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DEPBT as an Efficient Coupling Reagent for Amide Bond Formation with Remarkable Resistance to Racemization^{*,†}

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Abstract: 3-(Diethoxyphosphoryloxy)-1, 2, 3-benzotriazin-4(3H)-one (DEPBT) is an effective coupling reagent for synthesis of linear and cyclic peptides by both solution and solid-phase peptide synthesis. DEPBT mediates amide bond formation with remarkable resistance to racemization. When DEPBT is used as a coupling reagent, it is not necessary to protect the hydroxy group of the amino component (such as tyrosine, serine, and threonine) and the imidazole group in the case of histidine. The high efficiency of DEPBT and its utility have been demonstrated in the syntheses of complex natural products such as ramoplanin A2, ramoplanose aglycon, ustiloxin, and teicoplanin aglycon. © 2004 Wiley Periodicals, Inc. *Biopolymers (Pept Sci)* 80: 172–178, 2005

Keywords: DEPBT; coupling reagent; amide bond formation; racemization; linear peptides; cyclopeptides; peptido-alcohols; natural products

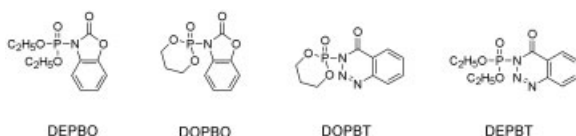
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*This article is dedicated to the memory of Dr. Murray Goodman. Murray was an outstanding and enthusiastic scientist who was

a true pioneer in peptide chemistry. He was a long-time colleague and friend, mentors of Haitao and Xiaohui. Part of the work described in this article was carried out in collaboration with Murray. We will always remember him.

†Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature (1984), *European Journal of Biochemistry*, Vol. 138, pp. 9–37.



SCHEME 1 The structures of the four organophosphorus compounds.

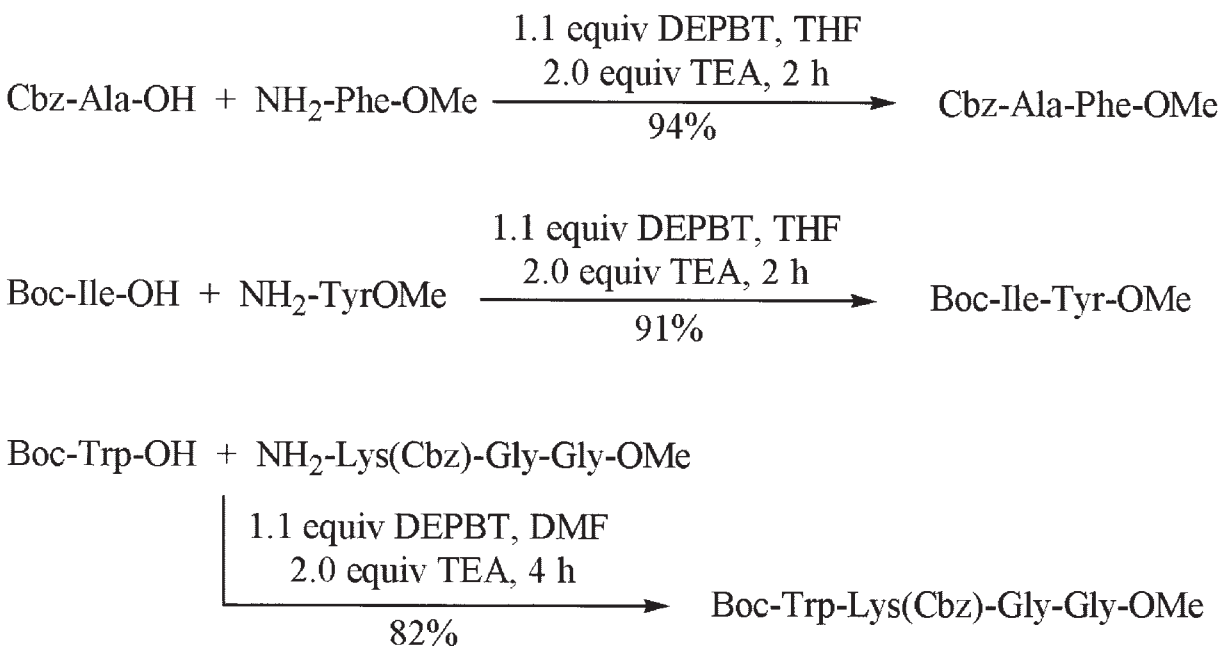
INTRODUCTION

The formation of the amide bond is one of the fundamental reactions in 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 for heterocycles.¹ 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 mini-

mizing racemization remains an important area of research. A number of organic and natural products with interesting biological activities have been discovered and they usually contain highly functionalized or noncoded amino acids as building blocks, which may not be easily constructed by the traditional peptide coupling reagents. For this reason, the development of new peptide coupling reagents has become a most fascinating field of research for many organic chemists with various background.² Accordingly, several organophosphorus compounds, DEPBO, DOPBO, DOPBT, and DEPBT, were developed in this laboratory as coupling reagents (Scheme 1).³ We used these coupling reagents in the syntheses of a number of bioactive peptides such as analogs of delta sleep inducing peptide,⁴ a group of γ -glutamyl oligopeptides isolated from *Panax* ginseng,⁵ cyclic pentapeptides^{6,7} and cyclic heptapeptides^{8–10} isolated from *Caryophyllaceae*, etc.

3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) demonstrated the best overall properties among all other organophosphorus compounds prepared. It not only is an effective coupling reagent for the formation of an amide bond, but it also has great resistance to the racemization of reaction product.

In this article, we describe the advantages^{11–13} and synthetic utility of DEPBT as a coupling reagent in the synthesis of bioactive peptides and complex natural products.^{14–17}



SCHEME 2 Peptide coupling reactions using DEPBT as the coupling reagent.

Table I Yields of Protected Dipeptides Containing Histidine with Unprotected Imidazole Group Synthesized by DEPBT

No.	Carboxyl Component	Product	Fast Atom Bombardment– Mass Spectroscopy m/z (M + H) ⁺	Yield (%)
1	Boc–Tyr(Bzl)–OH	Boc–Tyr(Bzl)–HisOMe	523	81.0
2	Boc–Ser(Bzl)–OH	Boc–Ser(Bzl)–HisOMe	447	54.6
3	Boc–Glu(OBzl)–OH	Boc–Glu(OBzl)–HisOMe	489	48.5
4	Boc–Asp(OBzl)–OH	Boc–Asp(OBzl)–HisOMe	475	47.3
5	Boc–Phe–OH	Boc–Phe–HisOMe	417	63.7
6	Boc–Pro–OH	Boc–Pro–HisOMe	367	56.1
7	Boc–Trp–OH	Boc–Trp–HisOMe	456	70.7
8	Boc–Arg(Tos)–OH	Boc–Arg(Tos)–HisOMe	580	64.7
9	Boc–Lys(2-Cl-Z)–OH	Boc–Lys(2-Cl-Z)–HisOMe	566	75.0
10	Z–Phe–OH	Z–Phe–HisOMe	451	70.0

RESULTS AND DISCUSSION

Synthesis of Linear Peptides

A typical peptide coupling reaction using DEPBT is shown in Scheme 2. It is worthwhile noting that the hydroxy group of the amino component (such as serine, threonine, and tyrosine) does not need to be protected. The side reaction of asparagine dehydration, easily caused by dicyclohexylcarbodiimide (DCC), was not observed in peptide couplings mediated by DEPBT. It has also been demonstrated that DEPBT can be used as the coupling reagent in solid-phase peptide synthesis.⁴ A number of peptides including delta sleep inducing peptide (DSIP), [γ -Abu]¹-DSIP,⁴ the precursor of Leu-enkephalin, (Z–Tyr–Gly–Gly–Phe–Leu–NH₂Ph)¹⁸ were synthesized using DEPBT as the coupling reagent.

For the use of DEPBT in the peptide synthesis involving histidine, a series of protected dipeptides

were made from different N-protected amino acids as the carboxyl components and HisOMe · 2HCl was used as the amino component (Table I). In the synthesis, the imidazole group of histidine was left unprotected. The results indicate that the application of DEPBT can be widely used for the synthesis of bioactive peptides containing histidine with unprotected imidazole group.¹³

Comparative Study of Racemization

Comparative studies were carried out to assess the racemization of reaction product in the peptide coupling using DEPBT.¹¹ An activated carboxyl intermediate was allowed to stand in the presence of tertiary amine for a short time (4–60 min). The intermediate was then allowed to react with benzylamine, and the resulting ratio of enantiomers was determined by chiral high performance liquid chro-

Table II Comparative Studies of Racemization During *in situ* Activation

$\text{Boc-Ser(Bzl)-OH} \xrightarrow[2.0 \text{ equiv DIEA, CH}_2\text{Cl}_2, 20^\circ\text{C}]{1.0 \text{ equiv or } 2.0 \text{ equiv activator}} \xrightarrow{\text{Delay}} \xrightarrow{\text{Benzylamine}} \text{Measure L : D Ratio by Chiral HPLC}$			
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 a long delay time, very little racemization was observed.

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

$\text{Boc-Cys(4MB)-X} \xrightarrow[\text{CH}_2\text{Cl}_2 \text{ or THF } 20^\circ\text{C}]{\text{1.0 equiv DIEA}} \xrightarrow{\text{Delay}} \xrightarrow{\text{Benzylamine}} \text{Measure L : D Ratio by Chiral HPLC}$ 0.33M				
X	Solvent	Delay Time (min)	L : D Ratio	Yield (%)
At	CH ₂ Cl ₂	5	87:13	94
OOBt	CH ₂ Cl ₂	5	98.5:1.5	85
OAt	THF	30	95:5	98
OOBt	THF	30	100:0	>99

matography (HPLC). To maximize sensitivity, experiments were carried out using the Boc-Ser(Bzl)-OH (Boc: *tert*-butyloxycarbonyl) and Boc-Cys(4MB)-OH as the carboxyl component, since these amino acids have the highest susceptibility toward racemization. Table II describes comparative rate of among DEPBT and the commonly used phosphonium reagents Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBroP) and benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP), and the uranium reagents N-[(dimethyl(amino)-1H-1,2,3-triazole[4,5-b] pyridin-1-yl-methylene)-N-methylmethanaminium hexafluorophosphate N-oxide (HATU), and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU).

As a complement to the *in situ* activation experiment, the 7-aza-1-hydroxy-benzotriazole (HOAt) and 3-hydroxy-1,2,3-benzotriazin-4(3H)-one (HOObt) esters of Boc-Cys(4MB)-OH were prepared in crystalline form and subjected to a similar experiment. The results are described in Table III.

Another experiment designed to examine the use of DEPBT in different solvents was carried out, and the results are described in Table IV.

More recently, in the synthesis of peptide nucleic acid (PNA) where T_{L-Phe} and L-Val-OMe are cou-

pled, different coupling reagents, DEPBT, *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazine-3-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TDBTU), HBTU, or HATU, were used and compared for the enantiomeric purity. Among them, the DEPBT-mediated reaction produced the best enantiomeric purity [DEPBT/diisopropyl ethyl amine (DIEA), 95.8% ee; TDBTU/DIEA, 91.8% ee; HBTU/DIEA, 83.6% ee; HATU/DIEA, 77.2% ee].¹⁹

The results shown above clearly indicate that DEPBT is a superior coupling reagent in terms of its ability to inhibit racemization when compared to other typical phosphonium and uranium coupling reagents.

Synthesis of Cyclic Peptides

Several natural cyclic peptides, including two pentapeptides, c(Pro-Tyr-Leu-Ala-Gly) and c(Ala-Tyr-Leu-Ala-Gly),^{6,7} and two heptapeptides, c(Gly-Tyr-Gly-Gly-Pro-Phe-Pro) and c(Gly-Ile-Pro-Tyr-Ile-Ala-Ala), all isolated from medicinal herbs, were synthesized successfully using DEPBT.^{8,10} The two cyclic pentapeptides, c(Pro-Tyr-Leu-Ala-Gly) and c(Ala-Tyr-Leu-Ala-Gly), were used to evaluate the cyclization yield of DEPBT, BOP, 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide (EDCI), HBTU, and diphenylphosphoryl azide (DPPA). The yields of

Table IV The Effect of the Solvent on Racemization and Yield of an *in situ* Activated Intermediate for Boc-Cys(4MB)-OH

$\text{Boc-Cys(4MB)-OH} \xrightarrow[\text{2.0 equiv DIEA CH}_2\text{Cl}_2 \text{ or THF } 20^\circ\text{C}]{\text{2.0 equiv activator}} \xrightarrow{\text{Delay}} \xrightarrow{\text{Benzylamine}} \text{Measure L : D Ratio by Chiral HPLC}$ 0.33M				
Activation	Solvent	Delay Time (min)	L : D Ratio	Yield (%)
DEPBT (2.0 equiv)	CH ₂ Cl ₂	15	100:0	73
DEPBT (2.0 equiv)	THF	30	100:0	>99

Table V The Isolated Yields of Different Coupling Reagents Mediated Peptide Cyclization of H-Pro-Tyr-Leu-Ala-Gly-OH and H-Ala-Tyr-Leu-Ala-Gly-OH

Cyclopeptide	Coupling Reagent	Yield (%)
c(Pro-Tyr-Leu-Ala-Gly)	DEPBT	54
	BOP	35
	EDC	25
	HBTU	40
	DPPA	52
c(Ala-Tyr-Leu-Ala-Gly)	DEPBT	52
	TBTU ^a	45
	DCC	15

^a TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.

the cyclization reactions are shown in Table V. It can be readily seen that DEPBT-mediated reactions gave the best yields.

In the synthesis of leuteinizing hormone-releasing hormone (LHRH) antagonists containing three non-coded amino acids, c[Trp(Boc)-Phg-Arg(Tos)-D-Aph(Boc)-D-Ala], the cyclization was carried out using DEPBT and HBTU/DMAP. The DEPBT-mediated reaction had a better yield (Table VI).²⁰

In another synthesis, DEPBT was used for the cyclization of the lanthionine analog of TT-232 (Scheme 3).²¹ Lanthionines are monosulfide analogs of cystine and constituents of peptide lantibiotics, which provides more constrained peptide structures with greater stability toward enzymatic degradation compared to the labile disulfide bridge of cysteine in natural or unnatural cyclic peptide sequences.

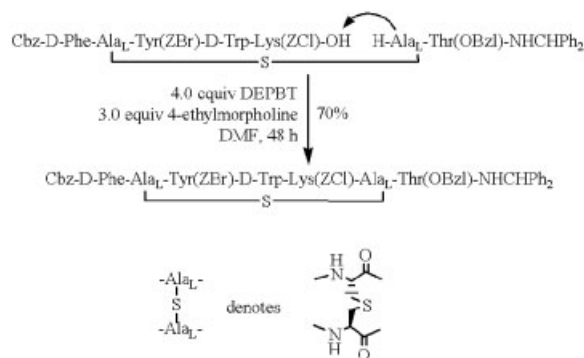
A Proposed Mechanism for DEPBT-Mediated Coupling

In Scheme 4, a proposed mechanism for DEPBT-mediated coupling is shown. A tertiary amine is required to generate the carboxylate of the N-protected

Table VI Cyclization Yields of H-Trp(Boc)-Phg-Arg(Tos)-D-Aph(Boc)-D-Ala-OH With Two Different Coupling Reagents

Reagent	Yield(%)*
DEPBT	22
HBTU/DMAP	12

* The yield of cyclic pentapeptide was based on the amount of product isolated by HPLC.

**SCHEME 3** Synthesis of lanthionine analog of TT-232.

amino acid, which then attacks the central phosphorus atom of DEPBT. The resulting transient intermediate **1** rearranges with a loss of diethyl phosphite to form the HOObt ester **2**. This strongly activated stable intermediate is attacked by the amino component to form the amide product. This mechanism is analogous to that by which the phosphonium reagent BOP generates HOBT esters.

In the recent attempt of synthesis D-biotin-Gly-Gly-OEt using DEPBT as the coupling reagent, D-biotin-OObt was indeed isolated rather than the desired peptide.²²

Synthesis of the Peptido-Alcohols and N-Glycopeptide

When DEPBT is used for peptide coupling, it is not necessary to protect the hydroxy group of the amino component. Therefore, DEPBT can be used to synthesize peptido-alcohols. The use of DEPBT has been reported for the synthesis of a series of peptido-alcohols and a glycopeptide derivative from N-protected amino acids and different amino components with one or more unprotected hydroxyl groups

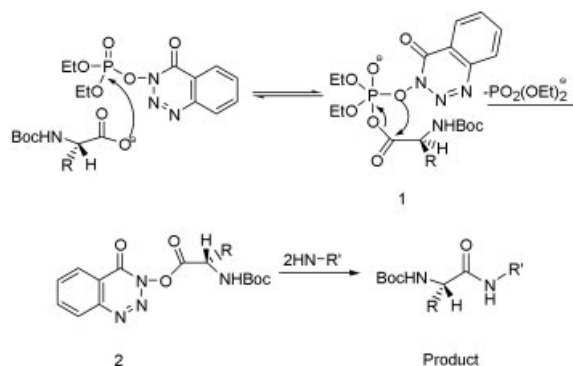

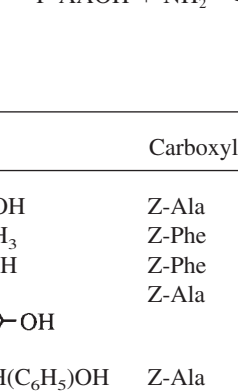
**SCHEME 4** A proposed mechanism for DEPBT-mediated coupling.

Table VII Synthesis of *N*-protected Peptido-alcohols using DEPBT
$$\text{P-AAOH} + \text{NH}_2\text{-}\overset{\text{R}}{\text{C}}\text{H-OH} \xrightarrow[\text{Solvent}^*]{\text{DEPBT}} \text{P-AA-NH-}\overset{\text{R}}{\text{C}}\text{H-OH}$$

Reaction Product	Carboxyl Component	Amino Component	Yield (%)
Z-Ala-NHCH(C ₂ H ₅)CH ₂ OH	Z-Ala	DL-2-aminobutanol	58
Z-Phe-NHCH ₂ CH(OH)CH ₃	Z-Phe	DL-1-amino-2-propanol	43
Z-Phe-NHCH(CH ₃)CH ₂ OH	Z-Phe	D-Alaol	51
Z-Ala-NH- 	Z-Ala	<i>p</i> -aminophenol	76
Z-Ala-NHCH(CH ₂ OH)CH(C ₆ H ₅)OH	Z-Ala	(1 <i>s</i> ,2 <i>s</i>)-(+)-2-amino-1-phenyl-1,3-propanediol	80
Z-Tyr(Bzl)-Thr-ol	Z-Tyr(Bzl)	Throl	76
Fmoc-Cys(Acm)-Thr-ol	Fmoc-Cys(Acm)	Throl	68
Z-Tyr(Bzl)-Ser-ol	Z-Tyr(Bzl)	Serol	77
Fmoc-Lys(Boc)-Thr-Cys(Acm)-Thr-ol	Fmoc-Lys(Boc)-Thr	Cys(Acm)-Throl	69
	Z-Ala	Glucosamine hydrochloride	47

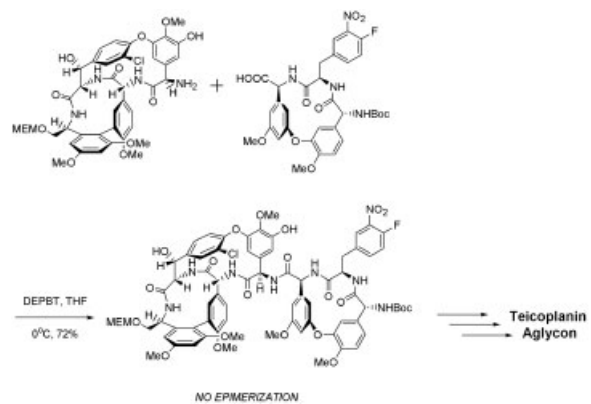
* Solvent selected depending on solubility. Solvents used were: Anhydrous CH₂Cl₂, THF, DMF or mixtures of CH₂Cl₂, THF, DMF, C₅H₅N and H₂O.

(Table VII).¹² When DEPBT is used as the coupling reagent, the results clearly show that the carboxyl component selectively reacts with the amino group of the amino alcohol substrate to form the amide bond. The hydroxyl group in the amino component did not react (i.e., no formation of the ester was observed). This is a unique property of DEPBT, since the hydroxy group of the amino alcohol must be protected when DCC or EDCI are used in the synthesis of peptido-alcohols.

Amide Bond Formation in Complex Natural Products

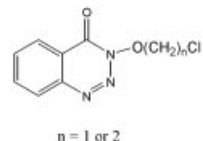
DEPBT has been demonstrated to be an excellent coupling reagent for amide bond formation in several natural product syntheses recently. In the key step of the total synthesis of teicoplanin aglycon, DEPBT was used in a number of amide bond formations involving phenylglycine. As with the syntheses of analogous substrates in the vancomycin series, the same coupling reactions with phenylglycine commonly resulted in low conversions or significant racemization of the product, depending on the reagents or reaction conditions employed. However, the use of

DEPBT [0°C, tetrahydrofuran (THF)] uniformly produced the best results, providing good to excellent conversion, and most importantly, with no detectable racemization (Scheme 5).¹⁴ In the total synthesis of (–)-tamandarin B, DEPBT was employed by Joullié et al. to couple the side chain to the macrocycle (Scheme 6).¹⁶ Moreover, DEPBT was employed in the total synthesis of ustiloxin D,¹⁵ ramoplanin A2 and ramoplanose,¹⁷ etc.

**SCHEME 5** Total synthesis of teicoplanin by Boger et al.

Side Reaction

A reaction between an intermediate substance (HOObt) derived from DEPBT and the solvent CH_2Cl_2 or $\text{ClCH}_2\text{CH}_2\text{Cl}$ was observed. The side pre-cuts were isolated (Scheme 7).²³

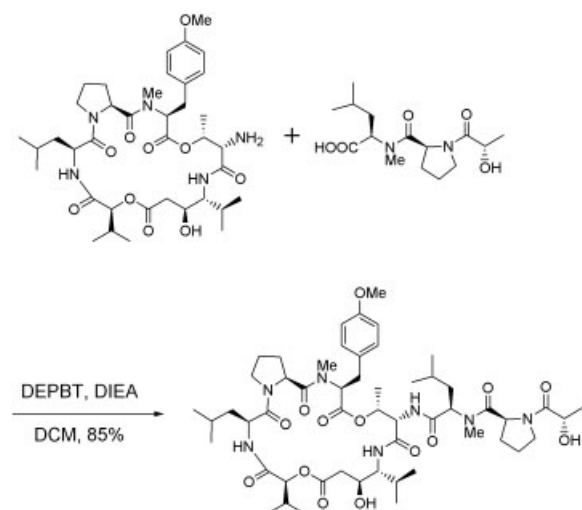


SCHEME 7 Side products from DEPBT-mediated reactions.

CONCLUSION

For the purpose of chiral synthesis, the development of a new coupling reagent with the ability to minimize racemization is one of the greatest challenges for organic chemists. The organophosphorus compound DEPBT has been demonstrated as an excellent coupling reagent. A number of bioactive peptides, cyclic peptides, and peptidomimetics were synthesized using DEPBT under mild conditions in good yield. DEPBT offers exciting properties for amide bond formation with a remarkable resistance to racemization. Its high efficiency and utility has been demonstrated in the key step of total synthesis of several complex natural products such as (-)-tamandarin, teicoplanin aglycon.

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SCHEME 6 Total synthesis of (-)-tamandarin B by Joullié et al.

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