TamiSolve™ NxG as a suitable solvent for cost-efficient green SPPS

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In recent years replacing hazardous solvents by greener alternatives has become a point of emphasis in chemical and pharma industries.[2] For example, in peptide synthesis great advances have been made in the past decades although it has been stated that more attention needs to be paid to the impact of solvents and reagents, used in this field, on health and environment.[3] Specifically, solid-phase peptide synthesis (SPPS) is a dominant synthetic methodology which has traditionally relied on use of large amounts of N,N-dimethyl formamide (DMF), a polar aprotic solvent with reprotoxic[4] and potentially carcinogenic characteristics.[5] Consequently, SPPS in which DMF is substituted for less harmful alternatives has become an active area of research in recent years.[6] Our aim with green SPPS is to put forth protocols suitable for large scale synthesis of pharmaceutical peptides that would not only replace DMF with green solvents, but improve the synthesis at the same time. To this end we set out to evaluate N-butylpyrrolidinone (NBP, cas nr. 3470-98-2) also known as TamiSolve™ NxG[7] as a solvent for green SPPS. NBP has been reported as a biodegradable alternative for DMF in several common synthetic transformations[8] and our interest in NBP was triggered by the fact that in NBP, Fmoc-Cys(Trt)-OH in the presence of coupling additive Oxyma was completely stable. This is a remarkable improvement over DMF, in which Fmoc-Cys(Trt)-OH/ Oxyma decomposes extensively, conceivably via an Oxyma N-oxyl radical induced mechanism (Figure 1).[9] During the course of our NBP evaluation a report on replacing DMF with NBP (1:1) in SPPS of Octreotide has been disclosed, in which NBP gave slightly inferior purity of the peptide (80 %) compared to DMF (86%).[10] Unfortunately, NBP as a stand alone SPPS solvent is too expensive to be considered suitable for cost-efficient peptide synthesis. We therefore set out to examine NBP in SPPS together with less polar, inexpensive green cosolvent(s). We envisioned that this approach would result in inexpensive solvent systems well suited for SPPS on PS resins commonly used in large scale manufacturing. As a model substrate we chose the Aib-ACP 10 mer **1**, which in a recent green synthesis on PS resin gave the target molecule in 54% purity.[6b]

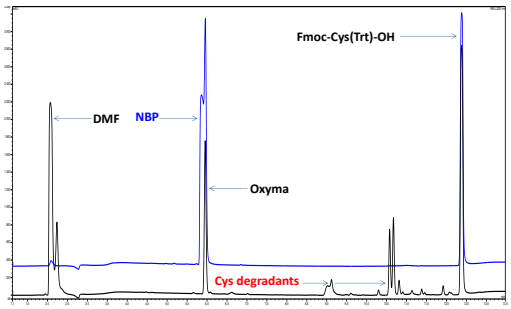


Figure 1. HPLC of 0.1M Fmoc-Cys(Trt)-OH/Oxyma in DMF (**in black**) and in NBP (**in blue**) after 72 h at rt. For identities of Cys degradants in the DMF sample see ref 6a.

H-Val[1]-Gln[2]-Aib[3]-Aib[4]-Ile[5]-Asp[6]-Tyr[7]-Ile[8]-Asn[9]-Gly[10]-NH_2 (**1**)

We chose to examine the most difficult part of the Aib-ACP SPPS (Val[1]-Aib[4]) for which we used a Fmoc-5-10 resin prepared by our earlier green SPPS protocol relying on the use of DMSO/EtOAc as the solvent system.[5a] Throughout this study we used 4-methylpiperidine (4-MP) for all de'Fmocings, Oxyma/DIC for all couplings and mild conventional heating (40 – 45 °C) to attain suitable SPPS kinetics.[11]

In our first experiment we examined four coupling solvents i) DMF ii) NBP iii) NBP/EtOAc (1:9) and iv) DMSO/EtOAc (1:9), keeping DMF for all de'Fmocings and washes (Table 1). This experiment revealed that both DMSO/EtOAc (entry 2) and NBP/EtOAc (entry 4) constitute better coupling solvents for Aib-ACP SPPS than DMF (entry 1) and NBP (entry 3) do. We next examined solvents for Fmoc deprotections, using NBP/EtOAc as the solvent for couplings but changing the NBP/EtOAc ratio from 1:9 to 1:1 to ensure suitable solubility of all common AA derivatives at sufficient concentrations (>~0.2 M). We tested DMF, NBP, NBP/EtOAc (1:1) and NBP/EtOAc (1:4) as de'Fmocing solvents (Table 2) and found that both NBP/EtOAc mixtures (entries 3 and 4) gave higher purities of Aib-ACP than DMF (entry 1) and NBP (entry 2) did. It is worth noting that the crude peptides from the NBP/EtOAc runs did not contain any peaks which were not present in the crudes using DMF and NBP as solvents (Figure 2). On the other hand, all four crudes contained comparable amounts of Ac-4-10-NH_2 (3 – 4 %), conceivably due to the background acetylation during the Aib[3] coupling caused by the presence of AcOH in EtOAc. In an attempt to suppress the Ac-4-10-NH_2 impurity we reexamined solvents for couplings, in which we tested 3 green coupling solvent mixtures NBP/EtOAc, NBP/*i*-PrOAc and NBP/MeCN respectively (Table 3). Importantly, AcOH content in all solvents in this experiment was determined to be quite negligible, below the LOQ of 50 ppm. We used NBP/EtOAc 1:1 as de'Fmocing solvent throughout and found that NBP/*i*-PrOAc and NBP/MeCN as coupling solvents gave comparable purity of Aib-ACP (entries 2 – 3),[12] both of which were higher than the purity obtained with NBP/EtOAc (entry 1). Compared to the amounts of Ac-4-10-NH_2 seen in Figure 2 the content of this Ac truncation in the three crudes in Table 3 was significantly reduced, down to the levels which can be explained by the presence of minute amounts of AcOH in AA derivatives.[13] Finally, having found that NBP/EtOAc constitutes a suitable solvent for de'Fmocings and NBP/*i*-PrOAc or NBP/MeCN are suitable solvents for couplings we set out to examine green solvent wash protocols. As washes account for the vast majority of solvent consumption in SPPS we chose not to investigate NBP, which would be too costly if used in the large amounts needed for the washes. Instead, we attempted to i) omit the washes after couplings ii) use EtOAc instead of DMF in washes after de'Fmocings (Table 4) to render the washing as simple and inexpensive as possible.[14] These experiments showed that solvent washes after couplings can be fully eliminated without any erosion in purity of Aib-ACP (entries 1 and 2 vs 3 and 4). On the other hand, replacing DMF with EtOAc as solvent after de'Fmocings resulted in a slight decrease in Aib-ACP purity (entries 1 and 3 vs 2 and 4). In fact, this observation is in a good agreement with our previous evaluation of green solvent washes after de'Fmocings in which we determined that neat EtOAc is inferior to DMF whereas using 2% DMSO in EtOAc comparable crude purities as with DMF can be achieved.[6a] It is worth stating that 86.3% attained in Table 4, entry 3 synthesis is to our knowledge the highest purity of Aib-ACP synthesized by a fully green SPPS protocol on a PS resin.

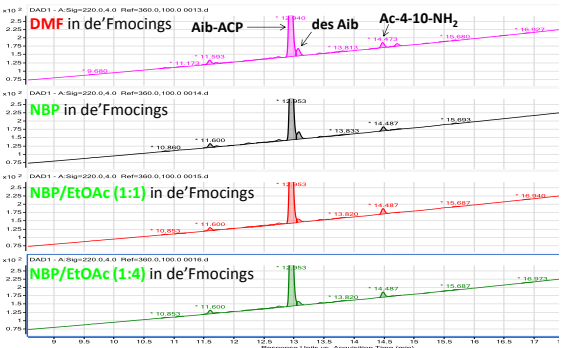
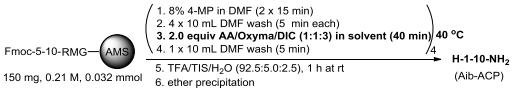


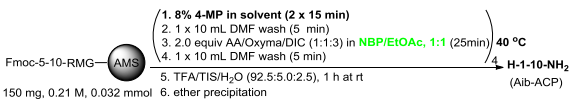
Figure 2. LC-HRMS overlay of four Aib-ACP crudes synthesized using different solvents in de'Fmocings, see Table 2.

Table 1. SPPS of Aib-ACP using different coupling solvents



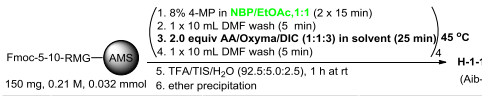
Entry	Coupling solvent	LC-HRMS purity (%)	des Aib (%)
1	DMF	75.2	12.8
2	DMSO/EtOAc, 1:9	**84.9**	**2.6**
3	NBP	76.4	10.4
4	NBP/EtOAc, 1:9	**83.6**	**1.4**

Table 2. SPPS of Aib-ACP using different de'Fmocing solvents



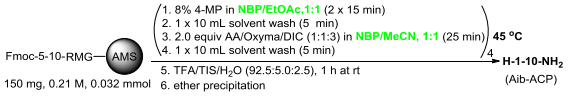
Entry	de'Fmocing solvent	LC-HRMS purity (%)	des Aib (%)
1	DMF	77.7	4.8
2	NBP	81.0	5.3
3	NBP/EtOAc, 1:1	**83.0**	**2.1**
4	NBP/EtOAc, 1:4	82.5	2.5

Table 3. SPPS of Aib-ACP using different coupling solvents II



Entry	Coupling solvent	LC-HRMS purity (%)	des Aib (%)
1	NBP/EtOAc, 1:1	83.0	2.5
2	NBP/*i*PrOAc, 1:1	88.8	1.9
3	NBP/MeCN, 1:1	**88.5**	**1.9**

Table 4. SPPS of Aib-ACP using different solvent wash protocols



Entry	Solvent wash after de'Fmocing	Solvent wash after coupling	LC-HRMS purity (%)[2]	des Aib (%)
1	DMF	DMF	89.5	3.2
2	EtOAc	DMF	84.4	2.5
3	DMF	none	90.5	1.2
4	EtOAc	none	**86.3**	**1.9**

In summary we examined TamiSolve™ NxG (NBP) as a solvent for green SPPS and found that NBP with inexpensive, common green solvents such as EtOAc and MeCN constitutes a highly competent solvent system which in Aib-ACP SPPS performs better than both DMF and NBP. As a solvent for washes after chemical steps in SPPS we propose to omit washes after couplings and use EtOAc with small amount of a polar cosolvent such as DMSO or NBP for washes after de'Fmocings.[6a] This green SPPS approach combines the use of solvents which work well in the chemical steps of the synthesis with inexpensive solvent washing, rendering the methodology well suited for cost-efficient peptide manufacturing.

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[1] Correspondence to jap@polypeptide.com.
[2] See e.g. Byrne, F. P.; Jin, S.; Paggiola, G.; Petchey, T. H. M.; Clark, J. H.; Farmer, T. J.; Hunt, A. J.; McElroy, C. R.; Sherwood, J. *Sustain. Chem. Process.* **2016**, *4*, 1.
[3] Datta, S.; Sood, A.; Török, M. *Current Org. Synth.* **2011**, *8*, 262.
[4] https://www.echa.europa.eu/documents/10162/5d7b909d-ef93-4fb3-8ca0-60e4e799e0e6
[5] http://monographs.iarc.fr/ENG/Publications/internrep/14-002.pdf
[6] See for example a) Pawlas, J.; Lundqvist, M.; Svensson, T.; Nilsson, M.; Rasmussen, J. H.; Poster P-280 at 25th American Peptide Symposium, June 2017, Whistler, BC, Canada. b) Kumar, A.; Jad, Y. E.; El-Faham, A.; de la Torre, B. G.; Albericio, F. *Tetrahedron Lett.* **2017**, *58*, 2986. c) Lawrenson, S. B.; Arav, R.; North, M. *Green. Chem.* **2017**, *19*, 1685. d) Kumar, A.; Jad, Y. E.; Collins, J. M., Albericio, F.; de la Torre, B. *ACS Sustainable Chem. Eng.*, **2018**, Just Accepted.
[7] Clark, J.H.; Hunt, A.; Topi, C.; Paggiola, G.; Sherwood, J. *Sustainable Solvents: Perspectives from Research, Business and International Policy (Green Chemistry Series), page 164;* Royal Society of Chemistry: London, 2017.
[8] Sherwood, J.; Parker, H. L.; Moonen, K.; Farmer, T. J.; Hunt, A. J. *Green. Chem.* **2016**, *18*, 3990.
[9] For an earlier assessment of stability of Fmoc-Cys(Trt)-OH under different conditions see ref 6a.
[10] Lopez, J.; Pletscher, S.; Aemissegger, Bucher, C.; Gallou, F. *Org. Process Res. Dev.* **2018**, *22*, 494.
[11] Experimental: A half of 4-MP and DIC was added at the outset of de'Fmocings and couplings respectively and the other half was added half way through; Aib[3] coupling was re-coupled; Boc-Val-OH was used for Val[1] coupling; Final Boc-1-10 resins were *i*-PrOH washed and dried *en vacuo* to constant weight prior to TFA cleavages. [2]LC-HRMS conditions: column, Waters CSH C18 1.7 um 2.1x150 mm; buffers: TFA/H_2O (0.1:100, A), TFA/MeCN (0.1:100, B); gradient: 94% B over 29 min, flow 0.25 mL/min, column temperature 30 °C.
[12] We chose to use NBP/MeCN as the coupling solvent in the ensuing experiments as MeCN is a more common, less expensive solvent than *i*-PrOAc.
[13] See f. ex. *Enhanced Specification Fmoc-amino Acids*, Novabiochem, **2014**.
[14] In the runs in which solvent wash after couplings was eliminated we quenched the coupling mixtures with 10% *i*-PrOH in EtOAc to prevent any interference of active esters from couplings with the subsequent de'Fmocings.