

Room temperature ionic liquids (RTIL's) are convenient solvents for peptide synthesis !

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Dedicated to Professor Armand Lattes on his 50th years teaching and research

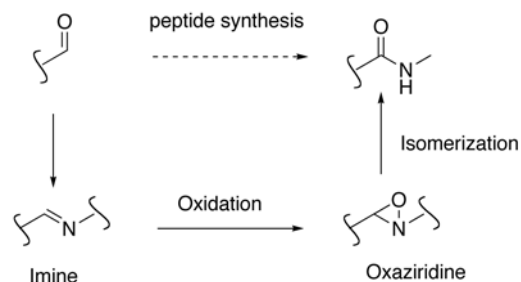
Abstract

The chemical peptide coupling with modern coupling agents is efficient in ionic liquids. The reaction rate is fast enough, and the method offers some interests in the case of hindered amino acids, which are not easy to couple under standard conditions. Highly pure crude peptides are obtained in most cases compared to the corresponding coupling in classical solvents. Di-, tetra-, octa- and cyclo-peptides are conveniently synthesized in good yields, and various cheap coupling agents may be used.

Keywords: Ionic liquids, peptide synthesis, amino acids, coupling reagents

Introduction

Some years ago, we embarked on the study of alternative methods for peptide coupling, considering that the "classical" methods still remain unsatisfactory in terms of atom economy, convergence and cost.¹ One of these methods relies on the oxaziridine-amide rearrangement, as we were inspired by this interesting and powerful reaction previously studied by Lattes and coworkers.^{2,3} Indeed, we supposed that this rearrangement would also occur starting from α -amino imines, thus leading to peptides after reaction (Scheme 1).

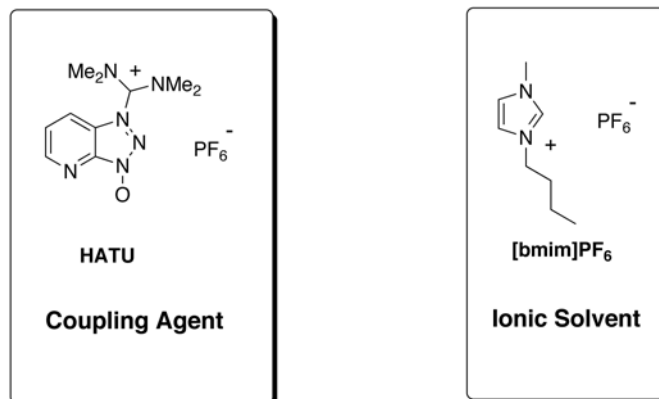


Scheme 1. The oxaziridine-amide rearrangement.

We were thus able to obtain various peptides, including aspartame, when using either ferrous ions, UV light, or silica gel as promoter of the rearrangement.⁴⁻⁶ Later, a variant led us to propose a mild method for the formylation of amino acids.⁷ Although not general, this method avoids the necessity to use any coupling agent and takes advantage of the reactivity of amino aldehydes to give easy access to the coupling of hindered amino acids.^{8,9} Inspired by this first set of results, we then examined other alternative methods, such as the *in situ* ring contraction of heterocyclic enamines yielding peptides of quaternary prolines,¹⁰ and copper (I) mediated formation of peptidic guanidines starting from amidines.¹¹

More recently, our interest for organic chemistry and asymmetric synthesis in ionic liquids¹²⁻¹⁷ prompted us to examine these new media in the context of peptide synthesis.¹⁸

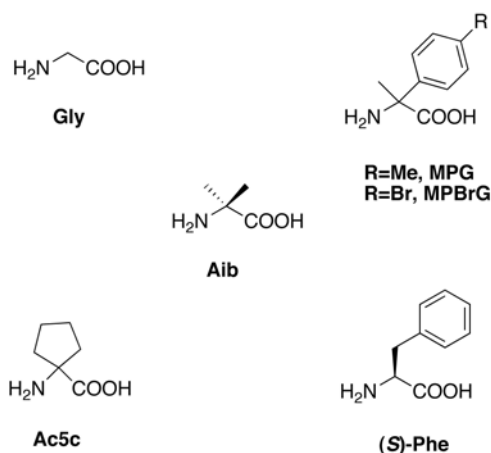
As well established today, the unique properties of room temperature ionic liquids (RTIL's) present many advantages in the context of green chemistry.¹⁹ At the beginning of our study, and rather curiously with regard to the importance of this topic, no report described chemical peptide synthesis in ionic liquids; only a study from Erbeldinger was devoted to enzymatic access to (*Z*)-aspartame.²⁰ During the course of our work, other powerful (or at least conceptually fascinating) approaches were described by other groups.²¹⁻²³ We decided to embark on this approach by considering "modern" coupling agents such as HATU (*O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate) and BOP ((Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate) as reagents of choice for this chemistry in ionic liquids, since they present structural similarities with ionic solvents, such as [bmim][PF₆] (Scheme 2). Thus we expected these coupling agents to be easily dissolved in ionic liquids, sufficiently stabilized by the solvent, to give slow and selective reactions (for the mechanism, *vide infra*).



Scheme 2. Comparison of the structures of coupling agent and the ionic solvent.

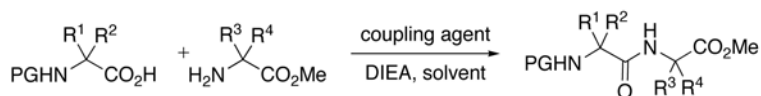
Results and Discussion

In a first set of experiments, we decided to realize the coupling of quaternary α -amino acids which are known to be more difficult to couple than their tertiary proteinogenic congeners.²⁴ The dipeptide formation was compared to results obtained in usual solvents.



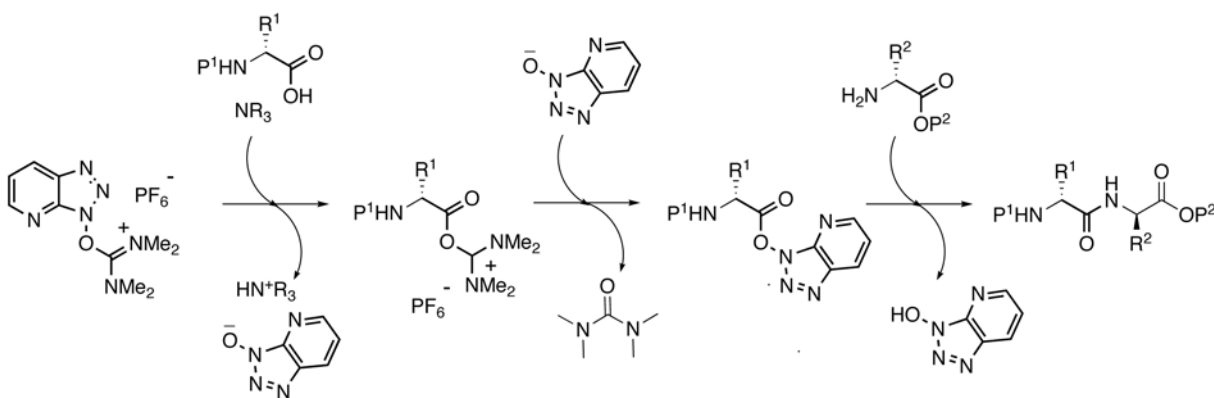
Scheme 3. Studied amino acids: Gly = glycine, Aib = aminoisobutyric acid, MPG = 2-methyl-2-(*p*-tolyl)-glycine, MPBrG = 2-methyl-2-(*p*-bromophenyl)-glycine, Phe = phenylalanine, Ac5c = Aminocyclopentanecarboxylic acid).

The studied amino acids are depicted in Scheme 3: all are commercially available except for MPG and MPBrG which were prepared according to a literature procedure.²⁵ The usual *Z* and *Boc* protecting groups were employed for the protection of the amine functions. The carboxylic function of the second amino acid was protected as methyl ester (Scheme 4).



Scheme 4. Synthesis of dipeptides in ionic liquids.

The main results for different coupling reactions are given in Table 1, along with a comparison with analogous reactions carried out in classical solvents (dichloromethane or THF). The simplified mechanism for such coupling reactions is presented in Scheme 5.²⁶⁻²⁸



Scheme 5. Simplified mechanism for peptide coupling using HATU as coupling reagent.

Table 1. Peptide couplings in [bmim][PF₆]

Dipeptide formed	Coupling Agent	T (°C)	Time (d)	Yield in [bmim][PF ₆] (%)	Yield in CH ₂ Cl ₂ (%)
ZGly-GlyOMe	HATU	65	3	93	93
ZGly-MPGOMe	HATU	65	3	99	93
ZGly-MPBrGOMe	HATU	65	3	99	88
ZGly-Ac5cOMe	HATU	65	3	93	-
BocAib-Ac5cOMe	HATU	65	3	43	-
BocPhe-PheOMe	HATU	65	3	99	-
BocPhe-GlyOMe	HATU	65	3	96	-
BocPhe-Ac5cOMe	HATU	65	3	99	-
BocMPG-GlyOMe	BOP	rt	1.5	82	66
BocMPG-MPGOMe	HATU	50	8	45	43
BocAib-MPGOMe	HATU	rt	4	52	59
BocMPG-AibOMe	HATU	rt	4	49	58

We first verified that experiments without any coupling agent, or using DCC, led to no conversion, and selected HATU as coupling reagent (anyway, BOP gave very similar results¹⁸).

After the optimization of the reaction conditions and the extraction procedure¹⁸ we were delighted to obtain very good yields in most cases, which compared favorably with that of peptide synthesis in « classical » solvents such as dichloromethane and THF. The main advantage was the higher purity of the crude product observed when the reaction was performed in an ionic liquid with respect to dichloromethane or THF. A more selective chemical pathway, due to the stabilization effect of the ionic liquid on both the coupling reagent and the charged intermediates, could explain this behavior (see Scheme 5). Also, we think that by-products resulting from the reaction of HATU, like tetramethylurea and HOAt, are not extracted and stay in the ionic phase (*vide infra*).

Moderate yields were observed for the coupling of two quaternary amino acids, but the efficiency was rather similar to molecular solvents. Interested by further studies in chiral recognition by means of hindered peptides,^{29,30} we succeeded in obtaining crystals of ZGly-(*R*)-MPGOMe which were suitable for X-ray analysis (slow crystallization from acetonitrile/water solution). The corresponding ORTEP is presented in Figure 1.

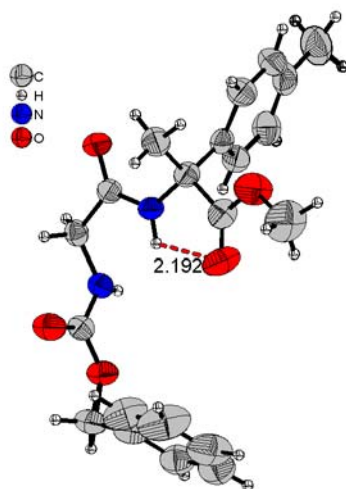
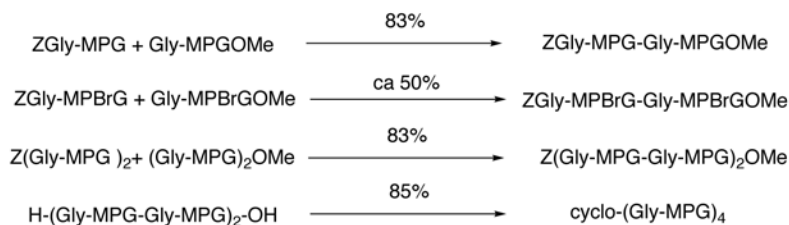


Figure 1. ORTEP of ZGly-(*R*)-MPGOMe. All non-hydrogen atoms are represented by their displacement ellipsoids drawn at the 50% probability level. Supplementary crystallographic data for CCDC 292584 are available free of charge via <http://www.ccdc.cam.ac.uk/conts/retrieving.html> (or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom; fax +44 1223 336033 or deposit@ccdc.cam.ac.uk).

In the crystalline state, this compound presents an intramolecular hydrogen bond that stabilizes the structure via a five-membered pseudo cycle. This behavior is rather common in small peptides including quaternary aminoacids.³¹

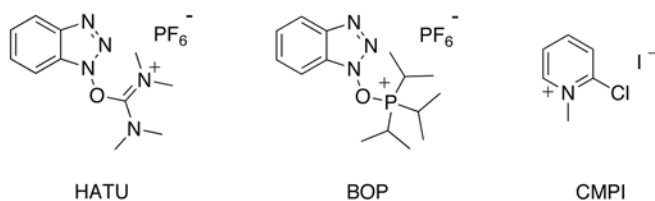
Having a method allowing easy access to various dipeptides (including examples with one or two hindered amino acids) in hands, we turned to the extension of the method to tetra-, octa- and cyclopeptides (Scheme 6).



Scheme 6. Synthesis of tetra-, octa- and cyclopeptides in [bmim][PF₆] (65° C, 4d, DIEA), HATU as coupling agent, except for cyclization carried out with BOP.

Interestingly, results for such couplings compared quite well with those observed in molecular solvents. For example, tetrapeptides were usually formed with ca 80% yield and octapeptide with ca 70% when synthesized in THF. Cyclooctapeptide was obtained in the 80-90% range depending on the concentration and conditions of the cyclization reaction.^{29,30}

As mentioned before, one interesting aspect of this method is the high purity of the obtained crude peptides. This was assumed to be due, at least in part, to the ability of ionic solvents to retain the residues of the activation agent in solution. In addition to the benefit for the purification procedure, this could open up the possibility to recycle the activation agent. To this purpose, we decided to examine a series of those reagents to compare both efficiency and reaction rate in [bmim][PF₆]. We choose the synthesis of dipeptide ZGly-MPBrGOMe as model reaction for this study. Typical results are shown in Table 2.

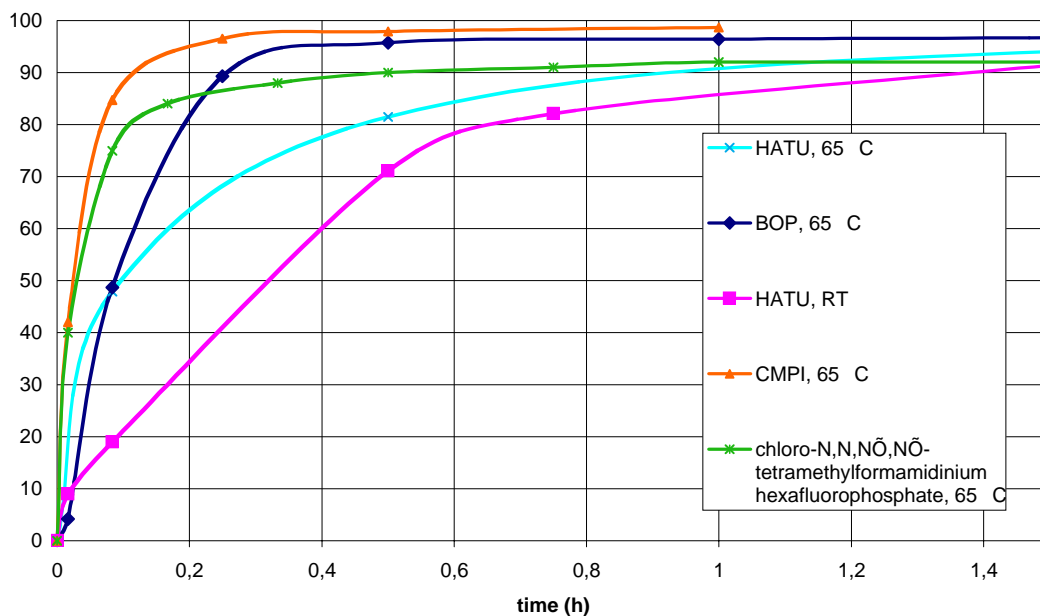


As previously reported,¹⁸ DCC failed to give any useful coupling but yielded mainly a crude mixture in which many impurities could be detected (not identified). On the other hand, HATU and BOP seem to be excellent reagents in these conditions, according to our working hypothesis (*vide supra*). In the fifth entry of table 2, the use of chloro-*N,N,N',N'*-tetramethylformamidinium hexafluorophosphate as coupling agent is reported, which is the precursor of HATU and much less expensive. Interestingly, a fairly good coupling yield was observed with this agent.

Table 2. Comparison of coupling reagents for synthesis of ZGly-MPBrGOMe in [bmim][PF₆] (65° C, DIEA, 3d).

Coupling reagent	Yield (%)
HATU	99
BOP	99
CMPI	97
DCC	No coupling
$\begin{array}{c} \text{Cl} \quad \text{NMe}_2 \\ \quad / \\ \text{C} \\ \quad \backslash \\ \text{NMe}_2 \quad \text{PF}_6^- \end{array}$	84

In the same series of experiments, we examined the reaction rate at the beginning of the coupling reaction. For this purpose, aliquots of the reaction mixture (2 drops) were quenched at various times with a water/acetonitrile/TFA mixture (55/45/0.1) and analyzed by HPLC (for chromatography conditions, see experimental part). This study showed a rapid coupling when using charged reagents, giving a conversion up to 90 % in 1.5h. Indeed, the efficiency (in terms of conversion rate) decreased in the order CMPI>BOP>HATU.

**Figure 2.** Kinetic study of the coupling reaction (synthesis of ZGly-MPBrGOMe in [bmim][PF₆], conversion >90% after 1h30). Comparison of various coupling reagents.

It is noteworthy that this kinetic behavior is not common: indeed, one could expect the opposite order of reactivity when constructing the peptide bond in classical solvents. Quite

interestingly, the less expensive coupling reagents seem to be the more efficient one in ionic liquids in terms of the rate of the reaction.

Conclusions

This work shows that peptide coupling using modern coupling agents is efficient in ionic liquids, especially for hindered amino acids that are not easy to couple under standard conditions. Interestingly, the crude peptides are obtained with higher purities with respect to coupling in classical solvents. Classical coupling agents like BOP or HATU can be used, but less expensive reagents like CMPI perform even better in these new media. Di-, tetra-, octa- and cyclopeptides are thus conveniently synthesized. Examination of a series of coupling reagents, both in terms of kinetics and efficiency shows unusual behavior as well as opens up the possibility of their recycling. Our ongoing studies focus on this research, and to evaluate the direct coupling of amino acid salts, since these salts can be dissolved into ionic liquids. If successful, this approach would avoid any protection / deprotection steps.

Experimental Section

General Procedures. The NMR spectra were recorded on a Brüker AVANCE 300 spectrometer (^1H at 300 MHz and ^{13}C at 75 MHz) in deuteriochloroform or deuteromethanol, the chemical shifts are quoted in ppm in δ -values and the coupling constants are quoted in Hz. Optical rotations were measured with a Perkin Elmer polarimeter, with a 1 dm length cell. HPLC were performed on a HP series 1100 apparatus (integrator HP 3395, column Capital HPLC LTD, C18-KL5-25091, 25 cm x 4.6 mm, 1 mL.min $^{-1}$, 20 μL , λ 215 nm).

Preparation of dipeptides. General procedure. The *N*-protected amino acid (25mg), the amino ester hydrochloride (1.1 eq.) and the coupling reagent (HATU or BOP) (1.1 eq.) were introduced in a 5 mL flask under nitrogen. 0.5 mL of [bmim][PF $_6$] was then added at room temperature. The reaction mixture was mixed and cooled at 0° C. Diisopropylethylamine (3.3 eq.) was then added dropwise. After stirring for 3 days at 65° C, the mixture was washed successively with NaHCO $_3$ (1M), citric acid (5%), and water. The dipeptide was extracted from the ionic phase with diethylether or toluene, using a continuous liquid-liquid extractor (24h).

Boc-(R)-MPG-(R)-MPG-OMe. White powder, m.p. 131°C; Retention time 9.16 min (HPLC, ACN/H $_2$ O/TFA: 80/20/0.1); ^1H NMR 1.38 (s, 9H, C(CH $_3$) $_3$), 1.88 (s, 3H, CH $_3$), 1.93 (s, 3H, CH $_3$), 2.32 (s, 3H, CH $_3$), 2.36 (s, 3H, CH $_3$), 3.63 (s, 3H, OCH $_3$), 6.10 (s, 1H, NH), 7.08-7.34 (m, 9H, Ar, NH); ^{13}C NMR 21.1, 21.2, 21.5, 24.3, 28.4, 53.2, 61.7, 61.8, 79.6, 125.4, 125.5, 129.1, 129.3, 136.9, 137.4, 137.9, 138.7, 154.3, 172.2, 173.4; $[\alpha]_D^{20}$ -30.6 (C = 0.5, MeOH); C $_{26}$ H $_{34}$ N $_2$ O $_5$ (454.57): calcd. C 68.70, H 7.54, N 6.16; found C 68.64, H 7.58, N 6.05; I.R. (cm $^{-1}$) 3398, 2977, 1728, 1681.

Boc-(R)-MPG-Aib-OMe. White powder, m.p. 134°C (dec.); Retention time 24.39 min (HPLC, ACN/H₂O/TFA: 50/50/0.1); ¹H NMR 1.30 (s, 9H, C(CH₃)₃), 1.37 (s, 6H, CH₃), 1.73 (s, 3H, CH₃), 2.26 (s, 3H, CH₃), 3.63 (s, 3H, OCH₃), 5.87 (s, 1H, NH), 6.65 (s, 1H, NH), 7.08-7.23 (m, 4H); ¹³C NMR 21.1, 24.6, 24.7, 25.3, 28.3, 52.6, 56.5, 62.0, 79.8, 125.6, 129.4, 137.3, 138.9, 154.5, 172.8, 174.8; [α]²⁰_D -14.6 (C = 0.5, MeOH); C₂₀H₃₀N₂O₅ (378.46): calcd. C 63.47, H 7.99, N 7.40; found C 63.38, H 8.07, N 7.52; I.R. (cm⁻¹) 3367, 3331, 2980, 1727, 1709, 1670.

Boc-(R)-MPG-Gly-OMe. Colorless oil; Retention time 7.01 min (HPLC, ACN/H₂O/TFA: 60/40/0.1); ¹H NMR 1.36 (s, 9H, C(CH₃)₃), 1.89 (s, 3H, CH₃), 2.33 (s, 3H, CH₃), 3.70 (s, 3H, OCH₃), 3.87-4.06 (m, 2H, CH₂), 6.00 (s, 1H, NH), 6.44 (s, 1H, NH), 7.15-7.33 (m, 4H); ¹³C NMR 21.4, 25.0, 28.6, 41.9, 52.7, 62.3, 80.1, 126.1, 129.8, 137.9, 138.4, 154.6, 170.3, 174.3; [α]²⁰_D -34.4 (C = 0.5, MeOH); C₁₈H₂₆N₂O₅ (350.18): calcd. C 61.70, H 7.48, N 7.99; found C 61.21, H 7.67, N 7.92; I.R. (cm⁻¹) 3389, 2977, 1755, 1722, 1670.

Boc-Aib-(R)-MPG-OMe. White needles, m.p. 129°C; Retention time 9.48 min (HPLC, ACN/H₂O/TFA: 60/40/0.1); ¹H NMR 1.37 (s, 9H, C(CH₃)₃), 1.39 (s, 6H, CH₃), 1.91 (s, 3H, CH₃), 2.24 (s, 3H, CH₃), 3.59 (s, 3H, OCH₃), 5.03 (s, 1H, NH), 7.05-7.27 (AA'BB', 4H, *J*=7.5 Hz), 7.55 (s, 1H, NH); ¹³C NMR 21.0, 22.0, 25.2, 25.6, 28.3, 52.9, 56.8, 61.6, 79.9, 125.8, 129.3, 137.4, 137.6, 154.6, 173.3, 173.6; [α]²⁰_D -36.2 (C = 0.5, MeOH); C₂₀H₃₀N₂O₅ (378.46): calcd. C 63.47, H 7.99, N 7.40; found C 63.19, H 8.26, N 7.31; I.R. (cm⁻¹) 3317, 2978, 1718, 1672.

Boc-Aib-Ac5c-OMe. White solid, m.p. 141°C; ¹H NMR 1.34 (s, 9H, C(CH₃)₃), 1.37 (s, 6H, CH₃), 1.64-1.69 (m, 4H, CH₂ cyclopentyl), 1.82-1.91 (m, 2H, CH₂ cyclopentyl), 2.04-2.11 (m, 2H, CH₂ cyclopentyl), 3.59 (s, 3H, OCH₃), 4.80 (s, 1H, NH), 7.40 (s, 1H, NH); ¹³C NMR 24.6, 25.6, 29.0, 37.2, 52.9, 56.8, 65.9, 80.0, 155.0, 174.3, 174.8; C₁₆H₂₈N₂O₅ (328.40): calcd. C 58.52, H 8.59, N 8.53; found C 58.75, H 8.70, N 8.93; I.R. (cm⁻¹) 3337, 2927, 1717, 1665.

Boc-Phe-Ac5c-OMe. White solid, m.p. 152°C; ¹H NMR 1.34 (s, 9H, C(CH₃)₃), 1.54-1.76 (m, 8H, cyclopentyl), 2.95-2.99 (m, 2H, CH₂), 3.62 (s, 3H, OCH₃), 4.24 (m, 1H, CH), 5.10 (s, 1H, NH), 8.17 (s, 1H, NH), 7.15-7.22 (m, 5H, Ar); ¹³C NMR 24.6, 28.4, 36.9, 38.3, 50.0, 52.6, 55.7, 80.2, 127.0, 128.7, 129.5, 136.9, 155.6, 170.9, 174.4; C₂₁H₃₀N₂O₅ (390.47): calcd. C 64.59, H 7.74, N 7.17; found C 64.28, H 8.02, N 7.36; I.R. (cm⁻¹) 3291, 2926, 1739, 1656.

Cyclo(Gly-(R)-MPG)₄. Colorless crystals, m.p. 330°C (DSC); Retention time 22.54 min (HPLC, ACN/H₂O/TFA: 50/50/0.1); ¹H NMR 1.82 (s, 12H, CH₃), 2.27 (s, 12H, CH₃), 3.61 (dd, 4H, CH₂, *J*=16.2 Hz, *J*=5.2 Hz), 3.80 (dd, 4H, CH₂, *J*=16.2 Hz, *J*=6.2 Hz), 7.07-7.29 (m, 16H), 8.17 (t, 4H, NH, *J*=5.6 Hz), 8.26 (s, 4H, NH); ¹³C NMR 20.6, 24.3, 43.6, 61.6, 126.2, 128.6, 136.2, 138.7, 168.8, 173.0; [α]²⁰_D -100 (C = 0.5, CH₂Cl₂), 2C₄₈H₅₆N₈O₈·1H₂O (873.01): calcd. C 65.26, H 6.60, N 12.78; found C 65.36, H 6.51, N 12.70; mass: calcd. 873.4, found [MH⁺] 873.7; I.R. (cm⁻¹) 3550, 3325, 1672, 1527; U.V. λ_{max} = 202nm.

Z-(Gly-(R)-MPG)₂-OMe. White powder, m.p. 226°C; Retention time 19.04 min (HPLC, ACN/H₂O/TFA: 40/60/0.1); ¹H NMR 1.92 (s, 3H, CH₃), 1.98 (s, 3H, CH₃), 2.23 (s, 3H, CH₃), 2.31 (s, 3H, CH₃), 3.64 (s, 3H, OCH₃), 3.85-3.99 (m, 4H, CH₂), 4.81 (s, 2H, OCH₂), 5.67 (t, 1H, NH, *J*=4.2 Hz), 6.68 (t, 1H, NH, *J*=4.9 Hz), 7.08-7.31 (m, 13H, Ar), 7.71 (s, 1H, NH), 8.02 (s, 1H, NH); ¹³C NMR 21.0, 21.1, 22.4, 23.4, 44.4, 45.2, 53.1, 62.9, 63.6, 67.8, 126.8, 127.3, 128.8,

129.0, 129.4, 129.9, 130.2, 138.1, 138.6, 138.9, 139.0 (2C), 159.0, 171.0, 171.7, 174.4, 175.3; $[\alpha]_D^{20}$ -38.4 (C = 0.5, CH₂Cl₂); C₃₃H₃₈N₄O₇ (602.68): calcd. C 65.77, H 6.36, N 9.30; found C 65.75, H 6.62, N 9.03; I.R. (cm⁻¹) 3312, 3064, 3030, 2997, 1725, 1665.

Z-(Gly-(R)-MPG)₄-OMe. White powder, m.p. 180°C (dec.); Retention time 17.07 min (HPLC, ACN/H₂O/TFA: 60/40/0.1); ¹H NMR 1.67 (s, 3H, CH₃), 1.70 (s, 3H, CH₃), 1.73 (s, 3H, CH₃), 1.80 (s, 3H, CH₃), 2.25-2.28 (m, 12H, CH₃), 3.51 (s, 3H, OCH₃), 3.59-3.77 (m, 8H, CH₂), 5.03 (s, 2H, OCH₂), 7.08-7.34 (m, 21H, Ar), 7.51 (t, 1H, NH, J=5.6 Hz), 7.95 (t, 1H, NH, J=5.6 Hz), 8.07-8.10 (m, 5H, NH), 8.35 (s, 1H, NH); ¹³C NMR 21.0 (4C), 23.5, 24.2, 24.8, 25.0, 44.5, 45.2 (3C), 53.1, 62.9, 63.6, 64.1, 64.2, 67.9, 126.9, 127.1 (2C), 127.4, 128.9, 129.1, 129.5, 129.9, 130.1, 130.2, 130.3, 137.9, 138.1, 138.6, 138.7, 138.8, 138.9, 139.0, 139.1, 159.1, 171.1, 171.7, 171.8, 174.4, 175.4, 175.7, 175.8; $[\alpha]_D^{20}$ -6.0 (C = 0.5, CH₂Cl₂); C₅₇H₆₆N₈O₁₁ (1039.18): calcd. C 65.88, H 6.40, N 10.78; found C 65.85, H 6.95, N 10.73; I.R. (cm⁻¹) 3305, 3025, 1664.

Z-Gly-(R)-MPG-OMe. White needles, m.p. 110 °C; Retention time 13.05 min (HPLC, CH₃CN/H₂O/TFA: 50/50/0.1); ¹H NMR 2.02 (s, 3H, CH₃), 2.32 (s, 3H, CH₃), 3.69 (s, 3H, OCH₃), 3.87-3.88 (m, 2H, CH₂), 5.12 (s, 2H, OCH₂), 5.83 (s, 1H, NH), 7.13-7.35 (m, 9H, Ar), 7.51 (s, 1H, NH); ¹³C NMR 20.9, 22.0, 44.6, 53.0, 61.7, 66.8, 125.6, 127.9, 128.0, 128.4, 129.2, 136.8, 137.8, 156.6, 168.1, 173.2; $[\alpha]_D^{20}$ -19.8 (c=0.5, CH₂Cl₂); C₂₁H₂₄N₂O₅ (384.43): calcd. C 65.61, H 6.29, N 7.29; found C 65.57, H 6.37, N 7.26; I.R. (cm⁻¹) 3322, 3031, 2950, 1731, 1678.

Z-Gly-(S)-MPBrG-OMe. White powder, m.p. 116°C; Retention time 20.35 min (HPLC, ACN/H₂O/TFA: 40/60/0.1); ¹H NMR 2.00 (s, 3H, CH₃), 3.68 (s, 3H, OCH₃), 3.85 (d, 2H, CH₂, J=5.3 Hz), 5.11 (s, 2H, OCH₂), 5.70 (t, 1H, NH, J=5.3 Hz), 7.28-7.56 (m, 9H, Ar), 7.59 (s, 1H, NH); ¹³C NMR 21.9, 44.8, 53.4, 61.6, 67.1, 122.2, 127.7, 128.0, 128.2, 128.5, 131.7, 136.2, 138.8, 156.7, 168.0, 173.0; $[\alpha]_D^{20}$ +9.6 (C = 0.5, MeOD); C₂₀H₂₁BrN₂O₅ (449.30): calcd. C 53.46, H 6.23, N 4.71; found C 53.49, H 6.37, N 4.89; I.R. (cm⁻¹) 3315, 2950, 1737, 1676.

Z-Gly-Ac5c-OMe. Colorless oil; ¹H NMR 1.73 (s, 4H, CH₂ cyclopentyl), 1.89-1.94 (m, 2H, CH₂ cyclopentyl), 2.15-2.22 (m, 2H, CH₂ cyclopentyl), 3.68 (s, 3H, OCH₃), 3.85 (d, 2H, CH₂, J = 5.3 Hz), 5.10 (s, 2H, CH₂), 5.69 (s, 1H, NH), 6.80 (s, 1H, NH), 7.33 (s, 5H, Ar); ¹³C NMR 24.7, 37.3, 44.5, 52.7, 66.0, 67.2, 128.1, 128.3, 128.6, 136.3, 156.8, 169.0, 174.7; C₁₇H₂₂N₂O₅ (334.37): calcd. C 61.07, H 6.63, N 8.38; found C 60.83, H 6.52, N 8.09; I.R. (cm⁻¹) 3327, 2954, 1729, 1674.

Spectral data for **Boc-Phe-Gly-OMe**,³² **Boc-Phe-Phe-OMe**³³ and **Z-Gly-Gly-OMe**³⁴ where found identical to literature values.

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