

Peptide Synthesis

Inverse Peptide Synthesis via Activated α -Aminoesters**

Jean-Simon Suppo, Gilles Subra, Matthieu Bergès, Renata Marcia de Figueiredo,* and Jean-Marc Campagne*

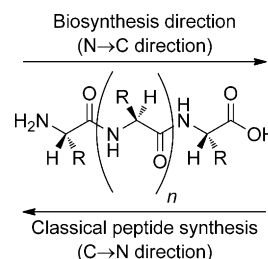
Dedicated to Professor Jacques Coste

Abstract: A mild, practical, and simple procedure for peptide-bond formation is reported. Instead of activation of the carboxylic acid functionality, the reaction involves an unprecedented use of activated α -aminoesters. The method provides a straightforward entry to dipeptides and was effective when a sensitive cysteine residue was used, as no epimerization was detected in this case. The applicability of this method to iterative peptide synthesis was illustrated by the synthesis of a model tetrapeptide in the challenging reverse $N \rightarrow C$ direction.

Besides their crucial physiological life functions, peptides are attracting increasing attention as drugs^[1] owing to their associated low toxicity and high specificity, but also as valuable auxiliaries for targeting,^[2] vectorization,^[3] and diagnostic purposes. Furthermore, as a result of their very nearly unlimited diversity of activity, structure, and functions, they are also now studied for other promising applications in materials science, such as artificial silks,^[4] hydrogels,^[5] supported catalysts, and biocompatible matrices for cell growth.

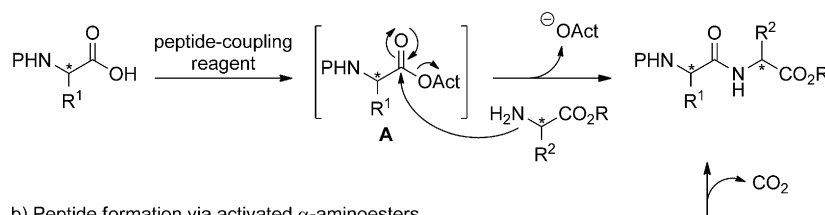
Whereas peptides are naturally built up in the $N \rightarrow C$ direction^[6] (ribosomal synthesis), they are chemically synthesized from the C-terminal to the N-terminal end (Scheme 1) either by means of solid-phase peptide synthesis (SPPS) or by classical solution synthesis.^[7] Traditionally, peptides are assembled through the use of coupling reagents,^[8] whereby the carboxylic acid moiety of

an amino acid is transformed into a good leaving group (X = O-acyl, urea, oxyphosphonium, guanidinium/uronium, F,

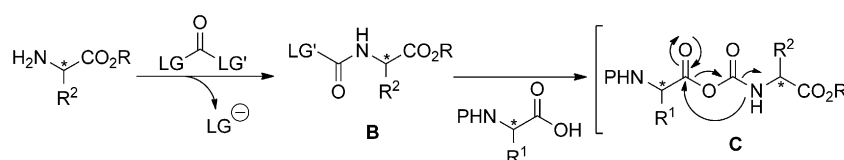


Scheme 1. Ribosomal and chemical peptide synthesis.

a) Traditional way to construct peptides through acid activation



b) Peptide formation via activated α -aminoesters



Scheme 2. a) Conventional peptide-bond formation through carboxylic activation. b) This study: peptide-bond formation via activated α -aminoesters. P = amine-protecting group, R^1 and R^2 = amino acid side chains, R = acid-protecting group, LG = leaving group, Act = activation agent.

[*] J.-S. Suppo, M. Bergès, Dr. R. Marcia de Figueiredo, Prof. Dr. J.-M. Campagne
Institut Charles Gerhardt Montpellier (ICGM), UMR 5253 CNRS-UM2-UM1-ENSCM, Ecole Nationale Supérieure de Chimie
8 Rue de l'Ecole Normale, 34296 Montpellier Cedex 5 (France)
E-mail: renata.marcia_de_figueiredo@enscm.fr
jean.marc-campagne@enscm.fr

Prof. Dr. G. Subra
Institut des Biomolécules Max Mousseron (IBMM)
UMR 5257 CNRS-UM2-UM1, LAPP-Faculté de Pharmacie
Avenue Charles Flahault, Bat E, 34093 Montpellier Cedex 5 (France)

[**] We gratefully thank the Agence Nationale de la Recherche for financial support (NIPS project ANR JCJC): ANR-12-JS07-0008-01 and Jonathan Brand for preliminary studies on this project.

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201402147>.

etc.), thus enabling the amine functionality of a second amino acid to react with it to form the amide bond (Scheme 2a). Although highly efficient, these coupling techniques suffer from some drawbacks. For example, epimerization could be observed during the introduction of sensitive amino acids (histidine and cysteine) and, more importantly, these conditions force peptide synthesis in the $C \rightarrow N$ direction. In fact, the synthesis of a whole peptide in the reverse way (i.e. the $N \rightarrow C$ direction) would imply the C-terminal activation of a peptide fragment highly prone to epimerization (through the formation of oxazolone^[9] intermediates) and thus the formation of an intractable diastereoisomeric mixture.

A number of innovative strategies for the synthesis of peptides have been reported: decarboxylative condensation

of *N*-alkyl hydroxylamines and α -ketoacids,^[10] the use of nitroalkanes as acyl-anion equivalents,^[11] and various strategies involving amino thioacids as the acyl donor with isonitriles,^[12] with azides,^[13] and very recently, with dithiocarbamate terminal amines.^[14,15] However, all these methods require the prior modification of both amino acids, which considerably limits their attractiveness. The reaction of carboxylic acids with isocyanates also constitutes an interesting alternative,^[16] although the need to prepare the sensitive isocyanate reaction partners hinders the application of this methodology in peptide synthesis.^[17]

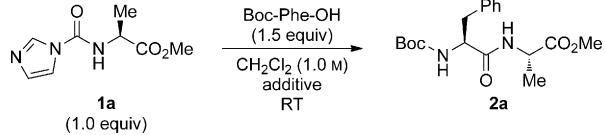
Herein, we describe our preliminary results towards a new route for the preparation of peptides from readily available, bench-stable, activated α -aminoesters under mild and neutral conditions (Scheme 2b). In contrast to all previously reported methodologies, we first activate the amino group, instead of the classical carboxylic activation; therefore, we expect to palliate the epimerization process that is generally observed in conventional coupling methods.

The starting point of our study was the synthesis of a suitable (simple, high-yielding, and stable) activated α -aminoester. After some unfruitful experiments involving *p*-nitrophenol derivatives,^[18] we turned to commercially available *N,N'*-carbonyldiimidazole (CDI),^[19,20] which has been broadly employed as a carboxyl-activating agent, and has proved its efficiency in peptide coupling over conventional peptide synthesis.

We prepared intermediates **1** in high yield by the treatment of free α -aminoesters with CDI and triethylamine in a CH_2Cl_2 /THF mixture (see the Supporting Information for details).^[21] Small amounts (< 7 %) of symmetrical ureas were occasionally observed as side products in the crude material. Compounds **1** were readily purified by a simple filtration through a short plug of silica gel. Moreover, they are stable for months when stored at 4 °C.

With these compounds in hand, we carried out a first experiment with compound **1a** and Boc-Phe-OH to validate our approach (Table 1). In the presence of triethylamine in CH_2Cl_2 , no dipeptide formation was observed (Table 1, entry 1). However, and quite intriguingly, when the reaction was carried out in the absence of a base, we observed the formation of the expected dipeptide **2a** in 63 % yield (Table 1, entry 2). Brief solvent screening revealed that the reaction proceeded smoothly in CH_2Cl_2 as well as in CH_3NO_2 , another polar aprotic solvent, whereas the solvent DMF, which is traditionally suitable for supported peptide synthesis, gave lower yields (Table 1, entries 3 and 4). Next, to improve the yield, we evaluated the addition of some additives (10 mol %). The same reaction profile was observed when imidazole was added to the reaction mixture (Table 1, entry 5). However, in the presence of HOBt or PTSA, we observed higher yields (Table 1, entries 6 and 7). After evaluating the use of Brønsted acids, we also tested the viability of the reaction in the presence of Lewis acid additives. Among many Lewis acids tested, CuBr_2 proved to be as efficient as HOBt or PTSA (Table 1, entry 8). Interestingly, when used together as additives, CuBr_2 and HOBt had a synergic effect on the condensation reaction, and the yield

Table 1: Peptide-bond formation by the use of activated α -aminoesters.^[a]



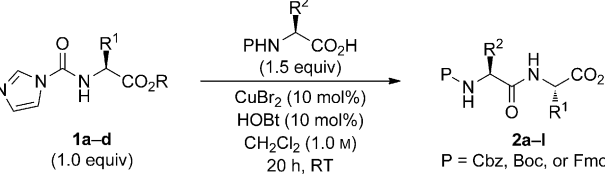
Entry	Additive	Reaction time [h]	Yield [%] ^[b]
1	Et_3N (1.0 equiv)	14	NR
2	—	14	63
3	—	20	23 ^[c]
4	—	20	62 ^[d]
5	imidazole (10 mol %)	20	53
6	HOBt (10 mol %)	20	75
7	PTSA (10 mol %)	20	77
8	CuBr_2 (10 mol %)	20	77
9	CuBr_2 /HOBt (10 mol %)	20	90

[a] Reactions were performed on a 0.51 mmol scale and proceeded to full conversion as judged by TLC. [b] Yield of the isolated product after purification by column chromatography. [c] DMF was used as the solvent (1.0 M). [d] CH_3NO_2 was used as the solvent (1.0 M). DMF = dimethylformamide, NR = no reaction, HOBt = 1-hydroxybenzotriazole, PTSA = 4-methylbenzenesulfonic acid.

of the expected dipeptide **2a** was improved to 90 % (Table 1, entry 9).

Having established that amide-bond formation can take place when activated α -aminoesters are used instead of activation of the acid functionality, we next focused on the scope of this reaction (Table 2) and attempted the synthesis of a range of dipeptides. Under the optimized conditions with CuBr_2 /HOBt, all reactions proceeded cleanly, and the expected dipeptides were isolated in moderate-to-good yields. Coupling efficiency was not significantly affected by the different side chains (Table 2, entries 4, 6, 8, and 11). Interestingly, the reaction proved to be fully compatible with

Table 2: Scope of the reaction for the synthesis of dipeptides.



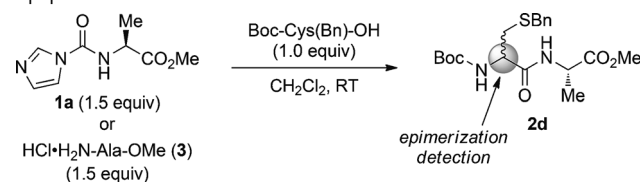
Entry	Imi-AA-OR 1	Dipeptide 2	Yield [%] ^[a]
1	Imi-Ala-OMe (1a)	Boc-Phe-Ala-OMe (2a)	90
2	1a	Fmoc-Phe-Ala-OMe (2b)	65
3	1a	Boc-Pro-Ala-OMe (2c)	61
4	1a	Boc-Cys(Bn)-Ala-OMe (2d)	67
5	1a	Cbz-Met-Ala-OMe (2e)	84
6	1a	Fmoc-Lys(Alloc)-Ala-OMe (2f)	54
7	Imi-Val-OMe (1b)	Boc-Phe-Val-OMe (2g)	62
8	Imi-Gly-OEt (1c)	Boc-Asp(OBn)-Gly-OEt (2h)	74
9	1c	Fmoc-Ala-Gly-OEt (2i)	66
10	Imi-Met-OMe (1d)	Cbz-Phe-Met-OMe (2j)	68
11	1d	Boc-Trp-Met-OMe (2k)	70
12	1d	Fmoc-Ala-Met-OMe (2l)	52

[a] Isolated yield after purification by column chromatography. Boc = *tert*-butoxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl, Bn = benzyl, Cbz = benzyloxycarbonyl, Alloc = allyloxycarbonyl.

Boc-, Fmoc- and Cbz-protected amino acids,^[22] an important issue for the method to be applied in routine peptide synthesis. Slightly lower, but still respectable, yields were observed with Fmoc derivatives, mainly owing to their lower solubility under these reaction conditions (Table 2, entries 6 and 12).

Having developed a straightforward route to dipeptides, we next investigated the use of our methodology in cases in which classical coupling reagents suffer from some limitations. If our hypothesis is right, and our method does not imply an activation of the carboxylate functionality, some of the side reactions commonly observed with classical peptide reagents should be alleviated. For example, it is well-known that the activation of cysteine residues may occur with some degree of epimerization as a result of the transient formation of a stabilized enol derivative.^[23] Indeed, in our hands, when Boc-Cys(Bn)-OH was coupled to H-Ala-OMe (**3**) after preactivation for 1 min in CH₂Cl₂ with BOP,^[24] 12 % epimerization was observed (35 % epimerization after preactivation for 5 min; Table 3, entries 2 and 3).^[25] As suggested by one

Table 3: Racemization study on the synthesis of Boc-Cys(Bn)-Ala-OMe dipeptide.

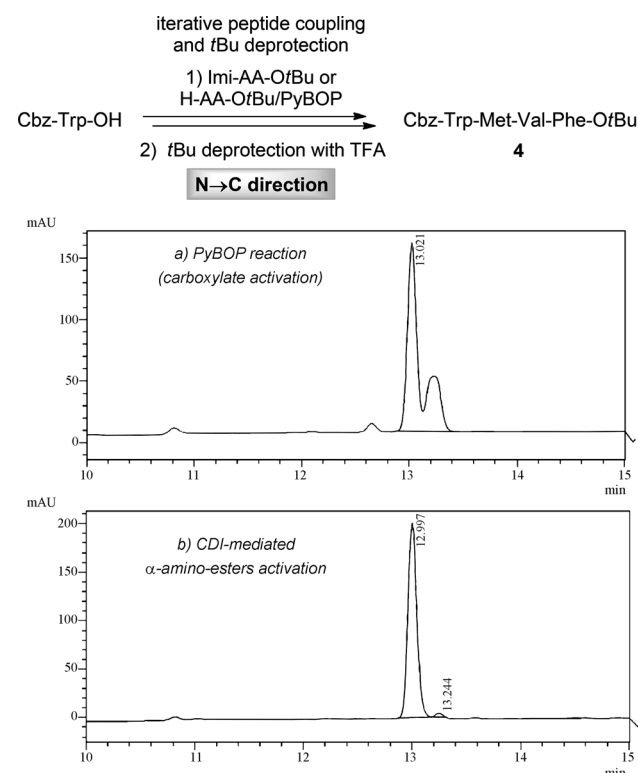
			
Entry	Conditions	Yield [%] ^[a]	Epimerization [%] ^[b]
1	this study ^[c]	67	0
2	BOP, DIPEA (4.0 equiv) ^[d]	87	35
3	BOP, DIPEA (4.0 equiv) ^[d,e]	80	12
4	BOP, DIPEA (2.0 equiv) ^[d]	41	7 ^[f]
5	BOP, DIPEA (1.0 equiv), H ₂ N-Ala-OMe ^[d]	27	1.5 ^[f]
6	CDI carboxylic acid activation ^[g]	80	8

[a] Yield of the isolated product after column chromatography. [b] The degree of racemization of the crude material was determined by HPLC on a chiral stationary phase (for details and chromatograms, see the Supporting Information). [c] Reaction conditions: **1a**, Boc-Cys(Bn)-OH, CuBr₂/HOBT (10 mol %), 20 h. [d] Reaction conditions: BOP (1.5 equiv), DIPEA, preactivation for 5 min, **3**, 2 h. [e] Preactivation for 1 min. [f] The epimerization level was estimated owing to the complexity of the HPLC chromatogram of the crude product. [g] Reaction conditions: Boc-Cys(Bn)-OH, CDI (1.0 equiv), preactivation for 5 min, **3**, 20 h. BOP = (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate.

referee, several amounts of base were surveyed, and indeed, lowering the amount of the base was very effective in minimizing epimerization, but also deleterious to the chemical yield of the BOP-mediated coupling reactions (Table 3, entries 4 and 5). Alternatively, the condensation of Imi-Ala-OMe (**1a**) with Boc-Cys(Bn)-OH in CH₂Cl₂ gave the expected dipeptide in 67 % yield with no detectable epimerization in the crude product (Table 3, entry 1). Notably, when CDI was used as the activating group of the carboxyl

functionality, 8 % epimerization was observed (Table 3, entry 6). This observation further corroborates our mechanistic proposal (Scheme 2b), as no cysteine epimerization was observed under our reaction conditions.

We next checked whether this protocol could be extended to longer peptides. As a proof of concept, the synthesis of Cbz-Trp-Met-Val-Phe-OTfBu (**4**) tetrapeptide was performed in the N→C direction. As mentioned above, N→C peptide synthesis is not recommended, since the activation of the C-terminal carboxylic acid of an elongating peptide fragment is a source of potential epimerization through oxazolone formation (see below). For the sake of comparison, the same tetrapeptide **4** was assembled by PyBOP^[26] activation in the same N→C direction (Scheme 3).



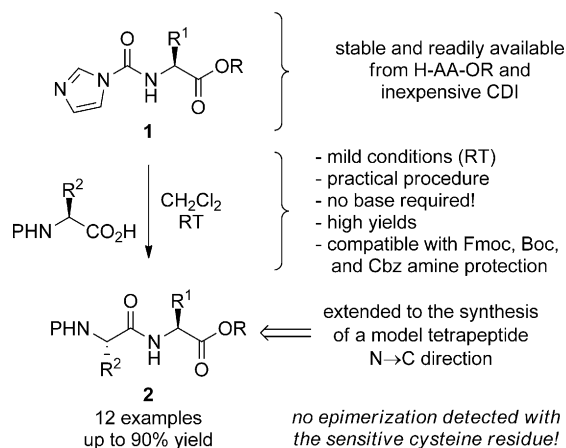
Scheme 3. Iterative N→C synthesis of tetrapeptide **4**. a,b) HPLC profiles of the tetrapeptides synthesized by the PyBOP strategy (carboxylate activation; a) and by CDI-mediated α-aminoester activation (b). For details, see the Supporting Information. PyBOP = (benzotriazol-1-yl-oxy)tripyrrolidinophosphonium hexafluorophosphate.

The synthesis proceeded in five linear steps in a straightforward manner (Scheme 3). Indeed, the reactions were very easy to set up and, conveniently, after each peptide coupling, a classical workup of the crude material, followed by filtration through a short plug of silica gel, was carried out to eliminate excess reagents and by-products. The crude compounds were clean enough to be used in the next step (i.e. TFA-mediated tBu deprotection; see the Supporting Information for details).

At the end of the synthesis, comparable yields and HPLC profiles of the crude product (see the Supporting Information) were observed for the two methods, carboxylic acid activation with PyBOP and CDI-mediated α-aminoester

activation. Purification of the product by preparative HPLC gave **4** in 35 and 25% overall yield for the PyBOP and CDI strategies, respectively. The HPLC profiles were similar and very clean (Scheme 3). A minor product (probably resulting from epimerization) was observed in the PyBOP synthesis, whereas this product was absent in the CDI synthesis.^[27]

In summary, we have presented herein a practical and simple procedure for the formation of peptide bonds on the basis of a new mode of activation of amino acids (Scheme 4). A prominent feature of this strategy is the unprecedented use



Scheme 4. CDI-mediated activation of α -aminoester derivatives.

of activated α -aminoesters instead of classical carboxylic activation. Our activated amino acid residues (i.e. Imi-AA-OR) are readily obtained from α -aminoesters and readily available and inexpensive CDI. Additionally, they are stable and can be stored at 4°C for several months. Peptide-bond formation proceeds under mild conditions in the absence of a base and is compatible with common *N*-urethane protecting groups (i.e. Fmoc, Boc and Cbz). In this study, preliminary investigations on the susceptibility to racemization were carried out with the sensitive amino acid residue cysteine [that is, with Boc-Cys(Bn)-OH]. The promising results obtained prompted us to further evaluate our method with the synthesis of a tetrapeptide in the reverse N→C direction. The desired tetrapeptide was isolated in good overall yield and purity. The extension of this new strategy to the synthesis of longer peptide targets and to solid-phase peptide synthesis (SPPS) is currently under investigation.

Experimental Section

Preparation of dipeptides **2**: Copper bromide (10 mol %), 1-hydroxybenzotriazole hydrate (10 mol %), and P-AA²-OH (1.5 equiv) were successively added to a solution of the Imi-AA¹-OR derivative (1.0 equiv) in CH₂Cl₂ (1.0 M) at room temperature (22°C), and the resulting blue suspension was stirred at this temperature for 20 h. After the completion of the reaction (TLC monitoring), the mixture was washed once with aqueous HCl (0.5 N). The aqueous phase was then extracted with CH₂Cl₂ (2 ×). The organic layers were combined, washed with saturated aqueous NaHCO₃ and brine, and dried over

MgSO₄, and the solvents were evaporated. Purification by column chromatography on silica gel afforded the corresponding dipeptide **2**.

Received: February 6, 2014

Published online: April 24, 2014

Keywords: amides · amino acids · epimerization · peptides · synthetic methods

- [1] a) “Therapeutic Peptides under the Spotlight”: C. Pichereau, C. Allary in *European Biopharmaceutical Review (EBR)*, January **2005**; b) V. Marx, *Chem. Eng. News* **2005**, 83, 17; c) L. M. Jarvis, *Chem. Eng. News* **2006**, 84, 23; d) P. Vlieghe, V. Lisowski, J. Martinez, M. Khrestchatsky, *Drug Discovery Today* **2010**, 15, 40; e) A. Sharman, S. Arora, *Res. J. Pharm. Biol. Chem. Sci.* **2011**, 2, 778.
- [2] N. Svensen, J. J. Díaz-Mochón, K. Dhaliwal, S. Planonh, M. Dewar, J. D. Armstrong, M. Bradley, *Angew. Chem.* **2011**, 123, 6257; *Angew. Chem. Int. Ed.* **2011**, 50, 6133.
- [3] C. M. Yamazaki, I. Nakase, H. Endo, S. Kishimoto, Y. Mashiyama, R. Masuda, S. Futaki, T. Koide, *Angew. Chem.* **2013**, 125, 5607; *Angew. Chem. Int. Ed.* **2013**, 52, 5497.
- [4] Y. Hidala, K.-I. Kontani, R. Taniguchi, M. Saiki, S. Yokoi, K. Yukuhiro, H. Yamaguchi, M. Miyazawa, *Biopolymers* **2011**, 96, 222.
- [5] R. P. Nagarkar, J. P. Schneider, *Methods Mol. Biol.* **2008**, 474, 61.
- [6] For selected references on inverse SPPS, see: a) A. Johansson, E. Åkerblom, K. Ersmark, G. Linderberg, A. Hallberg, *J. Comb. Chem.* **2000**, 2, 496; b) B. H. Lipshutz, Y.-J. Shin, *Tetrahedron Lett.* **2001**, 42, 5629; c) N. Thieriet, F. Guibé, F. Albericio, *Org. Lett.* **2000**, 2, 1815; d) W. G. Gutheil, Q. Xu, *Chem. Pharm. Bull.* **2002**, 50, 688; e) R. Sasubilli, W. G. Gutheil, *J. Comb. Chem.* **2004**, 6, 911; f) J. Martinez, P. Zajdel, M. Pawlowski, G. Subra, *PCT Int. Appl.*, WO2010023295 (A1) 20100304, **2010**.
- [7] a) J. Jones, *The Chemical Synthesis of Peptides*, Oxford Science Publications, Oxford, **1991**; b) “Synthesis of Peptides and Peptidomimetics”: M. Goodman, A. Felix, L. Moroder, C. Toniolo, *Houben-Weyl, Methods in Organic Chemistry, Vol E22a*, Thieme, Stuttgart, New York, **2002**; c) N. Sewald, H.-D. Jakubke, *Peptides: Chemistry and Biology*, Wiley-VCH, Weinheim, **2002**; d) F. Guzmán, S. Barberis, A. Illanes, *Electron. J. Biotechnol.* **2007**, 10, 279.
- [8] For selected reviews, see: a) F. Albericio, R. Chinchilla, D. J. Dodsworth, C. Nájera, *Org. Prep. Proced. Int.* **2001**, 33, 203; b) S.-Y. Han, Y.-A. Kim, *Tetrahedron* **2004**, 60, 2447; c) C. A. G. N. Montalbetti, V. Falque, *Tetrahedron* **2005**, 61, 10827; d) E. Valeur, M. Bradley, *Chem. Soc. Rev.* **2009**, 38, 606; e) A. El-Faham, F. Albericio, *Chem. Rev.* **2011**, 111, 6557; f) M. M. Joullié, K. M. Lassen, *ARKIVOC* **2010**, 189; g) M. Amblard, J.-A. Fehrentz, J. Martinez, G. Subra, *Mol. Biotechnol.* **2006**, 33, 239; h) R. M. Lanigan, T. D. Sheppard, *Eur. J. Org. Chem.* **2013**, 7453.
- [9] M. Goodman, L. Levine, *J. Am. Chem. Soc.* **1964**, 86, 2918, and references therein.
- [10] J. W. Bode, R. M. Fox, K. D. Baucom, *Angew. Chem.* **2006**, 118, 1270; *Angew. Chem. Int. Ed.* **2006**, 45, 1248.
- [11] B. Shen, D. M. Makley, J. N. Johnston, *Nature* **2010**, 465, 1027.
- [12] T. Wang, S. J. Danishefsky, *J. Am. Chem. Soc.* **2012**, 134, 13244.
- [13] a) R. V. Kolakowski, N. Shangguan, R. R. Sauers, L. J. Williams, *J. Am. Chem. Soc.* **2006**, 128, 5695; b) G. H. Hakmelah, G. Just, *Tetrahedron Lett.* **1980**, 21, 2119.
- [14] W. Chen, J. Shao, M. Hu, W. Yu, M. A. Giulianotti, R. A. Houghten, Y. Yu, *Chem. Sci.* **2013**, 4, 970.
- [15] For strategies involving the synthesis of nonpeptidic carbox-amides, see: a) S. H. Cho, E. J. Yoo, I. Bae, S. Chang, *J. Am. Chem. Soc.* **2005**, 127, 16046; b) M. P. Cassidy, J. Raushel, V. V.

- Fokin, *Angew. Chem.* **2006**, *118*, 3226; *Angew. Chem. Int. Ed.* **2006**, *45*, 3154; c) J. Gao, G.-W. Wang, *J. Org. Chem.* **2008**, *73*, 2955; d) W.-J. Yoo, C.-J. Li, *J. Am. Chem. Soc.* **2006**, *128*, 13064; e) L. U. Nordström, H. Vogt, R. Madsen, *J. Am. Chem. Soc.* **2008**, *130*, 17672; f) N. Gernigon, R. M. Al-Zoubi, D. G. Hall, *J. Org. Chem.* **2012**, *77*, 8386.
- [16] a) F. Jäger, P. Stelzel, P. Thamm, G. Wendlberger, *Houben-Weyl, Vol. 15/2*, Thieme, Stuttgart, **1974**, p. 184; b) A. C. Schuemaker, R. W. Hoffmann, *Synthesis* **2001**, 243; c) K. Sasaki, D. Crich, *Org. Lett.* **2011**, *13*, 2256; d) D. Crich, K. Sasaki, *Org. Lett.* **2009**, *11*, 3514; e) I. S. Blagbrough, N. E. Mackenzie, C. Ortiz, A. I. Scott, *Tetrahedron Lett.* **1986**, *27*, 1251.
- [17] For a few examples, see: a) S. Goldschmidt, M. Wick, *Justus Liebigs Ann. Chem.* **1952**, 575, 217; b) S. Goldschmidt, H. Lautenschlager, *Justus Liebigs Ann. Chem.* **1953**, 580, 68; c) G. Losse, H. Vietmeyer, *J. Prakt. Chem.* **1966**, *32*, 204.
- [18] Matthieu Bergès, Master's Thesis, Université Montpellier 2, **2010**.
- [19] For the use of *N*-methylimidazolium salt derivatives in the synthesis of ureas, thioureas, carbamates, and thiocarbamates, see: a) R. A. Batey, V. Santhakumar, C. Yoshina-Ishii, S. D. Taylor, *Tetrahedron Lett.* **1998**, *39*, 6267; b) R. A. Batey, C. Yoshina-Ishii, S. D. Taylor, V. Santhakumar, *Tetrahedron Lett.* **1999**, *40*, 2669; c) J. A. Grzyb, R. A. Batey, *Tetrahedron Lett.* **2003**, *44*, 7485; d) J. A. Grzyb, M. Shen, C. Yoshina-Ishii, W. Chi, R. S. Brown, R. A. Batey, *Tetrahedron* **2005**, *61*, 7153.
- [20] R. Paul, G. W. Anderson, *J. Am. Chem. Soc.* **1960**, *82*, 4596.
- [21] a) S. T. Heller, R. Sarpong, *Org. Lett.* **2010**, *12*, 4572; b) S. T. Heller, T. Fu, R. Sarpong, *Org. Lett.* **2012**, *14*, 1970.
- [22] A. Isidro-Llobet, M. Álvarez, F. Albericio, *Chem. Rev.* **2009**, *109*, 2455.
- [23] H. Li, X. Jiang, Y.-h. Ye, C. Fan, T. Romoff, M. Goodman, *Org. Lett.* **1999**, *1*, 91.
- [24] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, *16*, 1219.
- [25] For additional details, NMR spectra, and HPLC chromatograms for all reactions in the racemization study on the synthesis of Boc-Cys(Bn)-Ala-OMe dipeptide, see the Supporting Information.
- [26] a) J. Coste, D. Le-Nguyen, B. Castro, *Tetrahedron Lett.* **1990**, *31*, 205; b) E. Frérot, J. Coste, A. Pantaloni, M.-N. Dufour, P. Jouin, *Tetrahedron* **1991**, *47*, 259.
- [27] LC/MS chromatograms showed an isobaric profile for tetrapeptide **4** derived from the PyBOP reaction and its major impurity.