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BROP : A NEW REAGENT FOR COUPLING N-METHYLATED AMINO ACIDS

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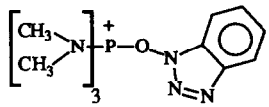
Summary: BroP (bromo tris(dimethylamino) phosphonium hexafluorophosphate) is a particularly suitable reagent for coupling N-methylated amino acids. It is stable and gives very high yields in short reaction times. Dipeptides are obtained without appreciable epimerization.

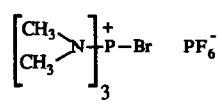
N-methylated amino acids are found in many natural peptides with interesting biological properties [1], such as cyclosporines [2] and didemnins [3].

Coupling N-methylated amino acids is difficult, and many peptide coupling reagents are ineffective [4a,5a]. For instance, BOP **1** [6], which is known to be one of the best peptide coupling reagents (see [7]), has been used in cyclosporine synthesis [1,8a], but is often ineffective [3,4a]. On the other hand, BOP-Cl [4, 5a] and Dpp-Cl [8b] give good results, but they too have certain disadvantages: long reaction times, preactivation at 0°C, and in the case of BOP-Cl, the storage and reaction conditions are restrictive, due to the relative instability of the reagent [5b]. It was therefore of interest to find other coupling reagents.

Since the work of König and Geiger [9], "non-N-methylated" amino acids have generally been coupled in the presence of hydroxybenzotriazole (HOBt), as in the case of DCC/HOBt. Similarly, Anteunis has obtained excellent results with BOP-Cl/HOBt [10]. It is thus surprising to find that in the case of N-methylated amino acids, BOP-Cl and Dpp-Cl are used without adding HOBt. Moreover, Anteunis has shown that for the latter couplings the addition of HOBt to BOP-Cl results in poor yields [5a]. Consequently, HOBt appears to be useless or even inadvisable in coupling N-methylated amino acids, and in the case of BOP **1**, the presence of the OBt residue in the molecule may produce the poor performances observed. Because of this, we decided to investigate the use of compound **2** . This brominated homologue



BOP **1**



BroP **2**

of BOP, which we designate as BroP (**Bro**mo tris(dimethylamino) **P**hosphonium hexafluorophosphate), is obtained from tris(dimethylamino) phosphine [11].

As can be seen in Table 1, identical results were obtained with BOP and BroP when two "non-N-methylated" amino acids were coupled or if only one of them was N-methylated (entries 1 to 8). On the other hand, when both amino acids were N-substituted, BroP still produced very good results, but BOP gave very poor yields (entries 9-10 and 11-12). The excellent behavior of BroP was confirmed by reactions 14-16.

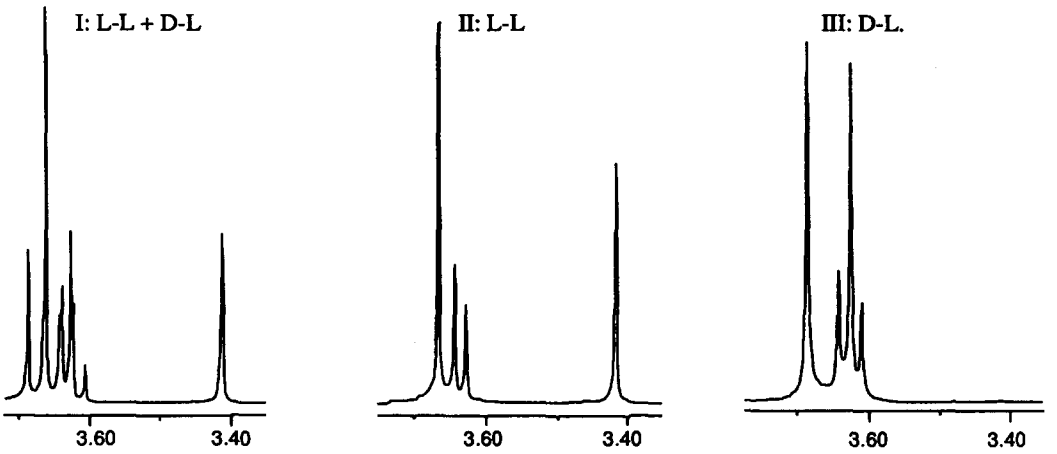
Table 1 : Coupling reactions with BOP and BroP [a]

Entry	Peptide	Reagent	Yield %	mp°C	$[\alpha]_D^{20}$ (c=1, MeOH)
1	Boc-Phe-Gly-OEt	BOP	88[b,c]	87-88[c]	-5 [c]
2	Boc-Phe-Gly-OEt	BroP	89[b]	87-88[c]	-5 [c]
3	Z-Val-MeVal-OMe	BOP	67	oil	-63
4	Z-Val-MeVal-OMe	BroP	71	"	-63
5	Z-D-Val-MeVal-OMe	BroP	80	"	-55
6	Z-MeVal-Val-OMe	BOP	87	"	-95
7	Z-MeVal-Val-OMe	BroP	90	"	-95
8	Z-D-MeVal-Val-OMe	BroP	85	"	+73
9	Boc-Pro-MeVal-OMe	BOP	25[d]	-	-
10	Boc-Pro-MeVal-OMe	BroP	82	54-55	-140
11	Z-MeVal-MeVal-OMe	BOP	5[e]	-	-
12	Z-MeVal-MeVal-OMe	BroP	70	oil	-195
13	Z-D-MeVal-MeVal-OMe	BroP	80	"	+2
14	Z-MeLeu-MeVal-OMe	BroP	89	"	-159
15	Z-D-MeLeu-MeVal-OMe	BroP	89	"	-11
16	Boc-Pro-MeLeu-OMe	BroP	81	112-114	-85

(a) One pot reaction for 1 h at room temperature: 1 equivalent of N-protected amino acid, 1.1 equivalent of C-protected amino acid hydrochloride, 1 equivalent of BroP (BOP), CH_2Cl_2 (1 cc/mmol), 3 equivalents of DIEA. Usual treatment and purification by chromatography on a silica gel column. $[\alpha]_D$ and mp concern the non-recrystallized compounds. Structures were verified by ^1H NMR.
(b) Reaction time: 30 min.
(c)$[\alpha]_D$: c = 2 (EtOH); litt.: see [7].
(d) Compound contaminated by hydroxybenzotriazolyl ester .
(e) Yield estimated on the basis of the ^1H NMR spectrum of the crude product.

In all cases, dipeptides were obtained without appreciable epimerization, as seen by comparing ^1H NMR spectra of diastereoisomer pairs. In all these comparisons, signals can be found with chemical shifts that differ in the two isomers. This is true for the OMe groups, whose sharp signals allow a precise observation of the absence of epimerization. As an example, Figure 1 shows the resonance signals of the OMe group in the two isomers L-L (spectrum II) and D-L (spectrum III) of Z-MeVal-MeVal-OMe obtained in experiments 12 and 13 (spectrum I shows the mixture of the two isomers).

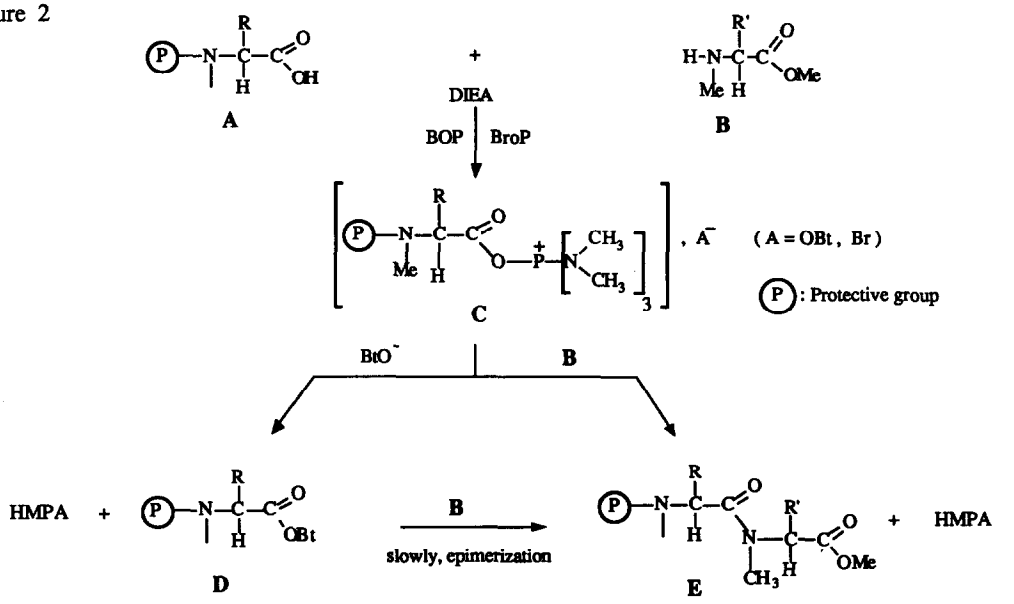
Figure 1: ^1H NMR spectra (δ:ppm, $CDCl_3$, 360 MHz). OMe signals of Z-(Me)Val-(Me)ValOMe [19].



In the case of BOP in experiments 9 and 10, the main product of the reaction was the active ester of oxybenzotriazole. This product is probably (based on ^1H NMR and IR spectra) a mixture of O and N-acylated forms [12]. This ester is not particularly reactive [13]: in the case of coupling 11, only 40% dipeptide was obtained after 16 h of reaction, and it was 35% epimerized. Thus, the poor results obtained with BOP can be explained by the formation and low reactivity of the "active" ester.

The following mechanism can be proposed (Fig. 2). In both cases (BOP and BroP) it is likely that a

Figure 2



transient acyloxyphosphonium salt **C** is first obtained by a reaction of amino acid **A** with the reagent [14]. In the case of BOP, two nucleophiles are in competition: the oxybenzotriazolyl anion and the amino group of amino acid **B**. Trapping of intermediate **C** by amino acid **B** is impeded by steric hindrance and higher reactivity of BtO^-: the oxybenzotriazolyl anion reacts to produce active ester **D**. For the same reasons, the latter is only slowly aminolyzed by **B**. The slowness of the reaction allows epimerization to occur, probably by the usual mechanism via oxazolone [15]. In the case of BroP, the BtO^- anion is absent and aminolysis of **C** results in the dipeptide **E**. It is also possible that other reaction intermediates are formed, such as acyl halide or symmetric anhydride [16].

These results raise a question as to the exact role of HOBt in peptide coupling reactions [17]. The problem has been discussed by Benoiton [18].

To summarize, BroP is an excellent reagent for coupling N-methylated amino acids. Its stability, ease of use, and the rapidity of the coupling reaction make it a very effective reagent [20]. It is currently being developed, along with its homologues, for use in other difficult coupling reactions (for ex. α, α'-disubstituted amino acids). The reaction mechanisms are also being investigated.

References and notes.

1- Yu. A. Ovchinnikov and V.T. Ivanov, Tetrahedron, 1975, **31**, 2177.
2- R.M. Wenger, Ang. Chem. Int. Ed., 1985, **24**, 77.
3- P. Jouin, J. Poncet, M-N. Dufour, A. Pantaloni and B. Castro, J. Org. Chem., 1989, **54**, 617.
4- a) R.D.Tung and D.H. Rich, J. Am. Chem. Soc. 1985, **107**, 4342.
b) R.D. Tung, M.K. Dhaon and D.H. Rich, J. Org. Chem., 1986, **51**, 3350.
5- a) C. Van der Auwera and M.J.O. Anteunis, Int. J. Peptide Protein Res., 1987, **29**, 574.
b) C. Van der Auwera and M.J.O. Anteunis, Bull. Soc. Chim. Belg., 1986, **95**, 203.
6- B. Castro, J-R. Dormoy, G. Evin and C. Selve, Tetrahedron Letters, 1975, 1219.
7- J. Coste, D. Le-Nguyen and B. Castro, Tetrahedron Letters, submitted.
8- a) I.J. Galpin, A.K.A. Mohammed and A. Patel, Tetrahedron, 1988, **44**, 1783.
b) I.J. Galpin, A.K.A. Mohammed and A. Patel, Tetrahedron, 1988, **44**, 1685.
9- W. König and R. Geiger, Chem. Ber., 1970, **103**, 788.
10- C. Van der Auwera, S.Van Damme and M.J.O. Anteunis, Int. J. Peptide Protein Res., 1987, **29**, 464.
11- B. Castro and J-R. Dormoy, Tetrahedron Letters, 1973, 3243.
12- Two absorptions (1820 and 1730 cm^{-1})were observed in the IR spectrum, corresponding to those described in [9] for the acylated O and N forms of oxybenzotriazole.
13- A similar observation was made by M.J. O Anteunis [5a, foot note p. 579] and by M.J.O. Anteunis and N.K. Sharma [Bull. Soc. Chim. Belg., 1988, **97**, 281, note 41].
14- a) D. Le-Nguyen and B. Castro, Peptide chemistry 1987, T. Shiba and S. Sakakibara Ed., 1988, 231.
b) J. Coste, M-N. Dufour, D. Le-Nguyen et B.Castro, Proceedings 11th Amer. Pept. Symp., in press.
15- Aminolysis can also occur on oxazolone.
16- The general mechanism of BroP action does not appear to involve the symmetric anhydride: J. Coste et B. Castro, in preparation and see [14b].
17- In the case of BOP used with "non-N-methylated" amino acids, we havé proposed a mechanism in which the active ester of oxybenzotriazole is not involved [14b].
18- N.L. Benoiton, Euchem. Conf., New trends in peptide Chemistry, Port Camargue, 25-29/04/1988 .
19- There are 4 singlets for OMe in each isomer. This arises from the presence of 4 conformers that are visible because of hindered rotation around the N-methyl substituted amide bonds. See, for example, B. Calas, R. Michelot, J.P. Lecaer, A. Cavé, J. Parello and P. Potier, Int. J. Peptide Protein Res., 1987, **29**, 170.
20- Caution: During the reaction the carcinogenic HMPA is formed.

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