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Synthesis of 2-acetamido-1,2-dideoxy-D-*galacto*-nojirimycin [DGJNAc] from D-glucuronolactone: the first sub-micromolar inhibitor of α-*N*-acetylgalactosaminidases

Daniel Best [a], Phoom Chairatana [a], Andreas F. G. Glawar [a,b], Elizabeth Crabtree [a,b], Terry D. Butters [b], Francis X. Wilson [e], Chu-Yi Yu [d], Wu-Bao Wang [d], Yue-Mei Jia [d], Isao Adachi [c], Atsushi Kato [c], George W. J. Fleet [a,*]

[a] *Chemistry Research Laboratory, Department of Chemistry, University of Oxford, Mansfield Road, Oxford OX1 3TA, UK*
[b] *Oxford Glycobiology Institute, University of Oxford, South Parks Road, Oxford OX1 3QU, UK*
[c] *Department of Hospital Pharmacy, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan*
[d] *CAS Key Laboratory of Molecular Recognition and Function, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China*
[e] *Summit PLC, 91, Milton Park, Abingdon, Oxon OX14 4RY, UK*

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ABSTRACT

2-Acetamido-1,2-dideoxy-D-*galacto*-nojirimycin [DGJNAc], prepared in 20% overall yield from D-glucuronolactone, is the first potent competitive sub-micromolar inhibitor of α-*N*-acetyl-galactosaminidases (K_i 0.081 μM from chicken liver, K_i 0.136 μM from *Charonia lampas*). DGJNAc is a good competitive—whereas the enantiomer L-DGJNAc is a very weak but non-competitive—inhibitor of β-hexosaminidases.

Iminosugars—in which the ring pyranose or furanose oxygen has been replaced by nitrogen—are the archetypes for interaction with carbohydrate processing enzymes.[1] However, among the myriad of sugar mimics reported, there is not a single example of efficient inhibition of α-*N*-acetyl-galactosaminidases (GalNAcases). This Letter reports DGJNAc [2-acetamido-1,2-dideoxy-D-*galacto*-nojirimycin] **1D** as the first potent, specific and competitive inhibitor of GalNAcases; D-glucuronolactone **2D**, a well-established chiron for the synthesis of many homochiral targets including amino acids[2] and iminosugars,[3] is the starting material for an efficient synthesis of DGJNAc **1D** in an overall yield of 20%. The L-enantiomers of many iminosugars have surprising biological activities compared to their D-natural products.[4] The synthesis of L-DGJNAc **1L** from the readily available[5] L-glucuronolactone **2L** is also reported. The only previous synthesis of **1D** starts from 1-deoxynojirimycin[6] and a racemic mixture of **1D** and **1L** has also been prepared;[7] no investigations of the glycosidase inhibitory properties of **1D** have hitherto been reported. The synthesis of DGJNAc **1D** requires introduction of nitrogen at C5 of the glucuronolactone with inversion of configuration (Scheme 1), epimerization of the hydroxy group at C3 and formation of the piperidine ring by introduction of nitrogen between C6 and C2 (with inversion of configuration).

Selective inhibition of β-hexosaminidases has potential in the study of osteoarthritis,[8] allergy,[9] Alzheimer's disease,[10] *O*-GlcNAcase inhibition,[11] cancer metastasis,[12] type II diabetes,[13] genetic diseases such as Tay-Sachs and Sandhoff diseases,[14] and of plant regulation.[15] The synthetic piperidine analogue of *N*-acetylglucosamine DNJNAc **3**[16] and its *N*-alkyl derivatives[17] are potent inhibitors of β-hexosaminidases. The natural product nagstatin **4**,[18] with a *galacto*-configuration, is not reported to inhibit GalNAcases even though it is a potent inhibitor of β-hexosaminidases.[19] The synthetic analogue with a *gluco*-configuration **5**[20] together with PUG derivative **6**[21] and GlcNAc–thiazoline **7**[22] are very potent inhibitors of β-hexosaminidases. A rare example of a potent pyrrolidine hexosaminidase inhibitor is LABNAc **8**;[23] the first pyrrolizidine β-hexosaminidase inhibitor, pochonicine **9** [or its enantiomer], was isolated from a fungal strain *Pochonia suchlasporia* var. *suchlasporia* TAMA 87 (Fig. 1).[24] Some seven-membered ring iminosugars also display potent inhibition.[25]

In contrast to the diversity of structure of β-hexosaminidase inhibitors, there are no compounds which show significant inhibition of GalNAcases. Studies on reversible binding to exo-GalNAcases may allow the design of chaperones for the treatment of Schindler–Kanzaki disease.[26] Inhibition of *exo*-GalNAcases provides a strategy for the treatment of cancer by the protection of macrophage activating factor.[27] *endo*-GalNAcases may have chemotherapeutic potential in the modification of a number of pathogens.[28]

For the synthesis of DJGNAc **1D** from D-glucuronolactone **2D**, the acetonide **10**[5] was esterified with trifluoromethanesulfonic (triflic) anhydride in dichloromethane in the presence of pyridine and the resulting crude triflate was treated with sodium azide in DMF to give the *ido*-azide **11**, mp 112–114 °C; $[\alpha]_D^{25}$ +261.4 (*c* 1.0,

* Corresponding author.
E-mail address: george.fleet@chem.ox.ac.uk (G.W.J. Fleet).

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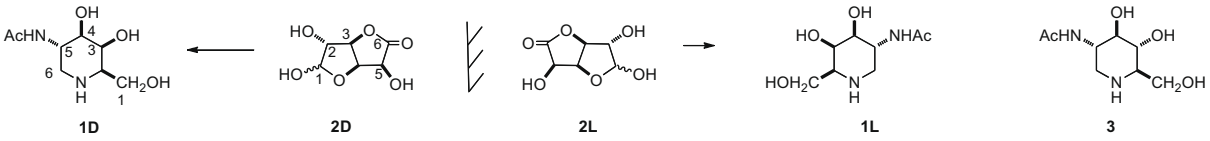


Scheme 1. Strategy for the synthesis of DGJNAc **1** enantiomers – numbering of C in DGJNAc derived from that in glucuronolactone **2**.

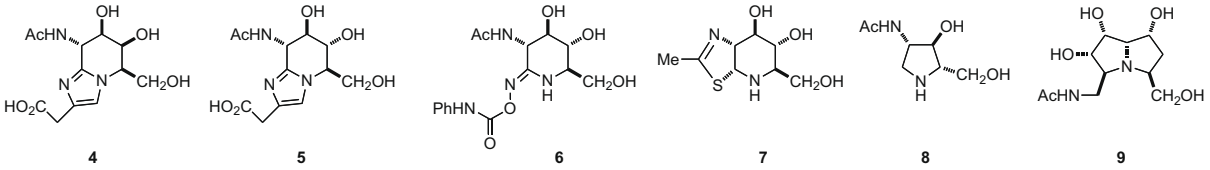
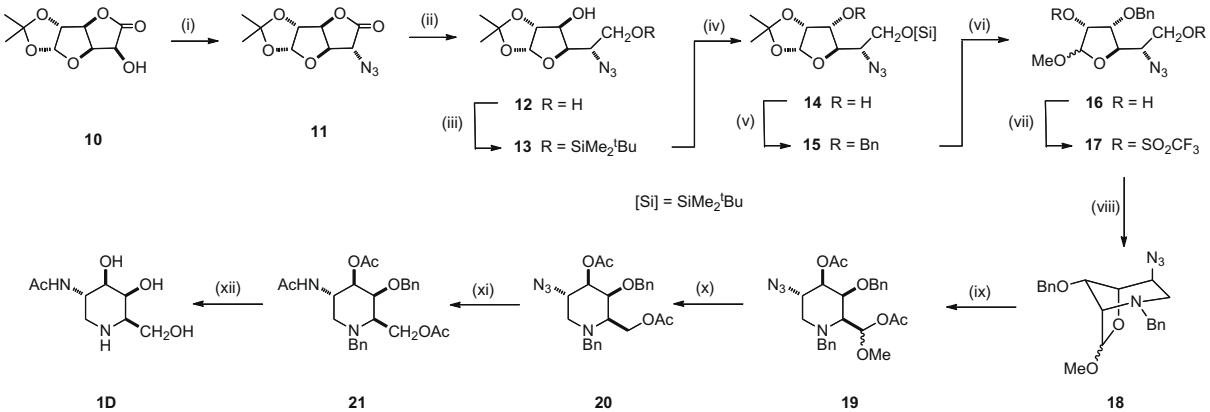


Figure 1. Potent β-hexosaminidase inhibitors.

$CHCl_3$) [lit.[29] mp 114–116 °C, $[\alpha]_D^{20}$ +243 (*c* 1.1, $CHC1_3$)], in 99% yield (Scheme 2). Direct conversion of the azidolactone **11** by a number of hydrides to the diol **12** gave only low yields; such α-azidolactones are extremely sensitive to base and commonly a two step reduction is necessary with initial reduction to the lactol. Accordingly DIBALH reduction of the azidolactone **11** in dichloromethane gave the corresponding lactol which was further reduced by sodium borohydride in methanol to afford the diol **12**, mp 120–122 °C, $[\alpha]_D^{25}$ −69.6 (*c* 0.94, $CHCl_3$), in 72% yield. Selective protection of the primary alcohol in **12** by reaction with *tert*-butyldimethylsilyl (TBDMS) chloride gave the corresponding TBDMS ether **13** as an oil, $[\alpha]_D^{25}$ −12.7 (*c* 1.1, $CHCl_3$) in 99% yield; the overall yield of **13** from glucuronolactone **2D** was 72% on a multigram scale and without any need for chromatographic purification until the final stage.

The synthesis of DGJNAc **1D** required inversion and subsequent protection of the remaining unprotected C3 OH in the silyl ether **13**. Oxidation of **13** with pyridinium chlorochromate in dichloromethane in the presence of molecular sieves afforded the corresponding ketone, which on reduction from the least hindered face of the carbonyl gave the inverted alcohol **14**, oil, $[\alpha]_D^{25}$ +74.9 (*c* 0.94, $CHCl_3$), in 79% yield. Treatment of **14** with benzyl bromide and sodium hydride in DMF formed the fully protected benzyl ether **15**, oil, $[\alpha]_D^{25}$ +101.5 (*c* 0.56, $CHCl_3$) in 97% yield. Both the acetonide and silyl protecting groups in **15** were removed by treatment with HCl in methanol to give a 5:1 α/β mixture of anomers of the methyl furanoside **16** (97%); reaction of **16** with triflic anhydride in dichloromethane in the presence of pyridine gave the ditriflate **17** which, with benzylamine in THF, gave the bicyclic pyrrolidine **18** as an oil, $[\alpha]_D^{25}$ +22.8 (*c* 1.11, $CHCl_3$), as the α-anomer only in an overall yield of 61%. Formation of a piperidine ring by cyclization of a ditriflate was thus efficient; examples of successful cyclizations of a ditriflate, such as the formation of a pyrrolidine,[30] are very rare.

Acetolysis of the furanoside **18** with boron trifluoride diethyl etherate in acetic anhydride gave a 4:1 mixture of the epimers **19** in 93% yield. The OMe group in **19** was reductively removed by sequential treatment with DIBALH in dichloromethane, followed by sodium borohydride in methanol; acetylation of the resulting diol allowed easy isolation of the diacetate **20** as an oil, $[\alpha]_D^{25}$ +79.8 (*c* 0.43, $CHCl_3$), in 83% overall yield from **19**. Rapid reduction of the azide in **20** by zinc powder in the presence of copper(II) sulfate in acetic acid–acetic anhydride–THF[31] with concurrent acylation of the corresponding amine gave the crystalline triacetate **21**, mp 112–114 °C, $[\alpha]_D^{25}$ +26.2 (*c* 1.1, Me_2CO) in 79% yield. Selective removal of the *O*-acetate protecting groups by treatment of **21** with catalytic sodium methoxide in methanol, followed by hydrogenolysis of the benzyl groups by palladium (10% on carbon) in 1,4-dioxane/aqueous hydrochloric acid gave, after purification by ion exchange chromatography, DGJNAc **1D**,[32] mp 150–154 °C, $[\alpha]_D^{25}$ +41.9 (*c* 0.67, H_2O) [lit.[6] oil, $[\alpha]_D^{20}$ +37 (*c* 1, MeOH)], in 98% yield. Unlike many iminosugars, the free base DGJNAc is



Scheme 2. Reagents and conditions: (i) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine; then NaN_3, DMF, 99%; (ii) DIBALH, CH_2Cl_2, ; then $NaBH_4$, MeOH, 72%; (iii) $^tBuMe_2SiCl$, pyridine, 99% [72% from **10**]; (iv) PCC, CH_2Cl_2, molecular sieves; then $NaBH_4$, EtOH, H_2O, 79%; (v) $PhCH_2Br$, NaH, DMF, 97%; (vi) MeOH, HCl, 97%; (vii) $(CF_3SO_2)_2O$, CH_2Cl_2, pyridine; (viii) $PhCH_2NH_2$, THF, 61% from **16**; (ix) $Et_2O{\cdot}BF_3$, Ac_2O, 93%; (x) DIBALH, CH_2Cl_2; then $NaBH_4$, MeOH; then Ac_2O, pyridine, 83%; (xi) Zn, $CuSO_4$ (aq), THF/AcOH/Ac_2O, 79%; (xii) MeONa, MeOH; then H_2, Pd (10% on C), HCl, 1,4-dioxane, H_2O, 98% [20% overall yield from **10**].

readily crystallized; the overall yield of DJNAc **1D** from D-glucuronolactone acetonide **10** was 20%. The enantiomer L-DGJNAc **1L**, mp 152–156 °C, $[\alpha]_D^{25}$ −46.6 (*c* 0.73, H_2O), was prepared by an identical procedure from L-glucuronolactone **2L**.

DGJNAc **1D** was a highly potent competitive inhibitor of GalNAcases (K_i 0.081 μM from chicken liver, K_i 0.136 μM from *Charonia lampas*), and a good but much less potent competitive inhibitor of β-hexosaminidases (IC_{50} 1.8 μM from Jack bean, IC_{50} 4.2 μM from bovine kidney, IC_{50} 8.3 μM from human placenta, IC_{50} 2.2 μM from HL-60). The enantiomer L-DGJNAc **1L**, showed no inhibition of α-*N*-acetylgalactosaminidases but was a very weak but non-competitive inhibitor of β-hexosaminidases [K_i 1100 μM—compared with K_i 2.2 μM for DGJNAc **1D**—from human placenta]. This result was in accord with Asano's hypothesis[33] that L-enantiomers show non-competitive inhibition whereas D-iminosugars usually are competitive inhibitors. DGJNAc **1D** showed modest inhibition of coffee bean α-galactosidase (IC_{50} 64 μM), whereas L-DGJNAc **1L** showed no inhibition of this enzyme. Both enantiomers of DGJNAc **1** were screened as inhibitors of a number of other glycosidases and neither enantiomer showed any significant inhibition [less than 50% inhibition at 1000 μM] against α-glucosidases (rice, yeast), β-glucosidases (almond, bovine liver), β-galactosidase (bovine liver), α-mannosidase (Jack bean), β-mannosidase (snail), β-glucuronidases (*E. coli*, bovine liver), α-L-rhamnosidase (*P. decumbens*), or α-L-fucosidase (bovine epididymis).

In summary, this Letter reports a convenient and scalable synthesis of DGJNAc **1D** from D-glucuronolactone **2D** in an overall yield of 20%. DGJNAc is the first highly potent and specific competitive inhibitor of GalNAcases. DGJNAc **1D** is a less potent but competitive—whereas L-DGJNAc **1L** is a very weak non-competitive—inhibitor of β-hexosaminidases. It is likely that ready access to DGJNAc **1D** as a potent and specific inhibitor of GalNAcases will allow useful investigation of a number of diseases.

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32. Selected data for DGJNAc **1D**: HRMS (ESI +ve): $C_8H_{16}N_2NaO_4$ found 227.1001; ($M+Na^+$) requires 227.1002; +41.9 (*c* 0.67, H_2O); mp 150–154 °C; ν_{max} (thin film, Ge): 3287 (br s, OH/NH), 1637 (s, amide I), 1561 (s, amide II); δ_H (D_2O, 400 MHz): 2.00 (3H, s, Me), 2.37 (1H, dd, H1a J_{gem} 12.9, $J_{1a,2}$ 11.6), 2.76 (1H, dt, H5 $J_{5,4}$ 1.3, $J_{5,6a} = J_{5,6b}$ 6.6), 3.08 (1H, dd, H1b J_{gem} 12.9, $J_{1b,2}$ 5.1), 3.58 (1H, dd, H3 $J_{3,2}$ 10.6, $J_{3,4}$ 3.0), 3.61 (1H, dd, H6a J_{gem} 11.1, $J_{6a,5}$ 6.3), 3.65 (1H, dd, H6b J_{gem} 11.1, $J_{6b,5}$ 6.6), 3.96 (1H, dt, H2 $J_{2,1a}$ 11.1, $J_{2,1b}$ 5.1, $J_{2,3}$ 11.1), 4.01 (1H, dd, H4 $J_{4,3}$ 3.0, $J_{4,5}$ 1.4); δ_C (D_2O, 100 MHz): 22.7 (Me), 47.7 (C1), 49.1 (C2), 59.4 (C5), 61.9 (C6), 68.9 (C4), 73.2 (C3), 175.2 (*C*OMe); LRMS (ESI +ve): 205 (77%, $M+H^+$), 431 (100%, 2 $M+Na^+$).
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