Peptidomimetics *via* copper-catalyzed azide–alkyne cycloadditions

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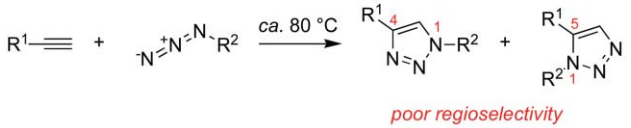
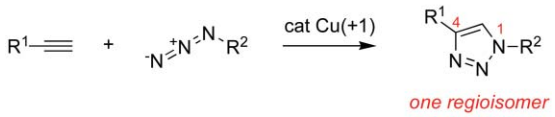
This *critical review* concerns the impact of copper-mediated alkyne–azide cycloadditions on peptidomimetic studies. It discusses how this reaction has been used to insert triazoles into peptide chains, to link peptides to other functionalities (*e.g.* carbohydrates, polymers, and labels), and as a basis for evolution of less peptidic compounds as pharmaceutical leads. It will be of interest to those studying this click reaction, peptidomimetic secondary structure and function, and to medicinal chemists.

A Introduction

Huisgen 1,3-dipolar cycloaddition reactions of alkynes and organic azides are well known and have numerous applications in synthetic organic chemistry.[1,2] This transformation has the advantage of high chemoselectivity since few functional groups react with azides or alkynes in the absence of other reagents. However, it has the limitations associated with any process that requires heat, and, more importantly, that produces two regioisomers (1,4 and 1,5).



(reaction1)



(reaction2)

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It is rare that contemporary science uncovers a very simple modification of a classical "named reaction" that dramatically improves its value, but in the case of the Huisgen cycloaddition, this happened. The groups of Meldal[3] then Sharpless,[4] independently, found copper(I) catalysts dramatically accelerate the reaction and make it totally regioselective for the 1,4-isomer (reaction 2).

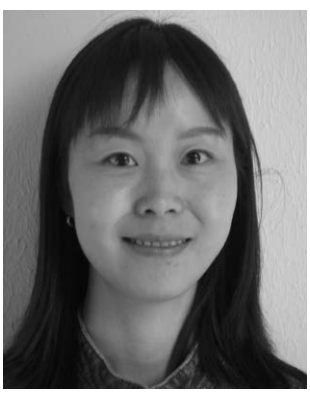
Sharpless and his co-workers have published many high quality papers to alert the scientific community to the value of reaction 2. They coined the term "click chemistry" to describe this and ones like it that proceed in very high yields and that almost invariably work. There are, in fact, too few transformations that fit these criteria in organic chemistry! Click reactions in general have been reviewed,[5,6] and there is one review specifically on copper-catalyzed azide–alkyne cycloadditions.[7]

Here, this review approaches the area with one focus: peptidomimetics. We are interested in combinatorial and/or high throughput syntheses of peptidomimetics of potential value in medicinal chemistry. Reaction 2 has several attributes that are highly attractive in this regard, specifically it:

• gives the 1,2,3-triazole nucleus that is a likely candidate for small molecule drugs;

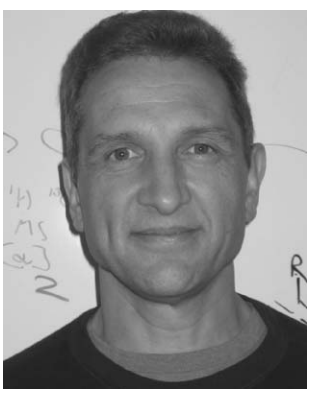
• is compatible with the side chains of all the amino acids, at least in protected form;

• can potentially give high yields of products with few by-products; and,



Yu L. Angell

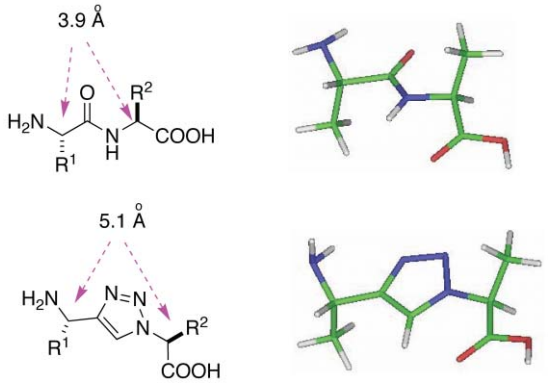
Yu L. Angell received her BS in Chemistry from Wuhan University in China in 2001. She joined the research group of Professor Kevin Burgess at Texas A & M University in 2001. Her research has focused on triazole-based beta-turn peptidomimetics.



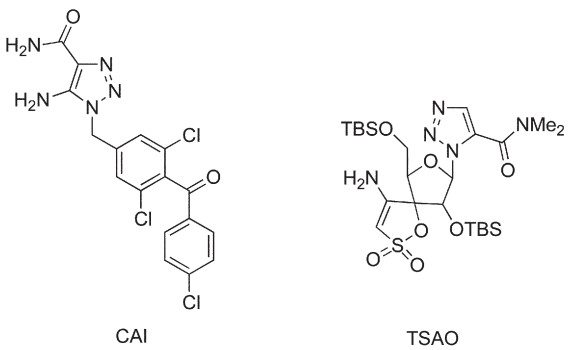
Kevin Burgess

Kevin Burgess is a professor at Texas A & M University where he has been since 1992. His research focuses on synthesis and synthetic methodology to tackle problems of relevance to medicinal chemistry and biotechnology. Specifically, one project is concerned with use of combinatorial libraries designed to disrupt or mimic protein–protein interactions, and it is that which motivated the preparation of this review.

• the molecular dimensions of the 1,4-disubstituted 1,2,3-triazoles are somewhat similar to amide bonds in terms of distance and planarity.

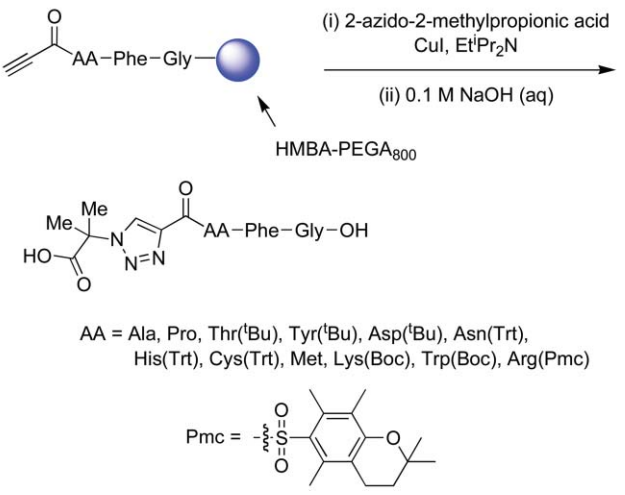


Potential pharmaceuticals based on 1,2,3-triazoles include the anti-cancer compound CAI[8] and the nucleoside derivative known as TSAO,[9] a non-nucleoside reverse transcriptase inhibitor. Both these compounds are (or have been) in clinical trials.



Chemoselectivity

Meldal and co-workers were the first to begin to address the compatibility of protected amino acid side-chains with the featured copper-mediated click reaction. Reaction 3 illustrates how various solid-supported tripeptides were reacted with an azide to give high product yields *via* HPLC analyses. These purities reflect the efficiencies of the cycloaddition and the base-mediated cleavage reaction together. To the best of our knowledge there has been no such systematic study featuring unprotected side chains, but research in our lab has indicated most amino acids side chains are compatible.



(reaction 3)

B Mechanism

The mechanistic interpretation shown in Fig. 1 is supported by kinetic studies,[10] product distributions for specialized substrates,[10] and DFT calculations.[11]

Kinetic studies indicate the reaction is second order in the Cu(1+) source when present at low concentrations; aggregates of the metal may form at higher concentrations. Under typical conditions, the reaction is not precisely first order in alkyne, but it is in the azide component. These observations lend support to the assertion that the reaction may involve rapidly equilibrating copper–alkyne complexes. Product inhibition does not tend to be prevalent in these reactions.

Experiments with constrained diazides show the reaction is difficult to stop even if only one equivalent of alkyne is used. This implies an intermediate involving only one triazole unit can be more reactive than the starting diazide. However, similar experiments with constrained alkynes do accumulate monotriazole intermediates. These observations should not be over-interpreted, but they do imply that monotriazole intermediates can react significantly faster if an azide is held proximal than in cases where an alkyne group is.

Postulated mechanisms have been tested using DFT calculations, and the theoretical data indicates the reaction pathway shown in Fig. 1. It is important to note that other evidence points to the possible involvement of dicopper (and higher order) species; this was not tested in the DFT calculations because it is computationally difficult to do so. Nevertheless, these calculations provided several valuable insights into the reaction. They indicate that π-complexation of terminal alkynes greatly increases the acidity of the ^{sp}C–*H* (“by up to 9.8 units”). Displacement of water ligands by alkynes is thermodynamically much more favorable than if an acetonitrile is liberated. Finally, theory supports the 6-membered

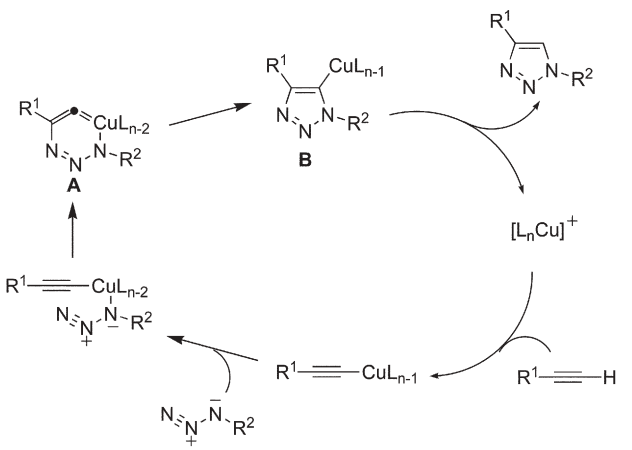


Fig. 1 Putative mechanism of the copper-mediated click reaction of alkynes and azides from DFT calculations.

metallocycle intermediate **A** and its contraction to the *C*-ligated triazole **B**.

C 1,2,3-Triazoles in close analogs of peptides

Some molecules have been made wherein a 1,2,3-triazole is a genuine surrogate for an amide bond, while others have an amide in cyclic structures where the analogy to a cyclic peptide maybe more tenuous. Both these types are covered in this section.

Acyclic compounds

Meldal's group prepared a huge library of compounds (over 400,000) *via* a split and mix approach, then screened them on resin.[12] Fig. 2 shows the basic structure of the compounds that they produced. The resin was first masked with *two* different protecting groups (Aloc- and Fmoc-based). It was the Fmoc-protected arm that was subjected to most of the chemical changes. This was functionalized with a photolabile linker (Fig. 2a), then with a peptide chain "Mis" (Fig. 2b) designed to enhance the response of the photocleaved material to MALDI-MS analyses. Two randomized amino acids were coupled onto the *N*-terminus of this chain, the last one to be coupled being a propiolic acid amide. Copper-mediated cycloadditions were then performed using the Fmoc-protected, amino acid derived azides shown in Fig. 2c. Finally, two more randomized amino acids were coupled. For each coupling step the Fmoc protected amino acids, and the azide in the click reaction, were doped with 10% of the corresponding Boc-protected materials. These were, of course, not deprotected in the piperidine-mediated steps, so a ladder of nested chain-terminated segments was produced as the synthesis evolved. Consequently, photocleavage and MS analysis of the chain revealed the sequence of monomers added *via* a series of mass differences. This so called "MS-laddering" technique[13–15] allows peptidomimetics on "active-beads" to be characterized. Thus the "Fmoc-arm" of the construct is shown in Fig. 2d.

The "Aloc-arm" of the construct was modified using orthogonal protecting group chemistry to give a peptide substrate for the target enzyme: a protease from the parasite *Leishmania mexicana*. This had an *N*-terminal 3-nitro-Tyr quencher and a *C*-terminal fluorescent group (2-aminobenzoic acid on a Lys side-chain) so that on cleavage by the protease the beads would fluoresce, *i.e.* when the quencher-containing *N*-terminal part was cleaved.

The beads in Meldal's construct function as "microreactors". When an inhibitor of the enzyme is produced in the split synthesis, the substrate is not cleaved, and the bead does not fluoresce. Most sequences in the split and mix procedure however give cleaved substrates and fluorescent beads. Screening gave a range of "hit" substances, which were characterized by MS. Statistical analysis of the structures of these then led to consensus sequences that were re-prepared and characterized in solution.

Data that emerged from the work shown in Fig. 2 revealed that the target protease had an affinity for the Mis sequence; this is unfortunate because the only role of that sequence was to enhance MS binding. In retrospect this design weakness could be avoided, but to rectify the flaw the whole library would have to be prepared again. Overall this teaches an

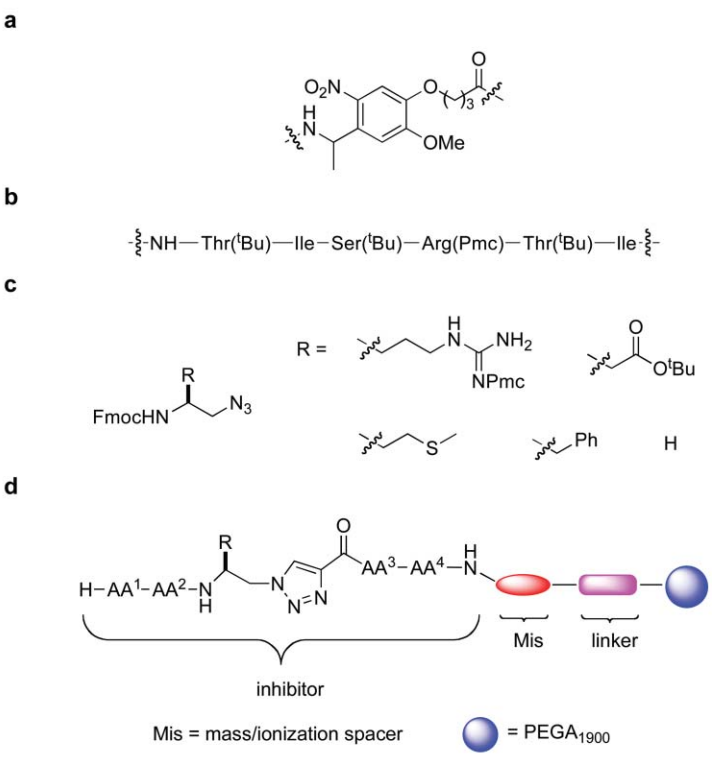
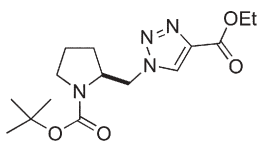


Fig. 2 Meldal's split and mix construct, showing the: **a** photolabile linker; **b** Mis chain for enhanced MS detection; **c** generic structure of the azide components in the click reaction; **d** "Fmoc-arm".

important lesson: simple encoding strategies tend to be best. If the complexity of the encoding molecules rivals that of the probe compound then this invites experimental difficulties.

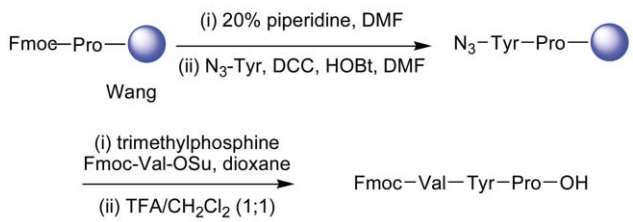
One of the motivations for interest in compounds containing triazole and peptide segments is to prepare synthetic analogs of peptides with perturbed secondary structures. A straightforward illustration of this idea is shown in Fig. 3.[16] In that work click chemistry was used to combine azido- and alkyne-functionalized peptides to give putative β-turn mimics. The secondary structures of these compounds were examined in chloroform, and showed NOE crosspeaks, temperature/concentration variations of N*H* chemical shifts, and FTIR data that are consistent with a small sheet-like structure. Of course, hydrogen-bonding effects are accentuated in non-protic media, so it remains to be established that these constructs are secondary structure mimics in aqueous solutions.

Proline-derived triazoles like **1** have been synthesized *via* click reactions. These molecules were of interest as surrogates for dipeptide segments to see how the triazole impacts *cis*/*trans* proline–amide bond ratios.[17]



1

Access to triazole-based peptidomimetics is greatly facilitated by a relatively convenient azo-transfer reaction[18] that generates 2-azido acids from α-amino acids[19] or protected derivatives of these (reaction 4). This azo-transfer reaction tends to proceed without racemization of the amino acid chirality, even for peptide segments with one free amine group. Further azido acids can be activated and coupled to the *N*-terminus of a peptide without significant racemization.[19]



(reaction4)

Using this azo-transfer reaction, Ghadiri and coworkers[20] explored click-combinations of *C*-terminal propargyl peptides with α-azido-acids to give dipeptide surrogates (Fig. 4). Thus the L-Leu-derived triazole ε^2-amino acid was substituted to give sequences in the pLI-GCN4 sequence to test if the mimics retained the native α-helical character. Mimic **2** in the solid state had a disordered structure about the triazole segment (X-ray), but **3** and **4** had helical structures in the peptide region. Like GCN4, peptide-mimics **2** and **4** formed tetramers in solution (as seen *via* gel permeation chromatography; presumably these are four-helix-bundles), whereas **3** self-assembled into structures with two helices packed against each other.

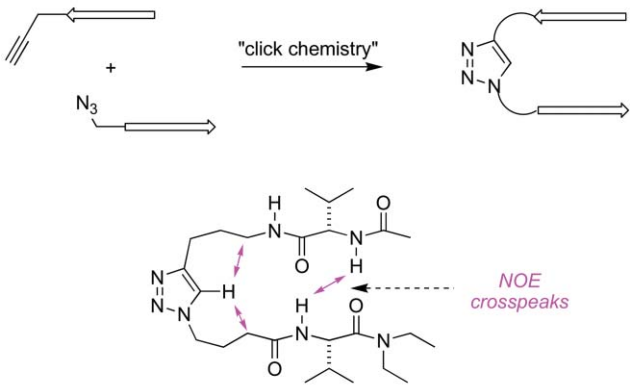


Fig. 3 Construction of a β-sheet mimic.

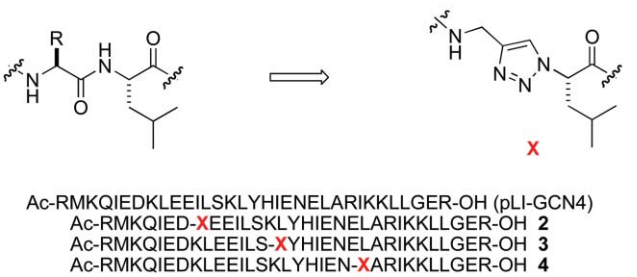


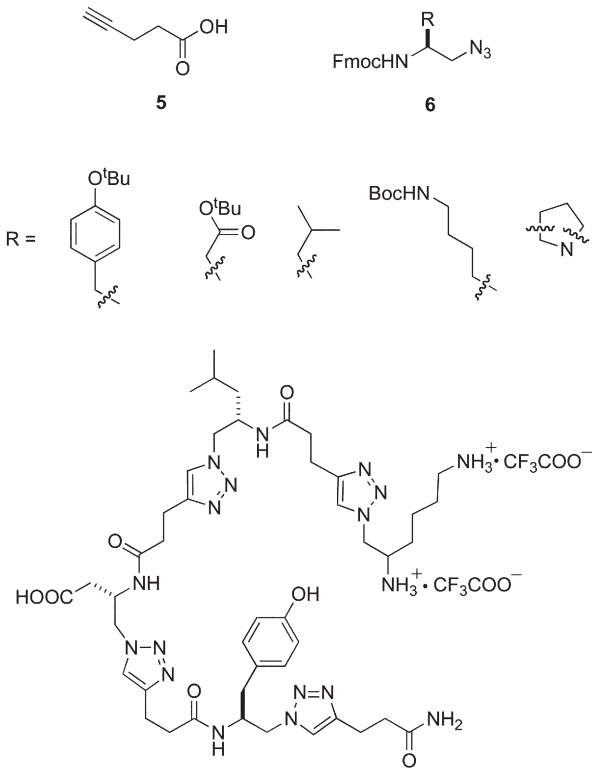
Fig. 4 Triazole-based dipeptide surrogates in GCN4 mimics.

The research described above investigates the effect of placing single triazole-based amino acid surrogates in large peptides. At the other extreme is the use of several triazole-based residues in series. Trimeric molecules of this kind have been made wherein both the alkyne and azide fragments are derived from amino acids (Fig. 5).[21] This is unlike the work above where the alkyne fragment was propargyl amine, *i.e* did not have an amino acid side chain. It was recognized that the triazole units have distinct dipole moments, and conformers of oligomers containing them could be termed *syn* or *anti* based on the orientation of these dipoles. In fact the tripeptide derivatives prefer "zig-zag" conformations in the solid state (X-ray) and in DMSO solution (NMR) wherein the dipoles are opposite.

In between the two extremes of isolated triazole-based residues and several in series, are molecules with alternating triazole and amide linkages: these have also been investigated, to some degree.[22] Using solid phase syntheses to connect pentynoic acid **5** and the Fmoc protected amino-acid azides **6** it proved possible to prepare systems like **7**. Copper(1+) iodide and ascorbic acid combinations (DMF/piperidine) were found to be the most effective for formation of the triazole units.

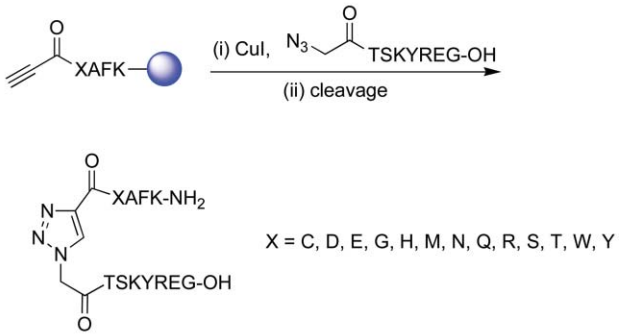
Formation of peptide–peptide linkages

Orthogonal chemoselectivities for amino acid coupling reactions and the copper-catalyzed click-reaction can be useful for joining two peptide fragments.[23] Reaction 5 shows a simple illustration where a supported alkyne was joined to a peptide with an *N*-terminal azide fragment. A more elaborate example of this ligation method is given in reaction 6. Here the cyclic



7

protected peptide **8** has peripheral nitro, NHivDde, and NHAloc groups. These were selectively converted to free amines under different conditions, coupled with alkyne-containing acid fragments, then "clicked" with *N*-terminal azidopeptides. Deprotection of the amino acid side chains and cleavage from the trityl-based resin gave the multimeric peptide **9**. The exact application of this particular product was not revealed.



(reaction5)

Cyclic compounds

One compelling advantage of the orthogonal nature of the Cu-mediated alkyne–azide couplings is that they can be used to prepare cyclic peptides. Meldal and coworkers have done this to make an analog **10** of a disulfide-containing cyclic peptide.[24] They used their poly(ethylene glycol)-based amino polymer resins, which have excellent swelling characteristics in water. Cyclic product **10** was obtained with high purity and yield *via* two methods. In the first, the side-chains were deprotected and the supported peptide was cyclized *via* the Cu-mediated reaction, then cleaved from the resin. In the alternative procedure, the side-chain protection was removed *after* the

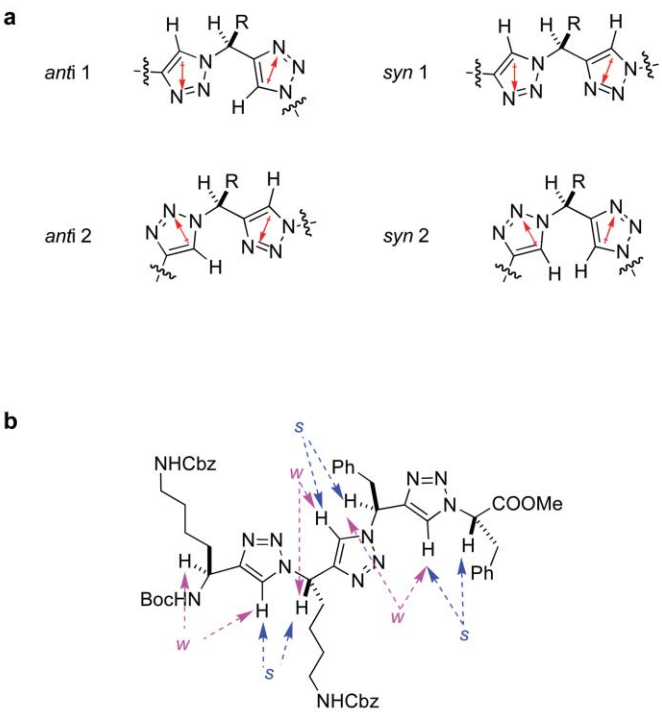
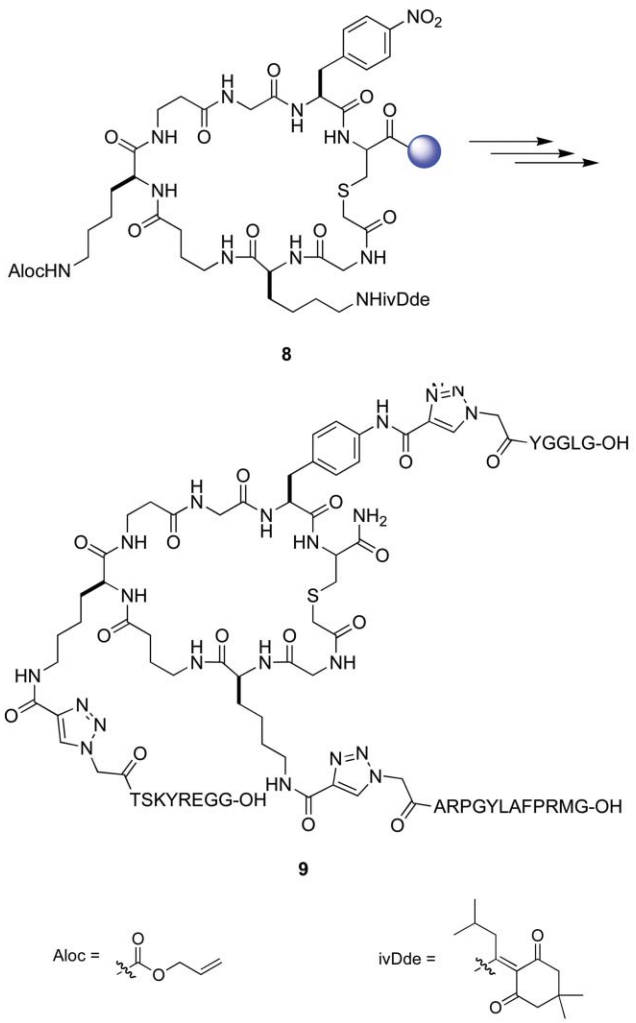
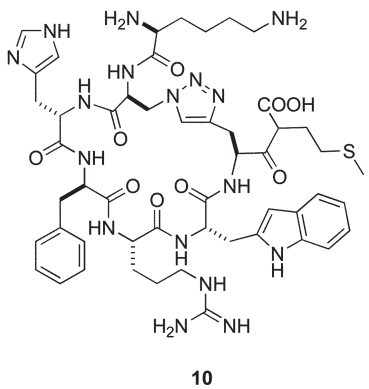


Fig. 5 Trimers of triazole-based amino acid surrogates: **a** must accommodate dipole moments of the triazole units; and, **b** appear to do this in zig-zag conformations that keep the dipoles *anti* (W = weak, S = strong).

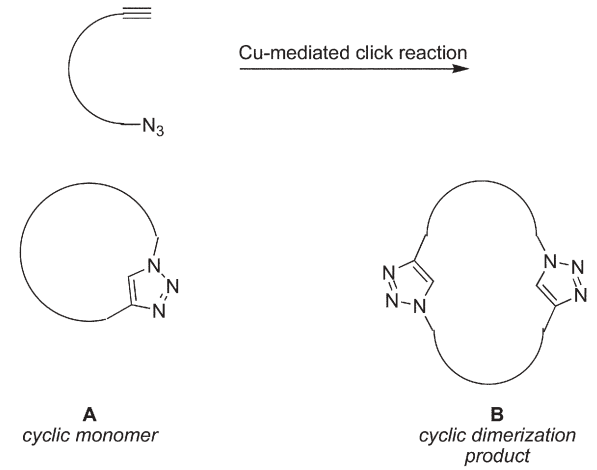


(reaction 6)

cyclization. Both approaches gave over 70% yield of HPLC-purified product. No mention of any dimerization products was made, even though such products can be prevalent, as described in the next section.



The mysterious dimerization effect. Compound **10** was the expected product (type **A**) from a click-mediated macrocyclization reaction. However, a surprising number of papers have reported macrocyclic dimers **B** as prevalent products in similar processes. This sub-section discusses those examples and the possible origins of this anomaly.



A spectacular demonstration of this macrocyclodimerization effect featured two resin-bound 19-amino acid peptide molecules **11** combining into a 38-residue cyclic peptide **12** where 36 amino acid residues are in the ring (Fig. 6).[25] This cyclization required a certain density of molecules on the resin; if the supported concentration was too dilute then it failed. Attempts to form a similar ring system but *via* closure with amide bond formation also were largely unsuccessful, indicating the peptide sequence itself is not predisposed to cyclize. Yields of the cyclodimer were reduced when more than 0.5 equivalents of copper were used, and the process was completely interrupted if an excess of a simple alkyne (but not

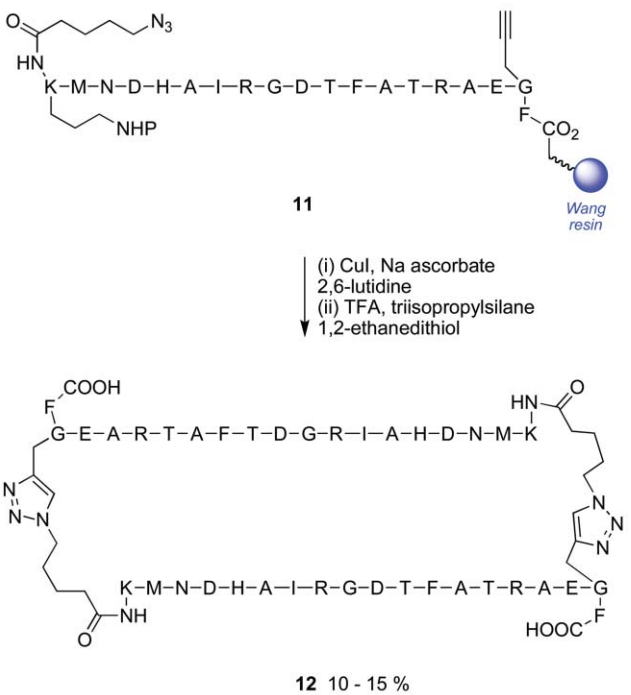
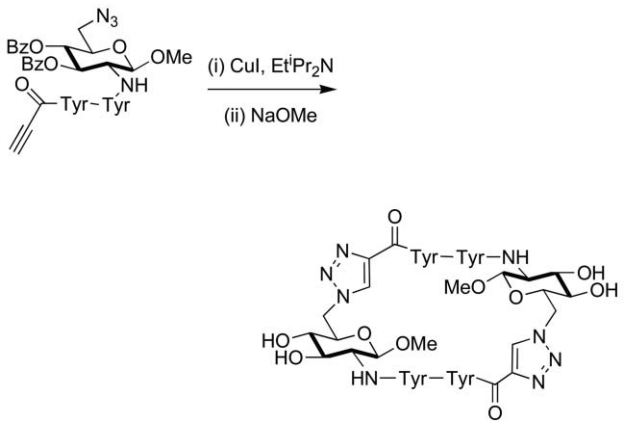


Fig. 6 Preparation of a 38-residue cyclic peptide from cyclodimerization reaction.

of an azide) was included. Thus the cyclodimerization seems to be favored by low catalyst concentrations, and bringing alkyne units into proximity was more important than it was for the azides. In **11** the azide and alkyne are on the *N*- and *C*-termini, respectively, but this did not seem to be critical because a model peptide with reversed orientation also cyclodimerized well (though some more monomer was formed, data not shown). On the basis of these observations it was proposed that the reaction proceeds *via* two alkynes bound to a dicopper intermediate where the azide units interact with the Cu-atom that is not attached to the alkyne of the same substrate.

Another surprisingly efficient macrocyclodimerization reaction occurred in syntheses of carbohydrate–amino acid hybrids as artificial receptors for small biomolecules. This time the click reactions were solution phase ones.[26] Various conditions were evaluated, and CuI combined with *N*,*N*-diisopropylethylamine in acetonitrile was best. Two similar substrates were investigated. These only differed by one amino acid residue, and both cyclized well (64 and 33% before deprotection); reaction 7 shows the most efficient (the yield for the other was 33%).

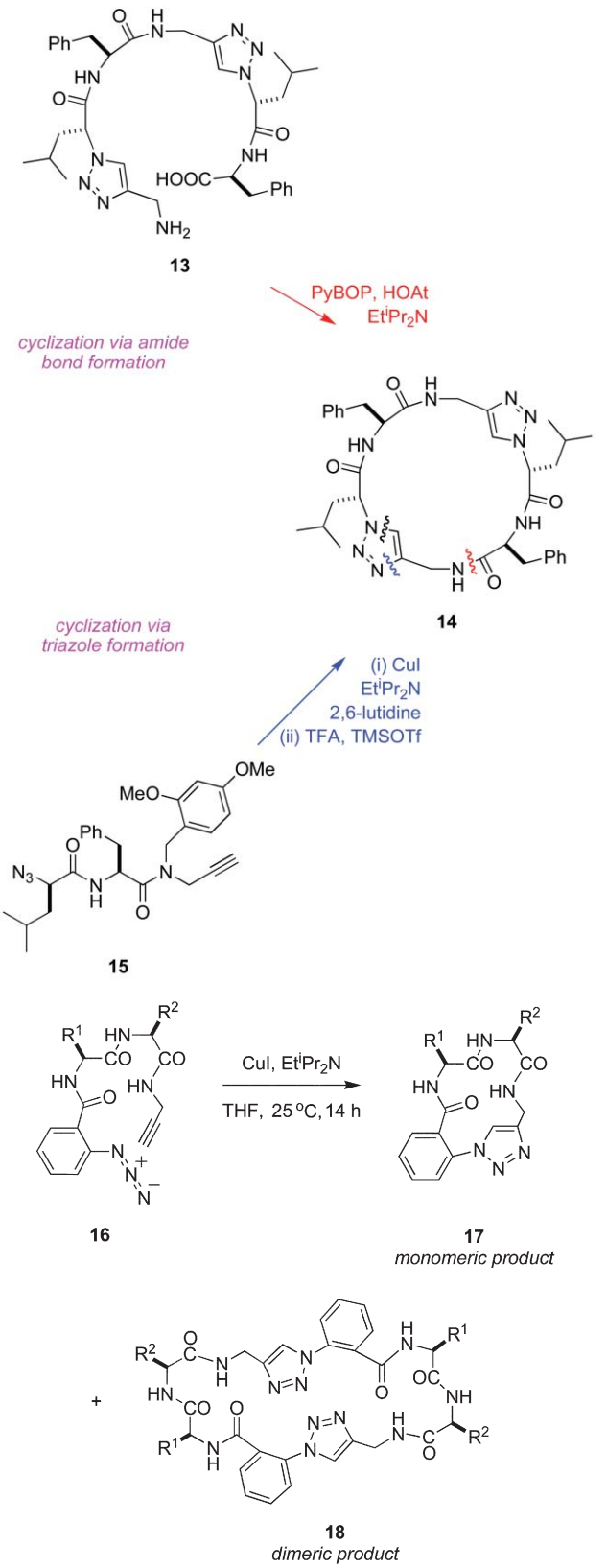


(reaction7)

Cyclic ditriazole **14** forms nicely organized nanotube structures in the solid state in which these doughnut-shaped molecules pack on top of each other *via* H-bonds. Originally monomers **14** were prepared *via* an amide bond forming reaction from linear peptidomimetic **13** (the yield for that step was 65%).[27] Conversion of the alkyne–azide **15** into that same product was vulnerable to simple cyclization and to formation of polymeric products. However, in the event, the macrocyclodimerization of the *N*-protected substrate **15** was shown to be quite efficient. A 94 : 6 mixture of the desired product **14** and the corresponding cyclotrimer was formed in this process, from which **14** was isolated in 80% yield.[28]

Another solution-phase illustration of macrocyclodimerization in this area featured eight different azidoalkyne dipeptide substrates **16**, prepared *via* solution phase methods.[29] Mixtures of the monomeric and dimeric products **17** and **18** formed when these substrates were subjected to the copper-mediated bis-triazole formation reaction. The ratios of monomer-to-dimer ranged from 54 : 46 to 18 : 82, and the dimeric product was prevalent in all but one case.

Fig. 7 shows a postulate to account for formation of macrocyclodimers over the corresponding monomeric forms.



The key point is that *exo*-like intermediates **F** will be favored over *endo*-like ones **E** because of the geometric constraints of forming 1,4-disubstituted triazoles. In other words, there will be somewhat larger entropy barriers to cyclic monomers *via*

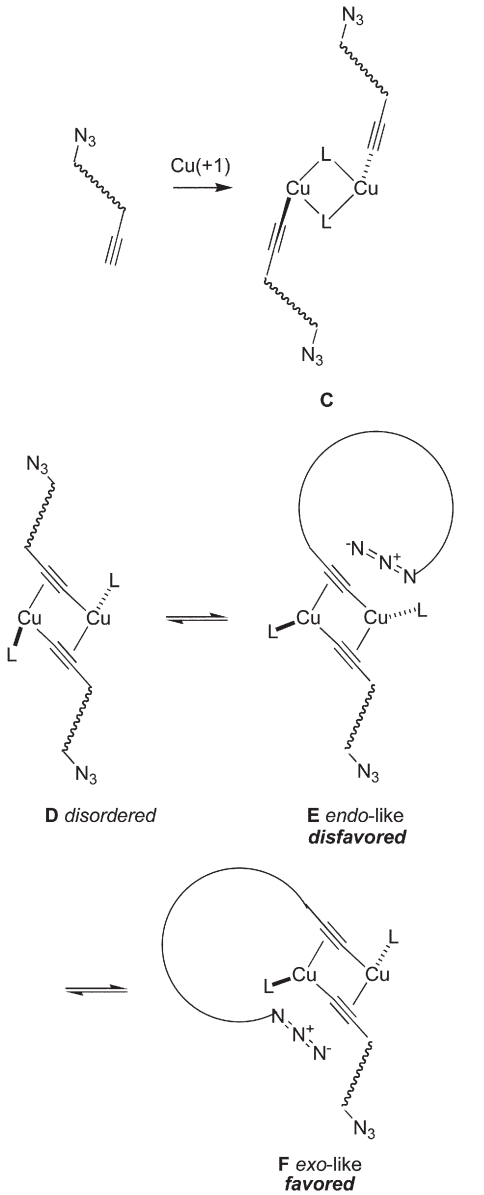
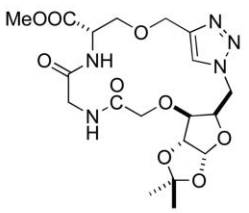


Fig. 7 Possible rationale for formation of macrocyclodimers.

intermediates **E** that can account for preferential formation of macrocyclic dimers.

Based on the examples given above, formation of macrocyclodimers can be expected, but it will not *always* occur. For example, compound **19** was one of four similar compounds prepared *via* Cu-mediated triazole formation to close 12- to 17-membered rings. No macrocyclodimers were observed in these cases, even though the researchers looked for them. Compound **20** is a 1,5-disubstituted triazole formed *via* the Cu-mediated process.[30] This is *highly* unusual for this type of reaction. It is tempting to assume that this product formed because of some particular constraint that prevented formation of the expected 11-membered ring. However, if that is the case, then a similar stereoelectronic constraint prevented formation of the macrocyclodimer from 1,4-triazoles. Clearly there are some unresolved issues surrounding this work.



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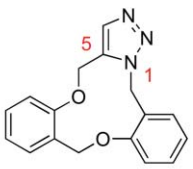
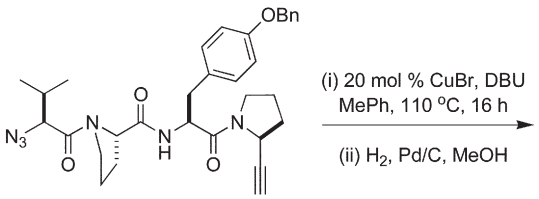
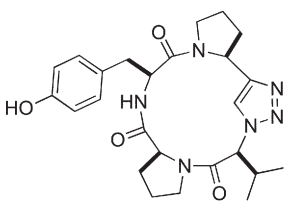


20

Another anomaly is the synthesis of the cyclic triazole tetrapeptide **21** (a potential tyrosinase inhibitor).[31] Attempted cyclization by peptide bond formation at room temperature failed to provide the desired product: mixtures of dimers and higher oligomers were obtained. The copper(I) catalyzed azide–alkyne coupling was successful but only at 110 °C; the triazole tetrapeptide **21** was isolated in 70% yield under these conditions. It is surprising to us that the reaction took such a high temperature (intermediate temperatures were also studied), and that no macrocyclodimer was observed.





21

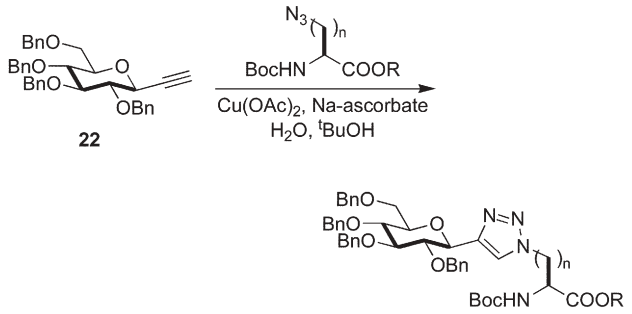
At least one other example of a macrocyclization reaction that gives only cyclic monomers has been reported. This did not involve peptides or peptidomimetics.[32]

D Compounds with triazole links to other functionalities

Carbohydrate- peptide

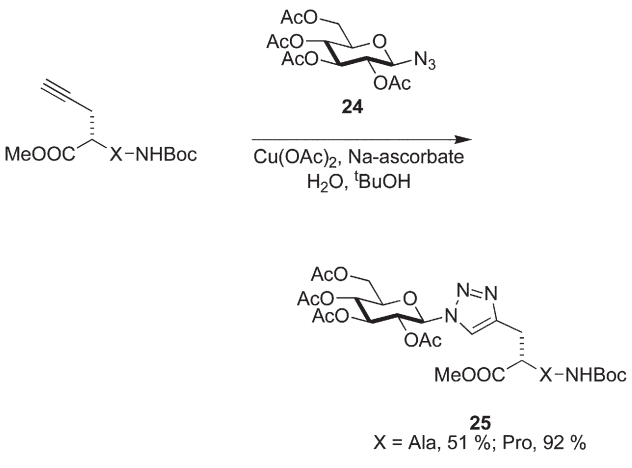
Glycopeptides are important compounds with a range of biological functions. Native *O*- and *N*-linked glycopeptides are relatively unstable to hydrolysis, but *C*-linked isosteres are far more robust to chemical and enzymatic degradation. They have potential as probes for glycopeptide biological activities and as drug candidates for diseases involving carbohydrate-based metabolic disorders.

C-Linked glycopeptide analogs like **23** can be formed very conveniently *via* Cu-mediated cycloadditions to carbohydrates with anomeric alkyne groups, *e.g.* **22**. This type of chemistry has been investigated by at least two groups using glucose- (reaction 8) and galactose-based starting materials.[33–35]



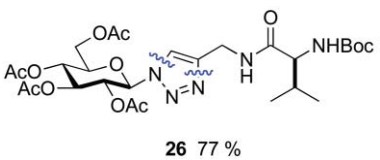
(reaction8)

N-Linked glycopeptides **25**, where the N-atom is part of the triazole, can be formed by reversing the orientation of the two functional groups in the Cu-mediated reaction. Thus, glycosidic azides, like **24**, can be coupled with alkyne-containing amino acids to give carbohydrates where the triazole is attached to the anomeric position (reaction 9).



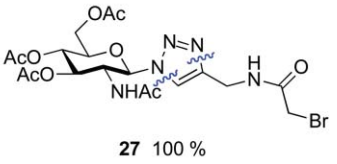
(reaction9)

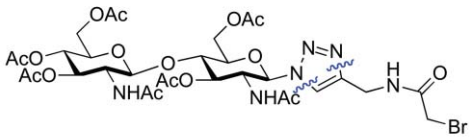
Anomeric azides[36–40] and unsaturated amino acid derivatives[41] are readily accessible so this chemistry has quite a wide scope. Indeed, one of the easiest ways to prepare unsaturated amino acids is to add an *N*-propargyl group; such substrates have also been investigated as click partners to make glycopeptide analogs like **26**.[42]



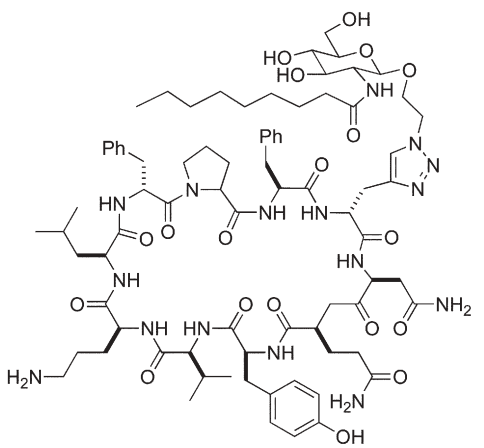
Mono- and disaccharide alkylating agents **27** and **28** have been prepared *via* the Cu-mediated click process, and used to couple with cysteine residues in a peptide. Glycopeptide analogs produced in this way are compatible with native ligation methods involving *C*-terminal thioesters and *N*-terminal Cys-residues. Large peptides containing several unnatural *S*-linkages to carbohydrates have been prepared in this way.[43]

One of the most elaborate routes to glycopeptide analogs has been reported by Lin and Walsh.[44] They were motivated to prepare glycosylated forms of the cyclic peptide antibiotic





known as tyrocidine or "Tyc". To do this, about 17 linear decapeptides were prepared on a solid phase having the Tyc sequence except that one, two or three propargyl glycine residues had been substituted at select positions. These linear peptides were derivatized as *N*-acetyl cysteamine esters to facilitate chemoenzymatic cyclization using the excised thioesterase domain of tyrocidine synthetase. This afforded a small library of cyclic peptides containing one to three propargyl side chains. These were reacted with 21 different azidomonosaccharide derivatives to give a range of glycosidated Tyc analogs having triazole-based linkages to the sugar parts (Fig. 8).



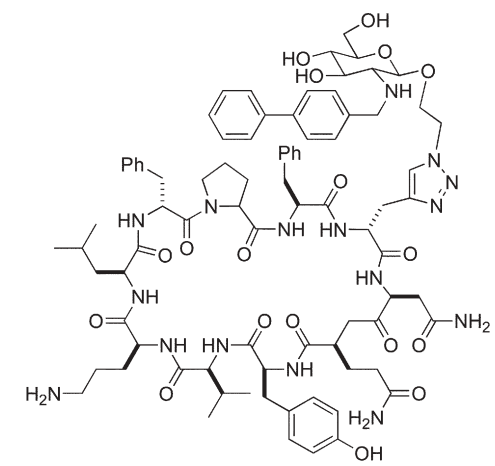
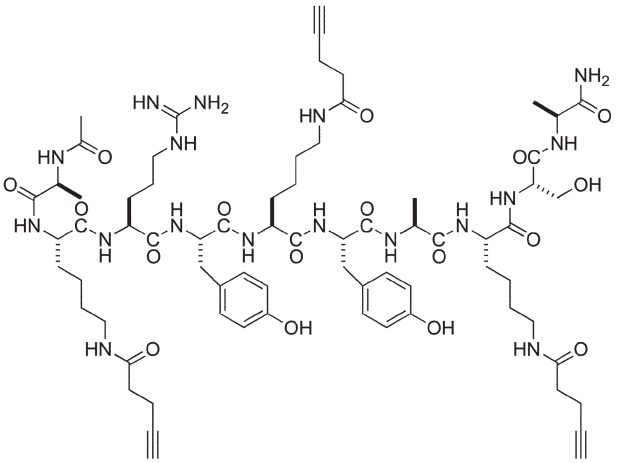


Fig. 8 **a** Tyrocidine; and **b** some of the analogs prepared.

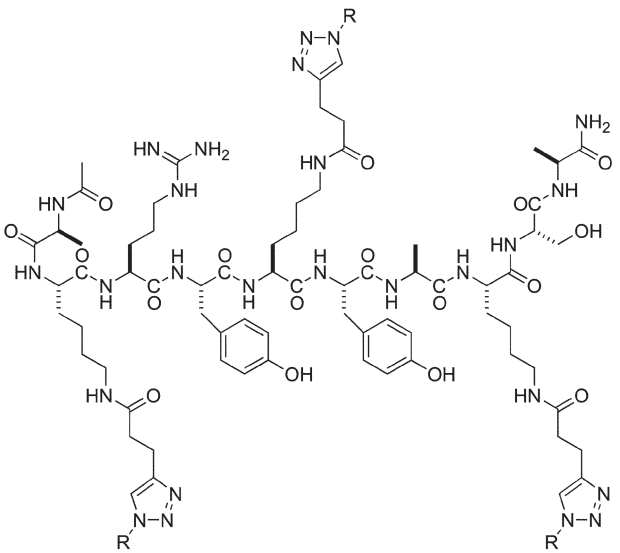
Another way to take advantage of the exquisite chemoselectivity of the copper-mediated cycloaddition reaction is to conjugate carbohydrate based antigens onto peptide substrates. The Danishefsky group has done this for a model peptide system, and infer that the same approach will work for proteins.[45] In this study, the model peptide system **29** was coupled with a trisaccharide antigen to provide the immunogenic conjugate **30**. If this approach is to be applied to proteins, it requires chemoselective introduction of alkyne or azide groups otherwise the labeling is non-uniform.



29

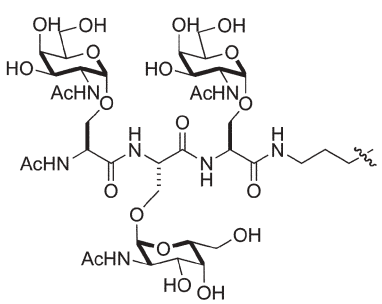
$R-N_3$, Cu nano powder

phosphate buffer, pH = 7.2, 2 h

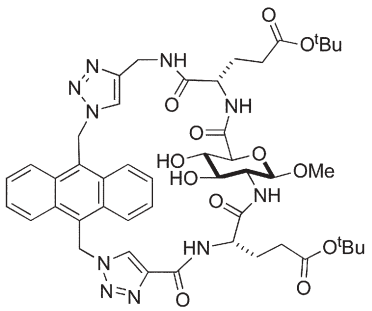


30

R =

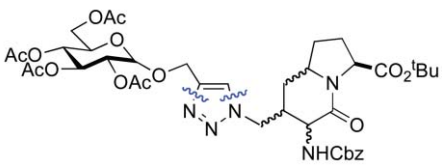


Carbohydrates can also form the basis of macrocyclic molecules for molecular recognition. Compound **31** is representative of a molecule prepared for this purpose.[46] It has a fluorescent reporter group (anthracene-based) which forms a hydrophilic face with the two triazole units. No specific target was suggested for this potential host molecule, though clearly the amino acid and sugar units might be varied to accommodate different hosts.



31

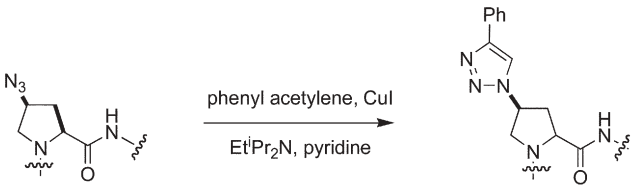
Finally, less peptidic peptidomimetics can also be fused to carbohydrate residues using the Cu-mediated reaction. An illustrative example is compound **32** wherein a conformationally restricted analog of the Ala-Pro dipeptide was joined with a glucose-based azide (and with fluorescein and biotin in other experiments, not shown).[47]



32 80 %

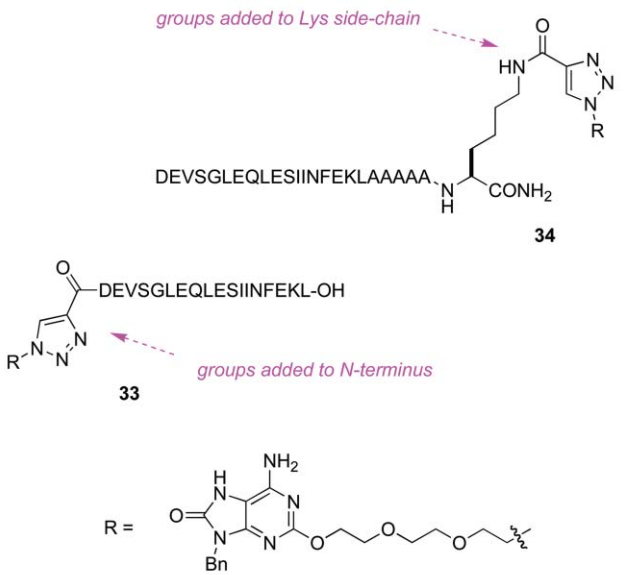
Organic- peptide

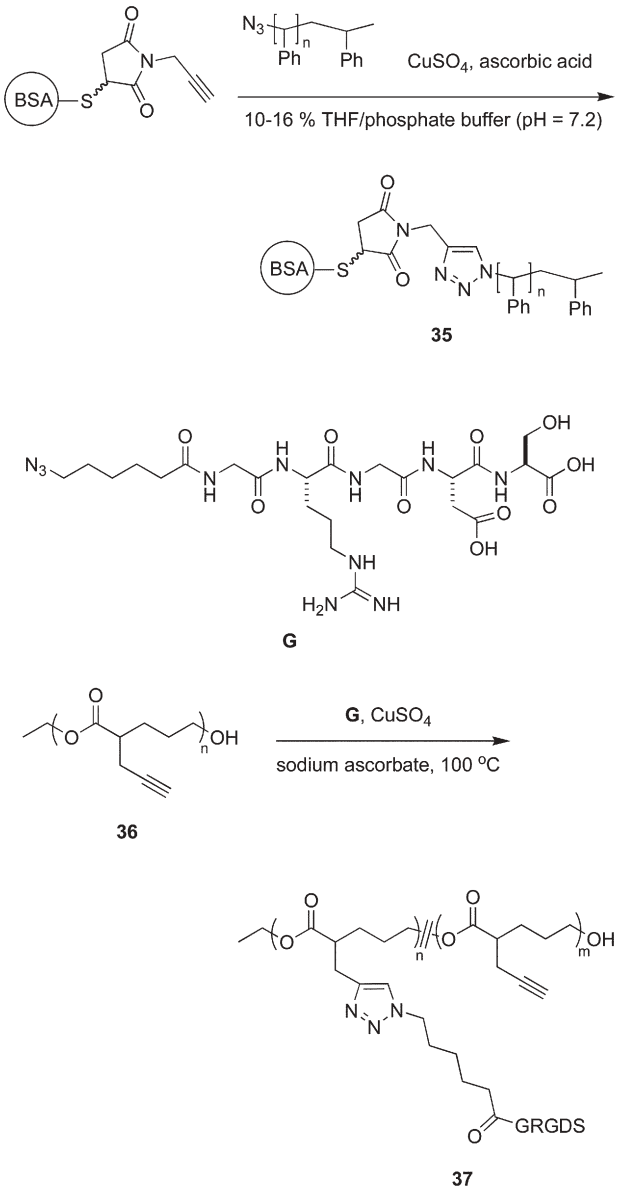
Alkyne- or azide-modified peptides can be transformed into a variety of structures beyond glycopeptide analogs. The last section briefly mentioned cases in which Cu-mediated cycloadditions were used to add biotin and fluorescein: these are clearly two very useful types of molecular probes for bioconjugation. The Cu-promoted process also provides convenient access to structural diversification for medicinal chemistry. For instance, *cis*-4-azidoproline was substituted for the Pro6 residue in the dodecapeptide RINNIPWSEAMM to allow for preparation of a range of analogs *via* the Cu-promoted reactions with azides. Routine peptide synthesis methods were used to incorporate this azido-residue. The parent sequence was discovered *via* phage display; it binds to the gp120 protein of HIV-1 and prevents the HIV virus docking with CD4 cells. Preparation of over 20 derivatives *via* Cu-mediated click chemistry, and testing these for gp120 binding revealed addition of phenyl acetylene as shown in reaction 10 gave approximately a 500-fold increase in binding.[48]



(reaction10)

Incorporation of azidoproline residues is quite a specialized modification; it is essentially only valuable for parent peptides containing proline and for modifications at that position. On the other hand, one of the simplest and most general of the approaches to peptidomimetics of extended peptides is to couple propiolic acid to either the *N*-terminus or to the side chains of Lys or Orn residues, then make modifications *via* the Cu-mediated cycloaddition process. This was done, for example, to peptide chains containing the SIINFEKL sequence (a major histocompatiblity {MHC} class 1 epitope) to access derivatives **33** and **34** with conjugated 2-alkoxy-8-hydroxyadenine entities.[49]





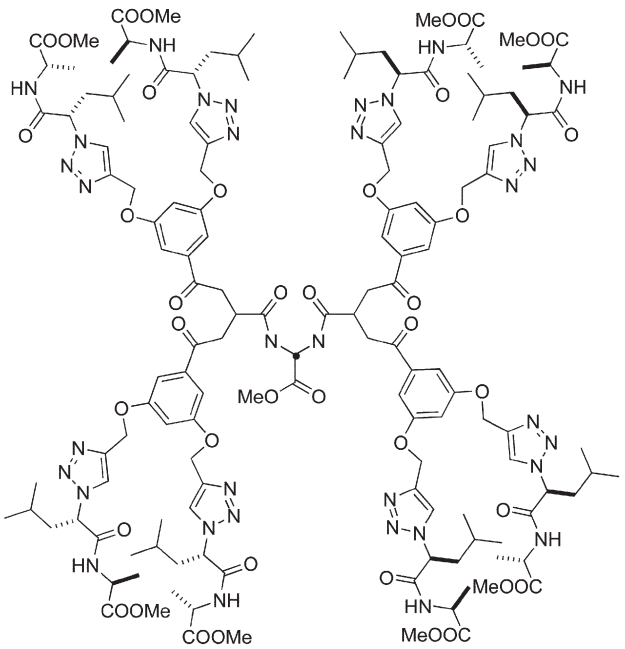
Polymer- and dendrimer-peptide

Most amphiphilic molecules are relatively small structures, like detergents. However, it is possible to make ''amphiphilic-nanostructures'' containing large, water-soluble, biomolecules attached to lypophilic polymers of a comparable size. Copper-mediated cycloaddition reactions provide a convenient way to do this. In the example shown in reaction 11, bovine serum albumin was attached to a polystyrene fragment *via* this approach. Aggregates of the product molecules **35** were visualized *via* various microscopy techniques (SEM and TEM).[50]

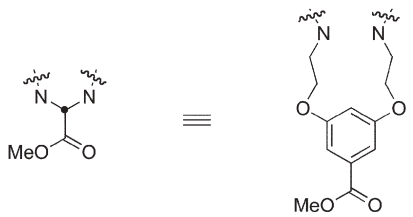
Polyesters are less chemically stable than polystyrene derivatives, but the conditions for Cu-mediated cycloadditions are sufficiently mild for their elaboration. Consequently, the polyester **36** from e-caprolactone was efficiently grafted with the RGD-containing azidopeptide **G** peptide to give the biomaterial **37** without a significant change in the polydispersity.[51]

Click reactions can be used to elaborate dendrimers. Sharpless, Fréchet and co-workers used alternating Cu-mediated cycloadditions and S_N2 substitutions to build up dendrimer cores.[52] So far, in the area of peptidomimetics, the Cu-mediated cycloadditions have only been used to add peptide residues to dendrimer surfaces. Thus, multivalent dendrimeric peptides like **38** were synthesized. The coupling reactions were slow and not high yielding under the regular conditions. However, microwave irradiation for 10 min to generate a temperature of 100 °C greatly accelerated the process and yields as high as 94% for a fourth generation system elaborated with eight end groups. Azide derived amino acids, linear- and cyclic peptides were coupled.[53]

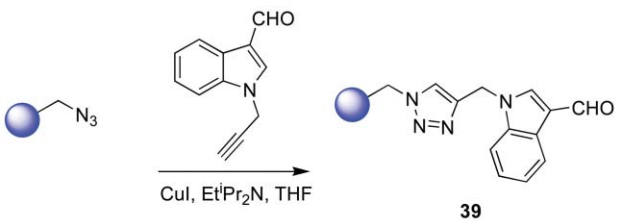
Chloromethylpolystyrene and similar supports are easily transformed into the corresponding azides *via* simple nucleophilic displacement reactions. The azido resins so produced can be further functionalized by click reactions to add linkers. Reaction 12 shows how this approach was



38



used to prepare a indole-based backbone amide linker **39** (BAL).[54,55]

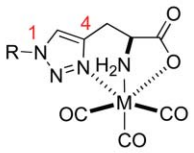


39

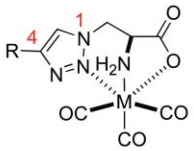
(reaction12)

Conjugates of metal complexes for radiolabeling

Triazoles are good ligands for transition metals. Peptides (or other biomolecules, in fact) can be *C*- or *N*-terminated with an amino acid derived alkyne or azide, then "clicked" with the complement in a copper catalyzed cycloaddition. This procedure fulfils two objectives simultaneously. First, it introduces a metal binding site onto the peptide. Second, the triazole linkage is itself part of the metal-ligand. The obvious application of this approach is in labeling bioactive peptides for imaging in cells or *in vivo*. Thus, complexes of the type **40** and **41** have been prepared, where the triazole units have different orientations but are still disposed to coordinate with metals. Technetium complexes of the derivatized peptide **42** have been prepared for this purpose.[56]



40



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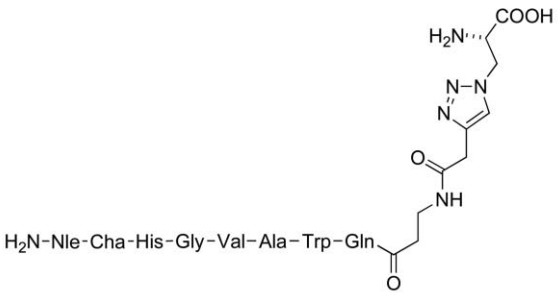
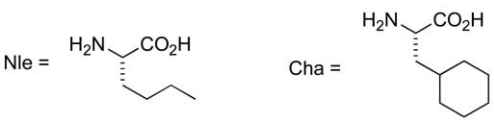


42



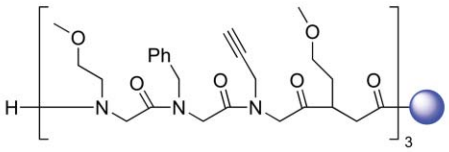
E 1,2,3-Triazoles in less peptidic compounds

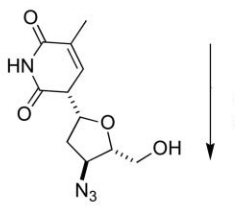
Peptoids

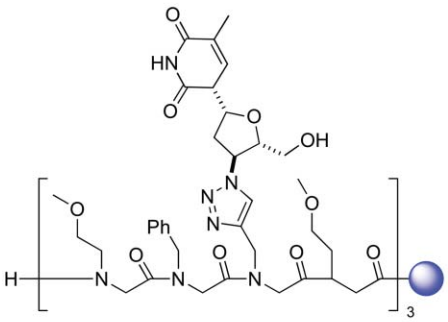
Peptoids (*N*-alkyl oligoglycines) are in some ways representative of molecules that bridge the gap between peptidic molecules and more organic based structures. The attractive feature of peptoids is that they are formed from primary amines, and a large selection of these is commercially available. If amines that are also functionalized with terminal alkynes are used, then Cu-promoted cycloadditions of azides can be used to increase the diversity even further. This elaboration could be done on each addition of an alkyne, or globally to every alkyne in the sequence prepared. The latter approach was taken in the one paper that has appeared so far on combining peptoids and catalyzed azide–alkyne cycloadditions.[57] In fact, the peptoids were derivatized while supported on a resin, then cleaved. Fluorophores, nucleobases and other peptoids were conjugated and yields of up 96% were obtained, *e.g.* reaction 13.

Non-peptidic mimics where triazoles replace amides

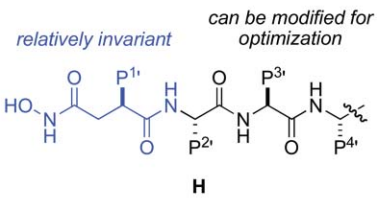
In medicinal chemistry, Cu-promoted cycloaddition reactions can be valuable in cases where a particular structural element is almost invariably required, but others are to be joined to it to promote high affinity, potency, and/or selectivity. A good example of this is in design of analogs of the matrix metalloprotease inhibitor type **H** where the hydroxamate dipeptide mimic is essential for binding metals in these enzymes, and the other part can be varied. Thus a library of mimics including compounds **43** and **44** was conveniently produced and tested. These two molecules were selected from the library on the basis of selective binding to, and inhibition of, the MMP-7 enzyme relative to thermolysin and collagenase.[58]



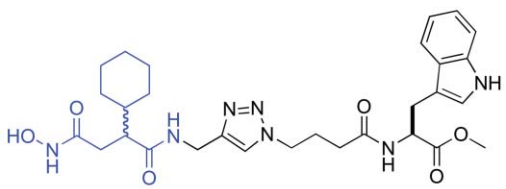




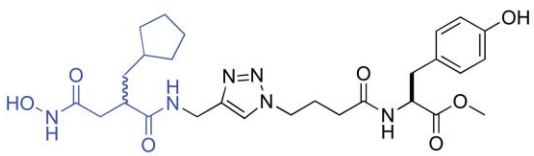
88 %



H

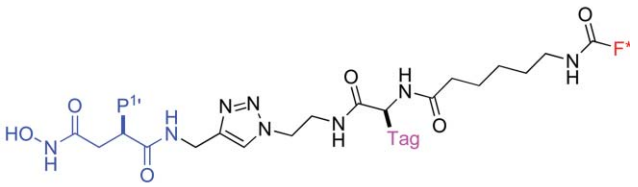


43

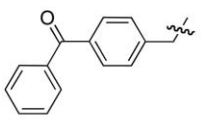


44

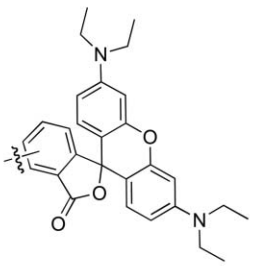
In a sequel to the work above, 12 compounds **I** were prepared to test the effects of the P$^{1'}$ substituents on binding and selectivity.[59] To this the Eastern portion of the molecule was engineered to include a photoaffinity tag and a fluorescent label. Irradiation of the compounds with the enzymes anchored the photoaffinity tag, and the extent of the bound fluorescence was taken as a measure of the affinity. This approach assumes that all the enzymes have identical affinities for the Eastern segment, and that the extent of photobleaching is uniform for all the substrates. If these are valid assumptions, then the method facilitates rapid assay of binding and selectivity.



I

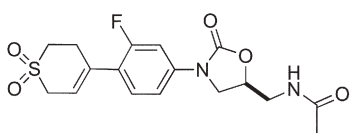


Tag
for photoaffinity labeling



F*
rhodamine reporter group

1,2,3-Triazoles with small 4-substituents can be useful as replacements for acetamide groups. A simple illustration of this is the lead structure **45** that was mimicked with triazole **46**. This was done to find analogs that would not display undesirable monoamine oxidase A side-effects but maintain the antibacterial properties. In the event, this was part of a much more extensive study and the compound formed by removal of TMS **46** emerged as an interesting lead.



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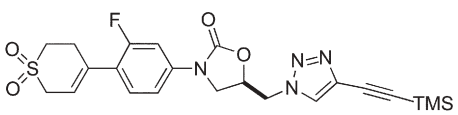
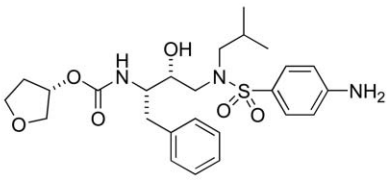


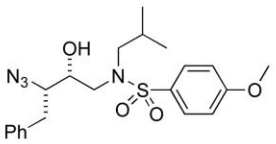
46

Amprenavir is one of the commercially available HIV-1 protease inhibitors. A library of 100 mimics of this structure was produced from the two starting azides **47** and **48** (accessible *via* diazo transfer chemistry to the amine, and *via* epoxide opening, respectively). These segments were joined with alkynes *via* Cu-mediated cycloadditions, and triazoles **49** and **50** emerged as potent inhibitors of both the wild type protease, and of three different mutants.[60] Intriguingly, when these compounds were co-crystallized with HIV-1 protease, the triazole unit revealed itself as an effective surrogate for the amide bond.[61] It has a large dipole moment (bisecting the ring plane near atoms N3 and C5) and the N2 and N3 electron lone pairs can function as hydrogen bond acceptors. In the specific case of **49**, the N2 atom serves as a H-bond acceptor surrogate

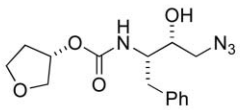
for the amide carbonyl of substrates in the protease. Further, the C5*H* serves as a hydrogen bond donor effectively mimicking the substrate amide N*H*.



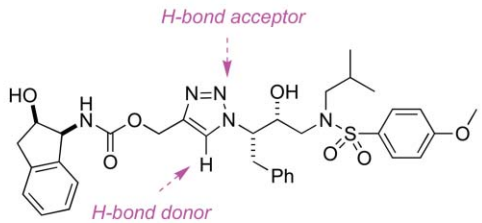
amprenavir



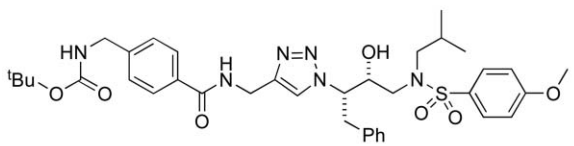
47



48

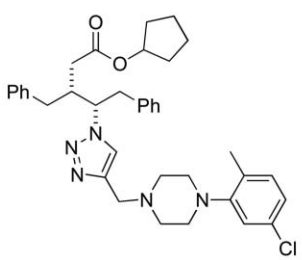


49

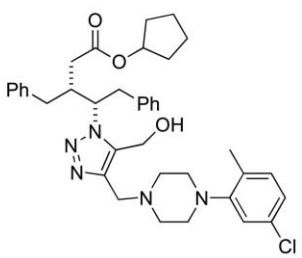


50

Sharpless and co-workers have prepared a series of compounds as potential HIV-1 protease inhibitors.[62] Molecular modeling indicated that triazole sub-units based on 1,2-amino azides, derived from amino acids, would be useful. Consequently, successive iterations of synthesis and testing led to the development of compounds **51** and **52**, which are nanomolar inhibitors. The latter compound was formed *via* a copper-mediated cycloaddition, followed by directed metalation and quenching with formaldehyde.



51
K_i 23 ± 4 nM

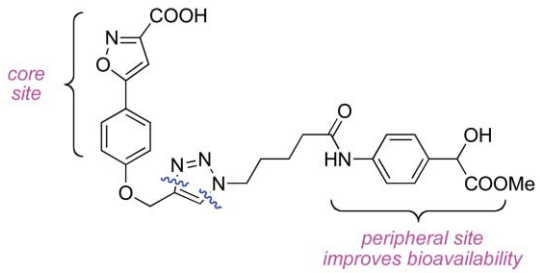


52
K_i 8 ± 0.5 nM

Other non-peptidic peptidomimetics

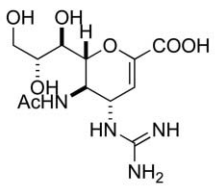
In many other branches of medicinal chemistry, triazoles from the click reaction have been used to extend molecules or replace similar functional groups.[63] This review focuses on peptidomimetics, so the discussion in this sub-section is restricted to compounds where the native substrate is a peptide, even if the synthetic analogs are not peptidic at all.

Inhibitors of tyrosine phosphatases may act *via* the active site or they may bind the enzyme peripherally. Moreover, some inhibitors have poor pharmacokinetic properties. Therefore, a logical approach to discovery of new inhibitors is to join two active segments into one molecule, and Cu-mediated cycloadditions are ideal for this.[64] Compound **53** emerged from a library of 66 ''bivalent'' compounds prepared in this way; it inhibits protein tyrosine phosphatase 1B with an IC_{50} of 4.7 mm.

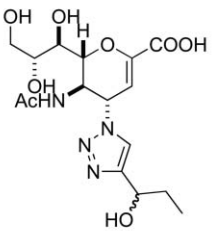


53

Compound **54** is one of 16 triazole-based zanamivir analogues that were formed *via* Cu-mediated cycloadditions. Zanamivir is a licensed pharmaceutical that acts *via* inhibition of neuramidases. In the analog, the triazole is acting as a mimic of the guanidine functionality. Compound **54** showed moderate inhibition against avian influenza virus (subtype H5N1) in a cellular assay.[65]



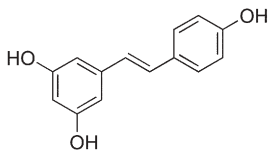
zanamivir



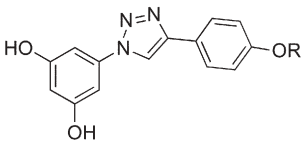
54

Resveratrol is a phytoalexin (antibiotic produced by plants under attack) displaying an array of biological activities. The problem with this compound is that it has a diverse array of activities at high concentrations. This has motivated research to find analogs by replacing the alkene with a triazole functionality, formed by the copper-mediated cycloaddition (and by other small heterocycles).[66] Compounds **55** were thus found to be more potent than the lead compound in cytotoxicity/antiproliferative assays.

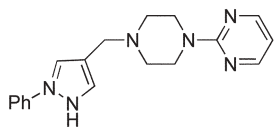
The lead compound NGD94-1 is a selective ligand for the D4 dopamine receptor. As part of a larger study, three compounds were prepared in which the triazole unit replaces the pyrazole part. The *N*-phenyl compound **56** had a K_i value of 3.2 nM for dopamine D4 receptor.[67]



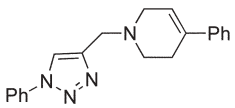
resveratrol



55
R = H , Me

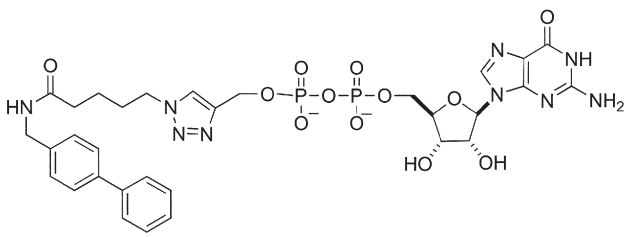


NGD 94-1



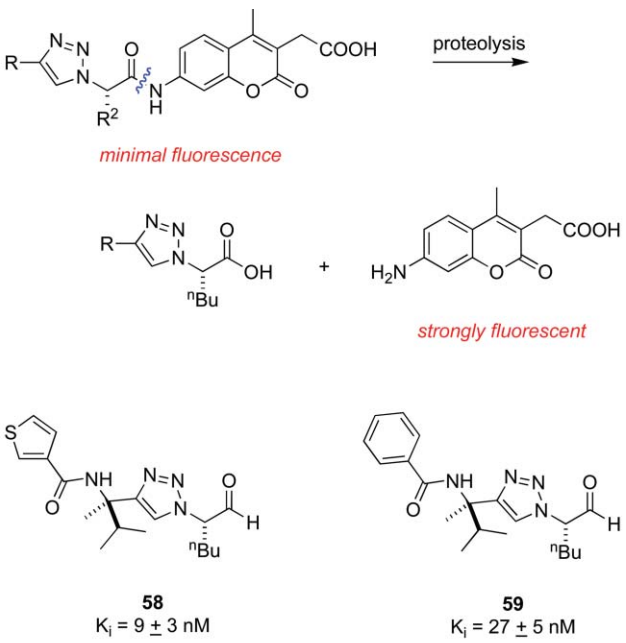
56

A library of 85 GDP-triazole compounds has been synthesized and screened for inhibition of fucosyltransferase activity.[68,69] It is known that the most important binding determinant was in the GDP part of inhibitors, but the azide function was added to screen for increased binding. Compound **57** was identified as a promising hit (IC_{50} 0.15–1.00 mM) depending on the enzyme used.



57

Ellman and coworkers have used a substrate-based screening method to identify key segments of inhibitors of the cysteine protease cathepsin S, which is implicated in autoimmune diseases.[70] In the first step, acylated amino coumarins



58
K_i = 9 ± 3 nM

59
K_i = 27 ± 5 nM

(reaction 14)

were screened to find the most appropriate functionality at the *N*-terminus *via* the method indicated in reaction 14. The preferred acyl groups contained some triazoles derived from click products. It was later shown that simple replacement of the coumarin with a hydrogen gave potent inhibitors, including compounds **58** and **59**.

Copper-mediated cycloadditions can be used to elaborate selected parts of libraries that contain terminal alkyne or azide functionalities. For instance, split syntheses were recently used to produce a 10,000-membered library of which 78 compounds contained triazoles.[71] Such applications are beyond the focus of this review.

F Conclusion

Copper-mediated cycloadditions proceed *via* pathways that feature activation of the alkyne part *via* coordination, then non-concerted cycloaddition of the azide component. This pathway is relatively unique, accounting for the superb chemoselectivity of the reaction. That chemoselectivity can be used in many ways. For instance, when alkyne or azido peptide units combine *via* this pathway the reaction is relatively insensitive to the amino acid side-chains. This serves as an excellent way to make peptidomimetics, particularly because there is some stereoelectronic similarity between 1,2,3-triazoles and amide bonds. Research on the three dimensional consequences of incorporating triazoles into peptides is at an early stage, and this is an exciting area for future studies. Further, Cu-mediated click processes can be used to conjugate peptides to carbohydrates, organic molecules, polymers, dendrimers, and labeling agents. Finally, 1,2,3-triazole cores may form the basis of small molecule pharmaceutical leads in which they fulfil some binding function of peptides, even though the molecular resemblance of these compounds to peptides is obscure.

There are some disadvantages of copper-mediated click reactions. They use azides and copper, both of which pose safety- and environmental-hazards. Further, macrocyclization reactions can be complicated by somewhat mysterious macrodimerization events. Nevertheless, the fact that there are convenient routes to amino-acid-derived azides and alkynes, and the lack of practical obstacles to executing these Cu-mediated reactions, both point to a strong future for the peptidomimetics formed *via* this route.

Acknowledgements

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