Bis(4,6-dimethoxy-1,3,5-triazin-2-yl) Ether as Coupling Reagent for Peptide Synthesis

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Dedicated to Professor *Ignacy Z. Siemion* on the occasion of his 80th birthday

Bis(4,6-dimethoxy-1,3,5-triazin-2-yl) ether (**4**) was prepared by treatment of 2-hydroxy-4,6-dimethoxy-1,3,5-triazine with 2-chloro-4,6-dimethoxy-1,3,5-triazine in 61% yield. Ether **4**, isoelectronic with pyrocarbonates, was found capable to activate carboxylic acids in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) to yield, under mild reaction conditions, superactive triazine esters. Versatility of this new coupling reagent was confirmed by condensation of lipophilic and sterically hindered carboxylic acids with amines in 71–98% yield, and by synthesis of peptides, including those containing Aib-Aib sequence, in solution with high yield and high enantiomeric purity.

Introduction. – In recent years, incorporation of an unnatural residue into the peptide chain became a standard synthetic practice. The main motivation for this approach arose from the expected increase of the resistance of unnatural structures toward metabolic degradation, prolonged activity, reduction of dosage, and expected less ambiguous interpretation of effects caused by incorporation of unnatural building blocks. There is also a substantially increased pool of naturally occurring peptides with non-coded amino acids or with additional structural elements active as antibiotics, toxins, and others [1]. In both cases, the available reagents and synthetic strategies turned out to be insufficient or even to fail completely in the case of poorly reactive and sterically hindered building blocks, the so-called difficult sequences or coupling building blocks with opposite disposition such as hydrophilicity–hydrophobicity. An example is the well-recognized group of peptides isolated from fungi, known as peptaibols, bearing several characteristic structural features [2]. These include the presence of multiple residues of α-aminoisobutyric acid (Aib) and amino alcohol at the C-terminal position. Due to the accumulation of α,α-disubstituted amino acid residues in sequences, synthesis of peptaibols has always been the synthetic challenge [3]. Synthetic problems caused by sterically hindered amino acids led to the search for the new, more-efficient coupling procedures and reagents, including phosphinium, iminium, and ammonium derivatives of 1-hydroxybenzotriazole [4], 8-azabenzotriazole [5], or oxyma (=ethyl 2-cyano-2-(hydroxyimino)acetate) [6].

Our efforts in this area were focused on the development of a new generation of triazine-based coupling reagents, designed according to the concept of 'superactive esters' [7]. Following the concept of 'superactive esters', increased efficiency of coupling reagents was achieved with an innovative strategy based on the introduction

of an additional synchronous and thermodynamically highly favored process. The accompanying process (*i.e.* an additional, fast, and energetically favored reaction or rearrangement of the co-products) synchronous with a release of the leaving group from tetrahedral intermediates should facilitate the rate-determining step [7]. By this way, the 'push' from the relatively mild activation and the 'pull' from additional stabilization of the products should afford better synthetic results than those obtained, when the same change of the thermodynamic potential of the reaction is solely due to more powerful activation. The versatility of the idea of acceleration of the coupling process in the case of 'superactive esters', based on facile departure of the leaving group by its rearrangement in an energetically favored, consecutive process to a stable, chemically inert, and neutral side product, has been thoroughly confirmed. The usefulness of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate (DMT/NMM/BF_4) was compared with standard coupling reagents, and its supremacy in peptide synthesis in solution and in solid phase was demonstrated [8]. Modification of substituents in the native structure of triazine-based coupling reagent afforded 4-[4,6-bis(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]-4-methylmorpholinium tetrafluoroborate, which was found highly efficient for incorporation of sterically hindered *N*-methylleucine and *N*-methylvaline into the peptide chain [9]. Replacement of 4-methylmorpholine (NMM) fragment by chiral tertiary amines provided highly enantioselective coupling reagents. These reagents, named 'traceless', when applied peptide synthesis from racemic *N*-protected amino acids for the first time, gave optically pure products with configuration and enantiomeric enrichment precisely predicted by a single model experiment [10].

Recently, we noticed the increased trends in application of strongly lipophilic building blocks used in modifications of the native peptide structures [11]. Moreover, there is an increasing number of new natural products with identified strongly lipophilic fragments crucial for the activity of the native molecules. Examples are peptaibols containing several α,α-disubstituted α-amino acid residues [12], *N*-alkylated amino acids, β-amino acids [13], lipidated peptides for gene delivery, fluorinated amino acids, and many others [14]. As the lipophilic nature of amino acids is essential for activity, there is increased demand for suitable methods of incorporation which reconciles high efficiency with tolerance of increased lipophilicity of reagents and reacting media.

Following the motto that '*similia similibus solvuntur*', we attempted to improve the synthetic versatility of triazine-based coupling reagents by modification of ionic structure of *N*-triazinylammonium salts in order to obtain more lipophillic analogs. Crystallographic analyses of structures of 'superactive' triazine esters [15], aryloxy-1,3,5-triazines [16], and bis(4,6-dimethoxy-1,3,5-triazin-2-yl) ether (**4**) suggested that the latter, due to unsymmetrical charge distribution [17], should be considered as potentially reactive intermediate (aza analog of pyrocarbonates) (see *Fig.*).

According to this assumption, bis-triazinyl ethers, after treatment with carboxylic acids, should yield 'superactive' triazine esters identical to active acylating species obtained under classic coupling conditions involving triazine-based coupling reagents. To verify this assumption, we prepared bis-1,3,5-triazine ether **4** and studied its potential for activation of the carboxylic group.

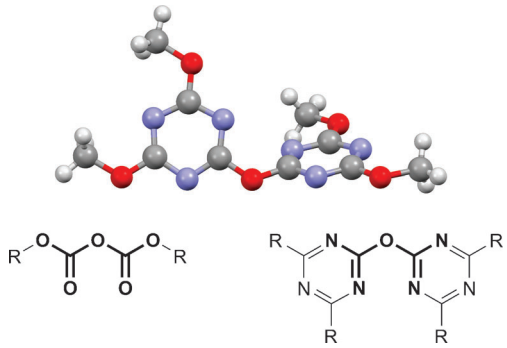
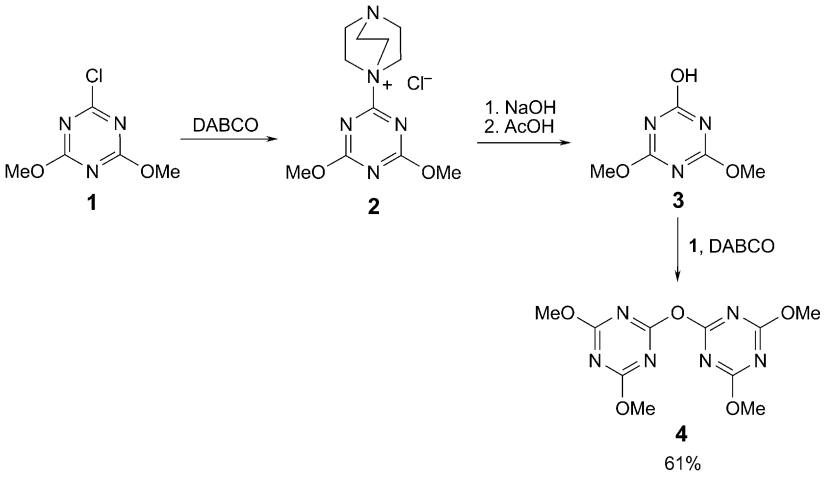


Figure. *Stick-and-ball representation of X-ray structure of bis(4,6-dimethoxy-1,3,5-triazin-2-yl) ether* (**4**) *demonstrating different arrangements of substituents in both triazine rings* [17] (*above*), *and analogous fragments of pyrocarbonates and triazine ethers in bold* (*below*)

Results and Discussion. – *Synthesis of Bis(4,6-dimethoxy-1,3,5-triazin-2-yl) Ether* (**4**). The procedure has been already described in [18]. In this work, however, expecting that ether synthesis according to *Williamson*-type procedure from aromatic substrates is not a trivial process [19], ether **4** was prepared in two separate steps (see *Scheme 1*).

Scheme 1. *Synthesis of Bis(4,6-dimethoxy-1,3,5-triazin-2-yl) Ether* (**4**)

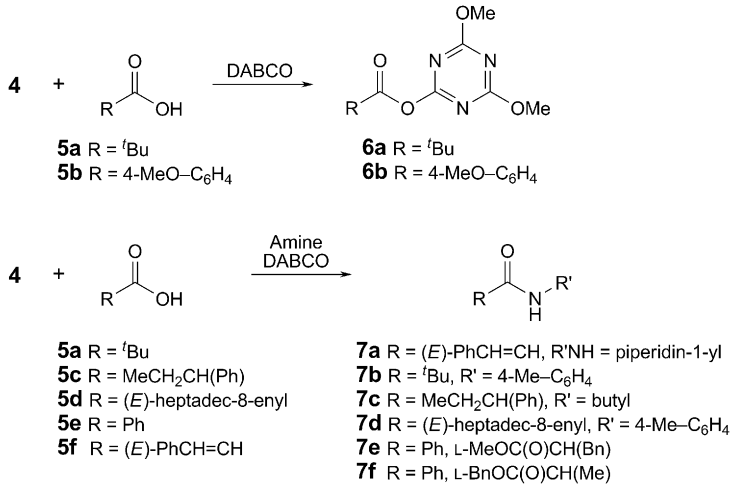


Substrate **3** was obtained in good overall yield by hydrolysis of **1**, presumably, *via* intermediate *N*-triazinylammonium salt **2**. The main advantage of this two-step procedure was reduced contamination of **3** with side-products formed by excessive cleavage of Me groups under strongly basic conditions. Then, the final product **4** was obtained in good overall yield by treatment of 2-hydroxy-4,6-dimethoxy-1,3,5-triazine (**3**) with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT; **1**) activated with 1,4-diazabicyclo[2.2.2]octane (DABCO) under anhydrous conditions.

Crystallographic studies of **4** confirmed asymmetry of structure with one ether C–O bond length of 1.371 Å and a dihedral angle of C–O–C–triazine ring of 40.77°, with the other C–O bond length being 1.365 Å and the corresponding dihedral angle 23.97° [17]. In spite of the observed alternation of ether C–O bonds and appropriate dihedral angles resulting from the strong AGIBA effect (Angular Group-Induced Bond Alternation) [20], preliminary experiments of carboxylic acid activation using **4** were not encouraging.

Activation of Carboxylic Acids Using **4**. Carboxylic acids were not prone to react with **4**. Moreover, an addition of equimolar amount of NMM or 4-(dimethylamino)-pyridine (DMAP) in order to generate a more nucleophilic carboxylate anion did not accelerate the process very much. However, it has been found that, in the presence of equimolar amount of DABCO, activation of carboxylic acids proceeded relatively fast, and that, under mild condition, **4** was completely consumed within 40 min. As the mechanism of this unique effect of DABCO is not known, it was essential to identify the reactive intermediate. The experiments involving 2,2-dimethylpropanoic acid (= pivalic acid; **5a**) and 4-methoxybenzoic acid (**5b**) treated with **4** in the presence of DABCO gave already known 2-(acyloxy)-4,6-dimethoxy-1,3,5-triazines (**6**; see *Scheme 2*) with characteristic IR signal of the active ester group at 1776 cm^{-1}. These compounds were spectroscopically and chromatographically identical with those prepared by standard procedure [7b].

Scheme 2. *Activation of Carboxylic Acids* **5** *Using* **4** *and 1,4-Diazabicyclo[2.2.2]octane* (DABCO) *to Yield Appropriate 2-Acyloxy-4,6-dimethoxy-1,3,5-triazines* **6** *and, in the Presence of Amines, the Corresponding Amides* **7**



This alternative pathway to 'superactive' triazine esters displays synthetic potential of **4** in the application for coupling strongly lipophilic substrates, because the approach based on **4** avoids ionic activators in contrast to all of the most advanced, typical condensing reagents (*N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU), *N*-[(1*H*-ben-

zotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide (TBTU), DMT/NMM/BF_4, *etc.*).

Formation of the peptide bond from amino acids is a complex, multistage process. Therefore, although it is known that activation of carboxylic function yields well known and highly reactive intermediates **6**, the versatility of **4** has to be cautiously evaluated. In the preliminary assessment, **4** was used as condensing reagent in the synthesis of amides. It has been found that the one-pot procedure is efficient for a broad range of substrates affording appropriate amides with 71–98% yield and satisfactory purity of the crude isolated products **7a**–**7f**. Ether **4** was found efficient in coupling amines with sterically hindered 2,2-dimethylpropanoic acid (**5a**), 2-phenylbutanoic acid (**5c**), and strongly hydrophobic (*Z*)-octadec-9-enoic acid (**5d**), as well as benzoic acid (**5e**) with amino acid esters.

Peptide Synthesis. In further studies, **4** was used as coupling reagent in the synthesis of protected peptides **8a**–**8i** (*Table*). Both hydrophobic as well as hydrophilic peptides protected with (*tert*-butoxy)carbonyl (Boc) or (benzyloxy)carbonyl (Z) group were prepared according to non optimized, standard procedure, with good yields, also in the case of sterically hindered Aib–Aib peptide bond useful in the synthesis of peptaibols. Their purities determined by HPLC at λ 220 nm were in the range of 61–97%, which is satisfactory considering strong UV absorption characteristics for any triazine-derived impurities at this wavelengths [21].

Table. *Peptide Synthesis Using* **4** *as Coupling Reagent*

Entry	Peptide	Yield [%]	Purity [%]	Racemization er [% L/% D][a])	
				N-terminal[b])	C-terminal[b])
1	ZLeu-PheOMe (**8a**)	87	75	96.5/3.5	97.4/2.4
2	ZAib-LeuOMe (**8b**)	83	96	–	99.0/1.0
3	ZAib-PheOMe (**8c**)	83	93	–	99.6/0.4
4	ZAib-AibOMe (**8d**)	69	87	–	–
5	BocVal-AibOMe (**8e**)	69	87	99.0/1.0	–
6	BocVal-LeuOMe (**8f**)	75	96	98.9/1.1	96.9/3.1
7	BocLeu-ValOMe (**8g**)	87	70	97.6/2.4	99.8/0.2
8	BocPro-PheOMe (**8h**)	91	97	100.0/0.0	99.9/0.1
9	Z-PheAib-LeuOMe (**8i**)	78	91	99.0/1/0	99.5/0.5

[a]) Enantiomeric ratio expressed as normalized ratio % L/% D determined by GC on *Chirasil-Val*™ capillary column after hydrolysis of peptides to amino acids and subsequent derivatization. [b]) N-Terminal and C-terminal amino acid residue.

Enantiomeric homogeneity at all stereogenic centers was confirmed by GC on a *Chirasil-Val*™ capillary column after total hydrolyses of peptides. In most of the cases, er % L/% D ratio exceeded 99 : 1 [22], even in the cases of difficult couplings involving Aib residue as a reaction components.

Conclusions. – In contradistinction to the well-known chemical inertness of all members of the ether family [23], bis(4,6-dimethoxy-1,3,5-triazin-2-yl) ether (**4**) was found surprisingly reactive. To our knowledge, these heterocyclic ethers, isoelectronic

with pyrocarbonates, have never been considered before as candidates for coupling reagents. Taking into account the advantages of **4** and a very broad range of possible modification of the parent heterocyclic structures, it seems that a brand new family of coupling reagents is accessible and careful studies on their properties are continued.

Experimental Part

General. HPLC: *Vydac C-18* column, detection at 220 nm, gradient from 10 to 50% B in 15 min, flow rate, 1 ml/min; *A:* 0.1% TFA in H_2O, *B:* 0.1% CF_3COOH (TFA) in mixture 90% MeCN and 10% H_2O. TLC: *Merck 'Silicagel-Alufolie'*; mobile phase, CH_2Cl_2, visualization, UV 254 nm and then spraying with 0.5% soln. of 4-(4-nitrobenzyl)pyridine (NBP) in EtOH. IR Spectra: KBr pellets or film; ν in cm^{-1}. ^{1}H-NMR Spectra: 250-MHz spectrometers; solvent peak as an internal reference; δ in ppm, J in Hz.

Elucidation of enantiomeric homogeneity of peptides was performed by GC equipped with a flame-ionization detector on *Chirasil-Val*™ column (25 m × 0.25 mm), H_2/air split, 1:50; carrier gas, He; pressure, 135 kPa. Hydrolysis of peptides (2–4 mg) was performed by treating with redistilled, constant-boiling aq. HCl (5 ml) in glass tube sealed under vacuum and heating to 100° in a water-bath for 24 h. After evaporation of the acids, the remaining residue was dried and derivatized according to the procedure described in [24] to obtain *N*-(trifluoroacetyl) amino acid methyl esters. In all cases, minor signal of D-enantiomer was identified by co-injection with appropriate racemate used as reference, and enantiomeric purity was calculated by normalization of signals intensities of both enantiomers.

4,6-Dimethoxy-1,3,5-triazin-2-ol (**3**). To a vigorously stirred soln. of 1,4-diazaybicyclo[2.2.2]octane (DABCO; 22.44 g, 200 mmol) and NaOH (8.80 g, 220 mmol) in H_2O (400 ml), cooled in an ice/water bath, *2-chloro-4,6-dimethoxy-1,3,5-triazine* (**1**; 35.10 g, 200 mmol) was added. Stirring was continued for 2 h, then the mixture was heated to 80° until all **1** was consumed (TLC). The clear soln. was cooled to r.t., and then acidified with AcOH to pH 6. The precipitate was filtered, washed with H_2O (2 × 30 ml), dried in vacuum desiccators, and recrystallized from EtOH to yield **3** (17.5 g, 55.8%). M.p. 144–146° ([25]: 183–184°). ^{1}H-NMR ($CDCl_3$): 3.86 (*s*, 2 Me). Anal. calc. for $C_5H_7N_3O_3$: C 38.22, H 4.49, N 26.74; found: C 38.55, H 4.35, N 27.00.

Bis(4,6-dimethoxy-1,3,5-triazin-2-yl) Ether (**4**). A soln. of **1** (17.60 g, 100 mmol) in THF (300 ml) was vigorously stirred and cooled to 0°, then DABCO (11.22 g, 100 mmol) was added dropwise. After 30 min, **3** (15.71 g, 100 mmol) was added. Stirring was continued at r.t. for 10 h, then the solvent was evaporated *in vacuo*, and the dry residue was dissolved in CH_2Cl_2 (100 ml). The org. phase was washed with H_2O (2 × 50 ml) and then with sat. aq. NaCl soln. (50 ml), dried ($MgSO_4$), filtered, and concentrated to dryness. Recrystallization of the solid residue from CH_2Cl_2/hexane gave **4** (18.1 g, 61%). R_f (CH_2Cl_2) 0.15. M.p. 148–152° ([18a]: 157–158°). IR (film): 1041, 1312, 1456, 1585, 1768, 1912, 2550, 2879, 2975, 3023, 3438, 3777. ^{1}H-NMR ($CDCl_3$): 4.02 (*s*, 4 Me). Anal. calc. for: $C_{10}H_{12}N_6O_5$: C 40.54, H 4.08, N 28.37; found: C 40.54, H 3.98, N 28.71.

4,6-Dimethoxy-1,3,5-triazin-2-yl 2,2-Dimethylpropanoate; **6a**). A vigorously stirred soln. of *2,2-dimethylpropanoic acid* (**5a**; 0.102 g, 1 mmol) in MeCN (5 ml) was cooled to 0–5°, then **4** (0.296 g, 1 mmol) and DABCO (0.112 g, 1 mmol) were added. Stirring and cooling was continued for 40 min, the solvent was evaporated *in vacuo* to avoid excessive heating, and the isolated oily residue crystallized when stored in a vacuum desiccators. M.p. 49–50° ([7b]: 50–52°). IR (film): 1096, 1199, 1366, 1585, 1696, 1776 (C=O), 2599, 2879, 2975, 3521.

4,6-Dimethoxy-1,3,5-triazin-2-yl 4-Methoxybenzoate (**6b**). Synthesis proceeded as described above, with *4-methoxybenzoic acid* (**5b**; 0.152 g, 1 mmol), MeCN (5 ml), **4** (0.296 g, 1 mmol), and DABCO (0.112 g, 1 mmol). After evaporation of solvent, the obtained oily residue crystallized slowly when stored under vacuum. M.p. 96–98° ([8]: 97–99°). IR (film): 1018, 1103, 1361, 1512, 1600, 1775 (C=O), 2584, 2846, 2953, 3640.

*(2*E*)-3-Phenyl-1-(piperidin-1-yl)prop-2-en-1-one* (**7a**). *General Procedure* (*GP*). The vigorously stirred soln. of cinnamic acid (**5f**; 0.148 g, 1 mmol) in MeCN (5 ml) was cooled to 0–5°, then **4** (0.296 g, 1 mmol) and DABCO (0.112 g, 1 mmol) were added, and stirring was continued for 40 min. Piperidine (0.085 g, 1 mmol) was added and stirring and cooling were continued for 2 h at 0–5°, and overnight at r.t.

After evaporation of the solvent, the residue was dissolved in AcOEt (20 ml) and washed successively with H_2O (15 ml), 1M $KHSO_4$ (2 × 15 ml), H_2O (2 × 15 ml), 1M $NaHCO_3$ (2 × 15 ml), H_2O (2 × 15 ml), and sat. aq. NaCl soln. (15 ml). The org. phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated to dryness *in vacuo* yielding crude **7a** (0.207 g, 98%). HPLC (gradient): purity 99%. M.p. 113–116° ([26]: 115–116°).^{1}H-NMR ($CDCl_3$): 1.61–1.67 (*m*, 3 CH_2); 3.62–3.63 (*m*, 2 CH_2); 6.91 (*d*, *J* = 15.5, CH); 7.36 (*m*, 3 arom. H); 7.52 (*m*, 2 arom. H); 7.64 (*d*, *J* = 15.5, CH).

2,2-Dimethyl-N-*(4-methylphenyl)propanamide* (**7b**). Condensation proceeded according to the *GP* with **5a** (0.102 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), and 4-toluidine (0.107 g, 1 mmol). After evaporation, crude **7b** was obtained (0.147 g, 77%). HPLC (gradient): purity 82%. M.p. 100–102° ([27]: 119–121°). ^{1}H-NMR ($CDCl_3$): 1.31 (*s*, 3 Me); 2.31 (*s*, Me); 3.99 (*s*, CO–NH); 7.14 (*d*, *J* = 8, 2 arom. H); 7.40 (*d*, *J* = 8, 2 arom. H).

N-*Butyl-2-phenylbutanamide* (**7c**). Condensation proceeded according to the *GP* with *2-phenylbutanoic acid* (**5c**; 0.164 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), and $BuNH_2$ (0.073 g, 1 mmol). After evaporation, crude **7c** was obtained (0.156 g, 71%) as an oil [28]. HPLC (gradient): purity 94%. ^{1}H-NMR ($CDCl_3$): 0.73–0.92 (*m*, *Me*CH_2CH, *Me*CH_2CH_2); 1.11–1.20 (*m*, MeC*H*$_2CH_2$); 1.20–1.35 (*m*, $MeCH_2CH_2$); 1.69–1.73 (*m*, 1 H, MeC*H*$_2$CH); 2.07–2.13 (*m*, 1 H, MeC*H*$_2$CH); 3.00–3.21 (*m*, $MeCH_2CH$, $NHCH_2$); 5.33–5.43 (br., CONH); 7.11–7.27 (*m*, 5 arom. H).

*(9*E*)*-N-*(4-Methylphenyl)octadec-9-enamide* (**7d**). Condensation proceeded according to the *GP* with *(*E*)-octadec-9-enoic acid* (**5d**; 0.322 g, 1.14 mmol), **4** (0.337 g, 1.14 mmol), DABCO (0.128, 1.14 mmol), and 4-toluidine (0.122 g, 1.14 mmol). After evaporation, **7d** was obtained (0.347 g, 93%). Oil ([29]: m.p. 42.5°). ^{1}H-NMR ($CDCl_3$): 0.95 (*t*, *J* = 6.5, Me); 1.12 (*s*, Me); 1.47–1.54 (*m*, 12 CH_2); 1.95–2.00 (*m*, C*H*$_2$CH = CHC*H*$_2$); 2.28–2.34 (*m*, CH = CH); 5.23–5.34 (*m*, NH); 7.09 (*d*, *J* = 8, 2 arom. H); 7.37 (*d*, *J* = 8, 2 arom. H).

Methyl N-*Benzoyl*-L-*phenylalaninate* (**7e**). Condensation proceeded according to the *GP* with *benzoic acid* (**5e**; 0.122 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), L-phenylalanine methyl ester hydrochloride (0.216 g, 1 mmol), and NMM (0.11 ml, 1 mmol). After evaporation, crude **7e** was obtained (0.257 g, 91%). HPLC (gradient): purity 85%. GC: t_R 18.42 (D-Phe), 18.71 min (L-Phe); % L/% D 97.9 : 2.1. M.p. 115–117° ([30]: 90–97°). ^{1}H-NMR($CDCl_3$): 3.26 (*t*, *J* = 5.5, C*H*$_2$Ph); 3.77 (*s*, MeO); 5.046–5.16 (*dd*, *J* = 5.5, C*H*CH_2Ph); 6.50–6.54 (br. *s*, NH); 7.12–7.30 (*m*, *Ph*CH_2); 7.38–7.54 (*m*, 3 H, *Ph*CONH); 7.70–7.74 (*m*, 2 H, *Ph*CONH).

Benzyl N-*Benzoyl*-L-*alaninate* (**7f**). Condensation proceeded according to the *GP* with **5e** (0.122 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), L-alanine benzyl ester 4-toluenesulfonate (0.351 g, 1 mmol), and NMM (0.11 ml, 1 mmol). After evaporation, crude **7f** was obtained (0.202 g, 71%). HPLC (gradient): purity 94%. GC: t_R 3.61 (D-Ala), 3.89 min (L-Ala); % L/% D 94.9 : 5.1. M.p. 55–57° ([31]: 97°). ^{1}H-NMR ($CDCl_3$): 1.53 (*d*, *J* = 7, Me); 4.85 (*q*, *J* = 7, MeC*H*); 5.21 (*s*, 1 H, CH_2); 5.22 (*s*, 1 H, CH_2); 6.77–6.79 (*m*, NH); 7.32–7.37 (*m*, *Ph*CH_2); 7.42–7.82 (*m*, *Ph*CONH).

Peptide Synthesis with **4** *as Coupling Reagent. Z-Leu-Phe-OMe* (**8a**; *Table*, *Entry 1*). Condensation proceeded according to the *GP* with Z-Leu-OH (0.265 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·PheOMe (0.216 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to afford crude **8a** (0.371 g, 87%). Oil ([32]: m.p. 87–88°). HPLC (gradient): purity 75%. GC: t_R 7.81 (D-Leu), 8.47 min (L-Leu); % L/% D 96.5 : 3.5; t_R 18.33 (D-Phe), 18.76 min (L-Phe); % L/% D 97.4 : 2.6. ^{1}H-NMR ($CDCl_3$): 0.84–0.93 (*m*, *Me*$_2$CH); 1.54–1.68 (*m*, Me_2CHCH_2); 1.71–1.88 (*m*, Me_2CHCH_2); 3.00–3.21 (*m*, $CHCH_2Ph$); 3.70 (*s*, MeO); 4.38–4.70 (*m*, C*H*CH_2Ph); 4.77–4.87 (*m*, Me_2CHCH_2CH); 5.09 (*s*, $PhCH_2O$); 5.25–5.38 (*m*, NH); 6.39–6.58 (*m*, CONH); 7.04–7.46 (*m*, 10 arom. H).

Z-Aib-Leu-OMe (**8b**; *Table*, *Entry 2*). Condensation proceeded according to the *GP* with Z-Aib-OH (0.237 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Leu-OMe (0.181 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to afford crude **8b** (0.301 g, 83%). HPLC (gradient): purity 96%. GC: t_R 3.22 (Aib), 7.77 (D-Leu), 8.44 min (L-Leu); % L/% D 99.0 : 1.0. M.p. 80–83° ([8]: 78–80°). ^{1}H-NMR ($CDCl_3$): 0.91 (*dd*, *J* = 6.5, *Me*$_2$CHCH$_2$); 1.53–1.55 (*s*, 2 Me); 1.57–1.68 (*m*, Me_2CHCH_2); 3.71 (*s*, MeO); 4.52–4.64 (*t*, *J* = 6, $CHCH_2CH$); 5.09 (*s*, OCH_2Ph); 5.32–5.35 (*m*, CONH); 6.69–6.77 (*m*, CONH); 7.33–7.36 (*m*, Ph).

Z-Aib-Phe-OMe (**8c**; *Table*, *Entry 3*). Condensation proceeded according to the *GP* with Z-Aib-OH (0.237 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Phe-OMe (0.216 g, 1 mmol), and

NMM (0.11 ml, 1 mmol) to give crude **8c** (0.301 g, 83%). HPLC (gradient): purity 93%. GC: t_R 3.23 (Aib), 18.21 (D-Leu), 18.49 min (L-Leu); % L/% D 99.6:0.4. M.p. 74–76° ([33]: 93–94°). ^{1}H-NMR ($CDCl_3$): 1.48 (*s*, Me_2C); 3.11 (*d*, *J* = 5.6, PhCH_2CH); 3.71 (*s*, MeO); 4.84 (*q*, *J* = 5.6, PhCH$_2$C*H*); 5.07 (*s*, PhCH_2O); 5.17–5.31 (*m*, CONH); 6.66–6.93 (*m*, CONH); 7.07–7.28 (*m*, *Ph*CH$_2$CH); 7.33–7.39 (*m*, *Ph*CH$_2$O).

Z-Aib-Aib-OMe (**8d**; *Table*, *Entry 4*). Condensation proceeded according to the *GP* with Z-Aib-OH (0.237 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Aib-OMe (0.154 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to furnish crude **8d** (0.255 g, 75%). M.p. 94–97° ([34]: 107–109°). HPLC (gradient): purity 87%. ^{1}H-NMR ($CDCl_3$): 1.49 (*s*, 4 Me); 3.76 (*s*, MeO); 5.08 (*s*, PhCH_2O); 5.22–5.27 (*m*, CONH); 6.86–6.91 (*m*, CONH); 7.28–7.36 (*m*, 5 arom. H).

Boc-Val-Aib-OMe (**8e**; *Table*, *Entry 5*). Condensation proceeded according to the *GP* with Boc-Val-OH (0.237 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Aib-OMe (0.154 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to yield crude **8e** (0.181 g, 57%). HPLC (gradient): purity 85%. GC: t_R 4.79 (D-Val), 4.99 min (L-Val); % L/% D 99.0:1.0; t_R 3.21 (Aib). M.p. 134–136° ([35]: 140°). ^{1}H-NMR ($CDCl_3$): 0.92 (*dd*, *J* = 6.8, Me_2CHCH); 1.42 (*s*, ^{t}Bu); 1.52 (*s*, Me_2C); 1.56–1.68 (*m*, Me_2C*H*CH); 3.70 (*s*, MeO); 3.77–3.85 (*m*, Me_2CHC*H*); 5.01–5.07 (*m*, CONH); 6.47–6.54 (*m*, CONH).

Boc-Val-Leu-OMe (**8f**; *Table*, *Entry 6*). Condensation proceeded according to the *GP* with Boc-Val-OH (0.237 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl Leu-OMe (0.182 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to affording crude **8f** (0.260 g, 75%). HPLC (gradient): purity 96%. GC: t_R 4.81 (D-Val), 5.04 min (L-Val); % L/% D 99.0:1.0; t_R 7.80 (D-Leu), 8.48 min (L-Leu); % L/% D 97:3. M.p. 115–117° ([36]: 121–124°). ^{1}H-NMR ($CDCl_3$): 0.92–0.99 (*m*, 2 Me_2CH); 1.45 (*s*, ^{t}Bu); 1.58–1.71 (*m*, Me_2C*H*CH$_2$); 2.09–2.23 (*m*, Me_2C*H*CH); 3.73 (*s*, MeO); 3.89 (*dd*, *J* = 9, Me_2CHC*H*); 4.58–4.67 (*m*, COCH); 4.96–5.11 (*m*, CONH); 6.11–6.25 (*m*, CONH).

Boc-Leu-Val-OMe (**8g**; *Table*, *Entry 7*). Condensation proceeded according to the *GP* with Boc-Leu-OH (0.231 g, 1mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl Val-OMe (0.168 g, 1 mmol),·and NMM (0.11 ml, 1 mmol) to yield crude **8g** (0.299 g, 87%). HPLC (gradient): purity 70%. GC: t_R 7.37 (D-Leu), 7.97 min (L-Leu); % L/% D 98.0:2.0; t_R 4.57 (D-Val), 7.76 min (L-Val); % L/% D 99.7:0.3. M.p. 112–115° ([37]: 116°). ^{1}H-NMR ($CDCl_3$): 0.89–0.96 (*m*, 4 Me); 1.44 (*s*, ^{t}Bu); 1.49–1.53 (*m*, Me_2C*H*CH$_2$); 1.61–1.64 (*m*, Me_2CHC*H*$_2$, Me_2C*H*CH); 3.74 (*s*, MeO); 4.03–4.16 (*m*, Me_2CHCH$_2$C*H*); 4.51–4.56 (*m*, CH); 4.85–4.88 (*m*, CONH); 6.52–6.58 (*m*, CONH).

Boc-Pro-Leu-OMe (**8h**; *Table*, *Entry 8*). Condensation proceeded according to the *GP* with Boc-Pro-OH (0.265 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Leu-OMe (0.181 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to give crude **8h** (0.245 g, 71%). HPLC (gradient): purity 97%. GC: t_R 10.41 min (L-Pro); % L/% D 100:0; t_R 7.81 (D-Leu), 8.46 min (L-Leu); % L/% D 98.1:1.9. M.p. 94–96° ([38]: 69–70°). ^{1}H-NMR ($CDCl_3$): 0.92 (*d*, *J* = 4.4, Me_2CH); 1.47 (*s*, ^{t}Bu); 1.54–1.75 (*m*, $(CH_2)_2$); 1.87–1.88 (*m*, Me_2CHC*H*$_2$CH); 2.09–2.37 (*m*, Me_2C*H*CH$_2$CH); 3.40–3.51 (*m*, CH_2); 3.72 (*s*, MeO); 4.20–4.33 (*m*, Me_2CHCH$_2$C*H*); 4.23–4.34 (*m*, CH); 4.50–4.67 (*m*, CONH); 6.36–6.44 (*m*, CONH).

Z-Phe-Aib-Leu-OMe (**8i**; *Table*, *Entry 9*). Condensation proceeded according to the *GP* with Z-Phe-Aib-COOH (0.384 g, 1 mmol), **4** (0.296 g, 1 mmol), DABCO (0.112, 1 mmol), HCl·Leu-OMe (0.182 g, 1 mmol), and NMM (0.11 ml, 1 mmol) to afford crude **8i** (0.470 g, 78%). HPLC (gradient): purity 91%. GC: t_R 18.18 (D-Phe), 18.54 min (L-Phe); % L/% D 99.2:0.8; t_R 3.22 (Aib), 7.69 min (D-Leu), 8.37 min (L-Leu); % L/% D 98.9:1.1. M.p. 96–98°. ^{1}H-NMR ($CDCl_3$): 0.92 (*d*, *J* = 6, Me_2CH); 1.35 (*s*, 3 H, Me_2C); 1.43 (*s*, 3 H, Me_2C); 1.51–1.71 (*m*, Me_2C*H*CH$_2$); 3.03 (*d*, *J* = 7.5, CHC*H*$_2$Ph); 3.71 (*s*, MeO); 4.25 (*q*, *J* = 7, Me_2CHCH$_2$C*H*); 4.51–4.50 (*m*, PhCH$_2$C*H*); 5.08 (*s*, CH_2Ph); 5.33 (*d*, *J* = 6, CONH); 6.04–6.06 (*m*, CONH); 6.83–6.89 (*m*, CONH); 7.18–7.39 (*m*, 10 arom. H).

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