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Synthesis and hydrolysis of a phenylalanyl adenylate pentacoordinated phosphorane

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Abstract

Amino acid-nucleotide conjugates have important biological functions and therapeutic applications. For example, aminoacyl adenylates are key intermediates in aminoacyl tRNA synthetase reactions. They may also be involved in the prebiotic synthesis of polypeptides. Finally, various amino acid carbomethoxy aryl phosphoramidates of nucleotide prodrugs may be activated through a mechanism involving a pentacoordinated phosphorane intermediates. In order to understand better the chemistry of these compounds, a phenylalanyl adenylate pentacoodinated phosphorane has been synthesized in 72% yield and its decomposition in aqueous solution studied. Hydrolysis gave 2′,3′-*O*-isopropylidene adenosine 5′-monophosphate, 2′,3′-*O*-isopropylidene adenosine, and phenylalanine. The results provide model chemistry for the enzymatic degradation mechanism of antiviral aryl amino acid phosphodiester amidates in cells, which leads to their activation.

Keywords: Phenylalanyl adenylate pentacoordinated phosphorane; Nucleotide prodrugs; Hydrolysis of phosphodiester amidates

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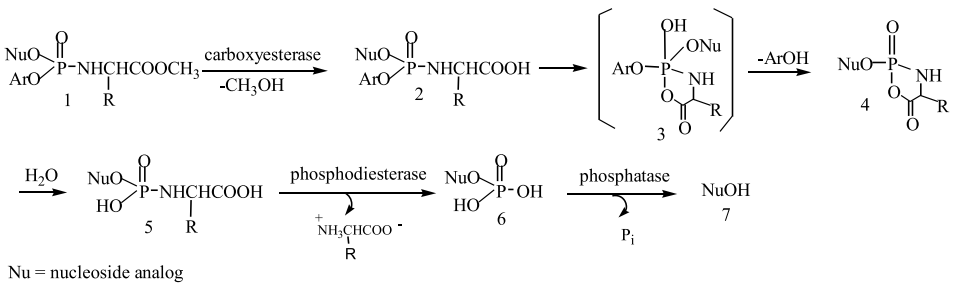
Compounds in which amino acids are linked to nucleotides are found in nature and have significant biological and medical importance. Aminoacyl adenylates are enzymatic reaction intermediates in aminoacyl tRNA synthetase reactions resulting from the reaction of amino acids with ATP in the biosynthesis of proteins [1]. They have also been implicated as intermediates in the prebiotic synthesis of polypeptides [2]. In our previous research, *N*,*O*-bis(trimethylsilyl)-α-amino acids, but not β-amino acids, could oligomerize into polypeptides in the presence of o-phenylene phosphorochloridate. The mechanism preceded through a five-membered cyclic pentacoordinated phosphoranes containing the α-amino acid residue and was a key step in the oligomerization reaction. This observation suggested that phosphates could preferentially choose α-amino acids in the prebiotic synthesis of polypeptides [3]. In addition, the activation mechanism for various amino acid carbomethoxy ester aryl phosphoramidate nucleotide prodrugs (**1**) in cells to form 5′-monophosphate (**6**) and nucleoside (**7**) most likely involves pentacoordinated phosphoranes (**3**) as shown in Scheme 1 [4–6]. Amino acid phosphoramidates of nucleosides have shown promise as potent antiviral agents, because in some cases they have exhibited enhanced antiviral activity and reduced cytotoxicity when compared to the parent nucleosides [7–9]. For these reasons it is very important to synthesize a pentacoordinated phosphorane containing amino acid and nucleoside components and study the hydrolysis pathway.

1. Materials and methods

All chemicals were purchased from Beijing Chemical Reagent in China. Proton, carbon, and phosphorus nuclear magnetic resonance (^{1}H, ^{13}C, and ^{31}P NMR) spectra were recorded on a Bruker AC spectrometer operating at 500, 125, and 81 MHz, respectively. Chemical shifts for ^{31}P NMR spectra are indicated in parts per million relative to an external 85% phosphoric acid standard. Field desorption mass spectrum (FDMS) was obtained on a Finnigan MAT 90 mass spectrometer. LC-ESI mass spectra were recorded on Bruker Esquire-LC electrospray ionization mass spectrometry.



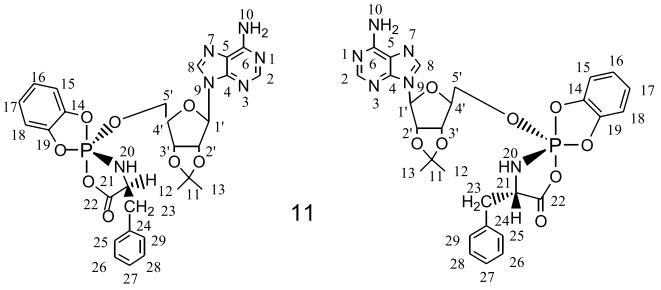
Scheme 1. Proposed amino acid decomposition pathway of antiviral aryl amino acid phosphodiester amidates [4–6].

1.1. Synthesis of 2′,3′-*O*-isopropylidene-5′-*O*-(trimethylsilyl)adenosine (**10**)

Compound **10** was obtained from the reaction of 2′,3′-*O*-isopropylidene adenosine and an excess of hexamethyldisilazane (HMDS, 1.5 eq) in dry CH_2Cl_2 by refluxing under a nitrogen atmosphere until a clear solution appeared (about 2.5 h). The excess HMDS and the solvent were removed by distillation leaving **10**. An ^1H NMR spectrum showed that the purity was greater than 95%. The product was not purified further. ^1H NMR (500 MHz, $CDCl_3$) of **10**: δ 0.18 (s, 9H, $SiMe_3$), 1.31, 1.53 (ss, 6H, H12, H13), 3.51–3.58 (m, 2H, H5′), 4.21 (m, 1H, H4′), 4.96 (d, 1H, H3′), 5.33 (m, 1H, H2′), 6.12 (d, 1H, H1′), 7.32 (s, 2H, H10), 8.16 (s, 1H, H8), 8.33 (s, 1H, H2).

1.2. Procedure for the preparation of **11**

To a stirring solution of 2,2,2-trichloro-1,3,2-benzodioxaphosphole (**8**) (1.23 g, 5 mmol) in dry CH_2Cl_2 (10 mL) at room temperature under a nitrogen atmosphere was added dropwise a solution of *N*,*O*-bis(trimethylsilyl)phenylalanine (5 mmol) in dry CH_2Cl_2 (5 mL). After 10 min, **8** was almost quantitatively converted into **9**, and a solution of 2′,3′-*O*-isopropylidene-5′-*O*-(trimethylsilyl)adenosine (**10**) (1.89 g, 5 mmol) was added dropwise to the resulting solution. The reaction was complete in 15 min. Subsequently, solvent was removed by evaporation in vacuo, and the target product **11** was obtained as a white solid after crystallization from CH_2Cl_2 and ethyl ether in 72% yield (2.2 g). ^{31}P NMR (81 MHz, CH_2Cl_2): δ −41.64, −41.92 ppm (integral area 1:1); ^1H NMR (500 MHz, $CDCl_3$): δ 1.07–1.56 (m, 6H, H12, H13), 3.22–3.36 (m, 2H, H23), 3.75 (m, 1H, H20, $^2J_{P-NH} = 8.2$ Hz), 3.91 (m, 1H, H21), 3.98 (m, 1H, H4′), 4.18 (m, 1H, H3′), 4.36 (m, 1H, H2′), 4.48 (m, 2H, H5′), 6.12 (m, 1H, H1′), 6.52–7.20 (m, 9H, H15, 16, 17, 18, 25, 26, 27, 28, 29), 7.42 (d, 2H, H10), 8.82 (d, 1H, H8), 9.36 (d, 1H, H2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.59, 27.00 (C12, C13), 37.05 (C23), 56.21 (C21), 68.45 (C5′), 81.60 (C2′), 85.26 (C3′), 85.88 (C4′), 91.93 (C1′), 109.94 (C15), 111.56 (C18), 114.49 (C11), 117.90 (C5), 120.89 (C17), 123.51 (C16), 127.15 (C27), 128.29 (C26, 28), 131.26 (25, 29), 135.45 (C8), 142.04 (C24), 142.53 (C19), 143.96 (C14), 144.46 (C4), 148.50 (C2), 151.07 (C6), 169.06 (C22, $^2J_{P-C} = 17.8$ Hz); Positive ion FDMS M^+ m/z 608. It was not possible to obtain a melting point because **11** was labile at high temperature.



11

1.3. Hydrolysis of compound **11**

Compound **11** (12.2 mg, 0.02 mmol) was dissolved in acetone (0.6 mL), and the solution was transferred into an NMR tube. Water (2 eq) was added to the solution and the tube was shaken. Subsequently, the hydrolysis of **11** was monitored by 81 MHz ^{31}P NMR spectroscopy at room temperature.

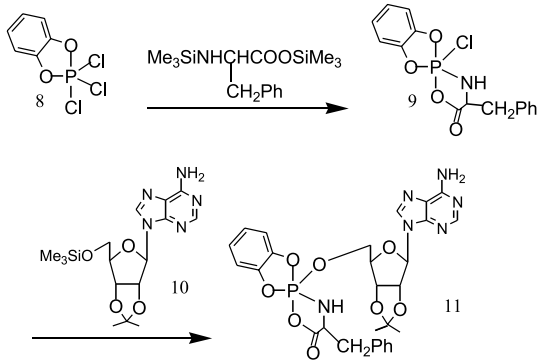
1.4. LC-ESI MS analytical conditions

LC-ESI MS analysis of the hydrolysis products of **11** was carried on Varian VISTA 5500 liquid chromatograph with a reversed-phase C18 column (ZORBAX SB-C18) and Bruker Esquire-LC-ESI mass spectrometer. Elution was accomplished with 20% acetonitrile and 0.1% acetic acid aqueous solution at a flow rate of 0.8 mL/min.

2. Results and discussion

The phenylalanyl adenylate pentacoordinated phosphorane (**11**) was synthesized by the sequential reaction of 2,2,2-trichloro-1,3,2-benzodioxaphosphole [10] (**8**) with *N*,*O*-bis(trimethylsilyl) phenylalanine [11] and 2′,3′-isopropylidene-5′-*O*-(trimethylsilyl)adenosine (**10**). The synthetic pathway is shown in Scheme 2.

There are two advantages using 2′,3′-*O*-isopropylidene-5′-*O*-(trimethylsilyl)-adenosine (**10**) instead of 2′,3′-*O*-isopropylidene adenosine/triethylamine. First, the isolation of the target product is easier by recrystallization in the absence of triethylamine hydrochloride. Second, nucleophilic attack of the 5′-hydroxyl of 2′,3′-*O*-isopropylidene adenosine to the carbonyl of the mixed anhydride is avoided. It is interesting to note that the reaction occurred on 5′-*O*-(trimethylsilyl)oxygen of 2′,3′-*O*-isopropylidene-5′-*O*-(trimethylsilyl)adenosine rather than on the free amino group of adenine. ^{31}P NMR integral areas of **11** showed that the product was a 1:1 mixture of diastereomers.



Scheme 2. Synthetic pathway of phenylalanyl adenylate pentacoordinated phosphorane (**11**).

The reaction of **11** with water in acetone was monitored by ^{31}P NMR spectroscopy at room temperature (Fig. 1). As the reaction progressed, the pair of peaks at −41.64 and −41.92 ppm, corresponding to the two diastereomers of **11** gradually decreased. At the same time, the peaks at −3.74 and −3.52 ppm, corresponding to phosphodiester (**15**) and phosphomonoester (**16**), respectively, increased. A small signal at 13.56 ppm, corresponding to the cyclicphosphotriester [12] (**14**), also appeared as shown in Fig. 1. After 12 h, **11** was completely converted to the final products **15–18**, catechol and nucleoside monophosphate (**19**). The structures of **15–18** were verified by LC-ESI-MS/MS. The peaks at 3.19, 3.62, 6.33, and 9.45 min (Fig. 2) correspond to compounds **17**, **16**, **18**, and **15**, respectively, whose protonated molecules $[M + H]^+$ are m/z 166, 191, 308, and 480 in ESI-MS. Based on these ob-

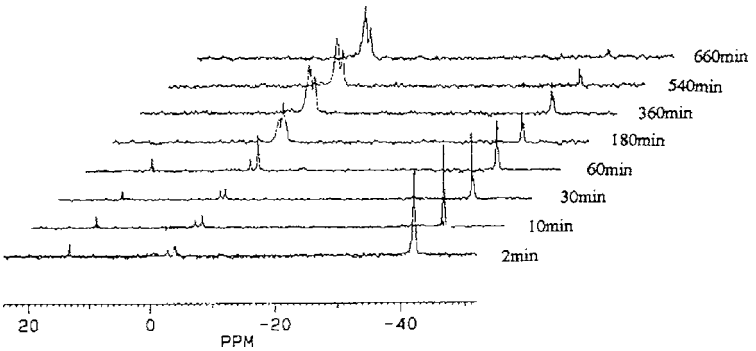


Fig. 1. The ^{31}P NMR spectra monitoring the hydrolysis reaction for phenylalanyl adenylate pentacoordinated phosphorane (**11**). The signals corresponding to the diastereomers of **11** (−41.64 and −41.92 ppm) are decreasing in intensity while the signals corresponding to **15**, **16**, and **19** (from −3.74 to −3.52 ppm) are increasing in intensity.

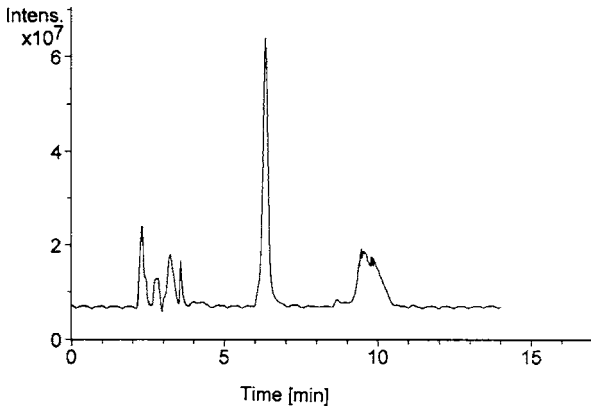
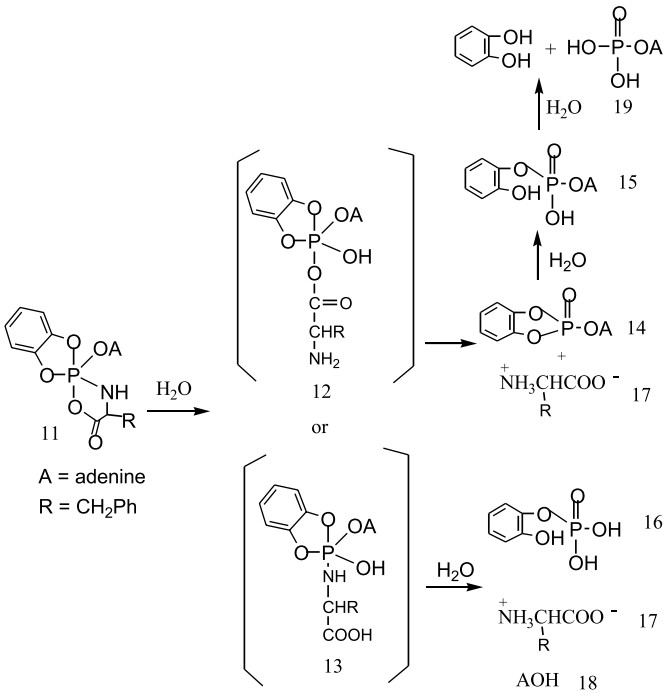


Fig. 2. A chromatogram of the hydrolysis products for phenylalanyl adenylate pentacoordinated phosphorane (**11**). The peaks at 3.19, 3.62, 6.33, and 9.45 min, corresponding to **17**, **16**, **18**, and **15**, were identified by ESI-MS.

servations, a hydrolysis pathway for **11** is proposed in Scheme 3. Nucleophilic attack of water on the phosphorus of **11** leads to the cleavage of the P–N bond or the P–O bond of the mixed anhydride bond to form intermediate **12** or **13**. Futher hydrolysis of **12** results in **14** with the loss of **17** while futher hydrolysis of **13** leads to **16** with the loss of **17** and adenosine (**18**). Subsequently, **14** was hydrolyzed into the phosphodiester (**15**), which, in turn, could produce catechol and **19**. The compounds correspond to two peaks within 3 min in Fig. 2. Unfortunately their protonated molecules $[M + H]^+$ were not determined because their sensitivity is low in positive ion ESI-MS.

The structure of compound **11** is similar to that of proposed intermediate **3** in Scheme 1, and the products of hydrolysis also are in agreement with the observed enzymatic degradation products of antiviral aryl amino acid phosphodiester amidates in cells [4–6].

In summary, we have developed a mild procedure for the production of aminoacyl adenylate pentacoordinated phosphoranes. Hydrolysis of the pentacoodinated phosphorus compound yields the phosphodiester, phosphomonoester, nucleoside, and amino acid. The hydrolysis pathway could provide model chemistry for enzymatic degradation mechanism of antiviral aryl amino acid phosphodiester amidates.



Scheme 3. Possible hydrolysis pathway for the phenylalanyl adenylate pentacoordinated phosphorane (**11**).

Acknowledgments

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