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Synthesis and Neuraminidase Inhibition Studies of 4-Azido, Amino, And Acetamido Substituted Sialosides

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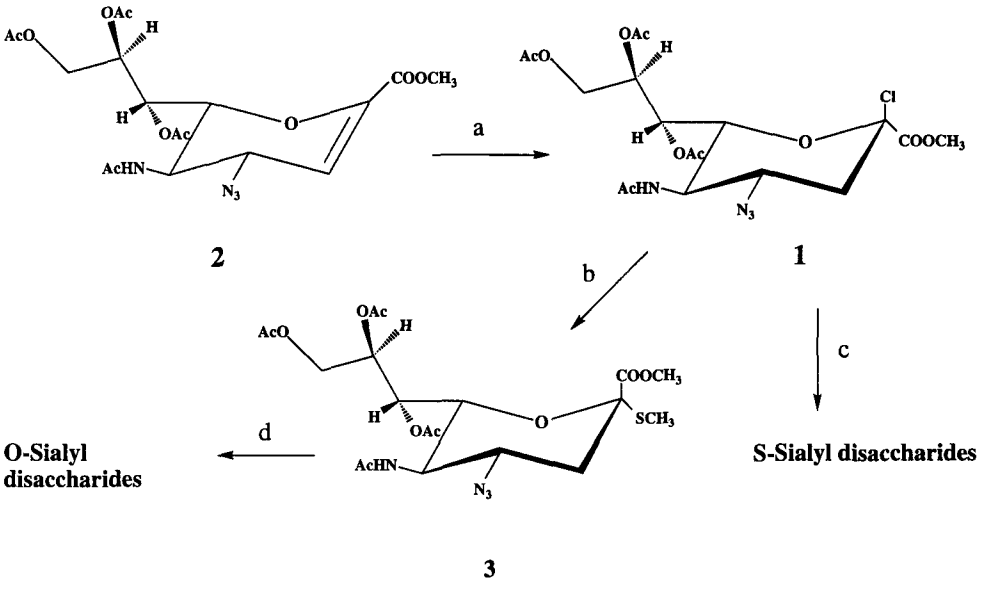
Abstract: Synthesis of 4-azido-NeuAc containing sialosides and thiosialosides has been developed via the hydrochlorination of an 4-azidoglycal. The azidosialosides have been converted into the amino and acetamido sialosides. Two of the aminosialosides show potent inhibition (K_i = 50-150 μM) of influenza neuraminidase.

The synthesis of sialoside based common inhibitors of influenza virus hemagglutinin and neuraminidase is of interest as anti viral drugs. Most of the synthetic sialopyranoside inhibitors of hemagglutinin are, however, poor inhibitors of the influenza neuraminidase. Recent crystal structure of the influenza neuraminidase complexed with α-sialic acid indicates[1] that the 4-substituent of sialic acid unit is close to two carboxyl groups (asp-151 and glu-119) at the neuraminidase active site. Introduction of amino or guanidino group at that site should, therefore, enhance the binding potencies of sialosides to neuraminidase through salt bridge formation.[2] To synthesize these 4-amino or guanidino sialosides for biological evaluations, a ready access to 4-azido or amino substituted sialic acid glycosyl donor was required. This report describes the first and a short synthesis of the 4- azido-chloride **1**, a reagent suitable for making 4-azido substitued O-and S- linked sialosides, and the transformation of the resultant sialoside into a 4-amino and acetamido substitued sialoside. Evaluation of these novel compounds with influenza virus neuraminidase shows that, indeed, the amino-substituted sialosides exhibit increased binding to neuraminidase, possibly through interaction with asp-151 and glu-119 carboxyl groups at neuraminidase binding site.

As a precursor to the azido glycosyl chloride **1**, we selected the azido glycal **2**, as it can be prepared in 4 steps from the commercially available N-acetyl-neuraminic acid.[3,4] As outlined in Scheme 1, the addition of hydrogen chloride to **2** in Markovnikov's mode should provide **1**. However, the hydrochlorination of acetylated sugar glycals is not a synthetically useful reaction, as the allylic substituent undergoes rearrangement leading to a number of products. We have now discovered that when the allylic substituent is an azide, glycal such as **2** undergoes facile hydrochlorination with anhydrous hydrogen chloride in acetonitrile or glacial acetic acid, preferably in the presence of lithium chloride.[5] After 4 days, greater than 80 to 90% of **2** was converted to the chloride **1** (the remaining 10% was the starting material) and the crude product obtained was sufficiently pure for subsequent glycosylation reactions. *This hydrochlorination was successful only with the azidoglycal **2** and failed with glycals containing other allylic substituents, such as an acetamido or an acetylthio group.* Fortunately, the azido group is the most desirable one, as it can be converted readily into an amino, guanidino or acetamido group.

Scheme 1



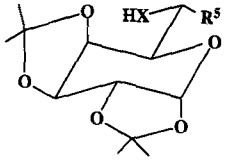
a) HCl gas - LiCl -CH_3CN or CH_3COOH, 6 days; b) CH_3SNa - CH_3CN[6]; c) sodium thiolate derivative of monosaccharides - CH_3CN[6]; d) monosaccharide alcohols - CH_3SOTf - CH_3CN, -38 C.[7]

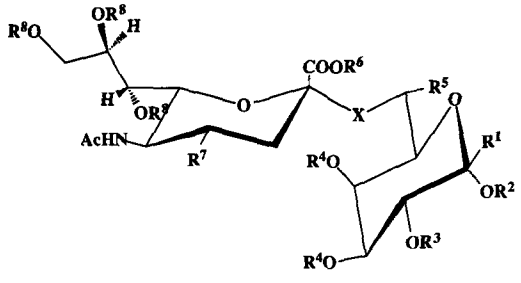
The availability of large amounts of glycosyl chloride **1** permitted the synthesis of many S- and O-linked 4-azido-sialyl disaccharides (Scheme 2). Thus, **1** was converted into the thio disaccharide **5**, by reacting with the sodium salt of the thiol derivative **4**[6] in greater than 45% yield (based on crude chloride **1**) or to the thioglycosyl donor **2** by reacting with sodium thiomethylate.[6] Condensation of thioglycoside **3** with alcohols such as **6** or **7** in presence of methylsulfenyl triflate afforded the O-linked α - sialosides **8** or **9** as the major products, in greater than 60% yield.[6,7] Using standard deprotection methodologies,[6] the 4-azido-sialosides **5**, **8**, and **9** were converted to the deprotected sugars **16**, **10**, and **11** (Scheme 2). The α-sialosidic configuration in these derivatives was established by measuring the coupling constant between C-1 and H-3ax of the NeuAc unit (J = 5.6 Hz).[8] The azido group was reduced with hydrogen in presence of Pd/C to obtain the 4-amino sialosides. This upon acetylation gave the 4-acetamido-sialosides. The complete NMR characterization of these compounds will be published separately.[9]

Evaluation[10] of the O-linked **azido** (**10** and **11**) and the **acetamido** sialosides (**12** and **13**) with influenza neuraminidase (WSN H1N1) showed that all of them were resistant to neuraminidase hydrolysis! Surprisingly, the corresponding thiosialoside analogs **16** and **17** were also poor inhibitors of the influenza

neuraminidase.[11] On this basis it can be concluded that the neuraminidase resistance of the azido and acetamido sialosides should arise due to their lack of binding to the enzyme. The 4-amino-O-linked sialoside **14** was also resistant to influenza virus neuraminidase hydrolysis. **However, it was a potent inhibitor (Ki = 150 uM), a first example of a potent inhibition of influenza neuraminidase by an O-linked sialoside.** The thioanalog **18** was even three times better an inhibitor of the influenza neuraminidase (***Ki* = 50 mM).**

Scheme 2





X = S, R^5 = Me, **4**
X = O, R^5 = H, **6**
X = O, R^5 = Me, **7**

X = S, R^1 = H, $R^2 = R^3 = R^4$ = isopropylidene, $R^5 = R^6$ = Me, $R^7 = N_3$, R^8 = Ac; **5**
X = O, R^1 = H, $R^2 = R^3 = R^4$ = isopropylidene, R^5 = H, R^6 = Me, $R^7 = N_3$, R^8 = Ac; **8**
X = O, R^1 = H, $R^2 = R^3 = R^4$ = isopropylidene, $R^5 = R^6$ = Me, $R^7 = N_3$, R^8 = Ac; **9**
X = O, $R^1 = R^2 = R^3 = R^4 = R^5 = R^8$ = H, R^6 = Na, $R^7 = N_3$; **10**
X = O, $R^1 = R^2 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, $R^7 = N_3$; **11**
X = O, $R^1 = R^2 = R^3 = R^4 = R^5 = R^8$ = H, R^6 = Na, R^7 = NHAc; **12**
X = O, $R^1 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, R^7 = NHAc, OR^2 = H, $R^1 = OCH_2CH_2SiMe_3$; **13**
X = O, $R^1 = R^2 = R^3 = R^4 = R^5 = R^8$ = H, R^6 = Na, $R^7 = NH_2$; **14**
X = O, $R^1 = R^2 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, $R^7 = NH_2$; **15**
X = S, $R^1 = R^2 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, $R^7 = N_3$; **16**
X = S, $R^1 = R^2 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, R^7 = NHAc; **17**
X = S, $R^1 = R^2 = R^3 = R^4 = R^8$ = H, R^5 = Me, R^6 = Na, $R^7 = NH_2$; **18**

The lack of hydrolysis of the amino sialoside **14**, in spite of its tight binding to the enzyme, indicates that the asp-151 may be an important residue required for the enzyme catalysis. Further modifications of the

amino group to a guanidino group following the work of Taylor *et al.* [2] should shed light into the mechanism of the neuraminiadse catalysis and in the design of tight binding influenza neuraminidase inhibitors.

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(5) Typical procedure for hydrochlorination of glycal **2**: Anhydrous hydrogen chloride was passed through an ice - cold solution of **2** (2.0 g) in acetonitrile (50 ml) containing lithium chloride (1.0 g) and 4 Å molecular sieves (5.0 g) for 20 min. The reaction mixture was sealed and stirred at ambient temperature for 4 days. The solution was cooled in ace - bath temperature and HCl gas was passed for 10 min and the reaction was continued for 2 more days. The reaction mixture was evaporated to dryness, suspended in dichloromethane, filtered over celite pad and the filtrate was washed with ice - cold water (3X) and saturated saturated sodium bicarbonate solution, dried over anhydrous magnesium sulfate and concentrated to dryness (1.6 g). ^{1}H-NMR ($CDCl_3$) ∂: 5.63 (d, 1 H, J = 9.6 Hz, NH), 5.46 (dd, 1 H, J = 2.8, 7.2 Hz, H-7), 5.19 (m, 1 H, H-8), 4.52 (dd, J = 2.8, 11.0 Hz, H-6), 4.40 (dd, J = 3.2, 12.8 Hz, H-9a), 4.26 (m, 1 H, H-4), 4.10 (dd, J = 5.6, 12.8 Hz, H-9b), 3.88 (s, C))C$\underline{H}_3$), 3.76 (m, H-5), 2.79 (dd, J = 4.8, 14.3 Hz, H-3eq), 2.14, 2.07, 2.06 and 2.03 (4xs, C$\underline{H}_3$COO-).

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(10) The enzymatic hydrolysis of azido and acetamido sialosides was monitored by thin layer chromatography (t. l. c.) on silica gel using ethyl acetate - ethanol - water (4:2:1) as eluant, followed by visualization with 5% sulfuric acid in ethanol spray and heating. αDNeuAc(2-6)ßDGal-OCH$_2$CH$_2$SiMe$_3$[5] was used as a standard. The t. l. c. eluant for amino sialoside **14** was ethyl acetate - ethanol - water (2:2:1) and the visualization of the compound was done with 1% ninhydrin solution in ethanol followed by heating. All of **14** remained, even after incubating with influenza neuraminidase for 24 h and based on this, a tentative conclusion about its resistance to hydrolysis was reached.

(11) The inhibition assays with thiosialosides were conducted as described in ref. 6.

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