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# Nitrosyl iron complexes with enhanced NO donating ability: synthesis, structure and properties of a new type of salts with DNIC dication $[\text{Fe}(\text{SC}(\text{NH}_2)_2)_2(\text{NO})_2]_2^+$

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Novel nitrosyl iron complexes  $[\text{Fe}(\text{SC}(\text{NH}_2)_2)_2(\text{NO})_2]\text{SO}_4 \cdot \text{H}_2\text{O}$  (**I**) and  $[\text{Fe}(\text{SC}(\text{NH}_2)_2)_2(\text{NO})_2]_2[\text{Fe}_2(\text{S}_2\text{O}_3)_2(\text{NO})_4]$  (**II**) have been synthesized by the reactions of  $\text{FeSO}_4$  and  $\text{Na}_2[\text{Fe}_2(\text{S}_2\text{O}_3)_2(\text{NO})_4]$ , respectively, with acidic solutions of thiocarbamide in water. The structure and properties of **I** and **II** were studied by X-ray analysis, Mössbauer, IR-, and EPR- spectroscopy and amperometry. Both complexes are characterized by a prolonged NO generation without additional activation in aqueous anaerobic solutions, similar to organic NO donor diethylene triamine; however, they are more effective: at pH 7 NO amount is ~32.6 and ~31.8 nM/mol of the complex for **I** and **II**, respectively. The obtained results show feasibility for the synthesis of water soluble hybrid nitrosyl NO-generating complexes, which contain the NO groups both in the cationic and anionic sublattices and provide the control of the NO release kinetics.

## Introduction

Recently, the search for medicines for NO therapy of socially significant diseases has been focused on two basic directions: i) anti NO therapy based on the use of traps for excess endogenous NO and inhibition of superactivity of cellular NO synthase and ii) NO therapy based on the use of exogenous NO donors.<sup>1</sup> The latter ones are of particular interest because this direction opens wide possibilities for using NO as a structurally small pharmacological molecule with a huge potential of anti-arteriosclerosis, wound healing, anticancer, antibacterial and other activities. Design of NO delivery systems, i.e., synthesis of new representatives of low molecular NO-donors (nitrates, nitrites, nitramines, 1,2-diazet-1,2-dioxides, guanidines, furoxans, oximes, sydnonimines, diazeniumdiolates (NONOates), S-nitrosothiols, nitrosyl metal complexes<sup>2</sup>) and development of macromolecular NO-releasing scaffolds (dendrimers, polymers/films, particle-type and coating platforms for NO delivery) becomes more and more important in NO biomedical application.<sup>3,4</sup>

The most of available low molecular NO donors have essential drawbacks: organic and inorganic nitrates induce nitrate tolerance;<sup>5,6</sup> sydnonimines yield superoxide anion together with NO release, thus forming carcinogenic peroxynitrite (ONOO<sup>-</sup>) and arising pathogenic conditions *in vivo*;<sup>7-10</sup> wide application of NO-ates,<sup>11-13</sup> effective class of organic NO donors, is limited by their high cost; S-nitrosothiols<sup>14,15</sup> are unstable upon storing and cytotoxic because in the presence of redox agents they form thiyl radicals recombining fast to form disulfides and nitrosonium ions, which hydrolyze to yield nitrite anions. Thus, with the exception of cyanonitrosyl metalates  $[\text{M}(\text{CN})_x(\text{NO})_y]^n$ ,<sup>16-18</sup> which might accumulate cyanides upon decomposition, the most of the available compounds and pharmaceutical substances able to control NO amount *in vivo* are synthetic organic compounds. However, the discovery of intracellular nitrosyl non-heme proteins participation in the NO biochemistry<sup>19-24</sup> brought a rapid increase in a new line of investigations based on the application of synthetic analogues of these highly reactive nitrosyl cellular intermediates as NO-releasing prodrugs.

Mono- and binuclear nitrosyl iron complexes with functional sulfur-containing ligands have some advantages over other low-molecular NO-donating agents: these compounds release NO at physiological pH values without any activation (photo, thermal or redox), they can be isolated in the crystalline state, and no toxic products form upon their decomposition, this being favorable for biological and medical investigations and applications.<sup>25-27</sup> These complexes are models of the active sites of nitrosyl non-heme iron-sulfur proteins existing in all living organisms, from bacteria to mammals.<sup>21,28</sup> Variation of functional sulfur-containing ligands (R) in the synthetic models of  $[\text{Fe}-2\text{S}]$  and  $[\text{2Fe}-2\text{S}]$  proteins,

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and of conditions of the synthesis provides more thorough fundamental knowledge of the structure of  $\{\text{RS}_2\text{Fe}(\text{NO})_2\}$  unit,<sup>25,29-44</sup> while investigation of these compounds pharmacological activity, in addition to the known NO properties, opens the possibilities for practical application of mimetics of nitrosyl non-heme proteins as effective NO-generating compounds for the therapy of socially significant diseases.<sup>45-52</sup>

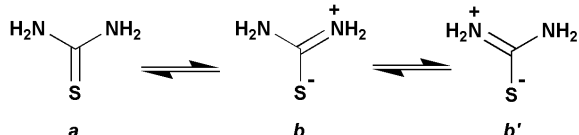
In the present work, thiocarbamide having a wide range of biological activity, including antituberculosis, antibacterial<sup>53,54</sup> and antithyroid<sup>55</sup> properties, was used as a functional sulfur-containing ligand. Besides, the thiocarbamide molecule has a complex resonance structure, and in the solution the equilibrium can shift either to the thiol or thione form depending on the acidity, this being responsible for structural variety of this ligand and the related complexes.

The work is aimed at the development of the method for synthesis of water soluble salts with mononuclear dinitrosyl cations  $[\text{Fe}(\text{SR})_2(\text{NO})_2]^+$  with R being thiocarbamide, and the examination of their structure and properties by X-ray method, Mössbauer, IR-, and EPR- spectroscopy and amperometry. NO-donating ability of complexes **I** and **II** was studied as compared to that of nitrosyl iron complexes  $\text{Q}_2[\text{Fe}_2(\text{S}_2\text{O}_3)_2(\text{NO})_4]$  with non-functional cation  $\text{Q} = \text{Na}^+$  (**III**);  $(\text{CH}_3)_4\text{N}^+$  (**IV**);  $(\text{C}_3\text{H}_7)_4\text{N}^+$  (**V**);  $(\text{C}_4\text{H}_9)_4\text{N}^+$  (**VI**) we obtained earlier,<sup>56,57</sup> and to commercial NO donor diethylene triamine (**VII**), a representative of NONO-ate class, which is widely used in clinical investigations.

## Results and discussion

### Description of preparation of **I** and **II** complexes

Acidic solutions of functional sulfur-containing ligand thiocarbamide were used for synthesis of complexes **I** and **II**. The structure of thiocarbamic acid diamide can be presented by resonance thioamido- and thioimido-forms (Scheme 1), with the latter one's contribution being predominant. This is responsible for its chemical properties, i.e., in reactions of thiourea the S atom, not N atom (like in urea), acts as a nucleophilic center.



Scheme 1 Thioamide (**a**) and thioimide (**b**, **b'**) forms of thiocarbamide

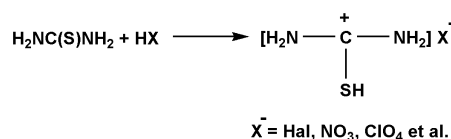
Thiocarbamide forms salts with halogens, mineral and organic acids, as well as with some bases (Scheme 2):

Single crystals of **I** were obtained by the interaction of the NO excess with aqueous mixture of iron (**II**) sulfate and thiourea hydrochloride according to Scheme 3 (a). The yield of single crystals was low. Complex **II** was obtained by the replacement of the thiosulfate ligands of sodium salt of tetranitrosyl binuclear anion by the functional ligand thiocarbamide in the presence of the reducing agent (sodium thiosulfate) in slightly acidic solution, according to Scheme 3 (b).

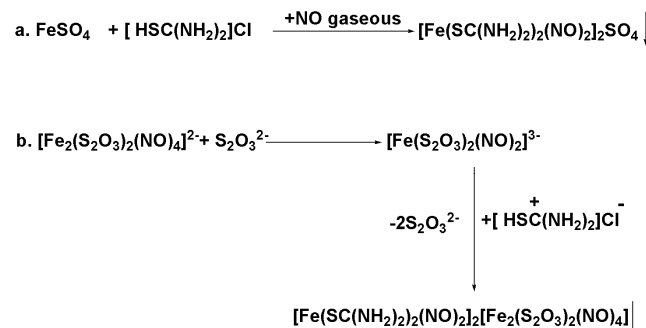
Complex **II** was isolated as single crystals suitable for X-ray analysis with high yield. Crystals **I** and **II** are stable upon storing in the inert atmosphere at  $T = -8$ – $-10^\circ\text{C}$  for long time ( $> 6$  months), this being confirmed by IR spectra of the samples. **I** and **II** are

soluble in water and other polar solvents.

Scheme 2



Scheme 3



### Description of the crystal structures

As follows from X-ray analysis data, the asymmetric unit of the structure of **I** (Fig. 1) is presented by two cations (**1** and **2**) (marked according to the numeration of the Fe atoms in Fig. 1) of the dinitrosyl iron complex (DNIC) with thiocarbamide ligand, sulfate anion and water molecule (O(w1)) with site occupancy  $\sim 0.43$ . The iron atoms in the cationic complexes are coordinated according to the numeration of the S atoms in Fig. 1) and two NO groups, and they have distorted tetrahedral structure. Thiocarbamide ligands **S1-S4** are planar and are in anti-orientation to each other with angles  $75.1^\circ$  (**S1**),  $57.0^\circ$  (**S2**),  $65.7^\circ$  (**S3**),  $54.9^\circ$  (**S4**) to planes  $\text{S}(1)\text{Fe}(1)\text{S}(2)$  and  $\text{S}(3)\text{Fe}(2)\text{S}(4)$ , respectively, as distinct from cationic DNIC with  $\text{Cl}^-$  counter ion we have obtained earlier.<sup>61</sup> The sulfur-carbon bond lengths lie in the range of 1.732(2)–1.739(2) Å, this being consistent with the length of single C-S bond, which is longer than double S=C bond (1.684 Å).<sup>62</sup>

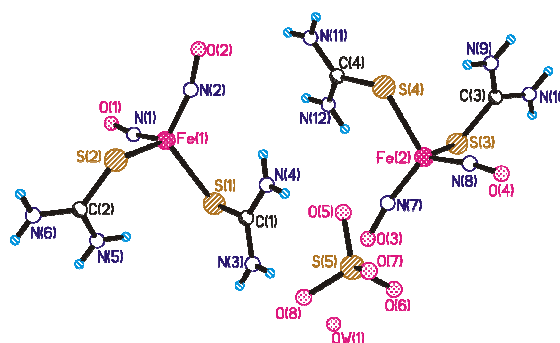


Fig. 1 The asymmetric unit of complex **I**. Selected bond distances (Å) and angles (deg): Fe(1)-N(1) 1.684(2), Fe(2)-N(8) 1.688(2), Fe(1)-N(2) 1.695(2), Fe(2)-N(7) 1.689(2), Fe(1)-S(2) 2.2907(7), Fe(2)-S(3) 2.2997(7), Fe(1)-S(1) 2.3250(7), Fe(2)-S(4) 2.3228(7), N(1)-O(1) 1.165(3), N(8)-O(4) 1.162(3), N(2)-O(2) 1.169(3), N(7)-O(3) 1.168(3), O(1)-N(1)-Fe(1) 168.9(2), O(4)-N(8)-Fe(2) 172.7(3), O(2)-N(2)-Fe(1) 164.9(2), O(3)-N(7)-Fe(2) 170.3(2), N(1)-Fe(1)-N(2) 117.7(1), N(8)-Fe(2)-N(7) 120.3(1), S(2)-Fe(1)-S(1) 108.83(3), S(3)-Fe(2)-S(4) 111.17(3).

**Table 1** X-ray crystallographic data **I** and **II**

Compounds	<b>I</b>	<b>II</b>
formula	C <sub>4</sub> H <sub>16</sub> N <sub>12</sub> O <sub>8.43</sub> S <sub>5</sub> Fe <sub>2</sub>	C <sub>4</sub> H <sub>16</sub> N <sub>16</sub> O <sub>14</sub> S <sub>8</sub> Fe <sub>4</sub>
formula weight	639.15	992.21
crystal system	orthorhombic	triclinic,
space group	Pbca	P -1
a, Å	12.6104(2)	6.2712(3)
b, Å	10.3501(2)	10.4132(5)
c, Å	37.1231(8)	12.1912(5)
α, deg	90.0	86.462(4)
β, deg	90.0	88.363(4)
γ, deg	90.0	87.613(4)
V, Å <sup>3</sup>	4845.3(2)	793.65(6)
Z	8	1
ρ <sub>caclcd</sub> , g/cm <sup>3</sup>	1.752	2.076
μ/mm <sup>-1</sup>	1.685	2.399
crystal size, mm	0.3 x 0.2 x 0.2	0.35 x 0.2 x 0.2
θ range, deg	3.03 to 34.00	3.25 to 36.09
R1(wR2) [I>2σ(I)]	0.0583(0.0908)	0.0415 (0.0787)
R1(wR2)	0.0725(0.0945)	0.0622 (0.0787)

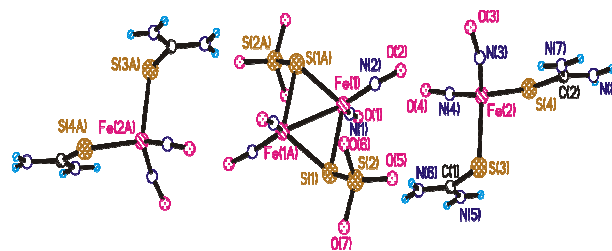
Furthermore, the average value of the C-N bond lengths in **S1-S4** ligands of complexes **1** and **2** is 1.313(3) Å (as compared to the length of single C-N bond 1.43 (aromatic) – 1.48 Å (alkyl)<sup>63</sup>). This points to the preferably zwitterion thioimide form of the thiocarbamide ligand (Scheme 1), as it was first shown earlier.<sup>61</sup>

The bond lengths in the nitrosyl groups in DNIC **1** and **2** differ inconsiderably (Table S1†), and average values of the Fe-N and N-O bond lengths are 1.689(2) and 1.166(3) Å, respectively. However, the valence angles at the nitrogen atoms of the nitrosyl groups differ more essentially (by 4.0° in **1** and 2.4° in **2**, this being outside of the experimental errors).

Complex **II** is the first compound containing two different structural types of sulfur-nitrosyl iron complexes in the same crystal (Fig. 2): the cations of mononuclear dinitrosyl iron with thiocarbamide ligand and the anion of binuclear tetranitrosyl iron with the thiosulfate ligand of “μ-S” type. In the unit cell, there is one centrosymmetric anion [Fe<sub>2</sub>(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub>(NO)<sub>4</sub>]<sup>2-</sup> per two cations [Fe(SC(NH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>(NO)<sub>2</sub>]<sup>+</sup>, which are connected with each other through the center of symmetry. The structure of the anionic complex is similar to that in previously studied complexes Q<sub>2</sub>[Fe<sub>2</sub>(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub>(NO)<sub>4</sub>], Q=Na<sup>+</sup>, Me<sub>4</sub>N<sup>+</sup>, n-Bu<sub>4</sub>N<sup>+</sup>, (PPh)<sub>4</sub>N<sup>+</sup>,<sup>56</sup> therefore, this work is focused on the analysis of the molecular structure of the cation containing thiocarbamide, and the crystalline structure of compound **II** as a whole. Similar to complex **I**, the iron atom in the cation of complex **II** has a distorted tetrahedral coordination. It is coordinated by two thiourea ligands (**A**, **B** in Fig. 2) and two NO groups.

The bond lengths in the nitrosyl groups coordinated to the Fe(2) atom are essentially similar (Table S1†), and are equal to those in the structure of **I**, while the valence angles at the nitrogen atoms differ considerably. The value of O(4)-N(4)-Fe(2) angle is close to the average value for similar angles in complex **I** (170.1(2)), while the other angle, O(3)-N(3)-Fe(2), is much less linear, 162.5(2)°, so the difference in the angles values is 7.6(2)°. This difference correlates with the increase of the N(3)-O(3) bond length to 1.175(2) Å as compared to the maximum value in

40



**Fig. 2** The general view of complex **II**. Selected bond distances (Å) and angles (deg): Fe(2)-N(3) 1.688(2), Fe(2)-N(4) 1.689(2), Fe(2)-S(4) 2.3130(5), Fe(2)-S(3) 2.3353(6), N(4)-O(4) 1.163(2), N(3)-O(3) 1.175(2), O(4)-N(4)-Fe(2) 170.1(2), O(3)-N(3)-Fe(2) 162.5(2), N(3)-Fe(2)-N(4) 114.18(9), S(4)-Fe(2)-S(3) 109.42(2).

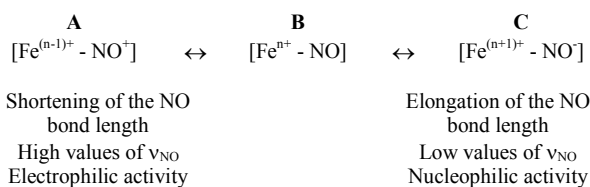
complex **I** (1.169(2)Å, Table S1†). This feature of the geometrical structure of the N(3)O(3) nitrosyl group can be due to a weak intramolecular H-bond N7-H7B...N3: d(N7...N3)=3.17 Å, d(H7B...N3)=2.39 Å, angle N7-H7B...N3 151.0°. The SC(NH<sub>2</sub>)<sub>2</sub> ligands are planar and in anti-orientation to the S(3)Fe(2)S(4) plane, similar to complex **I**, however, at different angles, 85.2° (**A**) and 86.1° (**B**). Considering a relative orientation of the thiocarbamide ligands in complexes **I** and **II**, in this type of cationic DNIC an essential freedom of rotation about the S-C bond can be noted. For example, as distinct from complex **II** with **A** and **B** ligands planes being non-parallel with angle 8.7°, the planes of ligands **S1**, **S2** and **S3**, **S4** in complex **I** lie at angles 89.6 and 70.6°, respectively. However, in the similar complex with Cl<sup>-</sup> counter ion,<sup>61</sup> the thiocarbamide ligands, in contrast to the complexes discussed in this work, are in syn orientation with respect to the S-Fe-S', at 34.5° angle to each other. Besides, the crystalline structure was shown to affect not only the molecule conformation of both cations [Fe(SC(NH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>(NO)<sub>2</sub>]<sup>+</sup>, but also the structure of the thiocarbamide ligands. The structure of **B** ligand is similar to that of such ligands in compound **I**, while in **A** the S(3)-C(1) bond is elongated, and the N(6)-C(1) bond is shortened (Table S1†). Apparently, this can be due to strong intermolecular H-bonds with participation of N(6)...O(5) 2.86 Å, N(6)...O(6') 2.88 Å. This is consistent with the elongation of all S-O distances in the thiosulfate ligand of the anionic complex (1.446(1)-1.466(2) Å) as compared to the average value of the S-O bond length for complexes of such type (1.43(1) Å). Other nitrogen atoms of the thiocarbamide ligands do not participate in such shortened intermolecular contacts.

The crystalline structures of both compounds have many intermolecular hydrogen bonds forming a continuous network, with the participation of the amino groups of the thiocarbamide ligands of cationic DNIC and the oxygen atoms of the sulfate anions (**I**) and the thiosulfate ligand in the complex anion (**II**) (Fig. S1, S3†). Both structures are also characterized by shortened S...S contacts between cationic DNIC, however, in the crystalline structure of **I** these contacts form a chain of the cationic complex molecules, while in the crystalline structure of **II** the cationic complex molecules form dimer (Fig. S2, S4†).

As mentioned earlier, in mononuclear neutral<sup>64</sup> and cationic<sup>61</sup> DNIC there is a difference in the Fe-S bond lengths. In **I** and **II** such difference was also detected. The Fe-S bond lengths

in cations **1** and **2** differ in the average of 0.029 Å, and in **II** of 0.022 Å, this being outside of the experimental error (0.0021 Å) in the determination of the Fe-S bond lengths (Table S1†). As follows from precision X-ray analysis and topological analysis of the electron density distribution<sup>65,66</sup>, the Fe-S bond in DNIC is an acceptor or a strongly polarized covalent bond.

Scheme 4



The elongation (shortening) of the Fe-S bond should be accompanied by shortening (elongation) of the adjacent S-C bond and by the increase of the contribution of the thione (thiol) forms in the ligand structure; however, this is not observed for the studied complexes. The obtained data suggest that the ligand structure is determined not only by donor-acceptor interactions, but also depends strongly on intermolecular and intramolecular interactions in the complex structure. In available sulfur-nitrosyl iron complexes, frequencies of the NO valence vibrations lie in the range of 1657–1807  $\text{cm}^{-1}$ <sup>25</sup> depending on the geometry and the electron density distribution in the Fe-NO fragment (Scheme 4).

The structure of the NO group was shown to be close to a neutral radical form.<sup>65,66</sup> The charge on the NO groups is almost zero (-0.2-0.3e) (type **B**, Scheme 4), the iron atom has a high-spin  $d^7 \text{Fe}^{+1}$  configuration, with a strong intramolecular exchange interaction with the NO radicals, thus leading to pairing the two of three  $\text{Fe}^{+1}$  unpaired electrons. Depending on the structural type of the nitrosyl groups and the nature of the functional sulfur containing ligand, a shift of the NO group absorption bands (AB) in the IR spectra is often experimentally observed to higher values (for type **A**) or to lower values (type **C**),<sup>67</sup> this being consistent with the bond lengths in the NO groups. For complex **I** the absorption bands lie in the frequency region of 1808-1734  $\text{cm}^{-1}$ . Complex **II** is characterized by the AB shift to a more positive region (1822 - 1732  $\text{cm}^{-1}$ ) as compared to complex **I** and the salts with the thiosulfate anion  $[\text{Fe}_2(\text{S}_2\text{O}_3)_2(\text{NO})_4]^{2-}$  (1787  $\text{cm}^{-1}$ , 1743  $\text{cm}^{-1}$ ).<sup>56</sup> Thus, the AB shift to more positive values points to the increase of the positive charge on the NO group in **II** and its higher electrophilic activity and nitrosylating ability as compared to complex **I**. The AB position in the IR spectra correlates with the lengths of the N-O bond: from X-ray analysis, the average N-O bond lengths in dinitrosyl mononuclear cations of **I** and **II** are 1.166 Å and 1.169 Å, respectively. For comparison, in anionic DNIC the average N-O bond length is 1.180 Å, while in neutral complexes it is 1.166 Å.

### Spectroscopic studies of **I** and **II** complexes

The Mössbauer spectrum for the crystals of **I** is a single doublet (Fig. S5†). This points to the structural equivalence of two iron atoms in **I** molecule. The values of the Mossbauer spectrum

parameters at 293 K (isomeric shift  $\delta_{\text{Fe}} = 0.184(1)$  mm/s, quadruple splitting  $\Delta E_Q = 1.165(1)$  mm/s, and width of absorption lines  $\Gamma = 0.246(1)$  mm/s) for **I** are close to those for the neutral DNIC with 1,2,4-triazole-3-thioly (  $\delta_{\text{Fe}} = 0.188(1)$  mm/s,  $\Delta E_Q = 1.118(1)$  mm/s)<sup>64</sup> and monocationic thiocarbamide DNIC with  $\text{Cl}^-$  counter ion<sup>61</sup> ( $\delta_{\text{Fe}} = 0.194(1)$  mm/s,  $\Delta E_Q = 1.167(1)$  mm/s) and <sup>68</sup>. The Mössbauer spectrum of complex **II** is a double symmetric quadrupole doublet (Fig. S6†) with parameters  $\delta_{\text{Fe}} = 0.219(1)$ ,  $\Delta E_Q = 1.08(2)$ ,  $\Gamma = 0.250(1)$  mm/s for the cationic part of the complex, and  $\delta_{\text{Fe}} = 0.115(1)$ ,  $\Delta E_Q = 1.33(1)$ ,  $\Gamma = 0.230(1)$  mm/s for the anionic part. These parameters are close to those for the series of previously studied binuclear thiosulfate complexes **III-VI**.<sup>56</sup> The ratio of the intensities of the spectral components is 1:1, this being in compliance with X-ray data.

The problem of correct description of the electronic structure of such type of DNIC by DFT methods was mentioned in <sup>68</sup>. The strong delocalization and correlation of electrons in the  $\text{S}_2\text{Fe}(\text{NO})_2$  structural unit, and unusual nature of the Fe-NO chemical bonds in these complexes, which is more similar to antiferromagnetic coupling, are a real challenge to the contemporary DFT methods of quantum chemistry.

Quantum chemical calculations were performed with the BP86, TPSS, B3LYP and TPSSh density functionals. All density functionals, besides in particular common B3LYP, predict the complex geometry in a good agreement with the experiment. The TPSSh/TZVP calculated geometries, Mössbauer (isomer shifts,  $\delta$ ) and quadrupole splittings ( $\Delta E_Q$ ) and EPR (anisotropic and isotropic g-tensor values) spectroscopic parameters are in a good agreement with the experiment (Table 2, S5 and S6†). The calculated IR frequencies of the NO groups are slightly overestimated, but it is not so crucial because it is a common trend.<sup>69</sup>

**Table 2** Comparison of the calculated structural and spectroscopic parameters for **X** with the experimental values

	Fe-N, Å	N-O, Å	Fe-S, Å	$\delta$ , mm/s	$ \Delta E_Q $ , mm/s	$\nu(\text{NO})$ , $\text{cm}^{-1}$	$g_x, g_y, g_z$ ( $g_{\text{iso}}$ )
Calc.	1.708, 1.709	1.165, 1.166	2.318, 2.343	0.217	1.029	1858*, 1807	2.013, 2.037, 2.043 (2.031)
Expt. <b>I</b>	1.684(2)- 1.695(2)	1.162(3)- 1.169(3)	2.2907(7)- 2.3250(7)	0.184(1)	1.165(1)	1808- 1734	2.015, 2.032, 2.038 (2.035)
Expt. <b>II</b>	1.688(2), 1.689(2)	1.163(2), 1.175(2)	2.3130(5), 2.3353(6)	0.219(1)	1.08(2)	1822- 1732	-

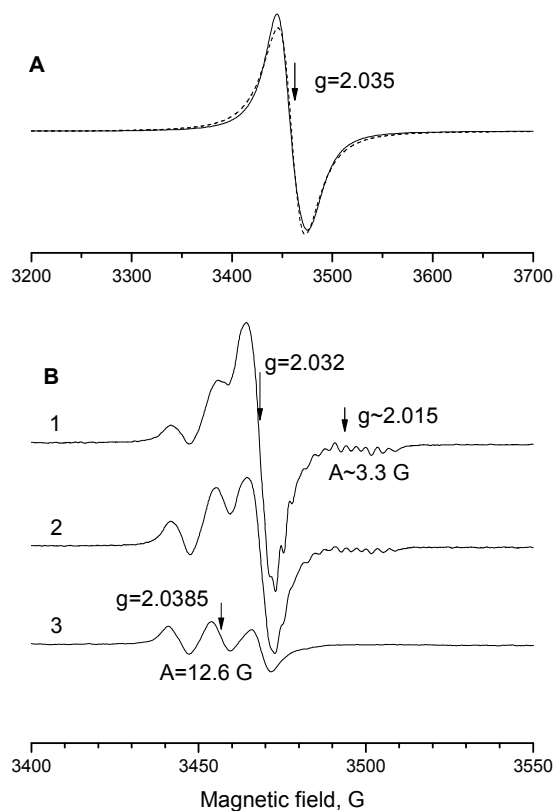
\*Usually, for DFT calculated IR frequencies use the scaling factors ~ 0.97-0.98 depending on the theory level (1802-1820, 1752-1770).

In general, the cationic DNIC under consideration has the Mössbauer spectroscopic parameters and the electronic structure close (Fig. S7†) to those in recently studied DNIC.<sup>68</sup> In our opinion, suggested<sup>68</sup> description (the superposition of  $\text{Fe}^{\text{III}}-(^3\text{NO})_2^{2-}$  and  $\text{Fe}^{\text{II}}-(^3/2\text{NO})_2^-$ ) of the electronic structure is not consistent with almost zero or slightly positive charged NO groups<sup>65,66</sup> and quasi-linear geometry of the Fe-NO fragments.<sup>67</sup> Perhaps, this problem can be solved by using a high-level

multiconfigurational MO *ab initio* calculations.

Fig. 3 shows the EPR spectra of the powder of **I** and its solution in DMSO at different time points after dissolution. The number of paramagnetic centers per a complex is 2.3 in the powder and 0.44, 0.30 and 0.08 in the solution immediately after dissolution and in 1.5 and 20 hours, respectively. The EPR line form for the powder is close to Lorentz one, and no anisotropy of g-factor is observed. This can be explained by considerable exchange interaction between the iron atoms in polycrystals. In the solution, the superposition of EPR spectra of at least three paramagnetic centers is observed with g-factors 2.0385, 2.032 and  $g \sim 2.015$ . The decrease of the intensity of EPR spectra is caused by detachment of at least one NO group accompanied by secondary reactions.

In Fig. 4 the EPR spectra of the powder of **II** and its solution in DMSO at different time points after dissolution are presented. The number of paramagnetic centers per a complex is 1.7 in the powder and 0.75, 0.45 and 0.15 in the solution immediately after dissolution and in 20 and 44 hours, respectively. In the EPR spectrum of **II** powder, unlike in **I** powder, the anisotropy of g-factor is observed, thus pointing to weaker exchange interactions between the paramagnetic centers as compared to **I**. This is in compliance with X-ray analysis data, i.e., in **I** the shortest distance between the Fe(2)···Fe(2) atoms is 5.06 Å, while in **II** structure the minimum distance between the Fe(2)···Fe(2) atoms is 5.86 Å.



**Fig. 3** The EPR spectra of **I** at room temperature. (A) - the polycrystalline powder (solid line) and simulation by Lorentz line, (B) - solution in

DMSO immediately after dissolution (1), in 1.5 hours (2) and in 20 hours (3).

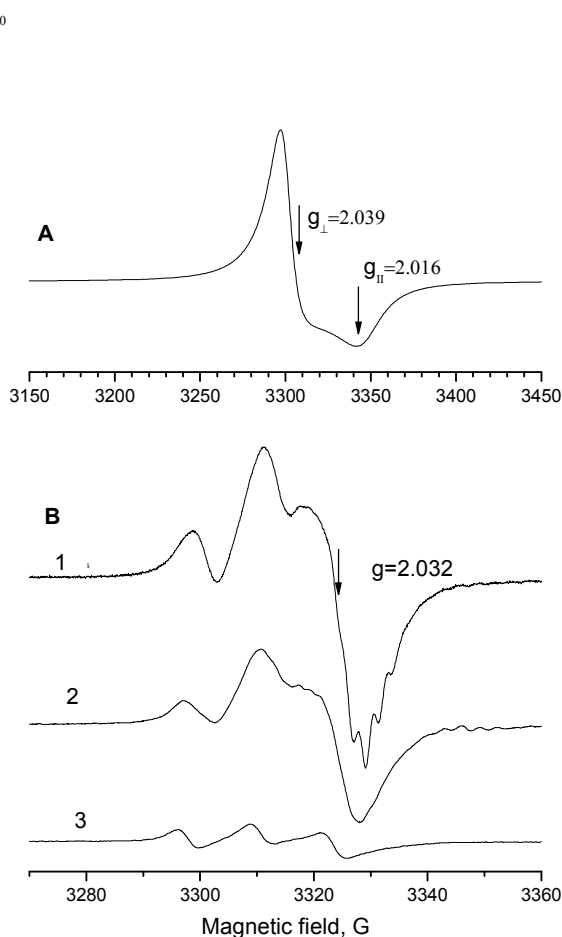
The Fe...Fe distances in **II** are close to those in the complex we had studied earlier,<sup>61</sup> which was also characterized by g-factor anisotropy. The EPR spectra of complexes **I** and **II** are similar, however, **II** decomposes slower.

### NO-releasing ability of complexes **I** and **II**

To analyze and interpret the experimental data on NO activity of complexes **I-VII**, the results of direct anaerobic electrochemical measurements of NO amount released under the complexes hydrolysis were considered. The experimental data were compared with those for diethylene triamine NO adduct (compound **VII**), the representative of NONO-ate family, effective NO donors that are used in clinics (Table 3).

**Table 3** The average maximum amount of NO generated by nitrosyl iron complexes **I-VII** in anaerobic water solutions at pH 7 and T=25°C

Complex	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>	<b>V</b>	<b>VI</b>	<b>VII</b>
NO amount, nM	32.6	31.8	3.3	10.3	4.5	6.8	4.0



**Fig. 4** The EPR spectra of **II** at room temperature. (A) - the

polycrystalline powder, (B) solution in DMSO immediately after dissolution (1), in 20 hours (2) and in 44 hours (3).

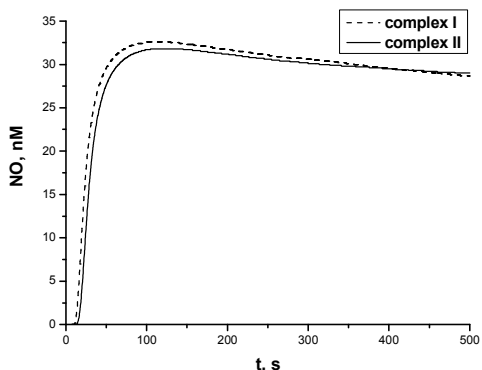


Fig. 5 The time dependence of NO amount generated by complexes **I** and **II** in aqueous solutions at pH 7 and  $T = 25^{\circ}\text{C}$  in anaerobic conditions (the complexes concentration is  $0.4 \cdot 10^{-5}\text{M}$ ).

Fig. 5 shows the characteristic time dependences of NO amount released into the solution upon the decomposition of the studied complexes in anaerobic water solutions at pH=7 and  $25^{\circ}\text{C}$ . It can be seen that complexes start to release NO not immediately but some time later (for **I** and **II** this time is  $\sim 10\text{-}12$  s).

It has been determined that 500 seconds after dissolution of the nitrosyl iron complexes, NO amount in solution for **I** and **II** is  $\sim 31\text{-}33$  nM/mol of the complex and is much higher than NO amount in solutions of NONO-ate. The kinetic dependences are characterized by the maximums. Both complexes are more effective donors than those studied earlier.

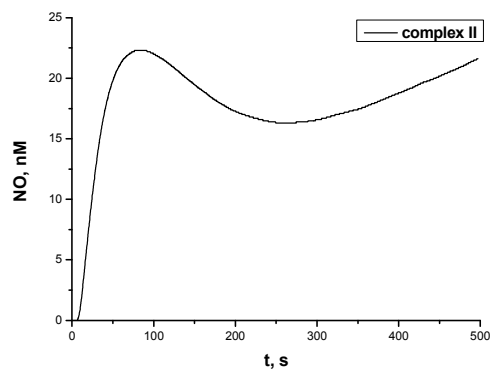


Fig. 6 The time dependence of NO amount generated by complex **II** in aqueous solution at pH 7 and  $T = 25^{\circ}\text{C}$  in anaerobic conditions (the complex concentration is  $0.4 \cdot 10^{-5}\text{M}$ ) upon incomplete dissolution of the sample (see the Experimental part).

Complex **II**, apparently, generates NO in two steps (Fig. 6), this being apparently due to the presence of four NO groups in the cation and four NO groups in the anion, both of them decomposing with different kinetics. Essentially absolute

coincidence of the kinetic curves for **I** and **II** in Fig. 5 suggests that the identical cations of **I** and **II** generate NO in case of complete dissolution of the complexes. Upon incomplete dissolution of sample **II** we managed to register NO release by the anion of complex **II**, thus supposing that **II** releases NO consecutively.

We can assume that in an aqueous solution of investigated dication iron complexes with thiourea **I** and **II** the mechanism of NO realization is similar to the NO generation in the solutions of monocation iron complex with thiourea  $[\text{Fe}(\text{SC}(\text{NH}_2)_2)_2(\text{NO})_2]\text{Cl} \cdot \text{H}_2\text{O}$ .<sup>61</sup> This mechanism involves the dissociation of Fe-NO bond and substitution of NO to the aqua ligand, by addition of water to the vacant iron coordination site. NO-donating activity was shown to preserve for 100 minutes of the experiment (the samples for analysis were taken from the same solution of the corresponding complex every 20 minutes), and the final amounts of the generated NO are  $\sim 5.0$  nM (for **I** and **II**). Apparently, in solutions there are stable nitrosyl intermediates forming upon the decomposition of **I** and **II**, which provide prolonged NO-donating properties of the complexes.

## Conclusions

For the first time water-soluble iron nitrosyl complexes with thiocarbamide containing mononuclear  $[\text{Fe}(\text{SR})_2(\text{NO})_2]_2^+$  cations have been obtained by the interaction of the iron salts  $\text{FeSO}_4$ ,  $\text{Na}_2[\text{Fe}_2(\text{S}_2\text{O}_3)_2(\text{NO})_4]$  and acidic solutions of thiocarbamide (R). Complex **II** is the first dinitrosyl iron complex with both the cation and anion being NO-donating ions. Their structure in the solid phase was studied by X-ray analysis, Mössbauer, IR- and EPR-spectroscopy. As follows from the comparative analysis of the molecular and crystalline structure of two compounds, the conformation of  $[\text{Fe}(\text{SR})_2(\text{NO})_2]_2^+$  cation changes depending on the nature of counter ions in the structure, this being due to easy rotation of thiocarbamide ligands about the Fe-S and S-C bonds. In addition, the type of intermolecular interactions in  $[\text{Fe}(\text{SR})_2(\text{NO})_2]_2^+$  salts with different counter ions affects the structure of thiocarbamide ligands, and, this being the most important, on the structure and reactivity of the nitrosyl groups. There are no differences in the EPR spectra of **I** and **II** powders, this being the evidence of similar paramagnetic centers in both samples, while the Mössbauer spectra of the powders suggest the presence of two different Fe centers, cation and anion, in the crystals of complex **II**. Considerable difference was determined for the decomposition of the complexes in aqueous solutions: **II** contains two NO-generating mononuclear cations and one NO generating binuclear anion per a molecule, as distinct from **I**, with only two cations being the NO donors. As follows from the EPR spectra of dimethyl sulfoxide solutions of **I** and **II**, **II** is more stable in the medium of highly coordinating solvent. Both complexes generate NO in water without additional thermal, photo or redox activation, and they are effective nitrosylating agents, with complex **II** releasing twice as much NO as complex **I**, whose NO-generating ability is more than twice as high as that of diethylenetriamine and various iron salts (+1) with tetranitrosyl dithiosulfate anion, which are isostructural with dianion of **II**, thus opening possibilities of a wide application of these new compounds in biomedical investigations. Furthermore, the obtained results demonstrate the possibility to increase the



amount of NO-generating centers due to the increase of the charge of a non-functional counter ion and the creation of hybrid NO donors containing NO-donating centers in both counter ions, with different kinetics of NO release by the cations and anions of these salts.

## Experimental section

### Synthesis

The following commercial chemicals were used: FeSO<sub>4</sub>·7H<sub>2</sub>O (Aldrich), Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O (Aldrich), thiocarbamide (Aldrich), diethylenetriamine NO-adduct (Sigma). Dimethylsulfoxide was purified using the technique.<sup>58</sup>

**[Fe(SC(NH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>(NO)<sub>2</sub>]<sub>2</sub>SO<sub>4</sub>·H<sub>2</sub>O (I).** 2.78 g of (0.01 mol) of FeSO<sub>4</sub>·7H<sub>2</sub>O was dissolved in 10 ml of H<sub>2</sub>O and placed in a three-necked flask under the inert atmosphere; then solution of 1.52 g (0.02 mol) of thiourea and 1 ml of HCl (conc., 0.012 mol) in 10 ml of H<sub>2</sub>O was added drop-wise with stirring at T=25°C. The excess of gaseous NO was passed through the light-pink reaction mixture for two hours. The solution was kept at T=5°C for 18 hours; the black single crystals were filtered and dried in the inert atmosphere. Yield: 0.66 g (34%). Elemental analysis of **I**. Found %: Fe-17.52; C-6.76; H-2.52; N-25.62; S-26.71; O-20.34; Calc. %: Fe-17.72; C-7.59; H-2.53; N-26.58; S-25.32; O-20.25. IR spectra (cm<sup>-1</sup>) of complex **I**: 3474 (w), 3393 (m), 3300 (m), 3124 (m), 2735 (w), 2351 (v.w.), 2077 (v.w.), 1808 (w), 1749 (w), 1734 (v.w.), 1637 (w), 1515 (w), 1433 (m), 1404 (m), 1126 (m), 1085 (w), 1062 (v.w.), 981 (w), 714 (s), ν<sub>NO</sub>=1808, 1748, 1734 cm<sup>-1</sup>.

**Fe(SC(NH<sub>2</sub>)<sub>2</sub>)<sub>2</sub>(NO)<sub>2</sub>][Fe<sub>2</sub>(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub>(NO)<sub>4</sub>] (II).** 0.88 g (1.5 mmol) of Na<sub>2</sub>[Fe<sub>2</sub>(S<sub>2</sub>O<sub>3</sub>)<sub>2</sub>(NO)<sub>4</sub>]·4H<sub>2</sub>O<sup>57</sup> and 0.76 g (3 mmol) of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O were dissolved in 30 ml of water, filtered, and the filtrate was placed in the three-necked flask under the inert atmosphere. Then the mixture of 0.53 g (7 mmol) of SC(NH<sub>2</sub>)<sub>2</sub> and 1 ml of HCl (conc., 0.012 mmol) in 20 ml of water was added drop-wise to the filtrate with stirring at T=25°C. The reaction mixture was kept at room temperature for a day and then filtered. The forming thin black-red long plates were filtered and dried in the inert atmosphere. Yield: 0.55 g (73%). Elemental analysis of **II**. Found %: Fe-23.00; C-4.87; H-1.62; N-21.80; S-26.10; O-22.52; Calc. %: Fe-22.60; C-4.80; H-1.60; N-22.60; S-25.80; O-22.60. IR spectrum (cm<sup>-1</sup>) of complex **II**: 3471(w), 3415 (w), 3375 (w), 3330 (m), 3215 (m), 3147 (m), 2733 (v.w.), 2405 (v.w.), 1822 (s), 1798 (v.s.), 1756 (v.s.), 1732 (v.s.), 1661 (m), 1636 (s), 1575 (w), 1532 (w), 1502 (w), 1434 (m), 1394 (m), 1216 (s), 1182 (s), 1107 (w), 1075 (w), 1006 (v.s.), 886 (w), 711 (m), 702 (m), ν<sub>NO</sub>=1822, 1798, 1756, 1732 cm<sup>-1</sup>.

### X-ray diffraction studies

X-ray diffraction analysis of monocrystals **I** and **II** was carried out on a CCD diffractometer Agilent XCalibur with EOS detector (Agilent Technologies UK Ltd, Yarnton, Oxfordshire, England). The data sets for crystals of both **I** and **II** were collected at 100.0(2) K using MoKα (λ=0.71073Å) radiation and treated by CrysAlisPro software for cell refinement, data collection, and data reduction with empirical absorption correction (Scale3AbsPack) of the experimental intensities.<sup>59</sup> The structures were solved by the direct methods. The positions and thermal parameters of non-hydrogen atoms were refined anisotropically

by the full-matrix least-squares method. Hydrogen atom positions were localized from the difference map and refined in the riding model. The crystallographic data for **I** and **II** are summarized in Table 1. The bond lengths and bond angles in both structures are listed in Table S1†. All calculations were performed with the SHELXTL program package.<sup>60</sup>

The X-ray crystal structure data have been deposited with the Cambridge Crystallographic Data Center, with reference codes CCDC 1001729 (**I**) and 1001730 (**II**).

### Physical measurements

IR spectra (cm<sup>-1</sup>) of complexes **I** and **II** were recorded on Perkin-Elmer Spectrum 100X at room temperature.

EPR spectra of powders and solutions of complexes **I** and **II** were recorded on ELEXSYS-II E500 radiospectrometer (Bruker) at room temperature. The spins number was determined on the assumption that spin is equal to 1/2.

Mössbauer <sup>57</sup>Fe absorption spectra of complexes **I** and **II** were recorded on WissEl operating in constant acceleration mode. Co in Rh matrix was used as the source. Spectra at low temperatures were measured using continuous flow helium cryostat CF-506 (Oxford Instruments) with controllable temperature. Mössbauer spectra were processed by the least square method assuming the Lorentzian form of the individual spectral components.

Electrochemical determination of NO. Sensor electrode “amiNO-700” of “inNO Nitric Oxide Measuring System” (Innovative Instruments, Inc., Tampa, FL, USA) was used for determination of the NO amount released by complexes **I** and **II** in the solution as compared to other NO donors. The NO concentration was recorded during ~500 seconds (with pace 0.2 s) in aqueous solution with the NO donor concentration 0.4·10<sup>-5</sup> M. For calibration of the electrochemical sensor, a standard NaNO<sub>2</sub> aqueous solution (100 μmol) was used, which was added to the mixture of 0.12 M KI and 2 ml of 1 M H<sub>2</sub>SO<sub>4</sub> in 18 ml of water. All experiments were performed in anaerobic solutions at 25°C and pH 7.0. Commercial buffer Hydrion (from Sigma-Aldrich, No.239089) was used (pH of the solutions was measured by membrane pH-meter “HI 8314” (HANNA instruments, Germany)).

The sample of **I** was dissolved in 10 ml of H<sub>2</sub>O during 1 minute, then its NO-donating ability was recorded after complete dissolution. Dissolution of **II** in 10 ml of H<sub>2</sub>O occurs during 10 minutes with some heating. At the first stage of dissolution the solution becomes light-yellow (within 3 minutes), then complete bleaching of the aqueous solution is observed, and at the last stage when the complex dissolves completely the solution becomes bright-yellow.

### Computational methods

All computations in this work were carried out with the ORCA program package.<sup>70</sup> The geometry optimization and frequency calculation were performed using the BP86,<sup>71</sup> TPSS,<sup>72</sup> B3LYP<sup>73</sup> and TPSSH<sup>74</sup> density functionals with TZVP<sup>75</sup> basis set. The RI<sup>76</sup> and RIJCOSX<sup>77</sup> approximations with the auxiliary basis sets TZV/J were used to accelerate the calculations. In order to calculate the electronic structure of cationic DNIC the BS(m,n)-DFT approach,<sup>68,78</sup> where m (n) denotes the number of unpaired spin-up (spin-down) electrons at the two interacting fragments,



was used (Fig. S7†).

The Mössbauer spectroscopic parameters were computed using the different density functional (Table S6). The CP(PPP)<sup>79</sup> basis set for Fe was employed, the TZVP<sup>75</sup> basis set for other atoms was used. Isomer shifts  $\delta$  were calculated from the electron densities at the Fe nuclei  $\rho_0$  employing the linear regression:

$$\delta = \alpha \cdot (\rho_0 - C) + \beta \quad (1)$$

Here, C,  $\alpha$  and  $\beta$  are the fit parameters, their values were taken from work<sup>80</sup> for corresponding density functional and basis set.

Quadrupole splittings  $\Delta E_Q$  were obtained from electric field gradients  $V_i$  ( $i = x, y, z$ ;  $V_i$  are the eigenvalues of the electric field gradient tensor) employing a nuclear quadrupole moment  $Q(^{57}\text{Fe}) = 0.16 \text{ barn}$ .<sup>81</sup>

$$\Delta E_Q = \frac{1}{2} e \cdot Q \cdot Vz \cdot \sqrt{1 + \frac{1}{3} \cdot \eta^2} \quad (2)$$

Here,  $\eta = (V_x - V_y)/V_z$  is the asymmetry parameter.

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