Efficient synthesis of 84-mer human Parathyroid hormone for the study of osteoporosis and hypoparathyroidism

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Abstract

Human parathyroid hormone (1-84) (PTH) (Figure 1) is produced by the parathyroid glands and regulates calcium and phosphate metabolism. PTH acts on PTHR1 receptors to stimulate bone formation and is used as a treatment for osteoporosis and hypoparathyroidism, a rare deficiency of parathyroid hormone [1,2]. There are limited published studies on full length PTH due the difficulty of obtaining the full sequence in high purity [2]. Others have used Boc-chemistry and combinations of Fmoc- based solid phase peptide synthesis (SPPS) with Native Chemical Ligation [3]. Here we explored PTH's complete synthesis using fast protocols on an automated peptide synthesizer, to obtain high purity PTH peptide and its analogs in a reduced amount of time which can be used to further understand PTH's role in SAR studies or enhancing bioavailability and stability of PTH based therapeutics.

SVSEIQLMHNLGKHLNSMERVEWLRKKLQDVHNFVAGALAPRDA
GSQRPRKKEDNVLVESHEKSLGEADKADVNVLTKAKSQ

Figure 1. *PTH structure.*

Method and Analysis

The peptide was synthesized in a 50 μmol scale using four different resins:

- H-Gln(Trt)-HMPB ChemMatrix® resin (0.35 mmol/g)
- Rink Amide ChemMatrix resin (0.47 mmol/g)
- H-Gln(Trt)- TentaGel® (0.18 mmol/g)
- Rink Amide TentaGel (0.19 mmol/g)

The synthesis was run with a 6X excess using PurePep™ reagents: HDMC/OxymaPure™/DIPEA, or HDMA/Oxyma Pure/DIPEA, using pre-packed Fmoc- amino acids in a 1:1:1:2 ratio AA/Activator/Additive/Base in duplicates on the Symphony® X. Deprotection was done 2 x 3 min at 25°C using 20% piperidine in DMF and the coupling reaction was run for 2 x 5 min at 25°C.

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 Symphony X followed by 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, 100 X 4.6 mm Acclaim column (Thermo), over 15 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 2.1 mm Kinetex column (Phenomenex), 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.

Results

Synthesis of PTH(1-84) on Rink Amide resins resulted in the highest purities and yields with the highest crude purity when using HDMC/OxymaPure (Table 1). Rink Amide TentaGel resin produced the highest purity (36.3%).

Synthesis with HDMA/OxymaPure (Table 2) produced similar results compared to HDMC/OxymaPure, with highest purities observed when Rink Amide resins were used. The highest purity, 37.7% (Figure 2C) was observed with Rink Amide ChemMatrix resin and the combination of HDMA/OxymaPure/DIPEA.

Table 1. *Percent of crude purity and yield of PTH synthesized with HDMC/OxymaPure/DIPEA.*

Resins	% Purity	% Yield
HMPB ChemMatrix	21.7	21.6
TentaGel	13.0	27.3
Rink ChemMatrix	33.4	31.2
Rink TentaGel	36.3	33.9

Table 2. *Percent of crude purity and yield of PTH synthesized with HDMA/OxymaPure/DIPEA.*

Resins	% Purity	% Yield
HMPB ChemMatrix	26.7	22.0
TentaGel	15.8	43.8
Rink ChemMatrix	37.7	34.3
Rink TentaGel	33.1	40.4

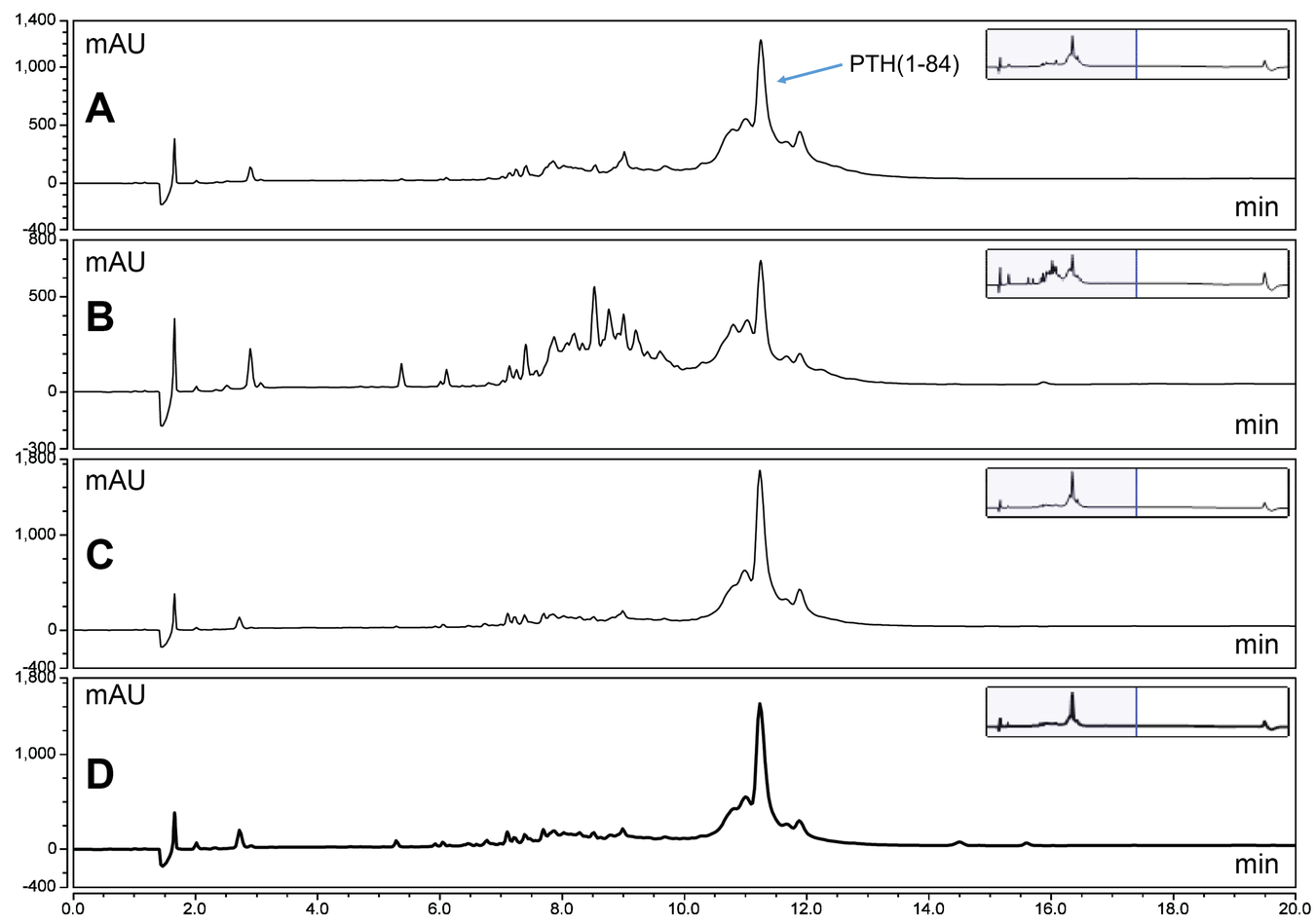

Figure 2. *Crude purity profiles of PTH(1-84) (RT: 11.2 min) synthesized on A) HMPB ChemMatrix, B) TentaGel, C) Rink Amide ChemMatrix, and D) Rink Amide TentaGel using HDMA/OxymaPure/DIPEA at 25°C.*

Conclusions

- Complete synthesis of 16 PTH(1-84) was successfully done including on instrument cleavage on the Symphony X using recently developed coupling reagents: HDMA and HDMC
- Rink Amide ChemMatrix resin in combination with HDMA/OxymaPure/DIPEA resulted in the best crude purity for PTH(1-84), showing HDMA's high coupling efficiency
- Further analysis using faster cycle times with Rink ChemMatrix/HDMA may provide suitable testing conditions for the synthesis of long difficult peptides in a significantly reduced amount of time
- The Symphony X provides ample flexibility for process optimization, here we tested 8 different conditions in duplicate simultaneously

References

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