

## <sup>31</sup>P NMR and ESI-MS Studies on Some Intermediates of the Peptide Coupling Reagents Triphenyl-chlorophosphoranes

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**Abstract:** The intermediates of the Appel coupling reagents were studied in acetonitrile, dimethoxyethane and dioxane by <sup>31</sup>P NMR, <sup>13</sup>C NMR spectrum and ESI-MS. In dioxane a new high coordinated phosphorous compound with <sup>31</sup>P NMR shift at -39 ppm was observed. The ESI-MS showed that it could be a penta-coordinated phosphorous compound containing dioxane. The carboxyl activated intermediates were also studied in three solvents.

**Keywords:** Triphenylphosphine, hexachloroethane, <sup>31</sup>P NMR, ESI-MS, penta-coordinated phosphorous compound.

The Appel coupling reagents hexachloroethane and triphenylphosphine for peptide synthesis had been studied<sup>1-2</sup> (Eq. 1). However, the reaction mechanism was not well investigated. In this paper, in order to understand the detail mechanism, the Eq. 1 was studied stepwise.

At first, the hexachloroethane was mixed with triphenylphosphine in different solvents under an atmosphere of dry nitrogen (Eq. 2), then checked by <sup>31</sup>P NMR spectroscopy separately. It was found that in different solvents the reaction intermediate (B) gave different chemical shifts (**Table 1**). For example, in acetonitrile the peak of B1 was observed at <sup>31</sup>P NMR 58.5 ppm, in dimethoxyethane (DME) B2 a peak at 1.5 ppm, in dioxane B3 a strong peak at -39 ppm (B3-1) and another weak peak at 61.0 ppm (B3-2). It is worth noting that in dioxane, the major peak was 97 ppm upfield shift than that in acetonitrile. So it was expected that in dioxane the major peak might be correspondent to a high coordinate phosphorous compound, such as penta-coordinated phosphorous compound<sup>3-6</sup>. Since in dioxane B3-1 was very unusual, it was studied further.

Formation of the activation intermediate B3-1 in dioxane was traced by <sup>31</sup>P NMR spectroscopy shown in **Figure 1**. When hexachloroethane was mixed with triphenyl-

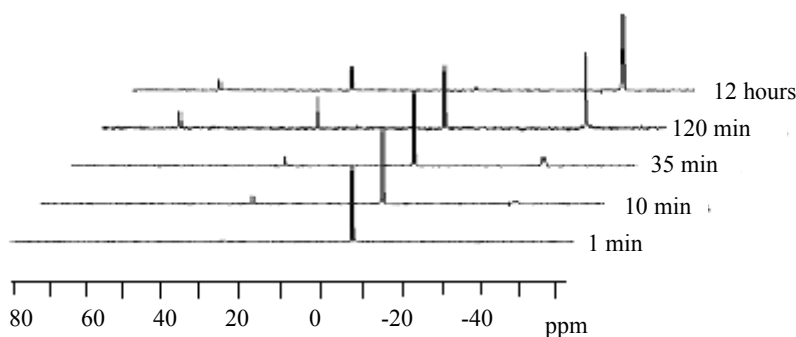
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phosphine in dioxane at room temperature, the peak of triphenylphosphine at  $^{31}\text{P}$  NMR  $-4.8$  ppm was decreasing, while a pair of new peaks at  $-39$ ,  $61.0$  ppm corresponding to intermediates B3-1 and B3-2 emerged, respectively. As the reaction continued, B3-1 reached its maximum within 12 h.

There was no attempt to isolate the reaction intermediates. To prove the composition of the B1, B2, B3, their MS were analyzed by the positive ion electrospray ionization mass spectrometry (ESI-MS) (**Table 1**). The  $m/z$  355 is consistent with the sodium adduct of B2 (Eq. 4), in which the dichloro atoms show the isotope effect. When  $\text{TiCl}_4$  was added into B2, a strong peak at  $64$  ppm was observed by  $^{31}\text{P}$  NMR. All these showed that a penta-coordinated phosphorous compound containing dichloro atoms was found in DME (B2). The peak  $m/z$  385 indicates that there is a dioxane incorporated into B3-1 (Eq. 4). The formation of B3-1 was proposed as Eq. 4, that one molecular of dioxane substituted the chlorine in B2. B1 had been studied by Appel<sup>7</sup> and confirmed by Beveridge<sup>8-10</sup>. Their difference of the B1, B2, B3 was also testified by  $^{13}\text{C}$  NMR. The  $^{13}\text{C}$  NMR of  $\alpha$  C in B1 was  $15$  ppm upfield shifted than that in  $\text{Ph}_3\text{P}$ , but the  $^{13}\text{C}$  NMR of  $\alpha$  C in B3-1 was  $2.3$  ppm downfield shifted than that in  $\text{Ph}_3\text{P}$ .

**Figure 1** The stack  $^{31}\text{P}$  NMR spectrum of formation of intermediate B3



**Table 1**  $^{31}\text{P}$  NMR ( $\delta$ : ppm) ESI-MS and  $^{13}\text{C}$  NMR data of intermediate (B) in different solvents

	$\text{Ph}_3\text{P}$	Acetonitrile (B1)	DME (B2)	Dioxane (B3-1)
$^{31}\text{P}$ NMR	$-4.8$	$58.5$	$1.5$	$-39$
ESI-MS	$263$	—	$355$ ( $357$ )	$385$ ( $387$ )
$^{13}\text{C}$ NMR $\text{C}_1$	$137.3$	$122.0$	$133.5$	$139.5$
$^1J_{\text{C-P}}$ (Hz)	$12.0$	$99.6$	$125.0$	$143.6$

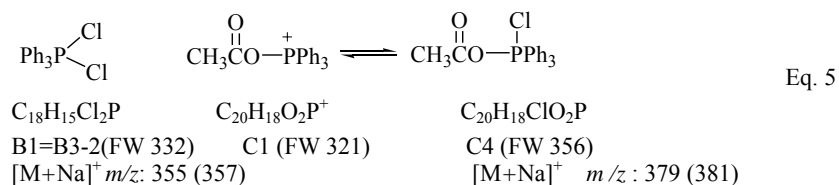
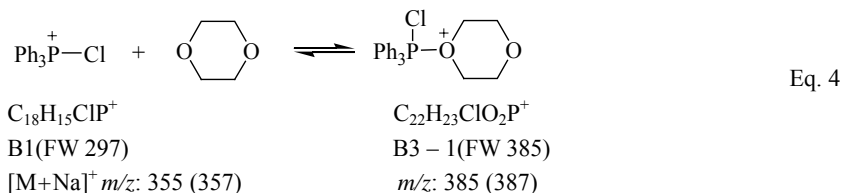
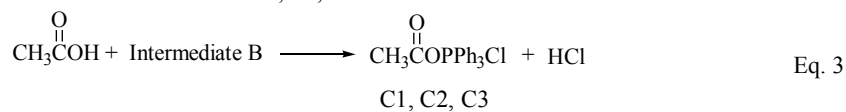
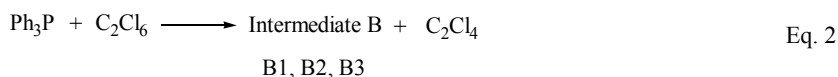
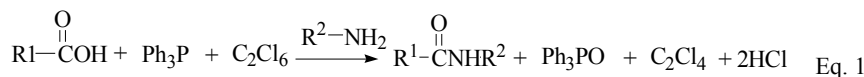
DME: dimethoxyethane

The next step is the activation of the carboxyl group, as shown in Eq. 3. When acetic acid was added to the solution of B1, B2 and B3, the peak of intermediate B disappeared right away and a new peak of intermediate C appeared at <sup>31</sup>P NMR spectroscopy (**Table 2**). It seems that the activated carboxyl intermediate C2 in DME is similar to C3 in dioxane as checked by <sup>31</sup>P NMR, <sup>13</sup>C NMR and ESI-MS. The ionic intermediate C1 had been studied by Appel<sup>1</sup>. The *m/z* 379 is the sodium adduct of a neutral intermediate C4 (Eq. 5). The <sup>31</sup>P NMR and <sup>13</sup>C NMR of C2 C3 was similar to the tetra-coordinated phosphorous compound. These showed that there is a fast equation between penta-coordinated and tetra-coordinated phosphorous compound (Eq. 5). The chemical compositions of C1, C2, C3 are showed in **Scheme 1**. The activated intermediates C1, C2, C3 could be coupled to the ester of amino acids to form peptide with good yield <sup>1-2</sup>.

**Table 2** <sup>31</sup>P NMR ( $\delta$ : ppm) and ESI-MS data of intermediate(C) in different solvents

	Acetonitrile (C1)	DME (C2)	Dioxane (C3)
<sup>31</sup> P NMR	40	31	31
ESI-MS	—	379(381)	379(381)
<sup>13</sup> C NMR C <sub>1</sub>	128.4	—	131.8
<sup>1</sup> J <sub>C-P</sub> (Hz)	105.0	—	103.3

**Scheme 1** Structures of B1, B2, B3, C1, C2, C3



In conclusion, in this paper the intermediates of the Appel coupling reagents were studied by  $^{31}\text{P}$  NMR,  $^{13}\text{C}$  NMR spectrum and ESI-MS in acetonitrile, DME and dioxane. A penta-coordinated phosphorous compound containing dichloro atoms was found in DME (B2). In dioxane (B3) a new high coordinated phosphorous compound with  $^{31}\text{P}$  NMR at 39 ppm was observed. The ESI-MS and  $^{13}\text{C}$  NMR showed that it could be a dioxane incorporated into the penta-coordinated phosphorous compound. The carboxyl activated intermediates were also studied in the three solvents.

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