Tetrahedron Letters 50 (2009) 4151–4153

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of new ribosylated Asn building blocks as useful tools for glycopeptide and glycoprotein synthesis

M. Angeles Bonache [a,b], Francesca Nuti [a,b], Alexandra Le Chevalier Isaad [a,b], Feliciana Real-Fernández [a,b], Mario Chelli [a,b], Paolo Rovero [a,c], Anna M. Papini [a,b,*]

[a] Laboratory of Peptide & Protein Chemistry & Biology (PeptLab), Polo Scientifico e Tecnologico, University of Florence, via della Lastruccia 13, I-50019 Sesto Fiorentino (FI), Italy
[b] Dipartimento di Chimica Organica 'Ugo Schiff' and CNR-ICCOM, Polo Scientifico e Tecnologico, University of Florence, via della Lastruccia 13, I-50019 Sesto Fiorentino (FI), Italy
[c] Dipartimento di Scienze Farmaceutiche, Polo Scientifico e Tecnologico, University of Florence, Via Ugo Schiff 6, I-50019 Sesto Fiorentino (FI), Italy

ARTICLE INFO

Article history:
Received 6 April 2009
Revised 24 April 2009
Accepted 29 April 2009
Available online 3 May 2009

Keywords:
N-Glycosylated Asn for SPGPS
α- and β-ribofuranose Asn
Triazine-based coupling reagent

ABSTRACT

We performed the first synthesis of new Asn derivatives bearing α- or β-ribose as pure anomers, linked by an *N*-glycosidic bond, on the side chain of the Asn residue orthogonally protected for Fmoc/^{t}Bu SPPS, by an efficient five-step strategy with a global yield of 73% starting from D-ribose. These building blocks are obtained in a large scale and can be useful tools for glycopeptide and glycoproteins synthesis.

Glycoproteins are fundamental for a variety of biological events, that is, folding, fertilization, neuronal development, hormone activities, immune surveillance, and inflammatory responses. However, investigating these processes is a challenge by the fact that naturally expressed glycoproteins often emerge as heterogeneous mixtures. In addition, post-translational modifications (PTMs) such as alterations of sugars on the amino acid side chains may in part lead to higher structural and functional protein diversity. Two main forms of protein glycosylation are naturally found: O- and N-glycosylation. The majority of N-linked motifs are Asn(GlcNAcβ) and O-linked forms are usually Ser/Thr(GlcNAcβ) or Ser/Thr(GlcNAcα). Although other unusual linkages are also observed[1] including Asn(GlcNAcα),[2] Asn(Glc),[3] Asn(GalNAc),[4] Asn(Rha),[5] Ser/Thr(Manα),[6] or even C-linked Trp(Manα).

Biosynthesis of the glycan portion is not DNA mediated as in the case of proteins; the glycan structure is subjected to competition between various enzymes and their substrates. Moreover, N-glucosylation, a PTM not common in eukaryotic proteins, has been detected only in bacterial glycoproteins.[3] This variable post-translational processing of glycans results in expression of an assortment of possible glycan structures called 'glycoforms', glycoproteins that possess the same protein backbone but differ in the oligosaccharide components and sites of glycosylation. Expression of glycoproteins in mammalian cell culture habitually leads to mixtures of glycoforms.

In light of the heterogeneity for each glycan, which limits the isolation, purification, and manipulation of natural glycoproteins, the development of an efficient synthetic chemistry for the protein–sugar assembly is essential to advance in understanding the biological role of glycoconjugates. The synthesis of glycopeptides requires a combination of synthetic methods from both carbohydrate and peptide chemistry. To determine their activity relationships not only sugars but also control of their configuration and of the linkage with amino acids are needed. Several reviews cover strategies for the preparation of N-linked and O-linked glycoaminoacids.[7]

Solid-Phase GlycoPeptide Synthesis (SPGPS) based on building block approach is an efficient method to obtain glycosylated peptides bearing different sugar moieties on the side chains of different amino acids. SPGPS requires an excess of building block units to achieve high yields, so it is necessary to prepare them in high quantity. For this reason it is fundamental to develop convenient synthetic pathways to glycosylated building blocks and efficient glycopeptide solid-phase synthetic pathways. In our laboratory several glycosylated building blocks orthogonally protected for SPGPS were developed in large scale, for example, Glc, Gal, Man, GlcβGlc, GlcNAc, and glycomimetics, on the side chains of Asp, Ser, Glu, Thr, and HyPro.[8] We identified Asn(β-Glc) as minimal epitope for Multiple Sclerosis (MS) autoantibody recognition, after evaluating in competitive and SP-ELISA a CSF114-type glycopep-

* Corresponding author. Tel.: +39 055 457 3561; fax: +39 055 457 3584.
E-mail address: annamaria.papini@unifi.it (A.M. Papini).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.04.124

tide library based on glyco-amino acid diversity.[9] The role of ribose moiety has not been previously studied.

It is well known that ribose (Rib) is produced in vivo from glucose and it plays a fundamental role in cellular energy metabolism and cellular signaling. Mono (ADP-ribosyl) transferases transfer single ADP-ribose to acceptor proteins.

Due to high interest in the glycan–protein linkages as *N*-(Asn) glycosides, now we are interested to obtain new N-linked ribofuranose Asn derivatives. We report herein an efficient synthesis of two N^{α}-Fmoc protected asparagine building blocks bearing α- or β-D-ribose, orthogonally protected for SPGPS: Fmoc-L-Asn[(2,3-*O*-isopropylidene,5-*O*Ac)α-Rib]-OH [Fmoc-L-Asn(RibCMe2Acα)-OH, **9α**] and Fmoc-L-Asn[(2,3-*O*-isopropylidene,5-*O*Ac)β-Rib]-OH [Fmoc-L-Asn(RibCMe2Acβ)-OH, **9β**] (Fig. 1).

Most of the protected glycosylamines are prepared by hydrogenation of an acetyL-β-glycosyl azide using different catalytic reagents.[10] Applying the catalyst $Pd(OH)_2$ to this synthetic strategy, ribosylamine **3** was obtained in a very low yield (6%) (Scheme 1).

In order to improve the yield of the reduction reaction, we investigated an alternative synthetic strategy, starting from ribose, protected in C-2 and C-3 with a rigid isopropylidene group that avoids the opening of the sugar ring. The new building blocks **9α** and **9β** were synthesized starting from the 2,3-*O*-isopropylidene-1,5-diacetate-protected ribosyl derivative **5**, obtained by reaction of **4** with acetone and subsequent acetylation with Ac_2O in pyridine.[11] Azide moiety was introduced by reaction with Me_3SiN_3, via $SnCl_4$ catalysis, affording a mixture of the anomers **6α**:**6β** (38:62). It was possible to easily separate the two anomers by FCC and to characterize them as pure compounds (Scheme 2). The next reduction was performed on the β-anomer in the presence of palladium black, obtaining in any case a mixture of amines **7α**:**7β** in good yield, as detected by ^1H NMR spectrum.

Coupling reaction of the anomeric mixture of the ribosyl amines **7α:7β** with Fmoc-L-Asp-OAll afforded the desired *N*-glycosidic bond, by using the new efficient triazine-based coupling reagent (TBCR).[12]

Allyl group protection was chosen for $^{\alpha}$COOH because it is orthogonal with the isopropylidene and acetyl groups on the hydroxyl functions of the ribosyl moiety. Activation of carboxylic function by the TBCR is particularly efficient because of the formation of triazine 'superactive ester', as recently confirmed with the synthesis of protected dipeptides and esters, sterically hindered amino acids, manual and automated solid-phase peptide synthesis (SPPS) of difficult peptide sequences, and head-to-tail constrained cyclopeptide analogs.[13] The two pure anomers **8α** and **8β** of the new protected ribosylated N^{α}-Fmoc-asparagine allyl esters were obtained after an efficient separation by FCC from the corresponding anomeric mixture (3:2) (Scheme 3).

By analyzing the ^1H and ^{13}C NMR spectra it is possible to assign the configuration of the compounds **8α**[14] and **8β**.[15] The anomeric configuration of **8α** was assigned by the $J_{1,2}$ spin coupling constant of H-1 that is usually bigger for the α-anomers than for the corresponding β-anomers.[16] In fact H-1 and H-2 are less eclipsed in the case of β-anomers. In our case, the α-anomer shows $J_{1,2}$ = 4.4 Hz and the β-anomer $J_{1,2}$ = 0. As Tam et al. described, the chemical shift of H-1 is further downfield for the α-anomers (δ 5.74 ppm) than for the corresponding β-anomers (δ 5.57 ppm). Moreover, it is possible to observe the difference in chemical shift of the C1 (Δδ) shown by the α-anomer (80.6 ppm) compared to the corresponding value for the β-anomer (88.6 ppm) in accordance with the literature.[17] Subsequent removal of the allyl group with $Pd(PPh_3)_4$ and triphenylsilane afforded the anomerically pure building blocks **9α** and **9β** in a very good yield (Scheme 3).

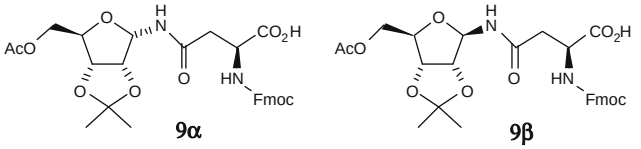
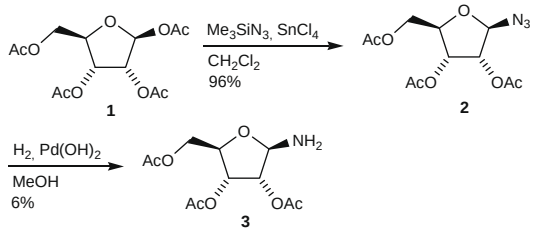
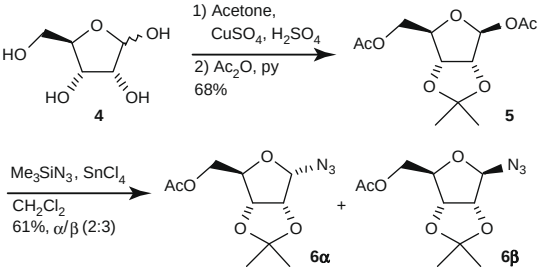


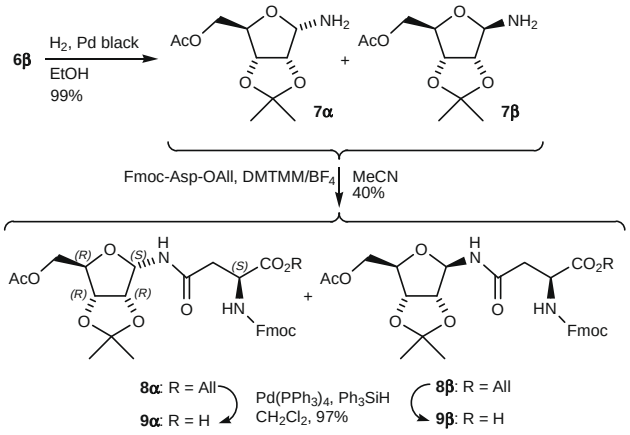
Figure 1. New building block N-linked ribofuranose Asn derivatives **9α** and **9β**.



Scheme 1. Synthesis of 2,3,5-triacetyl-β-D-ribofuranosylamine.



Scheme 2. Synthesis of the ribofuranosylazide **6α** and **6β**.



Scheme 3. Synthesis of the new building block N-linked ribofuranose Asn derivatives **9α** and **9β**.

Acknowledgments

This work was supported by Ente Cassa Risparmio di Firenze and FIRB Internazionalizzazione 2004 RBIN04TWKN, MIUR (Italy). M.A.B. thanks the Alfonso Martín Escudero Foundation (Spain) for her post-doctoral fellowship. We thank Dr. Marco Boccalini for his assistance with NMR spectroscopy.

Supplementary data

Supplementary data (in the supplementary material experimental procedures and characterization data of the products are available) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.124.

References and notes

1. Vliegenthart, J. F. G.; Casset, F. *Curr. Opin. Struct. Biol.* **1998**, *8*, 565–571.
2. Shibata, S.; Takeda, T.; Natori, Y. *J. Biol. Chem.* **1988**, *263*, 12483–12485.
3. Wieland, F.; Heitzer, R.; Schaefer, W. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5470–5474.
4. Paul, G.; Lottspeich, F.; Wieland, F. *J. Biol. Chem.* **1986**, *261*, 1020–1024.
5. Messner, P.; Sleytr, U. B. *FEBS Lett.* **1988**, *228*, 317–320.
6. Strahl-Bolsinger, S.; Gentzsch, M.; Tanner, W. *Biochim. Biophys. Acta* **1999**, *1426*, 297–307.
7. (a) Buskas, T.; Ingale, S.; Boons, G.-J. *Glycobiology* **2006**, *16*, 113R–136R; (b) Gamblin, D. P.; Scanlan, E. M.; Davis, B. G. *Chem. Rev.* **2009**, *109*, 131–163; (c) Ying, L.; Liu, R.; Zhang, J.; Lam, K.; Lebrilla, C. B.; Gervay-Hague, J. *J. Comb. Chem.* **2005**, *7*, 372–384; (d) Ameijde, J.; Albada, H. B.; Liskamp, R. M. *J. Chem. Soc., Perkin Trans.* **2002**, *1*, 1042–1049; (e) Taylor, C. M. *Tetrahedron* **1998**, *54*, 11317–11362.
8. (a) Nuti, F.; Paolini, I.; Cardona, F.; Chelli, M.; Lolli, F.; Brandi, A.; Goti, A.; Rovero, P.; Papini, A. M. *Bioorg. Med. Chem.* **2007**, *15*, 3965–3973; (b) Paolini, I.; Nuti, F.; Pozo-Carrero, M. C.; Barbetti, F.; Kolesinska, B.; Kaminski, Z. J.; Chelli, M.; Papini, A. M. *Tetrahedron Lett.* **2007**, *48*, 2901–2904.
9. (a) Lolli, F.; Mulinacci, B.; Carotenuto, A.; Bonetti, B.; Sabatino, G.; Mazzanti, B.; D'Ursi, A. M.; Novellino, E.; Pazzagli, M.; Lovato, L.; Alcaro, M. C.; Peroni, E.; Pozo-Carrero, M. C.; Nuti, F.; Battistini, L.; Borsellino, G.; Chelli, M.; Rovero, P.; Papini, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 10273–10278; (b) Papini, A. M. *Nat. Med.* **2005**, *11*, 13.
10. (a) Theim, J.; Wiemann, T. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 80–82; (b) Teshima, T.; Nakajima, K.; Takahashi, M.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*, 363–366; (c) Von dem Bruch, K.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 101–103; (d) Kunz, H.; Waldmann, H.; März, J. *Liebigs Ann. Chem.* **1989**, 45–49.
11. (a) Cruickshank, K. A.; Reese, C. B. *Synthesis* **1983**, 199–200; (b) Fürstner, A.; Radkowski, K.; Wirtz, C.; Goddard, R.; Lehmann, C. W.; Mynott, R. *J. Am. Chem. Soc.* **2002**, *124*, 7061–7069.
12. DMTMM/BF_4^-, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate.
13. (a) Kamiński, Z. J.; Kolesińska, B.; Kolesińska, J.; Sabatino, G.; Chelli, M.; Rovero, P.; Błaszczyk, M.; Główka, M. L.; Papini, A. M. *J. Am. Chem. Soc.* **2005**, *127*, 16912–16920; (b) Kaminski, Z.; Papini, A. M.; Kolesińska, B.; Kolesińska, J.; Jastrzabek, K.; Sabatino, G.; Bianchini, R. PCT/EP2005/055793 (2005).
14. Fmoc-L-Asn(2,3-*O*-Isopropyliden-5-*O*-acetyl-α-D-ribofuranosyl)-OAll (**8α**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, 2H, J = 6.8 Hz, Fmoc 4-H and 5-H), 7.59 (d, 2H, J = 6.4 Hz, Fmoc 1-H and 8-H), 7.39–7.27 (m, 4H, Fmoc 2-H, 7-H, 3-H and 6-H), 6.59 (d, 1H, J = 9.2 Hz, 1-NH), 6.06 (d, J = 7.2 Hz, NHFmoc), 5.94–5.84 (m, 1H, = CH), 5.74 (dd, 1H, J = 4.4, J = 9.6 Hz, 1-H), 5.32 (d, 1H, J = 17.0 Hz, $=CH_2$), 5.22 (d, 1H, J = 10.4 Hz, $=CH_2$), 4.67–4.58 (m, 5H, CH_2Fmoc, α-H Asn, 2-H, 3-H), 4.41–4.27 (m, 2H, CH_2OAll), 4.22–4.19 (9-H Fmoc, 4-H), 4.15–4.06 (m, 2H, 5-H), 3.08 (system ABMX, dd, 2H, J_{AB} = 16.2, J_{AM} = 4.4, J_{BM} = 3.6 Hz, β-H Asn), 2.05 (s, 3H, OAc), 1.50 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.47, 170.28, 169.75 (COOAll, 1-CONH, COOAc), 156.08 (CO, Fmoc), 143.83, 141.23, 127.67, 127.03, 125.20, 119.94 (12C, Ar Fmoc), 131.59 (CH-OAll), 118.69 (CH_2=), 113.26 ($C(CH_3)_2$), 81.85 (C-2), 80.60 (C-1), 79.48 (C-4), 79.21 (C-3), 67.24 (CH_2 OAll), 66.40 (OCH_2 Fmoc), 64.97 (C-5), 50.66 (C^{α}Asp), 47.06 (C-9 Fmoc), 37.79 ($C^{\beta}H_2$ Asn), 26.17, 24.54 (2C, CH_3), 20.89 (CH_3, OAc).
15. Fmoc-L-Asn-(2,3-*O*-isopropyliden-5-*O*-acetyl-β-D-ribofuranosyl)-OAll (**8β**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, 2H, J = 6.8 Hz, Fmoc 4-H and 5-H), 7.60 (d, 2H, J = 6.4 Hz, Fmoc 1-H and 8-H), 7.41–7.29 (m, 4H, Fmoc 2-H, 7-H, 3-H and 6-H), 6.76 (d, 1H, J = 4.8 Hz, 1-NH), 6.14 (d, J = 8.8 Hz, NHFmoc), 5.94–5.85 (m, 1H, =CH), 5.57 (d, 1H, J = 5.2 Hz, 1-H), 5.32 (d, 1H, J = 17.2 Hz, $=CH_2$), 5.22 (d, 1H, J = 10.4 Hz, $=CH_2$), 4.70–4.59 (m, 5H, CH_2Fmoc, α-H Asn, 3-H, 2-H), 4.44–3.97 (m, 4H, 9-H Fmoc, 4-H, and 5-H), 2.98 (system ABMX, dd, 2H, J_{AB} = 16.2, J_{AM} = 4.4, J_{BM} = 3.6 Hz, β-H Asn), 2.10 (s, 3H, OAc), 1.51 (s, 3H, CH_3), 1.32 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$) δ 170.41, 1 69.91, 169.82 (COOAll,1-CONH, COOAc), 156.12 (CO, Fmoc), 143.74, 141.15, 127.64, 127.03, 125.17 (12C, Ar-Fmoc), 131.49 (CH-OAll), 119.92 (CH_2=), 113.75 ($C(CH_3)_2$), 88.60 (C-1), 85.62 (C-3), 84.03 (C-4), 81.26 (C-2), 67.36 (CH_2-OAll), 66.45 (CH_2 Fmoc), 64.68 (C-5), 50.81 (C^{α}Asn), 47.18 (C-9 Fmoc), 37.72 ($C^{\beta}H_2$ Asn), 26.40, 25.24 (2C, CH_3), 21.13 (CH_3, OAc).
16. (a) Tam, S. Y. K.; Klein, R. S.; De las Heras, F. G.; Fox, J. J. *J. Org. Chem.* **1979**, *44*, 4854–4862; (b) Sokolova, T. N.; Yartseva, I. V.; Preobrazhenskaya, M. N. *Carbohydr. Res.* **1981**, *93*, 19–34; (c) MacCoss, M.; Robins, M. J.; Rayner, B.; Imbach, J.-L. *Carbohydr. Res.* **1977**, *59*, 575–579; (d) Numao, N.; Hemmi, H.; Naujokaitis, S. A.; Rabinovitz, M.; Beisler, J. A. *J. Med. Chem.* **1981**, *24*, 515–520.
17. Chavis, C.; de Gourcy, C.; Dumont, F.; Imbach, J.-L. *Carbohydr. Res.* **1983**, *113*, 1–20.