High-throughput parallel synthesis optimization of Glucagon-like Peptide 1 receptor agonists

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Introduction

GLP-1 related peptides are an important class of peptides due to their effects on metabolic diseases. GLP-1 receptor agonists are used for treatment and management of type 2 diabetes[1]. The GLP-1 agonist class improves glycemic control through multiple mechanisms with a low risk of hypoglycemia [1]. In 80-90% of type 2 diabetes cases, obesity is also present thus targeting multiple receptors to tackle both is of great interest[2]. Evers et al. have recently described peptides with dual agonist activity at the GLP-1 receptor and the glucagon receptor for this purpose [1].

Many SAR studies have been performed on GLP-1 related peptides in the search for stability and target affinity improvements, including introduction of cyclizations and attaching PEG based monomers. Published patents describe the SPPS of these type of peptides as being optimized by fragment synthesis and solution phase condensation in order to reduce impurities and maximize yields for pharmaceutical productions. Here we show the rapid automated synthesis optimization screen of two GLP-1 receptor agonists (Lixisenatide and Pramlintide; Figure 1 and 2) and one dual GLP-1 and glucagon receptor agonist (Figure 3).

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPSKKKKKK-NH_2

Figure 1. Lixisenatide

H-KCNTATCATQRLANFLVHSSNNFGPILPPTNVGSNTY-NH_2

Figure 2. Pramlintide

H-H-Aib-QGTFTSDLSKQK-(yE-yE-C16)-DEQRAKLFIEWL-Aib-AGGPPS-Aib- KPPPK-NH_2

Figure 3. Dual GLP/GlucagonR agonist [2]

Method & Analysis

GLP-1 agonists were synthesized by Fmoc-chemistry SPPS on a Symphony® X peptide synthesizer at a 25 µmol scale. Using the parallel synthesis capability on the Symphony X, three resins and two coupling reagents were tested in parallel. The resins tested were:

- R Ram Tentagel® Resin (0.19 mmol/g)
- Rink Amide ChemMatrix® resin (0.55 mmol/g)
- Low-loaded Rink Amide MBHA resin (0.22 mmol/g)

Deprotections were done using 20% piperidine in DMF at 25°C for 2 x 30 s. The coupling reactions were done at 25°C for 2 x 1 min with a 12 excess with final concentrations of 100 mM AA and 100 mM HCTU or COMU® with 200 mM DIPEA. For the synthesis of GLP/glucagonR agonist, the Fmoc-Lys(ivDde)-OH was coupled for 2 x 5 min.

Analysis

The cleavage was done using TFA/thioanisole/H_2O/phenol/EDT (84.5:5:5:5:2.5) for 2 h at 25°C on the Symphony X followed by precipitation in diethyl ether. The resulting peptides were dissolved in water and analyzed on a Thermo Scientific Ultimate 3000 HPLC using a C18, 100 Å, 2.6 um, 50 X 2.1 mm Kinetex Evo column (Phenomenex), over 4.5 or 5.5 min with a flow rate of 0.8 mL/min and a gradient of 5-95% B or 0-70% B, respectively; 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 30 min with a flow rate of 1 mL/min and a gradient of 5-95% B where A is 0.1% formic acid in water and B is 0.1% formic acid in acetonitrile.

Symphony X

- 24 parallel independent reaction vessels to run different scales, sequences and protocols on multiple RVs all at the same time or on-demand while running other projects
- 12 vessels with pre-activation chemistry
- Real-time UV monitoring of the reaction solution not the waste during mixing to control deprotection times and minimize excess waste for better purity and yields
- Rapid Infrared (IR) heating
- Single Shot™ additions with almost no dead volume

Results

PEG based Rink Amide ChemMatrix resin produced the best crude purities in the synthesis of all three peptides using either HCTU or COMU as the coupling reagent at room temperature. This confirms the advantage of using PEG based resins for the synthesis of long peptides.

In the synthesis of Lixisenatide the highest crude purity was achieved with COMU (Table 1) and for Pramlintide and Dual GLP-1/GluR agonist was achieved with HCTU (Table 2 and Table 3). Both HCTU and COMU are highly reactive coupling reagents able to provide high crude purity with coupling reactions of 1 min.

Table 1. *Crude purity of **Lixisenatide**.*

Resins	% Purity	
	HCTU	COMU
R Ram Tentagel	54.7	49.5
Rink ChemMatrix	56.4	68.2
Rink MBHA Low Loaded	45.1	41.0

Table 2. *Crude purity of **Pramlintide**.*

Resins	% Purity	
	HCTU	COMU
R Ram Tentagel	35.1	43.0
Rink ChemMatrix	62.7	47.0
Rink MBHA Low Loaded	62.3	42.2

Table 3. *Crude purity of **Dual GLP/GluR**.*

Resins	% Purity	
	HCTU	COMU
R Ram Tentagel	59.6	59.1
Rink ChemMatrix	63.0	49.6
Rink MBHA Low Loaded	62.1	57.6

Figure 4. *Crude purity profiles of Pramlintide using HCTU/DIPEA at 25°C and synthesized on different resins:*

A) Rink Amide MBHA,

B) R Ram TentaGel,

C) Rink Amide ChemMatrix resins

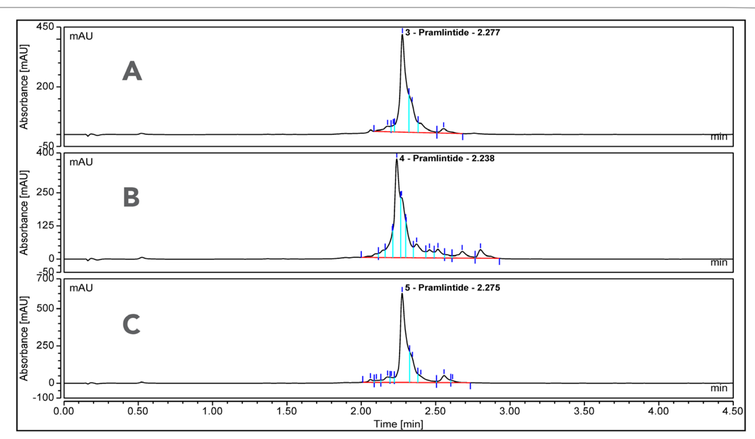
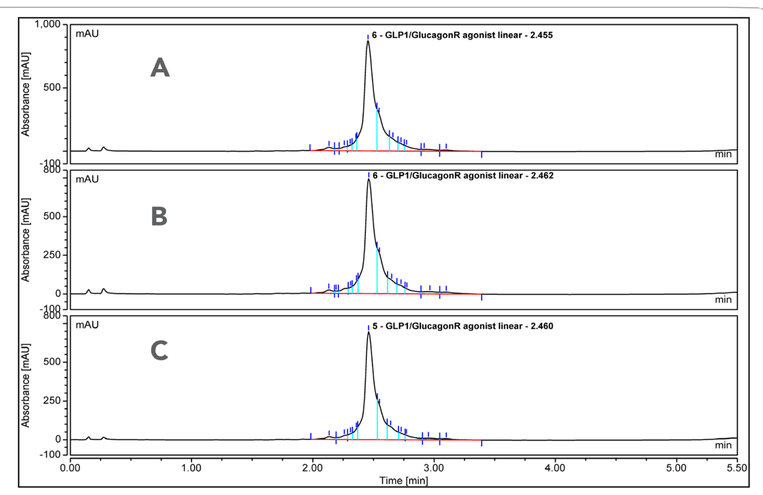


Figure 5. *Crude purity profiles of Dual GLP/GluR using HCTU/DIPEA at 25°C and synthesized on different resins:*

A) Rink Amide MBHA,

B) R Ram TentaGel,

C) Rink Amide ChemMatrix resins



Conclusions

- Multi-variable conditions were successfully tested in parallel for the high-throughput optimization of GLP-1 receptor agonists getting valuable synthesis information in ~ 2 days
- ChemMatrix resin provided the best crude purity in the synthesis of GLP-1 agonists
- HCTU and COMU are highly reactive coupling reagents effective in achieving high purity peptides with 2 x 1 min coupling reaction times
- Synthesis of Lixisenatide resulted in optimal crude purity using Rink Amide ChemMatrix resin and COMU as the coupling reagent with 2 x 1 min couplings
- Synthesis of Pramlintide and the linear dual GLP/GLuR agonist resulted in high crude purities with multiple conditions with the Rink ChemMatrix and HCTU combination providing crude purities above 62%
- Next steps include the optimization of the Lys^{14} elongation after ivDde removal of dual GLP-1/GluR agonist

References

1) Kalra, S. et al. Glucagon-like peptide-1 receptor agonists in the treatment of type 2 diabetes: Past, present, and future. Indian J. Endocrinol. Metab. 20, 254–67 (2016).

2) Evers, A. et al. Dual Glucagon-like Peptide 1 (GLP-1)/Glucagon Receptor Agonists Specifically Optimized for Multidose Formulations. J. Med. Chem. 1, (2018).

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