Synthesis of an HIV-targeted stapled peptide: Fully automated on-resin peptide cyclization via olefin metathesis

James Cain¹, Daniel Martinez¹, Andrew Kennedy², Cyf Ramos-Colon¹

¹Gyros Protein Technologies, United States

²Gyros Protein Technologies, United Kingdom

<https://doi.org/10.17952/35EPS.2018.113>

Introduction

Cyclic peptides are an important tool for development of peptide therapeutics. Their relative rigidity allows for increased membrane permeability and stability to proteases, addressing two major drawbacks of peptide drug development. Peptide cyclization can be achieved several ways, including head-to-tail cyclizations or side chain-to-side chain cyclizations, which can be done by lactam bridge formation, disulfide bonds or hydrocarbon stapling *via* olefin metathesis, to name a few. Hydrocarbon stapling stabilizes α -helical structures and thus increases cell permeability and stability. Other advantages of stapled peptides include the targeting of protein-protein interactions, important for targeting many disease states such as cancer.

Zhang *et al.* have described the synthesis and properties of an i, i+4 stapled peptide, NYAD-1 (Figure 1), which targets the capsid and inhibits HIV-1 in cell culture [1].

Figure 1: NYAD-1 stapled peptide structure. X = (S)-2-(4'-pentenyl)alanine

A small GTPase RAB25, has been found to have both pro-oncogenic and anti-oncogenic phenotypes in specific cellular contexts. Mitra *et al.* have shown several cell permeable peptides, including RFP14 peptide (Figure 2), that disrupt RAB25:FIP complex formation *in vitro* and *in situ* and compete with the context-specific phenotypes associated with RAB25 function in ovarian and breast cancer cell lines [2].

Figure 2: RFP14 peptide structure.

Here we show the fully automated synthesis, from linear, on-resin cyclization, and resin cleavage, of NYAD-1 and RFP14.

Methods

Protocol A: NYAD-1 was synthesized on Rink MBHA resin (0.33 mmol/g) at 10 μ mol scale. Deprotection was done with 20% piperidine in DMF for 2 x 5 min. The coupling reaction was done with 100 mM amino acid and HATU, 400 mM NMM for 2 x 20 min with ten-fold excess for natural amino acids, 1 x 30 min with five-fold excess for unnatural amino acid.

Protocol B: RFP14 was synthesized using Rink Amide ChemMatrix Resin (0.54 mmol/g substitution) at a 25 μ mol scale. Deprotection time was 2 x 1:30 min at 25°C, 60°C, or 90°C using 20% Piperidine in DMF. Amino acids were coupled using a six-fold excess and final concentration of 100 mM for amino acid and HCTU and 200 mM for DIEA. Coupling time was 2:30 min at 25°C, 60°C, or 90°C. A capping step was done after every coupling reaction for 5 min. DMF washes followed deprotect, coupling, and capping steps with 3 repetitions at 30 seconds each.

Metathesis Reactions: The resin bound Fmoc-protected peptide was treated with a 10 mM solution of Grubbs catalyst (Bis(tricyclohexylphosphine) benzylidene ruthenium(IV)dichloride) in 1,2-Dichloroethane (2 mL) for 2 X 2 hours.

Cleavage: Final cleave used TFA:TIS:EDT:Water (95:1:2.5:2.5) for 2 hours at 25°C on the instrument followed by precipitation in ether.

Analysis: Peptides were analyzed using a C18, 300 Å, 5 mm, 250 x 4.6 mm column (Varian Microsorb-MV) or C18, 180 Å, 4.6 um, 250 X 4.6 mm Polaris column (Agilent), with a gradient of 5-95%B in 60 or 30 min, respectively, using Water (0.1%TFA):ACN(0.1%TFA) at 1ml/min. A 1:10 dilution of a standard sample of 3 mg/ml was run on a Phenomenex Kinetex 2.6 um C18 100A 50x2.1 mm column for LCMS or a Polaris column with a gradient of 5-50%B in 7 or 30 min, respectively, using Water(0.1%FA):ACN(0.1%FA) at 1 ml/min.

Results

NYAD-1

NYAD-1 was successfully synthesized on an automated peptide synthesizer with a 31.4% crude purity using HATU and a conservative protocol (Protocol A).

Figure 3: Crude purity profile of stapled NYAD-1 using HATU and NMM.

RFP14

RFP14 was synthesized in parallel at three different temperatures, 25°C, 60°C, and 90°C using a fast protocol of 2:30 min coupling time using HCTU. The best linear crude purity was seen at the higher 90°C temperature (Table 2, Figure 4A).

Table 1: Effect of temperature on crude purity of linear RFP14.

Peptide	25°C	60°C	90°C
RFP14	28.7%	27.7%	59.6%

Figure 4: Crude purity profiles of: A) linear RFP14 (at 90°C) and B) stapled RFP14.

Conclusions

Therapeutic stapled peptides were successfully synthesized under variable conditions on automated peptide synthesizers in over 30% crude purity. Independent induction heating on the Prelude X allowed multiple temperatures to be screened simultaneously on the synthesis of the linear RFP14 peptide with improved purity with increase in temperature up to 90°C.

References

1. Zhang, H. *et al.* Retrovirology. 2013. 10, 1–20. 2013.
2. Mitra, S. *et al.* Nat. Commun. 2017. 8 (1).