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Deacetylation of N^{α}-methylated glycopeptides reveals that aza-enolates provide protection against β-elimination of carbohydrates *O*-linked to serine

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Abstract

Three glycopeptides Ac-Ala-Ser[β-Gal(OAc)$_4$]-Phe-NH$_2$, Ac-Ala-N^{α}-Me-Ser[β-Gal(OAc)$_4$]-Phe-NH$_2$ and Ac-Ala-Ser[β-Gal(OAc)$_4$]-N^{α}-Me-Phe-NH$_2$ have been prepared and treated with base in order to remove the *O*-acetyl protective groups. The glycopeptide which carried the *N*-methyl group on the glycosylated serine, was substantially more susceptible to β-elimination than the two others. This reveals that formation of an aza-enolate from the amide bond to a glycosylated serine provides protection against β-elimination under basic conditions.

Removal of *O*-acyl protective groups from the carbohydrate moiety is often the final step in synthesis of a glycopeptide. This is usually achieved using a moderately strong base such as sodium methoxide, ammonia[1] or hydrazine hydrate[2] in methanolic solutions. Since the α-protons of the amino acid residues are acidic, treatment with base is accompanied by risks of epimerization of α-stereocenters, or β-elimination if the carbohydrate is attached to serine or threonine.[1,3–10] Removal of acetyl groups is usually facile, but the increased concentration of base required for removal of *O*-benzoyl groups may cause β-elimination and slight epimerization.[1,7–10] It has been suggested that formation of aza-enolates by removal of the acidic amide protons protects the glycopeptide from these side-reactions.[9,11] To test this hypothesis we have synthesized three glycotripeptides, two of which are N^{α}-methylated either on the galactosylated serine or on the C-terminal phenylalanine, in order to prevent aza-enolate formation at these positions. The rate of β-elimination upon de-*O*-acetylation of the different glycopeptides was then investigated.

Synthesis of the three glycopeptides required preparation of building blocks **3** and **7** in which a tetra-*O*-acetylated galactose moiety is β-linked to serine and *N*-methylated serine, respectively (Scheme 1). Building block **3** was synthesized in 55% yield from pentaacetate **1** and Fmoc-Ser-OPfp (**2**), using boron trifluoride etherate as promoter.[12] *N*-Methylated serine **4** was Fmoc-protected

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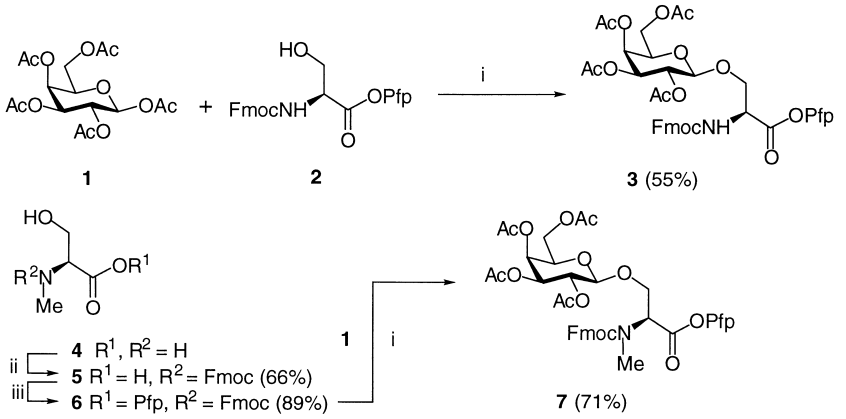
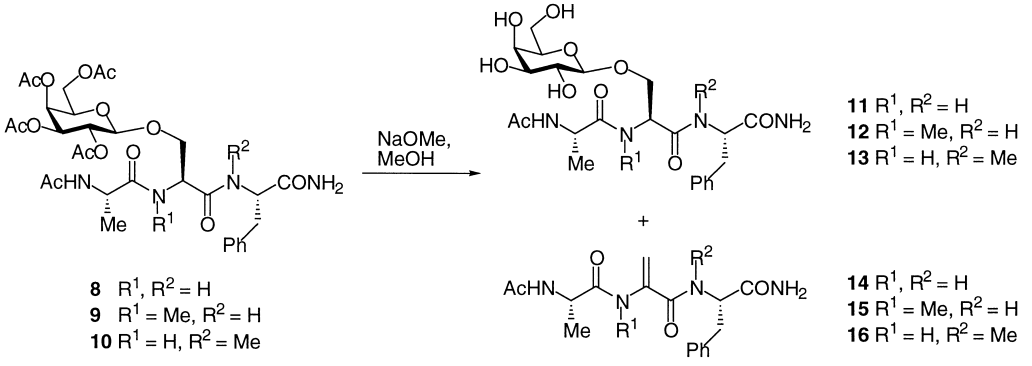
and then converted into the pentafluorophenyl (Pfp)-ester giving derivative **6**. This was galactosylated using the same conditions as for **3** to give the building block **7**[13] in 71% yield. The higher yield in the glycosylation of *N*-methylated serine **6**, as compared to **2**, is in agreement with previous observations made by Polt and co-workers.[14]



Scheme 1. Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt; (ii) Fmoc-Suc, Et_3N, H_2O:MeCN (1:2), rt; (iii) PfpOH, DIC, EtOAc, 0°C→rt

Building blocks **3** and **7** were then used for assembly of glycopeptides **8**–**10**[15] on a TentaGel S-NH_2^{TM} resin functionalized with the Rink linker.[16,17] The three glycopeptides were cleaved from the resin with TFA:water (95:5) and then purified by flash chromatography and reversed-phase HPLC-chromatography. Treatment of the glycopeptides with 10 mM methanolic sodium methoxide resulted in rapid (<30 min) removal of the *O*-acetyl groups to give **11**–**13** (Scheme 2).[18] As revealed by reversed-phase HPLC prolonged exposure to base (0.5–5 h) led to β-elimination of **11**–**13**, giving **14**–**16**[19] at different rates. In order that to avoid differences in the experimental conditions affected the rates of β-elimination, the non-*N*-methylated **8** was used as an internal control which, in two separate experiments, was treated with sodium methoxide in the same flask as **9** and **10**. After deacetylation of **8** and **10** the resulting **11** and **13** both underwent β-elimination slowly and at equal rates. This established that formation of an aza-enolate at the



Scheme 2.

serine-phenylalanine amide bond did not provide any significant protection against β-elimination. In contrast, glycopeptide **12**, in which the glycosylated serine is *N*-methylated, underwent β-elimination much more rapidly than **11**. As shown in Fig. 1, only a small amount of non-methylated **11** had been converted to dehydroalanine **14** when ~50% of **12** had been transformed to **15**. In fact, it was not possible to prepare *N*-methylated **12** from **9** without formation of **15** by β-elimination. This is in contrast to glycopeptides **8** and **10**, which could be deacetylated without any detectable β-elimination. Crude **12** could, however, be purified by reversed-phase HPLC, and NMR spectroscopy of the resulting pure **12** did not reveal any signs of epimerization of the α-stereocenters.

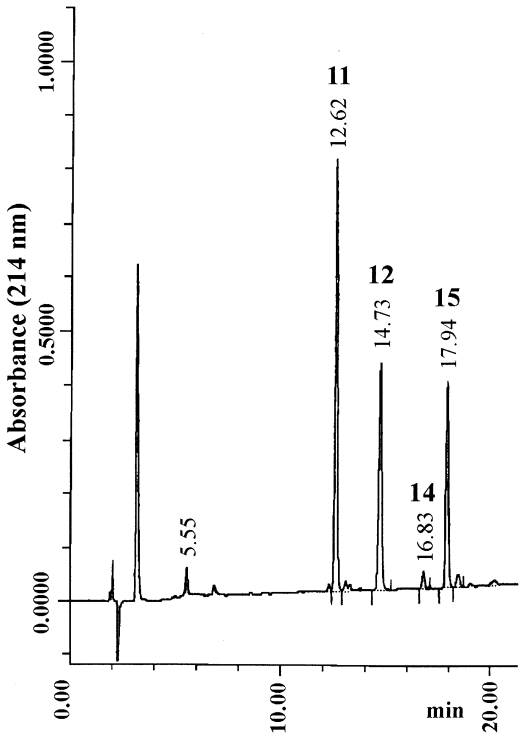


Figure 1. HPLC-chromatogram of the simultaneous de-*O*-acetylation of glycopeptides **8** and **9** which leads to formation of **11** and **12**, as well as their respective β-eliminated products **14** and **15** (conditions: Kromasil C-8 column. Linear gradient of 0–80% B in A over 60 min. A = 0.1% aqueous TFA, B = 0.1% TFA in MeCN. Flowrate 1.5 ml/min)

The possibility that *N*-methylation of the central serine residue increased the rate of β-elimination of **12** by influencing the conformation around the serine C_α–C_β bond was ruled out by inspection of the coupling constants between the H-α and H-β protons. Glycopeptide **12** populates two conformations for the alanine-serine amide bond. One rotamer displayed coupling constants of 6.8 Hz between the Serα- and the Serβ-protons, thus indicating free rotation around the C_α–C_β bond just as for non-methylated **11**. For the other rotamer the values of the $^3J_{\alpha,\beta}$ coupling constants were found to be 4.8 and 9.4 Hz, respectively. This reveals a predominant population of a conformation in which Ser *O*β and *H*α adopt a *gauche* orientation, i.e. one of the two conformations which do not allow β-elimination to occur.

In conclusion, we have shown that N^{α}-methylation of a glycosylated serine results in a substantial increase in the rate of β-elimination on base-catalyzed removal of *O*-acyl protective groups from glycopeptides. We interpret this result as being due to the fact that a protective aza-enolate cannot be formed adjacent to the carbohydrate moiety. Since β-elimination is often encountered on removal of benzoyl protective groups from *O*-linked glycopeptides,[1,7–10] and cannot be avoided if the carbohydrate is linked to a *N*-methylated serine, novel protective groups should find use in the synthesis of glycopeptides. Investigations of such protective groups are now in progress in our laboratory.

Acknowledgements

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13. Compound **7**: ^{1}H NMR (400 MHz, $CDCl_3$), rotamers (~2:1), δ (ppm): 5.37–5.42 (m, 2H, H-4,4′), 5.21 (dd, 1H, J = 7.9, 10.5 Hz, H-2′), 5.14 (dd, 1H, J = 8.0, 10.5 Hz, H-2), 4.58 (d, 1H, J = 7.9 Hz, H-1), 4.27 (d, 1H, J = 8.0 Hz, H-1′), 3.05 (s, 1H, Me), 2.99 (s, 1H, Me′); FABMS: $(M+Na)^+$ 860 calcd, 860 obsd.
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15. Glycopeptide **8**: yield 97%; ^{1}H NMR (400 MHz, Acetone-d_6), δ (ppm): Ala 4.21 (m, 1H, H-α), 1.32 (d, 3H, J = 7.2 Hz, Me); Ser 4.36 (m, 1H, H-α), 3.84 (dd, 1H, J = 7.3, 10.7 Hz, H-β), 3.76 (dd, 1H, J = 4.8, 10.7 Hz, H-β′); Phe 4.51 (m, 1H, H-α), 3.26 (dd, 1H, J = 4.4, 14.0 Hz, H-β), 2.99 (dd, 1H, J = 9.8, 13.9 Hz, H-β′); Gal 4.74 (d, 1H, J = 7.6 Hz, H-1); FABMS: $(M+H)^+$ 695 calcd, 695 obsd. Glycopeptide **9**: yield 86%; ^{1}H NMR (400 MHz, Acetone-d_6), major rotamer, δ (ppm): Ala 4.82 (m, 1H, H-α), 1.31 (d, 3H, J = 6.9 Hz, Me); Ser 5.16 (dd, 1H, J = 4.7, 9.4 Hz, H-α), 3.9–4.0 (m, 2H, H-β,β′); Phe 4.54 (ddd, 1H, J = 3.8, 8.7, 11.2 Hz, H-α), 3.28 (dd, 1H, J = 3.7, 14.0 Hz, H-β), 2.92 (dd, 1H, J = 11.3, 13.9 Hz, H-β′); Gal 4.75 (d, 1H, J = 7.9 Hz, H-1); minor rotamer, δ (ppm): Ala 1.19 (d, 3H, J = 6.8 Hz, Me); Phe 4.62 (m, 1H, H-α), 3.20 (dd, 1H, J = 5.1, 14.1 Hz, H-β), 2.95 (m, 1H, H-β′); Gal 4.72 (d, 1H, J = 7.6 Hz, H-1); FABMS: $(M+H)^+$ 709 calcd, 709 obsd. Glycopeptide **10**: yield 39%; ^{1}H NMR (400 MHz, Acetone-d_6), rotamers (~1:1), δ (ppm): Ala 4.38 (t, 1H, J = 7.2 Hz, H-α), 4.31 (t, 1H, J = 7.2 Hz, H-α); Ser 4.5–4.6 (m, 1H, H-α); Phe 5.20 (dd, 1H, J = 6.5, 9.0 Hz, H-α); Gal 4.80 (d, 1H, J = 7.9 Hz, H-1), 4.42 (d, 1H, J = 7.9 Hz, H-1); FABMS: $(M+H)^+$ 709 calcd, 709 obsd.
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18. Glycopeptide **11**: ^{1}H NMR (400 MHz, MeOH-d_4), δ (ppm): Ala 4.25–4.35 (m, 1H, H-α), 1.28 (d, 3H, J = 7.2 Hz, Me); Ser 4.54 (t, 1H, J = 5.4 Hz, H-α), 4.09 (dd, 1H, J = 5.1, 10.4 Hz, H-β), 3.7–3.8 (m, 1H, H-β); Phe 4.57 (m, 1H, H-α), 3.21 (dd, 1H, J = 4.9, 14.1 Hz, H-β), 2.97 (dd, 1H, J = 9.1, 14.0 Hz, H-β); Gal 4.27 (d, 1H, J = 7.2 Hz, H-1); FABMS: $(M+H)^+$ 527 calcd, 527 obsd. Glycopeptide **12**: ^{1}H NMR (400 MHz, MeOH-d_4), rotamers (∼1:1), δ (ppm): Ala 4.8–4.9 (m, 1H, H-α), 4.70 (q, 1H, J = 7.0 Hz, H-α), 1.36 (d, 3H, J = 7.2 Hz, Me), 1.23 (d, 3H, J = 7.0 Hz, Me); Ser 5.23 (dd, 1H, J = 4.8, 9.3 Hz, H-α), 5.14 (t, 1H, J = 6.7 Hz, H-α), 3.9–4.1 (m, 4H, H-β); Phe 4.50–4.65 (m, 2H, H-α), 3.2–3.3 (m, 2H, H-β), 2.9–3.0 (m, 2H, H-β); Gal 4.2–4.3 (m, 2H, H-1); FABMS: $(M+Na)^+$ 563 calcd, 563 obsd. Glycopeptide **13**: ^{1}H NMR (400 MHz, MeOH-d_4), rotamers (∼3:2), δ (ppm): Ala 4.34 (q, 1H, J = 7.1 Hz, H-α), 4.26 (q, 1H, J = 7.2 Hz, H-α), 1.29 (d, 3H, J = 7.2 Hz, Me), 1.22 (d, 3H, J = 7.2 Hz, Me); Ser 5.04 (t, 1H, J = 6.3 Hz, H-α), 4.63 (dd, 1H, J = 4.8, 9.3 Hz, H-α), 3.98 (dd, 1H, J = 5.9, 10.1 Hz, H-β), 3.75–3.85 (m, 1H, H-β), 3.3–3.4 (m, 1H, H-β), 2.30 (dd, 1H, J = 4.0, 10.2 Hz, H-β); Phe 5.25 (dd, 1H, J = 5.6, 10.3 Hz, H-α), 5.06 (dd, 1H, J = 4.0, 10.0 Hz, H-α), 3.25–3.40 (m, 2H, H-β), 2.95–3.05 (m, 2H, H-β); Gal 4.24 (d, 1H, J = 7.5 Hz, H-1), 3.92 (d, 1H, J = 7.3 Hz, H-1); FABMS: $(M+Na)^+$ 563 calcd, 563 obsd.
19. Peptide **14**: ^{1}H NMR (400 MHz, MeOH-d_4), δ (ppm): Ala 4.30 (q, 1H, J = 7.2 Hz, H-α), 1.34 (d, 3H, J = 7.2 Hz, Me); ΔApa 5.76 (s, 1H, H-β), 5.40 (s, 1H, H-α); Phe 4.63 (m, 1H, H-β), 3.27 (dd, 1H, J = 5.0, 14.0 Hz, H-β), 2.98 (dd, 1H, J = 10.0, 14.0 Hz, H-β); FABMS: $(M+H)^+$ 347 calcd, 347 obsd. Peptide **15**: ^{1}H NMR (400 MHz, MeOH-d_4), δ (ppm): Ala 4.41 (m, 1H, H-α), 1.25 (d, 3H, Me); ΔApa 6.19 (s, 1H, H-β), 5.72 (s, 1H, H-β); Phe 4.61 (m, 1H, H-α), 3.3–3.4 (m, 1H, H-β), 3.05 (br t, 1H, J = 12.4 Hz, H-β).