Pergamon

Tetrahedron Letters, Vol. 36, No. 2, pp. 299-302, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(94)02235-6

Synthesis and Influenza Virus Sialidase Inhibitory Activity of the 5-Desacetamido Analogue of 2,3-Didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid (GG167)

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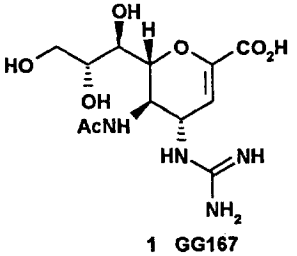
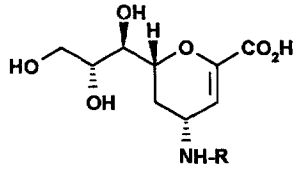
Abstract: The title compound **2b** has been synthesised in 8 steps from 2-deoxy-D-glucose (2-DG). Key transformations were an aldolase reaction utilising 2-DG to form the 9 carbon sugar **3**, and then introduction of the required unsaturation and a 4-amino substituent in a one-pot process. The poor inhibitory activity of **2b** demonstrates that the 5-acetamido substituent in 2,3-didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid (GG167) **1** is critical for sialidase binding.

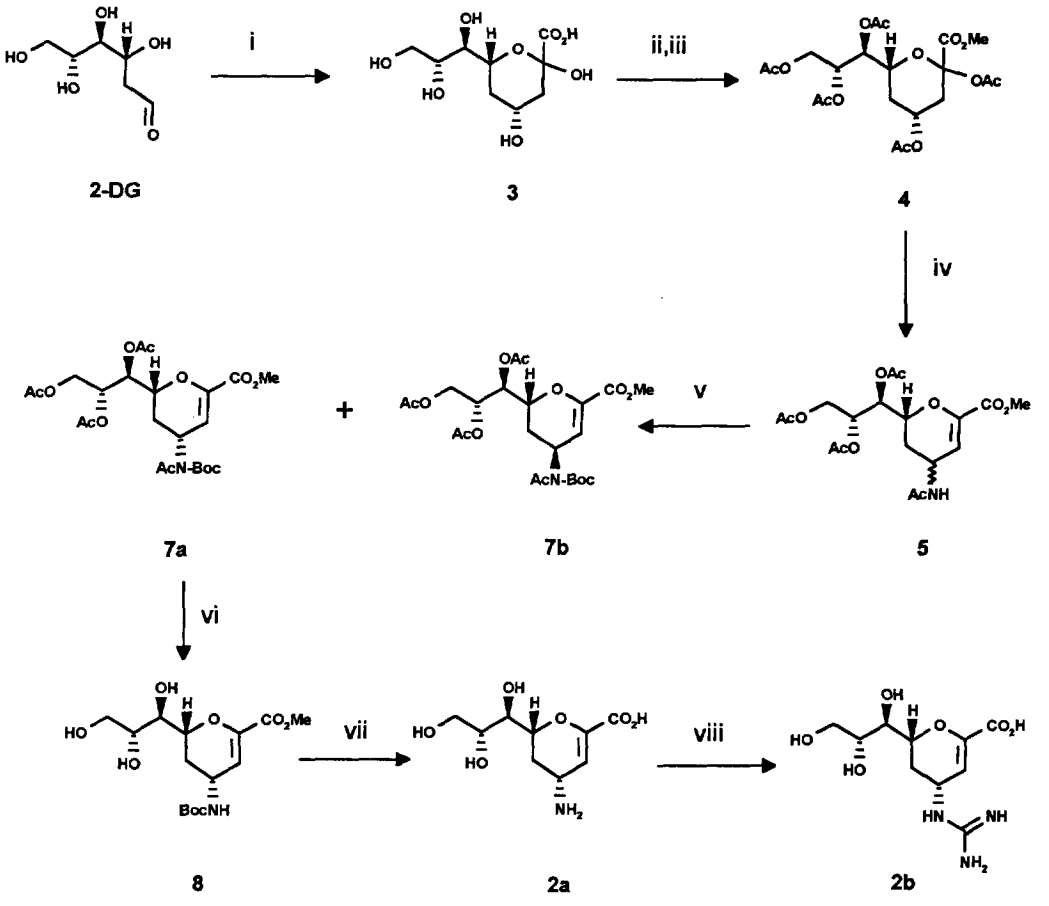
2,3-Didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid (GG167) **1** is a potent and selective inhibitor of influenza virus sialidase. It was rationally designed from the X-ray crystal structure of sialic acid bound to influenza sialidase with the aid of molecular modelling and computational chemistry techniques.[1,2] Recently the synthesis of **1** from N-acetyl-D-neuraminic acid (NANA) has been reported.[3-6] Formally, this represents a synthesis of **1** from N-acetyl-D-mannosamine (NAM) since NANA is available from NAM and pyruvate using an aldolase reaction.[7] In order to understand the contribution to sialidase binding made by each of the groups on the dihydropyran ring, we and others have embarked on the synthesis of analogues of **1**.[6,8,9] In this communication, we describe the synthesis and biological properties of **2a** and **2b** which lack a substituent at the 5-position of the dihydropyran ring. This has been achieved by utilising 2-DG as substrate in the aldolase reaction,[7] and subsequently introducing the required unsaturation and a 4-amino substituent in a one pot process involving an unexpected Ritter reaction.[10,*]



1 GG167

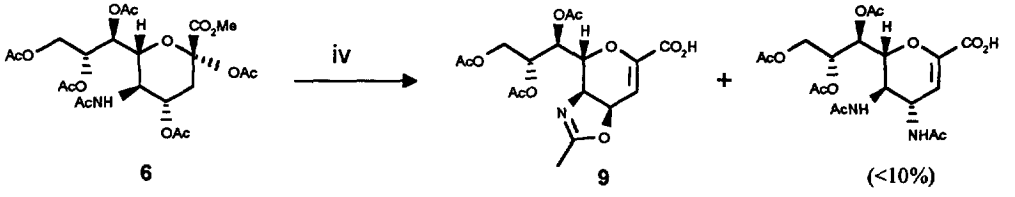


2 a: R = H
b: R = C(=NH)NH$_2$



Reagents and conditions: i) Aldolase, sodium pyruvate (41%) ii) MeOH / Dowex (H^+) (90%) iii) Ac_2O, Pyridine, DMAP (75%) iv) TMS-OTf, MeCN (2:1 β:α, 80%) v) Boc_2O, DMAP, dioxan, separate isomers vi) NaOMe, MeOH vii) NaOH aq then TFA/CH_2Cl_2 (95% over 3 steps) viii) bis-Boc-PCH then TFA/CH_2Cl_2.

Scheme 1



Scheme 2

Immobilised NANA-aldolase[11] catalysed the conversion of 2-DG to the key intermediate **3** (Scheme 1). Typically, bioconversions were carried out at 20°C in a 2L flask (250 ml working volume) containing 2-DG (25 g) and sodium pyruvate (69 g). The pH was adjusted to 7.5 with NaOH and reactions were started by the addition of washed immobilised beads (150 g wet weight). Upon completion of the reaction, enzyme beads were removed by filtration and washed with distilled water (250 ml). The combined filtrate (500 ml) was passed through a bed (400 ml) of Amberlite 200 (H^+) to remove sodium ions. The eluate was processed through a bed (500 ml) of Macro-Prep High Q on which 2-DG was not retained. The C-9 sugar **3** was then eluted with 0.5M acetic acid. Finally, the solution containing product was evaporated to low volume and then to dryness in the presence of 2-propanol to afford **3**.

Following established procedures, the carboxyl group in **3** was esterified using methanol and the hydroxyl groups peracetylated to give the fully protected intermediate **4**. Using methodology developed for the synthesis of **1**, compound **4** was next treated with trimethylsilyl triflate (TMSOTf) in acetonitrile.[6] In this reaction, whilst expecting elimination of the anomeric acetate, we also observed clean substitution of the newly formed allylic acetate by MeCN *via* a Ritter reaction to give a mixture of 4-acetamides **5** (2:1 ratio). A similar Ritter reaction was observed as a minor side reaction when compound **6** was subjected to the same conditions, although in this case the major product of the reaction was the oxazoline **9** formed *via* intramolecular attack at C-4 by the 5-acetamido group (Scheme 2).[6,12]

The individual isomers of **5** could not be separated at this stage, but after N-Boc protection, the bis-N-acylated derivatives **7a** and **b** were readily separated and the pure α-isomer **7a** (minor product) was further elaborated to the targets **2a** and **2b**. Thus, the major isomer produced in the Ritter reaction resulted from inversion of the stereochemistry of compound **4** at the C-4 position.[13]

The final stages of the synthesis were completed using standard reactions. Thus, deacetylation of **7a** with methanolic sodium methoxide afforded the triol **8**. This was deprotected by treatment with aqueous sodium hydroxide followed by TFA/CH_2Cl_2 to afford the amine **2a**. Guanylation of **2a** proceeded smoothly using N,N'-bis-Boc-1H-pyrazole-1-carboxamidine (bis-Boc-PCH)[14] and subsequent TFA deprotection afforded pure desacetamido GG167 **2b**.[15]

Compounds **2a** and **b** were evaluated as inhibitors of influenza A and B sialidase.[16] Both compounds displayed markedly reduced affinity for the viral enzymes when compared to **1** (Table 1).

Table 1: Inhibition of Influenza A and B sialidases by compounds 1,2a & 2b[17]

	IC_{50} (μM)	
Compound	**Flu A (Aichi)**	**Flu B (Victoria)**
1	0.005	0.004
2a	>500	-
2b	130	>400

In conclusion therefore, using a combination of enzyme mediated aldol chemistry and a novel one pot reaction for elimination and introduction of a nitrogen substituent, we have prepared **2a** and **2b** which are analogues of 2,3-didehydro-2,4-dideoxy-4-guanidinyl-N-acetylneuraminic acid (GG167) lacking a 5-substituent on the dihydropyran ring. Subsequent evaluation of these compounds against influenza virus

sialidase has established the critical importance of the 5-acetamido group for good binding affinity of GG167.

Acknowledgements: We wish to thank Richard Bethell and Safia Madar for performing the enzyme assays, and Richard Upton and Sean Lynn for running nOe experiments. Discussions with Dr MJ Dawson are also gratefully acknowledged.

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15. **Compound 2a:** ^{1}H NMR (D_2O): 6.04 (1H, dd, H-3, J 5.1, 1.7 Hz), 4.40 (1H, ddd, H-6, J 12.4, 2.3, 1.9 Hz), 4.15 (1H, m, H-4, J 5.8, 4.8, 1.5 Hz), 3.90 (1H, m, H-8, J 9.0, 6.1, 2.9 Hz), 3.82 (1H, dd, H-9, J 12.0, 2.9 Hz), 3.63 (1H, dd, H-9', J 12.0, 6.1 Hz), 3.56 (1H, dd, H-7, J 9.0, 1.9 Hz), 2.31 (1H, m, H-5 pseudo axial J 15.4, 12.4, 5.8 Hz), 2.03 (1H, dm, H-5 pseudo equatorial J 15.4, 2.3, 1.7, 1.5 Hz). High Res MS: Found MH^+ 234.09772, calcd for $C_9H_{16}NO_6$ 234.09776.
Compound 2b: ^{1}H NMR (D_2O): 5.97 (1H, bd, H-3), 4.58 (1H, ddd, H-6, J 13, 7.2, 2 Hz), 4.44 (1H, m H-4), 3.93 (1H, ddd, H-8, J 9, 7, 2.6 Hz), 3.88 (1H, dd, H-9, J 14, 2.6 Hz), 3.63 (2H, m, H-7,9), 2.23 (1H, dm, H-5 pseudoequatorial, J 13, 6.5, 1.8 Hz), 1.92 (1H, br t, H-5, pseudo axial, J 13 Hz). υ_{max} (KBr) 3600-3200 (br), 1668, 1612, 1202, 1145 cm^{-1}. High Res MS: Found MH^+ 276.11928, calcd for $C_{10}H_{18}N_3O_6$ 276.11956.
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✠. *Note added in proof:* Since the submission of this manuscript a report has appeared which describes a Ritter reaction on a heptulose sugar derivative similar to the one which we observe in the conversion **4** → **5**. Driguez P-A, Barrere B, Quash G and Doutheau A. *Carbohydr Res.* **1994**, *262*, 297.

(*Received in UK* 7 *October* 1994; *revised* 9 *November* 1994; *accepted* 11 *November* 1994)