Selective Transformations of the Anomeric Centre in Water Using DMC and Derivatives

David Lim and Antony J. Fairbanks

Abstract 2-Chloro-1,3-dimethylimidazolinium chloride (DMC) and its derivatives are useful for numerous synthetic transformations, which involve selective activation of the anomeric centre of unprotected reducing sugars in water. This chapter summarises research reported to date using DMC and derivatives, such as 2-azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP). DMC has been successfully employed for the synthesis of glycosyl oxazolines, 1,6-anhydro-, 1-azido-, and a variety of thioglycosides. The use of ADMP allows the one-pot synthesis of glycosyl triazoles in water *via* the Cu-catalysed azide-alkyne Huisgen cycloaddition reaction. This latter methodology can be applied to a wide variety of carbohydrates and is also amenable to convergent glycopeptide synthesis in which oligosaccharides are directly conjugated to peptides that contain propargyl glycine residues. Such protecting group free methodologies, particularly when applied to complex oligosaccharides isolated from natural sources, may allow ready access to a wide variety of biologically interesting glycoconjugates.

1 Introduction

Protecting groups are typically unavoidable in synthetic carbohydrate chemistry. However, their use inevitably involves additional synthetic steps, resulting in loss of materials, and the generation of a significant amount of waste; not only chemical waste, but also in terms of cost and time. Thus, there is an increasing need for the development of methods for the synthetic manipulation of carbohydrates that do not involve protecting groups, but yet still furnish the desired products in high yield and with the requisite selectivity.

D. Lim · A.J. Fairbanks (✉)
Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand
e-mail: antony.fairbanks@canterbury.ac.nz

© Springer International Publishing AG 2018

Z.J. Witczak and R. Bielski (eds.), *Coupling and Decoupling of Diverse Molecular Units in Glycosciences*, https://doi.org/10.1007/978-3-319-65587-1_5

2 Synthesis of Glycosyl Oxazolines in Water Using DMC

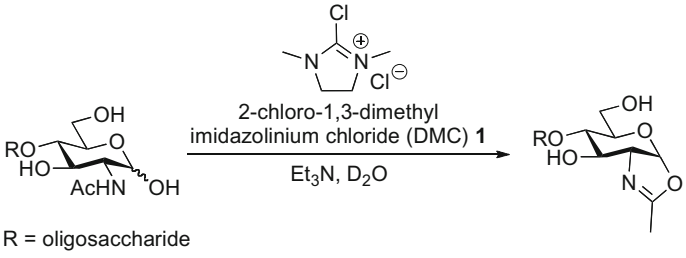
Glycosyl oxazolines have found wide application in carbohydrate chemistry, first in their protected forms as donors for oligosaccharide synthesis [1, 2], and subsequently, and perhaps more importantly as activated donor substrates for the enzymatic synthesis of glycopeptides and glycoproteins [3, 4].

In 2004, Kadokawa et al. [5] came close to developing a useful method for the direct synthesis of glycosyl oxazolines in water when they used a series of carbodiimides to activate the anomeric centre. However, these reactions only afforded the desired products in very low yield (<30%). Even so, the fact that this type of highly selective transformation was possible at all drew the attention of the Glycoscience community.

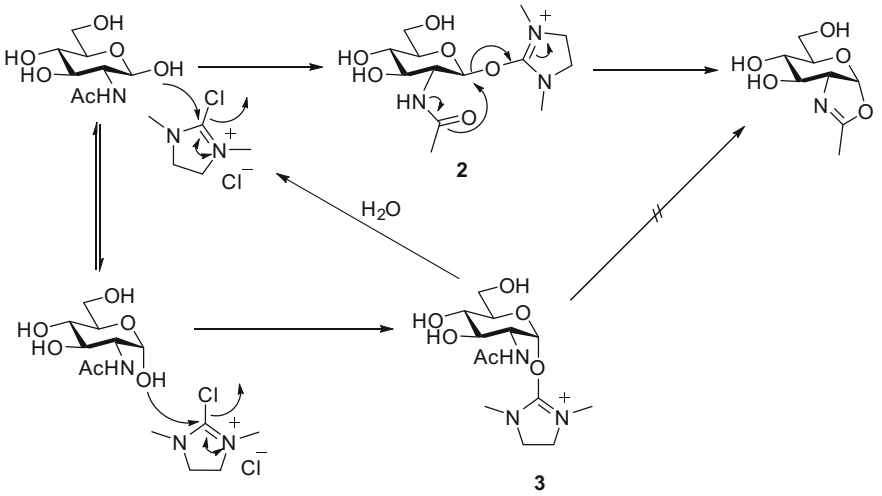
The dehydrative properties of the reagent, 2-chloro-1,3-dimethylimidazolium chloride **1** (DMC), were originally reported by Isobe and Ishikawa [6, 7]; however these reported transformations were all performed in organic solvents. In 2009, Shoda and co-workers [8] made a significant breakthrough in the field when they reported the use of DMC for the direct synthesis of glycosyl oxazolines from reducing sugars in D_2O in high yield (Scheme 1).

Prior to this report, the synthesis of glycosyl oxazolines typically required peracetylated 2-acetamido-2-deoxy sugars to be prepared. These could then be converted to the corresponding oxazolines using Lewis acids, such as ferric(III) chloride, tin(IV) chloride, boron trifluoride, or trimethylsilyl triflate, [9–12] before the final removal of acetate protecting groups under basic conditions. However, the use of strong Lewis acids can damage glycosidic linkages and, typically in the cases of larger oligosaccharides, may result in the formation complex reaction mixtures and low yields. This report, therefore, represented a significant advance in the production of *N*-glycan oxazolines, particularly as donor substrates for enzymatic synthesis.

The proposed mechanism [8] of oxazoline formation involves preferential attack of the hemiacetal hydroxyl on DMC; in the case of the β-anomer this yields reactive intermediate **2** (Scheme 2). Intramolecular attack of the 2-acetamido group at the anomeric centre, followed by abstraction of a proton by a suitable base, then affords



Scheme 1 Synthesis of glycosyl oxazolines directly from reducing sugars in water by Shoda et al. [8]



Scheme 2 Proposed mechanism for sugar oxazoline formation using DMC [8]

the oxazoline. The α-imidazolinium intermediate **3** can also be formed by attack of the α-hemiacetal hydroxyl on DMC. However, since **3** cannot directly form an oxazoline it is then probably hydrolysed to regenerate the β-anomer of the free sugar; this β-anomer then follows the reaction pathway *via* intermediate **2** to the oxazoline product. Although this mechanistic pathway is plausible, Shoda has stated [8] that the intermediacy of a β-glycosyl chloride during the conversion of **3** into the oxazoline product cannot be ruled out.

The use of DMC for the selective activation of reducing sugars in water has subsequently proved pivotal in facilitating the use of glycosyl oxazolines as donors for enzymatic glycosylation reactions catalysed by endo-β-*N*-acetylglucosaminidases (ENGases), such as those reported by the groups of Fairbanks [13–16], Wang [17–19], and Yamamoto [20–22].

Subsequently, Shoda and co-workers [23] reported another method for the direct synthesis of glycosyl oxazolines using the DMC analogue, 2-chloro-1,3-dimethyl-1*H*-benzimidazol-3-ium chloride **4**, (CDMBI) as the dehydrative agent (Fig. 1).

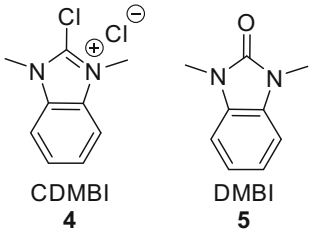
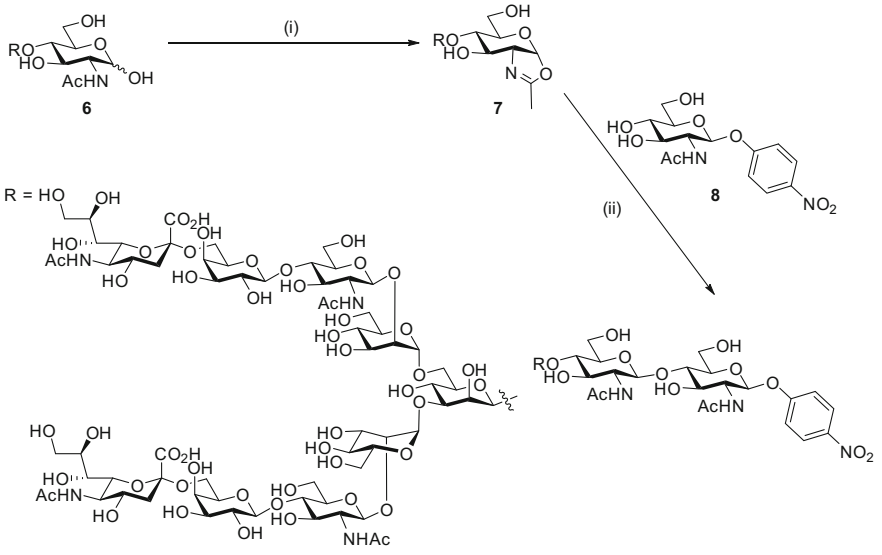


Fig. 1 DMC-analogue CDMBI **4**, and the urea DMBI **5** formed by its hydrolysis [23]

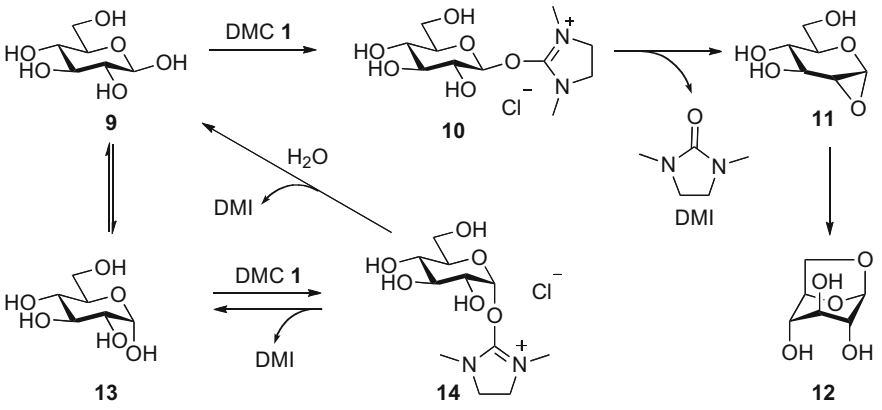
CDMBI was alleged to be superior to DMC, as it is less hygroscopic and easier to handle, although most applications still use the commercially available DMC for oxazoline formation. Shoda also stated that replacing the imidazolidine ring of DMC with the more electron-rich benzimidazole would reduce the reactivity of the chloroformamidinium group toward nucleophilic attack by water, and that additionally it made the reactive glycosyl-imidazolidinium intermediate more stable. It was also postulated that the introduction of the aromatic ring would also reduce the aqueous solubility of the hydrolysed product, namely the urea **5** (1,3-dimethylbenzimidazol-2-one, DMBI) (Fig. 1). Indeed, when the complex bi-antennary *N*-glycan decasaccharide **6** (derived from a sialyl glycopeptide isolated from egg yolks [24]) was converted to the corresponding oxazoline **7** using CDMBI **4**, DMBI **5** precipitated from the mixture as the reaction proceeded, and was easily removed by filtration (Scheme 3). Moreover, the filtrate containing the oxazoline could then be directly used for a subsequent enzymatic glycosylation of the acceptor *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside **8** using the glycosynthase, Endo-M N175Q [21], without the need for further purification or isolation of the oxazoline intermediate.



Scheme 3 Enzymatic glycosylation of GlcNAc-β-pNP with the sialoglycan-oxazoline without isolation of the intermediate oxazoline [23]. Reaction conditions: **i** CDMBI **4**, Na_3PO_4, H_2O; **ii 8**, Endo-M N175Q

3 Synthesis of 1,6-Anhydro Sugars in Water Using DMC

Subsequent to the first report on oxazoline formation, DMC and its derivatives have been employed for the synthesis of a variety of glycosides formed by intercepting the α-imidazolinium intermediate **3** (Scheme 2) with different nucleophiles. In the absence of any external nucleophile, the 6-hydroxyl group may attack the anomeric centre. Thus, Shoda et al. [25] reported the use of DMC for the formation of 1,6-anhydro sugars from unprotected glycopyranoses in water in almost quantitative yield. The DMC procedure mitigated the requirement for the harsh reaction conditions that had previously been used for the synthesis of 1,6-anhydro sugars, such as pyrolysis [26, 27] or thermal degradation [28, 29]. The proposed reaction mechanism is illustrated in Scheme 4, and is similar to that previously suggested [8] for the formation of glycosyl oxazolines. Herein the first step is a nucleophilic attack of the anomeric hydroxyl group of the predominant β-anomer of glucose **9** on DMC **1**, giving rise to intermediate **10**. Intramolecular attack of the 2-hydroxyl group of **10** at the anomeric carbon affords a 1,2-anhydro intermediate **11**, which is subsequently converted to the 1,6-anhydro sugar **12** via intramolecular nucleophilic attack by the 6-hydroxyl group. The α-anomer of glucose **13** also reacts with DMC to give the corresponding α-intermediate **14**. Although direct conversion of **14** into the 1,6-anhydro sugar **12** is possible by an attack of the 6-hydroxyl at the anomeric centre, Shoda suggests that in fact **14** is hydrolysed by an attack of water to regenerate β-glucose **9**. β-Glucose **9** then follows the above pathway via **11** to give the 1,6-anhydro sugar **12**. Evidence for this overall mechanistic pathway, and the requirement for a 1,2-anhydro intermediate such as **11** *en route* to the 1,6-anhydro sugar, was the fact that the corresponding 1,6-anhydro sugars were *not* formed when D-mannose, 2-deoxy-D-glucose, and 2-fluoro-2-deoxy-D-glucose were used as substrates.



Scheme 4 Proposed mechanism for 1,6-anhydro sugar formation [25]

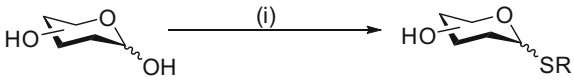
The published optimised method uses a large excess of triethylamine to drive 1,6-anhydro sugar formation. In cases where other nucleophiles are to be introduced at the anomeric centre, e.g., for glycosyl azide synthesis using DMC (see Sect. 5) [30], fewer equivalents of base are commonly used. However, in these instances, 1,6-anhydro sugar formation can still be an undesired competing side reaction, which reduces the yields of desired products and can be particularly problematic when oligosaccharides, synthesised using multi-step methods and obtained in small quantities, are used. In these cases, 1,6-anhydro sugar formation can typically be minimised by the use of a large excess of the external nucleophile. Alternatively, Shoda has also reported that acetonitrile may be used as a co-solvent to suppress 1,6-anhydro sugar formation [31].

4 Synthesis of Thioglycosides Using DMC

Thioglycosides have found widespread application throughout the carbohydrate field as glycosyl donors for chemical oligosaccharide synthesis [32–35]. There has also been interest in the synthesis of de-protected thioglycosides as stable analogues of *O*-glycosides and as potential enzyme inhibitors [36, 37]. The introduction of sulfur at the anomeric centre often involves the reaction of a peracetylated sugar with a thiol in the presence of a Lewis acid [38], or alternatively substitution of a glycosyl halide with a thiolate [39]. In general, these methods require multi-step reaction sequences, involving protection/de-protection strategies, and are usually performed in organic solvents. Although direct methods for the preparation of thioglycosides from hemiacetals have been reported using trifluoroacetic acid as an activator, these reactions gave poor anomeric selectivities and also resulted in the formation of dithioacetal by-products [40, 41].

4.1 Aryl Thioglycosides

In 2009, Tanaka et al. [31] reported a simple method for the direct synthesis of aryl thioglycosides from reducing sugars in an aqueous solvent system using DMC and excess triethylamine (Scheme 5).



Scheme 5 Synthesis of aryl thioglycosides from unprotected sugars [31]. Reaction conditions: **i** DMC **1**, R-SH, Et_3N, H_2O/MeCN

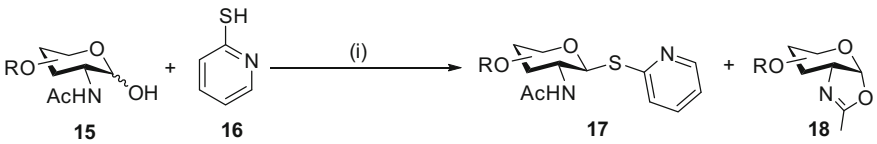
The use of benzenethiol, *p*-toluenethiol, and 4-methoxybenzenethiol as the thiol component afforded the corresponding aryl thioglycosides as mixtures of anomers, in high yield. However, when 4-nitrobenzenethiol was reacted with D-glucose and DMC, the corresponding thioglycoside product was formed exclusively as the β-anomer, in 90% yield. It was found that the procedure could also be applied to disaccharides, and the corresponding products were formed in quantitative yield, and as exclusively the β-anomer.

4.2 2-Pyridyl 1-Thio-Glycosides

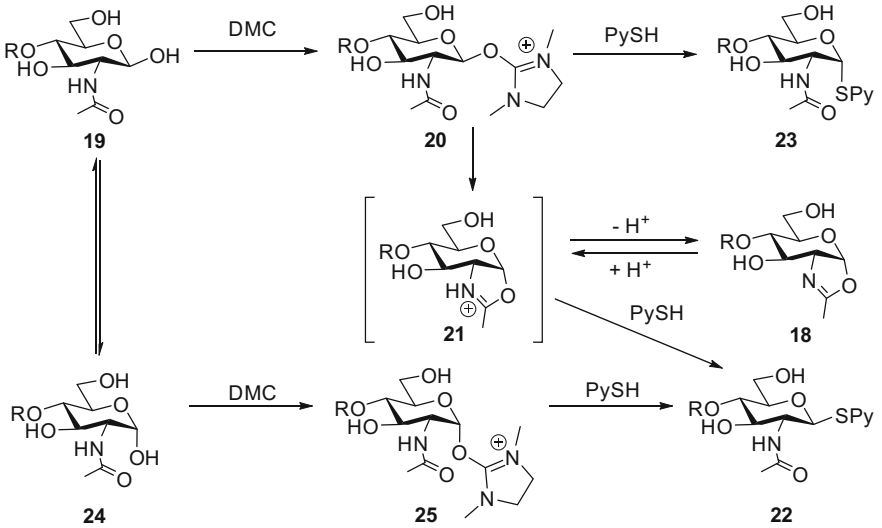
Shoda et al. [31, 42] then demonstrated that it was also possible to glycosylate 2-mercaptopyridine **16** with oligosaccharides having an *N*-acetylglucosamine unit at the reducing end **15** using DMC to give the corresponding 2-pyridylthioglycosides **17** (Scheme 6).

Interestingly, the glycosyl oxazoline **18** was obtained as a by-product from these reactions. However, it was found that treating oxazoline **18** with 1 M HCl led to the opening of the ring and an increased yield of the desired 2-pyridylthioglycoside **17**. A plausible mechanism, which explains the formation of both anomers of the 2-pyridylthioglycoside products as well as the oxazoline, was suggested and is shown in Scheme 7. Nucleophilic attack of the β-hemiacetal of **19** on DMC affords **20**, which may then cyclise to give the oxazolinium intermediate **21**. Attack on intermediate **21** by mercaptopyridine (PySH) will yield the β-2-pyridylthioglycoside **22**. However, if the proton on the nitrogen atom of **21** is abstracted by a base, the intermediate will be converted to the corresponding oxazoline **18**, which is itself unreactive towards nucleophilic opening. The addition of acid to oxazoline **18** leads to the re-protonation of the nitrogen to regenerate **21**, which can subsequently react with mercaptopyridine to give **22**. Alternatively, imidazolidinium intermediate **20** can react directly with mercaptopyridine to give the α-2-pyridylthioglycoside **23**.

The α-anomer of the starting material **24** can also react with DMC to give intermediate **25**, which can be directly attacked by mercaptopyridine to give the β-2-pyridylthioglycoside **22**.



Scheme 6 Glycosylation of mercaptopyridine **16** with unprotected sugars using DMC reported Shoda et al. [42] Reaction conditions: **i** DMC **1**, Et_3N, H_2O/MeCN (4:1), 0 °C, 1 h



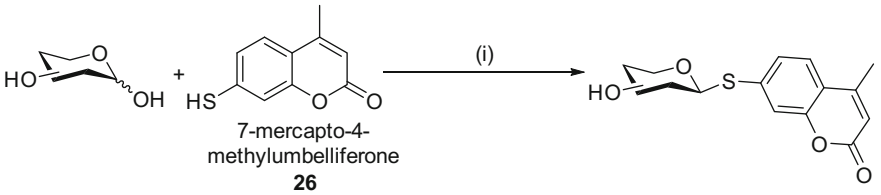
Scheme 7 Proposed mechanism for 2-pyridylthioglycoside formation using PySH and DMC [42]

4.3 4-Methyl-7-Thioumbelliferyl-Labelled Glycosides (MUS-Labelled Glycosides)

There is a growing need to develop quantitative ways to label sugars with chromophores. The most common current method of labelling is reductive animation, in which an oligosaccharide is reacted with an amine containing the chromophore, and the ensuing imine is then reduced by a source of hydride [43]. However, sugars labelled in this manner contain an open-chain 1-aminoalditol moiety, essentially rendering the reducing end inert for further useful transformations. This ring opening also reduces the structural relevance of that part of the oligosaccharide, for example with respect to binding or conformational studies. Furthermore, the conversion of 1-aminoalditols back into reducing sugars requires harsh conditions, such as the use of hydrogen peroxide [44, 45]. A method for introducing a detectable chromophore that could also be easily detached would, therefore, be highly advantageous.

In 2013, Shoda et al. [46] reported the direct introduction of 4-methyl-7-thioumbelliferone **26** (MUS) at the anomeric centre of monosaccharides and at the reducing terminus of oligosaccharides using DMC activation (Scheme 8). The fluorescence spectrum of the MUS-labelled glycosides produced indicated that the labelled derivatives showed a high sensitivity for fluorescence detection based on the maximum wavelengths for excitation and emission at 330 and 395 nm, respectively.

The yields of the MUS-sugars made by this DMC labelling method were sufficiently high to suggest its application in quantitative studies. Indeed, analysis of a mixture of laminari-oligosaccharides of known composition, which were then modified by both MUS-labelling and pyridylamination, showed very similar



Scheme 8 Synthesis of MUS-labelled sugars using DMC by Shoda et al. [46]. Reaction conditions: **i** DMC **1**, Et_3N, H_2O/MeCN (1:1), 0 °C, 1.5 h

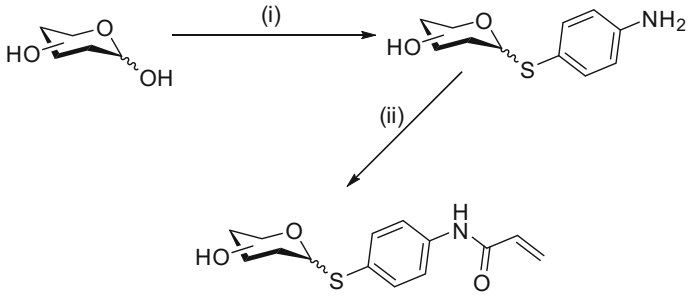
Table 1 Quantitative analysis of MUS-labelled lamnari-oligosaccharide structures by Shoda et al. [46]

DP	Actual amount (mol%)	MUS-labelling (area%)	Pyridylamination (area%)
1	36.9	34.5	37.6
2	19.4	21.6	19.0
3	13.2	14.4	12.7
4	10.0	10.6	10.0
5	8.0	8.2	8.2
6	6.7	6.2	7.0
7	5.8	4.5	5.5

quantitative values (Table 1). The MUS-glycosides were also easily de-protected by conventional halosuccinimide-mediated thioglycoside activation (e.g. *N*-bromo, *N*-chloro-, and *N*-iodosuccinimide) to re-form the corresponding reducing sugars, which could then be used for further functionalisation; a significant advantage compared to the pyridylamination labelling method.

4.4 Glycopolymerisation

Although the binding of carbohydrates to proteins is a fundamental process, with widespread importance throughout Biology, it is now appreciated that the majority of carbohydrate–protein interactions are of low affinity. Nature has compensated for this apparent paradox by the use of multivalent receptor-ligand presentation, which amplifies the affinity of single interactions. It is fair to say that all aspects of this so-called 'glycocluster effect' [47, 48] are not yet completely understood, although detailed discussions and theories have been presented [49, 50]. Despite shortcomings in our understanding, there have been many reports on the design and production of synthetic glycoclusters, such as glycopolymers [51], glycodendrimers [52], and glyconanoparticles [53]. Many of these synthetic constructs have demonstrated an amplification in carbohydrate-mediated binding, in a similar manner to that achieved by multivalent carbohydrate presentations of natural glycopeptides [54] and glycoproteins [55].



Scheme 9 One-pot synthesis of glycomonomers [58]. Reaction conditions: **i** DMC **1**, HS–C_6H_4–NH_2, Et_3N, H_2O/MeCN; **ii** acryloyl chloride, Et_3N, H_2O/THF

The preparation of glycopolymers often requires laborious, multi-step procedures involving protection and de-protection of a saccharide and which must also include the introduction of a polymerizable group, such as vinyl or norbornene, at the anomeric centre [56, 57]. Only after multiple synthetic steps can the resulting glycomonomers be used in a polymerization reaction. Clearly, more efficient synthetic methodologies for the preparation of glycomonomers would be advantageous.

In 2014, Tanaka et al. [58] reported a one-pot method for the production of glycomonomers directly from unprotected sugars, by the DMC-mediated synthesis of 4-aminophenyl 1-thio-glycosides in water in the presence of triethylamine, and their subsequent acrylamidation (Scheme 9).

These acylamide containing glycomonomers were then subjected to reversible addition-fragmentation chain transfer (RAFT) living radical polymerisation to give glycopolymers, which were subsequently immobilised onto gold nanoparticles for investigations into glycocluster effects.

4.5 S-*Linked Glycopeptides*

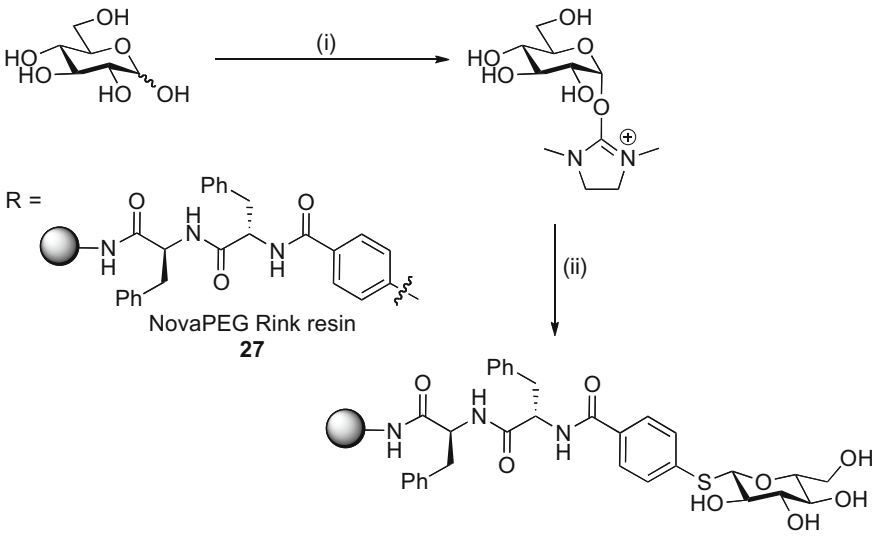
S-Linked peptide and protein glycosylation, where the anomeric oxygen of *O*-linked glycosides has been replaced by sulfur, has attracted a significant amount of interest from the Glycoscience community [59–61]. *S*-Linked glycopeptides are well known for their functional surrogacy, in addition to their chemical and biological stability as compared to their *O*-linked counterparts, especially towards glycosidase-catalysed cleavage [62].

S-Linked glycopeptides can be accessed by several strategies. The most common involve either conjugate addition or nucleophilic substitution reactions, with a glycosyl thiol acting as the nucleophile [63–66]. However, more recent approaches, such as free radical thiol-ene ‘Click’ reactions [67], desulfurative rearrangements [68], and the opening of 1,6-anhydrosugars [69], are alternatives that may possess certain advantages. Nevertheless, all of these methods still require multi-step methodologies in order to prepare the required glycosyl thiol.

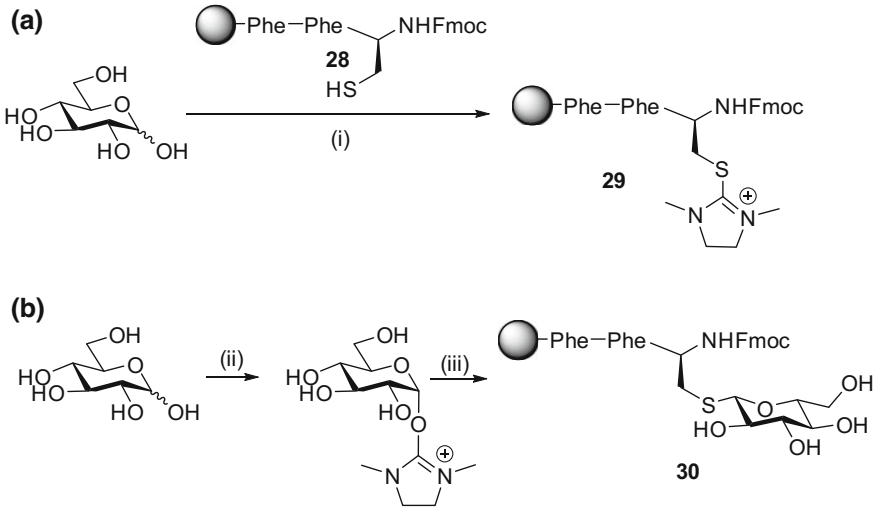
Winssinger et al. [70] recently reported the use of DMC for the solid phase synthesis of *S*-linked glycopeptides. By using a H_2O/dioxane solvent mixture and cooling to −10 °C, they demonstrated that unprotected sugars could be 'pre-activated' by DMC, and found the glycosyl-imidazolinium intermediate that was formed was stable for up to 1 h. The addition of a polymer-bound peptide containing a thiol to the reaction mixture during that time period then led to the formation of the desired polymer-bound *S*-linked glycopeptide **27** (Scheme 10).

An attempt was also made to glycosylate a polymer-bound tripeptide containing cysteine (Fmoc-Cys-Phe-Phe-Rink) with various mono- and disaccharides. Interestingly, direct reaction with D-glucose with DMC in the presence of the tripeptide **28** (i.e. without the pre-activation process) led to the exclusive formation of the thioimidazolidinium by-product **29** (Scheme 11a). However, when the conditions previously identified for pre-activation were used, the desired glycopeptide **30** was obtained in >90% yield (Scheme 11b).

The process was applied to other monosaccharides (D-galactose, D-mannose, L-fucose) and also to disaccharides (lactose, mellibiose, and Galα(1 → 4)Glc), though the yields were lower in the cases of the disaccharides. An elegant method, involving an iterative addition strategy, was found to increase efficiency of glycopeptide formation, and led to conversions to the desired product of >99%. The process was then exemplified by the solid phase synthesis of an analogue of the repeat unit of the cancer-associated MUC1 glycopeptide, in which the two natural *O*-linked carbohydrates sites were replaced with *S*-linked glycans.



Scheme 10 Glycosylation of a thiol-containing resin with D-glucose using the DMC methodology [70]. Reaction conditions: **i** DMC **1**, Et_3N, H_2O/dioxane (1:1), −10 °C; **ii** RSH



Scheme 11 Solid phase *S*-glycosylation of a resin-bound tripeptide **28** by Winssinger et al. [70]. Reaction conditions: **28**, DMC **1**, Et_3N, H_2O/dioxane (1:1), −10 °C; **ii** DMC **1**, Et_3N, H_2O/dioxane, −10 °C, 15 min; **iii** **28**

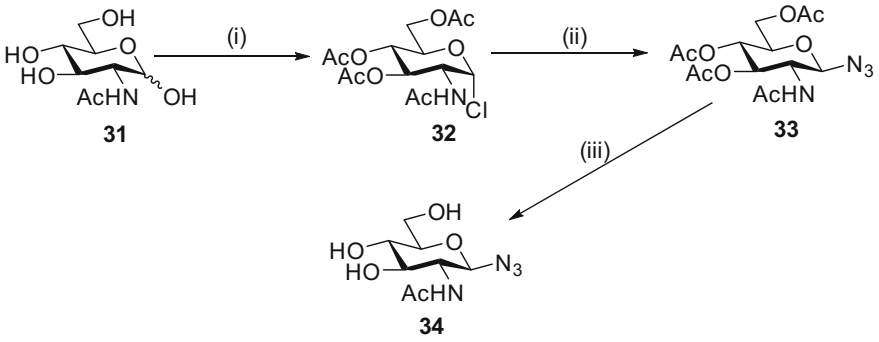
5 Synthesis of Glycosyl Azides in Water Using DMC

Glycosyl azides are highly useful synthetic intermediates in sugar chemistry [71]. The traditional method for their formation involves at least three or four steps, invariably involving protecting group manipulations, to furnish the desired de-protected product. For example, a typical sequence involves the conversion of the sugar, e.g. *N*-acetyl-D-glucosamine **31**, into a protected glycosyl halide **32**, followed by nucleophilic displacement of the anomeric leaving group by azide to give the glycosyl azide **33** (Scheme 12). Finally, the ester protecting groups are removed, for example by Zemplén de-acetylation, to give the de-protected glycosyl azide **34**.

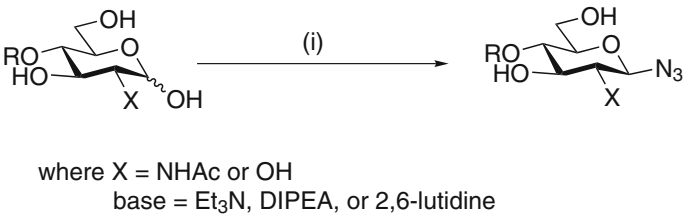
In addition to the inherent inefficiency of these multi-step reaction sequences, this type of synthetic route can also occasionally become problematic when applied to higher oligosaccharides due to cleavage of inter-glycosidic bonds during the synthetic pathway.

Following on from earlier studies in the Shoda group, Tanaka et al. [30] reported that glycosyl azides of monosaccharides could be formed directly when unprotected sugars were treated with excess DMC in water in the presence of a large excess (typically 10 equivalents of each) of azide and triethylamine (Scheme 13).

Interestingly, when the reaction was applied to disaccharides using triethylamine as the base, 1,6-anhydro sugars were formed as by-products. It was suggested that, for some reason, intramolecular nucleophilic attack of the 6-hydroxy group at the anomeric centre was increased in the presence of triethylamine [30]. A screen of various other bases was performed, and it was found that the use of either *N*,*N*-diisopropylethylamine (Hunig's base, DIPEA) or 2,6-lutidine led to reduced



Scheme 12 Synthesis of de-protected glycosyl azides. Reaction conditions: **i** AcCl; **ii** NaN_3, sat. aq. $NaHCO_3$, $tBuNH_4{\cdot}HSO_4$, DCM; **iii** Na, MeOH



Scheme 13 Direct synthesis of glycosyl azides from unprotected sugars using DMC [30]. Reaction conditions: **i** DMC **1**, NaN_3, base, H_2O

formation of the 1,6-anhydro derivative, presumably an effect that was related to their greater steric bulk. The use of 2,6-lutidine as the base was also found to be essential in the case of 2-acetamido sugars to avoid significant oxazoline formation.

Tanaka et al. [30] then demonstrated that the reaction could be applied to larger oligosaccharides, including a sialic acid terminated complex biantennary decasaccharide, which was converted to the corresponding glycosyl azide in 87% yield, though this reaction did require the use of 20 equivalents of DMC and 40 equivalents of 2,6- lutidine. Glycosyl azides were generally produced stereoselectively as the 1,2-*trans* glycosides, except in the cases of 2-deoxy sugars, which were formed as anomeric mixtures. To date, a detailed mechanism has not been presented, though the results suggest that reaction of sugars containing a 2-hydroxy group probably proceeds via 1,2-anhydro sugar intermediates.

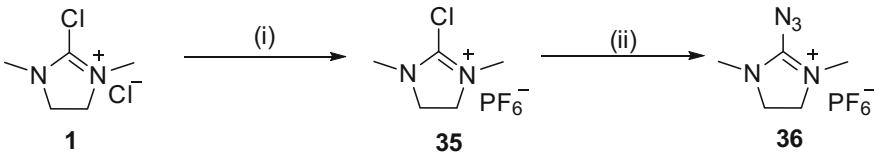
6 Synthesis of Glycosyl Triazoles in a One-Pot Reaction

The original Shoda method [30] for glycosyl azide synthesis, like all DMC activation procedures, potentially generates up to two equivalents of HCl per equivalent of DMC used; one equivalent is produced from de-protonation of the anomeric hydroxyl group, and a second one may additionally be produced from water in the

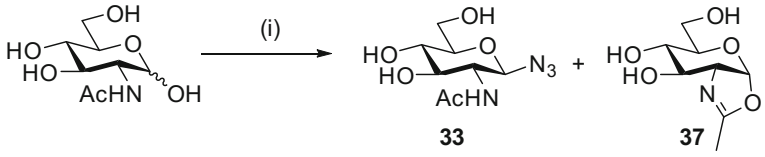
case of a hydrolysis reaction. This production of acid during the course of the reaction necessitates the use of excessive amounts of triethylamine. Furthermore, the use of a large excess of azide is also required.

As an alternative approach which would avoid the requirement for this large excess of reagents, we envisaged replacement of the chlorine on the dimethylimidazolinium ring of DMC with azide; such a reagent should still be able to activate a reducing sugar, would only result in the formation of one equivalent of acid, and would additionally itself act as the source of azide. 2-Azido-1,3-dimethylimidazolinium hexafluoro phosphate (ADMP), has been previously used as an efficient agent for diazo-transfers [72–75], migratory aminations [76, 77], and azide transfer [78] reactions. Although ADMP contains a significant amount of nitrogen, impact sensitivity and friction sensitivity tests have demonstrated that it is not explosive [73], making it in fact safer to handle than sodium azide. Additionally unlike DMC, which is hygroscopic [23, 73], ADMP is isolated as a stable crystalline solid and is easily handled. Although, unlike DMC, it is not currently commercially available, ADMP can be very easily synthesised, for example following procedures originally reported by Kitamura et al. [72, 79] (Scheme 14), simply by conversion of DMC **1** to the hexafluorophosphate salt **35**, and then treatment with sodium azide to give ADMP **36**.

In 2014, we [80] reported an alternative method for the synthesis of glycosyl azides in water. *N*-Acetylglucosamine was reacted with ADMP **36** and triethylamine in a D_2O/MeCN (4:1) solvent mixture to give the glycosyl azide **33**, as well as oxazoline **37** (Scheme 15). The use of D_2O as solvent, as first reported by Shoda in the original oxazoline formation study [8], resulted in higher yields of products, presumably due to reduced rates of competitive solvent-mediated hydrolysis.



Scheme 14 Synthesis of ADMP **36** [72, 79]. Reaction conditions: **i** $NaPF_6$, MeCN, rt, 30 min, 99%; **ii** NaN_3, MeCN, 0 °C, 3 h, quant



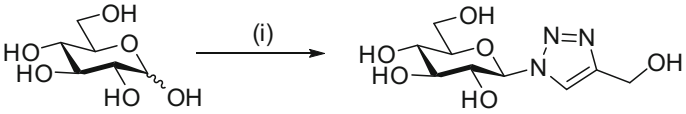
Scheme 15 Reaction of GlcNAc with ADMP to yield glycosyl azide **33** and oxazoline **37** [80]. Reaction conditions: **i** ADMP **36**, Et_3N, D_2O/MeCN (4:1), 0 °C

However, applying the idea reported by Shoda et al. [42] that the subsequent addition of acid would result in oxazoline ring opening, we found that the desired azide **33** was obtained as the sole reaction product when the crude reaction mixture was simply acidified with 1 M aqueous HCl.

Glycosyl azides are obvious substrates for further elaboration by the modified Huisgen cycloaddition [81, 82], the most well known 'Click' reaction. This high yielding transformation may be performed in water, leading to numerous applications, as reaction conditions are compatible with biological systems. In the carbohydrate field, 'Click chemistry' has already been applied to the synthesis of a wide range of sugar derivatives [83] having interesting biological properties [84], including inhibitory activity against glycosidases [85–90] and glycosyltransferases [91]. The development of a one-pot process for glycosyl azide and then triazole formation by Click reaction with an alkyne was, therefore, an obvious avenue for investigation.

We found that after the formation of the glycosyl azide was complete, the addition of propargyl alcohol, $CuSO_4 \cdot 5H_2O$, and L-ascorbic acid, and then heating at 50 °C for 14 h, led to the formation of the glycosyl triazole **38** in excellent yield and with complete stereoselectivity; the 1,2-*trans* glycosyl triazole being formed in all cases (Scheme 16). This procedure was then applied to a variety of sugars ranging from in size from mono- to trisaccharides [80]. In all cases, the use of D_2O as co-solvent led to a variable, but minor, the amount of deuterium incorporation into the triazole ring during the course of the reaction.

The generality of the one-pot process with respect to the alkyne was explored. The reaction was found to be widely applicable, including with interesting and potentially biologically relevant substrates such as propargyl glycine. All alkynes investigated were successfully Clicked with monosaccharides, and products formed in high yield and with complete stereoselectivity. The only limitation appeared to be in terms of the solubility of the alkyne-coupling partner in the aqueous reaction medium. The one-pot Click reaction also allowed the direct conjugation of reducing sugars to a variety of other carbohydrates which themselves contained an alkyne

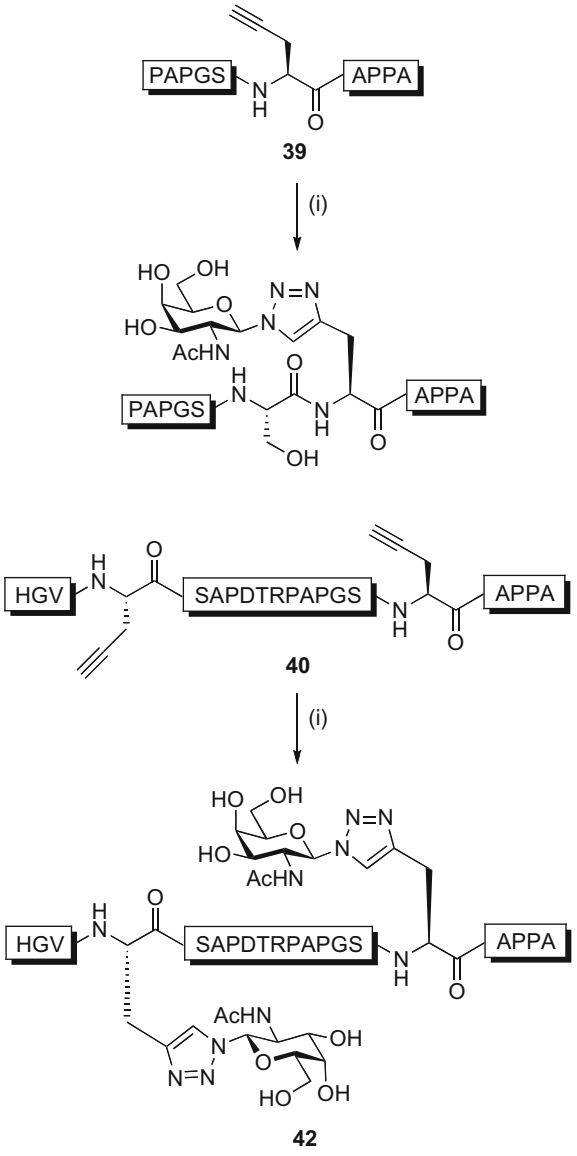


38

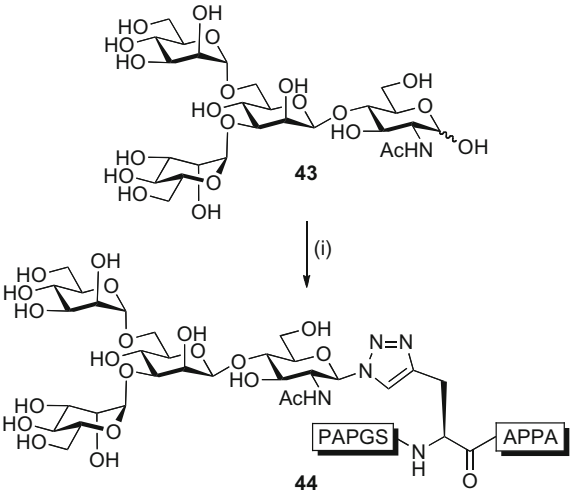
Scheme 16 Direct synthesis of glycosyl triazoles from reducing sugars in water by Fairbanks et al. [80]. Reaction conditions: **i** (1) ADMP **36**, Et_3N, D_2O/MeCN (4:1), 0 °C, 3 h, then add propargyl alcohol, $CuSO_4 \cdot 5H_2O$, L-ascorbic acid, 50 °C for 14 h

functionality, giving rise to a variety of di-, tri- and pentasaccharide mimics. All reactions were high yielding and completely stereoselective [80].

Click chemistry has previously been applied to access different glycopeptides, [92, 93] and also protein scaffolds decorated with oligosaccharides [94]. However,



Scheme 17 Conjugation of GalNAc with 10-mer **39** and 20-mer **40** by Fairbanks et al. [80]; Reaction conditions: **i** GalNAc, ADMP, Et_3N, D_2O, MeCN, 0 °C, 3 h, then add alkyne, $CuSO_4{\cdot}5H_2O$, L-ascorbic acid, and heat to 50 °C for 14 h [80]

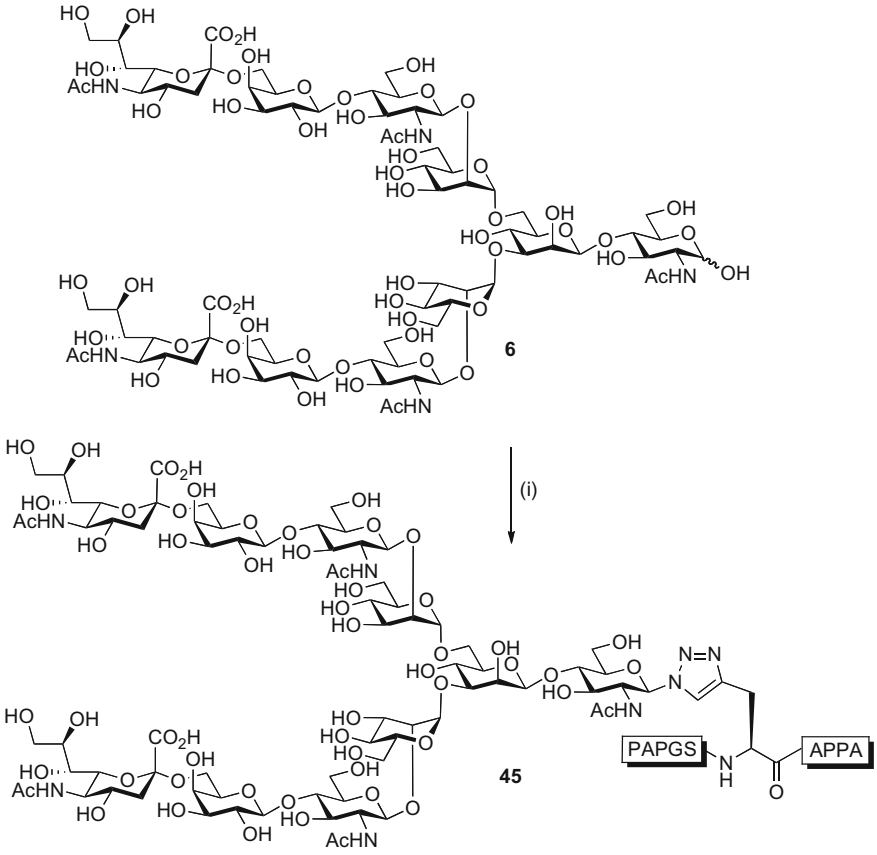


Scheme 18 Reaction of tetrasaccharide **43** with 10-mer **39** by Fairbanks et al. [80]. Reaction conditions: **i** ADMP, Et_3N, D_2O, MeCN, 0 °C, 3 h, then add 10-mer **39**, $CuSO_4{\cdot}5H_2O$, L-ascorbic acid, and heat to 50 °C for 14 h

reported procedures require multiple protecting group manipulations. In terms of a biologically interesting application, glycosylated versions of the tandem repeat domain of the cancer-associated mucin MUC1 [95, 96] have shown potential as components of synthetic anti-cancer vaccines [97, 98]. A further exemplification of the one-pot azide/Click reaction, we, therefore, conjugated a variety of sugars to two synthetic MUC1 peptides that incorporated propargyl glycine (Pra) residues. Conversion of GalNAc to its azide using ADMP and then direct reaction with peptides **39** and **40**, comprising one and two propargyl glycines, respectively, in the presence of $CuSO_4{\cdot}5H_2O$, gave the corresponding glycopeptides **41** and **42** in good yield (Scheme 17).

Similarly tetrasaccharide **43** [99], which corresponds to a core region of *N*-glycans, was also directly reacted with peptide **39** and gave glycopeptide **44** in 42% yield (Scheme 18).

As a final example of the utility of the method, the complex biantennary *N*-glycan **6** decasaccharide [21, 100] was converted to the glycosyl azide using ADMP, and then directly reacted with peptide **39** in the presence of $CuSO_4{\cdot}5H_2O$ to furnish glycopeptide **45** in 42% yield (Scheme 19).



Scheme 19 Conjugation of decasaccharide **6** to 10-mer **45** by Fairbanks et al. [80]. Reaction conditions: **i** **6**, ADMP, Et_3N, D_2O, MeCN, 0 °C, 3 h, then add 10-mer **39**, $CuSO_4{\cdot}5H_2O$, L-ascorbic acid, and heat to 50 °C for 14 h

7 Conclusion

Traditional methods of accessing glycoconjugates typically involve multi-step syntheses and require complex and protracted protecting group strategies. These methods are generally technically demanding, inefficient, expensive, and the production of significant amounts of the target material is usually logistically difficult to achieve.

Since the initial report by Shoda and co-workers on the use of 1,3-dimethylimidazolinium chloride (DMC) for the selective conversion of unprotected 2-acetamido sugars to glycosyl oxazolines in water, various synthetic targets have been accessed using this, or related, highly selective activating agent. In particular, selective activation of the anomeric centre under aqueous conditions

and direct reaction with a diverse range of nucleophiles allows ready access to a range of glycoconjugates without recourse to any protecting group manipulations. Additionally, the products of nucleophilic substitution at the anomeric centre may be further derivatised in the same reaction vessel, for example by Click chemistry.

As a wide variety of structurally complex reducing oligosaccharides are available from natural sources, protecting group free methods based on DMC and similar activating agents show significant promise for rapid access to a broad range of biologically interesting glycoconjugates.

References

1. Donohoe TJ, Logan JG, Laffan DDP (2003) Trichloro-oxazolines as activated donors for aminosugar coupling. Org Lett 5:4995–4998
2. Blatter G, Beau J-M, Jacquinet J-C (1994) The use of 2-deoxy-2- trichloroacetamido-D-glucopyranose derivatives in syntheses of oligosaccharides. Carbohydr Res 260:189–202
3. Fairbanks AJ (2011) Endohexosaminidase catalysed glycosylation with oxazoline donors: the development of robust biocatalytic methods for synthesis of defined homogeneous glycoconjugates. C R Chim 14:44–58
4. Fairbanks AJ (2013) Endohexosaminidase-catalyzed synthesis of glycopeptides and proteins. Pure Appl Chem 85:1847–1863
5. Kadokawa J, Mito M, Takahashi S et al (2004) Direct conversion of 2-Acetamido-2-deoxysugars to 1,2-Oxazoline derivatives by dehydrative cyclization in water. Heterocycles 63:1531–1535
6. Isobe T, Ishikawa T (1999) 2-Chloro-1,3-dimethylimidazolinium chloride. 2. Its application to the construction of heterocycles through dehydration reactions. J Org Chem 64:6989–6992
7. Isobe T, Ishikawa T (1999) 2-Chloro-1,3-dimethylimidazolinium chloride. 1. A powerful dehydrating equivalent to DCC. J Org Chem 64:6984–6988
8. Noguchi M, Tanaka T, Gyakushi H et al (2009) Efficient synthesis of sugar oxazolines from unprotected N-acetyl-2-amino sugars by using chloroformamidinium reagent in water. J Org Chem 74:2210–2212
9. Matta KL, Johnson EA, Barlow JJ (1973) A simple method for the synthesis of 2-acetamido-2-deoxy-β-D-galactopyranosides. Carbohydr Res 26:215–218
10. Srivastava VK (1982) A facile synthesis of 2-methyl-(3,4,6-tri-O-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-e]-2-oxazoline. Carbohydr Res 103:286–292
11. Nakabayashi S, Warren CD, Jeanloz RW (1986) A new procedure for the preparation of oligosaccharide oxazolines. Carbohydr Res 150:c7–c10
12. Colon M, Staveski MM, Davis JT (1991) Mild conditions for the preparation of high-mannose oligosaccharide oxazolines: entry point for β-glycoside and neoglycoprotein syntheses. Tetrahedron Lett 32:4447–4450
13. Kowalczyk R, Brimble MA, Tomabechi Y et al (2014) Convergent chemoenzymatic synthesis of a library of glycosylated analogues of pramlintide: structure–activity relationships for amylin receptor agonism. Org Biomol Chem 12:8142–8151
14. McIntosh JD, Brimble MA, Brooks AES et al (2015) Convergent chemo-enzymatic synthesis of mannosylated glycopeptides; targeting of putative vaccine candidates to antigen presenting cells. Chem Sci 6:4636–4642

15. Tomabechi Y, Krippner G, Rendle PM et al (2013) Glycosylation of pramlintide: synthetic glycopeptides that display in vitro and in vivo activities as amylin receptor agonists. Chem Eur J 19:15084–15088
16. Tomabechi Y, Squire MA, Fairbanks AJ (2014) Endo-β-N-acetylglucosaminidase catalysed glycosylation: tolerance of enzymes to structural variation of the glycosyl amino acid acceptor. Org Biomol Chem 12:942–955
17. Lomino JV, Naegeli A, Orwenyo J et al (2013) A two-step enzymatic glycosylation of polypeptides with complex N-glycans. Bioorg Med Chem 21:2262–2270
18. Orwenyo J, Huang W, Wang L-X (2013) Chemoenzymatic synthesis and lectin recognition of a selectively fluorinated glycoprotein. Bioorg Med Chem 21:4768–4777
19. Smith EL, Giddens JP, Iavarone AT et al (2014) Chemoenzymatic Fc glycosylation via engineered aldehyde tags. Bioconjug Chem 25:788–795
20. Umekawa M, Huang W, Li B et al (2008) Mutants of mucor hiemalis endo-β-N-acetylglucosaminidase show enhanced transglycosylation and glycosynthase-like activities. J Biol Chem 283:4469–4479
21. Umekawa M, Higashiyama T, Koga Y et al (2010) Efficient transfer of sialo-oligosaccharide onto proteins by combined use of a glycosynthase-like mutant of Mucor hiemalis endoglycosidase and synthetic sialo-complex-type sugar oxazoline. Biochim Biophys Acta Gen Subj 1800:1203–1209
22. Umekawa M, Li C, Higashiyama T et al (2010) Efficient glycosynthase mutant derived from mucor hiemalis endo-β-N-acetylglucosaminidase capable of transferring oligosaccharide from both sugar oxazoline and natural N-glycan. J Biol Chem 285:511–521
23. Noguchi M, Fujieda T, Huang WC et al (2012) A practical one-step synthesis of 1,2-oxazoline derivatives from unprotected sugars and its application to chemoenzymatic β-N-acetylglucosaminidation of disialo-oligosaccharide. Helv Chim Acta 95:1928–1936
24. Sun B, Bao W, Tian X et al (2014) A simplified procedure for gram-scale production of sialylglycopeptide (SGP) from egg yolks and subsequent semi-synthesis of Man3GlcNAc oxazoline. Carbohydr Res 396:62–69
25. Tanaka T, Huang WC, Noguchi M et al (2009) Direct synthesis of 1,6-anhydro sugars from unprotected glycopyranoses by using 2-chloro-1,3-dimethylimidazolinium chloride. Tetrahedron Lett 50:2154–2157
26. Köll P, Metzger J (1978) Thermal degradation of cellulose and chitin in supercritical acetone. Angew Chem Int Ed 17:754–755
27. Miura M, Kaga H, Yoshida T, Ando K (2001) Microwave pyrolysis of cellulosic materials for the production of anhydrosugars. J Wood Sci 47:502–506
28. Sasaki M, Takahashi K, Haneda Y et al (2008) Thermochemical transformation of glucose to 1,6-anhydroglucose in high-temperature steam. Carbohydr Res 343:848–854
29. Köll P, Borchers G, Metzger JO (1991) Thermal degradation of chitin and cellulose. J Anal Appl Pyrolysis 19:119–129
30. Tanaka T, Nagai H, Noguchi M, et al. (2009) One-step conversion of unprotected sugars to β-glycosyl azides using 2-chloroimidazolinium salt in aqueous solution. Chem Commun 3378–3379
31. Tanaka T, Matsumoto T, Noguchi M et al (2009) Direct Transformation of unprotected sugars to Aryl 1-Thio-β-glycosides in aqueous media using 2-Chloro-1,3-dimethylimidazolinium chloride. Chem Lett 38:458–459
32. Sarkar S, Sucheck SJ (2011) Comparing the use of 2-methylenenapthyl, 4-methoxybenzyl, 3,4-dimethoxybenzyl and 2,4,6-trimethoxybenzyl as N-H protecting groups for p-tolyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucosides. Carbohydr Res 346:393–400
33. Milhomme O, Dhénin SGY, Djedaïni-Pilard F et al (2012) Synthetic studies toward the anthrax tetrasaccharide: alternative synthesis of this antigen. Carbohydr Res 356:115–131
34. Ennis SC, Fairbanks AJ, Slinn CA et al (2001) N-Iodosuccinimide-mediated intramolecular aglycon delivery. Tetrahedron 57:4221–4230
35. Yasomanee JP, Demchenko AV (2014) Hydrogen bond mediated aglycone delivery: synthesis of linear and branched α-glucans. Angew Chem Int Ed 53:10453–10456

36. Rye CS, Withers SG (2004) The synthesis of a novel thio-linked disaccharide of chondroitin as a potential inhibitor of polysaccharide lyases. Carbohydr Res 339:699–703
37. Rempel BP, Withers SG (2008) Covalent inhibitors of glycosidases and their applications in biochemistry and biology. Glycobiology 18:570–586
38. Drouin L, Cowley AR, Fairbanks AJ, Thompson AL (2008) 4-Methoxyphenyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside. Acta Crystallogr E 64:o1401–o1401
39. Pei Z, Dong H, Caraballo R, Ramström O (2007) Synthesis of positional thiol analogs of β-D-galactopyranose. Eur J Org Chem 4927–4934
40. Funabashi M, Arai S, Shinohara M (1999) Novel syntheses of diphenyl and/or trimethylene dithioacetals of mono- and oligosaccharides in 90% trifluoroacetic acid. J Carbohydr Chem 18:333–341
41. Yanase M, Funabashi M (2000) Stereoselective 1,2-cis-1-thioglycosidation of aldohexoses with tert-butyl mercaptan in 90% trifluoroacetic acid. J Carbohydr Chem 19:53–66
42. Yoshida N, Noguchi M, Tanaka T et al (2011) Direct dehydrative pyridylthio-glycosidation of unprotected sugars in aqueous media using 2-chloro-1,3-dimethylimidazolinium chloride as a condensing agent. Chem Asian J 6:1876–1885
43. Hase S (2010) Pyridylamination as a means of analyzing complex sugar chains. Proc Jpn Acad Ser B 86:378–390
44. Kallin E, Lonn H, Norberg T (1988) Derivatization procedures for reducing oligosaccharides, part 2: chemical transformation of 1-Deoxy-1-(4-trifluoroacetamidophenyl)aminoalditols. Glycoconj J 5:145–150
45. Suzuki S, Fujimori T, Yodoshi M (2006) Recovery of free oligosaccharides from derivatives labeled by reductive amination. Anal Biochem 354:94–103
46. Yoshida N, Fujieda T, Kobayashi A et al (2013) Direct introduction of detachable fluorescent tag into oligosaccharides. Chem Lett 42:1038–1039
47. Lee YC, Lee RT (1995) Carbohydrate-protein interactions: basis of glycobiology. Acc Chem Res 28:321–327
48. Lundquist JJ, Toone EJ (2002) The cluster glycoside effect. Chem Rev 102:555–578
49. Lee RT, Lee YC (2000) Affinity enhancement by multivalent lectin-carbohydrate interaction. Glycoconj J 17:543–551
50. Dam TK, Brewer CF (2010) Multivalent lectin—carbohydrate interactions, pp 139–164
51. Le Droumaguet B, Nicolas J (2010) Recent advances in the design of bioconjugates from controlled/living radical polymerization. Polym Chem 1:563
52. Tanaka K, Siwu ERO, Minami K et al (2010) Noninvasive imaging of dendrimer-type N-glycan clusters. In Vivo dynamics dependence on oligosaccharide structure. Angew Chem Int Ed 49:8195–8200
53. Poonthiyil V, Nagesh PT, Husain M et al (2015) Gold nanoparticles decorated with sialic acid terminated Bi-antennary N-glycans for the detection of influenza virus at nanomolar concentrations. ChemistryOpen 4:708–716
54. Glunz PW, Hintermann S, Williams LJ et al (2000) Design and synthesis of Le y -bearing glycopeptides that mimic cell surface le y mucin glycoprotein architecture. J Am Chem Soc 122:7273–7279
55. Yamamoto N, Tanabe Y, Okamoto R et al (2008) Chemical synthesis of a glycoprotein having an intact human complex-type sialyloligosaccharide under the boc and fmoc synthetic strategies. J Am Chem Soc 130:501–510
56. Roy R, Tropper FD, Romanowska A (1992) New strategy in glycopolymer synthesis. Preparation of antigenic water-soluble poly(acrylamide-co-p-acrylamidophenyl beta-lactoside). Bioconjug Chem 3:256–261
57. Fraser C, Grubbs RH (1995) Synthesis of glycopolymers of controlled molecular weight by ring-opening metathesis polymerization using well-defined functional group tolerant ruthenium carbene catalysts. Macromolecules 28:7248–7255
58. Tanaka T, Inoue G, Shoda S-I, Kimura Y (2014) Protecting-group-free synthesis of glycopolymers bearing thioglycosides via one-pot monomer synthesis from free saccharides. J Polym Sci A 1(52):3513–3520

59. Gamblin DP, Garnier P, van Kasteren S et al (2004) Glyco-SeS: selenenylsulfide-mediated protein glycoconjugation—a new strategy in post-translational modification. Angew Chem Int Ed 116:846–851
60. Bernardes GJL, Marston JP, Batsanov AS et al. (2007) A trisulfide-linked glycoprotein. Chem Commun 3145–3147
61. Brimble MA, Edwards PJ, Harris PWR et al (2015) Synthesis of the antimicrobial s-linked glycopeptide, glycocin F. Chem Eur J 21:3556–3561
62. Driguez H (2001) Thiooligosaccharides as tools for structural biology. ChemBioChem 2:311–318
63. Levengood MR, van der Donk WA (2007) Dehydroalanine-containing peptides: preparation from phenylselenocysteine and utility in convergent ligation strategies. Nat Protoc 1:3001–3010
64. Galonić DP, van der Donk WA, Gin DY (2003) Oligosaccharide-peptide ligation of glycosyl thiolates with dehydropeptides: synthesis of S-linked mucin-related glycopeptide conjugates. Chem Eur J 9:5997–6006
65. Thayer DA, Yu HN, Galan MC, Wong C-H (2005) A general strategy toward S-linked glycopeptides. Angew Chem Int Ed 44:4596–4599
66. Bernardes GJL, Grayson EJ, Thompson S et al (2008) From disulfide- to thioether-linked glycoproteins. Angew Chem Int Ed 47:2244–2247
67. Dondoni A, Massi A, Nanni P, Roda A (2009) A new ligation strategy for peptide and protein glycosylation: photoinduced thiol-ene coupling. Chem Eur J 15:11444–11449
68. Crich D, Yang F (2008) Synthesis of neoglycoconjugates by the desulfurative rearrangement of allylic disulfides. J Org Chem 73:7017–7027
69. Zhu X, Dere RT, Jiang J et al (2011) Synthesis of α-glycosyl thiols by stereospecific ring-opening of 1,6-anhydrosugars. J Org Chem 76:10187–10197
70. Novoa A, Barluenga S, Serba C, Winssinger N (2013) Solid phase synthesis of glycopeptides using Shoda's activation of unprotected carbohydrates. Chem Commun 49:7608–7610
71. Györgydeák Z, Thiem J (2006) Synthesis and transformation of glycosyl azides. Adv Carbohydr Chem Biochem 60:103–182
72. Kitamura M, Tashiro N, Miyagawa S, Okauchi T (2011) 2-Azido-1,3-dimethylimidazolinium salts: Efficient diazo-transfer reagents for 1,3-dicarbonyl compounds. Synthesis 1037–1044
73. Kitamura M, Kato S, Yano M et al (2014) A reagent for safe and efficient diazo-transfer to primary amines: 2-azido-1,3-dimethylimidazolinium hexafluorophosphate. Org Biomol Chem 12:4397–4406
74. Kitamura M, Yano M, Tashiro N et al (2011) Direct synthesis of organic azides from primary amines with 2-Azido-1,3-dimethylimidazolinium hexafluorophosphate. Eur J Org Chem 2011:458–462
75. Kitamura K, Shigeta M, Maezawa Y et al (2013) Preparation of L-vancosamine-related glycosyl donors. J Antibiot 66:131–139
76. Kitamura M, Murakami K, Shiratake Y, Okauchi T (2013) Synthesis of α-arylcarboxylic acid amides from silyl enol ether via migratory amidation with 2-Azido-1,3-dimethylimidazolinium hexafluorophosphate. Chem Lett 42:691–693
77. Kitamura M, Miyagawa S, Okauchi T (2011) Synthesis of α, α-diarylacetamides from benzyl aryl ketones using 2-azido-1,3-dimethylimidazolinium hexafluorophosphate. Tetrahedron Lett 52:3158–3161
78. Kitamura M, Koga T, Yano M, Okauchi T (2012) Direct synthesis of organic azides from alcohols using 2-Azido-1,3-dimethyl-imidazolinium hexafluorophosphate. Synlett 23:1335–1338
79. Kitamura M (2015) Synthesis Of 2-Azido-1,3-dimethylimidazolinium hexafluorophosphate (ADMP). Org Synth 92:171–181
80. Lim D, Brimble MA, Kowalczyk R et al (2014) Protecting-group-free one-pot synthesis of glycoconjugates directly from reducing sugars. Angew Chem Int Ed 53:11907–11911

81. Tornøe CW, Christensen C, Meldal M (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J Org Chem 67:3057–3064
82. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A stepwise huisgen cycloaddition process: copper(i)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angew Chem Int Ed 41:2596–2599
83. Dondoni A (2007) Triazole: the keystone in glycosylated molecular architectures constructed by a click reaction. Chem-Asian J 2:700–708
84. Wilkinson BL, Long H, Sim E, Fairbanks AJ (2008) Synthesis of arabino glycosyl triazoles as potential inhibitors of mycobacterial cell wall biosynthesis. Bioorg Med Chem Lett 18:6265–6267
85. El Akri K, Bougrin K, Balzarini J et al (2007) Efficient synthesis and in vitro cytostatic activity of 4-substituted triazolyl-nucleosides. Bioorg Med Chem Lett 17:6656–6659
86. Rossi LL, Basu A (2005) Glycosidase inhibition by 1-glycosyl-4-phenyl triazoles. Bioorg Med Chem Lett 15:3596–3599
87. Wilkinson BL, Innocenti A, Vullo D et al (2008) Inhibition of carbonic anhydrases with glycosyltriazole benzene sulfonamides. J Med Chem 51:1945–1953
88. Wilkinson BL, Bornaghi LF, Houston TA et al (2006) A novel class of carbonic anhydrase inhibitors: glycoconjugate benzene sulfonamides prepared by "click-tailing". J Med Chem 49:6539–6548
89. De las Heras FG, Alonso R, Alonso G (1979) Alkylating nucleosides. 1. Synthesis and cytostatic activity of N-glycosyl(halomethyl)-1,2,3-triazoles. A new type of alkylating agent. J Med Chem 22:496–501
90. De las Heras FG, Camarasa M-J (1982) Synthesis of Alkylating 1-Glycosyl-5-substituted 1,2,4-Triazoles 1. Nucleos Nucleot 1:45–56
91. Yeoh KK, Butters TD, Wilkinson BL, Fairbanks AJ (2009) Probing replacement of pyrophosphate via click chemistry; synthesis of UDP-sugar analogues as potential glycosyl transferase inhibitors. Carbohydr Res 344:586–591
92. Li H, Aneja R, Chaiken I (2013) Click chemistry in peptide-based drug design. Molecules 18:9797–9817
93. Tomabechi Y (2015) Synthesis of glycopeptides by click chemistry. Trends Glycosci Glycotechnol 27:63–65
94. Wang H, Huang W, Orwenyo J et al (2013) Design and synthesis of glycoprotein-based multivalent glyco-ligands for influenza hemagglutinin and human galectin-3. Bioorg Med Chem 21:2037–2044
95. Hanisch F-G, Muller S (2000) MUC1: the polymorphic appearance of a human mucin. Glycobiology 10:439–449
96. Sherblom AP, Moody CE (1986) Cell surface sialomucin and resistance to natural cell-mediated cytotoxicity of rat mammary tumor ascites cells. Cancer Res 46:4543–4546
97. Kaiser A, Gaidzik N, Westerlind U et al (2009) A synthetic vaccine consisting of a tumor-associated sialyl-T N-MUC1 tandem-repeat glycopeptide and tetanus toxoid: induction of a strong and highly selective immune response. Angew Chem Int Ed 48:7551–7555
98. Lakshminarayanan V, Thompson P, Wolfert MA et al (2012) Immune recognition of tumor-associated mucin MUC1 is achieved by a fully synthetic aberrantly glycosylated MUC1 tripartite vaccine. Proc Natl Acad Sci 109:261–266
99. Rising TWDF, Heidecke CD, Moir JWB et al (2008) Endohexosaminidase-catalysed glycosylation with oxazoline donors: fine tuning of catalytic efficiency and reversibility. Chem Eur J 14:6444–6464
100. Seko A, Koketsu M, Nishizono M et al (1997) Occurrence of a sialylglycopeptide and free sialylglycans in hen's egg yolk. Biochim Biophys Acta - Gen Subj 1335:23–32