

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
GSQRPRKKEDNVLVESHEKSLGEADKADVNVLTAKKSQ

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/H₂O/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.

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

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%).

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

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 2D) was observed with Rink Amide ChemMatrix resin and the combination of HDMA/OxymaPure/DIPEA.

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

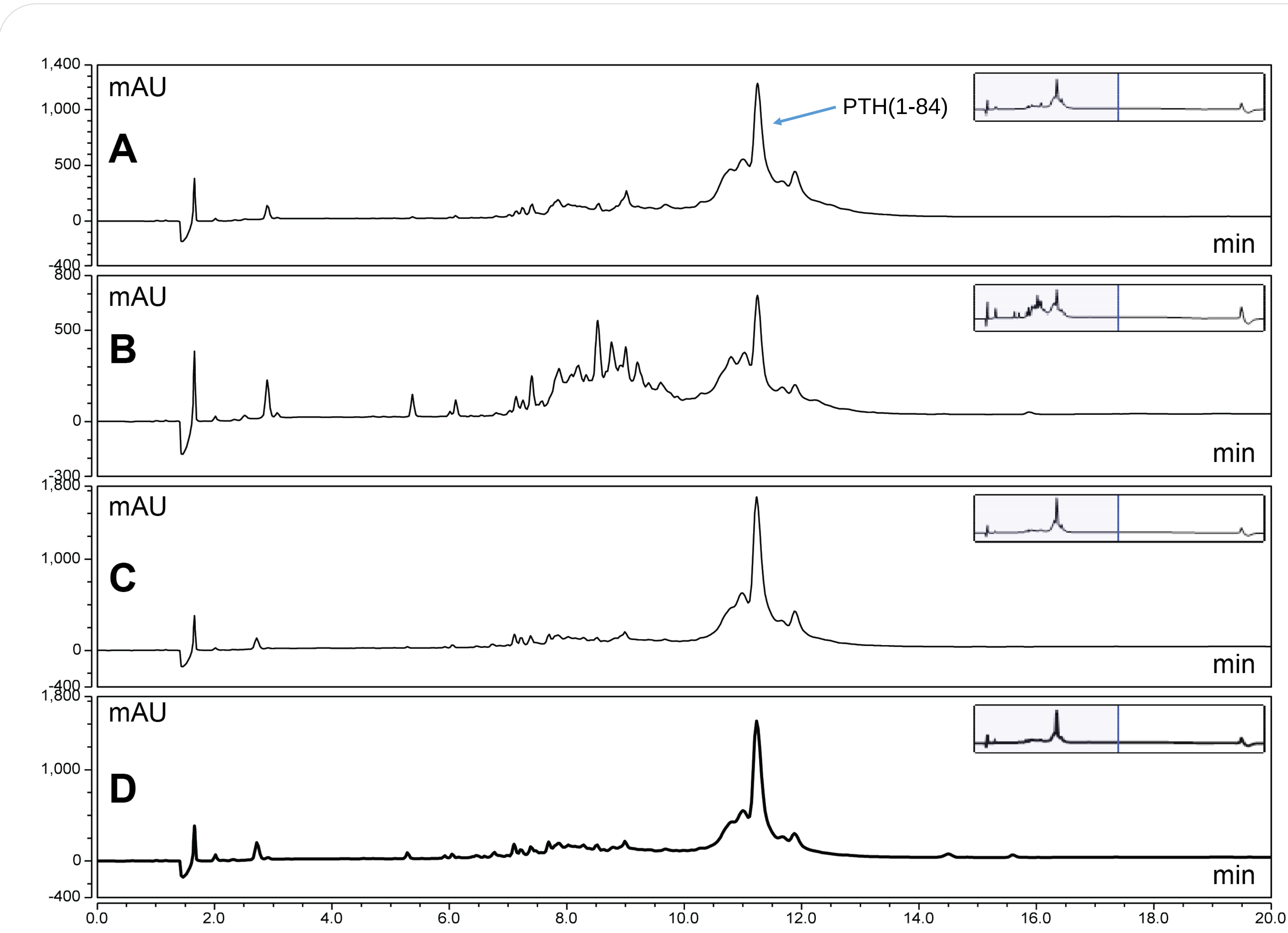


Figure 2. Crude purity profiles of PTH(1-84) (RT: 11.2 min) synthesized on A) HMPB ChemMatrix, B) TentaGel, C) Rink Amide ChemMatrix, and D) Rink Amide TentaGel using HDMA/OxymaPure/DIPEA at 25°C.

References

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