An Efficient Synthesis of the Glycosidase Inhibitor 1,6-Dideoxy-6,6-difluoronojirimycin

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1,6-Dideoxy-6,6-difluoronojirimycin (**1**) was prepared from an easily accessible and commercially available starting material, methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside. Key steps of this synthesis are a difluorination reaction using DAST and a reductive amination. The overall yield of the synthesis is 44 %. Compound **1** has been tested for its activity as an inhibitor of various glucosidases and galactosidases.

Key words: Deoxynojirimycin, Fluorination, Reductive Amination, Glycosidase Inhibitor

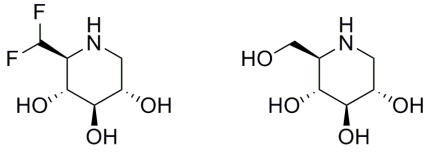
Introduction

Several polyhydroxylated iminosugars are strong and/or specific inhibitors of glycosidases and glycosyltransferases [1, 2]. 1-Deoxynojirimycin (**2**, Fig. 1) is a natural iminosugar, and it has been shown to be an effective inhibitor for various glycosidases [3, 4]. Glycosidases are critical for the normal cellular development of all organisms. Several iminosugar-based compounds have been discussed for the therapeutic treatment of diabetes mellitus, type 1 Gaucher disease and lysosomal storage disorders [5, 6].

Modification of a known iminosugar inhibitor is a promising strategy for obtaining better or more selective inhibitors. Fluorine's special properties (high electronegativity, small size) contribute to its importance in medicinal chemistry. Thus, the effects of fluorine substitution on the biological behavior of bioactive molecules have been used effectively in the development of new drugs. The germinal difluoromethyl group is a strong electron-withdrawing group, and it is an isopolar and isosteric substituent for oxygen [7, 8].

Results and Discussion

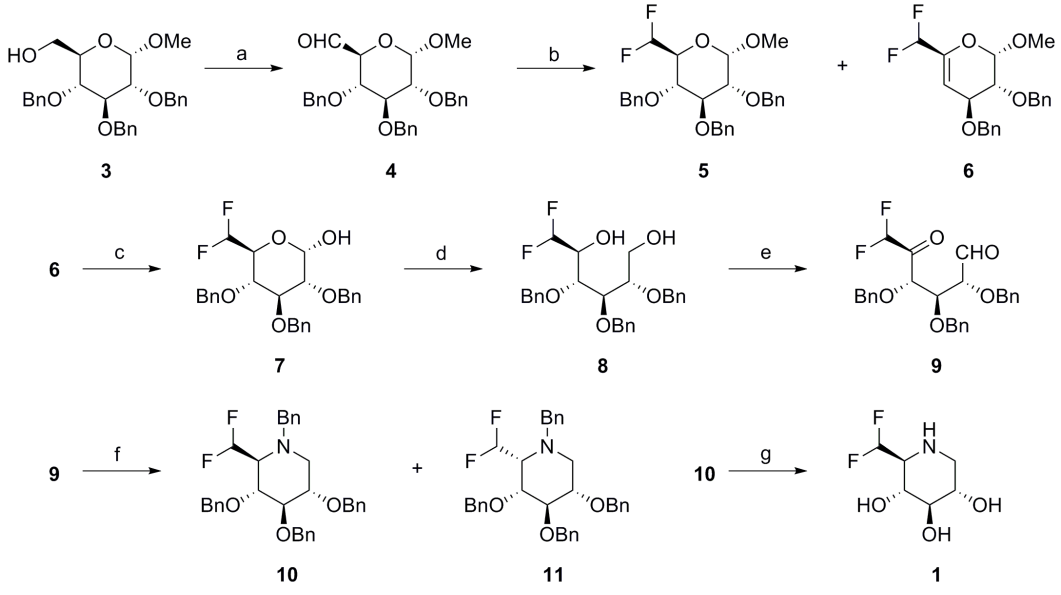
Several years ago, Szarek *et al.* [9] were able to synthesize 1,6-dideoxy-6,6-difluoro-nojirimycin (1,5,6-trideoxy-6,6-difluoro-1,5-imino-D-glucitol) (**1**) starting from L-sorbose; the overall yield of this synthetic approach was 8 %. We became interested in this compound since **1** is a competitive inhibitor [9] for an α-glucosidase (from yeast). To obtain significant amounts of **1** a new strategy for its synthesis had to be developed. Retrosynthetic analysis of **1** revealed a suitably protected gluco-configurated pyranoside having an unprotected primary hydroxyl group at C-6 as an ideal starting material for the synthesis of **1**.



1 **2**

Fig. 1. Structure of deoxynojirimycin (**2**) and its 6,6-difluoro-analog **1**.

Thus, Swern oxidation [10, 11] of commercially available methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**3**, Scheme 1) gave aldehyde **4**. Reaction of crude **4** with DAST in dichloromethane at 25 °C for 4 d yielded 85.5 % of the 6,6-difluoro compound **5**. As a by-product of this fluorination reaction generated in an elimination reaction, **6** was obtained in 6 % yield. Compound **5** is characterized in its ^{19}F NMR spectrum by the presence of two signals centered at δ = −132.46 and −134.97 ppm showing $^2J_{F,F}$ = 283.4 Hz. From this spectrum as well as from the corresponding ^{1}H NMR spectra the coupling constants $^2J_{F^A,H\text{-}6}$ = 54.5, $^2J_{F^B,H\text{-}6}$ = 54.4 Hz, $^3J_{F^A,H\text{-}5}$ = 18.3, and $^3J_{F^B,H\text{-}5}$ =



Scheme 1. a) DMSO, TFAA, −78 °C, 1 h, then NEt_3, −78 → 25 °C, 24 h, quant. (crude product); b) DAST, dichloromethane, 25 °C, 4 d, 85.5 % of **5** and 6 % of **6**; c) H_2SO_4 (4 N), HOAc, 85 °C, 9 d, 79.5 %; d) $LiAlH_4$, THF, 25 °C, 4 h, 99.6 %; e) DMSO, TFAA, −78 °C, 1 h, then NEt_3, −78 °C → 25 °C, 1 h, 75.6 %; f) $BnNH_2$, AcOH, MeOH, −78 °C, then $NaBH_3CN$, 88 % of **10** and 6.9 % of **11**; g) H_2, Pd/C, 35 °C, 4.64 atm, 48 h, 98.9 %.

8.8 Hz were determined. Carbon atom C-6 exhibits $^1J_{C,F}$ = 247.3 and 243.9 Hz couplings, whereas for C-5 $^2J_{C,F}$ = 19.9 and 20.2 Hz were measured.

Reacting **5** for 9 d with a mixture of 4 N sulfuric acid/acetic acid at 85 °C gave 79.5 % of the glucopyranose **7**. During the progress of the reaction the formation of both anomers could be detected by TLC. After work-up, however, only the α-anomer was isolated showing $^3J_{H\text{-}1,H\text{-}2}$ = 3.5 Hz in its ^{1}H NMR spectrum.

Reduction of **7** with $LiAlH_4$ in THF gave an almost quantitative yield of the diol **8** whose Swern oxidation [10, 11] using DMSO/TFAA/NEt_3 gave a 75.6 % yield of the hex-5-ulose **9**. This compound was immediatly used as a starting material for a reductive amination using benzylamine and sodium cyanoborohydride [12]. From this reaction, a mixture of the epimeric compounds **10** and **11** was obtained. The main epimer **10** was hydrogenated in the presence of Pd/C to yield target compound **1** in an almost quantitative yield.

Compound **1** exhibited no significant cytotoxicity as determined in colorimetric sulforhodamine B assays using 9 different human cancer cell lines. The results of an *in vitro* evaluation of **1** to act as an enzyme inhibitor using commercially avaliable glycosidases are compiled in Table 1.

Table 1. Inhibitory activity of **1**. IC_{50} values (in mM) using several representative glycosidases in a *p*-nitrophenolate assay.

Enzyme	Organism	IC_{50} (mM)
α-Glucosidase	*B. stearothermophilus*	1.46
α-Glucosidase	baker's yeast	4.36
β-Glucosidase	almonds	3.07
α-Galactosidase	green coffee beans	3.33
β-Galactosidase	*E. coli*	0.90

Whereas for the α-glucosidases and the galactosidases a competitive inhibition is found, inhibition of the β-galactosidase from almonds takes place by a mixed competitive inhibition.

Experimental Section

General methods

Melting points are uncorrected (Leica hot stage microscope). Optical rotations were obtained using a Perkin-Elmer 341 polarimeter (1 cm micro cell, 20 °C). NMR spectra were recorded using the Varian spectrometers Gemini 200, Gemini 2000 or Unity 500 (δ given in ppm, *J* in Hz, internal Me_4Si or internal CCl_3F), IR spectra (film or KBr pellet) on a Perkin-Elmer FT-IR spectrometer Spectrum 1000. MS spectra were taken on an Intectra GmbH AMD 402 (electron impact, 70 eV) or on a Thermo Electron Finnigan LCQ (electrospray, voltage 4.5 kV, sheath gas nitrogen) instrument. For

elemental analysis a Foss-Heraeus Vario EL instrument was used. TLC was performed on silica gel (Merck 5554, detection by UV absorption or by treatment with a solution of 10 % sulfuric acid, ammonium molybdate and cerium(IV) sulfate) followed by gentle heating. The solvents were dried according to usual procedures.

Biological testing: p-nitrophenolate assay [13]

The *p*-nitrophenolate assay was performed as a microtiter plate assay using a Tecan instrument. The α-glucosidase (from *Bacillus stearothermophilus*) was obtained from Sigma (96 U mg^{-1}; Sigma-Aldrich, Steinheim, Germany), β-glucosidase (almonds) from Fluka (8.92 U mg^{-1}; Sigma-Aldrich), α-galactosidase (green coffee beans) from Sigma (45.6 U mg^{-1}), and the β-galactosidase (165 U mg^{-1}) from Fluka. For all experiments, 0.06 M phosphate buffer (pH = 6.0) was used except for the β-glucosidase where an acetate buffer (0.05 M, pH = 5.0) was applied. Dilution factors of 6.0, 4.0, 3.0, 2.0, 1.0, 0.03, and 0.01 mg mL^{-1} were applied for the inhibitor and 1.0, 0.5, 0.1, 0.05, and 0.025 mM for the 4-nitrophenol. UV measurements were performed at λ = 415 nm at least in triplicate, resulting in an SD of 0.0188 and R^2 = 0.99988.

*Methyl 2,3,4-tri-O-benzyl-α-D-gluco-hexodialdo-1,5-pyranoside (**4**)*

To a solution of dry DMSO (0.93 g, 107.64 mmol) in dry dichloromethane (8.0 mL) at −78 °C, a solution of trifluoroacetic acid anhydride (1.99 mL, 8.49 mmol) in dry dichloromethane (2.0 mL) was added dropwise, and the mixture was stirred at this temperature for 45 min. A solution of **3** (1.00 g, 2.15 mmol) in dry dichloromethane (8.0 mL) was added dropwise maintaining the temperature at −78 °C. The mixture was stirred for 2 h, then a solution of triethylamine (2.2 mL, 15.78 mmol) in dry dichloromethane (8.0 mL) was added dropwise, and the mixture was allowed to warm to 25 °C. After stirring at 25 °C for 12 h, dichloromethane (200 mL) and water (100 mL) were added, the phases were separated, and the organic phase was extracted with water (3 × 100 mL). The combined organic phases were evaporated under reduced pressure, and the remaining residue was subjected to chromatography (silica gel, hexaneethyl acetate 5 : 3) to afford **4** (quant.) as a mixture of aldehyde and its corresponding hydrate; colorless oil. – $[\alpha]_D$ = +55.97° (c = 0.34, $CHCl_3$). – R_f = 0.45 (hexane-ethyl acetate, 5 : 3). – IR (film): ν = 3031m, 2932s, 1704s, 1640w, 1497w, 1454s, 1383m, 1096s, 739m, 698m cm^{-1}. – MS (ESI): *m/z* (%) = 498.1 (5) $[M+NH_4, H_2O]^+$, 503.3 (15) $[M+Na, H_2O]^+$, 512.1 (70) $([M+NH_4, MeOH]^+)$, 517.3 (100) $[M+Na, MeOH]^+$, 1010.8 (80) $([M_2+Na, (MeOH)_2]^+$. – $C_{28}H_{30}O_6$ (462.53): calcd. C 72.71, H 6.54; found C 72.55, H 6.80.

*Methyl 2,3,4-tri-O-benzyl-6-deoxy-6,6-difluoro-α-D-gluco-pyranoside (**5**) and methyl 2,3-di-O-benzyl-4,6-dideoxy-6,6-difluoro-β-L-threo-hex-4-enopyranside (**6**)*

To a solution of **4** (1.00 g, 2.16 mmol) in dry dichloromethane (40 mL), DAST (446 μL, 3.24 mmol) was added, and the mixture was stirred at 25 °C for 4 d. Methanol (5.0 mL) was carefully added, and the solvents were removed under reduced pressure. The oily residue was dissolved in dichloromethane (100 mL) and washed with water (50 mL). The aq. phase was reextracted with dichloromethane (3 × 100 mL); the organic phases were combined, and the solvent was evaporated under reduced pressure. The remaining residue was subjected to chromatography (silica gel, hexane-ethyl acetate, 5 : 3) to afford **5** (890 mg, 85.5 %) and **6** (48 mg, 6 %).

*Data for **5**:* colorless oil. – $[\alpha]_D$ = +10.46° (c = 0.45, $CHCl_3$) (lit.: +9.5° (c = 0.1, $CHCl_3$) [14]). – R_f = 0.80 (hexane-ethyl acetate, 5 : 3). – IR (film): ν = 3388m, 3089m, 3064s, 3032s, 3005m, 2919s, 1954m, 1877w, 1811w, 1732m, 1606m, 1586m, 1497s, 1454s, 1404s, 1361s, 1328s, 1196s, 1156s, 1051s, 918m, 821m, 788m, 737s, 697s, 631m, 554m, 531m, 463m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36 – 7.23 (m, 15 H, Ph), 5.89 (dd, 1 H, $^2J_{6,F}$ = 54.4, $^2J_{6,F}$ = 54.5 Hz, 6-H), 4.99 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.88 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.81 (d, 1 H, ^2J = 10.8 Hz, CH_2OBn), 4.79 (d, 1 H, ^2J = 12.0 Hz, CH_2OBn), 4.64 (d, 1 H, ^2J = 12.0 Hz, CH_2OBn), 4.63 (d, 1 H, $^3J_{1,2}$ = 3.5 Hz, 1-H), 4.59 (d, 1 H, ^2J = 10.8 Hz, CH_2OBn), 4.00 (dd, 1 H, $^3J_{4,3}$ = 9.2, $^3J_{4,5}$ = 9.2 Hz, 4-H), 3.84 (ddd, 1 H, $^3J_{5,F}$ = 8.8, $^3J_{5,4}$ = 9.2, $^3J_{5,F}$ = 18.8 Hz, 5-H), 3.57 (dd, 1 H, $^3J_{3,2}$ = 9.7, $^3J_{3,4}$ = 9.2 Hz, 3-H), 3.53 (dd, 1 H, $^3J_{2,1}$ = 3.5, $^3J_{2,3}$ = 9.7 Hz, 2-H), 3.38 (s, 3 H, OCH_3) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 138.4, 137.8, 137.6, 128.8, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.9, 127.7 (each C_{ar}), 113.7 (dd, $^1J_{6,F}$ = 247.3, $^1J_{6,F}$ = 243.9 Hz, C–6), 98.2 (C-1), 81.7 (C-4), 79.5 (C-2), 77.2 (C-3), 75.8, 75.1, 73.5 (each CH_2OBn), 68.8 (dd, $^2J_{5,F}$ = 19.9, $^2J_{5,F}$ = 20.2 Hz, C–5), 55.4 (OCH_3) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −132.46 (ddd, 1 F, $^3J_{F,5}$ = 8.81, $^2J_{F,6}$ = 54.4, $^2J_{F,F}$ = 283.4 Hz, F^A), −134.97 (ddd, 1 F, $^3J_{F,5}$ = 18.3, $^2J_{F,6}$ = 54.5, $^2J_{F,F}$ = 283.4 Hz, F^B) ppm. – MS (ESI): *m/z* (%) = 502.1 (200) $[M+NH_4]^+$, 507.2 (55) $[M+Na]^+$, 990.5 (20) $[M_2+Na]^+$. – $C_{28}H_{30}O_5F_2$ (484.53): calcd. C 69.41, H 6.24; found C 69.28, H 6.36.

*Data for **6**:* colorless oil. – $[\alpha]_D$ = +118.89° (c = 0.51, $CHCl_3$). – R_f = 0.80 (hexane-ethyl acetate, 5 : 3). – IR (film): ν = 3442m, 3065m, 3032s, 2934s, 1724w, 1694s, 1600w, 1585m, 1497m, 1454s, 1385s, 1346s, 1278s, 1197s, 1158s, 1106s, 1049s, 925m, 811m, 739s, 698s, 650m, 608m, 536m, 462m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 – 7.27 (m, 10 H, Ph), 5.90 (t, 1 H, $^2J_{6,F}$ = 54.4 Hz, 6-H), 5.34 (m, 1 H, 4-H), 4.89 (d, 1 H, $^3J_{1,2}$ = 2.5 Hz, 1-H), 4.80 (d, 1 H, ^2J = 12.2 Hz,

CH_2OBn), 4.73 (d, 1 H, ^2J = 12.2 Hz, CH_2OBn), 4.65 (m, 2 H, 1 H, CH_2OBn), 4.27 (m, 1 H, 3-H), 3.79 (dd, 1 H, $^3J_{2,1}$ = 2.5, $^3J_{2,3}$ = 7.3 Hz, 2-H), 3.48 (s, 3 H, OCH_3) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 143.9 (dd, $^2J_{5,F}$ = 23.5, $^2J_{5,F}$ = 24.0 Hz, C-5), 138.0, 137.9, 137.9, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7 (each C_{ar}), 110.4 (dd, $^1J_{6,F}$ = 239.4, $^1J_{6,F}$ = 239.3 Hz, C-6), 102.6 (dd, $^3J_{6,F}$ = 6.0, $^3J_{6,F}$ = 6.0 Hz, C-4), 99.9 (C-1), 76.0 (C-2), 73.3 ($CH_2(OBn)$), 72.7 (C-3), 71.8 (d, $^5J_{CH_2(OBn),F}$ = 10.6 Hz, ($CH_2(OBn)$), 56.7 (OCH_3) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −122.94 (d, 2 F, $^2J_{F,6}$ = 54.4 Hz, F) ppm. – MS (ESI): *m/z* (%) = 394.3 (100) $[M+NH_4]^+$, 399.3 (88) $[M+Na]^+$, 774.7 (64) $[M_2+Na]^+$. – $C_{21}H_{22}O_4F_2$ (376.39): calcd. C 67.01, H 5.89; found C 66.82, H 6.01.

*2,3,4-Tri-O-benzyl-6-deoxy-6,6-difluoro-α-D-glucopyranose (**7**)*

A mixture of **5** (350 mg, 7.22 mmol), acetic acid (2.56 mL) and 4 N H_2SO_4 (1.44 mL) was heated to 85 °C and the mixture was stirred for 9 d. After cooling to 25 °C the mixture was slowly poured into an ice-cold aqueous solution of sodium hydrogencarbonate (200 mL). The mixture was extracted with chloroform (4 × 100 mL), the combined organic phases were dried (Na_2SO_4), and the solvent was evaporated under reduced presure. The remaining residue was subjected to chromatography (silica gel, hexane-ethyl acetate, 5 : 3) to afford **7** (270 mg, 79.5 %) as a colorless solid. M. p. 118 – 120 °C. – $[\alpha]_D$ = +3.32° (*c* = 0.35, $CHCl_3$); R_f = 0.68 (hexane-ethyl acetate, 5 : 3). – IR (KBr): ν = 3425s, 3064m, 3031m, 2918m, 1748w, 1607w, 1498m, 1454m, 1364m, 1328m, 1218m, 1157m, 1132m, 1098m, 1040s, 910w, 792w, 728m, 694m, 632m, 552w, 528w, 458w cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.29 – 7.17 (m, 15 H, Ph), 5.84 (dd, 1 H, $^2J_{6,F}$ = 54.1, $^2J_{6,F}$ = 54.1 Hz, 6-H), 5.17 (d, 1 H, $^3J_{1,2}$ = 3.5 Hz, 1-H), 4.89 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.81 (d, 1 H, ^2J = 10.8 Hz, CH_2OBn), 4.77 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.71 (d, 1 H, ^2J = 11.8 Hz, CH_2OBn), 4.61 (d, 1 H, ^2J = 11.8 Hz, CH_2OBn), 4.55 (d, 1 H, ^2J = 10.8 Hz, CH_2OBn), 4.07 (dd, 1 H, $^3J_{3,2}$ = 9.4, $^3J_{3,4}$ = 9.2 Hz, 3-H), 3.94 (dd, 1 H, $^3J_{4,3}$ = 9.2, $^3J_{4,5}$ = 9.2 Hz, 4-H), 3.54 (ddd, 1 H, $^3J_{5,4}$ = 9.2, $^3J_{5,F}$ = 9.9, $^3J_{5,F}$ = 16.8 Hz, 5-H), 3.50 (dd, 1 H, $^3J_{2,1}$ = 3.5, $^3J_{2,3}$ = 9.4 Hz, 2-H) ppm. – ^{13}C NMR (100 MHz, $CDCl_3$): δ = 138.4, 137.6, 137.6, 130.8, 128.8, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (each C_{ar}), 113.9 (dd, $^1J_{6,F}$ = 242.8, $^1J_{6,F}$ = 242.8 Hz, C-6), 91.3 (C-1), 81.2 (C-2), 79.7 (C-4), 75.8, 75.1, 73.5 (each CH_2OBn), 71.8 (C-3), 69.3 (dd, $^2J_{5,F}$ = 20.3, $^2J_{5,F}$ = 20.3 Hz, C-5) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −132.23 (ddd, 1 F, $^3J_{F,5}$ = 9.9, $^2J_{F,6}$ = 54.1, $^2J_{F,F}$ = 283.1 Hz, F^A), −134.20 (ddd, 1 F, $^3J_{F,5}$ = 16.8, $^2J_{F,6}$ = 54.1, $^2J_{F,F}$ = 283.1 Hz, F^B) ppm. – MS (ESI): *m/z* (%) = 488.3 (100) $[M+NH_4]^+$, 493.3 (23) $[M+Na]^+$, 962.8 (52) $[M_2+Na]^+$. – $C_{27}H_{28}O_5F_2$ (470.51): calcd. C 68.92, H 5.99; found C 68.79, H 6.12

*2,3,4-Tri-O-benzyl-6-deoxy-6,6-difluoro-D-glucitol (**8**)*

To an ice-cold solution of **7** (370 mg, 0.79 mmol) in abs THF (20 mL), lithium aluminum hydride (118 mg, 3.15 mmol) was added in several portions and the suspension was stirred for 20 min at this temperature and for 4 h at 25 °C. Methanol (1 mL) was carefully added and stirring was continued for another 30 min. The solvents were evaporated under reduced pressure. The remaining residue was dissolved in dichloromethane (100 mL) and was washed with water (30 mL). The aqueous phase was reextracted with dichloromethane (4 × 100 mL), the combined organic phases were dried (Na_2SO_4) and the solvent was evaporated under reduced pressure. The residue was subjected to chromatography (silica gel, hexane-ethyl acetate, 5 : 3) to afford **8** (370 mg, 99.6 %) as a colorless oil. – $[\alpha]_D$ = +5.23° (*c* = 0.74, $CHCl_3$). – R_f = 0.12 (hexane-ethyl acetate, 5 : 3). – IR (KBr): ν = 3418m, 3064m, 3032m, 2929m, 1704m, 1604m, 1497m, 1454m, 1398m, 1211m, 1059s, 1028s, 915w, 737m, 699s, 606w, 462m cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 – 7.26 (m, 15 H, Ph), 5.84 (ddd, 1 H, $^3J_{6,5}$ = 2.7, $^2J_{6,F}$ = 54.9, $^2J_{6,F}$ = 54.9 Hz, 6-H), 4.70 (d, 1 H, ^2J = 11.3 Hz, CH_2OBn), 4.65 (s, 2 H, CH_2OBn), 4.65 (d, 1 H, ^2J = 11.3 Hz, CH_2OBn), 4.57 (s, 2 H, CH_2OBn), 4.01 (dddd, 1 H, $^3J_{5,6}$ = 2.7, $^3J_{5,4}$ = 7.1, $^3J_{5,F}$ = 9.0, $^3J_{5,F}$ = 16.3 Hz, 5-H), 3.90 (dd, 1 H, $^3J_{3,2}$ = 7.1, $^3J_{3,4}$ = 4.2 Hz, 3-H), 3.85 (dd, 1 H, $^3J_{4,3}$ = 4.2, $^3J_{4,5}$ = 7.1 Hz, 4-H), 3.81 (ddd, 1 H, $^3J_{2,1A}$ = 4.5, $^3J_{2,1B}$ = 4.7, $^3J_{2,3}$ = 7.1 Hz, 2-H), 3.79 (dd, 1 H, $^3J_{1B,2}$ = 4.7, $^2J_{1B,1A}$ = 11.9 Hz, 1-H^A), 3.64 (dd, 1 H, $^3J_{1B,2}$ = 4.5, $^2J_{1B,1A}$ = 11.9 Hz, 1-H^A) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 137.7, 137.2, 137.1, 128.8, 128.7, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6 (each C_{ar}), 115.1 (dd, $^1J_{6,F}$ = 243.3, $^1J_{6,F}$ = 243.7 Hz, C-6), 78.7 (C-3), 78.6 (C-4), 75.9 (C-2), 74.4 ($CH_2(OBn)$), 73.2 ($CH_2(OBn)$), 73.2 ($CH_2(OBn)$), 70.8 (dd, $^2J_{5,F}$ = 21.6, $^2J_{5,F}$ = 21.5 Hz, C-5), 61.6 (C-1) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −130.80 (ddd, 1 F, $^3J_{F,5}$ = 9.0, $^2J_{F,6}$ = 54.9, $^2J_{F,F}$ = 286.5 Hz, F^A), −133.28 (ddd, 1 F, $^3J_{F,5}$ = 16.3, $^2J_{F,6}$ = 55.9, $^2J_{F,F}$ = 286.5 Hz, F^B) ppm. – MS (ESI): *m/z* (%) = 473.2 (5) $[M+H]^+$, 490.3 (16) $[M+NH_4]^+$, 495.4 (38) $[M+Na]^+$, 966.8 (100) $[M_2+Na]^+$. – $C_{27}H_{30}O_5F_2$ (472.52): calcd. C 68.63, H 6.40; found C 68.50, H 6.61.

*2,3,4-Tri-O-benzyl-6-deoxy-6,6-difluoro-D-xylo-hexos-5-ulose (**9**)*

To a mixture of DMSO (1.05 g, 13.53 mmol) and dry dichloromethane (9.0 mL) at −78 °C a solution of

trifluoroacetic anhydride (1.91 mL, 9.36 mmol) in dry dichloromethane (2.2 mL) was added, and the mixture was stirred for 45 min at −78 °C. A solution of **8** (560 mg, 1.19 mmol) in dry dichloromethane (9.0 mL) was slowly added, and stirring was continued at −78 °C for 2 h. A solution of triethylamine (2.46 mL, 1.81 mmol) in dry dichloromethane (9.0 mL) was added, and stirring was continued for another 1 h. Dichloromethane (50 mL) and water (20 mL) were added, and the aqueous phase was extracted with dichloromethane (3 × 50 mL), the combined organic phases were dried (Na_2SO_4) and the solvents were evaporated under reduced pressure. The remaining residue was subjected to chromatography (silica gel, hexane-ethyl acetate, 5 : 3) to afford **9** (787 mg, 75.6 %) as a colorless oil that was used immediately for the next reaction step. – $[\alpha]_D$ = +31.15° (*c* = 0.27, MeOH). – R_f = 0.55 (hexane-ethyl acetate, 5 : 3). – IR (film): ν = 3033w, 2923w, 1770m, 1498w, 1455m, 1362w, 1211m, 1092s, 739m, 699w, 605w cm^{-1}. – MS (ESI, MeOH): *m/z* (%) = 486.3 (12) $[M+NH_4]^+$, 491.4 (18) $[M+Na]^+$, 504.2 (14) $[M+NH_4, H_2O]^+$, 509.4 (24) $[M+Na, H_2O]^+$, 518.3 (38) $[M+NH_4, MeOH]^+$, 523.4 (68) $[M+Na, MeOH]^+$, 958.7 (22) $[M_2+Na]^+$, 990.0 (20) $[M_2+NH_4, (H_2O)_2]^+$, 1008.8 (34) $[M_2+NH_4, (H_2O)_2]^+$, 1022.7 (100) $[M_2+Na, (MeOH)_2]^+$. – MS (ESI, EtOH): *m/z* (%) = 491.4 (25) $[M+Na]^+$, 531.5 (33) $[M+NH_4, EtOH]^+$, 537.5 (53) $[M+Na, EtOH]^+$, 553.3 (30) $[M+K, EtOH]^+$, 599.4 (100) $[M+K, (EtOH)_2]^+$.

*2,3,4-Tri-O-benzyl-1,6-dideoxy-6,6-difluoro-nojirimycin (**10**) and 2,3,4-tri-O-benzyl-1,6-dideoxy-6,6-difluoro-L-idonojirimycin (**11**)*

To a solution of **9** (250 mg, 0.53 mmol) in dry methanol (5.0 mL), sodium sulfate (650 mg) was added, and the solution was cooled to −78 °C. A solution of benzylamine (70 μL, 0.65 mmol) and acetic acid (111 μL, 1.92 mmol) in dry methanol (5.0 mL) was added, and the mixture was stirred for 2 h at this temperature. Sodium cyanoborohydride (74 mg, 1.17 mmol) was added in small portions, and the suspension was stirred for another 2 h at −78 °C. The solution was allowed to warm to 25 °C, and stirring was continued for additional 24 h followed by 48 h at 50 °C. Methanol (20 mL) was added, and the suspension was filtered through a thin layer of silica gel. The solvents were evaporated, the remaining residue was dissolved in dichloromethane (50 mL) and washed with an aqueous solution of sodium hydrogen carbonate and sodium chloride (satd., 20 mL each). The organic layer was dried (Na_2SO_4), the solvents were evaporated, and the remaining residue was subjected to chromatography (silica gel, hexane-ethyl acetate, 85 : 15) to afford **10** (220 mg, 88.0 %) and **11** (20 mg, 6.9 %).

*Data for **10**:* colorless oil. – $[\alpha]_D$ = +85.56° (*c* = 0.29, MeOH). – R_f = 0.56 (hexane-ethyl acetate, 85 : 15). – IR (film): ν = 3081w, 2883w, 2225w, 1919s, 1879s, 1653w, 1511w, 1323w, 1285m, 1229w, 969m, 735w, 617w, 577w, 564w, 509m, 451s cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.37 – 7.22 (m, 20 H, Ph), 6.22 (dt, 1 H, $^3J_{6,5}$ = 2.0, $^2J_{6,F}$ = 54.3 Hz, 6-H), 4.89 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.86 (d, 1 H, ^2J = 10.9 Hz, CH_2OBn), 4.71 (d, 1 H, ^2J = 11.5 Hz, CH_2OBn), 4.63 (d, 1 H, ^2J = 11.5 Hz, CH_2OBn), 4.61 (d, 1 H, ^2J = 11.5 Hz, CH_2OBn), 4.55 (d, 1 H, ^2J = 11.5 Hz, CH_2OBn), 4.02 (d, 1 H, ^2J = 14.2 Hz, CH_2NBn), 3.92 (d, 1 H, ^2J = 14.2 Hz, CH_2NBn), 3.84 – 3.77 (m, 2 H, 4-H, 3-H), 3.58 (ddd, 1 H, $^3J_{2,1A}$ = 7.0, $^3J_{2,1B}$ = 11.1, $^3J_{2,3}$ = 7.2 Hz, 2-H), 3.27 (m, 1 H, 5-H), 2.95 (dd, 1 H, $^3J_{1B,2}$ = 11.1, $^2J_{1B,1A}$ = 11.4 Hz, 1-H^B), 2.87 (dd, 1 H, $^3J_{1A,2}$ = 7.0, $^2J_{1A,1B}$ = 11.4 Hz, 1-H^A) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 139.0 (Ph (NBn)), 138.9, 138.4, 138.4, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2 (each C_{ar}), 119.1 (dd, $^1J_{6,F}$ = 245.7, $^1J_{6,F}$ = 248.1 Hz, C–6), 83.0 (C-3), 78.8 (d, $J_{C,F}$ = 5.3 Hz, C-4), 78.5 (C-2), 75.5 (CH_2OBn), 73.6 (CH_2OBn), 72.7 (CH_2OBn), 58.9 (t, $^1J_{5,F}$ = 18.2 Hz, C–5), 59.2 (CH_2NBn), 49.4 (d, $J_{1,F}$ = 3.4 Hz, C–1) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −119.93 (dt, 2 F, $^3J_{F,5}$ = 16.0, $^2J_{F,6}$ = 54.3 Hz, F) ppm. – MS (ESI): *m/z* (%) = 544.3 (100) $[M+H]^+$, 566.3 (18) $[M+Na]^+$, 1086.1 (1) $[M_2+H]^+$. – $C_{34}H_{35}O_3F_2N$ (543.64): calcd. C 75.12, H 6.49, N 2.58; found C 75.98, H 6.57, N 2.43.

*Data for **11**:* colorless oil. – $[\alpha]_D$ = +0.06° (*c* = 0.30, MeOH). – R_f = 0.52 (hexane-ethyl acetate, 85 : 15). – IR (film): ν = 2999w, 2887w, 2014w, 1649w, 1519w, 1407w, 1363m, 1272w, 1165w, 1109w, 956s, 920s, 800w, 692w, 617s, 578s cm^{-1}. – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.34 – 7.19 (m, 20 H, Ph), 6.04 (ddd, 1 H, $^3J_{6,5}$ = 1.8, $^2J_{6,F}$ = 55.5, $^2J_{6,F}$ = 55.3 Hz, 6-H), 4.87 (d, 1 H, ^2J = 10.7 Hz, CH_2OBn), 4.85 (d, 1 H, ^2J = 10.7 Hz, CH_2OBn), 4.74 (d, 1 H, ^2J = 11.1 Hz, CH_2OBn), 4.58 (d, 1 H, ^2J = 11.1 Hz, CH_2OBn), 4.50 (d, 1 H, ^2J = 11.7 Hz, CH_2OBn), 4.45 (d, 1 H, ^2J = 11.7 Hz, CH_2OBn), 4.14 (d, 1 H, ^2J = 13.6 Hz, CH_2NBn), 3.69 – 3.56 (m, 3 H, 4-H, 3-H, 2-H), 3.56 (d, 1 H, ^2J = 13.6 Hz, CH_2NBn), 2.98 (dd, 1 H, $^3J_{1B,2}$ = 4.6, $^2J_{1B,1A}$ = 12.1, 1-H^B), 2.93 (m, 1 H, 5-H), 2.23 (dd, 1 H, $^3J_{1A,2}$ = 9.5, $^2J_{1A,1B}$ = 12.1, 1-H^A) ppm. – ^{13}C NMR (125 MHz, $CDCl_3$): δ = 138.4 (Ph (NBn)), 138.4, 128.1, 137.8, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 127.2, 127.1 (each C_{ar}), 115.8 (dd, $^1J_{6,F}$ = 245.7, $^1J_{6,F}$ = 245.2 Hz, C–6), 84.9 (C–3), 77.7 (C-2), 76.8 (d, $J_{C,F}$ = 5.3 Hz, C-4), 74.6, 74.4, 72.1 (each CH_2OBn), 65.9 (dd, $^1J_{5,F}$ = 19.2, $^1J_{5,F}$ = 19.2 Hz, C–5), 58.0 (CH_2NBn), 51.4 (C-1) ppm. – ^{19}F NMR (188 MHz, $CDCl_3$): δ = −119.9 (ddd, 1 F, $^3J_{F,5}$ = 9.3, $^2J_{F,6}$ = 55.5, $^2J_{F,F}$ = 280.7 Hz, F^A), −129.4 (ddd, 1 F, $^3J_{F,5}$ = 18.1, $^2J_{F,6}$ = 55.3, $^2J_{F,F}$ = 280.7 Hz, F^B) ppm. – MS (ESI): *m/z* (%) = 544.3 (100) $[M+H]^+$, 566.3 (20) $[M+Na]^+$. – $C_{34}H_{35}O_3F_2N$ (543.64): calcd. C 75.12, H 6.49, N 2.58; found C 74.96, H 6.70, N 2.27

*1,6-Dideoxy-6,6-difluoro-nojirimycin (1,5,6-trideoxy-6,6-difluoro-1,5-imino-D-glucitol) (**1**)*

A solution of **10** (180 mg, 0.34 mmol) in dry methanol (25.0 mL) containing palladium on charcoal (10 %, 210 mg) was hydrogenated (35 °C, 4.64 atm, 48 h). The solution was filtered through a Celite pad, and this pad was washed with methanol (3 × 25 mL). The combined organic phases were dried ($MgSO_4$), the solvent was removed under reduced pressure, and the residue was subjected to chromatography (silica gel, methanol-ethyl acetate, 20 : 80) to afford **1** (60 mg, 98.9 %) as a colorless foam. – $[\alpha]_D$ = +29.48° (*c* = 0.3, MeOH); (lit.: $[\alpha]_D$ = +31.3° (*c* = 0.5, MeOH) [9]); R_f = 0.38 (methanol-ethyl acetate = 20 : 80). – IR (film): ν = 3457w, 2449w, 2171w, 2015w, 1563w, 1325s, 1245s, 1010m, 939w, 861m, 705m, 628w cm^{-1}. – ^{1}H NMR (500 MHz, CD_3OD): δ = 6.02 (ddd, 1 H, $^3J_{6,5}$ = 6.6, $^2J_{6,F}$ = 55.9, $^2J_{6,F}$ = 56.6 Hz, 6-H), 3.89 (m, 1 H, 2-H), 3.85 (m, 1 H, 3-H), 3.77 (m, 1 H, 4-H), 3.34 (m, 1 H, 5-H), 3.20 (dd, 1 H, $^3J_{1B,2}$ = 1.9, $^2J_{1B,1A}$ = 13.0 Hz, 1-H^B), 3.07 (dd, 1 H, $^3J_{1A,2}$ = 2.0, $^2J_{1A,1B}$ = 13.1 Hz, 1-H^A) ppm. – ^{13}C NMR (125 MHz, CD_3OD): δ = 116.6 (dd, $^1J_{6,F}$ = 240.9, $^1J_{6,F}$ = 240.4 Hz, C-6), 69.6 (d, $J_{C,F}$ = 4.9 Hz, C-4), 69.1 (C-3), 68.2 (C-2), 58.8 (dd, $^2J_{5,F}$ = 20.6, $^2J_{5,F}$ = 20.6 Hz, C-5), 46.7 (C-1) ppm. – ^{19}F NMR (188 MHz, CD_3OD): δ = −128.77 (dd, 1 F, $^2J_{F,6}$ = 55.2, $^2J_{F,F}$ = 297.7 Hz, F^A), −131.59 (dd, 1 F, $^2J_{F,6}$ = 57.1, $^2J_{F,F}$ = 298.5 Hz, F^B) ppm. – MS (ESI): *m/z* (%) = 184.3 (100) $[M+H]^+$. – $C_6H_{11}O_3F_2N$ (183.15): calcd. C 39.35, H 6.05, N 7.65; found C 39.27, H 6.18, N 7.56.

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