Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Research 342 (2007) 460–466
Carbohydrate RESEARCH

Direct oxidation of sugar nucleotides to the corresponding uronic acids: TEMPO and platinum-based procedures

Martin Rejzek,[a] Balaram Mukhopadhyay,[a,†] Cory Q. Wenzel,[b] Joseph S. Lam[b] and Robert A. Field[a,*]

[a]*Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK*
[b]*Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada N1G 2W1*

Received 6 July 2006; received in revised form 17 October 2006; accepted 17 October 2006
Available online 25 October 2006

Dedicated to the memory of Professor Nikolay K. Kochetkov

Abstract—The direct oxidation of UDP-α-D-glucose and UDP-*N*-acetyl-α-D-glucosamine to the corresponding uronic acids was explored using either TEMPO or platinum-catalysed oxidation with molecular oxygen. Whilst TEMPO-based procedures gave rise to substantial over-oxidation and/or degradation of UDP-glucose, oxidation of UDP-*N*-acetyl-glucosamine to UDP-*N*-acetyl-glucosaminuronic acid was achieved with >90% conversion and ca. 65% isolated yield using a platinum-catalysed procedure.

Keywords: Sugar nucleotide; Oxidation; Uronic acid; TEMPO; Platinum

1. Introduction

In connection with studies on sugar nucleotide biotransformations en route to enzymatic synthesis of natural and unnatural oligosaccharides, we had a need to prepare a range of UDP-uronic acids. In recent studies we have reported the flexibility of the Leloir pathway enzymes for the preparation of a wide range of sugar nucleotides,[1] and their use as a resource for antibiotic glyco-engineering.[2] To expand the repertoire of building blocks available to include uronic acids, it is necessary to develop a straightforward method for selective oxidation of the sole primary alcohol in hexopyranose-based sugar nucleotides to the corresponding carboxylic acids. Enzymatic oxidation procedures are effective and attractive: UDP-α-D-glucose (UDP-Glc) dehydrogenase[3] operating with in situ co-factor regeneration, for instance, gives the corresponding uronic acids efficiently, whilst galactose oxidase[4] in the presence of catalase can be coerced into converting UDP-α-D-galactose (UDP-Gal) into UDP-α-D-galacturonic acid (UDP-GalA). In part of our work we had a specific need to prepare UDP-*N*-acetyl-α-D-glucosaminuronic acid (UDP-GlcNAcA) in connection with studies on LPS biosynthesis in the human respiratory pathogens *Bordetella pertussis* and *Pseudomonas aeruginosa*.[5] The *P. aeruginosa* UDP-*N*-acetyl-α-D-glucosamine (UDP-GlcNAc) dehydrogenase-encoding gene, *wbpA*, has recently been cloned by our group, and the dehydrogenase enzyme has been overexpressed and characterised.[6] In order to prepare UDP-GlcNAcA, and analogues thereof, for studies of this and subsequent enzymes in the pathway leading to UDP-2,3-diacetamido-α-D-mannuronic acid (UDP-Man-diNAcA),[7] we aimed to develop a general approach that would be tolerant of a range of sugar substitutions and hence we resorted to the investigation of chemical oxidation methods.

Primary alcohols can also be selectively oxidised in the presence of secondary alcohols by catalytic dehydrogenation[8] and in particular by platinum-catalysed

* Corresponding author. Tel.: +44 1603 450720; e-mail: r.a.field@uea.ac.uk
† Present address: Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, PO Box 173, Lucknow 226 001, India.

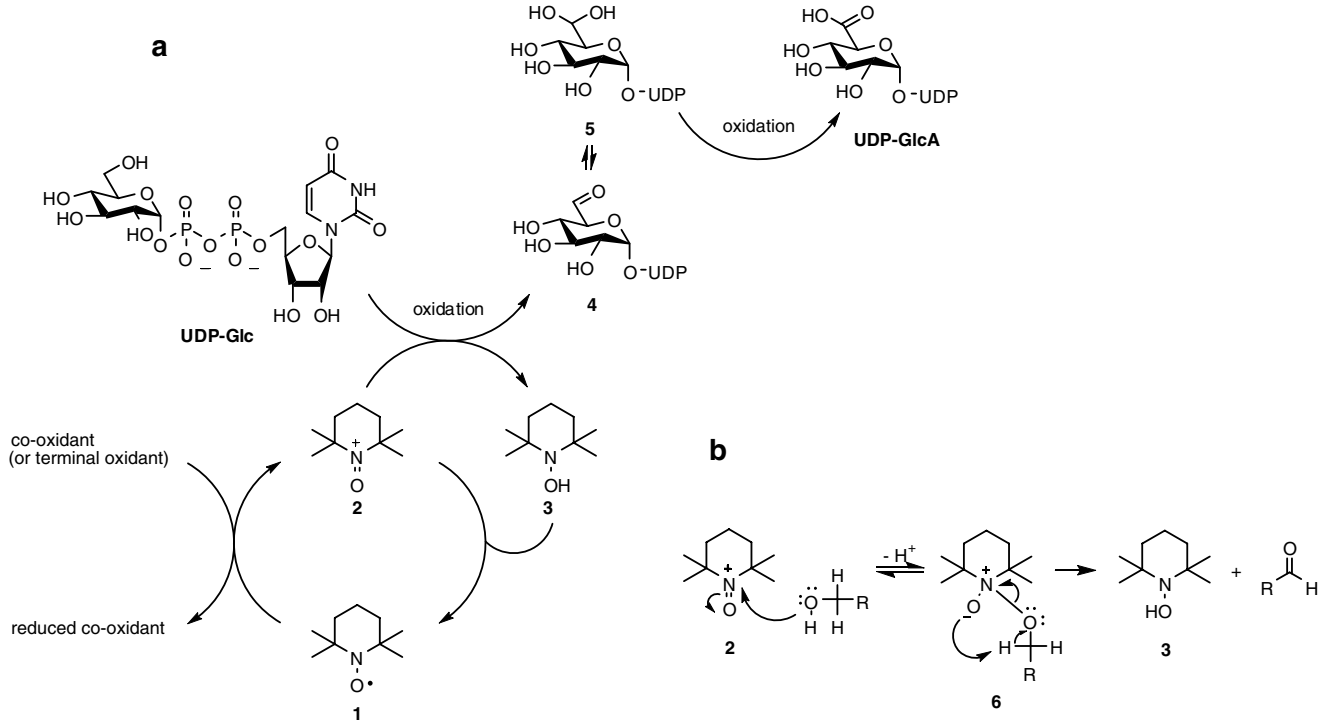
0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.10.016

oxidation using molecular oxygen.[9] A modification of this method has been successfully applied to oxidise derivatives of nucleosides[10] without the need to protect the secondary alcohol groups of the sugar moiety. More importantly, platinum-catalysed oxidation has been shown to selectively oxidise UDP-*N*-acetyl-α-D-mannosamine (UDP-ManNAc) to the corresponding uronic acid (UDP-ManNAcA), albeit in yields in the 12–20% range.[11] Further, Li and Bugg[12] have achieved the oxidation of UDP-*N*-acetyl-α-D-glucosamine (UDP-GlcNAc) to the corresponding uronic acid (UDP-GlcNAcA) using platinum and molecular oxygen. Such reactions are typically conducted in water at elevated temperatures, which provide limited scope for multiple parallel synthesis and are also a potential cause for concern when dealing with labile sugar derivatives. Positive experience with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) mediated procedures in connection with oxidation of synthetic, unprotected plant glycan fragments[13] attracted us to consider this reagent.[14] Selective oxidation of the C-6 primary alcohol of unprotected sugars[15–17] and of sugar-1-phosphates[18] with TEMPO is well documented. TEMPO has also been used to oxidise the C-5′ primary hydroxyl group of nucleosides[19] and 2′-deoxynucleosides[20] to give the corresponding nucleoside-5′-carboxylic acids. However, in these cases, 2′,3′-*O*-isopropylidene or 3′-*O*-*tert*-butyldiphenylsilyl protected nucleosides had to be employed in order to avoid oxidation of the secondary hydroxyl groups of ribose or 2′-deoxyribose, respectively. To the best of our knowledge, no direct TEMPO-mediated oxidation of an unprotected sugar nucleotide has been reported. Our plan was to assess sugar nucleotide oxidation with TEMPO (in combination with a co-oxidant)[21] against a procedure employing molecular oxygen–platinum metal.[22]

2. Results and discussion

Given the commercial availability of UDP-α-D-glucuronic acid (UDP-GlcA), initial studies were conducted on the oxidation of UDP-Glc. As reviewed by de Nooy and co-workers,[21] in TEMPO-mediated oxidation of alcohols the relatively stable nitroxyl radical **1** is first oxidised to an oxoammonium salt **2** by the action of an internal co-oxidant (typically sodium hypochlorite) (Scheme 1a). The oxoammonium salt **2** is the actual reactant in the alcohol oxidation process and itself undergoes reduction to the corresponding hydroxylamine **3**, which is then recycled to the initial nitroxyl species **1** by the action of the internal co-oxidant (Scheme 1a).

Although considerable effort has been made to elucidate the mechanism of the reaction between the oxoammonium salt **2** and alcoholic substrates, the detail remains unclear. As postulated by de Nooy and co-workers,[23] under alkaline conditions adduct **6** might be expected to undergo a Cope-like elimination involving a cyclic transition state (Scheme 1b). Consequently



Scheme 1. (a) Prospective mechanism for TEMPO mediated oxidation of UDP-Glc to UDP-GlcA. (b) Sterically confining cyclic mechanism for the oxidation of alcohols.

Table 1. Screening of conditions for TEMPO[a] mediated oxidation of UDP-Glc

Entry	Co-oxidant (equiv)	Co-catalyst (concn)	Solvent/additive	Time (h)	T (°C)	UDP-Glc:A:B[e]	pH	Ref.
1	NaOCl (2.4)[d]	NaBr (10 mol %)	H_2O	2	0	Decomposed	10[b]	21,24,15
2	NaOCl (3) + $NaHCO_3$ (s)	NaBr (10 mol %)	Satd aq $NaHCO_3$–Na_2CO_3	0.5	0	Decomposed	9.5	21,25
3	NaOCl (2.2)	NaBr (10 mol %)	CH_3CN/H_2O 9:1	23	−10	Decomposed	7[c]	21
4	NaOCl (2.2)	NaBr (10 mol %)	$CH_3CN/10^{-5}$ M NaOH	24	−10	Decomposed	9[c]	21
5	NaOCl (2.2)	NaBr (10 mol %)	CH_3CN/H_2O 9:1	4	−10 to rt	Decomposed	7[c]	21
6	NaOCl (2.2) + $NaHCO_3$ (s)	NaBr (10 mol %)	CH_3CN/satd aq $NaHCO_3$	23	−10	0:4.6:1	8.6	25
7	NaOCl (2.5) + $NaHCO_3$ (s)	NaBr (10 mol %)	CH_2Cl_2/H_2O and $Bu_4N{\cdot}HSO_4$	0.5	0	Decomposed	8.6	25
8	NaOCl (2.2)	—	$CH_3CN/10^{-5}$ M NaOH	48	rt	SM recovered	9[c]	21
9	NaOCl (3)	—	CH_3CN/H_2O 9:1	8	−10 to rt	2:1:1	7[c]	21
10	NaOCl (3)	—	CH_3CN/H_2O 9:1	28	−10	0:1:2	7[c]	21
11	NaOCl (3)	—	CH_3CN/H_2O 9:1	48	−10	Decomposed	7[c]	21
12	NaOCl (3)	—	$CH_3CN/10^{-5}$ M NaOH	48	−15	1:3:6	9[c]	21,16
13	NaOCl (3)	—	$CH_3CN/10^{-5}$ M NaOH	63	−15	0:1:4	9[c]	21,16
14	NaOCl (3)	—	$CH_3CN/10^{-5}$ M NaOH	72	−15	0:0:1	9[c]	21,16
15	NaOCl (3) + $NaHCO_3$ (s)	—	CH_3CN/satd aq $NaHCO_3$	17	−15	0:1:3.5	8.6	21,15
16	NaOCl (3)[d]	—	10^{-5} M NaOH	24	0	Decomposed	10[b]	21
17	DAIB (2.2) + $NaHCO_3$ (s)	—	CH_3CN/H_2O 1:1	4	0 to rt	5:4:1	9	19,20
18	DAIB (2.2) light excluded	—	CH_3CN/H_2O 1:1	4	0 to rt	2:7:1	7–5	19,20
19	NMO (2.2)	—	CH_3CN/H_2O 1:1	4	−10 to rt	SM recovered	—	

[a] 1 mol % was used in all reactions.
[b] Adjusted to given value by continuous addition of 0.5 M NaOH.
[c] Initial value before addition of NaOCl (pH 12.6).
[d] Adjusted to pH 10 by addition of 1 M HCl.
[e] Ratio determined by HPLC, A and B are peaks eluting after UDP-Glc (see Fig. 1) under conditions given in Section 3.1.

secondary alcohols are oxidised slower than their sterically less constrained primary alcohol counterparts.[‡] TEMPO-mediated reaction of UDP-Glc would therefore be expected to oxidise the C-6″ primary alcohol to give the corresponding aldehyde **4** initially. In aqueous solution, the aldehyde **4** will undergo a (reversible) hydration to give a *gem*-diol **5**, which on oxidation with a further equivalent of the oxoammonium salt should then lead to the C-6″ carboxylic acid, UDP-GlcA (Scheme 1a).

To investigate the TEMPO-mediated oxidation of UDP-Glc, a variety of conditions were employed (see Table 1). The course of the oxidation was followed by TLC or HPLC and reaction products were characterised by ^{1}H NMR spectroscopy. In two of these experiments (see Table 1, entries 8 and 19) the starting material, UDP-Glc, was recovered. Entry 8 confirms that the oxoammonium salt **2** required for the oxidation is not stable at room temperature and entry 19 suggests that NMO is not a strong enough oxidant to oxidise TEMPO to the oxoammonium salt **2**. In a number of experiments ^{1}H NMR spectroscopy analysis of the resulting mixture showed cleavage of the sugar–phosphate bond associated with degradation of the sugar nucleotide; this was particularly frequent in experiments employing NaBr as co-catalyst (entries 1–5 and 7).

In many oxidation reactions (e.g., entries 6, 9–10, 12–15) and in particular where (diacetoxyiodo)benzene (DAIB) was employed as co-oxidant (entries 17 and 18), after elution of UDP-Glc substrate on anion exchange HPLC (see Fig. 1), two newly formed peaks, A and B, were eluted. Using preparative anion exchange HPLC, both A and B were isolated and subsequently characterised.

Using ^{1}H NMR spectroscopy the materials isolated as peaks A and B were both shown to be complex mixtures. Employment of anion exchange chromatography facilitated separation of ionized carboxylic acids (peak B) from a mixture of aldehydes and their corresponding

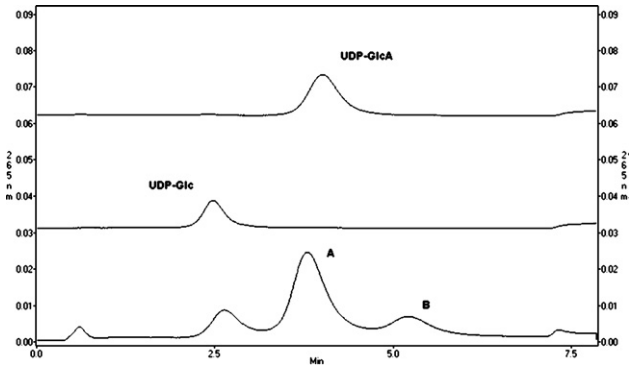


Figure 1. HPLC: strong anion exchange column Poros HQ 50. TEMPO mediated oxidation of UDP-Glc (see Table 1, entry 18). Top: authentic UDP-GlcA (t_R 4.06 min); middle: authentic UDP-Glc (t_R 2.46 min); bottom: oxidation mixture after 4 h, formation of two new peaks observed: A (t_R 3.80 min) and B (t_R 5.22 min).

[‡] Relative rates for TEMPO-mediated oxidation of cyclohexanol (0.52), cyclopentanol (2.1), methyl α-D-glucopyranoside (7.8) (Ref. 23).

hydrates (peak A). In spite of the complexity of the mixtures, it was possible to make some tentative structural conclusions from ^{1}H NMR data.

Peak A eluted with a retention time similar to that of authentic UDP-GlcA. However, ^{1}H NMR data of peak A suggested a mixture of compounds containing no UDP-GlcA. Most importantly, the absence (or strong suppression) of H-2′ and H-3′ signals in the ^{1}H NMR spectra was indicative of over-oxidation of the ribose moiety either to the corresponding 2′,3′-di-ketone or the 2′- or 3′-mono-ketones, which adopt the preferred 2′,3′-ene-diol form (Chart 1). This was further supported by the fact that the anomeric H-1′ of ribose collapsed into a singlet at 6.00 ppm (s, 1H) (doublet at 6.02 ppm in UDP-GlcA).[26] Moreover, a strong signal at 9.22 ppm (s, 1H) could be assigned to a non-hydrated C-6″ aldehyde,[27] probably stabilised by conjugation with a double bond arising from β-elimination reaction.[28,29] The corresponding alkene proton H-4″ appears at 5.61 ppm (d, $J = 3.6$ Hz).[30] The fact that aldehyde is not hydrated is also consistent with α,β-unsaturation. The signal of the non-hydrated aldehyde group was, however, absent in the ^{1}H NMR of the crude reaction mixture; its hydrated form was observed instead as a broad singlet at 5.55 ppm,[22] so it is likely that the β-elimination actually occurred during the chromatography process, giving compound **7** or **8**.

^{1}H NMR data for peak B again showed significant differences to that expected for UDP-GlcA. The absence of H-2′ and H-3′ ^{1}H NMR signals and the fact that the anomeric H-1′ signal of ribose collapsed into a singlet at 5.93 ppm (s, 1H) again support the over-oxidation of the ribose moiety to the corresponding mono- and/or di-ketones. Moreover, the presence of a multiplet at 5.56–5.60 ppm is consistent with an alkene proton β to a carboxylate moiety. Although the material isolated as peak B was clearly a mixture of products, ^{1}H NMR data suggest that the major component is likely compound **9** or **10**.

In summary, all attempts to date to selectively oxidise the C-6″ primary alcohol of UDP-Glc to the desired uronic acid with TEMPO failed, leading only to inseparable samples of mixed oxidation, over-oxidation, elimination and degradation products. The major complication seems to be the undesired oxidation of the ribose secondary alcohol groups to the corresponding mono- and/or di-ketones, which appears to proceed with rates similar to the oxidation of the glucose primary alcohol. It has been shown[23] that TEMPO-mediated oxidation of the primary alcohol in methyl α-D-glucopyranoside proceeds 15 times faster than the oxidation of cyclohexanol. This factor is only 3.7 in the case of cyclopentanol, making the selective oxidation of a glucose residue rather difficult in the presence of a ribose residue (as in UDP-Glc).

We then (re)focussed our attention on the platinum-catalysed oxidation of UDP-GlcNAc with molecular oxygen (Scheme 2). The course of the oxidation was followed by HPLC and by ^{1}H NMR spectroscopy, with authentic UDP-GlcNAcA (generated enzymatically with the *Pseudomonas* WbpA dehydrogenase)[6] used for comparison.

The oxidations were performed in water containing $NaHCO_3$ using an oxygen cylinder as a source of oxygen. Platinum black or standard quality PtO_2 hydrogenated in H_2O prior to the oxidation to give platinum were shown not to catalyse the required oxidation, even at elevated temperatures and prolonged reaction times. PtO_2 (Adams' catalyst) pre-hydrogenated in H_2O was used to give a high surface platinum catalyst initially, and a yield showed conversions of about 20% when the UDP-GlcNAc oxidation was performed at 100 °C for 24 h.

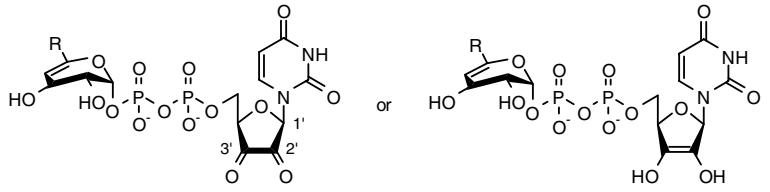
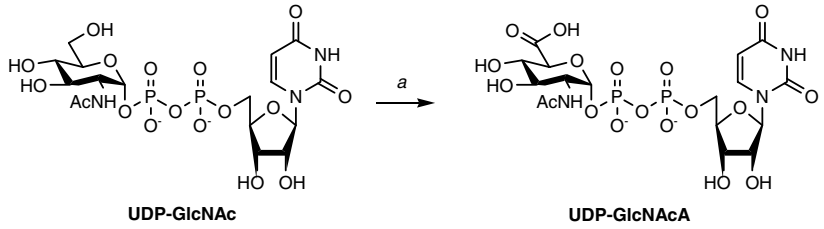


Chart 1. Tentatively assigned structures of the major components of mixtures arising from TEMPO-mediated oxidation of UDP-Glc.



Scheme 2. Reagents and conditions: (a) Pt, O_2, H_2O–$NaHCO_3$, reflux.

The conversion has gradually been improved by use of glassware designed to enable vigorous stirring of the reaction mixture whilst passing oxygen into the mixture through a porosity 1 sinter (see Supplementary data). Most importantly, further addition of fresh catalyst during the course of the reaction was essential to achieve high conversion of starting material to product. A combination of these measures eventually led to conversion of ca. 92%, as determined by HPLC on a Poros HQ 50 strong anion exchange column (Fig. 2).

Under conditions indicated in Section 3.1, UDP-GlcNAc eluted at 2.21 min and the product UDP-GlcNAcA at 3.73 min. On a larger scale (>100 mg) it was deemed necessary to prolong the reaction time or remove the old catalyst by filtration and use a new batch in order to achieve similar conversions. Finally, the product was isolated as its triammonium salt in a yield of 65% using preparative HPLC on a Poros HQ 20 anion exchange column. The purified sample was analysed using ion-pair reversed phase HPLC[31] with tetrabutylammonium hydrogen sulfate (TBAHS) as the counterion. The purity of the synthetic UDP-GlcNAcA was shown to be >99% (Fig. 2b). ^{1}H, ^{13}C and ^{31}P NMR spectra of the purified triammonium salt of UDP-GlcNAcA were in good agreement with published data[6] for UDP-*N*-acetyl-D-glucosaminuronic acid tris(triethylammonium) salt. Diagnostic ^{1}H NMR signals include H-6″a (3.80 ppm, dd, $J_{6''a,6''b} = 12.5$ Hz, $J_{5'',6''a} = 4.9$ Hz, 1H) and H-6″b (3.87 ppm, dd, $J_{6''a,6''b} = 12.5$ Hz, $J_{5'',6''b} = 2.3$ Hz, 1H) signals for UDP-GlcNAc,[32] which were lost on oxidation and H-5″ (3.93 ppm, ddd, $J_{5'',6''a} = 4.9$ Hz, $J_{5'',6''b} = 2.3$ Hz, $J_{4'',5''} = 10.1$ Hz, 1H), which on oxidation became a simple doublet (4.16 ppm, d, $J_{4'',5''} = 10.5$ Hz, 1H). In ^{13}C NMR spectra, the C-6″ (62.9 ppm) signal for UDP-GlcNAc[27] was lost on oxidation and became a signal at 177.0 ppm corresponding to the newly formed carboxylic acid in UDP-GlcNAcA.

In conclusion, the direct oxidation of two unprotected sugar nucleotides, UDP-Glc and UDP-GlcNAc, to the corresponding uronic acids (UDP-GlcA and UDP-GlcNAcA, respectively) was explored using either TEMPO or platinum-catalysed oxidation. In the case of TEMPO-mediated oxidation, the major complication turned out to be the competing oxidation of the ribose secondary alcohol groups to the corresponding diketone. Further studies are required in order to overcome this problem. On the other hand, despite initial concerns about reactions of sugar nucleotides requiring boiling water as the solvent, following optimisation of apparatus and experimental protocol, platinum-catalysed oxidation with molecular oxygen proved to be a convenient method for oxidation of UDP-GlcNAc to the corresponding uronic acid, producing highly pure synthetic material in 100 mg+ quantities.

3. Experimental

3.1. General methods

All chemicals were purchased as reagent grade and used without further purification. TLC was performed on precoated silica plates containing a fluorescence indicator. Silica gel (63 μm) was used for analytical TLC with 6:3:1 CH_2Cl_2–MeOH–water as mobile phase. Compounds were visualised under UV (254 nm) and by heating after dipping in a solution of 5% H_2SO_4 in EtOH.

HPLC was performed on a Biocad Sprint Perfusion Chromatography instrument. The mixtures were applied on a Poros HQ 50 strong anion exchange column (10 × 50 mm, CV ~ 4 mL) for small scale separations. The column was first equilibrated with 5 CV of 5 mM NH_4HCO_3 buffer, then the mixture was applied and eluted with linear gradient of NH_4HCO_3 from 5 to 250 mM in 15 CV, then with NH_4HCO_3 250 mM in 5 CV at a flow rate of 12 mL min^{-1} and detection with an on-line detector to monitor A_{265}. Finally, the column was washed with 3 CV of 1 M NH_4HCO_3 followed by 1 CV of 5 mM NH_4HCO_3 at the same flow rate after each

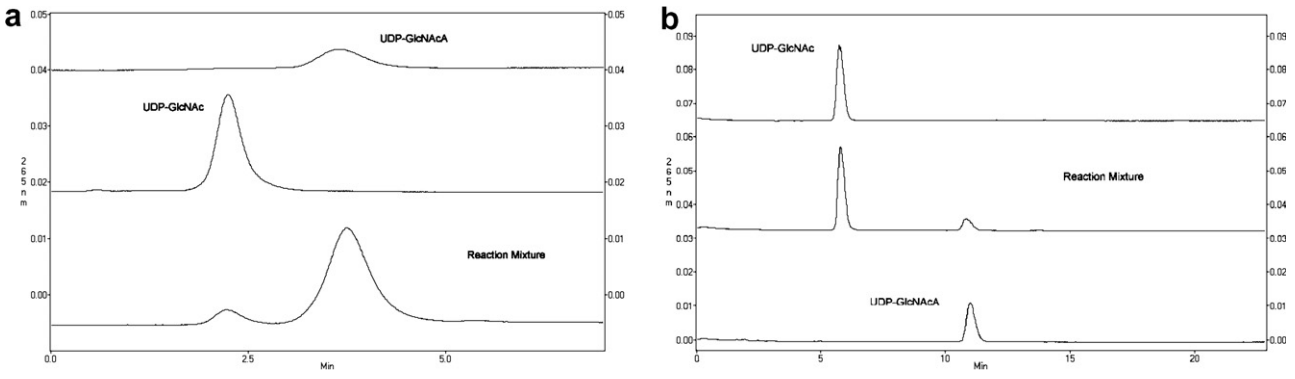


Figure 2. (a) HPLC: anion exchange column Poros HQ 50. Top: authentic UDP-GlcNAcA (t_R 3.73 min); middle: authentic UDP-GlcNAc (t_R 2.21 min); bottom: oxidation mixture after 48 h. (b) Ion-pair reversed phase HPLC. Top: authentic UDP-GlcNAc (t_R 5.76 min); middle: oxidation mixture after 4 h; bottom: UDP-GlcNAcA (t_R 10.89 min) following purification by anion exchange chromatography (>99% purity).

run. In the case of large scale separation, a Poros HQ 20 strong anion exchange column (16 × 100 mm, CV ~ 20 mL) was used under similar conditions (with different flow rate of 30 mL min^{-1} and detection with an on-line detector to monitor A_{280}).

Purified samples were analysed using ion-pair reversed-phase HPLC[26] on a Phenomenex Jupiter 5 μm C18 column (4.6 × 250 mm, CV ~ 4.2 mL). Mobile phase A: 50 mM potassium phosphate buffer pH 7.0 (50 mM K_2HPO_4 solution adjusted to pH 7.0 with a 50 mM KH_2PO_4 solution). The counterion, TBAHS, was added at the final concentration of 2.5 mM. Mobile phase B: 100 mM potassium phosphate buffer pH 7.0 (100 mM K_2HPO_4 solution adjusted to pH 7.0 with a 100 mM KH_2PO_4 solution). The counterion (TBAHS) was added at the final concentration of 2.5 mM. The resulting buffer was diluted with acetonitrile in a ratio 2:1 (buffer to acetonitrile). All eluents were degassed by a continuous stream of helium. The column was first equilibrated with 0.5 CV of 97.5% A and 2.5% B. The sample was applied and eluted with linear gradient of 97.5% A and 2.5% B to 70.0% A and 30.0% B in 4 CV, then with 1 CV of 70.0% A and 30.0% B and finally with 0.5 CV of 97.5% A and 2.5% B at a flow rate of 1 mL min^{-1} and detection with an on-line detector to monitor A_{265}. After a set of experiments the column was washed with Milli-Q water followed by a linear gradient from 0% to 15% of acetonitrile in potassium phosphate buffer pH 7.0 at a flow rate of 1 mL min^{-1}. Samples of sugar nucleotides were dissolved in Milli-Q water to give a concentration of about 1 mg mL^{-1}. The stock solutions were then further diluted to achieve working concentrations. The injected amount of each compound was approximately 4 nmol.

NMR spectra were acquired on a Bruker 300 MHz or a Varian 400 MHz spectrometer at 25 °C. Lyophilised sugar nucleotides were dissolved in 0.7 mL of D_2O. Chemical shifts are reported in ppm with respect to the methyl resonance of internal acetone at δ_H 2.22 ppm and δ_C 30.89 ppm. External 85% phosphoric acid was used for the ^{31}P chemical shift reference (0 ppm). ^1H and ^{13}C NMR spectra were assigned using gCOSY and gHSQC. Multiplicity of signals in ^{13}C NMR spectra was determined from gHSQC spectra. Authentic UDP-GlcNAcA was generated by enzymatic oxidation of UDP-GlcNAc with *P. aeruginosa* UDP-GlcNAc 6-dehydrogenase WbpA, as described previously.[6]

3.2. Representative methods for TEMPO-mediated oxidation of UDP-α-D-glucose

The procedures employed were adapted from Epp and Widlansky[19] (See Supplementary data Table 1, entry 17). DAIB (14.0 mg, 43.5 μmol), TEMPO (0.6 mg, 3.8 μmol), $NaHCO_3$ (6.7 mg, 79.8 μmol), and UDP-Glc, disodium salt (12.1 mg, 19.8 μmol) were combined in a reaction vessel, and to this mixture was added 1:1 MeCN–water (2 mL) at 0 °C. The reaction mixture was gradually allowed to warm up to room temperature. All the time the pH of the reaction mixture was ~9. The reaction course was monitored by HPLC. After 4 h, the volatiles were evaporated under reduced pressure (bath temperature 25 °C, membrane pump). The remaining solid was triturated with Et_2O (2 × 2 mL), acetone (2 × 2 mL) and the residue was dissolved in water (1 mL) and separated using anion exchange HPLC.

3.3. Oxidation of UDP-*N*-acetyl-α-D-glucosamine to UDP-*N*-acetyl-α-D-glucosaminuronic acid with platinum–oxygen

Adams' catalyst (Aldrich; 20 mg) was hydrogenated in water (5 mL) under atmospheric pressure for 2 h. The apparatus was evacuated and released under nitrogen 3 times. The freshly prepared platinum in water was added to a solution of UDP-GlcNAc·$2Na^+$ (240 mg, 0.37 mmol) and $NaHCO_3$ (62.4 mg, 0.74 mmol) in water (12 mL). The vigorously stirred mixture was heated to 100 °C and oxygen was passed into the mixture through porosity 1 sinter. After 4 h, the conversion reached ca. 19% by HPLC. A new batch of catalyst, prepared by hydrogenation of Adams' catalyst (20 mg) in water (5 mL), was added and the oxidation was continued overnight. After 48 h, the conversion reached ca. 92%. The catalyst was removed by filtration through two filter papers and a 0.45 μm filter (Millipore Whatman). If necessary, the pH of the filtrate was adjusted to 8.6 by addition of solid $NaHCO_3$ and the solution was freeze-dried. The resulting solid was dissolved in water (1 mL) and purified using anion exchange HPLC. Fractions containing the title compound were pooled and lyophilized to give incompletely neutralised material. Aqueous ammonia (0.1 mL) was added and the material was freeze-dried again to give triammonium salt of the title compound UDP-GlcNAcA (161 mg, 65%) as a white solid in a purity of >99% (as judged by HPLC); ^1H NMR (400 MHz, D_2O): δ 7.95 (1H, d, $^3J_{5,6}$ 8.2 Hz, H-6), 5.98 (1H, d, $^3J_{1',2'}$ 4.4 Hz, H-1′), 5.96 (1H, d, $^3J_{5,6}$ 8.2 Hz, H-5), 5.52 (1H, dd, $^3J_{1'',P\beta}$ 7.6 Hz, $^3J_{1'',2''}$ 3.2 Hz, H-1″), 4.38–4.34 (2H, m, H-2′ and H-3′), 4.29–4.27 (1H, m, H-4′), 4.24–4.20 (1H, m, H-5′a), 4.18–4.13 (1H, m, H-5′b), 4.16 (1H, d, $^3J_{4'',5''}$ 10.5 Hz, H-5″), 4.02 (1H, dt, $^3J_{2'',3''}$ 10.6 Hz, $^3J_{1'',2''}$ 3.2 Hz, $^4J_{2'',P\beta}$ 3.0 Hz, H-2″), 3.81 (1H, dd, $^3J_{3'',4''}$ 9.4 Hz, $^3J_{2'',3''}$ 10.6 Hz, H-3″), 3.58 (1H, dd, $^3J_{3'',4''}$ 9.4 Hz, $^3J_{4'',5''}$ 10.5 Hz, H-4″), 2.07 (3H, s, $CH_3C(O)NH$); ^{13}C NMR (100 MHz, D_2O): δ 177.0 (1C, s, COO^-), 175.5 (1C, s, $CH_3CO{\cdot}NH$), 167.0 (1C, s, C-4), 152.5 (1C, s, C-2), 142.3 (1C, d, C-6), 103.3 (1C, d, C-5), 94.9 (1C, dd, $^2J_{C\text{-}1'',P\beta}$ 6.1 Hz, C-1″), 89.0 (1C, d, C-1′), 83.9 (1C, dd, $^3J_{C\text{-}4',P\alpha}$ 9.2 Hz, C-4′), 74.4 (1C, d, C-2′), 73.8

(1C, d, C-5″), 72.8 (1C, d, C-4″), 71.3 (1C, d, C-3″), 70.3 (1C, d, C-3′), 65.7 (1C, td, $^{2}J_{C\text{-}5',P\alpha}$ 5.6 Hz, C-5′), 54.1 (1C, dd, $^{3}J_{C\text{-}2'',P\beta}$ 8.3 Hz, C-2″), 22.7 (1C, q, *C*H$_3$CO.NH); ^{31}P NMR (121 MHz, D$_2$O): δ −7.74 (P$_\beta$, d, $J_{P\alpha,P\beta}$ 20.5 Hz), −9.46 (P$_\alpha$, d, $J_{P\alpha,P\beta} = 20.5$ Hz); ESI-MS (*m*/*z*): for protonated acid 666 $(M-H+2Na)^+$ (9%), 644 $(M+Na)^+$ (22%), 622 (5%), 620 (7%), 449 (28%), 427 (89%), 218 (100%); HRMS: Calcd for protonated acid $C_{17}H_{26}N_3O_{18}P_2$ ($[M+H]^+$): 622.0681, found 622.0681. ^{1}H, ^{13}C and ^{31}P NMR data were in good agreement with data published[6] for UDP-*N*-acetyl-D-glucosaminuronic acid tris(triethylammonium) salt generated by enzymatic oxidation with *P. aeruginosa* UDP-GlcNAc 6-dehydrogenase WbpA.[6]

Acknowledgements

We thank the BBSRC and the EPSRC for financial support and the EPSRC Mass Spectrometry Service, University of Wales, Swansea for invaluable support. We also acknowledge the support from the Canadian Institutes of Health Research in the form of an operating grant (MOP-14687) to J.S.L. who holds a Canada Research Chair in Cystic Fibrosis and Microbial Glycobiology. Dr. A. H. Haines is thanked for informative discussions.

Supplementary data

^{1}H, ^{13}C, ^{31}P NMR and ES-MS spectra for UDP-GlcNAcA, and photographs of the apparatus used for the platinum catalysed oxidation. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2006.10.016.

References

1. Errey, J. C.; Mukhopadhyay, B.; Kartha, K. P. R.; Field, R. A. *Chem. Commun.* **2004**, 2707–2796.
2. Yang, M.; Proctor, M. R.; Bolam, D. N.; Errey, J. C.; Field, R. A.; Gilbert, H. J.; Davis, B. G. *J. Am. Chem. Soc.* **2005**, *127*, 9336–9337.
3. De Luca, C.; Lansing, M.; Martini, I.; Crescenzi, F.; Shen, G.-J.; O'Regan, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1995**, *117*, 5869–5870.
4. Basu, S. S.; Dotson, G. D.; Raetz, C. R. H. *Anal. Biochem.* **2000**, *280*, 173–177.
5. Burns, V. C.; Pishko, E. J.; Preston, A.; Maskell, D. J.; Harvill, E. T. *Infect. Immun.* **2003**, *71*, 86–94; Rocchetta, H. L.; Burrows, L. L.; Lam, J. S. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 523–553.
6. Miller, W. L.; Wenzel, C. Q.; Daniels, C.; Larocque, S.; Brisson, J.-R.; Lam, J. S. *J. Biol. Chem.* **2004**, *279*, 37551–37558.
7. Allen, A. G.; Thomas, R. M.; Cadisch, J. T.; Maskell, D. J. *Mol. Microbiol.* **1998**, *29*, 27–38; Wenzel, C. Q.; Daniels, C.; Keates, R. A. B.; Brewer, D.; Lam, J. S. *Mol. Microbiol.* **2005**, *57*, 1288–1303.
8. For a general review on oxidation by catalytic dehydrogenation, see, Haines, A. H. *Methods for the Oxidation of Organic Compounds*; Academic Press: London, 1988; pp 10–14.
9. For example: Marino, J. P.; Fernández, R.; de la, P.; Laborge, E. *J. Org. Chem.* **1987**, *52*, 4898–4913.
10. For example: Rosowsky, A.; Kim, S.-H.; Trites, D.; Wick, M. *J. Med. Chem.* **1982**, *25*, 1034–1040; Kim, K.-H.; Kim, J.-Y.; Lee, K.-H.; Noh, M.-J.; Kim, Y.-C.; Park, H.-J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 483–486.
11. Anderson, J. S.; Page, R. L.; Salo, W. L. *J. Biol. Chem.* **1972**, *247*, 2480–2485; Yamazaki, T.; Warren, C. D.; Herscovics, A.; Jeanloz, R. W. *Carbohydr. Res.* **1980**, *79*, C9–C12; Yamazaki, T.; Warren, C. D.; Herscovics, A.; Jeanloz, R. W. *Can. J. Chem.* **1981**, *59*, 2247–2252.
12. Li, J. J.; Bugg, T. D. H. *Chem. Commun.* **2004**, 182–183.
13. Chauvin, A.; Nepogodiev, S. A.; Field, R. A. *J. Org. Chem.* **2005**, *70*, 960–966.
14. Calderón, F. *Synlett* **2006**, 657–658.
15. Fraschini, C.; Vignon, M. *Carbohydr. Res.* **2000**, *328*, 585–589.
16. Söderman, P.; Widmalm, G. *Eur. J. Org. Chem.* **2001**, *18*, 3453–3456.
17. Lin, F.; Peng, W.; Xu, W.; Han, X.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1219–1223.
18. Heeres, A.; van Doren, H. A.; Gotlieb, K. F.; Bleeker, I. P. *Carbohydr. Res.* **1997**, *299*, 221–227.
19. Epp, J. B.; Widlanski, T. S. *J. Org. Chem.* **1999**, *64*, 293–295.
20. Jung, M. E.; Toyota, A. *J. Org. Chem.* **2001**, *66*, 2624–2635.
21. For a comprehensive review on the TEMPO-mediated oxidation of alcohols, see: de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Synthesis* **1996**, 1153–1174.
22. Fried, J.; Sih, J. C. *Tetrahedron Lett.* **1973**, 3899–3902.
23. de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Tetrahedron* **1995**, *51*, 8023–8032.
24. de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Carbohydr. Res.* **1995**, *269*, 89–98.
25. Anneli, P. L.; Biffi, C.; Montanari, F.; Quici, S. *J. Org. Chem.* **1987**, *52*, 2559–2562.
26. Breazeale, S. D.; Ribeiro, A. A.; Raetz, C. R. H. *J. Biol. Chem.* **2002**, *277*, 2886–2896.
27. For spectral data for uridine diphospho-α-D-gluco-hexodialdose hydrate, see Campbell, R. E.; Tanner, M. E. *Angew. Chem., Int. Ed.* **1997**, *36*, 1520–1521.
28. Schoevaart, R.; Kieboom, T. *Tetrahedron Lett.* **2002**, *43*, 3399–3400.
29. Huff, J. B.; Bieniarz, C. *J. Org. Chem.* **1994**, *59*, 7511–7516.
30. For an example of a β-elimination reaction in Swern and PCC oxidations of sugars leading to the corresponding α,β-unsaturated aldehyde, see: Levoirier, E.; Canac, Y.; Norsikian, S.; Lubinaeau, A. *Carbohydr. Res.* **2004**, *339*, 2737–2747.
31. Meynial, I.; Paquet, V.; Combes, D. *Anal. Chem.* **1995**, *67*, 1627–1631.
32. Sweet, C. R.; Ribeiro, A. A.; Raetz, C. R. H. *J. Biol. Chem.* **2004**, *279*, 25400–25410.