



**HAL**  
open science

## Solvent-Free Synthesis of Peptides

Valérie Declerck, Pierrick Nun, Jean Martinez, Frédéric Lamaty

► **To cite this version:**

Valérie Declerck, Pierrick Nun, Jean Martinez, Frédéric Lamaty. Solvent-Free Synthesis of Peptides. *Angewandte Chemie International Edition*, 2009, 48, pp.9318-9321. 10.1002/anie.200903510 . hal-00433100

**HAL Id: hal-00433100**

**<https://hal.science/hal-00433100>**

Submitted on 4 Jan 2021

**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.

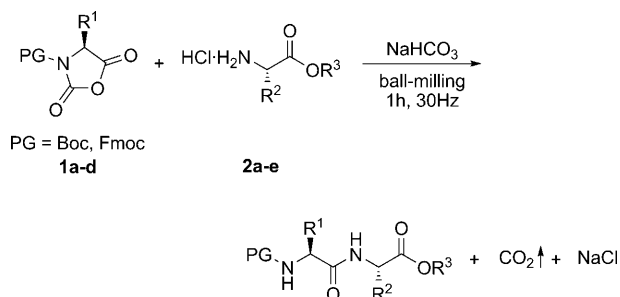
# Solvent-Free Synthesis of Peptides\*\*

Valérie Declerck, Pierrick Nun, Jean Martinez, and Frédéric Lamaty\*

The chemistry of peptides has been continuously growing during the last few decades. Peptides are now not only considered as pharmacological tools but also as active pharmaceutical ingredients, in connection to their high therapeutic index and low toxicity.<sup>[1]</sup> The market for therapeutic bulk peptides is expected to grow rapidly in the next few years.<sup>[2]</sup> In spite of the well-established procedures<sup>[3]</sup> for peptide synthesis by chemical ways, that is, stepwise synthesis in solution and solid-phase peptide synthesis, one of the major problems related to peptide preparation concerns the huge amount of solvent needed, particularly for synthesis on solid supports (2000–5000 kg for a large peptide). There is still a need to explore efficient, convenient, and environmentally friendly methods for peptide synthesis, particularly when the time for the scale-up of peptide production comes.

The field of “green chemistry” has recently grown at a rapid pace. Some major thematic areas have emerged: use of alternative feedstock and of innocuous reagents, employment of natural processes, use of substitute solvents, design of safer chemicals, development of alternative reaction conditions, and minimization of energy consumption.<sup>[4]</sup> One particularly active area is in the use of substitute solvents such as aqueous, ionic, fluoruous, or supercritical fluids to replace volatile organic and chlorinated solvents and to solve the problems of treating or recycling solvent waste.<sup>[5]</sup> An alternative approach would be to carry out chemical reactions in the absence of solvent.<sup>[6]</sup> Techniques such as mixing, grinding, or ball-milling have proved their efficiency in the field of organic chemistry in the solid state.<sup>[7]</sup> We report herein a new strategy for the preparation of peptides under solvent-free conditions by using ball-milling technology.<sup>[8]</sup> This strategy has been exemplified by the synthesis of the sweetener dipeptide H–Asp–Phe–OMe (aspartame).

We have studied the coupling of urethane-protected  $\alpha$ -amino acid *N*-carboxyanhydride (UNCA) derivatives **1** with  $\alpha$ -amino acids, amides, or esters, while keeping in mind that all of these compounds have to remain in the solid state under the ball-milling conditions<sup>[9]</sup> (Scheme 1). UNCAs are



**Scheme 1.** Synthesis of dipeptides under solvent-free conditions. Boc: *tert*-butoxycarbonyl; Fmoc: 9-fluorenylmethoxycarbonyl; PG: protecting group.

activated forms of amino acids that have proved to be useful in peptide<sup>[10]</sup> and organic synthesis.<sup>[11]</sup>

The reaction was tested for the coupling of Boc–Val–NCA (**1a**, 1 equiv) with HCl–H–Leu–OMe (**2a**, 1 equiv) in the presence of NaHCO<sub>3</sub> (1.5 equiv) in a hardened-steel vessel with steel balls. The vessel was agitated for 1 h at a frequency of 30 Hz. Analysis of the reaction mixture after this time showed the exclusive presence of the dipeptide Boc–Val–Leu–OMe, obtained in quantitative yield. To our knowledge, this represents the first example of peptide bond formation in solvent-free conditions. The results obtained with various UNCAs and amino acid derivatives are presented in Table 1.

The various UNCA derivatives do not present the same reactivity profile. Boc–Val–NCA (**1a**) reacted quantitatively with amino acid derivatives to yield the corresponding dipeptides (Table 1, entries 1–5) whereas Fmoc–Val–NCA (**1b**) gave lower conversions (Table 1, entries 6, 7, 9, and 10), except when coupling with HCl–H–Ala–OMe (**2c**; Table 1, entry 8). Excellent conversions and yields were achieved with Boc–Phe–NCA (**1c**; Table 1, entries 12–14), except for the reaction with HCl–H–Phe–OMe (Table 1, entry 15). Recovery of the product from the reaction vessel was less efficient in some cases and resulted in inferior yields (Table 1, entries 12 and 14). An amino ester, AcOH–H–Gly–O*t*Bu was also tested and gave satisfying results (Table 1, entry 16). By contrast, the free amino acid did not react. It is worth noting that better results were obtained with freshly prepared starting materials. By reaching the maximum capacity of the ball-mill used in this study, up to 500 mg of dipeptides could be prepared. The reaction mixture was recovered directly from the milling jar, washed with water to remove the inorganic salts, and dried to provide the clean dipeptide. In the case of an incomplete reaction, maybe due to the physicochemical state of the reaction mixture (Table 1, entry 15), addition of water to the reaction mixture resulted in the opening of the UNCA. Insoluble in water, the protected dipeptide Boc–Phe–Phe–

[\*] Dr. V. Declerck,<sup>[†]</sup> P. Nun, Prof. J. Martinez, Dr. F. Lamaty  
Institut des Biomolécules Max Mousseron (IBMM)  
UMR 5247 CNRS-UM1-UM2, Université Montpellier II  
Place Eugène Bataillon, 34095 Montpellier Cedex 5 (France)  
Fax: (+33) 4-6714-4866  
E-mail: frederic.lamaty@univ-montp2.fr

[†] Current address:  
Laboratoire de Synthèse Organique et Méthodologie (LSOM)  
ICMMO UMR 8182, Université Paris-Sud 11  
15 Rue Georges Clemenceau, 91405 Orsay Cedex (France)

[\*\*] We thank the MENRT, the CNRS, and the Fondation d'Entreprise EADS for financial support.

**Table 1:** Examples of dipeptides synthesized under solvent-free conditions.

Entry	UNCA	Amino ester	Dipeptide/Tripeptide	Conversion [%] <sup>[a]</sup>	Yield [%]
1	Boc-Val-NCA ( <b>1a</b> )	HCl·H-Leu-OMe ( <b>2a</b> )	Boc-Val-Leu-OMe	100	87
2		HCl·H-Leu-O $t$ Bu ( <b>2b</b> )	Boc-Val-Leu-O $t$ Bu	97	85
3		HCl·H-Ala-OMe ( <b>2c</b> )	Boc-Val-Ala-OMe	100	100
4		HCl·H-Ala-O $t$ Bu ( <b>2d</b> )	Boc-Val-Ala-O $t$ Bu	100	100
5		HCl·H-Phe-OMe ( <b>2e</b> )	Boc-Val-Phe-OMe	100	88
6	Fmoc-Val-NCA ( <b>1b</b> )	HCl·H-Leu-OMe ( <b>2a</b> )	Fmoc-Val-Leu-OMe	90	–
7		HCl·H-Leu-O $t$ Bu ( <b>2b</b> )	Fmoc-Val-Leu-O $t$ Bu	92	–
8		HCl·H-Ala-OMe ( <b>2c</b> )	Fmoc-Val-Ala-OMe	100	76
9		HCl·H-Ala-O $t$ Bu ( <b>2d</b> )	Fmoc-Val-Ala-O $t$ Bu	78	–
10		HCl·H-Phe-OMe ( <b>2e</b> )	Fmoc-Val-Phe-OMe	93	–
11	Boc-Phe-NCA ( <b>1c</b> )	HCl·H-Leu-OMe ( <b>2a</b> )	Boc-Phe-Leu-OMe	85	–
12		HCl·H-Leu-O $t$ Bu ( <b>2b</b> )	Boc-Phe-Leu-O $t$ Bu	100	70
13		HCl·H-Ala-OMe ( <b>2c</b> )	Boc-Phe-Ala-OMe	99	79
14		HCl·H-Ala-O $t$ Bu ( <b>2d</b> )	Boc-Phe-Ala-O $t$ Bu	100	73
15		HCl·H-Phe-OMe ( <b>2e</b> )	Boc-Phe-Phe-OMe	58	55
16		AcOH·H-Gly-O $t$ Bu ( <b>2f</b> )	Boc-Phe-Gly-O $t$ Bu	100	90
17		Boc-Val-NCA ( <b>1a</b> )	HCl·H-Ala-Gly-OMe	Boc-Val-Ala-Gly-OMe	100

[a] The reaction mixture was recovered in AcOEt and washed with water and analyzed by <sup>1</sup>H NMR spectroscopy. Calculated conversion numbers are given with a  $\pm 2\%$  error.

OMe was recovered by filtration and drying, with an excellent yield based on the conversion (Table 1, entry 15).

We showed unambiguously that coupling occurred in the solid state in the presence of the three solids (UNCA, amino ester, and base) and not during the analysis process. For this purpose and as an example, we analyzed in detail the ball-milling of Boc-Phe-NCA (**1c**), HCl·H-Ala-OMe (**2c**), and NaHCO<sub>3</sub> (Table 1, entry 13). Firstly, ball-milling of the starting materials in the absence of NaHCO<sub>3</sub> did not yield the dipeptide Boc-Phe-Ala-OMe (analyzed by IR and solid-state <sup>13</sup>C NMR spectroscopies). Furthermore, when the UNCA was ball-milled alone in the presence of NaHCO<sub>3</sub>, the reaction did not occur, nor was degradation observed. Consequently, all three solids were necessary for the coupling reaction. Two analytical samples that did not involve any solvent were used for analysis: the starting materials and the crude reaction mixture taken directly from the ball-mill jar. Solid-state IR analysis showed the disappearance of the characteristic bands of Boc-Phe-NCA (**1c**;  $\tilde{\nu} = 1817$  and  $1872\text{ cm}^{-1}$ ) and the formation of the peptide bond (characteristic amide carbonyl bands in the solid state at  $\tilde{\nu} = 1624$  and  $1655\text{ cm}^{-1}$ ). We then used cross-polarization/magic-angle spinning (CP/MAS) <sup>13</sup>C NMR analysis (Figure 1). The solid-state NMR analysis of the crude mixture, with no further treatment, clearly showed signals corresponding to the exclusive formation of the expected dipeptide, with consumption of the starting materials. The carbonyl region in Figure 1c shows the disappearance of the characteristic signal of **1c** (labeled as f in Figure 1a), which corresponds to the loss of CO<sub>2</sub> during the reaction. Complete conversion of the amino ester **2c** cannot be proved from Figure 1c because of overlapping of the signal, but complementary analysis (mass spectrometry) of the same sample confirmed the absence of **2c**. The dipeptide cannot be formed during this analysis because solid-state <sup>13</sup>C NMR spectroscopy has shown the absence of **1c**. Again, no reaction was detected in the absence of NaHCO<sub>3</sub>. These results establish without any ambiguity that the reaction took place in the solid state.

The reaction kinetics were studied at different frequencies (10, 20, and 30 Hz). Independently of the frequency, all reactions were completed, albeit with an expected longer reaction time at lower speeds (5 h at 10 Hz and 2.5 h at 20 Hz, compared with 1.25 h at 30 Hz). For each vibration speed, the profile showed apparent zero-order kinetics, in agreement with a solid-solid-state reaction mechanism.<sup>[12]</sup>

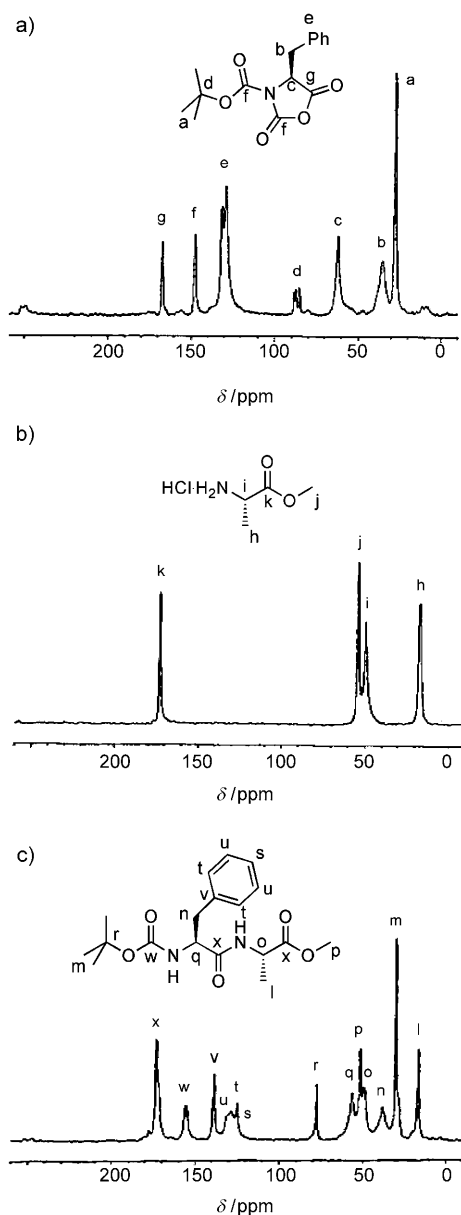
The possible epimerization that could occur during the solid-solid coupling reaction was evaluated. An HPLC analysis of an authentic sample of Boc-D-Phe-L-Ala-OMe prepared under known nonracemizing conditions was compared to the HPLC profile of the dipeptide product from the reaction of **1c** and **2c**. No racemization was detected by HPLC analysis.

As a proof of concept and to show the suitability of this method for making larger peptides, we synthesized the tripeptide Boc-Val-Ala-Gly-OMe from HCl·H-Ala-Gly-OMe, which was coupled to Boc-Val-NCA (**1a**), as depicted in Scheme 2. Complete conversion and a quantitative yield of the expected tripeptide (Table 1, entry 17, and Scheme 2) were obtained, with the coupling step being performed without solvent and according to the standard procedure.

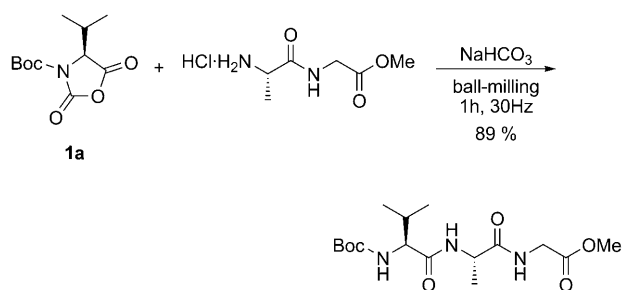
To demonstrate the application of this method, we prepared the commercially attractive dipeptide aspartame or  $\alpha$ -L-aspartyl-L-phenylalanine methyl ester. Aspartame is a nutritive sweetener approximately 150 times sweeter than sucrose.<sup>[13]</sup> To the best of our knowledge, aspartame had not been previously prepared by the UNCA route, either in the presence or absence of solvent.<sup>[14]</sup>

By using the method described above, we considered the coupling of Boc-Asp(O $t$ Bu)-NCA (**1d**) and HCl·H-Phe-OMe (**2e**). Acid-labile Boc and  $t$ Bu protecting groups were chosen because they can be simultaneously cleaved under acidic conditions.

First, UNCA **1d** was prepared according to the synthetic method depicted in Scheme 3. The key step in the preparation of **1d** was the cyclization of compound **3** by the Vilsmeier's salt method (Scheme 3).<sup>[15]</sup>

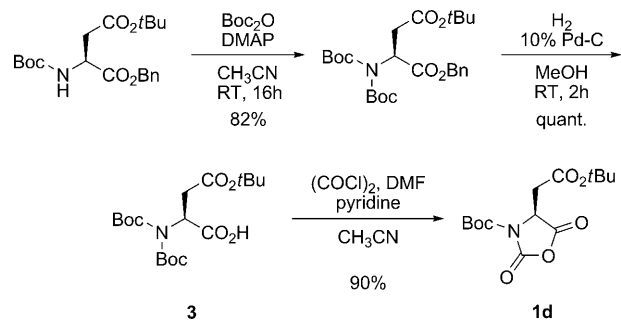


**Figure 1.** CP/MAS solid-state  $^{13}\text{C}$  NMR analysis of a) Boc-Phe-NCA (**1c**), b)  $\text{HCl}\cdot\text{H}_2\text{N-Ala-OMe}$  (**2c**), and c) crude Boc-Phe-Ala-OMe.

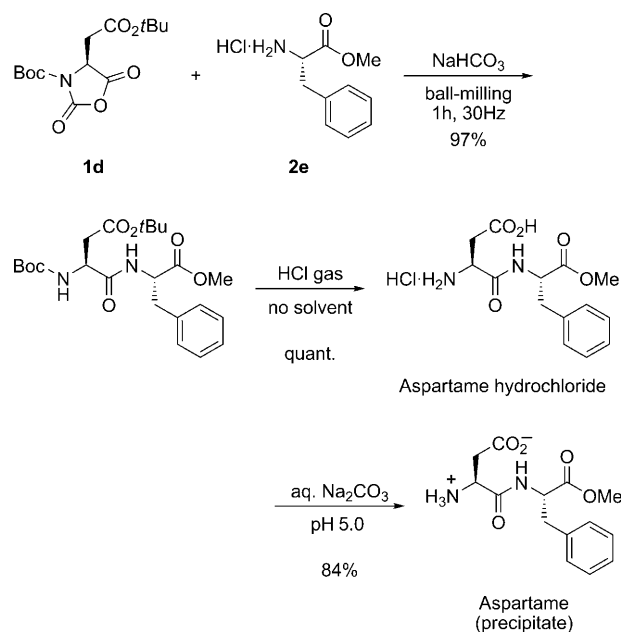


**Scheme 2.** Synthesis of a tripeptide under solvent-free conditions.

The procedure described above for the preparation of dipeptides was then used for the preparation of aspartame (Scheme 4). Ball-milling of Boc-Asp(OtBu)-NCA (**1d**) and



**Scheme 3.** Synthesis of UNCA **1d**. DMAP: 4-dimethylaminopyridine; DMF: *N,N*-dimethylformamide.



**Scheme 4.** Synthesis of the sweetener aspartame under solvent-free conditions.

$\text{HCl}\cdot\text{H-Phe-OMe}$  (**2e**) for 1 h yielded the protected dipeptide as the only product, along with inorganic salts. The Boc and *t*Bu protecting groups were then cleaved under acidic conditions in a gas-solid reaction, without solvent. The protected dipeptide in solid form was placed on a fritted glass, and HCl gas was blown through for 3 h in the absence of solvent to yield the hydrochloride form of aspartame. The yield for the two steps is quantitative, and removal of the Boc and *t*Bu protecting groups under acidic conditions afforded only volatile by-products. The hydrochloride of aspartame was dissolved in water and then neutralized to the isoelectric point (pH 5.0) with an aqueous solution of  $\text{Na}_2\text{CO}_3$ .<sup>[15]</sup> The resulting aspartame precipitated as a white powder and was filtered and dried in vacuo (84% yield).

In summary, a straightforward, high-yielding method for the preparation of peptides (including dipeptides and a tripeptide) with no epimerization and in the absence of solvent has been reported. This method involves the reaction of a UNCA with an amino ester (or amino acid amide). It was illustrated by the preparation of the aspartame sweetener.

Aspartame was obtained in pure form in three steps from Boc–Asp(OtBu)–NCA without the use of organic solvents, either for the coupling reaction or the removal of the protecting groups, and with the generation of volatile organic by-products and water-soluble inorganic salts. The only purification step consisted of a final precipitation from water to recover solid aspartame. This methodology was also exemplified by the synthesis of the tripeptide Boc–Val–Ala–Gly–OMe with good purity and excellent yield.

Received: June 29, 2009

Revised: September 8, 2009

Published online: October 30, 2009

**Keywords:** ball-milling · peptides · peptide synthesis · solid-state reactions · solvent-free reactions

- 
- [1] a) A. K. Sato, M. Viswanathan, R. B. Kent, C. R. Wood, *Curr. Opin. Biotechnol.* **2006**, *17*, 638–642; b) G. Mustata, S. M. Dinh, *Crit. Rev. Ther. Drug Carrier Syst.* **2006**, *23*, 111–135.
- [2] V. Marx, *Chem. Eng. News* **2005**, *83*, 17–24.
- [3] *Synthesis of Peptides and Peptidomimetics, Vol. E22* (Eds.: M. Goodman, A. Felix, L. Moroder, C. Toniolo), Thieme, Stuttgart, **2002**.
- [4] *Green Chemistry: Frontiers in Benign Chemical Syntheses and Processes* (Eds.: P. T. Anastas, T. C. Williamson), Oxford University Press, New York, **1999**.
- [5] J. H. Clark, S. J. Tavener, *Org. Process Res. Dev.* **2007**, *11*, 149–155.
- [6] K. Tanaka, F. Toda, *Chem. Rev.* **2000**, *100*, 1025–1074.
- [7] F. Toda, *Top. Curr. Chem.* **2005**, *254*, 1–305.
- [8] J. Martinez, F. Lamaty, V. Declerck, WO 2008125418, **2008**.
- [9] a) G. Kaupp, J. Schmeyers, J. Boy, *Tetrahedron* **2000**, *56*, 6899–6911; b) G. Kaupp, M. R. Naimi-Jamal, V. Stepanenko, *Chem. Eur. J.* **2003**, *9*, 4156–4160.
- [10] W. D. Fuller, M. Goodman, F. R. Naider, Y.-F. Zhu, *Biopolymers* **1996**, *40*, 183–205.
- [11] a) J. Hang, S.-K. Tian, L. Tang, L. Deng, *J. Am. Chem. Soc.* **2001**, *123*, 12696–12697; b) J. Hang, H. Li, L. Deng, *Org. Lett.* **2002**, *4*, 3321–3324; c) T. R. Elworthy, J. P. Dunn, J. H. Hogg, G. Lam, Y. D. Saito, T. M. P. C. Silva, D. Stefanidis, W. Woroniecki, E. Zhornisky, A. S. Zhou, K. Klumpp, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6344–6387; d) E. Dardennes, S. Labano, N. S. Simpkins, C. Wilson, *Tetrahedron Lett.* **2007**, *48*, 6380–6383; e) P. Audinn, C. Pothion, J.-A. Fehrentz, A. Loffet, J. Martinez, J. Paris, *J. Chem. Res. Synop.* **1999**, 282–283; f) P. Chevallet, J.-A. Fehrentz, K. Kiec-Kononowicz, C. Devin, J. Castel, A. Loffet, J. Martinez, *Letts. Pept. Sci.* **1996**, *2*, 297–300; g) J.-A. Fehrentz, C. Genu-Dellac, M. Amblard, F. Winternitz, A. Loffet, J. Martinez, *J. Pept. Sci.* **1995**, *1*, 124–131; h) J.-A. Fehrentz, C. Pothion, J.-C. Califano, A. Loffet, J. Martinez, *Tetrahedron Lett.* **1994**, *35*, 9031–9034; i) J.-A. Fehrentz, E. Bourdel, J. C. Califano, O. Chaloin, C. Devin, P. Garrouste, A.-C. Lima-Leite, M. Llinares, F. Rieunier, J. Vizavonna, F. Winternitz, A. Loffet, J. Martinez, *Tetrahedron Lett.* **1994**, *35*, 1557–1560; j) J.-A. Fehrentz, J.-C. Califano, M. Amblard, A. Loffet, J. Martinez, *Tetrahedron Lett.* **1994**, *35*, 569–571; k) M. Mc Kiernan, J. Huck, J.-A. Fehrentz, M.-L. Roumestant, P. Viallefont, J. Martinez, *J. Org. Chem.* **2001**, *66*, 6541–6544; l) M. Paris, J.-A. Fehrentz, A. Heitz, A. Loffet, J. Martinez, *Tetrahedron Lett.* **1996**, *37*, 8489–8492; m) M. Paris, J.-A. Fehrentz, A. Heitz, J. Martinez, *Tetrahedron Lett.* **1998**, *39*, 1569–1572; n) M. Paris, C. Pothion, C. Michalak, J. Martinez, J.-A. Fehrentz, *Tetrahedron Lett.* **1998**, *39*, 6889–6890; o) C. Pothion, J.-A. Fehrentz, A. Aumelas, A. Loffet, J. Martinez, *Tetrahedron Lett.* **1996**, *37*, 1027–1030; p) C. Pothion, J.-A. Fehrentz, P. Chevallet, A. Loffet, J. Martinez, *Letts. Pept. Sci.* **1997**, *4*, 241–244.
- [12] G. Kaupp, *CrystEngComm* **2003**, *5*, 117–133.
- [13] R. H. Mazur, J. M. Schlatter, A. H. Goldkamp, *J. Am. Chem. Soc.* **1969**, *91*, 2684–2691.
- [14] J. S. Tou, B. D. Vineyard, *J. Org. Chem.* **1985**, *50*, 4982–4984.
- [15] R. Mita, T. Katoh, A. Yamaguchi, T. Oura, C. Higuchi, EP 196866, **1986** [*Chem. Abstr.* **1987**, *107*, 7612].
-