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## Graphical Abstract

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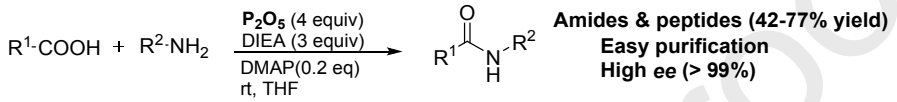

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# Phosphorus Pentoxide for Amide and Peptide Bond Formation with Minimal By-products

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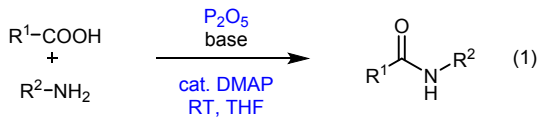
ABSTRACT

Phosphorus pentoxide and DMAP are used for amide bond formation from carboxylic acids and amines. Dipeptides and amides have been synthesized using this reagent in 42-77% yields and >99% ees. The protocol is attractive as it occurs at ambient temperature, the formation of organic by-products is minimal and the reagent can be readily quenched using water. Furthermore, excellent enantioselectivities are observed without the use of harsh triazole based additives.



## 1. Introduction

Coupling reagents or activating groups for carboxylic acids play an important role in peptide synthesis.[1] Carbodiimides[2] in presence of additives such as 1-hydroxy-1H benzotriazole (HOBt) and 1-Hydroxy-7-azabenzotriazole (HOAt) are popularly used coupling reagents.[3] However, benzotriazoles are explosive as well as carcinogenic.[4] Another class of coupling reagents is uronium/aminium salts that are triazole derivatives. 2-(1H-benzotriazol-1-yl)1,1,3,3-tetramethyluroniumhexafluoro-phosphate (HBTU)[5] and related reagents such as HCTU[6] and HATU[7] belong to this family. These reagents provide excellent yields with minimal racemization. They are relatively expensive and the guanidium by-product has to be removed by column chromatography. Phosphonium salts are yet another class of coupling reagents based on benzotriazoles.[8] (Benzotraizol-1-yloxy)tris(dimethylamino)phosphoniumhexa-fluorophosphate (BOP)[9] and PyBOP[10] are examples of phosphonium-based reagents. BOP reagents liberate hexamethylphosphoramide (HMPA), which is highly carcinogenic.[11] Recently hypervalent iodine (III) reagents[12] have also been used for peptide synthesis. With the growing applications of peptides, development of low-cost, efficient and non-toxic coupling reagents is highly desirable. Alkyl-phosphonic anhydrides such as T3P have been routinely used for peptide synthesis with minimal racemization.[13] Phosphorus pentoxide is a cheap and efficient dehydrating agent that has been used to synthesize anhydrides from the corresponding carboxylic acids.[14] $P_2O_5$ has also been used for synthesizing polymeric amides from activated carboxylic esters and cyclic diketopiperazine derivatives from the corresponding amino acid monomer.[15] N-Substituted phenylpropioloyl amides have also been synthesized using $P_2O_5$ as a dehydrating agent.[16] Significant racemization was observed when peptide synthesis was carried out using $P_2O_5$ in diethylphosphite at elevated temperatures.[17] Herein, we describe a mild method for synthesizing amides and peptides using $P_2O_5$ and catalytic DMAP at room temperature (Equation 1). The reaction does not require the use of hazardous solvents such as diethylphosphite. Minimal racemization and by-products are observed in the reaction as the carboxylic acid is "activated" as the corresponding symmetrical acid anhydride. The yields for amide/peptide bond formation are comparable to commonly used coupling reagents.




## 2. Results and Discussion

### *2.1 Synthesis of amides*

Amide bond formation using simple carboxylic acids and amines was first explored using phosphorus pentoxide (Scheme 1). The reaction was carried out in the presence of diisopropylethylamine (DIEA) and catalytic amounts of *N-N*-dimethylamino pyridine (DMAP). A variety of amides **3a-i** were synthesized by coupling aromatic or benzylic carboxylic acids with primary or secondary amines. The reported yields for the synthesis of these amides using other coupling reagents is also

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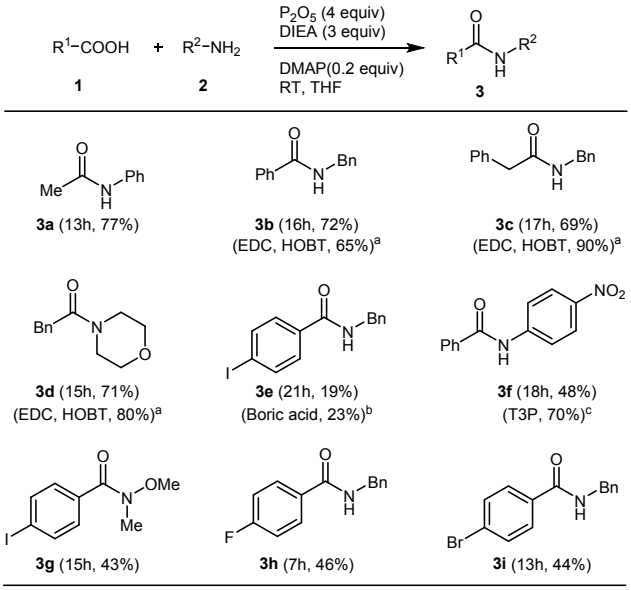
withdrawing groups were synthesized in good yields (~70%) that were comparable to reported procedures. Amides **3e-i** with electron withdrawing groups could also be obtained using $P_2O_5$ albeit with relatively lower yields (~40%).

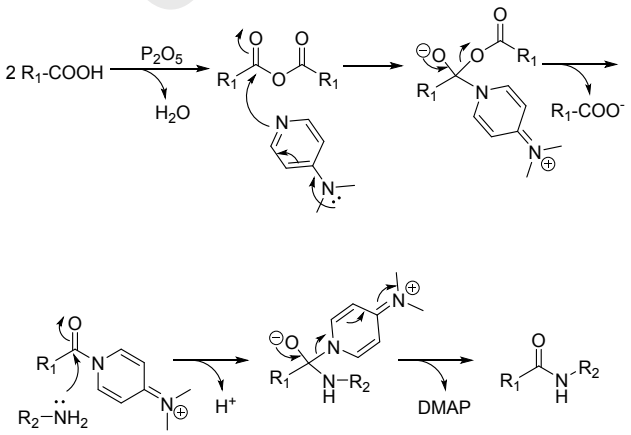



[a]Ref.18a, [b]Ref.18b, [c]Ref.18c.

**Scheme 1.** Substrate scope for amide synthesis using $P_2O_5$.[18]

The reaction presumably goes in a two-step fashion, wherein the first step is formation of the anhydride. The proposed mechanism for amide bond formation using $P_2O_5$ and DMAP is illustrated in Scheme 2. The first step involves formation of the symmetric anhydride by dehydration of the carboxylic acid in the presence of $P_2O_5$. Nucleophilic attack of DMAP to the anhydride and the subsequent expulsion of the carboxylate group provides a more activated carbonyl center. The amine adds to this intermediate to form the requisite amide after elimination of DMAP. As the formation of anhydride, followed by attack by DMAP is highly facile, one can envisage minimal racemization for peptide bond formation using this methodology without the use of toxic triazole based additives. Furthermore, because the acid is activated as the anhydride, the formation of unwanted by-products typically observed for peptide synthesis using coupling reagents is avoided. The lower yields for acids with electron withdrawing groups can be attributed to the lower efficiency of anhydride formation for these substrates.

**Scheme 2**. Proposed mechanism for amide bond formation.




$P_2O_5$ was utilized for coupling Fmoc glycine **4** and phenylalanine methyl ester. After completion of the reaction, the excess $P_2O_5$ was readily quenched by addition of water. As envisaged, other organic by-products were not observed during work-up of the reaction. A simple filtration column afforded the dipeptide Fmoc-NH-Gly-(L)Phe-OMe **5a** in 67% yield (Equation 2). The optical purity of the product was determined to be 99.6 % ee using chiral-HPLC (1% isopropyl alcohol/hexane) (Figure 1). Racemization *via* the oxazolone intermediate is presumably not operative as seen by >99% ee for dipeptide **5a** containing a chiral amino acid at the C-terminus.

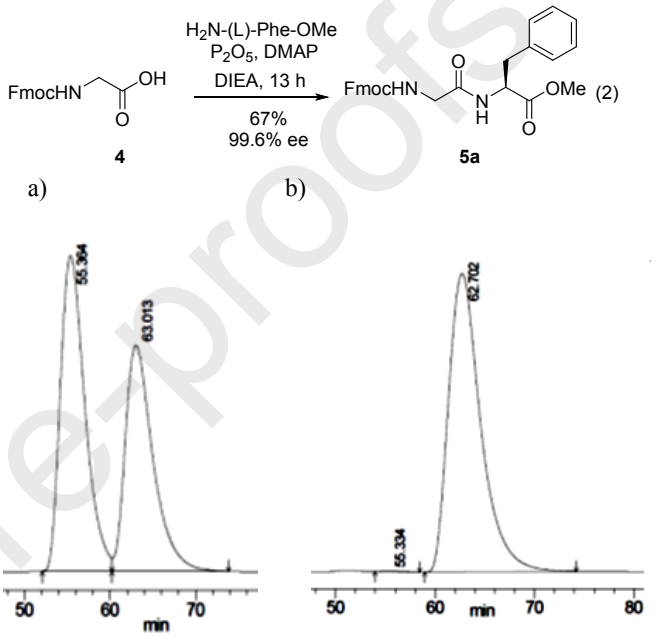



**Figure 1.** HPLC chromatogram of a) racemic mixture of Fmoc-NH-Gly-Phe-OMe; b) Fmoc-NH-Gly-(L)Phe-OMe **5a** synthesized.

The optical rotation of enantiomerically pure compound **5a** was used as a benchmark for optimizing the reaction conditions for dipeptide synthesis (Table 1). Lowering equivalents of $P_2O_5$ and/or performing the reaction under reflux decreased the yields as well as optical purity of dipeptide **5** (Table 1, entries 2-3). Using other solvents such as diethylphosphite did not improve the yields obtained using 1 equiv of $P_2O_5$ (Table 1, entries 4-7). Dicholoromethane was also not found to be a good solvent for the reaction with 4 equiv of $P_2O_5$ (entry 8). These led to racemization as reported in the literature.[17] The use of DIEA and DMAP was found to be crucial for this reaction (entries 9 & 10).

**Tab**

| No. | Solvent | Temp. (°C) | $P_2O_5$ (equiv) | Yield[a] (%) | ee[b] |
|---|---|---|---|---|---|
| 1 | THF | rt | 4 | 67 | >99%[c] |
| 2 | THF | reflux | 1 | 51 | 90% |
| 3 | THF | reflux | 2 | 54 | 85% |
| 4 | Diethyl-phosphite | rt | 1 | 53 | 84% |
| 5 | Diethyl-phosphite | reflux | 1 | 50 | 81% |
| 6 | Diethyl-Phosphite / THF | rt | 1 | 54 | 88% |
| 7 | Diethyl-Phosphite /THF | reflux | 1 | 59 | 87% |
| 8 | $CH_2Cl_2$ | rt | 4 | 62 | 89% |
| 9 | THF[d] | rt | 2 | 18 | 87% |
| 10 | THF[e] | rt | 2 | 13 | 87% |

[a]Isolated yields, [b] Determined using optical rotation *c* 0.1, MeOH, $[\alpha]_D^{25}$, [c]Determined using chiral HPLC, [d]without DIEA, [e]without DMAP.

A variety of dipeptides were synthesized using the optimized reaction condition (Scheme 3). The reagent was also found to be compatible with Cbz-protected amino acids and afforded dipeptides **6** in good yields and excellent ees. Dipeptide Fmoc-Ile-Phe-OMe **7** was also synthesized in good yields. Epimerization was not observed in the $^1$H NMR and $^{13}$C NMR spectrum of the peptide. Dipeptides **8-14** from a variety of amino acids such as isoleucine, proline, serine, threonine, phenyl alanine and valine were also synthesized in moderate yields of 42-65%. Epimerization was not observed in all these cases also.

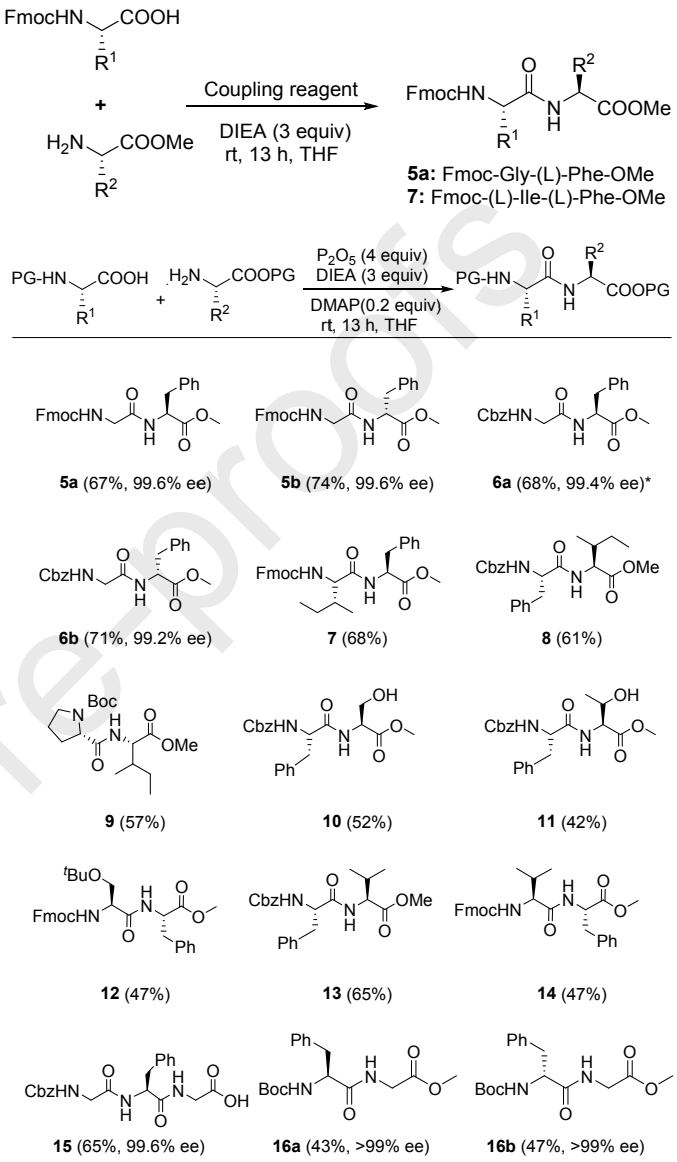
The tripeptide Cbz-Gly-(L)-Phe-Gly-OH **15** was synthesized in 65% overall yield by coupling of Cbz- Gly-(L)-Phe-OH and HCl.NH-Gly-OMe, followed by base hydrolysis. The optical purity of peptide Cbz-Gly-(L)-Phe-Gly-OH ($[\alpha]_D^{25}$ -13.1 $^o$ (c, 2, ethanol)) was found to be comparable to reported literature values ($[\alpha]_D^{25}$ -12.8 $^o$ (c, 2, ethanol)[17a] & $[\alpha]_D^{25}$ -13.2$^o$ (c, 2, ethanol))[17b] showing that **14** was obtained exclusively as the L isomer. The dipeptide **16a** containing a chiral amino acid at the N-terminus was also synthesized (in contrast to **5a**) to ensure that racemization was not observed in either case.

*2.3 Comparison with known coupling reagents*

The efficiency of $P_2O_5$ was compared with other coupling reagents for the synthesis of Fmoc-Gly-(L)-Phe-OMe **5a** & Fmoc-(L)-Ile-(L)-Phe-OMe **7** (Table 2). Coupling reagents that are commonly used for peptide synthesis, relatively less expensive and known to be efficient were chosen for this study. The reaction time was maintained the same for all reactions in order to compare their efficiencies. The equivalents of coupling reagents and additives used were based on known precedent for the coupling reagent used.

The yields with $P_2O_5$ were ~ 6-16% and 3-9% lower than the other coupling reagents for synthesis of **5a** and **7**, respectively. Despite the lower yields, an advantage of this reagent was that the TLC of the reaction mixture did not show formation of any by-product. The unreacted amino acids could be separated during work-up and a quick filtration through a silica column afforded is hygroscopic. Therefore, sufficient care must be taken while handling this reagent. However, given the fact that $P_2O_5$ is extremely cheap and the separation of the peptides is easy makes it an extremely attractive reagent for peptide synthesis.

**Scheme 3.** Peptides synthesized using protocol.



| No. | Peptide | Reagent (equiv) | DMAP (equiv) | Yield (%) |
|---|---|---|---|---|
| 1 | **5a** | $P_2O_5$ (4) | 0.2 | 67 |
| 2 | **5a** | HCTU (1.2) | 0 | 79 |
| 3 | **5a** | HBTU (1.2) | 0 | 83 |
| 4 | **5a** | DCC (3) | 0.2 | 73 |
| 5 | **5a** | DIC (3) | 0.2 | 78 |
| 6 | **7** | $P_2O_5$ (4) | 0.2 | 68 |
| 7 | **7** | HCTU (1.2) | 0 | 77 |
| 8 | **7** | HBTU (1.2) | 0 | 71 |
| 9 | **7** | DCC (3) | 0.2 | 72 |
| 10 | **7** | DIC (3) | 0.2 | 72 |

**Table 2.** Comparison with other coupling reagents

## 3. Conclusion

In conclusion, a cost-effective as well as mild protocol has been developed for amide and peptide synthesis using phosphorus pentoxide as a coupling reagent. Amides from carboxylic acids without electron withdrawing groups were synthesized in yields of 69-77% that were comparable to reported methods. $P_2O_5$ was also found to useful for peptide coupling and afforded dipeptides in 42-74% yields with minimal racemization. The protocol is extremely attractive as the reagent is cheap, no racemization is observed, formation of hazardous organic by-products is minimal and isolation of the desired product is quick. Efforts are underway to further improve the efficiency of this reagent for synthesis of peptides.

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## 5. Conflicts of interest

The authors declare the following competing financial interest(s): Indian patent application filing is in process. Patent application number 201821001972.

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- Minimal epimerization
- Minimal by-products