NBP is an equally good solvent as DMF for Microwave Assisted Solid Phase Peptide Synthesis[1]

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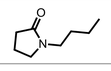
Introduction

Solid-Phase Peptide Synthesis (SPPS) is the prevailing method for synthesizing research peptides today. However, SPPS is associated with a significant environmental concern due to the utilization of hazardous solvents such as N,N-dimethylformamide (DMF) or N-methylpyrrolidone (NMP).[2-4] In light of this, our research endeavors to identify more environmentally friendly solvents for SPPS. In this study, we have assessed the suitability of five green solvents (Table 1) as alternatives to DMF in automated SPPS.

Table 1: Classification of the solvents in this study according to big pharma.

Solvent	Structure	Viscosity (MPa·s)	Pfizer	Sanofi	GSK	CHEM21/AstraZeneca
DMF (N,N-dimethylformamide)		0.80	Undesirable	Substitution requested	Major known issues	Hazardous
Cyrene (Dihydrolevoglucosenone)		13.80	-	-	Some known issues	Problematic
NBP (N-Butyl-pyrrolidone)		4.30	-	-	-	-
2-Me-THF (2-Methyltetrahydrofuran)		0.58	Usable	Recommended	Some known issues	Problematic
EtOAc (Ethyl acetate)		0.45	Preferred	Recommended	Few known issues	Recommended
DOL (1,3-Dioxolane)		0.59	-	-	Some known issues	Hazardous

It is worth noting that none of these selection guides have yet classified NBP and even though more research papers have investigated NBP since 2016, so far it is still considered green. Despite the similar structure to NMP. NBP is not toxic and display a completely different metabolic profile[5] and is non-reprotoxic, non-mutagenic, and biodegradable, with a lower skin and eye irritability compared to DMF[6-8].

To the best of our knowledge, all previous studies have had a smaller scope, focusing on binary mixtures, di-peptides, impurities, etcetera.[5,9-16] We believe that a more comprehensive study would be of benefit, especially focusing on finding a solvent or solvent mixture for peptide synthesis at elevated temperatures as that is the preferred method in our research.

Material and methods

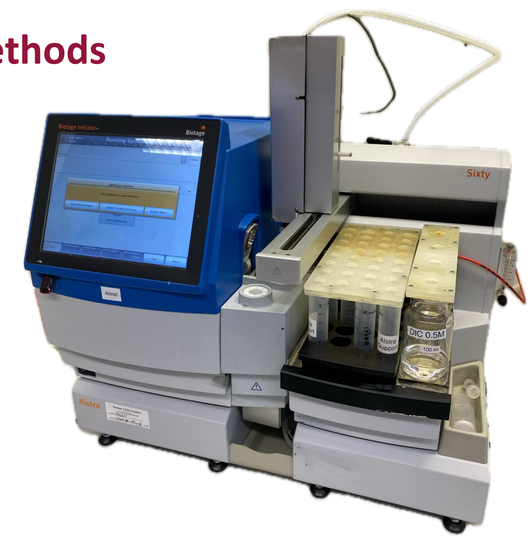


Figure 1: Biotage Initiator+ Alstra in our lab

Our investigation encompassed all stages of the synthesis process, from resin swelling using TentaGel S RAM (0.22 mmol/g), dissolution of reagents, and microwave-assisted synthesis of five diverse peptides using Biotage Initiator+ Alstra (Figure 1). For further method descriptions and synthesis conditions see QR-code.

This work

Based on:

References

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Result and discussion

In this study, we systematically evaluated 37 different solvents and solvent mixtures, including NBP, DOL, EtOAc, Cyrene, and 2-Me-THF, through a comparative analysis with DMF. The evaluation process encompassed initial resin swelling, where roughly half of the solvents/solvent mixtures proved ineffective. Subsequent dissolution of reagents and starting materials further narrowed down the selection. This resulted in the identification of eight solvents and solvent mixtures for testing in the microwave-assisted SPPS of our model peptide [Asp[5]]-Vasopressin.

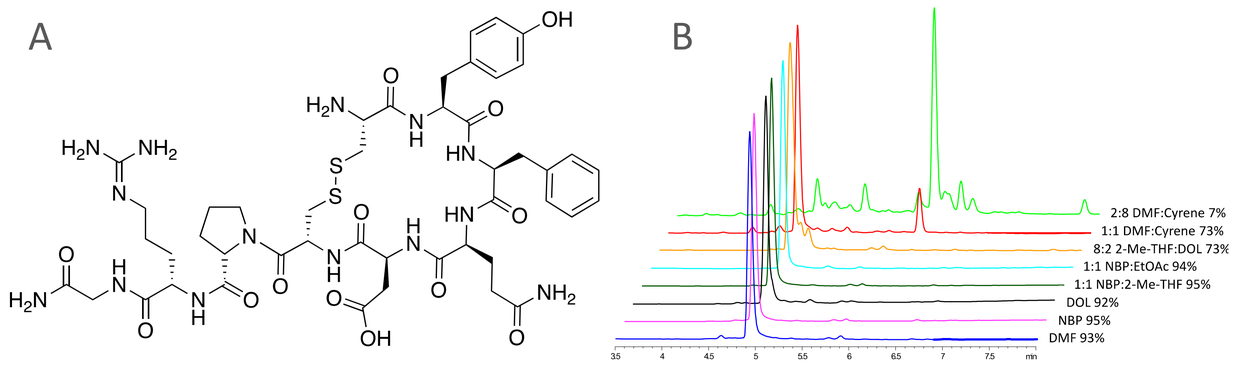

Figure 2A: Structure of the model peptide [Asp[5]]-Vasopressin. B: HPLC profile of crude [Asp[5]]-Vasopressin synthesized in different solvents. The main peak elutes at approximately 35% MeCN in H_2O.

Our findings illuminated that NBP, whether utilized in its pure form or in conjunction with EtOAc or 2-Me-THF, demonstrated performance on par with DMF in the synthesis of [Asp[5]]-Vasopressin (Figure 2 and Table 2).

With a successful protocol established, we tested NBP for synthesizing more complex peptides (Figure 3).

Table 2: Yields and purity of [Asp[5]]-Vasopressin synthesized in different solvents after one round of purification.

Synthesis solvent	Yield	Purity
DMF	85%	97%
1:1 DMF:Cyrene	39%	97%
2:8 DMF:Cyrene	-	-
DOL	79%	92%
NBP	88%	99%
8:2 2-Me-THF:DOL	86%	94%
1:1 NBP:EtOAc	87%	98%
1:1 NBP:2-Me-THF	87%	98%

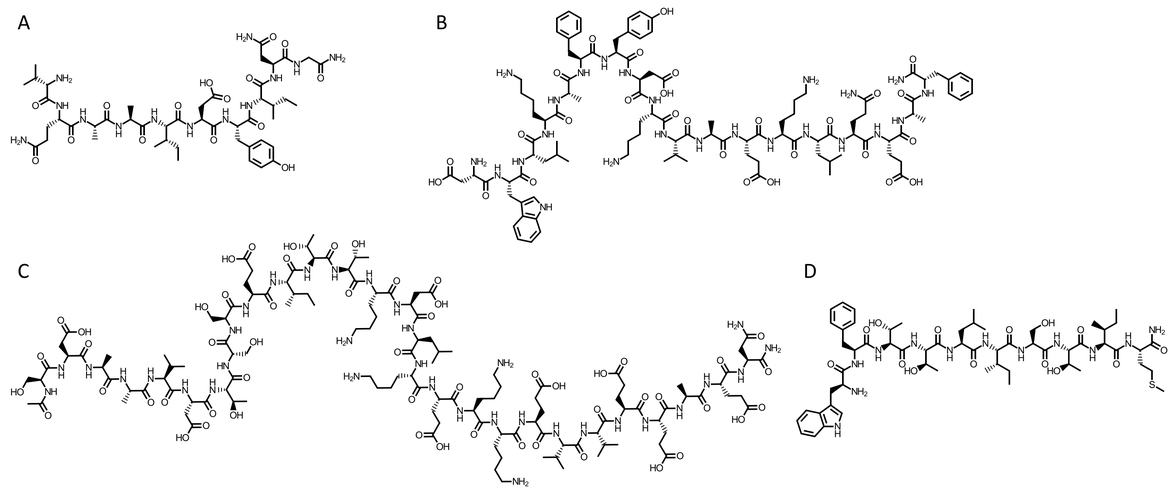

Figure 3: Structures of the four peptides synthesized with NBP. A: ACP(65-74); B: [Des-Ac]-18A; C: Thymosin a1; D: Jung-Redemann.

NBP proved comparable performance with DMF even in the microwave-assisted synthesis of the intricate peptides ACP 65-74, Peptide 18A, Thymosin α1, and Jung-Redemann (Figure 4).

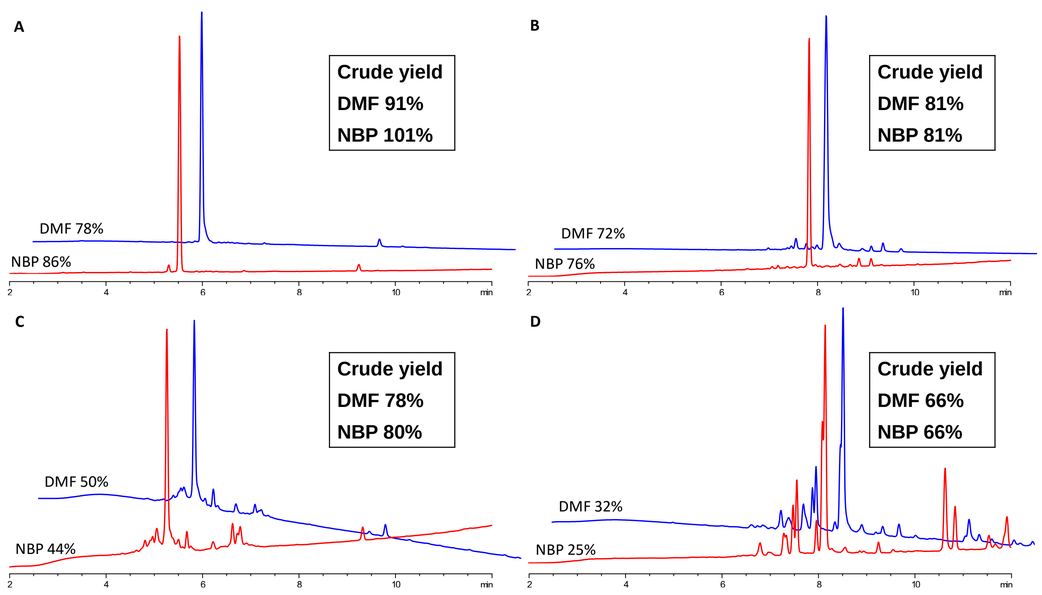

Figure 4A: HPLC profile of crude ACP (65-74). Synthesis in DMF in blue, NBP in red. The main peak elutes at approximately 43% MeCN in H_2O. B: HPLC profile of crude [Des-Ac]-18A. Synthesis in DMF in blue, NBP in red. The main peak elutes at approximately 58% MeCN in H_2O. C: HPLC profile of crude Thymosin a1. Synthesis in DMF in blue, NBP in red. The main peak elutes at approximately 41% MeCN in H_2O. D: HPLC profile of crude Jung-Redemann. Synthesis in DMF in blue, NBP in red.

Conclusion

Our findings indicate that NBP emerged as a strong contender, performing on par with DMF in all tested syntheses. With the insights gained from this study, we are positioned to phase out DMF from our SPPS processes, marking a significant step towards sustainability in our laboratory practices. This study not only informs our immediate practices but also propels us towards ongoing efforts to discover even greener methods for synthesizing peptides in the future.