ABDULRAHMAN BADR HASAN AL-KUBATI

SUBSTITUTION OF PROBLEMATIC SOLVENTS WITH GREENER OPTIONS FOR INDUSTRIAL APPLICATIONS

Universidade do Algarve

Faculdade de Ciências e Tecnologia

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Declaration of Authorship and Copyright

I declare that I am the author of this work, which is original. The work cites other authors and works, which are adequately referred in the text and are listed in the bibliography.

Abdulrahman Badr Hasan Al-Kubati

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List Of Abbreviations and Acronyms

DCC	1,3-Dicyclohexylcarbodiimide
COMU	(1-Cyano-2-Ethoxy-2-Oxoethylidenaminooxy) Dimethylamino-Morpholino-Carbenium Hexafluorophosphate
EDC.HCl	1-Ethyl-3-(3-(Dimethylamino)Propyl)-Carbodiimide. HCl
HOBt	1-Hydroxybenzotriazole
Triton X100	2-[4-(2,4,4-Trimethylpentan-2-Yl) Phenoxy]Ethanol
2-MeTHF	2-Methyltetrahydrofuran
PyAOP	7-Azabenzotriazol-1-Yloxy)Tripyrrolidinophosphonium Hexafluorophosphate
MeCN	Acetonitrile
APIs	Active Pharmaceutical Ingredients
AMPs	Antimicrobial Peptides
PyBOP	Benzotriazol-1-Yloxytripyrrolidinophosphonium Hexafluorophosphate
$CHCl_3$	Chloroform
CSPS	Classical Solution Peptide Synthesis
CMC	Critical Micelle Concentration
DNELs	Derived No Effect Levels
DoE	Design of Experiments
DCM	Dichloromethane
Cyrene	Dihydrolevoglucosenone
DMC	Dimethyl Carbonate
DMSO	Dimethyl Sulfoxide
TPGS-750-M	DL-Alpha-Tocopherol Methoxypolyethylene Glycol Succinate
EtOH	Ethanol
EtOAc	Ethyl Acetate
GSK	Glaxosmithkline
HATU	Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium
HBTU	Hexafluorophosphate Benzotriazole Tetramethyl Uronium
IPs	Interfering Peptides
LPPS	Liquid-Phase Peptide Synthesis
MeOH	Methanol
DMAc	N,N'-Dimethylacetamide

DMF	N,N-Dimethylformamide
Z-Phe-Leu-OEt	N-[(Phenylmethoxy)Carbonyl]-L-Phenylalanyl-L-Leucine Ethyl Ester
NMP	N-Methyl Pyrrolidone
NMR	Nuclear Magnetic Resonance
PbTs	Peptide-Based Therapeutics
PTS	Polyoxyethanyl-A-Tocopheryl Sebacate
BrijO10	Polyoxyethylene (10) Oleyl Ether
Tween 20	Polyoxyethylene (20) Sorbitan Monolaurate
Tween 80	Polyoxyethylene (20) Sorbitan Monooleate
PMI	Process Mass Intensity
PPIs	Protein-Protein Interactions
REACH	Registration, Evaluation Authorization and Restriction of Chemicals
SPPS	Solid-Phase Peptide Synthesis
SVHCs	Substances of Very High Concern
Boc	Tert-Butoxycarbonyl
THF	Tetrahydrofuran
ECHA	The European Chemicals Agency
HLB	The Hydrophilic-Lipophilic Balance
TLC	Thin Layer Chromatography
TEM	Transmission Electron Microscopy
GVL	γ-Valerolactone

Abstract

Peptide production stands as one of the most vital and rapidly growing sectors within the pharmaceutical industry, offering a wide array of therapeutic applications. The conventional methods of peptide synthesis rely heavily on organic solvents, such as N,N-dimethylformamide (DMF), N-methyl pyrrolidone (NMP), and dichloromethane (DCM), leading to significant generation of hazardous waste. However, their adverse effects on human health and the environment, as well as their substantial carbon footprint, have prompted the need for greener alternatives. Micellar chemistry, with water as the solvent, provides a promising alternative to traditional organic solvents, mitigating the adverse environmental impacts associated with conventional approaches. In this study, commercially available surfactants in water were investigated for their potential in facilitating a simple peptide coupling reaction. Different coupling agents and reaction parameters, such as time and surfactant solution amount, were explored. Co-solvents and binary surfactant systems were also examined to enhance reaction efficiency and selectivity. The success of the peptide coupling reactions was evaluated through product yields and analysis using proton nuclear magnetic resonance (H-NMR) spectroscopy, confirming the effectiveness of surfactant-based systems in promoting desired coupling reactions.

Key words: Peptide production, Micellar chemistry, Surfactants, Solvents.

Resumo

A química de peptídeos continua a desempenhar um papel crucial em várias áreas sendo um campo fundamental na indústria farmacêutica. No centro desse campo está a ligação peptídica, uma ligação fundamental na química orgânica, presente em diferentes tipos de moléculas, e que pode ser encontrada em produtos naturais ou agroquímicos, ou ainda em produtos industriais como detergentes, lubrificantes ou polímeros sintéticos. No entanto, este tipo de ligação é especialmente predominante nos princípios activos farmacêuticos (PAFs) mais vendidos e nos peptídeos terapêuticos, sendo por isso a base de muitos medicamentos.

A síntese de peptídeos em fase sólida (SPPS), introduzida por Bruce Merrifield, trouxe uma evolução significativa na química de peptídeos. Este tipo de síntese simplificou a eliminação de produtos secundários, como reagentes e solventes, tornando o processo mais eficiente. Além disso, possibilitou a automatização, permitindo acoplamentos iterativos por meio de um conjunto definido de operações unitárias, facilitando deste modo a síntese de peptídeos complexos e melhorando o rendimento da síntese.

Recentemente, tem havido interesse crescente no desenvolvimento de uma abordagem híbrida que combina as vantagens da SPPS com a síntese de peptídeos em fase líquida (LPPS). Ao contrário da SPPS, a LPPS realiza todas as reações em solução, semelhante à síntese de peptídeos em fase líquida convencional (CSPS), mas eliminando a necessidade de excesso de reagentes. Esses três métodos primários, CSPS, SPPS e LPPS, oferecem vantagens e apreciações distintas na síntese de peptídeos. Assim, o aperfeiçoamento contínuo desses métodos contribuiu para o avanço significativo na química de peptídeos e no sucesso da sua produção para várias aplicações na indústria farmacêutica.

Contudo, os métodos tradicionais de síntese de peptídeos estão dependentes e condicionados pela utilização de solventes orgânicos, entre os quais N,N-dimetilformamida (DMF), N-metil pirrolidona (NMP) e diclorometano (DCM).

Embora esses solventes tenham sido cruciais para o processo de síntese, também são responsáveis por gerar uma quantidade substancial de resíduos, cujo impacto na saúde humana e nos ecossistemas, juntamente com sua grande pegada de carbono, tem gerado preocupações e enfatizado a necessidade urgente de alternativas menos poluentes e mais e sustentáveis.

A síntese de peptídeos através de métodos e tecnologias sustentáveis, em resposta à crescente consciência ambiental, tornou-se por isso fundamental. A mudança em direção a práticas ecologicamente corretas não apenas aborda preocupações ecológicas, mas tem também implicações socioeconómicas mais amplas. No caso da indústria farmacêutica, a adoção de metodologias sustentáveis na síntese de peptídeos pode potencialmente conduzir a para um caminho mais responsável e mais ético.

Uma das abordagens sustentáveis mais promissoras é o desenvolvimento de métodos de síntese de peptídeos sem solventes ou utilizando como solvente a água. Essas técnicas inovadoras não apenas eliminam a necessidade de solventes orgânicos, mas também reduzem substancialmente a produção de resíduos. Além disso, os processos em base aquosa geralmente exibem maior seletividade, maior rendimento e maior grau de pureza dos produtos finais, contribuindo deste modo para uma elevada eficiência geral do processo.

A água, sendo ecologicamente amigável, é uma alternativa potencial pois possui inúmeras vantagens, entre as quais a abundância, baixo custo, não toxicidade e sustentabilidade. A água é conhecida como um solvente eficiente para possibilitar diferentes processos de transformação biológica. O uso da química micelar em água oferece uma estratégia aliciante para a síntese de peptídeos uma vez que reduz as implicações ambientais negativas associadas às abordagens tradicionais.

A substituição dos reagentes tradicionais por outros ambientalmente mais adequados é outra área importante de investigação e desenvolvimento. De facto, a implementação de reagentes verdes, com baixa toxicidade e alta eficiência, pode aprimorar significativamente a síntese de peptídeos com estruturas mais complexas. Esses reagentes devem puder ser utilizados em condições de síntese mais suaves, reduzindo deste modo o consumo de energia e a formação de subprodutos.

Este trabalho envolveu a realização de 49 diferentes procedimentos experimentais para produzir o dipeptídeo (Z-Phe-Leu-OEt). Na síntese foram utilizadas 8 soluções distintas de surfactante e três agentes de acoplamento diferentes (COMU, HBTU e EDC.HCl). Entre os agentes de acoplamento testados, a COMU demonstrou os maiores rendimentos em todas as soluções de surfactante, variando de 52,4% a 75,7%. Esse resultado indica que a COMU é um eficiente agente de acoplamento em meios micelares, especialmente quando combinado com o surfactante Brij O10, que apresentou o máximo de 75,7% de rendimento.

Ao comparar surfactantes tradicionais como Brij O10 e Tween 80 com surfactantes com design específico TPGS-750-M e PS-750-M, curiosamente os primeiros tiveram maiores rendimentos quando usados em conjunto com o agente de acoplamento COMU. Esse resultado inesperado sugere que surfactantes tradicionais podem potencialmente servir como opções menos dispendiosas, mas igualmente competentes, para a química micelar, reduzindo assim os custos gerais.

Além dos agentes de acoplamento e dos surfactantes, também foi estudada a influência do tempo de reação e dos co-solventes no rendimento da síntese de Z-Phe-Leu-OEt. Tempos de reação mais longos foram considerados melhores, pois os rendimentos foram significativamente mais altos quando usado 2 %m/m de TPGS-750-M em água com HBTU como agente de acoplamento. Por outro lado, reações mais diluídas levaram em geral a rendimentos de síntese do produto mais baixos .

A adição de co-solventes ao meio micelar, por outro lado, também conduziu a um aumento de rendimento da reação. Por exemplo, o THF foi identificado como melhor co-solvente para reações de acoplamento com HBTU, embora os rendimentos para os surfactantes usados com EDC.HCl tenham sido mais altos ou comparáveis quando o EtOAc foi usado como co-solvente. A preferência pelo EtOAc em relação a co-solventes polares apróticos mais nocivos, como THF ou tolueno, aumenta ainda mais o potencial ecológico desta abordagem. A utilização do EDC.HCl como agente de acoplamento levou a uma maior eficiência do processo de acoplamento na solução micelar composta por Tween 80/Brij O10. O rendimento obtido foi de 67,1%, superando ligeiramente os rendimentos alcançados quando cada surfactante foi usado individualmente.

Como trabalho futuro seria importante continuar a explorar algumas condições experimentais de síntese, utilizando diferentes combinações de surfactante e de agentes de acoplamento (tipo e concentração) no sentido de obter combinações ótimas para alcançar maiores rendimentos de reação na síntese deste ou outros peptídeos semelhantes.

1. Introduction

Peptide production stands as one of the most important and rapidly growing sectors within the pharmaceutical industry, offering a wide array of therapeutic applications (Ferrazzano *et al.*, 2022; Jad *et al.*, 2019). However, the conventional methods employed in peptide synthesis heavily rely on the use of organic solvents, which consequently generate substantial amounts of hazardous waste (Isidro-Llobet *et al.*, 2019). This predicament necessitates the urgent implementation of sustainable methods and technologies to address the escalating concerns associated with solvent waste in pharmaceutical manufacturing.

The environmental impact stemming from solvent usage in the pharmaceutical industry cannot be overstated. Organic solvents, such as N,N-dimethylformamide (DMF), N-methyl pyrrolidone (NMP), and dichloromethane (DCM), are commonly utilized in large quantities for their excellent solvating properties and compatibility with peptide synthesis processes (Procopio *et al.*, 2022). However, their adverse effects on human health, persistent toxicological effects, and substantial carbon footprint have prompted an indispensable need for greener alternatives. To tackle these challenges, the principles of green chemistry have emerged as a transformative approach. Green chemistry emphasizes the development and adoption of sustainable practices that minimize the use of hazardous substances, reduce waste generation, and prioritize the safety of human health and the environment (Ncube *et al.*, 2023).

The integration of sustainable methods and technologies in peptide production holds tremendous significance, not only from an environmental standpoint but also from a socio-economic perspective (Rosen & Kishawy, 2012). As the demand for peptides continues to soar, the adoption of sustainable practices becomes imperative to ensure long-term viability and resilience within the pharmaceutical industry. By minimizing solvent waste and embracing greener alternatives, the industry can enhance its environmental stewardship, reduce costs associated with waste management, and bolster its public perception as an advocate for sustainable development (Gao *et al.*, 2020).

Micellar chemistry, with water as the solvent, provides a promising alternative to traditional organic solvents, mitigating the adverse environmental impacts associated with conventional approaches (Gabriel *et al*., 2015; Hazra *et al*., 2022; Lipshutz *et al*., 2011). Water, being abundant, non-toxic, and environmentally benign, serves as an attractive solvent candidate (Cortes-Clerget *et al*., 2021). Moreover, the advent of micellar catalysis and the development of new designer surfactants have revolutionized the field, enabling efficient peptide synthesis in aqueous media.

In this contribution, commercially available surfactants in water were explored for the implementation of a simple peptide coupling reaction. The reaction involved the coupling of N-CBZ-L-Phenylalanine and L-leucine ethyl ester HCl, using different coupling agents including (1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium- hexafluorophosphate (COMU), hexafluorophosphate benzotriazole tetramethyl uronium (HBTU) and 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide.HCl (EDC.HCl) . The investigation aimed to assess the efficacy of these surfactant-based systems in facilitating the peptide coupling process. In addition, the influence of reaction time and surfactant solution amount were also studied. Furthermore, the effect of using co-solvents was examined to assess its influence on the peptide coupling reaction. The incorporation of co-solvent was explored as a potential means to enhance reaction efficiency and control the selectivity of the coupling process. Binary surfactant systems were employed to examine the possibility to enhance reaction yield.

In order to evaluate the success of the peptide coupling reactions, product yields were calculated, and the resulting products were analyzed using proton nuclear magnetic resonance (H-NMR) spectroscopy. The analysis of the products by high resolution H-NMR provided valuable insights into the chemical structure of the synthesized peptide, validating the efficiency of the surfactant-based systems in promoting the desired coupling reactions.

2. Literature Review

2.1 Peptide chemistry and pharmaceutical industry

Peptide chemistry has emerged as a pivotal discipline within the pharmaceutical industry, driven by the recognition of the diverse applications and therapeutic potential of peptides. The amide bond, a key linkage in organic chemistry, is found in a wide range of compounds, including natural products, agrochemicals, and industrial materials such as detergents, lubricants, and synthetic polymers (Pattabiraman & Bode, 2011). In recent years, the amide bond has emerged as a crucial structural motif in the pharmaceutical field, particularly in top-selling active pharmaceutical ingredients (APIs) and therapeutic peptides (Procopio *et al*., 2022).

The development of peptide therapeutics has witnessed significant progress over the years. The introduction of the first synthetic peptide therapeutic, oxytocin, in 1962 marked a milestone in this field (Mitchell, 2008). Currently, about 80 peptide-based therapeutics (PbTs) have been approved and launched on the market, with >150 peptides in clinical development and 400–600 peptides in preclinical studies (Muttenthaler *et al*., 2021). Despite the challenge of poor oral bioavailability associated with peptide therapeutics, alternative routes of administration, such as subcutaneous injection or inhalation, have been employed (Lau & Dunn, 2018). Furthermore, advancements in peptide formulation, such as the use of permeation enhancers for improved oral absorption, show promise for overcoming this limitation and accelerating the growth of therapeutic peptides (Karavasili *et al*., 2015).

The pharmaceutical industry's interest in peptide candidates for drug discovery and development programs stems from the potential of exploiting peptide drugs for a wide range of therapeutic indications. One significant area is the targeting of protein-protein interactions (PPIs) that regulate various diseases. Interfering peptides (IPs), which modulate PPIs, have found application in the treatment of conditions such as cancer, diabetes and obesity, cardiovascular disease, and gastrointestinal disorders (Bruzzoni-Giovanelli *et al*., 2018; Ferrazzano *et al*., 2022). Moreover, the urgent need for alternative treatments to combat antibiotic resistance has led to increased research on antimicrobial peptides (AMPs) (Chung & Khanum, 2017).

Additionally, the recent Covid-19 pandemic has further accelerated the search for novel therapeutic peptides against SARS-CoV-2 infection and associated diseases (Khavinson *et al.*, 2021)

The growth and potential of peptide therapeutics are reflected in the market value of this class of drugs. Currently valued at USD 23 billion, the market is projected to reach USD 57 billion by 2027 (Martin *et al.*, 2020) This upward trend underscores the pharmaceutical industry's recognition of the value and future prospects of peptide-based therapeutics. Continued research and development in peptide chemistry will undoubtedly lead to further advancements and innovations in drug discovery and development, benefiting patients worldwide.

2.1.1 Classical approaches to chemical peptide synthesis

In recent years, the field of peptide chemistry has made remarkable advancements, driven by the recognition of peptides' immense potential as therapeutic molecules. This has resulted in a substantial demand for peptides in various applications. Initially, classical solution peptide synthesis (CSPS) served as the primary method for peptide preparation (Eggen *et al.*, 2005). CSPS offers advantages such as the use of small excesses of reagents, aligning with green chemistry principles and providing financial benefits. This method involves isolating and characterizing intermediates, which ensures a high-quality crude product but at the expense of significant time and human effort (A. Sharma *et al.*, 2022).

The introduction of solid-phase peptide synthesis (SPPS) by Bruce Merrifield revolutionized peptide chemistry (Merrifield, 1963). SPPS simplified the removal of non-tethered entities like reagents and solvents, making the process more efficient (Isidro-Llobet *et al.*, 2019). It facilitated automation, allowing for iterative couplings through a defined set of unit operations. Consequently, SPPS enabled the synthesis of challenging peptides with greater ease and precision (Mitchell, 2008).

In recent years, there has been a growing interest in developing a hybrid approach that combines the strengths of SPPS and CSPS. Known as liquid-phase peptide synthesis (LPPS), this method carries out all reactions in solution, similar to CSPS, eliminating the need for excess reagents (Ferrazzano *et al.*, 2022; A. Sharma *et al.*, 2022).

However, in LPPS, the growing peptide chain is supported on a soluble tag, which imparts special properties to the chain, facilitating solution chemistry and simplifying the workup process after each step. The critical requirement for the LPPS strategy is the selection of a soluble tag that differs from the properties of the reagents and byproducts generated during the reactions, enabling their easy removal through precipitation, filtration, or extraction (Martin *et al*., 2020).

These three primary methods, namely CSPS, SPPS, and LPPS, offer distinct advantages and considerations in peptide synthesis. The continuous development and optimization of these peptide synthesis methods contribute significantly to the advancement of peptide chemistry and the successful production of peptides for various applications in the pharmaceutical industry.

2.1.1.1 Classical solution peptide synthesis

Classical solution peptide synthesis stands as the initial method employed for the synthesis of peptides (Eggen *et al*., 2005; Wu *et al*., 2022a). This technique allows for the utilization of minimal excess reagents. Nevertheless, the process of CSPS is laborious and time-intensive when preparing peptides for research purposes or on a large-scale, particularly those of medium and long lengths. CSPS operates on the principle of coupling individual amino acids in a solution. On the other hand, the fragment condensation approach has been adopted for the synthesis of elongated peptides. In this approach, short peptide fragments are initially synthesized and subsequently merged to yield a longer peptide. One notable advantage of CSPS in peptide synthesis lies in its ability to facilitate deprotection and purification of intermediate products, resulting in the production of a final peptide product with high purity (Carpino *et al*., 2003; Nishiuchi *et al*., 1998).

Several peptides have been successfully synthesized using CSPS, including oxytocin, a neuromodulating nonapeptide and crucial hormone in sexual reproduction (du Vigneaud *et al*., 1954; Du Vigneaud *et al*., 1953); porcine gastrin-releasing peptide, a hormone that stimulates gastric acid secretion in the stomach (Chandrudu *et al*., 2013); and human insulin, a peptide hormone comprising 51 amino acids responsible for regulating carbohydrate metabolism within the body (Akaji *et al*., 1993).

2.1.1.2 Solid-phase peptide synthesis

In 1963, Merrifield accomplished a significant milestone by successfully synthesizing an elongated peptide chain on an inert solid support known as resin (Merrifield, 1963). Since the advent of Merrifield's groundbreaking synthesis, the SPPS strategy has undergone substantial evolution, ultimately becoming the prevailing standard for peptide synthesis (Verlander, 2007). The initial step in SPPS involves the attachment of the first amino acid to the resin through a linker possessing a reactive functional group. This linker serves the crucial purpose of facilitating the subsequent cleavage of the synthesized peptide from the resin upon completion of the synthesis. Within the SPPS framework, peptide assembly typically proceeds from the C-terminal to the N-terminal end on the resin by employing side chain protected amino acids to mitigate any undesired side reactions during the coupling process (Kimmerlin & Seebach, 2005).

Another pivotal aspect of SPPS involves the use of a temporary alpha-amino (N^{α}) protecting group, which must be removable to enable the coupling of the next amino acid in the peptide chain, while preserving the integrity of the side chain protecting groups and the peptide resin linkage (Palomo, 2014). The 9-fluorenylmethoxycarbonyl solid-phase peptide synthesis (Fmoc-SPPS) technique has emerged as the prevailing method employed for peptide synthesis. In contrast to tert-butoxycarbonyl (Boc)-SPPS (known as Merrifield chemistry), which necessitates more rigorous acidic treatments during the ultimate global deprotection step, Fmoc-SPPS presents a more amiable approach for peptide synthesis.(Harris & Brimble, 2013; Jaradat *et al*., 2022).

The implementation of solid-phase peptide synthesis (SPPS) necessitates the utilization of excess reagents and solvents to ensure the completion of each coupling step (Wegner *et al*., 2021). One of the notable advantages of this method lies in the ease of removing the excess reagents and by-products through resin washing steps followed by filtration(Shelton & Jensen, 2013). Typically, hazardous polar aprotic solvents such as N,N'-dimethylformamide (DMF), 1-methyl-2-pyrrolidone (NMP), N,N'-dimethylacetamide (DMAc), and the chlorinated solvent dichloromethane (DCM) find application in SPPS (Castro *et al*., 2021).

However, the substantial use of these solvents during peptide synthesis generates a considerable volume of hazardous waste, posing environmental and safety concerns.

2.1.1.3 Liquid-phase peptide synthesis

Liquid-phase peptide synthesis was initially documented by Bayer and Mutter in the early 1970s (Mutter *et al*., 1971). LPPS involves the substitution of insoluble resins with a soluble polymeric support, which exhibits solubility in halogenated and ether solvents, while being less soluble in polar solvents like methanol (MeOH) or ethanol (EtOH). Consequently, the coupling and deprotection steps are performed in solvents such as dichloromethane (DCM), chloroform ($CHCl_3$), or tetrahydrofuran (THF), followed by precipitation using MeOH or acetonitrile (MeCN), or an aqueous workup (Takahashi *et al*., 2012a; Takahashi & Yamamoto, 2012). LPPS combines the benefits of SPPS and classical solution chemistry (Jad *et al*., 2019).

In this approach, all reactions take place in solution, similar to CSPS, thus eliminating the need for excessive reagent quantities (A. Sharma *et al*., 2022). However, in LPPS, the growing peptide chain is supported by a soluble tag that imparts unique properties facilitating solution chemistry and simplifying workup after each step (Wu *et al*., 2022b). The key criterion for the LPPS strategy lies in the distinct properties of the soluble tag, which must differ from those of the reagents and byproducts generated during reactions, thus enabling their straightforward removal through precipitation, filtration, or extraction (Wu & Yamamoto, 2023). The crucial difference between LPPS and SPPS lies in the support medium. While SPPS employs large solid polymers as support, LPPS predominantly employs small, well-defined molecules or soluble polymers.

SPPS, requires the use of excess amounts of amino acids and coupling agents to ensure complete conversion of couplings (Naoto Sugisawa *et al*., 2023). Additionally, substantial quantities of solvents like N,N-dimethylformamide (DMF) and N-methyl-2-pyrrolidone (NMP) are required for each reaction and subsequent washing steps (Martin *et al*., 2021). These factors pose significant challenges when scaling up the synthesis, leading to a substantial increase in overall costs. In this regard, LPPS exhibits advantages over SPPS as it allows for the minimization of required quantities of amino acids, coupling agents, and solvents (Okada *et al*., 2019).

LPPS presents a promising avenue for achieving enhanced crude purity and improved scalability compared to SPPS (Y. S. Lee, 2019). Nevertheless, the widespread adoption of LPPS has been limited due to the prevailing approach of employing precipitation or extraction techniques for the crucial separation of intermediates from reaction by-products. Regrettably, these methods are time-consuming and necessitate frequent optimization across cycles and target peptides, as the physical properties of growing peptide intermediates exhibit unpredictable variations (Frederick *et al*., 2021). Consequently, the overall LPPS process becomes slow and burdensome (Takahashi *et al*., 2012b).

2.2 Drawbacks of using conventional solvents in peptide synthesis approaches from the perspective of green chemistry

From the standpoint of green chemistry, the use of traditional solvents in peptide synthesis methods has considerable disadvantages. Firstly, the production and disposal of these solvents raise environmental problems because they are frequently compounds that are made from fossil fuels. Conventional solvents may also be highly toxic, endangering both human health and the environment. Additionally, their inefficient utilization results in increased waste generation and energy use, which increases the carbon footprint. The potential for environmentally friendly and sustainable peptide synthesis procedures is constrained by the reliance on conventional solvents. Researchers are exploring alternative solvent systems that are ecologically safe, commercially viable, and in line with green chemistry principles to resolve these issues.

2.2.1 Occupational health concerns and toxicity

The synthesis of peptides through amide bond formation using partially protected amino acid derivatives is highly inefficient and environmentally unsustainable (Varnava & Sarojini, 2019b). The process requires numerous auxiliary reagents in the form of protecting groups and coupling agents, resulting in low atom efficiency (Verlander, 2007). Additionally, peptide synthesis requires using toxic solvents such as DMF and NMP.

These concerns are amplified in solid-phase peptide synthesis, where excessive amounts of reagents are used, and resins are washed multiple times with toxic solvents (Lawrenson *et al*., 2017).

The use of polar aprotic solvents like DMF or NMP is of particular concern since they are known to be reprotoxic and cause environmental issues when present in waste (Parmentier *et al*., 2016).

These conventional solvents have been categorized as reproductive toxicants according to Article 57(c) of REACH (Registration, Evaluation Authorization and Restriction of Chemicals) (Wegner *et al*., 2021). Furthermore, they have been identified as SVHCs (Substances of Very High Concern). DMF was included in the Candidate List as a SVHC for Authorization in December 2012, and the Authorization process was proposed in 2014. In 2016, it was suggested that DMF, NMP, and DMAC should be collectively classified under the same category of polar aprotic solvents, a decision that was confirmed in February 2018. In Oct 2018, the public consultation for the revised DMF restriction proposal was launched. The final deadline for comments on the restriction report was June 2019. (Becker *et al*., 2021; Jordan *et al*., 2022; Sels *et al*., 2020; Wegner *et al*., 2021).

DMF, widely utilized in the pharmaceutical industry and peptide production due to its low volatility, molecular size, and chemical properties (acting as an electron donor and facilitating complex formation), has raised concerns regarding its toxicity since the 1980s (Kim & Kim, 2011; Marsella & Updated by Staff, 2013). Studies have revealed that DMF can be absorbed through the skin or inhalation, and it is known to cause hepatotoxicity, leading to liver damage (Lynch *et al*., 2003; Scailteur *et al*., 1987).

In 2001, approximately 285,000 tons of DMF were used globally (Chen *et al*., 2022). The toxicity of DMF is expected to eventually necessitate its elimination from the solvents used in the pharmaceutical industry by the REACH organization (REACH, 2006). As per the DMF restriction proposal of October 2018, the manufacturing and use of DMF should be limited to concentrations at or below 0.3% in industrial and professional settings. This restriction ensures that under normal operational conditions, exposure remains below The Derived No Effect Levels (DNEL).

For workers, the DNEL for DMF has been calculated as 3.2 mg/m^3 for long-term inhalation exposure and 0.79 mg/kg/day for dermal exposure (Wegner *et al*., 2021). The new limits for workers' long-term inhalation exposure were established at 6 mg/m^3, and for dermal exposure, the level is now 1.1 mg/kg/day (Coatings, 2023) .

Another solvent used extensively in peptide synthesis is NMP, which is currently classified as a “substance of very high concern” by the European Chemicals Agency (ECHA). There are impending measures to restrict the use of NMP in Europe and in the US (Schmied-Tobies *et al.*, 2021). In the workplace environment, NMP exposure primarily occurs through inhalation; however, its ability to permeate human skin suggests that dermal absorption is also a significant route of exposure (Meier *et al.*, 2013). NMP is readily absorbed through human skin, as well as the gastrointestinal and respiratory tracts. Experimental studies indicate significant absorption following inhalation (40–60%), oral (approximately 100%), and dermal (up to 100% under specific conditions) exposure. Dermal absorption has been extensively investigated due to its potential for human exposure (Saghir, 2014). Presently, the airborne occupational exposure limit value for NMP in the European Union is set at 40 mg/m^3 (equivalent to 10 ppm) in the workplace (ECHA, 2016). Additionally, NMP and formulations containing ≥5% NMP must be labeled as reproductive toxicants. The DNEL for NMP is calculated as 14.4 mg/m^3 for inhalation exposure and 4.8 mg/kg/day for dermal exposure (Schmied-Tobies *et al.*, 2019).

Studies conducted on pregnant animals have demonstrated that NMP poses harm to the developing fetus. It exhibits toxicity to the reproductive systems of both male and female test animals (Sitarek & Stetkiewicz, 2008). However, the reproductive effects of NMP have not been thoroughly investigated in humans. In a study conducted by Lee in 1987, pregnant rats were exposed to an aerosol-vapor mixture of NMP at concentrations of 0, 0.1, 0.5, and 1.0 mg/liter for 6 hours per day, 5 days per week, over a period of 4 weeks. Rats exposed to 1.0 mg/liter exhibited lethargy, respiratory difficulties, and excessive mortality. These rats also showed focal pneumonia, bone marrow hypoplasia, and atrophy of lymphoid tissue in the spleen and thymus(LEE, 1987b).

2.2.2 Environmental impacts of conventional solvents

The excessive consumption of nonrenewable, toxic solvents in peptide reactions, extractions, purifications, and cleaning processes is a notable example of unsustainable practices. It has been estimated that the annual industrial-scale production of organic solvents is close to 20 million metric tons (Clark *et al.*, 2015).

This overconsumption of solvents poses significant environmental risks and is detrimental to the ecosystem (Hojo *et al*., 2022). The environmental concerns related to solvents can be categorized into three areas: the source and synthesis of the solvents themselves, their properties during usage including accidental discharge, and their disposal (Welton, 2015).

The application of the E-factor, which measures waste generation, has revealed that the pharmaceutical industry generates a larger proportion of waste compared to the production of fine or bulk chemicals. More than 70% of the waste associated with pharmaceutical production is attributed to solvents, as estimated by GSK (Jiménez-González *et al*., 2004). This can be attributed to the complex synthesis process of pharmaceuticals, which involves numerous steps and isolations of intermediate products (Welton, 2015). The waste generated during the synthesis of a peptide with an average molecular mass between 1000 and 5000 Da is considerable, resulting in a typical PMI (Process Mass Intensity) ranging from 3000 to 15,000 kg kg^{-1} of Active Pharmaceutical Ingredient (API). Moreover, the usage of hazardous solvents and reagents further contributes to the overall environmental and health risks (Koenig *et al*., 2019).

Consequently, there has been a growing interest in seeking greener alternatives to substitute organic solvents. The health and environmental concerns associated with the use of solvents have prompted the exploration and development of more sustainable options.

2.2.3 Substitution of solvents by greener alternatives in peptide synthesis

Up to this point, the process of synthesizing peptides has been accompanied by the generation of significant quantities of toxic waste. As mentioned before, DMF, DCM, and NMP are commonly used in peptide synthesis. Furthermore, concerns have been raised about the hepatotoxic consequences of DMF exposure, as well as the toxic and allergic properties of the solvents used in peptide synthesis. As a result, there is a pressing requirement for the development of a peptide synthesis process that is safer and environmentally sustainable.

In recent years, a considerable body of research has been devoted to investigating more environmentally sustainable approaches for peptide synthesis. Notably, Albericios' laboratory has emerged as a key player in both traditional peptide synthesis and the development of greener, safer procedures (Varnava & Sarojini, 2019a). Their research has concentrated on replacing highly toxic solvents with less hazardous alternatives, thereby improving the green aspects of the synthesis process. Their research has demonstrated that using THF and MeCN as alternatives for DMF in peptide synthesis produces equivalent, and in some cases, superior results. Although THF and MeCN are not technically green solvents, they are more environmentally friendly than DMF and DCM (Acosta *et al*., 2009; Jad *et al*., 2015).

Shifting to a safer coupling reagent can have a significant impact on peptide manufacturing sustainability. The bulk of peptide bonds produced in the industrial setting are formed with 1-hydroxybenzotriazole (HOBt) or its derivatives. These compounds are frequently used in conjunction with a carbodiimide, such as EDC.HCl, or as a standalone reagent, such as immonium salts (HATU, HBTU) or phosphonium salts (PyAOP, PyBOP) (El-Faham *et al*., 2009a). However, the explosive properties of HOBt, as well as the toxicity of HATU, have encouraged Albericios' group to investigate and record the development of a third generation uronium salt, COMU (El-Faham & Albericio, 2010a; Wehrstedt *et al*., 2005). This new coupling reagent combines a morpholonium-based immonium moiety as a proton acceptor with Oxyma as a leaving group, resulting in a superior and safer reagent for peptide synthesis (El-Faham & Albericio, 2011).

Over the last 10 years, there has been increasing interest in the use of biomass-derived solvents as potentially less harmful and more sustainable solutions for peptide synthesis (Clark *et al*., 2015). Organic solvents derived from biomass, such as 2-Methyltetrahydrofuran (2-MeTHF), dihydrolevoglucosenone (Cyrene), p-Cymene, and γ-valerolactone (GVL), are chemical compounds that have several attractive properties, including abundant availability, renewability, low toxicity, biodegradability, and reasonable cost. These solvents are therefore recognized as environmentally benign alternatives, and their use is recommended by multiple selection guides (Procopio *et al*., 2022).

2-Methyltetrahydrofuran (2-MeTHF) has received the most comprehensive investigation as a bio-based solvent (Jad *et al*., 2017; Jad, Acosta, Govender, *et al*., 2016; Jad, Acosta, Khattab, *et al*., 2016). It is derived from furfural or levulinic acid and has been studied as a green alternative solvent since 2013, when McMillan *et al*. reported its use in amide synthesis for the first time. They examined several solvents as alternatives for dimethylformamide (DMF) and dichloromethane (DCM) in coupling reactions between alkyl and aryl acids and amines. The results showed that 2-MeTHF, along with ethyl acetate (EtOAc) and Dimethyl carbonate (DMC) , had potential as appropriate alternative solvents, particularly when (COMU) was used as the coupling reagent, producing comparable conversion rates to those reported with DCM or DMF (MacMillan *et al*., 2013).

The best solvent is no solvent even though the use of solvents is a standard practice in synthetic organic chemistry (Obst & König, 2018). Mechanochemistry is a promising field of study in the greening of amide/peptide synthesis (Avila-Ortiz & Juaristi, 2020). It is an ideal condition involving the absence of a solvent, which simplifies the reaction, makes it less hazardous and toxic, saves energy, and reduces the generation of by-products or solvent waste. This approach allows for a significant reduction in the E-factor associated with organic processes (Sahoo & Banik, 2020).

Declerck *et al*. successfully synthesized aspartame (a dipeptide sweetener) using the ball-milling approach in 2009, marking a significant milestone in solvent-free peptide synthesis (Declerck *et al*., 2009). Following that, the researchers extended their method to tripeptides, resulting in high product yields.

Pentapeptide Leu-enkephalin was successfully synthesized in a nine-step process with exceptionally good overall yields (46% overall yield) by improving the reaction conditions and using a minimal amount of ethyl acetate (EtOAc), which is considered safer solvent. Notably, none of the nine steps involved the use of potentially hazardous organic solvents (Bonnamour *et al*., 2013).

In line with the Green Chemistry philosophy, the pursuit of environmentally friendly and sustainable methods in chemical synthesis has become a major objective. The use of water as a solvent and the incorporation of surfactants have emerged as potential replacements for hazardous organic solvents.

Micellar chemistry not only adheres to Green Chemistry principles, but also provides several benefits such as enhanced reaction efficiency, greater selectivity, and reduced environmental effects.

2.3 Water as a reaction medium

Water serves as the ubiquitous solvent in nature, enabling biological transformation processes. It provides various advantages, including being inexpensive, economical, safe, non-toxic, and sustainable (La Sorella *et al*., 2015). In recent years, there has been a growing interest in aqueous phase reactions in the field of green chemistry, and several articles have been published to highlight the advances made in this field (Hazra *et al*., 2022; Shen *et al*., 2021b). Initially, two types of reactions were identified: on-water reactions of heterogeneous systems and in-water reactions of homogeneous systems. Sharpless first proposed the concept of on-water reactions in 2005; these reactions take place at the interface of oil and water, necessitating vigorous agitation (Narayan *et al*., 2005). Indeed, effective substance transmission becomes a major challenge in this case. In-water reactions, on the other hand, entail the dissolution of hydrophilic substrates in an aqueous solution. Nonetheless, due to the insolubility of most organic species in water, it is still quite limited.

2.3.1 Surfactants and micelle formation

Micelle-mediated synthesis has recently gained interest as a suitable approach for aqueous synthesis in the realm of industrial chemistry. Surfactants are utilized in almost all reported cases to create a micellar system, which either provides a reaction field with its surface in aqueous media or serves as a solubilizing agent (Hojo *et al*., 2022). Surfactants are amphiphilic molecules that self-assemble. Amphiphiles are organic molecules that have both hydrophobic and hydrophilic parts. The polar portion (hydrophilic) is called “head” and the apolar portion (hydrophobic) usually a long chain hydrocarbon is considered “tail” (figure 2.1). These compounds are most stable when each part is located in a suitable environment, which is only possible at the interface between two media. Therefore, amphiphilic compounds are also called surface-active agents, or in short, surfactants (Ghosh *et al*., 2020).

When the surfactant concentration in aqueous solution exceeds the critical micelle concentration (CMC), it self-aggregates to create nano-sized micelles (nanoreactors) with a hydrophobic interior and a hydrophilic exterior (Sheldon, 2019).

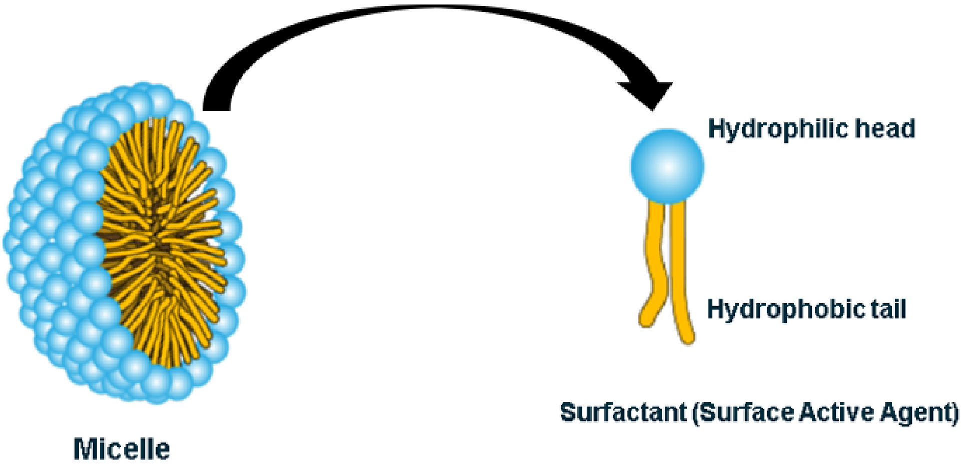


Figure 2. 1 Micelle and the structure of amphiphilic molecules.

Adopted from: https://psiberg.com/micelle-formation.

The choice of an amphiphile is critical since it determines the size, configuration, and internal hydrophobicity of nano-micelle reactors (Sheldon, 2019). Several factors influence the morphology and size of micelles, including: (i) the chemical composition of the surfactant; (ii) molecular geometry; (iii) the relative proportions of hydrophilic and hydrophobic segments; and (iv) experimental conditions such as temperature, pH, and ionic strength. Micelle size is important in facilitating chemical transformations. Inadequate sizes, whether too small (less than 50 nm) or too large (more than 500 nm), fail to facilitate the desired chemical reactions (Ansari *et al.*, 2020). When the particle size of micelles is too large, it impedes the exchange process and hence slows the reaction rate. If the micelle size is too small, the reactants may have difficulty penetrating the micelles, hindering reactions from occurring (Duong, 2021). The geometry of the surfactant influences the formation of different aggregates. Figure 2.2 shows some types of micelles that can form in an aqueous environment. Spherical micelles are the most basic form of aggregation (Ghosh *et al.*, 2020).

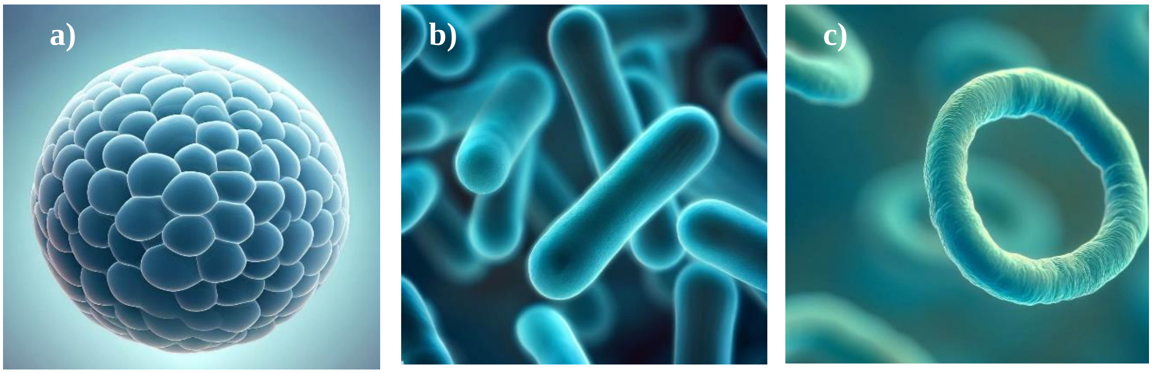


Figure 2. 2 Shapes of self-assemblies formed by amphiphiles in water: a) spherical, b) cylindrical and c) vesicle micelles.

The critical micelle concentration (CMC) is the lowest concentration required for assembling spherical micelles. This formation is characterized by often-sharp discontinuity in system properties including surface tension, conductivity, light scattering, self-diffusion, and dissolved component molality (Cornils & Herrmann, 2004). Several investigations have been conducted to determine the parameters that influence micelle formation, particularly in aqueous medium.

Some of the factors that influence CMC in aqueous solution are (i) surfactant structure, (ii) the presence of added electrolyte (in the case of ionic surfactants) in the solution, (iii) counterion nature (for ionic surfactants), (iv) the presence of various organic compounds in solution, and (v) solution temperature (Ghosh *et al.*, 2020).

The temperature at which the amphiphile concentration approaches the critical micelle concentration (CMC) is known as the Krafft point or Krafft temperature, and it is usually accompanied by a sudden rise in solubility (Dwars *et al.*, 2005). The effect of temperature on the CMC of surfactants in an aqueous environment is complex, with the initial tendency indicating a decrease in value up to a specific minimum, followed by a rise as temperature is raised further. The increase in temperature reduces the hydration of the hydrophilic group, boosting micellization, but also disturbing the structured water surrounding the hydrophobic group, limiting micellization(Gaines, 1972; Mukerjee & Mysels, 1955; Os *et al.*, 1993).

The hydrophilic head group can be cationic, anionic, zwitterionic, or non-ionic. The hydrophilic-lipophilic balance (HLB) represents the relationship between the hydrophilic head group and the hydrophobic chain (Myers, 2006). For micelle forma, the hydrophobic chain needs to be a particular length (>C10). The CMC decreases as the chain length increases (Dwars *et al*., 2005).

2.3.2 Designer surfactants

Surfactants are categorized according to their chemical structure, shape, charge, and application. One common classification criterion for surfactants is their head charge, which might be anionic, cationic, nonionic, or zwitterionic (Bordes & Holmberg, 2015). Surfactants can be classified based on their (HLB) values since these values influence surfactant functions such as emulsification, solubility, and detergency (figure 2.3) (Ohadi *et al*., 2020). In this study, utilized surfactants are divided into two categories: traditional and novel designer surfactants. Surfactants such as sodium dodecyl sulfate (SDS), Tween 20, Tween 80, Trito X 100, Brij O10, and others are examples of traditional surfactants.

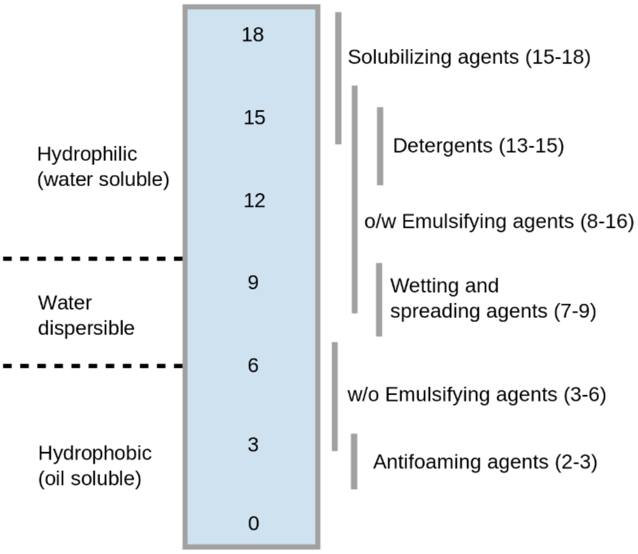


Figure 2. 3 HLB scale showing functions of surfactants along with their HLB range.

Adopted from: Aulton, M. E. (2002). Pharmaceutics: The science of dosage form design

On the other hand, new designer surfactants are tailored molecules that are specifically designed for desired functions and applications. They exceed traditional surfactants in terms of performance and distinctive properties. Over the last 12 years, the Lipshutz group has developed sustainable, practical, and user-friendly synthesis procedures (Isley, 2020). Lipshutz lab has been a pioneer in micellar chemistry, developing three generations of PEG-based surfactants (PTS, TPGS-750-M, NOK).Polyoxyethylene-a-tocopheryl sebacate (PTS) is a first generation non-ionic amphiphilic compound that belongs to the family of polyoxyethylene derivatives. PTS produced good results in terms of yields and reaction rates (Lipshutz *et al*., 2011).

TPGS-750-M is the most widely studied PEG-based surfactant of the second generation, and it has been proven to be very efficient for general application in metal-catalyzed cross-coupling reactions in water. This surfactant has been used to study several "name" reactions, including Heck, Suzuki-Miyaura, Sonogashira, Negishi-like couplings, and peptide couplings, as well as C-H activations and olefin metathesis reactions(Shen *et al*., 2021a).

SPGS-550-M("Nok"), a third generation of micelle based on phytosterol β-sitosterol rather than vitamin E derivatives, was developed in 2014 (Lorenzetto *et al*., 2020). They also reported another surfactant "Coolade" with low-foaming properties later in 2019 to lower the reaction vessel. This novel surfactant contains a PEG moiety, but the hydrocarbon tail is missing to prevent foaming(N. R. Lee *et al*., 2019).

Another designer surfactant was MC-1, a novel surfactant inspired by the widespread application of the environmentally hazardous dipolar aprotic solvent dimethyl sulfoxide (DMSO). MC-1 delivers a highly polar sulfone component into a surfactant's otherwise nonpolar region, and therefore into the inner cores of its generated nanomicelles. This has resulted not only in a solution to solubility problems, but also in higher yields, easier handling of reaction mixtures, and the elimination of co-solvents (Cortes-Clerget, Spink, *et al*., 2019).

Similar to the previously described "DMSO-like" surfactant MC-1, the "DMF-like" qualities of Savie, a recent designer surfactant from Lipshutz lab, with an improved emulsifying capacity when compared to "glyme-like" PEGylated TPGS-750-M. Savie provides for increased homogeneity of micellar reaction mixtures without the need for organic co-solvents, resulting in typically higher yields(Kincaid *et al*., 2022).

2.3.3 Micellar chemistry and green chemistry

Green chemistry is a conceptual system that aims to make chemical processes and reactions safer and more environmentally friendly. The application of green chemistry principles, which are comprised of twelve fundamental pillars (figure 2.4), presents significant challenges while also providing chances for progress across multiple disciplines (Anastas & Eghbali, 2010). Notably, micellar chemistry has made significant advancements in recent years, resulting in improved reaction yields, greater selectivity, easier catalyst recycling, and organic media substitution with surfactants in aqueous conditions (Lorenzetto *et al*., 2020). This revolutionary change in medium selection is accelerated by the numerous advantages offered by micellar chemistry (table 2.1) when viewed through the lens of the twelve principles of green chemistry(Cortes-Clerget, Lee, *et al*., 2019; Isley, 2020). The literature surveyed reveals that the usage of micellar media represents most of the green chemistry principles, often meeting multiple principles simultaneously(Fabris *et al*., 2023). Surfactants are typically utilized in small amounts, avoiding the use of large amounts of organic solvents, which are non-biodegradable and remain in the environment for long periods of time. Surfactants' chemical nature, however, raises significant concerns regarding their degradation and persistence in the environment (Mustahil *et al*., 2019).

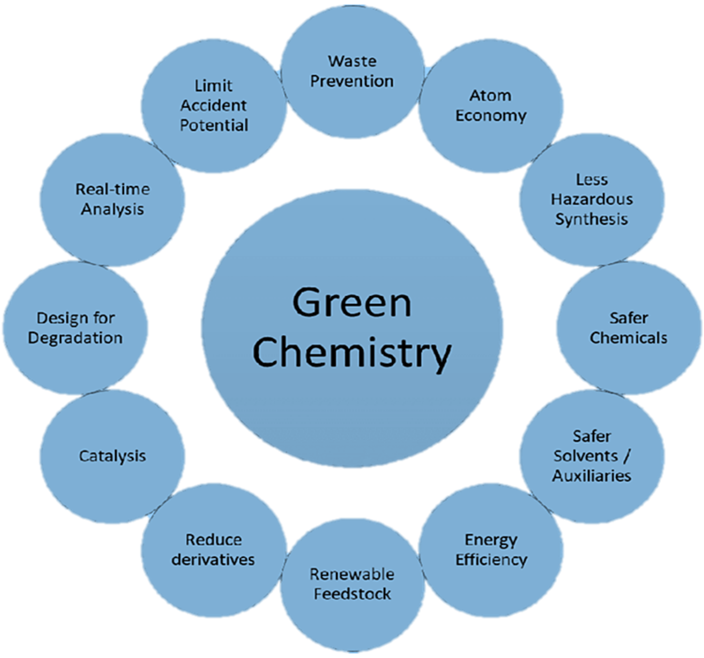


Figure 2. 4 Green chemistry 12 principles.

Adopted from: Istasse, T. (2021). Synthesis of furan derivatives from monosaccharides in reaction media based on choline chloride.

Table 2. 1 Advantages and disadvantages of micellar chemistry.

Advantages	Disadvantages
1. Reactions run in water 2. Commercially available reagents 3. Low E-factors 4. High yields 5. Fast reaction 6. Economical and sustainable	1. Not giving high yields in all cases 2. Low stability when encountering environmental changes. 3. There is a fundamental gap in literature about the basic rules of micellar chemistry

The extensive application of surfactants for detergency has led to a development of a diverse variety of commercially available surfactants with rapid degradation. Despite this, their presence in aquatic environments is unavoidable due to their high affinity for water. Therefore, optimal surfactants should be composed of hydrophilic and hydrophobic potions that are easily separated, leading to naturally occurring compounds that are rapidly degraded by microorganisms. Recent reviews have focused on evaluating the biodegradability and biocompatibility of synthetic surfactants (Infante *et al*., 2004; Katiyar *et al*., 2019; Madani *et al*., 2019).

2.3.4 Micellar chemistry and peptide synthesis

For many years, peptide coupling has been extensively utilized as a fundamental reaction in medicinal chemistry (Lau & Dunn, 2018). With respect to the choice of reaction solvent, peptide synthesis in terms of its environmental footprint is far from being benign and sustainable (Bonnamour *et al*., 2013). Even though water is the natural medium for peptide biosynthesis, organic solvents are still commonly employed due to the sequential nature of the synthesis process (Cortes-Clerget *et al*., 2017a). Lipshutz group established an environmentally friendly method for amide/peptide bond synthesis using an aqueous micellar medium. The reaction takes place within the core of the nanomicelles, formed by 2 wt.% aqueous solution of different designer surfactants.

Experimentation revealed that the coupling step between two protected amino acids at room temperature exhibited optimal efficiency when using a combination of COMU and 2,6-lutidine.

The workup procedure includes a simple extraction followed by aqueous washes with acidic and basic solutions, keeping the surfactant and any by-products in the initial aqueous layer. However, in some cases the synthesis of polypeptides containing polar amino acids occasionally resulted in the reaction mixture becoming thick and sticky, inhibiting stirring and, as a result, lowering conversion levels and overall isolated yields (Cortes-Clerget, Spink, *et al.*, 2019). To address this issue, 10% (v/v) THF as co-solvent is added, resulting in better solubility (Gabriel *et al.*, 2017b). The following table 2.2 presents reported examples of peptide synthesis in a micellar medium.

Table 2. 2 Some reported examples of peptide synthesis in a micellar medium.

No.	Study Description	Yield's Range (%)	Ref.
1	A novel method for synthesizing peptides in solution using a tandem deprotection/coupling sequence at room temperature, using micellar catalysis and **TPGS-750-M** surfactant, successfully synthesizes polypeptides with up to ten amino acid residues.	70-99	(Cortes-Clerget *et al.*, 2017a)
2	In this paper, **PS-750-M** was employed. The presence of 3° amides from the surfactant proline linker in the micelle of PS-750-M mimics DMF, DMAc, and NMP. As a result, the micellar characteristics facilitate fast amide couplings via EDC, removing the need for expensive and specialized coupling agents.	83-96	(S. Sharma *et al.*, 2020)
3	2 wt% **MC-1**/H_2O was used in several peptide coupling reactions, resulting in higher yields compared to TPGS-750-M. No co-solvent is needed	79-92	(Cortes-Clerget, Spink, *et al.*, 2019)
4	In this study, a new surfactant named (**Savie**) was introduced. Some peptide coupling reactions were carried out in water at room temperature with 2 wt% Savie, resulting in a nearly 10% higher isolated yield over TPGS-750-M.	71-88	(Kincaid *et al.*, 2022)

2.3.5 Scale-up of micellar chemistry

Green solvents and micellar chemistry have emerged as safe and sustainable solutions as a result of various triggers, such as regulatory measures and the potential ban of reprotoxic polar aprotic solvents through the Substance of Very High Concern (SVHC) process under the REACH framework (Bergkamp & Herbatschek, 2014) , or the pursuit of the United Nations' Sustainable Development Goals (Department of Economic and Social Affairs of United Nation, 2023)**.**

Designer surfactants, which allow a variety of reactions to occur in water at ambient temperature, was envisaged as an environmentally benign alternative to standard organic solvents (Lipshutz, 2017). These experiments have mostly occurred in academic laboratories, where reactions are normally carried out on a small scale of 100 mg or less. As a result, there has been a scarcity of experimental evaluation of their potential for large-scale applications (Hauk *et al*., 2021).

However, a breakthrough observation by industrial chemists engaged in process research at Novartis Pharma AG, led by Fabrice Gallou, has now enabled the scaling-up of micellar chemistry in the pharmaceutical industry (Hauk *et al*., 2021; Parmentier *et al*., 2020). Novartis relied significantly on TPGS-750-M, which benefited from the addition of an organic co-solvent, such as acetone ethyl acetate or THF. The use of such a co-solvent not only facilitated physical transfer, scale-up, and scale-down studies, but it also resulted in the growth of initially created "naked" micelles, thereby doubling their inner diameter, and improving reaction outcomes(Andersson *et al*., 2018).

2.3.6 Limitations of micellar chemistry

Micellar chemistry has received attention for its prospective advantages, however, there are some drawbacks that restrict its widespread implementation. One such disadvantage is the continuous reliance on organic solvents to isolate and purify reaction products in some reactions. Another key issue with micellar chemistry is the difficulty of effectively eliminating residual inorganic and organic compounds from the water used in these procedures. Water contaminated with such substances cannot be released untreated, necessitating additional purification measures. However, these purification approaches are still inefficient and further research is needed (Lipshutz, 2018).

The field of micellar chemistry faces a lack of knowledge of its fundamental concepts. Scientists readily admit that there is still much to learn about the basic principles that regulate micellar systems. To develop and optimize reactions in micellar settings, researchers now rely significantly on empirical knowledge. In this discipline, a lack of comprehensive theoretical understanding impedes the development of robust and predictable procedures.

Alessandro Scarso from Ca’ Foscari University of Venice mentioned this problem by saying: now is the time for micellar chemists to start learning those rules: “So people just mix things, and the catalysis works. Now we need more precise understanding of what’s going on in micellar aggregates.”(Sam Lemonick, 2020) .

Furthermore, when exposed to environmental changes, micelles as reaction media exhibit a basic restriction in terms of stability. However, when the concentration falls below the CMC, micelles can dissociate and lose their stability as a result, impacting the reaction performance negatively (Lu *et al*., 2018b). Addressing these obstacles is critical for advancing the future potential of micellar systems and expanding their applicability in industrial chemistry.

3. Experimental Design

In the procedure, the preparation of N-[(Phenylmethoxy)carbonyl]-L-phenylalanyl-L-leucine ethyl ester (Z-Phe-Leu-OEt) was described, which serves as an exemplary dipeptide model (Figure 3.1). The investigation showcases the practical applications of various coupling systems, namely COMU/2,6-lutidine, HBTU/2,6-lutidine, and EDAC/2,6-lutidine, in conjunction with distinct surfactant solutions. Considering the substantial concentrations employed (0.50 M for coupling), it is generally advantageous to occupy most of the vial volume with the reaction mixture to facilitate efficient stirring and, consequently, achieve improved yields. Typically, the reactions are conducted in dram glass vials at a scale of 0.2 mmol, employing mild conditions and maintaining room temperature.

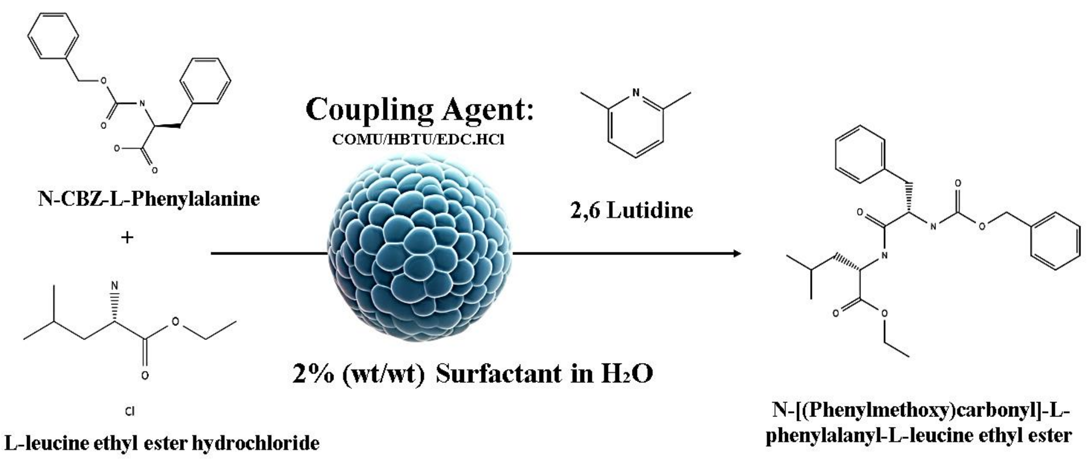


Figure 3. 1 Z-Phe-Leu-OEt synthesis in micellar medium.

3.1 Materials and equipment

3.1.1 Amino acids

- **N-CBZ-L-Phenylalanine**: N-CBZ-L-Phenylalanine is a protected amino acid with the molecular formula $C_{17}H_{17}NO_4$ and a molecular weight of 299.3 g/mol. It is a white amorphous powder. This compound is commonly stored at ambient temperature, making it convenient for handling and storage.
- **L-leucine ethyl ester HCl**: L-Leucine ethyl ester HCl is a chemical compound with the molecular formula $C_8H_{18}ClNO_2$. It has a molecular weight of 195.69 g/mol. This compound exists as a white crystalline powder. This compound is typically stored at room temperature.

3.1.2 Coupling agents

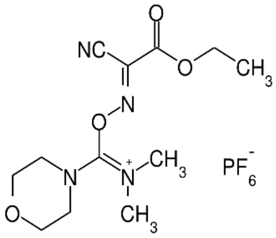
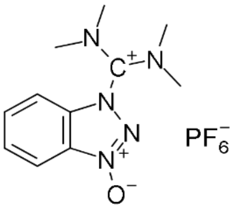
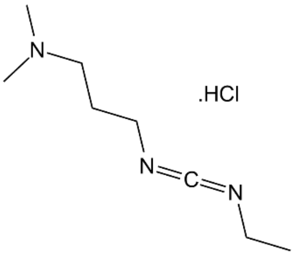
- **COMU**: COMU, which stands for (1-cyano-2-ethoxy-2-oxoethylidenaminooxy) dimethylamino-morpholino-carbenium hexafluorophosphate, represents a third generation uronium-type coupling reagent, incorporating ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma) and a morpholino carbon skeleton (El-Faham & Albericio, 2010b). The presence of the morpholino group significantly impacts the solubility, stability, and reactivity of this reagent. In comparison to benzotriazole-based reagents such as HATU and HBTU, COMU demonstrates a reduced level of hazardousness (Varnava & Sarojini, 2019a). The oxygen present in the imminium structure enhances the stability of COMU as opposed to its tetramethyl derivatives.

Moreover, Oxyma derivatives exhibit greater stability when compared to benzotriazole derivatives like HATU and HBTU (El-Faham *et al*., 2009b). Upon completion of the reaction, the solution transitions from colored to colorless or yellow. Specifically, when 2,6-lutidine is utilized, the solution becomes yellow (Withey & Bajic, 2015).

- **HBTU**: Hexafluorophosphate benzotriazole tetramethyl uronium , which contains carbodiimide and HOBt (1-Hydroxybenzotriazol), is commonly utilized as an activating reagent for carboxylic acids in peptide synthesis, in both solution and solid phase methodologies, with or without the addition of an additional equivalent of HOBt (Mali *et al*., 2014). The development of HBTU took place between the late 1970s and the early 2000s (McKnelly *et al*., 2020).

  The use of HBTU has a number of advantages, such as very low levels of racemization, simple reaction conditions, short reaction times, and good product yields (Sureshbabu *et al*., 2010).
- **EDC•HCl**: EDC•HCl, or 1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide, is one of the most common and inexpensive coupling reagents in chemical synthesis. This chemical compound is present as a white, crystalline powder (Wróblewska *et al*., 2017). EDC exhibits comparable reactivity to other carbodiimides such 1,3-Dicyclohexylcarbodiimide (DCC). However, EDC has an advantage over DCC in terms of the water solubility of the resultant urea, allowing for easier extraction. Contrarily, the dicyclohexylurea that results from the reaction with DCC has a limited solubility in different solvents and needs to be removed using filtration, which might not be as effective as extraction (Pottorf & Szeto, 2001).

Table 3. 1 Used coupling agents and their structures, and types.

No.	Coupling agent	Structure	Type	Ref.
1	COMU		Uronium	(El-Faham & Albericio, 2010b)
2	HBTU		Guanidinium salts	(Carpino *et al.*, 2002)
3	EDC•HCl		Carbodiimide	(Pottorf & Szeto, 2001)

3.1.3 Surfactants

Eight different surfactants (table 3.2) were ordered and used in basic peptide synthesis for this experimental study. In order to prepare the surfactant solutions, a 2% w/w concentration of the surfactant is dissolved in deionized water. The solution is stirred overnight until complete dissolution is achieved. The resultant surfactant solution can be stored at ambient temperature in an inert atmosphere for more than six months without losing activity.

Table 3. 2 Used surfactants and their main properties.

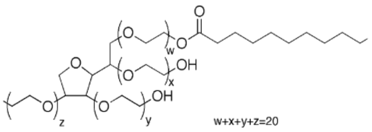
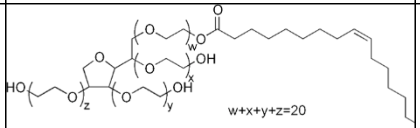
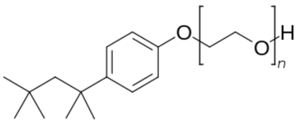
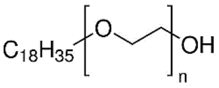
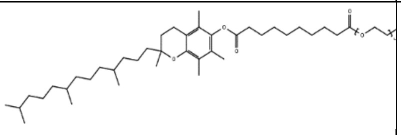
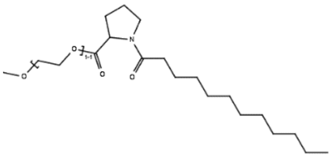
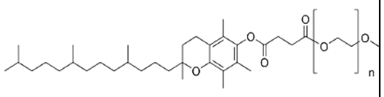
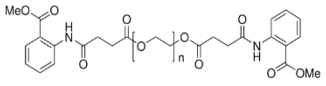
No.	**Commercial name**	**Structure**	**CMC (mM)**	**HLB**	**Micelle size (nm)**	**Applications**	**Ref.**
1	Tween 20	 Polyoxyethylene (20) sorbitan monolaurate	0.05	16.7	8.5	Emulsifier, detergent	(C. Tang & McInnes, 2022 ; Rodríguez Niño & Rodríguez Patino, 1998)
2	Tween 80	 Polyoxyethylene (20) sorbitan monooleate	0.019	15	11	Emulsifier, detergent	(Bide *et al.*, 2021; Rodríguez Niño & Rodríguez Patino, 1998)
3	Triton-X100	 2-[4-(2,4,4-trimethylpentan-2-yl) phenoxy]ethanol	0.24-0.27	13.4	5.5	Emulsifier, detergent	(Dharaiya *et al.*, 2015; Kapadia *et al.*, 2019; Singh & Tyagi, 2015)
4	Brij O10	 Polyoxyethylene (10)oleyl ether	0.94	12.4	–	Emulsifier, detergent	(J. Tang *et al.*, 2013)
5	PTS	 Polyoxyethanyl-α-tocopheryl sebacate	0.1	10	25	Solubilizing agent, designer surfactant	(C. Tang & McInnes, 2022b)
6	PS-750 M		0.001	14	50–150	Solubilizing agent, designer surfactant	(Kaur *et al.*, 2022; Smith *et al.*, 2018)

Table 3.2 Continuation.

No.	**Commercial name**	**Structure**	**CMC (mM)**	**HLB**	**Micelle size (nm)**	**Applications**	**Ref.**
7	TPGS-750 M	 DL-alpha-Tocopherol methoxypolyethylene glycol succinate	0.1	12.7	50-60	Solubilizing agent, designer surfactant	(Andersson *et al.*, 2018; Lorenzetto *et al.*, 2022; C. Tang & McInnes, 2022a)
8	Coolade		-	-	30-40	Antifoaming agent	(N. R. Lee *et al.*, 2019)

3.1.4 Solvents

- Ethyl acetate (EtOAc)
- Hexane
- Methyl t-butyl ether (MTBE)
- Tetrahydrofuran (THF)
- Acetone
- Dichloromethane (DCM)

3.1.5 Inorganic salts

- Sodium sulfate, anhydrous (Na_2SO_4)
- Sodium carbonate, anhydrous (Na_2CO_3)

3.1.6 Acids

- Hydrochloric acid (HCl)
- Acetic acid, glacial (CH_3COOH)

3.1.7 Equipment

Analytical balance, plate magnetic stirrer, 4 ml dram vials, rotavapor, water bath, vacuum/argon gas system, Nuclear magnetic resonance (NMR), narrow-mouth graduated Erlenmeyer flasks, graduated cylinders, round-bottom flasks (single neck), separatory funnel, sleeve stoppers, egg-shaped magnetic stir bars, weighting paper, spatula, parafilm m paraffin film, Thin Layer Chromatography (TLC) silica gel 60-gauge F254 glass plates and UV lamp.

3.2 Synthesis of dipeptide using surfactants solutions

A total of 27 dipeptide syntheses were performed in 8 different surfactant solutions employing three different coupling agents: COMU, HBTU, and EDC.HCl. The objective of these trials was to evaluate and compare the solubilizing efficiency of these solutions. The procedures are explained as the following:

1. For setting the reaction (figure 3.2), a 4 ml dram vial of adequate size is used. The vial is selected to hold a reaction volume that is at least one-third of its overall capacity. This choice allows for more efficient stirring and enhances the total yield of the process.
2. The amino acid N-Cbz-L-Phenylalanine (0.2 mmol, 1.0 equiv.) and L-Leucine ethyl ester HCl amino acid (0.21 mmol, 1.05 equiv.) are added to the vial.
3. The vial is filled with 0.4 mL of a 2% (wt/wt) solution of the surfactant in water to provide an appropriate reaction environment. The amino acid concentration in the reaction mixture is 0.50 M as a result. Stirring the solution for 1 minute ensures that the protected amino acids are properly dispersed, resulting in a transparent-to-milky solution, depending on the specific properties of the protected amino acids.
4. In the following step, 0.071 mL of 2,6-lutidine (0.61 mmol, 3.05 equiv.) is added to the vial.
5. The coupling agent (0.21mmol, 1.05 equiv.) is added to the vial. In the case of COMU, the solution should instantly turn brilliant yellow, while in the case of HBTU and EDC.HCl, it should turn milky white.
6. The mixture is stirred vigorously at room temperature for 1 h.

7. The reaction completion is monitored after 1 hour using thin-layer chromatography (TLC). The eluent is a 2:1 combination of ethyl acetate and hexane with a drop of acidic acid. The used staining reagent is phosphomolybdic acid.
8. The vial is filled with 2.5 mL of methyl tert-butyl ether (MTBE) after the TLC analysis. To guarantee better extraction gentle, shaking is performed. The upper layer is collected with a pipette into a separatory funnel. This procedure is repeated four times more for a total volume of 10 mL.
9. Next, 5 mL of a 1 M HCl solution is added to the separatory funnel. After that, the funnel is shaken to wash and separate the two layers. The lower layer, which contains HCl, is collected into a beaker.
10. Following that, 5 mL of a saturated aqueous Na_2CO_3 solution is added to the separatory funnel. Shaking the funnel encourages layer separation, and the lower layer is collected into a second beaker.
11. The organic layer is transferred to a beaker. In order to dry the organic phase, anhydrous sodium sulfate (Na_2SO_4) is added until some free powder appears, and the solution is filtered via a funnel.
12. To remove volatiles, the filtered solution is subjected to 30 minutes of rotary evaporation in a 50°C water bath under a 350 mbar vacuum.
13. ^{1}H NMR analysis (in $CDCl_3$) confirms the resultant product(see the annex), which can be used without further purification. The product is kept under argon between 0 and 8 °C, maintaining its stability for several months.

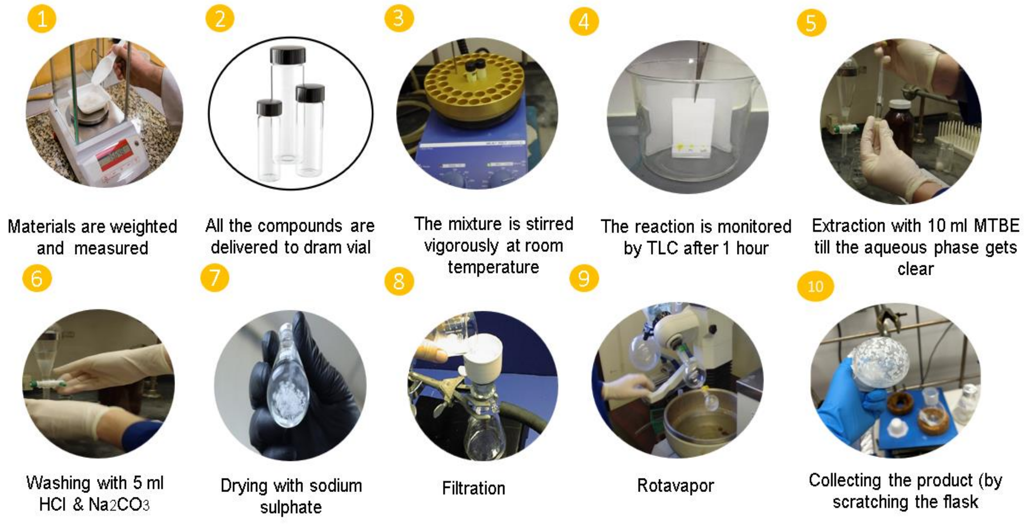


Figure 3. 2 Synthesis of dipeptide procedures.

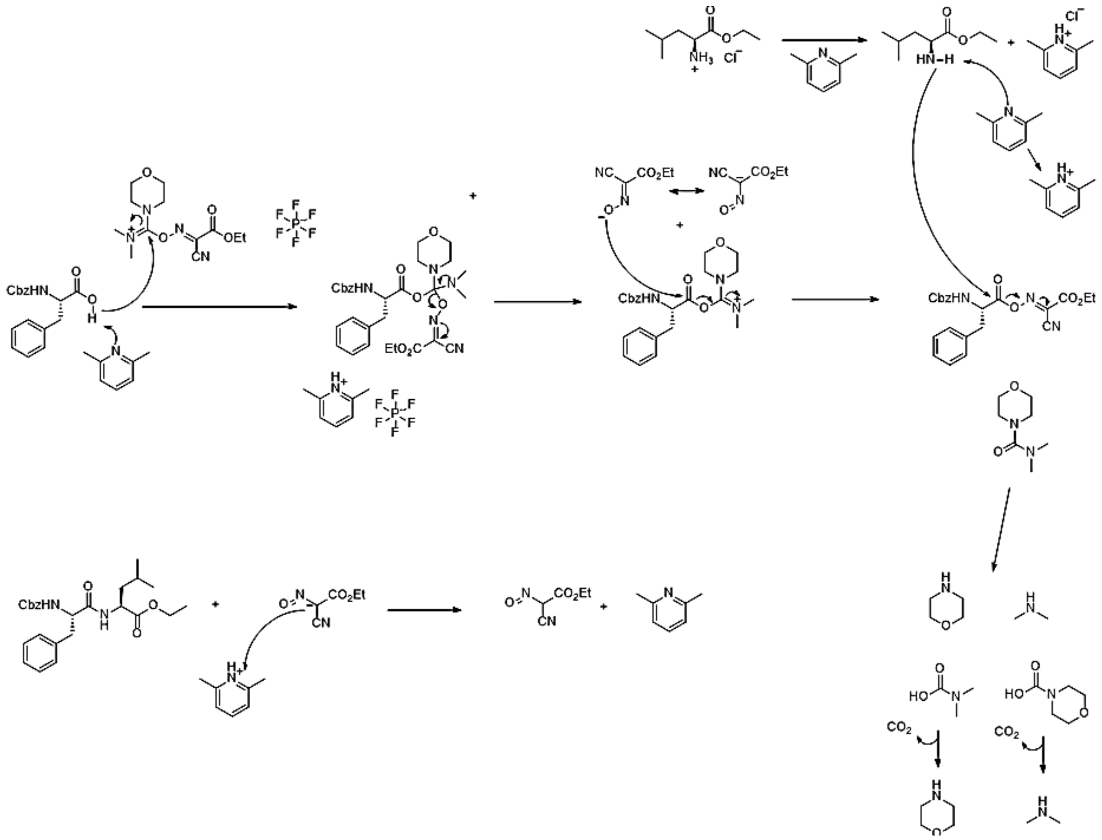


Figure 3. 3 Chemical transformation scheme for COMU as coupling agent.

3.3 Influence of reaction time

In order to study influence of reaction time on yield in micellar medium, the dipeptide synthesis was carried out in a 2% (wt/wt) TPGS-750-M-H_2O solution using the (HBTU/2,6 lutidine) system. Four entries (vials) were prepared, and four different reaction durations were attempted: 1 hour, 3 hours, 24 hours, and 48 hours. The procedures specified in section 3.2 (steps 1–13) were followed. The yields were calculated and compared.

3.4 Influence of surfactant solution amount

The dipeptide synthesis was carried out in a 2% (wt/wt) TPGS-750-M-H_2O solution using the (HBTU/2,6 lutidine) system. Three entries (vials) were prepared with different amounts of surfactant solution in order to change reaction concentration: 0.4 ml, 0.6 ml and 0.8 ml. The procedures specified in section 3.2 (steps 1–13) were followed. The yields were calculated and compared.

3.5 The effect of using co-solvents

In order to investigate the effect of using co-solvents, the surfactants with the highest and lowest yields were selected. Following that, 8 vials were prepared, and the dipeptide synthesis was carried out in these surfactant solutions along with co solvents (table 3.3): 10% (vol/vol) THF ,then 10% (vol/vol) EtOAc. The procedures specified in section 3.2 (steps 1–13) were followed. The yields were determined and compared.

Table 3. 3 The conducted dipeptide synthesis trials with co-solvents.

Coupling Agent	**Surfactant**	**Co-solvent 10% (vol/vol)**	
HBTU	Tween 20 (Lowest yield)	THF	EtOAc
	Brij O10 (Highest yield)		
EDC.HCl	Coolade (Lowest yield)		
	PTS (Highest yield)		

3.6 Synthesis of dipeptide using mixtures of surfactants solutions

In this experiment, the possibility of enhancing reaction yield by mixing different surfactant solutions was examined. 8 surfactant solutions were combined in a 1:1 ratio, taking into consideration their chemical structure and provided yields. 10 binary combinations were used in the synthesis (table 3.4). The coupling agent (HBTU or EDC.HCl) was selected based on the outcomes of the individual surfactant solutions. The reaction was conducted using amino acids concentration of 0.50 M, wherein 0.2 ml of surfactant solution A and 0.2 ml of surfactant solution B were introduced to the dram vials. The steps indicated in section 3.2 (steps 1-13) were followed.

Table 3. 4 Conducted dipeptide synthesis trials using mixtures of surfactants solutions.

Coupling agent	**Mixed surfactants (A/B 1:1)**	
	A	**B**
EDC.HCl	Brij O10	Tween 80
		PTS
	Tween 80	PS 750 M
		Tween 20
	Triton X100	Coolade
		Tween 20
HBTU	TPGS 750 M	PTS
		PS 750 M
		Brij O10
		Coolade

4. Results and Discussion

A commonly used dipeptide, Z-Phe-Leu-OEt, was synthesized employing several surfactants and coupling agents. The objective of this study was to evaluate these surfactants and coupling agents based on the yield of the Z-Phe-Leu-OEt synthesis. Furthermore, the effects of reaction duration and surfactant solution amount were investigated. Likewise, the effect of employing co-solvents on the peptide coupling reaction was examined. Binary surfactant systems were used to investigate the possibilities of increasing reaction yield. EDC.HCL and HBTU were examined extensively due to economic preference. The following discussion will compare the yields achieved with each coupling agent and surfactant solution.

4.1 Peptide Synthesis Using Different Surfactants Solutions and Coupling Agents

Table 4. 1 (Z-Phe-Leu-OEt) synthesis in different surfactants and coupling agents.

Coupling Agent	**Yield %**							
	Tween 20	**Tween 80**	**Brij O10**	**Triton X100**	**PTS**	**TPGS 750M**	**PS 750 M**	**Coolade**
COMU	52.4	73.1	**75.7**	53.5	67.5	67.2	52.4	36.9
HBTU	44.3	52.8	**65.1**	58.8	57.9	57.2	46.1	45.7
EDC.HCl	57.4	51.4	59.7	63.9	**65.1**	44.5	44.0	37.7

COMU demonstrated the highest yield percentages across all surfactants among the coupling agents evaluated, ranging from 52.4% to 75.7% (table 4.1). These results imply that COMU is an efficient coupling agent in micellar media. It regularly outperformed HBTU and EDC.HCl in terms of achieved yields and sustainability (El-Faham *et al*., 2009a; El-Faham & Albericio, 2010b). COMU exhibited numerous advantages over traditional benzotriazole-based coupling reagents, including greater solubility, a water-soluble byproduct, and color change monitoring (Kumar *et al*., 2017). COMU in combination with Brij O10 demonstrated the highest yield of 75.7%, indicating that the COMU/Brij O10 pair is the most effective coupling surfactant combination for achieving optimal yields in this study.

When the coupling agent HBTU was combined with Brij O10 as the surfactant, the highest yield of 65.1% was obtained among tested surfactant solutions had the greatest yield of 65.1% of the surfactants examined when EDC.HCl was employed as the coupling agent. On the other hand, surfactants such as Coolade and PS 750M resulted in comparatively lower yields, ranging from 36.9% to 52.4%.

In this study, the TPGS 750 M did not deliver the best results, contrary to the literature (Cortes-Clerget *et al*., 2017b; Cortes-Clerget, Spink, *et al*., 2019; Shen *et al*., 2021a). Traditional surfactants, such as Brij O10 and Tween 80, achieved higher yields when coupled with the coupling agent COMU. This unexpected result implies that these traditional surfactants could serve as less expensive and equally competent alternatives for micellar synthesis, potentially lowering the overall cost of the procedure.

4.2 The effect of reaction time

The results indicate that the reaction time impacts the yield of Z-Phe-Leu-OEt synthesis. A prolonged reaction time resulted in significantly greater yields in the case of %2 w/w TPGS -750-M in water with HBTU as the coupling agent. According to the results in figure 4.1, the yield was 56.3% after one hour of reaction and decreased to 55.6% after three hours due to an experimental error. When the reaction was allowed to run for 24 hours, the yield increased to 57.2%. After 48 hours of reaction time, the most noticeable rise in yield was found, with a yield of 79.7%. Addition of NaCl or applying higher temperatures to the reaction mixture can afford larger micelles and induces faster reactions (Minkler et al., 2011; Mohajeri & Noudeh, 2012).

This observation implies that some reactions take longer to complete and that longer reaction periods can lead to higher yields due to prolonged vigorous stirring.

Run	Sample running time (h)	Yield %
1	1	56.3
2	3	55.6
3	24	57.2
4	48	**79.7**

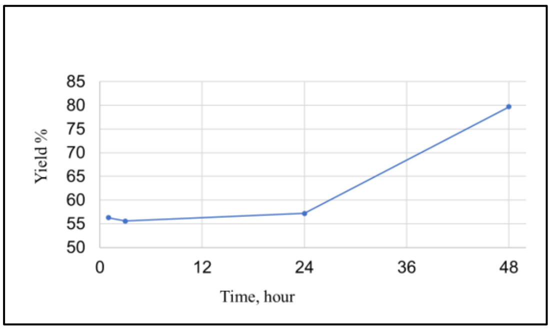


Figure 4. 1 Reaction time influence on achieved yields.

4.3 The effect of surfactant solution amount

Upon examining the effect of reaction dilution on the yield (figure 4.2), it was obvious that more diluted reactions resulted in lower overall yields of the target product in the case of using %2 w/w TPGS 750M in water with HBTU as the coupling agent. This observation is verified by results from runs 1, 2, and 3, in which the reaction concentration gradually decreased by increasing solution amount (0.4, 0.6, and 0.8 ml, respectively). This can be due to the surfactant content being not able to maintain same number or size micelle self-assembly. In case of CMC instability, they tend to diffuse along the air-water or aqueous-organic phase interface, resulting in micelle disintegration (Lu et al., 2018a).

Run	Solution amount, ml	Reaction conc. (M)	Yield %
1	0.4	0.50	**56.3**
2	0.6	0.33	**54.8**
3	0.8	0.25	**46.1**

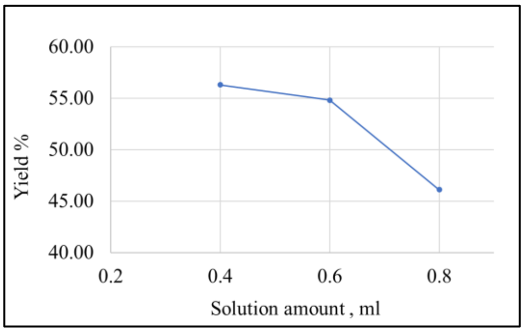


Figure 4. 2 solution amount influence on achieved yields, 1hr.

Understanding the behavior and efficiency of surfactant solutions and dilution can be difficult if micelle size and aggregation are not precisely measured utilizing techniques such as Transmission Electron Microscopy (TEM) or other relevant equipment. These studies can assist to visualize and analyze the structural arrangements and interactions within surfactant systems using these microscopic examinations.

The optimal reaction concentration is 0.50 M of the amino acids which was examined to provide higher yields (Cortes-Clerget, Lee, *et al.*, 2019b). The results presented above emphasize the significance of maintaining an appropriate reaction concentration in order to achieve higher yields in micellar-enabled peptide synthesis.

4.4 The effect of using co-solvents

The coupling reactions were carried out in this experiment utilizing separate agents (HBTU and EDC.HCl) and surfactants (Tween 20, Brij O10, PTS, and Coolade) with two distinct co-solvents, 10% THF (tetrahydrofuran) and 10% EtOAc (ethyl acetate) (table 4.2). The major focus of the discussion is to compare the performance of THF and EtOAc as co-solvents and to examine the impact of co-solvents on the yield of the coupling reactions. The surfactants with the highest and lowest yields were selected for each coupling agent.

Table 4. 2 The effect of using THF and ethyl acetate as co-solvents.

Coupling Agent	**Surfactant**	**Yield %**		
		Non co-solvent	**Co-solvent**	
			10% THF	**10% EtOAc**
HBTU	**Tween 20**	44.3	**56.9**	39.7
	Brij O10	65.1	**67.0**	**65.5**
EDC.HCl	**PTS**	65.1	58.6	**67.1**
	Coolade	37.7	**56.5**	**56.4**

Adding co-solvents to the micellar medium was demonstrated to not only serve as a processing aid for physical transfer but also indeed enlarge the initially formed micelles, essentially doubling the inner diameter and facilitating solubilizing of reaction components (Hauk *et al.*, 2021). The expansion of micelles in the presence of organic solvents is expected since co-solvent molecules can arrange themselves within the micellar framework, causing particle growth up until saturation, at which time no further swelling is detected (Gabriel *et al.*, 2017a). When THF was employed as co-solvent for the HBTU agent, the yield percentages were often higher than when EtOAc was used. This suggests that, when utilizing the surfactants examined in this experiment, THF is a better co-solvent for HBTU-mediated coupling processes.

In comparison to THF, the yield percentages for the EDC.HCl agent were higher or comparable with EtOAc as the co-solvent. Given that ethyl acetate (EtOAc) achieved comparable results to THF as a co-solvent, it may be preferred in situations where THF is unavailable or undesirable due to safety or environmental concerns. EtOAc is widely regarded as a less hazardous and more environmentally friendly alternative to THF.

4.5 Peptide synthesis using mixtures of surfactants solutions

The main objective of this experiment was to investigate the possibility of increasing reaction yield by using a mixed micellar solution composed of several surfactant solutions (table 4.3). Eight surfactant solutions were combined in a 1:1 ratio, considering their distinct chemical structures and the yields they provided in previous experiments (table 4.1). The synthesis involved the use of ten binary combinations of these surfactants. In general, homologous nonionic/nonionic surfactants may be ideally mixed, whereas two surfactants with different head groups are largely nonideally mixed (Jiang et al., 2011). Some binary mixtures were not able to mix and form one phase within the reaction.

Table 4. 3 (Z-Phe-Leu-OEt) synthesis in Binary Mixtures of Surfactants Solutions.

Coupling agent	**Mixed surfactants (A/B 1:1)**		**Yield %**	**Yield with A**	**Yield with B**
	A	**B**			
EDC.HCl	**Brij O10**	**Tween 80**	**67.1**	59.7	51.4
		PTS	57.0		65.1
	Tween 80	**PS 750 M**	50.2	51.4	44.0
		Tween 20	57.1		57.4
	Triton X100	**Coolade**	55.5	63.9	37.7
		Tween 20	54.5		57.4
HBTU	**TPGS 750 M**	**PTS**	44.8	57.2	57.9
		PS 750 M	50.5		46.1
		Brij O10	42.3		65.1
		Coolade	43.4		45.7

This experiment shows that a (Tween 80/Brij O10) micellar solution mixture boosts the efficiency of the coupling reaction when EDC.HCl was used as coupling agent (yield of 67.1%) slightly more than either surfactant alone.

It is notable that depending on the coupling agent used, the efficiency of the mixed micellar solution differed. In certain cases, the yields obtained with a single surfactant were higher than those obtained with the combined micellar solution. In a mixed surfactant solution, the presence of surfactants that have different Critical Micelle Concentrations (CMC) and head group structures can have a substantial impact on reaction stability and solubilizing performance (Patel *et al.*, 2017).

Further studies can reveal how mixing surfactants can affect their properties like micelle size, HLB and CMC. Additional research could be undertaken using Design of Experiments (DoE) to evaluate different concentrations of the surfactants. In this work, a surfactant concentration of 2% w/w in water was utilized. Examining various coupling agents with different surfactant solutions might lead to the identification of optimal combinations that provide larger percentages of the desired product. Better results can be achieved with EDC.HCL as the most common reagent for large-scale peptide couplings (S. Sharma *et al.*, 2021).This investigation will aid in the optimization of the synthesis process and may lead to the discovery of more efficient and cost-effective micellar chemistry methods.

5. Conclusions and Future Work

Peptide chemistry has evolved as an important discipline in the pharmaceutical sector, owing to the recognition of multiple applications and therapeutic potential. However, typical peptide synthesis processes mainly rely on organic solvents, resulting in significant volumes of hazardous waste. As the need for peptides increases, the pharmaceutical industry needs to embrace sustainable procedures to maintain long-term viability and resilience. In this context, the use of water as a solvent and the integration of surfactants have emerged as a promising alternative to hazardous organic solvents. Micellar chemistry adheres to Green Chemistry principles and provides several advantages, including increased reaction efficiency, improved selectivity, and lower environmental impact.

In this work, 49 experiments were conducted to synthesize (Z-Phe-Leu-OEt) dipeptide in 8 different surfactant solutions using three different coupling agents: COMU, HBTU, and EDC.HCl. COMU had the greatest yield percentages across all surfactants among the coupling agents tested, ranging from 52.4% to 75.7%. This result indicates that COMU is an efficient coupling agent in micellar media, especially when combined with Brij O10, where it achieved the maximum yield of 75.7%. When compared to other designer surfactants such as TPGS-750-M and PS-750-M, traditional surfactants such as Brij O10 and Tween 80 achieved higher yields when used together with the coupling agent COMU. This unexpected result suggests that these traditional surfactants could serve as less expensive and equally competent options for micellar chemistry, accordingly, lowering the overall cost.

Furthermore, the results showed that reaction time had a substantial impact on Z-Phe-Leu-OEt synthesis yield. Longer reaction times resulted in significantly higher yields when using %2 w/w TPGS-750-M in water with HBTU as the coupling agent (48 hours). In addition, more diluted reactions resulted in lower overall yields of the target product.

The addition of co-solvents to the micellar medium improved reaction yields. THF was found to be a better co-solvent for HBTU-mediated coupling reactions, though the yield percentages for the surfactants used together with EDC.HCl was either higher or comparable when EtOAc was used as the co-solvent.

The preference for EtOAc over hazardous polar aprotic co-solvents such as THF or Toluene can increase the green potential of this approach.

Finally, the results in table 4.3 showed that when EDC.HCl was employed as the coupling agent, (Tween 80/Brij O10) micellar solution mixture increased the efficiency of the coupling process, resulting in a yield of 67.1%, which was slightly higher than either surfactant alone. Future research endeavors may include examining the use of Design of Experiments (DoE) to evaluate several surfactant concentrations and examine various coupling agents in different surfactant solutions, potentially resulting in the identification of ideal combinations that produce higher percentages of the desired product.

Bibliography

Annex xv restriction report proposal for a restriction dimethylformamide (DMF) 05th oct 2018, https://echa.europa.eu/documents/10162/93384644-983c-9bf4-f7b3-b9d80348b8ca (accessed Jun 26,2023)

Aulton, M. E. (2002). Pharmaceutics: The science of dosage form design.

Acosta, G. A., Del Fresno, M., Paradis-Bas, M., Rigau-DeLlobet, M., Côté, S., Royo, M., & Albericio, F. (2009). Solid-phase peptide synthesis using acetonitrile as a solvent in combination with PEG-based resins. Journal of Peptide Science, 15(10), 629–633. https://doi.org/10.1002/PSC.1158

Akaji, K., Fujino, K., Tatsumi, T., & Kiso, Y. (1993). Total synthesis of human insulin by regioselective disulfide formation using the silyl chloride-sulfoxide method. Journal of the American Chemical Society, 115(24), 11384-11392.

Anastas, P., & Eghbali, N. (2010). Green chemistry: principles and practice. Pubs.Rsc.Org. https://pubs.rsc.org/en/content/articlehtml/2010/cs/b918763b

Andersson, M. P., Gallou, F., Klumphu, P., Takale, B. S., & Lipshutz, B. H. (2018). Structure of Nanoparticles Derived from Designer Surfactant TPGS-750-M in Water, As Used in Organic Synthesis. Chemistry - A European Journal, 24(26), 6778–6786. https://doi.org/10.1002/chem.201705524

Ansari, T. N., Gallou, F., & Handa, S. (2020). Cross-couplings in water - A better way to assemble new bonds. In Organometallic Chemistry in Industry: A Practical Approach (pp. 203–238). John Wiley & Sons, Ltd. https://doi.org/10.1002/9783527819201.ch8

Avila-Ortiz, C. G., & Juaristi, E. (2020). Novel methodologies for chemical activation in organic synthesis under solvent-free reaction conditions. In Molecules (Vol. 25, Issue 16, p. 3579). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/molecules25163579

Becker, J., Manske, C., & Randl, S. (2022). Green chemistry and sustainability metrics in the pharmaceutical manufacturing sector. Current Opinion in Green and Sustainable Chemistry, 33, 100562.

Bergkamp, L., & Herbatschek, N. (2014). Regulating Chemical Substances under REACH: The Choice between Authorization and Restriction and the Case of Dipolar Aprotic Solvents. Review of European, Comparative and International Environmental Law, 23(2), 221–245. https://doi.org/10.1111/reel.12083

Bide, Y., Fashapoyeh, M. A., & Shokrollahzadeh, S. (2021). Structural investigation and application of Tween 80-choline chloride self-assemblies as osmotic agent for water desalination. Scientific Reports, 11(1), 17068.

Bonnamour, J., Métro, T. X., Martinez, J., & Lamaty, F. (2013). Environmentally benign peptide synthesis using liquid-assisted ball-milling: Application to the synthesis of Leu-enkephalin. Green Chemistry, 15(5), 1116–1120. https://doi.org/10.1039/c3gc40302e

Bordes, R., & Holmberg, K. (2015). Amino acid-based surfactants–do they deserve more attention?. Advances in Colloid and Interface Science, 222, 79-91.

Bruzzoni-Giovanelli, H., Alezra, V., Wolff, N., Dong, C. Z., Tuffery, P., & Rebollo, A. (2018). Interfering peptides targeting protein–protein interactions: the next generation of drugs?. Drug Discovery Today, 23(2), 272-285.

Carpino, L. A., Ghassemi, S., Ionescu, D., Ismail, M., Sadat-Aalaee, D., Truran, G. A., Mansour, E. M. E., Siwruk, G. A., Eynon, J. S., & Morgan, B. (2003). Rapid, continuous solution-phase peptide synthesis: Application to peptides of pharmaceutical interest. Organic Process Research and Development, 7(1), 28–37. https://doi.org/10.1021/OP0202179/SUPPL_FILE/OP0202179_S.PDF

Carpino, L. A., Imazumi, H., Ferrer, F. J., Zhang, C., Lee, Y., Foxman, B. M., Henklein, P., Hanay, C., Clemens, M., Wenschuh, H., Klose, J., Beyermann, M., & Bienert, M. (2002). The Uronium/Guanidinium Peptide Coupling Reagents: Angewandte Chemie International Edition, 41(3), 441–445. https://onlinelibrary.wiley.com/doi/10.1002/1521-3773%2820020201%2941%3A3%3C441%3A%3AAID-ANIE441%3E3.0.CO%3B2-N

Castro, H. J., Krüger, T., Richner, F., Lechner, C., & Sejer, D. (2021). Replacing DMF in solid-phase peptide synthesis: varying the composition of green binary solvent mixtures as a tool to mitigate common side-reactions. 3312–3321. https://doi.org/10.1039/d1gc00604e

Chandrudu, S., Simerska, P., & Toth, I. (2013). Chemical methods for peptide and protein production. Molecules, 18(4), 4373-4388.

Chen, Y., Wang, M., Wang, W., Zeng, Y., Fan, X., Tu, M., Ma, Y., & Wei, D. (2022). Metabolic Engineering for the Comprehensive Utilization of N, N-Dimethylformamide-Containing Wastewater. Journal of Agricultural and Food Chemistry, 70(42), 13574–13582. https://doi.org/10.1021/acs.jafc.2c05240

Chung, P. Y., & Khanum, R. (2017). Antimicrobial peptides as potential anti-biofilm agents against multidrug-resistant bacteria. Journal of microbiology, immunology and infection, 50(4), 405-410.

Clark, J. H., Farmer, T. J., Hunt, A. J., & Sherwood, J. (2015). Opportunities for bio-based solvents created as petrochemical and fuel products transition towards renewable resources. International Journal of Molecular Sciences, 16(8), 17101-17159.

Coatings, E. (2023). DMF Restriction on the Horizon. European Coatings. https://www.european-coatings.com/news/legislation/dmf-restriction-on-the-horizon/, accessed date : 04.07.2023

Cornils, B., & Herrmann, W. A. (2004). Aqueous-Phase Organometallic Catalysis: Concepts and Applications, 2nd Edition | Wiley. https://www.wiley.com/en-gb/Aqueous+Phase+Organometallic+Catalysis%3A+Concepts+and+Applications%2C+2nd%2C+Completely+Revised+and+Enlarged+Edition-p-9783527605460 **,** accessed date : 04.07.2023

Cortes-Clerget, M., Berthon, J. Y., Krolikiewicz-Renimel, I., Chaisemartin, L., & Lipshutz, B. H. (2017a). Tandem deprotection/coupling for peptide synthesis in water at room temperature. Green Chemistry, 19(18), 4263–4267. https://doi.org/10.1039/C7GC01575E

Cortes-Clerget, M., Berthon, J. Y., Krolikiewicz-Renimel, I., Chaisemartin, L., & Lipshutz, B. H. (2017b). Tandem deprotection/coupling for peptide synthesis in water at room temperature. Green Chemistry, 19(18), 4263–4267. https://doi.org/10.1039/C7GC01575E

Cortes-Clerget, M., Lee, N. R., & Lipshutz, B. H. (2019). Synthetic chemistry in water: applications to peptide synthesis and nitro-group reductions. Nature Protocols, 14(4), 1108–1129. https://doi.org/10.1038/s41596-019-0130-1

Cortes-Clerget, M., Spink, S. E., Gallagher, G. P., Chaisemartin, L., Filaire, E., Berthon, J. Y., & Lipshutz, B. H. (2019). MC-1. A “designer” surfactant engineered for peptide synthesis in water at room temperature. Green Chemistry, 21(10), 2610–2614. https://doi.org/10.1039/c9gc01050e

Cortes-Clerget, M., Yu, J., Kincaid, J. R. A., Walde, P., Gallou, F., & Lipshutz, B. H. (2021). Water as the reaction medium in organic chemistry: From our worst enemy to our best friend. Chemical Science, 12(12), 4237–4266. https://doi.org/10.1039/d0sc06000c

Declerck, V., Nun, P., Martinez, J., & Lamaty, F. (2009). Solvent-free synthesis of peptides. Angewandte Chemie - International Edition, 48(49), 9318–9321. https://doi.org/10.1002/anie.200903510

Department of Economic and Social Affairs of United Nation. (2023). THE 17 GOALS | Sustainable Development. In Sustainable Development. https://sdgs.un.org/goals, accessed date : 07.07.2023

Dharaiya, N., Aswal, V. K., & Bahadur, P. (2015). Characterization of Triton X-100 and its oligomer (Tyloxapol) micelles vis-à-vis solubilization of bisphenol A by spectral and scattering techniques. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 470, 230–239. https://doi.org/10.1016/j.colsurfa.2015.01.053

du Vigneaud, V., Resslbr, C., Swan, J. M., Roberts, C. W., & Katsoyannis, P. G. (1954). The Synthesis of Oxytocin. Journal of the American Chemical Society, 76(12), 3115–3121. https://doi.org/10.1021/JA01641A004/ASSET/JA01641A004.FP.PNG_V03

Du Vigneaud, V., Ressler, C., Swan, J. M., Roberts, C. W., Katsoyannis, P. G., & Gordon, S. (1953). The Synthesis of an Octapeptide Amide With The Hormonal Activity of Oxytocin. Journal of the American Chemical Society, 75(19), 4879–4880. https://doi.org/10.1021/JA01115A553/ASSET/JA01115A553.FP.PNG_V03

Duong, Uyen T, "Micellar nanocatalysis for efficient reactions." (2021). Electronic Theses and Dissertations, 3718, https://doi.org/10.18297/etd/3718

Dwars, T., Paetzold, E., & Oehme, G. (2005). Reactions in micellar systems. Angewandte Chemie International Edition, 44(44), 7174-7199.

ECHA. (2016). ECHA Committee for Risk Assessment (RAC) and Scientific Committee on Occupational Exposure Limits (SCOEL) Joint Opinion to resolve differences in scientific opinion as regards exposure levels for Table of Contents. November, 1–19., accessed date : 08.07.2023

Eggen, I. F., Bakelaar, F. T., Petersen, A., Ten Kortenaar, P. B. W., Ankone, N. H. S., Bijsterveld, H. E. J. M., Bours, G. H. L., El Bellaj, F., Hartsuiker, M. J., Kuiper, G. J., & Ter Voert, E. J. M. (2005). A novel method for repetitive peptide synthesis in solution without isolation of intermediates. Journal of Peptide Science, 11(10), 633–641. https://doi.org/10.1002/psc.670

El-Faham, A., & Albericio, F. (2010). COMU: A third generation of uronium-type coupling reagents. Journal of Peptide Science, 16(1), 6–9. https://doi.org/10.1002/psc.1204

El-Faham, A., & Albericio, F. (2011). Peptide coupling reagents, more than a letter soup. Chemical Reviews, 111(11), 6557–6602. https://doi.org/10.1021/cr100048w

El-Faham, A., Funosas, R. S., Prohens, R., & Albericio, F. (2009a). COMU: A safer and more effective replacement for benzotriazole-based uronium coupling reagents. Chemistry - A European Journal, 15(37), 9404–9416. https://doi.org/10.1002/chem.200900615

El-Faham, A., Funosas, R. S., Prohens, R., & Albericio, F. (2009b). COMU: A safer and more effective replacement for benzotriazole-based uronium coupling reagents. Chemistry - A European Journal, 15(37), 9404–9416. https://doi.org/10.1002/chem.200900615

Fabris, F., Illner, M., Repke, J.-U., Scarso, A., & Schwarze, M. (2023). Is Micellar Catalysis Green Chemistry? Molecules, 28(12), 4809. https://doi.org/10.3390/molecules28124809

Ferrazzano, L., Catani, M., Cavazzini, A., Martelli, G., Corbisiero, D., Cantelmi, P., ... & Tolomelli, A. (2022). Sustainability in peptide chemistry: Current synthesis and purification technologies and future challenges. Green Chemistry, 24(3), 975-1020.

Frederick, M. O., Boyse, R. A., Braden, T. M., Calvin, J. R., Campbell, B. M., Changi, S. M., ... & Xia, H. (2021). Kilogram-scale GMP manufacture of tirzepatide using a hybrid SPPS/LPPS approach with continuous manufacturing. Organic Process Research & Development, 25(7), 1628-1636.

Gabriel, C. M., Keener, M., Gallou, F., & Lipshutz, B. H. (2015). Amide and Peptide Bond Formation in Water at Room Temperature. Organic Letters, 17(16), 3968–3971. https://doi.org/10.1021/ACS.ORGLETT.5B01812/SUPPL_FILE/OL5B01812_SI_001.PDF

Gabriel, C. M., Lee, N. R., Bigorne, F., Klumphu, P., Parmentier, M., Gallou, F., & Lipshutz, B. H. (2017a). Effects of co-solvents on reactions run under micellar catalysis conditions. Organic Letters, 19(1), 194–197. https://doi.org/10.1021/acs.orglett.6b03468

Gabriel, C. M., Lee, N. R., Bigorne, F., Klumphu, P., Parmentier, M., Gallou, F., & Lipshutz, B. H. (2017b). Effects of co-solvents on reactions run under micellar catalysis conditions. Organic Letters, 19(1), 194–197. https://doi.org/10.1021/acs.orglett.6b03468

Gaines, G. L. (1972). Critical micelle concentrations of aqueous surfactant systems. Journal of Colloid and Interface Science, 38(3), 671–672. https://doi.org/10.1016/0021-9797(72)90423-7

Gao, F., Bai, R., Ferlin, F., Vaccaro, L., Li, M., & Gu, Y. (2020). Replacement strategies for non-green dipolar aprotic solvents. Green Chemistry, 22(19), 6240–6257. https://doi.org/10.1039/d0gc02149k

Ghosh, S., Ray, A., & Pramanik, N. (2020). Self-assembly of surfactants: An overview on general aspects of amphiphiles. Biophysical Chemistry, 265, 106429. https://doi.org/10.1016/j.bpc.2020.106429

Harris, P. W. R., & Brimble, M. A. (2013). A comparison of Boc and Fmoc SPPS strategies for the preparation of C-terminal peptide α-thiolesters: NY-ESO-1 ^{39}Cys-^{68}Ala-COSR. Biopolymers, 100(4), 356–365. https://doi.org/10.1002/bip.22223

Hauk, P., Wencel-Delord, J., Ackermann, L., Walde, P., & Gallou, F. (2021). Organic synthesis in Aqueous Multiphase Systems — Challenges and opportunities ahead of us. Current Opinion in Colloid and Interface Science, 56, 101506. https://doi.org/10.1016/j.cocis.2021.101506

Hazra, S., Gallou, F., & Handa, S. (2022). Water: an underestimated solvent for amide bond-forming reactions. ACS Sustainable Chemistry & Engineering, 10(16), 5299-5306.

Hojo, K., Fujiwara, S., Inai, H., Manabe, Y., & Tsuda, Y. (2022). Environmentally Conscious In-Water Peptide Synthesis Using Boc Strategy. International Journal of Peptide Research and Therapeutics, 28(2), 1–10. https://doi.org/10.1007/s10989-021-10354-1

Infante, M. R., Pérez, L., Pinazo, A., Clapés, P., Morán, M. C., Angelet, M., García, M. T., & Vinardell, M. P. (2004). Amino acid-based surfactants. Comptes Rendus Chimie, 7(6–7), 583–592. https://doi.org/10.1016/j.crci.2004.02.009

Isidro-Llobet, A., Kenworthy, M. N., Mukherjee, S., Kopach, M. E., Wegner, K., Gallou, F., Smith, A. G., & Roschangar, F. (2019). Sustainability Challenges in Peptide Synthesis and Purification: From R&D to Production. Journal of Organic Chemistry, 84(8), 4615–4628. https://doi.org/10.1021/acs.joc.8b03001

Isley, N. A. (2020). Utilizing Micellar Catalysis for Organic Synthesis: A Desk Reference. 20. https://www.acsgcipr.org/wp-content/uploads/Micelle-catalysis-guide-sigma-aldrich.pdf, accessed date :25.06.2023

Jad, Y. E., Acosta, G. A., Govender, T., Kruger, H. G., El-Faham, A., De La Torre, B. G., & Albericio, F. (2016). Green Solid-Phase Peptide Synthesis 2. 2-Methyltetrahydrofuran and Ethyl Acetate for Solid-Phase Peptide Synthesis under Green Conditions. ACS Sustainable Chemistry and Engineering, 4(12), 6809–6814. https://doi.org/10.1021/acssuschemeng.6b01765

Jad, Y. E., Acosta, G. A., Khattab, S. N., De La Torre, B. G., Govender, T., Kruger, H. G., El-Faham, A., & Albericio, F. (2015). Peptide synthesis beyond DMF: THF and ACN as excellent and friendlier alternatives. Organic & Biomolecular Chemistry, 13(8), 2393–2398. https://doi.org/10.1039/C4OB02046D

Jad, Y. E., Acosta, G. A., Khattab, S. N., De La Torre, B. G., Govender, T., Kruger, H. G., El-Faham, A., & Albericio, F. (2016). 2-Methyltetrahydrofuran and cyclopentyl methyl ether for green solid-phase peptide synthesis. Amino Acids, 48(2), 419–426. https://doi.org/10.1007/S00726-015-2095-X/TABLES/6

Jad, Y. E., Govender, T., Kruger, H. G., El-Faham, A., De La Torre, B. G., & Albericio, F. (2017). Green Solid-Phase Peptide Synthesis (GSPPS) 3. Green Solvents for Fmoc Removal in Peptide Chemistry. Organic Process Research and Development, 21(3), 365–369. https://doi.org/10.1021/ACS.OPRD.6B00439/SUPPL_FILE/OP6B00439_SI_001.PDF

Jad, Y. E., Kumar, A., El-Faham, A., de la Torre, B. G., & Albericio, F. (2019). Green transformation of solid-phase peptide synthesis. ACS Sustainable Chemistry & Engineering, 7(4), 3671-3683.

Da'san MM, J., Al Musaimi, O., & Albericio, F. (2022). Advances in solid-phase peptide synthesis in aqueous media (ASPPS). Green Chemistry, 24(17), 6360-6372.

Jiang, Y., Chen, H., Mao, S., Luo, P., Du, Y., & Liu, M. (2011). Dynamics of mixed surfactants in aqueous solutions. Journal of Physical Chemistry B, 115(9), 1986–1990. https://doi.org/10.1021/jp107858y

Jiménez-González, C., Curzons, A. D., Constable, D. J. C., & Cunningham, V. L. (2004). Cradle-to-Gate Life Cycle Inventory and Assessment of Pharmaceutical Compounds. International Journal of Life Cycle Assessment, 9(2), 114–121. https://doi.org/10.1007/BF02978570

Jordan, A., Hall, C. G., Thorp, L. R., & Sneddon, H. F. (2022). Replacement of less-preferred dipolar aprotic and ethereal solvents in synthetic organic chemistry with more sustainable alternatives. Chemical reviews, 122(6), 6749-6794.

Kapadia, H., Brahmbhatt, H., Dabhi, Y., & Chourasia, S. (2019). Investigation of emulsion and effect on emission in CI engine by using diesel and bio-diesel fuel: A review. Egyptian Journal of Petroleum, 28(4), 323–337. https://doi.org/10.1016/j.ejpe.2019.06.004

Karavasili, C., Spanakis, M., Papagiannopoulou, D., Vizirianakis, I. S., Fatouros, D. G., & Koutsopoulos, S. (2015). Bioactive Self-Assembling Lipid-Like Peptides as Permeation Enhancers for Oral Drug Delivery. Journal of Pharmaceutical Sciences, 104(7), 2304–2311. https://doi.org/10.1002/JPS.24484

Katiyar, S. S., Kushwah, V., Dora, C. P., Patil, R. Y., & Jain, S. (2019). Design and Toxicity Evaluation of Novel Fatty Acid-Amino Acid-Based Biocompatible Surfactants. AAPS PharmSciTech, 20(5), 1–10. https://doi.org/10.1208/S12249-019-1396-X/FIGURES/7

Kaur, G., Kaur, K., & Handa, S. (2022). Efficient catalysis in dynamic nanomicelles of PS-750-M suspended in water. Current Opinion in Green and Sustainable Chemistry, 38, 100690. https://doi.org/10.1016/J.COGSC.2022.100690

Khavinson, V. K., Popovich, I. G., Linkova, N. S., Mironova, E. S., & Ilina, A. R. (2021). Peptide Regulation of Gene Expression: A Systematic Review. Molecules

2021, Vol. 26, Page 7053, 26(22), 7053. https://doi.org/10.3390/MOLECULES26227053

Kim, T. H., & Kim, S. G. (2011). Clinical Outcomes of Occupational Exposure to N,N-Dimethylformamide: Perspectives from Experimental Toxicology. Safety and Health at Work, 2(2), 97–104. https://doi.org/10.5491/SHAW.2011.2.2.97

Kimmerlin, T., & Seebach, D. (2005). “100 years of peptide synthesis”: Ligation methods for peptide and protein synthesis with applications to β-peptide assemblies. Journal of Peptide Research, 65(2), 229–260. https://doi.org/10.1111/j.1399-3011.2005.00214.x

Kincaid, J. R., Wong, M. J., Akporji, N., Gallou, F., Fialho, D. M., & Lipshutz, B. H. (2023). Introducing Savie: A Biodegradable Surfactant Enabling Chemo-and Biocatalysis and Related Reactions in Recyclable Water. Journal of the American Chemical Society, 145(7), 4266-4278.

Koenig, S. G., Bee, C., Borovika, A., Briddell, C., Colberg, J., Humphrey, G. R., ... & Sneddon, H. F. (2019). A green chemistry continuum for a robust and sustainable active pharmaceutical ingredient supply chain. ACS Sustainable Chemistry & Engineering, 7(20), 16937-16951.

Kumar, A., Jad, Y. E., de la Torre, B. G., El-Faham, A., & Albericio, F. (2017). Re-evaluating the stability of COMU in different solvents. Journal of Peptide Science, 23(10), 763–768. https://doi.org/10.1002/psc.3024

La Sorella, G., Strukul, G., & Scarso, A. (2015). Recent advances in catalysis in micellar media. Green Chemistry, 17(2), 644–683. https://doi.org/10.1039/c4gc01368a

Lau, J. L., & Dunn, M. K. (2018). Therapeutic peptides: Historical perspectives, current development trends, and future directions. Bioorganic and Medicinal Chemistry, 26(10), 2700–2707. https://doi.org/10.1016/j.bmc.2017.06.052

Lawrenson, S. B., Arav, R., & North, M. (2017). The greening of peptide synthesis. Green Chemistry, 19(7), 1685–1691. https://doi.org/10.1039/c7gc00247e

LEE, K. (1987). Toxicity of N-methyl-2-pyrrolidone (NMP): Teratogenic, subchronic, and two-year inhalation studies. Fundamental and Applied Toxicology, 9(2), 222–235. https://doi.org/10.1016/0272-0590(87)90045-5

Lee, N. R., Cortes-Clerget, M., Wood, A. B., Lippincott, D. J., Pang, H., Moghadam, F. A., Gallou, F., & Lipshutz, B. H. (2019). Coolade. A Low-Foaming Surfactant for Organic Synthesis in Water. ChemSusChem, 12(13), 3159–3165. https://doi.org/10.1002/cssc.201900369

Lee, Y. S. (2019). Gram-Scale Preparation of C-Terminal-Modified Enkephalin Analogues by Typical Liquid-Phase Peptide Synthesis. Current Protocols in Protein Science, 98(1), e97. https://doi.org/10.1002/cpps.97

Lipshutz, B. H. (2017). When Does Organic Chemistry Follow Nature’s Lead and “make the Switch”? Journal of Organic Chemistry, 82(6), 2806–2816. https://doi.org/10.1021/acs.joc.7b00010

Lipshutz, B. H. (2018). Catalyst: Imagine Doing Chemistry at No Cost … to the Environment! Chem, 4(9), 2004–2007. https://doi.org/10.1016/j.chempr.2018.08.031

Lipshutz, B. H., Ghorai, S., Abela, A. R., Moser, R., Nishikata, T., Duplais, C., Krasovskiy, A., Gaston, R. D., & Gadwood, R. C. (2011). TPGS-750-M: A second-generation amphiphile for metal-catalyzed cross-couplings in water at room temperature. Journal of Organic Chemistry, 76(11), 4379–4391. https://doi.org/10.1021/jo101974u

Lorenzetto, T., Berton, G., Fabris, F., & Scarso, A. (2020). Recent designer surfactants for catalysis in water. Catalysis Science & Technology, 10(14), 4492–4502. https://doi.org/10.1039/D0CY01062F

Lorenzetto, T., Frigatti, D., Fabris, F., & Scarso, A. (2022). Minimalistic β-sitosterol based designer surfactants for efficient cross-coupling in water. Journal of Organometallic Chemistry, 964, 122316. https://doi.org/10.1016/j.jorganchem.2022.122316

L Lu, Y., Zhang, E., Yang, J., & Cao, Z. (2018). Strategies to improve micelle stability for drug delivery. Nano research, 11, 4985-4998.

Lynch, D. W., Placke, M. E., Persing, R. L., & Ryan, M. J. (2003). Thirteen-week inhalation toxicity of N, N-dimethylformamide in F344/N rats and B6C3F1 mice. Toxicological Sciences, 72(2), 347-358.

MacMillan, D. S., Murray, J., Sneddon, H. F., Jamieson, C., & Watson, A. J. B. (2013). Evaluation of alternative solvents in common amide coupling reactions: replacement of dichloromethane and N,N-dimethylformamide. Green Chemistry, 15(3), 596–600. https://doi.org/10.1039/C2GC36900A

Madani, M., Zargar, G., Takassi, M. A., Daryasafar, A., Wood, D. A., & Zhang, Z. (2019). Fundamental investigation of an environmentally-friendly surfactant agent for chemical enhanced oil recovery. Fuel, 238, 186–197. https://doi.org/10.1016/j.fuel.2018.10.105

Mali, S. M., Ganesh Kumar, M., Katariya, M. M., & Gopi, H. N. (2014). HBTU mediated 1-hydroxybenzotriazole (HOBt) conjugate addition: Synthesis and stereochemical analysis of β-benzotriazole N-oxide substituted γ-amino acids and hybrid peptides. Organic and Biomolecular Chemistry, 12(42), 8462–8472. https://doi.org/10.1039/c4ob01548g

Marsella, J. A., & Updated by Staff. (2013). Dimethylformamide. Kirk-Othmer Encyclopedia of Chemical Technology, 1–9. https://doi.org/10.1002/0471238961.0409130513011819.A01.PUB2

Martin, V., Egelund, P. H. G., Johansson, H., Thordal Le Quement, S., Wojcik, F., & Sejer Pedersen, D. (2020). Greening the synthesis of peptide therapeutics: An industrial perspective. RSC Advances, 10(69), 42457–42492. https://doi.org/10.1039/d0ra07204d

Martin, V., Jadhav, S., Egelund, P. H. G., Liffert, R., Johansson Castro, H., Krüger, T., Haselmann, K. F., Thordal Le Quement, S., Albericio, F., Dettner, F., Lechner, C., Schönleber, R., & Pedersen, D. S. (2021). Harnessing polarity and viscosity to identify green binary solvent mixtures as viable alternatives to DMF in solid-phase peptide synthesis. Green Chemistry, 23(9), 3295–3311. https://doi.org/10.1039/d1gc00603g

McKnelly, K. J., Sokol, W., & Nowick, J. S. (2020). Anaphylaxis Induced by Peptide Coupling Agents: Lessons Learned from Repeated Exposure to HATU, HBTU, and HCTU. Journal of Organic Chemistry, 85(3), 1764–1768. https://doi.org/10.1021/acs.joc.9b03280

Meier, S., Schindler, B. K., Koslitz, S., Koch, H. M., Weiss, T., Käfferlein, H. U., & Brüning, T. (2013). Biomonitoring of exposure to N-methyl-2-pyrrolidone in workers of the automobile industry. Annals of Occupational Hygiene, 57(6), 766–773. https://doi.org/10.1093/annhyg/mes111

Merrifield, R. B. (1963). Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. Journal of the American Chemical Society, 85(14), 2149–2154. https://doi.org/10.1021/JA00897A025

Minkler, S. R. K., Lipshutz, B. H., & Krause, N. (2011). Gold catalysis in micellar systems. Angewandte Chemie - International Edition, 50(34), 7820–7823. https://doi.org/10.1002/anie.201101396

Mitchell, A. R. (2008). Bruce Merrifield and solid‐phase peptide synthesis: A historical assessment. Peptide Science, 90(3), 175-184.

Mohajeri, E., & Noudeh, G. D. (2012). Effect of temperature on the critical micelle concentration and micellization thermodynamic of nonionic surfactants: Polyoxyethylene sorbitan fatty acid esters. E-Journal of Chemistry, 9(4), 2268–2274. https://doi.org/10.1155/2012/961739

Mukerjee, P., & Mysels, K. J. (1955). A Re-evaluation of the Spectral Change Method of Determining Critical Micelle Concentration. Journal of the American Chemical Society, 77(11), 2937–2943. https://doi.org/10.1021/JA01616A003/ASSET/JA01616A003.FP.PNG_V03

Mustahil, N. A., Baharuddin, S. H., Abdullah, A. A., Reddy, A. V. B., Abdul Mutalib, M. I., & Moniruzzaman, M. (2019). Synthesis, characterization, ecotoxicity and biodegradability evaluations of novel biocompatible surface active lauroyl sarcosinate ionic liquids. Chemosphere, 229, 349–357. https://doi.org/10.1016/j.chemosphere.2019.05.026

Muttenthaler, M., King, G. F., Adams, D. J., & Alewood, P. F. (2021). Trends in peptide drug discovery. Nature Reviews Drug Discovery 2021 20:4, 20(4), 309–325. https://doi.org/10.1038/s41573-020-00135-8

Mutter, M., Hagenmaier, H., & Bayer, E. (1971). New Method of Polypeptide Synthesis. Angewandte Chemie International Edition in English, 10(11), 811–812. https://doi.org/10.1002/anie.197108112

Myers, D. (2005). Surfactant Science and Technology. https://doi.org/10.1002/047174607x

Naoto Sugisawa, Akira Ando, & Shinichiro Fuse. (2023). Rapid and column-chromatography-free peptide chain elongation via a one-flow, three-component coupling approach. Chemical Science. https://doi.org/10.1039/D3SC01333B

Narayan, S., Muldoon, J., Finn, M. G., Fokin, V. V., Kolb, H. C., & Sharpless, K. B. (2005). “On water”: Unique reactivity of organic compounds in aqueous suspension. Angewandte Chemie - International Edition, 44(21), 3275–3279. https://doi.org/10.1002/anie.200462883

Ncube, A., Mtetwa, S., Bukhari, M., Fiorentino, G., & Passaro, R. (2023). Circular Economy and Green Chemistry: The Need for Radical Innovative Approaches in the Design for New Products. Energies, 16(4), 1–21. https://doi.org/10.3390/en16041752

Nishiuchi, Y., Inui, T., Nishio, H., Bódi, J., Kimura, T., Tsuji, F. I., & Sakakibara, S. (1998). Chemical synthesis of the precursor molecule of the Aequorea green fluorescent protein, subsequent folding, and development of fluorescence. Proceedings of the National Academy of Sciences, 95(23), 13549–13554. https://doi.org/10.1073/PNAS.95.23.13549

Obst, M., & König, B. (2018). Organic synthesis without conventional solvents. European Journal of Organic Chemistry, 2018(31), 4213-4232.

Ohadi, M., Shahravan, A., Dehghannoudeh, N., Eslaminejad, T., Banat, I. M., & Dehghannoudeh, G. (2020). Potential use of microbial surfactant in microemulsion drug delivery system: a systematic review. Drug design, development and therapy, 541-550.

Okada, Y., Takasawa, R., Kubo, D., Iwanaga, N., Fujita, S., Suzuki, K., Suzuki, H., Kamiya, H., & Chiba, K. (2019). Improved Tag-Assisted Liquid-Phase Peptide Synthesis: Application to the Synthesis of the Bradykinin Receptor Antagonist Icatibant Acetate. Organic Process Research and Development, 23(11), 2576–2581. https://doi.org/10.1021/ACS.OPRD.9B00397/ASSET/IMAGES/LARGE/OP9B00397_0009.JPEG

Os, N. van, Haak, J., & Rupert, L. (1993). Physico-Chemical Properties of Selected Anionic, Cationic and Nonionic Surfactants. In Physico-Chemical Properties of Selected Anionic, Cationic and Nonionic Surfactants. https://doi.org/10.1016/c2009-0-09112-0

Palomo, J. M. (2014). Solid-phase peptide synthesis: an overview focused on the preparation of biologically relevant peptides. RSC Advances, 4(62), 32658–32672. https://doi.org/10.1039/C4RA02458C

Parmentier, M., Wagner, M. K., Magra, K., & Gallou, F. (2016). Selective amidation of unprotected amino alcohols using surfactant-in-water technology: A highly desirable alternative to reprotoxic polar aprotic solvents. Organic Process Research and Development, 20(6), 1104–1107. https://doi.org/10.1021/acs.oprd.6b00133

Parmentier, M., Wagner, M., Wickendick, R., Baenziger, M., Langlois, A., & Gallou, F. (2020). A general kilogram scale protocol for suzuki–miyaura cross-coupling in water with TPGS-750-M surfactant. Organic Process Research & Development, 24(8), 1536-1542.

Patel, U., Parekh, P., Sastry, N. V., Aswal, V. K., & Bahadur, P. (2017). Surface activity, micellization and solubilization of cationic gemini surfactant-conventional surfactants mixed systems. Journal of Molecular Liquids, 225, 888–896. https://doi.org/10.1016/j.molliq.2016.11.017

Pattabiraman, V. R., & Bode, J. W. (2011). Rethinking amide bond synthesis. Nature, 480(7378), 471-479.

Pottorf, R. S., & Szeto, P. (2001). 1-Ethyl-3-(3′-dimethylaminopropyl)carbodiimide Hydrochloride. Encyclopedia of Reagents for Organic Synthesis, 2–4. https://doi.org/10.1002/047084289x.re062

Procopio, D., Siciliano, C., Trombino, S., Dumitrescu, D. E., Suciu, F., & Di Gioia, M. L. (2022). Green solvents for the formation of amide linkages. Organic & Biomolecular Chemistry, 20(6), 1137-1149.

REACH. (2006). REGULATION (EC) No 1907/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL. Official Journal of the European Communities, 1907, 1–520.

Rosen, M. A., & Kishawy, H. A. (2012). Sustainable manufacturing and design: Concepts, practices and needs. Sustainability, 4(2), 154–174. https://doi.org/10.3390/su4020154

Saghir, S. A. (2014). Absorption. In Encyclopedia of Toxicology: Third Edition (pp. 1–6). Elsevier. https://doi.org/10.1016/B978-0-12-386454-3.00361-4

Sahoo, B. M., & Banik, B. K. (2020). Solvent-less reactions: Green and sustainable approaches in medicinal chemistry. In Green Approaches in Medicinal Chemistry for Sustainable Drug Design (pp. 523-548). Elsevier.

Lemonick,S. (2020). For organic chemists, micellar chemistry could be a revolution. C&EN Global Enterprise, 98(22), 20–23. https://doi.org/10.1021/cen-09822-feature1

Scailteur, V., Toxicology, R. L.-, & 1987, U. (1987). Dimethylformamide (DMF) hepatotoxicity. Toxicology, 43(3), 231–238. https://www.sciencedirect.com/science/article/pii/0300483X87900825

Schmied-Tobies, M. I., Murawski, A., Schmidt, L., Rucic, E., Schwedler, G., Apel, P., ... & Kolossa-Gehring, M. (2021). Pentachlorophenol and nine other chlorophenols in urine of children and adolescents in Germany–Human biomonitoring results of the German Environmental Survey 2014–2017 (GerES V). Environmental Research, 196, 110958.

European Chemicals Agency. (2019). How to Comply with REACH Restriction 71, Guideline for Users of NMP (1-Methyl-2-Pyrrolidone)pdf.,accessed date: 19.07.2023

Sels, H., De Smet, H., & Geuens, J. (2020). SUSSOL-Using artificial intelligence for greener solvent selection and substitution. Molecules, 25(13), 3037. https://doi.org/10.3390/molecules25133037

Sharma, A., Kumar, A., De La Torre, B. G., & Albericio, F. (2022). Liquid-Phase Peptide Synthesis (LPPS): A Third Wave for the Preparation of Peptides. Chemical Reviews, 122(16), 13516–13546. https://doi.org/10.1021/acs.chemrev.2c00132

Sharma, S., Buchbinder, N. W., Braje, W. M., & Handa, S. (2020). Fast Amide Couplings in Water: Extraction, Column Chromatography, and Crystallization Not Required. Organic Letters, 22(15), 5737–5740. https://doi.org/10.1021/acs.orglett.0c01676

Sharma, S., Kaur, G., & Handa, S. (2021). Insights into Fast Amide Couplings in Aqueous Nanomicelles. Organic Process Research and Development, 25(8), 1960–1965. https://doi.org/10.1021/acs.oprd.1c00203

Sheldon, R. A. (2019). The greening of solvents: Towards sustainable organic synthesis. Current Opinion in Green and Sustainable Chemistry, 18, 13–19. https://doi.org/10.1016/j.cogsc.2018.11.006

Shelton, P. T., & Jensen, K. J. (2013). Linkers, resins, and general procedures for solid-phase peptide synthesis. Methods in Molecular Biology, 1047, 23–41. https://doi.org/10.1007/978-1-62703-544-6_2

Shen, T., Zhou, S., Ruan, J., Chen, X., Liu, X., Ge, X., & Qian, C. (2021). Recent advances on micellar catalysis in water. Advances in Colloid and Interface Science, 287, 102299. https://doi.org/10.1016/j.cis.2020.102299

Singh, V., & Tyagi, R. (2015). Investigations of mixed surfactant systems of lauryl alcohol-based bissulfosuccinate anionic gemini surfactants with conventional surfactants: A fluorometric study. Journal of Taibah University for Science, 9(4), 477–489. https://doi.org/10.1016/j.jtusci.2015.01.010

Sitarek, K., & Stetkiewicz, J. (2008). Assessment of reproductive toxicity and gonadotoxic potential of N-methyl-2-pyrrolidone in male rats. International Journal of Occupational Medicine and Environmental Health, 21(1), 73–80. https://doi.org/10.2478/v10001-008-0006-z

Smith, J. D., Ansari, T. N., Andersson, M. P., Yadagiri, D., Ibrahim, F., Liang, S., Hammond, G. B., Gallou, F., & Handa, S. (2018). Micelle-enabled clean and selective sulfonylation of polyfluoroarenes in water under mild conditions. Green Chemistry, 20(8), 1784–1790. https://doi.org/10.1039/c7gc03514d

Sureshbabu, V. V., Lalithamba, H. S., Narendra, N., & Hemantha, H. P. (2010). New and simple synthesis of acid azides, ureas and carbamates from carboxylic acids: Application of peptide coupling agents EDC and HBTU. Organic and Biomolecular Chemistry, 8(4), 835–840. https://doi.org/10.1039/b920290k

Takahashi, D., & Yamamoto, T. (2012). Development of an efficient liquid-phase peptide synthesis protocol using a novel fluorene-derived anchor support compound with Fmoc chemistry; AJIPHASE®. Tetrahedron Letters, 53(15), 1936–1939. https://doi.org/10.1016/j.tetlet.2012.02.006

Takahashi, D., Yano, T., & Fukui, T. (2012). Novel diphenylmethyl-derived amide protecting group for efficient liquid-phase peptide synthesis: AJIPHASE. Organic Letters, 14(17), 4514–4517. https://doi.org/10.1021/ol302002g

Tang, C., & McInnes, B. T. (2022). Cascade Processes with Micellar Reaction Media: Recent Advances and Future Directions. Molecules, 27(17), 5611.. https://doi.org/10.3390/molecules27175611

Tang, J., Wang, Y., Wang, D., Wang, Y., Xu, Z., Racette, K., & Liu, F. (2013). Key structure of brij for overcoming multidrug resistance in cancer. Biomacromolecules, 14(2), 424–430. https://doi.org/10.1021/BM301661W

Varnava, K. G., & Sarojini, V. (2019). Making Solid-Phase Peptide Synthesis Greener: A Review of the Literature. Chemistry - An Asian Journal, 14(8), 1088–1097. https://doi.org/10.1002/asia.201801807

Verlander, M. (2007). Industrial applications of solid-phase peptide synthesis - A status report. International Journal of Peptide Research and Therapeutics, 13(1–2), 75–82. https://doi.org/10.1007/s10989-006-9075-7

Wegner, K., Barnes, D., Manzor, K., Jardine, A., & Moran, D. (2021). Evaluation of greener solvents for solid-phase peptide synthesis. Green Chemistry Letters and Reviews, 14(1), 153-164.

Wehrstedt, K. D., Wandrey, P. A., & Heitkamp, D. (2005). Explosive properties of 1-hydroxybenzotriazoles. Journal of Hazardous Materials, 126(1–3), 1–7. https://doi.org/10.1016/j.jhazmat.2005.05.044

Welton, T. (2015). Solvents and sustainable chemistry. Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences, 471(2183). https://doi.org/10.1098/rspa.2015.0502

Withey, J. M., & Bajic, A. (2015). Operationally simple synthesis of N, N -diethyl-3-methylbenzamide (DEET) Using COMU as a coupling reagent. Journal of Chemical Education, 92(1), 175–178. https://doi.org/10.1021/ed500488x

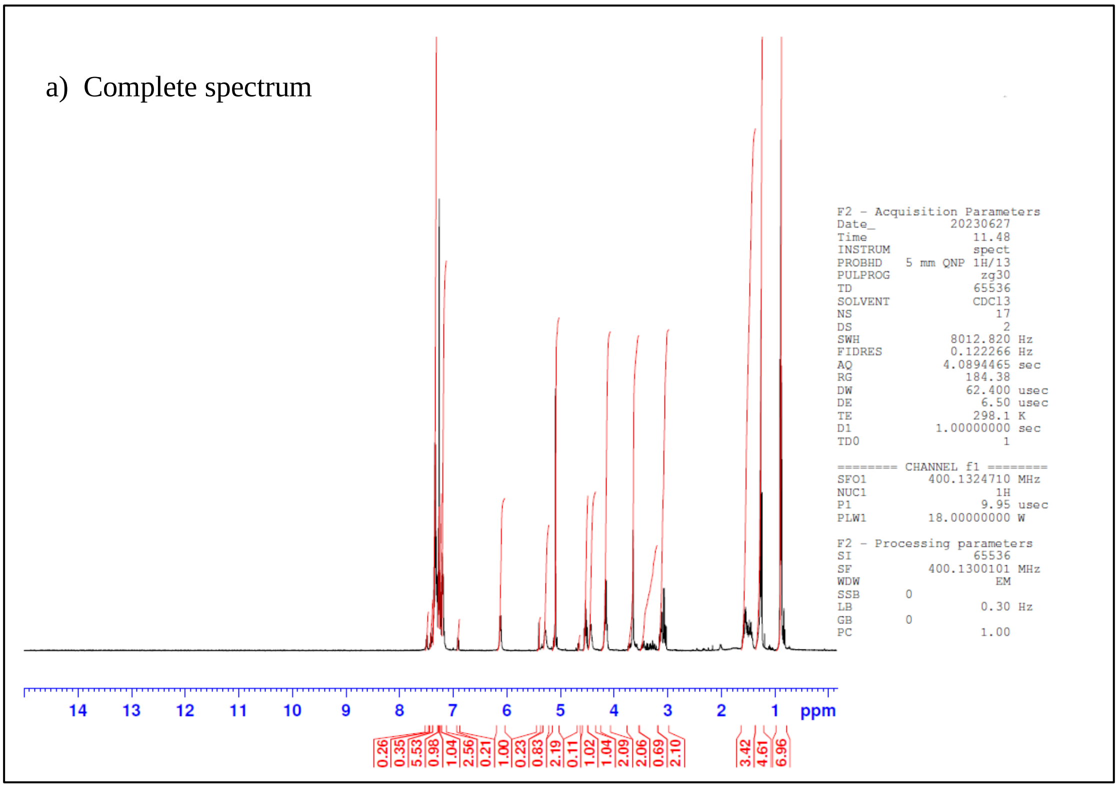
Wróblewska, A., Paluch, P., Wielgus, E., Bujacz, G., Dudek, M. K., & Potrzebowski, M. J. (2017). Approach toward the understanding of coupling mechanism for EDC reagent in solvent-free mechanosynthesis. Organic Letters, 19(19), 5360–5363. https://doi.org/10.1021/acs.orglett.7b02637

Wu, A., Ramakrishna, I., Hattori, T., & Yamamoto, H. (2022). Silicon-based hydrophobic tags applied in liquid-phase peptide synthesis: protected DRGN-1 and poly alanine chain synthesis. Organic & Biomolecular Chemistry, 20(44), 8685–8692. https://doi.org/10.1039/D2OB01795D

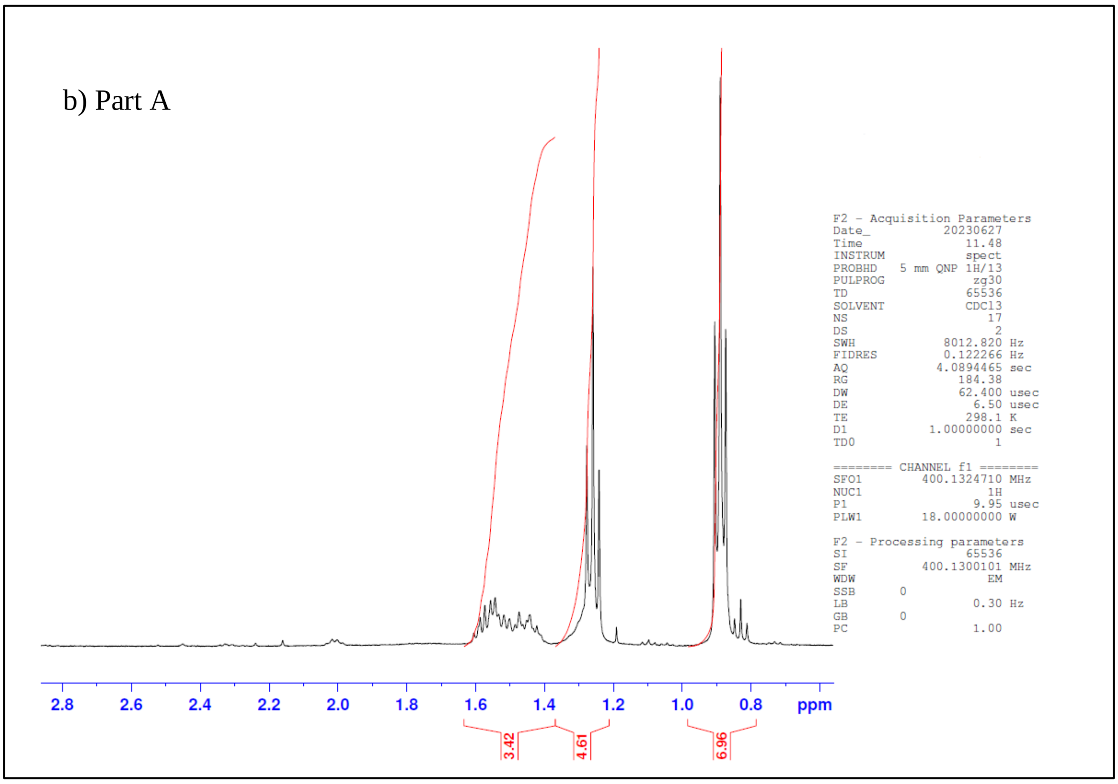
Wu, A., & Yamamoto, H. (2023). Super silyl-based stable protecting groups for both the C- and N-terminals of peptides: applied as effective hydrophobic tags in liquid-phase peptide synthesis. Chemical Science, 14(19), 5051–5061. https://doi.org/10.1039/d3sc01239e

Annex

Figure (a,b,c,d) H^1 NMR spectra of Z-Phe-Leu-OEt in $CDCl_3$.Figure (a,b,c,d) 1



a) Complete spectrum



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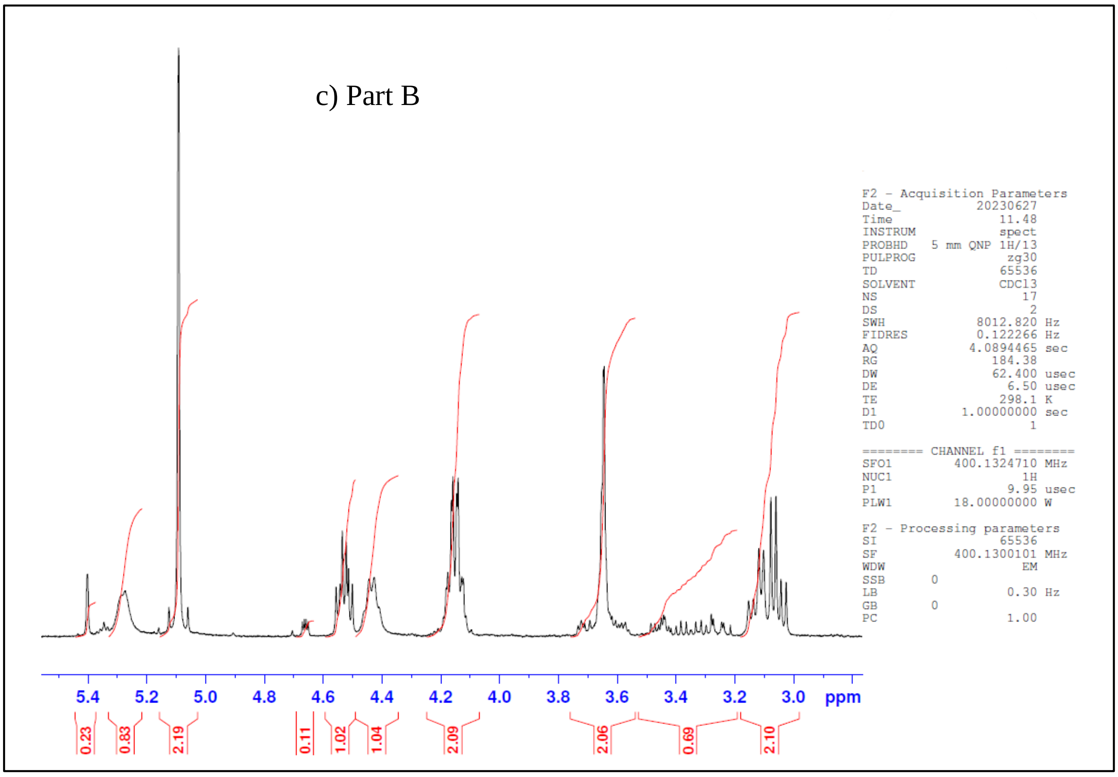
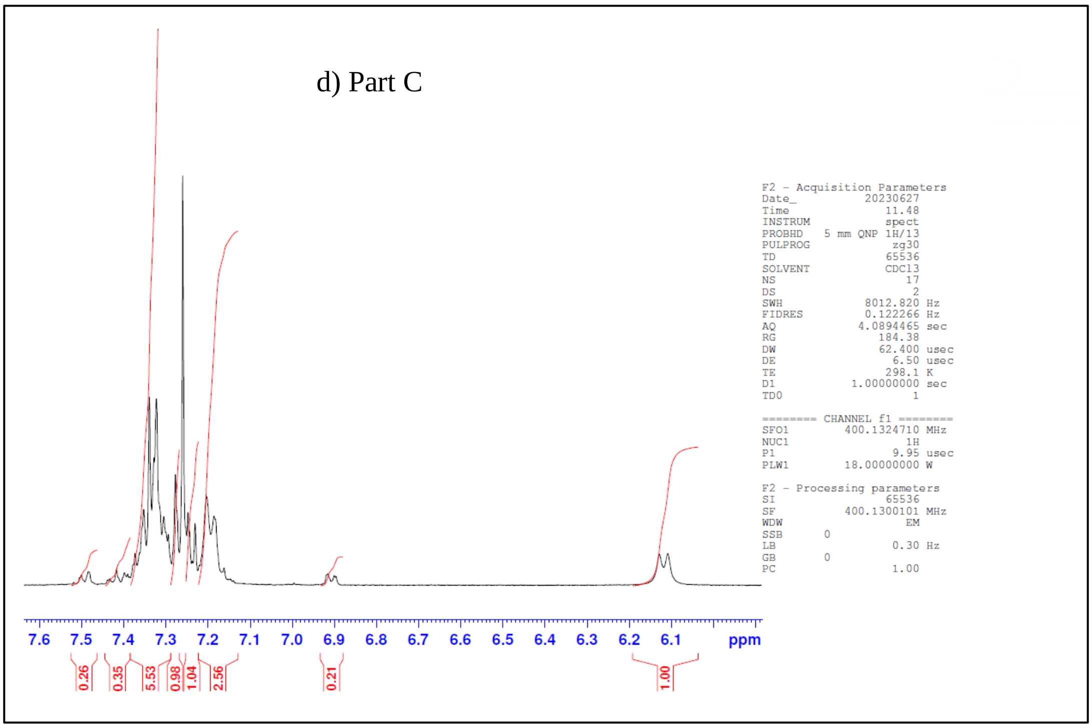


c) Part B

```
F2 - Acquisition Parameters
Date_              20230627
Time                  11.48
INSTRUM               spect
PROBHD    5 mm QNP 1H/13
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       17
DS                        2
SWH                8012.820 Hz
FIDRES             0.122266 Hz
AQ                4.0894465 sec
RG                   184.38
DW                   62.400 usec
DE                     6.50 usec
TE                    298.1 K
D1               1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            400.1324710 MHz
NUC1                     1H
P1                     9.95 usec
PLW1            18.00000000 W

F2 - Processing parameters
SI                    65536
SF              400.1300101 MHz
WDW                      EM
SSB         0
LB                     0.30 Hz
GB          0
PC                     1.00
```



d) Part C

```
F2 - Acquisition Parameters
Date_              20230627
Time                  11.48
INSTRUM               spect
PROBHD    5 mm QNP 1H/13
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                       17
DS                        2
SWH                8012.820 Hz
FIDRES             0.122266 Hz
AQ                4.0894465 sec
RG                   184.38
DW                   62.400 usec
DE                     6.50 usec
TE                    298.1 K
D1               1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1            400.1324710 MHz
NUC1                     1H
P1                     9.95 usec
PLW1            18.00000000 W

F2 - Processing parameters
SI                    65536
SF              400.1300101 MHz
WDW                      EM
SSB         0
LB                     0.30 Hz
GB          0
PC                     1.00
```