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Synthesis of a Potential Heparanase Inhibitor

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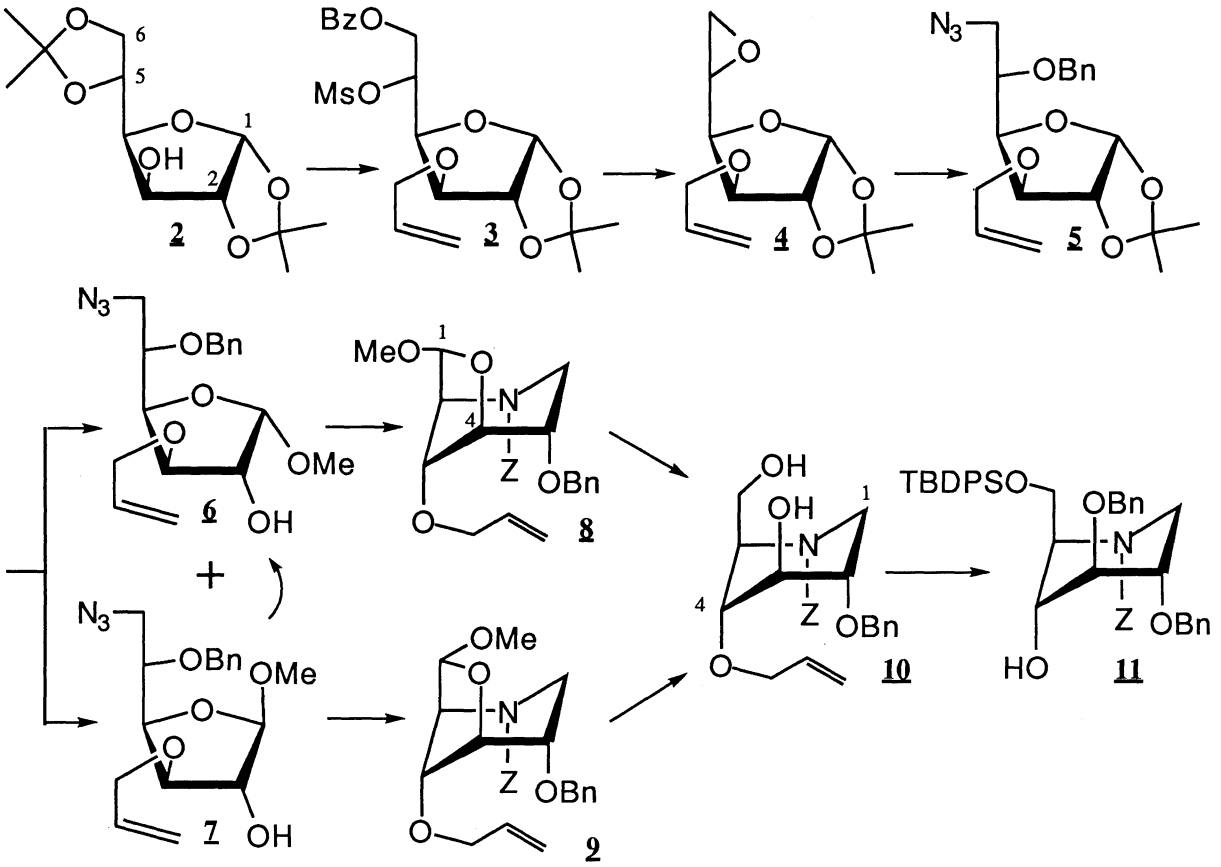
Aza-analog of the basic disaccharide unit in heparan sulfate was designed as a potential inhibitor against heparanase produced by solid tumor cells, and synthesized via a coupling reaction of a phenyl 2-azido-1-thio-D-glucopyranoside derivative with a partially protected 1-deoxynojirymycin derived from D-glucose, followed by manipulation such as imino acid formation, *O*-sulfation.

According to the paper by Nakajima et al.,[1] heparan sulfate-specific endo-β-D-glucuronidase (heparanase) plays an important role in cell invasion of some malignant solid tumors through basement membranes, suggesting that heparanase inhibitors have the possibility of preventing metastasis of such tumors. Such consideration prompted us to prepare compound **1**, a mimic of the basic repeating disaccharide unit of heparan sulfate, as a potential heparanase inhibitor. The compound **1** is composed of 2-acetamido-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl moiety and 2,6-dideoxy-2,6-imino-L-gulonic acid; i. e. a 1-deoxynojirimycin-like uronic acid.[2] 1-Deoxynojirimycin is well known as one of the potent D-glucosidase inhibitors and has been widely utilized for designing and synthesizing other glycosidase inhibitors.[3] However, such utilizations of the imino gulonic acid, a human liver β-D-glucuronidase inhibitor,[2] have not been reported.[4]

Our synthetic process directed towards **1** involved chemical conversion[5] of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**2**) into a nojirimycin derivative, 2,3-di-*O*-benzyl-*N*-benzyloxycarbonyl-6-*O*-*t*-butyldiphenylsilyl-1,5-dideoxy-1,5-imino-D-glucitol (**11**), as a glycosyl acceptor, its coupling with phenyl 2-azido-3,4-di-*O*-benzyl-2-deoxy-6-*O*-*p*-methoxybenzyl-1-thio-D-glucopyranoside (**12**), as a glycosyl donor, and regioselective manipulations such as imino acid formation and *O*-sulfation. 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**2**) was converted to the 6-*O*-benzoyl-5-*O*-mesyl derivative **3**; mp 133 °C, $[\alpha]_D$ -11°, in 70% overall yield by the sequence: i) allylation of the secondary hydroxyl group using allyl bromide and NaH in *N*,*N*-dimethylformamide (DMF) at 0 °C, ii) selective hydrolysis of the 5,6-*O*-isopropylidene group with 80%AcOH at 60 °C, iii) esterifications of two hydroxyl groups using 1.0 equiv. of benzoyl chloride, then 2.0 equiv. of methanesulfonyl chloride in pyridine-CH_2Cl_2 at -20–0 °C.[6] Methanolysis of **3** with NaOMe in MeOH, followed by oxirane formation with *t*-BuOK in DMF-tetrahydrofuran (THF) at 0–23 °C, gave **4**; $[\alpha]_D$ -52°, in 76 % yield. The epoxide in **4** was opened by the action of sodium azide in aqueous DMF containing NH_4Cl at 80 °C, and the resulting alcohol was benzylated with benzyl bromide (BnBr), NaH and tetrabutylammonium iodide (n-$Bu_4N^+I^-$) in DMF at 0 °C to give **5**; $[\alpha]_D$ -57°, in 85% yield. Compound **5** was treated with 3%HCl in MeOH at room temperature, affording an anomeric mixture of methyl glycosides, which was readily separated



into each isomer {for **6**; $[\alpha]_D$ +88°, δ_H ($CDCl_3$): 4.99 (d, $J_{1,2}$ = 4.9 Hz), 50% yield and for **7**; $[\alpha]_D$ -47°, δ_H: 4.85 (br. s), 47% yield} by silica-gel column chromatography. The isomer **7** was equilibrated (3%HCl-MeOH, rt, 8h) to the anomeric mixture, from which **6** was obtained in 52% yield. By repetition of this procedure two times, compound **6** was synthesized in 80% yield from **5**. Treatment of **6** with triflic anhydride in pyridine-CH_2Cl_2 at -50 °C gave the corresponding triflate, from which the piperidine ring system was formed *via* reduction of the azide group to amine (triphenylphosphine, CH_2Cl_2, 0–40 °C), followed by cyclization through intramolecular displacement of the triflate group {K_2CO_3, H_2O-THF-MeOH (2:2:1), rt}. The bicyclic amine so formed was isolated, upon treatment with benzyloxycarbonyl chloride at 0 °C, as carbamate **8**[7]; $[\alpha]_D$ +29°, in 69% yield from **6**. On the other hand, the stereoisomer **9**; $[\alpha]_D$ -40°, was obtained from **7** in only 8% yield under the same conditions. The formation of the bicyclic amine from **7** as monitored by TLC analysis was sluggish to decompose the intermediary triflate derivative under these conditions. This seems to be due to the severe interaction between the C(6) hydrogen and the methoxy group at C-1 position as well as the 5-*O*-benzyl and 3-*O*-allyl moieties in the transition state as shown in Figure 1. Acidic hydrolysis of **8** or **9** (trifluoroacetic acid, aq. dioxane, rt), followed by reduction ($NaBH_4$, ethanol, 0 °C) gave **10**[8]; $[\alpha]_D$ +7.7°, in 70–71% yield. The piperidine derivative **10** was transformed into the glycosyl acceptor **11**; $[\alpha]_D$ +27°, in 63% overall yield by the sequence: i) silylation of the primary hydroxyl group using *t*-butylchlorodiphenylsilane and imidazole in DMF at room temperature, ii) benzylation of the secondary hydroxyl group using BnBr, NaH and *n*-$Bu_4N^+I^-$ in DMF at 0 °C iii) cleavage of the allyl ether by $PdCl_2$ and NaOAc in aqueous AcOH at 50 °C.

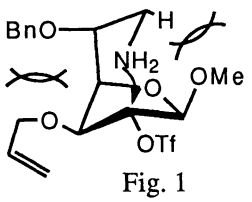
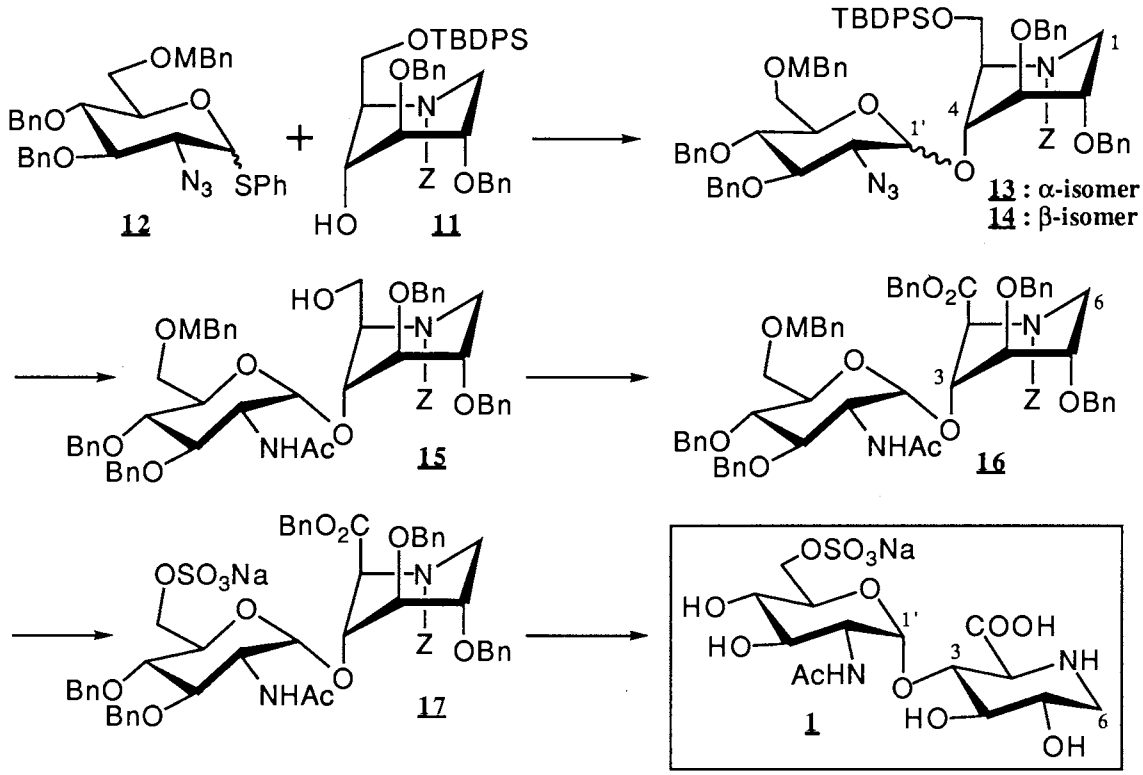


Fig. 1



Glycosyl donor **12** was prepared from 1,6-anhydro-2-azido-3,4-di-*O*-benzyl-2-deoxy-β-D-glucopyranose[9] in 2 steps {1. ZnI_2, (phenylthio)trimethylsilane, 1,2-dichloroethane, rt, 2. *p*-$MeOC_6H_4CH_2Cl$, NaH, *n*-$Bu_4N^+I^-$, DMF, 0 °C}. Coupling reaction of **11** and **12** was conducted with *N*-iodosuccinimide and triflic acid in ether-dichloromethane (5 : 1)[10] at -50 °C to afford α-glycoside **13**; $[\alpha]_D$ +42°, and the corresponding β-isomer **14**; $[\alpha]_D$ +15°, in 77 and 15% yield, respectively. Reaction of **13** with thioacetic acid in pyridine at room temperature gave 2'-acetamido derivative, in which silyl group at C-6 position was removed by treatment with tetrabutylammonium fluoride in the presence of AcOH in THF[11] to give **15**; $[\alpha]_D$ +20°, in 82% yield. Conversion of the hydroxyl group in **15** into the carboxyl function was achieved by Jones oxidation at room temperature, and, after benzylaton (BnBr, Cs_2CO_3, DMF, rt), the benzyl ester **16**, $[\alpha]_D$ +53°, was obtained in 72% yield from **15**. Deprotection of **16** (2,3-dichloro-5,6-dicyano-1,4-benzoquinone, aq. CH_2Cl_2, rt), followed by *O*-sulfation (SO_3·Me_3N, DMF, rt) gave **17**; $[\alpha]_D$ +19°, in 75% yield. Finally, all *O*-benzyl groups were removed by hydrogenolysis with 10% palladium carbon under hydrogen atmosphere in aq. MeOH-AcOH to give **1**[12] in high yield. The results of the examination about the inhibitory activity of **1** against a couple of heparanases will be reported elsewhere.

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6) All stable new compounds gave satisfactory data of elemental analysis. Values of $[\alpha]_D$ were measured in chloroform solution at 23±3 °C if not otherwise noted.

7) All ^{1}H-NMR (400 or 500 MHz, 23 °C) spectra of **8** -**11**, **13**-**17** appears as broadened and complicated signals probably due to the presence of some rotamers. This made difficult the ^{1}H-NMR analyses. However, the difficulty was circumvented by measuring such spectra in d$_6$-dimethyl sulfoxide at the elevated temperature (80-120 °C). **8**. δ_H (110 °C): 3.74 (1H, ddd, $J_{5,6a}$ = 6.8 Hz, $J_{5,6b}$ = 4.9 Hz, $J_{4,5}$ = 2.0 Hz, H-5), 4.36 (1H, ddd, $J_{3,4}$ = 3.9 Hz, $J_{2,4}$ = 2.0 Hz, H-4), 4.76 (1H, brs, H-1); **10**. δ_H (80 °C): 3.32 (1H, dd, $J_{1a,1b}$ = 14 Hz, $J_{1a,2}$ = 3.4 Hz, H-1a), 3.49 (1H, q, $J_{2,3}$ = 4.2 Hz, $J_{1b,2}$ = 3.7 Hz, H-2), 3.53 (1H, dd, $J_{3,4}$ = 5.2 Hz, $J_{4,5}$ = 4.9 Hz, H-4), 3.74 (1H, q, $J_{2,3}$ = 4.2 Hz, H-3), 3.92 (1H, dd, H-1b), 4.00 (1H, q, H-5); **11**. δ_H (80 °C): 3.21 (1H, dd, $J_{1a,1b}$ = 14 Hz, $J_{1a,2}$ = 2.9 Hz, H-1a), 3.54 (1H, dd, $J_{2,3}$ = 3.9 Hz, $J_{3,4}$ = 5.8 Hz, H-3), 3.70 (1H, q, $J_{1b,2}$ = 3.0 Hz, H-2), 3.86 (1H, dd, $J_{6a,6b}$ = 10 Hz, $J_{6a,5}$ = 4.9 Hz, H-6a), 3.92 (1H, dd, $J_{6b,5}$ = 5.4 Hz, H-6b), 3.98 (1H, m, H-4), 4.05 (1H, q, $J_{4,5}$ = 6.3 Hz, H-5), 4.12 (1H, dd, H-1b); **13**. δ_H (100 °C): 3.22 (1H, dd, $J_{1a,1b}$ = 14 Hz, $J_{1a,2}$ = 3.0 Hz, H-1a), 3.44 (1H, dd, $J_{2',3'}$ = 10 Hz, $J_{1',2'}$ = 3.9 H, H-2'), 3.63 (1H, dd, $J_{4',5'}$ = 9.7 Hz, $J_{3',4'}$ = 8.8 Hz, H-4'), 3.74 (1H, dd, H-3'), 3.85 (1H, brs, H-3), 3.92 (1H, $J_{1b,2}$ = 4.5 Hz, H-1b), 4.11 (1H, brt, H-4), 5.17 (1H, d, H-1'); **14**. δ_H (110 °C): 4.52 (1H, d, $J_{1',2'}$ = 7.8 Hz, H-1'); **15**. δ_H (120 °C): 1.58 (3H, s, NAc), 3.35 (1H, dd, $J_{1a,1b}$ = 14 Hz, $J_{1a,2}$ = 2.9 Hz, H-1a), 3.54 (1H, t, $J_{2',NH}$ =$J_{2',3'}$ =9.3 Hz, $J_{1',2'}$ = 3.4 H, H-2'), 4.00 (1H, brt, $J_{3,4}$ = $J_{4,5}$ = 3.0 Hz, H-4), 4.06 (1H, dd, $J_{1b,2}$ = 3.9 Hz, H-1b), 4.88 (1H, d, H-1'); **16**. δ_H (110 °C): 1.62 (3H, s, NAc), 3.54 (1H, t, $J_{2',3'}$ = $J_{3',4'}$ = 9.3 Hz, H-3'), 4.39 (1H, brs, H-3), 4.94 (1H, d, $J_{1',2'}$ = 3.9 Hz, H-1'), 4.99 (1H, brs, H-2).

8) The preferred conformation of a series of deoxynojirimycin derivatives having the benzyloxycarbonyl group seems to be near ^{1}C$_4$ rather than ^{4}C$_1$ form (vide supra, see also Ref. 3).

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11) Deprotection in the absence of AcOH resulted in the product contaminated with a cyclic *N,O*-carbonate produced by intramolecular attack of alkoxide anion to neighboring benzyloxycarbonyl group.

12) $[\alpha]_D$ +84° (H_2O); ^{1}H-NMR (400 MHz; D_2O) δ: 2.07 (3H, s, NAc), 2.98 (1H, dd, $J_{6a,6b}$ = 13 Hz, $J_{6a,5}$ = 8.9 Hz, H-6a), 3.56 (1H, dd, $J_{6b,5}$ = 4.0 Hz, H-6b), 3.61 (1H, dd, $J_{4',5'}$ = 10 Hz, $J_{3',4'}$ = 9.4 Hz, H-4'), 3.65 (1H, d, $J_{2,3}$ = 7.6 Hz, H-2), 3.76 (1H, dd, $J_{2',3'}$ = 10 Hz, H-3'), 3.81 (1H, dd, $J_{4,5}$ = 7.6 Hz, $J_{3,4}$ = 7.3 Hz, H-4), 3.89 (1H, ddd, H-5), 3.96 (1H, brd, H-5'), 3.98 (1H, dd, $J_{1',2'}$ = 3.7 Hz, H-2'), 4.08 (1H, dd, H-3), 4.26 (1H, brd, $J_{6'a,6'b}$ = 11 Hz, H-6'a), 4.34 (1H, dd, $J_{5',6'b}$ = 2.9 Hz, H-6'b), 5.42 (1H, d, H-1'); ^{13}C-NMR (100 MHz; dioxane δ_C 67.4 as an external reference) δ: 22.8 (CH_3), 44.9 (C-6), 54.1 (C-2'), 61.3 (C-2), 67.4 (C-6'), 67.5 (C-5), 69.9 (C-4'), 71.3 (C-5'), 71.6 (C-3'), 72.9 (C-4), 74.3 (C-3), 97.7 (C-1'), 171.4 (*C*OO), 175.2 (NH*C*O).

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