



# **Amine Activation: “Inverse” Dipeptide Synthesis and Amide Function Formation through Activated Amino Compounds**

Eleonora Tosi, Jean-Marc Campagne, Renata Marcia de Figueiredo

## **► To cite this version:**

Eleonora Tosi, Jean-Marc Campagne, Renata Marcia de Figueiredo. Amine Activation: “Inverse” Dipeptide Synthesis and Amide Function Formation through Activated Amino Compounds. *Journal of Organic Chemistry*, 2022, 87 (18), pp.12148-12163. <10.1021/acs.joc.2c01288>. <hal-03791611>

**HAL Id: hal-03791611**

**<https://hal.science/hal-03791611v1>**

Submitted on 29 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

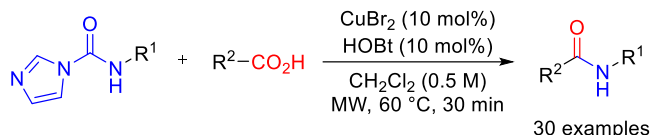


HAL Authorization

# Amine Activation: « Inverse » Dipeptide Synthesis and Amide Function Formation through Activated Amino Compounds

Eleonora Tosi, Jean-Marc Campagne,\* and Renata Marcia de Figueiredo\*

**ABSTRACT:** A copper(II)/HOBt-catalyzed procedure for the synthesis of dipeptides and “general” amides has been developed using microwave irradiation to considerably hasten the reaction. As an alternative of using traditional carboxylic acids activation, the method relies on the use of N-acyl imidazoles as activated amino partners. By doing so, a nonconventional way to reach dipeptides and amides has been proposed through the challenging and less studied N → C direction synthesis. A series of dipeptides and “general” amides has been successfully synthesized and the applicability of the method has been illustrated in gram-scale syntheses. The mild reaction conditions proposed are completely adequate for couplings in the presence of sensitive amino acids, affording the products without detectable racemization. Furthermore, experimental observations prompted us to propose a plausible reaction pathway for the couplings.



R<sup>1</sup>, R<sup>2</sup> = Alk, Ar, aminoacid residue  
compatible with Fmoc, Boc, Cbz protecting groups for amino acids

♦ no base ♦ no detectable racemization ♦ non-conventional amine activation

## INTRODUCTION

Amides are one of the most important functional groups in organic, medicinal, and bioorganic chemistry. Regardless of its vital role in sustaining life (the backbone of proteins), amides are also present in several natural products, polymers, and materials. Besides, they are one of the utmost flourishing entities in modern drug-like compounds<sup>1</sup> being present in around 25% of all pharmaceuticals currently in the market.<sup>2</sup> The omnipresence of amides in small molecule pharmaceuticals and the potential of peptide-based therapeutics in a large panel of diseases<sup>3</sup> expose a real need for novel methodologies to construct the amide function. Moreover, such developments might be selective, mild, and practical to fit the peptide chemistry requirements.

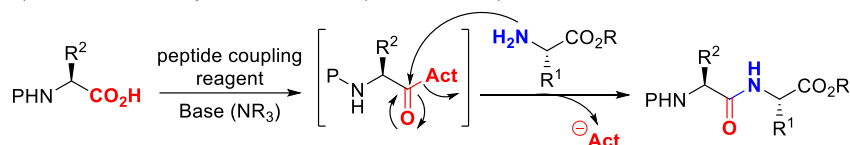
The biosynthesis (i.e., ribosomal synthesis) of peptides and proteins takes place from the N → C direction, whereas they are chemically constructed from the C → N direction either by means of classical solution synthesis or solid-phase peptide synthesis (SPPS). The traditional way to synthesize amides relies on the pre- or in situ activation of the carboxylic acid partner (i.e., the transformation of the OH group into a better leaving group) in the presence of both a base and an overstoichiometric amount of a coupling reagent (or a combination of reagents). Then, an SN<sub>2</sub>t reaction of the in situ activated carboxylic acid with the free-amine function of a second amino acid partner affords the required amide function (Scheme 1, eq (a)). Thanks to a wide-ranging and well-known library of such coupling reagents,<sup>4</sup> this method is very efficient and widespread used even though wasteful and generally costly. Also, amide

synthesis through classical carboxylic acid activation is often connected to intrinsic limitations associated with the nature of coupling reagents such as hazardous properties (e.g., potential explosive benzotriazole-based coupling reagents) and epimerization through enolization of sensitive amino acids (histidine and cysteine). Particularly, these conditions also impose both (i) the synthesis of peptides from the C → N direction to suppress (or at least minimize) oxazolone<sup>5</sup> formation and (ii) the one-by-one amino acid introduction into the growing peptide chain via the preactivation of a single carbamate N-protected amino acid each time. Indeed, coupling reagents-mediated peptide synthesis from the N → C direction would imply the C-terminal activation of a peptide segment that is highly prone to epimerization (with the notable exception of proline and glycine) through oxazolone formation. Such an issue will consequently afford a very complex and unextractable mixture of diastereomers.

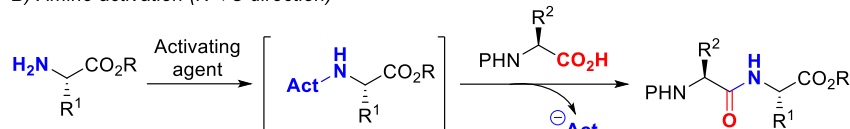
To tackle these challenges, novel nonclassical approaches for amide formation have been developed.<sup>6</sup> However, most of these strategies are not always compatible with peptides and, when applied, require the prior modification of at least one amino acid partner. In this context, we have been interested in developing new strategies for amide synthesis through amine activation instead of conventional carboxylic acid activation. Inspired by the natural way (i.e., ribosomal synthesis) of synthesizing peptides and proteins, the idea was to develop a chemical, in solution, method to reach amides through the N

## Scheme 1. (A) Traditional Peptide Synthesis and (B) Concept of Synthesis through Amine Activation

A) Traditional carboxylic acid activation (C→N direction)

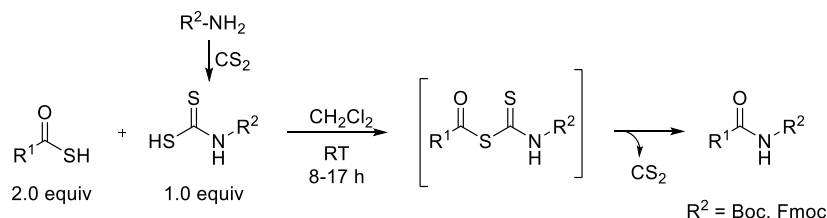


B) Amine activation (N→C direction)

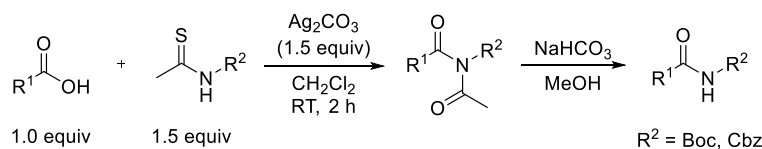


## Scheme 2. Synthetic Strategies Previously Reported for the Construction of Amides through Amine Activation

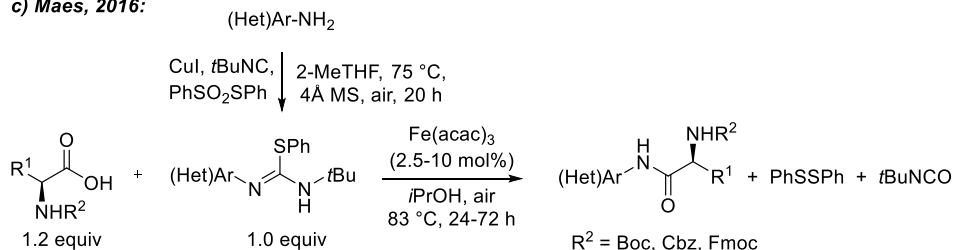
a) Houghten and Yu, 2013:



b) Hutton, 2014:



c) Maes, 2016:

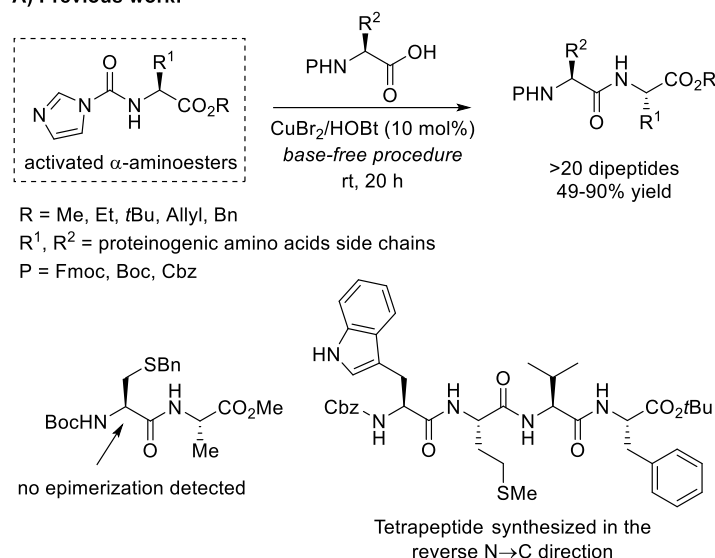


→ C direction. While countless methods of traditional carboxylic acid activation<sup>7</sup> with coupling reagents<sup>4</sup> (C → N direction; Scheme 1, eq (a)) have been developed, very little has been proposed through inverse N → C direction (Scheme 1, eq (b)). In 2013, Houghten and Yu described the combination of thioacids and dithiocarbamate-terminal amines toward amides and peptides (Scheme 2a).<sup>8</sup> Soon after, Hutton proposed a silver-promoted approach for inverse peptide synthesis by merging carboxylic acids and thioamides (Scheme 2b).<sup>9</sup> In 2017, Maes published a method for amide synthesis via the combination of isothioureas and carboxylic acids under iron catalysis (Scheme 2c).<sup>10</sup> In this context, we have described in 2014 preliminary results on inverse peptide synthesis using activated α-aminoesters.<sup>11,12</sup> Thanks to their readily availability and stability, the synthesis of several dipeptides and tripeptides and

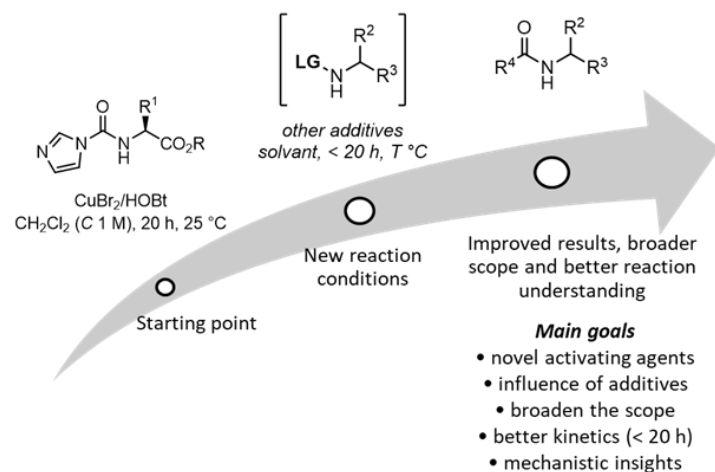
one tetrapeptide from the N → C direction was possible in a racemization-free manner even when sensitive amino acids (i.e., cysteine) were used (Scheme 3A). With the objective of giving a full account of this work and also proposing new conditions able to improve both reaction kinetics and scope, we describe therein our detailed work within this subject (Scheme 3B). We will focus on several points: (i) to describe the search for other compatible and efficient activating agents, (ii) to explore the influence of different additives, (iii) to broaden the previous scope on peptide synthesis and to extend it to “general” amides, (iv) to study the possibility of scaling up the more practical reaction conditions toward both dipeptide and “general” amide syntheses, (v) to evaluate racemization

**Scheme 3. (A) Previous Work on Amine Activation via CDI-Activated  $\alpha$ -Aminoesters. (B) Current Full Account on Our Initial Work and New Targeted Objectives**

**A) Previous work:**



**B) This work:**



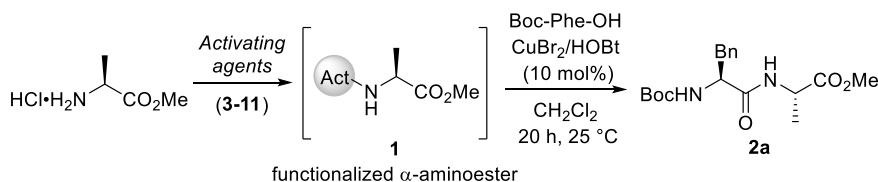
issues, (vi) to determine residual Cu traces on a dipeptide synthesis, (vii) to improve the kinetics, and (viii) to obtain some insights into the coupling mechanism.

## RESULTS AND DISCUSSION

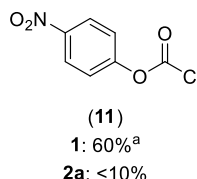
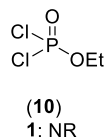
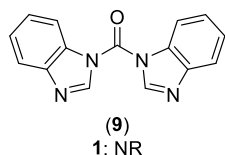
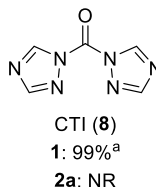
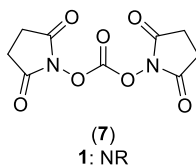
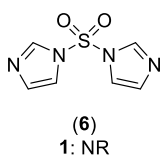
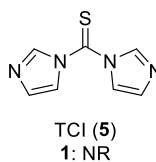
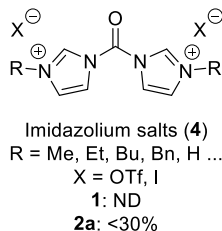
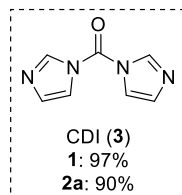
**Reaction Conditions Optimization.** *Activating Agent Choice for the Amine Function.* In our previous study,<sup>11</sup> the amine activation of a first amino ester with N,N'-carbonyldiimidazole (CDI, **3**) gave stable  $\alpha$ -aminoesters **1** that in combination with CuBr<sub>2</sub> and HOBt (10 mol % each) as additives afforded the best results during the synthesis of several dipeptides, including Boc-Phe-Ala-OMe **2a** (90%). Nevertheless, and despite the practical and mild aspects of the devised operational conditions, the reaction kinetic was still slow to be practically applied to peptide chemistry (20 h were needed to reach good yields). To reduce the reaction times, various activating agents were thus investigated (compounds **3–11**, Scheme 4). When using imidazo-

lium salts **4** that are known to be efficient in amide, carbamate, thiocarbamate, and urea syntheses,<sup>13,14</sup> only low yields (<30%) for dipeptide **2a** were observed.<sup>15</sup> In the presence of commercially available compounds **5–7** and carbonyl dibenzimidazole derivative **9** (prepared from benzimidazole and diphosgene), the formation of functionalized  $\alpha$ -aminoesters **1** did not proceed efficiently. Phosphorous-based activating agents, such as ethyl phosphorodichloridate (**10**), were ineffective. 1,1'-Carbonyl-di-(1,2,4-triazole) (**8**) and *p*-nitrophenyl chloroformate (**11**) were able to afford the corresponding activated  $\alpha$ -aminoester **1** (NMR yield of 99 and 60%, respectively); however, the subsequent coupling reaction with Boc-Phe-OH did not efficiently provide dipeptide **2a** (no reaction and <10% yield with **10** and **11**, respectively). Thus, in our hands, CDI displayed (i) the best results in terms of stability and yields for the synthesis of functionalized  $\alpha$ -aminoester **1a** and (ii) the unsurpassed yield

#### Scheme 4. Activating Agent Trials for the Preparation of Functionalized $\alpha$ -Aminoesters **1** and Dipeptide **2a**



Activating agents tried:



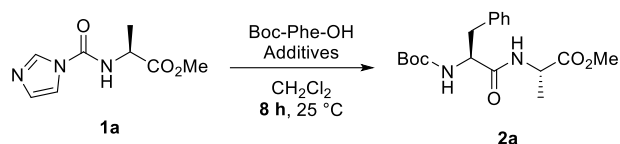
<sup>a</sup> Crude <sup>1</sup>H NMR yield. ND = Not Determined; reactions were performed in a sequential one-pot strategy without isolation of **1**. NR = No Reaction; the activation step failed and **1** could be neither isolated nor observed by <sup>1</sup>H NMR of the crude reaction mixture.

of 90% for dipeptide **2a**. It remains thus so far the N-activating agent of choice for the coupling reactions.

**Solvents and Additives Survey.** To gain a better understanding of the parameters governing the outcomes of this transformation and, ideally, to improve the reaction kinetics, we decided to survey the reaction conditions. First, a full range of solvents has been tested (for the complete screening, see the SI). If CH<sub>2</sub>Cl<sub>2</sub> is the one of choice for which **2a** was isolated in 90% yield, coupling reactions were also effective in CH<sub>3</sub>CN (78%), CH<sub>3</sub>NO<sub>2</sub> (74%), and ClCH<sub>2</sub>CH<sub>2</sub>Cl (80%) after 20 h at room temperature. Next, the possibility of changing the additives used under our standard conditions (CuBr<sub>2</sub> and HOBt) was investigated. Important to mention that this additives survey was realized for couplings run for 8 h instead of 20 h, and the main results are summarized in Table 1.

Entry 1 was run using the standard conditions [CuBr<sub>2</sub>/HOBt (10 mol % each)] for 20 h. Thus, a trial was run with a reduced reaction time of 8 h (entry 2). In this case, dipeptide **2a** was isolated in a reduced 62% yield. A blank reaction, in the absence of additives, was carried out as a benchmark, and dipeptide **2a** was obtained in 56% yield (entry 3). CuBr<sub>2</sub> combined with another Brønsted acid, the *p*-toluenesulfonic acid, afforded comparable

yields (entries 2 and 4). If the combination of CuBr<sub>2</sub>/HOBt afforded a synergistic effect for couplings run in 20 h,<sup>11</sup> interestingly, when CuBr<sub>2</sub> was used as the sole additive, a slightly improved yield of 68% was observed for couplings run in 8 h (entries 1, 2, and 5). The use of a more acidic additive, HBr (48 wt.% in H<sub>2</sub>O), slightly decreased the isolated yield to 48% (entry 6). Moving from a Cu(II) to a Cu(I) salt, using CuCN (10 mol %) without adding Brønsted acids, a comparable yield of 67% was obtained (entry 7). Alike copper-based Lewis acids, magnesium perchlorate<sup>16,17</sup> was also able to catalyze the couplings in the presence or absence of a Brønsted acid in similar yields (entries 8 and 9). Afterward, we evaluated the influence of boron-type Lewis acids. If catalytic amounts of BF<sub>3</sub>·OEt<sub>2</sub> enabled the couplings with similar outcomes (entries 10–12), a stoichiometric quantity was deleterious and **2a** was isolated in only 29% yield (entry 13). In this case, the starting material was not recovered, suggesting a possible degradation/deprotection during the course of the reaction. By merging BF<sub>3</sub>·OEt<sub>2</sub> with either imidazole or PTSA, no synergistic effect was observed and similar yields to the ones obtained when BF<sub>3</sub>·OEt<sub>2</sub> was used alone were obtained (entries 14 and 15). Moving to organoboron-type Lewis acids,<sup>18,19</sup> different outcomes were observed (entries 16–21). Best yields were

**Table 1. Additives survey.<sup>a</sup>**

entry	additives	mol %	yield <sup>c</sup> (%)
1	HOBt/CuBr <sub>2</sub> <sup>b</sup>	10/10	90
2	HOBt/CuBr <sub>2</sub>	10/10	62
3			56
4	PTSA/CuBr <sub>2</sub>	10/10	60
5	CuBr <sub>2</sub>	10	68
6	HBr (48%)	10	48
7	CuCN	10	67
8	Mg(ClO <sub>4</sub> ) <sub>2</sub> /HOBt	10/10	70
9	Mg(ClO <sub>4</sub> ) <sub>2</sub>	20	62
10	BF <sub>3</sub> ·OEt <sub>2</sub>	10	64
11	BF <sub>3</sub> ·OEt <sub>2</sub>	20	63
12	BF <sub>3</sub> ·OEt <sub>2</sub>	50	67
13	BF <sub>3</sub> ·OEt <sub>2</sub>	100	29
14	Imidazole/BF <sub>3</sub> ·OEt <sub>2</sub>	10/50	69
15	PTSA/BF <sub>3</sub> ·OEt <sub>2</sub>	10/10	64
16	Phenylboronic acid	20	49
17	Phenylboronic acid	50	71
18	Phenylboronic acid	100	75
19	Phenylboronic acid	150	78
20	<i>p</i> -Tolylboronic acid	100	41
21	<i>p</i> -Bromophenylboronic acid	100	82

<sup>a</sup>Reaction conditions: ImiAlaOMe (1.00 equiv), Boc-Phe-OH (1.50 equiv), CH<sub>2</sub>Cl<sub>2</sub> (C 1.0 M), 25 °C, 8 h. <sup>b</sup>Standard conditions: reaction run for 20 h. <sup>c</sup>Isolated yields.

attained when a stoichiometric amount or an excess was employed (75 and 78%, respectively; entries 18 and 19), and the couplings worked better when *p*-bromophenylboronic acid was used as a reaction promoter (82% yield; entry 21).<sup>20</sup>

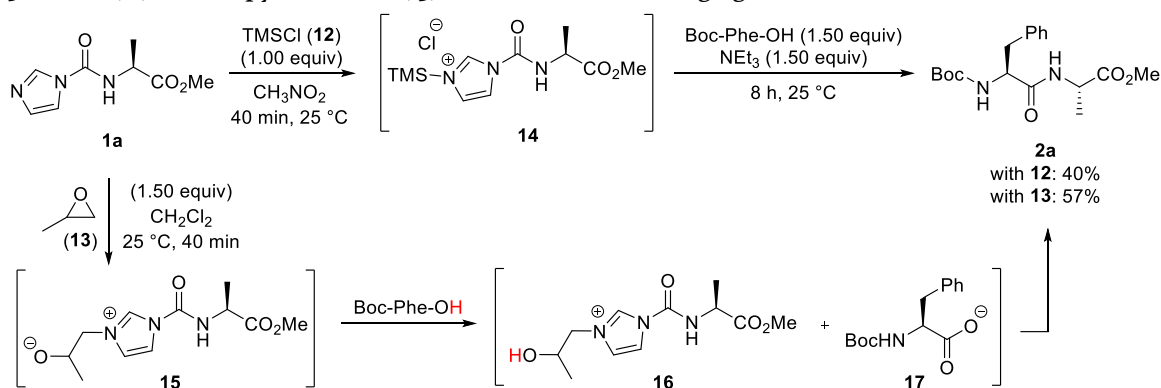
The use of stoichiometric quantities of trimethylsilylchloride (TMSCl, **12**) and propylene oxide (**13**) as reaction promoters was also evaluated (Scheme 5). In the case of **12**, the addition of a base was necessary to assure the carboxylate anion **17** formation to obtain the nucleophilic displacement of the imidazolium salt **14** derived from the TMSCl addition. The reaction furnished **2a** in a moderate 40% yield. The reaction was next attempted in the presence of propylene oxide (**13**). Indeed, **13** could act as both an imidazole

activating agent and next as a base to generate carboxylate **17** (Scheme 5). The yield for isolated **2a** was, however, quite disappointing (57%). The use of other silylating agents such as *N,O*-bis-(trimethylsilyl)acetamide (BSA) and *N*-methyl-*N-tert*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) as stoichiometric activating reagents for the imidazole ring was also studied. However, in these cases, dipeptide **2a** was only observed in low yields (<35%).

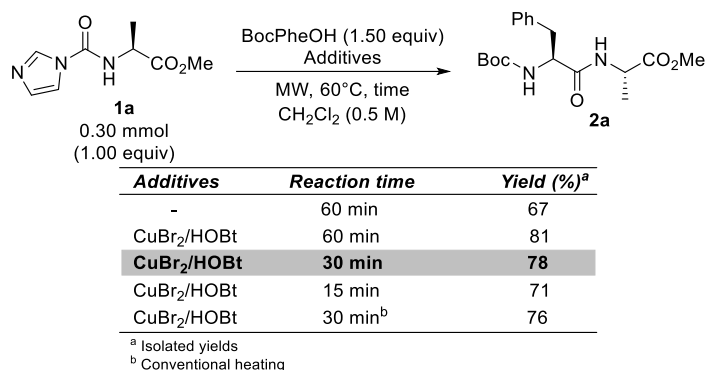
Overall, a set of additives (acidic and/or basic ones) has been examined in a catalytic fashion for 8 h instead of 20 h. However, none has stood out from the others in terms of efficiency (yields ranging from 60 to 70% in all cases). If it can be seen as a positive point, it might be mentioned that it makes understanding the reaction difficult and, consequently, the choice for further additives.

**Microwave Irradiation and/or Conventional Heating.** Consequently, we decided to investigate our coupling reactions under microwave irradiation, a mature technique compatible with peptide chemistry.<sup>21</sup> The first trials that have been performed (Table 2) showed that a remarkable improvement, in terms of reaction kinetics, can be reached through this technique (from 20 h to 30 min). By the way, no diastereomer formation for dipeptide **2a** was detected on the crude <sup>1</sup>H NMR. Thus, a selection of dipeptides **2** was synthesized to survey the scope of the couplings under microwave irradiation (Table 2; also see the SI for additional details). It is interesting to note that similar kinetics could also be observed under conventional heating. Comparable or slightly improved results were obtained when the reactions were performed in 30 min either in microwave irradiation or conventional heating instead of the classical method (room temperature, 20 h).

**Reaction Extension to General Amides.** In our preliminary communication, the synthesis of several dipeptides **2** (12 examples, 52–90% yields) was described by combining CDI-activated  $\alpha$ -aminoesters **1** and amino acids bearing free-carboxylic acid functions.<sup>11</sup> Later on, a sequential one-pot procedure was proposed, avoiding the need for prior formation and isolation of intermediates **1**.<sup>22</sup> In both works, dipeptides were isolated in very good yields, and commonly used *N*-urethane protecting groups are compatible with the reaction conditions. We then decided to move forward and try to

**Scheme 5. TMSCl (**12**) and Propylene Oxide (**13**) as Imidazole Activating Agents**

**Table 2. MW Trials and Subsequent Scope**



entry	Imi-AA-OR	dipeptide	yield (%) <sup>a</sup>
1	Imi-Ala-OMe ( <b>1a</b> )	Boc-Phe-Ala-OMe ( <b>2a</b> )	78
2	<b>1a</b>	Cbz-Met-Ala-OMe ( <b>2b</b> )	80
3	<b>1a</b>	Fmoc-Lys(Alloc)-Ala-OMe ( <b>2c</b> )	70
4	<b>1a</b>	Boc-Cys(Bn)-Ala-OMe ( <b>2d</b> )	77
5	<b>1a</b>	Boc-Pro-Ala-OMe ( <b>2e</b> )	47
6	<b>1a</b>	Fmoc-Phe-Ala-OMe ( <b>2f</b> )	73
7	Imi-Gly-OEt ( <b>1b</b> )	Boc-Asp(OBn)-Gly-OEt ( <b>2g</b> )	80
8	<b>1b</b>	Fmoc-Ala-Gly-OEt ( <b>2h</b> )	77
9	<b>1b</b>	Cbz-Trp-Gly-OEt ( <b>2i</b> )	66
10	Imi-Val-OMe ( <b>1c</b> )	Boc-Phe-Val-OMe ( <b>2j</b> )	70
11	Imi-Met-OMe ( <b>1d</b> )	Cbz-Phe-Met-OMe ( <b>2k</b> )	66
12	<b>1d</b>	Boc-Trp-Met-OMe ( <b>2l</b> )	72
13	Imi-Met-OrBu ( <b>1e</b> )	Cbz-Trp-Met-OrBu ( <b>2m</b> )	66

<sup>a</sup>Isolated yields.

transpose the reaction conditions to “general” amides synthesis (not bearing AAs). Indeed, small-to-medium molecules bearing the amide function are also omnipresent on various natural and pharmaceutical products.<sup>23</sup> In 2011, N-acylation of amines to amides was ranked the first position in the top 10 reactions most used in the pursuit of drug candidate development.<sup>24</sup> More recently, amide synthesis has been placed among the most used reactions in drug discovery.<sup>25</sup>

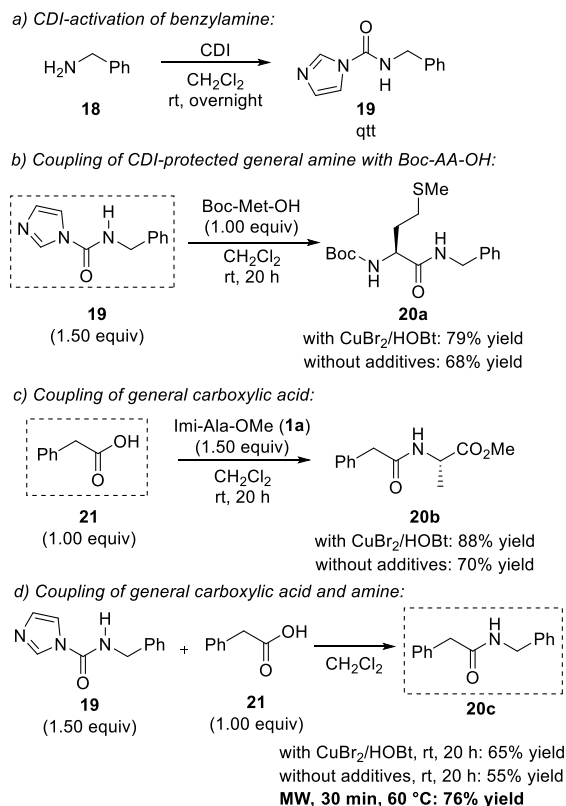
Before synthesizing “general” amides, the combination of at least one AA partner with a simple (non-AA) carboxylic acid or amine derivative was first studied (Scheme 6). As depicted in Scheme 6a, benzylamine (**18**) affords CDI-protected intermediate **19** in excellent yield. It is noteworthy to mention that compound **19** was prepared following the procedure (in the absence of a base) developed during the one-pot strategy to avoid, or at least considerably minimize, the formation of symmetrical urea (see the SI, scheme S1).<sup>22</sup> Next, we studied the behavior of **19** in the presence of Boc-Met-OH as a coupling partner (Scheme 6b). Either in the presence or absence of additives, the reaction works well and gives the amide compound **20a** in good yields. We have thus checked the combination of Imi-Ala-OMe **1a** and a “general” carboxylic acid **21**, and the coupling takes place in good yields (Scheme 6c). Finally, the combination of phenylacetic acid (**21**) and **19** gave the expected amide **20c** in good isolated yields (Scheme 6d). Knowing at this point that “general” amide **20c** can be synthesized under the developed classical conditions (20 h, rt), we have next evaluated the use of MW irradiation. By doing so, **20c** was isolated in an improved 76% yield in a reduced reaction time (30 min), showing that MW is also

compatible (and advantageous) with “general” amide synthesis.

Subsequently, the synthesis of other “general” amides was undertaken (Scheme 7). Merging amino acid and non-amino acid substrates afforded “hybrid” amides **20a,b,d–h** in good yields (62–86%). Less reactive substrates such as benzoic acid and aniline are compatible with the coupling conditions, but lower yields for amides **20d** and **20g** were obtained (45 and 25%, respectively). Moving to only non-amino acid substrates, the reactions also operated finely, giving the expected “general” amides **20c,i–r** in moderate to good yields (60–90%). Benzyl and alkyl chains on both amines and carboxylic acids are well tolerated. If the use of an  $\alpha,\beta$ -unsaturated carboxylic acid afforded the expected amide **20r** in 62%, couplings with aromatic ones (such as benzoic acid) failed under our conditions. When using phenylacetic acid as substrate, the expected amide **20i** was isolated in 86%, whereas the application of more acidic  $\alpha,\alpha$ -difluorophenylacetic acid did not allow the amide formation (**20s**).

**Quantification of Residual Cu Traces on Dipeptides.** In line with our interest in applying the coupling conditions developed into peptide chemistry, it was essential to quantify the possible residual traces of copper on our isolated dipeptides due to the use of a Cu(II) salt as an additive.<sup>26</sup> Indeed, potential contamination of APIs with heavy metals is a key issue in pharmaceutical R&D: see, for example, the largescale process for the preparation of Thymitaq involving copper-catalyzed Ullmann coupling.<sup>26a</sup> Thus, analysis [inductively coupled plasma mass spectrometry (ICP-MS), see

**Scheme 6. First Attempts toward “General” Amides Synthesis. (a) CDI Activation of Benzylamine. (b, c) Coupling of One AA Residue with a “General” Amine or Carboxylic Acid Compound (Non-AA Residue). (d) First Example of a “General” Amide Function Formation (with Non-AA Residues Involved in the Coupling Reaction)**



the SI] of a sample of Boc-Phe-Ala-OMe (from the large-scale synthesis and after purification via column chromatography) showed that our dipeptide contains an average of 1.53 ppm of copper.

**Benchmark Reactions Scale-Up.** The scale-up of the stepwise transformation was easily performed (87% yield, 71.6 mmol) but imposed some reaction conditions optimization to minimize the formation of undesired symmetrical urea (see the SI).<sup>12</sup> Later on, we also validated the microwave irradiation strategy for the preparation of dipeptide **2a** and “general” amide **20i** on a 5.50 mmol scale. In both cases, very good yields were attained (Scheme 8).

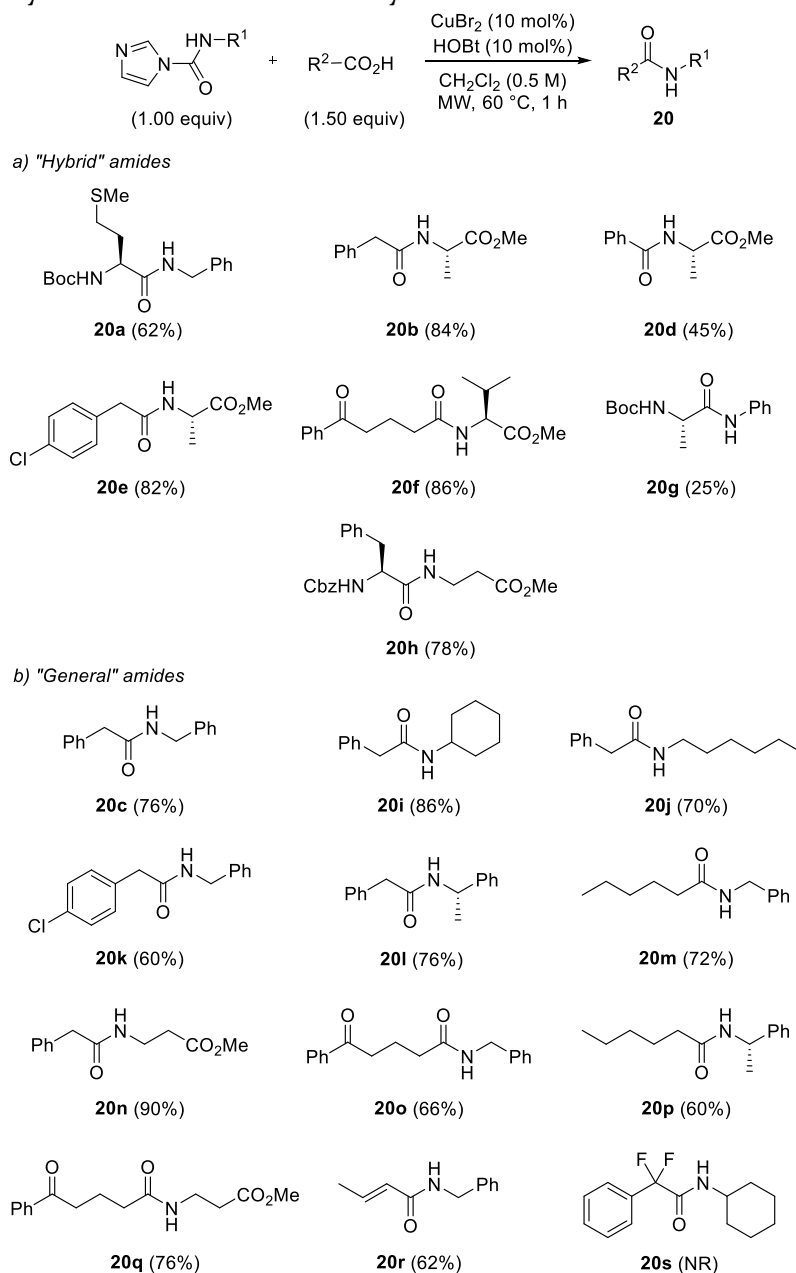
**Racemization Studies.** One important concern on peptide synthesis is racemization issues. The efficiency of our method was first evaluated during the synthesis of Boc-Cys(Bn)-Ala-OMe dipeptide **2d**. Indeed, cysteine is well known as being very prone to racemization during its carboxylate activation with coupling reagents and base due to the formation of a stabilized enol intermediate.<sup>27</sup> Under our former conditions (i.e., with additives, 20 h, rt), **2d** was formed with no detected epimerization.<sup>11</sup> When the same coupling was carried out under MW irradiation, for 30 min at 60 °C, **2d** was isolated in a slightly improved yield of 77% (Table 2), and no racemization was observed by HPLC analysis (Scheme 9) nor in the crude <sup>1</sup>H NMR spectra. We have also performed the Anderson test<sup>28,29</sup> with the evaluation of the developed conditions during the synthesis of

tripeptide Cbz-Gly-Phe-Gly-OEt **22**, which is also a sensitive case (Scheme 10). In this particular example, if racemization takes place with traditional peptide coupling reagents, it is imputed to oxazolone formation as a dipeptide is going to be activated instead of a single amino acid. In our hands, when the synthesis of **22** took place under classical conditions via PyBOP carboxylate activation, racemization of 40% was detected (Table 3, entry 1). In contrast, switching to the Oxyma Pure/DIC system developed by Albericio and co-workers, a very low level of racemization was observed (<2%) (Table 3, entry 2).<sup>30</sup> When Imi-Gly-OEt was used, in the presence of  $\text{CuBr}_2/\text{HOBt}$  (10 mol %) at room temperature and over 20 h, we were delighted to observe the isolation of tripeptide **22** in the absence of racemization (<1%) (Table 3, entry 5). This result supports our hypothesis that CDI-protected  $\alpha$ -aminoesters allow peptide couplings without involving a prior carboxylic acid activation. For comparison, when CDI was used to activate the carboxylic acid function of dipeptide Cbz-Gly-Phe-OH, with or without additives, 8.6 and 5.8% of racemization on the phenylalanine were, respectively, observed (Table 3, entries 3 and 4). The results obtained in the racemization studies, either during the preparation of **2d** or **22**, place our conditions among the least racemizing ones.

**Mechanistic Insights.** Concerning the mechanism, experimental observations have guided us to postulate some plausible reaction pathways. During our first studies, we observed that the dipeptide synthesis does not take place in the presence of a base (i.e., no dipeptide is formed).<sup>11</sup> Accordingly, we postulate that the imidazole ring on the Imi-AA-OR substrate is able to deprotonate the carboxylic acid function on the PHN-AA-OH partner giving rise to an imidazolium salt intermediate<sup>31</sup> and the carboxylate (Scheme 11). However, if imidazolium salt cannot be generated, the coupling with the carboxylate (formed by deprotonation with the external base) cannot take place as the imidazole ring is not a good leaving group compared to the imidazolium salt. On the other hand, in the absence of an external base, the imidazole ring might deprotonate the carboxylic acid function of Boc-Phe-OH, giving rise to an imidazolium salt, which can be displaced during the coupling reaction by the engendered carboxylate to give rise to the amide function. If the imidazole ring could deprotonate the carboxylic acid, the question is to understand how the coupling evolves from this point. We believe that a carbamic anhydride intermediate **23** might be formed, either via direct carboxylate addition into Imi-AA-OR or via the intermediacy of an isocyanate **24** obtained by simple elimination of imidazole (**25**) (Scheme 12). Then, three possibilities can be postulated: the first one supports the dipeptide formation by simple rearrangement with concomitant  $\text{CO}_2$  elimination (path A); the second one proposes the formation of an acyl imidazole intermediate **26** (path B), and the third route suggests the formation of an oxazolone **27** (path C).<sup>32</sup> Attempts to gain hints on the formation of intermediate **23** and evolution toward the amide function were realized by NMR monitoring.<sup>33</sup> If the studies realized did not allow us to



## Scheme 7. Scope for “Hybrid” and “General” Amides Synthesis<sup>a</sup>

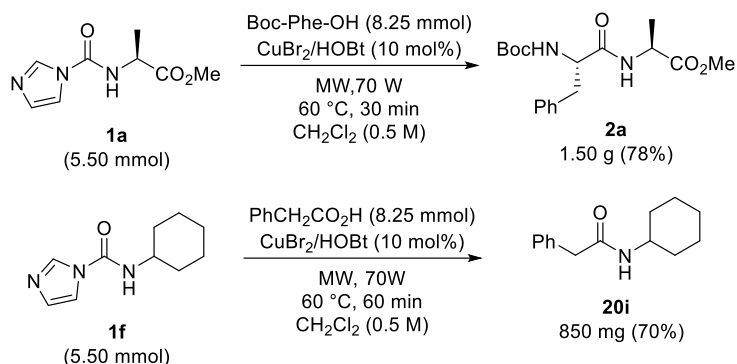


<sup>a</sup>NR = no reaction.

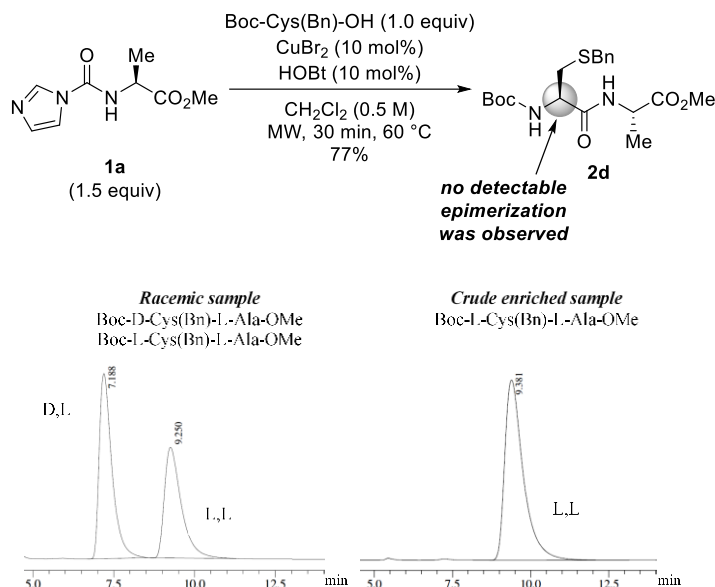
distinctly distinguish between the three pictured pathways, some points could be raised. From the experiences realized, any intermediate previously synthesized and/or observed (acyl imidazole **26**, isocyanate **24**, and carbamic anhydride **23**) were not detected on the <sup>13</sup>C spectra.<sup>34</sup> We can anticipate that either they are not formed or their inherent reactivity did not allow them to be perceived in situ. Nevertheless, a growing pic at 124.4 ppm could be detected, which can be credited to carbon dioxide that is released during the coupling.<sup>35</sup> It seems that the dipeptide arises directly from **23**, and its formation could be the rate-limiting step of the process. Once **23** in situ formed, a fast intramolecular rearrangement, with concomitant CO<sub>2</sub> extrusion, could provide the dipeptide. This observation could explain the lack of accumulation of the mixed anhydride in

the reaction, the nonobservance of acyl imidazole and isocyanate intermediates, and also the chemical shift of 124.4 ppm assigned to CO<sub>2</sub>. Moreover, an interesting study published in 2004 by Vaidyanathan and co-workers described the positive effect of carbon dioxide on the reaction kinetics between the coupling of an acyl imidazole and an amine toward the amide formation (Scheme 13a).<sup>36</sup> Also recently, following the publication of our first communication on the reactivity of N-acyl imidazoles in amide synthesis, Jiang and Bi have computationally studied the reaction highlighting a 1,3-

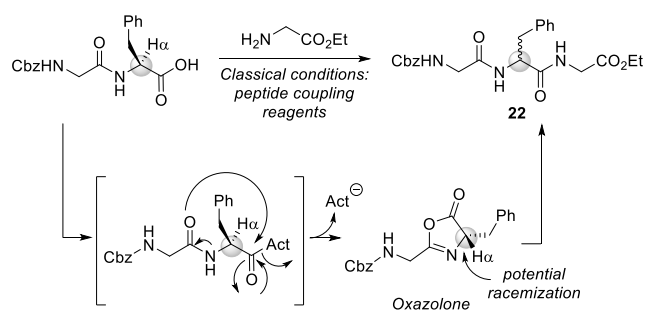
### Scheme 8. Large-Scale Synthesis of Dipeptide **2a** and Amide **20i** under Microwave Irradiation



### Scheme 9. Racemization Study on the Synthesis of Boc-Cys(Bn)-Ala-OMe **2d** under MW Irradiation



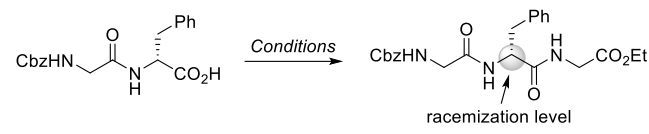
### Scheme 10. Anderson Test<sup>28,29</sup>



acyl transfer (among less favorable mechanistic alternatives) on the carbamic anhydride (Scheme 13b).<sup>37</sup> Gratifyingly, these studies corroborate our initial mechanistic assumptions, and altogether, path A (Scheme 12) is more likely to operate during the process. Another observation that supports this reaction pathway is that our dipeptides are isolated without racemization, eliminating thus the possible transient formation of both acyl imidazole **26** and oxazolone **27**.

### CONCLUSIONS

The catalytic system composed of CuBr<sub>2</sub>/HOBt first developed to synthesize dipeptides in mild conditions in 20 h has been reworked to improve the reaction kinetics, widen its field of application, and acquire better knowledge of the coupling mechanism. The use of unprecedented N-acyl imidazoles as activated amino partners paves the way to synthesize peptides from the challenging reverse N → C direction. Moreover, the ability to avoid traditional carboxylic acid activation combined with the absence of a base on the reaction conditions made possible the synthesis of dipeptides and one tripeptide in a process with no detectable epimerization. In this study, by reworking the reaction parameters, we were delighted to note that microwave irradiation considerably improved the reaction kinetics from 20 h to 30 min, keeping the same outcomes about yields. Also, the method could be fruitfully transposed into the synthesis of

**Table 3. Anderson Test Results**


entry	conditions	Rdt (%)	Rac (%) <sup>a</sup>
1	(i) PyBOP (1.50 equiv) DIPEA (4.00 equiv), 5 min (ii) HCl·H-Gly-OEt (1.50 equiv) CH <sub>2</sub> Cl <sub>2</sub> (c 0.5M), 2 h, rt	57	40
2	(i) Oxyma (1.50 equiv) DIC (1.50 equiv), 5 min (ii) HCl·H-Gly-OEt (1.50 equiv) DIPEA (1.50 equiv) CH <sub>2</sub> Cl <sub>2</sub> (c 0.5M), 2 h, rt	70	< 2%
3	(i) CDI (1.00 equiv), 5 min (ii) HCl·H-Gly-OEt (1.50 equiv) CH <sub>2</sub> Cl <sub>2</sub> (c 0.5M), 20 h, rt	54	8.6
4	(i) CDI (1.00 equiv) CuBr <sub>2</sub> /HOBt (10 mol%), 5 min (ii) HCl·H-Gly-OEt (1.50 equiv) CH <sub>2</sub> Cl <sub>2</sub> (c 0.5M), 20 h, rt CuBr <sub>2</sub> /HOBt (10 mol%)	61	5.8
5	Imi-Gly-OEt (1.50 equiv) CH <sub>2</sub> Cl <sub>2</sub> (c 1.0M), 20 h, rt	55	< 1

<sup>a</sup>The racemization level of phenylalanine on the crude material was determined by chiral HPLC on a chiral stationary phase (see the SI for details and HPLC profiles).

“general” amides (non-amino acid substrates), giving the possibility to apply it for the preparation of important active pharmaceutical ingredients (API molecules) that bear an amide function. Finally, a plausible reaction pathway could be identified based on our experimental observations and supported by precedents from the literature. Overall, the improved catalytic method described herein affords a nonconventional way to reach amides in mild and simple reaction conditions in a very short reaction time. The scope of both amino acids and “general” amine and carboxylic acid precursors are quite broad, and the expected amides are isolated in good yields in the absence of

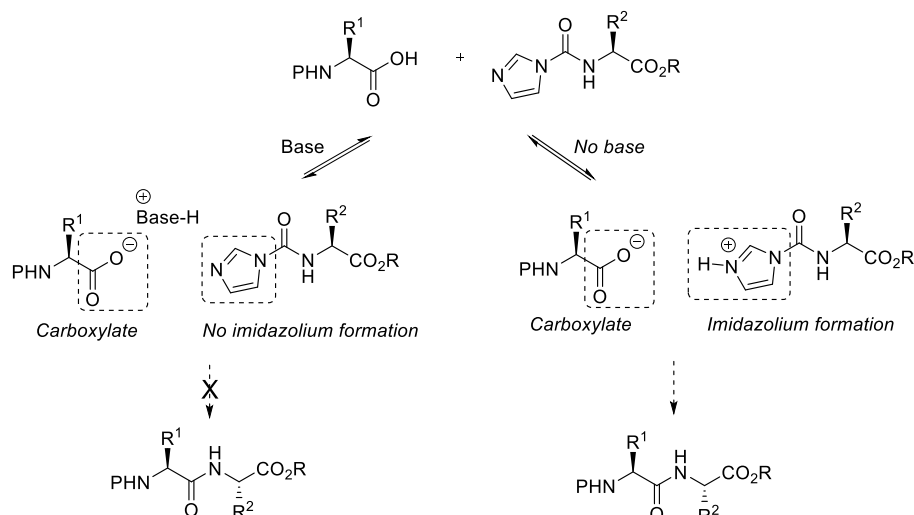
detectable racemization. Future work will be devoted to exploring further the methodology of peptide chemistry.

## EXPERIMENTAL SECTION

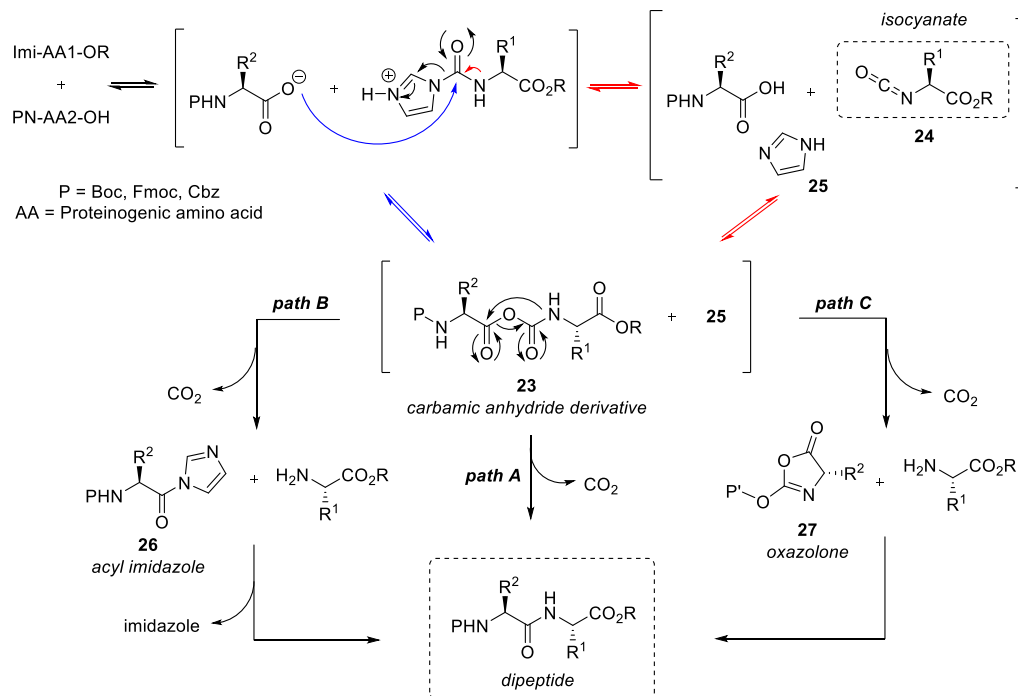
**General Information.** Unless otherwise specified, all commercial materials were used without further purification. Reactions were carried out in round-bottom flasks equipped with a magnetic stirring bar; there is no need for an argon atmosphere. Analytical thin-layer chromatography (TLC) of all reactions was performed on silica gel 60 F254 TLC plates. Visualization of the developed chromatogram was performed by UV absorbance (254 nm) and using ninhydrin stain. Flash chromatography was carried out on silica gel 60 A (35–70 mm). FT-IR spectra were recorded with a PerkinElmer Spectrum 1000; absorptions are given in wave numbers (cm<sup>-1</sup>). <sup>1</sup>H and <sup>13</sup>C{<sup>1</sup>H} NMR spectra were recorded with a Bruker Ultra shield 400 plus. <sup>1</sup>H chemical shifts are reported in delta (δ) units in parts per million (ppm) relative to the singlet at 7.26 ppm for d-chloroform (residual CHCl<sub>3</sub>). <sup>13</sup>C{<sup>1</sup>H} chemical shifts are reported in ppm relative to the central line of the triplet at 77.0 ppm for d-chloroform. Splitting patterns are designated as s = singlet; d = doublet; t = triplet; q = quartet; quin = quintuplet; m = multiplet; b = broad; bs = broad singlet; bd = broad doublet; and bt = broad triplet. All coupling constants (J values) are reported in Hertz (Hz). Microwave-assisted reactions were performed in a Microwave synthesizer CEM Discover SP (CEM Corporation, Matthews, NC). The system contains a single-mode microwave cavity that produces controlled irradiation at 2450 MHz. Reaction times refer to hold times at the indicated temperature (60 °C), not to total irradiation times. The temperature was measured through a volume-independent infrared (IR) sensor. The couplings were run under the pressure of 3 bar (range of pressure for the MW synthesizer: 0–30 bar) and a maximum power of 70 W in sealed reaction vessels (range of power for the MW synthesizer: 0–300 W). Optical rotations were measured with an ADP 440 Polarimeter (Bellingham + Stanley). Low-resolution mass spectra were recorded on a Waters QToF-I spectrometer using electrospray ionization. NMR studies for mechanism understanding purposes and high-resolution mass spectra were obtained using spectrometers operated by the Plateforme d'Analyse et de Caractérisation (PAC) of Pôle Chimie Balard de Montpellier.

**General Procedure for the Synthesis of “CDI-Activated” Amines (1,19).**<sup>13c</sup> These intermediates were prepared according to the method described in the literature.<sup>11,12</sup> To a solution of the

## Scheme 11. Experimental Observations

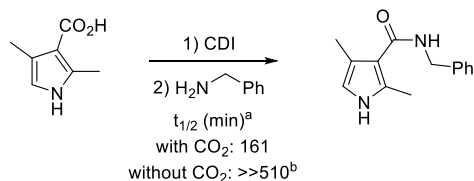


## Scheme 12. Possible Pathways toward the Amide Formation

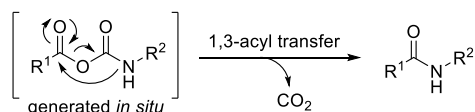


## Scheme 13. (A) Literature Studies on CO<sub>2</sub> Positive Effect on Amide Formation<sup>36</sup> and (B) 1,3-Acyl Transfer via DFT Calculations<sup>37</sup>

A) CO<sub>2</sub>-Rate enhancement in amidations (Vaidyanathan 2004)



B) 1,3-Acyl transfer of mixed carbamic anhydrides (Bi and Jiang 2018)



corresponding amine or H-AA-OR (4.00 mmol, 1.00 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL, C 0.2 M) at room temperature was added *N,N'*-carbonyldiimidazole (4.80 mmol, 1.20 equiv). The obtained suspension was thus stirred at this temperature overnight. After reaction completion (TLC monitoring), the mixture was washed once with H<sub>2</sub>O (20 mL). The organic phase was separated, and the aqueous phase was further extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 15 mL). The organic layers were combined, dried over MgSO<sub>4</sub>, and evaporated. Purification was performed by column chromatography on a short silica gel pad (100% ethyl acetate) to afford the desired intermediates. *The analytical data were identical in all respects to those previously reported in the literature.*<sup>11,12</sup>

**General Procedure for the Microwave-Assisted Synthesis of Dipeptides (2a–m).** In a 5 mL microwave vessel containing a solution of the Imi-AA<sub>1</sub>-OR derivative (0.30 mmol, 1.00 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (0.6 mL, C 0.5 M), copper(II) bromide (0.03 mmol, 0.10 equiv), 1-hydroxybenzotriazole hydrate (0.03 mmol, 0.10 equiv), and then P-AA<sub>2</sub>-OH (0.45 mmol, 1.50 equiv) were successively

added. The suspension was then heated at 60 °C under a microwave for 30 min. After reaction completion, the mixture was diluted with 10 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed once with aqueous HCl (0.5 N, 8 mL). Then, the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (1 × 10 mL). The organic layers were combined and washed with saturated aqueous NaHCO<sub>3</sub> (8 mL) and brine (8 mL). The organic phase was dried over MgSO<sub>4</sub>, and the solvent was removed under vacuum. Purification was performed by column chromatography on silica gel (pentane/ethyl acetate 7:3 to 1:1) to afford the products.

**General Procedure for the Synthesis of “Hybrid” Amides (20a,b,d–h).** In a 4 mL screw top vial containing a solution of the imidazole intermediate (0.50 mmol, 1.00 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL, C 1.0 M), copper(II) bromide (0.05 mmol, 0.10 equiv), 1-hydroxybenzotriazole hydrate (0.05 mmol, 0.10 equiv), and a carboxylic acid (0.75 mmol, 1.50 equiv) were successively added. The suspension was then heated at 60 °C under a microwave for 60 min. After reaction completion, the mixture was diluted with 15 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed once with aqueous HCl (0.5 N, 10 mL). Then, the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (1 × 15 mL). The organic layers were combined and washed with saturated aqueous NaHCO<sub>3</sub> (10 mL) and brine (10 mL). The organic phase was dried over MgSO<sub>4</sub>, and the solvent was removed under vacuum. Purification was performed by column chromatography on silica gel (pentane/ethyl acetate 7:3 to 1:1) to afford the expected products.

**General Procedure for the Synthesis of “General” Amides (20c,i–r).** In a 5 mL microwave vessel containing a solution of the imidazole intermediate (0.50 mmol, 1.00 equiv) in CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL, C 0.5 M), copper(II) bromide (0.05 mmol, 0.10 equiv), 1-hydroxybenzotriazole hydrate (0.05 mmol, 0.10 equiv), and a carboxylic acid (0.75 mmol, 1.50 equiv) were successively added. The suspension was then heated at 60 °C under a microwave for 60 min. After reaction completion, the mixture was diluted with 15 mL of CH<sub>2</sub>Cl<sub>2</sub> and washed once with aqueous HCl (0.5 N, 10 mL). Then, the aqueous phase was extracted with CH<sub>2</sub>Cl<sub>2</sub> (1 × 15 mL). The organic layers were combined and washed with saturated aqueous NaHCO<sub>3</sub> (10 mL) and brine (10 mL). The organic phase was dried

over MgSO<sub>4</sub>, and the solvent was removed under vacuum. Purification was performed by column chromatography on silica gel (pentane/ethyl acetate 7:3 to 2:3) to afford general amides.

(S)-Methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoate (**2a**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (82 mg, 78%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.18–7.30 (m, 5H), 6.54 (d, J = 7.0 Hz, NH), 5.06 (bs, NH), 4.51 (quin, J = 7.1 Hz, 1H), 4.37 (bs, 1H), 3.70 (s, 3H), 3.05 (d, J = 6.6 Hz, 2H), 1.39 (s, 9H), 1.33 (d, J = 7.2 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

(S)-Methyl 2-((2)-2-(((benzyloxy)carbonyl)amino)-4-(methylthio)butanamido)propanoate (**2b**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (87 mg, 80%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.29–7.38 (m, 5H), 6.60 (bd, J = 6.6 Hz, NH), 5.49 (bd, J = 7.7 Hz, NH), 5.11 (s, 2H), 4.57 (quin, J = 7.3 Hz, 1H), 4.37–4.42 (m, 1H), 3.75 (s, 3H), 2.61 (t, J = 7.2 Hz, 2H), 1.95–2.11 (m, 5H), 1.41 (d, J = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

(S)-Methyl 2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(((allyloxy)carbonyl)amino)hexanamido)propanoate (**2c**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (114 mg, 70%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.76 (d, J = 7.4 Hz, 2H), 7.58 (d, J = 7.6 Hz, 2H), 7.39 (td, J = 7.5, 0.5 Hz, 2H), 7.30 (td, J = 7.5, 1.3 Hz, 2H), 6.56 (d, J = 6.6 Hz, NH), 5.83–5.92 (m, 1H), 5.56 (d, J = 7.4 Hz, NH), 5.26 (d, J = 16.9 Hz, 1H), 5.17 (d, J = 10.4 Hz, 1H), 4.97 (bs, 1H), 4.54–4.61 (m, 2H), 4.38 (d, J = 6.8 Hz, 2H), 4.17–4.23 (m, 2H), 3.74 (s, 3H), 3.16–3.24 (m, 2H), 1.83–1.92 (m, 1H), 1.65–1.71 (m, 1H), 1.49–1.58 (m, 2H), 1.40 (d, J = 7.2 Hz, 3H), 1.23–1.31 (m, 2H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

(S)-Methyl 2-((S)-2-((tert-butoxycarbonyl)amino)-3-phenylpropanamido)propanoate (**2d**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (82 mg, 77%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.22–7.36 (m, 5H), 6.87 (d, J = 5.2 Hz, NH), 5.30 (bs, NH), 4.55 (quin, J = 7.2 Hz, 1H), 4.25 (bs, 1H), 3.75 (s, 3H), 3.74 (s, 2H) 2.88 (dd, J = 13.9, 5.7 Hz, 1H), 2.74 (dd, J = 14.0, 6.7 Hz, 1H), 1.46 (s, 9H), 1.40 (d, J = 7.2 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

tert-Butyl (R)-2-(((S)-1-methoxy-1-oxopropan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (**2e**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (42 mg, 47%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 4.51 (bs, 1H), 4.19–4.31 (m, 1H), 3.72 (s, 3H), 3.31–3.43 (m, 2H), 1.84–2.26 (m, 4H), 1.45 (s, 9H), 1.37 (d, J = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>38</sup>

(S)-Methyl 2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-phenylpropanamido)propanoate (**2f**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (105 mg, 73%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.77 (d, J = 7.5 Hz, 2H), 7.54 (t, J = 6.9 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.19–7.33 (m, 7H), 6.21 (bs, NH), 5.31 (bs, NH), 4.42–4.53 (m, 3H), 4.32–4.34 (m, 1H), 4.19 (t, J = 6.8 Hz, 1H), 3.71 (s, 3H), 3.12–3.15 (m, 1H), 3.01–3.06 (m, 1H), 1.34 (d, J = 7.0 Hz, 3H). The analytical

data were identical in all respects to those previously reported in the literature.<sup>11</sup>

Benzyl (S)-3-((tert-butoxycarbonyl)amino)-4-((2-ethoxy-2-oxoethyl)amino)-4-oxobutanoate (**2g**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (100 mg, 80%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.30–7.34 (m, 5H), 7.04 (t, J = 5.0 Hz, NH), 5.73 (d, J = 8.3 Hz, NH), 5.14 (d, J = 12.3 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 4.59 (bs, 1H), 4.18 (q, J = 7.1 Hz, 2H), 3.97 (d, J = 5.0 Hz, 2H), 3.03 (dd, J = 17.0 Hz, 3.7 Hz, 1H), 2.74 (dd, J = 17.0 Hz, 5.8 Hz, 1H), 1.43 (s, 9H), 1.25 (t, J = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

Ethyl (((9H-fluoren-9-yl)methoxy)carbonyl)-L-alanylglycinate (**2h**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (92 mg, 77%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.77 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.6 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.31 (dt, J = 11.2, 0.6 Hz, 2H), 6.44 (bs, NH), 5.30 (bs, NH), 4.42 (bs, 2H), 4.30 (bs, 1H), 4.22 (q, J = 7.1 Hz, 3H), 4.03 (d, J = 4.7 Hz, 2H), 1.42 (d, J = 6.2 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

Ethyl ((benzyloxy)carbonyl)-L-tryptophylglycinate (**2i**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (86 mg, 66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.43 (bs, NH), 7.60 (bs, 1H), 7.27–7.34 (m, 6H), 7.16 (bt, J = 7.5 Hz, 1H), 7.08 (bt, J = 7.3 Hz, 1H), 7.01 (bs, 1H), 6.51 (bs, NH), 5.66 (bs, NH), 5.03–5.11 (m, 2H), 4.57 (bs, 1H), 4.10 (q, J = 7.1 Hz, 2H), 3.79–3.90 (m, 2H), 3.19–3.30 (m, 2H), 1.22 (t, J = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>39</sup>

Methyl (tert-butoxycarbonyl)-L-phenylalanyl-L-valinate (**2j**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (81 mg, 70%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.19–7.29 (m, 5H), 6.42 (d, J = 7.8 Hz, NH), 5.07 (bs, NH), 4.45 (dd, J = 8.6, 5.1 Hz, 1H), 4.33–4.38 (m, 1H), 3.67 (s, 3H), 3.06 (d, J = 6.8 Hz, 2H), 2.03–2.13 (m, 1H), 1.40 (s, 9H), 0.86 (d, J = 6.8 Hz, 3H), 0.83 (d, J = 6.9 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

Methyl ((benzyloxy)carbonyl)-L-phenylalanyl-L-methioninate (**2k**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (88 mg, 66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.16–7.34 (m, 10H), 6.58 (bs, NH), 5.42 (bs, NH), 5.08 (s, 2H), 4.63 (dt, J = 12.7, 3.7 Hz, 1H), 4.45 (q, J = 6.4 Hz, 1H), 3.70 (s, 3H), 3.02–3.13 (m, 2H), 2.39 (t, J = 7.3 Hz, 2H), 2.05–2.13 (m, 1H), 2.03 (s, 3H), 1.85–1.96 (m, 1H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

Methyl (tert-butoxycarbonyl)-L-tryptophyl-L-methioninate (**2l**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (97 mg, 72%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.19 (bs, NH), 7.64 (d, J = 7.8 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.17–7.21 (m, 1H), 7.11–7.15 (m, 1H), 7.09 (bs, NH), 6.45 (bs, 1H), 5.16 (bs, NH), 4.55–4.60 (m, 1H), 4.44–4.47 (m, 1H), 3.65 (s, 3H), 3.35 (dd, J = 14.5, 5.4 Hz, 1H), 3.16 (dd, J = 14.5, 7.1 Hz, 1H), 2.23–2.27 (m, 2H), 1.99–2.04 (m, 4H), 1.82–1.89 (m, 1H), 1.43 (s, 9H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

*tert*-Butyl (S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-3-(1*H*-indolyl)propanamido)-4-(methylthio)butaneperoxoate (**2m**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 1:1, v/v) afforded the title compound as a white solid (107 mg, 66%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.34 (bs, NH), 7.63 (d, *J* = 7.6 Hz, 1H), 7.30–7.34 (m, 5H), 7.18 (m, 1H), 7.09–7.11 (m, 1H), 7.01 (bs, NH), 6.57 (bs, 1H), 5.52 (d, *J* = 6.7 Hz, 1H), 5.10 (s, 2H), 4.56 (d, *J* = 6.4 Hz, 1H), 4.44–4.47 (m, 1H), 3.36 (dd, *J* = 14.6, 4.4 Hz, 1H), 3.19 (dd, *J* = 14.6, 6.8 Hz, 1H), 2.23–2.28 (m, 2H), 2.01–2.06 (m, 1H), 1.98 (s, 3H), 1.81–1.88 (m, 1H), 1.42 (s, 9H). The analytical data were identical in all respects to those previously reported in the literature.<sup>11</sup>

*tert*-Butyl (S)-1-(benzylamino)-4-(methylthio)-1-oxobutan-2-yl)-carbamate (**2oa**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (104 mg, 62%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.24–7.33 (m, 5H), 6.64 (bs, NH), 5.23 (bs, NH), 4.43 (s, 2H), 4.28 (d, *J* = 6.3 Hz, 1H), 2.47–2.60 (m, 2H), 2.08–2.15 (m, 1H), 2.07 (s, 3H), 1.89–1.98 (m, 1H), 1.41 (s, 9H). The analytical data were identical in all respects to those previously reported in the literature.<sup>40</sup>

Methyl (2-phenylacetyl)-L-alaninate (**2ob**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (93 mg, 84%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.20–7.31 (m, 5H), 6.01 (bs, NH), 4.51 (quin, *J* = 7.2 Hz, 1H), 3.64 (s, 3H), 3.52 (s, 2H), 1.27 (d, *J* = 7.2 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>41</sup>

*N*-Benzyl-2-phenylacetamide (**2oc**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (87 mg, 76%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.17–7.37 (m, 10H), 5.67 (bs, NH), 4.42 (d, *J* = 5.8 Hz, 2H), 3.63 (s, 2H). The analytical data were identical in all respects to those previously reported in the literature.<sup>42</sup>

*N*-Benzoyl-L-alanine methyl ester (**2od**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (47 mg, 45%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.78–7.81 (m, 2H), 7.47–7.51 (m, 1H), 7.39–7.44 (m, 2H), 6.85 (d, *J* = 6.0 Hz, NH), 4.79 (quint, *J* = 7.2 Hz, 1H), 3.77 (s, 3H), 1.51 (d, *J* = 7.2 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>43</sup>

Methyl (2-(4-chlorophenyl)acetyl)-L-alaninate (**2oe**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (108 mg, 82%). mp 103–105 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.31–7.34 (m, 2H), 7.20–7.23 (m, 2H), 5.98 (bs, NH), 4.58 (quint, *J* = 7.2 Hz, 1H), 3.73 (s, 3H), 3.55 (s, 2H), 1.36 (d, *J* = 7.2 Hz, 3H). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 173.5, 170.0, 133.4, 133.1, 130.8 (2C), 129.2 (2C), 52.6, 48.3, 42.9, 18.5. FTIR (neat, cm<sup>-1</sup>) 3272, 3070, 3009, 1730, 1653, 1543, 1491, 1445, 1361, 1332, 1304, 1260, 1217, 1108, 1088, 1013, 962, 856, 803, 734, 708, 677. HRMS (ESI) *m/z*: [(M + H)<sup>+</sup>] Calcd for C<sub>12</sub>H<sub>15</sub>ClNO<sub>3</sub> 256.0735; Found 256.0742.

Methyl (5-oxo-5-phenylpentanoyl)-L-valinate (**2of**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (132 mg, 86%). mp 72–74 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.95–7.97 (m, 2H), 7.50–7.57 (m, 1H), 7.43–7.47 (m, 2H), 6.02 (d, *J* = 8.6 Hz, NH), 4.57 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.72 (s, 3H), 3.08 (t, *J* = 6.9 Hz, 2H), 2.35–2.39 (m, 2H), 2.06–2.19 (m, 3H), 0.93 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 200.0, 172.7, 172.6, 136.9, 133.2, 128.7 (2C), 128.2

(2C), 57.1, 52.2, 37.4, 35.5, 31.3, 20.3, 19.1, 18.0. FTIR (neat, cm<sup>-1</sup>) 3307, 2962, 1744, 1672, 1650, 1529, 1446, 1385, 1371, 1300, 1284, 1255, 1196, 1182, 1149, 1028, 994, 963, 734, 688, 659. HRMS (ESI) *m/z*: [(M – H)<sup>–</sup>] Calcd for C<sub>17</sub>H<sub>22</sub>NO<sub>4</sub> 304.1554; Found 304.1556.

*tert*-Butyl (S)-(1-oxo-1-(phenylamino)propan-2-yl)carbamate (**2og**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (33 mg, 25%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.41 (bs, NH), 7.42–7.45 (m, 2H), 7.19–7.27 (m, 2H), 7.00–7.04 (m, 1H), 4.99 (bs, NH), 4.25 (m, 1H), 1.39 (s, 9H), 1.37 (d, *J* = 7.1 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>10a</sup>

Methyl (S)-3-(2-(((benzyloxy)carbonyl)amino)-3-phenylpropanamido)propanoate (**2oh**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (148 mg, 78%). mp 116–119 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.22–7.37 (m, 8H), 7.15–7.17 (m, 2H), 6.18 (bt, *J* = 5.8 Hz, NH), 5.37 (bs, NH), 5.08 (s, 2H), 4.35 (bt, *J* = 6.3 Hz, 1H), 3.63 (s, 3H), 3.42–3.50 (m, 1H), 3.32–3.40 (m, 1H), 3.08–3.13 (m, 1H), 2.96–3.01 (m, 1H), 2.39–2.46 (m, 1H), 2.29–2.36 (m, 1H). <sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, CDCl<sub>3</sub>) δ 172.7, 170.8, 155.9, 136.5, 136.3, 129.4 (2C), 128.9 (2C), 128.7 (2C), 128.4, 128.2 (2C), 127.2, 67.2, 56.6, 51.9, 39.1, 34.8, 33.7. FTIR (neat, cm<sup>-1</sup>) 3284, 2956, 1734, 1688, 1644, 1535, 1495, 1435, 1372, 1329, 1253, 1230, 1199, 1136, 1086, 1039, 752, 697, 676. HRMS (ESI) *m/z*: [(M + H)<sup>+</sup>] Calcd for C<sub>21</sub>H<sub>25</sub>N<sub>2</sub>O<sub>5</sub> 385.1758; Found 385.1762.

*N*-Cyclohexyl-2-phenylacetamide (**2oi**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (93 mg, 86%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.21–7.37 (m, 5H), 5.19 (bs, NH), 3.70–3.80 (m, 1H), 3.54 (s, 2H), 1.80–1.84 (m, 2H), 1.53–1.63 (m, 3H), 1.25–1.37 (m, 2H), 0.95–1.13 (m, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>44</sup>

*N*-Hexyl-2-phenylacetamide (**2oj**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (77 mg, 70%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.24–7.37 (m, 5H), 5.38 (bs, NH), 3.56 (s, 2H), 3.16–3.21 (m, 2H), 1.36–1.41 (m, 2H), 1.19–1.28 (m, 6H), 0.85 (t, *J* = 6.9 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>45</sup>

*N*-Benzyl-2-(4-chlorophenyl)acetamide (**2ok**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (79 mg, 60%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.27–7.33 (m, 5H), 7.18–7.22 (m, 4H), 5.72 (bs, NH), 4.41 (d, *J* = 5.8 Hz, 2H), 3.57 (s, 2H). The analytical data were identical in all respects to those previously reported in the literature.<sup>46</sup>

(S)-2-Phenyl-*N*-(1-phenylethyl)acetamide (**2ol**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a pale-yellow oil (92 mg, 76%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.30–7.16 (m, 10H), 5.62 (bs, NH), 5.12 (quint, *J* = 7.2 Hz, 1H), 3.58 (s, 2H), 1.40 (d, *J* = 7.2 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>47</sup>

*N*-Benzylhexanamide (**2om**). Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (74 mg, 72%). mp 53–55 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ

7.36–7.26 (m, 5H), 5.66 (bs, NH), 4.44 (d,  $J = 5.7$  Hz, 2H), 2.20 (t,  $J = 7.6$  Hz, 2H), 1.62–1.70 (m, 2H), 1.28–1.34 (m, 4H), 0.89 (t,  $J = 7.0$  Hz, 3H).  $^{13}\text{C}\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$  173.0, 138.6, 128.8 (2C), 128.0 (2C), 127.6, 43.7, 36.9, 31.6, 25.6, 22.5, 14.1. FTIR (neat,  $\text{cm}^{-1}$ ) 3288, 2955, 2930, 2856, 1631, 1548, 1492, 1453, 1430, 1355, 1284, 1260, 1235, 1217, 1190, 1080, 1025, 748, 724, 693. HRMS (ESI)  $m/z$ :  $[(M + H)^+]$  Calcd for  $\text{C}_{13}\text{H}_{20}\text{NO}$  206.1500; Found 206.1500.

**Methyl 3-(2-phenylacetamido)propanoate (20n).** Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a pale-yellow oil (100 mg, 90%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.23–7.37 (m, 5H), 5.34 (bs, NH), 3.62 (s, 3H), 3.55 (s, 2H), 3.47 (q,  $J = 6.1$  Hz, 2H), 2.50 (t,  $J = 6.0$  Hz, 2H). The analytical data were identical in all respects to those previously reported in the literature.<sup>48</sup>

***N*-Benzyl-5-oxo-5-phenylpentanamide (20o).** Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (74 mg, 66%). mp 94–96 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.96–7.93 (m, 2H), 7.54–7.58 (m, 1H), 7.43–7.47 (m, 2H), 7.27–7.38 (m, 5H), 5.85 (bs, NH), 4.44 (d,  $J = 5.7$  Hz, 2H), 3.07 (t,  $J = 6.9$  Hz, 2H), 2.34 (t,  $J = 7.2$  Hz, 2H), 2.11 (quint,  $J = 7.1$  Hz, 2H).  $^{13}\text{C}\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  200.0, 172.4, 138.4, 136.9, 133.3, 128.9 (2C), 128.8 (2C), 128.2 (2C), 128.0 (2C), 127.7, 43.8, 37.5, 35.6, 20.4. FTIR (neat,  $\text{cm}^{-1}$ ) 3268, 3062, 2919, 1680, 1642, 1554, 1493, 1445, 1417, 1381, 1358, 1306, 1282, 1202, 1027, 977, 719, 699, 685, 656. HRMS (ESI)  $m/z$ :  $[(M + H)^+]$  Calcd for  $\text{C}_{18}\text{H}_{20}\text{NO}_2$  282.1489; Found 282.1494.

***(S)*-*N*-(1-Phenylethyl)hexanamide (20p).** Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (65 mg, 60%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.23–7.35 (m, 5H), 5.88 (bs, NH), 5.13 (quint,  $J = 7.2$  Hz, 1H), 2.15 (t,  $J = 7.7$  Hz, 2H), 1.62 (quint,  $J = 7.5$  Hz, 2H), 1.47 (d,  $J = 6.9$  Hz, 3H), 1.24–1.34 (m, 4H), 0.88 (t,  $J = 7.0$  Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>49</sup>

**Methyl 3-(5-oxo-5-phenylpentanamido)propanoate (20q).** Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (106 mg, 76%). mp 99–102 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.94–7.97 (m, 2H), 7.53–7.58 (m, 1H), 7.43–7.47 (m, 2H), 6.09 (bs, NH), 3.68 (s, 3H), 3.52 (q,  $J = 6.0$  Hz, 2H), 3.05 (t,  $J = 7.0$  Hz, 2H), 2.54 (t,  $J = 6.0$  Hz, 2H), 2.28 (t,  $J = 7.2$  Hz, 2H), 2.07 (quint,  $J = 7.0$  Hz, 2H).  $^{13}\text{C}\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ )  $\delta$  199.9, 173.2, 172.5, 136.9, 133.2, 128.7 (2C), 128.2 (2C), 51.9, 37.5, 35.6, 34.9, 34.0, 20.2. FTIR (neat,  $\text{cm}^{-1}$ ) 3300, 2949, 1728, 1680, 1644, 1549, 1441, 1423, 1382, 1319, 1198, 1175, 1103, 1000, 947, 888, 738, 690, 657. HRMS (ESI)  $m/z$ :  $[(M - H)^-]$  Calcd for  $\text{C}_{15}\text{H}_{18}\text{NO}_4$  276.1241; Found 276.1238.

***(E)*-*N*-Benzylbut-2-enamide (20r).** Purification by flash column chromatography on silica gel eluting with pentane/ethyl acetate mixture (7:3 to 2:3, v/v) afforded the title compound as a white solid (54 mg, 62%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.26–7.36 (m, 5H), 6.89 (dq,  $J = 15.2$ , 6.8 Hz, 1H), 5.80 (dq,  $J = 15.2$ , 1.6 Hz, 1H), 5.64 (bs, NH), 4.51 (d,  $J = 5.7$  Hz, 2H), 1.86 (dd,  $J = 6.8$ , 1.6 Hz, 3H). The analytical data were identical in all respects to those previously reported in the literature.<sup>50</sup>

## ■ ASSOCIATED CONTENT

### SI Supporting Information

$^1\text{H}$  and  $^{13}\text{C}\{^1\text{H}\}$  NMR spectra and HPLC profile

## ■ AUTHOR INFORMATION

### Corresponding Authors

Jean-Marc Campagne – ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier 34293, France; orcid.org/0000-0002-4943-047X; Email: [jean-marc.campagne@enscm.fr](mailto:jean-marc.campagne@enscm.fr)

Renata Marcia de Figueiredo – ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier 34293, France; orcid.org/0000-0001-5336-6071; Email: [renata.marcia.de.figueiredo@enscm.fr](mailto:renata.marcia.de.figueiredo@enscm.fr)

### Author

Eleonora Tosi – ICGM, Univ Montpellier, CNRS, ENSCM, Montpellier 34293, France

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This work was supported by the Agence Nationale de la Recherche (projet NOPS, number ANR-18-CE07-0038-01). The authors are grateful to the CNRS and the ENSCM. They are also very thankful to Aurélien Lebrun (NMR studies toward mechanism elucidation; Laboratoire de Mesures Physiques-Plateau Technique-Montpellier).

## ■ REFERENCES

- (a) Craik, D. J.; Fairlie, D. P.; Liras, S.; Price, D. The Future of Peptide-Based Drugs. *Chem Biol Drug Des.* **2013**, *81*, 136–147. (b) Uhlig, T.; Kyprianou, T.; Martinelli, F. G.; Oppici, C. A.; Heiligers, D.; Hills, D.; Calvo, X. R.; Verhaert, P. The emergence of peptides in the pharmaceutical business: From exploration to exploitation. *EuPA Open Proteomics* **2014**, *4*, 58–69.
- Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. A Knowledge-Based Approach in Designing Combinatorial or Medicinal Chemistry Libraries for Drug Discovery. 1. A Qualitative and Quantitative Characterization of Known Drug Databases. *J. Comb. Chem.* **1999**, *1*, 55–68.
- (a) Henninot, A.; Collins, J. C.; Nuss, J. M. The Current State of Peptide Drug Discovery: Back to the Future? *J. Med. Chem.* **2018**, *61*, 1382–1414. (b) Fosgerau, K.; Hoffmann, T. Peptide Therapeutics: Current Status and Future Directions. *Drug Discovery Today* **2015**, *20*, 122–128. (c) Albericio, F.; Kruger, H. G. Therapeutic peptides. *Future Med. Chem.* **2012**, *4*, 1527–1531. (d) Nestor Jr, J., Jr. The Medicinal Chemistry of Peptides. *Curr. Med. Chem.* **2009**, *16*, 4399–4418.
- For a selected review, see (a1) El-Faham, A.; Albericio, F. Peptide Coupling Reagents, More than a Letter Soup. *Chem. Rev.* **2011**, *111*, 6557–6602. For a selection of recent literature on coupling reagents, see: (a) Li, Y.; Li, J.; Bao, G.; Yu, C.; Liu, Y.; He, Z.; Wang, P.; Ma, W.; Xie, J.; Sun, W.; Wang, R. NDTP Mediated Direct Rapid Amide and Peptide Synthesis without Epimerization. *Org. Lett.* **2022**, *24*, 1169–1174. (b) Wang, Z.; Wang, X.; Wang, P.; Zhao, J. Allenone-Mediated Racemization/Epimerization-Free Peptide Bond Formation and Its Application in Peptide Synthesis. *J. Am. Chem. Soc.* **2021**, *143*, 10374–10381. (c) Ren, J.-W.; Tong, M.-N.; Zhao, Y.-F.; Ni, F. Synthesis of Dipeptide, Amide, and Ester without Racemization by Oxalyl Chloride and Catalytic Triphenylphosphine Oxide. *Org. Lett.* **2021**, *23*, 7497–7502. (d) Aspin, S. J.; Taillemaud, S.; Cyr, S.; Charette, A. B. 9-Silafluorenyl Dichlorides as Chemically Ligating Coupling Agents and Their Application in Peptide Synthesis. *Angew. Chem., Int. Ed.* **2016**, *55*,



13833–13837. (e) Hu, L.; Xu, S.; Zhao, Z.; Yang, Y.; Peng, Z.; Yang, M.; Wang, C.; Zhao, J. Ynamides as Racemization-Free Coupling Reagents for Amide and Peptide Synthesis. *J. Am. Chem. Soc.* **2016**, *138*, 13135–13138.

(5) Goodman, M.; Levine, L. Peptide Synthesis via Active Esters. IV. Racemization and Ring-Opening Reactions of Optically Active Oxazolones. *J. Am. Chem. Soc.* **1964**, *86*, 2918–2922.

(6) For a selection of recent reviews, see: (a) Pattabiraman, V. R.; Bode, J. W. Rethinking Amide Bond Synthesis. *Nature* **2011**, *480*, 471–479. (b) de Figueiredo, R. M.; Suppo, J.-S.; Campagne, J.-M. Nonclassical Routes for Amide Bond Formation. *Chem. Rev.* **2016**, *116*, 12029–12122. (c) Ojeda-Porras, A.; Gamba-Sanchez, D. Recent Developments in Amide Synthesis Using Nonactivated Starting Materials. *J. Org. Chem.* **2016**, *81*, 11548–11555. (d) Narendar Reddy, T.; Beatriz, A.; Jayathirtha Rao, V.; de Lima, D. P. Carbonyl Compounds' Journey to Amide Bond Formation. *Chem. Asian J.* **2019**, *14*, 344–388. (e) Sabatini, M. T.; Boulton, L. T.; Sneddon, H. F.; Sheppard, T. D. A Green Chemistry Perspective on Catalytic Amide Bond Formation. *Nat. Catal.* **2019**, *2*, 10–17. (f) Hollanders, K.; Maes, B. U. W.; Ballet, S. A New Wave of Amide Bond Formations for Peptide Synthesis. *Synthesis* **2019**, *51*, 2261–2277. (g) Massolo, E.; Pirola, M.; Benaglia, M. Amide Bond Formation Strategies: Latest Advances on a Dateless Transformation. *Eur. J. Org. Chem.* **2020**, *2020*, 4641–4651. (h) Todorovic, M.; Perrin, D. M. Recent Developments in Catalytic Amide Bond Formation. *Pept. Sci.* **2020**, *112*, 1–18. (i) Tosi, E.; de Figueiredo, R. M.; Campagne, J.-M. Enantioselective Catalytic C-H Amidations: An Highlight. *Catalysts* **2021**, *11*, 471–487.

(7) For selected reviews, see: (a) Han, S.-Y.; Kim, Y.-A. Recent Development of Peptide Coupling Reagents in Organic Synthesis. *Tetrahedron* **2004**, *60*, 2447–2467. (b) Montalbetti, C. A. G. N.; Falque, V. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61*, 10827–10852. (c) Valeur, E.; Bradley, M. Amide Bond Formation: Beyond the Myth of Coupling Reagents. *Chem. Soc. Rev.* **2009**, *38*, 606–631. (d) Joullie, M. M.; Lassen, K. M. Evolution of Amide Bond Formation. *ARKIVOC* **2010**, *2010*, 189–250. (e) Lanigan, R. M.; Sheppard, T. D. Recent Developments in Amide Synthesis: Direct Amidation of Carboxylic Acids and Transamidation Reactions. *Eur. J. Org. Chem.* **2013**, *2013*, 7453–7465. (f) Dunetz, J. R.; Magano, J.; Weisenburger, G. A. Large-Scale Applications of Amide Coupling Reagents for the Synthesis of Pharmaceuticals. *Org. Process Res. Dev.* **2016**, *20*, 140–177.

(8) Chen, W.; Shao, J.; Hu, M.; Yu, W.; Giulianotti, M. A.; Houghten, R. A.; Yu, Y. A Traceless Approach to Amide and Peptide Construction from Thioacids and Dithiocarbamate-Terminal Amines. *Chem. Sci.* **2013**, *4*, 970–976.

(9) (a) Pourvali, A.; Cochranet, J. R.; Hutton, C. A. A New Method for Peptide Synthesis in the N-C Direction: Amide Assembly through Silver-Promoted Reaction of Thioamides. *Chem. Commun.* **2014**, *50*, 15963–15966. (b) Hutton, C. A.; Shang, J.; Wille, U. Synthesis of Peptides by Silver-Promoted Coupling of Carboxylates and Thioamides: Mechanistic Insight from Computational Studies. *Chem.-Eur. J.* **2016**, *22*, 3163–3169. (c) Thombare, V. J.; Hutton, C. A. Rapid, Traceless, AgI-Promoted Macrocyclization of Peptides Possessing an N-Terminal Thioamide. *Angew. Chem., Int. Ed.* **2019**, *58*, 4998–5002.

(10) (a) Zhu, Y.-P.; Mampuy, P.; Sergeyev, S.; Ballet, S.; Maes, B. U. W. Amine Activation: N-Arylamino Acid Amide Synthesis from Isothioureas and Amino Acids. *Adv. Synth. Catal.* **2017**, *359*, 2481–2498. (b) Zhu, Y.-P.; Sergeyev, S.; Franck, P.; Orru, R. V. A.; Maes, B. U. W. Amine Activation: Synthesis of N-(Hetero)arylamides from Isothioureas and Carboxylic Acids. *Org. Lett.* **2016**, *18*, 4602–4605.

(11) Suppo, J.-S.; Subra, G.; Bergès, M.; de Figueiredo, R. M.; Campagne, J.-M. Inverse Peptide Synthesis via Activated  $\alpha$ -Aminoesters. *Angew. Chem., Int. Ed.* **2014**, *53*, 5389–5393.

(12) Suppo, J.-S.; de Figueiredo, R. M.; Campagne, J.-M. Dipeptide Syntheses via Activated  $\alpha$ -Aminoesters. *Org. Synth.* **2015**, *92*, 296–308.

(13) (a) Batey, R. A.; Santhakumar, V.; Yoshina-Ishii, C.; Taylor, S. D. An Efficient New Protocol for the Formation of Unsymmetrical Tri- and Tetrasubstituted Ureas. *Tetrahedron Lett.* **1998**, *39*, 6267–6270. (b) Batey, R. A.; Yoshina-Ishii, C.; Taylor, S. D.; Santhakumar, V. A New Protocol for the Formation of Carbamates and Thiocarbamates Using Carbamoyl Imidazolium Salts. *Tetrahedron Lett.* **1999**, *40*, 2669–2672. (c) Grzyb, J. A.; Batey, R. A. Carbamoylimidazolium Salts as Diversification Reagents: An Application to the Synthesis of Tertiary Amides from Carboxylic Acids. *Tetrahedron Lett.* **2003**, *44*, 7485–7488. (d) Grzyb, J. A.; Shen, M.; Yoshina-Ishii, C.; Chi, W.; Brown, R. S.; Batey, R. A. Carbamoylimidazolium and Thiocarbamoylimidazolium Salts: Novel Reagents for the Synthesis of Ureas, Thioureas, Carbamates, Thiocarbamates and Amides. *Tetrahedron* **2005**, *61*, 7153–7175. (e) Duspara, P. A.; Islam, Md. S.; Lough, A. J.; Batey, R. A. Synthesis and Reactivity of N-Alkyl Carbamoylimidazoles: Development of NMethyl-Carbamoylimidazole as a Methyl Isocyanate Equivalent. *J. Org. Chem.* **2012**, *77*, 10362–10368.

(14) Important to mention, during the synthesis of amides by Batey and co-workers, only secondary amines were used as substrates for the activation step with the imidazolium salt CBMIT giving rise to tertiary amides; see ref 13c.

(15) The synthesis of imidazolium salts **4** were somehow difficult to be achieved in our hands. In these particular cases, due to the instability of the intermediates formed, derivatives **1** were not isolated; the coupling reactions were performed in a sequential one-pot procedure.

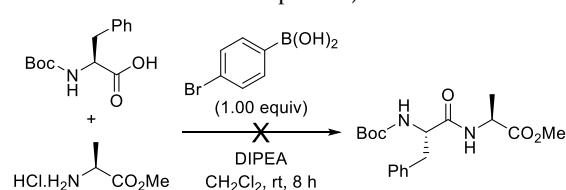
(16) Wu, J.; Sun, W.; Xia, H.-G.; Sun, X. A Facile and Highly Efficient Route to  $\alpha$ -Amino Phosphonates via Three-Component Reactions Catalyzed by  $\text{Mg}(\text{ClO}_4)_2$  or Molecular Iodine. *Org. Bioorg. Chem.* **2006**, *4*, 1663–1666.

(17) Bartoli, G.; Bosco, M.; Dalpozzo, R.; Marcantoni, E.; Mas-saccesi, M.; Rinaldi, S.; Sambri, L.  $\text{Mg}(\text{ClO}_4)_2$  as a Powerful Catalyst for the Acylation of Alcohols under Solvent-Free Conditions. *Synlett* **2003**, *1*, 39–42.

(18) Lawson, J. R.; Melen, R. L. Recent Developments and Applications of Lewis Acidic Boron Reagents. *Organometallic Chemistry*, **2017**; Vol. *41*, pp 1–27.

(19) Dimitrijević, E.; Taylor, M. S. Organoboron Acids and Their Derivatives as Catalysts for Organic Synthesis. *ACS Catal.* **2013**, *3*, 945–962.

(20) A control reaction was performed demonstrating that even though a stoichiometric amount of boronic acid was required, the amine function needed to be activated by CDI (the reaction does not work with free- $\text{NH}_2$  amine partner).



(21) For the first application of MW irradiation in solid-phase peptide synthesis, see: (a) Yu, H. M.; Chen, S. T.; Wang, K. T. Enhanced Coupling Efficiency in Solid-Phase Peptide Synthesis by



- Microwave Irradiation. *J. Org. Chem.* **1992**, *57*, 4781–4784. For selected reviews, see: (b) Pedersen, S. L.; Tofteng, A. P.; Malik, L.; Jensen, K. J. Microwave Heating in Solid-Phase Peptide Synthesis. *Chem. Soc. Rev.* **2012**, *41*, 1826–1844. (c) Caddick, S.; Fitzmaurice, R. Microwave Enhanced Synthesis. *Tetrahedron* **2009**, *65*, 3325–3355.
- (22) de Figueiredo, R. M.; Suppo, J.-S.; Midrier, C.; Campagne, J.-M. Sequential One-Pot Synthesis of Dipeptides through the Transient Formation of CDI-N-Protected  $\alpha$ -Aminoesters. *Adv. Synth. Catal.* **2017**, *359*, 1963–1968.
- (23) Carey, J. S.; Laffan, D.; Thomson, C.; Williams, M. T. Analysis of the Reactions Used for the Preparation of Drug Candidate Molecules. *Org. Biomol. Chem.* **2006**, *4*, 2337–2347.
- (24) Roughley, S. D.; Jordan, A. M. The Medicinal Chemist's Toolbox: An Analysis of Reactions Used in the Pursuit of Drug-Candidates. *J. Med. Chem.* **2011**, *54*, 3451–3479.
- (25) Bostrom, J.; Brown, D. G.; Young, R. J.; Keserü, G. Expanding the Medicinal Chemistry Synthetic Toolbox. *Nat. Rev. Drug Discovery* **2018**, *17*, 709–727.
- (26) (a) Malmgren, H.; Backstrom, B.; Solver, E.; Wennerberg, J. A Large Scale Process for the Preparation of Thymitaq. *Org. Process Res. Develop.* **2008**, *12*, 1195–1200. (b) For a review on the toxicity of metal compounds, see: Egorova, K. S.; Ananikov, V. P. Toxicity of Metal Compounds: Knowledge and Myths. *Organometallics* **2017**, *36*, 4071–4090.
- (27) Li, H.; Jiang, X.; Ye, Y.-h.; Fan, C.; Romoff, T.; Goodman, M. 3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT): A New Coupling Reagent with Remarkable Resistance to Racemization. *Org. Lett.* **1999**, *1*, 91–94.
- (28) (a) Anderson, G. W.; Young, R. W. The Use of Diester Chlorophosphites in Peptide Syntheses. Mixed Anhydrides. *J. Am. Chem. Soc.* **1952**, *74*, 5307–5309. (b) Anderson, G. W.; Blodinger, J.; Welcher, A. D. Tetraethyl Pyrophosphite as a Reagent for Peptide Syntheses. *J. Am. Chem. Soc.* **1952**, *74*, 5309–5312.
- (29) Paul, R.; Anderson, G. W. N,N'-Carbonyldiimidazole, a New Peptide Forming Reagent. *J. Am. Chem. Soc.* **1960**, *82*, 4596–4600.
- (30) (a) Subiros-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. Oxyma: An Efficient Additive for Peptide Synthesis to Replace the Benzotriazole-Based HOBt and HOAt with a Lower Risk of Explosion. *Chem.-Eur. J.* **2009**, *15*, 9394–9403. (b) El-Faham, A.; Al Marhoon, Z.; Abdel-Megeed, A.; Albericio, F. OxymaPure/DIC: an Efficient Reagent for the Synthesis of a Novel Series of 4-[2-(2-Acetylaminophenyl)-2-oxo-acetylamino] benzoyl Amino Acid Ester Derivatives. *Molecules* **2013**, *18*, 14747–14759.
- (31) (a) Otake, Y.; Shibata, Y.; Hayashi, Y.; Kawauchi, S.; Nakamura, H.; Fuse, S. N-Methylated Peptide Synthesis via Generation of an Acyl N-Methylimidazolium Cation Accelerated by a Brønsted Acid. *Angew. Chem., Int. Ed.* **2020**, *59*, 12925–12930. (b) Watson, R. B.; Butler, T. W.; DeForest, J. C. Preparation of Carbamates, Esters, Amides, and Unsymmetrical Ureas via Brønsted Acid-Activated N-Acyl Imidazoliums. *Org. Process Res. Dev.* **2021**, *25*, 500–506.
- (32) The oxazolone **27** can also be reversibly formed from intermediate acylimidazole **26**.
- (33) For NMR monitoring, reactions were carried out in the absence of both CuBr<sub>2</sub> and HOBt for solubility and overlap signals simplification.
- (34) Independently, for shift signals displacement identification, we have synthesized and isolated the isocyanate **24** and synthesized and observed on the crude <sup>1</sup>H NMR the acylimidazole intermediate **26**.
- (35) The chemical shift observed of 124.4 ppm is in agreement with the one described in the literature (124.9 ppm in CDCl<sub>3</sub>). Fulmer, G.R.; Miller, A. J. M.; Sherden, N. H.; Gottlieb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. NMR Chemical Shifts of Trace Impurities: Common Laboratory Solvents, Organics, and Gases in Deuterated Solvents Relevant to the Organometallic Chemist. *Organometallics* **2010**, *29*, 2176–2179.
- (36) Vaidyanathan, R.; Kalthod, V. G.; Ngo, D. P.; Manley, J. M.; Lapekas, S. P. Amidations Using N,N'-Carbonyldiimidazole: Remarkable Rate Enhancement by Carbon Dioxide. *J. Org. Chem.* **2004**, *69*, 2565–2568.
- (37) Jiang, Y.-Y.; Liu, T.-T.; Zhang, R.-X.; Xu, Z.-Y.; Sun, X.; Bi, S. Mechanism and Rate-Determining Factors of Amide Bond Formation through Acyl Transfer of Mixed Carboxylic-Carbamic Anhydrides: A Computational Study. *J. Org. Chem.* **2018**, *83*, 2676–2685.
- (38) Rao, I. N.; Boruah, A.; Kumar, S. K.; Kunwar, A. C.; Devi, A. S.; Vyas, K.; Ravikumar, K.; Iqbal, J. Synthesis and Conformational Studies of Novel Cyclic Peptides Constrained into a 310 Helical Structure by a Heterochiral D-Pro-L-Pro Dipeptide Template. *J. Org. Chem.* **2004**, *69*, 2181–2184.
- (39) Chen, H.; He, M.; Wang, Y.; Zhai, L.; Cui, Y.; Li, Y.; Li, Y.; Zhou, H.; Hong, X.; Deng, Z. Metal-Free Direct Amidation of Peptidyl Thiol Esters with  $\alpha$ -Amino Acid Esters. *Green Chem.* **2011**, *13*, 2723–2726.
- (40) George, J.; Kim, H. Y.; Oh, K. Substituted Pyrrololactams via Ring Expansion of Spiro-2H-pyrroles from Intermolecular Alkyne-Isocyanide Click Reactions. *Org. Lett.* **2017**, *19*, 628–631.
- (41) (a) Campagne, J.-M.; Coste, J.; Jouin, P. (1H-Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium Hexafluorophosphate- and (1H-Benzotriazol-1-yloxy)tripyrrolidinophosphonium Hexafluorophosphate-Mediated Activation of Monophosphonate Esters: Synthesis of Mixed Phosphonate Diesters, the Reactivity of the Benzotriazolyl Phosphonic Esters vs the Reactivity of the Benzotriazolyl Carboxylic Esters. *J. Org. Chem.* **1995**, *60*, 5214–5223. (b) Yu, W.; Yang, S.; Xiong, F.; Fan, T.; Feng, Y.; Huang, Y.; Junkai, F.; Wang, T. Palladium-Catalyzed Carbonylation of Benzyllic Ammonium Salts to Amides and Esters via C–N Bond Activation. *Org. Biomol. Chem.* **2018**, *16*, 3099–3103.
- (42) Nielsen, D. U.; Neumann, K.; Taaning, R. H.; Lindhardt, A. T.; Modvig, A.; Skrydstrup, T. Palladium-Catalyzed Double Carbonylation Using Near Stoichiometric Carbon Monoxide: Expedient Access to Substituted <sup>13</sup>C<sub>2</sub>-Labeled Phenethylamines. *J. Org. Chem.* **2012**, *77*, 6155–6165.
- (43) Alandini, N.; Buzzetti, L.; Candish, L.; Collins, K. D.; Favi, G.; Melchiorre, P.; Schulte, T. Amide Synthesis by Nickel/Photoredox-Catalyzed Direct Carbamoylation of (Hetero)Aryl Bromides. *Angew. Chem., Int. Ed.* **2020**, *59*, 5248–5253.
- (44) Charvieux, A.; Le Moigne, L.; Borrego, L. G.; Duguet, N.; Metay, E. Solvent-Free N-Alkylation of Amides with Alcohols Catalyzed by Nickel on Silica–Alumina. *Eur. J. Org. Chem.* **2019**, *2019*, 6842–6846.
- (45) Tam, E. K. W.; Rita, L. Y.; Chen, A. 2-Furanylborynic Acid as an Effective Catalyst for the Direct Amidation of Carboxylic Acids at Room Temperature. *Eur. J. Org. Chem.* **2015**, *2015*, 1100–1107.

- (46) Watson, A. J. A.; Wakeham, R. J.; Maxwell, A. C.; Williams, J.M.J. Ruthenium-Catalysed Oxidation of Alcohols to Amides using a Hydrogen Acceptor. *Tetrahedron* **2014**, *70*, 3683–3690.
- (47) Bai, J.; Zambron, B. K.; Vogel, P. Amides in One Pot from Carboxylic Acids and Amines via Sulfinylamides. *Org. Lett.* **2014**, *16*, 604–607.
- (48) Dumas, A. M.; Molander, G. A.; Bode, J. W. Amide-Forming Ligation of Acyltrifluoroborates and Hydroxylamines in Water. *Angew. Chem., Int. Ed.* **2012**, *51*, 5683–5686.
- (49) Chapman, R. S. L.; Tibbetts, J. D.; Bull, S. D. 1,1-Diacyloxy-1-Phenylmethanes as Versatile N-Acylating Agents for Amines. *Tetrahedron* **2018**, *74*, 5330–5339.
- (50) Ortega-Rojas, M. A.; Rivera-Ramirez, J. D.; Ávila-Ortiz, C. G.; Juaristi, E.; Gonzalez-Muñoz, F.; Castillo, E.; Escalante, J. One-Pot Lipase-Catalyzed Enantioselective Synthesis of (R)-(-)-N-Benzyl-3(benzylamino)butanamide: The Effect of Solvent Polarity on Enantioselectivity. *Molecules* **2017**, *22*, 2189.