五配位氧磷烷分子间配体交换反应-RNA 水解和融合过程的化学模型

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摘要 具有五元环和三配体结构的五配位氧磷烷(**ab₂**)在碱催化条件下自发进行分子间的配体交换反应, 产生不同配体组合的全部三种五配位氧磷烷(**a₃**, **b₃** 和 **a₂b**). 如果把其中 **a₃** 与 **b₃** 作为父代分子, 其配体交换产生的五配位氧磷烷 **a₂b** 和 **ab₂** 可以视作子代分子, 从而自发实现了分子结构的多样化. 因此, 五配位氧磷烷分子间配体交换反应可以作为研究生命过程中具有五配位磷中间体结构化学性质的模型, 对理解基因转录和生命信息储存等过程中涉及的 RNA 分子剪接、水解和融合等重要生命过程的分子机制提供了重要依据.

关键词 磷化学; 五配位氧磷烷; 配体交换; 有机化学模型

Intermolecular Ligand Exchange of Penta-oxy Phosphoranes: Potential Chemical Model for RNA Hydrolysis and Fusion

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Abstract Penta-coordinated phosphoranes (**ab₂**) with a five-member cycle and three ligands would simultaneously exchange with themselves under base catalysis to form three different penta-oxy phosphoranes with all the combinatorial ligands referred as **a₃**, **b₃**, and **a₂b**. If we consider **a₃** and **b₃** as parents, the products obtained from exchange, namely **a₂b** and **ab₂**, could be regarded as the offspring of the first generation, leading to the diversified chemical structures. Thus, these fascinating reactions could be considered as a promising chemical model for studying the unique chemistry of possible penta-coordinated phosphorus intermediates in the process of RNA self-splicing, hydrolysis and fusion for gene transcription and biological information storage.

Keywords phosphorus chemistry; penta-oxy phosphorane; ligand exchange; organic chemical model

1 Introduction

Serving as one of the fundamental elements of genetic materials in organisms, phosphorus plays an irreplaceable role in the physiological activity of organisms. At molecular level, due to their unique chemistry, phosphorus-containing compounds participate in many physiological processes such as ATP-assisted enzyme reactions, posttranslational modifications of proteins and transmembrane transport. Penta-coordinated phosphorus as key important intermediate involved in many biological process, such as phosphoryl group transfer,[1] ATP hydrolysis,[2] RNA hydroly-

sis via internal transesterification,[3] RNA self-splicing and twister ribozyme catalysis.[4] Recently, the discovery of RNA fusion pairs could be formed with direct RNA-RNA interactions to regulate the specific gene transcriptions.[5] Furthermore, it was found that RNA fusion was very closely to the development of human desease such as cancer. However, the molecular mechanisms of RNA hydrolysis and fusion with ligands exchange are unknown and should be understand firstly by using small chemical models, for example the penta-coordinated phosphorus compounds. Indeed, most of the phosphorus-containing bioactivity compounds which exist in a tetra-coordinated P(4) state would be activated firstly to produce penta- or hexa-coordinated phosphorus with or without enzyme catalysis in order to produce new structures with diverse functions. However, it is very difficult to synthesize and identify these very unstable intermediates under biological aqueous conditions. Decades of experimental and computational work has been made in order to determine the intrinsic chemistry of penta-coordinated phosphorus for the biological catalysis.[6] For example, metal fluorides (MF$_x$), such as MgF$_3^-$ and AlF$_3$, have been widely used as ligands with trigonal bipyramidal complexes to mimic the grometry of the phosphorus transition state for phosphoryl transfer process.[7] Several computational models proposed with five-coordinate intermediates showed associative-type phosphoryl transfers mechanisms, such as the tyrosyl-DNA phosphodiesterase I (Tdp1),[8] and phospholipase D superfamily with the five-coordinate phosphohistidine intermediate which could catalyze the cleavage of the headgroup of phosphatidylcholine to produce phosphatidic acid and choline.[9] In order to systematically understand the intrinsic chemistry of penta-coordinated phosphorus, in this work we are trying to synthesize these model compounds with controllable stability and then investigate them by using ^{31}P NMR.

Oxyphosphoranes, especially penta-coordinated P(5) compounds, have been successfully prepared and identified by several groups.[10,11] The relatively stable trigonal bipyramidal structure endows oxyphosphoranes with the possibility of being the intermediates in the displacement reaction of biochemically important pyrophosphates (tetra-coordinated phosphorus compounds) such as AMP, ADP, ATP and phospholipid. In 1975, Ramirez's group[12] set up an oxyphosphorane model to simulate the intermediate in the hydrolysis of ATP. From that time on, several groups have reported similar P(5)-centered chemical models for the study of biochemical reactions.[13] In the past decades, Ramirez's group and other scientists have successfully prepared various penta-coordinated oxyphosphoranes stabilized by diketone ligands. Moreover, the alcoholysis of oxyphosphoranes has also been discovered.[14] Herein, we identify an interesting intermolecular ligand-exchange reaction of one pentaoxyphosphorane for
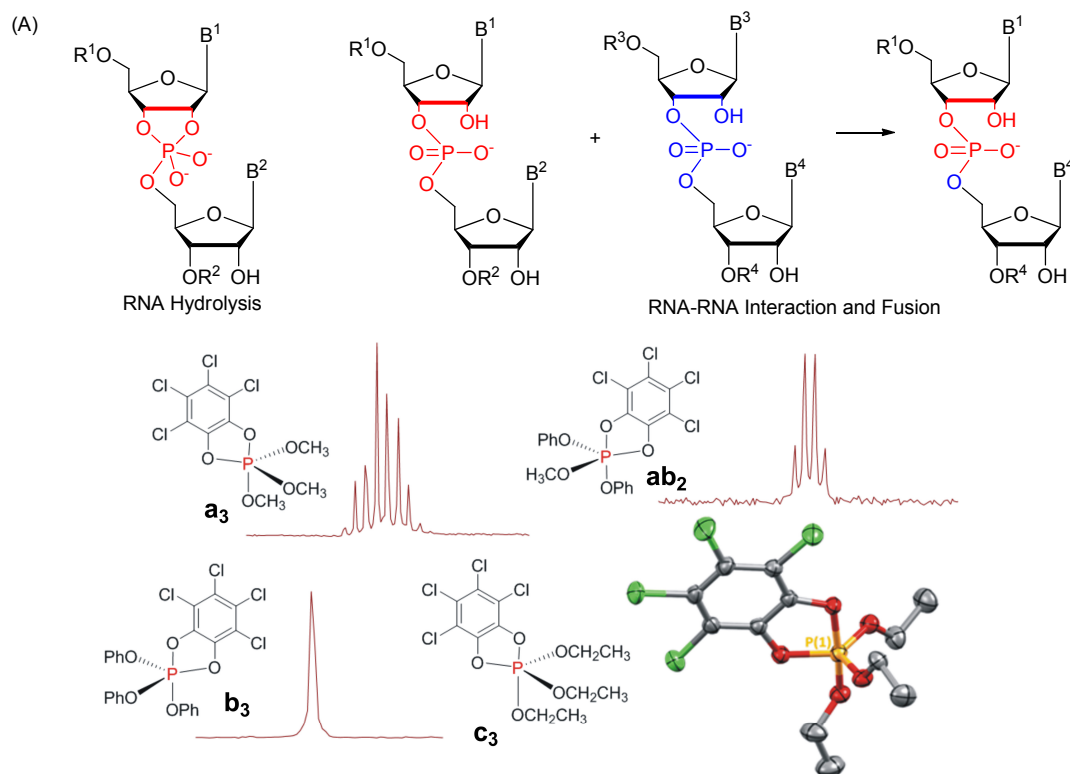
Scheme 1 (A) Biological models of penta-coordinate phosphorus intermediate, and (B) chemical structure of penta-coordinated phosphorus with ^{1}H-coulpled ^{31}P NMR spectra

Molecular structure of compound **c$_3$** with the aniso-tropic displacement parameters depicted at a 50% probability level. The hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°): P(1)—O(1) 1.767(1), P(1)—O(2) 1.573(1), P(1)—O(3) 1.656(1), P(1)—O(4) 1.609(1) P(1)—O(5) 1.573(1); O(1)—P(1)—O(3) 87.71(6), O(3)—P(1)—O(5) 123.85(6). The insert are ^{1}H-^{31}P NMR spectra.
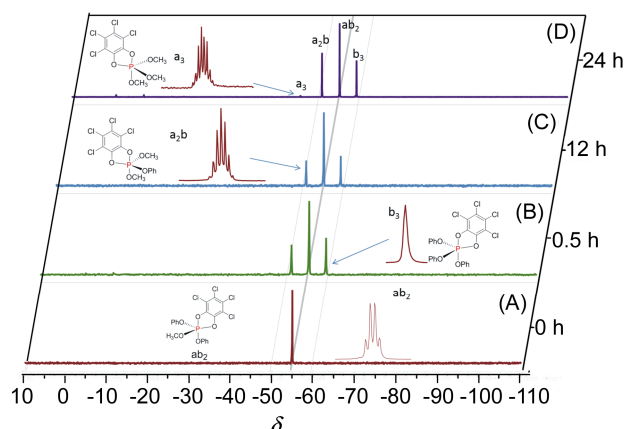
Figure 1 Time-dependent, stacked ^{31}P NMR spectra for the reaction of ligand exchange of **ab$_2$** (250 mmol/L) with 10% pyridine in D_6-benzene solution at 25 ℃ for 24 h

producing all three penta-coordinated phosphoranes with the combinatorial ligands under mild conditions with base catalysis, which might set up a chemical model for the possible intermediates of RNA hydrolysis and fusion (Scheme 1A).

2 Results and discussion

Four penta-oxy phosphoranes (**ab$_2$**, **a$_3$**, **b$_3$**, and **c$_3$**) have been prepared with five-member cyclic structures as colorless crystal in high yields after recrystallization in toluene (Scheme 1B). The chemical structures were determined by using NMR for **ab$_2$**, **a$_3$**, **b$_3$** and single crystal XRD analysis for **c$_3$**. For example, **ab$_2$** with one methoxyl and two phenoxyl ligands was determined at ^{31}P NMR δ −55 with four peaks (*t*), indicating that three hydrogen atoms are adjacent to phosphorus center. Compound **ab$_2$** was stable at room temperature in anhydrous toluene solution without new peak formed (Figure 1A). In contrast, when 10% equivalent pyridine was added into the ab$_2$ solution, two new peaks at δ −51 and −60 were quickly formed with different ratios (Figure 1B). After 24 h incubation at rt, the reaction reached its equilibrium and the third novel peak was produced at δ −47 ppm with about 0.1% (**a$_3$**) as shown in Figure 1D. In order to confirm their structures, ^{31}P NMR tests with ^{1}H-coupling were performed and three new penta-oxy phosphoranes, namely **a$_3$**, **a$_2$b** and **b$_3$** (*s*), could be simultaneously generated from the **ab$_2$** with different ratio without side reaction under mild conditions. For example, **ab$_2$** shows a quartet with $J_{H-P}=$ 14.5 Hz in ^{1}H-coupled ^{31}P NMR, indicating that there are only one methyl group linked to the phosphorus center of **ab$_2$**. Meanwhile, **a$_2$b** displays a heptet with $J_{H-P}=14.4$ Hz in ^{1}H-coupled ^{31}P NMR, indicating that there are two methyl groups linked to the phosphorus center of **a$_2$b**. The cleavage and formation of P—O ester bonds are atom economic with high efficiency. The diversities of chemical structures could be obtained for deserving more functions and information.

In order to identify the ligand-exchange mechanism, **a$_3$**

with three methoxyl groups (^{31}P NMR δ −45) was treated with **b$_3$** with three phenol groups (^{31}P NMR δ −60) in a ratio of 1∶1 in C$_6$D$_6$ solution and monitored *in situ* by using ^{31}P NMR. However, no new peaks could be formed even after 48 h incubation (Figure 2A). Addition of extra Lewis base will promote the transformation of penta-oxy phosphorus compounds.[13] Thus, 10 mol% equivalent fresh distilled pyridine was added into the reaction mixture. The formation of **a$_2$b** and **ab$_2$** could be obtained immediately in the ^{31}P NMR spectrum (Figure 2B). After 12 h, two singlets at δ −51 and −55 were obtained, which could be identified as **a$_2$b** and **ab$_2$**, respectively. The NMR spectra of **a$_2$b** and **ab$_2$** were also confirmed by comparing with authentic samples. The reaction proceeded smoothly with the addition of pyridine and more than 80% of **a$_3$** and **b$_3$** were transformed into **a$_2$b** and **ab$_2$** after 24 h incubation. Other Lewis base such as 2,6-lutidine showed similar behavior under the same reaction conditions. Similarly, **c$_3$** with three ethoxyl groups could also smoothly exchange ligands with **b$_3$** to form **c$_2$b** and **cb$_2$** under base catalysis. The above results suggested that intermolecular ligand exchanges were the intrinsic reaction of penta-oxy phosphoranes with the economic cleavage and formation of the P—O ester bonds under base catalysis (Scheme 2).
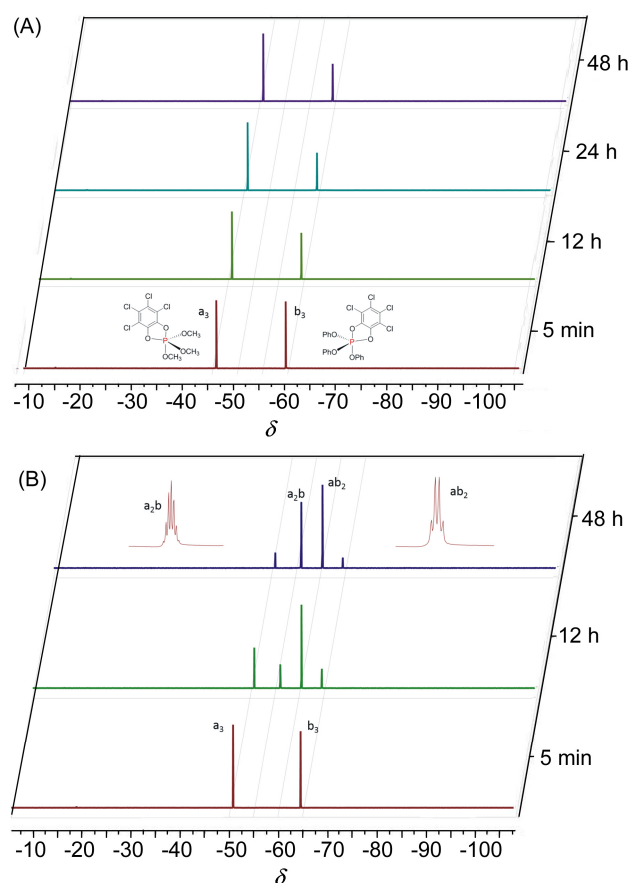
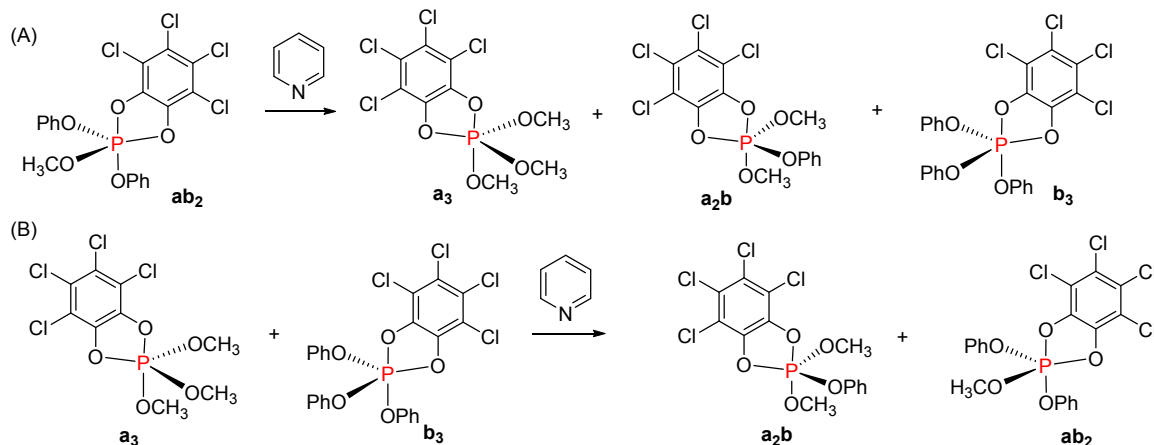**Figure 2** Time-dependent, stacked ^{31}P NMR spectra for the reaction of ligand exchange between **a$_3$** (250 mmol/L) and **b$_3$** (250 mmol/L) without base (A) or with 10% pyridine (B) for 48 h

To determine the ligand exchange whether or not a

unique phenomenon for penta-coordinated phosphorus, a_3 was treated with triphenyl phosphate **1b** in a C_6D_6 solution for 5 d at 25 ℃, no exchange product was detected even with the catalysis of 2,6-lutidine. Based on the experimental results, we assumed that the d_{sp3} orbitals of penta-coordinated phosphorus were key to ligand exchange. Thus, the regular tetra-coordinated phosphate with tetrahedron sp^3 orbitals could not exchange ligands with themselves or other d_{sp3} orbitals of phosphoranes.

To understand the mechanism, the reaction of a_3 (1 equiv.) with 2,6-lutidine (1 equiv.) in anhydrous C_6D_6 so-lution was investigated. The reaction mixture was moni-tored by ^{31}P NMR (Figure 3A). Two new peaks at δ 3 (**2a**) and -93 (**1a**) were obtained immediately with the gradi-ent disappearing of a_3. **1a** displays a heptet with $J_{H-P} = 15.2$ Hz in ^1H-coupled ^{31}P NMR, indicating that there are two methyl groups linked to the phosphorus center. The singlet -93 could be postulated as an intermediate with a pen-ta-coordinated phosphorous center as shown in Scheme 3. To determine the possible structures, 2D ^1H-^{31}P HMBC NMR experiment was performed (Figure 3B). The signal at

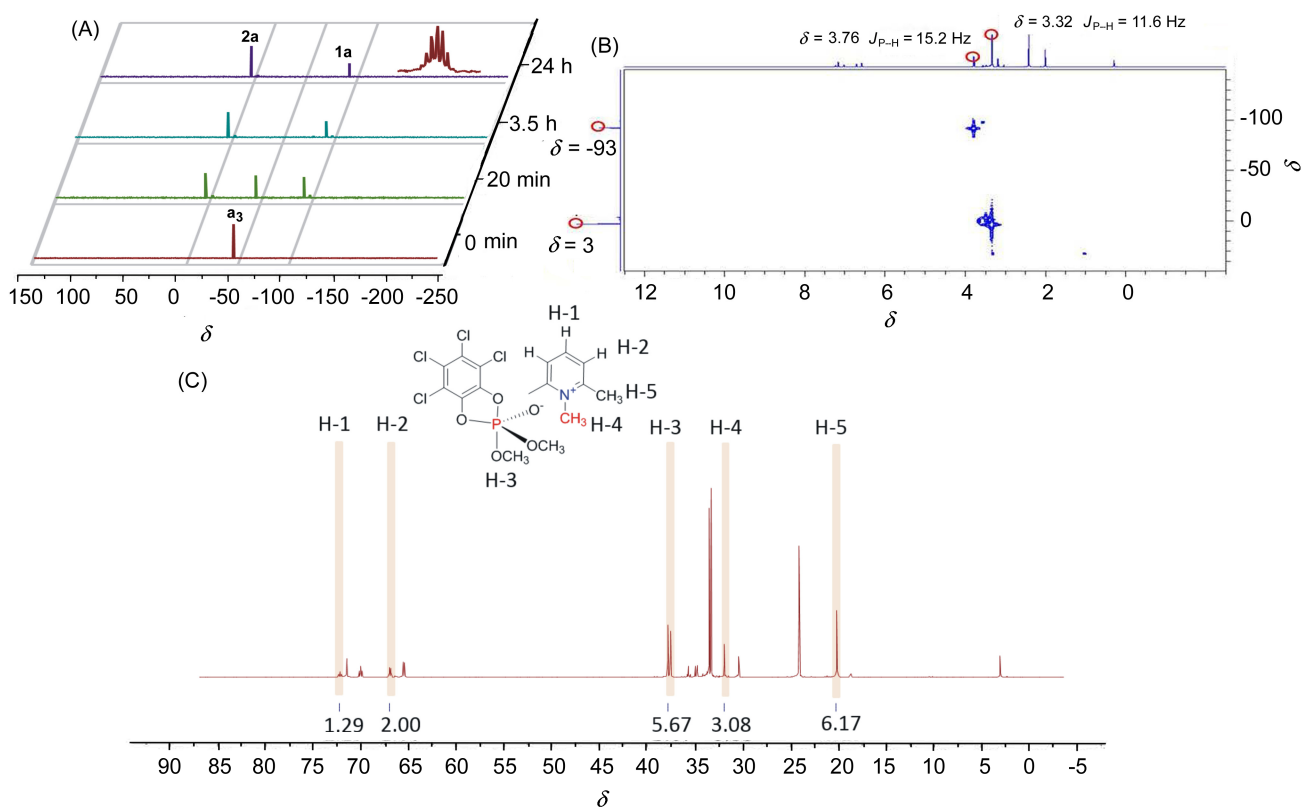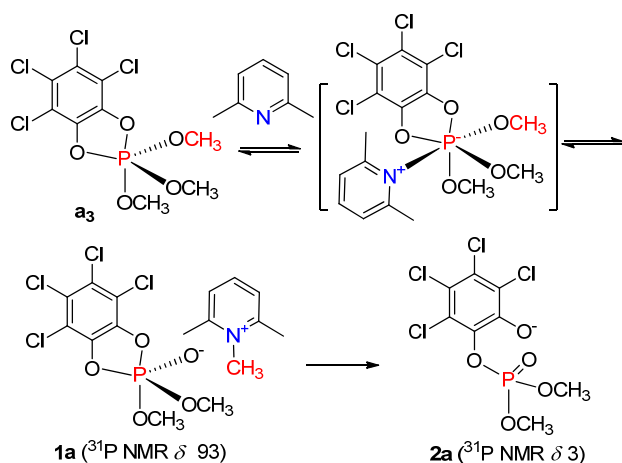**Scheme 2**　Ligand exchange of penta-coordinated phosphoranes with base catalysis

Figure 3　(A) Time-dependent, stacked ^{31}P NMR spectra for the reaction of a_3 with 1 equiv. of 2,6-lutidine in D_6-benzen solution at 25 ℃ for 24 h, (B) ^1H-^{31}P HMBC NMR spectrum of the mixture of a_3 with 1 equiv. of 2,6-lutidine after 24 h, and (C) ^1H NMR spectrum of reaction mixture of a_3 with 2,6-lutidine (1 equiv.) for 24 h
The marked peaks with integration area are assigned to **1a** with ^{31}P NMR signal at δ -93

Scheme 3 Possible mechanism of ligand exchange of penta-coordinated phosphoranes

δ -93 was correlated to the protons at δ 3.76 with $J_{P-H}=$ 15.2 Hz, which usually belongs to the penta-coordinated phosphorane. While the signal at δ 3 of the ^{31}P NMR was correlated to the protons at δ 3.32 with $J_{P-H}=11.6$ Hz, which usually belongs to the tetrahedron phosphates. Furthermore, ^{1}H NMR showed that two sets of spectra with specific integrated areas were well corresponded to **1a** with penta-coordinated phosphorus (Figure 3C) as compared with the ^{1}H NMR spectrum of the authentic 2,6-lutidine (Figure S3, Supporting Information). In conclusion, the new signals at δ -93 could be a penta-coordinated phosphorane **1a**, while the peak at δ 3 is a tetra-coordinated phosphate.

Inspired by the experimental results, a possible mechanism was proposed as shown in Scheme 3. The starting material **a₃** firstly reacted with pyridine or 2,6-lutidine to produce a transition states or intermediate with hexa-coordinated phosphorus center.[15] Then a slow demethylation of the unstable hexa-coordinated phosphorus afforded the ionic intermediate **1a** as methyl group donor, which was in turn quickly nucleophilically attacked by another penta-coordinated ionic intermediate, leading to the intra-molecular ligand exchange. Meanwhile, phosphate **2a** could be formed with the ring opening reaction of five-membered cycle when no other penta-coordinated phosphorus compounds were afforded.

3   Conclusions

In this work, we provide a new chemical model of penta-coordinated phosphoranes with inter-molecular ligand exchange. In general, the experimental results demonstrated that the first generation compounds with ligands **a₃** and **b₃**, could be hybridized and produced the second generation of compounds containing the mixed ligand units. The results show that penta-oxy phosphoranes **a₃/b₃** or **c₃/b₃** could inter-exchange their ligands of **a/b** or **c/b** to produce new chemical species with high efficiency. The self-ligand exchange within penta-oxy phosphorane **ab₂** molecules could

also produce three penta-oxy phosporanes with all of the ligands combination. Therefore, we wondered that if the uni-molecule **ab₂**, containing hetero-structure information could play the role of chemical information carrier. Furthermore, the reactivity of penta-oxy phosphorane could also be considered as a plausible chemical model for the study of the intermediates for phosphoryl group transfer, nucleoside hydrolysis, and RNA fusion at molecular level. It is possible that the gene transcription processes are closely related to RNA editing through the phosphate ligand exchange of the genetic bits. We then proposed that the ligand exchange reaction of the penta-coordinated phosphorus might be a general chemistry for translation of chemical information into biological signals as the phosphorus has done in living systems.

4   Experimental

4.1   General

All reactions were carried out in a glove box with Ar. Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. ^{31}P NMR (165 MHz), ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured on a Bruker AVIII 400M.

4.2   General preparation for the oxy-phosphoranes

Oxyphosphoranes were prepared from the corresponding trialkyl phosphite with 1 equiv. of tetrachloro-o-benzoquinone in the toluene solution (Scheme 4). The oxyphosphoranes were obtained as colorless crystal in high yield after recrystallization from toluene. The molecular structure of **c₃** was further characterized by single crystal XRD analysis.

a_3, $R^1 = R^2 = R^3 = Me$
b_3, $R^1 = R^2 = R^3 = Ph$
c_3, $R^1 = R^2 = R^3 = Et$
ab_2, $R^1 = R^2 = Ph$, $R^3 = Me$
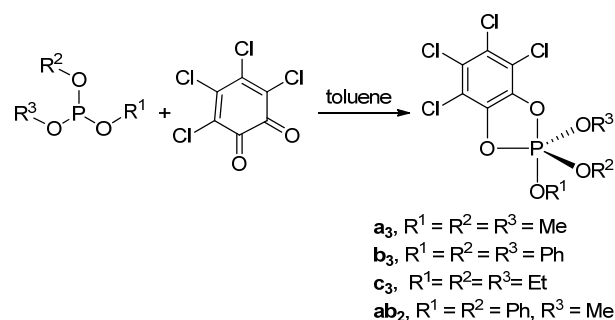
Scheme 4   Preparation of oxyphosphoranes

Penta-oxy phosphorane (**a₃**): Colorless solid; ^{1}H NMR (C_6D_6, 400 MHz) δ: 3.33 (d, $J_{H-P}=13.6$ Hz, 9 H); ^{13}C NMR (C_6D_6, 100 MHz) δ: 140.8 (d, $J_{C-P}=6.7$ Hz), 124.4, 114.4 (d, $J_{C-P}=17.35$ Hz), 56.0, (d, $J_{C-P}=11.36$ Hz); ^{31}P NMR (C_6D_6, 164 MHz) δ: -45.

Penta-oxy phosphorane (**b₃**): Colorless solid; ^{1}H NMR (C_6D_6, 400 MHz) δ: 7.06 (d, $J=8.1$ Hz, 6H), 6.97 (t, $J=7.7$ Hz, 6H), 6.83 (t, $J=7.2$ Hz, 3H); ^{13}C NMR (C_6D_6, 100 MHz) δ: 153.6 (d, $J_{C-P}=12.9$ Hz), 140.3 (d, $J_{C-P}=6.4$ Hz), 129.7, 125.4, 125.1 (d, $J_{C-P}=1.9$ Hz), 121.1 (d, $J_{C-P}=5.2$ Hz), 114.8 (d, $J_{C-P}=18.0$ Hz); ^{31}P NMR (C_6D_6, 164 MHz)

δ: -60.

Penta-oxy phosphorane (**ab$_2$**): Colorless solid; ^{1}H NMR (C$_6$D$_6$, 400 MHz) δ: 7.00~6.96 (m, 8 H), 6.84~6.81 (m, 2H), 3.43 (d, J=14.3 Hz, 3H); ^{13}C NMR (C$_6$D$_6$, 100 MHz) δ: 153.8 (d, J_{C-P}=12.4 Hz),140.4 (d, J_{C-P}=6.0 Hz), 129.6 (d, J_{C-P}=1.3 Hz), 125.1, 124.6 (d, J_{C-P}=1.8 Hz), 121.3 (d, J_{C-P}=5.3 Hz), 114.7 (d, J_{C-P}=17.8 Hz); ^{31}P NMR (C$_6$D$_6$, 164 MHz) δ: -55.

Penta-oxy phosphorane (**c$_3$**): Colorless solid; ^{1}H NMR (C$_6$D$_6$, 400 MHz) δ: 3.88~3.82 (m, 6 H), 1.00 (t, J=7.3 Hz, 9 H); ^{13}C NMR (C$_6$D$_6$, 100 MHz) δ: 141.1 (d, J_{C-P}=5.9 Hz), 124.2, 114.2 (d, J_{C-P}=17.4 Hz), 65.0 (d, J_{C-P}=11.5 Hz), 16.2 (d, J_{C-P}=8.3 Hz); ^{31}P NMR (C$_6$D$_6$, 164 MHz) δ: -48.

Supporting Information　　NMR spectral data for **a$_3$**, **b$_3$**, **ab$_2$** and **c$_3$**, and crystal data for **c3**. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn/.

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