J Mol Model (2007) 13:219–224
DOI 10.1007/s00894-006-0155-6

ORIGINAL PAPER

Investigation of the structure of 6-amino-4-methylamino-5-nitrosopyrimidine by X-ray diffraction, NMR and molecular modeling

Gintaras Urbelis · Inga Susvilo · Sigitas Tumkevicius

Received: 7 March 2006 / Accepted: 10 August 2006 / Published online: 20 September 2006

Abstract The structure of 6-amino-4-methylamino-5-nitrosopyrimidine in the solid state and dimethylsulfoxide solution was investigated using single crystal X-ray diffraction and ^{1}H, ^{13}C NMR spectroscopy methods. Hartree-Fock (HF) and density functional (DFT) levels of theory were used to interpret the experimental data obtained by X-ray and NMR methods.

Keywords HF · DFT · NMR · Chemical shifts · X-ray · Heterocycles

Introduction

Alkoxy- and amino-substituted 5-nitrosopyrimidines are important as potential inhibitors of the human DNA-repair protein O^6-alkylguanine-DNA-transferase [1–3] and of cyclin-dependent kinases [4]. Moreover, 5-nitrosopyrimidines with amino-acid residues attached to the pyrimidine nucleus have been shown to be able to form manganese (II) [5], zinc (II) and Cd(II) [6] complexes both in solution and in the solid state. Such complexes are of great interest because these metals are involved in numerous biological molecules [7, 8]. Recently we have found that *N*-methyl-*N*-(6-amino- or arylamino-5-nitropyrimidin-4-yl) glycinates treated with sodium alkoxides undergo rearrangement to form the corresponding 5-nitroso-4-methylaminopyrimidines [9]. Elucidation of their structure showed some interesting features, which required additional studies. In this paper, we present results of a more detailed investigation of the structure of 6-amino-5-nitroso-4-methylaminopyrimidine (**1**) using single crystal X-ray, ^{1}H, ^{13}C NMR and quantum chemical methods.

Electronic supplementary material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00894-006-0155-6 and is accessible for authorized users.

G. Urbelis · I. Susvilo · S. Tumkevicius (✉)
Department of Organic Chemistry, Faculty of Chemistry,
Vilnius University,
Naugarduko 24,
LT-03325 Vilnius, Lithuania
e-mail: sigitas.tumkevicius@chf.vu.lt

Materials and methods

The melting point of **1** was determined in an open capillary and is uncorrected. IR spectra were run in Nujol mulls on a Perkin-Elmer FT spectrophotometer Spectrum BX II. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA spectrometer (300 and 75 MHz, respectively) using tetramethylsilane as an internal standard. The purity of compound **1** was monitored by TLC using silica gel 60 F_{254} aluminum plates (Merck). Visualization was accomplished by UV light. Synthesis of compound **1** was carried out according to [9]. The characteristics of compound **1** are the following: M.p. 256 °C (dec) (from DMSO); IR (Nujol): 3320, 3329, 3331 cm^{-1} (NH_2, NH); ^{1}H NMR (DMSO-d_6): 2.90, 3.04 (3H, d, J=4.8 Hz, NCH_3); 8.02 (1H, s, C2–H); 8.08, 8.51, 9.11, 10.23 (2H, br.s, NH_2); 9.59, 11.11 (1H, q, J=4.8 Hz, $NHCH_3$); ^{13}C NMR (DMSO-d_6): 26.26, 27.66, 139.99, 140.7, 145.45, 146.01, 163.05, 164.52, 164.7, 165.9.

The computational study followed the scheme shown below. Geometry optimizations for compound **1** were performed using the PC GAMESS version [10] of the GAMESS (US) QC package [11]. The shielding calculations were performed with the GIAO method [12, 13] using the Gaussian 98 suite of programs [14]. Calculations were performed at the HF and B3LYP [15, 16] levels of theory. For the geometry optimization and the shielding calcula-

tions, the 6-31G [17] and 6-311G [18] split-valence basis sets augmented with diffuse and polarization functions were used. In order to compare isotropic shieldings with experimental chemical shifts, the NMR parameters for the reference molecule tetramethylsilane (TMS) were calculated for each basis set.

Crystal data for compound 1 $C_5H_7N_5O$, M_w 153.16, orthorhombic, space group Pca21; Z=4, a=16.4247(4), b=4.7518(2), c=8.3893(8) Å, $\alpha=\beta=\gamma=90°$; V=654.8(3) Å^3, $F(000)$=320, D_x=1.554 g/cm^3. Full crystallographic data are available as supplementary material.

Results and discussion

Structural analysis using X-ray and quantum chemical methods

The crystallographic data of **1** showed that in the solid state the molecule adopts a conformation in which the methyl C atom is directed away from the nitroso group and the nitroso group is turned towards the methylamino substituent (Fig. 1).

The intramolecular dimensions show a number of features of molecule **1** (Table 1). There is marked equalization of the C–C and C–N bonds, with bonds C2–N1, C2–N3 and C4–N7, C6–N11 being short despite the C4–N7 and C6–N11 bonds formally being single bonds in the classical representation. In addition, the C5–N9 and O10–N9 distances in the C-nitroso fragment differ by only about 0.08 Å (Table 1), whereas in simple neutral compounds, where there is no possibility of significant electronic delocalization, these distances normally differ by at least 0.20 Å [19] and the N–O distance rarely exceeds 1.25 Å. [19–22] The N7-methyl distance is as expected.

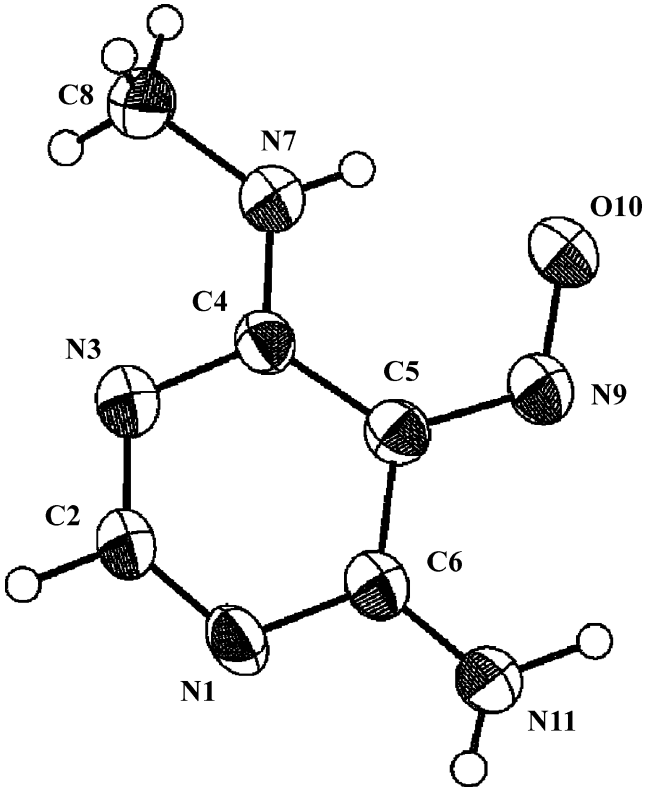


Fig. 1 ORTEP drawing of compound 1

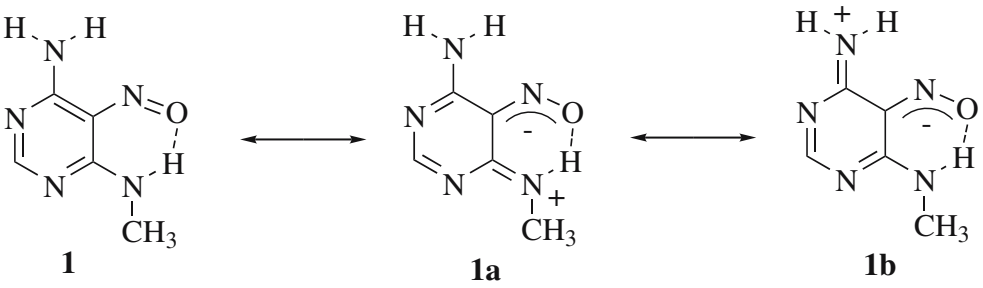
Table 1 Selected geometric parameters (Å, °) of compound **1** obtained from X-ray data

Bonds	Distance or angles	Bonds	Distance
N1-C2	1.323(4)	C5-C4	1.435(4)
N1-C6	1.362(3)	N7-C4	1.323(3)
N3-C2	1.316(4)	N7-C8	1.454(4)
N3-C4	1.362(3)	N11-C6	1.320(4)
C5-N9	1.357(3)	O10-N9	1.276(3)
C5-C6	1.431(4)		
C2-N1-C6	115.8(3)	N7-C8-H8C	107.9(19)
C2-N3-C4	115.3(2)	O10-N9-1C5	117.5(2)
N9-C5-C6	115.5(2)	N7-C4-N3	117.6(2)
N9-C5-C4	127.5(2)	N7-C4-C5	121.6(2)
C6-C5-C4	117.0(2)	N3-C4-C5	120.8(2)
C4-N7-C8	123.5(3)	N3-C2-N1	130.7(3)
C4-N7-H7	112(3)	N3-C2-H2	114.0(18)
C8-N7-H7	125(3)	N1-C2-H2	115.3(18)
C6-N11-H11A	118(2)	N11-C6-N1	116.8(3)
C6-N11-H11B	119(2)	N11-C6-C5	122.9(2)
N7-C8-H8A	110(3)	N1-C6-C5	120.3(2)
N7-C8-H8B	108(3)		

The distance N(7)...O(10) and the angle N(7)–H(7)–O(10) were found to be 2.654 Å and 139°, respectively, which indicates that in **1** an intramolecular hydrogen bond between the oxygen of the nitroso group and the hydrogen of the methylamino group is formed. The dimensions all point to the importance of the charge-separated forms **1a**,**b** (Scheme 1) as important contributors to the overall molecular-electronic structure.

In order to investigate the utility of *ab initio* methods for predicting bond lengths for molecule **1**, calculations at the HF and DFT levels of theory were performed. Bond length errors were calculated by subtracting experimental bond lengths from the calculated ones. Negative values mean that the calculated bond is shorter than the bond obtained from the X-ray analysis. The calculations performed at both the HF/6-31G(d,p) and B3LYP/6-31G(d,p) levels of theory showed a reasonable agreement of the computed structural parameters with the experimental X-ray data. Nevertheless, molecular structures determined by calculations at the DFT level of theory are more accurate than those obtained at HF level. The difference in accuracy is most marked for the O10–N9 bond length. It differs from experiment by

Scheme 1 The charge-separated forms of compound 1



1 **1a** **1b**

0.075 Å at HF/6-31G(d,p) and by 0.021 Å at B3LYP/6-31G (d,p) (Table 2). The calculations with double and triple split-valence basis sets gave quite similar results. Mean signed and unsigned errors (MSE and MUE) as well as the largest positive (Err_{max}) and negative (Err_{min}) errors of bond lengths calculated with the different methods are given in Table 3. Their values show that the basis set augmentation with additional polarization and diffuse functions has no significant influence on the accuracy of the calculated molecular structure. The detailed results are available as supplementary material.

NMR spectra and their evaluation by theoretical methods

The ^{13}C NMR spectrum of compound **1** recorded in dimethylsulfoxide solution contains two sets of signals for all carbon atoms. ^{1}H NMR spectrum are even more complex. For example, the amino group in the 6th position of the pyrimidine ring is characterized by four broadened singlets at δ=8.08, 8.51, 9.11 and 10.23 ppm. The NH proton of the methylamino group correspondingly gave two quartets at 9.59 and 11.11 ppm. Two doublets at 3.04 and 2.90 ppm can be attributed to the protons of the methyl group. However, for the C2–H proton, only one signal is observed at 8.02 ppm. One can see that these spectral data cannot be explained only by the structural features derived for molecule **1** from the crystallographic analysis. The observed NMR spectrum can be interpreted as being caused by a slow rotation of the amino and nitroso groups around the corresponding C–N bonds. A similar nitroso-group rotation effect for the ^{1}H NMR spectrum was studied in 4-dialkylaminonitrosobenzenes [23]. As a consequence, equilibrium between two conformers **1** and **1c**, stabilized by intramolecular hydrogen bonds, occurs in dimethylsulfoxide solution (Scheme 2), and therefore two sets of signals corresponding to the two forms are observed in the ^{1}H and ^{13}C NMR spectra.

Table 2 Differences (Å) between the calculated and experimental bond lengths

	Method	
Bond	HF/6-31G(d,p)	B3LYP/6-31G(d,p)
N1-C2	−0.008	0.013
N1-C6	−0.030	−0.016
N3-C4	−0.026	−0.010
N3-C2	−0.003	0.016
C5-N9	0.016	0.014
C5-C4	−0.008	0.005
C5-C6	−0.015	0.002
N7-C8	−0.005	−0.001
N7-C4	0.001	0.013
N11-C6	0.012	0.022
O10-N9	−0.075	−0.021
MUE	0.018	0.012

To assign which signal set in the ^{1}H NMR spectrum of **1** belongs to a definite conformer, a selective decoupling of all NH signals was performed. It was found that saturation of the NH proton at 9.59 ppm caused a change of the doublet of the methyl signal at 3.04 ppm into a singlet, which indicates that these signals are interrelated and belong to the same conformer. Saturation of the NH signal at 11.11 ppm caused conversion of the methyl doublet at 2.90 ppm into a singlet. Thus, singlets at 9.59 and 11.11 ppm belong to the N*H*Me group of different conformers. The NH signal of the methylamino group of conformer **1** due to the intramolecular hydrogen bond should be in a downfield region. Hence, we can conclude that a doublet of the methyl group at 2.90 ppm and the NH signal at 11.11 ppm belong to conformer **1**. Two other interrelated signals, the doublet of the methyl group at 3.04 ppm and NH signal at 9.59 ppm belong to another conformer **1c**. According to the integral intensities of the signals of the methyl groups, the ratio of conformers **1**:**1c** in dimethylsulfoxide solution is *ca* 63%:37%.

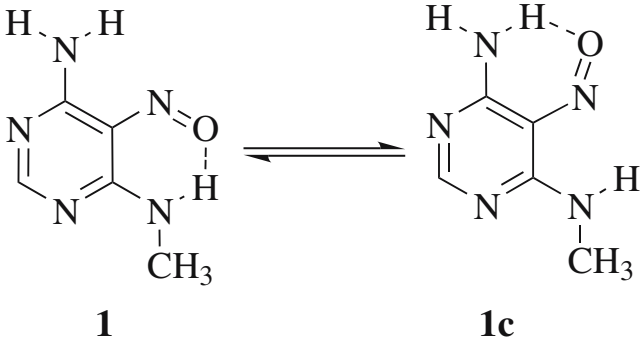
In order to unravel the reasons for the existence of conformers **1** and **1c** in solution, computational analysis to evaluate of their energy was performed. The data are summarized in Tables 4 and 5. The energy values are very dependent on the method used and the basis set. The calculations performed at the DFT level with double split-valence basis sets gave better results than those obtained at the HF level (Table 4). Basis set augmentation with additional polarization and diffuse functions at both levels of theory led to considerably worse results when compared with the experimental data. Calculations using triple split-valence basis sets also tend to overestimate the stability of

Table 3 Errors (Å) between the calculated and experimental bond lengths

Method	Errors			
	MSE	MUE	$Err._{max}$	$Err._{min}$
HF/6-31G(d,p)	−0.013	0.018	0.016	−0.075
HF/6-31G(2d,p)	−0.015	0.020	0.016	−0.081
HF/6-31+G(2d,p)	−0.014	0.019	0.013	−0.080
HF/6-31+G(2df,p)	−0.014	0.019	0.014	−0.081
B3LYP/6-31G(d,p)	0.003	0.012	0.022	−0.021
B3LYP/6-31G(2d,p)	0.001	0.012	0.021	−0.028
B3LYP/6-31+G(2d,p)	0.001	0.012	0.022	−0.025
B3LYP/6-31+G(2df,p)	0.001	0.012	0.021	−0.027
HF/6-311G(d,p)	−0.014	0.020	0.018	−0.084
HF/6-311G(2d,p)	−0.016	0.021	0.016	−0.082
HF/6-311+G(2d,p)	−0.015	0.020	0.013	−0.081
HF/6-311+G(2df,p)	−0.016	0.021	0.014	−0.084
B3LYP/6-311G(d,p)	0.012	0.015	0.026	−0.014
B3LYP/6-311G(2d,p)	−0.001	0.011	0.019	−0.028
B3LYP/6-311+G(2d,p)	0.000	0.011	0.020	−0.026
B3LYP/6-311+G(2df,p)	−0.002	0.011	0.019	−0.030

conformer **1c** (Table 5). Considering the data obtained, one can conclude that the molar ratio **1**:**1c** obtained by the B3LYP/6-31G(d,p) method is the closest to the experimental value derived from the ^{1}H NMR spectra.

Further, quantum theoretical methods were used to calculate ^{13}C and ^{1}H NMR spectra of conformers **1** and **1c**. Table 6 shows differences between the values computed using different approaches and experimental ^{13}C and ^{1}H chemical shifts. Calculated values of ^{13}C and ^{1}H chemical shifts are given in the supplementary material. Calculations of ^{13}C chemical shifts using HF and B3LYP methods with triple split-valence basis sets gave larger errors than the corresponding methods with double split-valence basis sets. Moreover, augmentation of the triple split-valence basis set with additional polarization and diffuse functions led to an overestimation of ^{13}C chemical shifts at both the HF and B3LYP levels of theory. More accurate values of ^{13}C chemical shifts were obtained from the calculations using double split-valence basis sets. The B3LYP/6-31G(d,p) method was found to give the most accurate results for ^{1}H chemical shifts for the methyl group and for the proton at the C2 atom of the pyrimidine ring.



Scheme 2 The equilibrium between two conformers in a solution

It is also noteworthy that calculations gave the same value of the C2–H chemical shift for the two conformers, as observed in the experimental ^{1}H NMR spectrum (Table 7).

The experimental ^{13}C NMR spectra for the two conformers are very similar, except for the chemical shifts for the C4 and C6 atoms. To assign which of these signals belong to the C4 or C6 atom was the main problem, which was solved by comparing the experimental spectra for both conformers with the calculated ones (Table 7). These data indicate that a signal in the high-field region belongs to the carbon atom that is connected to a group taking part in the intramolecular interaction with a nitroso group.

Conclusions

The structure of 6-amino-4-methylamino-5-nitrosopyrimidine (**1**) has been examined by X-ray, NMR and quantum chemical methods. It has been shown that in the solid state, partial separation of charge has a considerable contribution to the molecular-electronic structure of **1**, whereas in dimethylsulfoxide solution compound **1** exists as a mixture of two conformers, stabilized by intramolecular hydrogen bonds between the oxygen of the nitroso group and the amino or methylamino groups. The usefulness of computational methods for interpretating the experimental data and predicting their properties has been demonstrated. The molecular structure determined at the DFT level of theory is more accurate than that obtained using the HF method. For calculation of the relative energies and ^{1}H and ^{13}C chemical shifts for the conformers of compound **1**, the relatively inexpensive B3LYP/6-31G(d,p) method yielded the best accuracy.

Table 4 Relative energy values (kcal mol^{-1}) and molar ratio (%) based on the Boltzmann distribution law of conformer **1** in comparison with **1c** calculated with double split-valence basis sets

Method	6-31G(d,p)		6-31G(2d,p)		6-31+G(2d,p)		6-31+G(2df,p)	
	ΔE[a]	Amount of **1**	ΔE	Amount of **1**	ΔE	Amount of **1**	ΔE	Amount of **1**
HF	−0.0707	52.96	−0.0951	53.98	−0.1835	57.64	1.6148	6.25
B3LYP	−0.3343	63.66	−0.3689	64.99	−0.5156	70.37	7.7295	0.00

[a] $\Delta E = E_1 - E_{1c}$

Table 5 Relative energy values (kcal mol^{-1}) and molar ratio (%) based on the Boltzmann distribution law of the compound **1** in comparison with **1c** calculated with triple split-valence basis sets

Method	6-311G(d,p)		6-311G(2d,p)		6-311+G(2d,p)		6-311+G(2df,p)	
	ΔE	Amount of **1**	ΔE	Amount of **1**	ΔE	Amount of **1**	ΔE	Amount of **1**
HF	0.4799	30.90	2.0451	3.14	1.5317	7.11	2.1015	2.86
B3LYP	3.6237	0.23	3.8720	0.15	3.5415	0.26	5.3623	0.01

Table 6 Errors (ppm) between the calculated and experimental ^{13}C and ^{1}H chemical shifts for both conformers

		^{13}C				^{1}H		
Method	MSE	MUE	$Err._{max}$	$Err._{min}$	MSE	MUE	$Err._{max}$	$Err._{min}$
HF/6-31G(d,p)	2.6	2.6	5.7	–	0.20	0.31	0.47	−0.22
HF/6-31G(2d,p)	1.4	2.4	4.5	−2.4	0.08	0.23	0.30	−0.29
HF/6-31+G(2d,p)	1.4	2.5	4.5	−2.8	0.13	0.20	0.28	−0.14
HF/6-31+G(2df,p)	2.8	3.4	6.3	−1.7	0.11	0.18	0.27	−0.15
B3LYP/6-31G(d,p)	−4.0	4.3	7.3	−7.3	0.04	0.15	0.22	−0.22
B3LYP/6-31G(2d,p)	−3.4	4.0	7.7	−7.7	0.06	0.17	0.23	−0.23
B3LYP/6-31+G(2d,p)	−2.6	3.2	6.7	−6.7	0.13	0.16	0.24	−0.05
B3LYP/6-31+G(2df,p)	−1.0	2.3	5.2	−5.2	0.13	0.15	0.27	−0.04
HF/6-311G(d,p)	7.0	7.0	11.5	–	0.15	0.24	0.31	−0.18
HF/6-311G(2d,p)	6.3	6.4	11.1	−0.2	0.04	0.19	0.29	−0.29
HF/6-311+G(2d,p)	6.8	6.9	11.8	−0.4	0.11	0.21	0.29	−0.20
HF/6-311+G(2df,p)	6.9	6.9	12.3	−0.1	0.12	0.22	0.30	−0.19
B3LYP/6-311G(d,p)	4.0	4.0	7.7	–	0.13	0.19	0.26	−0.11
B3LYP/6-311G(2d,p)	3.7	3.9	7.9	−0.7	0.11	0.19	0.27	−0.17
B3LYP/6-311+G(2d,p)	4.5	4.5	7.9	–	0.19	0.21	0.35	−0.04
B3LYP/6-311+G(2df,p)	4.4	4.4	7.6	–	0.22	0.24	0.40	−0.03

Table 7 Experimental and calculated at the B3LYP/6-31G(d,p) level ^{13}C and ^{1}H chemical shifts (ppm) of **1** and **1c**

	1		1c	
Atom	Exp.	Calc.	Exp.	Calc.
C2	165.90	160.06	164.52	160.12
C4	139.99	137.85	163.05	157.68
C5	146.01	138.66	145.45	139.26
C6	164.70	159.10	140.70	136.17
C8	26.26	26.58	27.66	28.70
H(Me)	2.90	2.68	3.04	3.08
H(C2)	8.02	8.19	8.02	8.20

Acknowledgements The work was supported by the Lithuanian Science and Study Foundation, Grant No. T-05292.

We express our gratitude to Dr. O. Eicher-Lorka (Institute of Chemistry, Lithuania) for the possibility to use the GAUSSIAN suite of programs for the shielding calculations.

References

1. Marchal A, Melguizo M, Nogueras M, Sanchez A, Low JN (2002) Synlett 255–258
2. Quesada A, Marchal A, Melguizo M, Nogueras M, Sanchez A, Low JN, Cannon D, Farrell DMM, Glidewell C (2002) Acta Crystallogr B58:300–315

3. Chae MY, McDougall MG, Dolan ME, Swenn K, Pegg AE, Moschel RC (1995) J Med Chem 38:359–365
4. Davies TG, Pratt DJ, Endicott JA, Johnson LN, Noble ME (2002) M Pharmacol Ther 93:125–133
5. Lopez-Garzon R, Arranz-Mascaro P, Godino-Salido ML, Gutierrez-Valero MD, Cuesta R, Moreno JM (2003) Inorg Chim Acta 355:41–48
6. Lopez-Garzon R, Arranz-Mascaro P, Godino-Salido ML, Gutierrez-Valero MD, Perez-Cadenas A, Cobo-Domingo J, Moreno JM (2000) Inorg Chim Acta 308:59–64
7. Chung KH, Hong E, Do Y, Moon CH (1996) J Chem Soc Dalton Trans 3363–3369
8. Hughes MH (1981) The inorganic chemistry of biological processes. Wiley, New York
9. Susvilo I, Brukstus A, Tumkevicius S (2005) Tetrahedron Lett 46:1841–1844
10. Granovsky AA; http://classic.chem.msu.su/gran/gamess/index.html
11. Schmidt MW, Baldridge KK, Boatz JA, Elbert ST, Gordon MS, Jensen JJ, Koseki S, Matsunaga N, Nguyen KA, Su S, Windus TL, Dupuis M, Montgomery JA (1993) J Comput Chem 14:1347–1363
12. London FJ (1937) Phys Radium 8:397–409
13. Ditchfield R (1974) Mol Phys 27:789–807
14. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Zakrzewski VG, Montgomery JA Jr, Stratmann RE, Burant JC, Dapprich S, Millam JM, Daniels AD, Kudin KN, Strain MC, Farkas O, Tomasi J, Barone V, Cossi M, Cammi R, Mennucci B, Pomelli C, Adamo C, Clifford S, Ochterski J, Petersson GA, Ayala PY, Cui Q, Morokuma K, Salvador P, Dannenberg JJ, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Cioslowski J, Ortiz JV, Baboul AG, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Gomperts R, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Andres JL, Gonzalez C, Head-Gordon M, Replogle ES, Pople JA (2001) Gaussian 98, revision A11. Gaussian Inc, Pittsburgh Pennsylvania
15. Becke AD (1988) Phys Rev B 38:3098–3100
16. Lee C, Yang W, Parr RG (1988) Phys Rev B 37:785–789
17. Hehre WJ, Ditchfield R, Pople JA (1972) J Chem Phys 56:2257–2261
18. Krishnan R, Binkley JS, Seeger R, Pople JA (1980) J Chem Phys 72:650–654
19. Talberg HJ (1977) Acta Chem Scand Ser A 31:485–491
20. Davis MI, Boggs JE, Coffey D, Hanson HP (1965) J Phys Chem 69:3727–3730
21. Bauer SH, Andreassen AL (1972) J Phys Chem 76:3099–3108
22. Schlemper EO, Murmann RK, Hussain MS (1986) Acta Cryst C42:1739–1743
23. Buckley PD, Furness AR, Jolley KW, Pinder DN (1974) Austr J Chem 27:21–26