Advances in peptide synthesis with green chemistry and cutting-edge technologies

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EUROAPI
Active Solutions for Health

Partner with EUROAPI

- Extensive experience with highly modified oligonucleotides & peptides, challenging chemistries and sequences
- Time- and cost-efficient transition from small to commercial scale production
- Investment in capability, process optimization & innovation
- Comprehensive support for the preparation of regulatory CMC package
- Strong customer interaction and transparent project management

Innovation for SPPS

Several existing challenges in SPPS:

- Green chemistry
- DMF restriction, hazardous to the environment & human health
- Large excess of reagents needed; sometimes a coupling needs to be repeated
- Substantial solvent consumption, especially for the washing steps
- Process development time: complex processes with multiple steps

Greener solvents

Development of new green solvent mixtures to replace DMF in peptide synthesis: Ternary mixture (CMR free, class 3*)

→ Successful synthesis of Bivaluridin (20 mer), Thymosin alpha (29 mer) and a GLP1 agonist (39 mer)

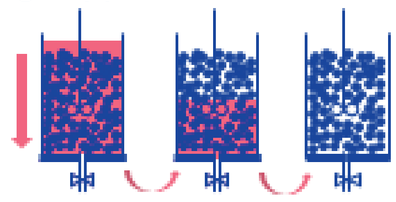
Origin of solvent mixture	Solvents	Tested ratio	Thymosin α purity (%)	Yield (%)
Standard	DMF	-	28	58
Pawlas et al. Schönleber, Pedersen et al.	DMSO/EtOAc	3:7	25	59
Pawlas et al.	NBP/EtOAc	1:3	51	69
Papini, Pacini, Rovero et al.	DMSO/BuOAc	3:7	23	58
EUROAPI	Ternary mixture	-	53	80

→ Enhance purity and yield of peptides while using class 3 solvents (low toxic potential to man) to promote sustainable development processes

Reduction of solvent consumption

Current SPPS process uses batch washes which require large volume of solvent and long operation time.

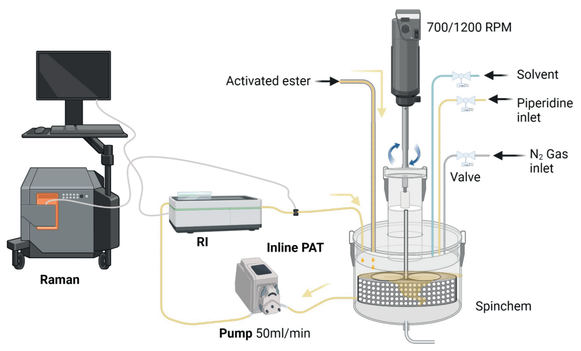
Simulated flow through approach for wash steps is proposed.

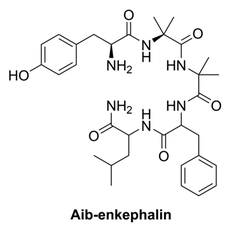


- 60% solvent consumption reduction due to more efficient wash process.
- 20% operation time reduction due to faster wash process in flow mode.
- Improved crude purity due to shorter holding time between coupling steps.
- Low regulatory impact. Drug Muster File does not describe wash process because during washes not chemical reaction occurs.
- Minimal CAPEX investment would be required.
- Timeline for lab scale POC 6 weeks.
  Successful POC: Thymosin alpha (29 mer), GLP1 agonist (39 mer)
- **Overall cost saving due to increased process intensity and reduce solvent use.**

Real time monitoring and Rotating bed reactor technology for SPPS

New experimental set-up for online monitored SPPS



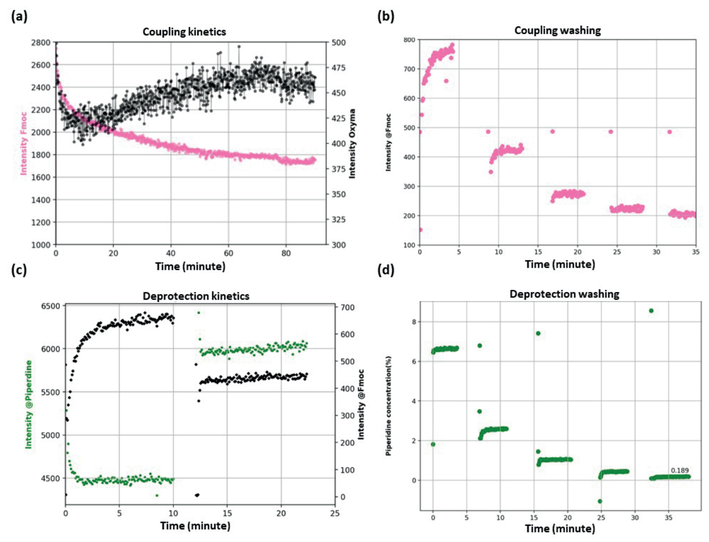


Aib-enkephalin

Aib-enkephalin
Classical short model peptide
Challenging synthesis: steric hindrance due to Aib residues

Investigating the responses of Raman spectrometry and Refractive index for all steps:

Raman spectroscopy monitoring of reaction and washing steps during Aib-enkephalin synthesis



(a) coupling kinetics of Fmoc-Phe-OH
(b) coupling washing of Fmoc-Phe-OH
(c) deprotection kinetics of Fmoc-Aib-OH (4th amino acid)
(d) deprotection washing of Fmoc-Aib-OH (4th amino acid)

	PMI kg/kg	Purity	
		Aib-enkephalin H-Tyr-Aib-Aib-Phe-Leu-NH2	DesAib-impurity H-Tyr-Aib-Phe-Leu-NH2
Optimized protocol SpinChem reactor	2541	78 %	15 %
Standard protocol CSBio synthesizer	3795	20 %	77 %

POC with synthesis of Gonadorelin (approved API):

Crude Gonadorelin purity	70 %
Purified Gonadorelin purity	91 %
Overall yield	60 %

Major results:
Instant flagging of deviation
Quick decision making
Enhanced efficiency
Improved quality
Improved PMI

Conclusion
PAT is a promising solution for accelerated peptide synthesis development

SpinChem is an attractive alternative technique to perform peptide synthesis

PAT is also applicable for standard peptide synthesis and oligonucleotides

References

Green Chem., 2019, 21, 5990-5998 - Green Chem., 2021, 23, 3312-3321 - Org. Process Res. Dev. 2020, 24, 1341-1349
J. Pept. Sci. 2024, e3605 - ACS Sustainable Chem. Eng. 2024, 12, 14629-14637