Rhodiasolv PolarClean – a greener alternative in solid-phase peptide synthesis

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**ABSTRACT**

PolarClean, a green solvent prepared through the valorization of a byproduct of Nylon-66 manufacturing, shows an excellent capacity to dissolve all Fmoc-amino acids and key coupling reagents and additives. It can also swell polystyrene and ChemMatrix, the two resins most widely used in solid-phase peptide synthesis. The synthesis of model peptides has been carried out, rendering the target peptide as a major component. The performance of PolarClean demonstrates its utility in the toolbox for Green Solid-Phase Peptide Synthesis.

1. Introduction

The industrial era focused on covering the needs of the population. In contrast, attention is now moving towards sustainability, in agreement with the 12 Principles of Green Chemistry (1) and the philosophy of the Circular Economy (2). In the chemistry arena, the use of solvents is probably the biggest challenge. The excessive use of solvents in chemical synthesis is ubiquitous and hence contributes immensely to industrial waste, accounting for 56% of the material used in the manufacturing of pharmaceutical agents (3). In this context, an ideal green solvent is non-toxic, biodegradable, recyclable, inexpensive, and non-volatile, and it is produced from renewable sources (4–7).

Solid-phase peptide synthesis (SPPS), the method of choice for the synthesis of peptides in tiny amounts for research purposes and also in multi Kg scale to cover the needs of the pharmaceutical industry, has many green characteristics (7). However, its main drawback is the use of solvents. In this regard, recent years have witnessed increasing research interest in green solvents compatible with the fluorenylmethoxycarbonyl (Fmoc)/\textsuperscript{\textit{t}}-butyl (tBu) SPPS scheme, like 2-Me-THF (8, 9), GVL (10, 11), NFM (12), NBP (13), PC (14) NOP (15) and also a mixture of solvents (16, 17).

In this context, here we studied the use of ethyl-5-(dimethylamino)-2-methyl-5-oxopentanoate (Rhodiasolv PolarClean, Figure 1) for all steps of SPPS. PolarClean is a green solvent industrially produced through the valorization of 2-methylglutaronitrile (MGN), which is a byproduct of Nylon-66 production (6). The burning of MGN, which is responsible for greenhouse gas emission, is overcome by chemical valorization of this compound. PolarClean is water-missile, biodegradable, eco-friendly, non-toxic, non-flammable, and non-volatile (6, 18, 19). It is commercially available and due to its high solvency property, it finds application as solvent, co-solvent for effective solubilization of chemicals, and...
crystal growth inhibitor in agrochemical formulations (18). PolarClean is not sensitizing for skin and a toxicological study found low acute toxicity (no observed adverse effect at 1000 mg kg\(^{-1}\) d\(^{-1}\) rat), and no evidence of carcinogenicity, genotoxicity, or mutagenicity (19, 20).

2. Experimental

2.1. Material and method

Rhodiasolv PolarClean, OxymaPure, and DIC were a gift from Luxembourg Biotech (Rehovot, Israel). All reagents and solvents were purchased from commercial suppliers and used without further purification. Fmoc amino acids and Fmoc-Rink Amide AM resin (loading 0.64 mmol/g) was supplied by Iris Biotech. Piperidine was purchased from Sigma-Aldrich. DMF, other organic solvents, and HPLC quality CH\(_3\)CN were purchased from Merck. Milli-Q water was used for RP-HPLC analyses. Analytical HPLC was performed on an Agilent 1100 system using a Phenomenex Aeris\textsuperscript{TM} C18 (3.6 μm, 4.6 × 150 mm) column, with a flow rate of 1.0 mL/min and UV detection at 220 nm. Chemstation software was used for data processing. Buffer A: 0.1% trifluoroacetic acid (TFA) in H\(_2\)O; buffer B: 0.1% TFA in CH\(_3\)CN. LC-MS was performed on a Thermo Scientific Dionex UltiMate 3000 using Phenomenex Aeris\textsuperscript{TM} C18 (3.6μm, 4.6 × 150 mm) column. Buffer A: 0.1% formic acid in H\(_2\)O; buffer B: 0.1% formic acid in CH\(_3\)CN.

2.2. Solubility

0.1 Mmol of protected amino acids, coupling reagents, additives were added to 1 mL of PolarClean solvent, with stirring until dissolution. Then, successive addition of 0.1 mmol of the reagent was added with stirring until reaching a concentration of 0.9 M or until the reagent could not be solubilized. In this case, successive amounts of 0.1 mL of PolarClean were added with stirring until complete solubilization.

2.3. Resin swelling

Resins (200 mg) were placed in a 5 mL syringe, treated with enough solvent to swell the resin, and allowed to stand for 12 h at RT / 3 h at 45°C. The swollen resin was compressed with the piston until no more solvent could be extracted. The piston was pulled slowly until the resin recovered its maximum volume in the syringe, and the volume of the resin was read (the void volume of the tip and the syringe was averaged to 0.15 mL). The swelling was calculated using the following formula: \( (\text{volume of the swelled resin} - 0.15 \text{mL}) / 0.2 \text{g} = \times(\text{mL/g}) \).

2.4. Procedure for SPPS

All model peptides were synthesized manually on a Fmoc-Rink amide AM-PS resin (0.1 mmol scale, loading 0.64 mmol/g) in polypropylene syringes fitted with a porous polyethylene disc, following a Fmoc/tBu strategy using DIC-OxymaPure as coupling method for 1 h. 20% piperidine in PolarClean or DMF (1 × 1 + 1 × 10 min) used to remove Fmoc. After coupling and Fmoc removal, the resin was washed with PolarClean or DMF (2 × 1 min after coupling and 3 × 1 min after Fmoc removal). The whole process was carried out at 45°C. Global deprotection and cleavage were done with TFA-triisopropylsilane (TIS)-H\(_2\)O (95:2.5:2.5) for 1 h at RT, and the peptide was precipitated with cold ether. After centrifugation, the solvent was removed by decantation and further cold ether was added, and the whole process was repeated three times. Crude peptides were analyzed by HPLC. HPLC gradients were: 5-60% B into A in 15 min for H-Tyr-Gly–Gly-Phe-Leu-NH\(_2\), 5-20% B into A in 15 min for H-Ala-Lys-Asp-Gly-Tyr-Ile-NH\(_2\), 0-5% B into A in 15 min for H-Lys-Thr-Thr-Lys-Ser-NH\(_2\).

3. Result and discussion

PolarClean is a polar aprotic solvent like DMF and NMP, both widely used in peptide synthesis, and other green solvents (NBP, GVL, etc.) (Table 1). Although, PolarClean (6 €/kg) is more expensive than DMF (1.5 €/kg) which is widely used solvent for SPPS, the increase in consumption of former will reduce the price as happens with all commodities. To the best of our knowledge, it has not been used for SPPS.

In terms of physical properties, PolarClean has a high boiling point (278–282°C) and low melting point (−60°C), and it is highly viscous (9.78 cP) (18, 21). The first two properties are ideal for SPPS because they allow for its use in automated syntheses with microwave-assisted or traditional heating. However, its high viscosity compared with other common solvents used in SPPS (Table 1) could impair its diffusion through the polymeric matrix and therefore preclude its use in this strategy.
Despite this adverse property and given the good performance of PolarClean in other chemical industrial applications (20–23), here we examined its compatibility in fluorenylmethoxycarbonyl (Fmoc)/tert-butyl (tBu) SPPS using PS resin, which is the most common and affordable solid support used. To be considered suitable for use in SPPS, solvents must show the good capacity to solubilize reagents and swell resin, and they must also be compatible with the conditions used for peptide coupling and protecting group removal. Reagent solubility is possibly the most limiting factor for the use of solvents in SPPS or any other chemical syntheses. With this in mind, we first evaluated the solubility of Fmoc-protected amino acids, coupling reagents and additives in this new green solvent. Most of the Fmoc-protected amino acids were highly soluble (>0.9 M), and all of them showed solubility >0.4 M (Table 2). The coupling additives, OxymaPure and HOBt, were also highly soluble (>0.9 M) while HOAt achieved a value of >0.54. Taking into consideration that 0.2 M is the concentration at which SPPS operates, only HBTU and HATU (<0.05 M) did not show compatibility, because COMU was also enough soluble (0.25 M) to be used.

Next, we investigated the swelling of resins for peptide synthesis, which is also a key parameter, because if the resin does not swell properly in solvents, accessibility of reaction site can be poor and reaction yield will drop down (8, 24). In the case of PolarClean, its high viscosity can hamper its diffusion inside the resin and thus jeopardize swelling. Maximum swelling was observed at 12 h at RT and at 3 h at 45°C. The polystyrene (PS) and ChemMatrix showed swelling values of

### Table 1. List of some solvents used for SPPS.

| Structure | Density (g/mL) | M.P. (°C) | B.P. (°C) | Viscosity (cP) | Dielectric constant |
|-----------|----------------|-----------|-----------|----------------|-------------------|
| PolarClean* | 1.04 | −60 | 278–282 | 9.78 | 29.9 |
| N,N-dimethylformamide (DMF)* | 0.95 | −31 | 153 | 0.92 | 38.0 |
| N-methyl-2-pyrrolidone (NMP) | 1.03 | −24 | 202 | 1.65 | 32.0 |
| N-Butylpyrrolidinone (NBP)* | 0.96 | <−75 | 241 | 4.00 | – |
| γ-Valerolactone (GVL) | 1.05 | −31 | 207–208 | 1.86 | 36.5 |
| 2-Methyl tetrahydrofuran (2-Me-THF) | 0.86 | −136 | 70–80 | 0.60 | 6.9 |
| N-Formylmorpholine (NFM) | 1.15 | 20–23 | 236–237 | 7.86 | – |
| Propylene carbonate (PC) | 1.20 | −48.8 | 242 | 2.80 | 65.0 |
| N-Octyl pyrrolidone (NOP) | 0.92 | −31.1 | 114–115 | 6.60 | – |

*Cost of polarClean (6 €/kg) is high compared to DMF (<1.5 €/kg), widely used solvent for SPPS but lower compared to that of NBP (∼8.5 €/kg).

### Table 2. Solubility of reagents (Fmoc-AA-OH and additive coupling reagents) in PolarClean at RT.

| Reagent | Solubility (M) | Reagent | Solubility (M) |
|---------|----------------|---------|----------------|
| Fmoc-Gly-OH | >0.9 | Fmoc-Glu(OrBu)-OH | >0.9 |
| Fmoc-Ala-OH | >0.9 | Fmoc-Arg(Pbf)-OH | 0.63 |
| Fmoc-Val-OH | >0.9 | Fmoc-Ser(tBu)-OH | >0.9 |
| Fmoc-Ile-OH | >0.9 | Fmoc-Thr(tBu)-OH | >0.9 |
| Fmoc-Phe-OH | 0.75 | Fmoc-Asp(OrBu)-OH | >0.9 |
| Fmoc-Leu-OH | >0.9 | Fmoc-Trp(Boc)-OH | >0.9 |
| Fmoc-Met-OH | >0.9 | Fmoc-Tyr(tBu)-OH | >0.9 |
| Fmoc-Pro-OH | >0.9 | HOAt | 0.54 |
| Fmoc-Lys(Boc)-OH | >0.9 | HOBt | >0.9 |
| Fmoc-Cys(Tt)-OH | 0.41 | OxymaPure | >0.9 |
| Fmoc-His(Tt)-OH | 0.41 | COMU | 0.25 |
| Fmoc-Asn(Tt)-OH | 0.41 | HATU | 0.05 |
| Fmoc- Gin(Tt)-OH | 0.63 | HBTU | 0.04 |
4.25 and 4.75 mL/g respectively, which are more than acceptable for SPPS (8, 25, 26) (Figure 2 and Table 3).

We next tested the efficiency of PolarClean (also compared with DMF) for SPPS. To this end, we targeted three model peptides: (A) H-Tyr-Gly-Gly-Phe-Leu-NH₂ (Leu enkephalinamide), (B) H-Ala-Lys-Asp-Gly-Tyr-Ile-NH₂ (fragment 1–6 of the toxin II from scorpion Androctonus australis Hector (27) and C) H-Lys-Thr-Thr-Lys-Ser-NH₂ (peptide-4 used as an anti-wrinkle agent in cosmetics) (28). These model peptides were synthesized on Fmoc-RinkAmide AM-PS (0.1 mmol scale, loading 0.64 mmol/g) using PolarClean for all synthetic steps, namely Fmoc removal, coupling, and washings. Coupling and Fmoc removal steps were performed at 45°C (because of the high viscosity of the solvent). Coupling was performed using a three-fold excess of equimolar Fmoc-AA-OH-N,N'-diisopropylcarbodiimide (DIC)-OxymaPure (1:1:1) with respect to resin, with 1 min pre-activation followed by coupling for 1 h. Fmoc removal was accomplished with a 20% piperidine solution in the respective solvent for 10 min. After global deprotection with trifluoroacetic acid (TFA)-triisopropylsilane-H₂O (9.5:2.5:2.5), the peptide was precipitated in ether and lyophilized. Analysis by high-performance liquid chromatography (HPLC) (Figure 3) showed that the target

### Table 3. Swelling of polystyrene and polyethylene glycol resins in PolarClean, NBP and DMF at RT.

| Entry | Solvents | Fmoc-Rink amide AMPS Swelling | H-Rink amide ChemMatrix Swelling |
|-------|-----------|-------------------------------|---------------------------------|
| 1     | PolarClean| 4.25                          | 4.75                            |
| 2     | NBP*      | 5.25                          | 5.25                            |
| 3     | DMF*      | 4.75                          | 5.75                            |

*Data extracted from ref 25.

Figure 2. Swelling of polystyrene and polyethylene glycol resins in PolarClean. AM-PS denotes aminomethyl polystyrene.

Figure 3. HPLC of peptide synthesis in PolarClean: (A) H-Tyr-Gly-Gly-Phe-Leu-NH₂ (5–60% B into A in 15 min), (B) H-Ala-Lys-Asp-Gly-Tyr-Ile-NH₂ (5–20% B into A in 15 min), (C) H-Lys-Thr-Thr-Lys-Ser-NH₂ (0–5% B into A in 15 min).
peptide was obtained as a major peak in all cases, with a clean chromatographic profile for further purification if required. In this regard, the performance of the synthesis using PolarClean was very similar to that using DMF. In the case of peptide B containing the sequence Asp-Gly which is used to study aspartimide formation (27), it is important to highlight that the absence of the β-peptide during analysis, which is the proof of the mentioned side-reaction. For peptides B and C (Figure 3), some small hydrophobic peaks were observed, which could correspond to the reaction of the methyl ester of PolarClean with the free amino group on the growing peptide chain during Fmoc removal, as also reported for other ester-based solvents such as PC (14, 29) and, to a lesser extent, GVL (10, 11). See Supplementary Information for HPLC comparison between PolarClean and DMF synthesis and mass spectra of the final peptides.

4. Conclusion

Here we have expanded the Green Solid-Phase Peptide Synthesis (GSPPS) toolbox. PolarClean is a suitable solvent for SPPS for short, hurdle-free peptides. It showed the excellent capacity to dissolve all 20 Fmoc derivatives of the proteinogenic amino acids and most of the coupling reagents and additives. Only HBTU and HATU were not compatible with PolarClean. Despite its high viscosity, this novel solvent showed the good capacity to swell the two resins most commonly used, namely polystyrene and ChemMatrix. The synthesis of model peptides using PolarClean as solvent rendered the target peptide as the major component, thus facilitating purification if required. In the context of green chemistry, we envisage that SPPS and other chemical processes will become ‘à la carte’ in the future, in the sense that more than one green solvent will be required. It might be great for the scale-up production of short peptides. Given this consideration, PolarClean is certain to find its place in the GSPPS toolbox.

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