Comparison of Different Heating Protocols For the Cyclization of Melanotan II on the *Prelude*® *X*

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INTRODUCTION

Heat-assisted synthesis has been shown to reduce the time necessary to produce high-purity linear peptides, and its application to the synthesis of cyclic peptides may provide similar advantages. To demonstrate the utility of heating in the preparation of a cyclic peptide, the potent melanocortin receptor agonist Melanotan II (MT-II)[1] has been synthesized. Multiple temperature profiles in parallel were tested for the optimization of the cyclization reaction of MT-II peptide utilizing induction heating on the *Prelude X* peptide synthesizer.

METHODS & ANALYSIS

Peptide Synthesis
The MT-II linear peptides were synthesized on the Tribute peptide synthesizer at 200 µmol scale using Rink Amide resin (loading 0.32 mmol/g). Deprotection was performed with 20% piperidine in DMF 2 x 3 for min at RT. Couplings were performed at a final concentration of 200 mM AA (10 eq.), 200 mM HCTU (10 eq.) and 400 mM NMM (20 eq.) for 30 min at RT. Aloc and Allyl side chain protection was used for Lys and Asp. For microcleavage, the cocktail used was TFA/EDT/water/TIS and the reaction was performed for 2 h at RT.
Cyclization: Following Pd-mediated removal of the side chain protecting groups and washing, a solution of PyClock (50 mM, 5 eq) and DIEA (100 mM, 10 eq) in DMF was added to the resin. After cyclization, the resin was washed with DMF and DCM, and cleaved using TFA/EDT/water/TIS for 2 h at RT.
The resulting crude peptide was dissolved in water and analyzed on a Varian ProStar HPLC using a C18, 180 Å, 5 um, 250 x 4.6 mm column (Agilent Polaris), over 60 minutes with a flow rate of 1 mL/min, and using a gradient of 5-95% B, where Buffer A is 0.1% TFA in water, and Buffer B is 0.1% TFA in acetonitrile. Detection was at 214 nm. Mass analysis was performed on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 um, 50 x 2.1 mm column (Phenomenex Kinetex), over 7 min with a flow rate of 1 mL/min and using a gradient of 5-50% B where Buffer A is 0.1% formic acid in water and Buffer B is 0.1% formic acid in acetonitrile

REFERENCES

Al-Obeidi, F., A.M., Hadley, M.E., Pettitt, B.M., and Hruby, V.J. *J. Am. Chem.Soc.* **111**, 3413-3416 (1989)

RESULTS

The synthesis of MT-II peptide using induction heating was found to improve cyclization times and the efficiency of the cyclization reaction. Heating at 55° C or 85° C was effective for accelerating the reaction, but the highest purity was achieved by heating to 85 ° C for 5 minutes (Table 1).

MT-II peptide sequence:
Ac-Nle-cyclo[Asp-His-D-Phe-Arg-Trp-Lys]-NH2

Temp °C	Cyclization Times	Purity %
55	1 min	68.7
85	1 min	69.5
55	5 min	67.8
85	5 min	72.6

Table 1. Effect on peptide crude purity of different temperature protocols during cyclization of MT-II

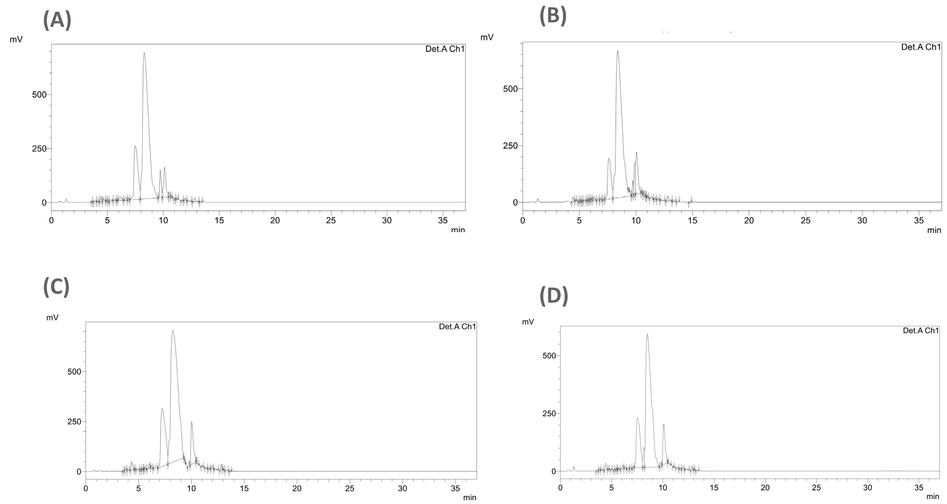

Figure 1. HPLC Traces using four different protocols
(A) MT-II peptide at 55 °C and 1 min cyclization times
(B) MT-II peptide at 55 °C and 5 min cyclization times
(C) MT-II peptide at 85 °C and 1 min cyclization times
(D) MT-II peptide at 85 °C and 5 min cyclization times

CONCLUSION

- Induction heating achieved a significant decrease of cyclization time without compromising peptide crude purity
- Efficient cyclization can be obtained from 1 min cyclization reaction times at 2 different temperatures
- Multi-variable conditions were tested in parallel for the process optimization of the MT-II pepide cyclization reaction

PRELUDE X

- 6 parallel independent heated reaction vessels
- 3 vessels with preactivation chemistry
- 30 seconds ramp up time to 90°C from RT of 20 mL DMF
- Real time UV monitoring
- Single Shot™ additions with almost no dead volume