Click Thiol–Ene Chemistry

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The Emergence of Thiol–Ene Coupling as a Click Process for Materials and Bioorganic Chemistry**

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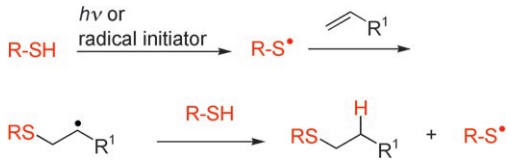
click chemistry · radical reactions · thiol–ene coupling

In the early years of this decade Sharpless and co-workers brought to light the archetypal concept of click chemistry.[1] Ever since, an exponentially increasing number of papers have appeared (>1000)[2] on the use of the quintessential click reaction represented by copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[3] The great success of this process as a ligation tool in fields of research as diverse as organic synthesis, polymer and material science, medicinal chemistry, molecular biology, and biotechnology[4] relies on the efficiency and selectivity (regio- and chemo-), the ready occurrence of the process under aerobic conditions, and its wide scope regardless of the molecular complexity of the reagents. The formation of a robust linker such as the 1,4-disubstituted 1,2,3-triazole ring (a molecular keystone[4c]) which displays biological and pharmacological activities of its own, supports the synthetic utility of the CuAAC reaction. The click chemistry universe, however, is populated by other well-known reactions[1] (e.g. the hetero-Diels–Alder reaction, nucleophilic ring-opening of strained heterocyclic electrophiles, carbonyl transformation into oxime ethers and hydrazones, addition to carbon–carbon multiple bonds such as epoxidation, dihydroxylation, aziridination, nitrosyl, and sulfenyl halide additions) and very likely many others are waiting to be disclosed in the vast repertoire of classical and modern synthetic methods. One reaction that is emerging as an attractive click process is the century-old addition of thiols to alkenes,[5] which is currently called thiol–ene coupling (TEC). The photochemically/thermally-induced version of this reaction is known to proceed by a radical mechanism to give an anti-Markovnikov-type thioether[6] (Scheme 1). The click status of this reaction is supported by it being highly efficient and orthogonal to a wide range of functional groups, as well as for being compatible with water and oxygen. Quite rewardingly, the reaction enables the establishment of a robust ligation motif between substrates by virtue of the stability of the thioether linkage in a wide range of chemical environments, such as strong acid and basic media as well as oxidizing and reducing conditions.



Scheme 1. The thiol–ene radical reaction.

Over the years, the TEC reaction has been extensively exploited in polymer chemistry.[7] The UV-induced cross-linking of unsaturated polymers (photocuring) by reaction with multifunctional thiols is currently employed in surface coating owing to a number of advantages over other curing methods, especially those employing heavy-metal catalysts.[8] Biomaterials for application in medicine, especially dentistry,[9] have been prepared by using this process. Only recently, however, has the click aspect of the TEC reaction been fully appreciated in the field of polymer science. Quite significantly a new term, "thio-click", was coined in a paper dealing with the modification of the backbone of poly[2-(3-butenyl)-2-oxazoline] by reaction with mercaptans.[10] In this way both hydrophobic fluoropolymers and water-soluble glycopolymers were prepared starting from the same readily available material. In the same vein, the functionalization of 1,2-polybutadiene was carried out by grafting an array of aryl thiols (which were generated in situ from acetyl or benzoyl thioesters) onto pendant vinyl groups. [11] In both cases the wide scope and modular nature of the TEC reaction as well as the absence of toxic transition-metal catalysts were emphasized. The products were well-defined polymers without giving side reactions such as thiyl radical coupling, which could lead to disulfide formation. The great potential of thiol–ene chemistry was exploited by Hawker and co-workers in the synthesis of poly(thioether) dendrimers.[12] In this work the key sulfur–carbon bond-forming reaction was used for the construction of both the dendritic backbone and the functionalization of the chain ends. Starting from a 2,4,6-triallyloxy-1,3,5-triazine core, the fourth generation dendrimer [G4]-OH_{48} (Figure 1) was constructed through iterative photoinduced TEC and alkene generation in each cycle. After the transformation of [G4]-OH_{48} into the ene-functional dendrimer [G4]-ene_{48}, the TEC reaction between this polyalkene substrate and monofunctionalized thiols enabled functionalization of the periphery of the dendrimer in an

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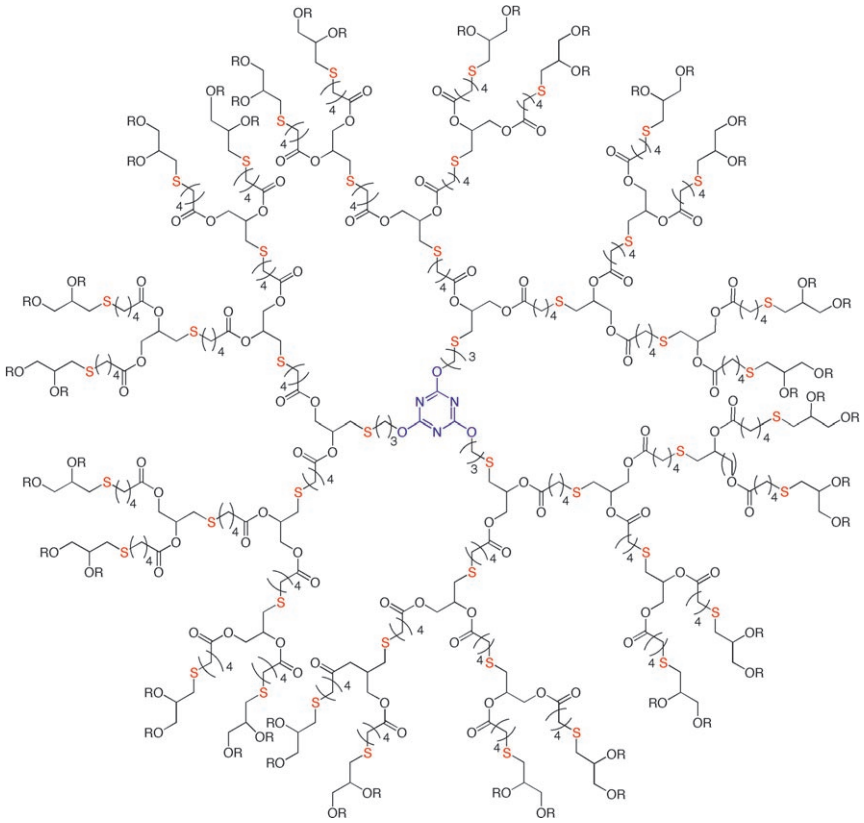

Figure 1. Thiodendrimer [G4]-OH_{48} (R = H) and esters (R = C(O)$(CH_2)_4SCH_2CO_2H$, C(O)-$(CH_2)_4SCH_2CO_2(CH_2)_4$-pyren-1-yl, C(O)$(CH_2)_4SCH_2CH(NHFmoc)CO_2H$. Fmoc = 9-fluorenylmethyloxycarbonyl.

essentially complete manner. The extensive use and fidelity of TEC in this synthetic endeavor led the authors to conclude that this reaction can be reasonably added to the list of reactions that fulfill the requirements of click chemistry.

Substantial modifications of synthetic polymers with bioorganic molecules such as amino acids, peptides, and carbohydrates through TEC have been carried out by Schlaad and co-workers.[13] They recognized the potential for this reaction to be comparable to CuAAC for the synthesis of polymers made of biologically and synthetically derived parts, that is, the so-called "biohybrids" or "molecular chimeras".

There are few recent synthetic applications of the TEC reaction described in bioorganic chemistry. Initial work was carried out in the laboratories of Vliegenthart and Fraser-Reid. By specifically targeting the synthesis of neoglycoconjugates, these researcher groups reported on the chain elongation of allyl glycosides by the radical additions of different thiols to give sulfide-spacer glycosides.[14] In a similar vein, Klaffke and co-workers described the photochemical coupling of allyl *N*-glycosides of various oligosaccharides with cysteamine to yield amino-terminated thioether spacers. These compounds were then transformed into neoglycopeptides by transglutaminase(TGase)-promoted coupling with the dipeptide benzyloxycarbonyl-glutamine-glycine (Cbz-Gln-Gly).[15] Glycodendrons featuring thioether linkages holding mannoside fragments were prepared by Heidecke and Lindhorst by very efficient thermal azobisisobutyronitrile-(AIBN)-promoted radical TEC.[16] The compounds were designed as oligomannoside mimetics and therefore were tested for their potential as inhibitors of bacterial adhesion. Hence the utility of TEC in glycobiology was demonstrated. Quite recently, the robust, efficient, and orthogonal nature of TEC as a ligation tool of complex multifunctionalized substrates was validated by Kunz and co-workers through the synthesis of vaccines for tumor-associated glycopeptide antigens.[17] Importantly, the thioether linkage to bovine serum albumin (BSA) was immunocompatible, this being a crucial property in programs directed toward the development of antitumor vaccines. After initial exploration of the applicability of the radical-induced TEC reaction to the conjugation of amino acids and peptides, preformed glycopeptides were introduced into the carrier protein BSA by multiple thioether linkage formation to give the BSA-glycopeptide vaccines. Figure 2 shows one of the materials that was prepared displaying a sialic acid residue.

The increasing interest in TEC as a tool for click-type processes in bioorganic chemistry was demonstrated earlier this year by Waldmann and co-workers.[18] The flexibility of the AIBN-initiated reaction was validated by the synthesis of S-alkylated cysteines from the coupling of cysteine with various alkenes. Notably, the coupling reaction occurred without any inconvenient racemization of the amino acid. Moreover, TEC was tolerant to densely functionalized alkenes as it was applied to the attachment of a fluorescent dansyl derivative and a biotin marker.

The few examples reported above are representative of important applications of thiol–ene coupling as an efficient ligation tool for the regioselective assembly of complex molecular fragments. The biologically friendly nature of the

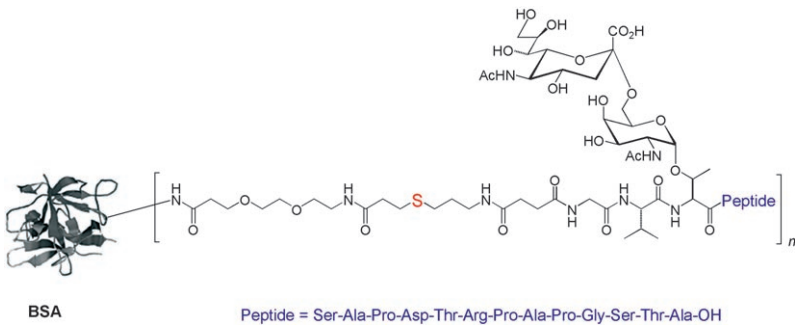

Figure 2. A glycopeptide vaccine containing a sialic acid residue.

coupling reaction (which excludes the use of noxious metal catalysts while being promoted by UV light radiation or radical initiators) and its complete atom economy are very attractive features that simplify both the reaction processing and the product isolation. The main side product of this process is the disulfide compound arising from thiol homocoupling. From this compound the thiol can be easily regenerated by using, for example, inexpensive and readily available dithiothreitol. A known drawback of TEC is the reversibility of the thiyl radical addition to the alkene double bond.[6b] This reversibility may vary substantially depending on the specific structure of both reagents and can be sensitive to the reaction temperature and the concentration of the thiol. Optimum reaction conditions have to be sought for each system under use to force the equilibrium toward the irreversible "locking step" wherein the thioalkyl radical captures an H radical from another thiol to give the final stable product. So far, much attention has been paid to this reaction by researchers operating in fields as diverse as polymer and bioorganic chemistry. It is likely that this interest will expand to researchers in related fields such as nanomaterials, molecular biology, and biomedicine. Accordingly, the absence of any metal catalyst in the TEC reaction suggests its usefulness for bioconjugation wherein CuAAC cannot be employed because of the toxicity of copper towards living cells.[19] It has to be considered, however, that experiments in vivo may be complicated by the lack of selectivity resulting from the large diffusion of thiol functions in the cells. Another point of concern can be the low tolerance of biomolecules to high-energy UV radiation. Fortunately enough, reactions can be performed with low-energy light at 365 nm in the presence of suitable photoinitiators.[12] In conclusion, it is likely that a deeper awareness by researchers of the great potential of this reaction will stimulate its use as a ligation tool complementary to the well-established click CuACC and other spring-loaded processes.[20]

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[20] Note added in proof (July 22, 2008): After the submission of this article, the author became aware of a very recent paper from the research groups of Niemeyer and Waldman (P. Jonkheijm, D. Weinrich, M. Köhn, H. Engelkamp, P. C. M. Christianen, J. Kuhlmann, J. C. Maan, D. Nüsse, H. Schroeder, R. Wacker, R. Breinbauer, C. M. Niemeyer, H. Waldmann, *Angew. Chem.* **2008**, *120*, 4493-4496; *Angew. Chem. Int. Ed.* **2008**, *47*, 4421 – 4424) which reported the photochemically induced thiol–ene coupling (TEC) for the covalent patterning of proteins with retention of their structure and activity. This work provides further evidence for the potential of TEC to be effective in bioconjugation.

