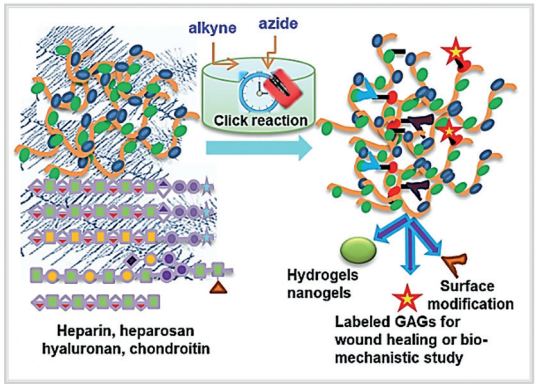
Click-Chemistry-Assisted Alteration of Glycosaminoglycans for Biological Applications

Smritilekha Bera*
Dhananjoy Mondal

School of Chemical Sciences, Central University of Gujarat, Gandhinagar, Gujarat-382030, India
lekha026@yahoo.com

Published as part of the Virtual Collection
Click Chemistry and Drug Discovery



Received: 03.03.2023
Accepted after revision: 08.05.2023
Published online: 19.06.2023 (Version of Record)
DOI: 10.1055/s-0040-1720072; Art ID: SO-2023-03-0024-RV

Crowd-Reviewed

Abstract This short review describes the assistance of click chemistry in the chemical modification of glycosaminoglycans. Through an alkyne-azide 1,3-dipolar cycloaddition reaction, the chemically and physiologically stable triazole unit connects glycosaminoglycans with other labelled or attached functionalities. The synthesized glycosaminoglycan (GAG) conjugates act as drug carriers, forming hydrogels or nanohydrogels for localized drug delivery or injectable GAGs and so on. These are used in research on antithrombotic agents, protein binding, and hepatocyte growth factors, as well as in mechanistic studies of glycosaminoglycans biosynthesis and wound healing.

Key words click reaction, triazole, glycosaminoglycans, biological activities, alkyne-azide 1,3-dipolar cycloaddition

1 Introduction

Naturally abundant anionic heteropolysaccharides, glycosaminoglycans (GAGs), are composed of an amino sugar (N-acetylated, or N-sulfated D-glucosamine and/or N-acetyl-D-galactosamine), uronic acid (D-glucuronic acid or L-iduronic acid), and galactose units, as shown in Figure 1.[1–6] The linkages are either 1,4- or 1,3- and the sulfation are at 4-, 6-, or 2-positions, depending on the GAG type, with the exception of hyaluronan, which is unsulfated. GAGs usually control cell–cell contacts, cell proliferation, enzyme inhibition, growth factor receptor activation and other metabolic processes. Apart from the organization of extracellular matrices, due to high charge density (retaining water molecules),[7] glycosaminoglycans have been reported to interact with different proteins, especially chemokines,[8] during inflammation[9–12] and for promoting chemotaxis.[13,14] Their contribution is also highly notable in the studies of blood anticoagulation,[15] fertilization,[16] and reproduction[17] etc. These are mostly found as part of a proteoglycan that is attached to a protein core.

Heparin, heparan sulfate, chondroitin sulfate, keratan sulfate, and hyaluronan are the most extensively used GAGs in medicine. One of the GAGs, heparan sulfate (HS),[18] is primarily found on the surface of cells for circulating leucocytes and regulating cell–cell and cell–extracellular matrix interactions. Heparin generally dissociates from the protein core and exists as free GAG chains, and it acts as an anticoagulant, in brain development, and as an anticancer agent.[19] Though structurally similar, the average heparin disaccharide contains 2.7 sulfate groups, whereas heparan sulfate contains one sulfate group per disaccharide, and L-iduronic acid predominates in heparin, whereas the D-glucuronic acid epimer is present in heparan sulfate.[20,21] Chondroitin sulfate (CS) contains a higher percentage of glucuronic acid units than iduronic acid. It is used as an alternative medicine in dietary supplements to treat osteoarthritis, brain development, learning and memory-related issues, joint pain and so forth. Dermatan sulfate, also known as chondroitin sulfate B, plays roles in coagulation, cardiovascular disease, carcinogenesis, infection, wound repair, skin shape maintenance, and fibrosis. The concentration of iduronic acid units is higher than that of glucuronic acid units in CS.[22] Keratan sulfate is the only GAG that does not contain uronic acid; it is composed of almost all glucosamine and some 6-sulfated galactose units.[23] It is one of the major components of the cornea and plays important roles in cor-

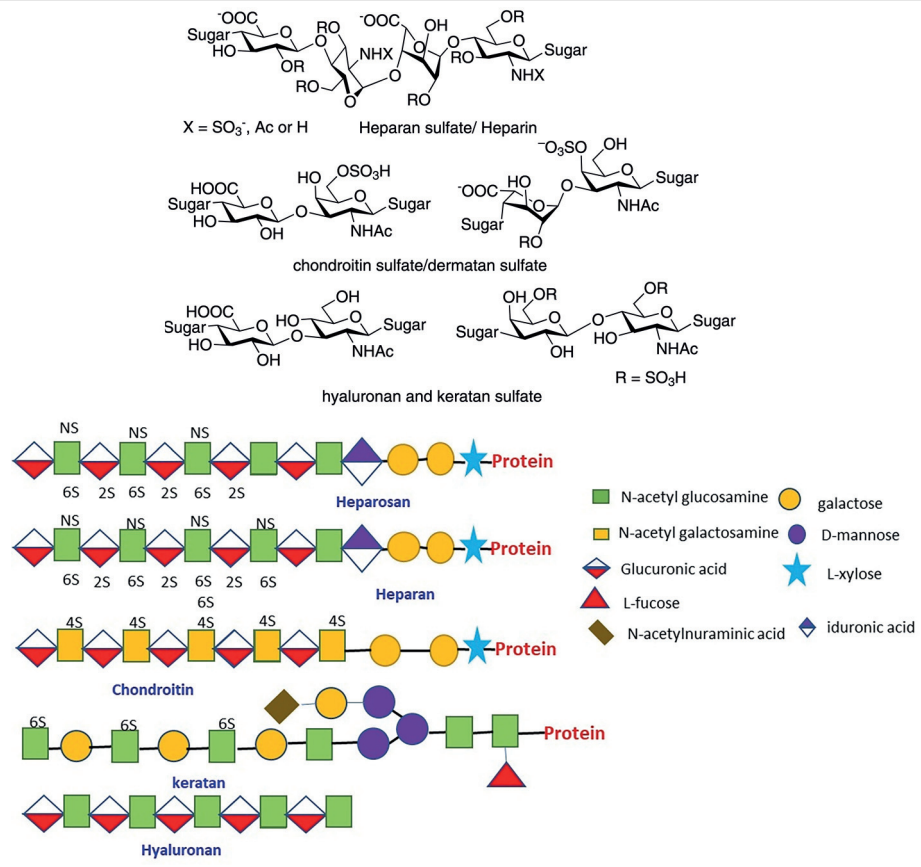


Figure 1 Structures and pictorial representation of glycosaminoglycans

Biographical Sketches

Dr. Smritilekha Bera achieved her Ph.D. at the National Chemical Laboratory in Pune, India, under the supervision of Dr. Mukund K. Gurjar. She started working as a DST scientist in the School of Chemical Sciences, Central University of Gujarat, India, after completing a postdoctoral research associateship for three years at the University of Manitoba in Canada and two years at the Rensselaer Polytechnic Institute (RPI) in New York. Her research works involve the synthesis of natural products, heterocyclic building blocks, aminoglycosides, and unnatural glycosaminoglycans (hyaluronan, and chondroitin sulfate) for medicinal purposes. As an independent researcher, she has been working on the synthesis and applications of photoswitchable molecules and organic nanoparticles. She has published internationally fifty peer-reviewed journals, six book chapters, and four patents.

Dr. Dhananjoy Mondal is an assistant professor in the School of Chemical Sciences at the Central University of Gujarat in Gandhinagar, India. Following his Ph.D. in synthetic organic chemistry from the National Chemical Laboratory in Pune, India, under the supervision of Dr. Mukund K. Gurjar, he worked as a postdoctoral research associate for more than four years at the University of Manitoba in Canada and Rensselaer Polytechnic Institute in the United States. His research interests include the synthesis of natural products, glycopeptidomimetics, and organo-nano conjugates for various biological applications. He has more than 49 research articles and six book chapters to his credit.

neal development and the maintenance of corneal transparency in the tissue. Hyaluronic acid (HA)[24] has the highest average molecular weight, with longer chains reaching 104 kDa; other GAGs fall between 30 and 40 kDa. HA has roles in tumor marking, eye and plastic surgery, and wound healing etc.

2 Synthetic Modification of GAGS

Though these GAGs are biologically and medicinally important polysaccharides obtained from natural sources (animal tissue or bacterial cells) with heterogeneity and structural variation, sufficient quantities of these well-defined polysaccharides are required for structure–activity relationship investigations as well as other biological studies. In recent times, it has been observed that polysaccharides can be modified to produce a wide range of derivatives such as nanogels, hydrogels, and micelles with unique architectural structures.[25–27] The structural modifications of heteropolysaccharide GAGs are also not far behind and are used for coatings, drug delivery, drug carriers, and biomedical materials etc.[28,29] Thus their modifications are a priority in many research communities.

One of the most common approaches is the chemical modification,[30] which provides the advantage of simple and well-defined structural modifications to tailor homogeneously dispersed GAGs or surface-modified GAGs with required functional groups and conjugation. For well-controlled structures, typical modification strategies include selective oxidation, esterification or etherification, nucleophilic displacement reactions, protection–deprotection etc. These chemical reactions may have several drawbacks, such as tedious steps, low yields, longer reaction times, and low-yielding coupling with large molecules, particularly protein or peptides or other bulky molecules. Though the methods mentioned above are still very useful and continue to be used by the scientific community, click chemistry in this case may be essential for conjugating with a macromolecule. According to this viewpoint, chemical modification using click chemistry may provide these polysaccharide materials in a well-defined manner, allowing researchers to gain a deeper understanding of the structure–property relationships of polysaccharide derivatives and other biomedical applications.

3 Click Chemistry

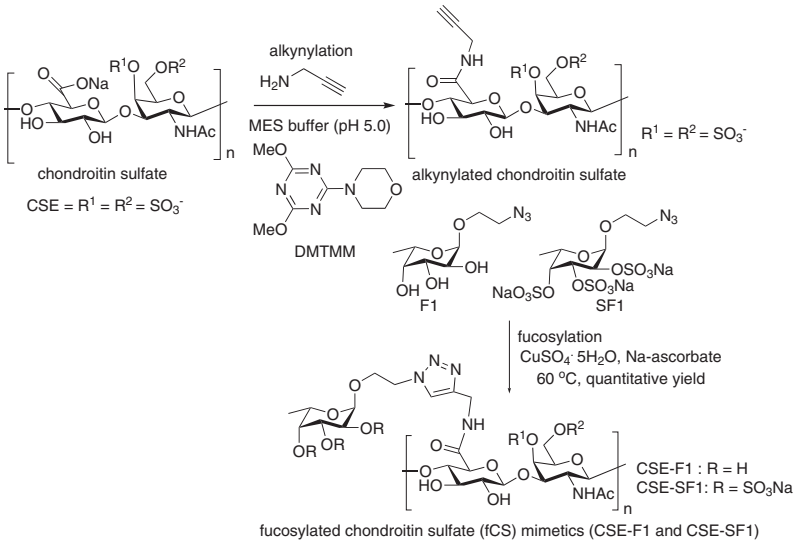
The idea of copper-catalyzed azide-alkyne 'click chemistry', which was first introduced by Sharpless and coworkers,[31] has had a significant influence on the conjugation chemistry employed by the macromolecule fraternity. The 'click' reactions are typically stereospecific, modular, widely applicable, and provide extraordinarily high yields. Furthermore, the reaction conditions and product isolation are straightforward, utilizing readily available chemicals and safe or benign solvents. Though 'click' reactions include the well-known azide–alkyne Huisgen cycloaddition, thiol-ene and thiol-Michael reactions, Diels–Alder reaction, and oxime click reaction, this review focuses primarily on the azide–alkyne Hüisgen cycloaddition.[32]

Click chemistry links together small molecules in a quick, efficient manner to yield covalently conjugated products. The simple process of click chemistry involves the chemical joining of small units together, for example, an azide and an alkyne group, to produce a triazole. Other than linear click chemistry, strain-promoted non-catalytic cycloaddition with cyclooctynes is highly applicable in biology due to the Fenton reaction developed by Bertozzi and coworkers.[33] Under acidic or basic conditions, metabolic degradation, and redox conditions, the biorthogonal triazole motif is generally stable to hydrolysis.

4 Modification of GAGS Applying Click Chemistry

A typical marine polysaccharide, derived from sea cucumber called fucosylated chondroitin sulfate (fCS) has a variety of biological properties, but its promising anticoagulant properties are incomparable. Here, a site-selective alkynylation on CS was reported with controlled ratios ranging from 0.15 to 0.78. With the aid of the Cu(I) stabilizing ligand tris(hydroxypropyl triazolylmethyl)amine (THPTA) and the copper-catalyzed azide–alkyne cycloadditions (CuAACs) 'click' reaction with azide substituted fucose, the fucose branches were successfully grafted onto alkynylated CS. The biological activities of a small library of 12 fCS mimics with different sulfation patterns and fucose branch densities were obtained. The findings demonstrated that fCS mimics having Chondroitin sulfate backbone (CSE) CSE-F1 and CSE-SF1 had anticoagulant properties through intrinsic pathways and antithrombin III (ATIII)-inhibited bovine 14 coagulation factor Xa (Fxa). The study found that the CSE backbone and free carboxyl groups are essential for the anticoagulant action of these compounds (Scheme 1).[34]

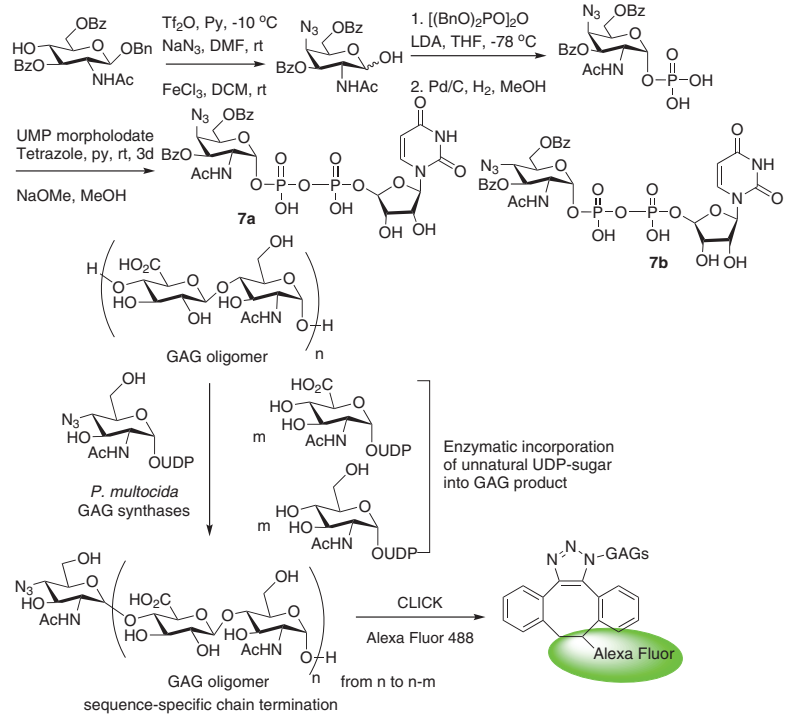
In the field of polysaccharide synthesis, Linhardt and coworkers extended their research into the development of unnaturally chemically modified nucleotide sugars, UDP-4-N_3-GlcNAc and UDP-4-N_3-GalNAc, to incorporate hyaluronan, heparosan, or chondroitin. To generate triazole-linked labelled-GAGs for detection and wound healing, the UDP-4-N_3-GlcNAc was first conjugated with Alexa Fluor 488 DBCO alkyne as a chain termination substrate for hyaluronan or heparosan synthase (Scheme 2). This enzymatic methodology provides a 'green' strategy to access regioselectively azide-functionalized GAGs, which would bring superior



Scheme 1 Synthesis of fucosylated chondroitin sulfate mimetics

control of the final structure and composition of these macromolecules compared to chemical methods to install azido groups into polysaccharides.[35]

A chondroitin and hyaluronan pseudo tetrasaccharide with 1,4-disubstituted 1,2,3-triazole rings as inter-glycosidic linkers were synthesized by exploiting very efficient CuAACs as key steps by Bera et al.[36]

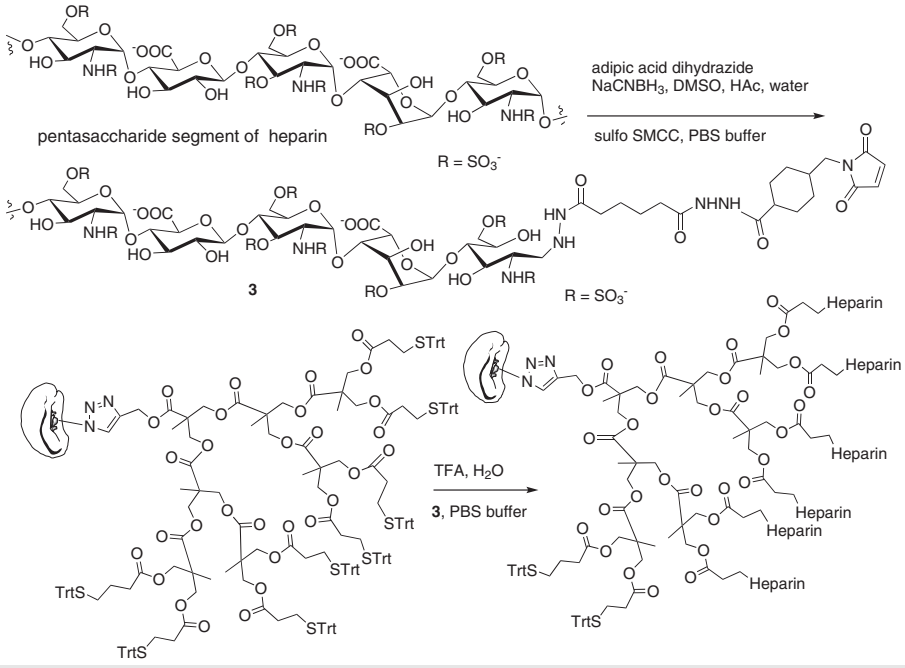


Scheme 2 Synthesis of Alexa Fluor 488-labelled GAGs

In another work, Linhardt and co-workers converted the amino functionalities on decellularized aortas into azido groups using 3-azido benzoic acid. The polyester-8-hydroxy-1-acetylene bis-MPA dendron was reacted with aortas azide through an alkyne azide click reaction to produce eight new attachment sites for each modified amine and to extend the end groups of the dendron towards the luminal side of the vessel. Next, end-on modified heparin chains were used to elaborate the dendron end groups. On the surface of native endothelial cells, heparin chains were positioned similarly to heparan sulfate groups (Scheme 3). Through the use of fluorescence microscopy and histological examination, the consistency of the heparin coating was confirmed. Studies on platelet adhesion and the factor Xa anti-thrombogenic assay were used to show the effectiveness of heparin linkage. According to the findings, the in vivo blood compatibility of decellularized aortas with other vessels may be enhanced using directed heparin immobilization.[37]

Heparanase (HPSE) is an endoglycosidase that breaks down HS in the extracellular matrix and cell membrane. A non-reducing-end labelling of HS via click chemistry was developed and used in this study for the HPSE assay. HS chains on recombinant human syndecan 4 were first labelled with GlcNAz at their non-reducing ends using dimeric HS polymerase EXT1/EXT2 (Figure 2). The labelled sample was then biotinylated using click chemistry, immobilized on a multi-well plate, and detected using ELISA. HPSE digestion of the biotinylated sample removed the label, reducing the signal in the ELISA assay. Nonreducing end labelling eliminated the interference in an HPSE reaction caused by retained internal HS labelling. The assay is very sensitive, requiring only 2.5 ng of labelled syndecan 4 in each reaction and is highly reproducible, with Z′> 0.6. Overall, this method is suitable for high-throughput drug screening on HPSE.[38]

For in vivo imaging research, Willén and co-workers produced the naphthoxylosides XylNapN$_3$ and XylNapN$_3$, tagged with the TAMRA fluorophore. XylNapN$_3$ was established as a substrate for 4GalT7 and galactosylation following an identical kinetic pattern to that of XylNap. It has been demonstrated that A549 cells take up XylNapN$_3$ and begin the manufacture of soluble GAGs with comparable size and disaccharide compositions to GAGs from the well-known primers XylNap and XylNapOH. These GAG chains successfully underwent a strain-promoted cycloaddition reaction with an Alexa Fluor 647 fluorophore containing DBCO (Scheme 4). After being successfully biotinylated using an azide derivative, GAGs obtained from primary fibroblasts stimulated with XylNapN$_3$ were employed in SPR assays to ascertain the kinetics of their interaction with hepatocyte growth factor.[39]



Scheme 3 Dendron-functionalized heparin linked through the triazole motif

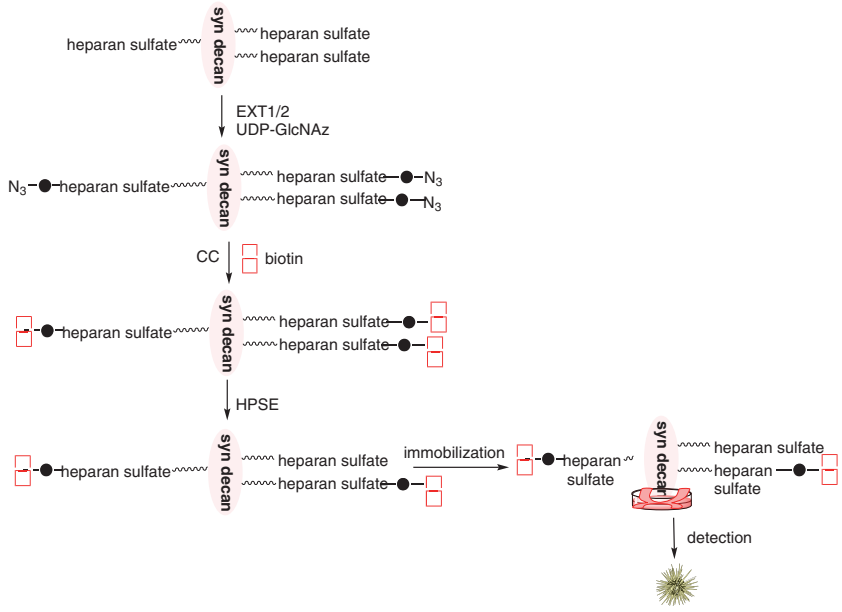
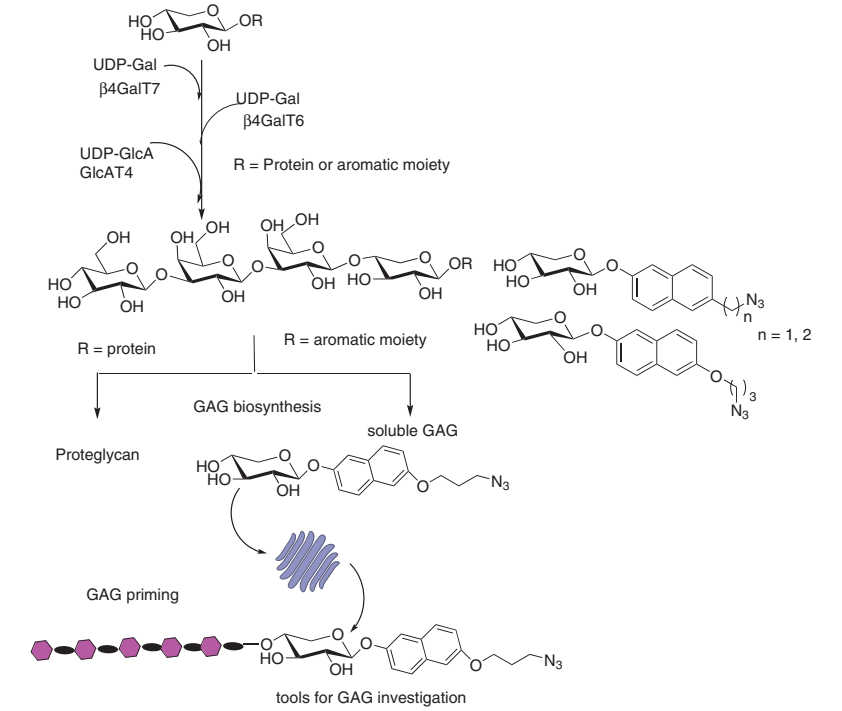
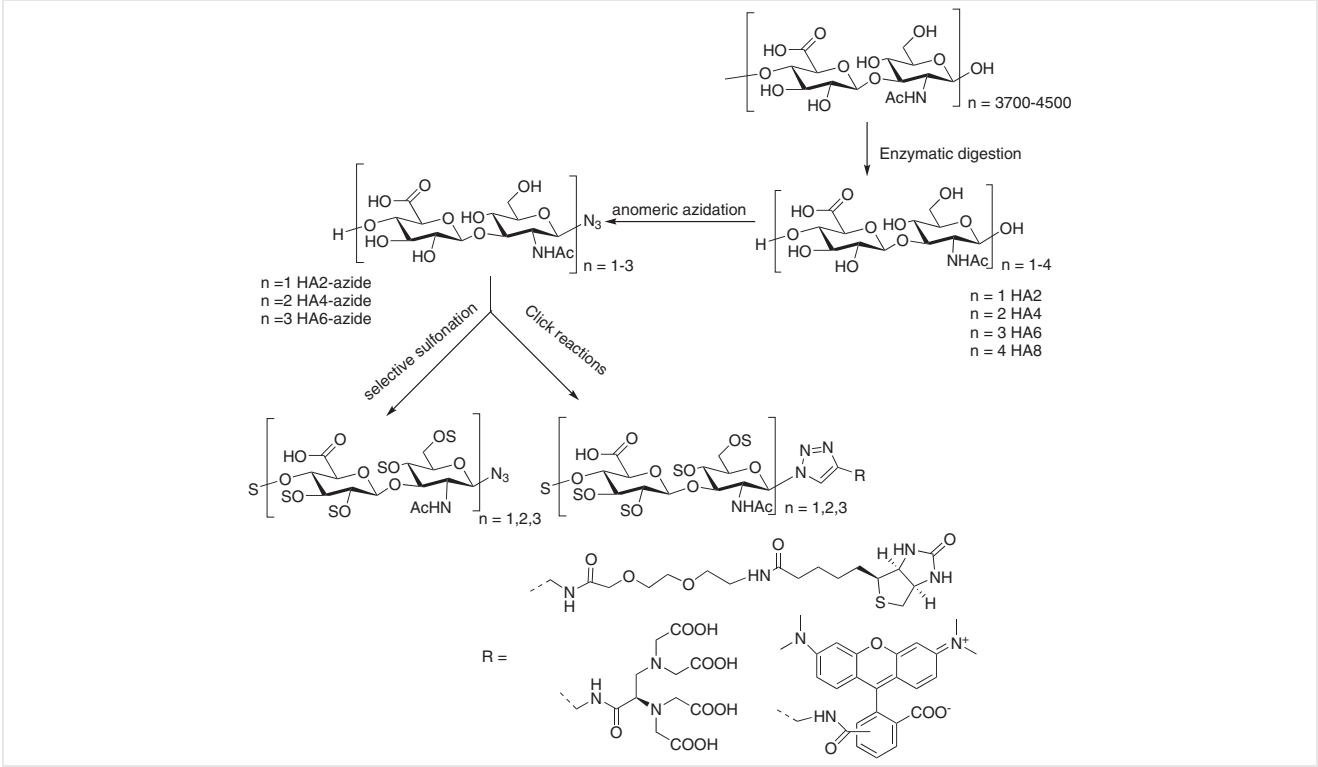


Figure 2 Non-reducing-end labelled-HS proteoglycan for HPSE assay



Scheme 4 Azide-labelled GAGs for conjugation to the Alexa Fluor 647 fluorophore

In order to get well-defined sulfated and anomerically modified GAG oligosaccharides of HA for GAG-protein binding studies, several sulfated azide derivatives, including pentasulfate 5s-HA4-azide, hepta-sulfated 7s-HA4-azide, pentasulfate 5s-HA2-azide, nonasulfated 9s-HA4-azide, and the trideca-sulfated13s-HA6-azide were prepared. To incorporate a metal-chelating label for elucidating the structure of a GAG-complex with interleukin 10 (IL-10) protein, HA4-azide was 'clicked' with the EDTA-derivative, *N*-propargyl-(2,2,3,3-tetra-methoxycarbonyl-methyl-2*R*,3-diamino)-propanamide in the presence of Cu(I) ions and ligand TBTA (tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine)



Scheme 5 Preparation of defined sulfated GAG with anomeric modifications

(Scheme 5). Saponification of the methyl esters of 1,2,3-triazole derivative followed by sulfation yielded the nonasulfate 9s-HA4-(triazolyl)-EDTA, which helped quantify binding to IL-10 by measuring chemical shift perturbations in HSQC-NMR spectra. The precise position of the metal chelating, sulfated GAG, complexed with copper-(II) by using paramagnetic resonance enhancement (PRE) NMR on the protein surface was assessed, indicating the structure of the IL-10-GAG complex.[40]

Balagurunathan and co-workers[41] designed and synthesized a library of click-xylosides having various aglycones to interpret the mechanism of GAG biosynthesis in a cellular system and assessment of glycosaminoglycan. In their program, click chemistry was explored for the connection of metabolically stable xyloside with various hydrophobic groups via triazole linkage (Figure 3). Their studies revealed that the structure of the aglycone moieties, sulfation pattern, disaccharide composition, and chain length of GAG chains affect the mechanism of GAG biosynthesis. In another work, RGD-conjugated xylosides via triazole linkage to prime GAG chains in various cell types were used to determine the mechanism of biosynthesis.[41b,42]

Piperno and co-workers covalently grafted β-cyclodextrin (CD) on the hyaluronic acid backbone by employing a triazole linkage formed by the reaction of mono-6-deoxy-6-azido-cyclodextrin (CDN_3) with alkyne-functionalized

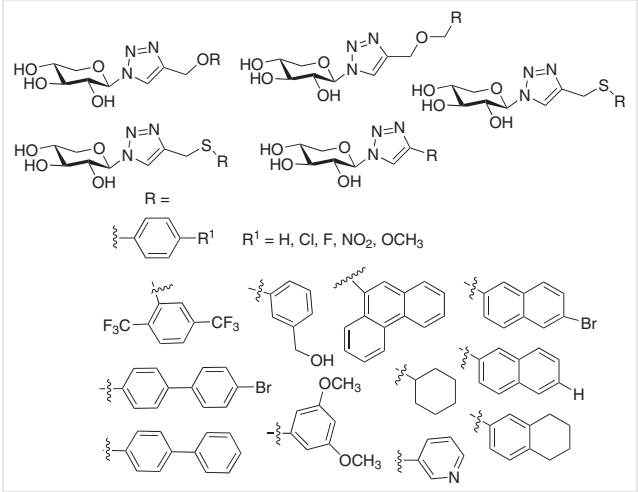
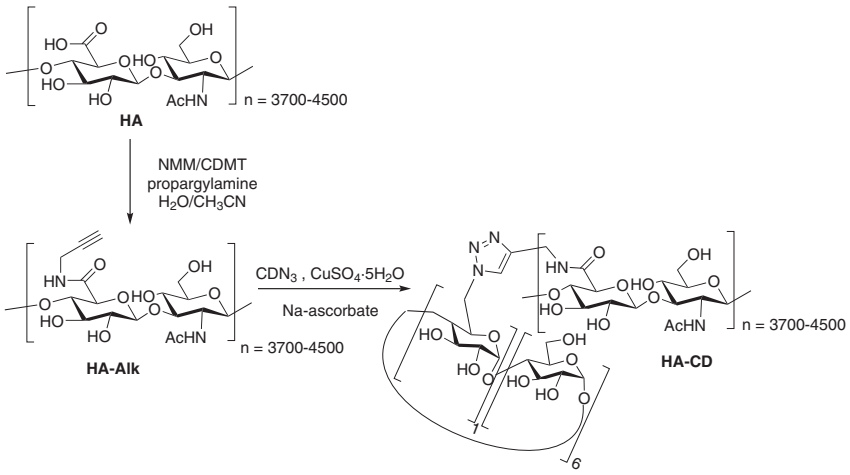


Figure 3 Few RGD-conjugated xylosides for conjugation to GAG

HA.[43] Alkyne fragments were added to the HA backbone by a coupling process known as 'triazine-activated amidation'. The interaction with acyclovir (Acy), a model antiviral drug, demonstrated that the biological features of the HA-CD/Acy complex (cytotoxicity and suppression of viral plaque formation) coupled with a delayed-release of Acy from HA-CD/Acy exhibit a good antiviral activity (Scheme 6).

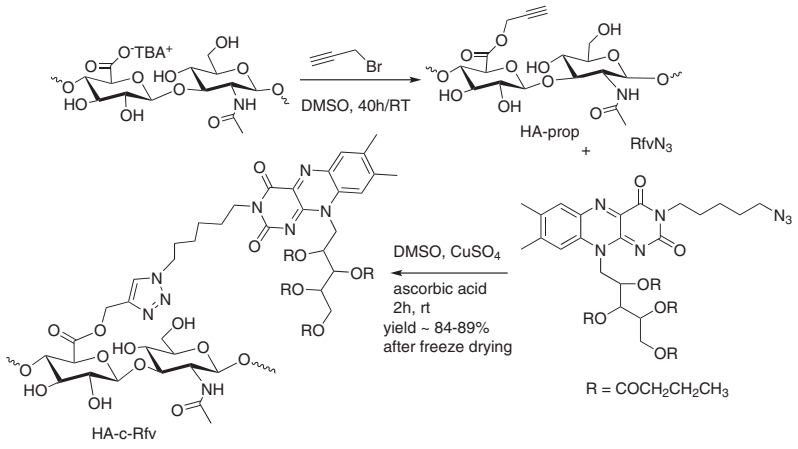


Scheme 6 Triazole-linked β-cyclodextrin (CD) grafted hyaluronic acid

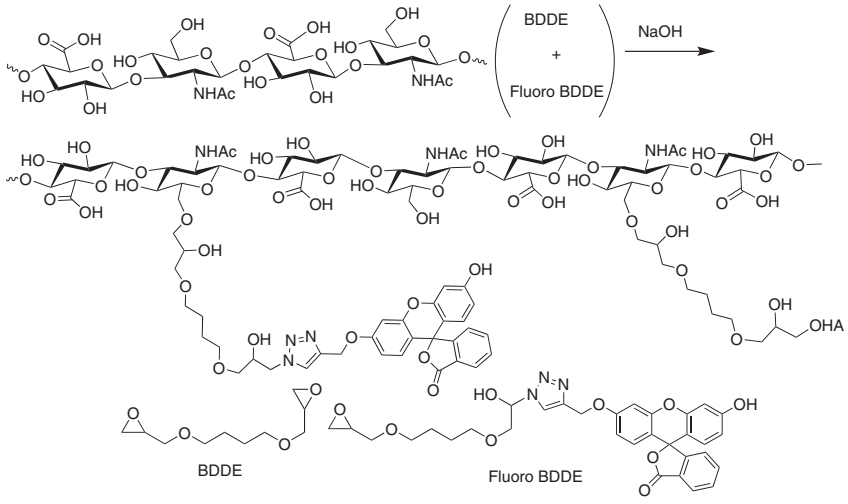
Meo and co-workers synthesized amphiphilic hyaluronic acid and riboflavin derivatives (HA-c-Rfv)-based self-assembled hydrogel nanoparticles by utilizing the copper sulfate-catalyzed azide-alkyne cycloaddition (CuAAC) reaction in the presence of ascorbic acid in DMSO for two hours at room temperature. With yields of between 84 and 89%, the products were collected by freeze-drying. The esterification of HA carboxylic groups with alkyne moieties produced polymeric chains that could couple with $RfvN_3$. Using an autoclave-based method, this amphiphilic material generated nanohydrogels (NHs) in the 150–200 nm range in water medium. By including dextrose as a cryoprotectant, the NHs formulation could be freeze-dried, and NHs were obtained that were exceptionally stable in aqueous solutions. As a case study for a drug-laden nanocarrier, dexamethasone, piroxicam, and paclitaxel were successfully loaded into NHs using the film-hydration approach (Scheme 7). PEG-N_3 was employed to obtain PEGylated NHs, and it was coupled with an HA-c-Rfv derivative that had an excess of propargylic sections, opening the door to a variety of functionalization options.[44]

Galderma and Boiteau prepared functionalized cross-linked hyaluronic acid (HA) in one step as shown in Scheme 8.[45] 1,4-Butanediol diglycidyl ether was used as cross-linker. This is advantageous because workup procedures performed prior to the cross-linking step could result in HA degradation. The click CuAAC reaction performed well with azido-epoxide and alkyne-fluorescein in both organic and aqueous media. The hydrogel formed by cross-linked HA is useful in cosmetic and medical surgery.

Saletti and co-workers recently described how to create hydrogels by cross-linking (CL) hyaluronan grafted (HA-FA-Pg) copolymers. In this instance, the clickable propargyl groups of the HA-FA-Pg graft copolymers were employed in a very mild CuAAC dimerization reaction in the presence of extremely small quantities of CuBr and DIPEA in THF. The



Scheme 7 Conjugation of hyaluronic acid and riboflavin derivatives for amphiphilic hydrogels

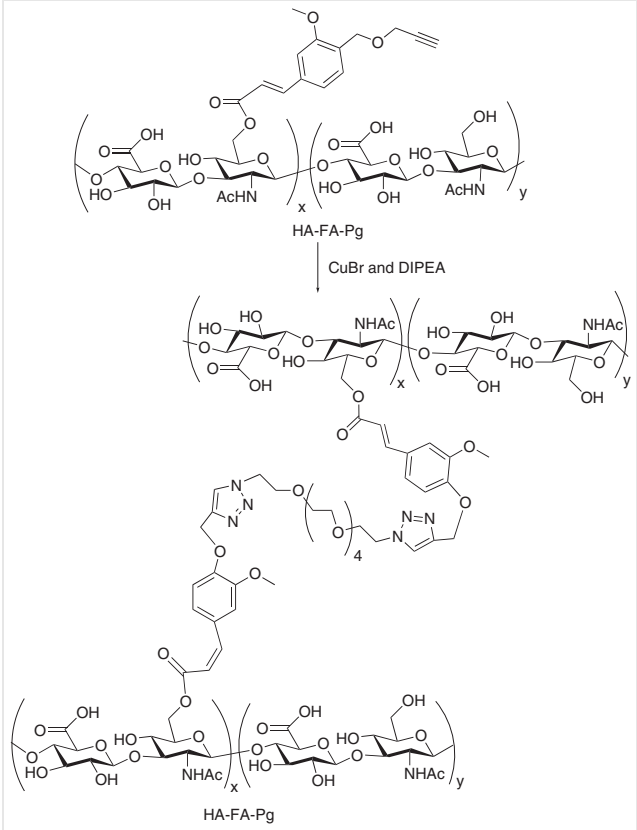


Scheme 8 Single-step preparation of cross-linked hyaluronic acid-based hydrogel

resulting HA-FA-HEG-CL materials interacted with water to produce hydrogels that exhibited a variety of gelation characteristics. Different rheological behaviors have been seen for HA-FA-HEG-CL materials in various biological domains due to the tuning potential afforded by the crosslinking degree (Scheme 9). Since its shear-thinning ratio exactly falls within the range of a healthy synovial fluid, HA(270)-FA-HEG-CL-10 appears to be a strong candidate for use in the treatment of osteoarticular disorders. Since its shear-thinning ratio exactly falls in the range of a healthy synovial fluid shear-thinning ratio (70–250), HA(270)-FA-HEG-CL-10 appears to be a good candidate for application in the treatment of osteoarticular diseases. It is also suitable as a viscosity enhancer for eye drops. The qualitative and quantitative cytotoxicity data show that the HA(270)-FA-Pg graft copolymer cross-linking process is fully biocompatible because the resulting HA(270)-FA-HEG-CL-10, HA(270)-FA-HEG-CL-20, and HA(270)-FA-HEG-CL-40 materials had no in vitro cytotoxic effects.[46]

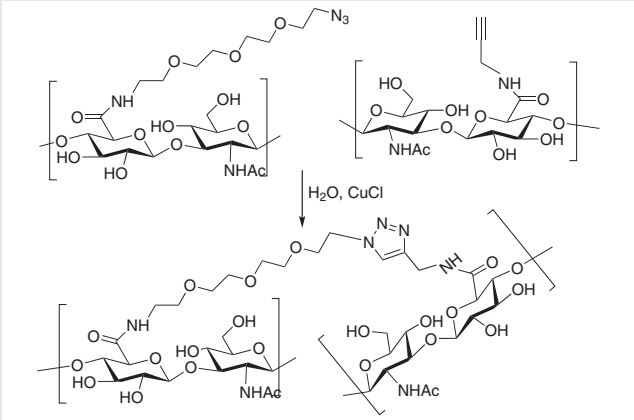
Using water-soluble hyaluronic acid (HA) with side chains including alkyne and azide moieties, Crescenzi and co-workers reported the production of HA-sodium salt based 'click' hydrogels. After gelation in aqueous solutions of benzydamine and doxorubicin, it was demonstrated that the polysaccharide-bearing nanogels function as a drug reservoir for the drug doxorubicin (Scheme 10). In fact, the authors showed that the hyaluronan networks enabled both the successful trapping of yeast cells as well as an effective controlled release of doxorubicin. In the latter instance, 60% of the cells demonstrated proliferative activity following the hydrogel scaffold's click creation.[47]

Martini and co-workers described the synthesis and determined the stability of HA-hydrogels, cross-linked via triazoles. Herein, a positively charged alkylated triazole ring



Scheme 9 HA-based platform using HA-FA-Pg graft copolymer

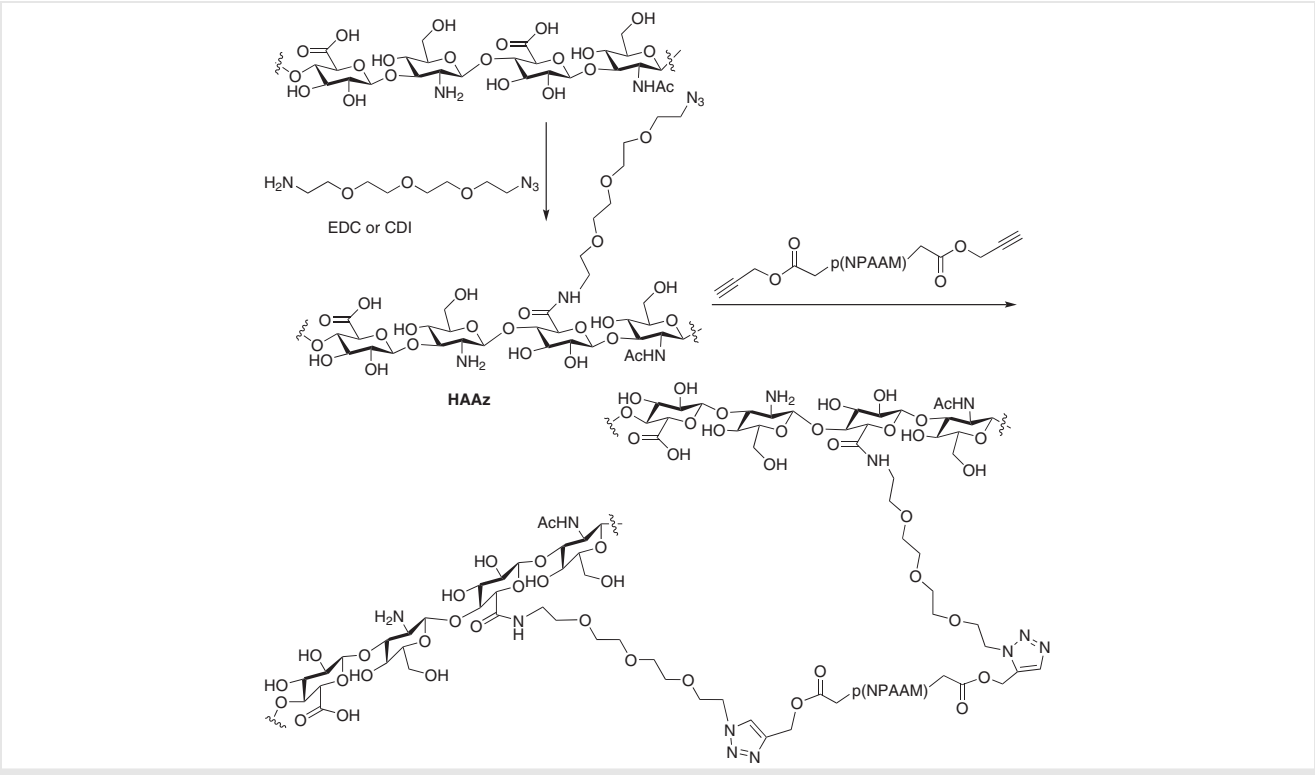
was reacted with thiolated hyaluronan via the thio-Michael addition to get neutral and charged triazole-based cross-linkers with respect to their ability to form hydrogels.[48]



Scheme 10 Schematic representation of triazole-linked HA-based hydrogels

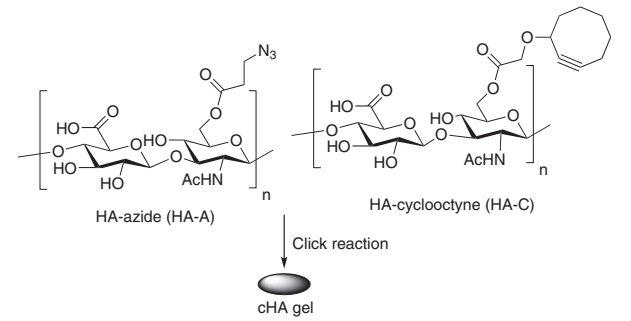
A biological hydrogel was formed by employing a click reaction of the hyaluronic acid (HA), chondroitin sulfate (CS), and gelatin in order to replicate the extracellular matrix found in natural cartilage, made up of core proteins and glycosaminoglycans. 11-Azido-3,6,9-trioxaundecan-1-amine (AA) was used to modify HA and CS, and propionic acid was used to modify gelatin (G-PA). A cross-linked hydrogel was formed when the azide ($-N_3$) groups of HA-AA and CS-AA reacted with the alkyne groups of G-PA in the presence of Cu(I). The synthesized hydrogel was extremely swollen and displayed elastomer-like properties with weight loss of up to 45% after 4 weeks of incubation in phosphate-buffered saline. The hydrogel released 10% CS and 20% gelatin in 2 weeks.[49] Thus, this hydrogel could boost chondrocyte adhesion and proliferation, according to in vitro cell culture results.

Another hydrogel was formed by exploring the click reaction of an azido-derivative of hyaluronate with propargylated p(NiPAAm). For that, the azido-hyaluronan derivative was synthesized using coupling reagent ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) or carbonyl diimidazole (CDI), followed by the addition of hyaluronan, *N*-hydroxysuccinimide (NHS) and 11-azido-3,6,9-trioxaundecan-1-amine to get HAAz derivative HAAz5 and HAAz33, respectively. Then the resulting azido derivative was coupled with propargylated p(NiPAAm) to form the hydrogel (Scheme 11). This hybrid system exhibits several behavioral responses to changes in temperature, pH, and ionic strength while preserving both cumulative and individual sensitivity to external stimuli.[50] An additional advantage is its ability to simulate extracellular hyaluronidase-catalyzed matrix degradation. On the surface of these hybrid hydrogels, HT-29 (tumor) cells proliferate more than NIH3T3 (healthy) cells, and it might work as a potential biopolymer for localized drug delivery.



Scheme 11 Cross-linking of hyaluronan azide and propargylated p(NiPAAm) for hydrogel formation

Ito and co-workers' research involved the reaction of hyaluronic acid-derived azide (HA–A) with cyclooctyne-attached HA (HA–C) using a double-barreled syringe to form a hydrogel via strain-promoted [3+2] cycloaddition without any catalyst, under physiological conditions. The hydrogel slowly degraded in PBS buffer over 2 weeks, and it was accelerated to 9 days by hyaluronidase, while it rapidly degraded in a cell culture media with fetal bovine serum within 4 days. Both HA–A and HA–C showed good biocompatibility with fibroblast cells in in vitro assays. This injectable HA-derived hydrogel is expected to be useful for tissue engineering and drug-delivery systems utilizing its orthogonality (Scheme 12).[51]



Scheme 12 Synthesis of injectable hyaluronan-based hydrogel

5 Conclusions

Glycosaminoglycans (GAGs) are essential for biological activities such as cell-cell communication and cell proliferation, in addition to influencing enzymes and activating growth factor receptors during numerous metabolic processes. The widespread applications of GAG chains in blood coagulation, wound healing, tumor biology, and cosmetic or medical surgery inspired the development of GAG-derived therapeutic medicines such as modified GAG chains, GAG binding peptides, GAG-based enzyme inhibitors etc. In the literature, no precise method for efficient chemical synthesis of these highly sulfated and complex polysaccharides such as glycosaminoglycans has been detailed as a preferred synthetic route. The click reaction, as a highly reactive and efficient method, has been established as a reliable tool for synthesizing or modifying functional biomacromolecules such as glycosaminoglycans. Its synthetic potential for structural modification may open up new avenues for the formation of important structural motifs for GAG function, as well as labelled bio-functional glycosaminoglycans and oligosaccharides to enhance GAG biological activity. These developments will be highly beneficial to the field of GAG syntheses covering the research on modern glycobiology, proteoglycans, as well as heparan sulfate, chondroitin sulfate and hyaluronan fragments in biological systems. Hence, oligosaccharide synthesis and the amplification of multivalency via the formation of the triazole unit employing click methods are simple and useful approaches that address the difficulties involved with total syntheses of GAGs using organic chemistry or biosynthesis.

We demonstrated the modification of GAGs through the connection of other important motifs using chemically and biologically stable triazole linkage via the click reaction with azide and alkyne in this review article. The development of GAG-derived therapeutic drugs, such as modified GAG chains, GAG binding peptides, and GAG-based enzyme inhibitors, was sparked by the extensive use of GAG chains in blood coagulation, wound healing, tumor biology, and aesthetic or medical surgery. The field of tissue engineering, injectable hyaluronan-based hydrogels, and localized drug-delivery research, including the synthesis of hydrogels and nanohydrogels, may all benefit from the use of the synthesized GAGs. These are employed in studies on hepatocyte growth factors, protein binding, and anti-thrombotic therapy. Drugs such as doxorubicin, dexamethasone, piroxicam, and paclitaxel can also be transported with the help of triazole-linked GAGs.

6 Abbreviations

CuAAC copper-catalyzed azide-alkyne cycloadditions
ATIII antithrombin III
UDP uridine diphosphate
EXT1/EXT2 exostosin 1 and 2 gene
ELISA enzyme-linked immunosorbent assay
GlcNAz *N*-azidoacetylglucosamine-tetraacylated
TAMRA tetramethylrhodamine
DBCO dibenzoazacyclooctyne
SPR surface plasmon resonance
EDTA ethylenediaminetetraacetic acid
RGD Arg-Gly-Asp
DIPEA *N*,*N*-diisopropylethylamine
PEG poly(ethylene glycol)
NIH3T3 National Institutes of Health, 3-day transfer, inoculum 3×10^5 cells
HT29 human colon adenocarcinoma

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

We are highly pleased with the support of the infrastructure facility from the Central University of Gujarat.

References

(1) (a) Antonio, J. D. S.; Iozzo, R. V. *Glycosaminoglycans: Structure and Biological Functions*, In *Encyclopedia of Life Sciences*; John Wiley & Sons: Weinheim, **2001**. (b) Gandhi, N. S.; Mancera, R. L. *Chem. Biol. Drug Des.* **2008**, *72*, 455.
(2) Esko, J. D.; Kimata, K.; Lindahl, U. *Proteoglycans and Sulfated Glycosaminoglycans*, In *Essentials of Glycobiology, 2nd ed*; Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etzler, M. E., Ed.; Cold Spring Harbour Laboratory Press: New York, **2009**.
(3) DeAngelis, P. L. *Anat. Rec.* **2002**, *268*, 317.
(4) Taylor, K. R.; Gallo, R. L. *FASEB J.* **2006**, *20*, 9.
(5) Gandhi, N. S.; Mancera, R. L. *Chem. Biol. Drug Des.* **2008**, *72*, 455.
(6) Raman, R.; Sasisekharan, V.; Sasisekharan, R. *Chem. Biol.* **2005**, *12*, 267.
(7) Schnabelrauch, M.; Scharnweber, D.; Schiller, J. *Curr. Med. Chem.* **2013**, *20*, 2501.
(8) Salanga, C. L.; Handel, T. M. *Exp. Cell Res.* **2011**, *317*, 590.
(9) Middleton, J.; Neil, S.; Wintle, J.; Clark-Lewis, I.; Moore, H.; Lam, C.; Auer, M.; Hub, E.; Rot, A. *Cell* **1997**, *91*, 385.
(10) Middleton, J.; Patterson, A. M.; Gardner, L.; Schmutz, C.; Ashton, B. A. *Blood* **2002**, *100*, 3853.
(11) Wang, L. C.; Fuster, M.; Sriramarao, P.; Esko, J. D. *Nat. Immunol.* **2005**, *6*, 902.
(12) Bishop, J. R.; Schuksz, M.; Esko, J. D. *Nature* **2007**, *446*, 1030.
(13) Luster, A. D.; Greenberg, S. M.; Leder, P. *J. Exp. Med.* **1995**, *182*, 219.
(14) Webb, L. M. C.; Ehrengruber, M. U.; Clark-Lewis, I.; Baggiolini, M.; Rot, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7158.
(15) Bourin, M. C.; Lindahl, U. *Biochem. J.* **1993**, *289*, 313.
(16) Ariga, T.; Miyatake, T.; Yu, R. K. *J. Neurosci. Res.* **2010**, *88*, 2303.
(17) Kano, K.; Miyano, T.; Kato, S. *Biol. Reprod.* **1998**, *58*, 1226.
(18) (a) Sasisekharan, R.; Raman, R.; Prabhakar, V. *Annu. Rev. Biomed. Eng.* **2006**, *8*, 181. (b) Sasisekharan, R.; Venkataraman, G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 626.
(19) (a) Lever, R.; Page, C. P. *Nat. Rev. Drug Discovery* **2002**, *1*, 140. (b) Sugahara, K.; Kitagawa, H. *IUBMB Life* **2002**, *54*, 163.
(20) Toida, T.; Yoshida, H.; Toyoda, H.; Koshiishi, I.; Imanari, T.; Hileman, R. E.; Fromm, J. R.; Linhardt, R. J. *Biochem. J.* **1997**, *322*, 499.
(21) Lindahl, U.; Kusche-Gullberg, M.; Kjellén, L. *J. Biol. Chem.* **1998**, *273*, 24979.
(22) (a) Silbert, J. E.; Sugumaran, G. *IUBMB Life* **2002**, *54*, 177. (b) Prabhakar, V.; Raman, R.; Capila, I.; Bosques, C. J.; Pojasek, K.; Sasisekharan, R. *Biochem. J.* **2005**, *390*, 395.
(23) (a) Funderburgh, J. L. *IUBMB Life* **2002**, *54*, 187. (b) Barry, F. P.; Neame, P. J.; Sasse, J.; Pearson, D. *Matrix Biol.* **1994**, *14*, 323. (c) Christner, J. E.; Distler, J. J.; Jourdian, G. W. *Arch. Biochem. Biophys.* **1979**, *192*, 548.
(24) (a) Papakonstantinou, E.; Roth, M.; Karakiulakis, G. *Derm.-Endocrinol.* **2012**, *4*, 253. (b) Gupta, R. C.; Lall, R.; Srivastava, A.; Sinha, A. *Front. Vet. Sci.* **2019**, *6*, 192. (c) Dovedytis, M.; Liu, Z. J.; Bartlett, S. *Engineered Regeneration* **2020**, *1*, 102.
(25) (a) Nilasaroya, A.; Poole-Warren, L. A.; Whitelock, J. M.; Martens, P. J. *Biomaterials* **2008**, *29*, 4658. (b) Ornell, K. J.; Lozada, D.; Phan, N. V.; Coburn, J. M. *J. Mater. Chem. B* **2019**, *7*, 2151.
(26) (a) Vassie, J. A.; Whitelock, J. M.; Lord, M. S. *Glycosaminoglycan Functionalized Nanoparticles Exploit Glycosaminoglycan Functions, in: Glycosaminoglycans. Methods in Molecular Biology, Vol. 1229*; Balagurunathan, K.; Nakato, H.; Desai, U., Ed.; Humana Press: New York, **2015**. (b) Niu, J.; Yuan, M.; Liu, Y.; Wang, L.; Tang, Z.; Wang, Y.; Qi, Y.; Zhang, Y.; Ya, H.; Fan, Y. *Front. Chem.* **2022**, *10*, 1028372. (c) Liu, Y.; Han, Y.; Zhu, T.; Wu, X.; Yu, W.; Zhu, J.; Shang, Y.; Lin, X.; Zhao, T. *Drug Delivery* **2021**, *28*, 2100.
(27) (a) Carvalho, A. M.; Teixeira, R.; Novoa-Carballal, R.; Pires, R. A.; Reis, R. L.; Pashkuleva, I. *Biomacromolecules* **2018**, *19*, 2991. (b) Cabral, H.; Miyata, K.; Osada, K.; Kataoka, K. *Chem. Rev.* **2018**, *118*, 6844.
(28) (a) Schnabelrauch, M.; Schiller, J.; Möller, S.; Scharnweber, D.; Hintze, V. *Biol. Chem.* **2021**, *402*, 1385. (b) Scharnweber, D.; Hübner, L.; Rother, S.; Hempel, U.; Anderegg, U.; Samsonov, S. A.; Pisabarro, M. T.; Hofbauer, L.; Schnabelrauch, M.; Franz, S.; Simon, J.; Hintze, V. *J. Mater. Sci.: Mater. Med.* **2015**, *26*, 232. (c) Schnabelrauch, M.; Scharnweber, D.; Schiller, J. *Curr. Med. Chem.* **2013**, *20*, 2501.
(29) (a) Hachim, D.; Whittaker, T. E.; Kim, H.; Stevens, M. M. *J. Controlled Release* **2019**, *313*, 131. (b) Neves, M. I.; Araújo, M.; Moroni, L.; da Silva, R. M. P.; Barrias, C. C. *Molecules* **2020**, *25*, 978. (c) Miura, Y.; Fukuda, T.; Seto, H.; Hoshino, Y. *Polym. J.* **2016**, *48*, 229.
(30) (a) Palhares, L. C. G. F.; London, J. A.; Kozlowski, A. M.; Esposito, E.; Chavante, S. F.; Ni, M.; Yates, E. A. *Molecules* **2021**, *26*, 5211. (b) Mende, M.; Bednarek, C.; Wawryszyn, M.; Sauter, P.; Biskup, M. B.; Schepers, U.; Bräse, S. *Chem. Rev.* **2016**, *116*, 8193.
(31) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *41*, 2596.
(32) (a) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565. (b) Tornoe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057. (c) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem. Int. Ed.* **2005**, *44*, 2210. (d) Rodionov, V. O.; Presolski, S. I.; Diaz, D. D.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12705. (e) Meldal, M.; Tornoe, C. W. *Chem. Rev.* **2008**, *108*, 2952. (f) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249.
(33) (a) Agard, N. J.; Baskin, J. M.; Prescher, J. A.; Lo, A.; Bertozzi, C. R. *ACS Chem. Biol.* **2006**, *1*, 644. (b) Lutz, J.-F.; Zarafshani, Z. *Adv. Drug Delivery Rev.* **2008**, *60*, 958. (c) Sletten, E. M.; Bertozzi, C. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974.
(34) (a) Kuang, L.; Damayanti, N. P.; Jiang, C.; Fei, X.; Liu, W.; Narayanan, N.; Irudayaraj, J.; Campanella, O.; Deng, M. *J. Appl. Polym. Sci.* **2019**, *136*, 47212. (b) Fan, F.; Zhang, P.; Wang, L.; Sun, T.; Cai, C.; Yu, G. *Biomacromolecules* **2019**, *20*, 3798.
(35) (a) Karst, N. A.; Linhardt, R. J. *Curr. Med. Chem.* **2003**, *10*, 1993. (b) Zhang, X.; Green, D. E.; Schultz, V. L.; Lin, L.; Han, X.; Wang, R.; Yaksic, A.; Kim, S. Y.; DeAngelis, P. L.; Linhardt, R. J. *J. Org. Chem.* **2017**, *82*, 9910.
(36) Bera, S.; Linhardt, R. J. *J. Org. Chem.* **2011**, *76*, 3181.
(37) Dimitrievska, S.; Cai, C.; Weyers, A.; Balestrini, J. L.; Lin, T.; Sundaram, S.; Hatachi, G.; Spiegel, D. A.; Kyriakides, T. R.; Miao, J.; Li, G.; Niklason, L. E.; Linhardt, R. J. *Acta Biomaterialia* **2015**, *13*, 177.
(38) Wu, Z. L.; Huang, X.; Ethen, C. M.; Tatge, T.; Pasek, M.; Zaia, J. *Glycobiology* **2017**, *27*, 518.
(39) Willén, D.; Mastio, R.; Söderlund, Z.; Manner, S.; Westergren-Thorsson, G.; Tykesson, E.; Ellervik, U. *Bioconjugate Chem.* **2021**, *32*, 2507.
(40) Schiller, J.; Lemmnitzer, K.; Dürig, J.-N.; Rademann, J. *Biol. Chem.* **2021**, *402*, 1375.
(41) (a) Tran, V. M.; Nguyen, T. K.; Sorna, V.; Loganathan, D.; Kuberan, B. *ACS Chem. Biol.* **2013**, *8*, 949. (b) Mausam, K.; Quintero, M. V.; Raman, K.; Tran, V. M.; Kuberan, B. *Methods Mol. Biol.* **2015**, *1229*, 517. (c) Tran, V. M.; Victor, X. V.; Yockman, J. W.; Kuberan, B. *Glycoconjugate J.* **2010**, *27*, 625.
(42) Victor, X. V.; Nguyen, T. K. N.; Ethirajan, M.; Tran, V. M.; Nguyen, K. V.; Kuberan, B. *J. Biol. Chem.* **2009**, *284*, 25842.

(43) Piperno, A.; Zagami, R.; Cordaro, A.; Pennisi, R.; Musarra-Pizzo, M.; Scala, A.; Sciortino, M. T.; Mazzaglia, A. *J. Inclusion Phenom. Macrocyclic Chem.* **2019**, *93*, 33.
(44) Manzi, G.; Zoratto, N.; Matano, S.; Sabia, R.; Villani, C.; Coviello, T.; Matricardi, P.; Di Meo, C. *Carbohydr. Polym.* **2017**, *174*, 706.
(45) Galderma, S. A.; Boiteau, J.-G. European Patent WO2015044455, **2017**.
(46) (a) Saletti, M.; Paolino, M.; Ballerini, L.; Giuliani, G.; Leone, G.; Lamponi, S.; Andreassi, M.; Bonechi, C.; Donati, A.; Piovani, D.; Schieroni, A. G.; Magnani, A.; Cappelli, A. *Pharmaceutics* **2022**, *14*, 1041. (b) Paolino, M.; Varvarà, P.; Saletti, M.; Reale, A.; Gentile, M.; Paccagnini, E.; Giuliani, G.; Komber, H.; Licciardi, M.; Cappelli, A. *J. Appl. Polym. Sci.* **2023**, *40*, e53300.
(47) Crescenzi, V.; Cornelio, L.; Di Meo, C.; Nardecchia, S. *Biomacromolecules* **2007**, *8*, 1844.
(48) Martini, M.; Hegger, P. S.; Schädel, N.; Minsky, B. B.; Kirchhof, M.; Scholl, S.; Southan, A.; Tovar, G. E. M.; Boehm, H.; Laschat, S. *Materials* **2016**, *9*, 810.
(49) Hu, X.; Li, D.; Zhou, F.; Gao, C. *Acta Biomaterialia* **2011**, *7*, 1618.
(50) Pasale, S. K.; Cerroni, B.; Ghugare, S. V.; Paradossi, G. *Macromol. Biosci.* **2014**, *14*, 1025.
(51) Takahashi, A.; Suzuki, Y.; Suhara, T.; Omichi, K.; Shimizu, A.; Hasegawa, K.; Kokudo, N.; Ohta, S.; Ito, T. *Biomacromolecules* **2013**, *14*, 3581.