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NOP/DMC as a Lead Solvent Platform for Lower-Burden Fmoc/tBu Peptide Manufacturing

Pavel Jungwirth[1,*]

Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, 160 00 Prague 6, Czech Republic

*Corresponding author: pavel.jungwirth@uochb.cas.cz

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Abstract

The Solid-phase Peptide Synthesis (SPPS) technology is required to manufacture all kinds of chemically-modified therapeutic peptides. However, the repetitive Fmoc/tBu procedure involves the consumption of vast amounts of swelling, coupling, deprotection, washing, cleavage, precipitation, purification, and cleaning solvents. The manufacturing problem solved in this paper is whether there is any greener solvent system which could minimize the liquid burden without compromising the quality of crude peptides, accessibility of resins, solubility of reagents, solvent recovery, and compatibility of the system with the equipment. The comparison includes traditional DMF/NMP/DCM approach and solvent systems comprising propylene carbonate, N-butylpyrrolidinone (NBP), DMSO/2-MeTHF, DMSO/DOL, N-octylpyrrolidone/dimethyl carbonate (NOP/DMC), neat NOP, PolarClean/water, dipropyleneglycol dimethylether (DMM), anisole/NOP, and N-formylmorpholine/anisole. The quantitative information encompasses peptide-process PMI values of about 3000–15000 kg kg^{-1} per the molecular mass of peptides of around 1000–5000 Da; contribution of solvents and washing to the total mass of process of about 80–90%; NOP/DMC 8:2 crude purity of 97.5% for Aib-enkephalin and >99% for linear octreotide; purity of neat NOP of Aib-enkephalin of 97.7% and 97.4% on Wang and trityl chloride resins; purity of NBP of >99% for AKDGYI-NH_2 at 45 °C; purity of DMSO/2-MeTHF of bivalirudin of 70.7%; purity of DMSO/DOL of bivalirudin of 72.7%; and purity of PolarClean/water of 86% for Leu-enkephalin amide. NOP/DMC is the best first choice for plant qualification due to the ability of the DMC fraction to reduce the viscosity of NOP; high peptide purity for both Aib-enkephalin and linear octreotide; and recovery of NOP, DMC, and piperidine. NBP, DMSO-based systems, PolarClean/water, DMM, anisole/NOP, and NFM/anisole retain the value for specific sequence classes and equipment. Nevertheless, their direct testing under resin-specific, impurity-specific, recovery-specific, cleaning-specific, and product contact-specific conditions is required.

Keywords: Solid-phase peptide synthesis; green solvents; process mass intensity; materials compatibility; solvent recovery; peptide purification; resin swelling; Fmoc/tBu chemistry; product-contact qualification.

1. Introduction

Peptides have emerged as a class of drugs with chemistry between small molecules and biologics [1]. Value of the peptides is provided by their specific binding, potency tunability, and possibility to address difficult-to-reach biological targets with the help of these agents [2]. Development trends for this class of therapeutics expand the scope of candidate molecules continuously [3]. Today many peptides include unnatural building blocks, modifications in their ends, lipid groups, disulfides, cyclization, N-methylation, PEGylation, stapling, and other modifications that improve exposure, targeting, and stability of the molecule [4]. These structural characteristics require chemical synthesis if no appropriate protecting group pattern can be delivered using recombinant protein expression [5]. Modern chemistry technologies allow also controlled introduction of non-natural amino acid residues and termini [6]. Manufacturing challenge of the peptides goes beyond the research scale and requires larger production quantities [7]. Peptide API manufacturing requires scalable and reproducible chemical route to produce peptide drug candidates [8].

SPPS serves as a key technology for the majority of the products due to the solid-supported peptide assembly that eliminates intermediate isolation as a need for separation by filtration and washing [9]. Subsequent developments resulted in broad application of the solid-phase principle in peptide synthesis [10]. Later research demonstrated applicability of the solid-phase assembly as a method for automated peptide manufacturing [11]. Introduction of Fmoc chemistry with a base-labile protecting group allowed combination of acid-labile side chain protection and the whole procedure of cleavage [12]. Further development of the Fmoc/tBu protocol established it as the method for routine synthesis [14]. Growing peptide chain remains immobilized on the resin, soluble impurities are washed away, and the process of Fmoc deprotection and amino acid coupling is repeated until the desired sequence is synthesized. This operational principle is convenient for automation, but it creates the environmental and material problem for peptide manufacture [15]. Every residue addition requires swelling solvent, deprotection solution with base, activated coupling mixture, drainage, and several wash steps [16].

Protected peptide will face with acidolytic cleavage, scavengers, precipitation solvent, purification mobile phase, and drying procedure [17].

Green chemistry metrics explain why the solvent part dominates the process. Atom economy defines how effectively reactant atoms become the part of the desired product [18]. Green chemistry framework includes this concept in the context of hazard prevention and waste minimization [19]. The E-factor defines how much waste is generated relative to the product mass [20]. Process mass intensity (PMI) is a particularly suitable metric for pharmaceutical processes as it defines material consumption relative to isolated product mass [21]. Holistic frameworks demonstrate how hazard and mass metrics should be used jointly to understand the process impact [22]. Green chemistry becomes the key concept in process research and development and not just an environmental calculation [23]. For the case of peptide production the product mass is relatively low compared with liquid volumes used for resin swelling, washing, cleavage, RP-HPLC, fraction concentration, and lyophilization. Assessments of peptide manufacturing processes have shown that peptide synthesis and purification can be characterized by extremely high material burdens [24]. Broader assessments of peptide sustainability reveal repetitive solvent use as a critical contribution [17]. PMI analysis has confirmed dominance of washing and solvent volumes in representative manufacturing process [25]. A solvent replacement that improves only the step of coupling has limited value if wash volume, crude impurity content, or purification demand will remain the same.

The current Fmoc/tBu SPPS relies strongly on N,N-dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), and dichloromethane (DCM). These solvents are widely used in peptide synthesis because of their capability to swell popular polystyrene and PEG-based resins, dissolve Fmoc-protected amino acids and coupling reagents, provide efficient Fmoc deprotection, and fit automatic synthesizers routines. Disadvantages of these solvents are significant. Solvent assessments in pharmaceutical industries define classic process solvents as primary replacement targets [26]. There are solvent selection guides for evaluation of the alternatives to the classic process solvents [27]. The CHEM21 guide expands comparison to the classic and more exotic solvents [28]. Additional tools provide evaluation criteria from environmental, safety, and practical points of view [29]. Industrial manufacturers continue identifying the solvent replacement as a key green chemistry research area [30]. Special reviews for greener SPPS arrive to the same conclusion [31]. Industrial perspective demonstrates that the solvent replacement should be assessed through the complete peptide process [32]. The solvent replacement problem is particularly challenging for SPPS since solvent is not only a reaction medium. It is also a swelling liquid, washing liquid, deprotection solvent, coupling solvent, drainage solvent, cleaning solvent, and recurrent liquid contact for process equipment.

Several solvent classes have been studied for this purpose. Propylene carbonate has been used as a solvent both for solution- and solid-phase synthesis and provides bradykinin purity close to DMF [33]. NBP has been studied as NMP-like solvent with high solvating power, useful properties of resins, and improved toxicological profile [34]. DMSO/2-MeTHF and DMSO/DOL are polarity-viscosity balanced solvent combinations [35]. NOP is a highly-efficient solvent for SPPS, and NOP/DMC is particularly valuable solvent combination since DMC reduces viscosity of neat NOP without loss of peptide purity and allows component recovery [36]. Circular aqueous Fmoc/tBu synthesis demonstrates applicability of the aqueous media for peptide manufacturing [37]. PolarClean demonstrates the opportunity to develop a greener solvent for some selected peptide sequences [38]. DMM provides additional solvent alternatives among ether-based systems [39]. Anisole/NOP is a solvent combination designed according to the coupling system compatibility [40]. NFM/anisole provides ultrasound-assisted and flow-compatible conditions [41].

Material perspective provides another criterion for solvent evaluation that pure synthetic studies often underestimate. Walls of reactor, filter frits, transfer tubes, valves, elastomeric seals, pump heads, column parts, storage vessels, and recovery systems are repeatedly wetted by fresh solvent, process- aged solvent, piperidine-containing deprotection solution, activated coupling solvent, cleavage cocktail, aqueous-organic mobile phase, and concentrated waste solvent. Such contacts can result in problems like swelling of elastomers and polymers, extraction, leaching, residue carrying-over, increased pressure, surface attack, increased difficulty of cleaning, and loss of dimensional tolerance. Immersion tests are the standard way to screen metals for corrosion [42]. Volumetric change of rubber samples provides similar test for rubber components when exposed to liquids [43]. Materials selection guidance is also available for corrosion-resistant alloys [44]. Corrosion resistance tables can be used for preliminary assessment of new solvent compatibility with certain materials before conducting route-specific test [45]. Residue limits must be established when new solvent is introduced to pharmaceutical manufacturing [46]. Good manufacturing practice requirements make cleaning, residue carrying-over, and contact with process fluids part of qualification process [47].

The research question is very narrow and manufacturing-oriented: which green solvent candidates will stay credible in Fmoc/tBu SPPS considering peptide purity, PMI lever, purification load, recoverability of solvents, and repetitive exposure of the equipment to these solvents? The paper will evaluate each solvent according to its actual tasks during the process execution. The solvent that demonstrates good hazard profile but decreases crude peptide purity or creates persistent

residues should not be considered as a better solvent. On the contrary, solvent that provides high crude purity and recoverable solvent streams will be a more powerful solvent despite the need of specific compatibility assessment prior scale-up.

This approach allows avoiding common weakness of many solvent substitution papers. Flash point, bio-based origin, and short-peptide chromatograms are good parameters, but they are insufficient. New manufacturing solvent must go through the same equipment repeatedly, dissolve the same reagents in process concentration, release the same residues to the waste or recovery system, and impact the volume of RP-HPLC solvent. The least risky route is the one that preserves peptide quality and reduces solvent volume, purification needs, and stress on the equipment.

2. Materials and Methods

2.1 Solvent Systems and Peptide Cases

The comparison is based on peer-reviewed literature of SPPS and green-solvent research that covers complete synthesis cycle, resin swelling, reagent solubility, purity of crude, solvent recovery, aqueous workup, process mass or material-specific handling aspects. The DMF-free green-solvent study provides essential pharmaceutical-grade peptide data [48]. Recent advancements in resins give difficult-sequence data relevant to scalable synthesis [49]. The solvent data comprises standard DMF/NMP/DCM, propylene carbonate, NBP, DMSO/2-MeTHF, DMSO/DOL, NOP/DMC, pure NOP, PolarClean/water, DMM, anisole/NOP, and NFM/anisole. The peptide data comprises bradykinin, Aib-enkephalin, linear octreotide, AKDGYI-NH_2, bivalirudin, Leu-enkephalin amide, Aib-ACP, ACP-derived peptides, and difficult sequence motifs to test peptide aggregation, side reaction, or deprotection behavior.

The numerical data cannot be averaged into universal solvent rank due to the differences between studies in peptide sequence, resin, resin loading, temperature, coupling reagent, deprotection base, solvent ratio, reaction time, chromatography, and calculation of purity of crude. Pooling data would lead to false precision. The data are thus employed as the record of the process associated with peptide and resin. Every solvent is evaluated by its ability to cover all the responsibilities of SPPS: resin swelling, amino acid solubility, solubility of coupling reagent, Fmoc removal, coupling, washing, compatibility with cleavage, crude purity, impurity control, purification effect, solvent recovery, cleaning efforts, and interaction with manufacturing materials.

2.2 Criteria for Solvent System Qualification

Solvent system can be considered as more qualified if it is capable of performing throughout the entire duty cycle of SPPS rather than being efficient in one particular reaction step. The qualitative calculation of solvent qualification can be defined as

$$S_s = w_1 C_s + w_2 Q_s + w_3 H_s + w_4 V_s + w_5 P_s + w_6 M_s, \tag{1}$$

where S_s is the total suitability of solvent system s, C_s is the capability for chemical cycle operations, Q_s is the crude quality and contaminant controls, H_s is the safety and handling improvements relative to DMF/NMP/DCM, V_s is the viscosity and drainage and automated operation considerations, P_s is the purification and recovery consideration, and M_s is the repeatability and compatibility with manufacturing materials. The weights are not constant because the proper weightage depends on the order, resin, scale, equipment train, and purification method. This calculation is a structured approach to making solvent selection multidimensional.

The interpretation of Eq. (1) is fairly obvious. Low hazard is not a substitute for poor resin swelling ability. Good swelling is not a substitute for poor removal and elastomer incompatibility. Purity of the crude solvent is not a substitute for a non-recoverable stream that results in residual contamination. The solvent is considered both as the chemical environment for the batch and as the repeating chemical environment for the wetted equipment.

2.3 Process Mass Intensity and Purification Assessment

PMI is calculated based on the standard formula

$$\mathrm{PMI} = \frac{\sum m_{\mathrm{input}}}{m_{\mathrm{isolated\ peptide}}}, \tag{2}$$

where $\sum m_{\mathrm{input}}$ represents solvent, reagents, resin, base, cleavage materials, purification materials, and other material inputs within the selected system boundary. The application of PMI is not meant to provide new calculations of proprietary plant values but to see which solvent changes affect the largest terms in the numerator and which are just material shifting between synthesis and purification/recovery.

The relationship expressed in Eq. (2) means that crude purity becomes a process variable, not simply an analysis result. High crude purity can alleviate load on RP-HPLC, gradient length, repeat purification, number of fractions, evaporation time, and need for water. Wash volume reduction can lead to PMI reduction, but only when carryover and impurity generation are under control. Solvent recovery will help to reduce total material input, but at the expense of additional separation, energy usage, residue control, and cleaning. Thus crude purity, wash burden, and recovery are interconnected rather than independent.

2.4 Product-Contact Material Assessment

The product-contact materials assessment included the site of contact and state of the material in question. The sites of contact were reactor wall, filter frit, tubing for transferring solution, valve, seal, pump head, column hardware, storage tank, and recovery device. The states were fresh solvent, solvent containing deprotection base, solvent containing activated amino acid and coupling additive, cleavage liquor, wash solvent, aged solvent, recovered solvent, aqueous-organic purification solution, and concentrated residue.

The evaluation criteria were deliberately conservative. If the direct compatibility test data are missing for the solvent candidate at SPPS conditions, the contact-material pair is considered unconfirmed, rather than confirmed. Chemical compatibility in small-scale synthesis does not mean that the solvent would be able to pass through pumps, seals, filters, column, and recovery device without swelling, extraction, pressure instability, surface modification, and failure to clean up. The manufacturing candidate should pass both tests.

3. Results

3.1 Liquid-Contact Cycle of SPPS

A ten-residue Fmoc/tBu synthesis cycle involves resin swelling, either first amino acid loading or resin functionalization, ten rounds of deprotection steps, ten rounds of coupling steps, several washing cycles, final deprotection, cleavage from resin by acidolysis, precipitation, and purification. This simple case already has significantly more liquid interactions than any single organic transformation. Solvent comes in contact with resin particles, porous frits, reactor walls, tubing, rubber components, and separation equipment numerous times before the final product isolation.

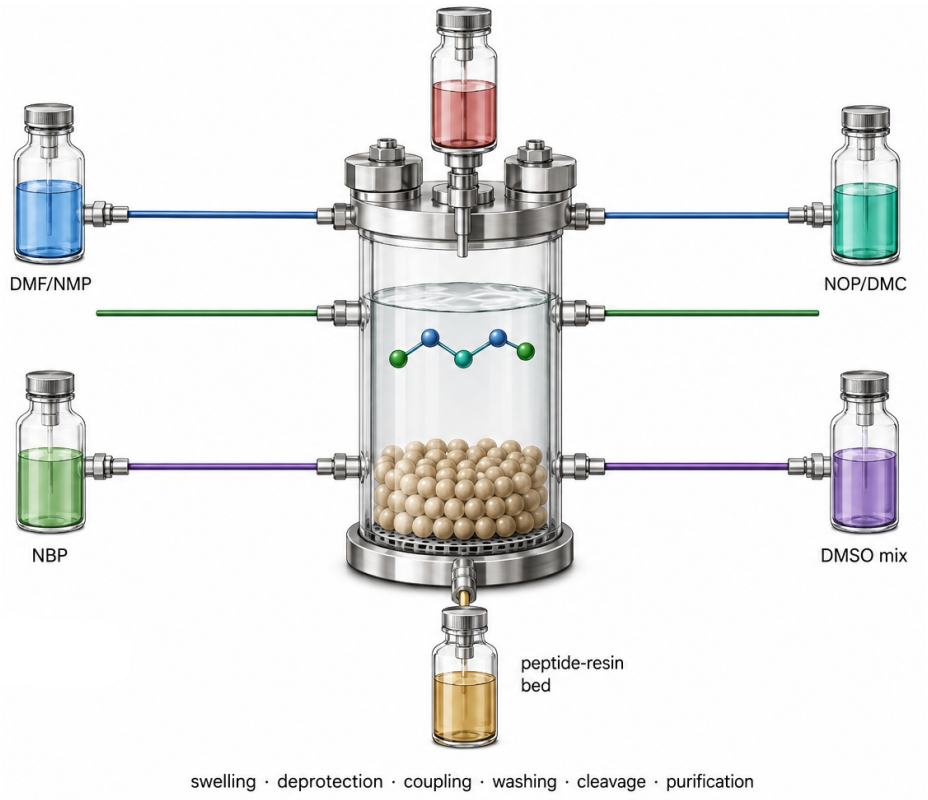

Figure 1: SPPS liquid-contact cycle.

The route process map in Figure 1 explains the reason that solvent selection is a manufacturing process choice. The chemical cycle takes up only one segment of the route. The contact layer specifies the wetted items that contain the same

solvent through swelling, deprotection, coupling, washing, cleavage, purification, and recovery. A solvent that is fine in the coupling step may still be weak in the overall route due to its slow drainage, retention of base, attack on elastomer, residues left by evaporation, and increase of purification solvent requirement. This figure thus links the chemistry of chain assembly in the chemical cycle with the physical route of each stream.

The above outcome changes the framing of the core challenge. An ideal solvent for green SPPS must sustain chain assembly and minimize the cumulative exposure load at the same time. It has to operate in neutral, basic, activated, acidic, aqueous-organic and high concentration waste environments. The best solvent choice is not only the most environmentally-friendly one used in bottles but also the liquid system which can support the process under the above conditions without passing any risk to the subsequent purification or equipment operation.

3.2 Process-Mass Burden of Liquid Operations

Peptide synthesis has been talked about using the chemistry of the coupling step, but process mass is actually dominated by liquid flows. In the PMI splitting selected for this study, solvent and washing are assigned the highest portions, while piperidine, TFA cleavage liquor, protected amino acids, coupling reagents and resin have lower fractions. Recent analysis based on the process-mass approach corroborates the dominance of washing and solvent usage [25].

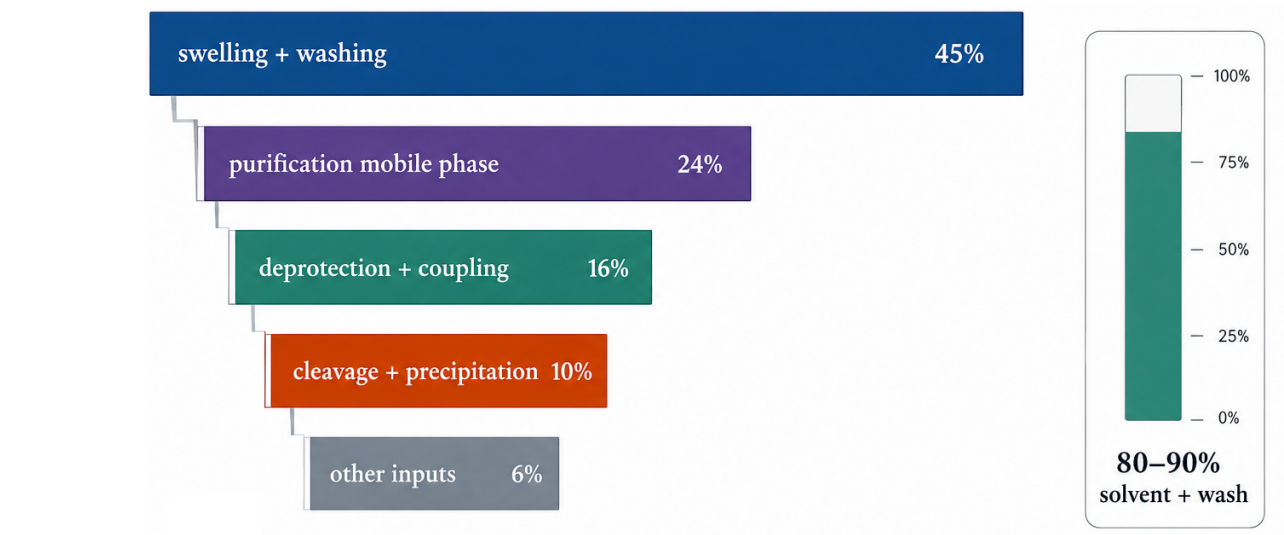

Figure 2: Process-mass burden.

Distribution in Fig. 2 implies that solvent and wash solvent streams carry almost 85% of the mass burden. The piperidine stream and the TFA cleavage stream each account for roughly 4.5% of the mass burden. Amino acids and the coupling reagents contribute a small share together, and resin contributes less than a small share of the mass total. The above pattern explains why switching from DMF or NMP to another solvent for the coupling solution alone is not enough unless it results in a smaller wash volume, a smaller cleavage transfer burden, a smaller purification burden, or a smaller solvent burden.

The above distribution also helps prevent another misunderstanding. Low resin mass does not mean the resin choice does not matter. Swelling and drainage define the solvent amount required to obtain diffusion and wash. They decide if incomplete deprotection, incomplete coupling, aggregation, or impurities caught within increase the purification burden. A solvent which increases the resin accessibility may reduce the downstream burden even if its mass fraction is considered only as wash solvent.

3.3 Peptide Manufacturing Routes and Dependence on Purification

Chemical synthesis processes are important for the production of peptide API, especially for modified peptide sequences. Continuous flow peptide synthesis has emerged from solid and solution synthesis processes [50]. Liquid-phase synthesis of peptides is also now available [51]. Modern continuous-flow SPPS enables rapid multigram-scale peptide supply [52]. Solid-phase and liquid-phase syntheses have a dominating share of the routes. Recombinant production and hybrid routes have a smaller share of the routes. The distribution is important as solvent switching in SPPS may become influential in peptide manufacturing only if it goes beyond a demonstration of a short peptide sequence synthesis.

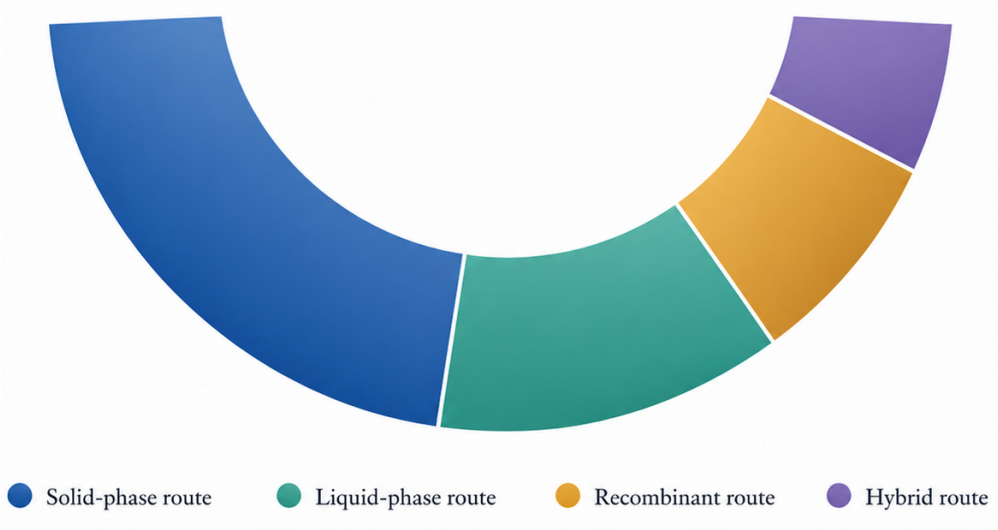

Figure 3: Manufacturing route distribution.

Distribution of the Routes from Figure 3 illustrates the industrial setting of the solvent issue. Solid-phase and liquid-phase synthesis are still important routes for peptides API, and recombinant and hybrid routes are smaller players. It does not mean that SPPS is better in all cases. Long, hydrophobic, aggregating or expensive-to-make sequences would benefit from fragment condensation, solution chemistry, hybrid production or expression. Significance of SPPS consists of its capacity to incorporate chemically elaborate fragments that are typical for drug peptides; hence, solvent choice is a manufacturing issue.

Purification is another industrial factor. Since peptide-related impurities are close in structure to the target substance, purification frequently requires RP-HPLC despite efficient coupling chemistry. Purification by solid-phase techniques can be used in selected workflows along with counterion exchange [53]. Total costs of purification include the organic mobile phase, water, buffer or counterion control, fraction collection, pooling, concentration, lyophilization, and waste disposal.

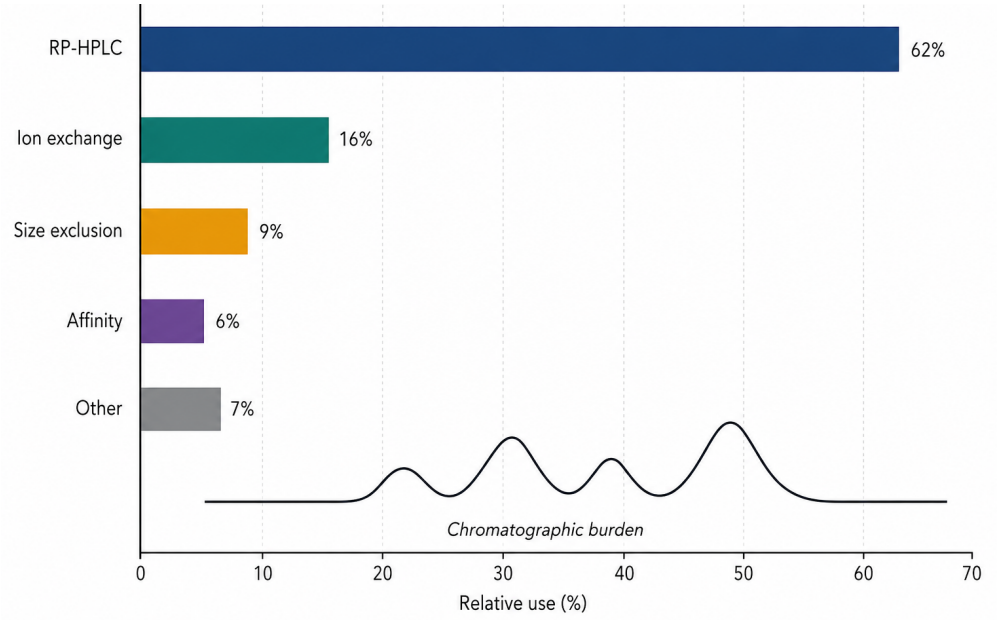

Figure 4: Purification method use.

The purification map in Figure 4 shows that the most common technique is RP-HPLC, while the others occupy smaller shares such as ion exchange, size exclusion, affinity and many others. The corresponding HPLC trace in Figure 4 under the map demonstrates that structurally similar peptide impurities cause downstream solvent requirements from upstream chemistry. The solvent for synthesis, which provides a high purity of crude, decreases the purification load more efficiently than a solvent with a preferable hazard rating but having deletion products, epimers or eluting side products. A greener synthesis solvent might become less efficient in case of gradient elongation, fraction increase, repeated purification step, etc.

The synthesis and purification data provide the indication to the only requirement at the route level. A preferable solvent will be not the one providing the maximum purity in synthesis in isolation or the most favorable one according to

the hazard diagram, but the one which preserves the compatibility of synthesis and purification and material recovery.

3.4 Performance of Solvents Comparison

Solvent replacement includes the analysis of resin swelling, solubility of reagents, deprotection, coupling, purity of the crude product, handling, recovery, materials exposure. The solvent profile provided in this paper groups the above-mentioned properties qualitatively due to the different peptides used in the underlying studies.

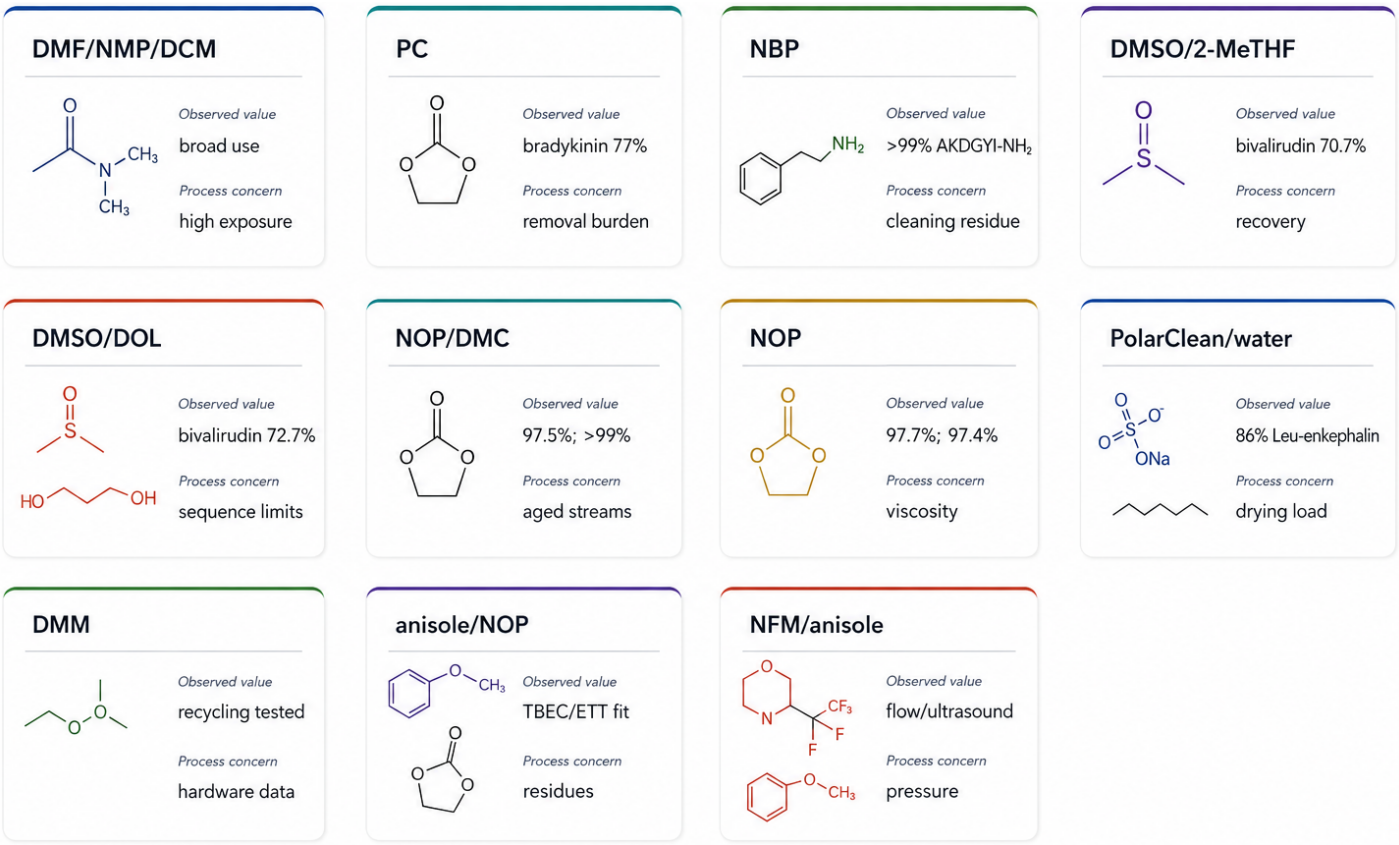

Figure 5: Candidate solvent profiles.

Figure 5 demonstrates the reason for which none of the candidates is chosen based on a single attribute. Traditional DMF/NMP/DCM is synthetic excellent since it enables swelling, solubility, deprotection, and coupling. However, its exposure profile is not desirable. Although propylene carbonate has better environmental position and proven ability to synthesize bradykinin, it has high boiling point and difficulties with recovery. NBP allows NMP's performance and can suppress selected side reactions. However, its high boiling point and cleaning validation issues are relevant too. DMSO/2-MeTHF and DMSO/DOL are examples of balanced polarity and handling of binary solvents. However, recovery and sequence-dependence issues are not solved for broad-scale manufacturing use. NOP/DMC has the best position due to high crude purity and viscosity reduction/recovery. PolarClean/water proves feasibility of aqueous process, but water processing and viscosity need further investigation.

Figure 5 also reflects the emergence of DMM, anisole/NOP, and NFM/anisole systems. DMM was investigated in terms of amino-acid solubility, resin swelling, deprotection kinetics, coupling, PMI, and solvent recycling [39]. Anisole/NOP is compatible with TBEC/ETT coupling and has high synthesis efficiency for Aib-enkephalin and Aib-ACP [40]. NFM/anisole allows ultrasound-assisted, flow-compatible, and natural-product-oriented peptide synthesis with OxymaB [41]. These systems deserve direct evaluation along with NOP/DMC and NBP since the limitation of these systems is not related to the synthetic potential. It is insufficient material exposure and recovery data.

The table presents the process-related information, which is not clear by crude purity only. NOP/DMC has the best position among other candidates because the record includes high purity, viscosity mitigation, suitability for automated protocols, and recovery. DNB and DMSO-based systems are still worth using because they can be adapted for specific sequence or resin classes, but they have other issues with residues and recovery. DMM, anisole/NOP, and NFM/anisole extend the solvent-design space and need to be investigated along with NOP/DMC; the limitation of these systems is not the absence of synthetic potential, but insufficient material exposure and recovery data.

Table 1: Solvent qualification records.

Solvent system	Best documented strength	Principal concern	Process decision
DMF/NMP/DCM	Broad operational history in Fmoc/tBu SPPS	Hazard, exposure, and regulatory pressure	Performance reference, not a sustainability endpoint
Propylene carbonate	Bradykinin synthesis close to DMF purity	Solvent removal, high boiling point, broader sequence range	Selected-sequence option requiring recovery verification
NBP	NMP-like screening performance; reduced side-reaction tendency in selected studies	Cleaning, residue, high-boiling behavior	Strong alternative when hardware and residue control are acceptable
DMSO/2-MeTHF and DMSO/DOL	Polarity-viscosity balancing; bivalirudin and Aib-enkephalin demonstrations	Mixed-solvent recovery and sequence dependence	Flexible development platform rather than universal replacement
NOP/DMC	High purities for Aib-enkephalin and linear octreotide; viscosity reduction; recoverable components	Full equipment-material compatibility across aged streams	Priority candidate for plant-scale qualification
NOP	Strong crude purities with compatible resins	Viscosity and automated drainage	Valuable parent solvent, improved by DMC blending
PolarClean/water	Aqueous Fmoc/tBu feasibility and solvent reuse demonstration	Water load, viscosity, drying, purification transfer	Low-amide route for selected sequences
DMM	Multi-step evaluation including swelling, solubility, kinetics, PMI, and recycling	Few industrial equipment-contact measurements	Candidate requiring hardware and long-sequence testing
Anisole/NOP	High peptide efficiencies with TBEC/ETT compatibility	Recovery and material-contact confirmation	Coupling-system-specific mixture needing route qualification
NFM/anisole	Ultrasound-assisted and flow-compatible green SPPS demonstration	Scale-up hardware exposure and residue profile	Intensification-oriented candidate after hardware screening

3.5 Crude Purity in Terms of Peptide Cases

Crude purity is not a comprehensive metric of process performance, but it is the easiest way to correlate synthesis and subsequent purification efforts. Crude purity can alleviate the burden on RP-HPLC load, enable gradient shortening, reduce re-purification steps, ease pooling, and minimize solvent consumption. Sequence dependent or moderate crude purity will negate the benefit of a green synthesis solvent through shifting of burden into purification.

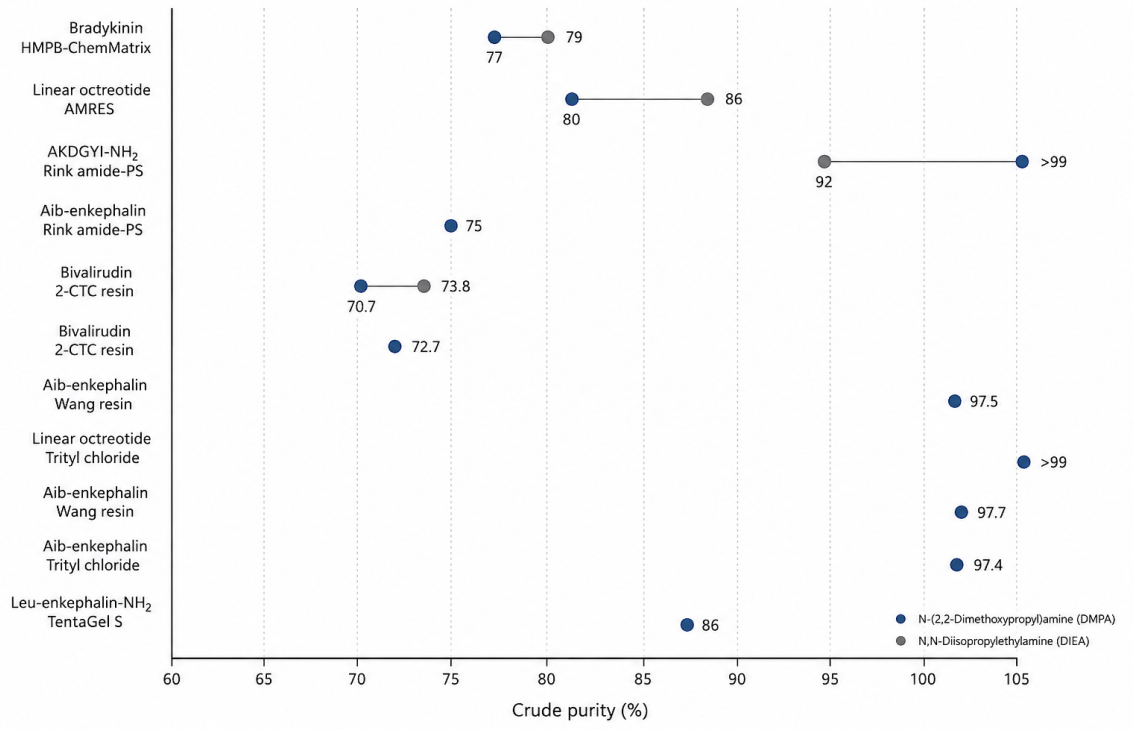

Figure 6: Crude purity by peptide case.

The comparison of the same peptide cases shown in Figure 6 highlights NOP/DMC and neat NOP as the best choices from the presented full-cycle data. NOP/DMC 8:2 achieves 97.5% crude purity for Aib-enkephalin and over 99% purity for linear octreotide, whereas neat NOP yields 97.7% and 97.4% Aib-enkephalin purities using Wang and trityl chloride

resins respectively. These numbers are significant because they represent the level higher than the replacement of DMF. This indicates a possibility of reducing purification burden through such approach if it is confirmed for the target peptides. The case with NBP is more complicated. Linear octreotide yields 80% of crude purity against 86% obtained with DMF in one case, whereas AKDGYI-NH_2 reaches over 99% of purity at 45 °C. A PolarClean/water score of 86% is attained for Leu-enkephalin amide, showing the aqueous nature of such peptides but not their ability to replace all others.

Table 2: Selected full-cycle crude purities.

Solvent system	Peptide	Support	Coupling system	Crude purity
Propylene carbonate	Bradykinin	HMPB-ChemMatrix	HBTU/HOBt/DIPEA	77% (DMF: 79%)
NBP	Linear octreotide	AMRES	DIC/HOBt	80% (DMF: 86%)
NBP	AKDGYI-NH_2	Rink amide-polystyrene	DIC/OxymaPure	>99% at 45 °C (DMF: 92%)
DMSO/2-MeTHF 3:7	Aib-enkephalin	Rink amide-polystyrene	DIC/OxymaPure	75%
DMSO/2-MeTHF 3:7	Bivalirudin	2-chlorotrityl chloride resin	DIC/OxymaPure	70.7% (DMF: 73.8%)
DMSO/DOL 3:7	Bivalirudin	2-chlorotrityl chloride resin	DIC/OxymaPure	72.7%
NOP/DMC 8:2	Aib-enkephalin	Wang resin	DIC/OxymaPure	97.5%
NOP/DMC 8:2	Linear octreotide	Trityl chloride resin	DIC/OxymaPure	>99% (DMF: >99%)
NOP	Aib-enkephalin	Wang resin	DIC/OxymaPure	97.7%
NOP	Aib-enkephalin	Trityl chloride resin	DIC/OxymaPure	97.4%
PolarClean/water 1:4	Leu-enkephalin-NH_2	TentaGel S	TCFH/collidine	86%

Selected purities reveal that solvent effectiveness depends upon peptide, resin, temperature, coupling chemistry, and deprotection method. One instance of high purity cannot be considered proof for all cases. Comparative analysis is the right way. NOP/DMC is a good solvent since its high purity has been found for more than one peptide and resin system. NBP is good since it works very well with adjusted conditions and could avoid racemization or aspartimide formation in specific cases [54]. DMSO-containing solvent systems still have to be used when polarity and viscosity are to be tuned; however, their purities are lower. PolarClean/water solvent system extends the operation limits due to decreased dependency on classical amide solvents, while implications of water usage have to be taken into account in overall process assessment.

Same values can provide a basis for qualification sequence. First, a solvent has to be tested for a reference short peptide, and then for a target-like peptide containing challenging amino acids, and finally at scale reflecting fraction number, gradient length, yield and solvent recovery. Otherwise, no correlation between crude purity and mass yield benefit can be established.

3.6 Exposure of Wetted Equipment

The material contact layer is not secondary to synthesis. Any solvent substitution alters the fluid conditions under which every wetted piece of equipment operates. It is especially relevant for high boiling polar solvents and for binary systems that behave differently when freshly made and when activated, base containing, acidic, or concentrated.

The equipment contact chain of Figure 7 ties each SPPS liquid stream to the possible material concern. Swelling solvent could interact with elastomers, base stream with frit pressure or seal operation, coupling mixture with activated reagents in residues, cleavage liquor with polymer pieces, purification mobile phase with column hardware, and recovered solvent with by-products. This chain should be traced through every batch and through every wetted component. Thus, compatibility cannot be derived from fresh solvent look or a successful vial synthesis experience.

The materials table alters the choice rationale. The highest purity of synthesis becomes unnecessary if the solvent affects product contact elastomers or cannot be cleaned. NOP/DMC continues being the primary candidate due to combination of high peptide purities with viscosity control and recovery possibilities, but its further implementation in the plant requires testing of 316L SS, PTFE or PFA tubing, FKM or EPDM elastomers, frit materials, pumps' hardware, and columns' hardware. Similar procedure is required for NBP and DMSO mixtures due to high boiling residues' potential cleaning and carry-over problems. PolarClean/water needs pressure, viscosity, drying, and bioburden related attention.

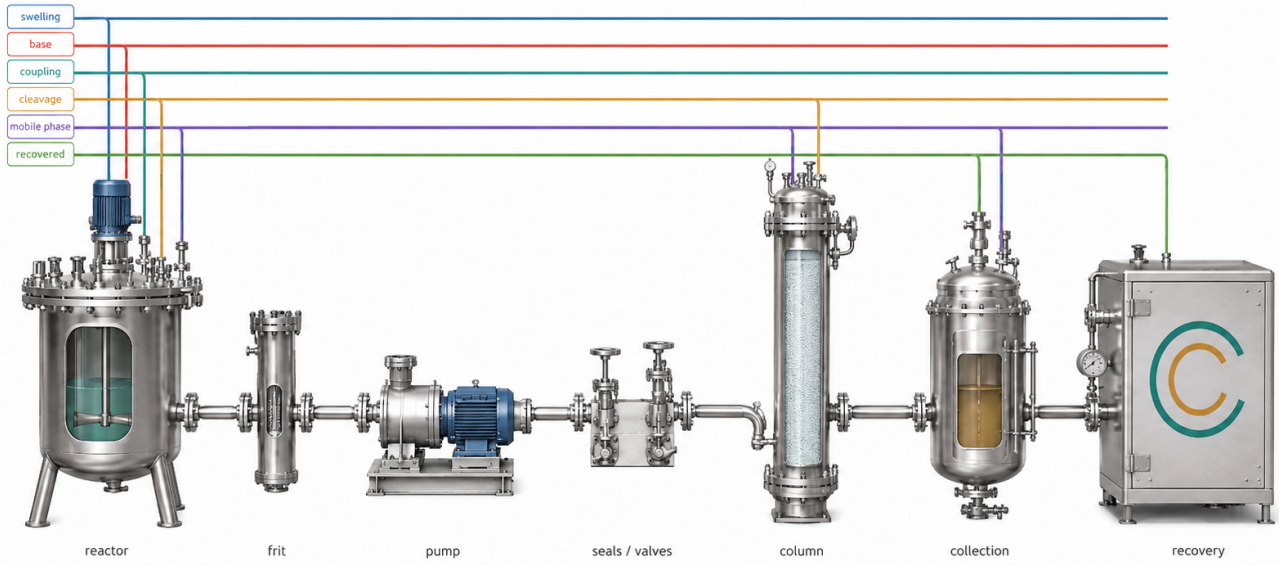

Figure 7: Wetted equipment contact.

It is especially important for corrosion and material studies. The standard coupon tests, elastomer swelling, hardness changes, surface inspection, pressure loop test, and leachable materials' analysis could transform solvent enthusiasm into measurable qualification. The most useful compatibility test for green SPPS is one that exposes materials to the same chemical state of the route as possible.

Table 3: Materials-exposure interpretation.

Solvent system	Synthetic advantage	Materials-exposure concern	Process interpretation
DMF/NMP/DCM	Strong swelling and broad reagent solubility	High-concern amide solvents; halogenated-solvent contact	Effective performance reference with unfavorable repeated exposure profile
Propylene carbonate	Green polar aprotic profile; bradykinin purity close to DMF	High boiling point and solvent-removal demand	Suitable for selected sequences when drying and recovery are verified
NBP	NMP-like solvating power and useful side-reaction behavior	High-boiling polar solvent; residue and cleaning control	Strong candidate where cleanability and product-contact residues are acceptable
DMSO/2-MeTHF	Polarity adjustment with a greener ether component	DMSO recovery and residual removal	Useful mixed system when purification benefits offset recovery complexity
NOP/DMC	High crude purities; DMC lowers viscosity limitation	Mixed-solvent recovery and elastomer behavior under aged streams	Best priority for plant-relevant compatibility trials
PolarClean/water	Aqueous-compatible, low-amide route	High viscosity and increased aqueous processing	Attractive but must control water, drying, and equipment-pressure effects
DMM	Recyclable green solvent with broad SPPS-step testing	Few measurements for seals, frits, pumps, and recovery residues	Candidate requiring equipment-contact testing
NFM/anisole	Green intensification medium for ultrasound and flow SPPS	Compatibility under ultrasound, flow pressure, and residue conditions	Candidate for intensified systems after hardware screening

3.7 Impurity Formation and RP-HPLC Burden

The initial SPPS impurity generation pathways are rooted in the general difficulties associated with the process of the formation of an amide bond [55]. Reagent selection for the coupling step influences the reaction efficiency and the side-product formation [56]. Protecting group choice provides yet another sequence-specific source of risks [57]. The addition of safe coupling reagents allows reducing the hazards while keeping the reaction efficient [58]. However, modern coupling reagents still need to be used carefully since their properties transcend mere activation efficiency [59]. NBP studies indicate that even the choice of solvent may affect the racemization and aspartimide formation [54]. Impurity formation pathways are therefore based on incomplete coupling, incomplete Fmoc deprotection, deletion sequences, truncated products, racemization, aspartimide formation, diketopiperazine formation, arginine lactamization, aggregation, side chain deprotection side products, cleavage by-products, and resin carry-over. These pathways are significant because the peptide impurities often

are similar to the desired product. Small sequence or stereochromatic differences may cause difficulty in the separation of impurities using RP-HPLC and additional solvent consumption.

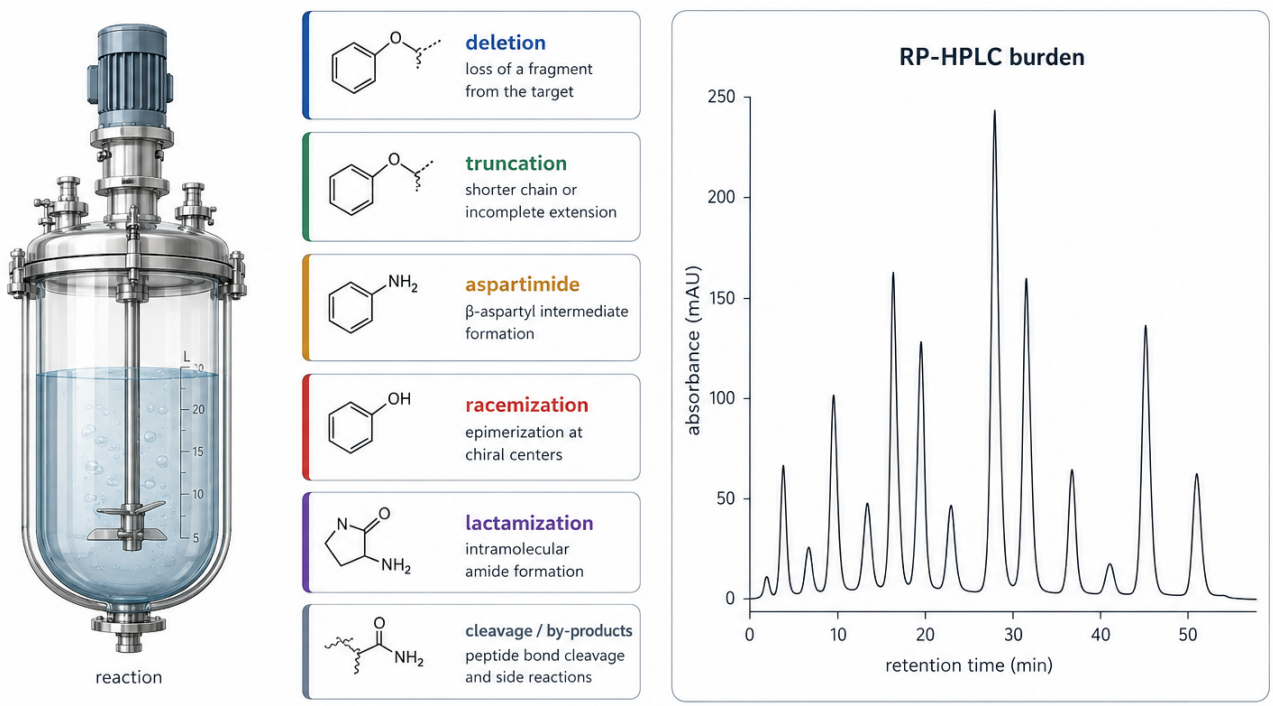

Figure 8: Impurity and purification burden.

The impurity pattern in Figure 8 shows how upstream conditions affect the downstream mass burden. Incomplete coupling generates deletion sequences, incomplete Fmoc deprotection generates truncated products, presence of Asp in the sequence generates aspartimides, presence of Arg in the sequence generates lactamization, and aggregation-prone regions reduce accessibility of reactive sites. Every type of impurity increases the purification burden via the increased overlap in chromatography, number of fractions, amount of solvent, and reprocessing required. Thus, the effect of the solvent on polarity, viscosity, base strength, solubility of the reagent, and swelling of the resin reaches beyond the synthesis yield and affects the purification costs.

The practical conclusion is that solvent qualification should be sequence-specific. Peptides rich in Asp residues should be tested for aspartimide formation, peptides containing Arg residues should be tested for lactamization, peptides with Cys, His and Ser should be tested for racemization and side chain sensitivity, and hydrophobic and aggregation prone sequences should be tested for reactive site accessibility. A solvent that does well for Aib-enkephalin is promising but inconclusive for a sequence that is long, hydrophobic, and functionally complicated. Some of the best solvents will be those that hold up to such stress tests rather than just being good on shorter peptides.

3.8 Scale-Up Qualification Strategy

NOP/DMC is the best choice for initial plant-qualification testing since it has the best overall profile regarding purity of the crude product, viscosity reduction, automation, and recovery of solvents. The typical order for qualification should not start with optimization of synthesis but rather postpone materials testing until the last moment since discovery of potential issues with seal swelling, pressure stability, and cleanability will make the route selection process invalid.

The qualification strategy shown in Figure 9 includes parallel testing for chemical performance and material compatibility. Testing of NOP/DMC should include comparison of different solvent ratios such as 7:3, 8:2, and 9:1 based on the target resin, intermediate loading, selected chemistry for coupling, and difficult residue panel. In addition, it should provide data on bed expansion, drainage, pressure drop, Fmoc removal, coupling efficiency, crude purity, impurity identification, fraction number, solvent consumption, recovered solvent composition, and residues after evaporation. At the same time, materials compatibility of 316L stainless steel, PTFE or PFA tubing, FKM or EPDM elastomers, seals, frits, and pump parts needs to be tested using fresh and process-aged streams.

Evidence Profile for NOP/DMC in Figure 10 illustrates the reason why NOP/DMC is placed at the first place in the qualification strategy. The process includes high peptide content in Aib-enkephalin and linear octreotide with reduction of handling steps due to DMC as well as recovery of NOP, DMC, and piperidine mixture. However, some decisions such as aged stream exposure, elastomer performance, cleanability residues, and residual solvent control remain unknown at this

stage.

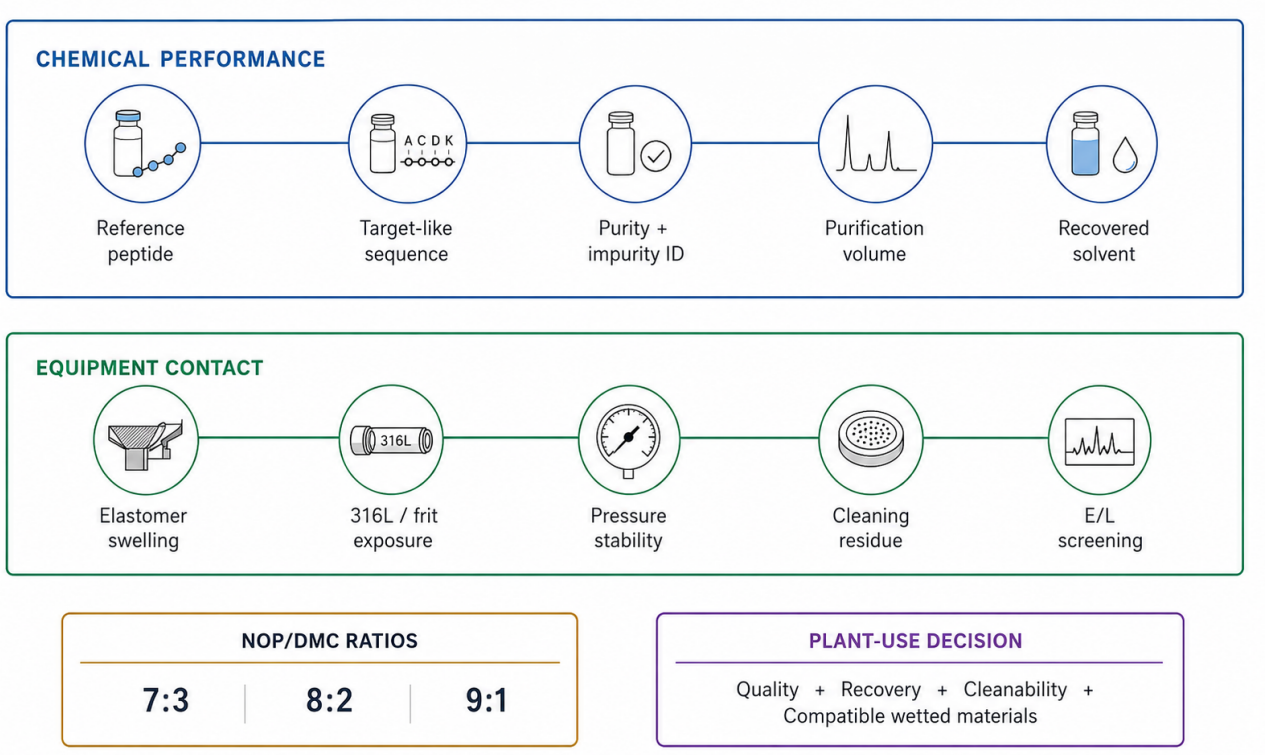

Figure 9: Parallel solvent qualification.

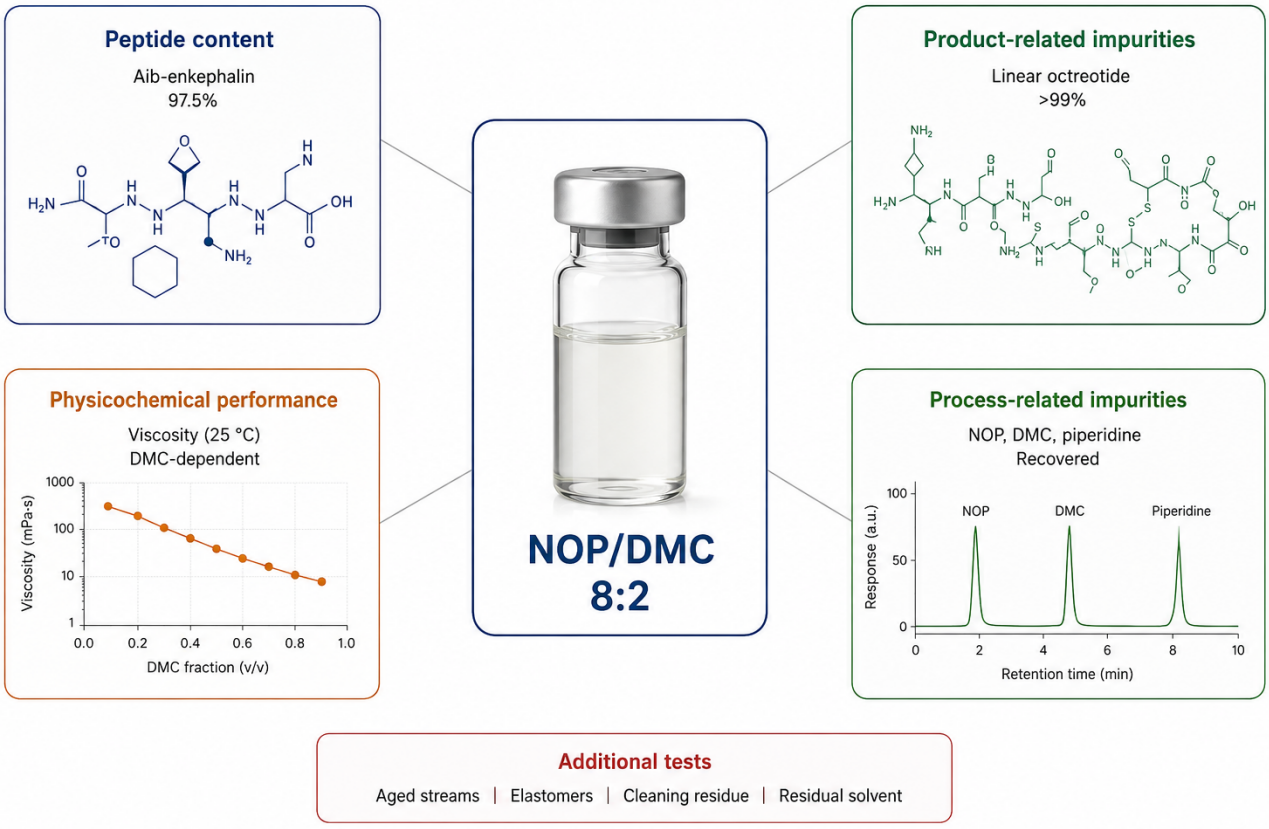

Figure 10: NOP/DMC evidence profile.

The data in Table 4 provide the minimum amount of information required for a manufacturing decision. Resin swelling and reaction efficiency address the question of whether or not the peptide is manufacturable. Hard sequence test addresses whether the solvent has generality or only short peptides capability. The materials test identifies whether or not the solvent is tolerable by the equipment. The recovery, cleanability and purification tests will identify whether low synthetic burden translates into low total burden. Solvent advancement will be linked to the quality of the final product. Solvent will not

be considered based on its crude chromatography alone.

Table 4: Scale-up measurements.

Validation area	Key measurement	Reason for inclusion
Resin behavior	Swelling volume, bed expansion, drainage time, pressure drop	Confirms reactive-site accessibility and identifies viscosity-related process limits
Reaction performance	Coupling completion, Fmoc removal rate, crude purity, impurity profile	Establishes whether solvent substitution preserves synthetic reliability
Difficult sequences	Asp-, Arg-, Cys/His/Ser-, and hydrophobic test peptides	Detects aspartimide, lactamization, racemization, aggregation, and incomplete coupling risks
Equipment materials	Mass change, dimensional change, hardness, surface inspection	Identifies swelling, softening, extraction, corrosion, or surface damage before scale-up
Recovery and cleaning	Recovered fraction, residue after evaporation, cleanability, odor, carryover	Determines whether the solvent can be recycled or removed without contaminating later batches
Purification effect	RP-HPLC gradient length, fraction count, solvent volume, water demand	Converts crude-purity improvements into process-mass and operating benefits
Regulatory and residual control	Residual-solvent method, specification proposal, extractables/leachables screen	Connects solvent substitution with release testing and product-contact material risk

The same qualification logic must be followed before declaring any advantage of the newer solvent systems. DMM, anisole/NOP, and NFM/anisole have promising track records, but they should be qualified against the same sequence panel, resin families, wash logic, and equipment-contact materials as NOP/DMC and NBP. An analogous qualification campaign will clarify if any potential solvent advantage comes from the solvent itself, the coupling system, the resin, the process intensification technology, or the particular peptide sequence.

4. Discussion

The results provide the answer to the research question by revealing the solvent suitability as an interaction of chemical, process, purification, recovery, and materials contact parameters. The highest suitability in the near term belongs to NOP/DMC as it performs well in the greatest number of process dimensions. The crude purities of 97.5% for Aib-enkephalin and >99% for linear octreotide are proof of chemical suitability, while the presence of DMC fraction reduces the viscosity concern about neat NOP and enables automation. Distillation-based recovery of NOP, DMC, and piperidine creates a process-mass suitability which is directly relevant to PMI [36]. The combination of those advantages makes NOP/DMC superior to all the candidates that are hazardous profile suitable, but incomplete in recovery, handling or materials contact qualifications.

NBP is a strong but conditional candidate. Its NMP-like solvating ability is helpful, and the >99% crude purity achieved for AKDGYI-NH_2 at 45 °C proves that excellent peptide quality is achievable with appropriate conditions. The 80% crude purity for linear octreotide compared to 86% for DMF shows that its advantage is not universal. Therefore, NBP should be seen as a sequence- and condition-dependent alternative that may be particularly helpful if its side reactions profile and resin performance match the particular sequence. Its high boiling point makes cleaning, residue management, and recovery crucial parts of qualification.

DMSO/2-MeTHF and DMSO/DOL are another kind of candidates. The value of these candidates is in tunability of polarity and handling, not in uniformly high crude purity. The bivalirudin crude purities of 70.7% in DMSO/2-MeTHF and 72.7% in DMSO/DOL are close to 73.8% in DMF, proving that these mixtures can approach traditional solvent performances for a challenging peptide. The limitation is not chemical impossibility but process complexity of DMSO recovery, residue management, odor concentration and purification compatibility. Therefore, these systems can be seen as development tools for peptides where polarity tuning resolves the otherwise hard to solve resin or solubility problem.

PolarClean/water gives conceptually important result, breaking the assumption that Fmoc/tBu SPPS has to be dominated by classical amide solvents. The 86% crude purity of Leu-enkephalin amide proves the aqueous-compatible synthesis and solvent reuse possibility [37]. The complementary study of PolarClean supports its use as a greener SPPS medium [38]. The process engineering trade-off is the high water volume, affecting drying, purification, waste treatment, microbial control, equipment pressure and energy consumption. Therefore, PolarClean/water has to be seen as a low-amide route for selected sequences requiring water balance evaluation.

DMM, anisole/NOP, and NFM/anisole show that solvent innovation moves in two directions. One direction aims to

develop a broad solvent replacement that can support the usual SPPS duties, as proven for DMM [39]. The other aims to create mixtures compatible with the particular coupling system, as in anisole/NOP with TBEC/ETT [40]. TBEC itself was evaluated as an efficient carbodiimide replacement in SPPS [60]. NFM/anisole develops the direction towards ultrasound or flow-compatible methods [41]. These systems must go through the same qualification as NOP/DMC, NBP and DMSO-based mixtures. Not only the synthetic efficiency is important, but recovery, residue control, aging, compatibility under aged stream, and purification compatibility should be evaluated before route can be selected.

The additional implication of the results is that solvent qualification should be assessed on the level of repeated campaigns, not a single batch. The industrial production requires repeated batches, repeated columns usage, repeated solvent recovery and repeated cleaning verification. Even if a single batch shows high crude purity, the route can fail if the residue accumulation changes the resin-bed hydrodynamics, if the recovered solvent gradually accumulates impurities, or if the elastomer swelling changes the pump metering. This becomes especially relevant for the higher boiling alternatives and mixed systems, as the easiness of the laboratory solvent replacement does not necessarily mean that the residue will be persistent in the equipment. The recycle loop and repeated contact experiments should accompany the first successful synthesis.

The difference between greener solvent discovery and greener process adoption is crucial. The solvent discovery answers the question of whether a candidate can perform the function of DMF, NMP, or DCM in the selected synthesis. The process adoption answers the question of whether the candidate improves the overall route after synthesis, purification, isolation, recovery and cleaning are taken into account. A solvent giving 95-99% crude purity but having a complex recovery train may still be useful if it reduces the RP-HPLC load enough to compensate the recovery burden. A solvent being benign and easily available may be inefficient if it increases the deletion sequences and makes the purification necessary. The best route is the one where the solvent choice reduces the chromatogram load and mass burden at the same time.

The process contact evaluation is the main contribution of this manuscript. Many solvent discussions are focused on chemical greenness, resin swelling or crude purity. These viewpoints are important, but not sufficient. An industrial solvent continuously contacts the hardware while carrying the reactive additives, bases, acids, peptide fragments, impurities, and concentrated residues. The solvent also determines the amount of purification solvent after synthesis. Therefore, the solvent candidate is not necessarily the least hazardous liquid on the selection chart. It is the solvent system that reduces the hazard and mass burden while maintaining the peptide quality, equipment reliability, recovery practicality and cleanability.

The conclusion is not a universal solvent mandate, as the peptide manufacturing is sequence-specific. A solvent being good for Aib-enkephalin can be inefficient for a hydrophobic precursor; a medium being good for aqueous compatibility can lose the attractiveness if the purification water and drying energy requirements become excessive; a solvent having strong swelling can be impractical if the cleaning residues interfere with the release testing. The values discussed in this manuscript are guidelines for the early route design and measurements required for the scale-up, not the replacement of the empirical development for the target peptide.

The limitations of the conclusions are also evident. The crude purities come from different peptides, resins, temperatures and coupling systems, so they cannot be averaged into a universal ranking. The materials compatibility is suggested as the requirement but was not proved for each candidate solvent under aged stream conditions relevant to SPPS. PMI closure is incomplete for the majority of solvent systems, as full solvent usage, recovery yield, purification volume and cleaning burden were not published for many systems. However, these limitations do not decrease the value of the manufacturing conclusion, but define the measurements required to transform a laboratory solvent success into a plant solvent decision.

The most informative next experiment would not compare a single simple peptide in DMF and a single alternative solvent. The meaningful qualification campaign should select a target-like sequence set, use identical resin loading and wash logic, measure the crude purity and impurity identity, record the actual solvent volumes, purify at comparable loading, quantify fraction volume and recovery, expose representative hardware materials to fresh and aged streams, measure solvent recovery and cleaning residues. This package allows a direct comparison between NOP/DMC, NBP, DMSO/2-MeTHF, PolarClean/water, DMM, anisole/NOP and NFM/anisole.

4.1 Selection of Route and Equipment

The values presented in this manuscript have direct implications for early route development. A peptide research team will start from having a chosen sequence, resin, protecting group scheme, and coupling strategy. The solvent should be decided on along with the other factors rather than later based on the first crude chromatogram. Propylene carbonate or PolarClean/water may be preferable for a short polar sequence due to lesser swelling and solubility constraints. If the sequence is hydrophobic or prone to aggregate formation, then NOP/DMC, NBP, or DMSO-containing systems will be relevant due to better solvating properties. For a sequence containing Asp-Gly, Asp-Ser, Arg-rich, Cys-rich, His-rich, or steric Aib motifs, the chosen solvent should be evaluated by impurity identity as well as purity level. These numbers explain

why: 97.5% Aib-enkephalin purity in NOP/DMC or >99% linear-octreotide purity in the same solvent system are stronger process signals than moderate purity obtained in milder conditions, as difficult residues and purification overlap are usually the sources of process burden.

The solvent selection also needs to consider the resin bed. Swelling values are not just a matter of convenience, but control over diffusion, deprotection reach, completion of coupling reactions, wash performance, and pressure drop. Even a safer solvent can create a burdensome route in case of poor resin swelling requiring longer contact times, more excess of reagents, and additional washing. A viscous solvent can increase hold-up in the bed and elongate drainage process especially in case of automation and large columns. This is the main reason why NOP/DMC is preferable over neat NOP in manufacturing development. Even though neat NOP provides excellent purity levels for Aib-enkephalin on both Wang and trityl chloride resins (97.7% and 97.4% correspondingly), adding DMC to the solvent mixture resolves the issue of its handling while retaining the high peptide quality. So DMC is not only a diluent but also a process-enabling solvent that increases the chances of automated operation success.

Purification consequences are also essential. The difference between 70–75% and 97–99% in crude purity is not an analytical detail in peptide manufacture. Lower crude purity will result in increased column loading, broader window of fractions, higher organic content in the mobile phase, higher demand for water, longer concentration time, and lower isolated yield after pooling. The route using solvent system with 70.7% bivalirudin purity may be valuable for solving the resin or handling issues, but cannot be considered as process-burden reduction route unless purification volumes are known. By contrast, the route maintaining crude purity over 97% can claim the reduction of the process burden unless the impurity profile is dominated by closely eluting impurities. This difference is why the manuscript considers crude purity and purification as one route parameter, rather than separate synthetic and downstream parameters.

The same rationale applies to the recovery of solvent. Recovery of NOP, DMC, and piperidine by distillation is important for attacking the most prominent PMI, namely liquid re-use. However, it needs to be analyzed thoroughly as solvent recycling will accumulate trace impurities, by-products, odor-active substances, base, water, and cleavage residues. A stream of recovered solvent working in one cycle, but slowly changing its coupling properties in further manufacturing cycles will not be acceptable. Practical qualification should include not only the fresh solvent, but also first and repeatedly recovered solvents. The peptide purity, identity of the trace impurities, and residue after evaporation should be determined for these different streams.

Equipment materials need to be selected with the same consideration. Stainless steel 316L reactor may withstand solvent causing unacceptable swelling for FKM seals or dimensional changes in EPDM gasket. PTFE/PFA tubing and pump diaphragms or frits will exhibit different behavior. Solvent-containing piperidine, activated amino acid, OxymaPure, DIC, TCFH/collidine, TFA, scavengers, water, acetonitrile, and residues concentrated during the process should not be treated the same way as the fresh solvent. In case of NOP/DMC the qualifying liquids include: 7:3, 8:2, and 9:1 solvent ratio, piperidine-containing deprotection solutions, DIC/OxymaPure coupling mixtures, post-wash solvent, cleavage-adjacent streams in case of possible carry-over, and recovered fractions. The measurements should include mass change, volume change, hardness, surface changes, pressure drop, extractables, leachables, and residue after cleaning procedure.

Control of residual solvents concludes the manufacturing development. DMF, NMP, DCM, DMSO, DMC, NBP, NOP, PolarClean, DMM, NFM, anisole, and 2-MeTHF are different by their volatility, detection, regulatory acceptance, and product retention properties. A solvent being easy to use in synthesis can become problematic in case of lack of sensitivity in residual testing or high-boiling traces remaining after lyophilization. At the same time, a solvent requiring new analytical methods can still be acceptable when such method is specific, validated, and supported by real removal steps. Thus, the solvent selection should include residual testing methods development before locking the route. The route cannot be called completed until synthesis, purification, drying, cleaning, and release testing all confirm the same solvent choice.

Taking into account the above, the order for manufacturing development can be proposed. NOP/DMC should be the first candidate for a target sequence in case of desire to reduce the usage of classical amides and chlorinated solvents without compromising the crude purity. NBP should be taken into consideration when its NMP-like behavior, side reaction prevention, or resin compatibility matches the sequence. DMSO/2-MeTHF and DMSO/DOL should be used in cases of predicted solvating power improvement. PolarClean/water can be applied in case of possibility to utilize the compatibility with water without the penalty of drying and purification process. DMM, anisole/NOP, and NFM/anisole should be tested in case of recyclable solvent behavior, TBEC/ETT chemistry, ultrasound, or flow operations are intended in the process.

5. Conclusions

The research question was what greener solvent alternatives would remain believable for the Fmoc/tBu solid phase peptide synthesis when taking into consideration synthesis performance, PMI effect, purification complexity, solvent recycling, and

product contact material exposure at once. The answer is that NOP/DMC has to be qualified for production first. NBP, DMSO solutions, PolarClean/water, DMM, anisole/NOP, and NFM/anisole have to be left in the development area where their particular strengths fit the peptide sequence, resin type, coupling reaction, and equipment configuration.

NOP/DMC has to be prioritized first because of the convergence of its advantages, rather than the reliance on one fortunate factor, shown in Figure 10. NOP/DMC 8:2 allows reaching 97.5% crude purity for Aib-enkephalin and >99% for linear octreotide, the DMC component solves viscosity problem for neat NOP, automation appears to be more feasible in comparison with neat high-viscosity NOP, and distillation can allow recovery of NOP, DMC, and piperidine. In this way, NOP/DMC tackles the most significant SPPS challenge – repetitive use of solvent and washing liquid – without immediate sacrifice of product quality. The process still needs tests for elastomer, alloy, frit, tubing, pump, column, recovery, residue, and cleanability under fresh, basic, acidic, activated, aged, and concentrated conditions.

No greener SPPS is possible by substituting DMF with a single solvent with promising properties. It has to decrease the liquid load and preserve the swelling of the resin, solubility of the reagents, kinetics of deprotection and coupling reactions, impurities control, purification, solvent recycling, and product contact material compatibility. Crude purity, PMI, and materials exposure form the integral part of the same manufacturing question – whether the solvent can decrease the process load without shifting the risks to other stages or equipment. According to the criteria presented in the paper, the answer is the qualification order priority, with NOP/DMC first and other candidates depending on the chemistry and equipment requirements for the target peptide.

Conflict of Interest

The author declares no conflict of interest.

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