4-nitrophenol was monitored for 20 min at 25 °C, measuring the absorbance (1 reading each minute) at 400 nm. A value of εl = 570,000 M^{-1} (pH 7.0), determined in the same conditions used for the enzyme assays, was used to convert absorbance into product concentrations. Initial velocities were calculated from the slopes of the Abs versus time graphs for each concentration of substrate, and these were used to construct Michaelis–Menten plots. The kinetic parameters K_M, K_{Mapp} and V_{max} were determined by fitting the experimental results to a rectangular hyperbole using the Origin 8 Graph Pad. The inhibition type was established as competitive for all enzymes and inhibitors tested, using 4 different concentrations of inhibitors (in triplicate). Individual K_i values were obtained from the linear regression plot of K_M/K_{Mapp} as a function of the inhibitor concentration, [*I*], where the slope corresponds to $1/K_i$. Reported K_i values are expressed as average of 3 independent K_i determinations.

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Supplementary data

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