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Protecting group free glycosylation: one-pot stereocontrolled access to 1,2-*trans* glycosides and (1→6)-linked disaccharides of 2-acetamido sugars†

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Unprotected 2-acetamido sugars may be directly converted into their oxazolines using 2-chloro-1,3-dimethylimidazolinium chloride (DMC), and a suitable base, in aqueous solution. Freeze drying and acid catalysed reaction with an alcohol as solvent produces the corresponding 1,2-*trans*-glycosides in good yield. Alternatively, dissolution in an aprotic solvent system and acidic activation in the presence of an excess of an unprotected glycoside as a glycosyl acceptor, results in the stereoselective formation of the corresponding 1,2-*trans* linked disaccharides without any protecting group manipulations. Reactions using aryl glycosides as acceptors are completely regioselective, producing only the (1→6)-linked disaccharides.

Introduction

Nature assembles di- and oligosaccharides from unprotected monosaccharide building blocks by the use of highly selective glycosyl transferase enzymes.[1] In many cases these require pre-activation of one component (the glycosyl donor) by first conversion to a glycosyl nucleoside di- (or mono) phosphate derivative. Using these activated substrates, glycosyl transferases, due to their evolution into highly substrate specific enzymes, are able to achieve glycosylation with complete control of both regio- and stereochemistry, and in water as the solvent.

Cloning, expression and purification of recombinant glycosyl transferases has therefore provided highly effective tools that may be used in a synthetic fashion to produce di- and oligosaccharide materials.[2] However, the donor substrates required for these enzymes are expensive and unstable. Furthermore, only a limited number of enzymes are currently commercially available, and the effort required to clone, express and purify a new enzyme from scratch is daunting. Additionally, the exquisite specificity of these enzymes often precludes their more widespread application, since they are highly specific to the formation of one particular type of glycosidic linkage.

The majority of oligosaccharide construction is therefore still undertaken by chemical synthesis, and this is unlikely to change in the near future. However, such methods are logistically extremely demanding. Reaction control can only be achieved after multiple protecting group manipulations of both donor and acceptor components. Indeed, despite advances in glycosylation methodology,[3] for example improvement of reaction efficiency by which the monosaccharide units are actually linked,[4] the vast majority of the effort expended during the synthesis of an oligosaccharide synthesis involves the production of selectively protected monosaccharide building blocks to act as donors and acceptors.

In order to circumvent these considerable logistical impediments, the direct chemical glycosylation of unprotected carbohydrates has become an area of significant interest.[5] In 2009, Shoda[6] and co-workers first introduced the dehydrating reagent 2-chloro-1,3-dimethylimidazolinium chloride (DMC)[7] into the carbohydrate field, and revealed its remarkable ability to selectively activate the anomeric hydroxyl group of unprotected reducing sugars in aqueous solution. A series[8] of highly useful protecting group-free processes based on the use of DMC and analogues[9] have since been developed, including: the synthesis of glycosyl oxazolines;[6] the production of 1,6-anhydro sugars;[10] the synthesis of glycosyl azides[11] and their one-pot conjugation to other species *via* "click" chemistry;[12] the synthesis of aryl[13] and pyridyl thioglycosides,[14] and the direct glycosylation of cysteine residues of peptides.[15] DMC activation has been expanded to allow the direct synthesis of glycosyl thiols,[16] cyanomethyl thioglycosides,[17] glycosyl thiosulfates,[18] glycosyl dithiocarbamates,[19] and can even effect selective acetylation of the anomeric hydroxyl group of unprotected sugars in aqueous solution.[20] In these examples, high levels of 1,2-*trans*

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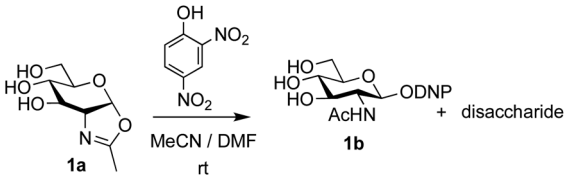
stereocontrol are generally observed, due to the intermediacy of either 1,2-anhydro sugars[21] or glycosyl oxazolines.

More recently interest has turned to intermolecular glycosylation of oxygen nucleophiles, including the one-step synthesis of *para*-nitrophenyl (PNP) and other aryl glycosides of both 2-hydroxy and 2-acetamido sugars directly from the corresponding unprotected reducing sugar.[22] An obvious next step into the further development of DMC-activation processes is an investigation into the possibility of disaccharide formation, ideally using both completely unprotected donors and acceptors.[23] Such protecting group free chemical glycosylation would be unprecedented,[24,25] and would in some respects be analogous to the ability of glycosyl transferases to assemble di- and oligosaccharides; *i.e.* synthesis without the requirement for any protracted reaction sequences involving protecting group manipulations. However, significant hurdles to overcome are avoidance of both self-condensation and hydrolysis of the glycosyl donor, and control of both the regio- and stereochemistry of the newly formed glycosidic linkage. Together these represent formidable obstacles.

In this study we chose to investigate the potential glycosylation of unprotected 2-acetamido sugar donors, using glycosyl acceptors that were unprotected other than at their anomeric centres. Disaccharides of 2-acetamido sugars are components of many biologically important oligosaccharides and glycoconjugates. For example the GlcNAcβ(1→6)Gal and GalNAcβ(1→6)Gal disaccharides are component parts of proteoglycans, glycolipids, and the blood group oligosaccharides,[26] whilst GlcNAcβ(1→6)GlcNAc is the component disaccharide of lipid A (or endotoxin),[27] a constituent part of the lipopolysaccharides that form the outer membranes of most Gram-negative bacteria. The production of these biologically important disaccharides by chemical means[28] was therefore the subject of extensive synthetic effort in the latter decades of the 20th century. Invariably the multi-step routes used to access them encompassed numerous protecting group manipulations in addition to the required glycosylation reaction. However, several of these syntheses were achieved using the 'oxazoline method'[29] – namely use of a glycosyl oxazoline as the glycosyl donor, which was activated to reaction with an acceptor by treatment with an acid. It was therefore envisaged that since unprotected 2-acetamido sugars can be converted into their corresponding pyranose oxazolines by DMC-mediated reaction,[6] (a very useful method for producing unprotected oxazolines as donors for enzyme catalysed glycosylations),[30] then perhaps oxazolines made in this fashion could then be used for chemical disaccharide formation, opening up a realistic possibility of protecting group free disaccharide formation *via* chemical means in the case of 2-acetamido sugars.

Results and discussion

The possibility of disaccharide formation *via* intermediate unprotected glycosyl oxazolines made by the DMC-method was first revealed during the attempted formation of the β-dinitrophenyl (DNP) glycoside of GlcNAc (Scheme 1). Here, using the previously developed method,[22b] namely conversion of



Scheme 1 Unexpected disaccharide formation during attempted conversion of GlcNAc oxazoline **1a** into its β-DNP glucoside **1b**.

GlcNAc **2a** to its oxazoline **1a** by treatment with DMC/Et_3N in water, freeze drying, dissolution of the residue in an appropriate anhydrous aprotic solvent system (*e.g.* a 10 : 1 mixture of MeCN and DMF) and addition of the phenol, unexpectedly led to disaccharide side products. It was therefore concluded that, in addition to direct reaction with the oxazoline to form the desired glycoside **1b**, di-nitrophenol (pK_a in water ∼4.1)[31] was capable of catalysing self-reaction of the GlcNAc oxazoline, leading to disaccharide formation. As previously mentioned, acid catalysis was one of the first examples of the 'oxazoline method' of glycosylation. There are multiple reports of this type of process in the older literature, for example using *p*-toluenesulfonic acid (TsOH) as the acid,[32] though they were not always particularly high yielding. Though largely superseded by alternative processes, there have also been several attempts at increasing the efficiency of the oxazoline method in more recent years.[33,34] Most relevantly, Bundle and co-workers reported the production of β-glycosides of GlcNAc *via* an intermediate furanosyl oxazoline, temporarily protected *in situ* as the 5,6-acetonide, which was reacted with an alcohol as solvent and either *p*-toluenesulfonic or camphorsulfonic acids.[35]

A study was therefore initiated in order to investigate the possible utility of this acid catalysed disaccharide formation in a more general sense. In particular the question arose as to whether this method of glycosylation could be applied using both unprotected oxazolines as the donors and unprotected glycosyl acceptors. Reaction between GlcNAc **2a** as the glycosyl donor and the β-pNP-glucoside **3a**, made in one step as previously described,[22a] as the glycosyl acceptor was investigated as a model system (Table 1). Firstly, GlcNAc was converted to its oxazoline by treatment with DMC/Et_3N in water, and the crude reaction mixture freeze dried. The residue was dissolved in a 10 : 1 mixture of MeCN and DMF as an anhydrous aprotic solvent system. An excess of acceptor **3a** (initially 10 equiv.) was then added; an excess was deemed necessary so that glycosylation of the acceptor **3a** would outcompete any self-reaction of GlcNAc oxazoline. The addition of an acid was then used to initiate glycosylation; reactions were monitored by TLC and run until all oxazoline had reacted.

Firstly, the ability of di-nitrophenol (DNPOH) to cause oxazoline opening and glycosylation was investigated. The use of a large excess of DNPOH (6 equiv.) and 10 equiv. of the acceptor **3a** led to the production of the GlcNAcβ(1→6)Glc disaccharide **4a** as the only reaction product, in 37% yield (Table 1, entry 1) after 24 h. Notably glycosylation was completely regioselective for the primary 6-hydroxyl group of the acceptor.[36] The product

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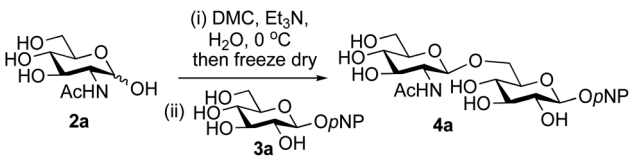
Table 1 Reaction development with GlcNAc **2a** as donor, and β-pNP glucoside **3a** as acceptor



Entry[a]	Acid (equiv.)	Time/h	Acceptor **3a**/equiv.	Isolated yield of **4a** (%)
1	DNPOH (6)	24	10	37
2	DNPOH (6)	24	5	39
3	DNPOH (6)	24	3	32
4	DNPOH (1)	24	5	29
5	PPTS (6)	1	5	39
6	PPTS (1)	4	5	22
7	Pyridinium triflate (6)	1	5	39
8	Pyridinium triflate (1)	4	5	18
9	TsOH (6)	3	5	56
10	TsOH (1)	3	5	50
11	CSA (1)	1	5	46
12[b]	TsOH (1)	3	5	31

[a] Reaction conditions: (i) DMC, Et_3N, H_2O, 0 °C, 1 h, then freeze-dry; **3a**, MeCN/DMF (10 : 1), 4 Å powdered molecular sieves, acid, rt. [b] Two-step process in which the oxazoline was specifically purified before glycosylation.

regiochemistry was confirmed by HMBC correlations between C-**1b** and H-**6a**/**6a**′ and H-**1b** and C-**6a**/**6a**′ (see ESI†). Although this 37% yield perhaps seems somewhat modest at first, this single step synthesis should be compared with other syntheses of protected versions of the GlcNAcβ(1→6)Glc disaccharide, which typically require at least seven synthetic steps.[28]

Reduction of the amount of acceptor **3a** used to 5 equivalents actually resulted in a slight increase (39%) in product yield (Table 1, entry 2); again, reaction required 24 h to reach completion. However, the use of only 3 equivalents of **3a** resulted in decreased isolated product yield (32%, Table 1, entry 3), so 5 equivalents of acceptor were used in subsequent reactions. A reduction in the amount of DNPOH used to only 1 equivalent correspondingly led to a significant reduction in yield (Table 1, entry 4, 29%).

Next alternative acids were investigated. Firstly, the use of 6 equivalents of pyridinium *p*-toluenesulfonate (PPTS) led to a faster reaction (complete after 1 h at rt) that was equally effective to DNPOH (39% yield, Table 1 entry 5), but the use of only 1 equiv. of PPTS resulted in a significantly decreased yield of **4a** (22% yield, Table 1 entry 6) and required 4 h to reach completion. Pyridinium triflate showed a similar profile of effectiveness (Table 1 entries 7 & 8). However, the use of *p*-toluenesulfonic acid (TsOH) was found to produce superior results: 6 equivalents of TsOH gave **4a** in 56% yield after 3 h at rt, whilst the use of only 1 equivalent of TsOH still gave **4a** in a comparable 50% yield. The use of 1 equivalent of camphorsulfonic acid (CSA) also produced **4a** in similar yield after 1 h at rt (Table 1, entry 11, 46%). However, given the lower cost of TsOH, the use of one equivalent of this acid was selected for all other applications. Finally, for comparative purposes a two-step process, in which the oxazoline was specifically purified before reaction with acceptor **3a**, was investigated (Table 1, entry 12). Notably the yield of product obtained in this case, (31%) was considerably lower than the equivalent one-step process (50%, Table 1, entry 10), indicating that oxazoline purification is not advantageous.

Application of the process to the one-pot stereoselective synthesis of β-alkyl glycosides of GlcNAc using some simple alcohols was investigated next (Table 2).[37] Initial studies using only 5 equivalents of *n*-pentenol as the acceptor and performing the reaction in an MeCN/DMF mixture produced the desired β-pentenyl glycoside **5a** in 48% yield (Table 2, entry 1). However, a process emulating Fischer glycosylation, in which the alcohol was itself used as the solvent, was found to be more efficient. The optimised procedure involved freeze-drying of the crude reaction mixture following DMC-mediated oxazoline formation in water. Then the residue was dissolved in the appropriate alcohol and stirred with 4 Å powdered molecular sieves for 30 min at room temperature, before oxazoline opening was initiated by the addition of 1 equivalent of TsOH (based on GlcNAc starting material). After stirring for 3 h at rt, the reaction was complete (as monitored by TLC), and the corresponding β-alkyl glycosides **5a–c** were isolated by column chromatography in good yield (73–76%, Table 2, entries 1–3). The process was also applicable to a tertiary alcohol acceptor, *tert*-butanol (Table 2, entry 4), although the yield of the product β-*tert*-butyl glycoside **5d** was decreased (45%). This yield is comparable to that reported by Bundle (51%, $\beta:\alpha$, 4 : 1),[35] but in contrast our reaction was completely stereoselective. Despite this slightly lower yield, ready protection of the anomeric centre of GlcNAc as an acid cleavable *tert*-butyl glycoside may find useful

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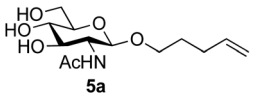
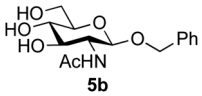
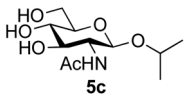
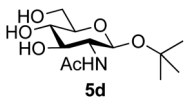
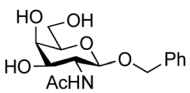
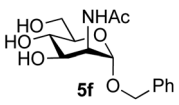
Table 2 One-pot production of alkyl glycosides[a]

Entry	Donor	Alcohol	Product	Isolated yield (%)
1	GlcNAc **2a**	*n*-Pentenol	 **5a**	73, 48[b]
2	GlcNAc **2a**	Benzyl alcohol	 **5b**	75
3	GlcNAc **2a**	Isopropanol	 **5c**	76
4	GlcNAc **2a**	*tert*-Butanol	 **5d**	45
5	GalNAc **2b**	Benzyl alcohol	 **5e**	43
6	ManNAc **2c**	Benzyl alcohol	 **5f**	57

[a] Reaction conditions: (i) DMC, Et_3N, H_2O, 0 °C, 1 h, then freeze-dry; (ii) alcohol as solvent, TsOH (1 equiv.), 4 Å powdered molecular sieves, rt, 3 h. [b] Reaction performed in MeCN/DMF in the presence of 5 equiv. of alcohol as acceptor.

application in synthesis. Furthermore, the procedure was also applicable to other unprotected 2-acetamido sugars; thus the benzyl glycosides of GalNAc **2b** and ManNAc **2c** were produced stereoselectively as the β- and α-glycosides **5e** and **5f** respectively (Table 2, entries 4 and 5). Interestingly the slightly lower yields mirror the generally lower yields observed for the DMC-mediated formation of the oxazolines of these monosaccharides.[6] Attention then turned to the use of unprotected glycoside acceptors in order to delineate the scope of the reaction (Table 3). Firstly, using GlcNAc **2a** as the glycosyl donor, a range of different unprotected glycosides (**3a–i**, **5d**) were trialled as acceptors. The previously optimised reaction using the pNP-glucoside acceptor **3a** gave the GlcNAcβ(1→6)Glc disaccharide **4a** in 49% yield on a larger scale (Table 3, entry 1). Next, the use of the *manno* configured pNP-acceptor **3b** gave the corresponding known GlcNAcβ(1→6)Man disaccharide **4b**[38] in a similar yield (46%), again in a reaction that was completely regioselective. This one-step synthesis compares very favourably with the previous reported[38] convergent synthesis of **4b** which required a total 6 steps (24% overall yield based on the linear sequence using the acceptor; see ESI Scheme S1†). However, surprisingly, glycosylation of the methyl mannopyranoside **3c** under identical conditions was not completely regioselective, and gave a mixture of 3 regioisomeric disaccharides, in which the β(1→6) isomer **4c** [38] predominated (50% combined yield, entry 3) and from which **4c** [38] could be readily purified by chromatography and isolated in pure form (25% isolated yield).

The reason for the observed reduction in regioselectivity was probed further. Evidence of the importance of the use of an aryl glycoside as the acceptor was the observation that glycosylation of the *para*-methoxyphenyl mannoside **3d** was also completely regioselective (40% yield, entry 4; reaction performed in DMF as the sole solvent for reasons of acceptor solubility). In contrast, reaction of the unprotected glycosyl acetate **3e** (entry 5) and fluoride **3f** (entry 6) were non-selective, and produced a mixture of 3 regioisomers (48% and 55% combined yields respectively) in which the (1→6)-linked disaccharides **4e** and **4f** predominated (36% and 33% isolated yields respectively). Use of the pNP-galactoside acceptor **3g** (Table 3, entry 7) at rt was also not completely regioselective (46% total yield), but again the (1→6) disaccharide **4g** [39] predominated (32% isolated yield). However, in this case, lowering the reaction temperature to 0 °C resulted in a completely regioselective reaction, and formation of the GlcNAcβ(1→6)Gal disaccharide **4g** as the sole glycosylation product in 44% yield. Again, this one-step synthesis compares very favourably with previous reported[39] 8-step convergent synthesis of **4g** (13% yield based on the linear sequence using the acceptor; see ESI, Scheme S2†). Next the use of the pNP-glycoside of GlcNAc **3h** as acceptor (Table 3, entry 8) gave the GlcNAcβ(1→6)GlcNAc disaccharide **4h** [40] as a single regioisomer in 39% yield. This particular reaction is notable as the product is the constituent disaccharide of lipid A. This one-step synthesis therefore compares extremely favourably with previous methods[40] of production of this disaccharide (6 or 8

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Table 3 Disaccharide formation using unprotected 2-acetamido sugars as donors, and unprotected glycosides as acceptors[a]

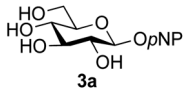
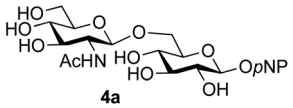
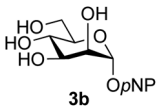
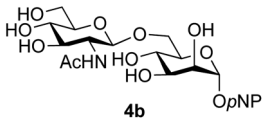
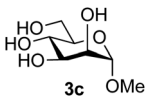
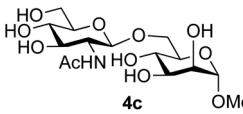
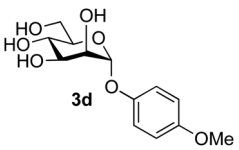
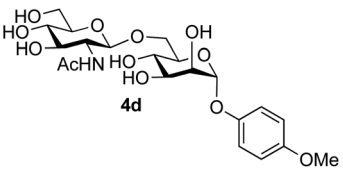
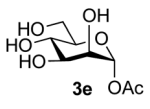
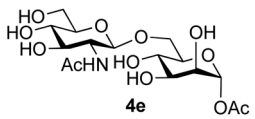
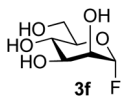
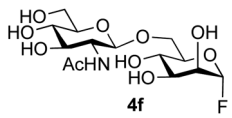
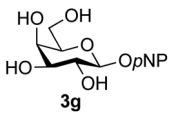
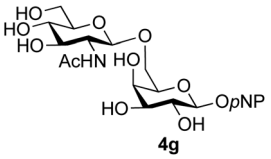
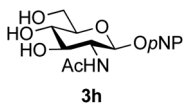
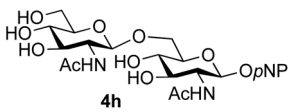
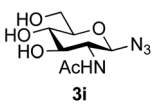
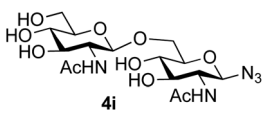
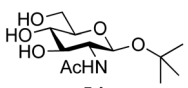
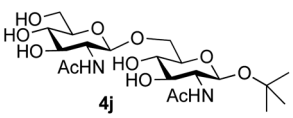
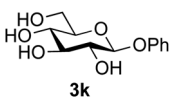
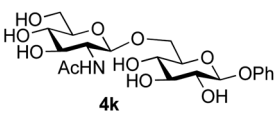
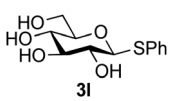
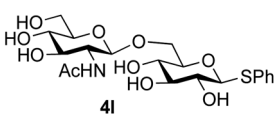
Entry	Donor	Acceptor	Product	Isolated yield of (1→6) linked disaccharide (%)	Regioselectivity/total combined yield
1	GlcNAc **2a**	 **3a**	 **4a**	49	(1→6) only
2	GlcNAc **2a**	 **3b**	 **4b**	46	(1→6) only
3	GlcNAc **2a**	 **3c**	 **4c**	25	3 regioisomers/50%
4	GlcNAc **2a**	 **3d**	 **4d**	40[e]	(1→6) only[e]
5	GlcNAc **2a**	 **3e**	 **4e**	36	3 regioisomers/48%
6	GlcNAc **2a**	 **3f**	 **4f**	33	3 regioisomers/55%
7	GlcNAc **2a**	 **3g**	 **4g**	32[a] 44[b]	2 regioisomers/46%[a] (1→6) only[b]
8	GlcNAc **2a**	 **3h**	 **4h**	39[e]	(1→6) only[e]
9	GlcNAc **2a**	 **3i**	 **4i**	31[a] 37[d]	3 regioisomers/62%[a] 3 regioisomers/54%[d]
10	GlcNAc **2a**	 **5d**	 **4j**	39[a] 26[b]	3 regioisomers/68%[a] 3 regioisomers/52%[b]
11	GlcNAc **2a**	 **3k**	 **4k**	45	(1→6) < 1% of any other regioisomer
12	GlcNAc **2a**	 **3l**	 **4l**	34[a] 39[b]	3 regioisomers/47%[a] 3 regioisomer/58%[b]

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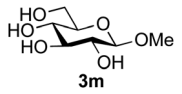
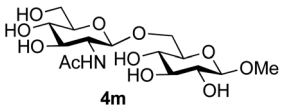
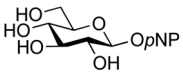
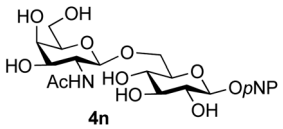
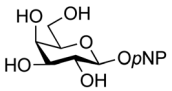
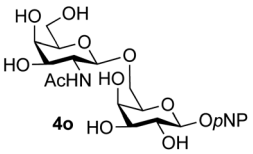
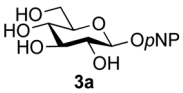
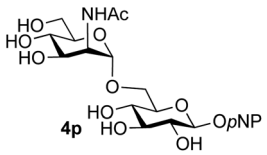
Table 3 (*Contd.*)

Entry	Donor	Acceptor	Product	Isolated yield of (1→6) linked disaccharide (%)	Regioselectivity/total combined yield
13	GlcNAc **2a**	 **3m**	 **4m**	18	3 regioisomers/51%
14	GalNAc **2b**	 **3a**	 **4n**	40	(1→6) only
15	GalNAc **2b**	 **3g**	 **4o**	26[a] 30[c]	2 regioisomers/40%[a] (1→6) only[c]
16	ManNAc **2c**	 **3a**	 **4p**	15	(1→6)/trace amount of another regioisomer

[a] Reaction conditions: (i) DMC **1**, Et_3N, H_2O, 0 °C, 1 h, then freeze-dry; acceptor (**3a–i** or **5d**), MeCN/DMF (10 : 1), 4 Å powdered molecular sieves, TsOH, rt, 3 h. [b] Reaction performed at 0 °C. [c] Reaction performed at −10 °C. [d] Reaction performed at −16 °C for 24 h. [e] Reaction performed in DMF as solvent.

steps, ∼15% yield based on the linear sequence using the acceptor; see ESI Scheme S3†). Further evidence that the complete (1→6)-regioselectivity was acceptor dependent was that the glycosylation of the GlcNAc glycosyl azide **3i** to give **4i**[41] was also not completely regioselective (entry 9), even when using the lowest reaction temperature available (−16 °C; 24 h required to reach completion). Reasoning that the origin of the high (1→6)-regioselectivity was perhaps due to the steric bulk of the anomeric substituent of the acceptor, the use of the *tert*-butyl glycoside **5d** was investigated. Surprisingly reaction of **5d** did not result in completely regioselective reaction (entry 10), though again the (1→6)-disaccharide product **4j** could be readily obtained in pure form by chromatography.

Further insight was sought into the origin of the reaction regioselectivity. To this point reactions of all the aryl glycoside acceptors had proven completely regioselective, albeit in the case of galactoside **3b** requiring reaction at 0 °C, whereas glycosylation of all other glycosides had not been completely selective. Interestingly, reactions of aryl glycosides comprising both electron withdrawing (*e.g.* **3b**) and electron donating (*e.g.* **3d**) aromatic substituents had proven equally regioselective. The origin of regioselectivity of glycosylation of an unprotected, or partially protected, acceptor is complex, as it may depend on both acceptor conformation, and electronic effects.[42] Although glycosyl acceptor reactivity has recently become an area of interest, it has undoubtedly been under-examined in comparison with the extensive studies into glycosyl donor reactivity and selectivity. For comparative and modelling (*vide infra*) purposes the outcomes of reactions of GlcNAc **2b** with the β-*gluco* configured phenyl **3k**, thiophenyl **3l** and methyl **3m** glycosides were examined. Reaction of phenyl glycoside **3k** was again completely regioselective (entry 11), but in contrast reaction of thiophenyl glycoside **3l** was not (entry 12); a mixture of three regioisomers was formed, even at 0 °C. Similarly, reaction of the methyl glycoside **3m** gave a mixture of three regioisomers (entry 13).

In order to possibly gain insight into these differences in reaction outcome, molecular structures for each of the acceptors **3k**, **3l** and **3m** were explored using computational methods (see ESI†). First, low energy conformers were identified using the Tinker software[43] by performing a general conformational search, where the energies were calculated using the MMFF94 force field.[44] The geometries and energies of these low energy conformers were then refined using density functional theory (DFT) to obtain the final structural, energetic and electronic information (see ESI, Tables S3–S5†). Composite images comprising superpositions of the low energy conformations (namely those within 10 kJ mol^{-1} of the lowest energy conformer) of glycosides **3k**,[45] **3l** and **3m**[46] are shown in Fig. 1 (for full details see ESI, Fig. S1–S3†).

The only significant differences observed were that the phenyl ring of SPh glycoside **3l** was found to adopt different

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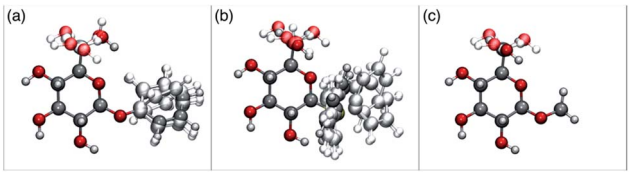

Fig. 1 Composite structures of low energy conformations of glycosyl acceptors; (a) OPh glycoside **3k**; (b) SPh glycoside **3l**; (c) OMe glycoside **3m**.

conformations than in the corresponding OPh glycoside **3k**, in line with previous observations,[47] presumably as result of the greater C–S bond length and differences in the endo anomeric effect. Low energy conformers the of methyl glycoside **3m** comprised rotamers around the C5–C6 bond. No exceptional conformations or data were observed for the OPh glycoside **3k** which may have explained the high preference for selective reaction of the 6-OH group. Furthermore, calculations revealed no differences in charge distribution at the reacting centre (O6) for any conformers of the three acceptors (Tables S3–S5†). Thus unfortunately, computational analysis did not provide any clear-cut rationalisation for the observed differences in acceptor regioselectivity.

Next, we investigated if the relative solubilities of the glycosyl acceptors may play a part in altering the regioselectivity of the glycosylation reaction.[48] Thus, we undertook an assessment of acceptor solubility for several acceptors (**3e**, **3f**, **3k**, **3l**, **3m**) by NMR in a mixture of CD_3CN and d7-DMF (10 : 1) at concentrations that matched those used for the glycosylation reactions, in the presence of glycosyl oxazoline **1a** (see ESI†). Although we found that methyl glycoside acceptor **3m** was marginally less soluble than OPh acceptor **3k**, all of the other acceptors studied displayed comparable solubility. We therefore concluded that acceptor solubility under the reaction conditions was not a significant contributing factor to reaction regioselectivity.

The lack of complete regioselectivity using alkyl glycoside acceptors intimated that extension of the procedure to trisaccharide synthesis would not be straightforward. Indeed attempted reaction using a pNP-maltoside as acceptor produced a mixture of two trisaccharides, each containing a GlcNAcβ(1→6) linkage (ESI, Table S1†). Other attempted trisaccharide synthesis resulted in non-regioselective reactions (ESI, Table S2†).

Finally, attention turned to the use of other 2-acetamido sugars as donors. Using GalNAc **2b** as the glycosyl donor the reaction outcome was found to closely mirror the results found using GlcNAc as donor. Firstly (Table 3, entry 14) the use of the pNP-glucoside acceptor **3a** produced the GalNAcβ(1→6)Glc disaccharide **4k** as the sole product in 40% yield. Next (entry 15), whilst the use of the pNP-galactoside **3g** as acceptor at rt was not completely regioselective, lowering the reaction temperature to −10 °C resulted in completely selective reaction and gave the GalNAcβ(1→6)Gal disaccharide **4l** as the sole product in 30% yield. Finally, the use of ManNAc **2c** as donor was investigated using acceptor **3a**. Although the product yield was significantly lower (15%, entry 16), the 1,2-*trans* α(1→6) linked disaccharide **4m** was isolated as the reaction product, demonstrating wider applicability of the basic process, though indicating that in this case further reaction optimisation is required.

Conclusions

A pragmatic method has been developed for the glycosylation of unprotected 2-acetamido sugars, allowing their direct conversion into either 1,2-*trans* alkyl glycosides, or (1→6)-linked disaccharides. In all cases the transformation is completely stereoselective, though absolute control of regioselectivity of disaccharide formation is dependent on the acceptor used. Whilst the major reaction product is invariably the (1→6)-linked disaccharide, reactions using aryl *O*-glycoside acceptors are completely regioselective. This simple glycosylation process supersedes traditional synthetic methods to access this type of material, which require multiple protecting group manipulations, and protracted reaction sequences.

Data availability

All data is provided the ESI,† including detailed synthetic procedures, compound characterization, and copies of NMR spectra.

Author contributions

X. Q. performed all of the synthetic chemistry and compound characterisation. A. L. G. performed all of the modelling and computational studies. A. J. F. conceived and directed the project, and wrote the paper, with input from X. Q. and A. L. G.

Conflicts of interest

There are no conflicts to declare.

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