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Amide and Peptide Bond Formation in Water at Room Temperature

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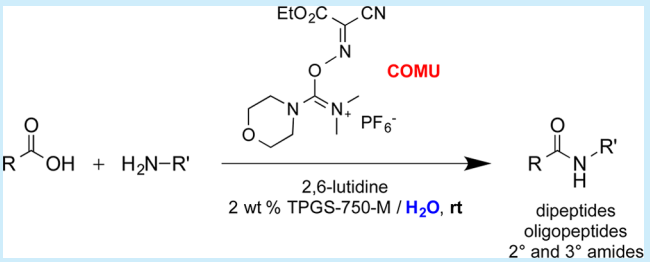
Supporting Information

ABSTRACT: A general and environmentally responsible method for the formation of amide/peptide bonds in an aqueous micellar medium is described. Use of uronium salt (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholinocarbenium hexafluorophosphate (COMU) as a coupling reagent, 2,6-lutidine, and TPGS-750-M represents mild conditions associated with these valuable types of couplings. The aqueous reaction medium is recyclable leading to low E Factors.



Currently, the most common strategies for generating amide bonds involve use of carbodiimide, uronium, or phosphonium reagents (e.g., EDCI, HATU, BOP, etc.), which rely on benzotriazole-based activators such as HOBt and HOAt, in order to increase reaction rates and suppress racemization.[1] Although these methods tend to be highly effective, especially in peptide bond formation, unpredictable autocatalytic decomposition makes reagents containing a benzotriazole scaffold undesirable from the standpoint of safety.[2] More recently, oxime-based activators such as Oxyma **1** (Figure 1) have been put forth as safer choices relative to

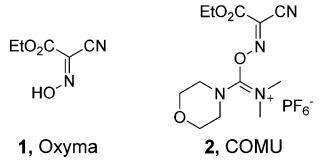


Figure 1. Structures of Oxyma and COMU.

triazole-based reagents.[3] The emerging popularity of Oxyma has led to the development of a highly active Oxyma-derived uronium salt (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)-dimethylaminomorpholino carbenium hexafluorophosphate (COMU), **2**, which is an attractive alternative to current coupling methods, in particular with regard to stereoretention.[4] Another approach to activated ester synthesis of amides comes in the form of boronic acid catalysis (BAC).[5] While BAC does offer the ability to form amide bonds catalytically, high catalyst cost and modest scope may be viewed as limitations of this method.

Notwithstanding these advances in amide/peptide bond constructions (*vide supra*), which lead to increased efficiency and, therefore, less organic waste, a "greener" protocol would likely be welcomed since nearly all coupling reactions are run in polar aprotic solvents (e.g., DMF, DCM).[1] Hence, issues such as safety, waste disposal, health, and lack of recyclability remain.[1] To date, very few methods have been developed which utilize greener alternatives, such as water, as the reaction medium.[6,7]

Our approach toward making a more environmentally sound process for the formation of amide and peptide bonds was to utilize TPGS-750-M (**3**, Figure 2), a surfactant designed by our

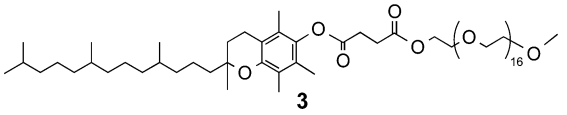


Figure 2. Structure of TPGS-750-M.

group[8] that, upon dissolution in water, spontaneously assembles into nanomicelles. These particles can then be utilized to solvate water-insoluble reagents and act as "nanoreactors" of the appropriate size (50−60 nm) and shape (spheres or worms) in an exclusively aqueous medium, thereby replacing commonly used organic solvents with water. Herein we report a robust general procedure for amide/peptide bond formation in water at ambient temperatures that (a) takes place in high yields; (b) offers considerable scope in terms of coupling partners; (c) shows high protecting group tolerance; (d) occurs with negligible racemization; (e) allows for facile recyclability of the reaction medium; and (f) produces very little organic waste as manifested by low E Factors.

Optimization focused on the coupling of *p*-toluic acid with L-Leu-OEt·HCl. Several tertiary amine bases,[9] concentrations, and surfactants[10] were screened. Eventually, the amide product could be formed in 93% yield after 13 h (Table 1, entry 2) using *N*-methylmorpholine as a base in 4 wt % TPGS-750-M/H_2O. Further screening with pyridyl bases (e.g., 2,6-lutidine,

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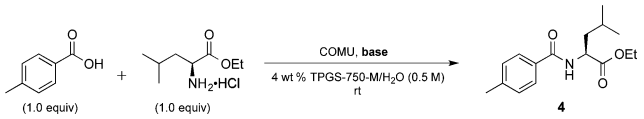
Table 1. Optimized Conditions for Amide Bond Formation



entry	base	time (h)	yield of **4** (%)[a]
1	*N*-methylmorpholine (2.0 equiv)	13	70
2	*N*-methylmorpholine (3.0 equiv)	13	93
3	2,6-lutidine (3.0 equiv)	0.25	>99
4	2,4,6-collidine (3.0 equiv)	4	>99

[a]Yields of isolated products.

2,4,6-collidine) resulted in quantitative yield of the desired product, as well as a substantial increase in reaction rate.

The optimized conditions of the model system leading to amide **4** translated well to the formation of peptide bonds. In fact, a lower surfactant concentration (2 rather than 4 wt %), while maintaining a global concentration in water of 0.5 M, could be employed, affording comparable yields and reaction times (Figure 3). The scope of this reaction proved to be quite broad, where tolerance to several common peptide *N*-terminus protecting groups such as Cbz, Boc, and Fmoc, along with several C-terminus protecting groups such as alkyl, allyl, and benzyl esters, was noted. Especially sterically hindered amino acids such as *N*-Cbz protected α-aminoisobutyric acid (*Z*-Aib-OH) coupled exceptionally well under these aqueous conditions (products **5**–**9**) with no obvious limitations involving coupling partners such as Phe-OMe HCl, **8**, and Pro-OBn HCl, **9**. Similarly, coupling to form Boc-Phg-Phg-OMe, **15**, was achieved in high yield (88%) after 4 h. Lipophilic product Fmoc-Val-Leu-OEt, **20**, was also formed in high yield (96%) under the optimized conditions, while the same reaction yielded only 53% product in the absence of surfactant, indicative of the role of TPGS-750-M in this technology. Fmoc deprotection, typically occurring under basic conditions,[11] was not observed. Successful couplings were also achieved for several important oligopeptide natural product intermediates including *Chlamydocin* precursor **21**,[12] and *Streptocidin C* precursor **23**[13] with comparable yields and reaction times analogous to those seen previously.

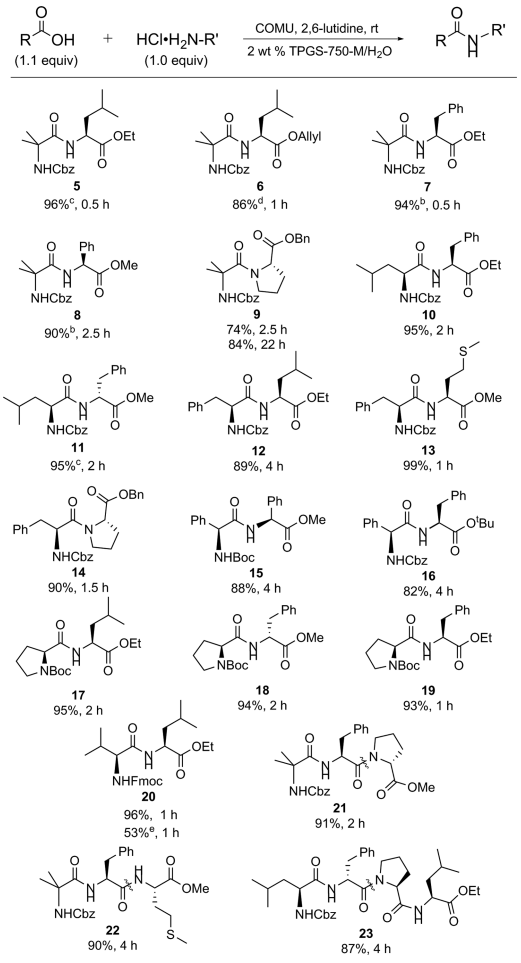
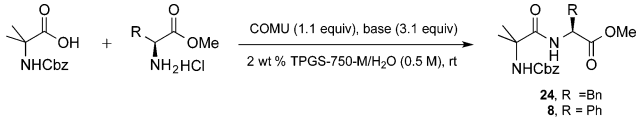


Figure 3. Peptide coupling products in TPGS-750-M/H_2O.[a] [a] Conditions (by order of addition): carboxylic acid (1.1 equiv), 2 wt % TPGS-750-M/H_2O (0.5 M), 2,6-lutidine (3.1 equiv), ammonium chloride (1.0 equiv), COMU (1.1 equiv). [b] Yields of isolated products are reported. [c] 5.0 mmol scale. [d] From ammonium tosylate salt of Leu-OAllyl. [e] Isolated yield in the absence of TPGS-750-M.

Use of 2,6-lutidine was originally pursued for its known ability as an effective base for amide couplings, while reducing the extent of epimerization/racemization due to steric bulk surrounding its basic site.[14] Under our standard conditions leading to products *Z*-Aib-Phg-OMe (**8**) and *Z*-Aib-Phe-OMe (**23**), the chiral integrity of these materials was maintained (Table 2). Interestingly, no epimerization was observed even when pyridine was used in its place. The low occurrence of epimerization is likely due to the mildly acidic to neutral conditions (pH = 6–7) involved, which is known to be insufficient for deprotonation even of the α-proton in residues such as phenylglycine.[15] When stronger bases such as DBU or DABCO were employed, a more alkaline reaction mixture resulted (pH = 9), and no detectable coupling product was observed. Furthermore, it was found that either increasing or decreasing the pH of a reaction where 2,6-lutidine is used as the base leads as well to lower overall yields.[16]

Table 2. Study of Epimerization under Standard Conditions



entry	product	base	time (h)	yield[a]	ee[b]
1	**24**	2,6-lutidine	0.5	93%	>99%
2	**24**	pyridine	0.5	94%	>99%
3	**8**	2,6-lutidine	2.5	90%	>99%

[a]Isolated yield. [b]Determined by HPLC analysis.

The correlation between pH and reaction yield may simply be due to amino acid solubility. Most coupling partners will dissolve readily into the aqueous surfactant solution containing 2,6-lutidine, which may solubilize each component as its corresponding carboxylate or ammonium salt. The reaction then could take place in water yielding the now insoluble carboxamide product, which may be solubilized within the micelles present in solution. This notion is supported in that these reactions do occur to varying extents in the absence of surfactant (i.e., on water), albeit, on occasion, with considerably lower product yields (e.g., Figure 3, product **20**).[17]

In order to further explore the generality of this method, products resulting from amide bond formation were generated,

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as illustrated in Figure 4. As expected, simple alkanoic acid-alkylamine-derived amides (products **25**–**27**), including

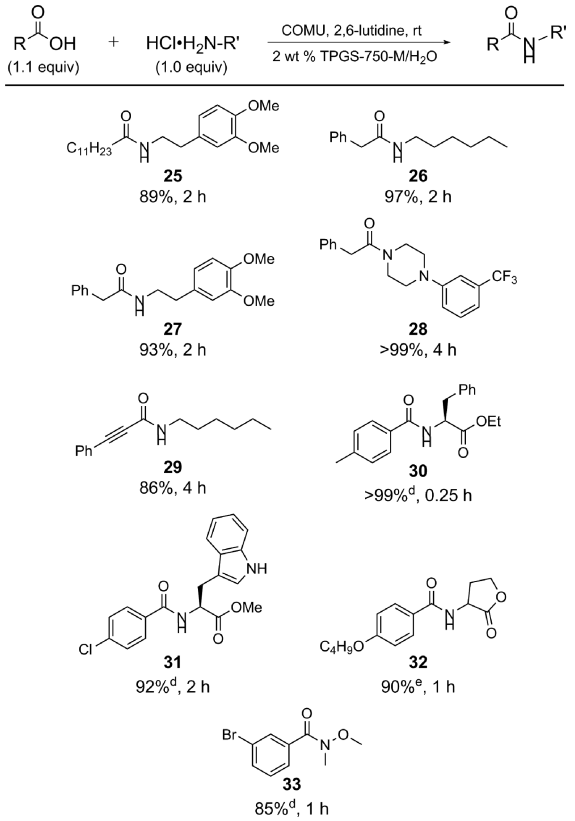
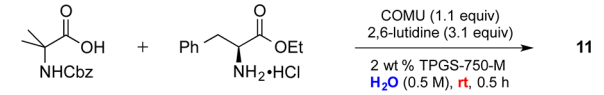


Figure 4. Amide products formed in TPGS-750-M/H_2O.[a−c] [a] Conditions (by order of addition): carboxylic acid (1.1 equiv), 2 wt % TPGS-750-M/H_2O (0.5 M), 2,6-lutidine (3.1 equiv), amine (1.0 equiv), COMU (1.1 equiv). [b] Yields of isolated products are reported. [c] 1.0 M. [d] From ammonium chloride salt of the amine. [e] From α-amino-γ-butyrolactone hydrobromide; reaction temperature: 45 °C.

secondary amines such as piperazine in **28**, are produced in high yields without modification to the general procedure. Amides from conjugated carboxylic acids can be produced as well under standard conditions including amide (**29**), as well as substituted benzamides (**30**–**33**). Reaction of *p*-chlorobenzoic acid with L-Trp-OMe·HCl afforded Benzotript[18] methyl ester **31** in high yield (92%) after only 2 h, while the unprotected indole nitrogen of tryptophan showed no influence on the coupling. More electron-rich benzoic acids such as 4-*n*-butoxybenzoic acid offered an unexpected challenge en route to racemic **32**, where only a 36% yield was obtained after 16 h, while the undesired Oxyma-activated ester was isolated in 57% yield. To circumvent the reduced electrophilicity of this activated ester, the reaction was heated to 45 °C to afford in 90% yield product **32**, with only trace amounts of the activated intermediate remaining. In addition, adduct **33** was isolated in 85% yield after only 1 h from the condensation of *m*-bromobenzoic acid and the *N*,*O*-dimethylhydroxylamine hydrochloride, this being the first reported synthesis of a Weinreb amide[19] in the absence of organic solvent.

An added benefit to performing these peptide couplings is realized given the high solubility of the urea and oxime byproducts of COMU in water. Extraction with a minimum volume of organic solvent (e.g., *i*-PrOAc) is sufficient in most cases to remove the coupling product from the reaction mixture, thereby leading to an exceptionally low E Factor[20] of 2.8. The remaining aqueous mixture can then be recycled at least four additional times without change in yield (Scheme 1).

Scheme 1. Recycling and E Factors[a]



initial reaction	1st recycle[b]	2nd recycle	3rd recycle	4th recycle
92%	95%	>99%	96%	>99%

E Factors (single run; *i*-PrOAc extraction)
organic solvent: 2.8[c]
organic solvent + water: 7.8

[a]Yields of isolated products are reported. [b]Recycle performed by introducing all components to the aqueous reaction mixture from the previous reaction, after extraction with MTBE. [c]96% isolated yield.

Impurities found in the organic extracts are easily removed using typical acid/base washes followed by filtration through a silica plug to yield pure peptides, eliminating the need for further purification in most cases.[21]

In summary, we have developed a protocol utilizing peptide coupling reagent COMU (**2**) and designer surfactant TPGS-750-M (**3**) in water as a general method for generating peptide and amide bonds. This methodology successfully addresses several environmental issues, including: replacement of organic solvent with very small amounts of water as the reaction medium, avoidance of benzotriazole activators, recycling of the aqueous surfactant solution, and minimal use of organic solvents for workup/purification. Further studies on this coupling approach that feature multiple bond-formations in aqueous nanomicelles in a single pot will be the subject of a future report from these laboratories.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01812.

Experimental procedures, characterization data, ^1H and ^{13}C NMR spectra, and HPLC chromatograms (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557. (b) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827.
(2) Wehrstedt, K. D.; Wandrey, P. A.; Heitkamp, D. *J. Hazard. Mater.* **2005**, *126*, 1.

DOI: 10.1021/acs.orglett.5b01812
Org. Lett. XXXX, XXX, XXX−XXX

(3) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem. - Eur. J.* **2009**, *15*, 9394.
(4) (a) El-Faham, A.; Subirós-Funosas, R.; Prohens, R.; Albericio, F. *Chem. - Eur. J.* **2009**, *15*, 9404. (b) El-Faham, A.; Albericio, F. *J. Pept. Sci.* **2010**, *16*, 6. (c) Subirós-Funosas, R.; Nieto-Rodriguez, L.; Jensen, K. J.; Albericio, F. *J. Pept. Sci.* **2013**, *19*, 408. (d) Cherkupally, P.; Acosta, G. A.; Nieto-Rodriguez, L.; Spengler, J.; Rodriguez, H.; Khattab, S. N.; El-Faham, A.; Shamis, M.; Luxembourg, Y.; Prohens, R.; Subiros-Funosas, R.; Albericio, F. *Eur. J. Org. Chem.* **2013**, *2013*, 6372.
(5) (a) Gernigon, N.; Al-Zoubi, R. A.; Hall, D. G. *J. Org. Chem.* **2012**, *77*, 8386. (b) Maki, T.; Ishihara, K.; Yamamoto, H. *Tetrahedron* **2007**, *63*, 8645. (c) Ishihara, K.; Ohara, S.; Yamamoto, H. *J. Org. Chem.* **1996**, *61*, 4196.
(6) MacMillan, D. S.; Murray, J.; Sneddon, H. F.; Jamieson, C.; Watson, A. J. B. *Green Chem.* **2013**, *15*, 596.
(7) (a) Wang, Q.; Wang, Y.; Kurosu, M. *Org. Lett.* **2012**, *14*, 3372. (b) Hojo, K.; Ichikawa, H.; Onishi, M.; Fukumori, Y.; Kawasaki, K. *J. Pept. Sci.* **2011**, *17*, 487. (c) Hojo, K.; Hara, A.; Kitai, H.; Onishi, M.; Ichikawa, H.; Fukumori, Y.; Kawasaki, K. *Chem. Cent. J.* **2011**, *5*, 49. (d) Hojo, K.; Ichikawa, H.; Fukumori, Y.; Kawasaki, K. *Int. J. Pept. Res. Ther.* **2008**, *14*, 373. (e) Hojo, K.; Ichikawa, H.; Maeda, M.; Kida, S.; Fukumori, Y.; Kawasaki, K. *J. Pept. Sci.* **2007**, *13*, 493. (f) Galanis, A. S.; Albericio, F.; Grøtli, M. *Org. Lett.* **2009**, *11*, 4488. (g) Salam, S. M. A.; Kagawa, K.; Kawashiro, K. *Tetrahedron: Asymmetry* **2006**, *17*, 22. (h) Kunishima, M.; Imada, H.; Kikuchi, K.; Hioki, K.; Nishida, J.; Tani, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 7254. (i) De Marco, R.; Tolomelli, A.; Greco, A.; Gentilucci, L. *ACS Sustainable Chem. Eng.* **2013**, *1*, 566.
(8) (a) Lipshutz, B. H.; Ghorai, S.; Abela, A. R.; Moser, R.; Nishikata, T.; Duplais, C.; Krasovskiy, A. *J. Org. Chem.* **2011**, *76*, 4379. (b) Lipshutz, B. H.; Ghorai, S. *Aldrichimica Acta* **2008**, *41*, 59.
(9) See Supporting Information for additional base screening data.
(10) See Supporting Information for surfactant screening data.
(11) Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*, 4th ed.; John Wiley & Sons, Inc.: NJ, 2007; Chapter 7.
(12) Newkirk, T. L.; Bowers, A. A.; Williams, R. W. *Nat. Prod. Rep.* **2009**, *26*, 1293.
(13) Gebhardt, K.; Pukall, R.; Fiedler, H.-P. *J. Antibiot.* **2001**, *54*, 428.
(14) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695. (b) Brieke, C.; Cryle, M. J. *Org. Lett.* **2014**, *16*, 2454.
(15) (a) Smith, G. G.; Sivakua, T. *J. Org. Chem.* **1983**, *48*, 627. (b) Stroud, E. D.; Fife, D. J.; Smith, G. G. *J. Org. Chem.* **1983**, *48*, 5368.
(16) See Supporting Information for pH adjustment study
(17) See Supporting Information for additional examples comparing reactions conducted in the absence of surfactant.
(18) Hahne, W. F.; Jensen, R. T.; Lemp, G. F.; Gardner, J. D. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, *78*, 6304.
(19) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.
(20) (a) Sheldon, R. A. *Chem. Ind.* **1992**, 903. (b) Lipshutz, B. H.; Ghorai, S. *Green Chem.* **2014**, *16*, 3660. (c) Lipshutz, B. H.; Isley, N. A.; Fennewald, J. C.; Slack, E. D. *Angew. Chem., Int. Ed.* **2013**, *52*, 10911.
(21) The only observed products of this reaction are the desired peptide/amide, byproducts from COMU decomposition, and, in some cases, trace amounts of the activated ester. Incomplete reactions show predominately unreacted SM. Addition of extra COMU is usually not an option due to the slurry-like property of the final reaction mixture.

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