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

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

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" [2-5] under demanding conditions on the new ResPep continuous flow (CF) synthesizer with real-time UV monitoring and feedback.

Test sequences:

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

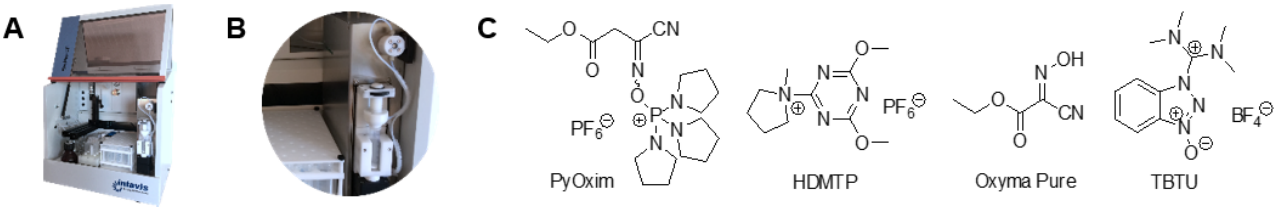

Figure 1: (A) ResPep CF synthesizer. (B) 1-Column module with piston pump and heating block. (C) Structures of applied coupling reagents used in this study.

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

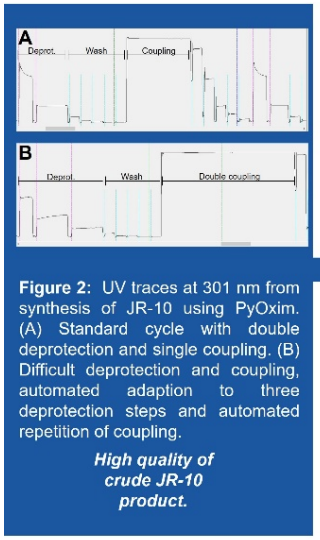

Figure 2: UV traces at 301 nm from synthesis of JR-10 using PyOxim. (A) Standard cycle with double deprotection and single coupling. (B) Difficult deprotection and coupling, automated adaption to three deprotection steps and automated repetition of coupling.

High quality of crude JR-10 product.

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

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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