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SYNTHESIS OF Δ^4-β-D-GLUCOPYRANOSIDURONIC ACIDS AS MIMETICS OF 2,3-UNSATURATED SIALIC ACIDS FOR SIALIDASE INHIBITION

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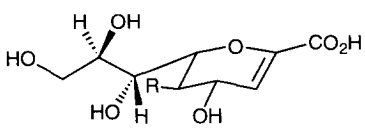
Abstract: Mimetics of Neu5Ac2en and KDN2en, based on Δ^4-β-D-glucopyranosiduronic acids, have been synthesised. The Neu5Ac2en mimetic **5** showed inhibition of both bacterial and viral sialidases, with inhibition of the viral sialidase being comparable to that of Neu5Ac2en itself.

Sialic acids are involved in a range of biological processes including cell–cell and cell–microbe interactions.[1] The importance of sialic acids in these processes, especially with respect to human disease states, has led to interest in the synthesis of natural and modified sialic acids[2] both as probes of sialic acid-recognising proteins, and as potential glycopharmaceuticals. Over recent years there has also been an increasing interest in the development of sialylmimetics, exemplified by the large number of sialyl-Le^x mimetics reported,[3] as well as sialyltransferase inhibitors,[4] and a number of inhibitors of influenza virus sialidase.[5] As part of our continuing research into sialic acid chemistry and biochemistry we have been interested in developing a 'family' of sialylmimetics that are readily accessible, and that have the potential for relatively facile functional group modification. Initially, with a view to increasing our current understanding of sialidases, we wished to mimic the 2,3-dehydro-sialic acids, 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (Neu5Ac2en **1**), and 2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (KDN2en **2**), which are natural sialidase inhibitors.[1]



1 Neu5Ac2en; R = NHAc
2 KDN2en; R = OH

A 2,3-dehydro-sialic acid is essentially a β-*C*-glycoside of a Δ^4-D-glucopyranosiduronic acid. The substituent at C-5 of the sialic acid corresponds to the C-2 substituent of the Δ^4-D-glucuronic acid such that Neu5Ac2en and KDN2en mimetics would be derived from 2-acetamido-2-deoxy-D-glucose and D-glucose, respectively. The glycerol side chain is represented by the β-substituent at the anomeric position which can be

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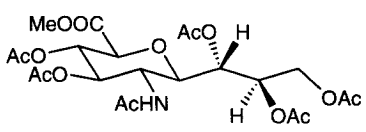
varied to introduce a range of functionalities with differences in size, hydrophobicity, and charge. Neu5Ac2en itself has been synthesised via the methyl 2-acetamido-2-deoxy-β-D-glucuronate *C*-glycoside **3**,[6] while glucuronic acid chemistry has also been used for the preparation of mimetics of the potent influenza sialidase inhibitor 4-amino-4-deoxy-Neu5Ac2en.[7] It has been shown in the development of the influenza sialidase inhibitor **4**, a carbocyclic mimetic of 4-amino-4-deoxy-Neu5Ac2en, that the glycerol side chain could be replaced with simple alkyl ethers.[8] Incorporation of a 3-pentanyl ether in the position representing the glycerol side chain produced an inhibitor of influenza virus sialidase with 100-fold higher activity than the corresponding Neu5Ac2en derivative. It was of interest to us to determine if such simple hydrophobic side chains would be tolerated by other sialidases. Our initial targets were therefore the *iso*-propyl Δ^4-β-D-glucopyranosiduronic acid derivatives **5** and **6**, mimetics of Neu5Ac2en **1** and KDN2en **2**, respectively.



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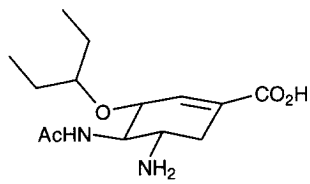

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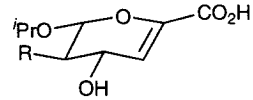
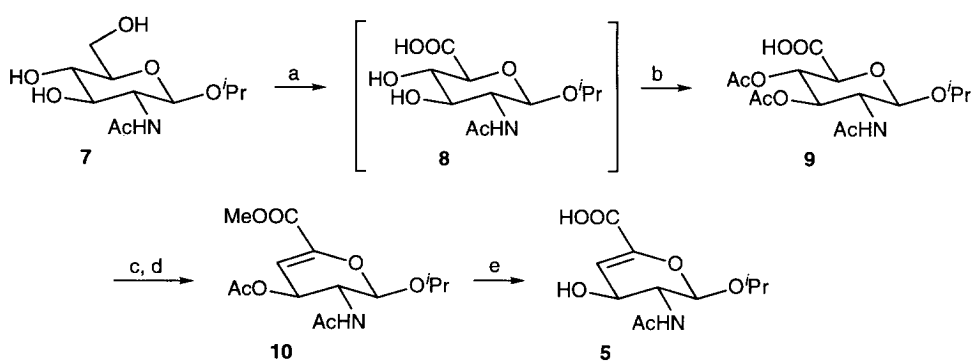


5 R = NHAc
6 R = OH

Synthesis Δ^4-D-glucopyranosiduronic acids[9]

The strategy applied to the synthesis of both the Neu5Ac2en and KDN2en mimetics **5** and **6**, respectively, is illustrated in Scheme 1 below for the Neu5Ac2en mimetic **5**. The β-*iso*-propyl glycoside **7** of 2-acetamido-2-deoxy-D-glucopyranose was prepared in 84% yield by reaction of peracetylated 2-acetamido-2-deoxy-D-glucopyranose with TMSOTf and then *iso*-propanol, according to a procedure reported for



Scheme 1. *Reagents and conditions:* (a) Pt(0), O_2, $NaHCO_3$ (pH 7.5), H_2O/*i*-PrOH, 80 °C, 4 h; (b) Ac_2O, py, rt, o'n; (c) PPh_3, DIAD, MeOH, rt, o'n; (d) DBU, CH_2Cl_2, 65 °C, 2 h; (e) NaOH, aq MeOH, rt, 2 h.

glycosidation of peracetylated chitobiose,[10] followed by base-catalysed de-*O*-acetylation. The corresponding β-*iso*-propyl glycoside of D-glucopyranose was prepared from peracetylated α-D-glucopyranosyl bromide by reaction with *iso*-propanol in the presence of Ag_2CO_3 in 68% yield. The glycoside **7** was selectively oxidized at the primary hydroxyl group using Pt(0) and oxygen.[11] The resulting acid **8** was then acetylated to give **9**, in order to avoid complications of competing lactone formation encountered when esterification was attempted on the free acid **8**. Esterification of **9** under Mitsunobu conditions gave a mixture of the methyl Δ^4-glucuronate **10**, and the non-eliminated methyl glucuronate (ratio approximately 80:20). Complete conversion to **10** was accomplished by reaction of the mixture with DBU. The yield, over 5 steps, from **7** to *iso*-propyl 2-acetamido-2-deoxy-Δ^4-β-D-glucopyranosiduronic acid (**5**) was 69%. The overall yield for the synthesis of the KDN2en mimetic *iso*-propyl Δ^4-β-D-glucopyranosiduronic acid (**6**) from *iso*-propyl β-D-glucopyranoside was 60%.

Biological evaluation

Compounds **5** and **6** were tested for inhibitory activity against various sialidases,[12] in comparison with the corresponding 2,3-didehydro-sialic acids.[13] Interesting results were observed with inhibition of influenza virus A (N9) and *Vibrio cholerae* sialidases; the Neu5Ac2en mimetic **5** showed a greater inhibition of the viral sialidase (100% at 0.1 mM; K_i 2.7×10^{-6} M) compared to the bacterial sialidase (25% at 0.1 mM; K_i 1.2×10^{-4} M), while Neu5Ac2en itself is a μM inhibitor of both sialidases (K_is 1.3×10^{-6} M and 3.4×10^{-6} M, respectively). Compound **5** also showed some inhibition of the sialidases from *Salmonella typhimurium* and *Clostridium perfringens* (~24% at 0.1 mM against each sialidase). It is interesting to note that examination[14] of the X-ray crystal structures of the sialidases from influenza virus A, *V. cholerae*, and *S. typhimurium* has revealed that interactions of the proteins with the glycerol side chain of *N*-acetylneuraminic acid are different.

The sialidase isolated from the hepatopancreas of the oyster (*Crassostrea virginica*) has been found to cleave both KDN and Neu5Ac glycosides, with a tenfold preference for the cleavage of 2-α-(4-methylumbelliferyl)-KDN over the corresponding Neu5Ac glycoside.[15] The KDN-sialidase from *C. virginica* was inhibited by KDN2en (95% at 1 mM), however the KDN2en mimetic **6** showed rather poor inhibition at the same concentration (6% at 1 mM). Neu5Ac2en was found to be a poor inhibitor of this sialidase (20% at 1 mM) while the Neu5Ac2en mimetic **5** showed no inhibition at all at 1 mM.

The lower inhibition of the bacterial sialidases by **5**, and of the KDN-sialidase from *C. virginica* by compounds **5** and **6**, compared to the corresponding 2,3-dehydro-sialic acids, suggests that replacement of the glycerol side chain with an *iso*-propyl group is not well tolerated. During the development of the influenza sialidase inhibitor **4** it was shown that the nature of the ether side chain could dramatically affect the inhibition obtained.[8] Although the initial inhibition results described above suggest that an *iso*-propyl ether is not an appropriate replacement for the glycerol side chain for recognition by some sialidases, it remains to be established if the non-viral sialidases will tolerate major structural modification of the glycerol side chain.

Conclusion

We have described the synthesis of simple mimetics, based on Δ^4-β-D-glucopyranosiduronic acids, of the 2,3-dehydro-sialic acids Neu5Ac2en and KDN2en. The compound **5**, containing a simple *iso*-propyl glycoside, shows inhibition of influenza virus A sialidase comparable with Neu5Ac2en, while producing lower inhibition of bacterial sialidases than Neu5Ac2en. Substitution around both templates is now being varied to probe the substructural binding requirements of a number of sialic acid-recognising proteins for these novel sialylmimetics.

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