Synthesis of Glucosylceramide Analogues: Imino-Linked 5a-Carbaglycosylceramides, Potent and Specific Glucocerebrosidase Inhibitors

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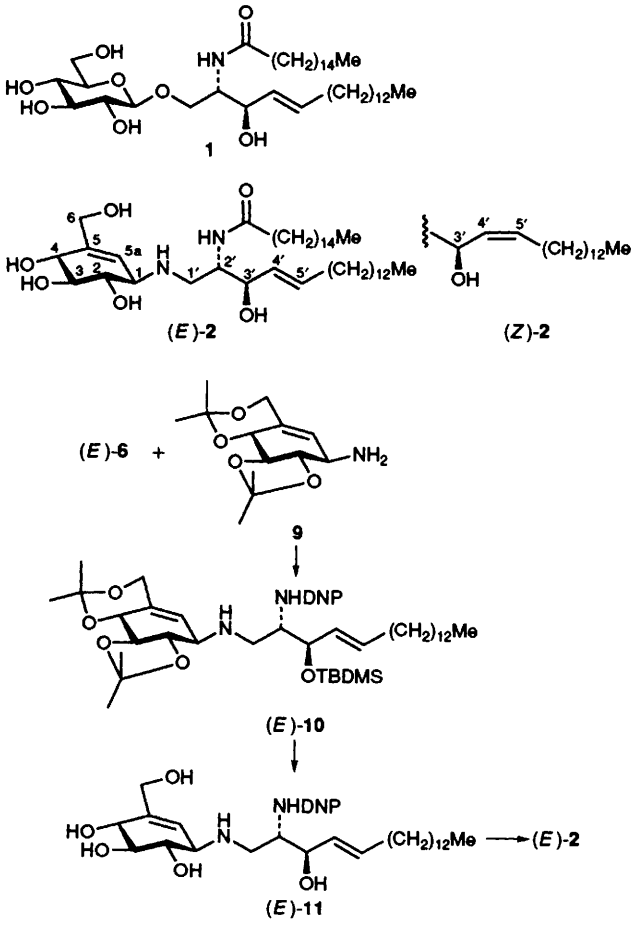
Imino-linked carbocyclic analogues (*E*)- and (*Z*)-**2** of glucosylceramide **1** have been synthesized and shown to be potent and specific inhibitors against glucocerebrosidase.

Glycosphingolipids play an important role in biological systems as essential constituents of membrane and cell walls. Their biological functions and biosynthesis from glucosylceramide have therefore been extensively studied. In order to gain further understanding of their biological functions, it is necessary to study structural analogues as well as naturally occurring compounds.[1] Recently, several carbocyclic analogues[2] of glycosylamides were shown to possess biological activity comparable to those of the parent compounds, suggesting that introduction of carba-sugar residues instead into glycolipids could possibly be carried out with retention of biological activity.

Here we describe a synthesis of the newly designed carbocyclic analogues (*E*)- and (*Z*)-**2** of glucosylceramide **1**, containing unsaturated 5*a*-carba-sugar residues bonded by way of imino-linkages. Interestingly, both compounds as expected have shown to be very potent and specific glucocerebrosidase inhibitors. The synthetic method involves reaction of the newly-prepared aziridines (*E*)- and (*Z*)-**6**, precursors of sphingosine-analogues, with protected unsaturated 5a-carba-β-D-hexopyranosylamine (β-valienamine) **9**, respectively, (Scheme 1).



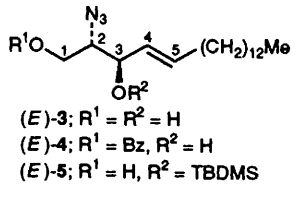
Scheme 1 For convenience, only the structural formulae of compounds (*E*)-**3**–(*E*)-**11** are shown

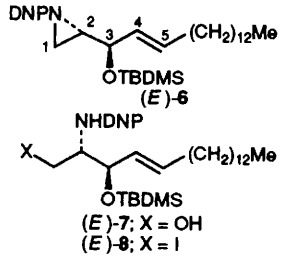
Selective benzoylation of the known azidosphingosine[3] (*E*)-**3** gave the 1-*O*-benzoyl derivative† (*E*)-**4** (65%), which is silylated with *tert*-butyldimethylsilyl chloride (TBDMS) followed by *O*-deacylation to give the 1-OH unprotected derivative (*E*)-**5**[4] (73%). Reduction of the azido group of (*E*)-**5** with triphenylphosphine and subsequent treatment with 2,4-dinitrofluorobenzene afforded the *N*-2,4-dinitrophenylaziridine derivative (*E*)-**6**, $[\alpha]_D^{26}$ −162 (*c* 1.2, $CHCl_3$) (35%), and the amino alcohol (*E*)-**7**, $[\alpha]_D^{26}$ −28 (*c* 1.0, $CHCl_3$) (45%), non-selectively. The ^1H NMR spectrum of (*E*)-**6** showed three signals due to the aziridino protons: δ_H (270 MHz, $CDCl_3$) 2.72 (1 H, d, *J* 4, 1a-H), 2.61 (1 H, ddd, *J* 3.6, 4 and 6.2, 2-H) and 2.14 (1 H, d, *J* 6.2, 1b-H), supporting the assigned structure. The latter (*E*)-**7** could readily be transformed into (*E*)-**6** by iodination with triphenylphosphine, imidazole and iodine [→(*E*)-**8**], and then treatment with silver fluoride in pyridine [→(*E*)-**6**, 83% overall yield]. Similarly, the aziridine (*Z*)-**6**, $[\alpha]_D^{25}$ −140 (*c* 0.93, $CHCl_3$), was prepared in a three-step sequence from (*Z*)-**3**.[3]

Coupling of the aziridine (*E*)-**6** and a slight excess (1.2 mol equiv.) of 2,3:4,6-di-*O*-isopropylidene-β-valienamine[5] **9** in propan-2-ol for 5 days at 120 °C afforded, after purification by silica gel chromatography with ethyl acetate–toluene (1:15), a 60% yield of the secondary amine (*E*)-**10**, the ^1H NMR spectrum of which fully supported the structure proposed. The protecting groups of (*E*)-**10** were removed by treatment with Bu^n_4NF in THF and then with 80% acetic acid (aq) to give the 2,4-dinitrophenyl (DNP) derivative (*E*)-**11** (75%). Compound (*E*)-**11** was treated with Amberlite IRA-400 (OH^-) resin in acetone–methanol–H_2O (3:5:1) and the resulting amine was acylated with hexadecanoyl chloride in sodium acetate (aq) to afford, after silica gel chromatography with chloroform–methanol (7:1), the glycosylceramide analogue (*E*)-**2**, $[\alpha]_D^{27}$ −21 (*c* 0.35, MeOH), in 58% yield; the assigned structure was confirmed by ^1H NMR spectroscopy.

The *Z*-isomer (*Z*)-**2**, $[\alpha]_D^{26}$ −38 (*c* 0.29, MeOH), was similarly prepared in 27% overall yield, starting from the coupling product (*Z*)-**10** obtained by condensation of (*Z*)-**6** and **9**.

Bioassays[6] of (*E*)- and (*Z*)-**2** indicated that they both possess strong inhibitory activity (IC_{50} *ca* 0.4 μg ml^{-1}) against glucocerebrosidase, and show almost no observable activity against other β-glucosidases tested so far. It is notable that the unnatural-type analogue *Z*-isomer (*Z*)-**2** possesses a rather higher inhibitory activity than the *E*-isomer (*E*)-**2**. The present results suggested that the newly designed carbocyclic anal-





ogues of glucosylceramide will be useful for elucidating the biological functions of glycosphingolipids.

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Footnote

† All new compounds described were homogeneous on TLC, and characterised on the basis of elemental and spectrometric analyses. Selected ^{1}H NMR spectral data (270 MHz, $CDCl_3$) for (*E*)-**6**: δ 8.86 (1 H, d, *J* 2.6, Ph), 8.27 (1 H, dd, *J* 9.2, Ph), 7.31 (1 H, d, Ph), 5.75 (1 H, dt, *J* 7, 7 and 15.5, 5-H), 5.48 (1 H, dd, *J* 7 and 15.4, 4-H), 4.38 (1 H, dd, *J* 3.6 and 7, 3-H), 2.72 (1 H, d, *J* 4, 1a-H), 2.61 (1 H, ddd, *J* 3.6, 4 and 6.2, 2-H), 2.14 (1 H, d, *J* 6.2, 1b-H), 2.07 (2 H, q, *J* 7, 6a,b-H), 1.25 (22 H, s, 11 CH_2), 0.89 (9 H, s, Bu^t), 0.88 (1 H, t, *J* 7, Me) and 0.06 (6 H, s, $SiMe_2$).

For (*Z*)-**6**: δ 8.85 (1 H, d, *J* 2.4, Ph), 8.27 (1 H, dd, *J* 9.3, Ph), 7.33 (1 H, *J* 9.3, Ph), 5.57 (1 H, dt, *J* 7.3, 7.3 and 10.7, 5-H), 5.40 (1 H, dd, *J* 8.3 and 10.3, 4-H), 4.78 (1 H, dd, *J* 3.4 and 8.3, 3-H), 2.72 (1 H, dd, *J* 3.4, 1a-H), 2.61 (1 H, ddd, *J* 3.4, 3.4 and 5.9, 2-H), 2.15 (1 H, d, *J* 5.9, 1b-H), 2.15–2.00 (2 H, m, 6a,b-H), 1.24 (22 H, s, 11 CH_2), 0.89 (9 H, s, Bu^t), 0.88 (3 H, t *J* 7, Me) and 0.063, 0.056 (3 H each, 2 s, $SiMe_2$).

For (*E*)-**10**: δ 9.14 (1 H, d, *J* 2.9, Ph), 9.08 (1 H, d, *J* 7.3, NH), 8.21 (1 H, dd, *J* 7.3 and 9.4, Ph), 7.03 (1 H, *J* 9.4, Ph), 5.72 (1 H, dt, *J* 7.3, 7.3 and 15.4, 5′-H), 5.44 (1 H, dd, *J* 6.8 and 15.4, 4′-H), 5.30 (1 H, br s, 5a-H), 4.62 (1 H, d, *J* 7.8, 4-H), 4.49 and 4.14 (each 1 H, 2 d, *J* 14.2, 6a, b-H), 4.33 (1 H, dd, *J* 4.4 and 6.8, 3′-H), 3.88–3.78 (1 H, m, 2′-H), 3.71 (1 H, dd, *J* 7.8 and 9, 3-H), 3.57 (1 H, d, *J* 9, 1-H), 3.45 (1 H, t, *J* 12.7, 2-H), 3.16 (1 H, dd, *J* 4.9 and 12.7, 1′a-H), 3.03 (1 H, dd, *J* 4.4 and 12.7, 1′b-H), 2.07–1.98 (2 H, m, 6′a,b-H), 1.57, 1.44, 1.42 and 1.40 (each 3 H, 4 s, 2 CMe_2), 1.25 (22 H, br s, 11 CH_2), 0.88 (3 H, t, *J* 7, CH_3), 0.86 (9 H, s, Bu^t) and −0.02, −0.05 (each 3 H, 2 s, $SiMe_2$).

For (*E*)-**2** [270 MHz, $CDCl_3$–CD_3OD (2:1)]: δ 5.73 (1 H, dt, *J* 6.4 and 15.3, 5′-H), 5.58 (1 H, br s, 5a-H), 5.44 (1 H, dd, *J* 6.9 and 15.3, 4′-H), 4.25–4.10 (3 H, m, 4-, 6a,b-H), 4.08 (1 H, dd, *J* 5.9 and 6.9, 3′-H), 3.89 (1 H, q, *J* 5.9, 2′-H), 3.55 (1 H, dd, *J* 7.4 and 9.9, 3-H), 3.34 (1 H, dd, *J* 8.4 and 9.9, 2-H), 3.27 (1 H, br d, *J* 8.4, 1-H), 2.98 and 2.81 (each 1 H, 2 dd, *J* 8.4 and 12.6, 1a,b-H), 2.20 (2 H, t, *J* 7, $COCH_2$), 2.04 (2 H, q, *J* 6.5, 6′a,b-H), 1.27 (48 H, br s, 24 CH_2) and 0.89 (6 H, t, *J* 6.5, 2 Me).

For (*Z*)-**2**: δ 5.59 (1 H, br s, 5a-H), 5.57 (1 H, dt, *J* 7.3, 11.0, 5′-H), 5.39 (1 H, dd, *J* 8.4, 11.0, 4′-H), 4.45 (1 H, *J* 6.6, 8.4, 3′-H), 4.18 and 4.12 (each 1 H, 2 d, *J* 14.3, 6a,b-H), 4.20–4.10 (1 H, m, 4-H), 3.91 (1 H, dt, *J* 5.1, 6.6, 6.6, 2′-H), 3.55 (1 H, dd, *J* 4.0, 9.9, 3-H), 3.47 (1 H, dd, *J* 8.1, 9.9, 2-H), 3.38 (1 H, br d, *J* ~8, 1-H), 3.05 (1 H, dd, *J* 6.6, 12.8, 1a′-H), 2.88 (1 H, dd, *J* 5.1, 12.8, 1b′-H), 2.20 (2 H, t, *J* 7.0, $COCH_2$), 2.15–2.00 (2 H, m, 6′a,b-H), 1.67–1.52 (2 H, m, 2″a,b-H), 1.26 (46 H, br s, 23 CH_2) and 0.89 (6 H, t, *J* 7.0, 2 Me).

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