dichloroethane and K_2CO_3. Under these iodide-free conditions, the reaction was successful and a 71% yield was recorded (Table 2, entry 4). In the case of scaled-up conditions, the iodide-free potassium salt of **1a** became accessible via an alternative method by careful observation during hydrolysis. When iodolactone **5** was hydrolyzed in ethanol, first, a precipitate appeared. This was proven by NMR analysis to be a potassium salt of iodohydrin carboxylic, an intermediate acid. If the hydrolysis was continued under prolonged heating at 70 °C, the precipitate disappeared. The resulting potassium salt and all of the by-products were soluble in ethanol. Then the reaction mixture was dried onto silica gel, and elution with methanol provided the desired carboxylate salt. Most of the contaminant, especially inorganic salts such as KI, was removed by adsorption on silica gel.

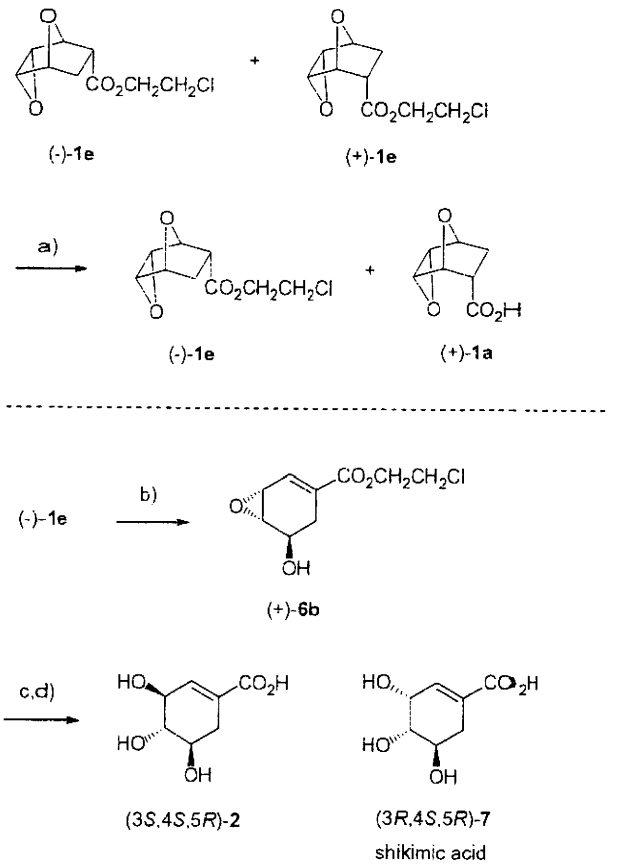
The PLE catalyzed hydrolysis in the scaled-up experiment proceeded in a reproducible manner, and (+)-**1a** (54.7% yield) and (−)-**1e** (42.7% yield) were obtained with only an extractive work-up separation. In this case, both ees of (+)-**1a** (90.6% ee) and (−)-**1e** (99.4% ee) were unambiguously determined (see Section 4) ($E = 116$). The simple recrystallization of acid (+)-**1a** from EtOAc enhanced the enantiomeric excess to 96.4%. It is noteworthy that acid (+)-**1a** has been reported as the starting material for oseltamivir phosphate by Terashima and Ujita.[12]

Our product, epoxyester **1e**, has the same level of oxygen functionality in suitable positions as shikimic acid and related compounds. Naturally occurring shikimic acid (3*R*,4*S*,5*R*)-**7** is essential for the industrial synthesis of (−)-oseltamivir phosphate.[13,14] The process for fermentative production has already been established,[15,16] as (3*R*,4*S*,5*R*)-**7** is a biosynthetic key intermediate for the well-known shikimate pathway. An epimeric form, (3*S*,4*S*,5*R*)-**2** (3-epishikimic acid), has recently gained attention as the starting material for vitamin D_3 precursors,[17] the synthon of natural products,[18,19] and as a template for combinatorial synthesis.[20] The availability of this epi-form, however, is very low due to no direct biosynthetic pathway. An attempted acid-catalyzed epimerization under harsh conditions only results in a stereoisomeric mixture with parent (3*R*,4*S*,5*R*)-shikimic acid **7**, accompanied with the dehydrated 4-hydroxybenzoic acid.[18] Otherwise, a tedious multi-step conversion was required, involving selective protection of 4,5-*trans* diol and inversion at C-3.[19]

The above-mentioned situation prompted us to establish a route from (−)-**1e** to epishikimate (3*S*,4*S*,5*R*)-**2**. Epoxy ester (−)-**1e** was submitted to LHMDS-mediated β-elimination[2] to give (+)-**6b** (Scheme 6). It is noteworthy that the desired reaction occurred in as high as 94% yield without any damage on the 2-chloroethyl ester, which would also suffer from β-elimination. For the transformation of **6b** to (3*S*,4*S*,5*R*)-**2**, the electron-withdrawing property of the chloroethyl group was quite advantageous. The alkaline hydrolysis of **6b** proceeded very smoothly, and the following stereoselective epoxide ring opening[21] gave (3*S*,4*S*,5*R*)-**2a** (79.7%); $[\alpha]_D^{25} = -33.1$ (*c* 0.34, H_2O) {lit.[21] $[\alpha]_D = -31.0$ (*c* 0.1, H_2O)}; whose spectroscopic data coincided with those reported previously.[21]

3. Conclusion

Based on the pig liver esterase-catalyzed kinetic resolution of 2-chloroethyl 3,8-dioxatricyclo[3.2.1.$0^{2,4}$]octane-6-carboxylate **1e**, an expeditious route for polyhydroxylated cyclohexenoids has been established. The design of the substrate structure was supported by conformational analysis and fitness in an enzyme catalytic site model. The reaction conditions for the synthesis of optimized substrate, excluding the formation of by-products necessary to simplify the workup procedure, which is indispensable for preparative-scale synthesis have been elucidated. 3-Epishikimic acid should be a more promising starting material in organic synthesis following our establishment of a scalable supply.



Scheme 6. Derivation of hydrolyzate to (3*S*,4*S*,5*R*)-3-epishikimic acid **2a**. Reagents and conditions: (a) pig liver esterase, 0.2 M phosphate buffer (pH 7.0), [42.7% for (1*R*,2*S*,4*R*,5*S*,6*R*)-(−)-**1e** (99.4% ee)], [54.7% for (1*S*,2*R*,4*S*,5*R*,6*S*)-(+)-**1a** (90.6% ee)]; (b) LHMDS, THF, −78 °C, 1 h, (96%); (c) KOH, THF, H_2O, 50 °C, 1 h; (d) TFA, H_2O, 50 °C, 3 h (79.7%).

4. Experimental

4.1. Materials and methods

Merck Silica Gel 60 F_{254} thin-layer plates (1.05744, 0.5 mm thickness) and Silica Gel 60 (spherical and neutral; 100–210 μm, 37560-79) from Kanto Chemical Co. were used for preparative thin-layer chromatography and column chromatography, respectively. The commercial PLE preparation was purchased from Sigma.

4.2. Analytical methods

All melting points are uncorrected. IR spectra were measured as thin films for oils or ATR for solid on a Jeol FT-IR SPX60 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ or D_2O at 270 MHz on a Jeol JNM EX-270 or at 400 MHz on a Jeol JNM GX-400 spectrometer or at 400 MHz on a VARIAN 400-MR spectrometer, and ^{13}C NMR spectra were measured in $CDCl_3$ or D_2O at 100 MHz on a Jeol GX-400 spectrometer or at 100 MHz on a VARIAN 400-MR spectrometer. High resolution mass spectra were recorded on a Jeol JMS-700 MStation spectrometer. HPLC data were recorded on Jasco MD-2010 multi-channel detectors and SHIMADZU SPD-M20A diode array detector. Optical rotation values were recorded on a Jasco P-1010 polarimeter. Silica Gel 60 (spherical, 100–210 μm, 37558-79) of Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with E. Merck Silica Gel 60 F_{256} plates (0.5 mm thickness, No. 5744).

4.3. Screening of hydrolytic enzymes

The screening of hydrolytic enzymes were performed as follows. A 2 mL sample tube was charged with an appropriate amount of racemic ethyl ester **1b** (10.0 mg) and potassium phosphate buffer (0.2 M, 0.25 mL, pH 7.0) at room temperature for 24 h in the presence of several lipases and protease at an amount of 80–100 mg/mL of phosphate buffer, in the case of pig liver esterase 0.2 mg/mL of phosphate buffer. The progress of the reaction was monitored by TLC analysis [silica gel, developed with hexane–EtOAc (1:1)]. The reaction mixture was quenched with citric acid to pH 2, and extracted with EtOAc. The combined organic phases were dried over Na_2SO_4 and concentrated in vacuo. Among the seven enzymes tested, only pig liver esterase showed the progress of hydrolysis.

4.3.1. (±)-7-*endo*-Oxabicyclo[2.2.1]hept-5-carboxylic acid 3

The known procedure[5] was slightly modified in regard to the reaction temperature. Furan (250 mL) and acrylic acid (250 mL) were mixed and kept for 6 days at room temperature, for 17 days at 4 °C, and then for 28 days at 7 °C. The precipitated solids were recovered by filtration to give carboxylic acid (±)-**3** (41.7 g, *endo*:*exo* = 8:2) as a colorless solid, mp 95–96 °C, lit.[6] mp 97–100 °C. To the above mentioned filtrate was added furan and acrylic acid and kept for one month at 7 °C to give another crop of crystal. The NMR spectrum was identical with that reported previously.[1,6]

4.3.2. (±)-(1*R**,2*R**,3*R**,6*R**,7*S**)-2-Iodo-4,8-dioxatricyclo[4.2.1.0^{3,7}]-nonan-5-one 5

To a solution of the acid (±)-**3** (20.0 g, 142 mmol) in $NaHCO_3$ aq solution (300 mL) was added dropwise a solution of I_2 (40.0 g, 157 mmol) in THF (80 mL) under ice-cooling, and the mixture was stirred for 68 h at room temperature. To the mixture was added saturated $Na_2S_2O_3$ aq solution, and the precipitates were collected in filtration to give crude iodolactone (±)-**5** (25.1 g). The filtration was extracted with EtOAc (three times). The organic layer was washed with brine, dried over Na_2SO_4 and concentrated in vacuo, to give another crop of iodolactone (±)-**5** (2.00 g) as the sample for NMR measurement. Its NMR spectrum was identical with that reported previously.[1] The combined yield of above-mentioned (±)-**5** (27.0 g) was 71%, and this was employed for the next step without further purification.

4.3.3. (±)-3,8-Dioxatricyclo[3.2.1.0^{2,4}]octane-6-carboxylic acid 1a

To a solution of iodolactone (±)-**5** (1.01 g, 3.80 mmol) in DMF (15 mL) was added KOH (0.54 g, 9.98 mmol) and stirred for 24 h at room temperature. After removal of water in vacuo, the residue was added 1 M HCl to pH 2. The mixture was extracted with EtOAc (10 times), and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (6:1) to afford carboxylic acid (±)-**1a** (193 mg, 33.5%) as a colorless solid, mp 153–154 °C. Its NMR spectrum was identical with that reported previously.[12]

4.3.4. Ethyl (±)-3,8-dioxatricyclo[3.2.1.0^{2,4}]octane-6-carboxylate 1b

To a solution of iodolactone (±)-**5** (0.51 g, 1.87 mmol) in DMF (10 mL) was added with KOH (0.37 g, 6.60 mmol) and stirred for 24 h at room temperature. After removal of water in vacuo, the residue was dissolved anhydrous DMF. The mixture was added EtI (0.96 g, 6.56 mmol) at 40 °C, and stirred for 6 h. After removal of volatile materials in vacuo, the reaction was quenched with NH_4Cl aq solution, and extracted with EtOAc (three times). The combined organic phases were washed with brine and dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography with hexane–EtOAc (2:1) to afford ethyl ester (±)-**1b** (316 mg, 91.8%) as a colorless solid, mp 52—53 °C. Its NMR spectrum was identical with that reported previously.[12]

4.3.5. Methyl (±)-3,8-dioxatricyclo[3.2.1.0^{2,4}]octane-6-carboxylate 1c

In a similar manner as described for **1b**, a solution of iodolactone (±)-**5** (0.80 g, 3.01 mmol) in DMF (10 mL) was treated with KOH (0.40 g, 7.13 mmol) and MeI (1.28 g, 6.56 mmol) to give methyl ester (±)-**1c** (390 mg, 78.3%) as a colorless solid; mp 75 °C. Its NMR spectrum was identical with that reported previously.[2]

4.3.6. 2-Chloroethyl (±)-3,8-dioxatricyclo[3.2.1.0^{2,4}]octane-6-carboxylate 1e

In a similar manner as described for **1b**, a solution of iodolactone (±)-**5** (266 mg, 1.00 mmol) in DMF (3 mL) was treated with KOH (132 mg, 2.35 mmol) and $ClCH_2CH_2I$ (300 mg, 1.58 mmol), to give 2-chloroethyl ester (±)-**1e** (171 mg, 78.5%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.97 (1H, ddd, J = 4.9, 11.3, 11.3 Hz, H-7$_{exo}$), 2.07 (1H, dd, J = 4.9, 11.3 Hz, H-7$_{endo}$), 2.93 (1H, dt, J = 4.9, 11.3 Hz, H-6), 3.69 (2H, t, J = 5.7 Hz, CH_2Cl), 4.01 (1H, dd, J = 2.4, 4.3 Hz, H-4), 4.11 (1H, dd, J = 2.2, 4.3 Hz, H-2), 4.32 (1H, dt, J = 5.7, 11.3 Hz, C*H*HCH$_2$Cl, Ha in Fig. 1), 4.44 (1H, dt, J = 5.7, 11.3 Hz, CH*H*CH$_2$Cl, Hb in Fig. 1), 4.50 (1H, dt, J = 2.2, 4.9 Hz, H-5), 4.70 (1H, dt, J = 2.2, 4.9 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.5, 41.8, 44.7, 64.1, 66.6, 66.7, 77.5, 78.2, 171.0; IR ν_{max} 3006, 2962, 2358, 1732, 1449, 1334, 1207, 1176, 883 cm^{-1}. Anal. Calcd for $C_9H_{11}ClO_4$: C, 49.44; H, 5.07. Found: C, 49.19; H, 5.04.

This ester was also able to be prepared by the action of $ClCH_2CH_2Cl$. To a solution of the acid (±)-**1a** (15.6 mg, 0.10 mmol) in anhydrous DMF was added K_2CO_3 (55.2 mg, 0.40 mmol) and $ClCH_2CH_2Cl$ (59.4 mg, 0.60 mmol), and the mixture was stirred at 40 °C for 24 h. The same workup as above provided (±)-**1e** (16.5 mg, 75.7%).

4.3.7. Scaled-up and preparative synthesis of 1e

A solution of iodolactone (±)-**5** (3.20 g, 12.0 mmol) in EtOH (20 mL) was added KOH (2.00 g, 35.6 mmol) and the mixture was stirred for 5 h at 70 °C. After removal of volatile materials in vacuo, the residue was re-dissolved in MeOH. To the mixture was added silica gel (50 g), and stirred for 30 min. After concentration in vacuo, the residual solid was charged on a glass column, and that was eluted with ethanol to give carboxylic acid (±)-**1a** (86.4 mg) as a colorless solid. Further elution with MeOH afforded potassium salt (±)-**1a** (2.40 g).

To a solution of the above potassium salt (±)-**1a** (2.07 g) in DMF (10 mL) was added $ClCH_2CH_2Cl$ (5.28 g, 53.4 mmol), and the mixture was stirred at 60 °C for 24 h. The same workup provided (±)-**1e** (1.55 g, 71.1%).

4.3.8. Carbamylmethyl (±)-3,8-dioxatricyclo[3.2.1.0^{2,4}]octane-6-carboxylate 1f

In a similar manner as described for **1b**, a solution of iodolactone (±)-**5** (0.80 g, 3.01 mmol) in DMF (5 mL) was treated with KOH (0.40 g, 7.13 mmol) and $ClCH_2CONH_2$ (0.84 g, 8.98 mmol), gave carbamylmethyl ester (±)-**1f** (423 mg, 65.9%) as a colorless solid. Further purification by recrystallization from EtOAc afforded (±)-**1f**: mp 131.0–133.0 °C: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.03 (1H, ddd, J = 4.6, 11.2, 11.6 Hz, H-7$_{exo}$), 2.09 (1H, dd, J = 4.3, 11.6 Hz, H-7$_{endo}$), 3.02 (1H, dt, J = 4.6, 11.2 Hz, H-6), 4.11 (1H, dd, J = 1.9, 4.4 Hz, H-4), 4.18 (1H, dd, J = 2.2, 4.4 Hz, H-2), 4.55 (1H, dt, J = 2.2, 4.6 Hz, H-5), 4.62 (1H, d, J = 15.6 Hz, C*H*HCONH$_2$, Hc in Fig. 1), 4.69 (1H, d, J = 15.6 Hz, CH*H*CONH$_2$, Hd in Fig. 1), 4.77 (1H, dt, J = 1.9, 4.4 Hz, H-1), 5.81 (1H, br s, NH$_2$), 6.69 (1H, br s,

NH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.6, 44.7, 62.8, 67.0, 67.7, 77.4, 78.1, 169.9, 170.4; IR ν_{max} 3400, 3190, 2958, 1753, 1680, 1417, 1306, 1197 cm^{-1}. Anal. Calcd for $C_9H_{11}NO_5$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.53; H, 5.15; N, 6.47.

4.3.9. 2,2,2-Trifluoroethyl (±)-3,8-dioxatricyclo[3.2.1.0^{2,4}]-octane-6-carboxylate 1g

A mixture of carboxylic acid (±)-**1a** (156 mg, 1.00 mmol), DMAP (245 mg, 2.00 mmol), EDC-Cl (384 mg, 2.00 mmol), CF_3CH_2OH (150 mg, 1.50 mmol), and triethylamine (202 mg, 2.00 mmol) in DMF (1 mL) was stirred at room temperature under argon. The reaction was monitored by silica gel TLC, developed with hexane–EtOAc (1:4). After stirring for 10 h at room temperature, the mixture was quenched by the addition of EtOAc–water. The organic materials were extracted with EtOAc, and the combined organic phases were washed with brine and dried over Na_2SO_4. The organic phase was concentrated in vacuo. The residue was purified by silca gel column chromatography with hexane–EtOAc (1:1) to afford trifluoroethyl ester (±)-**1g** (192 mg, 80.6%) as a colorless oil; ^1H NMR (270 MHz, $CDCl_3$): δ = 1.93 (1H, ddd, J = 4.6, 11.3, 11.6 Hz, H-7$_{exo}$), 2.04 (1H, dd, J = 4.3, 11.6 Hz, H-7$_{endo}$), 2.94 (1H, dt, J = 4.6, 11.3 Hz, H-6), 3.98 (1H, dd, J = 2.4, 4.6 Hz, H-4), 4.05 (1H, dd, J = 2.2, 4.6 Hz, H-2), 4.44 (1H, dddd, J = 8.4, 12.7 Hz, CH_2CF_3), 4.53 (1H, dt, J = 1.9, 4.9 Hz, H-5), 4.58 (1H, dddd, J = 8.4, 12.7 Hz, CH_2CF_3), 4.69 (1H, dt, J = 1.9, 4.9 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.5, 44.4, 60.2, 60.6, 66.5, 66.6, 77.4, 77.5, 121.5, 124.3, 169.7; IR ν_{max} 3010, 2969, 2368, 2337, 1747, 1411,1276, 1155, 879 cm^{-1}. HRMS (EI): calcd for $C_9H_9F_3O_4$: [M^-]: 238.0453; found: m/z = 238.0453.

4.3.10. PLE-catalyzed hydrolysis of esters 1b–1g

The hydrolysis of each substrate was carried out under the same conditions as described for the screening of enzymes with ethyl ester **1b**. The E-value of the each substrate was uniformly calculated from the conversion and ee(P) as follows. The conversion was determined by ^1H NMR analysis of crude reaction mixture. Ee(P) was determined by the HPLC analysis at the stage of **6a**, after methylation of hydrolyzate and following β-elimination as described later.

4.3.11. PLE-catalyzed hydrolysis of 2-chloroethyl ester 1e

To a stirred solution of 2-chloroethyl ester (±)-**1e** (373.3 mg, 1.71 mmol) in a phosphate buffer (0.2 M, pH 7.0; 8.5 mL), PLE (Sigma, E2884, 850 μL) was added and the mixture was stirred for 24 h at room temperature. The reaction was quenched with 1 M HCl to pH 2, and extracted with EtOAc (10 times). The combined organic layer was dried over Na_2SO_4 and concentrated in vacuo, and the ratio between unreacted recovery **1e** and hydrolyzate **1a** was determined by ^1H NMR measurement. The above mentioned crude mixture was washed with saturated $NaHCO_3$ aq solution. The organic layer was washed with brine and dried over Na_2SO_4, concentrated in vacuo to give (−)-**1e** (159.3 mg, 0.73 mmol) as the unreacted recovery. The aqueous layer was acidified to pH 3 and extracted with EtOAc (10 times). The extract was dried over Na_2SO_4 and concentrated in vacuo to give (+)-**1a** (145.4 mg, 0.93 mmol, mp 107–108 °C). These samples were employed for the next step without further purification.

Ester (−)-**1e**: $[\alpha]_D^{23}$ = −5.3 (c 1.02, $CHCl_3$), 99.4% ee as shown below. Its IR and NMR spectra were in good accordance with those of racemic sample. Acid (+)-**1a**: $[\alpha]_D^{23}$ = +11.7 (c 1.00, MeOH), 90.6% ee as shown below. This was further purified by recrystallization from EtOAc to give (+)-**1a** (93.6 mg, 71%, mp 114–115 °C) $[\alpha]_D^{23}$ = −14.4 (c 0.75, MeOH). The sample obtained by recrystallization as above (15.0 mg) was treated with CH_2N_2 to give (+)-**1c** (15.6 mg, 96%); mp 63–64 °C, $[\alpha]_D^{23}$ = +11.7 (c 0.75, MeOH). This was further converted to (−)-**6a** (11.1 mg, 74%, 95.6% ee); $[\alpha]_D^{23}$ = −207 (c 0.55, MeOH). HPLC analysis was performed in the same manner: t_R (min) = 15.1 (97.8%), 33.1 (2.2%).

Further recrystallization provided a sample of (+)-**1a** (49% recovery, mp 112–113 °C), $[\alpha]_D^{23}$ = −15.8 (c 0.76, MeOH). This sample was revealed to be 96.4% ee by the HPLC analysis at the subsequent stage of **6a** as below, and we concluded that the enantiomeric excess of the acid **1a** reaches constant value by repetition of the recrystallization from EtOAc.

The scaled-up experiment by applying (±)-**1e** (1.00 g, 4.59 mmol) worked well in a reproducible manner to give (−)-**1e**: (230 mg, 22.9%) $[\alpha]_D^{24}$ = −5.3 (c 1.00, $CHCl_3$); 99.7% ee after derivatization to **6b** and its HPLC anlalysis. Acid (+)-**1a**: (428 mg, 59.8%) $[\alpha]_D^{24}$ = +11.0 (c 1.00, MeOH); 77.3% ee by HPLC analysis of corresponding **6a**.

4.3.12. Methyl (1*S*,2*R*,4*S*,5*R*,6*S*)-(+)-3,8-dioxatricyclo[3.2.1.0^{2,4}]-octane-6-carboxylate 1c

To a solution of the acid (+)-**1a** (30.6 mg, 0.20 mmol) as above in anhydrous DMF was added Cs_2CO_3 (163 mg, 0.50 mmol) and CH_3I (85.1 mg, 0.60 mmol). The mixture was stirred at 50 °C for 24 h. After concentration to dryness in vacuo, the residue was extracted with EtOAc (three times), and the combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC with hexane–EtOAc (1:1) to afford methyl ester (+)-**1c** (20.1 mg, 59%) as a colorless solid. Mp 67–68 °C, $[\alpha]_D^{23}$ = +11.7 (c 0.57, MeOH). Its IR and NMR spectra were identical with that of the authentic specimen.[2]

4.3.13. Methyl (1*S*,5*S*,6*R*)-(−)-5-hydroxy-7-oxabicyclo[4.1.0]-hept-2-en-3-carboxylate 6a

To a solution of lithium hexamethyldisilazide [$(TMS)_2NLi$, 0.20 mL, 0.20 mmol] was added in THF (0.20 mL) at −78 °C. To a solution of methyl ester (−)-**1c** (20.1 mg, 0.13 mmol) in THF (0.20 mL) was added the LHMDS solution above dropwise at −78 °C, and the mixture was stirred for 1 h at that temperature. The reaction was quenched with saturated NH_4Cl aq solution, and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by preparative TLC with hexane–EtOAc (1:1) to afford methyl ester (−)-**6a** (14.0 mg, 70%, 90.6% ee) as a colorless solid. $[\alpha]_D^{23}$ = −200 (c 0.70, MeOH) [lit.:[2] $[\alpha]_D$ = +213 (c 0.56, MeOH), for (1*R*,5*R*,6*S*)-**6a**]. The product (−)-**6a** was analyzed by HPLC [column, Daicel Chiralcel OD-H, 0.46 cm × 25 cm; hexane–2-propanol (5:1); flow rate 0.5 mL/min]: t_R (min) = 15.1 (95.3%), 33.1 (4.7%).

Enantiomerically enriched acid (+)-**1a** (15.6 mg, 0.10 mmol) by recrystallization in twice was treated with CH_2N_2 to give (+)-**1c** (15.7 mg, 89%); mp 63–64 °C, $[\alpha]_D^{23}$ = +11.7 (c 0.75, MeOH). This was converted to (−)-**6a** (11.1 mg, 74%, 96.4% ee); $[\alpha]_D^{23}$ = −207 (c 0.55, MeOH). HPLC analysis was performed in the same manner: t_R (min) = 15.1 (98.2%), 33.1 (1.8%).

4.3.14. 2-Chloroethyl (1*R*,5*R*,6*S*)-(+)-5-hydroxy-7-oxabicyclo-[4.1.0]hept-2-en-3-carboxylate 6b

In a similar manner as described for (±)-**6b**, 2-chloroethyl ester (−)-**1e** (47.2 mg, 0.21 mmol) in THF (0.30 mL) was added with a solution of lithium hexamethyldisilazide [$(TMS)_2NLi$, 0.31 mL, 0.31 mmol] in THF (0.30 mL), gave (+)-**6b** (36.4 mg, 77%, 99.4% ee: $[\alpha]_D^{23}$ = −233 (c 1.08, MeOH); The product (+)-**6b** was analyzed by HPLC analysis [column, Daicel Chiralcel OD-H, 0.46 cm × 25 cm; hexane–2-propanol (5:1); flow rate 0.5 mL/min]: t_R (min) = 18.0 (0.3%), 38.0 (99.7%); ^1H NMR (400 MHz, $CDCl_3$): δ = 2.32 (1H, ddd, J = 3.3, 5.2, 17.6 Hz, H-6β), 2.80 (1H, dt, J = 2.1, 17.6 Hz, H-6α), 3.48 (1H, t, J = 3.9 Hz, H-3), 3.57 (1H, ddd, J = 2.1, 2.8, 3.9 Hz, H-4), 3.69 (2H, t, J = 5.7 Hz, CH_2Cl), 4.38 (2H, t, J = 5.7 Hz, CO_2CH_2), 4.57 (1H, br m, H-5), 7.19 (1H, dd, J = 3.3, 3.9 Hz, H-2); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 29.3, 41.5, 46.2, 56.1, 63.5, 64.5, 130.3, 134.4, 165.5; IR ν_{max} 3425, 2964, 1709, 1641, 1417, 1392, 1250,

1099, 918 cm^{-1}. Anal. Calcd for $C_9H_{11}ClO_4$: C, 49.44; H, 5.07. Found: C, 49.43; H, 5.36.

4.3.15. (3*S*,4*S*,5*R*)-(−)-3,4,5-Trihydroxy-1-cyclohexene-1-carboxylic acid 2

To a solution of (+)-**6b** (55.0 mg, 0.25 mmol) in THF and water (1:1, 4 mL) was added KOH (21.2 mg, 0.38 mmol). After stirring for 1 h at 50 °C, the mixture was neutralized with 1 M HCl to pH 3, and concentrated in vacuo. The solid was dissolved in water (1 mL) and trifluoroacetic acid (400 μL, 5.39 mmol) was added to the solution with stirring. The mixture was stirred for 3 h at 50 °C. The reaction mixture was concentrated in vacuo to remove volatile materials. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (10:1) to afford carboxylic acid (−)-**2**: $[\alpha]_D^{25} = -33.1$ (*c* 0.34, H_2O) [lit.:[20] $[\alpha]_D = -31.0$ (*c* 0.1, H_2O)]; ^1H NMR (400 MHz, D_2O): $\delta = 2.06$ (1H, dddd, $J = 2.8$, 4.0, 10.0, 16.8 Hz, H-6 β), 2.61 (1H, ddd, $J = 1.6$, 6.0, 16.8 Hz, H-6α), 3.33 (1H, dd, $J = 8.4$, 10.0 Hz, H-4), 3.62 (1H, dt, $J = 6.0$, 10.0, 10.0 Hz, H-5), 4.11 (1H, dddd, $J = 1.6$, 2.4, 4.0, 8.4 Hz, H-3), 6.51 (1H, dd, $J = 2.4$, 2.8 Hz, H-2); ^{13}C NMR (100 MHz, D_2O): $\delta = 31.7$, 68.6, 71.5, 76.2, 128.2, 139.2, 169.7; IR ν_{max} 3261, 1556, 1409, 1072 cm^{-1}. Its ^1H NMR spectrum was identical with that reported previously.[21] As this product **2** is a trihydroxy acid and shows highly hydrophilic property and is susceptible to an irreversible adsorption on silica gel, the yield was estimated to be 79.7%, at the stage just before the final purification, based on ^1H NMR with an internal standard [methyl β-D-glucoside, Tokyo Kasei Co., M709, analytically pure grade, standard signal at $\delta = 4.23$ (1H, d, $J = 7.6$ Hz)].

Acknowledgements

The authors thank Professor Kaoru Nakamura of Institute for Chemical Research, Kyoto University, for his valuable discussion and encouragement on this study. Meito Sangyo Co. Ltd and Nagase ChemteX Co. were acknowledged with thanks, for generous gift of enzymes. This work was supported both by a Grant-in-Aid for Scientific Research (No. 18580106) and 'High-Tech Research Center' Project for Private Universities: matching fund subsidy 2006–2011 from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and acknowledged with thanks.

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Design and Synthesis of 5a-Carbaglycopyranosylamime Glycosidase Inhibitors

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Abstract: 5a-Carba-α-D-glucopyranosylamine, validamine, and analogous compounds valienamine and valiolamine, have proved to be important lead compounds for development of clinically useful medicines, including the very strong α-glucosidase inhibitor, voglibose, *N*-(1,3-dihydroxyprop-2-yl)valiolamine, now used widely as a clinically important antidiabetic agent. In this review, we describe recent advances in development of glycosidase inhibitors on the basis of the ground-state mimics of the postulated glycopyranosyl cation, considered to be formed during hydrolysis of glycopyranosides, and introduce a new type of highly potent α-fucosidase inhibitor, 5a-carba-α-L-fucopyranosylamine, α-fuco validamine. Interestingly, the corresponding β-anomer, and in particular its D-enantiomer, has been shown to possess very strong cross-inhibitory activity toward β-galactosidase and β-glucosidase. Structure and inhibitory activity relationships concerning these α,β-fuco derivatives, as well as parent α,β-galacto validamines are discussed here with reference to our results.

1. INTRODUCTION

Acarbose (**1**) [1] and synthetic voglibose (**2**) [2,3], potent and specific α-glucosidase inhibitors, are clinically important for control of diabetes. These carbasugars [4] are carbocyclic analogues of glycofuranoses and pyranoses. The naturally occurring carbasugar 5a-carba-α-D-glucopyranosylamine (validamine, **4α**) [5] and some related compounds, 5,5a-unsaturated (valienamine, **5**) [6], 5-hydroxyl (valiolamine, **6**) [5], and 5a-hydroxyl derivatives (hydroxyvalidamine, **7**) [7], were first isolated from fermentation broth of the antibiotic validamycin A (**3**) [8] and then characterized as active components from the degradation products of **3** and its homologues. Their structures were fully established on the basis of spectroscopic data and total syntheses. Subsequently, **5** and its 5,5a-epoxy derivative **8** [9] were found to be components of the α-amylase inhibitors acarbose (**1**) and NS-504, the oxidized homologue of **1**, respectively, Fig. (**1**).

These carbaglycosylamines themselves possess more or less inhibitory activity toward α-glucosidases. Their activity is likely to be attributable to their structures mimicking the ground- and/or transition-state glucopyanosyl cations postulated to be formed during hydrolysis of α-glucopyranosides. Extensive efforts have been made for development of new type α-glucosidase inhibitors, leading to the discovery of very potent compound, voglibose [**2**, *N*-(1,3-dihydroxyprop-2-yl)valiolamine].

By analogy with the structural features of **4α** and **5**, some carbaglycosylamines, structurally related to the naturally occurring hexopyranoses involved in cell-surface oligosaccharide chains, might be valuable targets for exploration. In particular, 5a-carba-glucosyl (**4α,β**), galactosyl (**9α,β**), mannosyl (**10α,β**), and fucosylamines (**11α,β**), and *N*-acetyl-2-amino-2-deoxy-6a-carbaneuraminic acid (**12α,β**) could provide lead compounds for development of potent inhibitors active against the corresponding glycohydrolases, Fig. (**2**).

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2. CARBAGLYCOSYLAMINE GLYCOSIDASE INHIBITORS

2-1. Naturally Occurring Glycosidase Inhibitors: Validamycins and Acarbose

Agricultural antibiotic validamycins A-H have been widely used to control sheath bright disease of rice plants. The major and most active validamycin A (**3**) is the 5a,5a'-dicarbatrisaccharide, composed of validoxylamine A and β-linked D-glucopyranose. Trehalase inhibitory activity is due to the N-linked dicarbadisaccharide core mimicking the transition-state glycopyranosyl cation assumed to be formed during hydrolysis of αα-trehalose. In contrast, acarbose (**1**) is a carbatetrasaccharide, containing valienamine N-linked to C-4 of the 4,6-dideoxy-α-D-glucopyranose residue. The structural features of this carba-oligosaccharide core are very similar to the non-reducing end of amylose type α (1-4) D-glucan, and it's α-glucosidase inhibitory activity is attributable to mimics of the transition-state glycosyl cation thought to be formed on activation by α-glucosidase. In both cases, biological activity is apparently due to stereospecificity for certain glycosidases, and the structural core provided by the unsaturated carbaglycosylamine **5** has been shown to play important roles in their biological features.

Recent progress and increase in the scope of unsaturated carbaglycosylamine glycosidase inhibitors was reviewed [10] in a Mini-Review of Medicinal Chemistry. Here, we would like to summarize developments with regard to inhibitors related to the ground-state mimic validamine.

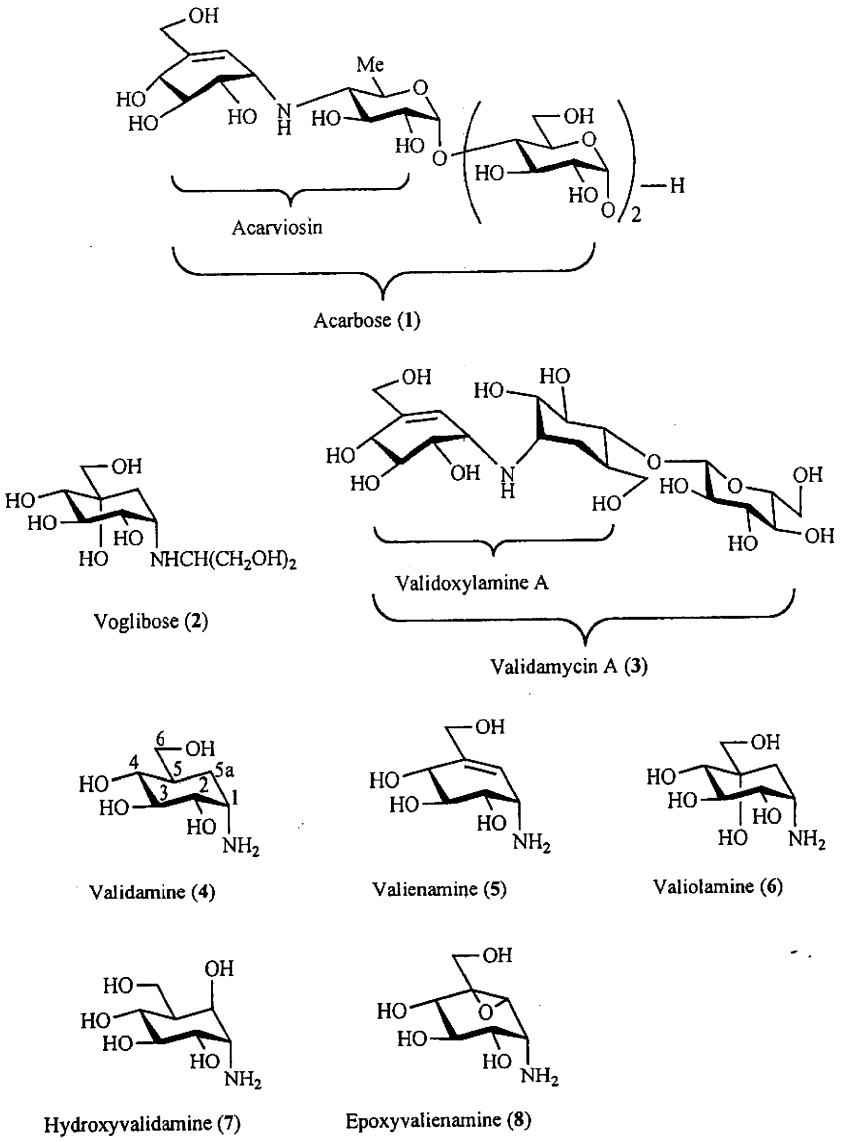


Fig. (1). Acarbose, validamycin A, and validamine, and related compounds derived from antibiotics and glycosidase inhibitors.

2–2. Synthesis of 5a-Carbaglycosylamines

We initially attempted to prepare 5a-carbasugars by incorporation of hydroxymethyl branches into the cyclitol rings, leading to the synthesis of two new 5a-carbahexoses with α-*altro* and β-*galacto* configurations [11]. However, owing to the practical difficulty in obtaining optically active compounds, we soon established that production of a large quantity of precursors and ready optical resolution to chiral carbasugars would be indispensable for further developments in carbasugar chemistry and biochemistry.

For this purpose, it appeared attractive to establish systematic routes to carbasugars, starting from readily available 1,4-anhydro-5a-carba-α-glucopyranose, the *endo*-adduct **13** of furan and acrylic acid. When ready formation of crystalline *endo*-adduct **13** from a reaction mixture was first observed [12], we soon realized that this compound might become a most versatile precursor for preparation of various kinds of carbasugars and derivatives.

We here briefly detail the systematic synthetic routes to several useful intermediates in Figs. (**3**-**5**). All preparative processing was elaborated using common reagents under conventional conditions as simply as possible [13,14]. Although optical resolution is difficult to overcome in any preparative design of carbasugars, optically pure samples of **13** could be obtained with readily available (*R*)- and (*S*)-phenylethylamines.

In synthetic carbohydrate chemistry, naturally abundant D-glucose, D-galactose, and D-mannose, for example, have effectively been applied for preparation of D-series sugar derivatives. However, elaboration of the corresponding L-series sugars is always difficult in practice. Since in our carbasugar synthesis, both enantiomers of **13** could be readily provided pure [15], optically pure compounds could

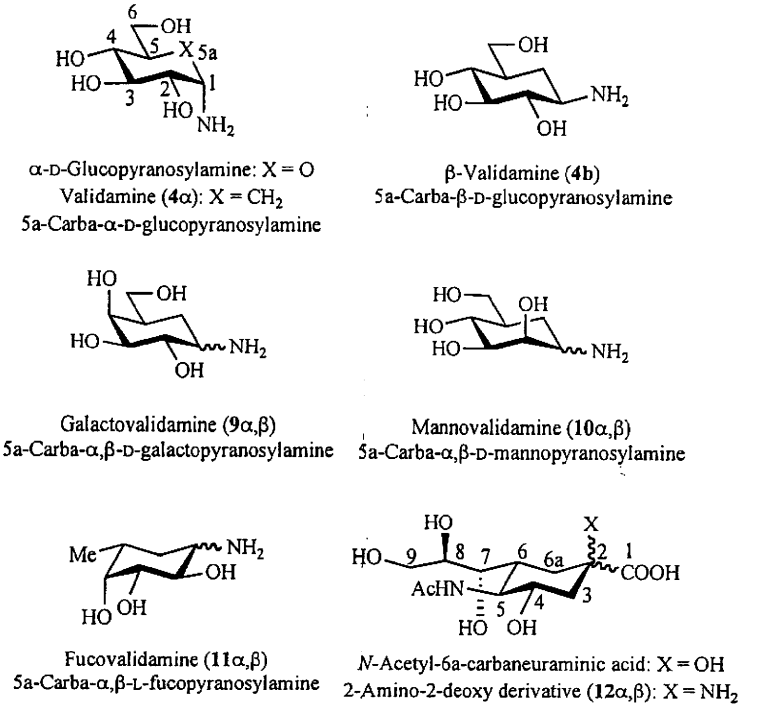


Fig. (2). Validamine and related carbaglycosylamines of biological interest.

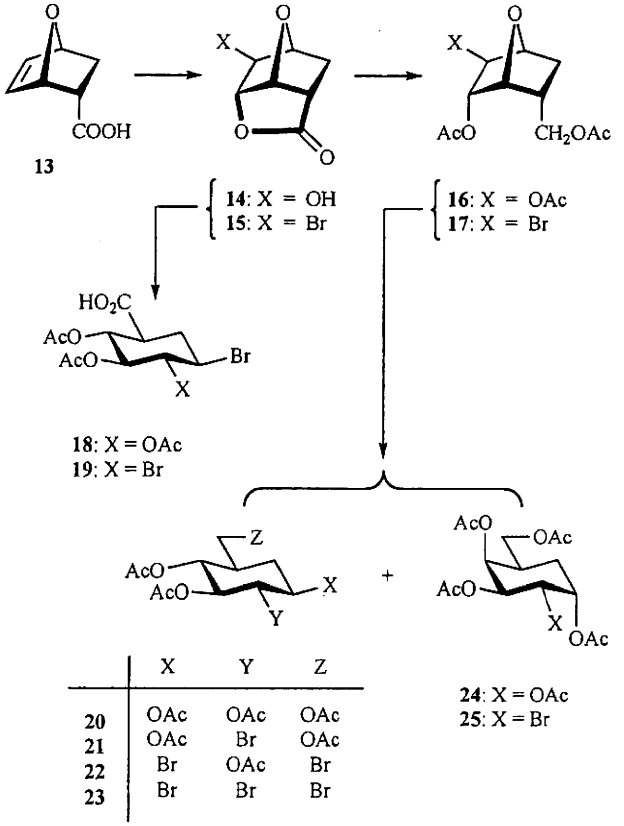


Fig. (3). Several synthetic precursors derived from the *endo*-adduct of furan and acrylic acid. Unless otherwise noted, for convenience, the formulae only depict only one of the respective enantiomers throughout in this article.

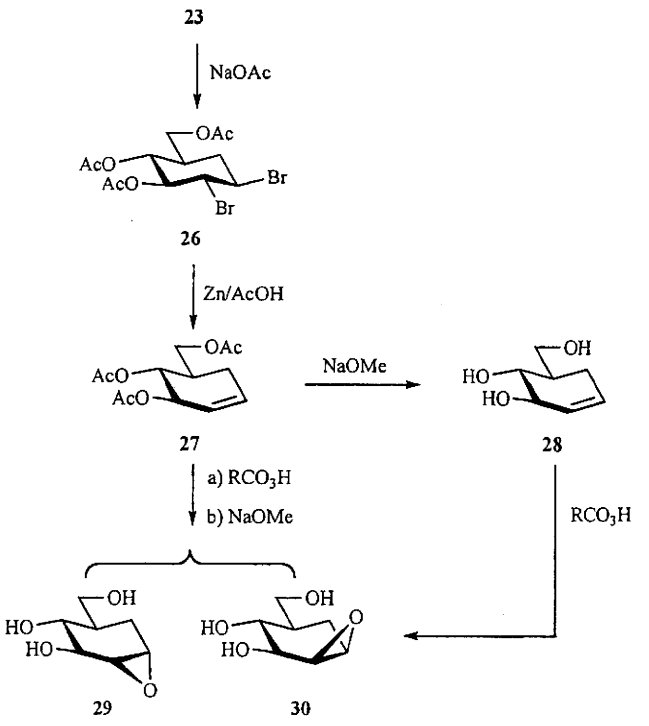


Fig. (4). Preparation of synthetically useful 1,2-anhydro-5a-carba-α-gluco and β-mannopyranoses.

be obtained when preparative routes for the racemates were established.

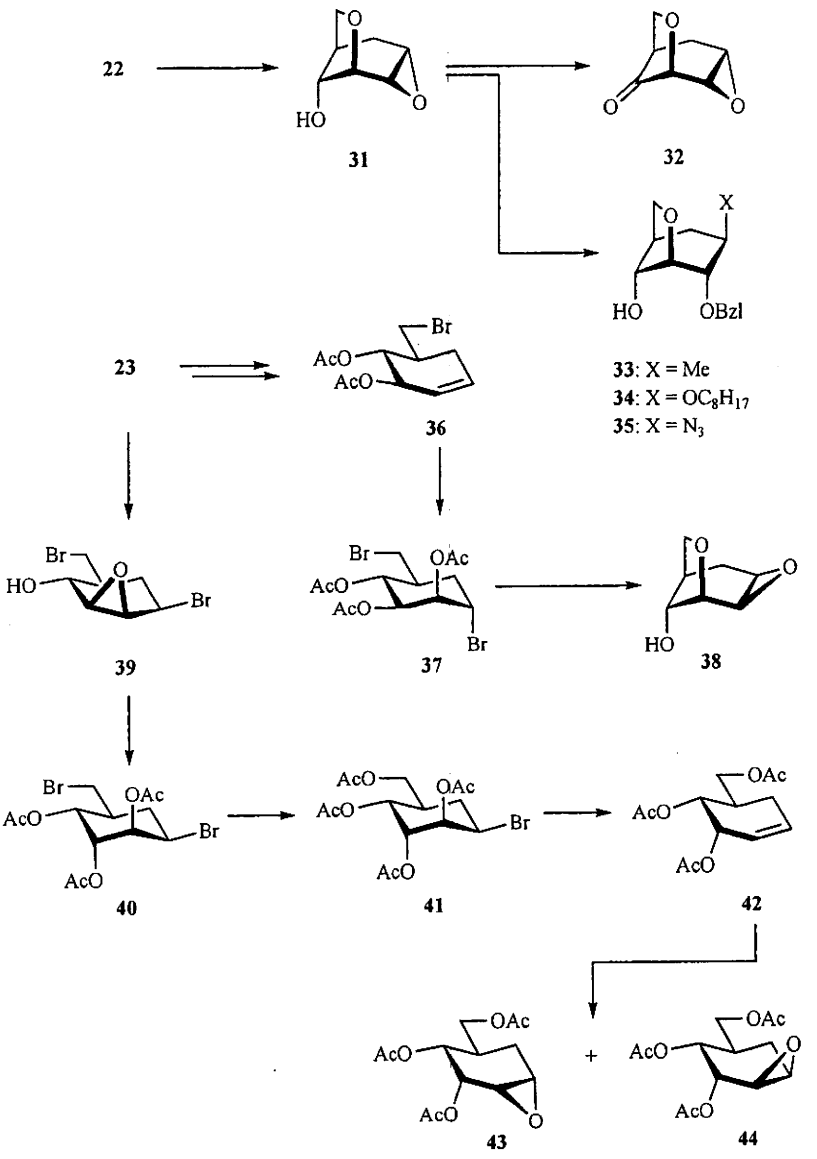


Fig. (5). Preparation of some anhydro and dianhydro-5a-carbahexopyranoses.

2-3. Systematic Synthesis from the *endo*-Adduct of Furan and Acrylic Acid

Peracid oxidation of the adduct **13** gave crystalline hydroxy lactone (**14**), which was reduced with LAH to give the triol, isolated as crystalline acetate (**16**) [16], Fig. (**3**). Similarly, through its bromo lactone (**15**), the bromodeoxy compound (**17**) was obtained. Acid cleavage of their 1,4-anhydro rings with H_2SO_4/AcOH afforded the corresponding 5a-carba-β-glucopyranose (**20** and **21**) and α-galacto-pyranose (**24** and **25**) in acceptable yields. Direct treatment of **14** and **15** with HBr/AcOH gave the bromodeoxy carba-uronic acids **18** and **19** [17]. On the other hand, similar bromination of **16** and **17** produced selectively the bromo-deoxy-5a-carbaglucopyranose derivatives **22** and **23** [18]. These bromo compounds underwent nucleophilic substi-tution with acetate, azide, and thiolate anions to produce precursors for aminodeoxy and deoxythio-5a-carbahexo-pyranoses. Stereochemical reaction courses were usually controlled on the basis of direct S_N1 in aprotic solvents and/or S_N2 reaction mechanisms through neighboring group participation. On other hand, elimination of **22** with DBU/ toluene afforded the conjugate 1,3-alkadiene, a versatile precursor for the 5,5a-unsaturated derivative, valienamine (**5**), and its analogues [19].

Anhydro and dianhydro derivatives, obtained by treatment of bromides with NaOMe/MeOH, provide access to new types of N- and O-linked carba-oligosaccharides. For example, coupling with protected carbasugars gave rise to a new type of 5a-carbadisaccharide, Fig. (**4**). Selective displacement of the 6-bromo group of **23** with an acetate ion gave the 1,2-dibromide **26**, which was debrominated with Zn/AcOH to afford the versatile carbaglucal-type derivative **27**. Peracid oxidation of **27** gave the α-gluco epoxide **29** as the major product, while the triol **28** gave the β-manno

epoxide **30** selectively through the *cis*-directing effect of the 3-hydroxyl group.

On treatment with NaOMe/MeOH, compounds **21** and **25** gave, through base-catalyzed epoxide-group migration, a hardly separable mixture of the epoxides.

Similar treatment of **22** with MeONa gave the versatile dianhydride **31**, which was transformed into the carbahexose intermediates **32–35**, Fig. (**5**). Starting from **23**, the isomeric dianhydride **38** was obtained through the alkene **36** and dibromide **37**. On the other hand, the isomeric dibromide **40** was provided through the epoxide **39**. The 1,2-anhydrides **43** and **44** were prepared through the alkene **42** derived from the bromide **41**.

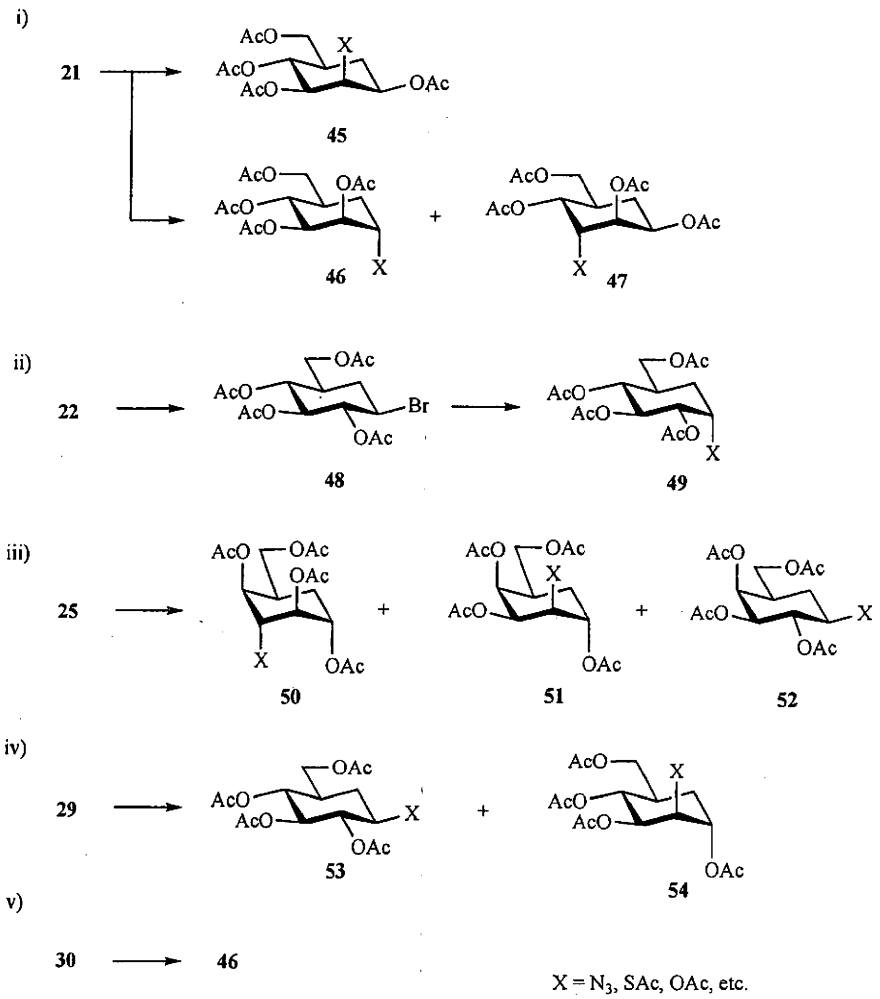
2-4. Design and Synthesis of Validamine and its Stereoisomers

These bromo and anhydro compounds can be used as reactive carbaglycosyl acceptors and/or donors in nucleophilic substitution reactions, as well as in coupling with NH_2 and OH unprotected carba and true sugar derivatives, affording N- and O-linked carba-oligosaccharides, Fig. (**6**).

Starting from the bromides **21**, **22**, and **25**, eight precursors **45-52** were generated for carbahexose derivatives, including α-manno **46**, α-gluco **49**, and β-galacto validamines **52**. The epoxy compounds **29** and **30** afforded β-*gluco* **53** and α-*manno* type compounds **46** and **54**.

Progressing from successful development of voglibose, Takeda Chemical Co. has been extensively engaged in chemical modification of the components supplied abundantly by degradation of validamycins.

The β-galacto [20], β-gluco [21], and α-manno validamines [22] (**4β**, **9β** and **10α**) were first synthesized by our group in racemic modification, starting from the *endo*-adduct **13**. Later, Kameda and coworkers prepared α-D-galacto and α-D-manno validamines (**D-9α** and **D-10α**) by chemical transformation of validamine, and assayed their inhibitory activity toward glycohydrolases in detail [23]. Compound **D-10α** was shown to be a moderate α-mannosidase inhibitor (IC_{50} = 56 μM, Jack beans; 36 μM, Almonds) and to possess high potency (K_i = 1.2 μM) against endoplasmic reticulum α-mannosidase. Furthermore, **D-9α** was found to be a weak α-galactosidase inhibitor.



* The reaction products were isolated and characterized as acetyl derivatives.

Fig. (6). Readily available 5a-carbahexo ecursors through nucleophilic substitution.

2-5. Design and Synthesis of N-Linked Carba-oligosaccharides and Carbaglycosylceramides

In order to determine biochemical features of carbaglycosylamines related to validamine (**4α**), we first carried out chemical modification of the bioactive core structures of validamycins and acarbose, Fig. (7). Concerning trehalase inhibition, synthesized α,α-trehalose type symmetric bis(validamine) (**55**) [24] was shown to possess similar activity to validoxylamine A, indicating that validamine residues play a role in mimicking carbaglycosyl cations generated from α,α-trehalose. However, maltose type 5a'-carbadisaccharide (**56**) [25a] was found not to be a potent α-glucosidase inhibitor, revealing the transition-state type unsaturated carbaglycosylamine valienamine (**5**) to be indispensable [25b]. In line with our preparative interest in carbasugars, synthesis of 2-acetamido-2-deoxyvalidamine and derivatives, related to *N*-acetyl-5a-carbaglucosamine, was attempted. Chitobiose type 5a'-carbadisaccahrides (**57** and **58**) might have been expected to possess some inhibitory activity, *e.g.* chitinase, but this was not the case [26]. Only 5a-carbamannopyranosylamine derivatives **59**–**61** were found to be weak to moderate α-mannosidase inhibitors [27]. The N-linked carbalactoside **62**, and *N*-acetyl-carba-lactosaminide **63** and isolactosaminide **64** were prepared and assayed for inhibitory activity against β-galactosidase. Rather interesting biological features were revealed as substrate analogues for fucosyltransferase [28].

In other attempts to replace hexopyranose residues with carbahexopyranose in bioactive compounds, Fig. (**8**), the 5a'-carbatrehazolin (**65**), derived from the trehalase inhibitor trehazolin, the α-glucosylamine residue of which was replaced with 5a-carbaglycosylamine, was shown to preserve strong activity [29]. In the case of bioactive glycosylamides, *N*-octadecyl-5a-carbaglucosylamide (**66**) was demonstrated to have similar activity to the parent, indicating that 5a-carbaglucose could be useful as a mimic of sugar moieties [30]. These successful results with carbaglycosylamides stimulated us to apply our preparative know-how to research on glycolipid chemistry.

First attempts to substitute glycopyranose residues of glycosylceramides with carbaglycopyranose provided us with 5a-carbaglucosyl (**67a**) and galactosylceramides (**67b**) [31]. Biological assays indicated these to possess weak to moderate inhibitory activity against gluco and galacto-cerebrosidases. The results further led to finding of the respective unsaturated analogues **67c**,**d**, possessing strong and specific potential toward the corresponding glycocere-

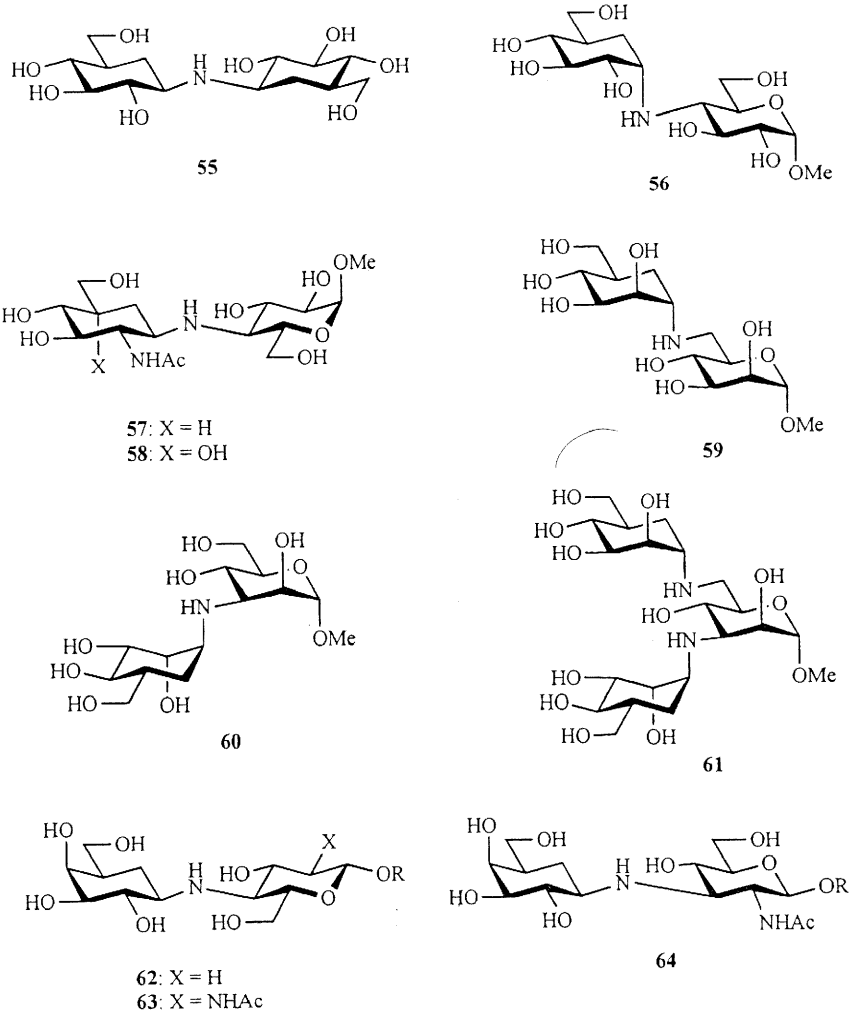


Fig. (7). Some N-linked 5a,5a'- and 5a'-carba-oligosaccharides classified as carbaglycosylamines.

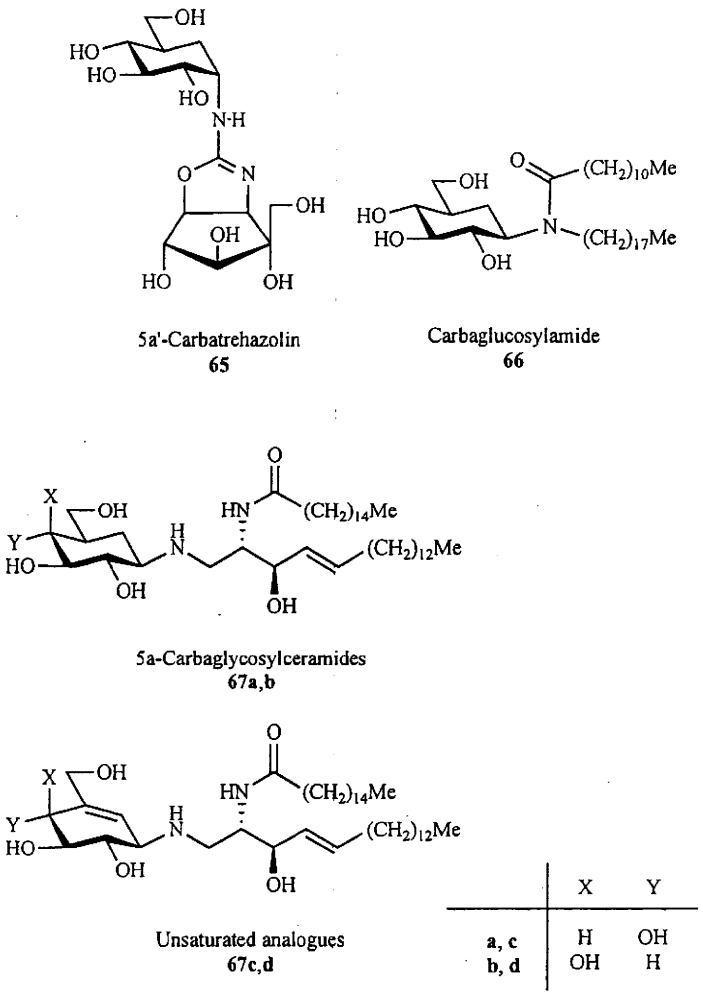


Fig. (8). 5a-Carbatrehazolin, carbaglucosylamide, and 5a-carbaglycosylceramides.

brosidases (mouse liver), IC_{50} 0.3 and 2.7 μM, respectively [32].

3. CARBAGLYCOSYLAMINE α-FUCOSIDASE INHIBITORS

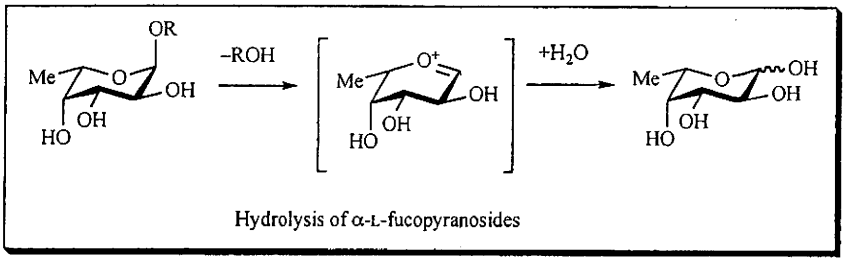
The inhibitory potency of validamine (**4**) stimulated our interest in studying biochemical features of other 5a-carbaglycosylamines with β-*gluco*-, α,β-*galacto*-, and αβ-*manno*- and α,β-*fuco* configurations, Fig. (**2**), expected to be potent inhibitors of some glycosidases. However, we were rather disappointed with the preliminary results that their free bases **4β**, **9α,β**, and **10α,β** did not possess any acceptable activity, except for the moderate α-mannosidase inhibitor 5a-carba-α-mannopyranosylamine **10α**. Especially, we were dissatisfied in that β-validamine **4β** and limited derivatives did not show any inhibitory activity toward β-glucosidase, contrary to expectations from analogy with the structural features of α-glucosidase inhibitors validamine **4α** and derivatives.

In the final stage of our work on 5a-carbaglycosylamine glycosidase inhibitors, α-fuco validamines (**11α**) remained for exploration owing to their synthetic inaccessibility. On the basis of the structure-inhibitory activity relationship as suggested by the postulated reaction mechanism for hydrolysis of α-fucopyranosides, two types of inhibitor were designed: the ground-state **11α,β** and the transition state mimics **68α,β**, Fig. (**9**). Furthermore, two analogues **69α,β** were added. These were 5a-carba-α- and β-fucopyranose derivatives featuring aminomethyl functions at C-1.

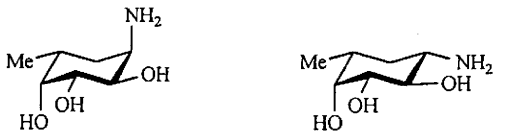
3-1. Synthesis of a α-Fuco Validamine, 5a-Carba-α-fucopyranosylamine

Synthesis of 5a-carba-α,β-fucopyranosylamines (**11α,β**) was planned in line with our systematic synthesis. Therefore, two or three preparative approaches [33,34] were carried out simultaneously for appropriate characterization of the intermediates appearing in the sequences.

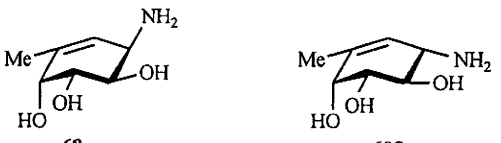
Compound **48** was prepared in a three-step process (**14→16→22→48**, overall yield 35%) from the *endo*-adduct **13** [35], Fig. (**10**). A mixture of **48** and NaN_3 in DMF was reacted at 90 °C to give an inseparable, ca. 4 :1 mixture of the azide **70** and the side product 5a,1-alkene. The mixture was de-*O*-acetylated (**→71**) under Zemplén conditions and subsequently treated with α,α-dimethoxytoluene and *p*-TsOH in DMF. After conventional acetylation, two products were found to be separable by silica gel chromatography,



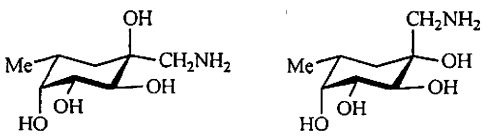
Hydrolysis of α-L-fucopyranosides



Ground-state mimics



Transition-state mimics



1-*C*-Aminomethyl-5α-carba-α,β-L-fucopyranoses

Fig. (9). Putative reaction mechanisms for hydrolysis of α-L-fucopyranosides, and some designed α-fucosidase inhibitors: fucose-type carbaglycosylamines.

giving **72** (60%). The direct S_N2 reaction occurred preferentially at C-1. Compound **72** was de-*O*-acetylated and then treated with NaH-benzyl bromide in DMF to give the dibenzyl ether **73** (89%). Treatment of **73** with 80% aq. AcOH gave the diol **74** (97%), which was mesylated to give the 4,6-dimesylate **75** (97%). Compound **75** was then treated with NaI in 2-butanone at 90 °C to afford selectively the 6-iodide **76** (85%), treatment of which with tributyltin hydride and AIBN in toluene at 120 °C gave the 6-deoxy derivative **77** (74%).

Compound **77** was treated with KOAc and 18-crown-6 ether in DMF to give the acetate **78** (99%) with an α-*galacto* configuration, the structure of which was confirmed on the basis of the ^{1}H NMR spectrum of its 4-OH derivative **79**. Hydrogenolysis of **79** in ethanol, containing 1 M HCl, in the presence of 10% Pd/C afforded, after purification by chromatography on a column of Dowex 50 W × 2 (H^+) resin with methanolic ammonia, syrupy **DL-11α** (8%).

As an alternative, the dianhydride **31** was benzylated to give the benzyl ether **80** (65%), Fig (**11**) [36]. Reaction of **80** with NaN_3 (→**81**), followed by tosylation, led to the azide tosylate **82** (75%), hydrogenation of which with Raney nickel in ethanol containing acetic anhydride gave the *N*-acetyl derivative **83** (80%). Cleavage of the anhydro ring, followed by simultaneous *O*-debenzylation, was conducted by treatment with HBr/AcOH to give the bromide **84** (80%), dehydrobromination of which with AgF in pyridine afforded the alkene **85** (48%), together with **84** (46%) unchanged. Hydrogenation of **85** with 5% Pd/C proceeded selectively to give the 6-deoxy derivative **86** (90%). Treatment of **86** with excess NaOMe/MeOH gave the 2,3-epoxide, which was hydrolyzed with acid, followed by acetylation, to afford the *N,O*-acetyl derivative **87** (80%). Removal of the protecting groups gave **11α** quantitatively.

3-2. Biological Assay and Chemical Modification of α-Fuco Validamine

Interestingly both 5a-carba-α-fucopyranosylamines **DL-** and **L-11α** were demonstrated to be very potent α-fucosidase inhibitors (K_i = 0.23 and 0.012 μM, bovine kidney), the effects being fully comparable to those of deoxyfuconojirimycin (DFJ) [37]. As expected, N-substitution with alkyl and phenylalkyl functions resulted in dramatic increase of their inhibitory potential. The 2-, 3-, and 4-deoxy

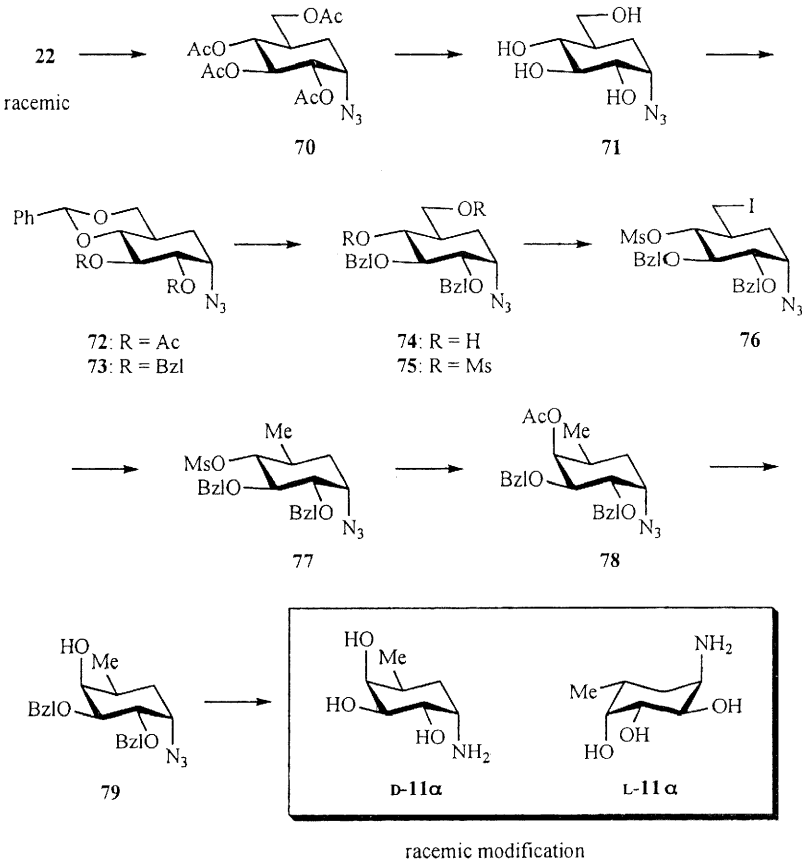


Fig. (10). Synthesis of 5a-carba-α-fucopyranosylamine.

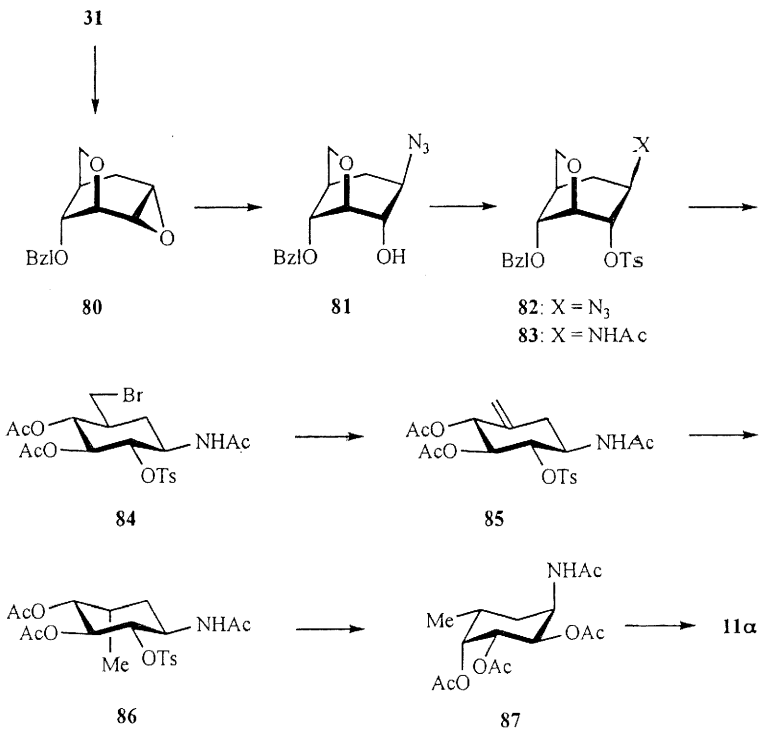


Fig. (11). Convenient synthesis of 5a-carba-α-fucopyranosylamine.

derivatives of 5a-carba-α-fucopyranosylamine were synthesized [38], and shown not to possess any detectable activity. The presence of all substituents with an α-*fuco* configuration, with one amino and three consecutive hydroxyl groups, constitutes the minimum core for α-fucosidase inhibitors.

Thus, the *N*-alkyl derivatives **90a-g** were initially prepared by LAH reduction of the corresponding amides **89a-g** from the protected amine **88** derived from the azide, Fig. (**12**). Alternatively, reductive alkylation of **88** with the corresponding aldehydes proceeded smoothly on treatment with sodium cyanoborohydride in THF under acidic conditions, leading to the *N*-alkyl and *N*-phenylalkyl derivatives in 45–70% yields.

Furthermore, an attempt was made to improve inhibitory potential by incorporation of a cyclic isourea function [36] with various N-substituents, thereby inducing change of the electron distribution and somewhat flattening the chair conformation without affecting the configurational arrangement of the two hydroxyl and methyl groups. Thus, treatment of **11α** with the corresponding alkyl and aryl isothiocyanates in aqueous 60% EtOH yielded the thioureas **91a-c** (ca. 100%), which, under influence of yellow mercuric oxide, were converted into the corresponding cyclic isoureas **92a-c**. (~100%).

Results of inhibition assay of several derivatives of **11α** toward α-L-fucosidase (bovine kidney) and four other glycosidases are listed in Table (**1**) [36]. None of the compounds, showed any significant activity against α-glucosidase (Baker's yeast and rat intestine), α-mannosidase (Jack beans), or α-galactosidase (green coffee beans and rat liver). As shown within the limited scope of racemic modifications of the compounds tested, the inhibitory activity against α-L-fucosidase was dramatically increased by incorporation of alkyl and phenylalkyl groups into the amino function of **11α**. Change of the *N*-ethyl on **11α** to a *N*-nonyl group improved the inhibitory potential, reaching a maximum with an aliphatic eight-carbon chain: *N*-octyl-5a-carba-α-DL-fucopyranosylamine (**90d**), shown to possess very strong and specific inhibitory activity against α-L-fucosidase, with *p*-nitrophenyl-α-L-fucopyranoside as the substrate. It is reasonable that the L-enantiomer should be several times more potent than DFJ, the strongest fucosidase inhibitor reported so far. The results suggest that the catalytic site of the enzyme can tolerate addition of various sizes of aliphatic chain to the basic portion involved in binding.

The *N*-octyl group seems to act as a structurally efficient hydrophobic spacer, leading to appropriate electron-release to the nitrogen atom for docking at the active site of the enzyme. Interestingly, the results are in line with those observed for inhibition of glucocerebrosidase by a series of *N*-alkyl-β-valienamines [39]. Thus, incorporation of *N*-alkyl functions could be clearly demonstrated to influence the activity of the inhibitors of this kind, suggesting that there is

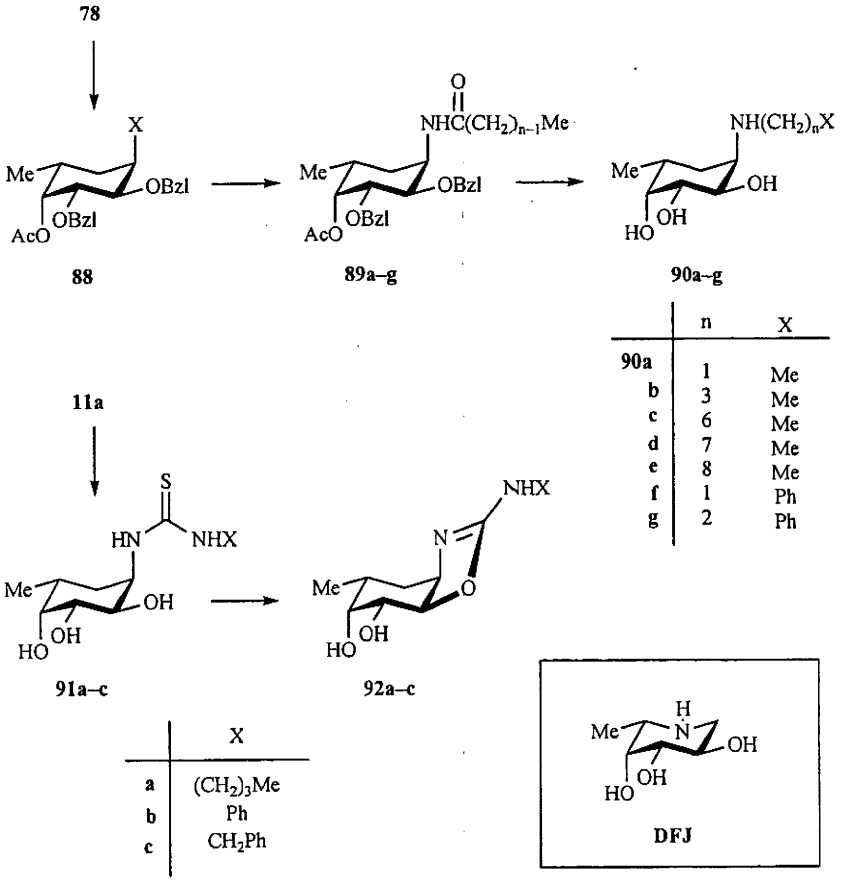
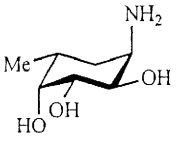
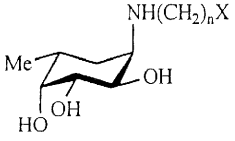


Fig. (12). Preparation of some N-substituted 5a-carba-α-DL-fucopyranosylamines and cyclic-isourea derivatives.

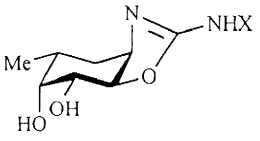
Table 1. Inhibitory Activity [IC_{50} (K_i) μM] of some 5a-Carba-α-DL-fucopyranose Derivatives against α-Fucosidase (Bovine kidney)



11α



90a–g



92a–c

Compd.	Inhibitory Activity
	IC_{50} (K_i) μM
11α	9.3 (1.0)
90a	21 (0.18)
b	0.92 (0.074)
c	0.27 (0.048)
d	0.11 (0.016)
e	0.24 (0.11)
f	1.0 (0.069)
g	0.24 (0.032)
92a	140
b	7.6
c	72
DFJ	0.41 (0.031)

All compounds except for **11α** and **DFJ** are racemic.

All compounds, except for **90d** (IC_{50} = 37 μM), were found to possess almost no activity toward β-galactosidase (bovine liver).

much room for development of potent new forms by further chemical modification.

On the other hand, the 1-*C*-aminomethyl analogues L-**69α,β** of **11α,β** were shown to possess strong inhibitory potential (K_i = 2.8 and 0.3 μM) toward α-fucosidase, implying their use as novel leads in chemical modification [34].

Similar change of the potential has been observed for three cyclic isourea derivatives **92a-c**. Although the *N*-phenyl derivative **92b** showed medium inhibitory activity, due to a possible stacking effect of the spacer phenyl group, both basic feature of nitrogen atoms and the free hydroxyl group at C-2 seem to play an important role in attaining high potential.

3-3. Synthesis and Inhibitory Activity of β-Fuco Validamine and Derivatives

The unexpected findings for biochemical features of the 1-*C*-aminomethyl-5a-carbafucopyranoses **69α,β** prompted us to prepare β-fuco validamine **11β** and evaluate its possible α-fucosidase inhibitory activity in detail.

Treatment of **23** with excess of Zn dust in DMF at 80 °C gave the alkene (**93**, 59%), together with the 6-debromo compound **94** (32%), Fig. (**13**) [44]. When the reaction time was prolonged, **94** was mainly obtained but in poor yield, owing to its simultaneous decomposition. Compound **93** isolated was debrominated with tributyltinhydride to give **94** (86%). *O*-Deacetylation of **93**, followed by selective benzoylation of the allylic hydroxyl group (→**94**) and subsequent mesylation, afforded the mesylate **95** (64%). Direct nucleophilic substitution of **95** with a benzoate anion in DMF and successive deprotection gave **96** (93%). Blocking the two hydroxyls with a cyclohexylidene group (→**97**, 85%), followed by epoxidation with *m*CPBA in a phosphate buffer solution (pH ca. 6), gave the single β-epoxide **98** (90%). Rear attack of the peracid seems to be restricted by the presence of a bulky cyclohexylidene group.

Reaction of the racemic **98** with alkylamines or phenylalkylamies in 2-propanol at 120 °C proceeded slowly but almost regio-selectively to afford the respective N-substituted 5a-carba-β-fucopyranosylamines **99a-f** (70–90%), which were deprotected with 80% aqueous AcOH, and the resulting amine acetates were purified over a column of Dowex 50 W × 2 (H^+) resin with 1% methanolic ammonia to give the free bases **100a-f** (ca. 100%). The optically active **100b-d,f** were similarly prepared from D- and L-**98**.

Compounds DL- and L-**11β**, and four *N*-alkyl and two *N*-phenylalkyl derivatives **100a-f** were assayed for activity against seven glycosidases: α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucosaminidase, α-mannosidase, and α-fucosidase [44]. All compounds were shown to exhibit appropriate inhibitory potential toward β-galactosidase, β-glucosidase, and α-fucosidase, as listed in Table (**2**). Compared to the corresponding derivatives of the α-anomers, they possessed about one tenth of the inhibitory activity against α-fucosidase and the potential was thought to be attributable to β-L-fucopyranose mimicking enantiomers, as verified by assaying newly prepared pure L-enantiomers L-**100b-d**.

It is worthy of note that all N-substituted derivatives possess very strong activity toward both β-galactosidase and β-glucosidase, reaching a maximum with an aliphatic twelve-carbon dodecyl group. Their high cross-inhibitory potential could be demonstrated to be largely due to the respective D-enantiomers, viz. *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-**100b**, L-**100c**, and L-**100f**. For example, DL-**100e** has been demonstrated to be a strong inhibitor possessing characteristic pH dependent activity against β-glucosidase (almond): K_i = 0.39 μM, at pH 5.5, e.g. calystegine [41] (K_i = 0.75 μM, pH independent). Thus, N-substituted 6-deoxy-5a-carba-β-D-

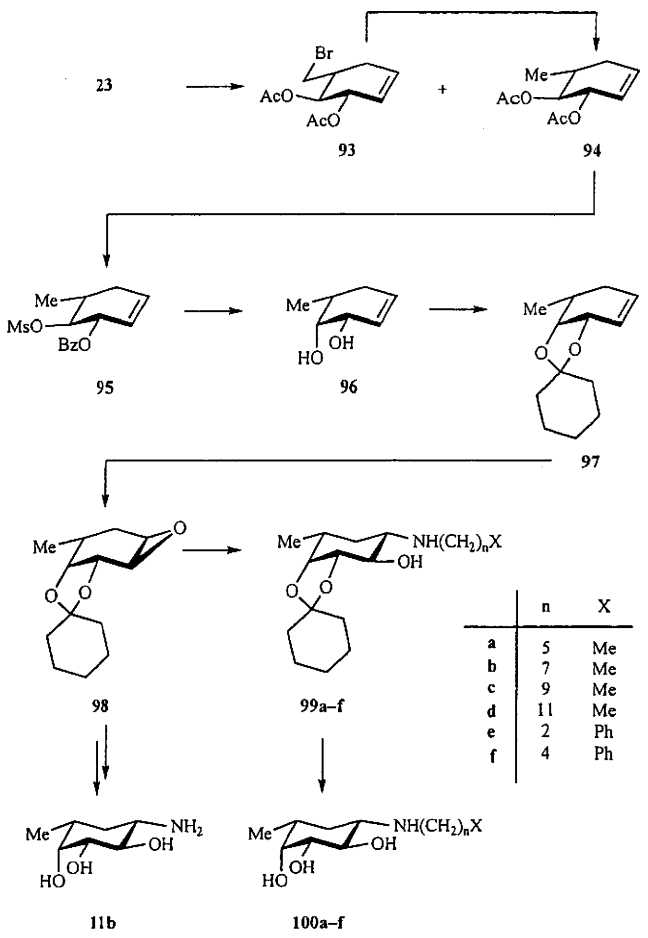


Fig. (13). Preparation of N-substituted 5a-carba-β-fucopyranosylamines.

galactopyranosylamines may be promising lead compounds for new β-galactosidase and β-glucosidase inhibitors.

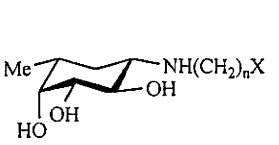
3-4. Synthesis and Inhibitory Activity of β-Galacto Validamine and Derivatives

The strong cross-inhibitory activity exhibited by β-fuco validamine (**11β**) stimulated our interest in biochemical features of its parent β-D-galacto validamine (**9β**). This compound had been assayed once for β-galactosidase, but its unexpected low potency did not point to advantages for further modification.

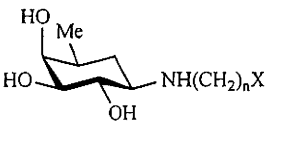
Epoxidation of the alkene **D-101**, derived from **21** through multi-step sequences, gave the α-galacto 1,2-epoxide **102** [30], Fig. (**14**). Treatment of **102** with some alkylamines, followed by deprotection, afforded four *N*-alkyl derivatives **D-103a-d**. Interestingly, as shown by e.g. the *N*-octyl derivative **D-103b**, they were found to be moderate inhibitors of α-galactosidase, as well as, β-galactosidase and β-glucosidase, Table **3**. In contrast, the L-enantiomers, as indicated by **L-103b**, were found to lack inhibitory activity against all enzymes [42].

It is noteworthy that the free bases **D-9β** and **L-11β** were found to be medium but specific inhibitors against α-galactosidase and α-fucosidase, matching the respective stereochemistry of the substrates. However, the *N*-alkyl derivatives **D-103b-d** are all moderate inhibitors of α- and β-galactosidases, and β-glucosidase, so that the N-substituents as well as the hydrophobic area conferred in by the 5-methyl branching on the carbocyclic ring are likely to control and enhance binding potential at the active sites of enzymes.

Table 2. Inhibitory Activity [IC_{50} (K_i), μM] of some *N*-Substituted 5a-Carba-β-fucopyranosylamines 100a-f toward Three Glycosidases*



L-100a–f



D-100a–f

Compd.	Inhibitory activity, IC_{50} (K_i) μM		
	α-Fuc	β-Gal	β-Glc
L-11β	4.3 (0.2)	NI	NI
DL-100a	8.2	3.7	0.73
DL-100b	5.5	0.7 (0.11)	1.5 (3.2)
L-100b	1.8	3.7	15
DL-100c	2.7	0.2 (0.009)	2.0 (0.4)
L-100c	0.7	1.0	17
DL-100d	3.8	0.02 (0.0045)	0.50 (0.53)
L-100d	0.91	0.46	6.1
DL-100e	7.5	5.7	0.38 (0.057)
DL-100f	5.1	0.9 (0.046)	1.4 (1.2)
L-100f	1.2	2.4	10

* α-Fucosidae (bovine kidney); β-Galactosidase (bovine liver); β-Glucosidase (rat intestine). NI: no inhibition < 10^{-3} M

Table 3. Inhibitory Activity (IC_{50}, μM) of some *N*-Substituted 5a-Carba-β-D-galactosylamines Against Three Glycosidases*

Compd.	n	Inhibitory activity, IC_{50} μM		
		α-Gal	β-Gal	β-Gul
D-9β	-	2.8	NI	NI
D-103a	3	39	NI	13
b	7	5.2	8	14
c	9	25	16	25
d	11	35	5.8	8.7

* α-Galactosidase (green coffee beans); β-Galactosidase (bovine liver); β-Glucosidase (rat intestine).
NI: no inhibition < 10^{-3} M

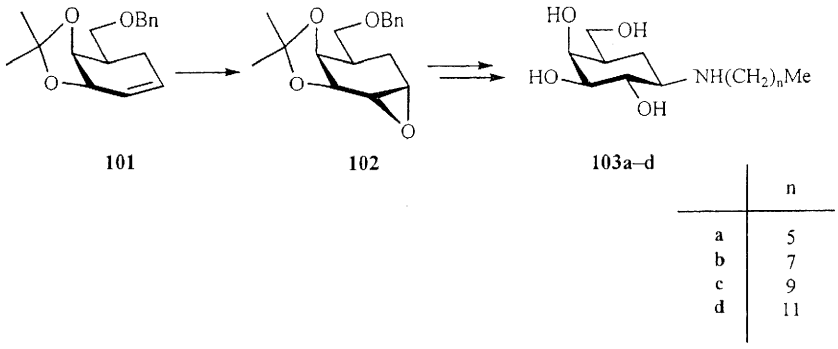


Fig. (14). Preparation of some N-substituted 5a-carba-β-D-galactopyranosylamines.

3-5. Synthesis of N-Linked Carbadisaccharides Composed of β-Fuco and β-Galacto Validamine Moieties

Carbadisaccharides containing the β-fuco and β-galacto validamine moieties were designed in order to elucidate the influence of the sugar and/or sugar like aglycones on the activity originating in the carbaglycosylamine moieties, Fig (**15**).

Coupling of **DL-11α** and the dianhydro azide **104** [33] in 2-propanol at 120°C afforded, after separation, new type carbadisaccharides **D-** and **L-105α** (48 and 44%), which were demonstrated to possess specific inhibitory activity (K_i = 3.1 and 0.13 μM) toward α-fucosidase [33]. The analogous β-carbadisaccharide **L-105β** (93%), derived from **L-11β** and **104**, was also shown to be a specific α-fucosidase inhibitor (IC_{50} = 3.9 μM), with a similar tendency as observed for **L-11β** [44]. It is promising that these disaccharide mimics may constitute a new group of specific α-fucosidase inhibitors suitable for further modification. Compounds **105α,β** were initially designed to provide precursors for carbadisaccharides of biological interest, e.g. N-linked *N*-acetyl-5a'-carbalactosaminide derivatives,

Reductive amination of the ketone **32** with the amine **9β** proceeded in stereoselective fashion, affording N-linked β(1→4)-5a,5a'-dicarbadisaccharide **106**, featuring a versatile 1,2:3,6-dianhydro-5a-carba-α-glucopyranose residue [43]. The free base **9β** was first converted into the hydrochloride and then subjected to reductive coupling with the ketone **32**. Thus, reaction of **32** (2 molar equiv) and **9β** HCl was conducted in aqueous methanol in the presence of sodium cyanoborohydride (2 molar equiv) and anhydrous $MgSO_4$ at reflux temperature. The product was readily isolated as the tetra-*O*-acetyl derivative (47%) of **106**. Therefore, the analogous ketones **107a–c** were prepared by oxidation of **33–35** and subjected to similar coupling with **9β**, giving the respective 5a,5a'-dicarbadisaccharide derivatives **108a-c** (60%, 41%, and 50%). *O*-Debenzylation of **108a** gave N-linked methyl 3,6-anhydro-5a,5a'-dicarba-β-D-lactoside **109** (91%), which could be shown to possess specific inhibitory activity against α-galactosidase (IC_{50} = 1.2 μM, green coffee beans). Acetolysis of **109** would be expected to give rise to the N-linked 5a,5a'-dicarba-β-lactoside derivative.

Further preparative utility of the dianhydride **31** is summarized in Fig. (**16**), demonstrating access to 5a-carba-galacto and glucopyranosylamine derivatives [43]. Compound **31** was converted into th through mesylation followed by azidolysis. Acid catalyzed cleavage of the 3,6-anhydride produced the azide **111** with β-gluco configuration. Acetolysis of **111** proceeded through a direct S_N2 mechanism to give rise to the azide **112** with β-*galacto* configuration. On the other hand, nucleophilic opening of the 1,2-anhydride was easily conducted by treatment with octylamine in 2-propanol to give the amine **113** (ca. 90%), treatment of which with HBr/AcOH, followed by *O*-deacetylation, afforded the *N*-octyl-6-bromo-6-deoxy-carbaglucopyranosylamine **114a**. Conventionally **114a** could be transformed into the 6-hydroxyl **114b** and 6-deoxy derivatives **114c**.

We seem to have taken a roundabout course to finally elucidate the biochemical interactions of *N*-alkyl derivatives of β-validamine **4β** with glycosidases. Compounds **114b,c** have been assayed for activity toward β-glucosidase and β-galactosidase [44]. Contrary to our expectation, these compounds were demonstrated to be medium β-galactosidase inhibitors (IC_{50} = 10 and 18 μM) and did not exhibit any activity against β-glucosidase. These results suggest that only the β-*galacto* configuration may be required for inhibitory interaction toward β-glucosidase. It seems difficult to explain rationally the fact that β-validamine lacked inhibitory activity toward the enzyme in spite of close resemblance of the substrate structures.

3-6. Transition-state Mimic 5,5a-Unsaturated Derivatives of α,β-Fuco Validamines

Compounds **68α** and **68β** were synthesized in a racemic modification for rough assays of activity toward α-L-fucosidase [40], Fig. (**17**). Compound **115** was prepared in 50–60% yield by treatment of **40** with NaOAc in HMPA at 120 °C. The protecting groups were initially replaced with methoxymethyl groups, in order to eliminate neighboring participation reactions, thus converting **115** into the trimethoxymethyl ether **116** (ca. 100%). Treatment of **116** with bromine in CCl_4 gave the 1,4-addition products (**117α** and **117β**) in 21 and 48% yields, respectively. Selective debromination of the major **117β** was conducted by treatment with NBH in HMPA to give a 1:2 mixture of the bromides (**118α,β**, 57%), together with **117α,β** (ca. 45% recovered). Direct substitution with a bromide ion generated in situ is likely to occur at allylic carbon atom, resulting in epimerization. Therefore, a mixture of **118α,β** should be furnished directly from a crude mixture of the dibromides. The mixture was treated with NaN_3 in DMF to give a 1:2

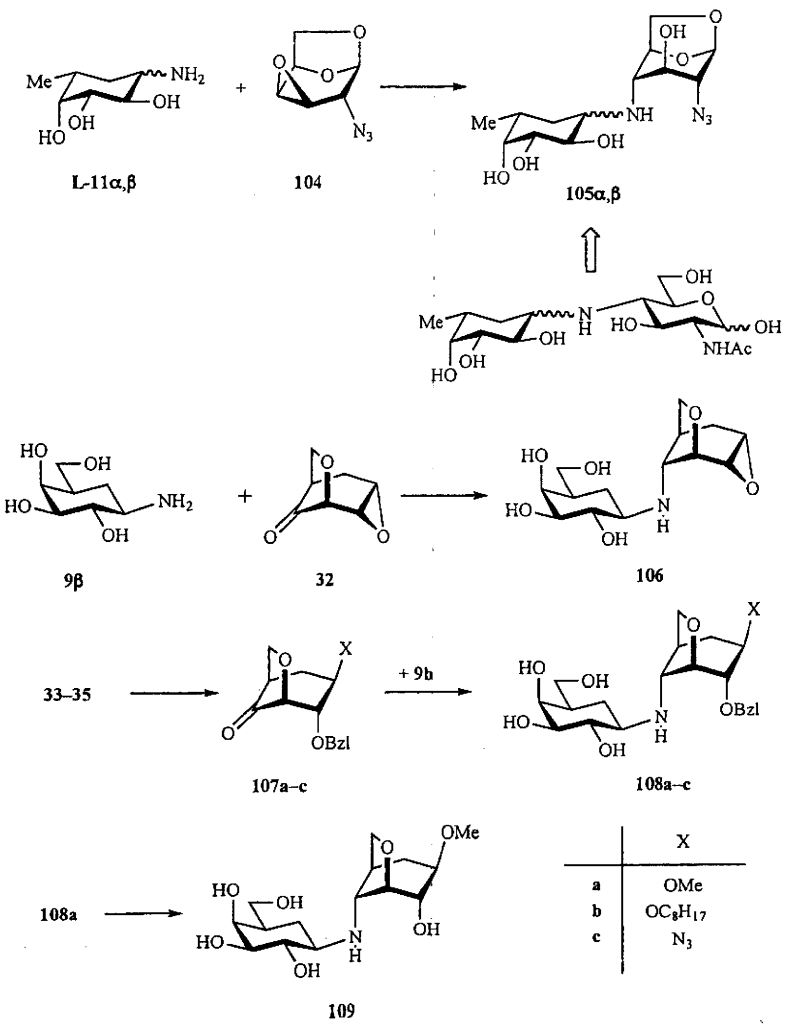


Fig. 15. Preparation of some precursors of N-linked 5a-carba-β-L-fucopyranosyl GlcNAc and 5a,5a'-dicarba-β-D-lactose derivatives.

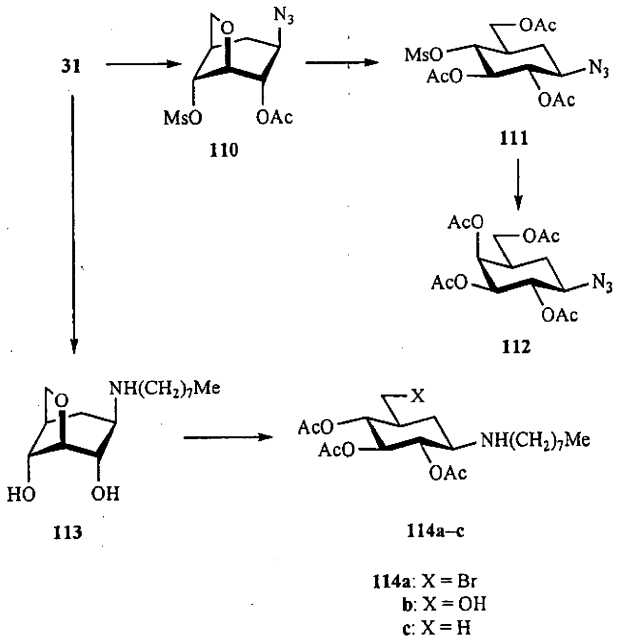


Fig. (16). Convenient preparation of 5a-carba-β-galacto and glucopyranosylamine derivatives from the dianhydride.

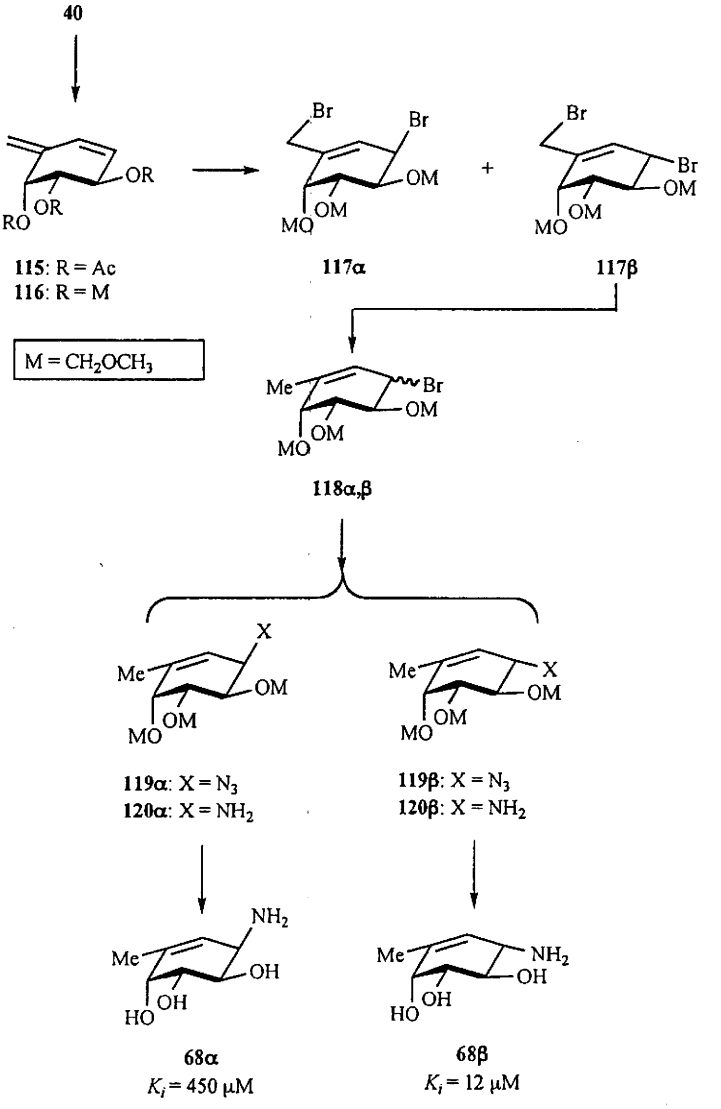


Fig. (17). Synthesis of transition state analogues of 5a-carba-α,β-fucopyranosylamines and constants for their enzyme inhibitory activity against α-fucosidase (bovine liver).

mixture of the azides **119α,β** (88%). Reduction with Ph_3P in aqueous THF gave a mixture of the amines **120α,β**, and subsequent treatment with 4 M HCl afforded the respective free bases **68α** and **68β** (ca. 100%). Contrary to expectation, racemic **68α** and **68β** were found to be rather weak fucosidase inhibitors, with K_i values of 45 and 1.2×10^{-5} M, respectively. Throughout our studies on carbaglycosylamine glycosidase inhibitors, these results are the first instance of transition-state mimics of postulated fucopyranosyl cations being less potent than ground-state mimics, owing to possible differences in binding to the active site of the enzyme. Hydrolysis of α-fucopyranosides seems to mechanistically provide a somewhat different stereoelectronic course, compared with other hexopyranosides having the hydroxyl function at C-6. The present results indirectly suggest that the hydrolytic reaction of the α-L-fucosidase (bovine kidney) features an S_N2-type mechanism with nucleophilic displacement rather than an S_N1-type one through an oxocarbenium ion intermediate

3-7. Related Aminocyclopentanetriol Glycosidase Inhibitors

(Hydroxymethyl)aminocyclopentitol glycosidase inhibitors **122a-d**, **123**, and **124** , and several derivatives thereof have already been synthesized and their biological features investigated extensively [45, 46]. Actually these compounds are considered to be ring-contract models derived from validamine (**4**), valiolamine (**6**), and their stereoisomers **9** and **121**, Fig (**18**), and their structure and inhibitory activity relationship has been discussed in detail [47]. In general their inhibitory potential appears to be comparable with that of structurally related parent 5a-carbaglycopyranosylamines, probably depending on close resemblance between their structures and those of the ground states of the corresponding substrates. Interestingly, cross-inhibitory activity toward β-galactosidase and β-glucosidase, exhibited by the β-galacto D-**4β** and β-fuco validamines D-**9β**, has also been

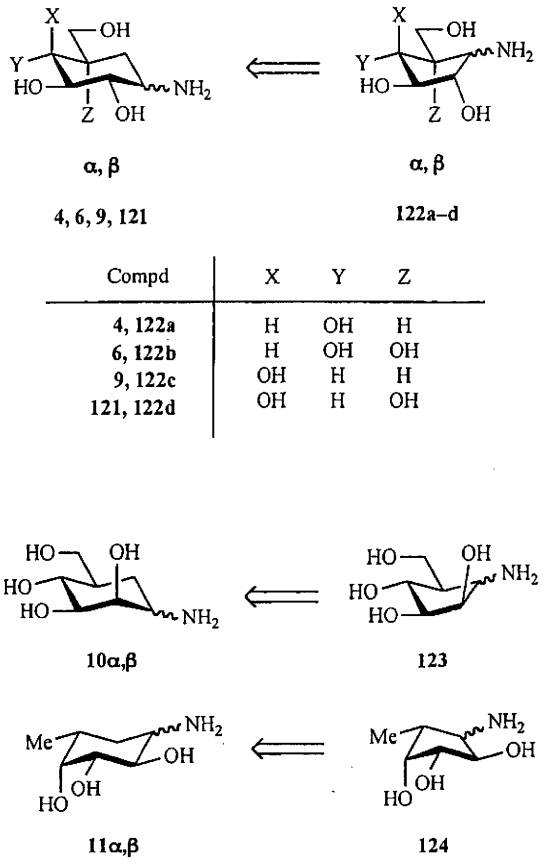


Fig. (18). Potent glycosidase inhibitors, amino(hydroxymethyl)-cyclopentanetriols, the ring-contract analogues derived from validamine and valiolamine, and their stereoisomers.

observed for the corresponding ring-contract analogues **122aβ** and **122cβ** [48], indicating that configuration of the 4-hydroxyl group is ignored by the enzymes. However, in the case of structurally more rigid carbahexopyranosylamine inhibitors, the configuration of the 4-hydroxyl seems to play a role of enhancing binding interaction toward the enzymes, although it is difficult to explain rationally solely on the close resemblance of the substrate structures. Therefore, enzymatic action of a certain glycosidase may depend on the structure and activity relationship of these characteristic inhibitors.

4. CONCLUSION

The door to the new possibility of carbasugars being developed as potent glycosidase inhibitor, is now open by analogy with the preceding discovery of very strong 5a-carba-α-fucopyranosylamine α-fucosidase inhibitor. Furthermore, the fact that α-fuco valienamines designed as related transition-state mimicking inhibitors of α-fucosidase unexpectedly possessed only moderate activity motivated us to compile this review article on validamines as ground-state type inhibitors. The interesting biochemical and biological features found for simple 5a-carbaglycopyranosylamines provide a promising basis for further development [51] of biologically active sugar mimics of this type.

Recently, *N*-octyl-β-valienamine (NOV) [39] and its 4-epimer (NOEV) [49] were found to induce remarkable expression of mutant lysosomal enzymes and to correct pathological effects of plasmic storage of substrates in some human disorders. This stimulated a systematic survey of such kind of glycosidase inhibitors has been carried out [50]. Very recently, NOEV was found to be a good candidate new molecular therapeutic (chemical chaperone therapy) for G_{M1}-gangliosidosis caused by β-galactosidase deficiency [52]. Since some derivatives of 5a-carbaglycosylamines with α-*fuco* and β-*galacto* configurations have been recognized as strong and specific inhibitors of fucosidase or/and nonspecific but very strong cross-inhibitors of β-galactosidase and β-glucosidase, our efforts should continue for development of medicines of this kind, adopting these substances as leads and target compounds.

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