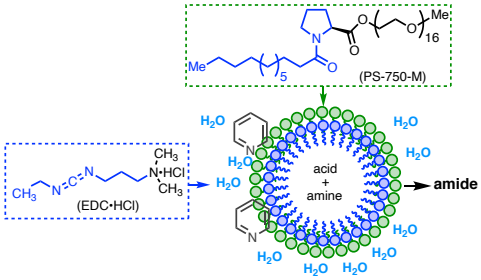
Insights into Fast Amide Couplings in Aqueous Nanomicelles

Sudripet Sharma,[†] Gaganpreet Kaur,[†] Sachin Handa[†,*]

[†]Department of Chemistry, University of Louisville, Louisville, Kentucky 40292, United States

Supporting Information Placeholder



(Fast amide couplings in possible mixed micelles)

ABSTRACT: 1-Ethyl-3-(3-(dimethylamino)propyl)-carbodiimide (EDC•HCl) has both the lipophilic and the hydrophilic regions, causing self-aggregation (also called nanoparticles formation) in an aqueous medium containing PS-750-M amphiphile. Kinetic and ^{1}H NMR studies probe the effect of different organic bases on the potential nanoparticle formation of EDC•HCl. It also reveals why the pyridine base works better under micellar conditions. The methodology was examined on multigram scale synthesis of bioactive molecules, where excellent reaction yields were obtained without product epimerization while maintaining the shorter reaction time.

KEYWORDS. amide couplings, micellar catalysis, chemistry in water, sustainability.

Introduction. The first Nobel Prize in the field of (poly)peptides was awarded to Vincent du Vigneaud in 1955 for work on the synthesis of sulfur compounds and the first bioactive polypeptide oxytocin.[1] Since then, the field of (poly)peptide synthesis has achieved excellence.[2] At present, *ca.* 100 bioactive (poly)peptides are on the market, and more than 400 are in clinical trials.[3,4] Peptides cover *ca.* 10% of the market of active pharmaceutical ingredients (APIs).[5,6] However, the growing need to produce more complex peptide-based APIs requires efficient synthetic strategies at reasonably low cost without product epimerization. It includes fine-tuning synthetic methodologies that cover protecting group-free chemistry,[7,8] the use of high-performance liquid chromatography (reverse-phase) for product purification[9] or the development of chromatography-free technologies,[10,11] and exploitation of more economical reagents.[12-14]

Along the same lines, in 2016, the ACS Green Chemistry Institute Pharmaceutical Roundtable (ACS GCIPR) identified the need for greener synthetic methods for peptide and oligonucleotide synthesis.[15] In 2018, amide couplings were included in the top 10 key research areas.[16] ACS GCIPR further identified greener peptide and peptide-conjugate synthesis as a key challenge for the pharmaceutical industry. The current state-of-the-art technologies for peptide couplings have a gap in reaction efficiency and sustainability, as evidenced by waste generation data from current peptide and oligonucleotide processes.[18] It includes 3000-15000 kg waste generation per kg API synthesis. The use of hazardous reagents and solvents generates substantial waste; most of the waste is produced by solvents, such as, DMF and NMP.[19-21] Recently, several reports disclosed greener alternatives for amide and peptide couplings.[10,21-25] The amide couplings in water could drastically reduce waste generation, potentially leading to environmentally benign and economic processes.[26,27] In this direction, our group has recently reported amide couplings in water, with spontaneous product precipitation after reaction completion, allowing isolation of pure product by filtration, avoiding column chromatography and organic solvents.[10] Although many reports state the advantages of water over other organic solvents (DMF, NMP, DCM) in amide couplings,[21,22,25] to the best of our knowledge, the systematic study on how amide couplings work in the micellar medium is still underexplored. Therefore, we intend to gain insights into how the reaction proceeds in an aqueous micellar medium, why are reactions generally fast, and why a specific base works well with EDC•HCl as a coupling agent? The fundamental understanding of these points could further advance the field of aqueous micellar catalysis, which could assist researchers in designing recyclable coupling agents compatible with micellar catalysis.

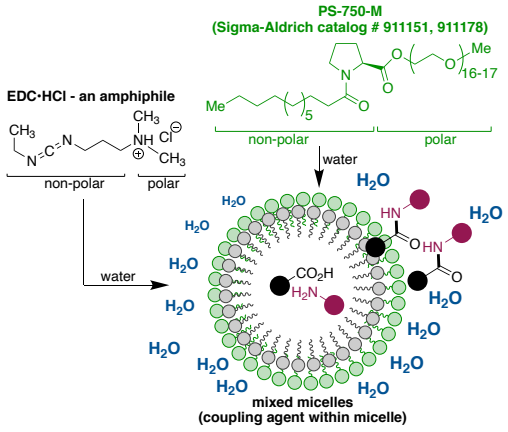
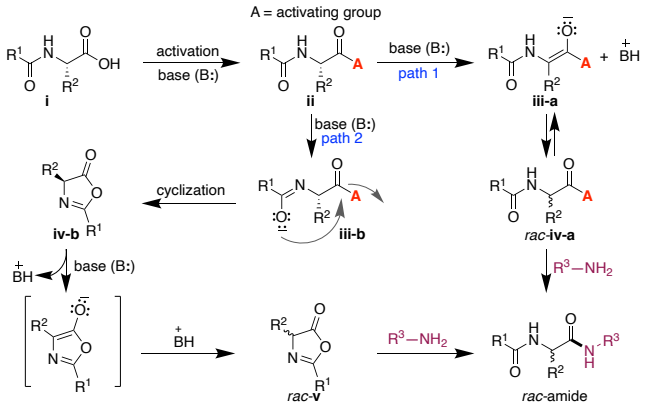


Figure 1. Mixed micelles leading efficient amide couplings.

As depicted in Scheme 1, the formation of an amide bond mainly involves two steps. The first step includes the formation

of activated complex **ii** after a reaction between acid **i** and the coupling agent. This step may require a base. The slower formation of an activated complex of carboxylic acid causes low conversions.[28,29] To overcome this problem, methods involving the use of better coupling agents have been developed. It includes the use of carbodiimides (DCC, DIC, EDC•HCl) with or without additives (HOBt, HOOBt, HOAt, DMAP).[30,31] The phosphonium reagents, such as, BOP, PyBOP, and PyAOP, have also been employed in these couplings.[5,32] Likewise, aminium/uronium reagents TBTU, HBTU, HATU, and COMU have also shown their unique importance in amide couplings.[5,22,33,34] Among many commercially available reagents, EDC•HCl is the most common reagent for large-scale amide couplings.[35] The urea byproduct formed from EDC•HCl is readily soluble in water, easing the product isolation.



Scheme 1. Plausible pathways for racemization.

The second step involves the attack of nucleophilic amine to the activated carboxylate. As depicted in Scheme 1, the presence of a base in the reaction medium and cyclization of activated complex **ii** is responsible for epimerization.[31,36] However, the base facilitates the formation of activated complex **ii**. In the presence of a base, two pathways could simultaneously operate. In pathway 1, a base causes enolate formation **iii-a**, leading to *rac*-**iv-a** followed by a reaction with an amine nucleophile forming *rac*-amide product. In pathway 2, the base promotes cyclization of **iii-b** to **iv-b**. Upon further reaction with the base, it epimerizes to *rac*-**v**, which provides *rac*-amide after reaction with an amine. This epimerization could be prevented if the reaction undergoes at faster rate, therefore, not allowing the undesired reaction pathways to operate. We have demonstrated that fast amide couplings are possible in an aqueous micellar medium of PS-750-M without epimerization.[10]

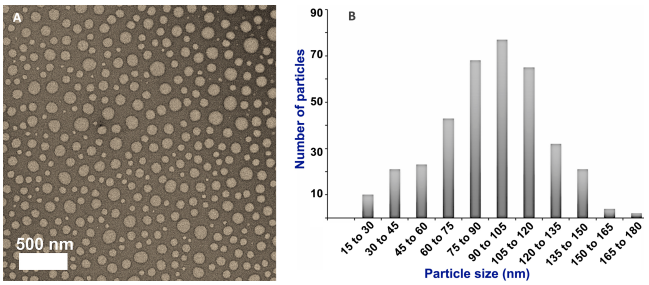
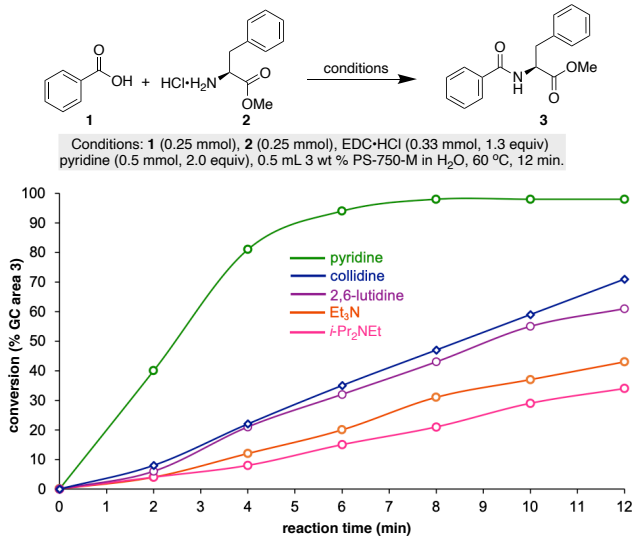


Figure 2. (A) HRTEM analysis of EDC•HCl in 3 wt % aqueous PS-750-M; (B) particle size distribution.

Results and Discussion. Notably, EDC•HCl has both lipophilic non-polar hydrocarbon chain and hydrophilic polar head (ammonium chloride residue). Therefore, it has the potential to behave as an ionic amphiphile in water (Figure 1). When dissolved in an aqueous medium containing amphiphile PS-750-M, it can self-aggregate to form unique nanoparticles containing a mixture of EDC•HCl and PS-750-M. The reactions in these nanoparticles take place under a higher local concentration than reactions in a homogeneous solution, potentially facilitating the rapid and efficient product formation in water. To confirm the existence of such particles, high-resolution transmission electron microscopy (HRTEM) imaging of EDC•HCl in an aqueous solution of PS-750-M was performed (Figure 2A). HRTEM analysis reveals particles of variable sizes (Figure 2A); the average particle size calculated from image 2 A is 92 nm (Figure 2B). These mixed micelles are potentially formed due to the self-aggregation of EDC•HCl and PS-750-M in an aqueous environment. A higher concentration of EDC•HCl within these particles readily activates the carboxylic acid for the desired amidation at faster rates. Notably, in HRTEM analysis, no such particles were observed for EDC•HCl solution in neat water.



Scheme 2. Effect of base on reaction kinetics (GC-MS conversions using 1,1,2,2-tetrachloroethane as an internal standard; for details see Supporting Information, pages S3, S4).

The nanoparticles containing EDC•HCl and PS-750-M are formed due to their amphiphilic nature, which could be diminished with the strong base by neutralizing the EDC•HCl salt. Therefore, we conducted a model kinetic study of coupling between **1** and **2** using different bases (Scheme 2). A coupling reaction was fast when a pyridine base was used (for details, see Supporting Information). Notably, a complete conversion to **3** was achieved in just 8 minutes (98% yield based on GC-MS analysis). However, when pyridine was replaced with *i*-Pr_2NEt, only 34% conversion to **3** was observed in 12 minutes. Likewise, the use of a Et_3N base affords 43% conversion to **3** after 12 minutes. These studies reveal that strong organic bases are not compatible with fast amide couplings, most likely due to the loss of the amphiphilic nature of the coupling agent. The effect of relatively another weak base (2,6-lutidine) on the reaction rate was further explored. The reaction rate was slightly fast compared to the cases when *i*-Pr_2NEt and Et_3N bases were used. 2,6-Lutidine affords 61% conversion, while collidine provides

71% conversion in the first 12 minutes. The data from kinetic studies suggest that reaction is more efficient with the use of a weaker base. Once again, this trend could be further correlated with the neutralization of EDC•HCl salt with Et_3N or *i*-Pr_2NEt. The neutral form of EDC no longer participates in the formation of mixed micelles, which are most likely responsible for reaction efficiency in water. Pyridine's better performance over collidine and 2,6-lutidine is attributable to its low pKa in aqueous solution (in water *ca*. 6) and water solubility, enabling more concentration of coupling partners in the dynamic nanoparticles. Such a weakly basic micellar environment and fast reaction rate also eliminate the possibility of epimerization.

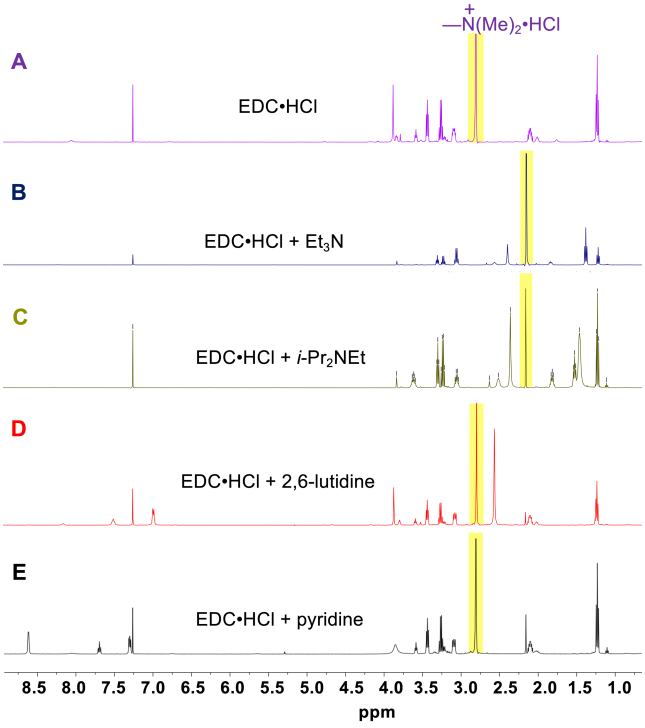
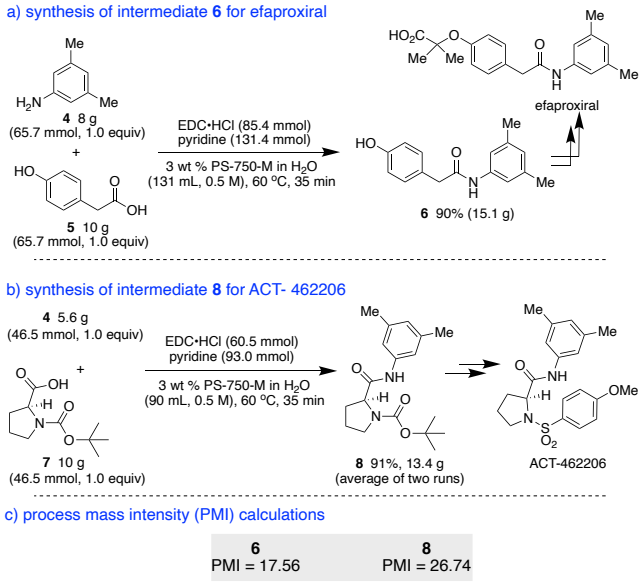


Figure 3. ^1H NMR study: Effect of base on EDC•HCl structure. (A) EDC•HCl. (B) EDC•HCl and Et_3N. (C) EDC•HCl and *i*-Pr_2NEt. (D) EDC•HCl and 2,6-lutidine. (E) EDC•HCl and pyridine (for details, see Supporting Information, pages S24, S25).

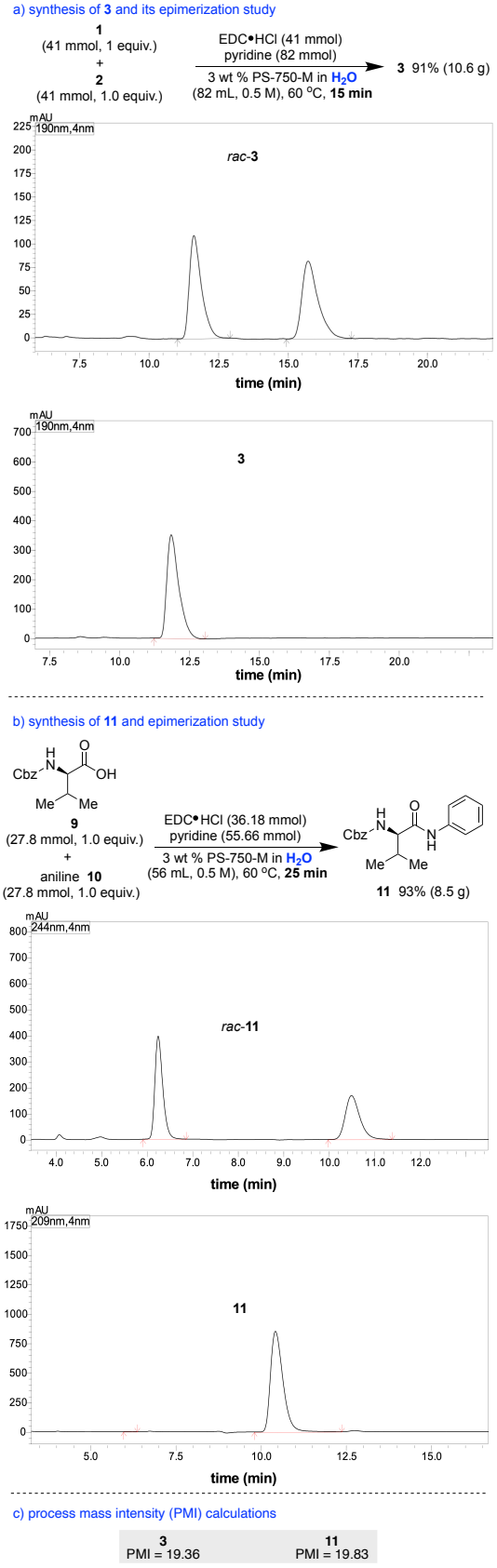
To further validate the effect of the base on the amphiphilic structure of EDC•HCl, ^1H NMR studies in $CDCl_3$ were conducted using different organic bases as additives (Figure 3A-E). Notably, $CDCl_3$ could potentially mimic the micellar interior in terms of polarity. A ^1H NMR analysis of EDC•HCl shows a singlet of six protons of *N,N*-dimethylammonium residue at 2.81 ppm (A). Upon neutralization with a base, this signal should show an upfield shift. Upon addition of 1.0 equivalent Et_3N, a proton signal of *N,N*-dimethyl residue shifts from 2.81 to 2.15 ppm (B). A similar trend was observed when EDC•HCl was treated with *i*-Pr_2NEt; a signal shows an upfield shift to 2.15 ppm (C). In contrast, upon the addition of pyridine or 2,6-lutidine bases, *N,N*-dimethylammonium residue remained intact as evidenced by no change of proton signal at 2.81 ppm (D, E). Likewise, no neutralization of EDC•HCl was detected in ^1H NMR analysis when collidine base was used (see Supporting Information, page S25). A similar trend was observed in D_2O as NMR solvent, i.e., no neutralization of EDC•HCl was observed using weak bases, such as, pyridine and 2,6-lutidine. In contrast, partial neutralization was observed with Et_3N and *i*-Pr_2NEt (see Supporting Information, pages S26, S27). Based on these results, pyridine assists in retaining the amphiphilic structure of EDC•HCl that further participates in the formation of mixed micelle.

Furthermore, the role of the pyridine base in reaction rate acceleration was confirmed by the control experiment. When **4** and **5** were exposed to the coupling conditions without any base (see Supporting Information, S28), only 39% **6** was observed in 35 minutes, compared to 91% under standard conditions. The control experiment further strengthened the critical role of pyridine in fast amide couplings. The need for PS-750-M for the fast coupling was also verified by kinetic study. The kinetics of a reaction between **1** and **2** in neat water revealed the slower reaction rate in the absence of amphiphile PS-750-M. Only 58% **3** was formed in 12 minutes, while the reaction was completed in 8 minutes in aqueous mixed micellar conditions (see Supporting Information, pages S5, S6).



Scheme 3. Synthesis of **6** and **8** – intermediates for bioactive compounds.

From above studies, we found that the EDC•HCl and PS-750-M potentially self-aggregates to form nanoparticles, the amphiphilic state of the coupling agent is required for efficient couplings, and pyridine as a water-soluble base promotes the reaction efficiency. We next tested the reaction efficiency on multi-gram scale examples. For this study, we first selected two important examples, which afford intermediates of bioactive compounds. Notably, in both examples, coupling partners were used in equimolar amounts that further reduce waste generation. The methodology was first tested to synthesize an intermediate of efaproxiral, a radiation enhancer useful to patients with brain metastases originating from breast cancer.[37] A reaction between amine **4** and unprotected phenolic carboxylic acid **5** affords **6** in excellent yield (90%) in 35 minutes. The obtained product is



Scheme 4. (a-c) Multigram scale reactions, epimerization study, and PMI calculations.

6% more than the 0.5 mmol or one-gram scale reactions (Scheme 3a).[10] Besides, no column chromatography or additional purification procedure was required, and pure product **6** was obtained only by filtration in 98% HPLC purity (for details, see Supporting Information, pages S7-S9). Notably, the phenolic OH group of **5** did not cause any byproduct formation, and the reaction was highly selective to desired amide coupling.

This efficient synthetic technology was then tested on a second multigram scale example that affords an intermediate (**8**) of ACT-462206, a potential candidate for the treatment of insomnia.[38] Notably, the synthesis of **8** was previously reported as problematic in the micellar medium by Lipshutz and co-workers.[21] With our technology, the reaction was completed within 35 minutes, providing an excellent yield, i.e., 91% (Scheme 3b). The product purity verified by HPLC was 98% (for details, see Supporting Information, pages S10-S13).

To further confirm the greenness of our methodology, process mass intensity (PMI) was calculated. The ideal PMI values are 10-40. PMI calculated for the synthesis of **6** and **8** came out to be 17.56 and 26.74, respectively, which meets greener and sustainable processes. Thus, this synthetic protocol offers a greener alternative and generates minimal waste based on scalability, high product purity, and low PMI values.

As previously noted, another challenge in amide couplings is undesired epimerization. However, the mild basic aqueous conditions and the fast reaction rates could result in no epimerization. To further validate this claim, we selected the synthesis of compounds **3** and **11** on multigram scales. These compounds are highly prone to epimerization (Scheme 4). The multigram scale synthesis of **3** results in excellent yield (91%), high product purity (HPLC purity = 97.7%), and no epimerization (Scheme 4a). Likewise, compound **11** was obtained in 93% yield and 98% purity. It was isolated only by simple filtration. No epimerization was observed in this case (Scheme 4b). Low PMI values in these examples also evidence greenness and sustainability (Scheme 4c).

Conclusion. In summary, we report insights into fast amide couplings in water. The amphiphilic nature of EDC•HCl and PS-750-M and the use of the water-soluble organic base are responsible for fast reaction rates. ^{1}H NMR studies support the retention of the amphiphilic nature of EDC•HCl in the presence of a pyridine base. As testified on multi-gram scale examples, the reaction efficiency and selectivity are proven critical aspects of this technology. This aqueous micellar technology has a great potential to be adopted by the pharmaceutical industry. Besides, the effect of the hydrocarbon chain length of EDC•HCl on reaction kinetics will be reported in due course.

ASSOCIATED CONTENT

Supporting Information

Supporting information contains the detailed experimental procedures for multigram scale synthesis of **3**, **6**, **8**, and **11**; analytical data including ^{1}H NMR, ^{13}C NMR, and compounds purity by HPLC analysis; kinetic experiments and epimerization studies.

AUTHOR INFORMATION

Corresponding Author

*sachin.handa@louisville.edu

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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