ORGANIC LETTERS

1999
Vol. 1, No. 1
91−93

3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT): A New Coupling Reagent with Remarkable Resistance to Racemization

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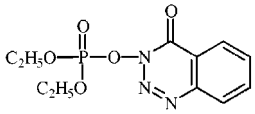
Received April 9, 1999

ABSTRACT



DEPBT

The new crystalline phosphate reagent 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) mediates amide bond formation with a remarkable resistance to racemization. Comparative racemization studies were carried out and DEPBT proved to be superior to typical phosphonium and uronium coupling reagents. DEPBT is easily prepared and is exceedingly stable with a shelf life of months at room temperature. The advantageous properties of DEPBT render it a useful and unique addition to the arsenal of coupling reagents.

The formation of the amide bond is one of the fundamental reactions of organic synthesis. Medicinal chemists have long recognized the great utility of amino acids as chiral synthons for the preparation of well-defined three-dimensional bioactive molecules. The traditional use of the amide bond in drug discovery has been for the synthesis of peptides, but with the recent explosion in the use of solid-phase organic synthesis, especially for the preparation of combinatorial libraries, the amide bond once again is playing a central role. Amino acids have been utilized as chiral building blocks in numerous solid-phase syntheses, particularly of heterocycles,[1] including β-sultams,[2] diketopiperazines,[3] and tetramic acids.[4]

Preservation of stereochemical integrity during solid-phase synthetic reactions is crucial for the rapid identification and scale-up of hit compounds. The development of amide bond forming reagents ("coupling reagents") that can be utilized with a diverse set of reactants while minimizing racemization remains an important area of research. Accordingly, we have prepared several organophosphorus compounds as coupling reagents.[5,6] Among them, 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) has the best overall properties.

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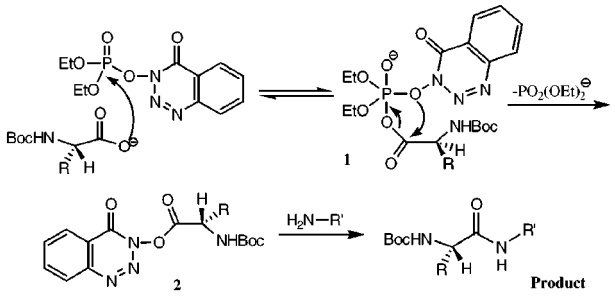
(1) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449.
(2) Gordeev, M. F.; Gordon, E. M.; Patel, D. V. *J. Org. Chem.* **1997**, *62*, 8177.
(3) Szardenings, A. K.; Burkoth, T. S.; Lu, H. H.; Tien, D. W.; Campbell, D. A. *Tetrahedron* **1997**, *53*, 6573−6593.
(4) Romoff, T. T.; Ma, L.; Wang, Y.; Campbell, D. A. *Synlett* **1998**, *12*, 1341.
(5) Fan, C.-X.; Hao, X.-L.; Ye, Y.-H. *Synth. Commun.* **1996**, *26*, 1455.
(6) Ye, Y.-H.; Fan, C.-X.; Zhang, D.-Y.; Xie, H.-B.; Hao, X.-L.; Tian, G.-L. *Peptides: Frontiers of peptide sciences, Proceedings of the Fifteenth American Peptide Symposium*; Tam, J. P.; Kaumaya, P. T. P., Eds.; Kluwer: Netherlands, 1999; p 337.
(7) Castro, B.; Dormoy, J.-R.; Dourtoglon, B.; Erin, G.; Selve, C.; Ziegler, J.-C. *Synthesis* **1976**, 751.
(8) Romoff, T. T.; Tran, T.; Goodman, M. *J. Pept. Res.* **1997**, *49*, 281.
(9) Chiralcel OD column, hexane/2-propanol solvent.
(10) 4MB = 4-methylbenzyl.
(11) Coste, J.; Frerot, E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437.
(12) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
(13) Dourtoglu, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett.* **1978**, *19*, 1269.

10.1021/ol990573k CCC: $18.00
Published on Web 05/24/1999

A proposed mechanism for DEPBT-mediated coupling is shown in Scheme 1. A tertiary amine is required to generate

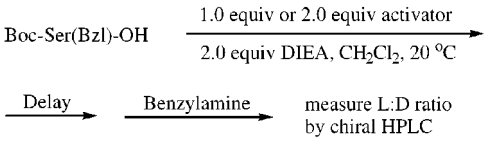
Scheme 1. Proposed Mechanism for DEPBT-Mediated Coupling



the carboxylate of the N-protected amino acid, which then attacks the central phosphorus atom of DEPBT. The resulting transient intermediate **1** rearranges with loss of diethyl phosphite to form the 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt) ester **2**. This strongly activated stable intermediate is attacked by the amine component to form the amide product. This mechanism is analogous to that by which the phosphonium reagent BOP[7] generates HOBt esters.

The recently described protocol by Romoff[8] was employed to assess the tendency for DEPBT to induce racemization. In this procedure, an activated carboxyl intermediate is allowed to stand in the presence of tertiary amine for a short time. The intermediate is then reacted with benzylamine, and the resulting ratio of enantiomers is determined by chiral HPLC.[9] To maximize sensitivity, experiments were carried out using Boc-Ser(OBzl)-OH and Boc-Cys(4MB)-OH[10] as the carboxyl components, since these amino acids have the highest susceptibility to racemization. Table 1 describes

Table 1. Comparative Studies of Racemization during in Situ Activation



activation	delay time, min	L:D ratio	yield, %
PyBrop (1.0 equiv)	4	65:35	81
HATU (1.0 equiv)	4	84:16	91
HBTU (1.0 equiv)	15	79:21	>99
BOP (1.0 equiv)	15	85:15	95
DEPBT (1.0 equiv)	60[a]	95:5	70
DEPBT (2.0 equiv)	60[a]	96:4	>99

[a] Even with such long delay time, very little racemization is observed.

comparative rates of racemization among DEPBT and the commonly used phosphonium reagents PyBroP[11] and BOP, and the uronium reagents HATU[12] and HBTU.[13] Structures of these reagents are shown in Figure 1.

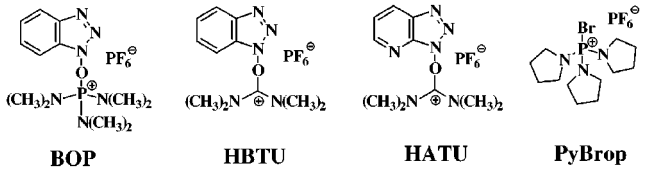
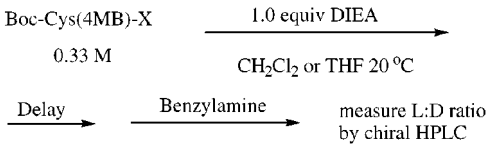


Figure 1. Structures of activating reagents utilized in this study.

As a complement to the in situ activation experiment, the 7-aza-1-hydroxybenzotriazole (HOAt) and HOOBt esters of Boc-Cys(4MB)-OH were prepared in crystalline form and subjected to a similar experiment. The results are described in Table 2.

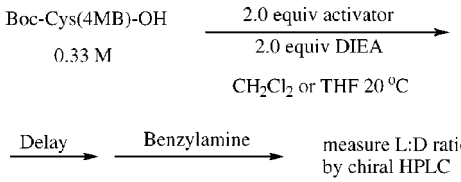
Table 2. Racemization Studies of Preactivated Intermediates for Boc-Cys(4MB)-OAt and Boc-Cys(4MB)-OOBt



X	solvent	delay time, min	L:D ratio	yield, %
OAt	CH_2Cl_2	5	87:13	94
OOBt	CH_2Cl_2	5	98.5:1.5	85
OAt	THF	30	95:5	98
OOBt	THF	30	100:0	>99

An experiment designed toward optimization of the use of DEBPT was carried out, and the results are described in Table 3.

Table 3. Effect of the Solvent on Racemization and Yield of an in Situ Activated Intermediate for Boc-Cys(4MB)-OH



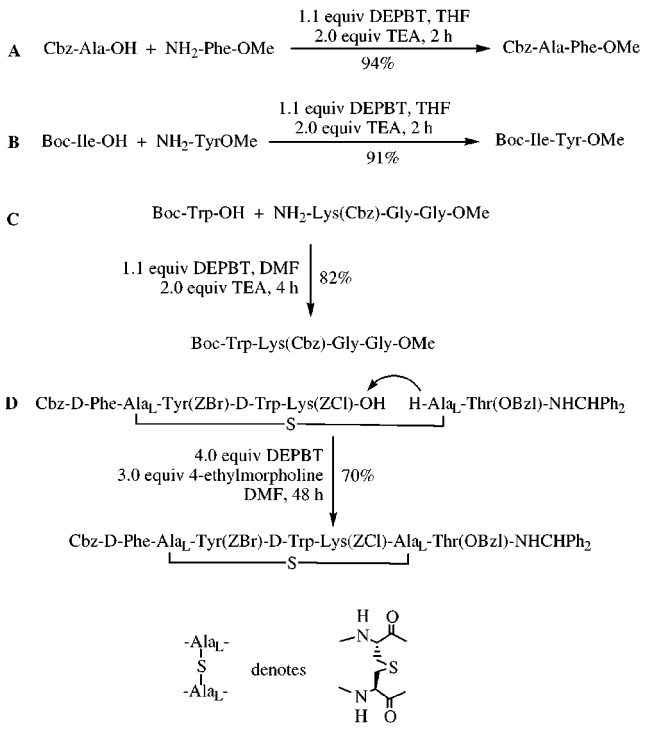
activation	solvent	delay time, min	L:D ratio	yield, %
DEPBT (2.0 equiv)	CH_2Cl_2	15	100:0	73
DEPBT (2.0 equiv)	THF	30	100:0	>99

A number of coupling reactions were carried out using DEPBT.[5,14] It is worthwhile noting that it is not necessary to protect the hydroxyl of the amino component (such as tyrosine). Examples of using DEPBT as the coupling reagent

(14) Ye, Y. H.; Fan, C. X.; Zhang, D. Y.; Xie, H. B.; Tian, G. L. *Chem. J. Chin. Univ.* **1997**, *18*, 1086.

in peptide synthesis are shown in Scheme 2. It has also been demonstrated that DEPBT can be used as the coupling reagent in solid-phase peptide synthesis.[14]

Scheme 2. Peptide Coupling and Cyclization Reactions Using DEPBT as the Coupling Reagent



It is remarkable that amide bond forming reactions mediated by DEPBT are so strongly resistant to racemization, even when a base as strong as DIEA is employed. DEPBT can be used under normal peptide coupling conditions. Ideal conditions utilize 2 equiv of DEPBT and 2 equiv of DIEA with THF as the solvent. These results are consistent with those reported by Sakakibara, who observed that HOOBt as an additive in peptide coupling reactions is superior to the commonly used HOBt.[15] The structure of DEPBT has been confirmed by X-ray crystallography (Figure 2).[16]

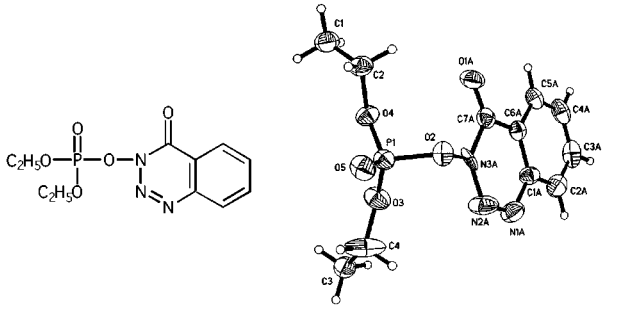


Figure 2. The chemical and X-ray structures of 3-(diethoxyphosphoryloxy)-1,2,3-benzotrazin-4(3*H*)-one (DEPBT).

The reagent DEPBT is readily prepared by reaction of 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt) with diethyl chlorophosphate in the presence of triethylamine in dichloromethane.[17] The compound is a crystalline colorless solid with a shelf life of months at room temperature. This stability relative to phosphonium and uronium activating reagents is no doubt a consequence of its existence as a neutral moiety; the other reagents exist as hygroscopic salts. The advantageous properties of DEPBT render it a useful and unique addition to the arsenal of coupling reagents.

Acknowledgment. The authors thank Joseph Taulane and Dr. Heather Sings for helpful discussions. The X-ray diffraction analysis of DEPBT was carried out by Dr. Peter Gantzel in UCSD X-ray facility. This work was supported by NIH-DK15410.

Supporting Information Available: Experimental protocols for the racemization experiments and syntheses of the HOAt and HOOBt esters, specifics and chromatograms of the chiral HPLC experiments, and crystal structure coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) (a) Kuroda, H.; Chen, Y. N.; Kimura, T.; Sakakibara, S. *Int. J. Pept. Protein Res.* **1992**, *40*, 294. (b) Sakakibara, S. *Biopolymers* **1995**, *37*, 17.

(16) There is some ambiguity about the uronium salt structures of HATU and HBTU: the crystal structures of these compounds have been shown to exist as guanidinium-1 *N*-oxide salts although the structures may be different in solution. See: Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57.

(17) Synthesis of DEPBT: To a solution of HOOBt (14.3 g, 0.088 mmol) and TEA (8.92 g, 0.088 mmol) in CH_2Cl_2 (100 mL) at 0 °C is added diethylphosphorochloridate (17.3 g, 0.1 mmol) in CH_2Cl_2 (50 mL) dropwise. The reaction mixture is stirred for 3 h. After the resulting triethylamine hydrochloride salt is removed by filtration, the solvent is removed. The residue is dissolved in EtOAc (150 mL); washed with 0.1 N HCl, water, and brine; dried over $MgSO_4$; and taken to dryness. The crude product is recrystallized from EtOAc/petroleum ether to give colorless crystals of DEPBT (21.5 g, 82%), mp 72−74 °C. MS (EI): 299 M^+. Anal. Calcd for C, 44.15; H, 4.72; N, 14.05. Found C, 44.20; H, 4.71; N, 14.30.