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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（ab_2）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（a_3, b_3 和 a_2b）。如果把其中 a_3 与 b_3 作为父代分子，其配体交换产生的五配位氧磷烷 a_2b 和 ab_2 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 Phospho-ranes—The Potential Chemical Model for RNA Hydrolysis and Fusion

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Abstract Penta-coordinated phosphoranes with a five-member cycle and three ligands (ab_2) would simultaneously exchange with themselves under base catalysis to form three different penta-oxy phosphoranes with all the combinatorial ligands refered as a_3, b_3, and a_2b. If we consider a_3 and b_3 as parents, the products obtained from exchange, namely a_2b and ab_2, 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 study 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 pro-

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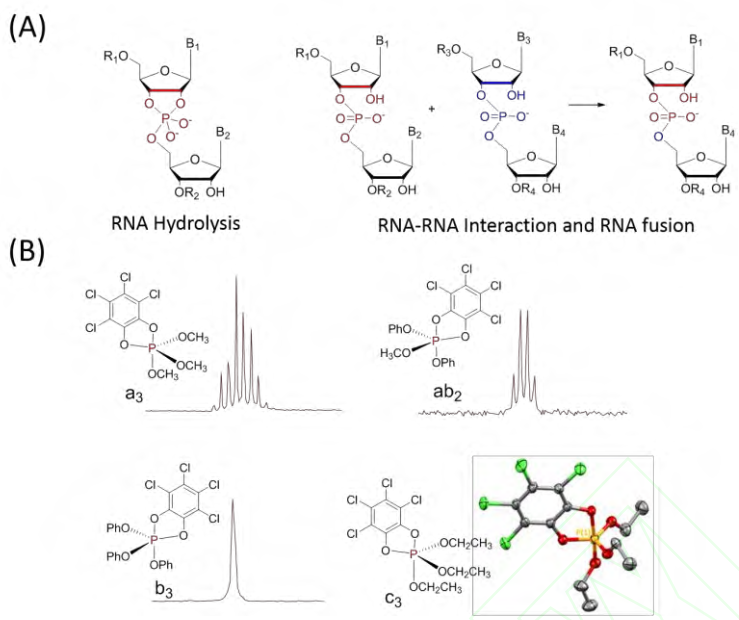
teins and transmembrane transport. Penta-coordinated phosphorus as key important intermediate involved in many biological process, such as phosphoryl group transfer,[1] ATP hydrolysis,[2] RNA hydrolysis via internal transesterification,[3] RNA self-splicing and twister ribozyme catalysis[4]. Recently, 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 were 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 bio-activity compounds 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 (MFx), 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 *et al.* set up an oxyphosphorane model to simulate the intermediate in the hydrolysis of ATP.[12] 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] Here, we identify an interesting intermolecular ligand-exchange reaction of one pentaoxyphosphorane for 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 (Table S1-S6, Supporting Information). For example, ab_2 with one methoxyl and two phenoxyl ligands was determined at ^{31}P NMR δ = -55 ppm 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 -51 ppm and -60 ppm 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 -47 ppm with about 0.1% (a_3) as shown in Figure 1D (Table S7, Supporting Information). In order to confirm their structures, ^{31}P NMR tests with ^{1}H-coupling were performed and deduce that three new penta-oxy phosphoranes, namely a_3, a_2b 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 $J_{H\text{-}P}$ = 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_2b displays a heptet with $J_{H\text{-}P}$ = 14.4 Hz in ^{1}H-coupled ^{31}P NMR, indicating that there are two methyl groups linked to the phosphorus center of a_2b. The cleavage and formation of P-O ester bonds are atom economic with very high efficient. The diversity of chemical structures could be improved responsible for deserving more functions and information.



Scheme 1 (A) Biological models of penta-coordinate phosphorus intermediate. (B)The chemical structure of penta-coordinated phosphorus ^{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 (deg): 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.

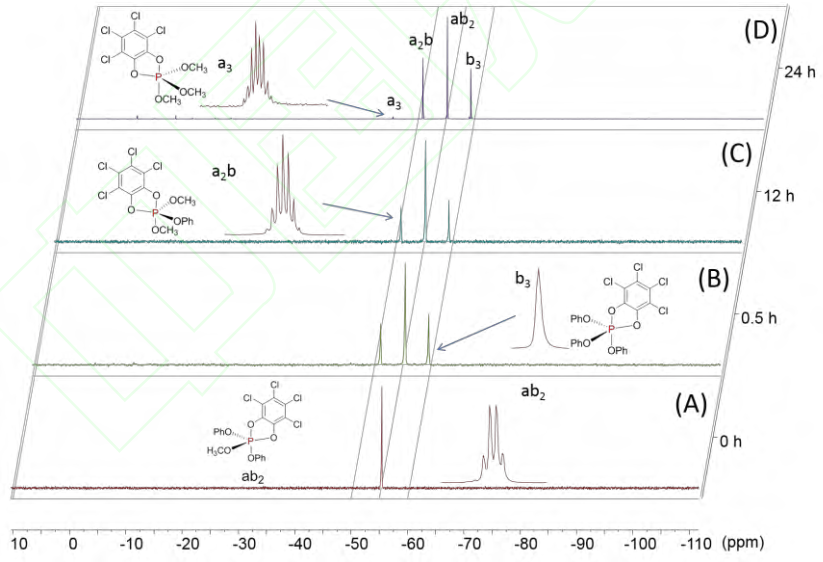


Figure 1 Time-dependent, stacked ^{31}P NMR spectra for the reaction of ligand exchange of ab_2 (250 mM) with 10% pyridine in D_6-benzen solution at 25 ℃ for 24 h. The insert are the chemical structures with corresponding ^{1}H-^{31}P NMR spectra.

In order to identify the ligand-exchange mechanism, we then treated a_3 with three methoxyl groups (^{31}P NMR δ = - 45 ppm) with b_3 with three phenol groups (^{31}P NMR δ = - 60 ppm) in a ratio of 1:1 in C_6D_6 solution and monitored by *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% equivalent fresh distilled pyridine was added into the reaction mixture. The formation of a_2b and ab_2 could be obtained immediately in the ^{31}P NMR spectrum (Figures 2B). After 12 hours, two singlet at -51 ppm and -55 ppm were obtained, which could be identified as a_2b and ab_2 respectively. The NMR spectra of a_2b 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_2b and ab_2 after 24 h

incubation. Other Lewis base such as 2,6-lutidine showed similar behavior under the same reaction conditions (Table S8, Supporting Information). Similarly, c_3 with three ethoxyl groups could also smoothly exchange ligands with b_3 to form c_2b and cb_2 under base catalysis (Table S9, Supporting Information). 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).

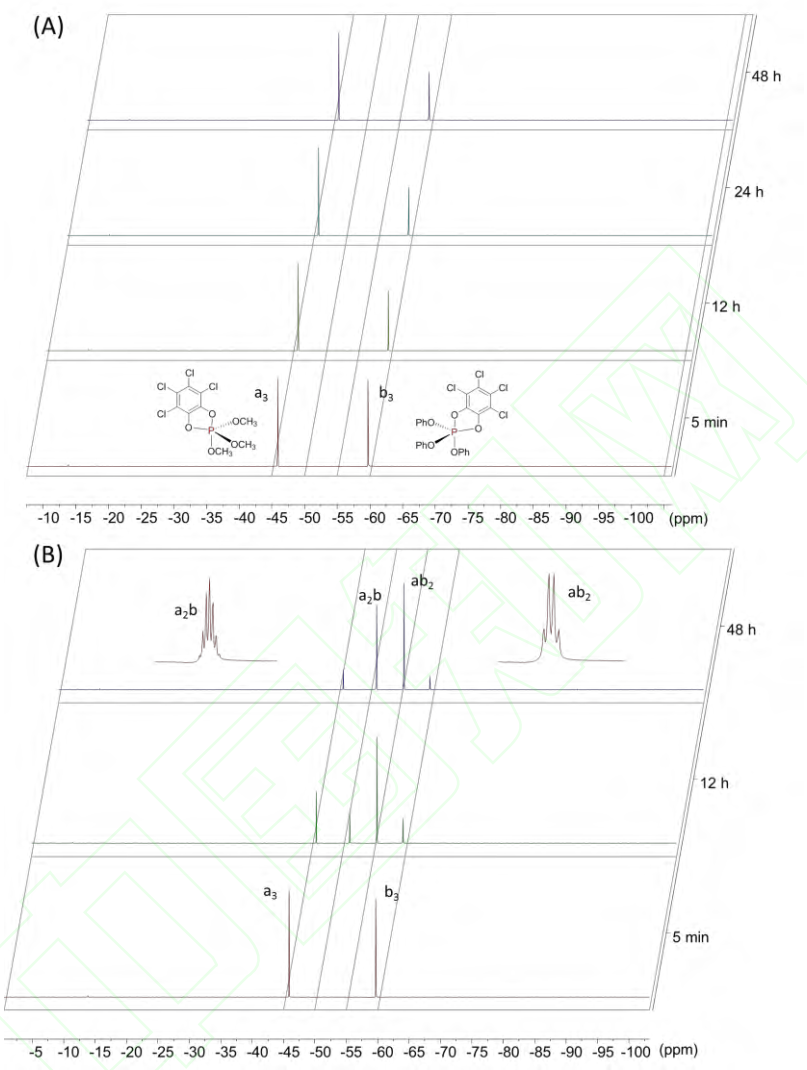
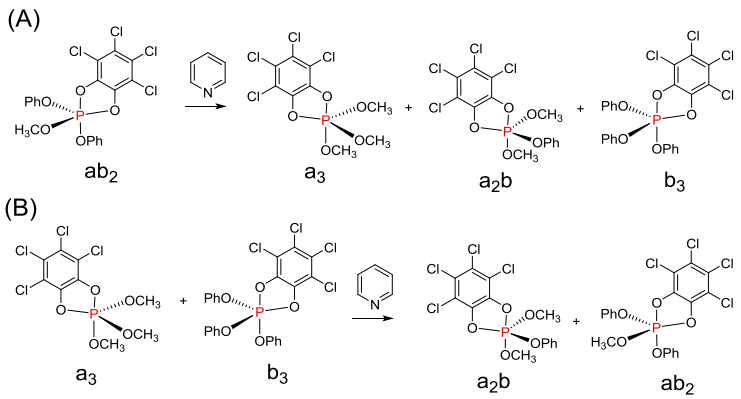


Figure 2 Time-dependent, stacked ^{31}P NMR spectra for the reaction of ligand exchange between a_3 (250 mM) and b_3 (250 mM) without base (A) or with 10% pyridine (B) for 48 h. The insert are ^{1}H-^{31}P NMR spectra.



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

To determine the ligand exchange whether or not a unique phenomenon for penta-coordinated phosphorus, a_3 was treated with triphenyl phosphate 1b and a_3 in a C_6D_6 solution for 5 days at 25 ℃, no exchange product was detected in ^{31}P NMR even with the catalysis of 2,6-lutidine (Figure S1 and S2, Supporting Information). Based on the experimental results, we assumed that the dsp^3 orbital of penta-coordinated phosphorus are key to ligand exchange. Thus, the regular tetra-coordinated phosphate with a tetrahedron sp^3 orbital could not exchange ligands with themselves or other dsp^3 phosphoranes.

To understand the mechanism, we investigated the reaction of a_3 (1 equiv.) with 2,6-lutidine (1 equiv.) in anhydrous C_6D_6 solution. The reaction mixture was monitored by ^{31}P NMR (Figure 3A). Two new peaks at 3 ppm (2a) and -93 ppm (1a) was obtained immediately with the gradient 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 a -93 ppm could be postulated as an intermediate with a penta-coordinated phosphorous center as shown in Scheme 3. To determine the possible structures, a 2D ^1H-^{31}P HMBC NMR experiment was performed (Figure 3B). The signal at -93 ppm was correlated to the protons at 3.76 ppm with J_{P-H} = 15.2 Hz, which usually belongs to the penta-coordinated phosphorane. While the signal at 3 ppm of the ^{31}P NMR was correlated to the protons at 3.32 ppm with J_{P-H} = 11.6 Hz, which usually belongs to the tetrahedron phosphates. Furthermore, ^1H NMR shown that two sets of spectra with specific integrated areas were well corresponded to 1a with penta-coordinated phosphorus as compared with the ^1H NMR spectrum of the authentic 2,6-lutidine (Figure 3C, Figure S3, Supporting Information). In conclusion, the new signals at -93 ppm could be a penta-coordinated phosphorane 1a, while the peak at 3 ppm is a tetra-coordinated phosphate.

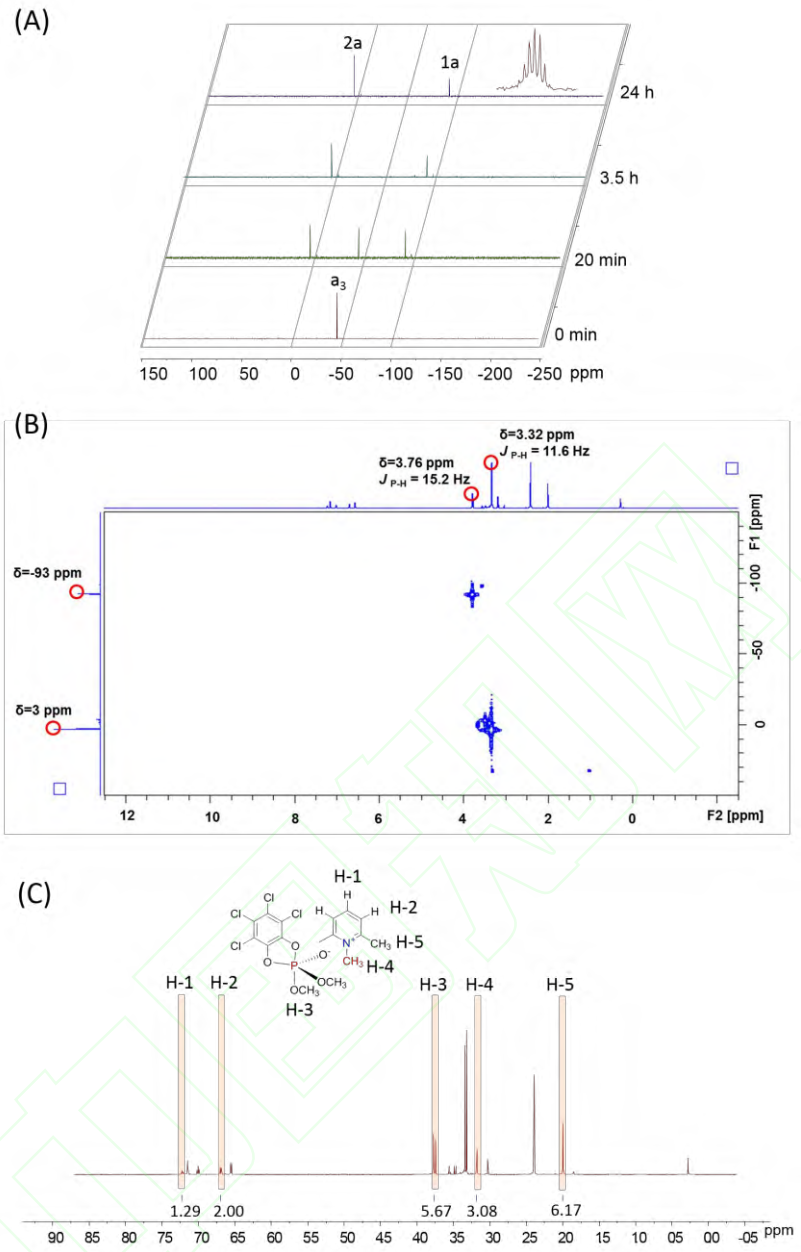
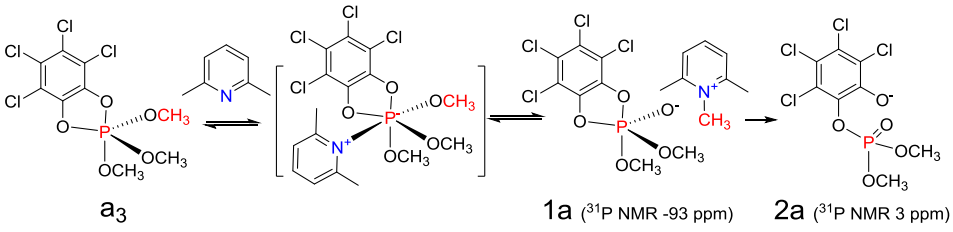


Figure 3 (A) Time-dependent, stacked ^{31}P NMR spectra for the reaction of a_3 with 1 equivalent 2,6-lutidine in D_6-benzen solution at 25 ℃ for 24 h. (B) ^{1}H-^{31}P HMBC NMR spectrum of the mixture of a_3 with 1 equivalent 2,6-lutidine after 24 h. (C) The ^{1}H NMR spectrum of reaction mixture of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



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

Inspired by the experimental results, a possible mechanism was proposed as shown in Scheme 3. The starting material a_3 firstly reacts with pyridine or 2,6-lutidine and produces a transition states or intermediate with a hexa-coordinated phosphorus center.[15] Then a slow demethylation of the unstable hexa-coordinated phosphorus to afford the ionic intermediate 1a and *N*-methylation of base as donor, which is 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

With this work, we provide a new chemical model of penta-coordinated phosphoranes with the inter-molecular ligand exchange. In general, the experimental results show that the first generation compounds with ligands a_3 and b_3, could be hybridized and produced the second generation of compounds containing mixed ligand units. The results show that penta-oxy phosphoranes a_3/b_3 or c_3/b_3 could inter-exchange their information units a/b or c/b to produce new chemical species with different relative ratios. The self-ligand exchange within penta-oxy phosphorane ab_2 molecules could also produce three penta-oxy phosporanes with all of the ligands combination. Therefore, we wondered that if the uni-molecule ab_2, 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 phosphorus intermediates of phosphoryl group transfer, nucleoside hydrolysis, and RNA fusion at molecular level. It is significant to point out that the genetic transcription mechanism should be performed by the genetic editing of DNA and RNA 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 have done in living systems.

Supporting Information Synthesis and NMR spectral data for a_3, b_3, ab_2 and c_3, Figure S1-S3, and Table S1-S9. The Supporting Information is available free of charge via the Internet at http://sioc-journal.cn/.

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图文摘要

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

Wang, Xun; Chen, Su; Wu, Yile; Wang, Xiaoyu; Tang, Guo; Liu, Yan; Xu, Pengxiang; Gao, Xiang*; Zhao, Yufen*

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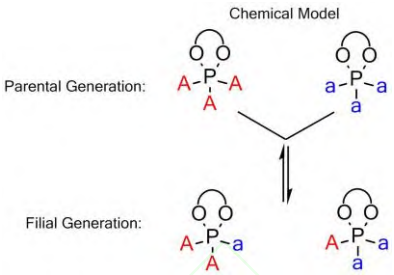

Here we find a chemical model through the ligand exchange between penta-oxy phosphoranes.

