EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Mechanochemical synthesis of dipeptides using Mg-Al hydrotalcite as activating agent under solvent-free reaction conditions

Authors: José M. Landeros and Eusebio Juaristi

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201601276

Link to VoR: http://dx.doi.org/10.1002/ejoc.201601276

Supported by
ACES Asian Chemical Editorial Society

WILEY-VCH

10.1002/ejoc.201601276

WILEY-VCH

Mechanochemical synthesis of dipeptides using Mg-Al hydrotalcite as activating agent under solvent-free reaction conditions

José M. Landeros,[a] and Eusebio Juaristi*[a,b]

Dedication ((optional))

Abstract: Given the high demand for green and sustainable synthetic methods for the formation of amides and peptidic bonds, herein we report the efficient, solvent-free mechanochemical synthesis of dipeptides from *N*-protected amino acids and amino acid methyl ester hydrochlorides in the presence of 1-hydroxy benzotriazole (HOBt) and *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as coupling reagents, and using Mg-Al hydrotalcite-like minerals as green activating agent. From commercial Mg-Al hydrotalcite (HT-S) we obtained its calcined (HT-C) and reconstructed (HT-R) modifications, which were evaluated as activating heterogeneous bases in amidation reactions, replacing commonly used toxic and/or corrosive bases (e.g., NEt_3, iPr_2NEt and NaOH). As a practical application of this strategy, various α,α- and α,β-dipeptides were prepared in good to excellent yields. Under optimized reaction conditions (vibrating mill at 25 Hz for 75 min) HT-S and HT-R showed higher activity (89% yield of the desired peptide) as compared with HT-C (57% yield). The present protocol offers several advantages, including the use of readily available reagents and inexpensive materials, easy work-up, simple recovery and recyclability of the hydrotalcite activator, and short reaction times.

Introduction

The formation of the peptidic (amide) bond is the key step in peptide and protein synthesis, and the relevance of this synthetic process is recognized both in industry and academia.[1] The amide functional group is present in about 25% of all drugs currently commercialized,[2] and this fact has motivated many recent advances in the area of synthesis of amides and peptides, including the development of new, racemization-suppressing coupling agents,[3] as well as methods for the direct activation of carboxylic acids for their condensation reaction with amines.[3b,4] Nevertheless, most synthetic methods for amide bond formation are performed in homogeneous phase, requiring the use of potentially hazardous organic solvents such as CH_2Cl_2 and DMF toxic catalysts such as tertiary amines, harsh reaction conditions long reaction times, and concomitant formation of large quantities of chemical waste. These facts are contrary to the principles of sustainable chemistry,[5] which mandate the minimization or even complete removal of solvents in chemical transformations, the use of efficient catalysts, as well as the development of energy-saving processes.

In this regard, in recent years several green strategies in organic chemistry that employ non-traditional energy sources, such as ultrasound irradiation, microwave, and high-speed ball-milling (HSBM) have been developed.[6] The latter technique (HSBM) also known as mechanochemistry, has proven to be a viable green alternative for the synthesis of amides and peptides under solvent-free conditions or liquid assisted grinding (LAG), achieving good yields at short reaction times, reducing production costs, and minimizing waste of valuable chemicals.[7]

In this context, the development of catalytic protocols and the use of heterogeneous activators is highly desirable in the quest of sustainable chemistry. In this regard, the replacement of homogeneous catalysts/activating agents by heterogeneous counterparts offers several advantages such as easy separation and recovery of the catalyst/activating agent from the reaction medium, no need for neutralization and extraction steps, possibility of recycling of the catalyst/activating agent, and reduction of waste formation.[8]

Among heterogeneous solid bases, hydrotalcite minerals (HT´s) have been used as replacement of classic bases (alkali metal hydroxides or amines) in large-scale synthesis of fine chemicals and in pharmaceutical processes. HT´s are commercially available, inexpensive synthetic or natural anionic clays. Naturally occurring HT has the general formula $[Mg^{2+}_{1-x}Al^{3+}_{x}(OH)_2]^{x+}(CO_3^{n-})_{x/n}.mH_2O$, where Mg^{2+} and Al^{3+} are di- and trivalent cations, respectively, $(CO_3^{n-})_{x/n}$ is an interlayer compensating anion, *x* corresponds to the fraction of trivalent cations in the brucite-type layers $[Mg(OH)_2]$ and *m* is the water of crystallization.[9] Numerous studies of the preparation and determination of the physicochemical properties of HT´s have been reported.[10] Among the three most important properties of HT´s are: (1) its composition, which gives rise to a solid material with basic character, modulated by the corresponding anion. (2) The formation of homogeneous mixed oxides after heat treatment (420-470 °C) with generation of MgO acid-base pairs. (3) When calcined hydrotalcite is rehydrated under a flow of water vapor at ambient temperature, the original layered structure is restored, in a so-called “memory effect”. These characteristics turn HT’s into interesting materials for application as heterogeneous activating agents.[9,11]

[a] J. M. Landeros, E. Juaristi
Departamento de Química
Centro de Investigación y de Estudios Avanzados,
Instituto Politécnico Nacional,
Avenida IPN No. 2508, 07360 Ciudad de México, Mexico
E-mail: juaristi@relaq.mx, ejuarist@cinvestav.mx
http://www.relaq.mx/RLQ/EusebioJuaristi.html
[b] E. Juaristi
El Colegio Nacional
Luis González Obregón No. 23, Centro Histórico,
06020 Ciudad de México, Mexico.

Supporting information for this article is given via a link at the end of the document.

Recently Morales-Serna and coworkers reported the synthesis of peptides and amides under activation by hydrotalcite, in the presence of EDC and HOBt, and under heterogeneous conditions using CH_2Cl_2 as solvent.[12] Although the observed yields varied from good to excellent, extended reaction times and use of CH_2Cl_2 as reaction medium demerit its application as a synthetic green chemistry strategy. On the other hand, Štrukil and coworkers have reported the mechanochemical synthesis of amides and dipeptides by carbodiimide-mediated coupling. Among several coupling agents, EDC turned out most suitable under neat or LAG conditions. Afterwards, Lamaty and coworkers were able to synthesize di-, tri- and tetra-peptides in good yields, using EDC as coupling agent and in the presence of Oxyme (ethyl hydroxy-iminocyanoacetate), NaH_2PO_4 and LAG conditions.

Taking these developments into account, we decided to adapt the protocol suggested by Morales-Serna et al.[12] to carry out peptide synthesis in a green mechanochemical strategy. Thus, we report here the mechanosynthesis of α,α-, α,β- and β,β-dipeptides by amidation between *N*-protected-amino acids and amino methyl ester hydrochlorides activated catalyzed by hydrotalcite-like minerals in the presence of appropriate coupling reagents and under solvent-free conditions.

The present work is part of our efforts to extend the applicability of mechanochemistry in organic synthesis.[13]

Results and Discussion

2.1. Characterization of hydrotalcite minerals

Powder XRD patterns of commercial Mg–Al hydrotalcite (HT-S), its calcined form (HT-C), and reconstructed form (HT-R) are shown in Figure 1. The HT-S sample display sharp and intense reflections at 11.6°, 23.3°, 60.7° and 62.0° [ascribed to diffraction by planes (0 0 3), (0 0 6), (1 1 0), and (1 1 3), respectively] characteristic of a well-crystallized Mg–Al hydrotalcite (JCPDS: 41-1428). The calcined hydrotalcite (HT-C, 450 °C) exhibit low reflections at 43.4°and 63.0° [ascribed to the diffraction (4 0 0), and (4 4 0) planes, respectively] associated with Mg(Al)O mixed oxide periclase-like structure, similar to XRD of MgO (JCPDS: 45-0946), owing to loss of interlayer water and decarbonation in a lamellar structure. Rehydration of HT-C in water vapor restored the layered structure as result of the memory effect. HT-R shows similar reflection pattern relative to HT-S; however, HT-R is less crystalline as reflected by intensity and sharpness of (0 0 3) and (0 0 6) planes, which are directly correlated to the crystallinity of the material.[14]

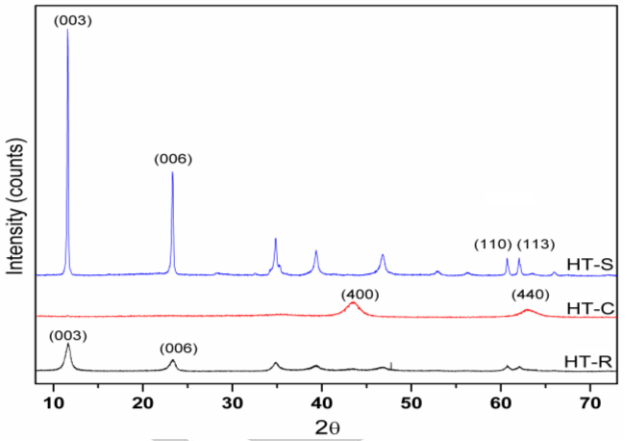


Figure 1. Powder XRD patterns of commercial hydrotalcite (HT-S), hydrotalcite calcined to 450 °C (HT-C) and reconstructed hydrotalcite (HT-R).

FTIR-ATR spectra of commercial (HT-S), calcined (HT-C) and reconstructed hydrotalcite (HT-R) are shown in Figure 2. The spectrum of HT-S reveals a broad band at 3413 cm^{-1}, characteristic of hydroxyl group stretching vibration, which is attributed to interlayer water and hydroxyl groups of the brucite-like layers. Hydrogen bonding between carbonate ions and water in the interlayer is detected as a shoulder close to 3027 cm^{-1}, whereas the bands at 1640 and 1365 cm^{-1} are associated with interlayer water molecules and carbonate anions, respectively.[15] The bands at 929 and 771 cm^{-1} are assigned to Al–O bonds.[16]

On the other hand, the bands 3413 and 1365 cm^{-1}, related to water bending vibrations and interaction of water–carbonate ions in the interlayer, are no longer present in the sample of HT-C, due to loss of water and carbonate ions during calcinations. The weak band at 1400 cm^{-1} is attributed to interaction between remaining CO_3^{2-} and Mg^{2+}.

Rehydration of HT–C in a decarbonated–water saturated gas flow induced a reconstruction of hydrotalcite structure. The HT-R exhibits bands attributed to hydroxyl groups and water around 3448 and 1640 cm^{-1}, respectively. Finally, the band at 1369 cm^{-1} corresponds to carbonate ions. [10c]

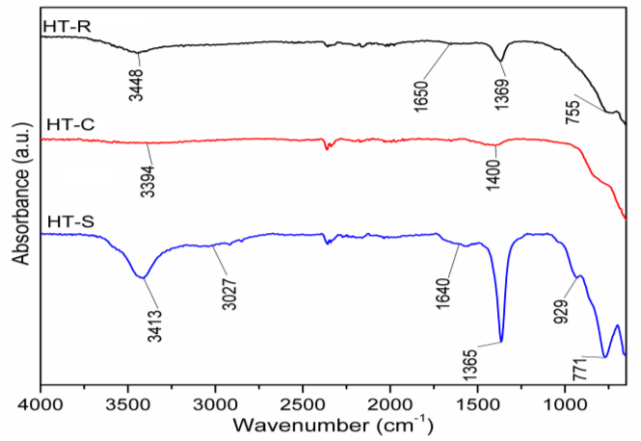


Figure 2. Infrared spectra of hydrotalcite-like materials.

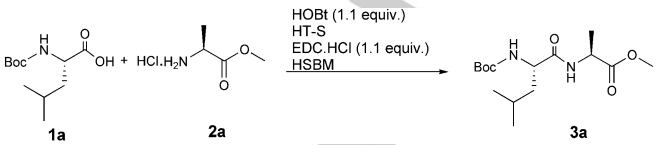
2.2. Mechanosynthesis of dipeptides catalyzed by hydrotalcite-like minerals

In order to evaluate the activating potential of the hydrotalcite-like materials (HT-S, HT-C and HT-R) in amidation reactions under high speed ball milling (HSBM) and solvent-free conditions, we began this study following the best experimental conditions described by Morales-Serna et al.,[12] that is employing *N*-Boc-L-leucine (**1a**, 0.25 mmol), L-alanine methyl ester hydrochloride (**2a**, 0.25 mmol), EDC (0.275 mmol), and HOBt (275 mmol). The parameters examined to optimize this procedure include the vibration frequency of ball milling (Hz); the type of material and size of the jar mill, the molar ratio of hydrotalcyte (HT-S), and the reaction time (Table 1).

The vibration frequency effect at 15, 20 and 25 Hz was examined in an agate jar mill (Jar-1) as shown in entries 1 to 3 (Table 1). Following a sequence of three cycles (90 min each), the highest yield (41%) was obtained at 25 Hz. Subsequently, the size and type of material of the jar mill were evaluated (Table 1, entries 4-7), finding that the stainless-steel Jar-3 (12 mL, 2.1 cm of diameter) at 25 Hz affords product **3a** in higher yield (65%). After selecting the operating frequency (25 Hz) and the type of reactor (Jar-3), the effect of the quantity of HT-S on the reaction was examined (Table 1, entries 8-12). Best results were observed when employing 250 mg (0.41 mmol) of HT-S, the reaction afforded the desired product **3a** in 89% yield. Finally, the reaction time and the number of milling balls were optimized. Best results were found with reactor Jar-3, with one or two balls, at 25 Hz, 75 min of reaction time, and 250 mg of HT-S (Table 1, entries 16 and 20).

A control experiment under the optimal reaction conditions but without HT-S as activating agent, afforded only traces of the expected product (entry 22 in Table 1). This result indicates that it neither HOBt nor EDC are sufficient for the condensation reaction to take place. That is, the presence of the HT-S as activating base is crucial for the reaction to proceed.

Table 1. Optimization of the Reaction Conditions for the Synthesis of *N*-Boc-L-Leu-L-Ala-OMe Dipeptidic Derivative **3a**[a].



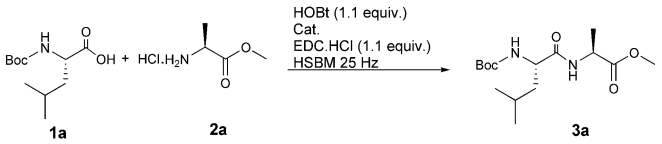
Entry	HT-S [mg]	Time [min] (Cycles)	Power [Hz]	No. balls (d [mm])	Reactor Type[b]	Yield %[c]
1	62.5	90 (3)	15	2 (5)	Jar-1	22
2	62.5	90 (3)	20	2 (5)	Jar-1	35
3	62.5	90 (3)	25	2 (6)	Jar-1	41
4	62.5	90 (3)	25	2 (6)	Jar-2	43
4	62.5	90 (3)	25	2 (6)	Jar-2	43
5	62.5	90 (3)	25	1 (6)	Jar-2	38
6	62.5	90 (3)	25	1 (9)	Jar-3	63
7	62.5	90 (3)	25	2 (9)	Jar-3	65
8	125	90 (3)	25	2 (9)	Jar-3	83
9	200	90 (3)	25	2 (9)	Jar-3	88
10	250	90 (3)	25	2 (9)	Jar-3	89
11	300	90 (3)	25	2 (9)	Jar-3	87
12	500	90 (3)	25	2 (9)	Jar-3	45
13	250	90 (3)	25	2 (9)	Jar-3	89
14	250	90 (2)	25	2 (9)	Jar-3	89
15	250	90 (1)	25	2 (9)	Jar-3	89
16	**250**	**75 (1)**	**25**	**2 (9)**	**Jar-3**	**89**
17	250	60 (1)	25	2 (9)	Jar-3	82
18	250	45 (1)	25	2 (9)	Jar-3	81
19	250	30 (1)	25	2 (9)	Jar-3	50
20	**250**	**75 (1)**	**25**	**1 (9)**	**Jar-3**	**89**
21	250	60 (1)	25	1 (9)	Jar-3	83
22	--	75 (1)	25	1 (9)	Jar-3	traces

[a] Experimental conditions: *N*-Boc-Leucine **1a** (0.25 mmol), L-alanine methyl ester hydrochloride **2a** (0.25 mmol), EDC (0.275 mmol), HOBt (0.275 mmol). [b] Reactor type: Jar-1 of agate (5 mL, 1.2 cm diameter) and ball of agate; Jar-2 of stainless steel (6 mL, 1.2 cm diameter) and ball of stainless steel; Jar-3 of stainless steel (12 mL, 2.1 cm diameter). [c] Yield of isolated product (**3a**) after chromatographic purification.

Once established the optimal conditions for amide bond formation with commercial HT-S as activating agent had been established, HT-C and HT-R forms were evaluated (Table 2). The results show that the HT-C mineral form affords product **3a** in moderate yield (55-57%), even with prolonged reaction times (Table 2, entries 2 and 3). By contrast, the material HT-R turned out to be comparable in efficiency to HT-S (Table 2, entries 4 and 5).

Interestingly, other representative bases such as $NaHCO_3$, Cs_2CO_3 and K_2CO_3 were evaluated in order to compare them with the hydrotalcites. The yields obtained with these inorganic salts were generally lower (Table 2, entries 6-10).

Table 2. Screening of Activating Agents for the Synthesis of *N*-Boc-L-Leu-L-Ala-OMe Dipeptidic Derivative **3a**[a].



Entry	Catalyst	mg	Time [min] (Cycles)	Yield[b] [%]
1	HT-S	250	75 (1)	89
2	HT-C	250	75 (1)	55
3	HT-C	250	90 (2)	57
4	HT-R	250	75 (1)	89
5	HT-R	250	90 (2)	89
6	$NaHCO_3$	250	75 (1)	76
7	$NaHCO_3$	250	90 (2)	78
8	$NaHCO_3$	34	75 (1)	60
9	Cs_2CO_3	250	75 (1)	67
10	K_2CO_3	250	75 (1)	15

[a] Reaction conditions: *N*-Boc-Leucine **1a** (0.25 mmol), L-alanine methyl ester hydrochloride **2a** (0.25 mmol), EDC (0.275 mmol), HOBt (0.275 mmol), Jar-3 of stainless steel (12 mL, 2.1 cm diameter). [b] Yield of isolated product (**3a**) after chromatographic purification.

According to the above observations, both the HT-S and HT-R materials present higher activity. Nevertheless, the lower cost of HT-S represents an advantage. Accordingly, we examined the scope of the HT-S promoting amidation reaction. A variety of amino methyl ester hydrochlorides were coupled with different *N*-protected-amino acids [including the protecting groups *tert*-butyloxycarbonyl (Boc); benzyloxycarbonyl (Cbz) and 9-fluorenylmethoxycarbonyl (Fmoc)] to give the corresponding dipeptide products in the yields summarized in Table 3.

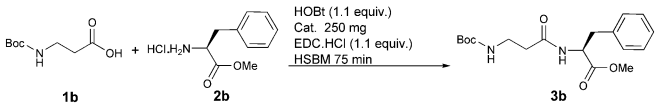
As part of the present study, recycling of the HT-S activating material was carried out in the synthesis of dipeptide **3b**. The HT-S material that had been recovered after filtration, was dried at 100 °C and reused. This procedure was repeated up to four cycles (Table 4). The results show a significant decrease in product yield after each cycle (Table 4, entries 1-4). Nevertheless, when the recovered material HT-S of the second cycle was calcined and rehydrated (see the Experimental Section), the corresponding reconstructed hydrotalcite HT-R was obtained and characterized (see Figures S4 and S5 in the Supporting Information), exhibiting total activity recovery (Table 4, entry 5).

Table 3. Scope of Dipeptide Synthesis Under Solvent-Free Conditions and with Hydrotalcite HT-S as Activating Agent.[a]

Entry	*N*-Protected Amino acid	Amino methyl ester [b]	Dipeptide *N*-Protected-aa-aa-OMe	Yield (%)[b]
1	Boc-Leu	Ala	*N*-Boc-Leu-Ala (**3a**)	89
2	Boc-β-Ala	Phe	*N*-Boc-β-Ala-Phe (**3b**)	89
3	Boc-β-Ala	Val	*N*-Boc-β-Ala-Val (**3c**)	85
4	Boc-β-Ala	Ala	*N*-Boc-β-Ala-Ala (**3d**)	79
5	Boc-β-Ala	Leu	*N*-Boc-β-Ala-Leu (**3e**)	89
6	Boc-β-Ala	Ile	*N*-Boc-β-Ala-Ile (**3f**)	82
7	Boc-β-Ala	Gly	*N*-Boc-β-Ala-Gly (**3g**)	78
8	Boc-β-Ala	His	*N*-Boc-β-Ala-His (**3h**)	70[c]
9	Boc-β-Ala	β-Ala	*N*-Boc-β-Ala-β-Ala (**3i**)	73
10	Cbz-Phe	Phe	*N*-Cbz-Phe-Phe(**3j**)	83
11	Cbz-Phe	Ala	*N*-Cbz-Phe-Ala (**3k**)	85
12	Fmoc-Val	Phe	*N*-Fmoc-Val-Phe (**3l**)	83
13	Fmoc-Val	Ala	*N*-Fmoc-Val-Ala (**3m**)	88

[a] Reaction conditions: *N*-protected-amino acid (0.25 mmol), amino methyl ester hydrochloride (0.25 mmol), EDC (0.275 mmol), HOBt (0.275 mmol), HT-S (250 mg, 0.41 mmol), Jar-3 of stainless steel (12 mL, 2.1 cm diameter). [b] Yield of isolated product after chromatographic purification. [c] HT-S (400 mg, 0.66 mmol) was used.

Table 4. Recycling of the Hydrotalcite Activating Agent in the Synthesis of α,β-Dipeptidic Derivative **3b**[a].



1b 2b 3b

Entry	Catalyst [250 mg]	Cycle [b]	Yield (%)[b]
1	HT-S	1	81
2	HT-S	2	75
3	HT-S	3	57
4	HT-S	4	43
5	HT-R[c]	3	83

[a] Reaction conditions: *N*-Boc-*β*-ala **1b** (0.25 mmol), L-phenylalanine methyl ester hydrochloride **2b** (0.25 mmol), EDC (0.275 mmol), HOBt (0.275 mmol). [b] Yield of isolated product (**3b**) after chromatographic purification. [c] Reconstructed hydrotalcite HT-S after second recycle.

Finally, when the synthesis of dipeptidic derivative **3a** was carried out at the larger scales of 1 and 2 mmol, the observed yields were 76 and 60%, respectively (for experimental details see Supporting Information). This decrease in yield can be attributed to an unfavorable reaction medium (sticky and viscous) and slow mass transfer, as previously discussed by Bonnamour and coworkers.[17] In order to improve the mixing of the reagents in the ball mill, a few drops of solvent ("minimal solvent") were added. This strategy is called solvent-assisted grinding (LAG).[18] In this context, a recent study by Porte and coworkers[7d] recommends the use of EtOAc and γ-valerolactone as green additives in α-peptide mechanosynthesis. Therefore, we examined to use EtOAc as liquid additive in the reaction. Furthermore, in view of the remarkable characteristics of *N,N′*-dimethyl-*N,N′*-propyleneurea (DMPU),[19] this solvent was also examined in LAG. Indeed, cyclic urea DMPU is a polar, aprotic solvent, water soluble in any ratio and can be used as replacement of toxic DMF. Besides DMPU has shown its effectiveness as a co-solvent in highly nucleophilic reactions.[19] With EtOAc and DMPU (0.5 µl per mg of reactants) as LAG additives the expected dipeptide was obtained in 84 and 83% yield, respectively (for experimental details see Supporting Information). Additional studies on the use of DMPU in LAG are on-going in our laboratories.

Conclusions

In summary, this work shows that Mg-Al hydrotalcite-like materials are efficient basic activators for amidation (peptide bond formation) of *N*-protected-amino acids in the presence of HOBt and EDC, under mechanochemical, solvent-free reaction conditions. Dipeptidic derivatives were obtained in good to excellent yields using commercial (HT-S) and reconstructed hydrotalcite (HT-R), whereas calcined hydrotalcite (HT-C) afforded lower yields. The present protocol offers several advantages including short reaction time (75 min), the use of low-cost, readily available reagents, mild reaction conditions, easy recovery of the catalyst, and minimization of waste.

Experimental Section

General Remarks: All reagents and solvents were obtained from Sigma–Aldrich and were used without further purification, unless otherwise stated. Commercial *N*-ethyl-*N*´-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 1-hydroxy benzotriazole (HOBt) and Hydrotalcite Synthetic® ($Mg_6Al_2(CO_3)(OH)_{16}{\cdot}4H_2O$) were also acquired from Sigma-Aldrich. Flash column chromatography was performed with Silica gel. Mechanochemical experiments were carried out in a Retsch MM200 ball mill that was equipped with one of three possible types of reactors: *Jar-1,* an agate mill jar (5 mL, 1.2 cm diameter) equipped with two balls of agate (diameter: 6 mm; mass: 0.44 g); *Jar-2,* stainless steel mill jar (6 mL, 1.2 cm diameter) equipped with one (or two) ball mill(s) (diameter: 6 mm, mass 1.52 g); *Jar-3,* a stainless steel mill jar (12 mL, 2.1 cm diameter) equipped with one (or two) stainless steel ball(s) (diameter: 9.0 mm; mass: 5.6 g). FTIR-ATR spectra were recorded with a Perkin–Elmer (PC16, Spectrum GX) FTIR/FIR spectrometer with attenuated total reflectance (ATR). The NMR spectroscopic data were recorded on Jeol ECA (500 MHz) and Bruker DP300 (300 MHz) spectrometers. ^{1}H NMR chemical shifts are in parts per million (ppm) and coupling constants in hertz (Hz). The multiplicities of the NMR signals are reported by using the following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Melting points (mp) were measured with a Büchi B-540 instrument. High-resolution mass spectra were recorded with an LC/MSD-TOF Agilent mass spectrometer. The hydrotalcites were characterized by powder XRD with Cu-Kα1 radiation, using a D-500 SIEMENS diffractometer (equipped with a graphite monochromator) in the range from 4 to 75◦ (2θ) and FTIR-ATR.

Activation of hydrotalcite: Calcined hydrotalcite (**HT-C**). This material was prepared from Hydrotalcite Synthetic® (**HT-S**) according to the procedure reported by Rao and coworkers.[20] HT-S was heated to 450 °C in a tubular furnace (heat rate 5°/min) under air flow for 8 h, and then cooled to room temperature under nitrogen flow to give **HT-C** as a white powder.

Reconstructed hydrotalcite (**HT-R**). This material was prepared from **HT-C** according to the procedure reported by Rao and coworkers.[20] The calcined hydrotalcite (**HT-C**) was rehydrated at room temperature under a flow of water vapor and nitrogen for 48 h. The resulting solid was dried at 100 °C in a tubular furnace under nitrogen flow for two hours to give **HT-R** as a white powder.

General procedure for the synthesis of dipeptides 3a-3m. A mixture of *N*-protected derivatives of α- or β-amino acids (0.25 mmol), α- or β-aminoester hydrochloride (0.25 mmol) and HOBt (0.275 mmol) was milled in a stainless steel grinding jar (12 mL, 2.1 diameter) equipped with one stainless steel ball (9 mm diameter, 5.6 g) for 2 min at 25Hz.

Commercial hydrotalcite **HT-S** (0.41 mmol) was added and milled for 2 min at 25 Hz. Finally EDC (0.275 mmol) was added and milled for 75 min at 25 Hz. The resulting crude product mixture was recovered with ethyl acetate (30 mL) and filtered to remove the hydrotalcite solid material. The organic phase was washed successively with 10% citric acid solution (2 x 5 mL), 10% $NaHCO_3$ solution (2 x 5 mL), 10% K_2CO_3 solution (2 x 5 mL), brine (2 x 5 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated in vacuum and the resulting residue was purified by flash column chromatography on silica gel (hexanes/EtOAc) to give dipeptides **3a-3m**.

***N*-Boc-Leu-L-Ala-OMe** (**3a**).[12] *N*-Boc-Leucine **1a** (0.25 mmol), L-alanine methyl ester hydrochloride **2a** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 70 mg of **3a** as a white solid (89% yield), mp = 110-114 °C; $[\alpha]^{25}_D$ = –46.8 (*c* 0.33 in MeOH), [lit.[12] mp 113-114 °C; $[\alpha]^{25}_D$ = –49.2 (*c* 0.0116 in MeOH)]. (FT-IR/ATR cm^{-1}) ν_{max} 3440, 2966, 2931, 1700, 1511. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.81 (1H, br s, Ala-NH), 5.02 (1H, br, Leu-NH), 4.52 (1H, q, *J* = 7.2 Hz), 4.12 (1H, br, $CHCO_2$), 3.70 (3H, s, OCH_3), 1.67-1.57 (2H, m, CH_2 *i*-Bu), 1.42-1.48 (1H, m, CH*i*-Bu), 1.40 (9H, s, *t*-Bu), 1.35 (3H, d, *J* = 7.1 Hz, CH_3), 0.90 (6H, t, CH_3*i*-Bu, *J* = 6.1 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.1, 172.1, 155.6, 80.1, 53.0, 52.4, 48.0, 41.3, 28.3, 24.7, 22.9, 22.0, 18.3. HR-ESI-TOF calcd for $C_{15}H_{28}N_2O_5$ [M + H]$^+$: 317.2070, found: 317.2071 (0.1 ppm error).

***N*-Boc-*β*-Ala-L-Phe-OMe** (**3b**).[21] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-phenylalanine methyl ester hydrochloride **2b** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 78.3 mg of **3b** as a white solid (89% yield), mp = 92–94 °C. $[\alpha]^{25}_D$ = + 58.8 (*c* 1.0, $CHCl_3$), [lit.[21] mp 91-92 °C. $[\alpha]^{25}_D$ = +51.0 (*c* 1.0, $CHCl_3$)] . (FT-IR/ATR cm^{-1}) ν_{max} 3359, 3313, 29763, 1739, 1681, 1646. ^{1}H NMR (350 MHz, $CDCl_3$) δ 7.30-7.23 (3H, m, H-Ar), 7.09-7.07 (2H, d, H_{ortho}-Ar, *J* = 7.0 Hz), 6.12 (^{1}H, br, Phe-NH), 5.12 (1H, br-s, *β*- Ala-NH), 4.86 (1H, m, CH), 3.72 (3H, s, OCH_3), 3.35 (2H, m, CH_2*N*Boc), 3.13, (1H, dd,CH_2Ph, *J* = 5.6, 13.8 Hz), 3.05 (1H, dd, CH_2Ph, *J* = 6.2, 13.8 Hz), 2.36 (2H, t, CH_2C=O, *J* = 5.6 Hz),1.44 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) 172.0, 171.2, 156.1, 135.8, 129.3, 128.7, 127.3, 79.4, 53.2, 52.5, 38.0, 36.6, 36.2, 28.5 ppm. HR-ESI-TOF calcd for $C_{18}H_{27}N_2O_5$ [M + H]$^+$: 351.1914, found: 351.1915 (0.1 ppm error).

***N*-Boc-*β*-Ala-L-Val-OMe** (**3c**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-valine methyl ester hydrochloride **2c** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 64.2 mg of **3c** as a gummy solid (85% yield). $[\alpha]^{25}_D$ = +9.0 (*c* 1.6, $CHCl_3$), [Lit.[13a] $[\alpha]^{25}_D$ = +12.0 (*c* 1.6, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3340, 3324, 2958, 2919, 1735, 1689, 1650. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (1H, br, Val-NH), 5.25 (1H, br-s, *β*-Ala-NH), 4.50 (1H, m, ($CHCO_2$)), 3.68 (3H, s, OCH_3), 3.36 (2H, m, CH_2NBoc), 2.44 (2H, m, CH_2C=O), 2.15 (1H, m, (CH)), 1.38 (9H, s, *t*-Bu), 0.92 (3H, d, CH_3, *J* = 6.8 Hz), 0.89 (3H, d, CH_3, *J* = 6.8 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 171.6, 156.0, 79.2, 57.0, 52.2, 36.6, 36.0, 31.10, 28.3, 18.9, 17.8 ppm. HR-ESI-TOF calcd for $C_{14}H_{27}N_2O_5$ [M + H]$^+$: 303.1914, found: 303.1915 (0.3 ppm error).

***N*-Boc-*β*-Ala-L-Ala-OMe** (**3d**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-alanine methyl ester hydrochloride **2d** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 54.1 mg of **3d** as a white solid (79% yield), mp = 76-79 °C. $[\alpha]^{25}_D$ = + 45.5 (*c* 0.1, $CHCl_3$) [lit.:[13a] mp = 78-80 °C. $[\alpha]^{25}_D$ = +55.0 (*c* 0.1, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3357, 3322, 2983, 1745, 1681, 1646. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.33 (1H, br, Ala-NH), 5.21 (1H, br-s, β-Ala- NH), 4.56 (1H, q, CH, *J* = 7.2 Hz), 3.73 (3H, s, OCH_3), 3.39 (2H, m, CH_2NBoc), 2.42 (2H, t, CH_2C=O, *J* = 5.8 Hz), 1.41 (9H, s, *t*-Bu), 1.38 (3H, s,CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 171.1, 156.1, 79.3, 52.5, 48.0, 36.5, 36.0, 28.3, 18.3 ppm. HR-ESI-TOF calcd for $C_{12}H_{23}N_2O_5$ [M + H]$^+$: 275.1601, found: 275.1604 (0.8 ppm error).

***N*-Boc-*β*-Ala-L-Leu-OMe** (**3e**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-Leucine methyl ester hydrochloride **2e** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give (70.3 mg, 89% yield) of **3e** as a white solid, mp = 49-52 °C. $[\alpha]^{25}_D$ = +12.9 (*c* 0.3, $CHCl_3$) [lit.[13a] mp = 50-52 °C. $[\alpha]^{25}_D$ = + 5.5 (*c* 0.1, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3351, 2956, 2933, 1753, 1691, 1650. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.68 (1H, br, Leu-NH), 5.31 (1H, br-s, *β*-Ala-NH), 4.49 (1H, m, ($CHCO_2$)), 3.61 (3H, s, OCH_3), 3.29 (2H, m, CH_2NBoc), 2.35 (2H, m, CH_2C=O), 1.60-1.49 (2H, m, CH_2 *i*-Bu), 1.48-1.41 (1H, m, CH*i*-Bu), 1.31 (9H, s, *t*-Bu), 0.88 (6H, d,CH_3*i*-Bu, *J* = 5.8 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.5, 171.5, 156.1, 79.2, 56.2, 52.6, 41.1, 36.6, 36.0, 28.3, 24.8, 22.7, 21.7 ppm. HR-ESI-TOF calcd for $C_{15}H_{29}N_2O_5$ [M + H]$^+$: 317.2070, found: 317.2073 (0.5 ppm error).

***N*-Boc-*β*-Ala-L-Ile-OMe** (**3f**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-isoleucine methyl ester hydrochloride **2f** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 64.8 mg of **3f** as a gummy solid (82% yield), $[\alpha]^{25}_D$ = +12.31 (*c* 0.3, $CHCl_3$), [lit.[13a] $[\alpha]^{25}_D$ = +5.0 (*c* 0.7, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3317, 2966, 2927,1743, 1693, 1634. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.23 (1H, br, Ile-NH), 5.20 (1H, br-s, *β*-Ala-NH), 4.53 (1H, m, NCH), 3.69 (3H, s, OCH_3), 3.36 (2H, m, CH_2NBoc), 2.41 (2H, m, CH_2C=O), 1.83 (1H, m, CH), 1.37 (10H, m, CH_2CH_3, *t*-Bu), 1.17-1.04 (1H, m, CH_2CH_3), 0.87 (6H, m, $2CH_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 171.5, 156.0, 79.1, 56.3, 52.0, 37.6, 36.6, 35.9, 28.3, 25.1, 15.4, 11.4 ppm. HR-ESI-TOF calcd for $C_{15}H_{29}N_2O_5$ [M + H]$^+$: 317.2070, found: 317.2071 (0.1 ppm error).

***N*-Boc-*β*-Ala-Gly-OMe** (**3g**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-glycine methyl ester hydrochloride **2g** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the

synthesis of dipeptides to give 50.7 mg **3g** as a liquid (78% yield). (FT-IR/ATR cm^{-1}) ν_{max} 3309, 2915, 2846, 1739, 1639, 1662. ^1H NMR (300 MHz, $CDCl_3$) δ 6.67 (1H, br, Gly-NH), 5.30 (1H, br-s, *β*-Ala- NH), 3.98 (2H, d, CH_2CO_2, *J* = 5.36 Hz), 3.70 (3H, s, OCH_3), 3.36 (2H, q, CH_2NBoc, *J* = 5.9 Hz), 2.43 (2H, t, $CH_2C=O$, *J* = 5.7 Hz), 1.37 (9H, s, *t*-Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 170.4, 156.1, 79.2, 52.3, 41.1, 36.5, 35.9, 28.3 ppm. HR-ESI-TOF calcd for $C_{11}H_{21}N_2O_5$ $[M + H]^+$: 261.1444, found: 261.1443 (0.5 ppm error).

***N*-Boc-*β*-Ala-L-His-OMe** (**3h**).[22] *N*-Boc-*β*-alanine **1b** (0.25 mmol), L-histidine methyl ester dihydrochloride **2h** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (400 mg, 0.66 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides (not washed with citric acid solution) to give 59.7 mg of **3h** as a white solid (70% yield), mp 77-81 °C. $[\alpha]^{25}_D$ = + 5.0 (*c* 0.35, CH_3OH), [lit.[22] mp 76–78 °C; $[\alpha]^{22}_D$ +5.85 (*c* 0.29, MeOH)]. (FT-IR/ATR cm^{-1}) ν_{max} 3318, 2977, 2915, 1737, 1685, 1646, 1527. ^1H NMR (500 MHz, $CDCl_3$) δ 7.50 (1H, s, N=C*H*NH), 7.35 (1H, br-s, His-NHCO), 6.74 (1H, s, C=C*H*NH), 5.60 (1H, t, *β*-Ala-NH, *J* = 5.7 Hz), 4.74 (1H, m, CH), 3.65 (3H, 2, OCH_3), 3.36 (2H, m, CH_2NBoc), 3.0 (2H,d, CH_2, *J* = 5.1 Hz), 2.39 (2H, t, $CH_2C=O$, *J* = 5.9 Hz), 1.39 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 171.8, 156.3, 135.4, 135.0, 115.9, 79.4, 52.8, 52.5, 36.9, 36.4, 29.0, 28.4 ppm. HR-ESI-TOF calcd for $C_{15}H_{25}N_4O_5$ $[M + H]^+$: 341.1823, found: 341.1822 (0.74 ppm error).

***N*-Boc-*β*-Ala-*β*-Ala-OMe** (**3i**).[13a] *N*-Boc-*β*-alanine **1b** (0.25 mmol), *β*-alanine methyl ester hydrochloride **2i** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 50 mg of **3i** as a white solid (73% yield), mp = 76-78 C, [lit.[13a] mp = 77-78 °C]. (FT-IR/ATR cm^{-1}) ν_{max} 3359, 2973, 2915, 1731, 1681, 1643. ^1H NMR(300 MHz, $CDCl_3$) δ 6.37 (1H, br, *β*-Ala-NH), 5.23 (1H, br-s, *β*-Ala-BocNH), 3.67 (3H, s, OCH_3), 3.48 (2H, q,CH_2NBoc, *J* = 5.9 Hz), 3.35 (2H, q,$CH_2C=O$, *J* = 5.9 Hz), 2.53 (2H, t, CH_2NHCO, *J* = 5.9 Hz), 2.36 (2H, t, CH_2CO_2, *J* = 5.9 Hz), 1.41 (9H, s, *t*-Bu) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 171.5, 156.0, 79.2, 51.8, 36.5, 36.1, 34.7, 33.7, 28.3 ppm. HR-ESI-TOF calcd for $C_{12}H_{23}N_2O_5$ $[M + H]^+$: 275.1601, found: 275.1614 (0.3 ppm error).

***N*-Cbz-Phe-L-Phe-OMe** (**3j**).[23] *N*-Cbz-L-Phenylalanine **1c** (0.25 mmol), L-phenylalanine methyl ester hydrochloride **2b** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 96.2 mg **3j** as a white solid (83% yield), mp = 136–138 °C. $[\alpha]^{25}_D$ = + 35.1 (*c* 0.33, $CHCl_3$), [lit.[23] mp = 134–136 °C. $[\alpha]^{25}_D$ = –21.9 (*c* 0.01, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3307, 3270, 3033, 2946, 1733, 1687, 1660; 698. ^1H NMR (500 MHz, $CDCl_3$) δ 7.10–7.34 (15H, m, Ar), 6.37 (1H, br s, Phe-NH), 5.35 (1H, br s, Phe-NH), 5.04 (2H, m, OCH_2Ph), 4.78 (1H, br s, CH), 4.45 (1H, d, CH, *J* = 6.4 Hz), 3.65 (3H, s, OCH_3), 2.94–3.09 (4H, m, $2CH_2Ph$). ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4, 170.5, 155.9, 136.3, 136.2, 135.6, 129.4, 129.3, 128.7, 128.6, 128.5, 128.3, 128.12, 127.2, 127.1, 67.15, 56.0, 53.4, 52.4, 38.4, 37.9. HR-ESI-TOF calcd for $C_{27}H_{28}N_2O_5$ $[M + H]^+$: 461.2070, found: 461.2077 (1.4 ppm error).

***N*-Cbz-Phe-L-Ala-OMe** (**3k**).[7e] *N*-Cbz-L-Phenylalanine **1c** (0.25 mmol), L-alanine methyl ester hydrochloride **2d** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 81.9 mg **3k** as a white solid (85% yield), mp 142-144 °C. $[\alpha]^{25}_D$ = + 7.2 (*c* 0.33, $CHCl_3$), [lit.[7e] mp 126.0-127.0 °C; $[\alpha]^{25}_D$ = –24.1 (*c* 1.10, EtOH)]. (FT-IR/ATR cm^{-1}) ν_{max} 3295, 3058, 3027, 2950, 1741, 1691, 1648, 1533, 1259, 1216. ^1H NMR (500 MHz, $CDCl_3$) δ 7.14-7.34 (10H, m, 2Ar), 6.54 (1H, d, *J* = 7.5 Hz, Ala-NHCO), 5.46 (1H, d, Phe-NHCO, *J* = 9.0 Hz), 5.0 (2H, m, OCH_2Ph), 4.52-4.44 (2H, m, 2CH), 3.69 (3H, s, OCH_3), 3.05 (2H, d, CH_2Ph, *J* = 6.4 Hz), 1.31 (3H, d, CH_3, *J* = 7.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.95, 170.59, 156.02, 136.36, 136.25, 129.47, 128.75, 128.63, 128.29, 128.12, 127.12, 67.13, 56.08, 52.57, 48.21, 38.63, 18.32. HR-ESI-TOF calcd for $C_{21}H_{24}N_2O_5$ $[M + H]^+$: 385.1763, found: 385.1759 (0.3 ppm error).

***N*-Fmoc-Val-L-Phe-OMe** (**3l**).[7e] *N*-Fmoc-L-valine **1d** (0.25 mmol), L-phe methyl ester hydrochloride **2b** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 103.0 mg **3l** as a white solid (83% yield), mp 182–184 ºC. $[\alpha]_D^{25}$ = + 9.8 ($CHCl_3$, *c* = 0.6, $CHCl_3$), [lit.[7e] mp = 182-183 ºC; $[\alpha]_D^{25}$ = +15.9 (*c* 1.02, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3281, 1727, 1685, 1651, 1533 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) *δ* 7.75 (d, *J* =7.6 Hz, 2H), 7.59 (d, *J* = 7.6 Hz, 2H), 7.39 (t, *J* = 7.2 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 2H), 7.25-7.14 (m, 3H), 7.06 (d, *J* = 3.6 Hz, 2H), 6.38 (d, *J* = 7.9 Hz, 1H), 5.41 (d, *J* = 7.2 Hz, 1H), 4.88 (m, 1H), 4.42 (m, 1H), 4.29 (t, *J* = 6.8 Hz, 1H), 4.203 (t, *J* = 6.8 Hz, 1H), 4.02 (m, 1H), 3.69 (s, 3H), 3.16-3.04 (m, 2H), 2.11-2.01 (m, 1H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 170.1, 170.9, 156.3, 143.9, 141.3, 135.6, 129.2, 128.7, 127.8, 127.3, 127.1, 125.2, 120.09, 67.1, 60.2, 53.1, 52.4, 47.2, 37.9, 31.3, 19.1, 17.9. HR-ESI-TOF calcd for $C_{30}H_{32}N_2O_5$ $[M + H]^+$: 501.2383, found: 501.2386 (0.5 ppm error).

***N*-Fmoc-Val-Ala-OMe** (**3m**).[7e] Fmoc-L-valine **1d** (0.25 mmol), L-ala methyl ester hydrochloride **2a** (0.25 mmol), HOBt (0.275 mmol), EDC (0.275 mmol) and commercial hydrotalcite HT-S (250 mg, 0.41 mmol) were allowed to react according to the general procedure for the synthesis of dipeptides to give 93.8 mg **3m** as a white solid (88% yield), mp = 208-211°C. $[\alpha]_D^{25}$ = –18.1 ($CHCl_3$, *c* = 1.05), [Lit.[7e] mp = 205-207 ºC; $[\alpha]_D^{26}$ = –18.8 (*c* 1.00, $CHCl_3$)]. (FT-IR/ATR cm^{-1}) ν_{max} 3340, 3120, 1750, 1660, 1520, 1440, 1370, 1345, 1260, 1220. ^1H-NMR (500 MHz, $CDCl_3$): *δ* 7.73 (d, *J* = 7.3 Hz, 2H), 7.60-7.55 (m, arom. 2H), 7.42-7.34 (m *arom.*, 2H) , 7.31-7.24 (m, *arom.* Hz, 2H), 6.67 (br s, 1H), 5.59 (d, 1H, *J* = 8.8 Hz), 4.57 (q, *J* = 7.4 Hz, 1H), 4.41 (m, 1H), 4.32 (m, 1H), 4.19 (m, 1H) 4.07 (m, 1H), 3.71 (s, 3H), 2.13-2.05 (m, 1H), 1.37 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H) ^{13}C NMR ($CDCl_3$): *δ* 173.2, 171.0, 156.5, 143.9, 143.8, 141.3, 127.8, 127.1,125.2, 125.1 120.0, 67.1, 60.2, 52.5, 48.1, 47.2, 31.5, 19.1, 18.1, 18.0. HR-ESI-TOF calcd for $C_{24}H_{28}N_2O_5$ $[M + H]^+$: 425.2076, found: 425.2070 (0.1 ppm error).

Acknowledgements

The authors thank Consejo Nacional de Ciencia y Tecnología (CONACyT, Mexico) for financial support via grant: CB-2013/220945 and a postdoctoral fellowship to J.M.L. We are also grateful to Gloria Reyes-Rangel and to Professor Guillermo Negrón-Silva for useful discussions.

Keywords: Amide bond formation • High speed ball milling • Solvent-free reaction • Hydrotalcite • Dipeptide synthesis

[1] M. M. Joullié, K. M. Lassen, *ARKIVOC*, **2010**, *viii*, 189-250.

[2] a) A. K. Ghose, V. N. Viswanadhan, J. J. Wendoloski, *J. Comb. Chem.* **1999**, *1*, 55-68; b) J. S. Carey, D. Laffan, T. Colin, M. T. Williams, *Org. Biomol. Chem*. **2006**, *4*, 2337-2347; c) S. D. Roughley, A. M. Jordan, *J. Med. Chem*., **2011**, 54, 3451-3479.

[3] a) S.-Y. Han, Y.-A. Kim, *Tetrahedron*. **2004**, 60, 2447-2467; b) V. R. Pattabiraman, J. W. Bode, *Nature*, **2011** , *480*, 471-479; c) T. I. Al-Warhi, H. M. A. Al-Hazimi, A. El-Faham, *J. Saudi. Chem. Soc*. **2012**, *16*, 97-116; d) A. El-Faham, F. Albericio, *Chem. Rev.* **2011**, *111*, 6557–6602; f) L. Zhang, X.-J. Wang, J. Wang, N. Grinberg, D. Krishnamurthy, C. H. Senanayake, *Tetrahedron Lett*. **2009**, *50*, 2964-2966.

[4] R. M. Lanigan, T. D. Sheppard, *Eur. J. Org. Chem*. **2013**, 7453-7465.

[5] P. T. Anastas, J. C. Warner, in *Green Chemistry: Theory and Practice*, Vol. 1, Oxford University Press, Oxford, **1998**.

[6] a) J. G. Hernández, C. G. Avila-Ortiz, E. Juaristi, *Useful Chemical Activation Alternatives in Solvent-Free Organic Reactions*, in *Comprehensive Organic Synthesis*, *Vol. 9*, Second Edition, (Eds.: G. A. Molander and P. Knochel), Elsevier, Oxford, **2014**, pp. 287-314; b) S. L. Pedersen, A. P. Tofteng, L. Malik,K. J. Jensen, *Chem. Soc. Rev*., **2012**, *41*, 1826–1844; c) A. Bruckmann, A. Krebs, C. Bolm, *Green Chem*. **2008**, 10, 1131-1141; d) S. L. James, C. J. Adams, C. Bolm, D. Braga, P. Collier, T. Friščić, F. Grepioni, 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*, 413-447. e) D. Margetic, V. Strukil, in *Mechanochemical Organic Synthesis,* Elsevier, Oxford, **2016;** f) R. Brindaban, A. Stolle (Eds.), in *Ball Milling Towards Green Synthesis: Applications, Projects, Challenges*. Royal Society of Chemistry, Cambridge, **2015**. g) T. Friščić, S. L. Child, S. A. A. Rizvi, W. Jones, *CrystEngComm* **2009**, *11*, 418−426; h) T. Friščić, I. Halasz, P. J. Beldon, A. M. Belenguer, F. Adams, S. A. J. Kimber, V. Honkimaki, R. E. Dinnebier, *Nature Chem.* **2013**, *5*, 66−73; i) G. A. Bowmaker, *Chem. Commun*., **2013**, 49, 334-348; S. A. Mitchenko, *Theor. Exp. Chem*. **2007**. *43*, 211-228.

[7] a) T.-X. Métro, E. Colacino, J. Martinez, F. Lamaty, "Amino Acids and Peptides in Ball Milling" in *Ball Milling Towards Green Synthesis: Applications, Projects, Challenges, Ch. 6* (Eds.: A. Stolle and B. Ranues), Royal Society of Chemistry, Cambridge, **2014**, pp.114-150. b) D. Margetić, V. Štrukil, "Carbon–Nitrogen Bond-Formation Reactions" *in Mechanochemical Organic Synthesis, Ch. 3* (Eds.: D. Margetić and V. Štrukil), Elsevier, Oxford, **2016**, pp 141–233. c) V. Štrukil, B. Bartolec, T. Portada, I. Đilović, I. Halasz, D. Margetić*, Chem. Commun*. **2012**, *48*, 12100–12102. d) V. Porte, M. Thioloy, T. Pigoux, T.-X. Métro, J. Martinez, F. Lamaty, *Eur. J. Org. Chem.* **2016**, *21*, 3505-3508. e) C. Duangkamol, S. Jaita, S. Wangngae, W. Phakhodee, M. Pattarawarapan. *RSC Adv*. **2015**, *5*, 52624–52628.

[8] I. Fechete, Y. Wang, J. C. Védrinec, *Catal. Today*, **2012**, *1*, 2-27.

[9] F. Cavani, F. Trifirò, A. Vaccari, *Catal. Today*, **1991**, 173-301.

[10] a) Z. P. Xu, J. Zhang, M. O. Adebajo, H. Zhang, C. Zhoud, *Appl. Clay Sci.* **2011**, *53*, 139-150; b) A. Corma, S. Iborra, *Adv. Catal*. **2006**, *49*, 239-302; c) D. Tichit, B. Coq, *CATTECH*, **2003**, *7*, 206-217;

[11] a) T. Baskaran, J. Christopher, A. Sakthivel, *RSC Adv.*, **2015**, *5*, 98853-98875; b) D. P. Debecker, E. M. Gaigneaux, G. Busca, *Chem. Eur. J*. **2009**, *15*, 3920-3935.

[12] J. A. Morales-Serna, M. A. Jaime-Vasconcelos, E. García-Ríos, A. Cruz, D. Angeles-Beltrán, L. Lomas-Romero, G. E. Negrón-Silva, J. Cárdenas, *RSC Adv*. **2013**, *3*, 23046-23050.

[13] a) J. G. Hernández, E. Juaristi, *J. Org. Chem*. **2010**, *75*, 7107-7111; b) J. G. Hernández, E. Juaristi, *Tetrahedron,* **2011**, *67*, 6953-6959; c) J. G Hernández, E. Juaristi, *J. Org. Chem.*, **2011**, *76*, 1464-1467; d) J. G. Hernández, V. García-López, E. Juaristi, *Tetrahedron* **2012**, *68*, 92-97; e) E. Machuca, Y. Rojas, E. Juaristi. *Asian J. Org. Chem*. **2015**, *4*, 46-53; f) L. A. Polindara-García, E. Juaristi, *Eur. J. Org. Chem*. **2016**, *6*, 1095-1102.

[14] S. Kannan, A. Narayanan, C. S. Swamy, *J. Mater. Sci.* **1996**, *31*, 2353-2360.

[15] a) S. K. Sharma, P. A. Parikh, R. V. Jasra, *Appl. Catal., A.* **2010**, *386*, 34-42; b) S. Abelló, F. Medina, D. Tichit, J. Pérez-Ramírez, J. C. Groen, J. E. Sueiras, P. Salagre, Y. Cesteros, *Chem. Eur. J.* **2005**, *11*, 728-739.

[16] P. Kuśtrowski, D. Sułkowska, L. Chmielarz, A. Rafalska-Łasocha, Dudek, B., Dziembaj. R. *Microporous Mesoporous Mater.* **2005**, *78*, 11-22.

[17] J. Bonnamour, T.-X. Métro, J. Martinez, F. Lamaty, *Green Chem.* **2013**, *15*, 1116-1120.

[18] a) T. Friščić, S. L. Child, S. A. A. Rizvi, W. Jones, *CrystEngComm* **2009** *11*, 418−426; b) T. Friščić, I. Halasz, P. J. Beldon, A. M. Belenguer, F. Adams, S. A. J. Kimber, V. Honkimaki, R. E. Dinnebier, *Nature Chem.* **2013**, *5*, 66−73.

[19] a) T. Mukhopadhyay, D. Seebach, *Helv. Chim. Acta*. **1982**, *65*, 385-391; b) E. Juaristi, P. Murer, D. Seebach, *Synthesis.***1993**, *12*, 1243-1246.

[20] K. K. Rao, M. Gravelle, J. Sanchez-Valente, F. Figueras, *J. Catal.* **1998**, *173*, 115-121.

[21] C. Giordano, G. Lucente, M. Nalli, G. Pagani-Zecchini, M. Paglialunga-Paradisi, K. Varani, S. Spisani, *Il Farmaco*, **2003**, *58*, 1121-1130.

[22] C. Wiles, P. Watts, *Synthesis*. **2007**, *17*, 2608–2610.

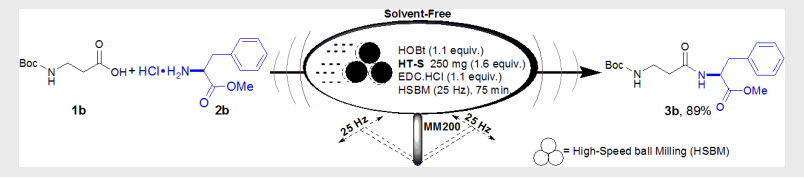
[23] R. R. Hill, D. Birch, G. E. Jeffs, M. North, *Org. Biomol. Chem*. **2003**, *1*, 965-972.

10.1002/ejoc.201601276

WILEY-VCH

Entry for the Table of Contents

FULL PAPER



An efficient protocol for the synthesis of several α,α-, α,β- and β,β-dipeptides under ball-milling activation and solvent-free conditions has been developed. The reaction takes place in the presence of coupling agents EDC, HOBt, and with Mg-Al hydrotalcite as activator.

Mechanochemistry *

*José M. Landeros and Eusebio Juaristi**

Page No. – Page No.

Mechanochemical synthesis of dipeptides using Mg-Al hydrotalcite as activating agent under solvent-free reaction conditions