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Thomas-Xavier Métro, Evelina Colacino, Jean Martinez, Frédéric Lamaty. Amino Acids and Peptides in Ball Milling. Achim Stolle; Brindaban Ranu. Ball Milling Towards Green Synthesis: Applications, Projects, Challenges, Royal Society of Chemistry, pp.114-150, 2014, 978-1-78262-348-9. 10.1039/9781782621980-00114 . hal-02364140

HAL Id: hal-02364140

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Submitted on 25 May 2021

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CHAPTER 6

Amino Acids and Peptides in Ball Milling[†]

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6.1 Introduction

For many years, pharmaceutical companies have focused their attention on the development of drugs based on the biological activity of small molecules. More recently, peptides have been recognized as efficient active pharmaceutical ingredients and new delivery systems have moved them forward in the modern therapeutic arsenal. Peptides also serve as pharmacological tools.¹

Peptides have many advantages over small drugs, the major ones being their high potency and selectivity. They can also be investigated over a broad range of targets, providing generally a better binding with fewer side-effects and avoiding accumulation in tissues. Moreover, at a time when small drugs and their metabolites have been recognized as pollutants in the environment, peptides are considered as less eco-toxic since their degradation pathways in nature lead to the generation of more innocuous molecules such as amino acids.²

[†]The contribution of Thomas-Xavier Métro and Evelina Colacino to this work is equivalent.

An inconvenience can be their poor metabolic stability and oral availability. In this regard, new practical delivery technologies have been investigated such as nasal spray or micro-needles. Furthermore, chemists have been involved in the design and synthesis of mimicking original molecules and preparing new structures that are more resistant to degradation by endogenous enzymes.

About 100 peptidic drugs have now reached the pharmaceutical market and many peptides are now in the pipeline of pharmaceutical companies. They are usually made of a 5–10 amino acid sequence but, in some cases, larger peptides up to 50 amino acids have been synthesized and commercialized.³ One can cite as an example Fuzeon (enfuvirtide), a 36-amino-acid antiretroviral.

As a consequence, the market for therapeutic bulk peptides is expected to grow rapidly in the next few years. It has been evaluated as more than \$40 billion. Some of these molecules are becoming blockbusters. Indeed, peptides such as Copaxone[®] (for multiple sclerosis therapy) and hormone-related products such as leuprolide, octreotide, and goserelin (Figure 6.1) have reached annual sales of more than \$1 billion each.⁴

The synthesis of peptides is now very well established with three major approaches, in solution, in the solid phase or using recombinant techniques for the larger peptides. The chemical preparation of these molecules consists of assembling amino acids by stepwise successive reactions consisting of a coupling reaction with a protected amino acid followed by a deprotection step. The synthesis of peptides has undergone strong developments with the discovery of solid phase supported synthesis.⁵ This technique, based on the use of an insoluble polymeric support to anchor a first amino acid, allows a stepwise synthesis, including washings to eliminate soluble excess of coupling and deprotection reagents and side products (Scheme 6.1). One of the major advantages is the possibility to fully automate such a process.^{6,7}

Nevertheless, while being extremely practical and efficient, these methods make use of large amounts of solvent. To produce 1 kg of peptide it is thought that 5000 kg of solvent are needed. From this point of view, there is a need to explore new methods for the scale up of peptide production that would avoid or decrease the use of solvents, all the more so given that the solvents recovered from the reaction and the washings are loaded with toxic compounds used during the coupling or the deprotection step.⁸

The building blocks that are used to make peptides are protected amino acids. Consequently, the preparation of amino acids and their protected derivatives are of the utmost importance in this area. Furthermore, amino acids and their derivatives may exert biological activities on their own.^{9,10} They are also important starting materials arising from the chiral pool for the preparation of heterocycles.¹¹

We describe in this chapter the application of ball-milling in peptide synthesis, including the preparation of amino acids and their protected derivatives.

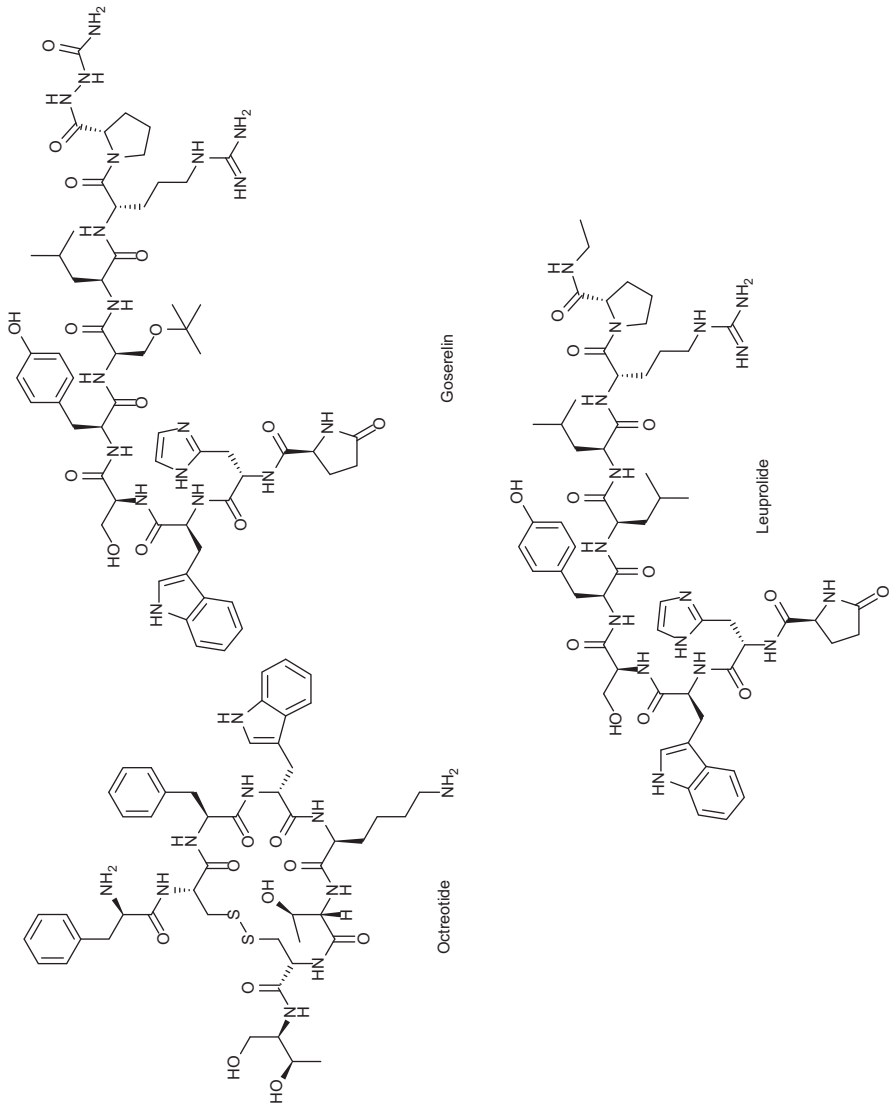
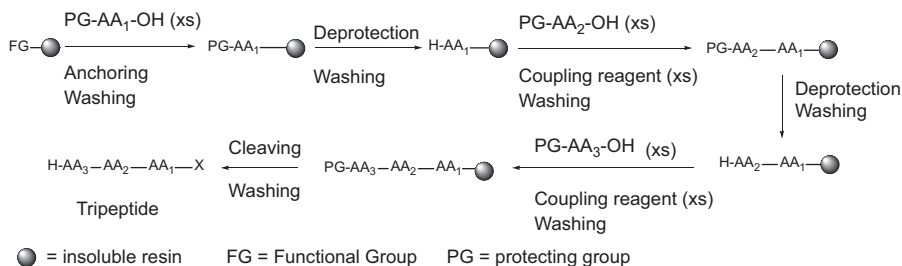


Figure 6.1 Examples of therapeutic peptides.



Scheme 6.1 Schematic synthesis of a tripeptide on solid-phase.

6.2 Mechanochemical Synthesis and Derivatization of Amino Acids

Amino acids are interesting targets for solid-state reactions, due to their intrinsic properties such as their zwitterionic nature and high melting points. However, their reactivity and uses in mechanochemical processes have not yet been fully explored and exploited.

6.2.1 Synthesis of Amino Acid Derivatives

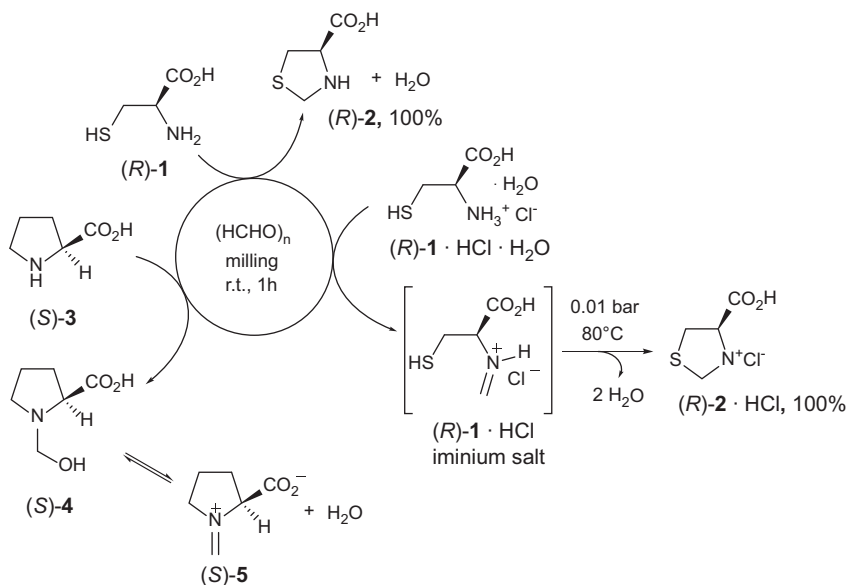
Historically, the first example of their use in organic mechanochemistry dates back to 2000, when *L*-cysteine^{12–14} (**1**) (together with its hydrochloride monohydrate derivative) and *L*-proline¹³ (**3**) were tested for their solid-state reactivity in the presence of stoichiometric quantities of paraformaldehyde (Scheme 6.2).

Solid paraformaldehyde (HCOH)_n (**6**) polymer is a handling-friendly and convenient alternative to access gaseous formaldehyde monomer (**7**) (formed *in situ* during mechanochemical milling by complete breakage of the weak polymer chain bonds). Thus, the solid-state condensation in the ball-mill with amino (or ammonium) group led to the corresponding methylene iminium salts,¹⁵ (*R*)-**1** · HCl and (*S*)-**5** quantitatively. However, they are extremely reactive and they can be easily trapped by nucleophiles, such as the thiol group on *L*-cysteine¹² (**1**) – leading to *L*-thiazolidine (or its hydrochloride)¹² (*R*)-**2** after removing *in vacuo* the water of the reaction – or water,¹³ leading to large-scale quantities (200 g)¹⁶ of stable *N*/*O*-hemiacetal (*S*)-**4**.^{16,17}

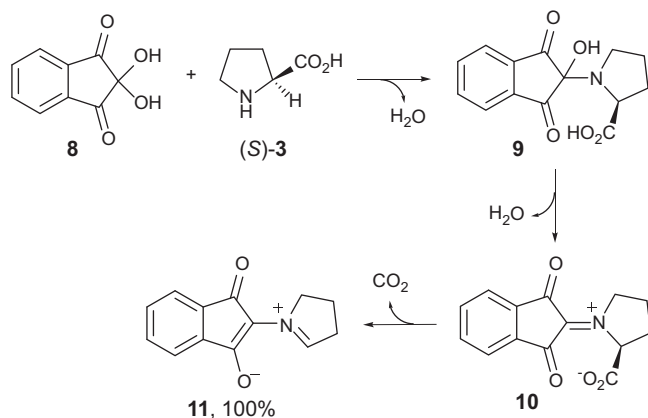
(*S*)-Proline (**3**) also served for the waste free, large scale and quantitative synthesis of azomethine ylide **11**^{14,16,18} using stoichiometric milling with ninhydrin, *via* a three-step solid-state cascade reaction (substitution/elimination/decarboxylation) without the need of purification,¹⁷ outperforming the synthesis in solution (82% yield) (Scheme 6.3).

6.2.2 Oxidation Reactions

As an alternative to a plethora of methods for the synthesis of disulfides in solution, the aerobic solid-state oxidation of thiol to symmetrical organo-disulfides under ball milling was achieved using *L*-cysteine (**1**) with iodine



Scheme 6.2 Quantitative methylene iminium cysteine and proline salts synthesis (and trapping).

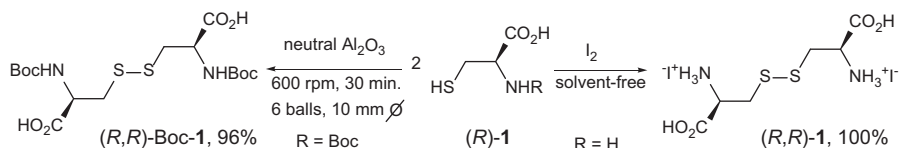


Retsch MM200 Swing Mill: 421 mg
 20-25 Hz, 1 h, 30°C,
 10 mL jar, 2 steel balls, 12 mm \varnothing

2 L Horizontal Rotor Ball Mill: 146 g
 Simoloyer, 1100 rpm, 40 min, 15-21°C,
 Steel balls: 100Cr6, 2kg, 5 mm \varnothing

Scheme 6.3 Cascade synthesis of azomethine ylide **11**.

(quantitatively)¹⁴ or over neutral aluminium oxide¹⁹ (grinding auxiliary) in the absence of any metal-catalyst, base or solvent (Scheme 6.4). In the last case, the product was isolated by washing the reaction mixture with ethanol, followed by evaporation of the solvent, while the grinding auxiliary could be recovered, dried and recycled.



Scheme 6.4 Solid-state oxidation to organo-disulfides.

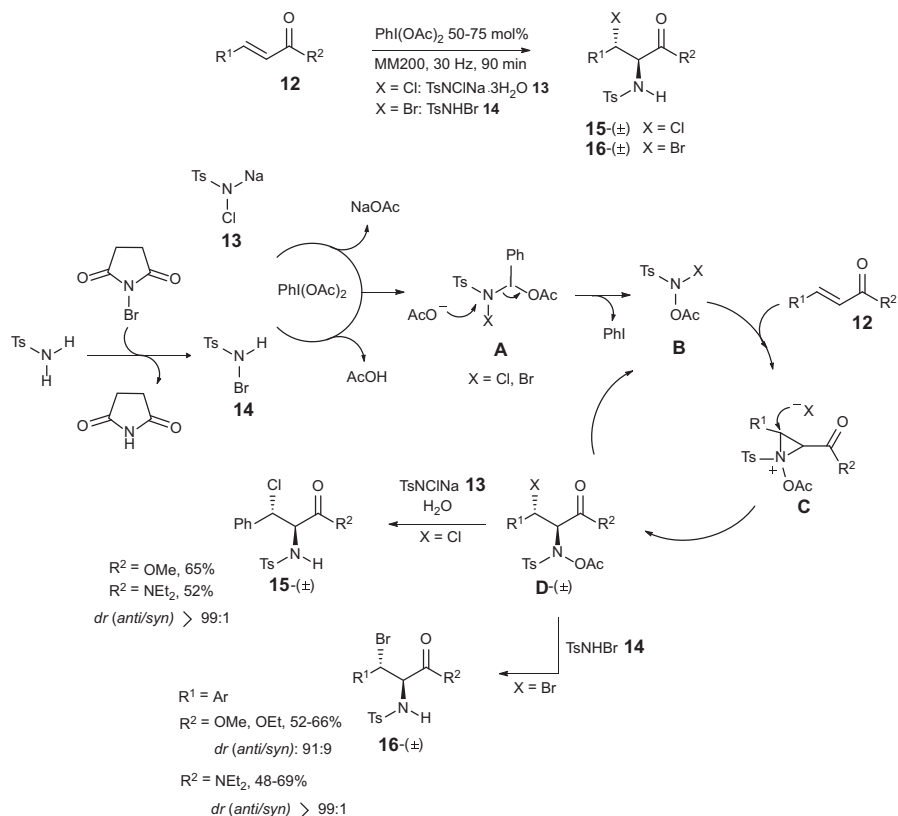
6.2.3 Asymmetric Synthesis of Amino Acids

Ball milling was also successfully applied to the preparation of amino acid derivatives in their chiral form, which remains an ongoing challenge for organic chemists.²⁰ For example, the metal-free aminohalogenation^{21,22} of electron-deficient olefins was described from cinnamate ($R^2 = \text{OMe}$) and cinnamide ($R^2 = \text{NET}_2$) **12**, promoted by (diacetoxyiodo)benzene [$\text{PhI}(\text{OAc})_2$], with high regio- and diastereo-selectivity (Scheme 6.5).

Commercially available and inexpensive chloramine-T trihydrate ($\text{TsNClNa} \cdot 3\text{H}_2\text{O}$) or tosylamide/*N*-bromosuccinimide (TsNH_2/NBS)²³ system were used as nitrogen and halogen sources, respectively. Although the reaction mechanism is not yet known, it was proposed that the oxidation of chloro- **13** or bromo-derivative **14** by $\text{PhI}(\text{OAc})_2$ led to a similar intermediate *N*-acetoxy-*N*-halogen-*p*-toluenesulfonamide **B**, *via* intermediate **A** after releasing iodobenzene. The electrophilic attack of **B** on α,β -unsaturated enones **12** afforded the highly reactive aziridinium intermediate **C** that underwent fast ring opening *via* $\text{S}_{\text{N}}2$ attack of the more electrophilic β -position by the *in situ* formed nearby halogen anion. High regio- and diastereoselectivity (up to *anti/syn* > 99:1) characterized the formation of intermediate **D**-(\pm), which reacted with the suitable halogeno-derivative (**13** or **14**) to afford the final product, regenerating the intermediate **B**. The mechanism involving the formation of a bridged halogenium ion intermediate (instead of aziridinium) was excluded because a reversed regio-stereoselectivity would have been observed (dominant formation of the *syn*-diastereoisomer).

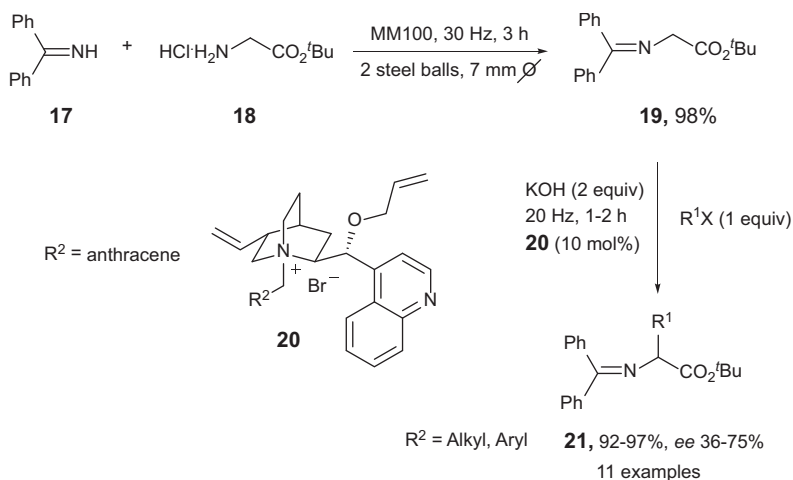
Chiral amino esters were also prepared by an asymmetric alkylation reaction starting from the Schiff base of glycine in the presence of chiral ammonium phase-transfer catalyst (PTC) **20** under basic conditions²⁴ (Scheme 6.6).

The transamination reaction between benzophenone imine (**17**) and stoichiometric quantities of glycine *tert*-butyl ester hydrochloride (**18**) led to Schiff base **19** in nearly quantitative yield (and up to two-gram scale), after washing the milling powder with water to eliminate ammonium chloride salt. The reaction time was shorter compared to the synthesis in solution (overnight) and no chlorinated solvent was needed. For the enantioselective reaction, the first step was a solid-state deprotonation occurring at the interface of the Schiff base and KOH – performed best among the various bases explored with M_2CO_3 or MOH ($\text{M} = \text{Na}, \text{K}, \text{Cs}$) – and leading to a



Scheme 6.5 Diastereoselective aminohalogenation and plausible reaction mechanism.

potassium enolate, which remained stable as shown by spectroscopic analysis. Glycine *t*-butyl ester Schiff base was selected for the alkylation reaction due to its higher stability to hydrolysis. In the second step, interaction with the chiral ammonium salt derived from cinchonidine **20** induced the reaction of the electrophile R^1X on a preferential face. Although excellent yields and exclusive monoalkylation were obtained, the enantiomeric excess (*ee* up to 75%) values were lower than those obtained in solution under PTC conditions. The enantiomeric excess could not be improved by increasing the amount of catalyst or varying the quantity of base. The ball-milling frequency showed little influence on the measured enantiomeric excess during the preparation of allylglycine *t*-butyl ester: at 10 or 20 Hz identical *ees* (64%) were obtained, with a further decrease of optical purity at 30 Hz (58% *ee*). Despite the difficulties associated with temperature control and a high concentration of reactants, the results demonstrated that asymmetric synthesis in a ball-mill is not a chimera.



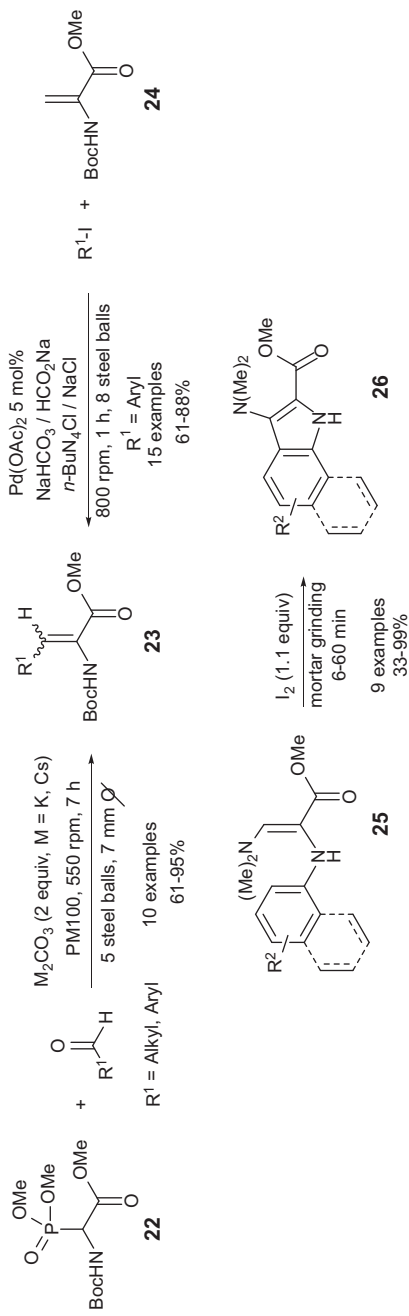
Scheme 6.6 Solvent-free synthesis of glycine Schiff base and enantioselective alkylation under PTC-conditions.

6.2.4 Synthesis of Unsaturated Amino Acids

Unsaturated unnatural amino acid derivatives **23** were also obtained using the ball-milling technique by performing a Horner–Wadsworth–Emmons reaction²⁵ or a Heck–Jeffery protocol,^{26,27} and used as platform to access 2-carbonylindoles **26** (Scheme 6.7).²⁸

High yields of Boc-protected unsaturated amino-esters were obtained by grinding amino-phosphoryl acetate **22** and various aromatic or aliphatic aldehydes in a planetary ball-mill based on a Horner–Wadsworth–Emmons reaction, with a conversion close or equal to 100% (Scheme 6.7).²⁵ The (*Z*)/(*E*) selectivity was always very high (100 : 0) with exclusive formation of the (*Z*)-isomer using aromatic aldehydes and hindered *ortho*-substituted substrates, except in one case (with naphthaldehyde the selectivity was lower, (*Z*)/(*E*) 82 : 18). Linear aliphatic aldehydes were also tested: in most cases high yields (up to 90%) and 100% selectivity in favor of the (*Z*)-isomer were obtained, while branched or sterically hindered aldehydes were less suitable, with incomplete conversion of starting material, while ketones were unreactive. A comparative experiment starting from aminophosphoryl acetate **22** and 3,5-dimethoxybenzaldehyde (with Cs_2CO_3 as base) was carried out in a flask with a stir bar. After 7 h, incomplete conversion (43%) was obtained, as well as by heating the mixture at 50 °C (45% conversion). Under the same conditions, mechanochemical activation led to full conversion and 94% yield for unsaturated Boc-protected amino ester **23** ($\text{R}^1 = 3,5\text{-dimethoxyphenyl}$), showing the importance of ball-milling activation for this transformation.

Exclusive (*Z*)-selectivity was also observed when dehydrophenylalanine derivatives **23** were prepared using solvent- and phosphine-free Heck–Jeffery protocol^{26,27} in a planetary mill under phase transfer conditions involving



Scheme 6.7 Solvent-free synthesis of unsaturated amino esters and 2-carboxy-substituted indoles.

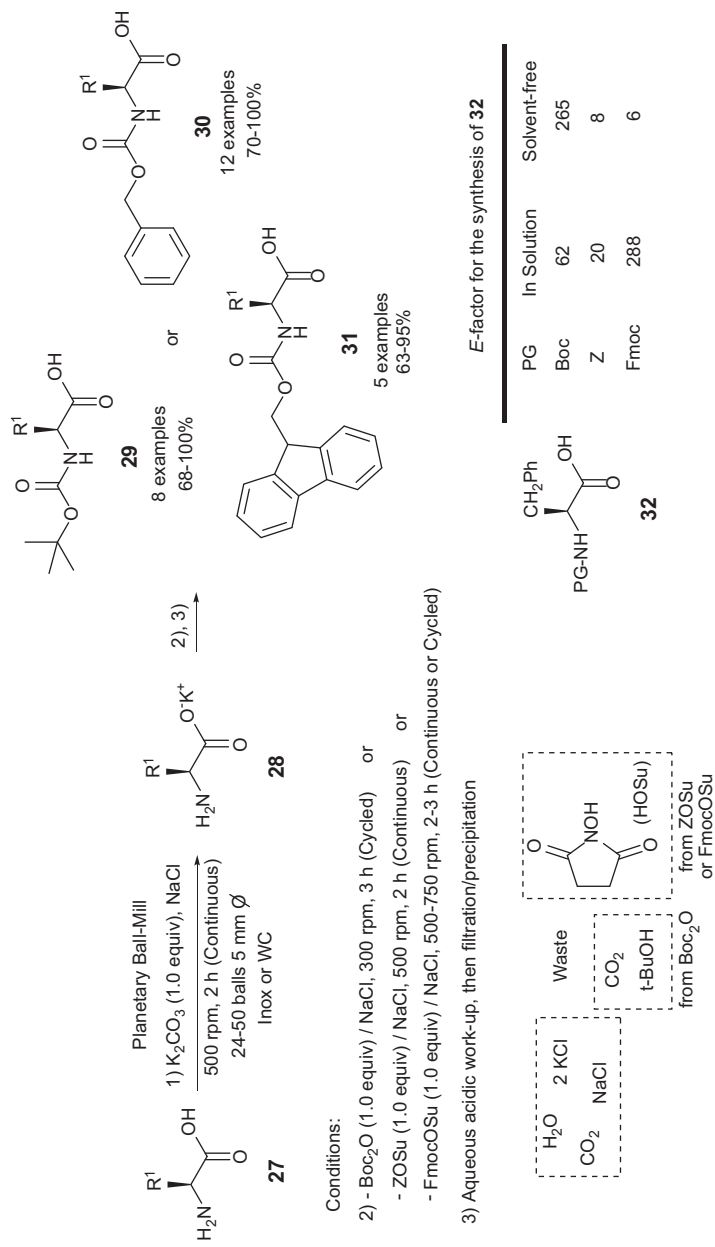
tetrabutylammonium chloride (Scheme 6.7). Various aryl iodo-anilines were coupled successfully (up to 5 mmol scale with 78% yield), in the presence of NaCl (5 mg mg⁻¹ aryl halide) as a grinding aid,^{29,30} while bromide derivatives failed to give the coupling products. The cross-coupling reaction outcome was influenced by steric and electronic effects of substituents on the aromatic ring: electron-withdrawing groups or the proximity of a heteroatom to the halide led to poor results. The results obtained under ball-milling conditions were compared to those obtained under usual conditions: (i) on heating with or without stirring, (ii) under microwave irradiation, (iii) under high static pressure conditions (200 kg cm⁻²), using an ordinary hydraulic press for making IR-tablets. The reaction mixture was heated in all cases, at 80 °C, a temperature that matches that generated during milling at full speed (800 rpm) for 1 h. The yields were always lower compared to ball-mill experiments (up to 33%), demonstrating that the conditions created during ball-milling by combination of pressure (rotation of the steel balls), heat, grinding and stirring were not easy to obtain under usual conditions, affirming the power of milling for the positive outcome of the reaction. This trend was also confirmed for the iodine-promoted intramolecular cyclization of substituted 2-anilinoenaminones **25** to prepare 2-carbonylated-3-dimethyl-amino-indoles **26** by solvent-free manual grinding in a mortar (Scheme 6.7).²⁸ The reactions were generally faster (6–60 min instead of 12 h) and higher yielding (often quantitative) compared to synthesis in solution using acetonitrile or in the presence of Lewis acids.

6.2.5 Synthesis of Protected Amino Acids

Ball-milling technology was also applied to the solvent-free synthesis of carbamate *N*-protected α - and β -amino acids³¹ as Boc-, Z-, and Fmoc-derivatives (Scheme 6.8).

The reactions were performed using two different planetary ball-mill apparatus and process parameters were also investigated: (i) stainless steel or tungsten carbide (WC) grinding jar material, (ii) number of grinding balls, (iii) rotation speed, (iv) mode of operation under cycled or continuous milling, and (v) grinding additives.

In the one-pot/two-step protocol, the first step relied on the inhibition of the reactivity of α - or β -carboxylic acid function through the *in situ* formation of a transient potassium internal salt, and then stoichiometric amounts of the suitable protecting group (Boc₂O, Z-OSu or Fmoc-OSu) were introduced in the second step (Scheme 6.8). This eco-friendly methodology was general for all the protecting groups tested in the study, and gave good to excellent yields of amino acid derivatives without any purification and in a scale from 50 mg up to 1 g of final product. The *N*-protected amino acid derivatives were not soluble in water: in the case of *N*-Fmoc- and *N*-Z-derivatives, after acidification, the pure product precipitated off the solution and was recovered, while liquid-liquid extraction was necessary for *N*-Boc-protected amino acids. The only by-products were water, CO₂ and *t*-BuOH (eliminated by



Scheme 6.8 Two step/one-pot *N*-protection of amino acids in a planetary ball-mill.

evaporation), together with water soluble compounds such as inorganic salts (MCl, with M = Na, K), traces of unreacted amino acid hydrochloride or hydroxysuccinimide (SuOH), which were washed away during aqueous acidic precipitation/filtration work-up (Scheme 6.8).

In the case of Boc-protection, cycled milling mode (at 300 rpm during three cycles of 1 h each, with 10 min pause between cycle) was the right approach to avoid both the formation of *N*-diacylated by-products and to promote a fast kinetics for *N*-protection, while diminishing the kinetics of Boc₂O degradation, which were otherwise observed under continuous milling, at higher rotation speeds (500 or 650 rpm cycled or not) and independently of the number of balls used (6, 12 or 24 stainless steel balls). In the case of Z- and Fmoc-protection, if the highly reactive Z-Cl, Z₂O or Fmoc-Cl were not suitable because of their fast decomposition, the hydroxysuccinimidyl derivatives Z-OSu and Fmoc-OSu, respectively, were used. In the case of *N*-Fmoc derivatives, yields and selectivity were higher at increased rotation speed (up to 750 rpm) and number of balls (up to 50), while the modulation of the energetic conditions applied to the grinding jar, using different milling materials, had no influence. Notice that in the case of Fmoc-protection the formation of side-products usually observed in solution (*i.e.* Fmoc-β-Ala-OH or dipeptides Fmoc-β-Ala-AA-OH *via* the Lossen rearrangement)^{32,33} was avoided under mechanochemical activation. From the point of view of environmental impact, especially in the case of Z- and Fmoc-protection, the *E*-factors³⁴ were greatly improved compared to classical syntheses in solution (Scheme 6.8). In the case of Boc-protection, the nature of waste for solution or solvent-free synthesis was the same, but liquid-liquid extraction (instead of precipitation) work-up was needed to recover the product, with a negative impact on the *E*-factor value.

In a similar approach, a vibrational ball-mill was used for the carbamoylation reaction of α-, β- or quaternary amino esters (Scheme 6.9),³⁵ sharing the same advantages as previously illustrated for the *N*-protection of amino acid derivatives in the planetary ball-mill³¹ (Scheme 6.8) in terms of: (i) ease of recovery of pure final products, (ii) entity and nature of waste, and (iii) full conversion and yield. In addition, Z-OSu and Fmoc-OSu could be replaced by the more convenient chloride derivatives. Independently of the incoming protecting group, the reaction kinetics depended on the nature of the *C*-terminal ester, which proved to be slower when *t*-butyl instead of methyl esters were used. The number of balls in the milling jar also seemed to have an impact on the outcome of the reaction: one ball instead of two led to incomplete conversion of substrates and much lower yields.

Notably, mechanochemical activation was particularly suitable for the *N*-Boc carbamoylation of hindered substrate such as H-Aib-OMe, affording impressive improved yield (68%) – three times more than in solution.³⁶ However, very low conversions were obtained for the *N*-carbamoylation reaction in the vibrational ball-mill from amino acids (instead of planetary milling, Scheme 6.8), or in the planetary ball-mill from amino esters (instead of vibrational apparatus, Scheme 6.9), probably because of the different

stress phenomena (horizontally vibrating or circularly shaking milling) and quantity of energy delivered to the sample, working at 30 Hz or at 450 rpm (which corresponded to 7.5 Hz), showing that the two apparatus were not necessarily interconvertible when performing the same reaction.

The same trend was observed during the solvent-less esterification of the C-terminal position of amino acids,³⁵ which is effective in the planetary and not in the vibrational ball-mill apparatus (Scheme 6.10).

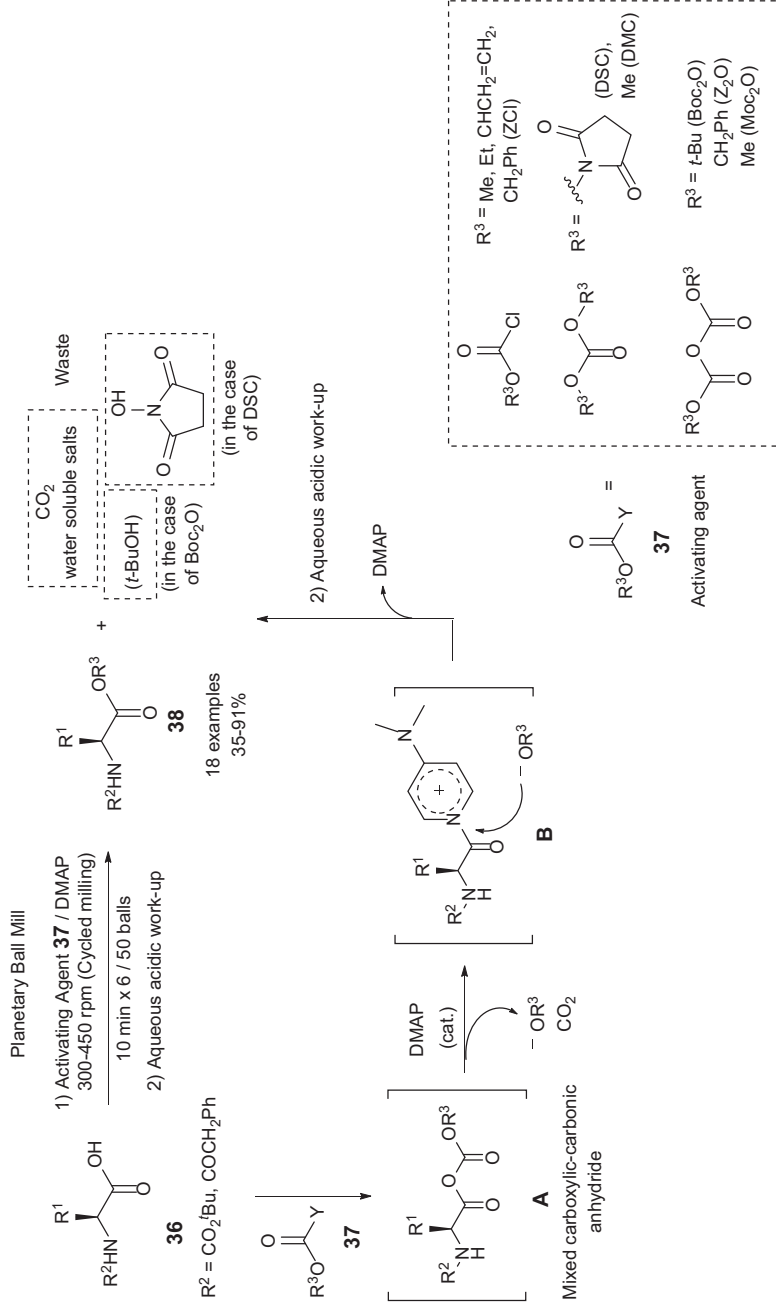
Cycled milling was suitable for the reaction of various dialkyl dicarbonates such as Boc₂O, Z₂O, Moc₂O [(ROCO)₂O, with R = O^tBu, Bn, Me, respectively], carbonates (RO₂CO, with R = succinimide, Me) or alkyl chloroformates (ROCOCl, with R = Bn, Me, Et, allyl) with N-protected α-amino acids, in the presence of DMAP as base. As already illustrated for the N-protection of amino acids³¹ (Scheme 6.8), continuous milling provoked the premature decomposition of activating agents **37** used in stoichiometric quantity. The decarboxylative esterification proceeded *via* formation of a mixed carboxylic-carbonic anhydride **A** (Scheme 6.10), which was converted into acylpyridinium derivative **B** by nucleophilic attack of DMAP. The evolution of carbon dioxide provided the driving force of the reaction.

Compared to solution synthesis, the preparation of *tert*-butyl esters from Boc₂O presented the advantages of shorter reaction times and reduced quantity of waste, also avoiding the use of expensive Boc₂O in excess, Lewis acids, solvents or *t*-BuOH to speed up the reaction. Benzyl or methyl ester derivatives using the highly reactive dicarbonates Z₂O, Moc₂O or dimethyl carbonate were not obtainable under solvent-less mechanochemical activation because of their fast decomposition. Alternatively, the corresponding chloroformates reacted straightforwardly, and with the succinimidyl esters prepared from *N,N'*-disuccinimidyl carbonate (DSC). In all cases, the pure products were recovered after simple and clean filtration/precipitation work-up, as already illustrated (Schemes 6.8 and 6.9), with lower environmental impact compared to solution syntheses.

For the spot-to-spot solvent-free mechanochemical derivatizations of *N*- or *C*-protected amino acids (Schemes 6.9 and 6.10, respectively) the temperature of the mixtures was 23–25 °C, after vibrational or planetary milling, ruling out a temperature increase into the milling jar³⁷ as responsible for the good yields.

The solvent-free derivatization of the *N*- or *C*-terminal position of amino acids was also reported with *N,N'*-carbonyldiimidazole (CDI) (Scheme 6.11).³⁸ In a first step, carboxylic acids were efficiently transformed into acylimidazoles by treatment with CDI as activating agent. Subsequent addition of amine in a ball-mill reactor furnished, through a highly stereoselective process, the amino acid derivatives **40** and **41** when using enantiopure HCl · NH₂-Phe-O^tBu or Boc-NH-Phe-OH, respectively (Scheme 6.11).

Notably, consumption of both the carboxylic acid in the first step and the acyl-imidazole in the second step were monitored by infrared analyses. Precipitation of pure final compounds in water, followed by filtration, afforded the amides **40** and **41** without the need of any organic solvent.



Scheme 6.10 C-Terminal esterification for protection/activation of *N*-protected amino acids in a planetary ball-mill.

A similar approach was developed by Margetić and coworkers to produce amino amides by using a mechanochemical process (Scheme 6.12).³⁹ Boc-Alanine was activated in the reaction media by addition of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) in the presence of 2 equivalent of dimethylaminopyridine (DMAP) as the base, 20 equivalents of NaCl as a solid grinding assistant and small quantities of nitromethane as a liquid grinding auxiliary. Subsequent reaction with *p*-anisidine or 4-chloroaniline provided the corresponding amino amides in 87% and 88% yield respectively.

6.3 Mechanochemical Synthesis of Peptides

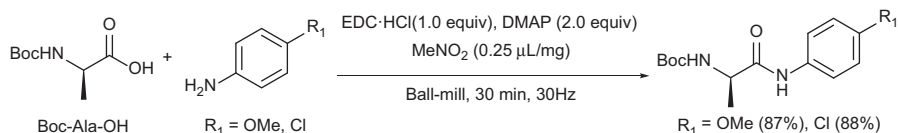
6.3.1 Synthesis of Di- and Tripeptides

In 2009, motivated by the desire to mitigate the environmental impact of classical peptide synthesis approaches, Lamaty and coworkers envisioned performing solvent-free peptide synthesis based on the use of the ball-milling technology.⁴⁰ Initial investigations were carried out on the coupling of the Boc-protected phenylalanine *N*-carboxyanhydride (Boc-Phe-NCA) with alanine methyl ester hydrochloride salt (HCl·H-Ala-OMe) in the presence of NaHCO₃ in a vibrating ball-mill (Scheme 6.13).

After 1 h of vigorous agitation (30 Hz) in a vibrating ball-mill, reaction media was recovered from the milling jar by using both EtOAc and water. Classical treatments of the organic phase such as water washings, drying and concentration furnished the pure dipeptide Boc-Phe-Ala-OMe in 79% yield without epimerization as observed by HPLC analysis. The low environmental impact of this reaction has to be noticed. The usual highly toxic solvents such as DMF, CH₂Cl₂, NMP or THF were not required and volatile and corrosive bases such as Et₃N and DIPEA were replaced by innocuous NaHCO₃. In addition, this approach furnished non-toxic CO₂ and NaCl as the only byproducts.

As one could argue that this reaction could have taken place during reaction media recovery with EtOAc and water, the authors performed solid-state IR and CP/MAS ¹³C NMR analysis of the reaction media before EtOAc recovery. These analysis revealed the disappearance of the characteristic signals of Boc-Phe-NCA ($\nu = 1817$ and 1872 cm⁻¹ in IR and $\delta = 147.6$ in CP/MAS ¹³C NMR) and the emergence of the typical signals of the desired product Boc-Phe-Ala-OMe ($\nu = 1624$ and 1655 cm⁻¹ in IR and $\delta = 156.0$ and 177.8 in CP/MAS ¹³C NMR). As these analytical samples were not involved in any solubilization process, these results proved that the reaction clearly occurred in the solid state.

Another particularity of these reaction conditions has been revealed by studying the kinetics of the reaction. As previously described by others on solid state reactions,⁴¹ measuring the conversion of Boc-Phe-NCA into Boc-Phe-Ala-OMe at different times of reaction revealed apparent zero-order kinetics (Figure 6.2). Notably, the reaction order seems to be independent of the frequency applied to the reaction media.



Scheme 6.12 EDC-mediated mechanochemistry of amides.

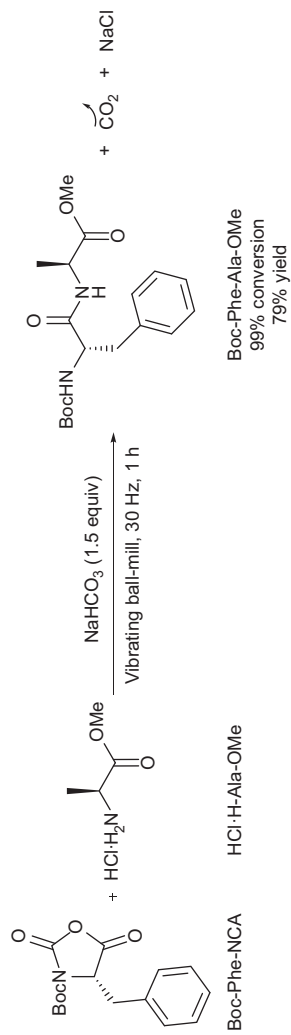
This approach was applied to the coupling of various α -UNCAs with α -aminoesters, furnishing numerous α,α -dipeptides with high yields (Table 6.1). When starting from Boc-Phe-NCA with alanine or leucine esters as the nucleophiles, high conversions and yields were obtained (Table 6.1, entries 1–3). Under the same conditions, treatment of Boc-Phe-NCA with phenylalanine methyl ester hydrochloride salt gave a low conversion of 58%, which was supposed by the authors to be related with the physicochemical state of the reaction mixture (Table 6.1, entry 4). Indeed, due to its insolubility in water, Boc-Phe-Phe-OMe could be recovered as pure material by simple trituration in aqueous media followed by filtration and drying. It was isolated in 55% yield, which can be considered as very satisfying compared to the relatively low conversion. Notably, the production of Boc-Phe-Phe-OMe under these conditions fits particularly well the 12 principles of Green Chemistry⁴² as it was produced without the use of any organic solvent from the reaction to the product recovery. In addition, aminoesters can also be used as their acetate salt as AcOH·H-Gly-OtBu was transformed into Boc-Phe-Gly-OtBu with high conversion and yield (Table 6.1, entry 5). Bulky electrophiles such as Boc-Val-NCA can also lead efficiently to the corresponding dipeptides with yields ranging from 85% to 100% (Table 6.1, entries 6–10). The urethane protecting group can also be switched to the widely used Fmoc group as treating Fmoc-Val-NCA with various amino-esters furnished the corresponding dipeptides with high conversions (Table 6.1, entries 11–15).

The same approach is applicable to the synthesis of tripeptides as reacting the dipeptide HCl·H-Ala-Gly-OMe with Boc-Val-NCA produced Boc-Val-Ala-Gly-OMe in 89% yield (Scheme 6.14).

6.3.2 Scale-up of Peptide Synthesis

The scalability of this approach was also studied. While the reaction conditions were optimized for a vibrating ball-mill, performing the synthesis of a dipeptide on a large scale was realized on a planetary ball-mill by using a 250 mL jar equipped with an in-line temperature and pressure monitoring system. The latter was filled with 3.85 g of Boc-Phe-NCA, 1.84 g of HCl·H-Ala-OMe and 1.7 g of NaHCO₃ and agitated for 2 h in the planetary ball-mill at 350 rpm, furnishing 4.3 g of the dipeptide Boc-Phe-Ala-OMe in an excellent yield of 94% (Scheme 6.15).⁴³

Measurement of the temperature indicated a slow but regular increase from 26 to 33 °C during the first 60 min of the reaction (Figure 6.3). In addition, the pressure increased during 50 min from atmospheric pressure



Scheme 6.13 Mechanochemical synthesis of Boc-Phe-Ala-OMe.

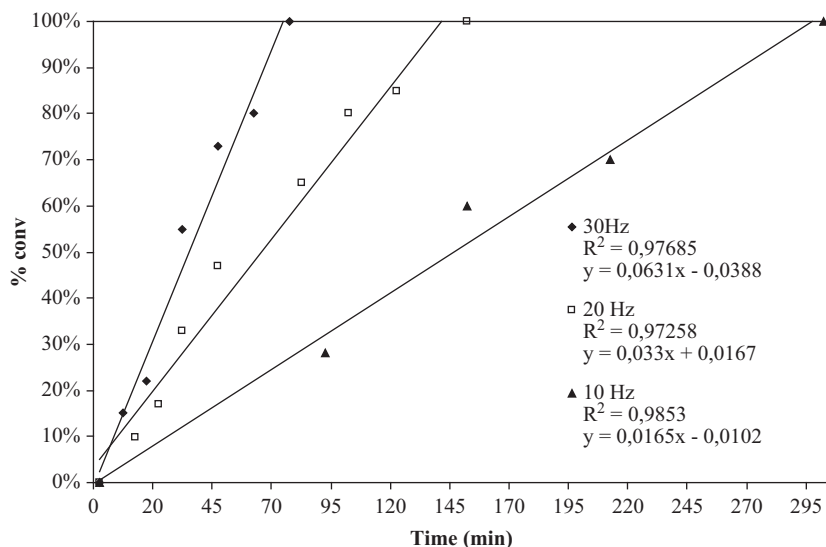


Figure 6.2 Conversion of Boc-Phe-NCA into Boc-Phe-Ala-OME as a function of time.

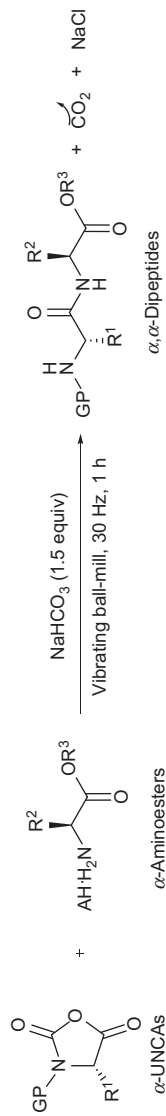
to a plateau at 3 bar that lasted 15 min, until the reactor was opened ($t=60$ min). This pressure increase is in complete accordance with the formation of CO_2 that occurs during the reaction. Opening the jar to follow completion of the reaction resulted in a pressure drop to atmospheric pressure. An additional hour of agitation at 350 rpm resulted in an insignificant rise in pressure and temperature indicating the end of the reaction, which could be confirmed by ^1H NMR analysis of the crude.

This approach was then applied to the synthesis of the dipeptide aspartame, a nutritive sweetener that is produced at the multi-ton scale every year. After preparation of Boc-Asp(*O**t*Bu)-NCA, the latter was treated with phenylalanine methyl ester hydrochloride during 1 h milling at 30 Hz to produce Boc-Asp(*O**t*Bu)-Phe-OME with an excellent yield of 97% (Scheme 6.16). Boc and *t*Bu protecting groups were then removed by gaseous HCl treatment in the absence of any solvent to furnish aspartame hydrochloride in quantitative yield. Notably, these two first reactions are performed with a close to quantitative global yield while producing either volatile or water-soluble side-products (CO_2 , isobutylene and NaCl). Finally, aspartame hydrochloride was solubilized in water and neutralization to the isoelectric point (pH 5.0) with an aqueous solution of Na_2CO_3 furnished aspartame as a white powder in 84% yield.

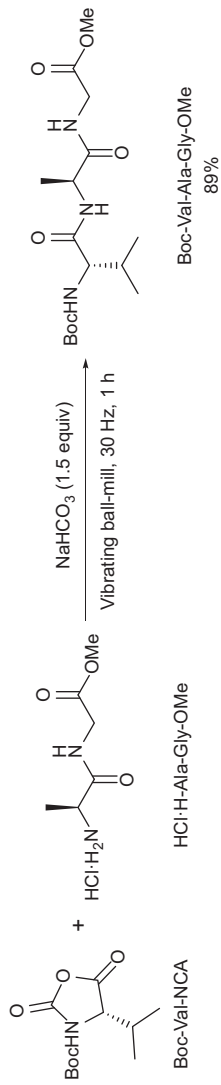
6.3.3 Synthesis of α,β - and β,β -Dipeptides

By using a very similar method, Juaristi and coworkers published a year later the solvent-free, ball-mill mediated synthesis of α,β - and β,β -dipeptides.⁴⁴ Indeed, β -UNCAs were used in place of the α -UNCAs utilized by Lamaty and

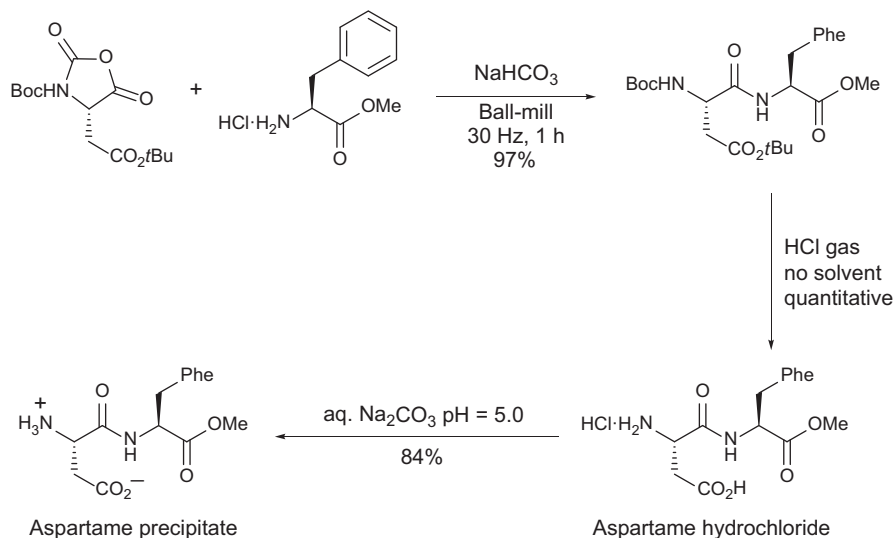
Table 6.1 Mechanosynthesis of α,α -dipeptides from α -UNCAs and α -aminoesters.



Entry	α -UNCA	α -Aminoesters	α,α -Dipeptides	Conversion (%)	Yield (%)
1	Boc-Phe-NCA	HCl · H-Ala-O <i>t</i> Bu	Boc-Phe-Ala-O <i>t</i> Bu	100	73
2		HCl · H-Leu-OMe	Boc-Phe-Leu-OMe	85	—
3		HCl · H-Leu-O <i>t</i> Bu	Boc-Phe-Leu-O <i>t</i> Bu	100	70
4		HCl · H-Phe-OMe	Boc-Phe-Phe-OMe	58	55
5		AcOH · H-Gly-O <i>t</i> Bu	Boc-Phe-Gly-O <i>t</i> Bu	100	90
6	Boc-Val-NCA	HCl · H-Leu-OMe	Boc-Val-Leu-OMe	100	87
7		HCl · H-Leu-O <i>t</i> Bu	Boc-Val-Leu-O <i>t</i> Bu	97	85
8		HCl · H-Ala-OMe	Boc-Val-Ala-OMe	100	100
9		HCl · H-Ala-O <i>t</i> Bu	Boc-Val-Ala-O <i>t</i> Bu	100	100
10		HCl · H-Phe-OMe	Boc-Val-Phe-OMe	100	88
11	Fmoc-Val-NCA	HCl · H-Leu-OMe	Fmoc-Val-Leu-OMe	90	—
12		HCl · H-Leu-O <i>t</i> Bu	Fmoc-Val-Leu-O <i>t</i> Bu	92	—
13		HCl · H-Ala-OMe	Fmoc-Val-Ala-OMe	100	76
14		HCl · H-Ala-O <i>t</i> Bu	Fmoc-Val-Ala-O <i>t</i> Bu	78	—
15		HCl · H-Phe-OMe	Fmoc-Val-Phe-OMe	93	—



Scheme 6.14 Mechanosynthesis of the tripeptide Boc-Val-Ala-Gly-OMe.



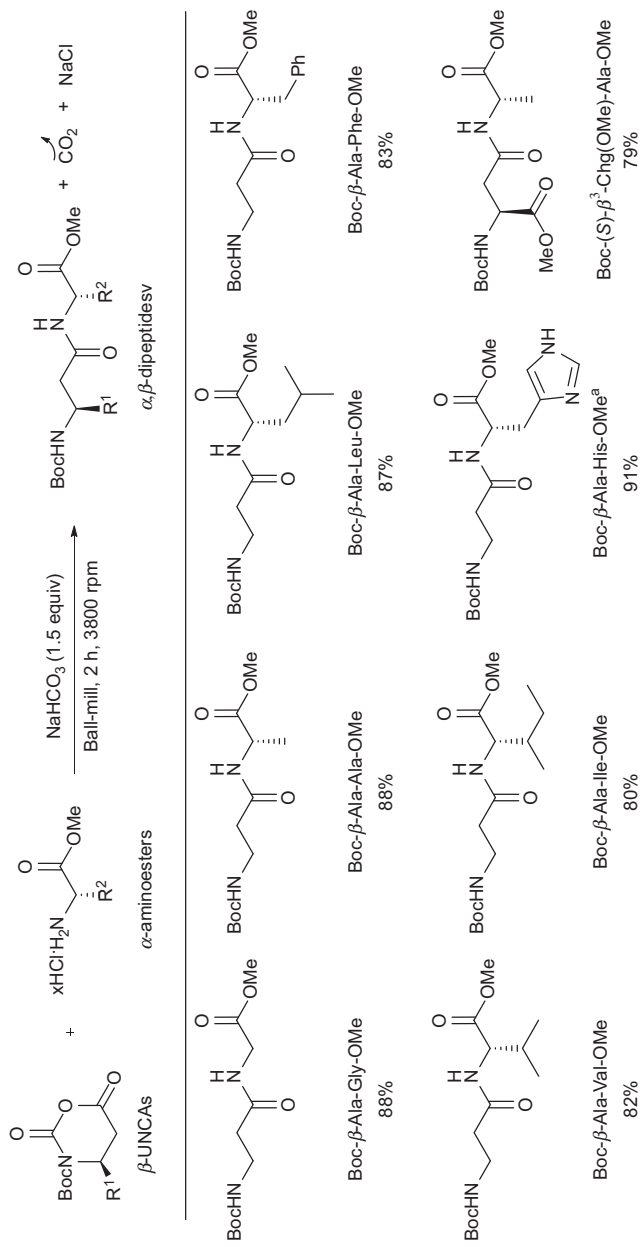
Scheme 6.16 Synthesis of aspartame by mechanochemistry.

anhydride (Boc-(*S*)- β^3 -Chg(OMe)-NCA) led to Boc-(*S*)- β^3 -Chg(OMe)-Ala-OMe in 79% yield when reacted with HCl · H-Ala-OMe (Scheme 6.17).

β,β -Dipeptides were also produced efficiently by treating β -UNCAs with β -aminoesters. Surprisingly, the yields were higher when using β -aminoesters than when using α -aminoesters. Indeed, treatment of HCl · H- β -Ala-OMe and HCl · H-(*S*)- β^3 -hPhg-OMe [(*S*)- β^3 -homophenylglycine methyl ester hydrochloride] with Boc- β -Ala-NCA furnished Boc- β -Ala- β -Ala-OMe and Boc- β -Ala-(*S*)- β^3 -hPhg-OMe with 96% and 91% yield, respectively (Scheme 6.18). When treated with the same β -aminoesters, Boc-(*S*)- β^3 -hPhg-NCA led to the production of Boc-(*S*)- β^3 -hPhg- β -Ala-OMe and Boc-(*S*)- β^3 -hPhg-(*S*)- β^3 -hPhg-OMe with excellent yields of 93% and 94%. Finally, treatment of Boc-(*S*)- β^3 -Chg(OMe)-NCA with HCl · H- β -Ala-OMe furnished Boc-(*S*)- β^3 -Chg(OMe)- β -Ala-OMe with a high yield of 91%.

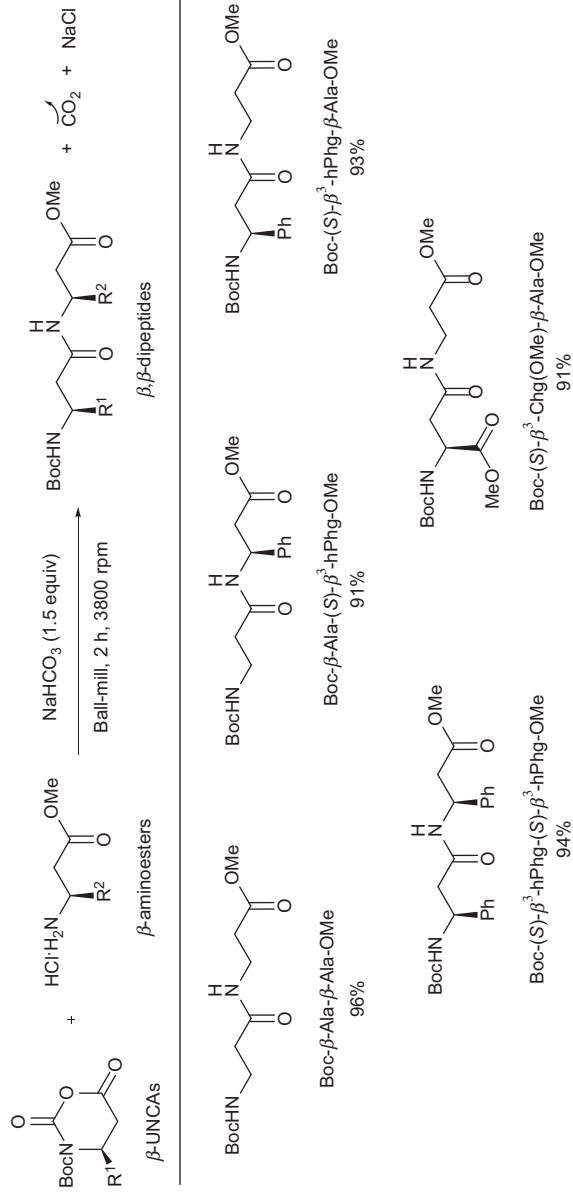
6.3.4 Synthesis of Peptides with a Longer Amino Acid Sequence

Subsequent development of the methodology was provided by Lamaty and coworkers with the aim of applying the later approach to the synthesis of longer peptide sequences.⁴⁵ As this objective requires the production of dipeptides on a large scale, efforts were directed to increase the productivity of the method. Indeed, whatever the size of the jar, the previously described operating procedure was performed using a relatively low milling load. Milling load can be defined as “the sum of the mass of the reactants per free volume in the jar”.⁴⁵ In other words, increasing the milling load will result in improving the productivity of the process.



^a 2 equiv of NaHCO₃ were used.

Scheme 6.17 Mechanosynthesis of α,β -dipeptides from β -UNCAs and α -aminoesters.

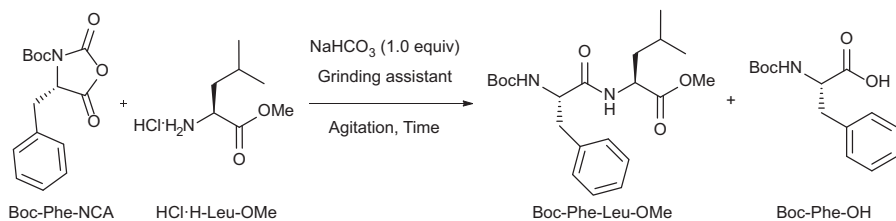


Scheme 6.18 Mechanosynthesis of β, β -dipeptides from β -UNCA and β -aminoesters.

Indeed, in a typical experiment involving a 10 mL jar filled with one 10 mm diameter metallic ball, Boc-Phe-NCA, HCl·H-Leu-OMe and NaHCO₃, up to a milling load of 5.9 mg mL⁻¹, gave a satisfying conversion of 59% reached after 10 min of agitation (Scheme 6.19, Figure 6.4). Unfortunately, when the milling load was increased up to 22.5 mg mL⁻¹, conversion into Boc-Phe-Leu-OMe dropped to 17% (Figure 6.4), which was attributed by the authors to the “highly viscous and sticky reaction media”. This observation was hypothesized to be related to mass transfer limitations resulting in a low speed of formation of Boc-Phe-Leu-OMe. To improve mass transfer of the reactants in the jar, the authors envisioned the use of small amounts of a liquid as a grinding assistant. Thus, adding 1.4 μL of EtOAc per mg of reactants to the reaction mixture ($\eta = 1.4 \mu\text{L mg}^{-1}$; η stands for the ratio of added liquid volume to the mass of solid reactants)⁴⁶ led to 91% conversion into Boc-Phe-Leu-OMe after the same grinding time. One could argue that adding EtOAc to the solid reactants and placing this reaction mixture under classical agitation could be enough to overcome the mass transfer limitations. The latter possibility was rejected by the fact that when the same reaction mixture was placed under classical magnetic agitation, the speed of the reaction was far slower than under ball-milling agitation. Indeed, when placed under classical agitation, the conversion into Boc-Phe-Leu-OMe reached 12% after 40 min (Figure 6.4). The latter experiment proved unambiguously that it is the combination of ball-milling with the presence of a grinding assistant that is responsible for the fast conversion into the dipeptide. Besides, switching from EtOAc to a predominant solvent used in peptide synthesis such as DMF did not match the performance of the ball-mill approach as 37% of conversion was reached after 40 min of agitation.

Another positive effect of using a liquid grinding assistant is the influence on Boc-Phe-NCA hydrolysis. Indeed, while conversion of Boc-Phe-NCA into Boc-Phe-OH reached 23% after 20 min of grinding with a milling load of 22.5 mg mL⁻¹, it declined to 2% after the same amount of time if EtOAc was present in the reaction media (Figure 6.5).

These conditions were then successfully applied to the synthesis of a wide range of di- to pentapeptides (Table 6.2). First of all, when previously optimized conditions were applied to the production of Boc-Phe-Leu-OMe, the latter could be isolated with 95% yield (Table 6.2, entry 1). No



Scheme 6.19 Liquid-assisted mechanosynthesis of Boc-Phe-Leu-OMe.

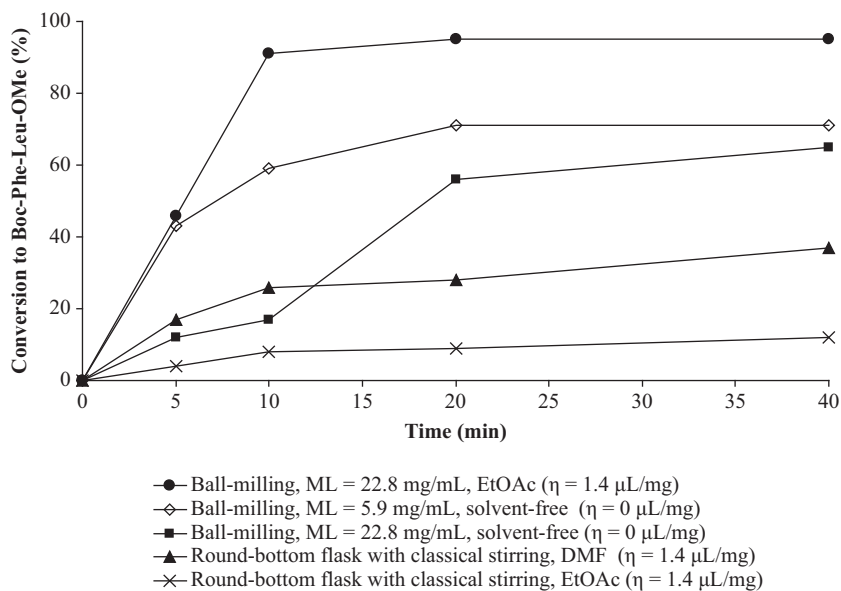


Figure 6.4 Influence of the composition of the reaction mixture and the type of agitation on the conversion into Boc-Phe-Leu-OMe.⁴⁵

epimerization occurred during the coupling as no traces of Boc-D-Phe-Leu-OMe could be observed by HPLC analysis. Other NCAs such as Boc-Leu-NCA, Boc-Val-NCA or Boc-Ile-NCA reacted efficiently with differently substituted α -amino esters (Table 6.2, entries 2–5). Indeed, replacing either the hydrochloride amino ester salt with a *p*-tosylate salt or the methyl ester with a benzyl ester did not affect the efficiency of the reaction as *p*-TsOH · H-Leu-OBn and HCl · H-Pro-OBn were transformed into Boc-Leu-Leu-OBn and Boc-Leu-Pro-OBn with 93% and 90% yield, respectively (Table 6.2, entries 2 and 3). Similarly, sterically challenging Boc-Val-NCA and Boc-Ile-NCA reacted with HCl · H-Phe-OME and HCl · H-Ile-OME to produce Boc-Val-Phe-OME and Boc-Ile-Ile-OME with high yields (Table 6.2, entries 4 and 5). Functionalities other than *N*-carboxyanhydride can be used to activate α -amino acid substrates. Indeed, *N*-hydroxysuccinimide esters of Boc-protected glycine, tyrosine or phenylalanine (Boc-AA-OSu) led to the efficient production of the corresponding peptides (Table 6.2, entries 6–11). This α -amino acid activation presents the advantage of producing the low toxicity and water-soluble *N*-hydroxysuccinimide (HOSu) as a side-product during the course of the reaction. Afterwards, HOSu can easily be eliminated during extractions. To evaluate the efficacy of liquid-assisted grinding on the elimination of the mass transfer limitations, the milling load of the reaction producing Boc-Phe-Phe-OME from Boc-Phe-OSu and *p*-TsOH · H-Leu-OBn was raised substantially to 188.8 mg mL⁻¹. While being slightly slower than with a milling load of 28.4 mg mL⁻¹, performing the reaction with a milling load of 188.8 mg mL⁻¹ allowed close to 800 mg

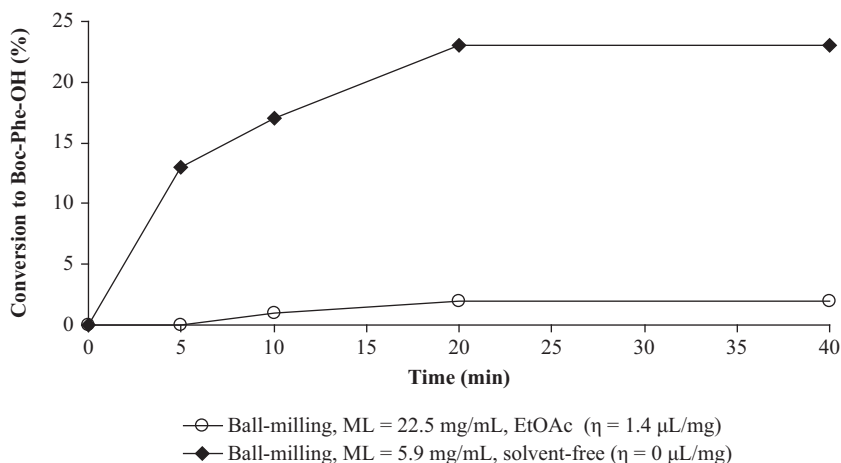


Figure 6.5 Influence of the composition of the reaction mixture on the hydrolysis of Boc-Phe-NCA.⁴⁵

of dipeptide to be obtained in a small 10 mL jar with 80% yield (Table 6.2, entry 11). Producing this amount of peptide enabled the synthesis of much longer peptides by using this approach. On this perspective, the Boc protecting groups of the mechanothesized dipeptides were cleaved using gaseous HCl treatment. This procedure has the advantages of being solvent-free and of furnishing the deprotected peptides as hydrochloride salts in quantitative yields.

The corresponding dipeptides were then engaged in another coupling step with activated α -amino acid derivatives. A wide variety of tripeptides were isolated with good to excellent yields starting from either Boc-AA-NCA or Boc-AA-OSu (Table 6.2, entries 12–19). Thus, hydrophobic Boc-Leu-Leu-Leu-OBn and Boc-Phe-Phe-Phe-OME were produced in 79% and 86% yield respectively (Table 6.2, entries 12 and 13). This approach perfectly tolerates the presence of a heteroatom in the side chain of the reactants as Boc-Lys(Boc)-Gly-Pro-OBn and Boc-Trp-Val-Phe-OME were isolated in 79% and 86% (Table 6.2, entries 14 and 15). Interestingly, sterically challenging Boc-Ile-NCA furnished both Boc-Ile-Leu-Pro-OBn and Boc-Ile-Ile-Ile-OME with 86% yield when treated with HCl·H-Leu-Pro-OBn and HCl·H-Ile-Ile-OME respectively (Table 6.2, entries 16 and 17). Similarly, less encumbered Boc-Gly-OSu also produced Boc-Gly-Gly-Phe-OME and Boc-Gly-Phe-Leu-OBn with good yields (Table 6.2, entries 18 and 19). The latter peptide could be produced at the gram scale as 937 mg of this tripeptide (94% yield) could be isolated by performing the corresponding reaction with a high milling load of 152.7 mg mL^{-1} (Table 6.2, entry 19). After having been deprotected by gaseous HCl, two tripeptides could be engaged in another mechano-mediated peptide bond coupling. The corresponding tetrapeptide Boc-Leu-Leu-Leu-Leu-OBn and Boc-Gly-Gly-Phe-Leu-OBn were both isolated with an excellent yield of 96% (Table 6.2, entries 20 and 21). Once again, increasing

Table 6.2 Mechanosynthesis of di- to pentapeptides.

Entry	Boc-AA-NCA or Boc-AA-OSu	AH · H-(AA) _x -OR ³	Mechanosynthesized peptides	Yield (%) ^a
1	Boc-AA-NCA	Boc-AA-OSu	Boc-Phe-Leu-OMe	95 ^b
2	Boc-Phe-NCA	HCl · H-Leu-OMe	Boc-Leu-Leu-OBn	93
3	Boc-Leu-NCA	<i>p</i> -TsOH · H-Leu-OBn	Boc-Leu-Pro-OBn	90
4	Boc-Leu-NCA	HCl · H-Pro-OBn	Boc-Val-Phe-OMe	84
5	Boc-Ile-NCA	HCl · H-Phe-OMe	Boc-Ile-Ile-OMe	95
6	Boc-Gly-OSu	HCl · H-Ile-OMe	Boc-Gly-Phe-OMe	96
7	Boc-Gly-OSu	HCl · H-Phe-OMe	Boc-Gly-Pro-OBn	97
8	Boc-Tyr(Bn)-OSu	HCl · H-Pro-OBn	Boc-Tyr(Bn)-Leu-OMe	98 ^c
9	Boc-Phe-OSu	HCl · H-Leu-OMe	Boc-Phe-Phe-OMe	92
10	Boc-Phe-OSu	HCl · H-Leu-OMe	Boc-Phe-Leu-OMe	83 ^b
11	Boc-Phe-OSu	<i>p</i> -TsOH · H-Leu-OBn	Boc-Phe-Leu-OBn	90 ^d , 80 ^e
12	Boc-Leu-NCA	HCl · H-Leu-OBn	Boc-Leu-Leu-OBn	79
13	Boc-Phe-OSu	HCl · H-Phe-OMe	Boc-Phe-Phe-OMe	86
14	Boc-Lys(Boc)-OSu	HCl · H-Gly-Pro-OBn	Boc-Lys(Boc)-Gly-Pro-OBn	79

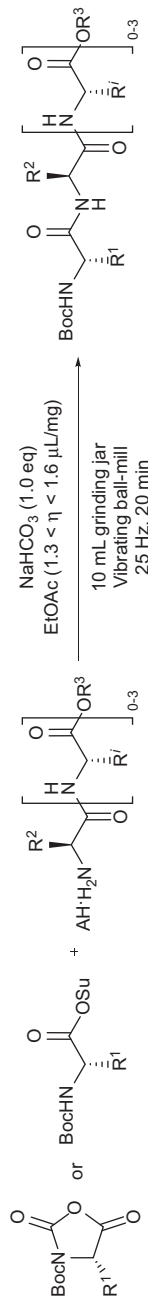


Table 6.2 (Continued)

Entry	Boc-AA-NCA or Boc-AA-OSu	AH · H(AA) _x -OR ³	Mechanosynthesized peptides	Yield (%) ^a
15	Boc-Trp-NCA	HCl · H-Val-Phe-OMe	Boc-Trp-Val-Phe-OMe	86
16	Boc-Ile-NCA	HCl · H-Leu-Pro-OBn	Boc-Ile-Leu-Pro-OBn	86
17	Boc-Ile-NCA	HCl · H-Ile-Ile-OMe	Boc-Ile-Ile-Ile-OMe	86
18	Boc-Gly-OSu	HCl · H-Gly-Phe-OMe	Boc-Gly-Gly-Phe-OMe	74
19	Boc-Gly-OSu	HCl · H-Phe-Leu-OBn	Boc-Gly-Phe-Leu-OBn	93 ^f (94) ^g
20	Boc-Leu-NCA	HCl · H-Leu-Leu-OBn	Boc-Leu-Leu-Leu-OBn	96
21	Boc-Gly-OSu	HCl · H-Gly-Phe-Leu-OBn	Boc-Gly-Gly-Phe-Leu-OBn	96 ^h (90) ⁱ
22	Boc-Tyr(Bn)-OSu	HCl · H-Gly-Gly-Phe-Leu-OBn	Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn	88 ^j

^aIsolated yield. 1.1 < η < 1.6 $\mu\text{L mg}^{-1}$ unless otherwise noted.

^b> 98% diastereomeric excess determined by chiral HPLC.

^cCompleted in 1 h.

^dML = 28.4 mg mL^{-1} , $\eta = 1.1 \mu\text{L mg}^{-1}$.

^eML (milling load) = 188.8 mg mL^{-1} , $\eta = \text{of } 1.1 \mu\text{L mg}^{-1}$. Completed in 2 h.

^fML = 22.9 mg mL^{-1} .

^gML = 152.7 mg mL^{-1} . Completed in 40 min with *t*BuOAc as the grinding auxiliary.

^hML = 22.2 mg mL^{-1} , $\eta = 1.1 \mu\text{L mg}^{-1}$.

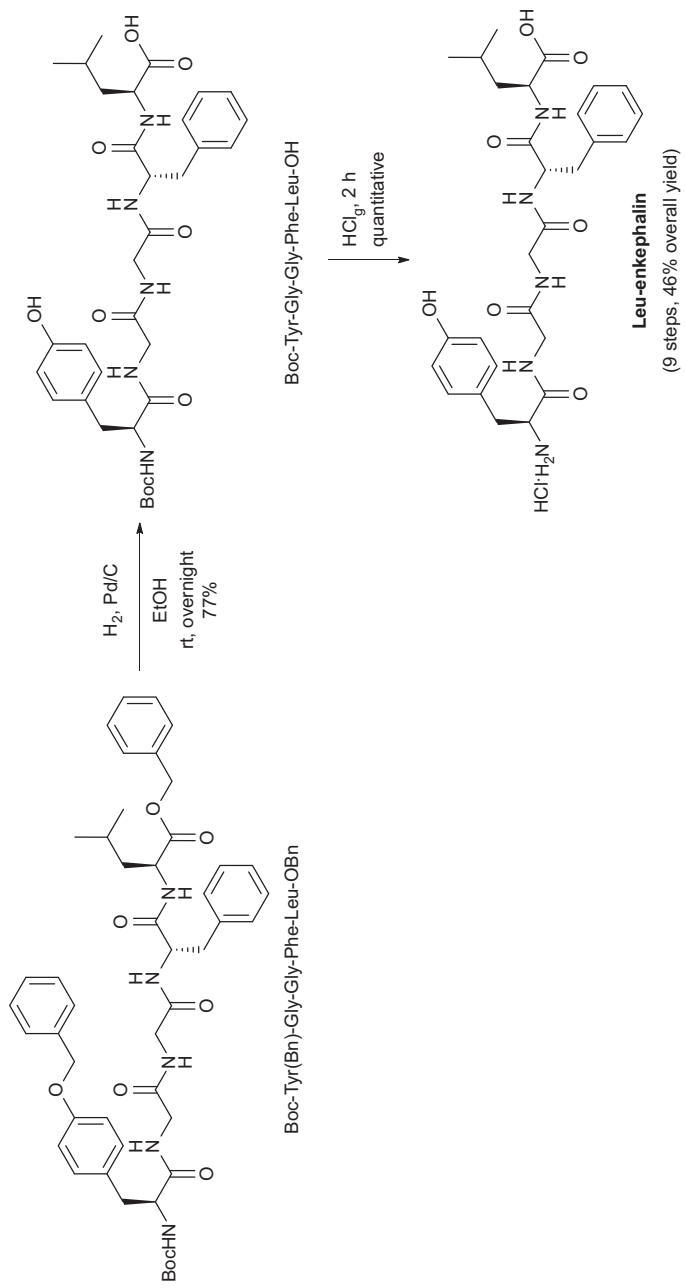
ⁱML = 60.6 mg mL^{-1} , $\eta = 1.1 \mu\text{L mg}^{-1}$. Completed in 40 min with *t*BuOAc as the grinding aid.

^jCompleted in 1 h.

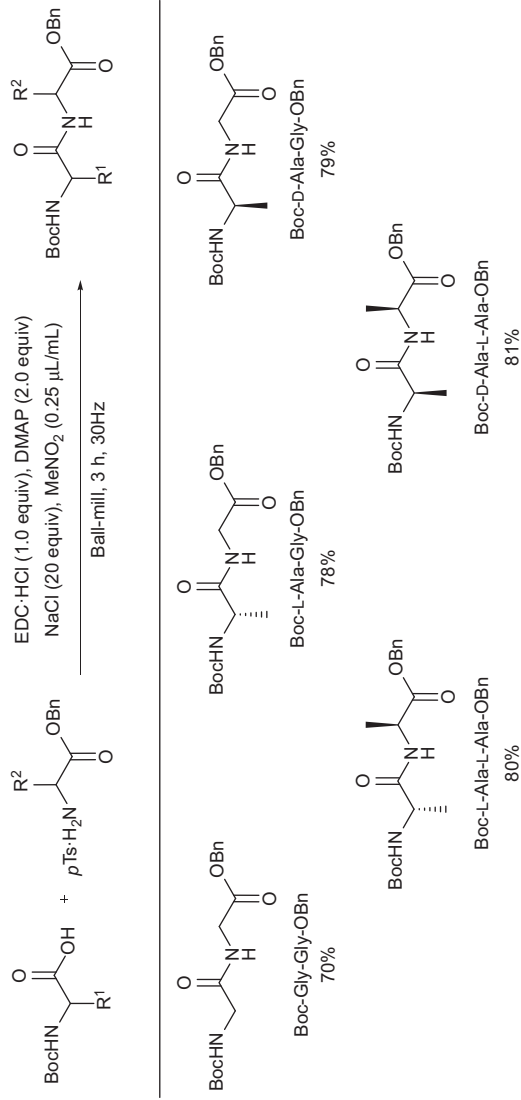
milling load from 22.2 to 60.6 mg mL⁻¹ did not dramatically hamper the efficacy of the reaction as the tetrapeptide Boc-Gly-Gly-Phe-Leu-OBn was produced in 96% and 90% yield, respectively. Finally, after solvent-free deprotection of the Boc group, the tetrapeptide HCl · H-Gly-Gly-Phe-Leu-OBn was reacted with Boc-Tyr(Bn)-OSu and furnished, after 1 h of grinding, the pentapeptide Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn in 88% yield (Table 6.2, entry 22). The synthesis of the last peptide involved four mechano-mediated peptide bond couplings realized in a row intersected by three solvent-free Boc deprotections. To the best of our knowledge, this is the longest linear organic synthesis to have been performed through ball-milling technology. This approach has allowed strong minimization of the use of organic solvents while avoiding the predominant toxic solvents such as DMF or DCM encountered in classical peptide synthesis. In addition, these reaction conditions require no more than stoichiometric quantities of non-hazardous reactants and transient Boc deprotections are realized without any solvent.

This approach was finally applied to the synthesis of the natural peptide Leu-enkephalin. The latter is an endogenous neurotransmitter presenting agonist activity at μ and δ opioid receptors, which was first reported and synthesized in 1975.⁴⁷ Thus, benzylic groups of Boc-Tyr(Bn)-Gly-Gly-Phe-Leu-OBn were cleaved by pallado-catalyzed hydrogenation to furnish Boc-Tyr-Gly-Gly-Phe-Leu-OH in 77% yield (Scheme 6.20). Then, the Boc protecting group was removed by gaseous HCl to produce Leu-enkephalin hydrochloride salt in quantitative yield. By using this strategy, Leu-enkephalin was efficiently produced in nine steps with a 46% overall yield.

Another interesting approach was developed in 2012 by Margetić³⁹ and coworkers to produce dipeptides by using a mechanochemical process. Instead of starting from pre-activated *N*-carboxyanhydrides or hydroxy-succinimide esters of α -amino acids substrates, Margetić and coworkers utilized Boc-protected α -amino acids as starting material. These α -amino acids were activated in the reaction media by addition of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC · HCl) in the presence of 2 equivalents of dimethylaminopyridine (DMAP) as the base, 20 equivalents of NaCl as a solid grinding assistant and small quantities of nitromethane as a liquid grinding auxiliary. Whereas originally optimized for coupling benzoic acid with *p*-anisidine (Section 6.2.5), these conditions were applied to the synthesis of a limited range of dipeptides with yields ranging from 70% to 81% (Scheme 6.21). It is worth mentioning that the dipeptides were recovered without using any organic solvent. Based on the low water-solubility of the products, a simple aqueous work-up followed by filtration allowed the isolation of the pure dipeptides. Thus, Boc-Gly-Gly-OBn was isolated with 70% yield starting from Boc-Gly-OH and *p*Ts · H-Gly-OBn. When treated with *p*Ts · H-Gly-OBn, α -substituted Boc-L-Ala-OBn and Boc-D-Ala-OBn furnished Boc-L-Ala-Gly-OBn and Boc-D-Ala-Gly-OBn with slightly improved yields of 78% and 79%, respectively. Similar yields were obtained when Boc-L-Ala-L-Ala-OBn and its diastereoisomer Boc-D-Ala-L-Ala-OBn were synthesized (80% and 81% respectively).



Scheme 6.20 Synthesis of Leu-enkephalin.



Scheme 6.21 Mechanosynthesis of dipeptides from non-protected α -amino acids.

This approach is advantageous in that it enables the production of dipeptide avoiding the use of pre-activated α -amino acids as substrates. Nevertheless, this particularity may be useful only in the very limited number of cases where the pre-activated α -amino ester is hardly synthesizable or not commercially available. Besides, the approach developed by Margetić and coworkers still requires the use of 2 equivalents of the highly toxic organic base DMAP. In addition, one can regret the use of an explosive liquid as grinding assistant such as nitromethane, which unfortunately hampers the environmental impact of the process.

In contrast, a very interesting point in this approach is that the peptide recovery is realized in the absence of any organic solvent. Organic solvent-free recovery and purification have been a scarcely studied theme in fine organic chemistry, but promising methods have been published recently.^{38,40,48–51} Mechanochemical peptide syntheses would gain even more interest in the organic chemist's community if solutions addressing this challenge would emerge.

6.4 Conclusion

Performing a reaction in a ball-mill is an efficient way of reaching more sustainable conditions in organic synthesis, especially if combined with innocuous solvent-assisted grinding and organic solvent-free recovery or purification. These approaches were highlighted in this chapter, demonstrating that amino acid derivatization or protection and peptide synthesis could greatly benefit from such processes.

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