Maxim Alexandrovich Lutoshkin* and Nicolay Nicolaevich Golovnev

Acid-base properties and keto-enol equilibrium of a 5-substituted derivative of 1,3-diethyl-2-thiobarbituric acid

DOI 10.1515/hc-2016-0011
Received February 19, 2016; accepted March 5, 2016; previously published online March 26, 2016

Abstract: This article deals with spectrophotometric and ab initio studies of 1,3-diethyl-7-hydroxy-5,5,7-trimethyl-2-thioxo-1,2,3,5,6,7-hexahydro-4*H*pyrano[2,3-*d*]pyrimidin-4-one (HDEAC). Acid-base properties for I = 0.25 and in a strongly acidic solution of HCl (I → 0) were investigated. The obtained value of pK_a (5.79±0.02) and $-pK_H$ (1.68±0.03) show that this compound is a weaker acid than thiobarbituric acid. For interpretation of the spectrophotometric data the ab initio methods with density functional theory at level PBE0/cc-pVDZ/SMD were used. The most energetically favorable structures for neutral and cationic forms of HDEAC were proposed.

Keywords: DFT; thiobarbituric acid; UV-vis.

Introduction

Thiobarbiturates, barbiturates and its substituted derivatives are coordination agents. Some of them exhibit useful medicinal properties such as antibacterial [1], anti-cancer [2], antitubercular [3] and other biological activities [4–6]. Thiobarbituric compounds have long been used in medicine and pharmacology. Phenobarbital has been placed on a WHO Model List of Essential Medicines, the most important medications needed in a basic health system [7]. Numerous complexes of thiobarbituric acid with some transition metals and lanthanides [8–11] have been described. The literature describes mainly 5,5-disubstituted thiobarbituric acids [12–15] and studies of bicyclic analogs thiobarbituric acids have been neglected.

***Corresponding author: Maxim Alexandrovich Lutoshkin,** Institute of Chemistry and Chemical Technology of the Siberian Branch of the RAS, Krasnoyarsk, Russian Federation, e-mail: maximsfu@yahoo.com
Nicolay Nicolaevich Golovnev: Institute of Non-Ferrous Metals and Materials Science, Siberian Federal University, Krasnoyarsk, Russian Federation

The bicyclic ligand investigated in this work, 1,3-diethyl-7-hydroxy-5,5,7-trimethyl-2-thioxo-1,2,3,5,6,7-hexahydro-4*H*pyrano[2,3-*d*]pyrimidin-4-one (HDEAC), was synthesized by Knoevenagel condensation of 1,3-diethyl-2-thiobarbituric acid with acetone [16] (Scheme 1).

Its structure has been confirmed by X-ray single crystal analysis [16].

The goal of this work was the experimental study of acid-base properties of HDEAC in a wide pH region and the theoretical study of its keto-enol equilibrium in aqueous solution.

Results and discussion

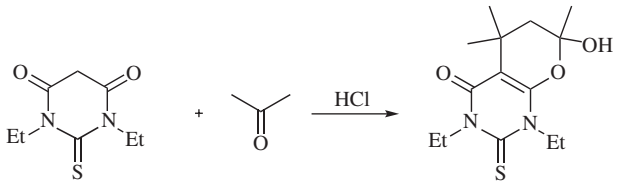
Figure 1 shows UV-vis spectra of various forms of HDEAC in aqueous solution. The ligand is stable over time under the indicated pH conditions. A linear relationship between absorbance and concentration indicates the absence of the molecular association in solution.

Only one maximum absorption peak for all absorbing forms of HDEAC can be observed (Figure 1). The maximum absorption is almost identical for neutral and anionic forms. A shift of maximum absorption to shorter wavelengths for the protonated form is observed. For all forms of HDEAC the absorption maxima exhibit similar values of extinction (Table 1). In comparison with other 1,3-substituted thiobarbituric acid these values of extinction are markedly lower [17].

Determination of the acid-base properties

Determination of pK_a was conducted in the pH range from 2 to 9, using three buffers. The spectral profile under different pH conditions for HDEAC is shown in Figure 2. Isosbestic points indicate the presence of two absorbing forms in solution.

This suggestion is consistent with the neutral and anionic forms of ligand being related predominantly to one specific tautomer. The analysis of the logI – pH relationship shows a single deprotonation with increasing



Scheme 1 Synthesis of the investigated ligand, HDEAC.

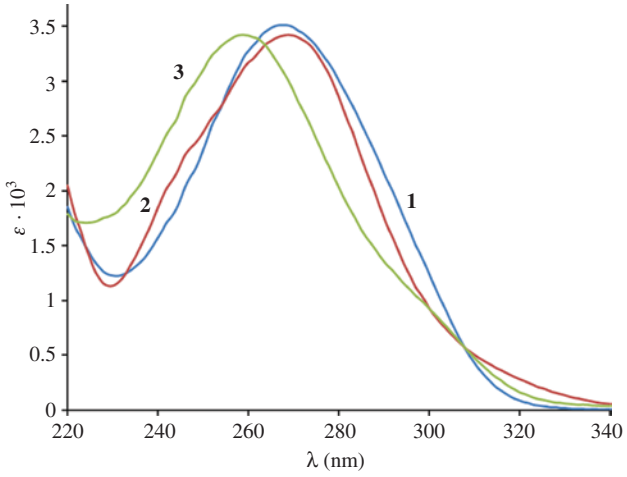


Figure 1 The UV-vis spectra of neutral (**1**; pH = 2), anionic (**2**; pH = 9) and protonated (**3**; [H⁺] > 9.5 M) forms of HDEAC.

pH (equation 2; Figure S1). All these facts suggest that the described process is dissociation of the neural form in the first step. All raw spectroscopic data are given in the Supplementary Material (Tables S1 and S2).

The obtained value of pK_a for HDEAC is 5.79±0.02. This value characterizes HDEAC as a weak acid. HDEAC is a much weaker acid than thiobarbituric acid (pK_a = 2.25) and barbituric acid (pK_a = 4.0) [18].

Study of the acid-base properties in strongly acidic solution

The study was conducted in HCl solutions. The results are shown in Table 2 and Figure 3. The presence of a single isosbestic point suggests the existence of the form H_2DEAC^+ derived from a single tautomer.

The value of the extinction of fully protonated form of HDEAC was not obtained because the maximum possible concentration of HCl does not provide full protonation of this ligand. Study of the acid-base properties HDEAC in other strong acids was not possible. In sulfuric acid, at any concentration, HDEAC undergoes restructuring [19, 20] with the formation of a yellow product. Nitric acid is not

Table 1 The UV-vis data for HDEAC in aqueous solution ($\varepsilon^{\lambda,nm} \pm 125$).

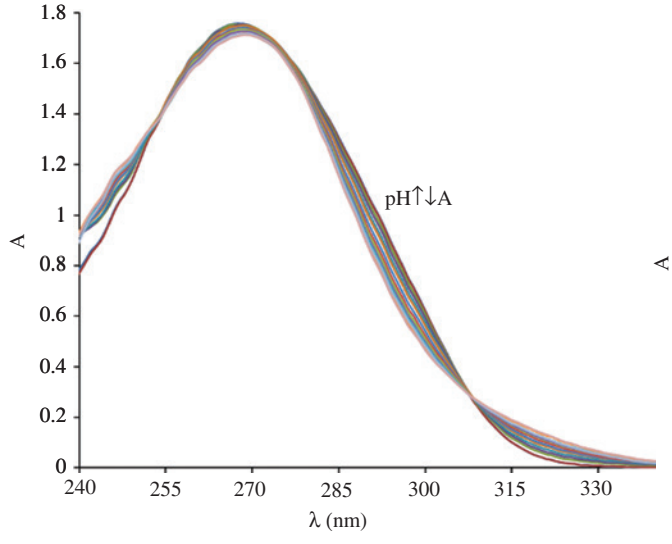
Form	$\varepsilon^{\lambda,nm}$
Anion ($DEAC^-$)	2534^{250}; 3504^{268}; 1568^{292}
Neutral (HDEAC)	2374^{250}; 3424^{269}; 1990^{292}
Cation (H_2DEAC^+)	1712^{225}; 3102^{250}; 3422^{258}

Superscripts bold values - wavelength, nm.

Table 2 The obtained $-pK_H$ values.

$-pK_H$ ±0.04	m^* ± 0.03[a]	λ, nm	I[b]
1.68	0.52	244	→0
1.74	0.48	288	

[a]Solvation coefficient, [b]ionization ratio.



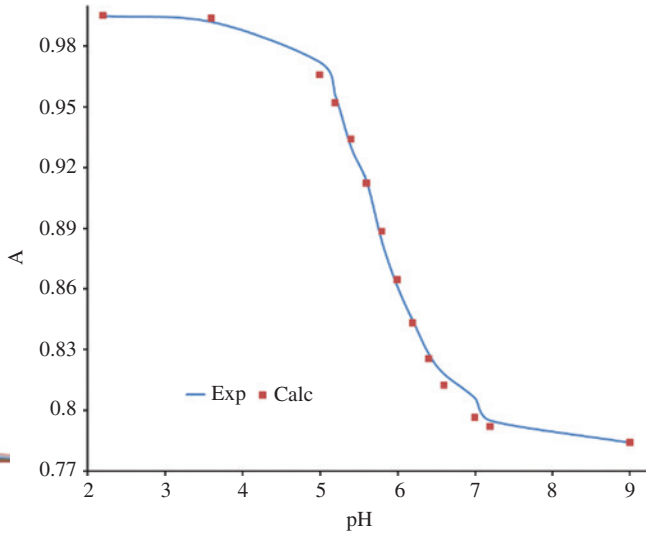


Figure 2 The UV-vis spectra of HDEAC at various pH values: 2.2; 3.6; 5.0; 5.2; 5.4; 5.6; 5.8; 6.0; 6.2; 6.4; 6.6; 7.0; 7.2; 9.0 and A^{292} – pH relationship. [HDEAC] = $5 \cdot 10^{-4}$ M, I = 0.25.

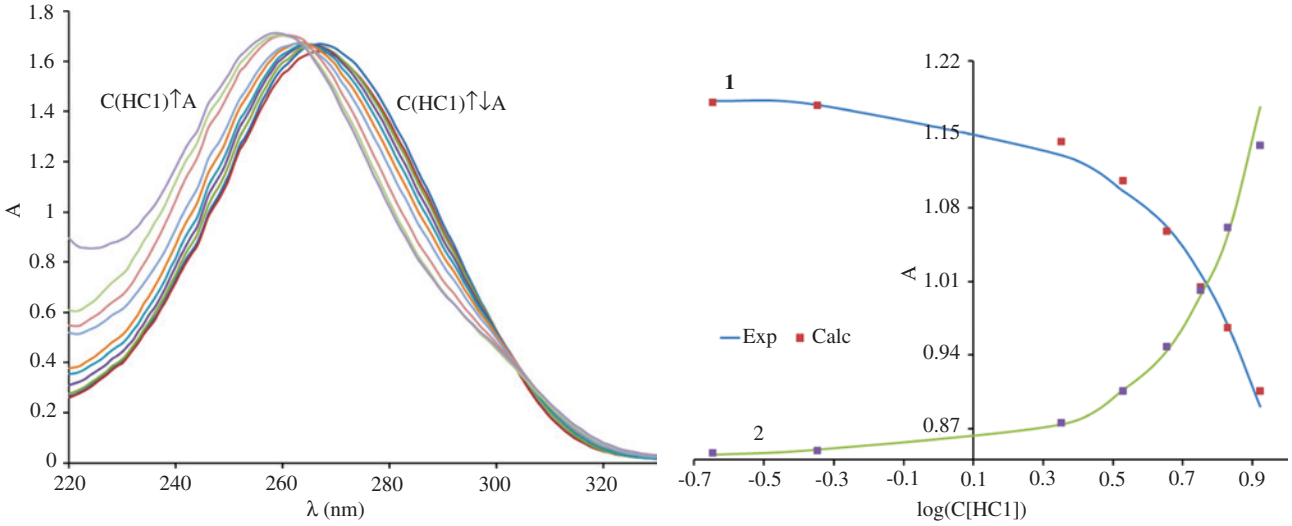


Figure 3 The UV-vis spectra of HDEAC obtained at various log([HCl]) values and absorbance as a function of log([HCl]): 2.30; 3.68; 4.60; 5.75; 6.90; 7.35; 8.27; 8.73; 9.66 м, [HDEAC] = $5 \cdot 10^{-4}$ м; Curve 1 – 288 nm, 2 – 244 nm.

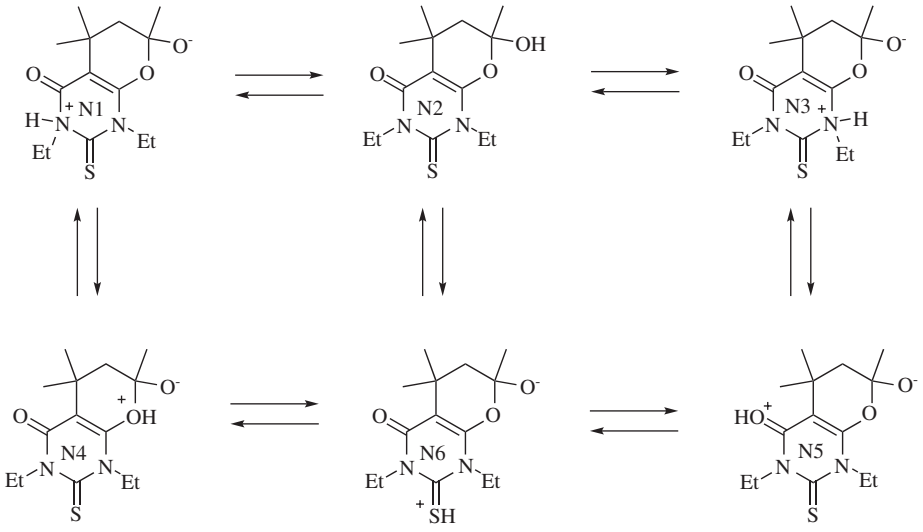
suitable because of its spectral characteristics. However, the high concentrations of HCl (>9.5 м) and the significant decrease in the absorbance relatively to the initial value indicate at dominance of the protonated form in solution.

As shown in Figure 3 the isosbestic point at 265 nm divides the spectra into two areas, first on the left with increases in the optical density (220–265 nm) due to increases in concentration of HCl and the second area on the right where the increases in HCl concentration lead to decreases in extinction (265–305 nm). Calculation at two wavelengths showed that the obtained value of pK_H (Table 2) is an invariant. Difference ($pK_H^{244\ nm} - pK_H^{288\ nm}$) equals to 0.06 logarithmic units and is associated with the presence of the neutral form of HDEAC.

The obtained value of pK_H characterizes HDEAC as a weak base, a substantial fraction of which remains not protonated even in strongly acidic solution ([H^+] > 10 м). The obtained value of the solvation coefficient m^* is smaller than 1 (0.52 for 244 nm and 0.48 for 288 nm). This indicates that HDEAC is a low-polarizable molecule with a small molecular volume [21].

Quantum-chemical calculation of the keto-enol equilibrium

As shown in Scheme 2, HDEAC may exist as six tautomers. Table 3 shows absolute and relative calculated energy



Scheme 2 Tautomerism of neutral form of HDEAC.

Table 3 Absolute and relative calculated energies of neutral tautomers of HDEAC shown in Scheme 2.

Tautomer	Absolute calculated energy (a.u.)	Relative calculated energy (kJ·mol^{-1})
N2	-1279.0911301	0.00
N1	-1279.0320577	155.09
N3	-1279.0328016	153.14
N5	-1279.0662033	65.45
N6	-1279.0659399	66.14
N4	-1279.0528350	100.54

for its tautomers. The form N2 is the most energetically favorable tautomer (Figure 4 – **2**). All other tautomers are of much greater energy.

Protonation of HDEAC can lead to the formation of 20 tautomers (Figure S2). However, as the calculation of neutral tautomers show, all isomers without proton at the terminal oxygen atom in non-thiobarbituric ring are extremely energetically unfavorable. Accordingly, only six of them (protonated isomers of N2) can actually exist (Scheme 3). Table 4 shows that the most stable protonated tautomer is P3 (Figure 4 – **1**).

Table 4 Absolute and relative calculated energies of tautomers of protonated forms of H_2DEAC^+.

Tautomer	Absolute calculated energy (a.u.)	Relative calculated energy (kJ·mol^{-1})
P3	-1279.5130177	0.00
P2	-1279.4782210	91.36
P1	-1279.4793925	88.28
P4	-1279.4954120	46.22
P5	-1279.5001458	33.80
P6	-1279.4765820	95.66

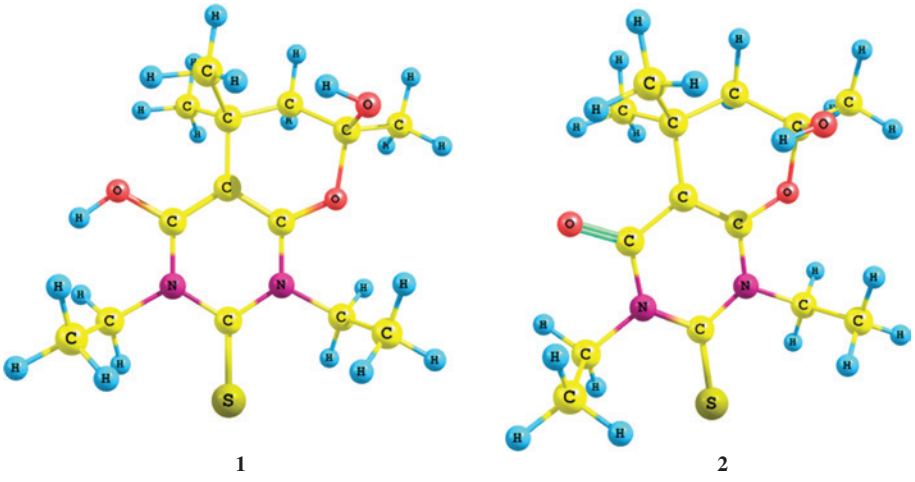
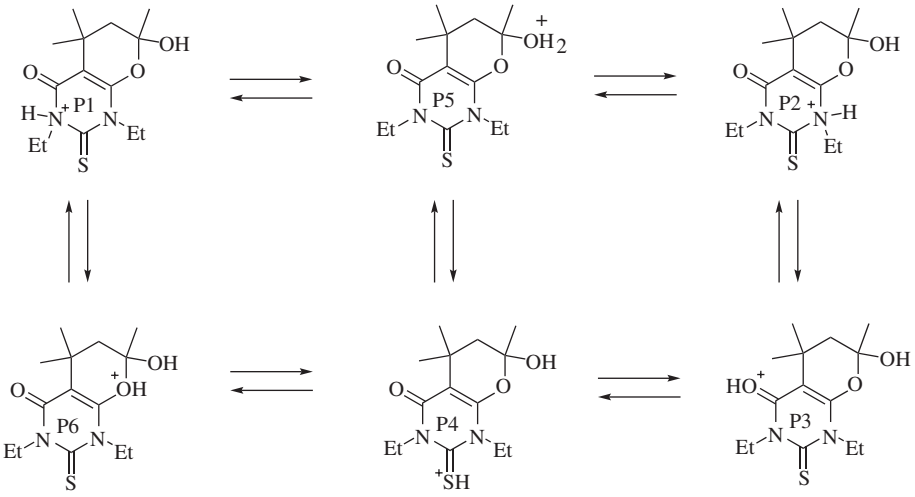


Figure 4 Optimization geometry of the protonated (**1**) and neutral (**2**) tautomers of HDEAC.



Scheme 3 Keto-enol equilibrium for protonated forms of H_2DEAC^+.

Conclusion

Acid-base properties of the bicyclic derivative of 1,3-diethyl thiobarbituric acid under various pH values were investigated. Quantum chemical calculations based on density functional theory (DFT) at level PBE0/cc-pVDZ/SMD suggest the most stable tautomers for neutral and protonated forms of the ligand.

Experimental

All chemicals were of analytical grade and used without purification. Buffer solutions in the pH range from 1.00 to 2.20 were prepared using HCl; in the pH range from 2.20 to 3.60 pH using NH_2CH_2COOH and HCl; in the pH range from 5.0 to 7.0 pH using $C_6H_8O_7$ (citric acid) and K_2HPO_4; in the pH range from 7.20 to 9.00 using Tris and HCl. The accurate desired pH values were obtained by adjusting the molarities of the buffer components in suitable amounts as previously described [22]. Synthesis of the ligand has been previously described [16].

The UV-Vis spectra were measured with the Evolution 300 scanning spectrophotometer (ThermoScientific, UK) using 1 cm quartz cells. Cell thermostating (±0.1 K) was performed with a Haake K15 thermostat connected to a Haake DC10 controller. The absorbance was measured in the range of 220–450 nm. The values of dissociation constant (pK_a) have been calculated using equation (1) [23] and the Henderson-Hasselbach equation (2) [24], where I is

$$A_i = \frac{C_{H_2L}(\varepsilon_{HL^-} \cdot K_a + \varepsilon_{H_2L}[H^+])}{K_a + [H^+]}, \quad (1)$$

$$pH = pK_a + \log I;\ I = \frac{A_i - A_{H_2L}}{A_{HL^-} - A_i}, \quad (2)$$

the ionization ratio. The Cox–Yates method (equation 3) [25] based on the excess acidity function χ [26] was used to determine the protonation constant K_H in strongly acidic solution,

$$A_i = \frac{A_{H_2L} - A_{H_3L^+}}{1 + (\frac{C_{H^+}}{K_H}) 10^{(m^*\chi)}} + A_{H_3L^+}; \quad (3)$$

where A_i, $A_{H_2L}(\varepsilon_{H_2L})$, $A_{H_3L^+}(\varepsilon_{H_3L^+})$, and $A_{HL^-}(\varepsilon_{HL^-})$ are the absorbances and molar extinction coefficients of the process solution, the free ligand, and its conjugate acid or base, respectively [27, 23]. Calculation of all equilibrium constants and molar extinction coefficients was performed using Scilab 5.5 software (http://www.scilab.org/) by means of nonlinear LSR analysis using equation (4) [28]:

$$\sum (A^{\lambda}_{exp} - A^{\lambda}_{calc})^2 \rightarrow \min. \quad (4)$$

Ab initio calculations were carried out using the GAMESS US program package [29] with a supercomputer at Moscow State University. Geometry optimization was performed by DFT with the hybrid functional PBE0 [30]. The cc-pVDZ basis set was applied to H, C, N, O, S. The solvent effects were evaluated using the SMD solvation model [31].

Acknowledgments: The research has been funded by state contract (No 3049) of Ministry of Education and Science of Russian Federation. The authors would also like to thank SFU CEJU for technical support.

References

[1] Hassan, M.; Khan, A. Synthesis and biological evaluation of new barbituric and thiobarbituric acid fluoro analogs of benzenesulfonamides as antidiabetic and antibacterial agents. *J. Fluorine Chem.* **2012**, *142*, 96–104.
[2] Laxmi, S.; Rajitha, G.; Rajitha, B. Photochemical synthesis and anticancer activity of barbituric acid, thiobarbituric acid, thiosemicarbazide, and isoniazid linked to 2-phenyl indole derivatives. *J. Chem. Biol.* **2015**, *15*, 1–7.
[3] Jaswal, A.; Sinha, N.; Bhadauria, M. Therapeutic potential of thymoquinone against anti-tuberculosis drugs induced liver damage. *Environ. Toxicol. Pharmacol.* **2013**, *36*, 779–786.
[4] Raufa, A.; Shahzada, S.; Design and synthesis of new barbituric- and thiobarbituric acid derivatives as potent urease inhibitors: structure activity relationship and molecular modeling studies. *Bioorg. Med. Chem.* **2015**, *17*, 6049–6058.
[5] Ezzaher, A.; Mouhamed, H.; Mechri, A. TBARs and non-enzymatic antioxidant parameters in Tunisian bipolar I patients. *Immuno-analyse & Biologie Specialisee*. **2012**, *27*, 315–324.
[6] Nowak, D.; Katucka, S.; Biatasiewicz, P. Exhalation of H_2O_2 and thiobarbituric acid reactive substances (TBARs) by healthy subjects. *Free Radical Biol. Med.* **2011**, *30*, 178.
[7] World Health Organization. WHO Model List of Essential Medicines. Retrieved 2013–12 (http://www.who.int/medicines/publications/essentialmedicines/en/).
[8] Tabassum, S.; Siddiqi, K.; Khan, N. H.; Kureshy, R. I.; Zaidi, S. A. A. Studies on complexes of thiobarbituric and 5,5-diethylbarbituric acid with Lanthanide ions. *Indian J. Chem.* **1987**, *26*(A), 489.
[9] Moamen, S. R.; El-Korashy, S. A.; Ahmed, A. S. A convenient method for the preparation of barbituric and thiobarbituric acid transition metal complexes. *Spectrochimica Acta Part A: Mol. Biomol. Spectr.* **2008**, *71*, 1084–1094.
[10] Masoud, M. S.; El-Dessouky, M. A. Transition metal (disubstitutedphenylazo)-barbituric and thiobarbituric acid complexes. *Transition Metal Chem.* **1990**, *15*, 443–448.
[11] Li, X.; Wu, Y.; Gu, D.; Gan, F. Synthesis, spectral and thermal properties of some transition metal(II) complexes with a novel ligand derived from thiobarbituric acid. *J. Therm. Anal. Calorim.* **2009**, *98*, 387–394.
[12] Golovnev, N. N.; Molokeev, M. S. The 5-(isopropylidene)-2-thiobarbituric acid: Preparation, crystal structure, thermal stability and IR-characterization. *J. Mol. Struct.* **2014**, *1068*, 216–221.
[13] Masouda, M. S.; El-Marghany, A. Spectral, coordination and thermal properties of 5-arylidene thiobarbituric acids. *Spectrochim. Acta Part A: Mol. Biomol. Spectr.* **2013**, *107*, 179–187.
[14] Akiyama, S.; Nakashima, K.; Nakatsuji, S.; Suzuki, M. Synthesis and properties of 5-substituted 2-thiobarbituric acid dyes. *Dyes Pigments*. **1987**, *8*, 459–463.
[15] Brewer, A. D.; Minatelli, J. A.; Plowman, J,; Paull, K. D. Narayanan VL.; 5-(N-phenylcarboxamido)-2-thiobarbituric acid (NSC

336628), a novel potential antitumor agent. *Biochem. Pharmacol.* **1985**, *34*, 2047–2050.
[16] Golovnev, N. N.; Molokeev, M. S. New class of bicyclic compounds derived from thiobarbituric acid with representative compound 1,3-diethyl-7-hydroxy-5,5,7-trimethyl-2-thioxo-1,2,3,5,6,7-hexahydro-4H-pyrano[2,3-d]pyrimidin-4-one. Preparation, crystal structure, mass spectrometry and IR spectroscopy. *J. Mol. Struct.* **2015**, *1102*, 101–107.
[17] Ivin, B. A.; Slesarev, V. I.; Smorigo, N. A. Prototropnie prevrascheniya i kislotno-osnovnie svoistva 2-tiobarbiturovoy kisloti. *Rus. J. Org. Chem.* **1974**, *10*, 1968–1973.
[18] Blais, M. S.; Enea, O.; Berthon, G. Relations structure-reactivite grandeurs thermodynamiques de protonation geterocycles satures et non satures. *Thermochim. Acta*. **1977**, *20*, 335.
[19] Maynert, E. W.; Washbur, E. The Reactions of some barbituric acid derivatives in concentrated sulfuric acid. *J. Am. Chem.* **1953**, *75*, 700–704.
[20] Street, H. V.; McMartin, C. Action of concentration sulphuric acid on 5,5-substined barbituric acid derivatives. *Clin. Chim. Acta* **1964**, *9*, 301–308.
[21] Petrov, A. I.; Lutoshkin, M. A.; Taydakov, I. V. Aqueous Complexation of Y^{III}, La^{III}, Nd^{III}, Sm^{III}, Eu^{III}, and Yb^{III} with some heterocyclic substituted β-diketones. *Europ. J. Inorg. Chem.* **2015**, *6*, 1074–1082.
[22] Eliseeva, S. V.; Bünzli, J.-C. G. Rare earths: jewels for functional materials of the future. *New J. Chem.* **2011**, *35*, 1165.
[23] Leggett, D. J. Computational Methods for the Determination of Formation Constants; Plenum Press; New York, **1985**, 478.
[24] Tiwari, S.; Ghosh, K. K.; Mixed micellization properties of cationic monomeric and gemini surfactants. *J. Chem. Eng. Data.* **2010**, *55*, 1153.
[25] Cox, R. Acidity functions: an update. *Can. J. Chem.* **1983**, 61, 2225–2229.
[26] Cox, R. The excess acidity of aqueous HCl and HBr media. An improved method for thecalculation of X-functions and H. *Can. J. Chem.* **1981**, *59*, 2023–2028.
[27] Celik, H.; Büyükaga, M.; Celebier, M.; Acar, T.;Bay-mak M. Determination of pKa values of some benzoxazoline derivatives and the structure-activity relationship. *J. Chem. Eng.* **2013**, *58*, 1589.
[28] Grebenyuk, S. A.; Perepichka, I. F.; Popov, A. F. Evaluation of the parameters of 1:1 charge transfer complexes from spectrophotometric data by non-linear numerical method. *Spectrochim. Acta Part A*. **2002**, *58*, 2913–2923.
[29] Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S. General atomic and molecular electronic structure system. *Comput. Chem.* **1993**, *14*, 1347.
[30] Adamo, C.; Barone, V. Toward reliable density functional methods without adjustable parameters: The PBE0 model. *J. Chem. Phys.* **1999**, *110*, 6158.
[31] Marenich, A. V.; Cramer, C. J.; Truhlar, D. G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B*. **2009**, *113*, 6378–6396.

Supplemental Material: The online version of this article (DOI: 10.1515/hc-2016-0011) offers supplementary material, available to authorized users.