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An efficient approach to *N*-acetyl-D-glucosaminuronic acid-based sialylmimetics as potential sialidase inhibitors

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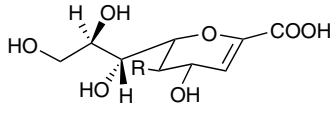
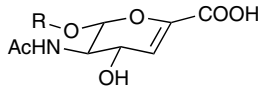
Abstract—A novel approach to the synthesis of β-glycosides of *N*-acetyl-D-glucosaminuronic acid, in six steps and good overall yield from *N*-acetyl-D-glucosamine, has been developed. The key synthetic step was the Lewis acid mediated *O*-glycosidation of methyl 1,3,4-tri-*O*-pivaloyl-*N*-acetyl-D-glucosaminuronate (**11**). Elaboration of glucosaminuronides **15** and **18** provided novel sialylmimetics **21** and **22**, which showed inhibition of *Vibrio cholerae* sialidase.

Sialidases form a large and widely studied group of enzymes, which catalyse the release of sialic acids from glycoconjugates by hydrolysis of the sialosyl glycosidic bond. The interest in sialidases has largely been due to their involvement in microbial pathogenesis, making them potential targets for therapeutic intervention.[1–3] The unsaturated sialic acid, 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enonic acid (Neu5Ac2en, **1**), is a naturally-occurring sialidase inhibitor. We have been interested in the synthesis of mimetics of Neu5Ac2en,[4] which are both readily accessible and have the potential for relatively facile functional group modification. Previously, we reported[4] the synthesis of a mimetic of Neu5Ac2en in which the glycerol side chain of the sialic acid was replaced by an isopropyl ether, to give the novel sialidase inhibitor **2**. Compound **2** was found to inhibit both influenza virus and *V. cholerae* sialidases.[4] This interesting result has led us to develop a method that facilitates rapid access to a series of mimetics **3** from a common, advanced intermediate. It was envisaged that this series would include derivatives with functionalities of varying size and hydrophobicity that replace the glycerol side chain of Neu5Ac2en (**1**), for evaluation as sialidase inhibitors. Described herein is our preliminary work towards the synthesis of a series of Neu5Ac2en mimetics **3**.



1 R = NHAc, Neu5Ac2en



2 R = $CHMe_2$
3 R = alkyl

The desired C-6 ether Neu5Ac2en mimetics **3** can be considered as β-*O*-glycosides of 4,5-unsaturated 2-acetamido-D-glucuronic acid, and are accessible via *N*-acetyl-β-D-glucosaminuronides **4** (Fig. 1). The most widely used approach to the preparation of β-D-glucosaminuronides, such as **4**, involves the selective oxidation[4–6] of the C-6 hydroxyl of a preformed *N*-acyl-β-D-glucosaminide, such as **5**. This strategy was previously applied to the synthesis of Neu5Ac2en mimetic **2** from *N*-acetyl-D-glucosamine.[4] An alternative approach is to begin with a uronic acid **6**, to introduce the nitrogen substituent at C-2, and then to form the glycoside. This is typically a more lengthy approach, but nevertheless has been used for the synthesis of various hexosaminuronide derivatives[7,8] in moderate yield from D-glucurono-6,3-lactone.

A strategy to form *N*-acyl-β-D-glucosaminuronides, such as **4**, which to the best of our knowledge has not been explored, is the direct *O*-glycosidation of an

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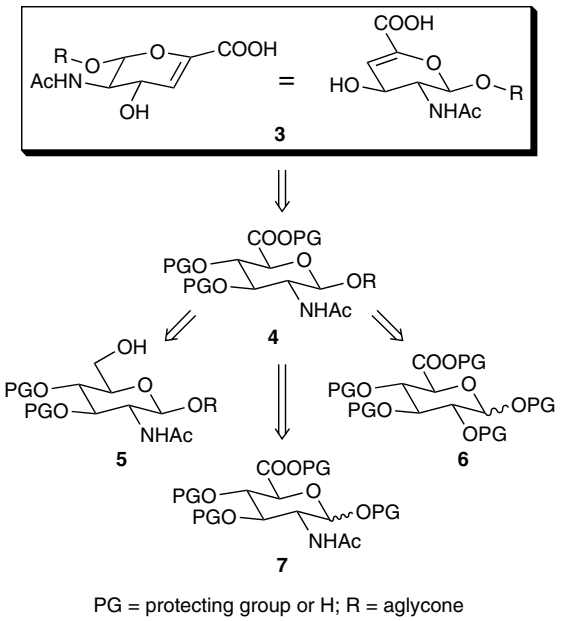


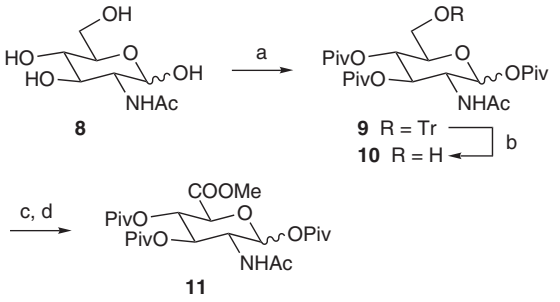
Figure 1. Approaches to C-6 ether Neu5Ac2en mimetics **3**.

N-acylglucosaminuronate, such as **7**. This template is readily accessible from an *N*-acylglucosamine (as described below) and is set up for β-glycosidation using anchimeric assistance from the C-2 acylamido group.[9] A glycosyl bromide derived from an *N*-acylglucosaminuronate was used for *N*-glycosidation by Timoshchuk and Kulinkovich in their synthesis of glucosaminuronic acid-based nucleoside analogues.[10,11] A strategy employing such an aminouronic acid-based intermediate offers advantages over the others described in that it is potentially more versatile, with the aglycone functionality being introduced at a later stage in the reaction sequence, allowing rapid access to a wide range of derivatives from a common glycosyl donor intermediate **7**.

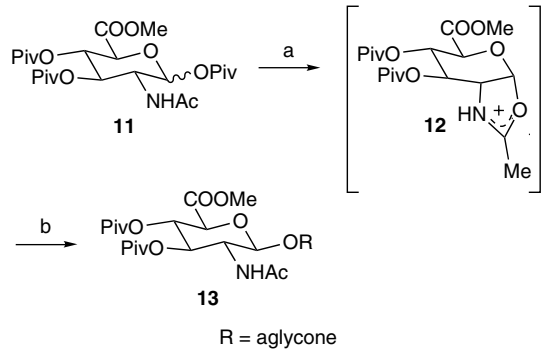
Our approach to the formation of C-6 ether Neu5Ac2en mimetics **3** from readily available *N*-acetyl-D-glucosamine (GlcNAc, **8**) is detailed in Schemes 1–3, with the key step being the β-selective *O*-glycosidation of the *N*-acetylglucosaminuronate intermediate **11**. The synthesis[†] of *N*-acetylglucosaminuronate intermediate **11** is outlined in Scheme 1. A sequence of selective tritylation of the primary hydroxyl group of GlcNAc (**8**), pivaloylation of the remaining hydroxyl groups to give **9**, and detritylation, led to the compound, **10**, with a free primary hydroxyl group. The pivaloylation reaction was found to be quite slow, even in the presence of DMAP, requiring six days for conversion of the 6-*O*-tritylated starting material to **9**. Monitoring of the reaction over time indicated that the C-4 hydroxyl group was the last to be pivaloylated.

Conversion of **10** to the corresponding uronic acid was carried-out using a TEMPO-mediated oxidation under basic conditions.[12] The pivaloyl protecting groups proved to be stable under the basic reaction conditions,

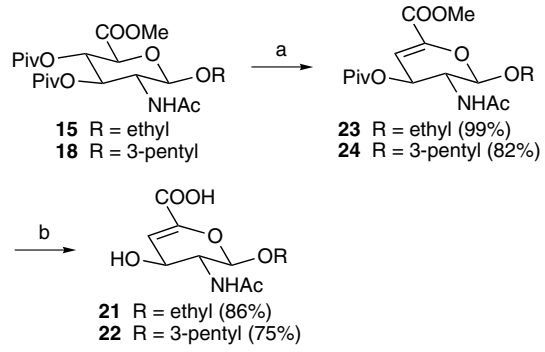
[†]All new compounds gave satisfactory spectral and analytical data.



Scheme 1. Reagents and conditions: (a) (i) Ph_3CCl (1.2 equiv), pyridine, ca. 100 °C, 20 min, (ii) ^{t}BuCOCl (3.6 equiv), DMAP, 0 °C → rt, 6 d (83%); (b) 80% aq AcOH, 65 °C, 1 h (92%); (c) cat. TEMPO, cat. KBr, Bu_4NBr, 10–15% NaOCl, satd aq $NaHCO_3$, sat aq NaCl, CH_2Cl_2, rt, 75 min; (d) $SOCl_2$ (0.6 equiv), $CH(OMe)_3$ (1.2 equiv), MeOH, rt, 30 min (76% over two steps).



Scheme 2. Reagents and conditions: (a) TMSOTf (1.1 equiv), $ClCH_2CH_2Cl$, 50 °C, 3 d; (b) (i) 3 Å MS, rt, 30 min, (ii) ROH (3 equiv), 24 h, rt (76–94%, based on recovered α-**11** or **14**).



Scheme 3. Reagents and conditions: (a) DBU (2 equiv), CH_2Cl_2, rt, 18 h; (b) 50% aq MeOH, aq NaOH, pH 13, 18 h.

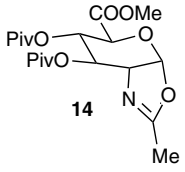
where the corresponding acetylated derivative showed some loss of acetate groups. The sequence of detritylation of **9** followed by TEMPO-mediated oxidation of the primary hydroxyl group was found to be significantly higher yielding (approximately 70% yield over two steps) than the previously reported[10] one-pot conversion of acetylated or benzoylated 6-*O*-trityl-GlcNAc to the corresponding uronic acid using CrO_3/H_2SO_4 (35% yield). The key intermediate **11** was thus obtained in 58% overall yield from GlcNAc (**8**) in a reaction sequence amenable to scale-up.

Table 1. β-Glycosidation of **11** with various alcohols

Alcohol	Product	Isolated yield (%)	Yield (%) based on recovered α-**11** and **14**
Ethanol	**15**	64	80
Isobutanol	**16**	77	88
Isopropanol	**17**	53	82
3-Pentanol[a]	**18**	78	94
Cyclopentanol[a]	**19**	56	76
1,2-*O*-Isopropylidene-glycerol	**20**	64	81

[a] Some co-elution with unreacted α-**11** during chromatographic purification.

Glycosidation[13] of **11** was carried-out in one-pot by in situ generation of the oxazolinium ion **12** from **11** (α/β ratio = 1.5:1.0) in the presence of TMSOTf[14] over three days, followed by reaction of **12** with an alcohol, as shown in Scheme 2. This procedure was used to produce a range of β-glycosides **13**, that were isolated in 53–78% yield (Table 1). Conversion of **11**–**13** was generally in the order of 80%, with unreacted **11** recovered solely as the α-anomer, and a small amount (~6%) of oxazoline **14** also produced. Based on the recovered α-**11** and **14**, yields ranged from 76% to 94% (Table 1). The use of freshly distilled TMSOTf and 1,2-dichloroethane resulted in optimal conversion of **11** (e.g., 88% conversion in reaction of **11** with isobutanol). The overall yield for the synthesis of *O*-glycosides **13** from GlcNAc (**8**) was 31–45% over six steps.



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Formation of the oxazolinium ion **12** from **11**, and particularly from the α-anomer of **11**, was found to be slower than the corresponding formation of oxazolinium ion from an anomeric mixture of peracetylated[4,14] or perpivaloylated GlcNAc. This is in line with the reported[15] destabilising effect of the C-5 alkoxycarbonyl group on the formation of a C-1 cation. The slower conversion of the α-pivaloate (α-**11**) to the intermediate oxazolinium ion **12** (as seen by ^{1}H NMR analysis of the reaction mixture over time) is consistent with observations on other 2-acetamido hexoses,[9] and is presumably due to the 1,2-*cis* relationship to the neighbouring C-2 acetamido group and consequent lack of anchimeric assistance.[9] Examination of the reaction conditions for the formation of the oxazolinium ion **12** from α-**11**, showed that neither an increase in the amount of TMSOTf, nor in the length of the reaction appreciably increased the production of **12** (or of **14** when the reaction was quenched with triethylamine).

The conversion of the glycosides **15** and **18** to the corresponding sialylmimetics **21** and **22**, was achieved in two steps (Scheme 3). β-Elimination of **15** and **18** using DBU in CH_2Cl_2 gave the unsaturated derivatives **23** and **24**, in 99% and 82% yield, respectively. Finally, base-catalysed deprotection gave **21** and **22**, in 86% and 75% yield, respectively, following HPLC purification of the crude products.

Compounds **21** and **22** were tested for inhibitory activity against *V. cholerae* sialidase, in comparison with Neu5Ac2en (**1**), using a fluorimetric assay.[16,17] Interestingly, the 3-pentyl derivative **22**, containing the larger hydrophobic side chain, showed greater inhibition (90% at 1 mM; estimated $K_i = 1 \times 10^{-4}$ M) compared to the ethyl derivative **21** (71% at 1 mM; estimated $K_i = 5 \times 10^{-4}$ M), although weaker inhibition than Neu5Ac2en (**1**) (97% at 1 mM; estimated $K_i = 3 \times 10^{-5}$ M). These results suggest that the glycerol side chain binding pocket of *V. cholerae* sialidase can accommodate a hydrophobic side chain of similar or slightly larger size compared to the glycerol side chain of Neu5Ac2en (**1**).

In conclusion, we have developed an efficient and versatile synthetic approach for the formation of C-6 ether Neu5Ac2en mimetics **3** from GlcNAc (**8**). Further work on a wider range of sialylmimetics and their biological evaluation will be reported in due course.

Acknowledgements

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Supplementary data

^{1}H and ^{13}C NMR data for all compounds can be found, in the online version, at doi:10.1016/j.bmcl.2004.08.064.

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13. Typical glycosidation procedure: TMSOTf (60 μL, 0.33 mmol, 1.1 equiv) was added to a stirred solution of an anomeric mixture of **11** (152 mg, 0.30 mmol) in anhydrous 1,2-dichloroethane (1.5 mL) under Ar. The clear yellow solution was warmed to an oil bath temperature of 50 °C. After 3 d, TLC analysis (EtOAc/hexane, 1:3) indicated that the starting material was nearly all consumed. The resulting brown reaction mixture was cooled to rt and 3 Å Molecular Sieves (0.5 g) were added. After 30 min, dry alcohol (3 equiv) was added and the reaction stirred at rt under Ar for 24 h. NEt_3 was added to adjust to pH 9, the reaction was filtered through Celite®, the residue was washed with $CHCl_3$/MeOH 10:1 (75 mL), and the filtrate was concentrated to give a brown gum. Flash chromatography gave the glycoside **13** as a clear colourless gum. Starting material **11** (α-anomer) and oxazoline **14** could also be isolated.
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