SYNTHESIS AND STRUCTURE OF α-AMINOPHOSPHATE AND ITS INTERACTION WITH DNA/BSA **

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*The reaction of terephthalaldehyde, p-chloroaniline, and diethyl phosphite in ethyl alcohol solution yields a new crystal (**1**), CCDC:1405044. This compound is characterized by X-ray single-crystal diffraction, IR, elemental analysis, ESI-MS, and ^1H and ^{13}C NMR. Compound **1** crystallizes in the triclinic system, space group P-1 with a = 11.0444(4) Å, b = 12.6468(4) Å, c = 12.7657(4) Å, V = 1627.46(9) Å^3, Z = 2, $C_{28}H_{36}Cl_2N_2O_6P_2$, M_r = 629.43, D_c = 1.284 g/cm^3, μ = 0.339 mm^{-1}, F(000) = 660, GOOF = 1.101, final R = 0.0875 and wR = 0.1082 for 7381 observed reflections (I > 2σ(I)). The X-ray analysis reveals that the planes of p-chloroaniline and terephthalaldehyde form a dihedral angle of 87.54(0.28)°. In the crystal of compound **1**, P atoms have tetrahedral geometries, made up by a double-bond O atom, one $C_α$ atom, and two O-ethyl groups. Besides, $C_α$ atoms are responsible for the existence of the optical activity. Five intramolecular hydrogen bonds helping to stabilize the crystal structure are observed. The interaction between compound **1** and DNA/BSA is also studied.*

Keywords: *crystal structure, interaction with DNA, binding constant, α-amionphosphate.*

СИНТЕЗ И СТРУКТУРА α-АМИНОФОСФАТА И ЕГО ВЗАИМОДЕЙСТВИЕ С ДНК/БСА

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*Реакция терефталевого альдегида, р-хлоранилина и диэтилфосфита в растворе этилового спирта дает новый кристалл (**1**), CCDC: 1405044. Соединение охарактеризовано с помощью рентгеновской дифракции, ИК спектроскопии, элементного анализа, ESI-MS и ^1H и ^{13}C ЯМР. Соединение **1** кристаллизуется в триклинной системе, пространственная группа P-1 с a = 11.0444(4) Å, b = 12.6468(4) Å, c = 12.7657(4) Å, V = 1627.46(9) Å^3, Z = 2, $C_{28}H_{36}Cl_2N_2O_6P_2$, M_r = 629.43, D_c = 1.284 г/см^3, μ = 0.339 мм$^{-1}$, F (000) = 660, GOOF = 1.101, конечное R = 0.0875 и wR = 0.1082 для 7381 наблюдаемых отражений (I > 2σ(I)). В результате рентгеноструктурного анализа показано, что плоскости р-хлоранилина и терефталевого альдегида образуют двугранный угол 87.54(0.28)°. В кристалле соединения **1** атомы Р имеют тетраэдрическую геометрию, которая состоит из атома кислорода двойной связи, одного атома $C_α$, двух О-этильных групп. Кроме того, атомы $C_α$ ответственны за существование оптической активности. Пять внутримолекулярных водородных связей помогают стабилизировать кристаллическую структуру. Изучено взаимодействие между соединением **1** и ДНК/БСА.*

Ключевые слова: *кристаллическая структура, взаимодействие с ДНК, константа связывания, α-аминофосфат.*

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Introduction. Due to their structural similarity to α-amino acids and significant biological activities, special attention is paid to the synthesis of α-aminophosphonates [1, 2]. It is reported that α-aminophosphonates act as peptidomimetic [3], antibiotics [4], anticancer [5], and pharmacological agents [6]. Several α-aminophosphonates act as potential enzyme inhibitors, namely (2-hydroxyphenyl)(4-hydroxyphenylamino)methylphosphonic acid, diethyl(2-chlorophenylamino) (2-hydroxyphenyl)methylphosphonate, diphenyl-1-(arylamino)-1-(pyridin-3-yl)ethylphosphonates, 6-(phosphono-difluoromethyl)-2-naphthoic acid, and the difluoro-methylenephosphonate group [7–11]. Moreover, Wang [12] reported that α-aminophosphonate N-derivatives with rigid structures can interact with DNA/BSA.

DNA is a widely used target for most chemotherapy drugs. It is reported that both covalent and noncovalent modes exist between drug molecules and DNA [13]. Cisplatin is an effective anticancer drug, as a $[Pt(NH_3)_2]^{2+}$ unit is formed by 1,2 intrastrand crosslinks between cisplatin and two adjacent guanine bases of DNA [14]. However, the application of platinum-based drugs is limited because of their toxic side effects. So the design of other hypotoxic metal complexes and organic compounds having tumor-inhibiting properties is of great interest [15–20]. Lerman [21] reported that the aromatic element in a molecule could help to interact with DNA. Besides, research on the mechanism of interaction between BSA (one of the most important carrier proteins in the plasma [22–24]) and drug could provide the basis for the design of new drugs.

In this paper, a new compound, α-aminophosphonate (**1**), was obtaned by employing terephthalaldehyde, *p*-chloroaniline, and diethyl phosphite in ethyl alcohol solution under mild reaction conditions. Its properties (in particularly, interaction with DNA/BSA) were investigated by such methods as IR spectroscopy, X-ray diffraction, elemental analysis, electrospray ionization mass spectra, and ^{1}H, ^{13}C, and ^{31}P NMR.

Experimental. *Materials and measurements.* All reagents and solvents were obtained from commercial sources without further purification. Deionized water was used in all chemical experiments, and double-distilled water was used to prepare the buffer solution and biological evaluation. A PHS-3TC pH meter was used to measure the pH value. A VARI-EL elemental analyzer was used to carry out elemental analyses (EAs). An IR-8300 spectrometer in KBr disks was used to record IR spectra (4000–400 cm^{-1}). A Bruker DRX-400 spectrometer with TMS as the internal standard was used to obtain ^{1}H and ^{13}C NMR spectra. ^{31}P NMR spectra (121.49 MHz) with H_3PO_4 (85%) as the external standard were obtained on a Bruker DRX-400 spectrometer. A Triple TOFTM 5600$^+$ system was applied for electrospray ionization mass spectra. A Cary Eclipse spectrophotometer was used to record the fluorescence spectrum. A SMART 1*K* CCD area detector single-crystal diffractometer with the ω-scan method and graphite monochromated MoK_α radiation (λ = 0.71073 Å) was used to obtain the X-ray data of **1**.

*Synthesis of α-aminophosphonates [$C_{28}H_{36}Cl_2N_2O_6P_2$] (**1**).* α-aminophosphonate **1** was synthesized using a modification of the known method [11, 12, 25]. A two-step procedure was used to synthesize **1**: first, 0.02 mol of *p*-chloroaniline and 0.02 mol of terephthalaldehyde were added to 20 mL of CH_3CH_2OH and refluxed for 4 h. When the reaction mixture was cooled to room temperature, the schiff base was crystallized from the solution, yield: **1a** 75%; a second 20 mmol of diethyl phosphonate was dissolved in 10 mL of CH_3CH_2OH and added into 10 mmol of **1a** dissolved in 10 mL of CH_3CH_2OH. Then the mixture was refluxed for 24 h with constant stirring. The oily residue was obtained by a rotary evaporator and then dissolved in 10 mL CH_3CH_2OH; a block crystal was collected after 3 days. Yield: 0.22 5g, 35%. EAs: calcd/found (%) for $C_{28}H_{36}Cl_2N_2O_6P_2$ (**1**): C 54.43/54.41, H 5.76/5.79, N 4.45/4.45. IR (cm^{-1}): 3240 ν(O-H, N-H), 2986 ν(C_α-H), 1227 ν(P=O), 1042, 1025 ν(P-O-C), 973 (C-P). ^{31}P NMR (D_2O/CD_3OD, ppm): δ 23.268. ^{1}H NMR (CD_3OD, ppm): *δ* 1.02-1.24 (-CH_3, 6H), 6.65-7.48 (Ar-H, 12H), 4.97 (-OCH_2-, 8H), 4.86 (-NH-, 2H), 3.89-4.10 (-CH-, 2H). ^{13}C NMR (CD_3OD, ppm): 135.65, 128.27, 128.18, 123.93, 122.19, 114.96, 63.28, 63.01, 55.62, 54.09, 15.30, 15.290, 15.233, 15.06. Exact mass for **1**: 629.43, ESI-MS: [**1**-H]$^-$ (m/z, 628.1236).

Results and discussion. *Crystal structure of compound **1**.* Compound **1** is triclinic, with space group is *P-1*. Crystallographic data, selected bond lengths, and bond angles are shown in Tables 1 and Table 2. From Fig. 1, we find that P atoms have tetrahedral geometries, made up of one C_α atom, a double-bond O atom, and two O-ethyl groups. Compound **1** has optical activity, which is provided by the chiral centers of C_α atoms. The bond lengths of C-P and P=O are almost comparable with similar structures [11, 12, 25, 26]. The planes of *p*-chloroaniline and terephthalaldehyde form a dihedral angle of 87.54(0.28)°. The dihedral angle between 2-hydroxyphenyl and pyridine rings is 54.9(1)° when the ethyl group and one of the two O bonded to the P atoms are displayed by the phenyl groups, also an intramolecular hydrogen bond is formed by the N of the pyridine ring and the O-H of 2-hydroxyphenyl, when they are connected close together [27], so the substitution of the functional groups around P will affect the arrangement of the other functional groups. The

early reported structure, named ethyl(2-hydroxyphenyl)(pyridinium-2-ylamino)methylphosphonate, synthesized via pyridinium-2-amino, 2-hydroxyphenyl, and diethyl phosphite, displays three intramolecular hydrogen bonds, and the intramolecular hydrogen bonds lead to the formation of one five-membered S(5) ring motif and one eight-membered S(8) ring motif [26].

Structure determination. A yellow single crystal of compound **1** was collected by the ω-scan method on a SMART 1K CCD area detector single-crystal diffractometer that used graphite monochromated MoK_α radiation (λ = 0.71073 Å). A crystal with a suitable size (0.42×0.32×0.22 mm) was selected for data collection. The structure was solved by direct methods with the program package [28]. After all the non-H atoms were refined in anisotropy, the hydrogen atoms of C and N were theoretically added and treated as riding on the related atoms.

The final cycle of full matrix least-squares refinement followed. For compound **1**, a total of 26368 reflections were obtained in the range 1.63 < θ < 27.47° with 26368 unique ones (R_{int} = 0.0261). The final R = = 0.0875, wR = 0.2427, $(\Delta\rho)_{max}$ = 0.988, $(\Delta\rho)_{min}$ = –0.973 e/Å^3, and S = 1.101. Tables 2 and 3 show the selected bond lengths, bond angles, and H-bonds.

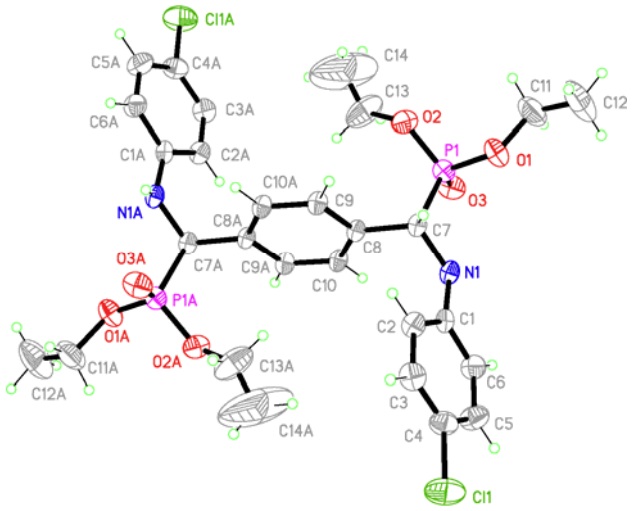

Fig. 1. X-ray structure of the compound **1**. Symmetry codes: (A) –x+2, –y+1, –z+2.

TABLE 1. Crystallographic data for Compound **1**

Empirical formula	$C_{28}H_{36}Cl_2N_2O_6P_2$	a, Å	11.0444(4)	F(000)	660
CCDC	1405033	b, Å	12.6468(4)	Goodness of fit	1.101
Formula weight	629.43	c, Å	12.7657(4)	Reflections collect.	26368
Temperature, K	296(2)	α, degree	81.177(2)	Reflections unique	7381
Wavelength, Å	0.71073	β, degree	12.7657(4)	R_{int}	0.0261
Crystal system	Triclinic	γ, degree	84.082(2)	R_1, wR_2 [$I > 2\sigma(I)$]	0.0875, 0.2427
space group	P-1	V, Å^3	1627.46(9)	R_1, wR_2 (all data)	0.1082, 0.2778
D_{calc}, g/cm^3	1.284	Z	2	Completeness, %	98.8%

TABLE 2. Selected Bond Lengths (Å) and Bond Angles (degree) for Compound **1**

Bond					
P(1)–O(1)	1.563(2)	P(1)–C(7)	1.818(3)	N(1)–C(1)	1.388(4)
P(1)–O(2)	1.569(3)	Cl(1)–C(4)	1.744(4)	N(1)–C(7)	1.451(4)
P(1)–O(3)	1.465(2)	C(7)–C(8)	1.519(3)	C(8)–C(10)	1.387(4)
O(1)–C(11)	1.459(5)	O(2)-C(13)	1.456(6)	C(8)–C(9)	1.393(3)
Angle					
O(3)–P(1)–O(1)	116.80(16)	O(1)–P(1)–O(2)	103.63(15)	O(1)–P(1)–C(7)	101.64(
O(3)–P(1)–O(2)	113.73(16)	O(3)–P(1)–C(7)	113.68(14)	O(2)–P(1)–C(7)	105.97(14)
C(1)–N(1)–C(7)	120.6(2)				

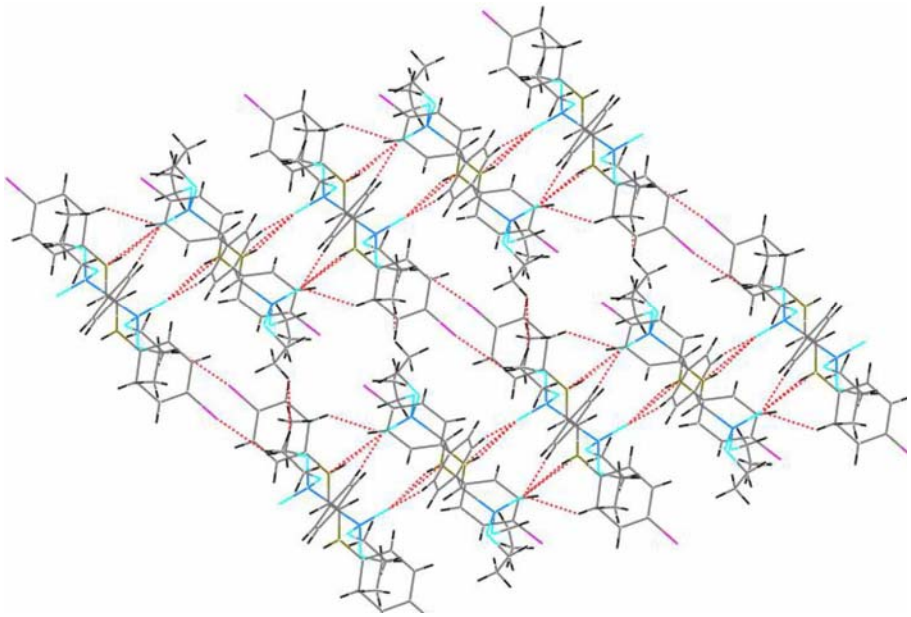

Fig. 2. Hydrogen bonds network for the compound **1**.

From Fig. 2 and Table 3 the intermolecular interactions of compound **1** can be found. Compound **1** molecules are connected by two intermolecular hydrogen bonds in which N1 and C22 are donors and O4 is the acceptor, giving rise to hydrogen-bonded $R_1^2(5)$ rings and a hydrogen-bonded $R_1^2(4)$ ring. The hydrogen bond interactions are unequal. For example, the distance of N1···O4 is 3.000 (3) Å. This interaction is stronger than for the case C22···O4 (the distance is 3.432(4) Å). Besides, the Cl atom also links the dimers through C19-H19A...Cl2 hydrogen bonds, the distance of C19···Cl2 is 3.574(6)Å, and the angle is 147°. Thus, two hydrogen-bonded rings, namely $R_1^2(4)$ and $R_1^2(5)$, and three hydrogen bonds, C19-H19A···Cl2, C27-H27A···O3, and N(2)-H(2A)···O(3), form a complicated hydrogen-bonding network related to the solid crystal structure (Fig. 2).

TABLE 3. Hydrogen Bond Lengths (Å) and Bond Angles (degree) of Compound **1**

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	∠DHA
N(1)–H(1A)···O(4)[(a)]	0.71(4)	2.32(4)	2.999(3)	159(4)
N(2)–H(2A)···O(3)[(b)]	0.80(4)	2.22(4)	2.961(3)	153(4)
C19–H19A···Cl2[(c)]	0.97	2.72	3.574(6)	147
C22–H22···O4	0.93	2.52	3.432(4)	167
C27–H27A···O3[(b)]	0.97	2.55	2.987(7)	108

Symmetry codes: (a) –*x*+2,–*y*+1, –*z*+2; (b) –*x*+1,–*y*+1,–*z*+2; (c) *x*–1, *y*, *z*+1.

DNA-binding studies. An anticancer complex must induce damage to DNA in cancer cells, resulting in the blocking and death of cancer cells [18, 19]. So, the interaction between compound **1** and DNA was studied using the fluorescence spectrum. Ethidium bromide (EB) can be intercalated by adjacent DNA base pairs to give a strong fluorescent light [29]. If another molecule could be inserted in DNA by replacing the EB, the fluorescence intensity would be quenched. As shown in Fig. 3, a significant reduction of the fluorescence intensity at 585 nm (510 nm excitation) with increasing of compound **1** indicates that the EB competes with compound **1** in the DNA binding sites [30]. Thus, the interaction of **1** and DNA is intercalation.

Now we will use the equation $F_0/F = 1 + K_{SV}[Q]$, where F_0 and F are the fluorescence intensity in the absence and in the presence of DNA, respectively; K_{SV} is the Stern–Volmer binding constant; $[Q]$ is the concentration of **1**. The calculated binding constant of **1** and DNA is 5.65×10^4 M^{-1}. The bonding constant is smaller than 10^7 M^{-1} thus a medium bonding effect exists between **1** and DNA [31].

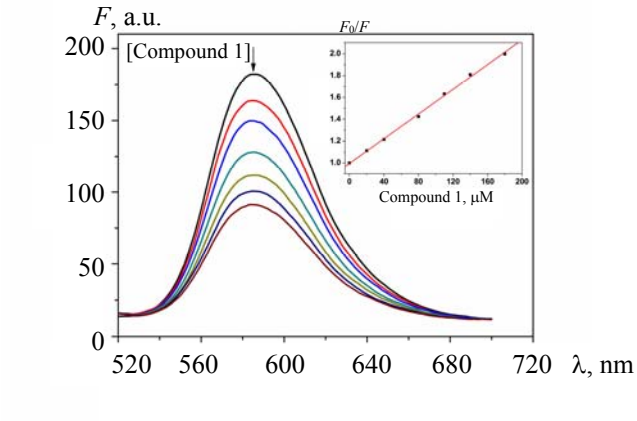

Fig. 3. Emission spectra of EB-CT-DNA in the absence (back line) and in the presence (other lines) of **1** with increasing amounts (0, 2.5, 5.0, 7.5, 10.0, 12.5, 15, 17.5, 22.5, 25.0 μM). Inset: plot of F_0/F versus [compound **1**] showing linearity.

Protein binding studies. Similarly, interaction could occur between complexes and protein (BSA) [23]. From Fig. 4 it is seen that with increasing compound **1**, the fluorescence of BSA is reduced gradually.

The value of K_{SV} is 1.01×10^4 M^{-1}, and the k_q is 1.01×10^4 (10^{12} $M^{-1} \cdot s^{-1}$) [32], as calculated from the slope of the plot of F_0/F vs. [**1**] (this dependence is determined by the equation $F_0/F = 1 + K_q\tau_0[Q] = 1 + K_{SV}[Q]$, where F_0 and F are the fluorescence intensities in the absence and in the presence of **1**, k_q is the quenching rate constant, τ_0 is the average lifetime of the biomolecule without the quencher (about 10^{-8} s), and K_{SV} is the quenching constant). The static quenching mechanism is obtained, because k_q is higher than the maximum static quenching constant [33]. The results are comparable with our previous reports [11, 12, 30].

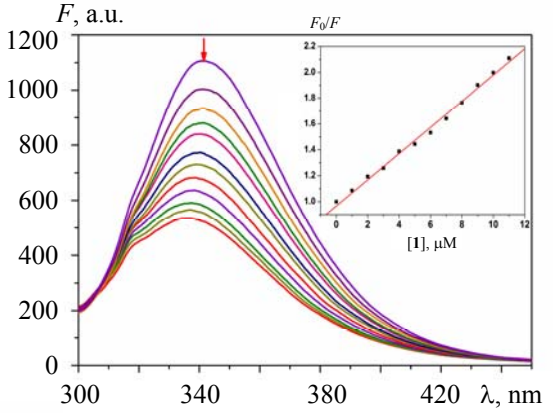

Fig. 4. Fluorescence emission spectra of the BSA (1.0 μM) system in the absence and presence of compound **1** (0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12 μM). Inset: plot of F_0/F versus **1** concentration.

Conclusion. A new α-aminophosphonate (**1**) has been constructed successfully. The X-ray analysis revealed that the dihedral angle between *p*-chloroaniline and terephthalaldehyde is 87.54(0.28)°. The P atoms of compound **1** are located in a tetrahedral geometry, formed by one C_α atom, two O-ethyl groups, and a double-bond O atom. In these structures, C_α atoms are chiral centres responsible for the existence of the optical activity. There are many intramolecular hydrogen bonds that help to stabilize the crystal structure of compound **1**. The interaction between compound **1** and DNA indicated that compound **1** could replace EB from the DNA binding sites with a binding constant of 5.65×10^4 M^{-1}. Further, the binding to BSA has also been investigated. The quenching mechanism of BSA was static, with $K_{SV} = 1.01 \times 10^4$ M^{-1} and a binding ratio of 1:1. The results showed that a good interaction between compound **1** and DNA/BSA was found.

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