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PyBOP® [1] AND PyBroP: TWO REAGENTS FOR THE DIFFICULT COUPLING OF THE α,α-DIALKYL AMINO ACID, Aib.

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Summary The difficult coupling of α-aminoisobutyric acid (Aib) was carried out using PyBOP® and PyBroP in a comparative study with BOP and BroP These reagents gave good results under simple conditions (one pot, r t , 1 h) Coded amino acids could be coupled with Aib using PyBOP under standard conditions of peptide synthesis without racemization whereas the coupling of two Aib residues required PyBroP/DMAP A fragment containing an Aib C-terminal could be coupled without epimerization of the penultimate residue

Introduction

Aib (α-aminoisobutyric acid) is a noncoded amino acid present in peptaibol antibiotics, which are isolated from fungi[2] and have a capacity to form voltage-dependent ion channels in bilayer membranes[3] The introduction of Aib into a peptide can result in modifications that are of interest conformationally (formation of α or 3_{10} helices, or ß-turns)[4] and pharmacologically[5] However, the introduction of Aib into a peptide remains difficult[6-11] The classic reagent DCC (or DCC/HOBt) gives mediocre yields despite long reaction times[12,13] Other methods have been used, i e mixed anhydrides[6,7], active esters[12,14], Aib oxazolone[7], but none of them gives consistently satisfactory results Coupling reagents, such as 2-chloro 4,6-dimethoxy-1,3,5-triazine[15] or diethyl phosphorobromidate[16] have been used in rare cases

An original method using an azirine as the synthetic equivalent of Aib has recently been developed[11] Specifically, it amounts to coupling with the Aib amine, which, after two steps, produces a peptide having an acidic Aib C-terminal Further coupling with this carboxyl must be carried out using classical methods[11] involving the limitations described above

Because of the difficulty of this coupling and the presence of many Aib residues in peptaibol sequences, these compounds have only rarely been synthesized, and in these cases in solution[13,17,18] For the same reasons, SPPS (solid-phase peptide synthesis) is unsuitable, although it has been used to obtain alamethicin[10]

We recently developed new peptide coupling reagents of the BOP[19] family PyBOP® **2**, BroP **3**, and PyBroP **4**. PyBOP[20] **2** gives results that are at least as good as BOP, and it avoids the use and formation of carcinogenic HMPA BroP[21] **3** et PyBroP[22] **4** give better results than reagents **1** and **2** when coupling N-methylated amino acids The results we have obtained when coupling N-methylated amino acids with BroP and PyBroP, as well as the corresponding differences in reactivity observed between reagents **1** and **2**, containing the oxybenzotriazole residue, and the brominated reagents **3** and **4**, led us to study their behavior during Aib coupling In the present paper, we report results obtained using reagents **1** to **4** in Aib coupling during the synthesis of di- and tripeptides

Results and Discussion

I/ Yields

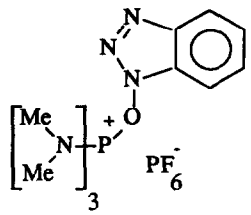
In a first series of reactions (table I, compounds **5-11**), the four reagents **1** to **4** gave high yields (80 to 95% in 1 h of reaction) even with hindered residues (compounds **6-11**) The results were identical whether Aib was in the C- or N-terminal position Moreover, there were no differences among the four reagents A second series of reactions (compounds **12-15**) involved the difficult coupling of two Aib residues The decrease in reactivity is particularly important with the bulky Boc protective group using

TABLE I: Aib COUPLINGS WITH 1-4 REAGENTS[a,b]

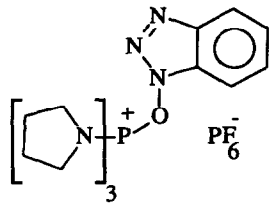
PEPTIDE	BOP	PyBOP	BroP	PyBroP	Literature		
	Yield%	Yield%	Yield%	Yield%	Ref	Yield%	Conditions
5 Z-Aib-Gly-OEt	92	87	89	87	12	55	DCC (for Me ester)
6 Z-Aib-Val-OMe	88	-	87	84	12	85	AE/Imidazole
7 Z-Val-Aib-OMe	-	-	90	-			
8 Z-Aib-Pro-OtBu	82	-	84	95[c]	14	5	AE/Imidazole
					17	80	DCC/12h (for Me ester)
9 Boc-Pro-Aib-OMe	-	88	-	83	18	78	MA/1h(-15°C),1h(-5°C)
10 Z-Aib-Ile-OMe	-	80	-	-			
11 Boc-Ile-Aib-OMe	-	83	-	-			
12 Z-Aib-Aib-OMe	-	89(16 h)	-	77(16h)	7	64	DCC/2 days
				80[c,d]	14	5	AE/Imidazole
					6	89	MA/3h(60°C),12h(rt)
13 Boc-Aib-Aib-OMe	80(16h)	86(16h)	25(24 h)	25(24 h)	18	54	MA/3h(60°C),12h(rt)
			76[d]	77[d]			
15 Boc-Aib-Aib-OBzl	-	-	65[d]	61[d]	23	~0	BOP-Cl
					13	63	DCC/HOBt/2eq./2 days
16 Z-Aib-Benzocaine	30(24h)	-	66[d](2h)	65[d](2h)	24	0	CDI/60h
					24	53	MA/30h

[a]Reactions for 1h at room temperature, except where otherwise stated. [b]See list of abbreviations[1]. [c]Obtained with 20% PyBroP excess. [d]With DMAP

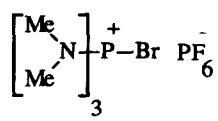
BroP and PyBroP (compound **13** 25%, 24 h) However, with the two latter reagents, catalysis with DMAP



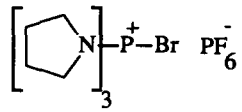
BOP **1**



PyBOP® **2**



BroP **3**



PyBroP **4**

made it possible to obtain good yields in only 1 h of reaction, although synthesis of Boc-Aib-Aib-OBzl **15** remained difficult even with DMAP catalysis For coupling of Z-Aib with a very weakly nucleophilic amine, benzocaine (p-NH_2-Ph-COOEt), the use of BroP(PyBroP)/DMAP made it possible to obtain 66% (65%) amide **16** in 2 h

Comparison of these results with those in the literature shows that reagents **1-4** are clearly more effective than DCC with respect to yield (compound **5**), and reaction time (compounds **8,12,15**) Compared to active esters in the presence of imidazole, the results are either similar (compound **6**) or better (compounds **8,12**) The same observation is also true for mixed anhydride methods (compounds **9,12,16** and **13**) With regard to other peptide coupling reagents diethyl phosphobromidate[16] gives results (compound **12** 85%, 2 h) comparable to those we obtained Dipeptide **15** could not be obtained with BOP-Cl[23], which was also true of CDI in the case of compound **16** [24] Nevertheless, our results were not as good as those obtained by the azirine method For example, Z-Aib-Aib-OH is obtained in two steps with 91% yield[25] whereas PyBroP/DMAP yielded 80% of the corresponding methyl ester **12** Similarly, Z-Val-Aib-OH is obtained with 95% yield[11] as opposed to 90% Z-Val-Aib-OMe **7** with BroP It should be noted, however, that in the azirine method, the precursor has to be prepared beforehand

The reagents we tested showed certain advantages Compounds **2**, **3** and **4** are, like BOP, stable solids that are easy to handle Moreover, PyBOP and PyBroP are now commercially available[26] The reactions can be carried out one pot, at room temperature C-protected amino acids can be coupled in the form of a free amine (compound **8**), chlorhydrate (compounds **5**, **6**, **7**, etc), tosylate (compound **15**), or trifluoroacetate (compound **18**) In the case of BroP (and BOP) HMPA is eliminated during the work-up in the acid washes of the reaction mixture With PyBOP and PyBroP, tris(pyrrolidino)phosphine oxide remaining in the crude product is easily eliminated by rapid chromatography on silica gel

II/ Epimerization

It has been demonstrated that when a urethane-protected amino acid is coupled with PyBOP[20], there is no epimerization Moreover, when N-methylated amino acids are coupled with BroP and PyBroP, epimerization is undetectable by ^{1}H-NMR at 360 MHz[21,22,27]

In the case of Aib, coupling with PyBOP also occurred without epimerization (Table II) The absence of diastereoisomer **24** (eq 3) during the synthesis of tripeptide **20** (eq 1), as measured by HPLC, showed that the coupling of Z-L-Leu with Aib-OAllyl (eq 1, compound **18**) did not involve racemization Similarly, the chirality of Z-L-Leu was preserved during coupling with Aib-Pro-OtBu (eq 2, compound **20**)

On the other hand, slight epimerization did occur when coupling with BroP and PyBroP, resulting in compounds **18** (eq. 1) and **20** (eq 2) (Table II). Moreover, epimerization was measurable by ^{1}H-NMR during the coupling of Z-Ile with Aib-benzocaine (eq 4, compound **26**) Signals related to α protons of L-Ile and the epimerization product D-allo-Ile are separate[28]. Thus, epimerization was evaluated at 0 5% with BroP and was not detectable with PyBroP

We considered it of interest to study the coupling of a peptide fragment containing an Aib at the C-terminal position In peptide synthesis, fragment coupling that extends the peptide in the direction N → C

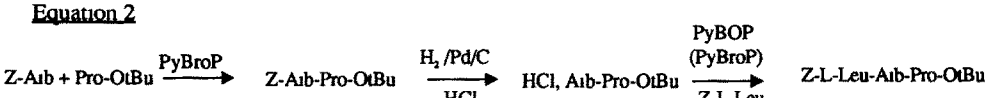
TABLE II % EPIMER BY STEP TO STEP COUPLING

Coupling	Compound	PyBOP	BroP	PyBroP	Method
Z-Leu + Aib-OAllyl	**18**	0*	1 5	-	HPLC
Z-Leu + Aib-Pro-OtBu	**20**	0*	-	1	HPLC
Z-Ile + Aib-Benzocaine	**26**	-	0.5	0**	NMR

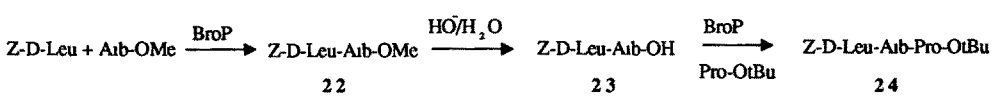
*not detectable on HPLC **not detectable on ^{1}H NMR

Equation 1

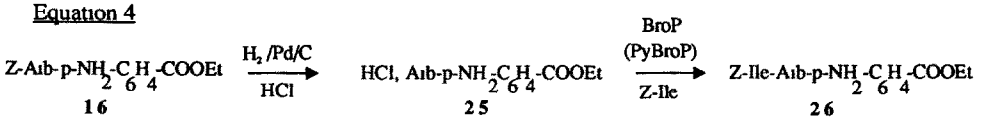
Equation 2



Equation 3



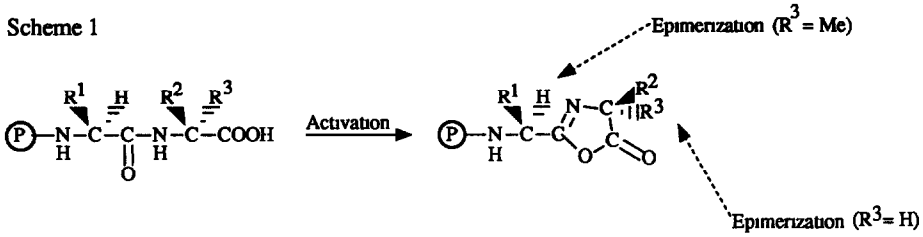
Equation 4



is used only rarely (except with Gly and Pro C-terminals) because it results in epimerization in the intermediate oxazolone (scheme 1 $R^3 = H$)

If an Aib carboxylate is activated (Scheme 1 $R^2 = R^3 = CH_3$), this epimerization mechanism does not occur and fragment coupling would appear to be advantageous since it allows convergent syntheses However, Heimgartner[28] and Bruckner[29] have shown that in this case the penultimate amino acid may be epimerized Heimgartner obtained as much as 50% epimer when coupling Z-Ile-Aib with benzocaine using

Scheme 1



DCC/HOBt Chirality was preserved when the reaction was carried out in the presence of 10-camphorsulfonic acid at 0°C Bruckner proposed a rule he called the "do's and don'ts of Aib peptide-coupling" the coupling of a P-Xxx-Aib fragment is formally forbidden if the chirality of the amino acid Xxx is to be preserved

We studied the coupling of the fragment Z-Leu-Aib with Pro-OtBu (eq 1), a group present in alamethicin This coupling falls into the category of "don'ts" in Bruckner's rule This author obtained as much as 49 3% epimer while preparing the oxazolone of peptides terminated by Leu-Aib[29], which shows the capacity of the Leu fragment to epimerize according to the mechanism considered here

TABLE III Z-Leu-Aib + Pro-OtBu COUPLING

Reagent	Yield % (2h reaction)	% Epimer (HPLC)
PyBOP	96	0*
PyBroP	74	0*
PyBroP/DMAP	79(1h)	0*
DCC	86	0*
DCC/HOBt	85	0*

*not detectable on HPLC

The results in Table III show that epimerization was not detectable under any of the conditions studied This was verified by HPLC in comparison with the diastereoisomer Z-D-Leu-Aib-Pro-OtBu **24** The absence of epimerization with PyBOP and PyBroP (even in the presence of DMAP) led us to carry out the same coupling with DCC and DCC/HOBt Here again, the chirality of the Leu residue was preserved These results are in agreement with the low percentages, i e 0 7% D-Leu and 0 6% D-Val, present in the alamethicin synthesized by Schmitt and Jung[18] who coupled Aib-terminated fragments with these amino acids in the penultimate position Although it is likely that oxazolone is involved during fragment coupling with Aib C-terminal activation[17,24], we do not consider that epimerization of the penultimate residue can occur under the usual conditions of peptide coupling

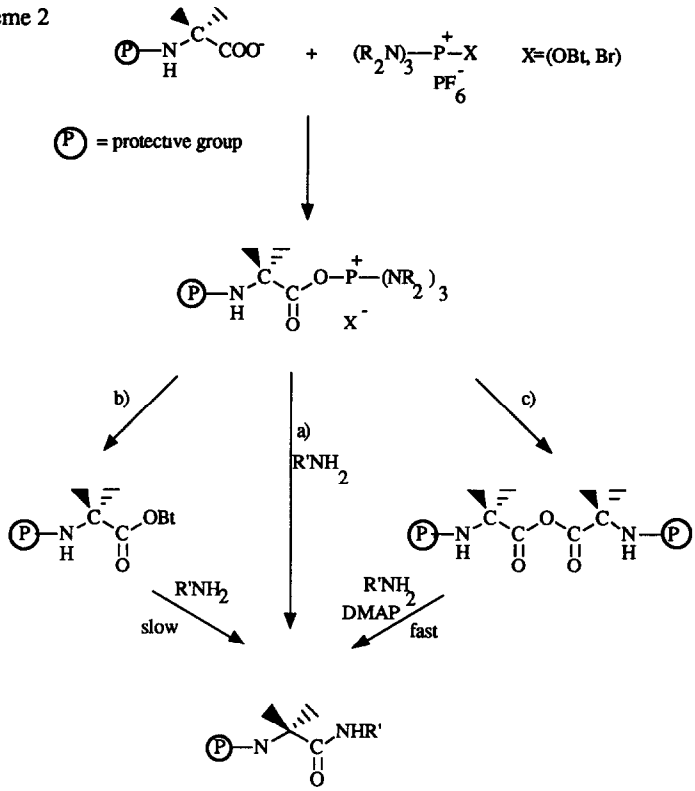
III/ Mechanism

The very difficult couplings (compounds **12, 13, 15, 16**) are of particular interest in terms of the mechanism involved In the cases of Z- and Boc-Aib couplings with Aib-OMe (compounds **12,13**) using BOP or PyBOP, thin layer chromatography revealed the immediate formation of an intermediate that slowly disappeared in favor of the dipeptide This kind of intermediate was obtained (TLC) by the action of PyBOP on Boc-Aib Its ^{1}H-NMR spectrum showed it to be the oxybenzotriazole ester of Boc-Aib **14** During the coupling of Z-Aib with benzocaine (compound **16**), the oxybenzotriazole ester of Z-Aib was also formed It reacted even more slowly because of the low nucleophilicity of the amine

In the general case of BOP coupling, we have proposed[30] the following mechanism. an acyloxyphosphonium salt is first formed, which is aminolyzed by the C-protected amino acid (Scheme 2,

pathway a) The results given above show that in the case of Aib, if the steric hindrance is large (compounds **12, 13**) or the amine is weakly nucleophilic (compound **16**), the anion $^-$OBt reacts with the acyloxyphosphonium salt to produce the oxybenzotriazole ester (Scheme 2, pathway b) The latter then reacts slowly to yield the expected product This result is perfectly comparable with those obtained in the coupling of two hindered N-methylated amino acids[21]

Scheme 2



For difficult couplings of Aib with BroP and PyBroP, a pathway via a symmetric anhydride can be considered (Scheme 2, pathway c) These reactions are markedly accelerated by DMAP, a reagent known to catalyze the aminolysis of anhydrides (It has also been shown that BroP can be used to prepare anhydrides[31]) This result differs from those obtained[21,22] with hindered N-methylated amino acids for which DMAP catalysis was unnecessary

Conclusion:

The results obtained and the practical advantages reported here indicate that reagents **1-4** are very suitable for Aib coupling No difference was found between BOP and PyBOP or BroP and PyBroP However, in view of the toxicity of HMPA, PyBOP and PyBroP may be preferable. PyBOP provides good results in most cases and produces peptides that are free of epimerization In the case of difficult coupling involving two Aib residues, the reaction is slow with PyBOP, but it can be carried out with PyBroP in the presence of DMAP The results obtained with these two reagents suggest that they can be used in solid-phase synthesis of peptaibols Studies on this subject are now in progress in our laboratory

Experimental Section

Melting points are uncorrected ^{1}H NMR spectra were recorded on a Bruker WM-360 instrument at 360 MHz Optical rotations were taken at 20°C on a Schmidt & Haensch Polartronic D apparatus and are at ± 1° Elemental analyses were obtained from the "Service Central d'Analyse du CNRS" FAB mass measurements were done by the "Laboratoire de mesures physiques", USTL, Montpellier TLC were performed on silica gel GF254 aluminium sheets (0 2 mm thick; Merck) Column chromatographies were performed using silica gel (0 063-0 200 mm, Merck) Analytical HPLC was carried out on a Beckman System Gold When ^{1}H NMR and elemental analyses are not given, the products are identical to those previously described in the literature

Coupling methods

BOP, PyBOP, BroP and PyBroP. General procedure: 2 mmol of DIEA (3 mmol if amine salt was present) were added to a solution of 1 mmol N-protected acid component , 1 1 mmol of the C-protected amino acid (or amine salt) and 1 mmol coupling reagent in 1 ml of CH_2Cl_2 (filtered on Alumina) The reaction was stirred at r t for 1 h (except where otherwise stated), evaporated, redissolved in 20 ml of EtOAc and washed 3 x with 5% $KHSO_4$, brine, 3 x 5% $NaHCO_3$, brine, dried on Na_2SO_4, filtered and evaporated All dipeptides obtained were purified by column chromatography on silica gel using hexane/EtOAc (except when racemization was studied)

Boc-Aib was synthesized from Aib (3 g) with 3 eq Boc-F (tert-butyl fluorocarbonate, 50%wt in monoglyme, a gift from Propeptide) in 1N NaOH/dioxan (100 ml) at pH=9,5 Dioxan was removed *in vacuo*, the solution acidified, and the product extracted from EtOAc, washed 3x with brine, dried on Na_2SO_4, evaporated Yield 69% M p =121-122°C (lit [12] m p = 118°C) Rf = 0 4 (hexane EtOAc, 50 50 1%AcOH)

HCl.H-Aib-OMe was synthesized according to Boissonnas et al [29] From Aib (4 g, 39mmol) Yield 93% . M p = 179-182°C (lit [12] m p = 179-183°C)

Tos.H-Aib-OBzl: A solution of Aib (2 g, 19 mmol), APTS H_2O (3 75 g, 19 mmol) and benzylalcohol (8 ml) in toluene (20 ml) was azeotropically refluxed for 16 h. The colourless solid was filtered and washed with ether Yield 78% M p = 152-153°C (lit [12] m p = 154-156°C)

Z-Aib-Gly-OEt (5).

With BOP. Z-Aib-OH (0 474 g, 2 mmol), HCl Gly-OEt (0 307 g, 2 2 mmol), (0 884 g, 2 mmol) BOP and DIEA (1 04 ml, 6 mmol) in CH_2Cl_2 (2 ml) Column chromatography (hexane EtOAc, 1 1) Yield 0 57 g (92%). Oil Rf = 0 5 (hexane EtOAc, 1 1) ^{1}H NMR ($CDCl_3$) δ 1 25 (3H, t, J = 7 1 Hz, O CH_2CH_3), 1 52 (6H, s, CH_3 Aib), 3 97 (2H, d, J = 4 9 Hz, CH_2 Gly), 4 18 (2H, q, J = 4 1 Hz, OCH_2CH_3), 5 07 (2H, s, CH_2 Z), 5 35 (1H, s, NH Aib), 6 79 (1H, broad s, NH Gly), 7 26-7 35 (5H, m, C_6H_5 Z) Anal calc for $C_{16}H_{22}N_2O_5$ 1/2 H_2O %C = 57 99, %H = 7 00, %N = 8 45 Found %C = 58 00, %H = 6 85, %N = 8 53

With PyBOP. From Z-Aib-OH (2 mmol) Yield 87%

With BroP. From Z-Aib-OH (0 5 mmol) Yield 89%

With PyBrOP. From Z-Aib-OH (4 mmol) Yield 87%

Z-Aib-Val-OMe (6):

With BOP: Z-Aib-OH (0 237 g, 1 mmol), HCl H-Val-OMe (0 184 g, 1 1 mmol), BOP (0 442 g, 1 mmol) and DIEA (0 52 ml, 3 mmol) in CH_2Cl_2 (1 ml) Column chromatography (hexane EtOAc, 60 40) Yield 0 294 g (88%) M p = 74°C (lit [12] m p = 100-102°C [1]) $[\alpha]_D$= -3° (c=1, EtOH) Rf = 0 55 (hexane EtOAc, 60 40) ^{1}H NMR ($CDCl_3$) δ 0 83 (3H, d, J = 6 8 Hz, CH_3 Val), 0 90 (3H, d, J = 6 4 Hz, CH_3 Val), 1 51 (3H, s, CH_3 Aib), 1 55 (3H, s, CH_3 Aib), 2 09-2 18 (1H, m, H-β Val), 3 70 (3H, s, OCH_3), 4 99 (1H, dd, J = 4 9 Hz, J = 8.8 Hz, H-α Val), 5 06-5 10 (2H, AB, J = 12 2 Hz, CH_2 Z), 5 28 (1H, broad s, NH Aib), 6 79 (1H, broad, NH Val), 7 27-7 37 (5H, m, C_6H_5 Z) Anal calc for $C_{18}H_{26}N_2O_5$ %C = 61 70, %H = 7 48, %N = 7 99. Found %C =61 90, %H =7 33, %N = 7 79

With BroP. From Z-Aib-OH (0 5 mmol) Yield 87 %

With PyBroP: From Z-Aib-OH (2 mmol) Yield 84%

Z-Val-Aib-OMe (7):

With BroP. Z-Val-OH (0 251 g, 1 mmol), HCl H-Aib-OMe (0 151 g, 1 1 mmol), BroP (0 388 g, 1 mmol) and DIEA (0 52 ml, 3 mmol) in CH_2Cl_2 (1 ml) Column chromatography (hexane EtOAc, 60 40) Yield 0 31 g (90%) M p = 83-84°C $[\alpha]_D$ = 24° (c=1, EtOH) ^{1}H NMR (DMSO d_6) δ 0.83 (3H, d, J = 6 8 Hz, CH_3 Val), 0 86 (3H, d, J = 6 8 Hz, CH_3 Val), 1 32 (3H, s, CH_3 Aib) , 1 34 (3H, s, CH_3 Aib), 1 84-1 98 (1H, m, H-β Val), 3.53 (3H, s, OCH_3), 3 85 (1H, dd, J~9 Hz, H-α Val), 5 03 (3H, s, CH_2 Z), 7 10 (1H, d, J = 9 3 Hz, NH Val), 7 27-7 38 (5H, m, C_6H_5 Z), 8 21 (1H, s, NH Aib) Anal calc for $C_{18}H_{26}N_2O_5$ %C = 61 70, %H = 7 48, %N = 7 99 Found %C = 61 23, %H = 7 56, %N = 7 91

Z-Aib-Pro-OtBu (8):

With BOP Z-Aib-OH (1 896 g, 8 mmol), H-Pro-OtBu (1 505 g, 8 8 mmol), BOP (3 54 g, 8 mmol) and

DIEA (4 6 ml, 27 mmol) in CH_2Cl_2 (8 ml). Column chromatography (hexane· EtOAc,40 60) Recrystallized from MeOH/H_2O Yield 2 65g (82%) M.p.= 105°C Rf = 0 3 (hexane EtOAc, 50 50) $[\alpha]_D$ = -91° (c=1,EtOH) ^1H NMR (DMSO d_6): δ 1.30 (6H, s, CH_3 Aib), 1.35 (9H, s, tBu), 1.62-1 75 (2H, m, H-β Pro), 1 75-1.96 (2H, m, H-γ Pro), 3.22-3 34 (1H, m, H-δ Pro), 3.60 (1H, broad, H-δ Pro), 4 09 (1H, broad d, H-α Pro), 4.96, 5 05 (2H, AB, J = 14.4 Hz, CH_2 Z), 7.27-7.38 (5H, m, C_6H_5 Z), 7.70 (1H, s, NH Aib) Anal. calc for $C_{21}H_{30}O_5N_2$ %C = 64 62, %H = 7.69, %N = 7 18 Found %C = 64.80, %H = 8 14, %N=7.24.

With BroP: From Z-Aib-OH (1 mmol). Yield: 84%.

With PyBroP: From Z-Aib-OH (10 mmol), PyBroP (12 mmol). Yield 95%

Boc-Pro-Aib-OMe (9):

With PyBOP. Boc-Pro-OH (0.43 g, 2 mmol), HCl H-Aib-OMe (0 337 g, 2 2 mmol), PyBOP (1 04 g, 2 mmol) and DIEA (1 04 ml, 6 mmol) in CH_2Cl_2 (2 ml). Column chromatography (hexane:EtOAc; 1.1). Yield· 88%. M p = 78°C (lit.[18] m p = 80°C); Rf = 0 4 (hexane EtOAc, 50 50) $[\alpha]_D$= -56° (c=1,EtOH) Anal calc for· $C_{15}H_{26}N_2O_5$ %C = 57 31, %H = 8 34, %N = 8 91 Found: %C = 57 14; %H = 8 53, %N = 8.89.

With PyBroP. From Boc-Pro-OH (2 mmol) Yield 83%

Z-Aib-Ile-OMe (10):

With PyBOP: Z-Aib-OH (0.474 g, 2 mmol), HCl H-Ile-OMe (0 399 g, 2 2 mmol), PyBOP (1 04 g, 2 mmol) and DIEA (1 04 ml, 6 mmol) in CH_2Cl_2 (2 ml) Column chromatography (hexane EtOAc, 50 50). Yield. 80 % M p = 48-50°C. Rf = 0 4 (hexane.EtOAc, 1 1) $[\alpha]_D$= +2° (c=1,EtOH) ^1H NMR ($CDCl_3$) δ 0.85 (3H, d, J = 6 8 Hz, CH_3 Ile), 0 87 (3H, t, J = 7 3 Hz), 1.04-1.16 (1H, m, H-γ Ile), 1 31-1 43 (1H, m, H-γ Ile), 1 50 (3H, s, CH_3 Aib), 1 53 (3H, s, CH_3 Aib), 1 81-1 92 (1H, m, H-β Ile), 3 68 (3H, s, OCH_3), 4 53 (1H, dd, J = 4 4 Hz, J = 8 3 Hz, H-α Ile), 5 06, 5 08 (2H, AB, J = 12 7 Hz, CH_2 Z), 5 31 (1H, broad s, NH Aib), 6 81 (1H, broad, NH Ile), 7 25-7 48 (5H, m, C_6H_5 Z) Anal calc for $C_{19}H_{28}O_5N_2$ %C = 62 64, %H = 7 69, %N = 7 69 Found· %C = 62.91, %H = 7 63; %N = 7 72

Boc-Ile-Aib-OMe (11)

With PyBOP. Boc-Ile-OH (0 462 g, 2 mmol), HCl H-Aib-OMe (0 338 g, 2 2 mmol), PyBOP (1 04 g, 2 mmol) and DIEA (1 04 ml, 6 mmol) in CH_2Cl_2 (2 ml) Column chromatography (hexane.EtOAc, 1 1) Yield 83% m p = 168°C. $[\alpha]_D$= -28° (c=1, EtOH) Rf = 0 8 (hexane EtOAc, 50 50). ^1H NMR ($CDCl_3$) δ 0 88 (3H, t, J = 7 3 Hz, CH_3 Ile), 0 90 (3H, d, J = 6 8 Hz, CH_3 Ile), 1 04-1 17 (1H, m, H-γ Ile), 1.42 (9H, s, CH_3 Boc), 1.40-1 50 (1H, H-γ Ile), 1 507 (3H, s, CH_3 Aib), 1 513 (3H, s, CH_3 Aib), 1.77-1 88 (1H, t, H-β Ile), 3.69 (3H, s, OCH_3), 3 85 (1H, ~t, J ~ 7 3 Hz, H-α Ile), 5 00 (1H, broad, NH Ile), 6.49 (1H, s, NH Aib) Anal calc for $C_{16}H_{30}N_2O_5$ %C = 58 18, %H = 9 09, %N = 8 48 Found %C = 58 54, %H = 9 32, %N = 8 44

Z-Aib-Aib-OMe (12) :

With PyBOP. Z-Aib-OH (0 237 g, 1 mmol), HCl H-Aib-OMe (0 169 g, 1 1 mmol), PyBOP (0 52 g, 1 mmol) and DIEA (0 52 ml, 3 mmol) in CH_2Cl_2 (1 ml) TLC monitoring showed immediate formation of one product (Rf = 0 4, hexane EtOAc, 70·30) which disappeared slowly in favor of **12** Reaction time 16 h Column chromatography (hexane EtOAc, 60 40) Yield 89% M p = 106-108°C (lit [6] m.p = 107-109°C) Rf = 0 35 (hexane EtOAc, 60 40)

With PyBroP. From Z-Aib-OH (1 mmol) Reaction time 16 h Yield 77%

With PyBroP/DMAP: Z-Aib-OH (0 4587 g, 1 935 mmol), HCl H-Aib-OMe (0 327 g, 2 13 mmol), PyBroP (1 082 g, 2 32 mmol = 1 2 eq), DIEA (0 8 ml, 2 4 eq), DMAP (0 142 g, 1 161 mmol = 0 6 eq) in CH_2Cl_2 (2 ml) Reaction time 1 h Yield 80%

Boc-Aib -Aib-OMe (13):

With BOP: Boc-Aib-OH (0 203 g, 1 mmol), HCl H-Aib-OMe (0 169 g, 1 1 mmol), BOP (0 442 g, 1 mmol) and DIEA (0.51 ml, 3 mmol) in CH_2Cl_2 (1 ml) Immediate formation of **14** (TLC) which disappeared slowly in favor of **13** Reaction time 16 h Column chromatography (hexane EtOAc, 1 1) Yield 80% M p = 91-92°C (lit [18] m p = 86-87°C) Rf = 0 4 (hexane EtOAc, 1 1)

With PyBOP. From Boc-Aib-OH (2 mmol) Immediate formation of **14** Reaction time 16 h Yield 86%

With BroP. From Boc-Aib-OH (1 mmol) Reaction time 24 h Yield 25%

With PyBroP. From Boc-Aib-OH (1 mmol) Reaction time. 24 h Yield 25%

With BroP/DMAP. Boc-Aib-OMe (0 203 g, 1 mmol), HCl H-Aib-OMe (0 169 g, 1 1 mmol), BroP (0 388 g, 1 mmol), DIEA (0.408 ml, 2 4 mmol) and DMAP (0 073 g, 0 6 mmol) in CH_2Cl_2 (1 ml) Reaction time 1 h Column chromatography (hexane.EtOAc, 1 1) Yield 76%

With PyBroP/DMAP. Boc-Aib-OH (0 322 g, 1 583 mmol), HCl-H-Aib-OMe (0 2673 g, 1.741 mmol), PyBroP (0 885 g, 1 899 mmol), DIEA (0 65 ml, 3 8 mmol = 2 4 eq) and DMAP (0 116 g, 0 94 mmol = 0 6 eq) in CH_2Cl_2 (1 6 ml) Reaction time 1h Column chromatography (hexane EtOAc, 1·1) Yield 77%

Boc-Aib-OBt (14): Boc-Aib-OH (0 203 g, 1 mmol), BOP (0 442 g, 1 mmol) and DIEA (0 34 ml, 2 mmol) in CH_2Cl_2 (1 ml) were stirred at R T TLC (revealed at 254 nm) showed immediate formation of one

product. After 30 min, CH_2Cl_2 was evaporated, the residue was dissolved in EtOAc, washed with $KHSO_4$ 5%, brine, dried on Na_2SO_4, filtered, evaporated. Yield of crude product: 0.24 g (oil). Rf = 0.65 (hexane:EtOAc; 1:1) . ^1H NMR ($CDCl_3$): δ 1.46 (s) and 1 5(s) (9H, Boc); 1 71 (6H, s, CH_3 Aib); 5 35 (1H, broad s, NH Aib); 7 3-8.1 (4H, OBt).

Boc-Aib-Aib-OBzl (15):

With BroP/DMAP: Boc-Aib-OH (0 812 g, 4 mmol), Tos Aib-OBzl (1 608 g, 4.4 mmol), BroP (1 552 g, 4 mmol), DIEA (1.63 ml, 9 6 mmol = 2.4 eq) and DMAP (0 293 g, 2 4 mmol = 0 6 eq) in CH_2Cl_2 (4 ml) Column chromatography (hexane·EtOAc, 1 1) Yield 65% M p = 121°C (lit.[13]: m p. = 121-122°C) Rf = 0.6 (hexane.EtOAc, 1:1)

With PyBroP/DMAP: From Boc-Aib-OH (1 mmol) Yield 61%

Z-Aib-Benzocaïne (16):

With BOP. Z-Aib-OH (0.474 g, 2 mmol), Benzocaine (0 363 g, 2 2 mmol), BOP (0.884 g, 2 mmol) and DIEA (0 68 ml, 4 mmol) in CH_2Cl_2 (2 ml) TLC monitoring showed immediate formation of one product (Rf = 0 4, hexane EtOAc, 70:30) which reacted slowly to give **16** Reaction time. 24 h. Work-up as general procedure Yield of crude product· 1.1 g TLC still showed the same product, benzocaine and **16** ^1H NMR ($CDCl_3$). about 30% of **16**; numerous signals at δ = 7 2-8 ppm revealed the presence of Z-Aib oxybenzotriazolyl ester.

With BroP/DMAP. Z-Aib-OH (3.92 g, 16 5 mmol), Benzocaine (2 48 g, 15 mmol), BroP (6 40 g, 16.5 mmol), DIEA (2.8 ml, 16.5 mmol) and DMAP (2 02 g, 16 5 mmol) in CH_2Cl_2 (15 ml) Reaction time· 2 h Column chromatography (hexane. EtOAc; 60:40). Yield 66% M p.= 126-7°C (lit.[24]: m p = 131.0-131.2°C). Rf = 0.4 (hexane:EtOAc; 60:40).

With PyBroP/DMAP. From Z-Aib-OH (1 mmol). Reaction time· 2h Yield. 65%

Z-L-Leu-Aib-Pro-OtBu (eq.1):

Tfa.H-Aib-OAllyl (17):

a) Boc-Aib-O-Allyl : Boc-Aib-OH (6 7 g, 33 mmmol) were dissolved in DMF (15 ml); allyl bromide (2 86 ml, 33 mmol) and Cesium carbonate (5 376 g, 16.5 mmol) were successively added and the reaction mixture stirred for 2 h at room temperature CsBr was filtered and the solvent removed *in vacuo*. The crude product was dissolved in CH_2Cl_2, washed with water, dried on Na_2SO_4, evaporated and recrystallized from MeOH/H_2O Yield 79% M.p = 50-51°C Rf = 0 7 (hexane EtOAc, 70·30) ^1H NMR (DMSO d_6) δ 1.32 (6H, s, CH_3 Aib), 1 35 (9H, s, CH_3 Boc), 4 51 (2H, d, J = 5 4 Hz, O-CH_2-CH=CH_2), 5 18 (1H, d, J = 10.8 Hz, O-CH_2-CH=CH_2), 5 30 (1H, dd, J = 17.1 Hz, J = 1 9 Hz, O-CH_2-CH=CH_2), 5.87 (1H, m, O-CH_2-CH=CH_2), 7 22 (1H, broad s, NH Aib) Anal calc for $C_{12}H_{21}NO_4$ %C = 59 24; %H = 8.70, %N = 5 76 Found: %C = 59 22; %H = 8.71; %N = 5.83

b) Tfa-Aib-O-Allyl Boc-Aib-OAllyl (6 g, 24 69 mmol) was treated with trifluoroacetic acid (12 35 ml) for 35 minutes Excess of TFA was removed *in vacuo* and the product crystallized from ether/hexane Yield 88% M p = 65-68°C. ^1H NMR (DMSO d_6) δ 1 48 (6H, s, CH_3 Aib) , 4.71 (2H, dt, J= 5 9 Hz, J = 1 4 Hz, O-CH_2-CH=CH_2), 5.28 (1H, dt, J = 11 0 Hz, J = 1.2 Hz, O-CH_2-CH=CH_2), 5.36 (1H, dt, J = 19 0 Hz, J = 1 4 Hz, O-CH_2-CH=CH_2), 5 94 (1H, m, O-CH_2-CH=CH_2), 8 51 (3H, NH_3^+ Aib). Anal. calc for $C_9H_{14}NO_4F_3$ %C = 42.02 ; %H = 5 45 ; %N = 5 45. Found: %C = 42 00, %H = 5.50; %N=5 23

1) Epimerization study with PyBOP .

Z-L-Leu-Aib-O-Allyl (18): 17 (3.115 g, 12.1 mmol), Z-L-Leu-OH (3.57 g, 13.4 mmol), PyBOP (6 41 g, 12.3 mmol), DIEA (6.2 ml, 36.3 mmol) in CH_2Cl_2 (12 ml) Column chromatography (hexane·EtOAc; 70:30). Yield: 87%. Rf = 0.3 (hexane EtOAc ; 70.30) M p. = 51-52°C. $[\alpha]_D$ = -25°. ^1H NMR (DMSO d_6): δ 0.85 (3H, d, J = 6.8 Hz, CH_3 Leu), 0.87 (3H, d, J = 6.8 Hz, CH_3 Leu), 1 34 (3H, s, CH_3 Aib), 1 37 (3H, s, CH_3 Aib), 1 32-1.38 (2H, m, H-β Leu), 1.62 (1H, m, H-γ Leu), 4 06 (1H, ~q, J ~ 8.8 Hz, H-α Leu), 4.46 (2H, d, J = 4 9 Hz, O-CH_2-CH=CH_2), , 5 02 (2H, s, CH_2 Z), 5.15 (1H, J = 10.7 Hz, O-CH_2-CH=CH_2), 5.27 (1H, J = 17.6 Hz, O-CH_2-CH=CH_2), 5.83 (1H, m, O-CH_2-CH=CH_2), 7.24 (1H, d, J =8 8 Hz, NH Leu), 7.28-7.40 (5H, m, C_6H_5 Z), 8.21 (1H, s, NH Aib). Anal calc. for $C_{21}H_{30}N_2O_5$: %C = 64 60 ; %H = 7 74, %N = 7.17 , Found %C = 64 69 , %H = 7.72 , %N = 7 13.

Z-L-Leu-Aib-OH (19): 18 (4.1 g, 10 5 mmol) and morpholin (2 75 ml, 31.5 mmol) in THF (200 ml) were stirred for 5 minutes in the presence of tetrakis-(triphenylphosphine)palladium(0) (1 21 g, 1 05 mmol) according to [33] THF was evaporated, the yellow product dissolved in EtOAc, washed 3 x with 5% $KHSO_4$, extracted with 5% $NaHCO_3$, the aqueous solution was acidified with 6N HCl and extracted with CH_2Cl_2 The product was crystallized from Et_2O/hexane Yield 63% M p =113-115°C $[\alpha]_D$= -25° (c=1, EtOH) ^1H NMR (DMSO d_6) δ 0.85 (3H, d, J = 6 4 Hz, CH_3 Leu), 0 87 (3H, d, J = 6.3 Hz, CH_3 Leu), 1 32 (3H, s, CH_3 Aib), 1 34 (3H, s, CH_3 Aib), 1 37-1 46 (2H, m, H-β Leu), 1 54-1.71 (1H, m, H-γ Leu), 4 05 (1H, ~q, J ~ 8 8 Hz, H-α Leu), 5 02 (2H, s, CH_2 Z), 7 26 (1H, d, J = 8 3 Hz, NH Leu), 7.28-7 40 (5H, m, C_6H_5 Z), 7 97 (1H, s, NH Aib), 12.1 (1H, broad s, COOH). Anal calc for $C_{18}H_{26}N_2O_5$· %C = 61 70, %H = 7 48, %N = 7 99 Found· %C = 61 39; %H = 7 26, %N = 8.08.

Z-L-Leu-Aib-Pro-OtBu (20): 19 (0 175 g, 0 5 mmol), HCl H-Pro-OtBu (0 114 g, 0 55 mmol), PyBOP (0.26 g, 0 5 mmol) and DIEA (0.264 ml, 1.5 mmol) in CH_2Cl_2 (0 5 ml) Reaction time: 2 h. Yield 96% The crude product contained no D-Leu diastereoisomer **24** (Yield and epimerization were studied by HPLC see below)

2) Epimerization study with BroP:

Z-L-Leu-Aib-OAllyl (18): 17 (5.02 g, 20 mmol), Z-L-Leu-OH (5 83 g, 22 mmol), BroP (7 76 g, 20 mmol) and DIEA (10.2 ml, 60 mmol) in CH_2Cl_2 (20 ml) Column chromatography (hexane·EtOAc, 70:30) Yield 74% M p = 49-52°C. $[\alpha]_D$= -25° (c=1, EtOH)

Z-L-Leu-Aib-OH (19): 18 (3.0 g, 7 7 mmol), morpholin (2 01 ml, 23 1 mmol) in THF (150 ml) and tetrakis-(triphenylphosphine)palladium(0) (0 92 g, 0 8 mmol) according to [33] Yield· 74%. M p.= 115°C $[\alpha]_D$= -25° (c=1,EtOH)

Z-L-Leu-Aib-Pro-OtBu (20): From **19** (0 5 mmol) with PyBOP Reaction time 1 h Yield 81 % % diastereoisomer **24**: 1 5 % (Yield and epimerization were studied by HPLC see below)

Z-L-Leu-Aib-Pro-OtBu (eq.2):

HCl.H-Aib-Pro-OtBu (21): 8 (2 5 g, 6 4 mmol) from PyBroP, Pd/C (0 5 g) and concentrated HCl (0 534 ml) in MeOH (60 ml) were stirred under H_2 for 1 h 45 After filtration, MeOH was evaporated and the product crystallized from MeOH/ether. Yield. 87%. M p = 160°C (decomposition) $[\alpha]_D$= -77° (c=1, EtOH) ^{1}H NMR (DMSO d_6). δ 1 38 (9H, s, CH_3 tBu), 1 56 (3H, s, CH_3 Aib), 1 59 (3H, s, CH_3 Aib), 1 72-1 82 (1H, m, Pro), 1.84-1 98 (2H, m, Pro), 2 07-2 17 (1H, m, Pro), 3 65 (2H, m, H-δ Pro),4 25 (1H, m, H-α Pro), 8 41 (3H, s, NH_3^+ Aib) Anal calc for $C_{13}H_{24}N_2O_3Cl$· %C = 53.51, %H = 8 29, %N = 9 60, %Cl = 12 15 Found. %C = 53.71, %H = 8 59, %N = 9 56, %Cl = 11 87

1) Epimerization study with PyBOP:

Z-L-Leu-Aib-Pro-OtBu (20): 21 (0 585 g, 2 mmol), Z-L-Leu-OH (0 584 g, 2 2 mmol), PyBOP (1 144 g, 2 mmol) and DIEA (1 02 ml, 6 mmol) in CH_2Cl_2 (2 ml) The crude product contained no Z-D-Leu-Aib-Pro-OtBu **24** (HPLC· see below) Column chromatography (hexane EtOAc, 1·1) Yield: 81% M p = 150-152°C $[\alpha]_D$ = -89° (c=0 7, EtOH) Rf =0 40 (hexane EtOAc, 50.50) (lit [11]· M p = 144-145°C, $[\alpha]_D$ = -82 2 (c=0.7; EtOH)). ^{1}H NMR (DMSO d_6) δ 0 86, 0 88 (6H, 2d, J = 7 4 Hz, CH_3 Leu), 1.29, 1 31, 1 36 (15H, 3s, CH_3 Aib, tBu), 1.39-1.50 (2H, m, H-β Leu), 1 54-1 87 (5H, 2m, H-β Pro, H-γ Pro, H-γ Leu), 3 49 (2H, m, H-δ Pro), 4.07 (2H, m, H-α Pro, H-α Leu), 4 97, 5 06 (2H, AB, J = 12.6 Hz, CH_2Z), 7 26-7 46 (6H, m, C_6H_5Z, NH Leu), 8 24 (1H, s, NH Aib)

2) Epimerization study with PyBroP:

Z-L-Leu-Aib-Pro-OtBu (20): 21 (0 585 g, 2 mmol), Z-Leu-OH (0 584 g, 2 2 mmol), PyBroP (1 03 g, 2 2 mmol) and DIEA (1.02 ml, 6 mmol) in CH_2Cl_2 (2 ml) The crude product contained 1% Z-D-Leu-Aib-Pro-OtBu **24** (for HPLC assay see below) Column chromatography (hexane EtOAc, 1 1) Yield 77% M p = 149-151°C $[\alpha]_D$ = -89° (c=0 7, EtOH)

Z-D-Leu-Aib-Pro-OtBu (24) (eq.3):

Z-D-Leu-Aib-OMe (22): Z-D-Leu-OH (4 1 g, 15 47 mmol), HCl H-Aib-OMe (3 4 g, 22 mmol), BroP (7 76 g, 20 mmol) and DIEA (10 2 ml, 60 mmol) in CH_2Cl_2 (20 ml) Column chromatography (hexane.EtOAc, 70 30) Yield 75% M p = 86-87°C $[\alpha]_D$ = +25° (c=1, EtOH) Anal calc for $C_{19}H_{28}N_2O_5$ %C = 62 64 , %H = 7 74 , %N = 7 64 Found %C = 62 31 , %H = 7 86 , %N = 7 69

Z-D-Leu-Aib-OH (23): 22 (2 07 g, 5 68 mmol) were stirred with 2N NaOH (5 7 ml) in MeOH (6 5 ml) for 1h The reaction mixture was then solved in EtOAc (100 ml), the carboxylate extracted with H_2O, acidified with 1N HCl and finally extracted with CH_2Cl_2 Yield 72% M p = 110-111°C $[\alpha]_D$ = +25° (c=1, EtOH)

Z-D-Leu-Aib-Pro-OtBu (24): 23 (0 35 g, 1 mmol), H-Pro-OtBu (0 197 g, 1 1 mmol) , BroP (0 388 g, 1 mmol) and DIEA (0 34 ml, 2 mmol) in CH_2Cl_2 (1 ml) Reaction time 1 h The crude product contained 1 3% Z-L-Leu-Aib-Pro-OtBu **20** (HPLC see below) Column chromatography (hexane EtOAc, 60 40) Yield 70% The product still contained 0 3% D-isomer after purification M p = 150-151°C $[\alpha]_D$= -36° (c=1, EtOH) Rf = 0 36 (hexane EtOAc, 50 50) ^{1}H NMR (DMSO d_6) δ 0 85 (3H, d, J = 7 4 Hz, CH_3 Leu), 0 87 (3H, d, J = 6 6 Hz, CH_3 Leu),1 32 (3H, s, CH_3 Aib), 1 34 (3H, s, CH_3 Aib), 1 36 (9H, s, tBu), 1 30-1 54 (2H, broad, H-β Leu), 1 54-2 03 (5H, 3 m, H-β Pro, H-γ Pro, H-γ Leu), 3 44 (2H, m, H-δ Pro), 4 06 (2H, m, H-α Pro, H-α Leu), 5 00, 5 06 (2H, AB, J = 12 5 Hz, CH_2Z), 7 27-7 39 (6H, m, C_6H_5Z, NH Leu), 7 95 (1H, s, NH Aib) Anal calc for $C_{27}H_{42}N_3O_6$ %C = 64 26, %H = 8 39, %N= 8 33 Found %C = 64 58, %H = 8 58, %N = 8 34.

Z-Ile-Aib-Benzocaine (eq.4):

HCl.H-Aib-Benzocaine (25): 16 (1 921 g, 5 mmol), concentrated HCl (0 625 ml, 7 5 mmol) and Pd-C (150 mg) in MeOH (40 ml) were allowed to react with H_2 The product was recrystallized from

MeOH/Et_2O Yield. 90% M p. = 146-150°C (decomposition) ^1H NMR ($CDCl_3$): δ 1.31 (3H, t, J =6 9 Hz, OCH_2CH_3), 1.66 (6H, s, CH_3 Aib), 4 29 (2H, q, J = 6 9 Hz, OCH_2CH_3), 7 88, 7 95 (4H arom , AA'BB', J = 8 1 Hz), 8 49 (3H , broad s, NH_3^+ Aib), 10 56 (1H, broad s, NH benzocaine) Anal calc for $C_{13}H_{19}N_2O_3Cl$· %C = 54 45; %H = 6 68, %N = 9 77, %Cl = 12.36 Found %C = 54 14, %H = 6 71, %N = 9 47, %Cl = 12 31.

Z-Ile-Aib-Benzocaïne (26):

With BroP. Z-Ile-OH (0 265 g, 1 mmol), **25** (0 321 g, 1 1 mmol), BroP (0 388 g, 1 mmol) and DIEA (0 51 ml, 3 mmol) in CH_2Cl_2 (1 ml) Reaction time· 1 h The crude product contained ~0 5% Z-D-allo-Ile-Aib-Benzocaine (NMR) Column chromatography (hexane EtOAc,1 1) Yield 60% M p = 79-80°C $[\alpha]_D$= +46° (c=1 1,EtOH) (lit.[11] m.p.= 78 8-79 5°C, $[\alpha]_D$ = 46 4° (c=1 1, EtOH)) Rf = 0 5 (hexane.EtOAc, 1 1) ^1H NMR ($CDCl_3$) showed· H-α Ile (δ 3 81, t, J = 6 5 Hz) and 0 5% H-α D-allo-Ile (δ 3 95) (see [28])

With PyBroP. Z-Ile-OH (0.3076 g, 1.159 mmol), **25** (0 3008 g, 1 049 mmol), PyBroP (0 7458 g, 1 6 mmol) and DIEA (0 54 ml, 3.15 mmol) in CH_2Cl_2 (1 05 ml) Reaction time 1 h The crude product contained no D-diastereoisomer (NMR). Column chromatography (hexane·EtOAc, 60 40) Yield 60% M p = 77-79°C $[\alpha]_D$ = +47° (c=1 1, EtOH) Rf = 0.4 (hexane : EtOAc , 60 40) ^1H NMR ($CDCl_3$) showed H-α Ile (δ 3 81, t, J = 6 5 Hz) and no signal corresponding to D-allo-Ile isomer (δ 3.95 ppm) (see [28]) The recording conditions made it possible to confirm that D-isomer was <0 2%

Z-L-Leu-Aib-Pro-OtBu (20): Fragment coupling (table 3):

Z-L-Leu-Aib-OH **19** used for this study was synthesized from PyBOP (see above) Yields and % epimer were measured on the reaction mixture without further work-up

1) Epimerization: The peaks corresponding to the diastereoisomers **20** and **24** were well separated (R T = 5 7 and 6 5 min respectively) using the following conditions

- normal phase column Ultrasphere Si 5µ 250 x 4 6 mm (Beckman)
- 2 ml/min flow of hexane EtOAc, 1 1 (isocratic)
- visualisation at 254 nm Integration using Beckman system Gold program

2) Yield: The yields of **20** were determined using the commercially available Z-Pro-Leu-OEt as internal reference under the following conditions:

- Ultrabase C8 5 µ 150 x 4 6 mm column (SFCC)
- visualization at 214 nm
- 2 ml/min flow of acetonitrile water· TFA, 50 50 1°/oo

Z-Pro-Leu-OEt R T = 3 9 min, Z-Leu-Aib-Pro-OtBu **20** (and **24**) R T = 4 8 min

Coupling with PyBOP. this reaction is described above (eq 1) Reaction time 2 h Yield 96% No epimerization

With PyBroP: **19** (0 175 g, 0 5 mmol), HCl H-Pro-OtBu (0 114 g, 0 55 mmol), PyBrOP (0 233 g, 0 5 mmol) and DIEA (0 26 ml, 1 5 mmol) in CH_2Cl_2 (0 5 ml) Reaction time 2 h Yield 74% No epimerization

With PyBroP/DMAP. **19** (0 175 g, 0 5 mmol), HCl H-Pro-OtBu (0 114 g, 0 55 mmol), PyBroP (0 233 g, 0 5 mmol), DIEA (0 237 ml, 1 4 mmol) and DMAP (0 189 g, 0 14 mmol) in CH_2Cl_2 (0 5 ml) Reaction time 1 h Yield 79% No epimerization

With DCC. **19** (0 175 g, 0 5 mmol), HCl H-Pro-OtBu (0 114 g, 0 55 mmol), DCC (0 104 g, 0 5 mmol), DIEA (0 264 ml, 1 5 mmol) in CH_2Cl_2 (0 5 ml) Reaction time 5 min at 0°C, 2 h at RT Yield 86% No epimerization

With DCC/HOBt. **19** (0 175 g, 0 5 mmol), HCl H-Pro-OtBu (0 114 g, 0 55 mmol), DCC (0 104 g, 0 5 mmol), HOBt (20% H_2O) (0 0822 g, 0 5 mmol), DIEA (0 264 ml, 1 5 mmol) in CH_2Cl_2 (0 5 ml) Reaction time. 5 min at 0°C, 2 h at RT Yield 85% No epimerization

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References and notes

1-Abbreviations and symbols follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (Eur J Biochem 1984, 138, 9) In addition the following abbreviations are used **BOP:** (1H-1, 2, 3-benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium hexafluorophosphate, **PyBOP®:** (1H-1, 2, 3-benzotriazol-1-yloxy)-tris(pyrrolidino)-phosphonium hexafluorophosphate, **BroP:** bromo-tris(dimethylamino)-phosphonium hexafluorophosphate, **PyBroP:** bromo-tris(pyrrolidino)-phosphonium hexafluorophosphate, **DCC:** dicyclohexyl-carbodiimide, **BOP-Cl:** bis(2-oxo-3-

oxazolidinyl)phosphinic chloride, **HOBt:** 1, 2, 3-benzotriazole-1-hydroxyde, **DIEA:** diisopropylethylamine; **DMAP:** 4-dimethylaminopyridine, **CDI:** carbonyl diimidazole, **MA:** mixed anhydride, **AE** active ester; **Aib:** α-aminoisobutyric acid

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