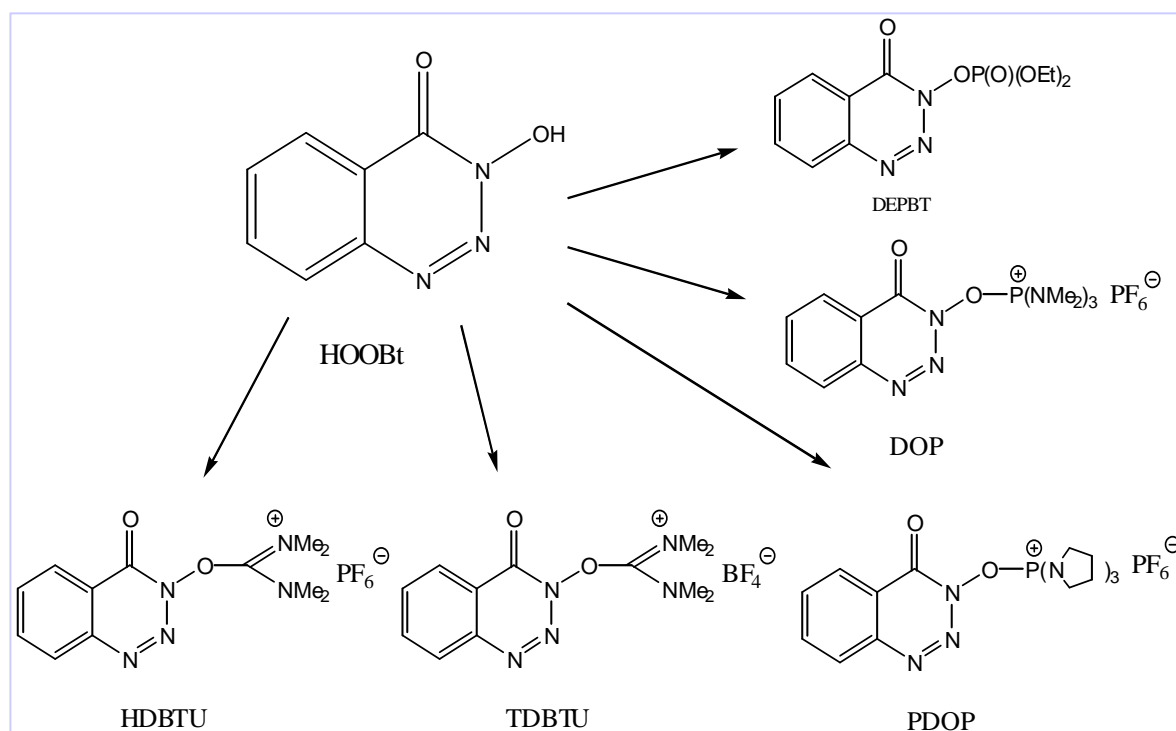


3-HYDROXY-4-OXO-1, 2, 3-TRIAZINE (HOObt)

AND ITS DERIVATIVES FOR AMIDE AND ESTER BONDS FORMATION



Introduction

Currently, the most common peptide coupling additive used during peptide coupling for both solutions and solid phase syntheses is 1-hydroxybenzotriazole (HOBt). This reagent has been used either in combination with a carbodiimide or other coupling agent or built into a stand-alone reagent, such as 1-benzotriazolyl-oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), as a phosphonium salt or an analogous uronium salt such as HBTU or TBTU. HOBt is applicable to both stepwise and segment condensations. However, many cases have been encountered in which HOBt is ineffective. In particular, in segment couplings at amino acid units other than glycine or proline the problem of racemization may be severe.

3-hydroxy-1, 2, 3-benzotriazin-4(3H)-one (HOOBt or HODhbt), a related compound, is a well-known reagent for peptide synthesis as an additive for coupling of amino acids in the presence of carbodiimides [1,2]. This compound provides high yield of peptide product and inhibits racemization, especially in difficult coupling and segment condensation (the most racemization sensitive cases in peptide chemistry). In this respect, HOOBt is superior to HOBt and its derivatives, and is comparable in activity to (and in some cases even better than) 1-hydroxy-7-azabenzotriazole (HOAt) [3].

As polypeptides become of increasing medicinal importance, there is a growing incentive to improve the methods by which they are synthesized. Since HOOBt is a highly efficient coupling reagent, the design and development of novel HOOBt derivatives and analogues, which are effective as peptide coupling additives in both stepwise (batch and continuous flow) and segment condensations, is highly desirable and urgently needed in the art.

BIO-LAB offers a broad selection of coupling reagents for most synthetic needs, especially for peptide synthesis (BOP, HBTU, TBTU, TSTU) and performs intensive investigations in the field of new coupling reagents and additives, aiming to answer problematic steps faced by the peptide chemist at industrial scale, for examples, substituted HOOBt (3-hydroxy-1,2,3-benzotriazin-4(3H)-one) derivatives [US Provisional Patent Application No 60/487962 “3-HYDROXY-4-OXO-1,2,3-TRIAZINES AND DERIVATIVES THEREOF FOR AMIDE AND ESTER BONDS FORMATION”].

HOOBt: The use of HOOBt is much less common than HOBt and HOAt, since the carbodiimide-mediated coupling in the presence of HOOBt is accompanied by formation of a by-product, which itself can react with N-alpha-amino groups to terminate the chain elongation [2]. Since it has then been reported that than the use of particular solvents and conditions can guarantee clean peptide forming reaction in high yield without by-products and racemization [4-6], the advantage of using HOOBt is clearer.

HOOBt/Carbodiimide coupling: Combination N-hydroxy compounds with carbodiimides have become widely adopted for peptide coupling to avoid loss of chirality and side product formation. In the Konig study [2] of racemization during peptide synthesis over 30 N-hydroxy compounds, including HOBt, 6-Cl-HOBt, 6-nitro-HOBt were described [7], and only one, HOOBt, proved to be generally superior to HOBt. Comparison between HOOBt and HOAt did not indicate the superiority of one over another [3]. In some cases, involving DMF as solvent and Collidine as base, the advantage of HOAt-derived reagents was higher, whereas for Carbodiimide couplings in TFE/ TCM, HOOBt and HOAt systems were about equally effective. In contrary, for synthesis of the model Z-Phe-Val-Ala-OMe peptide HOOBt was generally more efficient than HOAt for Carbodiimide couplings in DMF [3]. The same results were observed in the coupling mediated by EDC between Boc-Phe-Ile-OH and H-Phe-OBzl [4].

Fragment (segment) condensation: To synthesize large peptides and proteins, one of the most advantageous procedures is the fragment (segment) condensation method. It was demonstrated significant advantages of EDC/HOOBt in the condensation of long segments in solution for construction of β -Amyloid peptide (42 AAs) [4], human's Adrenomedullin (56 AAs) [8], Midkine (121 AAs) [9] and Pleiotrophin (136 AAs) [10], membrane protein (F1F0 ATP synthase subunit c) (79 AAs) [11], Phosphorylated urodilatin (32 AAs) [12]. The DIC/HOOBt system was effectively used for condensation of fragments on resin in solid phase peptide synthesis. For example, hydrophobic fragment of Elastin, VGVAPG, was six time consecutively coupled for successful preparation of hexamer (36 AAs) [5].

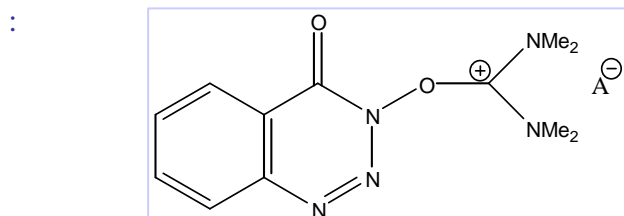
It should be mentioned the efficient use of HOOBt/carbodiimide reagent for synthesis of glucopeptide [13] and PNA [14, 15].

Several derivatives of HOOBt have been reported as useful additives for amide bond formation. For example, ammonium salts of HOOBt are used as additives for amidation of protected amino acids [16].

Addition of HOOBt to BOP-mediated peptide formation, using Boc-Asn-OH gave no cyano side products whereas in a similar experiment using BOP along cyano side-products were formed [17].

HOOBt- Active Esters: The applications of HOOBt - active esters of amino acids for peptide synthesis have been reported [18-23]. Fmoc amino acid OOBt esters have been prepared as crystalline solids and their use was demonstrated through the synthesis of the carrier protein decapeptide sequence 65-74 and a nonadecapeptide sequence [21]. OOBt esters have generally higher reactivity compared to OPfp esters and require no further activation, although HOBt is normally added with the pentafluorophenyl esters to speed up the coupling rate [23].

HDTU and TDTU: Carpino et al and Knorr et al disclosed the use of uronium coupling reagents derived from HOOBt, for peptide coupling A = PF₆ (HDTU) [3]; BF₄ (TDTU or TDhbTU) [24].



The model coupling of Fmoc-Ile-OH to H-Ile-resin, using different coupling reagents (HDTU, BOP, DEPBT, HATU, HBTU, TBTU, PBOP) and Lutidine as a base shows that the complete coupling was achieved after 1h reaction period only with HDTU [25].

HDTU was successfully used in the synthesis of Bipartite NLS (Nucleoplasmin) peptide with the better result (activity, purity, yield) than usual coupling reagents (HBTU, BOP, HCTU, HATU) [26].

HDTU was very effective in the synthesis of PNA monomers and polymers. When BOP, DEPBT, PDOP, HBTU and HATU failed, the reaction with HDTU provided complete coupling with all kind of used PNA monomers [25, 26].

DOP and PDOP: DOP and PDOP are new analogs of BOP and PyBOP, which combine the advantages of phosphonium coupling reagents and OOBt group: high reactivity, suppression of racemization, possibility to use some Fmoc amino acids without protection of side-chains (serine, threonine, tyrosine), absence of guanidine-containing by-products. These reagents can be suitable in the preparation of peptides by segment condensation or cyclization [25].

DEPBT: A phosphoryloxy derivative of HOBT, namely 3-(Diethoxyphosphoryloxy)-1, 2, 3- benzotriazin-4(3H)-one (DEPBT), has been reported [27-29] and a number of peptide coupling reactions (in solution and on solid phase) were carried out using this reagent with some important advantages. For example, it is not necessary to protect the hydroxyl group of the amino component such as serine, threonine and tyrosine. The side reaction of asparagines dehydration, easily caused by DCC, was not observed with DEPBT [30]. The high efficiency of DEPBT for amide bond formation have been demonstrated in the synthesis of complex molecules such as Tamandarin B or Glycopeptide teicoplanin aglycon and, especially, for peptide cyclization. The competitive study of different coupling reagents for preparation of cyclic peptide shows that DEPBT is among the best.

Product Data Sheet

HOObt

Product name and synonyms	HODhBt, 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine; 3-Hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine; 3-Hydroxy-1,2,3-benzotriazin-4(3H)-one
Product Number	
CAS Number	28230-32-2
Molecular Formula	C7H5N3O2
Molecular Weight	163.1
Appearance	Off-white to light pink powder
Assay by titration (NaOH 1N) by HPLC	98-101% 98% min.
Melting point	179-185°C
Solubility (0.1 g in 10 ml of dioxane)	Clear, yellow
Risk and safety phases	R 36/37/38 May be harmful if absorbed through the skin, if swallowed or inhaled S 26-36 Avoid breathing dust, vapor, mist, or gas. Avoid contact with skin and eyes.
Chemical Stability	Stable under normal temperatures and pressures
Stability in solution (closed vial)	Stable in DMF and NMP at least 5 days.
Conditions to Avoid	Incompatible materials (Strong oxidizing agents) Risk of explosion if heated under confinement
Handling and storage	Product remains stable at least 12 months when stored in dry place at room temperature in a tightly closed container. Prolonged exposure to elevated temperature and light should be avoided.

Product Data Sheet

Product name and synonyms	HDTU, HD_bTU, 2-(3,4-Dihydro- 4-oxo-1,2,3-benzotriazin-3-yl)-1,1,3,3 – tetramethyluronium hexafluorophosphate O-(3, 4-Dihydro-4-oxo-1, 2, 3-benzotriazin-3-y1)-N, N, N', N' -tetramethyluronium hexafluorophosphate
Product Number	
CAS Number	105832-38-0
Molecular Formula	C ₁₂ H ₁₆ F ₆ N ₅ O ₂ P
Molecular Weight	407.2
Appearance	white to off-white powder
Assay by HPLC	98% min
Melting point	134-138°C (dec)
Solubility	0.4 g (1 mmol) completely soluble in 1 ml of DMF 0.3 g – in 1 ml of NMP 0.25 g – in 1 ml of acetonitrile Practically insoluble in ethyl acetate, methylene chloride, hexane, ether
Risk and safety phases	R 36/37/38 May be harmful if absorbed through the skin, if swallowed or inhaled S 26-36 Avoid breathing dust, vapor, mist, or gas. Avoid contact with skin and eyes.
Chemical Stability	Stable under normal temperatures and pressures
Stability in solution (closed vial)	Stable in DMF and NMP at least 1 week.
Conditions to Avoid	Incompatible materials (Strong oxidizing agents) Risk of explosion if heated under confinement
Handling and storage	Product remains stable at least 12 months when stored in dry place at room temperature in a tightly closed container. Prolonged exposure to elevated temperature and light should be avoided

REFERENCES:

1. Koenig W. et al. DE 1939187 (1971)
2. Koenig W., Geiger R. Chem. Ber., 1970, v. 103 (7), 2034-40
3. Carpino L.A., El-Faham A., Albericio F. J. Org. Chem., 1995, v.60, p.3561
4. Inui T., et al. Letters in Peptide Science, 2002, v.8, p.319
5. Mihala N., et al. J. Peptide Science, 2001, v.7, p. 565
6. Nozaki S. J. Pep. Research, 1999, v.54, p.162
7. Koenig W., Geiger R. US 3725380 (1973)
8. Kawakami T., e.a., Bull. Chem. Soc. Jpn., 1996, v. 69, p. 3331
9. Inui T., et al. J. Peptide Science, 1996, v.2, p. 28
10. Inui T., et al. J. Pep. Research, 2000, v.55, p.384
11. Sato T., e.a. J. Pept Sci. 2002, v. 8(4), p.172.
12. Mostafavi H., Austermann S., Forssmann W.G, Adermann K. Int.J. Pept. Protein Res. 1996, v. 48(2), p. 200
13. Mizuno M. Trends in Glycoscience and Glycotechnology, 2001, v. 13, p. 11.
14. Uhlmann E., e.a. Angew. Chem. Int. Ed. 1998, v. 37, p. 2796
15. Ferrer E., Eritja R. Letters in Peptide Science, 2000, v.7, p.195
16. Somlai C., et al. Synthesis, 1992, p.285
17. JP Patent 6145195 (1994).
18. Cameron L.R et al. J. Chem. Soc., Perkin Trans. I., 1988, p. 2895
19. Koenig W. et al. DE 3618218 (1987)
20. Carey R.I. US. 5952497
21. Atherton E., et al. J. Chem. Soc., Perkin Trans. I., 1988, p. 2887
22. Karup G., et al. Int. J. Peptide Protein Res., 1988, v.32, p.331
23. Carey R.I. US 6075141 (2000).
24. Knorr R., e.a. Tetrahedron Lett., 1989, v.30, p.1927
25. US Provisional Patent Application No 60/487962 "3-HYDROXY-4-OXO-1,2,3-TRIAZINES AND DERIVATIVES THEREOF FOR AMIDE AND ESTER BONDS FORMATION"
26. Katzhendler J., Klauzner Y. Unpublished results.

27. Li H., et al. *Organic Letters* (1999), 1(1), 91-93
28. Ye, Yun-Hua et al. *Gaodeng Xuexiao Huaxue Xuebao* (1997), 18(7), 1086-1092
29. Fan, Chong-Xu et al. *Synthetic Communications* (1996), 26(7), 1455-60
30. Goodman M., Zapf C., Rew Y. *Biopolymers (Peptide Science)*, 2001, v. 60, p. 229