

Synthesis and glycosidase inhibitory activity of some N-substituted 5a-carba- β -fuco- and β -galactopyranosylamines, and selected derivatives[☆]

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Abstract—In the course of chemical modification of α -fucosidase inhibitors of 5a-carba-fucopyranosylamine type, an *N*-dodecyl derivative of the enantiomer 6-deoxy-5a-carba- β -D-galactopyranosylamine demonstrated very strong inhibition of β -galactosidase and β -glucosidase. This finding led us to synthesize corresponding 6-hydroxy compounds, in order to elucidate structure–activity relationships for inhibitors of this type. Among four *N*-alkyl-5a-carba- β -D-galactopyranosylamines prepared, the *N*-octyl derivative could be demonstrated to possess moderate activity toward α - and β -galactosidases, and β -glucosidase.

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1. Introduction

Recently, the *N*-octyl derivative¹ **2** of 5a-carba- α -fucopyranosylamine² (**1**), α -5a-CFucamine, was demonstrated to be a very strong α -L-fucosidase inhibitor (bovine kidney) with *p*-nitrophenyl- α -L-fucopyranoside, essentially comparable with deoxyfuconojirimycin (DFJ) in this regard. Although oxocarbenium ion transition-state analogs, the (5,5a)-unsaturated derivatives of **1**, were shown to possess weak activity, contrary to expectation, it is of interest to note that the β -anomer³ **3** also possessed high activity against α -L-fucosidase. These results are in contrast with the α -glucosidase inhibitory activity relationship⁴ observed between the ground-state mimic validamine, and a transition-state mimic, its unsaturated derivative, valienamine.

The present paper documents details for synthesis of some *N*-substituted derivatives⁵ of **3** with both racemic

and *L*-enantiomeric modifications, and a comparison of their inhibitory activity regarding four glycosidases. In addition, the corresponding 6-hydroxy derivatives of **3** were prepared in order to elucidate roles of C-5 substituents in displaying inhibition potential (Fig. 1).

Chemical modification of the β -anomer **3** was carried out by incorporation of hydrophobic alkyl and phenylalkyl portions into the amino group by direct treatment of the carba-sugar epoxide **13**, a newly prepared versatile precursor, with alkyl and phenylalkylamines. For convenience, rough screening of the activity of several racemic compounds **5a–f** readily available was performed, to allow estimation of the approximate enantiomeric contribution to the activity, based on comparison of data observed for *D*- and/or *L*-enantiomers (Fig. 2). The antipode of 5a-carba- β -L-fucopyranosylamine should be 6-deoxy-5a-carba- β -D-galactopyranosylamine. Therefore, inhibitory activity of the racemic modification of 5a-carba-hexopyranosylamine should be a sum of those of its components. This consideration is based on the assumption that each enantiomer does not interfere with the competitive inhibitory action of the others. For example, with racemic *DL*-**5d**, activity (IC₅₀ 3.8 μ M) against *L*-fucosidase should be largely due to the

Keywords: Glycosidase inhibitor; Galactopyranosylamine.

[☆] In this paper, the nomenclature of carba-sugar derivatives follows the IUPAC-IUB Recommendations 1996 (*Carbohydr. Res.* **1997**, 297, 1).

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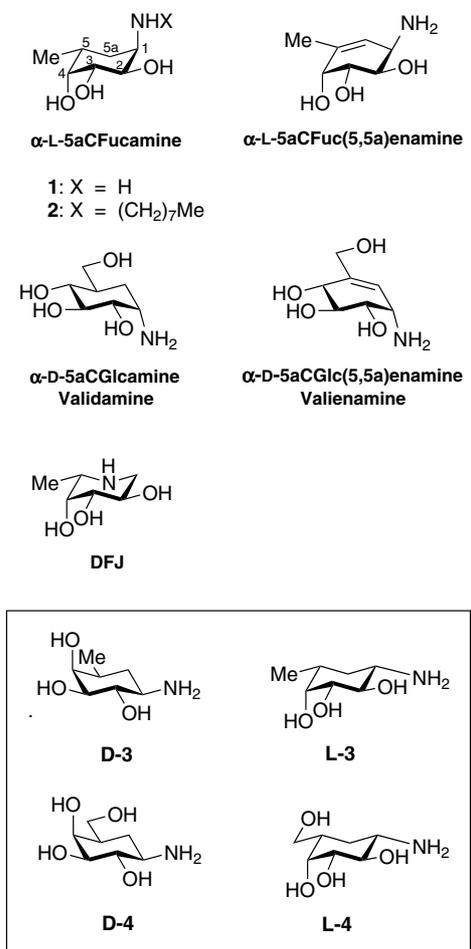


Figure 1. 5a-Carba- β -fucopyranosylamine (**3**) and β -galactopyranosylamine (**4**).

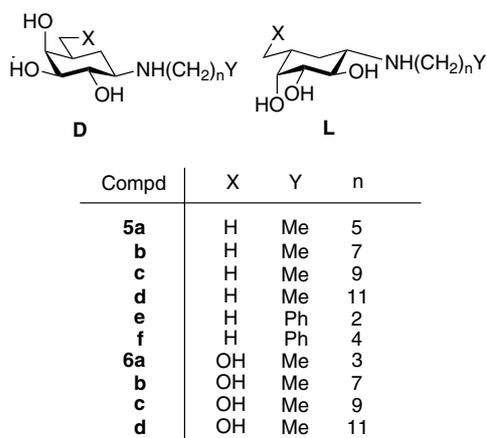


Figure 2. N-Substituted 5a-carba- β -fucopyranosylamines and β -galactopyranosylamines, assayed for inhibitory activity against seven glycosidases.

L-enantiomer, and high inhibition toward β -galactosidase (IC₅₀ 0.02 μ M) and β -glucosidase (IC₅₀ 0.50 μ M) would be probably attributable to the D-enantiomer (Table 1). This conclusion is partially supported by data

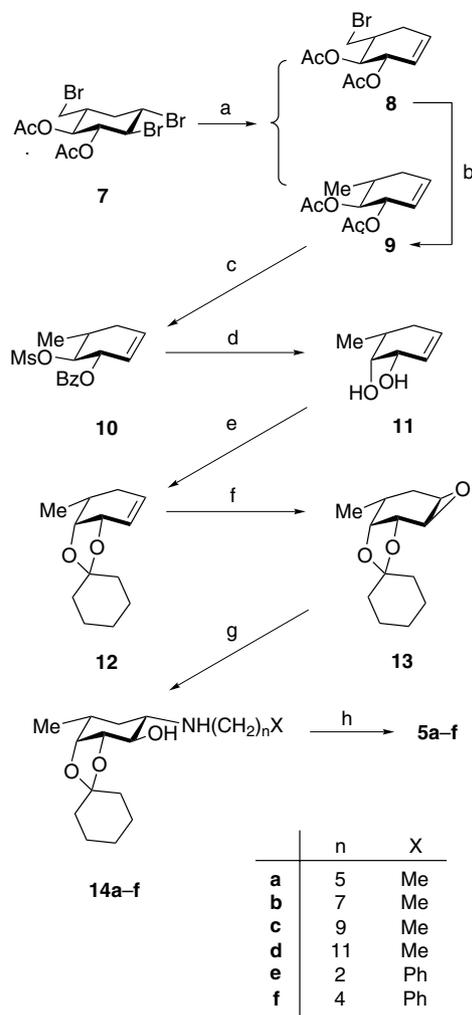
obtained for the newly synthesized optically active L-**5d** (IC₅₀ 0.91 μ M, α -fucosidase) (Table 2). Therefore, these results stimulated us to synthesize the corresponding parent 6-hydroxy compounds **6a–d** in order to elucidate structure–inhibitory activity relationships for inhibitors of this type, especially regarding the hydrophobic region due to C-5 substituents. As indicated by N-substitution¹ of the α -anomer **1**, chemical modification⁵ of the β -anomer **3** and its parent might be expected to increase its potential.

2. Results and discussion

Treatment of 3,4-di-*O*-acetyl-2,6-dibromo-2,6-dideoxy-5a-carba- β -L-glucopyranosyl bromide⁶ (L-**7**) with slight excess of zinc dust in DMF for 2 h at 80 °C gave 1,2-diacetoxy-6-bromomethyl-3-cyclohexene (D-**8**, 59%), together with the 6-debromo compound D-**9** (32%). When the reaction time was prolonged at a similar temperature, compound D-**9** was mainly obtained but in poor yield due to its simultaneous decomposition. Compound D-**8** isolated by fractionation was conventionally debrominated with tributyltinhydride in toluene in the presence of AIBN to give D-**9** (86%). Zemplén *O*-deacetylation of D-**9** with methanolic sodium methoxide, selective benzylation of the allylic hydroxyl group, and subsequent mesylation afforded the 1-mesylate D-**10** (64%). Direct nucleophilic substitution of D-**10** with an benzoate anion in DMF at 110 °C and successive deprotection with methanolic sodium methoxide gave (1*R*,2*S*,6*R*)-1,2-dihydroxy-6-methyl-3-cyclohexene L-**11** (93%). Conventional protection of L-**11** with cyclohexylidene group (\rightarrow L-**12**, 85%) and epoxidation with *m*-chloroperbenzoic acid in a phosphate buffer solution (pH \sim 6) gave the single β -epoxide L-**13** selectively in 90% yield. Rear attack of the peracid seems to be adequately restricted by the presence of a bulky cyclohexylidene group.⁷ The corresponding isopropylidene derivatives also gave similar results, but isolation of the products was somewhat difficult, compared to those for L-**12** and L-**13**.

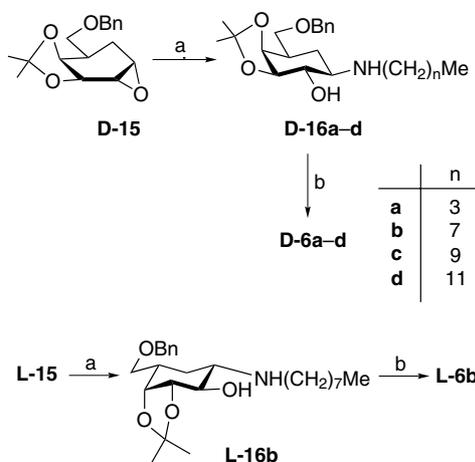
Reaction of the racemate⁸ DL-**13** with alkylamines or phenylalkylamines in 2-propanol in sealed tubes at 120 °C proceeded slowly but almost regio-selectively to afford the respective N-substituted 5a-carba- β -galactopyranosylamines DL-**14a–f**, in 70–90% yields, which, without isolation, were treated with 80% aqueous acetic acid at 80 °C, and the resulting amine acetates were purified over a column of Dowex-50W \times 2 (H⁺) resin with 1% methanolic ammonia to give the free bases DL-**5a–f** almost quantitatively. The optically active L-**5b–d,f** were similarly prepared from L-**13** (Scheme 1).

Since N-substituted 6-deoxy-5a-carba- β -galactopyranosylamines have been shown to possess strong inhibitory activity⁵ (see Section 4.28) against β -galactosidase and β -glucosidase, the findings stimulated our evaluation of inhibitory potential of the corresponding 5a-carba- β -galactopyranosylamines **4**, the structures of which more resemble those of the substrates.



Scheme 1. Synthesis of N-substituted 5a-carba- β -DL- and L-fucopyranosylamines **5a–f**. For convenience, the formulae only depict those of the L-enantiomers. Reagents and conditions: (a) Zn powder, AcOH, 80 °C; (b) Bu_3SnH , AIBN, toluene; (c) methanolic NaOMe; BzCl (1.2 molar equiv), pyridine; MsCl, DMAP, pyridine; (d) NaOBz, 90% aqueous DMF, 110 °C; methanolic NaOMe; (e) $(\text{MeO})_2\text{C}_6\text{H}_{10}$, TsOH, DMF; (f) *m*CPBA, CH_2Cl_2 , phosphate buffer; (g) alkyl or phenylalkylamines (molar equiv), 2-propanol, 120 °C, 4–10 days; (h) 80% aq AcOH, Dowex-50W \times 2 (H^+) resin, 1% aq NH_3 .

1,2-Anhydro-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- α -D-galactopyranose⁹ (**D-15**) was chosen as the synthetic precursor of *N*-alkyl-5a-carba- β -galactopyranosylamines. Diequatorial cleavage at C-1 with alkylamines was expected to occur preferentially in consideration of the previous synthesis of *N*-acetyl-5a'-carba-lactosaminide.¹⁰ Thus, a mixture of a molar equivalent of **D-15** and alkylamines was heated in a sealed tube at 120 °C for 2–3 weeks, producing the corresponding secondary amines **D-16a–d** in 50–60% yields. The protected amine **16a** was *O*-deisopropylidened with aqueous acetic acid, followed by hydrogenolysis with Pd/C, to give, after purification by an acid resin column, the amine **D-6a** (93%) as a white solid. Similarly, compounds **D-16b–d** were converted into the corresponding amines **D-6b–d**. Attempted isolation of the di-*N,O*-acetyl derivative of **D-16b** was successful, but its NMR spectrum was com-



Scheme 2. Synthesis of N-substituted 5a-carba- β -galactopyranosylamines **6a–d**. Reagents and conditions: (a) alkylamines (molar equiv), 2-propanol, 120 °C, 2–6 days; (b) 80% aq AcOH; H_2 , EtOH, 10% Pd/C, purified by a column of Dowex-50W \times 2 (H^+) resin, with 1% methanolic ammonia, or that of silica gel.

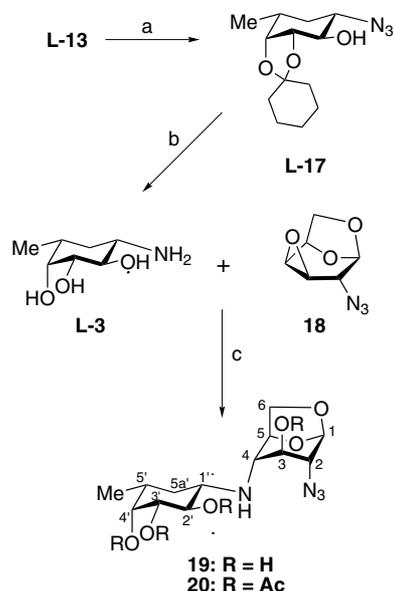
plex due to the restricted rotation around the secondary amido group, so that confirmation of the structure was rather difficult. The *N*-acetyl derivative was hydrolyzed with 1 M hydrochloric acid and the resulting hydrochloride was purified similarly to afford the amine **D-6b** (Scheme 2).

The enantiomeric epoxide **L-15** was prepared from 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-5a-carba-D-xylo-hex-1-enitol according to the standard procedure,⁹ in a 15% over-all yield. Reaction of **L-15** with a molar equivalent of octylamine gave the *N*-octyl derivative, which was deprotected usually to give **L-6b**.

Taking advantage of the readily available free base **L-3**, the *N*-linked carba-disaccharides¹¹ with carba- β -fucopyranosylamine residues were prepared in order to ascertain enzyme-inhibitory functions of the derivatives, the alkyl chains of which were replaced with sugar residues.

The epoxide **L-13** was treated with sodium azide in DMF to give a sole azide **17**, having a 5a-carba- β -fucopyranose structure. This compound was catalytically reduced with Pd/C to give, after elution through an acid column with methanolic ammonia, the free base **L-3** (72%). Coupling of **L-3** and 1,6:3,4-dianhydro-2-azido-2-deoxy- β -D-galactopyranose¹² **18** (1.6 molar equiv), in 2-propanol for two weeks 120 °C gave a single condensate **19** (93%), the structure of which was confirmed with reference to the ¹H NMR spectrum of its tetra-*O*-acetyl derivative **20** conventionally prepared (Scheme 3).

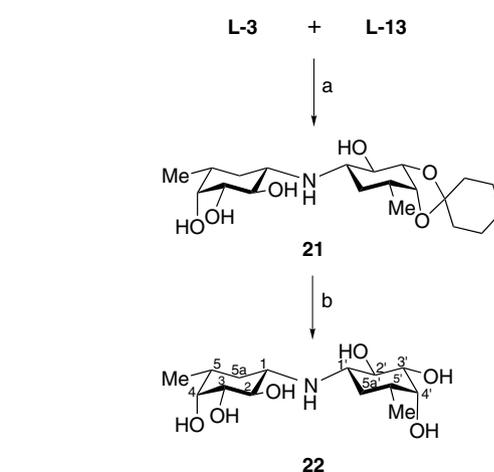
Synthesis of symmetric *N*-linked bis(5a-carba- β -fucopyranosyl) disaccharide was attempted by conventional coupling of **L-3** and **L-13**, affording the protected dicarbadisaccharide **21** (40%), which was *O*-decyclohexylidened to give a symmetric compound **22** (~100%). The structure was fully supported by ¹H NMR spectral data (Scheme 4).



Scheme 3. Synthesis of N-linked carba-disaccharide **19** containing 5a-carba- β -L-fucopyranose residue. Reagents and conditions: (a) excess NaN_3 , DMF, 110°C ; (b) H_2 , EtOH, 10% Pd/C; (c) **18** (1.6 molar equiv), 2-propanol, 120°C , two weeks.

3. Enzyme inhibitory activity

Four *N*-alkyl and two *N*-phenylalkyl derivatives **5a–f** of 5a-carba- β -fucopyranosylamine (**3**) and four *N*-alkyl derivatives **6a–d** of 5a-carba- β -galactopyranosylamine



Scheme 4. Synthesis of N-linked 5a,5a'-dicarbadisaccharide: bis(5a-carba- β -L-fucopyranosyl)amine **22**. Reagents and conditions: (a) L-13 (1.5 molar equiv), 2-propanol, 120°C , three weeks; (b) 80% aq AcOH, 80°C .

(**4**) were assayed for activity in a standard manner,¹³ against seven glycosidases: α -galactosidase (green coffee beans), β -galactosidase (bovine liver), α -glucosidase (Baker's yeast), β -glucosidase (almond), β -glucosaminidase (bovine kidney), α -mannosidase (Jack beans), and α -fucosidase (bovine kidney), as listed in Tables 1 and 2. None of the compounds were found to be inhibitors of α -glucosidase, β -glucosaminidase, or α -mannosidase.

Table 1. Inhibitory activity [IC_{50} (K_i) μM] of N-substituted 5a-carba- β -DL-fucopyranosylamines against three glycosidases

| Compound ^a | | | | Inhibitory activity, IC_{50} (K_i), (μM) | | |
|-----------------------|----|---|----|--|---------------------------------------|-------------------------------|
| <i>n</i> | X | Y | | α -Fucosidase (bovine kidney) | β -Galactosidase (bovine liver) | β -Glucosidase (almond) |
| DL-5a | 5 | H | Me | 8.2 | 3.7 | 0.73 |
| DL-5b | 7 | H | Me | 5.5 | 0.7 (0.11) | 1.5 (3.2) |
| DL-5c | 9 | H | Me | 2.7 | 0.2 (0.009) | 2.0 (0.4) |
| DL-5d | 11 | H | Me | 3.8 | 0.02 (0.0045) | 0.50 (0.53) |
| DL-5e | 2 | H | Ph | 7.5 | 5.7 | 0.38 (0.057) |
| DL-5f | 4 | H | Ph | 5.1 | 0.9 (0.046) | 1.4 (1.2) |

^a All compounds did not show any notable inhibitory activity against α -galactosidase, α -glucosidase, β -glucosaminidase, and α -mannosidase.

Table 2. Inhibitory activity (IC_{50} , μM) of 5a-carba- β -fuco- and β -galactopyranosylamines and its N-substituted derivatives against four glycosidases

| Compound ^a | | | | Inhibitory activity, IC_{50} (μM) | | | |
|-----------------------|----|----------|----|---|--|---------------------------------------|-------------------------------|
| X | Y | <i>n</i> | | α -Fucosidase (bovine kidney) | α -Galactosidase (green coffee beans) | β -Galactosidase (bovine liver) | β -Glucosidase (almond) |
| L-3 | H | H | 0 | 4.3 | NI | NI | NI |
| D-4 | OH | H | 0 | NI | 2.8 | NI | NI |
| L-5b | H | Me | 7 | 1.8 | NI | 3.7 | 15 |
| L-5c | H | Me | 9 | 0.7 | NI | 1.0 | 17 |
| L-5d | H | Me | 11 | 0.91 | NI | 0.46 | 6.1 |
| L-5f | H | Ph | 4 | 1.2 | NI | 2.4 | 10 |
| D-6a | OH | Me | 3 | NI | 39 | NI | 13 |
| D-6b | OH | Me | 7 | NI | 5.2 | 8 | 14 |
| L-6b | OH | Me | 7 | 105 | NI | 87 | 210 |
| D-6c | OH | Me | 9 | NI | 25 | 16 | 25 |
| D-6d | OH | Me | 11 | NI | 35 | 5.8 | 8.7 |

NI: no inhibition $<10^{-3}\text{M}$.

^a All compounds did not show any notable inhibitory activity against α -glucosidase, β -glucosaminidase, and α -mannosidase.

Compared to the corresponding derivatives² of the α -anomers, DL-5a–f possessed about one tenth of the inhibitory activity against α -fucosidase and the potential was actually thought to be mainly due to the β -L-fucose-mimicking enantiomers, as verified by assaying L-5b–d and L-5f newly prepared.

It is interesting to note that compounds DL-5a–f possess very strong activity toward both β -galactosidase and β -glucosidase, showing marked cross-reactivities with both enzymes as observed for aminocyclopentitol glycosidase inhibitors.^{14,15} Their high potential could be demonstrated to be largely attributable to the respective D-enantiomers, that is, N-alkyl-6-deoxy-5a-carba- β -D-galactopyranosylamines. In fact, the L-enantiomers, N-alkyl-5a-carba- β -L-fucopyranosylamines had decreased activity toward these two enzymes as shown for L-5b–d and L-5f, displaying rather potent cross-reactivities with both α -fucosidase and β -galactosidase. Among the six derivatives, compound DL-5d, actually D-5d, was the strongest inhibitor of β -galactosidase: $K_i < 4.5$ nM. Furthermore, compound DL-5e was demonstrated to be a very potent inhibitor, possessing characteristic pH-dependent activity against β -glucosidase: K_i 0.39 μ M at pH 5.5; K_i 0.057 μ M at pH 6.8. These results would indicate that optically active D-5e is an effective β -glucosidase inhibitor fully compatible with results for isofagomine¹⁶ (K_i 0.11 μ M, at pH 6.8) and calystegine¹⁷ (K_i 0.75 μ M, pH independent). Thus, N-substituted 6-deoxy-5a-carba- β -D-galactopyranosylamines may be promising lead compounds for new carba-sugar-type β -galactosidase and β -glucosidase inhibitors. The inhibitory potential of 5a-carba-glycosylamines and derivatives appears to be comparable to those of structurally related 4-amino-5-(hydroxymethyl)cyclopentane-1,2,3-triols.¹⁵ It may probably depend on close resemblance between the structures of 5a-carba-hexopyranosylamine and those of ground states of the corresponding substrates. However, in the case of 5a-carba-hexopyranosylamines, the configuration of the 4-hydroxyl function seems to be very important for the generation of activity, although it is difficult to explain rationally the fact that, the corresponding β -gluco isomers, the 4-epimers of D-5b, had rather decreased³ the activity toward β -glucosidase, in spite of close resemblance of the substrate structures.

It is noteworthy that the free bases L-3 and D-4¹⁸ were found to be not very strong but selective inhibitors against α -fucosidase and α -galactosidase, corresponding to the respective stereochemistry of the substrates (Table 2). As demonstrated for N-octyl derivative D-6b, N-alkyl-5a-carba- β -D-galactopyranosylamines are moderate α - and β -galactosidases, and β -glucosidase inhibitors, so that the N-substituents and the hydrophobic area conferred by the 5-methyl branching on the carbocyclic ring are likely to control and enhance binding to the active sites of enzymes. The L-enantiomers, as indicated by L-6b, almost lacked inhibitory activity against all enzymes.

Newly prepared carba-disaccharide-type compounds 19 and 22 only showed inhibitory activity (IC_{50} 3.9 and

39 μ M) against α -fucosidase, suggesting selectivity along the lines observed for L-3. Since, the former compound was initially designed for the purpose of providing a synthetic precursor for N-linked N-acetyl-5a'-carba-lactosaminide derivatives, it is promising that this kind of disaccharide mimic may give selective α -fucosidase inhibition through further chemical modification.

4. Experimental

4.1. General methods

Optical rotations were measured with a JASCO DIP-370 polarimeter,³ and $[\alpha]_D$ values are given in 10^{-1} deg cm² g⁻¹. ¹H NMR spectra were recorded for solutions in deuteriochloroform and deuteriomethanol with internal tetramethylsilane (TMS) as a reference with a JEOL JNM LAMBDA-300 (300 MHz) instrument. Mass spectra were determined with HITACHI M-8000 ion trap mass spectrometer. TLC was performed on silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for a column chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, 200–300 mesh) or silica gel 60 KO (Katayama Kagaku Kogyo Co., Osaka, 70–230 mesh). Organic solutions were dried over anhydrous Na₂SO₄ and concentrated at >45 °C under diminished pressure.

Unless otherwise noted, when both racemic and optically active compounds were prepared, the processing for the later were only described.

4.2. 3,4-Di-O-acetyl-1,5-anhydro-6-bromo-2,6-dideoxy-5a-carba-D-xylo-hex-1-enitol (D-8) and 3,4-di-O-acetyl-1,5-anhydro-2,6-dideoxy-5a-carba-D-xylo-hex-1-enitol [(1S,2S,3R)-1,2-di-O-acetyl-3-methylcyclohex-5-ene-1,2-diol] (D-9)

(a) 3,4-Di-O-acetyl-2,6-dibromo-2,6-dideoxy-5a-carba-L- β -glucopyranosyl bromide⁶ (L-7, 46.2 g, 0.10 mol) was dissolved in acetic acid (370 mL), to which zinc powder (33.5 g, 0.51 atom) was added. The suspension was stirred vigorously for 2 h at 80 °C. Formation of two components was observed by TLC (1:6 EtOAc/hexane). Excess zinc dust and precipitates were removed by filtration, and the filtrate was co-evaporated with toluene. The products were fractionated over a silica gel column (500 g, 1:10 EtOAc/hexane) to give D-9 (7.0 g, 32%) and D-8 (17.8 g, 59%) as a colorless syrup. Data for D-8: TLC: R_f 0.28 (1:6 EtOAc/hexane). The compound was characterized by comparison with an authentic racemate.⁶

Data for D-9: TLC: R_f 0.37 (1:6 EtOAc/hexane); $[\alpha]_D^{22} +12$ (c 1.7, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 5.80 (m, 1H, H-1), 5.44–5.51 (m, 2H, H-2, H-3), 4.94 (dd, 1H, $J_{3,4} = 7.8$ Hz, $J_{4,5} = 11.0$ Hz, H-4), 2.27 [ddd, 1H, $J_{1,5a(eq)} = J_{5,5a(eq)} = 4.3$ Hz, $J_{5agem} = 13.8$ Hz, H-5a(eq)], 2.04 and 2.08 (2s, each 3H, 2 \times Ac), 1.89–2.04 [m, 2H, H-5a(ax)], 0.97 (d, 3H, $J_{5,6} = 6.3$ Hz, H-6); ITMS-ESI (positive mode): m/z 235 [M+Na]⁺, 251 [M+K]⁺.

(b) To a solution of **D-8** (0.10 g, 0.34 mmol) in toluene (8.0 mL) were added in turn AIBN (8 mg, 0.048 mmol) and tributyltinhydride (0.27 mL, 1.0 mmol), and the mixture was stirred for 10 min at 120 °C. The reaction mixture was evaporated to dryness, and the residue was chromatographed on a silica gel column (7.0 g, hexane → 1:10 EtOAc/hexane) to give **D-9** (62 mg, 86%).

4.3. 3-*O*-Benzoyl-1,5-anhydro-2,6-dideoxy-4-*O*-mesyl-5a-carba-*D*-xylo-hex-1-enitol [(1*S*,2*S*,3*R*)-1-*O*-benzoyl-2-*O*-mesyl-3-methylcyclohex-5-ene-1,2-diol] (**D-10**)

A solution of **D-9** (0.94 g, 4.4 mmol) in methanol (5.0 mL) was treated with 1 M methanolic sodium methoxide (1.0 mL) for 1 h at room temperature, and, after neutralization with Amberlite IR-120B (H⁺) resin, concentrated to dryness. The residual diol was dissolved in dry pyridine (6.0 mL) and a solution was stirred at –15 °C, to which a solution of benzoyl chloride (0.62 mL, 5.3 mmol) in pyridine (3 mL) was added dropwise for 0.5 h. The mixture was stirred for 15 h at 0 °C. TLC (1:10 MeOH/CHCl₃) showed disappearance of the diol and formation of a new component. To the reaction mixture were added mesyl chloride (1.0 mL, 12.9 mmol) and a catalytic amount of DMAP under stirring at 0 °C, and then the mixture was stirred for 17 h at room temperature. Formation of one major and one minor components was detected by TLC. After the reaction was quenched by addition of methanol at 0 °C, the mixture was diluted with EtOAc (150 mL). The solution was washed thoroughly with 1 M hydrochloric acid, saturated aqueous NaHCO₃, and water, and dried. The organic layer was evaporated and the residue was chromatographed on a silica gel (100 g, 1:7 EtOAc/hexane) to afford **D-10** (0.87 g, 64%) as a colorless syrup: $[\alpha]_D^{22} +144$ (*c* 0.67, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.44–8.11 (m, 5H, Ph), 5.87 [dddd, 1H, *J*_{1,3} = 2.0 Hz, *J*_{1,5a(ax)} = *J*_{1,5a(eq)} = 4.9 Hz, *J*_{1,2} = 10.0 Hz, H-1], 5.77 (ddd, 1H, *J*_{1,3} = 2.0 Hz, *J*_{2,3} = 3.5 Hz, *J*_{3,4} = 7.8 Hz, H-3), 5.62 [dddd, 1H, *J*_{2,5a(ax)} = *J*_{2,5a(eq)} = 1.8 Hz, *J*_{2,3} = 3.5 Hz, *J*_{1,2} = 10.0 Hz, H-2], 4.83 (dd, 1H, *J*_{3,4} = 7.8 Hz, *J*_{4,5} = 11.2 Hz, H-4), 2.91 (s, 3H, Ms), 2.40 [ddd, 1H, *J*_{1,5a(eq)} = *J*_{5,5a(eq)} = 4.9 Hz, *J*_{5,agem} = 17.8 Hz, H-5a(eq)], 2.19 [dddd, 1H, *J*_{5,5a(eq)} = 4.9 Hz, *J*_{5,6} = 6.3 Hz, *J*_{5,5a(ax)} = 10.0 Hz, *J*_{4,5} = 11.2 Hz, H-5], 2.03 [m, 1H, H-5a(ax)], 1.20 (d, 3H, *J*_{5,6} = 6.3 Hz, H-6); ITMS-ESI (positive mode): *m/z* 333 [M+Na]⁺, 349 [M+K]⁺.

4.4. 1,5-Anhydro-2,6-dideoxy-5a-carba-*L*-lyxo-hex-1-enitol [(1*S*,2*R*,3*R*)-3-methylcyclohex-5-ene-1,2-diol] (**L-11**)

A mixture of **D-10** (0.87 g, 2.8 mmol) and sodium benzoate (2.0 g, 14 mmol) in 90% aqueous DMF (13 mL) was stirred for 15 h at 110 °C. After cooling, the mixture was diluted with ethyl acetate (150 mL) and the solution was washed with water. The organic layer was evaporated and the residue was treated with 1 M methanolic sodium methoxide (2 mL) in methanol (4 mL) for 3 h at room temperature. After neutralization with Amberlite IR-120B (H⁺) resin, the mixture was evaporated and the

residue was chromatographed on a silica gel column (30 g, 1:2 EtOAc/hexane) to give **L-11** (0.34 g, 93%) as a syrup: $[\alpha]_D^{22} +29$ (*c* 0.99, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 5.73 (br d, 1H, *J*_{1,2} = 10.3 Hz, H-1), 5.47 (br d, 1H, *J*_{1,2} = 10.3 Hz, H-2), 4.19 (br s, 1H, H-3), 3.77 (br s, 1H, H-4), 3.36 (br s, 2H, 2 × OH), 1.75–1.92 (m, 3H, H-5, 2 × H-5a), 1.03 (d, 3H, *J*_{5,6} = 6.3 Hz, H-6); ITMS-ESI (positive mode): *m/z* 151 [M+Na]⁺.

4.5. 1,5-Anhydro-3,4-*O*-cyclohexylidene-2,6-dideoxy-5a-carba-*L*-lyxo-hex-1-enitol (**L-12**)

A mixture of the triol **L-11** (2.00 g, 15.6 mmol), 1,1-dimethoxycyclohexane (10 mL), TsOH·H₂O (0.53 g, 3 mmol), and DMF (40 mL) was stirred for 2 h at room temperature. The mixture was then neutralized with triethylamine and evaporated to dryness. The product was purified with a silica gel column (20 g, 1:100 EtOAc/hexane) to give **L-12** (2.83 g, 85%) as a syrup, TLC: *R*_f 0.47 (1:10 EtOAc/hexane); $[\alpha]_D^{24} +37$ (*c* 0.9, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 5.71 [br ddd, 1H, *J*_{1,5a(ax)} = 2.0 Hz, *J*_{1,5a(eq)} = 2.4 Hz, *J*_{1,2} = 6.1 Hz, H-1], 5.51 (br d, 1H, *J*_{1,2} = 6.1 Hz, H-2), 4.44 (m, 1H, H-3), 4.08 (br d, 1H, *J*_{4,5} = 5.2 Hz, H-4), 1.77–1.85 (m, 2H, 2 × H-5a), 1.75 (br dd, 1H, *J*_{4,5} = 5.2 Hz, *J*_{5,6} = 6.3 Hz, H-5), 1.42 (m, 10H, C₆H₁₀), 1.05 (d, 3H, *J*_{5,6} = 6.3 Hz, Me); ITMS-APCI (positive mode): *m/z* 209 [M+H]⁺.

4.6. 1,2-Anhydro-3,4-*O*-cyclohexylidene-5a-carba- α -*L*-fucopyranose (**L-13**)

To a stirred suspension of compound **L-12** (2.83 g, 13.3 mmol) and a mixture of 1 M aqueous NaH₂PO₄ (1.0 mL) and 1 M aqueous Na₂HPO₄ (1.0 mL) in dichloromethane (42 mL), was added *m*CPBA (2.98 g, 17.2 mmol), and it was agitated vigorously for 3 h at 0 °C. The mixture was then diluted with chloroform (600 mL), and the solution was thoroughly washed successively with saturated aqueous sodium thiosulfate, sodium hydrogen carbonate, and water, dried, and evaporated. The residue was chromatographed on a silica gel column (30 g, 1:80 → 1:60 EtOAc/hexane) to give the epoxide **L-13** (1.57 g, 90%) as a crystalline solid, TLC: *R*_f 0.67 (1:10 EtOAc/hexane); $[\alpha]_D^{24} +19$ (*c* 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 4.31 (br d, 1H, *J*_{3,4} = 5.6 Hz, H-3), 3.98 (br d, 1H, *J*_{3,4} = 5.6 Hz, H-4), 3.23 (br s, 1H, H-1), 2.97 (br d, 1H, *J*_{2,3} = 3.2 Hz, H-2), 1.84 (m, 3H, H-5, 2 × H-5a), 1.25–1.78 (m, 10H, C₆H₁₀), 1.04 (d, 3H, *J*_{5,6} = 4.4 Hz, Me); ITMS-APCI (positive mode): *m/z* 225 [M+H]⁺.

4.7. *N*-Hexyl-5a-carba- β -DL-fucopyranosylamine (**DL-5a**)

A mixture of the epoxide **DL-13** (50 mg, 0.22 mmol), hexylamine (28.7 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for four days at 120 °C, and then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 100:1 → 20:1 CHCl₃/MeOH) to give the protected amine **DL-14a** (47 mg, 66%). It was treated with 80% aqueous acetic acid (1.0 mL) for 2 h at 80 °C, and the mixture was evaporated. The product was purified on a column of Dow-

ex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **DL-5a** (16 mg, 92%) as a white powder, TLC: R_f 0.57 (5:1 $CHCl_3/MeOH$); 1H NMR (300 MHz, CD_3OD): δ 3.80 (br s, 1H, H-1), 3.59 (dd, 1H, $J_{1,2} = J_{2,3} = 9.5$ Hz, H-2), 3.43 (br d, 1H, $J_{2,3} = 9.5$ Hz, H-3), 2.75 (dt, 1H, $J = 7.1$ Hz, $J_{gem} = 11.0$ Hz, $NHCH_2$), 2.39–2.51 (m, 2H, $NHCH_2$, H-1), 1.59–1.70 [m, 2H, H-5, H-5a(eq)], 1.45–1.51 (m, 2H, $NHCH_2CH_2$), 1.29–1.36 [m, 7H, $(CH_2)_3CH_3$, H-5a(ax)], 1.04 (d, 3H, $J_{5,6} = 6.5$ Hz, H-6), 0.88 (t, 3H, $J = 6.6$ Hz, CH_2CH_3); ITMS-ESI (positive mode): m/z 246 $[M+H]^+$.

4.8. N-Octyl-5a-carba- β -L-fucopyranosylamine (L-5b)

A mixture of the epoxide **L-13** (50 mg, 0.22 mmol), octylamine (36.1 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for four days at 120 $^\circ$ C, and then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 100:1 \rightarrow 20:1 $CHCl_3/MeOH$) to give the protected amine **L-14b** (47 mg, 66%). It was treated with 80% aqueous acetic acid (1.0 mL) for 3 h at 80 $^\circ$ C, and evaporated. The product was purified on a column of Dowex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **L-5b** (33 mg, \sim 100%) as a white powder, TLC: R_f 0.58 (5:1 $CHCl_3/MeOH$); $[\alpha]_D^{24} +21$ (c 1.4, MeOH); 1H NMR (300 MHz, CD_3OD): δ 3.80 (br s, 1H, H-4), 3.59 (dd, 1H, $J_{1,2} = J_{2,3} = 9.5$ Hz, H-2), 3.21 (br d, 1H, $J_{2,3} = 9.5$ Hz, H-3), 2.75 (dt, 1H, $J = 7.1$ Hz, $J_{gem} = 11.0$ Hz, $NHCH_2$), 2.39–2.51 (m, 2H, H-1, $NHCH_2$), 1.59–1.70 [m, 2H, H-5, H-5a(eq)], 1.29–1.36 [m, 7H, $(CH_2)_3CH_3$, H-5a(ax)], 1.04 (d, 3H, $J_{5,6} = 6.5$ Hz, H-6), 0.88 (t, 3H, $J = 6.6$ Hz, CH_2CH_3); ITMS-ESI (positive mode): m/z 274 $[M+H]^+$.

4.9. N-Decyl-5a-carba- β -L-fucopyranosylamine (L-5c)

A mixture of the epoxide **L-13** (50 mg, 0.22 mmol), decylamine (42.9 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for eight days at 120 $^\circ$ C, and then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 100:1 \rightarrow 20:1 $CHCl_3/MeOH$) to give the protected amine **L-14c** (53 mg, 88%). It was treated with 80% aqueous acetic acid (1.0 mL) for 3 h at 80 $^\circ$ C, and evaporated. The product was purified on a column of Dowex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **L-5c** (24 mg, \sim 100%) as a white powder, TLC: R_f 0.60 (5:1 $CHCl_3/MeOH$), $[\alpha]_D^{24} +1.4$ (c 0.8, MeOH); 1H NMR (300 MHz, CD_3OD): δ 3.61 (br s, 1H, H-4), 3.45 (dd, 1H, $J_{2,3} = 9.3$ Hz, $J_{1,2} = 9.5$ Hz, H-2), 3.21 (br d, 1H, $J_{2,3} = 9.3$ Hz, H-3), 2.73 (dt, 1H, $J = 7.3$ Hz, $J_{gem} = 11.5$ Hz, $NHCH_2$), 2.46–2.60 (m, 2H, H-1, $NHCH_2$), 1.45–1.57 [m, 4H, $NHCH_2CH_2$, H-5, H-5a(eq)], 1.21–1.34 [m, 15H, $(CH_2)_7CH_3$, H-5a(ax)], 1.05 (d, 3H, $J_{5,6} = 6.3$ Hz, Me); ITMS-ESI (positive mode): m/z 302 $[M+H]^+$.

4.10. N-Dodecyl-5a-carba- β -L-fucopyranosylamine (L-5d)

A mixture of the epoxide **L-13** (50 mg, 0.22 mmol), dodecylamine (40.0 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for 10 days at 120 $^\circ$ C, and

then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 80:1 \rightarrow 20:1 $CHCl_3/MeOH$) to give the protected amine **L-14d** (68 mg, 93%). It was treated with 80% aqueous acetic acid (1.0 mL) for 4 h at 80 $^\circ$ C, and evaporated. The product was purified on a column of Dowex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **L-5d** (35 mg, 69%) as a white powder, TLC: R_f 0.69 (5:1 $CHCl_3/MeOH$); $[\alpha]_D^{24} +0.2$ (c 1.4, $CHCl_3$); 1H NMR (300 MHz, CD_3OD): δ 3.70 (br s, 1H, H-4), 3.48 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{1,2} = 9.7$ Hz, H-2), 3.29 (dd, 1H, $J_{3,4} = 3.0$ Hz, $J_{2,3} = 9.6$ Hz, H-3), 2.69 (dt, 1H, $J = 7.3$ Hz, $J_{gem} = 11.2$ Hz, $NHCH_2$), 2.36–2.51 (m, 2H, H-1, $NHCH_2$), 1.49–1.66 [m, 4H, H-5, H-5a(eq), $NHCH_2CH_2$], 1.28–1.40 [m, 19H, $(CH_2)_9CH_3$, H-5a(ax)], 1.01 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6), 0.89 (t, 3H, $J = 6.6$ Hz, CH_2CH_3); ITMS-ESI (positive mode): m/z 330 $[M+H]^+$.

4.11. N-(2-Phenylethyl)-5a-carba- β -DL-fucopyranosylamine (DL-5e)

A mixture of the epoxide **DL-13** (50 mg, 0.22 mmol), 2-phenylethylamine (27.5 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for five days at 120 $^\circ$ C, and then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 100:1 \rightarrow 20:1 $CHCl_3/MeOH$) to give the protected amine **DL-14e** (55 mg, 72%). It was treated with 80% aqueous acetic acid (1.0 mL) for 3 h at 80 $^\circ$ C, and evaporated. The product was purified on a column of Dowex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **DL-5e** (10 mg, 50%) as a white powder, TLC: R_f 0.60 (5:1 $CHCl_3/MeOH$); 1H NMR (300 MHz, CD_3OD): δ 7.16–7.31 (m, 5H, Ph), 3.69 (br s, 1H, H-4), 3.50 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{1,2} = 9.7$ Hz, H-2), 3.30 (br d, 1H, $J_{2,3} = 9.6$ Hz, H-3), 2.82–3.06 [m, 4H, $NH(CH_2)_2$], 2.53 [ddd, 1H, $J_{1,5a(eq)} = 2.2$ Hz, $J_{1,2} = 9.7$ Hz, $J_{1,5a(ax)} = 11.9$ Hz, H-1], 1.59–1.68 [m, 2H, H-5, H-5a(eq)], 1.39 [m, 1H, H-5a(ax)], 1.02 (d, 3H, $J_{5,6} = 6.6$ Hz, H-6); ITMS-ESI (positive mode): m/z 266 $[M+H]^+$.

4.12. N-(4-Phenylbutyl)-5a-carba- β -L-fucopyranosylamine (L-5f)

A mixture of the epoxide **L-13** (50 mg, 0.22 mmol), 4-phenylbutylamine (34.5 μ L, 0.22 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for six days at 120 $^\circ$ C, and then evaporated. The residue was chromatographed on a silica gel column (5.0 g, 100:1 \rightarrow 20:1 $CHCl_3/MeOH$) to give the protected amine **L-14f** (55 mg, 66%). It was treated with 80% aqueous acetic acid (1.0 mL) for 3 h at 80 $^\circ$ C, and evaporated. The product was purified on a column of Dowex-50W \times 2 (H^+) resin (1.0 g, 1% aqueous NH_3) to give the amine **L-5f** (10 mg, 54%) as a white powder, TLC: R_f 0.60 (5:1 $CHCl_3/MeOH$); $[\alpha]_D^{24} +0.4$ (c 1.5, CD_3OD); 1H NMR (300 MHz, $CDCl_3$): δ 7.04–7.17 (m, 5H, Ph), 3.60 (br s, 1H, H-4), 3.38 (dd, 1H, $J_{1,2} = 9.3$ Hz, $J_{2,3} = 9.5$ Hz, H-2), 3.18 (br d, 1H, $J_{2,3} = 9.5$ Hz, H-3), 2.29–2.69 [m, 5H, $NH(CH_2)_2$, H-1], 1.45–1.62 [m, 6H, $(CH_2)_2Ph$, H-5, H-5a(eq)], 1.24 [br dd, 1H, $J_{1,5a(ax)} = 8.7$ Hz, $J_{5agem} = 12.6$ Hz, H-5a(ax)], 0.92 (d, 3H, $J_{5,6} = 6.5$ Hz, H-6); ITMS-ESI (positive mode): m/z 294 $[M+H]^+$.

4.13. *N*-Butyl-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- β -D-galactopyranosylamine (D-16a)

A mixture of 1,2-anhydro-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- α -D-galactopyranose⁹ **D-15** (53 mg, 0.18 mmol), butylamine (18 μ L, 0.18 mmol), and 2-propanol was heated in a sealed tube for two days at 120 °C, and then evaporated to dryness. The products were chromatographed on a silica gel column (7 g, CHCl₃ \rightarrow 20:1 CHCl₃/MeOH) to give the amine **D-16a** (25 mg, 38%) as a syrup, together with **D-15** (13 mg, 25%) recovered, $[\alpha]_D^{24}$ -3.8 (*c* 0.26, MeOH); ¹H NMR (300 MHz, CDCl₃): δ 7.27–7.35 (m, 5H, Ph), 4.54 (s, 2H, CH₂Ph), 4.29 (dd, 1H, $J_{4,5} = 4.2$ Hz, $J_{3,4} = 4.5$ Hz, H-4), 3.89 (dd, 1H, $J_{3,4} = 4.5$ Hz, $J_{2,3} = 7.6$ Hz, H-3), 3.47 and 3.64 (dd, each 1H, $J_{5,6} = 7.3$ Hz, $J_{6gem} = 9.0$ Hz, H-6a, H-6b), 3.41 (dd, 1H, $J_{2,3} = 7.6$ Hz, $J_{1,2} = 10.7$ Hz, H-2), 2.68 (br s, 1H, OH), 2.46 and 2.77 (2dt, each 1H, $J = 7.2$ Hz, $J_{gem} = 11.1$ Hz, NHCH₂), 2.32 [ddd, 1H, $J_{1,5a(eq)} = 3.1$ Hz, $J_{1,2} = 10.7$ Hz, $J_{1,5a(ax)} = 11.6$ Hz, H-1], 2.05–2.17 (m, 1H, H-5), 1.97 [ddd, 1H, $J_{1,5a(eq)} = 3.1$ Hz, $J_{5,5a(eq)} = 3.4$ Hz, $J_{5agem} = 12.5$ Hz, H-5a(eq)], 1.35 and 1.49 (2s, each 3H, CMe₂), 1.25–1.52 (m, 4H, H-2', H-3'), 1.14 [ddd, 1H, $J_{1,5a(ax)} = 11.6$ Hz, $J_{5,5a(ax)} = 12.2$ Hz, $J_{5agem} = 12.5$ Hz, H-5a(ax)], 0.91 (t, 3H, $J_{3',4'} = 7.2$ Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 364 [M+H]⁺.

4.14. *N*-Octyl-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- β -D-galactopyranosylamine (D-16b) and *N*-octyl-5a-carba- β -D-galactopyranosylamine (D-6b)

A mixture of the epoxide **D-15** (98 mg, 0.34 mmol), octylamine (50 μ L, 0.30 mmol), and 2-propanol was heated in a sealed tube for two days at 120 °C, and then evaporated to dryness. The products were chromatographed on a silica gel column (10 g, 60:1 CHCl₃/MeOH) to give the protected amine **D-16b** (73 mg, 51%) as a syrup and **D-15** (41 mg, 42%) recovered. Compound **D-16b** (73 mg, 0.17 mmol) was treated with acetic anhydride (0.36 mL) and pyridine (0.73 mL) overnight at room temperature.

The reaction mixture was evaporated, the residue was diluted with ethyl acetate (20 mL), and the solution was washed with 1 M hydrochloric acid, saturated aqueous NaHCO₃, and water, dried, and evaporated. The residue was chromatographed on silica gel (9 g, 2:3 EtOAc/hexane) to give the *N,O*-acetyl derivative (70 mg, 82%) of **D-16b**.

Hydrolysis of the *N,O*-acetyl derivative (70 mg, 0.14 mmol) with 1 M hydrochloric acid for 6 h at 100 °C gave, after chromatography on Dowex-50W \times 2 (H⁺) resin with 1% methanolic ammonia as eluent, the amine **D-6b** (38 mg, 71%) as a white powder, $[\alpha]_D^{24}$ -36 (*c* 0.44, MeOH); ¹H NMR (300 MHz, CD₃OD): δ 3.97 (br s, 1H, H-4), 3.63 (dd, 1H, $J_{1,2} = 7.3$ Hz, $J_{2,3} = 10.7$ Hz, H-2), 3.50 (dd, 1H, $J_{3,4} = 6.3$ Hz, $J_{2,3} = 10.7$ Hz, H-3), 3.28 and 3.51 (2dd, each 1H, $J_{5,6} = 2.7$ Hz, $J_{6gem} = 9.5$ Hz, H-6a, H-6b), 2.47 and 2.71 (2dt, each 1H, $J_{1',2'} = 7.5$ Hz, $J_{gem} = 11.1$ Hz, NHCH₂), 2.41 [br dd, 1H, $J_{1,2} = 7.3$ Hz,

$J_{1,5a(ax)} = 9.6$ Hz, H-1], 1.73 [ddd, 1H, $J_{1,5a(eq)} = 3.0$ Hz, $J_{5,5a(eq)} = 3.5$ Hz, $J_{5agem} = 12.2$ Hz, H-5a(eq)], 1.49–1.66 (m, 3H, H-5, H-2'), 1.24–1.46 [m, 11H, H-5a(ax), (CH₂)₅CH₃], 0.89 (t, 3H, $J_{7',8'} = 6.6$ Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 290 [M+H]⁺.

4.15. 1,2-Anhydro-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- α -L-galactopyranose (L-15)

This compound was prepared from 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-5a-carba-D-xylo-hex-1-enitol: $[\alpha]_D^{23} +22$ (*c* 0.7, CHCl₃); ITMS-ESI (positive mode); *m/z* 293 [M+Na]⁺, 309 [M+K]⁺, according to the standard four-step reactions,⁹ $[\alpha]_D^{23} +28$ (*c* 0.8, CHCl₃); ITMS-APCI (positive mode): *m/z* 291 [M+H]⁺.

4.16. *N*-Octyl-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- β -L-galactopyranosylamine (L-16b)

A mixture of the epoxide **L-15** (52 mg, 0.19 mmol), octylamine (30.2 μ L, 0.19 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for six days at 120 °C, and then evaporated to dryness. The residue was chromatographed on a silica gel column (8.0 g, 50:1 \rightarrow 10:1 CHCl₃/MeOH) to give the amine **L-16b** (50 mg, 63%) as a pale yellow syrup, TLC: *R*_f 0.58 (1:3 EtOH/toluene); $[\alpha]_D^{24} +10.6$ (*c* 0.79, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.26–7.35 (m, 5H, Ph), 4.54 (br s, 1H, H-4), 4.28 (dd, 1H, $J_{4,5} = 3.9$ Hz, $J_{3,4} = 4.4$ Hz, H-4), 3.89–3.93 (m, 1H, H-3), 3.62–3.67 (m, 1H, H-6a), 3.45–3.51 (m, 2H, H-2, H-6b), 2.51 and 2.82 (2dt, each 1H, $J = 4.1$ Hz, $J_{gem} = 11.0$ Hz, NHCH₂), 2.39 [br dd, 1H, $J_{1,5a(eq)} = 3.2$ Hz, $J_{1,2} = 8.5$ Hz, H-1], 2.13 [br dd, 1H, $J_{4,5} = J_{5,5a(eq)} = 3.9$ Hz, H-5], 1.99 [br dd, 1H, $J_{1,5a(eq)} = 3.2$ Hz, $J_{5,5a(eq)} = 3.9$ Hz, H-5a(eq)], 1.35 and 1.49 [2s, each 3H, CMe₂], 1.18–1.32 [m, 11 H, (CH₂)₅CH₃, H-5a(ax)], 0.88 (t, 3H, $J = 6.5$ Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 420 [M+H]⁺.

4.17. *N*-Decyl-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- β -D-galactopyranosylamine (D-16c)

A mixture of the epoxide **D-15** (54 mg, 0.18 mmol), decylamine (40 μ L, 0.20 mmol), and 2-propanol was heated in a sealed tube for two days at 120 °C, and then evaporated to dryness. The products were chromatographed on a silica gel column (8 g, CHCl₃ \rightarrow 25:1 CHCl₃/MeOH) to give the amine **D-16c** (49 mg, 60%) as a syrup, $[\alpha]_D^{20} -4.2$ (*c* 0.85, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 7.30–7.35 (m, 5H, Ph), 4.54 (s, 2H, CH₂Ph), 4.29 (dd, 1H, $J_{4,5} = 4.2$ Hz, $J_{3,4} = 4.5$ Hz, H-4), 3.90 (dd, 1H, $J_{3,4} = 4.5$ Hz, $J_{2,3} = 7.7$ Hz, H-3), 3.47 and 3.64 (dd, each 1H, $J_{5,6} = 7.5$ Hz, $J_{6gem} = 8.5$ Hz, H-6a, H-6b), 3.44 (dd, 1H, $J_{2,3} = 7.7$ Hz, $J_{1,2} = 10.4$ Hz, H-2), 3.29 (br s, 1H, OH), 2.48 and 2.78 (2dt, each 1H, $J = 7.3$ Hz, $J_{gem} = 11.2$ Hz, NHCH₂), 2.35 [ddd, 1H, $J_{1,5a(eq)} = 2.9$ Hz, $J_{1,2} = 10.4$ Hz, $J_{1,5a(ax)} = 11.4$ Hz, H-1], 2.05–2.18 (m, 1H, H-5), 1.98 [ddd, 1H, $J_{1,5a(eq)} = 2.9$ Hz, $J_{5,5a(eq)} = 3.4$ Hz, $J_{5agem} = 12.5$ Hz, H-5a(eq)], 1.35 and 1.49 (2s, each 3H, CMe₂), 1.10–1.52 [m, 17H, H-5a(ax), H-2', H-9'], 0.88 (t, 3H, $J_{9',10'} = 6.5$ Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 448 [M+H]⁺.

4.18. *N*-Dodecyl-6-*O*-benzyl-3,4-*O*-isopropylidene-5a-carba- β -D-galactopyranosylamine (**D-16d**)

A mixture of the epoxide **D-15** (48 mg, 0.17 mmol), dodecylamine (31 mg, 0.17 mmol), and 2-propanol was heated in a sealed tube for two days at 120 °C, and then evaporated to dryness. The products were chromatographed on a silica gel column (8 g, CHCl₃ → 25:1 CHCl₃/MeOH) to give the amine **D-16d** (58 mg, 74%) as a syrup, $[\alpha]_D^{24}$ -4.1 (*c* 0.33, MeOH); ¹H NMR (300 MHz, CDCl₃): δ 7.29–7.35 (m, 5H, Ph), 4.54 (s, 2H, CH₂Ph), 4.29 (dd, 1H, *J*_{4,5} = 4.2 Hz, *J*_{3,4} = 4.5 Hz, H-4), 3.90 (dd, 1H, *J*_{3,4} = 4.5 Hz, *J*_{2,3} = 7.6 Hz, H-3), 3.47 and 3.64 (dd, each 1H, *J*_{5,6} = 7.2 Hz, *J*_{gem} = 9.0 Hz, H-6a, H-6b), 3.38 (dd, 1H, *J*_{2,3} = 7.6 Hz, *J*_{1,2} = 10.7 Hz, H-2), 2.22 (br s, 1H, OH), 2.45 and 2.76 (2dt, each 1H, *J* = 7.2 Hz, *J*_{gem} = 11.1 Hz, NHCH₂), 2.30 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{1,2} = 10.7 Hz, *J*_{1,5a(ax)} = 12.2 Hz, H-1], 2.05–2.17 (m, 1H, H-5), 1.98 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{5,5a(eq)} = 3.4 Hz, *J*_{5agem} = 12.5 Hz, H-5a(eq)], 1.44–1.46 (m, 2H, H-2'), 1.35 and 1.49 (2s, each 3H, CMe₂), 1.25 [br m, 18H, (CH₂)₉CH₃], 1.12 [ddd, 1H, *J*_{1,5a(ax)} = 12.2 Hz, *J*_{5,5a(ax)} = 12.2 Hz, *J*_{5agem} = 12.5 Hz, H-5a(ax)], 0.88 (t, 3H, *J*_{11',12'} = 6.7 Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 476 [M+H]⁺.

4.19. *N*-Butyl-5a-carba- β -D-galactopyranosylamine (**D-6a**)

A mixture of **D-16a** (14 mg, 40 μ mol) and 80% aqueous acetic acid (2 mL) was stirred for 16 h at 80 °C, and then evaporated to dryness. The residue was hydrogenated in ethanol (2 mL) in the presence of 10% Pd/C under atmospheric pressure of hydrogen for 3 h at room temperature. A catalyst was removed by filtration, and the filtrate was evaporated. The residual product was chromatographed on a column of Dowex-50W \times 2 (H⁺) resin (0.9 g, 1% methanolic NH₃) to give the amine **D-6a** (8.6 mg, 93%) as a white powder, $[\alpha]_D^{20}$ +20 (*c* 0.42, MeOH); ¹H NMR (300 MHz, CD₃OD): δ 3.97 (br, 1H, H-4), 3.63 (dd, 1H, *J*_{1,2} = 7.3 Hz, *J*_{2,3} = 10.7 Hz, H-2), 3.50 (dd, 1H, *J*_{3,4} = 6.3 Hz, *J*_{2,3} = 10.7 Hz, H-3), 3.28 and 3.51 (dd, 1H, *J*_{5,6} = 2.7 Hz, *J*_{gem} = 9.5 Hz, H-6a, H-6b), 2.47 and 2.71 (2dt, each 1H, *J* = 7.5 Hz, *J*_{gem} = 11.1 Hz, NHCH₂), 2.41 [br dd, 1H, *J*_{1,2} = 7.3 Hz, *J*_{1,5a(ax)} = 9.6 Hz, H-1], 1.73 [ddd, 1H, *J*_{1,5a(eq)} = 3.0 Hz, *J*_{5,5a(eq)} = 3.5 Hz, *J*_{5agem} = 12.2 Hz, H-5a(eq)], 1.49–1.66 (m, 3H, H-5, H-2'), 1.24–1.46 [m, 11H, H-5a(ax), (CH₂)₅CH₃], 0.89 (t, 3H, *J*_{7',8'} = 6.6 Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 234 [M+H]⁺.

4.20. *N*-Octyl-5a-carba- β -L-galactopyranosylamine (**L-6b**)

A mixture of **L-16b** (52 mg, 0.19 mmol) and 80% aqueous acetic acid (1.0 mL) was stirred for 2 h at 80 °C, and then evaporated to dryness. The residue was dissolved in ethanol (1.0 mL) and the solution was hydrogenated in the presence of a catalytic amount of 10% Pd-C in the atmospheric pressure of hydrogen for 2 h at room temperature. The product was chromatographed on a silica

gel column (3 g, 50:1 → 10:1 CHCl₃/MeOH) to give the amine **L-6b** (14.5 mg, 62%) as a pale yellow syrup, TLC: *R*_f 0.28 (1:2 EtOH/toluene); $[\alpha]_D^{24}$ +37 (*c* 0.89, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ 3.97 (br s, 1H, H-4), 3.64 (dd, 1H, *J*_{5,6a} = 7.4 Hz, *J*_{gem} = 10.4 Hz, H-6a), 3.47–3.55 (m, 2H, H-3, H-6b), 3.27–3.34 (m, 1H, H-2), 2.72 (dt, 1H, *J* = 3.9 Hz, *J*_{gem} = 11.3 Hz, NHCH₂), 2.39–2.53 (m, 2H, H-1, NHCH₂), 1.71–1.78 (m, 1H, H-5), 1.47–1.63 [m, 3H, NHCH₂CH₂, H-5a(eq)], 1.32 [m, 11H, (CH₂)₅CH₃, H-5a(ax)], 0.89 (t, 3H, *J* = 6.4 Hz, CH₂CH₃); ITMS-APCI (positive mode): *m/z* 290 [M+H]⁺.

4.21. *N*-Decyl-5a-carba- β -D-galactopyranosylamine (**D-6c**)

A mixture of **D-16c** (38 mg, 86 μ mol) and 80% aqueous acetic acid (2 mL) was stirred for 4 h at 80 °C, and then evaporated to dryness. The residue was hydrogenated in ethanol (2 mL) in the presence of 10% Pd/C under atmospheric pressure of hydrogen for 3 h at room temperature. A catalyst was removed by filtration, and the filtrate was evaporated. The residual product was chromatographed on a column of Dowex-50W \times 2 (H⁺) resin (2.7 g, 1% methanolic NH₃) to give the amine **D-6c** (20 mg, 73%) as a white powder, $[\alpha]_D^{24}$ ~0 (*c* 0.28, MeOH); ¹H NMR (300 MHz, CD₃OD): δ 3.97 (br, 1H, H-4), 3.52 (dd, 1H, *J*_{1,2} = 9.0 Hz, *J*_{2,3} = 9.2 Hz, H-2), 3.50 and 3.64 (dd, each 1H, *J*_{5,6} = 7.2 Hz, *J*_{gem} = 10.6 Hz, H-6), 3.29 (dd, 1H, *J*_{3,4} = 2.8 Hz, *J*_{2,3} = 9.2 Hz, H-3), 2.48 and 2.72 (2dt, each 1H, *J* = 7.1 Hz, *J*_{gem} = 13.3 Hz, NHCH₂), 2.43 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{1,2} = 9.0 Hz, *J*_{1,5a(ax)} = 11.2 Hz, H-1], 1.74 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{5,5a(eq)} = 3.7 Hz, *J*_{5agem} = 12.2 Hz, H-5a(eq)], 1.57–1.67 (m, 1H, H-5), 1.52 (br t, 1H, *J*_{2',3'} = 6.2 Hz, H-2'), 1.24–1.37 [m, 15H, (CH₂)₇CH₃, H-5a(ax)], 0.89 (t, 3H, *J*_{9',10'} = 6.6 Hz, CH₂CH₃); ITMS-ESI (positive mode): *m/z* 318 [M+H]⁺.

4.22. *N*-Dodecyl-5a-carba- β -D-galactopyranosylamine (**D-6d**)

A mixture of **D-16d** (49 mg, 0.12 mmol) and 80% aqueous acetic acid (2 mL) was stirred for 16 h at 80 °C, and then evaporated to dryness. The residue was hydrogenated in ethanol (2 mL) in the presence of 10% Pd/C under atmospheric pressure of hydrogen for 3 h at room temperature. A catalyst was removed by filtration, and the filtrate was evaporated. The residual product was chromatographed on a column of Dowex-50W \times 2 (H⁺) resin (3.6 g, 1% methanolic NH₃) to give the amine **D-6d** (25 mg, 69%) as a white powder, $[\alpha]_D^{24}$ -45 (*c* 0.17, MeOH); ¹H NMR (300 MHz, CD₃OD): δ 3.97 (br, 1H, H-4), 3.47–3.55 (m, 2H, H-2, H-6), 3.64 (dd, 1H, *J*_{5,6} = 7.2 Hz, *J*_{gem} = 10.6 Hz, H-6), 3.29 (dd, 1H, *J*_{3,4} = 2.8 Hz, *J*_{2,3} = 9.5 Hz, H-3), 2.48 and 2.72 (2dt, each 1H, *J* = 7.4 Hz, *J*_{gem} = 11.4 Hz, NHCH₂), 2.42 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{1,2} = 9.5 Hz, *J*_{1,5a(ax)} = 9.8 Hz, H-1], 1.74 [ddd, 1H, *J*_{1,5a(eq)} = 3.1 Hz, *J*_{5,5a(eq)} = 3.5 Hz, *J*_{5agem} = 12.2 Hz, H-5a(eq)], 1.57–1.67 (m, 1H, H-5), 1.52 (br t, 2H, *J*_{2',3'} = 6.2 Hz, H-2'), 1.28–1.36 [m, 19H, (CH₂)₉CH₃, H-5a(ax)], 0.89 (t, 3H,

$J_{11',12'} = 6.7\text{ Hz}$, CH_2CH_3); ITMS-ESI (positive mode): m/z 346 $[\text{M}+\text{H}]^+$.

4.23. 3,4-*O*-Cyclohexylidene-5a-carba- β -L-fucopyranosyl azide (**L-17**)

A mixture of the epoxide **L-13** (152 mg, 0.66 mmol) and sodium azide (650 mg, 25 molar equiv) in DMF (3.0 mL) was stirred for two days at 110 °C. The reaction mixture was then diluted with ethyl acetate (60 mL), and the solution was washed with saline and water, dried, and evaporated to dryness. The residue was chromatographed on silica gel (18 g, 1:6 ethyl acetate/hexane) to give **L-17** (174 mg, 98%) as a solid, TLC: R_f 0.58 (1:3 ethyl acetate/hexane); $[\alpha]_D^{24} -3.1$ (c 0.39, MeOH); ^1H NMR (300 MHz, CDCl_3): δ 4.07 (t, 1H, $J_{4,5} = 3.9\text{ Hz}$, $J_{3,4} = 4.7\text{ Hz}$, H-4), 3.89 (dd, 1H, $J_{3,4} = 4.7\text{ Hz}$, $J_{2,3} = 7.4\text{ Hz}$, H-3), 3.51 (dd, 1H, $J_{2,3} = 7.4\text{ Hz}$, $J_{1,2} = 10.3\text{ Hz}$, H-2), 3.25 [ddd, 1H, $J_{1,5a(\text{eq})} = 4.0\text{ Hz}$, $J_{1,5a(\text{ax})} = 8.0\text{ Hz}$, $J_{1,2} = 10.3\text{ Hz}$, H-1], 2.78 (s, 1H, OH), 2.14 (s, 3H, Ac), 1.93 [dddd, 1H, $J_{5,5a(\text{eq})} = 3.4\text{ Hz}$, $J_{4,5} = 3.9\text{ Hz}$, $J_{5,6} = 6.8\text{ Hz}$, $J_{5,5a(\text{ax})} = 9.0\text{ Hz}$, H-5], 1.37–1.96 [m, 12H, C_6H_{10} , 2 \times H-5a], 1.15 (d, 3H, $J_{5,6} = 6.8\text{ Hz}$, Me); ITMS-APCI (positive mode): m/z 268 $[\text{M}+\text{H}]^+$.

Data for the 2-*O*-acetyl derivative: TLC: R_f 0.40 (1:5 ethyl acetate/hexane); ^1H NMR (300 MHz, CDCl_3): δ 5.01 (dd, 1H, $J_{2,3} = 7.8\text{ Hz}$, $J_{1,2} = 10.7\text{ Hz}$, H-2), 4.08 (dd, 1H, $J_{3,4} = J_{4,5} = 4.0\text{ Hz}$, H-4), 3.96 (dd, 1H, $J_{3,4} = 4.0\text{ Hz}$, $J_{2,3} = 7.8\text{ Hz}$, H-3), 3.28 [ddd, 1H, $J_{1,5a(\text{eq})} = 3.9\text{ Hz}$, $J_{1,5a(\text{ax})} = 8.0\text{ Hz}$, $J_{1,2} = 10.7\text{ Hz}$, H-1], 2.14 (s, 3H, Ac), 1.79 [ddd, 1H, $J_{1,5a(\text{eq})} = J_{5,5a(\text{eq})} = 4.0\text{ Hz}$, $J_{1,2} = 10.7\text{ Hz}$, H-5a(eq)], 1.63 [m, 2H, H-5, H-5a(ax)], 1.19–2.01 (m, 10H, C_6H_{10}), 1.17 (d, 3H, $J_{5,6} = 6.3\text{ Hz}$, Me).

4.24. 5a-Carba- β -L-fucopyranosylamine (**L-3**)

A solution of **L-17** (250 mg, 0.94 mmol) in ethanol (5 mL) was hydrogenolyzed in the presence of a catalytic amount of 10% Pd/C for 4 h at room temperature. The product was *O*-decyclohexylidened similarly to give, after purification by acid resin column to give the amine **L-3** (109 mg, 72%) as a white powder, $[\alpha]_D^{24} -2.2$ (c 0.29, MeOH); ^1H NMR (300 MHz, D_2O): δ 3.77 (br s, 1H, H-4), 3.40 (m, 2H, H-2, H-3), 2.78 (m, 1H, H-1), 1.71 (m, 1H, H-5), 1.62 [ddd, 1H, $J_{1,5a(\text{eq})} = J_{5,5a(\text{eq})} = 4.0\text{ Hz}$, $J_{5a(\text{gem})} = 12.7\text{ Hz}$, H-5a(eq)], 1.30 [ddd, 1H, $J_{1,5a(\text{ax})} = J_{5,5a(\text{ax})} = J_{5a(\text{gem})} = 12.7\text{ Hz}$, H-5a(ax)], 0.92 (d, 3H, $J_{5,6} = 6.8\text{ Hz}$, H-6); ITMS-APCI (positive mode): m/z 162 $[\text{M}+\text{H}]^+$.

4.25. 1,6-Anhydro-2-azido-4-(5a-carba- β -L-fucopyranosyl-1-yl)amino-2,4-dideoxy- β -D-glucopyranose (**19**)

A mixture of **L-3** (11.8 mg, 0.07 mmol) and 1,6:3,4-dianhydro-2-azido-2-deoxy- β -D-galactopyranose¹² (**18**, 18.6 mg, 0.11 mmol) in 2-propanol (1 mL) was heated for two weeks at 120 °C, and then evaporated to dryness. The residue was chromatographed on a silica gel (2.0 g, 1:10 MeOH/ CHCl_3) to give the amine **19** (22.5 mg, 93%) as a syrup: $[\alpha]_D^{24} -37$ (c 0.18, MeOH);

^1H NMR (300 MHz, CD_3OD): δ 5.38 (br s, 1H, H-1), 4.44 (br d, 1H, H-5), 4.09 (br d, 1H, H-6a), 3.82 (br s, 1H, H-3), 3.61–3.67 (m, 2H, H-6b, H-4'), 3.52 (br s, 1H, H-2), 3.17–3.35 (m, 2H, H-2', H-3'), 2.66 (br s, 1H, H-4), 2.46–2.54 (m, 1H, H-1'), 1.51–1.74 (m, 2H, H-5', H-5a'eq), 1.05 (dd, 1H, $J_{\text{gem}} = 13.4\text{ Hz}$, $J_{5,5a'ax} = 12.0\text{ Hz}$, H-5a'ax), 0.82 (d, 3H, $J_{5,6} = 6.6\text{ Hz}$, H-6); ITMS-APCI (positive mode): m/z 331 $[\text{M}+\text{H}]^+$.

Compound **19** (11.4 mg) was converted into the acetyl derivative by treatment with acetic anhydride (0.5 mL) and pyridine (1.0 mL) for 10 h at room temperature. The product was chromatographed on a silica gel (1.0 g, 1:7 acetone/hexane) to give the tetra-*O*-acetyl derivative **20** (13.5 mg, 78%) as a colorless syrup: ^1H NMR (300 MHz, CD_3OD): δ 5.44 (br s, 1H, H-1), 5.31 (br s, 1H, H-4'), 5.19 (dd, 1H, $J_{1',2'} = J_{2',3'} = 10.0\text{ Hz}$, H-2'), 4.83 (br s, 1H, H-2), 4.56 (br s, 1H, H-5), 4.09 (br d, 1H, $J_{\text{gem}} = \sim 7.0\text{ Hz}$, H-6a), 3.82 (br dd, 1H, H-6b), 3.51 (br s, 1H, H-3), 3.02–3.10 (m, 1H, H-1'), 2.71 (br s, 1H, H-4), 2.14, 2.12, 2.09, and 1.97 (4s, each 3H, 4 \times Ac), 1.75–1.82 [m, 2H, H-5, H-5a(eq)], 1.48–1.55 [m, 1H, H-5a(ax)], 0.93 (d, 3H, $J_{5,6} = 6.6\text{ Hz}$, H-6); ITMS-APCI (positive mode): m/z 499 $[\text{M}+\text{H}]^+$.

4.26. *N*-(5a-Carba- β -L-fucopyranosyl-1-yl)-3,4-*O*-cyclohexylidene-5a-carba- β -L-fucopyranosylamine (**21**)

A mixture of **L-3** (13.2 mg, 0.08 mmol) and **L-13** (28.2 mg, 0.12 mmol) in 2-propanol (1 mL) was heated in a sealed tube for three weeks at 120 °C, and then evaporated to dryness. The residual product was eluted from a column of silica gel (1.5 g, 1:10 MeOH/ CHCl_3) to give **21** (12.9 mg, 40%) as a colorless syrup: ^1H NMR (300 MHz, CD_3OD): δ 3.99–4.02 (m, 1H, H-4'), 3.73–3.78 (br d, 1H, $J_{3',4'} = 4.4\text{ Hz}$, H-3'), 3.60 (br s, 1H, H-4), 3.20–3.37 (m, 3H, H-2, H-3, H-2'), 2.37–2.48 (m, 2H, H-1, H-1'), 1.79–1.85 (m, 1H, H-5'), 1.26–1.68 [m, 13H, C_6H_{10} , H-5, H-5a(eq), H-5a'eq], 1.16–1.22 (m, 1H, H-5a'ax), 1.12 [br d, 1H, $J_{5,5a(\text{ax})} = 4.1\text{ Hz}$, H-5a(ax)], 1.01 (d, 3H, $J_{5',6'} = 6.8\text{ Hz}$, H-6'), 0.92 (d, 3H, $J_{5,6} = 6.6\text{ Hz}$, H-6).

4.27. *N*-(5a-Carba- β -L-fucopyranosyl)-5a-carba- β -L-fucopyranosylamine (**22**)

Compound **21** (42 mg, 0.11 mmol) was treated with 80% aqueous acetic acid (0.5 mL) for 5 h at 80 °C, and then co-evaporated with ethanol and toluene. The residue was chromatographed on a column of Dowex-50W \times 2 (H^+) (1.0 g, 1% aqueous NH_3) to give **22** (12.2 mg, $\sim 100\%$) as a white powder: $[\alpha]_D^{24} +16$ (c 0.38, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 3.68 (br s, 2H, H-4, H-4'), 3.46 (dd, 2H, $J_{1,2} = J_{1',2'} = 9.3\text{ Hz}$, $J_{2,3} = J_{2',3'} = 9.7\text{ Hz}$, H-2, H-2'), 3.32 (dd, 2H, $J_{3,4} = J_{3',4'} = 2.5\text{ Hz}$, $J_{2,3} = J_{2',3'} = 9.7\text{ Hz}$, H-3, H-3'), 2.80 (ddd, 2H, $J_{1,5a(\text{eq})} = J_{1',5a'eq} = 3.9\text{ Hz}$, $J_{1,2} = J_{1',2'} = 9.3\text{ Hz}$, $J_{1,5a(\text{ax})} = J_{1',5a'ax} = 12.2\text{ Hz}$, H-1, H-1'), 1.57–1.69 [m, 4H, H-5, H-5', H-5a(eq), H-5a'eq], 1.22 [br dd, 2H, $J_{1,5a(\text{ax})} = J_{1',5a'ax} = 12.2\text{ Hz}$, $J_{5a(\text{ax}),5a(\text{eq})} = J_{5a'ax,5a'eq} = 12.4\text{ Hz}$, H-5a(ax), H-5a'ax], 0.84 (d, 6H, $J_{5,6} = J_{5',6'} = 6.6\text{ Hz}$, H-6, H-6'); ITMS-ESI (positive mode): m/z 306 $[\text{M}+\text{H}]^+$.

4.28. Biological assay

(a) Determination of IC_{50} : the IC_{50} values were determined for all inhibitors and correspond to the inhibition concentration required for 50% inhibition of the enzyme in our experimental conditions. α -Fucosidase (bovine kidney) was assayed using *p*-nitrophenyl α -L-fucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 37°C. α -Galactosidase (green coffee beans) was assayed using *p*-nitrophenyl α -D-galactopyranoside as a substrate at pH 6.8 (20 mM, phosphate buffer) at 27°C. β -Galactosidase (bovine liver) assayed similarly using *p*-nitrophenyl β -galactopyranoside at 37°C. α -Glucosidase (Baker's yeast) was assayed similarly using *p*-nitrophenyl α -D-glucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27°C. β -Glucosidase (almond) was assayed similarly using *p*-nitrophenyl β -D-glucopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27°C. β -Glucosaminidase (bovine kidney) was assayed using *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 37°C. α -Mannosidase was assayed using *p*-nitrophenyl α -D-mannopyranoside (4 mM) as a substrate at pH 5.5 (30 mM CH_3COONa) at 27°C.

Test compound was added to each reaction mixture described above and it was incubated for 1 h at 27 or 37°C. After 1 h, the reaction was quenched by addition of three volumes of aqueous 0.2 M sodium carbonate, and the absorbance of the liberated *p*-nitrophenol was measured at 410 nm. The percentage inhibition was calculated by the formula $BA \times 100$, where *A* is the *p*-nitrophenol liberated by the enzyme without an inhibitor and *B* is that with an inhibitor.

(b) Determination of K_i : the K_i values were determined for especially potent inhibitors using Dixon graphical method. The effects of pH on the inhibition were investigated for DL-5e and reference inhibitor calystegine C,¹⁷ at pH 5.5 and 6.8, in order to compare their K_i values with those reported for isofagomine.¹⁶ The following substrate concentrations were applied: 4, 2, 1, and 0.5 mM, and the inhibitor concentrations: 0.25, 0.1, 0.05, and 0.025 $\mu\text{g mL}^{-1}$ or 0.025, 0.01, 0.005, and 0.0025 $\mu\text{g mL}^{-1}$.

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