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CF_3-BOP, CF_3-PyBOP and CF_3-HBTU: New peptide coupling reagents specially suited for α-aminoisobutyric acid condensations [a]

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Abstract. The peptide-coupling reagents CF_3-BOP (**1b**), CF_3-PyBOP (**1c**) and CF_3-HBTU (**2a**) with built-in 1-hydroxy-6-(trifluoromethyl)benzotriazole proved to be highly efficient for the coupling of α-aminoisobutyric acid residues.

Introduction [b]

The isolation of an increasing number of naturally occurring peptides bearing α-aminoisobutyric acid (Aib)[1] residues has stimulated the chemical synthesis of this type of biologically interesting peptides. However, it became apparent that introduction of Aib into peptides using well-established coupling reagents in solid-phase syntheses, *e.g.* dicyclohexylcarbodiimide/1-hydroxybenzotriazole and BOP[2] (**1a**), was a slow and low-yielding process[3]. In order to solve this problem much effort has been directed to the development of more powerful reagents[4].
We now wish to report that the CF_3-OBt-containing reagents CF_3-BOP (**1b**), CF_3-PyBOP (**1c**) and CF_3-HBTU (**2a**) are promising tools for the coupling of Aib.

Results and discussion

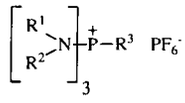
Some years ago,[5] we presented the bifunctional phosphorylating reagent *O*-(2-chlorophenyl)-*O*,*O*-bis(benzotriazol-1-yl)phosphate **3a** for the introduction of 3′ → 5′ internucleotide phosphotriester linkages. The rate of the two-step phosphorylation could be substantially enhanced by application of the CF_3 analogue **3b**[6]. On the basis of this information it was expected that the readily accessible CF_3-substituted reagents **1b,c** and **2a** would exert a beneficial effect on the introduction of the Aib moiety into peptides.
In order to assess this assumption, we first examined the coupling of isoleucine methyl ester with Z-protected Aib. It can be seen in entry 1 (Table I) that *in-situ* activation with both BOP (**1a**) and CF_3-BOP (**1b**) proceeded in an excellent yield. On the other hand, the yield of the CF_3-BOP-mediated coupling of the more hindered[7] proline methyl ester with Z-Aib-OH (entry 2) was much higher than with BOP. The difference in efficacy between the latter reagent and CF_3-BOP was even more pronounced in the peptide bond formation between Z-Aib-OH and H-Aib-OMe (entry 3). In this respect, it is interesting to note that *Coste* et al.[3b] used the highly active bromine-containing phosphonium salts BroP[8] (**1d**) and PyBroP[9] (**1e**) for the preparation of Z-Aib-Aib-OMe (**4c**). However, the coupling efficiency of reagents **1d,e** did not deviate substantially from those with BOP and its pyrrolidine homologue PyBOP[9] (**1f**). In addition, *Coste* et al.[3b] provided evidence that the (Py)BOP-mediated condensation of Z-Aib-OH with the sterically hindered Aib methyl ester proceeded via the benzotriazolyl (OBt) ester of the *N*-protected Aib. In order to substantiate the effect of OBt and CF_3-OBt esters on the formation of Z-Aib-Aib-OMe (**4c**), coupling was conducted after preactivation of the *N*-protected Aib with BOP and CF_3-BOP. It is evident from the results in entry 4 that the Aib dipeptide **4c**

[a] Dedicated to Professor *G.I. Tesser* on the occasion of his 65th birthday.
[b] **Abbreviations** are in accordance with the recommendations of the IUPAC-IUB as set forth in J. Biol. Chem. **260**, 14 (1985). Additional abbreviations:
Aib = α-aminoisobutyric acid
HOBt = 1-hydroxybenzotriazole
BOP = (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BroP = bromotris(dimethylamino)phosphonium hexafluorophosphate
Bz = benzoyl
CF_3-BOP = [6(trifluoromethyl)benzotriazol-1-yloxy]-tris(dimethylamino)phosphonium hexafluorophosphate
CF_3-HBTU = 2-[6(trifluoromethyl)benzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate
CF_3-PyBOP = [6(trifluoromethyl)benzotriazol-1-yloxy]tris(pyrrolidino)phosphonium hexafluorophosphate
DIEA = *N*,*N*-diisopropylethylamine
Fmoc = fluoren-9-ylmethoxycarbonyl
HATU = 2-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
= 2-(triazolo[4,5-*l*]pyridin-3-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
MeVal = *N*-methylvaline
PyBOP = (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate
PyBroP = bromotris(pyrrolidino)phosphonium hexafluorophosphate
Z = benzyloxycarbonyl.

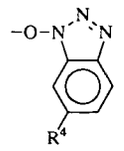
*Table I Relevant data on the formation of Aib-containing dipeptides with BOP (**1a**), CF_3-BOP (**1b**), CF_3-PyBOP (**1c**) and CF_3-HBTU (**2a**)*

Entry	Dipeptide		Yield (%) [a,b]			
			1a	**1b**	**1c**	**2a**
1	Z-Aib-Ile-OMe	**4a**	90	95	n.d. [c]	n.d. [c]
2	Z-Aib-Pro-OMe	**4b**	76	95	n.d. [c]	n.d. [c]
3	Z-Aib-Aib-OMe	**4c**	47	82	83	66
4	Z-Aib-Aib-OMe	**4c**	52 [c,d]	83 [c]	n.d. [e]	70 [f]
5	Z-Aib-Aib-OMe	**4c**	67 [g]	94 [g]	n.d. [e]	85 [g]
6	Fmoc-Aib-Aib-OMe	**4d**	51	86	82	73

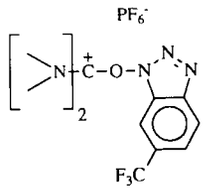
[a] The yields were determined after 1h coupling at room temperature (see Experimental section). [b] In case of incomplete reaction (entries 2–6) the sole side-product was the (CF_3-)OBt-ester of the *N*-protected amino acid. [c] Amino acid ester hydrochloride was added after 5 min preactivation. [d] Addition of CF_3-HOBt (0.5 eq), DIEA (0.5 eq) and amino acid hydrochloride (1.0 eq), after 5 min preactivation, furnished **4c** in 53% yield. [e] n.d. = not determined. [f] Amino acid ester was added after 20 min preactivation. The long preactivation time was necessary due to the low solubility of CF_3-HBTU in CH_2Cl_2. [g] DMF as solvent



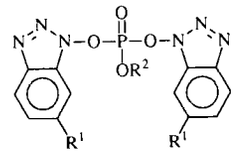
1 a: $R^1 = R^2 = CH_3$, R^3 = OBt (BOP)
b: $R^1 = R^2 = CH_3$, $R^3 = CF_3$-OBt (CF_3-BOP)
c: $R^1, R^2 = (CH_2)_4$, $R^3 = CF_3$-OBt (CF_3-PyBOP)
d: $R^1 = R^2 = CH_3$, R^3 = Br (BroP)
e: $R^1, R^2 = (CH_2)_4$, R^3 = Br (PyBroP)
f: $R^1, R^2 = (CH_2)_4$, R^3 = OBt (PyBOP)



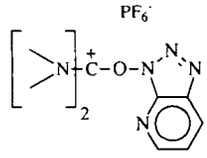
OBt: R^4 = H
CF_3-OBt: $R^4 = CF_3$



2 a: CF_3-HBTU



3 a: R^1 = H, R^2 = 2-chlorophenyl
b: $R^1 = CF_3$, R^2 = 2-chlorophenyl



5: HATU

could be isolated in comparable yields with those resulting from *in-situ* activation of Z-Aib-OH with BOP and CF_3-BOP, thus indicating that the acylation of an amine with decreased reactivity proceeds predominantly via the OBt or the more activated CF_3-OBt ester. Apart from this, it has been established[10] that the solvent DMF may significantly enhance the efficiency of peptide bond formation. The latter effect was confirmed by the near quantitative formation of **4c** (entry 5) using CF_3-BOP as the activating agent.

At this stage, we were anxious to find out whether the pyrrolidine analogue CF_3-PyBOP (**1c**) and the uronium derivative CF_3-HBTU (**2a**) would be as effective as CF_3-BOP. It can be seen in entry 3 and 6 that CF_3-PyBOP gave similar results in the synthesis of **4c** and **4d**. However, CF_3-HBTU-assisted condensation of two Aib residues (entries 3–6) gave lower yields than with CF_3-BOP. Interestingly, the recently introduced[11] and highly advocated[12] 7-azabenzotriazole-based reagent HATU (**5**) gave a lower yield (*i.e.* 69%) than CF_3-BOP in the coupling of Z-Aib-OH with H-Aib-OMe. The diminished efficacy of the uronium salts CF_3-HBTU and HATU might be attributed[13] to the *in-situ* generated strongly basic tetramethylurea.

Stimulated by the results thusfar obtained, we turned our attention to the rather troublesome[15] coupling of *N*-methylvaline (MeVal). In accordance with expectation, we found that both BOP- and CF_3-BOP-mediated condensation of valine methyl ester with Z-protected MeVal (entry 1 in Table II) gave the desired dipeptide **4e** in high yield. In contrast, reaction of Z-Val-OH with MeVal methyl ester (entry 2) proceeded unsatisfactorily, even at 35°C (entry 3)[16]. Nonetheless, it is clear that CF_3-BOP is also superior in this particular case to BOP (*cf.* the high-yielding CF_3-BOP-assisted couplings in entries 2–6 in Table I). On the other hand, acylation of *N*-methylated valine under the agency of BroP and PyBroP afforded higher yields than with (Py)BOP or CF_3-BOP[15].

Finally, it is of interest to note that loss of chiral integrity using CF_3-BOP (see note [e] in Table II) was, as gauged by the *Davies* test[17], in the same order as for BOP[18].

In conclusion, the results presented in this paper indicate that shelf-stable CF_3-BOP, CF_3-PyBOP and CF_3-HBTU are efficient coupling reagents for the introduction of Aib residues. The application of these promising condensing reagents in the solid-phase synthesis of Aib-containing peptides will be reported in due course.

Experimental

General methods and materials

DMF was stirred with CaH_2 and distilled under reduced pressure. CH_2Cl_2 was distilled from P_2O_5 and stored over molecular sieves (4

*Table II Relevant data on the formation of dipeptides with BOP (**1a**) and CF_3-BOP (**1b**)*

Entry	Dipeptide		Yield (%) [a]	
			1a	**1b**
1	Z-MeVal-Val-OMe	**4e**	92	95
2	Z-Val-MeVal-OMe [b,c]	**4f**	4	32
3	Z-Val-MeVal-OMe [b,c]	**4f**	12 [d]	49 [d]
4	Bz-Val-Val-OMe [e]	**4g**	32	46

[a] The yields were determined after 1h coupling at room temperature (see Experimental section). [b] The sole side-product was the (CF_3-)OBt-ester of Z-Val-OH. [c] After 1 h, hydrazine monohydrate (0.1 ml, 2 mmol) was added to facilitate purification. [d] Conducted at 35°C. [e] %DL diastereoisomer: 53% and 50%, for BOP and CF_3-BOP, respectively.

Å). DIEA was distilled from KOH. BOP reagent was purchased from Richelieu Biotechnologies. HATU was purchased from Millipore. Fmoc-Aib-OH was obtained from NovaBiochem. MeVal was prepared according to a procedure described by *McDermott* and *Benoiton*[19]. Reactions were run at ambient temperature unless noted otherwise. TLC analysis was performed on Schleicher and Schüll DC Fertigfolien F1500 LS254 employing the following solvent systems: A (ethyl-acetate/light-petroleum-(b.p. 40–60°C), 1/1, v/v), B (ethyl-acetate/light-petroleum-(b.p. 40–60°C), 1/2, v/v). Compounds were visualized by UV (254 nm) and 4,4'-methylenebis(*N*,*N*-dimethyl-aniline) reagent[20]. Column chromatography was performed on Kieselgel 60, 230–100 mesh (Merck). Melting points were measured on a Büchi melting-point apparatus and are uncorrected. Optical rotations were determined with a PROPOL automatic polarimeter at 20°C. Mass spectra were obtained with a Finnigan MAT SSQ 710 (Finnigan MAT, San José) spectrometer equipped with an electro-spray interface. 1D- and 2D-(^{1}H–^{1}H COSY) ^{1}H-NMR spectra were recorded at 300 MHz on a Bruker WM-300 spectrometer interfaced with an ASPECT 2000 computer. ^{13}C- and ^{31}P-NMR spectra were recorded on a Jeol JNM-FX 200 spectrometer, operating at 50.1 and 80.7 MHz, respectively. Chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si for ^{1}H, and to the signal for internal chloroform (δ 77.0) or acetone (δ 29.8) for ^{13}C. ^{31}P chemical shifts are given relative to 85% H_3PO_4 as external standard.

[(6-Trifluoromethyl)benzotriazol-1-y-oxy]tris(dimethylamino)phosphonium hexafluorophosphate, CF_3-BOP (**1b**). A solution of 1-hydroxy-6-(trifluoromethyl)benzotriazole[21] (1.02 g, 5.0 mmol) and triethylamine (0.70, 5.0 mmol) in CH_2Cl_2 (10 ml) was carefully added to a mixture of chlorotris(dimethylamino)phosphonium hexafluorophosphate[22] (1.72 g, 5.0 mmol) in acetone (25 ml) at 0°C. After stirring for 30 min at room temperature, the mixture was concentrated and acetone (40 ml) was added followed by the addition of water (200 ml) upon which the phosphonium salt precipitated. The white precipitate was collected by filtration and redissolved in CH_2Cl_2 (100 ml). The solution was washed with water (2×30 ml), dried ($MgSO_4$) and concentrated to give **1b** (2.25 g, 88%) as a white solid which was used without further purification. The obtained phosphonium salt, recrystallized from CH_2Cl_2/light-petroleum-(b.p. 40–60°C), decomposes at $T >$ 140°C. MS (*m*/*z*): 365 [**1b**-PF_6]$^+$. ^{31}P NMR (acetone-d_6): δ 45.9 (*s*, P^+), −141.1 (septet, PF_6^-, $J_{P,F}$ 710.5 Hz). ^{13}C NMR (acetone-d_6): δ 144.9, 128.1 (C_q), 132.2 (q, *C*-CF_3, $^2J_{C,F}$ 32.7 Hz), 124.3 (q, C-*C*F_3, $^1J_{C,F}$ 272.6 Hz), 123.4, 123.3, 123.0, 107.8, 107.7 (CH-arom.), 37.7 (CH_3-N, $^2J_{C,P}$ 4.4 Hz).

[(6-Trifluoromethyl)benzotriazol-1-yloxy]tris(pyrrolidino)phosphonium hexafluorophosphate, CF_3-PyBOP (**1c**). Prepared as described for the preparation of **1b** starting from bromotris(pyrrolidino)-phosphonium hexafluorophosphate[23] (3.60 g, 7.7 mmol); yield 3.13 g (69%). Phosphonium salt **1c**, recrystallized from CH_2Cl_2/light-petroleum-(b.p. 40–60°C), decomposes at $T >$ 140°C. MS (*m*/*z*): 258 [O=P-$(N(CH_2)_4)_3$+H]$^+$, 443 [**1c**-PF_6]$^+$, 515 [2O=P-$(N(CH_2)_4)_3$+H]$^+$. ^{31}P NMR (acetone-d_6): δ 33.1 (s, P^+), −141.1 (septet, PF_6^-, $J_{P,F}$ 708.8 Hz). ^{13}C NMR (acetone-d_6): δ 145.0, 128.2 (C_q), 132.1 (q, *C*-CF_3, $^2J_{C,F}$ 32.7 Hz), 124.5 (q, C-*C*F_3, $^1J_{C,F}$ 272.6 Hz), 123.4, 123.1, 108.0 (CH-arom.), 49.0 (CH_2-*C*H_2-N, $^2J_{C,P}$ 4.4 Hz), 26.6 (*C*H_2-CH_2-N, $^3J_{C,P}$ 8.8 Hz).

2-[6(Trifluoromethyl)benzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate, CF_3-HBTU (**2a**). Prepared starting from tetramethylchloroformamidinium hexafluorophosphate[24] (1.40 g, 5.0 mmol) in an analogous method to that used for **1b**; yield 1.74 g (78%). Uronium salt **2a**, recrystallized from acetone/CH_2Cl_2, decomposes at $T >$ 160°C. MS (*m*/*z*): 302 [**2a**-PF_6]$^+$. ^{31}P NMR (acetone-d_6): δ −141.2 (septet, PF_6^-, $J_{P,F}$ 708.5 Hz). ^{13}C NMR (acetone-d_6): δ 151 (C^+), 136.3, 134.2 (C_q), 129.4 (q, *C*-CF_3, $^2J_{C,F}$ 33.7 Hz), 124.3 (q, C-*C*F_3, $^1J_{C,F}$ 272.6 Hz), 130.6, 116.9, 115.0 (CH-arom.), 42.8, 42.4 (CH_3).

General procedure for BOP, CF_3-PyBOP- and CF_3-HBTU-mediated dipeptide formation

To a mixture of Z-amino acid (0.50 mmol), amino acid methyl ester hydrochloride (0.50 mmol) and either BOP (220 mg, 0.50 mmol), CF_3-BOP (255 mg, 0.50 mmol), CF_3-PyBOP (293 mg, 0.50 mmol) or CF_3-HBTU (224 mg, 0.50 mmol) in CH_2Cl_2 (3 ml) was added DIEA (0.26 ml, 1.50 mmol). After 1 h, the mixture was diluted with ethyl acetate (100 ml), washed with water (2×30 ml), 1M $NaHCO_3$ (2×30 ml), water (2×30 ml), 1M $KHSO_4$ (2×30 ml), brine (2×30 ml), dried ($MgSO_4$) and concentrated. When needed, purification of the residue by column chromatography (eluent: light-petroleum-(b.p. 40–60°C)/ethyl-acetate, 2/1 → 1/1, v/v) gave **4a–g**.

N-[α-(Benzyloxycarbonylamino)isobutyryl]-L-isoleucine methyl ester, Z-Aib-Ile-OMe (**4a**). R_f 0.75 (system A); $[\alpha]_D$ +18.2° (*c* 1, $CHCl_3$). MS (*m*/*z*): 365 [M+H]$^+$, 387 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$): δ 7.4–7.3 (m, 5H, H-arom.), 6.86 (bd, 1H, NH Ile, $J_{H\alpha NH}$ 6.4 Hz), 5.41 (s, 1H, NH Aib), 5.09 (AB, 2H, CH_2 Z), 4.56 (dd, 1H, Hα Ile, *J* 4.7 and 8.5 Hz), 3.70 (s, 3H, OCH_3), 1.9 (m, 1H, Hβ Ile), 1.55 and 1.52 (2 s, each 3H, Hβ Aib), 1.4–1.3 and 1.2–1.1 (2 m, each 1H, Hγ Ile), 0.89 (t, 3H, Hδ Ile, $J_{\gamma,\delta}$ 7.3 Hz), 0.87 (d, 3H, Hγ Ile, $J_{\beta,\gamma}$ 6.9 Hz). ^{13}C NMR ($CDCl_3$): δ 174.0, 172.0 (C=O Aib, Ile), 154.8 (C=O Z), 136.0 (C_q, Z), 128.1, 127.7, 127.6 (CH-arom.), 66.3 (CH_2 Z), 56.6 (Cα Aib), 56.2 (Cα Ile), 51.6 (OCH_3), 37.4 (Cβ Ile), 25.5 (Cβ Aib), 24.7 (Cγ Ile), 15.1 (β-CH_3 Ile), 11.2 (Cδ Ile).

N-[α-(Benzyloxycarbonylamino)isobutyryl]-L-proline methyl ester, Z-Aib-Pro-OMe (**4b**). R_f 0.19 (system A); $[\alpha]_D$ −63.8° (*c* 1, $CHCl_3$). MS (*m*/*z*): 349 [M+H]$^+$, 371 [M+Na]$^+$. ^{13}C NMR ($CDCl_3$): δ 172.7, 171.9 (C=O Aib, Pro), 154.1 (C=O Z), 136.3 (C_q Z), 128.1, 127.9, 127.8 (CH-arom.), 66.0 (CH_2 Z), 60.4 (Cα Pro), 56.4 (Cα Aib), 51.7 (OCH_3), 47.5 (Cδ Pro), 27.4 (Cβ Pro), 25.4 (Cγ Pro), 24.7, 24.1 (Cβ Aib).

N-(N-Benzyloxycarbonyl-α-aminoisobutyryl)-α-aminoisobutyric acid methyl ester, Z-Aib-Aib-OMe (**4c**). R_f 0.18 (system B). MS (*m*/*z*): 337 [M+H]$^+$, 359 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$): δ 7.4–7.3 (m, 5H, H-arom.), 6.92 and 5.42 (2 s, each 2H, NH), 5.10 (s, 2H, CH_2 Z), 3.71 (s, 3H, OCH_3), 1.50 (s, 12H, Hβ). ^{13}C NMR ($CDCl_3$): δ 174.8, 173.5 (C=O Aib), 155.0 (C=O Z), 136.2 (C_q Z), 128.3, 127.9, 127.8 (CH-arom.), 66.5 (CH_2 Z), 56.7, 56.2 (Cα), 52.3 (OCH_3), 25.2, 24.3 (Cβ).

N-(N-Fluoren-9-ylmethoxycarbonyl-α-aminoisobutyryl)-α-aminoisobutyric acid methyl ester, Fmoc-Aib-Aib-OMe (**4d**). R_f 0.41 (system A). MS (*m*/*z*): 425 [M+H]$^+$, 447 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$): δ 7.75 and 7.59 (2 d, 4H, H-arom., *J* 7.4 and 7.5 Hz), 7.6–7.3 (m, 4H, H-arom.), 6.90 and 5.50 (2 s, each 1H, NH Aib), 4.40 (d, 2H, CH_2 Fmoc, *J* 6.6 Hz), 4.20 (t, 1H, CH Fmoc, *J* 6.8 Hz), 3.71 (s, 3H, OCH_3), 1.53 (s, 12H, Hβ). ^{13}C NMR ($CDCl_3$): δ 174.8, 173.5 (C=O Aib), 155.0 (C=O Fmoc), 143.7, 141.2 (C_q Fmoc), 127.5, 126.9, 124.9, 119.8 (CH-arom.), 66.4 (CH_2 Fmoc), 56.7, 56.3 (Cα), 52.4 (OCH_3), 47.1 (CH Fmoc), 25.1, 24.4 (Cβ).

N-[N(Benzyloxycarbonyl)-L-N-methylvalyl]valine methyl ester, Z-MeVal-Val-OMe (**4e**). R_f 0.54 (system B). $[\alpha]_D$ −93.4° (*c* 1, MeOH); lit.[15] $[\alpha]_D$ −95° (*c* 1, MeOH). MS (*m*/*z*): 379 [M+H]$^+$, 401 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$): δ 7.34 (bs, 5H, H-arom.), 6.53 and 6.01 (d, and 'bs', ratio 4/1, 1H, NH, $J_{H\alpha NH}$ 8.3 Hz), 5.17 (AB, 2H, CH_2 Z), 4.49 (dd, 1H, Hα Val, $J_{\alpha,\beta}$ 5.7 Hz, $J_{H\alpha NH}$ 8.7 Hz), 4.15 and 4.05 (d and 'bd', ratio 4/1, 1H, Hα MeVal, $J_{\alpha,\beta}$ 11.1 Hz), 3.72 (s, 3H, OCH_3), 2.90 (s, 3H, NCH_3), 2.3 (m, 1H, Hβ MeVal), 2.1 (m, 1H, Hβ Val), 0.96 and 0.89 (2 d, each 3H, Hγ MeVal, $J_{\beta,\gamma}$ 6.4 Hz), 0.83 and 0.80 (2 d, each 3H, Hγ Val, $J_{\beta,\gamma}$ 6.8 Hz). ^{13}C NMR ($CDCl_3$): δ 171.7, 169.9 (C=O MeVal, Val), 157.2 (C=O Z), 136.3 (C_q Z), 128.2, 127.7, 127.4 (CH-arom.), 67.2 (CH_2 Z), 64.9, 56.6 (Cα MeVal, Val), 51.7 (OCH_3), 30.6, 29.6 (Cβ MeVal, Val), 25.8 (NCH_3), 19.2, 18.6, 18.4, 17.3 (Cγ MeVal, Val).

N-[N(Benzyloxycarbonyl)-L-valyl]-L-N-methylvaline methyl ester, Z-Val-MeVal-OMe (**4f**). R_f 0.52 (system B). $[\alpha]_D$ −110.0° (*c* 1, MeOH); lit.[15] $[\alpha]_D$ −63° (*c* 1, MeOH). MS (*m*/*z*): 347 [M+H-CH_3OH]$^+$, 379 [M+H]$^+$, 401 [M+Na]$^+$. ^{13}C NMR ($CDCl_3$): δ 173.0, 171.1 (C=O MeVal, Val), 156.3 (C=O Z), 136.3 (C_q Z), 128.4, 127.9, 127.7 (CH-arom.), 66.7 (CH_2 Z), 65.0, 61.4, 55.8, 55.6 (Cα MeVal, Val), 51.7 (OCH_3), 31.4, 31.2 (Cβ MeVal, Val), 27.4, 26.9 (NCH_3), 19.7, 19.1, 18.6, 17.4 (Cγ MeVal, Val).

N-(N-Benzoylvalyl)valine methyl ester, Bz-Val-Val-OMe (**4g**) *(diastereoisomeric mixture).* MS (*m*/*z*): 335 [M+H]$^+$, 357 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$): δ 7.9–7.8 and 7.5–7.4 (2 m, 5H, H-arom.), 7.2–7.1 (m, 2H, NH), 4.8–4.7 and 4.6–4.5 (2 m, each 1H, Hα), 3.75 and 3.67 (2 s, 3H, OCH_3 LL and DL form respectively), 2.1–2.3 (m, 2H, Hβ), 1.0–0.9 (m, 12H, Hγ). ^{13}C NMR ($CDCl_3$): δ 172.0, 171.8, 171.7, 171.5 (C=O Val), 167.3 (C=O Bz), 131.5, 128.4, 127.1 (CH-arom.), 58.6, 57.4 (Cα), 51.9 (OCH_3), 31.4, 30.9, 30.7 (Cβ), 19.3, 19.1, 19.0, 18.9, 18.3, 18.1, 17.9, 17.7 (Cγ).

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