Reviews

Structure and properties of iron nitrosyl complexes with functionalized sulfur-containing ligands

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The review summarizes the results of studies aimed at developing the fundamentals for the design of a new class of nitric oxide donors, *viz.*, iron nitrosyl complexes with functionalized sulfur-containing ligands, which are structural analogs of the active sites of non-heme nitrosyl iron-sulfur proteins. The structures, reactivities, and pharmacological activity *in vitro* and *in vivo* of these complexes are considered.

Key words: iron nitrosyl complexes, NO donors, thiols, X-ray diffraction study, IR spectroscopy, mass spectrometry, Mössbauer spectroscopy.

Physiological role of nitric oxide (NO)

In the early 1980s, it was found that NO can be released from stimulated endothelial cells accompanied by the dilation of blood vessels, and this fact led to the discovery of a new messenger molecule. Thus, nitric oxide was identified as an integral component of the cardiovascular system and the immune response to pathogens. These discoveries have resulted in an explosion in the study of nitric oxide chemistry in biological systems, which is currently one of the largest and fastest growing areas in biomedical science. An analysis of the published data shows that fundamental changes in the understanding of NO as a regulator of cell metabolism have occurred in the last decade. Nitric oxide is a free radical gas. It is a short-lived compound, which readily undergoes various chemical transformations, has different actions, which in many cases remain unclear, is continuously formed in mammals, functions as a signal molecule in diverse physiological and biochemical processes, including the regulation of gene expression at the level of mRNA transcription and translation, and plays a key role in the activity of living organisms by interacting with cellular substrates.[1—22]

According to *http://apps.isiknowledge.com*, 1184 papers were published in the period from 1980 to 1990, which were devoted mainly to problems of NO and environmental factors; in 1990—2000, the number of publications on the biochemistry and pharmacology of NO increased to 62680, and about 26 millions of studies concerning the problems of molecular biology and pharmacology of NO were published in the last decade. Nitric oxide has a broad range of biological activities. It is involved in the regula-

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tion of blood vessel tone, inhibits platelet aggregation and adhesion to blood vessel walls, is present in the central and autonomic nervous systems, regulates the respiratory system, the gastrointestinal tract, and the urogenital system, and mediates the neuronal signal transduction, immune reactions, antimicrobial and antitumor activities, the reproductive function, and so on.

The effects of NO on biomolecules are generally divided into three categories:[23]

1) regulatory (vascular permeability, vascular tone, cell adhesion, inhibition of platelets, regulation of the immune system, neurotransmission, liver metabolism, bronchodilation, memory and learning, synaptic adaptation, kidney function, erection);

2) protective (leukocyte inhibition, blood pressure reduction, the antitumor, antioxidant, antibacterial, and antimalarial activities);

3) deleterious (enhancement of the susceptibility to metal-mediated toxicity, alkylation, and radiation, inhibition of mitochondrial respiration, lipid peroxidation, DNA damage, enzyme inhibition, a decrease in the antioxidant level, septic shock, reperfusion injury, myocardial injury).

At low concentrations, NO acts as a signal transducer in many physiological processes (for example, in the regulation of blood flow, smooth muscle relaxation, iron homeostasis, and platelet activity and as a neurotransmitter). At high concentrations, NO is cytotoxic to the pathogenesis and tumors. An excess of NO leads to the initiation and development of pathological processes. An understanding of the mechanisms of these biological effects is complicated by the chemistry of NO and its intermediates, but the curb of this simple molecule provides impressive possibilities for establishing new fundamental mechanisms of its action and achieving therapeutic results in the treatment of some socially significant diseases (arterial pressure, ischemic heart disease, myocardial infarction, bronchial asthma, septic shock, acute respiratory distress syndrome, thromboembolic disease, renal failure, bronchospasm, encephalopathy, epilepsy, neurotic depression, neurodegenerative disorders (Alzheimer´s disease, Parkinson´s disease), erectile dysfunction, diabetes, immunodeficiency, pulmonary hypertension, and cancer). The intensive search for new therapeutic ways of delivery (or removal of an excess) of NO has stimulated the understanding of the causes of most diseases and the treatment of disorders in the formation, the metabolism, and functions of NO under physiological conditions.

There are two main pathways of the *in vivo* NO formation: non-enzymatic and enzymatic. By the non-enzymatic pathway is generally meant the reduction of nitrites and nitrates to NO.[24–31] This reaction proceeds either as the disproportionation of nitrite or nitrous acid in an acidic medium or as the direct reduction of nitrite (for example, by Fe ions). The disproportionation of nitrite occurs to a substantial degree at pH < 5. However, this mechanism takes place only in the states accompanied by the oxidation of the medium, for example, in the case of ischemia or inflammation, whereas the direct reduction of nitrite in the presence of heme proteins occurs at neutral pH. Heme-containing proteins having nitrite reductase activity include hemoglobin (Hb), myoglobin, cytochrome *c* oxidase, and cytochrome P450.

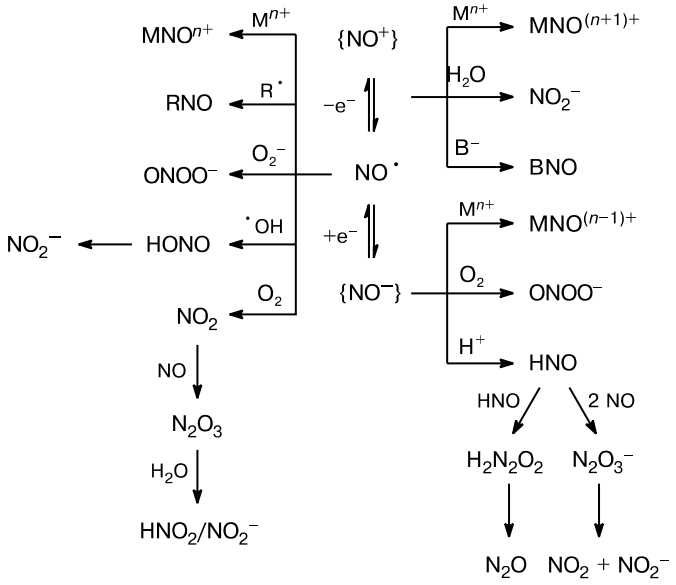
The enzymatic NO synthesis in cells is catalyzed by cytochrome P450-like heme proteins, *viz.*, NO synthases (NOSs). Three NOSs are currently known. Two of them are the main, constitutive, isoforms of heme-containing enzymes catalyzing the NO synthesis from L-arginine. These are Ca^{2+}-regulated isoforms (cNOSs), which were initially identified in neuronal tissues (nNOS or NOSI) and endothelial cells (ecNOS or NOSIII). The third NOS is the inducible and Ca^{2+}-independent isoform (iNOS or NOSII) found in macrophages. It forms homodimers, which catalyze the transformation of the terminal guanidine nitrogen atom of L-arginine to form NO and citrulline (through N^{ω}-hydroxy-L-arginine as the intermediate) in the complex reaction involving molecular oxygen and NADPH as co-substrates and FMN, FAD, BH4, and the enzyme-bound heme as cofactors. The NO formation depends also on L-arginine, whose presence is, in turn, determined by the activity of competing enzymes (for example, of arginase) and the L-arginine transport in cells *via* the cationic amino-acid transport (CAT) system.[32]

In all forms, endogenous NO (its redox forms are the nitroxyl anion NO^- and the nitrosonium cation NO^+) (Scheme 1) can interact with low-molecular-weight cellular targets, such as the superoxide anion, oxygen, the hydroxonium ion, thiols, *etc.* to form reactive intermediates, for example, peroxynitrite or nitrosothiols (reaction products of NO with thiols), which are NO carriers extending the period of its endogenous action. Nitric oxide reacts also with high-molecular-weight cellular targets, *viz.*, the active sites of metalloproteins, to form metal nitrosyl complexes, which can act as nitrosating agents by themselves. The interaction with metal-containing proteins and enzymes is considered as playing the main role in many physiological and pathophysiological effects of NO (guanylate cyclase, hemoglobin, and NOSs by themselves are specific examples).

The local NO concentration is another important factor. At low concentrations (lower than 150 nmol), NO acts as the signal transducer in many physiological processes, such as the regulation of blood flow, smooth muscle relaxation, iron homeostasis, platelet activity, and neurotransmission; at high concentrations, NO produces a cytotoxic effect on the tumor pathogenesis.[33]

Therefore, when developing innovative therapeutic approaches, two main factors should be taken into account: the reactivity and the amount of local NO. Currently, there are two strategies for the treatment of a series of diseases associated with changes in the cellular NO level:

Scheme 1



M^{n+} are enzyme metals

1) anti-NO therapy (in this approach, the central role is in designing drugs acting as scavengers of an excess of NO/NOS, inhibitors of NOS activity, and repressors of NOS expression) and 2) the use of exogenous NO donors under conditions, when a deficiency of NO leads to pathological changes. There are particular requirements for NO donors:[34]

1) target products of the NO donor synthesis should be isolated in the pure solid state;

2) NO donors should rapidly decompose in aqueous solutions at physiological pH accompanied by the NO release without additional activation;

3) the decomposition of NO donors should give nitric oxide in quantitative yield.

Exogenous NO donors

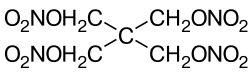
Figure 1 shows all known classes of compounds capable of generating NO. Most of the currently available compounds, which can change the NO level *in vivo*, are of organic nature. The main advantages and drawbacks of these NO-donor agents were described in the literature.[35—51] However, once it was found that metal nitrosyl complexes (primarily, iron complexes) are involved in the biochemistry of NO, a new field has appeared based on the use of transition metal complexes for the regulation of the NO level in biological systems.

Nitrosyl iron-sulfur complexes as promising NO donors

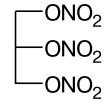
As mentioned above, proteins containing metal ions, in particular, iron ions, in the active sites are the major targets of NO *in vivo* (Fig. 2). These metal ions can easily coordinate the reactive NO molecule. It is known that iron-regulatory proteins play a key role in the regulation of redox homeostasis. They are activated by the superoxide radical, iron, and NO and influence, in particular, the transcription of superoxide dismutase and a number of other anti-stress proteins. In a typical iron-sulfur regulon, the active site consists of two [2Fe-2S] clusters. Among the majority of structurally and functionally alternating cofactors, protein-bound [Fe-S] clusters are known in biology. In addition to [2Fe-2S]-, [3Fe-4S]-, and [4Fe-4S]-type clusters known since 1960s, the following clusters have been found in recent years: 1) high-nuclearity clusters (nitrogenase); 2) [4Fe-4S] clusters containing other Fe centers (siroheme in sulfite reductase and binuclear non-heme iron in hydrogenase); 3) heteronuclear [Fe-S] complexes containing Mo, Ni, or other metal atoms (nitrogenase and acetyl-CoA synthase). Sulfur provided by cysteines generally serves as protein ligands for these clusters. It should be noted that the number of examples of the coordination by the oxygen atom of aspartate, glutamine, or tyrosine, as well as of the coordination by the nitrogen atom provided by histidine or arginine residues, increases. The coordination of exogenous ligands (water, enzyme substrates (citrate in the case of aconitase) or cofactors (such as *S*-adenosylmethionine (SAM) in the case of radical SAM enzymes) was also found.

The [Fe-S] clusters, which were initially characterized as electron-transport systems (photosynthesis and respiration), are involved in various chemical processes, including the sulfur and ligand exchange. Clusters with dif-

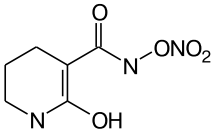
Nitrates



Pentaerythrityl tetranitrate

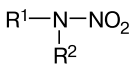


Nitroglycerin

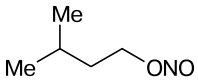


Nicorandil

Nitramines



Nitrites



Isoamyl nitrite

Organometallic nitrosyls

$M_x(NO)_y(CO)_z$; $M_x(NO)_y[R]$; $M_x(NO)_y(CO)_z[R]$

R = Alk, Ar; M = V, Mn, Fe, Co, Ir

Carbonyls and isocyanates

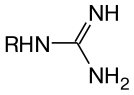
$CpM(NO)$; $Cp_nM_m(NO)_p$; $Cp_nM_m(NO)_p(CO)_2$;

$CpM(NO)(CO)[R]$; $CpM(NO)[R]$

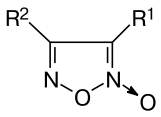
M = Cr, Mn, Mo, W, Pt, Pd, Ni

Organic cyclopentadienyl nitrosyls

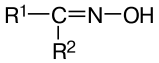
Guanidines



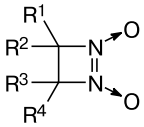
Furoxans



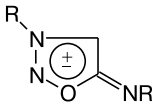
Oximes



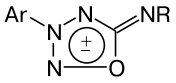
1,2-Diazete 1,2-dioxides



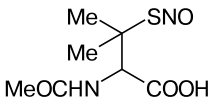
Sydnone imines



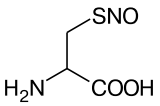
Mesoionic oxatriazoles



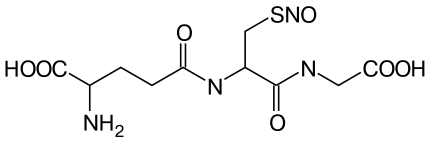
***S*-Nitrosothiols**



S-Nitroso-*N*-acetyl-penicillamine

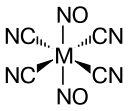


S-Nitrosocysteine



S-Nitrosoglutatione

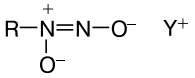
Cyanonitrosylmetallates



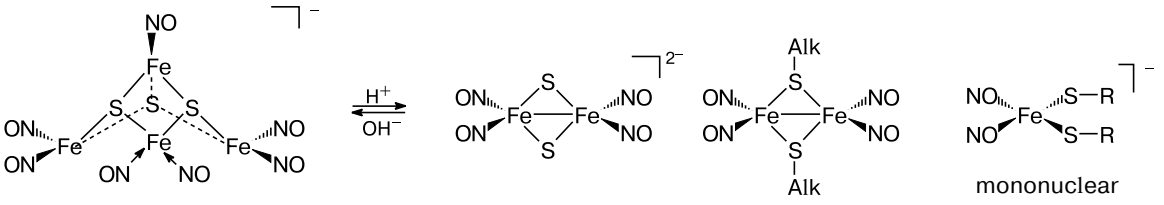
$Na_2[Fe(CN)_5NO]$

M = Cr, Fe, Re, V, Mo, Mn, Co, Ru, Ni

Diazenium-1,2-diolates (NONOates)



Iron-sulfur nitrosyl complexes



tetranuclear

binuclear

mononuclear

R = Ph

Glutathione, cysteine

Fig. 1. Exogenous NO donors.

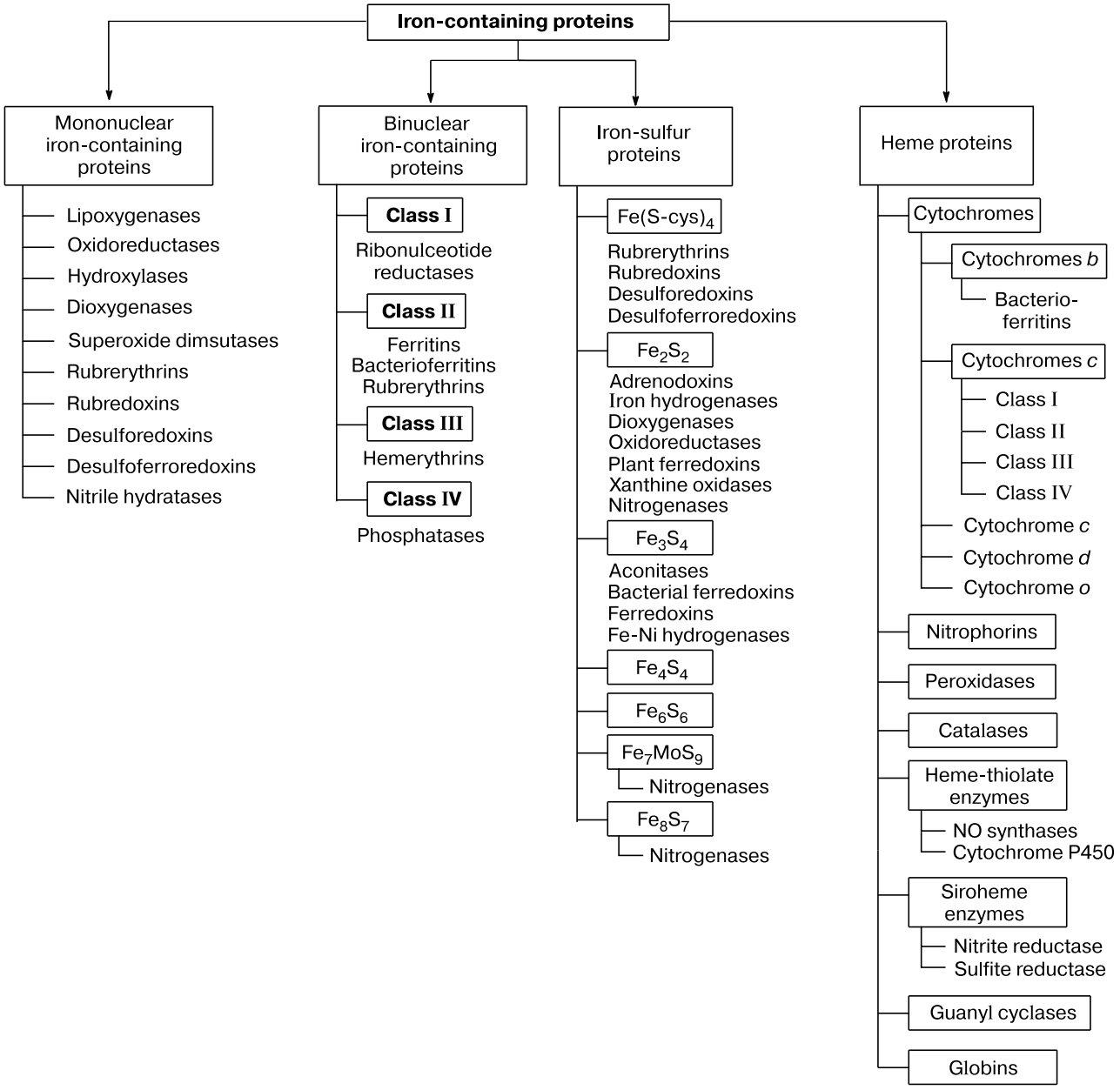


Fig. 2. Classification of iron-containing proteins.[23]

ferent nuclearities undergo reversible interconversions and serve as bridges between polypeptide chains. They are sensitive to molecular oxygen and oxidants, such as hydrogen peroxide, superoxide, and NO, which is functionally significant, and it is these chemical properties of non-heme [*n*Fe-*m*S] enzymes that are responsible for their additional catalytic and biological role.[52]

The reactions of endogenous NO with active sites of non-heme [*n*Fe-*m*S] proteins give iron nitrosyl complexes. Such proteins as glutathione transferase[53] serve as natural depots (reservoirs) of nitric oxide.[54,55] It was reliably established that iron nitrosyl complexes with thiol-containing ligands (cysteine, glutathione) fulfill important functions in the body, such as the storage and transport of cellular NO.[56—63] Being intermediates in the formation and decomposition of natural thiols (RSH) catalyzed by cellular iron, these complexes, like RSNO, induce vasodilation and are responsible for cytotoxicity of NO.[64—66]

It should be noted that, while the reactions of NO with low-molecular-weight cellular substrates, such as the superoxide anion, oxygen, amines, and thiols, have been studied in-depth, the biomimetics of the reactions of NO with proteins, particularly with non-heme proteins, is complicated by the problems associated with the isolation

of real nitrosyl adducts of iron-sulfur proteins. This is due to their extreme instability both *in vivo* and *in vitro* and the associated difficulties in investigating their physicochemical characteristics. Therefore, the synthesis and studies of structural analogs of nitrosyl adducts of non-heme iron are important for the understanding of the nature of the bond in the {S-Fe-NO} moiety and the mechanisms of reactions of generated NO for its *in vivo* targeted delivery, which is of great significance for the design of new-generation drugs based on natural sources.

Compounds belonging to this class are versatile and simultaneously selective regulators of vitally important processes. Due to low stability and high rates of the reactions with active forms of oxygen, the fundamental mechanisms of their reactions with cellular targets are poorly known. This class of NO donors is little studied in spite of the fact that these compounds have considerable advantages. For example, the use of iron nitrosyl complexes with sulfur-containing ligands as adjuvants to chemo- and radiotherapy[67–69] opens new prospects for the efficient treatment of malignant tumors. This class of compounds can initiate the synthesis of stress proteins enhancing the immunogenicity of the body[70] and can be used for the design of a new class of cardiovascular drugs because the administration of these drugs in animals causes vascular dilation.[71]

In the present review, methods for the synthesis and the structures and properties of bi- and mononuclear nitrosyl iron-sulfur complexes, which are synthetic models of the active sites of nitrosyl [2Fe-2S] and [1Fe-2S] proteins serving as natural reservoirs of nitric oxide, are considered. Unlike toxic polynuclear nitrosyl iron-sulfur clusters (containing three or more iron atoms), these compounds hold promise as a class of organometallic NO donors for biological and medicinal studies.[72]

In spite of the structural diversity of iron-sulfur clusters found in biological systems, all these clusters consist of simple units, most often of $[Fe_2S_2]$ rhombs, which are linked to proteins by cysteine or histidine ligands. There are four main structure types of Fe-S sites in the nature: mono-, bi-, tri-, and tetranuclear (by the nuclearity is meant the number of iron atoms coordinated by sulfur atoms) (Fig. 3). All other structure types of polynuclear active sites are combinations of these four known types.

First synthetic analogs of non-heme nitrosyl iron-sulfur proteins, *viz.*, the black salt $[Fe_4S_3(NO)_7]^-$ and the red salt $[Fe_2S_2(NO)_4]^{2-}$, were synthesized by Roussin,[73] and these synthetic methods were further improved[74] (Scheme 2).

Upon photoactivation, both salts release nitric oxide. However, experiments in cell cultures and two cell lines, *viz.*, the human neoplastic cell line (SK-MEL188), and the mouse neoplastic cell line (S91),[75] showed that the black salt is cytotoxic. The use of polynuclear iron-sulfur clusters as NO donor has no practical interest[23] because of

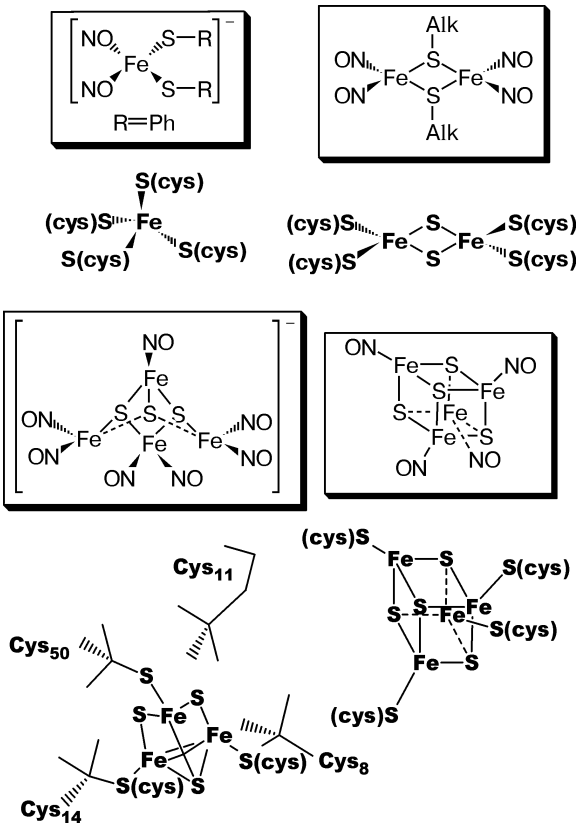


Fig. 3. Structures of active sites of natural iron-sulfur proteins and their synthetic models (in frames).

Scheme 2

$$FeSO_4 + NaNO_2 + (NH_4)_2S \xrightarrow{NH_4OH} NH_4[Fe_4S_3(NO)_7] \xrightarrow{ROH} R_2[Fe_2S_2(NO)_4]$$

$$NH_4[Fe_4S_3(NO)_7] \xrightarrow{Alk_4NHal} (Alk_4N)_2[Fe_2S_2(NO_4)]$$

R = Na, K, Me, Et

their toxicity; however, these clusters are important for investigation of the coordination modes and the nature of the bond in the coordination unit $\{(NO)_m\text{-}Fe_n\text{-}S\}$. For example, neutral trinuclear iron thiolate nitrosyl, $[(NO)Fe(\mu\text{-}S,S\text{-}C_6H_4)]_3$, and its oxidation product, $[(NO)Fe(\mu\text{-}S,S\text{-}C_6H_4)]_3[PF_6]$, were synthesized and studied by X-ray diffraction, X-ray absorption spectroscopy, IR and ESR spectroscopy. The magnetic properties of these compounds were investigated.

The binuclear complex, *viz.*, Roussin´s red salt, is less toxic and more photoactive. However, the use of this salt as an NO donor is limited because of its extreme instabili-

ty. Thus, this complex is immediately transformed in solution into the black salt[76] (Scheme 3).

Scheme 3

$$2\,H^+ + 4\,Fe_2S_2(NO)_4^{2-} \xrightarrow{h\nu} 2\,Fe_4S_3(NO)_7^- + 2\,S^{2-} + N_2O + H_2O$$

The so-called Roussin´ red salt esters, unlike the red salt, are more stable in solution. The method for their synthesis is based on the reaction of Roussin´s red salt with alkyl halides[77—80] (Scheme 4).

Scheme 4

$$[Fe_2S_2(NO)_4]^{2-} + 2\,RHal \longrightarrow [Fe_2(SR)_2(NO_4)]$$

R = Me, Et, CH_2Ph, CH_2CH_2OH, $CH_2CH_2SO_3$

The reactions of sulfur-containing ligands (thioglycolate, ethanethiol, and 2-methyl-2-propanethiol) with $NaNO_2$ and $FeSO_4$ also produce neutral binuclear nitrosyl complexes. These compounds are isostructural with the thiosulfate iron nitrosyl complex, which was synthesized for the first time in 1895 by Hoffman and Weide in yields of up to 65% (Scheme 5).

Scheme 5

$$2\,FeSO_4 + 4\,K_2S_2O_3 + 4\,NO \longrightarrow K_2[Fe_2(S_2O_3)_2(NO)_4] + K_2S_4O_4 + 2\,K_2SO_4$$

More recently,[81] $NaNO_2$ was used as the nitrosating agent, but the method proved to be less efficient because it gave iron(III) hydroxide as the by-product, and the yield of the nitrosyl product was 30%.

"Esters" containing alkyl substituents were also synthesized from the thiosulfate nitrosyl complex and the corresponding thiols[82] according to Scheme 6.

Scheme 6

$$[Fe(S_2O_3)_2(NO)_4]^{2-} + 2\,S_2O_3^{2-} \longrightarrow 2\,[Fe(S_2O_3)_2(NO)_2]^{3-} + 2\,RS^- \longrightarrow 4\,S_2O_3^{2-} + [Fe_2(SR)_2(NO)_4]$$

R = Me, Et, Pr^i, Bu^i, Pr^n, Bu^n, $(CH_2)_4Me$

The nitrosyl complexes were isolated from the reaction mixture in yields of up to 60%. Unfortunately, this method is inapplicable to complexes insoluble in CH_2Cl_2. The complexes $[Fe_2(SR)_2(NO)_4]$ can also be synthesized in yields of up to 80% by the Brauer method;[82] however, the reaction produces Fe_2O_3 and this method cannot be used for thiols with low basicity constants (pK_a) (Scheme 7).

Scheme 7

$$4\,FeSO_4 + 8\,KOH + 2\,RSH + 4\,NO \longrightarrow [Fe_2(SR)_2(NO)_4] + 4\,K_2S_4O_4 + Fe_2O_3 + 5\,H_2O$$

Neutral "esters" can also be synthesized by the reaction of AlkSH with the complex $[Fe_2I_2(NO)_4]$ in the presence of weak bases or by the reaction of Alk_2S_2 with the carbonyl complex $[Fe(CO)_2(NO)_2]$. The complex $[Fe(SCH_2CH(NH_2)COOH)_2(NO)_2]$ was synthesized by the reaction of mercaptopropionic acid with $[Fe(CO)_2(NO)_2]$ in tetrahydrofuran followed by the precipitation of the major product with hexane. According to the ESR data, these reactions proceed *via* the formation of mononuclear dinitrosyl iron complexes (DNIC).

The quantum yields of the NO release from Roussin´ salt esters containing alkyl substituents are 0.02—0.13. It was shown[16] that 4 moles of NO are generated from 1 mole of the complex, as opposed to the black salt, which gives 3.7 moles of NO from 1 mole of the salt. Data on the study of the cytotoxicity of these compounds are lacking in the literature, except for the information on the cancerogenic properties[52] of the neutral binuclear complex $[Fe_2(SMe)_2(NO)_4]$.

In recent years, main attention has been given to the synthesis of binuclear complexes with functionalized R. However, there are a few examples of these complexes. For instance, a salt containing protoporphyrin-IX (PRIX) as the ligand R, which is the structural analog of porphyrin, has been recently synthesized and characterized.[83,84]

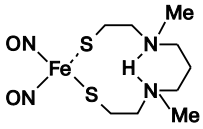
Numerous evidence of the active involvement of metal (primarily, iron) nitrosyl complexes in the biochemistry of nitric oxide *in vivo* have stimulated intense interest in biologically active metal complexes, particularly, in those, which are biomimetics of cellular nitrosyl intermediates. Iron nitrosyl complexes *in vivo* exist in the following two forms: mononuclear $[Fe(SR)_2(NO)_2]^-$ and binuclear $[Fe_2(SR)_2(NO)_4]$. These forms are in a dynamic equilibrium, which depends on the thiol concentration under physiological conditions.[85] It was reliably established that endogenous NO can be stabilized and stored in the form of dinitrosyl iron complexes (DNIC) with protein thiols, thus extending its lifetime and retaining biological activity.

According to the ESR data, these non-heme iron nitrosyl complexes belong to paramagnetic $Fe(NO)_2$-type complexes, which are more well-known as $g = 2.03$ complexes due to their characteristic isotropic g factor.[86] Complexes DNIC were found for the first time in tissues and were classified as mononuclear iron nitrosyl complexes

$[Fe(NO)_2(L)_2]^n$ (L = SR, NR, OR). Many compounds of the composition $Fe(NO)_2(L)_2$ containing derivatives of amino acids, peptides, or proteins as the ligand L were found by ESR spectroscopy, including in ferritin, which serves as the iron storage depot in the human body,[87] but these compounds were not isolated in the crystalline state. The main aim of the above-mentioned investigations was to detect these compounds because their isolation and study by direct structural methods present considerable difficulties. Taking into account the insufficient assortment of synthetic analogs, the latter are particularly interesting in view of investigations of the reactivity of DNIC, including the processes of their formation by the reactions of NO with iron-sulfur complexes in the case of pronounced biological activity of DNIC.

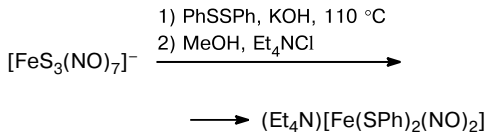
Solutions of dinitrosyl iron complexes with thiols of natural origin (cysteine or glutathione) can be used as NO donors in biochemical and medical studies. Generally, DNIC are synthesized by bubbling gaseous NO through a mixture of iron(II) sulfate and the corresponding water-soluble thiol taken in a molar ratio of 1 : 2 in the form of unstable aqueous solutions[88–91] or as lyophilized composites of these solutions with water-soluble polymers.[92] This limits the wide use of DNIC for applied purposes because of the uncontrolled initial composition.

The first synthetic analog of DNIC with a sulfur-containing ligand was prepared by the reaction of $(FeL)_2$ (L is the *N*,*N*-dimethyl-*N*,*N*-bis(2-mercaptoethyl)-1,3-propanediamine dianion) with $NOPF_6$ in dichloromethane.[93]



Anionic DNIC of the composition $[NEt_4][Fe(NO)_2(SPh)_2]$ was synthesized[94] by the reaction of Roussin´s black salt with diphenyl sulfide (Scheme 8).

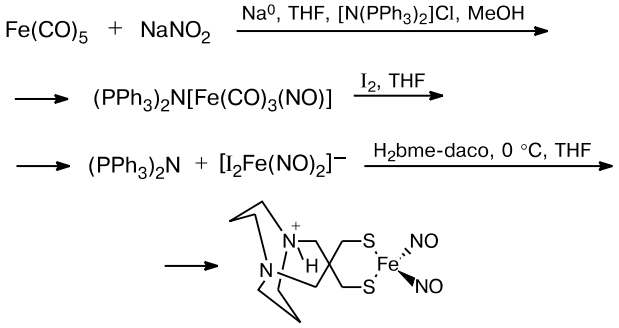
Scheme 8



Cationic DNIC of the composition $[Fe(NO)_2(SC(NH_2)_2)]X$ and $[Fe_2(S(CH)_2NH_3)_2(NO)_4]X$ (X = Cl, I) were synthesized by the multistep reaction from $[Fe(NO)_2(CO)_2]$ or $[Fe(NO)_2I]_2$ in tetrahydrofuran and were studied by X-ray diffraction.[95] A series of the anionic complexes $[Fe(NO)_2(n\text{-}C_3H_7S)_2]^-$, $[Fe(NO)_2(4\text{-}NO_2\text{-}C_3H_3N_2)_2)]^-$, $[Fe(NO)_2(NCS)_2]^-$, and $Na[Fe(NO)_2L_2]$ (L = MeO^-, EtO^-, $(NH_2)_2CO$, F) was characterized[95] by ESR spectroscopy in ethanol and CH_2Cl_2. Solutions of DNIC were prepared by the addition of an excess of the anionic ligand to a solution of $FeCl_2$ at a concentration of 10 mmol L^{-1} followed by the bubbling of gaseous NO for 5 min.

The crystallization and investigation of the structures and physicochemical properties of DNIC in the solid state present considerable difficulties because of their instability. Active research performed in recent years has resulted in the isolation and investigation of single crystals of a number of new sulfur-containing DNIC. The reaction with the use of *N*,*N*´-bis(2-mercaptoethyl-1,5-diazocyclooctane) (H_2bme-daco) according to the method[96] led to the isolation of a complex stable at $T = -35$ °C (Scheme 9). The storage of the mononuclear iron complex in air or an increase in the temperature (above −35 °C), as well as the presence of moisture, resulted in the decomposition of the complex.

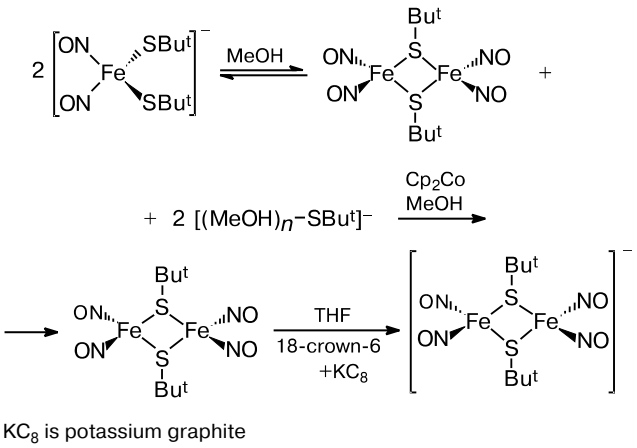
Scheme 9



H_2bme-daco is *N*,*N*´-bis(2-mercaptoethyl-1,5-diazocyclooctane)

Recently,[97] anionic dimeric DNIC $[(NO)_2Fe(\mu\text{-}SBu^t)_2Fe(NO)_2]_2^-$ with the $[K^+—18\text{-crown-6}]$ cation was synthesized (Scheme 10).

Scheme 10



KC_8 is potassium graphite

The anionic complex of the composition [PPN]-$[(PhS)_2(Fe(NO)_2]$ (PPN is bis(triphenylphosphoranylid-

ene)ammonium) was synthesized by bubbling gaseous NO through a solution of [PPN][$Fe(SPh)_3NO$] in THF followed by the precipitation of the product with hexane.[98] The complex [PPN][$(EtS)_2(Fe(NO)_2$] was synthesized and studied by X-ray diffraction.[98] The latter complex was synthesized as follows: the complex [PPN]-[$(PhS)_2(Fe(NO)_2$] and [PPN][EtS] were dissolved in MeCN under an atmosphere of N_2 and treated with diethyl ether, then undissolved [PPN][PhS] and an excess of [PPN][EtS] were filtered off, and hexane was added to the filtrate to precipitate a red-brown powder of [PPN][$(EtS)_2$-$(Fe(NO)_2$].

The dinitrosyl iron complex with the $[(SC_7H_4SN)_2Fe(NO)_2]^-$ anion was synthesized by the reaction of $[S_5Fe(NO)_2]^-$ with bis(2-benzothiazolyl) disulfide.[99] In the synthesis of analogous dinitrosyl compounds, stronger, compared with $[SC_7H_4SN]^-$, electron-donating thiolates $\{RS\}^-$ (R = C_6H_4-*o*-NHCOMe, C_4H_3S, $C_6H_4NH_2$, Ph) initiate the thiolate-ligand substitution to form $[(SC_6H_4$-*o*-$NHCOMe)_2Fe(NO)_2]^-$, $[(SC_4H_3S)_2$-$Fe(NO)_2]^-$, and $[(SPh)_2Fe(NO)_2]^-$, respectively.

According to the results of the study,[88] solutions of anionic DNIC can be prepared by the replacement of CO ligands in $[Fe_2(CO)_2(NO)_2]$ by nitrogen-containing ligands, such as imidazole (Im), 1- and 4-methylimidazoles, benzimidazole (BenzIm), 5-methylbenzimidazole, and L-histidine ($FeN_{Cycl}N_{Cycl}NN$). The complexes were identified by ESR and IR spectroscopy. Quantum chemical calculations were performed for the $NNFe(NO)_2$ moiety in the complexes DNIC with phosphine and tetracyanoethylene ligands. The latter complex was characterized also by X-ray diffraction.

Therefore, sulfur nitrosyl complexes have attracted growing attention due to the experimental discovery of the formation of protein-bound dinitrosyl iron complexes (protein DNIC *in vivo*), the use of metal nitrosyl complexes as compounds delivering NO to biological targets, and the universal properties of the bond between metals and NO.[100—117]

Nitric oxide can act as NO^+, NO^-, and the paramagnetic neutral NO· radical. Hence, the determination of the formal oxidation state of transition metals (in particular, of iron) and NO in dithiolate iron nitrosyl complexes is a difficult problem. Thus, according to Enemark and Feltham,[100] the metal-NO unit can be represented as $\{Fe(NO)_x\}^n$, where $n = x\pi_{NO} + (n - x)d_{Fe}$. The structures and properties of binuclear nitrosyl iron-sulfur complexes are actively discussed. For example, it was suggested that the binuclear iron complexes $[Fe_2(SR)_2(NO)_4]$ exist in solution in the form of dimeric associates of mononuclear dinitrosyl iron complexes.[118,119] In spite of the fact that the electronic and geometric structures of some paramagnetic DNIC were studied both experimentally and theoretically, there is no unified description of the electronic configuration of the iron atom in crystalline DNIC. The electronic structure of the $\{Fe(NO)_2\}^9$ center is generally described as the $\{Fe^{3+}(NO^-)_2\}^9$, $\{Fe^{1+}(NO^{\cdot})_2\}^9$, or $\{Fe^{1-}(NO^+)_2\}^9$ configuration. The first structure is the S = 1/2 state for d^5Fe^{3+} with NO^- ligands, the second structure may be the S = 1/2 low-spin state for d^7Fe^{1+} or the S = 3/2 high-spin state for d^7Fe^{1+} antiferromagnetically coupled with two NO· ligands with S = 1/2 to give the S_{gen} = 1/2 ground state, and the third structure is the simple S = 1/2 state for d^9Fe^{1-} with NO^+ ligands (Fig. 4). It is difficult to determine the oxidation state of metal in metal nitrosyl complexes, including DNIC, because of the very small gap between the d orbital of the transition metal and the π^* orbital of NO. One of the commonly used methods for the determination of the effective charges on NO and the iron atom is based on the position of the NO stretching band in the IR spectra. Thus, the NO^+ complexes give ν_{NO} at 1650—1985 cm^{-1}, whereas the band ν_{NO} for NO^- complexes is observed at 1525—1590 cm^{-1}. However, it is widely accepted that the effective charge on metal cannot always be adequately assigned based only on the ν_{NO} value in the IR spectrum.

Another observation is based on the ESR data. The paramagnetic four-coordinate DNIC $[Fe(NO)_2L_2]^{x+}$ are distinguished due to their characteristic isotropic values g = 2.03 (as discussed above) with various L, which substantially differ from thiols. Based on the ESR data, most of researchers choose the d^7 configuration of the Fe^{1+} ion,

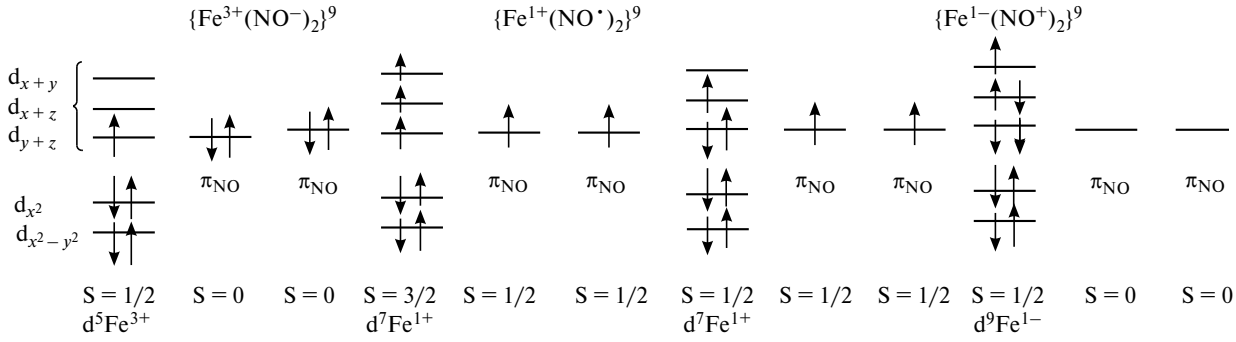


Fig. 4. Possible structures of the paramagnetic $\{Fe(NO)_2\}^9$ center.

but an alternative opinion is that the ESR parameters of DNIC can be better explained by the d^9-electronic configuration of the Fe^{1-} ion. However, no final conclusions can been drawn as yet. The determination of the charges on iron and NO is important for the elucidation of the reactivity of nitric oxide generated from DNIC *in vivo*.

Therefore, the synthesis of new synthetic bi- and mononuclear iron nitrosyl complexes with sulfur-containing ligands in the crystalline state and investigation of their properties are necessary for the fundamental study of the structures and the nature of the bond in the structural unit of the iron nitrosyl complexes $\{Fe(NO)_2\}$ with S ligands, as well as for the understanding of the mechanism of action of this class of compounds as NO donors.

In addition, it should be taken into account that one of approaches to the improvement of the efficacy of drugs, including NO donors, is based on the search for new hybrid drugs, whose molecules contain simultaneously two pharmacophores to provide at least the dual (or synergistic) therapeutic effect.[120] Some hybrid drugs are aimed at interacting with multiple targets as a single molecule, whereas other drugs should be destroyed to deliver active ingredients of the hybrid to the targets. In particular, these hybrids can be used in chemotherapy. As an example of polyfunctional hybrid compounds containing the ONO_2 group as the nitric oxide donor, let us refer to hybrids having inhibitory activity in the treatment of colon cancer *in vitro* and *in vivo*. These compounds contain aspirin and the nitrate group (ONO_2). In most cases, when designing these drugs, the NO-donor moiety is bound to the molecule bearing the major pharmacophore *via* a linker. The linker may be pharmacologically inert or active (antiinflammatory, antineurodegenerative, antioxidant, and cardiotropic agents, carnitine and triterpenoids, prostaglandins and polyunsaturated fatty acids, β_2-adrenoreceptor agonists, *etc.*). In the opinion of the authors of the review,[121] hybrid compounds containing the ONO_2 group can be designed based on a large number of known drugs.

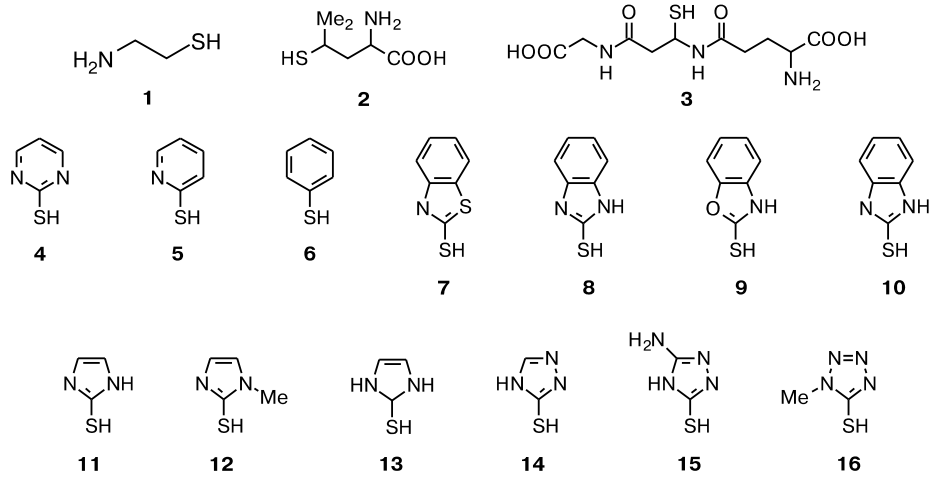
In the present review, we consider new synthetic approaches and procedures for the isolation of nitrosyl [2Fe-2S] and [1Fe-2S] complexes as hybrid compounds in the crystalline state, which were developed in the Institute of Problems of Chemical Physics of the Russian Academy of Sciences. In the methods for the synthesis of the complexes, functionalized aliphatic and azaheterocyclic thiols **1**—**16** were used as sulfur-containing ligands. These thiols are widely used in biochemical and medical experiments for the growth inhibition of malignant tumors of different genesis[122—124] and the cell genome protection an also as antitumor and antibacterial agents.

The structural unit $\{Fe(NO)_2\}$ containing NO, which is the key signal molecule serving as a mediator of various physiological processes *in vivo*, is the second pharmacologically important ingredient of this hybrid.

Among functionalized thiols having antibacterial and inhibitory activities, noteworthy are benzimidazole-2-thiol (**8**) (the cAMP phosphodiesterase inhibitor) and benzothiazole-2-thiol (**7**) (the polyphenol oxidase inhibitor) exhibiting antimicrobial properties. Azaheterocyclic thiols, in particular compound **8** and its derivatives, which are structural analogs of purine bases in DNA, are known as reversible inhibitors of cellular DNA and RNA synthesis and, along with benzoxazole and benzothiazole derivatives, are widely used for the growth inhibition of malignant tumors of different genesis.[125]

Triazole- and tetrazolethiols exhibit a broad spectrum of antimicrobial and fungicidal properties, cause arrest of S-ribosome and DNA formation, and inhibit the riboflavin biosynthesis.[126—127] It is known that pyridine-2-thiol (**5**) is a potential antimetabolite of pyrimidine bases of nucleic acids and its pharmacological effect is similar to that of 6-thioguanine and 6-mercaptopurine, which are used in clinical practice for the acute leukemia treatment.[128]

Imidazolidine-2-thione is the structural analog of ergothioneine. Mercaptohistidines (small aromatic thiols) dif-



fer in many aspects from other naturally occurring thiols, most of which are cysteine derivatives. The aromatic rings of imidazole in mercaptohistidines impart unusual reactivity to the thiol group, whose active form (the thiolate anion) exists in a wide pH range. The *in vitro* studies of model mercaptohistidines provided evidence that these compounds are efficient radical acceptors and can protect against damages induced by peroxynitrite and active oxygen intermediates, as well as have potential antioxidant[129] and antitumor[130] activities.

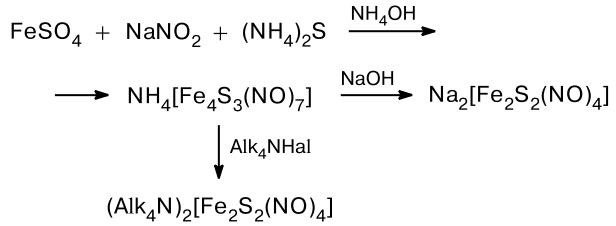
Natural aliphatic thiols also exhibit a wide range of biological activities. Cysteamine (**1**) is used in clinical practice as a detoxifying agent.[131] This compound can protect cells against DNA damage and can be used in the treatment of particular cancer forms;[132] it efficiently acts on human lymphocyte cells[133] and neoplastic nerve cells and induces apoptosis in leukemia cell subpopulations. Penicillamine (**2**), which has no antibiotic activity, is used in medicine as a chelating agent to accelerate the removal of heavy metals that are present at high non-physiological concentrations from the body (therapy for Wilson—Konovalov disease) and non-physiological heavy metals (an antidote to mercury and lead poisoning).[134] Only pure D-penicillamine is therapeutically important, because the L isomer is toxic (the difference between the D and L isomers is attributed to their antagonistic activity against Vitamin B_6). Glutathione is a natural tripeptide that is present in living eukaryotic cells at high concentrations (up to 8 μmol L^{-1}).[135] The main function of glutathione is to maintain the intracellular redox potential and protect cells against chemically active oxygen. A decrease in the level of glutathione causes the development of Parkinson´s and Alzheimer´s diseases due to the accumulation of free radicals.[136]

Bidentate nitrogen-containing heterocyclic thiols have a high coordination ability due to the presence of the (N—C—S) structural unit, and the functional properties of iron nitrosyl complexes can be varied depending on the nature of the ligand. The synthesis of nitrosyl iron-sulfur complexes with azaheterocyclic thiols is important also in view of investigations of the coordination modes of iron to thiol in the presence of NO. The ligand binding to the iron atom can occur as follows: *in a monodentate mode* through the sulfur atom η^1-S or through the nitrogen atom η^1-N, *in a bridging mode* both through the sulfur atom μ_2-S (η^2-S) and through the sulfur and nitrogen atoms μ_2-S,N (η^2-S; η^1-N), *in a chelate mode* μ_1-S,N (η^1-S, η^1-N), and *in combined modes* μ_3-S,N (η^2-S, η^1-N) and μ_2-S,N (η^2-S, η^1-N). Besides, the presence of substituents (NH_2, COOH, OH, *etc.*) in heterocyclic thiols can additionally extend the coordination ability of the ligand. It should be noted that the tautomerism and, correspondingly, the presence of a particular tautomer and, in some cases, of their mixture, plays an important role in the existence of a particular coordination mode of a heterocyclic ligand and the formation of a metal—ligand bond. Taking into account the tautomerism of thiols, it is particularly interesting to know the form (thiol or thione) in which aliphatic and azaheterocyclic thiols are coordinated to iron ions that are already bound in a complex with NO. Since the exchange interaction in iron nitrosyl complexes, their full spin, and the spin density distribution are responsible for the reactivity of the complexes, we analyzed also the relationship between the structure of the complexes and their physicochemical and biological properties. This makes it possible to investigate the mechanisms of reactions of nitric oxide and its reactive intermediates *in vivo* and, simultaneously, holds promise for the design of new NO donors of this class having desired biological properties determined by functionalized thiols. The latter can facilitate the enhancement of the effect induced by NO as the polyfunctional regulator messenger, and, consequently, hybrid complexes will be more efficient drugs for the treatment of various pathologies associated with a deficiency of endogenous NO.

Synthesis of nitrosyl iron-sulfur complexes

Synthesis of anionic nitrosyl iron-sulfur complexes. The anionic tetranuclear complex $[Fe_4S_3(NO)_7]^-$ with the ammonium cation is formed by the reaction of aqueous solutions of iron(II) sulfate and sodium nitrate in the presence of ammonium sulfide as the reducing agent in an alkaline medium[137] (Scheme 11). Salts with tetraalkylammonium cations were synthesized by the metathesis. Single crystals of the ammonium salt were prepared by the slow crystallization (48 h) from a water-methanol solution (1 : 1) of the complex in air at ~20 °C. The yields were 57—62%. In the solid state, the complexes are stable in air and are soluble in water and polar solvents.

Scheme 11



Alk = Pr^n, Bu^n

The anionic binuclear sulfide complexes $[Fe_2S_2(NO)_4]^{2-}$ were synthesized by the treatment of dry crystals of $NH_4[Fe_4S_3(NO)_7] \cdot H_2O$ with a 10% aqueous NaOH solution with heating and storage of the reaction mixture on a water bath until the ammonium smell completely disappeared (see Scheme 11). In the case of salts with organic cations, the reaction mixture was treated with a stoichiometric amount of the corresponding tetraalkylammonium

halide in water. Then the solvent was evacuated from the reaction mixture over $CaCl_2$ until crystals formed.[136] The yields were 35—70%. The crystals of the complexes are unstable in air, and the stability decreases with increasing volume of the cation in the series of salts with the cations $Na^+ \rightarrow Cs^+ \rightarrow (Bu)_4N^+$. The complexes are readily soluble in water and polar solvents. It was found that the anionic complexes with the sulfide anion are immediately transformed into complexes with the tetranuclear anion

$$2\,H^+ + 4\,[Fe_2S_2(NO)_4]^{2-} \longrightarrow$$
$$\longrightarrow 2\,[Fe_4S_3(NO)_7] + 2\,S_2^- + N_2O + H_2O.$$

This is evidenced by cyclic voltammograms (CV curves) of their tetrabutylammonium salts, which are almost identical in the shape and peak potentials in tetrahydrofuran and acetonitrile solutions.[137,138] The tetranuclear anions are stable in solution. According to the quantum chemical calculations, the lowest unoccupied molecular orbital of the cluster with the tetranuclear anion, with the orbitals of the Fe—Fe bond making the major contribution, is antibonding. Nevertheless, the one-electron reduction of the anion is reversible. This is indicative of stability of the radical dianion $[Fe_4S_3(NO)_7]^{\cdot 2-}$, at least on the cyclic voltammetry time scale. The total two-electron reduction of the tetranuclear anion is irreversible

$$[Fe_4S_3(NO)_7]^- \xrightarrow{e^-} [Fe_4S_3(NO)_7]^{2-} \xrightarrow{e^-}$$
$$\longrightarrow [Fe_4S_3(NO)_7]^{3-} + P,$$

where P are products.

Anionic complexes with the thiosulfate anion $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ proved to be more stable in solution than those with sulfide anions. The complexes were synthesized[139—142] by the reaction of iron(II) sulfate with sodium thiosulfate in the presence of gaseous NO at 20 °C for 1—1.5 h (Scheme 12). The reaction mixture was kept at 6–8 °C for 15—20 h. Then the crystalline precipitate that formed was separated by filtration and dried in air. Salts with organic cations were synthesized by the treatment of the sodium salt with a stoichiometric amount of the corresponding tetraalkylammonium halide in water. All operations associated with the preparation and mixing of solutions were performed under an inert atmosphere.

Scheme 12

$$2\,FeSO_4 + 4\,Na_2S_2O_3 + 4\,NO\uparrow \longrightarrow$$
$$\longrightarrow Na_2[Fe_2(S_2O_3)_2(NO)_4] + Na_2S_4O_6 + 2\,Na_2SO_4$$
$$\downarrow Alk_4NBr$$
$$(Alk_4N)_2[Fe_2(S_2O_3)_2(NO)_4]$$

Alk = Me, Et, Pr^n, Bu^n

The yields were 34—72%. In the solid state, the complexes are stable in air and are soluble in water and polar solvents.

The CV curves of the thiosulfate complex show two cathodic peaks almost identical in height. The stepwise two-electron reduction of the complex is completely reversible in the first step, which is indicative of stability of the one-electron reduction product (the trianion). The subsequent one-electron reduction of the mixed-valence Fe^0Fe^I complexes is irreversible. The scheme of the reduction of the thiosulfate nitrosyl complex can be written as follows:

$$[Fe_2(\mu\text{-}S_2O_3)_2(NO)_4]^{2-} \xrightarrow{e^-} [Fe_2(\mu\text{-}S_2O_3)_2(NO)_4]^{3-} \xrightarrow{e^-}$$
$$\longrightarrow [Fe_2(\mu\text{-}S_2O_3)_2(NO)_4]^{4-} + P.$$

The ESR parameters of the chemically reduced thiosulfate dianion (g = 2.031 (H_2O), 2.030 (EtOH), 2.032 (CH_2Cl_2)) are similar to the ESR parameters of the quintet of mononuclear DNIC with thiol-containing ligands, which were found in miroorganisms and animal tissues.[140]

Synthesis of neutral nitrosyl iron-sulfur complexes. The presence of stable thiosulfate DNIC in an aqueous solution allowed the exchange reactions of thiosulfate ligands with functionalized heterocyclic thiols (RS^-) to be performed (Scheme 13), resulting in the development of original methods for the synthesis of neutral binuclear iron nitrosyl complexes in aqueous alkaline media (at room temperature under anaerobic conditions)[143—155] (Scheme 13).

Scheme 13

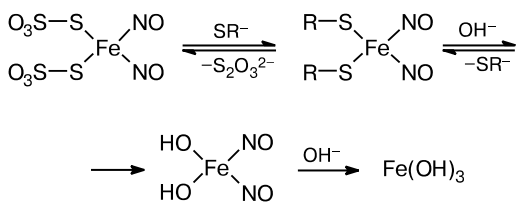
$$[Fe_2(S_2O_3)_2(NO)_4]^{2-} + S_2O_3^{2-} \longrightarrow$$
$$\longrightarrow [Fe(S_2O_3)_2(NO)_2]^{3-} \xrightarrow[-2\,S_2O_2^{2-}]{2\,RS^-,\ nOH^-}$$
$$\longrightarrow [Fe_2(SR)_2(NO)_4]$$

The experimental pH values of the reaction mixtures are given in Table 1. Generally, the pH values of the reaction are close to pK_a of the corresponding thiols. It should be noted that the reaction performed in the presence of an excess of OH^- gave iron(III) hydroxide as the major product (Scheme 14).

Table 1. Experimental pH of the reaction mixtures used for the synthesis of neutral iron-sulfur nitrosyl complexes

S ligand	pK_a	S ligand	pK_a
4	7.1	**11**	10.4
5	9.9	**14**	7.8
7	7.2	**15**	7.5

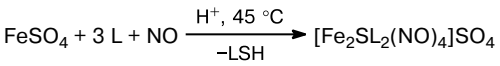
Scheme 14



According to the Mössbauer spectroscopy data, the powders prepared by the reaction presented in Scheme 13 contain an octahedral Fe^{III} phase as an impurity. For example, the Mössbauer spectroscopy parameters for the binuclear iron complex with imidazol-2-yl are $\delta = 0.399(2)$ mm s^{-1} and $\Delta E_Q = 0.798(2)$ mm s^{-1}. These are compounds of the composition $Fe(R)_3$, where R are thiol-2-ates, used for the synthesis. The impurity phase in the reaction mixture accounted for no more than 2—5%. The exception is the complex $[Fe_2(SC_3H_5N_2)_2(NO)_4]$ (**Imid**), for which the content of the impurity phase in some synthesis was 50%. To purify the reaction product from the impurity Fe(III) phase and isolate single crystals of neutral binuclear nitrosyl complexes, the powders were recrystallized from mixtures of organic solvents. The yields were 56—82%.

Synthesis of cationic nitrosyl iron-sulfur complexes. Cationic iron nitrosyl complexes were synthesized[156] by the reduction (Scheme 15) of an anaerobic aqueous solution of iron(II) sulfate with an excess of natural thiol (L-cysteamine, penicillamine, or glutathione) under an NO gas flow at pH 6.5—6.8.

Scheme 15



The reaction was performed with vigorous stirring of the reaction mixture until crystals of the nitrosyl complex formed. Then the reaction mixture was kept at 6—8 °C for 15 h, and the crystalline precipitate that formed was filtered off and dried in air. The yields were 11—30%. The reaction gives single-phase products, which do not require additional purification. The complexes are stable in the absence of an inert atmosphere during a long period of time.

Structures of iron nitrosyl complexes with functionalized ligands

The crystallographic parameters of iron nitrosyl complexes with functionalized S ligands, which are analogs of natural thiamine and histidine, as well as of pyrimidine and purine bases of DNA, are given in Tables 2 and 3.

In the $[Fe_4S_3(NO)_7]^-$ anion (Fig. 5), there are the following two types of contacts: between the apical Fe_a atom and the Fe_b atoms of the basal plane of the trigonal pyramid and between the sulfur-bridged Fe_b atoms of the basal plane (Fe_a—S—Fe_b groups). The sulfur atoms of these bridges form also bonds with the apical Fe_a atom. The average Fe_a—S and Fe_b—S distances are 2.205 and 2.256 Å, respectively. The Fe_a atom is bound to one NO ligand and three bridging S atoms, whereas each Fe_b atom is coordinated by two nitrosyl ligands and two bridging S atoms. The Fe_a—N bond (1.651 Å) is shorter than the Fe_b—N bonds with the peripheral atoms (1.661—1.675 Å). The Fe—N—O angles are nearly linear. The differences in the angles are associated with the formation of intermolecular hydrogen bonds between the cation of the complex and the nitrosyl groups of the anion. The complexes with the $[Fe_4S_3(NO)_7]^-$ anion are stabilized by three-center bonds formed by the bridging sulfur atoms.

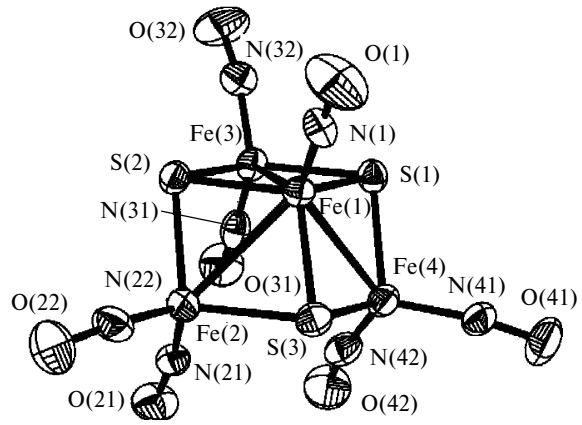


Fig. 5. Structure of the $[Fe_4S_3(NO)_7]^-$ anion in the tetranuclear iron nitrosyl complex **BSR-NH$_4$**. The bond lengths: Fe_a—S, 2.205 Å; Fe_b—S, 2.256 Å; Fe_a—N, 1.651 Å; Fe_b—N, 1.661—1.675 Å; $(N—O)_a$, 1.160 Å; $(N—O)_b$, 1.66 Å; the Fe_a—N—O angle is 176.5°; the Fe_b—N—O angle is 168.1°; Fe_a...Fe_b, 2.697 Å.

In the $[Fe_2S_2(NO)_4]^{2-}$ anion (Fig. 6, *a*), two iron atoms are linked by two bridging sulfur atoms. Each iron atom is tetrahedrally coordinated by two sulfur atoms and two NO groups. The Fe—N—O fragments are linear. The Fe—N—O angles are similar to the Fe_b—N—O angles in the $[Fe_4S_3(NO)_7]^-$ anion.

In the $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ anion (Fig. 6, *b*), each iron atom is linked to another iron atom by two μ-sulfur atoms and two nitrogen atoms of two NO groups, and the bridging sulfur atom is bound to the SO_3 group. The distribution of the bond lengths in the series of complexes with the Na^+ cation and tetraalkyl cations (Me_4N^+, $(Bu^n)_4N^+$) is similar to that in tetranuclear Fe_b iron complexes. The cations occupy channels in the blocks of thiosulfate anions formed by the negatively charged oxygen atoms of SO_3 groups. The presence of SO_3 groups at the bridging sulfur atoms, on which the negative charge is localized, leads to the electron density redistribution in the thiosulfate complexes compared with the sulfide complexes

Table 2. Principal crystallographic data for anionic and cationic μ-S-type iron complexes

Parameter	**BSR-NH$_4$**	**RSR-Na**	**RSR-Pr**	**TNIC-Na**	**TNIC-Me**	**TNIC-Bu**	**Cys**	**Pen**
Molecular formula	NH_4-$[Fe_4S_3(NO)_7]$ · H_2O	$Cs_2[Fe_2S_2(NO_4]$ · $2H_2O$	$((Pr^n)_4N)_2$-$[Fe_2S_2(NO)_4]$	Na_2-$[Fe_2(S_2O_3)_2(NO)_4]$ · $4H_2O$	$(Me_4N)_2$ $[Fe_2(S_2O_3)_2(NO)_4]$	$((Bu^n)_4N)_2$ $[Fe_2(S_2O_3)_2(NO)_4]$	$[Fe_2(S(CH_2)_2$-$NH_3)_2(NO)_4]$-SO_4 · $2.5H_2O$	$[Fe_2(S(C_5H_{11}NO_2)_2$-$(NO)_4]$ SO_4 · $5H_2O$
Empirical formula	$Fe_4S_3N_8O_8H_6$	$Cs_2Fe_2S_2N_4O_6H_4$	$Fe_2S_2N_6O_4C_{24}H_{56}$	$Na_2Fe_2S_4O_{14}N_4H_8$	$Fe_2S_4O_{10}N_6C_8H_{24}$	$Fe_2S_4O_{10}N_6C_{32}H_{72}$	$Fe_2S_3N_6O_{10.5}C_4H_9$	$Fe_2S_3C_{10}H_{32}N_6O_{17}$
Molecular weight	566	597	668	574	604	780	527	716
Crystal system	Triclinic	Monoclinic	Monoclinic	Monoclinic	Triclinic	Monoclinic	Triclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/c$	$P2_1/n$	$P2_1/c$	$P\bar{1}$	$P2_1/c$	$P\bar{1}$	$P2_1$
a/Å	9.451(2)	9.608(2)	10.455(2)	11.22(4)	7.719(2)	20.332(4)	6.850(1)	6.1878(4)
b/Å	10.000(2)	11.402(2)	13.647(1)	10.44(4)	12.272(2)	13.070(3)	10.575(2)	28.2739(17)
c/Å	10.577(2)	12.601(3)	12.504(3)	7.62(2)	6.513(1)	18.009(4)	13.723(2)	7.8615(5)
α/deg	59.02(3)	90	90	—	83.78(3)	90	90.884(3)	90
β/deg	68.57(3)	107.13(3)	92.02(3)	92.2(2)	86.30(3)	91.07(3)	95.900(3)	102.456(14)
γ/deg	79.05(3)	90	90	—	73.48(3)	90	90.635(3)	90
V/Å^3	797.9(3)	1319.2(5)	1781.6(7)	892(5)	587.6(2)	4785(2)	988.6(3)	1343.02(15)
Z	2	4	2	2	1	4	2	2
d_{calc}/g cm^{-3}	2.353	3.009	1.246	2.11(2)	1.708	1.205	1.771	1.771
R factors based on all reflections								
R_1	—	—	—	—	—	—	0.1204	0.0392
wR_2	0.0512	0.0394	0.036	0.091	0.116	0.096	0.0548	0.0811
R factors based on reflections with $I > 2(\sigma)(I)$	—	—	0.089	0.116	0.265	0.254	—	—

Table 3. Principal crystallographic data for neutral μ-S- and μ-N-C-S-type iron complexes

Parameter	**Py**	**Pym**	**Triaz**	**AmTriaz**	**Im**	**Mim**	**Imid**	**Bim**	**Tetraz**
Molecular formula	$[Fe_2(SC_5H_4N)_2(NO)_4]$	$[Fe_2(SC_4H_3N_2)_2(NO)_4]$	$[Fe(SC_2H_3N_3)(SC_2H_2N_3)(NO)_2]\cdot 1/2H_2O$	$[Fe_2(SC_2H_3N_4)_2(NO)_4]\cdot 2H_2O$	$[Fe_2(SC_3H_3N_2)_2(NO)_4]$	$[Fe_2(SC_4H_5N_2)_2(NO)_4]$	$[Fe_2(SC_3H_5N_2)_2(NO)_4]$	$[Fe_2(SC_7H_5N_2)_2(NO)_4]\ 2C_3H_6O$	$[Fe_2(SC_2H_3N_4)(NO)_4]$
Empirical formula	$Fe_2S_2N_6O_4C_{10}H_8$	$Fe_2S_2N_8O_4C_8H_6$	$FeS_2N_8O_{2.5}C_4H_6$	$Fe_2S_2N_{12}O_6C_4H_{10}$	$Fe_2S_2N_8O_4C_6H_6$	$Fe_2S_2O_8O_4C_8H_{10}$	$Fe_2S_2N_8O_4C_6H_{10}$	$Fe_2S_2N_8O_5C_{20}H_{22}$	$Fe_2S_2C_4H_6N_{12}O_4$
Molecular weight	452	454	326	498	430	458	434	630	462
Space system	Monoclinic	Triclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Triclinic	Triclinic	Monoclinic
Space group	*C*2/*c*	$P\bar{1}$	*C*2/*c*	$P\bar{1}$	*P*2(1)/*n*	*C*2/*c*	$P\bar{1}$	$P\bar{1}$	*P*2(1)/*c*
a/Å	20.935(4)	6.4170(10)	18.789(4)	8.006(2)	7.473(1)	14.455(3)	6.422(2)	8.737(1)	7.587(2)
b/Å	7.964(2)	7.6200(10)	9.528(2)	7.809(2)	12.928(3)	9.848(2)	7.618(2)	9.072(1)	13.347(3)
c/Å	13.697(3)	8.348(2)	13.623(3)	8.471(3)	8.173(1)	13.108(3)	8.194(2)	9.083(1)	16.406(3)
α/deg	90	75.550(10)	90	64.42(3)	90	—	100.93(2)	74.82(1)	103.25(3)
β/deg	132.65(3)	80.800(10)	99.73(3)	71.46(3)	105.35(3)	116.36(3)	100.93(2)	73.10(1)	—
γ/deg	90	85.200(10)	90	67.01(3)	90	—	90.08(3)	86.62(1)	—
V/Å^3	1679.6(6)	397.3(1)	2403.7(9)	432.6(2)	761.4(3)	1671.9(6)	386.2(2)	664.70(13)	1671.1(6)
Z	4	1	8	1	2	4	1	1	4
d_{calc}/g cm^{-3}	1.756	1.934	1.802	1.912	1.85	1.82	1.866	1.615	1.898
R factors based on all reflections									
R_1	—	—	—	—	—	—	—	—	0.0863
wR_2	0.1316	0.0356	0.0304	0.0394	0.0400	0.0408	0.0321	0.0274	0.2728
R factors based on reflections with $I > 2(\sigma)(I)$	0.2893	0.0732	0.0806	0.1023	—	0.0884	0.0503	0.0722	—

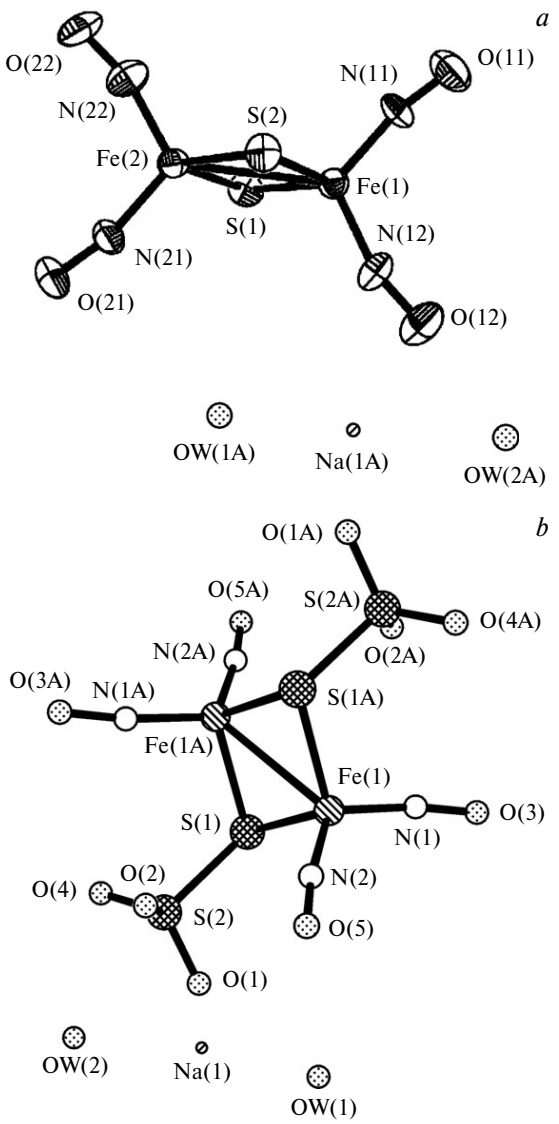


Fig. 6. Structures of the anions in the binuclear iron nitrosyl complexes $[Fe_2S_2(NO)_4]^{2-}$ (*a*) and $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ (*b*). The average interatomic distances in the sulfide anion: Fe—S, 2.230 Å; N—O, 1.170 Å; Fe—N, 1.659 Å; Fe—Fe, 2.702 Å; the Fe—N—O angle is 164.6°. The average interatomic distances in the thiosulfate anion: Fe—S, 2.256 Å; N—O, 1.158 Å; Fe—N, 1.670 Å; Fe—Fe, 2.702 Å; the Fe—N—O angle is 169.6°.

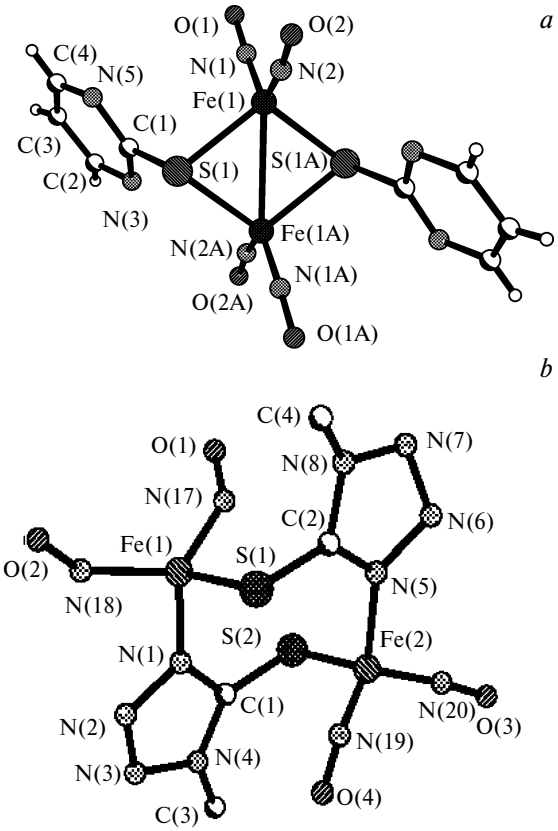


Fig. 7. Structures of the neutral binuclear iron nitrosyl complexes: *a*, $[Fe_2(SC_4H_3N_2)_2(NO)_4]$ (**Pym**); *b*, $[Fe_2(SC_2H_3N_4)_2(NO)_4]$ (**Tetraz**). In the complex **Tetraz**, the bond lengths are: Fe—S, 2.229 Å; Fe—N(3), 2.010 Å; Fe—N, 1.674 Å; N—O, 1.159 Å; C—S, 1.736 Å; C—C, 1.365 Å; the Fe—N—O angle is 167.3°. The Fe...Fe distances in the complexes **Pym** and **Tetraz** are 2.727 and 4.06 Å, respectively.

$[Fe_2S_2(NO)_4]^{2-}$. The SO_3 groups cause steric hindrance to the transformation of the binuclear complex into the tetranuclear complex $[Fe_4S_3(NO)_7]^-$. The complexes are more stable both in the solid state and solution than the sulfide complexes due to the formation of three-center bonds by the bridging sulfur atoms.

The geometry of neutral binuclear complexes, for example, of **Pym** (Fig. 7, *a*), is similar to that of thiosulfate complexes. The average bond lengths are as follows: Fe—S, 2.265 Å; Fe—N, 1.663 Å; N—O, 1.162 Å; the Fe—N—O angle is 170.0°. The C—S bond length is 1.805(5) Å, which indicates that aromatic thiols are coordinated to iron in the thiol form. The X-ray diffraction studies of the anionic and neutral complexes revealed that the bond lengths in nitrosyl groups vary in a wide range.

In neutral binuclear complexes, for example, in **Tetraz** (Fig. 7, *b*), heterocyclic ligands are bound to iron atoms through the bridging sulfur and nitrogen atoms (μ-S-C-N mode). The iron atoms have a tetrahedral configuration but are spaced, on the average, by ~4 Å. The bond lengths between the iron atom and the nitrogen atom of the heterocyclic ligand, Fe—N(3), in paramagnetic binuclear sulfur nitrosyl complexes is 2.010 Å; the Fe—S bond length is 2.299 Å. According to the X-ray diffraction data, the average C—S bond length in heterocycles is 1.736 Å, which is larger than the length of the C=S double bond (1.684 Å), *i.e.*, the distribution of the bond lengths and bond angles in the aromatic ligands of all μ-S-C-N-type complexes is better consistent with the thiol form of the ligand. An analysis of the bond lengths in the structural unit $\{Fe(NO)_2\}$ in all complexes shows that they are only slightly

different. It is assumed that the $\{Fe(NO)_2\}$ moiety can undergo internal disproportionation up to the complete oxidation/reduction of NO groups. The partial oxidation/reduction in the Fe—NO moiety can occur due to the easy $d_{Fe}\rightarrow\pi^*_{NO}$ donation and back donation accompanied by the shortening/elongation of Fe—N bonds and the elongation/shortening of the N—O bonds. In the complex $[Fe_2(SC_3H_5N_2)_2(NO)_4]$ (**Imid**), the Fe—N—O group tends to be more linear compared with those in the complexes $[Fe_2(SC_3H_3N_2)_2(NO)_4]$ (**Im**) and $[Fe_2(SC_4H_5N_2)_2(NO)_4]$ (**Mim**). The observed substantial decrease in the length of the bond between the iron atom and the nitrogen atom of the heterocycle in the complex **Imid** accounts for its higher stability in protic media. The C—S bond lengths in the complexes under consideration (1.740(4), 1.729, and 1.741 Å for **Im**, **Mim**, and **Imid**, respectively) are larger than the average length of the C=S double bond (1.684 Å), which is evidence in favor of the C—S single bond and the coordination of the ligand in the thiol form in spite of the fact that the reaction giving the complex **Imid** proceeds with the involvement of the ligand in the tautomeric thione form.

In the neutral mononuclear complex $[Fe(SC_2H_3N_3)(SC_2H_2N_3)(NO)_2]$ (**Triaz**) (Fig. 8), the Fe atom is characterized by a weakly distorted tetrahedral configuration with the angles in the range of 102.74(2)—119.79(3)°. The NO groups in **Triaz** are weakly bent (the Fe—N—O angles are 169.02(7)—172.84(6)°). A comparison of the geometry of the heterocycles A and B shows that, in spite of the protonation, the bond lengths in the five-membered rings are very similar (differ by only 0.01 Å). The C(2)—N(3) and C(4)—N(7) bond lengths for the protonated and nonprotonated nitrogen atoms of the rings A and B have similar values (1.3561(8) and 1.3585(8) Å, respectively) due to the short inter- and intramolecular N(6)—H(6)...N(3) and N(7)—H(7)...N(5′) distances and the short intramolecular N—O(2)...C(2A) (O(2)...C(2A), 2.995(1) Å) and N—O(1)...N(6B), N—O(1)...N(8B) (3.046(1)—3.047(1) Å) contacts. An analysis of the electron density revealed that both five-membered rings have almost identical configurations, *i.e.*, the similar charge density in the region of the chemical bond and the lone electron pairs in the vicinity of the hydrogen and sulfur atoms. The maxima corresponding to the electron pairs are only slightly different for the S(1) and S(2) atoms due apparently to their coordination to the metal atom. In the S(1)S(2)Fe (see Fig. 8, *b*) and N(1)N(2)Fe (Fig. 8, *c*) planes, the electron density is localized in the vicinity of the Fe atom and is characterized by the cross-type distribution. In the region of Fe—NO interactions, the maxima on the nitrogen atoms are oriented toward the depletion region around the Fe atom and, consequently, these bonds can be described as peak—hole interactions. On the contrary, the Fe—S bonds and, in particular, the Fe—S(2) bond, belong to peak—peak interactions. The Fe—S(1) and Fe—S(2) interactions are similar and are characterized by the same chemical bond type. Substantial differences are observed for the NO groups. Negative charges are localized on the nitrogen atoms of the rings, the sulfur atoms, and the oxygen atoms of the NO ligands. It should be noted that the nitrosyl nitrogen atoms are almost neutral; the net charges on the N(1)O(1) and N(2)O(2) groups are in the range from −0.25 to −0.37 e. The charges on the S(1) and S(2) atoms are similar to that (−0.20 e) on the exocyclic sulfur atom in the 1,2,4-triazole-5-thiolate anion. The average bond length in the complex **Triaz** is 1.725(2) Å, which indicates that the ligands are involved in the coordination in the thiol form.

The salt with the $[Fe_2(S(CH)_2NH_3)_2(NO)_4]^{2+}$ dication in the complex **Cys** (see Fig. 9) crystallizes with 2.5 water molecules, and the salt with the $[Fe_2(S(C_5H_{11}NO_2)_2(NO)_4]^{2-}$ dication in the complex **Pen** crystallizes with five water molecules.[156] In the dications, the iron atoms are bound to two nitrogen atoms of the nitrosyl ligands and two μ-S atoms of the protonated ligands. The iron atoms have distorted tetrahedral geometry with the maximum devia-

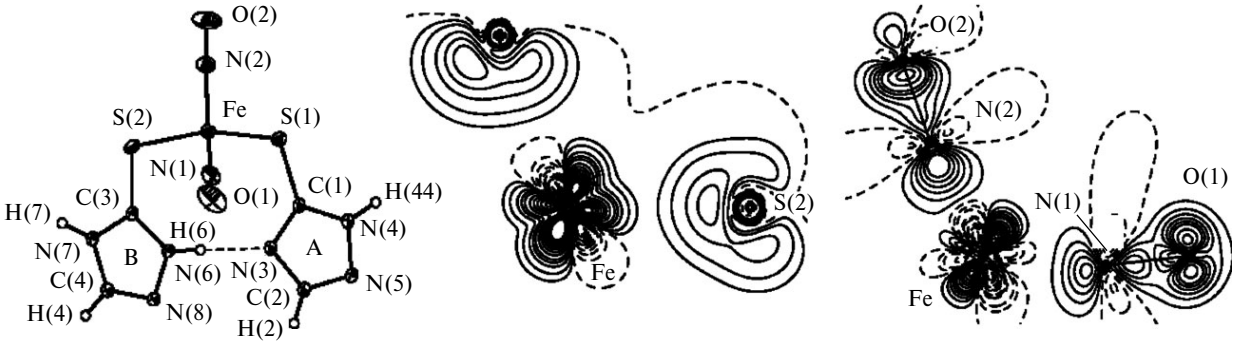


Fig. 8. Molecular structure of the mononuclear iron nitrosyl complex $[Fe(SC_2H_3N_3)(SC_2H_2N_3)(NO)_2]\cdot 1/2H_2O$ (*a*), and the deformation electron density maps in the FeS(1)S(2) (*b*) and FeN(1)N(2) (*c*) planes of this complex. The contour intervals are at 0.1 e $Å^{-3}$; the negative values are plotted as dashed lines. The bond lengths are: Fe—S, 2.302 and 2.322 Å; Fe—N, 1.669 and 1.677 Å; N—O, 1.164 and 1.163 Å; C—S, 1.731 and 1.707 Å; the Fe—N—O angles are 169.0 and 172.8°.

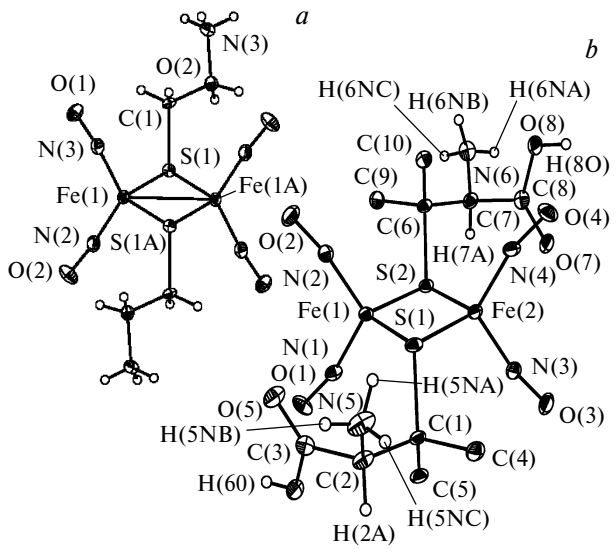


Fig. 9. Structures of the cations in the iron nitrosyl complexes with natural aliphatic thiols, $[Fe(S(CH_2)_4NH_3)_2(NO)_2]SO_4 \cdot 2.5H_2O$ (**Cys**) (*a*) and $[Fe(S(C_5H_{11}NO_2)_2(NO)_4]SO_4 \cdot 5H_2O$ (**Pen**) (*b*). The bond lengths in the complex **Cys**: Fe—S, 2.244—2.252 Å; Fe—N, 1.665—1.675 Å; N—O, 1.164—1.180 Å; the Fe—N—O angles are 171.4—173.7°, the N—Fe—N angles are 121.6°; Fe...Fe, 2.672 Å. The bond lengths in the complex **Pen**: Fe—S, 2.255—2.262 Å; Fe—N, 1.666—1.699 Å; N—O, 1.163—1.172 Å; the Fe—N—O angles are 165.8—169.1°, the N—Fe—N angles are 131.95°; Fe...Fe, 2.709 Å.

tion of the NO—Fe—NO angles to 121.3(2)°, except for the Fe(1)—Fe(1A) bond. Although two independent dications have almost identical geometry, the Fe...Fe distances in these dications are different (2.672 (1) and 2.682 (1) Å). The structures of both complexes are similar to that of the thiosulfate anion and belong to the structure type of Roussin´ red salt esters.

The geometric and electronic structures of the neutral binuclear tetranitrosyl iron complexes with μ-S-type aromatic thiolyls, **Ph**, **Py**, and **Pym**,[157] the complexes **AmTriaz**, **Im**, **Mim**, and **Imid** with μ-N-C-S-coordinated bridging ligands,[158] and the mononuclear dinitrosyl iron complex **Triaz** (see Ref. 159) in different isomeric states were calculated by the density functional theory methods at the B3LYP and PBE levels of theory. Both theoretical approaches give good agreement between the geometric structures of the complexes and the experimental values; the rms differences in the bond lengths and bond angles are 0.02—0.04 Å and 2—3°, respectively.

It was found that the ground state of the system in the μ-S-type complexes **Ph**, **Py**, and **Pym** is diamagnetic. This state arises for the $\{Fe(NO)_2\}$ units with the antiparallel orientation of the local spins 1/2. The NO group bears a small negative charge localized primarily on the O atom, and the Fe—NO bond should be considered as homopolar.

For the complexes **AmTriaz**, **Im**, **Mim**, and **Imid**, the μ-N-C-S-coordination mode is energetically more favorable compared with the μ-S-coordination mode. As a result, the Fe...Fe distances are longer due to which the intramolecular exchange coupling is insignificant, and at $T = 296$ K the complexes are paramagnetic with $\mu_{eff} = 2.5\ \mu_B$. The interaction between the spins of the Fe atoms is antiferromagnetic.

The neutral mononuclear complex **Triaz** is characterized by the presence of an intramolecular N—H...N hydrogen bond between the ligands A and B (see Fig. 8), resulting in the equalization of the Fe—S and S—C bond lengths. The spin of the ground state of the system is 1/2. Each NO group bears a small negative charge on the O atom, and the Fe—NO bond is nearly homopolar. The optimized geometry of the dication **Cys** in the singlet state[160] is also in good agreement with the experimental structural data. The typical deviations of the bond lengths and bond angles are not larger than 0.1 (B3LYP) and 0.03 Å (PBE) and 4°, respectively. Each $Fe(NO)_2$ group possesses an unpaired electron. Therefore, in all types of complexes the electronic configuration of the $Fe(NO)_2$ unit with one unpaired electron is formed upon coupling of the spin 3/2 of the Fe atom to the opposite spins 1/2 of two NO groups, which corresponds to the oxidation state Fe^{+1} (d^7). The theoretical calculations not only give good agreement with the experimental structures of the complexes but also adequately describe their IR spectra. The classification of the electron density distributions for the metal—nitric oxide bond (M—NO) is shown in Scheme 16.

Scheme 16

$$[M^{(n-1)+}—NO^+] \leftrightarrow [M^{n+}—NO] \leftrightarrow [M^{(n+1)+}—NO^-]$$
$$\mathbf{I} \qquad\qquad \mathbf{II} \qquad\qquad \mathbf{III}$$

It is known that the type **I** of the electron density is characterized by short metal—NO bonds, high stretching frequencies of NO groups (1650—1985 cm^{-1}) in the IR spectra, and the electrophilic activity.[161] The type **III** is characterized by an elongation of the M—NO bonds, a decrease in the stretching frequencies of NO groups (1525—1590 cm^{-1}), and the nucleophilic activity. The M—NO bond is characterized by diverse geometry.[57]

The stretching frequencies of the NO group in the complexes under study are in the range of 1657—1807 cm^{-1}. The bond and the angle at the apical iron atom in the tetranuclear anion $[Fe_4(\mu_3\text{-}S)_3(NO)_7]^-$ most closely correspond to the linear geometry (ν_{NO} 1738.7—1725.3 cm^{-1}). The characteristic stretching frequencies of NO in the IR spectra of the complexes with the sulfide anion $[Fe_2S_2(NO)_4]^{2-}$ are in the range of 1657.0—1719.0 cm^{-1}. In the salts with the thiosulfate anion $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$, there is the difference between the NO groups. Thus, one of the groups is less linear than another (ν_{NO} 1741.0 and 1794.0 cm^{-1}, respectively). This difference is confirmed

by the X-ray diffraction data and is observed also for the neutral complexes with aromatic μ-S- and μ-N-C-S-type thiolyls (ν_{NO} 1723.0—1797.0 and ν_{NO} 1725.0—1807.0 cm^{-1}, respectively) and the cationic complexes with aminothiolyls (ν_{NO} 1723.0—1773.0 cm^{-1}).

According to the Mössbauer spectroscopy data, the isomer shifts δ_{Fe_a} for the complexes with the $[Fe_4(NO)_7S_3]^-$ anion are similar to those obsereved for the neutral cubane-type complex $[Fe_4(NO)_4S_4]$.[137] In the salts with the $[Fe_2S_2(NO)_4]^{2-}$ anion, the role of the cation is not simply to compensate the negative charge of the cluster. An increase in the size of the cation leads to a substantial decrease in the quadrupole splitting ΔE_Q, *i.e.*, the total charge distribution in the valence shell of iron and the surrounding atoms becomes more symmetrical. The isomer shift (δ) also substantially decreases, which is evidence of an increase in the s-electron density on the Fe^{57} nuclei in the series from Na^+ to Bu_4N^+ and is consistent with a decrease in the Fe—S and Fe—Fe bond lengths. The shifts δ in $[Fe_2(S_2O_3)_2(NO)_4]^{2-}$ are almost two times larger (Fig. 10) than the δ values for the isoelectronic complexes with the $[Fe_2S_2(NO)_4]^{2-}$ dianion. This is indicative of a decrease in the 4s-electron density on the iron atom due apparently to the electron-withdrawing properties of SO_3 groups. In the thiosulfate complexes, the Fe—S bond lengths and the Fe—N—O angles are larger, whereas the N—O bond lengths are smaller than the corresponding parameters in the sulfide complexes. The charge on NO in the thiosulfate anion can be formally considered as more positive than that in the sulfide anion. The δ parameters for the neutral μ-S-type complexes slightly (by 0.02 mm s^{-1}) differ from those for the thiosulfate complexes. The δ values for the μ-N-C-S-type complexes are larger (by almost a factor of two) compared with the corresponding μ-S-type complexes, which is indicative of a decrease in the 4s-electron density on the iron atom in this type of complexes. A comparison of the Fe—N—O structural units in the μ-N-C-S-type complexes with those in the μ-S-type complexes also showed that they are nonequivalent. Thus, the N—O and Fe—N bonds in the Fe—N(2)—O(2) group are shorter (N(2)—O(2), 1.169(7) Å; Fe—N(2), 1.661(6) Å), and this group is more linear (the angle is 171.5(6)°). The bond lengths in another group, Fe—N(1)—O(1), are longer (N(1)—O(1), 1.187(7) Å; Fe—N(1), 1.681(5) Å), and the Fe—N—O angle (158.1(5)°) is smaller than the corresponding angles in all known complexes. The difference in the bond angles in the Fe—N—O groups in these complexes is substantial (13.5°), as opposed to the μ-S-type complexes, in which this difference is, on the average, 2—4°. Presumably, this difference in the structure of the iron nitrosyl moieties is attributed to the charge redistribution in the μ-N-C-S-type iron complexes, resulting in that one of NO groups becomes more positively charged. In the μ-N-C-S complexes, the Fe—N(2) bond (1.661(6) Å) is substantially shorter than another bond (1.681(5) Å), and

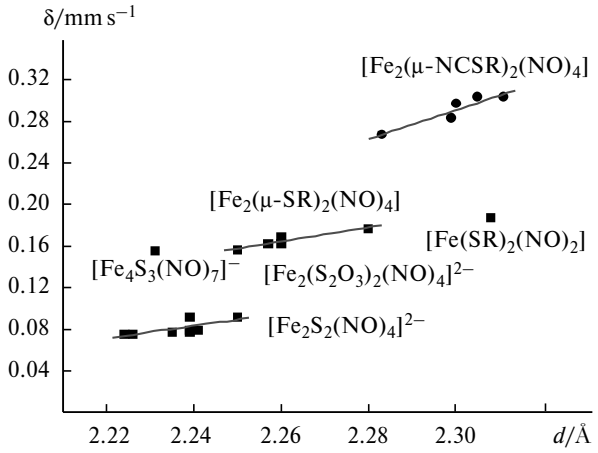


Fig. 10. Isomer shifts (δ) and the Fe—S bond lengths (*d*) in iron nitrosyl complexes with S-functionalized ligands.

the Fe—N(2)—O(2) is more linear (171.5(6)°). The stretching frequencies of nitrosyl groups in the IR spectra of this type of complexes are high, the difference between two absorption bands being 73 cm^{-1}, whereas this value for the μ-S complexes is 20—43 cm^{-1}. The observed substantial splitting of the bands is apparently associated with the nonequivalence of NO groups in Fe—N—O moieties.

In the nitrosyl dications $[Fe_2(SR)_2(NO)_4]^{2+}$, the isomer shifts are in the range characteristic of anionic and neutral μ-S-type complexes. In going from the $[Fe_2S_2(NO)_4]^{2-}$ salts with different charge-compensating cations to neutral complexes, the δ parameter undergoes a jump by approximately 0.1 mm s^{-1}. It is difficult to determine the oxidation state of iron in nitrosyl complexes because of a small energy difference between the d orbitals of the metal and the π* orbitals of NO. Hence, the total charge distribution in one of two equivalent moieties of the binuclear complex $\{Fe(NO)_2\}^x$, where *x* is the sum of the d electrons on the metal and the π* electrons on NO, can be taken into account. It should be noted that the $\{Fe(NO)_2\}$ moiety in the dimer contains nine valence electrons regardless of the type of the bridging ligands, $(S)^{2-}$, $(SR)^-$, or $(SR)^0$. Then, the electronic state of the iron atom should remain unchanged without account for the difference in the geometry of the Fe—NO bonds (the Fe—N—O angles are in the range of 167—173°). In fact, this change in the formal charge of the bridging sulfur atom from −2 to −1 leads to an increase in the Fe—S bond length, on the average, by 0.03 Å and, correspondingly, to an increase in the isomer shift. Since the positively charged R substituents in this series are also acceptors, the isomer shifts are in the range characteristic of $(S)^-$-bridged Roussin´ salt esters. A slight increase in the isomer shift can be related to an increase in the electron-withdrawing ability and the length of R (in going from cysteamine to penicillamine and glutathione).

Physicochemical properties of iron nitrosyl complexes with functionalized S ligands in the solid state and solution

An analysis of the mass spectra of the gas phase upon heating of anionic and neutral complexes at $T = 25$ °C showed that the gas phase consists of water, CO, and CO_2 molecules captured by samples from atmospheric air. After heating to 70—120 °C, the spectra show peaks corresponding to $[NO]^+$ (the most intense), $[CO]^+$, $[H_2O]^+$, $[N]^+$, and $[CS]^+$ ions, which are decomposition products of the ligands. The cationic complexes are more stable. The heating of these complexes to 70 °C does not initiate their decomposition, and the spectra have peaks belonging to molecular ions of the gases that are present in the air.

The studies of the magnetic properties of iron nitrosyl complexes provide direct information on their spin state.[162] Figure 11 presents the temperature dependences of the effective magnetic moment $\mu_{eff}(T)$ for neutral μ-N-C-S-type complexes. The magnetic susceptibility curves $\chi(T)$ for the complexes have a maximum characteristic of the dimers at 63 and 83 K (Fig. 12). The presence of the maximum in the curves $\chi(T)$ is attributed to the competition between the spin relaxation and the exchange interaction of negative sign. For the complex **AmTriaz**, the magnetic moment μ_{eff} is almost constant up to ~50 K due to weaker intramolecular exchange interactions compared to those in the complexes **Im** and **Mim**. The curves $\mu_{eff}(T)$ for all complexes at high temperatures approach ~2.5 μ_B indicating that each Fe atom possesses an unpaired electron, *i.e.*, each metal center is in the low-spin state $S = 1/2$, like in the mononuclear paramagnetic complex **Triaz**. The theoretical value for noninteracting spins is $g\sqrt{2S(S+1)} = 2.45\ \mu_B$ at $g = 2$. At low temperatures, the magnetic susceptibility slightly increases due apparently to the presence of monomeric impurities in the samples. As a result, the magnetic moment μ_{eff} is not zero at $T \to 0$. The experimental data for the complexes **Im**, **Mim**, and **Imid** are adequately described by the curves $\chi(T)$ fitted with the use of the Bleaney—Bowers dimer model taking into account the exchange interactions between the dimers and the presence of a paramagnetic impurity. For the complex **AmTriaz**, this model is less accurate due, apparently, to spin-orbital interactions. The optimal parameters of this model were reported in the study.[159] The calculated hyperfine coupling constants of the nitrogen atoms involved in the coordination sphere of the Fe atoms and the constants of the ^{17}O nuclei of the nitrosyl ligands are of the same order of magnitude as those of the N atoms directly bound to the Fe atoms. The experimental observation of the hyperfine structure of the spectra (hss) for the ^{17}O atoms is important for the understanding of the bonding nature of NO ligands, because these values are governed by the character of the bonding molecular orbital of the O—N—Fe group. The simulation of the ESR spectra with the calculated hcc values gives the same line width as that observed

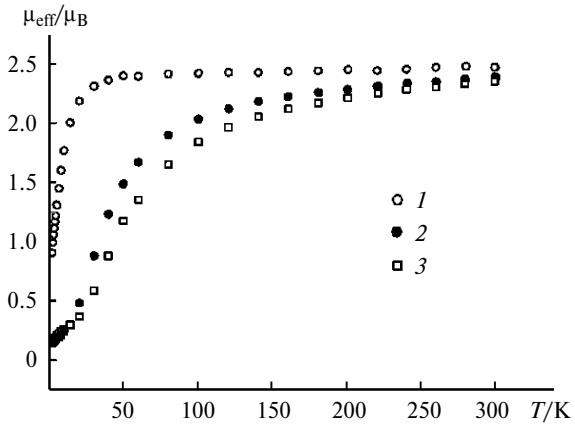


Fig. 11. Temperature dependences of the effective magnetic moments for the complexes **AmTriaz** (*1*), **Im** (*2*), and **Mim** (*3*) in a magnetic field of 5 kOe.

in the experimental spectra of powders of **AmTriaz**, **Im**, **Mim**, **Imid** with unresolved hss. The calculated Mulliken charges on the NO group have small negative values (from −0.26 to −0.31). The sign of the charge and a decrease in the NO vibrational frequency indicate that the NO groups are medium-strength acceptors. However, the almost linear coordination of the NO ligands indicates that the value of the accepted electron density is small.

At room temperature, the crystalline complex **Triaz** was characterized by the Lorentzian-shaped ESR signal peaked at $g = 2.04$ with a half-width of 1.7 mT.[163] The dissolution of the complex in methanol led to the appearance of a more narrow signal at the center of this ESR signal peaked at $g = 2.35$ with a half-width of 0.7 mT, whose intensity increased as the crystals were dissolved until the wider signal completely disappeared. For solutions of the binuclear complex **Tetraz**, hss was observed[153] due to magnetic interactions of the spins of unpaired elec-

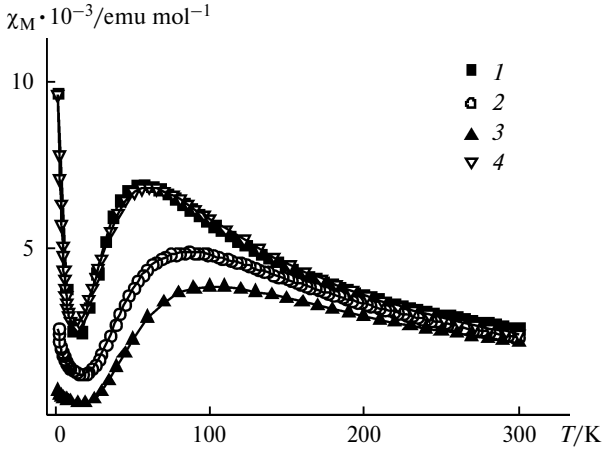


Fig. 12. Temperature dependences of the magnetic susceptibility for the complexes **Bim** (*1*), **Mim** (*2*), **Imid** (*3*), and **Im** (*4*) in a static magnetic field of 1 kOe.

trons with protons and nitrogen nuclei. For polycrystals, hss is not observed. It was found that the spectrum corresponds to the starting complex **Tetraz**. The fact that the identical ESR spectra were obtained in different solvents attests to the binuclear nature of the paramagnetic species, because the dissociation of the complex **Tetraz** into two monomers results in the appearance of the coordination vacancy on the iron atom. This vacancy is occupied by a solvent molecule, which should have an effect on the parameters of the ESR spectrum. In addition, the slower attenuation of the ESR signal of the complex **Tetraz** in DMSO compared with its solutions in EtOH, which correlates with a substantial difference in the stability of **Tetraz** in these media, also confirms this assignment. The decrease in the intensity of the ESR spectra of **Tetraz** solutions with time indicates that the stable decomposition products of this complex are diamagnetic, *i.e.*, the primary decomposition products of the complex undergo rapid transformation accompanied by the NO release.

An analysis of the isotope distributions of the ion peaks and the measurements of the precise ion masses enabled the identification of all main detected ions in solutions of the complexes under study.

The mass spectra of solutions of **Tetraz** and other neutral complexes[164] show ions of mononuclear nitrosyl intermediates. In DMSO, the complexes are much more stable than in methanol. The small number of spins per complex in all solutions indicates that the mononuclear complexes are also further decomposed to give NO and other products. The cationic complexes are the most stable in solution, but the stability decreases in the series **Cys** > **Pen** > **Glu**.

Nitric oxide-donor activity of iron nitrosyl complexes with functionalized S ligands

The amount of NO generated into solution by complexes after their hydrolysis was studied with the use of an amiNO700 sensor electrode. A few seconds after the dissolution of neutral complexes in aerobic aqueous solutions, the amount of the released NO is substantially larger than that for the reference organic donor NONOate (Fig. 13, *a*) and anionic sulfide complexes. The NO release from the cationic complexes is a slower process (Fig. 13, *b*).

For all the complexes under study, the kinetic curves display maxima, which may be attributed to further transformations of the generated NO.[151]

$$\text{Complex} \xrightarrow{k_1} \text{FeL} + \text{NO}, \qquad (1)$$

$$\text{NO} \xrightarrow{k_2} \text{P}, \qquad (2)$$

where $L = [Fe(SR)_2(NO)_3]^{n-}$.

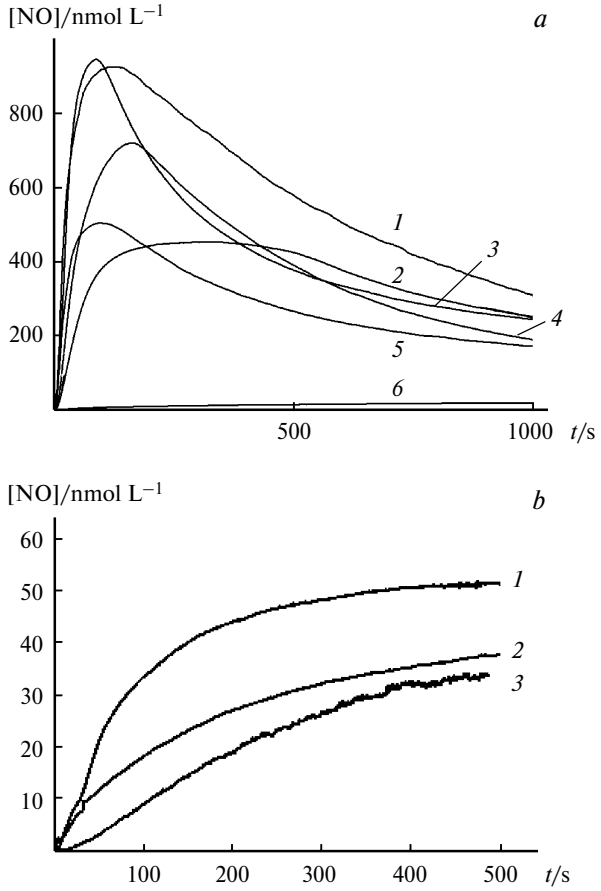


Fig. 13. Amount of NO generated by iron nitrosyl complexes ($c = 0.4 \cdot 10^{-5}$ mol L^{-1}) in a 1% aqueous DMSO solution at pH 7.00 and $T = 25$ °C *versus* the time under aerobic conditions: *a*, the complexes **Tetras** (*1*), **Triaz** (*2*), **Imid** (*3*), **AmTriaz** (*4*), **Melm** (*5*), and **RSR-Na** (*6*); *b*, the complexes **Pen** (*1*), **Cys** (*2*), and **Glu** (*3*).

This scheme of transformations corresponds to the following functional time dependence of the NO concentration:

$$[NO] = ck_1[\exp(-k_2t) - \exp(-k_1t)]/(k - k_2), \qquad (3)$$

where the constant c has a physical meaning of the maximum NO concentration, which would be reached in the absence of further transformations of NO. It is known that N_2O is the final transformation product of NO in solutions of dinitrosyl iron complexes. Apparently, the nitrosyl iron-containing intermediate, which is formed through dissociation of the Fe—NO bond in the starting complex and the coordination of a water molecule at the free coordination site, acts as a reducing agent to transform NO into the NO^- anion. The stronger reducing properties of the intermediate compared with the initial complex result from an increase in the electron density on the Fe atom due to coordination of a water molecule. The decomposition of nitrosyl complexes under aerobic conditions leads

to an increase of the amount of the released NO by several times (compared with the processes under anaerobic conditions), which may be attributed to the faster electron transfer to the oxygen molecule from the reduced complex. This competing redox process results in a decrease in the fraction of reduced NO molecules, which undergo transformations in subsequent reactions and, consequently, in an increase in the detected NO concentration. In addition, like in the case of NONOate, NO from solution can directly interact with the negatively charged NO^- ligand of the iron nitrosyl complex, resulting in the formation of the hyponitrite ligand in the coordination sphere. In fact, this process is accompanied by the electron transfer to NO but without its elimination from the coordination sphere. Evidently, in the case of sufficiently fast reactions giving the hyponitrite ligand, this mechanism will lead to a decrease in the constant *c*, *e.g.*, in the maximum amount of NO molecules.

The energy of the dissociation of the Fe—NO bond and the replacement of NO by an aqua ligand in the complexes **Pym** and **Ph** were studied by quantum chemical modeling using the density functional theory methods.[154] The theoretical investigation showed that the observed pH effect (Fig. 14) may be attributed to the dissociation of the complex into two mononuclear complexes as a result of the electrophilic attack of the proton on the S atom in an acidic medium and the associative mechanism of the replacement of NO by the OH^- ion in an alkaline medium. A more detailed analysis of alternative mechanisms requires further studies.

Reactions of nitrosyl complexes with heme proteins.[153,165–174] When reacting with NO that is released from complexes upon hydrolysis, hemoglobin (Hb) forms HbNO. The absorption spectrum of Hb showing the characteristic band in the visible region at λ_{max} = 556 nm (ε = 12.5 mmol L^{-1} cm^{-1}) is transformed into the spectrum with bands at λ_{max} = 545 nm (ε = 12.6 mmol L^{-1} cm^{-1}) and λ_{max} = 575 nm (ε = 13.0 mmol L^{-1} cm^{-1}). Figure 15 displays the time-course kinetic curves for the formation of HbNO in the reaction of the complex **Pym** with Hb. The experimental points were obtained from the time-resolved difference absorption spectra. All kinetic curves for the reactions of complexes with Hb are well described in terms of the formalism of the pseudo-first-order reactions. The theoretical single-exponential curves were fitted to the data using the Origin software and the function $y = a(1 - e^{-kt})$, where k is the effective first-order rate constant and a is the final concentration of HbNO. The formation of HbNO can be described by two sequential reactions: 1) the decomposition of the complex resulting in the NO release and 2) the formation of HbNO. The rate of the formation of HbNO is equal to the rate of the NO release into solution. The final HbNO concentration in experiments is determined by the initial Hb concentration because the binding constant of NO to Hb is $3 \cdot 10^{10}$ L mol^{-1}. Since the rate of the reaction of Hb with NO is similar to the diffusion rate (the second-order rate constant k_2 = $= 1.02 \cdot 10^8$ L mol^{-1} s^{-1}), the experimental constants k (Table 4) indicate that the NO release from the complexes into solution is the rate-determining step in the formation of HbNO in the experiments. The rate of the decomposition of the complexes **Bim**, **Btz**, **Py**, and **Pym** in solutions containing Hb is lower (by an order of magnitude) than the rates of the decomposition of the other complexes, *i.e.*, these complexes more slowly generate NO into solution due to different stabilization of the complexes under consideration by hemoglobin, which is attributed to the

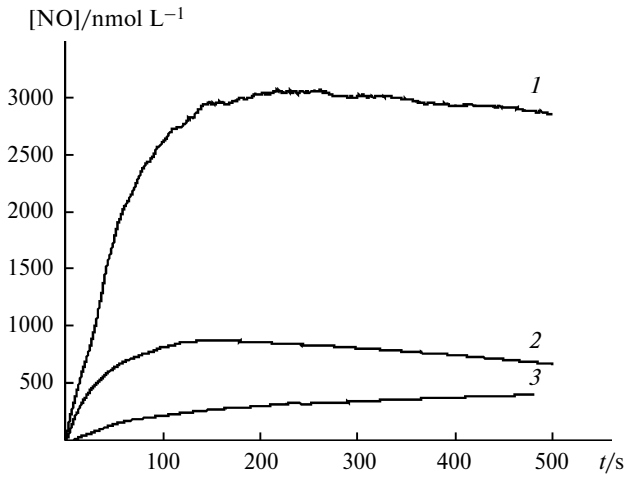


Fig. 14. Amount of NO generated by the complex **Pym** ($c = 0.4 \cdot 10^{-5}$ mol L^{-1}) *versus* the time at different pH for aqueous solutions under anaerobic conditions at 25 °C: pH 6.00 (*1*), 7.43 (*2*), and 7.00 (*3*).

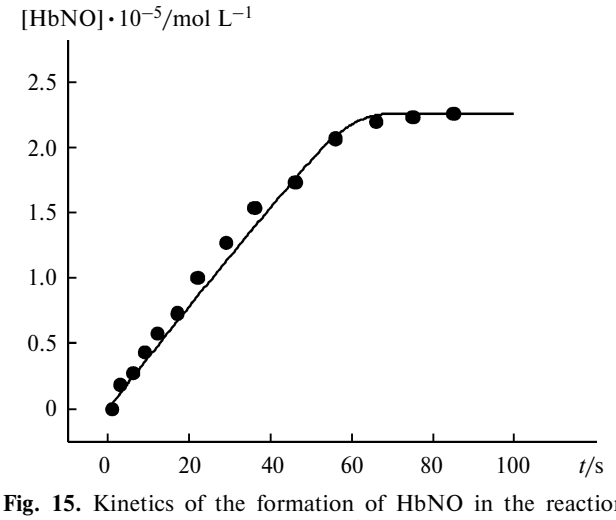


Fig. 15. Kinetics of the formation of HbNO in the reaction of the complex **Pym** ($c = 2 \cdot 10^{-4}$ mol L^{-1}) with Hb (c = $= 2.26 \cdot 10^{-5}$ mol L^{-1}). The points correspond to the experimental data, the theoretical single-exponential curve fitted to the experimental data is represented by the solid line.

Table 4. Effective first-order rate constants (k) for the reactions of iron nitrosyl complexes with Hb

Complex	$k \cdot 10^3/s^{-1}$	Complex	$k \cdot 10^3/s^{-1}$
TNIC	4.5±0.45	**Bim**	0.16 ± 0.16
RSR-Na	0.061±0.006	**Btz**	0.33±0.3
AmTriaz	1.72±0.17	**Py**	0.62±0.06
Tetraz	7.4±0.74	**Pym**	0.37±0.03
Im	8.85±0.9	**Pen**	3.30±0.3
Mim	3.46±0.35	**Cys**	28.6±0.3
Triaz	1.84±0.18	NO-at	0.08 ± 0.08
MBim	0.12±0.12		

different basicity of the S ligands. The reaction rate of the NO generation by the anionic complex **RSR—Na** is almost two orders of magnitude smaller than the rates for all other complexes and is, apparently, determined by the fact that Roussin´s red salt is transformed in solution into Roussin´s black salt, which is stable in protic media, and, consequently, the additional activation (photo-, thermo-, *etc*.) is required for the NO generation.

The reactions of Hb, oxygenated hemoglobin (HbO_2), and methemoglobin (metHb) with **Pym** resulting in the nitrosylation of the free SH group of 93β cysteine were investigated. It was found that HbO_2 reacts with NO that is released from **Pym** to form metHb. The complex **Pym** reduces metHb to Hb at a high rate ($k = 6.7 \cdot 10^{-3}$ s^{-1}) followed by the formation of HbNO ($k = 6.5 \cdot 10^{-3}$ s^{-1}). The oxidized complex **Pym** releases NO at a higher rate than the starting complex. The reactions of HbO_2 and metHb with **Pym** ($c = 0.02$ mmol L^{-1}) produce nitrosothiols at micromolar concentrations during 5 min; Hb does not form nitrosothiol in the reaction with **Pym**. Therefore, it can be hypothesized that, under the conditions of the metabolism, complexes of this new class of NO donors can produce a sufficient amount of nitrosothiol for the regulation of blood flow along with the formation of HbNO.[169]

In the studies of the complex **Cys**, it was shown for the first time[172,173] that the hydrolysis of NO donors in the presence of ferrocytochrome *c* (cyt c^{2+}) can give the iron nitrosyl complex NO-cyt c^{2+}, which plays a role of the NO depot. The rate constant of the NO release from **Cys** can be determined from the kinetics of the formation of NO-cyt c^{2+}. At pH 3.0, the rate constant is $(2.7\pm0.1) \cdot 10^{-3}$ s^{-1}. It was found that, unlike deoxyhemoglobin (Hb), cyt c^{2+} exerts a weaker stabilizing effect on **Cys**. In the presence of Hb, the complex **Cys** ($c = 2 \cdot 10^{-4}$ mol L^{-1}) releases NO during 40 h, whereas in the presence of cyt c^{2+} the reaction is completed in 1 h.

The influence of the cationic iron nitrosyl complexes (**Cys** and **Pen**) and the neutral iron nitrosyl complex (**Bim**) on the enzymatic activity of hydrolytic enzymes, *viz.*, cyclic guanosine monophosphate phosphodiesterase (cGMP PDEase) and sarcoplasmic reticulum (SR) $Ca^{2+}Mg^{2+}$-dependent ATPase, was studied.[174] The complexes are, to a different degree, modulators of activity of both enzymes, they weakly retard the cGMP PDEase activity and are pronounced inhibitors of the SR Ca^{2+}-ATPase-driven active transport. The complexes have virtually no effect on the hydrolytic center of SR Ca^{2+}-ATPase and completely retard the active transport of calcium in the concentration range of $1 \cdot 10^{-6}$—$1 \cdot 10^{-4}$ mol L^{-1}, thus separating the hydrolytic and transport functions of the enzyme. Hence, at concentrations corresponding to the ratio enzyme : inhibitor = 1 : 1, the complexes can induce structural-functional changes of SR Ca^{2+}-ATPase, which can mediate the antimetastatic effect of the compounds under consideration.

Biological activity of iron nitrosyl complexes with functionalized S ligands

Cardiotropic activity of anionic and cationic iron nitrosyl complexes. Recent studies in the field of molecular cardiology have shown that NO plays a key role in the regulation of blood vessel tone and myocardial metabolism.[175] It was found that a deficiency of nitric oxide leads to the development of endothelial dysfunction, which, in turn, causes an increase in the coronary vessel tone, as well as an increase in the platelet aggregation and adhesiveness. In the case of ischemic reperfusion injury of the heart, this leads to the development of "no reflow" syndrome, resulting in the progressive decline in blood flow and finally in the cardiomyocyte death.[176] Organic nitrates and nitroprusside are widely used as drugs for cardiovascular disorders. However, as mentioned above, these drugs have drawbacks and adverse side effects: 1) nitrate tolerance and cyanide poisoning, 2) the necessity of the additional activation (thermo-, photo-, or enzymatic), which limits their use in clinics.

In the Russian Cardiology Research and Production Complex, the cardiotropic properties of nitrosyl [2Fe-2S] complexes, which are potentially able to prevent the increase in the coronary vessel tone and regulate the metabolism of the ischemic heart, were studied for the first time. The aim was to design original drug prototypes, which have hypotensive properties and reduce myocardial ischemia and reperfusion injury. The injection of the tetranitrosyl iron complex with sodium (**TNIC-Na**), **Pen**, and sodium nitroprusside before global ischemia was studied on the isolated perfused rat heart. The recovery of heart function and coronary vessels on reperfusion was estimated, and the influence of NO donors on the metabolic state of the the reperfused heart was evaluated. It was found that **TNIC-Na** and **Pen** have a protecting action on the isolated rat heart function after ischemia combined with the improvement of the recovery of the aerobic myocardial metabolism during reperfusion. The functional and

metabolic efficiency of the NO donors increases in the series **Pen** < nitroprusside < **TNIC-Na**. In *in vivo* experiments on rats, it was shown that the intravenous administration of NO donors before myocardial ischemia causes a decrease in the mean arterial pressure followed by the recovery to the end of reperfusion, but the injection of **TNIC-Na** and **Pen** at concentrations of 1 μmol kg^{-1} decreases the size of experimental myocardial infarction in rats (Fig. 16). In the limitation period of coronary blood flow, all tested NO donors do not exhibit arrhythmogenic action capable of causing death of experimental animals, due to which these compounds can be used for the design of original drugs for the therapy of acute coronary syndrome.[177—180]

Antitumor activity of iron nitrosyl complexes. An analysis of the published data shows that NO influences the level of tumor cell apoptosis, the activity of the p53 gene, and the neoplasm of tumor-feeding blood vessels. The molecular genetic mechanisms for antitumor activity of NO-containing compounds were not systematically studied. The carcinolytic activity of NO donors may be manifested both in the case of their individual use and in the complex therapy (as adjuvants) with known antineoplastic agents. The carcinolytic activity may be a consequence of either one or a sequential series of molecular genetic mechanisms: 1) the binding of NO to SH groups of proteins and enzymes (primarily, of proteases) and their inhibition; 2) the inhibition of the most important repair protein engaged in DNA alkylation damage, *viz.*, human *O*-6-methylguanine-DNA methyltransferase, in the complex therapy with alkylating carcinolytic agents (mono- and bifunctional nitrosoalkylureas), cisplatin, *etc.*; 3) the induction of DNA single- and double-strand breaks resulting in the apoptosis (programmed cell death); 4) the formation of DNA-DNA and DNA-protein cross-links, including with repair proteins, *viz.*, DNA-DNA glycosylases and UvrABC exonucleases, which are necessary for the repair of such cross-links.[181—184]

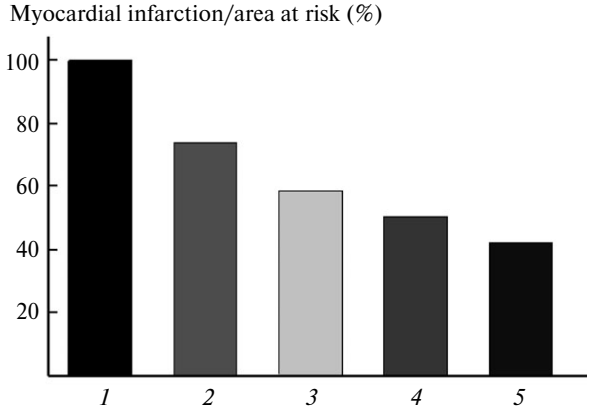


Fig. 16. Limitation of the myocardial infarct size by NO donors after intravenous administration to rats compared to the control group (*1*): the complex **TNIC-Na** in a dose of 5 (*2*) and 1 μmol kg^{-1} (*5*), the complex **Pen** in a dose of 1 μmol kg^{-1} (*3*), and Nitroglycerin in a dose of 3.2 μmol kg^{-1} (*4*).

The main aim in the development of therapeutic approaches for the treatment of oncological diseases by modern chemotherapeutic agents (NO donors) is to study in detail the mechanisms for their geno- and cytotoxic activities.

In the N. M. Emanuel Institute of Biochemical Physics of the Russian Academy of Sciences, the molecular genetic mechanisms for cytotoxic and mutagenic activity of crystalline iron-sulfur nitrosyl complexes were studied using *E. coli* as a model system. Thus, the gene expression of DNA repair responses and mutation activity, SOS and SoxRS regulons (oxidative stress), and Ada-repair response (cell resistance to alkylating compounds) was investigated.[185—189] The induction of DNA double-strand breaks in mammalian blood leukocytes by complexes was quantified. The formation of unrepaired DNA double-strand breaks is, apparently, one of the mechanisms of antitumor activity of this class of NO donors because it is DNA double-strand breaks in mammalian cells that act as signal structures, *viz.*, precursors of the programmed cell death (apoptosis).

In the N. N. Blokhin Russian Cancer Research Center of the Russian Academy of Medical Sciences, comprehensive investigations of the antitumor activity of nitrosyl complexes *in vitro* and *in vivo* were carried out.[190—193] The complexes were tested in four human tumor cell lines (SKOV-3 ovarian cancer cell line, MCF-7 breast cancer cell line, A549 non-small-cell lung cancer cell line, and K562 myeloid leukemia cell line). The complexes **Im**, **Btz**, **RSR-Na**, and **Cys** cause the 75—92% inhibition of the tumor cell growth. For the complex **Cys**, the highest cytotoxicity levels were found in four cell lines (the 50% growth inhibitory concentration GI_{50} for the K562, SKOV-3, MCF-7, and A549 cells is 17, 43, 20, and 90 μmol L^{-1}, respectively). Since the complex **Cys** is known to be stable during storage, readily soluble in water, capable of generating NO in solution over a long period of time, and consisting of components devoid of cytotoxicity, it was tested in detail in nine cell lines of different histogenesis (in K562 myeloid leukemia cells, SKBR-3 breast cancer cells, MCF-7 breast cancer cells, A293 immortalized kidney cancer cells, SKOV-3 ovarian cancer cells, LS174T colon cancer cells, DU145 prostate cancer cells, PC-3 prostate cancer cells, and A549 non-small-cell lung cancer cells). The breast cancer cell lines were most sensitive to **Cys**. The study of the action of the complex **Cys** on the distribution of K562 leukemia cells over the cellular cycle phases by flow cytometry based on DNA ploidy revealed that **Cys** caused the 16% increase in the amount of cells in the S phase, a 8% decrease in the G1 phase, and the 8% decrease in the G2/M phase. It was also found that the complex **Cys** induces the apoptosis in K562 myeloid leu-

kemia cells and LS174T colon cancer cells. The complexes **Btz** and **Ph** also initiate the activity of caspases-3 and -7 of K562 leukemia. For the complex **Btz**, it was shown that the amount of cells in the apoptosis depends on the concentration. The ability of the complex **Ph** at the same concentration to induce the apoptosis was lower (23%) compared with the complexes **Btz** and **Cys**.

The antitumor activity of iron nitrosyl complexes *in vivo* was tested in four mouse tumor models included in the set of necessary animal tumors used for the selection of new antitumor agents: B-16 melanoma, epidermoid Lewis lung carcinoma (LLC), Ca-755 breast adenocarcinoma, and P-388 lymphocytic leukemia. The tumor growth retardation TGR (%) and the life span extension LSE (%) served as the evaluation criteria for the antitumor effect. Investigations were performed with the use of the active compounds, **Im**, **Btz**, **RSR-Na**, and **Cys**, revealed based on *in vitro* assays. It was found that the compounds **Im** and **Btz** have a short-term antitumor effect after the intraperitoneal administration daily for five days. The complex **RSR-Na** has the antitumor effect corresponding to the efficiency of the new class of original agents. In B-16 melanoma, the administration of the agent for ten days resulted in TGR of 70, 66, and 74%, respectively, 13, 18, and 22 days after the tumor inoculation, and LSE was 28%; in LLC cells, TGR was 83, 63, 69, and 65% after 7, 13, 16, and 23 days, respectively, and LSE was 27%. The complex **Cys** also has the antitumor effect corresponding to the efficiency criteria of a new class of original agents: in Ca-755 cells, the injection of the complex for nine days resulted in TGR of 71, 76, 63, and 64%, respectively, 4, 7, 11, and 15 days after the tumor inoculation, and LSE was 66%; in the LLC tumor, the administration of the agent for ten days resulted in TGR of 86, 67, and 61%, respectively, 7, 13, and 16 days after the tumor inoculation, and LSE was 7%.

Conclusions

The discovery of nitric oxide (NO) as an important polyfunctional regulator of various physiological processes *in vivo*, including neurotransmission, immune system regulation, blood pressure regulation, smooth muscle relaxation, platelet aggregation, and macrophage cytotoxicity, is a significant achievement in science in the last decades.

A search for and investigation of new compounds, NO donors, are necessary for the understanding of the action of NO and its reactive cellular nitrosyl intermediates, as well as for the development of therapeutic strategies in the treatment of socially significant diseases. Iron nitrosyl complexes with functionalized S ligands are of particular interest because they are formed in cells of living organisms (bacteria, plants, and mammals) due to the effect of endogenous NO on the active sites of non-heme iron-sulfur proteins and, along with nitrosothiols, are biological reservoirs for NO. The design of new iron nitrosyl complexes with functionalized S ligands is a topical problem enabling the fundamental study of the mechanisms of action of NO and the design of drugs with desired biological properties, which will facilitate the enhancement of the effect induced by the polyfunctional regulator messenger (NO) and will be more efficient in the treatment of various pathologies caused by a deficiency of endogenous NO.

The review summarizes data on the methods for the synthesis of mono- and binuclear iron nitrosyl complexes with functionalized sulfur-containing ligands, which are structural analogs of the active sites of non-heme iron-sulfur proteins, investigations of their structures, the reactivity, and pharmacological (mainly, cardiotropic and antitumor) activities *in vitro* and *in vivo*.

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