Redox-active ferrocene-modified pyrimidines and adenine as antitumor agents: structure, separation of enantiomers, and inhihibition of the DNA synthesis in tumor cells

L. V. Snegur,[a] S. I. Zykova,[a,b] A. A. Simenel,[a] Yu. S. Nekrasov,[a†] Z. A. Starikova,[a†] S. M. Peregudova,[a] M. M. Il´in,[a] V. V. Kachala,[c] I. K. Sviridova,[d] and N. S. Sergeeva[a]*

[a]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 119991 Moscow, Russian Federation. Fax: +7 (499) 135 5083. E-mail: snegur@ineos.ac.ru*

[b]*Branch of the K. G. Razumovsky Moscow State University of Techology and Management, 2 ul. Shulaikinoi, 142601 Orekhovo-Zuevo, Moscow region, Russian Federation. E-mail: svetlana-zykova@mail.ru*

[c]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation.*

[d]*P. A. Gertsen Moscow Oncological Research Institute, 3, 2-oi Botkinskii pr., 125284 Moscow, Russian Federation. Fax: +7 (495) 945 7415. E-mail: prognoz.06@mail.ru*

The structures, electrochemical properties, enantiomeric separation of ferrocenyl-(alkyl)pyrimidines and ferrocenyl(ethyl)adenine and their effects on the DNA synthesis in tumor cells were studied. Enantiomeric mixtures were separated by HPLC on modified cellulose as the chiral selector. The electrochemical properties of compounds were studied by cyclic voltammetry. All compounds have reversible single-electron redox transition in the region of 0.52—0.60 V, which belongs to ferrocene—ferrocenium with a positive shift compared to ferrocene (0.52 V). The molecular structure of 1-*N*-(ferrocenylbenzyl)-5-iodocytosine was studied by X-ray diffraction. 1-*N*-(Ferrocenylethyl)adenine was studied for ability to inhibit the DNA synthesis in the human ovarian cancer cell culture by the ^{3}H-thymidine test.

Key words: ferrocene, ferrocenyl pyrimidines, ferrocenyl adenine, thymine, cytosine, adenine, enantiomeric separation, HPLC, X-ray diffraction study, DNA synthesis inhibition, ^{3}H-thymidine test, ovarian cancer.

In pharmacology, the study of interactions between drug compounds and DNA play a key role in the design of novel drugs. Nucleic bases, nucleosides, nucleotides, and DNA became subjects of research in organometallic chemistry since 1980 when the ferrocene-modified (Fc) adenine was synthesized.[1] Later, the uridine-based ferrocene compounds were obtained.[2] Even these first synthetic works dealed with the biological aspects of novel ferrocene-containing compounds. For example, N^6-(ferrocenylmethyl)adenine was found to inhibit mitosis[1] and 5-(2-ferrocenylethenyl)uridine was inefficient towards leukosis L1210.[2] Later, we noted that ascitic tumors, such as leukoses L1210 and P388, are less sensitive to the action of ferrocene compounds.[3]

During two last decades, ferrocene compounds attract attention of chemists, biologists, and health professionals as a novel class of potential anticancer agents,[4—6] which is caused by their pronounced antiproliferative activity in combination with a low toxicity. The latter is quite important in the design of novel drugs. For example, the therapeutic indices TI = LD_{50}/ED, which reflect the differences between therapeutically efficient doses (ED) and lethal doses (LD_{50}), for ferrocene compounds are significantly higher than those for drugs used in clinical practice, which suggests a specific effect of ferrocenes.

Recently, several reviews[4,5] devoted to the biochemical properties of ferrocenes have been published. In Ref. 6, we have presented data on the antitumor activities of ferrocene compounds. Such great attention to the bioinorganic chemistry of ferrocenes is caused by their unique properties. Ferrocenes can be oxidized readily at physiologic pH and reduced also readily.[4a] They possses lipophilicity and easily penetrate through cell and nuclear membranes. The ferrocene fragments are readily introduced to different N and C positions of purine and pyrimidine bases, nucleosides, nucleotides, oligonucleotids, and DNA.[3d,4] Finally,

† Deceased.

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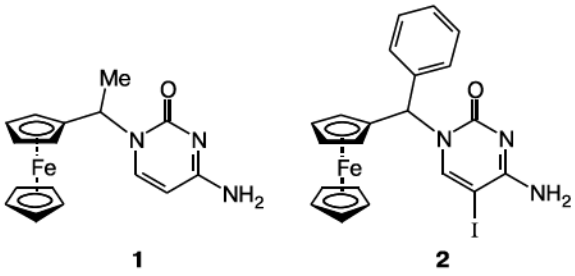
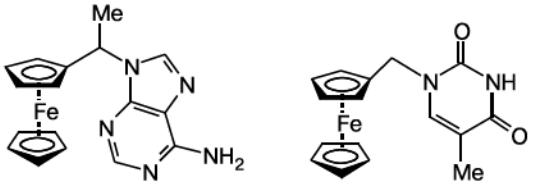
a series of ferrocene compounds are commercially available. Our interest for such type of derivatives is caused, on the one hand, by the mechanistic aspects and, on the other hand, by a potential medical application.[3,6]

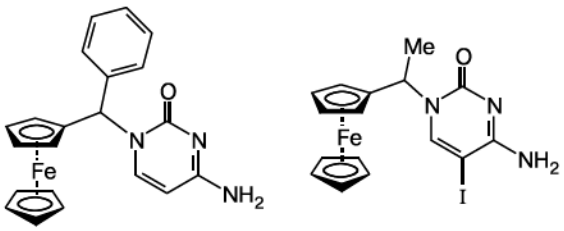
In the present work, the redox potentials of ferrocene-modified nucleic bases **1**—**6** were determined by cyclic voltammetry (we have described their syntheses recently[3d]) and the conditions for enantiomeric separation of racemic mixtures were found; the structure of compound **6** was established by X-ray diffraction. The ability of compound **3** to inhibit the DNA synthesis of human ovarian cancer cells was evaluated by the ^{3}H-thymidine test.



1 **2**



3 **4**

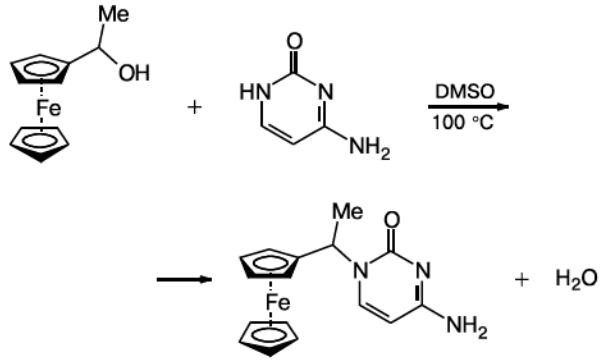


5 **6**

Results and Discussion

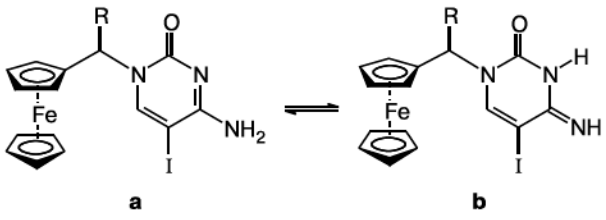
Compounds **1**—**6** were prepared by the reaction of equimolar amounts of the corresponding ferrocene alcohols FcCH(R)OH (R = H, Me, Ph) and cytosine, 5-iodocytosine, adenine, and thymine in DMSO at 100 °C (Scheme 1). After column chromatography, the compounds were isolated in good yields (Experimental presents the modified synthetic procedure).[3d] It should be noted that this procedure requires no inert atmosphere, since DMSO under experimental conditions can act as the reducing agent as it has been established for the ferrocenium salts.[7] The ferrocene alcohols in the neutral medium are weaker and, consequently, more specific ferrocenyl alkylating agents than the ferrocenyl ferrocenylammonium salts.[8a] Therefore, ferrocenylalkylation of nucleic bases (see Scheme 1) as exemplified by the cytosine proceeds regioselectively. No polyferrocenylalkylated products are produced in this reaction (see also preparation of ferrocenylalkylated imidazoles using carbonyldiimidazole).[9]

Scheme 1



The structures of 1-*N*-ferrocenylpyrimidines and 9-*N*-ferrocenyladenine were determined based on the ^{1}H and ^{13}C NMR spectra and the ^{1}H—^{13}C heteronuclear correlations. When analyzing the spectral data for ferrocenyl-5-iodocytosines (compounds **2** and **6**), both compounds in DMSO-d_6 were found to be in the form of tautomeric structure **b** (Scheme 2). Indeed, two broadened singlets of nitrogen protons (exocyclic and cyclic) were recorded at 6.58 and 7.80 (compound **2**) and at 6.11 and 7.64 (compound **6**), respectively.

Scheme 2



a **b**

R = Ph (**2**), Me (**6**)

Introduction of the ferrocenyl fragment and different bridging groups (from methyl to benzyl) binding the heterocyclic and ferrocenyl fragments into the nucleic base molecule allows varying the lipophilicites of final compounds.

The electrochemical properties of ferrocene-modified pyrimidines **1**, **2**, **4**—**6** and adenine **3** were studied by cyclic voltammetry. Table 1 gives the electrode potentials for 1-(ferrocenylmethyl)thymine (**4**) where the ferrocenyl fragment is linked with the heterocyclic N(1) atom through the methylene bridge. In compounds **1**, **3**, and **6**, the bond is realized through the substituted methylene bridge

Me—CH. In compounds **2** and **5**, the briding unit is the benzylidene group PhCH. All compounds under study were found to undergo reversible single-electron oxidation. For compounds **1**—**5**, the electrode potentials are shifted to the positive region compared to that of ferrocene to be 0.60, 0.59, 0.59, 0.57, and 0.53 V, respectively, which suggests the electron-withdrawing properties of *N*-heterocycles. For compounds **6** as for ferrocene, E^{ox} = 0.52 V. The data obtained are in agreement with those for the Fc-methylated nucleic bases.[8]

Thus, we have a tool for varying electrode potentials. By changing both heterocycles themselves or substituents therein and linking bridging units, we obtain different patterns. For example, 1-(1-ferrocenylethyl)cytosine (**1**) has E^{ox} = 0.60 V, which is the highest oxidation potential in a series of compounds under study. Upon introduction of the iodine atom to the cytosine ring, the electrode potential decreases by 80 mV and is E^{ox} = 0.52 V for 1-(1-ferrocenylethyl)-5-iodocytosine (**6**), which is the lowest potential in this series (Fig. 1). If the methyl group in compound **6** is replaced with the phenyl one, the potential increases and is E^{ox} = 0.59 V for 1-(α-ferrocenylbenzyl)-5-iodocytosine (**2**) (Fig. 1). It is clear that in the latter case the dominating factor is electron-withdrawing properties of the phenyl fragment.

Such delicate tuning of the electrochemical behavior is quite beneficial in the design of drugs, as well as in the synthesis of redox active DNAs for the design of molecular devices.[8b,c] Recently,[10] the electrochemical behaviors of disubstituted ferrocenes with adenine and thymine as substituents, as well as with the C_2-saturated and C_2-unsaturated (double bond) bridge has been studied. The oxidation of Fc-adenine compounds having ethyl or ethylene bridge was found to proceed more difficult than that of the corresponding thymine compounds. Moreover, the presence of double bonds in the bridge considerably increases the oxidation potential (>200 mV).[10] When studying the electrochemistry of ferrocene-modified oligonucleotides containing 7-deazaadenine and uracil, it was found that the potentials of Fc-conjugates are shifted by 50—75 mV relative to that of ferrocene, which allows electrochemical differentiation of these conjugates.[11] Thus, the ferrocene compounds can be oxidized easily to the ferrocenium salts, which are transported in the aqueous media (blood, cytoplasm). Such processes are carried out most easily for the compounds whose oxidation potential is close to that of ferrocene.

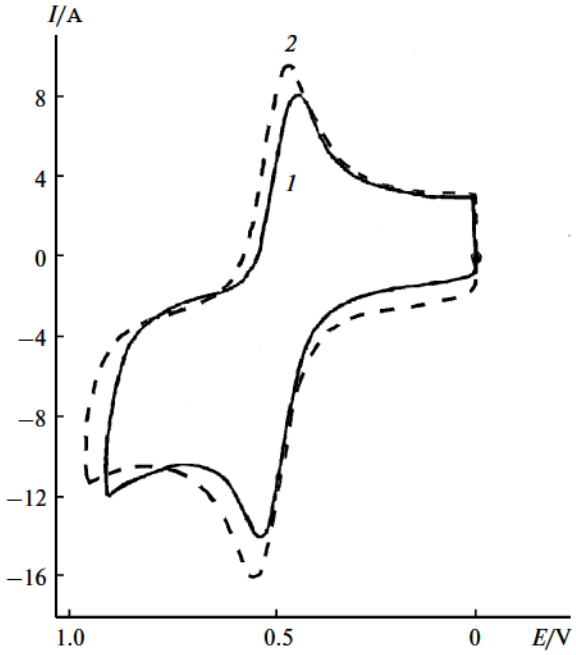


Fig. 1. Cyclic voltammograms of compounds **6** (*1*) and **2** (*2*) (DMF, $C = 2 \cdot 10^{-3}$ *M*, 0.1 *M* $[(n\text{-Bu})_4N]PF_6$).

The reduction potentials were determined for the iodocytosine-based compounds **2** and **6** (Table 1). At E^{red} = = −1.88 and −1.92 V, respectively, these compounds are reduced irreversibly. The analogous results were obtained for ferrocenyl(ethyl)benzotriazole, FcCH(Me)BTr (E^{red} = −2.43 V), and ferrocenyl(butyl)imidazole, $FcCH(CH_2CH_2Me)Im$ (E^{red} = −2.34 V)[3a].

The structures of ferrocenylmethylated adenine, thymine, cytosine, substituted purines,[8a,12] as well as C(5)-ferrocenylthymidine[8c] and C(5)-ethynylferrocenylcytidine[8b] has been studied earlier by X-ray diffraction. These compounds are methyl-, rarer ethyl-, or ethynyl-linked ferrocenes. In the present work, we performed X-ray diffraction study to establish the structure of 1-(α-ferrocenylbenzyl)-5-iodocytosine (**2**) (Fig. 2, Table 2). It should be noted that the structure presented is the first example of compound where the ferrocene fragment is linked with the nucleic base through the benzyl bridge. The cyclopentadienyl rings in the ferrocene fragment of compound **2** has the eclipsed conformation. The planes of cyclopentadienyl rings (Cp) are almost parallel and the dihedral angle is 1.4°.

Table 1. Redox potentials of ferrocenylalkylated pyrimidines and adenine

Compound	Formula	E^{ox}	E^{red}
		V	
1	FcCH(Me)Cyt	0.60	—
2	FcCH(Ph)-5-I-Cyt	0.59	−1.88
3	FcCH(Me)Ade	0.59	—
4	$FcCH_2Thy$	0.57	—
5	FcCH(Ph)Cyt	0.53	—
6	FcCH(Me)-5-I-Cyt	0.52	−1.92
7	FcH	0.52	—

Note. FcH is ferrocene; Fc is ferrocenyl, CytH is cytosine ($C_4H_5N_3O$), 5-I-CytH is 5-iodocytosine ($C_4H_4IN_3O$), AdeH is adenine ($C_5H_5N_5$), ThyH is thymine ($C_5H_5N_2O$); argon.

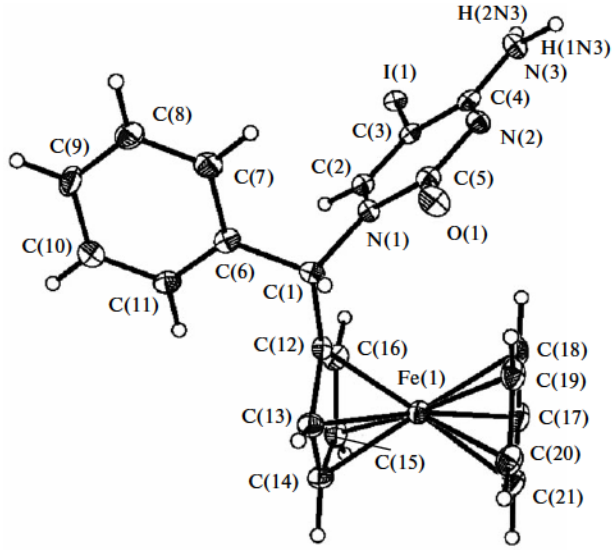


Fig. 2. Molecular structure of 1-(α-ferrocenylbenzyl)-5-iodocytosine (**2**).

The iron atom is located between rings and closer to the substituted ring. The distances Fe—Cp_{unsub} and Fe—Cp_{sub} are 1.647 Å and 1.641 Å, respectively (the average bond lengths are Fe—C_{unsub} 2.044 Å, Fe—C_{sub} 2.040 Å, and Fe—C_{ipso} 2.022(4) Å). Such tendency to be near the substituted ring is observed for the ferrocene-modified adenine, cytosine, and thymine.[8a,12a] The dihedral angle between the phenyl ring and cytosine ring is close to the right angle and equal to 88.30(9)° (see Fig. 2). The dihedral angle between the cytosine ring and substituted Cp ring is equal to 113.4°. The bond between the heterocycle and ferrocene fragment is realized through the atomic thriad N(cytosine)—C(bridge)—C(ferrocenyl). The exocyclic N(1)—C(1) bond length between the cytosine N(1) atom and the bridging C(1) atom in compound **2** is equal to 1.485 Å and exceeds the statistically average value (1.469 Å) typical of the C(sp^3)—N bonds with the three-coordinated nitrogen atom.[13]

Table 2. Selected bond lengths (*d*) and angles for compound **2**

Parameter	Value
Bond	*d*/Å
C(1)—C(12)	1.504(5)
C(1)—N(1)	1.485(5)
C(1)—C(6)	1.521(5)
N(1)—C(2)	1.369(5)
N(1)—C(5)	1.412(5)
C(3)—I(1)	2.091(4)
C(4)—N(3)	1.327(5)
Angle	ω/deg
C(12)—C(1)—C(6)	114,1(3)
C(12)—C(1)—N(1)	113.4(3)
C(6)—C(1)—N(1)	110.5(3)
C(1)—N(1)—C(2)	119.5(3)
C(1)—N(1)—C(5)	120.1(3)
C(2)—N(1)—C(5)	120.4(3)
C(2)—C(3)—C(4)	117.0(3)

As we noted earlier when considering the structure of $FcCH_2$-adenine and FcCH(Me)-adenine, these structural features agree good with the increased lability of the N—C bonds and can be associated with the exhibition of antitumor effects by ferrocene compounds.[3b] It is pertinent to remind that, in the DNA molecule, the N—C glycoside bond between the nucleic base and deoxyribose is realized by involving the N(1) atom for thymine and cytosine and the N(9) atom for adenine. It should be emphasized that during tumor transformations it is this N(9)adenine—deoxyribose bond which breaks upon depurination of DNA. Going back to the structure of compound **2**, note that the change in the N(cytosine)—C(bridge) bond length correlates with the electronic properties of heterocycles and their substituents, in our case iodine. The C(bridge)—C(ferrocenyl) bond lengths for compounds **2** (1.504 Å) and related compounds are virtually close.[8a,12a]

The molecule of **2** contains several H-acceptors and the standard NH_2 group; therefore, hydrogen bonding is possible in crystal. Indeed, two indepedent molecules in the crystal of **2** are linked into the centrosymmetric dimer through the N...H—N-type hydrogen bonds formed by the cytosine nitrogen atoms and one of the hydrogen atoms of the exocyclic amino group (Fig. 3). The parameters of the N(2)···H(1NB)—N(3B) hydrogen bond are N···H 2.09 Å, N—H 0.90 Å, and N···N 2.978(5); the NHN angle is 171°. The second hydrogen atom of the NH_2 group forms a weak intramolecular hydrogen bond H(2N3)···I(1), the interatomic distance H···I is equal to 2.92 Å. The dimeric fragments are combined through the intermolecular dipole-dipole interactions (I(1)···O(1) = 2.956(4) Å) (Fig. 3).

Compounds **1**—**6** under study, except for compound **4**, are racemic mixtures, since the carbon atom being in the α-position towards the ferrocene nucleus is a chiral center.

With regard to the drugs whose molecules contain chiral centers, there are certain requirements of the Pharmaceutical Committee of the Russian Federation. According to these requirements, when studying novel drugs, the toxicity and bioactivity must be studied for each enantiomer taken alone.

We performed analytical HPLC separation of racemic mixtures into enantiomers. Earlier, this separation methods have been applied successfully for the enantiomeric mixtures of ferrocene compounds with different simple substituents,[14] as well as for ferrocenyl(alkyl)azoles[15,16] and ferrocene-modified thiopyrimidines[8e]. With regard to nucleic bases, there was only one not quite successfull

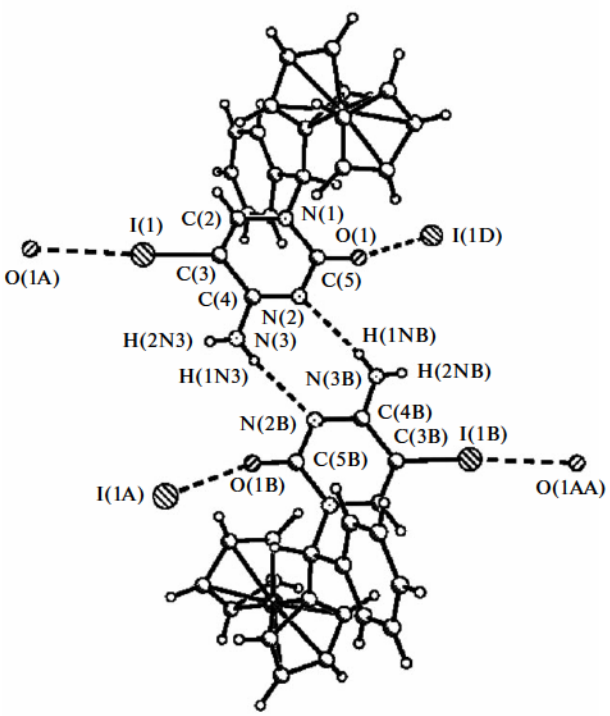


Fig. 3. Intermolecular interactions in the crystal of 1-(α-ferrocenylbenzyl)-5-iodocytosine (**2**).

attempt to separate 9-(ferrocenylethyl)adenine by HPLC,[12b] where β- and γ-cyclodextrins were chosen as chiral selectors, but were found to be insufficiently efficient. As the chiral stationary phase for separation of enantiomeric mixtures of ferrocene derivatives with bulky purine and pyrimidine substituents, we chose modified cellulose. The analytical data on enantiomeric separation are summarized in Table 3.

Four pairs of enantiomers **2**, **3**, **5** and **6** under study were separated with success. The most efficient separation was carried out in the case of compounds **2** and **5** having bulky phenyl substituent ($\alpha = 1.53$ for the cytosine derivatives **5** and $\alpha = 1.44$ for the 5-iodocytosine derivative **2**). For separation of 1-(1-ferrocenylethyl)cytosine (**1**) and the methyl analog of compound **5**, different columns, including Chiralcel OJ, were used. However, we failed to achieve satisfactory resolution of enantiomers, which

Table 3. Enantiomeric separation of the racemic mixtures of ferrocene-modified nucleic bases

Compounds	Column	k'_1	k'_2	α
2	Chiralcel OD	6.69	9.61	1.44
3	Chiralpak AS-H	5.87	6.38	1.09
5	Chiralcel OD	12.37	18.92	1.53
6	Chiralcel OJ-H	4.86	5.87	1.21

Note. k' is the retention factor; α is the separation factor.

is likely due to the formation of strong intermolecular associates.

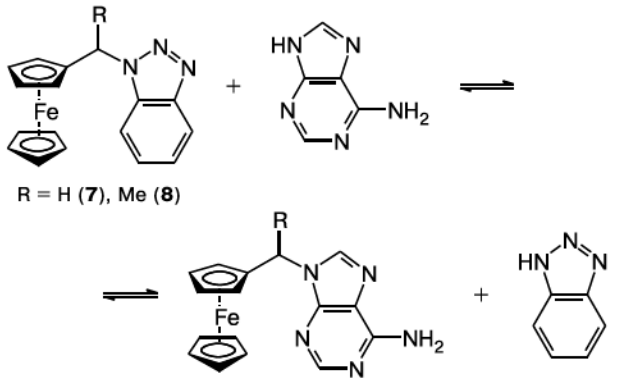
We assumed that the chiral recognition mechanism on modified cellulose involves the formation of specific hydrogen bonds as for ferrocenyl(alkyl)azoles.[15,16] However, in contrast to the azole derivatives, the chromatograms of compounds **2**, **3**, **5**, and **6** under study display broadened peaks of enantiomers. Peak broadening can be due to additional hydrogen bonding of the purine and pyrimidine amino groups with the carbamate ones. Such bonding is kinetically slow and the broadening is pronounced for the peaks of compounds containing the primary amino groups.[14]

Thus, we found conditions for the analytical separation of racemic mixtures of ferrocene-containing nucleic base derivatives.

In medical practice, antimetabolic drugs, such as mercaptopurine, thioguanin, and fluorouracil, which are derivatives of nucleic bases, *viz.*, purine and pyrimidine are well known.[17] Along with that, these compounds were found to have side toxic effects, mainly haemotoxicity and hepatotoxicity. This severe problem associated with the absence of specificity has been existing for a long time in oncology and, till now, is one of the most important problems in chemotherapy. For this reason, novel efficient anticancer drugs with a low toxicity towards normal cells and tissues are being searched actively. Ferrocene compounds as antitumor agents are being studied intensively during the last decade[3,4c,4c,4f,5,6,18,19] and proved themselves as low-toxicity compounds. We have discovered earlier the antitumor effects of some ferrocene derivatives of nucleic bases and pyrimidines in *in vivo* experiments, *i.e.* in experiments on animal (mice) models.[3d,3e] For example, for *S*-ferrocenylethyl-2-thiopyrimidine inhibition of the tumor growth on the carcinoma strain 755 was 95% compared to the control.[3e] Such result demonstrates strongly pronounced effect, which is comparable with that of the antitumor agent cisplatin. It was found that 1-(ferrocenylmethyl)thymine (**4**) also inhibites the growth of carcinoma 755.[3d] Moreover, the combination of 1-(ferrocenylmethyl)thymine and the well-known cytostatic drug cyclophosphamide was found to have synergic effect on carcinoma 755 and Lewis lung carcinoma on animal model.[3d] Recently, the ferrocenylthymine conjugate was found to inhibit the growth of estrogen receptors and T-blast-like tumor cells.[20]. Also, worthy of mention is the migration phenomenon of ferrocenylalkyl fragments from benzotriazole to adenine (Scheme 3) and, *vice versa*, from adenine to benzotriazole under both acid conditions and conditions close to the physiological ones (in water).[3b] A low toxicity of ferrocene compounds is interpreted to be a conseqeunce of such "soft" ferrocenylalkylation.[3b] Note also that the subcapsular test on animal model showed that $FcCH(CH_3)$-benzotriazole (**8**) causes the tumor regression down to 45% on the surgical material (squamous cell

lung carcinoma).[3b] All these results suggest that a novel class of medicaments for chemotherapy of cancerous diseases can be designed based on ferrocene.

Scheme 3



In some reviews, DNA is regarded as the most probable primary cell target for ferrocene compounds.[21,4c] The ferrocene derivatives have been shown to have effect on the DNA synthesis in tumor cells[22] and to inhibit the catalytic activity of topoisomerase IIβ, *viz.*, enzyme regulating cleavage of both DNA chains.[23] There are also other point of views where the biological effects are not related to the effect on DNA, but exhibit indirectly.[4f,18]

While elaborating our biological studies, in the present work we studied the effect of 9-(1-ferrocenylethyl)adenine (**3**) on the inclusion of tritium-labeled thymidine into DNA. This so called thymidine test was applied successfully for monoacetylferrocene, *sym*-diacetylferrocene, and other ferrocene compounds,[24] as well as for 1-(1-ferrocenylethyl)benzotriazole (**8**) (see Scheme 3).[22] As noted above, compouind **3** can be regarded as the ferrocenylalkylating agent (see Scheme 3). The effect of compound **3** on the DNA synthesis was studied on human ovarian cancer cells in a wide dosage range from 0.5 to 300.0 g mL^{-1}. Fig. 4 shows the study results in the graph form for the low-dosage range from 0.5 to 25.0 g mL^{-1}. All experiments were performed in triplicate. For each point, the standard deviation is ±5—7%. As the negative control, we studied incorporation of ^{3}H-thymidine in the absence of compound **3** (solvent only) and these data were taken as 100%. The ovarian cancer cells were used in the logarithmic phase of growth.

Let us consider the low concentration range. At the lowest concentrations under study (0.5 and 5.0 g mL^{-1}), three hours after incubation (see Fig. 4) a slight stimulation of the synthetic processes in DNA up to 115 and 103%, respectively, occurs compared to the control. The activation effects have been noted also for 1-(ferrocenylethyl)benzotriazole (**8**)[22] and Fc-modified thiamine and

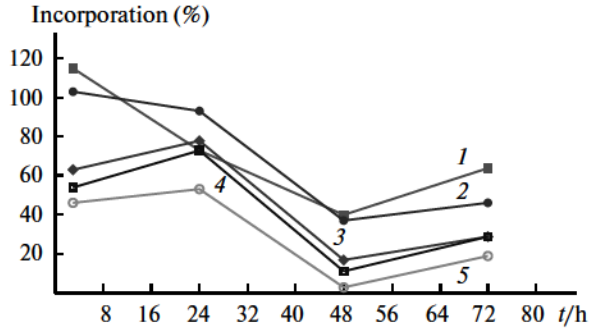


Fig. 4. ^{3}H-Thymidine incorporation into DNA. The human ovarian cancer cells (surgical material) were treated with a solution of 1-(1-ferrocenylethyl)adenine (**3**) with concentrations of 0.5 (*1*), 5.0 (*2*), 10.0 (*3*), 20.0 (*4*), and 25.0 (*5*) g mL^{-1}. The data given are the average of three independent experiments performed in triplicate.

hydroxythiamine[25] at early steps of experiments. At higher doses and the same incubation time (3 h), compound **3** suppresses the synthetic processes in DNA down to 63, 54, and 46% (the corresponding doses of 10, 20, and 25 g mL^{-1} in Fig. 4). Thus, about 50% of cells undergo death at the doses of 20 and 25 g mL^{-1} at early incubation stages (3—24 h). Therefore, compound **3** at these doses possesses acute cytotoxicity. Note that the completion of one cell cycle requires 24 h. The time point of 3 h was chosen to evaluate the effect of compound **3** as a cellular poison.

With increasing the incubation time to 48 h, the DNA synthesis decreases from 73% to 40% (0.5 g mL^{-1} dose) and from 53% (24 ч) to 3% (25 g mL^{-1} dose). Then, the DNA synthesis intensifies again up to 64% (0.5 g mL^{-1} dose, 72 h) and 19% (25 g mL^{-1} dose, 72 h).

At high concentrations of 50, 100, 200, and 300 g mL^{-1}, the function efficiency—time appears as curves which are almost parallel to the X axis, *i.e.* the survival rate of tumor cells at high doses does not depend on the incubation time. Inhibition of the DNA synthesis is about 30% at the concentration of 100 g mL^{-1} and decreases at the concentrations of 200 and 300 g mL^{-1}.

Thus, compound **3** upon addition to the tumor cell culture shows dose-dependent inhibition of the ^{3}H-thymidine inclusion into DNA, *i.e.* the DNA synthesis in ovarian cancer cells is delayed. These results allow consideration of DNA as one of the most probable primary cell targets for the ferrocene-modified compounds exhibiting acute toxicity at high doses and antitumor effects at low doses, which increase with increasing the doses.

Thus, in the present work, we studied the electrochemical properties of six ferrocenylalkylated pyrimidines and adenine. Enantiomeric separation of the ferrocene compounds was performed by HPLC. The structure of one pyrimidine derivatives was established by X-ray diffraction. In the experiments on the human ovarian cancer cell

culture, the inhibiting effect of 1-(1-ferrocenylethyl)-adenine (**3**) on the DNA synthesis in tumor cells was assessed.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance-600 (600.13 MHz) and Bruker DRX-500(A) (500.13 MHz and 125.76 MHz) spectrometers in DMSO-d_6 at 30 °C. Electon impact mass spectra were obtained on a Kratos MS-890 instrument at 70 eV. IR spectra were recorded on a UR-20 (Karl Zeiss) spectrometer.

Nucleic bases were purchased from Acros Organics and used without additional purification. Ferrocenylmethanol was prepared from trimethyl ferrocenyl ammonium iodide pre-synthesized according to a known procedure.[26] 1-Ferrocenylethanol and α-ferrocenylphenylmethanol were synthesized from the corresponding acyl derivatives by reduction with lithium aluminium hydride in THF.[16,27] Compounds **1**—**6** have been described recently and their analytical data correspond to the literature ones.[3d] For full characterization of compound **1** ^{13}C NMR spectral data are provided and for compound **2** refined melting point is provided.

Synthesis of compounds 1—6 (general procedure). The main procedure used was that described in Ref. 3d with slight modifications and revisions.[3d] To a solution of ferrocenylcarbynol (1.0 mmol) in DMSO (10—15 mL), nucleic base (1.0 mmol) was added. The mixture was stirred until dissolution of the nucleic base. If the nucleic base does not dissolve at ~20 °C, DMSO was heated slightly. The reaction solution was heated to 100 °C and kept at this temperature until the mixture becomes dark, the course of the reaction being monitored by TLC. The mass was cooled to ~20 °C and water (50 mL) was added. The mixture was extracted with chloroform (3 × 25 mL) and the combined organic fractions were washed with water until a specific odor of DMSO disappears and dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo*. The residue was chromatographed on a silica gel column using dichloromethane and methanol as eluents.

1-(1-Ferrocenylethyl)cytosin (1), FcCH(Me)Cyt. The yield was 80% Yellow crystals, m.p. 207 °C decomp., m.p. 207 °C.[3d] ^{1}H NMR (DMSO-d_6), δ: 1.31 (d, 3 H, Me, J = 6.9 Hz); 3.98—4.16 (m, 9 H, Fc); 5.35—5.42 (m, 1 H, CH); 6.72 (br.s, 2 H, NH_2); 7.07 (s, 1 H, CH); 7.11 (s, 1 H, CH). ^{13}C NMR (DMSO-d_6), δ: 19.60 (Me); 49.99 (CH); 67.01, 67.98, 68.20, 68.86 (C_5H_4); 69.21 (C_5H_5); 89.15 (*ipso*-C_5H_4); 94.04 (C(5)); 142.36 (C(6)); 155.81 (C(2)); 165.57 (C(4)).

1-(α-Ferrocenylbenzyl)-5-iodocytosine (2), FcCH(Ph)-5-I-Cyt. The yield was 65%. Powder-like orange substance, m.p. 169—170 °C decomp., m.p. 190 °C.[3d] ^{1}H NMR (DMSO-d_6), δ: 4.06 (s, 1 H, C_5H_4); 4.11 (s, 5 H, C_5H_5); 4.27 (s, 1 H, C_5H_4); 4.30 (s, 1 H, C_5H_4); 4.33 (s, 1 H, C_5H_4); 6.58 (br.s, 1 H, NH); 6.67 (s, 1 H, CH); 7.28 (d, 2 H, Ph, J = 7.6 Hz); 7.32 (t, 1 H, Ph, J = 7.3 Hz); 7.37—7.40 (m, 2 H, Ph); 7.63 (s, 1 H, HC(6)); 7.80 (br.s, 1 H, NH).

9-(1-Ferrocenylethyl)adenine (3), FcCH(CH$_3$)Ad. The yield was 65%. Yellow-orange crystals, m.p. 194—195 °C decomp., m.p. 194—195 °C.[3d,12a] ^{1}H NMR (DMSO-d_6), δ: 1.67 (d, 3 H, Me, J = 7.1 Hz); 4.01—4.37 (m, 9 H, Fc); 5.54 (m, 1 H, CH); 7.01 (br.s, 2 H, NH_2); 7.91 (s, 1 H, CH); 7.94 (s, 1 H, CH).

1-(Ferrocenylmethyl)thymine (4), FcCH$_2$Tm. The yield was 60%. Yellow crystals, m.p. 215 °C decomp., m.p. 215 °C.[3d] ^{1}H NMR (DMSO-d_6), δ: 1.71 (s, 3 H, Me); 1.76 (d, 3 H, Me, J = 7.5 Hz); 4.06—4.31 (m, 4 H, C_5H_4); 4.20 (s, 5 H, C_5H_5); 4.55 (s, 2 H, CH_2); 7.55 (s, 1 H, HC(6)); 11.14 (s, 1 H, NH).

1-(α-Ferrocenylbenzyl)cytosine (5), FcCH(Ph)Cyt. The yield was 70%. Powder-like orange substance, m.p. 185—187 °C decomp., m.p. 185—187 °C.[3d] ^{1}H NMR (DMSO-d_6), δ: 4.07 (s, 1 H, C_5H_4); 4.12 (s, 5 H, C_5H_5); 4.24 (s, 1 H, C_5H_4); 4.27 (s, 1 H, C_5H_4); 4.31 (s, 1 H, C_5H_4); 5.68—5.69 (d, 1 H, HC(5), J = 7.1 Hz); 6.77 (s, 1 H, CH); 7.02 (br.s, 1 H, NH); 7.11 (br.s, 1 H, NH); 7.22—7.23 (d, 2 H, Ph, J = 7.3 Hz); 7.30—7.31 (t, 1 H, Ph, J = 7.0 Hz); 7.35—7.36 (m, 3 H, Ph, HC(6)).

1-(1-Ferrocenylethyl)-5-iodocytosine (6), FcCH(CH$_3$)-5-I-Cyt. The yield was 80%. Yellow-orange crystals, m.p. 158—159 °C decomp., m.p. 158—159 °C.[3d] ^{1}H NMR (DMSO-d_6), δ: 1.58 (d, 3 H, Me, J = 5,0 Hz); 4.10—4.35 (m, 4 H, C_5H_4); 4.18 (s, 5 H, C_5H_5); 5.62 (q, 1 H, CH, J = 5.0 Hz); 6.11 (br.s, 1 H, NH); 7.52 (s, 1 H, HC(6)); 7.64 (br.s, 1 H, NH).

Electrochemistry. The electrochemical properties of compounds **1**—**6** were studied by cyclic voltammetry (CV) on a PI-50-1 (Gomel) potentiostat using an XY recorder. The scan rate was 200 mV sec^{-1}. The concentrations of compounds under study were $2.0 \cdot 10^{-3}$ mol L^{-1}. The measurements were performed at ~20 °C in argon atmosphere in deoxygenated DMF (the solvent was pre-dried over KOH and distilled *in vacuo* over K_2CO_3) containing 0.1 M $[(Bu^n)_4N]PF_6$ as the supporting electrolyte, in a three-electrode electrochemical cell with unseparated cathode and anode compartments. Potentials were measured relative to aqueous saturated calomel electrode separated from the solution under study in the cell through a bridge filled with a solution of supporting electrolyte. The auxiliary electrode was platinum plate located in the cell. The working electrode was the frontal part of glass-sealed glassy carbon electrode (the disc area is 2.0 mm^2).

X-Ray diffraction study of compound 2. The crystals of 1-(α-ferrocenylbenzyl)-5-iodocytosine (**2**) were grown by slow diffusion from a solution in DMSO-d_6 at ~20 °C. The crystallographic parameters and experimental characteristics are given in Table 4. After averaging the equivalent reflections, the experimental data set of independent reflections was used for solving and refining the structure. The structure was solved by the direct method and refined over F^2_{hkl} by the full-matrix least-squares method in the anisotropic approximation for non-hydrogen atoms and in the isotropic approximation for hydrogen atoms. The absolute configuration was determined by the Flack parameter. All calculations were performed by the SHELXTL PLUS 5 program complex.[28] The crystallographic data for compound **2** are deposited at the Cambridge Crystallographic Data Center (CCDC 801187).

Enantiomeric separation. The analytical separation of enantiomers was performed by HPLC on Chiralcel columns (250×4.6 mm, 5 m). The stationary phases were modified cellulose (Chiralcel OD, Chiralcel AS-H, Chiralcel OJ-H) or amylose (Chiralpak AS-H), the eluent was hexane—propan-2-ol, 9 : 1; 1.0 mL min^{-1}; UV detector 254 nm.

Antiproliferative test. To study the effect of 9-(1-ferrocenylethyl)adenine (**3**) on the human tumor cell proliferation, an attempt based on inclusion of radioactive label (^{3}H-thymidine) to DNA (classic radiometric test allowing estimation of

Table 4. Main crystallographic data and refinement parameters for compound **2**

Parameter	Value
Molecular formula	$C_{21}H_{18}FeIN_3O$
Molecular weight	511.13
Crystal color and habit	Orange, lamellar
Crystal dimensions/mm	0.37×0.25×0.15
Crystal system	Monoclinic
Space group	$P2_1/n$
T/K	100(2)
a/Å	11.1467(13)
b/Å	11.8969(13)
c/Å	14.0192(16)
α/deg	90
β/deg	91.337(2)
γ/deg	90
V/Å^3	1858.6(4)
Z	4
d_{calc}/g m^{-3}	1.827
μ/mm^{-1}	2.489
F(000)	1008
θ $_{max}$/deg	26.00
Number of reflections	16921
Number of independent reflections	3631
R_{int}	0.0460
Number of parameters refined	244
R_1 ($I > 2\sigma(I)$)	0.0313(2899)
wR_2	0.0718
GOOF	1.011
Residual electron density, (max/min)/e A^{-3}	1.771/−0.795

the *in vitro* effects of chematherapeutic agents on the intensity of DNA synthesis in cells). The experiments were performed on human ovarian cancer cells, which were incubated with radioactive ^{3}H-thymidine followed by determination of its inclusion into DNA. Compouind **3** was preliminary dissolved in 70% aqueous ethanol and the solution was diluted with water to give the required concentration. The concentrations were in the range of 0.5—300.0 g mL^{-1}. Cancer cells were incubated for 3, 24, 48, and 72 h at 37 °C (5% CO_2) in 96-well plates containing compound **3** at increasing dosage concentrations. At the last incubation hour, each well was supplemented with ^{3}H-thymidine. After appropriate treatment (washing out of thymidine, cell fixation on glass-fiber filters, drying, and application of solid scintillation coating), the degree of isotope inclusion into cells was determined. Fig. 4 shows data averaged for three independent experiments.

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