Pergamon

Tetrahedron Letters 39 (1998) 7471–7474

TETRAHEDRON LETTERS

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

Chun P. Chow and Clifford E. Berkman*

Department of Chemistry & Biochemistry, San Francisco State University, 1600 Holloway Ave., San Francisco, CA 94132, USA

Received 16 July 1998; revised 29 July 1998; accepted 30 July 1998

Abstract: *N*-Phosphoryl amino acids were prepared rapidly and in high yield via phosphoramidite amine-exchange in the presence of 1*H*-tetrazole followed by oxidation with *m*CPBA. 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.

Keywords: phosphoramidite amine-exchange; phosphoramidate; *N*-phosphoryl amino acids; 1*H*-tetrazole

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.

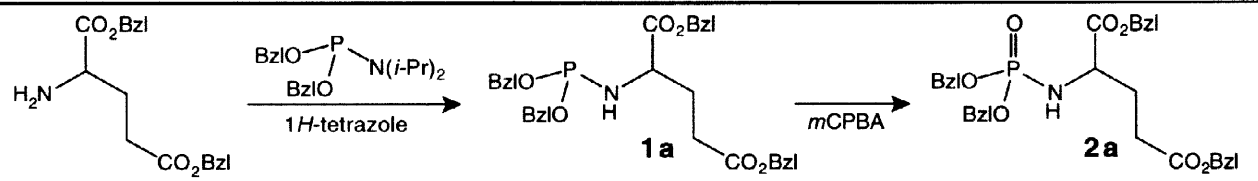
With a desire to prepare phosphoramidates as putative transition-state analog inhibitors of a glutamyl metalloprotease, we were interested in exploiting the nucleoside solid-phase phosphoramidite amine-exchange chemistry for use in analogous solution-phase chemistry involving amino acids.[4] As a model 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 I_2 in H_2O to chemically promote the oxidation of the second step. Thus,

0040-4039/98/$ - see front matter
PII: S0040-4039(98)01673-6

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 ^{31}P 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 *m*CPBA. Longer reaction times resulted in lower yields due to considerable formation of side products as noted by ^{31}P 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



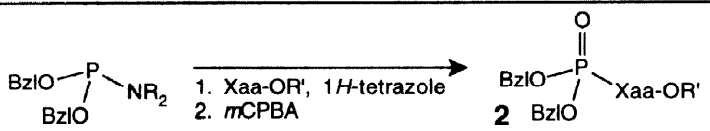
Entry	Solvent	Amine-Exchange Reaction Time (min)	1*H*-Tetrazole (equiv.)	2a Yield (%)*
1	CH_3CN	60	0.2	9
2	CH_3CN	60	1.0	64
3	CH_3CN	45	1.0	74
4	CH_3CN	30	1.0	80
5	CH_3CN	15	1.0	89
6	CH_3CN	5	1.0	82
7	CH_2Cl_2	60	1.0	36
8	Dioxane	60	1.0	< 1
9	THF	60	1.0	< 1

*spectroscopic yield based on ^{31}P NMR

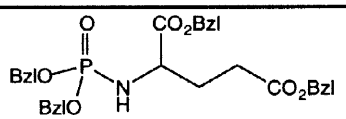
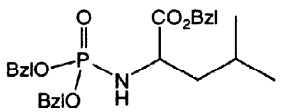
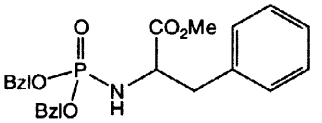
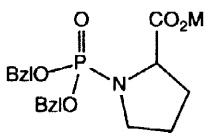
The mechanism and role of 1*H*-tetrazole in phosphoramidite amine-exchange reactions remain conjectural although it has been postulated that it functions as a proton donor to promote the cleavage of the amino ligand of the initial phosphoramidite in order to facilitate substitution by an incoming amine.[6] 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 1*H*-tetrazole in acetonitrile. Interestingly, no such complex precipitated upon the addition of H-Glu(OBzl)-OBzl to a solution of 1*H*-tetrazole, perhaps due to greater solubility or weaker interaction of the ion pair. It can be concluded, however, that 1*H*-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**).

Table 2. *N*-Phosphoryl Amino Acids via Phosphoramidite Amine-Exchange



2

Entry	R	Xaa-OR'*	Product	Yield(%)†
1	$CH(CH_3)_2$	Glutamic acid dibenzyl ester	**2a** 	74
2	CH_2CH_3	Glutamic acid dibenzyl ester	**2a**	74
3	CH_3	Glutamic acid dibenzyl ester	**2a**	83
4	CH_3	Leucine benzyl ester	**2b** 	89
5	CH_3	Phenylalanine methyl ester	**2c** 	65‡
6	CH_3	Proline methyl ester	**2d** 	83
7	CH_2CH_3	Proline methyl ester	**2d**	81

*C-protected amino acid †isolated yield[8] ‡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 phoshphoramidates (**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. *Typical experimental procedure* (**2a**): 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 CH_3CN (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 CH_2Cl_2 and 10% wt. aqueous Na_2CO_3, and the aqueous layer was extracted three times. The combined organic layers were washed with brine, dried over $MgSO_4$, 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).

Acknowledgments

This investigation was supported by a 'Research Infrastructure in Minority Institutions' award from the National Center for Research Resources with funding from the Office of Research on Minority Health, National Institutes of Health (Grant #RR11805-02). The authors would like to extend their gratitude for the Graduate Equity Fellowship to C. Chow sponsored by San Francisco State University. Endless assistance from Roberta Neuman is also gratefully acknowledged and to whom, in fond memory, this publication is dedicated.

References and notes

[1] Gryaznov, S.; Skorski, T.; Cucco, C.; Nieborowska-Skorska, M.; Chiu, C. Y.; Lloyd, D.; Chen, J. K.; Koziolkiewicz, M.; Calabretta, B. *Nucleic Acids Res.* **1996**, *24*, 1508-1514.

[2] Zhang, Z.; Tang, J. Y. *Tetrahedron Lett.* **1996**, *37*, 331-334.

[3] McCurdy, S. N.; Nelson, J. S.; Hirschbein, B. L.; Fearon, K. L. *Tetrahedron Lett.* **1997**, *38*, 207-210.

[4] Rodriguez, C. E.; Holmes, H. M.; Mlodnosky, K. L.; Lam, V. Q.; Berkman, C. E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1521-1524.

[5] Bannwarth, W.; Trzeciak, A. *Helv. Chim. Acta* **1987**, *70*, 175-186.

[6] Hayakawa, Y.; Kataoka, M. *J. Am. Chem. Soc.* **1997**, *119*, 11758-11762.

[7] ^{1}H 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] ^{31}P NMR (300 MHz, $CDCl_3$, externally referenced to H_3PO_4): **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 Na_2CO_3 with CH_2Cl_2. 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 ^{31}P NMR with those reagents were 34 and 21 %, respectively.