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Synthesis of N-Phosphoryl Amino Acids via Phosphoramidite Amine-Exchange

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Abstract: N-Phosphoryl amino acids were prepared rapidly and in high yield via phosphoramidite amine-exchange in the presence of 1H-tetrazole followed by oxidation with mCPBA. Formation of dibenzyl phosphoramidates incorporating the representative C-terminal protected amino acids leucine, phenylalanine, glutamic acid, and proline were demonstrated to be most successful with dibenzyl N,N-dimethylphosphoramidite although the N,N-diethyl and N,N- diisopropyl analogs also provided high yields. © 1998 Elsevier Science Ltd. All rights reserved.

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Phosphoramidates are continuing to be an important class of rationally designed therapeutics especially as oligonucleotide analogs employed as antisense and antigene agents.¹ Increasing interest in such compounds has provided the impetus for the synthetic development of nucleoside phosphoramidites for use in solid phase amine-exchange chemistry.² In principle, the phosphoramidite amine-exchange reaction is promoted by 1*H*-tetrazole, which facilitates the displacement of a dialkylamino ligand on the phosphitylating reagent by an incoming amine. Owing to the flowing process required for solid-phase nucleotide synthesis, a large excess 1*H*-tetrazole has been used historically, in some cases up to 200 equivalents with acetonitrile being the most common reaction solvent.³ Following solid phase-nucleotide amine-exchange on phosphoramidite, rapid *in situ* oxidation is commonly promoted by I_2 in H_2O to generate a desired phosphoramidate.

With a desire to prepare phosphoramidates as putative transition-state analog inhibitors of a glutamyl metalloprotease, we were interested in exploiting the nucleoside solid-phase phosphoramidite amine-exchange chemistry for use in analogous solution-phase chemistry involving amino acids.⁴ As a model for our phosphoramidate transition-state inhibitors, as well as crucial synthetic intermediates, we chose to prepare *N*-(dibenzyl phosphoryl)glutamate **2a** (**Table 1**). Initially, we chose the common phosphitylating reagent dibenzyl *N*,*N*-diisopropylphosphoramidite to establish the synthetic conditions for the amine-exchange.⁵ Because of the lipophilic nature of the C-terminal-protected amino acids chosen to incorporate into our target phosphoramidates, we were not necessarily limited to polar reaction solvents such as acetonitrile that are commonly employed in reactions with nucleotides.³ Furthermore, due to the reduced water solubility of our products, we chose 3-chloroperoxybenzoic acid (*m*CPBA) rather than I₂ in H₂O to chemically promote the oxidation of the second step. Thus, we examined the amine-exchange reaction involving glutamic acid dibenzyl ester [H-Glu(OBzl)-OBzl] in a variety of conditions and solvents as detailed in Table 1.

Yields for the reactions (Table 1) based on product (2a) formation as monitored by ³¹P NMR were generally favored when carried out in acetonitrile. Maximal product formation was observed when the amine-exchange step to form intermediate 1a was terminated after 15 min by addition of mCPBA. Longer reaction times resulted in lower yields due to considerable formation of side products as noted by ³¹P NMR. Dichloromethane was identified as a suitable solvent for the phosphoramidite amine-exchange although such reactions suffered lower yields. The extent of amine-exchange to 1a and the subsequent formation of product 2a in dioxane and THF was minimal (<1%).

Table 1. Phosphoramidite Amine-Exchange Conditions									
H ₂ N	BzIO BzIO 1H-te CO ₂ BzI	$ \begin{array}{c} & & & \\ & & & \\ & & \\ \hline N(i-Pr)_2 & & \\ $	mCPBA Bzk						
Entry	Solvent	Amine-Exchange Reaction Time (min)	1 <i>H-</i> Tetrazole (equiv.)	2a Yield (%)*					
1	CH ₃ CN	60	0.2	9					
2	CH ₃ CN	60	1.0	64					
3	CH ₃ CN	45	1.0	74					
4	CH ₃ CN	30	1.0	80					
5	CH ₃ CN	15	1.0	89					
6	CH ₃ CN	5	1.0	82					
7	CH ₂ Cl ₂	60	1.0	36					
8	Dioxane	60	1.0	< 1					
9	THF	60	1.0	< 1					

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*spectroscopic yield based on ³¹P NMR

The mechanism and role of 1H-tetrazole in phosphoramidite amine-exchange reactions remain conjectural although it has been postulated that it functions as a proton donor to promote the cleavage of the amino ligand of the initial phosphoramidite in order to facilitate substitution by an incoming amine.⁶ Limited evidence of this proposed mechanism has been documented, however, during the course of our investigation, we observed a significant formation of a white precipitate shortly after the addition of the phosphoramidite reagent (see Experimental details). Characterization of this precipitate by ¹H NMR and MS was suggestive of a diisopropylammonium tetrazolide ion pair.⁷ It was noted that this precipitate also formed upon the sole addition of 1 equivalent diisopropylamine to 1H-tetrazole in acetonitrile. Interestingly, no such complex precipitated upon the addition of H-Glu(OBzl)-OBzl to a solution of 1H-tetrazole, perhaps due to greater solubility or weaker interaction of the ion pair. It can be concluded, however, that 1H-tetrazole was not recycled during the course of a phosphoramidite amine-exchange as indicated by the low yield resulting from the addition of only 0.2 equivalents 1*H*-tetrazole (Table 1, Entry 1).

To establish that this methodology was applicable to a broader range of phosphoramidites, amine-exchange reactions with the N,N-dimethyl- and N,N-diethylphosphoramidite analogs were examined under the optimized conditions identified for the generation of phosphoramidate **2a** (Table 2, Entries 1-3).

		BzIO P NR ₂ BzIO 2. mCPBA	BzIO ^P Xaa-OR' 2 BzIO	
Entry	R	Xaa-OR'*	Product	Yield(%)†
1	CH(CH ₃) ₂	Glutamic acid dibenzyl ester	2a BzIO P N CO ₂ BzI BzIO H CO ₂ BzI	74
2	CH ₂ CH ₃	Glutamic acid dibenzyl ester	2a	74
3	CH,	Glutamic acid dibenzyl ester	2a	83
4	CH ₃	Leucine benzyl ester	2b BziO P N BziO H	89
5	CH ₃	Phenylalanine methyl ester	2c Bzio H	65‡
6	CH ₃	Proline methyl ester	2d BzIO	83
7	CH ₂ CH ₃	Proline methyl ester	2d	81

Table 2.	N-Phosphoryl	Amino	Acids	via	Phosphoramidite	Amine-Exchange
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*C-protected amino acid †isolated yield⁸ ‡spectroscopic yield of 89 %

Among the three dibenzyl N,N-dialkylphosphoramidite reagents examined with H-Glu(OBzl)-OBzl, the highest yield was observed with dibenzyl N,N-dimethylphosphoramidite (**Table 2, Entry 3**). This reagent was thus selected for amine-exchanges with several representative C-terminal protected amino acids (Xaa-OR') to form the corresponding phosphoramidates **2b-2d** (**Entries 4-6**). Reactions with both leucine benzyl ester (H-Leu-OBzl) and phenylalanine methyl ester (H-Phe-OMe) resulted in high spectroscopic yields of the respective phosphoramidates (**2b** and **2c**) although chromatographic resolution of **2c** from side products was difficult resulting in a lower isolated yield (65%). More surprising were the successful results for amine-exchanges with proline methyl ester (H-Pro-OMe; **Table 2, Entries 6 and 7**). These results may suggest that the kinetics of formation and precipitation of a dialkylammonium tetrazolide ion pair may drive the amine-exchange.

In conclusion, phosphoramidite amine-exchange is a successful approach to synthesize *N*-phosphoryl amino acids. This methodology has proved to be superior to the formation of similar phosphoramidates from the reaction of primary amines and dialkyl phosphites.⁹ Acetonitrile has been confirmed as a superior solvent for this reaction, however,

dichloromethane was identified as an alternative solvent albeit with lower yields. The successful results from the reactions of H-Pro-OMe indicate that this methodology appears to be a promising route for preparing phosphoramidates of secondary amines.

Experimental details

All solvents and *N*,*N*-diisopropylethylamine (DEA) were freshly distilled prior to use. All other reagents were obtained from commercial sources and used without further purification. <u>Typical experimental procedure (2a)</u>: A flask was charged with 1*H*-tetrazole (0.028 g, 0.40 mmol) and H-Glu(OBzl)-OBzl•p-tosylate (0.240 g, 0.48 mmol) which were dissolved in CH₃CN (10 mL) under Ar_(g) at room temperature. DEA (0.084 mL, 0.48 mmol) and dibenzyl *N*,*N*-dimethylphosphoramidite (0.106 mL, 0.40 mmol) were sequentially added via syringe, the later dropwise.¹⁰ The reaction stirred 15 min after which *m*CPBA (0.296 g, ~ 1.2 mmol) was added as a solid and the reaction mixture stirred an additional 45 min.¹¹ The solvent was removed *in vacuo*, the crude reaction mixture was partitioned between CH₂Cl₂ and 10% wt. aqueous Na₂CO₃, and the aqueous layer was extracted three times. The combined organic layers were washed with brine, dried over MgSO₄, and evaporated *in vacuo* to give a dark brown oil. The product was purified by flash silica gel chromatography (hexane:ethyl acetate 6:4, *v:v*; R_f = 0.14) to give the desired phosphoramidate **2a** as a viscous yellow oil (0.235 g, 83% yield).

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- [7] ¹H NMR (300 MHz, D_2O): δ 8.56 (s, 1 H), 3.43-3.56 (sep, 2 H), 1.28 (d, 12 H, J = 6.6); ESI-MS in the cation mode m/z 69 and in the anion mode m/z 102.
- [8] ³¹P NMR (300 MHz, CDCl₃, externally referenced to H₃PO₄): 2a δ 7.93; 2b δ 8.04; 2c δ 7.67; 2d δ 6.94.
- [9] Luo, J; Ganem, B. Tetrahedron Lett. 1991, 32, 3145-3146.
- [10] In order to obtain the high reported yield with H-Leu-OBzl, its tosylate salt was neutralized prior to use by extraction from 10% wt. aqueous Na₂CO₃ with CH₂Cl₂. Subsequent addition of DEA was thus unnecessary and therefore omitted.
- [11] Magnesium monoperoxyphthalate (MMPP) and sodium meta-periodate were also examined as alternative oxidizing agents. However, the spectroscopic yields based on ³¹P NMR with those reagents were 34 and 21 %, respectively.