Supplementary Material for

Stereoselective Synthesis of 2-Acetamido-1,2-dideoxynojirimycin (DNJNAc) and Ureido-DNJNAc Derivatives as New Hexosaminidase Inhibitors

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1.- Experimental part.

1.- Synthesis of 3-acetamido-1,3-dideoxyaltronojirimycin (29)

3-Azido-2,4-di-O-benzyl-5N,6O-(cyclic carbamate)-1,3-dideoxyaltronojirimycin (26)

A solution of 20 (278 mg, 0.91 mmol) in DMF (5 mL) was added via cannula to a suspension of NaH (51 mg, 2.01 mmol) in DMF (5 mL) cooled at 0°C. After 10 min, benzyl bromide (0.17 mL, 1.37 mmol) was added dropwise, and the reaction was allowed to stir at r.t. until no starting material was observed by TLC. H2O (5 mL) was then added and the crude was extracted with CH2Cl2 (3x 5 mL), dried over MgSO4, and purified by chromatography on silica gel using hexane/EtOAc to give 26 (306 mg, 84%) as a colorless oil. [α]20D = +31.4 (c=0.64, CHCl3). 1H-NMR (400 MHz, CDCl3, δ/ppm): 7.40 − 7.27 (m, 10H), 4.73 (d, J=12.0 Hz, 1H), 4.65 (d, J=11.5 Hz, 1H), 4.48 (d, J = 11.5 Hz, 1H), 4.36 (d, J = 12.0 Hz, 1H), 4.32 (m, 1H), 4.11 (m, 1H), 4.07 – 4.00 (m, 1H), 3.94 (d, J = 15.0 Hz, 1H), 3.88 (m, 2H), 3.63 (m, 1H), 3.06 (dd, J = 15.0, 1.5 Hz, 1H). 13C-NMR (100 MHz, CDCl3, δ/ppm): 157.7 (CO), 137.1 (C), 136.7 (s), 128.7 (s), 128.4 (CH), 128.4 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 75.0 (CH), 73.6 (CH), 71.9 (CH2), 70.9 (CH2), 65.4 (CH2), 59.3 (CH), 52.9 (CH), 38.0 (CH2). IR (film, νmax / cm⁻¹): 3031, 2912, 2107, 1755, 1454, 1418, 1235, 1070. HRMS (ES): calcd. for C23H27N2O5: 395.17138, found 395.17222

3-Acetamido-2,4-di-O-benzyl-5N,6O-(cyclic carbamate)-1,3-dideoxyaltronojirimycin (27)

Pd/C (49 mg, 0.05 mmol) was added to a solution of 26 (305 mg, 0.77 mmol) in EtOAc (7 mL) and the reaction was charged with H2 (5 barg) and stirred at r.t. for 4h. Palladium was filtered over Celite and solvents were removed under low pressure. The colorless oil obtained was dissolved in pyridine (2 mL) and Ac2O (122 μL, 1.16 mmol) was added. The reaction was stirred at r.t. for 16h. H2O (5 mL) was then added and the crude was extracted with CH2Cl2 (3x 5 mL), dried over MgSO4, and purified by chromatography on silica gel using hexane/EtOAc to give 27 (258 mg, 81%) as a white foam. [α]20D = +19.5 (c=0.2, CHCl3). Mp: 70-72 °C. 1H-NMR (400 MHz, CDCl3, δ/ppm): 7.38-7.22 (m, 10H), 5.86 (d, J = 5.5 Hz, 1H), 4.72 (d, J = 11.5 Hz, 1H), 4.61 (q, J = 4.5 Hz, 1H), 4.57 (d, J = 11.5, 1H), 4.45 (d, J = 11.5 Hz, 1H), 4.37 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 11.5 Hz, 1H), 4.05 (m, 2H), 3.95 (d, J = 14.5 Hz, 1H), 3.90 (dd, J = 10.0, 4.5 Hz, 1H), 3.67 (ddd, J = 10.0, 8.0, 4.5 Hz, 1H), 3.06 (dd, J = 14.5, 1.5 Hz, 1H), 2.00 (s, 3H). 13C-NMR (100 MHz, CDCl3, δ/ppm): 171.1 (CO), 157.7 (CO), 137.5 (C), 136.7 (C), 128.7 (CH), 128.4 (CH), 128.4 (CH), 128.2 (CH), 127.9 (CH), 73.0 (CH), 72.5 (CH), 71.3 (CH2), 70.7 (CH2), 66.2 (CH2), 52.9 (CH), 48.0 (CH), 38.7 (CH2), 23.3 (CH3). IR (film, νmax / cm⁻¹): 3325, 2918, 1758, 1658, 1547, 1104, 1071. HRMS (ES): calcd. for C23H27N2O5: 411.19145, found 411.19126
3-Acetamido-2,4-di-O-benzyl-1,3-dideoxyaltronojirimycin (28)

NaOH 6M (0.25 mL, 1.53 mmol) was added to a solution of 27 (63 mg, 0.15 mmol) in MeOH : H2O 9:1 (5 mL) and the reaction was stirred at reflux for 4 h. H2O (5 mL) was then added and the crude was extracted with EtOAc (3x 5 mL), dried over MgSO4 and purified by chromatography on silica gel using CH2Cl2/MeOH to give 28 (56 mg, 94%) as a colorless oil. \([\alpha]^{20}_D = -5.4 \text{ (c}=0.55, \text{ CH}_3\text{OH}).\]

\(^1\)H-NMR (400 MHz, CD3OD, \(\delta/\text{ppm}\)): 7.40 – 7.25 (m, 10H), 4.65 (m, 3H), 4.54 (d, \(J = 11.5\) Hz, 1H), 4.44 (d, \(J = 11.5\) Hz, 1H), 3.93 (m, \(J = 6.5\) Hz, 3H), 3.74 (dd, \(J = 11.5, 6.5\) Hz, 1H), 3.41 (m, 1H), 3.27 (dd, \(J = 13.5, 2.0\) Hz, 1H), 3.17 (d, \(J = 13.5\) Hz, 1H), 1.99 (s, 3H).

\(^13\)C-NMR (100 MHz, CD3OD, \(\delta/\text{ppm}\)): 173.8 (CO), 138.9 (C), 138.5 (CH), 129.5 (CH), 129.4 (CH), 129.2 (CH), 129.1 (CH), 129.0 (CH), 73.7 (CH), 73.0 (CH2), 72.0 (CH2), 70.6 (CH), 59.6 (CH2), 57.0 (CH), 47.5 (CH), 43.9 (CH2), 22.6 (CH3). IR (film, \(\nu_{\text{max}}/\text{cm}^{-1}\)): 3641, 3212, 3065, 1653, 1454, 1247, 1169, 1030. HRMS (ES): calcd. for \(\text{C}_{22}\text{H}_{29}\text{N}_{2}\text{O}_{4}\): 385.2122, found 385.2111

3-Acetamido-1,3-dideoxyaltronojirimycin (29)

Pd/C (44 mg, 0.04 mmol) was added to a solution of 28 (200 mg, 0.52 mmol) in previously degassed MeOH (10 mL) was added Pd/C (44 mg, 0.04 mmol). The reaction was charged with \(\text{H}_2\) (20 barg) and stirred at 60ºC for 20h. Palladium was then filtered through Celite and the crude was purified by chromatography on silica gel using CH2Cl2/MeOH/NH3 72.5:25:2.5 to give 29 (82 mg, 77%) as a slightly yellow sticky foam. \([\alpha]^{20}_D = -16.5 \text{ (c}=0.42, \text{ MeOH}).\]

\(^1\)H-NMR (400 MHz, D2O, \(\delta/\text{ppm}\)): 4.13 (dd, \(J = 6.0, 4.0\) Hz, 1H), 4.00 (dd, \(J = 7.5, 4.0\) Hz, 1H), 3.88 (td, \(J = 6.0, 3.0\) Hz, 1H), 3.82 – 3.74 (m, 2H), 3.04 – 2.89 (m, 2H), 2.78 (dd, \(J = 14.0, 6.0\) Hz, 1H), 2.06 (s, 3H). \(^13\)C-NMR (100 MHz, D2O, \(\delta/\text{ppm}\), d6-DMSO internal reference): 176.1 (CO), 68.6 (CH), 66.8 (CH), 61.4 (CH2), 58.7 (CH), 54.5 (CH), 46.4 (CH2), 23.6 (CH3). IR (film, \(\nu_{\text{max}}/\text{cm}^{-1}\)): 3311, 2930, 1650, 1549, 1376, 1299, 1068. HRMS (ES): calcd. for \(\text{C}_8\text{H}_{17}\text{N}_{2}\text{O}_{4}\): 205.11828, found 205.11806
2.- Preparation of compounds 32b-d.

2-Acetamido-3,4,6-tri-O-acetyl-1,2-dideoxy-5-N-(N’-octylaminocarbonyl)nojirimycin (32b)

TFA (0.53 mL, 6.92 mmol) was added to a solution of 31 (99 mg, 0.23 mmol) in CH₂Cl₂ (8 mL) and the reaction was stirred at r.t. until no starting material was observed by TLC. Solvent was removed under low pressure and the resulting oil was dissolved in CH₂Cl₂ (8 mL). TEA (0.25 mL, 1.79 mmol) and octyl isocyanate (122 µl, 0.69 mmol) were added and the reaction was heated at reflux for 4h. H₂O (5 mL) was then added and the reaction was extracted with CH₂Cl₂ (3x 5 mL), dried over MgSO₄, and purified by chromatography on silica gel using CH₂Cl₂/MeOH to give 32b (71 mg, 85%) as a colorless oil. [α]²⁰°D = -57.2 (c=2.01, CHCl₃). ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 6.52 (d, J = 7.5 Hz, 1H), 5.10 – 4.98 (m, 2H), 4.91 (m, 1H), 4.47 (dd, J = 11.0, 7.5 Hz, 1H), 4.26 (td, J = 7.0, 2.0 Hz, 1H), 4.20 – 4.11 (m, 1H), 4.07 (m, 1H), 3.98 (d, J = 14.0 Hz, 1H), 3.30 (dd, J = 14.5, 3.0 Hz, 1H), 3.22 (m, 2H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 1.98 (s, 3H), 1.50 (t, J = 7.0 Hz, 2H), 1.29 (m, 10H), 0.88 (t, J = 6.5 Hz, 3H). ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 171.0 (CO), 169.6 (CO), 168.8 (CO), 168.7 (CO), 159.1 (CO), 68.1 (CH), 67.0 (CH), 61.0 (CH₂), 53.9 (CH), 46.6 (CH), 41.1 (CH₃), 39.1 (CH₂), 31.7 (CH₃), 30.0 (CH₂), 29.2 (CH₂), 29.19 (CH₂), 26.8 (CH₂), 23.2 (CH₂), 22.5 (CH₂), 20.8 (CH₃), 20.8 (CH₃), 20.7 (CH₃), 14.0 (CH₃). IR (film, νmax / cm⁻¹): 3359, 2936, 2846, 1758, 1649, 1521, 1373, 1213, 1040. HRMS (ES): calcld. for C₂₃H₄₆N₄O₈: 486.28099, found 486.28081

2-Acetamido-3,4,6-tri-O-acetyl-1,2-dideoxy-5-N-(N’-phenylaminocarbonyl)nojirimycin (32c)

TFA (0.41 mL, 5.18 mmol) was added to a solution of 31 (75 mg, 0.17 mmol) in CH₂Cl₂ (8 mL) and the reaction was stirred at r.t. until no starting material was observed by TLC. Solvent was removed under reduced pressure and the resulting oil was dissolved in CH₂Cl₂ (8 mL). TEA (0.19 mL, 1.35 mmol) and phenyl isocyanate (56 µl, 0.52 mmol) were added and the reaction was heated at reflux for 4h. H₂O (5 mL) was then added and the reaction was extracted with CH₂Cl₂ (3x 5 mL), dried over MgSO₄, and purified by chromatography on silica gel using CH₂Cl₂/MeOH to give 32c (63 mg, 80%) as a white solid. [α]²⁰°D = -73.0 (c=0.24, CHCl₃). Mp: 79-82 °C. ¹H-NMR (400 MHz, CDCl₃, δ/ppm): 7.44 (m, 2H), 7.30 (m, 3H), 7.07 (tt, J =7.0, 1.0 Hz, 1H), 6.46 (d, J = 7.5 Hz, 1H), 5.07 (t, J = 3.5 Hz, 1H), 4.97 (m, 1H), 4.54 (dd, J = 11.5, 7.0 Hz, 1H), 4.42 (t, J = 7.5 Hz, 1H), 4.24 (dd, J = 11.5, 7.5 Hz, 1H), 4.17 (dt, J=14.5, 1.5 Hz,1H) 4.12 (q, J = 3.5 Hz, 1H), 3.37 (dd, J = 14.5, 3.0 Hz, 1H), 2.15 (s, 6H), 2.07 (s, 3H), 1.98 (s, 3H). ¹³C-NMR (100 MHz, CDCl₃, δ/ppm): 171.7 (CO), 170.0 (CO), 169.0 (CO), 168.8 (CO), 156.4 (CO), 138.9 (C), 129.1 (CH), 123.6 (CH), 119.7 (CH), 68.2 (CH), 67.3 (CH), 61.5 (CH₂), 54.1 (CH), 46.8 (CH), 39.4 (CH₂), 23.5 (CH₃), 21.1 (CH₃), 21.0 (CH₃), 20.9 (CH₃). IR (film, νmax / cm⁻¹): 3333, 3013, 2928, 1746, 1662, 1537, 1444, 1370, 1232. HRMS (ES): calcld. for C₂₃H₂₈N₄O₈: 450.18709, found 450.18715

2-Acetamido-3,4,6-tri-O-acetyl-5-N-(N’-benzylaminocarbonyl)-1,2-dideoxynojirimycin (32d)

TFA (0.39 mL, 5.15 mmol) was added to a solution of 31 (74 mg, 0.17 mmol) in CH₂Cl₂ (8 mL) and the reaction was stirred at r.t. until no starting material was observed by TLC. Solvent was removed under
low pressure and the resulting oil was dissolved in CH$_2$Cl$_2$ (8 mL). TEA (0.19 mL, 1.33 mmol) and benzyl isocyanate (63 µL, 0.52 mmol) were added and the reaction was heated at reflux for 4h. H$_2$O (5 mL) was then added and the reaction was extracted with CH$_2$Cl$_2$ (3x 5 mL), dried over MgSO$_4$ and purified by chromatography on silica gel using CH$_2$Cl$_2$/MeOH to give 32d (56 mg, 70%) as a colorless oil. [α]$^{20}_{D}$ = -55.0 (c=1.30, CHCl$_3$). \( ^1H \)-NMR (400 MHz, CDCl$_3$, δ/ppm): 7.36 – 7.25 (m, 5H), 6.46 (d, \( J = 7.5 \) Hz, 1H), 5.40 (t, \( J = 5.5 \) Hz, 1H), 5.01 (t, \( J = 3.5 \) Hz, 1H), 4.90 (m, 1H), 4.50 – 4.42 (m, 2H), 4.36 (dd, \( J = 15.0, 5.0 \) Hz, 2H), 4.14 (dd, \( J = 11.5, 6.5 \) Hz, 1H), 4.06 (dd, \( J = 7.0, 3.5 \) Hz, 1H), 4.00 (d, \( J = 15.0 \) Hz, 1H), 3.33 (dd, \( J = 15.0, 3.5 \) Hz, 1H), 2.11 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H), 1.91 (s, 3H). \( ^13C \)-NMR (100 MHz, CDCl$_3$, δ/ppm): 170.0 (CO), 168.8 (CO), 168.0 (CO), 168.0 (CO), 159.0 (CO), 138.3 (C), 127.7 (CH), 126.7 (CH), 126.5 (CH), 67.2 (CH), 66.2 (CH), 60.0 (CH$_2$), 53.2 (CH), 45.7 (CH), 44.1 (CH$_2$), 38.4 (CH$_2$), 22.3 (CH$_3$), 19.9 (CH$_3$), 19.9 (CH$_3$), 19.7 (CH$_3$). IR (film, \( \nu_{max} / \text{cm}^{-1} \)): 3359, 2927, 1746, 1651, 1532, 1370, 1225, 1043. HRMS (ES): calcd. for C$_{22}$H$_{30}$N$_3$O$_8$: 464.20270, found 464.20274.
3.- Preparation of compounds 10b-d.

2-Acetamido-1,2-dideoxy-5-N-(N’-octylaminocarbonyl)nojirimycin (10b).

32b (71 mg, 0.20 mmol) was dissolved in a NH3 saturated MeOH solution (4 mL) and the reaction was stirred at r.t. for 18 h. Solvent was removed under low pressure, and the crude was purified by chromatography on silica gel using CH2Cl2/MeOH to give 10b (47 mg, 75%) as a slightly yellow solid. [α]20D = +26.9 (c=2.0, CH3OH). Mp: 57-59 °C. 1H-NMR (400 MHz, CD3OD, δ/ppm): 3.95 (m, 1H), 3.92 – 3.80 (m, 3H), 3.79 – 3.70 (m, 2H), 3.61 (t, J = 4.5 Hz, 1H), 3.35 (dd, J = 14.0, 3.0 Hz, 1H), 3.21 – 3.05 (m, 2H), 1.95 (s, 3H), 1.49 (m, 2H), 1.35 – 1.27 (m, 10H), 0.90 (t, J = 7.0 Hz, 3H). 13C-NMR (100 MHz, CD3OD, δ/ppm): 172.6 (CO), 161.7 (CO), 71.6 (CH), 70.2 (CH), 61.9 (CH), 61.7 (CH2), 51.6 (CH), 41.8 (CH2), 40.9 (CH2), 33.0 (CH2), 31.1 (CH2), 30.5 (CH2), 30.4 (CH2), 28.0 (CH2), 23.7 (CH3), 22.9 (CH2), 14.4 (CH3). IR (film, νmax / cm⁻¹): 3333, 2917, 2853, 1623, 1533, 1373. HRMS (ES): calcd. for C17H34N3O5: 360.24930, found 360.24925

2-Acetamido-1,2-dideoxy-5-N-(N’-phenylaminocarbonyl)nojirimycin (10c)

32c (60 mg, 0.13 mmol) was dissolved in a NH3 saturated MeOH solution (4 mL) and the reaction was stirred at r.t. for 18 h. Solvent was removed under low pressure, and the crude was purified by chromatography on silica gel using CH2Cl2/MeOH to give 10c (25 mg, 58%) as a slightly yellow solid. [α]20D = +21.5 (c=1.2, CH3OH). Mp: 61-63 °C. 1H-NMR (400 MHz, CD3OD, δ/ppm): 7.33 – 7.22 (m, 4H), 7.02 – 6.96 (m, 1H), 4.13 (m, 1H), 4.04 – 3.97 (m, 2H), 3.92 (q, J = 3.5 Hz, 1H), 3.82 (dd, J = 11.5, 3.5 Hz, 1H), 3.73 (t, J = 5.0Hz, 1H), 3.66 (t, J = 5.0 Hz, 1H), 3.42 (dd, J = 14.0, 3.5 Hz, 1H), 1.97 (s, 3H). 13C-NMR (100 MHz, CD3OD, δ/ppm): 172.87 (CO), 159.6 (CO), 140.9 (C), 129.6 (CH), 123.8 (CH), 121.4 (CH), 71.5 (CH), 70.4 (CH), 62.5 (CH), 61.9 (CH2), 51.7 (CH), 40.8 (CH2), 22.9(CH3). IR (film, νmax / cm⁻¹): 3227, 2923, 1636, 1533, 1444. HRMS (ES): calcd. for C18H32N3O5: 324.15540, found 324.15551

2-Acetamido-5-N-(N’-benzylaminocarbonyl)-1,2-dideoxy- nojirimycin (10d)

32d (56 mg, 0.12 mmol) was dissolved in a NH3 saturated MeOH solution (4 mL) and the reaction was stirred at r.t. for 18 h. Solvent was removed under low pressure, and the crude was purified by chromatography on silica gel using CH2Cl2/MeOH to give 10d (31 mg, 76%) as a slightly yellow solid. [α]20D = +39.5 (c=1.4, CH3OH). Mp: 56-58 °C. 1H-NMR (400 MHz, CD3OD, δ/ppm): 7.35 – 7.26 (m, 4H), 7.21 (m, 1H), 4.35 (dd, J = 15.0 Hz, 2H), 4.08 – 4.03 (m, 1H), 3.94 – 3.85 (m, 3H), 3.80 – 3.71 (m, 2H), 3.63 (t, J = 4.5 Hz, 1H), 3.40 (dd, J = 15.0, 4.0 Hz, 1H), 1.89 (s, 3H). 13C-NMR (100 MHz, CD3OD, δ/ppm): 172.6 (CO), 161.6 (CO), 141.3 (C), 129.3 (CH), 128.1 (CH), 127.8 (CH), 71.4 (CH), 70.2 (CH), 61.8 (CH), 61.6 (CH2), 51.5 (CH), 45.3 (CH2), 40.8 (CH2), 22.9 (CH3). IR (film, νmax / cm⁻¹): 3353, 2917, 1617, 1533, 1450, 1264, 1059. HRMS (ES): calcd. for C18H24N3O5: 338.17105, found 338.17116.
4. NMR spectra
32c
Lineweaver-Burk Plot for $K_i$ determination (2.1 $\mu$M) of 10c against $\beta$-N-acetylglicosaminidase (human placenta, pH 5.5).
Lineweaver-Burk Plot for $K_i$ determination (4.1 μM) of 10c against β-N-acetylglicosaminidase (bovine kidney, pH 5.5).
Lineweaver-Burk Plot for $K_i$ determination (1.5 µM) of 10c against $\beta$-N-acetylglucosaminidase (Jack bean, pH 5.5).