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Quantum mechanical calculations of the cephalosporin nucleus

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ABSTRACT

A computational study using several ab initio methods, HF, DFT and MP2 at different basis set levels (6-31G*, 6-31G**, 6-311G** and 6-311++G**) has been performed to determine the possible stable conformations attained by the cephalosporin nucleus. The calculations were carried out in three stages by studying the conformational analysis of the 3-cephem nucleus, the 3-cephem-4-carboxylic acid nucleus and the acetylamino group of the 7-acetylamino-3cephem-4-carboxylic acid. In the first two stages, the potential energy surfaces indicated two minima that correspond to the S1-up and C2-up conformations, with the S1-up being more stable. The energy required for the interconversion of the S1-up to the C2-up is around 7 kcal/mol, indicating the feasibility of interconversion between the two conformers. In the third stage, the acetylamino group attained two conformations with respect to the 3-cephem nucleus. All the geometric parameters obtained in this study were found to be in reasonably good agreement with available X-ray diffraction data, even upon using a simple basis set.

1. Introduction

Since the discovery of β-lactam antibiotics, the study of β-lactam systems has increased spectacularly due to their powerful effect against a wide variety of bacterial infections. Penicillins and cephalosporins constitute the most representative group of β-lactam antibiotics. Their mechanism of action relies on the inhibition of a group of enzymes named penicillin-binding proteins (PBP). These enzymes are essential to complete bacterial cell wall synthesis by a cross-linking of peptidoglycan chains [1]. When the biosynthesis of the bacterial cell walls is interrupted, the wall is weakened, becomes permeable to water, and the cell swells, bursts and dies. However, bacteria protect themselves against antibiotics primarily via the production of β-lactamases, which catalyze the hydrolysis of β-lactam antibiotics into harmless products [2]. As the number of bacterial strains acquiring resistance to the current antibiotic drugs grows, efforts are devoted to the development and the discovery of new antibiotics capable of overcoming such resistance. Those efforts focus on the quantitative structure activity analysis of these antibiotics [3–12], their biosynthesis [13] and the mechanism of the alkaline hydrolysis of β-lactam antibiotics [2c,14]. However, for developing and designing a new series of penicillins and cephalosporins, deep knowledge of the β-lactam, the thiazolidine and the dihydrothiazine rings is essential.

Molecular orbital calculations provide a complementary way for studying molecular systems that acquire different conformational structures. In the last years, several theoretical studies employing semiempirical [8,15–17], molecular mechanics [18] and ab initio [3–7,19] methods have been devoted to studying the structure of β-lactams. However, while many of these studies have been devoted to the study of the monocyclic β-lactam ring [19] and of the penicillin nucleus [3,17b], comparatively few have been devoted to the study of cephalosporin nucleus [17a].

The aim of this work is to give a thorough and unified description of cephalosporin nucleus, its conformational variations and factors that affect the stability of the cephalosporin nucleus. Ab initio methods, HF, DFT and MP2 were employed at different basis set levels to deduce the most appropriate and the least expensive ab initio method; that could be employed in the study of β-lactam systems.

Cephalosporin's basic bicyclic structure consists of a four membered β-lactam nucleus fused with a dihydrothiazine nucleus. Fig. 1 represents a typical cephalosporin with the numbering employed in this study. The pyramidal character of the β-lactam nitrogen atom and the tetrahedral character of C6 necessitate the puckering of the cephalosporin molecule at the fusion site. Moreover, early crystallographic measurements on the sodium salt of cephalosporin C indicated that the C2, C3, C4 and N5 atoms lie nearly in a plane within the limits of experimental error, with the sulphur atom of about 0.61 Å above the plane and C6 0.61 Å below it [20]. This results in a pronounced puckering in the dihydrothiazine nucleus. X-ray analysis of cephalosporin compounds indicated that the extent of the overall puckering depends on the nature of the amide group on C7, substituent on C3 and molecular packing in the crystal lattice that differs according to the

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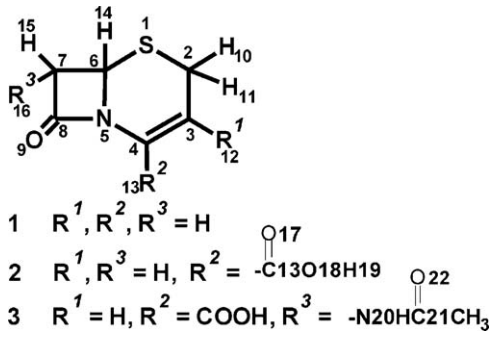

Fig. 1. Atomic numbering employed in the study.

chelating agent. With respect to cephalosporin's structure activity analysis, reported studies revealed that the reactive β-lactam ring is the important structure moiety for their antimicrobial activity. This is attributed to the strain attained by the ring; lack of resonance of the amide endocyclic system caused by the pronounced pyramidal character of the β-lactam nitrogen atom [21], and possibilities for electron delocalization outside the lactam ring [22].

In the present study, due to the complex nature of cephalosporins, the conformational analysis of cephalosporins proceeded in several stages. In the first stage, the study focused on the conformational characteristics of the basic bicyclic unit of the cephalosporin system, i.e. 3-cephem nucleus 1, where substituents at C3, C7 as well as the carboxylic group are replaced by hydrogen atoms. In the second stage of the conformational analysis, the carboxylic group was introduced to the system 2. In the final stage, the different orientations of the acylamino side chain in 3 with respect to the basic unit were studied.

2. Computational method

All calculations in this study were carried out employing the Gaussian98 program, Revision A.9 package [23]. The methods employed included HF, B3LYP and MP2 using the 6-31G*, 6-31G**, 6-311G** and 6-311++G** basis sets. The conformational analysis was carried out in three stages. First, by carrying out a series of partial optimizations constraining the concerned dihedral angle step by step within the appropriate range, with a step size of 10°. Next, the geometries of the located minima were optimized at the corresponding level of calculations. Finally, by locating a transition state, which connects the located minima, and then characterizing the transition state by vibrational frequency evaluations at the appropriate levels of calculations. HF/6-31+G* model was employed at certain stages of the study.

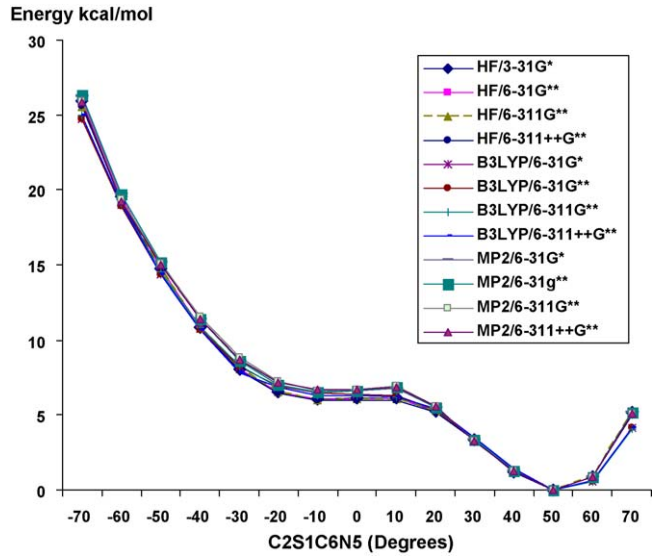

Fig. 2. Conformational analysis curves for the dihydrothiazine ring in 1, representing the energies relative to the lowest energy conformer found by each model chemistry.

3. Results and discussion

3.1. Conformational analysis of 3-cephem nucleus 1

Crystallographic studies indicated that the highly rigid and puckered bicyclic system of cephalosporins could take two different conformations [24]. These are, the "Sl-up" conformation, in which, the sulphur atom lies above the plane formed by the other atoms in the ring; and the "C2-up" conformation, with its C2 atom above the plane formed by the other atoms in the ring. In the crystalline state, cephalosporins occur mostly in the Sl-up conformation [24]. In the present work, the interconversion of the cephem nucleus 1 between these conformations is investigated using the dihedral angle C2S1C6N5 – that determines the C2 relative position with respect to the sulphur atom – as a reaction coordinate. The angle is varied from −70° to 70°, with a step of 10° and the geometry is completely optimized throughout this reaction path except for the C2S1C6N5 dihedral angle.

Regardless of the method used or the basis set employed in the calculation, the 3-cephem 1 molecule follows a similar potential energy profile, on which, two energy minima are located (Fig. 2). These minima are in accordance with the reported crystallographic results and correspond to the S1-up 1a and C2-up 1b conformations, where the S1-up being the more stable conformer (*cf.* Fig. 3).

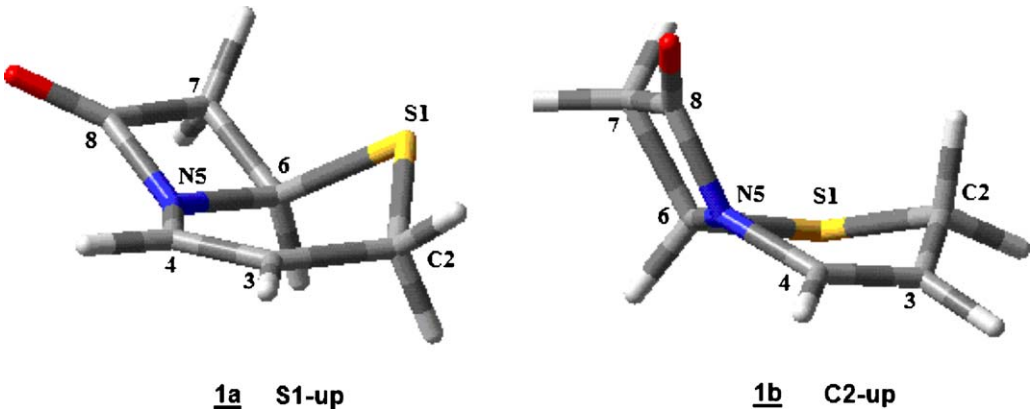

Fig. 3. The S1-up and C2-up conformations of 1 at the B3LYP/6-311++G** level.

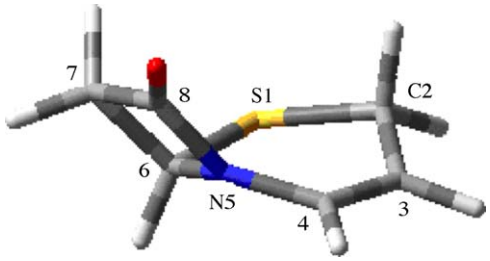

Fig. 4. Transition state TS for the interconversion between S1-up and C2-up conformers of 1 at the B3LYP/6-311++G** level.

At the corresponding level of calculations, the located minima are fully optimized with no geometrical constrains and the transition state that connects the S1-up and the C2-up conformers is located and characterized by vibrational frequency evaluations (*cf.* Fig. 4). The optimized value of the dihedral angle C2S1C6N5 for the S1-up and C2-up is greatly dependent on the theoretical method used, it ranges between 51° and 55° and between −4° and −15°, respectively. The transition state is located around 6.2°, 3° and 16° for HF, B3LYP and MP2, respectively. Depending on the calculation method employed, **ΔE**, the energy difference between the S1-up and C2-up conformers ranges from 5.63 to 6.46 kcal/mol. In addition, the energy barriers $\mathbf{E}^{\neq}$ between the most stable conformer – S1up – and the transition state are around 6.2, 6.4 and 7.3 kcal/mol for HF, B3LYP and MP2, respectively (*cf.* Table 1). On the other hand, the reverse energy barriers $\mathbf{E_r}$ between the less stable conformer – C2 up – and the transition state do not exceed 0.14, 0.03 and 1.73 kcal/mol for HF, B3LYP and MP2, respectively (*cf.* Table 1). These values agree with the fact that, in the crystalline state, cephalosporins occur mostly in the S1-up conformation [24] and with the readiness of the interconversion between the two conformations. Reported molecular mechanics calculations [18], CNDO, AM1 and MNDO semiempirical calculations [15,17a] indicated similar behaviour, however, PM3 predicted the C2-up conformer to be more stable than the S1-up conformer by about 0.55 kcal/mol [16,17b]; and MINDO/3 method only detected one minimum corresponding to the S1-up conformation [17a].

Tables 2–4 present some significant bond lengths, bond angles and dihedral angles predicted for 1 by HF, B3LYP and MP2 at the smallest and the largest basis sets employed in this study [25] as

Table 1
ΔE and $\mathbf{E}^{\#}$ activation energy (in kcal/mol) involved in the interconversion process of 1 and 2.

Method	Basis set	1			2		
		ΔE = S1 − C2	$\mathbf{E}^{\#}$	$\mathbf{E_r}$	**ΔE** = S1 − C2	$\mathbf{E}^{\#}$	$\mathbf{E_r}$
HF	6-31G*	6.07	6.20	0.13	3.09	3.77	0.68
	6-31G**	6.07	6.20	0.14	3.13	3.79	0.67
	6-311G**	6.01	6.12	0.11	3.03	3.69	0.65
	6-311++G**	5.99	6.09	0.10	2.85	3.56	0.72
B3LYP	6-31G*	6.46	6.47	0.02	3.84	4.22	0.37
	6-31G**	6.46	6.48	0.03	3.89	4.25	0.36
	6-311G**	6.35	6.37	0.03	3.61	4.03	0.42
	6-311++G**	6.28	6.31	0.03	3.33	3.86	0.52
MP2	6-31G*	5.90	7.28	1.38	4.06	5.78	1.72
	6-31G**	5.84	7.23	1.39	4.08	5.78	1.69
	6-311G**	5.63	7.12	1.49	3.90	5.74	1.84
	6-311++G**	5.64	7.36	1.72	3.55	5.71	2.16
Semiempirical [17]	AM1		2.00			2.00	
	MNDO		0.30			0.30	
	PM3		1.57			1.57	

*Refers to the inclusion of "d" and/or "p" functions to the basis sets.

Table 2
Optimized bond length (in Å) predicted at the specified level and X-ray reported values for 3-cephem 1.

	HF				B3LYP				MP2				Semiempirical[a]	X-ray
	6-31G*		6-311++G**		6-31G*		6-311++G**		6-31G*		6-311++G**			
	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1/C2-UP	S1/C2-UP
S1C2	1.828	1.833	1.827	1.833	1.855	1.863	1.853	1.861	1.830	1.837	1.827	1.835	1.829/1.821	1.82/1.79[b]
S1C6	1.812	1.823	1.812	1.824	1.835	1.844	1.833	1.842	1.812	1.819	1.809	1.818	1.822/1.815	1.79/1.83[b]
C2C3	1.509	1.507	1.509	1.506	1.507	1.504	1.505	1.502	1.503	1.497	1.505	1.499	1.489/1.490	1.51/1.50[c]
C3C4	1.321	1.318	1.322	1.318	1.342	1.338	1.339	1.335	1.346	1.344	1.350	1.347	1.353/1.352	1.32/1.29[b]
C4N5	1.392	1.409	1.393	1.410	1.391	1.408	1.392	1.408	1.394	1.412	1.395	1.413	1.428/1.436	1.41/1.41[b]
N5C6	1.449	1.458	1.450	1.457	1.463	1.473	1.463	1.473	1.462	1.482	1.465	1.483	1.512/1.513	1.51/1.48[b] 1.45[d]
C6C7	1.546	1.547	1.546	1.548	1.552	1.555	1.550	1.554	1.545	1.546	1.548	1.550	1.571/1.576	1.59/1.60[c] 1.566[d]
C7C8	1.529	1.528	1.529	1.527	1.546	1.544	1.542	1.541	1.537	1.535	1.540	1.538	1.551/1.550	1.57/1.53[b] 1.52/1.52[c]
C8N5	1.377	1.376	1.377	1.376	1.399	1.398	1.397	1.395	1.401	1.402	1.406	1.403	1.479/1.474	1.35/1.43[b] 1.40/1.38[c] 1.382[d]
C8O9	1.182	1.183	1.177	1.178	1.205	1.205	1.199	1.200	1.213	1.214	1.205	1.206	1.195/1.196	1.21/1.25[b] 1.18/1.21[c]

[a] PM3 calculations on cephalotin [17b].
[b] Cephalotin sodium salt [24f].
[c] 3((1-Methyl-lH-tetrazolyl)thiomethyl)-7-(thien-2-ylacetamido)-3-cephem-2-carboxylic acid [24d].
[d] 4-Acetyl-3-methyl-7p-phenoxyacetamido-Δ^3-cephem [24i].

Table 3
Optimized bond angles (in°) predicted at the specified level and X-ray reported values for 3-cephem 1.

	HF				B3LYP				MP2				Semiempirical[a]	X-ray
	6-31G*		6-311++G**		6-31G*		6-311++G**		6-31G*		6-311++G**			
	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1/C2-UP	S1/C2-UP
C6S1C2	95.85	100.24	95.99	100.44	94.61	99.41	94.55	99.43	93.94	96.98	93.72	96.58	98.7/101.4	92/93[b] 95/93[c]
S1C2C3	113.91	114.18	113.80	114.21	114.17	114.77	114.13	114.80	113.34	111.63	112.97	111.26		
C2C3C4	124.93	121.55	125.04	121.75	124.87	121.43	124.90	121.51	124.28	119.23	124.06	118.87	124.4/122	123/127[b] 124/124[c]
C3C4N5	121.85	118.82	121.83	118.79	121.56	118.42	121.47	118.25	121.02	116.70	121.05	116.33		
C4N5C6	125.80	126.51	125.77	126.49	125.99	126.68	125.96	126.62	125.37	125.62	125.00	125.07	123.9/122.9	123/127[b] 125.8[d]
N5C6S1	111.93	114.03	111.80	113.93	111.79	114.18	111.62	114.03	111.87	113.67	111.79	113.80	1.512/1.513	
N5C6C7	88.05	87.84	88.08	87.85	88.49	88.17	88.42	88.06	88.57	88.24	88.74	88.29	1.571/1.576	
C6C7C8	85.33	85.50	85.31	85.46	85.67	85.86	85.72	85.88	85.85	86.12	85.62	85.93	87.6/87.5	84/87[b] 86/83[c]
C7C8N5	91.44	91.67	91.49	91.72	91.06	91.35	91.17	91.45	91.12	91.67	91.29	91.73	90.6/90.8	93/89[b] 92/95[c]
C7C6S1	117.40	120.32	117.09	120.16	118.06	121.15	117.89	121.09	116.68	119.69	115.84	119.07		
C7C8O9	136.61	136.49	136.43	136.35	137.15	136.95	136.83	136.73	137.05	136.77	136.82	136.68		
C8N5C6	95.02	94.87	94.91	94.84	94.77	94.62	94.67	94.60	94.24	93.64	93.91	93.58	92.5/92.8	97/95[b] 95/93[c]
C8N5C4	131.79	131.39	131.66	131.53	133.83	134.13	133.86	134.40	131.94	130.73	131.12	131.17	126/125	131.2[d] 132.6[d]
O9C8N5	131.95	131.82	132.06	131.90	131.79	131.69	131.98	131.81	131.81	131.55	131.88	131.57	130.8/130.3	134/131[b] 130/129[c]

[a] PM3 calculations on cephalotin [17b].
[b] Cephalotin sodium salt [24f].
[c] 3((1-Methyl-lH-tetrazolyl)thiomethyl)-7-(thien-2-ylacetamido)-3-cephem-2-carboxylic acid [24d].
[d] 4-Acetyl-3-methyl-7p-phenoxyacetamido-Δ^3-cephem [24i].

well as reported crystallographic [18,24,26,27] and semiempirical values [17]. In accordance with X-ray results, some of the predicted values are sensitive to the orientation adopted by the dihydrothiazine ring. Thus, Table 2 reveals that, regardless of the model chemistry used, C2-up conformer has slightly longer S1C2 and S1C6 bond lengths than the S1-up conformer does. In addition, all bond angles including the S1 and C2 atoms or the double bond are affected by the conformation adopted by the dihydrothiazine ring. Thus, the C2-up conformation has wider C6SlC2, N5C6S1, C7C6S1, S1C2H11 and C2C3H12 bond angles than does the S1-up conformation, while; the S1-up conformer has wider C2C3C4 and C3C4N5 bond angles. On the other hand, the C4N5C8, C4N5C6 and C6N5C8 bond angles, which are related to the pyramidal character of the β-lactam nitrogen atom, are found to be independent of the conformation attained by the dihydrothiazine ring. With regard to dihedral angles, those involve S1 and C2 atoms show great sensitivity to the conformation of the dihydrothiazine nucleus (*cf.* Table 4). These include: the C2S1C6N5 dihedral angle; C2S1C6C7, C8N5C6C2 dihedral angles, which determine the dihydrothiazine ring orientation with respect to the β-lactam ring and the degree of puckering at the fusion site and S1C6N5C4 dihedral angle that determines the degree of puckering exerted by the sulphur atom on the dihydrothiazine ring. In addition, the C3C4N5C8 and H13C4N5C8 dihedral angles are of great importance and are sensitive to the dihydrothiazine nucleus' conformation. The C3C4N5C8 dihedral angle determines the degree of resonance achieved by the endocyclic amide system through the dihydrothiazine nucleus and accounts for the stability of the S1-up conformer. Values predicted for the S1-up conformer range between −137.55° and −143.50° depending on the level of calculation, while those for C2-up conformer range between −92.12° and −111.21°. These values indicate higher possibility for the amide resonance in the case of S1-up than for the C2-up conformer. The importance of the H13C4N5C8 dihedral angle is

Table 4
Optimized dihedral angles (in°) predicted at the specified level and X-ray reported values for 3-cephem 1.

	HF				B3LYP				MP2				Semiempirical[a]	X-ray
	6-31G*		6-311++G**		6-31G*		6-311++G**		6-31G*		6-311++G**			
	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1/C2-UP	S1/C2-UP
C2SlC6N5	51.74	−7.04	51.83	−6.32	52.65	−4.37	52.90	−3.88	54.76	−15.82	55.70	−15.55	38.4/−14.6	54.5/−9.8[b]
C2S1C6C7	151.38	95.26	151.27	95.82	153.07	98.96	153.05	99.16	154.63	86.37	155.34	86.46		154.60[c]
C8N5C6C2	147.71	115.10	147.06	115.29	151.90	120.27	151.60	120.44	148.86	106.40	146.98	105.16		146.40[d]
S1C6N5C4	−37.05	−32.91	−37.21	−33.41	−38.00	−35.41	−38.26	−36.07	−36.36	−30.89	−36.24	−32.26		
C3C4N5C8	−138.38	−103.58	−137.55	−103.80	−143.50	−110.94	−142.91	−111.21	−138.87	−93.55	−136.43	−92.12		138.80[e]
H13C4N5C8	41.60	78.70	42.29	78.23	35.42	71.89	35.79	71.37	40.08	90.69	42.31	92.01	59.1/88.6	61.9/80.9[b]
C4N5C8C7	153.31	153.25	153.12	153.50	154.74	156.33	154.69	156.76	151.56	150.14	150.59	150.34	141.9/139.6	152.72[e]
C4N5C8O9	−25.55	−25.25	−25.67	−25.03	−24.33	−22.74	−24.20	−22.15	−27.59	−28.53	−28.45	−28.25		−23.80[d]
C6N5C8O9	−175.55	−175.82	−175.20	−175.76	−178.18	−178.72	−177.78	−178.56	−175.47	−174.12	−173.80	−173.28		−174.46[d]

[a] PM3 calculations on cephalotin [17b].
[b] (3*R*)-3-(3-methyl-7-phenoxyacetamido-3-cephem-4-yl)-3-hydroxybutanoicacid [16].
[c] Cephradine bis(dimethylformamide) clathrate [26].
[d] bis(Cephradine) 4-hydroxybenzoic acid tetrahydrate [26].
[e] bis(Cefadroxil)-β-naphthol octahydrate clathrate [27].

that, it defines the relative orientation of the expected carboxylic group in cephalosporins. Apart from the aforementioned dihedral angles, other dihedral angles of significant importance do not show sensitivity to the conformation of the dihydrothiazine ring. Among these is the C7C8-N5C4 dihedral angle, which is related to the pyramidal character of the β-lactam nitrogen atom.

Regarding the effect of the model chemistry employed on the predicted parameters, our results show that, for the same calculation method, an improvement of the basis set used does not markedly influence either the trend or the predicted values. However, changing the theoretical method has significantly changed the predicted values of the dihedral angles, especially upon inclusion of electronic correlation effect at the MP2 level. This effect is more pronounced for the C2 conformation. Thus, a difference of 10° is encountered between values predicted for the dihedral angle C2S1C6C7 on going from HF to MP2 method and of 15° between values predicted for the dihedral angle C8N5C6C2 on going from the B3LYP and MP2 methods.

Generally, in comparison with the available X-ray diffraction data, the predicted structural parameters for the 3-cephem nucleus 1 are in good agreement with the experimental values [24,26–28] even with the smallest basis set employed in this study. Previously reported values of the semiempirical methods PM3, AM1, and MNDO [17] are far from the experimental values.

The pyramidal character of the β-lactam nitrogen atom is expressed by **h**, which is the distance between the nitrogen atom of the lactam ring and the plane formed by the three carbon atoms bonded to it. Cephalosporins typically have **h** values between 0.19 and 0.24 Å [28]. In agreement with experimental values, HF method predicts values of 0.22 and 0.21 Å for the S1-up and C2-up, respectively. B3LYP method predicts slightly weaker pyramidal character for C2-up conformer; **h** values are 0.18–0.19 Å for the S1-up and 0.16–0.17 Å for the C2-up. On the other hand, MP2 predicts **h** values that range between 0.23 and 0.25 Å for the S1-up and comparatively higher values 0.26–0.28 Å for the C2-up conformer. Reported PM3 overestimated the pyramidal character of the β-lactam nitrogen, where **h** is equal to 0.33–0.36 Å for the Sl-up form and 0.37–0.38 Å for the C2-up form, while MINDO/3 greatly underestimated the pyramidality; **h** equals to 0.07 Å [17]. On the other hand, values that range between 0.23–0.25 Å and 0.27–0.32 Å are predicted by MNDO and AM1, respectively.

3.2. Conformational analysis of the dihydrothiazine ring of 3-cephem-4-carboxylic acid 2

The conformational analysis of the dihydrothiazine ring of 3-cephem-4-carboxylic acid 2 is performed taking the dihedral angle C2S1C6N5 as a reaction coordinate. The employed methods included HF, DFT and MP2 methods at the 6-31G*, 6-31G**, 6-311G** and 6-311++G** levels of calculation. Two minima that correspond to the S1-up and C2-up conformations were located at all calculation levels. The optimized values of C2S1C6N5 dihedral angle predicted for the S1-up and the C2-up conformations range between 50.78–55.87° and −8.58° to −16.91°, respectively. The corresponding X-ray diffraction values for cephalotin are 54.5° and −8.9°/−9.8° for the S1-up and C2-up, respectively [29]. In both conformers, the carboxylic group is oriented with the carbonyl oxygen facing the H12 atom on C3, with O17–H12 bond distance around 2.5 Å, thus stabilizing both conformers. The energy gap **ΔE** between the two conformers ranges from 2.85 to 4.08 kcal/mole (*cf.* Table 1). At each level of calculation employed in this study, the transition state that connects the S1-up and C2-up conformers is located and characterized by vibrational frequency evaluation. The value of the C2S1C6N5 dihedral angle associated with the transition state are around 13.3°; 10.3° and 17.32° for HF, B3LYP and MP2, respectively. The energy barriers $\mathbf{E}^{\neq}$ between the most stable conformer – S1up – and the transition state are around 3.8, 4.2 and 5.78 kcal/mol; while the reverse energy barriers $\mathbf{E}_r$ reach 0.72, 0.52 and 2.16 kcal/mol for HF, B3LYP and MP2, respectively. It should be noted that, although the introduction of the carboxylic group did not affect the stability order of the two conformers, it reduces the energy gap **ΔE** between them as well as the energy barrier $\mathbf{E}^{\neq}$ and increases the reverse energy barriers $\mathbf{E}_r$ (*cf.* Table 1).

Table 5 presents some of the most significant parameters predicted for the S1-up and C2-up conformers of 2 by HF, B3LYP and MP2 at the 6-31G* and 6-311++G** level of calculation [25]. Generally, the improvement of the basis set used did not influence either the trend or the value of the predicted parameters; however, the predicted values of the dihedral angles are sensitive to the theoretical method employed especially upon inclusion of electronic correlation effect at the MP2 level.

Although the introduction of the carboxylic group has a negligible effect on the bond length of the 3-cephem nucleus, it has affected its bond angles. Thus, for the S1-up conformer, changes of +1.42°, −2.96° and −1.13° are introduced in the bond angles of C2C3C4, C7C6S1 and C4N5C6, respectively. Nearly all bond angles of the C2-up conformer are reduced. In addition, the dihedral angle C8N5C6C2 is noticeably affected; a decrease of about 8° and 4° is recorded for the S1-up and C2-up conformers with the three methods employed. In general, the sensitivity of some bond angles and dihedral angles to the conformation of the dihydrothiazine nucleus is unaffected by the introduction of the carboxylic group. All values predicted for 2 using the HF, B3LYP and MP2 methods are in reasonably good agreement with experimental values.

The degree of pyramidalization **h** predicted by the HF, B3LYP and MP2 methods for 2 ranges between 0.23–0.25 Å, 0.20–0.22 Å and 0.27–0.29 Å, respectively.

3.3. Conformational analysis of 7-acetylamino-3cephem-4-carboxylic acid

As a final stage in studying the structure of cephalosporins, the orientation of the acylamino group with respect to the cephem nucleus is investigated by studying the rotation of the acetylamino group – introduced at position 7 – around the cephem nucleus at the HF/6-31+G* level of calculation. The conformational analysis is carried on the S1-up 3 and C2-up 4 conformers taking the C8C7N20C21 dihedral angle as the reaction coordinate in the region −180° to 180°. The potential energy curves (Fig. 5) show

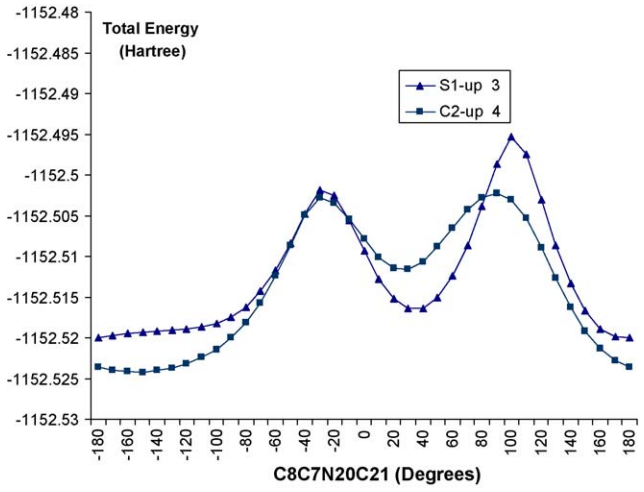

Fig. 5. Conformational analysis of the acetyl group in 3 and 4 obtained by HF/6-31+G*.

Table 5
Optimized bond length (in Å), bond angles and dihedral angles (in°) predicted at the specified level and X-ray reported values for 3-cephem-4-carboxylic acid 2.

	HF				B3LYP				MP2				Semiempirical[a]	X-ray
	6-31G*		6-311++G**		6-31G*		6-311++G**		6-31G*		6-311++G**			
	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1-UP	C2-UP	S1/C2-UP	S1/C2-UP
S1C2	1.820	1.832	1.845	1.862	1.855	1.863	1.842	1.861	1.824	1.838	1.821	1.837	1.829/1.821	1.82/1.79[b]
S1C6	1.816	1.824	1.839	1.845	1.835	1.844	1.838	1.843	1.814	1.819	1.812	1.818	1.822/1.815	1.79/1.83[b]
C4N5	1.399	1.406	1.402	1.408	1.401	1.409	1.401	1.409	1.397	1.407	1.398	1.407	1.428/1.436	1.41/1.41[b]
C8N5	1.385	1.379	1.387	1.380	1.412	1.403	1.409	1.401	1.415	1.405	1.416	1.406	1.479/1.474	1.40/1.38[c] 1.382[d]
C6S1C2	97.05	98.65	97.30	99.68	95.83	98.64	95.90	98.45	95.00	97.06	94.81	96.55	98.7/101.4	92/93[b] 95/93[c]
C2C3C4	125.89	120.66	126.08	120.70	126.20	120.68	126.18	120.51	124.99	118.89	124.76	118.43	124.4/122	123/127[b] 124/124[c]
C4N5C6	124.67	126.06	124.84	125.92	124.49	126.15	124.61	125.94	123.80	125.33	123.82	124.86	123.9/122.9	125.8[e]
C7C6S1	114.71	119.78	114.42	119.54	115.06	120.58	114.87	120.44	114.10	119.59	113.54	118.99		
C8N5C4	131.65	130.73	131.67	130.65	134.01	132.80	133.99	132.96	131.18	129.74	130.66	130.08	126/125	131.2[d] 132.6[d]
C2SlC6N5	50.91	−9.94	50.78	−10.05	52.03	−9.45	51.96	−9.94	54.92	−16.91	55.87	−16.91	38.4/−14.6	54.5/−9.8[b] 51.36[c]
C2S1C6C7	149.36	91.56	149.00	91.24	151.44	93.20	151.01	92.27	153.82	85.06	154.42	84.73		154.60[f]
C8N5C6C2	139.12	110.62	138.46	109.87	143.15	113.90	142.61	112.88	140.44	104.58	139.41	103.04		146.40[c]
S1C6N5C4	−41.69	−33.17	−42.20	−33.32	−42.36	−33.78	−42.95	−34.31	−39.72	−29.85	−40.25	−31.32		−40.91[g]
C13C4N5C8	57.35	85.60	58.29	86.82	52.14	81.21	53.07	83.08	56.11	92.79	57.16	95.08	59.1/88.6	61.9/80.9[b]
C4N5C8C7	154.31	152.70	154.46	152.50	154.72	153.78	155.11	153.84	151.27	149.18	151.37	149.54	141.9/139.6	156.0[f]
N5C4C13O17	−165.24	−176.85	−163.92	−175.57	−167.92	−177.79	−164.73	−175.58	−165.33	−178.75	−161.93	−175.33		

[a] PM3 calculations on cephalotin [17b].
[b] Cephalotin sodium salt [24f].
[c] 3((1-Methyl-lH-tetrazolyl)thiomethyl)-7-(thien-2-ylacetamido)-3-cephem-2-carboxylic acid [24d].
[d] 4-Acetyl-3-methyl-7p-phenoxyacetamido-Δ^3-cephem [24i].
[e] bis(Cephradine) 4-hydroxybenzoic acid tetrahydrate [26].
[f] Cephalexin β-naphthol clathrate [30].
[g] bis(Cefadroxil) 2,6-dihydroxynaphthalene clathrate nonahydrate [30].

Table 6
Optimized parameters predicted for 7-acetylamino-3cephem-4-carboxylic acid conformers 3a and 4a.

Parameter	HF/6-31+G*		Semiempirical[a]	Experimental	Parameter	HF/6-31+G*		Semiempirical[a]	Experimental
	3a	4a				3a	4a		
S1C2	1.821	1.831	1.819	1.82[b]	N5C6C7	87.39	87.04		87.77[f]
S1C6	1.814	1.819	1.822	1.79[b]	C6C7C8	84.48	84.91	87.6	84[b], 86[c]
C2C3	1.504	1.503	1.489	1.55[b], 1.51[c]	C7C8N5	91.14	91.85	90.6	93[b], 92[c]
C3C4	1.325	1.324	1.353	1.32[b]	C8N5C6	94.22	94.67	92.5	97[b], 95[c]
C4N5	1.402	1.409	1.428	1.41[b]	C8N5C4	130.23	129.95	126	131.2[d]
N5C6	1.453	1.464	1.512	1.51[b], 1.45[d]	O9C8N5	133.04	132.83	130.8	134[b]
C6C7	1.558	1.561	1.571	1.59[c], 1.566[d]	C2SlC6N5	52.26	−7.34	38.4	54.5[b], 51.36[c]
C7C8	1.531	1.527	1.551	1.57[b], 1.52[c]	C2S1C6C7	149.71	93.43		149.96[d]
C8N5	1.381	1.369	1.479	1.35[b], 1.382[d]	C8N5C6C2	134.62	107.54		146.40[c]
C8O9	1.181	1.185	1.1956	1.21[b], 1.18[c]	S1C6 N5C4	−43.74	−35.91		−40.91[f]
C6S1C2	96.64	100.09	98.7	92[b], 95[c]	N5C8C7N20	−135.19	−131.23	−126.4	−134.9[b]
S1C2C3	115.11	113.58		116.19[e]	C8N5C4C13	62.07	89.38	59.1	61.9[b]
C2C3C4	125.81	120.70	124.4	123[b], 124[c]	C4N5C8C7	156.49	156.10	141.9	156.0[g]
C3C4N5	121.75	118.23		120.20[e]	N5C4C13O17	−167.72	−177.82		
C4N5C6	124.24	125.66	123.9	123[b], 125.8[f]	C8C7N20C21	−155.19	−179.14	−145.5	−149.4
N5C6S1	111.65	113.11		111.39[f]	C4N5C8O9	−19.11	−19.69		−19.60[f]

[a] PM3 calculations on cephalotin [17b].
[b] Cephalotin sodium salt [24f].
[c] 3((1-Methyl-lH-tetrazolyl)thiomethyl)-7-(thien-2-ylacetamido)-3-cephem-2-carboxylic acid [24d].
[d] 4-Acetyl-3-methyl-7p-phenoxyacetamido-Δ^3-cephem [24i].
[e] Cephradine bis(dimethylformamide) clathrate [26].
[f] bis(Cephradine) 4-hydroxybenzoic acid tetrahydrate [26].
[g] Cephalexin β-naphthol clathrate [30].

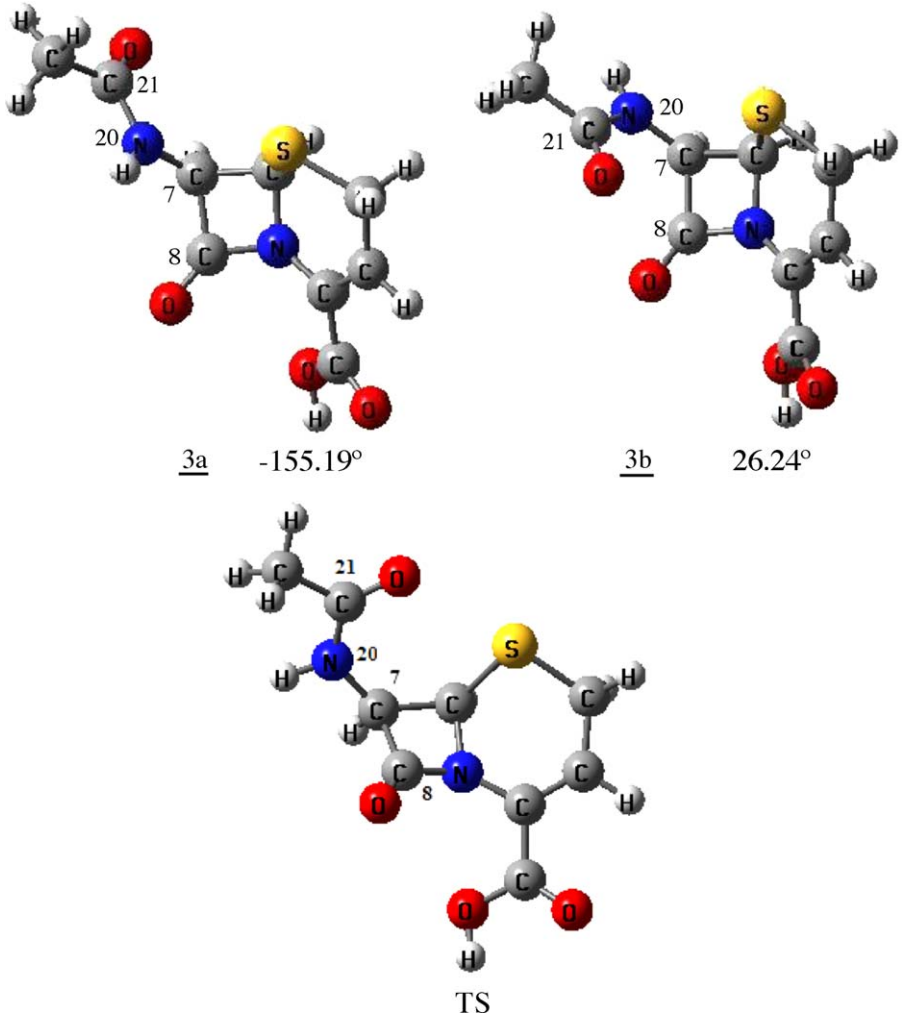

Fig. 6. The optimized structure of 3a, 3b and the transition state at HF/6-31+G* level.

two minima, indicating the existence of two stable orientations for the acetylamino group. The optimized orientations of the acetylamino group as expressed by the dihedral angle C8C7N20C21 for the S1-up conformer 3 correspond to the values of −155.19° 3a and 26.24° 3b, where the former is more stable by 7.82 kcal/mol. These orientations are in reasonably good agreement with previously reported crystallographic results: −156.79° for bis(cephradine)-2-acetonaphthone clathrate hydrate [30,31], −158.91° for bis(cefadroxil) 2,6-dihydroxynaphthalene clathrate nonahydrate [30], 30.63° for cephradine methyl 3-hydroxybenzoate clathrate [26] and 32.89° for cephradine bis(dimethylformamide) clathrate [26]. Previously reported semiempirical values for cephalotin are −145.4°, −128.7°, −160.3° and −107.7° at the PM3, AM1, MNDO and MINDO level of calculation, respectively [17]. The activation energy required to rotate the acetylamino group from its orientation in 3a to 3b is 14.10 kcal/mol. Fig. 6 represents the structure of the two optimized orientations 3a, 3b and the structure of the located transition state.

With respect to the C2-up conformer 4, the optimized orientations of the acetylamino group correspond to the values of −179.14° 4a and 35.10° 4b. The energy difference between the two orientations is 2.17 kcal/mol in favour of 4a. The activation energy required to transform 4a to 4b is 11.62 kcal/mol. Examination of the energy potential curves for 3 and 4 reveals that, although 3a is more stable than 4a by 3.45 kcal/mole, 3b is less stable than 4b by 2.19 kcal/mol. The stability of 4b over 3b is attributed to the orientation attained by 0.22 with respect to H11 with only 2.31 Å separating them (Fig. 7). This stabilizing effect is also reflected on the low energy barrier between 4a and 4b as well as the low activation energy required for their interconversion, as compared with values calculated for the 3 conformers.

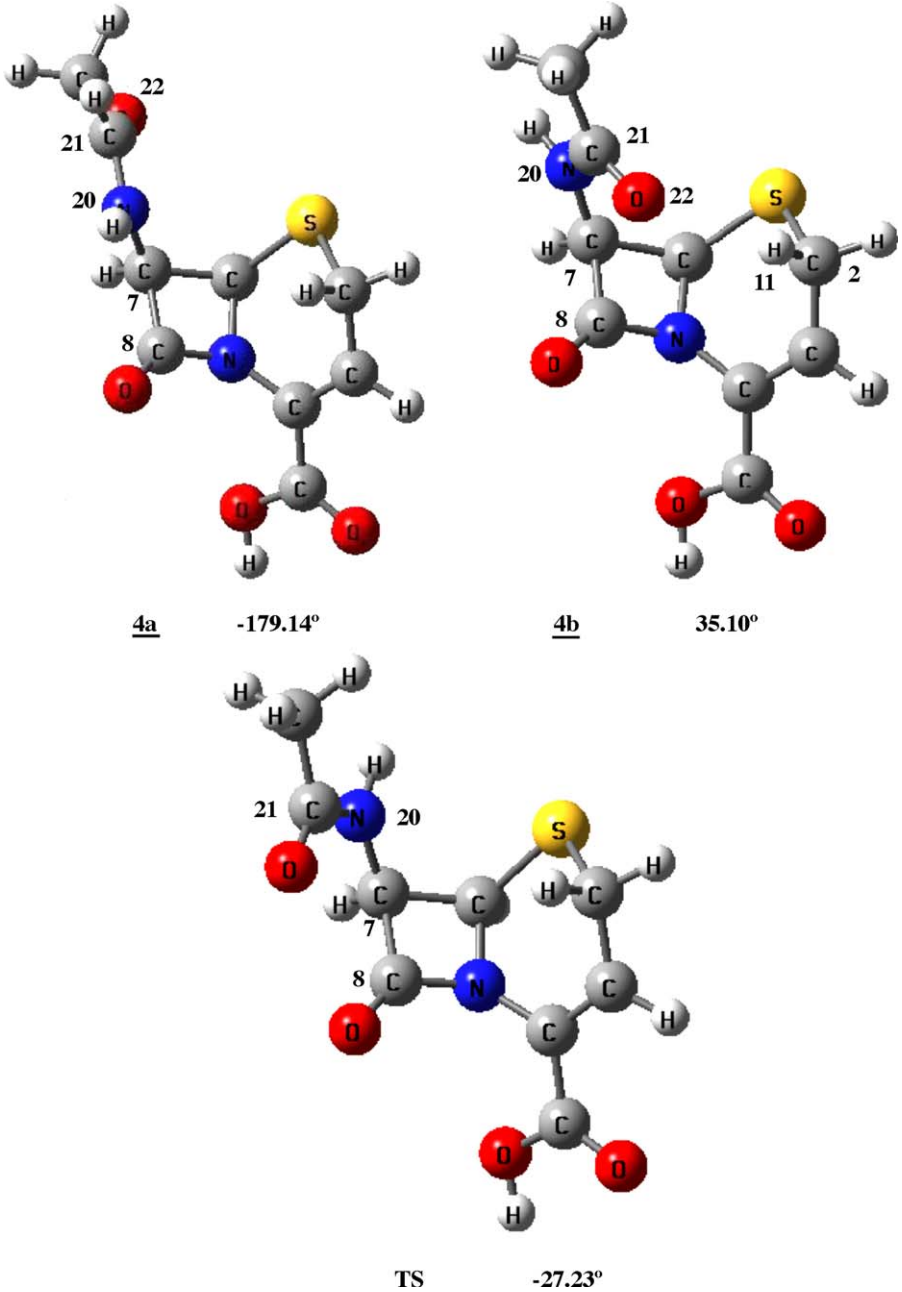

Fig. 7. The optimized structure of 4a, 4b and the transition state at HF/6-31+G* level.

The transition state that correlates the more stable S1-up conformer 3a to the more stable C2-up conformer 4a is located at −27.23°. The energy $\mathbf{E}^{\neq}$ required for such interconversion is quite small, 3.98 kcal/mole; and accounts for the feasibility of this interconversion and the existence of both conformations in the solid state.

Examining Table 6 reveals that, HF/6-31+G* method, provides reasonably acceptable results that are in good agreement with the X-ray diffraction results. The predicted **h** for 3a is 0.26 Å, which is slightly higher than experimental values.

4. Conclusion

Conformational analysis study has been performed on cephalosporin nucleus using HF, B3LYP and MP2 methods at different basis set with increasing complexity. In accordance with X-ray diffraction method, all calculations have predicted two stable conformers, the S1-up and the C1-up. Unlike semiempirical methods, the stability order of the two conformers is independent on the level of calculation. The S1-up conformer with the carboxyl–carbonyl group adopting *trans* orientation with respect to the lactam-nitrogen was found to be the more stable conformation. The energy difference $\Delta\mathbf{E}$ between the two conformers and the energy barrier $\mathbf{E}^{\neq}$ for the interconversion process are small. They ranged between 2.85–4.08 kcal/mol and 3.8–5.78 kcal/mol, respectively, depending on the method of calculation. These values account for experimental findings, that, S1-up being the readily detected conformer and for the readiness of the interconversion between the two conformers. The **h** values predicted for all the studied conformers are in reasonable agreement with the reported experimental values. With regard to the adequacy of ab initio methods for predicting the properties of the cephalosporins, we conclude that, increasing the complexity of the model chemistry employed did not induce further improvement on the predicted properties. Generally, all results were in good agreement with the reported experimental results. Moreover, the simple HF/6-31+G* model chemistry is quite adequate for predicting the properties of cephalosporins.

References

[1] (a) G. Lancini, F. Parent, Antibiotics: an Integrated View, Springer, New York, 1982;
(b) D.M. Livermore, J.D. Williams, β-Lactams: Mode of Action and Mechanisms of Bacterial Resistance—Antibiotics in Laboratory Medicine, fourth ed., William & Wilkins, Baltimore, 1996;
(c) D.J. Waxman, J.L. Strominger, Annu. Rev. Biochem. 52 (1983) 825;
(d) E.H. Flyn, Cephalosporins and Penicillins: Chemistry and Biology, Academic Press, New York, 1972

[2] (a) J.M. Ferére, M. Nguyen-Distéche, J. Coyette, B. Joris, M.I. Page, The Chemistry of β-Lactams, Blackie Academic, Glasgow, 1992;
(b) M.I. Page, A.P. Laws, M.J. Slater, J.R. Stone, Pure Appl. Chem. 67 (1995) 711;
(c) M.I. Page, Adv. Phys. Org. Chem. 23 (1987) 165;
(d) H. Christensen, M.T. Martin, S.G. Waley, J. Biochem. 266 (1990) 853.

[3] A. Peña-Gallego, E.M. Cabaleiro-Lago, A. Fernández-Ramos, J.M. Hermida-Ramón, E. Martínez-Núñez, J. Mol. Struct. (Theochem.) 491 (1999) 177.

[4] M.A. Palafox, J.L. Núñez, M. Gill, J. Phys. Chem. 99 (1995) 1124.

[5] (a) J. Pitarch, M.F. Ruiz-López, J.-L. Pascual-Ahuir, E. Silla, I. Tuñón, J. Phys. Chem., B 101 (1997) 3581;
(b) J. Pitarch, M.F. Ruiz-López, E. Silla, J.-L. Pascual-Ahuir, I. Tuñón, J. Am. Chem. Soc. 120 (1998) 2146.

[6] M.-S. Kim, W.K. Park, S.C. Park, J. Mol. Struct. (Theochem.) 630 (2003) 265.

[7] H.S.R. Gilson, M. Krauss, J. Am. Chem. Soc. 121 (1999) 6984.

[8] C.I. Williams, M.A. Whitehead, J. Mol. Struct. (Theochem.) 491 (1999) 93.

[9] N.C. Cohen, J. Med. Chem. 26 (1983) 259.

[10] H.R. Pfaendler, J. Gosteli, R.B. Woodward, G. Rihs, J. Am. Chem. Soc. 103 (1981) 4526.

[11] S. Hanessian, M.J. Rozema, J. Am. Chem. Soc. 118 (1996) 9884.

[12] C. Fuganti, P. Grasselli, M. Mendozza, S. Servi, G. Zucchi, Tetrahedron 53 (1997) 2617.

[13] (a) C. Ghiron, E. Piga, T. Rossi, B. Tamburini, R.J. Thomas, Tetrahedron Lett. 37 (1996) 3891;
(b) T. Rossi, C. Marchioro, A. Paio, R.J. Thomas, P. Zarantonello, J. Org. Chem. 62 (1997) 1653;
(c) M. Panunzio, R. Camerini, R. Pachera, D. Donati, C. Marchioro, A. Perboni, Tetrahedron Asymmetry 7 (1996) 2929;
(d) D. Andreotti, T. Rossi, G. Gaviraghi, D. Donati, C. Marchioro, E.D. Modugno, A. Perboni, Bioorg. Med. Chem. Lett. 6 (1996) 491;
(e) C. Bismara, R.D. Fabio, D. Donati, T. Rossi, R.J. Thomas, Tetrahedron Lett. 36 (1995) 4283;
(f) R. Camerini, M. Panunzio, G. Bonanomi, D. Donati, A. Perboni, Tetrahedron Lett. 37 (1996) 2467;
(g) M. Anada, S.-I. Hashimoto, Tetrahedron Lett. 39 (1998) 9063;
(h) T. Matsumoto, T. Murayama, S. Mitsuhashi, T. Miura, Tetrahedron Lett. 40 (1999) 5043;
(i) Y.-Y. Ke, T.-H. Lin, Biophys. Chem. 114 (2005) 103.

[14] (a) T. Yamana, A. Tsuji, J. Pharm. Sci. 65 (1976) 1563;
(b) A. Tsuji, E. Nakashima, Y. Deguchi, K. Nishide, T. Shimizu, S. Horiuchi, S. Ishikawa, T. Yamana, J. Pharm. Sci. 70 (1981) 1120;
(c) P. Proctor, N.P. Gensmantel, M.I. Page, J. Chem. Soc., Perkin Trans. 2 (1982) 1185;
(d) D. Boyd, J. Med. Chem. 26 (1983) 1010;
(e) K. Bowden, K. Bromley, J. Chem. Soc., Perkin Trans. 2 (1990) 2111;
(f) J. Frau, J. Donoso, F. Muntioz, F. García Blanco, J. Comp. Chem. 13 (1992) 681;
(g) J. Frau, J. Donoso, F. Muñoz, F. García-Blanco, Helv. Chim. Acta 79 (1996) 353;
(h) J. Frau, J. Donoso, F. Muñoz, B. Vilanova, F. García-Blanco, Helv. Chim. Acta 80 (1997) 739.

[15] W.C. Topp, B.G. Christensen, J. Med. Chem. 17 (1974) 342.

[16] N.V. Joshi, V.S.R. Rao, J. Biol. Sci. 4 (1982) 209.

[17] (a) J. Frau, P. Donoso, F. Munoz, F. Garcia-Blanco, J. Mol. Struct. (Theochem.) 251 (1991) 205;
(b) A. Lambert, A. Cartier, J. Mol. Struct. (Theochem.) 370 (1996) 1;
(c) J. Frau, M. Coll, J. Donoso, F. Munoz, F. Garcia Blanco, J. Mol. Struct. (Theochem.) 231 (1991) 109.

[18] D.B. Boyd, J. Med. Chem. 22 (1979) 533.

[19] J.Cz. Dobrowolski, J. Sadlejac, A.P. Mazurek, J. Mol. Struct. (Theochem.) 684 (2004) 181.

[20] D.C. Hodgkin, E.N. Maslen, Biochem. J. 79 (1961) 393.

[21] J.R. Johnson, R.B. Woodward, R. Robinson, in: D. Crowfoot, C.W. Bunn, B.W. Rogers-Low, A. Turner-Jones (Eds.), The Chemistry of Penicillin, Princeton University Press, Princeton, 1949.

[22] J.M. Ghuysen, J.M. Frère, M. Leygh-Bouille, J. Coyette, J. Dusart, M. Nguyen-Disteche, Annu. Rev. Biochem. 48 (1979) 73.

[23] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, V.G. Zakrzewski, J.A. Montgomery, Jr., R.E. Stratmann, J.C. Burant, S. Dapprich, J.M. Millam, A.D. Daniels, K.N. Kudin, M.C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G.A. Petersson, P.Y. Ayala, Q. Cui, K. Morokuma, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J. Cioslowski, J.V. Ortiz, A.G. Baboul, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, J.L. Andres, C. Gonzalez, M. Head-Gordon, E.S. Replogle, J.A. Pople, Gaussian98 program, Revision A.9 package, Gaussian, Inc., Pittsburgh, PA, 1998.

[24] (a) A. Balsamo, P. Domiano, B. Macchia, F. Macchia, M. Nardelli, Eur. J. Med. Chem.-Chim. Ther. 15 (1980) 559;
(b) J.P. Declercq, G. Germain, C. Moreaux, M. Van Meerssche, Acta Crystallogr. B 33 (1977) 3868;
(c) B. Tinant, B. Coene, J.P. Declercq, G. Germain, M. Van Meerssche, Cryst. Struct. Commun. 10 (1981) 259;
(d) E.F. Paulus, Acta Crystallogr. B 30 (1974) 2915;
(e) M. Van Meerssche, G. Germain, J.P. Declercq, B. Coene, C. Moreaux, Cryst. Struct. Commun. 8 (1979) 281;
(f) R.M. Sweet, L.F. Dahl, J. Am. Chem. Soc. 92 (1970) 5489;
(g) M. Van Meerssche, G. Germain, J.P. Declercq, B. Coene, C. Moreaux, Cryst. Struct. Commun. 8 (1979) 287.

[25] Detailed tables including predicted values by the three methods at the 6-31G*, 6-31G**, 6-311G** and 6-311++G** basis sets levels are provided as supplementary material.

[26] G.J. Kemperman, R. de Gelder, F.J. Dommerholt, P.C. Raemakers-Franken, A.J.H. Klunder, B. Zwanenburg, Eur. J. Org. Chem. (2001) 3641.

[27] G.J. Kemperman, R. de Gelder, F.J. Dommerholt, P.C. Raemakers-Franken, A.J.H. Klunder, B. Zwanenburg, Chem. Eur. J. (1999) 2163.

[28] D.B. Boyd, in: R.B. Morin, M. Gorman (Eds.), Chemistry and Biology of β-Lactam Antibiotics, vol. 1, Academic Press, New York, 1982, , Chapter 5.

[29] P. Domiano, A. Balsamo, B. Macchia, F. Macchia, J. Chem. Soc., Perkin Trans. 2 (1987) 841.

[30] G.J. Kemperman, R. de Gelder, F.J. Dommerholt, P.C. Raemakers-Franken, A.J.H. Klunder, B. Zwanenburg, J. Chem. Soc., Perkin Trans. 2 (2000) 1425.

[31] G.J. Kemperman, R. de Gelder, F.J. Dommerholt, P.C. Raemakers-Franken, A.J.H. Klunder, B. Zwanenburg, J. Chem. Soc., Perkin Trans. (2001).