Coupling *N*-Methylated Amino Acids Using PyBroP[1] and PyCloP Halogenophosphonium Salts: Mechanism and Fields of Application

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Received October 26, 1993®

PyBroP (1) and PyCloP (2), two halotripyrrolidinophosphonium hexafluorophosphates, are peptide-coupling reagents highly efficient for coupling *N*-methylated amino esters, in contrast with PyBOP (3), the hydroxybenzotriazolyl analogue. These halogenophosphonium salts 1 and 2 are convenient (one-pot reactions) stable solids soluble in conventional solvents. Use of them gave an excellent peptide yield with essentially no epimerization. Activation with these reagents probably involves the formation of an (acyloxy)phosphonium, as shown in the case of 2,4,6-trimethylbenzoic acid activation. In the case of reagents 1 and 2, oxazolone and/or a symmetrical anhydride were intermediates which were rapidly aminolyzed. In contrast, the benzotriazolyl ester intermediate which was formed with PyBOP (3) was poorly reactive with *N*-methylated amino esters. PyBroP (1) and PyCloP (2) were less efficient in the coupling of some Boc-amino acids because of *N*-carboxyanhydride formation; this was particularly the case when Boc-Val-OH or Boc-MeVal-OH was coupled with MeVal-OMe.

Introduction

Many peptides and depsipeptides isolated from plant strains, microorganisms, and marine organisms contain *N*-methylated α-amino acids. Some of these pseudopeptides such as cyclopsporine,[2] didemmines,[3] and dolastatines[4] have therapeutically proven or promising biological properties. The addition of an *N*-methylated amino acid to a peptide can also produce analogues with modified conformations and activities.[5]

In peptide synthesis, coupling reactions with hindered *N*-methylated amino acids are difficult, and standard reagents are often inefficient.[6] Of all the reagents used, the best yields and epimerization results have been obtained (see ref 6) with Dpp-Cl[7] and BOP-Cl.[8] However, these reagents are water sensitive. Moreover, since Dpp-Cl also reacts with amines,[9] the *N*-protected amino acid must first be preactivated; this is also the case for BOP-Cl with primary amines.[10,11] Furthermore, the degree of purity is very important in the use of this latter reagent.[12]

In preliminary reports,[13,14] we demonstrated that halogenophosphonium reagents BroP (bromotris(dimethylamino)phosphonium hexafluorophosphate), PyBroP (1),[15] and PyCloP (2)[15] permit efficient coupling between *N*-methylated amino esters and Z-*N*-methylated amino acids, in contrast to PyBOP (3)[15,16] or BOP[17] which are commonly used in peptide synthesis. These halogenophosphonium reagents, which have been used by us[18a] and others[18b] for the synthesis of natural products bearing *N*-methylamino acids, can also be employed for difficult coupling of α,α-disubstituted amino acids such as α-aminoisobutyric acid (Aib)[19] or α-methylcysteine[20] and for coupling *N*-methyl-α,α-dialkylamino acids.[21] However, we observed a limitation on the use of the reagents 1 and

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† This article was taken in part from the Ph.D. Dissertation Eric Frérot, of University of Montpellier II, 1992.

® Abstract published in *Advance ACS Abstracts*, April 1, 1994.

(1) Nomenclature, abbreviations and symbols follow the recommendations of *Nomenclature of Organic Chemistry* (*Nomenclature of Organic Chemistry*; Pergamon: Oxford, 1979; Sections A–F and H and of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9–37). In addition, the following abbreviations are used: BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; PyBOP, (1*H*-benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate; PyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; PyCloP, chlorotripyrrolidinophosphonium hexafluorophosphate; BOP-Cl, *N*,*N*-bis(2-oxo-3-oxazolidinyl)phosphinic chloride; Dpp-Cl diphenylphosphoryl chloride; BroP, bromotris(dimethylamino)phosphonium hexafluorophosphate; HBTU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HBPyU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium hexafluorophosphate; TBPyU, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-bis(tetramethylene)uronium tetrafluoroborate; TPyClU *N*,*N*,*N*′,*N*′-bis(tetramethylene)chlorouronium tetrafluoroborate; EDC, *N*-[3-(dimethylamino)propyl]-*N*′-ethyl-carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; DIEA, diisopropylethylamine, NMM, *N*-methylmorpholine; NMP, *N*-methylpyrrolidone; DCM, dichloromethane; NCA, *N*-carboxyanhydride; SPPS, solid-phase peptide synthesis. When using the three-system for amino acids or peptides, the abbreviation MeXaa means *N*-Me amino acid.

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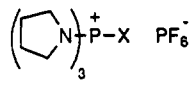
Table 1. Coupling of *N*-Methyl Amino Acids with Reagents 1, 2, and 3. Method,[a] Reaction Time (h), and Yield (epimer %[b])

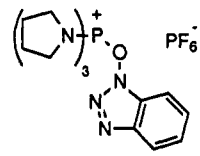
entry	peptide	PyBroP (1)	PyCloP (2)	PyBOP (3)
1	Z-MeVal-Val-OMe 4	A, 1, 85 (0.3)	A, 1, 88 (0.15)	A, 1, 90 (0.2)
2	Z-Val-MeVal-OMe 5	A, 1, 70[c] (0)	A, 1, 85[c]	A, 1, 11[c]
3	Z-Val-MeVal-OMe 5	A, 24, 96[c] (0.5)	A, 24, 96[c] (0.15)	A, 24, 76[c] (0)
4	Z-Val-MeVal-OMe 5	B, 3, 100[c] (0.15)	B, 3, 100[c] (0.15)	
5	Z-MeVal-MeVal-OMe 6	A, 1, 61 (≤0.3)	A, 1, 59 (≤0.2)	A, 1, <5[d]
6	Z-MeVal-MeVal-OMe 6	B, 3, 87 (≤0.3)	B, 3, 84 (≤0.3)	A, 72, 58 (18)
7	Fmoc-MeVal-Val-OMe 7	B, 3, 92		
8	Fmoc-Val-MeVal-OMe 8	B, 3, 84		A, 24, 30
9	Fmoc-MeVal-MeVal-OMe 9	B, 3, 83 (≤0.3)	B, 3, 75 (≤0.3)	
10	Boc-MeVal-Val-OMe 10	A, 1, 64		A, 1, 76
11	Boc-MeVal-Val-OMe 10	B, 3, 81		
12	Boc-Val-MeVal-OMe 11	B, 3, 44	B, 3, 58	A, 24, 45
13	Boc-MeVal-MeVal-OMe 12	B, 3, 34	B, 3, 36	A, 24, 26[d]
14	Boc-Pro-MeVal-OMe 13	A, 1, 79 (≤0.1)	A, 1, 85 (≤0.2)	A, 1, 26[d]
15	Boc-MeLeu-MeVal-OMe 14	B, 3, 88 (≤0.6)[e]	B, 3, 97 (≤0.6)	

[a] Method A: *N*-protected amino acid (1 equiv), *C*-protected amino acid hydrochloride (1.1 equiv), and coupling reagent (1 equiv) at 0 °C in DCM (1 mL/mmol), then 3 equiv of DIEA, 1 min at 0 °C, then rt. Method B: *N*-protected amino acid (1.5 equiv), *C*-protected amino acid hydrochloride (1 equiv), and coupling reagent (1.5 equiv), at 0 °C in DCM (1 mL/mmol), then 4 equiv DIEA, 1 min at 0 °C, then rt. [b] Percent of epimer determined by HPLC (peptides 4, 5, 13) or ^{1}H NMR (peptides 6, 9, 14), by comparison with the corresponding DL diastereomer. For peptides 7, 8, 10, and 12, attentive examination of ^{1}H NMR spectra showed no other diastereoisomer. [c] Yields determined by HPLC (internal standard). [d] Yield estimated by ^{1}H NMR, on the crude product. [e] Results obtained for Boc-D-MeLeu-MeVal-OMe (14').

2 due to the formation of *N*-carboxyanhydride when coupling Boc-amino acids.[22]



PyBroP (1) : X = Br
PyCloP (2) : X = Cl



PyBOP (3)

We present here a comprehensive study on the use of PyBroP (1) and PyCloP (2) for coupling *N*-methylated amino acids, including the fields of application and limitations of these reagents in comparison with PyBOP (3). The mechanisms of amino acid activation promoted with all three reagents are also discussed.

Reagents 1 and 2 for Coupling *N*-Me-amino Acids. 1. Z Protection. Valine was chosen as a model amino acid because of its high steric hindrance and the common occurrence of MeVal residues in natural pseudopeptides.[2,4] We first investigated coupling valine esters and *N*-methylvaline esters with benzyloxycarbonyl-protected valine or *N*-methylvaline.

Table 1 gives the yields and percentages of epimerization obtained during synthesis of *N*-methylated dipeptides 4–6 with reagents 1–3, according to the following optimized conditions:

a. Reagent Stoichiometry. With reagents 1 and 2, by use of stoichiometric quantities of reactants (method A), some of the coupling reactions were difficult (entries 2, 3, and 5). In these cases, improved yields could be obtained with shorter reaction times (entries 4 and 6) using a slight excess of *N*-protected amino acid and coupling reagent (1.5 equiv, method B).

b. Base Quantities. The importance of the base quantity used was already discussed for BOP.[23] Accordingly, for reagents 1 and 2, we used 3 equiv of DIEA with method A and 4 equiv with method B. Indeed, during synthesis of dipeptide 5 with PyBroP according to method B but using only 3 equiv DIEA (instead of 4 equiv, Table 1, entry 4) the reaction was incomplete even after 24 h (91%).

c. Solvent. High yields and low epimerization were obtained with PyBroP for the synthesis of dipeptide 5 using different common solvents (DCM, DMF, and NMP) in the presence of DIEA or NMM (see Experimental Section). The best result was obtained when DCM/DIEA was used (Table I, entry 4).

d. Temperature. The observed temperature increase, at the onset of the coupling reactions, during base addition, enhanced epimerization. Peptide 5 obtained in this way (stoichiometric conditions of method A, PyBroP, 16 h, yield = 93%) contained 1.6% of the 5' diastereoisomer. This could be avoided by using a cooling bath (0 °C) during the first few minutes of the reaction. Under these conditions, only 0.5% of the 5' diastereoisomer was obtained (Table 1, entry 3, PyBroP).

e. One-Pot Reaction. In contrast to Dpp-Cl and BOP-Cl (see above), we verified that PyCloP did not react with the primary amine function in the amino esters, thus allowing coupling without preactivation. For example, H-Ala-OMe did not react with PyCloP in the presence of DIEA, even after 72 h at room temperature (^{31}P NMR monitoring, data not shown).

The present results (Table 1) demonstrated that the following occur after dipeptide synthesis: first, when the *C*-protected amino acid was not *N*-methylated, coupling was equally efficient with the three reagents 1–3 (entry 1), and second, when the amino ester was *N*-methylated (entries 2–6), coupling was more difficult and reagents 1 and 2 were much more effective than PyBOP (3). Indeed, the results with this latter reagent were discouraging with dipeptide 6 (entries 5 and 6), both in terms of yield (58% after 72 h) and epimerization (18% DL epimer).

When PyBroP or PyCloP was used, the reaction times, yields, and epimerization percentages were comparable to previous results obtained with Dpp-Cl[24] and BOP-Cl.[25,26]

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However, reagents 1 and 2 have the advantage of being stable solids, soluble in standard peptide synthesis solvents (DCM, DMF) and usable without preactivation. Accordingly, dolastatin 15 could be synthesized with PyCloP using the Z/OtBu strategy and method B.[18a]

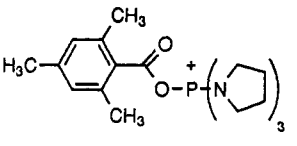
2. Fmoc Protection. The results of Fmoc-amino acid coupling under these conditions were similar to those obtained with Z-amino acids (Table 1, entries 7–9). The inefficiency of PyBOP for coupling an *N*-Me amino ester (entry 8) was also confirmed.

3. Boc Protection. The results obtained with Boc protection differed from those we obtained with Z and Fmoc protection. Although, as expected, poor results were obtained when PyBOP was used to couple *N*-MeVal-OMe (Table 1, entries 12–14); no increase in yield was obtained using PyBroP or PyCloP (entries 12 and 13). Similarly, only modest yields were obtained for dipeptide **10** (Table 1, entry 10) with PyBOP or PyBroP, while these two reagents were very efficient for analogues **4** and **7**. Difficulties have already been encountered in coupling Boc-amino acids,[6] in particular with BOP-Cl.[10,25,26] Analysis of activation reactions with reagents 1 and 2 (see below) demonstrated that these results were due to the formation of *N*-carboxyanhydride (NCA) **15** or **16**. However, the Boc protection should not be systematically ruled out since results obtained in coupling *N*-MeVal-OMe with Boc-Pro-OH (Table 1, entry 14) or Boc-MeLeu-OH (entry 15) were identical to or better than Z(Fmoc)-MeVal reactions (entries 5, 6, and 9).

Activation Mechanisms with Reagents 1–3. We investigated the mechanism of *N*-protected amino acid activation induced by the phosphonium salts PyBroP (1), PyCloP (2), and PyBOP (3) to explain the efficiency differences between 1 or 2 versus 3 and the limitations when using reagents 1 and 2 for coupling Boc-Val-OH and Boc-MeVal-OH.

1. (Acyloxy)phosphonium. It was assumed[27] that the first step in carboxylic acid activation by phosphonium salt reagents such as BOP,[28] Ph_3P/CCl_4,[29a] or $(Me_2N)_3P/CCl_4$[29b] involves formation of an (acyloxy)phosphonium salt. This type of intermediate has been obtained, for instance, by reaction of an acid chloride with HMPA in the presence of $SbCl_5$.[30] However, attempts to detect this intermediate by ^{31}P NMR in reactions with amino acids and phosphonium salts such as BOP[31] or Bates reagent ($[(Me_2N)_3P^+]_2O, 2BF_4^-$),[32] or in reactions with phenylacetic acid and the $(Me_2N)_3P/CCl_4$ system,[30] have been unsuccessful.

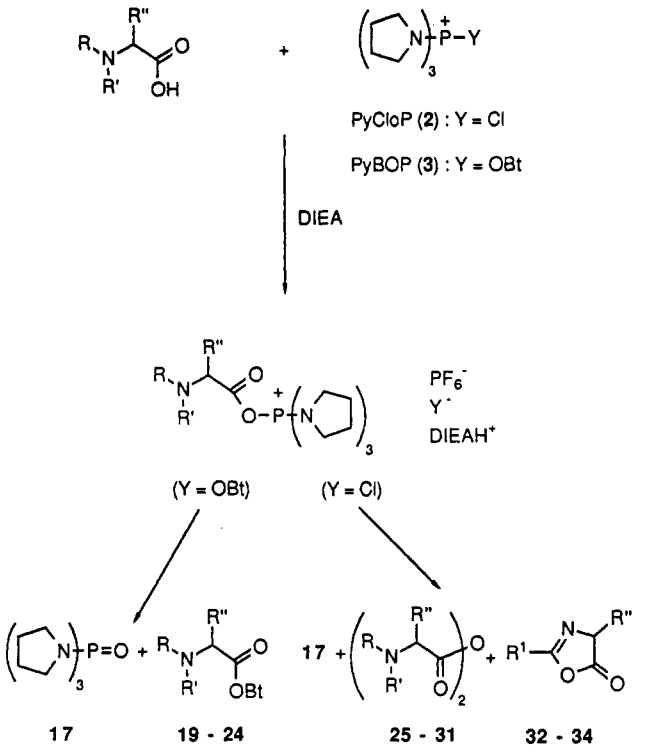
We carried out a study of activation reactions with PyBOP and PyCloP using ^{31}P NMR at low temperature. Tripyrrolidinophosphine oxide (**17**) was immediately formed during the reaction of PyCloP/DIEA with Z-Val-OH at 0 °C, without any detectable intermediate formation. However, during the reaction of the sterically hindered 2,4,6-trimethylbenzoic acid, with PyBOP (^{31}P NMR δ = 31.3 ppm) at −20 °C, or with PyCloP (δ = 36.3 ppm) at 0 °C, we observed the formation of an intermediate (δ = 22.3 ppm) which then disappeared to give rise to **17** (δ = 14.7 ppm). The chemical shift of the observed intermediate was similar to that of hexachloroantimonate **18a** (δ = 22.0 ppm) prepared, using Teichmann's method,[30] by the action of 2,4,6-trimethylbenzoyl chloride on **17** in the presence of $SbCl_5$. This intermediate was therefore consistent with the corresponding hexafluorophosphate structure **18b**.



18a: X = $SbCl_6^-$
18b: X = PF_6^-

It is reasonable to assume that an unstable (acyloxy)-phosphonium salt intermediate was actually formed during peptide coupling with a phosphonium salt of the BOP family. This intermediate released phosphine oxide **17** with concomitant formation of an amino acid activated species, whose nature (oxybenzotriazole ester, symmetric anhydride or oxazolone, cf. Scheme 1) depended on the reactants used.

Scheme 1. *N*-Protected Amino Acid Activation Using PyCloP or PyBOP Reagents[a]



R = Z; R' = H; R" = *i*-Pr	**19**	**25**	R^1 = OBzl **32**
R = Fmoc; R' = H; R" = *i*-Pr	**20**	**26**	OFm[a] **33**
R = Boc; R' = H; R" = *i*-Pr	**21**	**27**	O*t*-Bu **34**
R = Boc; R',R" = $(CH_2)_3$	**22**	**28**	
R = Z; R' = Me; R" = *i*-Pr	**23**	**29**	
R = Fmoc; R' = Me; R" = *i*-Pr	-	**30**	
R = Boc; R' = Me; R" = *i*-Pr	**24**	**31**[b]	

[a] Key: (a) OFm: *O*-fluorenylmethyl; (b) Me-NCA **16** was also formed, see text and Scheme 3.

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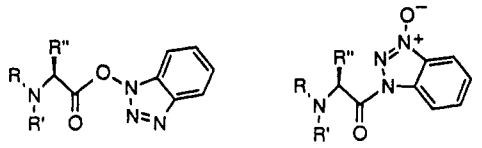
Chart 1. O- and N-Acylated Forms of Benzotriazolyl Esters



2. Activation with PyBOP Reagent: Formation of Benzotriazolyl Esters. With PyBOP, the initially-formed (acyloxy)phosphonium was attacked by the oxybenzotriazolyl anion to form a benzotriazolyl ester (Bt ester). Bt esters have already been characterized and are assumed to be the aminolyzed intermediates in coupling reactions with DCC/HOBt[33] or BOP.[28,31b] These esters are found in the *O*-acylated and *N*-acylated forms (Chart 1).[34]

In our cases, the formed Bt esters were not stable enough to be purified. However, analysis of the reaction mixture or the crude reaction product by RP-HPLC, IR, and ^{1}H NMR confirmed the presence of both *O*- and *N*-acylated forms. The RP-HPLC analysis (using a diode array UV detector) revealed two compounds whose UV spectra were in accordance with the proposed structures.[35] In all cases, the IR analysis showed an absorption band at 1810–1820 cm^{-1}, corresponding to the *O*-acylated form.[36] The *N*-acylated form (ν = 1730–1750 cm^{-1})[36] could not be identified with Z(Fmoc, Boc)-Val-OBt because it was masked by the absorption of carbamate. However, this absorption band was visible for Z(Boc)-MeVal-OBt and Boc-Pro-OBt, with the carbamate band located at 1690–1700 cm^{-1}. The ^{1}H NMR analysis revealed numerous signals corresponding to benzotriazole residues from the two forms of Bt esters in the δ = 7–8.5 ppm region. It should also be pointed out that with Boc-MeVal-OH the activation reaction led to formation of Me-NCA **16** (see below) in addition to Bt esters. Thus, PyBOP-mediated activation of *N*-protected (Z(Fmoc, Boc)-Val-OH, Boc-Pro) and *N*-methylated (Z(Boc)-MeVal-OH) amino acids afforded formation of the corresponding oxybenzotriazolyl esters **19–24**; with Z-MeVal, the results were almost identical for PyBOP, DCC/HOBt, and BOP.

During the synthesis of Z-Val-Val-OMe with PyBOP, at room temperature, HPLC analysis did not reveal the formation of Z-Val-OBt. However, Bt esters were detected when the reaction was performed at −75 °C (data not shown). Thus, it is reasonable to imagine that the Bt esters are the intermediates which are aminolyzed during coupling with PyBOP.[37]

During slow coupling reactions, i.e., when the C-protected amino acid is N-methylated, the Bt esters are indeed the intermediates which are aminolyzed. HPLC analysis of the PyBOP-mediated synthesis of Z-Val-MeVal-OMe (**5**) showed immediate formation of both Z-Val-OBt forms. This coupling reaction was very slow; it took more than 48 h for the Bt ester to react (81% yield). The presence of Bt esters during coupling reactions with PyBOP, and their slow reaction rates, were also noted with dipeptides **6** and **11–13**.[37]

Such results were not limited to the use of PyBOP; we have noted identical results in previous investigations[13,14] when coupling *N*-methylated amino acids with reagents containing HOBt or the OBt residue: DCC/HOBt, BOP, and the uronium reagent HBPyU. Similarly, in regard to coupling of *N*-methylated amino acids with BOP-Cl, Anteunis[26b,38] suggested that the poor yields obtained with BOP-Cl/HOBt are due to low reactivity of Bt esters. However, the results of Rich show no marked effect of HOBt during coupling of Boc-Val with MeLeu-Ala-OBzl (67% yield with BOP-Cl and 57% with BOP-Cl/HOBt),[10] while Benoiton noted[39] that addition of HOBt to DCC resulted in better yields when coupling Z-MeXaa and X'aa-OR. Our results clearly confirm that the poor reactivity of Bt esters was responsible for the coupling difficulties. More precisely, coupling was difficult when the *C*-protected amino acid was *N*-methylated.[40] We also noted[19] the slow reaction of Z(Boc)-Aib-OBt with Aib-OMe, and the recent results of Goodman[21] confirmed the low reactivity of Boc-Phe-OBt with *N*-methylated α,α-dialkylated amino esters.

3. Activation with PyBroP and PyCloP Reagents. Since identical results were obtained with both PyBroP (**1**) and PyCloP (**2**) reagents (Table 1), the activation mechanism was studied with reagent **2**. The intermediate species (symmetrical anhydrides, oxazolones, NCA) present in the reaction media were identified by IR and ^{1}H NMR analysis in comparison to authentic samples or published data.[41] These different species showed characteristic and very distinct IR absorption bands (anhydrides 1820 and 1750 cm^{-1}, oxazolones 1835 and 1685 cm^{-1},[45,47,48] and NCA 1850 and 1785 cm^{-1} [50]) and could be identified in the spectrum of the reaction medium. In addition, carbamate absorption, at 1720 cm^{-1} for Val derivatives and 1690–1700 cm^{1} for MeVal or Pro derivatives, was also observed.

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(34) Katritzky, A. R.; Malhotra, N.; Fan, W.-Q.; Anders, E. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1545–1747 and references cited therein.
(35) UV spectra for the highest retention time peak showed λ_{max} = 254 and 283 nm, which could correspond to the less polar *O*-acylated form presenting less conjugations than the *N*-acylated form (lowest retention peak, λ_{max} = 247, 327, and 340 nm).
(36) Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *Int. J. Peptide Protein Res.* **1984**, *23*, 300–305.
(37) It is still possible that the OBt ester rapidly reached equilibrium with oxazolone, or oxazolonium in the case of *N*-Me amino acid, and that this latter species was the aminolyzed intermediate. This equilibrium would agree with the epimerization observed in Z-MeVal-Val-OMe (Table 1, entry 6, PyBOP-mediated coupling reaction) and with the formation of NCA **16** from Boc-MeVal-OBt (see below).
(38) Anteunis, M. J. O.; Sharma, N. K. *Bull. Soc. Chim. Belg.* **1988**, *97*, 281–292, see note 41.
(39) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem.* **1977**, *55*, 911–915, see note added in proof.
(40) In the case of Boc-amino acids, the formation of NCA (see below) probably interferes, thus explaining the results of Rich.
(41) Z(Fmoc, Boc)-Val and Boc-Pro symmetrical anhydrides **25–28** were previously described;[42,43,44] we prepared them using DCC. We also synthesized Z(Fmoc)-MeVal anhydrides **29** and **30** using EDC as described by Benoiton.[42] Since the EDC method proved inefficient with Boc-MeVal,[42] we used DCC which led to formation of an anhydride **31** and NCA **16** mixture (86/14). Oxazolone **32** was prepared according to Jones.[45] Oxazolones of Fmoc-Val (**33**)[46,47] and Boc-Val (**34**)[48] were identified in activation reaction mixtures by comparison of the IR and ^{1}H NMR data with those in the literature. NCA **15** was obtained as described by Hirschmann,[49] and *N*-Me-NCA **16** was synthesized by reaction of phosgene with Boc-MeVal in the presence of DIEA.
(42) Chen, F. M. F.; Kuroda, K.; Benoiton, N. L. *Synthesis* **1978**, 928–929.
(43) Heimer, E. P.; Chang, C.-D.; Lambros, T.; Meienhofer, J. *Int. J. Peptide Protein Res.* **1981**, *18*, 237–241.
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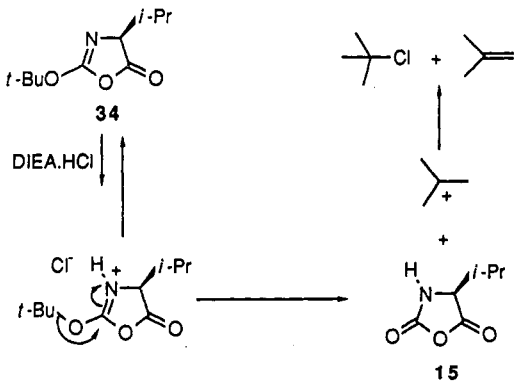
Table 2. PyCloP-Mediated Activation of R-Val-OH, Percent Yield of the Observed Species[a]

	R-Val-O[b]		$(R\text{-Val})_2O$		R-Val-Ox	
R	10	40	10	40	10	40
Z	25	15	55	55	20	30
Fmoc	20	5	50	50	30	45
Boc	30	10	25	10	45	80

[a] Percentages determined by ^{1}H NMR after 10 and 40 min reaction. [b] Unreacted carboxylate.

Scheme 2. Formation of NCA 15



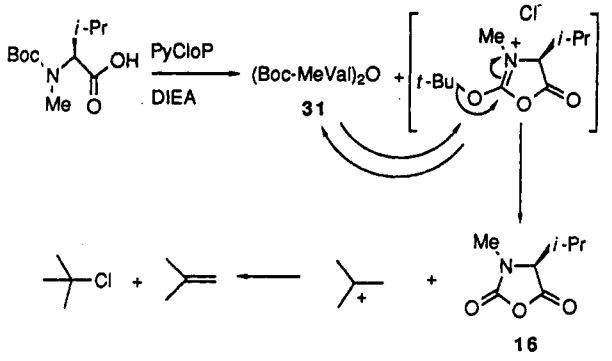
PyCloP-mediated activation of Z(Fmoc,Boc)-Val-OH resulted in a mixture of symmetrical anhydride (**25**, **26**, and **27**, respectively) and oxazolone (**32**, **33**, and **34**, respectively) in the proportions (^{1}H NMR determination) given in Table 2 at 10 and 40 min. There were no significant differences between Z, Fmoc, and Boc protection. Nevertheless, under preactivation conditions of Boc-Val-OH, and IR absorption at 1780 cm^{-1} appeared after 1.5 h of reaction and slowly increased. This IR band corresponds to the *N*-carboxyanhydride (NCA) **15** which was isolated by silica gel chromatography.

PyCloP-mediated activation of Z(Fmoc)-MeVal only produced the corresponding symmetrical anhydride (**29**, **30**).[51]

PyCloP-mediated activation of Boc-MeVal-OH afforded a mixture of symmetrical anhydride **31** and *N*-Me-NCA **16** in proportions that varied over the time-course of the activation reaction. The percentage of **16**, as determined by ^{1}H NMR, increased from 60% (after 10 min) to 80% (40 min).

NCA formation from Boc-amino acids was previously reported during Boc-amino acid activation with a variety of activation methods.[48,53-55] In agreement with the proposed mechanism,[55] oxazolone **34** is quite likely protonated by the DIEA hydrochloride present in the medium (Scheme 2). The formed oxazolonium ion then loses a *tert*-butyl cation, as shown by the presence of *tert*-butyl chloride and isobutene identified by ^{1}H NMR in the reaction medium.

Scheme 3. Formation of *N*-Me-NCA 16 from Boc-MeVal-OH



N-Me-NCA **16** was formed along with *tert*-butyl chloride and isobutene (^{1}H NMR), and the mechanism of the activation (Scheme 3) was therefore probably the same as with Boc-Val. Although no oxazolonium ions were observed during Boc-MeVal-OH activation,[51b] this ion could be an intermediate and form NCA through loss of the *tert*-butyl cation. The oxazolonium ion could be formed directly from (acyloxy)phosphonium and/or from the symmetrical anhydride.

Boc-MeVal-OH produced *N*-Me-NCA **16** much faster than Boc-Val produced NCA **15**. In the latter case, the weak side reaction could be explained by only partial oxazolonium formation, in the presence of the DIEA excess.[56]

We also observed NCA formation during PyCloP-mediated activation for amino acids other than Boc-Val-OH or Boc-MeVal-OH. We noted here also that Me-NCA was more easily formed than NCA. IR studies showed that the 1780 cm^{-1} characteristic absorption band appeared after 5 min activation for Boc-MeAla-OH and Boc-MeLeu-OH instead of 3 and 1.5 h for Boc-Ala-OH and Boc-Leu-OH, respectively.

We also observed *N*-Me-NCA **16** formation when Boc-MeVal-OH was activated using other reagents (PyBroP, DCC, DCC/HOBt, PyBOP, or HBTU[57]).[58]

With Fmoc-MeVal-OH, PyCloP-mediated activation did not afford the NCA, and with Z-MeVal-OH, only small amounts were observed (IR) after 16 h. The NCA formation from Boc-protected MeVal-OH was probably due to the greater stability of the *tert*-butyl cation compared with the benzyl or fluorenylmethyl cations.

For the Boc-amino acids, NCA formation was not only obtained during activation but also during the coupling reactions (without preactivation) of peptides **11** (TLC

(51) (a) We did not detect an ^{1}H NMR signal corresponding to *N*-CH_3 protons of an oxazolonium (δ = 3.71 ppm for that of Bz-MeVal).[52] (b) This is in agreement with the report that Z(Boc)-MeAla-OH does not produce an oxazolonium ion in the same activation conditions (DCC) in which it is afforted by Bz-MeAla.[52]

(52) Davies, J. S.; Mohammed, A. K. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2982-2990.

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(55) Benoiton, N. L.; Lee, Y. C.; Chen, F. M. F. *Int. J. Peptide Protein Res.* **1993**, *41*, 587-594 and references cited therein.

(56) In this context, Benoiton[55] showed that addition of base reduced the amount of NCA during activation of nonmethylated Boc-amino acids.

(57) Dourtoglu, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, 1269-1272.

(58) PyBroP yielded the same results as PyCloP. After 30 min activation, amounts of **16** were (^{1}H NMR analysis) as follows 2.5% (DCC), 5% (DCC/HOBt), and 13% (PyBOP); with HBTU, we obtained the same results (IR analysis) as with PyBOP. Furthermore, with all these reagents, we noted by IR analysis (up to 24 h) the transformation of the first observed intermediate (anhydride or Bt ester) into *N*-Me-NCA **16**. The rate of this transformation was related to the experimental conditions. Hence, DCC-mediated activation of Boc-MeVal-OH resulted in 2.5% NCA **16** in the reaction mixture and 15% after treatment (filtration, evaporation). The same behavior was noted with PyBOP: 13% Me-NCA **16** in the reaction mixture and almost 100% after standard treatment (washing and solvent evaporation). Similarly, for Boc-Val-OH, PyBOP-mediated activation only produced the OBt ester in the reaction mixture (IR analysis), but this ester was accompanied by NCA **15** (14%) after standard treatment of the reaction mixture. We also observed spontaneous formation of NCA **15** from $(Boc\text{-Val})_2O$. Formation of *N*-Me-NCA **16** was also observed during the synthesis of a Boc-MeVal mixed anhydride.[55]

analysis of the crude product) and 12 (isolated by column chromatography). Thus, the low yields obtained when coupling Boc-amino were due to the formation of NCA or N-Me-NCA.[59]

The yield of coupling did not depend exclusively on the ease of formation of NCA or Me-NCA (compare entry 11 with entry 13), and the influence of the amino ester is determinent. Indeed, when Boc-Val-OH or Boc-MeVal-OH was coupled with Me-Val-OMe, yields were dramatically lower than when the Z or Fmoc protection was used (Table 1, compare entry 12 with entries 4 or 8 and entry 13 with entries 6 or 9); conversely, coupling Boc-MeVal-OH with Val-OMe resulted in yields comparable to those obtained when Z(Fmoc)-MeVal was coupled (compare entry 10 with entry 1 and entry 11 with entry 7). In the case of Z-Val-OH or Z-MeVal-OH, the low reactivity of MeVal-OMe resulted in long coupling times (see above); when Boc-Val-OH or Boc-MeVal-OH was coupled, because of NCA formation, the effect of this reactivity resulted in poor yields.[60] In addition, we verified that MeVal-OMe was poorly reactive toward $(Boc\text{-}MeVal)_2O$ (31) (25% dipeptide 12 yield after 6 h).

However, the case of valine is particular since coupling Boc-MeLeu-OH with MeVal-OMe gave a very good yield using method B (entry 15), despite rapid formation of NCA during the preactivation reaction (30% after 10 min). Boc-Pro-OH showed a very specific behavior; for this amino acid the formation of an oxazolonium ion is difficult because of the cycle strain (see ref 6); indeed, we did not observe NCA formation during PyCloP-mediated activation, and coupling yields were good, using PyBroP or PyCloP, even with method A (Table 1, entry 14).

Conclusion

The coupling reagent, chlorotris(dimethylamino)phosphonium perchlorate (the chlorinated analogue of BOP), was previously investigated[62] but abandoned in favor of BOP. However, the results we obtained with the PyBroP and PyCloP reagents raise the issue of their general use in peptide synthesis. With PyCloP, we obtained satisfactory results for solution synthesis of dipeptides,[63] but SPPS of the ACP 65-74 fragment was disappointing.[61] These results were similar to those we obtained with TPyClU (an analogue of PyCloP), whereas TBPyU (an analogue of PyBOP) proved to be very efficient.[64] It has also been shown that better results are obtained with BOP-Cl/HOBt than with BOP-Cl in coupling common amino acids.[11a] In this context, DCC/HOBt is better than DCC. Thus, the presence of HOBt, or the OBt residue in the coupling reagent, appears to be favorable for the synthesis of unmodified peptides.

In contrast, when N-methylated amino esters were coupled, better results were obtained in the absence of this additive. In this case, PyBroP and PyCloP were quite suitable reagents. Despite their limited use for coupling some Boc-amino acids,[65] they are choice reagents when coupling N-methylated amino acids.

Experimental Section

General Procedures. Usual workup consists of the following: washing the organic phase with 5% $KHSO_4$, 5% $NaHCO_3$, and brine, drying over Na_2SO_4, filtration, and concentration of the solvent in vacuo. Analytical TLC were performed on silica gel $60F_{254}$ aluminum sheets (0.2-mm thick). Column chromatography was performed using silica gel 63–200 μm. Mp were determined using a Buchi melting point apparatus. Optical rotations were measured at 20 °C. ^1H, ^{13}C, and ^{31}P NMR data were recorded at, respectively, 360, 90, and 81 MHz in $CDCl_3$ unless specified otherwise, δ ppm are given versus TMS (^1H, ^{13}C) or H_3PO_4 (^{31}P), and J values are given in Hz. IR data (ν cm^{-1}) were obtained in $CHCl_3$. HPLC analyses were performed with an apparatus equipped with a diode array UV detector (UV data; λ_{max} are given in nm); the following conditions were used: (1) Ultrasphere Si 5-μm column (Beckman), 250 × 4.5 mm, hexane/AcOEt (70:30), 2 mL/min, detection at 254 nm; (2) Ultrabase C_8 5 μm (Société Française de Chromatographie Colonne: SFCC), 150 × 4.6 mm, CH_3CN/H_2O/TFA (35:65:0.1), 2 mL/min, detection at 214 nm; (3) Ultrabase C_8 5 μm (SFCC), 150 × 4.6 mm, CH_3CN/H_2O/TFA (0.1%), gradient 10 to 40% CH_3CN in 30 min, 1.5 mL/min, detection at 214 nm; (4) Ultrabase C_8 5 μm (SFCC), 150 × 4.6 mm, CH_3CN/H_2O/TFA (50:50:0.1), 2 mL/min, detection at 214 nm; (5) Ultrabase C_8 5 μm (SFCC), 150 × 4.6 mm, CH_3CN/H_2O/TFA (50:50:0.1), 1.5 mL/min, detection at 214 nm; and (6) Ultrabase C_8 5 μm (SFCC), 150 × 4.6 mm, CH_3CN/H_2O/TFA (0.1), gradient 30 to 90% CH_3CN in 30 min, 1.5 mL/min, detection at 214 nm. Mass spectra were performed, unless specified otherwise, in the FAB+ mode by the Department of Physical Measurements at the University of Montpellier II. Elemental analyses were performed by the CNRS, at the Ecole Nationale Supérieure de Chimie of Montpellier.

Commercially available compounds were used as received, unless otherwise stated. Diethyl ether and THF were distilled under argon from sodium/benzophenone. DCM was filtered, under argon, on alumina.

Z(Boc)-N-methyl amino acids were synthesized by the procedure of Benoiton;[66,67] Fmoc-MeLeu-OH was synthesized using the procedure of Freidinger.[68]

Bromotripyrrolydinophosphonium Hexafluorophosphate (PyBroP) (1). To a stirred solution of phosphoryl tribromide (28.7 g, 0.1 mol) in acetonitrile (25 mL) was added dropwise, at room temperature, over a 20-min period, a solution of tripyrrolidinophosphine oxide[69] (27.7 g, 0.108 mol) in acetonitrile (15 mL). The temperature rose to 37 °C. The mixture was then

(59) In the case of Boc-MeVal-OH, the very rapid formation of Me-NCA 16 accounts for the observed low yield using PyBroP or PyCloP (Table 1, entry 13). However, we noted (see above) that the formation of NCA 15 from Boc-Val-OH preactivation was slow. Thus, this side reaction does not seem to explain *a priori* the low yield obtained when synthesizing the dipeptide 11 with the reagents 1 and 2 (entry 12). The fact that NCA formation was induced by DIEA hydrochloride (Scheme 2) suggests, in agreement with Benoiton,[55] that it could be accelerated by an additional quantity of amine hydrochloride. Indeed, when Boc-Val-OH was preactivated in the presence of 1 equiv of triethylamine hydrochloride, NCA 15 formation was markedly accelerated (strong IR band at 1780 cm^{-1} after 30 min, instead of a weak band after 1.5 h). Since the Boc-Val-MeVal-OMe dipeptide (Table 1, entry 12) was prepared from MeVal-OMe hydrochloride, substantial NCA could be formed during the coupling reaction itself.

(60) PyCloP-mediated activation of Boc-Aib-OH also produces the corresponding NCA,[61] thus explaining, in reactions without DMAP catalysis, the worse result for Boc-Aib-Aib-OMe synthesis (25% yield after 24 h reaction) than for Z-Aib-Aib-OMe synthesis (77% after 16 h).[19] With DMAP catalysis, the increased coupling reaction rate led to results that were independent of the Boc- or Z-protecting group (77% after 1 h reaction).[19]

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(65) Moreover, during BroP-mediated coupling reactions, an N-trifluoroacetamide was observed when the TFA salt of an amine was used (Poncet, J. Personal communication, 1993).

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stirred for 30 min at room temperature. The mixture was then added dropwise to KPF_6 (18.5 g, 0.1 mol) in water (800 mL): a solid was formed. The solid was filtrated, washed with water, and dissolved in acetone; the solution was added dropwise, under stirring, to water (10× the acetone volume), to furnish a white solid which was filtrated, washed with water, and dissolved in DCM. The solution was dried (Na_2SO_4) and the solvent removed under reduced pressure. After recrystallization (AcOEt), a white solid was obtained (31.8 g, 68%): mp 138–139 °C; ^{1}H NMR δ 2.03 (m, 12 H), 3.34 (m, 12 H); ^{31}P NMR δ 27.9 (s), −143.9 (hept, J = 713); MS m/z 320, 322 (1:1). Anal. Calcd for $C_{12}H_{24}BrF_6P_2N_3$: C, 30.92; H, 5.19; N, 9.01. Found: C, 31.13; H, 5.26; N, 8.93.

Chlorotripyrrolydinophosphonium Hexafluorophosphate (PyCloP) (2). To a stirred solution of phosphoryl trichloride (freshly distilled under nitrogen) (23 mL, 0.1 mol) in DCM (15 mL) was added dropwise, at room temperature, over a 20-min period, a solution of tripyrrolidinophosphine oxide[69] (25.7 g, 0.1 mol) in DCM (15 mL). The temperature rose to 30–35 °C. The mixture was then stirred for 30 min at room temperature. KPF_6 (18.5 g, 0.1 mol) in water (150 mL) was rapidly added under stirring. The mixture was decanted and extracted with DCM. The organic phase was washed with water and dried (Na_2SO_4) and the solvent removed under reduced pressure. After recrystallization (DCM/Et_2O), a white solid was obtained (34.4 g, 82%): mp 150–151 °C; ^{1}H NMR δ 2.02 (m, 12 H), 3.34 (m, 12 H); ^{31}P NMR δ 36.5 (s), −143.7 (hept, J = 713); MS m/z 276, 278 (3:1). Anal. Calcd for $C_{12}H_{24}ClF_6P_2N_3$: C, 34.16; H, 5.69; N, 9.96. Found: C, 34.12; H, 5.79; N, 9.97.

N-Methylvaline Methyl Ester Hydrochloride. Z-MeVal-OH (5 g, 18.87 mmol) was esterified with CH_2N_2 in Et_2O solution. After removal of the solvent, the crude product (5.26 g, oil) was dissolved in a mixture of MeOH (100 mL) and 12 N HCl (2.4 mL) and hydrogenolyzed (4 h) over 10% Pd on charcoal. After filtration, the solvent was removed under reduced pressure. After recrystallization (DCM–Et_2O), 2.66 g (78%) was obtained: mp 136–138 °C; $[\alpha]_D$ +30° (c 1, EtOH); ^{1}H NMR δ 1.11 (d, J = 6.8, 3 H), 1.17 (d, J = 6.8, 3 H), 2.59, (br, 1 H), 2.76 (br s, 3 H), 3.58 (br, 1 H), 3.74 (s, 3 H), 9.57 (br, 1 H), 10.25 (br, 1 H). Anal. Calcd for $C_7H_{16}ClNO_2$: C, 46.28; H, 8.81; Cl, 19.52; N, 7.71. Found: C, 46.48; H, 8.98; Cl, 19.79; N, 7.84.

General Procedure for Coupling N-Methylamino Acids with Reagents of the PyBOP Family. Method A. A solution (or suspension) of the N-protected amino acid (1 equiv), amino ester hydrochloride (1.1 equiv), and coupling reagent (1 equiv) in DCM (1 mL/mmol) was treated under stirring with DIEA (3 equiv) at 0 °C. The ice bath was removed after 1 min and the stirring continued at room temperature as long as needed. The mixture was poured into AcOEt (50× the DCM volume) and the solution treated according to the usual workup. The crude product was purified by column chromatography on silica gel.

Method B. Identical to procedure A, except for the stoichiometry: *N-protected amino acid (1.5 equiv), amino ester* hydrochloride (1 equiv), coupling reagent (1.5 equiv), DIEA (4 equiv).

HPLC Determination of the Diastereoisomeric Purity. The diastereoisomeric purity of dipeptides **4**, **5**, and **13** was determined by HPLC in comparison wtih the DL diastereoisomers **4′**, **5′** (previously obtained),[13] and **13′**. We verified that the absorption coefficient was identical for each pair of diastereoisomers; when analysis was done on a product purified by column chromatography, we verified that the diastereoisomers were not separated during the purification.

[N-(Benzyloxycarbonyl)-N-methyl-L-valyl]-L-valine Methyl Ester (4). With PyBOP, method A, with Z-MeVal-OH (0.265 g, 1 mmol). Reaction time: 1 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.5) gave an oil (0.34 g, 90%): $[\alpha]_D$ −90° (c 1, EtOH); ^{1}H NMR, two conformers, δ 0.77 (d, J = 6.6, 3 H), 0.81 (d, J = 6.8, 3 H), 0.86 (d, J = 6.5, 3 H), 0.93 (d, J = 6.3, 3 H), 2.05–2.13 (m, 1 H), 2.21–2.31 (m, 1 H), 2.87 (s, 3 H), 3.69 (s, 3 H), 4.03 (br, 0.2 H), 4.12 (d, J = 11.3, 0.8 H), 4.46 (dd, J = 5.0, J = 8.9, 1 H), 5.11 and 5.19 (br AB, J = 12.6, 2 H), 5.93 (br, 0.2 H), 6.46 (br d, $J \approx$ 8.1, 0.8 H), 7.24–7.32 (m, 5 H). Anal. Calcd for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 7.99; N, 7.40. Found: C, 63.23; H, 7.98; N, 7.57. HPLC (conditions 1, **4**, t_R 3.2 min; **4′**, t_R 2.9 min) showed epimer **4′** (0.2%). For reactions with PyBroP and PyCloP, see Table 1.

[N-(Benzyloxycarbonyl)-L-valyl-N-methyl]-L-valine Methyl Ester (5). Z-Val-OH (0.502 g, 2 mmol), MeVal-OMe,HCl (0.182 g, 1 mmol), and PyBroP (0.932 g, 2 mmol) in DCM (1 mL) were treated with DIEA (0.783 mL, 4.5 mmol) as indicated above. Reaction time: 3 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.5); oil (0.34 g, 90%); $[\alpha]_D$ −114° (c 1, EtOH); ^{1}H NMR, two conformers, δ 0.82 (d, J = 6.7), 0.88 (d, J = 6.8), 0.91 (d, J = 6.7), 0.95 (d, J = 6.8), 0.99 (d, J = 6.5), total 12 H, 1.93–2.05 (m, 1 H), 2.13–2.25 (m, 1 H), 2.86 (s, 0.5 H), 3.04 (s, 2.5 H), 3.60 (s, 0.5 H), 3.67 (s, 2.5 H), 4.16 (d, J = 10.5, 0.15 H), 4.49 (dd, J = 6.8, J = 9.1, 0.85 H), 4.62 (br dd, 0.15 H), 4.91 (d, J = 10.5, 0.85 H), 5.07 (s, 2 H), 5.45 (d, J = 9.2, 1 H), 7.25–7.35 (m, 5 H). Anal. Calcd for $C_{20}H_{30}N_2O_5$: C, 63.47; H, 7.99; N, 7.40. Found: C, 63.74; H, 8.00; N, 7.43.

Z-Val-MeVal-OMe (5): Study of the Optimal Coupling Conditions. Reactions with 0.5 mmol of Val-OMe, HCl. All measurements were made on the reaction mixture itself. Coupling reagent, reaction time, and yield (epimerization) are given for each case. The yields of **5** were determined by HPLC (conditions 4) with Z-Pro-Leu-OEt as internal standard: **5** and **5′**, t_R 4.5 min, standard t_R 3.7 min. The diastereoisomeric purity was determined by HPLC (conditions 2): **5**, t_R 33.7 min; **5′**, t_R 36.5 min.

Coupling Using Method A. With PyBOP, 1 h, 11% (the main product was Z-Val-OBt (**19**), HPLC t_R 3.1 and 5.5 min); 24 h, 76% (0%); 48 h, 81%. With PyBroP, 1 h, 70% (0%); 24 h, 96% (0.5%). With PyBroP, in the same conditions, but without cooling at the beginning of the reaction, 16 h, 93% (1.6%). With PyCloP, 1 h, 85%; 24 h, 96% (0.15%).

Coupling Using Method B. With PyBroP, 0.5 h, 71%; 1 h, 81%; 3 h, 100% (0.15%). With PyCloP, 3 h, 100% (0.15%).

Quantity of Base. With PyBroP, method B, but using 3 equiv of DIEA, 0.5 h, 67%; 1 h, 73%; 3 h, 83% (0%), 24 h, 91%. With PyBroP, method B (i.e., 4 equiv of DIEA), see above.

Effect of Base and Solvent. With PyBroP, method B, 3 h. DCM/DIEA, see above; DCM/DIEA/DMAP (DMAP (0.4 equiv), DIEA (3.6 equiv)), 71% (15.7%); DCM/NMM, 100% (0.7%); DMF/DIEA, 100% (0.3%); DMF/NMM, 91% (0.95%); NMP/DIEA, 100% (0.45%).

[N-(Benzyloxycarbonyl)-N-methyl-L-valyl]-N-methyl-L-valine Methyl Ester (6). Using method A, with PyCloP (and 1 mmol of Z-MeVal-OH). Reaction time: 1 h. Column chromatography (hexane/AcOEt, (70:30), R_f 0.5): oil (0.23 g, 59%); $[\alpha]_D$ −191° (c 1, EtOH); ^{1}H NMR, four conformers (40:25:20:15), δ 0.64 (d, J = 6.5), 0.73 (≈d), 0.75 (d, J = 6.6), 0.83 (d, J = 6.8), 0.84 (d, J = 6.5), 0.86 (d, J = 6.9), 0.88 (d, J = 6.8), 0.90 (d, J = 6.8) 0.96 (d, J = 6.8), 0.98 (d, J = 6.8), 1.03 (d, J = 6.5), total 12 H, 2.07–2.49 (m, 2 H), 2.72 (2 s, 1 H), 2.78 (s, 1 H), 2.79 (s, 0.5 H), 2.82 (s, 0.4 H), 2.85 (s, 0.5 H), 2.87 (s, 1.2 H), 3.02 (s, 1.4 H), 3.41 (s, 0.8 H), 3.63 (s, 0.4 H), 3.65 (s, 0.5 H), 3.67 (s, 1.3 H), 4.10 (d, J = 10.6, 0.1 H), 4.39 (d, J = 10.4), 4.41 (d, $J \approx$ 10), total 0.35 H, 4.48 (d, J = 10.6, 0.15 H), 4.62 (d, J = 10.6, 0.3 H), 4.71 (d, J = 10.8, 0.5 H), 4.78 (d, J = 10.4, 0.1 H), 4.82 (d, J = 10.5, 0.5 H), 5.10 and 5.21 (AB, J = 12.2, 0.6 H), 5.11, 5.17 (AB, J = 12.8, 1.4 H), 7.25–7.34 (m, 5 H). Anal. Calcd for $C_{21}H_{32}N_2O_5$: C, 64.26; H, 8.22; N, 7.14. Found: C, 64.14; H, 8.53; N, 7.60. The diastereoisomeric purity (DL epimer ≤ 0.2%) was determined using ^{1}H NMR, by comparison with the ^{1}H NMR spectrum of the DL isomer **6′** (see ref 13). Measurements were performed on the OMe (δ 3.67 for **6** and 3.70 for **6′**) and N-Me signals (δ 3.02 for **6** and 3.00 for **6′**). With PyBroP (and 1 mmol of Z-MeVal-OH), reaction time 1 h, 61% (≤0.3%). With PyBOP (and 1 mmol of Z-MeVal-OH), reaction time 1 h, ^{1}H NMR of the crude product showed numerous signals in the range 7.20–8.40 ppm indicating the presence of Z-MeVal-OBt (**23**) as major product, estimated dipeptide yield <5%; after 16 h, Z-MeVal-OBt (**23**) was still present, estimated dipeptide yield 35% (7% epimerized); after reaction for 72 h and the usual workup, the dipeptide was purified by column chromatography, yield 58% (18% epimerized). Using method B, with PyBroP or PyCloP (and 1 mmol of MeVal-OMe,HCl), see Table 1.

[N-[[(9-Fluorenylmethyl)oxy]carbonyl]-N-methyl-L-valyl]-L-valine Methyl Ester (7). With PyBroP (and 1.0 mmol of Val-OMe,HCl), method B, reaction time 3 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.3) gave an oil (0.43 g, 92%): $[\alpha]_D$ −83° (c 1, EtOH); ^{1}H NMR (DMSO-d_6), several conformers, δ 0.75 (d, J = 6.5, 2 H), 0.83 (d, J = 6.9, 2 H), 0.86

(d, J = 6.5, 3 H), 0.88 (d, J = 6.5, 5 H), 1.95–2.11 (m, 2 H), 2.79 (s, 2.1 H), 2.83 (s, 0.9 H), 3.60 (s, 2.1 H), 3.64 (s, 0.9 H), 4.00–4.08 (m, 0.6 H), 4.10–4.22 (m, 0.6 H), 4.25–4.45 (m, 3.8 H), 7.31 (td, J = 7.4, J = 1.1, 2 H), 7.41 (br t, J = 7.3, 2 H), 7.62 (d, J = 7.1, 1.5 H), 7.70 (br d, 0.5 H), 7.89 (d, J = 7.4, 1.5 H), 8.02 (br d, 0.5 H), 8.29 (d, J = 7.2, 1 H); MS m/z 467. Anal. Calcd for $C_{27}H_{34}N_2O_5$: C, 69.51; H, 7.35; N, 6.00. Found: C, 69.03; H, 7.71; N, 6.08.

[*N*-[[(9-Fluorenylmethyl)oxy]carbonyl]-L-valyl]-*N*-methyl-L-valine Methyl Ester (8). With PyBroP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.5): oil (0.39 g, 84%); $[\alpha]_D$ −107° (c 0.14, EtOH); ^{1}H NMR (DMSO-d_6), several conformers, δ 0.72 (d, J = 6.7, 3 H), 0.84 (d, J = 6.8, 3 H), 0.92 (d, J = 6.4, 3 H), 0.93 (d, J = 6.5, 3 H), 1.92–2.05 (m, 1 H), 2.09–2.21 (m, 1 H), 2.75 (s, 0.1 H), 3.00 (s, 2.9 H), 3.48 (s, 0.1 H), 3.62 (s, 2.9 H), 4.19–4.36 (m, 4 H), 4.76 (d, J = 10.5, 1 H), 7.30 (m, 2 H), 7.41 (t, J = 7.4, 2 H), 7.66 (d, J = 8.5, 1 H), 7.70 (d, J = 7.4, 2 H), 7.88 (d, J = 7.5, 2 H); MS m/z 467. Anal. Calcd for $C_{27}H_{34}N_2O_5$: C, 69.51; H, 7.35; N, 6.00. Found: C, 69.29; H, 7.33; N, 5.85. With PyBOP (and 0.75 mmol of Fmoc-Val-OH), method A, reaction time 24 h, yield: 0.14 g (30%).

[*N*-[[(9-Fluorenylmethyl)oxy]carbonyl]-*N*-methyl-L-valyl]-*N*-methyl-L-valine Methyl Ester (9). With PyBroP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.6): oil (0.40 g, 83%); $[\alpha]_D$ −158° (c 1, EtOH); ^{1}H NMR (DMSO-d_6), four conformers (40:35:15:10), δ 0.06 (d, J = 6.8, 1.3 H), 0.11 (d, J = 7.0, 0.3 H), 0.34 (d, J = 6.4, 1.3 H), 0.47 (d, J = 6.6, 0.3 H), 0.55 (d, J = 6.7, 1.3 H), 0.58 (d, J = 6.8, 0.3 H), 0.67 (d, J = 6.7, 0.9 H), 0.68 (d, J = 7.1, 0.6 H), 0.70 (d, J = 6.9, 0.9 H), 0.73 (d, J = 6.6, 0.3 H), 0.79 (d, J = 6.4, 1.3 H), 0.82 (d, J = 6.8, 0.5 H), 0.87 (d, J = 6.5, 1.3 H), 0.92 (d, J = 6.5, 0.5 H), 0.93 (d, J = 6.5, 0.9 H), 1.81–1.91 (m, 0.5 H), 1.93–2.05 (m, 0.5 H), 2.08–2.28 (m, 1 H), 1.96 (s, 1.2 H), 2.35 (s, 0.3 H), 2.44 (s, 1.2 H), 2.52 (s, 0.4 H), 2.54 (s, 1.1 H), 2.69 (s, 0.4 H), 2.72 (s, 0.3 H), 2.84 (s, 1.1 H), 3.41 (s, 0.3 H), 3.42 (s, 0.2 H), 3.45 (s, 0.2 H), 3.56 (s, 1.1 H), 3.58 (s, 0.2 H), 3.60 (s, 1 H), 3.68 (d, $J \approx 10$, 0.1 H), 3.73 (d, $J \approx 10$, 0.1 H), 4.17 (d, J = 10.3, 0.1 H), 4.19 (d, J = 10.3, 0.1 H), 4.26–4.32 (m, 1.5 H), 4.39–4.62 (m, 2.0 H), 4.71 and 4.80 (AB of ABM, J = 10.5, J = 3.1, 1 H), 4.97, 5.02 (2 d, 0.1 H), 7.29–7.35 (m, 2 H), 7.37–7.44 (m, 2 H), 7.60–7.68 (m, 2 H), 7.87–7.91 (m, 2 H); MS m/z 481. Anal. Calcd for $C_{28}H_{36}N_2O_5$: C, 69.98; H, 7.55; N, 5.83. Found: C, 69.69; H, 7.74; N, 6.06. The diastereoisomeric purity (DL epimer ≤0.3%) was estimated by comparison with the ^{1}H NMR spectrum of the mixture 9 + 9′ (see below). Synthesis with PyCloP, see Table 1.

Fmoc-DL-MeVal-MeVal-OMe (9 + 9′). Coupling reaction was performed, during 3 h, on Fmoc-L-MeVal-OH (0.265 g, 0.75 mmol) with PyBroP, using method B, but without cooling and using DIEA (0.261 mL, 1.5 mmol) and DMAP (0.061 g, 0.5 mmol). Column chromatography (hexane/AcOEt (70:30), R_f 0.6) furnished an oil (0.14 g, 58%). ^{1}H NMR (DMSO-d_6) showed numerous additional signals besides those of 9, in particular for N-Me (δ 1.92, 2.40, 2.83, 2.87, 2.89) and for O-Me (δ 3.53, 3.66). The intensive signals δ 2.87 and 3.53 were absent in the spectrum of 9.

[*N*-(*tert*-Butyloxycarbonyl)-*N*-methyl-L-valyl]-L-valine Methyl Ester (10). With PyBOP and Boc-MeVal-OH (0.231 g, 1 mmol), method A, reaction time 1 h. Column chromatography (hexane/AcOEt (70:30), R_f 0.5) furnished (0.26 g, 76%): mp 61–63 °C; $[\alpha]_D$ −112° (c 1, EtOH); ^{1}H NMR δ 0.84 (d, J = 7.0), 0.85 (d, J = 6.7), 0.87 (d, $J \approx 8$), 0.91 (d, J = 6.5), total 12 H, 1.45 (s, 9 H), 2.09–2.18 (m, 2 H), 2.19–2.30 (m, 2 H), 2.76 (s, 3 H), 3.70 (s, 3 H), 3.95 (br, 0.2 H), 4.03 (br d, J = 10.6, 0.8 H), 4.49 (dd, J = 4.9, J = 8.9, 1 H), 6.15 (br, 0.2 H), 6.52 (br, 0.8 H); MS m/z 345. Anal. Calcd for $C_{17}H_{32}N_2O_5$: C, 59.28; H, 9.36; N, 8.13. Found: C, 59.29; H, 9.31; N, 8.08. Reaction with PyBroP (methods A and B), see Table 1.

[*N*-(*tert*-Butyloxycarbonyl)-L-valyl]-*N*-methyl-L-valine Methyl Ester (11). With PyBOP (and 0.5 mmol of Boc-Val-OH), method A, reaction time 24 h. The reaction mixture analyzed by HPLC (conditions 6), up to 7 h, showed the presence of Boc-Val-OBt (21) (t_R 11.60 and 15.80 min, see below). Column chromatography (hexane/AcOEt (80:20), R_f 0.4) furnished an oil (0.08 g, 45%): $[\alpha]_D$ −140° (c 0.25, EtOH); ^{1}H NMR (DMSO-d_6), two conformers, δ 0.74 (d, J = 6.7, 2.4 H), 0.81 (d, J = 6.7, 3.3 H), 0.83 (d, J = 7.0, ≈ 0.5 H), 0.89 (d, J = 6.7, 2 H), 0.92 (d, J = 6.5, 3.3 H), 0.96 (d, J = 6.5, ≈ 0.5 H), 1.35 (s, 8.1 H), 1.37 (s, 0.9 H), 1.84–1.96 (m, 1 H), 2.06–2.22 (m, 1 H), 2.62 (s, 0.3 H), 3.01 (s, 2.7 H), 3.61 (s, 3 H), 4.10 (t, J = 8.8, 1 H), 4.39 (d, J = 10, 0.1 H), 4.79 (d, J = 10.3, 0.9 H), 6.96 (d, J = 8.6, 1 H); MS m/z 345. Anal. Calcd for $C_{17}H_{32}N_2O_5$: C, 59.28; H, 9.36; N, 8.13. Found: C, 59.21; H, 9.48; N, 8.28. With PyBroP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h, TLC analysis (hexane/AcOEt (50:50)) of the crude product showed 17 ($R_f \approx 0$), NCA 15 (R_f 0.5), and dipeptide 11 (R_f 0.8). After column chromatography (hexane/AcOEt (80:20)), pure 11 (44%) was obtained. With PyCloP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h, TLC of the crude product was the same as in the case of the reaction with PyBroP; yield was 58% after column chromatography.

[*N*-(*tert*-Butyloxycarbonyl)-*N*-methyl-L-valyl]-*N*-methyl-L-valine Methyl Ester (12). With PyBroP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h. Column chromatography (hexane/AcOEt (70:30)) fournished two products: dipeptide 12 and Me-NCA 16. Dipeptide 12: oil (R_f 0.7); 0.12 g, 34%; $[\alpha]_D$ −200° (c 0.27, EtOH); ^{1}H NMR, four conformers, δ 0.77 (d, J = 6.8), 0.80 (d, J = 6.9), 0.81 (d, J = 6.9), 0.84 (d, J = 7.2), 0.84 (d, J = 6.6), 0.86 (d, J = 6.4), 0.87 (d, J = 6.5), 0.88 (d, J = 6.8), 0.98 (d, J = 6.5), 1.00 (d, J = 6.5), 1.01 (d, J = 6.5), total 12 H, 1.416 (s), 1.419 (s), 1.45 (s), 1.46 (s), total 9 H, 2.16–2.38 (m, 2 H), 2.59 (s, 0.5 H), 2.62 (s, 0.8 H), 2.73 (s, 0.5 H), 2.74 (s, 1.2 H), 2.77 (s, 0.8 H), 2.85 (s, 0.5 H), 3.00 (s, 0.5 H), 3.03 (s, 1.2 H), 3.64 (s), 3.65 (s), 3.656 (s), 3.66 (s), total 3 H, 4.22 (d, J = 10.5, 0.2 H), 4.32 (d, J = 10.3, 0.2 H), 4.41 (d, J = 10.2, 0.3 H), 4.57 (d, J = 10.5, 0.2 H), 4.65 (d, J = 10.8, 0.5 H), 4.81 (d, J = 10.4, 0.2 H), 4.82 (d, J = 10.5, 0.4 H); MS m/z 359. Anal. Calcd for $C_{18}H_{34}N_2O_5$: C, 60.31; H, 9.56; N, 7.81. Found: C, 60.41; H, 9.77; N, 7.61. N-Methylcarboxyanhydride 16 (see below): oil (R_f 0.4); 0.08 g (34% yield, calculated from Boc-MeVal-OH). With PyCloP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h: 12 (0.13 g, 36%); 16 (0.07 g, 30%, calculated from Boc-MeVal-OH). With PyBOP (and 1 mmol of Boc-MeVal-OH), method A, reaction time 24 h: HPLC (conditions 6) of the reaction mixture showed t_R 4.70 (16), 14.95 (24) and 17.70 min (12); ^{1}H NMR of the crude product (0.40 g) showed 12 (estimated yield 26%), N-carboxyanhydride 16 (estimated yield 26%), 17, and HOBt.

[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-*N*-methyl-L-valine Methyl Ester (13). With PyBroP (and 1 mmol of Boc-Pro-OH), method A, reaction time 1 h. Column chromatography (hexane/AcOEt (60:40), R_f 0.6) furnished (0.27 g, 79%): mp 51–53 °C; $[\alpha]_D$ −139° (c 1 EtOH); ^{1}H NMR, several conformers, δ 0.90 (d, J = 6.7), 0.91 (d, J = 6.7), 0.96 (d, J = 6.6), 1.00 (d, J = 6.6), total 6 H, 1.32 (s, 0.9 H), 1.39 (s, 3.6 H), 1.41 (s, 4.5 H), 1.79–1.89 (m, 2 H), 1.91–2.25 (3 m, 3 H), 2.91 (s, 0.1 H), 2.97 (s, 0.3 H), 2.98 (s, 1.2 H), 3.07 (s, 1.4 H), 3.32–3.66 (m, 2 H), 3.66 (s, 1.4 H), 3,67 (s, 1.2 H), 3.71 (s, 0.3 H), 3.75 (s, 0.1 H), 3.78 (d, J = 10.6, 0.1 H), 4.59 (m, 0.5 H), 4.67 (m, 0.5 H), 4.87 (d, J = 10.2, 0.4 H), 4.89 (d, J = 10.3, 0.5 H), minor signals were observed at 3.80 (d, $J \approx 10$) and 4.78 (m). Anal. Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18. Found: C, 59.51; H, 9.06; N, 8.24. The diastereoisomeric purity was studied by HPLC (conditions 3): 13, t_R 27.3 min; 13′, t_R 26.8 min; ≤0.1% 13′ was present. With PyCloP (and 1 mmol of Boc-Pro-OH), method A, reaction time 1 h, yield 85% (13′, ≤0.2%). With PyBOP (and 1 mmol of Boc-Pro-OH), method A, reaction time 1 h, ^{1}H NMR of the crude product (0.29 g) showed 13 (26% estimated yield), Boc-Pro-OBt (22) and 17.

[*N*-(*tert*-Butyloxycarbonyl)-D-prolyl]-*N*-methyl-L-valine Methyl Ester (13′). With PyBroP (and 1 mmol of Boc-Pro-OH), method A, reaction time 1 h. Column chroamtography (hexane/AcOEt (60:40), R_f 0.6) gave a solid (0.25 g, 73%): mp 121–123 °C; $[\alpha]_D$ −58° (c 1, EtOH); ^{1}H NMR, several conformers, δ 0.83 (d, J = 6.7, 2.5 H), 0.96 (d, J = 6.5, 3.3 H), 1.01 (d, J = 6.6, 0.2 H), 1.36 (s, 5.5 H), 1.38 (s, 0.5 H), 1.43 (s, 3 H), 1.78–1.88 (m, 2 H), 1.92–2.06 (m, 1 H), 2.15–2.27 (m, 2 H), 2.86 (0.3 H), 2.89 (s, 0.3 H), 3.01 (s, 1.6 H), 3.06 (s, 0.8 H), 3.35–3.65 (m, 2 H), 3.67 (s, 2.3 H), 3.71 (s, 0.7 H), 3.88 (d, J = 10.5, 0.1 H), 4.15 (d, J = 10.5, 0.1 H), 4.52 (br), 4.57 (dd, J = 8.0, J = 3.5), total 0.6 H, 4.63–4.76 (br), 4.70 (d, J = 10.2), total 0.65 H, 4.91 (d, J = 10.6,

0.55 H). Anal. Calcd for $C_{17}H_{30}N_2O_5$: C, 59.63; H, 8.83; N, 8.18. Found: C, 59.71; H, 8.92; N, 8.32. HPLC (see above) showed LL-epimer, ≤0.1%.

[*N*-(*tert*-Butyloxycarbonyl)-*N*-methyl-L-leucyl]-*N*-methyl-L-valine Methyl Ester (14). With PyCloP (and 0.826 mmol MeVal-OMe,HCl), method B, reaction time, 3 h. Column chromatography (hexane/AcOEt (80:20), R_f 0.45) gave an oil (0.30 g, 97%): $[\alpha]_D$ −177° (*c* 1, EtOH); ^{1}H NMR (DMSO-d_6), four conformers (35:35:15:15), δ 0.71–1.01 (12 H), 1.35 (s, 0.7 H), 1.40 (s, 6.5 H), 1.44 (s, 1.8 H), 1.30–1.65 (m, 3 H), 2.12–2.28 (m, 1 H), 2.45 (s, 0.5 H), 2.54 (s, 0.5 H), 2.64 (s, 1 H), 2.65 (s, 1 H), 2.69 (s, 0.5 H), 2.80 (s, 0.5 H), 2.96 (s, 1 H), 2.97 (s, 1 H), 3.60 (s, 0.6 H), 3.62 (s, 1.8 H), 3.65 (s, 0.6 H), 4.10 (d, J = 10.2, 0.15 H), 4.26 (d, J = 10.1, 0.15 H), 4.55 (d, J = 10.3, 0.65 H), 4.68 (d, J = 10, 0.05 H), 4.74 (≈t, J ≈ 7, 0.15 H), 4.86 (m, 0.35 H), 4.91–5.30 (2 m, 0.5 H); MS *m/z* 373. Anal. Calcd for $C_{19}H_{36}N_2O_5$: C, 61.26; H, 9.74; N, 7.52. Found: C, 61.25; H, 9.65; N, 7.51. The diatereoisomeric purity (DL-epimer, ≤0.6%) was determined by comparison with the ^{1}H NMR spectrum of the DL isomer 14′; measurements were performed on the *O*-Me and *N*-Me signals.

[*N*-(*tert*-Butyloxycarbonyl)-*N*-methyl-D-leucyl]-*N*-methyl-L-valine Methyl Ester (14′). With PyBroP (and 1 mmol of MeVal-OMe,HCl), method B, reaction time 3 h. Column chromatography (hexane/AcOEt (80:20), R_f 0.45) gave an oil (0.33 g, 88%): $[\alpha]_D$ −2° (*c* 1, EtOH); ^{1}H NMR (DMSO-d_6), four conformers, δ 0.70 (d, J = 6.7, 0.8 H), 0.73 (d, J = 6.6, 0.6 H), 0.78 (d, J = 6.5, 1.5 H), 0.81–0.92 (7.6 H), 0.95 (d, J = 6.5, 1.5 H), 1.40 (s, 4.5 H), 1.43 (s, 2.6 H), 1.46 (s, 1.9 H), 1.28–1.68 (m, 3 H), 2.13–2.27 (m, 1 H), 2.54 (s, 0.5 H), 2.59 (s, 1.1 H), 2.64 (br s, 1.4 H), 2.71 (s, 1.1 H), 2.79 (s, 0.5 H), 2.92 (s, 0.7 H), 2.93 (s, 0.7 H), 3.61 (s, 1.5 H), 3.68 (s, 1 H), 3.69 (s, 0.5 H), 4.07 (d, J = 10.5, 0.2 H), 4.34 (d, J = 10.6, 0.4 H), 4.58 (d, J = 10.3, 0.2 H), 4.63 (d, J = 10.4, 0.2 H), 4.82 (t, J = 7.3, 0.3 H), 4.99 (m, 0.4 H), 5.2 (t, J = 7.2, 0.3 H); LL-epimer, ≤0.6%. Anal. Calcd for $C_{19}H_{36}N_2O_5$: C, 61.26; H, 9.74; N, 7.52. Found: C, 61.47; H, 9.76; N, 7.63.

4-Isopropyl-1,3-oxazolidine-2,5-dione (15). To Val-OH (5.86 g, 50 mmol) in anhydrous THF (100 mL) was added, under N_2, a 1.93 M solution of phosgene in toluene (52 mL, 100 mmol). The stirred mixture was heated at 45–50 °C (1 h). After elimination of the phosgene under a N_2 stream, the solvent was evaporated in vacuo. The residue was dissolved in AcOEt; addition of hexane gave white crystals (2.1 g, 30%): mp 63–64 °C (lit.[49] mp 58–59 °C); ^{1}H NMR δ 1.00 (d, J = 6.8, 3 H), 1.07 (d, J = 6.9, 3 H), 2.23 (d hept, J = 4.3, J = 6.9, 1 H); 4.19 (dd, J = 4.3, J = 0.95, 1 H); 6.56 (br s, 1 H); IR ν 3455 (m), 1850 (s), 1780 (vs).

3-Methyl-4-isopropyl-1,3-oxazolidine-2,5-dione (16). To Boc-MeVal-OH (0.924 g, 4 mmol) and a 1.93 M solution of phosgene in toluene (2.2 mL, 4.2 mmol) in DCM (6 mL) was added, under stirring at 0 °C, DIEA (0.696 mL, 4 mmol). After the solution was stirred for 1 h at rt, the solvents were evaporated in vacuo and the residue was dissolved in AcOEt. After the usual workup, evaporation in vacuo produced an oil (0.52 g, 82%): TLC (AcOEt/hexane (30:70)) R_f 0.65; $[\alpha]_D$ −8° (*c* 1, DCM); HPLC (conditions 6) t_R 4.70 min; ^{1}H NMR δ 0.97 (d, J = 7.0, 3 H), 1.17 (d, J = 7.0, 3 H), 2.26 (d hept, J = 3.4, J = 7.0, 1 H), 2.97 (s, 3 H), 3.99 (d, J = 3.4, 1 H); ^{13}C NMR δ 15.99; 17.08; 28.46; 28.64; 66.05; 152.17; 167.49; IR ν 1846 (m), 1778 (s). Anal. Calcd for $C_7H_{11}NO_3$: C, 53.49; H, 7.05; N, 8.91. Found: C, 54.24; H, 7.80; N, 8.17.

Tripyrrolidino[(mesitylcarbonyl)oxy]phosphonium Hexafluoroantimonate (18a). 2,4,6-Trimethylbenzoic acid (5.0 g, 30,5 mmol) and thionyl chloride (7.26 g, 61,0 mmol) were refluxed 5 h under N_2. Distillation of the reaction mixture, under N_2, furnished 5.0 g (90%) of the acyl chloride; bp 87 °C (2 mmHg). According to Teichmann's method,[30] to a cold (−20 °C) solution of 2,4,6-trimethylbenzoyl chloride (1.82 g, 10 mmol) and tripyrrolydinophosphine oxide (17)[69] (2.57 g, 10 mmol) in anhydrous DCM (20 mL) was added dropwise, under N_2, a solution of $SbCl_5$ (2.99 g, 10 mmol) in anhydrous DCM (10 mL). The mixture was stirred for 18 h at rt. The solvent was removed under reduced pressure; the residue washed with anhydrous ether furnished, after filtration, a solid (5 g, yellow). The solid was dissolved in AcOEt and precipitated with ether and the operation was repeated (3 times), giving 1.6 g (22%) of white crystals: mp 83–86 °C; ^{31}P NMR δ 22.0 (s, relative intensity I = 74), impurities were observed at δ 22.4 (s, I = 11, unknown) and 10.7 (s, I = 15, 17); MS *m/z* 404; MS FAB (neg mode) *m/z* 331, 333, 335, 337, 339, 341, 343, 345 (relative intensities 39, 92, 100, 64, 25, 7, 3, 2). Anal. Calcd for $C_{22}H_{35}Cl_6N_3O_2PSb$: C, 35.75; H, 4.77; Cl, 28.78; N, 5.68; O, 4.33. Found: C, 35.62; H, 4.92; Cl, 28.36; N, 5.67; O, 4.69.

^{1}H NMR Observation of Tripyrrolidino[(mesitylcarbonyl)oxy]phosphonium Hexafluorophosphate (18b). From PyCloP: 2,4,6-trimethylbenzoic acid (0.164 g, 1 mmol) and PyCloP (0.422 g, 1 mmol) were dissolved in $CDCl_3$ (1 mL); DIEA (0.348 mL, 2 mmol) was added at 0 °C; after 10 min, the ^{31}P NMR spectrum, recorded at 0 °C, showed δ 36.3 (s, relative signal intensity I = 10, PyCloP), 22.3 (s, I = 5, **18b**), 14.7 (s, I = 2, 17), −143.9 (hept, J = 713, PF_6^-); after 20 min at the same temperature, δ 36.3 (I = 10), 22.3 (I = 3), 14.4 (I = 5). From PyBOP: 2,4,6-trimethylbenzoic acid (0.164 g, 1 mmol) and PyBOP (0.52 g, 1 mmol) were dissolved in $CDCl_3$ (1 mL); DIEA (0.348 ml, 1 mmol) was added at −20 °C; after 10 min, the ^{31}P NMR spectrum, recorded at −20 °C, showed δ 31.3 (s, I = 10, PyBOP), 22.4 (s, I = 1, **18b**), 14.2 (s, I = 6, 17), −143.8 (hept, J = 713, PF_6^-); after 20 min, the temperature was increased to 0 °C; after 10 additional min at 0 °C, δ 31.7 (I = 10), 22.3 (I = 0.5), 14.0 (I = 17); 20 min further, temperature was increased to 24 °C, δ 31.8 (I = 10), 13.9 (I = 120).

Activation Reactions with PyBOP. General conditions are described for Z-Val-OH activation. For the other compounds, see supplementary material.

***N*-(Benzyloxycarbonyl)-L-valine 1*H*-Benzotriazol-1-yl Ester (19).** To a solution of Z-Val-OH (0.126 g, 0.5 mmol) and PyBOP (0.260 g, 0.5 mmol) in DCM (0.5 mL) was added DIEA (0.087 mL, 0.5 mmol). After being stirred for 30 min at rt, the reaction mixture was diluted in AcOEt (50 mL) and worked up as usual. An oil (0.310 g) was obtained. HPLC (condition 6) showed two pics: t_R 12.3 min (intensity I = 1), UV λ_{max} 247, 315 (sh), 327, 340, t_R 16.1 min (I = 3), UV λ_{max} 254, 283; ^{1}H NMR showed 17 (δ 1.80 (m), 3.12 (m)), Z-MeVal-OH (traces), and **19** (δ 1.15 (d, J = 6.8, 3 H), 1.20 (d, J = 6.8, 3 H), 2.41 (hept, J = 6.8, 1 H), 4.64 (≈ t, J = 6.8, 1 H), 5.17 (br s, 2 H), 5.48 (br d, J = 7.0, 1 H), 7.20–8.40 (numerous signals, 9 H)); IR ν 3442 (w), 1818 (m), 1725 (vs).

Activation of *N*-Protected Amino Acids with PyCloP. Reference Compounds. Anhydrides **25**,[42] **26**,[43] **27**,[44] and **28**[42] are known. We synthesized them using DCC (more or less DCU was present in the obtained products).

Bis-(*S*)-[*N*-(benzyloxycarbonyl)-*N*-methyl-2-amino-3-methylbutanoic] Anhydride (29). Synthesized according to Benoiton's method.[42] To Z-MeVal-OH (1.060 g, 4 mmol) in DCM (20 mL) was added EDC (2 mmol). After the solution was stirred for 2 h at rt, the solvent was evaporated in vacuo; the residue was dissolved in AcOEt and washed with cold solutions of 10% citric acid, brine, 5% HCO_3Na, and brine. After being dried over Na_2SO_4, the solution was evaporated in vacuo to an oil (0.90 g, 87%): $[\alpha]_D$ −86° (*c* 1, DCM); ^{1}H NMR (several conformers) δ 0.9–1.05 (4 d, 12 H), 2.20 (m, 2 H), 2.86, 2.89 and 2.90 (3 s, relative intensities 25, 47, 28, 6 H), 4.16 (d, J = 10.0, 0.25 H), 4.27 (d, J = 10.0, 0.55 H), 4.51 (m, 1.2 H), 5.13 (m, 4 H), 7.25 – 7.40 (10 H); IR ν (cm^{-1}) 1821 (s), 1782 (vw), 1745 (sh), 1698 (vs). Anal. Calcd for $C_{28}H_{36}N_2O_7$: C, 65.61; H, 7.08; N, 5.46. Found: C, 65.75; H, 7.31; N, 5.33. This product contained **16** (IR; ^{1}H NMR, ≈1%) and Z-MeVal-OH (^{1}H NMR, ≈1%). Using DCC, **16** was not observed, but DCU was present.

Bis-(*S*)-[*N*-[[(9-fluorenylmethyl)oxy]carbonyl]-*N*-methyl-2-amino-3-methylbutanoic] Anhydride (30). From Fmoc-MeVal-OH (1.059 g, 3 mmol), using EDC as for **29** synthesis, a foam (0.69 g, 67%) was obtained: $[\alpha]_D$ −108° (*c* 2, DCM); ^{1}H NMR (several conformers) δ 0.6–1.05 (12 H), 2.07 (m, 0.6 H), 2.19 (m, 1.4 H), 2.81, 2.83, 2.86, 2.87 (4 s, relative intensities 1, 1, 2, 2, 6 H), 3.9–4.6 (8 H), 7.26 (m, 4 H), 7.35 (m, 4 H), 7.53 (≈ d, 4 H), 7.73 (m, 4 H); IR ν 1822 (m), 1751 (sh), 1699 (s). Anal. Calcd for $C_{42}H_{44}N_2O_7$: C, 73.24; H, 6.44; N, 4.07. Found: C, 72.00; H, 6.84; N, 4.61.

Bis-(*S*)-[*N*-(*tert*-butyloxycarbonyl)-*N*-methyl-2-amino-3-methylbutanoic] Anhydride (31). Boc-MeVal-OH (0.462 g, 2 mmol) and DCC (0.206 g, 1 mmol) in DCM (2 mL) were stirred for 30 min at 0 °C. After the mixture was allowed to stand 2 h at −30 °C, DCU was filtered and the solvent evacuated in vacuo. An oil (0.39 g) was obtained: ^{1}H NMR ($CDCl_3$) δ 0.87 (br, 6 H),

1.01 (d, J = 6.5, 6 H), 1.42 (s, 18 H), 2.22 (br, 2 H), 2.79 (s, 3 H), 2.85 (s, 3 H), 3.90–4.05 (br, 1 H), 4.47 (br, 1 H); IR ν 1818 (m), 1745 (w), 1687 (s). NCA **16** was present (14%): ^{1}H NMR δ 0.96 (d), 1.16 (d), 2.97 (s), 3.99 (d); IR ν 1842 (vw), 1780 (m). For the same reaction (solvent being $CDCl_3$), when the reaction mixture was only filtered (solvent was not evacuated) and the ^{1}H NMR spectrum immediately recorded, only 2.5% of NCA **16** was present.

(Boc-MeVal)$_2$O Reactivity Versus MeVal-OMe. Boc-MeVal-OH (0.231 g, 1 mmol) and DCC (0.0515 g, 0.5 mmol) in $CHCl_3$ (1 mL) were stirred for 30 min at 0 °C. The IR spectrum of the reaction mixture was in accordance with **31** containing traces of NCA **16** (ν 1780 (vw)). MeVal-OMe·HCl (0.091 g, 0.5 mmol) and DIEA (0.174 mL, 1 mmol) were added at rt. After 5 h reaction, the IR spectrum still showed **31** (IR, ν 1818) and an increased proportion of **16** (ν 1780 (m)). After 6 h, to confirm that **31** was still present, excess pyrrolidine was added, and the IR spectrum showed the immediate disappearance of the 1818 cm^{-1} absorption band. After filtration and the usual workup, column chromatography (hexane/AcOEt (80:20)) furnished **12** (0.045 g, 25%) and an oil (0.035 g) whose ^{1}H NMR spectrum was consistent with Boc-MeVal pyrrolidinylamide (≈30% yield) containing NCA **16** (≈5% yield).

General Procedure of Activation. To a solution of the amino acid (0.5 mmol) and of PyCloP (0.211 g, 0.5 mmol) in 0.5 mL of $CHCl_3$ (IR study) or $CDCl_3$ (^{1}H NMR study) was added DIEA (0.174 mL, 1 mmol). Spectra of the activation mixture were recorded after dilution to 0.1 M (IR) or 0.2 M (^{1}H NMR). See supplementary material.

Acknowledgment. We are indebted to ANVAR for financial support. We would like to thank Professor Bertrand Castro for the interest he showed for this work. We are grateful to Dr. Lynn Salhi for her help in the revision of this manuscript.

Supplementary Material Available: ^{1}H NMR, IR, UV, and HPLC data of compounds **20–24**, the PyBOP(BOP, DCC/HOBt)-activation reaction of Z-MeVal-OH, and the PyBOP(DCC/HOBt, HBTU)-activation reaction of Boc-MeVal-OH, ^{1}H NMR and IR data of compounds **25–28** and **32**, the PyCloP-activation mixture of Z(Fmoc, Boc)-Val-OH, Z(Boc)-MeVal-OH, Boc-Ala-OH, Boc-Leu-OH, Boc-Pro-OH, Boc-MeAla-OH, and Boc-MeLeu-OH, and the PyBroP-activation mixture of Boc-MeVal-OH (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.