Synthesis of labelled 1-azafagomine

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(3,4-*trans*-4,5-*trans*)-4,5-Dihydroxy-3-(hydroxymethyl)hexahydro-(3-^{13}C)pyridazine (azafagomine, **1a**) was synthesised in 7 steps starting from (2-^{13}C) malonic acid **5a** through conversion to pentadienoic acid and Diels–Alder reaction with 4-phenyl-1,2,4-triazole-3,5-dione, reduction, epoxidation, epoxide hydrolysis and deprotection.

Introduction

Biomolecules labelled with stable isotopes are useful probes in NMR studies of intermolecular interactions.[1] By labelling with NMR-active nuclei that occur at low natural abundance it is possible to tremendously enhance signals that are otherwise difficult to observe. This may allow the gathering of new information about electronic or conformational properties during host–guest binding, particularly when the host is a protein.

Recently we discovered the glycosidase inhibitor 1-azafagomine **1**.[2] This compound inhibits both α- and β-glucosidase

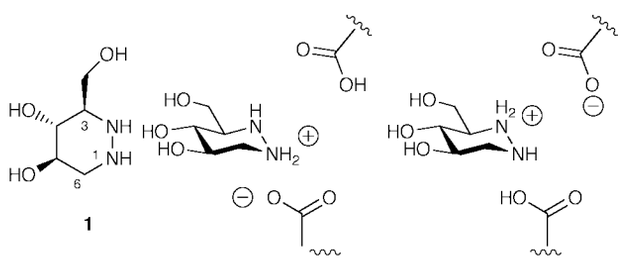
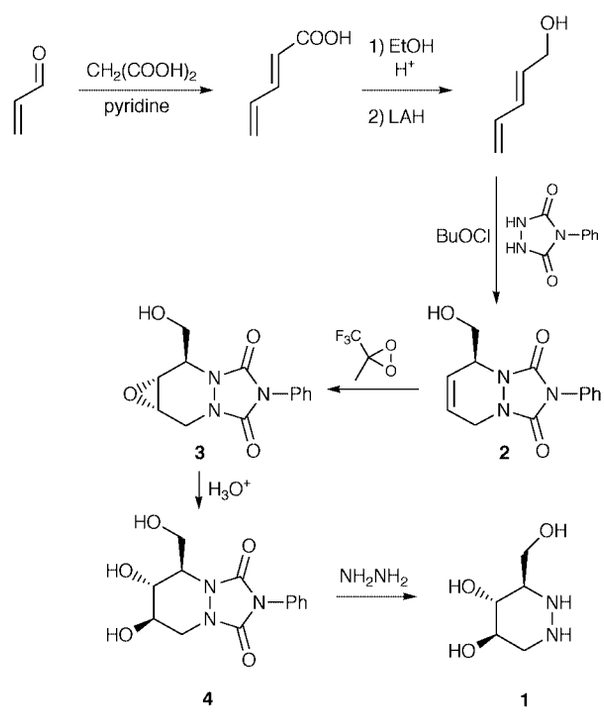


Fig. 1 The chemical structure of 1-azafagomine and its anticipated interaction with a glycosidase.

potently and was expected to be a transition state analogue. While **1** was designed to mimic, when protonated, an oxocarbenium ion and anomeric carbocation at the same time, the observation that **1** was a very weak base (pK_a 3.9)[2] cast some doubt on whether **1** could be protonated by the enzyme inside its active site. It therefore became interesting to try to elucidate whether **1** would become protonated when being bound to α- or β-glucosidase. This we anticipated might be possible with ^{13}C-labelled **1** containing the label in a position that would be affected by protonation of the nitrogens. These positions could be C-3 or C-6 since the chemical shifts of these differed considerably.[2] In this paper we report a synthesis of (±)-**1** containing ^{13}C at C-3.

Results and discussion

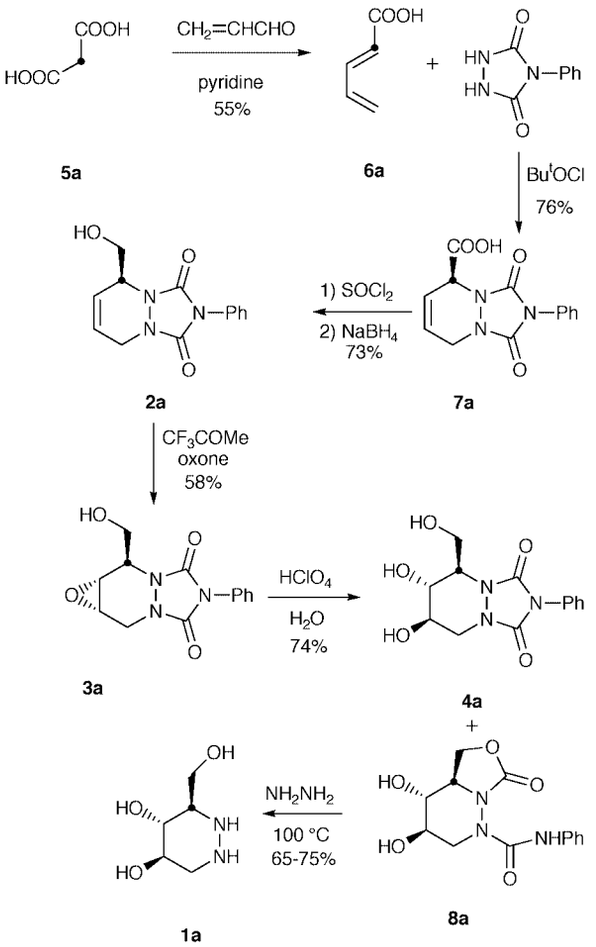
Though our previous synthesis of (±)-**1** (Scheme 1) became the basis for the preparation of 3-(^{13}C)-**1**, it was clear that the synthesis had to undergo some adjustments before it could be used. The synthesis relied on the use of penta-2,4-dien-1-ol as an intermediate, the synthesis of which consisted of esterification and reduction of pentadienoic acid. Both these reactions were carried out on relatively sensitive compounds and were also difficult to carry out on a small scale. These steps were therefore unattractive in a synthesis with costly materials. We therefore planned to carry out the Diels–Alder reaction earlier in the



Scheme 1 Previously published synthesis of (±)-**1**.[2]

synthesis and delay the reduction of the carboxylic acid. In the previous work, we had carried out the Diels–Alder reaction with pentadienoic acid, but never solved the problem of reduction of the adduct to **2**.

2-(^{13}C)-Labelled pentadienoic acid **6a** was synthesised in 55% yield by reaction[3] of 2-(^{13}C)malonic acid **5a** with acrolein (acrylaldehyde) in excess (Scheme 2). The acid **6a**, a relatively unstable compound, was promptly treated with 4-phenyl-1,2,4-triazole-3,5-dione, which was obtained *in situ* by preoxidation of 4-phenylurazole with *tert*-butyl hypochlorite. This reaction gave the labelled Diels–Alder adduct **7a** in 76% yield.[2] A sequence of reactions was found that could reduce the acid: **7a** was converted ($SOCl_2$) to the acid chloride, which was treated with $NaBH_4$ in 1,4-dioxane–water[4] to give the labelled alcohol **2a**. Carrying out the reduction of the acid chloride in DMF was also possible, but direct reduction of the acid **7a** with $LiAlH_4$ gave a low yield. Epoxidation of **2a** was carried out as in the unlabelled case[2] using methyl(trifluoromethyl)dioxirane giving labelled *trans*-epoxide **3a** in 58% yield. The hydrolysis of **3a** to triol **4a** was also carried out essentially as with **3**, but in some runs an interesting, previously undetected, by-product **8a** was observed in occasionally up to 40% yield. Compound **8a** was identified from changes in the ^{1}H and ^{13}C NMR spectra as



Scheme 2 Synthesis of ^{13}C labelled (±)-**1** using a modified synthesis. Dot (·) depicts ^{13}C.

compared with **4a**. In **8a** the ^{13}C signals of the *ipso*, *para* and *ortho* positions of the aromatic group (δ_C 138.6, 122.4 and 119.7) differ widely from those of **3a** or **4a** (δ_C 131.2, 127.4 and 125.7). These new signals fit those of an anilide and resemble those of a similar compound **9** previously synthesised by us.[5] Furthermore, ^{13}C NMR spectroscopy shows two carbonyl groups are present in **8a**, and the ^{1}H chemical NMR spectrum in DMSO shows only 2 OH groups are present, in addition to an NH group. For comparison, 3 OH signals are clearly seen in the DMSO spectrum of **4a**.

By-product **8a** was formed in amounts varying from 0 to 40%. Its formation appears to depend on the reaction time, because when pure triol **4a** was subjected to hydrolysis conditions **8a** was formed. Interestingly, no hydrolysis of the phenylurazole moiety has been observed in this reaction, so apparently intramolecular attack on the carbonyl group by the hydroxy group is much more favorable than attack by water. Thus the by-product is formed from the product, and to avoid its formation it is important not to prolong the reaction time.

Nevertheless, both **4a** and **8a** can be converted to labelled azafagomine. Hydrazinolysis of **4a**, by the method described for **4**,[2] gave 3-labelled 1-azafagomine **1a** in 65% yield. Similarly, hydrazinolysis of **8a** gave **1a** in 75% yield, so it is not necessary to separate mixtures of **4a** and **8a** before hydrazinolysis.

Conclusions

We have described a succesful synthesis of the first ^{13}C-labelled derivative of a 1-azasugar. NMR studies of the inhibitor–enzyme complexes are underway. The synthesis described is also an improvement on the previous synthesis of (±)-**1**, since it reduces the work with sensitive dienes.

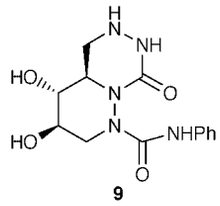


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Fig. 2 Dihydrazino derivative obtained in previous work, resembling by-product **8a**.[5]

Experimental

General

^{13}C NMR and ^{1}H NMR spectra were recorded on a Varian Gemini 200 instrument. Mass spectra were obtained on a Micromass LCT-QTOF instrument. Concentrations were performed on a rotary evaporator at a temperature below 40 °C.

(*E*) (2-^{13}C)-Penta-2,4-dienoic acid 6a

(2-^{13}C)Malonic acid **5a** (0.95 g, 9.05 mmol, 99% ^{13}C, Cambridge Isotope Laboratories, Inc.) was dissolved in 1.4 ml of dry pyridine and heated to 80 °C. Acrolein (0.775 ml, 16.4 mmol) was added in one portion, and the mixture was stirred for 30 min. First ice (6 g) and then sulfuric acid 96% (0.8 ml) was added with stirring, followed by extraction (3 × 15 ml $CHCl_3$), drying ($MgSO_4$) and evaporation of the solvent. The crude product **6a** was obtained as a yellow solid, which was used immediately without further purification. Yield: 490 mg (55%); ^{13}C NMR (d_6-DMSO) δ 171.8 (d, C-1, $J_{1,2}$ 293 Hz), 146.4 (d, C-3, $J_{3,2}$ 275 Hz), 133.9 (C-5), 126.2 (d, C-4, $J_{4,2}$ 37 Hz), 120.7 (intensity 100×, C-2); ^{1}H NMR ($CDCl_3$) δ 9.85 (br s, 1H, H-1), 7.28 (ddd, 1H, H-3, $J_{3,2}$ 15.4 Hz, $J_{3,4}$ 11.0 Hz, $J_{H3,C2}$ 2.2 Hz), 6.42 (dddt, 1H, H-4, $J_{4,5c}$ 10.6 Hz, $J_{4,5t}$ 16.8 Hz, $J_{H4,C2}$ 3.7 Hz, $J_{4,2}$ 0.7 Hz), 5.84 (ddd, 1H, H-2, $J_{H2,C2}$ 164 Hz), 5.59 (d, 1H, H-5*t*), 5.49 (d, 1H, H-5*c*).

(±)-7,9-Dioxo-8-phenyl-1,6,8-triaza-(2-^{13}C)bicyclo[4.3.0]non-3-ene-2-carboxylic acid 7a

4-Phenylurazole (0.927 g, 5.2 mmol) was suspended in EtOAc (7 ml) at 0 °C and *tert*-butyl hypochlorite (0.567 g) was added to give a red homogeneous solution. After 10 min, **6a** (0.49 g, 4.95 mmol) was added, and the solution was stirred for 18 h at room temperature. The precipitated adduct **7a** was isolated by filtration, and washed with pentane. Yield: 1.03 g (76%); ^{13}C NMR (d_6-DMSO) δ 168.0 (d, C-2, $J_{2',2}$ 218 Hz), 152.3, 151.5 (C-7, C-9), 130.9 (Ar *ipso*), 128.6 (*meta*), 127.6 (*para*), 125.4 (*ortho*), 122.9 (C-4), 119.2 (d, C-3, $J_{3,2}$ 166 Hz), 55.2 (intensity 100×, C-2), 42.5 (C-5); ^{1}H NMR (DMSO) δ 7.49 (m, 5H, ArH), 6.12 (m, 2H, H-3, H-4), 5.22 (br d, 1H, H-2, $J_{H2,C2}$ 147 Hz), 4.32 (br d, 1H, H-5eq, $J_{5eq,5ax}$ 16.5 Hz), 4.03 (br d, 1H, H-5 ave.); MS (ES) *m*/*z* 297.0719 ($M + Na^+$). Calc. for $C_{12}{}^{13}CH_{11}N_3O_4 + Na^+$: *m*/*z*, 297.0676.

(±)-2-Hydroxymethyl-8-phenyl-1,6,8-triaza-(2-^{13}C)bicyclo-[4.3.0]non-3-ene-7,9-dione 2a

To freshly distilled $SOCl_2$ (5 ml) was added **7a** (1.00 g, 3.65 mmol) and the suspension was refluxed for 90 min, giving a homogeneous solution. The remaining $SOCl_2$ was removed by evaporation and the crude acid chloride dried under reduced pressure. The acid chloride was then dissolved in dry 1,4-dioxane (12 ml) and added over 30 min to a solution of water–1,4-dioxane (5 ml : 5 ml) containing $NaBH_4$ (400 mg) and keeping the temperature at 10 °C. After stirring the mixture for 2 h, water (20 ml) was added. Extraction with CH_2Cl_2 (4 × 25 ml), drying ($MgSO_4$) and evaporation gave a crude product (0.938 g). This was purified by flash chromatography, using EtOAc as the eluent, giving **2a** (0.696 g, 73%); ^{13}C NMR (DMSO) δ 151.9, 150.2 (C-7, C-9), 131.1 (Ar *ipso*), 128.4

(*meta*), 127.4 (*para*), 125.6 (*ortho*), 123.0 (d, C-3, $J_{3,2}$ 175 Hz), 121.2 (C-4), 60.5 (d, C-2, $J_{2',2}$ 149 Hz), 54.6 (intensity 100×, C-2), 42.8 (C-5); ^{1}H NMR (DMSO) δ 7.47 (m, 5H, ArH), 6.03 (m, 2H, H-3, H-4), 5.06 (br s, 1H, 2′-OH), 4.46 (br d, 1H, H-2, $J_{H2,C2}$ 144 Hz), 4.18 (dd, 1H, H-5ax, $J_{5ax,5eq}$ 16.5 Hz, $J_{5ax,4}$ 2.2 Hz), 4.03 (d, 1H, H-5eq), 3.68 (d, 2H, H_2-2′, J 4.8 Hz); MS (ES) *m*/*z* 283.0927 (M + Na^+). Calc. for $C_{12}{}^{13}CH_{13}N_3O_3$ + Na^+: *m*/*z*, 283.0884.

(2,3-*trans*-3,4-*cis*)-3,4-Epoxy-2-hydroxymethyl-8-phenyl-1,6,8-triaza(2-^{13}C)bicyclo[4.3.0]non-3-ene-7,9-dione 3a

Alcohol **2a** (0.50 g, 1.92 mmol) was dissolved in a mixture of MeCN (15 ml) and water (10 ml). The solution was cooled to 0 °C, and 1,1,1-trifluoroacetone (2 ml) and $NaHCO_3$ (1.3 g) were added, followed by Oxone® (6.15 g) in small portions over a period of 10 min. The mixture was stirred 15 h at room temperature. Another charge of $NaHCO_3$ (0.65 g), 1,1,1-trifluoroacetone (1 ml) and Oxone (3 g) was added, and after 2 h the reaction mixture was worked up by the addition of water (100 ml) and extraction with $CHCl_3$ (5 × 50 ml). The combined organic layers were dried ($MgSO_4$) and concentrated to give a solid mixture of *trans* and *cis* epoxide (468 mg) in a 3:1 ratio. On addition of $CHCl_3$ (18 ml) pure **3a** crystallized out (308 mg, 58%); ^{13}C NMR (DMSO) δ 150.1 (C-7, C-9), 131.0 (Ar *ipso*), 128.4 (*meta*), 127.5 (*para*), 125.6 (*ortho*), 59.0 (d, C-2′, $J_{2',2}$ 152 Hz), 53.5 (intensity 100×, C-2), 49.6 (d, C-3, $J_{3,2}$ 178 Hz), 47.3 (C-4), 40.6 (C-5); ^{1}H NMR (DMSO) δ 7.46 (m, 5H, Ar), 5.39 (dt, 1H, 2′-OH, $J_{2'\text{-OH},2'}$ 5.9 Hz, $J_{2'\text{OH},C2}$ 1.5 Hz), 4.46 (dt, 1H, H-2, $J_{H2,C2}$ 145 Hz, $J_{2,2a'}$ 4.0 Hz), 4.13 (d, 1H, H-5ax, $J_{5ax,5eq}$ 13.9 Hz), 3.92 (dd, 1H, H-5eq, $J_{5eq,4}$ 2.2 Hz), 3.80 (m, 2H, H-2′), 3.63 (m, 2H, H-3, H-4). MS (ES): *m*/*z* 299.0891 (M + Na^+). Calc. for $C_{12}{}^{13}CH_{13}N_3O_4$ + Na^+: *m*/*z*, 299.0833.

(2,3-*trans*-3,4-*trans*)-3,4-Dihydroxy-2-hydroxymethyl-8-phenyl-1,6,8-triaza(2-^{13}C)bicyclo[4.3.0]non-3-ene-7,9-dione 4a and (4,5-*trans*-5,6-*trans*)-4,5-dihydroxy-8-oxo-2-(*N*-phenyl)carbamoyl-1,2-diaza-8-oxa-bicyclo[4.3.0]nonane 8a

Epoxide **3a** (0.236 g, 0.855 mmol) was dissolved in water (25 ml) and $HClO_4$ (70%; 0.59 ml) was added. The solution was heated to 100 °C for 5 h, then neutralised with $KHCO_3$ (0.70 g) and concentrated. Flash chromatography in EtOAc gave pure **4a** (184 mg, 74%), while **8a** was also obtained (44 mg, 18%).

4a: ^{13}C NMR (DMSO) δ 152.9, 150.8 (C-7, C-9), 131.2 (Ar *ipso*), 128.4 (*meta*), 127.4 (*para*), 125.7 (*ortho*), 65.9 (C-4), 65.3 (d, C-3, $J_{3,2}$ 155 Hz), 60.3 (intensity 100×, C-2), 57.8 (d, C-2′, $J_{2',2}$ 149 Hz), 46.1 (C-5); ^{1}H NMR (DMSO) δ 7.46 (m, 5H, ArH), 5.62 (d, 1H, 4-OH, $J_{4\text{-OH},4}$ 3.7 Hz), 5.49 (t, 1H, 3-OH, $J_{3\text{-OH},3} = J_{3\text{-OH},C2}$ = 3.7 Hz), 4.98 (dt, 1H, 2′-OH, $J_{2'\text{-OH},2'}$ 5.9 Hz, $J_{2'\text{-OH},C2}$ 1.5 Hz), 4.00 (dt, 1H, H-2, $J_{2,C2}$ 144 Hz, $J_{2,2'}$ 5.8 Hz), 3.79 (m, 4H, H-2′, H-3, H-4), 3.66 (dd, 1H, H-5ax, $J_{5ax,5eq}$ 15.0 hz, $J_{5ax,4}$ 2.6 Hz), 3.51 (dd, 1H, H-5$_{eq}$, $J_{5eq,4}$ 2.6 Hz); MS (ES) *m*/*z* 317.0988 (M + Na^+). Calc. for $C_{12}{}^{13}CH_{15}N_3O_5$ + Na: *m*/*z*, 317.0939.

8a: ^{13}C NMR (DMSO) δ 155.7 (C-9), 153.7 (C-2′), 138.6 (Ar *ipso*), 128.0 (*meta*), 122.4 (*para*), 119.7 (*ortho*), 71.6 (d, C-5, $J_{5,6}$ 152 Hz), 67.3 (C-4), 64.4 (d, C-7, $J_{6,7}$ 135 Hz), 56.4 (intensity 100×, C-6), 46.0 (C-3); ^{1}H NMR (DMSO) δ 9.21 (s, 1H, NH), 7.54 (d, 2H, o-ArH, $J_{o,m}$ 8.1 Hz), 7.30 (t, 2H, m-ArH, $J_{m,o} = J_{m,p}$ = 7.7 Hz), 7.02 (t, 1H, p-ArH), 5.64 (br s, 1H, 4-OH), 5.40 (s br, 1H, 5-OH), 4.41 (m, 3H, H-4, H-5, H-7a), 3.55 (ddd, 1H, H-6, $J_{6,C6}$ 128 Hz, $J_{6,7a}$ 6.5 Hz, $J_{6,7b}$ 9.0 Hz), 3.21 (m, 3H, H_2-3, H7b); MS (ES) *m*/*z* 317.0951 (M + Na^+). Calc. for $C_{12}{}^{13}CH_{15}N_3O_5$ + Na^+: *m*/*z*, 317.0939.

The by-product **8a** was also obtained from **4a** (40 mg), when treated with $HClO_4$ (75%, 100 µl) in 4 ml of water for 50 h at 100 °C. After neutralisation ($KHCO_3$) and concentration a residue containing a 2:1 mixture of **4a** and **8a** was obtained.

(3,4-*trans*-4,5-*trans*)-4,5-Dihydroxy-3-(hydroxymethyl)hexahydropyridazine 1a

Triol **4a** (83 mg, 0.28 mmol) was dissolved in hydrazine hydrate (2 ml) and heated at 100 °C for 18 h. The solution was concentrated to give a syrup. Flash chromatography in EtOH–NH_4OH (25%) 9:1 gave pure **1a** (27 mg, 65%); ^{13}C NMR (D_2O) δ 70.2 (d, C-4, $J_{4,3}$ 155 Hz), 69.7 (C-5), 61.4 (intensity 100×, C-3), 58.0 (d, C-3′, $J_{3',3}$ 160 Hz), 49.9 (C-6); ^{1}H NMR (D_2O) δ 3.58 (ddd, 1H, H-3′a, $J_{3'a,3'b}$ 12.1 Hz, $J_{3'a,3}$ 2.9 Hz, $J_{3'a,C3}$ 1.8 Hz), 3.42 (ddd, 1H, H-3′b, $J_{3'b,3}$ 5.9 Hz, $J_{3'b,C3}$ 2.2 Hz), 3.32 (dd, 1H, H-5, $J_{5,6ax}$ 10.3 Hz, $J_{5,6eq}$ 5.1 Hz), 3.09 (dt, 1H, H-4, $J_{4,3,5}$ 9.9 Hz, $J_{4,C3}$ 2.6 Hz), 2.97 (dd, 1H, H-6$_{eq}$, $J_{6eq,6ax}$ 12.8 Hz, $J_{6eq,5}$ 5.1 Hz), 2.47 (dddd, 1H, H-3, $J_{3,C3}$ 137 Hz, $J_{3,4}$ 9.9 Hz); MS (ES) *m*/*z* 172.0776 (M + Na^+). Calc. for $C_4{}^{13}CH_{12}N_2O_3$ + Na^+: *m*/*z* 172.0775.

Alternatively **8a** (29 mg) was subjected to hydrazinolysis in hydrazine hydrate (2 ml) at 100 °C for 18 h. After work-up as above **1a** (11 mg, 75%) was obtained.

Acknowledgements

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