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Synthesis of *S*-glycosyl amino acids and *S*-glycopeptides via photoinduced click thiol–ene coupling

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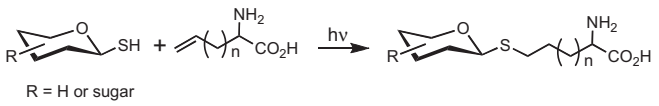
ABSTRACT

We report on the photoinduced addition of glycosyl thiols to alkenyl glycines (thiol–ene coupling) to give *S*-glycosyl amino acids in high yields (53–90%). One of the amino acids thus prepared was used in the construction of a tripeptide via solution phase chemistry. Moreover, a one-pot two-step approach to an *S*-glycopeptide was developed using glutathione (GSH) as model starting material. This approach involved GSH *S*-alkenylation followed by photoinduced hydrothiolation by a glycosyl thiol.

While native *O*- and *N*-glycosidic bonds of glycopeptides are prone to hydrolytic cleavage by *O*- and *N*-glycosidases, synthetic *C*- and *S*-analogs are expected to be stable toward such enzymatic degradation. Therefore, much effort has been devoted in the last decades to synthesizing *C*- and *S*-glycosyl amino acids and their assembly in glycopeptides.[1,2] Glycopeptides with these non-native linkages can be used as probes for biochemical studies and leads in drug discovery, such as, for example, vaccines. It has to be noticed, however, that the replacement of the oxygen atom of an *O*-linked glycopeptide with a sulfur atom is the most straightforward way for isosteric mimicry because the differences existing between the C–S and C–O bond lengths as well as the C–S–C and C–O–C bond angles result in small variation between the positions of carbon atoms in C–O and C–S glycosidic linkages.[3] Nevertheless, the longer C–S bond and the weaker stereoelectronic effect in *S*-linked derivatives allow greater flexibility. Among the various methods for *S*-glycosyl amino acid and peptide synthesis,[1c] the coupling of an activated sugar derivative with a protected cysteine residue is most widely exploited.[4] Cyclic *S*-glycopeptide, however, have been preferentially prepared by peptide synthesis with *S*-glycosylated amino acid building blocks.[5] Substantial advancements have been made in very recent years. A method reported by Davis and coworkers involves coupling of a carbohydrate thiosulfonate with a cysteine residue to give a disulfide glycosyl amino acid that in turn is desulfurized by a phosphine.[6] This approach was applied to a protein containing a single cysteine residue to give the thioether-linked glycoprotein. Reported from Davis laboratory is also another method that appeared in a very recent publication[7] when our work described below was in progress.[8] It involves thermally or photochemically induced free-radical addition of glycosyl thiols to homoallylglycine in a protected and unprotected form to give alkyl-tethered *S*-glycosyl glycines. Also this work was propaedeutic to the synthesis of *S*-linked protein glycoconjugates.

The recent disclosures from the Davis group prompted us to report here on a similar approach to *S*-glycosyl amino acids, that is, based on the photoinduced hydrothiolation of alkenyl glycines by glycosyl thiols (Scheme 1). This photoreaction, currently referred to as thiol–ene coupling (TEC),[9] is known to proceed by a radical mechanism and, therefore, allows to add thiols to non-activated alkenes in an anti-Markownikov fashion.[10] Main features of photoinduced TEC include the occurrence by irradiation at close to visible light under aerobic conditions at room temperature in aqueous solvents and orthogonality to a wide range of functional groups. Owing to its efficiency and specificity, TEC has been recently exploited by us for the glycosylation of natural cysteine containing peptide such as glutathione (GSH) and the protein bovine serum albumin (BSA).[11]



R = H or sugar

Scheme 1. General equation for *S*-glycosyl amino acid synthesis via photoinduced TEC.

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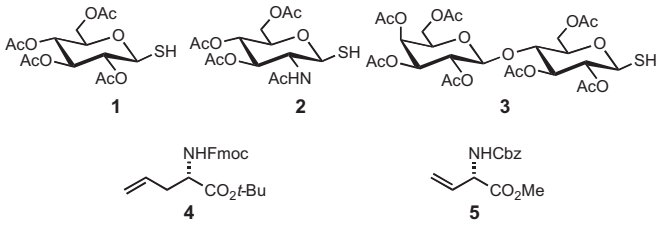


Figure 1.

To perform the reaction shown in Scheme 1, we selected the model glycosyl thiols **1–3** and allyl and vinyl glycines **4** and **5**, respectively, as substrates (Fig. 1). Carbohydrates **1–3** and amino acids **4** and **5** were known compounds and, therefore, were prepared by literature methods (see Supplementary data). *O*-Acetyl protecting groups in the sugar thiols were used because of their compatibility with the planned photoreaction.[12] Moreover orthogonal protective groups in the glycinyl moiety were employed in order to obtain target *S*-glycosyl amino acids suitable for peptide synthesis.

Taking advantage of the optimized conditions established in our recent works in which TEC was conveniently exploited for *S*-disaccharide synthesis,[12] peptide and protein glycosylation,[11] and calixarene glycoclustering,[13] we performed the photoreactions of a slight excess of thiols **1–3** (1.2 equiv) with alkenes **4** and **5** by irradiation at λ_{max} 365 nm in the presence of 2,2-dimethoxy-2-phenylacetophenone (DPAP) as the photoinitiator (0.1 equiv).

Reactions were carried out in dichloromethane as a solvent at room temperature in glass vials using a hand-held UVA generating apparatus. No care was taken to exclude air and moisture. Much to our delight all reactions went to completion in 1 h as shown by the total disappearance of the alkene proton signal at $\delta = 5.7$ ppm in the ^{1}H NMR spectrum of the crude reaction mixture. Yields of isolated *S*-glycosyl amino acids **6–11** that are presented in Table 1 demonstrate the high efficiency of TEC in each reaction. Noteworthy is the fact that no epimerization occurred in all cases being the sugar disulfide the only observed side-product that arised from the thiyl radical homocoupling. Thus it appeared quite reasonable to conclude that the configuration of all asymmetric carbons of both reagents was preserved under the photochemical conditions and consequently assign the stereochemistry of isolated products as identical to that of the reactants.

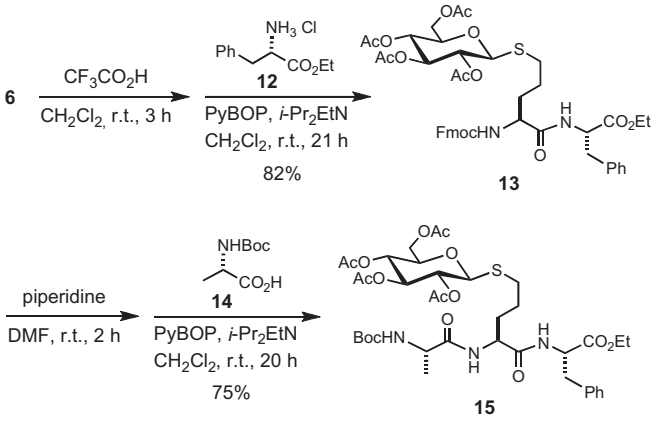
In order to demonstrate the viability of the approach to *S*-glycopeptides by the use of the *S*-glycosyl amino acids thus prepared, the synthesis of a tripeptide was performed by solution phase chemistry (Scheme 2). To this end, the amino ester **6** was transformed into the free carboxylic acid by selective removal of *tert*-butyl group and the crude product was coupled with phenyl alaninate **12** under basic conditions (*i*-Pr$_2$EtN) and in the presence of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as a condensing agent. The *N*-Fmoc protected dipeptide **13** thus obtained was liberated of the Fmoc protective group by basic treatment and the free amine was condensed with *N*-Boc alanine **14** under basic conditions in the presence of PyBOP. The pure final tripeptide **15** featuring a pendant glucoside residue linked through a robust thioether linkage was isolated by chromatography in 75% yield. This compound turned out to be a single diastereomer by NMR analysis, thus indicating that all stereocenters of the reactants employed remained unaltered throughout the whole synthetic sequence.

In a second instance we set out to develop a TEC-based post-synthetic approach to *S*-glycopeptides. To this end we envisaged introducing a chemical handle bearing an ene tag on a cysteine free sulfhydryl group and then performing the photoinduced TEC with

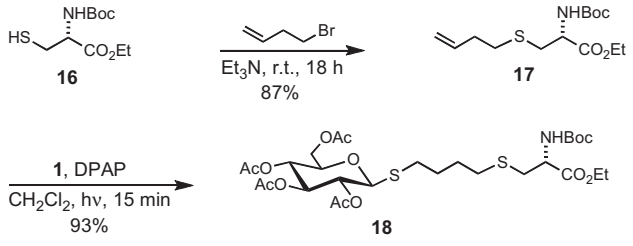
Table 1
Photoinduced reactions[a] of sugar thiols **1–3** with alkenyl glycines **4** and **5**

Thiol	Alkene	Product (yield)[b]
1	**4**	**6** (73%)
2	**4**	**7** (53%)
3	**4**	**8** (80%)
1	**5**	**9** (90%)
2	**5**	**10** (89%)
3	**5**	**11** (89%)

[a] All reactions were carried out with 1.2 equiv of thiol and 0.1 equiv of DPAP in CH_2Cl_2 under irradiation at 365 nm for 30 min.
[b] The products were isolated by column chromatography on silica gel.



Scheme 2.



Scheme 3.

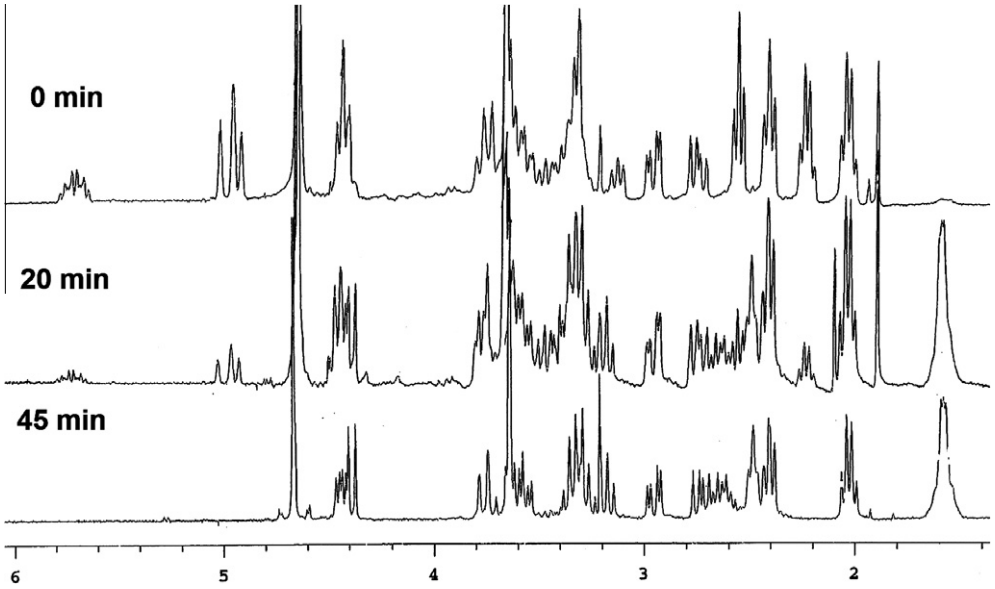
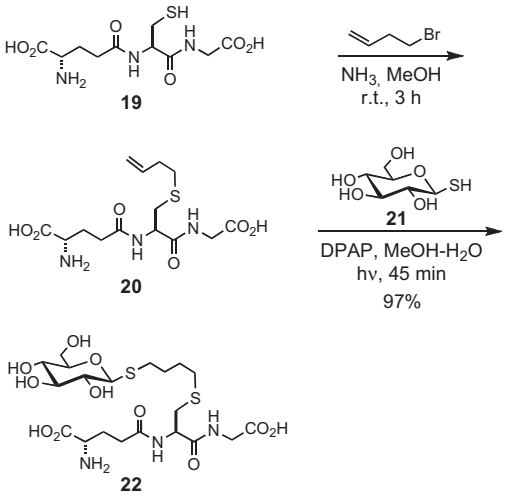


Figure 2. ^{1}H NMR spectra of the reaction mixture between **20** and **21** (Scheme 4) monitored at different times.



Scheme 4.

a glycosyl thiol. In a model study serving to validate this idea, we introduced a homoallyl chain in L-cysteine **16** by reaction with 4-bromo-1-butene in the presence of Et_3N. Then, the *S*-butenyl cysteine **17** thus formed was submitted to the photoinduced reaction (λ_{max} 365 nm) with glycosyl thiol **1** in the presence of DPAP (Scheme 3). The TEC reaction worked beautifully as it afforded in only 15 min irradiation the *S*-thioglycosylbutyl cysteine **18** in excellent isolated yield (93%). It has to be noted that the choice of the butenyl as a chemical handle was made after having carried out some experimentation with the use of the allyl group. In fact the photoinduced reaction of **1** with *S*-allyl cysteine gave the expected thioether in very low yield together with by-products derived from coupling of various thiyl radicals (see Fig. S1 in Supplementary data).

Encouraged by this result we extended the approach to the *S*-glycosylation of glutathione **19** (Scheme 4). The selective *S*-homoallylation of **19** by butenyl bromide in NH_3/MeOH proceeded readily and specifically due to the superior nucleophilicity of the free sulfhydryl group with respect to terminal amino group of glutamic acid.[14] Thus, the *S*-homoallyl glutathione **20** formed in almost quantitative yield. Then, this crude product was coupled with excess of unprotected sugar thiol **21** (1.2 equiv) under the above photoinduced conditions with the only change that a mixture of MeOH–H_2O was employed as the solvent.

The NMR spectrum of the reaction mixture recorded after 20 min irradiation revealed the presence of unreacted alkene **20** while this was completely consumed after 45 min (Fig. 2).

Thus, to our satisfaction, the target *S*-glycopeptide **22** was isolated by chromatography in 97% yield. It is noteworthy that the coupling of **20** with **21** gave **22** in similar yield upon exposure (8 h) to unfocused sunlight. This and our earlier finding[12] on the use of sunlight for performing TEC reactions, demonstrate the ability of this click process to proceed under very mild and benign conditions with the need of minor amounts of energy.

In summary, two routes have been paved to *S*-glycopeptides. One route consists of *S*-glycosyl amino acid synthesis from photoinduced addition of sugar thiols to alkenyl glycine followed by incorporation of the amino acid into a peptide. The second route, that is, specific for a cysteine containing peptide such as glutathione, involves peptide *S*-homoallylation followed by TEC with sugar thiol. Thus, extension of this process to larger peptides and proteins now becomes of interest. Finally, the observation that TEC can proceed under ambient processing conditions and upon sunlight irradiation, opens up the way for future applications particularly for bioconjugation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.11.097.

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