Cu-Catalyzed Azide−Alkyne Cycloaddition

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1. Introduction

The Huisgen 1,3-dipolar cycloaddition reaction of organic azides and alkynes[1,2] has gained considerable attention in recent years due to the introduction in 2001 of Cu(1) catalysis by Tornøe and Meldal,[3] leading to a major improvement in both rate and regioselectivity of the reaction, as realized independently by the Meldal and the Sharpless laboratories.[4,5] The great success of the Cu(1) catalyzed reaction is rooted in the fact that it is a virtually quantitative, very robust, insensitive, general, and orthogonal ligation reaction, suitable for even biomolecular ligation[6] and in vivo tagging[7,8] or as a polymerization reaction for synthesis of long linear polymers.[9] The triazole formed is essentially chemically inert to reactive conditions, e.g. oxidation, reduction, and hydrolysis, and has an intermediate polarity with a dipolar moment of ∼5 D.[10] The basis for the unique properties and rate enhancement for triazole formation under Cu(1) catalysis should be found in the high ΔG of the reaction in combination with the low character of polarity of the dipole of the noncatalyzed thermal reaction, which leads to a considerable activation barrier. In order to understand the reaction in detail, it therefore seems important to spend a moment to consider the structural and mechanistic aspects of the catalysis. The reaction is quite insensitive to reaction conditions as long as Cu(1) is present and may be performed in an aqueous or organic environment both in solution and on solid support.

This review will focus mainly on the Cu(1) catalysis in the Huisgen reaction, broadly known as the azide/alkyne-"click"-reaction or CuAAC-reaction, and will not consider the noncatalyzed thermal versions at any great length. The thermal version of the reaction first described by Michael[11] and later investigated in detail by Huisgen[1,2] has been reviewed in great detail and analyzed by Frontal Orbital−Pertubation theory by Lwowski.[12]

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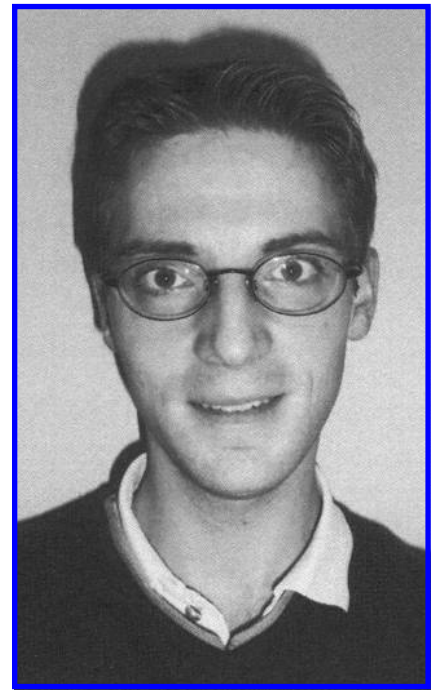
Morten Meldal is the leader of synthesis at Carlsberg Laboratory in Copenhagen and Directs a Centre of Combichem and Molecular Recognition. He holds an Adjunct Professorship at Copenhagen University and has a Ph.D. degree in Chemistry of Oligosaccharides from Technical University of Denmark. He did his PostDoc in peptides with R.C. Sheppard at M. R. C. in Cambridge. He has received 13 awards and is a member or board member of a large variety of scientific societies. He founded Society of Combinatorial Sciences, which he is currently chairing. He has over 300 publications and 21 patents, and his research areas include the following: combinatorial chemistry, "click" chemistry, polymer chemistry, organic synthesis, automation in synthesis, artificial receptors and enzymes, nanoassays, biomolecular recognition, enzyme activity, cellular assays, molecular immunology, nanoscale MS/NMR and polymer encoding. Throughout his career, Prof. Meldal has had an outstanding influence on contemporary methodology, particularly in peptide and combinatorial chemistry, and has always contributed with innovative and practical solutions to the currently most pressing general problems in these scientific fields.



Christian W. Tornøe studied Chemistry at the University of Copenhagen, Denmark, and later received his Ph.D. degree within the subject of CuAAC click chemistry in 2002 from the University of Pharmaceutical Sciences in Denmark under the supervision of Prof. Morten Meldal. He moved to the pharmaceutical industry research environment at H. Lundbeck A/S, where he received training as a medicinal chemist. He has published several patents in the potassium ion channel-field and is currently working on Alzheimer related research. His research interests include copper-catalyzed reactions, microwave chemistry, and the field of Alzheimer research.

to apologize in advance for any references which have not been retrieved through the search profiles and techniques employed.

2. Reviews on Cu-Catalyzed Azide−Alkyne Cycloaddition (CuAAC)

The different applications of the triazole chemistry have previously been extensively reviewed in a range of excellent reviews. However, only a few can be considered key publications for the Cu(1) catalyzed reaction. In 2003 Kolb and Sharpless et al.[13] presented a review outlining the special nature of the triazole chemistry with an emphasis on the potential use of the reaction in biochemical studies and drug discovery. Bock et al. presented a review with an impressive in-depth analysis of the reaction in 2006 including all essential mechanistic and methodological aspects at the time.[14] Binder et al.[15] and Lutz[16] described the polymer and materials science applications in excellent reviews. Gil et al.,[17] Li et al.,[18] Moses and Moorhouse,[19] and Wu and Fokin[10] have reviewed the general synthetic utility of click chemistry across the fields.

Other reviews mention the CuAAC as essential in particular important fields, e.g. as one out of many useful in 1,3-dipolar cycloaddition reactions[20] and as important in the catalytic conversions of acetylenes[21] and azides,[22] in dendrimer and polymer grafting[23–25] as well as synthesis,[26,27] and in chemical ligation.[28,29] It has been described as a "green" aqueous reaction.[30] Reviews also describe application in synthesis of peptidomimetics,[31,32] and in bioconjugations.[33–35] It has been compared to the Staudinger ligation[29] and used in profiling of proteases,[36] in pull down assays, in carbohydrate cluster and dendrimer synthesis,[37] and in combinatorial drug discovery.[38,39]

3. Mechanistic Considerations on the Cu(1) Catalysis

The role of copper in the catalysis of the triazole formation has been subject to many disputes and revisions since the discovery of this extremely potent cycloaddition, in which the catalyst accelerates the rate of reaction with 7 orders of magnitude.

Recent quantum mechanical calculations of the noncatalyzed reaction between HN_3 and H_2C_2, HCN, H_4C_2, and H_3CN, respectively, show that the transition state is largely nonpolarized in all cases.[40] The polarity increased marginally by alkyl or aryl substitution of the azide; however, in the uncatalyzed reaction the alkyne remains a poor electrophile.[41] The most important factor determining the activation energy barrier is the so-called distortion energy of the azide constituting 18.1 of the 29.9 kcal/mol $\Delta G^{\ddagger}$ for the reaction with acetylene. For HCN the same energies are 21.2 and 34.6, respectively. The most significant difference between the two reactions is the ΔG's, which are 52 kcal/mol for the acetylene and 11 kcal/mol for the HCN. This large ΔG for

the triazole formation is partly responsible for the click property of this reaction.

Another report supported by DFT calculations described the catalysis to be mediated by a single copper atom in the +1 oxidation state. These calculations assumed (probably incorrectly) an "end on" orientation of Cu^+ to the alkyne in the transition state. It was concluded that the rate enhancement is due to a stepwise process lowering the transition state energy 11 kcal/mol compared to the uncatalyzed concerted cycloaddition.[42] The Cu^+ coordinated first with the acetylene π-electrons, thereby lowering the pK_a of the acetylene proton followed by exothermal formation the acetylide. The Cu^+−acetylide complex coordinated the azide followed by rearrangement of the complex into a 6-membered metallocycle and further into the copper-metallated triazole. The Cu−triazole complex eventually releases the free triazole and the $L_nCu(1)$ by protonation or reaction with other electrophiles. Although the calculations were informative, this course of events may be incorrect since kinetic measurements indicated that the reaction was at least second order with respect to the concentration of $L_nCu(1)$. In fact, at intermediate concentrations, the reaction is second order both with respect to [Cu(1)] and [alkyne],[43] and it is most likely that more than one Cu atom is directly involved in the transition state of the reaction. Considering this observation and structural evidence retrieved from the Cambridge Crystal Database, it is unlikely that a single Cu(1) atom aligned with the C−C bond of the alkyne is responsible for catalysis, and it seems additional DFT calculations are required to complete the picture. Bock et al.[14] have provided an excellent review with an in-depth analysis of the current information.

In order to shed light on the mechanism of the CuAAC, it was worthwhile to pay a visit to the Cambridge Crystal Database. The structural information revealed that the coordination of acetylide to Cu(1) is a complex affair. Approximately 35 structures containing Cu(1)−acetylene complexes can be found. Typical architectures of the crystalline complexes are presented in Figure 1. In more than 90% of all Cu(1)−alkyne complexes in the database, each C−C-triple bond coordinates three Cu-atoms (e.g., Figure 1E and F), and thus, this type of coordination appears to be the energetically more favored coordination number of the acetylide. The coordination number and the three almost equivalent bond angles, C−C−Cu, indicate that the π-electrons of the alkyne are strongly involved in the Cu(1) coordination, rendering the secondary carbon with a large partial positive charge. The second most abundant type of structure is one with a coordination number of 2. In all these structures, the C−C−Cu bond angles are ∼130−140°. Only a few structures (e.g., the complex of acetylene with CuCl) exist in which one Cu(1) is coordinated end on with a bond angle of 180°, probably induced by symmetry and crystal forces.

The picture is completely different for Cu(1) complexes with nitriles (Figure 1A) and isonitriles (Figure 1G). Here the coordination is always 1:1 and the orientation of the complex is linear, i.e. with a C−N−Cu(1) or N−C−Cu(1) bond angle of ∼180°. This would not involve development of significant partial charge on the secondary atom (C or N, respectively) and could explain why a similar Cu catalysis is not observed in the reaction between nitriles and azides to form tetrazoles.

Azides, on the other hand, coordinate to Cu(1) in two different ways. Most common is the end-on coordination, in which the terminal azide nitrogen is coordinated to the central Cu atom with an ∼180° bond angle. The alternative coordination of the carbon linked nitrogen atom seems to require a second intramolecular coordination partner such as the 1,2-pyrazole in Figure 1I in order to direct the azide coordination, which has a N−N−Cu(1) bond angle of ∼120°.

A crystal structure of CuI that substantiates CuI may occur as solvated clusters in solution has been included in Figure 1A.

Considering the complexity of ligand interaction with Cu(1) and particularly that of the alkyne complexation as indicated by the retrieval from CCDB, it is therefore possible that the detailed structural secrets of the transition state responsible for the extreme rate enhancement and selectivity in the Cu(1) catalyzed triazole formation will not be unambiguously determined in any near future.

Figure 1A presents the crystal structure of $Cu_5I_6(CH_3CN)_2$ clusters isolated from acetonitrile. Figures 1B, C, and D present various minor modes of binding of the alkynes to Cu(1) while E and F represent 90% of the alkyne−Cu(1) structures retrieved from the database and show an almost tetrahedral arrangement of three Cu(1) atoms around the alkyne. In contrast, nitrile and isonitrile unambiguously bind Cu(1) end-on in a linear arrangement (Figure 1A and G). The azides bind to Cu(1) in crystal structures either with the terminal nitrogen (Figure 1H) or with the substituted imine nitrogen in the case of additional binding (Figure 1I).

The presence of Cu_nI_n clusters in CuI solution has for the purpose of this review been substantiated by a negative mode ESI-MS (Figure 2). In purified CuI, the main cluster observed is $Cu_4I_5^-$ in negative ion mode immediately after dissolving the crystals in CH_3CN. The MS spectrum of the pure cluster is best obtained at rather low energy of the collision gas. As the energy was ramped, both smaller and larger clusters from CuI_2^- up to $Cu_6I_7^-$ were formed, and at very high energy, CuI_2^- is dominant. I^- was not observed under any conditions. The ionization was most likely by loss of Cu^+ but may also be by I^- complexation with a cluster. Upon addition of phenylacetylene (10 equiv), peaks related to Cu(1) clusters are selectively and almost completely suppressed in the spectrum.

Considering the second order kinetics for the [Cu(1)] observed by Rodionov et al.[43] and the structural evidence retrieved from the Cambridge Crystal Database, it is unlikely that a single Cu(1) atom aligned with the C−C bond of the alkyne is responsible for catalysis, and it seems more DFT studies on transition models including those depicted in Figure 3 are required to complete the picture. However, based on the structural details above, it may be suggested as in Scheme 1, **3B**, that the acetylide and the azide are not necessarily coordinated to the same Cu atom in the transition state. This possibility was also indicated by Bock et al.,[14] in mechanistic studies by Rodionov et al.,[43] and in calculations performed by Straub.[44] One possibility in the case of, e.g., CuI catalysis could be formation of clusters such as that shown in Figure 3A or more likely Figure 3B, in which the favored coordination mode from the crystal data is maintained while providing a six-membered transition state for the reaction between the terminal azide nitrogen and the positively charged secondary carbon of the acetylide. The intermediate **3B** (Scheme 1) with the two Cu(1) atoms in the cyclic transition state is tempting because it is the only

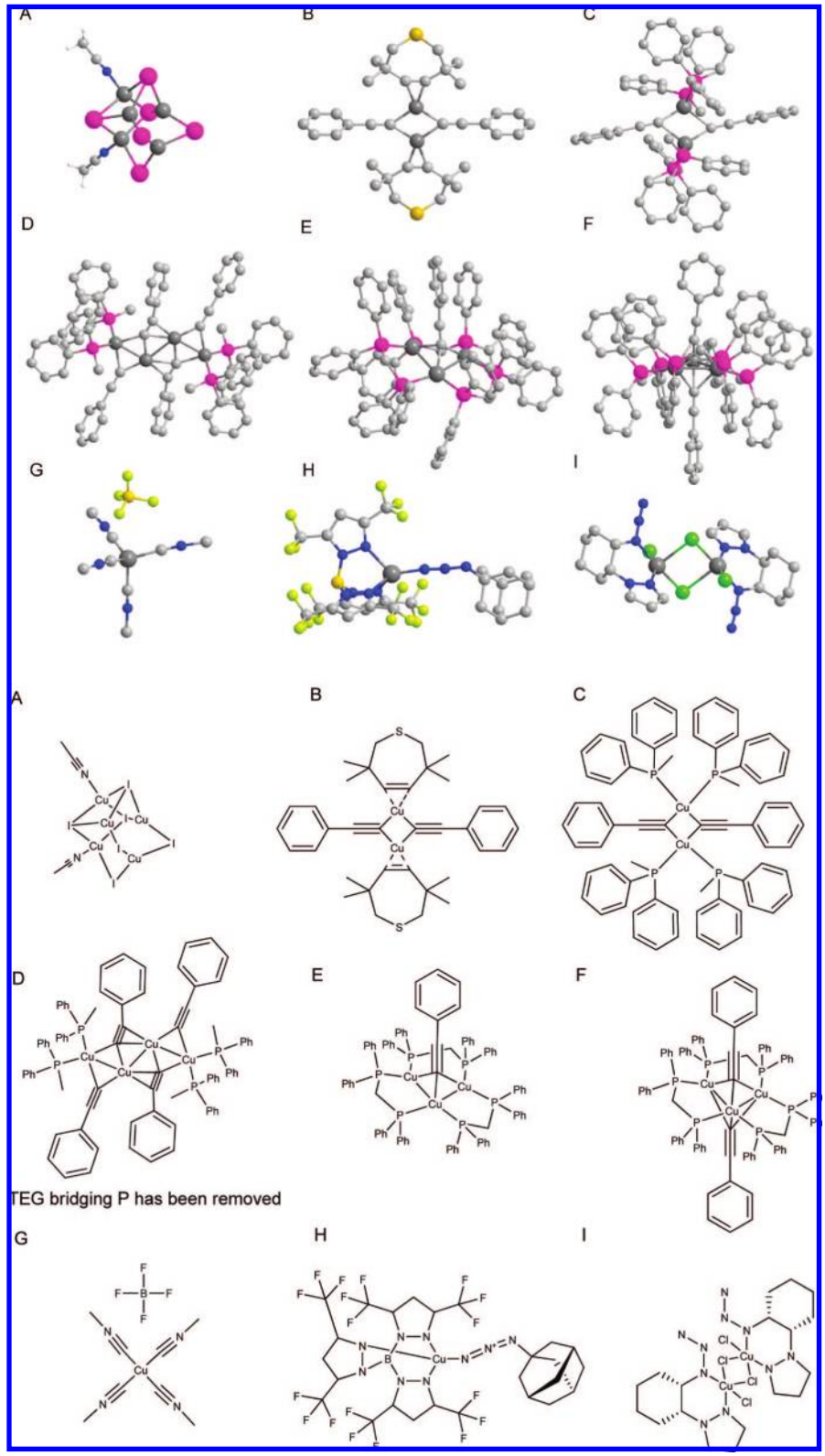


Figure 1. Snapshots from the Cambridge Crystal Database and the associated structural drawings. A: The structure of an abundant CuI cluster, $Cu_5I_6(CH_3CN)_2$ in CH_3CN solution. In CCDB a large variety of clusters for both Cu_mBr_n and Cu_mI_n may be found and Cu_4I_4 clusters are predominant. B: Shows the difference in coordination of terminal (productive) and internal (nonproductive) alkynes. C: Bivalent coordination of terminal acetylene as in B. D: Complex where the central Cu(1) atoms each coordinates three acetylenes, which in turn coordinate two Cu(1) atoms terminally and for two of the acetylenes one Cu(1) at the π-orbital (triethyleneglycol connecting the phosphines has been removed for clarity). E and F: By far the most abundant arrangement of the Cu(1) with respect to the acetylene in which three Cu(1) atoms have an almost tetrahedral arrangement around the terminal carbon of the acetylene. G: Shows the exclusive terminal coordination of isonitrile around Cu. H and I: Represents the two modes of azide coordination to either the terminal (H) or the imine-nitrogen (I) that also involve anchimeric assistance in the coordination.

one that unambiguously can explain the absolute regioselectivity of the reaction. It would also explain the unexpected poor results obtained in the resolvation of gem-diazides using chiral ligands to the Cu(1).[45] Scheme 1

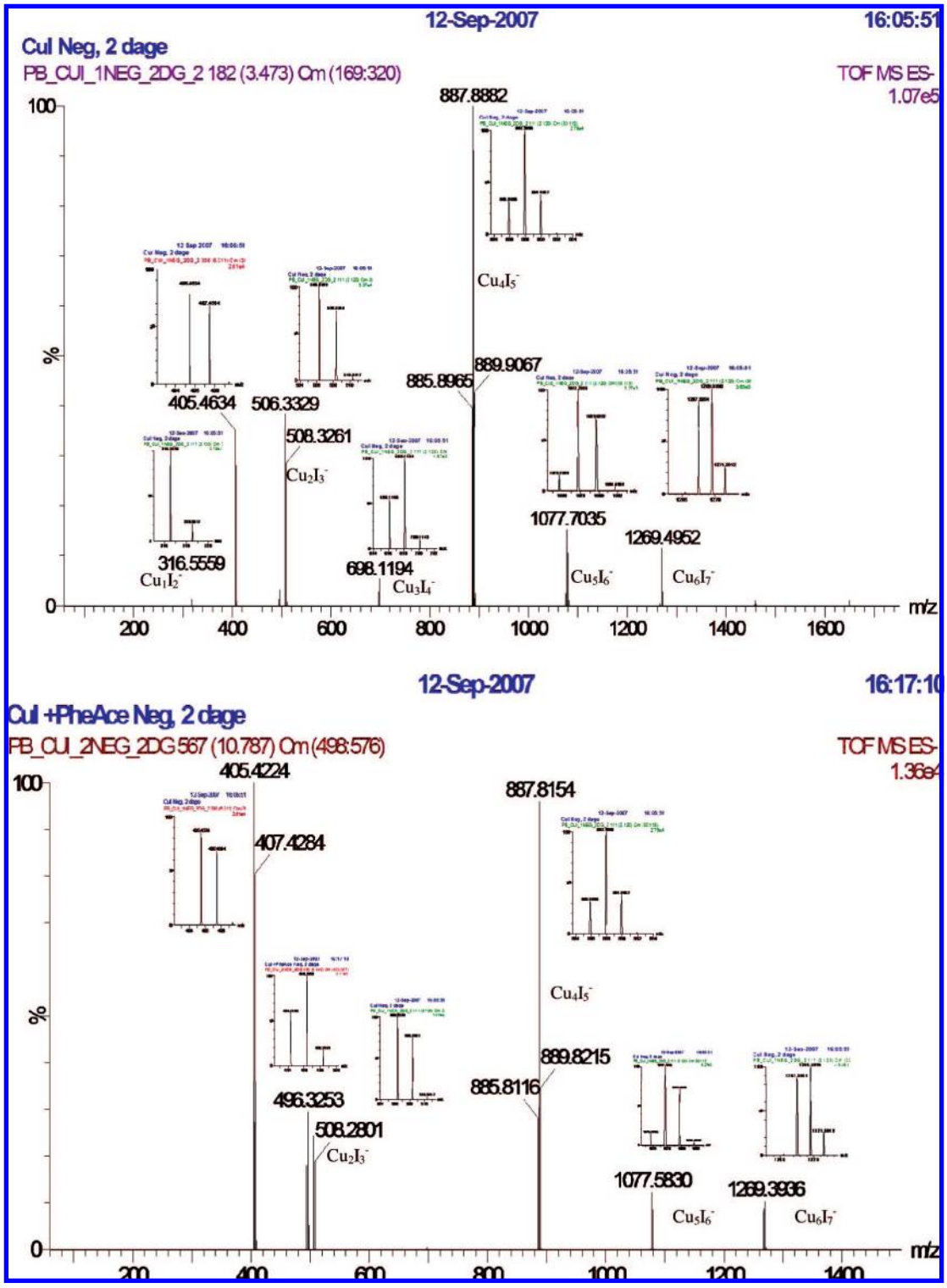


Figure 2. ESI-MS (Micromass QTOF-Ultima, negative mode, collision energy 10) of a 100 μM solution of CuI in degassed acetonitrile after 2 days under N_2. The charged species were observed with a TIC of 10^5, which was reduced 10-fold by addition of phenylacetylene. A similar spectrum of ascorbic acid/$CuSO_4$ (1−1) in H_2O only shows $CuAsc_2$, indicating the presence of Cu_2Asc_2. No Asc-ion was observed.

provides a suggestion including both possibilities for coordination and delivery of azide to the alkyne during the transition state of the reaction. The same metallocene intermediate is formed in the two mechanisms and results in ring contraction to give the metallated triazoles, **5**. In reaction of $Ph_3P-Au-N_3$ with phenylacetylene, the metallated Au-triazole is the end product that has been crystallized.[46] Since a Cu(1) cluster can coordinate more than 1 alkyne (see Figure 1), this would also provide an alternative explanation to the extreme rate enhancement for the second azide in reactions of bis-azides,[43] since the cluster is likely to coordinate a second alkyne, thus increasing the local concentration of copper acetylide. The second order kinetics with respect to both Cu(1) and alkyne combined with the fact that low equivalents (see below) of pure Cu(1) in inert atmosphere provide the cleanest reactions suggests that optimal conditions are either at high concentrations with [alkyne]/[Cu(1)] > 10 in a solvent that dissolves all

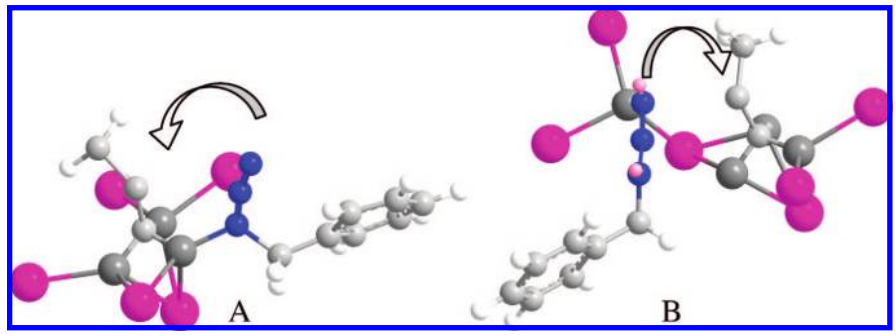
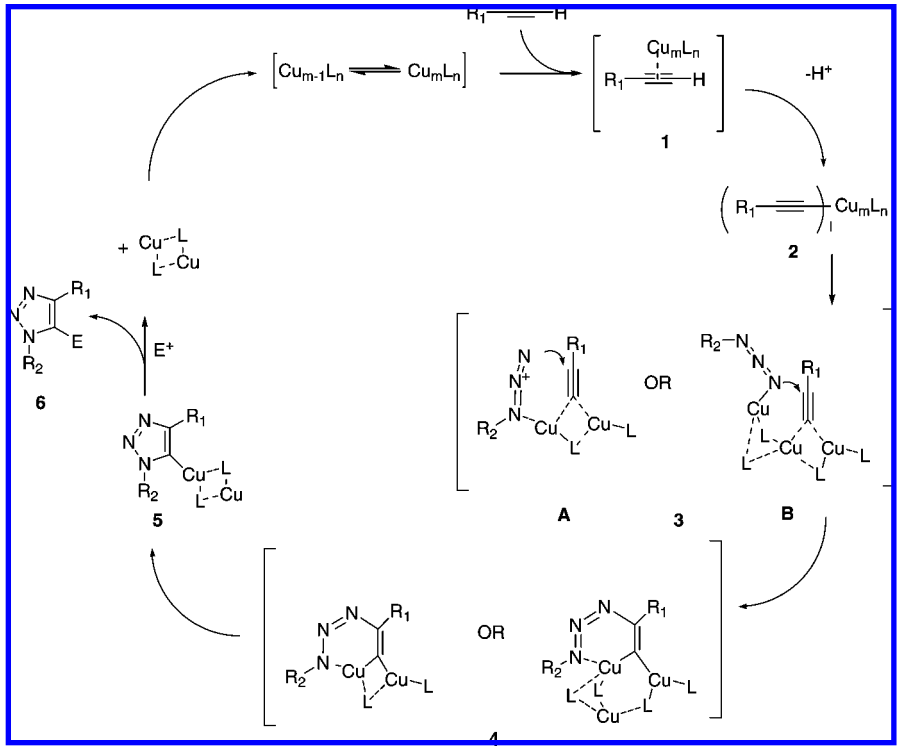


Figure 3. Tentative models of intermediates A and B in Scheme 1 illustrate the two mechanistically different precursors of six-membered transition states. Only transition states according to model B may explain the extreme 1,4-selectivity and second order kinetics for [Cu(1)].

Scheme 1. Outline of Plausible Mechanisms for the Cu(1) Catalyzed Reaction between Organic Azides and Terminal Alkynes[a]



[a] Intermediate A is generally assumed to be the intermediate; however, it fails to explain much of the observed behavioral data of the reaction, and alternatively, intermediate B could explain most observations.

components or at conditions where high pseudoconcentrations are achieved at the interface between different phases (e.g., CH_2Cl_2/H_2O, polymers, or liposomes). In the presence of a large excess of Cu(1), the reaction becomes slower toward the end.[47,48]

The effect of Cu(1) is not exclusive to the formation of 1,4-substituted triazoles or to the use of terminal alkynes. Under forcing conditions where azide and alkyne are kept in a specific arrangement in 2-alkyne-substituted benzyl azide, CuI is essential as a cocatalyst. Chowdhury et al.[49] suggested a transition state where the π-electrons of the alkyne and the terminal nitrogens of the azide are all coordinated to a Cu(1) atom.

4. The Cu(1) Source

The formation of triazoles from azides and terminal alkynes catalyzed by Cu(1) is an extraordinarily robust reaction, which could be performed under a wide variety of conditions and with almost any source of solvated Cu(1).[14] Provided the reactants are maintained in solution or even as a mixture in a glassy state[50] or aggregate[51,52] and the Cu(1) has not been removed by disproportion or oxidation to Cu(2), the product is usually formed in very high yields. The most important factor seems to be that of maintaining the [Cu(1)] at a high level at all times during reaction. This is why the use of a Cu(2) source with addition of a reducing agent in a large excess has been one of the preferred methods. The presence of reducing agent renders the reaction much less susceptible to oxygen, and such reactions have often been carried out under open-air conditions. However, as detailed below, this is not always without problems, due to potential oxidative side reactions.

The sources of Cu(1) used since 2001 are tabulated in Tables 1–5. In many of the reports, the procedures have been optimized using a variety of conditions and only the procedures that produced the best results are listed.

The most common conditions are the aqueous conditions employing $CuSO_4$ and a reducing agent listed in Table 2.

Table 1

Cu source (equiv)	reducing agent (equiv)	solvent	base	ligand	temp/°C, (h)	yield (%)	application	ref
CuI (0.01−0.1)		THF	DIPEA		25	70−98	A, B, C, O, Q, P, L	3, 53–56
		pyridine					A, B, C, O, Q	4
		THF	DIPEA		23	76	P, O, Q, A	57
		DMSO			25		H	58
		DMSO			80	85−97	A, C, M	59, 60
		THF	DIPEA	TBTA	20 (2)		L, C, A, Q, O	61, 62
		DMF	DBU		60		E	63
		toluene			80	61−98	B, J, I, E	64–66
		NMP	DIPEA		23	3−11	D, I, O	67
		toluene	DIPEA		23 or 40 (10−16)	49−97	I, J, P, Q, C	68–73
	NaAsc	CH_3CN/H_2O/tBuOH	DIPEA		23	∼80	B, D, P	74
		CH_3CN/H_2O/DMSO	Lut		23	15−20	B, D, P, O	75
	NaAsc	DMF or DMF/iBuOH	pyridine		23 (72)	up to 96	O, Q, C	76
0.1 M in pyridine		THF	DIPEA		23 (5)	up to 89	I, O, Q, R, C	77
		DMSO	Et_3N	proline	65		O, C	78
		H_2O/EtOH	Et_3N		60	45−90	E, C	79–81
purified (0.01)		H_2O/(CH_3CN or MeOH)	Et_3N		25	up to 90	A, C, J	82, 83
		THF	Et_3N		35 (2−4)	53	H, E	84, 85
		CH_3CN	DIPEA		23 (144), 45 (78)	22	C, Q, P, J	86, 87
	NaAsc	DMSO/H_2O		BMAH	23 (1.5)	54−99	A, C	88
		THF or toluene	DBU		35 or 50	80	H, P, Q	89–91
		CH_3CN		∓TBTA	23 (48)		H, E	92, 93
		$CHCl_3$	Lut,		0 (12)	56−95	A, B, C	94
(1)		CH_3CN/H_2O	DIPEA		23 (24)	60	Q, C	95
(+ $Pd(PPh_3)_2Cl_2$)		DMF	Et_3N		115	33−66	A, B, C	49
	NaAsc	DMF	piperidine		23 (5)	56 (crude pure)	A, O, Q	96
	NaAsc	CH_3CN/H_2O/DMSO	DIPEA		23 (0.16)	(crude pure)	A, Q, D, I	97
	- or -Air	CH_3CN	DIPEA		23 (2)	86−96	Q, C, J, N	98, 99
		pyridine			23	85	A, B	100
		CH_3CN	DIPEA		45 (72)	64	A, P, Q	101
		toluene/tBuOH	DIPEA		23 (16)	96	L, C	102
		CH_3CN/H_2O	...(Et_3N)		23 (20)	17−90	A, C	82
(1.3)		THF	DIPEA		23 (?)	17−56	I, C, O	103, 104
	(Ar)	CH_3CN	Lut		23 (12)	80	I, C, E, Q	105–108
		DMF			70−80 (18−48)	91−93	H, C	109
	ICl, RX (E^+)	THF	Et_3N		23 (20)		A, C	110, 111
	N_2	THF			23 (24)	80	L, C, E	50, 112
		toluene			23 (120)	93	G, C	113
	HAsc	DMF/pyridine	DIPEA		23 (18)	75 (4 triazoles)	Q, C, D	114
PS-NMe_2:CuI		CH_2Cl_2	PS-NMe_2		23 (12)	61−99	A, C, O	115
		CH_3CN	DIPEA	Lut	23 (−)	70−80	P, Q, E	116
		$CHCl_3$ or H_2O	Et_3N		25 (7−24)	31−98	A, C	117, 118
		CH_3CN/H_2O/DMSO	DIPEA		23 (0.15)	54−99	M, Q, D	97
		DMF/THF	DIPEA		23 (−)	100	O, C, Q	119, 120
		THF	Et_3N		23 (−)	80−84	G, L, C	121
(∼0.01/alkyne)		THF	DIPEA		60 (24)		O, J, I, D	122
		MeOH	DIPEA		23 (8)	∼90	N, Q, D	123, 124
		DMSO/H_2O			70 (12)	25−90	J, I, R	125
		CH_2Cl_2		chiral Lig's	23 (−)	23−25 mono	B, C, L	45
	NaAcs	NMP/H_2O	Lut		23 (48−96)	4−8	P, Q, O	126
		CH_3CN/H_2O	DIPEA	pyridine	23 (16)	95	Q, O	127
		dioxane/H_2O	$CuSO_4$		23 (12−24)	40−78	A, C	128
		DMF		His	23 (12)	66−100	B, Q	129
ultrasound		H_2O			23 (0.25−0.50)	52−95	C	130

Other frequently used Cu(1) sources are CuI (Table 1) or CuBr, which is often preferred in polymer reactions (Table 4). Table 3 lists the usage of Cu(0) (wire, turnings, powder, or nanoparticles) with or without addition of $CuSO_4$. Table 5 lists a variety of Cu(1) salts with special properties, e.g. improved solubility in organic solvents or increased rate of reaction compared to the $CuSO_4$/ascorbate or CuI standards.

A large difference is observed between the dependence of base in the application of $CuSO_4$ and Cu(1)-halide salts. While the catalytically active Cu(1) species is directly generated by reduction with ascorbate and immediately forms Cu-acetylides, the CuI and CuBr salts require at least an amine base (TBTA and other nitrogen heterocycles do not provide sufficient basicity) or high temperature to form the Cu-acetylide complexes. This difference may be due to the fact that e.g. CuI initially occurs in stable clusters such as that of Figure 1 A and requires a certain concentration of acetylide anion before the reactive complex can form. Ultrasonication greatly enhances the CuI catalyzed reaction even in the absence of base.[130,358] In any event, the many discrepancies in the literature on the reactivity of CuI nicely correlate with the presence or absence of base in the reaction medium.

The robustness of the reaction allows for many manipulations of conditions, and this is obvious when inspecting the tables. Most frequently, the reaction has been performed with CuI in THF, CH_3CN, or DMSO or with $CuSO_4$/ascorbate in water/alcohol mixtures. There is no obvious correlation between method used and yield of reaction, and there is a tendency to perform a case-to-case optimization. In some instances, the $CuSO_4$ is preferred due to ease of workup and purity of products, while, in other reactions, the Cu(1) halides are superior, particularly in rate of reaction.

In the original report by Tornøe and Meldal of the CuAAC,[3] CuI was used as a source due to its partial

Table 2

Cu source (equiv)	reducing agent (equiv)	solvent	base	ligand	temp/°C, (h)	yield (%)	application	
$CuSO_4$ (0.01−0.2)	NaAsc (0.04−0.4)	H_2O/tBuOH			23 (12−46)	82−100	A, B, C, M, J, P, Q, H	5, 131–158
	NaAsc	H_2O/tBuOH			23		H, E, K, G, R, I, J	159–165
	NaAsc	H_2O/tBuOH			23	70−96	L, C	61, 133, 166, 167
	NaAsc	H_2O			23 (2)	∼60 and ∼95	I, J, D, M	62, 168, 168–175
	NaAsc	CH_2Cl_2/H_2O/MeOH			23		A	176
	NaAsc	DMF			25 (36)	up to 89	E	177
	NaAsc	acetone/H_2O			80 (16)	76−78	H, C	178, 179
	NaAsc	HEPES/NaCl buffer	pH 6.5	Batho	23		K	51
	NaAsc	PBS/(tBuOH or DMF)		TTA	4 (72 or 16)	(90)	N, A, B, D, F	180–182
	NaAsc	H_2O/tBuOH			23 (24)	up to 98	G, Q, J	183–185
	NaAsc	H_2O/THF			30−80	70−95	I, C	186, 187
	NaAsc	H_2O/DMF			μw 100 (0.33, 0.1)	84−96	C, G, Q, I	188, 189
	NaAsc	H_2O/MeOH (or tBuOH)		TBTA	23 (24)	47, 78−96	M, D, N, J	190–192
(1.8 eqv)	NaAsc	H_2O/tBuOH	DIPEA		23	18−25 (Cu in excess)	M	48
	NaAsc	H_2O/EtOH			23 (24)	62−84	E, C, N, D, I	193–197
	NaAsc	DMF/H_2O			μw 80 (0.33) ;μw 90 (0.17)	89	M, E	198, 199
	NaAsc	DMF or DMF/H_2O			23 (3−16)	63−88	C, J	200–202
	NaAsc	H_2O/THF			23	80−92	E, C, G, Q, J, H	203–214
	NaAsc	H_2O/THF/(tBuOH)			60 (24 or38)	80−90	G, E, C	215, 216
	NaAsc	H_2O/MeOH			23 (18)	- or 63−96	N, Q, C, A	217–219
	NaAsc	PBS	pH 6.5		23 (24)	30−74	D, F	220
	Asc-H	DMF			23 or −10 (12)	41 or 72−82	E, C	221–223
(0.2 mM)	TCEP	PBS	pH 8				D, M, N	224
	TCEP (0.4 mM)	SFM					D, F, M	225
	NaAsc	borate buffer/tBuOH	pH 8.2, pH 8		23	90	Q, C, J, M	226
	TCEP	PBS/(tBuOH)/(EtOH)		TBTA	23 (1) or 4 (17)	precip	D, C, K, F	6, 227–229
	NaAsc	H_2O/DMSO		TBTA	23 (20 or 0.5)	44 (two steps)	C, Q, J, R, N	230–232
	NaAsc	PB/TFH	pH 7.2		23		D, H	233
	NaAsc	H_2O/tBuOH/EtOH, H_2O/DMF, or H_2O/THF		TBTA	23 (24)	87−96	I, C	192
(0.1)	NaAsc	H_2O			50−80 (1−24)	86−97	G, C	234–239
	NaAsc (0.2)	PB/tBuOH/DMSO			70 (2)	89	Q, C	240, 241
	TCEP	H_2O/MeOH		TBTA	23		D, M, F	242
	NaAsc	H_2O/DMSO			μw 60 (03)	100 by LC	Q, D	242
	NaAsc	H_2O/MeOH/AcOEt	Lut		23 (16)	80−92	C, Q, O	243
	NaAsc	H_2O/DMSO			23 (16)		Q, H, M	244
(0.05)	NaAsc, N_2	THF/H_2O			23−25 (24−42) or 70 (24)	75−98	G, C, H, Q	245
(40 times excess Cu)	NaAsc	PB	TBAF		50 (48)	76	H, E, C	249
	TCEP	H_2O/tBuOH	pH 7.9		37 (2)	50	D, Q	250
	NaAsc	H_2O			35−40 (0.16−48)	58−96	Q, C	251–254
	NaAsc	H_2O/EtOH	pH 7.9		μw (80 W, 0.5)	49	E, C, H	255
	NaAsc	H_2O/DMSO			70 (15−20)	51−70	C	256
	NaAsc	$CHCl_3/H_2O$/EtOH	K_2CO_3	Pro	70 (14)	26−71	A, C	257
	NaAsc	H_2O/tBuOH/DMSO			37 (16)	91	Q, C	258
	NaAsc	CH_3CN					I, D, R	259
	NaAsc	H_2O/DMSO			23 (16)	88	A, C	260
	N_2 (no red.)	CH_2Cl_2/H_2O	DIPEA		37 (12)	54	D, Q	261
(0.15)	NaAsc (0.45)	H_2O			23 (1−12)	83−99	A, C, Q	52
	NaAsc	H_2O/tBuOH	pH 3.5		23 (0.3)		E, N	262
	NaAsc	PIPES			μw 130 (30)	100 crude	C, D	263
(1)	Asc-H	DMSO/Tris	pH 7.2		37 (0.1−0.8)	90	A, B, M	47
	NaAsc	H_2O/DMSO		Batho	23 (0.07)	80	A, B, R	264
	NaAsc	$CHCl_3/H_2O$/EtOH	Na_2CO_3	Pro	65 (16)	66−94	A, C	265
	NaAsc	DMSO/H_2O	Na_2CO_3		23 or 40 (16)	53−98	A, Q, C	266
	Asc-H (Ar)	saline	$Et_3NH^+,{}^-OAc$		23 (24)		Q, M	267
100-fold excess	NaAsc	H_2O		TBTA	23 (2)	∼90 by HPLC	Q, P	268, 269
5×10^{-5} to 10^2	NaAsc	CH_2Cl_2/H_2O/tBuOH		Bim	24 (0.2−24)	up to 100	B	270
	NaAsc			TBTA	23	up to 100	L, C	271

solubility in solvents of intermediate polarity such as acetonitrile, THF, acetone, pyridine, and DMSO (Table 1). It may be obtained in a highly pure form, and the purities of CuI as well as of CuBr have a large influence on both reaction rate and completion of the reaction.[82,83,322,323] The copper halides are broadly applicable to preparative, ligation, polymer, and biological chemistries. CuI has often been selected, as a Cu(1) source when special anhydrous conditions were required.[94,100] CuI was furthermore employed in a highly optimized protocol for repetitive triazole formation on solid support. Here a high concentration of piperidine and addition of ascorbate was found to be essential for the formation of triazole based oligomers resembling peptides.[96]

Recently, the superior performance of CuBr in aqueous in vivo ligation has been demonstrated.[7,8,225,322] CuBr is also preferred in polymer ligation, particularly in combination with PMDETA (Table 4). Similar conditions are often used in the ATRP polymerizations which may be the origin of this preference; however, the conditions seem robust and lead to high molecular weights when employed for e.g. polymerizations based on repetitive triazole formation.[9] The use of polymer bound CuI[115] described by Girard et al. is an

Table 3

Cu source (equiv)	ox. agent (equiv)	solvent	base	ligand	temp/°C, (h)	yield (%)	application	ref
Cu(0)	$CuSO_4$ (0.01−0.1)	PB	pH 8		4 (24)		K	8
	$CuSO_4$	CH_3CN/H_2O			30	75−78	M, A	272
	$CuSO_4$	DMF		TBTA	23 (16)	50	G	273
(ox. coupling)	$CuSO_4$, air	CH_3CN/H_2O	Na_2CO_3		25 (18)	23−87	A, C	206
	$CuSO_4$, NaAsc	DMF			μw 90	60	E, J, A	199
	$CuSO_4$	PB	pH 8	TBTA	23 (1)		D, M	274
	$CuSO_4$	H_2O/tBuOH			23 (48)	100 by LC (∼73)	I, R, Q, J, C	275, 276
	$CuSO_4$	H_2O/THF		TBTA	μw 85 (0.33)	27−92	Q, C	277
	$CuSO_4$	H_2O/tBuOH			μw 125 (0.15)	84−93	A, C	278
	$CuSO_4$	PB	pH 8		37 (1)	75	D, M, Q	279
	$CuSO_4$ (0.05)	H_2O/tBuOH		TBTA	μw 100 (0.05−0.4)	40−84	A, C, I, R	280
	$CuSO_4$	EtOH			23 or 50 (5−10)	80−94	A, C	281
	$CuSO_4$	H_2O/EtOH/tBuOH			23 (18)	∼100	R, Q, D	282
	$CuSO_4$	EtOH			80 (12)	48−85	P, C	283
	air ?	H_2O/tBuOH			23 (168)	91 (100 by anal.)	Q, C, E	284
Cu(0) nano	in situ Cu_2O	H_2O/tBuOH or PBS			25 (18)	80−99	A, B, C, Q	285, 286
(air protected)	in situ Cu_2O	toluene			23 (2−4)	87−100	A, C	287
(powder, Aldrich)	in situ Cu_2O	H_2O/tBuOH or H_2O/tBuOH	Et_3N·HCl		23 (2)	83−96	A, C	288
	in situ Cu_2O	H_2O			23 (96)	92	L, C	289
(on Al_2O_3)	in situ Cu_2O				23 (3−8)	40−92	A, C	290

Table 4

Cu source (equiv)	red. agent (equiv)	solvent	base	ligand	temp/°C, (h)	yield (%)	application	ref
$CuBr(Ph_3P)_3$ (0.1)		THF	DIPEA		50		L, K, H, G	291, 292
		CH_2Cl_2	DIPEA		23 (48)	82	I	178
		CH_3CN		Phen	50		A	293
(1.8 equiv)		$(CH_2Cl)_2$	DIPEA		60	54	Q, C	294
		THF or dioxane	DIPEA		23 (48−72)		E, H, M	295–297
		toluene	DBU		110	57−73	Q, C, P, A	298–300
		THF	DIPEA		μw 140 (0.3) or 23 (48)	87−96	G, C	301
	(-air)	DMF	DIPEA		50 (48)	92	H, C	302
		toluene	DIPEA		23 (12)	27−65	Q, C	303
		DMSO	Et_3N		23	100 (NMR)	H, C, M, Q	304
CuBr	NaAsc	DMF	Lut	Bipy	23		O, E, N	305
CuBr/$Cu(OAc)_2$ (ox)		THF			50 (16)	97	A, C	306
		H_2O			23		D, M	307
(purified)		HEPES buffer/DMF	pH 8.5	Batho	4 (24)		D, K, M	308
		CH_3CN (anh)	Et_3N		23 (12−24)	26−78	A, B, C	309, 310
	(Ar or N_2)	DMF or THF		PMDETA	23 (24)	82−87	H, C	311–314
	(Ar)	DMF		PMDETA	23 (2 mono, 12 bis)	55, 100	H, C	315, 316
	(Ar)	THF		dNbipy	23 (24)		H, C	80
	(-air, N_2)	DMF or DMSO	±DBU		23 (2)	>95	A, H, C	317
	(-air, N_2)	DMF		PMDETA	23 (0.5)		A, B, H	9, 318, 319
		H_2O/DMSO/tBuOH		TBTA	23		Q, D	320
		toluene	DBU		110 (16)		A, Q, P, C	300
	NaAsc	DMF		PMDETA	80 (0.1)		H, C	321
(purified)		PBS		TBTA	4 (16)	high	D, F	322, 323
	(-air, Ar)	DMF			60 (16)	46−92	H, C	324
$CuBr_2$	AscH	DMSO or NMP	$PrNH_2$		23 (12)	63−78	Q, M	325, 326
(+$Pd(OAc)_2$)	PPh_3	toluene, $AllOCO_2Me$, TMS-N_3			80 (2−48)	35−88	A, C	327

attractive alternative that allows catalyst recycling. The paper also contains a semiquantitative solubility study for copper halides.

Aqueous conditions introduced originally by Rostovtsev et al.[5] are extremely useful in biochemical conjugations, where it is the most used procedure; however, these conditions can often be used with great success even in organic preparations, provided a sufficient concentration of substrates in solution can be obtained (Table 2). The aqueous conditions ensure easy isolation and high purity of products.

Conditions utilizing a mixture of Cu(0) in the form of wire, turnings, powder, or nanoparticles[287] with or without addition of a Cu(2) source such as $CuSO_4$ are also quite useful in the aqueous environment although there seem to be a latency period for the active catalytic species to form[285] (Table 3). The removal of solid Cu facilitates the product isolation.

Finally, there is a range of other Cu(1) sources (Table 5) that have been introduced for a variety of reasons, e.g. for increased solubility in organic solvents ($[Cu(CH_3CN)_4]PF_6$, $(EtO)_3P$:CuI, $Cu(CH_3CN)_4OTf$) or for $Cu(OAc)_2$ to improve reactivity as compared to $CuSO_4$.[219] Two accounts report on the favorable activity of solid supported CuI,[115,338] one on Cu(1) zeolites,[354] and one Cu supported on Al_2O_3 nanoparticles.[290] $Cu(CH_3CN)_4OTf$ was found to be particularly reactive when used with bathophenanthroline ligand for in vivo surface conjugation of molecular probes onto viral particles.[355] $Cu(OAc)_2$ has been reported to catalyze the formation of triazole from alkyne and azide almost as effectively as Cu(1) salts.[351] Considering the large body of evidence from other studies that formation of Cu(1) is essential for catalysis and that Cu(1) efficiently catalyzes the reaction at less than 0.01 equiv, it is tempting to ascribe catalysis to small amounts of Cu(1) present in the reaction mixture. In order to unambiguously prove activity of Cu(2) in the reaction, catalysis should be performed in the presence of a strong oxidant that effectively removes all adventurous Cu(1).

Table 5

Cu source (equiv)	red. agent (equiv)	solvent	base	ligand	temp/°C, (h)	yield (%)	application	ref
$[Cu(CH_3CN)_4]PF_6$ (0.01)		H_2O/tBuOH or acetone			23 (24)	94	A, E	328, 329
(0.1)	(N_2)	DMF	DIPEA	TBTA	23 (16)	50−90	L, H, C	330
		CH_2Cl_2	Lut		23 (48 or 12)	87	J, C, O	331, 332
	(-air)	CH_2Cl_2/CH_3CN	Na_2CO_3		23 (21)	62	E, C	333
		DMSO			μw (0.01)	91 (LC)	Q, C, D, O	334
(ox., air, NMO)		CH_2Cl_2		TRMEDA	23 (0.3)	31−68	A, B C	335
		CH_2Cl_2			25 (24) or 70	up to 80	E, C	336
PS-NMe_2:CuI		CH_2Cl_2 or CH_3CN			23	up to 99	A, O	115, 337
silica:CuI		silica, no solvent	DIPEA		μw <115 (0.05)	91−98	Q, I, C	338
$(EtO)_3P$:CuI (0.2)		THF	DIPEA DIPEA		23	52−72	A, J, I	339
		toluene			μw 90 (0.2−1) or $(850W)_n$	86−99	N, G, Q	340, 341
		toluene			110 (0.8)	88−95	Q, E, C	342
$CuCl/Pd_2(dba)_3$	$P(OEt)_3$	dioxane			100 (3−24)	20−63	A, B, C	343
$CuBF_4$	hydroquinone	H_2O/DMSO		TBTA	23 (0.2)		N, E	344
CuCl		H_2O			23 (96)	92−99	Q, C	345
$CuCl_2$	NaAsc	iPrOH			23 (24)	62−68	I, C	346
$Cu(AcO)_2$ (0.2)	NaAsc (0.4)	H_2O/tBuOH ($CHCl_3$)			23	67−88	Q,C	347–349
	Cu(0)	CH_3CN/THF		TBTA	25		H, E, C	350
Cu(2) catalysis!		H_2O			23 (20)	71−100	A, C	351
		CH_3OH	iPr_2NH		25 (16)	95	J, C, M	352
TTA:$CuSO_4$	NaAsc or TCEP	bicarbonate buffer/tBuOH		TTA	23		A, Q, D	353
Cu(1) zeolite (USY)		toluene			23 (15)	52−87	A, C	354
$Cu(CH_3CN)_4OTf$ (1 mM)		Tris buffer	pH 8.5	Batho	23 (12)	60−85	A, D, K	355
CuOTf	(N_2)	Tris buffer	pH 8	Batho	23 (16)		D, K, M	356
Cu(2):bis-batho	e^- (−300 mV)	H_2O	KPF_6	Batho	23 (~0.1)	100	E, N, A	357

It should be remembered that the noncatalyzed Huisgen reaction occurs at elevated temperature and in the case of reactive substrates even at ambient temperature with significantly prolonged reaction times. However, Cu(1) catalyzes the reaction with a rate enhancement of $\sim 10^7$ even in the absence of auxiliary ligands and provides a clean and selective conversion to the 1,4 substituted triazoles even under microwave or elevated temperature conditions. The use of microwave irradiation has significantly shortened reaction times, to minutes, with excellent yields and purities and exclusive formation of the 1,4 isomer.[338]

Except for decomposition due to substrate instability, the triazole-formation is essentially insensitive to steric bulk and electronic properties of the alkyne and azide, although the rates may differ and conditions may have to be optimized in particular cases. An example of steric factors and complexation influencing the triazole formation was described in the synthesis of rotaxanes,[336] where it was found that the counterion in the Cu(1) source had a major influence on the outcome, and in nonpolar solvent, $Cu(CH_3CN)_4PF_6$ was found to be far superior to other copper salts such as CuOTf and CuI, leading to excellent yields of rotaxanes.

5. The Influence of Ligands on Cu(1) Catalysis

Although ligands are by no means required for the catalytic effect of Cu(1) in triazole formation, they are often employed both to enhance the rate of reaction and to protect the Cu(1) from oxidation in the presence of adventurous oxygen. There may be a range of mechanistic effects of the use of ligands that coordinate the Cu(1) catalysts in the triazole formation. The protection from oxidation of the catalyst is of course important to maintain a good concentration of the catalytically active complexes throughout reaction. However, the direct effects on catalysis can be considerable. These effects can be due to a direct influence on the catalytic complex involved in the reaction; that is, the ligand is coordinated to Cu(1) during the catalytic process. While this is the general understanding, it is important in all mechanistic considerations to keep in mind the second order kinetics observed with respect to [Cu(1)] that is not easily explained in a model with a ligand, e.g. **7** or **8** that saturate the coordination of Cu(1) with four chelating lone-pairs. In fact, a rate reducing effect has been observed by addition of tetravalent ligands to click-polymer reactions catalyzed by CuBr,[9] and particularly so by ligands containing multiple pyridines. The ligands, which have been found to be the most effective in catalysis, are presented in Figure 4.

One aspect of the catalysis is the presence of a variety of Cu(1)-ligand clusters in the reaction medium. The equilibrium between these clusters is slow and may depend significantly on the exact reaction conditions at all times. When purified CuI is dissolved in acetonitrile, the major component is initially Cu_5I_5; however, this is in equilibrium with smaller quantities of Cu_2I_2−Cu_7I_7. If the more catalytically active cluster is different from Cu_5I_5, the presence of a ligand could affect the rate of reaction. The ligand effect could also differ depending on the counterions involved in cluster formation, as sometimes observed.[250,328] Similar observations have been made both for partially oxidized Cu nanoparticles[287] and for the $CuSO_4$/ascorbate reaction where a delay in onset of catalysis was ascribed to formation of appropriate catalytic clusters.[285] The presence of ligands may, in addition to directly promoting catalysis, also have an effect on both the equilibrium distribution and the rate of equilibration among the clusters to substantially enhance the rate of reaction on its own by promoting the presence of the most active clusters. Interestingly, the catalytic effect of e.g. the bathophenanthrolinedisulfonate ligand **16** is optimal with addition of 2 equiv of catalyst while addition of excess is suppressing the catalytic effect in a [Cu(1)] dependent manner.[264] Similar effects are observed with the most popular ligand TBTA introduced by Sharpless.[359] Recently, the oligobenzimidazole ligands e.g. **18** corresponding to TBTA in architecture were shown to be extremely efficient to protect Cu(1) against disproportionation and effect catalysis.[270] More than 1 active species was involved in catalysis according to the response to variations in salt and the pH of the reaction. Histidine has also been found to have a significant effect as

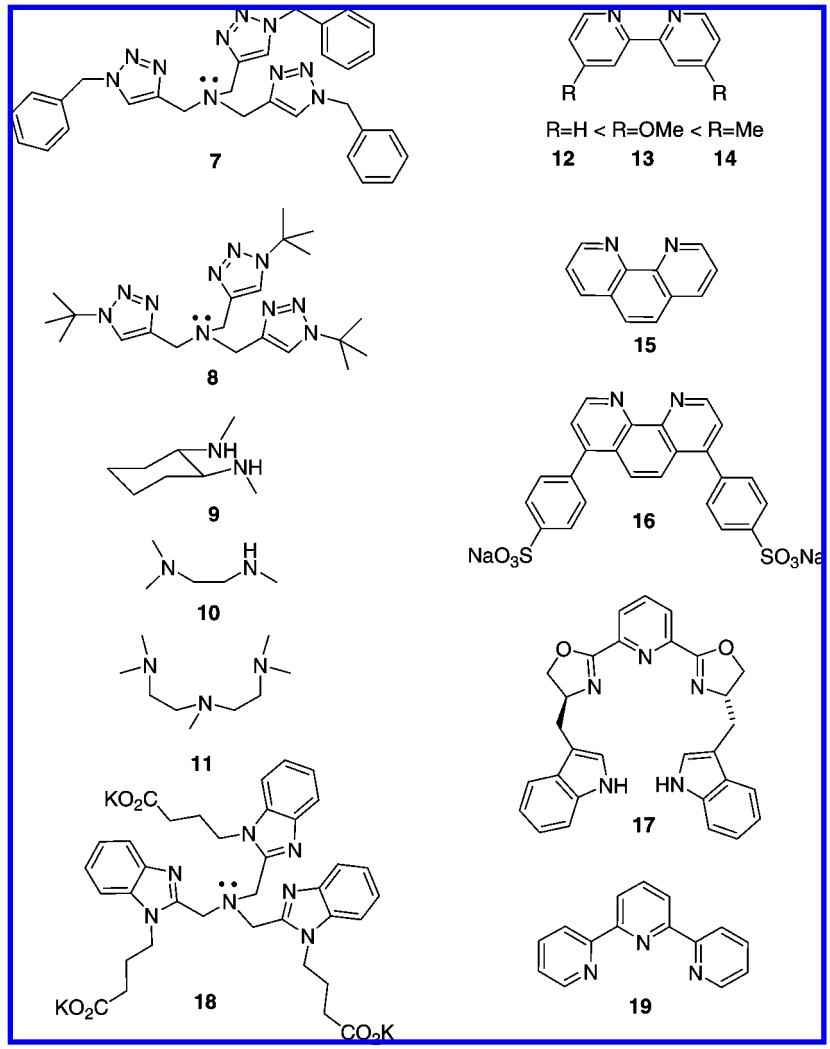


Figure 4. Effective ligands used to promote the Cu(1) catalyzed triazole formation. TBTA, **7**, and bathophenanthroline, **16**, are mostly used for organic synthesis and bioconjugations while PMEDTA, **11**, is predominant in polymer chemistry.

a catalyst, which should be considered in peptide triazole assembly, where results may depend on the presence or absence of histidines.[129]

6. Reactivity of the Alkyne and Azide Substrates

The unhindered terminal coordination of the two reactants to the catalytic Cu(1) cluster as a starting point for the reaction provides for catalysis of triazole formation almost independently of the substitutions. The most significant are the electronic effects that influence the formation of the Cu(1) acetylides and the establishment of the transition state of the reaction. However, apart from substrate decomposition, there seems to be only one report where an alkyne and an organic azide (1,1-diphenyl azido acetic acid) could not be forced to form the triazole.[4] Similarly to the Huisgen reaction,[360] α-carbonyl-alkynes are more reactive than akyl-alkynes, while the aromatic alkynes are similar or marginally less reactive. Keeping the reactants soluble throughout the reaction is a key requirement for a successful outcome.[59] Direct substitution of the alkyne with heteroatoms gives some substrate instability, particularly toward hydrolysis, but they do react to give triazoles under appropriate conditions.

With increasing size of both alkyne and azide substrates in e.g. conjugation of azide containing proteins with large fragments of DNA containing alkyne with the inherently more dilute reaction conditions used in such reactions, conversions have been observed to decrease and be less than optimal.[250] This indicates that there could be limiting cases of bioconjugation where the utility of this ligation reaction may not be optimal, although new improved conjugation conditions are continuously being developed.[355]

Organic azides are considerably more reactive than the azide anion itself, as explained above. This has allowed the development of one-pot procedures in which NaN_3 is first reacted with an arylhalide followed by formation of a 1,4-substituted triazole.[88] The azides are generally reactive, and only carboxylazides and sulfonylazides require special attention due to substrate instability, as detailed below.[94] Somewhat lower reactivity is observed for perfluoroalkylmethylazides.[82]

A special situation is that of substituted allylic azides, since these are very prone to 1,3-sigmatropic rearrangements at significant rates exceeding those of triazole formation.[150] The allylic azides have a preference for triazole formation from the primary and secondary azides compared to the tertiary, thus indicating some steric effect on the rate of triazole formation. On the other hand, only little difference was observed in the rate difference between primary and secondary azides, indicating again the terminal coordination of azide to Cu in the transition state.

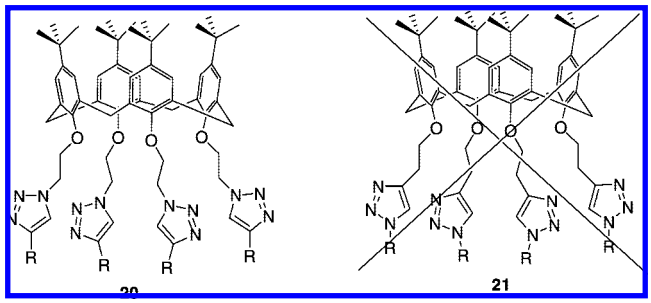


Figure 5. For calixarenes decorated with azidoethyl ethers the Cu(1) catalyzed reaction with alkynes forms four triazoles simultaneously in a total yield of 80% while the same calixarene decorated with alkynes failed to give the expected product.

7. Proximity Effects in the Efficiency and Rate of the Triazole Formation

Several groups have reported significant rate enhancements in the formation of triazole when the azides are linked together on a polymer,[317] dendrimer, or calixarene.[215] The effect is much less pronounced if it is the alkynes that are grouped together on a scaffold, and in the particular case of high density of alkyne, even suppression of triazole formation can be observed.[215] This effect was also studied from a mechanistic point of view,[43] as reviewed by Bock et al.[14] The proximity effects are particularly large in an arrangement of scaffolded azides where the catalytic Cu(1) complex leaving a completed cycle of triazole formation and already recruiting and coordinating the next alkyne from the solution encounters a very high local azide concentration in preparation of, e.g., **20** (Figure 5).[14,43] In contrast, complete failure was observed in producing the triazole product, **21**, from scaffolded alkyne, and this is most likely due to three important effects. The alkyne is ideally situated to engage in Cu(1) catalyzed homocoupling, the alkyne is also reactive as an electrophile in coupling to the newly formed Cu(1)-coordinated neighboring triazole, and finally, the presence of four alkynes may saturate the coordination sphere of the catalytic Cu(1) complex. The formation of byproducts by the first two routes probably prohibits the use of increased temperature as a means to overcome reduced catalysis. Although this is a special case of preorganization of alkyne, it should call for special consideration in situations with high density of alkyne.

Even in polymers containing multiple statistically distributed azides, where molecular tumbling is slow and a decrease in reactions rate is expected as compared to the rate of small molecule monomers, considerable rate enhancements (e.g., 4-fold) can be observed. This demonstrates the generality of the conformation independent pseudoconcentration effect for templated azides.[215]

Extreme rate enhancement was observed for formation of the second triazole in compounds **22** and **23** (Figure 6) that results in near complete suppression of the monotriazole. Whether this is of a similar nature as the general effect above or if transfer of the second azide to prebound acetylene occurs intramolecularly in a complex such as **29** in Figure 6 has not yet been determined. In any event, a high degree of preorganization of intermediates is required for significant suppression of monotriazole, since even structurally similar diazides such as **25** show an almost statistical distribution of products. It is important to note that the situation with bis-alkyne **24** is completely different from that of the bis-azide **22**, since the formation of the acetylide does not seem to be rate determining in the Cu(1) catalyzed triazole formation and a statistical distribution of products is to be expected.

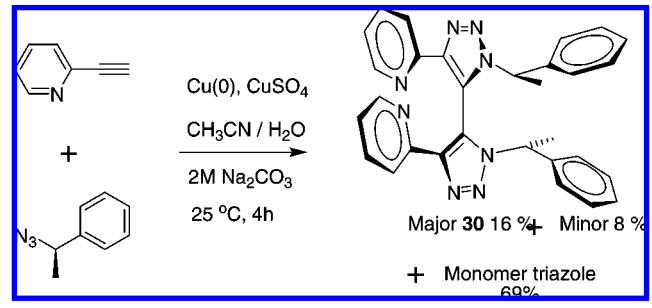
A special case of proximity is that observed in the formation of dimers during attempts to cyclize peptides either on solid support[75] or in solution.[74] Although cyclic monomers have been obtained in high yield in some cases,[57] the situation where the peptide ends does not easily meet, provide for reaction of the alkynes of one peptide with the azide of another. This results in a linear dimer. Dimer cyclization can either be due to proximity of reaction partners through peptide alignment once the first triazole is formed or, as suggested by Punna et al.,[75] because the two alkynes coordinate strongly to the same Cu cluster, thus facilitating internal formation of two triazoles in one complex containing two peptides. The effect was shown to be highly concentration dependent[74] and occurs only at peptide concentrations above 1 mM. The fact that only dimers formed is not easy to explain if this reaction is not controlled by peptide specific interactions.

8. Side Reactions of the Cu(1) Catalyzed Triazole Formation

8.1. Side Reactions and Oxidative Couplings of Cu(1) Triazole Complexes

Usually the quantitative formation of the 1,4-disubstituted triazole is ensured through hydrolytic cleavage of the Cu(1)-bound end product **5** (Scheme 1) of the catalytic cycle to regenerate active catalyst and liberate the triazole. Tiny amounts of a byproduct found to be the bistriazole formed by oxidative coupling of **5** were observed by Rostovtsev et al.[5] during development of the aqueous $CuSO_4$/ascorbate version of the reaction.

Scheme 2. Oxidative Coupling To Yield up to ~90% Bis-triazoles Can Be Promoted by the Presence of Inorganic Base and Air[a]



[a] Chiral bis-triazole cores of interest for catalysis may be obtained. This reaction constitutes a potential side-reaction in the mono-triazole formation.

Very elegantly, Angell and Burgess[361] recently brought this oxidative coupling to the preparative stage. They reduced the proton activity of the reaction medium by addition of inorganic base, e.g. 1.5 M K_2CO_3, and obtained the oxidatively coupled bis-triazoles in up to 95% yield at 0.3 M substrate concentration. The use of carbonate was not essential but gave superior yields, and it has been speculated that carbonate favorably coordinates two organocuprates **5** to promote the coupling.[361] The barrier to rotation in the core bis-triazole of e.g. **30** is high (stable at 115 °C in DMSO), and chiral compounds are not unlike the biaryls used extensively in catalysis. Yields vary, but the reaction

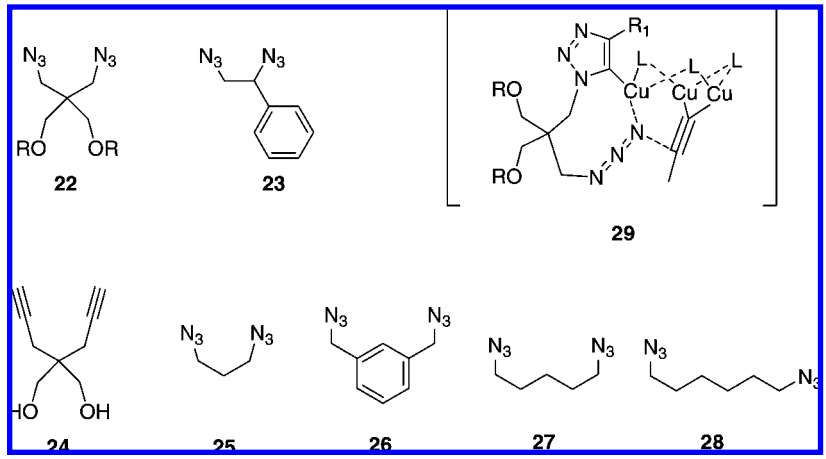
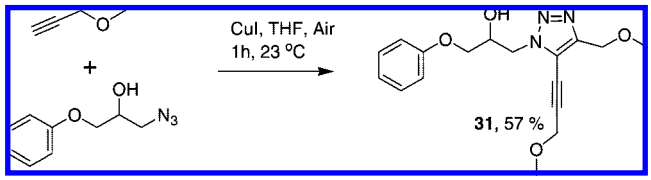
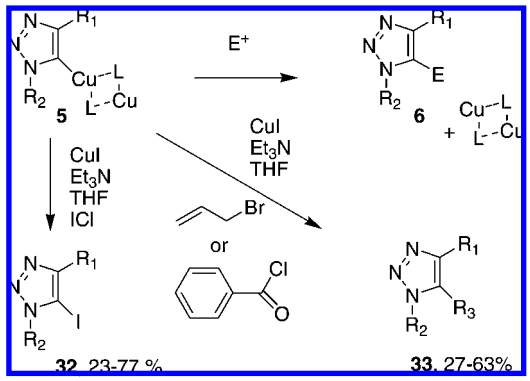


Figure 6. Bis-azides **22** and **23** gave considerable rate enhancement for formation of the second triazole. Compounds **24**−**28** do not have this ability to any significant extent. The rate enhancement could occur via intramolecular coordination of the second azide to the Cu(1) cluster, leaving the first formed triazole.

Scheme 3. Oxidative Coupling of Excess Alkyne with Copper-Triazole Adduct 5



Scheme 4. Reaction of Electrophiles with Copper-Triazole Adduct 5

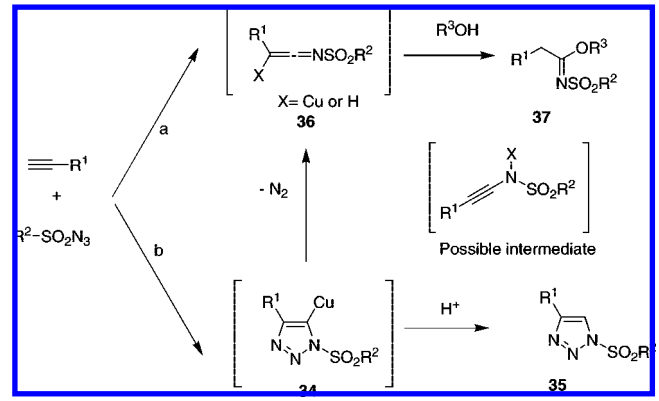


is quite sensitive to steric bulk, and they are best with the less bulky propargyl ethers, which allowed the oxidative coupling product to be formed in excellent yields. During the oxidative coupling under these conditions, a considerable amount of trisubstituted triazole carrying an acetylene at C-5 was observed in varying amounts and was formed in 50% yield when using KOH.[361] The oxidative coupling of Cu(1) complexed acetylides is a putative side reaction in all Cu(1) catalyzed triazole couplings and has also been seen in CuI couplings when air was present[335] (**31**, Scheme 3); however, all oxidative couplings are bimolecular, and formation of byproducts by this route is expected to rapidly wear off with dilution, leading to essentially quantitative formation of triazole.

8.2. Competing Electrophiles in the Demetallization of Copper-Triazole 5

The presence of electrophiles other than protons can lead to high yields of 1,4,5-trisubstituted 1,2,3-triazoles. ICl has been used extensively as an additive to the CuI catalyzed

Scheme 5. In CuAAC with Sulfonyl Azides, the Cu-Triazole 34 May Decompose To Form the Sulfonylketimine 36



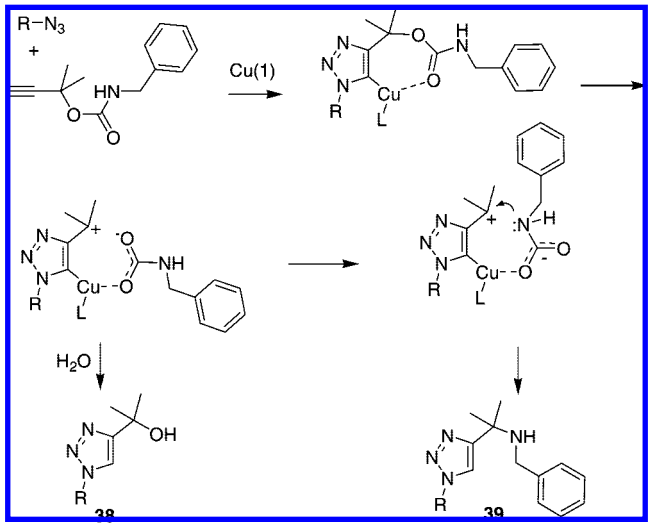
reaction to obtain substitution with iodide in the 5 position (**32**)[110,111] for further derivatization.[110,306] The 5-substituted halide can also be obtained by direct 1,3-cycloaddition using terminal alkyne halide.[306] Alkyl halides and acyl halides can also act as electrophiles to give a yield of 27−63% of the 5-alkylated or 5-acylated triazoles (**33**).[111] Terminal bromoalkynes may also enter into the CuI or CuBr catalyzed reaction and afford 5-bromo-substituted triazole. Here CuBr catalysis is preferred due to formation of a small amount of the iodide-substituted analog when using CuI.[110] Demetallization in the presence of excess alkyne may be used preparatively to obtain the 5-alkyne substituted triazole.[128]

8.3. Side Reactions with Sulfonylazides

Upon reaction of sulfonylazides (and potentially also of other activated azides such as carbonyl- and phosphorylazides) with Cu(1) acetylide, an *N*-sulfonylated amide bond was formed and this product was isolated in high yields under aqueous conditions.[117,362,363] There seems to be a consensus understanding that the sulfonylated amide bond is derived by decomposition of Cu-complex **34** (Scheme 4) through irreversible loss of N_2 and formation of the sulfonylketimine, **36**. The ketimine may react with hydrolytic solvents to give imidates, **37**, which in the case of water rearrange to give the amide bond.[42,117]

Recently, the Sharpless group has established anhydrous conditions with CuI in $CHCl_3$/2,6-lutidine at 0 °C to rescue the product **34** from decomposition and provide selective

Scheme 6. Byproducts May Form from Alkynes Containing a Leaving Group in the α-Position, Particularly if the Formed Carbenium Ion Is Stabilized[a]



[a] The side reactions are suppressed in pyridine.

formation of the desired 1-sulfonyltriazoles, **35**.[94] It should be noted that similar conditions at 25 °C gave the byproduct in the hands of Cho et al.[363] and the selectivity may well be achieved mainly through temperature control, allowing the completion of the catalytic cycle before the irreversible N_2 loss can occur.

8.4. Alkynes with a Leaving Group in the α-Position

Alkynes substituted with α-carbamate were investigated by Bertrand and Gesson[100] as substrates in the CuAAC, and they found considerable formation of unwanted products derived through carbenium ion intermediate generated at the α-position by loss of carbamate. This was particularly serious in the α,α-dimethyl substituted alkyne, where carbenium ion formation is favored but is a potential side reaction whenever leaving groups are introduced α to the alkyne.

The byproducts formed included the expected alkene derived by proton abstraction; as the major product the hydroxyl compound **38** from reaction with water and the secondary amine **39** formed by trapping of the liberated amine. The side reaction could be suppressed almost completely with use of CuI and anhydrous pyridine as a solvent.

8.5. Hydrolytic Side Reactions of Ynamides

The ynamides (Figure 7) constitute a valuable class of heteroatom substituted alkynes for the synthesis of highly functionalized triazoles.[257,309,348] Initially, *N*-alkyne-substituted imidazolinones, e.g. **40**, and oxazolinones, e.g. **41**, mainly afforded decomposition products, indicating a need for reduction of the electron donating character of the nitrogen by sulfonylation e.g. by use of *N*-benzyl-*N*-acetylene sulfonamides as the alkyne substrate.[348]

These side reactions occur due to high electrophilicity of the heteroatom substituted carbon of the alkyne, as demonstrated in the preferential hydroazidation of oxazolinone substituted acetylene over that of phenyl acetylene (Scheme 7) to yield *N*-alkene substituted triazole byproducts in a homo- (**44**) or hetero- (**45**) tandem reaction with 2 equiv of

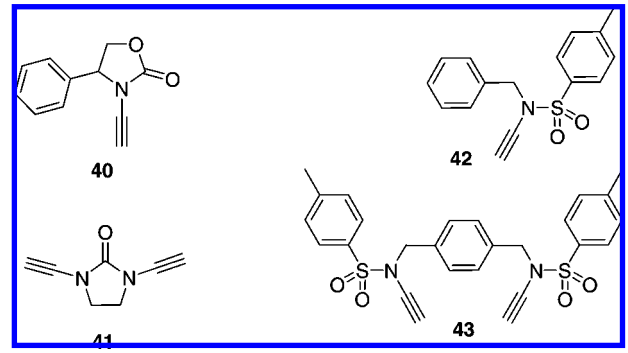
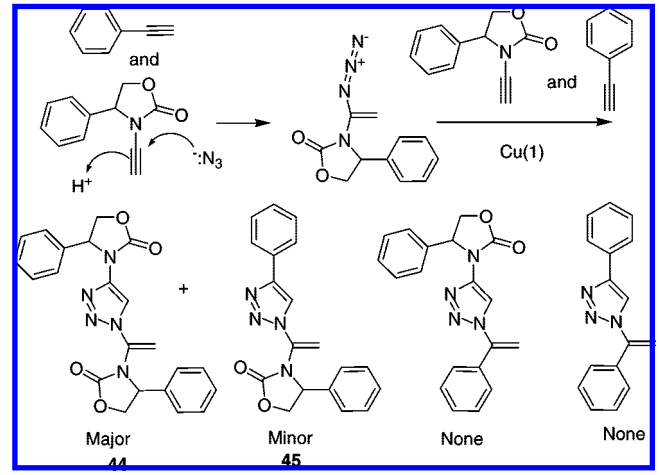


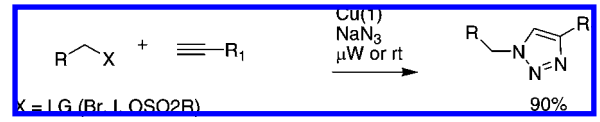
Figure 7. Typical ynamides used in CuAAC. The sulfonamides present the best performance.

Scheme 7. The Competing Hydroazidination Reaction, Which Is Observed as a Side Reaction in Reactions of Ynamides with Azides (the Normal Azide/Alkyne Reaction Is Not Shown)[a]



[a] Only the ynamide reacts with the azide nucleophile.

Scheme 8. One-Pot Reaction with Azide Formation and CuAAC[a]



[a] The two reactions can run simultaneously due to the lack of reactivity of N_3^- in CuAAC.

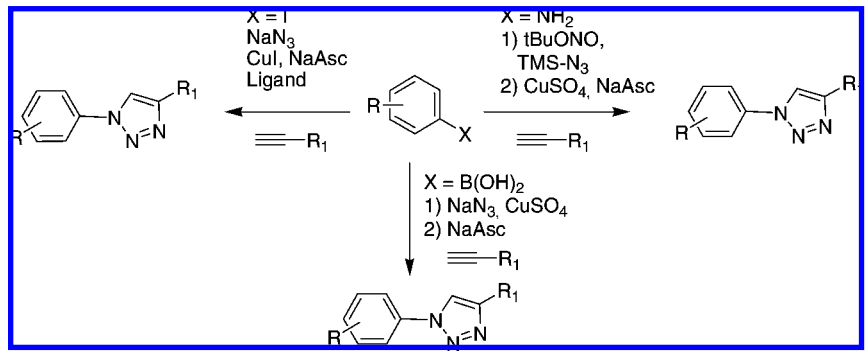
alkyne.[257] However, with careful orchestration of the reaction conditions, e.g. syringe pump addition of the alkyne[257] or use of anhydrous CH_3CN/CuBr, high yields of triazole substituted imidazolin-2-ones could be obtained even with the bis-*N*-alkynylated ynamides **41** and **43**.[309]

9. Applications of the CuAAC in "Click" Chemistry

9.1. Cu(1) in Preparative Organic Synthesis of 1,4-Substituted Triazoles

The Cu(1) catalyzed formation of triazoles is a selective transformation and has allowed a range of multicomponent or one-pot reactions in which the azide and alkyne precursors are formed in situ from simple starting materials prior to 1,3-cycloaddition. The most obvious precursor reaction is the formation of the azide from alkyl halide and sodium azide under the conditions of triazole formation.[130,247,364] The

Scheme 9. One-Pot Azide Formation and CuAAC[a]



[a] The two reactions can run simultaneously due to the lack of reactivity of N_3 in CuAAC.

reaction of inorganic azide with alkyne is quite slow and is generally not competing unless the reaction of the alkyl halide is very sluggish. High yields can be achieved in 10 min with use of microwave conditions.[278]

The presence of Cu(1 or 2) even catalyzes the transformation into azide and also allows the direct catalytic transformation of aryl and vinyl halides into azides.[88,265] The rate of conversion catalyzed by CuI has been shown to depend on the presence of sodium ascorbate and e.g. 1,2-*trans*-dimethylamino cyclohexane as a ligand.[365] When the reaction was performed with $CuSO_4/Na_2CO_3$/Pro/NaN_3 and PEG-supported propargyl ester, a significant amount of the Sonogashira product from reaction of the aryl iodide with the alkyne was formed in addition to the triazoles.[239] Similarly, aryl and vinyl boronic acids may be converted to azide in the presence of Cu(1) or Cu(2) salts and coupled with alkyne. The conversion of the boronic acids to azide is faster than that of the halides, and the reaction with alkyne can apparently occur with $CuSO_4$ in the absence of any extra reducing agents.[218] Aromatic amines have been used in one-pot reactions involving diazotation with tBu-ONO in the presence of TMS-azide followed by in situ cycloaddition of the formed azide.[260]

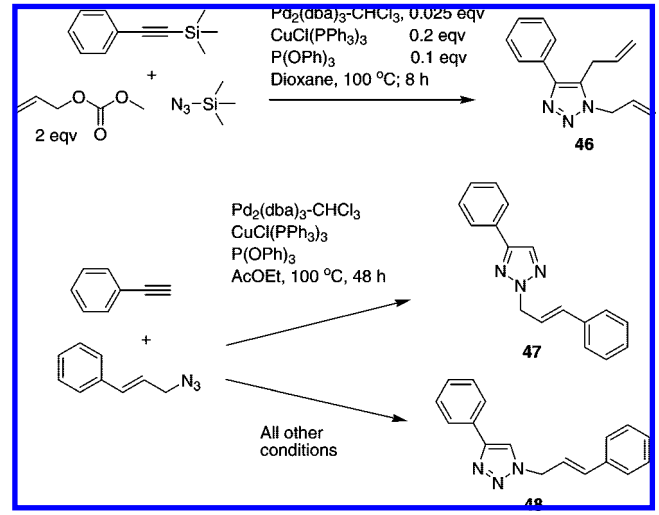
Structural variation of the alkyne may be derived in one-pot from propargyl bromide by reaction with amines. The reaction between the halide and the amine is not required for the triazole formation and may occur before, during, or after cycloaddition.[213]

As mentioned above (Scheme 4), the Cu-triazole intermediate may undergo cleavage from Cu(1) with a variety of electrophiles other than H^+. Wu et al. used ICl, allyl bromide, and acylchlorides as electrophiles to obtain the iodo, allyl, and keto triazoles, respectively.[111] The 5-halide substituted triazoles may alternatively be obtained through use of terminal halide substituted alkynes useful as synthons for further modification to yield more complex triazoles.[306]

Kamijo et al.[327] have developed a Cu(1)−Pd(0) bimetallic catalysis of allyl transfer from 2 equiv of allylic methyl carbonates to TMS-azide, and the allylic azides formed react in situ with alkyne under Cu(1) catalysis. Furthermore, the excess allyl-palladium complex acts as an electrophile that quenches the Cu(1) triazole intermediate, **5**, and yields the 1,4,5-trisubstituted triazoles.[343] With less allyl donor, the substitution in the 5 position can be circumvented (Scheme 10);[327] however, partial 1−2 rearrangement of the allyl group may occur, depending on the exact conditions.[366]

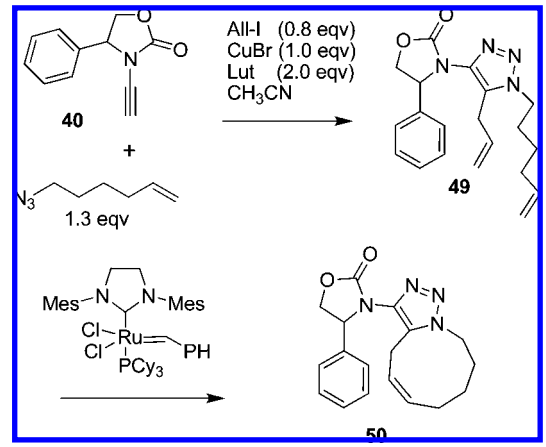
Similarly, Zhang et al. used allyl iodide as an added quenching electrophile in the 1,3-cycloaddition to introduce allylic groups in the 5 position of the triazole, which was

Scheme 10. The Pd/Cu Couple Catalyzed Formation of Trisubstituted Triazoles[a]



[a] It is not yet clear whether the allyl azide is formed prior to cycloaddition or whether the palladium is able to catalyze the direct reaction between alkyne and azide ion followed by allyl transfer. In any event, the palladium is capable of catalyzing the conversion of e.g. **47** to **48**.

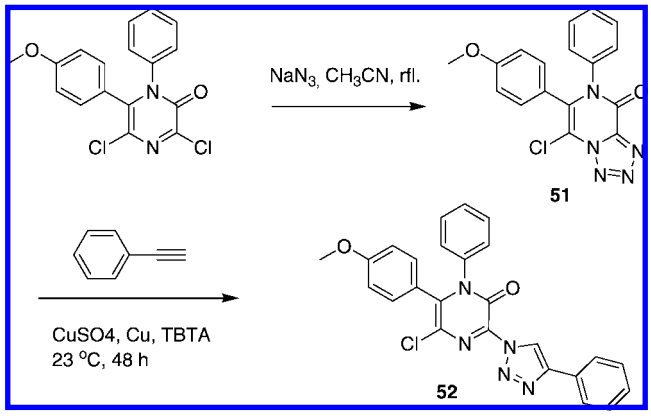
Scheme 11. Addition of Allyl Iodide to the CuAAC Allows Electrophilic Trapping of the Intermediate, 5[a]



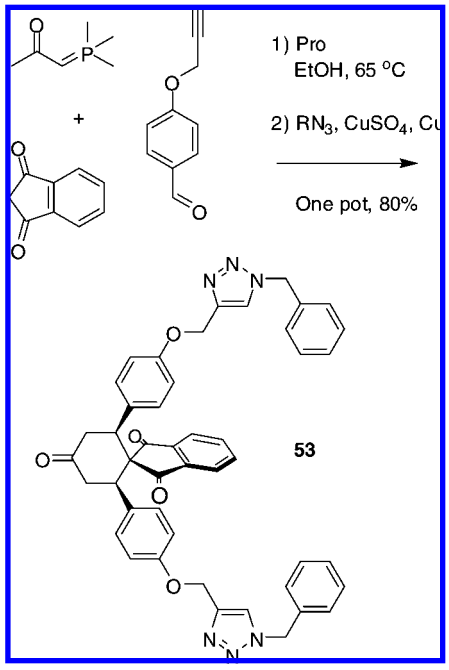
[a] When using alkenylazides, the product **49** may enter into RCM to yield interesting bicyclic scaffolds, e.g. **50**.

formed from alkene substituted ynamides and/or azides for further modification by metathesis (Scheme 11).[310] The cycloaddition reactions of ynamides with azides is prone to side reactions as described above.[257,309,348]

Scheme 12. Intermediate Tetrazole Formation in the Synthesis of 3-Triazolo-pyrazinones



Scheme 13. One-Pot Reaction Involving Wittig, Knoevenagel, Diels−Alder, and CuAAC Reactions



Triazoles, which are not substituted at the nitrogens, are the putative reaction products of the cycloaddition between hydrazoic acid and alkynes. This reaction is not readily performed, and methods based on temporarily protected azides have been developed. Loren et al.[367] used acyloxymethyl and aminocarbonyloxymethyl azides, and upon triazole formation with terminal alkynes the protecting group was removed with base. The base labile 2-tosylethyl group has also been used for azide protection to produce compounds with free NH in the triazole by deprotection with $tBuO^-$ at low temperature. The standard cycloaddition with TMS-azide is quite slow; however, conditions has been described for high yields in the transformation with alkynes followed by TMS cleavage.[140]

Ring opening of substituted methylenecyclopropanes with CuI/I_2 affords the 2,4-diiodobut-1-enes, which may be selectively substituted at the primary iodide with azide. After Cu(1) catalyzed cycloaddition with alkynes, an intramolecular $Pd(OAc)_2$ catalyzed Heck reaction with the vinyl iodide yields the fused pyrrolotriazoles.[256]

The $CuSO_4$/NaAsc catalyzed synthesis of a large array of novel fluorogenic compounds from azide and alkyne fluorophore precursors was described by Sivakumar et al.[193] They coupled coumarin-based azides with aromatic alkynes and obtained a range of fluorophores with interesting emission spectra toward biochemical detection of triazole formation. Zhou and Fahrni did a similar study using the reaction of coumarinacetylenes and arylmethyl azide.[47]

The triazole formation is an easy reaction to perform, and W. D. Sharpless et al. described procedures for carrying out the reaction in an educational environment.[157] The use of Cu(0) derived Cu(1) for the catalysis provides easy product isolation. Orgueira et al. found that addition of amine:HCl salts to Cu(0) facilitated formation of soluble Cu(1) from the oxidized surface, resulting in significant rate enhancements.[288] The rate enhancements obtained with μw-techniques are very significant, and in most cases reaction times can be reduced to a few minutes or even seconds without loss of the 1,4-selectivity characteristic for the Cu(1) catalyzed reaction.[188,189,200,280] This could be particularly important if the reactants are less reactive or if less reactive intermediates are formed. As an example of the latter, Kaval et al. observed the formation of interesting tetrazole intermediates (**51**) in the one-pot reaction of 3,5-dichloro-1-alkyl-2-(1*H*)pyrazinones with azide and alkyne.[280]

The increased temperature under μw-conditions shifted the equilibrium toward ring opening of tetrazole to allow the azide to react with a Cu−alkyne complex, affording **52**. Also, conventional heating can be used to increase the rate of reaction with such less reactive species as employed in the synthesis of 1,2,3−1,2,4-*bis*-triazoles by Xia et al.[238] In a catalyzed bimolecular reaction such as the Cu(1) catalyzed reaction between alkyne and azide, bringing the reactants together artificially in a phase transfer scenario may enhance reaction rates significantly and the choice of solvent system should always be considered carefully when settling for the preparative reaction conditions. Lee et al. found that mixtures of water and dichloromethane were particularly useful in this regard.[52]

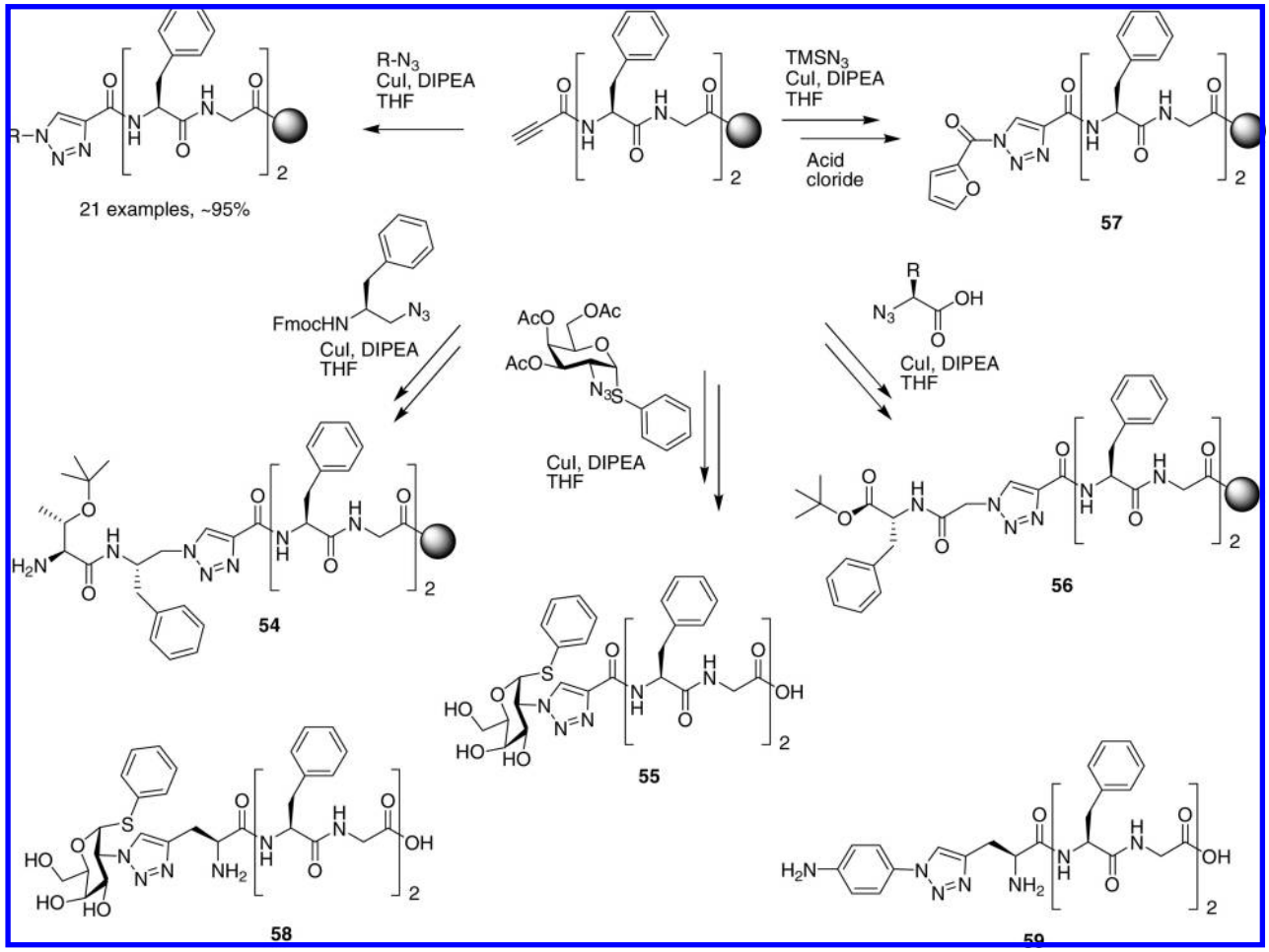
In planning synthetic schemes for triazole preparation, it is often an advantage to introduce the triazole(s) as late as possible in the process due to the high degree of orthogonality of this reaction with both functional groups and chemical conditions. One example of this is the elegant synthesis of a range of large complex spiro compounds by sequential application of aldol, Wittig, Knoevenagel, Michael addition, and Diels−Alder reactions followed by clean transformation to products containing two triazoles, e.g. **53**.[281]

In synthesis of compounds containing multiple triazoles, it should be considered that the selectivity of the reaction toward terminal alkynes in combination with application of temporary protection of alkynes with different labile silyl groups allows the sequential and easy introduction of several differently decorated triazoles in one molecule.[252] Similarly, the azide may be masked as an amine that is readily and selectively converted into azide by a diazo transfer reaction.[176] These properties of the triazole coupling should hold a lot of promise for sequential one-pot reactions and for diverse library synthesis of complex multitriazole molecules.

9.2. Solid Phase Synthesis of Triazoles

The first account on Cu(1) catalyzed triazole synthesis was a synthesis of peptidotriazoles and triazole-linked neoglycopeptides on solid support.[3,4] The alkyne was first linked to resin bound peptides in the form of an *N*-terminal propynoyl group or internally as a propargylglycine (**58**, **59**).

Scheme 14. The Versatility of the CuAAC Was Demonstrated through Synthesis of a Large Variety of Differently Linked Peptidic Triazoles, e.g. 54−59 on Solid Support in Essentially Quantitative Yield



Catalyzed by CuI/DIPEA, a large variety of azides, including azido sugars (**55**) and azide derivatives derived from amino acids (**56**), protected amino alcohols (**54**), and TMS-azide (**57**), were coupled in THF at 25 °C in close to quantitative yields. The reaction was found to be truly catalytic, requiring as little as 0.01 equiv of CuI.[4] The method was used to produce split/mix libraries of protease inhibitors.[77]

Fan and Zhang described the first repetitive solid phase synthesis where the CuI catalyzed triazole formation was used as key-coupling reaction alternated with peptide couplings.[96] They coupled 4-pentynoic acid to the preceding amino acid. The resin bound alkyne was reacted under Cu(1) catalysis in 20% piperidine/DMF with chiral, Fmoc-protected, 2-substituted amino ethyl azides derived from amino acids. The reaction occurred with simultaneous Fmoc-cleavage. The use of piperidine in the triazole coupling was crucial for obtaining the quantitative yields required for repetitive chemistries and provided compound **62** containing four triazoles in almost quantitative yield.

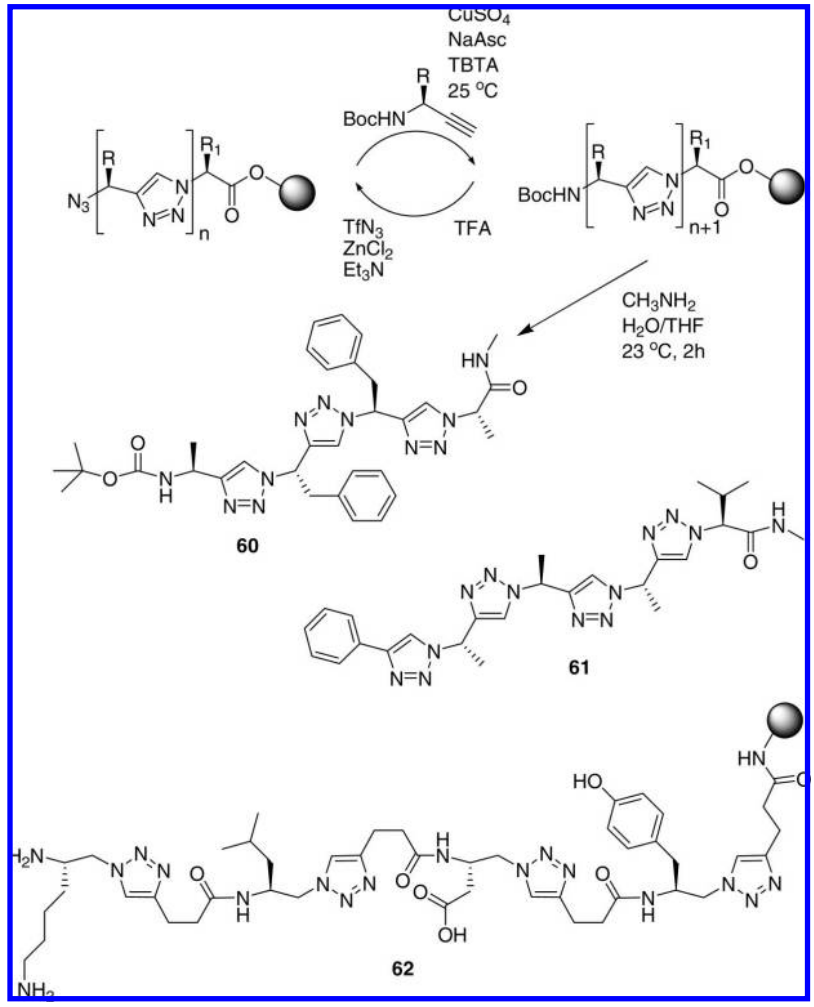
Angelo and Arora recently described an elegant synthesis of a true peptide mimetic triazole oligomer on solid support.[368,369] Substituted, chiral, and Boc-protected propargylamines derived from amino acids were coupled repetitively to the growing azidomethyltriazole oligomer under catalysis with $CuSO_4$/NaAsc/TBTA in NMP/H_2O. Between couplings, terminal azide was efficiently produced from the deprotected amine in high yield by $ZnCl_2$ catalyzed diazo transfer using triflic azide. The average yield/coupling obtained for synthesis of a tetratriazole oligomer was 97% and they produced the compounds **60** and **61** on solid support.

Solid phase synthesis of small molecule triazoles could be accomplished on a selenium linker.[78] Triazole was formed from the propargylselenide resin and enabled C-allylation of the α-methylene for further decoration prior to oxidative elimination of the selenium linker.

Cu(1) catalyzed triazole coupling has been used for attachment of complex linker functionality often including the first building blocks to solid supports.[62,119,332,370,371] These immobilization reactions have invariably been performed by converting amino resins to azido resins by diazo transfer followed by coupling of the complex functionality containing an alkyne. The diazo transfer is uneventful and quantitative on gel supports while aminopropyl-CPG requires some heating. The method has been used to prepare resin for DNA synthesis,[62] a resin for synthesis of aminooxyalkyl triazoles useful for preparation of e.g. metallo-enzyme inhibitors,[332] resins containing new backbone amide linkers such as the acid labile 3-formylindol-1-ylmethyltriazole,[370] and recyclable resins containing acryloyloxymethyltriazole as a linker for synthesis of tertiary amines.[119]

Triazole immobilizations have also been used for the attachment of functionality to supports for chromatographic separations and for affinity chromatography. Azide was introduced through nucleophilic substitution on cross-linked epoxide containing glycidylpolymethacrylate beads. For

Scheme 15. Solid Phase Triazole Oligomer Synthesis Presents a True Opportunity to Mimic Peptide Structure at Large without Peptide Bonds[a]



[a] Cycle yields above 99% were obtained during synthesis of **62**. The triazole containing peptides has been found to assume structures that are compatible with e.g. α-helices.

comparison, an alkyne resin was prepared by including propargyl acrylate in the polymerization.[92] By CuI mediated triazole coupling with fatty alkynes, the azide resins gave the best chromatographic reversed phase material compared to that obtained with alkyne derivatized supports and fatty azide.

Furthermore, alkyne-containing protein could be immobilized through triazole. Punna et al. converted amino agarose into azido pentanoyl and pentynoyl agarose via amide bond formation using PNP esters.[305] A variety of ligands were efficiently coupled using both the azide and the alkyne resins and conditions with CuBr/NaAsc/2,2′-bipyridine/2,6-lutidine.

An impressive fragment based solid phase synthesis of polyproline, combined with intermediate CuAAC (CuI, DIPEA, DMF/THF), provided polyprolines, derivatized with different triazoles at every third Pro residue (**63**; Figure 8).[120] 4-Azido prolines were used to install triazoles in the 4-position of the proline rings. Both 4-S and 4-R azido prolines were synthesized and used in assembly on solid support in order to investigate the influence on conformation. The triazole peptides formed by CuAAC were compatible with formation of both PPI and PPII helical structures.

Smith et al.[337] have demonstrated inverted solid phase continuous flow chemistry where the CuI catalyst is immobilized while reagents are in the mobile phase. Excess reagents and leaking Cu were trapped on scavenger resins, and high yields of pure materials could be obtained up to the 1.5 g scale.

9.3. Modification of Peptide Function with Triazoles

Peptide inhibitors based on incorporation of triazoles are described in a separate paragraph.

Local structural motifs in protein loops are crucial to their interaction with other protein receptors, and often β-turn motifs are incorporated into peptide mimetics to maintain bioactive conformations. Oh and Guan[184] have investigated the triazole as a β-turn mimetic structure (Figure 9). They found that the 1,4-substituted triazole formed an efficient mimetic with four atom extensions of both substitutions, i.e. a triazole formed by Cu(1) catalyzed cycloaddition of 4-pentynylamine and 4-azidobutanoic acid.

Using CuAAC, Horne et al.[108] incorporated triazoles into peptides that form four helix bundles and solved the crystal structure of the bundled peptides. The triazoles were shown

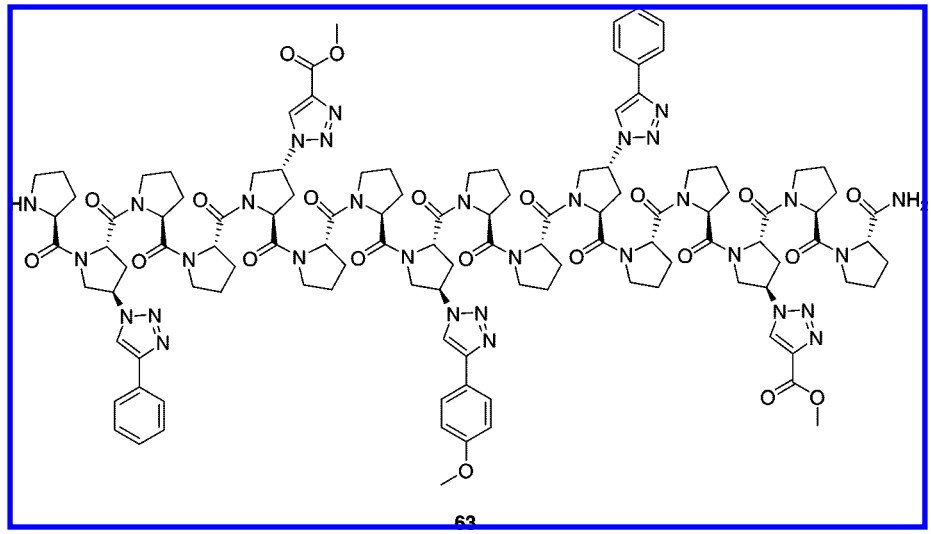


Figure 8. Solid phase synthesis of polyproline with in situ CuAAC on solid support.

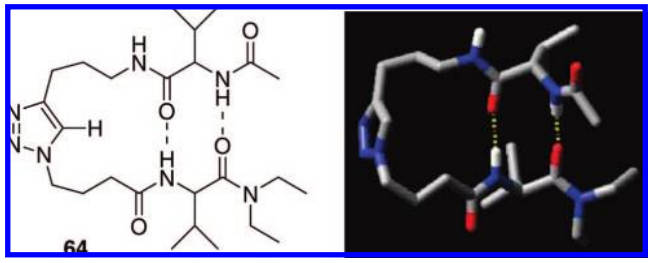


Figure 9. Optimized configuration for a triazole induced β-turn mimetic structure.

to form an $i - (i + 4)$ hydrogen bond in the individual α-helix and stabilized bundle formation through a water HB-bridge to the neighboring helix as well, thus demonstrating the compatibility of the triazole as a peptide bond surrogate.

Paul et al.[166] investigated the proline cis/trans isomery of one of all four structural isomers containing a triazole as a proline peptide bond mimetic. The most interesting isomer is the readily available forward 1,4-isomer obtained by conversion of amino acid carboxylate to alkyne and the amino group to azide, also used in repetitive synthesis of triazole oligomers.[368,369] It was found that the content of cis increased from 10 to 30% by introduction of this mimetic, and unlike in Ac-Pro-Gly-NHMe, where a hydrogen bond between the Ac-CO and the HNMe eliminate the cis conformer, the cis/trans ratio in the mimetic was unaffected by the presence of the methylamide, indicating absence of a hydrogen bond.

Peptoids (Figure 10F and G) are useful peptide mimetics where a large range of functionality can easily be incorporated by introduction of alkyl appendages to nitrogen atoms in an oligo-glycine. This technology was employed to attach both propargyl (F) groups and alkylazides (G) for further modification by click reactions to introduce fluorophores, steroids, ferrocenes, fluorous, and lipophilic tags to the peptoid in a very efficient molecular lego approach.[76,114] Franke et al.[55] used a similar approach to attach different peptides to a central cyclic peptide scaffold by sequential unmasking of amino groups, coupling with propiolic acid, and Cu(1) catalyzed cycloaddition to azidoacetylated peptides (Figure 11).

The triazole cycloaddition has also been used to couple antigens with adjuvants and less successfully to TLR ligands.[67] In the future, peptide modification by triazoles will be highly dependent on our ability to synthesize appropriate chiral building blocks containing azides and alkynes. One such procedure involves the use of a nitrile hydratase/amidase enzyme preparation for optical resolution of α-substituted β-azidocyanides to mimic β-amino acids.[144]

9.4. Triazole Containing Enzyme Inhibitors and Receptor Ligands

The triazole moiety is an excellent stable mimetic of the peptide bond that is not susceptible to proteolytic processing and may provide metabolically stable and efficient inhibitors of key mammalian, bacterial, and viral proteases. Thus, it could have potential for drug development. Tornøe et al.[77] produced and screened solid phase combinatorial libraries containing a triazole targeted to bind at the active site of *Leishmania mexicana* cysteine protease CP2.8. Inhibitors with nanomolar activity were identified (e.g., **77**), but curiously the triazole in these bound at the S3−S2 sites rather than at the S1−S1′ sites.

Based on replacement of the central part of known HIV inhibitors, novel compounds (e.g., **76**) in which the triazole actively participated in the crucial binding to the water nucleophile were obtained by Brik et al.[275,372] By parallel cycloaddition reactions, Wang et al. prepared 96 hydroxamic acid metalloprotease inhibitors (Figure 12) using 8 alkyne containing hydroxamic acids and 12 azide building blocks.[162] Using the inhibitors in an ELISA format, they produced inhibition fingerprints of 3 different metalloproteases including MMP7 involved in proliferation of cancer. The triazole did not contribute significantly to inhibitor binding; however, micromolar MMP7 selective inhibitors (e.g., **73**) were identified.

In a substrate activity screening approach by Patterson et al.,[103,104] small molecule fluorogenic substrates as well as micromolar triazolonitrile and triazolo-chloromethylketone inhibitors of cathepsins (e.g., **75**) were prepared by solid phase CuI/DIPEA catalyzed triazole formation in a four step synthesis. The developed inhibitors were more selective than their parent aldehydes toward cathepsin K and S over B and L. An elegant chiral synthesis of the required building blocks for construction of the true triazole based peptide bond surrogate has been developed.[103] Wang et al.[373] synthesized

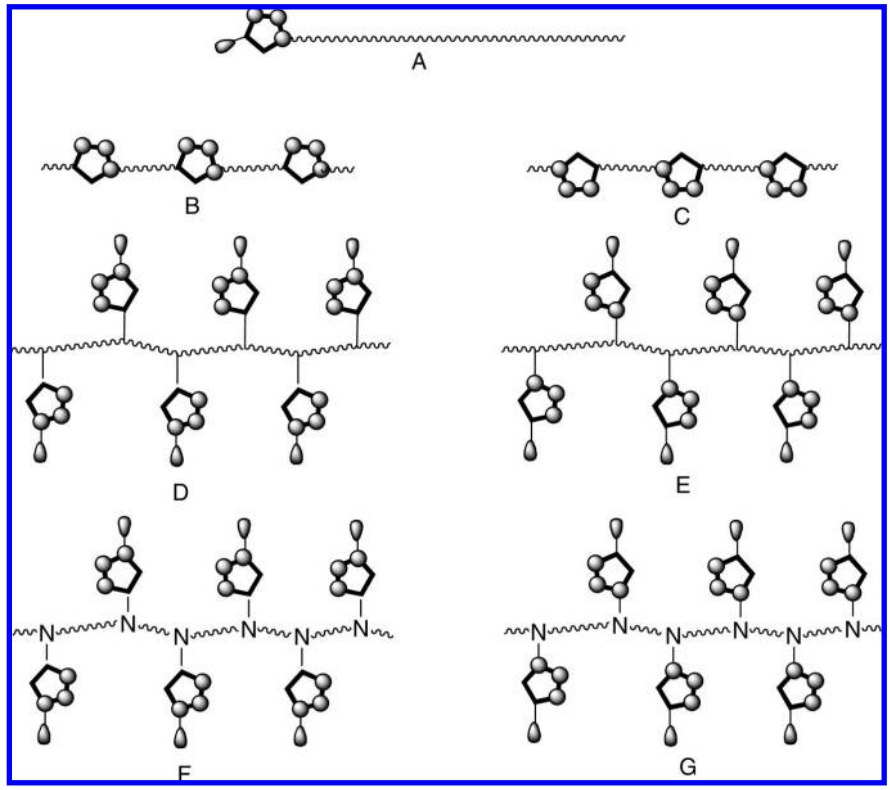


Figure 10. Different types of peptidic triazoles that have been prepared either in solution or on solid support.

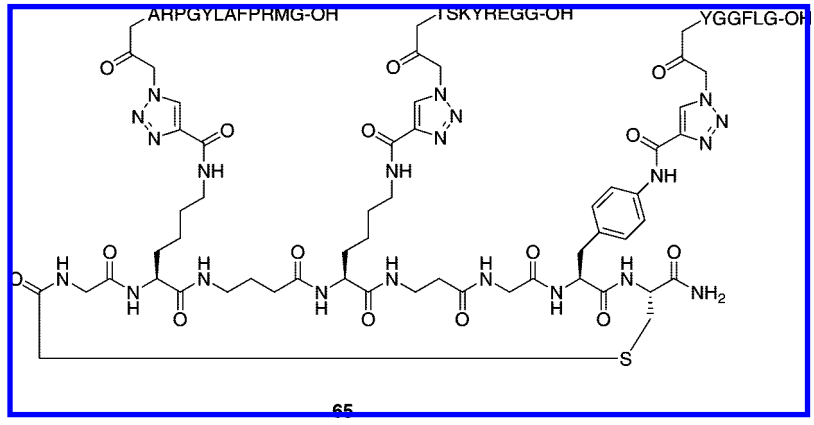


Figure 11. Peptides on peptides by CuAAC. Amino groups on the cyclic scaffold were protected as -NO_2, Aloc, and Dde, respectively.

a small library of hydroxamic acid metalloprotease inhibitors containing a photoaffinity probe and a fluorescence tag for metalloprotease fingerprinting. The probe and tag were attached as a last synthetic step to the deprotected inhibitor by CuAAC.

Srinivasan et al.[259] have developed phosphatase inhibitors with selectivity toward PTP1B involved in diabetes and obesity (e.g., **67**). Based on a bidentate known inhibitor from Abbott, 14 azides and 5 alkynes were linked by triazole formation to form 66 putative inhibitors. A potent and selective inhibitor was formed by reaction of 5-(4-propargyloxyphenyl)-1,2-oxazol-3-carboxylic acid with methyl 4-(5-azidopentanoylamino)phenyl-glycolate. In a similar approach,[192] cycloaddition of a large array of aromatic alkynes to methyl 4-azidophenyl ketocarboxylate provided micromolar inhibitors of different phosphatases including PTP1B. A subset of these inhibitors (e.g., **66**) contained as many as four triazoles formed in two consecutive cycloadditions.

A sterically congested insect selective GABA receptor antagonist, **72** was synthesized in quantitative yield from 2,6-dichloro-4-trifluoromethylphenyl azide and phenylacetylene. The two bisected aromatic rings were forced 90° away from planarity due to the bulky chlorine atoms in the ortho-positions.[132]

Highly selective dopamine D4-receptor ligands (e.g., **71**) were synthesized by Löber et al.[346] by reaction of *N*-propargyl 3,4-didehydro-4-phenylpiperidine or *N*-propargyl-*N*′-phenyl piperazine with phenylazide in yields of 62−68% in the presence of $CuCl_2$ and ascorbate.

Inhibitors of glycosylating enzymes have been targeted with triazole chemistry. Thus, propargyl phosphoric esters of GDP were coupled to organic azides with Cu(1) in an array format and screening of the products provided a selective fucosyl transferase inhibitor (**74**) carrying only a biphenylmethylaminocarbonyl group as a functional mimetic of fucose.[282] Salameh et al.[69] derivatized the

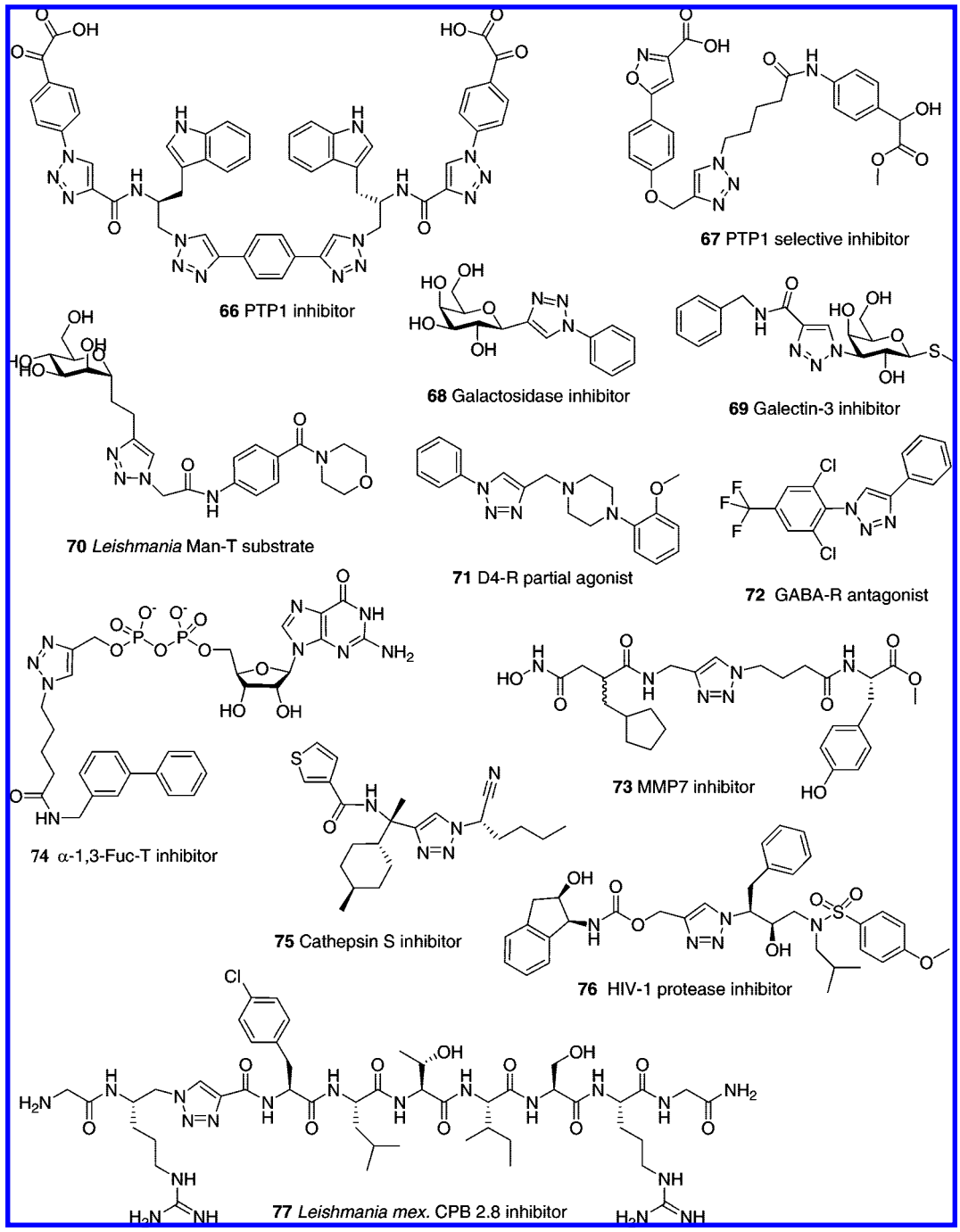


Figure 12. Superior enzyme inhibitors and receptor ligands identified from compound libraries where the diversity was achieved by facile CuAAC. Only the best compounds are presented, and they illustrate the general acceptance of the triazole as a central scaffold in biomolecular interactions.

acetylated β-thioglycoside of 3-azido galactopyranose with alkynes including propiolic acid methyl ester by CuI/DIPEA catalysis in high yields. Aminolysis of the resulting triazole ester with benzylamine gave a reasonably good ligand **69** for galectin-3.

1,2-β-Mannosyltransferase is a relatively safe target in antileishmania drug development, and van der Peet et al.[230] have produced substituted triazolyl methyl α-1-mannopyranosides as substrates (e.g., **70**) in order to define the substrate requirements. Rossi and Basu[68] used phenyl β-gluco- and β-galactopyranosyl triazoles produced by CuI/DIPEA catalysis as moderate inhibitors (e.g., **68**) of the corresponding glycosidases.

9.5. Modification of Natural Products and Pharmaceuticals

There are three major types of modification of natural products and drug compounds that have been performed through Cu(1) catalyzed triazole formation. One type involves the attachment of property modifying chemical entities to the drug in order to enhance e.g. solubility or bioavailability.[141,163,185,201,331] A second type involves formation of triazole in order to attach a fluorophore or a biotin label to the drug at a late stage of the synthesis.[134,135,171,272,276] The predominant uses of the triazole coupling in drugs and natural products are directed toward diversifying the drug structure in order to exploit the

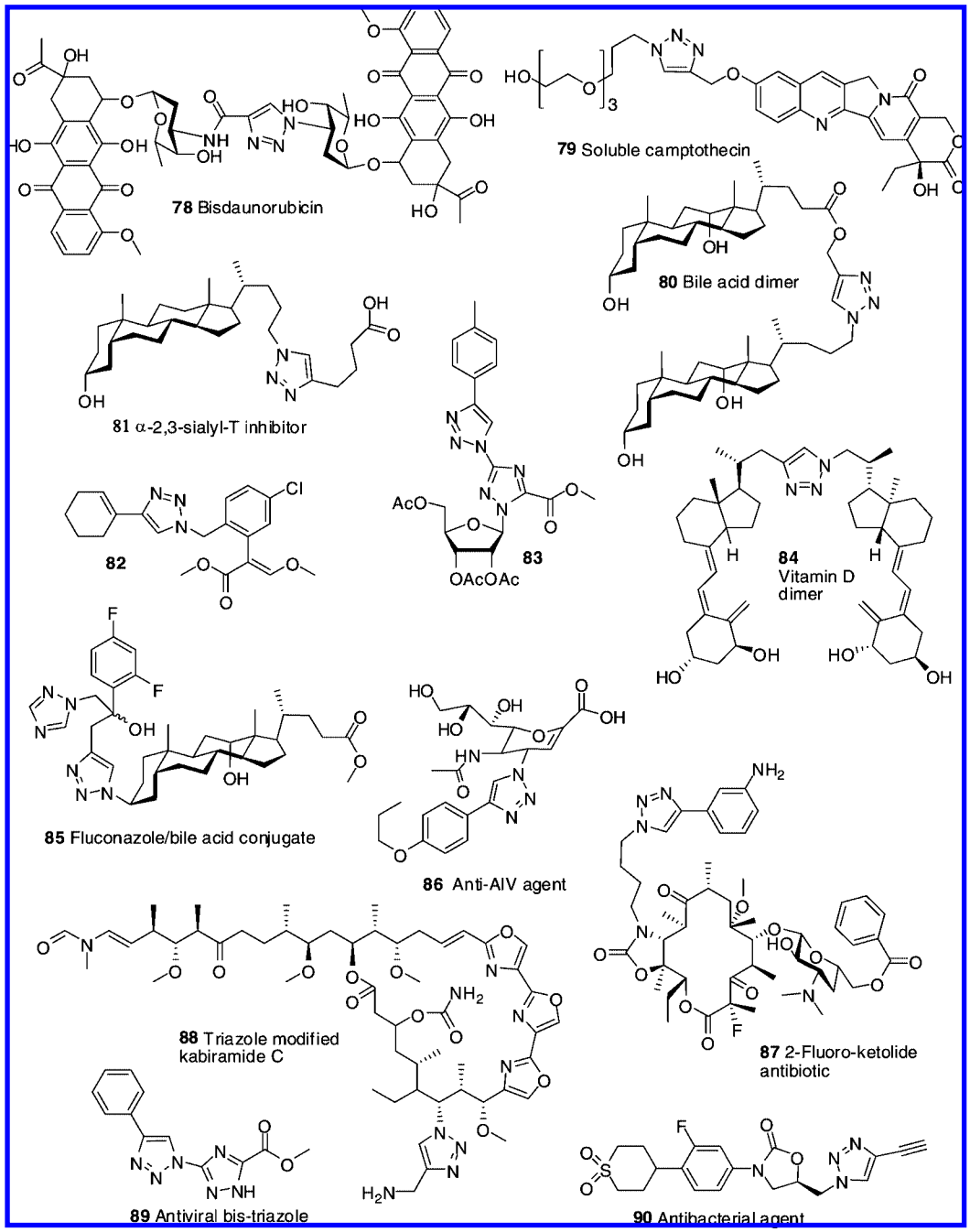


Figure 13. Activity and selectivity of existing drugs and natural products have been modified through facile CuAAC, compatible with the complexity of these compound classes.

chemical space around a compound with a known activity (Figure 13).[64,65,83,105,122,125,151,152,172,186,189,197,205,339]

With a few exceptions[151,152] in vitamin D synthesis (e.g., **84**) advantage has been taken of the exquisite orthogonality and specificity of the triazole coupling and the triazole has been performed in the last step of synthesis in the presence of unprotected functional groups. The reactions have mainly been performed by standard CuI or $CuSO_4$/ascorbate conditions with the appropriate provision for solubility and reactivity problems and in generally high yields of 70−100% of isolated compound.

Xu et al. modified the antitumor alkaloid camptothecin with a propargyl ether in the 6-position of the quinoline ring and formed a triazole with monoazido OEG of different length to significantly improve the solubility.[201] The triethylene glycol derivative **79** showed significantly increased solubility while maintaining activity.

The membrane adhesion properties of polyamines for transfer of DNA over the cell membrane were modified by attachment of a lipdated propargyl amine to an azide containing branch in the center of the polyamine molecule using $Cu(CH_3CN)_4PF_6$ as catalyst.[331] Cinchona alkaloids often attached to a stationary phase for chiral separations were modified with an azide in the 9-position, and a range of alkynes were coupled to the resulting secondary azide in good yields.[185] Cholic acid has been transformed into the propargyl ester and coupled to cholic acid derivatives containing azide substitutions of hydroxylated positions to produce tweezer-like receptor molecules (e.g., **80**) containing two cholic acid moieties around a central triazole.[163]

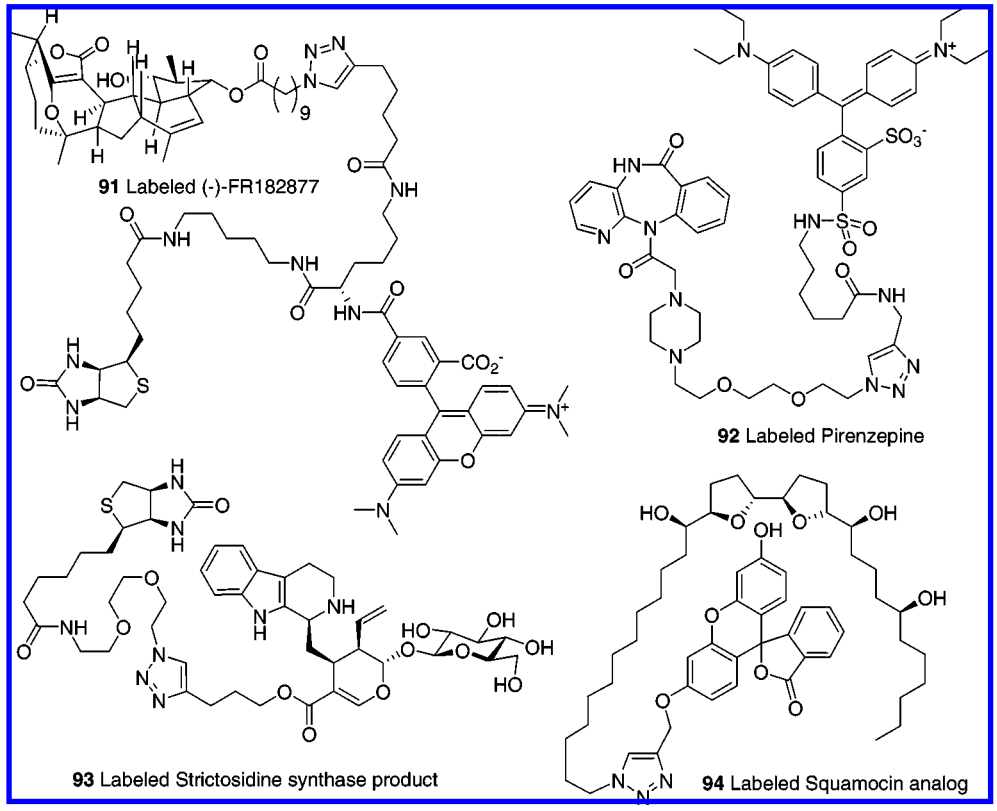


Figure 14. Labeling of unprotected and complex natural products and drugs with biotin and fluorophores in high yields is feasible through CuAAC.

Quader et al.[276] modified Neomycin B in various positions with Cu(1) catalyzed triazole couplings after transformation of different hydroxyl groups to the azide using double inversion via epoxide.

During the synthesis of a diversity oriented library of 10000 carpanone-like structures, Goess et al.[122] included 78 triazoles by Cu(1) catalysis at the last step of synthesis; however, these proved to be less active in a phenotypic assay involving disintegration or blocking of the exit of the golgi compared to simpler aromatic amine substitutions.

On the other hand, extension and modification of the acidic side chain in lithocholic acid by introduction of triazoles after conversion of the acid into an azide were found to provide potent α-2,3-sialyltransferase inhibitors (e.g., **81**).[205]

Triazole chemistry has been used extensively to modify and potentiate antitumor,[83,172,339] antibacterial,[64,65,105,125] antifungal,[189,374] and antiviral[186,197] compounds. The macrocyclic and Actin binding Kabiramide C was derivatized in the 7-position with azide and reacted with Fmoc-protected propargyl amine with Cu(1) catalysis. The product after Fmoc cleavage, **88**, was a potent binder of Prodan-G-Actin, indicating the potential use as an anticancer agent. It was not clear from the report whether the Fmoc-protection was a prerequisite for the successful outcome of the synthesis.[83] Dimers of daunorubicins, a well-known anthracycline antitumor agent, linked through the carbohydrates were prepared. The amino groups of glucosamines were converted to azide or propynoic amide and reacted with alkyne or azide spacers, respectively, using the $(EtO)_3P$:CuI/DIPEA conditions.[339] Only compounds (e.g., **78**) with a short spacing of the interchelators were active, and less so than the monomers.

In the macrocyclic antibiotic telithromycin, the side chain was converted into an azide acting as a protecting group during modification of the 5-*O*-glycosylated-hydroxyl group by hydrolytic cleavage of the natural desosamine and reglycosylation with a variety of deoxyamino sugars. A functional 3-aminophenyltriazole or 2-pyridyltriazole was then formed by CuI catalysis at elevated temperature to give a variety of antibiotic analogs (e.g., **87**) with sugar dependent activities.[64,65]

Linezolid is an antibacterial drug containing a 5-acetamidomethyl oxazolidinone showing a side effect of monoamine oxidase A inhibition often leading to hypertensive crisis. By replacement of the acetamide with an ethynyl triazole generated by reaction of the azidomethyl analog with buta-1,3-diynyl(trimethyl)silane in the presence of CuI and 2,6-lutidine, a 1,4-substituted ethynyltriazole analog **90** with significantly improved selectivity against the bacteria was obtained.[105] In a seminal study, vancomycin was deglycosylated and 6-azido-β-D-glucopyranose was attached enzymatically. Using CuI at elevated temperature and DMSO/H_2O as a solvent to ensure a broad spectrum of solubility, the 6-azide was converted into a large variety of 1,4-substituted triazoles, including the 4-heptanyltriazole showing a 2-fold lower MIC compared to vancomycin against three different Staphylococcus and Enterococcus bacteria.[125]

CuI catalyzed and microwave assisted triazole coupling was also used to link together bile acid with an analog of the antifungal agent, fluconazole, to form a bioconjugate antifungal agent, **85**, with the amphiphilic nature of the bile acid. The MIC's for the best conjugate were 3−10-fold higher than those of fluconazole, and the compound was active against 5 out of 6 fungal infections investigated, thus showing a profile deviating from that of the parent drug.[189] Chen et al.[374] prepared a small library of analogs of the antifungicide Azoxystrobin by CuAAC using 5 different 2-azidomethylaryl-3-methoxyacrylates and 22 aromatic

alkynes. Several compounds (e.g., **82**) were as active as Azoxystrobin.

Bis-1,2,3−1,2,4-triazoles (e.g., **89**) were found to act as antiviral agents against the tobacco mosaic virus by direct inhibition of the reproductive cycle of the virus. Methyl 5-azido-1,2,4-triazo-3-yl carboxylate was reacted with a range of alkynes, and products obtained by reaction with phenylacetylene and cyclohexen-1-ylacetylene were found to be significantly more active than the commercial antifungal agents ribavirin and DHT, known to have systemic action to enhance plant resistance rather than interacting with the virus.[186] A similar and active nucleotide analog **83** was also prepared.[187]

Triazole analogs of the AVI-neuramidase inhibitor Zanamivir, a neuraminic acid derivative carrying an equatorial guanidine in the 4-position, have been prepared. These analogs, where the guanidine was replaced with a triazole, were prepared by reaction of 4-azido-neuramic acid with a range of alkynes using $CuSO_4$/ascorbate catalysis. The 4-(4-propyloxyphenyl)triazole derivative **86** was almost as active as Zanamivir.[197]

The orthogonal nature of CuAAC has allowed the impressive labeling of unprotected, complex and even reactive drug molecules and natural products with various probes.

Chen et al.[171] isolated an enzymatic product formed from the propargyl ester of the secologanin substrate by a strictosidine synthase mutant. The enzyme catalyzed a Pictet−Spengler type of conversion of secologanin, and the propargylated product formed preferentially by the mutant enzyme was modified by triazole coupling to a PEG spaced biotin label for isolation of the product **93**. The authors present the product as the 1,5-substituted triazole; however, considering the overwhelming evidence for 1,4-selectivity described above this is likely to be a mistake.

The antitumor compound squamocin targeting mitochondria was derivatised with a peripheral azide, and this was modified to compound **94**, containing a peripheral azide, and this was in a triazole coupling with the 3-*O*-propargyl ether of fluorescein for investigation of the targeting process.[134] Adam et al.[135] labeled the bidental natural product, FR182877, covalently binding to various proteins via a strained vinyl bridgehead and a strained lactone, with alkyne containing rhodamine and biotin labels. FR182877 was first selectively modified at the most reactive alcohol as the 10-azidodecanoate and subsequently reacted with the tags under $CuSO_4$/ascorbate conditions. The product **91** specifically labeled carboxyesterase-1 in the mouse heart proteome (Figure 14).

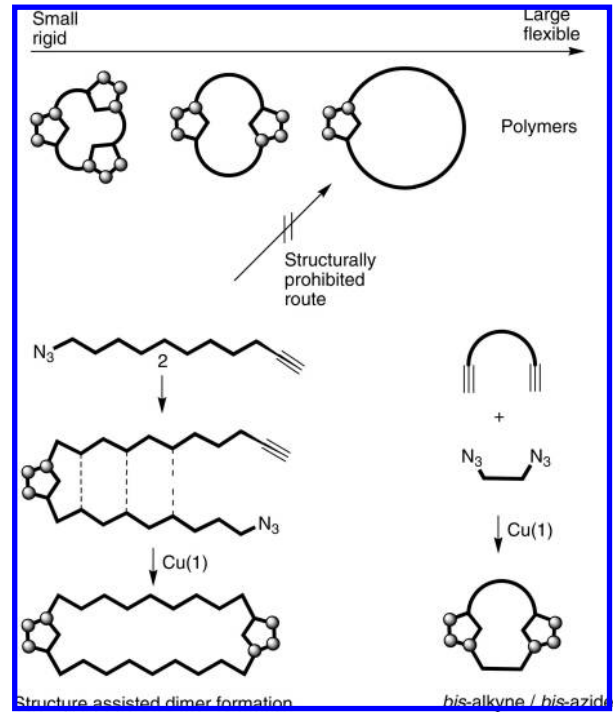
Bonnet et al.[272] similarly tagged a muscarinic M1 receptor antagonist with fluorescent probes, e.g. **92**, and with biotin.

The Cu(1) catalyzed 1,2,3-triazole modification of drugs is an orthogonal reaction that is easily carried out at a late stage of synthesis with minimal requirement for protection of functional groups. It provides high yields and pure products and the triazole moiety is generally quite well tolerated in drugs. It may therefore be expected that the Cu(1) catalysis will facilitate property modification for many of the existing drugs to overcome drug resistance and ADME problems.

9.6. Macrocyclizations Using Cu(1) Catalyzed Triazole Couplings

The outcome of macrocyclization reactions of linear structures containing an azide and an alkyne is highly structure dependent. From peptide cyclizations it is well-known that macrocycles containing from 8 to 14 atoms are extremely difficult to achieve and mostly cyclic dimers and trimers as well as larger linear oligomers are obtained. Cyclic pentapeptides−octapeptides form primarily the monomer if these are structurally favored by the peptide stereochemistry. This picture is even more pronounced in the structurally rigid carbohydrate oligomers where monomer cyclization is extremely structure dependent and for the cyclodextrins is only feasible with penta-, hexa-, and, less favorably, heptasaccharides. Thus, the most important factor determining the ability of a molecule to enter monomer or dimer cyclization is the probability of a contact encounter of the two reactive groups involved in cyclization, and it is very difficult to draw general conclusions about the outcome of a reaction without considering the conformational populations of the linear precursor.[283] The use of a *bis*-alkyne/*bis*-azide approach will ensure a macrocycle with at least two triazoles and, if the building blocks are rigid, formation of a linear polymer.

Scheme 16. Structural Influence on the Formation of Macrocycles by CuAAC



The triazole mediated cyclization reaction is different from cyclization by peptide bond formation in the sense that three

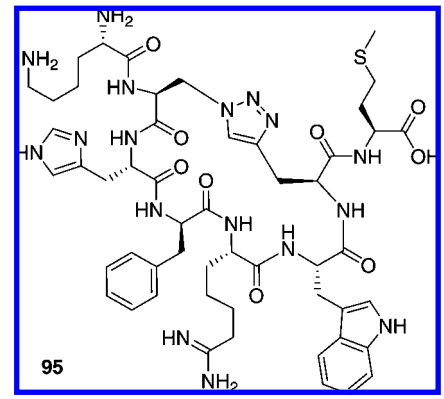


Figure 15. The first macrocyclization by CuAAC was the solid phase synthesis of cyclic melanocortin 4 receptor ligand **95**, showing high picomolar agonistic activity at the receptor.

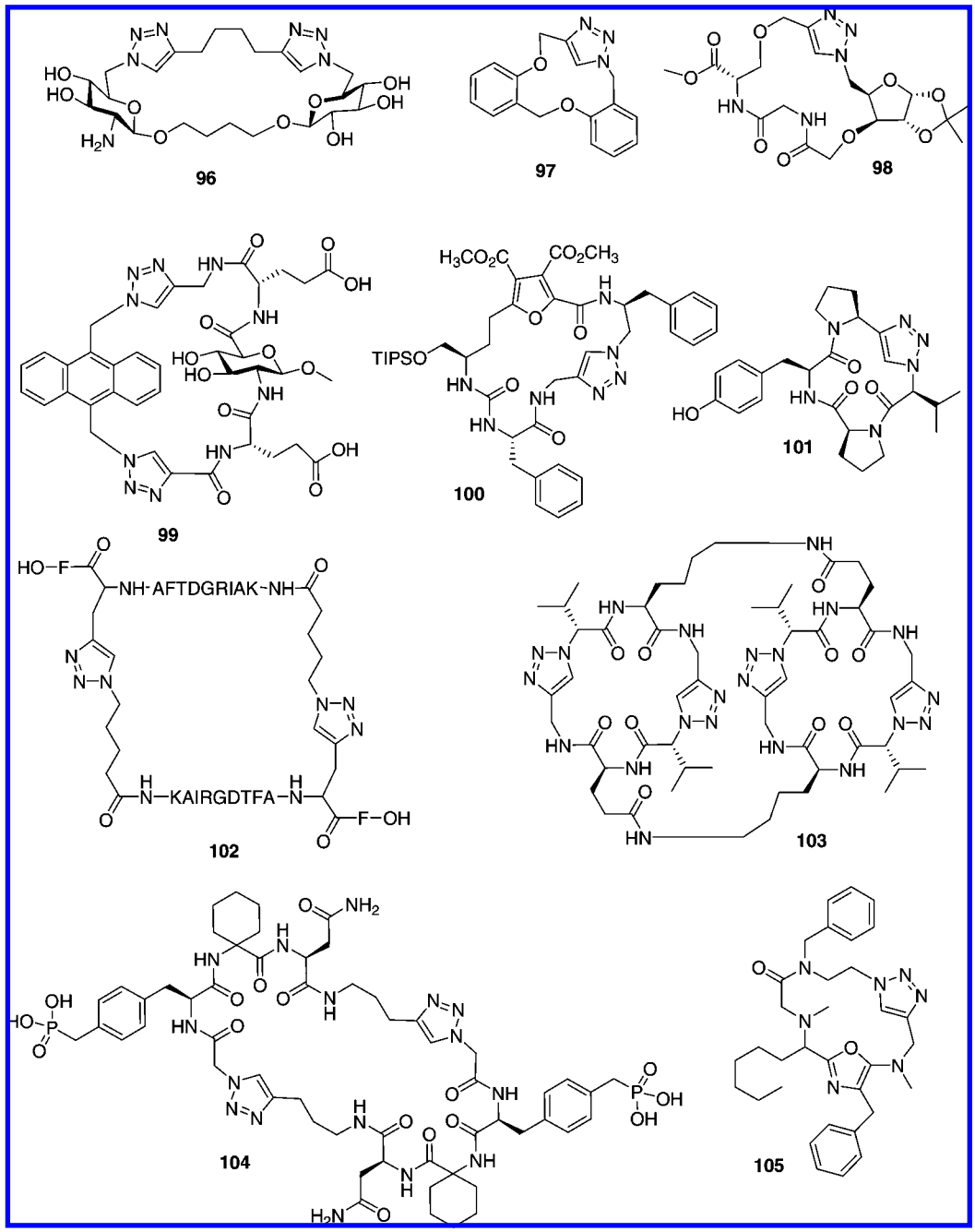


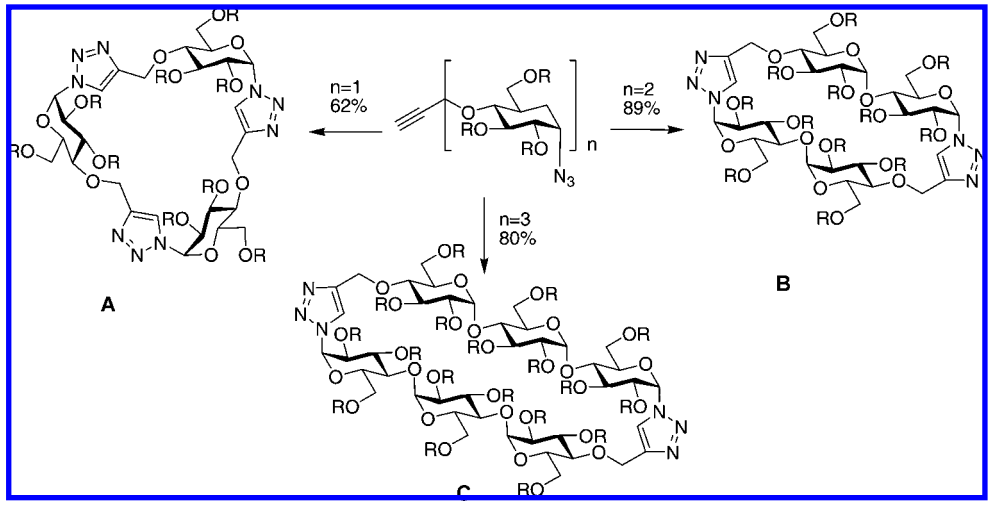
Figure 16. Small molecule and peptide macrocycles which have been synthesized by CuAAC.

components come together in the transition state, i.e. the alkyne, the catalytic copper cluster, and the azide. Whether the first cycloaddition is intermolecular or intramolecular is controlled mainly by structural probability (Scheme 16). Furthermore, in the transition state, the Cu(1) cluster has the ability to coordinate second alkyne residues. If an intramolecular reaction is favored, the cyclic monomer is formed. Once an intermolecular triazole has been formed, the probability of intramolecular formation of the second triazole increases significantly, often leading to clean formation of the dimer, when this is structurally favored. This is not surprising considering the large increase in pseudoconcentration of reactive groups upon formation of the first triazole and the ability of the Cu cluster to simultaneously recruit more alkynes and azides.

The first intramolecular monomer cyclization via triazole formation was the cyclization of an MCR4 active hexapeptide ligand, **95** performed in ~80% yield with CuI on solid support by Roice et al.[57] The reaction could be performed equally well with the protected and the unprotected peptide, it was clean, and no dimer was obtained.

Bock et al.[299,300] cyclized a structurally favored tetrapeptide with one proline and one *C*-terminal alkyne modified proline to the 13-atom cyclic monomer, **101**, of *cyclo*-[Pro-Tyr-Pro-Val], at high temperature using CuBr/DBU in refluxing toluene. The equivalent peptide cyclization was not feasible, and it was concluded that this is due to the fact that the macrocyclic complex leading to the transition state of the reaction, depending on which mechanism is invoked, is in fact 16 or 18 atoms in size. Ring contraction then occurs in a stepwise manner. The cyclic compounds were more active than the parent cyclopeptide in inhibiting mushroom tyrosinase.

Scheme 17. Macroyclization of Propargylated Azido Sugars May Be Achieved by CuAAc To Mimic Cyclodextrins[a]



[a] The preferred ring size is highly dependent on the size and structure of the glycan.

Small molecule intramolecular triazole cyclization to form a cyclic monomer has been performed on several small molecule structures. Ray et al.[154] prepared a variety of monomer cyclized triazolophanes (e.g., **97** and **98)** with a ring size ranging from 11−17 atoms in ∼30% yield using optimized conditions with CuI. There were no dimers isolated, and the moderate yields were ascribed to formation of larger oligomers, which could not be isolated. Similar results were obtained in the formation of flexible macrocycles (e.g., **105**) obtained through a tandem three-component reaction (amines, aldehydes, and isonitriles)/triazole macrocyclization.[54] Looper et al. prepared structurally more defined 17−20 atom macrocycles (e.g., **100**) in excellent yields of ∼50−80% using CuI/DIPEA in toluene.[71]

Dimer macrocyclization was first described by Bodine et al. in the synthesis of a cyclodextrin analog by linking two trimaltosyl azides carrying a propargyl ether at the 4″ position together via triazoles to mimic the receptor property of β-cyclodextrin. In this case there was no possibility for monomer macrocyclization due to the rigid carbohydrate structure and the cyclization of the dimer is very favored due to the dimension of the linear dimer resembling that of the maltoheptaose in which the ends readily meet. Compounds **B** and **C** in Scheme 17 are therefore readily formed. When azide and alkyne cannot react with each other in the dimer, e.g. in the linear dimer of 4-propargyl-α-D-glucopyrannosyl azide, a second round of triazole formation providing the linear trimer occurs prior to macrocyclization to yield the cyclic trimer **A** in 62% yield.[90,91]

The exceptional rigidity of saccharides is also apparent in the lack of monomer formation observed by Billing and Nilsson in cyclization of *N*-(propynoyldipeptidyl)-6-azidoglucosamines and the isolation of 64% macrocyclic dimer with CuI/DIPEA/CH_3CN at optimized conditions.[101] They also observed that yields of dimer were best at dilute concentrations, indicating loss of product due to oligomerization at higher monomer concentration. In a similar study by the same group, a tripeptide containing a central sugar amino acid and derivatized with alkyne at both termini was coupled with 9,10-bis(azidomethyl)anthracene to yield **99**.[86] There is no possibility for cyclic trimer formation in this case. The cyclization was followed in detail, and no detectable amount of the linear dimer could be observed as an intermediate for the cyclization, indicating the great rate acceleration for the formation of the second triazole.

Choi et al.[74] synthesized a triazole containing a phosphotyrosine 1-β-turn mimetic structure acting as an inhibitor for the Grb2 SH2 domain interaction using CuI/ascorbate/DIPEA. They observed formation of both cyclic monomer (18 atom macrocycle) and dimer (e.g., **104**), and the ratio was very dependent on the precursor concentration. Both compounds were biologically active, the monomer with micromolar and the dimer with nanomolar K_d's. However, interestingly, the most active compound was a linear analog of the cyclic dimer. Maarseveen et al.[116] cyclized a dipeptide containing an *N*-terminal azide and a *C*-terminal propargylamide with formation of 70−80% of macrocyclic dimer. In this case, no cyclic monomer (ring size 10 atoms) was observed. They compared the bis-triazole formation with cyclization by lactamization and found that the triazole formation was significantly better, even though two simultaneous reactions were required, and only one for lactam formation.[107] Compound **103** was obtained through consecutive CuAAC, peptide coupling, and a macrolactamization reaction. As mentioned, Punna et al. also compared the solid phase bis-triazole reaction with lactamization in the peptides $N_3(CH_2)_4$CO-K(Pg)AIRGD(tBu)TFAG(propargyl)−F−O-Resin and H−K(Boc)AIRGD(tBu)TFADF-O-Resin.[75] Although, in the latter peptide, lactamization is expected to give a number of byproducts due to the less than optimal protection of functional groups as well as oligomer formation, it was noted that no cyclic dimer was observed, in contrast to the bis-triazole formation, where the cyclic product **102** (72-membered ring) was isolated in 15−20% yield. They even dimerized a 19 amino acid peptide via formation of two triazoles, yielding a 124 membered cyclic dimer in 10−15% yield. The authors did not include any structural influence of the particular peptide sequence in their mechanistic considerations.

Another well established macrocyclization reaction, which has resemblances to the Cu(1) catalyzed triazole cyclization and is driven by biscoordination to a central metal atom, is the RCM reaction of dienes catalyzed by Grubbs ruthenium catalysts. Dörner and Westermann used this reaction to prepare macrocyclic natural product mimetics of cyclic glycolipids containing two triazoles (e.g., **B** in Scheme 17)

for the linkage of the functional sugars.[137] 6-Azido-glucopyranose derivatives containing lipid precursors for the RCM were linked together by bis-triazole formation with octadiyne Cu(1) prior to cyclization.

Recently, Hu et al.[375] synthesized triazole analogs of the cyclodepsipeptide, jasplakinolide. They compared lactamization with triazole coupling and found that under the optimized conditions of CuI, DIPEA, 2,6-lutidine, and CH_3CN/THF the macrocyclization via triazole was far superior to lactam cyclization and gave the cyclic monomer in high yields.

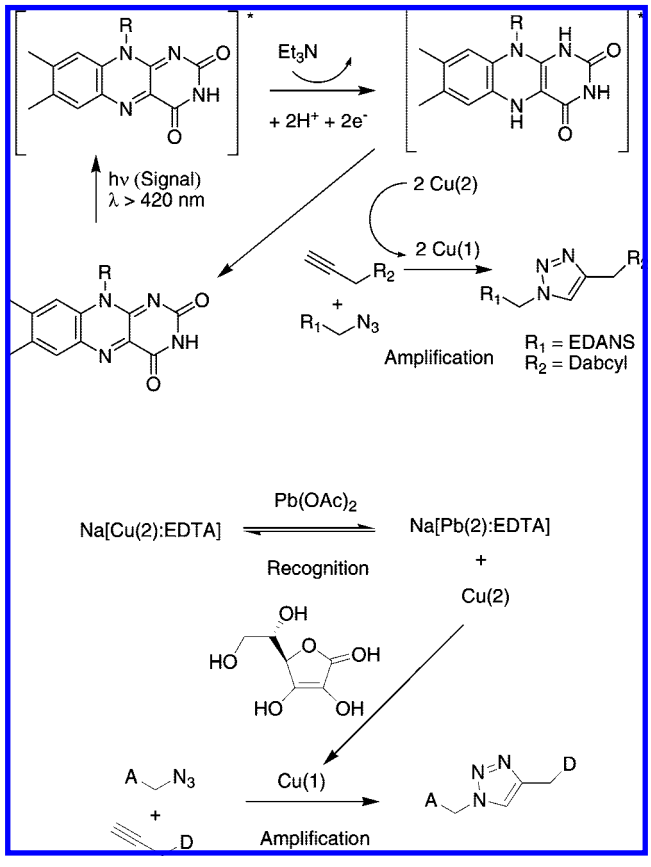
Mono-, di-, and trimer macrocycles were obtained through CuAAC of azide and alkyne containing furanoses.[283] The favored ring size was very sensitive to small changes in the structure of the furanose.

9.7. Catalytic Events Involving Cu(1) Catalyzed 1,2,3-Triazole Formation

A recent publication by Ritter and König[376] describes the coupling of riboflavin/triethylamine photoreduction with the reduction of Cu(2) to Cu(1) and transfer of the photon count to an amplified signal. The signal is produced through Cu(1) catalyzed triazole formation of a compound containing a FRET pair of dansyl and Dabcyl chromophores. The turnover for Cu(1) catalysis was ~70 in 20 min, and the response to the number of photons approached linearity (Scheme 18).

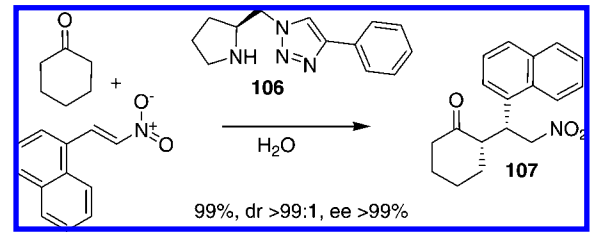
In a similar study, Zhu et al.[219,377] uses the capture of Cu(2) ions by EDTA and release with addition of Pb(2) to construct a sensitive sensor for Pb(2) based on allosteric catalysis. Cu(2) released by replacement with Pb is reduced to Cu(1) by excess ascorbate, and signal amplification is achieved through the triazole formation and FRET-quenching of a fluorescence signal.

Scheme 18. CuAAC Can Be Used as a Coupled Amplification Reaction To Detect a Signal or a Molecular Recognition Resulting in Formation of Cu(1)



Frequently, the catalytic and asymmetric properties of known catalysts and catalyst ligands have been modified through the easy coupling of acetylene and azide.[61,102,133,143,378] Highly diastereo- and enantioselective organocatalysts are readily available from Pro by conversion into *N*-Boc-(*S*)-2-azidomethyl pyrrolidine and coupling with e.g. phenylacetylene in the presence of CuI to yield **106**. In 1,4-addition of ketones to nitrovinyl compounds producing e.g. **107**, the catalyst generally gave ~99% ee and a dr in the range of 10/1 to 99/1.[102,143]

Scheme 19. Enantioselective 1,4-Addition of Ketones to Nitrovinyl Compounds by Organocatalysis with Pyrrolidine Containing Triazole 106



Similarly, coupling of diphenylpropargyl phosphine with a variety of organic azides provided the methylene bridged "clickphines" (e.g., **110**) used in palladium catalyzed highly regioselective (98%, 90% yield) *C*-allylation with cinnamyl acetate of sodium diethyl methylmalonate as a *C*-nucleophile.[61] The simple synthesis of complex chiral bis-triazole described recently by Angell and Burgess[361] mentioned above, using oxidative coupling of the intermediate **5,** could facilitate a number of new triazole applications in the field of asymmetric catalysis.

Gheorghe et al.[112] found that alkylation of 4-hydroxy-TEMPO with fluorous tags was not possible; on the other hand, alkylation with propargylbromide was straightforward and the reaction with fluorous alkyl azide and CuI gave 80% "F_{17}-Click-TEMPO", **108**, which performed excellently in the oxidation of alcohols to carbonyl compounds and could be recovered through four cycles, albeit by silica gel chromatography.

The Cu(1) catalyzed triazole coupling is particularly useful for immobilizing catalysts on e.g. a solid support or a functional molecule to facilitate selective catalyst regeneration. Font et al.[56] reacted *O*-propargylated hydroxyproline with azide containing polystyrene beads to afford a recyclable organocatalyst resin, **109**, for chiral aldol reactions performed with reasonable dr's and good yields and ee's. The catalyst could also be used with excellent enantioselectivity for chiral 2-aminooxylations of cyclic ketones with nitrosobenzene.[379] A polystyrene supported proline-type organocatalyst was synthesized by Alza et al.[380] The best results were obtained when 4-ethynylbenzyloxy-PS was reacted with Boc-L-azidomethyl pyrrolidine in the presence of CuI, DIPEA in DMF/THF at 40 °C. The catalysts obtained showed quantitative conversions as well as good diastereoselectivity and enantioselectivity in Michael addition of ketones to nitrovinyl compounds.

Bastero et al.[381] produced a chiral organo-Zn coordinating resin by CuAAC of 1,1,2-triphenyl-2-(4-(prop-2-ynyl)piperazin-1-yl)ethanol with PS-azide. They demonstrated that the resin was much less enantioselective in phenylation of

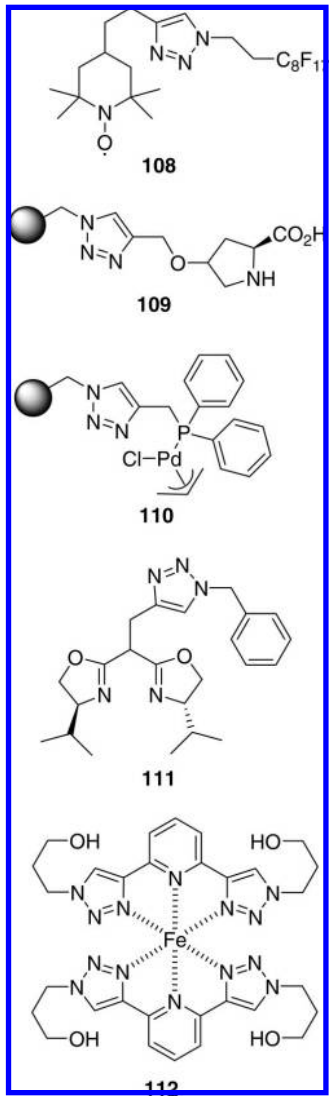


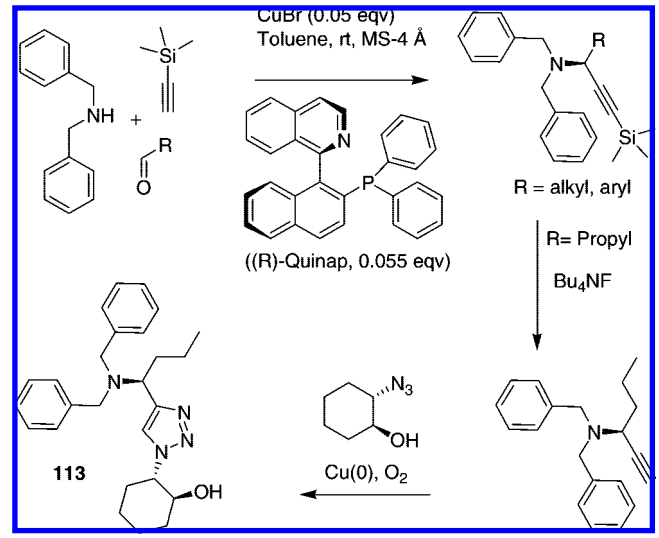
Figure 17. Catalysts and metal ligands derived through CuAAC.

aldehydes than the catalyst in solution and implied that the triazole may act as a nonchiral catalyst coordinating the zinc reagent. Similar interference from the triazole was observed for catalyst ligand **111**.

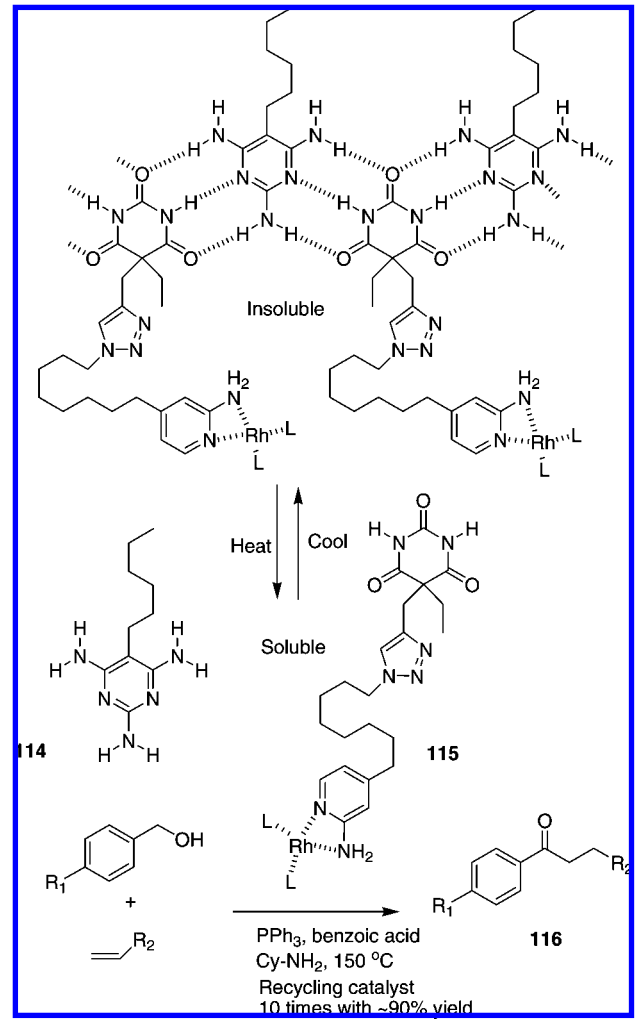
The chemical orthogonality of CuAAC, the mild reaction conditions employed, and the selectivity for terminal alkynes have facilitated decoration of complex alkyne containing organometallic compounds such as chelated diruthenium alkynyls by triazole formation on specifically installed ethynylphenyl groups to modify the structure without changing the coordination geometry around the ruthenium.[156] Li et al. reacted 2,6-diethynylpyridine with azides to afford the 2,6-triazole substituted pyridine (**112**) for strong coordination to Fe(2), Ru(2), and Eu(3), and the coordination of the triazoles was determined by crystal structure elucidation and investigated in cyclic voltammetry.[145] Chiral ligands for catalysis containing a phosphine and a triazole were synthesized by Dolhem et al.[167] using CuAAC between borane protected chiral azidoethyl phosphines and a variety of alkynes. The performance in catalysis has yet to be demonstrated.

Chiral 1-alkyl propargyl amine analogs of amino acids may be obtained through a Mannich-type reaction between dibenzyl amine, TMS-acetylene, and aldehydes by catalysis

Scheme 20. Chiral Catalytic Preparation of 1-Alkyl Amine Analogs of Amino Acids

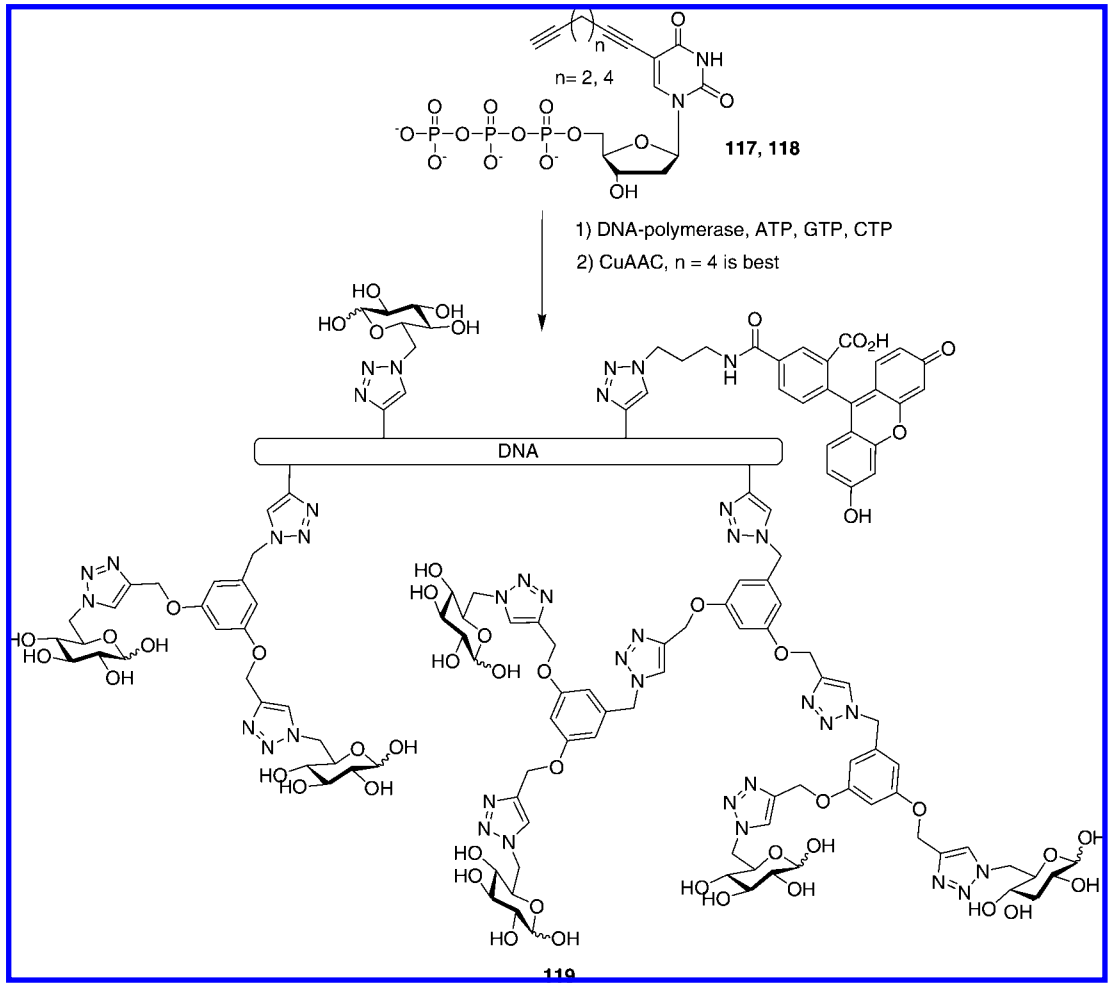


Scheme 21. Recycling of a Rhodium Catalyst Was Achieved through CuAAC Mediated Attachment of an Affinity Tag That Facilitated Isolation by Self Assembly and Precipitation at Ambient Temperature



with CuBr and the chiral ligand (R)-Quinap. After removal of TMS, the alkyne may be reacted with azides in the presence of copper powder to give triazole surrogate amino acid and peptide analogs such as **113**.[289]

Scheme 22. The Selectivity for Thymine Is Quite Promiscuous in the 5 Position and Allows the Incorporation of Alkyne in DNA[a]



[a] These were used for CuAAC with glycan clusters, which themselves were produced by CuAAC.

Gruitjters et al.[293] tagged a Cu(1) binding phenanthroline with the four-hydrogen-bond ureido[1*H*]pyrimidinone affinity tag. This binds to itself in chloroform but not in DMF/MeOH, and by attaching ureido[1*H*]pyrimidinone to a solid support, the catalyst could be fully recovered after completion of a Cu(1) catalyzed triazole synthesis by addition of the resin in chloroform and elution of bound catalyst with polar solvent. In a similar approach, Kim et al.[382] used the triazole ligation reaction to synthesize temperature-sensitive self-aggregating ligands **114** and **115** for a rhodium catalyst that remained in solution during the reaction, giving **116** at elevated temperature, but precipitated due to hydrogen bonding to effect catalyst rescue, when the reaction mixture was cooled to room temperature.

Recently, Meudtner et al.[271] reported the synthesis of 2,6-bistriazolopyridines by $CuSO_4$/ascorbate/TBTA catalyzed CuAAC of a new class of metal coordinating ligands binding to e.g. Fe^{2+} and Eu^{3+} with a great potential for use in catalysis.

The triazole coupling has a huge potential for stitching sensitive molecular entities together and could have a large potential in synthesis of complex catalyst ligands; however, the Cu(1) used in preparation of triazoles may itself coordinate the ligands and be difficult to remove from effective chelators before use.

9.8. Fluorous Triazoles

The rapid conversion and clean reaction observed when alkynes and azides are reacted in the presence of Cu(1) is ideal for the preparation of ^{18}F fluorous PET-scanning reagents, which have to be prepared cleanly within a short period of time to accomodate the fast decay of ^{18}F. This method has been used to ^{18}F label peptides,[97] carbohydrates, and nucleic acids.[142] Using optimized conditions (CuI/ascorbate/DIPEA), it was possible to quantitatively label e.g. Leu-enkephalin containing azide at the *N*-terminal by triazole formation with freshly prepared fluorous alkyne in 10 min.[97] A high concentration of $CuSO_4$/ascorbate and use of DMSO/water mixtures also gave fast 10 min conversions and were found superior for radiolabeling of carbohydrates.[142]

Larger fluorous tags employed in fluorous purification techniques may also be incorporated into molecules by triazole synthesis. If the fluorine atoms are separated from the reactive alkyne and azide by three or more carbon atoms, the inductive effect is negligible and the only necessary precaution is to ensure appropriate solubility of the tag and reagents.[352] If, on the other hand, fluorous azides with only one C−C bond between the fluorine atoms and the azide are used, the inductive effect is significant and influences the reactivity of the azide. In this case, purified CuI and Et_3N were particularly promising, giving reasonable yields with

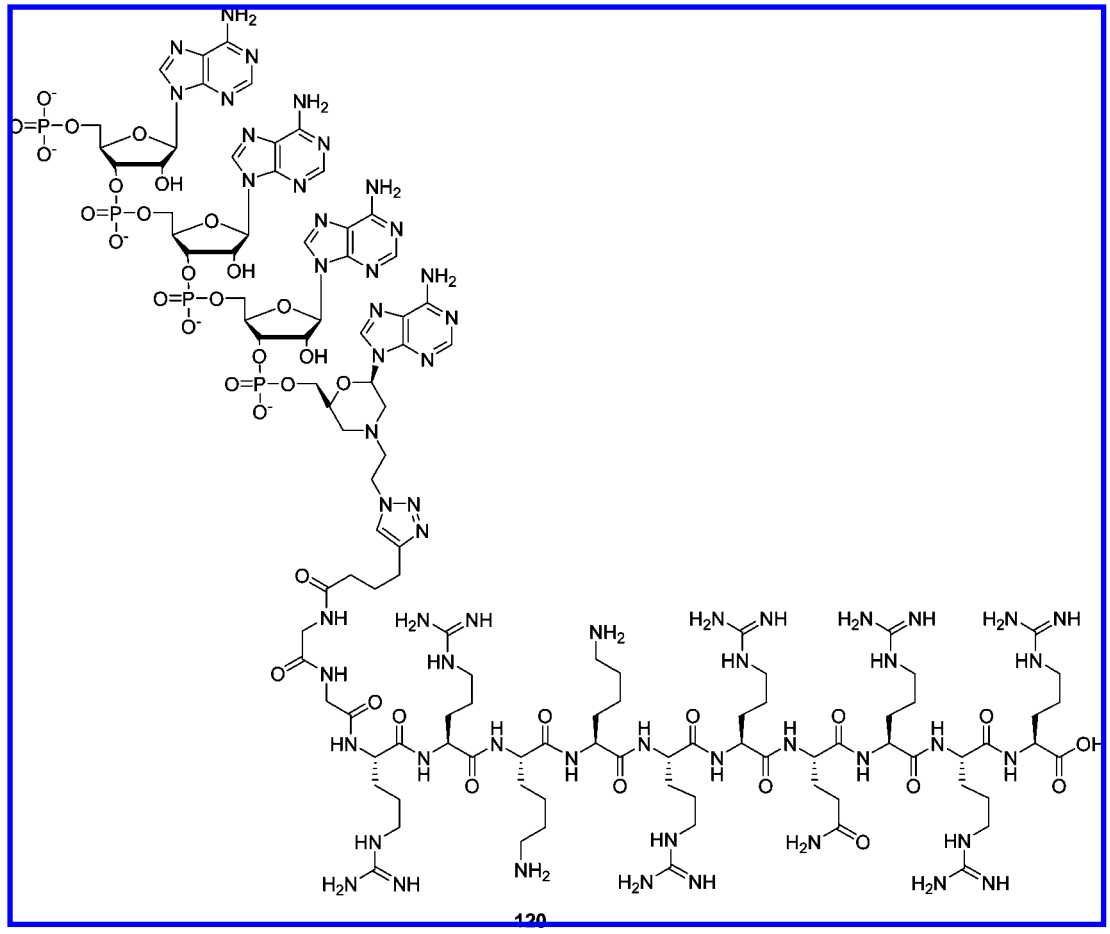


Figure 18. The Rnase L activating RNA tetramer sequence 2−5A was conjugated to the cell penetrating peptide TAT by triazole formation.

a large variety of fluorous azides.[82] CuI in DMSO at elevated temperature was also the preferred conditions for the preparation of triazoles containing organic potassium trifluoroborates.[59]

Zhu et al. prepared fluorine containing polymers by polymerization of 1,2-bis(4-ethynylphenoxy)-perfluorocyclobutane with bis-azido-PEG catalyzed by Cu(1). CuI and $CuSO_4$/ascorbate/triethylamine were both investigated as catalyst, and the latter was found to provide the best yield and purity, as well as the highest molecular weight of the final polymer.[165]

9.9. Modification of DNA and Nucleotides by Triazole Ligation

CuAAC has been used in several different ways to attach functionality to DNA molecules. Importantly, the triazole reaction is fully compatible with completely deprotected DNA and the reaction may be used as a true ligation reaction. The prevalent method used has been that of aqueous $CuSO_4$/ascorbate conditions in order to comply with the requirement of solvation of unprotected DNA.

One of the most important modifications is that of ligation of labels to terminals or internally in DNA, which provides the means to monitor distribution and binding of the DNA intracellularly and to investigate DNA−DNA interaction.[146,267,268,337,383]

Sela and Sirivolu[384,385] and Carell's group[155,320] prepared a range of nucleotides, e.g. **117** and **118**, linked through the bases to terminal alkyne via Sonogashira cross-coupling with the iodonucleoside. These building blocks were incorporated into DNA, and a variety of functionalities were ligated to the DNA, including azidonucleosides (**126**),[385] azidosugars (**119**),[155] and azide containing fluorophores.[320]

In a similar work, Weller and Rajski[353] linked 5′-aminoadenosine to the base of adenosine through a bis-aminoethylene bridge alkylated with a propargyl group. After incorporation into DNA, a pyrenyl fluorophore was ligated to the alkyne using $CuSO_4$, TBTA, and TCEP. A simpler construct using the acetylenebenzylether of glycerol for incorporation into the DNA chain was developed by Géci et al.[383] They attached pyrenylazide under microwave conditions in DMSO/acetate-buffer using $CuSO_4$/ascorbate catalyst to study the interchelation and triple helix stabilization. Similar conditions but at room temperature were employed to label DNA with 3-(perylen-3-yl)propylazide at the 5′-end which had been tagged with arylacetylene.[267]

The phosphoric diester backbone of DNA may be modified using phosphoric diester-amide linkages to propargylamine. This approach allowed Bouillon et al.[243] to decorate the DNA with several azidoalkyl glycosides (**125**) on solid support using $CuSO_4$/ascorbate conditions and microwave heating.

DNA, functionalized at the 5′-end with azide, was coupled thermally with small activated alkynes in the absence of Cu(1); however, the reactions with terminal alkynes were significantly accelerated by addition of CuCl or CuI and base.[95]

The Rnase L activating RNA tetramer sequence called 2-5A, which does not penetrate cells on its own, was conjugated with the membrane penetrating TAT peptide for

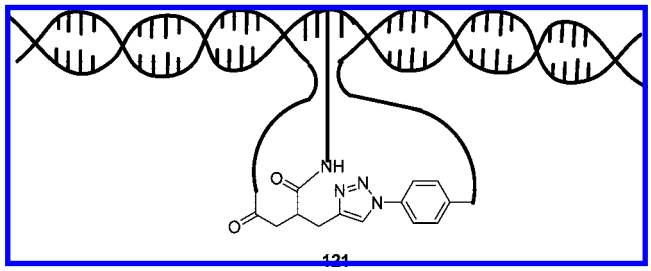


Figure 19. DNA templated CuAAC reaction product.

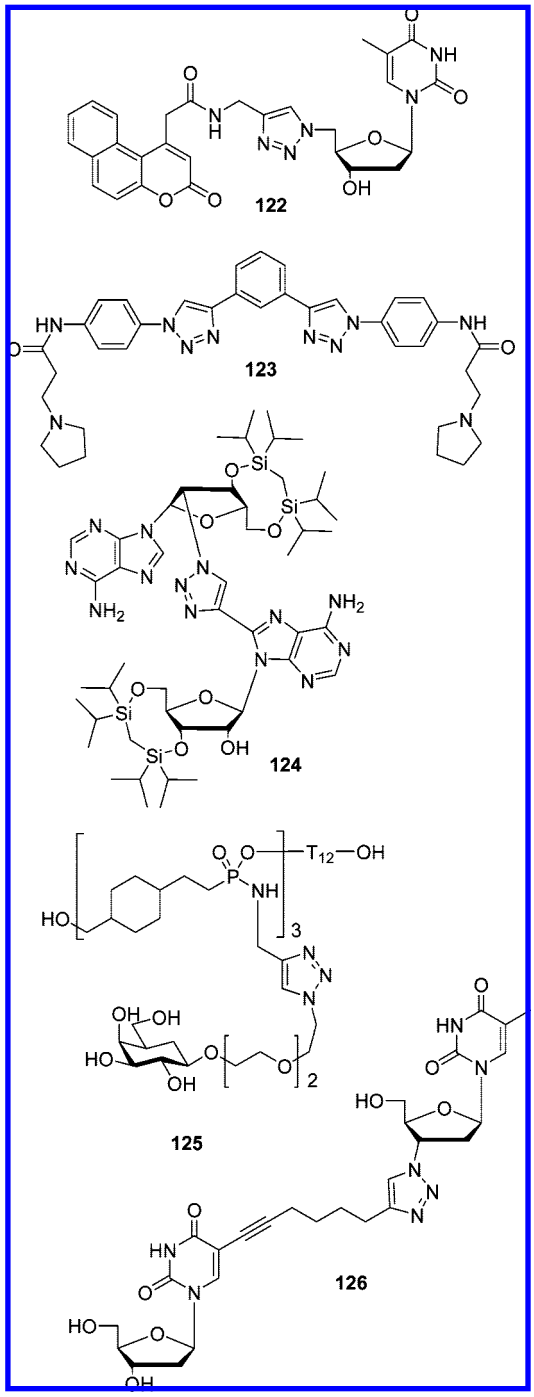


Figure 20. Modification of DNA, RNA, and nucleotides through CuAAC.

import into cells.[170] The conjugation under $CuSO_4$/ascorbate conditions of the unprotected peptide, *N*-terminally acylated with 6-pentynoic acid, with the unprotected 2-5A sequence, periodate oxidized and reductively aminated with amino-propylazide, gave a conjugate **120** that could traverse the membrane and activate the enzyme. Triazole coupling has also been performed as a DNA templated reaction (**121**) at very low concentrations using $CuSO_4$/ascorbate conditions.[136] Nucleotides have been modified with triazole chemistry by Xia et al. to provide antiviral agents as mentioned above.[187]

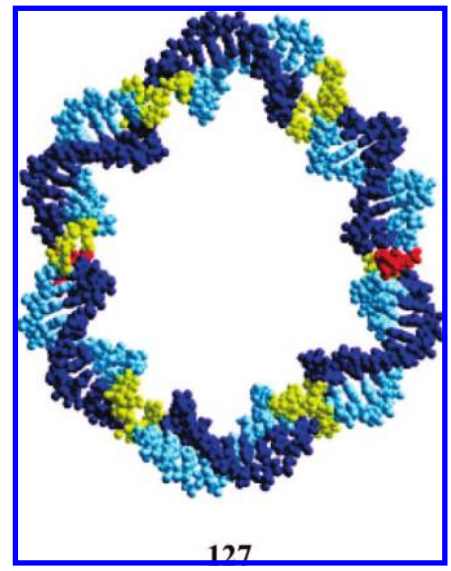


Figure 21. Formation of a double-stranded pseudohexagon from a single stranded cyclic template generated by triazole coupling (red) and linear precursor to entwine and cyclize the second strand with a triazole coupling (red). The single-strands are entwined six times. Unpaired TpT/TpT hinge segments are shown in yellow and double-stranded regions in light and dark blue. Reprinted with permission from ref 269. Copyright 2007 American Chemical Society.

Fluorescent building blocks in which coumarin-propargylamide derivatives were linked to 3′- and 5′-azido nucleosides (**122**) have been synthesized by $CuSO_4$/ascorbate conditions for the fluorescence labeling of the 5′- and 3′-ends of DNA.[146] Diadenosine nucleosides, **124** linked through triazole, were obtained through Cu(1) catalysis of the reaction of 2′-azido- with 8-alkyne-adenosine derivatives using $CuSO_4$/ascorbate conditions.[148] Lin et al.[386] have shown that CuAAC is indeed orthogonal to both nucleic acid triphosphate and boronic acid in preparation of boronic acid containing nucleotides for incorporation into DNA.

Ligands (e.g., **123**) that are interchelating with DNA and stabilize quadruplex DNA have been synthesized via double triazole chemistry, and the formed triazoles were found to be crucial for the interaction.[263] Another way of stabilizing DNA double strands is by cross-linking of the strands. This was achieved by Brown et al.,[268] who developed a 20 and a 24 atom phosphodiester cross-linker formed through CuAAC. The same technique was used to prepare large cyclic double strand DNA-catenanes **127** by cyclization of one strand followed by templated triazole cyclization of the second strand (Figure 21).[269]

9.10. Materials, Calixarenes, Rotaxanes, and Catenanes

Yilmaz et al.[387] described an efficient light harvesting molecule **128** in which a core perylenediimide was linked via triazole coupling to four antennae containing BODIPY. The BODIPY is a fluorophore in itself (ex. 526 nm em. 588 nm), but when linked to the perylenediimide core, its

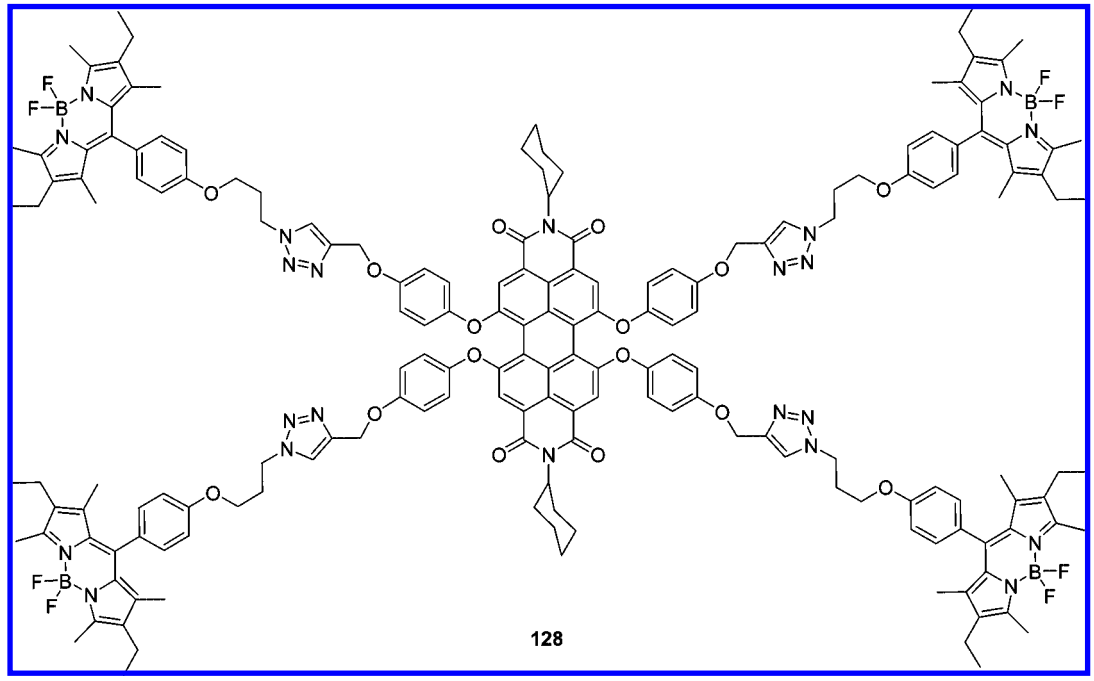


Figure 22. This impressive light harvesting antennae based on resonance energy transfer from the BODIPY branches to the core fluorophore was constructed in good yield using CuAAC.

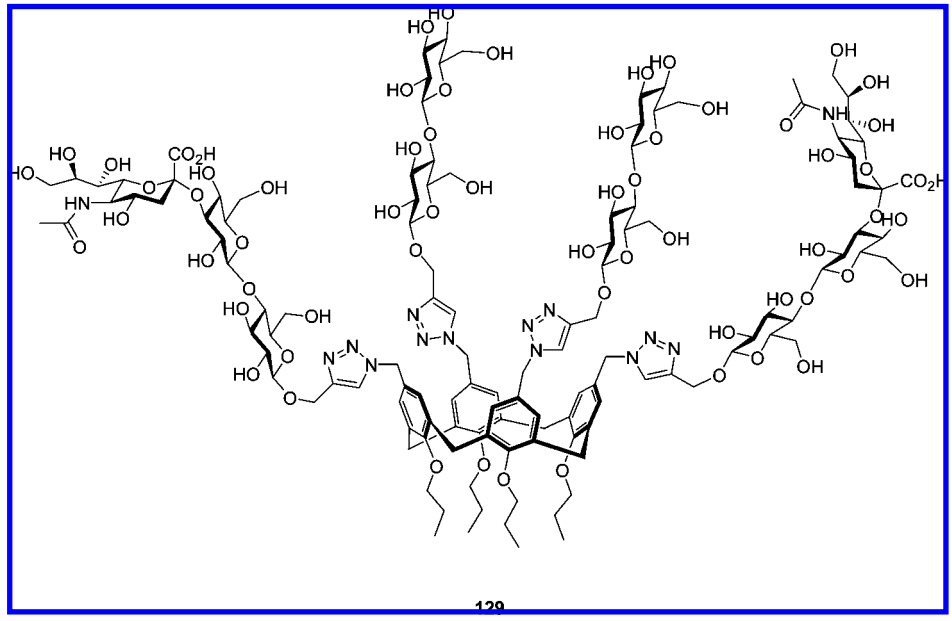


Figure 23. Azide modified calixarene was used in CuAAC to template a glycan cluster that in turn was used as a substrate for sialyl transferase.

fluorescence is completely quenched by energy transfer to the core, which emits at 615 nm.

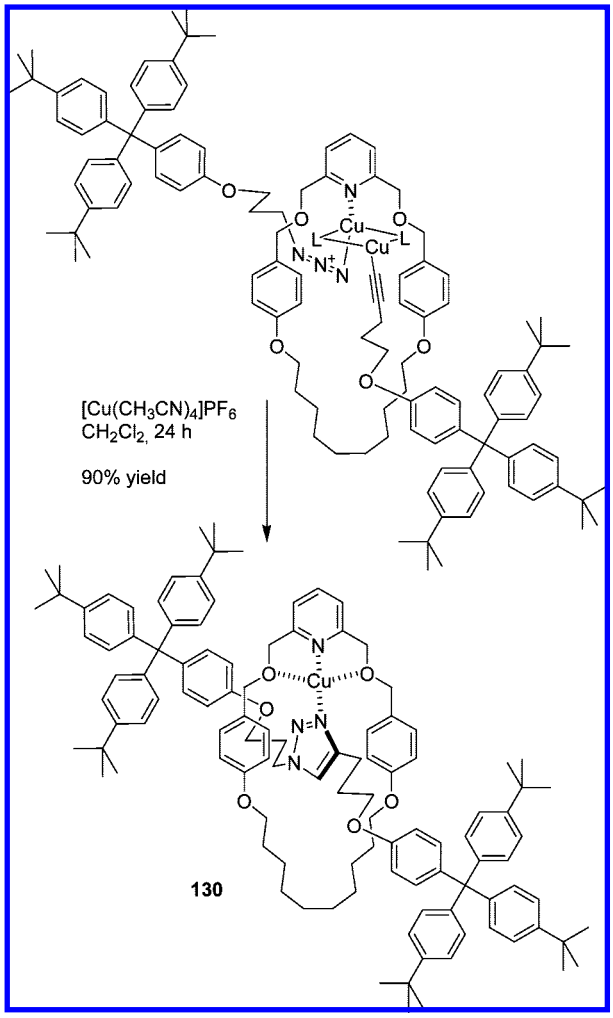
Calixarenes have been used as templates for peptide and carbohydrate clusters (see Figure 23). Bew et al.[199] attached *O*-propargylated Ala-Tyr-OMe and propargyl glycosides to the upper rim of azidomethylated calixarene by triazole couplings and were able to transfer sialic acid to the sugar enzymatically to give **129**.

As explained in Figure 5, the application of propargylated calixarenes is less successful; however, by modifying the oxygens with azido ethyl groups, triazoles readily formed at the lower rim of the calixarene and allowed quantitative incorporation of a range of functionalities.[215]

The conjugation of cryptophane, a cage composed of two tris-ethylene-interlinked cyclotriguaiacylenes and derivatized with a single propargyl group, to a MMP-7 substrate via Cu(1) catalyzed triazole coupling provided a substrate that bound ^{129}Xe in the cage and allowed monitoring substrate cleavage by Xe-NMR.[244]

The triazole formation is ideally suited for assembly of large fragments and even for polymerizations. The reaction is mild and tolerates a large range of conditions. Even supramolecular complexes can be joined with this chemistry, and it has therefore been found to be extremely useful for construction of e.g. rotaxanes.[177,222,255,329,333,336,388] Furthermore, during triazole mediated addition of the stoppers in the assembly of rotaxanes, the Cu(1) catalyst, in addition to coordinating the alkyne and azide in the transition state of the reaction, has frequently been found to assist threading

Scheme 23. The Synthesis of Rotaxanes Has Been Facilitated Greatly through CuAAC That Helps Coordinate the Two Reaction Partners in the Arch of the Macrocycle by Auxiliary Coordination of the Catalytic Cu(1)



through coordination of the catalyst to heteroatoms in the macrocycle (Scheme 23).[222,329,333,336]

This leads to significantly increased yields of functional rotaxanes that may now be obtained in practical amounts (e.g., **130** obtained in 90% yield) for application in material science.[333,336]

The importance of Cu(1) clusters in this reaction is revealed through the fact that, even on very short threads of only 10 atoms, two macrocycles can be assembled on the same thread because the azide and the alkyne are coordinated to separate Cu(1) atoms in the cluster.[336] The coordination of the macrocycle to Cu(1) was demonstrated through kinetic studies in which the reaction in the presence of macrocycle showed a significant 4−5-fold decrease in reaction rate and yet yielded 57% of the threaded product. The product obtained through coupling of bis-azido alkanes with stoppers provided rotaxanes, which were switchable by coordination with metal ions such as Cu(1) or $PdCl_2$ through coordination at the formed triazoles. Other bistable rotaxanes have been reported. A redox active, bistable rotaxane containing TTF and dioxanaphthalene was prepared by CuAAC and investigated for its switching property.[388] A functional pH switchable pseudorotaxane was prepared by Ooya et al.[255] through triazole coupling with Cucurbit(7)uril (CB) and 2,6-di-*O*-methyl-*β*-cyclodextrin (CD) threading on a polymer thread composed of CB/*N*,*N*′-3-phenylenebis(methylene)dipropargylamine and $(N_3)_2$-PPG(400). The position of the CB unit shifted between pH 2 and 11. Similar results were obtained with PEG threaded CD.[177] Dichtel et al.[222] formed a tribranched rotaxane **132** in 62% yield by performing triazole couplings with a core containing three alkynes and the prethreaded stopper-thread-azide building block. Using the same type of chemistry, they also prepared a catenane.[221]

Recently, Miljanić et al.[223] prepared catenanes (e.g., **133**) with donor acceptor properties through CuAAC and through Eglinton coupling of two terminal alkynes. The azide and alkynes were linked via different length oxyethylenes to the naphthalene core situated by complexation in the cyclobis(paraquat-*p*-phenylene) acceptor, and cyclization was effected with CuI (no base) or $CuSO_4$/ascorbic acid. The best yield was 42%; however, the Eglinton coupling of two ethynyl groups in 97% yield was much superior to the triazole formation, probably due to structural differences in the reactive intermediates.

9.11. Dendrimer Architecture Built on Triazole Formation

Assembly of dendrimers through cycloaddition reactions has been reviewed by Voit.[389] Dendrimer structures have been obtained through specific use of CuAAC in a number of conceptually different approaches (Scheme 24), including a sequential approach, a convergent approach, and a divergent approach, which essentially is a combination of the former two.

One of the determining factors in the dendrimer architecture is the design of the core. Putative core structures for use in CuAAC construction of dendrimers, many of which have been used, are presented in Figure 27.

Common for all approaches is that they require quantitative chemical transformations in order to obtain pure dendrimers in sufficient yield. This requirement becomes increasingly important with each generation of dendrimer reaction. This is particularly important in the event of dendrimers decorated on the surface with functionality, e.g. carbohydrate, peptide, electron transfer donor acceptors, catalysts, etc., where a chemistry, different from that used to construct the dendrimer core, is often necessary. The Cu(1) catalyzed reaction between azides and alkynes has the true nature of a ligation reaction and is ideally suited for the construction of dendrimers. Unlike nucleophilic substitutions, where the electrophile is usually subject to competing reactions, the reactants of the triazole ligation can be used in equimolar amounts and even large reactants selectively assemble on the Cu(1) cluster by coordination of the functional groups to facilitate triazole formation. Being orthogonal to most other chemical reaction conditions, the triazole formation can be conducted at any stage of the dendrimer synthesis. The most prevalent applications of the triazole are those of the convergent assembly where the triazoles are formed as a branching point at the core of the dendrimer[113,208,210,212,216,234–237,292] and of the surface modification or decoration.[121,183,188,273,301] Both applications draw upon the favorable quantitative formation of triazole in the former because reaction of two fairly large reaction partners is facilitated and in the latter because complete conversion at many reactive sites is necessary to obtain an acceptable yield and purity. Lee et al. have synthesized both Fréchet type (3,5-dihydroxybenzyl alcohol, Figure

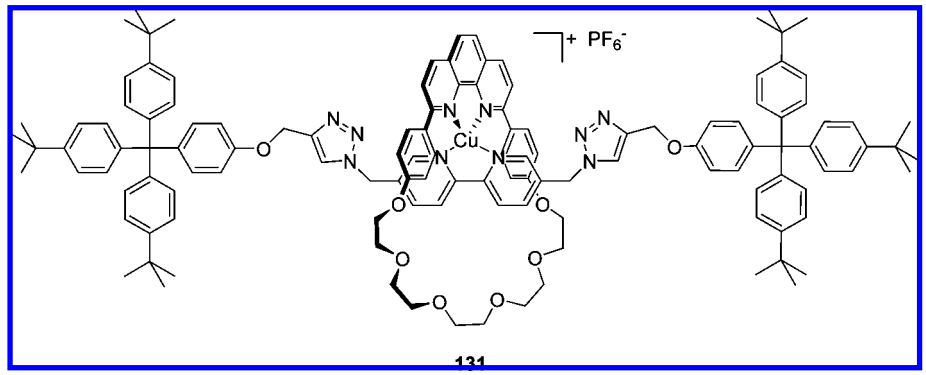


Figure 24. During stopper conjugation through CuAAC the bis-azide was held in place by bipyridine coordination, giving a high yield of roxatane **131**.

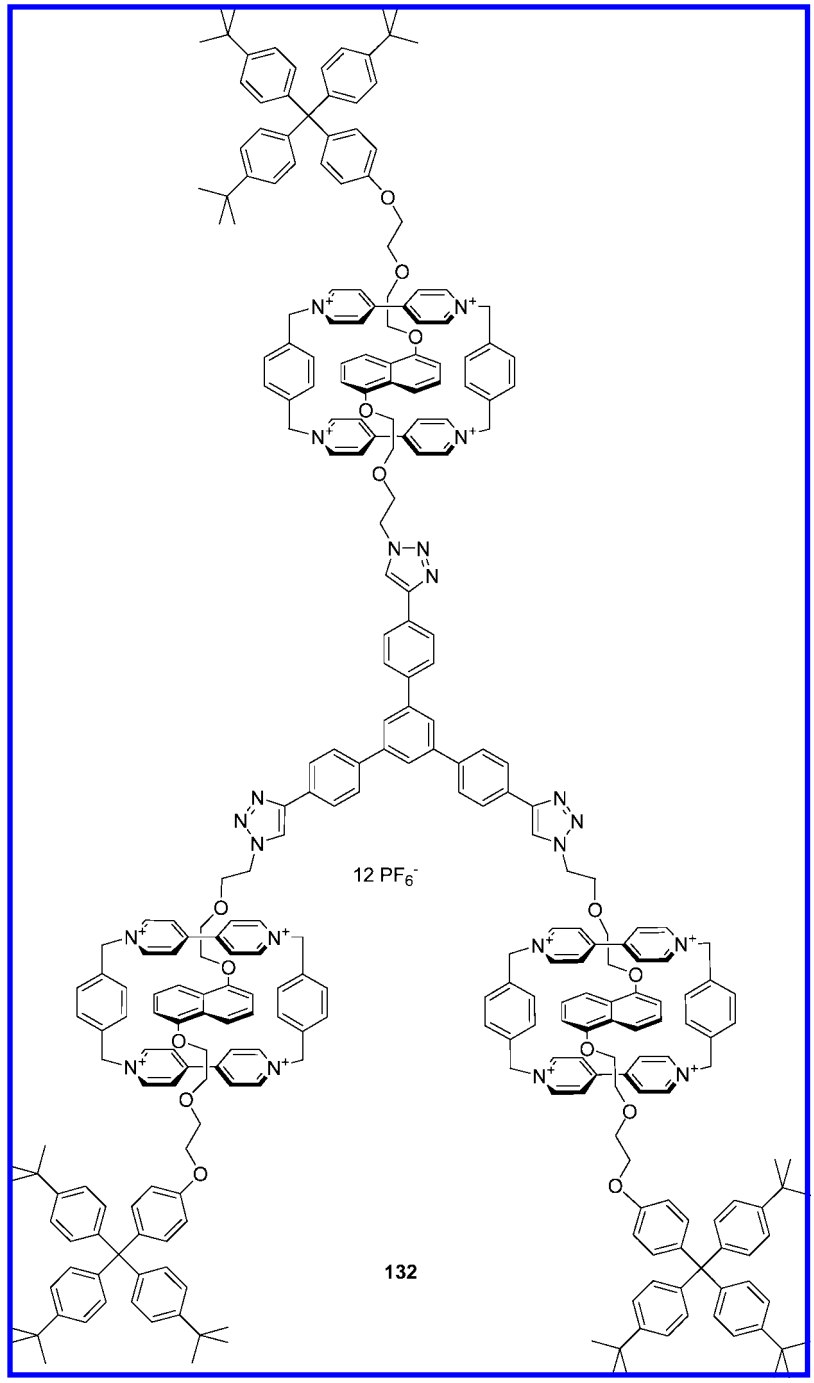


Figure 25. Tripodal rotaxane obtained in 62% yield through CuAAC.

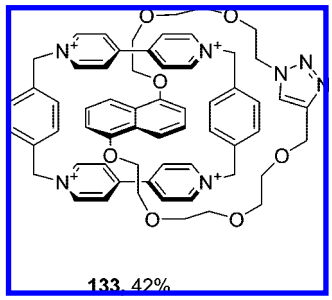
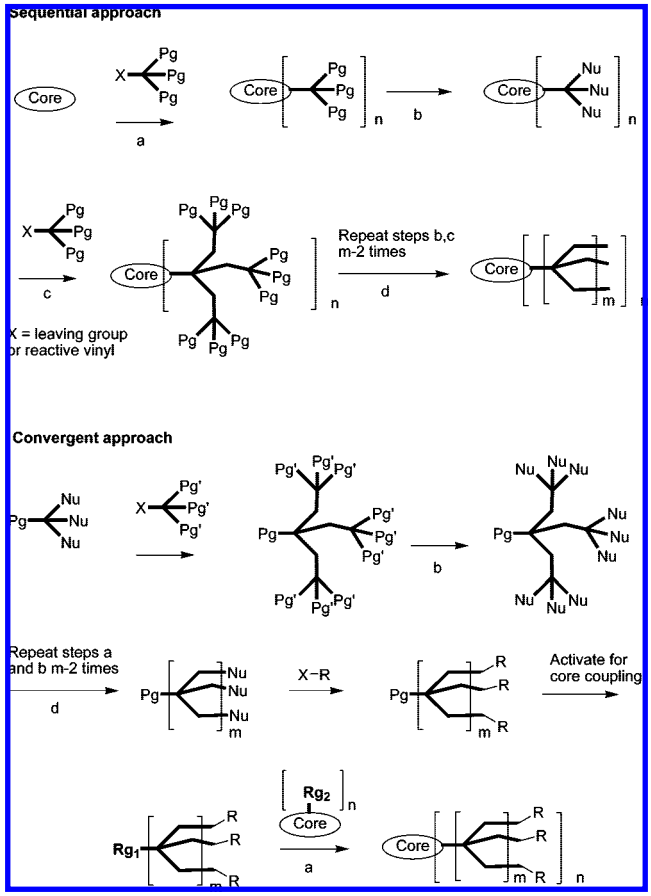


Figure 26. Catananes may be synthesized by CuAAC from precursors similar to those for compound **132**.

Scheme 24. Sequential and Convergent Approaches Used in Dendrimer Synthesis[a]



[a] In principle, the dendrons themselves could also be constructed in a convergent manner.

29) and PAMAM[211,212,216,236] (aliphatic, alternating ethylenediamine and methylacrylate, Figure 30) based[234,235,237] dendrimers by linking together the dendrons terminating in azide or alkyne with di-,[208,216] tri-,[234–236] and tetravalent[216] alkyne or di-[348] and trivalent[237] azide core structures, respectively. The Sharpless conditions have been used with the most success in these coupling reactions, often performed in DMF/water mixtures to enhance fragment solubility.

They also prepared mixed PAMAM/Fréchet diblock dendrimers containing up to four generations in each dendron.[113,210] The couplings were efficient with almost quantitative yields.

In a special construct, Fréchet-type dendrons were coupled by Cu(1) catalyzed triazole formation to a bis(alkyne)diruthenium complex by which the metal center became embedded in the dendrimer environment.[203]

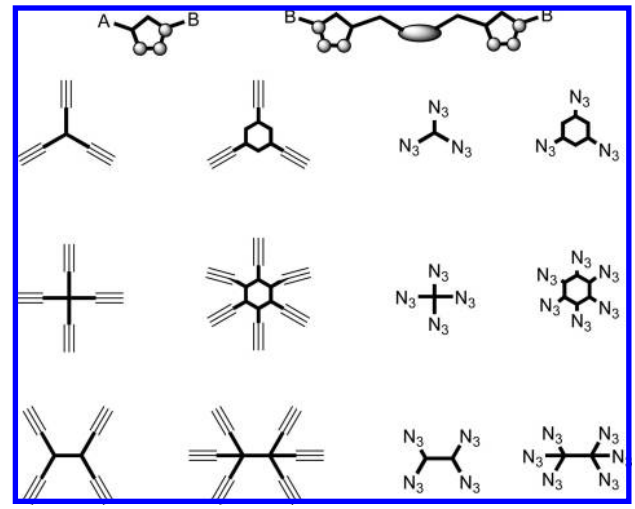


Figure 27. Putative core architectures for CuAAC. Many of these have been used in dendrimers synthesis.

Fréchet et al.[207,213] synthesized mixed dendrimers linked to a polystyrene backbone in which eight Fréchet dendrons were coupled to core dendrimers composed by branching to G3 with 2,2-di(hydroxymethyl)propanoic esters and esterification of terminal hydroxyls with pentynoic acid. The resulting G6 dendronized polystyrene had the expected M_w, thus proving the extreme efficiency of the Cu(1) catalyzed reactions in dendrimer preparation. Wu et al.[292] used a very similar technology to prepare a G4 2,2-di(hydroxymethyl)-propanoic esters dendron and convert both core and shell via two successive triazole couplings. During core modification with a branched fluorescent bis-coumarin tag via triazole formation, the dendron hydroxyls were protected by acetalization, and upon cleavage of the acetals, the surface could be esterified with pentynoic acid and reacted with azidoethyl-α-D-mannopyranose in the presence of $CuSO_4$/ascorbate to form a monodisperse product with a single peak in MS.

Malkoch et al.[301] investigated the CuAAC for decoration of a variety of alkyne dendrimers, including Fréchet-type, polyester-type, and DAB-polyamine dendrimers, with a variety of azide containing functional ligands such as organic residues, fluorophores, nucleosides, sugars, and Fréchet-type dendrons. According to MS, chromatographic separation, and NMR-spectroscopy, high yields and purities could be obtained, again demonstrating the efficiency of the reaction. In another approach, Fernandez-Megia et al. inverted the reaction and decorated flexible PEG dendrimers containing surface azido groups to which alkyne-glycosides were attached.[183] Rijkers et al.[188] attached a variety of peptidic structures such as azide derivatives of RGD- and Leu-enkephalin-peptides to alkynes on the surface of a dendrimer based on 3,5-bis(aminoethyloxy)benzamide branching. PEG-azide was coupled to alkynes on a dendrimer prodrug for selective delivery of an insoluble antitumor agent, in order to solvate the dendrimer and effect hydrolysis of the terminally attached camptothecin with penicillin G amidase.[273]

Catalytic dendrimers based on a hexavalent core of triphosphazin and branching with dichloro thiophosphoroushydrazone of 4-hydroxybenzaldehyde were terminated by reaction with 4-hydroxybenzyl azide and were then very efficiently decorated on the surface with propargylated azabis(oxazolines) using CuAAC.[121] The dendrimers were chelated with $CuCl_2$, and the second generation dendrimer showed very high enantioselectivities in chiral benzoylation

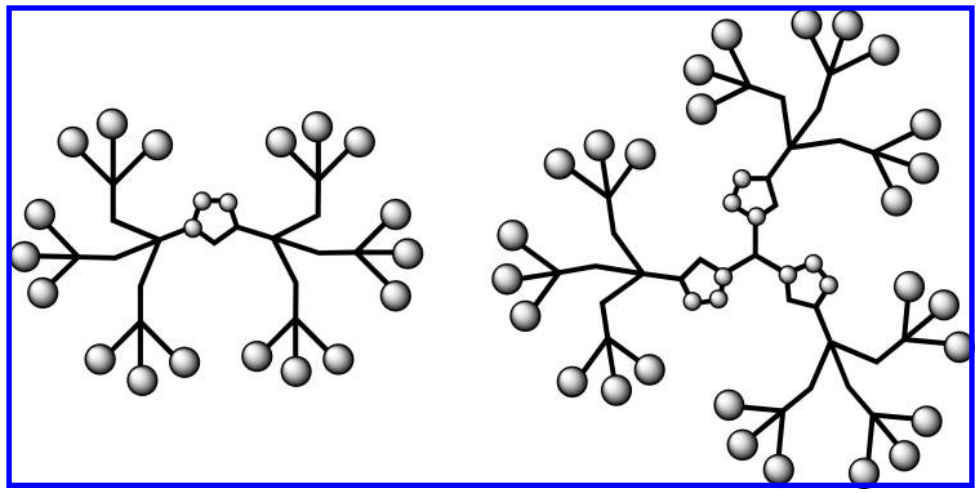


Figure 28. Dendron ligation by CuAAC. Here the CuAAC reaction is used to assemble preconstructed dendrons terminating in azide and/or alkyne. This permits ligation of two different dendrons or the assembly on a branched core.

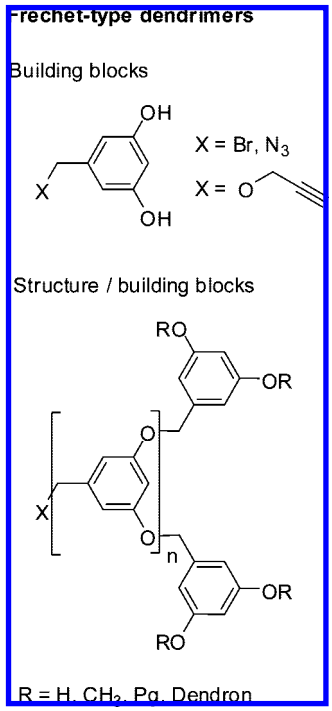


Figure 29. The Fréchet-type of dendrimer structure.

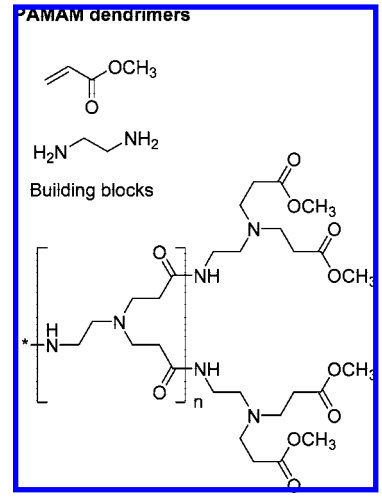


Figure 30. The PAMAM dendrimer structure.

of diols. The extreme efficiency of the triazole coupling was challenged by Wu et al.[161] and by Joralemon et al.,[158] who, by convergent and divergent approaches, respectively, prepared dendrimers exclusively by formation of triazoles as the branching reaction. In the convergent strategy, dendrons were constructed with building blocks containing a chloromethyl group and two alkyne groups. In the coupling cycle, the chloride of the growing dendron was substituted with azide and coupled with 1/2 equiv of the parent building block. The synthesis was efficient up to 4G dendrons, and these were attached to bis- and tris-alkyne cores. According to GPC-MS, highly pure dendrimers of M_w of up to 6345 were obtained. In the divergent approach,[158] 3,5-bis(hydroxymethyl)-1-prop-2-ynoxybenzene was employed for building the dendrimer from a bis(azidoethyl)glycol core. Between coupling steps, the hydroxyl groups were converted to chloride and substituted with azide. Pure G3 dendrimers were obtained.

Recently, Ornelas et al.[214] reported the incredible triazole based synthesis (Scheme 25) of dendrimer **134** with 81 ferrocene functions installed.

9.12. Carbohydrate Clusters and Carbohydrate Conjugation by Cu(1) Catalyzed Triazole Ligation Reactions

Carbohydrates are a prime class of signaling molecules in nature, and this function is highly dependent on the ability of sugars to form well defined clusters or antennary topology. For this reason, synthetic carbohydrate mimetics in which sugars are presented in a well-defined manner on a molecular template have been an important contribution to glycobiology. In this regard, the CuAAC has proven particularly useful. Not only is it quantitative and orthogonal to carbohydrate reactions, the structures obtained contain the fairly rigid 1,4-substituted triazole moiety, well suited to represent the structural mimic of a sugar ring. The carbohydrate ligations may be divided into five categories: modification of sugars with organic entities, formation of clustered

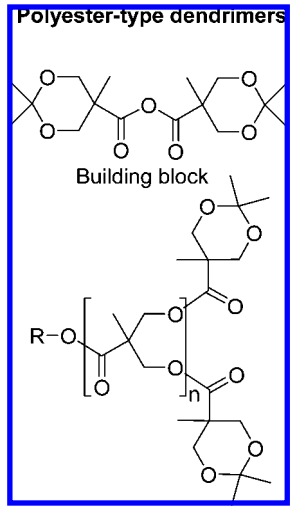


Figure 31. The polyester-type of dendrimer structure synthesized from the acetonide of 2,2-di(hydroxymethyl)propanoic ester.

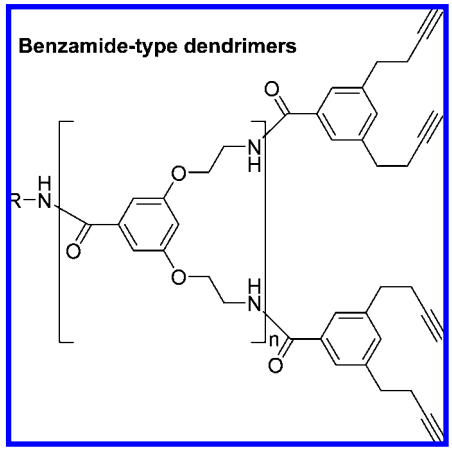


Figure 32. The benzamide-type of dendrimer structure.

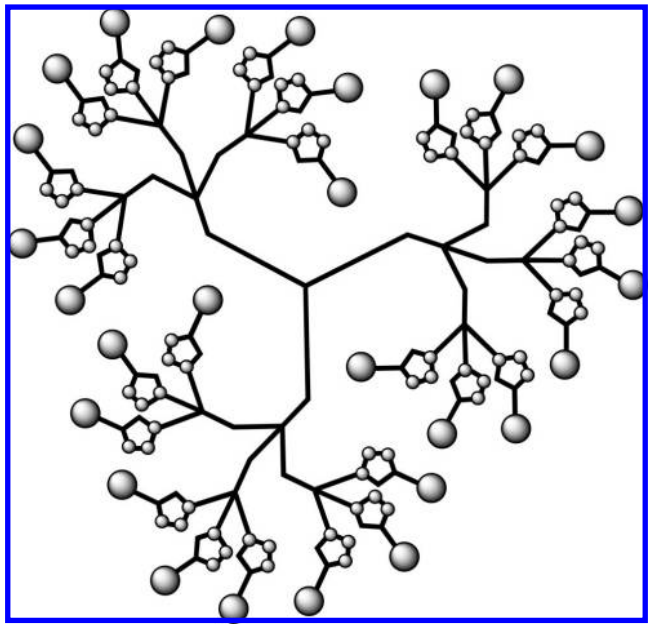


Figure 33. Dendrimer surface decoration by CuAAC.

molecules, ligation of sugars with other biologically functional molecules, glycopeptide mimicry, and oligosaccharide mimicry. The prevalent method for linking sugars with organic molecules has been to introduce an azide in the 1-, 2-, or 6-position of the sugar and react this with an array of organic alkynes,[248,251,253,277,345] although the inverse approach in which a propargyl glycoside was coupled with 4-azidophenylsulfonamide has also been employed.[254] The products of the latter were excellent and selective inhibitors of carbonic anhydrase. Carbonic anhydrase was also targeted with a variety of 1-azido sugars, linked to *N*-propargyl amidosulfonylbenzamide by the $CuSO_4$/ascorbate conditions.[253] Similar coupling conditions using organic alkynes and glycosyl azides gave e.g. steroidal glycosyltriazoles.[251]

Upon substitution of cellulose polymer in the 6-position with azide at a degree of substitution of 60−88%, it was possible to modify the overall polymer properties through triazole formation with a range of alkynes.[245,248,326] Ermolatév et al.[277] synthesized a series of glycosyltriazol-4-ylpyrazinones using microwave heating and Cu(0)/Cu(2) catalysis. Acyclic phosphonic acid analogs of the antiviral agent ribavirin were prepared through coupling of sugar derived dibenzyl 4-azido-1,2,3-trihydroxy-2,3-*O*-isopropylidenebutylphosphonates with methyl propiolate, which upon clean triazole formation was converted into the amide.[345]

In the formation of glycosyl clusters, the azide has also primarily been attached to the carbohydrate. Glycosylazides are easy to produce; however, it has been recognized that the direct attachment of the triazole to the carbohydrate[53,266,347,349] may interfere with the recognition of the sugar by receptors, and therefore, shorter[66] or longer[87,169] alkyl-spacers have been inserted between the triazole formed at the core of the cluster and the sugar.

Bearing this in mind, it becomes equally easy to prepare the propargyl glucosides[204,341] or even 4-pentynyl or 5-hexynyl glycosides[334] and couple these to azide containing core molecules (in e.g. **135**). Following the latter strategy, a large library of differently spaced glycans on 35 different bivalent core molecules was prepared and screened for specific interaction with DNA hairpin loops.[334] The increased reactivity of CuI catalysis over that of CuBr was demonstrated in a study where seven triazoles were formed on the rim of β-cyclodextrin. Use of microwave and $(EtO)_3P$:CuI or Ph_3P:CuBr with addition of CuI both in the presence of DIPEA greatly enhanced the reaction rate to give a near to quantitative yield of the heptavalent carbohydrate cluster.[341] Mannopyranose clusters were prepared on a swastika type of core structures (e.g., **137**) with or without PEG spacing and with triazole directed both 1,4- (azide on the core) and 4,1- (azide on the sugar) from the core.[204] Initial bioassays inhibiting the agglutination of baker's yeast with *E. coli*-x7122 indicate greatly enhanced cluster effects of these compounds.

Similarly, a 400-fold enhancement of binding to a plant lectin, RCA_{120}, was achieved with a tetravalent cluster obtained through triazole coupling of a methyl tetra-*O*-propargyl-β-D-galactopyranose core with the azidoethyl β-lactoside.[169] Tejler et al.[87] also used this glycoside to couple to a range of mono-, di-, and trivalent propiolic amide core structures. Yields of the triazole couplings were less optimal, possibly due to effects such as those described in Figure 5. Divalent clusters showed selectivity and increased potency as inhibitors for galectin-1 compared to methyl lactoside; however, when compared to appropriate reference compounds, the cluster effect was not very pronounced. Selective galectin-3 and 9N inhibitors were synthesized from β-D-mannopyranosyl azide by CuI/DIPEA catalyzed reaction

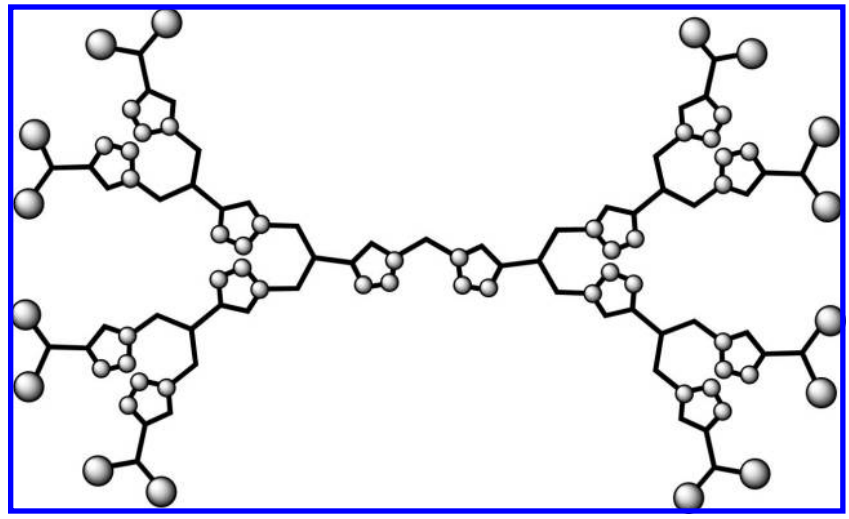


Figure 34. Dendrimer branching by CuAAC.

with propiolic esters and amides. The mannopyranose structurally mimics the galactose binding with the axial OH-2 in the same position of the binding site as the axial OH-4 in galactose.[73]

Dondoni et al.[66,72] prepared a panel of *C*-linked glucopyranose and galactopyranose clusters (e.g., **136** and **138**) using different types of aromatic, calixarene, and adamantane based core molecules to provide a flat, directed, and globular distribution of the sugars in space. Both glycosyl acetylenes and azidomethyl *C*-glycosides were used to couple to the azide and alkyne containing core structures, respectively. Attachment of the sugar via other ring atoms such as the 3-[53] or 4-position[347,349] has also been achieved (e.g., in **140**).

Chittaboina et al.[266] have described a one-pot conversion of glycosylhalides into glycosyltriazole clusters in high yield via glycosylazide generated under aqueous phase transfer conditions in the presence of $CuSO_4$/ascorbate. *N*,*N*,*N*′,*N*′-Tetrapropargyl ethylenediamine, tetrakis(propargyloxy)-methane, and 1,3,5-tri(propargyloxy)benzene (**141**) were used as core structures. One of the most significant applications of CuAAC is that of connecting a functional probe with a molecule that carries a particular property, e.g., for detection. As described above,[245,248,326] ligation to azide modified polysaccharides leads to new polysaccharides with special properties, including saccharide branching (e.g., **144**), attachment of fluorophores, changed solubility and charge, and electrochemical and charge transfer properties.[325] Hepta-(6-azido)-β-cyclodextrin (and methyl 6-azido-α-D-glycopyranoside) was reacted in high yield by CuAAC at all seven azides with 2-ethynylpyridine using only Cu(0) as catalyst for 7 days. The products had interesting fluorescence properties and acted as sensor molecules.

However, the CuAAC reaction really becomes useful when selective labeling of a probe in a biological environment at extremely low concentration is required. Ballell et al.[198,274] described a galectin-3 selective photoactive bivalent lactose probe (**148**) including a PEG-azide spacer that was used to label the protein in cells. The lysate with the immobilized probe was reacted with azido-derivatized rhodamine (**149**) to give a clean detection of galectin 3 at low levels of protein. In a very elegant process, Yamaguchi et al.[261] transferred the sugar part of proteoglycan enzymatically onto propargyl alcohol. The large sugar structure was ligated onto e.g. BSA that had been modified on lysine with 4−6 azidobenzamide residues to form a neoproteoglycan. Strangely, the reaction was performed in the absence of reducing agents and the yield seems to be very high, so it is likely that adventurous reducing agent has been present in the glycan preparation.

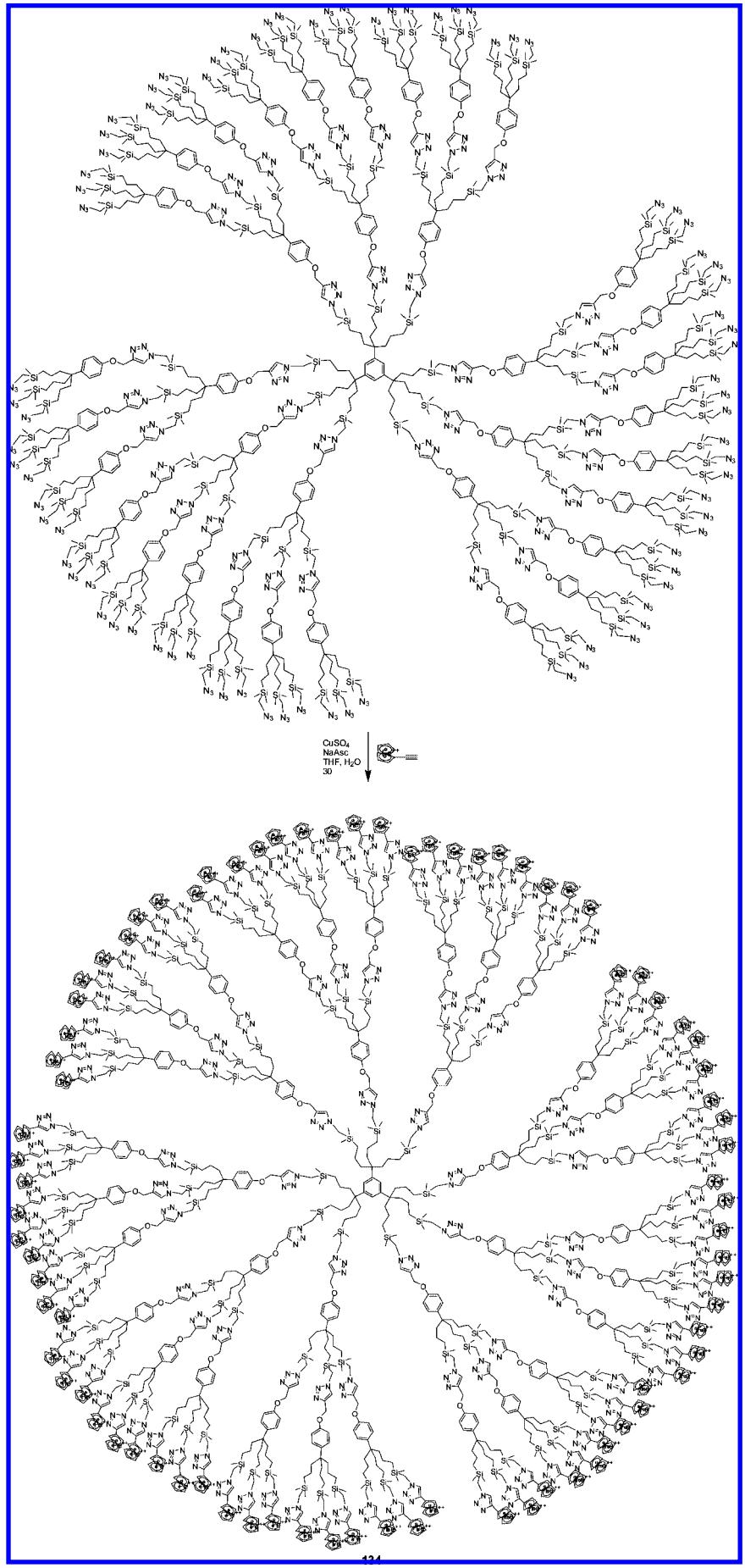
Triazole ligation has also been used to link carbohydrate functionality to different carbohydrates (**142**, **143**, **145**)[202] and amino acids (**147**)[98] and to ferrocene (**139**), which significantly modified the cyclic voltammetry.[342]

An important type of ligation is that of immobilizing complex biological molecules to a support or a surface. Propargylic maltose, glucose, and cyclodextrin were immobilized on azidopropyl-functionalized silica using $CuSO_4$/ascorbate to provide chiral columns with excellent separation profiles for nucleotides and sugars.[217] It was also possible to ligate complex sugars to lipids (**146**) for immobilization in titer wells for a glycosyl transferase assay[123] and in liposomes for studying carbohydrate protein interaction.[51]

Glycopeptides are a particularly interesting class of naturally occurring compounds consisting of two important types of biomaterial that are connected through a glycosidic bond. *N*-Propargyl-bromoacetamide could be coupled with chitobiosyl azides in the presence of Cu(1) to yield a precursor for attachment as thioethers to cysteine containing peptides. This was combined with native chemical ligation to yield a 32 amino acid neoglycopeptide from EPO.[258] In an impressive demonstration of the triazole coupling, Wan et al. [286] linked azide containing complex saccharides and even glycopeptides to a central peptide carrier in which lysine residues had been acylated with 4-pentynoic acid. Similarly, the macrocyclic antibiotic peptide, tyrocidine, containing alkyne substitutions, was ligated with a library of azidosugars and even small, branched triazole dendrons carrying modified sugars could be attached.[174] Kuijpers et al.[138] prepared an array of *C*- and *N*-linked glycosyltriazole amino acids and peptides in intermediate yields using $Cu(OAc)_2$/ascorbate as a catalyst.

One particular use of the triazole in carbohydrate synthesis is that of linking sugars together repetitively to mimic complex glycans (e.g., **145**).[70,240,241,294,298] A 1,6-linked mimetic structure of penta-mannopyranose was prepared by reaction of 2,3,4-tri-*O*-benzyl α-D-mannopyranosylacetylene with a *C*-glycoside derivative of 6-azido 2,3,4-*O*-benzyl mannopyranose, and after conversion of the free 6-hydroxyl

Scheme 25. Ultimate Triazole Based Dendrimer, Where the Core Attachment, Branching, and Installment of 81 Ferrocene Moieties Was Performed by CuAAC



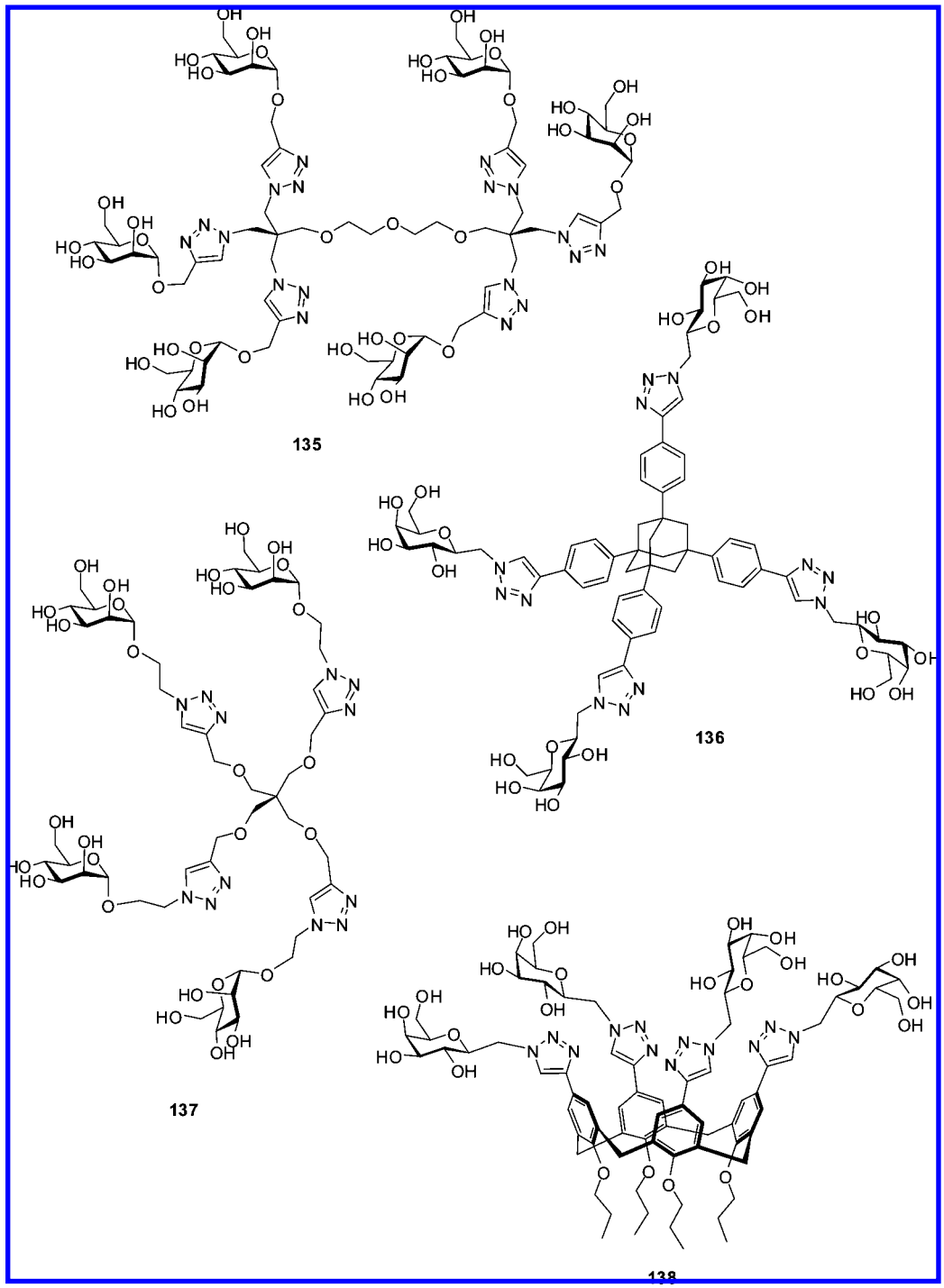


Figure 35. Flexible (**135**, **137**) and rigid (**136**, **138**) carbohydrate clusters obtained through multiple triazoles formed by CuAAC.

in the product to azide using DPPA, the triazole reaction was repeated. This cycle was carried out to form four consecutive triazole linkages to give **142**.[70] In a similar approach, the neooligosaccharide was build from the nonreducing end. Here, the azide was generated at the anomeric center and coupled with 6-*N*-propargylamide of the next glucuronic acid tetra acetate.[241,294]

Acetal protected mannofuranosyl azides have also been conjugated via triazoles with 3,5-propargylated arabinofuranose to form a neo-furanosyltrisaccharide **143**.[298]

Triazoles may conveniently be used for introduction of branching of linear fragments of polysaccharides (**144**).[303] Thus, large neosaccharides **145** mimicking starch fragments were synthesized by ligation of peracetyl oligomaltosyl-azides onto 4′,6′- and 6,6′-propargylated para-methoxyphenyl penta-*O*-benzyl-*β*-maltoside using $(Ph_3P:)_3CuBr$/DIPEA.[240]

9.13. Polymers and CuAAC

9.13.1. Cu(1) Catalyzed Triazole Formation in Polymer Chemistry

Application of CuAAC in polymers may be divided into three major categories: the use for derivatization and functionalization of linear and branched polymers, the preparation

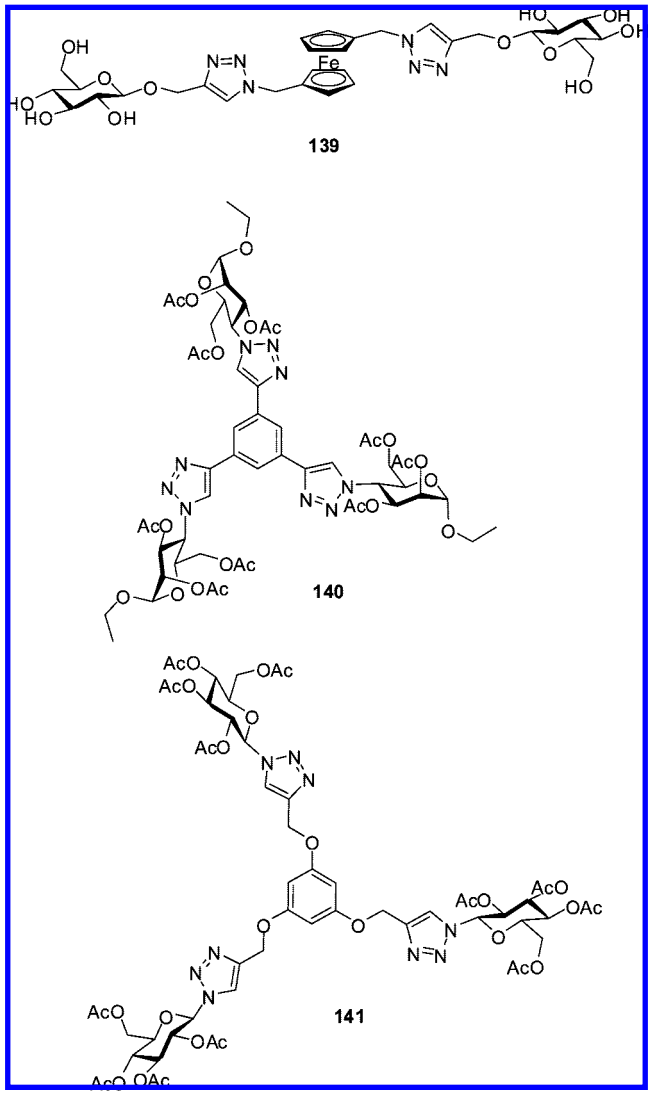


Figure 36. Carbohydrate clusters based on an aromatic core structure.

of linear polymers based on triazole formation, and the formation of triazole based solid supports. Particularly, the second category imposes strict requirements for a quantitative chemical transformation in order to obtain a high degree of polymerization and a useful distribution of size. Polymerization reactions have predominantly been taking advantage of the CuBr/PMDETA catalyst system with rigorous removal of oxygen, a key to success in these reactions. This may relate to the same catalyst often being used in living ATRP (or NMRP) polymerizations rather than to the efficiency of the catalyst.[85] Frequently, the CuBr has been protected by use in the form of $(Ph_3P:)_3CuBr$.

The application of triazoles for functionalization of polymers has been quite popular. When performing atom transfer radical polymerization, the terminal of the polymer is terminated with a leaving group, e.g. Br, which can easily be substituted with azide. The azide terminated polymers have been used to add functionality to polymers by CuAAC using e.g. CuI, CuBr, or $(PPh_3:)_3CuBr$ without ligand or with ligands such as PMDETA, **11**, or BiPy, **12**.[63,312,315,316,390] Linear block copolymers may be obtained by use of TMS-propargyl α-bromoisobutyrate as an initiator for the ATRP followed by triazole ligation with the monoazide macromonomers as described above.[89] The TMS group may be omitted in some cases as described by van Camp et al.,[391] who employed the propargylated polymer directly for CuAAC synthesis of both graft and diblock copolymers. Hasneen et al.[392] studied the rate of reaction for copolymer formation through CuAAC ligation of azidopolyacrylate and propargylated-polystyrene and found that under bipy(**12**)/CuBr conditions the reaction was almost quantitative in 24 h. Durmaz et al.[393] used the orthogonal Diels−Alder and CuAAC ligation reactions in "one pot" under CuBr/PMDETA conditions to prepare a triblock copolymer comprising PMA, PS, and PEG. The central block was PS containing anthracene at the initiator end and azide at the termination end. Liu et al.[394] employed bis(propargylaminocarbonyl)PEG and 2,2-bis(azidomethyl)propane-1,3-diol in a synthesis of long PEG polymers. By prederivatization of the diol with complex functional groups, a functionalization at all ligation sites of the final polymer was achieved. The CuAAC ligation has also been used to ligate different inorganic polyoxotungstate clusters in water.[395] Starting from a tris-functional initiator containing α-bromoisobutyrate, phenylethyl α-nitroxide, and a propargyl branch, Altintas et al.[313] managed to synthesize a well defined star copolymer **151** containing PEG, polystyrene, and poly(methyl methacrylate) by orthogonal activation for ATRP of MMA, then NMP of styrene, and finally triazole ligation of PEG-monoazide. Another trifunctional initiator for star polymer synthesis comprising PEG, PS, and PCL was synthesized by Deng et al.[448] Gao and Matyjaszewski[396] combined star polymer synthesis with block copolymer synthesis by polymerizing styrene onto a tris-bromoisobutyrate core, substituting bromine with azide, and "clicking" PEG-pentynoate on one, two, or three arms using CuAAC. Lutz et al. functionalized PEG-polyacrylate monoazide terminally with the RGD-peptide 4-pentynoyl-GGRGDG-$NH(CH_2)_2)NH_2$.[315] The same reaction was used to produce macromonomers from polymethacrylates and polystyrenes by terminal cycloaddition of propargyl methacrylate.[312] Polymerization of this kind of macromonomer provided large comb-type block copolymers containing PEG, polystyrene, and polymethacrylates.[314]

Polymerization from a central dimethyl 2,6-dibromoheptanedioate as an initiator allowed Gao et al. to prepare a polystyrene that carried bromine at both ends. After conversion to bisazide, the polymer was reacted with propargylalcohol.[316] Polystyrene only containing azide at one terminal was used to modify propargylated nanotubes by CuAAC.[63]

Binder et al.[330] modified a nitroxide initiator by triazole coupling prior to use in controlled radical polymerization of *N*-isopropyl acrylamide (NIPAM) to provide PNIPAM that could be grafted onto Fe_2O_3 nanoparticles via the terminal functional diol. Initiators containing the azide may also be used postpolymerization in triazole ligation.[319] Fleischmann et al.[397] have described the synthesis of a range of useful NMP initiators for polystyrene that is terminally modified with alkyne or azide. A special case of functionalization of the monoazide polymers is that of installing a tri- to hexaalkyne core which quantitatively forms the triazole in the presence of Cu(1) and provides star type polymers.[311,318] This can be further extended by grafting the monoazides to a polymer containing randomly distributed alkyne substituents to form brush type polymers.[84,179,304,312] Another application is the general modification of the polymer properties by modification at multiple random sites throughout the polymer chain.[139,296,302,317,324,398,399] Methacrylic

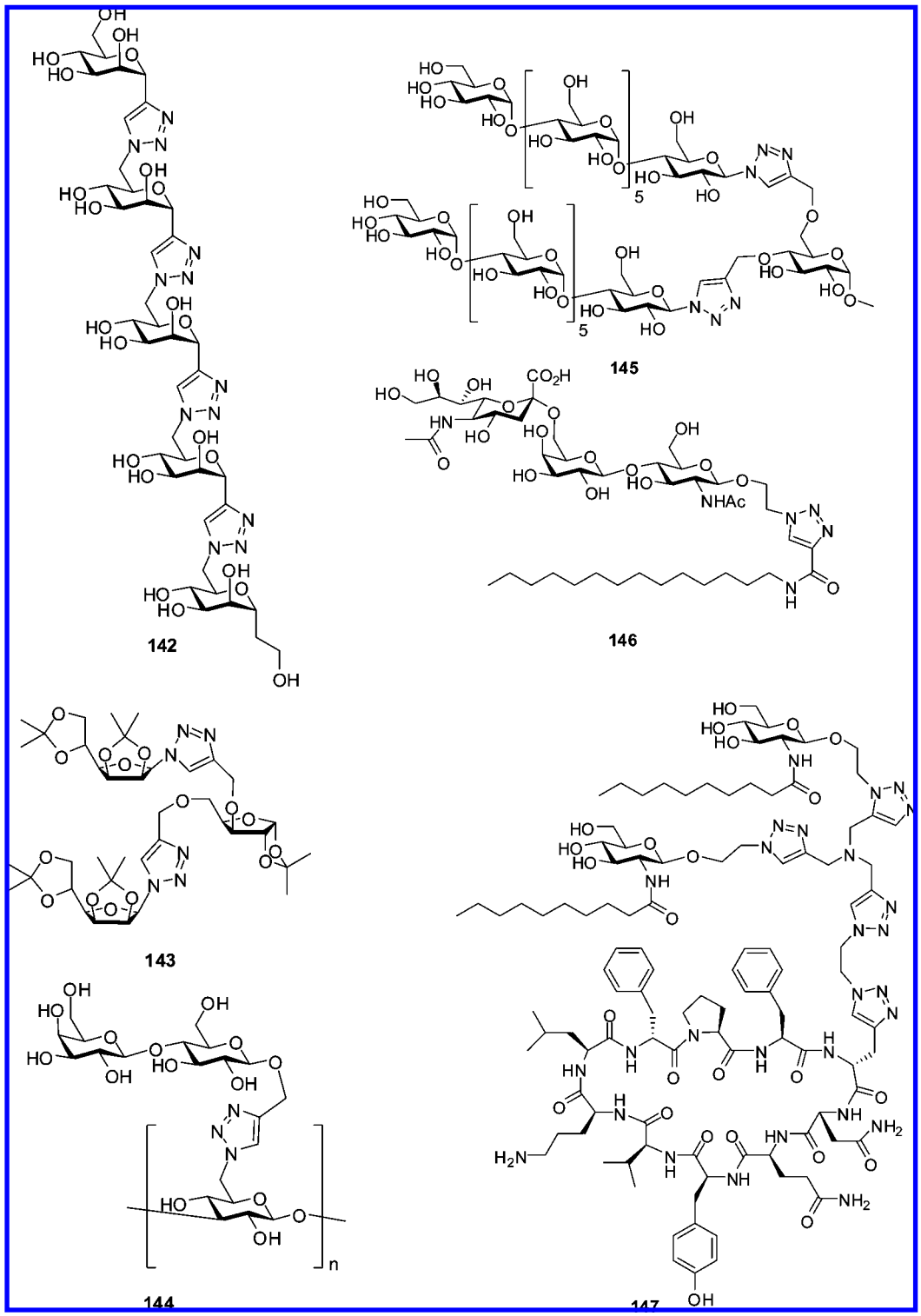


Figure 37. Ways to interconnect sugar residues via CuAAC to form mimetics of oligosaccharides, glycolipids, and glycopeptides.

esters of azidoalcohols may be polymerized and postfunctionalized with a variety of alkynes, e.g. propargyltriphenyl phosphonium bromide, to yield reactive polymers with a high loading of functional groups.[317] Propargyl methacrylates were synthesized by Quémener et al.[400] with and without TMS protection of the alkyne, and these were used to prepare polypropargyl methacrylates for PVA-grafted comb polymer synthesis. A different method was used by Gao and Matyjaszewski,[401] who polymerized hydroxyethyl methacrylate and postpolymerization acylated the polymer at the hydroxyl groups with 4-pentynoic acid using DCC and DMAP. This was followed by grafting of PEG-N_3 in 3 h using CuBr/PMDETA.

In another approach, poly(4-propargyloxystyrene) was reacted with azide containing aliphatic and aromatic diacids to yield a highly acidic polymer.[324] A similarly propargylated polystyrene was also derivatized through triazole cycloaddition with probes that should facilitate lithographic printing on gold surfaces.[296]

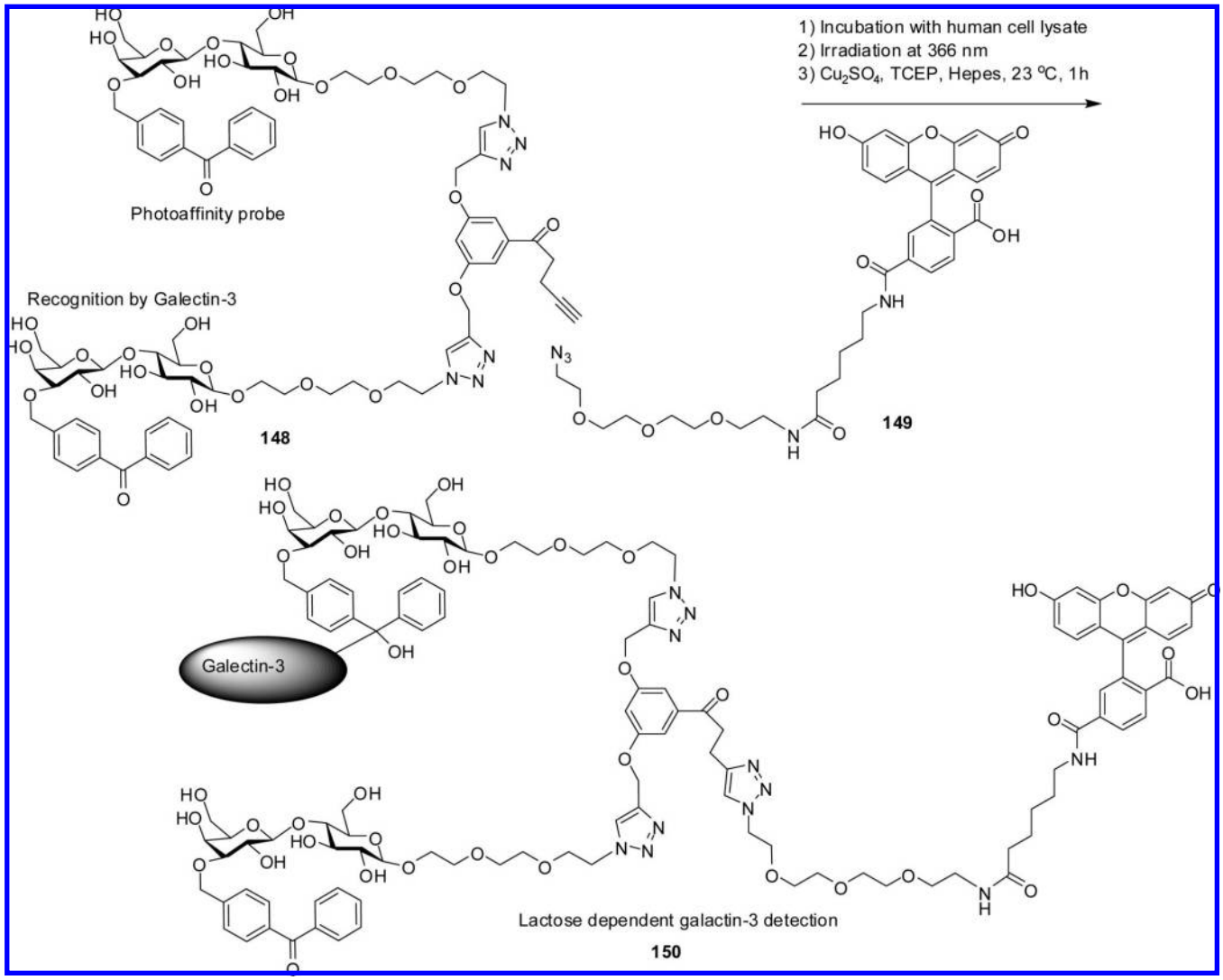


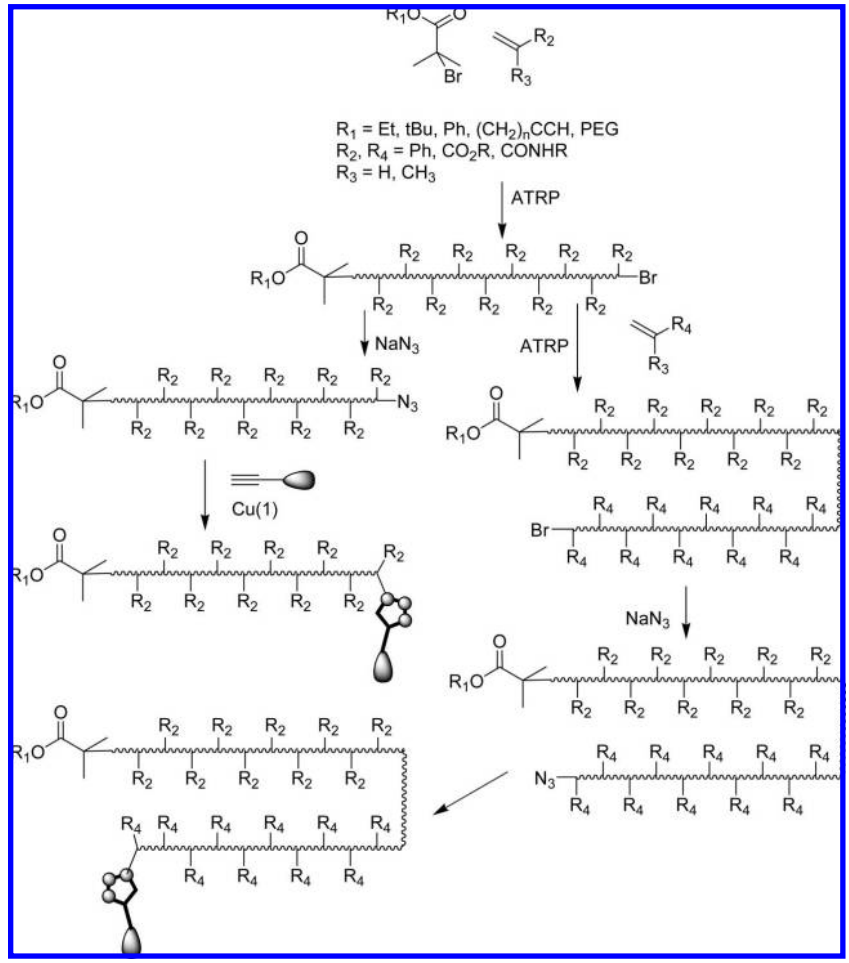
Figure 38. Selective labeling of galectin-3 in cell lysate. The photoaffinity probe reacts primarily with galectins, and separation by SDS-PAGE provides the bands for galectin-3 with selective suppression by increasing amounts of added lactose.

CuAAC using $CuSO_4$/Asc on hex-5-yn-1-yl acrylate monomers allowed preparation of polyacrylates with a high density of triazoles and hydroxymethyl triazoles. These polymers showed conductive properties.[402] Poly(phenylacetylenes) carrying azidopropyl groups were prepared quantitatively and functionalized with a variety of donor/acceptor groups using CuAAC to provide second order nonlinear optical PA's.[403]

Li et al. functionalized poly(azidoethylmethacrylate) with phenylacetylene using CuAAC and compared pre- and postfunctionalization. Both methods gave well defined polymers with quantitative formation of triazole.[404] Jung et al.[405] prepared polyglycidylazide from the chloride by azide substitution and reacted the polymer quantitatively with different substituted phenylacetylenes using $CuSO_4$/Asc. A large variety of complex hydrogen bond donor/acceptor moieties and other functional groups were attached to ROMP polymers alkylated at the succinimide nitrogen with propargyl or azidohexyl groups. The method was demonstrated to be highly divergent and provided many functional polymers.[406]

Incorporating 2-(pent-4-ynyl)-2-oxazoline in an 2-methyl-2-oxazoline polymerization provided a peracylated polyimine polymer carrying 4-pentynoic amides for further transformation via triazole formation.[139] Conventional ROMP polymerization in which the exo-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboximide monomer was propargylated or bromoalkylated on the nitrogen also lead to polymers, which were easily modified by CuAAC either by pre- or postpolymerization modification. Polymers were derivatized with thymine, long fatty amines, or a bifocal H-bond tweezers molecule.[302] Nonlinear-optical polyurethane polymers with altered optical properties were prepared from monomers which had been modified by triazole formation directly on the optical probe prior to polymerization.[398] Addition of α-chloro-ε-caprolactone in a polyester polymerization gave a linear polymer containing α-chloro esters at random positions, and these were substituted with azide. The grafting process was optimized with propargylbenzoate, and the optimal conditions of CuI/DBU were used to graft a range of functionalities onto the polyester by triazole formation.[85] Attachment of PEG mono-4-pentynoate to the azide substituted polymer provided a micelle forming copolymer.[84] Similarly, Parrish et al.[179] performed a ring opening homocopolymerization of a mixture of α-propargyl-δ-valerolactone and ε-caprolactone, and the properties of the resulting polyester were modified with PEG-azide and the peptide 6-azidohexanoyl-GRGDS. More elaborate functionalities such as redox active flavin[58] and highly fluorescent iridium complexes[407] with three aromatic *C*,*N*-ligands have been quantitatively attached

Scheme 26. Controlled Living Polymerizations Allowing Both Block Copolymers and Specific Terminal Modification of the Polymer by CuAAC



to azidomethyl polystyrene through CuI/DMSO catalyzed triazole formation with an alkyne attached to the functional molecule.

Several methods have been developed for grafting two or more functionalities to a polymer via triazoles.[109,178,249,297,304] Nitroxide catalyzed homocopolymerization of 4-vinylphenyl trimethylsilylacetylene, styrene, and trimethylsilyloxoethyl methacrylate and removal of the silyl groups provided a polymer with an alkyne and an alcohol that could be simultaneously grafted with a mixture of an organic azide and a carboxylic acid anhydride or an active ester in the presence of $CuBr(PPh_3)_3$ and DIPEA. 4-Methoxybenzoate and 1-(2,3,4,6-tetra-*O*-Ac-*β*-D-Glc)-azide were grafted in this manner. The two grafting reactions were completely orthogonal and could be used in a cascade fashion where one component was bifunctional (e.g., azidoalkyl amine or propargylamine) and the other monofunctional and consecutively formed either ester or amide followed by triazole formation or visa versa to provide composite graftings.[297] Ladmiral et al.[304] carried out random grafting with a mixture of azidoalkyl mannopyranoside and galactopyranoside and also with a mixture of the same derivatives with an azidoalkyl coumarin derivative to provide a biofunctional fluorescent polymer. Englert et al.[249] developed a pre- and a postpolymerization functionalization scheme for the decoration of poly(*p*-phenyleneethynylene)s. 2,5-Di(TIS-propargyloxy)-1,4-diiodobenzene and 2,5-di-2-ethylhexyl-1,4-diethynylbenzene were polymerized. Under simultaneous removal of TIS groups with TBAF, the alkynes were quantitatively derivatized with a range of functional azides including long chain aliphatic and fluorous azides, crown ethers, and glucoside through triazole formation. Alternatively, functionalization of the monomers via triazoles prior to polymerization also provided the fully derivatized polymers. Polyester prepared from α-propargyl-*δ*-valerolactone and *δ*-valerolactone by Parrish and Emrick[178] was partially grafted with the insoluble anticancer drug camptothecin, and residual alkynes were grafted with azido-PEG to increase water solubility. Coady and Bielawski[109] similarly converted the azido groups of poly(4-azidomethylstyrene) into two different functionalities by first reacting some azides randomly with alkyne using CuI in DMF at 70 °C and then reacting the residual azides with a carbene to give the triazene functionality.

9.13.2. CuAAC Polymerization Reactions

Polymerization by CuAAC is the obvious extension of the studies on preparation of functional polymers described above. Golas et al.[9] used their bis-azido polystyrene[316] as a macromonomer in a Cu(1) catalyzed polymerization with dipropargyl ether in the presence of 2,2′:6′2″-terpyridine ligand (tpy, **19**, Figure 4).

The tpy ligand was not giving the fastest conversion but secured almost complete conversion to triazole. The high molecular weight obtained was probably a result of protection of the Cu(1) catalyst against disproportionation. Although the reaction between macromonomers is par-

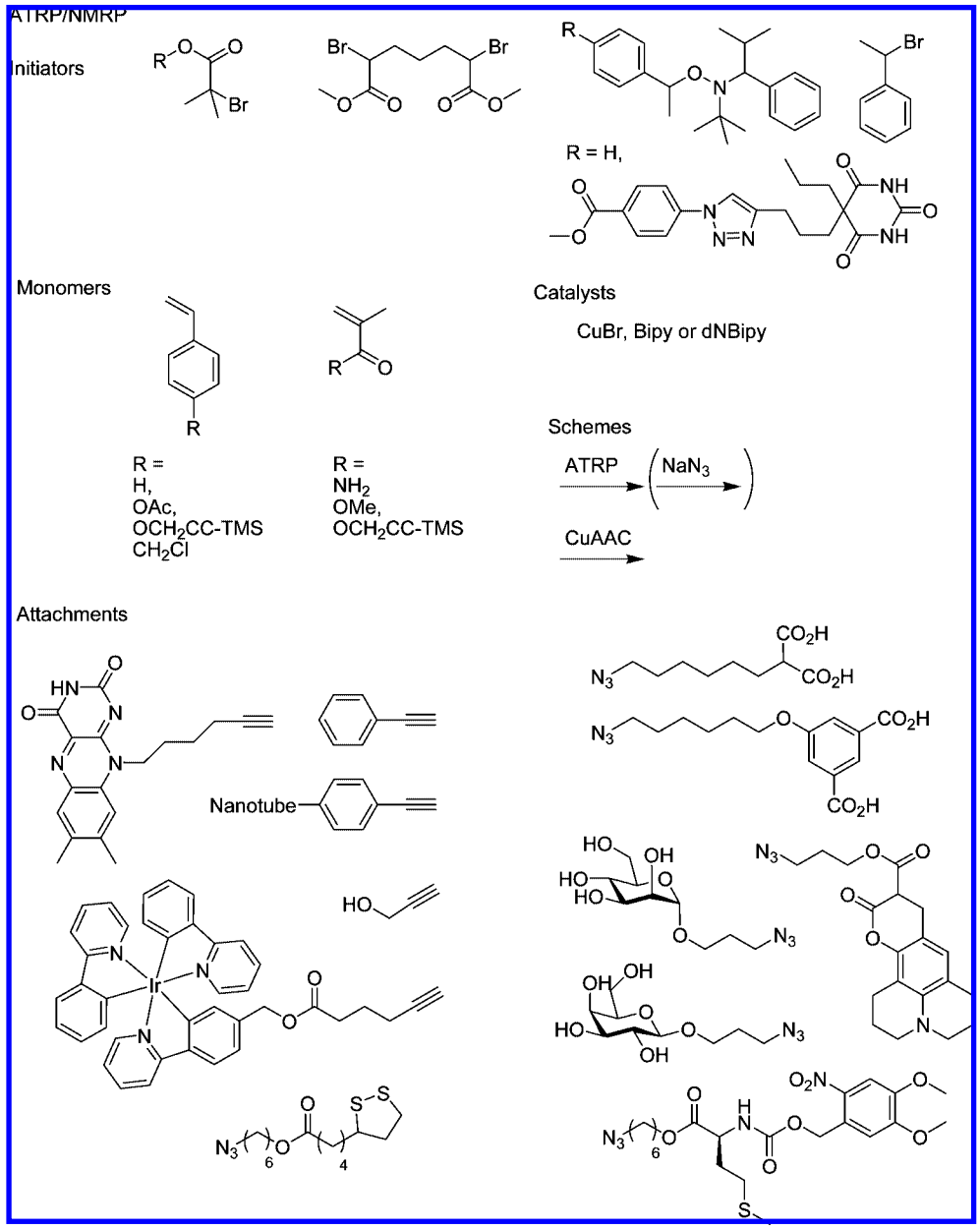


Figure 39. Chemical conditions and reagents used for production of polymers by controlled living polymerizations, e.g. ATRP or NMP. The polymers were decorated by attachments using CuAAC.

ticularly difficult, due to the low concentration of the reaction partners during reaction of the second propargyl group with azide-macromonomer, it was possible to increase the molecular weight ~10-fold by polymerization. A fraction of small molecular weight cyclic polymers were also formed. By using pseudodilution by syringe pump addition, Laurent and Grayson[408] utilized this to deliberately form the polymer macrocycles.

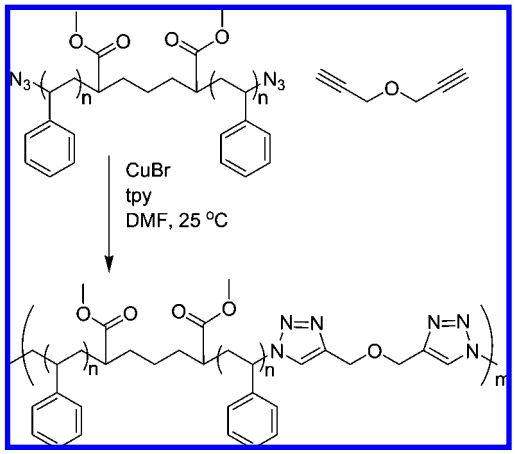
Cu(1) catalyzed polymerization of small bis-azides with bis-alkynes yielding linear conjugated polymers with a regular triazole backbone has been achieved by van Steenis et al.[350] They prepared 2,7-diazido fluorene and a variety of bis-acetylenes including 2,7-diethynyl fluorene and 1,4-diethynylbenzene. The polymerization of the former with the latter in the presence of Cu(0), $Cu(OAc)_2$, and TBTA at intermediate dilution and low temperature (−10 °C) provided a M_w of up to 396.000, indicating the efficiency and robustness of the reaction. Bakbak et al. took a very similar approach.[211]

Lu et al.[206] have described a stepwise iterative construction of a very regular triethylenglycol oligomer using a tosyl group for the intermediate protection and activation for substitution with azide. Thus, PhthN-$CH_2(CH_2OCH_2)_2$-CH_2-N_3 reacted with propargyl tosyl triethyleneglycol under $CuSO_4$/ascorbate conditions, and after nucleophilic displacement with azide, the coupling cycle could be repeated twice to yield the trimer in 39% overall yield. A highly organized dendrimeric polymer structure was synthesized by Liu et al.[409] as outlined in Scheme 29 by alternate living radical polymerization of styrene and CuAAC with a branching unit.

9.13.3. Cross-linked Polymers by CuAAC

Three-dimensional polymer networks, acting as strong adhesives, were produced by polymerization of mixtures of small bis-azide and tris- and quad-alkyne monomers.[93,410] The property for gluing copper materials even outperformed commercial glues. The adhesive strength greatly depended

Scheme 27. CuAAC Bisazide−Bisalkyne Polymerization of Polystyrenes Preformed by Controlled Living Polymerization[a]



[a] Large polymers can be formed in this manner.

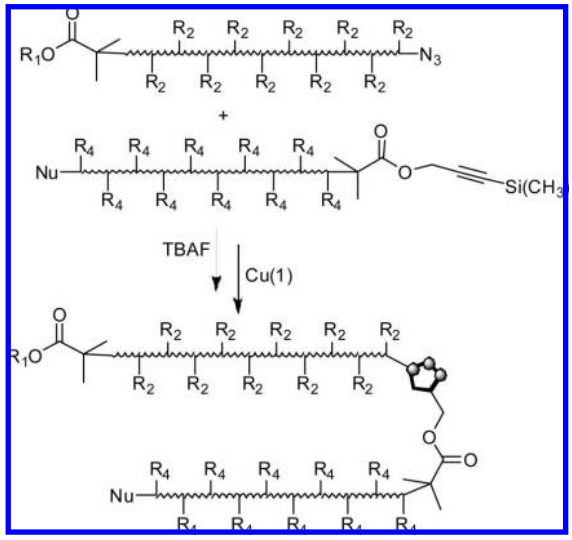


Figure 40. Linking two polymers specifically through terminal alkyne and azide functionality is feasible through CuAAC.

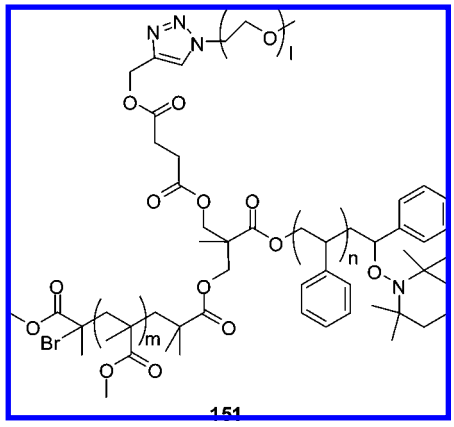


Figure 41. A star-triblock copolymer with three different polymer branches can be prepared by combination of the technology of initiator design ATPR, NMP, and CuAAC.

on the use of amine containing azides and alkynes due to the formation of longer polymer chains resulting in a stronger adhesive. This was ascribed to the amine assisting the acetylide complex to the catalytic Cu(1). A large range of monomer architectures were investigated, and tris(azido-

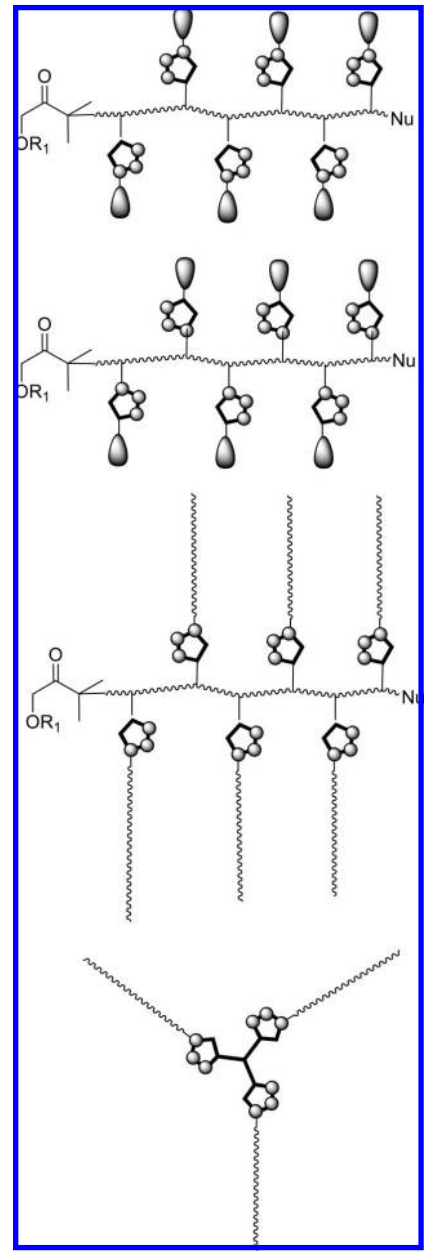


Figure 42. Functionalization at randomly distributed sites in a mixed polymer with functional groups by CuAAC is essentially quantitative. The triazole reaction also provides for construction of templated star polymers and brush-block copolymers.

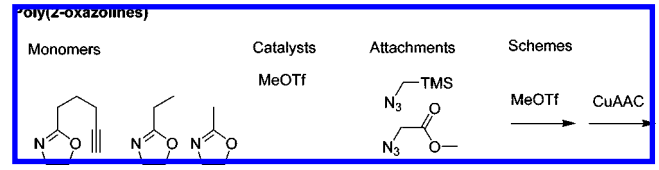


Figure 43. The acid catalyzed polymerization of 2-oxazolines produced an acrylated poly(ethylene imine) carrying hexynoic amides for derivatization by CuAAC.

methyl)aminomethane and tripropargylamine showed the best performance. Ten mole percent of CuI was added to initiate the polymerization reaction, and the Cu remained bound in the polymer (i.e., **152**) upon curing. The authors argued that the triazoles, which formed at the Cu surfaces of glued items, remained bound to the Cu(0). This could explain the strong adhesion observed. The 3-dimensional high molecular weight and high glass transition temperature polymer (ter-

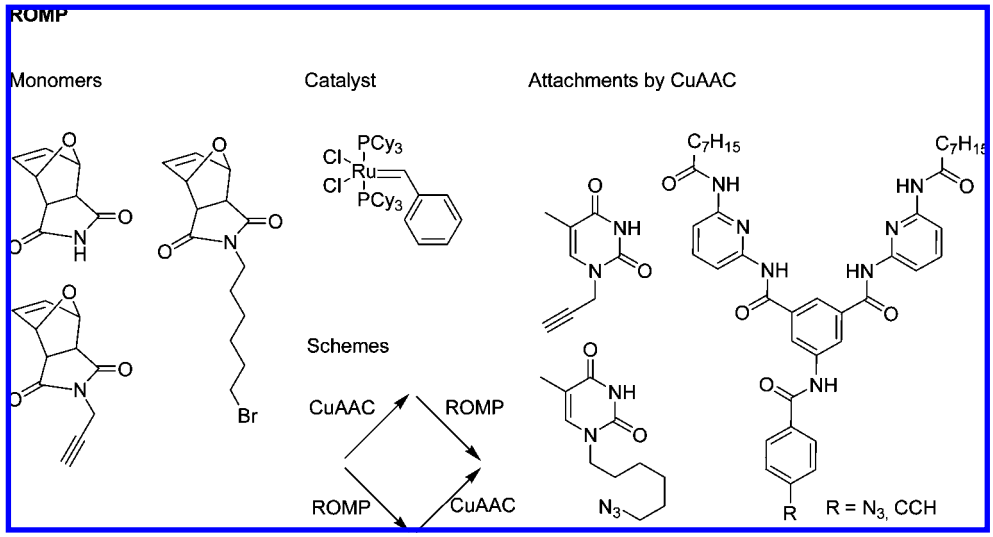


Figure 44. ROMP is an orthogonal polymerization technique that is very useful for preparation of polymers carrying a variety of functional groups including alkynes and alkyl halides easily converted to azide for CuAAC.

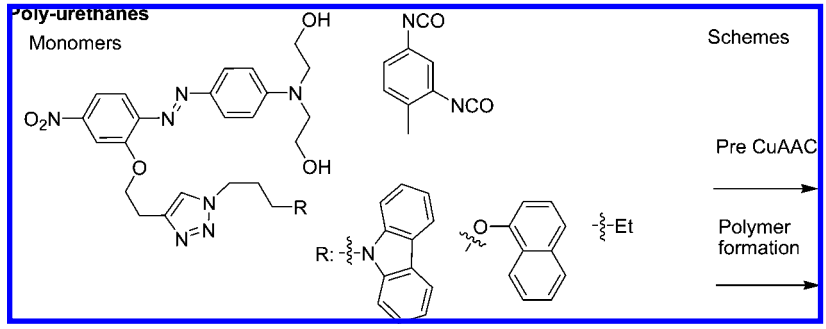


Figure 45. Poly(urethanes) with various nonlinear optical properties were synthesized by application of diol building blocks modified by CuAAC.

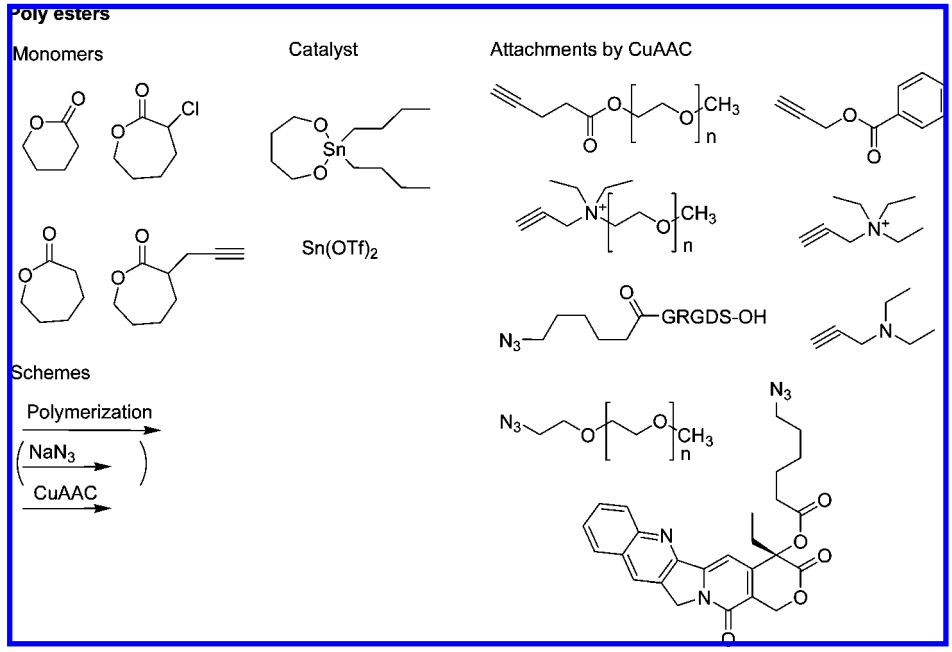


Figure 46. Polyesters can be formed by ring opening polymerization reactions starting from α-substituted caprolactones. The chloro-substituted polymer can be converted to the azide containing polymer in high yield, and the azide can be used for CuAAC functionalization.

moset) **153** produced in this manner show high T_g up to 200 °C.[50] A more swellable triazole polymer was formed by reaction of a 3:2 mixture of tripropargylamine with 1,6-diazidohexane under more dilute conditions.[164] Swelling of up to 20-fold was observed, which is not expected considering the theoretical structure of the polymer. However, the polymer precipitates during polymerization, and the presence of inaccessible azide in the final polymer indicates a spatial

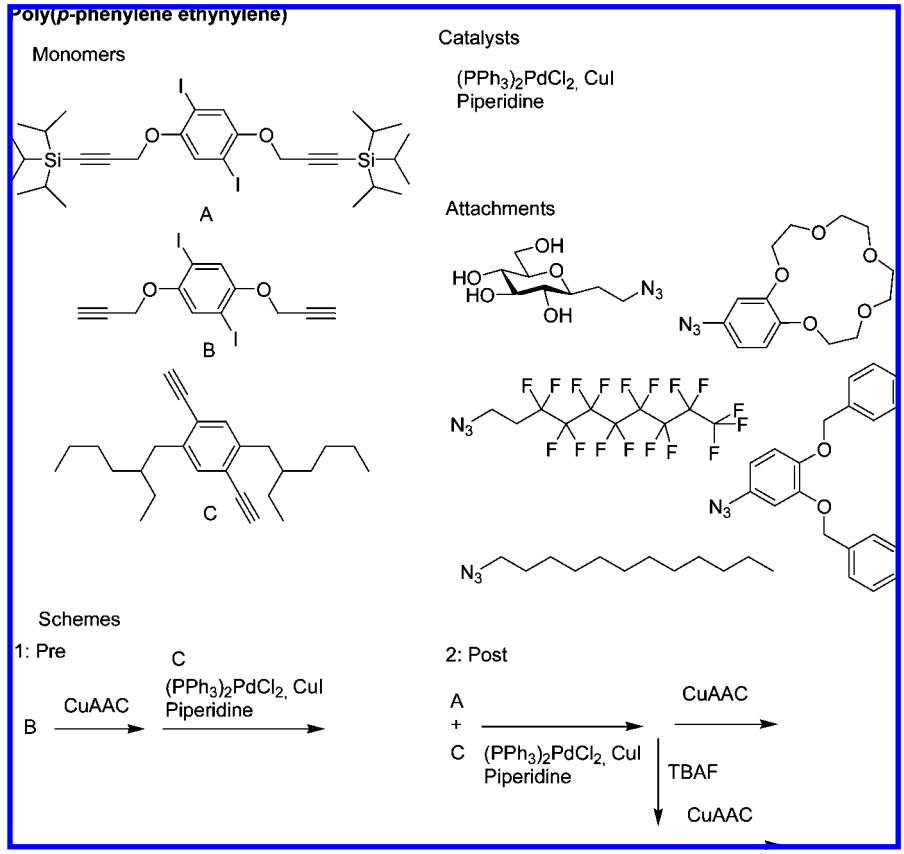


Figure 47. Pre- and postmodification (see schemes) of poly(*p*-phenylene ethynylenes) using CuAAC both lead to high yields of functionalized polymers.

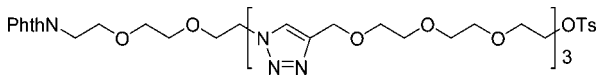


Figure 48. Stepwise approach to build well-defined oligomers of triethylene glycol by CuAAC. OTs acts as a temporary protection group that is readily replaced by azide.

separation of azide and alkyne, leading to a heterogeneous polymer composed of dense and inaccessible polymer nucleation sites and more loosely interconnected regions.

When 1,2-bis(bromoisobutyryloxy)-2-butene was used as an initiator for ATRP of *tert*-butyl acrylate and the terminal bromines were substituted with azide, an ozonolyzable linear polymer was obtained. This could be incorporated into a 3D-polymer network by quantitative triazole formation with e.g. tetrapropargyloxymethyl methane catalyzed under optimized conditions under argon with CuBr, PMDETA, and ascorbate in DMF. The solid polymer **154** could be cleaved and dissolved by ozonolysis.[321] Malkoch et al.[159] produced PEG-based hydrogels **155** with 11-fold swelling and incredible tensile strength and elasticity by polymerizing PEG_{10000} bis(4-pentynoate) and tetraethyleneglycol bis(2,2-di(azidomethyl)-propanoate) in a ratio of 2:1 with addition of $CuSO_4$ (0.4) and ascorbate (1). These conditions were optimal, and monitoring residual functional groups in the polymer with fluorescent alkynes and azides showed that the triazole formation was greater than 98.8%. Poly(vinyl alcohols) have been derivatized with carbonyl diimidazole and reacted with either propargylamines or aminoethylazide to afford azide or alkyne functionalized PVA's, respectively.[246] Mixing these and performing the triazole formation by addition of $CuSO_4$/ascorbate in DMSO gave up to 89% yield of a resin **156** with a swelling of 3−4-fold. Substituting bis-azido PEG for the PVA-azide resulted in only 64% gel **157** with a swelling of 10−12-fold in DMSO.

Curable linear polystyrenes, quantitatively functionalized by CuAAC with benzoxazines, were synthesized by Ergin et al.[411] They demonstrated that the benzoxazine functionality was stable to the conditions of triazole formation and the cured and cross-linked polystyrene had a high thermal stability.

Derivatization of polymers with bromo-isobutyrate and polymerization of trimethylsilyl propargylmethacrylate off the surface of the cross-linked polymer allowed the attachment of azidoethylglycosides in clusters by CuAAC along the grafted polymer chains. The beads competitively bound Con A, a mannose recognizing lectin. The growing implication of and the increased complexity of the problems challenged with the CuAAC in polymer chemistry indicate that this reaction may well take the prominent position alongside with radical, ring opening, and other mainstream polymerization techniques. The polymers will contain the stable triazole ring, which is chemically inert for most purposes and yet may play functional roles for the polymer properties. It will be exciting to follow the directions triazole chemistry will take in the world of polymers.

The special nature of this reaction, i.e. the quantitative yield, the orthogonality with most other chemistries, the fact that it is catalyzed by minute amounts of a large variety of Cu(1) catalysts in almost any solvent, and the robustness, simplicity, and temperature range, allow the polymer chemist to build highly complex and well defined polymer architectures from readily available LEGO macromonomers and polymer fragments in a completely new fashion. The polymer fragments obtained by living radical polymerizations may

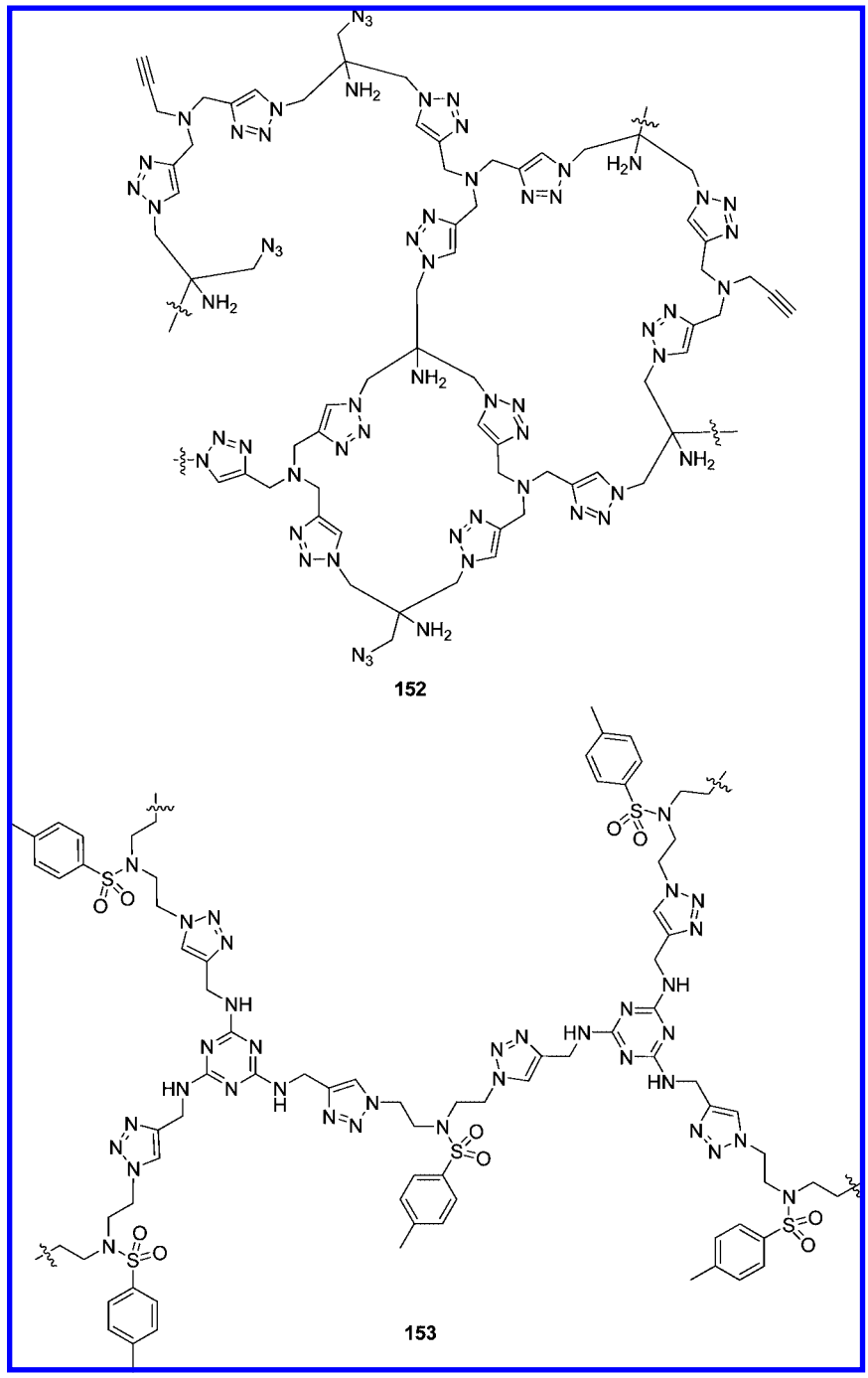


Figure 49. Adhesives produced through high density cross-linking in CuAAC polymerization of di- and trifunctional azides and alkynes. This adhesive is particularly useful for organic "welding" of copper items, since the triazole products remain covalently attached to the Cu surface.

be combined or attached to core structures to form heterostar polymers and dendritic polymers. Large polymer fragments may be polymerized to give high molecular weight and materials otherwise not achievable. The outcome of the reaction is reliable and predictable, leading in all cases to exclusive formation of the 1,4-isomer of the triazole formed during the reaction. Another benefit of the CuAAC reaction is that there exist a large variety of precursors and precursor reactions that provide access to the reactive alkyne and azide at the exact time when they are required and under mild reaction conditions.

In polymer chemistry, there has been a preference for using CuBr/PMDETA; however, results from other fields suggest that other selections of catalysts and ligands may function equally well or even better. The most important issues for a successful and quantitative reaction are those of establishing completely anaerobic conditions and protect the Cu(1) catalyst against disproportionation, e.g. by using the auxiliary ligand that is optimal under the specific reaction conditions. The optimal ligand is not always the ligand that provides the fastest reaction rate but rather the ligand that provides the best protection against disproportionation. However, the ligands also influence the structure and equilibria of Cu(1) cluster composition and may promote the formation of the most catalytically active Cu(1) complexes.

Disproportionation of Cu(1) to Cu(2) and Cu(0) is a particularly serious problem in the case of less reactive

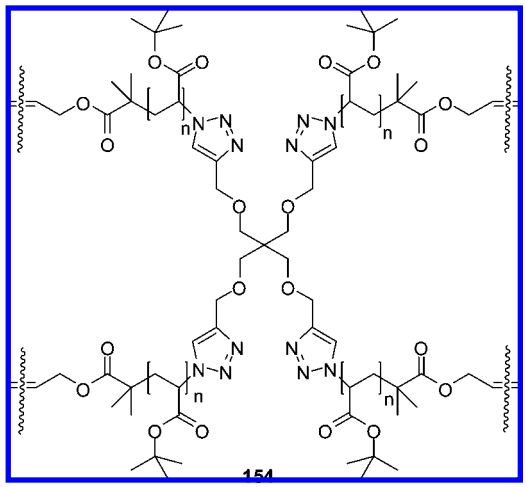


Figure 50. A cross-linked polymer that forms soluble star polymers by ozonolysis was synthesized by reacting bis-azide polymers formed from a divalent initiator with a tetravalent azide cross-linking agent using CuAAC.

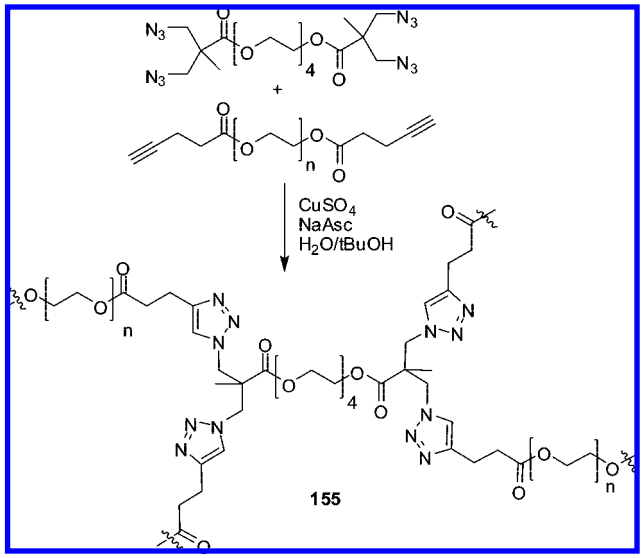


Figure 51. Cross-linked PEG-based polymers have been broadly used for solid phase synthesis and biomolecular screening. Now very well defined 3D-polymer networks may be obtained through the orthogonal CuAAC reaction.

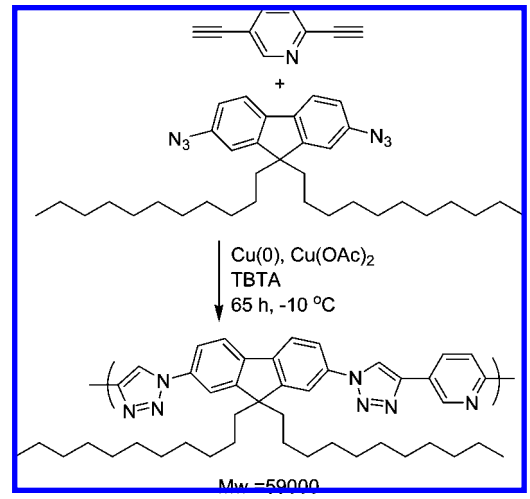
reaction components or dilute reactions where the addition of a reducing agent may facilitate conversion from Cu(2) to Cu(1).

The application of other "click" reactions in combination with the CuAAC reaction can furthermore widen the scope for preparation of complex and structurally well-defined polymers in the future. The challenge for the polymer chemist is in the design of macromolecular LEGO's that may enter into these chemical ligations in a sequential and controlled manner.

9.14. Surface Modification by CuAAC

Due to the orthogonal nature of the CuAAC, it holds great promise for surface immobilization of complex molecules in their unprotected and functional form. Surfaces that may be modified using this reaction include polystyrene, polyethylene, glass, silica, silica gels, gold, etc. Virtually any material, to which an alkyne or azide can be attached, may be functionalized by CuAAC. Chen et al.[412] modified resins and the surface of cotton fibers with azide and alkyne and

Scheme 28. Conjugated Polymers Were Formed through CuAAC of Aromatic Bisazides with Aromatic Bisalkynes[a]



[a] However, the conjugation/fluorescence properties of the polymer were not optimal.

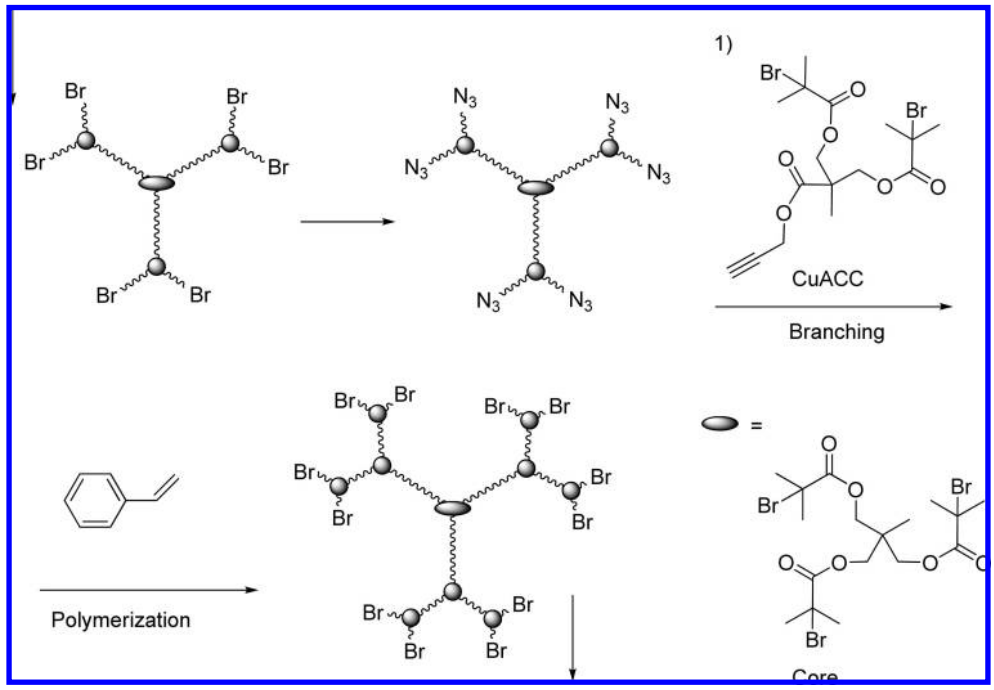
"clicked" fluorescence labeled poly(methacrylates) to their surfaces by CuAAC using CuBr/*N*-(propyl)-2-pyridylmethanimine.

Microtiter plates are important utensils in biochemistry and biology, and Wongs group has demonstrated the ease of functionalizing titer plates with complex carbohydrates for enzyme substrate and inhibition studies. The alkyne may first be immobilized either by reaction of an alkyne-isothiocyanate with amino functionalized plates or by attaching an alkyne with a lipidic tail by hydrophobic interaction with the polystyrene surface. Complex azidoethyl or azidopentyl glycosides[124] and transferase substrates[413] were immobilized using the CuI, DIPEA conditions. A second quite useful immobilization is that of preparation of silica gel based affinity purification materials. A chiral column has been prepared by immobilizing propargylated cinchona alkaloid on 0.87 mmol/g azidopropyl modified silica gel using optimized conditions of 5% CuI/DIPEA in acetonitrile. The product presents a high loading of the alkaloid of 0.75 mmol/g.[99]

Ortega et al.[340] used tripropargyloxymethyl hydroxymethyl methane as the core for preparing silica immobilized glycan dendrons. After alkylation with 2,2′-dichlorodiethylether (carcinogen!) it was reacted in 88% yield with 2-azidoethyl α-D-mannopyranose by μw assisted catalysis with $(EtO)_3P$: CuI and DIPEA in toluene. The chloride was converted into azide and alkyne derivatives, and the alkyne gave the superior result when coupled under the same conditions to azidopropyl derivatized silica. High yields and purity were achieved when ConA was purified on the affinity column. Ranjan and Brittain[414] synthesized propargylated polyacrylamide or polystyrene by RAFT and used CuAAC to conjugate these to azide modified silica gel for chromatography.

Regular HPLC columns with a variety of functional groups have been prepared in a similar manner using $CuSO_4$ ascorbate conditions.[195] Meng et al.[149] employed CuAAC to capture the peptide containing the active site thiol of GST onto silica via avidin−biotin interaction using a complex cleavable linker for DIOS-MS. Glass slides were modified by Sun et al.[180] in a two step approach during which malimido derivatized aminopropyl silanized (EMC) glass was first reacted with cyclopentadiene linked to propargylated

Scheme 29. Dendrimeric Polymer by Alternating Polymerization and Branching by CuAAC



PEG followed by CuAAC reaction with a variety of protein affinity ligands and even azide labeled protein using $CuSO_4$ and TCEP or Cu wire and TBTA ligand in PBS buffer.

Silicon wafers have also been used as a substrate for CuAAC.[194] After surface treatment with HF, the surface was reacted with nonane-1,8-diyne at high temperature. $CuSO_4$/accorbate catalyzed reaction of the remaining alkyne monolayer with azides gave high yields of triazole linked functionality on the surface.

Two reports describe the alternating layer by layer assembly of azide and alkyne derivatized polyacrylamides, obtained through living radical acrylate/acrylamide polymerization with addition of propargyl acrylate or an acrylate azide precursor, respectively. The layer by layer attachment to polyethylene,[415] quartz, silicon, or gold[262] was performed with $CuSO_4$/ascorbate conditions.

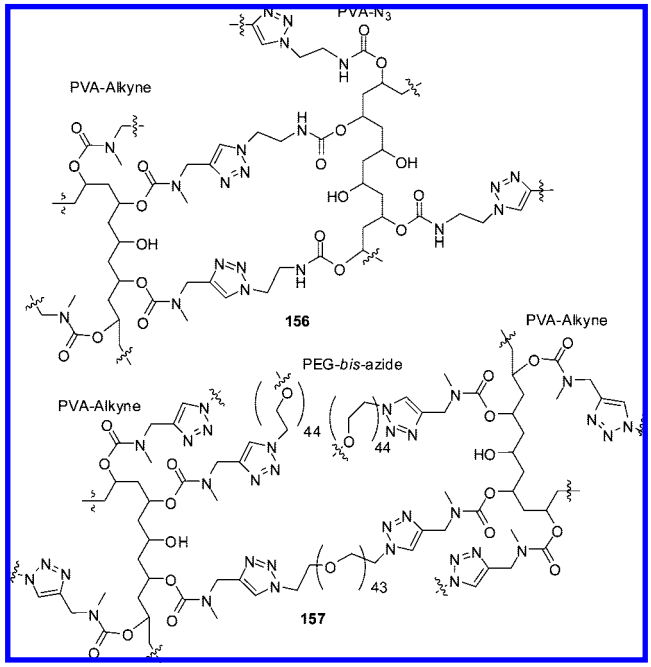


Figure 52. Cross-linked polymers were synthesized from linear poly(vinyl alcohols) and PEG polymers. The property of the final polymer may be tailored by substitution density of functional groups and polymer length.

A very interesting report by Devaraj et al.[357] describes the use of arrays of gold microelectrodes to control a spatially selective CuAAC reaction. By applying a negative potential of −300 mV to specific electrodes while maintaining the rest at +250 mV in the presence of a Cu(2) solution, Cu(1) is selectively formed at these electrodes and selectively promotes cycloaddition at sites with negative potential to effect local immobilization of ethynyl ferrocene. The functionalization was performed versus an Ag/AgCl/NaCl electrode in KPF_6 electrolyte containing Cu(2)bis(batho) and the ethynyl ferrocene.

CuAAC has also been used to control electron transfer rates at the gold electrode surface by systematic variation of the alkyl thiol monolayer thickness and the alkyne substituted redox molecules attached to surface bound azidoalkyl thiols.[231,344] Transfer rates varied 3−4 orders of magnitude. $CuBF_4$, TBTA, and hydroquinone as reducing agent were used for the immobilization. Reactions were orthogonal, clean, and highly reproducible, which led the authors to predict a great future for CuAAC in the field of surface chemistry. Ligands that have been immobilized on alkyne modified gold surfaces include nucleotides[232] and carbohydrates.[196] In the latter, *N*,*N*′-(dithiodidecane-1,10-diyl)bispropiolamide was used to prepare a monolayer on a plasmon-resonance gold chip and the resulting alkyne monolayer was reacted with azidoPEG glycosides. The reactions as well as the binding of proteins to the immobilized sugars could be followed directly by plasmon resonance.

In conclusion, it may be quite significant for the future that a new surface chemistry based on CuAAC with superior performance with respect to immobilization yields and complexity of immobilized structures has been introduced.

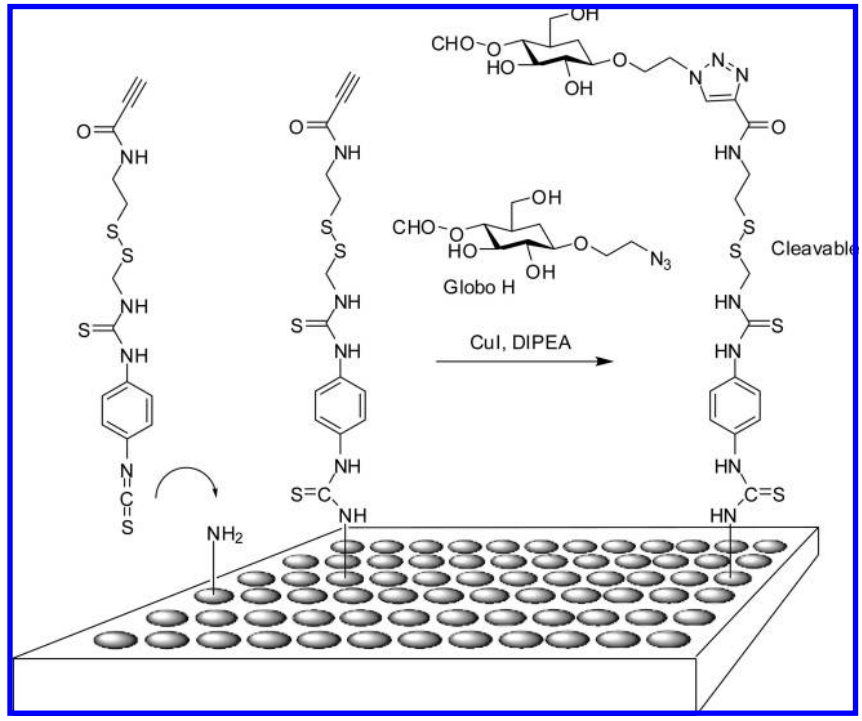


Figure 53. Immobilization by covalent attachment to titer wells is an important technique, and CuAAC may add considerably to this field by its virtue of being quantitative and selective.

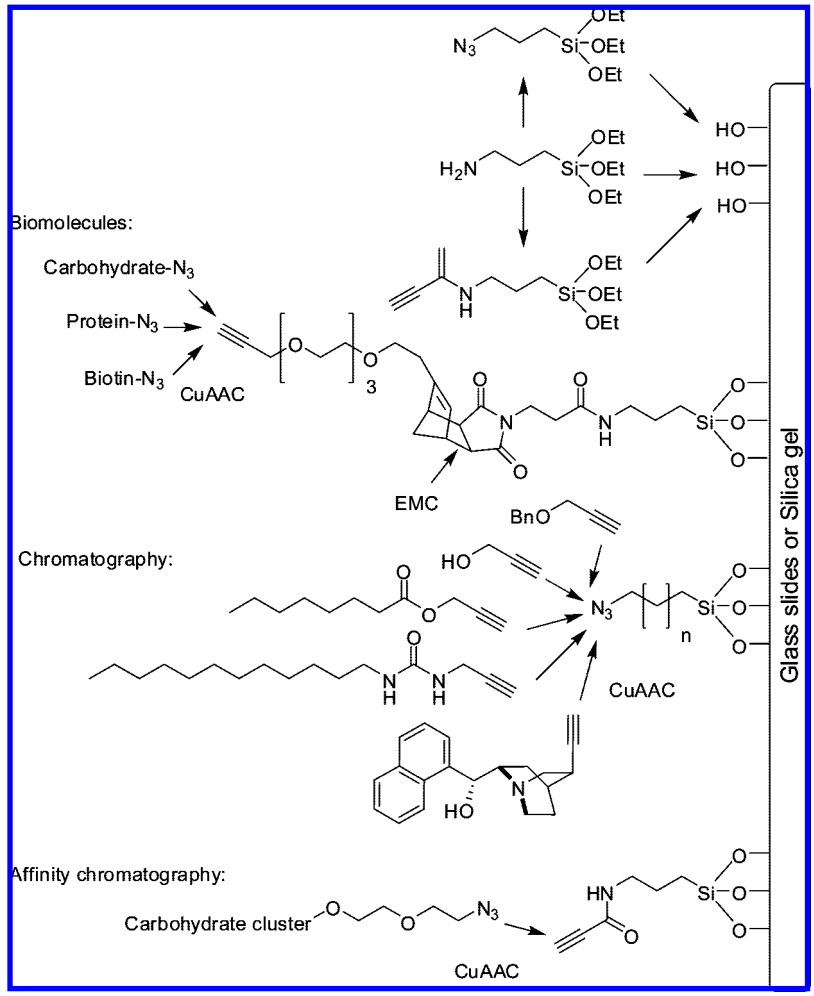


Figure 54. Glass surfaces are used extensively to produce microarrays of compounds. Both these and surface modified silica gels are available through CuAAC.

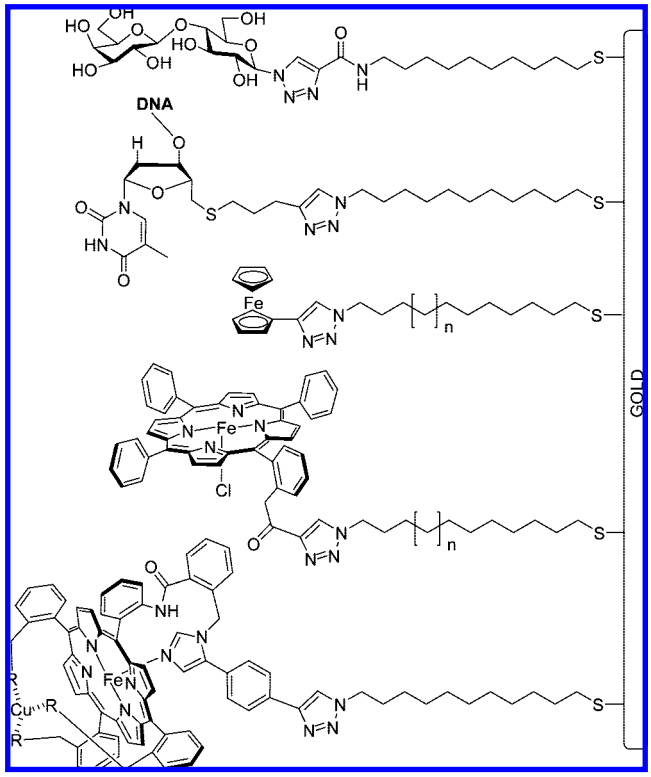
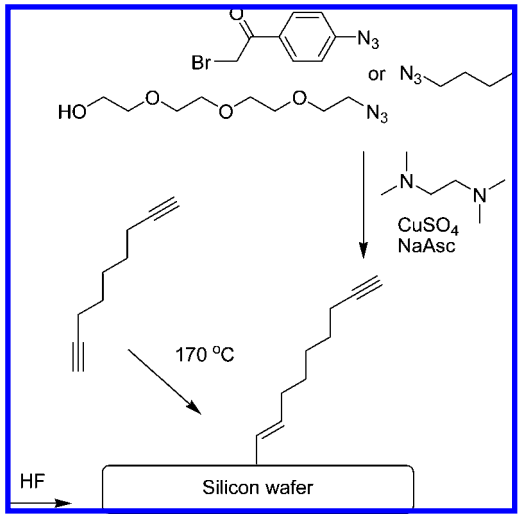


Figure 55. Gold surfaces have been modified with complex molecular entities for voltametric measurements and plasmon resonance. A special application is that of controlling the derivatization locally through reduction of Cu(2) to Cu(1) over a microelectrode kept at −300 mV with reference to the solution.

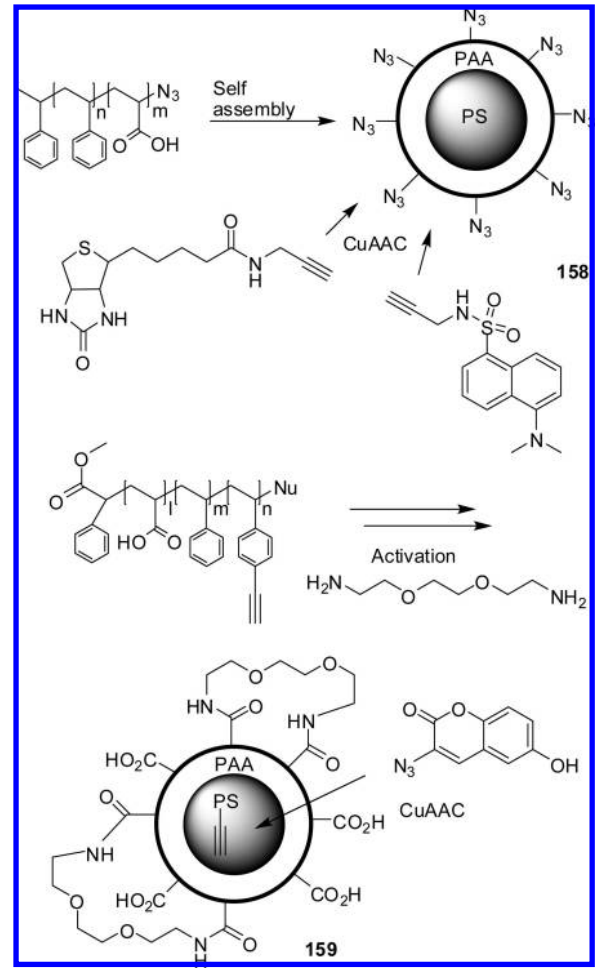
9.15. Nanostructures by CuAAC

Organic core shell nanoparticles may be synthesized with linear amphipatic block copolymers formed by two-step living radical polymerization of alkyl acrylates and styrene. Upon ester cleavage, these forms compact micelles in water with a PS-core and a solvated acrylate shell which may be partially functionalized with propargylamine or azidopropylamine and further cross-linked with e.g. bis-amino-PEG or 2,2′-(ethylenedioxy)bis(ethylamine).[295,416] If the nitroxide initiator contains a chlorobenzyl group, each chain will display a chloro benzyl functionality at the surface of the nanoparticle, and after azide substitution of the chloride, a CuAAC reaction may be performed with complex functional ligands at these selective sites.[173] Similar results were obtained starting with polystyrene ATRP with α-bromoethylbenzene followed by *tert*-butyl acrylate, azide substitution, and acrylate deprotection for synthesis of the block copolymer.[191] The CuAAC reaction on the nanoparticles was demonstrated by immobilization of an alkyne linked fluorescent dye to the relatively uniform nanoparticles **158**.

Scheme 30. Silicon Wafers (Si(100)) May Be Cleaned from SiO_2 by HF Treatment and Subjected to Reaction with Diynes at High Temperature To Provide an Alkyne Substituted Alkenyl Silylated Surface for CuAAC Reactions



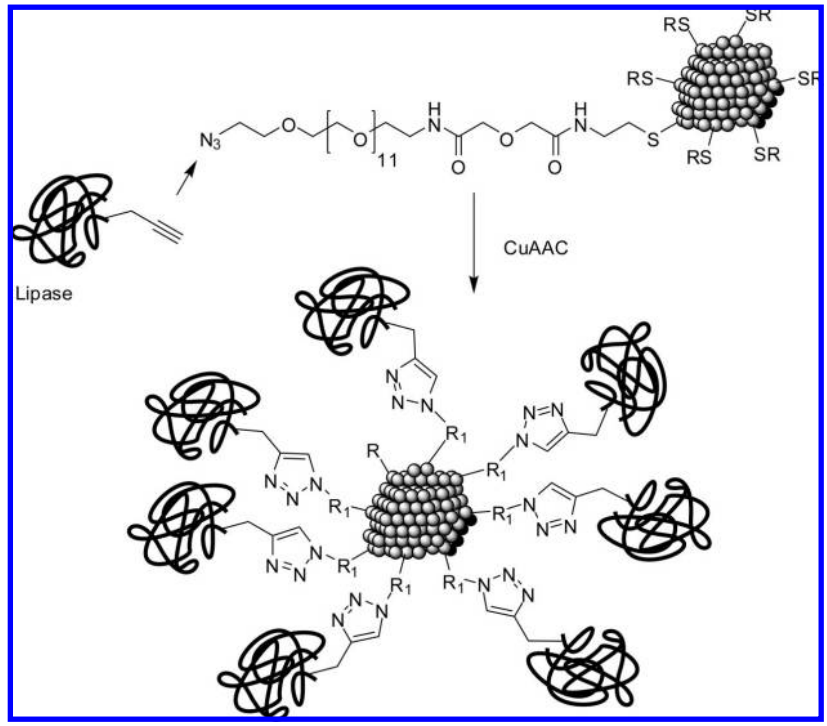
Scheme 31. Core−Shell Nanoparticles Produced by Self-assembly of Amphiphilic Block Copolymers May, if They Contain Azide or Alkyne in the Core or Shell, Be Modified Selectively at These Sites



Alternatively, when the linear polymer was formed with addition of either propargyl acrylamide to the acrylate or 4-(chloromethyl)styrene or 4-(TMS-ethynyl)styrene to the styrene in the two step polymerization, an alkyne substituted core or shell or an azide substituted core could be obtained, respectively. [295,416] Fluorescent labels could be diffused into the shell or the core to get selective shell or core labeling by CuAAC using $CuBr(PPh_3)_3$/DIPEA in THF/H_2O for access to the hydrophobic environment of the core in **159**.

The propargylamide containing core−shell nanoparticles described above were also cross-linked using dendritic azides[158] and $CuSO_4$/ascorbate. Small dendrimer-tetra azides were superior for the cross-linking, and when using excess of the dendrimer, unconsumed azide could be employed for shell labeling.[175]

Scheme 32. Gold Nanoparticles and Nanorods Have Been Surface Modified with Biologically Active Protein and Signalling Peptides for Targeting



Azide functionalized linear polystyrene has been encapsulated in polyurea microcapsules, and upon diffusion of alkyne containing small molecule catalysts, e.g. TEMPO and DMAP, into the capsules, they have been immobilized using $CuBr(PPh_3)_3$/DIPEA as a catalyst and thereby trapped on the polystyrene to produce stable catalytic microcapsules.[291]

Liposomes have also been efficiently labeled with fluorophore using CuAAC. The lipid anchor DOPE inserts into a phosphatidyl choline bilayer of micelles and by incubation of micelles with the propiolic amide derivative of DOPE, alkyne labeled micelles were formed. These could be almost quantitatively labeled in 5 h with the fluorescent derivative N_3-Lys(NBD)-NH_2 in the presence of CuBr.[307]

Fibrous nanostructures are formed when phosphatidyl choline derived lipids are cross-linked by CuAAC to form bolaamphiphiles with two phosphate headgroups. These molecules (e.g., **161**) formed in quantitative yield in a two phase system from lipid alkyne, and bis-azides may or may not disrupt the membrane integrity, depending on the exact structure of the lipid azide insert.[168] Replacement of the phosphate head groups with 1,2-diaminohexane provided materials (e.g., **160**) that form stable fibrous organogels upon CuAAC cross-linking with bis-azides.[106] Fibrous nanogels were also formed by the small molecule 2′-deoxyuridine after introduction of an acetylene at C5 followed by triazole formation with a range of alkyl substituted benzyl azides.[417] Gold nanorods have been labeled with nuclear localization signal-peptide which had been modified at the *N*-terminal in moderate yield through triazole coupling of 5-hexynoic amide with 11-azidoundecanethiol in the presence of CuI

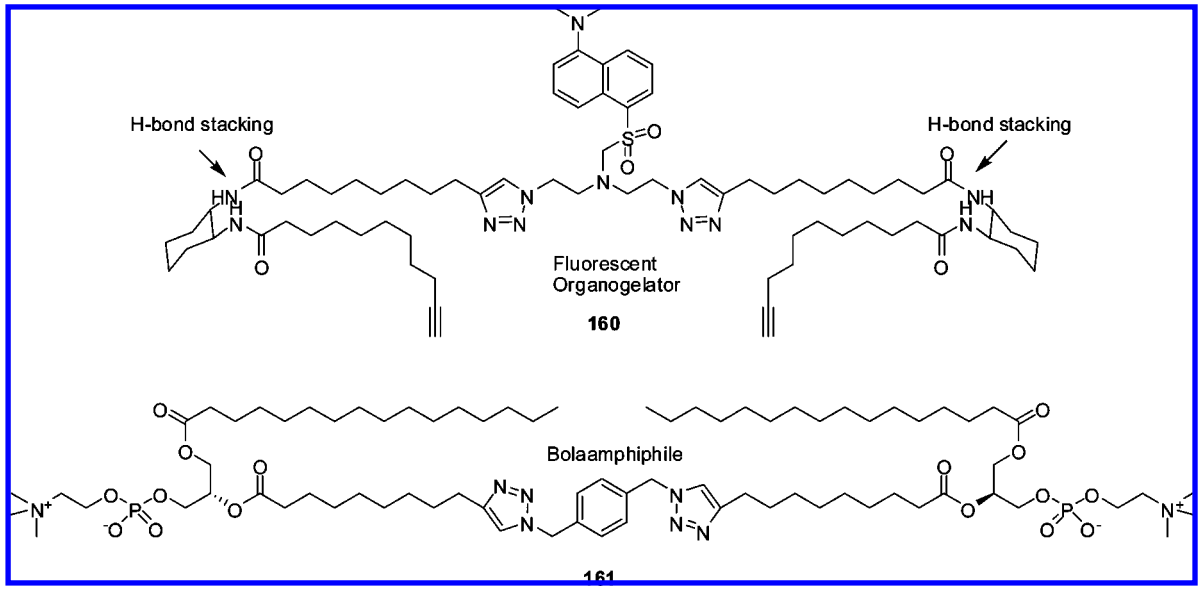


Figure 56. Cross-linked organogelators and membrane spanning bolaamphiphiles are now readily available through CuAAC chemistry.

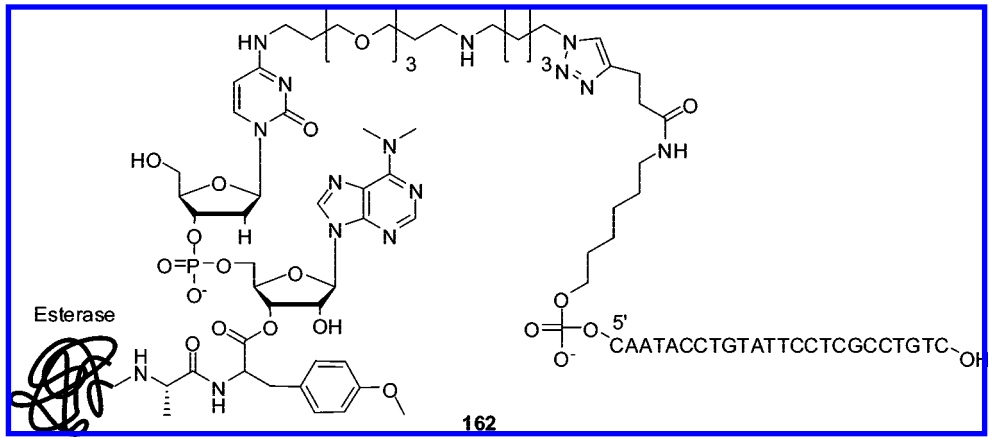


Figure 57. Terminal protein modification by pyromycin inhibition with an azide containing pyromycin derivative, followed by CuAAC ligation with DNA, is a selective procedure for constructing protein−DNA chimeras.

and TBTA with no addition of base.[418] Functionalized rods transferred to the nucleus of cells. A similar approach was taken by Brennan et al.[160] to label gold nanoparticles with a lipase, modified on a single surface exposed lysine using carbodiimide and a terminal alkynoic acid. An azido-PEG-thiol was attached to the nanoparticles via the thiol, and the lipase was attached by CuAAC.

The group of Gallardo et al. has utilized the CuI catalyzed triazole formation to produce liquid crystalline materials with a broad-temperature SmC phase.[80] They compared different conditions and found that, probably due to the lipidic character of the reacting components, the CuI catalysis was superior.[79,81]

9.16. Use of CuAAC for Bioconjugation and in Vivo Labeling

The ultimate challenge for a ligation reaction is that of selectively interconnecting macro-biomolecules in the extra- or intracellular environment. During ligations, large reaction partners often present at very low concentrations should be brought together and form a covalent bond selectively in the presence of all the functionality of the cell. Albeit selective intracellular chemical protein ligation has not yet been achieved, *in vivo* ligation is certainly within the scope of the CuAAC reaction. The advantage of CuAAC is that it can profit from the in vivo incorporation of unnatural amino acids containing azides or alkynes during translation and expression of proteins. Only very few other reactions, e.g. the native chemical ligations[419,420] derived from the intein process in biology and the Staudinger ligation reaction as developed by the Bertozzi group,[421] present comparable suitable properties.

The CuAAC has been used extensively for fluorescence labeling in cellular systems. Speers and Cravatt[6,242,422] developed an activity based protein profiling method involving CuAAC. They incubated living cancer cells and whole mice with alkyne and azidoalkyl benzenesulfonates that enter the cell and react with the active site of a variety of enzymes. After lysis of the cells, the enzyme immobilized probe is labeled with a fluorophore using $CuSO_4$/TCEP/TBTA catalysis. Thus, the process comprises an in vivo step of tagging the active sites of enzymes and an in vitro step of fluorescence labeling the tag with CuAAC. The best results were obtained using alkyne for tagging and azide for labeling. A trifunctional labeling reagent was developed containing rhodamine fluorophore, biotin for purification of labeled material, and azide for CuAAC. Probes that are more specific for a particular class of enzymes may also be employed.[422]

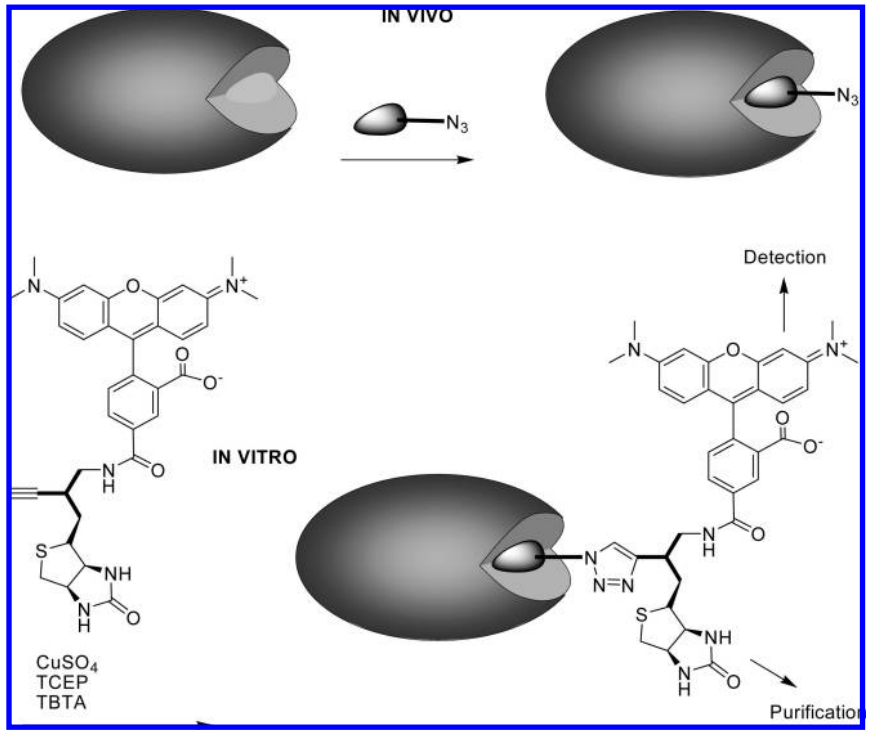
Luo et al.[423] described a specific profiling assay for the arginine deiminase, PAD4. A similar process for monitoring fucosyl transferase activity, during which the CuAAC was carried out in living cells, has been described by Sawa et al.[323] By employing 6-azido GDP-L-fucose or the equivalent 5-ethynyl analog for glycosyl transfer and by incubation with the cell penetrating fluorophores, 4-azido or 4-ethynyl *N*-ethyl 1,8-phthalimide, they could selectively identify the intracellular sites for fucosylation. In agreement with other reports, CuBr was found to be superior to other Cu-sources for in vivo labeling. The method was used for imaging of fucosyl transferase activity.

The amino acid depletion/replacement method developed by the Tirrells group has been used extensively for incorporation of alkyne and azido α-amino acids into proteins. By expression of a particular outer-membrane-bound protein, an engineered methionine rich OmpC, in mature *E. coli* cells by use of a medium shift procedure, the labeling occurs predominantly in this protein by substitutions of Met.[229] Initially, they used $CuSO_4$/TCEP/TBTA to label the proteins. However, the application of high purity CuBr/TBTA showed 7.5-fold increases in fluorescence over $CuSO_4$ labeling when analyzed by flow cytometry.[322]

A similar approach with incorporation of homopropargyl glycine (Hpg) instead of Met was used for monitoring newly synthesized proteins inside a range of mammalian cells.[225] Intracellular labeling was achieved with 3-azido-3-hydroxy-coumarin, and labeling was only efficient with a [Hpg]/[Met] $\sim$ 500 due to less active transfer of unnatural Hpg compared to Met. As a control, protein synthesis inhibitors abolished labeling. Interestingly, the $CuSO_4$/TCEP was again preferred in these intracellular labeling experiments.

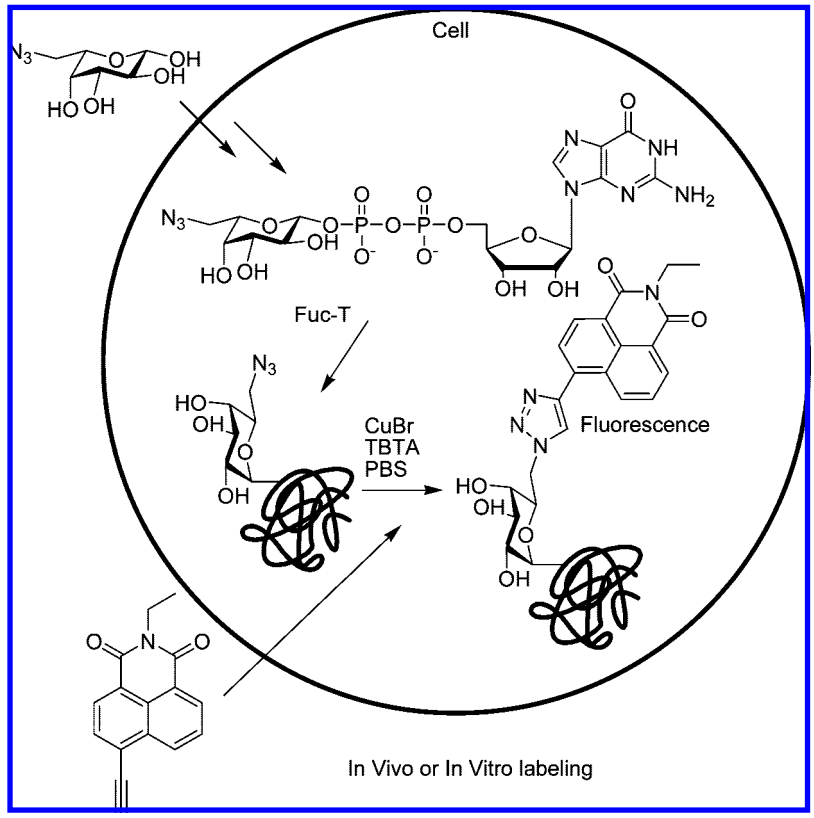
The group of Schultz has developed a method for the expansion of the genetic code to accommodate encoded unnatural amino acids in protein synthesis.[424] The method comprises the conversion of codons for particular amino acids in the GAL4 transcriptional activator with amber nonsense codons (TAG), and suppression of these in MaV203:pGADGAL4(2TAG) yeast strain was used to drive the selection of mutant synthetases specific for added alkyne- and azido-amino acids. After a positive−negative−positive selection scheme, specific mutant synthetases for the unnatural amino acids were produced. By introduction of the TAG codon in 6xHis tagged SOD, a protein containing

Scheme 33. Activity Based Protein Profiling Employs Specific Covalent in Vivo Attachment of a Small Molecule Containing an Azide or Alkyne to the Active Site of a Protein Family or Class[a]



[a] These may be subsequently labeled in vitro for affinity purification and fluorescence detection.

Scheme 34. In Vivo CuAAC May Facilitate the Monitoring of Fuc-T Activity in the Cell[a]

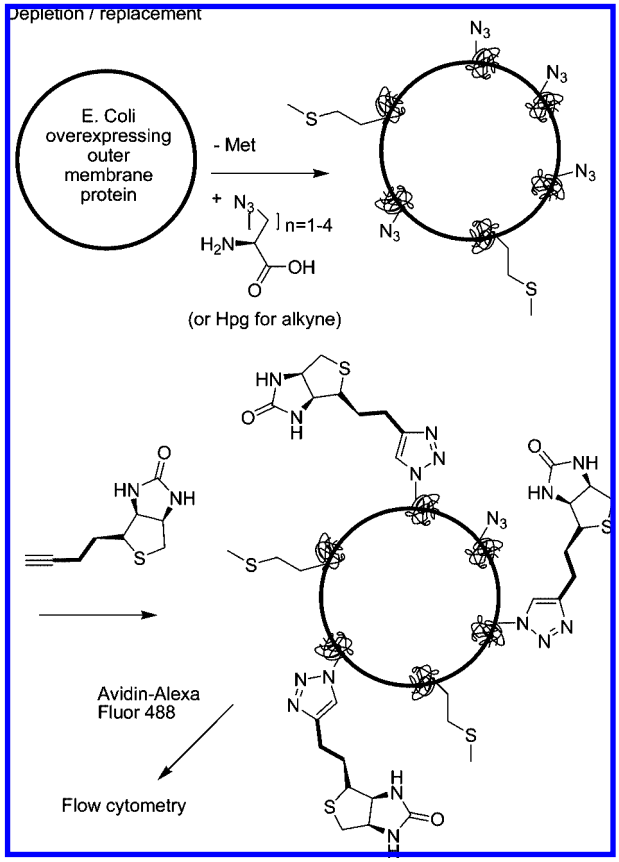


[a] The method should be broadly applicable to follow the intracellular conversion of specific substrates for transferase, synthase, and ligase.

alkyne- and azido-amino acids instead of Trp[33] could be obtained. Clean labeling of the purified protein was performed with dansyl and fluorescein fluorophores linked to either alkyne or azide using $CuSO_4$/Cu(0) catalysis.

The same conditions but with addition of TBTA were used to selectively PEGylate the modified SOD.[8] The labeling of alkyne containing myoglobin expressed in *E. coli* was performed in the same manner, and here it was found that

Scheme 35. Labeling of Proteins at the Cell Surface or Intracellularly Is Feasible through CuAAC[a]



[a] Proteins are derivatized either by depletion replacement or by supplementing the genetic code and developing mutant T-RNA synthase.

the addition of TBTA did not have a significant influence on the outcome of the labeling.[8] The TAG encoding has also been transferred to a phage display system where a range of unnatural amino acids were incorporated and azido-phenylalanine containing phages were labeled with dye under dilute $CuSO_4$/TCEP/TBTA conditions to avoid precipitation of phage.[182]

Iida et al.[425] took a similar approach to selectively attach viologen, a powerful electron donor, to cytocrome c_3 in order to study the electron transfer driven by photoexcitation of the Ru(2) complex. The ligation of the viologen azide to the purified *O*-propargyl tyrosine containing cytocrome c_3 was performed with $CuSO_4$/ascorbate in the presence of bathophenanthroline disulfonic acid.

Finn's group has worked extensively on the ligation of ligands and even protein to the capsules of viral particles. Initially, this was performed on cowpea mosaic virus by oxidative coupling of bis-(3-azidopropyl) cystine amide to the 3 position of tyrosines on the surface of the capsid, and dye coupling to the incorporated azides via CuAAC could be demonstrated.[228] In seminal work, less complex functionalization of the capsid with an NHS ester of an azido acid spacer allowed the attachment of large neoglycoconjugates obtained through ATRP of methacryloxyethyl-β-D-glucopyranoside. A fluorescein-bis-alkyne was used to label the glycoconjugate through CuAAC prior to formation of the second triazole with the azide on the virus particles.[356] Zhan et al.[181] labeled the same virus in a similar manner with a variety of hemicyanine dyes and found that the labeling result was highly dependent on the dye structure. However, the viral particles are also sensitive to the exact nature of the CuAAC conditions employed.

Complex glycans and even transferrin were linked to the virus using the optimized conditions of 1 mM of a Cu(1) source, e.g. $[Cu(CH_3CN)_4]OTf$, $[Cu(CH_3CN)_4]PF_6$, or CuBr with 3 equiv of bathophenanthroline disulfonate to protect both Cu(1) and the viral particle against decomposition. A larger excess of the catalyst slowed down the reaction significantly.[355] Horse spleen ferritin (HSF) is a capsular 24 subunit iron storage protein complex, which was acylated with propiolic acid and labeled with 3-azido-7-hydroxycoumarin on four exposed lysines/subunit in a similar manner, taking advantage of the CuBr/bathophenanthroline disulfonate conditions developed by Finn's group.[308]

Chemical protein−protein ligation to produce multivalent proteins or homo- and heterodimers mimics important biological functions and may be used in a manner different from the natural dimerization process. The selective ligation of proteins by formation of a single bond by chemical means is a difficult task. Natarajan et al.[220] dimerized scFv from monoclonal antibodies toward MUC1 on human breast and prostate cancer cell lines. Bromoacetamidoethyl azidoethyl diethyleneglycol was linked by reaction with a free cysteine to scFv. Tetra(propargyloxymethyl) methane was also reacted by CuAAC with the linker and subsequently attached to scFv. The two proteins were ligated in 74% yield using $CuSO_4$/ascorbate, and the dimer showed significantly improved binding to MUC1 and selectively to the cancer cell lines investigated.

Site specific *C*-terminal ligation and greatly improved microarray immobilization of green fluorescent protein (EGFP) and maltose binding protein (MBP) were achieved using intein technology and cysteine propargyl and azidopropyl amide as nucleophiles.[224] The propargylated proteins were ligated with fluorescein, biotin, glycosides, and glycopeptides. Most significantly, microarraying on azide modified glass slides of the alkyne modified proteins using $CuSO_4$/TCEP in PBS provided high quality microarrays with excellent performance in fluorescence intensity (EGFP) and binding of biotinylated maltose (MBP) compared to conventional unspecific immobilization techniques. Kalia and Raines[227] used a similar approach to introduce *N*-(2-azidoethyl)-2-hydrazino-acetamide and *N*-(2-azido-ethyl)-4-hydrazino-butyramide as nucleophiles at the *C*-terminal of RNase A−intein constructs. The reaction of nucleophile with thioester was performed while the ester was immobilized on chitin by a chitin binding fragment fused to the intein, and the azido labeled RNase A was retrieved in almost pure form by simple elution. The protein labeling was performed with $CuSO_4$/TCEP/TBTA in EtOH/PBS.

Conjugation of protein to polymers, DNA, lipids, and other proteins and peptides facilitates construction of neoproteins with specific biological performance that may be useful in e.g. biochemical investigations, vaccine development, and biomolecular targeting.

BSA has been reacted on a free thiol with *N*-propargyl maleimide and coupled by CuAAC ($CuSO_4$/ascorbate, THF/PB, pH 7.2) to polystyrene, terminated with azide as described in the polymer section, to form giant amphiphiles with micellar structure in aqueous buffer.[233]

Humenik et al.[250] have utilized a system for in vitro production of protein with *C*-terminally retained azido derivatized puromycin by inhibition of release factor 1 during

expression for conjugation of protein to oligodeoxynucleotides (ODN). 10- and 24-mer ODN's were derivatized as 5′-aminohexyl phosphodiesters, and the amino group was reacted with 4-pentynoic acid NHS ester to install the alkyne. $CuSO_4$/TCEP in PB, pH 7.9, was used to ligate protein and DNA. The shorter ODN gave a quantitative ligation reaction while the 24-mer afforded ∼50% of **162**. The protein−DNA conjugate was hybridized to DNA on a gold electrode, and binding was measured electrochemically. Musiol et al.[226] quantitatively conjugated azidoethyl phospholipids to a 17-mer homopropargylglycine containing peptide from human prion protein by CuAAC. After fluorescence labeling, the distribution of the peptide-lipid conjugate in HeLa cells was investigated.

Galactosylated neo-glycospingolipids, in which the lipid amide was replaced by an azido group and coupled with various lipidic-alkynes by CuAAC, were used in immunization studies. Triazole-conjugates from long alkyne chains were active in IFN-γ and IL-4 production when injected into mice.[426]

Photoaffinity (PA) probes for biochemistry are valuable tools for investigation of protein ligand interaction. Ballell et al.[198] produced a multivalent PA-ligand for galectin-3. To this end, 2-[2-(2-azido-ethoxy)-ethoxy]-ethyl 2,3,6,2′,4′,6′-hexa-*O*-acetyl-β-D-lactoside carrying a 4-benzoylphenylmethyl PA-group in the 3′ position was linked to 3,5-dipropargyloxybenzoic acid by CuAAC using microwave heating and $CuSO_4$/ascorbate. After deacetylation and amidation with propargylamine, the divalent probe was used to label galectin-3 and attach fluorescein-azide to the labeled protein via the propargylamide using CuAAC again.

Similarly, Zhao et al.[48] prepared HIV-1 integrase-PA-ligands containing biotin, linked via $(amidohexanoyl)_n$ amidomethyl triazole and the benzoylbenzene photoaffinity core to the inhibitory 2,4-dioxobutanoic acid. They demonstrated that the length of the linker influenced the inhibition of the integrase.

Li and Bittman[60] labeled a cholesterol-azide with BODIPY fluorophores through CuAAC for membrane studies. The BODIPY was linked through C5 to 4-vinylphenylacetylene or through C9 to 4-phenylacetylene, which were coupled to the cholesterol-azide using CuI, affording the triazole products, giving rise to fluorescence emission at ∼580 or ∼505 nm, respectively.

10. Other Methods for Triazole Synthesis

A range of intramolecular reactions have been described in which the arrangement of alkyne and azide is determined on the scaffold to which they are attached directing the thermal reaction toward the 1,5-substituted triazole.[153,427–429] A similar principle is at work in protein templated assembly of triazoles in which the directionality of the triazole formation is determined by the architecture of the binding pocket and the substrate.[430–433]

However, general selective methods for synthesis of 1,5-substituted triazoles are also available. These may be divided into four types: the reaction of terminal alkyne-Grignard reagents with azides,[430,434–437] thermal reaction driven by the bulkiness of TMS-alkyne compounds,[438] the Harvey approach[439] of thermal reaction of azides with (3-chloroacetonylidede)triphenylphosphorane,[440] and importantly, the recently developed direct ruthenium catalyzed reaction between alkynes and azides by Zhang et al.,[441] also investigated by Majireck et al.[442]

In the Grignard approach, which is the most established method, the terminal nitrogen of the azide reacts in a stepwise manner with the terminally metallated alkyne followed by ring closure to form the 4-magnesiotriazole.[436] This may be followed by a transmetalation reaction with $ZnCl_2$ and arylation at the 4-position using arylhalides and a palladium catalyst.

The Ru catalyzed reaction does not involve ruthenium acetylides, and catalysis is observed for both terminal and internal[442] alkynes. Ruthenium also catalyzes the trimerization of alkynes to form aromatic compounds, and the triazole formation is considered an intercept of this reaction by addition of the azide.[441]

Raghavendra and Lam[443] have reported an elegant solid phase method for selective synthesis of 1,4-triazoles. Polystyrenesulfonyl hydrazide was reacted with e.g. dichloromethylketones, and the resulting hydrazone reacted with amines to form the 1,4-substituted triazole with simultaneous cleavage off the resin.

Reaction of *Z*-disubstituted nitrovinyl compounds with $TMS\text{-}N_3$[444] or NaN_3[445] provides the 4,5-disubstituted triazoles in high yield.

The reaction of internal α-chloroalkynes with NaN_3 provides the 5-(α-azidoalkyl) substituted triazole through a proximity driven stepwise reaction of azide substitution, allelic rearrangement, triazole formation, and, finally, nucleophilic substitution with excess azide. If other nucleophiles are added at the second step of the reaction, these may react and replace the second azide.[367]

A palladium catalyzed three-component reaction between internal alkynes, $TMS\text{-}N_3$, and allylcarbonate[446] also led to 4,5-disubstituted triazoles. In this palladium cycle, the allyl-azide-palladium complex is initially formed and reaction with the alkyne gives the 1-allyl-triazole, which via the allyl palladium intermediate rearranges to the thermodynamically more stable 2-allyl-4,5-disubstituted triazole.

Baskin et al.[447] have developed a promising ligation method, which takes advantage of the steric strain of cyclooctyne to lower the $\Delta G^{\ddagger}$ of the transition state of uncatalyzed triazole formation and therefore allow biochemical ligation in the absence of Cu(1). This could be a potentially very useful reaction for future in vivo chemical ligations.

11. Conclusion

The CuAAC reaction is one of the most versatile organic reactions available to the scientific community at large, from biology to material sciences, and may be used to "click" fragments together as molecular LEGO's to form complex molecular architectures. The simplicity and robustness of the reaction has led to application in almost any field of chemistry and biochemistry. The outcome of the reaction is reliable and predictable, leading in all cases to exclusive formation of the 1,4-isomer. The reaction is complex and has been demonstrated to involve clusters of Cu(1) and ligands. The extreme specificity for 1,4-triazole formation may be explained through simple six-membered transition states involving two Cu(1) atoms on a Cu-cluster. The importance of maintaining a good concentration of Cu(1) throughout the reaction cannot be emphasized enough, and this calls for complete exclusion of oxygen from the reaction medium, particularly in the case of less reactive reaction components or dilute reactions. The role of auxiliary ligands in protecting the Cu(1) from oxidation or disproportionation

is very important. The ligands also influence the structure and equilibria of Cu(1) clusters and may promote the formation of the most catalytically active Cu(1) complexes.

Importantly, the CuAAC is extremely selective and the conditions of the reaction may be designed to accommodate nearly all types of protecting groups and reactive intermediates. This orthogonality extends into biological systems where alkyne and azide are essentially absent, and therefore, CuAAC-based ligations can be performed with exquisite selectivity even in the living cell. Only the Staudinger ligation shows equivalent performance; however, the ease with which both azide and alkyne amino acids are incorporated into proteins holds a lot of promise for the biochemical CuAAC ligation, and the specific and direct chemical ligation of two or more proteins within the cellular environment should be feasible in the near future.

The continuously ongoing development of new ligands and conditions for the CuAAC is an important prerequisite for the development of the reaction. The recently discovered activity of histidine peptides for self-catalysis may be an important discovery for the introduction of highly specific catalytic constructs into the structure of proteins in the cell.

The unique property of the CuAAC as a quantitative ligation reaction is facilitated through the large ΔG of reaction and the significant lowering of $\Delta G^{\ddagger}$ in the presence of Cu(1). This in turn is caused formation of the Cu-acetylide, which provides extreme polarization of the triple bond in combination with coordination of the azide on the Cu(1) cluster. In the search for new ligation reactions, these parameters as well as chemical selectivity therefore seem to be crucial for success.

12. Abbreviations

Asc	ascorbate
Batho	bathophenanthrolinedisulphate
Bipy	2,2′-bipyridine
Bim	tripotassium tris(1-(4-carboxybutyl)benzimidazo-2-ylmethyl)amine
BMAH	1*S*,2*S*-bis(methylamino)hexane
DIPEA	diisopropylethylamine
dNbipy	4,4′-di(5-nonyl)-2,2′-bipyridine
Lut	2,6-lutidine
PB	phosphate buffer
Phen	1,10-phenanthroline
PMDETA	*N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine
SFM	serum free medium
TCEP	tri(carboxyethyl)phosphine
TBTA	tri(1-benzyl-[1,2,3]-triazol-4-ylmethyl)amine
TRMEDA	*N*,*N*,*N*′-trimethylethylenediamine
TTA	tris(triazoyl)amine, most likely = TBTA

13. Acknowledgments

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14. References

(1) Huisgen, R. *Pure Appl. Chem.* **1989**, *61*, 613.
(2) Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1967**, *100*, 2494.
(3) Tornøe, C. W.; Meldal, M. *Peptidotriazoles: Copper(I)-catalyzed 1,3-dipolar cycloadditions on solid-phase, Peptides 2001, Proc. Am. Pept. Symp.*; American Peptide Society and Kluwer Academic Publishers: San Diego, 2001; pp 263−264.
(4) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057.
(5) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, B. K. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596.
(6) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686.
(7) Beatty, K. E.; Xie, F.; Wang, Q.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 14150.
(8) Deiters, A.; Schultz, P. G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1521.
(9) Golas, P. L.; Tsarevsky, N. V.; Sumerlin, B. S.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 6451.
(10) Wu, P.; Fokin, V. V. *Aldrich Chim. Acta* **2007**, *40*, 7.
(11) Michael, A. *J. Prakt. Chem.* **1893**, *48*, 94.
(12) Lwowski, W. *Azides and Nitrous Oxide, 1,3-Dipolar Cycloaddition Chemistry*; J. Wiley and Sons: New York, 1984; pp 559−651.
(13) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128.
(14) Bock, V. D.; Hiemstra, H.; Van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51.
(15) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007**, *28*, 15.
(16) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018.
(17) Gil, M V.; Arévalo, M. J.; López, Ó. *Synthesis* **2007**, 1589.
(18) Li, Y.; Ju, Y.; Zhao, Y. F. *Chin. J. Org. Chem.* **2006**, *26*, 1640.
(19) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249.
(20) Harju, K.; Yli-Kauhaluoma, J. *Mol. Diversity* **2005**, *9*, 187.
(21) Cozzi, P. G.; Hilgraf, R.; Zimmermann, N. *Eur. J. Org. Chem.* **2004**, 4095.
(22) Bräse, S.; Gil, C.; Knepper, K.; Zimmermann, V. *Angew. Chem., Int. Ed.* **2005**, *44*, 5188.
(23) Lecomte, P.; Riva, R.; Schmeits, S.; Rieger, J.; Van Butsele, K.; Jérôme, C.; Jérôme, R. *Macromol. Symp.* **2006**, *240*, 157.
(24) Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200.
(25) Read, E. S.; Armes, S. P. *Chem. Commun.* **2007**, 3021.
(26) Goodall, G. W.; Hayes, W. *Chem. Soc. Rev.* **2006**, *35*, 280.
(27) Evans, R. A. *Aust. J. Chem.* **2007**, *60*, 384.
(28) Yeo, D. S. Y.; Srinivasan, R.; Chen, G. Y. J.; Yao, S. Q. *Chem.−Eur. J.* **2004**, *10*, 4664.
(29) Köhn, M.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2004**, *43*, 3106.
(30) Chen, L.; Li, C. J. *Adv. Synth. Catal.* **2006**, *348*, 1459.
(31) Kaiser, J.; Kinderman, S. S.; Van Esseveldt, B. C. J.; Van Delft, F. L.; Schoemaker, H. E.; Blaauw, R. H.; Rutjes, F. P. J. T. *Org. Biomol. Chem.* **2005**, *3*, 3435.
(32) Angell, Y. L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674.
(33) Breinbauer, R.; Köhn, M. *ChemBioChem* **2003**, *4*, 1147.
(34) Dong, W. L.; Zhao, W. G.; Li, Y. X.; Liu, Z. X.; Li, Z. M. *Chin. J. Org. Chem.* **2006**, *26*, 271.
(35) Durek, T.; Becker, C. F. W. *Biomol. Eng.* **2005**, *22*, 153.
(36) Evans, M. J.; Cravatt, B. F. *Chem. Rev.* **2006**, *106*, 3279.
(37) Dondoni, A. *Chem.−Asian J.* **2007**, *2*, 700.
(38) Brik, A.; Wu, C. Y.; Wong, C. H. *Org. Biomol. Chem.* **2006**, *4*, 1446.
(39) Groth, T.; Renil, M.; Meinjohanns, E. *Comb. Chem. High Throughput Screening* **2003**, *6*, 589.
(40) Jones, G. O.; Ess, D. H.; Houk, K. N. *Helv. Chim. Acta* **2005**, *88*, 1702.
(41) Pérez, P.; Domingo, L. R.; Aurell, M. J.; Contreras, R. *Tetrahedron* **2003**, *59*, 3117.
(42) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Noodleman, L.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 210.
(43) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2210.
(44) Straub, B. F. *Chem. Commun.* **2007**, 3868.
(45) Meng, J. C.; Fokin, V. V.; Finn, M. G. *Tetrahedron Lett.* **2005**, *46*, 4543.
(46) Partyka, D. V.; Updegraff, J. B., III; Zeller, M.; Hunter, A. D.; Gray, T. G. *Organometallics* **2007**, *26*, 183.
(47) Zhou, Z.; Fahrni, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 8862.
(48) Zhao, X. Z.; Semenova, E. A.; Liao, C.; Nicklaus, M.; Pommier, Y.; Burke, J. *Bioorg. Med. Chem.* **2006**, *14*, 7816.
(49) Chowdhury, C.; Mandal, S. B.; Achari, B. *Tetrahedron Lett.* **2005**, *46*, 8531.
(50) Baut, N. L.; Díaz, D. D.; Punna, S.; Finn, M. G.; Brown, H. R. *Polymer* **2007**, *48*, 239.
(51) Hassane, F. S.; Frisch, B.; Schuber, F. *Bioconjugate Chem.* **2006**, *17*, 849.
(52) Lee, B. Y.; Park, S. R.; Jeon, H. B.; Kim, K. S. *Tetrahedron Lett.* **2006**, *47*, 5105.
(53) Giguére, D.; Patnam, R.; Bellefleur, M. A.; St Pierre, C.; Sato, S.; Roy, R. *Chem. Commun.* **2006**, 2379.
(54) Pirali, T.; Tron, G. C.; Zhu, J. *Org. Lett.* **2006**, *8*, 4145.
(55) Franke, R.; Doll, C.; Eichler, J. *Tetrahedron Lett.* **2005**, *46*, 4479.
(56) Font, D.; Jimeno, C.; Pericás, M. A. *Org. Lett.* **2006**, *8*, 4653.
(57) Roice, M.; Johannsen, I.; Meldal, M. *QSAR Comb. Sci.* **2004**, *23*, 662.

(58) Carroll, J. B.; Jordan, B. J.; Xu, H.; Erdogan, B.; Lee, L.; Cheng, L.; Tiernan, C.; Cooke, G.; Rotello, V. M. *Org. Lett.* **2005**, *7*, 2551.
(59) Molander, G. A.; Ham, J. *Org. Lett.* **2006**, *8*, 2767.
(60) Li, Z.; Bittman, R. *J. Org. Chem.* **2007**, *72*, 8376.
(61) Detz, R. J.; Heras, S. A.; De Gelder, R.; Van Leeuwen, P. W. N. M.; Hiemstra, H.; Reek, J. N. H.; Van Maarseveen, J. H. *Org. Lett.* **2006**, *8*, 3227.
(62) Oyelere, A. K.; Chen, P. C.; Yao, L. P.; Boguslavsky, N. *J. Org. Chem.* **2006**, *71*, 9791.
(63) Li, H.; Cheng, F.; Duft, A. M.; Adronov, A. *J. Am. Chem. Soc.* **2005**, *127*, 14518.
(64) Liang, C. H.; Yao, S.; Chiu, Y. H.; Leung, P. Y.; Robert, N.; Seddon, J.; Sears, P.; Hwang, C. K.; Ichikawa, Y.; Romero, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1307.
(65) Romero, A.; Liang, C. H.; Chiu, Y. H.; Yao, S.; Duffield, J.; Sucheck, S. J.; Marby, K.; Rabuka, D.; Leung, P. Y.; Shue, Y. K.; Ichikawa, Y.; Hwang, C. K. *Tetrahedron Lett.* **2005**, *46*, 1483.
(66) Dondoni, A.; Marra, A. *J. Org. Chem.* **2006**, *71*, 7546.
(67) Weterings, J. J.; Khan, S.; van der Heden, G. J.; Drijfhout, J. W.; Melief, C. J. M.; Overkleeft, H. S.; van der Burg, S. H.; Ossendorp, F.; van der Marel, G. A.; Filippov, D. V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3258.
(68) Rossi, L. L.; Basu, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3596.
(69) Salameh, B. A.; Leffler, H.; Nilsson, U. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3344.
(70) Cheshev, P.; Marra, A.; Dondoni, A. *Org. Biomol. Chem.* **2006**, *4*, 3225.
(71) Looper, R. E.; Pizzirani, D.; Schreiber, S. L. *Org. Lett.* **2006**, *8*, 2063.
(72) Dondoni, A.; Giovannini, P. P.; Massi, A. *Org. Lett.* **2004**, *6*, 2929.
(73) Tejler, J.; Skogman, F.; Leffler, H.; Nilsson, U. J. *Carbohydr. Res.* **2007**, *342*, 1869.
(74) Choi, W. J.; Shi, Z. D.; Worthy, K. M.; Bindu, L.; Karki, R. G.; Nicklaus, M. C.; Fisher, R. J.; Burke, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5265.
(75) Punna, S.; Kuzelka, J.; Wang, Q.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2215.
(76) Jang, H.; Fafarman, A.; Holub, J. M.; Kirshenbaum, K. *Org. Lett.* **2005**, *7*, 1951.
(77) Tornøe, C. W.; Sanderson, S. J.; Mottram, J. C.; Coombs, G. H.; Meldal, M. *J. Comb. Chem.* **2004**, *6*, 312.
(78) Xu, W. M.; Huang, X.; Tang, E. *J. Comb. Chem.* **2005**, *7*, 726.
(79) Conte, G.; Ely, F.; Gallardo, H. *Liq. Cryst.* **2005**, *32*, 1213.
(80) Cristiano, R.; Santos, D. M. P. D.; Conte, G.; Gallardo, H. *Liq. Cryst.* **2006**, *33*, 997.
(81) Gallardo, H.; Ely, F.; Bortoluzzi, A. J.; Conte, G. *Liq. Cryst.* **2005**, *32*, 667.
(82) Wu, Y. M.; Deng, J.; Fang, X.; Chen, Q. Y. *J. Fluorine Chem.* **2004**, *125*, 1415.
(83) Petchprayoon, C.; Suwanborirux, K.; Miller, R.; Sakata, T.; Marriott, G. *J. Nat. Prod.* **2005**, *68*, 157.
(84) Li, H.; Riva, R.; Jérôme, R.; Lecomte, P. *Macromolecules* **2007**, *40*, 824.
(85) Riva, R.; Schmeits, S.; Jérôme, C.; Jérôme, R.; Lecomte, P. *Macromolecules* **2007**, *40*, 796.
(86) Ågren, J. K. M.; Billing, J. F.; Grundberg, H. E.; Nilsson, U. J. *Synthesis* **2006**, 3141.
(87) Tejler, J.; Tullberg, E.; Frejd, T.; Leffler, H.; Nilsson, U. J. *Carbohydr. Res.* **2006**, *341*, 1353.
(88) Kacprzak, K. *Synlett* **2005**, 943.
(89) Opsteen, J. A.; Van Hest, J. C. M. *Chem. Commun.* **2005**, *11*, 57.
(90) Bodine, K. D.; Gin, D. Y.; Gin, M. S. *Org. Lett.* **2005**, *7*, 4479.
(91) Bodine, K. D.; Gin, D. Y.; Gin, M. S. *J. Am. Chem. Soc.* **2004**, *126*, 1638.
(92) Slater, M.; Snauko, M.; Svec, F.; Fréchet, J. M. J. *Anal. Chem.* **2006**, *78*, 4969.
(93) Díaz, D. D.; Punna, S.; Holzer, P.; Mcpherson, A. K.; Sharpless, K. B.; Fokin, V. V.; Finn, M. G. *J. Polym. Sci.* **2004**, *42*, 4392.
(94) Yoo, E. J.; Ahlquist, M.; Kim, S. H.; Bae, I.; Fokin, V. V.; Sharpless, K. B.; Chang, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 1730.
(95) Li, Z.; Seo, T. S.; Ju, J. *Tetrahedron Lett.* **2004**, *45*, 3143.
(96) Zhang, Z.; Fan, E. *Tetrahedron Lett.* **2006**, *47*, 665.
(97) Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett.* **2006**, *47*, 6681.
(98) Hotha, S.; Kashyap, S. *J. Org. Chem.* **2006**, *71*, 364.
(99) Kacprzak, K. M.; Maier, N. M.; Lindner, W. *Tetrahedron Lett.* **2006**, *47*, 8721.
(100) Bertrand, P.; Gesson, J. P. *J. Org. Chem.* **2007**, *72*, 3596.
(101) Billing, J. F.; Nilsson, U. J. *J. Org. Chem.* **2005**, *70*, 4847.
(102) Luo, S.; Xu, H.; Mi, X.; Li, J.; Zheng, X.; Cheng, J. P. *J. Org. Chem.* **2006**, *71*, 9244.
(103) Patterson, A. W.; Wood, W. J. L.; Hornsby, M.; Lesley, S.; Spraggon, G.; Ellman, J. A. *J. Med. Chem.* **2006**, *49*, 6298.
(104) Wood, W. J. L.; Patterson, A. W.; Tsuruoka, H.; Jain, R. K.; Ellman, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 15521.
(105) Reck, F.; Zhou, F.; Girardot, M.; Kern, G.; Eyermann, C. J.; Hales, N. J.; Ramsay, R. R.; Gravestock, M. B. *J. Med. Chem.* **2005**, *48*, 499.
(106) Díaz, D. D.; Rajagopal, K.; Strable, E.; Schneider, J.; Finn, M. G. *J. Am. Chem. Soc.* **2006**, *128*, 6056.
(107) Horne, W. S.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 9372.
(108) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366.
(109) Coady, D. J.; Bielawski, C. W. *Macromolecules* **2006**, *39*, 8895.
(110) Wu, Y. M.; Deng, J.; Chen, Q. Y. *Synlett* **2006**, 645.
(111) Wu, Y. M.; Deng, J.; Li, Y.; Chen, Q. Y. *Synthesis* **2005**, 1314.
(112) Gheorghe, A.; Cuevas, Y.; Horn, J.; Bannwarth, W.; Narsaiah, B.; Reiser, O. *Synlett* **2006**, 2767.
(113) Lee, J. W.; Kim, B. K. *Synthesis* **2006**, 615.
(114) Holub, J. M.; Jang, H.; Kirshenbaum, K. *Org. Biomol. Chem.* **2006**, *4*, 1497.
(115) Girard, C.; Önen, E.; Aufort, M.; Beauvière, S.; Samson, E.; Herscovici, J. *Org. Lett.* **2006**, *8*, 1689.
(116) Van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503.
(117) Yoo, E. J.; Bae, I.; Cho, S. H.; Han, H.; Chang, S. *Org. Lett.* **2006**, *8*, 1347.
(118) Yan, Z. Y.; Zhao, Y. B.; Fan, M. J.; Liu, W. M.; Liang, Y. M. *Tetrahedron* **2005**, *61*, 9331.
(119) Löber, S.; Gmeiner, P. *Tetrahedron* **2004**, *60*, 8699.
(120) Kümin, M.; Sonntag, L. S.; Wennemers, H. *J. Am. Chem. Soc.* **2007**, *129*, 466.
(121) Gissibl, A.; Padié, C.; Hager, M.; Jaroschik, F.; Rasappan, R.; Cuevas-Yanez, E.; Turrin, C. O.; Caminade, A. M.; Majoral, J. P.; Reiser, O. *Org. Lett.* **2007**, *9*, 2895.
(122) Goess, B. C.; Hannoush, R. N.; Chan, L. K.; Kirchhausen, T.; Shair, M. D. *J. Am. Chem. Soc.* **2006**, *128*, 5391.
(123) Fazio, F.; Bryan, M. C.; Blixt, O.; Paulson, J. C.; Wong, C. H. *J. Am. Chem. Soc.* **2002**, *124*, 14397.
(124) Bryan, M. C.; Fazio, F.; Lee, H. K.; Huang, C. Y.; Chang, A.; Best, M. D.; Calarese, D. A.; Blixt, O.; Paulson, J. C.; Burton, D.; Wilson, I. A.; Wong, C. H. *J. Am. Chem. Soc.* **2004**, *126*, 8640.
(125) Fu, X.; Albermann, C.; Zhang, C.; Thorson, J. S. *Org. Lett.* **2005**, *7*, 1513.
(126) Goncalves, V.; Gautier, B.; Regazzetti, A.; Coric, P.; Bouaziz, S.; Garbay, C.; Vidal, M.; Inguimbert, N. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5590.
(127) Gopi, H. N.; Tirupula, K. C.; Baxter, S.; Ajith, S.; Chaiken, I. M. *ChemMedChem* **2006**, *1*, 54.
(128) Yang, D.; Fu, N.; Liu, Z.; Li, Y.; Chen, B. *Synlett* **2007**, 278.
(129) Tanaka, K.; Kageyama, C.; Fukase, K. *Tetrahedron Lett.* **2007**, *48*, 6475.
(130) Sreedhar, B.; Reddy, P. S. *Synth. Commun.* **2007**, *37*, 805.
(131) Malow, M.; Wehrstedt, K. D.; Neuenfeld, S. *Tetrahedron Lett.* **2007**, *48*, 1233.
(132) Alam, M. S.; Kajiki, R.; Hanatani, H.; Kong, X.; Ozoe, F.; Matsui, Y.; Matsumura, F.; Ozoe, Y. *J. Agric. Food Chem.* **2006**, *54*, 1361.
(133) Gissibi, A.; Finn, M. G.; Reiser, O. *Org. Lett.* **2005**, *7*, 2325.
(134) Derbré, S.; Roué, G.; Poupon, E.; Susin, S. A.; Hocquemiller, R. *ChemBioChem* **2005**, *6*, 979.
(135) Adam, G. C.; Vanderwal, C. D.; Sorensen, E. J.; Cravatt, B. F. *Angew. Chem., Int. Ed.* **2003**, *42*, 5480.
(136) Gartner, Z. J.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370.
(137) Dörner, S.; Westermann, B. *Chem. Commun.* **2005**, 2852.
(138) Kuijpers, B. H. M.; Groothuys, S.; Keereweer, A. R.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; Van Delft, F. L.; Rutjes, F. P. J. T. *Org. Lett.* **2004**, *6*, 3123.
(139) Luxenhofer, R.; Jordan, R. *Macromolecules* **2006**, *39*, 3509.
(140) Loren, J. C.; Krasinski, A.; Fokin, V. V.; Sharpless, K. B. *Synlett* **2005**, 2847.
(141) Zinzalla, G.; Milroy, L. G.; Ley, S. V. *Org. Biomol. Chem.* **2006**, *4*, 1977.
(142) Sirion, U.; Kim, H. J.; Lee, J. H.; Seo, J. W.; Lee, B. S.; Lee, S. J.; Oh, S. J.; Chi, D. Y. *Tetrahedron Lett.* **2007**, *48*, 3953.
(143) Yan, Z. Y.; Niu, Y. N.; Wei, H. L.; Wu, L. Y.; Zhao, Y. B.; Liang, Y. M. *Tetrahedron: Asymmetry* **2006**, *17*, 3288.
(144) Ma, D. Y.; Wang, D. X.; Zheng, Q. Y.; Wang, M. X. *Tetrahedron: Asymmetry* **2006**, *17*, 2366.
(145) Li, Y.; Huffman, J. C.; Flood, A. H. *Chem. Commun.* **2007**, 2692.
(146) Kosiova, I.; Kovackova, S.; Kois, P. *Tetrahedron* **2007**, *63*, 312.
(147) Yap, A. H.; Weinreb, S. M. *Tetrahedron Lett.* **2006**, *47*, 3035.
(148) O'Mahony, G.; Ehrman, E.; Grøtli, M. *Tetrahedron Lett.* **2005**, *46*, 6745.
(149) Meng, J. C.; Siuzdak, G.; Finn, M. G. *Chem. Commun.* **2004**, *10*, 2108.

(150) Feldman, A. K.; Colasson, B.; Sharpless, K. B.; Fokin, V. V. *J. Am. Chem. Soc.* **2005**, *127*, 13444.
(151) Suarez, P. L.; Gándara, Z.; Gómez, G.; Fall, Y. *Tetrahedron Lett.* **2004**, *45*, 4619.
(152) Suh, B. C.; Jeon, H.; Posner, G. H.; Silverman, S. M. *Tetrahedron Lett.* **2004**, *45*, 4623.
(153) Gracias, V.; Darczak, D.; Gasiecki, A. F.; Djuric, S. W. *Tetrahedron Lett.* **2005**, *46*, 9053.
(154) Ray, A.; Manoj, K.; Bhadbhade, M. M.; Mukhopadhyay, R.; Bhattacharjya, A. *Tetrahedron Lett.* **2006**, *47*, 2775.
(155) Burley, G. A.; Gierlich, J.; Mofid, M. R.; Nir, H.; Tal, S.; Eichen, Y.; Carell, T. *J. Am. Chem. Soc.* **2006**, *128*, 1398.
(156) Xu, G. L.; Ren, T. *Organometallics* **2005**, *24*, 2564.
(157) Sharpless, W. D.; Wu, P.; Hansen, T. V.; Lindberg, J. G. *J. Chem. Educ.* **2005**, *82*, 1833.
(158) Joralemon, M. J.; O'Reilly, R. K.; Matson, J. B.; Nugent, A. K.; Hawker, C. J.; Wooley, K. L. *Macromolecules* **2005**, *38*, 5436.
(159) Malkoch, M.; Vestberg, R.; Gupta, N.; Mespouille, L.; Dubois, P.; Mason, A. F.; Hedrick, J. L.; Liao, Q.; Frank, C. W.; Kingsbury, K.; Hawker, C. J. *Chem. Commun.* **2006**, 2774.
(160) Brennan, J. L.; Hatzakis, N. S.; Tshikhudo, T. R.; Dirvianskyte, N.; Razumas, V.; Patkar, S.; Vind, J.; Svendsen, A.; Nolte, R. J. M.; Rowan, A. E.; Brust, M. *Bioconjugate Chem.* **2006**, *17*, 1373.
(161) Wu, P.; Feldman, A. K.; Nugent, A. K.; Hawker, C. J.; Scheel, A.; Voit, B.; Pyun, J.; Fréchet, J. M. J.; Sharpless, K. B.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2004**, *43*, 3928.
(162) Wang, J.; Uttamchandani, M.; Li, J.; Hu, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 3821.
(163) Aher, N. G.; Pore, V. S. *Synlett* **2005**, 2155.
(164) Li, C.; Finn, M. G. *J. Polym. Sci.* **2006**, *44*, 5513.
(165) Zhu, Y.; Huang, Y.; Meng, W. D.; Li, H.; Qing, F. L. *Polymer* **2006**, *47*, 6272.
(166) Paul, A.; Bittermann, H.; Gmeiner, P. *Tetrahedron* **2006**, *62*, 8919.
(167) Dolhem, F.; Johansson, M. J.; Antonsson, T.; Kann, N. *J. Comb. Chem.* **2007**, *9*, 477.
(168) O'Neil, E. J.; DiVittorio, K. M.; Smith, B. D. *Org. Lett.* **2007**, *9*, 199.
(169) Gao, Y.; Eguchi, A.; Kakehi, K.; Lee, Y. C. *Bioorg. Med. Chem.* **2005**, *13*, 6151.
(170) Zhou, L.; Thakur, C. S.; Molinaro, R. J.; Paranjape, J. M.; Hoppes, R.; Jeang, K. T.; Silverman, R. H.; Torrence, P. F. *Bioorg. Med. Chem.* **2006**, *14*, 7862.
(171) Chen, S.; Galan, M. C.; Coltharp, C.; O'Connor, S. E. *Chem. Biol.* **2006**, *13*, 1137.
(172) Duval, R. A.; Poupon, E.; Romero, V.; Peris, E.; Lewin, G.; Cortes, D.; Brandt, U.; Hocquemiller, R. *Tetrahedron* **2006**, *62*, 6248.
(173) O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *J. Polym. Sci.* **2006**, *44*, 5203.
(174) Lin, H.; Walsh, C. T. *J. Am. Chem. Soc.* **2004**, *126*, 13998.
(175) Joralemon, M. J.; O'Reilly, R. K.; Hawker, C. J.; Wooley, K. L. *J. Am. Chem. Soc.* **2005**, *127*, 16892.
(176) Beckmann, H. S. G.; Wittmann, V. *Org. Lett.* **2007**, *9*, 1.
(177) Loethen, S.; Ooya, T.; Choi, H. S.; Yui, N.; Thompson, D. H. *Biomacromolecules* **2006**, *7*, 2501.
(178) Parrish, B.; Emrick, T. *Bioconjugate Chem.* **2007**, *18*, 263.
(179) Parrish, B.; Breitenkamp, R. B.; Emrick, T. *J. Am. Chem. Soc.* **2005**, *127*, 7404.
(180) Sun, X. L.; Stabler, C. L.; Cazalis, C. S.; Chaikof, E. L. *Bioconjugate Chem.* **2006**, *17*, 52.
(181) Zhan, W. H.; Barnhill, H. N.; Sivakumar, K.; Tian, H.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 1691.
(182) Tian, F.; Tsao, M. L.; Schultz, P. G. *J. Am. Chem. Soc.* **2004**, *126*, 15962.
(183) Fernandez-Megia, E.; Correa, J.; Riguera, R. *Biomacromolecules* **2006**, *7*, 3104.
(184) Oh, K.; Guan, Z. *Chem. Commun.* **2006**, 3069.
(185) Kacprzak, K.; Migas, M.; Plutecka, A.; Rychlewska, U.; Gawronski, J. *Heterocycles* **2005**, *65*, 1931.
(186) Xia, Y.; Fan, Z.; Yao, J.; Liao, Q.; Li, W.; Qu, F.; Peng, L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2693.
(187) Xia, Y.; Li, W.; Qu, F.; Fan, Z.; Liu, X.; Berro, C.; Rauzy, E.; Peng, L. *Org. Biomol. Chem.* **2007**, *5*, 1695.
(188) Rijkers, D. T. S.; Van Esse, G. W.; Merkx, R.; Brouwer, A. J.; Jacobs, H. J. F.; Pieters, R. J.; Liskamp, R. M. J. *Chem. Commun.* **2005**, 4581.
(189) Pore, V. S.; Aher, N. G.; Kumar, M.; Shukla, P. K. *Tetrahedron* **2006**, *62*, 11178.
(190) Hosoya, T.; Hiramatsu, T.; Ikemoto, T.; Aoyama, H.; Ohmae, T.; Endo, M.; Suzuki, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1289.
(191) Opsteen, J. A.; Brinkhuis, R. P.; Teeuwen, R. L. M.; Löwik, D. W. P. M.; Van Hest, J. C. M. *Chem. Commun.* **2007**, 3136.
(192) Xie, J.; Seto, C. T. *Bioorg. Med. Chem.* **2007**, *15*, 458.
(193) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603.
(194) Ciampi, S.; Böcking, T.; Kilian, K. A.; Jasmes, M.; Harper, J. B.; Gooding, J. J. *Langmuir* **2007**, *23*, 9320.
(195) Guo, Z.; Lei, A.; Liang, X.; Xu, Q. *Chem. Commun.* **2006**, 4512.
(196) Zhang, Y.; Luo, S.; Tang, Y.; Yu, L.; Hou, K. Y.; Cheng, J. P.; Zeng, X.; Wang, P. G. *Anal. Chem.* **2006**, *78*, 2001.
(197) Li, J.; Zheng, M.; Tang, W.; He, P. L.; Zhu, W.; Li, T.; Zuo, J. P.; Liu, H.; Jiang, H. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5009.
(198) Ballell, L.; Scherpenzeel, M. v.; Buchalova, K.; Liskamp, R. M. J.; Pieters, R. J. *Org. Biomol. Chem.* **2006**, 4387.
(199) Bew, S. P.; Brimage, R. A.; L'Hermite, N.; Sharma, S. V. *Org. Lett.* **2007**, *9*, 3713.
(200) Khanetskyy, B.; Dallinger, D.; Kappe, C. O. *J. Comb. Chem.* **2004**, *6*, 884.
(201) Xu, C. Y.; Huang, M. Z. *Chin. Chem. Lett.* **2006**, *17*, 883.
(202) Gouin, S. G.; Bultel, L.; Falentin, C.; Kovensky, J. *Eur. J. Org. Chem.* **2007**, 1160.
(203) Chen, W. Z.; Fanwick, P. E.; Ren, T. *Inorg. Chem.* **2007**, *46*, 3429.
(204) Touaibia, M.; Shiao, T. C.; Papadopoulos, A.; Vaucher, J.; Wang, Q.; Behamioud, K.; Roy, R. *Chem. Commun.* **2007**, 380.
(205) Chang, K. H.; Lee, L.; Chen, J.; Li, W. S. *Chem. Commun.* **2006**, *6*, 629.
(206) Lu, G.; Lam, S.; Burgess, K. *Chem. Commun.* **2006**, 1652.
(207) Mynar, J. L.; Choi, T. L.; Yoshida, M.; Kim, V.; Hawker, C. J.; Fréchet, J. M. J. *Chem. Commun.* **2005**, 5169.
(208) Lee, J. W.; Kim, J. H.; Kim, B. K. *Tetrahedron Lett.* **2006**, *47*, 2683.
(209) Seela, F.; Sirivolu, V. R. *Chem. Biodiversity* **2006**, *3*, 509.
(210) Lee, J. W.; Kim, B. K.; Kim, J. H.; Shin, W. S.; Jin, S. H. *J. Org. Chem.* **2006**, *71*, 4988.
(211) Bakbak, S.; Leech, P. J.; Carson, B. E.; Saxena, S.; King, W. P.; Bunz, U. H. F. *Macromolecules* **2006**, *39*, 6793.
(212) Lee, J. W.; Kim, B. K.; Kim, H. J.; Han, S. C.; Shin, W. S.; Jin, S. H. *Macromolecules* **2006**, *39*, 2418.
(213) Helms, B.; Mynar, J. L.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020.
(214) Ornelas, C.; Ruiz Aranzaes, J.; Cloutet, E.; Alves, S.; Astruc, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 872.
(215) Ryu, E. H.; Zhao, Y. *Org. Lett.* **2005**, *7*, 1035.
(216) Lee, J. W.; Kim, J. H.; Kim, B. K.; Kim, J. H.; Shin, W. S.; Jin, S. H. *Tetrahedron* **2006**, *62*, 9193.
(217) Gou, Z.; Lei, A.; Zhang, Y.; Xu, Q.; Xue, X.; Zhang, F.; Liang, X. *Chem. Commun.* **2007**, 2491.
(218) Tao, C. Z.; Cui, X.; Li, J.; Liu, A. X.; Liu, L.; Guo, Q. X. *Tetrahedron Lett.* **2007**, *48*, 3525.
(219) Zhu, L.; Lynch, V. M.; Anslyn, E. V. *Tetrahedron* **2004**, *60*, 7267.
(220) Natarajan, A.; Du, W.; Xiong, C. Y.; DeNardo, G. L.; DeNardo, S. L.; Gervay-Hague, J. *Chem. Commun.* **2007**, 695.
(221) Miljanic, O. S.; Dichtel, W. R.; Mortezaei, S.; Stoddart, J. F. *Org. Lett.* **2006**, *8*, 4835.
(222) Dichtel, W. R.; Miljanic, O. S.; Spruell, J. M.; Heath, J. R.; Stoddart, J. F. *J. Am. Chem. Soc.* **2006**, *128*, 10388.
(223) Miljanić, O. S.; Dichtel, W. R.; Khan, S. I.; Mortezaei, S.; Heath, J. R.; Stoddart, J. F. *J. Am. Chem. Soc.* **2007**, *129*, 8236.
(224) Lin, P. C.; Ueng, S. H.; Tseng, M. C.; Ko, J. L.; Huang, K. T.; Yu, S. C.; Adak, A. K.; Chen, Y. J.; Lin, C. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 4286.
(225) Beatty, K. E.; Liu, J. C.; Xie, F.; Dieterich, D. C.; Schuman, E. M.; Wang, Q.; Tirrell, D. A. *Angew. Chem., Int. Ed.* **2006**, *45*, 7364.
(226) Musiol, H. J.; Dong, S.; Kaiser, M.; Bausinger, R.; Zumbusch, A.; Bertsch, U.; Moroder, L. *ChemBioChem* **2005**, *6*, 625.
(227) Kalia, J.; Raines, R. T. *ChemBioChem* **2006**, *7*, 1375.
(228) Meunier, S.; Strable, E.; Finn, M. G. *Chem. Biol.* **2004**, *11*, 319.
(229) Link, A. J.; Tirrell, D. A. *J. Am. Chem. Soc.* **2003**, *125*, 11164.
(230) Van Der Peet, P.; Gannon, C. T.; Walker, I.; Dinev, Z.; Angelin, M.; Tam, S.; Ralton, J. E.; McConville, M. J.; Williams, S. J. *ChemBioChem* **2006**, *7*, 1384.
(231) Decréau, R. A.; Collman, J. P.; Yang, Y.; Yan, Y.; Devaraj, N. K. *J. Org. Chem.* **2007**, *72*, 2794.
(232) Devaraj, N. K.; Miller, G. P.; Ebina, W.; Kakaradov, B.; Collman, J. P.; Kool, E. T.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **2005**, *127*, 8600.
(233) Dirks, A. J.; Van Berkel, S. S.; Hatzakis, N. S.; Opsteen, J. A.; Van Delft, F. L.; Cornelissen, J. J. L. M.; Rowan, A. E.; Van Hest, J. C. M.; Rutjes, F. P. J. T.; Nolte, R. J. M. *Chem. Commun.* **2005**, 4172.
(234) Lee, J. W.; Kim, B. K.; Jin, S. H. *B. Kor. Chem. Soc.* **2005**, *26*, 833.
(235) Lee, J. W.; Kim, B. K. *B. Kor. Chem. Soc.* **2005**, *26*, 658.
(236) Lee, J. L.; Jung, H. K.; Kim, B. K.; Ji, H. K.; Won, S. S.; Jin, S. H.; Kim, M. *B. Kor. Chem. Soc.* **2006**, *27*, 1795.
(237) Lee, J. W.; Kim, J. H.; Kim, B. K.; Shin, W. S.; Jin, S. H. *Tetrahedron* **2006**, *62*, 894.

(238) Xia, Y.; Fanqi, Q.; Wei, L.; Qiongyou, W.; Peng, L. *Heterocycles* **2005**, *65*, 345.
(239) Shang, Y. J.; Ren, L. B.; Wang, D. M. *Chin. J. Chem.* **2007**, *25*, 1202.
(240) Nepogodiev, S. A.; Dedola, S.; Marmuse, L.; de Oliveira, M. T.; Field, R. A. *Carbohydr. Res.* **2007**, *342*, 529.
(241) Akula, R. A.; Temelkoff, D. P.; Artis, N. D.; Norris, P. *Heterocycles* **2004**, *63*, 2719.
(242) Speers, A. E.; Cravatt, B. F. *Chem. Biol.* **2004**, *11*, 535.
(243) Bouillon, C.; Meyer, A.; Vidal, S.; Jochum, A.; Chevolot, Y.; Cloarec, J. P.; Praly, J. P.; Vasseur, J. J.; Morvan, F. *J. Org. Chem.* **2006**, *71*, 4700.
(244) Wei, Q.; Seward, G. K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; Dmochowski, I. J. *J. Am. Chem. Soc.* **2006**, *128*, 13274.
(245) Hafrén, J.; Zou, W.; Córdova, A. *Macromol. Rapid Commun.* **2006**, *27*, 1362.
(246) Ossipov, D. A.; Hilborn, J. *Macromolecules* **2006**, *39*, 1709.
(247) Andersen, J.; Bolvig, S.; Liang, X. *Synlett* **2005**, 2941.
(248) Liebert, T.; nsch, C.; Heinze, T. *Macromol. Rapid Commun.* **2006**, *27*, 208.
(249) Englert, B. C.; Bakbak, S.; Bunz, U. H. F. *Macromolecules* **2005**, *38*, 5868.
(250) Humenik, M.; Huang, Y.; Wang, Y.; Sprinzl, M. *ChemBioChem* **2007**, *8*, 1103.
(251) Wilkinson, B. L.; Bornaghi, L. F.; Poulsen, S. A.; Houston, T. A. *Tetrahedron* **2006**, *62*, 8115.
(252) Aucagne, V.; Leigh, D. A. *Org. Lett.* **2006**, *8*, 4505.
(253) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocente, A.; Supuran, C. T.; Poulsen, S. A. *J. Med. Chem.* **2006**, *49*, 6539.
(254) Wilkinson, B. L.; Bornaghi, L. F.; Houston, T. A.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Poulsen, S.-A. *J. Med. Chem.* **2007**, *50*, 1651.
(255) Ooya, T.; Inoue, D.; Choi, H. S.; Kobayashi, Y.; Loethen, S.; Thompson, D. H.; Ko, Y. H.; Kim, K.; Yui, N. *Org. Lett.* **2006**, *8*, 3159.
(256) Chen, W. L.; Su, C. L.; Huang, X. *Synlett* **2006**, 1446.
(257) Zhang, X.; Hsung, R. P.; You, L. *Org. Biomol. Chem.* **2006**, *4*, 2679.
(258) MacMillan, D.; Blanc, J. *Org. Biomol. Chem.* **2006**, *4*, 2847.
(259) Srinivasan, R.; Uttamchandani, M.; Yao, S. Q. *Org. Lett.* **2006**, *8*, 713.
(260) Barral, K.; Moorhouse, A. D.; Moses, J. E. *Org. Lett.* **2007**, *9*, 1809.
(261) Yamaguchi, M.; Kojima, K.; Hayashi, N.; Kakizaki, I.; Kon, A.; Takagaki, K. *Tetrahedron Lett.* **2006**, *47*, 7455.
(262) Such, G. K.; Quinn, J. F.; Quinn, A.; Tjipto, E.; Caruso, F. *J. Am. Chem. Soc.* **2006**, *128*, 9318.
(263) Moorhouse, A. D.; Santos, A. M.; Gunaratnam, M.; Moore, M.; Neidle, S.; Moses, J. E. *J. Am. Chem. Soc.* **2006**, *128*, 15972.
(264) Lewis, W. G.; Magallon, F. G.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 9152.
(265) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897.
(266) Chittaboina, S.; Xie, F.; Wang, Q. *Tetrahedron Lett.* **2005**, *46*, 2331.
(267) Ustinov, A. V.; Korshun, V. A. *Russ. Chem. B* **2006**, *55*, 1268.
(268) El-Sagheer, A. H.; Kumar, R.; Findlow, S.; Werner, J. M.; Lane, A. N.; Brown, T. *ChemBioChem* **2008**, *9*, 50.
(269) Kumar, R.; El-Sagheer, A.; Tumpane, J.; Lincoln, P.; Wilhelmsson, L. M.; Brown, T. *J. Am. Chem. Soc.* **2007**, *129*, 6859.
(270) Rodionov, V. O.; Presolski, S. I.; Gardinier, S.; Lim, Y. H.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12696.
(271) Meudtner, R. M.; Ostermeier, M.; Goddard, R.; Limberg, C.; Hecht, S. *Chem.−Eur. J.*, in press
(272) Bonnet, D.; Ilien, B.; Galzi, J. L.; Riché, S.; Antheaune, C.; Hibert, M. *Bioconjugate Chem.* **2006**, *17*, 1618.
(273) Gopin, A.; Ebner, S.; Attali, B.; Shabat, D. *Bioconjugate Chem.* **2006**, *17*, 1432.
(274) Ballell, L.; Alink, K. J.; Slijper, M.; Versluis, C.; Liskamp, R. M. J.; Pieters, R. J. *ChemBioChem* **2005**, *6*, 291.
(275) Brik, A.; Muldoon, J.; Lin, Y. C.; Elder, J. H.; Goodsell, D. S.; Olson, A. J.; Fokin, V. V.; Sharpless, K. B.; Wong, C. H. *ChemBioChem* **2003**, *4*, 1246.
(276) Quader, S.; Boyd, S. E.; Jenkins, I. D.; Houston, T. A. *J. Org. Chem.* **2007**, *72*, 1962.
(277) Ermolatév, D.; Dehaen, W.; Van Der Eycken, E. *QSAR Comb. Sci.* **2004**, *23*, 915.
(278) Appukkuttan, P.; Dehaen, W.; Fokin, V. V.; Van Der Eycken, E. *Org. Lett.* **2004**, *6*, 4223.
(279) Deiters, A.; Cropp, T. A.; Mukherji, M.; Chin, J. W.; Anderson, J. C.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 11782.
(280) Kaval, N.; Ermolat'ev, D.; Appukkuttan, P.; Dehaen, W.; Kappe, C. O.; Van Der Eycken, E. *J. Comb. Chem.* **2005**, *7*, 490.
(281) Ramachary, D. B.; Barbas, C. F., III *Chem.−Eur. J.* **2004**, *10*, 5323.
(282) Lee, L. V.; Mitchell, M. L.; Huang, S. J.; Fokin, V. V.; Sharpless, K. B.; Wong, C. H. *J. Am. Chem. Soc.* **2003**, *125*, 9588.
(283) Chandrasekhar, S.; Rao, C. L.; Nagesh, C.; Reddya, C. R.; Sridhar, B. *Tetrahedron Lett.* **2007**, *48*, 5869.
(284) David, O.; Maisonneuve, S.; Xie, J. *Tetrahedron Lett.* **2007**, *48*, 6527.
(285) Pachón, L. D.; Van Maarseveen, J. H.; Rothenberg, G. *Adv. Synth. Catal.* **2005**, *347*, 811.
(286) Wan, Q.; Chen, J.; Chen, G.; Danishefsky, S. J. *J. Org. Chem.* **2006**, *71*, 8244.
(287) Molteni, G.; Bianchi, C. L.; Marinoni, G.; Santo, N.; Ponti, A. *New J. Chem.* **2006**, *30*, 1137.
(288) Orgueira, H. A.; Fokas, D.; Isome, Y.; Chan, P. C. M.; Baldino, C. M. *Tetrahedron Lett.* **2005**, *46*, 2911.
(289) Gommermann, N.; Gehrig, A.; Knochel, P. *Synlett* **2005**, 2796.
(290) Kantam, M. L.; Jaya, V. S.; Sreedhar, B.; Rao, M. M.; Choudary, B. M. *J. Mol. Catal.* **2006**, *256*, 273.
(291) Mason, B. P.; Bogdan, A. R.; Goswami, A.; McQuade, D. T. *Org. Lett.* **2007**, *9*, 3449.
(292) Wu, P.; Malkoch, M.; Hunt, J. N.; Vestberg, R.; Kaltgrad, E.; Finn, M. G.; Fokin, V. V.; Sharpless, K. B.; Hawker, C. J. *Chem. Commun.* **2005**, 5775.
(293) Gruijters, B. W. T.; Broeren, M. A. C.; Van Delft, F. L.; Sijbesma, R. P.; Hermkens, P. H. H.; Rutjes, F. P. J. T. *Org. Lett.* **2006**, *8*, 3163.
(294) Temelkoff, D. P.; Zeller, M.; Norris, P. *Carbohydr. Res.* **2006**, *341*, 1081.
(295) O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *Chem.−Eur. J.* **2006**, *12*, 6776.
(296) Sieczkowska, B.; Millaruelo, M.; Messerschmidt, M.; Voit, B. *Macromolecules* **2007**, *40*, 2361.
(297) Malkoch, M.; Thibault, R. J.; Drockenmuller, E.; Messerschmidt, M.; Voit, B.; Russell, T. P.; Hawker, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 14942.
(298) Miner, P. L.; Wagner, T. R.; Norris, P. *Heterocycles* **2005**, *65*, 1035.
(299) Bock, V. D.; Speijer, D.; Hiemstra, H.; Van Maarseveen, J. H. *Org. Biomol. Chem.* **2007**, *5*, 971.
(300) Bock, V. D.; Perciaccante, R.; Jansen, T. P.; Hiemstra, H.; Van Maarseveen, J. H. *Org. Lett.* **2006**, *8*, 919.
(301) Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C. J.; Russell, T. P.; Wu, P.; Fokin, V. V. *Macromolecules* **2005**, *38*, 3663.
(302) Binder, W. H.; Kluger, C. *Macromolecules* **2004**, *37*, 9321.
(303) Marmuse, L.; Nepogodiev, S. A.; Field, R. A. *Org. Biomol. Chem.* **2005**, *3*, 2225.
(304) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823.
(305) Punna, S.; Kaltgrad, E.; Finn, M. G. *Bioconjugate Chem.* **2005**, *16*, 1536.
(306) Kuijpers, B. H. M.; Dijkmans, G. C. T.; Groothuys, S.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; Van Delft, F. L.; Rutjes, F. P. J. T. *Synlett* **2005**, 3059.
(307) Cavalli, S.; Tipton, A. R.; Overhand, M.; Kros, A. *Chem. Commun.* **2006**, 3193.
(308) Zeng, Q.; Li, T.; Cash, B.; Li, S.; Xie, F.; Wang, Q. *Chem. Commun.* **2007**, 1453.
(309) Zhang, X.; Li, H.; You, L.; Tang, Y.; Hsung, R. P. *Adv. Synth. Catal.* **2006**, *348*, 2437.
(310) Zhang, X.; Hsung, R. P.; Li, H. *Chem. Commun.* **2007**, 2420.
(311) Altintas, O.; Yankul, B.; Hizal, G.; Tunca, U. *J. Polym. Sci.* **2006**, *44*, 6458.
(312) Vogt, A. P.; Sumerlin, B. S. *Macromolecules* **2006**, *39*, 5286.
(313) Altintas, O.; Hizal, G.; Tunca, U. *J. Polym. Sci.* **2006**, *44*, 5699.
(314) Liu, Q.; Chen, Y. *J. Polym. Sci.* **2006**, *44*, 6103.
(315) Lutz, J. F.; Börner, H. G.; Weichenhan, K. *Macromolecules* **2006**, *39*, 6376.
(316) Gao, H.; Louche, G.; Sumerlin, B. S.; Jahed, N.; Golas, P.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 8979.
(317) Sumerlin, B. S.; Tsarevsky, N. V.; Louche, G.; Lee, R. Y.; Matyjaszewski, K. *Macromolecules* **2005**, *38*, 7540.
(318) Gao, H.; Matyjaszewski, K. *Macromolecules* **2006**, *39*, 4960.
(319) Gondi, S. R.; Vogt, A.; Sumerlin, B. S. *Macromolecules* **2007**, *40*, 474.
(320) Gierlich, J.; Burley, G. A.; Gramlich, P. M. E.; Hammond, D. M.; Carell, T. *Org. Lett.* **2006**, *8*, 3639.
(321) Johnson, J. A.; Lewis, D. R.; Díaz, D. D.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 6564.
(322) Link, A. J.; Vink, M. K. S.; Tirrell, D. A. *J. Am. Chem. Soc.* **2004**, *126*, 10598.
(323) Sawa, M.; Hsu, T. L.; Itoh, T.; Sugiyama, M.; Hanson, S. R.; Vogt, P. K.; Wong, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12371.
(324) Thomsen, A. D.; Malmström, E. V. A.; Hvilsted, S. *J. Polym. Sci.* **2006**, *44*, 6360.
(325) Hasegawa, T.; Umeda, M.; Numata, M.; Li, C.; Bae, A. H.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Carbohydr. Res.* **2006**, *341*, 35.

(326) Hasegawa, T.; Umeda, M.; Numata, M.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2006**, *35*, 82.
(327) Kamijo, S.; Jin, T.; Huo, Z.; Yamamoto, Y. *J. Org. Chem.* **2004**, *69*, 2386.
(328) Chan, T. R.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853.
(329) Aucagne, V.; Hänni, K. D.; Leigh, D. A.; Lusby, P. J.; Walker, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 2186.
(330) Binder, W. H.; Gloger, D.; Weinstabl, H.; Allmaier, G.; Pittenauer, E. *Macromolecules* **2007**, *40*, 3097.
(331) Jean, M.; Le Roch, M.; Renault, J.; Uriac, P. *Org. Lett.* **2005**, *7*, 2663.
(332) Su, S.; Giguere, J. R.; Schaus, S. E.; Porco, J. *Tetrahedron* **2004**, *60*, 8645.
(333) Mobian, P.; Collin, J. P.; Sauvage, J. P. *Tetrahedron Lett.* **2006**, *47*, 4907.
(334) Thomas, J. R.; Liu, X.; Hergenrother, P. J. *J. Am. Chem. Soc.* **2005**, *127*, 12434.
(335) Gerard, B.; Ryan, J.; Beeler, A. B.; Porco, J. *Tetrahedron* **2006**, *62*, 6405.
(336) Aucagne, V.; Berna, J.; Crowley, J. D.; Goldup, S. M.; Hänni, K. D.; Leigh, D. A.; Lusby, P. J.; Ronaldson, V. E.; Slawin, A. M.; Viterisi, A.; Walker, D. B. *J. Am. Chem. Soc.* **2007**, *129*, 11950.
(337) Smith, C. D.; Baxendale, I. R.; Lanners, S.; Hayward, J. J.; Smith, S. C.; Ley, S. V. *Org. Biomol. Chem.* **2007**, *5*, 1559.
(338) Guezguez, R.; Bougrin, K.; El Akri, K.; Benhida, R. *Tetrahedron Lett.* **2006**, *47*, 4807.
(339) Zhang, G.; Fang, L.; Zhu, L.; Sun, D.; Wang, P. G. *Bioorg. Med. Chem.* **2006**, *14*, 426.
(340) Ortega, M.; Lopez-Jaramillo, J.; Hernandez-Mateo, F.; Santoyo-Gonzalez, F. *Adv. Synth. Catal.* **2006**, *348*, 2410.
(341) Pérez-Balderas, F.; Ortega, M. M.; Morales-Sanfrutos, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asín, F. G.; Isac-García, J.; Santoyo-González, F. *Org. Lett.* **2003**, *5*, 1951.
(342) Casas-Solvas, J. M.; Vargas-Berenguel, A.; Vallvey, L. F.; Santoyo, G. *Org. Lett.* **2004**, *6*, 3687.
(343) Kamijo, S.; Jin, T.; Yamamoto, Y. *Tetrahedron Lett.* **2004**, *45*, 689.
(344) Devaraj, N. K.; Decréau, R. A.; Ebina, W.; Collman, J. P.; Chidsey, C. E. D. *J. Phys. Chem. B* **2006**, *110*, 15955.
(345) Wróblewski, A. E.; Glowacka, I. E. *Tetrahedron: Asymmetry* **2005**, *16*, 4056.
(346) Löber, S.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2955.
(347) De Oliveira, R. N.; Sinou, D.; Srivastava, R. M. *Synthesis* **2006**, 467.
(348) IJsselstijn, M.; Cintrat, J. C. *Tetrahedron* **2006**, *62*, 3837.
(349) Oliveira, R.; Sinou, D.; Srivastava, R. *J. Carbohydr. Chem.* **2006**, *25*, 407.
(350) Van Steenis, D. J. V. C.; David, O. R. P.; Van Strijdonck, G. P. F.; Van Maarseveen, J. H.; Reek, J. N. H. *Chem. Commun.* **2005**, 4333.
(351) Reddy, K. R.; Rajgopal, K.; Kantam, M. L. *Synlett* **2006**, 957.
(352) Kaleta, Z.; Egyed, O.; Soós, T. *Org. Biomol. Chem.* **2005**, *3*, 2228.
(353) Weller, R. L.; Rajski, S. R. *Org. Lett.* **2007**, *7*, 2141.
(354) Chassaing, S.; Kumarraja, M.; Sido, A. S. S.; Pale, P.; Sommer, J. *Org. Lett.* **2007**, *9*, 883.
(355) Gupta, S. S.; Kuzelka, J.; Singh, P.; Lewis, W. G.; Manchester, M.; Finn, M. G. *Bioconjugate Chem.* **2005**, *16*, 1572.
(356) Gupta, S. S.; Raja, K. S.; Kaltgrad, E.; Strahle, E.; Finn, M. G. *Chem. Commun.* **2005**, 4315.
(357) Devaraj, N. K.; Dinolfo, P. H.; Chidsey, C. E. D.; Collman, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1794.
(358) Sreedhar, B.; Reddy, P. S. *Synth. Commun.* **2007**, *37*, 3259.
(359) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192.
(360) Qin, A.; Jim, C. K. W.; Lu, W.; Lam, J. W. Y.; Häussler, M.; Dong, Y.; Sung, H. H. Y.; Williams, I. D.; Wong, G. K. L.; Tang, B. Z. *Macromolecules* **2007**, *40*, 2308.
(361) Angell, Y.; Burgess, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 3649.
(362) Cassidy, M. P.; Raushel, J.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 3154.
(363) Cho, S. H.; Yoo, E. J.; Bae, I.; Chang, S. *J. Am. Chem. Soc.* **2005**, *127*, 16046.
(364) Han, X. *Tetrahedron Lett.* **2007**, *48*, 2845.
(365) Andersen, J.; Madsen, U.; Björkling, F.; Liang, X. *Synlett* **2005**, 2209.
(366) Kamijo, S.; Jin, T.; Huo, Z.; Yamamoto, Y. *J. Am. Chem. Soc.* **2003**, *125*, 7786.
(367) Loren, J. C.; Sharpless, K. B. *Synthesis* **2005**, 1514.
(368) Angelo, N. G.; Arora, P. S. *J. Org. Chem.* **2007**, *72*, 7964.
(369) Angelo, N. G.; Arora, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 17134.
(370) Löber, S.; Rodriguez-Loaiza, P.; Gmeiner, P. *Org. Lett.* **2003**, *5*, 1753.
(371) Loaiza, P. R.; Löber, S.; Hübner, H.; Gmeiner, P. *J. Comb. Chem.* **2006**, *8*, 252.
(372) Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *ChemBioChem* **2005**, *6*, 1167.
(373) Wang, J.; Uttamchandani, M.; Li, J.; Hu, M.; Yao, S. Q. *Chem. Commun.* **2006**, 3783.
(374) Chen, H.; Taylor, J. L.; Abrams, S. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1979.
(375) Hu, T. S.; Tannert, R.; Arndt, H. D.; Waldmann, H. *Chem. Commun.* **2007**, 3942.
(376) Ritter, S. C.; König, B. *Chem. Commun.* **2006**, 4694.
(377) Zhu, L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 1190.
(378) Suijkerbuijk, B. M. J. M.; Aerts, B. N. H.; Dijkstra, H. P.; Lutz, M.; Spek, A. L.; van Koten, G.; Klein Gebbink, R. J. M. *Dalton Trans.* **2007**, 1273.
(379) Font, D.; Bastero, A.; Sayalero, S.; Jimeno, C.; Pericás, M. A. *Org. Lett.* **2007**, *9*, 1943.
(380) Alza, E.; Cambeiro, X. C.; Jimeno, C.; Pericás, M. A. *Org. Lett.* **2007**, *9*, 3717.
(381) Bastero, A.; Font, D.; Pericás, M. A. *J. Org. Chem.* **2007**, *72*, 2460.
(382) Kim, D.-W.; Lim, S.-G.; Jun, C.-H. *Org. Lett.* **2006**, *8*, 2937.
(383) Géci, I.; Filichev, V. V.; Pedersen, E. B. *Chem.—Eur. J.* **2007**, *13*, 6379.
(384) Gheorghe, A.; Matsuno, A.; Reiser, O. *Adv. Synth. Catal.* **2006**, *348*, 1016.
(385) Seela, F.; Sirivolu, V. R. *Helv. Chim. Acta* **2007**, *90*, 535.
(386) Lin, N.; Yan, Y.; Huang, Z.; Altier, C.; Li, M.; Carrasco, N.; Suyemoto, M.; Johnston, L.; Wang, S.; Wang, Q.; Fang, H.; Caton-Williams, J.; Wang, B. *Nucleic Acids Res.* **2007**, *35*, 1222.
(387) Yilmaz, M. D.; Bozdemir, O. A.; Akkaya, E. U. *Org. Lett.* **2006**, *8*, 2871.
(388) Aprahamian, I.; Dichtel, W. R.; Ikeda, T.; Heath, J. R.; Stoddart, J. F. *Org. Lett.* **2007**, *9*, 1287.
(389) Voit, B. *New J. Chem.* **2007**, *31*, 1139.
(390) Opsteen, J. A.; Van Hest, J. C. M. *J. Polym. Sci.* **2007**, *45*, 2913.
(391) Van Camp, W.; Germonpré, V.; Mespouille, L.; Dubois, P.; Goethals, E. J.; Du Prez, F. E. *React. Funct. Polym.* **2007**, *67*, 1168.
(392) Hasneen, A.; Kim, S. J.; Paik, H. J. *Macromol. Res.* **2007**, *15*, 541.
(393) Durmaz, H.; Dag, A.; Altintas, O.; Erdogan, T.; Hizal, G.; Tunca, U. *Macromolecules* **2007**, *40*, 191.
(394) Liu, X. M.; Thakur, A.; Wang, D. *Biomacromolecules* **2007**, *8*, 2653.
(395) Micoine, K.; Hasenknopf, B.; Thorimbert, S.; Lacôte, E.; Malacria, M. *Org. Lett.* **2007**, *9*, 3981.
(396) Gao, H.; Min, K.; Matyjaszewski, K. *Macromol. Chem. Phys.* **2007**, *208*, 1370.
(397) Fleischmann, S.; Komber, H.; Appelhans, D.; Voit, B. I. *Macromol. Chem. Phys.* **2007**, *208*, 1050.
(398) Li, Z.; Zeng, Q.; Li, Z.; Dong, S.; Zhu, Z.; Li, Q.; Ye, C.; Di, C.; Liu, Y.; Qin, J. *Macromolecules* **2006**, *39*, 8544.
(399) Wang, X. Y.; Kimyonok, A.; Weck, M. *Chem. Commun.* **2006**, 3933.
(400) Quémener, D.; Le Hellaye, M.; Bissett, C.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *J. Polym. Sci.* **2008**, *46*, 155.
(401) Gao, H.; Matyjaszewski, K. *J. Am. Chem. Soc.* **2007**, *129*, 6633.
(402) Martwiset, S.; Woudenberg, R. C.; Granados-Focil, S.; Yavuzcetin, O.; Tuominen, M. T.; Coughlin, E. B. *Solid State Ionics* **2007**, *178*, 1398.
(403) Zeng, Q.; Li, Z.; Li, Z.; Ye, C.; Qin, J.; Tang, B. Z. *Macromolecules* **2007**, *40*, 5634.
(404) Li, Y.; Yang, J.; Benicewicz, B. C. *J. Polym. Sci.* **2007**, *45*, 4300.
(405) Jung, J. H.; Lim, Y. G.; Lee, K. H.; Koo, B. T. *Tetrahedron Lett.* **2007**, *48*, 6442.
(406) Kluger, C.; Binder, W. H. *J. Polym. Sci.* **2007**, *45*, 485.
(407) Wang, Z. X.; Qin, H. L. *Chem. Commun.* **2003**, *9*, 2450.
(408) Laurent, B. A.; Grayson, S. M. *J. Am. Chem. Soc.* **2006**, *128*, 4238.
(409) Liu, Q.; Zhao, P.; Chen, Y. *J. Polym. Sci.* **2007**, *45*, 3330.
(410) Liu, Y.; Díaz, D. D.; Accurso, A. A.; Sharpless, K. B.; Fokin, V. V.; Finn, M. G. *J. Polym. Sci.* **2007**, *45*, 5182.
(411) Ergin, M.; Kiskan, B.; Gacal, B.; Yagci, Y. *Macromolecules* **2007**, *40*, 4724.
(412) Chen, G.; Tao, L.; Mantovani, G.; Ladmiral, V.; Burt, D. P.; MacPherson, J. V.; Haddleton, D. M. *Soft Matter* **2007**, *3*, 732.
(413) Bryan, M. C.; Lee, L. V.; Wong, C. H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3185.
(414) Ranjan, R.; Brittain, W. J. *Macromolecules* **2007**, *40*, 6217.
(415) Bergbreiter, D. E.; Chance, B. S. *Macromolecules* **2007**, *40*, 5337.
(416) O'Reilly, R. K.; Joralemon, M. J.; Wooley, K. L.; Hawker, C. J. *Chem. Mater.* **2005**, *17*, 5976.
(417) Park, S. M.; Lee, Y. S.; Kim, B. H. *Chem. Commun.* **2003**, *9*, 2912.
(418) Oyelere, A. K.; Chen, P. C.; Huang, X.; El Sayed, I. H.; El Sayed, M. A. *Bioconjugate Chem.* **2007**, *18*, 1490.
(419) Liu, C. F.; Tam, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 6584.
(420) Dawson, P. E.; Muir, T. W.; Clarck-Lewis, I.; Kent, S. B. *Science* **1994**, *266*, 776.

(421) Kiick, K. L.; Saxon, E.; Tirrell, D. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 19.
(422) Speers, A. E.; Cravatt, B. F. *ChemBioChem* **2004**, *5*, 41.
(423) Luo, Y.; Knuckley, B.; Bhatia, M.; Pellechia, P. J.; Thompson, P. R. *J. Am. Chem. Soc.* **2006**, *128*, 14468.
(424) Chin, J. W.; Cropp, T. A.; Chu, S.; Meggers, E.; Schultz, P. G. *Chem. Biol.* **2003**, *10*, 511.
(425) Iida, S.; Asakura, N.; Tabata, K.; Okura, I.; Kamachi, T. *ChemBioChem* **2006**, *7*, 1853.
(426) Lee, T.; Cho, M.; Ko, S. Y.; Youn, H. J.; Dong, J. B.; Cho, W. J.; Kang, C. Y.; Kim, S. *J. Med. Chem.* **2007**, *50*, 585.
(427) Yanai, H.; Taguchi, T. *Tetrahedron Lett.* **2005**, *46*, 8639.
(428) Akritopoulou-Zanze, I.; Gracias, V.; Djuric, S. W. *Tetrahedron Lett.* **2004**, *45*, 8439.
(429) Röper, S.; Franz, M. H.; Wartchow, R.; Hoffmann, H. M. R. *Org. Lett.* **2003**, *5*, 2773.
(430) Krasinski, A.; Radic, Z.; Manetsch, R.; Raushel, J.; Taylor, P.; Sharpless, K. B.; Kolb, H. C. *J. Am. Chem. Soc.* **2005**, *127*, 6686.
(431) Whiting, M.; Muldoon, J.; Lin, Y. C.; Silverman, S. M.; Lindstrom, W.; Olson, A. J.; Kolb, H. C.; Finn, M. G.; Sharpless, K. B.; Elder, J. H.; Fokin, V. V. *Angew. Chem., Int. Ed.* **2006**, *45*, 1435.
(432) Barr, L.; Lincoln, S. F.; Easton, C. J. *Supramol. Chem.* **2005**, *17*, 547.
(433) Bourne, Y.; Radic, Z.; Kolb, H. C.; Sharpless, K. B.; Taylor, P.; Marchot, P. *Chem.-Biol. Interact.* **2005**, *157−158*, 159.
(434) Dai, Q.; Gao, W.; Liu, D.; Kapes, L. M.; Zhang, X. *J. Org. Chem.* **2006**, *71*, 3928.
(435) Liu, D.; Gao, W.; Dai, Q.; Zhang, X. *Org. Lett.* **2005**, *7*, 4907.
(436) Krasinski, A.; Fokin, V. V.; Sharpless, K. B. *Org. Lett.* **2004**, *6*, 1237.
(437) Akao, A.; Tsuritani, T.; Kii, S.; Sato, K.; Nonoyama, N.; Mase, T.; Yasuda, N. *Synlett* **2007**, 31.
(438) Coats, S. J.; Link, J. S.; Gauthier, D.; Hlasta, D. J. *Org. Lett.* **2005**, *7*, 1469.
(439) Harvey, G. R. *J. Org. Chem.* **1966**, *31*, 1587.
(440) Alajarin, M.; Cabrera, J.; Pastor, A.; Villalgordo, J. M. *Tetrahedron Lett.* **2007**, *48*, 3495.
(441) Zhang, L.; Chen, X.; Xue, P.; Sun, H. H. Y.; Williams, I. D.; Sharpless, K. B.; Fokin, V. V.; Jia, G. *J. Am. Chem. Soc.* **2005**, *127*, 15998.
(442) Majireck, M. M.; Weinreb, S. M. *J. Org. Chem.* **2006**, *71*, 8680.
(443) Raghavendra, M. S.; Lam, Y. *Tetrahedron Lett.* **2004**, *45*, 6129.
(444) Amantini, D.; Fringuelli, F.; Piermatti, O.; Pizzo, F.; Zunino, E.; Vaccaro, L. *J. Org. Chem.* **2005**, *70*, 6526.
(445) Quiclet-Sire, B.; Zard, S. Z. *Synthesis* **2005**, 3319.
(446) Kamijo, S.; Jin, T.; Huo, Z.; Yamamoto, Y. *Tetrahedron Lett.* **2002**, *43*, 9707.
(447) Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, I. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16793.
(448) Deng, G.; Ma, D.; Xu, Z. *Eur. Polymer Journal* **2007**, *43*, 1179.

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