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Asparagine surrogates for the assembly of *N*-linked glycopeptide mimetics by chemoselective ligation

Stéphane Peluso and Barbara Imperiali*

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

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Abstract—Alanine-β-hydroxylamine (Aβx) and alanine-β-hydrazide (Aβz) have been developed as asparagine surrogates for the assembly of *N*-linked glycopeptide mimetics by chemoselective ligation. The utility of these residues is illustrated with the synthesis of the oligosaccharyl transferase substrate mimetics **1** and **2**, and conjugation thereof with GlcNAc to obtain the *N*-glycopeptide mimetics **3** and **4**.

The synthesis of glycopeptide mimetics has recently received significant attention with the development of C-glycoside and S-glycoside analogs as well as chemoselective ligation techniques.[1] The latter is based on the use of saccharides and peptides bearing mutually and selectively reactive functionalities. This strategy is particularly attractive as it allows the convergent synthesis of neoglycopeptides without the need for protecting groups or activating agents. Recent examples include reaction of reducing sugars with peptides fea-

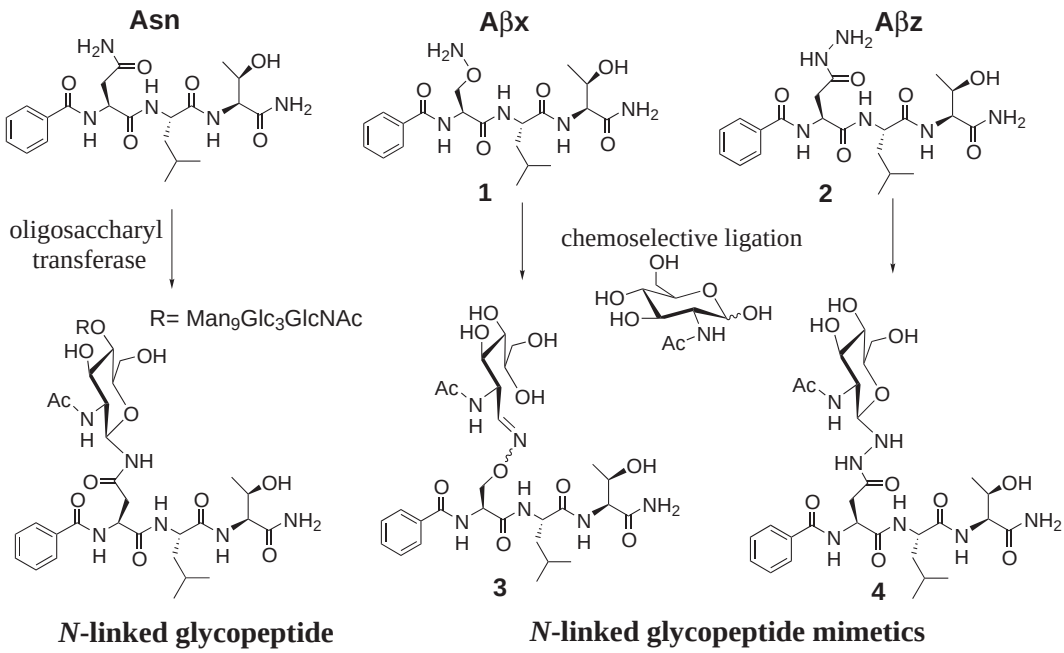

Figure 1. Alanine-β-hydroxylamine (Aβx) and alanine-β-hydrazide (Aβz) as asparagine surrogates for the assembly of *N*-linked glycopeptide mimetics by chemoselective ligation.

Keywords: glycopeptide mimetic; solid-phase synthesis; chemoselective ligation.

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

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turing an aminooxy group[2] and hydrazide- and thiosemicarbazide-functionalized saccharides with peptides featuring a carbonyl group.[3]

As part of an ongoing program focused on understanding enzyme catalyzed *N*-linked protein glycosylation,[4] we have developed alanine-β-hydroxylamine and alanine-β-hydrazide as asparagine surrogates for the assembly of *N*-glycopeptide mimetics by chemoselective ligation. This approach is illustrated with the synthesis of the oligosaccharyl transferase substrate mimetics **1** and **2**, and the *N*-linked glycopeptide mimetics **3** and **4** (Fig. 1).

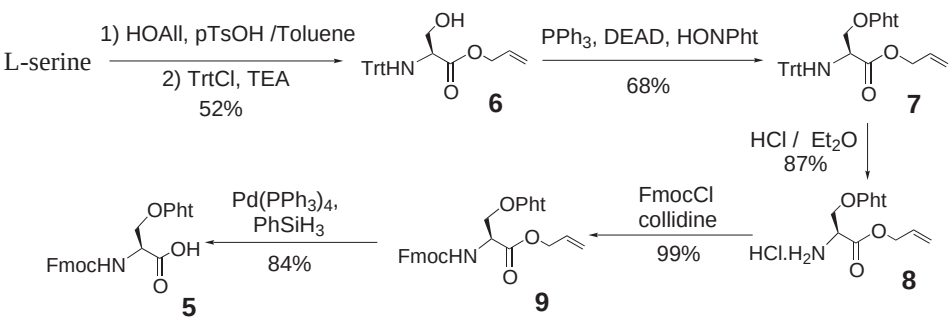
Alanine-β-hydroxylamine is related to the natural product canaline and to the antibiotics D- and L-cycloserine. The syntheses of canaline[5] and homocanaline[6] have been reported. Specifically, hydrolysis of L-cycloserine affords alanine-β-hydroxylamine[7] which can then be sequentially protected to access the FmocAβx-(Boc)OH[8] building block. However, this semi synthetic approach suffers from the price of commercially available L-cycloserine (Aldrich, $ 20/25 mg).

In order to generate more abundant quantities of a versatile building block for solid phase peptide synthesis (SPPS) we have chosen to target FmocAβx(Pht)OH. The Fmoc protecting group is compatible with the Fmoc/*t*Bu SPPS strategy. The phthalimide protecting group was selected over carbamate protecting groups because carbamate protected hydroxylamines have been shown to be vulnerable to alkylation or acylation at the remaining amide proton.[9] FmocAβx(Pht)OH **5** was synthesized in five steps from serine, in an overall yield of 25%. The key step of the synthetic strategy is a Mitsunobu reaction of *N*-hydroxyphthalimide with an *N*-α-trityl protected serine (Scheme 1). Serine was protected as the *N*-trityl and allyl ester in a single pot procedure to give **6** in 52% overall yield. The allyl ester was selected over a methyl ester since all attempts to remove the latter in Aβx(Pht)OMe led excusively to elimination side products. The use of the *N*-trityl protecting group has been shown to prevent elimination in Mitsunobu reactions of serine,[10] and **7** was obtained from **6** in 68% yield. Removal of the *N*-α-trityl protecting group was achieved by treating **7** with HCl/Et_2O. After trituration and washing with diethyl ether, pure **8** was isolated by centrifugation in 87% yield. Introduction of the *N*-α-Fmoc protecting group was performed in quantitative yield by using FmocCl and collidine in water/dioxane (1:1) to afford **9**. The weak base collidine was used to prevent elimination of the susceptible α-amino ester. Finally, the allyl ester was removed by using a catalytic amount of $Pd(PPh_3)_4$ and $PhSiH_3$, as scavenger, in DCM. The crude product was purified by flash chromatography followed by size exclusion chromatography to remove all traces of palladium impurities and acetic acid to obtain **5**.[11]

Use of **5** in SPPS was illustrated in the synthesis of **1** from H_2N-Leu-Thr(*t*Bu)-XAL-PS. Coupling of **5** was realized using PyAOP and collidine as a weak base in order to prevent elimination of the hydroxyphthalimide group. Fmoc deprotection was performed using piperidine/DMF (1:4) buffered with dinitrophenol followed by the N-terminal capping with benzoic anhydride and pyridine as base. At this stage, release from the solid support using TFA/DCM/H_2O/triisopropylsilane (90:5:2.5:2.5) afforded the phthalimide-protected peptide. Removal of the protecting group proceeded smoothly and quantitatively (HPLC analysis) using methylhydrazine in acetonitrile/water (4:1) to afford **1**.[12] Alternatively, the phthalimide protecting group can be removed on solid support using hydrazine:allyl alcohol:trifluoroethanol (1:3:46). Trifluoroethanol was essential; attempts with DMF, DCM or MeCN/H_2O as solvent gave inferior yields.

In contrast to the building block approach of Aβx, Aβz-containing peptide **2** was prepared directly on solid support by modification of the aspartic acid side chain of Bz-Asp(OAll)-Leu-Thr(*t*Bu)-XAL-PS. Upon palladium-catalyzed removal of the allylic ester using catalytic $Pd(PPh_3)_4$ and $PhSiH_3$, as scavenger, in DCM, the hydrazide functionality was obtained by coupling of *t*-butylcarbazate in the presence of PyAOP and collidine. Release from the solid support and deprotection were achieved using TFA/DCM/H_2O/triisopropylsilane (90:5:2.5:2.5) to afford **2**.[13]

Conjugation of **1** and **2** with GlcNAc was realized according to chemoselective ligation protocols (Fig. 1).[14] For example, a solution of GlcNAc 1 M in buffer (AcONa 0.1 M pH 5.6; 60 μl, 5 equiv.) was added to a solution of **1** (12 μmol) in DMSO (50 μl). After 24 h at room temperature, the solution was concentrated in vacuo. HPLC analysis showed the total conversion of **1** to afford neoglycoconjugate **3**.[15] Neoglycoconjugate **4**[15] was obtained from **2** following the same procedure. As



Scheme 1. Synthesis of FmocAβx(Pht)OH.

expected, ^{1}H NMR analysis of **3** in DMSO showed two sets of peaks arising from the *E*- and *Z*-acyclic glycosyl oxime isomers.[2a,16] Likewise, ^{1}H NMR analysis of **4** in DMSO showed two sets of peaks arising from the *cis* and *trans* β-glycosyl hydrazide isomers.[17]

In conclusion, we have developed two asparagine surrogates, alanine-β-hydroxylamine and alanine-β-hydrazide, for the assembly of *N*-glycopeptides mimetics by chemoselective ligation. These surrogates are currently being used for biochemical studies of oligosaccharyl transferase. The results of these studies will be reported elsewhere.

Acknowledgements

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11. Characterization of FmocAβx(Pht)OH **5**: $[\alpha]_D^{25}=+37.0$ ($c=1$, $CHCl_3$); mp = 88°C; $R_f=0.62$ (DCM:MeOH: AcOH, 90:10:1); ^{13}C NMR ($CDCl_3$) δ (ppm): 47.21, 53.38, 67.90, 77.88, 120.15, 124.13, 125.56, 127.34, 127.92, 128.83, 135.11, 141.45, 143.95, 156.52, 163.78, 172.45; ^{1}H NMR (CDCl3) δ (ppm): 7.86 (2H, dd, $J=3.0$ Hz, $J=5.5$ Hz), 7.77 (4H, m), 7.67 (2H, m), 7.41 (2H, t, $J=7.3$ Hz), 7.33 (2H, t, $J=7.3$ Hz), 6.48 (1H, d, $J=8.2$ Hz), 4.86 (1H, dd, $J=3.0$ Hz, $J=11.0$ Hz), 4.68 (1H, m), 4.44–4.35 (3H, m), 4.28 (1H, m).
12. Characterization of **1**: $C_{20}H_{31}N_5O_6$ ($M_w=437.52$), HPLC: $t_R=16.52$ min (C_{18} 7–100% B in 28 min), ESMS: $[M+H]^+=438.2$; ^{1}H NMR (DMSO-d_6) δ (ppm): 8.70 (1H, d, $J=7.6$ Hz), 8.31 (1H, d, $J=7.9$ Hz), 7.87 (2H, m), 7.62 (1H, d, $J=8.8$ Hz), 7.55 (1H, m), 7.49 (2H, m), 7.14 (1H, s), 7.13 (1H, s), 4.82 (1H, td, $J=7.6$ Hz, $J=5.2$ Hz), 4.36 (1H, dd, $J=7.9$ Hz, $J=14.9$ Hz), 4.16 (br, 1H), 4.10 (2H, m), 4.09 (1H, m), 1.62 (1H, m), 1.52 (2H, m), 1.00 (3H, d, $J=6.4$ Hz), 0.87 (3H, d, $J=6.7$ Hz), 0.82 (3H, d, $J=6.7$ Hz).
13. Characterization of **2**: $C_{21}H_{32}N_6O_6$ ($M_w=464.54$); HPLC: $t_R=16.70$ min (7–100% B in 28 min); ESMS: $[M+H]^+=465.21$; ^{1}H NMR (DMSO-d_6) δ (ppm): 10.29 (1H, br s), 8.71 (1H, d, $J=7.9$ Hz), 8.17 (1H, d, $J=8.2$ Hz), 7.85 (2H, m), 7.62 (1H, d, $J=8.8$ Hz), 7.55 (1H, m), 7.48 (2H, m), 7.13 (1H, s), 7.11 (1H, s), 4.87 (1H, td, $J=5.8$ Hz, $J=8.1$ Hz), 4.32 (1H, td, $J=5.9$ Hz, $J=8.4$ Hz), 4.08 (1H, dd, $J=3.7$ Hz, $J=8.8$ Hz), 4.01 (1H, m), 2.80 (1H, dd, $J=5.8$ Hz, $J=15.2$ Hz), 2.64 (1H, dd, $J=8.5$ Hz, $J=15.2$ Hz), 1.60 (1H, m), 1.50 (2H, m), 1.01 (3H, d, $J=6.1$ Hz), 0.85 (3H, d, $J=6.5$ Hz), 0.81 (3H, d, $J=6.5$ Hz).
14. Chemoselective ligation through oxime and hydrazone bond formation is commonly used in peptide and protein synthesis and has been found to be highly selective in the presence of competing amino acid side chain functionality. For an example see: Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893–3899.
15. Characterization of **3**: $C_{28}H_{44}N_6O_{11}$ ($M_w=640.72$); HPLC: $t_R=16.22$ min (7–100% B in 28 min); ESMS: $[M+H]^+=641.26$. Characterization of **4**: $C_{29}H_{45}N_7O_{11}$ ($M_w=667.54$); HPLC: $t_R=16.40$ min (7–100% B in 28 min); ESMS: $[M+H]^+=668.30$.
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