Optimized crude purity of cyclic melanocortin receptor agonist melanotan II using induction heating

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Introduction

Methods for solid phase peptide synthesis (SPPS) of linear peptides have been improved by the use of heat (>50°C), resulting in shorter coupling cycles and higher crude purity. Cyclic peptides are increasingly popular as therapeutic agents due to their potential membrane permeability as well as proteolytic and metabolic stability [1]. While cyclization chemistry has traditionally been performed in solution, there are challenges to this method that compromise cyclization efficiency, such as ring size and sequence character. The use of higher temperatures may provide advantages in the synthesis of cyclic peptides and to demonstrate this the potent, cyclic melanocortin receptor agonist Melanotan II (MT-II) [2] has been synthesized using induction heating at 50-85°C on the Prelude® X peptide synthesizer.

MT-II peptide sequence:

Ac-Nle-Asp-His-D-Phe-Arg-Trp-Lys-NH2

Method & Analysis

MT-II was synthesized by Fmoc-chemistry SPPS on a Prelude X peptide synthesizer at a 20 µmol scale using low-loaded Rink Amide MBHA resin (0.22 mmol/g). The full length linear MT-II was synthesized first using a 10-fold excess of coupling reagents, at a 200 mM HCTU and 400 mM NMM and 200 mM concentration of amino acid at room temperature. Deprotections were done using 20% piperidine in DMF at room temperature. Orthogonal protecting groups, Alloc and OAll, were used on Lys and Asp respectively to enable the on resin cyclization.

Alloc/OAll deprotection

0.39 equivalents of Pd(PPh3)4 in the presence of 29 equivalents of PhSiH were used for the side chain deprotections. The side chain deprotections were done for 45 min at room temperature followed by washings: 3X DCM, 3X DMF, 2X 0.5% DIPEA in DMF, 1X 6 equivalents HOBt in DMF, 1X 0.5% Sodium diethyldithiocarbamate (DEDTC), 3X DMF, 3X DCM, all wash solutions were added from the extra AA positions available on the Prelude X.

Cyclization

The on resin cyclization via lactam bridge was performed with a coupling solution of PyClock (50 mM, 5 eq) and DIEA (100 mM, 10 eq) in DMF. Two temperatures, 55°C and 85°C, for different cyclization times of 1 or 5 min were tested in parallel.

Analysis

After cyclization the resin was washed with DMF and DCM and dried on the instrument. The cleavage was done using TFA/EDT/H2O/TIS for 2 h at 25°C on the Prelude X followed by precipitation in diethyl ether. The resulting peptide was dissolved in water and analyzed on a Shimadzu Prominence HPLC using a C18, 180 Å, 5 um, 250 X 4.6 mm Polaris column (Agilent), over 30 min with a flow rate of 1 mL/min and a gradient of 5-95% B, where A is 0.1% TFA in water and B is 0.1% TFA in acetonitrile. Detection was done at 214 nm. Mass analysis was done on a Shimadzu LCMS-2020 Single-Quad mass spectrometer, equipped with a C18, 100 Å, 2.6 um, 50 x 2.1 mm Kinetex column (Phenomenex), over 7 min with a flow rate of 1 mL/min and a gradient of 5-50% B where A is 0.1% formic acid in water and B is 0.1% formic acid in acetonitrile.

Prelude X

- 6 parallel independently heated reaction vessels
- 3 vessels with preactivation chemistry
- Real time IntelliSynth™ UV deprotection monitoring
- Single Shot™ additions with almost no dead volume

Results

Use of induction heating during the cyclization reaction of MT-II led to improved results showing higher efficiency of the cyclization reaction. Heating at 55° or 85°C was effective in reducing the reaction time needed for completion with optimal results observed by heating at 85°C for 5 min (Table 1). It is worth noting that traditional cyclization reactions are run for longer than 2 h.

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

MT-II		
Cyclization temp °C	Cyclization times	Purity %
55	1 MIN	68.7
55	5 MIN	69.5
85	1 MIN	67.8
85	5 MIN	72.6

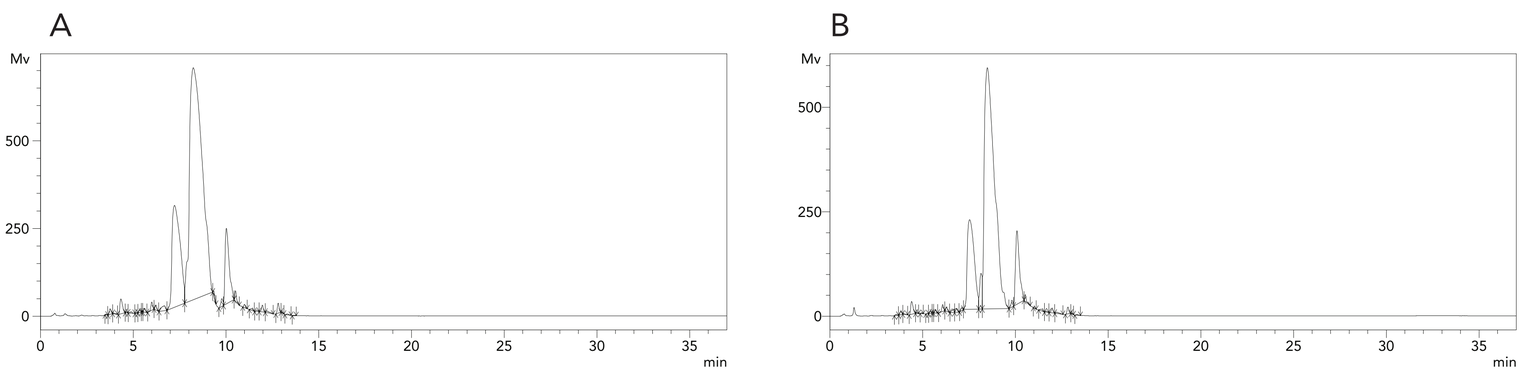

***Figure 1.** MT-II peptide cyclization at 55°C for A) 1 min and B) 5 min.*

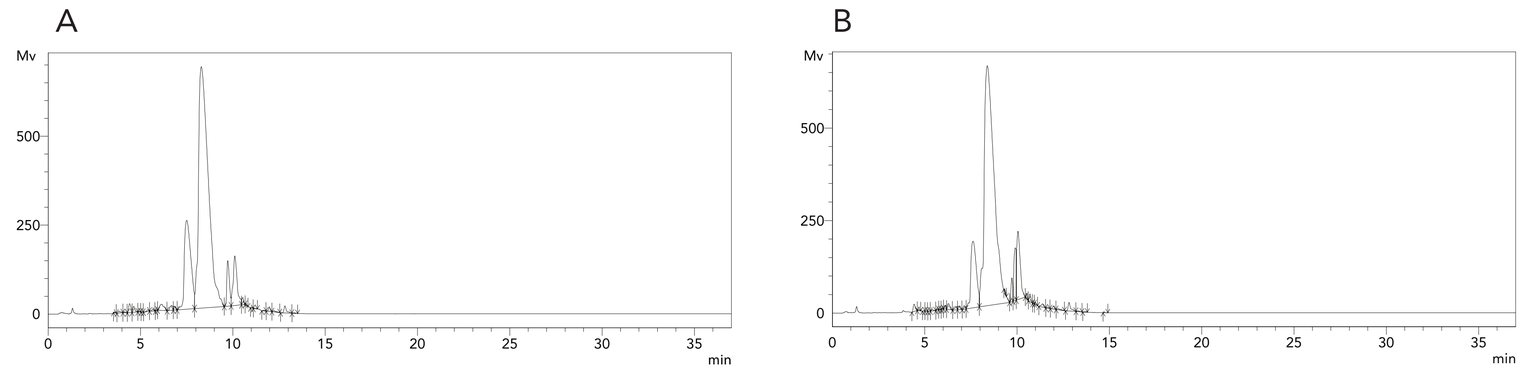

***Figure 2.** MT-II peptide cyclization at 85°C for A) 1 min and B) 5 min.*

Conclusion

- Induction heating provided increased efficiency decreasing the total cyclization time of MT-II without compromising purity.
- Efficient cyclization can be achieved after 1 min cyclization reaction in the presence of heat, 55°C or 85°C.
- With the capability to use 6 RVs on one synthesizer, multi-variable conditions were tested in parallel for the process optimization of the MT-II peptide cyclization reaction.

References

1. Joo, S. Biomol Ther (Seoul). 2012; 20(1): 19–26.
2. Al-Obeidi, F., A.M., Hadley, M.E., Pettit, B.M., and Hruby, V.J. J. Am. Chem. Soc. 1989, 111, 3413-3416

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