Research Article

Structure and Stability of Chemically Modified DNA Bases: Quantum Chemical Calculations on 16 Isomers of Diphosphocytosine

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We studied for the first time 16 tautomers/rotamers of diphosphocytosine by four computational methods. Some of these tautomers/rotamers are isoenergetic although they have different structures. High-level electron correlation MP2 and MP4(SDQ) *ab initio* methods and density functional methods employing a B3LYP and the new M06-2X functional were used to study the structure and relative stability of 16 tautomers/rotamers of diphosphocytosine. The dienol tautomers of diphosphocytosine are shown to be much more stable than the keto-enol and diketo forms. The tautomers/rotamers stability could be ranked as $PC3 = PC12 < PC2 = PC11 < PC1 < PC10 < PC8 < PC9 < PC15 < PC16 < PC6 \sim PC7 < PC13 < PC4 \sim PC14 < PC5$. This stability order was discussed in the light of stereo and electronic factors. Solvation effect has been modeled in a high dielectric solvent, water using the polarized continuum model (PCM). Consideration of the solvent causes some reordering of the relative stability of diphosphocytosine tautomers: $PC3 \sim PC12 \sim PC2 \sim PC11 < PC1 < PC10 < PC8 < PC9 < PC15 \sim PC16 < PC13 < PC6 \sim PC7 \sim PC14 < PC4 < PC5$.

1. Introduction

DNA is a naturally occurring biological macromolecule, containing thousands of nucleic acid bases, and it is of prime importance in genetic determination [1]. The five nucleic acid bases, cytosine, thymine, uracil, adenine, and guanine, found in DNA and RNA control the replication of DNA, store information required to synthesize proteins, and translate this information to the protein. Tautomerism is a well-known phenomenon occurring in nucleic acid bases [2–16], in which proton transfer from the heterocyclic ring center to an exocyclic oxo- or imino- group leads to the formation of either an –OH or an –NH₂ groups. These processes are known as keto-enol or imino-amino tautomerism,

respectively. Tautomerism, a sort of isomerism, plays an important role in organic chemistry, medicinal chemistry, pharmacology, and molecular biology. Tautomerism partially explains the structure of nucleic acids and their mutations [17]. In DNA bases, tautomerization results in altered base pairing configurations or mispairing due to changes in hydrogen-bonding capabilities. DNA mutations are likely to be caused by such alterations. Cytosine is one of the building pyrimidine nucleobases of RNA. A large amount of experimental [18–34] and theoretical work [35–58] has been carried out in order to elucidate the structure of cytosine and its tautomers. Chemically modified bases have attracted extensive interest due to their numerous pharmacological, biochemical, and biological capabilities [59–62]. Replacing

nitrogen in pyrimidine and purine DNA bases by the biological element of interest, phosphorus, may produce new synthesizable bases of special interest. Modification of cytosine base to phosphorus-containing cytosine analogues, diphosphocytosine, is a good start point in this topic. Also, their existence in many tautomeric forms, like other nucleoside bases, seems to be crucial in order to explain the mutation occurring during DNA duplication [63–66]. Searching the literatures confirms that no previous studies include all numbers of cytosine tautomers. This is the first time to include 16 isomers of cytosine. Similar to cytosine, the diphosphocytosine should also have 16 tautomers. There are many interesting questions on the diphosphocytosine tautomers concerning, for example, their relative stability order, energy criteria, proton transfer processes, electronic structure, and chemical properties. In an attempt to answer some of these questions using computational studies, some DFT methods (B3LYP, M06-2X) and *ab initio* MP2 and MP4(SDQ) methods are chosen to study the stability order and tautomerization processes of all the possible 16 diphosphocytosine tautomers in the gas phase as well as in water. Compared to the popular B3LYP method, studies of tautomerism using the recent method M06-2X are very rare and it is worthwhile to consider them here in this work to assess their accuracy compared to MP4(SDQ) data.

Among cytosine tautomers, experimental investigations performed in gas phase, solid state, or in solution indicate that the canonic amino-oxo tautomer of cytosine is the most stable one [67]. One of our interesting computational results [67, 68] of diphosphocytosine tautomers is the reverse stability order as compared to the parent analogue, cytosine. The origin of this stability order needs to be interpreted as it is not fully discussed. One of the main objectives of this work is to investigate this behavior on the basis of the unique bonding properties of the phosphorous atom.

2. Computational Methods

All quantum chemical calculations were performed using the Gaussian 09 suite of programs [69]. Geometry optimizations for all tautomers have been performed using *ab initio* density functional theory (DFT) at the B3LYP functional [70–72] and the new M06-2X functional in conjunction with the 6-31G(d) basis set. For each stationary point, we carried out a force constant harmonic frequency calculation at the same levels to characterize their nature as minima or transition states and to correct energies for zero-point energy and thermal contribution. The frequencies are scaled by a factor of 0.98. The vibrational modes were animated using the ChemCraft program [73]. Natural charges were computed within full Natural Bond Orbital Analysis, using NBO implemented in Gaussian 09 [69]. The vibrational modes were examined by using the ChemCraft program [73]. Partial charge distributions were calculated using the natural population analysis (NPA) method [74]. Solvation effect has been modeled in water using the polarized continuum model (PCM) of Tomasi et al. [75–78] at the B3LYP/6-31+G(d,p) level for all tautomers/rotamers.

3. Results and Discussion

3.1. Geometrical Aspects of Diphosphocytosine Tautomeric Forms. Geometry of the 16 tautomers and rotamers was optimized at four different levels. Optimized structures of the 16 diphosphocytosine tautomers (at MP4(SDQ)/6-31+G(d)) are presented in Figure 1. For the notations of the tautomers and rotamers of diphosphocytosine, we use **PC1**, **PC2**, **PC3**, ... **PC16** for the sixteen forms. All structures with their notations and atomic numbering are shown in Figure 1. Structure geometrical parameters of all the 16 tautomers are listed in Table 1. Bond length changes among tautomers show the same trend as expected from formal bond orders. In tautomers **PC2**, **PC3**, **PC11**, and **PC12**, all CC and CP bond lengths are in the range characteristic for aromatic rings. C1-C2 bond length changes among tautomers depending on the amount of π -bond character the tautomer has. In tautomers **PC2**, **PC3**, **PC11**, and **PC12** this bond distance is about 1.40 Å while in the rest of the tautomers this distance increased as shown in Table 1. In all the dienol forms, the P3-C2 and P3-C4 and also P5-C6 and P5-C4 distances are nearly similar while those for the other keto-enol and diketo forms show considerable differences of about 0.147 Å. This reflects the extent of conjugation and the increasing aromatic character of the six-membered ring. An inspection of the dihedral angles in Table 1 proves that all the tautomers/rotamers structures are nonplanar. It is reported that [79] results of electron correlation calculations (MP2/6-31G*) give nonplanar geometries for several nucleotide bases specifically, for cytosine, which showed a significant pyramidalization of the amino group from MP2/6-31G* calculations. Using enormous computer resources, a complete MP2/6-31G** frequency calculation was carried out [80] for cytosine, confirming that the planar structure is a saddle point. Bond angles in the ring show quite dramatic changes between tautomers and rotamers, by up to more than 10°. Owing to the lack of polarization functions on hydrogens, geometry parameters of the latter are expected to show larger differences, but these less important parameters were not listed in.

In the dienol form of diphosphocytosine, there are three double bonds giving it the six π -electrons needed to gain its aromatic character as in case of benzene. The phosphorous atom has an additional lone pair of electrons but not involved in the conjugated system. Once the keto form is formed in the diphosphocytosine, one resonance form is lost as the phosphorus atom is strongly pyramidal in the keto forms and its lone pairs are no longer available for conjugation. The extent of pyramidalization emerges from the sum of bond angles around the phosphorus atom. An extra destabilization occurs upon forming the second keto tautomer. Accordingly, diketo form is accompanied by destruction of conjugated π -electrons system in the ring of the diphosphocytosine. In case of cytosine, on the other hand, formation of keto or diketo forms still keeps planarity at the nitrogen atoms where they still able to resonate with the adjacent double bonds. Aromaticity is lost in both cytosine and diphosphocytosine, but the number of resonance structures decreased in the case of the latter system.

FIGURE 1: Optimized structures of the 16 PC isomers at MP4(SDQ)/6-31+G(d).

TABLE 1: Computed geometrical data of the 16 diphosphocytosine tautomers at MP4(SDQ)/6-31+G(d).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
R(1,2)	1.469	1.403	1.409	1.472	1.475	1.476	1.479	1.478	1.487	1.364	1.403	1.409	1.477	1.481	1.477	1.481
R(1,6)	1.350	1.39	1.385	1.351	1.351	1.351	1.351	1.349	1.348	1.443	1.390	1.385	1.345	1.345	1.346	1.346
R(1,9)	1.093	1.093	1.094	1.091	1.094	1.091	1.093	1.091	1.093	1.093	1.094	1.094	1.091	1.094	1.091	1.094
R(2,3)	1.741	1.658	1.766	1.863	1.854	1.858	1.847	1.868	1.855	1.834	1.766	1.766	1.859	1.854	1.858	1.851
R(2,7)	1.377	1.399	1.397	1.290	1.290	1.290	1.289	1.290	1.287	1.393	1.399	1.397	1.294	1.294	1.293	1.293
R(3,4)	1.856	1.752	1.755	1.844	1.848	1.834	1.836	1.875	1.877	1.876	1.752	1.756	1.701	1.701	1.705	1.705
R(4,5)	1.867	1.759	1.754	1.426	1.427	1.422	1.418	1.420	1.417	1.420	1.759	1.754	1.836	1.836	1.832	1.833
R(4,8)	1.229	1.382	1.383	1.701	1.702	1.704	1.707	1.871	1.882	1.868	1.382	1.383	1.382	1.387	1.373	1.373
R(5,6)	1.813	1.744	1.746	1.382	1.381	1.374	1.372	1.223	1.224	1.227	1.744	1.746	1.818	1.818	1.821	1.821
R(5,10)	1.420	1.092	1.092	1.831	1.831	1.829	1.829	1.834	1.831	1.708	1.092	1.092	1.426	1.426	1.423	1.423
R(6,11)	1.091	1.016	1.016	1.092	1.092	1.091	1.092	1.421	1.421	1.094	1.016	1.016	1.091	1.091	1.091	1.091
R(7,12)	1.014	1.016	1.016	1.028	1.031	1.028	1.03	1.092	1.092	1.017	1.016	1.016	1.029	1.030	1.029	1.030
R(8,13)	1.015	0.977	0.977	0.976	0.976	0.978	0.978	1.028	1.030	1.016	0.977	0.977	0.976	0.976	0.978	0.978
A(2,1,6)	126.1	125.9	126.0	125.5	125.5	125.7	124.9	127.8	123.7	125.8	125.9	125.9	126.8	126.4	126.2	125.8
A(2,1,9)	115.6	116.6	116.6	114.3	115.3	114.3	115.8	112.9	116.4	117.8	116.6	116.6	113.5	114.9	113.7	114.9
A(6,1,9)	118.3	117.5	117.4	120.2	119.2	119.9	119.2	119.2	119.9	116.4	117.4	117.4	119.7	118.7	120.0	119.2
A(1,2,3)	129.4	126.3	127.5	120.4	121.4	117.5	117.9	119.9	116.9	122.5	126.3	127.5	125.0	125.3	123.2	123.7
A(1,2,7)	112.8	117.6	116.8	118.1	124.6	117.5	124.8	116.6	124.9	122.1	117.6	116.8	115.6	122.4	116.4	123.1
A(3,2,7)	117.9	115.9	115.6	121.4	113.9	124.5	116.9	123.3	117.6	115.3	115.9	115.6	119.2	112.1	120.0	112.9
A(2,3,4)	101.6	103.7	102.5	102.9	102.6	101.6	100.7	100.1	101.0	102.4	103.7	102.6	102.8	101.8	102.9	101.9
A(3,4,5)	119.9	131.9	132.1	96.1	96.0	97.7	97.8	97.6	98.0	98.3	131.9	132.1	130.5	130.9	129.6	130.0
A(3,4,8)	119.3	116.3	110.4	97.2	96.4	97.3	97.7	96.1	95.9	97.3	116.3	110.4	115.3	115.3	121.6	121.5
A(5,4,8)	120.2	111.7	117.5	130.1	131.1	128.2	128.5	114.3	121.4	119.7	111.7	117.5	114.1	113.7	108.6	108.2
A(4,5,6)	100.3	100.7	101.7	113.7	113.2	108.9	109.1	122.4	119.8	119.4	100.6	101.7	102.2	101.8	100.8	100.5
A(4,5,10)	97.9	131.4	130.0	116.2	115.7	122.4	121.9	122.8	118.8	120.6	131.3	130.1	96.7	97.1	96.8	97.3
A(6,5,10)	98.9	115.5	116.2	101.8	101.4	101.7	101.6	99.5	102.3	99.9	115.5	116.2	98.5	97.9	98.9	98.4
A(1,6,5)	124.8	113.1	113.7	131.5	131.5	130.9	130.7	95.9	95.6	134.6	113.1	113.7	126.9	126.4	126.5	126.0
A(1,6,11)	118.4	115.3	115.7	116.6	116.5	116.8	116.9	98.4	98.8	113.3	115.2	115.7	118.7	118.8	118.5	118.7
A(5,6,11)	116.5	116.2	116.3	111.8	111.9	112.0	112.2	130.1	126.2	112.0	116.1	116.3	114.1	114.6	114.5	114.9
A(2,7,12)	118.8	111.6	111.9	111.4	110.4	112.0	110.7	116.9	118.4	115.2	111.6	111.8	111.4	110.6	111.5	110.7
A(4,8,13)	118.5	110.6	109.9	110.3	110.2	110.2	110.4	112.9	114.8	116.5	110.5	109.9	110.9	110.8	110.4	110.2
D(6,1,2,3)	114.4	0.1	-0.1	24.8	23.7	28.9	31.9	112.3	110.7	111.5	-0.1	-0.1	17.9	22.2	24.9	27.4
D(6,1,2,7)	-18.9	176.7	176.7	-159.5	-160.2	-158.8	-155.1	14.6	-45.5	-7.8	-176.7	176.7	-165.5	-161.3	-160.6	-157.9
D(9,1,2,3)	162.8	-179.2	-179.3	-158.3	-158.5	-154.7	-151.5	-169.5	142.2	174.7	179.2	-179.3	-163.1	-158.7	-156.6	-153.7
D(9,1,2,7)	161.9	-2.7	-2.6	17.5	17.5	17.7	21.5	-167.7	135.2	172.3	2.7	-2.6	13.4	17.7	17.9	21.0
D(2,1,6,5)	-16.9	-0.4	-0.5	3.2	1.8	3.8	2.4	8.1	-37.1	-5.2	0.4	-0.5	4.4	3.4	5.9	4.9
D(2,1,6,11)	-5.4	179.9	179.9	177.5	177.5	177.9	177.4	-4.5	-7.9	-11.7	-179.9	179.9	178.3	177.8	177.7	177.5
D(9,1,6,5)	-179.2	178.9	178.8	-173.6	-175.9	-172.4	-174.1	178.8	-178.4	171.8	-178.9	178.7	-174.4	-175.6	-172.5	-173.9
D(9,1,6,11)	174.4	-0.8	-0.8	0.8	-0.1	1.6	0.8	177.9	171.4	168.2	0.8	-0.8	-0.6	-1.2	-0.7	-1.4
D(1,2,3,4)	0.5	0.2	0.5	-29.8	-26.7	-41.3	-43.4	1.3	0.9	-8.3	-0.2	0.5	-15.7	-18.9	-22.2	-23.6
D(7,2,3,4)	3.9	-176.3	-176.3	69.1	71.2	-140.4	-142.9	-39.2	52.8	32.2	176.4	-176.3	167.9	164.3	163.5	161.2
D(1,2,7,12)	-177.9	27.9	28.6	154.5	156.8	146.9	143.0	-136.9	150.4	131.6	-27.9	28.7	-179.2	1.0	-179.0	1.4
D(3,2,7,12)	21.1	161.3	162.9	-106.6	-105.2	47.8	43.6	145.2	-134.3	-150.2	-161.3	162.9	-2.5	177.9	-4.3	176.6
D(2,3,4,5)	167.7	-155.1	-154.3	178.8	-0.9	179.8	-0.2	47.6	-36.6	-50.8	155.1	-154.1	-5.5	-5.8	-6.5	-7.3
D(2,3,4,8)	-157.3	-21.8	-19.9	-5.5	175.4	-8.4	172.9	-179.8	-0.9	17.4	21.8	-19.9	177.3	176.8	179.3	179.3
D(3,4,5,6)	-10.7	-0.3	-0.5	14.9	11.4	33.1	33.2	-4.1	-173.2	150.9	0.3	-0.6	20.2	22.7	26.9	28.6
D(3,4,5,10)	31.3	-179.9	179.9	-165.9	-168.3	-153.9	-153.9	58.7	-18.3	-160.2	179.9	179.8	120.4	122.3	127.4	128.6
D(8,4,5,6)	-157.3	0.2	0.2	-83.1	-86.4	132.5	132.7	-129.5	161.9	-26.8	-0.1	0.2	-162.5	-159.9	-158.3	-157.3
D(8,4,5,10)	-46.8	179.8	179.8	96.0	93.9	-54.5	-54.4	157.6	-117.7	-44.4	-179.8	179.8	-62.3	-60.3	-57.8	-57.3
D(3,4,8,13)	-147.4	-0.9	179.4	3.8	5.5	-10.1	-9.4	-30.6	62.6	142.1	1.0	179.4	158.0	151.8	0.2	0.1
D(5,4,8,13)	141.9	179.3	-0.3	-175.3	-174.8	177.8	178.5	-50.1	-17.0	-144.6	-179.3	-0.3	-19.7	-26.1	-175.1	-174.6
D(4,5,6,1)	41.3	0.3	0.4	18.6	16.7	-173.9	-173.8	-149.6	-117.3	41.9	-0.2	0.4	-20.8	-22.5	-28.4	-28.9
D(4,5,6,11)	34.5	179.9	179.9	-162.2	-163.1	-0.6	-0.4	138.1	162.7	31.2	-179.9	179.9	165.2	162.9	159.7	158.2

TABLE 2: Thermodynamic parameters (kcal/mol)^a for diphosphocytosine tautomers/rotamers at different levels in gas phase.

Species	B3LYP				M06-2X				MP2				MP4(SDQ) ^b	
	ΔE_0	ΔE_0^b	ΔH_{298}	ΔG_{298}	ΔE_0	ΔE_0^b	ΔH_{298}	ΔG_{298}	ΔE_0	ΔE_0^b	ΔH_{298}	ΔG_{298}	ΔE_0	
PC1	3.70	7.01	5.78	4.66	6.84	10.26	8.99	7.83	9.90	13.27	11.61	11.38	7.19	
PC2	0.00	0.62	0.50	0.46	0.00	0.72	0.58	0.50	0.40	0.42	0.42	0.38	0.46	
PC3	0.47	0.00	0.00	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
PC4	24.73	28.43	26.96	25.99	25.98	29.52	28.11	27.05	27.46	31.80	30.46	30.01	24.82	
PC5	24.55	28.53	26.87	25.63	26.02	29.68	28.21	27.01	28.17	32.75	26.78	26.68	25.51	
PC6	22.28	25.73	24.30	23.46	23.58	26.75	25.49	24.67	24.72	28.90	26.94	26.91	22.23	
PC7	22.11	25.64	24.15	23.54	23.14	26.49	25.13	24.44	24.87	29.09	26.94	0.04	22.17	
PC8	9.97	16.68	13.65	12.64	12.76	19.53	16.50	15.40	15.91	23.12	19.50	0.03	13.96	
PC9	12.00	19.21	15.95	14.82	14.16	21.46	18.21	16.73	17.82	25.48	21.66	21.11	15.68	
PC10	5.37	8.90	7.56	6.44	8.83	12.65	11.21	9.95	11.78	15.27	13.56	13.24	9.63	
PC11	0.92	0.48	0.50	0.46	0.967	0.56	0.58	0.50	0.40	0.42	0.42	0.38	0.46	
PC12	0.47	0.00	0.00	0.00	0.400	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
PC13	23.47	27.01	25.57	24.70	24.31	27.87	26.48	25.13	26.13	30.61	28.41	27.86	23.42	
PC14	25.18	29.14	27.50	26.56	25.96	29.81	28.25	27.26	27.68	32.49	30.12	0.00	25.17	
PC15	20.56	23.71	22.39	21.86	21.26	24.50	23.17	22.55	22.98	26.92	24.90	0.00	20.05	
PC16	21.76	25.23	23.75	23.23	22.43	25.76	24.39	23.81	23.96	28.21	26.04	25.99	21.24	

^aCorrected for zero-point energy and thermal energy; ^buncorrected energy.

TABLE 3: NPA atomic charges at MP2/6-31+G(d).

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
C1	-0.327	-0.343	-0.338	-0.267	-0.311	-0.265	-0.308	-0.267	-0.302	-0.443	-0.343	-0.338	-0.227	-0.267	-0.245	-0.283
C2	-0.041	-0.068	-0.077	-0.025	-0.024	-0.017	-0.016	-0.015	-0.033	0.017	-0.068	-0.077	-0.061	-0.064	-0.041	-0.045
P3	0.393	0.503	0.584	0.594	0.657	0.680	0.704	0.580	0.624	0.613	0.503	0.584	0.578	0.668	0.475	0.559
C4	0.029	-0.336	-0.338	-0.352	-0.326	-0.293	-0.268	0.061	0.057	0.033	-0.335	-0.338	-0.322	-0.361	-0.280	-0.314
P5	0.636	0.615	0.531	0.640	0.605	0.517	0.485	0.621	0.625	0.509	0.614	0.531	0.669	0.667	0.713	0.711
C6	-0.468	-0.533	-0.523	-0.555	-0.521	-0.545	-0.514	-0.535	-0.525	-0.515	-0.533	-0.523	-0.559	-0.530	-0.553	-0.525
N7	-0.893	-0.903	-0.904	-0.646	-0.655	-0.683	-0.690	-0.675	-0.663	-0.896	-0.903	-0.904	-0.676	-0.671	-0.677	-0.677
O8	-0.661	-0.774	-0.776	-0.771	-0.771	-0.773	-0.770	-0.628	-0.635	-0.645	-0.774	-0.776	-0.771	-0.775	-0.772	-0.778
H9	0.234	0.232	0.232	0.256	0.237	0.257	0.239	0.262	0.248	0.233	0.232	0.232	0.259	0.239	0.258	0.240
H10	-0.021	0.259	0.261	0.254	0.254	0.257	0.256	-0.019	-0.022	-0.013	0.259	0.261	-0.037	-0.036	-0.029	-0.030
H11	0.261	0.414	0.413	0.373	0.517	0.367	0.522	0.261	0.261	0.262	0.414	0.413	0.261	0.260	0.259	0.258
H12	0.421	0.419	0.421	0.515	-0.025	0.523	-0.006	0.371	-0.003	0.423	0.419	0.421	0.371	0.359	0.370	0.361
H13	0.436	0.513	0.515	-0.016	0.365	-0.026	0.365	-0.017	0.367	0.423	0.513	0.515	0.514	0.511	0.522	0.521

3.2. *Relative Energies and Thermodynamic Parameters of Tautomers.* Since our major objective of this study was to obtain information on the relative stabilities of diphosphocytosine tautomers and rotamers, we tried to go as high as possible, with the level of theory and use both DFT and high correlation *ab initio* MP2 and MP4(SDQ) methods. The results on the relative stabilities of diphosphocytosine tautomers and rotamers using different computational levels are listed in Table 2. For a better view, this is also shown in Figure 2. The results of the four computational methods are consistent with slight differences. The 16 diphosphocytosine tautomers and rotamers could be classified, on the basis of total relative energy, into three groups: (1) low-energy tautomers group containing four tautomers namely, **PC2**, **PC3**, **PC11**, and **PC12**. These tautomers: are those satisfying the aromaticity of the pyrimidine ring (the benzene-like

structure), (2) moderate-energy tautomers group containing also four tautomers, **PC1**, **PC8**, **PC9**, and **PC10**. (3) high energy tautomers group containing the rest eight tautomers, **PC4**, **PC5**, **PC6**, **PC7**, **PC13**, **PC14**, **PC15**, and **PC16**. DFT methods predict **PC2** to be the most stable lowest-energy tautomer. High correlation *ab initio* methods, on the other hand, predict the most stable is the hydroxy-amino form **PC3**. Energy difference is less than 0.50 kcal/mol. The structural difference between the two tautomers/rotamers is the proximity of the positively charged H to the positively charged phosphorus atom. The same argument is applied to **PC11** and **PC12**, where the positively charged H is repulsively interacting with P3 (0.503, Table 3) in **PC11** and P5 (0.531, Table 3) in **PC12**. Previous studies suggest that the canonical amino-oxo structure is the most stable one [81]. **PC5** is the most unstable highest-energy tautomer as predicted by

FIGURE 2: Relative energy of different diphosphocytosine tautomers at different levels.

FIGURE 3: MP2 corrected for zero-point and thermal energy versus uncorrected one for all the 16 diphosphocytosine tautomers.

all methods. This could be due to the destruction of the aromaticity and also attributed to the repulsive interaction of the positively charged H and the great positive charge accommodated on P3 (0.657, Table 3). As could be seen from Table 2 that including zero-point energy and thermal energy correction has a considerable effect on the molecular energy. The corrected energy is lower than the uncorrected by about 4.0 kcal/mol. Also, considering these corrections causes reordering of the tautomers stability. Figure 3 shows the MP2-corrected for zero-point and thermal energy versus uncorrected one for all the 16 diphosphocytosine tautomers. Based on the corrected energy, both B3LYP and M06-2X have

FIGURE 4: Tautomers' order by DFT and MP2 methods.

TABLE 4: Thermodynamic parameters for different tautomers (kcal/mol) at B3LYP/6-31+G(d) in water.

	ΔE_0^a	ΔE_0^b	ΔH_{298}	ΔG_{298}
PC1	3.531	0.658	2.021	22.773
PC2	0.177	0.122	0.137	0.275
PC3	0.000	0.000	0.000	0.000
PC4	27.936	23.999	25.904	25.843
PC5	28.035	23.887	25.919	25.612
PC6	26.048	22.526	24.159	24.496
PC7	26.193	22.510	24.229	24.550
PC8	16.902	10.067	13.404	13.409
PC9	19.178	11.994	15.528	15.411
PC10	5.515	2.203	3.824	3.970
PC11	0.177	0.126	0.137	0.287
PC12	0.000	0.000	0.000	0.000
PC13	25.953	22.167	23.990	24.103
PC14	26.648	22.727	24.617	24.804
PC15	24.025	20.722	22.223	22.721
PC16	24.613	21.245	22.773	23.353

^aUncorrected energy; ^bcorrected for zero-point and thermal energy.

the same order as $PC2 < PC3 = PC12 < PC11 < PC1 < PC10 < PC8 < PC9 < PC15 < PC16 < PC7 < PC6 < PC13 < PC5 \sim PC4 \sim PC14$. On the other hand, the relative stability order as predicted by *ab initio* Moller Plesset MP2 and MP4(SDQ) is $PC3 = PC12 < PC2 = PC11 < PC1 < PC10 < PC8 < PC9 < PC15 < PC16 < PC6 \sim PC7 < PC13 < PC4 \sim PC14 < PC5$. However, MP4(SDQ) method shows slight differences like $PC15 \sim PC16$ and $PC4 < PC14 \sim PC5$. Figure 4 presents tautomers' order by DFT and MP2 methods. Enthalpy and free energy are consistent with the total energy as can be detected from Table 2. One of the electronic factors that affect the stability of the tautomers is the repulsion between the neighboring -OH and -PH or -CH groups, while the

TABLE 5: NPA atomic charges at B3lyp/6-31+G(d)//B3lyp/6-31+G(d) PCM.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
C1	-0.298	-0.316	-0.297	-0.385	-0.302	-0.263	-0.297	-0.269	-0.303	-0.367	-0.308	-0.296	-0.238	-0.271	-0.251	-0.282
C2	-0.147	-0.106	-0.152	-0.057	-0.052	-0.056	-0.057	-0.049	-0.061	-0.061	-0.126	-0.151	-0.096	-0.088	-0.067	-0.073
P3	0.441	0.462	0.591	0.530	0.577	0.614	0.648	0.516	0.563	0.539	0.484	0.591	0.571	0.655	0.474	0.556
C4	-0.066	-0.375	-0.403	-0.361	-0.369	-0.348	-0.338	-0.007	-0.011	-0.033	-0.383	-0.403	-0.374	-0.402	-0.347	-0.369
P5	0.591	0.585	0.545	0.627	0.597	0.513	0.498	0.561	0.564	0.534	0.605	0.544	0.603	0.589	0.647	0.645
C6	-0.495	-0.559	-0.568	-0.158	-0.526	-0.536	-0.521	-0.515	-0.517	-0.587	-0.567	-0.568	-0.535	-0.515	-0.530	-0.516
N7	-0.819	-0.835	-0.846	-0.585	-0.616	-0.636	-0.645	-0.629	-0.620	-0.836	-0.840	-0.845	-0.631	-0.630	-0.633	-0.635
O8	-0.562	-0.702	-0.706	-0.662	-0.692	-0.697	-0.693	-0.535	-0.539	-0.545	-0.698	-0.705	-0.698	-0.707	-0.699	-0.704
H9	0.237	0.232	0.236	0.293	0.243	0.262	0.245	0.268	0.253	0.238	0.232	0.235	0.265	0.244	0.263	0.245
H10	0.010	0.263	0.267	-0.157	0.261	0.264	0.263	0.013	0.011	0.018	0.262	0.2665	-0.003	0.001	0.004	0.003
H11	0.266	0.420	0.412	0.379	0.508	0.363	0.511	0.267	0.266	0.266	0.415	0.411	0.266	0.264	0.264	0.263
H12	0.415	0.428	0.417	0.507	0.011	0.512	0.025	0.366	0.029	0.415	0.421	0.417	0.365	0.355	0.365	0.357
H13	0.427	0.504	0.506	0.029	0.361	0.006	0.361	0.012	0.363	0.418	0.502	0.506	0.506	0.504	0.510	0.511

other factor is the attraction between the O–H or P–H bonds and the lone pair of the neighboring phosphorous or oxygen atoms. Another factor is the attractive interaction between the O–H or P–H bonds, on one side, and the lone pair(s) of the adjacent P or O atoms, on the other one [82].

The amino N accommodates the highest negative charge (up to -0.904); then the O atom comes in the second place (up to -0.778). Positive charges are accommodated on the P and H atoms. Very little negative charge on H bonded to P atoms as appeared in **PC4**, **PC6**, and **PC8**. The values of the NPA charges at hydrogen atoms of –OH groups are larger than the values at the hydrogen atom of the –CH group (see Table 3). As a result, the repulsion between the neighboring –OH and –CH groups is stronger than the repulsion of the neighboring –PH and –CH groups.

3.3. Solvation Study. One of the most crucial factors determining the tautomer distribution in the biological media is the environment. Solvent preferentially stabilizes structures with localized charges. Solvation effect has been explored in water ($\epsilon = 78.54$) using the polarized continuum model (PCM) at the B3LYP/6-31+G(d) level for all the 16 tautomers. Table 4 collects the relative energies for different tautomers/rotamers (kcal/mol) at B3LYP/6-31+G(d) in water. In water, the most stable diphosphocytosine tautomers are **PC3** and **PC12** and the most unstable tautomers/rotamers are **PC4** and **PC5**. As seen from Table 4, the relative stabilities in water go in the order **PC3** ~ **PC12** ~ **PC2** ~ **PC11** < **PC1** < **PC10** < **PC8** < **PC9** < **PC15** ~ **PC16** < **PC13** < **PC6** ~ **PC7** ~ **PC14** < **PC4** ~ **PC5** at B3LYP/6-31+G(d) (PCM/water). This order is slightly different from that predicted at the same level in gas phase ($\epsilon = 1$). For example, **PC3** ~ **PC12** ~ **PC2** ~ **PC11**. This result is easily understandable on the basis of the calculated dipole moment of these tautomers. Polar tautomers/rotamers are favored in polar solvents.

The NPA charges computed for tautomers/rotamers geometry optimized in water are given in Table 5.

The NPA charges computed for tautomers/rotamers geometry optimized in water are generally smaller than those computed for tautomers/rotamers optimized in gas

phase. This is understandable as a role of the high dielectric constant solvent, water. The amino N accommodates the highest negative charge (up to -0.846), then the O atom comes in the second place (up to -0.707). Positive charges are accommodated on the P and H atoms. Very little negative charge on H bonded to P atoms as appeared in **PC4**, **PC6**, and **PC8**. The values of the NPA charges at hydrogen atoms of –OH groups are larger than the values at the hydrogen atom of the –CH group (see Table 5). As a result, the repulsion between the neighboring –OH and –CH groups is stronger than the repulsion of the neighboring –PH and –CH groups.

4. Summary

On the basis of the present theoretical study, we may confirm the following conclusions.

- (i) We studied for the first time 16 tautomers/rotamers of diphosphocytosine by four computational methods. Some of these tautomers/rotamers are isoenergetic although they have different structure.
- (ii) From the total energy point of view, the 16 tautomers/rotamers are categorized into three main groups. These are low-energy difference group, moderate-energy difference group, and high-energy difference group.
- (iii) **PC3** and **PC12** are the most stable tautomers/rotamers.
- (iv) Our systematic calculations show that the energy difference between components of a rotamer pair is very stable, independent of the level of theory. This leads to the important conclusion that besides the dominant **PC3** form, its pair **PC2** may lie not higher than $\sim 0.4 \text{ kcal mol}^{-1}$; therefore, any experimental study should consider this possibility when evaluating spectroscopic experimental studies.
- (v) Benzene-like structures are the most stable tautomers/rotamers.

- (vi) Charge is distributed on the ring atoms; –ve charges are on N and O, while +ve charges on P and H.
- (vii) Solvation does not affect the order significantly; **PC3 ~ PC12 ~ PC2 ~ PC11 < PC1 < PC10 < PC8 < PC9 < PC15 ~ PC16 < PC13 < PC6 ~ PC7 ~ PC14 < PC4 ~ PC5.**

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