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PERSPECTIVE

Thiol–yne coupling: revisiting old concepts as a breakthrough for up-to-date applications†

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Radical thiol–yne coupling (TYC) has emerged as one of the most appealing click chemistry procedures, appearing as a sound candidate for replacing/complementing other popular click reactions such as the thiol–ene coupling (TEC) and the Cu-catalysed azide–alkyne cycloaddition (CuAAC). Radical TYC is indeed a metal-free reaction suitable for biomedical applications, and its mechanistic features often make it more efficient than its TEC sister reaction and more suitable for multifaceted derivatisations in the materials chemistry and bioconjugation realms. This article reviews the fascinating results obtained in those fields in very recent years.

Introduction

Radical alkenes hydrothiolation is already more than a century old.[1] This reaction, which involves a variety of 'enes' and sulfhydryl-containing molecules (*e.g.* thiols, thiophenols, thiolcarboxylic acids) in the presence of radical sources, such as peroxides, or irradiation with UV light, entails addition of sulfanyl radicals (RS˙) to carbon–carbon double bonds and hence results in the anti-Markovnikov addition of the sulfur compound to the alkene. By the turn of the century, this is one of those historic reactions that has come to a new life when Sharpless initiated the 'click chemistry' realm.[2] Indeed, this reaction, now known as 'thiol–ene coupling' (TEC), possesses most of the characteristics typical of click reactions, *i.e.* orthogonality with other common synthetic procedures (definitely one of the most notable aspects), very mild reaction conditions, use of benign catalysts and solvents, high reaction rates, insensitivity to water and (often) oxygen, complete regio- (also stereo-, but this is not the case) selectivity, ready availability of both thiols and enes, ease of workup, and (usually) high yields. However, for mechanistic reasons that we are going to deal with below, this last feature is sometimes difficult to achieve, unless other click-chemistry conditions are overcome, for example by using a sizeable excess of either reagent. It should be also worth pointing out that, contrary to what is probably the most popular click reaction, *i.e.* the Cu-catalysed azide–alkyne cycloaddition (CuAAC), thiol click chemistry does not need any potentially toxic metal catalyst and can therefore be conveniently used in those areas where biotoxicity could be an important issue.

It should be emphasised that the high (both hetero- and homolytic) reactivity of thiols towards many functional groups could in principle spoil the requirement of orthogonality that is one of the main requisites for efficient click-chemistry procedures. On the other hand, modern mechanistic knowledge allows an advanced tuning of the reactivity of thiols and alkenes in such a way as to achieve complete selectivity and hence orthogonality. From this point of view, the radical TEC has been a crucial breakthrough, since, like many other radical reactions, it can be efficiently carried out in the presence of polar functional groups that react very slowly under radical conditions and are therefore not involved in such a click-process. In addition, the radical TEC is very fast and can usually be accomplished at room temperature under UV irradiation (and typically in the presence of a photoinitiator): although this may be a drawback in a few selected cases,[3] light-induced TEC entails a very attractive opportunity for carrying out both spatially and temporally conducted bioconjugations, materials surface modifications, and photolithographic patternings through simple control of light exposure, even in microfluidic devices.[4] Thiol click reactions (which include radical TEC as well as its ionic companion, the thiol-Michael addition) have been now recognised as an exceptional tool in organic synthesis and a huge number of applications to materials chemistry and bioderivatisation can be found in the literature.[5]

There is however a sister reaction that is not as old as radical TEC, but that dates back to more than eighty years ago and can definitely belong to that category of aged reactions that deserve new life and up-to-date applications after decades of more or less exploratory investigations. That is radical addition of thiols to alkynes, named, by analogy with the 'ene'-counterpart, 'thiol–yne coupling' (TYC).

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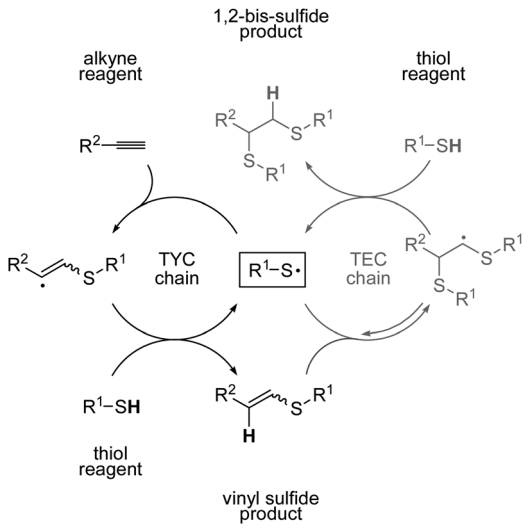
† This paper is heartily dedicated to Professor Piero Spagnolo on the occasion of his retirement.



Scheme 1 Mechanism of TYC and TEC radical chains.

Thiol–yne coupling: an overview

The first (apparently independent) examples of this reaction were reported in the 1930s by Finzi[6] and Kohler,[7] although both studies were not intended to be methodological reports on this novel kind of reaction. More thorough accounts were instead reported in the subsequent two decades, for example by Bader,[8a,b] who observed the so-called 'abnormal' (*i.e.* anti-Markovnikov) addition of thiolacetic acid to monosubstituted acetylenes affording vinyl sulfides: as in the case of alkenes, the reaction was catalysed by organic peroxides or UV light.[8]

Since the very beginning, all authors realised what makes radical TYC different from the TEC counterpart and hence its potential advantages and drawbacks. As shown in Scheme 1, the first reaction step is again addition of a sulfanyl radical to the carbon–carbon multiple bond to afford a β-sulfanyl-substituted vinyl radical: hydrogen transfer from the starting thiol affords a vinyl sulfide and a new sulfanyl radical that sustains the chain. The whole process, although *regio*selective, at least with terminal alkynes, is usually scarcely *stereo*selective, since the vinyl sulfide products are often formed as mixtures of both *E*- and *Z*-stereoisomers: one of the main requirements of click-chemistry-reactions, *i.e.* stereoselectivity, is therefore generally lost. Conversely, it is worth pointing out that radical additions to alkenes are generally faster than those to alkynes,[9] but the former usually occur in a reversible fashion,[10] whereas the latter are substantially irreversible, at least with *alkane*sulfanyl radicals.[11] This means that radical TECs often require a significant excess of alkene–thiol and/or high thiol concentrations[12] to shift the equilibrium towards the sulfide products. As a consequence, additional workup is necessary to get rid of that excess, hence losing one of the main advantages of click-reactions. On the contrary, most of radical TYCs are normally much more efficient when carried out with equimolar amounts of reagents, since the intermediate vinyl radicals are formed in a virtually irreversible manner and moreover abstract a hydrogen atom from the thiol reagent more rapidly than their alkyl counterparts. However, care should be always taken of reagents concentration, since high dilutions might result in appreciable side reactions, for example dimerisation of sulfanyl radicals to give the corresponding disulfide.

Anyway, the most important point is that, contrary to the products of TEC (*alkyl* sulfides), the products of TYC (*vinyl* sulfides) are reactive species that can undergo a subsequent addition of another sulfanyl radical to afford bis-sulfide bis-adducts through the intermediacy of α,β-disulfanyl-disubstituted alkyl radicals (TYC–TEC sequence, Scheme 1).[13] From a click-chemistry point of view, this additional process brings about extra drawbacks related to both regio- and stereoselectivity. Indeed, the bis-sulfides possess a new chiral centre that is created without any stereoselectivity; more crucially, although 1,2-bis-addition is the rule of thumb and it is observed, for example, with all terminal alkyl acetylenes, 1,1-bis-addition can compete with certain alkynes (especially arylacetylenes and aryl-propiolic acid derivatives), giving rise to notable regioselectivity problems. This issue was already observed in the earliest studies[6] and it has been dealt with in our recent methodological study on the main factors influencing thiol–yne couplings.[14] On the other hand, the possibility of a TYC–TEC sequence is the feature of the thiol–yne reaction that has contributed most to its popularity amongst materials chemists, since, when performing TYCs in such conditions as to optimise formation of bis-adducts, it allows for the efficient, straightforward construction of highly cross-linked or hyperbranched polymers and highly functionalised materials such as dendrimers:[15] it might be avowed that this is the result of the 'double' functionality of the alkyne moiety under radical thiol coupling conditions.

Two methodological studies have recently appeared dealing with the influence of substrates structure and reaction conditions (*e.g.* temperature and solvent) on the TYC outcome, in terms of both substrate reactivity (kinetics) and product distribution. In the former,[16] a kinetic investigation allowed for reporting a reactivity scale following (to some extent) what should have been expected on the basis of the general reactivity of sulfanyl radicals with alkynes:[11c] like most radical reactions, TYC is very sensitive to steric hindrance and hence internal alkynes react more slowly with respect to terminal ones; in addition, sulfanyls are electrophilic in nature and react more readily with electron-rich alkynes. However, the reactivity scale is not completely in line with the electron-density properties of the employed alkynes and also the unexpected results obtained with an alkyne such as *N*-methyl-*N*-propargylamine have not been accounted for yet. More interestingly, it was found that thiols react with cyclooctyne very rapidly and also without any initiation: this is most likely the result of relief of ring strain by dissolution of one π-bond by radical addition, a behaviour already encountered for the copper-free strain-promoted azide–alkyne cycloaddition (SPAAC).[17] Unfortunately, the lack of reactivity of the resulting cyclooctenyl sulfide, namely its inability to undergo further thiol addition, makes this alkyne not suitable as a monomer for network photopolymerisations. Furthermore, the high, spontaneous reactivity of cyclooctyne with thiols suggests limitations to the orthogonality of TYC in the presence of azide groups.

In our recent paper,[14] we focussed our attention instead on the influence of the experimental conditions, *i.e.* thiol–alkyne molar ratio (and concentrations), temperature, and, primarily, solvent, on the TYC reaction outcome, showing that a proper choice of

those conditions can favour highly selective occurrence of either mono- or bis-sulfide coupling products. This is not a crucial issue in the field of materials chemistry, where obtaining cross-linked polymers or highly functionalised materials by one-pot complete addition of two equivalents of *the same* thiol to the alkyne of interest (by what we can call a TYC–TEC *homo*-sequence) is the only attractive target. It could be instead an extremely appealing matter in the domain of bioconjugation, where selective formation of a vinyl sulfide mono-adduct can, on one hand, be a valid alternative to TEC for functionalisation of biomolecules under true click-chemistry conditions, and, on the other hand, pave the way to bis-functionalisations with two *different* sulfide moieties through TYC–TEC *hetero*sequences performed in succession with two *different* thiols. The results obtained will be described below in the section dedicated to bio-derivatisations, but we can anticipate here that it is definitely possible to influence the products ratio by changing the reaction conditions and the alkyne structure, with arylacetylene tags playing a key role in attaining mono-adducts in a highly selective fashion and under conditions much closer to click-chemistry than those of analogous TEC procedures.

Of course, radical TYCs can be performed under conditions different from those normally employed, namely in the presence of radical initiation methods diverse from photoinitiation. These include peroxides,[8] other thermal initiators such as azo-compounds (mainly AIBN),[18] and triethylborane,[18,19] a widespread reagent that can be used to generate a variety of radical species at rt or at any temperature below.[20] Single electron transfer (SET) processes can be also exploited to perform radical hydrothiolation of alkynes, for example starting from diphenyl disulfide and tertiary amines: this method has been recently applied to cyclisation of eneynes.[21] Even thiols can afford sulfanyl radicals and hence TYC reactions through SET mechanisms, for example by employing Mn(III) salts.[22]

Furthermore, although nowadays TYC reactions are usually intended as the radical processes shown in Scheme 1, the literature contains many examples of thiol–yne couplings carried out under ionic[23] (thiol-Michael additions) or transition-metal-catalysed conditions, right as in the case of TECs.[24] For example, it has been reported that cesium-carbonate-catalysed nuclephilic addition[25] of thiols to aryl acetylenes affords vinyl sulfide adducts with good yields and *Z*-selectivity (*anti*-addition),[23a,b] although stereo- and regioselectivity deteriorate with electrophilic aryl acetylenes and aliphatic alkynes, respectively.[26] Thiol–alkyne ionic coupling can also be catalysed by PhSeBr, although this method does not appear to be of broad application.[27] More interestingly, nucleophilic couplings between cysteine-containing peptides and electron-poor alkynes (propiolic acid derivatives and ethynyl ketones) have been carried out in buffered, slightly basic water–acetonitrile solutions as a bioconjugation tool for modification of those peptides.[28] The reaction results in *anti*-addition, affording the corresponding anti-Markovnikov vinyl sulfides with a good *Z*-stereoselectivity and it is not affected by the presence of other unprotected nucleophilic aminoacids in the peptide chain.

As far as metal catalysis is concerned, this has been carried out with a variety of organometal complexes, usually under homogeneous conditions: the metals employed comprise gold,[29] rhodium,[30] iridium,[31] palladium,[30a] actidinides,[32] zirconium,[33] and nickel,[34] and the results range from regio- and (at least partially) stereoselective anti-Markovnikov *syn*-addition[29,30a,e,f,31] to complete Markovnikov regioselectivity.[30a–d,32–34] Additional examples include the use of the Wilkinson catalyst,[35] which showed selective anti-Markovnikov *syn*-addition, and indium tribromide,[36] which, depending on the nature of the alkyne, afforded either anti-Markovnikov vinyl sulfides or 2,2-dithioacetals with, respectively, aryl or alkyl acetylenes.

A few papers have also appeared lately dealing with hydrothiolation of alkynes performed with heterogeneous systems under solvent-free conditions.[37] TYC is there achieved through either Al_2O_3-supported KF,[37a] affording good yields (but low stereochemistry control) independently of the nature of the thiols–alkynes employed, or (starting from disulfides) Al_2O_3-supported $NaBH_4$,[37b,c] allowing better stereocontrol but with a very limited variety of alkynes. The latter method can be notably accelerated by microwave irradiation.

There are finally a couple of papers that do not properly fit in any of the above categories and deserve a distinct discussion.[38] Starting from virtually equivalent amounts of reagents, both studies entail simple hydrothiolation in water at rt to give the anti-Markovnikov vinyl sulfide products, but an argument exists as far as the need for an additive is concerned. Indeed, the older paper[38a] established that β-cyclodextrin (1 equiv) was absolutely necessary in order to attain the coupling and no product was observed in the absence of that additive; on the contrary, the more recent one,[38b] although aware of the previous results, asserts that high yields of coupling products can be obtained in water alone, without any additive, under conditions strictly comparable to the previous ones. To make things even more puzzling, the reaction carried out with β-cyclodextrin appears to work only with thiophenols and arylacetylenes, yielding the corresponding vinyl sulfides with good *E*-stereoselectivity,[38a] whereas, in the absence of the additive, it seems that all kinds of thiols and alkynes (both aromatic and aliphatic) can efficiently couple, although with reduced and sometimes inverted stereoselection.[38b] Both papers suggest that either β-cyclodextrin or water itself can weaken the S–H bond strength through H-bonding, hence enhancing thiol nucleophilicity: thorough mechanistic explanations have not however been put forward by either authors. It is highly desirable that a full rationale of those results could be soon attained, since, as everyone can envisage, the possibility of performing TYCs under so straightforward conditions could open novel, very exciting scenarios in applications of thiol–yne chemistry.

In the last years, development of derivatisation procedures performed through thiol–yne coupling has been entirely monopolised by its radical version, most likely because this procedure appears as the most orthogonal of all and the closest to click-chemistry requisites. Nonetheless, the above discussion on hydrothiolation variants was intended to throw some more light on this subject and to suggest that advantageous alternatives to the standard photoinitiated radical TYC exist, in terms of both radical initiation and non-radical substitutes.

Until a handful of years ago, radical TYCs have been the content of reports dealing with either the synthesis of vinyl sulfides, which are quite an interesting class of organic compounds,[39] or kinetic–mechanistic issues on the involved radical species, or synthetic procedures (*e.g.* cascade cyclisations)

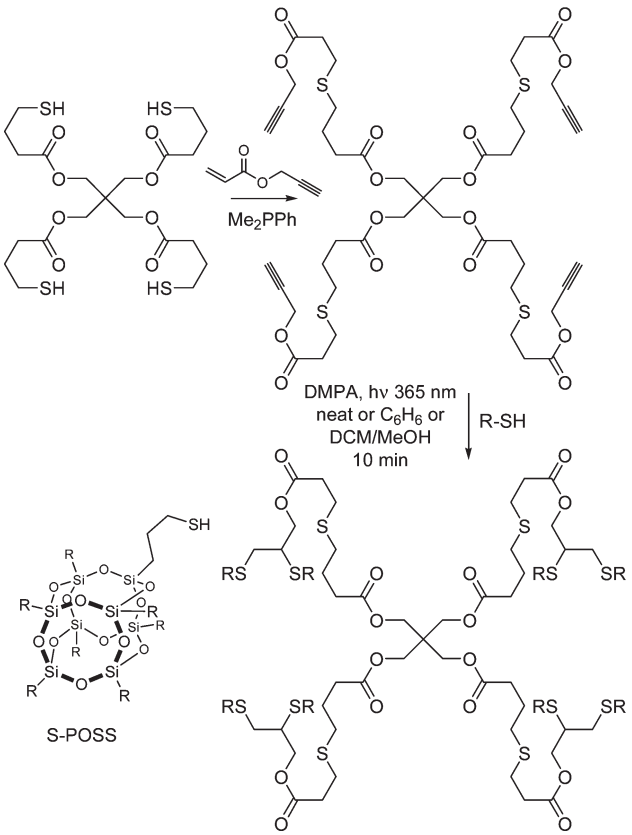
entailing intermediacy of β-sulfanyl-substituted vinyl radicals.[10a,11b,18,19c,40,41] It was indeed not until 2009 that this reaction started to be explored as a powerful tool for polymerisations, materials synthesis, and bioconjugation. The subsequent two sections will give an account on some remarkable results obtained in those fields basically in the last three years. In the materials chemistry section, polymers will be normally dealt with only when TYC is employed for post-functionalisation of the polymeric material, since TYC-induced polymerisations have been already reviewed.[13,15b,c,42,43]

Materials chemistry applications

Post-polymerisation techniques

Although use of thiol–yne coupling in materials chemistry has shown an impressive upsurge only since 2009, actually the first application of radical TYC in that field was reported in 2004,[44] when potentially interesting functional materials were synthesised starting from dodecanethiol and thermosetting resins containing side alkyl- or aryl-alkynyl chains under thermal conditions (AIBN, 60 °C). The hydrothiolation ratio, *i.e.* the percentage of free alkynyl groups remaining on the resin, was found to depend on the alkynyl moiety type, with butylalkynyl groups being more prone to hydrothiolation than the phenylalkynyl analogs, probably as a result of their relative bulkyness. Since addition of sulfanyl radicals to *aryl*-susbtituted alkynes is assumed to be more favoured with respect to their *alkyl*-counterparts,[9b,14] this is quite an interesting result that suggests that steric demand can well balance thermochemical requirements, at least in such a complicated environment as a polymeric chain. No formation of bis-adducts was observed with either alkynyl moieties and it should be emphasised that the structural formula reported in the paper is most likely wrong, since the displayed structure derives from Markovnikov, instead of the expected anti-Markovnikov, addition of the thiol to the C–C triple bond.

In the year 2009, that has been the milestone for application of TYC to materials chemistry, Hoyle and Lowe, together with their first paper on step-growth radical photopolymerisation of dithiols and dialkynes,[15a] published the first example of thiol–ene/thiol–yne (TEC–TYC) sequence as a route to prepare highly funcionalised materials through a very straightforward orthogonal synthesis.[45] The sequence entails a phosphine-catalysed thiol-Michael addition of a tetrathiol to propargyl acrylate (TEC), followed by a radical TYC–TEC sequence reaction on the resulting tetraalkyne affording bis-adducts of several thiols, including some with potential biomedical significance (Scheme 2). The overall process can therefore be regarded as a triple TEC–TYC–TEC sequence, with the first step ionic and the remaining two radical in nature. The TYC–TEC sequence was carried out under the conditions that were going to become the standard for this kind of procedures, *i.e.* at rt with 2,2-dimethoxy-2-phenylacetophenone (DMPA or Irgacure 651) as a photoinitiator and irradiation at 365 nm.[46] All reactions proceeded with high yields and very easy workup. No care was of course given to mono- or bis-adduct selectivity, since, as it had to be regular in the materials realm, the aim was to obtain a high funcionalisation degree in the simplest, shortest way. All



Scheme 2 TEC–TYC sequence for preparation of functional materials.

reactions were hence carried out with a two-fold excess of thiol to assure complete formation of 1,2-bis-sulfide products.

This method was successfully applied to functionalisation of RAFT (Reversible Addition Fragmentation chain Transfer) polymers.[45b] Specifically, a RAFT-produced homopolymer of *N*-isopropylacrylamide (PNIPAm) was mono- and bis-functionalised through its thiol ends to give, when the TEC–TYC sequence was adopted, bis-sulfide adducts with interesting lower critical solution temperatures (LCST), a parameter that can be crucial for application of the derived materials, particularly in the biomedical field. Radical TYCs were carried out with DMPA at 350 nm. Interestingly, when PNIPAm was derivatised with a 3-mercaptopropyl polyhedral oligomeric silsesquioxane (S-POSS, Scheme 2), a novel polymer–nanomaterial hybrid was obtained with highly bulky, hydrophobic ends.

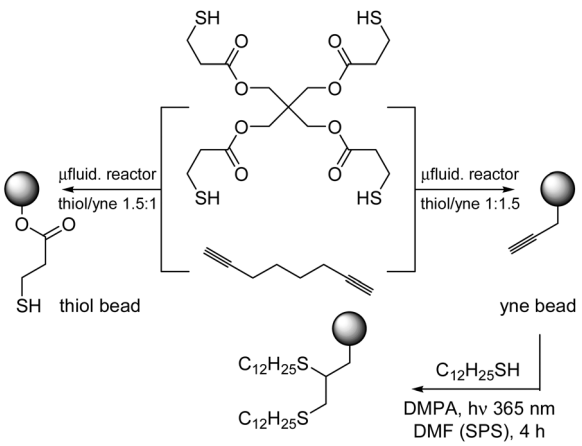
The potential affinity of TYCs for microfluidic setups, in analogy with TECs,[4] was exploited by Du Prez for the production of monodisperse thiol- and yne-beads, which were subsequently derivatised with several click-type reactions to manufacture functional resins (Scheme 3).[47,48] The purpose of the work was to compare a series of click-reactions under solid phase synthesis (SPS) conditions in order to determine the most efficient one and hence to choose the best ligation strategy (*i.e.* that requiring the least excess of reagent in the mobile phase) for beads functionalisation, *e.g.* for catalysts or enzymes immobilisation.

After UV continuous irradiation, TYC carried out on yne-beads with 1-dodecanethiol was found to be faster than CuAAC

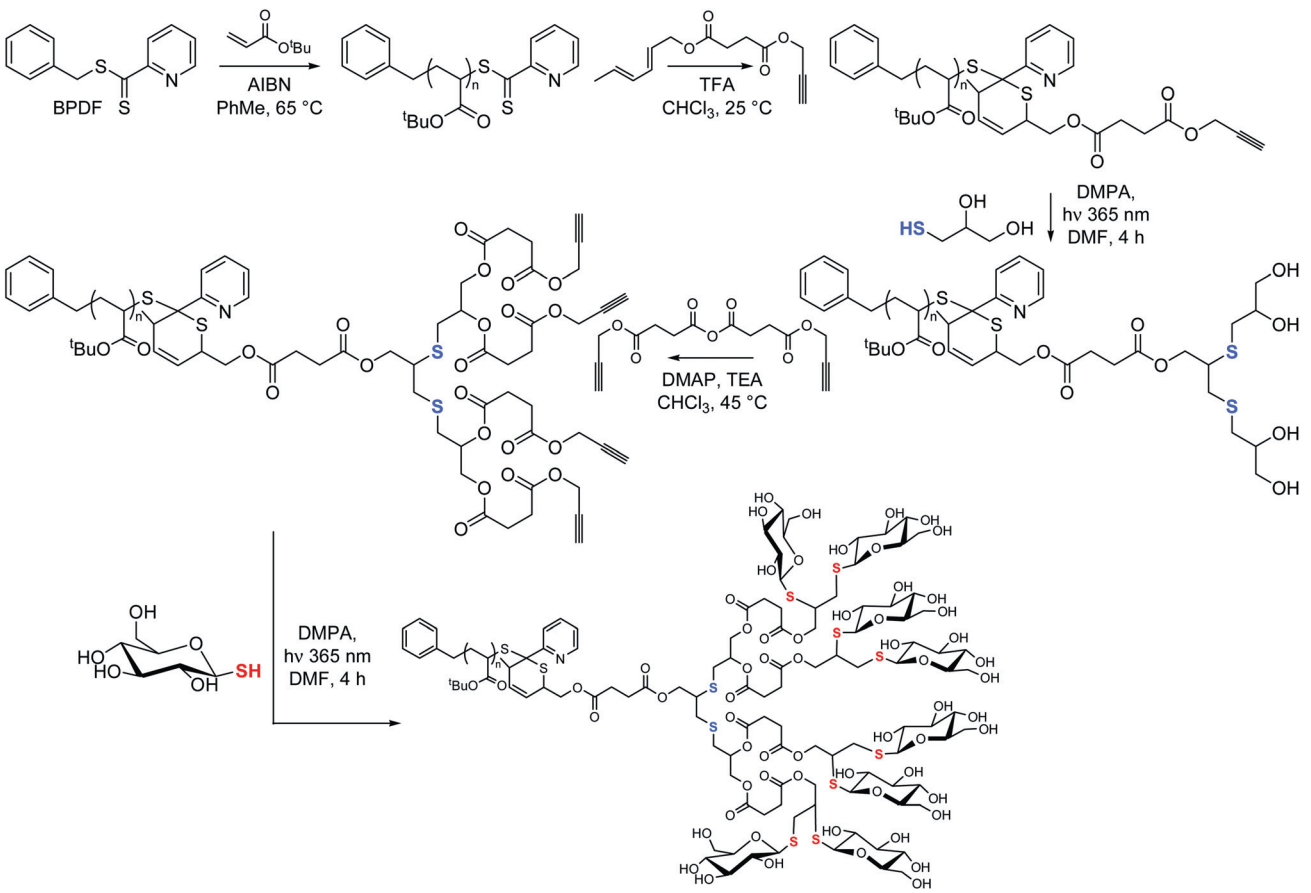
with 1-dodecaneazide and proceeded with higher conversions. TYC appears therefore superior to CuAAC in this kind of ligations, although many doubts remain as far as formation of either mono- or bis-adducts is concerned.[49]

Among the thiols that can be employed for polymer modifications, sugar thiols play an important role for the synthesis of glycopolymers, a very attractive class of materials for biomedical applications, for example as drug carriers.[50] These materials can in principle be obtained by direct polymerisation of sugar-containing monomers, but this reaction, although feasible, is somewhat discouraged by the relatively small library of available glycomonomers. Furthermore, some of them are not compatible with living radical polymerisation (LRP) techniques, which, if a hyperbranched polymer is the target, are among the best ones to achieve control over primary chain length and polydispersity. It is therefore crucial to devise novel methodologies for post-functionalisation of such polymers. In 2010 Perrier, after having reported that TYCs can be exploited to produce hyperbranched polymers,[15h] employed TYC chemistry for the first synthesis of branched glycopolymers following a post-polymerisation modification method combining RAFT-LRP techniques with click-chemistry.[51] In particular, a highly branched alkynyl-group-containing polymer was 'clicked' with glucothiose to give, under the usual conditions, a bis-functionalised derivative contaminated by *ca.* 10% of its mono-sulfide adduct, despite a 2.6-fold excess of thiolsugar. Once again, steric demand seems to play an important role in TYC selectivity, at least in bulky, highly cross-linked materials.

A particular glycopolymer with a glycodendric end-functionality was synthesised by Stenzel by standard radical TYC chemistry as a protein binder (Scheme 4).[52] Specifically, a poly(*tert*-butyl acrylate) obtained by radical RAFT polymerisation of *tert*-butyl acrylate in the presence of benzyl 2-pyridinyldithioformate (BPDF) was reacted through a reaction series entailing (i) hetero-Diels–Alder cycloaddition with an alkynyl diene, (ii) TYC–TEC



Scheme 3 TYC derivatisation of yne-beads.



Scheme 4 Synthesis of a glycodendric polymer by TYC chemistry.

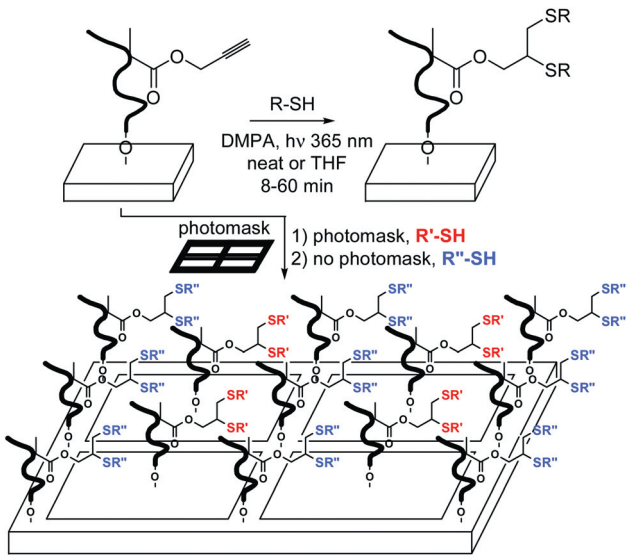
sequence with thioglycerol, (iii) exhaustive esterification, and (iv) eventual TYC–TEC sequence with β-thioglucose to afford the final glycodendric polymer, containing eight sugar units per structure. An analogous procedure was applied to the synthesis of a linear glycopolymer. The protein binding characteristics of the glycodendric and linear polymers self-assembled into micelles in water were compared and the glycodendron exhibited significantly better binding properties towards Concanavalin A.

Post-polymerisation techniques were recently exploited by Kang[53] for functionalisation of PVDF-*g*-PPMA polymeric membranes obtained by graft polymerisation of propargyl methacrylate (PMA) with ozone-pretreated poly(vinylidene fluoride) (PVDF). Through their pendant propargyl moieties, those membranes are like 'clickable platforms' that were reacted with 3-mercapto-1-propanesulfonic acid sodium salt (and other commercially available thiols) to give new membranes exhibiting electrolyte-dependent permeability to aqueous solutions. On the basis of the ratio between the surface concentration of sulfonic acid groups and the graft concentration of the corresponding starting copolymer membranes, and taking as granted that only bis-addition took place, it was assumed that only about 80% of the alkyne groups had undergone the TYC reaction. The possibility that percentage could not arise from 80% of complete double addition (formation of 1,2-bis-sulfide products) but instead from a mixed mono- and bis-addition mode is an issue that the authors did not address.

Surfaces modifications

The first example of application of TYC for engineering the chemistry and topography of surfaces dates back to 2009. This issue is decisive for technological developments related to applications such as biosensors and microelectronics, and, as we already stated in the above introduction, it can definitely take advantage of the main feature of radical photoinitiated TYC, *i.e.* the opportunity for carrying out both *spatially*- and *temporally*-conducted materials surface modifications merely by keeping light exposure under control. On this premise, Patton[54] reported the photopatterning of polymer-brushes-covered surfaces through two sequential TYCs carried out on propargyl derived polymer brushes anchored on the SiO_2 surface of silicon wafers with two different thiols in the presence and in the absence of a photomask (Scheme 5). Depending on the thiol nature, this procedure allows for production of surfaces characterised by the simultaneous presence of patterns with very different properties, for example hydrophilicity and hydrophobicity. As usual, TYCs were carried out at 365 nm with DMPA and the authors observed sometimes a non complete double addition of the thiol, especially when increasing the molecular weight of the latter. It is also worth noting that all reactions can be performed using sunlight as a radiation source, although in slightly longer times: this opens doors to the possibility of large-scale surface modifications using more green, renewable energy resources.

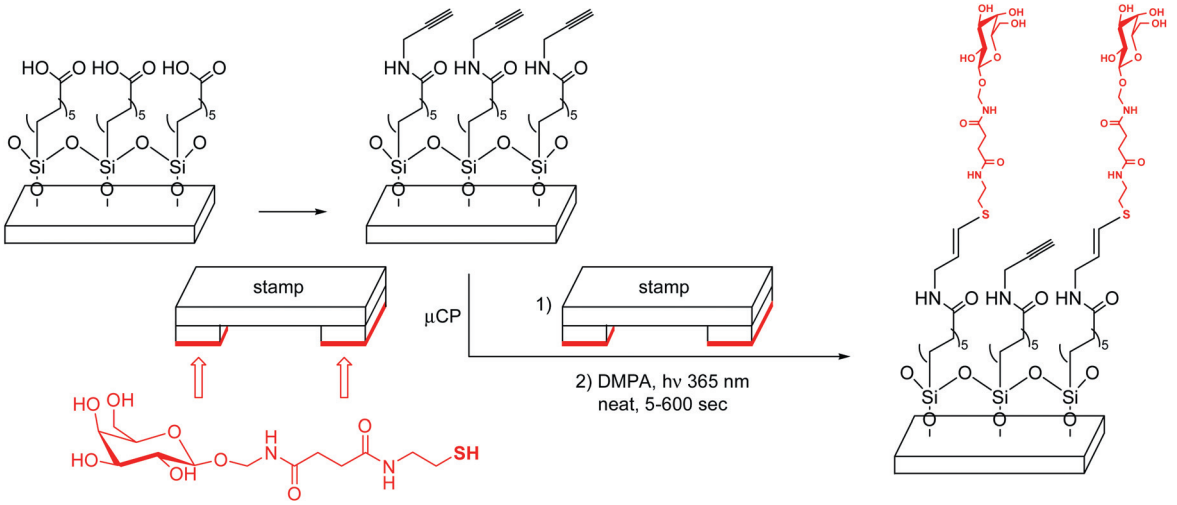
Very recently, the same author described the fabrication of complex multifunctional surfaces through a bulk post-polymerisation strategy entailing two different approaches.[55] In the former, alkyne-functionalised homopolymer brushes were statistically one-pot modified by radical TYC with mixtures of different (two



Scheme 5 Photopatterning of polymer brushes covered surfaces.

or even three) thiols with diverse properties: this robust, versatile procedure allows for construction of surfaces with, for example, a controlled level of hydrophilicity or coated with a specific percentage of biologically relevant molecules. In the latter approach, statistical copolymer brushes containing two different reactive centres were click-derivatised with two orthogonal thiol-click reactions, *i.e.* nucleophilic attack of a thiol to isocyanate, epoxy, or bromo moieties, followed by radical addition of another thiol to a C–C triple bond. The formation of bis-adducts by the radical TYC step was taken for granted and production of both 1,2-homo- and 1,2-hetero bis-sulfide adducts was observed in statistically conducted TYCs in the presence of mixtures of different thiols. This strategy could be extremely useful for production of complex polymer brush architectures as well as multiplexed biomolecules, which are ubiquitous in natural biological systems.

On the same premise of spatially conducted modifications that inspired Patton's first work, Ravoo reported selective immobilisation of thiols on surfaces by microcontact printing (μCP), a method that is emerging as a valid alternative to photochemical lithography for patterning of surfaces.[56] In his papers,[57] alkyne-terminated silicon- or glass-supported self assembled monolayers (SAMs) were photochemically patterned with a galactoside-thiol-conjugate solution spread on the surfaces of oxidised PDMS (polydimethylsiloxane) stamps together with DMPA activator: irradiation at 365 nm for a few minutes of the 'thiol-inked' stamps kept into contact with the SAMs led to galactoside immobilisation with both high efficiency and resolution (Scheme 6).[57a] The absence of any significant difference between the properties of the materials obtained by thiol–yne and corresponding thiol–ene procedures suggested selective binding of one equivalent of thiol to give mono-adducts: since in μCP the grafting density is limited not by the number of the surface reactive groups but rather by the size of the grafted molecules, this is probably the result of the major bulkyness of the sugar–thiol compared with that of the alkyne moieties. The overall procedure can be reversed by printing fluorescent alkynes



Scheme 6 Immobilisation of thiols on surfaces by microcontact printing.

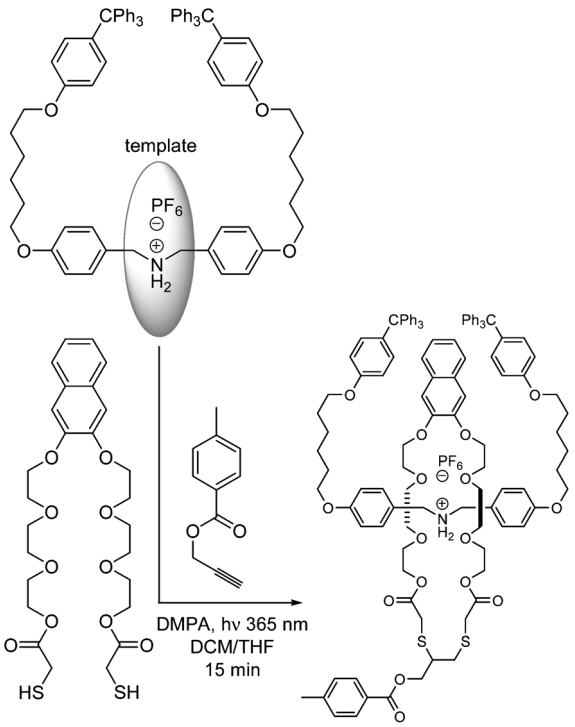
on thiol SAMs:[57b] in this case an unexpected reactivity scale was observed, *i.e.* electrophilic alkynes seemed to react faster than their nucleophilic analogs. Although the authors admit that it was very difficult to quantify those reactivities, due to the very fast reactions (the half life is always less than 1 min), that scale is not in agreement with the known electrophilicity of sulfanyl radicals and might suggest a concomitant occurrence of a thiol-Michael TYC, at least to some extent, in the presence of electrophilic alkynes. Unfortunately, in this latter paper[57b] we could not find any comments upon formation of mono- or bis-adducts: this should have been instead an important issue to deal with, since the steric requirements quoted in the former report[57a] should have been much less demanding when starting from the employed thiol SAMs and linear alkynes. Nevertheless, these studies could be of pivotal significance for the preparation of an assortment of biomolecular microarrays.

Surfaces modification can be also applied to electrodes for biosensing, as very recently shown by Szunerits,[58] who described the photoinitiated TYC functionalisation of boron-doped diamond (BDD) interfaces to be used as electrodes for the electrochemical detection of DNA-DNA hybridisation events by electrochemical impedance spectroscopy (EIS). BDD surfaces were modified by reaction with several thiols, including thiolated oligonucleotide strands, by rt irradiation at 365 nm apparently in the absence of any initiator (DMPA). Formation of bis-sulfide bis-adducts by TYC–TEC sequence was kept for granted on the basis of the known TYC reactivity. Reaction conditions were nonetheless suitably tuned in order to optimise surface coverage, which was indeed often found to depend on irradiation time and thiol concentration. No information was given about the actual surface structures obtained under the diverse conditions, simply assuming that bis-sulfides moieties were formed to different extents.[59] Although probably of no importance as far as applications of the modified surfaces is concerned, an investigation of the real product structure would be interesting from a merely chemical point of view. As a matter of fact, some of the thiols employed (6-ferrocenylhexanethiol and, above all, the thiolated oligonucleotide) possess a significant bulkiness and their 1,2-bis addition to the alkyne moiety should not be an easy process at all.

Although TYC-induced polymerisations are not the object of this review, we would like to mention a very interesting, peculiar example of surface derivatisation attained by direct polymerisation techniques recently reported by Oriol, Sánchez, and De la Fuente.[60] A photolithographic TYC process brought about by direct laser writing (DLW) was indeed used to manufacture patterned polymeric materials starting from a hyperbranched alkyne-functionalised macromonomer and a tetrafunctional thiol comonomer under irradiation with a 405 nm diode laser in the presence of 2-benzyl-2-dimethylamino-1-(4-morpholinophenyl)-1-butanone (Irgacure 369) as a photoinitiator. The process yielded stripes of cross-linked material whose width (10–200 μm) was controlled by focusing the laser beam and that can be used as a biocompatible polymer network, for example for cell growth and tissue engineering applications.

Supramolecular chemistry

To end this Section it is worth mentioning a paper where TYC was used as a 'clipping' method to synthesise macrocycles and rotaxanes. In that study, Li and Li[61] exploited an *inter*molecular-TYC–*intra*molecular-TEC radical sequence to close a macrocyclic ring through clipping of an alkyne with a dithiol. In the presence of a dumbbell-shaped molecular 'thread' containing a suitable ionic template the reaction led to an interlocked rotaxane, *i.e.* one of the most typical structures for the design of artificial molecular machines (Scheme 7). The couplings were conducted under the usual photoinitiated conditions and, in these cases, there is no need for speculations about mono- or bis-addition, since the intramolecular nature of the second coupling certainly makes formation of the bis-sulfide product more feasible and quantitative.[62] On the light of these results, TYC methodologies could undoubtedly be a viable candidate for attaining useful, efficient synthetic procedures also in the realm of supramolecular chemistry.



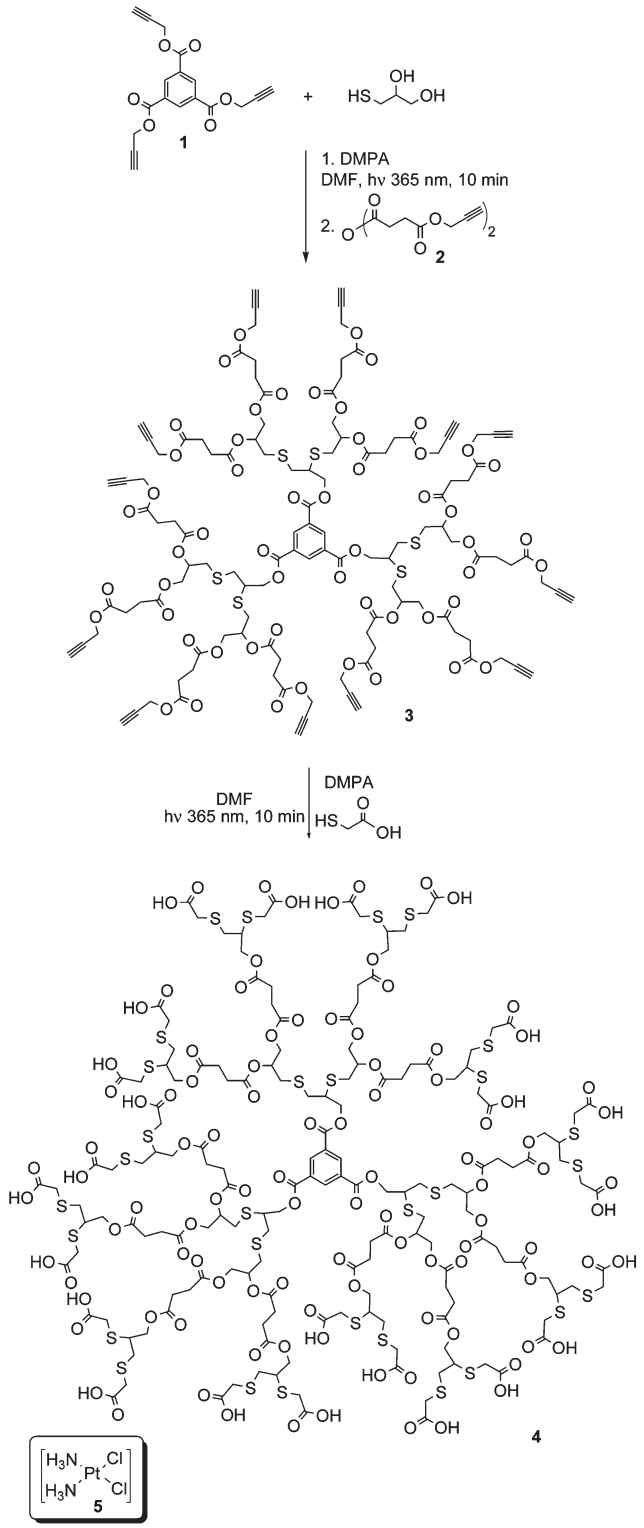
Scheme 7 Synthesis of an interlocked rotaxane by TYC clipping.

Bio-conjugations and derivatisations

The development of metal-free click procedures has recently been the object of intense research in the fields of medicinal chemistry and chemical biology because the potential toxicity of metal catalysts may constitute a major drawback when target molecules are designed for biomedical applications. Additionally, though the triazole moiety derived from the 'almost perfect' azide–alkyne coupling (CuAAC) has similarities to the ubiquitous amide moiety found in nature, it is not always a passive linker. Triazole, in fact, can be engaged in hydrogen bonding and stacking interactions with amino acid residues of proteins,[63] and this may complicate in some instances the interpretation of the recognition process between proteins and the triazole-containing ligand/material. Nevertheless, it is not surprising that, despite the vast repertoire of organic transformations available and the pressing need for novel metal-free click ligation procedures, only a handful of reactions has emerged as a real alternative to CuAAC in the fields of bioconjugation and chemical biology. Indeed, in addition to the fulfilment of the click requirements,[2] central to the development of efficient ligation strategies for biomolecules is also the utilisation of biorthogonal functionalities in the coupling reagents. These functionalities should warrant chemoselective ligations for avoiding cross-reactivity with other molecules, form covalent linkages under physiological conditions, and be non-toxic to the biological system.[64] Biorthogonal copper-free click reactions have been the object of dedicated reviews[65] and include the Staudinger ligation of an azide with a modified phosphine,[66] the strain-promoted azide–alkyne cycloaddition (SPAAC) of cyclooctynes,[17,65] the inverse electron-demand [4 + 2] Diels–Alder cycloaddition of tetrazines with alkenes,[67] the 1,3-dipolar cycloaddition of nitrile oxides with strained alkenes,[68] and the photoinduced 1,3-dipolar cycloaddition of *in situ* generated nitrile imines with alkenes to give pyrazoline cycloadducts (the so called 'photoclick' chemistry).[65a,69] The light-catalysed thiol–ene coupling (TEC) has also gained the status of click reaction and has been demonstrated to be useful for biomedical applications as well.[5] It is our opinion that each of the aforementioned reactions possesses strengths and drawbacks, which make them suitable for specific applications. Significantly, click reactions have also been engaged in orthogonal ligation sequences for dual and multiple functionalisations of biomolecules and materials.[70] In this regard, thiol–yne click chemistry appears perfectly suited to rapidly create multifunctional structures with high levels of complexity. As a matter of fact, TYC emerged in 2009 as a useful click process for biomedical applications with a first investigation on dendritic drug delivery systems, whereby the maximum degree of functionalisation of the macromolecule was achieved by means of repetitive thiol–yne reactions (TYC–TEC *homo*sequences).[71] Since then, however, different studies on peptide and protein modification, surface glycosylation, and lipid mimetic synthesis have demonstrated that TYC benefits from peculiar advantages other than the easy access to molecular complexity. Additional advantages of TYC comprise the possibility of approaching mono- or bis-addition conjugates in a selective manner, the utilisation of the readily available alkyne building blocks of CuAAC chemistry, the orthogonality with many of the other click reactions (including the thiol–ene reaction in its nucleophilic variant), and the typical inertia of thioether linkers towards non-specific interaction with proteins. In this Section of the review we provide a survey of the biomedical applications based on the use of TYC with the intent to highlight strengths but also limitations of this recently rediscovered methodology.

As anticipated, the group of Stenzel reported in 2009 the successful conjugation of the anti-cancer drug *cis*-platinum with a carboxylate terminated dendrimer suitably synthesised by an alternating sequence of thiol–yne and hydroxy–anhydride coupling reactions.[71] Following the approach to dendrimer synthesis *via* thiol–ene chemistry previously disclosed by the group of Hawker,[72] the mixture of the trifunctional alkynyl core molecule **1** and 1-thiogycerol was irradiated for 10 minutes under the usual conditions (rt, λ_{max} 365 nm, DMPA) to obtain the first generation of the dendrimer [G1]-OH_{12}. This intermediate was then subjected to esterification with acetylene anhydride **2** affording the alkyne end-functional dendrimer [G1]-yne_{12} **3** with twelve alkynyl functionalities (Scheme 8). Subsequent photoinduced thiol–yne reaction of **3** with thioglycolic acid gave the [G2] dendrimer **4** with twenty-four carboxylic acid terminal functionalities. The potential application of **4** as a delivery agent of platinum-based drugs was finally ascertained by its effective conjugation with *cis*-dichlorodiammineplatinum(II) **5**. More recently, the same group employed a similar TYC-based approach to generate different polymer architectures with carboxylate ligands serving as platinum drug delivery carriers.[73]

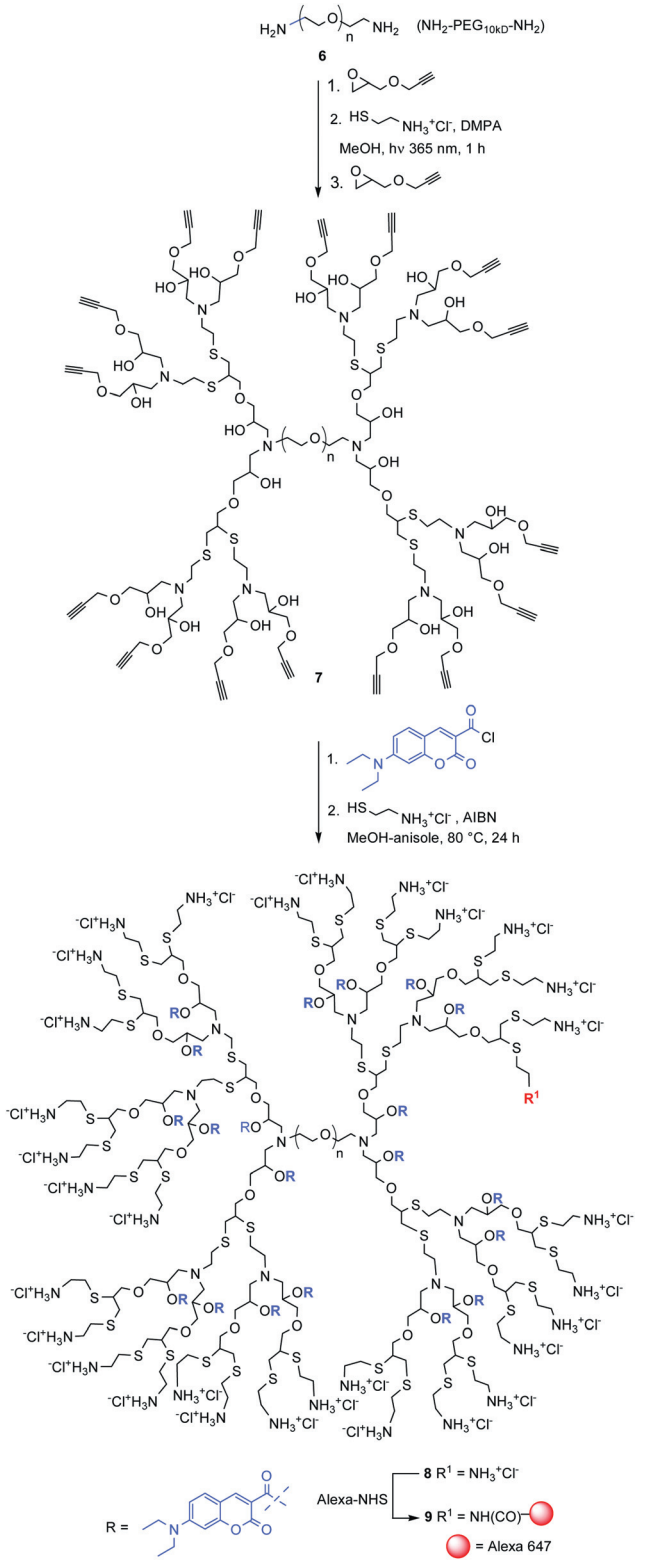
A fascinating piece of chemistry in the area of drug delivery was disclosed by Hawker and Albertazzi with their study on multifunctional trackable dendritic scaffolds.[74] Actually, an important limitation occurring when large amounts of hydrophobic drugs or dyes are bound to the chain ends of dendrimers is the alteration of dendrimer surface properties, which results in



Scheme 8 Synthesis of carboxylic terminal dendrimers as delivery vehicles of *cis*-platinum.

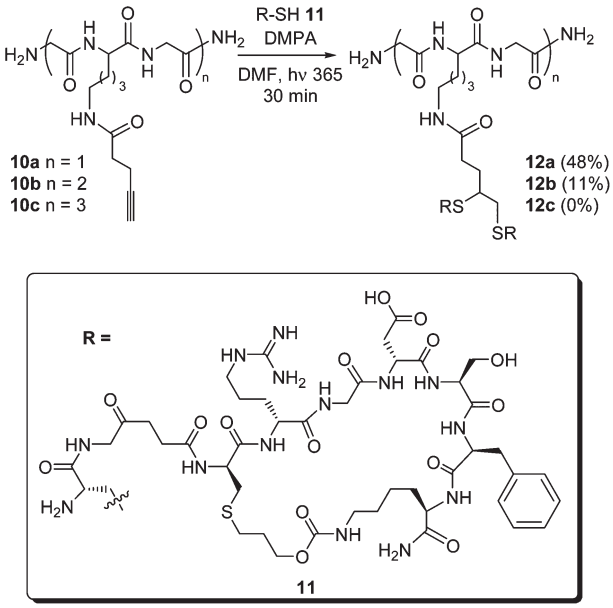
a decrease of solubility and bio-compatibility of the dendritic carrier. An alternative strategy consists in covalently attaching the cargo molecule to the interior of the dendrimer on condition that the suitable enzymatic system is capable to access and hydrolyse the linkages connecting the payload and the dendritic scaffold. The possibility of simultaneous monitoring inside living cells of both the dendrimer and its cargo molecule upon enzymatic cleavage would definitely complete the design of an ideal delivery strategy. The feasibility of such a challenging approach was investigated by the groups of Hawker and Albertazzi in a proof-of-concept study, in which they disclosed an effective synthesis of multifunctional hybrid dendritic delivery systems. These complex macromolecules were suitably designed to incorporate orthogonal functionalities at the chain ends and to the interior of the dendrimer and achieve chemoselective ligation of the dye and the payload, respectively. From a synthetic point of view, an alternating sequence of amine–epoxy and thiol–yne coupling reactions have been utilised to build up the multifunctional dendritic scaffold displaying internal hydroxyl groups and free protonated amino groups at the chain ends. The presence of the latter functionalities was also planned in advance to induce cell internalisation of the dendrimer through endocytosis. The water-soluble 10 kD bis-amine polyethylene glycol (PEG) **6** was selected as the core structure to enhance the solubility of the resulting hybrid dendritic-linear macromolecule (Scheme 9). The addition of propargyl glycidyl ether to **6** followed by photoinduced TYC (1 h) with cysteamine hydrochloride gave a second-generation octaamine intermediate, which in turn was reacted again with propargyl glycidyl ether to yield the hexadecyl-alkyne **7** bearing 20 internal hydroxy groups. At this stage of the synthesis, coumarin, a blue dye chosen as model cargo molecule, was loaded internally through a cleavable ester linkage to afford a coumarin poly-functionalised intermediate, which was subsequently subjected to AIBN-promoted *thermal* TYC (80 °C, 24 h) with cysteamine hydrochloride to yield the fourth-generation macromolecule **8** with *ca.* 20 internal coumarin units and 32 primary amino groups at the chain ends. It is worth noting that further photoinduced thiol–yne reaction was seemingly precluded by the presence of coumarin units in the dendritic structure. This experimental evidence is in agreement with previous observations on the capability of some aromatic compounds such as tryptophan to act in their photoexcited state as radical sponge and/or induce side-reactions by an electron transfer mechanism.[12] This issue, that is marginal in the present context, may instead complicate the execution of photoinduced TYC with some peptides and proteins (*vide infra*). The study of Hawker and Albertazzi was then completed by conjugating a single unit of the red dye Alexa647 to the surface of **8** through a stable amide bond. Gratifyingly, the resulting multifunctional dendritic scaffold **9** underwent intracellular enzymatic cleavage of the coumarin payload. Moreover, as a result of the dual labelling strategy adopted, the release of coumarin into the cytoplasm was successfully monitored inside living cells simultaneously to the dendritic scaffold.[74]

Besides dendrimer fabrication, another prolific field of research involving thiol–yne click chemistry is undoubtedly that of peptide–protein conjugation. In the middle of 2010 the group of Anseth reported on a straightforward approach to multivalent cyclic peptides based on sequential thiol–ene/thiol–yne photoreactions.[75] Peptide macrocyclisation and peptide clustering onto a core molecule are well-established strategies aimed at overcoming some of the inherent disadvantages related to peptide therapeutics such as high instability *in vivo* and low affinity for the intended target. Both cyclisation and multiple conjugations of



Scheme 9 Strategy for the fabrication of multifunctional dendritic scaffolds.

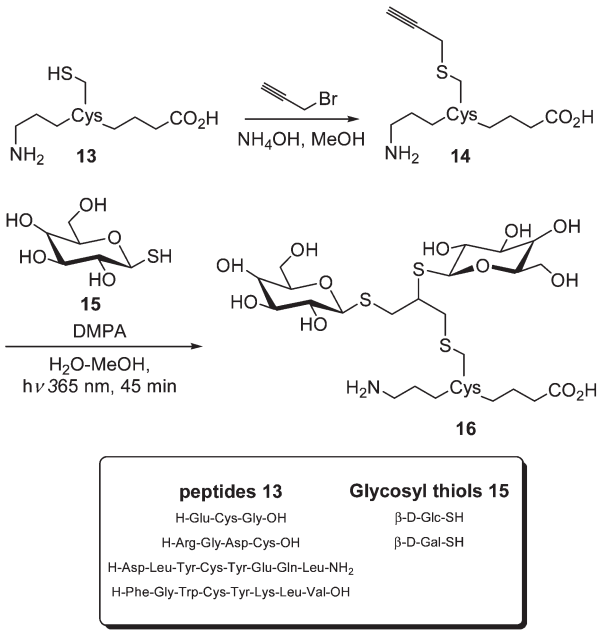
peptides necessitate, however, the execution of highly efficient and chemoselective reactions. In their study, they firstly employed the photoinduced thiol–ene reaction to induce the on-resin cyclisation of the growing peptide chain and obtain, after



Scheme 10 Synthesis of cyclic, multivalent peptides featuring the biologically active RGD sequence.

cleavage from support, the target peptide **11** featuring the active Arg-Gly-Asp (RGD) sequence and the clickable Cys residue (Scheme 10). The thiol–yne photoreaction was subsequently utilised for clustering **11** onto the alkyne-functionalised peptidic core molecules **10a–c** (valency = 2^n). Modest yields were detected for the RGD dimer **12a** (n = 1) and tetramer **12b** (n = 2), while formation of the RGD hexamer **12c** (n = 3) was not observed (in this case, TYC terminated at the level of mono-addition products). Much higher yields were obtained by clustering a linear peptide on the same core molecules **10a–c** (not shown here), thus indicating a strict dependence of TYC efficiency on the steric hindrance of the peptidic reactants. Nevertheless, the usefulness of this challenging approach to peptide therapeutics was finally validated by bioactivity studies, which showed enhanced potency of clustered peptides **12a,b** relative to the monomeric species **11**.[75]

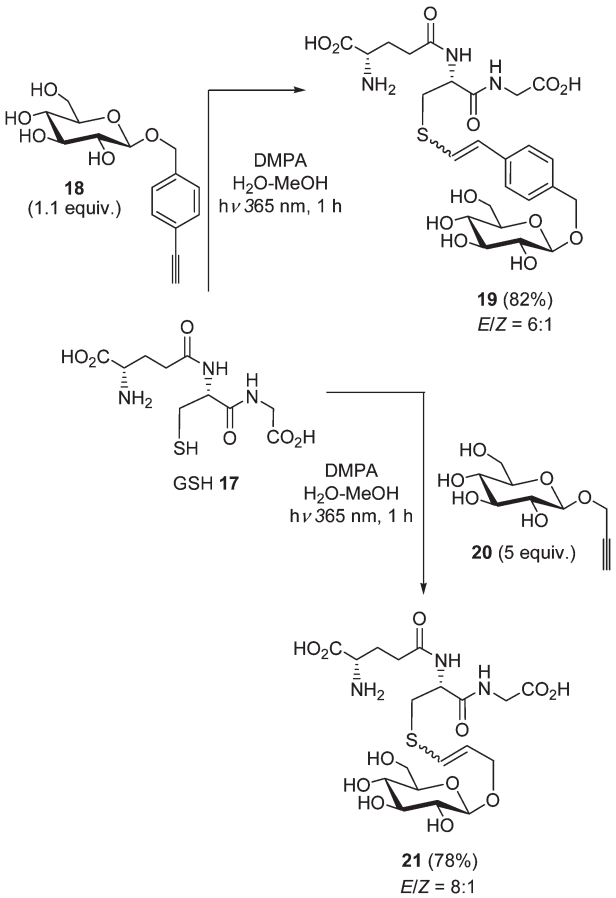
The implementation of radical thiol–yne reaction was investigated as a click process for the site-selective glycosylation of peptides and proteins as well.[14,76,77] Undeniably, synthetic glycopeptides with well-defined structures are attractive species owing to their potential utilisation as novel drug candidates and probes in glycobiology studies. Nevertheless, their preparation is complicated by the presence of the many reactive functional groups in the side chains of amino acid residues. Thus, there is a continued interest in the development of effective methods for the selective modification of peptides and proteins under mild and physiological conditions.[78] Paralleling the work of Davis on direct allylation of Cys to enable glycosylation of cysteine-containing proteins *via* olefin metathesis,[79] Dondoni utilised the 'tag-and-modify' approach[78b] for the synthesis of dually glycosylated peptides by photoinduced TYC.[76] Hence, installation of the propargyl tag on the Cys residue of peptide **13** by means of propargyl bromide was the first step of the synthetic plan (Scheme 11). Then, photoinduced hydrothiolation of the crude *S*-propargyl peptide **14** with an excess (4 equiv.) of glycosyl



Scheme 11 'Tag-and-modify' approach for the double glycosylation of cysteine-containing peptides.

thiol **15** produced the target glycosylated dithioether **16** as an inseparable mixture of diastereoisomers. The scope of the disclosed methodology was explored by using different glycosyl thiols and peptides but modest isolated yields were detected in the majority of the reported examples. The authors claimed that this unsatisfactory result was essentially due to the difficult purification of hydrophilic glycopeptides **16**. It is important to emphasise, however, that a tryptophan(Trp)-containing glycopeptide of type **16** could be recovered in only trace amounts for characterisation purposes. As the authors typically reported the conversion of **14** (>95%) but not the selectivity of its coupling with **15** (*i.e.* the purity of target glycopeptides), it is difficult to establish whether side-reactions induced by Trp occurred during the photoinduced glycosylation step. In any case, the synthetic sequence consisting of alkynyl tag installation and sequential hydrothiolation of the resulting alkynyl peptide by glycosylated and biotinylated sulfanyl radicals, though quite laborious, was definitely a major attractive item in this work.[76]

Overall, it clearly appears from the above study that, in the field of bioconjugation, isolation of the target molecules from complex reaction mixtures containing excess substrates and by-products may become a difficult task. Moreover, molecular complexity can be considered in many instances a secondary issue compared to finding a truly click way of derivatisation of valuable biological substrates. This could be the case of peptide glycosylation. Our group has been interested in this matter and investigated the effectiveness of direct photoinduced thiol–yne coupling of free cysteine-containing peptides *in their native form* with sugar alkynes to obtain mono-glycosylated peptides as vinyl sulfide adducts.[14] As anticipated, the basic idea was that the virtually irreversible nature of sulfanyl radical addition to C–C triple bonds could guarantee a click thiol–yne reaction with equimolar amounts of peptide–glycoside reagents. Crucial for succeeding in this project was, however, the accomplishment of an explorative study with model thiol and alkyne substrates.



Scheme 12 Direct glycosylation of unmodified glutathione with sugar aryl- and alkyl-alkynes.

Typically, it was observed that aromatic alkynes work as a better trap towards sulfanyl radicals, furnishing addition products in higher yields compared to aliphatic alkynes and with a distinct propensity to the formation of monosulfide rather than bis-sulfide adducts. Satisfyingly, these findings were successfully exploited for the direct glycosylation of the *unmodified* natural tripeptide glutathione **17** (γ-L-Glu-Cys-Gly, GSH) (Scheme 12). Indeed, the photoinduced coupling of **17** with a virtually equimolar amount (1.1 equiv.) of ethynylbenzyl β-D-glucopyranoside **18** in H_2O–MeOH for 1 h resulted in the quantitative formation of glycoconjugate **19**, as judged by ^{1}H NMR and LC-MS analyses of the crude reaction mixture. As required by a true click reaction, these optimal coupling conditions did not involve any significant excess of either reagents, and then allowed for a simple, rapid purification process of **19**. Despite previous concerns about the difficult isolation of hydrophilic glycopeptide species,[76] derivative **19** was readily recovered by short column chromatography with Sephadex LH20 in 82% yield as a 6 : 1 mixture of *E*/*Z* isomers. In full agreement with our previous observation on the lower reactivity of aliphatic alkynes with sulfanyl radicals, it was found that full conversion of GSH **17** to **21** could be achieved under irradiation only when using a 5-fold excess of sugar alkyne **20** (Scheme 12).

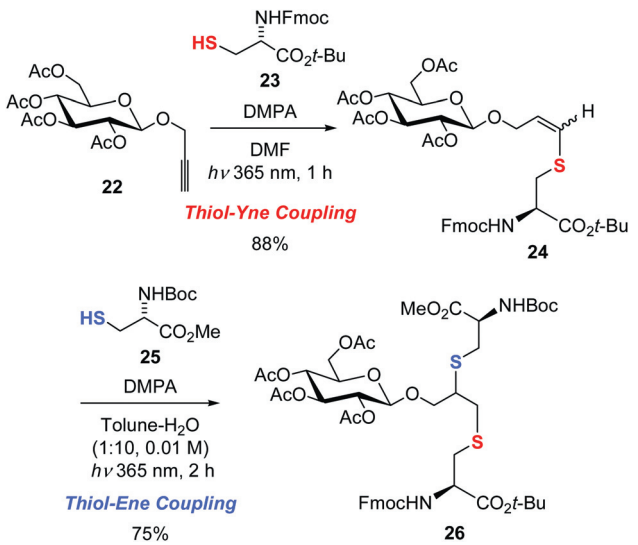
The compatibility of the optimised thiol–yne procedure with physiological conditions (phosphate buffer pH 7.4) was next established by coupling with good efficiency the sugar

arylalkyne **18** and the cysteine-containing synthetic nonapeptide TALNCNDSL (not shown here). Additionally, a proof-of-principle experiment was performed to determine the potential of our strategy for the dual modification of sugar alkynes through photoinduced TYC–TEC *hetero*sequences. Once again, this study was greatly facilitated by the previous discovery of suitable reaction windows for both mono- and double hydrothiolation of model alkynes. Accordingly, the vinyl thioether **24** was prepared in high yield by photoinduced TYC of the sugar alkyne **22** with the protected cysteine **23** in DMF as the solvent (Scheme 13). The intermediate **24** was then subjected to photoinduced TEC with the orthogonally protected cysteine **25** in diluted water–toluene (10 : 1 v/v) to give the target asymmetrically functionalised sugar derivative **26** in 66% overall yield. It is worth pointing out that the use of heterogeneous conditions significantly improved the efficiency of the TEC step, very likely because of micellar effects resulting in spots of very high reagents concentration.[14]

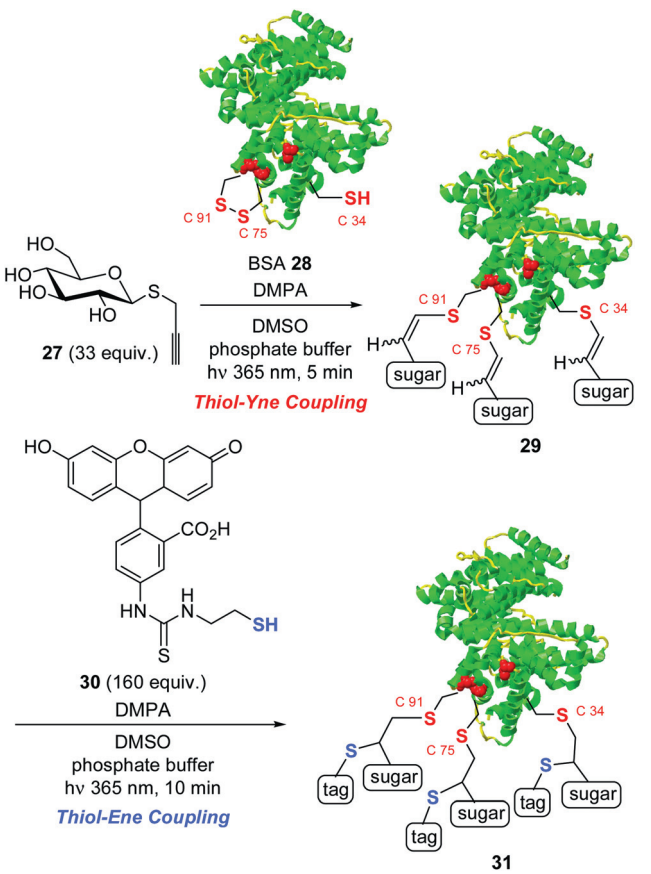
Later on, the groups of Davis and Dondoni explored the utilisation of the above dual modification strategy for achieving glycoconjugation and fluorescent labeling of bovine serum albumin (BSA) **28**, a 66 kDa globular protein featuring one free Cys residue at position 34.[77] Reproducing the experimental conditions reported in an earlier contribution on BSA glycoconjugation *via* photoinduced TEC,[12] the mixture of BSA **28** and sugar alkyne **27** (33 equiv.) was irradiated for 5 min (Scheme 14). The resulting mixture containing the intermediate **29** was then purified by size-exclusion centrifugation and again irradiated for 10 min in the presence of a large excess (160 equiv.) of fluorescein thiol **30**. Mass (MALDI-TOF) analysis of the resulting conjugate **31** indicated, however, that dual modification of BSA had occurred not only with SH-free cysteine 34 but also with the sulfhydryl groups formed by cleavage of the 75 ↔ 91 disulfide bridge, as already observed in the TEC-based study.[12] On the other hand, it is known that prolonged near-UV irradiation of goat α-lactalbumin or hen egg white lysozyme induces tryptophan residue excitation and subsequent electron transfer to some nearby cystine S–S bonds with consequential cleavage into thiol radical and thiolate anion.[80]

As the chemical integrity of disulfide bridges is often critical for protein structure and activity maintenance, the availability of an irradiation-free methodology for the direct modification at Cys residue of native proteins would be highly desirable. In our opinion, the almost ignored and underestimated peptide and protein modification strategy disclosed by Wong and Che is particularly well suited to this purpose.[28] As already mentioned, it was demonstrated that ionic thiol–yne reactions of free cysteine-containing peptides and electron-deficient alkynes proceed smoothly in aqueous media (pH 8.0 buffer, rt, 1–6 h) furnishing the corresponding vinyl sulfide conjugates in high yields and with complete chemoselectivity. The practicality and effectiveness of the above bioconjugation reaction were successfully established through selective ligation of the single free cysteine 34 of BSA **28** with the fluorescent dansyl-linked alkyne **32**, as confirmed by trypsin digestion and MALDI-TOF analysis of the resulting conjugate **33** (Scheme 15).[28]

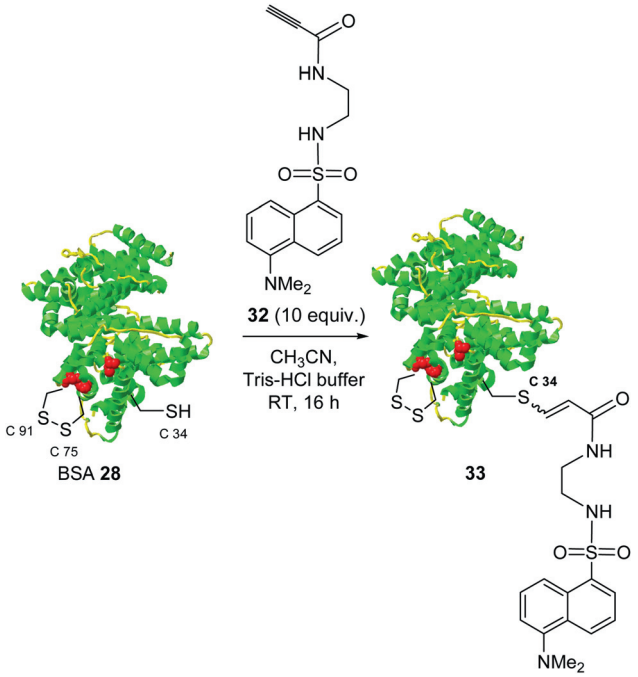
In a parallel area of research involving peptide functionalisation, the radical photoinduced thiol–yne reaction was applied to the facile synthesis of peptide-based double-hydrophilic block copolymers (DHBCs).[81] These hybrid materials are currently attracting enormous interest as biomimetic materials and their fabrication is typically achieved through direct ring-opening polymerisation (ROP) of *N*-carboxyanhydrides (NHCs) by



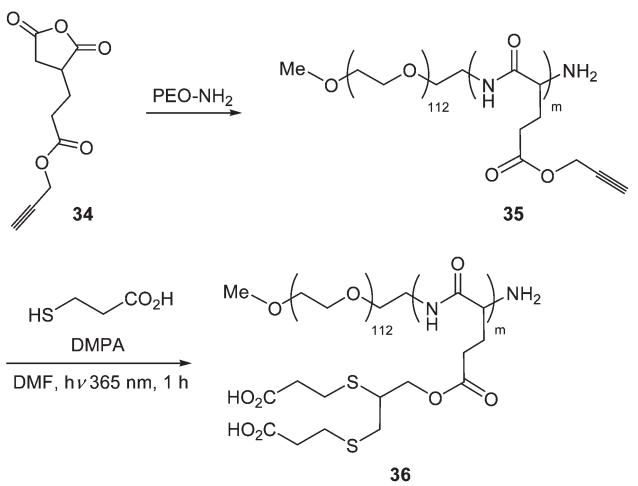
Scheme 13 TYC–TEC *hetero*sequence for the dual modification of sugar alkynes.



Scheme 14 Glycosylation and fluorescent labelling of bovine serum albumin (BSA).

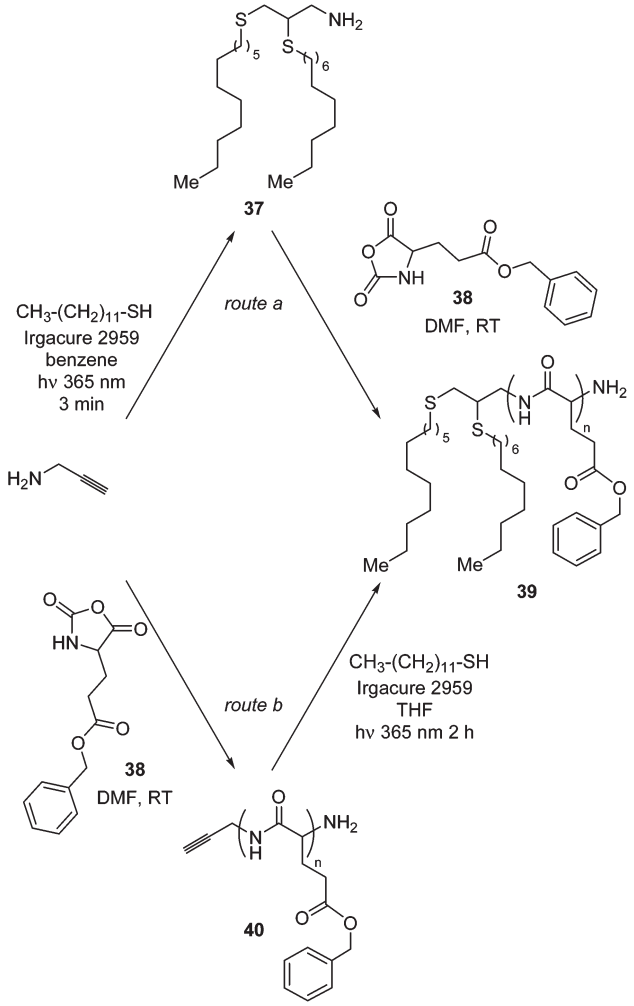


Scheme 15 Selective conjugation of BSA with a fluorescent tag.



Scheme 16 Synthesis of peptide-based double-hydrophilic block copolymers (DHBCs).

primary amine end-functionalised macroinitiators.[82] Nevertheless, this methodology suffers from an important limitation, that is the difficult side-chain functionalisation of the polypeptide segment. Although CuAAC and thiol–ene reactions proved to be successful in this endeavour, Zhu envisaged thiol–yne click chemistry as a better functionalisation strategy owing to the absence of any cytotoxic metal promoter in the coupling step and the easier access to chemical complexity in polypeptide segments.[81] As a representative example of the disclosed approach to DHBCs, the synthesis of copolymer **36**, a biomaterial utilised by the authors to control $CaCO_3$ biomineralisation, is shown in Scheme 16. Thus, the amine-terminated polyethylene glycol ($PEO–NH_2$) was used to initiate the ROP of the newly prepared monomer of γ-propargyl-L-glutamate *N*-carboxyanhydride



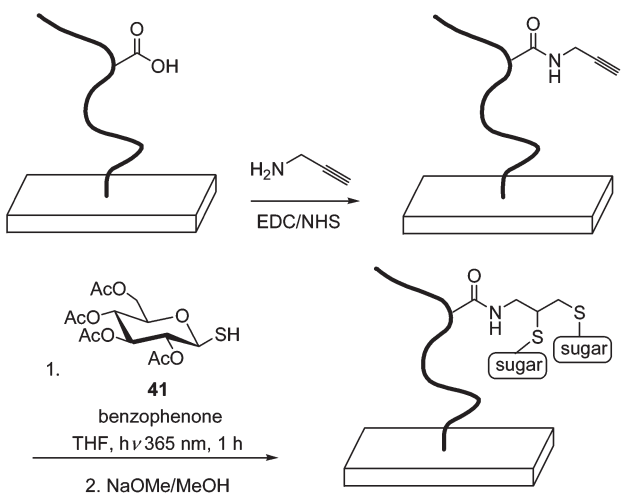
Scheme 17 Synthesis of polypeptide-based A_2B lipid mimetics.

(PLG-NCA) **34**. Then, mercaptopropionic acid was grafted onto the polypeptide backbone of intermediate **35** by photoinduced TYC giving after 1 h the polypeptide-based copolymer **36** in quantitative yield, as established by gravimetric analysis. It is worth stressing that no evidence of mono-addition product formation was detected in this study. Indeed, double hydrothiolation occurred with high efficiency in virtue of the α-helix secondary structure of **35** that induced a favourable spatial arrangement of alkyne groups along the polypeptide backbone.

In parallel, using the amphiphilic poly((propylene oxide) bis (2-aminopropyl ether)) ($NH_2–PPO–NH_2$) macroinitiator and suitable thiols in the TYC step, it was demonstrated that the disclosed strategy is also effective for the synthesis of polypeptide-based amphiphiles (not shown here).[81] These polymers serve as lipid mimetics and the production of this class of molecules *via* thiol–yne click chemistry was earlier reported by the group of Savin.[83] More precisely, A_2B 3-arm star polymers mimicking phospholipid structures (the A blocks correspond to lipophilic chains and the B block represents the polar head group) were synthesised by two different approaches both involving photoinduced TYC as the key ligation reaction (Scheme 17). In the divergent approach (*route a*), a mixture of propargyl amine, dodecanethiol (DDT), benzene solvent, and photoinitiator

(Irgacure 2959) was firstly irradiated (λ_{max} 365 nm) to afford the 2,3-bis-dodecylsulfanyl-propyl amine (DDT_2–NH_2) **37** in almost quantitative yield. Then amine **37** was used to initiate the ROP of benzyl glutamate NCA (BLG NCA) **38** and obtain the poly (benzyl glutamate) polymer **39** functionalised with two DDT 'legs' (DDT_2-PBLG; target DP_n = 20). In the convergent approach (*route b*), the DDT_2-PBLG star polymer **39** was obtained by photoinduced TYC of the propargyl amine-initiated poly(benzyl glutamate) **40** (target DP_n = 10) with dodecanethiol. These A_2B star polymers, together with those obtained using carbobenzyloxy lysine NCA (DDT_2-P(*Z*-Lys) polymers, not shown here) were subsequently deprotected, duly characterised, and demonstrated to self-assemble in aqueous solution.[83]

One of the most effective uses of click thiol–yne reaction has regarded the construction of glycosylated membranes for bioaffinity and bioseparation studies. In this field of research, in fact, fabrication of carbohydrate-decorated surfaces with high density of saccharide units is crucial for mimicking the 'glycoside cluster effect'. This effect is at the basis of many carbohydrate–protein interactions mediating fundamental biological processes such as inflammation and immune response.[84] Recently, click CuAAC was applied in the Xu laboratory to construct the cluster effect on the surface of glycosylated microporous polypropylene membranes (MPPMs) designed for lectin recognition.[85] Nevertheless, non-specific interactions between proteins and 1,2,3-triazole groups were detected along with the consequent decrease of recognition capability of membranes. Remarkable improvements were achieved by the same group through a similar approach based on photoinduced thiol–yne reaction.[86] This click process greatly facilitated realisation of the glycoside cluster effect affording surfaces with high level of functionalisation and without non-specific interactions between the thioether groups and proteins. The synthetic sequence adopted for fabricating the glycosylated MPPM surface is depicted in Scheme 18. Initially, acrylic acid (AA) is grafted by UV-induced polymerisation onto MPPM sample to give the polyAA-grafted membrane surface, which in turn is transformed into the alkyne-modified MPPM by carboxylic acid activation and condensation with propargyl amine. Subsequently, photoinduced TYC of the yne-functionalised surface with peracetylated β-D-glucopyranosyl thiol **41** followed by hydroxy groups deprotection furnishes the target glycosylated membrane with a high loading of sugar units, as established by XPS analysis. Even though the authors could not establish the completeness of the double hydrothiolation process for all alkyne groups because of the microporous nature of the membranes, they amply demonstrated the effectiveness of this novel approach. In fact, excellent affinity adsorption and significant recognition specificity towards model lectins were detected as the result of the high density of saccharides on the surface and the absence of non-specific carbohydrate–protein interactions.[86]



Scheme 18 Surface glycosylation of microporous polypropylene membranes (MPPMs) for lectin recognition.

Conclusions

Radical addition of thiols to alkynes is more than eighty years old but only in recent times has been rediscovered as a click process for the facile preparation of multifunctional polymer structures. While the efficacy of TYC-induced polymerisation has been well-documented elsewhere,[13,15b,c,42] in this review several examples have been highlighted to attest the potential of the photoinitiated thiol–yne radical reaction as a click ligation process for the post-synthetic functionalisation of polymers and materials as well as for biomolecules conjugation. In the former field of research, radical TYC has been shown to exhibit the same features of its thiol–ene counterpart, including ease of implementation, high conversion, efficiency, and orthogonality with other common click procedures, with the invaluable additional benefit of creating molecular complexity in a very straightforward manner, typically by means of TYC–TEC *homo*-sequences with the same thiol molecule. Steric bulkiness of coupling partners seems, however, to play an important role in this endeavour influencing mono- or bis-addition selectivity, which remained unascertained in several examples. A number of emerging applications have been described where TYC photoinitiation capability is successfully exploited for both spatially- and temporally-controlled functionalisations of surfaces. Undoubtedly, from this point of view, thiol–yne click chemistry might offer new, unique opportunities for the efficient fabrication of sensors and microelectronic devices.

In the field of bioconjugation, significant examples have been reported in which TYC–TEC *hetero*sequences performed in succession with two different thiols take place on the same substrate to give dually functionalised conjugates. Importantly, taking advantage of the peculiar reactivity of *aryl*alkynes in photoinduced TYCs, installation of arylacetylene tags onto biomolecules has proven to be an effective click strategy for attaining monoadduct conjugates in a highly selective manner and with marked advantages over analogous TEC procedures, in particular the requirement of merely equimolar amounts of coupling reagents.

As demonstrated by the majority of the examples herein reported, thiol–yne click chemistry is today intended as a radical process catalysed by light, though ionic and metal-promoted variants as well as different radical initiation methods are known. Photoinitiation, however, may result troublesome in some instances, as in the case of certain peptides and proteins modification.[77] Hence, the search for equally effective alternatives and the deeper understanding of the conditions affecting TYC

outcomes still remain fundamental challenges to further extend in the next future the range of valuable applications of thiol–yne click-chemistry.

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