Impact of glycoclustering on the structure of mucin peptides

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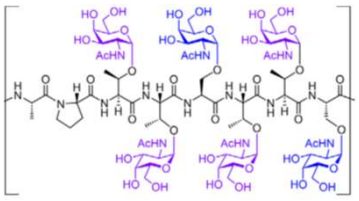
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https://doi.org/10.17952/38EPS.2026.P422

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Introduction

Glycosylation is thought to play a key role in rigidifying the peptide backbone of secreted mucins such as MUC5AC. However, its is not known how many of the potential O-glycosylation sites need to be occupied in order to impart rigid rod character. In MUC5AC, each of the Ser/Thr residues of the PTTSTTSX consensus octad (X: Ala, Thr) can carry α-O-GalNAc or larger glycans. Challenges in the synthesis of glyco-clustered peptides made it difficult to elucidate the impact of O-GalNAcylation on the length and flexibility of peptides. Here, we report glyco-economic syntheses of peptides featuring a previously unattained degree of glycoclustering. Circular dichroism (CD) spectroscopy and Electron Spin Resonance (EPR) and gas phase FRET measurement revealed that peptide stiffening depends on GalNAc clustering. Interestingly, GalNAcylation induces compaction upon desolvation.

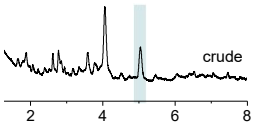


Fully GalNAcylated tandem repeat of MUC5AC

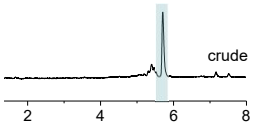
Replacing DMF by DOL + 1% Tween-20 improves efficiency of SPPS of MUC5AC tandem repeats

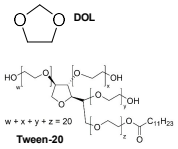
Ac-C-(APTTSTTS)$_5$-C-NH$_2$

20% Piperidine in **DMF**
5 eq AA/DIC/Oxyma, **DMF**, 75°C, 5 min
Ac_2O/DIPEA, **DMF**



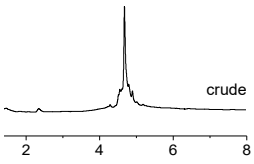
20% Pyrrolidine in **1% Tween-20/DOL**
5 eq AA/ DIC/Oxyma, **1% Tween-20/DOL**, 20 °C, 5 min
Ac_2O/DIPEA, **DOL**

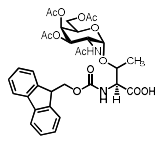




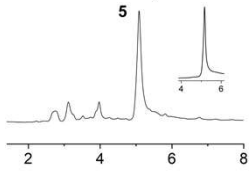
H_2N-CO-**PTTSTTS-(APTTSTTS)$_2$-NH$_2$**
HSe

Coupling glyco AA: 60 mM (Fmoc-Ser/Thr(Ac_3GalNAc) + Oxyma), 430 mM DIC in **1% Tween-20/DOL**, 10 min





Ac-C-(APTTSTTS)$_2$-N_2H_3



Ligation techniques to obtain long mucin glycopeptides

✓ C-terminal glycoserine does not interfere with neither ligation nor dechalcogenation methods

Native Chemical Ligation with C-terminal glycoamino acid[1]

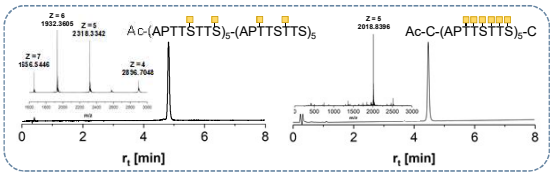
Ac-(APTTSTTS)$_5$-NH-NH$_2$ → 1. MPAA, acac, Gd-HCl buffer; 2. MPAA extraction with EtOAc, then $NaBEt_4$/TCEP → CPTTSTTS-(APTTSTTS)$_4$

Ac-C-(APTTSTTS)$_5$-(APTTSTTS)$_5$
with 80 aa, 20 GalNAc, yield 17%

Diselenide-Selenoester Ligation with C-terminal glycoamino acid

Ac-C-(APTTSTTS)$_2$-NH-NH$_2$ → 1. Ph_2Se_2, TCEP, acac, buffer A, pH 3, 37°C; 2. Buffer A, pH 7, RT; 3. Ph_2Se_2 extraction with hexane, then TCEP/DTT → (UPTTSTTS-(APTTSTTS)$_2$-C)$_2$

Ac-C-(APTTSTTS)$_5$-C
with 42 aa, 30 GalNAc, yield 22%

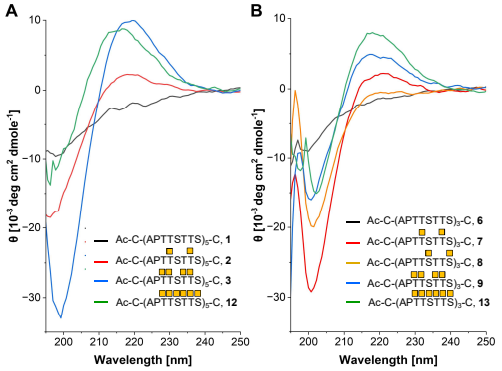


Conformational effect of clustered GalNAcylation – CD

GalNAcylation of two out of six Ser/Thr residues sufficient to induce PPII helix
→ Compare **2** with **1** or **7** with **6**

GalNAcylation of Thr more potent in inducing PPII helix than GalNAcylation of Ser
→ Compare **7** with **8**

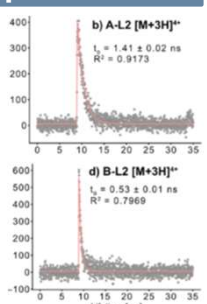
PPII conformation progressively stabilized with increasing degree of GalNAcylation



CD spectra of peptides containing A) five tandem repeats (**1**-**3**,**12**) or B) three tandem repeats (**6**-**9**, **13**). Conditions: 50 µM peptide, 150 mM NaCl, 25 mM Tris-HCl, pH 6.9. Spectra are corrected with calibration to pure GalNAc.

Compaction upon desolvation – Gas-phase FRET

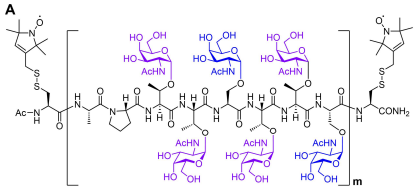
	FRET efficiency	
	gas phase ([M+3H]$^{3+}$)	solution phase H_2O, 50 mM NH_4OAc
CR6G QSY7 Ac-K-(APTTSTTS)$_3$-C, **A-L2**	77.5 %	40.3 %
CR6G QSY7 Ac-K-(APTTSTTS)$_3$-C, **B-L2**	92.4 %	21.3 %
CR6G QSY7 Ac-K-(APTTSTTS)$_3$-C, **C-L2**	93.1 %	2.7 %



Gas-phase fluorescence decay curves in the charge state [M + 3H]$^{3+}$ and [M + 3H]$^{4+}$. Gray circles represent single data points and the fit through these data points is represented by a red curve. The fluorescence lifetime is obtained through the fit. The error is the statistical error of the fit.

Stiffening effect of clustered GalNAcylation – PELDOR

A) Representative example of MTSL-labelled, multi-GalNAcylated MUC5AC tandem repeats. B-K) Primary 4-pulse and/or 5-pulse PELDOR data (left) and the corresponding distance distributions (right) determined using the DeerLab program. The error bounds indicate a 95% confidence interval. Conditions: 15 - 25 µM peptide, 150 mM NaCl, 25 mM Tris-HCl + 15% deuterated glycerol (v/v).



Ac-C*-(APTTSTTS)$_n$-C*, n=5 (**1'**), 3 (**6'**)

→ non-glycosylated peptides sample large conformational space

Ac-C*-(APTTSTTS)$_n$-C*, n=5 (**2'**), 3 (**7'**)

→ shift towards longer distances with two GalNAcs on Thr, particularly for longer peptide
→ broad distribution indicates backbone flexibility

Ac-C*-(APTTSTTS)$_n$-C*, n=3 (**8'**)

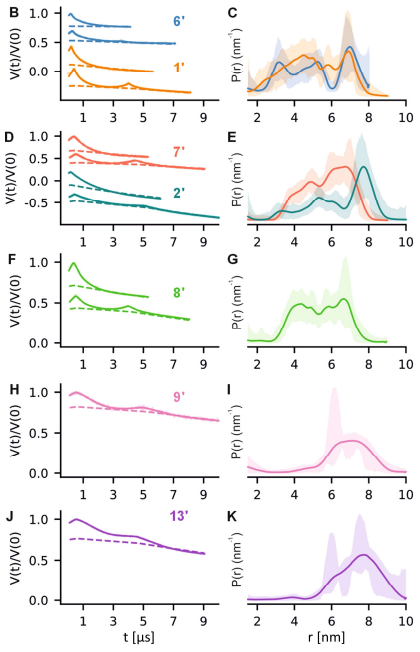
→ broad distance distribution with two GalNAcs on Ser

Ac-C*-(APTTSTTS)$_n$-C*, n=3 (**9'**)

→ significant stiffening of peptide backbone with four GalNAcs on Thr

Ac-C*-(APTTSTTS)$_n$-C*, n=3 (**13'**)

→ significant stiffening of peptide backbone at 100 % occupancy of glycosylation sites
→ technical limitations prevent accurate measurement of end-to-end distance



Conclusions

- ✓ 1% Tween-20 in DOL is an excellent solvent for SPPS of GalNAcylated MUC5AC tandem repeats.
- ✓ NCL proceeds smoothly when C-terminl amino acid is GalNAcylated.
- ✓ Two GalNAcs on Thr (but not Ser) induce PPII helix conformation, but this is not sufficient for stiffening.
- ✓ Four GalNAcs on Thr (50% of all amino acids glycosylated) required for stiffening of peptide backbone.
- ✓ GalNAcylation induces compaction after desolvation (perhaps due to intramolecular interactions between glycans), in contrast to stiffening in solution.

References

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Acknowledgements

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project ID 431232613 – SFB 1449.

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