

Green solvents and Safe Reagents in SPPS taking advantage of induction heating and automation: Synthesis and HPLC-free Purification of Difficult Sequences

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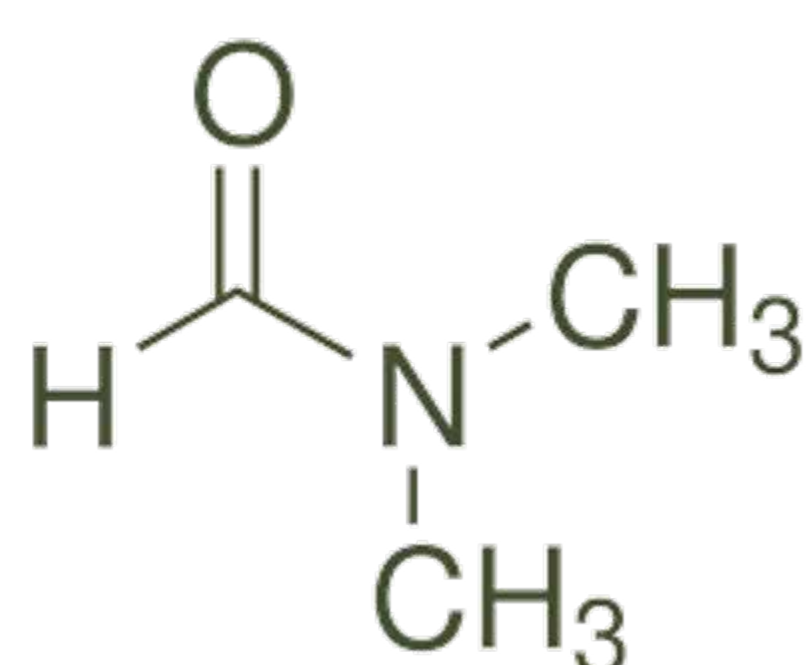
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DMF Issue: a high toxic gold standard solvent for SPPS



Flammable liquid and vapor



May damage the unborn child



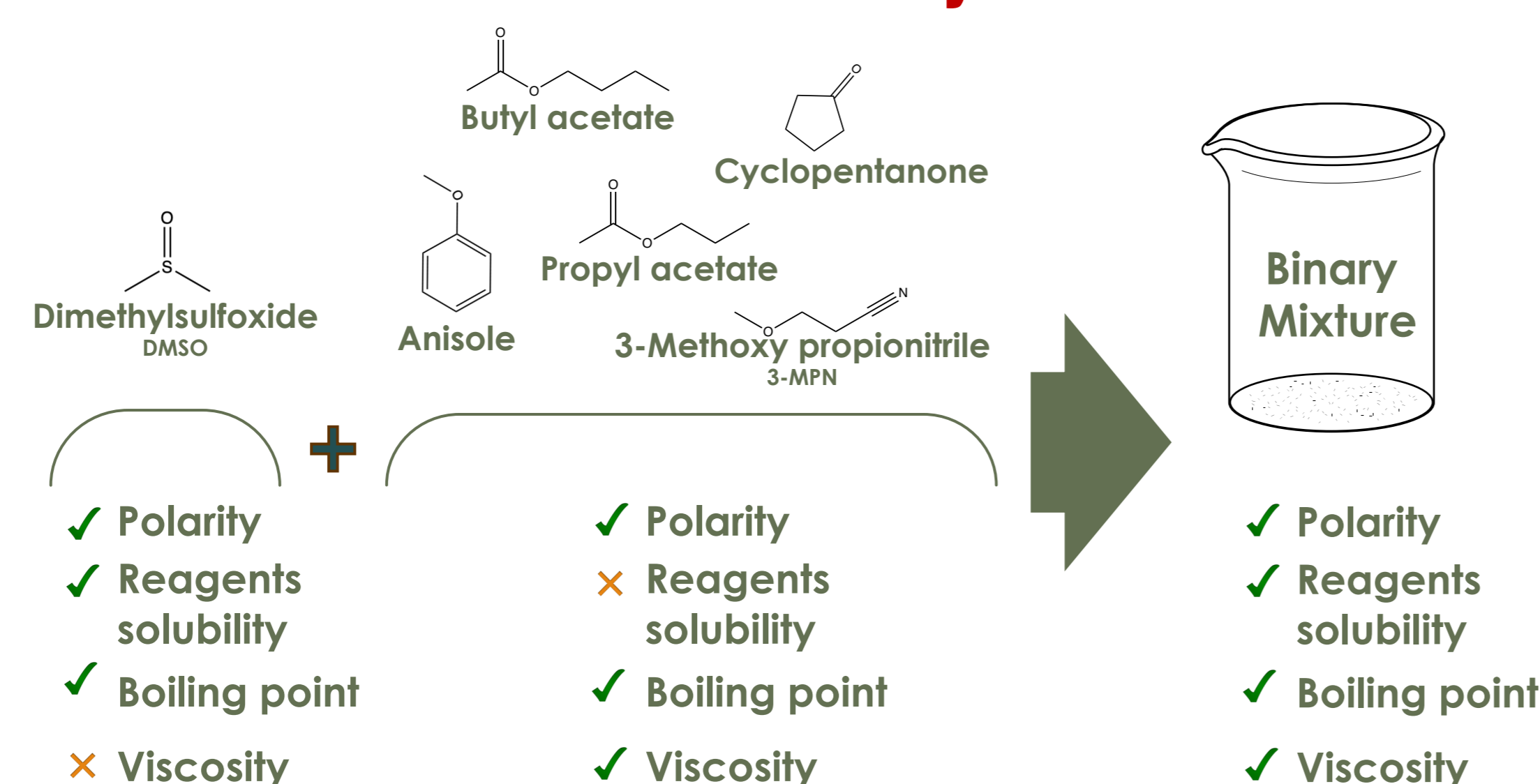
Harmful in contact with skin or if inhaled. Causes serious eye irritation

Thus, on December 12th, 2023, the European Commission has taken regulatory action to amend Annex XVII of REACH (Registration, Evaluation, Authorization and Restriction of Chemicals), imposing restrictions on the utilization of the solvent *N,N*-dimethylformamide (DMF) within the EU market, owing to its well-established high toxicity.

DMF replacement: Search of Neat or Binary Solvents

Selection Criteria

- Non-toxic
 - Polarity
 - Reagent Solubility
 - Boiling point
 - Viscosity
 - Polar & Aprotic
- ↓
- Sustainable efficient Green SPPS



Binary Mixtures - Solubility and Swelling test

Neat Solvents – Fmoc Amino Acids solubility

	Fmoc-His(Trt)-OH (M)	Fmoc-Gln(Trt)-OH (M)	Fmoc-Gly-OH (M)	Fmoc-Phe-OH (M)	Fmoc-Arg(Pbf)-OH (M)
cyclopentanone	<0.1	≥0.4	0.3	<0.1	<0.1
3-methoxypropionitrile	<0.1	≥0.4	<0.1	<0.1	<0.1
anisole	<0.1	<0.1	<0.1	<0.1	<0.1
propyl acetate	<0.1	0.2	<0.1	<0.1	<0.1
butyl acetate	<0.1	<0.1	<0.1	<0.1	<0.1
DMSO	≥0.4	≥0.4	≥0.4	≥0.4	≥0.4

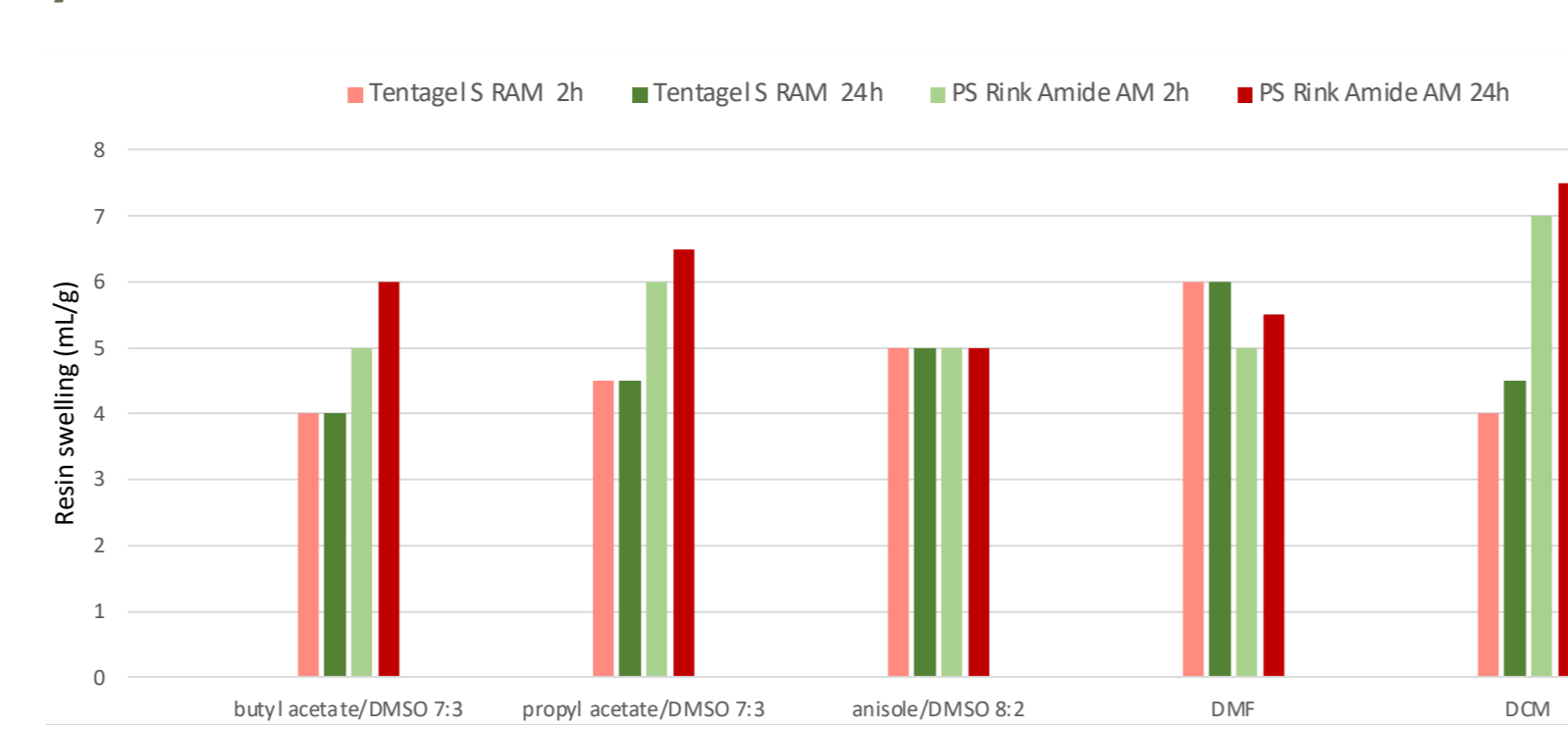
Binary Solvent Mixtures – Fmoc Amino Acids solubility

	Fmoc-His(Trt)-OH (M)	Fmoc-Arg(Pbf)-OH (M)	Fmoc-Gln(Trt)-OH (M)	Fmoc-Gly-OH (M)	Fmoc-Phe-OH (M)
cyclopentanone/DMSO 7:3	≥0.4	≥0.4	≥0.4	≥0.4	≥0.4
butyl acetate/DMSO 7:3	≥0.4	≥0.4	≥0.4	≥0.4	≥0.4
propyl acetate/DMSO 7:3	≥0.4	≥0.4	≥0.4	≥0.4	≥0.4
3-MPN/DMSO 7:3	0.2	≥0.4	≥0.4	0.2	<0.1
anisole/DMSO 8:2	≥0.4	≥0.4	≥0.4	≥0.4	≥0.4

Binary Solvent Mixtures – Coupling Reagents solubility

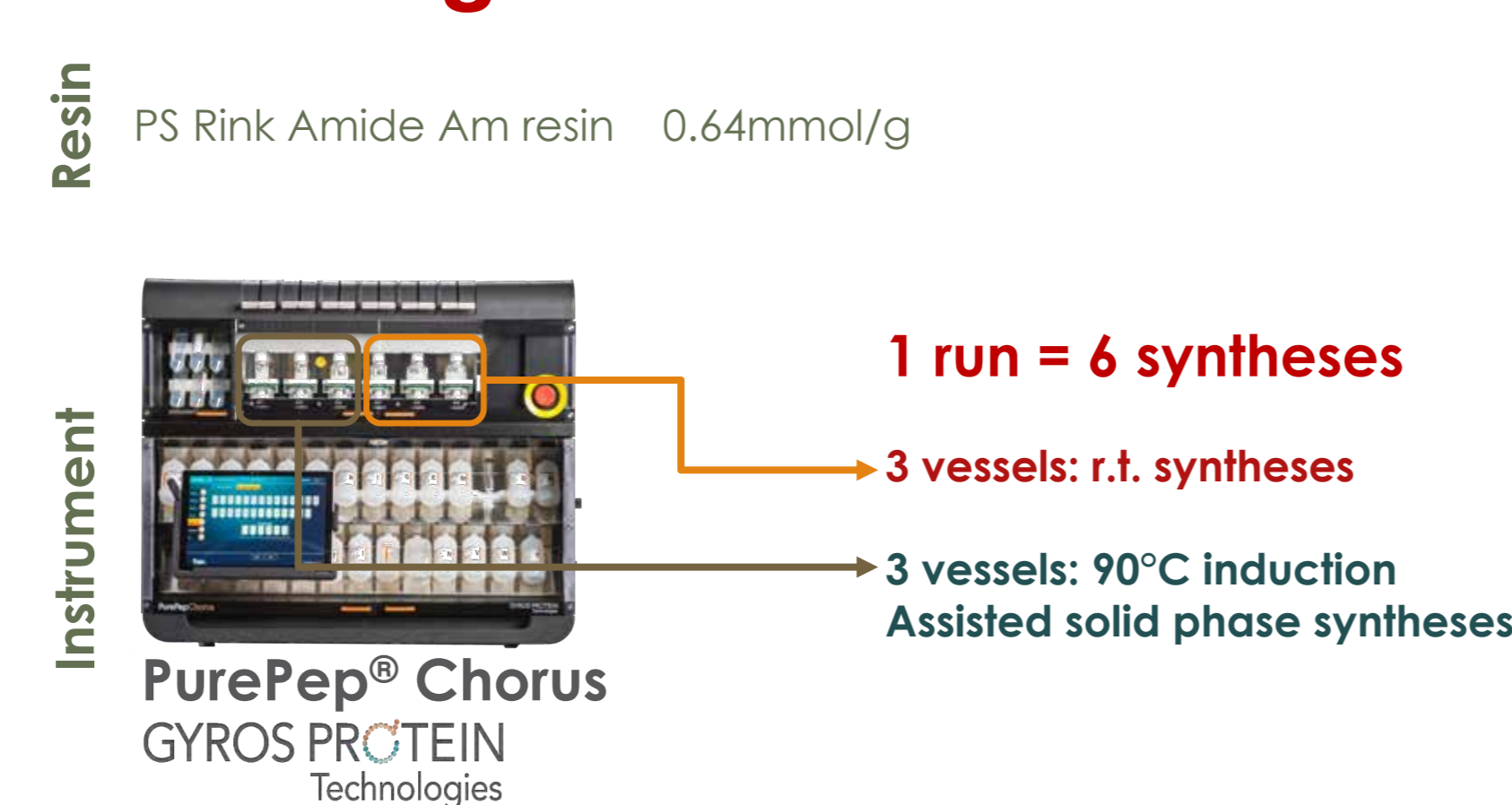
	HATU (M)	Oxyma Pure (M)	DIC (M)
Neat Solvents			
anisole	<0.1	≥1	≥1
butyl acetate	<0.1	≥1	≥1
propyl acetate	<0.1	≥1	≥1
Binary Mixtures			
anisole/DMSO 8:2	0.2	≥1	≥1
butyl acetate/DMSO 7:3	<0.1	≥1	≥1
propyl acetate/DMSO 7:3	<0.1	≥1	≥1

Binary Solvent Mixtures – Resin Swelling test



SPPS Experiment Design

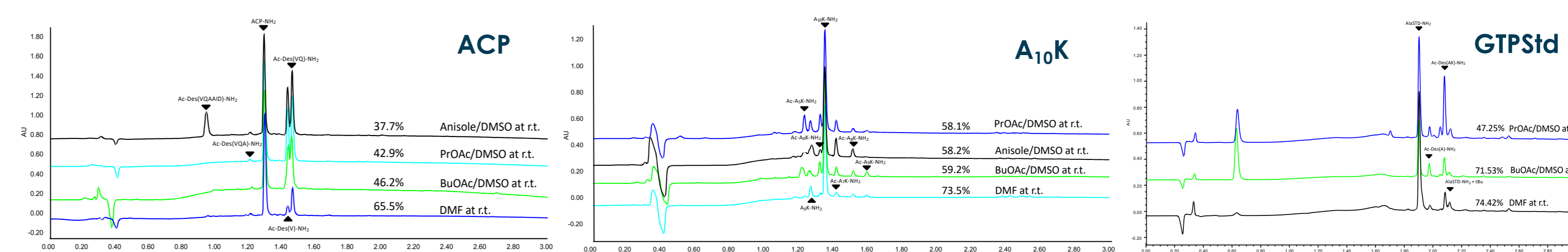
Coupling: Fmoc-AA/DIC/Oxyma Pure 5/5/5 equivalents
Fmoc- deprotection: 20% (v/v) Piperidine in Binary Mixture(1:1)
Coupling: r.t., 45' vs 90°C, 2'
Fmoc- deprotection: r.t., 5'+5' vs 90°C, 1'
ACP(Acyl Carrier Protein) (65-74): H-VQAAIDYING-NH₂
A₁₀K: H-AAAAAAAAAAK-NH₂
GTPStd: H-AKADEVSLHKWYG-NH₂



Operation	Solvent mixture	ratio	Solvent mixture	ratio	Solvent mixture	ratio
Fmoc-deprotection	PrOAc/DMSO/Piperidine	2:2:1	BuOAc/DMSO/piperidine	2:2:1	anisole/DMSO/piperidine	2:2:1
Coupling*	PrOAc/DMSO	7:3	BuOAc/DMSO	7:3	anisole/DMSO	8:2
Capping	PrOAc/pyridine/ acetic anhydride	15:16:19	BuOAc/pyridine/ acetic anhydride	15:16:19	anisole/pyridine/ acetic anhydride	15:16:19
Washing	EtOAc/DMSO	8:2	EtOAc/DMSO	8:2	EtOAc/DMSO	8:2

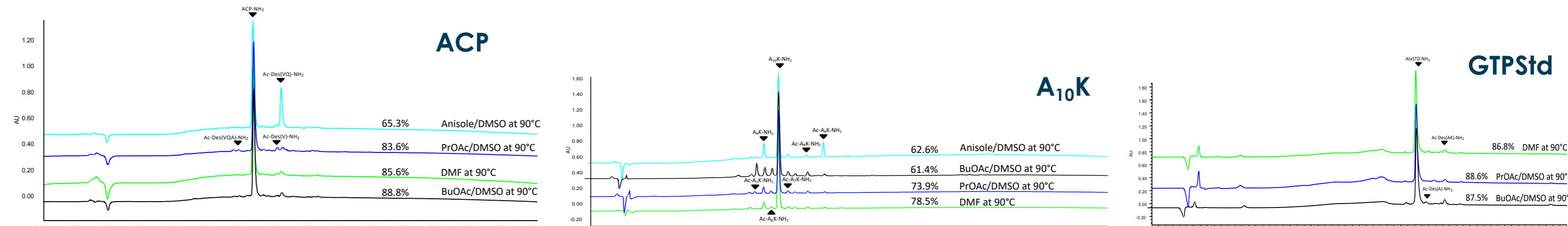
* Fmoc-amino acid 0.4M, DIC 1M, Oxyma pure 1M. Fmoc-His(Trt)-OH and Fmoc-Asn(Trt)-OH were solubilized in 6:4 acetate/DMSO and 7:3 anisole/DMSO mixtures because we observed precipitation after few hours from complete solubilization.

Model Peptides: Results at r.t.



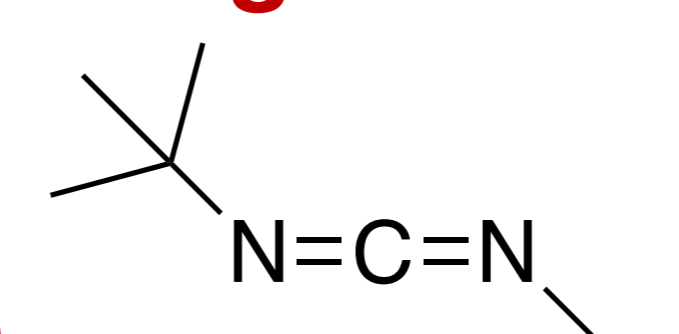
At r.t. results obtained using DMF are better, especially for ACP and A₁₀K, slightly better for GTPStd.

Model Peptides: Results at 90°C



At 90°C, results obtained with DMF and with PrOAc/DMSO or BuOAc/DMSO are comparable, even better for ACP and GTPStd.

Testing T-Bec, A safe and green SPPS reagent



Peptide	DMF	BuOAc	Anisole	PrOAc
	90°C, 2'	DMSO 90°C, 2'	DMSO 90°C, 2'	DMSO 90°C, 2'
ACP VQAAIDYING-NH ₂	89.1	77.0	78.6	78.4
A10K AAAAAAAK-NH ₂	77.4	72.4	79.9	62.1
GTPStd AKAEVSLHKWYG-NH ₂	89.6	93.3	83.9	74.1

Let's stress the system with difficult sequences!

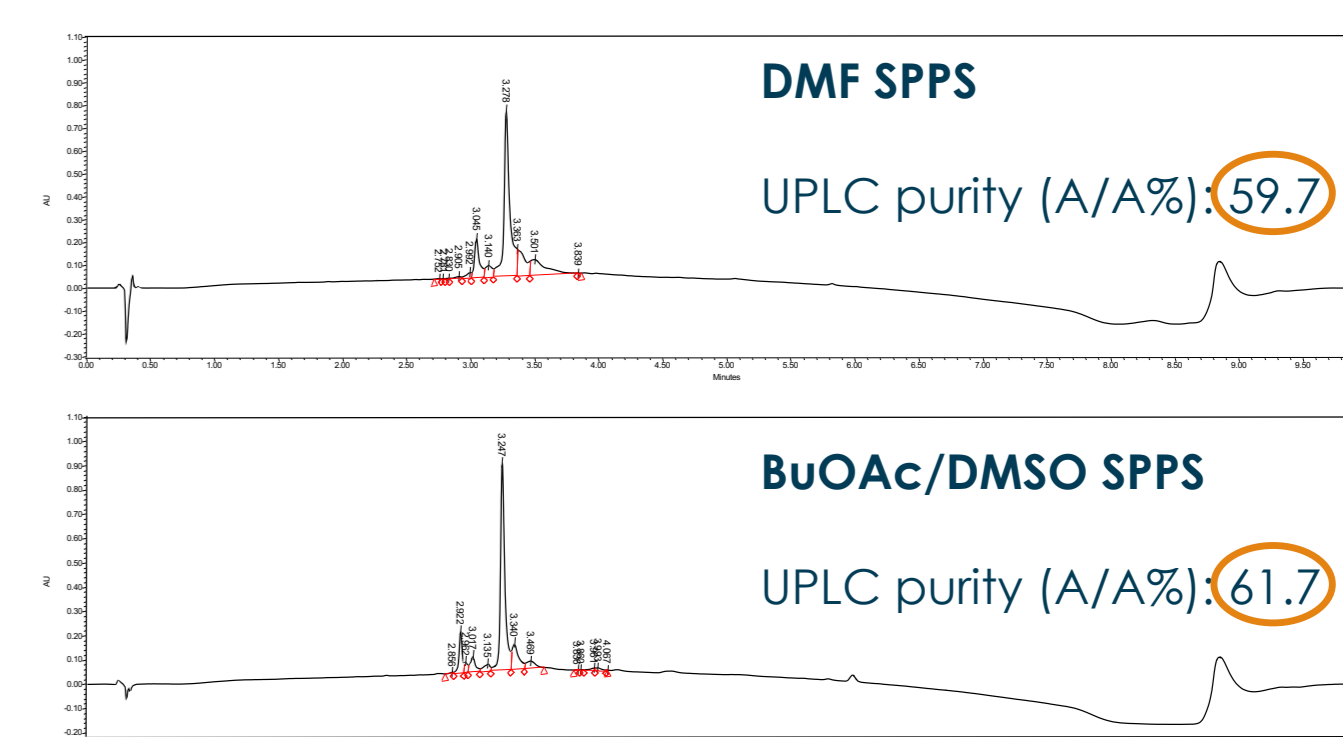
β-Amyloid(1-42)

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-NH₂

Length: 42 amino acids

Resin: Tentagel S RAM 0.20mmol/g

Synthesis at 90°C using BuOAc/DMSO or DMF



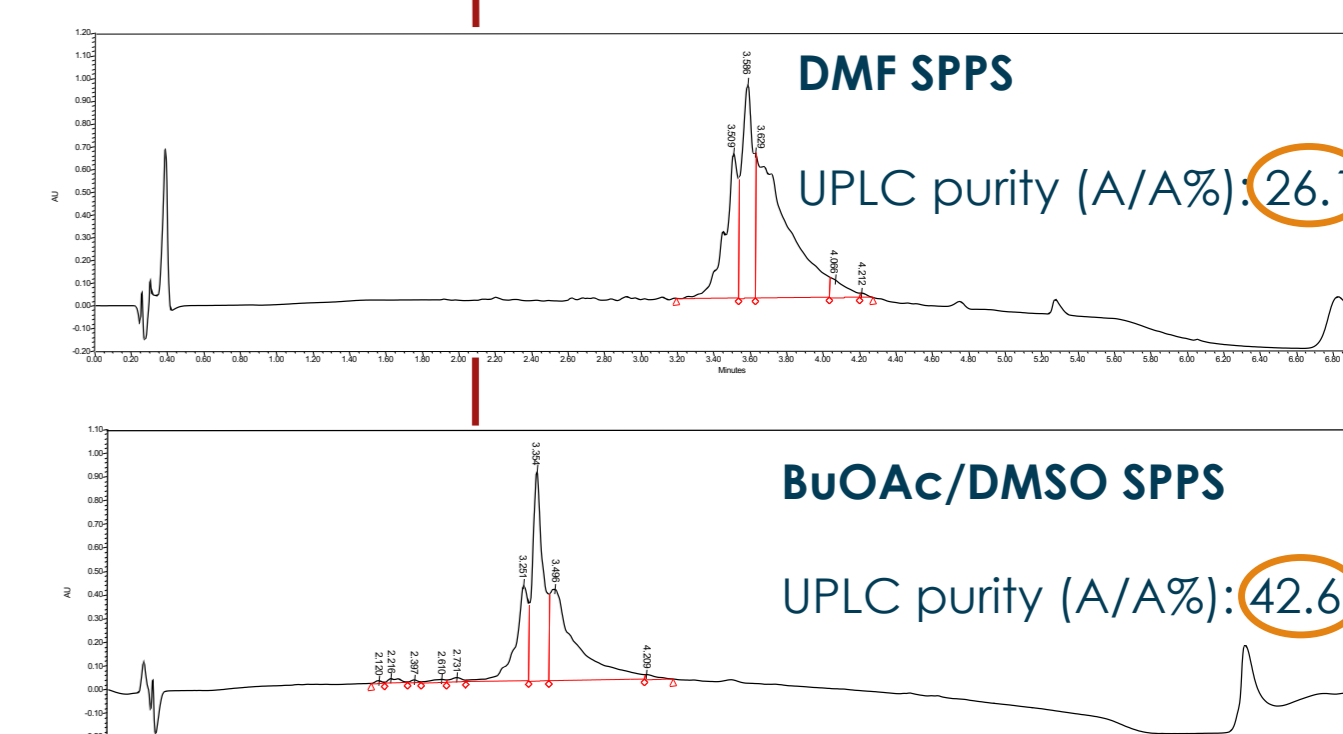
Sars CoV 2 Spike RBM₄₃₆₋₅₀₇

WNSNLDKSGVGGVNYLYRFLKSLNPKFERDISTEIVQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVYQP-NH₂

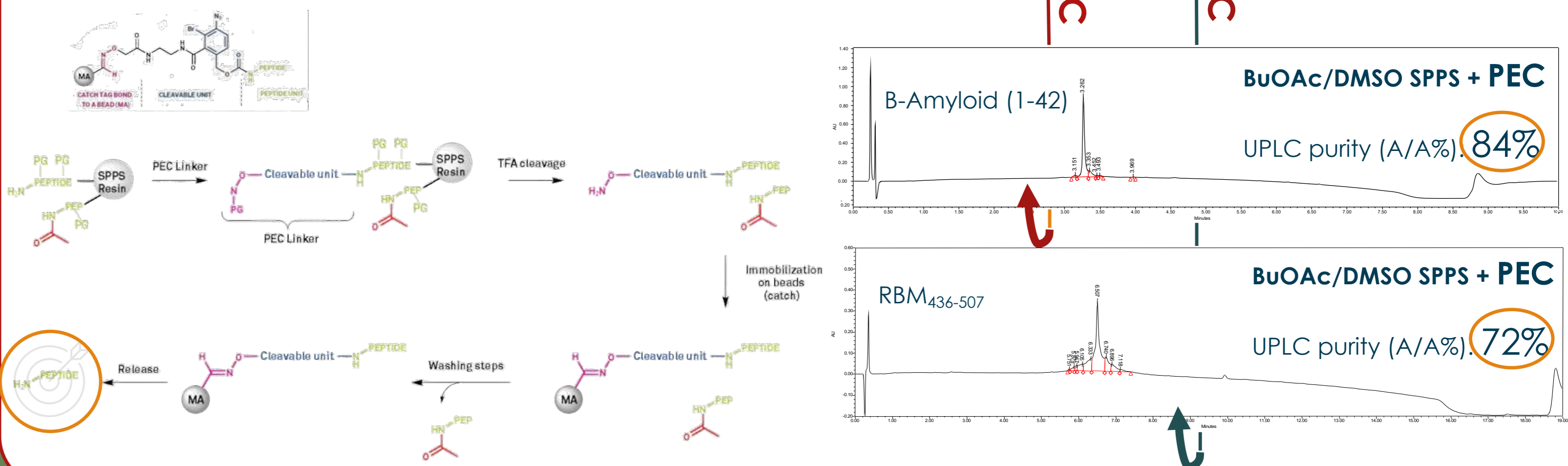
Length: 72 amino acids

Resin: Tentagel R RAM 0.19mmol/g

Synthesis at 90°C using BuOAc/DMSO or DMF



PEC - a catch and release HPLC-free purification



References & Acknowledgments

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