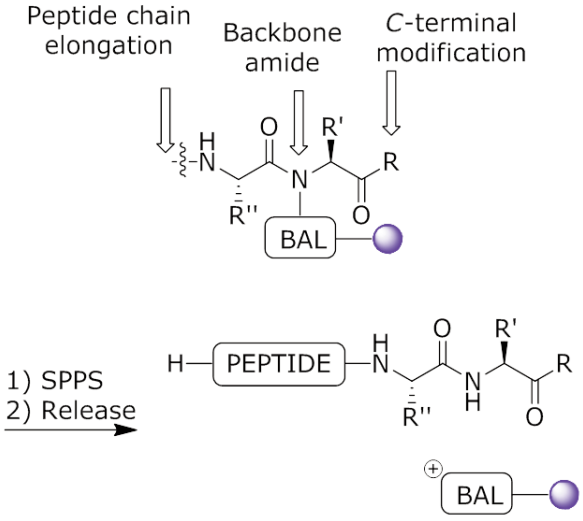
Synthesis of a Glycopeptide Aldehyde Using the *o*-BAL Strategy on the Initiator+ SP *Wave*

Application Note

Introduction

C-terminal peptide aldehydes are key components in oxime and hydrazine ligations and their synthesis is therefore very desirable. A well established method for the synthesis of *C*-terminal modified peptides including peptide aldehydes, is the backbone amide linker (BAL) strategy (Scheme 1).[1] In this strategy, the first amino acid is anchored by reductive amination followed by acylation of the newly formed secondary amine. Thus, the growing peptide chain is anchored *not* through the *C*-terminal carboxyl but through a backbone amide nitrogen giving access to, in principle, any *C*-terminal modification.[1]



Scheme 1: *Schematic outline of SPPS using the BAL strategy* [1]

The glycosylated peptide aldehyde Fmoc-L-Ser(α-D-GalNAc(Ac)$_3$)-Glu-Gly-Gly-H **(1)** was synthesized on a Biotage Initiator+ SP *Wave* microwave peptide synthesizer using the backbone amide linker (BAL) strategy.

Fmoc-L-Ser(α-D-GalNAc(Ac)$_3$)-Glu-Gly-Gly-H (1)

This sequence has previously been synthesized on a 'custom made' semi-automated system with success.[2]

Experimental

Materials

All materials were obtained from commercial suppliers; Sigma-Aldrich (acetonitrile, formic acid, diethyl ether, aminoacetaldehyde dimethyl acetal and sodium cyanoborohydride), Merck KGaA (1,2-dichloroethane (DCE) and acetic acid), Bachem (*N*-Ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide · HCl (EDC)), Iris Biotech GmbH (Fmoc-amino acids, Fmoc-L-Ser(α-D-GalNAc(Ac)$_3$-OH, DMF, NMP, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), trifluoroacetic acid (TFA), piperidine and *N,N*-diisopropylethylamine (DIEA), Peptides International Inc (5-(2-formyl-3,5-dimethoxyphenoxy)pentanoic acid (*o*-PALdehyde or *o*-BAL linker)) and Rapp Polymere GmbH (TentaGel S Rink Amide resin). Milli-Q (Millipore) water was used for LC-MS analysis.

Synthesis and Analysis

Anchoring of the linker (scale 100 µmol, 10 mL reactor vial): Tentagel S RAM resin (loading 0.28 mmol/g) was washed with NMP (5 mL). 5-(2-Formyl-3,5-dimethoxyphenoxy)pentanoic acid (*o*-PALdehyde) (2 eq.), HBTU (1.9 eq.), HOBt (2 eq.) and DIEA (3.9 eq.) in DMF (4 mL) were added and reacted for 10 min at 75 °C using microwave irradiation. The resin was washed with NMP (3 x 4 mL) and the anchoring step was repeated.

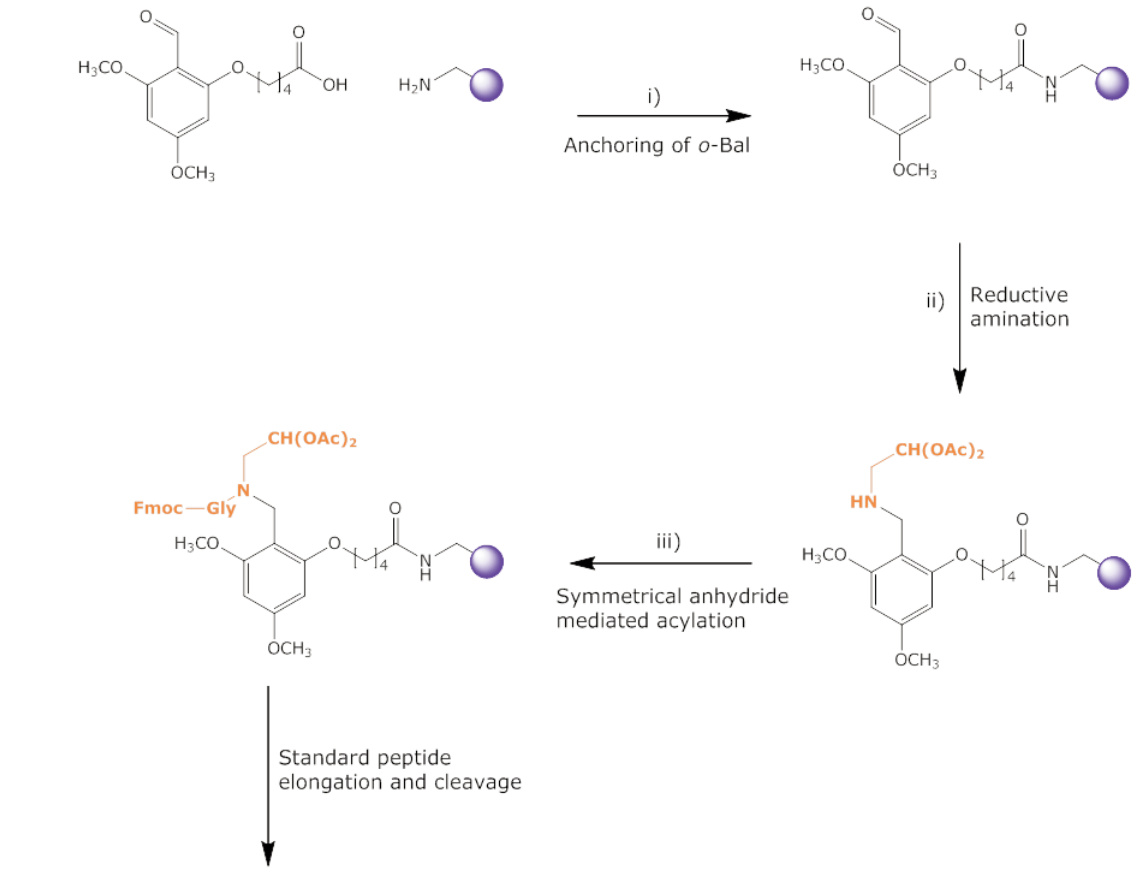
Reductive amination: Aminoacetaldehyde dimethyl acetal 99% (10 eq.) and $NaBH_3CN$ (10 eq.) were suspended in DMF-AcOH (99:1, 4 mL) and added to the *o*-BAL-resin and reacted for 10 min at 60 °C (microwave heating). The resin was washed with NMP (3 x 4 mL) and the reductive amination step was repeated.

Symmetrical anhydride: Fmoc-Gly-OH (10 eq.) and EDC (5 eq.) were suspended in DCE-DMF (10:1, 5 mL) and pre-activated for 10 min. The activated amino acid was then added to the resin and reacted for 10 min at 60 °C (microwave heating). The resin was washed with NMP (3 x 4 mL) and the step was repeated.

Standard peptide elongation (scale 25 µmol, 2 mL reactor vial): N^{α}-Fmoc deprotection was performed at room temperature (RT) in two stages by treating the resin with 40% piperidine/NMP (0.75 mL) for 3 min followed by 20% piperidine/NMP (0.75 mL) for 10 min. The resin was washed with NMP (4 x 0.75 mL). Fmoc-Glu(OtBu)-OH (4 eq.), HBTU (3.8 eq.), HOBt (4 eq.) and DIEA (7.8 eq.) in DMF (1 mL) were added to the resin and reacted for 10 min at 75 °C (microwave heating). The resin was washed with NMP (4 x 0.75 mL) and treated with 40% piperidine/NMP (0.75 mL) for 3 min followed by 20% piperidine/NMP (0.75 mL) for 10 min. The resin was then washed with NMP (4 x 0.75 mL). Fmoc-L-Ser(α-D-GalNAc$(Ac)_3$-OH (2 eq.), HBTU (1.9 eq.), HOBt (2 eq.) and DIEA (3.9 eq.) in DMF (1 mL) were added to the resin and reacted for 15 min at 75 °C (microwave heating). A part of the resin was treated with TFA-H_2O (19:1) for 2 hours at RT, filtered, precipitated with diethyl ether and the crude peptide was dissolved in acetonitrile-H_2O (1:1). Analytical HPLC was performed on a Dionex UltiMate 3000 with Chromeleon 6.80SP3 software. The peptide was analyzed on a Phenomenex Gemini 110 Å C18 column (3 µm, 4.6 × 50 mm) with a flow rate of 1.0 mL/min. The following solvent system was used: solvent A, water containing 0.1% formic acid; solvent B, acetonitrile containing 0.1% formic acid. The column was eluted using a linear gradient with increasing amount of buffer B over 12 min. Identification was carried out by ESI-MS (MSQ Plus Mass Spectrometer, Thermo).

Results and Discussion

The BAL strategy involves a number of difficult operations (Scheme 2) *(i)* coupling of the o-PALdehyde linker, *(ii)* a reductive amination for attachment of the first residue and *(iii)* a symmetrical anhydride mediated acylation for attachment of the second residue and then elongation by standard SPPS. The *C*-terminal is masked as an acetal during solid-phase synthesis and upon TFA treatment is released as an aldehyde. These steps have been thoroughly optimized[3,4] and by using microwave irradiation the reaction times have been decreased for all three steps. We have described the synthesis of a glycopeptide aldehyde on the Initiator+ SP *Wave* microwave peptide synthesizer. The semi-automated mode of operation, where the Fmoc deprotection and washing steps are automated with manual addition of other reagents is ideally suited for this type of synthesis. Vortex mixing of reagents in the microwave cavity ensured homogeneous heat distribution.



Scheme 2: ***i)*** *HBTU, HOBt, DIEA, o-PALdehyde in DMF, 2 x 10 min 75 °C,* ***ii)*** *$NaBH_3CN$, $NH_2CH_2CH(OCH_3)_2$ in DMF-AcOH (99:1), 2 x 10 min 60°C,* ***iii)*** *Fmoc-Gly-OH, EDC in DCE-DMF (9:1), 2 x 10 min 60 °C*

The glycopeptide aldehyde Fmoc-S(α-D-GalNAc(Ac)$_3$)-EGG-H (**1**) was synthesized successfully using microwave irradiation with a crude purity of 73% *(Figure 1*) and confirmed by ESI-MS (*Figure 2*), calculated average isotopic composition for $C_{41}H_{47}N_4O_{18}$, 883.83 Da. Found: m/z 884.50 $[M+H]^+$.

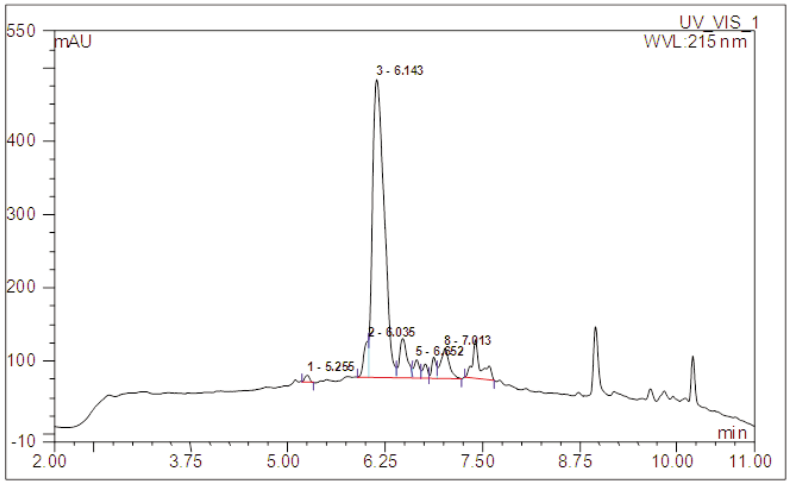


Figure 1: *RP-HPLC chromatogram of Fmoc-S(α-D-GalNAc(Ac)$_3$)-EGG-H (1)*

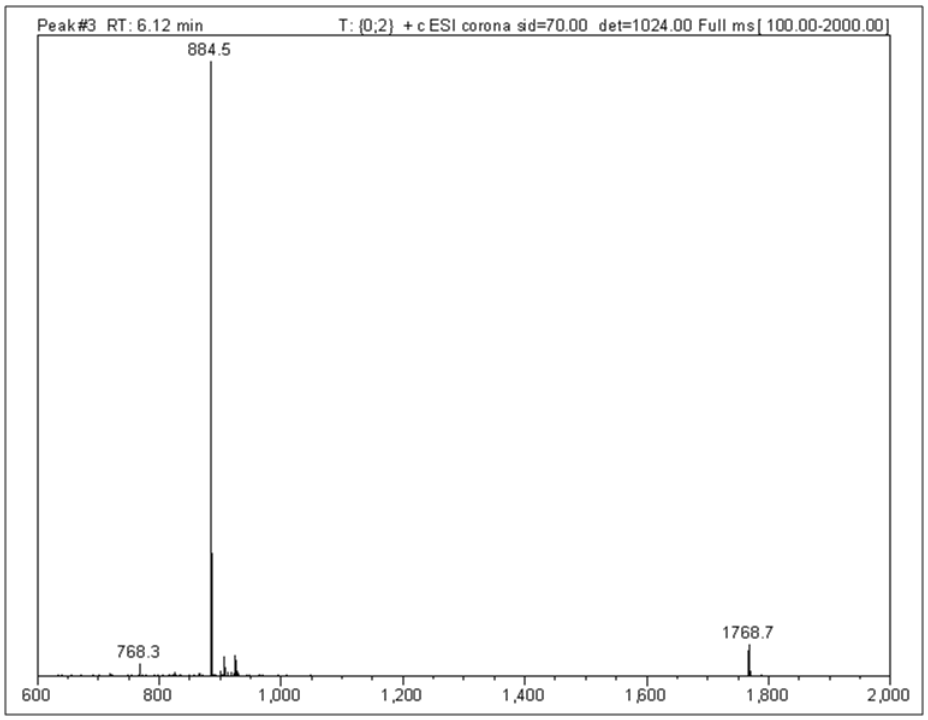


Figure 2: *ESI-MS of Fmoc-S(α-D-GalNAc(Ac)$_3$)-EGG-H* ***(1)***

Conclusion

The glycopeptide aldehyde Fmoc-L-Ser(α-D-GalNAc(Ac)$_3$)-Glu-Gly-Gly-H **(1)** was successfully synthesized on a Biotage Initiator+ SP *Wave* microwave peptide synthesizer using the backbone amide linker (BAL) strategy. The use of the small 2 mL reactor vial allowed the incorporation of the glycosylated amino acid on a small scale without wasting expensive reagents. The semi-automated mode of operation of the synthesizer, where the Fmoc deprotection and washing steps are automated with manual addition of other reagents, enabled total control of the synthesis. We have demonstrated the advantages of using this semi-automated synthesizer as a flexible platform and powerful development tool for the synthesis of non-standard peptides and peptidomimetics where valuable building blocks or precursors are to be used.

References

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