Organic LETTERS

Letter

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Straightforward Entry to *S*-Glycosylated Fmoc-Amino Acids and Their Application to Solid Phase Synthesis of Glycopeptides and Glycopeptidomimetics

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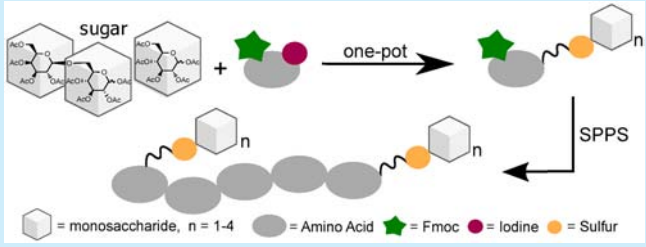
Supporting Information

ABSTRACT: Streamlined access to *S*-glycosylated Fmoc-amino acids was developed. The process provides diverse glycosylated modified amino acids in high yield and stereoselectivity taking advantage of the *in situ* generation of a glycosylthiolate obtained from carbohydrate acetates in a few steps. Mild basic conditions make the conjugation reaction compatible with Fmoc-iodo-amino acids. To validate the strategy the glycosylated building blocks were used for SPPS and the unprecedented incorporation of a long thio-oligosaccharide to the peptide chain was demonstrated.



Drug discovery based on peptides and proteins is a widely explored area in biomedical research that very often relies on the "peptidomimetic approach". As a matter of fact, introduction of modified amino acids or higher homologues thereof results in an expanded molecular diversity that can be exploited as a very effective tool in medicinal chemistry.[1] Indeed, peptidomimetics based on β- and γ-amino acids feature enhanced *in vivo* stability, but can also be of valuable interest for the investigation of new highly ordered architectures.[2,3]

Over the past years, the critical role played by the protein glycosylation in biology has been widely unveiled, as witnessed by the involvement of glycoproteins in a wide set of events such as cell adhesion and proliferation, trafficking, cell−cell recognition, inflammation, virulence and host immune response.[4] Glycosylation also plays a pivotal role in protein folding and proteolytic stability, and it is widely investigated for tuning the biological activity of non-naturally glycosylated peptides and proteins.[5]

In this regard, synthetic access to homogeneous natural or modified glycosylated peptides is a relevant topic in organic chemistry and different synthetic conjugation methods have been developed,[6] providing naturally occurring *O*- and *N*-linked, and uncommon *C*- and *S*-linked, glycopeptides. In particular, the thioglycoside bond is well-known to be chemically and enzymatically more stable than the *O*- linked counterpart, and it is well tolerated in biological systems because of its isosteric mimicry.[7] To date, several methods for the assembly of *S*-glycosylated peptides have been reported,[8] but very few of them are compatible with use of Fmoc-protected amino acids.[9] As to the saccharide moiety of the reported glycosylated Fmoc-amino acids and peptides, the known synthetic strategies entail preliminary generation and purification of a glycosylthiol,[9b,c,10] a glycosylthioacetate,[9a] or a glycosylthiomethylating agent[11] via the corresponding glycosyl bromides. Access to these 1-thiosugar derivatives is a tedious, time-consuming process and requires harsh acidic conditions for the generation of the 1-bromo intermediate, which can be an especially demanding issue when applying the method to longer oligosaccharides. In fact, a postsynthetic strategy of trisaccharide *S*-conjugation with a peptide was already described,[12] but saccharide sequences longer than two residues were never thio-anchored to Fmoc-amino acids prior to their solid-phase incorporation into a growing chain.

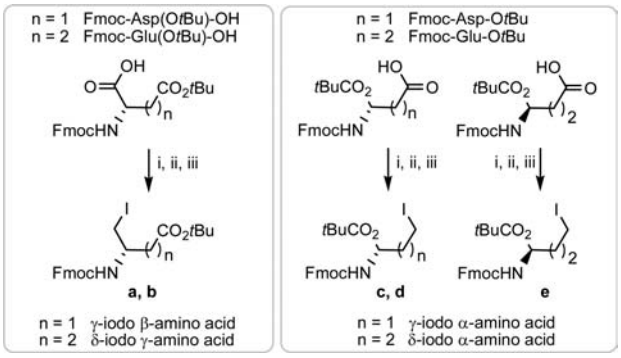
On this basis, we directed our effort toward the implementation of an operationally simple synthetic approach to *S*-glycosylated Fmoc-protected amino acids, which is endowed with both the compatible direct use of commercial Fmoc-amino acids and the applicability to carbohydrates more complex than mono- or disaccharides. To this aim, we have suitably adapted a straightforward strategy recently described for the synthesis of thioglycosides, entailing the conversion of per-*O*-acetylated carbohydrates into a glycosyl thiolate intermediate which is entrapped *in situ* with a suitable electrophile.[13] The synthetic sequence is advantageous because the sequential generation of the two requisite intermediates (namely, a glycosyl iodide and a glycosylthiouronium) takes very short reactions, and no purification of any saccharide intermediate is needed.

First, we have obtained iodo β- and γ-amino acids starting from Fmoc-L-aspartic and Fmoc-L-glutammic acids 4- and 5-*tert*-butyl esters respectively (**a**−**b**, Scheme 1) and iodo α-amino acids starting from corresponding 1-*tert*-butyl esters (**c**−**d**, Scheme 1), one of which belongs to the D-series (**e**, Scheme 1).[9c] The free

Received: December 19, 2014
Published: January 27, 2015

DOI: 10.1021/ol503664t
Org. Lett. 2015, 17, 640−643

Scheme 1. Preparation of Iodo-Amino Acids[a]



[a]Reagents and conditions: (i) Isobuthylchloroformate, NMM, THF, 0 °C, 1 h; (ii) $NaBH_4$, THF/H_2O, 0 °C, 20 min; (iii) I_2, PPh_3, imidazole, THF, rt, 1 h.

acidic functions were reduced to the corresponding alcohols and then converted into iodides[14] (Scheme 1).

The saccharide moiety was instead suitably elaborated through the very fast three-step procedure shown in Scheme 2. In the first step, the per-*O*-acetylated sugar precursor was treated with iodine and triethylsilane in dichloromethane under reflux to afford the corresponding anomeric iodide[15] in quantitative yield and within a few minutes. After a simple extractive workup, the crude glycosyl iodide was quickly converted into the corresponding isothiouronium salt by treatment with thiourea in acetonitrile at 60 °C; to the same vessel, the Fmoc-protected amino acid derivative was then added along with triethylamine to perform the *S*-conjugation in a *one-pot* fashion through a nucleophilic attack of the sugar thiolate anion to an Fmoc-iodo-amino acid, a process occurring with both high anomeric selectivity and yield (Table 1) and without the generation of disulfide side products.[16]

Optimization of the results required a thorough screening of conditions for the critical last step of the sequence by examining several parameters such as the solvent, sugar-to-amino acid stoichiometric ratio, temperature, and amount of the base. Not unexpectedly, the amount of TEA was the most critical parameter to affect the yield; when a large excess of the base (4−12 equiv) was used, the conjugation yield was drastically reduced by the Fmoc cleavage and subsequent generation of the adduct between the thiolate and dibenzofulvene arising from the Fmoc release. On the other hand, use of a moderate excess of the

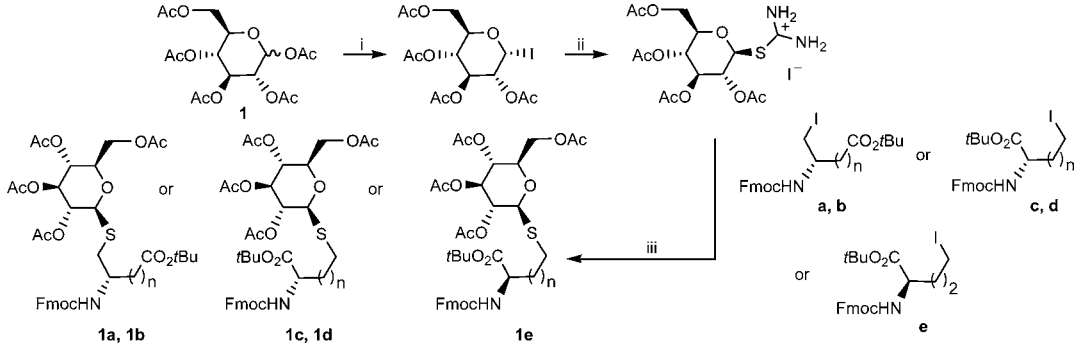
Table 1. *S*-Glycoconjugation Reactions of Various Sugar Acetates with Iodo-Amino Acids

		product and yield[a] (%)	
entry	sugar	*β*- and *γ*-iodo aa	*α*-iodo aa
1	D-glucose (**1**)	**1a** (60 *β* only)	**1c** (82 *β* only)
		1b (66 *β* only)	**1d** (88 *β* only)
			1e (80 *β* only)
2	D-mannose (**2**)	**2a** (65 *α* only)	**2c** (90 *α* only)
		2b (68 *α* only)	**2d** (83 *α* only)
3	D-galactose (**3**)	**3a** (70 *β* only)	**3c** (90 *β* only)
		3b (74 *β* only)	**3d** (86 *β* only)
4	L-fucose (**4**)	**4a** (60 *β* only)	**4c** (78 *β* only)
		4b (58 *β* only)	**4d** (82 *β* only)
5	glucosamine[b,c] (**5**)		**5c** (60 *β* only)
6	lactose (**6**)	**6a** (53 *β* only)	**6c** (60 *β* only)
		6b (55 *β* only)	**6d** (62 *β* only)
7	maltotriose[c] (**7**)	**7b** (69 *β* only)	**7c** (83 *β* only)
8	maltotetraose[c] (**8**)	**8b** (46 *β* only)	

[a]Isolated overall yields. [b]See Supporting Information for experimental details. [c]The reactions reported are the only ones carried out.

base (2 equiv) proved sufficient for promoting the desired coupling process without any loss of the Fmoc protecting group. The optimization study also indicated acetonitrile as a good solvent for the final step and that the glycoconjugation is fast enough to proceed at room temperature within 3−4 h. Remarkably, the overall conversion of the per-*O*-acetylated precursor to the *S*-glycosylated Fmoc-amino acid did not exceed 5 h and required a single purification step by column chromatography. In addition, all reactions of the sequence in Scheme 2 were performed under air without any special experimental precaution. The wide versatility of the proposed strategy was demonstrated by both the successful application to a wide range of mono- (**1**−**5**) and disaccharides (**6**) and the unprecedented glycoconjugation of Fmoc-amino acids with longer sequences such as the maltotriose trisaccharide **7** and maltotetraose tetrasaccharide **8** (Figure 1). Only for *N*-acetyl glucosamine, the relatively stable anomeric chloride **5**[17] was used instead of the corresponding iodide to react with thiourea in refluxing acetone to give the thiouronium salt.[16] In this case, the best yield was achieved by exchanging acetone for acetonitrile prior to performing the *S*-alkylation step. As proof of concept, the conjugation reactions for sugars **5**, **7**, and **8** have been carried out only with iodo-amino acids **b** and **c**.

Scheme 2. Synthetic Sequence for *S*-Glycosylated Amino Acids Formation[a]



[a](i) I_2, Et_3SiH, CH_2Cl_2, reflux, 5 min; (ii) thiourea, CH_3CN, 60 °C, 60 min; (iii) 0.7 equiv of **a**−**e**, 1.5 equiv of Et_3N, rt, 3−4 h. Yields reported below in Table 1.

DOI: 10.1021/ol503664t
Org. Lett. 2015, 17, 640−643

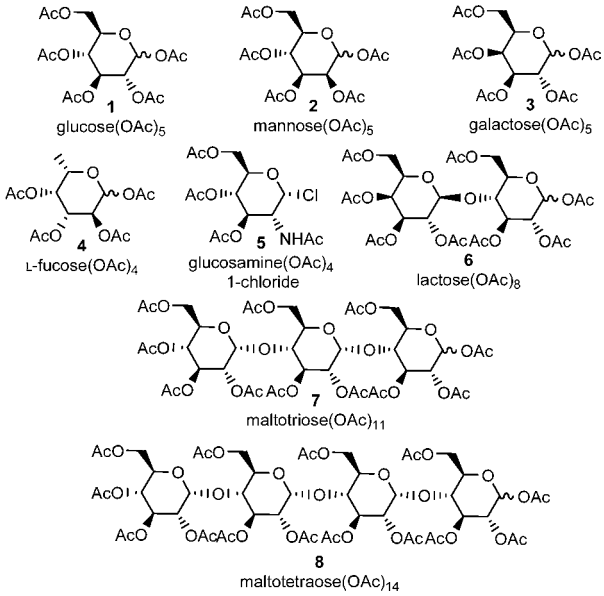


Figure 1. Starting peracetylated carbohydrates.

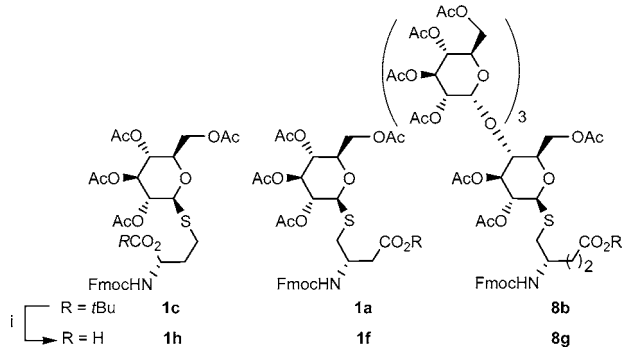
All the *S*-glycosyl amino acids were obtained with high 1,2-*trans* stereoselectivity; namely, pure β-anomers were obtained from *gluco* (**1a**−**e**, **5d**, **7b**−**c**, and **8b**) or *galacto*-configured sugars (**3a**−**d**, **4a**−**d**), whereas pure α-anomers were obtained from a *manno*-precursor (**2a**−**d**).

It is worth noting that the nucleophilic substitution reactions to more accessible electrophilic centers of iodo α-amino acids (**c**−**e**) are significantly more high-yielding (60−90%) than in the case of β- (**a**) and γ- (**b**) amino acid halides (46−74%) bearing the electrophilic carbon much closer to the bulky 9-fluorenylmethoxycarbonyl group.

It should be outlined that acetyl protecting groups play a beneficial role at multiple stages of the synthesis. First, they guarantee the above-mentioned 1,2-*trans* selectivity due to their possible participation effect serving in the generation of the thiouronium intermediate; in addition, their electron-withdrawing nature allows the oligosaccharide chains to be unaltered by the acidic conditions needed both in the early stage of anomeric iodination of the reducing terminus and in all the acidic conditions to which the *S*-glycosyl Fmoc-amino acids are exposed prior to and after its incorporation into the peptide sequence. Last, their presence ensures the protection of hydroxyl groups toward esterification when a high concentration of amino acid was used during the peptide elongation.[18]

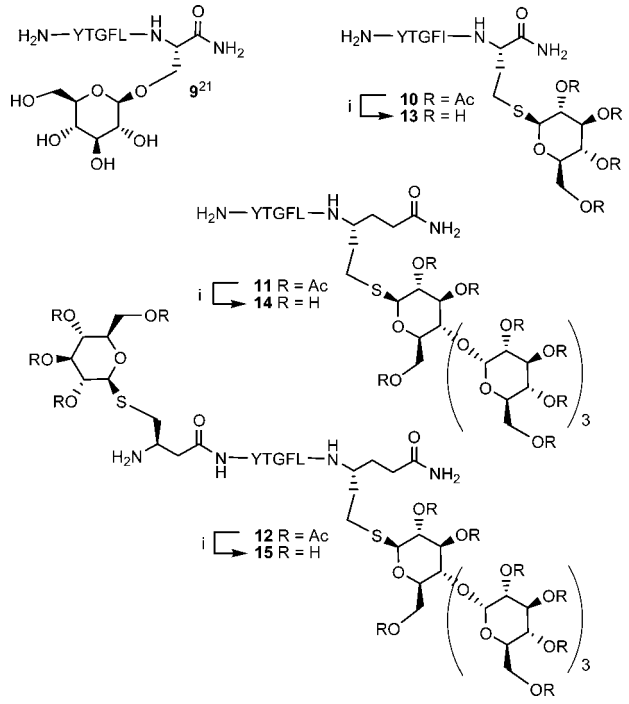
To validate the scope of our strategy, some of the obtained building blocks were employed in Fmoc solid-phase peptide synthesis. For this purpose, amino acids **1c**, **1a**, and **8b** were treated with TFA in dichloromethane to hydrolyze the *tert*-butyl ester function affording the final Fmoc-protected amino acids **1h**, **1f**, and **8g** (Scheme 3) in good yield (see Supporting Information). We designed our glycopeptides on an enkephalin analogue,[19] a pharmacophore contained in many opioids,[20] which possesses an improved analgesic effect due to a more efficient uptake through the blood−brain barrier arising from a simple glucosylation of a serine residue (**9**, Scheme 4).[21] Inspired by this model, we synthesized three *S*-glycosylated modified peptides highlighting the versatility of the strategy through the feasible introduction of glycosylated α-, β-, and γ-amino acids (**10**−**12**, Scheme 4). The glycoamino acid building blocks so

Scheme 3. Unmasking of Carboxyl Group[a]



[a]Reagents and conditions: (i) CH_2Cl_2/TFA 4:1, 0 °C, 2 h.

Scheme 4. Glycopeptides by SPPS and Deacetylation[a]



[a]Reagents and conditions: (i) MeOH/NH_4OH 4:1, rt, overnight.

prepared were in turn incorporated into the peptide sequence by a standard SPPS technique.

Notably, the solid-phase synthesis was found effective even with amino acids bearing a tetrasaccharide moiety and was also compatible with the assembly of glycopeptides with different sugars appended to the same peptide backbone.

In conclusion, we have developed a new general strategy for the linear assembly of *S*-glycopeptides and *S*-glycopeptidomimetics by standard Fmoc solid-phase synthesis. Upon incorporation of glycosylated α-amino acids, the pendant sugar moiety is linked to a homocysteine or homocysteine-like residue, whereas in the case of the incorporation of the corresponding β- and γ-glycoamino acids the sugar moiety looks linked to a cysteine side chain.

The method relies on a very fast and operationally simple synthesis of Fmoc-protected *S*-linked glycosyl amino acid building blocks starting from commercially available per-*O*-acetylated sugars and Fmoc/*tert*-butyl ester protected amino acids, both converted into the corresponding iodides. In a *one-pot*

DOI: 10.1021/ol503664t
Org. Lett. 2015, 17, 640−643

fashion the glycosyl iodide was turned into the anomeric thiolate via a thiouronium intermediate and then coupled with the iodo-amino acid to quickly afford the conjugated product with high stereoselectivity and yield. The Fmoc-protective group was found to be compatible with the optimized reaction conditions, and no protecting-group manipulation is needed except for the unmasking of the carboxylic function. Furthermore, the entire process requires just one purification step and short experimental times in comparison with other methods. The strategy proved to be applicable to a wide range of carbohydrates namely mono-, di-, and oligosaccharides, and it could be a useful tool in biomedical and medicinal chemistry for the streamlined construction of new glycopeptide targets of biological interest.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details, characterization of all compounds, ^{1}H and ^{13}C NMR spectra of sugar conjugates, NMR spectra, and LC-MS profiles of glycopeptides are reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Mr. Leopoldo Zona (National Research Council, Naples) for NMR technical assistance and Mr. Luca De Luca (National Research Council, Naples) for computer assistance. This study has been supported by the Italian Ministry of University and Research (PRIN2010-2011-prot. 2010C4R8M8_007).

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DOI: 10.1021/ol503664t
Org. Lett. 2015, 17, 640−643