Synthesis of [des-Arg^7]-Dynorphin A peptoid analogue: faster methods using parallel automated synthesis and induction heat

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Abstract

Peptide-based therapeutics are increasingly at the forefront in addressing new challenges due to their high target selectivity and potency with low toxicity. However, peptides are subject to high enzymatic degradation and decreased membrane permeation. These drawbacks can be overcome by peptidomimetics such as peptoids, which have decreased susceptibility to enzymatic degradation and improved membrane permeability resulting in increased bioavailability. Automated solid phase peptide synthesis (SPPS) increases reliability, efficiency, and crude purity for peptides and peptoids. For example, introducing heat (>50°C) in the SPPS of linear peptides has shown improved results with shorter coupling cycles and higher crude purity.

Dynorphin A (Dyn A, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln) is one of three endogenous opioid peptides with high affinities on the μ (MOR), δ (DOR), and κ opioid receptors (KOR) in the central nervous system, inhibiting nociception. It has been shown that many of the adverse effects related to opiate therapy, such as tolerance and addiction, are mediated through the KOR, while nociception is mainly mediated through the MOR. Thus, developing potent KOR antagonists will lead to potential chronic pain treatments with reduced addictive properties [1]. Ramos-Colon et al. found that [des-Arg^7]-Dyn A-(1−9)-NH_2 (Figure 1) was a minimum KOR antagonist with a duration of action of less than 30 min in mice[2]. Here, the synthesis optimization of a [des-Arg^7]-Dyn A-(1-9)-NH_2 peptoid analog prepared by the submonomer method is tested in parallel using an automated peptide synthesizer.

H-Tyr-Gly-Gly-Phe-Leu-Arg-Ile-Arg-NH_2

Figure 1. *[des-Arg^7]-Dyn A-(1-9)-NH_2*

Method and Analysis

The peptide was synthesized in a 25 μmol scale using Rink Amide MBHA (0.22 mmol/g) in duplicates on the Prelude® X. The synthesis was run using the monomer method with 1 M Bromoacetic acid and 1.2 M DIC for the acylation and the corresponding amine at 1 M for the displacement as shown on Table 1. The target peptide was synthesized as a peptide-peptoid hybrid using PurePep™ reagents: HDMA/OxymaPure/DIPEA, using pre-packed Fmoc-amino acids in a 1:1:1:2 ratio AA/Activator/Additive/Base for Arg and Gly residues. 20% piperidine in DMF was used for Fmoc-deprotections.

Table 1. *Protocol descriptions.*

Protocols	Peptoid		Peptide	
	Acylation	Displacement	Fmoc-dep	Coupling
A	2 x 30 min	1 x 60 min	2 x 10 min	2 x 30 min
B	2 x 15 min	1 x 40 min	2 x 10 min	2 x 30 min
C (75°C, Arg-25°C)	2 x 6 min	1 x 6 min	Gly:2 x 3 min	2 x 6 min
			Arg:2 x 10 min	2 x 30 min

Cleavage and Analysis

The cleavage was done using TFA/EDT/H_2O/TIS (94:2.5:2.5:1) for 2 h at 25°C on the Prelude X followed by evaporation and precipitation in diethyl ether. The resulting peptide was dissolved in water and analyzed on a Thermo Scientific Ultimate 3000 HPLC using a C18, 180 Å, 5 μm, 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 μm, 50 x 4.6 mm Microsorb column (Varian), over 15 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.

Summary

- Synthesis of [des-Arg^7]-Dyn A-(1-9)-NH_2 peptoid-peptide hybrid was successfully completed, including on-instrument resin cleavage, on the Prelude X using the monomer method and coupling reagent combination: HDMA/Oxyma Pure
- A faster protocol at 25°C resulted in similar purities to that of the standard peptoid monomer method resulting in faster automated synthesis, saving ~ 5 h
- Using heat resulted in faster synthesis, 6 h, with comparable purity to the longer protocols
- Arg δ-lactam formation leads to significant Arg deletions, adding a longer mix time at 25°C followed by a short heated mix might reduce the deletion potential

Results

Several protocols were tested for the synthesis of a peptoid-peptide hybrid of [des-Arg^7]-Dyn A-(1-9)-NH_2 (Figure 2). Using a shorter but still conservative protocol at room temperature (Protocol B) resulted in similar purities to that of standard peptoid monomer addition (Table 2) on Protocol A (14 h) in a reduced amount of time (11 h). Including heat allowed for even shorter reaction times with the synthesis done in 6 h (Protocol C). On all protocols an Arg deletion was observed as a secondary peak.

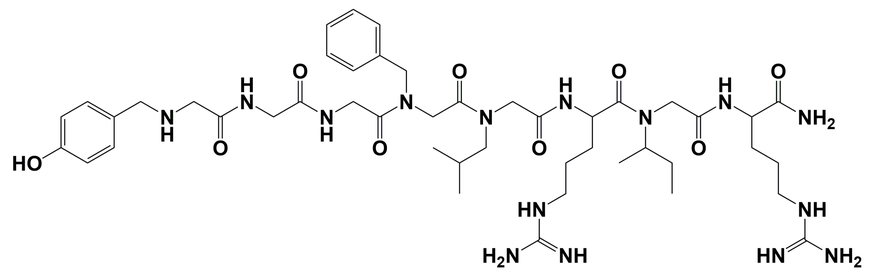

Figure 2. *[des-Arg^7]-Dyn A (1-9)-NH_2 peptoid-peptide hybrid.*

Table 2. *Percent of crude purity and yield of [des-Arg^7]-Dyn A-(1-9)-NH_2 peptoid hybrid*.

Protocol	% Purity	% Yield
A	43.5	68.6
B	45.6	42.9*
C	35.7	68.9

*Some product was lost during transfer after evaporation

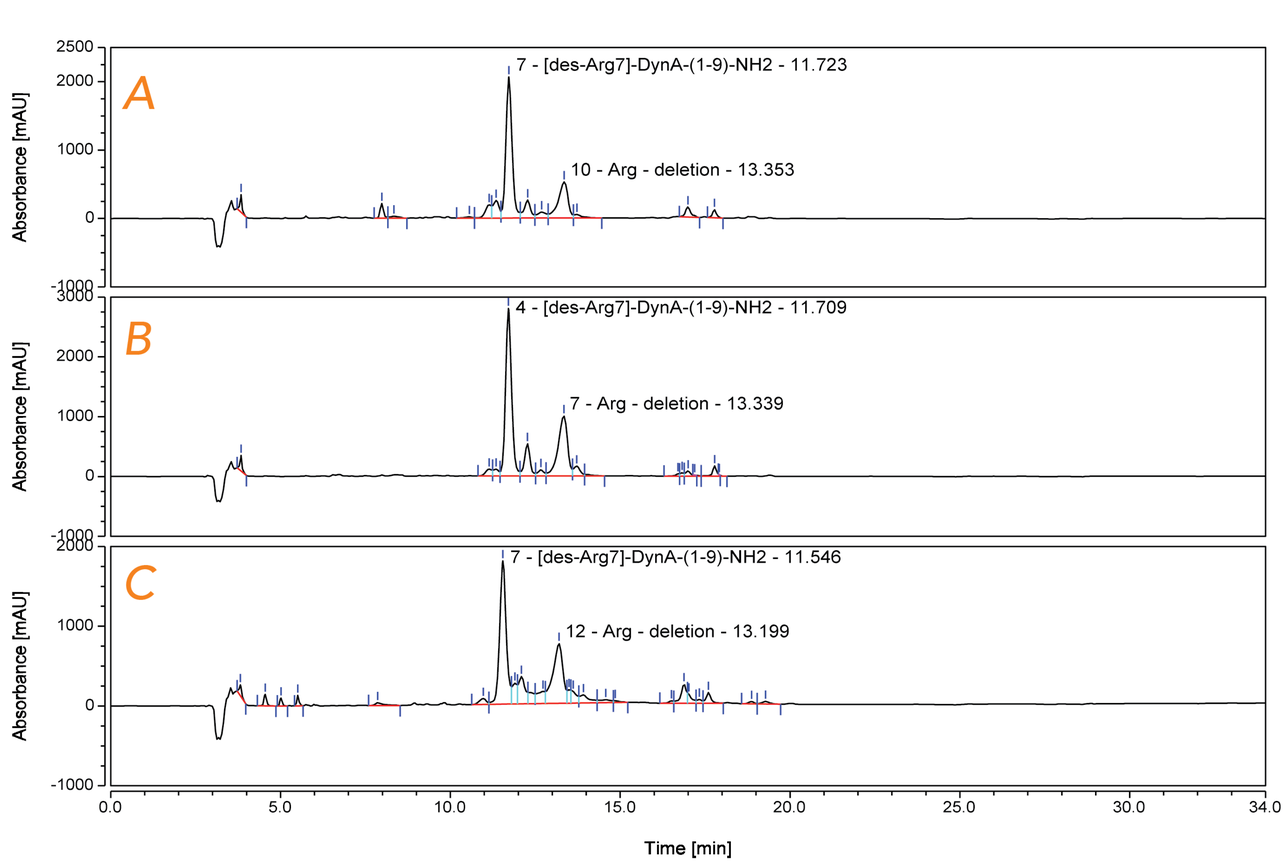

Figure 3. *Crude purity profiles of peptide-peptoid hybrid of [des-Arg^7]-Dyn A-(1-9)-NH_2 (RT: 11.7 min) synthesized using Protocol A **(A),** Protocol B (**B**), and Protocol C (**C**) on Rink Amide MBHA resin*.

References

[1] Shippenberg TS, Zapata A, Chefer VI. Dynorphin and the pathophysiology of drug addiction. Pharmacol Ther (2007);116:306–21.

[2] Ramos-Colon C.N., Lee Y.S., V.J. Hruby et al., Structure–Activity Relationships of [des-Arg ^{7}]Dynorphin A Analogues at the κ Opioid Receptor. J. Med. Chem. (2016); 59:10291–10298.

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