Pergamon

Tetrahedron Letters 42 (2001) 7207–7210

TETRAHEDRON LETTERS

Stereoselective synthesis of β-linked TBDMS-protected chitobiose-asparagine: a versatile building block for amyloidogenic glycopeptides

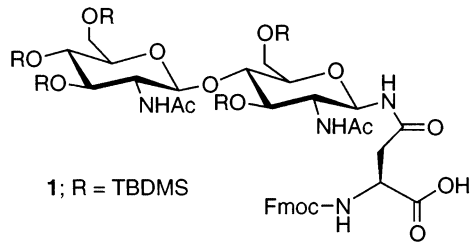
Carlos J. Bosques, Vincent W.-F. Tai and Barbara Imperiali*

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA

Received 27 June 2001; revised 7 August 2001; accepted 8 August 2001

Abstract—A simple and efficient synthesis of a Fmoc-L-Asn[β-chitobiose(TBDMS)$_5$]-OH with selectivity for the β-linked carbohydrate is described. The utility of this building block is illustrated in the synthesis of a glycosylated amyloidogenic peptide representing the 175-195† fragment of the mouse prion protein (**PrPGP**). © 2001 Elsevier Science Ltd. All rights reserved.

Glycopeptides and glycoconjugates have been widely used as targets for therapeutic agents and as models for biologically relevant systems.[1,2] The need for homogeneous samples of desired glycopeptides has generated significant interest in the development of different synthetic approaches including the chemoenzymatic, the convergent and the building block approaches.[3] Among these methods, the building block approach is particularly attractive due to the ease of incorporating the desired glycosylated amino acid through the solid-phase peptide synthesis (SPPS).[3–5] Acetyl protecting groups have been used extensively for the synthesis of carbohydrate building blocks and have proved to be successful for the preparation of many important glycoconjugates.[5,6] Despite many advantages, this approach also suffers some drawbacks. One of the main difficulties encountered when using this approach is the requirement for a final basic treatment for the deacetylation of the carbohydrate after the peptide has been cleaved from the resin. Through the preparation of glycosylated amyloidogenic peptides we found that the acetate protecting group strategy was complicated by the natural tendency of some amyloidogenic peptides to aggregate under basic conditions. Previously, Kihlberg and coworkers have shown the use of acid-labile protecting groups for the synthesis of *N*-linked and *O*-linked glycopeptide building blocks.[7,8] The main advantage of using acid-labile protecting groups for glycopeptide synthesis is that a concomitant deprotection of the saccharide moiety can be achieved during the acidic cleavage of the peptide from the resin without the need of extra exposure to basic conditions. Unfortunately, in the generation of *N*-linked chitobiose building blocks using *tert*-butyldimethylsilyl (TBDMS) protecting groups, anomerization of the *N*-acetylglucosamine proximal to the amino acid is a major side reaction.[7] Herein, the synthesis of β-linked TBDMS-protected chitobiose asparagine building block **1** is described together with the incorporation into the 175-195 fragment of the mouse prion protein (**PrPGP**). Our approach offers the advantage of an enhanced selectivity for the β-anomer and most importantly, the use of the TBDMS protection provides an impressive increase in the purity and total yield in the **PrPGP** synthesis.



1; R = TBDMS

The anomerization of the first *N*-acetylglucosamine during the synthesis of the Fmoc-L-Asn[β-chitobiose(TBDMS)$_5$]-OH seems to be induced by the bulky TBDMS groups during the reduction of the protected chitobiosyl-azide and the subsequent coupling of the chitobiosylamine to the aspartic acid.[7,8] In our hands, using the same (and modified) conditions used by others[7] for the reduction of the chitobiosyl-azide **2** and

Keywords: glycopeptide synthesis; amyloidogenic peptide; protein glycosylation.

* Corresponding author. Tel.: (617) 253-1838; fax: (617) 452-2419; e-mail: imper@mit.edu

† Human sequence numbering is used for the mouse **PrPGP**.

PII: S0040-4039(01)01524-6

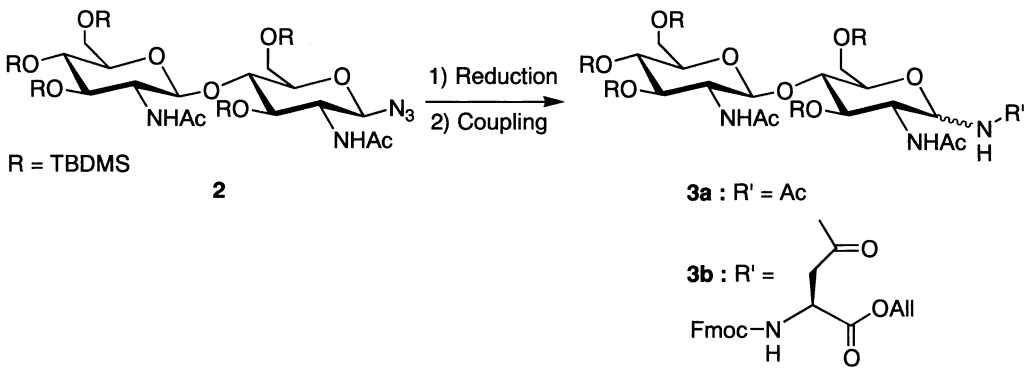
coupling between the chitobiosylamine and the activated aspartic acid resulted in anomerization of the carbohydrate to yield the α-anomer (**3bα**) as the major product (55% yield, α:β ~ 10:1). In an attempt to reduce the level of anomerization, different reduction conditions and preactivation of the aspartic acid (instead of in situ activation) were used (Scheme 1, Table 1). Unfortunately, these conditions did not show any significant improvement in the anomeric ratio. Staudinger reactions have also been attempted by others,[7] but these approaches have proved unsuccessful.

Since the anomerization appeared to be promoted by the bulky TBDMS groups, we elected to carry out the coupling with the free chitobiosylamine prior to carbohydrate protection (Scheme 2). Chitobiose octaacetate **6** was prepared as described previously[9] and deacetylated using NaOMe in MeOH to give the free chitobiose. Chitobiosylamine **7** was then generated by stirring the free chitobiose in a saturated ammonium hydrogen carbonate solution at 45°C for 2 days.[6,10,11] For the preparation of the building block, Fmoc-L-Asp(OH)-α-OAll **4** was activated in quantitative yield as the 1-oxo-2-hydroxydihydrobenzotriazene (Dhbt) ester **5**[6,12] and reacted with the free chitobiosylamine **7** in the presence of diisopropylethylamine in DMSO. This transformation afforded predominantly the β-linked building block (>10:1 β:α). Silylation of the coupling product using *tert*-butyldimethylsilyl triflate (TBDMS-OTf) and catalytic amounts of dimethylaminopyridine (DMAP) in pyridine afforded **8** in a 50% overall yield after flash column chromatography.[13] Finally, the α-carboxylic acid was deprotected in quantitative yield using $Pd(PPh_3)_4$ and $PhSiH_3$ in CH_2Cl_2.[14] The use of $PhSiH_3$ helped to minimize undesired Fmoc deprotection observed when using morpholine.[7,15]

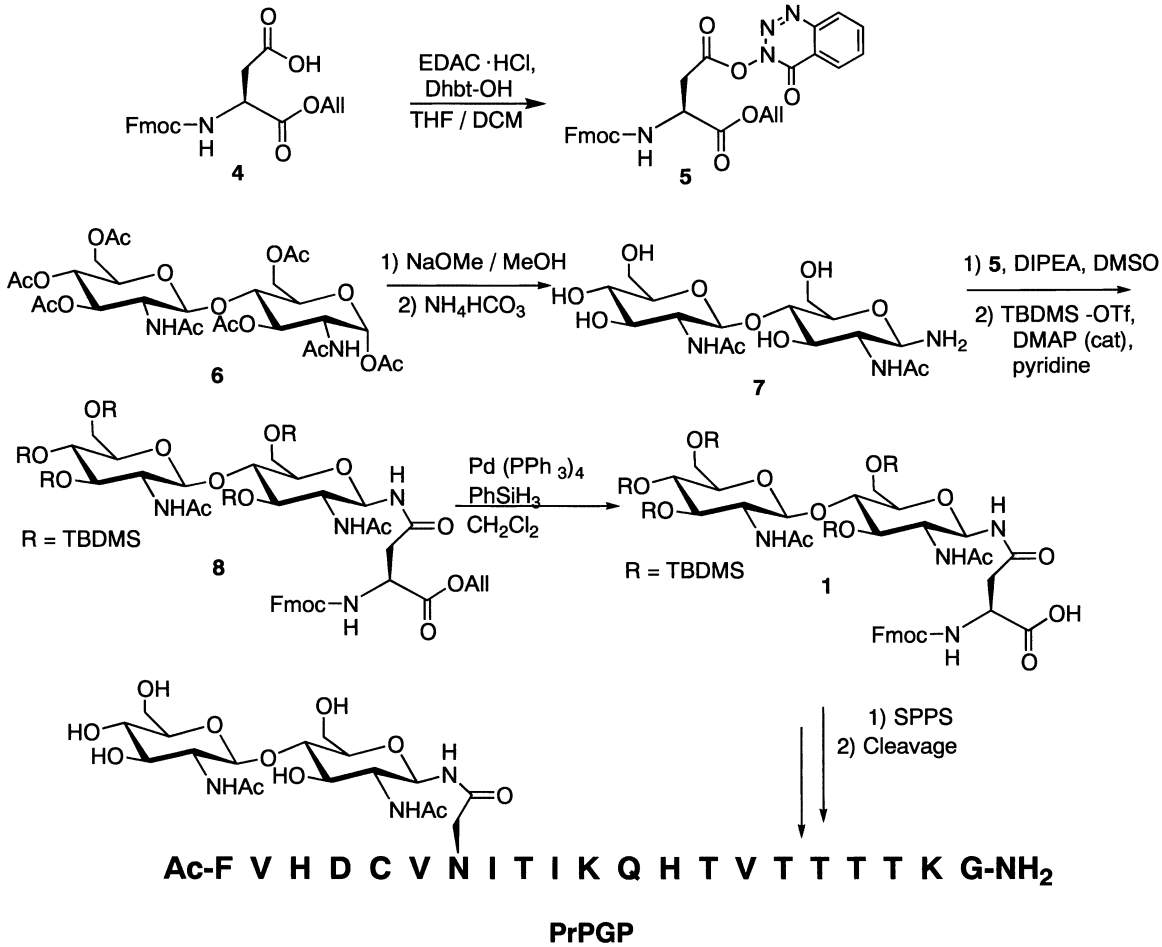
The use of the Fmoc-L-Asn[β-chitobiose(TBDMS)$_5$]-OH building block for the synthesis of amyloidogenic peptides is illustrated in the preparation of a glycopeptide of the 175-195 fragment of the mouse prion protein (**PrPGP**). This peptide fragment is stable in aqueous solution at low pH; however, electron microscopy (EM) analysis has demonstrated that **PrPGP** forms fibrils at high pH. Therefore, this peptide fragment represents a good test case for the use of acid-labile protected building block **1** in an amyloidogenic peptide. The incorporation of the TBDMS-protected building block into the peptide and the final cleavage and deprotection processes showed many advantages compared to the acetylated building block approach. First, the solubility of the TBDMS-protected building block allowed the coupling to be performed in dichloromethane compared to the acetylated counterpart which required *N*-methylpyrrolidinone for the best coupling conditions. Also, the coupling of the building block was more efficient in this case; in addition to proceeding efficiently, no peptide truncation products were observed after cleavage from the resin. On the other hand, the coupling for the acetylated counterpart was very difficult and many deletion products were observed after triple coupling with 1.5 building block equivalents for each coupling (Fig. 1). Most importantly, this approach provided higher yields and fewer side products; particularly because no basic treatment was required for the complete deprotection of the glycopeptide (which causes irreversible aggregation and precipitation) as it is the case when using the acetylated building block. After cleaving the peptide from the resin, only two major products were observed: the expected glycosylated product **PrPGP** (49% peptide after purification) and **PrPGP** with an extra TBDMS protecting group (11%) (Fig. 1). This approach provided enough material to carry out a panel of biophysical studies on the system; these studies will be the subject of a detailed structural study. The addition of an extra 0.8% water and additional shaking for 45 minutes after the standard 3 hours of cleavage produced the best results for the removal of the protecting groups.

Table 1. Survey of experimental conditions for synthesizing TBDMS-protected building block

Reduction	Coupling	α:β (%)
H_2, Pd/C in THF	Pyridine/Ac_2O (2:1)	75:25
H_2, Pd/$CaCO_3$ in EtOH	Pyridine/Ac_2O (2:1)	62:38
Raney Ni in EtOH	$Ac_2O/CHCl_3$	43:57
Raney Ni in EtOH	Fmoc-Asn(Dhbt)-OAll/$CHCl_3$	>91:9
H_2, Pd/C in THF	Fmoc-Asn(Dhbt)-OAll/$CHCl_3$	91:9



Scheme 1. Anomerization of the TBDMS-protected carbohydrate during the reduction and coupling conditions.



Scheme 2. Synthesis of the Fmoc-L-Asn [β-chitobiose(TBDMS)$_5$]-OH and incorporation into **PrPGP**.

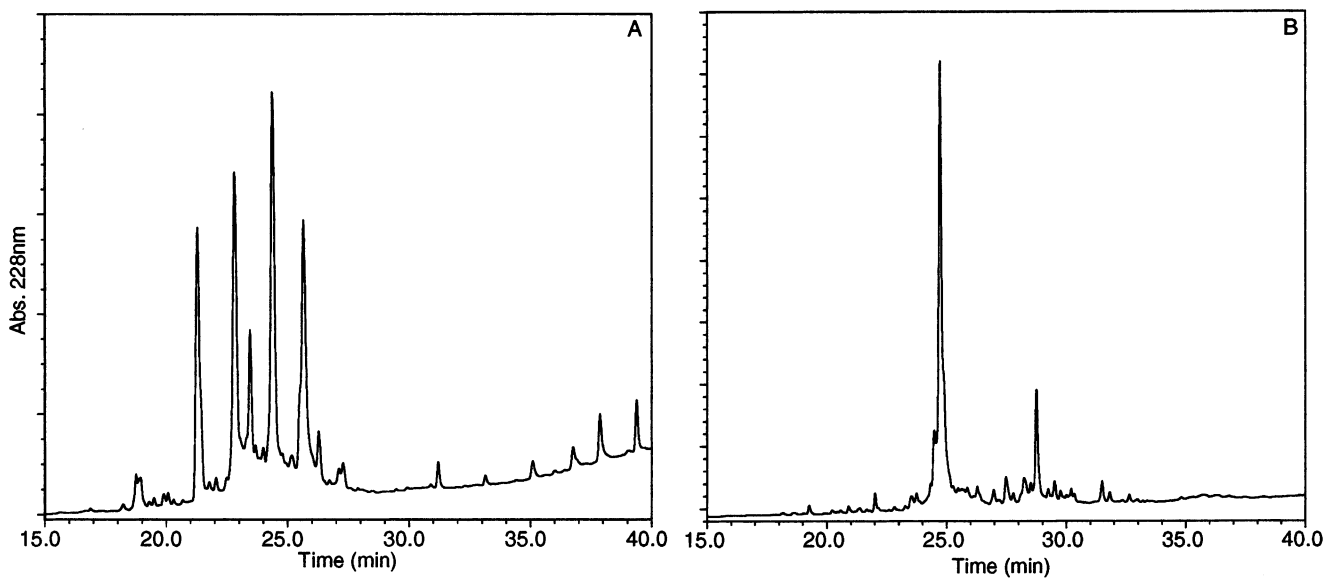


Figure 1. Comparison of the purity of the crude glycopeptides using the acetylated (**A**) and the silylated (**B**) building blocks. The HPLC analysis was performed on a C_{18}-reversed-phase column directly after acidic cleavage from resin and trituration. (**A**) Chromatogram shows acetylated glycopeptide ($t_R = 24.2$ minutes, [M+3H$^+$]/3 1001.2 (obsd); 1001.1 (calcd) and many truncation products. Gradient: 0–90% CH_3CN in H_2O and 0.1%TFA in 25 minutes. (**B**) Chromatogram shows completely deprotected **PrPGP** ($t_R = 24.71$ minutes, [M+2H$^+$]/2 1396.8 (obsd); 1396.6 (calcd) and **PrPGP** with an additional TBDMS ($t_R = 28.75$ minutes, [M+2H$^+$]/2 1453.4 (obsd); 1453.7 (calcd). Gradient: 15–70% CH_3CN in H_2O and 0.1% TFA in 30 minutes.

In conclusion, an efficient synthesis of a Fmoc-L-Asn[β-chitobiose(TBDMS)$_5$]-OH building block has been achieved via the coupling of a free chitobiosylamine and Fmoc-L-Asp(Dhbt)-α-OAll followed by TBDMS protection. This approach avoids the anomerization of the proximal GlcNAc residue previously encountered. The use of this building block in the synthesis of amyloidogenic peptides was illustrated by its incorporation into a fragment of the prion protein, that has been shown to aggregate into fibrils under basic conditions. The use of the acid-labile TBDMS-protected building block showed significant improvement on the synthesis of **PrPGP** when compared to the acetylated building block due to higher coupling efficiency and the elimination of the basic deprotection step after SPPS.

Acknowledgements

This research was supported by the NIH (GM-39334). C.B. is the recipient of a NIH traineeship in Biotechnology (1999–2001).

References

1. Danishefsky, S. J.; Allen, J. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 836–863.
2. Lohof, E.; et al. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 2761–2763.
3. Arsequell, G.; Valencia, G. *Tetrahedron: Asymmetry* **1999**, *10*, 3045–3094.
4. Jobron, L.; Hummel, G. *Angew. Chem., Int. Ed. Engl.* **2000**, *39*, 1621–1624.
5. Christiansen-Brams, I.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1461–1471.
6. Meinjohanns, E.; Meldal, M.; Paulsen, H.; Dwek, R. A.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 549–560.
7. Holm, B.; Linse, S.; Kihlberg, J. *Tetrahedron* **1998**, *54*, 11995–12006.
8. Broddefalk, J.; Bergquist, K.; Kihlberg, J. *Tetrahedron* **1998**, *54*, 12047–12070.
9. Terayama, H.; Takahashi, S.; Kuzuhara, H. *J. Carbohydr. Chem.* **1993**, *12*, 81–93.
10. Likhosherstov, L.; Novikova, O.; Derevitskaja, V.; Kochetkov, N. *Carbohydr. Res.* **1986**, *146*, C1–C5.
11. Due to the instability of glycosylamines in general, **7** was used for the next step without further purification.
12. Characterization of **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.45–7.89 (m, 4H, Dhbt), 7.78–7.33 (m, 8H, Fmoc-arom.), 6.15 (d, 1H, NαH), 5.90 (m, 1H, OCH_2-C$\underline{H}$=CH_2), 5.40–5.28 (m, 2H, CH=C$\underline{H}_{2\ cis,trans}$), 4.90–4.75 (m, 3H, $OC\underline{H}_2$-CH=CH_2, Hα), 4.45 (m, 2H, FmocC$\underline{H}_2$), 4.30 (t, J=7.10 Hz, 1H, FmocC$\underline{H}$), 3.49 (m, 2H, Hβ).
13. Characterization of **8**: ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.76–7.33 (m, 8H, Fmoc-arom.), 6.86 (d, J=7.5 Hz, 1H, γNH,), 6.60 (d, J=9.0 Hz, 1H, N$\underline{H}$Ac,), 6.14 (d, J=9.0 Hz, 1H, NαH), 5.90 (m, 1H, OCH_2-C$\underline{H}$=CH_2), 5.75 (d, J=10.4 Hz, 1H, N$\underline{H}$Ac′), 5.34 (dd, J=17.4, 1.5 Hz, 1H, CH=CH$\underline{H}_{trans}$), 5.24 (dd, J=10.3, 1.2 Hz, 1H, CH=C$\underline{H}$H$_{cis}$), 4.96 (t, J=7.6 Hz, 1H, H-1), 4.65 (m, 3H, $OC\underline{H}_2$-CH=CH_2, Hα), 4.55 (d, J=7.0 Hz, 1H, H-1′), 4.43 (dd, J=10.0, 7.0 Hz, 1H, FmocC$\underline{H}_2$), 4.29–4.21 (m, 2H, FmocC$\underline{H}_2$, FmocC$\underline{H}$), 4.13 (m, 1H, H-2′), 4.02–3.98 and 3.85–3.65 (m, 11H), 3.02 (dd, J=16.5, 4.3 Hz, 1H, Hβ), 2.68 (dd, J=16.5, 4.0 Hz, 1H, Hβ′), 2.05 (s, 3H, NAc), 1.96 (s, 3H, NAc), 0.93–0.87 (5s, 9H each, tBu), 0.17–0.02 (10s, 3H each, Si-CH_3).
14. Dessolin, M.; Guillerez, M. G.; Thieriet, N.; Guibe, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741–5744.
15. Characterization for compound **1** is in complete agreement with previous reports (see Ref. 7).