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Microwave irradiation and COMU: a potent combination for solid-phase peptide synthesis

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ABSTRACT

Here we demonstrate the compatibility of Oxyma-based uronium-type coupling reagent COMU with microwave-assisted peptide synthesizers. Consistent with previous reports, COMU displayed higher efficiency than benzotriazole classical immonium salts HATU and HBTU in the demanding synthesis of the Aib derivative of Leu-Enkephalin pentapeptide and did not yield Oxyma-based byproducts. Thus, the combination of microwave irradiation and COMU resulted in a similar performance in considerably shorter time to that achieved by manual synthesis.

Uronium-type coupling reagent 1-[(1-(cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholinomethylene)] methanaminium hexafluorophosphate, **1** (COMU, Fig. 1) is a more efficient alternative to classical benzotriazole immonium salts in terms of racemization suppression, coupling effectiveness, stability, and solubility.[1] In addition to the morpholino moiety, this superiority is attributed to the introduction of ethyl 2-cyano-2-(hydroxyimino)acetate, **2** (Oxyma, Fig. 1) in its structure. Oxyma displays high control of optical purity and extension of coupling in demanding sequences.[2]

Both Oxyma and its related uronium salt can be handled with a considerably lower risk than its explosive benzotriazole counterparts, as determined by calorimetry techniques. However, the thermal stability of Oxyma is relatively low.[1,2] Nonetheless, no incident has been reported during extreme coupling stability assays, carried out using microwave irradiation at 80 °C.[2]

Previous studies examined the stability of Oxyma toward the nucleophilic N-terminus of the growing peptide chain in extreme experiments. To enhance the likelihood of this side reaction, these experiments used a high excess of the additive in the absence of carbodiimide and Fmoc-AA-OH.[2] In the assay conducted at room temperature overnight, no Oxyma-based byproduct was found. In contrast, a similar experiment with microwave irradiation gave rise to some of these undesired byproducts (Fig. 2). In that demanding experiment, the main byproduct resulted from the formylation of the amino function (**3**, 47.9%), attack of the amino group on the carboxylate (**4**) and the carbonyl of the oxime (**5**), which was also hydrolyzed (**6**). In all the cases, impurities were detected in the range 2–4%.[2]

In order to unequivocally determine the compatibility of the Oxyma-derived coupling reagents with microwave-assisted solid-phase peptide synthesis (SPPS), we compared the performance of COMU with that of classical immonium salts HBTU **7** and HATU **8** in the synthesis of a true peptide model conducted in an automated peptide synthesizer with microwave irradiation (Fig. 3).[3]

Since its first appearance in 1986, microwave irradiation has been established as a highly useful tool for solid-phase organic

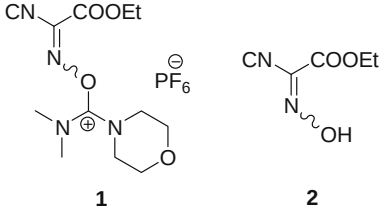

Figure 1. Structure of COMU and Oxyma.

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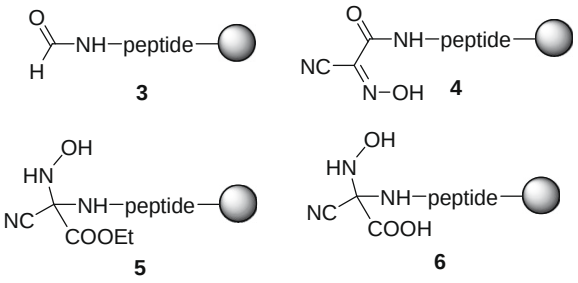


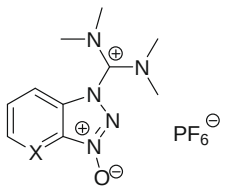
Figure 2. Side products obtained by reaction of Oxyma with a peptide in the absence of carbodiimide and Fmoc-AA-OH.

synthesis, both in academy and in industry, by increasing product yield and purity and reducing reaction times, in combination with thermal effects.[4–6] However, few studies have reported the application of this approach in the field of peptide synthesis since the early report of Wang and co-workers in 1991.[7] This low general acceptance could be partly attributed to the widespread belief that the acceleration rate of coupling and deprotection steps also leads to instability of coupling reagents and an increase in typical side reactions, such as racemization and aspartimide formation. Nevertheless, recent studies have confirmed that these drawbacks can be controlled with an adequate choice of reaction conditions, and also that resin loading is critical for optimal microwave-assisted peptide synthesis.[8,9] The availability of peptide synthesizers equipped with microwave irradiation has undoubtedly contributed to the sudden increasing popularity of this tool in SPPS, which has proved compatible with both Fmoc and Boc approaches in the assembly of difficult sequences including phospho and glycopeptides, cymantrene-peptide bioconjugates, and cyclotides.[10–16]

One of the key advantages offered by microwave irradiation (which cannot be explained by thermal effects) derives from the dipolar polarization mechanism, which provides extra energy for the rotation of the molecules with a dipolar moment, such as peptides.[8,12] This effect is highly relevant during the elongation of long and hydrophobic peptides, since the polar peptide backbone constantly aligns with the electric field, thereby disrupting chain aggregation. Thus, peptides of up to 30-mer can be prepared easily overnight.[8]

To further demonstrate the strong performance of microwave-assisted SPPS in challenging sequences, here we report the assembly of the highly demanding Aib-analog of Leu-enkephalin pentapeptide (H-Tyr-Aib-Aib-Phe-Leu-NH_2) by means of a Liberty CEM Microwave Peptide Synthesizer. The Aib-Aib sterically hindered coupling is an excellent scenario to compare the performance of COMU **1** and benzotriazole-based HBTU **7** and HATU **8**,[17] and also to study the formation of the side reactions associated with the presence of Oxyma during the coupling.

The synthesis was carried out in a 0.1 mmol scale of H-RinkAmide-ChemMatrix resin (loading = 0.42 mmol/g), a totally PEG-based solid support that shows good swelling properties in both polar and non-polar solvents.[18–21] A conditioning protocol was followed to remove any remaining base from the commercial resin before its transfer with DMF into a suitable-sized polypropylene vial. Solutions of reagents for coupling and deprotection steps were placed in appropriate vessels (Table 1).



HBTU, X=CH (**7**)
HATU, X=N (**8**)

Figure 3. Structure of benzotriazole-based immonium salts HBTU and HATU.

Short single couplings were applied to introduce the five residues (6 min at 80 °C), using 1 mmol of Fmoc-aminoacid (0.6 mmol for Aib, in order to enhance relative differences), 1 equiv of coupling reagent, and 2 equiv of base, relative to amino acid.[22] Deprotection steps were conducted with a solution of piperidine in DMF (Table 1) at 75 °C (3 min × 2). Once automated synthesis of the pentapeptide was completed (proceeding safely with COMU **1** under the abovementioned conditions), the final cleavage from the resin was accomplished with a 2-h treatment with 90% TFA/10% H_2O at room temperature. After precipitation with cold Et_2O and lyophilization, the samples were analyzed by reverse-phase HPLC-PDA and HPLC–MS. An example of the crude mixtures obtained is shown for the synthesis with COMU **1** (Fig. 4).

Two main compounds were observed in the crude mixtures: the desired pentapeptides (H-Tyr-Aib-Aib-Phe-Leu-NH_2) and des-Aib (H-Tyr-Aib-Phe-Leu-NH_2). Only traces of other possible deletion peptides such as des-Tyr (H-Aib-Aib-Phe-Leu-NH_2) and des-Aib,Tyr (H-Aib-Phe-Leu-NH_2) were detected. Some minor peaks were observed and these were not related to the coupling reagents or deletion peptides. The relative percentages of pentapeptide/des-Aib for each coupling reagent are shown in Table 2. The highest percentage of pentapeptide was obtained with COMU **1** (92%). This performance was better than that observed for HATU **8** (79%) or HBTU **7**, (23%). The manual synthesis with COMU at room temperature afforded a higher percentage of the Aib-enkephalin pentapeptide (99.7%);[1] however, coupling times were much longer (60 min, double coupling) than those in the abovementioned microwave synthesis (6 min). These results therefore highlight the enhanced efficiency of the method presented.

In our experiment with COMU, carried out in more realistic conditions than those abovementioned, no byproducts (**4–6**) related to

Table 1
Reagents and solvents used for microwave-assisted Fmoc-SPPS

	Reagent	Concentration	Solvent
Amino acid	Fmoc-AA-OH	0.2 M	DMF
Activator	HBTU/HATU/COMU	0.5 M	DMF
Base	DIEA	2 M	DMF
Deprotection	Piperidine	20% v/v	DMF

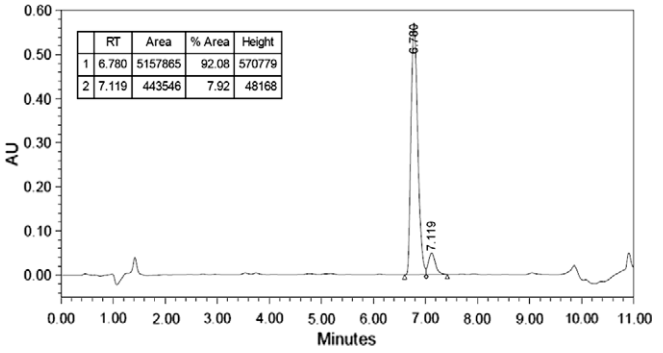


Figure 4. HPLC analysis of the crude obtained from the experiment using COMU. HPLC conditions: Waters SunFire C18 column (3.5 μm, 4.6 × 100 mm); linear gradient 20–30% of 0.036% TFA in CH_3CN/0.045% TFA in H_2O over 8 min; flow = 1.0 ml/min; detection at 220 nm; t_R (penta) = 6.780 min, $[M+H]^+$ = 611.35; t_R (des-Aib) = 7.119 min, $[M+H]^+$ = 526.30.

Table 2
Relative percentages of pentapeptide/des-Aib with various coupling reagents

Coupling reagent	Pentapeptide (%)	Des-Aib (%)	Yield (%)
COMU (**1**)	92.1	7.9	88.5
HBTU (**7**)	23.1	76.9	20.5
HATU (**8**)	79.5	20.5	76.0

the Oxyma moiety contained in the coupling reagent were detected. However, traces of guanidylation were found at the dipeptide stage[23] with each coupling reagent, during the introduction of the first Aib residue.

In summary, here we demonstrate the compatibility of Oxyma-based COMU **1** with microwave-assisted SPPS, during the automated synthesis of Aib-enkephalin pentapeptide. The Oxyma moiety contained in COMU also displayed high nucleophilic stability to the N-terminus of the growing peptide chain, as shown by the absence of Oxyma-based byproducts. Consistent with the previous reports, COMU showed better performance than classical immonium salts HATU and HBTU, which yielded lower percentages of the desired pentapeptide. On the basis of our findings, we conclude that the combination of COMU and microwave irradiation is an efficient approach for SPPS, as it yielded more than 90% of Aib-enkephalin, a highly demanding pentapeptide, in considerably less time than the conventional manual synthesis.

Acknowledgments

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References and notes

1. El-Faham, A.; Subirós-Funosas, R.; Prohens, R.; Albericio, F. *Chem. Eur. J.* in press. doi:10.1002/chem.200900615.
2. Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem. Eur.J.* in press. doi:10.1002/chem.200900614.
3. Carpino, L. A.; Imazumi, H.; El-Faham, A.; Ferrer, F. J.; Zhang, C.; Lee, Y.; Foxman, B. M.; Henklein, P.; Hanay, C.; Mügge, C.; Wenschuh, H.; Klose, J.; Beyermann, M.; Bienert, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 441–445.
4. Giguere, R. J.; Bray, T. L.; Duncan, S. M.; Majetich, G. *Tetrahedron Lett.* **1986**, *27*, 4945–4948.
5. Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. *Tetrahedron* **2001**, *57*, 9225–9283.
6. de la Hoz, A.; Diaz-Ortiz, A.; Moreno, A. *Chem. Soc. Rev.* **2005**, *34*, 164–178.
7. Chen, S. T.; Chiou, S. H.; Wang, K. T. *J. Chin. Chem. Soc.* **1991**, *38*, 85–91.
8. Palasek, S. A.; Cox, Z. J.; Collins, J. M. *J. Pept. Sci.* **2007**, *13*, 143–148.
9. Coantic, S.; Subra, G.; Martinez, J. *Int. J. Pept. Res. Ther.* **2008**, *14*, 143–147.
10. Bacsa, B.; Desai, B.; Dibo, G.; Kappe, C. O. *J. Pept. Sci.* **2006**, *12*, 633–638.
11. Katrizky, A. R.; Khashab, N. M.; Yoshioka, M.; Haase, D. N.; Wilson, K. R.; Johnson, J. V.; Chung, A.; Haskell-Luevano, C. *Chem. Biol. Drug Des.* **2007**, *70*, 465–468.
12. Cemazar, M.; Craik, D. J. *J. Pept. Sci.* **2008**, *14*, 683–689.
13. Brandt, M.; Gammeltoft, S.; Jensen, K. J. *Int. J. Pept. Res. Ther.* **2006**, *12*, 349–357.
14. Nagaike, F.; Onuma, Y.; Kanazawa, C.; Hojo, H.; Ueki, A.; Nakahara, Y.; Nakahara, Y. *Org. Lett.* **2006**, *8*, 4465–4468.
15. Peindy N'dongo, H. W.; Ott, I.; Gust, R.; Schatzschneider, U. *J. Organomet. Chem.* **2009**, *694*, 823–827.
16. Leta Aboye, T.; Clark, R. J.; Craik, D. J.; Goransson, U. *ChemBioChem* **2008**, *9*, 103–113.
17. El-Faham, A.; Albericio, J. *Org. Chem.* **2008**, *73*, 2731–2737.
18. Garcia-Martin, F.; Quintanar-Audelo, M.; Garcia-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Côté, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213–220.
19. García-Martín, F.; White, P.; Steinauer, R.; Côté, S.; Tulla- Puche, J.; Albericio, F. *Biopolymers (Pept. Sci.)* **2006**, *84*, 566–575.
20. de la Torre, B. G.; Jakab, A.; Andreu, D. *Int. J. Pept. Res. Ther.* **2007**, *13*, 265–270.
21. Frutos, S.; Tulla-Puche, J.; Albericio, F.; Giralt, E. *Int. J. Pept. Res. Ther.* **2007**, *13*, 221–227.
22. Common microwave protocols were used, showing the compatibility of COMU, HATU, and HBTU with those protocols.
23. Albericio, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678–9683.