Recent applications of the Cu^{I}-catalysed Huisgen azide–alkyne 1,3-dipolar cycloaddition reaction in carbohydrate chemistry

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This article surveys recent applications of Cu^{I}-catalysed 1,3-dipolar cycloaddition of azides and alkynes in carbohydrate chemistry, highlighting developments in the preparation of simple glycoside and oligosaccharide mimetics, glyco-macrocycles, glycopeptides, glyco-clusters and carbohydrate arrays.

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Introduction

1,3-Dipolar cycloaddition reactions in general have long been popular in the generation of carbohydrate mimetics,[1] with thermally-induced Huisgen azide–alkyne cross-coupling[2] being used for the synthesis of *N*-glycosyl triazoles,[3] as a means of effecting conversion of anomeric azides to glycosyl fluorides,[4] for the

Simone Dedola comes from Sardinia, where he received his degree in Chemistry from the University of Sassari in 2004. In 2005 he became a PhD student in Rob Field's group at the University of East Anglia in Norwich. His current research focuses on the synthesis of starch fragment analogues and enzyme inhibitors using "click chemistry". These studies are aimed at developing new tools with which to study starch structure and metabolism.

Sergey Nepogodiev was born in Russia. He studied chemistry at the Moscow State University and received his PhD under the guidance of Professor N. K. Kochetkov at the Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences in Moscow. In 1994, he moved to the UK to join the research group of Professor J. F. Stoddart at the University of Birmingham. Following short spells as a research officer at the University of Warwick (1999) and the University of Bath (2000), in 2001 he became a senior research fellow collaborating with Professor R. A. Field at the University of East Anglia and later at the John Innes Centre. His current research concerns a variety of aspects of plant carbohydrate chemistry, in particular the synthesis of complex cell wall oligosaccharides.

Rob Field is a University of East Anglia graduate (1986) and PhD (1989; Dr A. H. Haines). Following postdoctoral work in Oxford (1989–1991; Professor J. E. Baldwin), Dundee (1992–1994; Professor M. A. J. Ferguson and Professor S. W. Homans) and Alberta (1994; Professor O. Hindsgaul), he was appointed to the faculty at the University of St Andrews in 1994, where he was promoted through the ranks to Professor (1999). He returned to Norwich in 2001, initially at UEA and latterly at the John Innes Centre. His research programme focuses on plant and microbial carbohydrate chemistry, in particular cell wall biosynthesis, starch metabolism and the enzymology of antibiotic biosynthesis. He is also involved in the development of analytical tools (glyco-arrays, nanoparticles and quantum dots) and chemical genetics approaches to study the role of glycosylation in biological systems.

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preparation of cyclodextrin mimetics[5] and *S*-neoglyconjugates,[6] for instance. The robustness of this cycloaddition process has led to its inclusion under the 'click chemistry' banner, where it is arguably the 'cream of the crop'.[7] Latterly, this class of reaction has attracted substantial attention following the independent identification by Meldal[8] and Sharpless[9] that the classical 1,3-dipolar cycloaddition of azides and terminal alkynes can be catalysed by Cu^{I} salts (Fig. 1).

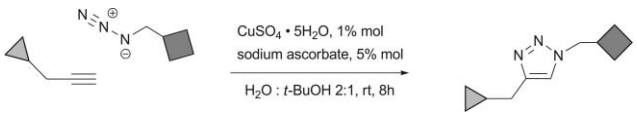

Fig. 1 The Cu^{I}-catalysed Huisgen azide–alkyne 1,3-dipolar cycloaddition reaction.

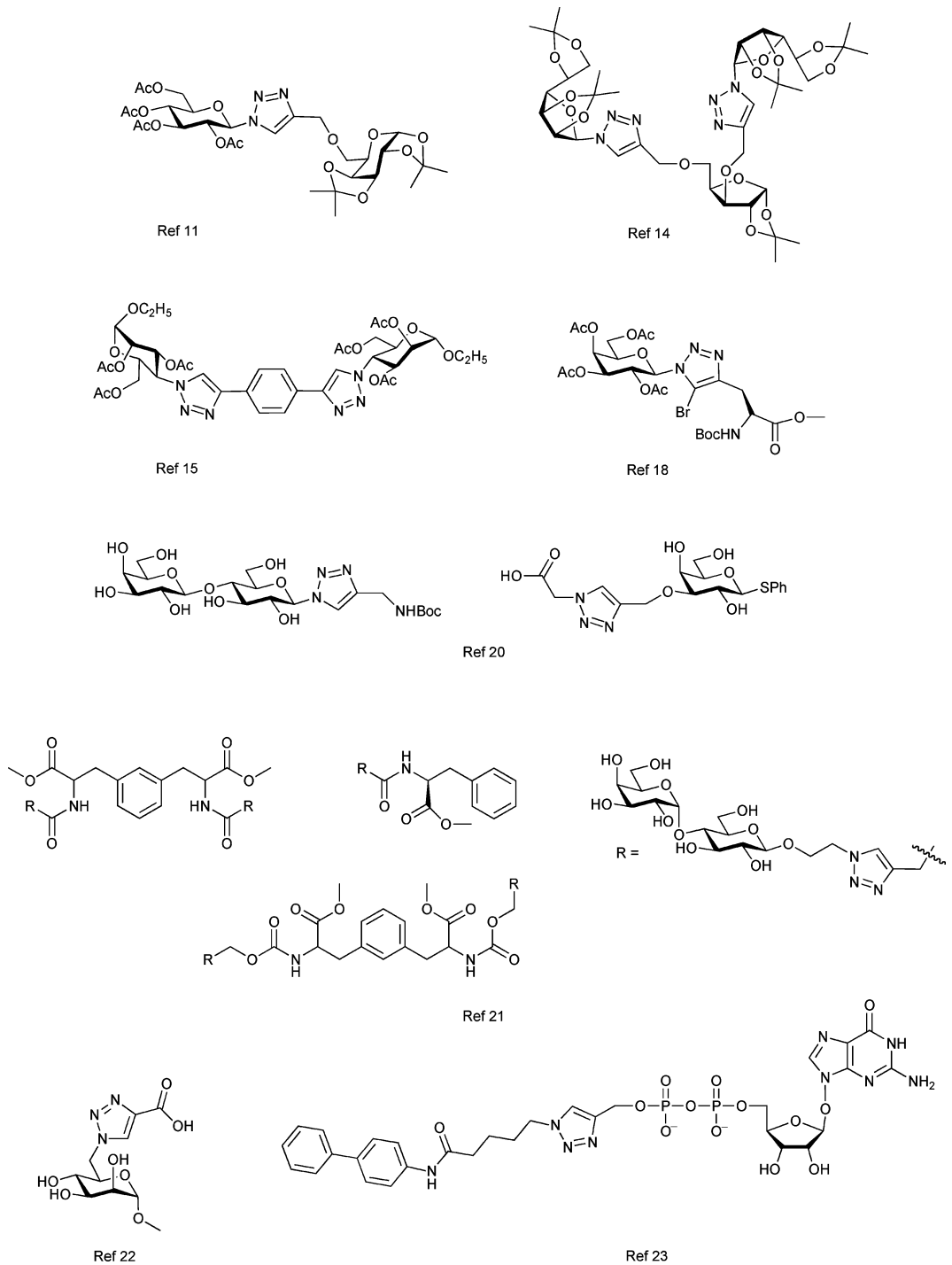

Fig. 2 Glycoside mimetics from azide–alkyne cycloaddition reaction.

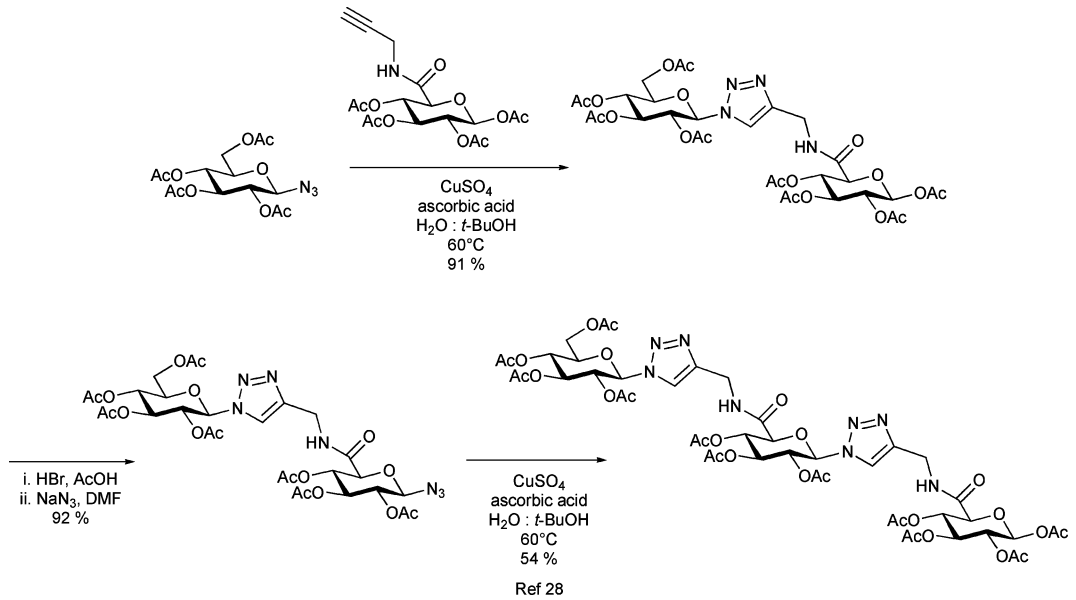

Fig. 3 Neoglycotrimer synthesis by iterative 1,3-dipolar cycloaddition and anomeric functional group interconversion.

This article highlights recent examples of the Cu^{I}-catalysed azide–alkyne 1,3-dipolar cycloaddition reaction from the carbohydrate literature, including the synthesis of simple glycoside and oligosaccharide mimetics, glyco-macrocycles, glycopeptides, glyco-clusters and carbohydrate arrays. However, the Cu^{I}-catalysed reaction is mild, regioselective (in contrast to the thermal process) and tolerant of diverse functionality, opening the way to diverse applications across many classes of organic molecules.[10]

Glycosides

The generation of *N*-glycosyl-triazoles from simple acetylenes,[11–13] sugar,[14–17] amino acid[18] and steroid-derived[17] terminal acetylenes (*C*-linked alkynes, *O*-propargyl ethers, ynamides) has been reported (Fig. 2). In terms of biological and medicinal applications, the functional group tolerance of the Cu^{I}-catalysed procedure is exemplified in the synthesis of triazole-based analogues of the neuraminidase inhibitor zanamavir.[19] Triazole-substituted sugars have also been explored as potential monovalent and multivalent galectin ligands,[20,21] and for investigation of substrate recognition by[22] and inhibition of[23] glycosyltransferases. Microwave-assisted, solvent-free synthesis of triazolyl-nucleosides has also been shown to be effective.[24]

On a broader front, azide–alkyne 1,3-dipolar cycloaddition has been used to generate water soluble carbohydrate derivatives of ferrocene[25] and azidopropyl-silica has been coupled with organic and sugar alkyne modifiers to generate novel triazole-based HPLC packings for carbohydrate[26] and for protein[27] separation.

Oligosaccharides

The efficiency and simplicity of azide–alkyne dipolar cycloaddition for coupling organic fragments has proved attractive. Neoglycotrimers have been derived from protected glucopyranosyl azide and *N*-propargyl glucuronamide, with subsequent manipulation of the reducing terminus of the neoglycodimer to install an azide group, thus permitting iteration of the coupling procedure (Fig. 3).[28] Triazole-linked (1,6)-α-D-oligomannoses have been prepared in a similar vein.[29]

With a view to interrogation of carbohydrate-active enzymes, triazole-linked pseudo-starch fragments have been prepared from protected sugar building blocks.[30] This approach was rapidly superseded by the same authors, relying on the chemoselectivity of the azide–alkyne dipolar cycloaddition to couple unprotected maltoheptaosyl azide and a di-*O*-propargyl glucoside in water in 89% yield (Fig. 4).[31] Related triazole-linked structures based on the natural product anti-diabetic drug acarbose have also been synthesised by dipolar cycloaddition chemistry (Fig. 4).[32]

This form of click chemistry is not restricted to low molecular weight materials. Applications in carbohydrate polymer chemistry include: the attachment of redox active or fluorescent tags to the 6-azido-6-deoxy derivative of the linear β-1,3-glucan curdlan;[33] coupling of hexynoate esters of cellulose to give fluorescent triazole derivatives of the polymer;[34] generation of triazoles from 6-azido-6-deoxy cellulose and simple organic alkynes.[35] Carbohydrate 'labels' for oligonucleotide synthesis on solid support have been introduced using click chemistry,[36] whilst trehalose click polymers have been used to promote plasmid DNA delivery.[37]

Glyco-polycycles and macrocycles

Acyclic and monosaccharide-derived azido-alkynes have been used to prepare multi-cyclic structures through dipolar cycloaddition.[38–42] Similar intermolecular coupling of carbohydrate-derived bis-alkynes with organic diazides affords access to macrocycles.[43] These studies follow on from key work on the development of cyclodextrin mimetics (Fig. 5),[44,45] which itself builds on earlier work on the thermal dipolar cycloaddition process.[5]

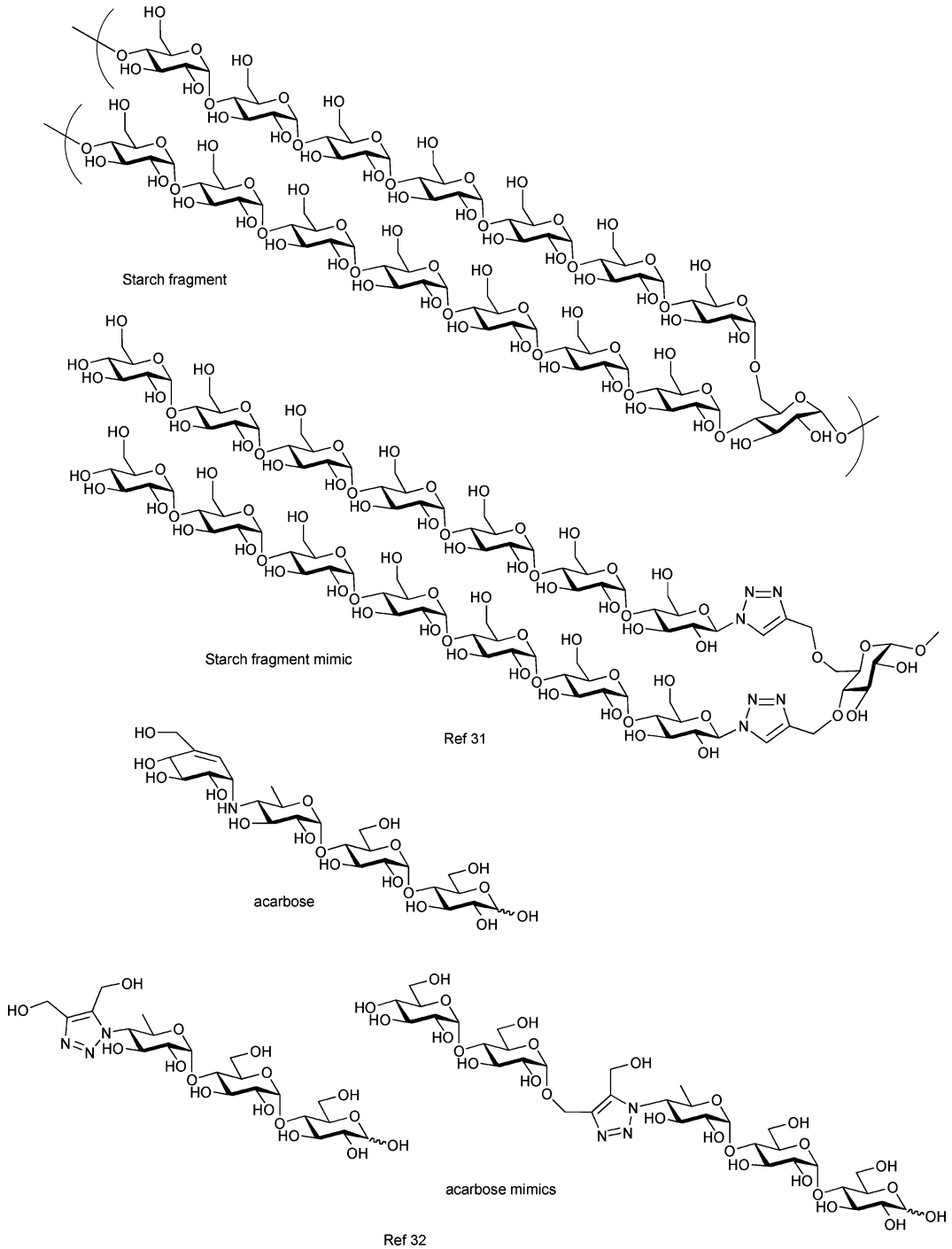

Fig. 4 Starch, triazole-based starch mimetic. Amylase inhibitor acarbose and triazole-based mimetics.

Glycopeptides

Triazole-linked glycosyl amino acids have been prepared from glycosyl azides[46] and glycosyl alkynes,[46,47] and from the corresponding propargyl and azidoethyl glycosides.[48] Click chemistry has also been used for the decoration of 2(1*H*)-pyrazinones[49] and azabicycloalkane amino acids[50] *en route* to glycopeptidomimetics. In connection with the synthesis of potential anti-cancer vaccines, pentynoic acid amides of peptide-based lysine side chains provide reactants for elaboration by cycloaddition with glycosyl amino acid-derived azides (Fig. 6).[51]

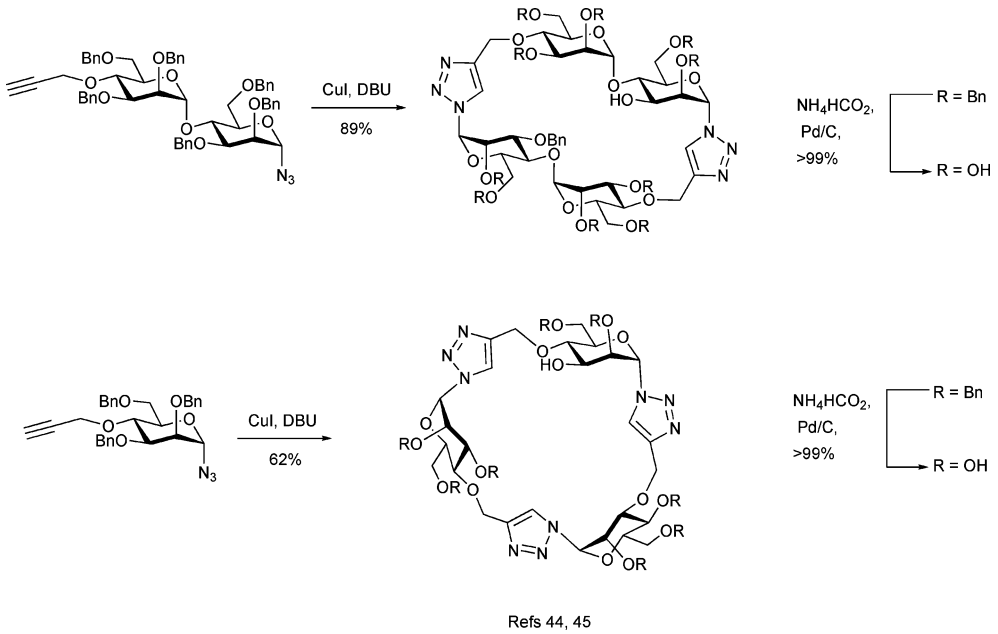

Fig. 5 Synthesis of carbohydrate-based macrocycles by sequential 1,3-dipolar cycloaddition.

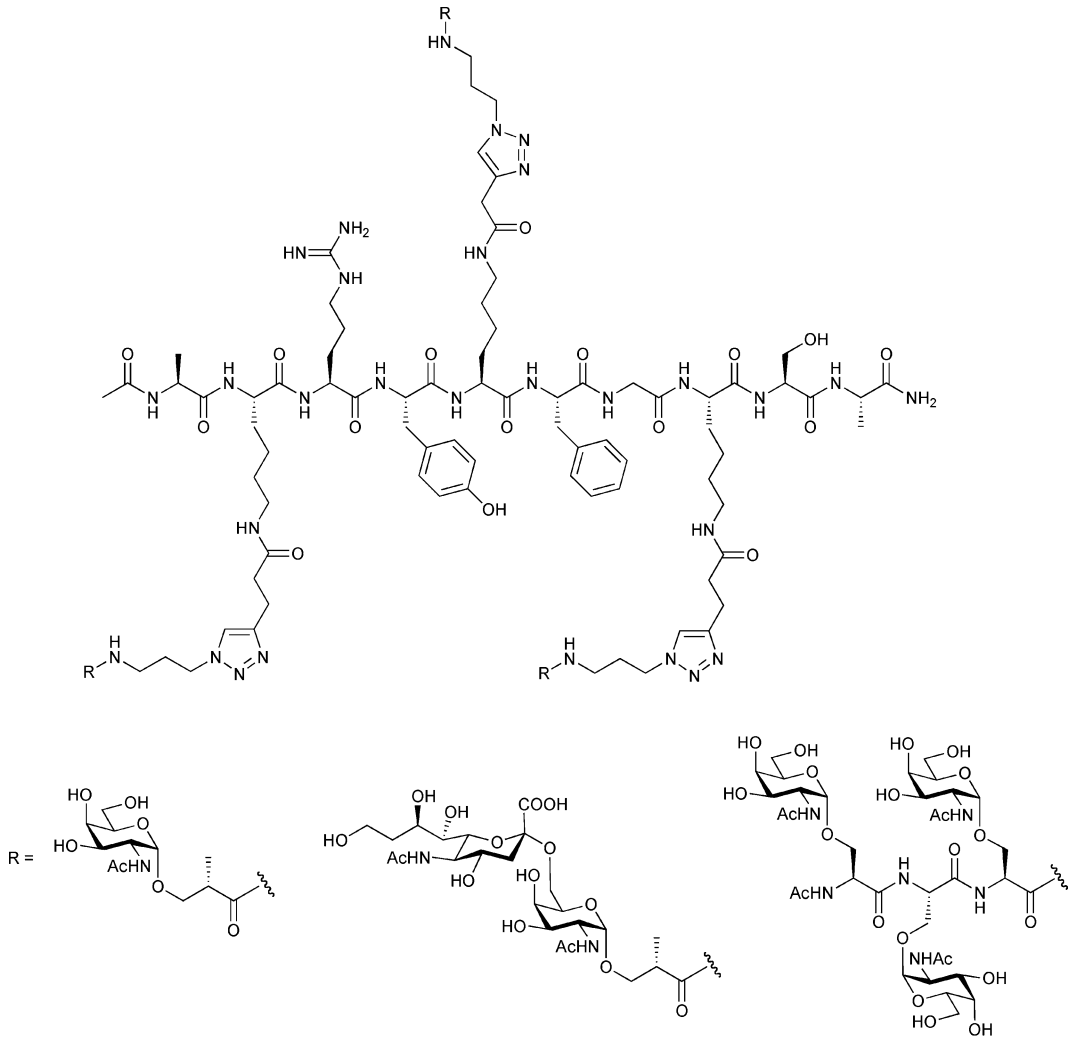

Fig. 6 Triazole-linked glycopeptide mimetics derived from azide–alkyne click chemistry.

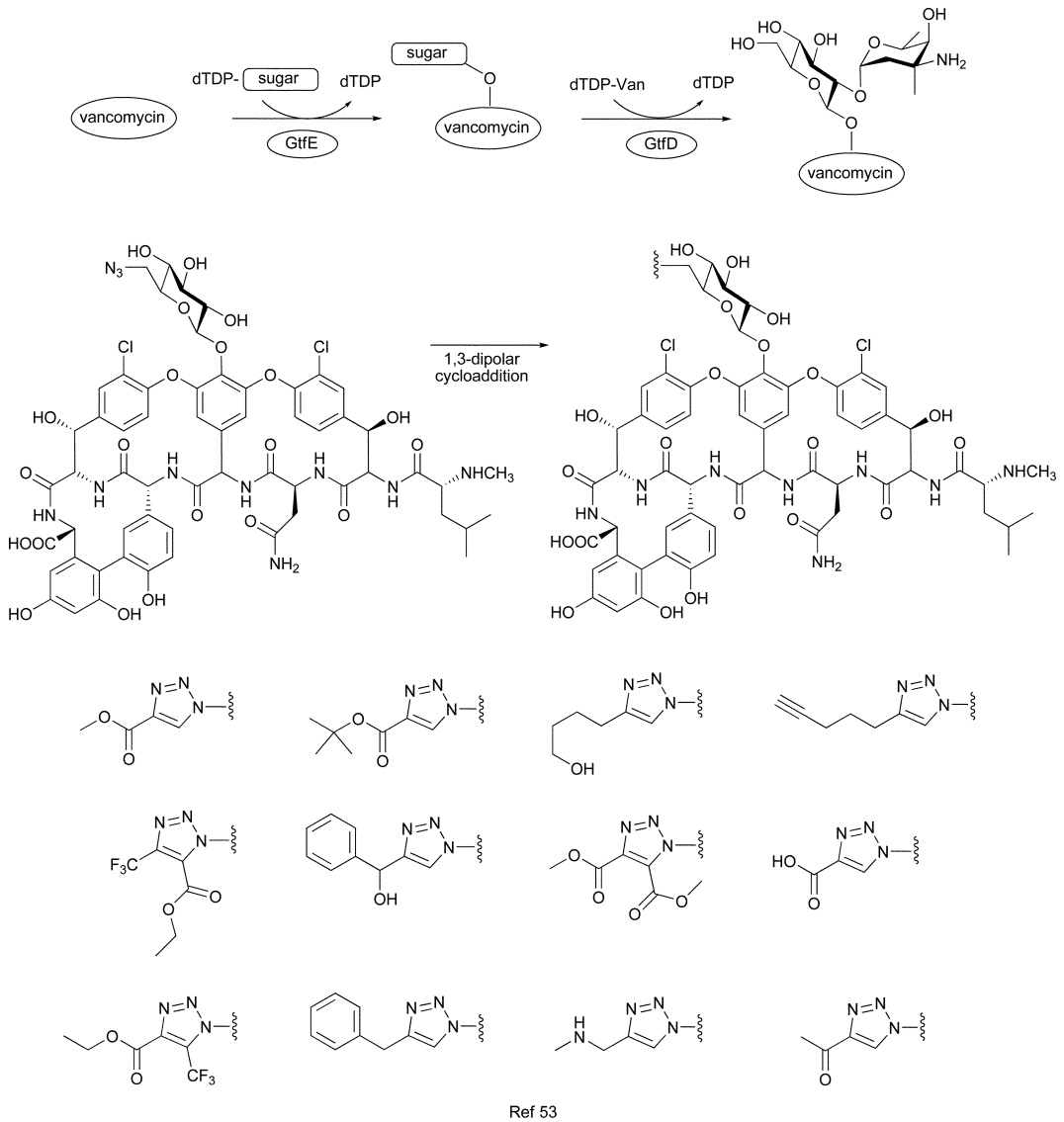

Fig. 7 Diverse library of vancomycin-like antibiotics arising from azide–alkyne cycloaddition.

Enzymatic synthesis of peptides derived from the unnatural amino acids propargylglycine and azidopropylglycine provides templates with which to react either glycosyl acetylenes or glycosyl azides, giving novel glycopeptide analogues.[52] Making further use of nature's elaborate biosynthetic machinery, and the modest substrate specificity of some glycosyltransferases in particular, *in vitro* derivatisation of the vancomycin peptide core with a range of sugars—'glycorandomisation'—has been demonstrated.[53] Specifically, the regio- and stereo-selective introduction of a 6-azido-6-deoxy-glucose unit provides an opportunity for chemoselective ligation with alkynes, generating an array of vancomycin analogues (Fig. 7). Other studies show the utility of the thioesterase domain of tyrocidin synthetase in the *in vitro* synthesis of cyclic decapeptides containing one to three propargylglycine units. Conjugation of these novel peptides with twenty one azido sugars gave a library of natural product-like molecules, some with better activity than tyrocidin itself (Fig. 8).[54]

Glyco-clusters

Multivalent display of neoglycoconjugates, to mimic natural presentation of carbohydrate structures, attracts increasing applications of azide–alkyne cycloaddition chemistry. Exploitation of propargyl glycosides and a range of organic cores possessing numerous azide groups has been demonstrated (Fig. 9),[55] as has the use of glycosyl azides[56] and azidoalkyl glycosides[57] with cores containing multiple propargyl ether groups. Calixarene-derived

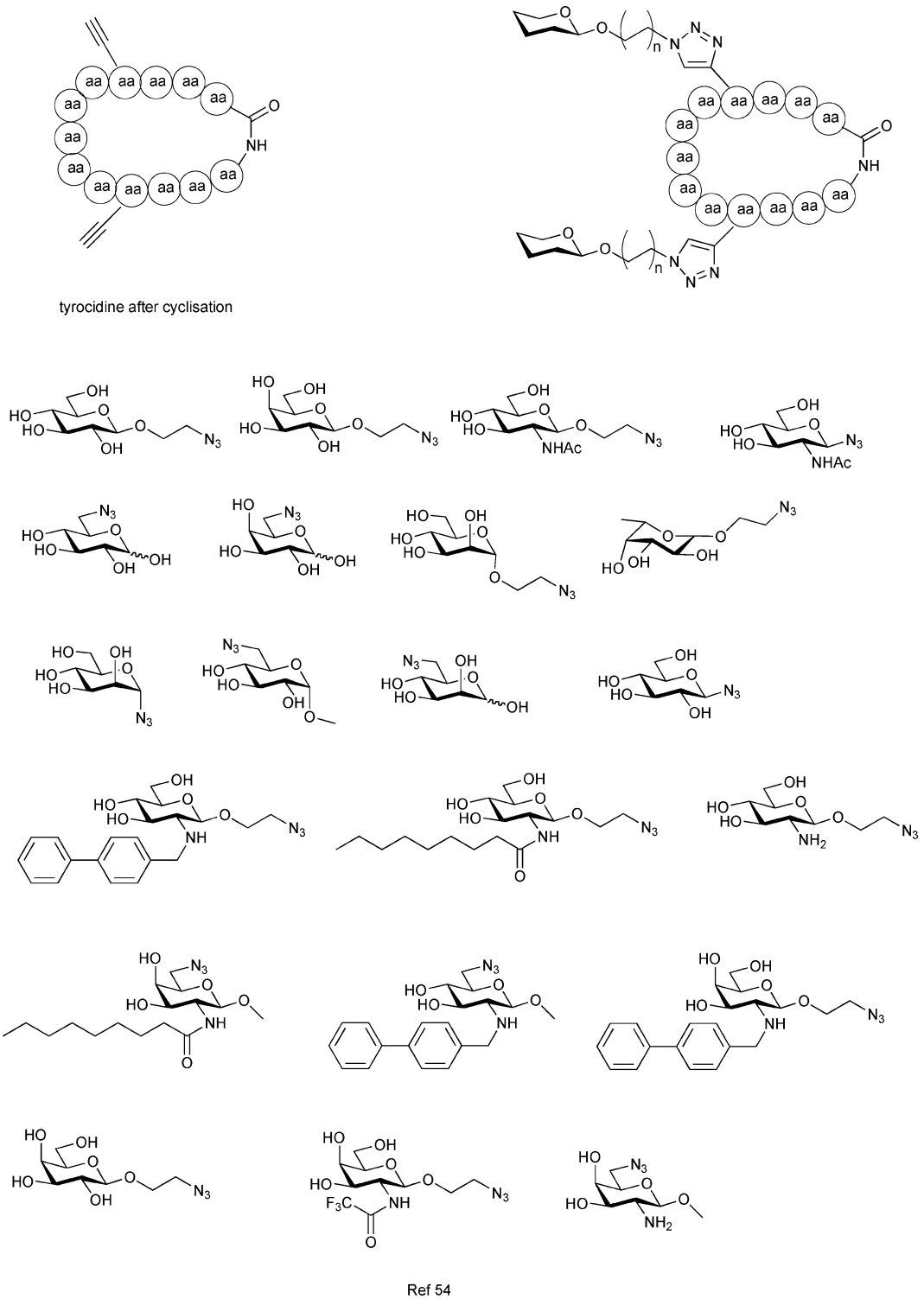

Fig. 8 Exploitation of click chemistry in the chemoenzymatic synthesis of cyclic oligopeptide antibiotics appended with modified monosaccharides.

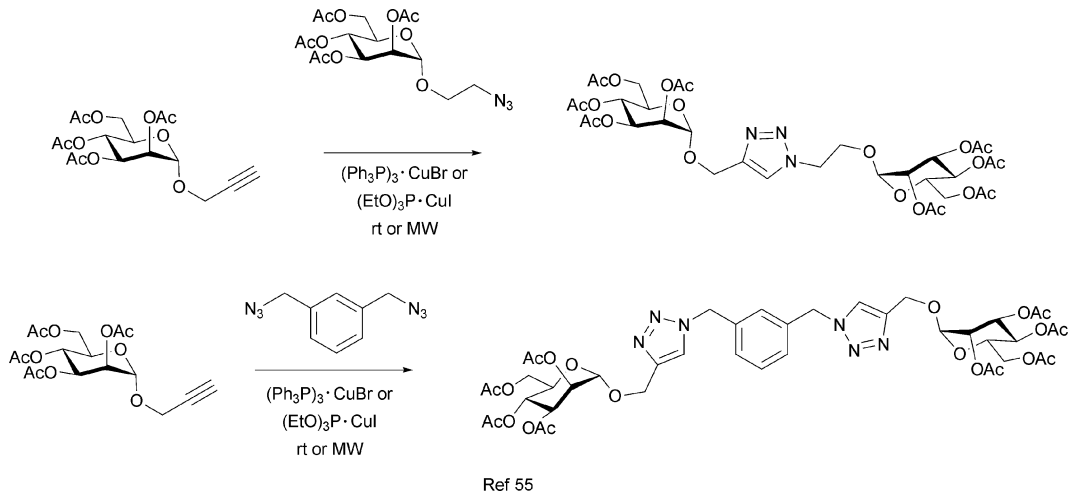

Fig. 9 Triazole-linked cluster neoglycoconjugates.

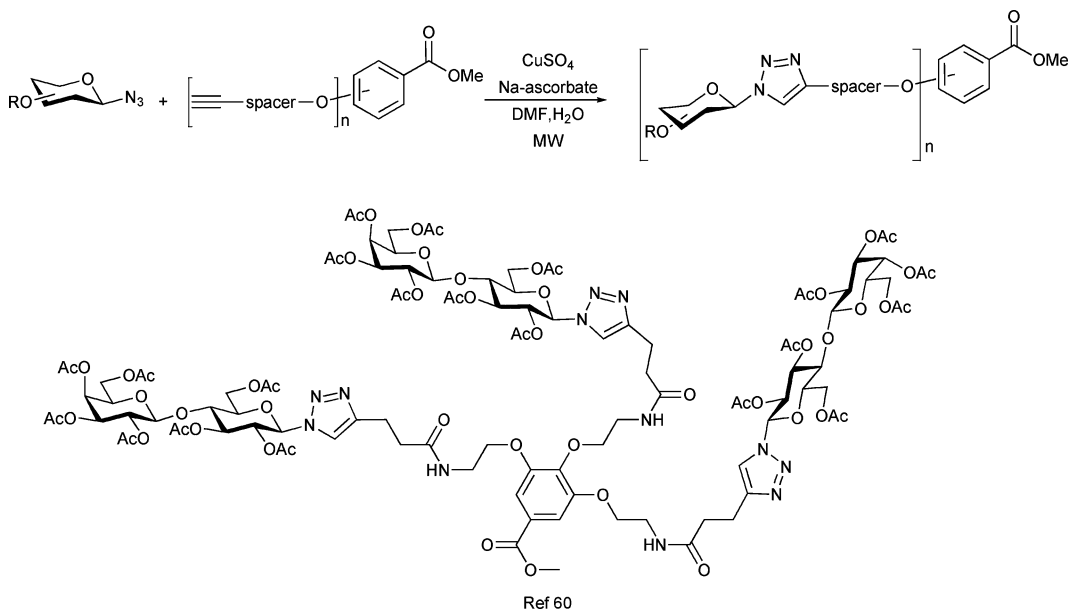

Fig. 10 Triazole-linked cluster glycoclusters.

azides have also been coupled with glycosyl acetylenes to give multivalent constructs.[58]

Carbohydrate-centred multivalent glycoclusters can be accessed from per-*O*-propargylated methyl galactoside.[59] Similar approaches from multiply hydroxylated benzoic acid, with either azide- or alkyne-based cores, gives rise to glycodendrimers (Fig. 10 and 11)[60,61] with similar chemistries giving PEG-dendritic block co-polymers.[62] Clickable alkyne polymers from living radical polymerisation have also been investigated as neoglycopolymers.[63]

Neoproteoglycans have been accessed from chondroitin sulfate using enzymatic cleavage of the glycan chain from protein in the presence of propargyl alcohol, which gives rise directly to the desired propargyl glycosides of the released oligosaccharides. Subsequent click ligation to azidobenzoic acid-modified bovine serum albumin gives neo-proteoglycans, with glycan chains presented in multivalent form from the protein surface (Fig. 12).[64]

Carbohydrate arrays

Array technologies have revolutionised molecular biology, and seem set to do the same for glycobiology.[65,66] Again, azide–alkyne dipolar cycloaddition has a role to play, with glyco-triazoles derived from hydrophobic propargylic amides being exploited for

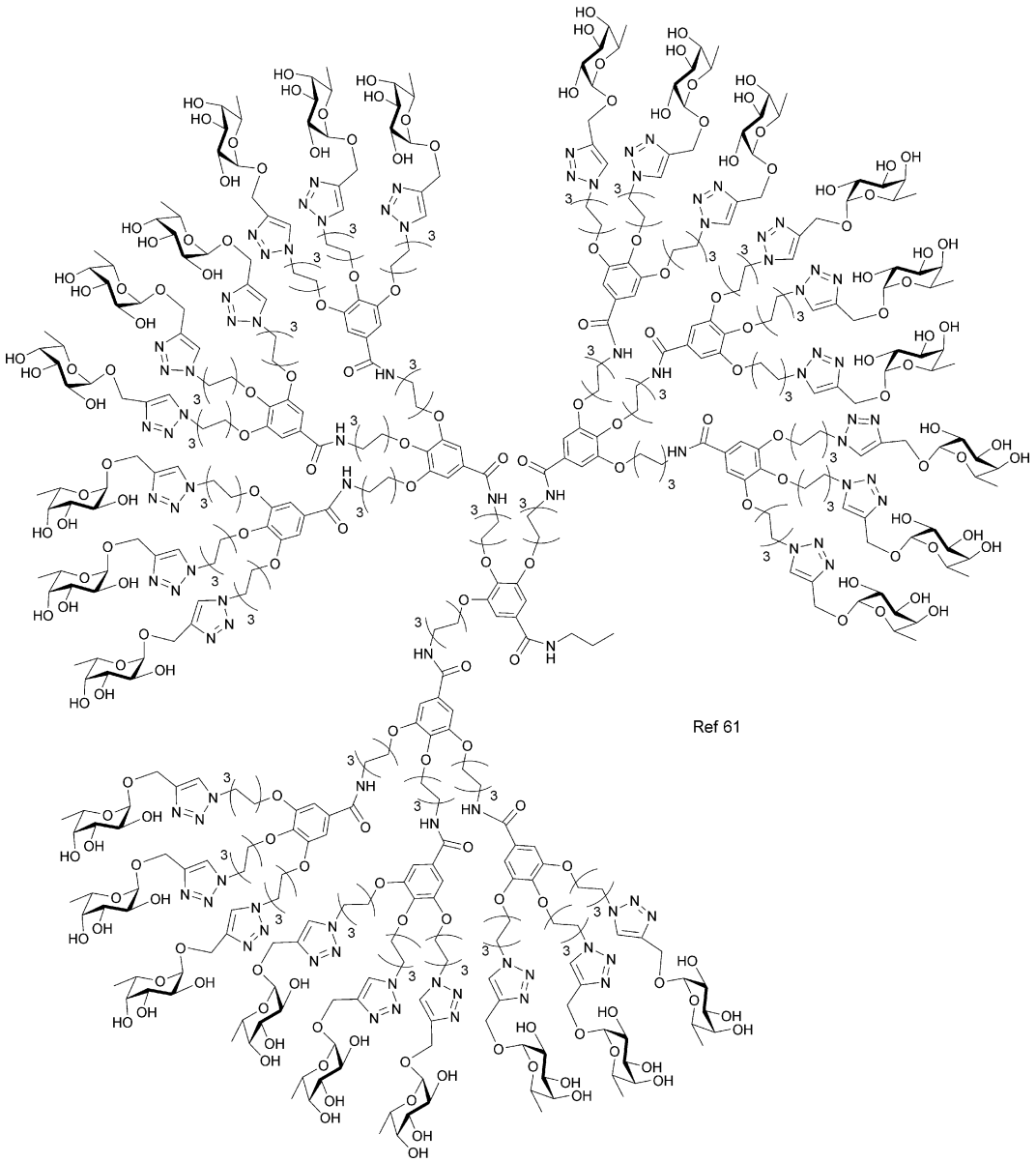

Fig. 11 Glycodendrimers from azides sugar and multivalent alkynes.

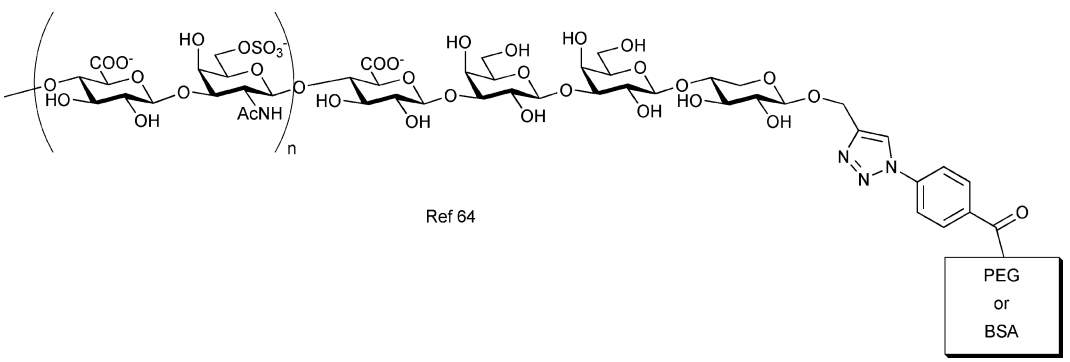

Fig. 12 Enzymatically derived oligosaccharides functionalised with BSA or PEG give access to triazole-linked neoproteoglycans.

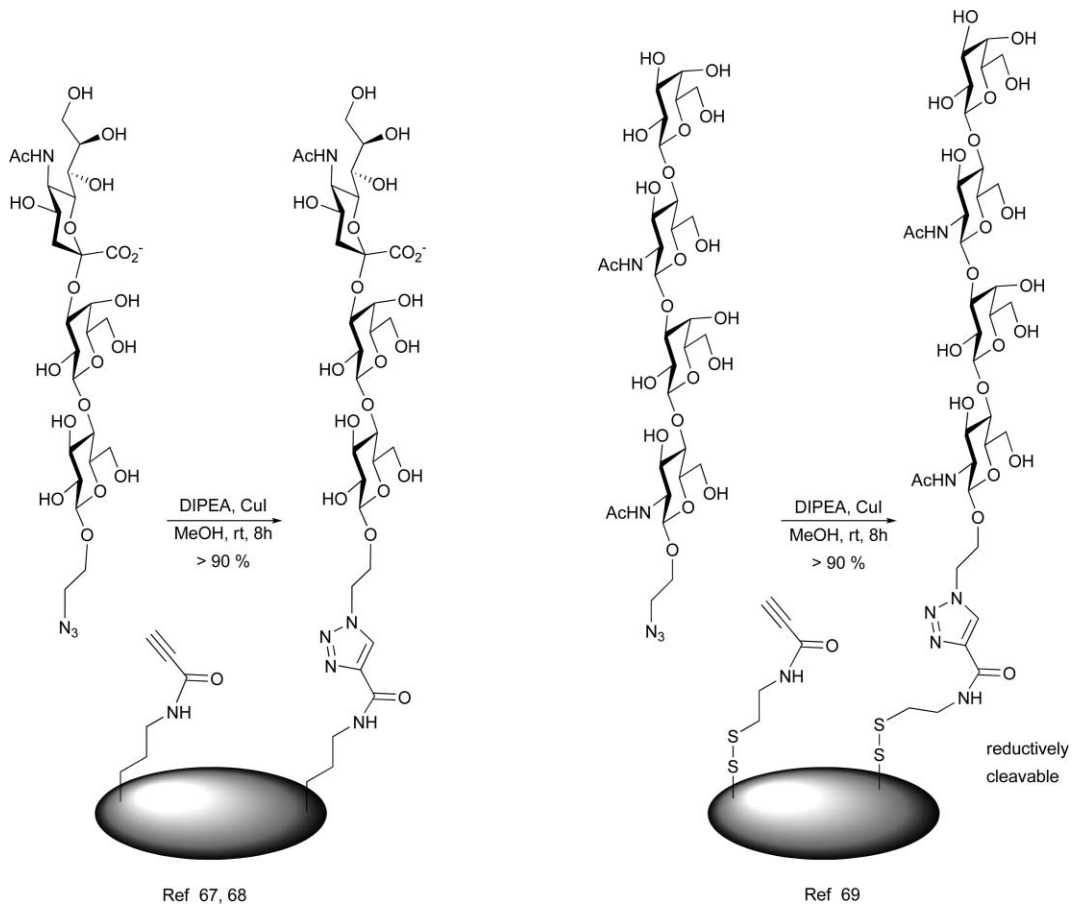

Fig. 13 New methods for fabrication of immobilised carbohydrates.

ligand immobilisation in plastic microtitre plates.[67,68] Disulfide exchange has also been used to generate reductively cleavable variants (Fig. 13).[69]

For more detailed spectroscopic and biophysical studies (*e.g.* infra-red spectroscopy, atomic force microscopy, surface plasmon resonance spectroscopy, quartz crystal microbalance), azide–alkyne dipolar cycloaddition has been explored for the attachment of sugars directly to pre-formed self-assembled monolayers (SAMs) on gold surfaces.[70–72] This approach has been investigated with the SAM displaying an alkyne for ligation with carbohydrate-derived azides, although the inverse approach with azido-functionalised SAMs has been used for pentynyl-nucleoside immobilisation.[73]

Summary

This Huisgen 1,3-dipolar cycloaddition reaction is particularly attractive in carbohydrate chemistry where, despite major advances in the past decade, the ever-present issue of glycosylation efficiency, stereo- and regio-control still hampers progress. Perhaps the ultimate in macro-scale click chemistry of this type concerns the rapid and specific covalent labelling of cellular glycans following biosynthetic incorporation of 5-azido-fucose into cell surface glycoproteins. Subsequent reaction with, for instance, alkynylated fluorophore provides the opportunity for *in vivo* imaging of fucosylated glycans (Fig. 14).[74] With improvements to methodology, including the introduction of polytriazolylamines ligands to stabilise Cu^{I}, reactions can be performed efficiently at room temperature.[75] Not only is this chemistry versatile, it is mild enough and selective enough to be compatible with intact cells.† Applications of the Cu^{I}-catalysed azide–alkyne dipolar cycloaddition chemistry in the carbohydrate field to date are many and varied: this situation is set to continue for some time to come.

† A comparative study of bioorthogonal reactions with azides (cycloaddition, Staudinger reaction) has recently been reported (ref. 76).

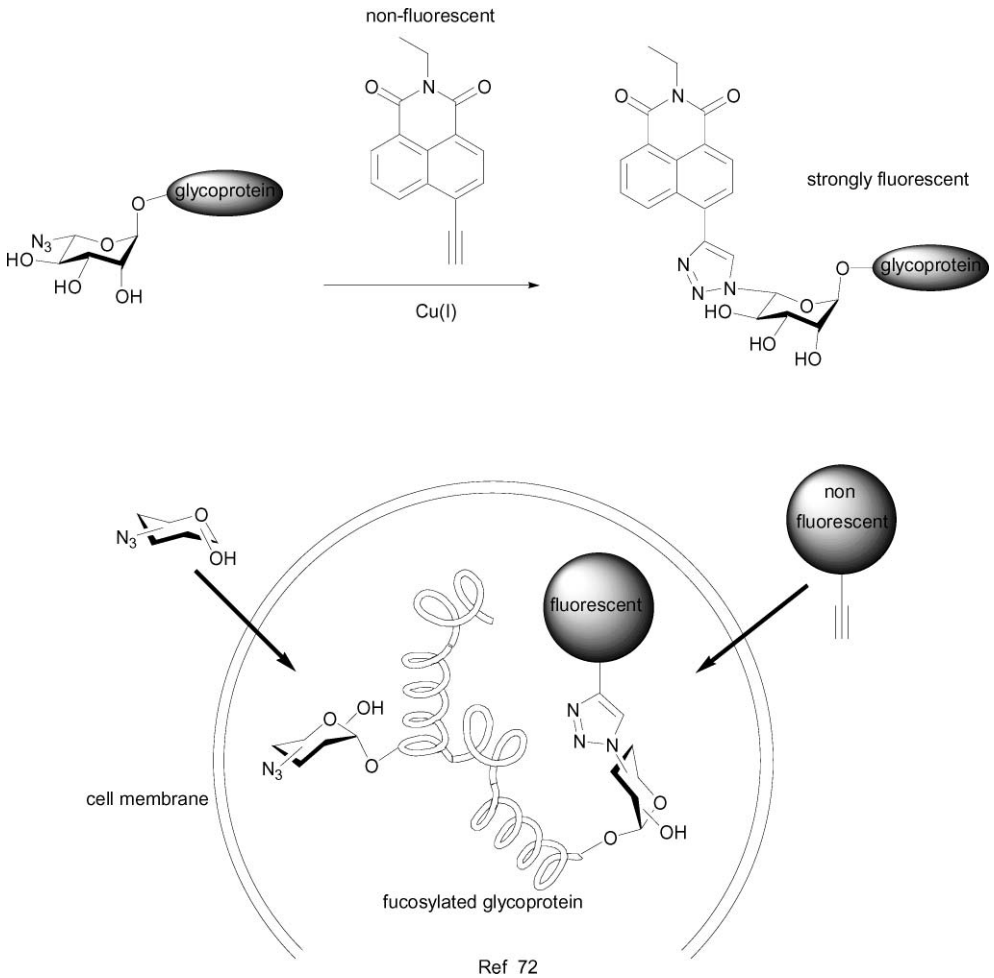

Fig. 14 Detection of fucosylated glycoconjugates at cell surfaces and inside the cell after biosynthetic incorporation of azidofucose into glycoproteins.

Acknowledgements

A short article such as this can only hope to be illustrative, not comprehensive. We apologise in advance to those authors whose work we have been unable to include. The Field group is supported by the BBSRC, EPSRC and the Weston Foundation.

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