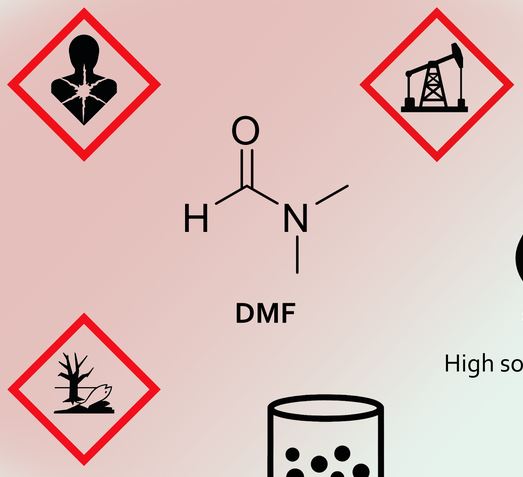
Glycerol-derived drop-in solvents for sustainable and more efficient peptide synthesis

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Background



- The growing demand for therapeutic peptides[1] calls for more **sustainable** synthetic approaches. **Solid-phase peptide synthesis** (SPPS) remains the method of choice due to its reliability and versatility.[2]
- *N*,*N*-Dimethylformamide (DMF), the most commonly used solvent, is classified as a substance of very high concern (SVHC) under REACH, emphasizing the **urgent need for greener, safer alternatives** in peptide manufacturing.[3]

Preliminary SPPS compatibility studies

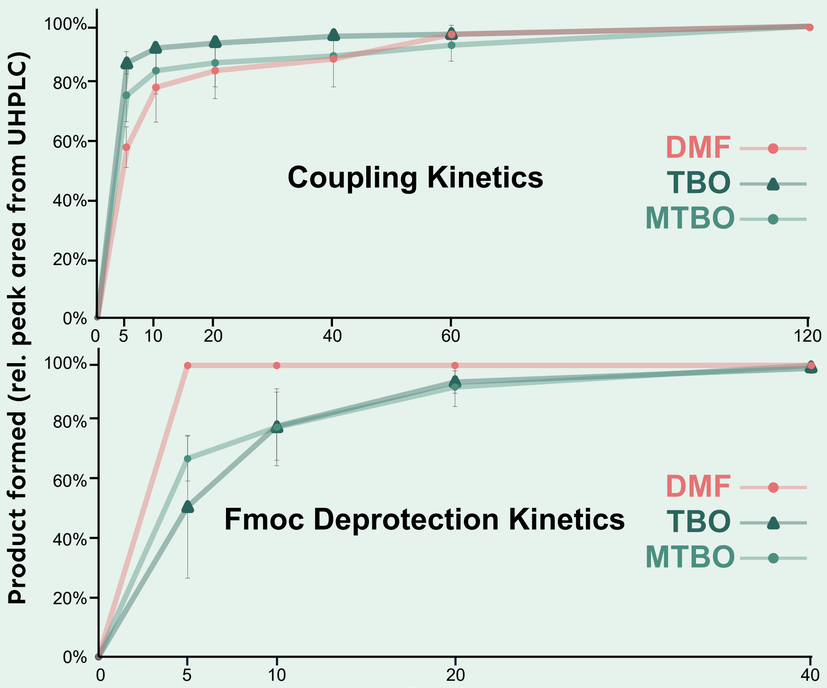
Reagent Solubility

20 canonical Fmoc amino acids (0.4 M)
Fmoc Rink Amide linker (0.2 M)
PyAOP (0.4 M)
HCTU (0.4 M)
DIC (1 M)
Oxyma (1 M)
Piperidine (20% *v/v*)
Pyrrolidine (20% *v/v*)

Resin Swelling

Polystyrene aminomethyl-RAM
Polystyrene-2CTC
TentaGel-RAM
TentaGel-PHB
NovaPEG-RAM
Li-RAM

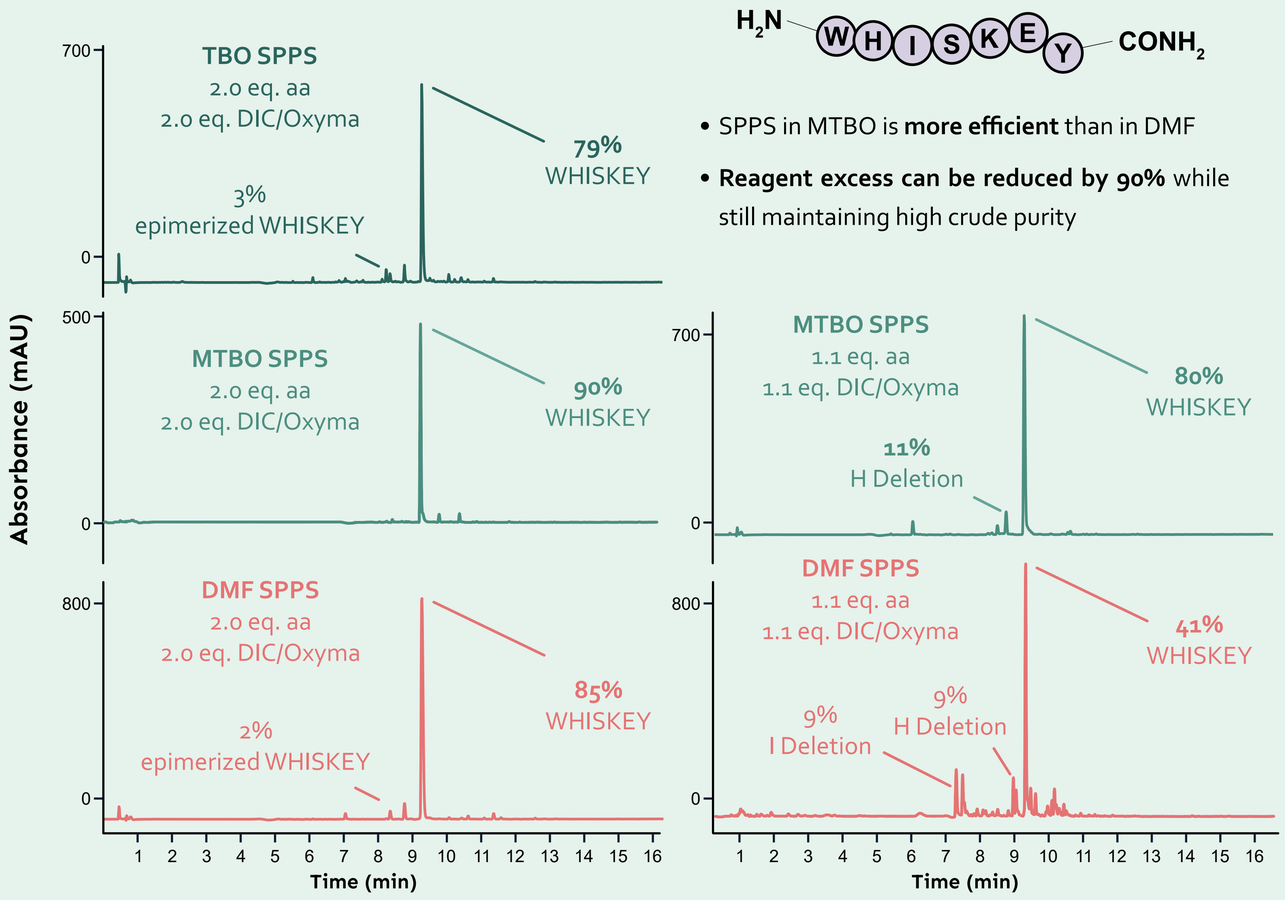
Reaction kinetics: Coupling and Fmoc deprotection



- Slightly **faster in (M)TBO** compared to DMF
- Standard protocol of 1 h per coupling remains unchanged

- Slower in (M)TBO compared to DMF
- Standard protocol of 2 x 5 min incubation for deprotection changed to **3 x 10 min**

SPPS with low reagent excess: MTBO outperforms DMF

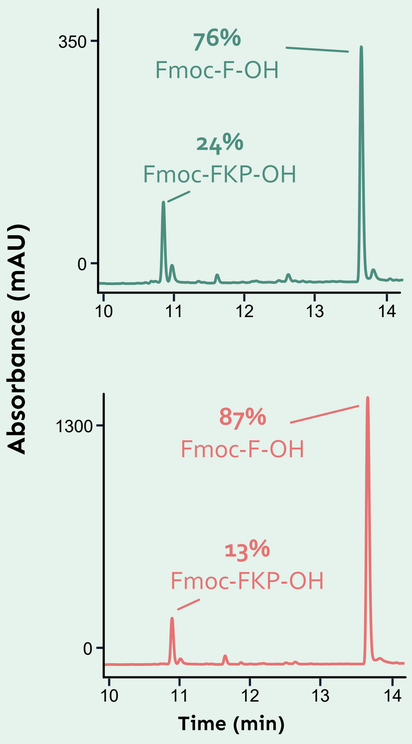


- SPPS in MTBO is **more efficient** than in DMF
- **Reagent excess can be reduced by 90%** while still maintaining high crude purity

Diketopiperazine formation

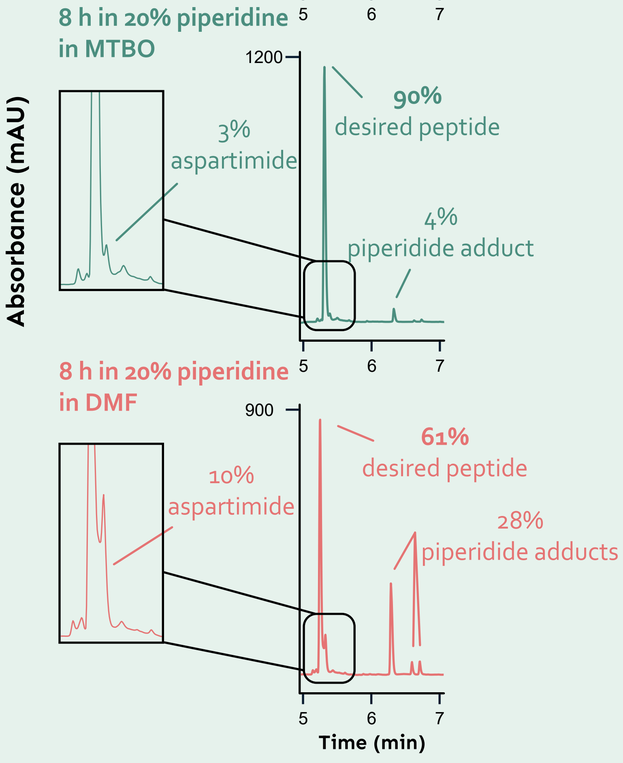


- Synthesis of **DKP prone motif** (Lys-Pro) on **TentaGel-PHB** resin
- Ratio of recovered full peptide (Fmoc-Phe-Lys-Pro) vs. liberated resin sites esterified with Fmoc-Phe for quantification

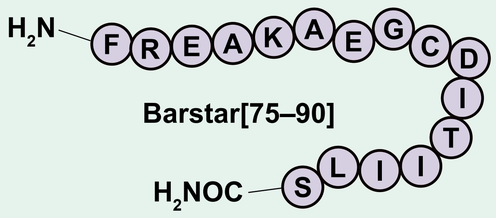


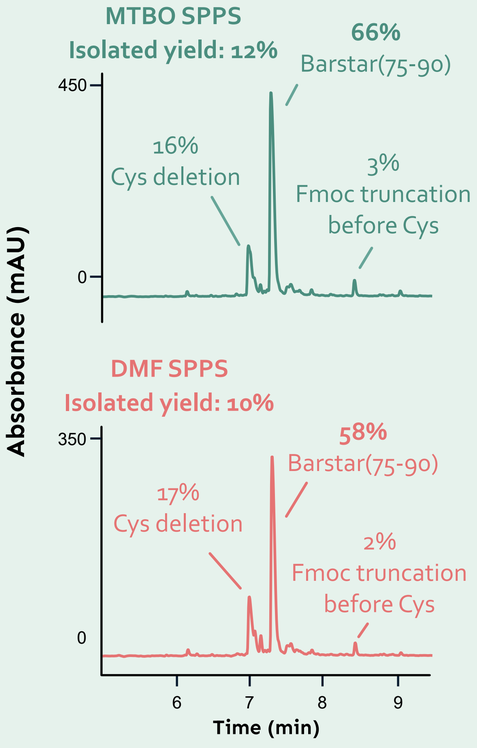
Aspartimide formation



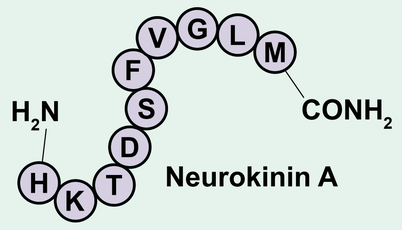


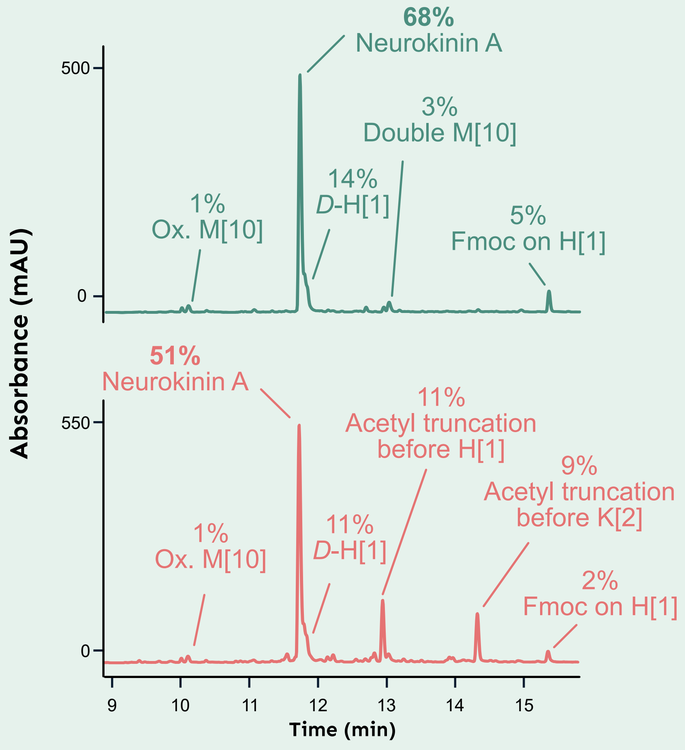
SPPS of a longer, aggregating peptide





Fully automated flow SPPS on PeptiPilot system





1 Topouzis, S *et al.*, *Br. J. Pharmacol.*, **2025,** 182, 1416–1445.

2 Merrifield, B., *J. Am. Chem. Soc.*, **1963**, 85 (14), 2149–2154.

3 Sherwood, J.; Albericio, F.; de la Torre, B. G. , *ChemSusChem*, **2024**, 17 (8).

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Conclusions

- Novel, bio-based & sustainable glycerol-derived solvents **compatible with SPPS**
- Ultra efficient coupling reaction enables **reduction of reagent excess**
- **Supression of notorious SPPS side-reactions** improves crude purity
- **MTBO outperforms DMF** in the synthesis of aggregating sequences & is **fully compatible with automated peptide synthesizers**