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An Insight into the Radical Thiol/Yne Coupling: The Emergence of Arylalkyne-Tagged Sugars for the Direct Photoinduced Glycosylation of Cysteine-Containing Peptides

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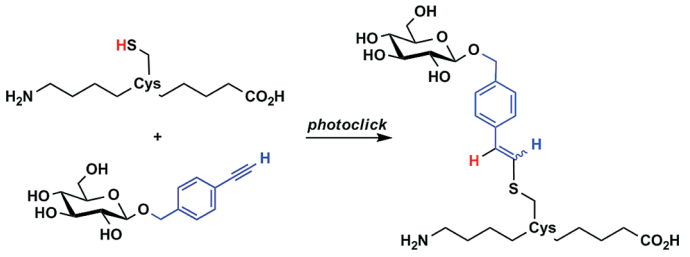

An explorative study of the Thiol−Yne Coupling (TYC) reaction has been carried out using an aliphatic (1-octyne) and an aromatic alkyne (phenylacetylene) and two alkanethiols (methyl thioglycolate and *N*-acetyl-L-cysteine methyl ester). The outcomes of the TYC reactions strongly depend on the experimental conditions (e.g., temperature, solvent, and alkyne/thiol ratio), but these can be properly adjusted to achieve selective production of either mono- or bis-coupling products. With respect to 1-octyne, phenylacetylene undergoes notably easier radical hydrothiolation, further showing a notably higher aptitude for monohydrothiolation exclusive of bis-hydrothiolation. The overall findings were exploited in glycosylation of cysteine derivatives as well as of cysteine-containing peptides. A sugar featuring an arylacetylene moiety gave rise to a true click-reaction, that is, glycosylation of the tripeptide glutathione in its native form, by means of virtually equimolar amounts of reagents. This reaction was successfully applied, under physiological conditions, to a cysteine-containing nonapeptide with marked advantages over the analogous Thiol−Ene Coupling (TEC) derivatization. A TYC/TEC sequence affording bis-armed cysteine derivatives through dual functionalization of an alkynyl sugar was additionally devised.

Introduction

In recent years, organic synthesis has witnessed a dramatic improvement of click-chemistry methods both in the biochemistry field (in particular for bioconjugation)[1] and in material science (for material derivatization).[2] Among the most popular 'click'-procedures, the thermally or photochemically induced radical addition of thiols to alkenes (the Thiol−Ene Coupling, TEC) has become an outstanding tool for functionalization of both molecules of biological interest[3] and many assorted materials,[4] owing to its special features such as chemoselectivity, versatility, and the absence of any need for metal catalysts.[3,5] Recently, it has been shown that thiol−ene couplings can be also accomplished in green solvents such as ionic liquids.[6] The main drawback of TEC is that, on many occasions, an excess of either reagents is

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(4) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* **2010**, *49*, 1540–1573.
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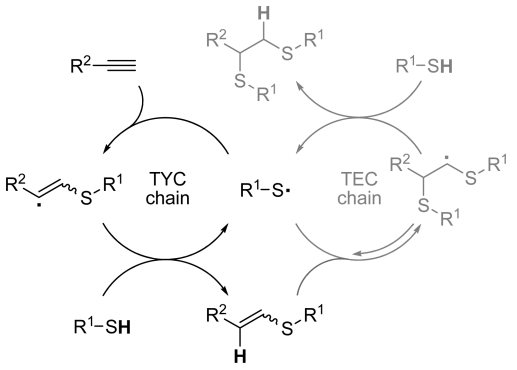
required in order to obtain complete conversions, a problem due to the reversibility of the attack of sulfur-centered radicals to C−C double bonds.[7] In those cases, additional workup is necessary to get rid of the excess of alkene/thiol and one of the main advantages of click-reactions is therefore lost. In principle, a possible way of overcoming this drawback could be to perform the reactions in the presence of alkynes instead of alkenes, that is, to carry out Thiol−Yne Couplings (TYCs). Indeed, although reported as a slower process, sulfanyl radical addition to C−C triple bonds is known to be virtually irreversible, at least with alkanesulfanyl radicals.[8] This breakthrough has actually been developed in a group of very recent papers where the authors exploited this idea in the field of polymer chemistry.[9] It should, however, be emphasized that those authors aimed at creating very highly functionalized materials (polymers and dendrimers) by means of a one-pot reaction and under very mild conditions. Hence, they drove the reaction to full completion by letting the initially formed vinyl sulfide adducts to react with additional thiol through a consecutive thiol−ene coupling. On the other hand, the issue of the selective formation of the primary vinyl sulfide TYC products could be crucial in the field of bioconjugation, where molecular complexity might be a secondary concern with respect to finding a reliable, 'clickable' way of derivatization of valuable biological substrates. As far as we know, only two papers have so far appeared focusing somewhat on this matter: the more recent one[10a] deals with dual glycosylation of peptides, thus, concentrating again on bis-addition coupling products, whereas the other one[10b] actually focuses on vinyl sulfide adducts, which

SCHEME 1. Mechanisms of TYC and TEC Radical Chains



are nevertheless attained through an ionic addition of thiols to suitable electron-deficient alkynes.

Scheme 1 shows the radical chain reactions that can operate under TYC conditions. Addition of a sulfanyl radical to the terminal carbon of the alkyne C−C triple bond gives a β-sulfanylvinyl radical that readily abstracts hydrogen from the thiol to regenerate the chain-carrier, sulfur-centered radical intermediate with concomitant formation of the vinyl sulfide monoadduct (TYC chain). Under certain circumstances, the monoadduct can undergo further addition of another sulfanyl radical to give an α,β-disulfanyl-disubstituted alkyl radical that can subsequently abstract hydrogen from the thiol to give the final bis-sulfide bis-adduct (TEC chain). Unlike the primary vinyl radical, the successive alkyl-counterpart occurs in a reversible fashion: this allows for properly tuning reaction conditions in order to favor formation of the bis-adduct (TYC-TEC sequence) or to stop the reaction at the vinyl sulfide stage (TYC).

In our opinion, the latter outcome is that deserving thorough attention. Indeed, the (virtually) irreversible occurrence of vinyl sulfide adduct from the radical TYC process could be exploited in a more 'clickable' reaction than the TEC one, since it could provide easier access to assorted sulfur-tethered products employing equivalent amounts of starting materials. This latter point could be of extreme importance in the field of bioconjugation, where the isolation of target molecules from complex reaction mixtures containing excess substrates is often a difficult task.[10a] Furthermore, synthesis and activity of bioconjugates containing the vinyl sulfide linkage are highly worthy of being explored and, to date, only a unique example dealing with this issue has been reported.[10b] Finally, the feasibility of selective syntheses of monoadducts can open the door to TYC-TEC sequences leading to nonsymmetric bis-functionalizations, hence, paving the way to very attractive dual-labeling investigations.[11]

Here, we report an explorative study on model substrates that aimed at getting more insight into the conditions affecting the coupling reaction outcome, particularly the ensuing mono- and bis-adduct ratio. Both classical organic solvents and water (or water/solvent mixtures) were taken into account for carrying out the reactions, since the aqueous medium would be crucial for applying Thiol−Yne Couplings as a ligation strategy, for instance, for peptide glycosylation,

(7) (a) Benati, L.; Montevecchi, P. C.; Spagnolo, P. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2103–2109. The reversibility of the addition of sulfanyl radicals to C−C double bonds precludes, for example, the radical addition of *disulfides* to C−C double bonds, which is inefficient unless either by employing alkynes instead of alkenes (see refs 7a above and 13a below) or in the presence of a very good radical trap such as a diselenide, see: (b) Ogawa, A.; Tanaka, H.; Yokoyama, H.; Obayashi, R.; Yokoyama, K.; Sonoda, N. *J. Org. Chem.* **1992**, *57*, 111−115 or a very efficient H-donor, see: (c) Taniguchi, T.; Fujii, T.; Idota, A.; Ishibashi, H. *Org. Lett.* **2009**, *11*, 3298–3301.

(8) (a) Heiba, E. I.; Dessau, R. M. *J. Org. Chem.* **1967**, *32*, 3837–3840. (b) Montevecchi, P. C.; Navacchia, M. L. *J. Org. Chem.* **1997**, *62*, 5600–5607. (c) Melandri, D.; Montevecchi, P. C.; Navacchia, M. L. *Tetrahedron* **1999**, *55*, 12227–12236. For a kinetic study on the addition of *benzene*sulfanyl radicals to alkynes, see: (d) Ito, O.; Omori, R.; Matsuda, M. *J. Am. Chem. Soc.* **1982**, *104*, 3934−3937. where that addition has been suggested to be slightly reversible. It is worth noting that addition of sulfur-centered radicals to alkynes is not always necessarily an irreversible process, provided that the radicals possess additional stabilization with respect to sulfanyls: sulfonyl radicals, for example, have been reported to add reversibly to C−C triple bonds, see: (e) Rosenstein, I, J. In *Radicals in Organic Synthesis*; Renaud, P., Sibi, M. P., Eds.: Wiley-VCH: Weinheim, Germany, 2001; Vol. 1, Chapter 1.4, pp 50−71. (f) Bertrand, M. P.; Ferreri, C. In *Radicals in Organic Synthesis*; Renaud, P., Sibi, M. P., Eds.: Wiley-VCH: Weinheim, Germany, Vol. 2, Chapter 5.5, pp 485−504. (g) Chatgilialoglu, C.; Ferreri, C. In *The Chemistry of Triple-Bonded Functional Groups*; Patai, S., Ed.; Wiley: Chichester, U.K., 1994; Vol. 2, pp 917−944. (h) Chatgilialoglu, C.; Bertrand, M. P.; Ferreri, C. In *S-Centered Radicals*; Alfassi, Z. B., Ed.; Wiley: Chichester, U.K., 1999; pp 311−354.

(9) (a) Fairbanks, B. D.; Scott, T. F.; Kloxin, C. J.; Anseth, K. S.; Bowman, C. N. *Macromolecules* **2009**, *42*, 211–217. (b) Chen, G.; Kumar, J.; Gregory, A.; Stenzel, M. H. *Chem. Commun.* **2009**, 6291–6293. (c) Chan, J. W.; Hoyle, C. E.; Lowe, A. B. *J. Am. Chem. Soc.* **2009**, *131*, 5751–5753. (d) Hensarling, R. M.; Doughty, V. A.; Chan, J. W.; Patton, D. L. *J. Am. Chem. Soc.* **2009**, *131*, 14673–14675. (e) Konkolewicz, D.; Gray-Weale, A.; Perrier, S. *J. Am. Chem. Soc.* **2009**, *131*, 18075–18077. (f) Lowe, A. B.; Hoyleb, C. E.; Bowman, C. N. *J. Mater. Chem.* **2010**, *20*, 4745–4750. (g) Fairbanks, B. D.; Sims, E. A.; Anseth, K. S.; Bowman, C. N. *Macromolecules* **2010**, *43*, 4113–4119. (h) Chan, J. W.; Shin, J.; Hoyle, C. E.; Bowman, C. N.; Lowe, A. B. *Macromolecules* **2010**, *43*, 4937–4942. (i) Hoogenboom, R. *Angew. Chem., Int. Ed.* **2010**, *49*, 3415–3417.

(10) (a) Lo Conte, M.; Pacifico, S.; Chambery, A.; Marra, A.; Dondoni, A. *J. Org. Chem.* **2010***75*, 4644–4647. (b) Shiu, H.-Y.; Chan, T.-C.; Ho, C.-M.; Liu, Y.; Wong, M.-K.; Che, C.-M. *Chem.—Eur. J.* **2009**, *15*, 3839–3850.

(11) For an outstanding example, see: van Kasteren, S. I.; Kramer, H. B.; Jensen, H. H.; Campbell, S. J.; Kirkpatrick, J.; Oldham, N. J.; Anthony, D. C.; Davis, B. G. *Nature* **2007**, *446*, 1105–1109.

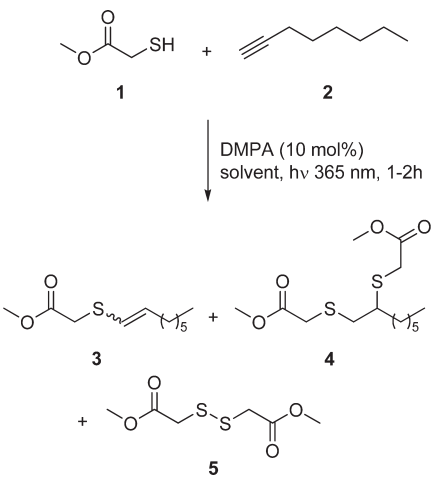
similarly to what has been done with TEC procedures.[3] In addition, we are going to confirm that, compared to alkylacetylenes, C−C triple bonds linked to aromatic substrates (arylacetylenes) work as a better trap toward sulfanyl radicals:[12] to the best of our knowledge, this enhanced behavior has been exploited neither in the domain of bioconjugation nor in that of material derivatization and could entail interesting consequences in both fields. Finally, some preliminary results will be reported showing that the data obtained from the explorative study could be successfully applied to some bioconjugation examples including glycosylation of the native form of glutathione and of an unmodified nonapeptide containing a cysteine residue.

Results and Discussion

Our long, earlier interest in the radical reactions of thiols with alkynes[13] led us to ascertain that, under thermal conditions (80−100 °C) and in hydrocarbon solvents (e.g., benzene or toluene), derived sulfanyl radicals usually lead to vinyl sulfide adducts only. Consistent with our original evidence, methyl thioglycolate **1** was presently found to react with equimolar amounts of 1-octyne **2** in toluene solution at 80 °C in the presence of AIBN as a radical initiator, affording vinyl sulfide **3** (62%) and disulfide **5** (28%) as the only identifiable reaction products (Table 1, entry 1). Nevertheless, the reaction outcomes alter to a considerable extent by changing conditions (i.e., solvent, temperature, concentration, and initiation method), as proved by a series of experiments (Tables 1−4) carried out between two thiols (methyl thioglycolate **1** and *N*-acetyl-L-cysteine methyl ester **10**) and two alkynes (1-octyne **2** and phenylacetylene **6**). The parallel behavior of thiols with aromatic and aliphatic alkynes has never been addressed in the recently reported TYC studies, but in light of our previous investigations, we thought that this point should deserve adequate consideration. Different reaction solvents were chosen on the basis of their potential employment in bioconjugation procedures (water and 95:5 water/DMSO mixtures) or material derivatization (DMSO, DMF). All the reactions were carried out at room temperature (r.t.)[14] for 1−2 h by irradiation with UV-lamp (λ_{max} 365 nm) using 2,2-dimethoxy-2-phenylacetophenone (DMPA, 10 mol %) as a radical initiator, that is, the conditions normally employed in this kind of radical couplings.[9] Thus, the model reaction of thiol **1** with alkyne **2** (1 equiv) in toluene (0.5 M) solution afforded, under these conditions, both mono- (**3**) and bis-adduct (**4**) in comparable amounts (45:55 ratio, 76% overall yield), together with very minor amounts (< 5%) of disulfide **5** (Table 1, entry 2).

As far as the **3/4** ratio is concerned, almost identical results were obtained by substituting toluene with DMSO or DMF, albeit with lower overall yields. It would therefore seem that temperature plays a pivotal role in affecting the outcoming sulfide-**3**/bis-sulfide-**4** ratio (by comparison of entries 1 and 2), as an expected result of a faster or slower back fragmentation of the dithioalkyl radical precursor of the bis-adduct. Interestingly, unlike the aliphatic acetylene **2**, under the same conditions phenylacetylene **6** gave only vinyl sulfide **7** in 90% yield (Table 2, entry 1).[15]

TABLE 1. Thiol−Yne Couplings of Methyl Thioglycolate 1 with 1-Octyne 2[a]



entry	**1**/**2**	solvent (*c* [M])	**3**/**4** [%][b,c]	yield **3** + **4** [%][d]
1	1:1	Toluene (0.5)[e]	100/−	62
2	1:1	Toluene (0.5)[f]	45/55	76
3	1:1	DMSO (0.02)	80/20	55
4	1:1	H_2O−DMSO (0.5)[g]	45/55	90
5	1:1	H_2O (0.5)	11/89	85
6	1:1	[bmim][PF_6]	25/75	52
7	2:1	DMSO (0.5)	−/100	75
8	2:1	H_2O−DMSO (0.5)[g]	−/100	90
9	2:1	H_2O (0.5)	−/100	89

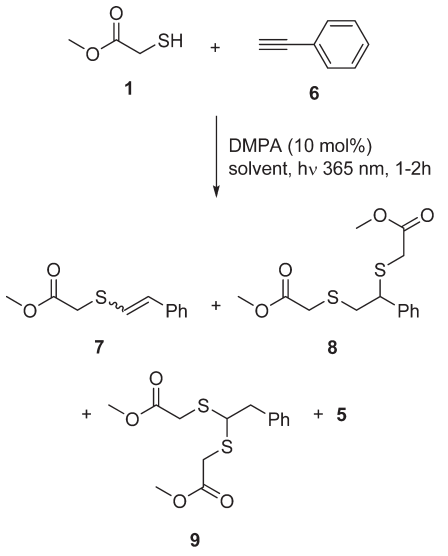
[a]Photoinduced reactions were carried out at r.t. with a household UVA lamp apparatus at λ_{max} 365 nm (see the Experimental Section for equipment setup). Reactions performed with **1** normally gave the corresponding disulfide **5** in < 5% yield; only the reaction of entry 3 afforded **5** in ca. 10% yield. [b]Values are relative percentages and were determined by GC−MS and ^{1}H NMR analysis. [c]Adduct **3** was a 1.2:1 mixture of *E* and *Z* isomers. [d]Isolated yield calculated on the basis of the starting alkyne. [e]Reaction performed under thermal conditions (80 °C) with AIBN as the radical initiator. [f]Similar results were obtained in DMSO or DMF. [g]H_2O/DMSO 95:5.

(14) Under these conditions, the reaction vessel actually warmed up to 35−40 °C. Anyway, strictly identical results were obtained with reaction mixtures kept at 25 °C by simultaneous air-cooling with compressed air.

(12) For comparisons between the reactivities of alkyl- and arylacetylenes, see refs 8d above, 13a, 13b, below, and (a) Ogawa, A.; Obayashi, R.; Ine, H.; Tsuboi, Y.; Sonoda, N.; Toshikazu, H. *J. Org. Chem.* **1998**, *63*, 881–884. For another recent example of radical monothiolation of arylacetylenes, see: (b) Beauchemin, A.; Gareau, Y. *Phosphorus, Sulfur, Silicon Relat. Elem.* **1998**, *139*, 187–192. It is worth emphasizing that a higher reactivity (kinetic constant of more than one order of magnitude higher) of phenylacetylene with respect to an alkyl congener (1-propyne) has been also reported for addition of alkyl (methyl) radicals, see: (c) Fischer, H.; Radom, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 1340–1371.

(13) (a) Benati, L.; Montevecchi, P. C.; Spagnolo, P. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2103–2109. and references therein. (b) Benati, L.; Montevecchi, P. C.; Spagnolo, P. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1659–1664. (c) Benati, L.; Capella, L.; Montevecchi, P. C.; Spagnolo, P. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1035–1038. (d) Benati, L.; Capella, L.; Montevecchi, P. C.; Spagnolo, P. *J. Org. Chem.* **1995**, *60*, 7941–7946. (e) Montevecchi, P. C.; Navacchia, M. L. *J. Org. Chem.* **1997**, *62*, 5600–5607. (f) Montevecchi, P. C.; Navacchia, M. L. *J. Org. Chem.* **1998**, *63*, 537–542. (g) Montevecchi, P. C.; Navacchia, M. L.; Spagnolo, P. *Tetrahedron* **1998**, *54*, 8207–8216. (h) Montevecchi, P. C.; Navacchia, M. L.; Spagnolo, P. *Eur. J. Org. Chem.* **1998**, 1219–1226. (i) Leardini, R.; Nanni, D.; Zanardi, G. *J. Org. Chem.* **2000**, *65*, 2763–2772. We recently exploited addition of sulfanyl radicals to alkynes as a novel tin-/metal-free method to generate assorted kinds of radicals, see: (j) Benati, L.; Calestani, G.; Leardini, R.; Minozzi, M.; Nanni, D.; Spagnolo, P.; Strazzari, S. *Org. Lett.* **2003**, *5*, 1313–1316. (k) Benati, L.; Leardini, R.; Minozzi, M.; Nanni, D.; Scialpi, R.; Spagnolo, P.; Zanardi, G. *Synlett* **2004**, 987–990. (l) Benati, L.; Bencivenni, G.; Leardini, R.; Minozzi, M.; Nanni, D.; Scialpi, R.; Spagnolo, P.; Zanardi, G. *J. Org. Chem.* **2006**, *71*, 3192–3197. (m) Bencivenni, G.; Lanza, T.; Leardini, R.; Minozzi, M.; Nanni, D.; Spagnolo, P.; Zanardi, G. *Org. Lett.* **2008**, *10*, 1127–1130.

TABLE 2. Thiol−Yne Couplings of Methyl Thioglycolate 1 with Phenylacetylene 6[a]

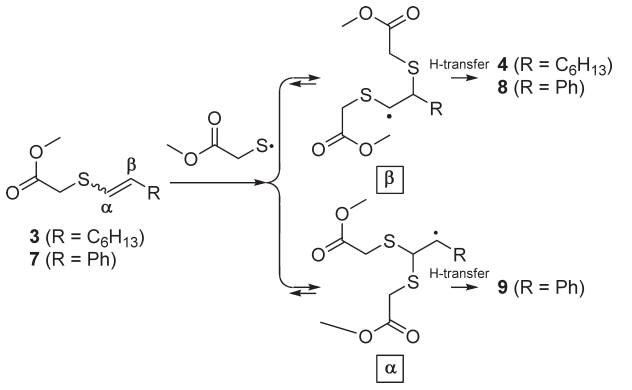


entry	**1**/**6**	solvent (*c* [M])	**7**/**8** + **9** [%][b,c]	yield **7** + **8** + **9** [%][d]
1	1:1	Toluene (0.5)[e]	100/−	90
2	1:1	DMSO (0.02)	100/−	65
3	1:1	H_2O−DMSO (0.5)[f]	100/−	87
4	1:1	H_2O (0.5)	90/10[g]	91
5	1:1	[bmim][PF_6]	90/10[g]	58
6	2:1	DMSO (0.5)	40/60[g]	69
7	2:1	H_2O−DMSO (0.5)[f]	22/78[g]	88
8	2:1	H_2O (0.5)	5/95[g]	91

[a]Photoinduced reactions were carried out at r.t. with a household UVA lamp apparatus at λ_{max} 365 nm (see the Experimental Section for equipment setup). Reactions performed with **1** normally gave the corresponding disulfide **5** in < 5% yield; only the reaction of entry 2 afforded **5** in ca. 10% yield. [b]Values are relative percentages and were determined by GC−MS and ^{1}H NMR analysis. [c]Adduct **7** was a 2:1 mixture of *Z* and *E* isomers. [d]Isolated yield calculated on the basis of the starting alkyne. [e]Similar results were obtained in DMSO or DMF. [f]H_2O/DMSO 95:5. [g]**8**/**9** ~2:1.

Dilution of the reaction mixture from 0.5 to 0.02 M led, with 1-octyne in DMSO, to preferential formation of monoadduct **3** (**3**/**4** ratio ca. 4:1; Table 1, entry 3), whereas the reaction with alkyne **6** was not substantially affected, yielding again the monoadduct **7** (65%) as the only coupling product (Table 2, entry 2); both reactions also afforded disulfide **5** as a byproduct (ca. 10%). Since, in general, dilution was found to affect negatively the reaction outcome in terms of lower overall yields, formation of byproducts, and lower conversions, all the subsequent reactions were performed in 0.5 M solutions. When the reaction of **1** with **2** was carried out in water/DMSO 95:5, no change in product distribution was observed (Table 1, entry 4), whereas pure water[16] strongly favored occurrence of bis-adduct **4** over the monoadduct **3** (**3**/**4** ratio ca. 1:8; Table 1, entry 5). So strong seems this effect of water that with this solvent also phenylacetylene **6** afforded small amounts (10%) of bis-adduct as a mixture of regioisomers **8** and **9** in a 2:1 ratio (Table 2, entry 4).

SCHEME 2. Reaction Mechanism of Formation of Bis-Adducts 4, 8, and 9



The outcoming difference in the regiochemistry of the bis-sulfide adducts arising from octyne **2** and phenylacetylene **6** probably reflects the relative stability of the alkyl radicals α and *β* that could in principle arise from further addition of sulfanyl radical to the respective double bond of vinyl sulfides **3** and **7** (Scheme 2). With sulfide **3**, the sulfanyl would strictly prefer to form the more stable radical *β*, R = C_6H_{13}, owing to back-donation stabilization provided by the attached sulfur atom; with sulfide **7**, the formation of the corresponding radical *β*, R = Ph, would be discouraged to some extent in favor of the resonance-stabilized benzylic radical α, R = Ph (Scheme 2).

It is worth noting that an analogous effect in favor of bis-hydrothiolation was observed by changing water with an ionic liquid ([bmim][PF_6]): with this solvent, 1-octyne gave adducts **4** and **3** in a 3:1 ratio (Table 1, entry 6) and phenylacetylene afforded again monoadduct **7** accompanied with minor amounts of bis-adducts **8** and **9**[17] (Table 2, entry 5). Preferential occurrence of bis-sulfide **4** at the expense of vinyl sulfide **3** would possibly entail especially fast H-transfer from thiol **1** to the intermediate dithioalkyl radical. Under these circumstances, in fact, that radical intermediate could be (seriously) discouraged to suffer usual *β*-elimination of sulfanyl radical yielding back vinyl sulfide **3** (see Scheme 1). Thus, our present findings with octyne **2** in the above ionic liquid seem to substantiate previous chemical evidence that thiols in ionic liquid solvents could act as very strong H-donors.[6] Moreover, the corresponding findings achieved in pure water first suggest that in such medium thiols should interestingly become even stronger H-donors. Whether the enhanced H-donor properties of thiol in ionic liquid and, especially, water would result from some solvent stabilization of the H-transfer transition state, as previously suggested,[6] or would just be a consequence of 'neat' reactions occurring inside organic droplets[18] is a debated question that will be dealt with in future studies.

Taking into account the overall results obtained with thiol **1** (Tables 1 and 2), we can infer that it is possible to modify properly the reaction outcome by tuning the reaction conditions. Starting from equivalent amounts of thiol and alkyne,

(15) All the vinyl sulfides were formed as mixtures of *E*- and *Z*-isomers: see the Experimental Section for details. Studies on the stereoselective formation of the kinetic (*Z*) or thermodynamic (*E*) product are currently underway.

(16) Reactions carried out in water were heterogeneous mixtures, whereas those carried out in water/DMSO 95:5 were normally homogeneous.

(17) Both in water and in the ionic liquid the **8**/**9** ratio is ca. 2:1.

(18) For a recent discussion about organic synthesis "on water", see: Chanda, A.; Fokin, V. V. *Chem. Rev.* **2009**, *109*, 725–748.

with 1-octyne **2** (Table 1), formation of monoadduct **3** is favored at higher temperatures (AIBN-initiated reaction in toluene) or diluted mixtures (0.02 M in DMSO), whereas formation of bis-adduct **4** is strongly favored in an ionic liquid such as [bmim][PF_6] and, particularly, in water. With phenylacetylene **6** (Table 2), the monoadduct **7** is always the exclusive product, with the exception of the small amounts of the regioisomeric bis-adducts **8** and **9** isolated in water or [bmim][PF_6]. If total production of bis-sulfide is desired, this can be readily achieved by using a 2-fold excess of thiol reagent. Indeed, the photolytically initiated reaction of alkyne **2** in the presence of 2 equiv of thiol **1** was found to afford virtually quantitative amounts of bis-sulfide **4** irrespective of the solvent employed (DMSO, H_2O/DMSO, or H_2O) (Table 1, entries 7−9). Even in the presence of the same excess of thiol **1**, the aromatic alkyne **6** behaved in a different fashion, since it could still afford significant amounts of monoadduct **7** both in DMSO and H_2O/DMSO (Table 2, entries 6 and 7). In pure water, however, **6** succeeded in forming virtually exclusive amounts of the bis-sulfides **8** and **9**, in line with the discovered effect of water solvent (Table 2, entry 8).

As far as the cysteine thiol **10** is concerned (Tables 3 and 4), its reactions appear slower than those of the congener **1** as a possible result of a higher steric hindrance.[19] This probably justifies the preferential formation of monoadduct **11**,[9d] which is the major product both in DMSO, water/DMSO, and pure water (Table 3, entries 1−3).

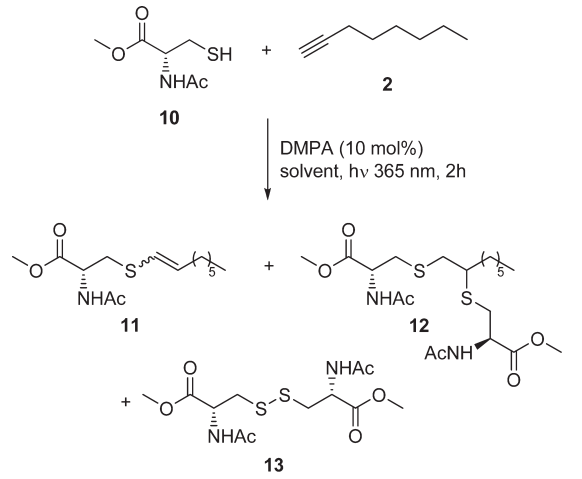
It is worth noting that highly selective production of the monoadduct **11** could be achieved by carrying out the reaction under diluted (0.02 M DMSO) conditions (35% yield of **11**, with only 50% conversion; not reported) or using a 2-fold excess of the alkyne **2** (**11**/**12** ratio ∼19:1; Table 3, entry 4). Conversely, the use of a 2-fold excess of cysteine **10** allowed highly selective occurrence of the bis-sulfide **12** (**11**/**12** ratio > 1:19 both in water/DMSO and water) in very good yield (> 90%) (Table 3, entries 5 and 6). In parallel, the TYC of the same cysteine **10** with equimolar phenylacetylene **6** afforded the monoadduct **14** in a very selective fashion under all conditions employed (Table 4, entries 1−3). No evidence of formation of a bis-adduct was observed either with 2 equiv of thiol (entries 4 and 5).

It is noteworthy that our explorative study with octyne **2** and phenylacetylene **6** clearly revealed a deeply different behavior of these two alkynes toward their radical coupling reactions with thiols **1**, **10**. Indeed, phenylacetylene **6** usually gave mono/bis-sulfide adducts in notably higher (overall) yields and, furthermore, showed a distinct propensity to form monosulfide rather than bis-sulfide product. It is therefore plausible that the aromatic alkyne **6** could act as a much stronger trap for sulfur-centered radicals than the aliphatic congener **2**. This point was actually substantiated by our additional finding that the usual reaction of cysteine **10** with equimolar amounts of both alkynes **2** and **6** in DMSO could basically afford the phenylvinyl sulfide **14** at the expense of the alkylvinyl one **11** (**11**/**14** ∼ 1:15, Scheme 3).

As anticipated, the ultimate goal of this study was to establish the potential role of photoinduced TYC as a click ligation tool for the direct monoglycosylation of unmodified

(19) We cannot exclude that polar factors may also play an additional role in the overall thiolation reaction, see: Escoubet, S.; Gastaldi, S.; Vanthuyne, N.; Gil, G.; Siri, D.; Bertrand, M. P. *J. Org. Chem.* **2006**, *71*, 7288–7292.

TABLE 3. Thiol−Yne Couplings of *N*-Acetyl-L-cysteine Methyl Ester 10 with 1-Octyne 2[a]



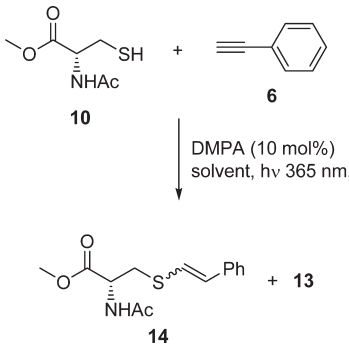
entry	**10**/**2**	solvent (*c* [M])	**11**/**12** [%][b,c]	yield **11** + **12** [%][d]
1	1:1	DMSO (0.5)	83/17	62
2	1:1	H_2O−DMSO (0.5)[e]	67/33	85
3	1:1	H_2O (0.5)	55/45	82
4	1:2	H_2O (0.5)	95/5	81
5	2:1	H_2O−DMSO (0.5)	< 5/ > 95	91
6	2:1	H_2O (0.5)[e]	< 5/ > 95	92

[a]Photoinduced reactions were carried out at r.t. with a household UVA lamp apparatus at λ_{max} 365 nm (see the Experimental Section for equipment setup). Reactions performed with **10** normally gave the corresponding cystine **13** in < 5% yield; only the reaction of entry 1 afforded **13** in ca. 20% yield. [b]Values are relative percentages and were determined by GC−MS and ^{1}H NMR analysis. [c]Adduct **11** was a 1.5:1 mixture of *Z* and *E* isomers. [d]Isolated yield calculated on the basis of the starting alkyne (entries 1−3 and 5−6) or the starting thiol (entry 4). [e]H_2O/DMSO 95:5.

peptides. The usefulness of this radical reaction in glycopeptide chemistry has been recently validated by the synthesis of dually glycosylated peptides by a two-step strategy, which involved first the *S*-propargylation of a cysteine containing peptide, and then the photoinduced coupling with excess of glycosyl thiol.[10a] Undoubtedly, if peptide mono glycosylation is the target, the direct TYC of sugar alkynes with peptides bearing a free cysteine residue appears as a more straightforward strategy. Our investigation on this complementary approach took advantage of the information gained from the above explorative study and involved peptide portions of increasing complexity. Accordingly, the coupling between the peracetylated *O*-propargyl β-glycoside **15** with a single cysteine residue, that is, the *N*-Fmoc cysteine *tert*-butyl ester **16**, was initially considered to establish optimal conditions for the selective monohydrothiolation pathway of such more complex substrates (Table 5).

The photoinduced TYC (λ_{max} 365 nm, DMPA 10 mol %) of equimolar **15** and **16** proceeded smoothly under homogeneous conditions with DMF (0.5 M) as the solvent to give exclusively the monoadduct **17** (25%) as a 1.5:1 mixture of *E*/*Z* isomers (Table 5, entry 1), albeit in low yields. Complete conversion of cysteine **16** was conveniently achieved by using a 3-fold excess of sugar alkyne **15**, thus, obtaining **17** in 88% isolated yield (entry 2). It is worth noting that, owing to the set of orthogonal protective groups, the glycosyl amino acid

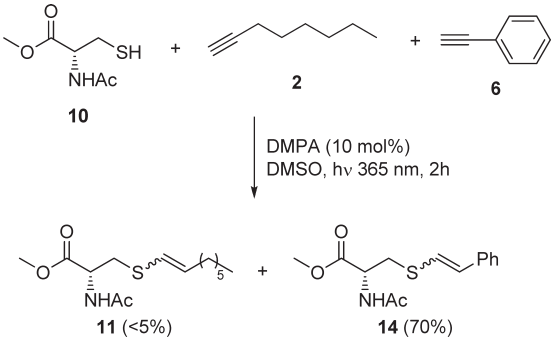
TABLE 4. Thiol−Yne Couplings of *N*-Acetyl-L-cysteine Methyl Ester 10 with Phenylacetylene 6[a]



entry	**10**/**6**	solvent (*c* [M])	yield **14** [%][b,c]
1	1:1	DMSO (0.5)	60
2	1:1	H_2O−DMSO (0.5)[d]	78
3	1:1	H_2O (0.5)	88
4	2:1	DMSO (0.5)	86
5	2:1	H_2O−DMSO (0.5)[d]	91

[a]Photoinduced reactions were carried out at r.t. with a household UVA lamp apparatus at λ_{max} 365 nm (see the Experimental Section for equipment setup). Reactions performed with **10** normally gave the corresponding cystine **13** in <5% yield; only the reaction of entry 1 afforded **13** in ca. 20% yield. [b]Adduct **14** was a ~1:1 mixture of *Z* and *E* isomers. [c]Isolated yield calculated on the basis of the starting alkyne. [d]H_2O/DMSO 95:5.

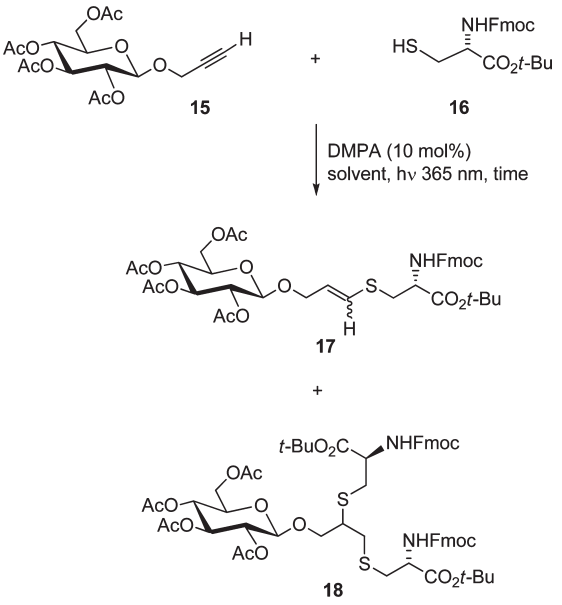
SCHEME 3. Competitive Reaction of *N*-Acetyl-L-cysteine Methyl Ester 10 with 1-Octyne 2 and Phenylacetylene 6 (1:1:1 Ratio)



17 appeared to be a suitable substrate for a co-translational approach[20] to glycopeptides through *N*-Fmoc-based peptide synthesis.

From a mechanistic point of view, possible formation of the bis-adduct **18** seemed to be strongly inhibited by steric factors in agreement with the results reported above and previous observations on photoinduced TYC of bulky thiols.[9d] Nevertheless, due to the relevance of 'bis-armed' amino acids of type **18** in peptide chemistry,[21] the double hydrothiolation of sugar alkyne **15** with cysteine **16** was actively pursued. Thus, bearing in mind the beneficial effect of heterogeneous

TABLE 5. Synthesis of Glycosyl Cysteine 17 and Bis-Armed Cysteine 18[a]



entry	**15**/**16**	solvent (*c* [M])	time (h)	yield **17**/**18** [%][b]
1	1:1	DMF (0.5)	1	25[c]/−
2	3:1	DMF (0.5)	1	88[c]/−
3	1:1	H_2O (0.5)	2	−/−[d]
4	1:1	H_2O-PhMe (0.01)[e]	2	6[c]/81[f]
5	1:2	H_2O-PhMe (0.01)[e]	2	−/85[f]

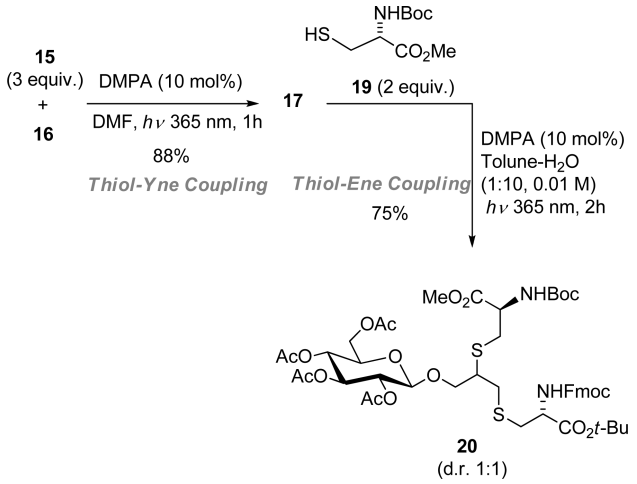
[a]Photoinduced reactions were carried out at r.t. with a household UVA lamp apparatus at λ_{max} 365 nm (see the Experimental Section for equipment setup). [b]Isolated yield calculated on the basis of the starting alkyne (entries 1, 3−5) or the starting thiol (entry 2). [c]Adduct **17** was a 1.5:1 mixture of *E* and *Z* isomers. [d]Cysteine **16** stuck on reaction vessel walls. [e]Toluene (10% v/v) was used to disperse reagents and catalyst in water. [f]d.r. ~1:1.

conditions in the model couplings of 1-octyne (Table 1, entry 5 and Table 3, entry 3), the photoinduced coupling (λ_{max} 365 nm, DMPA 10 mol %) of sugar alkyne **15** and cysteine **16** was initially performed in H_2O (0.5 M). Unfortunately, these conditions did not produce any results, very likely because of the 'sticky' nature of **16**, which resulted in agglomeration and hence precluded the intimate contact between the reaction partners (entry 3). On the other hand, when equimolar **15**, **16**, and the sensitizer DMPA (10 mol %) were previously dissolved with minimal toluene, the subsequent addition of H_2O (0.01 M) resulted in the formation of organic droplets which dispersed under vigorous magnetic stirring. Irradiation of that mixture for 2 h afforded, after concentration and column chromatography, the bis-glycosylated cysteine derivative **18** as the main product (81%, d.r. ~1:1) along with small amounts of the monoadduct **17** (6%; entry 4). The selective formation of the bis-adduct **18** was finally achieved by simply using 2 equiv of cysteine **16** under the same conditions (entry 5). Although a detailed analysis of this reaction outcome goes beyond the object of this research, it can be speculated that reactants concentration into organic droplets by means of hydrophobic interactions is responsible for the observed rate acceleration of the double hydrothiolation reaction.[18,22]

(20) McGarvey, G. J.; Benedum, T. E.; Schmidtmann, F. W. *Org. Lett.* **2002**, *4*, 3591–3594.

(21) For leading references on the synthesis of bis-amino acids, see: (a) Li, C.; Tang, J.; Xie, J. *Tetrahedron* **2009**, *65*, 7935–7941. For an interesting application, see: (b) Schafmeister, C. E.; Brown, Z. Z.; Gupta, S. *Acc. Chem. Res.* **2008**, *41*, 1387–1398.

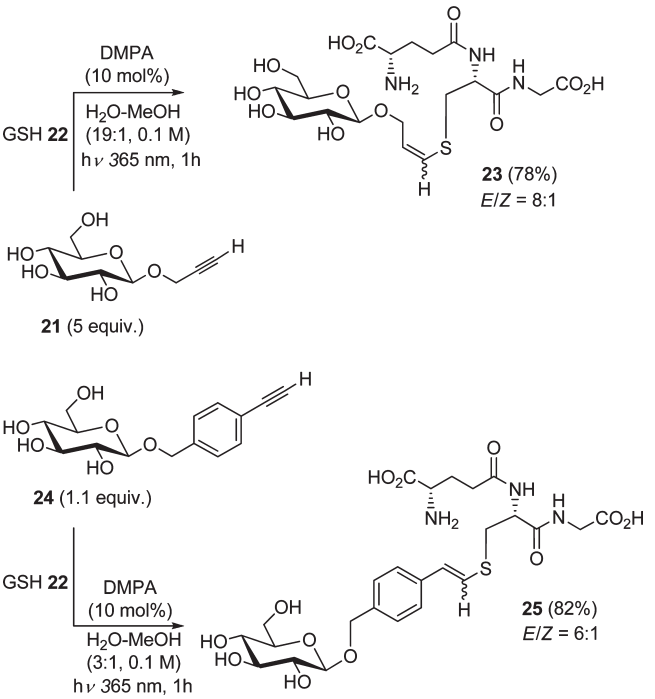
SCHEME 4. Synthesis of Orthogonally Protected Bis-Armed Cysteine 20



The discovery of a reaction window for both mono- and double-hydrothiolation of sugar alkyne **15** prompted us to investigate the sequential hydrothiolation of **15** using the two different cysteine derivatives **16** and **19** (Scheme 4). Indeed, we thought this proof-of-principle experiment might be of interest to establish the potential of photoinduced TYC/TEC sequences in dual-labeling investigations.[11] Thus, the vinyl thioether intermediate **17** was first prepared by TYC of alkyne **15** with cysteine **16** under optimized conditions (Table 5, entry 2), and then subjected to the photoinduced TEC (λ_{max} 365 nm, 2 h, DMPA 10 mol %) with cysteine **19** (2 equiv) in diluted water/toluene (10:1 v/v) (0.01 M) to give the target bis-adduct **20** (d.r. ~1:1) in 66% overall yield (Scheme 4). Noteworthy, the orthogonal protection of the bis-amino acid **20** allows for differential peptide chain elongation via Boc- and Fmoc-based peptide synthesis.[21]

The investigation of 'click' equimolar glycosylation of cysteine-containing peptides via photoinduced TYC was the next step in our program. To this aim, the readily available glycosyl alkyne **21** and the natural tripeptide glutathione **22** (γ-L-Glu-L-Cys-Gly, GSH) in its native form were considered suitable substrates for testing the efficiency of this approach (Scheme 5). After some experimentation, full conversion of GSH **22** could be achieved under irradiation (λ_{max} 365 nm, DMPA 10 mol %, 1 h) in a 19:1 H_2O−MeOH mixture[23] when using at least a 5-fold excess of sugar alkyne **21**, as it was established by LC−MS analyses (see Supporting Information). This rather disappointing, even though successful, result prompted us to synthesize an aromatic counterpart of the alkyne **21** (Scheme 5) in view of our previous discovery that an aromatic alkyne should be more effective than an aliphatic one in photoinduced TYC (Scheme 3). Accordingly, the unknown ethynylbenzyl β-D-glucopyranoside **24** was obtained by quantitative hydroxyl groups deprotection (NaOMe/MeOH) of the corresponding peracetylated derivative, which in turn was prepared, under nonoptimized conditions (45% yield), by $BF_3 \cdot OEt_2$-promoted glycosylation of β-D-glucose pentaacetate with ethynylbenzyl alcohol (Scheme S1, Supporting Information). Gratifyingly, irradiation for 1 h

SCHEME 5. Optimized Conditions for the Complete Glycosylation of Glutathione 22 by Sugar Alkynes 21 and 24

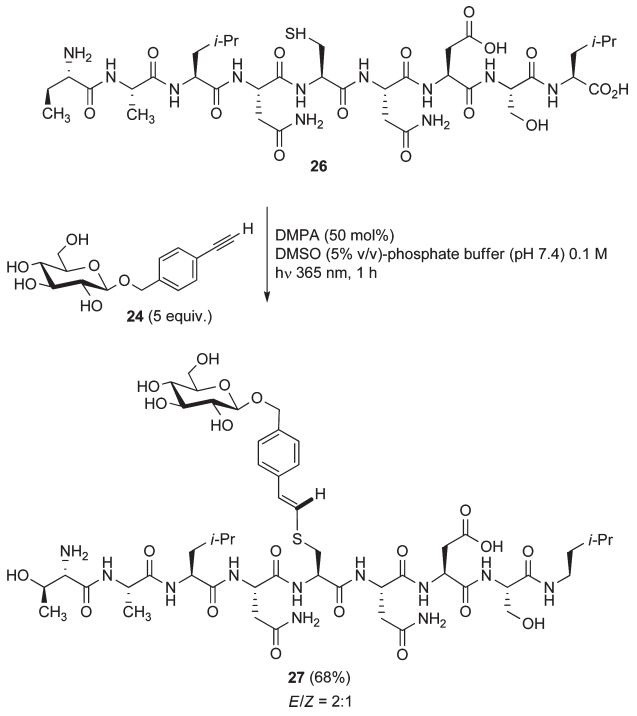


of a mixture of glutathione **22**, DMPA (10 mol %), and sugar alkyne **24** (1.1 equiv) in H_2O−MeOH 3:1 (v/v) (0.1 M)[23] resulted in quantitative formation of the glycoconjugate **25** as judged by ^1H NMR and LC−MS analyses of the crude reaction mixture (see Supporting Information). These optimal coupling conditions did not involve any significant excess of either reagents as required by a true 'click' reaction, and then allowed for a simple, rapid purification process of **25**. This compound was readily isolated by short-column chromatography with Sephadex LH20 in 82% yield as a 6:1 mixture of *E*/*Z* isomers (Scheme 5). With the pure glycopeptide **25** in hand, the stability of the vinyl thioether linkage in aqueous medium was next investigated by recording ^1H NMR spectra of a D_2O solution of **25** (0.03 M) over the time. Rewardingly, no degradation occurred during a week, as it was also confirmed by a final LC−MS analysis of the same solution.

Paralleling the previous study on peptide glycosylation via photoinduced thiol−ene coupling,[24] the efficacy of the optimized thiol−yne coupling was evaluated in aqueous solutions at physiological pH with the higher synthetic nonapeptide TALNCNDSL **26**,[25] which displays a single cysteine residue as well (Scheme 6). Hence, the coupling of **24** with **26** was optimized by adding a solution of DMPA (50 mol %) and **24** (5 equiv) in DMSO (5% final volume content) to a solution of **26** in 20 mM phosphate buffer (pH 7.4) and maintaining irradiation (λ_{max} 365 nm) for 1 h. Under these conditions, peptide **26** was quantitatively converted into the glycoconjugate **27**, which was obtained in 68% isolated yield (Sephadex LH20) as a 2:1 mixture of *E*/*Z* isomers. The selective attachment of **24** to the sulfhydryl group of **26** was duly confirmed by LC−MS/MS

(22) Pirrung, M. C.; Das Sarma, K.; Wang, J. *J. Org. Chem.* **2008**, *73*, 8723–8730.
(23) Homogeneous conditions were required to achieve good conversions of GSH **22**.
(24) Dondoni, A.; Massi, A.; Nanni, P.; Roda, A. *Chem.—Eur. J.* **2009**, *15*, 11444–11449.
(25) This compound was kindly provided as trifluoroacetic salt by UFPeptides S.r.L. (University of Ferrara).

SCHEME 6. Preparation of Glycopeptide 27



analyses of both (*E*) and (*Z*) isomers of glycopeptide **27** (Supporting Information). Inevitably, the use of a 5-fold excess of sugar alkyne **24** was required to drive the coupling to completion (LC−MS analysis). This result, however, appears quite satisfactory if compared to that obtained in the parallel TEC study.[24] In that occasion, even a 30-fold excess of a peracetylated allyl *C*-glycoside was required to achieve complete conversion of the same peptide **26**. It should be noted, however, that a detailed comparison between the two methodologies is complicated at this stage by too many variables, and therefore, it will be the object of a dedicated study.

Conclusions

Our explorative study showed that the radical alkanethiol/terminal alkyne coupling reactions are strongly affected by the adopted experimental conditions, including thiol/alkyne molar ratio, temperature and, above all, solvent. A proper choice of the reaction conditions can hence favor highly selective occurrence of either mono- or bis-sulfide coupling product. This study also showed that an aromatic alkyne, besides being much more reactive than an aliphatic congener, has a notably enhanced propensity to form the monocoupling product exclusive of the double-coupling one. Our present observations, which are unprecedented in the reported studies of TYC reactions in the fields of bioconjugation and/or material derivatization, may possibly pave the way to new important applications of the TYC strategy in those fields. The findings were preliminarily exploited in successful glycosylation of cysteine derivatives as well as in the production of a bis-armed cysteine through dual labeling of an alkynyl sugar in a TYC/TEC sequence. Further, the appealing behavior of aromatic alkynes in TYC was rewardingly applied to glycosylation of the native form of GSH just using virtually equimolar amounts of a sugar bearing an arylacetylene moiety and, more importantly, to a similar glycosylation of a cysteine-containing nonapeptide under physiological conditions. Aromatic alkynes have hence become a new, attractive tag for bioconjugation studies based on the TYC ligation strategy.

Experimental Section

Photoinduced reactions were carried out in a glass vial (diameter, 1 cm; wall thickness, 0.65 mm), sealed with a natural rubber septum, located 2.5 cm away from the UVA lamp (irradiation on sample: 365 nm, 1.04 W/m^2).

General Procedure for the Thiol/Yne Radical Couplings Reported in Tables 1−4. A mixture of alkyne (0.50 mmol), thiol (0.50 mmol unless otherwise stated), 2,2-dimethoxy-2-phenylacetophenone (13 mg, 0.05 mmol), and the stated solvent (1 mL unless otherwise stated) was irradiated at room temperature for 1−2 h under magnetic stirring. The crude mixtures of reactions performed in DMSO, H_2O/DMSO, or DMF were diluted with H_2O (5 mL) and extracted with AcOEt (3 × 5 mL). The combined organic phases were washed several times with H_2O, dried (Na_2SO_4), and concentrated. The crude mixtures of the reactions carried out in [bmim][PF_6] were extracted with AcOEt (3 × 5 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. For the reactions performed in toluene, the solvent was simply evaporated off. The resulting residues were eluted from a column of silica gel with the suitable elution system. Yields are reported in Tables 1−4.

(*E*/*Z*)-Methyl 2-(Oct-1-enylthio)acetate (3). Column chromatography with 5:1 hexanes−AcOEt afforded **3** as a 1.2:1 mixture of *E* and *Z* isomers. GC−MS: *Z*-**3**, r.t. 8.18, *m*/*z* 216 (M^+, 25), 145 (59), 143 (39), 109 (59), 85 (47), 71 (100), 55 (44); *E*-**3**, r.t. 8.23, *m*/*z* 216 (M^+, 39), 145 (74), 143 (40), 109 (50), 85 (42), 71 (100), 55 (37). ^{1}H NMR (400 MHz): δ = 6.02−5.94 (m, 1 H, H-1′), 5.77 (ddd, 0.55 H, $J_{2',3'a}$ = 7.0 Hz, $J_{2',3'b}$ = 7.1 Hz, $J_{1',2'}$ = 14.9 Hz, H-2′(*E*)), 5.65 (ddd, 0.45 H, $J_{2',3'a}$ = 7.3 Hz, $J_{2',3'b}$ = 7.4 Hz, $J_{1',2'}$ = 9.5 Hz, H-2′(*Z*)), 3.74 (s, 3 H, OMe), 3.35 (s, 2 H, 2H-2), 2.17−2.04 (m, 2 H, 2H-3′), 1.42−1.20 and 0.92−0.80 (2 m, 11 H, 2H-4′, 2H-5′, 2H-6′, 2H-7′, 3H-8′). ^{13}C NMR (100 MHz): δ = 170.3, 134.3, 131.9, 122.7, 120.7, 52.5, 52.4, 35.1, 35.0, 33.0, 31.9, 31.7, 31.6, 29.7, 29.3, 29.0, 28.9, 28.7, 22.7, 22.6, 14.1, 14.0. ESI MS (216): 239 ($M + Na^+$). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{11}H_{20}NaO_2S$ [M + Na]$^+$, 239.1082; found, 239.1087.

(*R*/*S*)-Methyl 2-[(1-[(2-Methoxy-2-oxoethyl)sulfanyl]methylheptyl)sulfanyl]acetate (4). Column chromatography with 5:1 hexanes−AcOEt afforded **4** as a yellow oil. GC−MS: r.t. 11.52, *m*/*z* 322 (M^+, <1%), 263 (7), 216 (40), 203 (47), 146 (19), 143 (73), 110 (26), 107 (29), 97 (35), 87 (27), 69 (46), 55 (100). ^{1}H NMR (600 MHz): δ = 3.71 (s, 3 H, OMe), 3.70 (s, 3 H, OMe), 3.32 and 3.21 (2 d, 2 H, J = 14.5 Hz, CH_2CO), 3.25 and 3.21 (2 d, 2 H, J = 14.7 Hz, CH_2CO), 3.00−2.91 (m, 2 H, H-1a, H-2), 2.81−2.75 (m, 1 H, H-1b), 1.80−1.70, 1.50−1.40, 1.38−1.10 (3 m, 10 H, 2H-3, 2H-4, 2H-5, 2H-6, 2H-7), and 0.85 (t, 3 H, J = 7.0, 3H-8). ^{13}C NMR (150 MHz): δ = 171.2, 171.0, 51.5, 51.4, 44.8 (C-2), 37.1 (C-1), 32.7, 32.2, 31.5, 30.6, 28.0, 25.5, 21.5, 13.0. ESI MS (322): 345 ($M + Na^+$). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{14}H_{26}NaO_4S_2$ [M + Na]$^+$, 345.1170; found, 345.1173.

(*R*/*S*)-Methyl 2-(2-[(2-Methoxy-2-oxoethyl)sulfanyl]-1-phenylethylsulfanyl)acetate (8). Column chromatography with 5:1 hexanes−AcOEt afforded **8** as a yellow oil. GC−MS: r.t. 12.05, *m*/*z* 314 (M^+, <1), 208 (55), 195 (100), 177 (18), 149 (38), 135 (29), 121 (97), 115 (20), 104 (27), 91 (25), 77 (18). ^{1}H NMR (400 MHz): δ = 7.36−7.24 (m, 5 H, Ph), 4.26 (t, 1 H, $J_{1,2}$ = 7.8 Hz, H-1), 3.71 (s, 3 H, OMe), 3.67 (s, 3 H, OMe), 3.15 (d, 2 H, 2H-2), 3.14 and 3.08 (2 d, 2 H, J = 14.7 Hz, CH_2CO), 3.13 and 2.98 (2 d, 2 H, J = 15.1 Hz, CH_2CO). ^{13}C NMR (100 MHz): δ = 170.4 (2C), 139.4, 129.1, 128.5, 128.2, 128.0, 127.8, 52.2 (2C), 49.3, 37.7, 33.4, 32.6. ESI MS

(314): 337 (M + Na^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{14}H_{18}NaO_4S_2$ [*M* + Na]$^+$, 337.0544; found, 337.0548.

Methyl 2-(1-[(2-Methoxy-2-oxoethyl)sulfanyl]-2-phenylethylsulfanyl)acetate (9). Column chromatography with 5:1 hexanes−AcOEt afforded **9** as a yellow oil. GC−MS: r.t. 11.80, *m*/*z* 314 (M^+, <1), 241 (27), 223 (50), 209 (26), 177 (48), 149 (100), 135 (50), 115 (38), 103 (14), 91 (51). ^{1}H NMR (400 MHz): δ = 7.35−7.22 (m, 5 H, Ph), 4.38 (t, 1 H, $J_{1,2}$ = 7.1 Hz, H-1), 3.70 (s, 6 H, OMe), 3.45 and 3.22 (2 d, 4 H, *J* = 15.2 Hz, 2 CH_2CO), 3.13 (d, 2 H, 2H-2). ^{13}C NMR (100 MHz): δ = 170.6 (2C), 137.5, 129.3 (2C), 128.4 (2C), 127.0, 53.8, 52.4 (2C), 42.2, 32.4 (2C). ESI MS (314): 337 (M + Na^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{14}H_{18}NaO_4S_2$ [*M* + Na]$^+$, 337.0544; found, 337.0549.

(2*R*,*E*/*Z*)-Methyl 2-Acetamido-3-(oct-1-enylthio)propanoate (11). Column chromatography with 2:1 hexanes−AcOEt afforded **11** as a 1.5:1 mixture of *Z* and *E* isomers. GC−MS: *Z*-**11**, r.t. 9.41, *m*/*z* 287 (M^+, 1), 228 (22), 177 (8), 144 (16), 111 (13), 87 (26), 81 (11), 69 (38), 55 (32), 43 (100); *E*-**11**, r.t. 9.56, *m*/*z* 287 (M^+, 2), 228 (18), 177 (5), 144 (14), 111 (13), 87 (14), 81 (15), 69 (33), 55 (34), 43 (100). ^{1}H NMR(400 MHz): δ = 6.33 (bd, 1 H, *J* = 7.0 Hz, NH), 5.90−5.70 (m, 1.4 H, H-1′(*E*), H-1′(*Z*), H-2′(*E*)), 5.58 (ddd, 0.6 H, $J_{2',3a'}$ = 7.2 Hz, $J_{2',3'b}$ = 7.3 Hz, $J_{1',2'}$ = 9.3 Hz, H-2′(*Z*), 4.91−4.83 (m, 1 H, H-2), 3.75 (s, 3 H, OMe), 3.23−3.04 (m, 2 H, 2H-3), 2.17−2.00 (m, 2 H, 2H-3′), 1.42−1.20 and 0.98−0.84 (2 m, 11 H, 2H-4′, 2H-5′, 2-H6′, 2H-7′, 3-H8′). ^{13}C NMR (100 MHz, selected data): δ = 170.8, 169.7, 134.2, 131.4, 123.8, 121.5, 52.6, 52.5, 52.1, 36.0, 35.3, 33.1, 31.6, 31.5, 29.0, 28.9, 28.8, 28.7, 23.1, 22.6, 22.5, 14.0. ESI MS (287): 310 (M + Na^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{14}H_{25}NNaO_3S$ [*M* + Na]$^+$, 310.1453; found, 310.1457.

Dimethyl (4*R*,11*R*)-7-Hexyl-2,13-dioxo-6,9-dithia-3,12-diazatetradecane-4,11-dicarboxylate (12). Column chromatography with 1:2 hexanes−AcOEt afforded **12** as a 1:1 mixture of C2 diastereoisomers. GC−MS: r.t. ∼25 (very broad peak), *m*/*z* 320 (M^+−*N*-Ac-Cys, 2%), 288 (18), 287 (13), 246 (14), 232 (17), 228 (28), 176 (34), 144 (9), 43 (100). ^{1}H NMR (400 MHz): δ = 6.64 (t, 1 H, *J* = 7.2 Hz, NH), 6.54 (t, 1 H, *J* = 7.9 Hz, NH), 4.89−4.79 (m, 2 H, H-2′, H-2″), 3.78 (s, 6H, OMe), 3.10−2.91 and 2.84−2.65 (2 m, 7 H, 2H-1, H-2, 2H-3′, 2H-3″), 2.07 and 2.06 (2 s, 6 H, C(O)Me), 1.78−1.60, 1.52−1.40, and 1.39−1.20 (3 m, 10 H, 2H-3, 2H-4, 2H-5, 2H-6, 2H-7), 0.88(t, 3 H, *J* = 6.7 Hz, 3H-8). ^{13}C NMR (100 MHz, selected data): δ = 171.3, 171.2 (2C), 170.0, 169.9, 52.7, 52.6, 52.3, 52.1, 52.0, 46.0, 38.7 (2C), 35.1, 35.0, 34.0, 33.9, 32.9, 32.7, 31.6, 29.0, 26.7, 26.6, 23.0, 22.5, 14.0. ESI MS (464): 487 (M + Na^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{20}H_{36}N_2NaO_6S_2$ [*M* + Na]$^+$, 487.1912; found, 487.1921.

(2*R*,*E*/*Z*) Methyl 2-Acetamido-3-(styrylthio)propanoate (14). Column chromatography with AcOEt afforded **14** as a ∼1:1 mixture of *E* and *Z* isomers (reported NMR spectra are for a 4:1 *Z*/*E* mixture). GC−MS: *Z*-**14**, r.t. 10.27, *m*/*z* 279 (M^+, 14), 220 (45), 161 (23), 144 (63), 134 (43), 116 (28), 115 (54), 98 (72), 91 (46), 84 (17), 77 (23), 43 (100); *E*-**14**, r.t. 10.52, *m*/*z* 279 (M^+, 14), 220 (49), 161 (25), 144 (62), 134 (47), 116 (28), 115 (61), 98 (74), 91 (51), 84 (17), 77 (25), 43 (100). ^{1}H NMR (400 MHz): δ = 7.46−7.16 (m, 5 H, Ph), 6.64 and 6.56 (2 d, 1 H, $J_{1',2'}$ = 15.6 Hz, H-1′(*E*), H-2′(*E*), 6.42 and 6.12 (2 d, 1 H, $J_{1',2'}$ = 10.7 Hz, H-1′(*Z*), H-2′(*Z*), 6.38 (d, 1 H, *J* = 7.1 Hz, NH), 4.97−4.88 (m, 1 H, H-2), 3.77 (s, 1.5 H, OMe), 3.72 (s, 1.5 H, OMe), 3.38−3.21 (m, 2 H, 2H-3), 2.03 (s, 3 H, C(O)Me), 2.00 (s, 3 H, C(O)Me). ^{13}C NMR (100 MHz, selected data): δ = 171.0 (*E*), 170.7 (*Z*), 170.5 (*E*), 169.8 (*Z*), 136.3, 129.4, 128.6, 128.5, 128.1, 127.3, 126.9, 126.5, 126.2, 125.5, 123.7, 60.3, 52.6, 52.4, 52.2, 37.7, 35.0, 22.9, 20.9, 14.0. ESI MS (279): 302 (M + Na^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{14}H_{17}NNaO_3S$ [*M* + Na]$^+$, 302.0827; found, 302.0830.

(2*R*,*E*/*Z*)-*tert*-Butyl 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(3′-(2″,3‴,4‴,6‴-tetra-*O*-acetyl-*β*-D-glucopyranosyl)oxyprop-1-enylthio)propanoate (17). A mixture of propargyl glycoside **15** (300 mg, 0.78 mmol), cysteine derivative **16** (103 mg, 0.26 mmol), 2,2-dimethoxy-2-phenyl-acetophenone (7 mg, 0.026 mmol), and DMF (1.5 mL) was irradiated at room temperature for 1 h under magnetic stirring, and then concentrated. The resulting residue was eluted from a column of silica gel with (i-Pr)$_2$O and then with 9:1 (i-Pr)$_2$O/AcOEt to give **17** (179 mg, 88%) as 1.5:1 mixture of *E* and *Z* isomers. ^{1}H NMR (300 MHz, DMSO-d_6, 120 °C, mixture of *E* and *Z* isomers): δ = 7.86 (d, 2 H, *J* = 7.4 Hz, Ar), 7.68 (d, 2 H, *J* = 7.4 Hz, Ar), 7.46−7.39 and 7.38−7.30 (2 m, 4 H, Ar), 7.14 (bs, 1 H, NH), 6.36 (ddd, 0.6 H, $J_{1',3a'}$ = 0.5 Hz, $J_{1',3b'}$ = 0.6 Hz, $J_{1',2'}$ = 15.0 Hz, H-1′(*E*)), 6.32 (ddd, 0.4 H, $J_{1',3a'}$ = 0.5 Hz, $J_{1',3b'}$ = 0.6 Hz, $J_{1',2'}$ = 9.5 Hz, H-1′(*Z*)), 5.75−5.60 (m, 1 H, H-2′), 5.28−5.15 (m, 1 H, H-3″), 4.94 (dd, 0.4 H, $J_{3'',4''}$ = 9.0 Hz, $J_{4'',5''}$ = 9.5 Hz, H-4″(*Z*)), 4.92 (dd, 0.6 H, $J_{3'',4''}$ = 9.0 Hz, $J_{4'',5''}$ = 9.5 Hz, H-4″(*Z*)), 4.85−4.70 (m, 1 H, H-1″, H-2″), 4.40−4.32 (m, 2 H, FmocCH_2), 4.30−4.06 and 4.00−3.88 (2 m, 7 H, FmocC*H*, H-2, 2 H-3′, H-5″, 2 H-6″), 3.20−2.80 (m, 2 H, 2 H-3), 2.05−1.92 (8 s, 12 H, CH_3), 1.45 (s, 9 H, *t*-Bu). ^{13}C NMR (75 MHz, mixture of *E* and *Z* isomers and conformers, selected data): δ = 170.3, 169.3, 115.6, 143.8, 141.3,128.5, 127.7, 125.1, 123.7, 120.0, 99.2, 83.2, 72.8, 71.7, 713, 69.3, 68.4, 67.2, 65.5, 61.9, 54.8, 54.1, 47.1, 37.0, 35.2, 27.9, 20.7. ESI MS (785): 803 (M + NH_4^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{39}H_{51}N_2O_{14}S$ [*M* + NH_4]$^+$, 803.3050; found, 803.3061.

(2*R*,2′*R*/*S*,2″*R*)-*tert*-Butyl 2-(((9*H*-Fluoren-9-yl)methoxy)carbonylamino)-3-(1′-(2″-((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3″-*tert*-butoxy-3″-oxopropylthio)-3′-((2‴,3‴,4‴,6‴-tetra-*O*-acetyl-*β*-D-glucopyranosyl)oxypropan-2′-ylthio)propanoate (18). To a mixture of cysteine derivative **16** (155 mg, 0.39 mmol), propargyl glycoside **15** (75 mg, 0.20 mmol), and 2,2-dimethoxy-2-phenyl-acetophenone (10 mg, 0.039 mmol) in toluene (2 mL) was added H_2O (19 mL). The resulting dispersion was irradiated at room temperature for 2 h under vigorous magnetic stirring, then concentrated, and eluted from a column of silica gel with (i-Pr)$_2$O and then with 9:1 (i-Pr)$_2$O/AcOEt to give **18** (206 mg, 85%) as a ∼1:1 mixture of C2′-diastereoisomers. ^{1}H NMR (300 MHz, DMSO-d_6, 120 °C, mixture of diastereoisomers): δ = 7.86 (d, 4 H, *J* = 7.4 Hz, Ar), 7.68 (d, 4 H, *J* = 7.4 Hz, Ar), 7.45−7.36 and 7.35−7.28 (2 m, 8 H, Ar), 7.06 (bs, 2 H, NH), 5.22 (dd, 0.5 H, $J_{3''',4'''}$ = 9.0 Hz, $J_{2''',3'''}$ = 9.5 Hz, H-3‴(diast. I)), 5.20 (dd, 0.5 H, $J_{3''',4'''}$ = 9.0 Hz, $J_{2''',3'''}$ = 9.5 Hz, H-3‴(diast. II)), 4.93 (dd, 0.5 H, $J_{4''',5'''}$ = 9.0 Hz, H-4‴-(diast. I)), 4.92 (dd, 0.5 H, $J_{4''',5'''}$ = 9.0 Hz, H-4‴(diast. II)), 4.86−4.74 (m, 2 H, H-1‴, H-2‴), 4.44−3.89 (m, 12 H, 2 FmocC*H*, 2 FmocCH_2, H-2, H-2″, H-3′$_a$, H-5‴, 2 H-6‴), 3.82 (dd, 0.5 H, $J_{2',3'b}$ = 5.0 Hz, $J_{3'a,3'b}$ = 10.5 Hz, H-3′b (diast. I), 3.70 (dd, 0.5 H, $J_{2',3'b}$ = 5.5 Hz, $J_{3'a,3'b}$ = 10.5 Hz, H-3′b (diast. II), 3.20−2.70 (m, 5 H, 2 H-3, 2 H-1″, H-2′), 2.02−1.92 (8 s, 12 H, CH_3), 1.46−1.42 (4 s, 18 H, *t*-Bu). ^{13}C NMR (75 MHz, mixture of diastereoisomers and conformers, selected data): δ = 170.6, 169.4, 155.8, 143.8, 141.3, 127.7, 127.0, 125.1, 120.0, 101.2, 100.8, 83.0, 82.9, 72.7, 72.6, 71.8, 71.0, 68.3, 67.2, 61.8, 54.6, 53.4, 47.0, 34.4, 28.0, 20.7, 20.6. ESI MS (1184): 1203 (M + NH_4^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{61}H_{76}N_3O_{18}S_2$ [*M* + NH_4]$^+$, 1202.4560; found, 1202.4551.

(2*R*,2′*R*/*S*,2″*R*)-Methyl 2-((*tert*-Butoxycarbonylamino)-3-(1′-(2″-((9*H*-fluoren-9-yl)methoxy)carbonylamino)-3‴-*tert*-butoxy-3‴-oxopropylthio)-3′-(2‴,3‴,4‴,6‴-tetra-*O*-acetyl-*β*-D-glucopyranosyl)oxypropan-2′-ylthio)propanoate (20). To a mixture of alkene **17** (150 mg, 0.19 mmol), cysteine derivative **19** (90 mg, 0.38 mmol), 2,2-dimethoxy-2-phenyl-acetophenone (10 mg, 0.038 mmol) in toluene (2.0 mL) was added H_2O (19 mL). The resulting dispersion was irradiated at room temperature for 2 h under magnetic stirring, and then concentrated. The resulting residue was eluted from a column of silica gel with 8:1 (i-Pr)$_2$O/AcOEt to give **20** (146 mg, 75%) as a ∼1:1 mixture of C2′-diastereoisomers. ^{1}H NMR (300 MHz, DMSO-d_6, 120 °C, mixture of diastereoisomers): δ = 7.86 (d, 2 H, *J* = 7.4 Hz, Ar), 7.70 (d, 2 H, *J* = 7.4 Hz, Ar), 7.45−7.38 and 7.37−7.30 (2 m, 4 H, Ar), 7.14 (bs, 1 H, NH), 6.60 (bs, 1 H, NH), 5.35 (dd, 0.5 H, $J_{3''',4'''}$ = 9.0 Hz, $J_{2''',3'''}$ = 9.5 Hz, H-3‴(diast. I)), 5.90 (d, 0.5 H, $J_{1''',2'''}$ = 7.5 Hz, H-1‴ (diast. I), 5.24 (dd, 0.5 H, $J_{3''',4'''}$ = 9.0 Hz, $J_{2''',3'''}$ = 9.5 Hz, H-3‴(diast. II)),

5.00−4.75 (m, 2.5 H, H-2‴, H-4‴, H-1‴ (diast. II), 4.40−4.32, 4.28−4.06, 4.00−3.90, and 3.80−3.64 (4 m, 13 H, FmocC*H*, FmocCH_2, H-2, H-2″, 2 H-3′, H-5‴, 2 H-6‴, OCH_3), 3.10−2.80 (m, 5 H, H-3, H-1″), 2.05−1.94 (8 s, 12 H, CH_3), 1.46 (s, 9 H, *t*-Bu), 1.43 and 1.42 (2 s, 9 H, *t*-Bu). ^{13}C NMR (75 MHz, mixture of diastereoisomers and conformers, selected data): δ = 170.7, 169.4, 155.8, 155.2, 143.8, 141.3, 127.7, 127.1, 125.2, 120.0, 101.2, 100.8, 91.7, 83.0, 72.8, 72.6, 71.9, 71.0, 68.3, 68.2, 67.2, 61.8, 61.4, 60.4, 54.7, 53.4, 52.6, 47.0, 46.8, 45.8, 40.3, 35.9. 29.7, 28.3, 28.0, 21.0, 20.9. ESI MS (1020): 1038 (M + NH_4^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{48}H_{68}N_3O_{18}S_2$ [*M* + NH_4]$^+$, 1038.3934; found, 1038.3922.

Glycopeptide 23. A mixture of sugar alkyne **21** (272 mg, 1.25 mmol), reduced glutathione **22** (77 mg, 0.25 mmol), 2,2-dimethoxy-2-phenyl-acetophenone (6 mg, 0.025 mmol), MeOH (0.1 mL), and H_2O (1.9 mL) was irradiated at room temperature for 1 h under magnetic stirring, and then concentrated. The resulting white solid residue was suspended in MeOH (2 mL) and triturated with portions of MeOH (3 × 2 mL) which were pipetted off. The residue left after trituration was eluted from a column of Sephadex LH20 with 3:1 H_2O−MeOH to give **23** (102 mg, 78%) as a 8:1 mixture of (*E*)- and (*Z*)-isomers (reported NMR spectra are for a 2:1 *E*/*Z* mixture). The occurrence of the *E*-isomer as the major compound was confirmed by analysis of the vinyl protons region of the ^{1}H NMR spectrum of the crude reaction mixture. ^{1}H NMR (300 MHz, D_2O, (*E*/*Z*)-isomers): δ = 6.25 (d, 0.66 H, *J* = 15.0 Hz, C*H*═CH (*E*)), 6.19 (d, 0.33 H, *J* = 10.5 Hz, C*H*═CH (*Z*)), 5.70−5.60 (m, 1 H, CH═C*H*), 4.48−4.40 (m, 1 H, H-5), 4.30 (d, 0.66 H, $J_{1''',2'''}$ = 8.0 Hz, H-1‴(*E*)), 4.28 (d, 0.33 H, $J_{1''',2'''}$ = 8.5 Hz, H-1‴(*Z*)), 4.22−4.00 (m, 2 H, 2 H-3′), 3.80−3.50 (m, 6 H, 2 H-2, H-10, H-5‴, 2 H-6‴), 3.34−3.04 (m, 4 H, H-1′a, H-2‴, H-3‴, H-4‴), 2.94−2.82 (m, 1 H, H-1′b), 2.40−2.30 (m, 2 H, 2 H-8), 2.02−1.94 (m, 2 H, 2 H-9).^{13}C NMR (75 MHz, D_2O, (*E*/*Z*)-isomers; selected data): δ = 174.7, 174.6, 173.8, 173.6, 172.1, 172.0, 129.7, 129.0, 128.3, 124.8, 124.4, 101.1, 100.7, 75.9, 75.8, 75.7, 72.9, 69.5, 69.4, 65.6, 60.6, 53.7, 52.9, 41.7 31.1, 25.9. ESI MS (525): 526 (M + H^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{19}H_{32}N_3O_{12}S$ [*M* + H]$^+$, 526.1707; found, 526.1701. The LC−MS analysis of the reaction mixture was performed to establish the full conversion of glutathione and confirm the *E*/*Z* ratio of **23** (see Figure S1).

Glycopeptide 25. A mixture of sugar alkyne **24** (79 mg, 0.27 mmol), reduced glutathione **22** (75 mg, 0.24 mmol), 2,2-dimethoxy-2-phenyl-acetophenone (6 mg, 0.024 mmol), and MeOH (0.5 mL), and H_2O (1.5 mL) was irradiated at room temperature for 1 h under magnetic stirring, and then concentrated. The resulting residue was eluted from a column of Sephadex LH20 with 1:1 H_2O−MeOH to give **25** (120 mg, 82%) as a 6:1 mixture of (*E*)- and (*Z*)-isomers. The occurrence of the *E*-isomer as the major compound was confirmed by analysis of the vinyl protons region of the ^{1}H NMR spectrum of the crude reaction mixture. ^{1}H NMR (400 MHz, D_2O, (*E*)-isomer): δ = 7.40−7.20 (m, 4, H, Ar), 6.72 and 6.54 (2 d, 2 H, *J* = 15.6 Hz, C*H*═C*H*), 4.74 and 4.57 (2 d, 2 H, *J* = 11.7 Hz, 2 H-2′), 4.56−4.50 (m, 1 H, H-5), 4.36 (d, 1 H, $J_{1''',2'''}$ = 8.0 Hz, H-1‴), 3.77 (dd, 1 H, $J_{5''',6'''a}$ = 2.0 Hz, $J_{6'''a,6'''b}$ = 12.5 Hz, H-6‴a), 3.74 (s, 2 H, 2H-2), 3.58 (dd, 1 H, $J_{5''',6'''b}$ = 5.5 Hz, H-6‴b), 3.57 (t, 1 H, *J* = 7.0 Hz, H-10), 3.40−3.10 (m, 5 H, H-2‴, H-3‴, H-4‴, H-5‴, H-1′a), 3.03 (dd, 1 H, $J_{1'b,5}$ = 8.0 Hz, $J_{1'a,1'b}$ = 14.5 Hz, H-1′b), 2.36−2.28 (m, 2 H, 2 H-8), 2.05−1.90 (m, 2 H, 2 H-9). ^{1}H NMR (400 MHz, D_2O, (*Z*)-isomer; selected data): δ = 6.49 and 6.22 (2 d, 2 H, *J* = 10.8 Hz, C*H*═C*H*), 4.37 (d, 1 H, $J_{1''',2'''}$ = 8.0 Hz, H-1‴). ^{13}C NMR (75 MHz, D_2O, (*E*/*Z*)-isomers; selected data): δ = 174.9, 173.8, 172.4, 116.7, 135.9, 129.5, 129.0, 127.3, 126.0, 124.3, 101.3, 76.1, 76.0, 73.3, 71.3, 69.9, 61.0, 54.0, 53.7, 41.8, 33.8, 31.4, 26.1. ESI MS (601): 619 (M + NH_4^+). HRMS (ESI/Q-TOF): calcd *m*/*z* for $C_{25}H_{39}N_4O_{12}S$ [*M* + NH_4]$^+$, 619.2280; found, 619.2275. The LC−MS analysis of the reaction mixture was performed to establish the full conversion of glutathione and confirm the *E*/*Z* ratio of **25** (see Figure S2). The LC MS/MS analysis of **25** was also performed to confirm the selective glycosylation of the cysteine residue of glutathione (see Figure S3).

Glycopeptide 27. A solution of sugar alkyne **24** (13.8 mg, 0.047 mmol) and DMPA (1.2 mg, 4.71 μmol) in DMSO (0.25 mL) was added to a solution of peptide **26** (10.0 mg, 9.41 μmol) in 20 mM phosphate buffer (pH 7.4, 5.0 mL). The resulting solution was irradiated at room temperature for 1 h under magnetic stirring, and then lyophilized. The resulting residue was eluted from a column of Sephadex LH20 with H_2O to give **27** (8.0 mg, 68%) as a 2:1 mixture of (*E*)- and (*Z*)-isomers but slightly contaminated by excess sugar alkyne (reported ^{1}H NMR spectrum is for a ∼1:2 *E*/*Z* mixture). The occurrence of the *E*-isomer as the major compound was confirmed by analysis of the vinyl protons region of the ^{1}H NMR spectrum of the crude reaction mixture. ^{1}H NMR (400 MHz, D_2O, (*E*/*Z*)-isomers, selected data): δ = 6.64 and 6.58 (2 d, 066 H, *J* = 13.5 Hz, C*H*═C*H*(*E*)), 6.44 and 6.165 (2 d, 1.32 H, *J* = 9.5 Hz, C*H*═C*H*-(*Z*)). The LC−MS analysis of the reaction mixture was performed to establish the full conversion of peptide **26** and confirm the *E*/*Z* ratio of **27** (see Figure S4). The LC MS/MS analyses of peptide **26** and glycopeptide **27** were performed to confirm the selective glycosylation of the cysteine residue of **26** (see Figures S5 and S6).

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Supporting Information Available: General experimental methods, synthesis of **24**, LC ESI-IT MS analyses of glycopeptides **23**, **25**, and **27**; copies of ^{1}H and ^{13}C NMR spectra of **3**, **4**, **7**−**9**, **11**, **12**, **14**, **17**, **18**, **20**, **23**, **peracetylated-24**, **24**, **25**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.