Click Chemistry – What’s in a Name? Triazole Synthesis and Beyond

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Abstract: The environmentally amiable route to carbon–heteroatom bond formation, described by Sharpless as ‘click chemistry’, has become known as a fast, efficient, and reliable approach to the synthesis of novel compounds with desired functionalities. Readily available starting materials must be used in this methodology and they should be essentially inert to most biological and organic conditions, including water and molecular oxygen. In this review, we cover reactions included in this label such as cycloadditions, nucleophilic ring-opening reactions of strained cycles, and amide synthesis, as well as their applications in organic synthesis, molecular biology, macromolecular chemistry and materials science.

1 Introduction

Modern research in organic chemistry and pharmacology requires the preparation of structurally complex molecules through a rational design; furthermore, if these molecules are to have any practical applications, the synthetic route should be both synthetically and economically viable. Although combinatorial chemistry emerged as a powerful tool to provide extensive libraries of compounds, applications in the pharmaceutical field usually depend strongly on the success of individual reactions. In that sense, a change in the way of considering synthetic challenges was needed in order to provide reliable reactions; this change took place in 2001 when the term ‘click chemistry’ was coined by Sharpless in an attempt at obviating the need for reliable reactions; they should also make use of readily available starting materials, environmentally friendly conditions (water as solvent, or solvent-free conditions), and should avoid chromatographic isolations. Usually, reactions included in such a group have a large thermodynamic driving force and involve the formation of a carbon–heteroatom bond. A few synthetically useful reactions fulfill such criteria: opening of strained rings such as epoxides and aziridines, oxygen additions to carbon–carbon double bonds (epoxidations), and 1,3-dipolar cycloadditions.

Click chemistry has been especially successful in the preparation of 1,2,3-triazoles by a 1,3-dipolar cycloaddition between azides and alkynes. The original reaction, known as the Huisgen cyclization, involved a thermal treatment of both reagents and afforded the corresponding triazoles with a complete lack of regioselectivity, as a 1:1 mixture of the 4- and 5-substituted derivatives. Nevertheless, the discovery that this reaction can be efficiently catalyzed with copper(I) salts to give exclusively the 4-substituted regioisomer resulted in an excellent procedure for the preparation of such heterocycles.

The interesting properties of the triazole scaffold, not only from a synthetic point of view, but also in the context of biological and pharmacological applications, led to a significant increase in click chemistry research. Furthermore, the triazole ring was shown to be compatible with a plethora of functional groups, as well as exhibiting good stability under several reaction conditions. Thus, much effort has been devoted to the preparation of triazole-containing peptides, oligosaccharides, and natural product analogues, and an enormous development in click chemistry has been achieved in the last few years. The preparation of dendrimers, polymeric materials, liquid crystals and potential pharmacophores has been achieved through this revolutionary concept. Not surprisingly, this modified triazole synthesis has become the paradigm of click chemistry in modern literature and both terms are now invariably associated, even though Sharpless’ seminal ideas offer promising perspectives on other synthetic platforms.

In this review, we cover recent applications of click chemistry, particularly those involving the opening or formation of strained rings, as well as 1,2,3-triazole formation,
while highlighting their connection to macromolecular chemistry and drug-candidate searches.

2 **Click Synthesis of Epoxides and Aziridines**

Epoxides and aziridines are highly valuable intermediates in organic synthesis, obtained mainly via oxidative processes from olefins. The reaction of such intermediates with nucleophiles in a selective fashion is a typical example of a click chemistry reaction.

α,β-Unsaturated acids and amides also undergo aminohydroxylation and dihydroxylation processes in a *click* fashion (good yields and extremely easy isolation) to afford the corresponding vicinal aminoalcohols or diols, respectively.

A *click* example for the preparation of aziridines starting from olefins was reported by Siu and Yudin (Scheme 1).

Thus, a series of aziridines were prepared by an electrochemical process, in moderate to good yields, and the reaction could be scaled up to a multigram level. With this green procedure, hazardous oxidants and metal additives are avoided.

Scheme 1

Voronkov et al. accomplished the practical and scaleable synthesis of isomeric limonene aziridines starting from commercially available limonene oxides, compounds of interest as key chiral intermediates. A 1:1 mix-
ture of commercially available limonene oxides 1 was subjected to sodium azide mediated stereoselective opening of the epoxide ring, affording the corresponding isomeric α-azido hydroxy derivatives 2 and 3 (Scheme 2). Treatment of the mixture with triphenylphosphine at room temperature via a pseudo-Staudinger reaction\(^{29}\) allowed a kinetic resolution, as the tertiary azide underwent a much slower reaction. Acid–base extraction followed by vacuum distillation afforded pure 4; harsher conditions (refluxing 1,4-dioxane) gave access to the diastereoisomeric azide 5.\(^{28}\)

![Scheme 2](image)

A different approach for the preparation of aziridines was reported by Fioravanti and co-workers,\(^{30}\) using, as starting materials, the functionalized enones 6, which were either commercially available or readily synthesized (Scheme 3). The aziridination step was carried out by treatment of the enones with arylsulfonyloxycarbamates in the presence of an inorganic base such as calcium oxide. These reactions proceeded in high yield, with high purity, and the results were not dependent upon the substituents of the aminating reagent; furthermore, aziridines 7 could be used without chromatographic purification.

![Scheme 3](image)

The prepared aziridines were then transformed into the corresponding alkenyl aziridines 8 via a Wittig reaction with high conversion and excellent \(E\)-stereoselectivity (up to 99%).\(^{30}\)

### 3 Nucleophilic Opening of Strained Rings: Epoxides and Aziridines

The stereoselective opening of strained rings, such as epoxides, aziridines and aziridinium ions, is a valuable synthetic tool for the preparation of more complex derivatives. In this context, Wong’s group reported a versatile methodology for the rapid discovery of enzymatic inhibitors, where the key step is an amine-mediated opening of epoxides in an aqueous medium.\(^{31}\)

The epoxide-containing core, 12, was prepared from commercially available epoxide 9 as depicted in Scheme 4. The epoxide moiety in the starting material underwent ring opening upon treatment with \(p\)-thiocresol in basic medium to afford derivative 10; attack of the thiolate reagent took place selectively at the less-hindered carbon of the epoxide moiety. Next, the Boc protective group was removed under acidic conditions and the transient free amino group was converted into the tetrahydrofuran-3-yl carbamate 11. Finally, the epoxide moiety was regenerated by use of trimethylxonium tetrafluoroborate, followed by treatment with potassium carbonate to give 12.\(^{31}\)

Epoxy 12 was subjected to ring opening with a series of primary amines to afford a small library of derivatives 13 that were screened as enzymatic inhibitors against HIV-1 PR and mutant V82A proteases. Compounds bearing either 2-amino-\(p\)-cresol or 4-phenoxyanilino moieties showed good inhibitory properties against both wild and mutant proteases.

Bulkier substituents in the amino residue were prepared starting from commercially available 9 and following a modified synthetic approach (Scheme 5),\(^{31}\) where one of the key steps was again an amine-mediated epoxide opening. In this case, the first step was the epoxide opening of 9 using a primary amine, followed by tosylation of the amino residue of 14 with \(p\)-methoxybenzenesulfonyl chloride. Removal of the protective group of 15, followed by introduction of the tetrahydrofuranyl moiety as indicated in Scheme 4, afforded derivatives 16 (Scheme 5), which exhibited potent viral protease inhibition in the nanomolar range (Table 1).

Fringuelli’s group has carried out an exhaustive study of the epoxide opening with numerous nucleophiles and reaction conditions, such as pH and Lewis acid catalysts. Thus, aminolysis,\(^{32}\) iodolysis, bromolysis,\(^{33,34}\) azidolysis\(^{33,35,36}\) and thiolysis\(^{37-42}\) of this ring have all been reported by these authors, with the background of these reactions fulfilling the click chemistry criteria.\(^{2}\) In particular, the thiolysis of epoxides was accomplished in a water medium.\(^{40,42}\)
For example, an easy and environmentally friendly thiolysis of substituted epoxides 17 by aromatic thiols 18 was carried out in basic medium and in the absence of a metal catalyst (Scheme 6). Under these conditions, the reaction took place in a regioselective fashion on the less hindered carbon of the epoxide; furthermore, the vicinal diol, a side-product (roughly 5%) obtained by attack of the hydroxide nucleophile, is water soluble, whereas most targeted β-hydroxy aryl sulfides 19 can be recovered by simple filtration in good yields (Table 2).

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To date, aziridines and aziridinium ions have received less attention in organic synthesis than their isomeric epoxides, although this situation seems to be changing. Under the appropriate reaction conditions, these intermediates can afford vicinal diamino moieties, a structural motif of particular relevance.

Usually, the opening of aziridines that bear no electron-withdrawing group on the nitrogen atom involves the use of acidic activating agents. Recently, Krasnova and Yudin reported the preparation of novel chelating agents where the key step was the opening of an aziridine ring with nitrogen nucleophiles such as hydrazine or a tetrazole derivative (Scheme 8). Racemic aziridine derivative 23 was prepared from the corresponding epoxide in a manner similar to that described in Scheme 2, in a two-step process: opening of the epoxide with sodium azide was followed by ring closure to the aziridine motif by treatment with triphenylphosphine.

Compound 23 was subjected to opening using two different nucleophiles. Treatment with hydrazine afforded transient 24 that was used for the next step without further purification; reaction with pentan-2,4-dione in refluxing ethanol afforded pyrazole derivative 25. Resolution of this compound was carried out using (S)-mandelic and (S)-camphorsulfonic acids, and enantiomerically pure 25 could be condensed with different aldehydes; however, the corresponding ligands showed limited stability.

When the opening of aziridine 23 was carried out with 5-phenyl-2H-tetrazole, derivative 26 was obtained as a racemic mixture; enantiomerically pure forms of 26 could be isolated upon resolution with (S)-mandelic and (S)-camphorsulfonic acids. Condensation of the S,S enantiomer with 2,6-diformyl pyridine afforded the dimeric ligand 27 (Scheme 8), which was complexed with copper salts and showed high diastereoselectivity in the styrene cyclopropanation reaction. Reaction of (R,R)-26 with 1,4-dibromopropane gave access to tertiary amine 28 in moderate yield.

Sharpless and co-workers have exploited the click opening of aziridinium ions with different nucleophiles. Unlike aziridine moieties, these useful synthetic intermediates can be opened at moderate temperatures, and at neutral and even basic pH. For example, pyrazolidin-3-ones were prepared using a spontaneous aziridinium ring opening as the key step. Reaction of the oxirane-containing ester 29 with secondary amines afforded derivatives 30 (major) and 31 (minor), that could be separated by crystallization. However, separation of the regioisomers is not necessary, as mesylation of the free hydroxyl group in the crude reaction leads, in both cases, to the aziridinium ion 32. The

**Table 2**

<table>
<thead>
<tr>
<th>Epoxide 17</th>
<th>Aryl sulfide 18</th>
<th>Yield (%)</th>
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**Scheme 8**

![Diagram](image11.png)
latter undergoes a spontaneous nucleophilic displacement by chloride ion to afford the building block 33 (Scheme 9), which is a valuable starting material that has been used in the preparation of pyrazolidin-3-ones, β-lactams or benzodiazepines (Figure 1).

![Scheme 9](image)

Figure 1

NR₂ = morpholine, diallylamino
R₁ = t-Bu, CH₂C=CH, n-Bu

4 Huisgen 1,3-Dipolar Cycloadditions

The 1,3-dipolar cycloaddition that involves azido and alkyne (terminal or internal) derivatives is known as the Huisgen cyclization, and gives access to 1,2,3-triazoles as a mixture of 1,4- and 1,5-regiosomers (Scheme 10). The lack of selectivity can be explained by the similarity in activation energies for both processes.

Such intrinsic features of the Huisgen cycloaddition make it unsuitable for being considered as a click reaction. Nevertheless, the observation that copper(I) salts promote faster (up to 10⁷ times) and regiospecific couplings between terminal alkynes and azides allowed for the rapid development of this reaction; these results were reported independently by the groups of Sharpless and Meldal. Moreover, the cycloaddition is usually carried out at room temperature in aqueous media, and is compatible with most common functional groups present in the molecules.

Usually, the source of copper(I) is the reduction of copper(II) sulfate in the presence of sodium ascorbate, although some other conditions have been reported, such as copper(I) salts, copper(I) complexes, and copper(I)-stabilized derivatives.

The accepted mechanism, shown in Scheme 11, involves the initial formation of a π complex between a terminal alkyne and copper(I), thereby lowering the pKₐ value of the alkyne. Under these conditions, the acetylene derivative is acidic enough to be deprotonated in aqueous medium.

![Scheme 11](image)

This copper(I) acetylide evolves to a copper(I) adduct upon attack of the organic azide, and then undergoes an intramolecular cyclization to give a copper-containing 1,2,3-triazole. Final protonation regenerates the catalyst and gives the 4-substituted 1,2,3-triazole derivative. Overall, the postulated mechanism is a stepwise, not a concerted, pathway.

Recently, Sharpless and co-workers demonstrated that ruthenium complexes such as Cp*RuCl(PPh₃)₂ catalyzed the Huisgen 1,3-dipolar cycloaddition; interestingly, the observed regioselectivity was reversed when compared with related copper(I) catalysis. A tentative mechanism suggested by the same authors involves a six-membered ruthenacycle intermediate, which undergoes reductive elimination, affording a 5-substituted triazole derivative. Unlike in the case of the copper(I)-mediated reaction, both terminal and internal alkynes can participate, which would mean that the formation of ruthenium acetylides can be discarded as a hypothesis.

Weinreb’s group reported the preparation of 5-substituted 1,2,3-triazoles by reaction of β-tosylethylazide with alkynes in the presence of ruthenium catalysts in refluxing benzene (Scheme 13). When the standard copper(I)-catalyzed protocol was used, it was found that reaction of β-tosylethylazide with terminal alkynes led to the expected 4-substituted 1,2,3-triazoles.
As an attempt at improving the catalysis for 1,3-dipolar cycloaddition reactions between azides and terminal alkynes, active and stable nanometric copper clusters have been proved to be efficient ligand-free catalysts. These clusters are prepared with a narrow cluster-size distribution as a stable solution by reducing copper(I) chloride in solution with tetraoctylammonium formate.

To prove the efficiency of this new catalyst, the kinetics of a model reaction between benzyl azide and prop-2-yn-1-ol catalyzed by copper shavings, copper powder, copper nanoclusters, and copper(II) sulfate/ascorbate were monitored. Copper nanocluster catalysis displayed the highest activity of the four systems tested, affording 100% conversion after 18 hours, and this result was in good agreement with the increased surface area of the nanoclusters. The specific surface area of the clusters and the powder was 168 m²/g and 0.15 m²/g, respectively.

An extended application of this reaction to the synthesis of electronic and optoelectronic materials is the coupling of azides with ruthenium-containing alkynyl biosensors. The corresponding di(ruthenium)-containing click product is obtained in excellent yields.

With the aim of exploring the feasibility of the click chemistry with ynamides and azides, coupling between N-benzyl-N-tosylynamide and N-Boc-2-azidoethyamine was also tested. This approach was broadened to potentially bioactive compounds, and carbohydrates and amino acids containing azido groups were clicked with a series of ynamides to yield the corresponding cycloadducts. Nevertheless, when the click Huisgen cycloaddition was attempted between copper(I) acetylides and sulfonyle azides, N-acylsulfonamides were isolated in aqueous medium, whereas amidines were obtained in the presence of secondary amines.

Another unexpected reaction was reported by Whiting and Fokin to take place between phenylacetylene and p-toluenesulfonyl azide. Despite obtaining the corresponding 1-sulfonyl-4-phenyl-1,2,3-triazole, the only isolated product was a cyclobutene derivative. Furthermore, when the reaction was accomplished in the presence of N-benzylideneaniline, an azetidinimine was obtained, together with a small amount of the 1,4-disubstituted triazole.

A catalyst-free alternative to carry out the covalent modification of biomolecules in living systems consists of a strain-promoted [3+2] cycloaddition. Following this methodology, Agard et al. reported the synthesis of triazole compounds by reaction of azides and cyclooctyne derivatives under physiological conditions.

The click Huisgen 1,3-dipolar cycloaddition has also been extended to the synthesis of tetrazoles when organic azides and nitriles are used. Upon simple heating of neat p-toluenesulfonyl or acyl cyanides and with one equivalent of various unhindered azides, quantitative conversion to the corresponding 1-alkyl-tetrazole derivatives was achieved (Scheme 14). The reaction was run neat, there were no side products, yields were quite high and isolation was simple. In all cases, the reaction was highly regioselective and only one isomer was observed. Unfortunately, bulky azides and aryl azides are not appropriate for this reaction, and only certain highly electron-deficient nitriles are good enough as dipolarophiles to engage organic azides in an intermolecular fashion.

### 4.1 Supramolecular Click Chemistry

#### 4.1.1 Dendrimers

Dendrimeric materials are extremely attractive candidates for a variety of surface-active applications, such as multivalent binding sites for interaction with biological receptors and cells surfaces in the construction of targeted drug delivery systems. Dendrimers contain three distinct...
structural parts, namely the core, end-groups, and branched units connecting core and periphery. These materials have been synthesized by methodologies based on both convergent and divergent routes. The convergent approach, introduced by Fréchet’s group, introduces the core scaffold in the final step. This method, on the one hand, enables the introduction of the desired functionalities in the structural building blocks, and on the other hand, implies fewer coupling reactions, more exact macromolecular architectures, greater monodispersity, and greater control over the placement of desired functionalities. In contrast, the divergent approach allows for the formation of the dendrimeric structure by way of iterative additions to the core scaffold.

It happens that the Huisgen 1,3-dipolar cycloaddition is a simple and reliable procedure for the efficient synthesis of chemically differentiated dendrimers. The methodology is characterized by 1,4-regiospecific 1,2,3-triazole formation, water tolerance, and acceptance of a wide range of functionalities. There are three strategies for triazole dendrimers: coupling reaction between a dendron azide and a dendron alkyne, between a dendron azide and polyalkynes or between a dendron alkyne and polyazides. In this context, polyvalent dendrimeric peptides that may be useful in the preparation of synthetic vaccines have been accessed via an efficient Huisgen 1,3-dipolar cycloaddition. Thus, Rijkers and co-workers accomplished the microwave-promoted coupling of polyvalent alkynes and azides in the presence of copper(II) and sodium ascorbate (Scheme 15). Using these conditions, the tetravalent dendron 46 was coupled with the azido-containing amide 47 to afford derivative 48. Dendrimeric cycloadducts were isolated in moderate to good yields (50–95%).

Sharpless, Hawker, and co-workers used the Huisgen 1,3-dipolar cycloaddition of dendrons containing azide (49) and alkyne (50) moieties to obtain third-generation dendrimer 51 as a biocompatible building block (Scheme 16). It is noteworthy that, as indicated above, Huisgen cycloaddition reactions are compatible with a plethora of functional groups; for instance, as depicted in Scheme 4, the reaction is not hindered by the presence of free hydroxyl groups and ester moieties.

The introduction of mannose and coumarin units to the periphery allowed for the preparation of unsymmetrical dendrimers that proved to be efficient agents for the inhibition of hemagglutination.

Propargyl-functionalized Fréchet-type dendrons 52, that is, poly(benzyl ether) dendrons, have been applied to the convergent synthesis of dendrimers using triiodal azide core 53 or azide-focal-point-functionalized Fréchet-type dendrons 55. Reactions were carried out in aqueous N,N-dimethylformamide, under the standard copper-catalyzed Huisgen reaction conditions, and the corresponding symmetrical and unsymmetrical triazole-containing dendrimers were obtained in good yields (Scheme 17).

Polyamidoamine-based dendrimers, so-called PAMAMs, represent a new class of macromolecular structures, and they are sometimes referred to as ‘dense star’ polymers.

A standard synthesis of these polymers is based on a divergent approach that involves a Michael addition of methyl acrylate and the amine core to afford the ester moiety, which is subjected to saponification and coupling with a diamine; subsequent iteration leads to the title compounds. These polymers were recently found to solubilize water-insoluble drugs and to promote their transport through biological membranes. Furthermore, the presence of a large number of exo-functional groups might allow their use as molecular biosensors. A high-yielding synthesis of this interesting family of dendrimers was recently achieved by Lee et al., in which the azido moieties were efficiently connected to the bis(alkyne) core unit 58 via a copper(I)-catalyzed Huisgen reaction to furnish PAMAM dendrimers 59 in 99% yield (Scheme 18).

Introduction of carbohydrate units to dendrimeric structures might lead to a glycoconjugate that could participate in recognition processes with biological receptors. However, synthetic routes described so far for the preparation of glycodendrimers usually involve long syntheses that afford unprotected coupled sugar scaffolds. In this context, Riguera’s group accomplished the anchoring of a series of carbohydrate-derived acetylenes into azido-terminated dendrimers through regioselective 1,2,3-triazole formation (Scheme 19); the reported dendrimers incorporated up to 27 monosaccharidic units (L-fucose, mannose, and lactose), with yields up to 92%. The inherent conditions of this reaction (environmentally friendly, regioselective, high-yielding) make this dendrimer derivatization a good candidate for the preparation of glycoconjugates of potential biological and pharmacological interest.
Scheme 16

Cu(Ph₃P)₃Br (2 mol%)/acetylene + Ph₂NEt, THF, 50 °C

Scheme 17

Synthesis 2007, No. 11, 1589–1620 © Thieme Stuttgart · New York
Fréchet’s group has also carried out the preparation of linear dendrimer-containing polymers using click chemistry. The sizes and shapes of these polymers can be modulated; the existence of a linear architecture might improve their use in some nanoscale applications, such as catalysis or molecular electronics. Furthermore, sometimes the spherical shape of a classical dendrimer is not appropriate for some applications. The approach used by
this group was to start from a linear polymer with pendant reactive groups (for example, alkyne moieties) and to carry out the coupling with an azido derivative. A dendronized polymer was obtained from the first-generation dendron 65 and poly(vinyl acetylene) 66 in quantitative yield, as depicted in Scheme 20. The same kind of reaction was also reported to occur in almost quantitative yield (> 98%) for third-generation azide-containing dendrons.

Scheme 20

4.1.2 Polymers

The control of the molecular structure, and particularly the chemical functionalities, of polymers is a pivotal task in modern polymer synthesis, because complex macromolecules are needed in the rapidly growing fields of nanotechnology and nanobiotechnology. In this context, click chemistry provides a powerful tool for the selective modification of copolymers, and the copper-catalyzed [2+3] Huisgen cycloaddition has provided access to triazole-containing derivatives.

Carroll et al. reported the preparation of flavin-derived polymers with potential analytical applicability, for example as potential sensors. Nucleophilic displacement of the chlorine atoms in chloromethylstyrene copolymer 68 with sodium azide, followed by copper(I)-mediated Huisgen cycloaddition with alkyne derivative 70 afforded the flavin-functionalized polymer 71 (Scheme 21). It is remarkable that the triazole scaffold has a strong dipolar moment and subsequently can establish hydrogen bonds with hydrogen donors; at the same time, they provide some hydrophilicity in the system while being stable under biological conditions. These specifically flavin-functionalized polymers displayed reversible redox-modulated recognition with complementary 2,6-diamidopyridine (DAP)-derived units, the binding association between the polymer and DAP being quantified through the use of fluorescence spectroscopy.

Polystyrene derivatives analogous to 68 have been converted into a triazolymethyl acrylate (TMA) resin as a solid support for the efficient parallel synthesis of differently substituted tertiary amines. The nucleophilic substitution of a Merrifield-type resin with sodium azide allowed for the formation of azide-substituted polystyrene 72. The subsequent 1,3-dipolar cycloaddition with commercially available propargyl acrylate was conducted in N,N-dimethylformamide/tetrahydrofuran in the presence of N,N-diisopropylethylamine and a catalytic amount of copper(I) iodide, leading to the TMA resin 73 (Scheme 22). Then, N-benzyl-N-methyamine was added in N,N-dimethylformamide and the aminopropionate 74 was alkylated with 2,6-dichlorobenzyl bromide. Final cleavage to the TMA resin, induced by basic medium, led to the tertiary amine 75 in a 68% yield, with the progression of the cycloaddition being monitored by IR spectroscopic methods.

Electrooptic polymers have been generated from the click Diels–Alder reaction between maleimide-containing nonlinear optical (NLO) chromophores and polymers that possess pendant anthryl-containing diene moieties. This synthetic approach is very mild, versatile, quantitative, and free of ionic species and catalysts.

The bromine chain-ends of a polystyrene prepared using atom-transfer radical polymerization were successfully transformed into various functional end-groups (ω-hydroxy, ω-carboxyl and ω-methylyvinyl) by a two-step path-
The derivatization of aliphatic polyesters by click reactions under very mild conditions allowed for the grafting of a wide range of functional groups onto poly(ε-caprolactone) without any protection or deprotection reactions.105 α-Chloro-ε-caprolactam (82) was converted into azido-containing polyester 84 through two different synthetic routes (Scheme 24): by copolymerization with ε-caprolactam followed by displacement of the chlorine atom by sodium azide, or by initial displacement of the chlorine atom in the monomer to give 85 and final copolymerization of the latter with ε-caprolactam to afford 84.

This azido-containing polymer was then subjected to click cycloaddition with propargyl acid derivatives in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to afford grafted triazoles 86 under mild conditions and in short reaction times, with IR spectroscopic monitoring of reaction progression. Thus, this methodology provides a robust synthetic pathway for grafting a plethora of functional groups onto a polymeric structure, either cationic, anionic or neutral, including labile lactic acid. The authors claim that the title polymers might be of interest in drug delivery and gene therapy.105
properties differed as a result of varying degrees of polymerization and perhaps a different distribution of triazole regioisomers.

Another important tool for the synthesis and use of macromolecules in fields ranging from biomedical devices to nanotechnology is the development of selective synthetic approaches that are orthogonal to the diverse array of functional groups present in many polymeric systems. Thus, by combining copper(I)-catalyzed 1,3-dipolar cycloaddition with other families of chemical transformations, the synthesis of macromolecule 96 and the cascade methodology for the preparation of 102 were recently simultaneously reported by Hawker and co-workers. 108

In the first case, an 8:1:1 terpolymer was prepared from styrene, p-(trimethylsilylacetylene)styrene, and 2-(trimethylsiloxyethyl)methacrylate using living-free-radical polymerization, and deprotection of both protecting groups with tetrabutylammonium fluoride (TBAF) gave difunctional macromolecule 95 \[ M_n = 31900 \text{ amu; } \text{PDI} = 1.16 \text{ (PDI = polydispersity index)} \]. 108 The acetylene and hydroxyl functionalities present in 95 afforded reactive sites that could couple simultaneously with both methyl 4-(azidomethyl)benzoate and the acetonide-protected bis-MPA [MPA = 2,2'-bis(hydroxymethyl)propionic acid] anhydride in the presence of copper(I) and N,N-diisopropylethylamine (Scheme 26). The orthogonally functionalized macromolecule, 96 \( (M_n = 39900 \text{ amu; \text{PDI} = 1.26}) \)
PDI = 1.19), was obtained by transforming the terminal acetylene group into a methyl benzoate substituted triazole motif and by esterification of the hydroxyl group (Scheme 26).\textsuperscript{108}

For the synthesis of 102 (Scheme 27),\textsuperscript{108} to a solution of a 9:1 random copolymer of \textit{tert}-butyl acrylate and \textit{N}-acyryloxy succinimide (97) in tetrahydrofuran were added propargylamine (98) and azo compound 99. In this case, copper(I) acts as a catalyst for the \textit{click} reaction between 98 and 99, while the \textit{N},\textit{N}-diisopropylethylamine catalyzes the amidation reaction of 97 with either 98 or the intermediary \textit{click} adduct 101. This sequence of reactions was monitored by GPC/HPLC and \textsuperscript{1}H NMR spectroscopy, which showed that all reactions reached completion after 16 hours at 50 °C and that the amidation chemistry and \textit{click} reactions occurred at approximately the same rate.\textsuperscript{108}

Conjugated polymers have been synthesized through the copper(I)-catalyzed 1,3-dipolar cycloaddition of azides and alkynes.\textsuperscript{109} The novel polymers obtained, linked via 1,4-disubstituted 1,2,3-triazole units, have interesting luminescent properties. Fluorene-derived 103 reacted with derivatives 104 and 105 in a degassed tetrahydrofuran–acetonitrile solution. The reaction started with the addition of copper(II)/copper(0) and trisbenzyltriazolylamine as a ligand (Scheme 28). The polymerization reactions were stopped at both ends of the growing chain by the ad-

![Scheme 27](image-url)
dition of excess azidobenzene as stopper, and after two hours, 2-ethynyl pyridine. The reactions were sufficiently fast to proceed at lower temperatures; when the reaction was accomplished at these low temperatures and under dilute conditions, polymers with high molecular mass and a more transparent appearance were obtained.\(^{109}\)

Copper(I)-catalyzed reactions have also been used successfully in macromolecular chemistry for the preparation of stable and thermoreversible cross-linked organogels through coupling between alkynes and azide-containing organogelators in a 10:1 ratio (Scheme 29).\(^{110}\) This method allows for the modification of some of the parent gel’s properties, while maintaining the overall structure and the thermoreversibility.
Poly(vinyl alcohol)-based hydrogels have been prepared using this biocompatible copper(I)-catalyzed version of Huisgen [3+2] cycloaddition by two different cross-linking strategies. Firstly, poly(ethylene glycol) diazide can be used as a cross-linker for the alkyn-functionalized poly(vinyl alcohol). Moreover, a multicomponent reaction involving two poly(vinyl alcohol)s plus amine-functionalized alkyn and azide substrates can also be considered (Scheme 30). Hydrogel properties are expected to be highly dependent upon the structure of the polymer components. The level of 100% gelation was not achieved, probably because of incomplete 1,3-cycloaddition cross-linking reactions, or perhaps because of some side reactions that could occur as a result of the difficulty in achieving the proper mixing of reagents. The reaction appears to be quite fast, and the gels were formed essentially immediately upon addition of the copper catalyst to the polymer solution.

Scheme 30

Synthetic glycopolymers are currently the subject of increasing attention; the reason is that, while simple monosaccharides can only establish weak interactions with protein receptors and elicit only a weak response to in vivo events, these interactions can be enhanced if carbohydrates are used in the form of macromolecular glycoconjugates. In fact, natural carbohydrate–protein interactions take place through higher-order oligomeric structures, and this observation is known as the 'cluster glycoside effect'.

Du’s group reported the convergent synthesis of C$_3$-symmetric (1→6)-N-phthalimido-β-D-glucose-derived octadecasaccharide 116 in a 62% yield (Scheme 31); the title compound was obtained by the coupling of azide 114, comprised of a hexasaccharide and an aliphatic spacer, with alkyn 115 in the presence of copper(II) sulfate (2–5 mol%) and sodium ascorbate (5–10 mol%) in aqueous tetrahydrofuran at 50–60 °C. The final octadecasaccharide 116 showed good antitumor activity in preliminary mouse tests.

Scheme 31

Triazole rings were also made in the direct assembly of functionalized sugars by reaction of alkynyl and azido glycosides. The assembly proceeded to completion in reaction times no longer than 45 minutes when organic-soluble copper(I) complexes such as tris(triphenylphosphine)copper(I) bromide [(Ph$_3$P)$_3$·CuBr] and triethylphosphite–copper(I) iodide complex [(EtO)$_3$P·CuI] were used under microwave irradiation.

The functionalization of polyalkyne materials by co-clicking reactions of appropriate mixtures of mannose- and galactose-based azides has been reported as a simple and efficient route to synthetic libraries of materials that differ only in the nature of the sugar moiety. In this procedure, (PPh$_3$)$_3$CuBr and N$_3$N-diisopropylethylamine were used as the catalytic system. $^1$H NMR and FT-IR analysis confirmed that the conversion of the alkyn groups of 117
into triazoles was achieved in nearly quantitative yield, and the molecular weight distribution of glycopolymers 120 was not significantly variable (Scheme 32).

A visibly fluorescent tag was also co-clicked into a polymeric scaffold via fluorescent azide 121 (Scheme 33).

The resulting polyvalent ligands 123 and 124 are quite useful in protein–carbohydrate binding interaction studies. The reported glycopolymers were subjected to binding studies with model lectins, that is, a series of plant proteins that selectively binds to carbohydrates; in particular, concanavalin A and *Ricinus communis* agglutinin bind to mannose and galactose, respectively.

6-Azido-6-deoxycellulose (125), obtained by low-temperature tosylation of parent cellulose and subsequent nucleophilic displacement by sodium azide in *N*,*N*-dimethylformamide, was subjected to copper(I)-catalyzed Huisgen cycloaddition with a series of acetylene derivatives. Thus, cellulose analogues 126–128, bearing methoxycarbonyl, 2-aniline, and 3-thiophene motifs (Figure 2), were prepared in a selective and efficient manner.

Tetrazole-containing polymers are also of interest for the production of ion-exchange and chelating resins and superabsorbers. This novel class of polymers can be obtained by means of click chemistry as a post-polymerization modification of polyacrylonitrile derivatives. The starting polymeric materials can be poly(ho-
macroinitiators for the synthesis of block copolymers or as precursors of other chain-end-functionalized polymeric materials.

4.2 Liquid Crystals

The first example of a liquid crystal that incorporated a regioisomeric 4-substituted 1,2,3-triazole in its structure was recently reported. Target compounds were designed to present cholesteric and ferroelectric mesophases through the incorporation of units in the mesogenic core that had the potential to confer such properties. In order to obtain the desired 1,4-disubstituted triazole, azide 129 (or 134) was added to a suspension of alkyne 130 (or 135), copper(I) iodide, and triethylamine in refluxing aqueous ethanol (Scheme 34). After 48 hours, the corresponding chiral liquid crystal was isolated in moderate to good yield (60–90%).

Thermal properties of the final compounds were investigated by using polarizing optical microscopy (POM) and differential scanning calorimetry (DSC). The analysis of the mesomorphic behavior showed that by displacing the 1,2,3-triazole ring to a central core position, the melting point is substantially lowered, thereby favoring the smectic phases.

4.3 Self-Assembled Monolayers

Self-assembled monolayers (SAMs) are of significant interest in interfacial reactions and many technological applications such as sensors, catalysis, microarrays, and molecular electronics. A wider use of SAMs in technology requires, as a first important step, the search for versatile organic reactions that can be performed on them, allowing for the introduction of required functionalities to the surface. For this purpose, click chemistry seems to be a good option.

The 1,3-dipolar cycloaddition of azides and alkynes to form 1,2,3-triazoles has been used to couple monolayer-covered azide-terminated silica substrates with acetylenes. The development of this reaction would permit the attachment of desired functionalities needed for specific surface properties in these alkylsiloxane monolayers. Azide-terminated monolayers were prepared in situ from 11-bromoundecylsiloxane monolayers and were subsequently reacted with three different acetylene compounds, as depicted in Scheme 35. The reactivity of acetylenyl-terminated SAMs on gold towards click chemistry has also been investigated. To a reaction mixture containing the acetylenyl-terminated SAM-coated gold substrates in aqueous ethanol were added an azido compound, copper(II) sulfate, and sodium ascorbate, and the mixture was stirred at room temperature for 12 hours (Scheme 36). This procedure proved to be a mild and efficient way of tailoring surface functionalities.

In a similar manner, the azido groups existing in mixed self-assembled monolayers on gold electrodes from various functionalized alkane thiols and azido alkane thiols, in the presence of copper(I) catalysts, reacted rapidly and quantitatively with terminal acetylenes to form 1,2,3-triazoles via click chemistry (Scheme 37). The coupling reaction proceeded in high yield and under very mild conditions.
Surfaces containing organic azides are excellent platforms for further modification. It is possible to easily control and measure the amount of a surface-bound azide that, once incorporated, reacts quantitatively with acetylene. This action is carried out by monitoring the intense infrared stretching mode of organic azides. The major limitation in these reactions appears to be steric hindrance.

### 4.4 Multicomponent Reactions

The increasing demand for novel biologically active small molecules and the laborious process of lead discovery have made the synthetic efficiency of multicomponent reactions very attractive to organic chemists. In these reactions where more than two starting materials react to form a product which incorporates essentially all of the reagent atoms, the selectivity of procedures and the compatibility between different functional groups represent key aspects to be considered.

Click chemistry, and more specifically, the Huisgen 1,3-dipolar cycloaddition, has emerged as a simple, fast, and efficient approach to synthesize functionalized 1,2,3-triazoles. For obvious reasons, the combination of both these techniques could be an important strategy for the generation of novel compound libraries.

One of the first contributions to the multicomponent variant of the copper-catalyzed Huisgen cycloaddition was reported by van der Eycken. In this process, azides were generated in situ from the corresponding halides, and underwent cyclization with copper(I) acetylenes, furnishing the expected 1,4-disubstituted 1,2,3-triazoles (Scheme 38). Performing both steps under microwave irradiation (100 W and 125 °C) significantly reduced the reaction time. The copper(I) catalyst was prepared by the disproportionation of the copper(0) and copper(II) species. The final products were obtained in roughly 90% yield.

![Scheme 38](image)

Barbas and Ramachary accomplished the preparation of a proline/copper(I)-catalyzed spiropyrimidione-triazole in a 90% yield. This synthesis is based on the stereospecific assembly of simple substrates like phosphorane, aldehydes, cyclic 1,3-diketones, and azides under organo-copper(I) catalysis by means of Wittig/Knoevenagel/Diels-Alder/Huisgen cycladdition reaction sequences in a one-pot fashion (Scheme 39).

The Huisgen cycloaddition has also been combined with the Biginelli multicomponent reaction for the efficient synthesis of libraries of compounds such as 149. This was carried out by performing rapid and high-yielding microwave-assisted azide-acetylene couplings. For this purpose, readily available 6-(azidomethyl)dihydropyrimidones were prepared as key intermediates using a multicomponent approach, and then reacted with a series of terminal acetylenes under copper(I) catalysis. The best results were obtained when microwave irradiation was used, heating at 80–100 °C. This led to an attractive linkage of two important N-heterocyclic pharmacophores (Scheme 40) with four points of diversity that can easily be accessed by choice of the appropriate building blocks.

### 4.5 Creating Drug Candidates under Click Conditions

The traditional process for the design and discovery of a new pharmaceutical drug is both time-consuming and expensive, despite the development of combinatorial chemistry. Thus, the revolutionary click chemistry concept is being exploited in drug and biomedical research as click chemistry constitutes a powerful tool for the rapid discovery of target enzyme inhibitors that exhibit great potency and specificity. In this context, Lee and co-workers prepared a library of triazole compounds that were shown to be potent inhibitors of human \(a\)-1,3-fucosyltransferase VI, a pivotal enzyme which is involved in the catalysis of the final glycosylation step in the biosynthesis and expression of many important saccharides.

Srinivasan and co-workers accomplished the preparation of a vast series of enzymatic inhibitors in processes related to obesity and diabetes. Furthermore, click adducts have been employed, not only for the development of new drug candidates, but also in glycobiochemical studies, such as DNA sequencing or the coupling of ligands to vesicles and liposomes.

The triazole scaffolds are expected to be more than just passive linkers, as they might be associated with biological targets through intermolecular hydrogen-bonding and dipole interactions.

It is reasonable to postulate that the combination of heterocyclic pyrazinones and triazoles could lead to compounds with pharmacological interest, as both structural motifs have been reported to exhibit biological activities. In this context, Kaval et al. considered two different synthetic approaches, the first of which was 1,3-dipolar cycloadditions with acetylene-containing 2(1H)-pyrazinones. In this case, the active catalyst was generated in situ from copper wire and copper(II) sulfate, and the reaction was carried out with microwave irradiation in aqueous tert-butyl alcohol (Scheme 41).

The other approach was a 1,3-dipolar cycloaddition with azide-containing 2(1H)-pyrazinones, which are in equilibrium with the corresponding tetrazoles via an intramolecular cyclization that involves the vicinal pyrimidinic nitrogen (Scheme 42). This equilibrium was found to be shifted to the bicyclic derivative, thus it was assumed that an increase in temperature should reverse the equilibrium to the unstable azide. Nevertheless, microwave-
mediated reaction attempts resulted either in no reaction or in reagent decomposition.

Surprisingly, when the reaction was carried out at room temperature using a tertiary amine as a copper(I) stabilizing agent, the desired substituted triazoles were obtained in moderate to good yields (Scheme 42).

The Huisgen [3+2] cycloaddition has also been employed in the search for inhibitors of acetylcholinesterase (AChE), a pivotal central nervous system neurotransmitter. For this purpose, a special technique, named target-guided synthesis (TGS), has been used; this approach is based on the assembly of two building blocks, with weak affinity for different structural features of the target enzyme, to produce a bivalent inhibitor.

Thus, in situ click chemistry between alkynes and azides with chains of differing length was used, without catalyst but instead in the presence of the enzyme as a template. Under these conditions, only those building blocks which are involved in an interaction with the active site of the en-

Scheme 41
zyme are close enough to each other to react and produce the potent inhibitor. It is remarkable that only syn-derivatives, that is, 1,5-disubstituted triazoles, were formed from among all the possible combinations in the presence of the enzyme; this is in contrast to what happens under thermal Huisgen conditions, wherein, as indicated above, a roughly 1:1 mixture of regioisomers is obtained.48

Some of these compounds exhibit femtomolar inhibition of AChE, the lowest dissociation constants reported to date for non-covalent inhibition of this enzyme.147,148 Triazole derivatives such as 158 (Figure 3) simultaneously mimic the structure of three AChE inhibitors: propidium and decamethronium ions, and tacrine.150,151

The in situ click chemistry approach has also been successfully applied to the formation of HIV-1 protease inhibitors; for this purpose, alkyne 159 and azide 160 were incubated in the presence of the protease, HIV-1-PR (Scheme 43). This protease, which plays an important role in the inhibition of viral replication, itself acts as a template for the reaction and even increases the rate of formation of the anti 1,4-triazole product 161.152

Other examples of an enzyme-mediated preparation of triazole derivatives were reported by the groups of Sharpless153 and Gmeiner154 for the preparation of carbonic anhydrase II inhibitors and dopaminergic agonists, respectively.

Another way of fighting against AIDS infection is the blockage of a viral envelope protein to antigens in the host T-cell surface.155 Gopi et al. carried out the modification of a proline unit with 4-phenyl-1,4-disubstituted 1,2,3-triazole, formed through a click [3+2] cycloaddition reaction.156 This led to a peptide (Figure 4) that binds to the viral protein with an affinity two orders of magnitude greater than that of the parent peptide and, thus, strongly disrupts the interaction of this glycoprotein with the host cell surface.156
In the search for inhibitors of carboxylesterase I (CE-I), an enzyme involved in some important biological processes such as drug metabolism, a series of rhodamine–biotin-tagged forms of (−) and (+)-166 were prepared (Scheme 44). These compounds were obtained by the copper(I)-catalyzed reaction of 164 with an alkyne-derivatized rhodamine–biotin agent 165. Binding studies revealed that association of (−)-166 with CE-I was stable even to protein denaturation.157

Click chemistry has also been applied toward the synthesis of resveratrol (167), a polyphenolic compound found in some plants, such as in the skin of red grapes.158 This compound exhibits some beneficial properties, including cardioprotective, neuroprotective, antiviral, and anti-inflammatory properties,159 so derivatives of resveratrol are of interest as potential biologically active compounds. Pagliai et al. accomplished the preparation of a library of resveratrol derivatives 168–172, wherein the carbon–carbon double bond of the stilbene moiety was replaced with a triazole ring (Figure 5).160 After the evaluation of the cytotoxicity of these compounds, the preliminary biological analysis suggested that some of the compounds screened were more potent cytotoxic and antiproliferative agents than resveratrol itself.

The broad spectrum of biological activities exhibited by vitamin D161 has prompted researchers to carry out the synthesis of analogues such as the triazole-bearing vitamin D.162 Click chemistry allowed for the cycloaddition of a vitamin D side-chain terminal acetylene with phenyl azide, and separately with a vitamin D side-chain azide, to yield the corresponding 1,2,3-triazole analogues of 1-α-hydroxy-vitamin D3 and a dimeric vitamin D derivative (173), in which the monomeric units are linked through a triazole tether (Figure 6).163

Taking advantage of the click strategy, several functionalized resins were prepared and evaluated for the parallel solid-phase synthesis of a series of aryl carboxamides, which frequently serve as key pharmacophoric elements in drugs.164 In this context, Gmeiner and co-workers reported the preparation of a library of solid-supported N-benzyl 1,2,3-triazole carboxamides.165 These compounds...
were prepared as depicted in Scheme 45, in four steps: reductive amination of a benzaldehyde derivative, N-acylation with alkynoic acids, [3+2] cycloaddition, and a final acidic cleavage. All target compounds thus obtained were screened for G-protein coupled-receptor binding, and some demonstrated excellent receptor recognition.165

Another target for click reactions is daunorubicin, one of the most important drugs employed in cancer chemotherapy, whose possible mechanisms of action include, among others, DNA intercalation.166 In order to increase the drug’s DNA binding affinity, dimeric species derived from daunorubicin derivatives offered the possibility of preparing a novel series of anticancer analogues.137 Thus, through click reactions, several dimeric derivatives such as 174 (Figure 7), bearing triazole tethers with differing chain lengths and varying flexibility, were successfully synthesized using (EtO)3PCuI as catalyst. Cytotoxicity studies revealed that shorter linkers presented a stronger activity against cancer cells, with flexibility being another key aspect.

Bitriazolyl compounds 177, which are of interest because of potential use in agrochemical research as well as in materials science, were synthesized through a copper(I)-catalyzed Huisgen cycloaddition by using the azidotriazole 175 and several terminal acetylenes as starting materials, followed by aminolysis of the ester moiety (Scheme 46). Tobacco mosaic virus was used as a model system for testing the antiviral activity of these bitriazolyl products, and some were shown to be even more potent than commercial products used in the treatment of this agricultural pest.167

Another interesting target for the design of new pharmacological drugs is the modulation of protein–carbohydrate interactions; for example, inhibitors of galectins (β-galactoside-binding lectins), might be potential active agents in...
cancer treatment.\textsuperscript{168} Pieters developed a method for the detection of galectins, even mixed with other proteins (Scheme 47).\textsuperscript{169} For this purpose, he used a benzophenone-based photoaffinity label, in which the benzophenone group was bound to the C-3 position of the galactose residue of lactose, and the glucose moiety of lactose was derivatized with a polyether chain that had a terminal azido group. When binding of galectin and the modified disaccharide took place, irradiation of the system resulted in an interaction of the photoaffinity label with the protein, followed by triazole formation, thus enabling galectin detection.\textsuperscript{169}

Another example of protein–carbohydrate interaction studied with triazole derivatives was reported by van der Peet et al.\textsuperscript{170} The authors proved that only \( \alpha \)-configured mannose derivatives bearing triazole substituents could act as substrates for \textit{Leishmania} \( \beta \)-1,2-mannosyltransferases.

4.6 Click Chemistry with Peptides and Proteins

Methods are needed for profiling the activity of enzymes in vivo in order to understand the role that these proteins play in physiological and pathological processes. Activity-based protein profiling (ABPP)\textsuperscript{171} uses active-site-directed chemical probes to determine the functional state of enzymes in complex proteomes, distinguishing active enzymes from their inactive precursors and/or inhibitor-bound form. Scheme 48 illustrates a standard ABPP probe, consisting of a reactive group and one or more reporter tags, the size of the latter being the main limitation for the in vivo application of ABPP probes.\textsuperscript{172} In contrast, click chemistry ABPP allows for the profiling of living cells and organisms by treating them with tag-free azide- or alkyne-modified probes, which are then conjugated \textit{in vitro} to the complementary alkyne- or azide-modified tag under cycloaddition reaction conditions to visualize probe-labelled proteins (Scheme 48).

Cycloaddition-based ABPP is a versatile method that allows, for example, for the in vivo and in vitro labelling of enzymes and for their detection from among the whole proteome; this methodology gives access to in vivo studies to determine whether a certain drug inhibits its target.\textsuperscript{173} The combination of both approaches, ABPP and click chemistry, has contributed to the design and synthesis of potent and selective fatty acid amide hydrolase inhibitors.\textsuperscript{174}

A further approach to aid in the study of protein binding and function is the incorporation of synthetically modified amino acids or peptides into the parent structure.\textsuperscript{175,176} In this sense, model studies have also been performed for the \( C \)-terminal lipidation of proteins by exploiting the Huisgen cycloaddition.\textsuperscript{177}

For instance, Marik and Sutcliffe reported the \( \text{[18F]} \)-labeling of peptides via click conjugation of \( \omega \)-[\( \text{[18F]} \)]-fluoroalkynes to several peptides that bear azide moieties, to furnish the corresponding \( \text{[18F]} \)-labelled target peptides (Scheme 49).\textsuperscript{178} Derivatives such as 180 are widely used as in vivo imaging agents of various physiological and pathological processes, using positron emission tomography.\textsuperscript{178}
An additional example that demonstrates the enormous potential of click chemistry connected with proteins is the preparation of small cyclic peptide analogues that are too strained for ring closure via lactamization. In order to demonstrate such a cyclization of peptides, the triazole analogue 182 of tyrosinase inhibitor 181 (Figure 8) was synthesized.\(^{179}\)

Thus, compound 182 was prepared starting from compounds 185 and 186 (Scheme 50), which were deprotected and coupled to afford the linear tetrapeptide analogue 184 that was then subjected to click conditions and thereby yielded 182.

Carbohydrate-derived azides have also been used in the structural modification of peptides (Scheme 51).\(^{180}\) Thus, coupling of per-O-acetylated glycopyranosyl azides 187 with bromoalkynamide 188 gave the corresponding glycopyranosyl triazole derivative 189.

![Figure 8](image_url)

**Figure 8**

Scheme 50

Scheme 51
The bromine atom in 189 was displaced through nucleophilic attack by the thiol groups of the cysteine units in peptide 190 (Scheme 52). Subsequent deprotection steps yielded the target glycotriazole-functionalized peptide 191.

Biomolecules can be subjected to immobilization in their native state by the use of some click reactions like Diels–Alder and [3+2] cycloadditions. In this context, Sun and co-workers reported the use of a bifunctional poly(ethylene glycol) linker with an alkyne terminal group (Scheme 53) for the immobilization of both proteins and carbohydrates.

### 4.7 DNA as Participant in Click Reactions

Click chemistry has successfully been evaluated as an easy and efficient method for DNA alkylation. The interest in this chemical modification is because of the importance of biological methylation in regulatory mechanisms of gene transcription. Thus, the coupling of the nucleoside analogue 192, which bears an alkyne moiety, with an organic azide, accompanied by covalent bond formation with a DNA fragment mediated by a methyltransferase enzyme, proceeded in an efficient manner and effectively demonstrated the possibility of using DNA in click modifications (Scheme 54).

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**Scheme 52**

**Scheme 53**
The modification of either a 3'- or 5'-terminus or an internal position of an oligonucleotide with a primary alkylamine group is a widely used method for the introduction of additional functional groups into DNA. In this sense, the oligonucleotide 5'-amino-GTT TTC CCA GTC ACG ACG-3' was used to prepare the azido-labeled DNA 194, which by coupling with the alkynyl 6-carboxyfluorescein 195 led to a fluorescent oligonucleotide with high selectivity, yield and stability (Scheme 55). This kind of derivative can be detected with laser-induced fluorescence techniques.

In the search for DNA metallation procedures, in order to increase the conductivity of these modified DNA nanostructures, an efficient and selective method for the depo-

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**Scheme 55**
sition of silver(0) around aldehyde-containing DNA has been achieved.185 Acetylene-labelled nucleotide triphosphates were inserted by using DNA polymerases and the product DNA was employed in a click approach with aldehydo azides; the latter process can be efficiently performed on a polyacrylamide gel.185

5 Concluding Remarks

Click chemistry refers to a revolutionary chemical concept concerning only reliable and efficient reactions. The reason for such a term is the requirement for using valuable, high-yielding, and low-cost reactions as an optimal method for the development of substances of biological interest.

In this context, the copper-catalyzed Huisgen cycloaddition, in particular, has proven to be the best example of click reactions, and there is already an extensive list of reports that describe the use of this practical reaction in the preparation of glycoconjugates, potent glycosidase inhibitors, and even protein and DNA modifications. It follows, then, that the reactions described herein can help researchers to accelerate the comprehension of biological systems and to develop new active substances such as vaccines and pharmaceutical drugs. Significant advances in click chemistry are anticipated.

As additional evidence for the current vitality of this field, after completing the writing of this review, the authors became aware of two recent and insightful reviews focused on selected applications of the archetypal click reaction of azides and alkynes to polymer and materials science.136,187

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