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Oxybenzotriazole Free Peptide Coupling Reagents for N-Methylated Amino Acids

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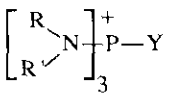
Abstract: *The main product of N-methylated amino acid coupling, when oxybenzotriazole is present in the reagent (BOP, PyBOP, HBPyU) or as an additive (DCC/HOBt), is the weakly reactive benzotriazolyl ester. On the other hand, the corresponding new halogenated reagents PyBroP, PyCloP and PyClU give very good results and can be recommended.*

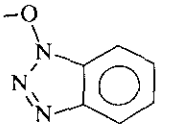
The coupling of N-methylated amino acids is a difficult reaction in which the usual reagents are often inefficient[1-5]. These amino acids are present in many natural peptides exhibiting important biological properties, such as cyclosporines[3] and some pseudopeptides of marine origin including didemnins[6] and dolastatins[7]. The latter are currently being synthesized in our laboratory, which led us to test new coupling reagents.

In a previous study[1], we showed that the reagent BroP **2**[8], unlike BOP **1**[9], allows easy coupling of N-methylated amino acids. This reagent compares favorably with BOP-Cl [4,5,10,11] and Dpp-Cl[12] and has the advantage of being stable and easy to use. However, like BOP, it yields HMPT during the coupling reaction, whose toxicity (see[13]) could make its use impossible.



PF_6^-

$R = R' = CH_3$	Y = OBt	BOP	**1**
$R = R' = CH_3$	Y = Br	BroP	**2**
$R , R' = (CH_2)_4$	Y = OBt	PyBOP	**3**
$R , R' = (CH_2)_4$	Y = Br	PyBroP	**4**
$R , R' = (CH_2)_4$	Y = Cl	PyCloP	**5**



OBt

BOP can be favorably replaced by its pyrrolidino homologue, PyBOP® **3**[13], in which case the reaction produces tripyrrolidino phosphine oxide, an *a priori* noncarcinogenic by-product. This prompted us to study the coupling of N-methylated amino acids by pyrrolidino homologues of BroP: PyBroP **4**[14] (bromo tripyrrolidino phosphonium hexafluorophosphate) and PyCloP **5**[14] (chloro tripyrrolidino phosphonium hexafluorophosphate).

Z-MeVal-Val-OMe	Z-Val-MeVal-OMe	Boc-Pro-MeVal-OMe	Z-MeVal-MeVal-OMe
6	**7**	**8**	**9**

Table I : Coupling reactions[a] - % yield (% epimerization)[b]

Peptide	PyBOP	PyBroP	PyCloP	HBPyU	PyClU
6	90 (0.2)	85 (0.3)	87 (0.15)	87 (0.15)	90 (0.2)
8	26 (-)[c]	79 (0)	85 (0.2)	21(-)[c]	82 (0.15)
9	<5 [c] 58 (18)[d]	61(≤ 0.3) 87 (≤ 0.3)[e]	59 (≤ 0.2)	<5 [c] 64 (13)[d]	66 (≤ 0.3)

(a) Unless otherwise indicated, DIEA (3 eq) was added to a mixture (cooled to 0°C) of N-protected amino acid (1 eq), C-protected amino acid chlorhydrate (1.1 eq), and coupling reagent (1 eq) in CH_2Cl_2 (1 ml/mmol), and stirred for 1 min cold and for 1 h at room temperature. N-methylated amino acids were obtained as described in[21]. Elemental analysis and ^{1}H-NMR spectra were consistent with the structures proposed.

(b) Yields after column chromatography. Epimerization percentages were determined in crude products by comparison with the dipeptide diastereoisomer[22] using HPLC (compounds **6** and **8**), and ^{1}H-NMR (360 MHz) (compound **9**).

(c) The main product was the benzotriazolyl ester of the N-protected amino acid[18].

(d) Reaction time: 72 h.

(e) 1.5 eq of N-protected amino acid, 1 eq of C-protected amino acid chlorhydrate, 1.5 eq of PyBroP, and 4 eq of DIEA. Reaction time 3 h.

*Table II : Z-Val-MeVal-OMe **7** - % yield (% epimerization)[a]*

Reaction time (h)	Py BOP	PyBroP	PyCloP	HBPyU	PyClU	DCC/ HOBt	DCC
1	11[b](-)	70 (0) 100 (0.15)[d]	85 (-)	12[b](-)	57 (0)	8[b](-)	78(-)
24	76 (0)[c]	96 (0.5)	96 (0.15)	77 (0)[c]	100 (0.8)	20 (0)[c]	87 (< 0.1)

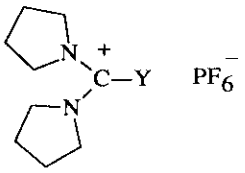
(a) Same conditions as (a) in Table I. In the case of DCC and DCC/HOBt : 1 eq of DIEA. Yield (internal standard) and epimerization (comparison with dipeptide diastereoisomer[22]) were determined by HPLC in the reaction mixture.

(b) Main product: benzotriazolyl ester of Z-Val.

(c) A small % of benzotriazolyl ester remained. After total disappearance of this ester, the following yields were obtained: PyBOP: 81% (48 h); HBPyU : 77% (90 h); DCC/HOBt : 22% (48 h)[19].

(d) Excess reagent, reaction time 3 h (see (e) in Table I).

The formation of the amide bond between the carboxyl of an N-methylated amino acid and the primary amine of a C-protected amino acid does not present any particular difficulties: the reagents PyBOP, PyBroP and PyCloP showed comparable efficiency in the synthesis of dipeptide **6** (Table I). In contrast, when the C-protected amino acid was N-methylated or when both amino acids were N-alkylated (compounds **7**, **8**, **9**: Tables I and II), PyBOP gave very low yields whereas PyBroP and PyCloP remained efficient. With PyBOP, yields were only moderate after 24 h (**7**: 76%) or 72 h (**9**: 58%) and there was even 18% epimerization in the last case. On the other hand, with the brominated homologue PyBroP **4**, yields were excellent after 24 h of reaction (compound **7**: 96%) and even after only 3 h when excess (1.5 eq) N-protected amino acid and reagent were used (compounds **7**: 100% and **9**: 87%). In most cases epimerization was absent or negligible (≤ 0.3%).



Y = OBt HBPyU **10**

Y = Cl PyClU **11**

These results are similar to those we previously obtained[1] when we compared the reagents BOP and BroP[15]. They confirm the hypothesis of a deleterious effect of hydroxybenzotriazole in the coupling of N-methylated amino acids, which has previously been observed for BOP-Cl[5]. To show the generality of this effect, we compared DCC/HOBt with DCC and HBPyU **10** with PyClU **11**. Compounds **10** (2-(1H-benzotriazol-1-yl)-1,1,3,3-bis(tetramethylene)-uronium hexafluorophosphate) and **11** (1,1,3,3-bis(tetramethylene)-chlorouronium hexafluorophosphate) are new reagents in the HBTU family[16], which we obtained by classical methods[17]. PyBOP, HBPyU and DCC/HOBt showed low efficiency in the synthesis of dipeptide **7** (Table II). The benzotriazolyl ester of Z-Val[18] was formed rapidly and its reactivity was weak. Even with long reaction times, the yields were unsatisfactory[19]. In the formation of dipeptides **8** and **9**, HBPyU and PyBOP behaved in a similar manner (Table I). On the other hand, with DCC or PyClU, as with PyBroP or PyCloP (i.e. in the absence of hydroxybenzotriazole), the yields of dipeptide **7** were high. This result was confirmed with PyClU in the synthesis of compounds **8** and **9** (Table I). Moreover, comparable results obtained for compound **6** with HBPyU and PyClU (Table I) confirm that the coupling reaction easily occurs if only the N-terminal amino acid is N-methylated.

To summarize, the weak reactivity of the benzotriazolyl ester which is formed is responsible for the deficient coupling of N-methylated amino acids carried out in the presence of HOBt or reagents containing an oxybenzotriazole residue. In contrast, the halogenated reagents PyBroP, PyCloP, and PyClU proved to be efficient in these couplings. Moreover, these compounds are solid, stable, and easy to use. PyBroP has the advantage of being commercially available[20]. Lastly, these results raise the problem of the utility of HOBt in peptide coupling reactions carried out on an amino acid protected in carbamate form.

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15. In the formation of compound **7**, the difference in yields between BOP (67%)[1] and PyBOP (11%) [present communication] can be attributed to different experimental conditions. Under the conditions used here (Table I), a yield of 10% was obtained with BOP after 1 h of reaction, and the main product was the benzotriazolyl ester. Compound **7** was then obtained with a 73% yield after 24 h of reaction.
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17. Compound **10** (F = 200-2°C(dec)) was obtained by the action of HOBt/NEt_3 on compound **11**. We designate it as HBPyU to comply with[16]. Its homologue tetrafluoroborate (TBPyU) was recently described by Henklein, P.; Beyermann, M.; Bienert, M.; Knorr, R. (Poster at the 21st European Peptide Symposium, Platja d'Aro, Spain, September 2-8, 1990). Compound **11** (F = 151-3°C) was obtained by the action of $POCl_3$ and then KPF_6/H_20 on the corresponding urea. We designate it as PyClU (**Py**rrolidino **Cl**oro**U**ronium) to comply with names used for homologues of the BOP family. This reagent is not hygroscopic, in contrast with the corresponding chloride (Henklein, P.; Beyermann, M.; Bienert, M.; Knorr, R. Poster at the 21st European Peptide Symposium, Platja d'Aro, Spain, September 2-8, 1990). Elemental analysis, Fab MS, and ^{1}H-NMR of compounds **10** and **11** are consistent with the proposed structures.
18. The benzotriazolyl esters were obtained by the action of BOP (1 eq) and DIEA (2 eq) on the N-protected amino acid, followed by the usual treatment. Although these esters are sensitive to hydrolysis they were detected by TLC, HPLC and ^{1}H NMR.
19. The very low yield obtained with DCC/HOBt could be due to hydrolysis of the benzotriazolyl ester by water contained in commercial HOBt.
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22. The diastereoisomers of compounds **6**, **7** and **9** have previously been obtained[1]; that of compound **8** was synthesized with PyBroP.

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