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Organophosphorus Compound DEPBT as a Coupling Reagent for Oligopeptides and Peptoids Synthesis: Studies on Its Mechanism

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Abstract: Some oligopeptides and peptoids were synthesized by applying the organophosphorus compound DEPBT as a coupling reagent. D-Biotin-OOBt was obtained unexpectedly. A proposed reaction mechanism for DEPBT-mediated coupling was proved.

Keywords: Amino acids, peptides, phosphorus, coupling.

We have developed an organophosphorus coupling reagent, 3-(diethoxyphosphoryloxy)-1,2,3-benzotrazin-4(3H)-one (DEPBT)[1,2]. It has been shown that this new coupling reagent is efficient as BOP and HBTU, both in solid phase and solution peptide synthesis[3]. It also showed great efficiency for peptide reaction without racemization[4].

Table 1 The physical constants of some Oligopeptides and peptoids

	Yield (%)		m.p.(°C)	$[\alpha]_D^{20}$(c, sol)	
	Obs.	Lit.[5]	Obs.	Lit.[5]	Obs.
Z-Ala-Ser-OMe	66	137-138	134-135	-20.0(1,MeOH)	-17(1,MeOH)
Z-Ala-Phe-OMe	94	101-102.5	99-101	-11.5(1,EtOH)	-13(1,EtOH)
Z-Asn-Phe-OMe	73	199-201	195-197	-3.0(1,DMF)	-1.6(1,dmf)
D-Biotin-Phe-OMe	70	92-96	------	+8.7(1,DMF)	-------
Boc-His(Bom)-Phe-OMe	57	138-140	45-47	-1.9(0.5,DMF)	-10.4(0.5,DMF)
Indol-Gly-Gly-OEt	58	93-97	-------	0	--------

As shown in the **Table 1**, all the reactions afford peptides (or peptoids) in fair to good yield. During the coupling reaction, the hydroxyl group in the amino components (serine) need not be protected. But when we synthesized D-Biotin-Gly-Gly-OEt, D-Biotin-OOBt[4] was got unexpectedly (**Scheme 1**). To explore this somewhat unusual reaction, we carried out the following reaction (**Scheme 1**) in which DCC was used as coupling reagent. The similar intermediate N-acyl urea was

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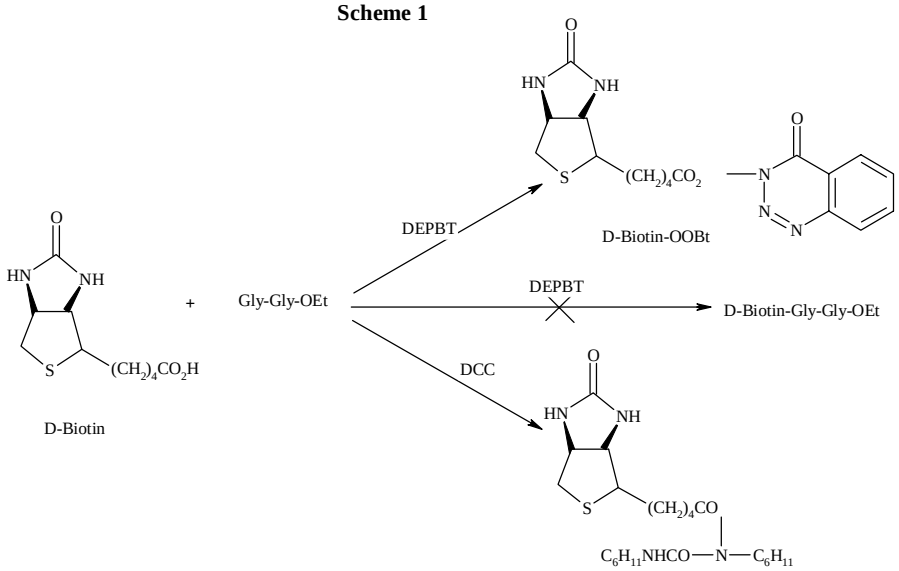
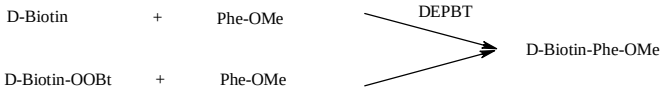
observed. Those results confirmed the assumption that Gly-Gly-OEt was self-coupled to be piperazinedione which was separated and purified thereafter.

In order to explain the formation of D-Biotin-OOBt, the other two parallel experiments were designed which were showed in **Scheme 2**. Both reactions gave the same product D-Biotin-Phe-OMe. This confirmed that D-Biotin-OOBt is an intermediate in the reaction of synthesis of D-Biotin-Phe-OMe, and it also explained the reason that the yield of D-Biotin-OOBt in the reaction of D-Biotin with Gly-Gly-OEt was too low, if the reaction was going on, D-Biotin-OOBt would generate more, and consume more. But in our reaction condition, Gly-Gly-OEt was self-coupled, therefore D-Biotin-OOBt did not consume, so the yield of it was low.

Scheme 1



Scheme 2



This result made us to study the possible mechanism of reactions in which DEPBT was used as a coupling reagent. H. T. Li[6] had suggested two possible mechanisms for DEPBT-mediated coupling, presented in **Figure 1** and **Figure 2**. But they could not prove it by experimental data. In the mechanism 1, the carboxylate of amino acid attacks the phosphorus in DEPBT to form the highly activated intermediate **1**, which is quickly transformed to the OOBt ester **2** *via* an intramolecular reaction. The incoming amine component initiates a nucleophilic attack leading to the desired amide bond formation. The possibility of proton abstraction from Cα of this intermediate is not favored because of the high electron deficiency about the HOOBt moiety making it difficult to accommodate another negative charge (compound **2′**). It is also possible

that intermediate **1** collapses to the highly activated anhydride **3**. **3** may react with the amine group to form the desired amide bond directly or it can react with HOOBt to form the ester **2**. The activated ester **2** can react with the incoming amine to form the desired product. Our experimental results favor the first mechanism because we obtained the activated intermediate D-Biotin-OOBt and also the second mechanism involves the formation of a highly activated anhydride which is prone to racemization. And in our experiment, the racemization is too low to emphasis it.

Figure 1 A proposed mechanism 1 for DEPBT-mediated coupling

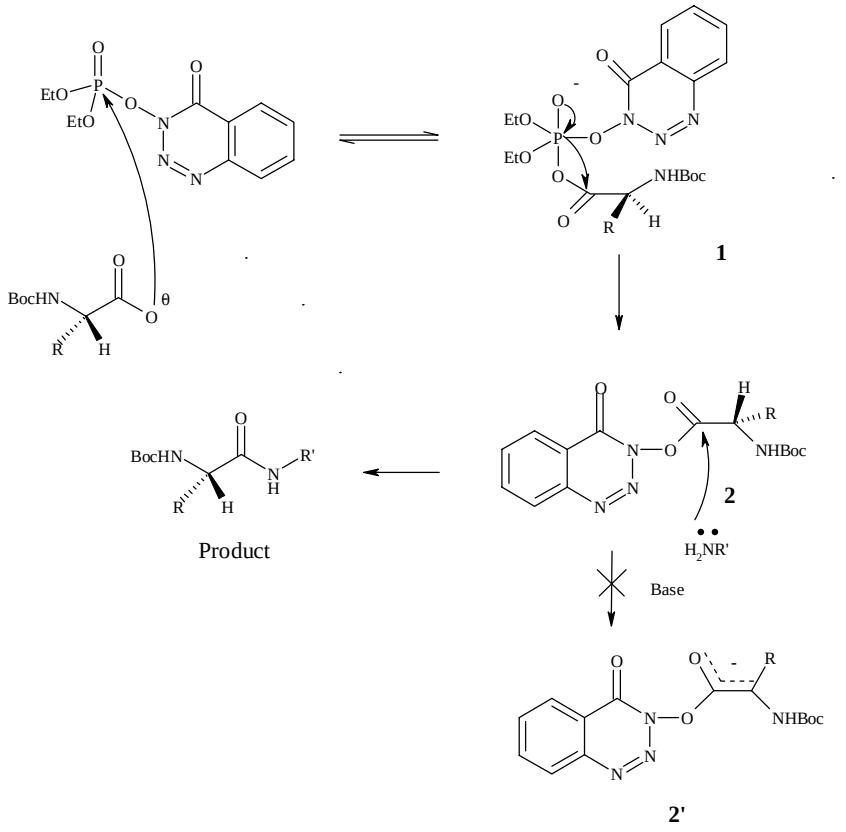
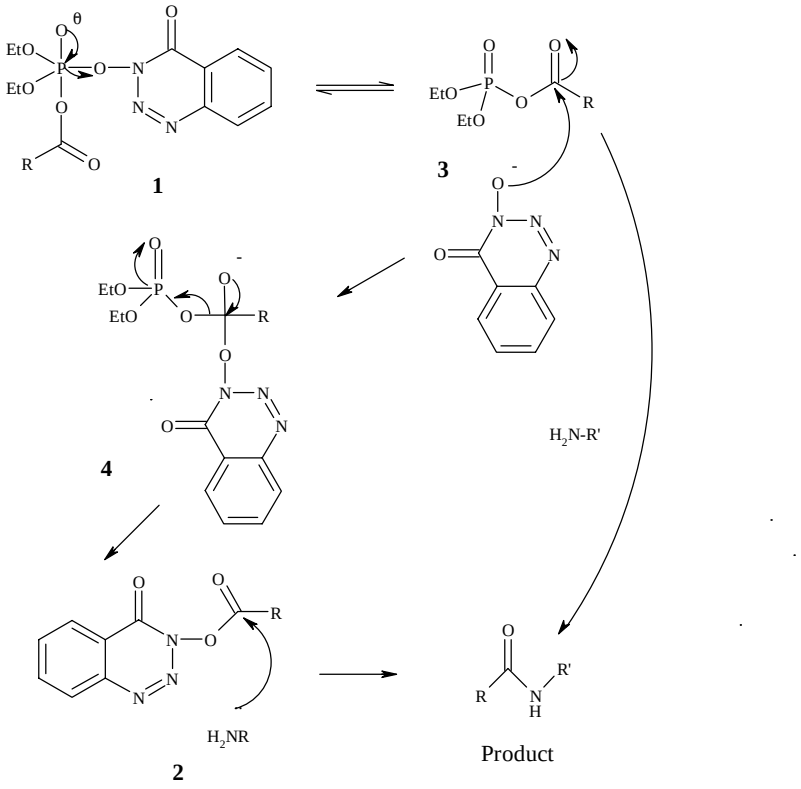


Figure 2 A proposed mechanism 2 for DEPBT-mediated coupling



In summary, we think that the mechanism in the reaction of DEPBT as coupling reagent is that the phosphorus moiety in DEPBT promoted the formation of the HOOBt active ester. The HOOBt ester in turn forms the peptide bond efficiently with the retention of optical integrity.

References and Notes

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2. Y. H. Ye, C. X. Fan, D. Y. Zhang, *et al*, *Chem. J. Chin. Univ.* **1997**, *7* (in Chinese),1086.
3. Standard abbreviation for amino acids and protecting groups follow the IUPAC-IUB Joint Commission on Biochemistry Nomenclature *J. Biol. Chem.* **1971**, *247*, 977.
4. H. T. Li, Racemization Studies on a New Coupling Reagent 3-(Diethoxy- phosphoryloxy) -1,2,3 benzotrazin-4(3H)-one (DEPBT). (Unpublished data)
5. G. R. Pettit, "*Synthetic Peptides*", Vol. 1-6, **1970, 1971, 1975, 1976, 1980, 1982**, Van Nostrand Rainhold Co. N.Y.
6. DEPBT was prepared according to a general procedure. D-Biotin-OOBt Yield: 14.5%. m.p.: 207-210°C. ^{1}HNMR(DMSO-d_6/TMS) δ :1.63-1.66(m,2H,-$(CH_2)_4$-),1,79-1,84(m,2H,-$(CH_2)_4$-),1.88- 1.98(m,2H,-$(CH_2)_4$-), 2.63-2.74 (m,1H,-CH-), 2.84-2.87 (m,2H,-$(CH_2)_4$-), 2.95-2.99 (m,1H,-(CH_2)- S-), 3.22-3.23 (m,1H,-(CH_2)-S-), 4.45-4.48 (m, 1H,-CH-NH-), 4.64-4.67 (m,1H,-CH-NH-), 7.87-7.91 (m,1H,Ar-H), 8.02-8.06 (m,1H,Ar-H), 8.24-8.26 (m,1H,Ar-H), 8.40-8.42 (m,1H,Ar-H); FAB- MS(*m/z*): 389 (M^+), 91 (base peak).

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