Evaluation of the "*ResPep continuous flow synthesizer*" with real-time UV-monitoring, automated feedback & heating in solid phase peptide synthesis

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Introduction

Automation in assembly of peptides via solid phase synthesis following the Fmoc-strategy [1] is a well-established method and commonly used for peptide synthesis today. Mostly, due to length of the peptide, the presence of hydrophobic stretches or sterically hindered amino acids, difficulties during peptide synthesis are sequence inherent.

Therefore, the choice of the proper conditions like coupling reagent and temperature have tremendous influence on the stepwise amide bond formation yielding the crude product in the highest purity possible. Here, we present examples of automated peptide synthesis of so called "difficult sequences" [2], [3], [4], [5] under demanding conditions on the new ***ResPep continuous flow (CF)*** synthesizer with real-time UV monitoring and feedback.

Experimental setup

Test sequences

1. ACP-10mer: H-VQAAIDYING-NH_2
2. JR-10mer: H-WFTTLISTIM-NH_2
3. Exenatide: H-HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2
4. Bivalirudin: H-(*D*)-Phe-PRPGGGGNGDFEEIPEEYL-NH_2
5. Asn15-FBP28: H-YYNNRTLESTWEKPQELK-OH

Applied method

Peptides were synthesized at a 100 µmol scale on the ***ResPep CF*** system in a one-column module connected to a real-time UV monitoring flow cell. Couplings were mediated under room temperature (RT) and 90 °C using standard Fmoc-building blocks and different coupling reagents (Figure 1C) in a final concentration of 0.24 M (PyOxim, HDMTP, TBTU) or 0,34 M (Oxyma Pure) for 8 min. Fmoc-deprotection was performed for 90 seconds with 20 % (v/v) piperidine and 1 % (v/v) formic acid in DMF. The minimum number of repetitions for deprotection reactions was set to two, maximum repetitions to three, respectively. If deprotection was detected to be difficult (slow deprotection rates), the deprotection time was automatically elongated as well as the following coupling reaction got repeated (Figure 2). As solid support low loaded Fmoc-Lys(Boc)-TCP-resin for Asn15-FBP28 (0,28 mmol/g) or Ramage-AM-PS-resin for all other peptides (0,12 mmol/g) were used. Cleavage was performed by treating the peptidyl-resin with reagent K for two hours. After precipitation in diethyl ether and following washing steps, the crude peptides were lyophilized and analyzed by mass spectrometry and RP-HPLC at 214 nm.

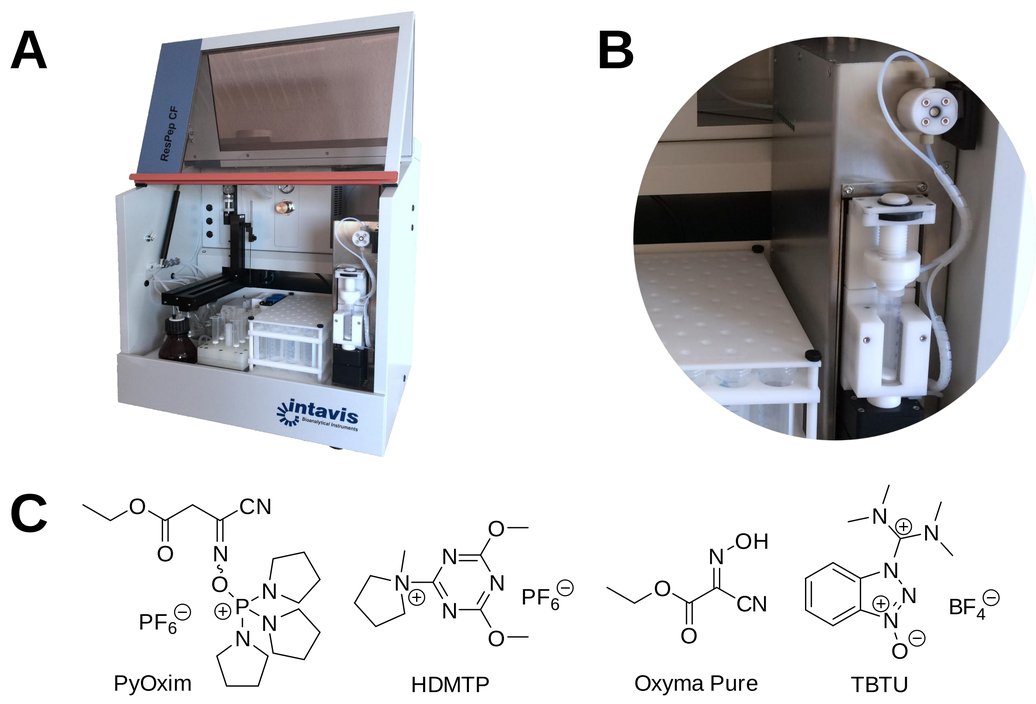

Figure 1: (A) ***ResPep CF*** synthesizer. (B) 1-Column module with piston pump and heating block. (C) Structures of applied coupling reagents used in this study.

Results

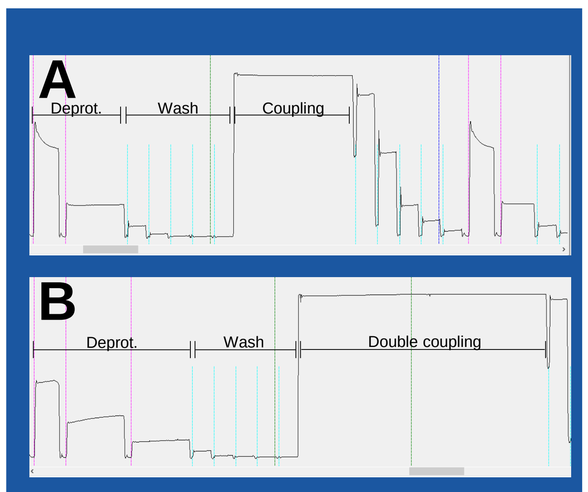

Figure 2: UV traces at 301 nm from synthesis of JR-10 using PyOxim. (A) Standard cycle with double deprotection and single coupling. (B) Difficult deprotection and coupling, automated adaption to three deprotection steps and automated repetition of coupling.

High quality of crude JR-10 product.

Table 1: Synthesis conditions: coupling reagents, RP-HPLC-purity of crude, lyophilized peptides.

Peptide	Coupling reagent	Deprotection [min][1]	Coupling time [min][2]	T [°C]	HPLC purity [%]
ACP-10mer	PyOxim	1,5	8	RT	71
	PyOxim	1,5	8	90	89
	PyOxim	3	8	90	90
	PyOxim	1,5	15	90	90
	HDMTP	1,5	8	90	82
	Oxyma Pure	1,5	8	90	79
	TBTU	1,5	8	90	71
JR-10mer	PyOxim	1,5	8	90	78
	HDMTP	1,5	8	90	72
	Oxyma Pure	1,5	8	90	66
Exenatide	PyOxim	1,5	8	90	52
	HDMTP	1,5	8	90	47
	Oxyma Pure	1,5	8	90	42
Bivalirudin	PyOxim	1,5	8	90	84
	HDMTP	1,5	8	90	82
	Oxyma Pure	1,5	8	90	78
Asn15-FBP28	HDMTP	1,5	8	90	70
	PyOxim	1,5	8	90	83
	Oxyma Pure	1,5	8	90	86

[1] deprotection times were automatically elongated through feedback based on online UV monitoring; [2] coupling got automatically repeated if deprotection rates are slow (based on online UV monitoring)

RT vs. 90 °C
Heating has tremendous effect on purity.

Deprotection time 1,5 min vs. 3 min
No significant improvement.

Coupling time 8 min vs. 15 min
No improvement.

Influence of choice of coupling reagent on quality

PyOxim > HDMTP > Oxyma Pure > TBTU

BUT:
For synthesis of Asn15-FEP8:
Performance of Oxyma Pure is significant better compared to the PyOxim

Choice of most effective reagent is dependent on sequence.

References

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