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Synthesis of Glycosylated Hydroxyproline Building Blocks

Gemma Arsequell*, Núria Sàrries and Gregorio Valencia

Unitat de Química de Glicoconjugats
Centre d'Investigació i Desenvolupament (C.I.D.-C.S.I.C.)
Jordi Girona 18-26, 08034 Barcelona (Spain).

Abstract[1]:
The synthesis of a series of new glycosylated N^{α}-Fmoc hydroxyproline building blocks has been accomplished for the first time with two approaches. First, by glycosylation of N^{α}-Fmoc Hyp OH, having unprotected carboxyl group with different peracetylated glycosyl derivatives. Alternatively, by glycosylation of its derivative N^{α}-Fmoc Hyp Opfp with different acylated glycosyl derivatives under silver triflate promotion. The use of benzoyl groups instead of acetyl as protecting groups for the carbohydrate moiety boosted the yield of glycosidation of N^{α}-Fmoc Hyp Opfp. These building blocks are ready for their incorporation into solid-phase glycopeptide synthesis protocols.

Carbohydrate parts of glycoproteins[2] play many important biological functions. The synthesis of both *natural glycopeptides*, derived as synthetic fragments from natural glycoproteins, for the purpose of supplying structurally well-defined derivatives as models for biochemical and immunochemical studies and *neoglycopeptides*[3], aimed at a specific biochemical target in order to achieve more potent analogues and to further understand structure-function extracellular matrix, is still challenging.

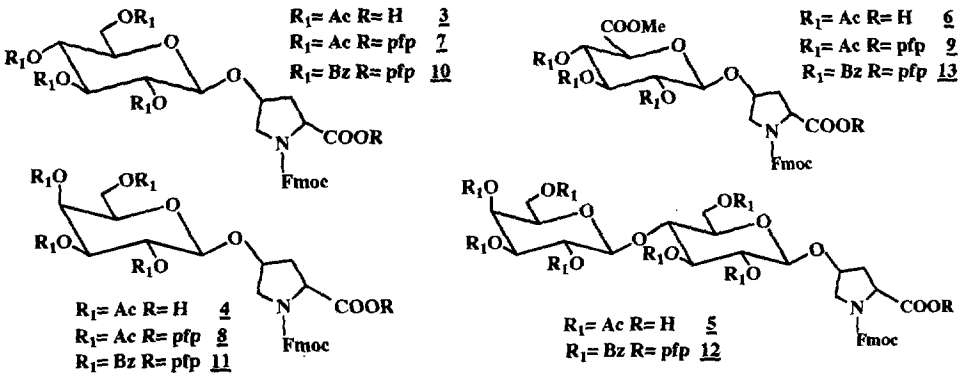
Protein glycosylation takes a variety of forms. The glycosidic linkage between D-galactose and L-arabinose with the hydroxyproline (Hyp) is found in nature in some glycoproteins of plant cell walls[4]. A novel type of hydroxyproline-containing glycoproteins in plants, recently found in chitinases involves a glycosidic linkage between the pentose sugar xylose and hydroxyproline[5].

A number of different strategies for the preparation of a large variety of *N*- and *O*-linked glycopeptides with well defined and predetermined structure have been reported either by **chemical**[6] or **enzymatic**[7] synthesis. The currently most versatile and general approach to the chemical synthesis is the stepwise solid-phase synthesis using glycosylated N^{α}-fluoren-9-ylmethoxycarbonyl (Fmoc[8]) amino acid derivatives as building blocks in standard or multiple synthesis protocols. The preparation of these glycopeptides is based on the techniques developed using solid-phase methodology combined with the appropriate choice of protecting groups of these building blocks, both in the carbohydrate and the peptide moieties.

Although the synthesis of several glycosides of Ser, Thr and Tyr have been published, only a few reports on

the successful glycosylation of Hyp are available. Common in all these procedures are the *Z* protection scheme[9] and the use of benzyl[10], methyl[11] or allyl[12] esters for the α-carboxyl protection of Hyp in the glycosylation reaction. We report here for the first time the glycosylation of N^{α}-Fmoc hydroxyproline for the synthesis of glycosylated Fmoc N^{α}-L-hydroxyproline derivatives as building blocks.

Following Eloffson's approach[13] for glycosylation of carboxyl unprotected Ser, Thr and Cys, in a first series of experiments we have attempted the glycosylation[14] of N^{α}-FmocHypOH (**1**), under Lewis acid promotion (Boron trifluoride etherate) with different peracetylated glycosylated donors (such as Glucose:Glc, Galactose:Gal, Lactose:Lac and Glucuronic acid methyl ester: Glc-ic COOMe) (See Scheme). The corresponding β-glycosyl amino acids: N^{α}-FmocHyp[Ac_4-β-D-Glc]OH (**3**), N^{α}-FmocHyp[Ac_4-β-D-Gal]OH (**4**), N^{α}-FmocHyp[Ac_7-β-D-Lactose]OH (**5**) and N^{α}-FmocHyp[Ac_3-β-D-Glc-ic COOMe]OH (**6**) were obtained in a 51%, 67%, 45% and 2% yield, respectively[15].



The Koenigs-Knorr reaction[16] is one of the oldest methods for the preparation of 1,2-*trans*-glycosides involving per-*O*-acylated glycopyranosyl halides as donors and silver salts as promoters. This reaction is influenced by some parameters such as the structure of the glycosyl halide and the alcohol, the nature of the promoter (halide ion acceptor) and the solvent. As far as to the structure of the glycosyl halide is concerned, acetates are a common choice for the protection of the carbohydrate hydroxyl groups. However, several authors have shown that improved yields are often obtained in glycosylations with benzoyl, instead of acetyl, protection of the glycosyl donor[17]. Owing to these facts, in a second series of experiments we have also assayed the glycosylation of an Hyp derivative under Koenigs-Knorr conditions. First of all, the starting activated ester N^{α}-Fmoc Hyp Opfp (**2**) was prepared for the first time, in a 88% yield after CC purification on silica gel from N^{α}-FmocHypOH, pfp-OH and diisopropylcarbodiimide (DIC) in THF. By analogous procedures involving the

pentafluorophenyl (Pfp) group[18] for the temporary protection of the α-carboxyl group in the *O*-glycosylation of Ser and Thr, we have later examined the glycosylation[19] of this new useful derivative **2**. Koenigs-Knorr conditions using silver triflate as promoter and different peracetylated 1-α-glycosyl bromides as donors ($Glc(OAc)_4Br$, $Gal(OAc)_4Br$ and Glc-ic $COOMe(OAc)_3Br$) (See Scheme) gave the corresponding protected β-glycosyl amino acids activated esters (pfp esters): N^{α}-FmocHyp[Ac_4-β-D-Glc]Opfp (**7**), N^{α}-FmocHyp[Ac_4-β-D-Gal]Opfp (**8**) and N^{α}-FmocHyp[Ac_3-β-DGlc-ic COOMe]Opfp (**9**) in 23%, 18% and 6% yield, respectively.

In order to further optimize the yield, the glycosylation of the activated ester **2** was performed with different benzoylated 1-α-glycosyl bromides as donors ($Glc(OBz)_4Br$, $Gal(OBz)_4Br$, $Lac(OBz)_8Br$, Glc-ic $COOMe(OBz)_3Br$) under the same conditions as reported above (See Scheme). The corresponding protected β-glycosyl amino acids activated esters: N^{α}-FmocHyp[Bz_4-β-D-Glc]Opfp (**10**), N^{α}-FmocHyp[Bz_4-β-D-Gal]Opfp (**11**), N^{α}-FmocHyp[Bz_7-β-D-Lactose]Opfp (**12**) and N^{α}-FmocHyp[Bz_3-β-D-Glc-ic COOMe]Opfp (**13**) were obtained in a 68%, 73%, 69% and 30% yield, respectively. The results show a determinant influence of the protecting grup (Bz *vs* Ac) of the carbohydrate moiety in the glycosylation reaction yields.

In summary, the synthesis of novel hydroxyproline glycosylated derivatives is here reported by following two reaction pathways which present several advantages over other proposed schemes. First, the need of simultaneous use of Z and ester temporarily potecting groups during the glycosylation step is avoided. Second, protection of amino function is accomplished by the N^{α}-Fmoc group, which is a labile group to mild organic bases[20] such as morpholine or piperidine without ß-elimination. Third, sodium methoxide[21] removable hydroxyl protecting groups, such as acetyl or benzoyl, can always be used. However, when benzoyl protected glycosyl halides are employed, cleaner and higher yield glycosylation reactions take place. And lastly, known methods for Ser/Thr glycosylation which are based either on unprotected α-carboxyl group of the amino acid (Eloffson's approach) or in pfp activated ester protections (Meldal's approach) can also be applied to Hyp.

These structural features makes these glycosylated L-hydroxyproline derivatives suitable to be used as building blocks for the solid-phase synthesis (SPGPS) of glycopeptides and neoglycopeptides containing sugars β-linked to Hydroxyproline. These building blocks may well be of use for multiple techniques and even synthesis of glycopeptide libraries.

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14. **In a typical experiment for glycosylation of FmocHypOH 1:**
The Lewis acid (BF_3Et_2O, 3 equiv.) was added at OoC to a suspension of the Fmoc amino acid 1 (1 equiv) and the 1,2-*trans* peracetylated glycosyl donor (1 equiv.) in dry acetonitrile and 4A molecular sieves. The reaction is monitored by TLC ($CHCl_3$/ MeOH / AcOH 80:10:1). After overnight stirring at rt Et_3N (3 equiv) is added and the crude is filtered through Celite, the organic layer is concentrated and the residue is chromatographed on silica gel ($CHCl_3$/ MeOH 8:1).
15. NMR (^1H and ^{13}C) and ES-MS data in accordance with the proposed structures.
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19. **In a typical experiment of glycosylation of FmocHypOPfp 2:**
The Fmoc amino acid activated ester 2 (1 equiv) is stirred in dry CH_2Cl_2 in the presence of silver triflate (1.1 equiv) and 4A molecular sieves for 10 min at rt. The peracylated 1-α-glycosyl halide (1.1 equiv.) was added at -40oC to the suspension. The reaction is monitored by TLC (Hexane/ Ethyl acetate 3:2). After 6 h stirring between -40oC and -20oC, Et_3N (1 equiv) is added and the reaction is left to reach rt. The crude is filtered through Celite, the organic layer is concentrated and the residue is chromatographed on silica gel under anhydrous conditions using dry solvents (Hexane/ Ethyl acetate 2:1).
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