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Methyl esters: an alternative protecting group for the synthesis of *O*-glycosyl amino acid building blocks

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Abstract

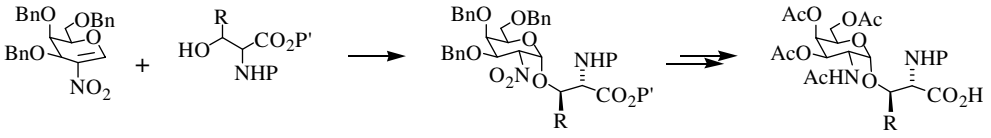
The glycosyl amino acids α-GalNAc-Ser and α-GalNAc-Thr are fundamental building blocks for glycopeptide synthesis, Schmidt's synthesis method often being chosen for this purpose. Methyl esters used as orthogonal carboxylic acid protecting group in this procedure were found to be an efficient and inexpensive alternative to other groups. The mild selective methyl ester deprotection by LiI improved the efficiency of the synthesis method.

Keywords: Glycosyl amino acid; Glycopeptide synthesis; Methyl esters; Protecting group

Glycoproteins are known to be involved in a vast array of biological events.[1] It has become evident that important recognition processes depend on the interplay between peptide and saccharide constituents.[2] For glycopeptide synthesis, the preparation of glycosyl amino acid building blocks is of primary importance, the α-glycosidic linkage between 2-acetamido-2-deoxy-D-galactopyranose and the hydroxyl group of L-serine or L-threonine being a very common motif in glycoproteins.[3]

As part of a program directed toward the conformational study of glycosides, glycomimetics, and glycopeptides,[4] the chemical preparation of *N*-Fmoc-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine and L-threonine was required. Two well-established methods have been reported for this purpose: (i) addition of amino acids to 2-azido-2-deoxy glycosyl donors[5] and (ii) Michael-type addition of amino acids to 2-nitroglycals as developed by Schmidt.[6] The latter procedure was chosen since it is more efficient and overcomes the latent difficulties of the former. Thus, 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal[7] was prepared and then used as a glycosyl donor according to Scheme 1.

Regarding the amino acids used, we show herein that L-serine and L-threonine methyl esters are very efficient glycosyl acceptors, the protection of their carboxylic acids as methyl esters and their deprotection with LiI[8] being a



Scheme 1. Reaction pathways for glycosyl amino acid synthesis following Schmidt's procedure (R = H or Me; P and P′ = different protecting groups).

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useful alternative for the preparation of glycosyl amino acids, which are key intermediates in glycopeptide synthesis.

Protection and deprotection steps are critical in many synthesis schemes, such as that of complex *O*-glycosyl amino acids. Appropriate choice of protector for the carboxyl group of the amino acid is crucial, because it must tolerate the reaction conditions and also be removable without affecting the rest of the molecule. Amino acid methyl esters are easy to prepare[9] and are commercially available. However, in spite of its stability, the methyl group has not been used as carboxylic acid protecting group in glycopeptide synthesis because of the deprotection conditions. Epimerization[10] of the α-carbon of the amino acid moiety during the saponification step by basic hydrolysis is a well-known problem as is the rapid deprotection of acetyl groups.

To overcome these drawbacks, lithium iodide was used for the deprotection of methyl esters.[8] Thus, the glycosyl amino acid methyl esters **1**–**4**[11] were treated with 6 equiv of lithium iodide under reflux in anhydrous ethyl acetate.[12] This mild neutral reagent showed itself to be compatible with the presence of acetyl and N-protecting groups, giving rise to free carboxylic acids in excellent yields (around 90–95%) (Table 1).[13] The reaction mechanism is probably the nucleophilic attack of the iodide ion on the amino acid methyl ester and displacement of the carboxylate ion as the leaving group, as occurs with cyanides.[14]

Very recently, a gentle procedure has been reported for the hydrolysis of esters having an α- or a β-heteroatom with respect to the ester carbonyl group by amine bases and lithium salts in wet solvents.[15]

The protection of the carboxylic acid as methyl ester must also be compatible with all reaction conditions of the other steps of the Schmidt procedure.[6] Among them, the most crucial step is the reduction of the nitro group, for which the usual procedure was the usage of hydrogen and platinized Raney nickel T4[16] as catalyst. This requires prolonged reaction times and careful preparation of the rather unstable catalyst. For these reasons, a cheaper procedure is to replace the former reagent with Zn[17] and 1 N HCl in aqueous acetic acid, for which the useful alternative has already been successfully tested with some carbohydrates[18] and more recently in glycosyl peptides.[19]

This nitro group reduction with Zn in acid solution worked very fast and efficiently with compounds **9**–**12**, giving rise to the corresponding glycosyl amino acid derivatives, which were submitted to hydrogenolysis and acetylation without any purification, providing the peracetylated 2-acetamido glycosyl amino acids in very good yields (Table 2).[20] It is noteworthy that this reduction in the presence of Zn is compatible with the sensitive protecting groups on the molecule. In addition, the different behavior of the α- and β-anomer was striking, the latter, **13** and **14**, were obtained in higher yields (around 90%, three steps) than the α, **1** and **2** (around 70%, three steps).

In summary, the preparation of complex glycosyl amino acid derivatives, building blocks necessary for the glycopeptide synthesis, requires the use of orthogonal protecting groups. The protection of carboxylic acids as methyl esters is shown to be an adequate alternative to other carboxylic acid protecting groups. The preparation of the amino acid methyl esters is very easy and inexpensive to carry out, in particular easier than other alkyl esters. Furthermore, this

Table 1
Deprotection of the methyl esters with lithium iodide

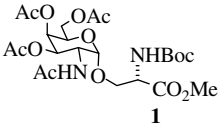
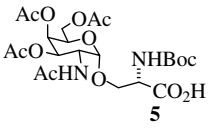
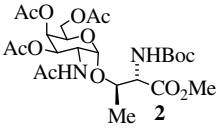
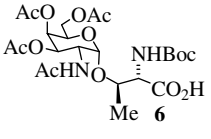
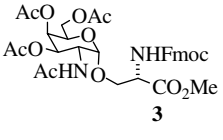
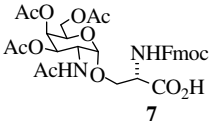
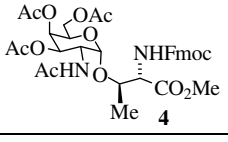
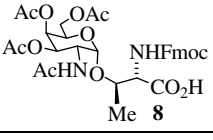
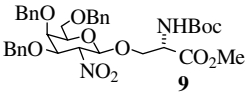
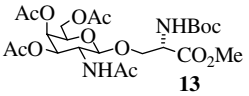
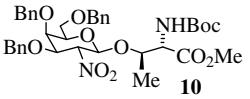
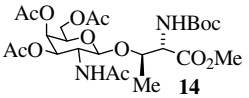
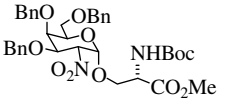
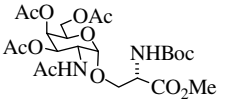
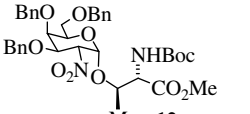
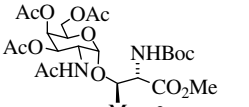
Entry	Methyl esters	Products	Yield (%)
1	 **1**	 **5**	90
2	 **2**	 **6**	90
3	 **3**	 **7**	96
4	 **4**	 **8**	95

Table 2
Reduction of the 2-nitro group, deprotection of the benzyl groups and acetylation of glycosyl amino acid methyl esters

Entry	Nitro-compounds	Products	Yield (%)
1	 **9**	 **13**	95
2	 **10**	 **14**	90
3	 **11**	 **1**	74
4	 **12**	 **2**	70

protecting group is very stable and compatible with the different reagents used in the synthesis of glycosyl amino acid derivatives (Schmidt's method) and its removal by LiI is very effective.

Acknowledgments

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Supplementary data

Spectroscopic data of new compounds **1**–**4**, **9**–**11**, **13**, and **14**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.12.078.

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11. Compounds **3** and **4**, having the useful Fmoc protecting group for the synthesis of peptides in solid phase, were easily obtained from **1** and **2**, respectively, by treating with TFA in CH_2Cl_2 and then with FmocONSu in THF–water in the presence of sodium bicarbonate. See Ref. 6c.
12. *Procedure for demethylation.* Lithium iodide (6 equiv) was added to a stirred solution of the methyl ester in dry ethyl acetate (10 mL/mmol) under nitrogen atmosphere, and the mixture heated under reflux for 20 h. Then the reaction was quenched by the addition of 10% hydrochloric acid solution and extracted with EtOAc. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated in vacuum. The product was purified by flash chromatography (CH_2Cl_2/MeOH 95:5 to 80:20).
13. The ^{1}H NMR data of target products **5**–**8** (Table 1) were identical with those reported by Toyokuni, T.; Hakomori, S.; Singhal, A. K. in *Bioorg. & Med. Chem.* **1994**, *2*, 1119–1132 (compounds **5** and **6**) or with those obtained from commercially available samples (**7** and **8**).
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20. *Procedure for reduction, debenzylation and acetylation.* Zn dust (24 equiv) was added to a solution of the nitro-compound in THF/H_2O (100 mL/mmol, 7:3) containing HCl (3 mL/mmol) and acetic acid (15 mL/mmol) cooled at 0 °C. After stirring for 2 h the reaction mixture was filtered, concentrated and the crude was dissolved in MeOH/AcOH (30 mL/mmol, 1:1), treated with 5% Pd–C (700 mg/mmol) and hydrogen, and then left overnight. The solvent was eliminated and a mixture of Py-Ac_2O (40 mL/mmol, 2:1) was added to the flask and stirred till the protection of the hydroxyl groups was completed. Elimination of the excess reagents and chromatography of the crude in CH_2Cl_2/MeOH (95:5) gave the corresponding amino acid derivatives (see Supplementary data).