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Green Synthesis of α,β- and β,β-Dipeptides under Solvent-Free Conditions

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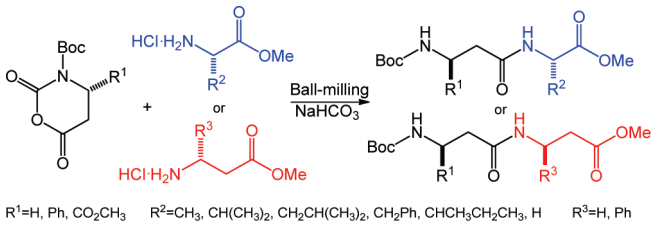

The reactivity of *N*-*tert*-butyloxycarbonyl-*N*-carboxyanhydrides derived from β-alanine, (*S*)-β^3-homophenylglycine, and (*S*)-β^3-carboxyhomoglycine with different α- and β-amino ester hydrochlorides was examined under ball-milling activation. In particular, good to excellent yields of several relevant α,β- and β,β-dipeptides were obtained. An illustrative application of this methodology consisted in the high-yield synthesis of the mammalian α,β-dipeptide *N*-Boc-L-carnosine-OMe.

Introduction

In the past decade, interest in Green Chemistry has expanded, and it now encompasses wide areas of the chemical enterprise. Of particular interest are developments with potential impact on industry and laboratory research as a means to continue chemical development in a more sustainable manner.

A way to evaluate how "green" a process is consists of determining how closely it fulfills the guidelines suggested by the so-called principles of Green Chemistry. In particular, within the "twelve principles" of Green Chemistry proposed by Anastas and Warner in the mid-1990s,[1] both the use of safer solvents and the design of more energy-efficient processes are key concepts.

High-speed ball-milling (HSBM) is a sustainable mechanochemical technique, which has commonly been used for milling minerals into fine particles, as well as in the synthesis and modification of inorganic solids and other organometallic materials.[2] Furthermore, in the area of synthetic organic chemistry, this technique has been successfully used to promote several solvent-free reactions. Reported applications include: Heck-type cross-couplings,[3a] asymmetric aldol reactions,[3b] Knoevenagel condensation reactions,[3c] Baylis−Hillman reactions,[3d] Michael additions,[3c] functionalization of fullerenes,[3e] synthesis of nitrones,[3f] and others.

Recently, Lamaty and co-workers reported a novel strategy for the synthesis of α-peptides under solvent-free conditions by means of ball-milling activation.[4] The methodology of Lamaty and co-workers requires no solvent, and it is based on mechanochemical mixing of the starting amino acids, thus fulfilling the aforementioned principles for a green synthesis.

As part of our current interest in the chemistry of β-amino acids and β-peptides,[5] we deemed it of interest to apply Lamaty's strategy to the synthesis of α,β- and β,β-dipeptides.

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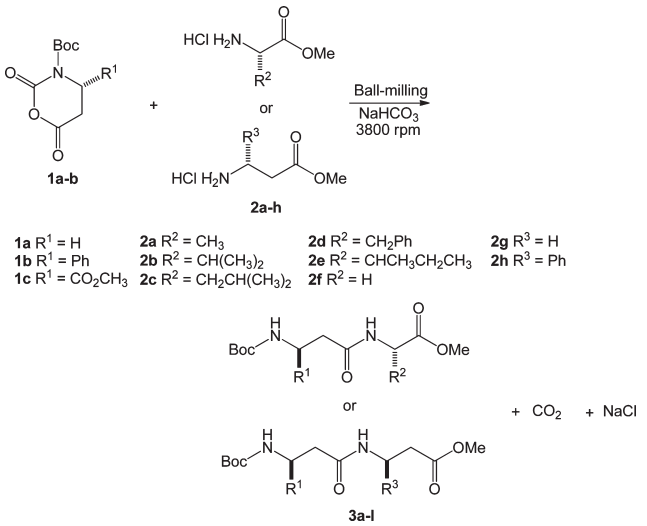
SCHEME 1. Synthesis of α,β- and β,β-Dipeptides under Solvent-Free Conditions



This required the coupling of urethane-protected β-amino acid *N*-carboxyanhydride (UNCA) derivatives with hydrochloride salts derived from α- and β-amino esters. In this regard, the scientific literature records the preparation and reactivity (in liquid phase) of UNCAs derived from β^2- and β^3-amino acids.[6a−c]

Extension of Lamaty's strategy to β-amino acid substrates is not trivial since the reactivity of β-UNCAs is usually limited relative to the α analogues. Indeed, Roumestant and co-workers[6a] examined the reaction between *N*-Boc-α-methyl-β-alanine *N*-carboxyanhydride and **1a** with benzylamine, benzyl alcohol, and the lithium enolate of ethyl acetate as nucleophiles. Although these reactions proceeded rapidly, yields were only modest (54−79%). By contrast, the same reaction with α-amino acid *N*-carboxyanhydrides afforded better yields of the expected products.[6d] Furthermore, the reactivity of methyl glycinate hydrochloride with *N*-Boc-α-methyl-β-alanine-NCA in CH_2Cl_2, in the presence of NEt_3, was evaluated, obtaining the expected dipeptide in 80% yield.[6c] Nevertheless, the synthesis of α,β- and β,β-dipeptides from β-UNCAs in solvent-free conditions has not been reported. In view of this, we proceeded to study the coupling reaction of *N*-*tert*-butyloxycarbonyl *N*-carboxyanhydrides derived from β-alanine **1a**, (*S*)-β^3-homophenylglycine **1b**, and (*S*)-β^3-carboxyhomoglycine **1c** with various α- or β-aminoester hydrochlorides **2a**−**h** (Scheme 1).

Results and Discussion

To do this, we synthesized β-UNCAs **1a**−**c** according to the method described by Roumestant,[6a] starting with commercial β-alanine, the (*S*)-β^3-homophenylglycine [(*S*)-β^3-hPhg], which was prepared according to the methodology reported by Juaristi and co-workers,[5f] or natural L-aspartic acid acting as β-amino acid, (*S*)-β^3-carboxyhomoglycine [(*S*)-β^3-chg]. The structure and solid-state conformation of

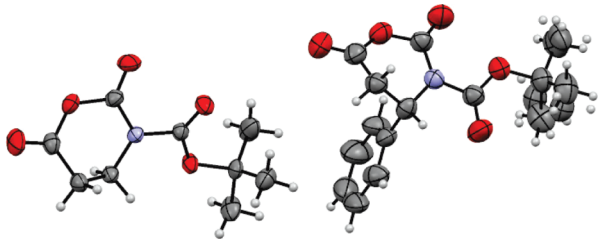


FIGURE 1. X-ray structure of crystal **1a** and **1b**.

1a and **1b** were determined by single-crystal X-ray diffraction analysis (Figure 1). Particularly interesting is the axial orientation of the phenyl group in **1b** as a consequence of the allylic $A^{1,3}$ strain.[7] Complete spectroscopic analysis and X-ray diffraction crystal structure determination of **1a**,**b** are presented in the Supporting Information.

Following the preparation of the required β-UNCAs, we carried out the coupling reaction between **1a** and α-amino ester hydrochlorides **2a**−**f**, in the presence of $NaHCO_3$ (1.5 equiv) and using the high-energy ball-milling process. The capsule containing the mixture of solid substrates was shaken at a frequency of 3800 rpm. The change in mass of the capsule was quantified during the shaking process until the recorded difference in mass was constant. This indicated that the reaction had ended; i.e., no more CO_2 was being liberated. In general, the required reaction time was 2 h.

The reaction mixture was removed from the capsule, dissolved in EtOAc, washed with brine, and dried to afford the desired α,β-dipeptides as white solids, except for **3f** (oil) in good yields (Table 1, entries 1−6).

All reactions were repeated at least twice to confirm the outcome of the procedure. Furthermore, in some assays the reaction time was varied, observing that with less time of milling the yield of the produced dipeptide decreased significantly. On the other hand, longer times did not lead to increased yields. The physical and spectroscopic properties of the isolated products **3a**−**e** are in agreement with previous reports of their synthesis in liquid phase.[8]

The coupling of Boc-β-Ala-NCA **1a** and Boc-(*S*)-β^3-hPhg-NCA **1b** with β-aminoester hydrochlorides **2g**−**h** was also carried out under the described conditions affording the four β,β-dipeptides **3g**−**j** in good yield (Table 1, entries 7−10). Interesting α,β- and β,β-dipeptides **3k**−**l** were obtained by coupling the α-amino ester hydrochlorides **2a** and **2g** with the novel β-UNCA **1c** (Table 1, entries 11 and 12).

Thus, all α,β- and β,β-dipeptides were obtained with yields that ranged in from 79% to 96% (Table 1). The best yields were obtained for the coupling reaction between **1a**−**c** and the β-amino ester hydrochlorides **2g**−**h**.

The absence of racemization or epimerization in the preparation of α,β-dipeptides using ball-milling activation was demonstrated by Lamaty and co-workers.[4] In this regard, we compared the specific optical rotations of dipeptides **3d** and **3m** with those reported in the literature. The recorded values showed

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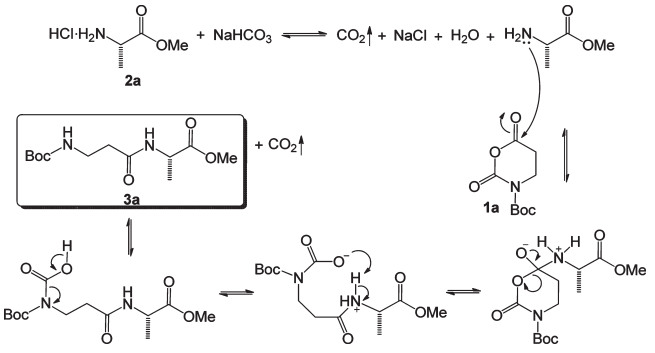
TABLE 1. α,β- and β,β-Dipeptides Prepared under Solvent-Free Conditions in This Work

entry	*N*-Boc-β-NCA	amino methyl ester hydrochloride	dipeptide *N*-Boc-aa-aa-OMe	yield (%)
1	β-ala	Ala	β-ala-Ala (**3a**)	88
2	β-ala	Val	β-ala-Val (**3b**)	82
3	β-ala	Leu	β-ala-Leu (**3c**)	87
4	β-ala	Phe	β-ala-Phe (**3d**)	83
5	β-ala	Ile	β-ala-Ile (**3e**)	80
6	β-ala	Gly	β-ala-Gly (**3f**)	88
7	β-ala	β-ala	β-ala-β-ala (**3g**)	96
8	β-ala	(*S*)-β^3-hPhg	β-ala-(*S*)-β^3-hPhg (**3h**)	91
9	(*S*)-β^3-hPhg	β-ala	(*S*)-β^3-hPhg-β-ala (**3i**)	93
10	(*S*)-β^3-hPhg	(*S*)-β^3-hPhg	(*S*)-β^3-hPhg-(*S*)-β^3-hPhg (**3j**)	94
11	(*S*)-β^3-chg-(CO_2CH_3)	Ala	(*S*)-β^3-chg(CO_2CH_3)-Ala (**3k**)	79
12	(*S*)-β^3-chg-(CO_2CH_3)	β-ala	(*S*)-β^3-chg(CO_2CH_3)-β-ala (**3l**)	91

SCHEME 2. Proposed Mechanism for the Synthesis of Dipeptides under Solvent-Free Conditions



total agreement with those reported, indicating that no racemization or epimerization took place.[9]

An efficient procedure for synthesizing peptides consists of the ring-opening polymerization of α-amino acid-*N*-carboxyanhydrides (NCAs). The mechanism of the ring opening in this process has been well estudied.[10] On the basis of those studies, we propose the mechanism depicted in Scheme 2 for the formation of the dipeptide Boc-β-Ala-L-Ala-OMe **3a**.

The importance of the incorporation of β-amino acids into peptides is recognized not only in view of their increased stability against proteolytic degradation in vitro and in vivo but also because β-peptides offer the potential for the design of drugs based on β-peptidic architecture.[11]

Although not many examples of α,β-dipeptides exist in nature, L-carnosine, a mammalian dipeptide composed of the amino acids β-alanine and L-histidine and found in muscle and brain tissues, is one salient example (Figure 2).

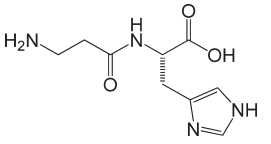
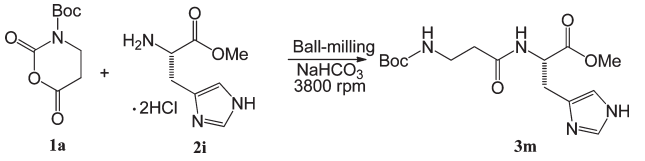


FIGURE 2. Natural L-carnosine.

SCHEME 3. Synthesis of Dipeptide Boc-β-Ala-L-His-OMe under Solvent-Free Conditions



L-Carnosine has proven to be an antiglycating agent, antioxidant-based on in vitro tests, and exhibits hydroxyl-radical scavenger properties. As a result, L-carnosine is presently an active topic of study in chemistry.[12]

Given the importance of L-carnosine, and to demonstrate the general applicability of the present synthetic procedure for the preparation of α,β-dipeptides under solvent-free conditions, we deemed it of interest to synthesize dipeptide Boc-β-Ala-L-His-OMe **3m** as an approach to the architecture of L-carnosine (Scheme 3).

The aminoester hydrochloride **2i** was prepared from commercial L-histidine. Ball-milling of **1a**, **2i**, and $NaHCO_3$ for 2 h yielded the protected dipeptide **3m** in excellent yield. It is worth mentioning that the general procedure implemented in this work usually involves the use of 1.5 equiv of $NaHCO_3$ to ensure the total liberation of the amino ester. However, when the reaction of **1a** and **2i** was carried out by applying this stoichiometric relationship, the yield of **3m** was low and the reaction generated many byproducts. Thus, we used 2 equiv of $NaHCO_3$, finding that the coupling was clean and the yield was 91%. Under these conditions, the 2 equiv of $NaHCO_3$ liberated both amino functions of the amino ester. All spectra (^1H, ^{13}C NMR, COSY, HETCOR and HMBS spectroscopy) of **3m** are presented in the Supporting Information.

Conclusion

In summary, a "green" protocol for the high-yield synthesis of α,β-dipeptides and several novel β,β-dipeptides under solvent-free conditions, starting with urethane-protected β-amino acid *N*-carboxyanhydrides and α- or β-aminoesters, is reported. As an illustrative application of this strategy,

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N-Boc-L-Carnosine-OMe, a protected derivative of natural α,β-dipeptide L-carnosine, was prepared in high yield.

Research toward the synthesis of other unnatural dipeptides and evaluation of its potential organocatalytic activity is already in progress in our laboratory.

Experimental Section

General Procedure for the Synthesis of the UNCAs Derived from β-Amino Acids. To a cooled (−20 °C) solution of DMF (1.18 mL, 15.3 mmol) in freshly distilled acetonitrile (7.5 mL) was added dropwise oxalyl chloride (1.34 mL, 15.3 mmol). After 30 min of stirring at −20 °C, a cooled solution (−20 °C) of *N*-Bis-Boc-β-alanine (740 mg, 2.56 mmol) and pyridine (207 μL, 2.56 mmol) in acetonitrile (4.5 mL) was added dropwise. The solution was stirred at −20 °C for 2 h and after that allowed to warm to room temperature (over 1 h) and stirred for a further 4 h. The reaction was quenched by pouring onto ice and the product extracted into AcOEt (3 × 30 mL). The combined organics were washed with $NaHCO_3$ solution (pH 8) and brine (50 mL). The organic phases were dried ($MgSO_4$) and evaporated under vacuum to give a yellowish oil; recrystallization from ethyl acetate gave the respective UNCA **1a**−**c**.

3-*tert*-Butyloxycarbonyl-4,5-dihydro-1,3-oxazine-2,6-dione (*N*-Boc-β-alanine-*N*-carboxyanhydride), 1a. 320 mg (58%) of white crystals, mp = 122−124 °C. (FT-IR/ATR cm^{-1}) ν_{max} 2985, 2939, 1824, 1790, 1739. ^1H NMR (500 MHz, $CDCl_3$) δ 3.90 (2H, t, CH_2N, J = 6.6 Hz), 2.87 (2H, m, CH_2CO), 1.54 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.7, 150.5, 144.5, 85.7, 39.2, 29.5, 27.7 ppm.

(*S*)-3-*tert*-Butyloxycarbonyl-4-phenyl-4,5-dihydro-1,3-oxazine-2,6-dione ((*S*)-*N*-Boc-β^3-hPhg-*N*- carboxyanhydride) 1b. 208 mg (60%) of white crystals. ^1H NMR (500 MHz, $CDCl_3$) δ 7.41−7.33 (3H, m, ArH), 7.22−7.20 (2H, m, ArH), 5.62 (1H, m, CH−Ph), 3.23 (1H, dd, J = 16.4, J = 6.4 Hz, CH_2), 3.13 (1H, dd, J = 16.4, J = 6.4 Hz, CH_2), 1.48 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.7, 150.3, 144.8, 137.1, 129.6, 129.1, 125.2, 86.0, 53.6, 36.8, 27.9 ppm.

(*S*)-3-*tert*-Butyloxycarbonyl-4-carboxymethyl-4,5-dihydro-1,3-oxazine-2,6-dione ((*S*)-*N*-Boc-β^3-chg-(CO_2CH_3)-*N*-carboxyanhydride), 1c. 250 mg (62%) as a gummy solid. ^1H NMR (500 MHz, $CDCl_3$) δ 4.55 (1H, dd, J = 8.0, J = 2.6 Hz, CH−CO_2CH_3), 3.66 (3H, s, −OCH_3), 3.20 (1H, dd, J = 19.4, J = 8.0 Hz, CH_2), 2.92 (1H, dd, J = 19.4, J = 2.6 Hz, CH_2), 1.53 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.2, 148.1, 147.1, 118.3, 85.9, 58.5, 53.0, 35.8, 28.0 ppm. (FT-IR/ATR cm^{-1}) ν_{max} 2982, 1833, 1805, 1738. HR-ESI-TOF: Calculated for $C_9H_{10}N_7O_5Na$ $[M+Na]^+$: 296.0737. Found: 296.0741 (1.0 ppm error).

General Procedure for the Coupling of UNCAs 1 with Aminoester Hydrochloride 2. A mixture of UNCA **1** (0.1 mmol), aminoester hydrochloride **2** (0.1 mmol), and $NaHCO_3$ (0.15 mmol) was vigorously milled for 2 h at 3800 rpm in a digital Mixer/Amalgamator used with a reactor made of Nylamid (cylinder, 25 mm long and with a diameter 10 mm) containing one stainless steel ball with a 5 mm diameter. The solid residue (or the melted mixture) was dissolved in AcOEt and washed with brine. The organic phase was dried over $MgSO_4$ and concentrated to yield the dipeptide.

Boc-β-Ala-L-Ala-OMe (3a). (24 mg, 88%) as a white solid. mp = 78−80 °C. $[\alpha]^{25}_D$ = +55.0 (*c* 0.1, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3356, 3321, 2983, 1745, 1682, 1648. ^1H NMR (500 MHz, $CDCl_3$) δ 6.30 (1H, br, Ala-NH), 5.19 (1H, br-s, β-Ala-NH), 4.55 (1H, q, CH, J = 7.2 Hz), 3.73 (3H, s, OCH_3), 3.38 (2H, m, CH_2NBoc), 2.41 (2H, t, CH_2C=O, J = 5.8 Hz), 1.40 (9H, s, *t*-Bu), 1.38 (3H, d, CH_3, J = 7.1 Hz) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 171.2, 156.1, 79.3, 52.9, 48.1, 36.6, 36.1, 28.4, 18.3 ppm. HR-ESI-TOF Calculated for $C_{12}H_{23}N_2O_5$ $[M + H]^+$: 275.1601. Found: 275.1609 (2.7 ppm error).

Boc-β-Ala-L-Val-OMe (3b). (25 mg, 82%) as a gummy solid. $[\alpha]^{25}_D$ = +12.0 (*c* 1.6, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3321, 2968, 2933, 1736, 1693, 1655. ^1H NMR (500 MHz, $CDCl_3$) δ 6.15 (1H, br, Val-NH), 5.18 (1H, br-s, β-Ala-NH), 4.53 (1H, m, ($CHCO_2$)), 3.73 (3H, s, OCH_3), 3.40 (2H, m, CH_2NBoc), 2.45 (2H, m, CH_2C=O), 2.15 (1H, m, (CH)), 1.41 (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 (125 MHz, $CDCl_3$) δ 176.6, 171.8, 156.2, 79.3, 57.2, 52.2, 36.8, 36.2, 31.1, 28.4, 19.0, 17.9 ppm. HR-ESI-TOF Calculated for $C_{14}H_{27}N_2O_5$ $[M + H]^+$: 303.1914. Found: 303.1921 (2.1 ppm error).

Boc-β-Ala-L-Leu-OMe (3c). (28 mg, 88%) as a white solid. mp = 50−52 °C. $[\alpha]^{25}_D$ = +5.5 (*c* 0.1, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3347, 2960, 2932, 1752, 1691, 1648. ^1H NMR (500 MHz, $CDCl_3$) δ 6.35 (1H, br, Leu-NH), 5.24 (1H, br-s, β-Ala-NH), 4.56 (1H, m, ($CHCO_2$)), 3.68 (3H, s, OCH_3), 3.36 (2H, m, CH_2NBoc), 2.41 (2H, m, CH_2C=O), 1.65−1.55 (2H, m, CH_2*i*-Bu), 1.54−1.44 (1H, m, CH*i*-Bu), 1.38 (9H, s, *t*-Bu), 0.89 (6H, d, CH_3*i*-Bu, J = 6.0 Hz) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.5, 171.5, 156.1, 77.4, 52.3, 50.7, 41.3, 36.7, 36.1, 28.4, 24.9, 22.8, 21.9 ppm. HR-ESI-TOF Calculated for $C_{15}H_{29}N_2O_5$ $[M + H]^+$: 317.2070. Found: 317.2075 (1.2 ppm error).

Boc-β-Ala-L-Phe-OMe (3d). (29 mg, 83%) as a white solid. mp = 89−91 °C. $[\alpha]^{25}_D$ = +52.0 (*c* 1.0, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3320, 2976, 2931, 1735, 1691, 1654. ^1H NMR (500 MHz, $CDCl_3$) δ 7.31−7.20 (3H, m, H−Ar), 7.11−7.08 (2H, d, H^{orto}−Ar, J = 7.0 Hz), 6.04 (1H, br, Phe-NH), 5.10 (1H, br-s, β-Ala-NH), 4.88 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.36 (2H, m, CH_2NBoc), 3.16, (1H, dd, CH_2Ph, J = 6.3, 13.8 Hz), 3.07 (1H, dd, CH_2Ph, J = 5.7, 13.8 Hz), 2.38 (2H, t, CH_2C=O, J = 5.5 Hz), 1.44 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 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 Calculated for $C_{18}H_{27}N_2O_5$ $[M + H]^+$: 351.1914. Found: 351.1923 (2.4 ppm error).

Boc-β-Ala-L-Ile-OMe (3e). (25.2 mg, 80%) as a gummy solid. $[\alpha]^{25}_D$ = +5.0 (*c* 0.7, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3345, 2958, 1755, 1689, 1651. ^1H NMR (500 MHz, $CDCl_3$) δ 6.05 (1H, br, Ile-NH), 5.17 (1H, br-s, β-Ala-NH), 4.58 (1H, m, NCH), 3.74 (3H, s, OCH_3), 3.42−3.39 (2H, m, CH_2NBoc), 2.47−2.44, (2H, m, CH_2C=O), 1.90−1.80 (1H, m, CH), 1.47−1.37 (1H, m, CH_2CH_3), 1.22−1.12, (1H, m, CH_2CH_3), 1.43 (9H, s, *t*-Bu), 0.86−0.96 (6H, m, 2×CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.5, 171.5, 156.2, 79.4, 56.5, 52.3, 38.0, 36.8, 36.4, 28.5, 25.4, 15.6, 11.7 ppm. HR-ESI-TOF Calculated for $C_{15}H_{29}N_2O_5$ $[M + H]^+$: 317.2070. Found: 317.2076 (1.6 ppm error).

Boc-β-Ala-Gly-OMe (3f). (23 mg, 88%) as a liquid. (FT-IR/ATR cm^{-1}) ν_{max} 3316, 2957, 1743, 1688, 1656. ^1H NMR (500 MHz, $CDCl_3$) δ 6.49 (1H, br, gly-NH), 5.25 (1H, br-s, β-Ala-NH), 4.01 (2H, d, CH_2CO_2, J = 5.37 Hz), 3.73 (3H, s, OCH_3), 3.38 (2H, q, CH_2NBoc, J = 6.0 Hz), 2.45 (2H, t, CH_2C=O, J = 5.8 Hz), 1.39 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 170.5, 156.2, 79.4, 52.5, 41.2, 36.6, 36.1, 28.5 ppm. HR-ESI-TOF Calculated for $C_{11}H_{21}N_2O_5$ $[M + H]^+$: 261.1444. Found: 261.1447 (0.7 ppm error).

Boc-β-Ala-β-Ala-OMe (3g). (28.5 mg, 96%) as a white solid. mp = 77−78 °C. (FT-IR/ATR cm^{-1}) ν_{max} 3357, 3288, 2972, 1732, 1683, 1643. ^1H NMR (500 MHz, $CDCl_3$) δ 6.19 (1H, br, β-Ala-NH), 5.16 (1H, br-s, β-Ala-BocNH), 3.69 (3H, s, OCH_3), 3.51 (2H, q, CH_2NBoc, J = 6.1 Hz), 3.38 (2H, q, CH_2C=O, J = 6.1 Hz), 2.53 (2H, t, CH_2NHCO, J = 6.1 Hz), 2.36 (2H, t, CH_2CO_2, J = 5.9 Hz), 1.41 (9H, s, *t*-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 171.6, 156.2, 79.3, 51.9, 36.7, 36.2, 34.9, 33.9, 28.4 ppm. HR-ESI-TOF Calculated for $C_{12}H_{23}N_2O_5$ $[M + H]^+$: 275.1601. Found: 275.1609 (2.7 ppm error).

Boc-β-Ala-(*S*)-β^3-hPhg-OMe (3h). (32 mg, 91%) as a white solid. mp = 82−84 °C. $[\alpha]^{25}_D$ = −47.0 (*c* 1.5, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3369, 3336, 2976, 2927, 1736, 1678, 1645. ^1H NMR (500 MHz, $CDCl_3$) δ 7.33−7.24 (5H, m, H−Ar), 6.80 (1H, br,

β^3-hPhg-NH), 5.42 (1H, m, CH), 5.22 (1H, br-s, β-Ala-NH), 3.62 (3H, s, OCH_3), 3.38 (2H, dd, CH_2NBoc, J = 5.8, J = 6.3 Hz), 2.88 (1H, dd, CH_2CO_2, J = 6.3, J = 15.9 Hz), 2.84 (1H, dd, CH_2CO_2, J = 6.3, J = 15.9 Hz), 2.43 (2H, t, $CH_2C{=}O$, J = 6.3 Hz), 1.43 (9H, s, t-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5, 170.8, 156.1, 140.4, 128.8, 127.7, 126.2, 79.3, 51.9, 49.7, 40.0, 36.7, 36.3, 28.4 ppm. HR-ESI-TOF: Calculated for $C_{18}H_{27}N_2O_5$ $[M + H]^+$: 351.1914. Found: 351.1920 (1.5 ppm error).

Boc-(S)-β^3-hPhg-β-Ala-OMe (3i). (16.3 mg, 93%, (0.05 mmol of **1b** and **2g** were used)) as a white solid. mp = 140−142 °C. $[\alpha]^{25}_D$ = −2.2 (c 0.3, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3346, 2961, 2925, 1734, 1680, 1645. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32−7.21 (5H, m, H−Ar), 6.18 (1H, br-s, β^3-hPhg-NH), 6.01 (1H, br-s, β-Ala-NH), 5.01 (1H, br-s, CH), 3.64 (3H, s, OCH_3), 3.64−3.28 (2H, m, CH_2N), 2.71−2.55 (2H, m, $CH_2C{=}O$), 2.41−2.27 (2H, m, CH_2CO_2), 1.41 (9H, s, t-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.0, 170.5, 155.4, 141.6, 128.7, 127.4, 126.2, 79.6, 51.9, 51.9, 43.0, 34.7, 34.7, 33.6, 28.5 ppm. HR-ESI-TOF Calculated for $C_{18}H_{27}N_2O_5$ $[M + H]^+$: 351.1914. Found: 351.1915 (0.1 ppm error).

Boc-(S)-β^3-hPhg-(S)-β^3-hPhg-OMe (3j). (40.0 mg, 94%) as a white solid. mp = 154−156 °C. $[\alpha]^{25}_D$ = −77.1 (c 0.8, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3308, 2929, 1736, 1683, 1650. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.21 (10H, m, H−Ar), 6.50 (1H, d, NHCO, J = 8.5 Hz), 6.19 (1H, br-s, NHBoc), 5.27 (1H, m, CHNHCO), 5.06 (1H, br-s, CHNHBoc), 3.54 (3H, s, OCH_3), 2.80−2.50 (4H, m, 2xCH_2), 1.40 (9H, s, t-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 169.7, 155.4, 141.7, 140.1, 128.8, 128.8, 127.8, 127.5, 126.3, 126.2, 79.7, 52.0, 51.9, 49.4, 43.0, 39.4, 28.5 ppm. HR-ESI-TOF Calculated for $C_{24}H_{31}N_2O_5$ $[M + H]^+$: 427.2227. Found: 427.2226 (0.3 ppm error).

Boc-(S)-β^3-chg-(CO_2CH_3)-L-Ala-OMe (3k). (28.8 mg, 79%) as a gummy solid. $[\alpha]^{25}_D$ = 16.5 (c 0.37, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3311, 2920, 1755, 1741, 1691, 1650. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.15 (1H, d, J = 6.6 Hz, Ala-NH), 5.70 (1H, br-d, J = 6.8 Hz, β^3-chg-NH), 4.58−4.52 (1H, m, CH−CO_2CH_3), 4.58−4.52 (1H, m, CH−CH_3), 3.75 (6H, s, 2×−OCH_3), 2.92 (1H, dd, J = 16.1, J = 4.5 Hz, CH_2), 2.72 (1H, dd, J = 15.6, J = 4.4 Hz, CH_2), 1.44 (9H, s, t-Bu), 1.39 (3H, d, J = 7.2 Hz, CH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 171.8, 169.4, 155.6, 80.0, 52.6, 52.5, 50.3, 48.1, 37.9, 28.3, 18.4 ppm. HR-ESI-TOF Calculated for $C_{14}H_{25}N_2O_7$ $[M + H]^+$: 333.1656. Found: 333.1656 (0.1 ppm error).

Boc-(S)-β^3-chg-(CO_2CH_3)-β-Ala-OMe (3l). (33.2 mg, 91%) as a gummy solid. $[\alpha]^{25}_D$ = +2.3 (c 0.95, $CHCl_3$). (FT-IR/ATR cm^{-1}) ν_{max} 3357, 2929, 1734, 1711, 1653. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.21 (1H, br-s, β-Ala-NH), 5.72 (1H, d, J = 8.1 Hz, β^3-chg-NH), 4.50 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.51−3.48, (2H, m, CH_2N), 2.86 (1H, dd, J = 15.9, J = 4.4 Hz, CH_2CH), 2.68 (1H, dd, J = 15.9, J = 4.4 Hz, CH_2CH), 2.53 (2H, t, CH_2CO_2, J = 5.7 Hz), 1.43 (9H, s, t-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.9, 171.9, 169.8, 155.6, 79.9, 52.7, 51.8, 50.3, 37.9, 34.7, 33.6, 28.2 ppm. HR-ESI-TOF Calculated for $C_{14}H_{25}N_2O_7$ - $[M + H]^+$: 333.1656. Found: 333.1663 (2.0 ppm error).

Boc-β-Ala-L-His-OMe (Boc-Carnosine-OMe) (3m). (62 mg, 91%, (0.2 mmol of **1a** and **2i** were used)) as a white solid. mp = 78−82 °C. $[\alpha]^{25}_D$ = 6.0 (c 0.3, CH_3OH). (FT-IR/ATR cm^{-1}) ν_{max} 3283, 2977, 2931, 1738, 1686, 1655, 1523. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (1H, s, H-2), 7.30 (1H, br-s, His-NHCO), 6.79 (1H, s, H-4), 5.61 (1H, t, β-Ala-NH, J = 5.7 Hz), 4.79 (1H, m, CH), 3.70 (3H, 2, OCH_3), 3.40 (2H, m, CH_2NBoc), 3.10 (2H, d, CH_2, J = 5.1 Hz), 2.43 (2H, t, $CH_2C{=}O$, J = 5.8 Hz), 1.43 (9H, s, t-Bu) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9 (CO_2CH_3), 171.7 (CONH), 156.2 (CO-Carbamate), 135.3 (C-2), 134.0 (C-5), 116.0 (C-4), 79.4 (C-t-Bu), 52.7 (CH), 52.4 (OCH_3), 36.9 (CH_2NBoc), 36.4 ($CH_2C{=}O$), 28.9 (CH_2), 28.4 (CH_3-t-Bu) ppm. COSY correlation [δ_H/δ_H]: 7.65/6.45 [2-H/4-H], 7.30/4.78 [His-NH/CH], 6.45/7.65 [4-H/2-H], 5.60/3.40 [β-Ala-NH/CH_2NBoc], 4.78/7.30/3.10 [CH/His-NH/CH_2], 3.40/5.60/2.43 [CH_2NBoc/β-Ala-NH/$CH_2C{=}O$], 3.10/4.78 [CH_2/CH], 2.43/3.40 [$CH_2C{=}O$/CH_2NBoc]. HETCOR correlation [δ_H/δ_C]: 7.55/135.3 [H-2/C-2], 6.79/116.0 [H-4/C-4], 4.79/52.7 [CH/CH], 3.70/52.4 [OCH_3/OCH_3], 3.40/36.9 [CH_2NBoc/CH_2NBoc], 3.10/28.9 [CH_2/CH_2], 2.43/36.4 [$CH_2C{=}O$/$CH_2C{=}O$], 1.43/28.4 [CH_3-t-Bu/CH_3-t-Bu]. HMBC correlation [δ_H/δ_C]: 7.55/116.0/134.0 [H-2/C-4/C-5], 6.79/135.3 [H-4/C-2], 4.79/28.9/134.0/171.9 [CH/CH_2/C-5/CO_2CH_3], 3.70/171.9 [OCH_3/CO_2CH_3], 3.40/36.4/156.2/171.7 [$CH_2NBoc/CH_2C{=}O$/CO−Carbamate/CONH], 3.10/52.7/116.0/134.0/171.9 [CH_2/CH/C-4/C-5/CO_2CH_3], 2.43/36.9/171.7 [$CH_2C{=}O/CH_2NBoc$/CONH], 1.43/28.4/79.4[CH_3-t-Bu/CH_3-t-Bu/C-t-Bu]. HR-ESI-TOF Calculated for $C_{15}H_{25}N_4O_5$ $[M + H]^+$: 341.1823. Found: 341.1819 (1.0 ppm error).

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Supporting Information Available: Copies of NMR spectra of all compounds, X-ray crystal structure, and crystallographic information files (CIFs) of **1a**,**b**. This material is available free of charge via the Internet at http://pubs.acs.org.