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PyBOP®: A NEW PEPTIDE COUPLING REAGENT DEVOID OF TOXIC BY-PRODUCT

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Summary: PyBOP® (benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate), an analog of BOP where dimethylamino groups are replaced with pyrrolidino, is the only analog exhibiting equivalent properties in peptide bond formation. It can be used instead of BOP for the sake of safety.

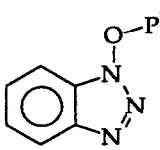
BOP **1**[1] is an excellent peptide coupling reagent that we have used either in solution[2] or in solid phase[3] peptide synthesis. Several authors have recently outlined its great interest[4]. However, the manufacture of BOP, as well as its utilization, involve the use or the formation of hexamethylphosphoric triamide (HMPA). HMPA's toxicity (carcinogenicity) has been the subject of numerous reports[5] and it has been recently included in the "Seveso list" of dangerous chemicals[6].

Hence it was essential to find a replacement product for BOP. Two main criticisms could be made on HMPA: its relative volatility and the presence of numerous methyl groups that may confer to it alkylating properties under metabolization conditions.

The replacement of the phosphorus atom by a carbon could be interesting ; however the corresponding compound HBTU and its analogs[7] are related to tetramethylurea which is relatively volatile and contains methyl groups. In fact, tetramethylurea has been reported to be cytotoxic[8].

The very straightforward replacement of the methyl groups by ethyl, that we tried first, resulted in the EtBOP analog with very poor coupling properties; for instance, the coupling of Boc-Phe-OH with H-Gly-OEt requires 5 hours instead of a few minutes. The same behavior was observed in BOP analogs where dimethylamino groups were substituted by morpholino or piperidino groups. However, the use of pyrrolidino groups on phosphorus resulted in a new reagent, nicknamed "PyBOP®" **2**, yielding coupling rates as good as, if not better than, those observed with BOP.

PyBOP® was prepared from the non volatile tris[pyrrolidino]-phosphine oxide[9], phosphorus oxychloride and hydroxybenzotriazole through the same procedures used for BOP[10]. The hexafluorophosphate salt proved to be the most convenient: it is crystalline, stable, non hygroscopic, and can be stored at room temperature (mp = 156-7°C ; NMR $^{31}P(CDCl_3)$: s (∂ = 31. 8 ppm), heptuplet (∂ = - 143. 7 ppm, J = 713 Hz).



1 $R_1 = R_2 = CH_3$ (BOP)

2 $R_1, R_2 = (CH_2)_4$ (PyBOP®)

Coupling tests using PyBOP® in solution are shown in Table 1. The conditions are identical to those we usually use for BOP. In the DCM molar solution containing 1 equ. N-protected amino acid, the following compounds were added: 1 equ. coupling reagent, 1.1 equ. C-protected amino acid hydrochloride and 2.75 equ. DIEA. TLC monitoring showed an immediate reaction, even in the case of hindered amino acids. The extent of racemization was 50% lower than that obtained with BOP in the Young's test (run 6), and compares favorably in the Anteunis' test (run 7, 8).

Table 1 : Solution couplings with PyBOP[a]

N°	Peptide (Yield %)	m.p.(°C)	$[\alpha]_D^{20}$(conc., solv.)	% epimerization
1	Boc-Phe-Gly-OEt[b](90)	87-8	-5 (2,EtOH)	
2	Boc-Ile-Val-OMe[c] (90)	166-7	-15 (1,AcOEt)	
3	Boc-Val-Val-OMe[d](95)	165-6	-10 (2,AcOEt)	
4	Boc-(D)Val-Val-OMe[e](90)	107-8	+7 (2,AcOEt)	
5	Z-Val-Val-OMe[f] (96)	108-10	-28 (1, EtOH)	
6	Bz-Leu-Gly-OEt[g](80)	143	-24 (3.1, EtOH)	15
7	Z-Gly-Phe-Val-OMe[h,i] (97)			3.3
8	Z-Gly-(D)Phe-Val-OMe[h](89)			2.5

a) Reaction time 30 min except run 6 (60 min). Crude products were purified by silicagel filtration. Structures were controled by NMR spectroscopy.

b) Litt.[11]: mp = 88 - 89.5°C ; $[\alpha]_D^{25}$ = -4.2 (2, EtOH). With BOP under the same conditions, the yield was 88% (this work).

c) Litt.[12]: mp = 166-7°C ; $[\alpha]_D^{20}$ = -15 (1, AcOEt)

d) Litt.[13]: F = 166-7°C ; $[\alpha]_D^{18}$ = -9.3 (2, AcOEt). Not contaminated by the product of run 4 (HPLC).

e) Not contaminated by the product of run 3 (HPLC).

f) Litt.[14]: mp = 107-9°C. Litt.[15]: $[\alpha]_D^{20}$ = -21.0 (1, EtOH). No D-L stereoisomer detected by ^{1}H NMR (360 MHz, d_6 acetone). Litt.[16]: coupling with DCC/HOBt yields 4% of D-L.

g) Young's test [17]. Litt.[17]: mp = 156.5-7°C ; $[\alpha]_D^{20}$ = -34.0 (3.1, EtOH). Litt.[18]: BOP gave 30% of D enantiomer.

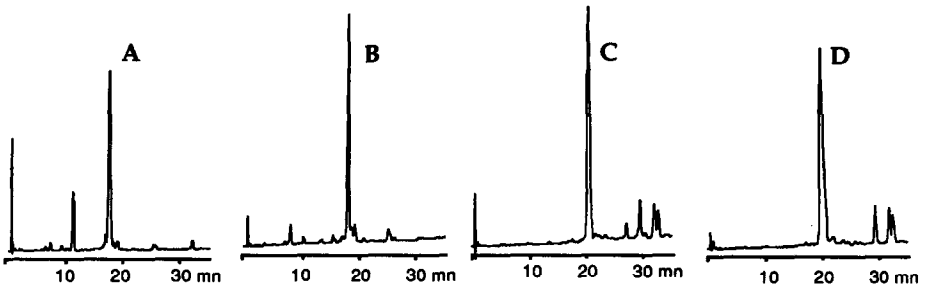
h) Anteunis' test [19]. ^{1}H NMR in agreement with litt.[19]. Stereoisomers determined by ^{1}H NMR (Val side chain methyl groups) and HPLC.

i) BOP gave 3.9% of D-L isomer (this work).

For a solid phase synthesis test, we used two model peptides, the acyl carrier protein (65-74) decapeptide (ACP(65-74): Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly) and the rat renin substrate tetradecapeptide (RRS: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Tyr-Tyr-Ser). These two peptides were synthesized in parallel, first with BOP and then with PyBOP under comparable conditions (solvent, total excess of acylating agents); the coupling times were carefully monitored. The coupling protocol was identical to that previously described[3d]: the starting Boc-amino acid chloromethylated resin was treated twice with TFA 50% in dichloromethane (first treatment for 1min., then for 30 min.), it was then allowed to react with the next Boc-amino acid and BOP or PyBOP (the latter compound is in equal amount as the Boc-amino acid) in the presence of DIEA (6 equ.). Neutralization steps were skipped as described previously; deblocking steps and washings of the resin were carried out as usual. The completion of coupling steps was ensured by the Kaiser test.

After the synthesis was accomplished, the peptide resins were treated by liquid HF in the presence of anisole. After lyophilization, the crude peptides were analyzed by HPLC. The HPLC profile of the crude products is given in the following Figure.

Figure: HPLC profile of the crude products
A: ACP(65-74)/BOP;B: ACP(65-74)/PyBOP; C: RRS/BOP; D: RRS/PyBOP



The synthesis of ACP(65-74) was initiated with Boc-Gly-CM resin (0.60mmol/g). The results are reported in Table 2. In the case of RRS, we started with Boc-Ser(Bzl)-CM resin (0.45 mmol/g). The results are reported in Table 3.

Tables 2 and 3 clearly show that using PyBOP for coupling is definitely time-saving. This is in particular the case with RRS where all the coupling steps were completed within 10 min.

Table 2 : comparison between BOP and PyBOP synthesis of ACP (65-74)

Step	equiv.	BOP[a]	PyBOP[a]
Boc-Asn (Xan)	1.5	20	10
Boc-Ile, 1/2H2O	2	30	10
Boc-Tyr (Dcb)	2	30	10
Boc-Asp (OcHx)	2	30	10
Boc-Ile, 1/2H2O	2	30	30
Boc-Ala	2	15	15
Boc-Ala	2	15	15
Boc-Gln(Xan)	2	30	30
recouple	2	30	15
Boc-Val	2	30	30
recouple	2	30	15

[a] time (min.)

Table 3 : comparison between BOP and PyBOP synthesis of RRS

Step	equiv.	BOP[a]	PyBOP[a]
Boc-Tyr (Dcb)	2	20	10
Boc-Tyr (Dcb)	2	20	10
Boc-Leu,H2O	2	15	10
Boc-Leu,H2O	2	15	10
Boc-His (Boc)[b]	2	20	10
Boc-Phe	2	20	10
Boc-Pro	2	20	10
Boc-His (Boc)[b]	2	30	10
Boc-Ile, 1/2H2O	2	55	10
Boc-Tyr (Dcb)	2	30	10
Boc-Val	2	15	10
Boc-Arg (Tos)	2	40	10
Boc-Asp (OcHx)	2	30	10

[b] DCHA salt

The Figure shows that in the case of ACP (65-74), PyBOP couplings resulted in the suppression of a side peak which was well observed in the BOP synthesis (RT ≈ 11 min.).

From these data it can be seen that PyBOP is superior to BOP for peptide coupling.

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References and notes:

1 B. Castro, J-R. Dormoy, G. Evin and C. Selve, Tetrahedron Letters, (1975), 1219.

2 a) G. Evin, J. Devin, J. Ménard, P. Corvol and B. Castro. Peptides, structure and functions,V.J. Hruby Ed., (1983), 591.
b) F. Cumin, G. Evin, J.-A. Fehrentz, R. Seyer, B. Castro, J. Ménard and P. Corvol. J. Biol. Chem., (1985), **260**, 9154 .
c) D. Le Nguyen, R. Seyer, A. Heitz and B. Castro, J. Chem. Soc. Perkin Trans. I, (1985), 1025 .

3 a) P. Rivaille, J.P. Gautron, B. Castro and G. Milhaud, Tetrahedron, (1980), 36, 3413.
b) M.P. Audousset-Puech, M. Dufour, A. Kervran, C. Jarrousse, B. Castro, D. Bataille and J. Martinez, FEBS Letters, (1986), **200**, 181.
c) J. Bouhnik, F-X. Galen, J. Ménard, P. Corvol, R. Seyer, J-A. Fehrentz, D. Le Nguyen, P. Fulcrand and B. Castro, J. Biol. Chem., (1987), **262** , 2913 .
d) D. Le Nguyen, A. Heitz and B. Castro, J. Chem. Soc. Perkin Trans. I , (1987), 1915.
e) C.F. Liu, J.A. Fehrentz, A. Heitz, D. Le Nguyen, B. Castro, F. Heitz, C. Carelli, F.X. Galen and P. Corvol, Tetrahedron, (1988), **44**, 675.
f) J.A. Fehrentz, A. Heitz, R. Seyer, P. Fulcrand, R. Devilliers, B.Castro, F. Heitz, and C. Carelli, Biochemistry, (1988), **27**, 4071.

4 a) D. Hudson, J. Org. Chem., (1988), **53**, 617.
b) A. Fournier, C-T. Wang and A.M. Felix, Int. J. Peptide Protein Res., (1988), **31**, 86.
c) A.M. Felix, C-T. Wang, E.P. Heimer and A. Fournier, Int. J. Peptide Protein Res., (1988), **31**, 231.
d) A. Fournier, W. Danho and A.M. Felix, Int. J. Peptide Protein Res., (1989), **33**, 133 .

5 a) R.Z. Schmidt , Gesamte Hyg. Ihre Grenzgeb., (1979), **25**, 662.
b) J .W. Lloyd, J.Am. Ind. Hyg. Assoc., (1975), **36** , 917.
c) N.V. Steere , J. Chem. Educ., (1976), **53** , A12.
d) L .D. Shott , A.B. Borkovec, W .A. Knapp Jr. , Toxicol. Appl. Pharmacol., (1971), **18**, 499.

6 Journal Officiel de la République Française, Paris, November 24, 1988.

7 a) V. Dourtoglou, J.C. Ziegler, and B. Gross, Tetrahedron Letters, (1978), 1269.
b) V. Dourtoglou, B. Gross, V. Lamproglou, and C. Ziodrou, Synthesis, (1984), 572.
c) R. Knorr, A. Tzeciak, W. Bannwarth and D. Gillessen, Tetrahedron Letters, (1989), **30**, 1927.

8 a) R. M. Rowell, Appl. Biochem. Biotechnol., (1984), **9**, 447.
b) M .L. Oustrin, C. Moisand, M.L. Cros and J. Bonnefoux , Ann. Pharm. Fr., (1972), **30** , 685.
c) A. Moisand, C. Moisand and G. Pitet, Ann. Pharm. Fr., (1970), **28** , 575.

9 The toxicity tests were not performed with tris[pyrrolidino]-phosphine oxide.

10 a) J-R. Dormoy and B. Castro, Tetrahedron Letters, (1979), 3321 .
b) B. Castro and J. Coste, Fr. P., 89.02.361.

11 R. Paul and G. W. Anderson, J. Amer. Chem. Soc., (1960), **82**, 4596 .

12 K. Lloyd and G.T. Young, J. Chem. Soc. (C), (1971), 2890 .

13 S. Kim, H. Chang and Y.K. Ko, Tetrahedron Letters, (1985), **26**, 1341 .

14 W. König and R. Geiger, Chem. Ber., (1970), **103**, 2034 .

15 J.W. Hinman, E.L. Caron and H.N. Christensen, J. Amer. Chem. Soc., (1950), **72**, 1620 .

16 J-P. Gamet, R. Jacquier and J. Verducci, Tetrahedron, (1984), **40**, 1995 .

17 M.W. Williams and G.T. Young, J. Chem. Soc.,(1963), 881 .

18 B. Castro, J-R. Dormoy, G. Evin and C. Selve, J. Chem. Res. , (1977), 182 .

19 C. Van der Auwera, S. Vandamme and M.J.O. Anteunis, Int. J. Peptide Protein Res., (1987), **29**, 464.

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