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Synthesis of a New Fucosidase Inhibitor, 1,5-Dideoxy-1,5-imino-L-talitol, via Cyanotrimethylsilanolysis of a β-D-Ribofuranoside and Its Inhibitory Activities

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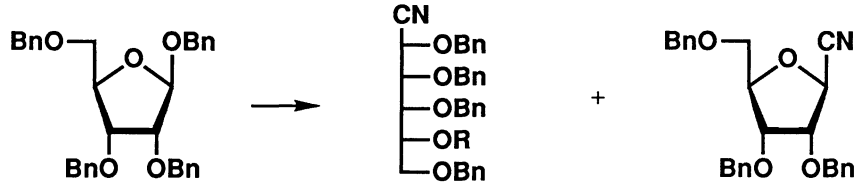
A new polyhydroxypiperidine of L-talo configuration was synthesized from benzyl tri-O-benzyl-β-D-ribofuranoside via ring-cleavage reaction with cyanotrimethylsilane and found to be an effective fucosidase inhibitor (Ki 10^{-5} – 10^{-6} M).

Many polyhydroxylated piperidines and pyrrolidines have been shown to be powerful and specific glycosidase inhibitors. Among them 1,5-dideoxy-1,5-iminohexitol, azapyranose analog of hexose, shows a remarkable inhibitory activity against a glycosidase which hydrolyzes a hexopyranosyl residue having the same configuration as that of the 1,5-iminohexitol.[1-8] In this communication the analog of L-talo configuration was first synthesized and the specific activity as inhibitor against α-L-fucosidase was found.

The reaction of cyanotrimethylsilane (TMSCN) with acetal in the presence of Lewis acids was first applied[9] to a glycoside, i.e., methyl tri-O-acetyl-β-D-ribofuranoside, giving the corresponding gluconononitrile. This acyclic derivative is appropriate for construction of piperidine ring because nucleophilic amino-methyl group can be easily produced by reduction of the nitrile and 5-trimethylsilyl ether can be converted into a suitable leaving group.

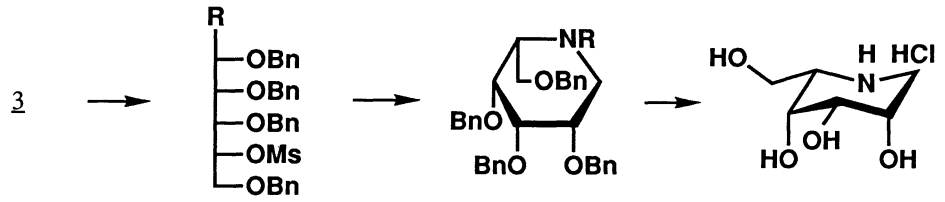
Reaction of benzyl 2,3,5-tri-O-benzyl-β-D-ribofuranoside (1) with TMSCN (5 equiv.) in the presence of trifluoroborane etherate (0.1 equiv.) at room temperature for 2 h gave a mixture of acyclic (2 and 3) and cyclic (4) cyanides in a total yield of 96% with the ratio of 4 : 1. The amount of the de-O-silylated derivative 3 depends on conditions employed. Treatment of the mixture with iron (II) chloride gave 3[10] in 77% yield. The configuration of 2 proved to be D-allo by the chemical conversions as shown below, indicating that the ring cleavage of 1



1 2 R = TMS 3 R = H 4

occurred via an S_N2 mechanism. On the other hand, the cyclic cyanide 4 was formed via a stereoselective S_N1 reaction.

A conventional sulfonylation of 3 with methanesulfonyl chloride in pyridine gave the 5-mesylate[11] 5 in 97% yield. Reduction of 5 with lithium almimun hydride in ether at -13 °C for 12 h gave 1-amino-1-deoxy-D-allitol derivative 6, which was further heated under reflux in ethanol saturated with sodium acetate to give a 1,5-dideoxy-1,5-iminohexitol derivative 7 in 46% yield. The iminohexitol 7 was characterized as the corresponding N-acetyl derivative[12] 8.



5 R = CN
6 R = CH_2NH_2

7 R = H
8 R = Ac
9 R = H HCl

10

De-O-benzylation of 7 was performed smoothly by hydrogenolysis in the presence of palladium/carbon after conversion into the corresponding hydrochloride 9 to give 1,5-dideoxy-1,5-imino-L-talitol hydrochloride[13] (10) in 70% yield. All coupling constants of the ring protons of 10 indicate axial-equatorial or equatorial-equatorial relation, and the L-talo configuration and the 1C conformation were confirmed. On the other hand, the compound 8 exists in a skew boat conformation, $^2S_3$.

The inhibitory activity of 10 toward the following glycosidases was examined: α-glycosidase from brewers yeast, β-glucosidase from almond, α-galactosidase from green-coffee bean, β-galactosidase from Aspergillus niger, α-mannosidase from jack bean, α-fucosidase from bovine epididymis and bovine kidney. The corresponding p-nitrophenyl glycosides were used as substrates except the o-nitrophenyl glycoside for β-galactosidase.

The concentrations of 10 for 50% inhibition of hydrolysis are summarized in Table 1. An unexpectedly strong inhibitory activity was observed toward α-fucosidases, while weak inhibitory activities were observed toward α-glucosidase and β-galactosidase. The mode of inhibition for β-glucosidase is uncompetitive,[14] while those for α-fucosidases are competitive,[14] giving Ki = 1.1 x 10^{-5} M (bovine epididymis) and Ki = 7 x 10^{-6} M (bovine kidney).

It is noteworthy that the iminohexitol 10 shows the remarkable and specific inhibitory activities toward α-fucosidases, although the structure of 10 is different from L-fucose except the ring atom in two points, i.e., non-deoxygenation at C-6 and inverted configuration at C-2.

Table 1. Inhibitory activity[15] of 1,5-dideoxy-1,5-imino-L-talitol toward several glycosidases

Glycosidase	Origin	50% inhibition M	Ki M
α-glucosidase	brewers yeast	3×10^{-3}	uncompetitive
β-glucosidase	almond	NI[a]	----
α-galactosidase	green-coffee bean	NI	----
β-galactosidase	Aspergillus niger	NI[b]	----
α-mannosidase	jack bean	NI	----
α-L-fucosidase	bovine epidydimis	2.7×10^{-5}	1.1×10^{-5}
α-L-fucosidase	bovine kidney	8×10^{-5}	7×10^{-6}

a) Less than 50% inhibition at 5.3×10^{-3} M.
b) Slightly lower than 50% inhibition at 5.3×10^{-3} M.

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1) The inhibitory activities of 1,5-dideoxy-1,5-iminohexitols having the following configurations were reported: D-gluco against α-D-glucosidase[2,3] (IC^{50} 1.9×10^{-4} M) and β-D-glucosidase[2,3] (IC^{50} 8.1×10^{-5} M), and its 2-acetamido analog against β-N-acetylglucosaminidase[4] (Ki 2.3×10^{-7} M) D-manno against α-D-mannosidase[5] (IC^{50} 1.5×10^{-4} M) and α-L-fucosidase[5] (IC^{50} 2.2×10^{-5} M), D-galacto against α-D-galactosidase[6,7] (Ki 1.6×10^{-9} M), 6-deoxy-L-galacto against α-L-fucosidase[8] (Ki 4.8×10^{-9} M).
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10) When the riboside 1 was treated with 5 equiv. TMSCN and 0.1 equiv. BF_3OEt_2, 2 and 3 were obtained in 25% and 52% yields, respectively. All new compounds were characterized by elemental analysis and spectroscopic data. Compound 3: $[\alpha]_D$ +59.6° (c 1.7, $CHCl_3$); ^{1}H-NMR data: δ 3.36 (dd, $J_{5,6a}$=5.7 Hz, H-6a), 3.46 (dd, $J_{5,6b}$=4.2 Hz, H-6b), 3.70 (t, $J_{3,4}=J_{4,5}$=4.7 Hz, H-4), 4.04 (dt, H-5), 4.05 (dd, $J_{2,3}$=9.0 Hz, H-3), 4.56 (d, H-2), 4.56, 4.80; 4.60, 4.84 (each ABq, CH_2 in Bn), 4.40 and 4.60 (each s, CH_2 in Bn), 7.26-7.30 (Ph); ^{13}C-NMR data: δ

78.77, 78.62, 70.13, 69.79 (each CH), 74.19, 73.79, 73.31, 72.57, 70.87 (each CH_2), 117.14 (CN), and 137.77-127.77 (aromatic carbons).

11) Compound 5: ^{1}H-NMR data (500 MHz): δ 4.55 (d, $J_{2,3}$=3.8 Hz, H-2), 3.97 (dd, $J_{3,4}$=6.7 Hz, H-3), 3.89 (dd, $J_{4,5}$=3.1 Hz, H-4), 5.07 (ddd, $J_{5,6}$=7.3 Hz, $J_{5,6'}$=3.4 Hz, H-5), 3.67 and 3.55 (each dd, $J_{6,6}$=11.2 Hz, H-6 and H-6'), 2.93 (s, MS), 4.71-4.39 (ABq x4, CH_2 in Bn) and aromatic protons.

12) Compound 8: $[\alpha]_D$ -13.5° (c 1.5, $CHCl_3$); ^{1}H-NMR data: δ 2.88 (t, $J_{1,1'}$= $J_{1,2}$=12.0 Hz, H-1), 3.25 (ddd, $J_{1',2}$=5.0 Hz, $J_{1',3}$=2.1 Hz, H-1'), 4.72 (dd, H-2), 4.09 (dd, $J_{3,4}$=2.2 Hz, H-3), 3.38 (dd, $J_{4,5}$=6.0 Hz, H-4), 4.19 (ddd, $J_{5,6}$=11.0 Hz, $J_{5,6'}$=2.1 Hz, H-5), 3.89 (dd, $J_{6,6'}$=11.0 Hz, H-6), 4.00 (dd, H-6'), 2.10 (s, Ac), 4.85-4.37 (ABq x4, CH_2 in Bn) and aromatic protons.

13) Compound 10: 2.63 (d, $J_{1,1'}$=14 Hz, H-1), 2.89 (dd, $J_{1',2}$=3.4 Hz, H-1'), 3.21 (t, $J_{2,3}$=3.4 Hz, H-2), 3.52 (bd, H-3), 3.60 (s, H-4), 2.77 (t, $J_{5,6}$=7.0 Hz, H-5), 3.24 (d, 2H, H-6 and H-6').

14) The modes of inhibition were ascertained from the Dixon plots.

15) The assay method of glycosidase activity was essentially that reported by Evans et al.[16] A typical procedure in the case of α-glucosidase (Sigma G-4634): 200 μl enzyme in 50 mM citrate buffer, pH 6.0 (7 mg/ml); 200 μl 2.2 – 3.6 mM p-nitrophenyl α-D-glucoside; 200 μl 1.6×10^{-2} – 10^{-6} M 10. Incubated 12 min, 25 °C. Added 1 ml 50 mM glycine-NaOH buffer, pH 10.1. Read 400 nm. β-Glucodidase (Sigma G-8625): 200 μl enzyme in 50 mM citrate buffer, pH 4.8 (1 mg/ml); 200 μl 1.9 mM p-nitrophenyl β-D-glucobside; followed by the same procedure as described above. α-Galactosidase (Sigma G-8507): 200 μl enzyme in 50 mM citrate buffer, pH 4.0 (2 mg/ml); 200 μl 4.4 mM p-nitrophenyl α-D-galactoside; 200 μl 10. Incubated 10 min, 25°. Followed by the same procedure as described above. β-Galactosidase (Sigma G-9132): 200 μl enzyme in 50 mM citrate buffer, pH 4.0 (6 mg/ml); 200 μl 2.1 mM o-nitrophenyl β-D-galactoside; Followed by the same procedure as described for α-galactosidase. α-Mannosidase (Sigma M-7257): 200 μl enzyme in 50 mM citrate guffer, pH 4.5 (2.5 mg/ml); 200 μl 3 mM p-nitrophenyl α-D-mannoside; Followed by the same procedure as described for α-glucosidase. α-L-Fucosidase (Sigma F-7753): 200 μl enzyme in 50 mM citrate buffer, pH 5.8 (5 mg/ml); 200 μl 2 mM p-nitrophenyl α-D-fucoside; Followed by the same procedure as described for α-galactosidase. α-L-Fucosidase (Sigma F-5884): 200 μl emzyme in 50 mM citrate buffer, pH 5.5 (1 mg/ml); 200 μl 3 mM p-nitrophenyl α-D-fucoside; Followed by the same procedure as described for α-galactosidase. All substrates were purchased from Seikagaku Kogyo Co. Ltd., dissolved in dimethyl sulfoxide (DMSO) and diluted (0.5% DMSO).

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