NIH Public Access
Author Manuscript
Carbohydr Chem. Author manuscript; available in PMC 2011 July 22.

Published in final edited form as:
Carbohydr Chem. 2010 ; 36: 152–167. doi:10.1039/9781849730891-00152.

Bioorthogonal chemical reporter methodology for visualization, isolation and analysis of glycoconjugates

Geert-Jan Boons[a]

[a] Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Road, Athens Georgia 30606, USA

Abstract

The recent development of metabolic oligosaccharide engineering combined with bioorthogonal reactions is providing unique opportunities to detect, image, and isolate glycoconjugates of living cells, tissues, and model organisms. In this methodology, exogenously-supplied non-natural sugars are fed to cells and employed by the biosynthetic machinery for the biosynthesis of neoglycoconjugates. In this way, reactive functional groups such as ketones, azides, and thiols have been incorporated into sialic acid, galactosamine, glucosamine, and fucose moieties of glycoconjugates. A range of bioorthogonal reactions have been described that functionalize the chemical 'tags' for imaging, isolation, and drug delivery.

1. Introduction

There is a growing appreciation that posttranslational modifications, such as protein glycosylation, dramatically increase polypeptide complexity and function.1¯10 For example, almost all cell surface and secreted proteins are modified by covalently-linked carbohydrate moieties and the glycan structures on these glycoproteins have been implicated as essential mediators in processes such as protein folding, cell signaling, fertilization, embryogenesis, neuronal development, hormone activity, and the proliferation of cells and their organization into specific tissues.11 In addition, over-whelming data supports the relevance of glycosylation in pathogen recognition, inflammation, innate immune responses, and the development of autoimmune diseases and cancer.12¯16 The importance of protein glycosylation is also underscored by the developmental abnormalities observed in a growing number of human disorders known as Congenital Disorders of Glycosylation caused by defects in the glycosylation machinery.17

Almost all naturally occurring protein glycosylations can be classified as either *N*-glycosides whereby *N*-acetyl glucosamine is linked to the amide side chain of an asparagine, or as *O*-glycosides whereby a saccharide is linked to the hydroxyl of serine, threonine or tyrosine.18 The biosynthesis of *N*-linked oligosaccharides occurs in the endoplasmic reticulum (ER) and Golgi complex. In the ER, a dolichol-linked $Glc_3Man_9GlcNAc_2$ oligosaccharide precursor is biosynthesized and transferred *en bloc* to an Asn-X-Ser/Thr sequon on newly synthesized polypeptides through the action of the multi-subunit oligosaccharide transferase complex.19,20 Subsequent trimming and processing of the transferred oligosaccharide results in a Man_3-$GlcNAc_2$ core structure, which is transported to the medial stacks of the Golgi complex where maturation of the oligosaccharide gives rise to extreme structural diversity.21¯24 This complexity of *N*-glycan structures is largely based on the cell-specific expression of a collection of glycosyl transferases that specify the extension of oligosaccharide structures onto the trimmed $Man_3GlcNAc_2$ core structure. The switch from structural uniformity in the ER to diversification in the Golgi complex coincides with a marked change in glycan function. In the early secretory pathway, the glycans have a common role in the promotion of protein folding, quality control, and certain sorting events. This is in contrast

NIH-PA Author Manuscript
NIH-PA Author Manuscript
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

to their roles in the Golgi complex, in which they are modified to perform a wide spectrum of functions displayed by the mature glycoproteins.

The biosynthesis of most *O*-glycans commences by diversification of the common core 1 precursor βGal1-3GalNAcα-Ser/Thr, or T-antigen, which is formed in the Golgi by extension of the Tn-antigen by action of T-synthase. The folding of the T-synthase is dependent on the chaperon *Cosmc* that resides in the ER. Mutations in *Cosmc* result in a dysfunctional chaperone, which leads to abrogation of the *O*-glycan synthesis. This can be seen in an accumulation of the Tn- and STn-antigen, which is highly associated with diseases such as cancer.

Despite their importance, in most cases the precise roles that glycans play in biological systems is not well understood because of their underpinning complexity. The past decade has seen development of integrated approaches, which are broadly termed glycomics, aimed at unraveling structure–function relationships of complex carbohydrates. Key glycomics technologies include mass spectrometric profiling of glycan structures isolated from cells and tissues,[25-27] glyco-gene microarray technology for measuring the expression levels of glycoenzymes and glycan-binding proteins, and screening for glycan-protein interactions using glycan and lectin array technologies.[28-33] The diverse data sets generated by the use of these technologies are beginning to provide an understanding of the fundamental structure–function relationships of glycans. Critical components that enable this process are bioinformatics platforms that store, integrate, process, and disseminate the data in a meaningful way.[34-36]

The use of tandem MS for glycomics is driven by the need to obtain structural information of glycans present in serum/plasma or tissue samples to understand metabolic or disease processes and discover new biomarkers.[25-27] Strategies for acquisition and interpretation of multistage MS have been most fully developed for permethylated glycans. The advantage to this approach is that tandem mass spectrometric dissociation of a glycosidic bond leaves a site that lacks a methyl group that is clearly indicated by mass. It is possible to differentiate some types of positional isomers based on the formation of specific product ion types. However, the assignment of glycan structures is very challenging due to the isobaric nature of glycans (*i.e.* different structures with the identical molecular weights).

Glycoarray technology is a key tool for glycomics that has the distinct advantage that only minute amounts of precious oligosaccharides are required while allowing fast, quantitative, systematic identification and characterization of carbohydrate-protein interactions.[28-33] In addition, a glycoarray format presents glycans in a multivalent fashion, which is often an important requirement for high affinity binding. In particular, the glycan array developed by the consortium of functional glycomics (CFG) has found wide utility in profiling interactions with carbohydrate binding proteins, growth factors, pathogen- and cancer-induced antibodies, viruses, and bacteria.[34,35] The saccharides of this array are prepared by chemoenzymatic approaches and comprise the most common terminal saccharide motifs found on *N*- and *O*-linked saccharides.

An emerging technology that makes it possible to isolate and visualize glycans exploits promiscuity of the biosynthetic machinery, which makes it possible to incorporate monosaccharides that have a unique chemical functionality (the reporter) into glycoconjugates of living cells. The chemical reporter of these glycans can then be reacted with a probe linked to a complementary bio-orthogonal functional group. The mutually selective chemical reactivity of the two functional groups ensures that only the metabolically labeled glycans are targeted for reaction. Bertozzi and coworkers have coined this

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

technology “bio-orthogonal chemical reporter” strategy, which is the topic of this review.[37-41]

2. Ketone based bioorthogonal labeling

In the late eighties it was found that sialyltransferases were remarkably permissive for substrates bearing certain functional groups and for example, even bulky fluorophores appended to CMP-Neu5Ac could be biosynthetically incorporated into glycans.[42,43] These modified nucleotide sugars were, however, highly polar and hence not membrane permeable and therefore could not readily be employed to label oligosaccharides of living cells.

Reutter and coworkers demonstrated that synthetic *N*-acyl-modified D-mannosamines can be taken up by cells and efficiently metabolized to the respective *N*-acyl-modified neuraminic acids, which can then be incorporated into cell surface glycoconjugates.[44] For example, derivatives such as *N*-propanoyl, *N*-butanoyl, *N*-pentanoyl, *N*-hexanoyl, and *N*-crotonoyl can be metabolized by the promiscuous sialic acid biosynthetic pathway with an efficiency of 10–85%. The use of this technology has revealed the unexpected functions of the *N*-acyl side chain of sialic acids in biological processes such as host-pathogen interactions, cell adhesion and proliferation and immune responses.[45-48]

An important conceptual advance was the discovery that monosaccharide analogs having a unique chemical functionality can also be employed in metabolic glycoengineering. The attraction of the use of such derivatives is the ability to perform chemoselective ligations on surfaces of living cells.[49] A stringent requirement of these reactions is the need of two participating functional groups to react under physiological conditions without interference with coexisting functionalities.[50]

It has been shown that the ketone-containing ManNLev can be incorporated into the sialic acid pathway and then employed for bioorthogonal reactions.[51] Although aldehydes and ketones are intracellular biosynthetic precursors, they are absent from cell surfaces and hence in this environment can serve as unique chemical reporters. For example, keto sugars expressed on cell surfaces can be reacted with aminooxy and hydrazide probes to form oximes and hydrazones, respectively.[51,52] In this way, various tags such as FLAG peptides, biotin, and fluorescent or fluorogenic molecules have been linked to the surface of living cells. The attraction of these conjugation approaches is that the tagged glycans can be enriched for further analysis by for example mass spectrometry, detected by flow cytometry for quantitative studies or visualized by confocal microscopy to study localization and trafficking.

It has been found that hydrazones hydrolyze when internalized into vesicular compartments where the pH is relatively low. This chemical lability can be exploited for drug,[53] gene[54] or toxin delivery.[51] It can also be employed for loading cell with an MRI contrast agent.[55] On the other hand, oximes are much more acid resistant and can be recycled after internalization.[53]

The use of ketones as chemical reporter has a number of drawbacks such as the requirement to perform the ligation reaction under mild acidic conditions and a limited possibility to extend the technology to a wide range of sugar derivatives. Paulson and co-workers have pioneered an alternative approach for introducing aldehydes into cell-surface sialic acid residues by mild periodate oxidation followed by an aniline catalyzed reaction with aminooxy-biotin to give biotinylated glycoconjugates (Scheme 1). Importantly, it has been found that aniline accelerates oxime formation by approximately 10-fold, and furthermore can be performed under neutral conditions.[56,57] Reagents to implement the periodate oxidation aniline catalyzed oxime ligation (PAL) are commercially available. Furthermore,

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

the use of aniline makes it possible to reduce the amount of aminooxy reagent by 10- to 50-fold compared to what typically is employed in uncatalyzed reactions.

Selective oxidation of sialic acids has also been employed for enrichment of glycoproteins for analysis by mass spectrometry. In the most advanced approach, sialylated glycoproteins were selectively periodate-oxidized, captured on hydrazide beads, trypsinized and the released peptides analyzed by mass spectrometry for protein identification.58 Next, the remaining glycopeptides were released by acid hydrolysis of sialic acid glycosidic bonds and the released compounds analyzed by MS for glycan identification. It is to be expected that this approach will be more facile when aniline is employed to catalyze the oxime formation.

3. Azido-based strategies for bioorthogonal labeling

Azides, which are extremely rare in biological systems, are emerging as a particularly powerful chemical reporter.37¯39,41,59,60 The azido group is small and therefore only minimally perturbs a modified substrate. Furthermore, a number of attractive reaction partners for azides have been developed that are compatible with biological systems.

A number of modified monosaccharides have been employed to introduce azides into cell surface glycoconjugates (Fig. 1).38 In this respect, sialic acid-containing glycans have been labeled by feeding cells *N*-azidoacetylmannosamine (ManNAz),50 which is an analog of the biosynthetic precursor *N*-acetylmannosamine (ManNAc) or by employing *N*-azidoacetylneuraminic acid (SiaNAz)61 or 9-azido-*N*-acetyl acetylneuraminic acid.62 Mucin-type glycans have been labeled with azido functions using *N*-azidoacetylgalactosamine (GalNAz).63 This unnatural sugar is processed by the GalNAc salvage pathway to form the intermediate uridine diphospho (UDP)-GalNAz, which is utilized by a family of polypeptide GalNAc transferases in the Golgi compartment, resulting in the formation of mucins that have a GalNAz moiety. Several derivatives of fucose modified by a chemical reporter have been employed for metabolic labeling.64¯66 In this case, the salvage pathway converts the fucose analogs into the corresponding GDP-fucose, which is then employed by fucosyltransferases for the biosynthesis of glycoconjugates. It has been found that the salvage pathway and fucosyl transferases tolerate modifications at C-6 of fucose.

6-Azido-6-deoxy-fucose was the first sugar to be investigated as a chemical reporter, however, it was found that this derivative exhibited some cytotoxicity. Below, it will be discussed that 6-alkynylfucose (alkynyl fucose) is also accepted by the fucose salvage pathway and exhibits lower toxicity. Finally, *O*-GlcNAc-modified proteins, which occur in the cytosol and nucleus, have been labeled with bioorthogonal chemical reporters by using either per-*O*-acetylated *N*-azidoacetylglucosamine (Ac_4GlcNAz).67 This compound is modified by the GlcNAc salvage pathway enzymes to form UDP-GlcNAz, which is used as a substrate by the cytosolic *O*-GlcNAc transferase. A more attractive approach to study *O*-GlcNAc modification of proteins exploits an engineered galactosyltransferase enzyme to selectively label *O*-GlcNAc proteins with an azide-biotin tag.68,69 The tag permits enrichment of low-abundance *O*-GlcNAc species from complex mixtures and localization of the modification to short amino acid sequences. Using this approach, changes in *O*-GlcNAc glycosylation on several proteins involved in the regulation of transcription and mRNA translocation, were detected. Also, it provided evidence that *O*-GlcNAc glycosylation is dynamically modulated by excitatory stimulation of the brain *in-vivo*.

The first example of the use of an azide as a chemical reporter was disclosed by the Bertozzi group and exploited a modified Staudinger ligation.50 The genius of this approach was a strategic placement of an ester on one of the phosphane aryl substituents (Scheme 2), which

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

ensured that the intermediate aza-ylide undergoes intramolecular amide formation rather than hydrolysis. It has been shown that the novel phosphane reagent could label Jurkat cells incubated with the *O*-acetylated *N*-azidoacetylmannosamine (Ac_4ManNAz; 20 μM) for 3 days. Thus, reaction of the cells with the phosphane reagent at a concentration of 1 mM followed by staining with fluorescein isothiocyanate (FITC)-avidin, showed a marked increase in fluorescence compared to control cells that were not metabolically labeled. The fluorescence signal was reduced by the addition of tunicamycin during incubation of Jurkat cells with the azido sugar, which is in agreement with previous observations that sialic acids on Jurkat cells reside mainly within *N*-linked glycans.

The Staudinger ligation technology has been extended to more elaborate phosphanes, which can be employed for imaging biomolecules in real-time (Scheme 3). The fluorescent probe of the reagent is quenched by the presence of the diazenyl moiety. However, upon reaction of the phosphane with an azide, the ester linkage is hydrolyzed resulting in removal of the diazenine and fluorescent activation. This approach gave much lower background labeling compared to an earlier strategy in which quenching was achieved by the lone pair of the phosphane reagent.70

The Cu(I)-catalyzed 1,3-dipolar cycloaddition of azides with terminal alkynes to give stable triazoles71,72 is emerging as one of the most powerful technologies for tagging a variety of biomolecules,73–77 activity-based protein profiling,78 and the chemical synthesis of microarrays and small-molecule libraries.79 Not surprisingly, this chemical technology has also been employed for labeling and isolation of glycoconjugates. The cellular toxicity of the Cu(I) catalyst has, however, precluded applications wherein cells must remain viable.80

Alkynes can be activated by ring strain and for example, alkynes constrained within an eight-membered ring undergo [3+2] cyclcoadditions with azides at ambient temperature without the need for a metal catalyst (Fig 2).81,82 The increased reactivity of cyclooctynes has been attributed to ground-state destabilizing effect of the triple bond, however, this model has been challenged with the introduction of the concept of dipole distortion.83–85 The strain-promoted cycloaddition has been used to label biomolecules without observable cytotoxicitiy.82 The scope of the approach has, however, been limited because of the slow rate of reaction.86

It has been found that the rate of strain-promoted cycloadditions can be increased by appending electron-withdrawing groups to the octyne ring. For example, it was shown that the reaction of difluorinated cyclooctyne (DIFO) with azides proceeds 60-times faster compared to an unsubstituted cyclooctyne.87 The rate enhancement by the *gem*-difluoro substitution has been attributed to the greater LUMO-lowering effect of the fluoro substituent.87 DIFO linked to Alexa Fluor has been successfully employed to investigate the dynamics of glycan trafficking and it was found that after incubation for 1 h, labeled glycans colocalize with markers for endosomes and Golgi.

Second-generation DIFO reagents (Fig. 2), which retain the difluorinated cyclooctyne core but possess a C–C bond to a linker substituent rather than a C–O linkage, could be prepared by a simplified synthetic scheme without impacting the reaction kinetics or bioorthogonality of the cycloaddition.88 Furthermore, an azacyclooctyne, which could be prepared in nine chemical steps from a glucose derivative, reacts with azido-labeled proteins and cells similarly to cyclooctynes. However, its superior polarity and water solubility reduced nonspecific binding, thereby improving the sensitivity of azide detection.

DIFO-fluorophore conjugates have been used for imaging azido-labeled biomolecules within complex biological systems such as living cells87 and zebrafish embryos,89 with low background fluorescence. Background labeling has, however, been observed when samples

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

were analyzed by Western blotting, possibly due nonspecific hydrophobic interactions or as yet uncharacterized reactions with protein functions.38

Recently, we have demonstrated that derivatives of 4-dibenzocyclooctynol (DIBO, Fig. 2) react exceptionally fast in the absence of a metal catalyst with azido-containing saccharides and amino acids and can be employed for visualizing glycoconjugates of living cells that are metabolically labeled with azido-containing monosaccharides.90 The attraction of DIBO is that its synthesis is extremely simple, it is nontoxic, and its hydroxyl can be employed to attach a variety of probes.

We have further extended the DIBO-based bioorthogonal reporter strategy by developing a photochemically triggered click reaction (Scheme 4). Such an approach provides opportunities for spatial and temporal control of labeling of the target substrates. In fact, photochemical release or generation of an active molecule is a widely employed strategy to deliver bioactive compounds to addressable target sites in a time-controlled manner.91–94 To achieve this goal, we have explored photochemical generation of reactive dibenzocyclooctynes. In this respect, it is known that single95–100 or two-photon101 excitation of cyclopropenones results in the formation of corresponding acetylenes. Photochemical decarbonylation of thermally stable diaryl-substituted cyclopropenones is especially efficient (Φ=0.2–1.0) and produces alkynes in a quantitative yield.100 This reaction is also extremely fast and is complete within a few hundred picoseconds after excitation.102,103 Thus, we anticipated that the cyclopropenone derivative would not react with azides under ambient conditions in the dark but efficiently produce reactive dibenzocyclooctynes upon irradiation. Indeed, the latter type of compound could be employed for labeling of living cells modified with azido-containing cell surface saccharides.

4. Metabolic labeling with alkyne-containing sugars

The alkyne group provides a bioorthogonal reporter that can be chemoselectively labeled with azido-containing probes using Cu(I) catalyzed [3+2] cycloadditions.64–66 Alkynyl monosaccharides derived from fucose and ManNAc have been incorporated into fucosylatated and sialylated glycans and in particular, the use of the 6-alkynyl fucose was attractive since it has a greatly reduced toxicity to cells compared with its azido counterpart. Furthermore, it has been shown that 4-pentynoyl mannosamine (Ac_4ManNAl) is metabolically incorporated in cultured cells and mice with greater efficiency than Ac_4 ManNAz.104 This result underscores the sensitivity of sialic acid biosynthetic enzymes to subtle differences in the *N*-acyl structures of ManNAc analogs.

Alkynyl labeled glycoconjugates have been coupled with azido containing probes such as biotin, click-activated fluorogenic coumarins (Scheme 5), and other fluorescent probes allowing the selective isolation or fluorescent imaging.66 In particular, the use of click-activated fluorogenic coumarins is attractive as it addresses the problem of background labeling. In this approach, the click product generates a strong detectable signal, whereas the starting materials remain traceless. The fluorogenic probe design was based on 4-amino-1,8-naphthalimide, which absorbs light in the visible region and emits at long wavelengths (λ_{max} 540–550 nm).66 It was postulated that the fluorescent signal of such a compound could be modulated by the formation of a triazole ring because an electron-donating group at the C-4 position was known to strongly affect fluorescent properties. Indeed, an 1,8-naphthalimide derivative, which has an azido moiety at the C-4 position, has a very low fluorescence intensity. However, the emission maximum of the click product is at 422 nm with a quantum yield of 0.29 when excited at 357 nm.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

Metabolic labeling with alkynyl-containing monosaccharides has also been employed for glycoprotein identification and glycan mapping.105 In this approach, glycoconjugates of cells are metabolically labeled with an alkynyl sugar probe, which can be selectively reacted with a biotin azide reagent using a Cu(I)-catalyzed [3+2] azide-alkyne cycloaddition. Introduction of a biotin moiety permits enrichment of glycoproteins by affinity capture with immobilized streptavidin. Tandem protein ID and glycan site mapping were carried out on-bead. Thus, first non-glycosylated peptides were harvested by tryptic digestion, allowing protein identification. The remaining resin bound glycopeptides were analyzed by treatment with *N*-glycosidase F (PNGase), which hydrolyzes the amide bond between the biotinylated glycan and Asn residue of the bound peptide. The shift from Asn to Asp at formerly glycosylated sites can be identified by a differential modification of + 1 Da on Asn in SEQUEST searches of MS data. The approach, which was applied to analyze the sialylated *N*-linked glycoproteins of prostate cancer cells, offers a method to identify proteins and map glycosylation sites of glycoproteins that carry a specific monosaccharide such as sialic acid. However, it does not enable the elucidation of the nature of the glycan at a particular glycosylation site.

5. Metabolic labeling with photoactivatable crosslinking sugars

Photoactivatable crosslinking reagents such as aryl azides and diazirine have been employed to covalently linking protein complexes for further analysis. Inspired by these chemical reactions, metabolic labeling strategies have been developed that employ monosaccharides modified by photoactivatable crosslinking moiety. The use of such a reagent makes it possible to analyze glycoconjugate-protein complexes.

Paulson and coworkers reported an attractive example of the use of a photo-activated aryl azido group to uncover *cis*-glycoprotein interactions on the surface of B-cells.106 In this study, cells were metabolically labeled with sialic acid modified at C-9 with a photo-activated aryl azide group.

Inspired by the successful use of the photo-activated aryl azide group, the Kohler group developed monosaccharides containing diazirine crosslinkers (Fig. 3) for metabolic incorporation onto cell surfaces, and demonstrated the utility of this approach by detecting multimerization of a cell surface glycoprotein.107 The diazirine crosslinker was selected because its small size was expected to minimize interference with carbohydrate-protein interactions. It was shown that peracetylated 5-SiaDAz and ManNDAz could be metabolically incorporated into cellular glycoproteins. The latter was demonstrated by employing a cell line (KD20) that is impaired in sialic acid biosynthesis by a lack of UDP-GlcNAc 2-epimerase that converts UDP-GlcNAc to ManNAc. Incubation of the cells with the sugar derivatives resulted in cell surface expression of sialic acid as demonstrated by flow cytometry using FITC-labeled *Sambucus nigra* agglutinin (FITC-SNA). As expected, untreated cells display low FITC-SNA binding. The photo-reactive sugars could be used to capture a carbohydrate-mediated interaction by sialic acid-dependent multimerization of CD22 when irradiated with 350 nm light for 20 min and their contents analyzed by Western blot using an anti-CD22 antibody.

6. Metabolic labeling with thiol containing sugars

Thiol groups have been installed into the glycocalyx by metabolic labeling with thioglycolyl analogs of ManNAc (Ac_5ManNTGc).108 Thiols are common within cells but are not found in the glycocalyx. Surprisingly, by locating this versatile functional group on the outer periphery of normally nonadhesive human Jurkat cells, we obtained spontaneous cell-cell clustering and attachment to complementary maleimide-derivatized substrates. When analyzed in human embryoid body–derived (hEBD) stem cells, Ac_5ManNTGc induced

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

catenin expression and altered cell morphology, consistent with neuronal differentiation. Notably, these effects were modulated by the growth substrate of the cells, with a stronger response observed on a gold surface than on glass. These results establish a novel approach for tissue engineering and offers a means to influence stem cell fates.

7. Concluding remarks

Bioorthogonal reactions are beginning to open new avenues for the investigation of biological processes in which carbohydrates are involved. Attention needs to be focused on possible perturbations in oligosaccharide structures arising from metabolic labeling with monosaccharides. In this respect, labeling experiments generally use relatively high concentration of monosaccharide derivative, which may perturb the metabolic flux, which in turn may influence glycoconjugate biosynthesis. Furthermore, monosaccharides are generally fed as acylated derivatives and it has been suggested that the acyl moieties may influence biological processes.41 Despite attractive features of currently reported probes and reagents, there is a need for new bioorthogonal functional groups that exhibit faster kinetics and minimal background reactions. Computational chemistry has been employed to rationalize reaction mechanism of bioorthogonal reactions and this insight is being exploited for the design of new reagents.109

Acknowledgments

This work is supported by the National Cancer Institute of the National Institutes of Health, Grant No R01CA088986.

References

1. Rudd PM, Elliott T, Cresswell P, Wilson IA, Dwek RA. Science. 2001; 291:2370. [PubMed: 11269318]
2. Varki A. Glycobiology. 1993; 3:97. [PubMed: 8490246]
3. Dwek RA. Chem. Rev. 1996; 96:683. [PubMed: 11848770]
4. Roth J. Chem. Rev. 2002; 102:285. [PubMed: 11841244]
5. Ritchie GE, Moffatt BE, Sim RB, Morgan BP, Dwek RA, Rudd PM. Chem. Rev. 2002; 102:305. [PubMed: 11841245]
6. Zachara NE, Hart GW. Chem. Rev. 2002; 102:431. [PubMed: 11841249]
7. Bertozzi CR, Kiessling LL. Science. 2001; 291:2357. [PubMed: 11269316]
8. Kleene R, Schachner M. Nat. Rev. Neurosci. 2004; 5:195. [PubMed: 14976519]
9. Bucior I, Burger MM. Curr. Op. Struct. Biol. 2004; 14:631.
10. Schmidt MA, Riley LW, Benz I. Trends Microbiol. 2003; 11:554. [PubMed: 14659687]
11. Taylor ME, Drickamer K. Curr. Opin. Cell. Biol. 2007; 19:572. [PubMed: 17942297]
12. Ohtsubo K, Marth JD. Cell. 2006; 126:855. [PubMed: 16959566]
13. Brockhausen I. EMBO Rep. 2006; 7:599. [PubMed: 16741504]
14. Brown JR, Crawford BE, Esko JD. Crit. Rev. Biochem. Mol. Biol. 2007; 42:481. [PubMed: 18066955]
15. Crocker PR, Paulson JC, Varki A. Nat. Rev. Immunol. 2007; 7:255. [PubMed: 17380156]
16. van Kooyk Y, Rabinovich GA. Nat. Immunol. 2008; 9:593. [PubMed: 18490910]
17. Freeze HH. Curr. Mol. Med. 2007; 7:389. [PubMed: 17584079]
18. Buskas T, Ingale S, Boons GJ. Glycobiology. 2006; 16:113R.
19. Dempski RE, Imperiali B. Curr. Opin. Chem. Biol. 2002; 6:844. [PubMed: 12470740]
20. Weerapana E, Imperiali B. Glycobiology. 2006; 16:91R.
21. Ellgaard L, Helenius A. Nat. Rev. Mol. Cell Biol. 2003; 4:181. [PubMed: 12612637]
22. Helenius A, Aebi M. Annu. Rev. Biochem. 2004; 73:1019. [PubMed: 15189166]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

23. Helenius A, Aebi M. Science. 2001; 291:2364. [PubMed: 11269317]
24. Mast SW, Moremen KW. Methods Enzymol. 2006; 415:31. [PubMed: 17116466]
25. Haslam SM, North SJ, Dell A. Curr. Opin. Struct. Biol. 2006; 16:584. [PubMed: 16938453]
26. Nana K, Satsuki I, Noritaka H, Akira H, Daisuke T, Teruhide Y. Trends Glycosci. Glycotech. 2008; 20:97.
27. Zaia J. Mass Spectrom. Rev. 2004; 23:161. [PubMed: 14966796]
28. Paulson JC, Blixt O, Collins BE. Nat. Chem. Biol. 2006; 2:238. [PubMed: 16619023]
29. Miyamoto S. Curr. Opin. Mol. Ther. 2006; 8:507. [PubMed: 17243486]
30. Feizi T, Fazio F, Chai WC, Wong CH. Curr. Opin. Struct. Biol. 2003; 13:637. [PubMed: 14568620]
31. de Paz JL, Horlacher T, Seeberger PH. Methods Enzymol. 2006; 415:269. [PubMed: 17116480]
32. Alvarez RA, Blixt O. Methods Enzymol. 2006; 415:292. [PubMed: 17116481]
33. Hirabayashi J. J. Biochem. 2008; 144:139. [PubMed: 18390573]
34. Raman R, Raguram S, Venkataraman G, Paulson JC, Sasisekharan R. Nat. Methods. 2005; 2:817. [PubMed: 16278650]
35. Raman R, Venkataraman M, Ramakrishnan S, Lang W, Raguram S, Sasisekharan R. Glycobiology. 2006; 16:82R.
36. Aoki-Kinoshita KF. Exp. Opin. Drug Discov. 2008; 3:877.
37. Du J, Meledeo MA, Wang ZY, Khanna HS, Paruchuri VDP, Yarema KJ. Glycobiology. 2009; 19:1382. [PubMed: 19675091]
38. Sletten EM, Bertozzi CR. Angew. Chem. Int. Ed. 2009; 48:6974.
39. Laughlin ST, Bertozzi CR. Proc. Natl. Acad. Sci. U. S. A. 2009; 106:12. [PubMed: 19104067]
40. Prescher JA, Bertozzi CR. Cell. 2006; 126:851. [PubMed: 16959565]
41. Elmouelhi N, Aich U, Paruchuri VDP, Meledeo MA, Campbell CT, Wang JJ, Srinivas R, Khanna HS, Yarema KJ. J. Med. Chem. 2009; 52:2515. [PubMed: 19326913]
42. Gross HJ, Rose U, Krause JM, Paulson JC, Schmid K, Feeney RE, Brossmer R. Biochemistry. 1989; 28:7386. [PubMed: 2510824]
43. Gross HJ, Brossmer R. Glycoconj. J. 1995; 12:739. [PubMed: 8748149]
44. Keppler OT, Horstkorte R, Pawlita M, Schmidts C, Reutter W. Glycobiology. 2001; 11:11R. [PubMed: 11181557]
45. Liu TM, Guo ZW, Yang QL, Sad S, Jennings HJ. J. Biol. Chem. 2000; 275:32832. [PubMed: 10976100]
46. Hayrinen J, Jennings H, Raff HV, Rougon G, Hanai N, Gerardyschahn R, Finne J. J. Infect. Dis. 1995; 171:1481. [PubMed: 7769282]
47. Wu J, Guo Z. Bioconjug. Chem. 2006; 17:1537. [PubMed: 17105234]
48. Wang QL, Ekanayaka SA, Wu J, Zhang JP, Guo ZW. Bioconj. Chem. 2008; 19:2060.
49. Lemieux GA, Bertozzi CR. Trends Biotechnol. 1998; 16:506. [PubMed: 9881482]
50. Saxon E, Bertozzi CR. Science. 2000; 287:2007. [PubMed: 10720325]
51. Mahal LK, Yarema KJ, Bertozzi CR. Science. 1997; 276:1125. [PubMed: 9173543]
52. Hang HC, Bertozzi CR. J. Am. Chem. Soc. 2001; 123:1242. [PubMed: 11456684]
53. Nauman DA, Bertozzi CR. Biochim. Biophys. Acta. 2001; 1568:147. [PubMed: 11750762]
54. Lee JH, Baker TJ, Mahal LK, Zabner J, Bertozzi CR, Wiemer DF, Welsh MJ. J. Biol. Chem. 1999; 274:21878. [PubMed: 10419507]
55. Lemieux GA, Yarema KJ, Jacobs CL, Bertozzi CR. J. Am. Chem. Soc. 1999; 121:4278.
56. Zeng Y, Ramya TNC, Dirksen A, Dawson PE, Paulson JC. Nat. Methods. 2009; 6:207. [PubMed: 19234450]
57. Kohler JJ. ChemBioChem. 2009; 10:2147. [PubMed: 19637152]
58. Nilsson J, Ruetschi U, Halim A, Hesse C, Carlsohn E, Brinkmalm G, Larson G. Nat. Methods. 2009; 6:809. [PubMed: 19838169]
59. Akira S. J. Biol. Chem. 2003; 278:38105. [PubMed: 12893815]
60. Hanson SR, Greenberg WA, Wong CH. QSAR Comb. Sci. 2007; 26:1243.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

61. Luchansky SJ, Goon S, Bertozzi CR. ChemBioChem. 2004; 5:371. [PubMed: 14997530]
62. Kosa RE, Gross HJ. Biochem. Biophys. Res. Commun. 1993; 190:914. [PubMed: 8439340]
63. Hang HC, Yu C, Kato DL, Bertozzi CR. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:14846. [PubMed: 14657396]
64. Hsu TL, Hanson SR, Kishikawa K, Wang SK, Sawa M, Wong CH. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:2614. [PubMed: 17296930]
65. Rabuka D, Hubbard SC, Laughlin ST, Argade SP, Bertozzi CR. J. Am. Chem. Soc. 2006; 128:12078. [PubMed: 16967952]
66. Sawa M, Hsu TL, Itoh T, Sugiyama M, Hanson SR, Vogt PK, Wong CH. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:12371. [PubMed: 16895981]
67. Vocadlo DJ, Hang HC, Kim EJ, Hanover JA, Bertozzi CR. Proc. Natl. Acad. Sci. U. S. A. 2003; 100:9116. [PubMed: 12874386]
68. Clark PM, Dweck JF, Mason DE, Hart CR, Buck SB, Peters EC, Agnew BJ, Hsieh-Wilson LC. J. Am. Chem. Soc. 2008; 130:11576. [PubMed: 18683930]
69. Khidekel N, Ficarro SB, Clark PM, Bryan MC, Swaney DL, Rexach JE, Sun YE, Coon JJ, Peters EC, Hsieh-Wilson LC. Nat. Chem. Biol. 2007; 3:339. [PubMed: 17496889]
70. Hangauer MJ, Bertozzi CR. Angew. Chem. Int. Ed. 2008; 47:2394.
71. Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. Angew. Chem. Int. Ed. 2002; 41:2596.
72. Tornoe CW, Christensen C, Meldal M. J. Org. Chem. 2002; 67:3057. [PubMed: 11975567]
73. Chin JW, Cropp TA, Anderson JC, Mukherji M, Zhang ZW, Schultz PG. Science. 2003; 301:964. [PubMed: 12920298]
74. Wang Q, Chan TR, Hilgraf R, Fokin VV, Sharpless KB, Finn MG. J. Am. Chem. Soc. 2003; 125:3192. [PubMed: 12630856]
75. Kho Y, Kim SC, Jiang C, Barma D, Kwon SW, Cheng JK, Jaunbergs J, Weinbaum C, Tamanoi F, Falck J, Zhao YM. Proc. Natl. Acad. Sci. U. S. A. 2004; 101:12479. [PubMed: 15308774]
76. Gierlich J, Burley GA, Gramlich PME, Hammond DM, Carell T. Org. Lett. 2006; 8:3639. [PubMed: 16898780]
77. Link AJ, Vink MKS, Agard NJ, Prescher JA, Bertozzi CR, Tirrell DA. Proc. Natl. Acad. Sci. U. S. A. 2006; 103:10180. [PubMed: 16801548]
78. Speers AE, Adam GC, Cravatt BF. J. Am. Chem. Soc. 2003; 125:4686. [PubMed: 12696868]
79. Sun XL, Stabler CL, Cazalis CS, Chaikof EL. Bioconjugate Chem. 2006; 17:52.
80. Link AJ, Tirrell DA. J. Am. Chem. Soc. 2003; 125:11164. [PubMed: 16220915]
81. Turner RB, Goebel P, Mallon BJ, Jarrett AD. J. Am. Chem. Soc. 1972; 95:790.
82. Agard NJ, Prescher JA, Bertozzi CR. J. Am. Chem. Soc. 2004; 126:15046. [PubMed: 15547999]
83. Bach RD. J. Am. Chem. Soc. 2009; 131:5233. [PubMed: 19301865]
84. Schoenebeck F, Ess DH, Jones GO, Houk KN. J. Am. Chem. Soc. 2009; 131:8121. [PubMed: 19459632]
85. Ess DH, Jones GO, Houk KN. Org. Lett. 2008; 10:1633. [PubMed: 18363405]
86. Agard NJ, Baskin JM, Prescher JA, Lo A, Bertozzi CR. ACS Chem. Biol. 2006; 1:644. [PubMed: 17175580]
87. Baskin JM, Prescher JA, Laughlin ST, Agard NJ, Chang PV, Miller IA, Lo A, Codelli JA, Bertozzi CR. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:16793. [PubMed: 17942682]
88. Codelli JA, Baskin JM, Agard NJ, Bertozzi CR. J. Am. Chem. Soc. 2008; 130:11486. [PubMed: 18680289]
89. Laughlin ST, Baskin JM, Amacher SL, Bertozzi CR. Science. 2008; 320:664. [PubMed: 18451302]
90. Ning XH, Guo J, Wolfert MA, Boons GJ. Angew. Chem. Int. Ed. 2008; 47:2253.
91. Pelliccioli AP, Wirz J. Photochem. Photobiol. Sci. 2002; 1:441. [PubMed: 12659154]
92. Mayer G, Heckel A. Angew. Chem. Int. Ed. 2006; 45:4900.
93. Ellis-Davies GCR. Nat. Methods. 2007; 4:619. [PubMed: 17664946]
94. Song W, Wang Y, Qu J, Lin Q. J. Am. Chem. Soc. 2008; 130:9654. [PubMed: 18593155]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

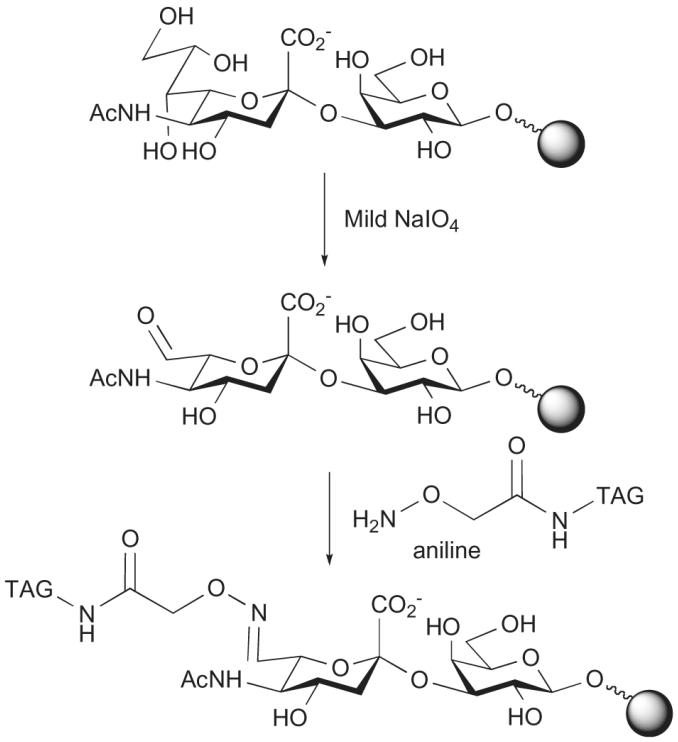
NIH-PA Author Manuscript

95. Chapman OL, Gano J, West PR, Regitz M, Maas G. J. Am. Chem. Soc. 1981; 103:7033.
96. Dehmlow EV, Neuhaus R, Schell HG. Chem. Ber. 1988; 121:569.
97. Murata S, Yamamoto T, Tomioka H. J. Am. Chem. Soc. 1993; 115:4013.
98. Chiang Y, Kresge AJ, Paine SW, Popik VV. J. Phys. Org. Chem. 1996; 9:361.
99. Kuzmanich G, Natarajan A, Chin KK, Veerman M, Mortko CJ, Garcia-Garibay MA. J. Am. Chem. Soc. 2008; 130:1140. [PubMed: 18183985]
100. Vladimir AP, Popik VV. J. Org. Chem. 2003; 68:7833. [PubMed: 14510563]
101. Urdabayev NK, Poloukhtine A, Popik VV. Chem. Commun. 2006; 454
102. Poloukhtine A, Popik VV. J. Phys. Chem. A. 2006; 110:1749. [PubMed: 16451004]
103. Takeuchi S, Tahara T. J. Chem. Phys. 2004; 120:4768. [PubMed: 15267337]
104. Chang PV, Chen X, Smyrniotis C, Xenakis A, Hu TS, Bertozzi CR, Wu P. Angew. Chem. Int. Ed. 2009; 48:4030.
105. Hanson SR, Hsu TL, Weerapana E, Kishikawa K, Simon GM, Cravatt BF, Wong CH. J. Am. Chem. Soc. 2007; 129:7266. [PubMed: 17506567]
106. Han S, Collins BE, Bengtson P, Paulson JC. Nat. Chem. Biol. 2005; 1:93. [PubMed: 16408005]
107. Tanaka Y, Kohler JJ. J. Am. Chem. Soc. 2008; 130:3278. [PubMed: 18293988]
108. Sampathkumar SG, Li AV, Jones MB, Sun ZH, Yarema KJ. Nat. Chem. Biol. 2006; 2:149. [PubMed: 16474386]
109. Chenoweth K, Chenoweth D, Goddard WA. Org. Biomol. Chem. 2009; 7:5255. [PubMed: 20024122]

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

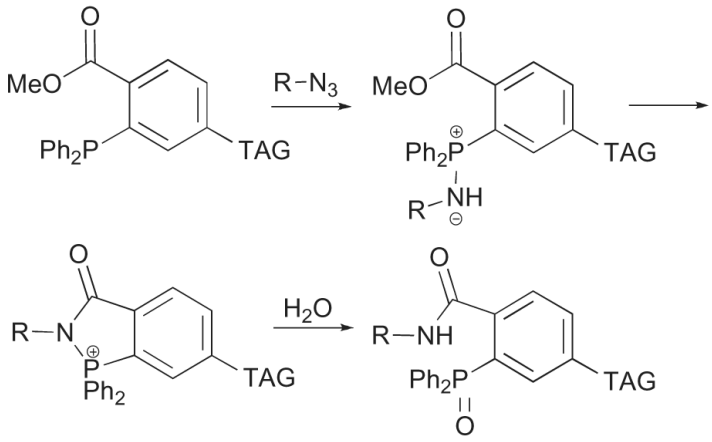


Scheme 1.
Covalent labeling of sialylated glycoconjugates by mild periodate cleavage followed by aniline-mediated oxime ligation. The tag can be biotin or a fluorescent probe.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

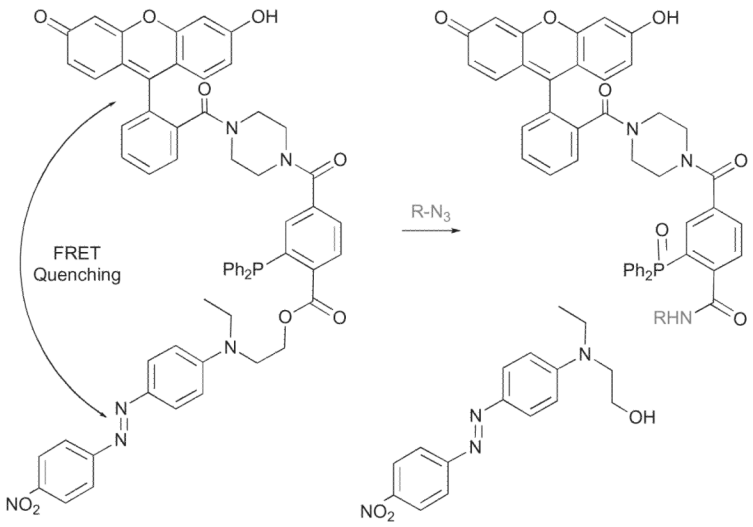


Scheme 2.
The Staudinger ligation of azides with triarylphosphanes.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

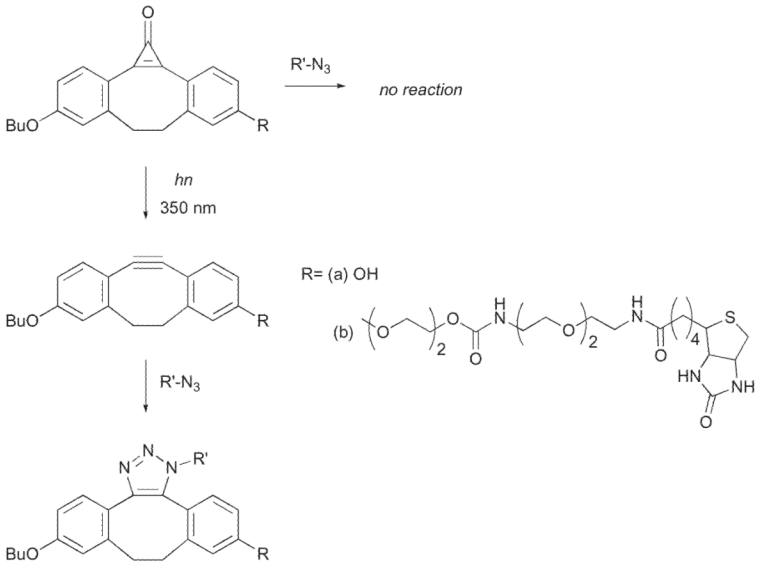


Scheme 3.
The Staudinger ligation of azides with triarylphosphanes using FRET quenching to minimize background labeling.

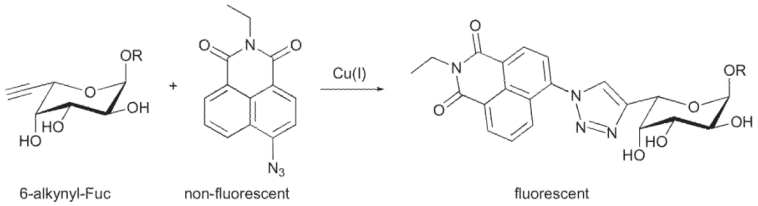
NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript



Scheme 4.
Photoactived metal free click reaction.



Scheme 5.

Fluorescence of 4-azido-1,8-naphthalimide is quenched by the azido moiety. Upon formation of a triazole, the derivative becomes fluorescent.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

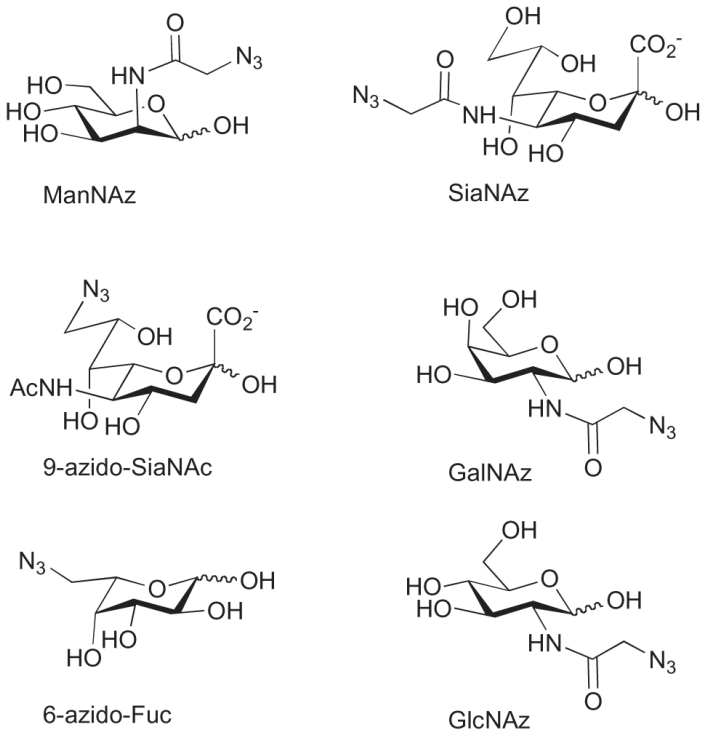


Fig. 1.
Azide bearing monosaccharides that as their acetylated derivatives have been employed for metabolic labeling of glycans.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

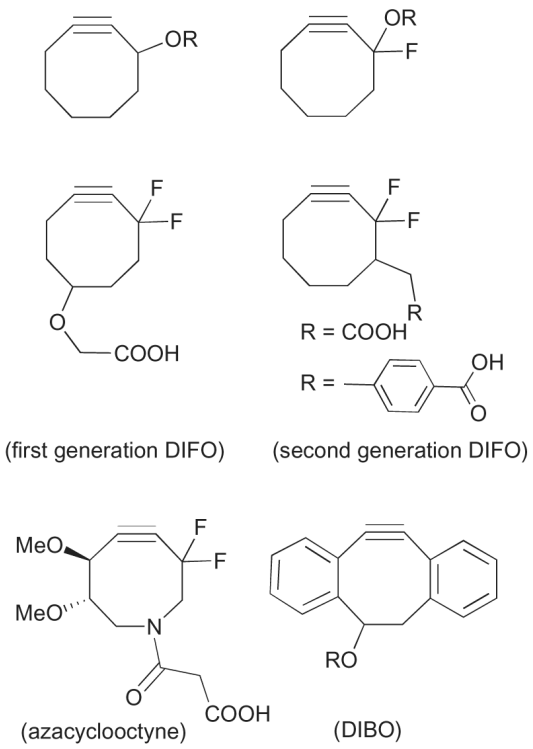


Fig. 2.
Cyclooctyne derivatives for metal free click reactions with azides.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

NIH-PA Author Manuscript

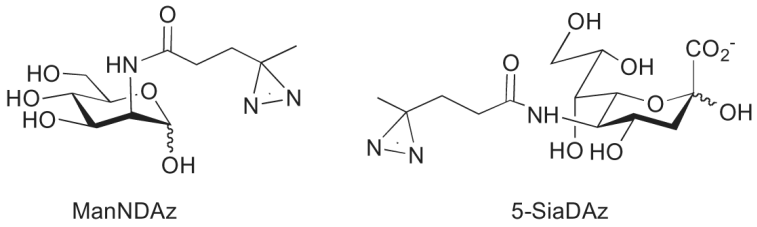


Fig. 3.
Photoactivatable crosslinking sugars.