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Chemical Modification of the β-Glucocerebrosidase Inhibitor N-Octyl-β-valienamine: Synthesis and Biological Evaluation of 4-Epimeric and 4-O-(β-D-Galactopyranosyl) Derivatives

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Abstract—N-Octyl-β-valienemine (**1**), a potent β-glucocerebrosidase inhibitor, was chemically transformed into two biologically interesting compounds: the 4-epimer **2**, β-*galacto*-type N-octyl-valienamine, and the 4-O-(β-D-galactopyranosyl) derivative **3**, a carba-lactosylceramide analogue. The former, interestingly, could be demonstrated to act as a very effective inhibitor (IC$_{50}$ = 0.3 μM) of human β-galactosidase. The latter exhibited moderate inhibitory activity (IC$_{50}$ = 20 μM) against β-glucocerebrosidase (mouse liver). © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

We previously reported that, among six N-alkyl[1] and six N,N-dialkyl-β-valienamines,[2] prepared in continuation of a chemical-modification program, the N-octyl derivative **1** exerts the strongest inhibition (IC$_{50}$ = 0.03 μM) of β-glucocerebrosidase (mouse liver). Since the glucosylceramide analogue composed of unsaturated 5a-carba-β-D-glucopyranose residue has been shown to be a strong and specific β-glucocerebrosidase inhibitor,[3] N-octyl β-valienamine having a β-*galacto* configuration, by analogy would be expected to show strong activity against β-galactocerebrosidase. This possibility was therefore examined in the present study. Furthermore, incorporation of a β-galactopyranose residue at C-4 of **1** was attempted to generate a lactosylceramide analogue, as a candidate[4] exo-β-galactoceramidase inhibitor.

Results and Discussion

The secondary amino function of N-octyl-2,3:4,6-di-O-isopropylidene-β-valienamine[1] (**4**) was first protected to give the N-*tert*-butoxycarbonyl derivative[5] (**5**), the isopropylidene groups of which were then removed with aqueous acetic acid (Fig. 1). The crude tetrol was then treated with α,α-dimethoxytoluene in DMF to give the 4,6-O-benzylidene derivative (**7**), which was further protected with methoxymethyl ether groups (→**8**). The benzylidene group was then removed and the resulting 6-hydroxyl group was selectively silylated to give the *tert*-butyldimethylsilyl derivative (**9**). The remaining free 4-hydroxyl group was then oxidized with pyridinium chlorochromate in CH$_2$Cl$_2$ and the resulting α,β-unsaturated ketone was reduced with 1 M lithium tri-*sec*-butyl borohydride in THF at −78 °C to give almost selectively the epimeric alcohol **10** in 66% yield. The ^{1}H NMR spectrum of **9** indicated a doublet of doublets (J = 9.9 and 7.9 Hz) at δ 3.53, attributable to 3-H, whereas, in the spectrum of **10**, the signal due to 3-H appeared as a narrow doublet of doublets (J = 10.0 and 2.0 Hz) at δ 3.46, confirming the structure proposed. This compound was finally deprotected by treatment with a mixture of 4 M hydrochloric acid and THF at 65 °C to afford, after purification over a column of Dowex 50W×2 (H$^+$) resin with 1% ammonia, to give N-octyl β-*galacto*-valienamine **2** in 91% yield.

For fear of difficulty of removal of the N-protecting group in the presence of an acid-labile glycoside linkage, a N-trifluoroacetyl group was selected for protection of compound **4**. The N-trifluoroacetyl derivative **6** was

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obtained quantitatively by treatment with trifluoro-acetic anhydride in pyridine (Schemes 1 and 2). Compound **11**, obtained by similar deprotection of **6** followed by benzylidenation, was first treated with 6 molar equivalent of benzoyl chloride in pyridine at room temperature to yield the dibenzoate **12**, which, after removal of the benzylidene group, subjected to a selective benzoylation of the primary hydroxyl group with 1.5 molar equivalent of the reagent at −15 °C, affording the 2,3,6-tribenzoate **13** in 97% overall yield. Incorporation of a β-galactose residue at C-4 was effected, however, in a poor yield by coupling **13** with 3 molar equivalent of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate[6,7] in dichloro-methane in the presence of BF$_3$ etherate at 0 °C to room temperature, affording a carba-disaccharide derivative **14** in 19% yield, together with **13** (58%) recovered. The product was treated with methanolic potassium carbonate for 1h at room temperature to give, after purification by a resin column as in the preparation of **2**, free *N*-octyl-5a′-carba-β-lactosylamine **3** in 73% yield. The structure was established on the basis of the ^{1}H NMR spectrum.

Biological Assay

Biological assays of compound **2** (Table 1) showed[9] medium inhibitory activity (IC$_{50}$ = 5.0 × 10^{-6} M) toward β-galactocerebrosidase (mouse liver). Interest-ingly, it was also demonstrated to possess strong activity (IC$_{50}$ = 3 × 10^{-7} M) against β-galactosidase (human).[8] Therefore, chemical modification of **2** as a lead com-pound has been carried out extensively in our labora-tory. We expect that the compounds of this group with a potent inhibitory activity against human β-galacto-sidase will be used in the near future for a new ther-apeutic trial of genetic β-galactosidase deficiency disorders as suggested in a previous report for Fabry disease with α-galactosidase deficiency.[4]

Table 1. Inhibitory activity (%) of compund **2** against β-galacto-sidase (human)

Inhibitor concentration (μm)	β-Galactoside activity (%)
0.000	100
0.050	77
0.125	60
0.250	43
0.500	36
1.000	36
2.500	19
5.000	14

Compound **3** was found to possess inhibitory activity (IC$_{50}$ = 2 × 10^{-5} M) only against β-glucocerebrosidase (mouse liver).[3] The above results might be understood in terms of its original potential and/or formation of the strong inhibitor, *N*-octyl-β-valienamine, generated by partial enzymatic hydrolysis of **3**.

Experimental

General methods

Melting points were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. Silica gel column chromatography was performed on silica gel 200–300 mesh (Wakogel C-300, Wako Junyaku Kogyo Co., Osaka), and analytical TLC on silica-gel 60 F-254 (E. Merck, Darmstadt). ^{1}H NMR spectra (270 and 300 MHz) were recorded on JEOL GSX-270 or JEOL GSX-300 instruments. Chemical shifts are expressed as δ values with reference to Me$_4$Si (δ 0.00) in CDCl$_3$, methanol-d_4, and DMSO-d_6, respec-tively. IR spectra were recorded on JASCO IR-810 of Hitachi FTS-65 spectrometers. Solutions were dried over anhydrous Na$_2$SO$_4$ and concentrated at < 45 °C under diminished pressure.

***N*-Octyl-*N*-*tert*-butoxycarbonyl-2,3:4,6-di-*O*-isopropyl-idene-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylamine (5).** To a solution of *N*-octyl-2,3:4,6-di-*O*-isopropylidene-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylamine[1] (**4**, 644 mg, 1.75 mmol) in dichloromethane (13 mL) were added triethylamine (0.97 mL) and di-*tert*-butyldicarbonate (0.81 mL, 3.50 mmol), and the mixture was stirred at 25 °C. After 1.5 h, similar amounts of the reagents were again added and stirring continued for 1h. The mixture was diluted with ethyl acetate (180 mL), washed with saturated aqueous sodium hydrogen carbonate (60 mL) and brine (2×60 mL), dried, and evaporated. The resi-dual product was purified by silica gel chromatography (50 g, EtOAc:hexane, gradient elution 1:11→1:8) to give **5** (807 mg, 99%) as a syrup, [α]$_D^{22}$ −61° (*c* 0.90; CHCl$_3$); IR (neat) ν 1695 cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 110 °C] δ 5.22 (br s, 1H, 5a-H), 4.59 (br d, 1H, $J_{3,4}$ = 9.3 Hz, 4-H), 4.57 (br d, 1H, $J_{1,2}$ = 9.3 Hz, 1-H), 4.40 and 4.13 (2 d, each 1H, J_{gem} = 13.7 Hz, 6,6-H), 3.74 (dd, 1H, $J_{2,3}$ = 9.3 Hz, 2-H), 3.59 (dd, 1H, 3-H), 3.11 and 2.93 (2 m, each 1H, NCH$_2$), 1.54–1.18 (m, 12H, 6×CH$_2$), 1.47, 1.37, 1.36, and 1.29 (4 s, each 3H, 2×CMe$_2$), 1.40 (s, 9H, *t*-Bu), 0.86 (t, 3H, J = 6.6 Hz, CH$_2$C*H*$_3$). Anal. calcd for C$_{26}$H$_{45}$NO$_6$: C, 66.78; H, 9.70; N, 3.00. Found: C, 66.57; 10.10; N, 3.11.

***N*-Octyl-*N*-*tert*-butoxycarbonyl-4,6-*O*-benzylidene-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylamine (7).** A mixture of **5** (768 mg, 1.64 mmol) and aqueous 60% acetic acid (16 mL) was stirred for 30 min at 60 °C, and then concentrated. The residue was co-evaporated with ethanol several times and eluted from a silica gel column (50 g, EtOH/toluene, gradient elution 1:10→1:4) to give a crude tetrol (540 mg) as a syrup. To a solution of a 242 mg-portion (0.62 mmol) of this compound in DMF (6 mL) were added α,α-dimethoxytoluene (112 μL, 0.75 mmol) and *p*-toluenesulfonic acid monohydrate (12 mg, 0.06 μmol), and the mixture was stirred for 3.5 h at 45 °C under diminished pressure provided by a water aspirator. Additional α,α-dimethoxytoluene (50 μL, 0.33 mmol) was then introduced, and the stirring con-tinued for 2 h. The mixture was diluted with ethyl ace-tate (60 mL), washed with water and saturated aqueous sodium hydrogen carbonate, dried, and evaporated.

Formation of two products was detected by TLC (R_f 0.33 and 0.74 in acetone/toluene, 1:5; R_f 0.05 and 0.70 in EtOAc/toluene, 1:5). The residue was purified by silica gel chromatography (16 g, EtOAc/toluene, gradient elution 1:24→1:3) to give, first, the di-O-benzylidene compound (159 mg, 45%), and then **7** (164 mg, 55%) as a syrup. The former compound was dissolved in methanol (5 mL) and treated with *p*-toluenesulfonic acid monohydrate (3 mg) for 10 min at 0 °C. After neutralization with a drop of triethylamine, the mixture was evaporated and the residue was eluted from a silica gel column (7 g) with acetone:toluene (1:7) to give **7** (110 mg, combined yield 82%). $[\alpha]_D^{22}$ −57° (*c* 0.97, CHCl₃); IR (neat) ν 3420, 1695 cm⁻¹; ¹H NMR [300 MHz, (CD₃)₂SO, 110 °C] δ 7.46–7.27 (m, 5H, Ph), 5.65 (s, 1H, C*H*Ph), 5.31 (br s, 1H, 5a-H), 4.39 (br s, 2H, 6,6-H), 4.36 (br d, 1H, $J_{3,4}$ = 7.6 Hz, 4-H), 4.16 (br s, 1H, 1-H), 3.64 (dd, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.3 Hz, 2-H), 3.52 (dd, 1H, 3-H), 3.25–2.80 (m, 2H, NCH₂), 1.57–1.17 (m, 12H, 6× CH₂), 1.39 (s, 9H, CMe₃), 0.87 (t, 3H, *J* = 6.2 Hz, CH₂C*H*₃). Anal. calcd for C₂₇H₄₁NO₆·0.5H₂O: C, 66.92; H, 8.73; N, 2.89. Found: C, 66.94; H, 8.85; N, 2.91.
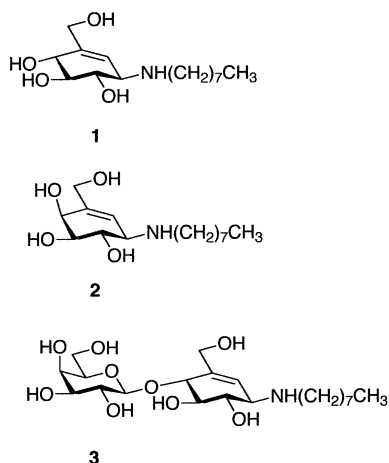

Figure 1.

N-Octyl-N-tert-butoxycarbonyl-4,6-O-benzylidene-2,3-di-O-methoxymethyl-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (8). To a solution of **7** (269 mg, 0.566 mmol) in 1,2-dichloroethane (7 mL) were added chloromethyl ether (0.43 mL, 5.66 mmol) and *N,N*-diisopropylethylamine (1.97 mL, 11.3 mmol), and the mixture was stirred for 3 h at 60 °C. After dilution with chloroform (60 mL), the solution was washed with 1 M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and water, dried, and evaporated. The residue was purified by silica gel chromatography (20 g, EtOAc/toluene, gradient elution 1:14 → 1:9) to give **8** (315 mg, 99%) as a syrup: IR (neat) ν 1695 cm⁻¹; ¹H NMR [300 MHz, (CD₃)₂SO, 110 °C] δ 7.45–7.28 (m, 5H, Ph), 5.70 (s, 1H, C*H*Ph), 5.38 (br s, 1H, 5a-H), 4.79 and 4.77 (2 d, each 1H, J_{gem} = 6.1 Hz) and 4.75 and 4.63 (2 d, each 1H, J_{gem} = 6.3 Hz) (2 × OCH₂), 4.57 (br d, 1H, $J_{3,4}$ = 7.7 Hz, 4-H), 4.43 (br s, 2H, 6,6-H), 4.27 (br s, 1H, 1-H), 3.99 (dd, 1H, $J_{1,2}$ = 9.3, $J_{2,3}$ = 10.0 Hz, 2-H), 3.78 (dd, 1H, 3-H), 3.28 and 3.27 (2 s, each 3H, 2× OMe), 3.07–2.87 (m, 2H, NCH₂), 1.62–1.20 (m, 12H, 6× CH₂), 1.39 (s, 9H, *t*-Bu), 0.87 (t, 3H, *J* = 6.5 Hz, CH₂C*H*₃). Anal. calcd for C₃₁H₄₉NO₈: C, 66.05;H, 8.76; N, 2.48. Found: C, 65.80;H, 8.99; N, 2.42.

N-Octyl-N-tert-butoxycarbonyl-2,3-di-O-methoxymethyl-6-O-tert-butyldimethylsilyl-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (9). A solution of **8** (315 mg, 0.56 mmol) and aqueous 60% acetic acid (8 mL) was stirred for 2 h at 60 °C, and concentrated. The residue was co-evaporated several times with ethanol and toluene, and then chromatographed on a silica gel column (20 g). Gradient elution with acetone/toluene (1:6 to 1:5) gave the diol (220 mg) as a syrup. A 160-mg-portion of this compound was dissolved in DMF (5 mL), to which *tert*-butylchlorodimethylsilane (100 mg, 0.67 mmol) was added. The mixture was stirred for 1h at room temperature, and then diluted with ethyl acetate (60 mL). The solution was washed with water, dried, and evaporated. The residual compound was purified by silica gel chromatography (20 g, EtOAc/toluene, 1:6) to give **9**
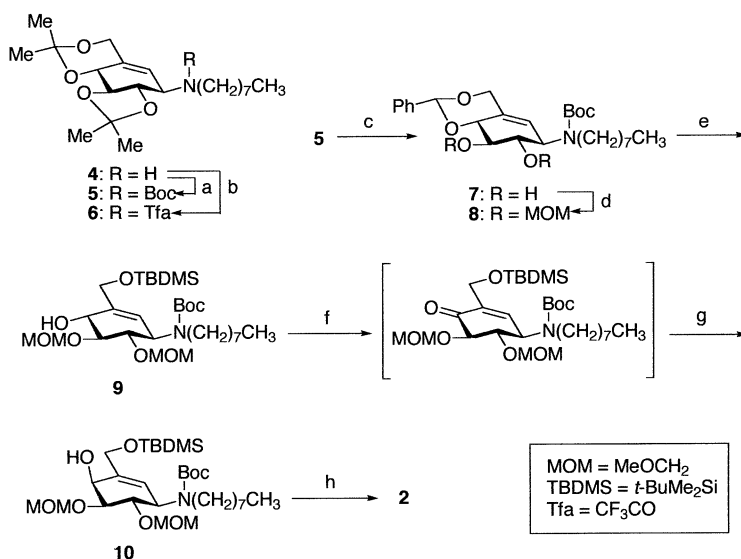
Scheme 1. Reagents and conditions: (a) (*t*-BuOCO)₂O, Et₃N, CH₂Cl₂, rt; (b) (CF₃CO)₂O, pyridine, rt; (c) 60% aq AcOH, 60 °C; PhCH(OMe)₂, TsOH, DMF, 45 °C; (d) MeOCH₂Cl, ClCH₂CH₂Cl, *i*-Pr₂EtN, 60 °C; (e) 60% aq AcOH, 60 °C; *t*-BuMe₂SiCl, DMF, rt; (f) PCC, CH₂Cl₂, rt; (g) *s*-Bu₃LiBH, THF, −78 °C→0 °C; (h) 4 M HCl, THF, 65 °C; Dowex 50×2 (H⁺) resin, 1% NH₃/MeOH.

(194 mg, 98%) as a syrup; $[\alpha]_D^{23}$ −101° (*c* 0.95, CHCl$_3$); IR (neat) ν 3445 (OH), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 110 °C] δ 5.36 (br s, 1H, 5a-H), 4.83 and 4.76 (2 d, each 1H, J_{gem} = 6.1 Hz), and 4.73 and 4.60 (2 d, each 1H, J_{gem} = 6.3 Hz) (2×OCH$_2$), 4.21 and 4.11 (ABq, J_{gem} = 13.9 Hz, 6,6-H), 4.12–4.04 (m, 2H, 1-H, 4-H), 3.87 (dd, 1H, $J_{1,2}$ = 9.3, $J_{2,3}$ = 9.9 Hz, 2-H), 3.53 (dd, 1H, $J_{3,4}$ = 7.9 Hz, 3-H), 3.35 and 3.26 (2 s, each 3H, 2×OCH$_3$), 3.05–2.88 (m, 2H, NCH$_2$), 1.60–1.20 (m, 12H, 6×CH$_2$), 1.38 (s, 9H, OCMe$_3$), 0.89 (s, 9H, CCMe$_3$), 0.86 (t, 3H, J = 6.8 Hz, CH$_2$CH_3), 0.05 (s, 6H, SiMe$_2$). Anal. calcd for C$_{30}$H$_{59}$NO$_8$Si: C, 61.08; H, 10.08; N, 2.37. Found: C, 60.82; H, 10.38; N, 2.45.

N-Octyl-*N*-*tert*-butoxycarbonyl-2,3-di-*O*-methoxymethyl-6-*O*-*tert*-butyldimethylsilyl-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosylamine (10).

To a solution of compound **9** (75 mg, 0.13 mmol) in CH$_2$Cl$_2$ (2 mL) were added powdered 4 A molecular sieves (75 mg) and pyridinium chlorochromate (41 mg, 0.19 mmol), and the mixture was stirred for 1h at room temperature. Additional pyridinium chlorochromate (41 mg, 0.19 mmol) was added, and stirring continued for 1h. The mixture was filtered through a Celite bed and the filtrate was chromatographed on a short column of silica gel with diethyl ether as an eluent to give a crude ketone. This compound was dissolved in THF (0.7 mL) and the solution was treated with 1 M lithium tri-s-butyl borohydride/THF solution (0.51 mL, 0.51 mmol) under argon for 30 min at −78 °C. The reaction mixture was allowed to warm to 0 °C and the reaction was quenched by addition of saturated aqueous ammonium chloride. After addition of magnesium sulfate, the solution was filtered through a Celite bed and the filtrate was evaporated. The crude product was purified by silica gel chromatography (7 g, EtOAc:toluene, gradient elution 1:9→1:6) to give **10** (49 mg, 66%) as a syrup; $[\alpha]_D^{23}$ −68° (*c* 1.13, CHCl$_3$); IR (neat) ν 3460 (OH), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 110 °C] δ 5.36 (br s, 1H, 5a-H), 4.71 and 4.59 (2 d, each 1H, J_{gem} = 5.6 Hz) and 4.75–4.66 (m, 2H) (2 × OCH$_2$), 4.38 (br s, 1H, 1-H), 4.19 and 4.12 (2 d, each 1H, J_{gem} = 13.2 Hz, 6,6-H), 4.13–4.02 (m, 2H, 2-H, 4-H), 3.46 (dd, 1H, $J_{2,3}$ = 10.3, $J_{3,4}$ = 2.0 Hz, 3-H), 3.33 and 3.25 (2 s, each 3H, 2 × OMe), 3.10–2.86 (m, 2H, 1′,1′-H), 1.62–1.16 (m, 12H, 6 × CH$_2$), 1.39 (s, 9H, CCMe$_3$), 0.89 (s, 9H, SiCMe$_3$), 0.86 (t, 3H, J = 7.3 Hz, CH$_2$CH_3), 0.05 (s, 6H, SiMe$_2$).

Anal. calcd for C$_{30}$H$_{59}$NO$_8$Si: C, 61.08; H, 10.08; N, 2.37. Found: C, 60.80; H, 10.37; N, 2.53.

N-Octyl-5a-carba-α-L-*arabino*-hex-5(5a)-enopyranosylamine (2).

A solution of **10** (29 mg, 0.049 mmol) in a mixture of THF (0.5 mL) and 4 M hydrochloric acid (1.5 mL) was stirred for 1.5 h at 65 °C, and then evaporated and co-evaporated with ethanol. The product was purified over a Dowex-50W×2 (H$^+$) resin column with 1% methanolic ammonia as an eluent to give **2** (13 mg, 91%) as a white solid: mp 126–128 °C; R_f 0.76 (MeOH:CHCl$_3$:H$_2$O, 35:60:5); $[\alpha]_D^{23}$ +16° (*c* 0.64, MeOH); IR (KBr) ν 3485 (OH), 3250 (amine) cm^{-1}; ^{1}H NMR (300 MHz, CD$_3$OD/CDCl$_3$, 1:2) δ 5.73 (d, 1H, $J_{1,5a}$ = 1.8 Hz, 5a-H), 4.16 (d, 1H, $J_{3,4}$ = 4.2 Hz, 4-H), 4.16 (br. s, 2H, 6,6-H$_2$), 3.64 (dd, 1H, $J_{1,2}$ = 8.1, $J_{2,3}$ = 10.0 Hz, 2-H), 3.48 (dd, 1H, 3-H), 3.12 (dd, 1H, 1-H), 2.78 (ddd, 1H, $J_{1'a,2'}$ = 7.4, J_{gem} = 11.2 Hz) and 2.57 (ddd, 1H) (NCH$_2$), 1.64–1.21 (m, 12H, 6 × CH$_2$), 0.89 (t, 3H, J = 6.7 Hz, CH$_2$CH$_3$). Anal. calcd for C$_{15}$H$_{29}$NO$_4$: C, 62.69; H, 10.17; N, 4.87. Found: C, 62.67; H, 10.47; N, 5.01.

N-Octyl-*N*-trifluoroacetyl-2,3:4,6-di-*O*-isopropylidene-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylamine (6).

To a solution of **4** (2.60 g, 7.07 mmol) in pyridine (40 mL) was added trifluoroacetic anhydride (2.0 mL, 14 mmol), and the mixture was stirred for 1h at room temperature and then evaporated. The residue was dissolved in ethyl acetate (360 mL) and the solution was washed with water, dried, and evaporated. The product was purified by silica gel chromatography (150 g, EtOAc:hexane, 1:10) to give **6** (3.26 g, 99%) as a colorless syrup; $[\alpha]_D^{30}$ −72° (*c* 1.20, CHCl$_3$); IR (neat) ν 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 110 °C] δ 5.30 (br s, 1H, 5a-H), 4.79–4.61 (m, 2H, 1-H, 4-H), 4.43 and 4.16 (2 d, each 1H, J_{gem} = 13.9 Hz, 6,6-H), 3.86 (br s, 1H, 2-H), 3.72 (dd, 1H, $J_{2,3}$ = 8.3, $J_{3,4}$ = 7.8 Hz, 3-H), 3.33 and 3.12 (2 m, 1H, NCH$_2$), 1.67–1.17 (m, 12H, 6 × CH$_2$), 1.49, 1.38, 1.30, and 1.27 (4 s, each 3H, 2 × CMe$_2$), 0.86 (t, 3H, J = 7.1 Hz, CH$_2$CH_3). Anal. calcd for C$_{23}$H$_{36}$F$_3$NO$_5$: C, 59.60; H, 7.83; N, 3.02. Found: C, 59.79; H, 7.79; N, 2.96.

N-Octyl-*N*-trifluoroacetyl-4,6-*O*-benzylidene-5a-carba-β-D-*xylo*-hex-5(5a)-enopyranosylamine (11).

A solution of **6** (3.08 g, 6.64 mmol) and aqueous 80% acetic acid (50
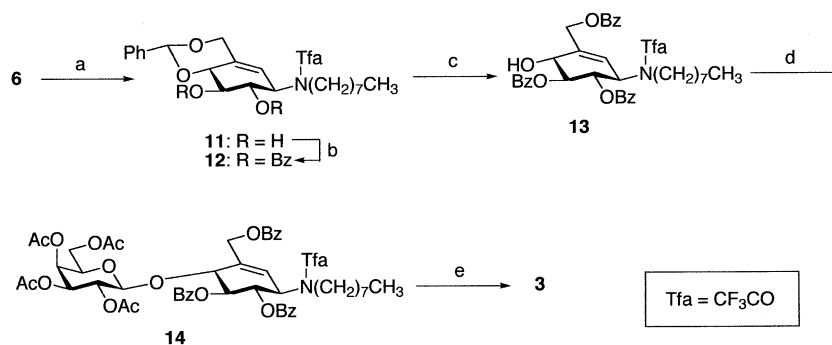
Scheme 2. Reagents and conditions: (a) 80% aq AcOH, 80 °C; PhCH(OMe)$_2$, TsOH, DMF, 45 °C; (b) BzCl (6 mol equiv), pyridine, rt; (c) 80% aq AcOH, 80 °C; BzCl (1.5 mol equiv), pyridine, −15 °C; (d) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate (3 mol equiv), BF$_3$/diethyl ether, molecular sieves 4 A, CH$_2$Cl$_2$, 0 °C, 0.5 h; (e) aq K$_2$CO$_3$, 1H, rt; Dowex 50 × 2 (H$^+$) resin, 1% NH$_3$/MeOH.

mL) was stirred for 30 min at 80 °C. The residue was concentrated and co-evaporated with ethanol three times and then with toluene. The residual product was dissolved in DMF (40 mL), to which were added α,α-dimethoxytoluene (2.0 mL, 13 mmol) and *p*-toluenesulfonic acid monohydrate (130 mg, 0.67 mmol). The mixture was stirred for 2 h at 45 °C under diminished pressure (water aspirator). The mixture was diluted with ethyl acetate (330 mL) and the solution was washed with water, saturated aqueous sodium hydrogen carbonate, dried, and evaporated. The products (R_f 0.15 and 0.83, EtOAc:toluene, 1:5) were chromatographed on a silica gel column (150 g) with EtOAc:toluene (gradient elution 1:7→1:6) to give **11** (1.96 g, 63%) as a syrup, together with a mixture (1.58 g, 37%) of isomeric di-*O*-benzylidene derivatives. A 1.5 g portion of the latter was dissolved in methanol (50 mL) and treated with *p*-toluenesulfonic acid monohydrate (47 mg) at 0 °C. After 10 min, the mixture was neutralized with triethylamine and evaporated. The residue was purified on a silica gel column (30 g, EtOAc:toluene, 1:6) to give **11** (0.93 g, 30%); $[\alpha]_D^{29}$ −78° (*c* 1.1, CHCl$_3$); IR (neat) ν 3440 (OH), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 150 °C] δ 7.48–7.27 (m, 5H, Ph), 5.68 (s, 1H, C*H*Ph), 5.38 (br s, 1H, 5a-H), 4.49–4.34 (m, 4H, 1-H, 4-H, 6,6-H), 3.77 (br s, 1H, 2-H), 3.60 (dd, 1H, $J_{2,3}$ = 9.5, $J_{3,4}$ = 7.6 Hz, 3-H), 3.50–3.05 (m, 2H, NCH$_2$), 1.61–1.21 (m, 12H, 6 × CH$_2$), 0.87 (t, 3H, J = 6.5 Hz, CH$_2$C*H*$_3$). Anal. calcd for C$_{24}$H$_{32}$F$_3$NO$_5$: C, 61.13; H, 6.84; N, 2.97. Found: C, 61.06; H, 6.83; N, 2.97.

N-Octyl-N-trifluoroacetyl-2,3-di-O-benzoyl-4,6-O-benzylidene-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (12). A solution of **11** (1.88 g, 3.99 mmol) in pyridine (30 mL) was treated with benzoyl chloride (2.77 mL, 23.9 mmol) for 5 h at room temperature. The mixture was then diluted with ethyl acetate (230 mL) and the solution was thoroughly washed with water, dried, and evaporated. The residue was purified by silica gel chromatography (200 g, EtOAc:hexane, gradient elution 1:9→1:6) to give **12** (2.69 g, 99%) as a white solid; mp 173–175 °C; $[\alpha]_D^{23}$ −69° (*c* 1.1, CHCl$_3$); IR (neat) ν 3460 (OH), 1730 (ester), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 150 °C] δ 7.95–7.26 (m, 15H, 3×Ph), 6.00 (br s, 1H, 2-H), 5.78 (dd, 1H, $J_{2,3}$ = 10.7, $J_{3,4}$ = 7.8 Hz, 3-H), 5.78 (s, 1H, C*H*Ph), 5.70 (br s, 1H, 5a-H), 5.10 (br d, 1H, 4-H), 5.02 (br d, 1H, $J_{1,2}$ = 7.8 Hz, 1-H), 4.58 (br s, 2H, 6,6-H), 3.52 and 3.31 (2 m, each 1H, NCH$_2$), 1.70–1.18 (m, 12H, 6×CH$_2$), 0.85 (t, 3H, J = 6.8 Hz, CH$_2$C*H*$_3$). Anal. calcd for C$_{38}$H$_{40}$F$_3$NO$_7$: C, 67.15; H, 5.93; N, 2.06. Found: C, 67.12; H, 5.93; N, 2.00.

N-Octyl-N-trifluoroacetyl-2,3,6-tri-O-benzoyl-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (13). A solution of **12** (2.63 g, 3.87 mmol) and aqueous 80% acetic acid (85 mL) was stirred for 1.5 h at 80 °C, and then concentrated. The residue was co-evaporated with ethanol and toluene. The product was dissolved in pyridine (45 mL), to which benzoyl chloride (0.67 mL, 5.8 mmol) was added at −15 °C, and the mixture was stirred for 2 h at −15 °C. It was then diluted with ethyl acetate (300 mL), and the solution was thoroughly washed with

water, dried, and evaporated. The product was purified by column chromatography (150 g, EtOAc:toluene, 1:15) to give **13** (2.61 g, 97%) as a syrup; R_f 0.56 (EtOAc:toluene, 1:5); $[\alpha]_D^{23}$ −29° (*c* 0.95, CHCl$_3$); IR (neat) ν 1730 (ester), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 150 °C] δ 8.03–7.30 (m, 15H, 3×Ph), 5.87 (br s, 1H, 2-H), 5.76 (br s, 1H, 5a-H), 5.57 (dd, 1H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 8.1 Hz, 3-H), 5.07–4.98 (m, 1H, 1-H), 5.03 and 4.92 (2 d, each 1H, J_{gem} = 12.9 Hz, 6,6-H), 4.74 (br d, 1H, 4-H), 3.37–3.27 (m, 2H, NCH$_2$), 1.62–1.12 (m, 12H, 6×CH$_2$), 0.83 (t, 3H, J = 6.3 Hz, CH$_2$C*H*$_3$). Anal. calcd for C$_{38}$H$_{40}$F$_3$NO$_8$: C, 65.60; H, 5.80; N, 2.01. Found: C, 65.68; H, 5.78; N, 2.05.

N-Octyl-N-trifluoroacetyl-4-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2,3,6-tri-O-benzoyl-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (14). To a stirred mixture of **13** (94 mg, 0.135 mmol), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate[5] (200 mg, 0.406 mmol) and powdered 4 A molecular sieves (100 mg) in dichloromethane (2 mL) was added 47% trifluoroborane etherate (110 μL, 0.41 mmol) at 0 °C under argon. The mixture was stirred for 30 min at room temperature, then neutralized with sodium hydrogen carbonate, and filtered through a Celite bed. The filtrate was diluted with ethyl acetate (60 mL) and the solution was washed with saturated aqueous sodium hydrogen carbonate and brine, dried, and evaporated. The residual products were purified by column chromatography on silica gel (27 g, EtOAc/toluene, gradient elution 1:11→1:9) to give **14** (26 mg, 19%) as a syrup, together with **13** (54 mg, 58%); R_f 0.18 (AcOH:toluene, 1:5); $[\alpha]_D^{23}$ −43° (*c* 1.1, CHCl$_3$); IR (neat) ν 1730 (ester), 1695 (amide) cm^{-1}; ^{1}H NMR [300 MHz, (CD$_3$)$_2$SO, 110 °C] δ 8.07–7.27 (m, 15H, 3×Ph), 6.04–5.84 (m, 1H, 2-H), 5.93 (br s, 1H, 5a-H), 5.74 (dd, 1H, $J_{2,3}$ = 8.8, $J_{3,4}$ = 8.1 Hz, 3-H), 5.11–4.81 (m, 8H, 1-H, 4-H, 6,6-H, 1′-H, 2′-H, 3′-H, 4′-H), 4.01–3.22 (m, 3H, 5′-H, 6′,6′-H), 3.40–3.22 (m, 2H, NCH$_2$), 2.00, 1.92, 1.87, and 1.85 (4 s, each 3H, 4×Ac), 1.60–1.03 (m, 12H, 6×CH$_2$), 0.82 (t, 3H, J = 6.5 Hz, CH$_2$C*H*$_3$). Anal. calcd for C$_{52}$H$_{58}$F$_3$NO$_{17}$: C, 60.87; H, 5.70; N, 1.37. Found: C, 60.44; H, 5.56; N, 1.41.

4-O-(β-D-Galactopyranosyl)-N-octyl-5a-carba-β-D-xylo-hex-5(5a)-enopyranosylamine (3). To a solution of **14** (26 mg, 0.025 mmol) in methanol (2 mL) was added potassium carbonate (60 mg, 0.43 mmol), and the mixture was stirred for 1h at room temperature. The mixture was taken up on a column of Dowex 50W×2 (H$^+$) resin and eluted with 1% methanolic ammonia to give **3** (11 mg, 73%) as a white solid; R_f 0.34 (MeOH: CHCl$_3$:H$_2$O, 35:60:8); $[\alpha]_D^{23}$ −65° (*c* 0.44, MeOH); IR (KBr) ν 3440 (OH) cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD/ CDCl$_3$, 1:2) δ 5.62 (br s, 1H, 5a-H), 4.43 (d, $J_{1′,2′}$ = 7.3 Hz, 1′-H), 4.34 and 4.02 (2 d, each 1H, J_{gem} = 13.4 Hz, 6,6-H), 4.25 (br d, 1H, $J_{3,4}$ = 7.7 Hz, 4-H), 3.92–3.47 (m, 5H, 3′-H, 4′-H, 5′-H, 6′,6′-H), 3.69 (dd, 1H, $J_{2,3}$ = 10.3 Hz, 3-H), 3.63 (dd, 1H, $J_{2′,3′}$ = 9.5 Hz, 2′-H), 3.51 (dd, 1H, $J_{1,2}$ = 8.8, 2-H), 3.23 (br d, 1H, 1-H), 2.74 and 2.53 (ddd, each 1H, J = 7.3, J_{gem} = 11.4 Hz, NCH$_2$), 1.62–1.20 (m, 12H, 6×CH$_2$), 0.89 (t, 3H, J = 6.6 Hz, CH$_2$C*H*$_3$).

Biological assay

The knockout mouse fibroblasts[10] transformed by SV-40 cDNA and expressing human β-galactosidase were cultured in serum-free Dulbecco's modified Eagle's medium (DMEM) containing 10 mM NH$_4$Cl for 24 h.[11] The culture medium was collected, dialyzed against 10 mM phosphate buffer, pH 6.5, concentrated, and used for the inhibition experiment. The enzyme activity was assayed with 4-methylumbelliferyl β-galactopyranoside as substrate as reported previously[12] in the presence of varying amounts of the inhibitor.

Acknowledgements

References and Notes

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