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A Facile Synthesis of N-Phosphoryl Amino Acids Containing Hydroxy Group

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Abstract: three kinds of N-(diisopropyloxyphosphoryl) amino acids containing hydroxyl group were prepared in high yield by using diisopropyl phosphite as the phosphorylating agent, sodium hypochlorite as the chlorinating agent and tetrabutyl ammonium bromide as the phase transfer catalyst in basic aqueous media.

Keywords: Amino acids, N-phosphoryl amino acid, phosphorylation, synthesis.

Derivatives of N-phosphorylamino acids are of pharmaceutical and biological interest[1,2]. Previously, we had successfully prepared N-(diisopropyloxy-phosphoryl)-amino acids (DIPP-AA) from diisopropyl phosphite (DIPPH) and amino acids in a mixture of water, ethanol, carbon tetrachloride and triethyl amine in good yield[3]. Considering that carbon tetrachloride is a known carcinogen, Brands et al. provided a method that substituted it for sodium hypochlorite[4]. But the yield was low (35~61%). Recently, this method was partly improved through adding tetrabutyl ammonium bromide (TBAB) as phase transfer catalyst and the yield was increased (58~68%)[5]. However, synthesis of DIPP-AA containing hydroxy group was not concerned.

Here we synthesized N-(diisopropyloxyphosphoryl)-serine(DIPP-Ser) **3a**, N-(diisopropyloxyphosphoryl)-threonine(DIPP-Thr) **3b** and N-(diisopropyloxy phosphoryl)tyrosine(DIPP-Tyr) **3c** *via* reaction of serine **2a**, threonine **2b** or L-threonine **2c** with DIPPH **1** in the presence of sodium hypochlorite (**Scheme 1**). The reaction mechanism was interpreted according to the reaction phenomena. The reaction conditions were optimized and the yields were remarkably increased.

The reactions were carried out by dropping 1.3 equivalents of **1** to basic aqueous mixture of 1.0 equivalents of **2** at 0℃ over 2 h. After 4 hours' stirring at 0℃, acidification and extraction **3a-c** were isolated in 89~95 yields[6]. Besides the influence of temperature (0℃) and pH(9~9.5), it was found that the reactivity of the intermediate diisopropyl phosphorochloridate **4** (**Scheme 2**) was one of the key factors. Adding a little TBAB as phase transfer catalyst and using excess of **1** could be enhanced yields as shown in **Table 1**.

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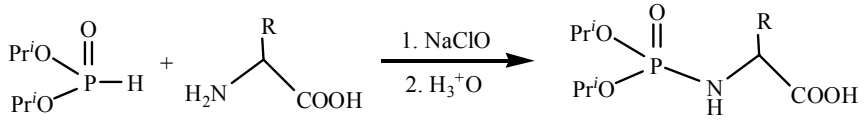
All of them were characterized by ^{1}HNMR, ^{13}CNMR, ^{31}PNMR, and ESI-MS spectral analysis as well as compared with authentic samples.

We traced the reaction procedure of **1** and NaClO by ^{31}PNMR. It was found that the peak of **1** (4.60 ppm) was gradually decreased; meanwhile the peak of **4** (1.96 ppm), which was validated with the authentic sample, was appeared and gradually increased. The experimental results revealed that produced **4** was an intermediate of the reaction procedure. In addition to the fact that hydrolysis of **4** would compete with the main reaction. If one equivalent of **1** was added, the amino acid could not be completely transformed as checked by the coloration analysis of ninhydrin. It is worth noting that the pH of solution did not obviously change during the reaction procedure. Thus, it was possible that the base released during the formation of **4** was neutralized by the hydrogen chloride generated in the main reaction step. Hence possible reaction mechanism of produced intermediate **4** was proposed as shown in **Scheme 2**.

Scheme 1



1

2a R= -CH_2OH, Ser
2b R= -$CH(CH_3)OH$, Thr
2c R= -CH_2PhOH, Tyr

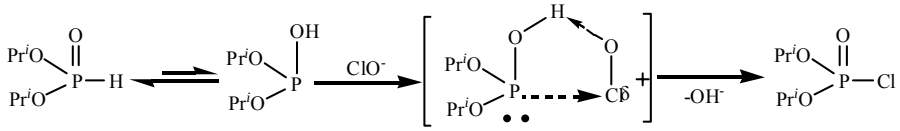
3a R= -CH_2OH, N-DIPP-Ser
3b R= -$CH(CH_3)OH$, N-DIPP-Thr
3c R= -CH_2PhOH, N-DIPP-Tyr

Table 1 Phosphorylation reaction conditions

Entry	Material	Product	TBAB (equiv.)	DIPPH (equiv.)	Yield*/%
1	L-Ser (**2a**)	DIPP-Ser (**3a**)	-	1.0	43
2	L-Ser (**2a**)	DIPP-Ser (**3a**)	0.007	1.0	65
3	L-Ser (**2a**)	DIPP-Ser (**3a**)	0.007	1.3	89
4	L-Thr (**2b**)	DIPP-Thr (**3b**)	-	1.0	45
5	L-Thr (**2b**)	DIPP-Thr (**3b**)	0.007	1.0	70
6	L-Thr (**2b**)	DIPP-Thr (**3b**)	0.007	1.3	92
7	L-Tyr (**2c**)	DIPP-Tyr (**3c**)	-	1.0	63
8	L-Tyr (**2c**)	DIPP-Tyr (**3c**)	0.007	1.0	78
9	L-Tyr (**2c**)	DIPP-Tyr (**3c**)	0.007	1.3	95

Yield of isolated pure product

Scheme 2



1 **1a** **4**

In conclusion, according to the reaction mechanism, adding a little TBAB as phase transfer catalyst and using excess of **1** could be enhanced the reactivity of intermediate **4**, which could remarkably increase the yields. This method is a simple and practical pathway for the synthesis of N-phosphoryl amino acids containing hydroxy group. It could be applied for the phosphorylation of any other amino acids. The work is in progress.

Acknowledgment

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Reference and Notes

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6. Typical reaction procedure: 0.1 mol amino acid **2a-c** was dissolved in 40 mL of water at ambient temperature. The solution was cooled to 0°C and the pH was adjusted to 9.0~9.5 by adding sodium hydroxide solution (about 4 mL). 21.6 g (0.13 mol) **1** and 0.24 g TBAB were added in one portion. 50 mL (12%) sodium hypochlorite was added over 2.0 h to the resulting mixture. The stirring was continued for an additional 4.0 h. The reaction mixture was extracted with ether twice. The water layer was acidified to pH 3~4 with concentrate hydrochloric acid (2.5 mL) at 0~5°C. Sodium chloride (8 g) was added and the resulting solution was extracted with *t*-butyl alcohol: ethyl acetate (1:1.5 v/v, 50 mL×3). The organic extracts were combined and dried over anhydrate $MgSO_4$ overnight and then concentrated to dryness in vacuum.

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