CuAAC: An Efficient Click Chemistry Reaction on Solid Phase

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ABSTRACT: Click chemistry is an approach that uses efficient and reliable reactions, such as Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC), to bind two molecular building blocks. CuAAC has broad applications in medicinal chemistry and other fields of chemistry. This review describes the general features and applications of CuAAC in solid-phase synthesis (CuAAC-SP), highlighting the suitability of this kind of reaction for peptides, nucleotides, small molecules, supramolecular structures, and polymers, among others. This versatile reaction is expected to become pivotal for meeting future challenges in solid-phase chemistry.

KEYWORDS: Click Chemistry, CuAAC, solid-phase, azide, alkyne

1. INTRODUCTION

In 1963, Merrifield1 introduced the concept of solid-phase peptide synthesis (SPPS), reporting the first efficient production of a tetrapeptide on a solid matrix, wherein the peptide chain was grown by covalent attachment of one end to the functionalized support. Thanks to Merrifield’s pioneering work, this concept has become a fully established method in peptide synthesis; however, many other known organic reactions have also been applied on solid phase (SP) supports to address synthetic problems and generate new molecular entities.2,3

Click chemistry4,5 promotes the use of organic reactions that allow the connection of two molecular building blocks in a facile, selective, high-yield reaction under mild conditions with few or no byproducts.4,5 Diels–Alder, Michael addition, pyridyl sulﬁde reaction, oxyme, thiolene, strain-promoted azide–alkyne cycloaddition (SPAAC), and Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC) have all been reported as Click chemistry reactions.5

In the past decade, the CuAAC reaction has emerged as an efﬁcient alternative in SP to replace amide bonds in peptides28 and to generate amino acid triazole derivatives,39 cyclic peptides,30 nucleotides,31–33 and new resins.34,35 Furthermore, this chemistry has the capacity to promote bioconjugation and peptide ligation, stemming from the properties of the triazole linkage as a peptide mimic.

This Review describes the general features and applications of CuAAC on SP (CuAAC-SP) and reveals the suitability of this kind of reaction for the modiﬁcation of peptides36–38 nucleotides,31–33 small molecules,39 supramolecular structures,40,41 and polymers.42

2. GENERAL CONSIDERATIONS REGARDING Cu(I)-CATALYZED AZIDE–ALKyne CYCLOADDITION ON SOLID PHASE (CuAAC-SP)

CuAAC is a type of Huisgen1,3-dipolar cycloaddition based on the formation of 1,4-disubstituted [1,2,3]-triazoles between a terminal alkyne and an aliphatic azide in the presence of copper43,44 and is classiﬁed as a Click Chemistry reaction.5 Click Chemistry was deﬁned by Sharpless et al.4,5 as any chemical reaction that allows high yields, generates no side-products or ones that are easily removed, is stereospeciﬁc, gives physiologically stable products, exhibits a large thermodynamic driving force, and has simple reaction conditions. Research into the synthesis of biomolecules via CuAAC-SP has emerged because of the stability of triazole scaffolds against metabolic degradation.4,5 In this Review, we use the terms CuAAC and Click Chemistry interchangeably.

In 2001, Meldal et al.25,26 developed a method for preparing 1,4-disubstituted 1,2,3-triazoles using Cu(I) salts as a catalyst for the 1,3-dipolar cycloaddition of terminal alkynes to azides on SP at room temperature using organic solvents such as ACN, THF, DCM, toluene, and DMF. Shortly after and using protic polar solvents such as t-butyl alcohol, ethanol or water, Sharpless et al.27 independently reported the same reaction in solution, naming it CuAAC (Scheme 1).

The CuAAC reaction was a breakthrough in triazole chemistry. The reactions of organic azides with terminal alkynes were shown to be accelerated by copper ions and to proceed regioselectively under these conditions, giving the 1,4-disubstituted 1,2,3-triazole regioisomer exclusively. The for-
mation of the 1,4-disubstituted 1,2,3-triazole ring occurs rapidly in the presence of Cu(I). The cycloaddition product is chemically inert and stable toward redox reactions and it has strong dipole moment of $\sim 5$ D, as shown by experimental studies, hydrogen bond accepting capacity, and aromatic character.

Table 1 summarizes the main features of the reaction. In general, several resins with a broad range of hydrophobicity have been used to prepare compounds such as peptides, peptoids, PNA, and nucleotides. Most of these reactions were carried out using a Cu(I) source and polar solvents. CuI, CuBr, CuCl, CuBr(PPh$_3$)$_3$, and [Cu(CH$_3$CN)$_4$]PF$_6$ are the most widely used copper sources, whereas DMF, ACN, THF, DCM, H$_2$O, tBuOH, NMP, DMSO, CHCl$_3$, and MeOH are the most frequently used polar solvents. In some cases, a Cu(II) salt, such as CuSO$_4$$\cdot$$5$H$_2$O, is used instead of Cu(I) salt, and the addition of a reductive reagent such as sodium ascorbate is required to reduce Cu(II) to Cu(I). These reactions can be carried out with or without a base, with reaction times ranging between 4 and 63 h. The application of microwave radiations between 40 and 60 °C decreases the reaction time considerably. The yield of CuAAC-SP is often reported as quantitative. In many cases, the process has been monitored by IR spectros-copy, tracking the disappearance of the characteristic azide band in the IR spectrum. The broad range of reaction conditions has allowed the production of a great variety of final products via CuAAC-SP (Table 1), thereby revealing the versatility of this kind of reaction and its huge potential.

The main variables in CuAAC-SP are the choice of resin, copper catalyst, solvent, and the presence or absence of a base;

Table 1. General Conditions of CuAAC-SP

<table>
<thead>
<tr>
<th>resin</th>
<th>Cu(I) cat.</th>
<th>Cu (equiv)</th>
<th>base</th>
<th>$t_k$ (h)</th>
<th>final product</th>
<th>ref</th>
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<td>peptide</td>
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<td>peptide</td>
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<td>CuBr$^b$</td>
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<td>DIEA, 2,6-lutidine</td>
<td>16-48</td>
<td>resin, peptide, PNA</td>
<td>34, 35, 49, 50</td>
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<td></td>
<td></td>
<td>0.1-2.5</td>
<td>DIEA</td>
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<td>peptide, PNA</td>
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<td>glycodendrimer, nucleotide</td>
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<td>16</td>
<td>resin</td>
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<td>piperidine</td>
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<td>CuSO$_4$$\cdot$$5$H$_2$O$^b$</td>
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<td>TBTA</td>
<td>0.5-5$^c$</td>
<td>nucleotide</td>
<td>109-112</td>
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<td>PNA</td>
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$^a$Polar solvents were used in the most reactions. $^b$Sodium ascorbate was used as Cu(I) stabilizer. $^c$Temperature between 40 and 60 °C or microwave reaction conditions. S.S.: Supramolecular structure.
Table 1 provides an indication of the variables in each category. Both hydrophobic and hydrophilic resins can be used; however, in all cases a polar solvent has been utilized to obtain quantitative results. The copper catalyst is indispensable, and a Cu(I) stabilizer is widely used. Finally, although a base (i.e., DIEA, piperidine, and others) is not imperative, it has been used in most cases to improve performance.

To better understand CuAAC-SP, we describe the current understanding of its mechanism and outline various methods used to synthesizing azide reaction partners. Preparation of the terminal alkyne is not included in this review because this group is usually introduced through conventional SP coupling procedures.48

2.1. Cu(I)-Catalyzed Azide–Alkyne Cycloaddition (CuAAC) Mechanism. The last generally accepted mechanism of the CuAAC reaction is shown in Scheme 2.117,118

![Scheme 2. Cu(I)-Catalyzed Azide–Alkyne Cycloaddition (CuAAC) Mechanism](image)

While the involvement of two Cu(I) ions in the reaction has been appreciated for some time, a detailed mechanism of this reaction has remained elusive. Worrell et al. recently showed that two Cu(I) atoms of the catalytic complex become π-complexes but the mode of binding of the azide to the Cu(I) π-complexes has not been demonstrated experimentally.

2.2. Azide Formation. Azides for the CuAAC-SP can be prepared by various methods,120,121 substitution or diazo-transfer being those most commonly used. Aliphatic azides are readily accessible by SN2-type substitution with the highly nucleophilic azide ion.121 These azide derivatives have long been appreciated as an important class of compounds (Table 2).122 Sodium azide (Na+·N− = N+ = N−) is the most widely used azide source.123

The diazo-transfer reaction allows the synthesis of organic azides from primary amines using a diazo-transfer reagent (Scheme 3). The most widely used diazo-transfer reagents are shown in Table 2. The procedure is well suited to SP, since side reactions involving the sensitive aliphatic diazonium ions can be circumvented. As an example, Barral et al.124 performed a particularly efficient conversion of aromatic amines into azides on SP, using trimethylsilyl azide as diazo-transfer reagent (Table 2, entry 1).

Wong et al.125–129 studied the diazo-transfer of aliphatic amines using Cu (II), Ni (II), Zn (II), tetrabutylammonium hydrogen sulfate, or 18-crown-6 as catalysts or additives, and trifluoromethanesulfonyl azide (triﬂyl azide) as diazo-transfer reagent. In most cases, the latter is prepared in situ by reaction of trifluoromethanesulfonic anhydride and sodium azide (Table 2, entry 2). Diversely substituted [1,2,3]-triazoles were achieved by treating resin-bound 3-amine-2-butenoic acid amides with tosyl azide in the presence of DMF and DIPEA130 (Table 2, entry 3).

In 2010, Katritzky et al.131 synthesized the crystalline benzoazolone-1-y1-sulfonfyl azide (Table 2, entry 4) as a new stable and widely available diazo-transfer reagent that efficiently provides N-(α-azidoacyl) benzoazolones with CuSO4·5H2O as copper catalyst. Crystalline 2-ethylimidazole-sulfonfyl azide (Table 2, entry 5) was designed by Schottenberger et al.132 as a convenient reagent to improve thermal stability for the azidation of electrophilic carbonatons.

Similarly, Goddard et al.133,134 developed imidazole-1-sulfonyl azide hydrochloride (Table 2, entry 6), an affordable and effective diazo-transfer reagent using K2CO3 and MeOH in the presence of CuSO4·5H2O as copper catalyst source.133 This reagent has proven to equal triﬂyl azide in its capacity as a "diazo donor" to promote the conversion of primary amines into azides and of activated methylene substrates into diazo compounds.

However, there are some safety concerns regarding the potential risk of explosion of this reagent. To identify safer-to-handle forms of this compound, several types of imidazole-1-sulfonyl azide salts (Table 2, entry 6) were prepared, and their sensitivity to heat, impact, friction, and electrostatic discharge were quantitatively determined. A number of these salts exhibited improved properties and can be considered safer than the aforementioned hydrochloride.134 In addition, Wang et al.135 reported a facile approach to synthesize this diazo-transfer reagent by optimizing the procedure to diminish potential risk of explosion. Some reports support the use of this reagent to prompt efficient diazo-transfer reactions, always using polar solvents or aqueous media.136–139

The introduction of azide groups on SP has been accomplished mainly through direct nucleophilic displacement of chloromethyl-derivatized solid supports by the azide ion.48,87,88,140 Some of these methods are sluggish under the harsh conditions used and they lack reproducibility. To overcome these limitations, a diazo-transfer reaction using triflyl azide (TfN3) was developed to convert amines into azides on SP, allowing the synthesis of azido peptides,141,142 oligonucleotides,143,144 and glycodendrimers.56 However, TfN3 is unstable and potentially explosive and it requires in situ preparation. To avoid this problem, Hansen et al.145 used imidazole-1-sulfonfyl azide hydrochloride (ISA-HCl) as a diazo-transfer reagent for the efficient preparation of azido peptides on SP. With the aim to generate azide resins, we recently described the application of the ISA-HCl-based diazo-transfer method to a set of four resins covering a broad range of hydrophobicity.49

3. Cu(I)-CATALYZED AZIDE–ALKYNE CYCLOADDITION ON SOLID-PHASE (CuAAC-SP)

3.1. History. In 1996, Zaragoza et al.130 published the first synthesis of diversely substituted [1,2,3]-triazoles on a solid
support through a cyclization. This synthesis was achieved by treating resin-bound 3-amino-2-butenoic acid amides with tosyl azide in the presence of a tertiary amine. A few years later, in 2002, Meldal et al.\textsuperscript{26} reported the first mild, efficient, and regiospecific copper(I)-catalyzed 1,3-dipolar cycloaddition of terminal alkynes into azides on SP, using hydrophilic PEGA 800 and SPOCC resins to obtain diversely 1,4-substituted [1,2,3]-triazoles in peptide backbones or side chains.

Given that CuAAC-SP has been successfully introduced in many fields of science, and to gain a better understanding of this topic, we have classified its use with regard to peptides,\textsuperscript{36−38} nucleotides,\textsuperscript{31−33} small molecules,\textsuperscript{39} supramolecular structures,\textsuperscript{40,41} and polymers,\textsuperscript{42} thus demonstrating its potential in SP synthesis over the last ten years.

### 3.2. Peptides

Peptides as drug candidates have some limitations that are related to their aqueous solubility, lipophilicity, H-bond formation, chemical stability, and metabolic stability (proteolytic or enzymatic degradation). Many strategies, including the introduction of non-natural amino acids, terminal protection, cyclization, and backbone modifications,\textsuperscript{37,144} have been used to increase the stability of drug candidate peptides.

The 1,2,3-triazole has attracted increasing attention as a bioisostere of the amide bond of peptides. Tron et al.\textsuperscript{28} reported the physicochemical properties of 1,2,3-triazoles and compared them with the amide bond. The 1,4-disubstituted triazole scaffold shows similarity to a Z-amide bond (Figure 1). This similarity is appreciated by the following features: the distances between substituents; R to R’ are 3.9 Å in amide and 5.0 Å in 1,4-disubstituted 1,2,3-triazole; the lone pair of 3-nitrogen mimics that of the carbonyl oxygen of the amide bond (Figure 1, blue remarked); the polarized C(5)-H bond can act as a hydrogen bonding donor, just like the amide N-H bond (Figure 1, red remarked); and the electrophilic and polarized 4-carbon is electronically similar to the carbonyl carbon (Figure 1, green remarked). The overall dipolar moment of the triazole system is larger than that of the amide bond: amide ∼4 D, 1,4-disubstituted 1,2,3-triazole ∼5 D, and its hydrogen bonding donor and acceptor properties are more marked than those of an amide bond, improve the properties of peptide mimics.\textsuperscript{28,145}

For instance, SP synthesis of 1,4-disubstituted 1,2,3-triazole via Click Chemistry has been used to replace the amide bond by 1,4-disubstituted 1,2,3-triazoles to form peptidotriazoles,\textsuperscript{60} peptide mimics,\textsuperscript{146} cyclic peptides,\textsuperscript{30} and amino acid modifications.\textsuperscript{29} Additionally, it has also been applied in radiotracer conjugation,\textsuperscript{147} peptide ligation,\textsuperscript{52} and bioconjugation.\textsuperscript{68} Most of these uses promote the introduction of contact groups to enhance affinity or the formation of glycopeptides,\textsuperscript{148} PEGylated peptides,\textsuperscript{82} and peptide nucleic acids (Figure 2).

The replacement of amide bonds by 1,4-disubstituted 1,2,3-triazoles on resin was reported in analog formation of peptidomimetics, such as Leu-enkephalin.\textsuperscript{146} Furthermore, RGD-based peptidomimetics with high activity and selectivity toward \(\alpha_v\beta_3\) and \(\alpha_5\beta_1\)\textsuperscript{149} a matriptase inhibitor mimic and\textsuperscript{46} a small peptide mimic of the Grb2-SH2 domain respectively,\textsuperscript{57} were also reported.

Furthermore, the formation of 1,2,3-triazoles as a substitute of 3-oxoalkanoic acids on Wang resin,\textsuperscript{130} the preparation of N-substituted histidine and tripeptide analogs from propargylglycine to form peptidotriazoles on PEGA 800 and SPOCC...
resins, and the formation of triazolamer (Figure 2, 2a) under different conditions of CuAAC were established.

A triazole scan of a biologically relevant peptide and its utility for the identification of novel peptidomimetics with improved properties was also achieved via a CuAAC strategy. Also, on-resin “Click” construction of peptide diphenyl phosphonates has been reported as a valuable method for the rapid diversification of serine protease activity-based probes (ABPs). CuAAC-SP has been used as a macrocyclization tool for the formation of triazole scaffold by substituting an amide bond via Click Chemistry. This reaction yielded the following: cyclic peptide mimetic model to optimize PEG based resins, cyclic tetra-, penta-, hexa-, and hepta-peptides (Figure 2, 2b), as a new family of cyclopeptide cyclo[-Arg-Gly-Asp-Ψ(triazole)-Gly-Xaa-] analogs, new cyclic RGD and NGR peptide analogs, two asymmetrical cyclopeptides (CP1 and CP2), side chain to side chain macrocyclization for the synthesis of a series of 21 amino acid helical peptides, cyclic peptide C2BL3C based on membrane penetration C2B loop 3 of Syt1, cyclic peptidotriazoles derived from the antimicrobial cyclic peptide c(Lys-Lys-Leu-Lys-Phe-Lys-Lys-Leu-Gln) (BPC194), and a cyclic peptidosteroid via convergent peptide ligation and macrocyclation.

Head-to-tail oligopeptide cyclodimerization was also reported when precursors containing azide and alkyne groups were exposed to Cu(I) ions on polystyrene supports. Cyclic lipopeptidotriazoles, which has proven to be a useful approach to improve the antibacterial activity of the parent peptides, as well as to endow them with antifungal properties, were also prepared using CuAAC-SP.

CuAAC was also used on azidoproline (Azp) (Figure 2, 2c) and on Azp-containing collagen model peptides (CMPs) to study their functionality and conformational properties. Click chemistry was also applied to attach [18F]fluoroalkynes to peptides functionalized with 3-azidopropionic acid (Figure 2, 2d), and also to introduce hydrophilic carbohydrate linker moieties into the stabilized BBS (7–14) sequence previously synthesized on Rink-amide resin bearing the (NαHis) Ac-chelator labeled with 99mTc using the tricarbonyl technique. Several peptide sequences, which contained modified amino-acid propargylglucose (Pra), were attached to red-fluorescent tetramethylrhodamine azide (TAMRA) via CuAAC-SP, in order to generate wide range of fluorescent substances, thus allowing the identification of cyclic peptidyl inhibitors against...
the calcineurin/NFAT interaction through high-throughput screening of one-bead-one-compound libraries.152,153

In addition, this Click Chemistry has been used to produce assembled and scaffolded peptides from peptide and scaffold precursors N-terminally modified with azido and alkyne moieties, respectively, using 2-CTC resin.52 An efficient self-purifying N-to-C iterative triazole ligation strategy was also applied to the synthesis of a polypeptide with 160 residues on ChemMatrix, PEGA800 and PEGA1900 resins, achieving high purity without the need of chromatographic purification.154

CuAAC-SP was also used as the key step to obtain valuable aminoacyl-(peptidyl)-penicillins, and this reaction showed general applicability and excellent regioselectivity using Wang resin as solid support (Figure 2, 2e).69 Two biotin linkers of different lengths bearing the activated p-(N-propynoylamino) toluic acid (PATA) were incorporated in biotinylated oligopeptides and C-myc peptide both in solution and on a solid support, achieving excellent yields of conversion.68

In this regard, peptides labeled with short-lived positron emitters were synthesized by CuAAC-SP for later use in molecular imaging by positron emission tomography.85 Another example is peptides loaded with a dendron via CuAAC-SP; these peptide mimotopes are promising candidates as multivalent ligands for antibody B13-DE1 recognition.155

3.3. Peptoids. Peptoids have become an important class of peptide mimics because of their structural and functional properties.157 Examples of peptoids formed via CuAAC-SP include glycosylated peptoids by global on-resin Click glycoconjugation of alkynyl substituted peptoids (Figure 2, 2f).148 Other highly functionalized peptoid oligomers were generated by sequential CuAAC-SP.158 Also, modular synthesis of glycosylated peptoids and polyamines,63 or amphiphilic peptoid transporters used for cell-penetration66 were performed using Rink-amide resin.

A focused dipeptide conjugated azidothymidine (AZT) library has been synthesized, and a convenient and efficient CuAAC-SP has been described using Wang resin as solid support.70 Versatile approaches for the efficient synthesis of PEGylated lipo-peptides via CuAAC “Click” conjugation between alkyne-bearing solid-supported lipopeptides and...
azido-functionalized PEGs was described using polystyrene or TentaGel resins (Figure 2, 2h). Most of the studies cited demonstrated the potential of CuAAC in chemical ligation strategies. Other examples of peptides were described by Zabrodski et al., reporting a fast and efficient incorporation of the pyridine ligands derivatives into N-substituted glycine peptoid oligomers via CuAAC-SP.

3.4. Peptide Nucleic Acid. Concerning peptide nucleic acids (PNAs), organometallic PNA oligomers were synthesized by Click Chemistry on SP through the insertion of ferrocene into PNA oligomers via 1,2,3-triazole formation on PEGA800 resin. Azidoferrocene, ethynylferrocene, and DEPA-ferrocene derivatives were introduced into TentaGel resin via Click Chemistry by reaction with PNA trimer conjugates. Other organometallic compounds, such as azidomethyl-ruthenocene, and their application via Click Chemistry with PNA oligomers immobilized on TentaGel resin have also been reported. Furthermore, a new azido derivative of 2,2'-dipicolyamine (Dpa) was successfully coupled on SP to a PNA oligomer (H-4-pentyinoic acid–spacer–spacer–tgca–tgca–tgca–lys–NH₂ spacer (–NH–(CH₂)₂–O–(CH₂)₂–O–(CH₂)–CO–) using CuAAC-SP to give Dpa-PNA oligomer. Finally, a fluorescent rhenium-containing PNA bioconjugate (Re-PNA) was prepared by means of conjugation of rhenium tricarbonyl complex of a bis(quinoline)-derived ligand (2-azido-N,N-bis((quinolin-2-yl)methyl)ethanamine, L–N₃), namely, ([Re(CO)₃(L–N₃)]Br), to a PNA oligomer via CuAAC-SP (TentaGel resin).

The synthesis of PNA bearing a triazole in place of the amide bond assembled via CuAAC-SP (Rink-amide resin) has been achieved (Figure 2, 2g). Click attachment of peptide to oligonucleotides on solid support (Wang resin) was also reported. Several peptide-siRNA conjugates were also obtained by a CuAAC-SP strategy, using an alkynyl nucleoside analog “clicked” onto a peptide-derivatized CPG (controlled pore glass) as solid support. Finally, fluorescent molecules were attached to pyrrolidinyl peptide nucleic acid bearing a D-prolyl-2-aminocyclopentane carboxylic acid backbone (acpcPNA) as a base surrogate via a triazole linker and using a CuAAC strategy on TentaGel resin as solid support.

3.5. Nucleotides. The structural diversity of active nucleosides proves that nucleoside analogs do not need to resemble their natural counterpart, and these new structures are worth exploring. In this regard, researchers have moved their attention to the possible benefits of innovative and new synthetic approaches, such as CuAAC-SP, for the synthesis of modified nucleosides, in which the phosphodiester linkage is replaced by 1,2,3-triazole moiety. This moiety is able to form cyclic nucleotides or assists in bioconjugation, such as in the case of glyconucleotides, glycoclusters, and contact groups (Figure 3).

To discover new derivatives with potential biological activity, CuAAC-SP has been applied to the loading of alkyne-functionalized leader nucleoside monomers suitable for SP oligonucleotide synthetic applications (Figure 3, 3a). In relation to the replacement of phosphodiester linkage by 1,2,3-triazole moiety, Isobe et al. designed and synthesized a new
triazole-linked analog of DNA on TentaGel resin (Figure 3, 3b). On the other hand, Morvan et al. designed an azido linker to prepare a DNA oligonucleotide, bearing both 3’-azide and 5’-alkyne functions on CPG resin. Cycloadditions were performed (Figure 3, 3c).

CuAAC-SP has also been applied to the synthesis of new nucleoside bioconjugates and to the generation of small libraries of nucleoside derivatives on resin. Indeed, the efficiency and simplicity of this reaction make it an attractive choice for the covalent linkage of two molecular entities to provide biomolecules with novel properties, such as biological activity, altered hydrophobicity, increased bioaffinity, or the capacity to carry metal ions. For instance, glyconucleotides were synthesized by 1,3-dipolar cycloaddition not only of T12-trispropargylphosphoramidate oligonucleotide with galactosyl azide on SP (CPG resin) but also of manose and galactose-alkyne for the preparation of oligonucleotide conjugates.

Glycodendrimer G(0) Dimer

Mannose and galactose oligonucleotide conjugates have been prepared by bi-Click Chemistry (two successive CuAAC-SP). The efficient synthesis of RNA conjugates with non-nucleoside building blocks has been described by Click Chemistry on CPG resin (Figure 3, 3e). The introduction of contact groups for recognition was also assisted by Click Chemistry. Biotin linker (p-N-propynoylaminomethyl acid, PATA) was conjugated to oligonucleotides. The site-specific incorporation of diamondoids on DNA via CuAAC on CPG resin was achieved (Figure 3, 3f). Facile one-step SP synthesis of multtopic organic-DNA hybrids on GPC beads was performed via CuAAC.

Another example is the use of CuAAC-SP to prepare fluorescent DNA probes containing internal xanthenes and cyanine dyes. Meyer et al. described the synthesis of heteroglyco 5’-oligonucleotide conjugates via CuAAC-SP with the formation of phosphoramidite derivatives bearing orthogonal function alkyne/thioacetyl, and an azide-bearing carbohydrate. Tänginen et al. reported the synthesis of Neomycin-conjugated homopyrimidine oligo 2’-deoxyribonucleotides on SP using Click Chemistry as these compounds are potential therapeutic agents since they recognize the DNA and the double helical RNA.

Figure 5. CuAAC-SP supramolecular structures and polymer applications.
3.6. Small Molecules. The combinatorial synthesis of libraries based on the so-called small molecules as bioactive entities has led to increased pressure on chemical synthesis to enhance throughput. SPOS has become an area of huge interest in organic and medicinal chemistry; the application of SPOS comprises a broad variety of organic reactions. For instance, CuAAC is a specific reaction that has been successfully applied on SP for small molecules (Figure 4).

Initially, Gmeiner et al.87–89,166 developed an efficient 1,3-dipolar cycloaddition of alkynyl-substituted handles with azidomethyl polystyrene that allowed the preparation of the formyloxy- and azidomethyltriazole (FAMT) handle and the formylazidomethyl polystyrene that allowed the preparation of the Rink resin and propargyl chemistry was carried out by means of azide-functionalized hydrophilic magnetic beads (Dynabeads M-270) via Click Chemistry on polystyrene resin.71 Recently, the SPOS of 1-vinyl and 1-allyl substituted 1,2,3-triazoles was achieved on a selenium matrix.77 on Rink-amide resin. Goethel et al.168 developed the synthesis of dopamine and serotonin synthesized with short PEG ethers, which were azide-functionalized to promote coupling onto alkyne-modified magnetic beads (Dynabeads M-270) via Click Chemistry on SP.168 Also, Pericas et al.94 used the Click strategy to immobilize MacMillan organocatalyst onto polymers and magnetic nanoparticles.94 Portnoy et al. studied one-pot esterification−Click reactions for the functionalization of Wang resin via CuAAC.93 Recently, the SPOS of 1-vinyl and 1-allyl substituted 1,2,3-triazoles was achieved on a selenium linker on solid support via Click Chemistry.75

3.7. Supramolecular Structures and Polymers. CuAAC-SP has been successfully applied to supramolecular structures, polymers, and affinity chromatography fields (Figure 5). The synthesis of lysine-based glycodendrimers as antagonists against Escherichia coli (Figure 5, a) using Click Chemistry was carried out by means of azide-functionalized Rink resin and propargyl α-D-mannopyranoside.86 Moreover, Hartmann et al.48 developed heteromultivalent glycooligomers by CuAAC for application in several fields such as antiviral drugs, vaccines and biosensors.88 The synthesis of molecularly encoded oligomers using a chemoselective CuAAC-SP was also carried out.72 Furthermore, the design and synthesis of a series of solid-tethered rotaxanes utilizing crown ether-naphthalene diimide or crown ether-bipyridinium host guest interactions were described (Figure 5, b).59 TentaGel polystyrene resins were initially modified into azide-functionalized beads in a two-stage procedure before the target supramolecular architectures were attached.59

The modification of polymers after the successful achievement of polymerization is a central task in macromolecular chemistry.95,169 CuAAC-SP has been applied to polymeric synthesis. Haddleton et al.95,169 developed the living radical polymerization of methyl methacrylate (MMA) and a fluorescent comonomer with 2-bromo-2-methylpropionic acid 3-azido- or halogen-terminated poly(methyl methacrylate) (PMMA) with average number of molar mass (Mn) close to that predicted, PDI < 1.20, and good first order kinetics, as expected for a living polymerization.

Cho et al.96 prepared multiwalled carbon nanotubes (MWCNTs) functionalized with poly(styrene-b-(ethylene-co-butylene)-b-styrene) triblock copolymer (SEBS) using Click Chemistry. In this case, various compositions of SEBS-functionalized MWCNTs were obtained from the reaction of azide moiety-containing SEBS on styrene units with alkynedeckorated MWCNTs (Figure 5, c). Girard et al.97 developed the polystyrene-supported triazoles via CuAAC on Merrifield resin.

Affinity chromatography is crucial for separating or analyzing specific target compounds in samples and for studying biological interactions. The immobilization of biomolecules is a mainstay in biological fields and related areas, with many potential applications such as the characterization of their functions and their interaction with other biomolecules, the analysis and purification of mixtures of biomolecules, and the design of SP-based assays or bioactive implant surfaces. The CuAAC has been used on silica supports and the modified supports have been successfully applied in affinity chromatography.

For instance, the covalent immobilization of suitable alkyne/azide carbohydrate derivatives on complementarily functionalized azide/alkyne silica was performed by Click ligation through CuAAC reaction of such compounds. New glyco-silicas have shown to be efficient and valuable bioselective affinity chromatographic supports for the purification of lectins, as well as for the one-pot fluorescent labeling of these proteins. Yang et al.171 developed a method, namely hydrophilic interaction chromatography (HILIC), which used a support prepared by clicking aspartic acid onto silica gel (termed as Click AA). This material was used as SP extraction sorbent for selective enrichment of glycopeptides.171 Another example reported was the building of 2-oxoglutaric acid receptor attached to resin via CuAAC-SP. This scaffold showed high affinity and specificity to separate the established 2-OG binding protein NtcA.172

4. CONCLUSIONS

CuAAC has made a significant contribution to Click Chemistry but has also been greatly extended to SP in recent years. This
review reveals the potential of this kind of Click reaction for the rapid construction of several molecules. We focused on CuAAC-SP, taking to account the capacity of this method to prepare a wide range of molecular entities, such as peptides, nucleotides, small molecules, supramolecular structures, and polymers. CuAAC-SP has been performed in the presence of the alkyne and the azide (one of them attached to the SP), a Cu(I) source, and the adequate solvent under basic conditions. In most reports either CuI, DIEA, and DMF or CuSO4·SH2O, Asc, and water/tBuOH were the reagents used as copper source, base, and solvent, respectively. CuAAC-SP allowed the replacement of amide bonds in peptides, thus facilitating the synthesis of cyclic or mimetic peptides. In addition to its application in bioconjugation, this reaction has also been used to generate new resins and amino acid-triazole derivatives. Favorable conditions to carry out the CuAAC-SP open up new avenues to tackle future synthetic challenges.

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The authors declare no competing financial interest.

**ABBREVIATIONS**
DMF dimethylformamide; ACN acetonitrile; THF tetrahydrofuran; DCM dichloromethane; NMP N-methylpyrrolidone; DMSO dimethyl sulfoxide; DIEA N,N-diisopropylethylamine; TEA triethylamine; TBT tris (benzyltriazolylmethyl)amine; PDMTA N,N’,N,N’’,N’’-pentamethyldiethylenetriamine; CuAAC Cu(I)-catalyzed azide–alkyne cycloaddition; SPOCC (solid-phase organic and combinatorial chemistry) resin; PEGA poly(ethylene glycol) amide backbone resin

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