



HAL
open science

Peptide Mechanosynthesis by Direct Coupling of N -Protected α -Amino Acids with Amino Esters

Vincent Porte, Marion Thioly, Titouan Pigoux, Thomas-Xavier Métro, Jean
Martinez, Frédéric Lamaty

► **To cite this version:**

Vincent Porte, Marion Thioly, Titouan Pigoux, Thomas-Xavier Métro, Jean Martinez, et al.. Peptide Mechanosynthesis by Direct Coupling of N -Protected α -Amino Acids with Amino Esters. European Journal of Organic Chemistry, 2016, 2016 (21), pp.3505-3508. 10.1002/ejoc.201600617 . hal-02364118

HAL Id: hal-02364118

<https://hal.science/hal-02364118>

Submitted on 8 Feb 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.

Peptide Mechanochemistry by Direct Coupling of *N*-Protected α -Amino Acids with Amino Esters

Vincent Porte,^[a] Marion Thioly,^[a] Titouan Pigoux,^[a] Thomas-Xavier Métro*^[a]
Jean Martinez,^[a] and Frédéric Lamaty*^[a]

Abstract: In view of developing alternatives to classical peptide synthesis strategies that suffer from low efficacy and negative environmental impact, the reactivity of *N*-protected α -amino acids, amino esters, and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide was studied under liquid-assisted grinding (LAG) conditions. The optimal reaction conditions enabled the intensive and environmentally benign mechanochemistry of a

wide range of dipeptides that could be produced up to the multigram scale. The critical influence of the nature of the liquid additive on the course of the reaction was revealed. Combined with solvent-free *tert*-butoxycarbonyl deprotection steps, this approach was applied to the synthesis of tri- and tetrapeptides, thus opening the way to the synthesis of much longer peptides.

Introduction

Peptides are a promising class of drug candidates owing to their high efficiency, selectivity, and biodegradability.^[1] Unfortunately, their classical ways of production in solution or on solid support require huge amounts of volatile and toxic organic solvents (e.g., DMF, CH₂Cl₂, and 1-methylpyrrolidin-2-one) and bases (e.g., Et₃N, *N,N*-diisopropylethylamine, and piperidine).^[2] To lower the environmental impact of peptide synthesis, several strategies have been developed, including those involving the use of water as the solvent,^[3] the use of microwave heating under solvent-free conditions,^[4] the design of coupling reagents with improved safety profile,^[5] and the use of continuous-flow conditions.^[6] In addition to these strategies, we developed an original approach based on the use of ball-milling technology.^[7] Ball milling in organic synthesis has regained interest in recent years owing to the possibility, among others, to avoid the use of problematic organic solvents, to reduce the time of the reaction, and to isolate otherwise elusive reaction intermediates.^[8] Our latest developments in the utilization of ball-milling technology applied to the synthesis of peptides was based on the utilization of activated *N*-protected α -amino esters (e.g., *N*-carboxyanhydrides or hydroxysuccinimide esters),^[7] but the low commercial availability of these chemicals hampered the scope of the approach.

Results and Discussion

Continuing our efforts to provide the organic chemist with environmentally friendly and easily scalable methods,^[9] we envisioned to study the mechanically mediated coupling of non-activated α -amino acids with amino ester salts under elevated milling-load conditions to implement an intensive and environmentally sound peptide synthesis approach. Preliminary screening of coupling agents [e.g., *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC), (1-cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylaminomorpholinocarbenium hexafluorophosphate (COMU[®]), *N,N'*-diisopropylcarbodiimide (DIC), 1,1'-carbonyldiimidazole (CDI)] revealed the superiority of EDC, which was confirmed by the work of Štrukil and co-workers.^[10] Indeed, they utilized EDC·HCl to produce dipeptides in a ball mill by coupling α -amino acids with α -amino esters. Unfortunately, this approach was limited to the synthesis of four easily accessible dipeptides on a small scale (<100 mg of isolated dipeptide) and low milling load (ML: <83 mg mL⁻¹).^[11] In addition, the reaction required an extended length of time (3 h) and the use of corrosive and toxic 4-(dimethylamino)pyridine (DMAP) as the base and explosive nitromethane as the liquid additive. Similarly, Duangkamol and co-workers described the synthesis of dipeptides on a small scale (ca. 100 mg) by grinding *N*-protected α -amino acids with cyanuric chloride and CH₂Cl₂.^[12] Yet, both of these chemicals present highly undesirable safety profiles that are not compatible with an efficient and sustainable strategy for peptide synthesis.^[13] For our part, we selected the difficult coupling of two valine residues as a first model reaction in our quest for optimal reaction conditions. Thus, *p*TosOH·H-Val-OBn (*p*TosOH = *p*-toluenesulfonic acid), Z-Val-OH (1.2 equiv.) (Z = benzyloxy-carbonyl), NaH₂PO₄ (4.0 equiv.), and Oxyma (ethyl hydroxyiminocynoacetate, 1.2 equiv.) were introduced in a stainless steel (SS) grinding jar with one polytetrafluoroethylene (PTFE) ball and, if needed, a liquid additive (Figure 1).

[a] Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, Université de Montpellier, CNRS, ENSCM, Place Eugène Bataillon, 34095 Montpellier cedex 5, France
E-mail: thomas-xavier.metro@umontpellier.fr
frederic.lamaty@umontpellier.fr
<http://www.greenchem.univ-montp2.fr>

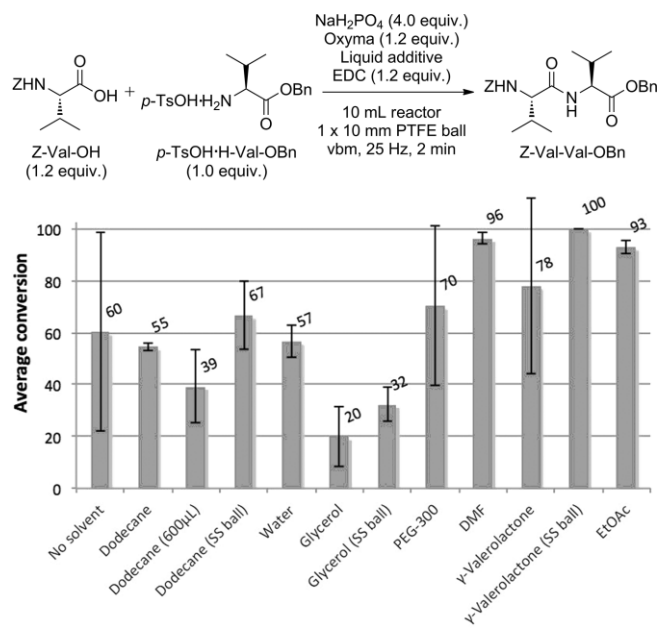


Figure 1. Liquid additive influence on average conversion and chemical homogeneity during the mechanochemistry of Z-Val-Val-OBn.

Oxyma was chosen as the epimerization suppressant owing to its advantageous safety profile.^[14,15] The use of a ball made of PTFE instead of SS was decided to avoid sparks that could arise from shocks between the metallic ball and the jar, and this allowed the scope of the reagents to be extended to those with very low flash points. The mixture was grinded for 2 min in a vibrating ball mill (vbm), EDC (1.2 equiv.) was added, and the jar was closed and agitated for another 2 min. To evaluate the chemical homogeneity inside the jar, the mixture was sampled in three different spots. Each of these samples was subjected to HPLC analysis to determine the average conversion. The standard deviation was calculated to serve as an indicator

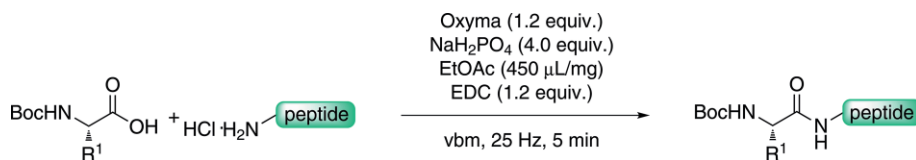
of chemical homogeneity. Without any liquid additive, the average conversion was low (60 %) and the standard deviation was very high (38 %), which indicated very low homogeneity (Figure 1). The utilization of a liquid additive was then envisaged to improve the homogeneity of the mixture. Liquid additives have been widely used in recent years,^[16] and yet the influence of their chemical nature on the course of a reaction has been studied in a very limited number of cases.^[17] First, the addition of apolar, aprotic dodecane (400 μ L) during the coupling led to a low conversion (55 %, Figure 1). The use of a larger volume of dodecane or a ball of higher density (i.e., SS) did not improve significantly the course of the reaction. Then, highly polar and protic liquids such as water, glycerol, and polyethylene glycol (PEG)-300 were envisaged to promote the reaction, but unfortunately, our attempts were met with no success. Polar and aprotic liquid additives were then evaluated: DMF furnished both an excellent average conversion (96 %) and a standard deviation (2 %). Unfortunately, DMF is a highly problematic solvent,^[18] for which other solvents with preferable safety profiles have been identified.^[19] Two of them, γ -valerolactone (GVL) and EtOAc, were envisaged under our conditions. To our delight, 100 % average conversion was obtained upon using GVL as the liquid additive along with a SS ball (Figure 1). Similarly, excellent average conversion (93 %) and standard deviation (3 %) were obtained upon using EtOAc as the liquid additive. Unlike GVL, the low flash point of EtOAc (-3 °C) led us to perform this reaction with a PTFE ball under an inert atmosphere (N_2) to prevent the formation of sparks (see above). These results are a clear indication that liquid-assisted grinding (LAG) is a very powerful strategy for the development of environmentally sound organic syntheses, as it allows merging of the best of a solvent-free synthesis (high speed of reaction) with the best of a solution-based synthesis (absence of mass-transfer limitations) while avoiding their respective drawbacks (mass-transfer limitations for solvent-free synthesis; slow kinetics and toxic solvents for solution-based synthesis).

Table 1. Examples of dipeptide synthesis by mechanochemistry.

Entry	N-Protected amino acid	Amino ester	Liquid additive	Dipeptide	Yield ^[a] [%]
1	Z-Val-OH	<i>p</i> TosOH·H-Val-OBn	GVL	Z-Val-Val-OBn	73
2	Boc-Phe-OH	HCl·H-Leu-OMe	GVL	Boc-Phe-Leu-OMe	62 ^[b]
3	Boc-Phe-OH	HCl·H-Gly-OMe	EtOAc	Boc-Phe-Gly-OMe	91 ^[c] (66) ^[d]
4	Boc-Phe-OH	HCl·H-Phe-OMe	EtOAc	Boc-Phe-Phe-OMe	77 ^[c]
5	Boc-Leu-OH·H ₂ O	<i>p</i> TosOH·H-Leu-OBn	EtOAc	Boc-Leu-Leu-OBn	70 (88) ^[d]
6	Boc-Leu-OH·H ₂ O	<i>p</i> TosOH·H-Leu-OBn	PEG-300	Boc-Leu-Leu-OBn	69 ^[e]
7	Boc-Ala-OH	HCl·H-Ala-OMe	EtOAc	Boc-Ala-Ala-OMe	63
8	Boc-Ile-OH·0.5H ₂ O	HCl·H-Ile-OMe	EtOAc	Boc-Ile-Ile-OMe	77
9	Z-Aib-OH	HCl·H-Val-OMe	EtOAc	Z-Aib-Val-OMe	89 ^[f]
10	Z-Aib-OH	HCl·H-Aib-OMe	EtOAc	Z-Aib-Aib-OMe	73
11	Z-Aib-OH	DBS·H-Pro-OrBu ^[g]	EtOAc	Z-Aib-Pro-OrBu	90 ^[h]
12	Boc-Arg(NO ₂)-OH	HCl·H-Leu-OMe	EtOAc	Boc-Arg(NO ₂)-Leu-OMe	88 ^[c]

[a] Yield of isolated product. ML = 105 mg mL⁻¹. [b] >99 % *de*. [c] EDC (1.7 equiv.), 15 min agitation. [d] ML = 432.5 mg mL⁻¹, 25 mL PTFE reactor, 20 mm PTFE ball, 5 min agitation. [e] 60 min agitation. [f] EDC (1.8 equiv.), 20 min agitation. [g] DBS = dibenzenesulfonamide. [h] EDC (1.8 equiv.), 10 min agitation.

Table 2. Tri- and tetrapeptides mechanosynthesis.



Entry	Boc amino acid	Amino ester	Product	Yield ^[a] [%]
1	Boc-Leu-OH·H ₂ O	HCl·H-(Leu) ₂ -OBn	Boc-(Leu) ₃ -OBn	65
2	Boc-Arg(Tos)-OH	HCl·H-(Leu) ₂ -OBn	Boc-Arg(Tos)-(Leu) ₂ -OBn	65
3	Boc-Glu(OCy)-OH	HCl·H-(Leu) ₂ -OBn	Boc-Glu(OCy)-(Leu) ₂ -OBn	51
4	Boc-Trp-OH	HCl·H-(Leu) ₂ -OBn	Boc-Trp-(Leu) ₂ -OBn	47
5	Boc-Ala-OH	HCl·H-Phe-Gly-OMe	Boc-Ala-Phe-Gly-OMe	52
6	Boc-Phe-OH	HCl·H-Phe-Gly-OMe	Boc-Phe-Phe-Gly-OMe	57
7	Boc-Pro-OH	HCl·H-(Leu) ₃ -OBn	Boc-Pro-(Leu) ₃ -OBn	74
8	Boc-Leu-OH·H ₂ O	HCl·H-(Leu) ₃ -OBn	Boc-(Leu) ₄ -OBn	91 ^[b]

[a] Yield of isolated product. [b] EDC (1.8 equiv.), 25 min agitation.

Upon using the best conditions (GVL along with a SS ball), Z-Val-Val-OBn was isolated in 73 % yield after only 10 min ball milling, classical acid–base extractions, followed by chromatography on silica gel to eliminate GVL (B.p. 207–208 °C) (Table 1, entry 1). Similarly, Boc-Phe-Leu-OMe was produced in 62 % yield with >99 % diastereomeric excess, as determined by HPLC analysis on a chiral stationary phase (Table 1, entry 2).

Once again, the use of GVL made purification on column chromatography inevitable. In contrast, the use of EtOAc for the coupling of Boc-Phe-OH with HCl·H-Gly-OMe furnished Boc-Phe-Gly-OMe in a very good yield of 91 % without requiring purification on silica gel (Table 1, entry 3). Upon performing the reaction at a much higher milling load (ML = 432.5 mg mL⁻¹), this reaction furnished 1.5 g of Boc-Phe-Gly-OMe in 66 % yield (Table 1, entry 3). The same strategy enabled the production of 2.2 g of Boc-Leu-Leu-OBn in 88 %, providing enough material to envisage synthesis of much longer peptides by using this approach (Table 1, entry 5). Other nonpolar dipeptides such as Boc-Ala-Ala-OMe and Boc-Ile-Ile-OMe were isolated in yields of 63 and 77 %, respectively (Table 1, entries 7 and 8). Furthermore, the production of Z-Aib-Val-OMe and Z-Aib-Aib-OMe in yields of 89 and 73 %, respectively, showed the efficiency of this approach, even for highly hindered substrates (Table 1, entries 9 and 10). These reaction conditions are more sustainable and much simpler to implement than previous strategies utilized for coupling two Aib residues.^[20] A secondary amine and a highly polar amino acid were also used as substrates, and Z-Aib-Pro-OtBu and Boc-Arg(NO₂)-Leu-OMe were isolated in yields of 90 and 88 %, respectively (Table 1, entries 11 and 12). Of note, the excess amounts of reactants required for the synthesis of all the peptides described herein are generally much lower than those in classical strategies. To produce longer peptides, Boc-Leu-Leu-OBn and Boc-Phe-Gly-OMe were quantitatively deprotected by gaseous HCl treatment without solvent.^[7] Then, treatment of HCl·H-(Leu)₂-OBn with Boc-Leu-OH·H₂O and Boc-Arg(Tos)-OH furnished the corresponding tripeptides in a satisfying yields of 65 % (Table 2, entries 1 and 2). Similarly, Boc-Glu(OCy)-(Leu)₂-OBn (Cy = cyclohexyl) and Boc-Trp-(Leu)₂-OBn were isolated, yet in moderate yields of 51 and 47 %, respectively (Table 2, entries 3 and 4). Notably, the undesirable forma-

tion of diketopiperazines was not detected by LC–MS analysis of the crude materials. Finally, upon treating the tripeptide HCl·H-(Leu)₃-OBn with Boc-Pro-OH and Boc-Leu-OH·H₂O, the tetrapeptides Boc-Pro-(Leu)₃-OBn and Boc-(Leu)₄-OBn were produced in excellent yields of 74 and 91 %, respectively (Table 2, entries 7 and 8), which clearly indicated the high potential of this approach to produce even longer peptides.

Conclusion

In summary, liquid-assisted grinding enabled the intensive, scalable, and environmentally benign production of various dipeptides by direct coupling of non-activated N-protected α-amino acids with amino esters mediated by EDC. The success of this strategy relied on the association of ball milling, a judiciously selected liquid additive, and reagents with reduced safety profiles. Combined with solvent-free deprotection steps, tri- and tetrapeptides could also be synthesized in good to excellent yields. Further applications of this liquid-assisted grinding strategy to the synthesis of longer peptides are currently underway.

Acknowledgments

The authors thank the Centre National de la Recherche Scientifique (CNRS) and Université de Montpellier for financial support. François Métro is warmly acknowledged for producing graphical abstract artwork.

Keywords: Mechanochemistry · Ball milling · Green chemistry · Amino acids · Peptides

- [1] K. Fosgerau, T. Hoffmann, *Drug Discovery Today* **2015**, *20*, 122.
- [2] Y. E. Jad, G. A. Acosta, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham, F. Albericio, *Org. Biomol. Chem.* **2015**, *13*, 2393.
- [3] a) C. M. Gabriel, M. Keener, F. Gallou, B. H. Lipshutz, *Org. Lett.* **2015**, *17*, 3968; b) R. De Marco, A. Tolomelli, A. Greco, L. Gentilucci, *ACS Sustainable Chem. Eng.* **2013**, *1*, 566.
- [4] A. Mahindra, N. Patel, N. Bagra, R. Jain, *RSC Adv.* **2014**, *4*, 3065.

- [5] a) D. Dev, N. B. Palakurthy, K. Thalluri, J. Chandra, B. Mandal, *J. Org. Chem.* **2014**, *79*, 5420; b) A. El-Faham, R. S. Funosas, R. Prohens, F. Albericio, *Chem. Eur. J.* **2009**, *15*, 9404.
- [6] S. Fuse, Y. Mifune, T. Takahashi, *Angew. Chem. Int. Ed.* **2014**, *53*, 851; *Angew. Chem.* **2014**, *126*, 870.
- [7] a) J. Bonnamour, T.-X. Métro, J. Martinez, F. Lamaty, *Green Chem.* **2013**, *15*, 1116; b) V. Declerck, P. Nun, J. Martinez, F. Lamaty, *Angew. Chem. Int. Ed.* **2009**, *48*, 9318; *Angew. Chem.* **2009**, *121*, 9482.
- [8] a) S. L. James, C. J. Adams, C. Bolm, D. Braga, P. Collier, T. Friščić, F. Griponi, K. D. M. Harris, G. Hyett, W. Jones, A. Krebs, J. Mack, L. Maini, A. G. Orpen, I. P. Parkin, W. C. Shearouse, J. W. Steed, D. C. Waddell, *Chem. Soc. Rev.* **2012**, *41*, 413; b) V. Štrukil, D. Gracin, O. V. Magdysyuk, R. E. Dinnebier, T. Friščić, *Angew. Chem. Int. Ed.* **2015**, *54*, 8440; *Angew. Chem.* **2015**, *127*, 8560.
- [9] a) T.-X. Métro, X. J. Salom-Roig, M. Reverte, J. Martinez, F. Lamaty, *Green Chem.* **2015**, *17*, 204; b) T.-X. Métro, J. Bonnamour, T. Reidon, A. Duprez, J. Sarpoulet, J. Martinez, F. Lamaty, *Chem. Eur. J.* **2015**, *21*, 12787; c) A. Beillard, E. Golliard, V. Gillet, X. Bantreil, T.-X. Métro, J. Martinez, F. Lamaty, *Chem. Eur. J.* **2015**, *21*, 17614; d) E. Colacino, P. Nun, F. M. Colacino, J. Martinez, F. Lamaty, *Tetrahedron* **2008**, *64*, 5569.
- [10] a) V. Štrukil, B. Bartolec, T. Portada, I. Đilović, I. Halasz, D. Margetić, *Chem. Commun.* **2012**, *48*, 12100; b) D. Tan, V. Štrukil, C. Mottillo, T. Friščić, *Chem. Commun.* **2014**, *50*, 5248.
- [11] Milling load (ML) is defined as the sum of the mass of the reactants per free volume in the jar.
- [12] C. Duangkamol, S. Jaita, S. Wangngae, W. Phakhodee, M. Pattarawarapan, *RSC Adv.* **2015**, *5*, 52624.
- [13] CH₂Cl₂ is classified as toxic and carcinogenic, and cyanuric chloride is classified as toxic, corrosive, and a skin sensitizer according to Regulation (EC) No 1272/2008.
- [14] Oxyma is not a hazardous substance according to Regulation (EC) No 1272/2008.
- [15] R. Subirós-Funosas, R. Prohens, R. Barbas, A. El-Faham, F. Albericio, *Chem. Eur. J.* **2009**, *15*, 9394.
- [16] G. A. Bowmaker, *Chem. Commun.* **2013**, *49*, 334.
- [17] a) T. Friščić, S. L. Childs, S. A. A. Rizvi, W. Jones, *CrystEngComm* **2009**, *11*, 418; b) T. Friščić, A. V. Trask, W. D. S. Motherwell, W. Jones, *Cryst. Growth Des.* **2008**, *8*, 1605; c) M. Tireli, M. Juribašić Kulcsár, N. Cindro, D. Gracin, N. Biliškov, M. Borovina, M. Čurić, I. Halasz, K. Užarević, *Chem. Commun.* **2015**, *51*, 8058.
- [18] DMF is classified as presenting reproductive toxicity according to Regulation (EC) No 1272/2008.
- [19] D. Prat, A. Wells, J. Hayler, H. Sneddon, C. R. McElroy, S. Abou-Shehadeh, P. J. Dunn, *Green Chem.* **2016**, *18*, 288, and references cited therein.
- [20] a) K. G. Jastrzabek, R. Subiros-Funosas, F. Albericio, B. Kolesinska, Z. J. Kaminski, *J. Org. Chem.* **2011**, *76*, 4506; b) J. Clayden, A. Castellanos, J. Solà, G. A. Morris, *Angew. Chem. Int. Ed.* **2009**, *48*, 5962; *Angew. Chem.* **2009**, *121*, 6076; c) P. Rossi, F. Felluga, P. Tecilla, F. Formaggio, M. Crisma, C. Toniolo, P. Scrimin, *J. Am. Chem. Soc.* **1999**, *121*, 6948.