Tetrahedron Letters, Vol. 33, No. 43, pp. 6445-6448, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Solid Phase Peptide Synthesis of Mucin Glycopeptides

S. Peters[a], T. Bielfeldt[a], M. Meldal[b], K. Bock[b] and H. Paulsen[a]*

a) Institut für Organische Chemie der Universität Hamburg, Martin-Luther-King-Platz 6, D-2000 Hamburg 13, Germany

b) Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500, Valby, Copenhagen, Denmark

Abstract: The synthesis of the new glycosylamino acid building block N^{α}-Fmoc-Thr(Ac_4-β-D-Galp)-(1→3)-(Ac_2-α-D-GalpN_3)-OPfp **3** and its use in a solid phase synthesis of triple glycosylated peptides from human intestinal mucin is described. The azide reduction was performed with thioacetic acid on the polymere bound glycopeptides.

In recent years the interest in glycoproteins has increased because the carbohydrates located on glycoproteins on the cell membranes are involved in many biological processes[1]. For a chemical synthesis of glycopeptides a suitable protected glycosylated amino acid is required as a building block for incorporation into a peptide chain by either solution or solid phase techniques[2]. It was demonstrated that a protecting group scheme with the fluoren-9-yl-methoxycarbonyl (Fmoc) group for the α-amino, pentafluorophenyl (Pfp)[3] for the α-carboxyl and acetyl for the carbohydrate hydroxy groups fulfill the requirements for a successful glycopeptide synthesis as shown by a multiple peptide synthesis of 40 different *O*-glycopeptides[4]. The synthesis of mucin glycopeptides, which carry a N-acetylgalactosamine (GalNAc) residue α-linked to the hydroxy groups of serine or threonine is best acchieved by application of a sugar donor with an azido substituent as a non participating group at C-2 in the glycosylation reaction[5]. The transformation of the azido group into the N-acetyl function is usually performed before the peptide synthesis. Recently, we developed a new strategy for a mucin glycopeptide synthesis in which the azido group was reduced by reaction of thioacetic acid with the polymer bound glycopeptide after completion of the peptide synthesis[6]. The method takes advantage of the Pfp-group which is sufficiently stable under the acidic conditions of the glycosylation reaction to act as a α-carboxyl protecting group and yet efficient as a highly activating leaving group during the peptide synthesis.

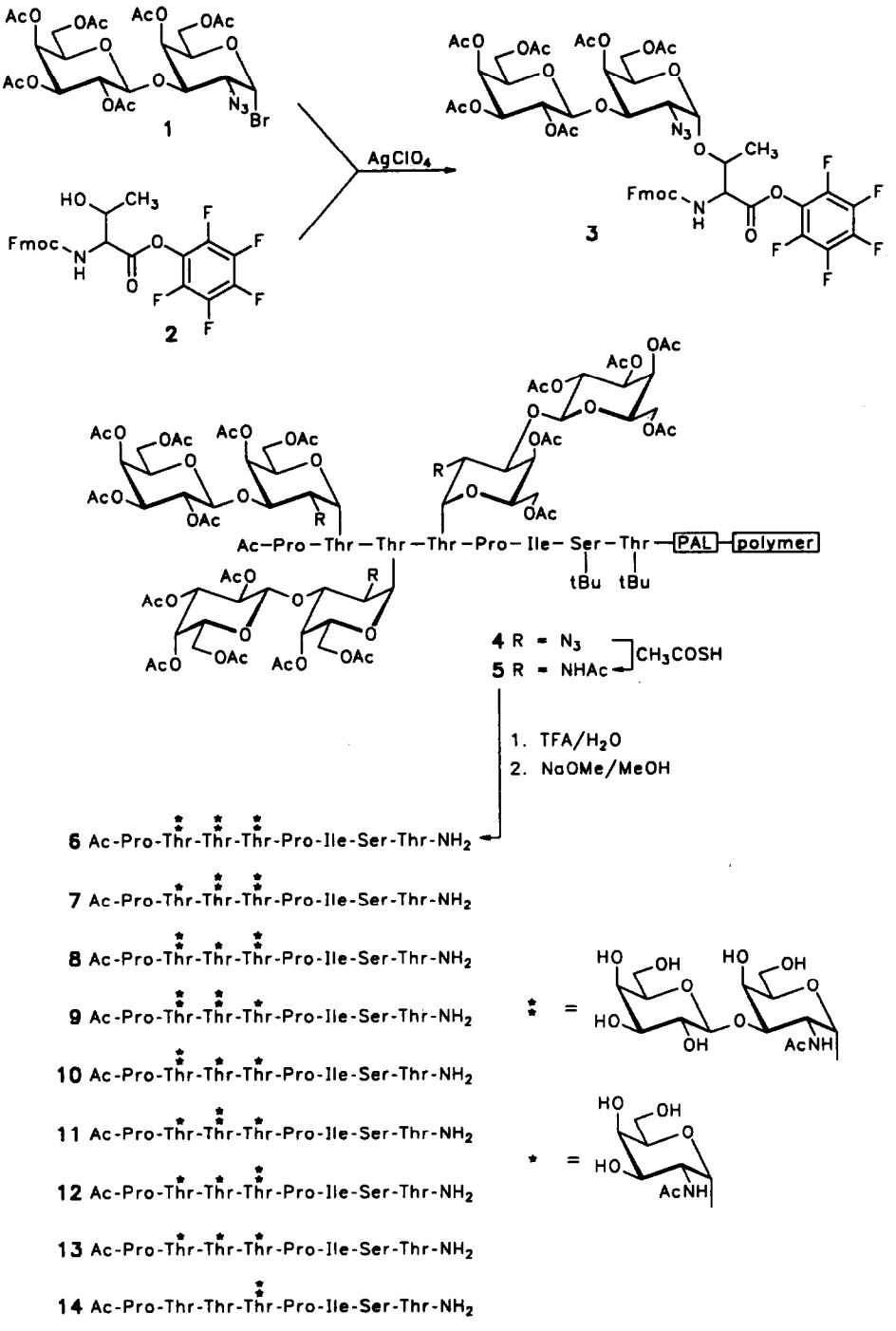
This work describes the application of the new azide strategy in the solid phase synthesis of a series of triple *O*-glycosylated peptides from human intestinal mucin[7] carrying monosaccharide (GalNAc) and disaccharide[8] (β-D-Gal-(1→3)GalNAc) glycans as model substrates for glycosyltransferases. For the incorporation of the disaccharide containing glycosylamino acid the Fmoc/Pfp protected threonine derivative **3** was used. The hydroxy groups of the carbohydrate were protected as acetates allowing a simple deprotection in the final step. Glycosylation of the Fmoc-Thr-OPfp **2**[9] with the bromide **1**[10] and silver perchlorate as catalyst afforded

the azido building block **3** and its β-anomer with an α:β ratio of 5:1. The α-anomer was isolated by reversed phase HPLC in a 55% yield without hydrolysis. Precipitation from diethyl ether/n-pentane afforded a stable solid material. Compound **3** was characterized by ^{1}H NMR spectroscopy and FAB mass spectroscopy[11].

The application of the azido building block **3** was exemplified by the solid phase glycopeptide synthesis of the N-acetyl octapeptide amide **6** carrying three vicinal disaccharide glycosylation sites. The synthesis was performed on a fully automatic peptide synthesizer using a kieselguhr supported polydimethyl acrylamide resin (Macrosorb) derivatized with a peptide amide linker (PAL)[12]. Non glycosylated Fmoc amino acids were activated as Dhbt-esters[13] and for the coupling reactions with the building block **3** Dhbt-OH was added as auxiliary nucleophile. The Dhbt-method allowed the peptide bond formation to be followed by the disappearance of the bright yellow color of the ion pair formed between unreacted amino groups and Dhbt-OH[14]. The decrease of the color was measured with a solid phase photo meter and the data were recorded by computer allowing the automation of the whole peptide synthesis. It should be noted that only a 1.5 fold excess of the valuable glycosylamino acid **3** was used for each coupling reaction stressing the nescessary high reactivity of the Pfp-ester. The reaction time of the glycosylated amino acid **3** was as fast as the unglycosylated amino acids (3-10 h) except the coupling of glycosylated Thr(5) to Pro(4) which required an extended reaction time (22 h). All Fmoc deprotections were performed with 50% morpholine in DMF[15]. Upon completion of the stepwise assembly the three azido groups on the polymer bound glycopeptide **4** were transformed into N-acetyl groups by reaction with thioacetic acid to give the polymer bound glycopeptide **5**. The thioacetic acid should be distilled several times before use and analyzed by GC to be free of dithioacetic acid to avoid the formation of thioacetates. The progress of the reduction was measured by IR spectroscopy following the disappearance (48 h) of the azido absorption band at ν(KBr) = 2117 cm^{-1}. The glycopeptide was cleaved off the resin with 95% aqueous trifluoroacetic acid (TFA) and deacetylated with a catalytic amount of sodium methoxide in methanol. After HPLC purification compound **6** was isolated in 42% yield[16] with excellent purity. Racemisation or β-elimination were not observed. The fully deprotected *O*-glycopeptide amide **6** was characterized by ^{1}H NMR spectroscopy and FAB-MS[17].

The building block **3** and the corresponding monosaccharide derivative N$^{\alpha}$-Fmoc-Thr(Ac$_3$-α-D-GalpN$_3$)-OPfp[6] were further used for the synthesis of the *O*-glycopeptides **7-14** in a multiple column peptide synthesizer[18]. The simultaneous assembly of these compounds was performed in analogy to the preparation of compound **6** but with a two fold excess of glycosylated amino acids. The decrease in coloration of the resin during the peptide bond formation was in this case followed visually. The azide reductions could also easily be carried out in the multiple column synthesizer. The pure glycopeptides **7-14** were obtained in quantities of 12 ~mg (34-40%) after praprative HPLC and all analyzed by HPLC, ^{1}H NMR and FAB-MS.

In this work it was demonstrated that the combined use of Fmoc and Pfp for the protection of the amino acid and the reduction of the azide on solid support results in a very effective strategy for the synthesis of *O*-glycopeptides containing complex carbohydrates with N-acetyl groups. The synthetic route is applicable for solid phase syntheses of poly-glycosylated mucin glycopeptides in an automatic or a multiple peptide synthesizer. Compounds **6-14** are a part of a series of *O*-glycopeptides which were required for enzymatic studies with glycosyltransferases[19].


1
2
3
AgClO4
Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-PAL-polymer
tBu tBu
4 R = N3
5 R = NHAc
CH3COSH
1. TFA/H2O
2. NaOMe/MeOH
6 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
7 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
8 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
9 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
10 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
11 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
12 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
13 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2
14 Ac-Pro-Thr-Thr-Thr-Pro-Ile-Ser-Thr-NH2

Acknowledgements: This work was supported by the Stiftung Stipendien-Fonds des Verbandes der Chemischen Industrie and by the Studienstiftung des Deutschen Volkes.

REFERENCES AND NOTES

1. J. Montreuil, Adv. Carbohydr. Chem. Biochem. **37**, 157 (1980).
2. H. Kunz, Angew. Chem. **99**, 297 (1987); Angew. Chem. Int. Ed. Engl. **26**, 294 (1987).
3. a) M. Meldal and K.J. Jensen, J. Chem. Soc. Chem. Commun., 483 (1990).
   b) A.M. Jansson, M. Meldal and K. Bock, Tetrahedron Lett. **31**, 6991 (1990); J. Chem. Soc. Perkin Trans. 1, in press.
   c) M. Meldal and K. Bock, Tetrahedron Lett. **31**, 6987 (1990).
4. S. Peters, T. Bielfedt, M. Meldal, K. Bock and H. Paulsen, J. Chem. Soc. Perkin Trans. 1, 1163 (1992).
5. H. Paulsen, Angew. Chem. **94**, 184 (1982); Angew. Chem. Int. Ed. Engl. **21**, 155 (1982).
6. T. Bielfeldt, S. Peters, M. Meldal, K. Bock and H. Paulsen, Angew. Chem., in press.
7. J.R. Gum, J.C. Byrd, J.W. Hicks, N.W. Toribara, D.T.A. Lamport and Y.S. Kim, J. Biol. Chem. **264**, 6480 (1989).
8. A different approach for solid phase glycopeptide syntheses with the same disaccharide core structure has recently been published by: B. Lüning, T. Norberg, C. Rivera-Baeza and J. Tejbrant, Glycoconjugate J. **8**, 450 (1991).
9. I. Schön and C. Kisfaludy, Synthesis, 303 (1986).
10. H. Paulsen and M. Paal, Carbohydr. Res. **135**, 71 (1984).
11. Compound **3**: m.p. 103 °C (not corr.); $[\alpha]_D^{23}$ = + 51.0 (c = 1.0, $CHCl_3$); 400 MHz ^1H NMR; δ, ppm; J, Hz; (in $CDCl_3$, ref Me_4Si, 300 K): 5.49 (d, 3.0 Hz, H-1), 4.73 (d, 7.8 Hz, H-1´).
    FAB-MS found MH^+:1109.6; calc. for $C_{49}H_{49}F_5N_4O_{20}$: 1108.3.
12. F. Albericio, N. Kneib-Cordonier, S. Biancalana, L. Gera, R.I. Masada, D. Hudson and G. Barany, J. Org. Chem. **55**, 3730 (1990).
13. E. Atherton, J.L. Holder, M. Meldal, R.C. Sheppard and R.M. Valerio, J. Chem. Soc. Perkin Trans. 1, 2887 (1988).
14. L.R. Cameron, J.L. Holder, M. Meldal and R.C. Sheppard, J. Chem. Soc. Perkin Trans. 1, 2895 (1988).
15. P. Schultheiss-Reimann and H. Kunz, Angew. Chem. **95**, 64 (1983); Angew. Chem. Int. Ed. Engl. **22**, 62 (1983).
16. Yields are calculated based on the loading of the resin and the degree of coupling of the first amino acid. Both were determined by quantitative amino acid analyses with norleucin as internal standard.
17. Compound **6**: $[\alpha]_D^{27}$ = + 7.6 (c = 1.0, H_2O);
    FAB-MS found MH^+: 1954.0; calc. for $C_{79}H_{132}N_{12}O_{44}$: 1952.9.
18. M. Meldal, C.B. Holm, G. Bojesen, M.H. Jakobsen and A. Holm, Int. J. Pept. Protein Res., in press.
19. I. Brockhausen, G. Möller, G. Merz, K. Adermann and H. Paulsen, Biochemistry **29**, 10206 (1990).

(Received in Germany 11 June 1992)