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CF_3-NO_2-PyBOP[1]: A New and Highly Efficient Coupling Reagent for *N*-Methyl Amino Acids

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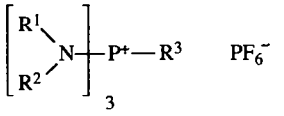
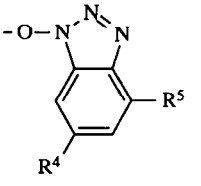
Abstract: *1-Hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole-containing phosphonium salt CF_3-NO_2-PyBOP (**1h**) proved to be a powerful reagent for in situ coupling of N-methylated amino acids.*

N-methyl amino acids are important constituents of a number of naturally-occurring peptides (*e.g.* cyclosporines,[2] didemnins[3] and dolastatins[4]) which exhibit interesting biological functions. It has also been established[5] that the metabolic stability of biologically active peptides can be enhanced by the introduction of *N*-methylated residues. Unfortunately, incorporation of hindered *N*-methyl amino acids into peptides under the agency of the standard condensing reagents DCC/HOBt and BOP (**1a**) is not completely satisfactory.[6] Recently, it was shown[7] that the efficacy of the latter condensation could be improved substantially by using the halogenophosphonium derivatives PyBroP (**1b**) and PyCloP (**1c**) as the coupling reagents.





1a BOP: $R^1 = R^2 = CH_3$; R^3 = OBt
b PyBroP: R^1, $R^2 = (CH_2)_4$; R^3 = Br
c PyCloP: R^1, $R^2 = (CH_2)_4$; R^3 = Cl
d PyBOP: R^1, $R^2 = (CH_2)_4$; R^3 = OBt
e CF_3-BOP: $R^1 = R^2 = CH_3$; $R^3 = CF_3$-OBt
f CF_3-PyBOP: R^1, $R^2 = (CH_2)_4$; $R^3 = CF_3$-OBt
g NO_2-PyBOP: R^1, $R^2 = (CH_2)_4$; $R^3 = NO_2$-OBt
h CF_3-NO_2-PyBOP: R^1, $R^2 = (CH_2)_4$; $R^3 = CF_3$-NO_2-OBt

OBt: $R^4 = R^5$ = H
CF_3-OBt: $R^4 = CF_3$; R^5 = H
NO_2-OBt: $R^4 = NO_2$; R^5 = H
CF_3-NO_2-OBt: $R^4 = CF_3$; $R^5 = NO_2$

An earlier study[8] from our laboratory revealed that replacement of the benzotriazol-1-yl (OBt) moiety in the coupling reagents BOP (**1a**) or PyBOP (**1d**) by CF_3-OBt had a beneficial effect, in terms of yield and reaction time, on peptide bond formation. The latter was illustrated in the highly effective coupling of sterically congested α-aminoisobutyric acid (Aib) residues in the presence of the new reagents CF_3-BOP (**1e**) and CF_3-PyBOP (**1f**). However, CF_3-BOP-mediated condensation of Z-protected valine with the highly hindered *N*-methylvaline (MeVal) methyl ester proceeded in a low yield.[8] Apart from this, Høeg-Jensen *et*

al.[9] demonstrated that CF_3-PyBOP (**1f**) as well as its NO_2-analogue **1g** are the reagents of choice for the preparation of endothiopeptides.

On the basis of the foregoing results it was expected that the coupling efficiency of **1e-f** could be improved further by the introduction of an additional electron-withdrawing NO_2-group in the CF_3-OBt moiety.

We here report the use of the novel reagent CF_3-NO_2-PyBOP (**1h**) in the synthesis of *N*-methyl amino acid-containing dipeptides.

The required CF_3-NO_2-HOBt derivative was readily accessible by a slight modification of the procedure of Reese and Pei-Zhuo.[10] Reaction of commercially available reagent PyBroP (**1b**) with CF_3-NO_2-HOBt furnished, after extractive work-up and crystallization, CF_3-NO_2-PyBOP (**1h**) in an excellent yield.[11]

In order to explore the potential usefulness of reagent **1h**, the synthesis of the dipeptides **2a-h** was undertaken. The results of this study are summarized in the Table. In addition, the yields of the CF_3-NO_2-PyBOP-mediated condensation were compared with those obtained using the highly advocated reagent PyBroP (**1b**). To this end, the condensations recorded in the Table were executed under the same conditions (*i.e.* one-pot reaction for 1 h at 20 °C using equimolar amounts of the appropriate amino acids and condensing reagent in the presence of *N,N*-diisopropylethylamine) as applied for the preparation of Aib-containing peptides.[8]

Table Relevant Data on the Synthesis of Dipeptides **2a-h**[12] using PyBroP[13] (**1b**) and CF_3-NO_2-PyBOP (**1h**) as the Condensating Reagents[14,15]

Entry	Dipeptide		Yield (%)[a]		$[\alpha]_D^{b,c}$	lit. $[\alpha]_D^{b,c}$	
			PyBroP	CF_3-NO_2-PyBOP			
1	Z-Val-Val-OMe	**2a**	89	98	-29	-28	(14)
2	Z-Val-MeVal-OMe	**2b**	49	76	-113	-110	(8)
3	Z-Pro-MeVal-OMe	**2c**	43	92	-103		
4	Fmoc-Val-MeVal-OMe	**2d**	57	85	-105	-107	(7)
5	Boc-Val-MeVal-OMe	**2e**	25	62[d]	-139	-140	(7)
6	Boc-MeLeu-MeLeu-OBzl	**2f**	47	87	-109	-107	(15)
7	Boc-MeLeu-MeVal-OMe	**2g**	40	76	-181	-177	(7)
8	Z-MeVal-MeVal-OMe	**2h**	22	71	-190	-191	(7)

a) Isolated yields. *b)* T = 20 °C. *c)* Solvent systems: **2a,f-h**: *c* 1, EtOH; **2b**: *c* 1, MeOH; **2c**: *c* 1, $CHCl_3$ **2d**: *c* 0.14, EtOH; **2e**: *c* 0.25, EtOH. *d)* Reaction time of 4 h gave **2e** in 92% yield.

It can be seen in the Table (entry 1) that amide bond formation between two 'non-methylated' valines under the agency of PyBroP (**1b**) or CF_3-NO_2-PyBOP (**1h**) is in both cases a high-yielding process. On the other hand, the yields of the CF_3-NO_2-PyBOP-assisted acylations of the more hindered *N*-methylvaline (MeVal) methyl ester with either Z-Val-OH (entry 2) or Z-Pro-OH (entry 3) were, in comparison with PyBroP, substantially higher. In this respect, it should be noted that application of the earlier reported

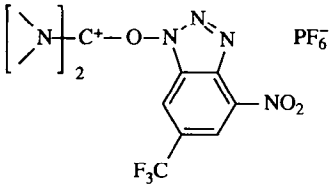
monosubstituted reagent CF_3-PyBOP furnished Z-Val-MeVal-OMe (**2b**) in 32% yield.[8] Moreover, the same dipeptide could be attained in 57% yield using the recently devised reagent HATU.[16] A higher coupling efficiency was also found in the CF_3-NO_2-PyBOP-mediated preparation of the Fmoc-protected dipeptide **2d** (entry 3). Apart from this, an interesting phenomenon was observed in the condensation of Boc-Val-OH with *N*-methylated valine (entry 5). Thus, the PyBroP-mediated peptide bond formation resulted in a low yield of Boc-Val-Val-OMe (**2e**). The poor yield of **2e** may be attributed to the conversion of activated Boc-valine into the corresponding *N*-carboxyanhydride (NCA).[7] In contrast, the occurrence of the undesired NCA adduct could not be detected in the CF_3-NO_2-PyBOP-assisted synthesis of dipeptide **2e**. The latter was endorsed by the nearly quantitative formation of the desired Boc-protected dipeptide within 4 h. The high potency of CF_3-NO_2-PyBOP (**1h**) is demonstrated further in the successful synthesis of di-*N*-methylated dipeptides **2f-g** (entries 6-7). Furthermore, it is evident that the rather difficult coupling of two MeVal residues (entry 8) under the agency of reagent **1h**, instead of **1b**, results in a threefold increase in yield of dipeptide **2h**.

In conclusion, the results presented in this paper clearly indicate that the crystalline and shelf-stable reagent CF_3-NO_2-PyBOP shows great promise[17] for the acylation of *N*-methyl amino acids. It is also not excluded that CF_3-NO_2-PyBOP may open the way to a solid phase directed synthesis of oligopeptides bearing *N*-alkylated amino acids.

REFERENCES AND NOTES

1. Abbreviations: Boc, *tert*-Butyloxycarbonyl; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; CF_3-BOP, [6-(trifluoromethyl)benzotriazol-1-yloxy]tris(dimethylamino)phosphonium hexafluorophosphate; CF_3-PyBOP, [6-(trifluoromethyl)benzotriazol-1-yloxy]tris(pyrrolidino)phosphonium hexafluorophosphate; CF_3-NO_2-HBTU, 2-[4-nitro-6-(trifluoromethyl)benzotriazol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate; CF_3-NO_2-HOBt, 1-hydroxy-4-nitro-6-(trifluoromethyl)benzotriazole; CF_3-NO_2-PyBOP, [4-nitro-6-(trifluoromethyl)benzotriazol-1-yloxy]tris(pyrrolidino)phosphonium hexafluorophosphate; DCC, *N,N'*-dicyclohexylcarbodiimide; Fmoc, fluoren-9-ylmethoxycarbonyl; HATU, 2-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MeLeu, *N*-methylleucine; PyBroP, bromotris(pyrrolidino)phosphonium hexafluorophosphate; PyCloP, chlorotris(pyrrolidino)-phosphonium hexafluorophosphate; Z, benzyloxycarbonyl
2. Wenger, R.M. *Angew. Chem. Int. Ed. Engl.* **1985**, *24*, 77-85
3. Jouin, P.; Poncet, J.; Dufour, M.-N.; Pantaloni, A.; Castro, B. *J. Org. Chem.* **1989**, *54*, 617-627 and references cited therein
4. Pettit, G.R.; Singh, S.B.; Herald, D.L.; Lloyd-Williams, P.; Kantoci, D.; Burkett, D.D.; Barkóczy, J.; Hogan, F.; Wardlaw, T.R. *J. Org. Chem.* **1994**, *59*, 6287-6295 and references cited therein
5. Fauchère, J.-L. In *Advances in Drug Research*; Testa, B., Ed.; Academic Press: London, 1986; Vol. 15, pp. 29-69
6. See for instance: Galpin, I.J.; Mohammed, A.K.A.; Patel, A.; Priestley, G. *Tetrahedron* **1988**, *44*, 1763-1772
7. Coste, J.; Frérot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437-2446
8. Wijkmans, J.C.H.M.; Kruijtzer, J.A.W.; Van der Marel, G.A.; Van Boom, J.H.; Bloemhoff, W. *Recl. Trav. Chim. Pays-Bas* **1994**, *113*, 394-397

9. Høeg-Jensen, T.; Olsen, C.E.; Holm, A. *J. Org. Chem.* **1994**, *59*, 1257-1263
10. Reese, C.B.; Pei-Zhuo, Z. *J. Chem. Soc. Perkin Trans. I* **1993**, 2291-2301
11. *Synthesis of CF_3-NO_2-PyBOP (**1h**)*: A solution of the triethylammonium salt of CF_3-NO_2-HOBt (2.31 g, 6.6 mmol) in CH_2Cl_2 (10 mL) was added in 30 min to a solution of PyBroP (3.10 g, 6.6 mmol) in acetone (17 mL) at 0 °C. After stirring for 1 h at room temperature, the mixture was concentrated, redissolved in CH_2Cl_2 (75 mL), washed with H_2O (2 x 25 mL), dried ($MgSO_4$) and evaporated to dryness. The obtained orange oil crystallized upon addition of ethyl acetate. The pale yellow crystals were collected by filtration and dried *in vacuo* to give **1h** (3.77 g, 85%). Phosphonium salt **1h** decomposes at T > 115 °C; plasma desorption MS (*m/z*): 489.6 $[M\text{-}PF_6]^+$; ^{31}P NMR (80.7 MHz, acetone-d_6): δ 33.3 (s, P^+), -141.3 (septet, PF_6^-, $J_{P,F}$ 708.9 Hz); ^1H NMR (200 MHz, acetone-d_6): δ 8.9 (m, 1H, H-arom.), 8.72 (dd, 1H, H-arom., *J* 0.5 and 1.4 Hz), 3.6 (m, 12H, CH_2-CH_2-N), 2.0 (m, 12H, CH_2-CH_2-N); ^{13}C NMR (50.1 MHz, acetone-d_6): δ 139.7, 137.0, 130.2 (C_q), 131.1 (q, C-CF_3, $^2J_{C,F}$ 34.7 Hz), 122.0 (q, C-CF_3, $^1J_{C,F}$ 273.0 Hz), 120.0, 114.7 (CH-arom.), 48.6 (d, CH_2-CH_2-N, $^2J_{C,P}$ 5.9 Hz), 26.1 (d, CH_2-CH_2-N, $^3J_{C,P}$ 8.8 Hz).
12. The dipeptides were fully characterized by ^{13}C and ^1H NMR spectroscopy as well as electrospray mass spectrometry. Selected analytical data of **2e**: ESMS (*m/z*): 485 $[M+Na]^+$, 501 $[M+K]^+$; ^1H NMR (300 MHz, $CDCl_3$): δ 2.94, 2.91, 2.87, 2.76, 2.73, 2.57, 2.53 (7 x s, 1.2, 1, 0.4, 0.4, 2.2, 0.4 and 0.4H, respectively, NCH_3); ^{13}C NMR (50.1 MHz, $CDCl_3$): δ 171.9 - 170.2 (C=O MeLeu), 155.3 - 153.7 (C=O Boc), 135.3, 135.0 (C_q OBzl), 128.1, 127.9, 127.7 (CH-arom.), 80.3, 79.8, 79.3 (C_q Boc), 66.8, 66.6, 66.3 (CH_2 OBzl), 57.2, 56.7, 54.4, 53.7, 53.5, 52.0, 51.8 (Cα), 38.0, 37.6, 37.4, 36.7 (Cβ), 30.7, 30.3, 29.1, 28.6, 27.9 (NCH_3, CH_3 Boc), 24.5 - 20.8 (Cγ, Cδ).
13. PyBroP was purchased from Novabiochem (Switzerland).
14. Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205-208
15. Tung, R.D.; Rich, D.H. *J. Am. Chem. Soc.* **1985**, *107*, 4342-4343
16. (a) Carpino, L.A.; El-Faham, C.; Minor, A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201-203; (b) Angell, Y.M.; García-Echeverría, C.; Rich, D.H. *Tetrahedron Lett.* **1994**, *35*, 5981-5984
17. A preliminary study indicated that the uronium derivative CF_3-NO_2-HBTU[18] may be as effective as CF_3-NO_2-PyBOP. For instance, condensation of Z-protected valine with H-MeVal-OMe afforded dipeptide **2b** in 75% yield.



CF_3-NO_2-HBTU

18. Treatment of tetramethylchloroformamidinium hexafluorophosphate[19] with CF_3-NO_2-HOBT, under the same conditions as mentioned for the synthesis of **1h**, gave CF_3-NO_2-HBTU(1.99 g, 60%) as pale yellow crystals. CF_3-NO_2-HOBT, decomposes at T > 115 °C; plasma desorption MS (*m/z*): 347.2 $[M\text{-}PF_6]^+$; ^1H NMR (300 MHz, acetone-d_6): δ 9.0 (m, 1H, H-arom.), 8.74 (d, 1H, H-arom., *J* 1.0 Hz), 3.54 (s, 12H, CH_3); ^{13}C NMR (50.1 MHz, acetone-d_6): δ 161.3 (C^+), 140.5, 137.7 (C_q), 132.1 (q, C-CF_3, $^2J_{C,F}$ 34.7 Hz), 130.3 (C_q), 123.6 (q, C-CF_3, $^1J_{C,F}$ 273.0 Hz), 120.5, 115.4 (CH-arom.), 41.6 (NCH_3).
19. Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, C. *Synthesis* **1984**, 572-574

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