2

Common Synthons for Click Chemistry in Biotechnology

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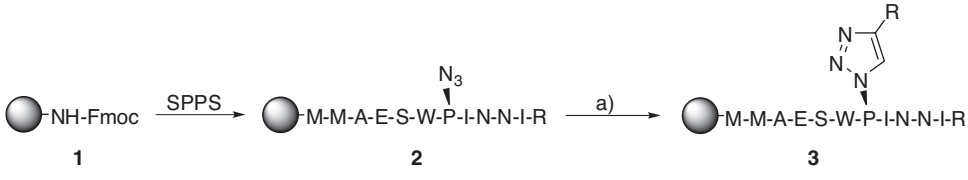
2.1 Introduction – Click Chemistry

The most widely used method for the synthesis of libraries of biologically-active molecular frameworks – particularly for the regioselective synthesis of 1,2,3-triazoles – is the copper-catalyzed 1,3-dipolar cycloaddition (click reaction) of azides[1–4] with alkynes. Meldal *et al.* first published this method in 2002 for triazole synthesis on solid phases,[5,6] closely followed by the report of Sharpless and Fokin *et al*. dealing with the water-based catalyzed reaction with copper sulfate and sodium ascorbate.[7] Many different conditions have been established for cycloaddition reactions.[8] Moreover, this reaction fulfills certain criteria that are particularly advantageous for biological uses: the alkyne group as well as the azide functionality are inert within molecules that exist in living systems, and the click reaction is generally compatible with water-containing systems (aqueous media near physiological pH can be used at ambient temperature and in short reaction times to prevent, for example, the denaturation of proteins). While other reactions include the use of nucleophiles or electrophiles, the components of a click reaction do not react in an undesirable way within biological systems. These advantages facilitate reactions in biological systems and even in living cells. As such, the click reaction is used as an alternative to other established methods described in the literature for the production of biomolecules[9,10] (for example, olefin metathesis of glycosides, intermolecular enyne metathesis of alkynyl and alkenyl glycosides, native chemical ligation, glycosylation of diols and coupling of alkynyl glycosides).

Click Chemistry for Biotechnology and Materials Science Edited by Joerg Lahann
 ISBN: 978-0-470-69970-6

2.2 Peptides and Derivatives

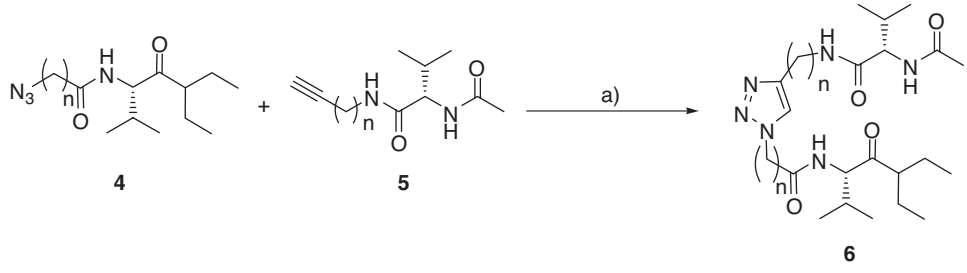
The click reaction has been used to synthesize diverse derivatives of bioactive compounds via substitution of the originally appearing functionality with the resulting triazole unit, in order to improve the activity of the compounds. Gopi *et al*. investigated the structure–activity correlation of peptide conjugates that act as receptor site antagonists of HIV-1 gp120.[11] The group synthesized derivatives of the original bioactive peptide on solid phases through the reaction of an immobilized azide-modified proline residue with alkynes containing different side chains (Scheme 2.1).



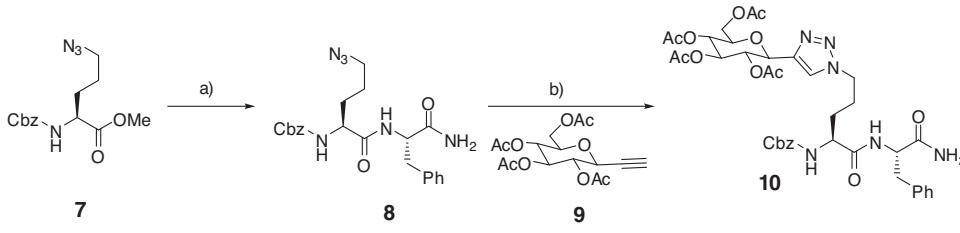
Scheme 2.1 *Synthesis of various triazole-based covalently modified peptide derivatives of an antagonist of HIV-1 gp120 through click chemistry. (a) CH_3CN–H_2O–DIEA–pyridine, alkyne (5 equiv.), CuI (1 equiv.), overnight.*[11]

The introduction of a triazole functionality offers the possibility of producing novel β-turn mimics.[12] The click reaction of an alkyne-containing dipeptide **5** and the azide-functionalized dipeptide **4** generates different tetrapeptides **6** in a convergent synthesis (Scheme 2.2). While the syntheses of Guan *et al*. are based on the reaction of terminally modified azide-containing peptides, there are many approaches using peptidic structures that bear azides in their side chains. Similar to the approach of Gopi *et al*. (Scheme 2.1), these strategies yield peptides with diverse triazole derivatives as side chain substituents.

Following the click strategy, peptides can be turned into glycopeptides as shown by Rutjes *et al*.[13] Different routes to these target glycopeptides have been explored: the coupling of amino acids can be performed traditionally via chemical- or alternatively enzyme-catalyzed coupling. The click reaction itself can be undertaken before, as well as after, peptide formation. Furthermore, azide-modified sugar-moieties and azide-containing peptides **7** have been used to perform the click reaction with alkynylated peptides as well as in the



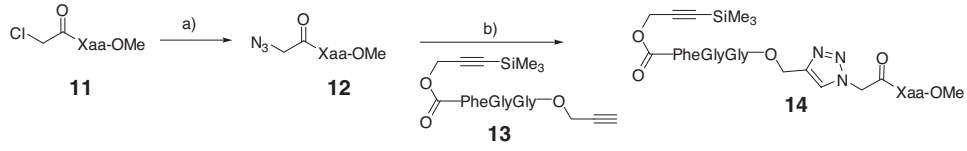
Scheme 2.2 *Synthesis of tetrapeptides **6** with* n = *1–4 through click reaction. (a) $CuSO_4$ (1 mol %), sodium ascorbate, H_2O/^{t}BuOH (1:1), r.t.*[12]



Scheme 2.3 *Enzymatic peptide coupling to give azide-containing dipeptide* ***8*** *and subsequent click reaction. (a) Phe–NH_2 or Gly–NH_2,* alcalase, *^{t}BuOH–DMF; (b) CuI, TBTA, NEt_3, MeCN, 40 °C.*[13]

inverse manner. It was ascertained that coupling through an enzymatic protocol followed by a click reaction with either the azide- or alkyne-containing sugar yields very good results, in terms of generating the triazole-modified dipeptides **10** (Scheme 2.3).

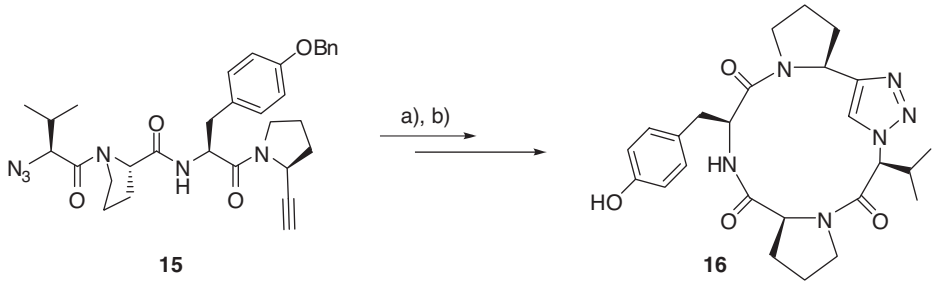
Aucagne and Leigh[14] demonstrated that the click reaction can be performed chemoselectively (Scheme 2.4). This approach was used to selectively generate two triazole linkages in a one-pot method. The first reaction is traditionally performed in the presence of a copper(I)-species with a dialkynyl-derivative **13**, which contains one free and one TMS-protected triple bond. The second click reaction is carried out in the presence of Cu(I) and Ag(I), which catalyze the deprotection of the second alkynyl functionality. The starting material **12** for those click reactions is easily prepared through the substitution of α-chlorinated peptides **11** with sodium azide.



Scheme 2.4 *Synthesis of azide-containing dipeptide analogs* ***12*** *and chemo-selective click cycloaddition. (a) NaN_3, DMF; (b)* ***13****, $CuSO_4$, sodium ascorbate, ^{t}BuOH/H_2O, 20 °C, 72 h, 98%; Xaa = Phe, Leu, Pro, Lys(Boc).*[14]

Besides the aforementioned methodologies for intermolecular click reactions, there are examples of intramolecular approaches that result in cyclic peptides. One example is found in the research conducted by Maarseveen *et al.*,[15,16] in which intramolecular click reactions were applied to the synthesis of cyclic tetrapeptides (Scheme 2.5). These tetrapeptides are of interest in their function as tyrosinase inhibitors, because the derivatives produced are established bioactive compounds. The synthesis of the triazole-containing cyclic tetrapeptides can theoretically be successful via cyclization through peptide coupling or, on the other hand, through triazole formation in the final step. It has been demonstrated that compound **16** is only accessible through ring closure via click reaction, underlining the importance of click chemistry for the formation of triazole-containing peptidomimetics (Scheme 2.5).

In subsequent investigations of peptide-bond isosteres, the Maarseveen group synthesized cyclotetrapeptide mimics containing two triazole moieties. Although these substrates



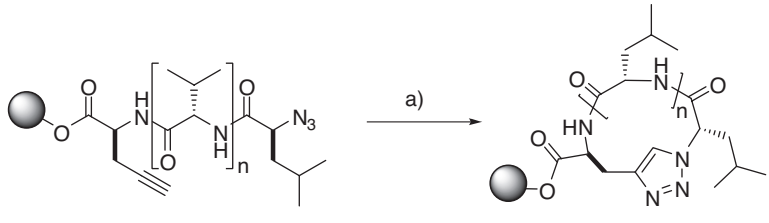
15 16

Scheme 2.5 *Synthesis of cyclo-[Pro–Val–ψ(triazole)-Pro–Tyr] by click cyclization. (a) CuBr, DBU, toluene, reflux, 70%; (b) H_2, Pd–C, MeOH, 93%.*[15,16]

were synthesized in an analogical manner via click cyclization, only moderate yields were attained.

Lokey *et al.*[17] performed similar cyclizations to generate small cyclic peptide derivatives on solid phases. The starting material **17** was initially built up with a propargyl-containing amino acid. After standard peptide coupling on bead, the last amino acid of the linear chain bearing the azide functionality was attached. Cyclization of **17** was performed in the presence of CuBr, sodium ascorbate and a diamine as a ligand, followed by cleavage from the resin achieved with TFA in methylene chloride. The successful synthesis was demonstrated for cyclic peptides **18** containing two to five leucine units. The major drawback of the solid phase synthesis was the appearance of varying amounts of the dimeric peptides as a result of the incomplete conversion of the click reaction.

Another modification on solid phases involves solid-phase peptide synthesis with orthogonal *N*-protected alkylamines as side chains. These can be deprotected, selectively acylated with propiolic acid as shown by Eichler *et al.*, and then 'clicked' via the addition of azide-modified peptides.[18] Another click reaction with resin-bound terminal alkynes has been illustrated by Meldal *et al.*[5,6] This group investigated the formation of triazole derivatives after solid-phase peptide synthesis. In the last step on bead, the terminal alkyne was reacted with different primary, secondary and tertiary alkyl and arylazides and an azido sugar moiety.[19]



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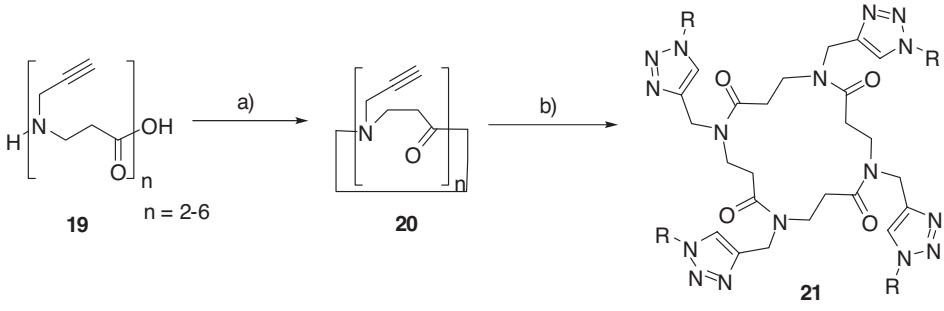
Scheme 2.6 *Click macrolactamization of propargyl glycine azido leucine on solid phases. a) CuBr (1 equiv.), sodium ascorbate (3 equiv.), DIEA (10 equiv.), 2,6-lutidine (10 equiv.), DMF, 25 °C, 6 h.*[17]

2.3 Peptoids

As triazole-containing mimics of peptides have been extensively investigated, there are other groups who have synthesized peptoids modified through the click reaction of azides with alkynes. Taillefumier *et al*. performed the first synthesis of functionalized β-peptoid macrocycles via linear synthesis of polyalkyne-containing linear β-peptoids and subsequent macrocyclization.[20] The functionalized cyclic peptoids **20** were then modified by the addition of azides to produce polytriazole-containing cycles **21** with different residues on the triazole side chain (Scheme 2.7).

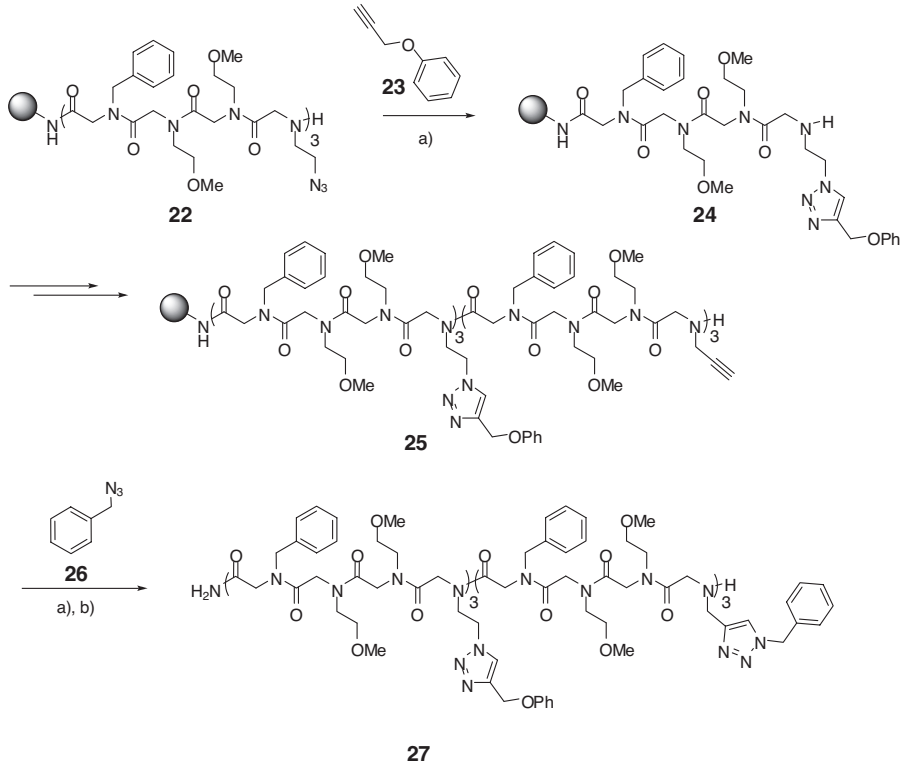


Scheme 2.7 *Macrolactamization of linear peptoids* ***19*** *and functionalization via click chemistry. (a) DPPA, $NaHCO_3$, MeCN–DMF; (b) RN_3, $CuSO_4$, sodium ascorbate, TBTA [tris-(benzyltriazolylmethyl)amine].*[20]

Noncyclic peptoid derivatives generated by click reaction can also be found in the literature. Kirshenbaum *et al*. generated *N*-substituted glycine peptoid oligomers **22** on bead that were used for click reactions on solid phases. The group incorporated azides into the oligomer and treated them with acetylenes **23** to generate triazole derivatives **24**. In subsequent steps, the oligomer was elongated and the inverse click reaction was performed, through the immobilization of acetylenes and reaction with azides **26**. Using this conjugation technique, complex peptidomimetic products were synthesized, containing different triazole substituents in specific positions. After peptoid-chain elongation, Kirshenbaum *et al*. were able to isolate 24-meric products and dodecamers containing two different triazole groups and up to four different triazole ring substituents respectively (Scheme 2.8).[21]

2.4 Peptidic Dendrimers

Peptidic dendrimers are biologically relevant structures, for example, as channels for drug delivery.[22] The functionalization of these dendrimers enables the modulation of the surface to generate special properties, which are essential for their specific interactions. Click chemistry has been used by several groups to design novel dendrimeric structures.[23–26] Haridas *et al*. synthesized triazole-based dendrimers **30** from $(Lys)_2$Lys-alkyne **28** and the azide-containing compound **29**. Moreover, the lysine-based dendron **28** has been coupled with cysteine cores and dendron **29** has been attached to a Lys-Asp-scaffold (Scheme 2.9).

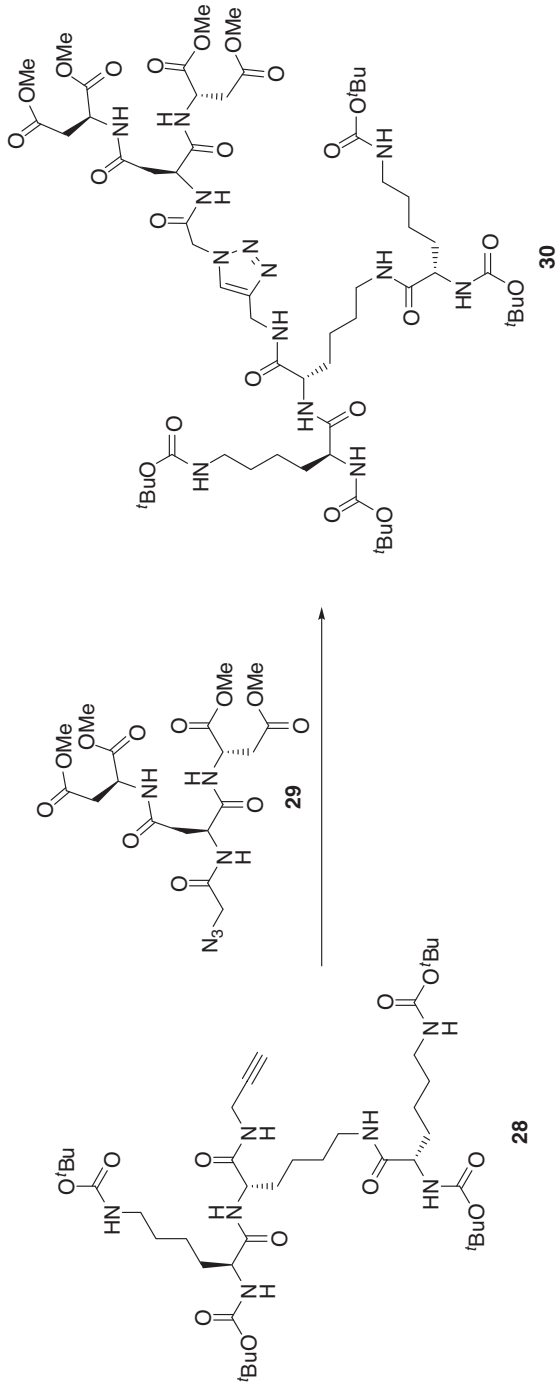


Scheme 2.8 *Modification of peptoid side chains by a sequential series of click reaction and oligomerization. (a) Coupling partner, CuI, sodium ascorbate, DIPEA in DMF–pyridine, r.t., 18 h; (b) 95% TFA in H_2O, r.t., 10 min.*[21]

The groups of Liskamp *et al.*[25] and Pieters *et al.*[23] constructed dendrimers that contain polyalkyne building blocks and coupled those to produce polytriazolic dendrimers. Furthermore, through the application of click chemistry, the latter group successfully synthesized a series of pore-forming compounds in order to build up magainin-based compounds.

2.5 Oligonucleotides

The application capacity of oligonucleotides and their derivatives is seemingly boundless. Modified oligonucleotides have the potential to widen existing diagnostic capabilities, while also fulfilling therapeutic applications. In genomic studies, they act as primers and probes for DNA detection and sequencing,[27,28] as primers in the polymerase chain reaction and as molecular beacons for detecting genetic mutations. As well as being used as anti-sense agents, oligonucleotides serve as probes for measuring gene expression in DNA microarrays and gene chips.[29] The wide variety of structural modifications of oligonucleotides is a result of the substantial number of known bases, sugar residues[30]



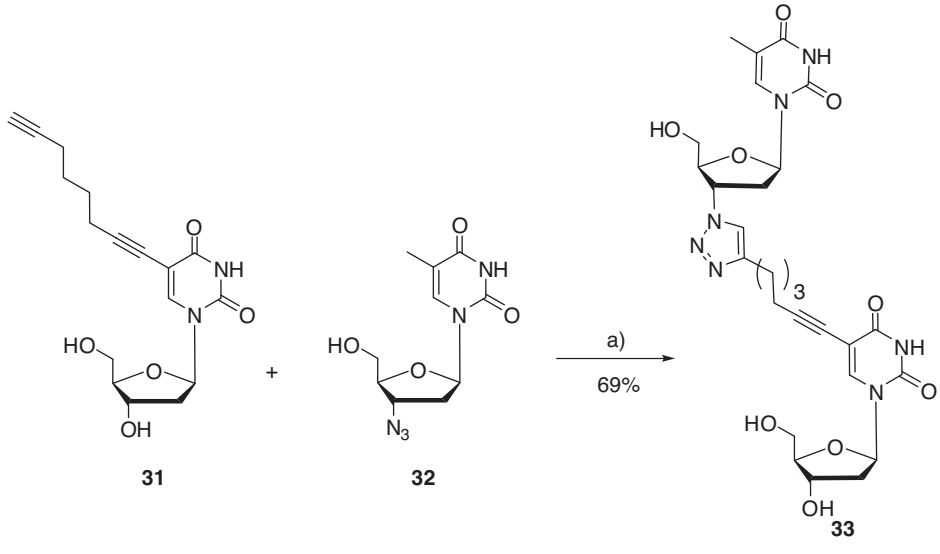
Scheme 2.9 *Synthesis of triazole-based dendrimers by click reaction. (a) 29, Cu(I), DIEA, 68%.*[24]

and altered phosphodiester backbones that can influence hybridization strength, binding affinity, mismatch discrimination, nuclease resistance and cellular uptake.[27,28]

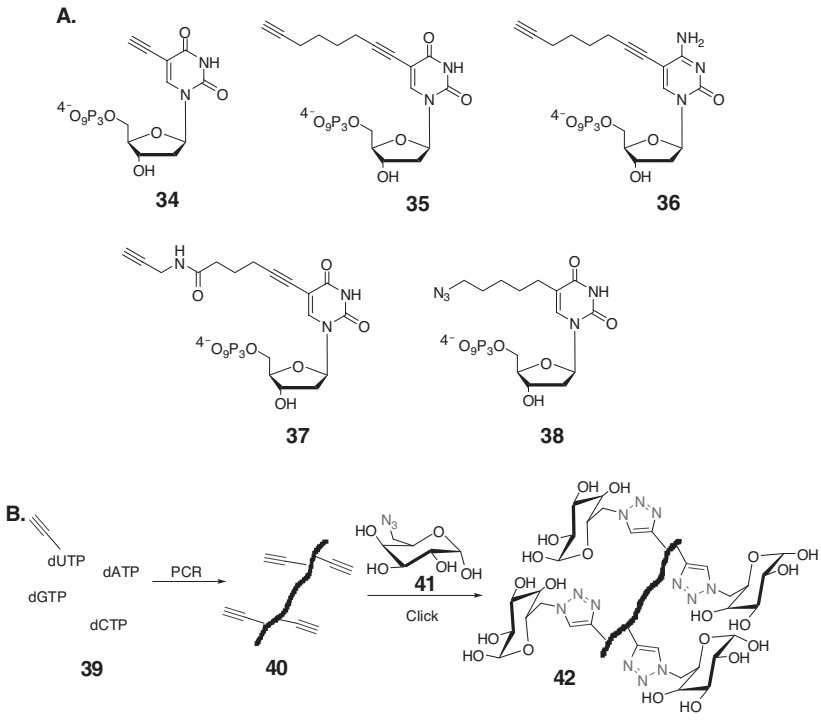
Introduced by Sharpless and coworkers, the click reaction is one of the most powerful methods of labeling oligonucleotides.[31,32] It has been performed not only on phosphoramidate-modified oligonucleotides,[33] but also on triple bonds and azides mostly attached through the 5′- or 3′-terminal conjugation[34] of the oligonucleotides. Because the 5-position of pyrimidines and the 7-position of purines lie in the major groove of the B-DNA duplex and have steric freedom for additional functionalities, most of the reported DNA modifications take place in these positions.[27]

Seela *et al*. reported on some modified nucleosides and oligonucleotides, which were synthesized through the Cu^{I}-catalyzed azide–alkyne cycloaddition. The group introduced reporter groups as well as duplex stabilizing residues into nucleic acids, one example of which is the cycloaddition of compound **31** and azidothymidine (AZT, **32**) to form the triazole product **33** (Scheme 2.10).[27]

The preceding methods facilitate the synthesis of a variety of lengths and structures of the side chains and nucleobases. Numerous cycloadditions work best using a spacer between the alkyne and the nucleobase.[35,36] Carell *et al*.,[37] for example, reported on the click reaction performed directly on PCR products of alkyne-modified uridine nucleosides **34**–**37** instead of on natural thymidine triphosphate (Scheme 2.11). The resulting high-density alkyne-modified DNA was subsequently derived to investigate the efficiency of the sugar labeling of DNA under mild conditions, in which galactose azide **41** was 'clicked' onto single- and double-stranded DNA to generate nucleic acids of type **42**.[37]

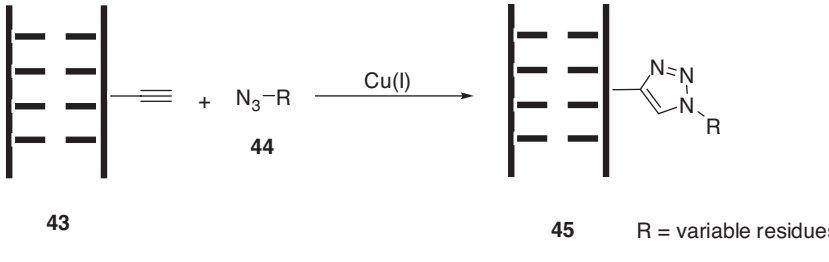


Scheme 2.10 *Click reaction of a thymidine derivative* ***31*** *at the 5-position with AZT (****32****): (a) $CuSO_4$, sodium ascorbate, THF/H_2O, r.t., 30 h.*[27]



Scheme 2.11 *(A) 5-Modified pyrimidine triphosphates* ***34–38*** *used in this study; (B) schematic description of DNA functionalization using click chemistry.*[37]

The principle of the alkyne-derived formation of nucleic acids shown later on (Scheme 2.12), as well as subsequent click reaction, led to a high potential for nanotechnology, including gold surface and nanoparticle functionalization.[27] Furthermore, in the field of nanotechnology, the magnetic properties and electrical conductivity of the primary sequence and the secondary structure of DNA are currently of particular interest in the preparation of functional self-assembled nanostructures.[38] Beyond the investigations of the Carell group,



Scheme 2.12 *Schematic view of the cycloaddition reaction performed on duplex DNA.*

Morvan *et al*.[39] developed an approach to sugar-modified oligonucleotides through the use of microwave-assisted click chemistry.

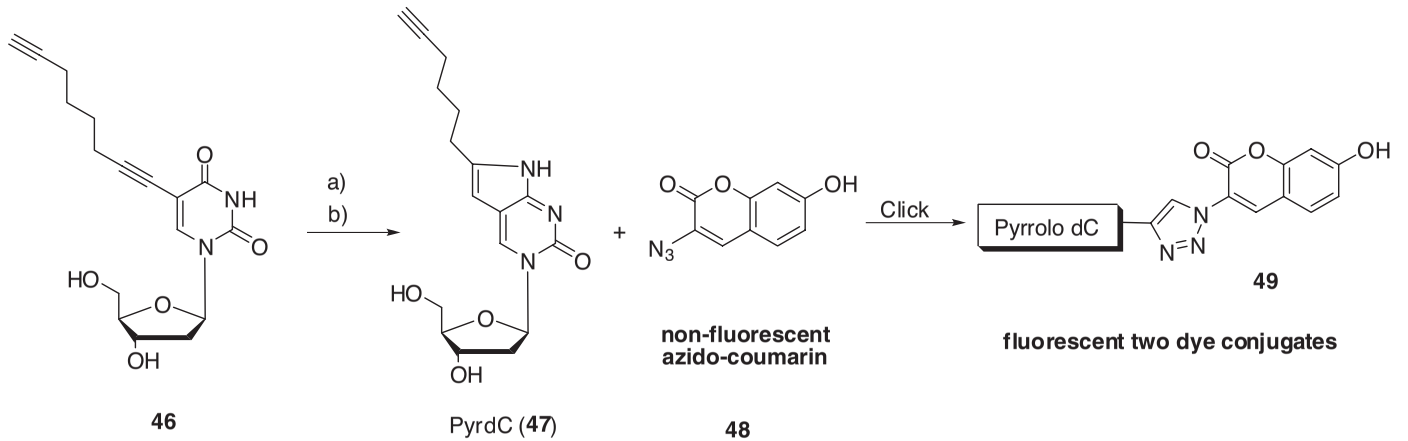
Seela *et al*. converted 5-(octa-1,7-diynyl)-2′-deoxyuridine (**46**)[36] into strongly fluorescent 1*H*-1,2,3-triazole conjugates **49** that incorporated two fluorescent reporters – the pyrrolo[2,3-*d*]pyrimidine derivative (Pyrrolo dC **47**) and the coumarin moiety **48** – via the click reaction, amongst other methods (Scheme 2.13).[40] The photophysical properties of the Cu(I)-catalyzed azide–alkyne cycloaddition product **49** differ from those of pyrdC. Thus, the cycloaddition reaction enabled longer excitation and emission wavelengths (excitation, 340 nm, emission, 466 nm vs excitation, 327 nm, emission, 410 nm for pyrdC). Furthermore, ds-DNA containing the click nucleotide **49** displayed a strong fluorescence increase compared with PyrdC **47** integrated into duplex DNA.[40]

Nielsen and coworkers worked on triazole stacking in the major groove, which produced increased nucleic acid duplex stability.[33] Moreover, Pederson *et al*.[41] reported that incorporated 1,2,3-triazoles stabilize parallel triplexes by twisting intercalating nucleic acids. Furo[2,3-d]pyrimidines conjugated to carbohydrates were synthesized by Ju *et al*. in 2008 via Sonogashira coupling and click chemistry to enhance the efficiency of structural diversity for structure–activity relationship studies.[30] Beyond the approaches concerning the labeling of oligonucleotides, click chemistry has been used to cross-link two ends of a DNA strand, through the reaction of alkynes and azides positioned at the base on the 3′- and 5′-termini.[42] Further examples were illustrated by Ju *et al*. in 2003, such as the synthesis of fluorescent single-stranded DNA (ss-DNA) via 1,3 dipolar cycloaddition between alkynyl 6-carboxyfluorescein (FAM) and 5′-azido-labeled ss-DNA. The resulting fluorescent ss-DNA is applicable as a primer in the Sanger dideoxy chain termination reaction, in order to produce DNA sequencing fragments (for other examples of biomolecule-labeling see Chapter 15).[29]

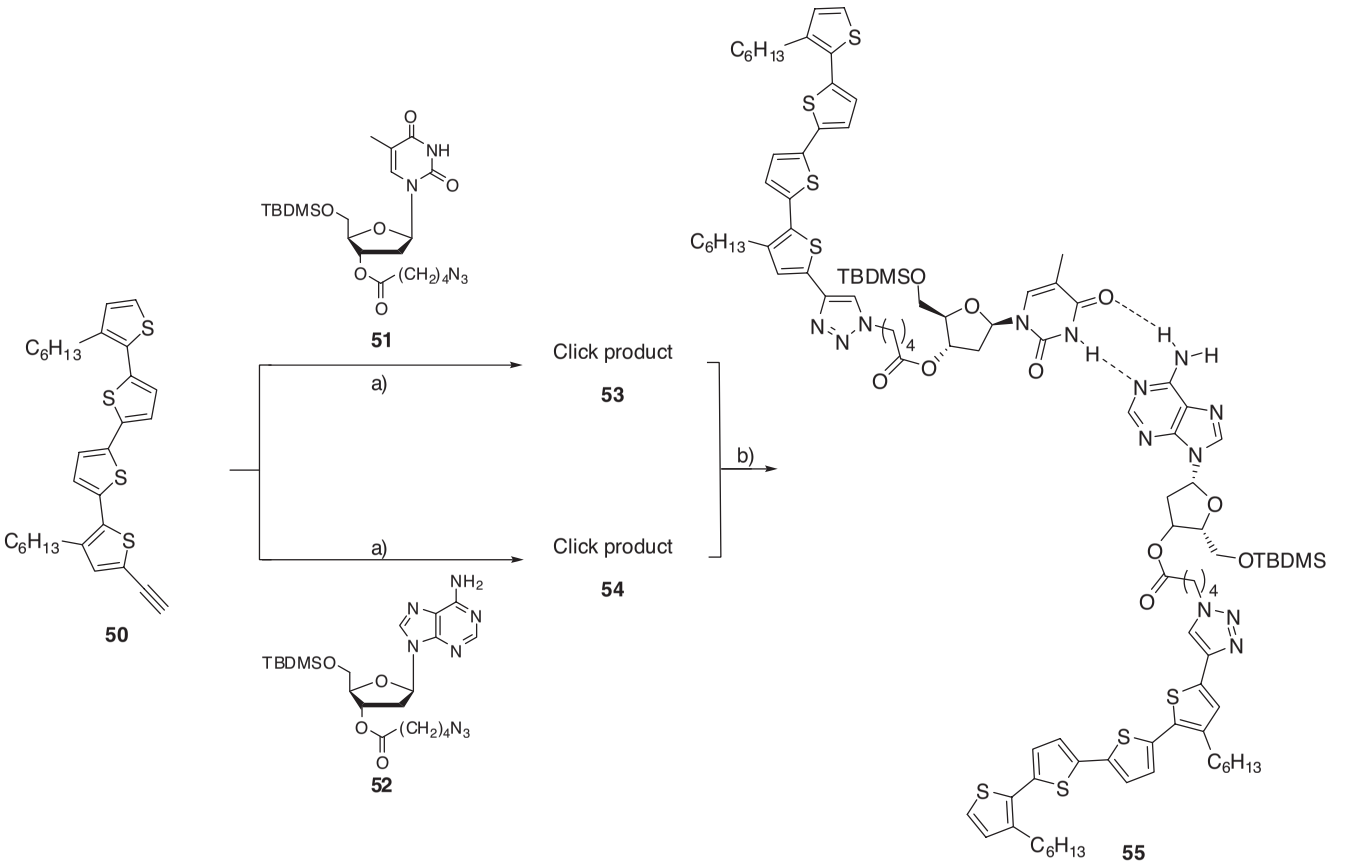
In the field of nanoarchitectures, nature offers an efficient tool to self-assemble artificial and biological systems via hydrogen bonding. This property was used by Bäuerle *et al*., who combined semi-conducting oligothiophenes **50** with complementary nucleosides **51** and **52** to create novel recognition-driven self-aggregated superstructures **55** based on the click reaction (Scheme 2.14).[43]

2.6 Carbohydrates

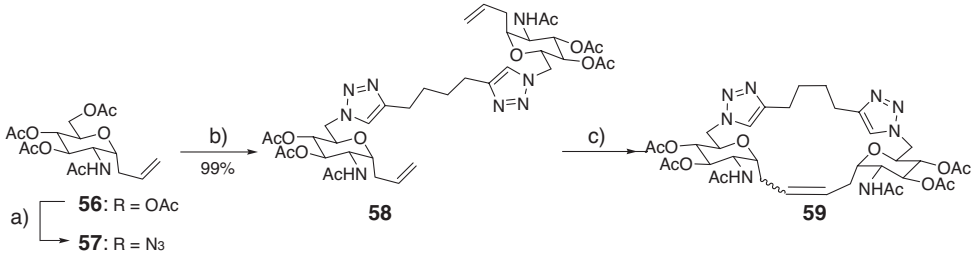
In biology, carbohydrates are a vitally important class of compounds. Oligosaccharides, glycolipids and glycopeptides – displayed on the cell surface – play a crucial role in cellular recognition events, including signal transduction, inflammation, immune response, apoptosis, tumor metastasis and viral and bacterial infections.[44,45] The study of carbohydrate–protein interactions is complicated, hindering efforts to develop a mechanistic understanding of the structure and function of carbohydrates. The weak binding affinities of carbohydrate–protein interactions and the availability of structurally complex carbohydrates remain two major challenges.[46] Thus, it is highly desirable to undertake the construction of mimics of complex saccharides, which can imitate the interaction of a given saccharide with its receptor,[47] as well as rapid screening of target saccharides through the development of modular fluorescent sensors.[48] Interest persists in different research



Scheme 2.13 *Synthesis of fluorescent two-dye conjugates via Cu(I)-catalyzed azide–alkyne cycloaddition of 3-azido-7-hydroxycoumarin (**48**) and PyrdC (**47**). (a) Et_3N/MeOH, CuI, reflux 70 °C; (b) 25 % aq. NH_3, overnight, r.t.*[40]



Scheme 2.14 *(a) Click reaction with $Cu(CH_3CN)_4PF_6$–Cu^0, THF; (b) self-assembly of the click products* ***53*** *and* ***54****, thymidine and 2′-desoxyadenosine-functionalized oligothiophenes.*[43]



Scheme 2.15 *Click-dimerization-ring-closing metathesis for the synthesis of carbohydrate-containing macrocycles. (a) NaOMe, MeOH, r.t.; TsCl, pyridine, 0 °C, r.t. then Ac_2O; NaN_3, DMF, 90 °C; (b) 1,7-octadiyne, ^tBuOH, ascorbate, $Cu(OAc)_2$, H_2O, r.t., 12 h; (c) Grubbs I catalyst (5 mol %), reflux, 12–24 h.*[49]

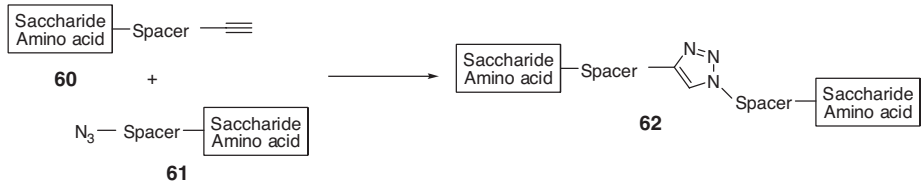
groups for dimeric[6] or amino acid glycoconjugates as well as pseudo-oligosaccharides, glycosylated β-peptides,[47] glucosamine derivatives[46] and many more. Beyond the general advantages of click chemistry outlined in the introduction, the formation of triazole mimics of existing carbohydrates is of interest because the heterocyclic ring can participate in hydrogen bonding – an excellent property in the context of biomolecular targets and solubility. 1,2,3-Triazoles containing dimeric saccharides and amino acid glycoconjugates may exhibit a broad spectrum of biological activities, including anti-bacterial, herbicidal, fungicidal, anti-allergic and anti-HIV properties (Scheme 2.15).

Thus, new sugar-containing macrocyclic compounds were developed via click-dimerization-ring-closing metathesis.[49] Following one of the syntheses, the olefinic functions were introduced at the anomeric center either as an *O*-allyl side chain via Königs Knorr-reaction or via Keck-allylation, leading to the *C*-glycosidic compound. The azide functionality of compound **57** was incorporated after deacetylation at the C-6 position of the sugar core through tosylation of the primary hydroxyl group, which was followed by substitution with sodium azide.

The carbohydrate dimers were then generated after the cycloaddition of the corresponding carbohydrate azide **57** and 1,7-octadiyne in *tert*-butanol and after the addition of an aqueous copper(II)acetate–sodium ascorbate mixture. The resulting diolefinic molecules were reacted for 12–24 h in order to accomplish ring-closing metathesis. In addition, 5 mol % of Grubbs' ruthenium catalyst were used at 40 °C to prevent homodimerization. Unfortunately, nonseparable mixtures of both *E/Z*-isomers were observed, thus the double bonds were reduced hydrogenolytically.

Glycodendrimers are mostly used for the study of biological processes that rely on carbohydrate–receptor interactions such as fertilization, pathogen invasion, toxin and hormone mediation, and cell-to-cell interactions, as well as on a variety of inhibitors.[50] In these cases, the syntheses of azido-terminated dendrimers were preferred over those incorporating terminal alkynes, because of the potential affinity of the latter ones to Cu(II)-catalyzed intradendritic oxidative coupling.

Kovensky *et al*. have developed another procedure for the connection of saccharides by click chemistry techniques. With or without carbon chains as spacers, different sugar-bearing azides on C-2, C-5 or C-6 can be used as building blocks.[6,51] This is also true of



Scheme 2.16 *Synthesis of pseudo-oligosaccharides and amino acid glycoconjugates via 1,3-dipolar cycloaddition (click reaction).*[9]

C-1 alkynylated saccharides or oligosaccharides, in the presence of a range of protecting groups, commonly used in oligosaccharide synthesis.[52]

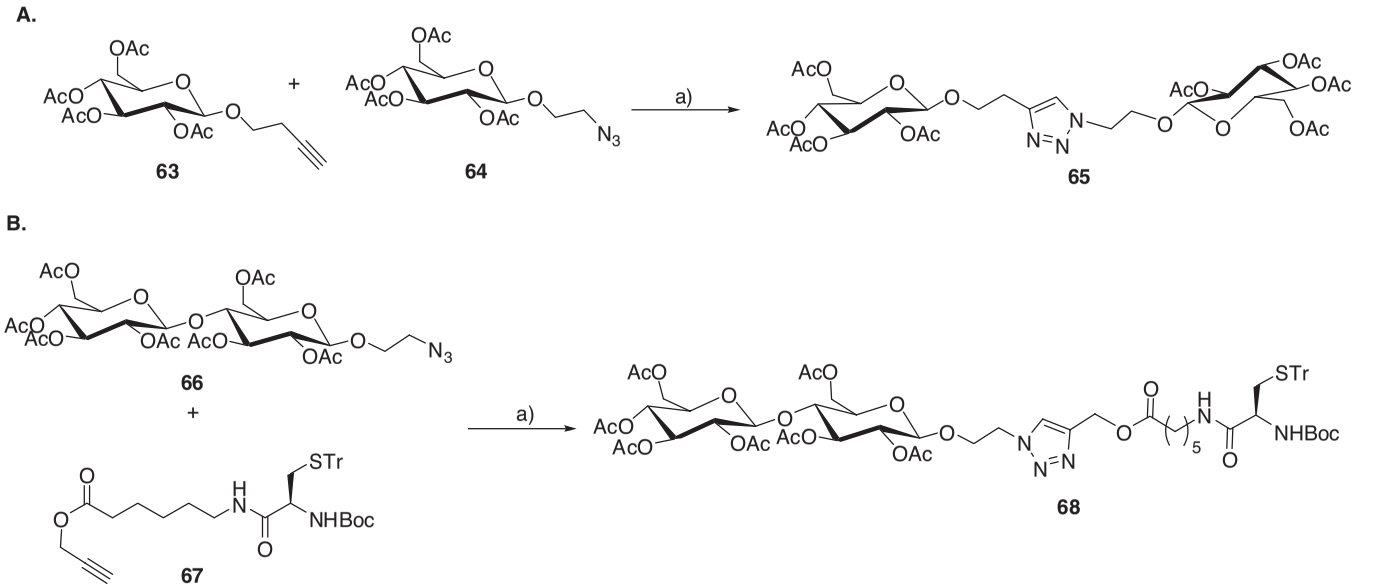
The efficient coupling of oligosaccharides to oligosaccharide–peptide conjugates **60** and **61** leads to homo- and hetero- dimeric glycoconjugates **62** (Scheme 2.16), which can act as potent reversible cross-linking reagents useful in measuring the distances between carbohydrate binding sites in polyvalent recognition sites. Several pseudo-oligosaccharides **65** and amino-acid glycoconjugates **68** were synthesized using easily accessible carbohydrate **63** and compound **67** in combination with azides **64** and **66** as building blocks for 1,3-dipolar cycloaddition (Scheme 2.17).[9]

Another field of complex oligosaccharides deals with the synthesis of highly glycosylated β-peptides **73** and **76**. Ziegler *et al*. started their synthesis with Fmoc-Asp(*O*-^{t}Bu)OH (**69**) to prepare (*S*)-tert-butyl 3-{[(9H-fluoren-9-yl)methoxy]carbonylamino}-4-azidobutanoate (**71**) in four steps [Scheme 2.18(A)][53] or alternatively with an alkynylated amino acid analog, the *tert*-butyl-protected Fmoc-asparaginic acid propargylamide **74** [Scheme 2.18(B)].[47]

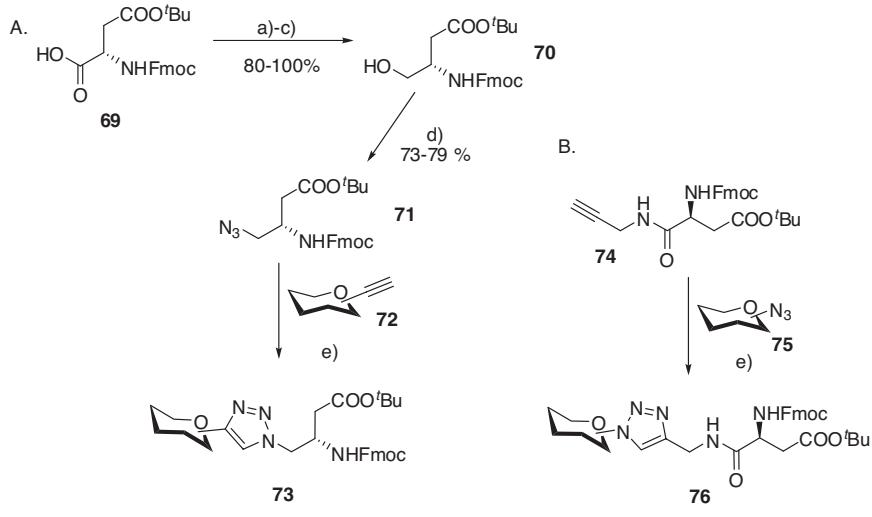
Following the copper-catalyzed coupling reaction, these asparaginic derivatives generated triazole-linked glycosylated amino acids **73** and **76**. Another example was illustrated by Du *et al*., who described a synthesis for C_3-symmetric glucosamine oligosaccharides using click chemistry for studies of interactions between carbohydrates and proteins.[46]

A new approach, the domino-click synthesis, was reported by Kumar *et al*. (Scheme 2.19), based on a series of consecutive intramolecular organic reactions with resonance-stabilized allenylmagnesium bromide **78**, in order to generate 1,2,3-bistriazoles **81**.[10] By coding mRNAs for proteins in drug discovery, based on size-specific mRNA hairpin loop binding agents, bistriazoles promise to be a good target. The addition of sugar azide **77** to the Grignard reagent **78** resulted in the formation of the novel 5-butynylated triazole **79** in good yield. A subsequent Cu(I)-catalyzed 1,3-dipolar cycloaddition with another equivalent of sugar azide **80a** or **80b** generated novel unsymmetrical bis-1,2,3-triazoles **81a,b**.

The proposed mechanism of this reaction is based on a similar reaction previously published by Sharpless and coworkers. The group investigated the conversion of azides and metalized alkynes into aryl azides through the addition of bromomagnesium acetylides, leading to the desired 1,5-disubstituted triazole regioisomers.[10] Based on these results, the mechanism in the case of the allenylmagnesium bromide addition can be explained via nucleophilic attack of the allenylmagnesium bromide species on the terminal nitrogen of the azide, followed by a simultaneous ring closure through N–C heterocyclization driven



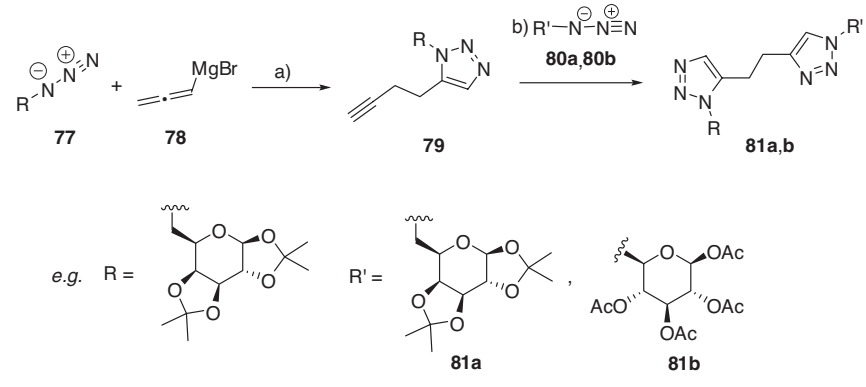
Scheme 2.17 *(a) CuI, EtN^iPr_2, MeCN, 2 h. (A) Synthesis of pseudo-oligosaccharide* ***65*** *(96% yield). (B) Conjugation of disaccharide* ***66*** *to amino acid derived alkyne* ***67*** *(91% yield).*[9]



Scheme 2.18 *Synthesis of triazole-glycosylated β-peptides via A. (S)-tert-butyl 3-{[(9H-fluoren-9-yl)methoxy]carbonylamino}-4-azidobutanoate (**71**) and (B) alkynylated compound **74**. (a) NMM; (b) $NaBH_4$; (c) H_2O; (d) PPh_3, DEAD, HN_3; (e) cat. $(EtO)_3P$–CuI, toluene, 80 °C, microwave, 30 min.*[47,53]

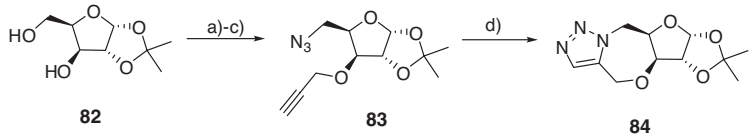
by the excess reagent. The final product is generated through the subsequent attack by a second equivalent of allenylmagnesium.

Another way to produce 1,2,3-triazoles is the intramolecular Huisgen-reaction on carbohydrate-derived azido-alkynes. The azido substrates were synthesized by an S_N2 displacement of the corresponding tosylates using NaN_3. Hotha *et al.*[54] and Mandal *et al.*[55] simultaneously illustrated a procedure for intramolecular cycloaddition without using any copper catalysts, ligands or other reagents (Scheme 2.20).

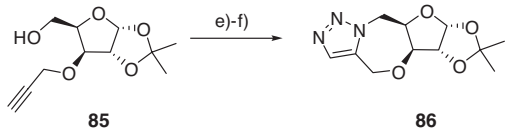


Scheme 2.19 *Domino-click approach to novel bistriazoles **81**. (a) dry THF, r.t.; (b) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, ^{t}BuOH/H_2O (1:1), r.t.*[10]

Hotha *et al.*:



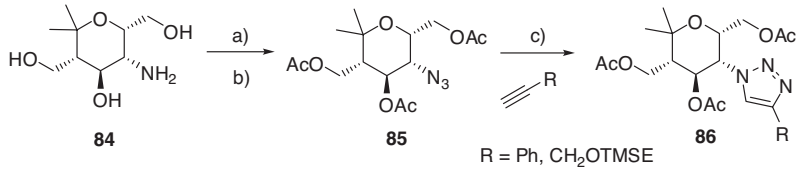
Mendal *et al.*:



Scheme 2.20 *Hotha et al.: (a)* p-*TsCl, pyridine, 0 °C, r.t., 10 h, 91%; (b) NaN_3, DMF, 90 °C, 8 h, 95%; (c) NaH, propargyl bromide, DMF, 0 °C, r.t., 2 h, 93%; (d) toluene, 100 °C, 2 h, 95%; Mendal et al.: (e) MsCl, Et_3N, CH_2Cl_2, r.t., 2.5 h; (f) NaN_3, DMF, 90 °C, 6 h, N_2.*[54,55]

Very similar structures were achieved by Bräse *et al*. through the addition of Me_3SiN_3 to a resin-bound thiopropargyl ether.[56] Cleavage from the resin and the simultaneous intramolecular click reaction of the liberated azide produced a triazolobenzothiazine in 14% yield over four steps.

The synthesis of a secondary azide for enantiopure carbohydrate mimetics has also been established. The synthesis was performed using Nf-N_3 (nonafluorobutanesulfonyl azide) and copper sulfate for the substitution of an amino function and the *in situ* acetylation of hydroxyl groups (Scheme 2.21).[57]



Scheme 2.21 *Azidation with Nf-N_3 of enatiopure carbohydrate mimetics. (a) NfN_3, K_2CO_3, $CuSO_4{\cdot}5H_2O$, MeOH/H_2O, 24 h; (b) Ac_2O, pyridine, DMAP; (c) alkyne, CuI, TBTA, Et_3N, MeCN, 40 °C, 24 h.*[57]

2.7 Conclusion

Click chemistry is a well-established method of generating various derivatives of biomolecules. This article has presented modifications of peptides, peptoids, dendrimers, oligonucleotides and carbohydrates that were synthesized according to their naturally occurring examples. Moreover, several synthetic methods for the formation of the azide functionality on the precursor molecules, as well as applications of the target molecules, have been described herein.

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