Searching for faster and efficient solid phase peptide synthesis methods for increased crude purity in reduced time

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Abstract

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).

H-HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPSKKKKKK-NH_2

Figure 1. Lixisenatide

H-KCNTATCATQRLANFLVHSSNNFGPILPPTNVGSNTY-NH_2

Figure 2. Pramlintide

For less synthetically difficult sequences, including many neoantigen peptides, the key challenge is rapid production of small libraries. This is addressed by multi-channel parallel synthesis, possibly accelerated by elevated temperature. Metastatic melanoma patients have been successfully treated by adoptive transfer of tumor infiltrating lymphocytes (TILs), with regression seen in 72% of patients in clinical trials (1). Mini gene library screenings led to identification of novel TILs targets. Here we used the example of mutant KIF2C peptides that were used for the study by Lu, as an example of the quick synthesis using elevated temperatures on an automated synthesizer.

Method & Analysis

GLP peptides on Symphony X 25°C	
Resins	Rink Amide MBHA LL, 0.22 mmol/g Rink Amide ChemMatrix, 0.55 mmol/g R Ram Tentagel, 0.19 mmol/g
Scale	25 umol
Deprotection	2 x 30 s
Couplings 12x-excess	2 x 1 min

Neoantigens on PurePep Chorus™ 75°C	
Scale & resin	25 umol Rink Amide MBHA LL (0.27 mmol/g)
Deprotection	2 x 2 min
Couplings 6x-excess	3 min

Cleavage: Final cleave used TFA:TIS:Water (95:2.5:2.5) or Reagent K for 2 hours at 25°C on the instrument.

Analysis: Peptides were analyzed using a C18 Kinetex Evo, 2.1 um, 50 x 4.6 mm column with a gradient of 5-95%B in 5 min using H_2O (0.1%TFA):ACN(0.1%TFA) at 0.8 ml/min on a Thermo Scientific U3000RS. A 1:10 dilution of a standard sample of 3 mg/ml was run on a Shimadzu LCMS-2020 Single-Quad mass spectrometer using a C18, 300 Å, 5 um, 50 x 4.6 mm column (Varian Microsorb-MV), with a gradient of 5-95%B in 9 min using H_2O (0.1%FA):ACN(0.1%FA) at 1 ml/min.

PurePep Chorus™

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- Single Shot™ additions with almost no dead volume
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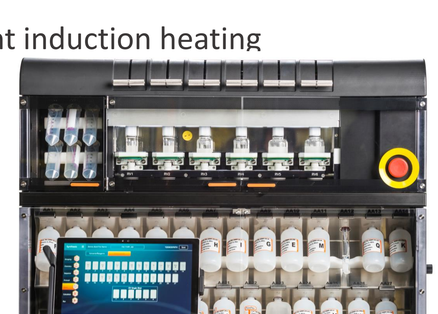


Results

GLP-related peptides synthesis optimization

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 was achieved with HCTU (Table 2). Both HCTU and COMU are highly reactive coupling reagents able to provide high crude purity with coupling reactions of 1 min.

Table 1. *Effect of coupling reagents and resin on crude purity of **Lixisenatide** at 25°C.*

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

Table 2. *Effect of coupling reagents and resin on crude purity of **Pramlintide** at 25°C.*

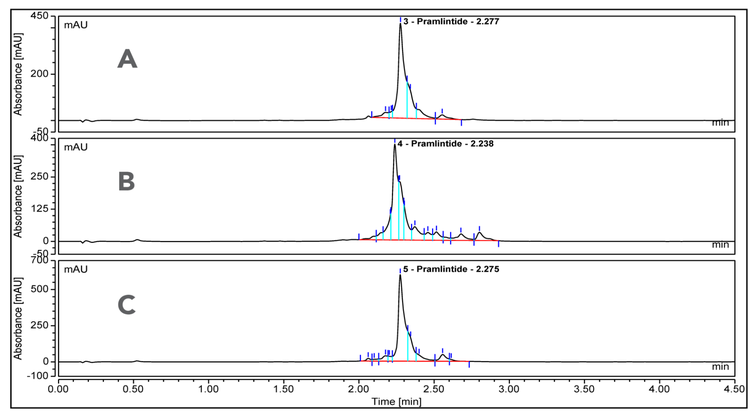
Resins	HCTU	COMU
R Ram Tentagel	35.1%	43.0%
Rink ChemMatrix	62.7%	47.0%
Rink MBHA LL	62.3%	42.2%

Figure 3. *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



Neoantigen Synthesis

The high purity synthesis of five peptides comprising the mutated region of the KIF2C gene were synthesized on the PurePep Chorus in parallel using a fast heated method showing the advantage of elevated temperatures and automated synthesis in the neoantigen research area (Table 3).

Table 3. *Crude purity of KIF2C peptides.*

Peptides	Crude Purity
RLFPGLTIKI	86.6%
RLFPGLTI	98.6%
LTIKIQRSNGL	98.1%
LQARLFPGLTI	96.9%
LQARLFPGLT	98.3%

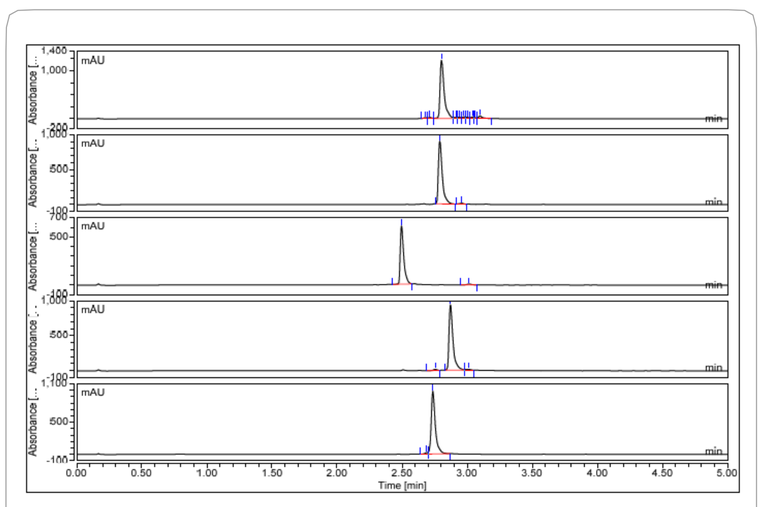


Figure 4. *Crude purity profile of KIF2C peptides synthesized at 75°C A) RLFPGLTIKI, B) RLFPGLTI, C) LTIKIQRSNGL, D) LQARLFPGLTI F) LQARLFPGLT.*

Figure 5. Parallel *UV deprotection monitoring graph.*

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
- Independent induction heating on the **PurePep Chorus** allowed multiple temperatures to be screened on the synthesis of neoantigen peptides in high crude purity in a reduced synthesis time

References

1 Lu, Y. et al. Clin Cancer Res. 2014, 20(13): 3401–3410.

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