

Reagents for specific modification of biopolymers

8.* Synthesis of 2,7-diaminophenazinium salts by nucleophilic substitution of hydrogen

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Direct nucleophilic substitution in quaternary 2-*N*-alkylacetamidophenazinium salts with primary and secondary amines can serve as a convenient method for the introduction of two different substituents under mild conditions.

Key words: phenazine, phenazinium salts, nucleophilic substitution of hydrogen.

One of the most urgent tasks of modern bioorganic chemistry is increasing the efficiency and selectivity of complementary-directed modification of nucleic acids (NA). A possible approach to the solution of this task is to decrease the reactivity of modifying groups to a level that would enable the occurrence of reactions in complementary complexes and not of side reactions with biopolymers in solutions.² A prerequisite for the realization of this approach is a considerable increase in the stability of complexes of an NA-target with an oligonucleotide that carries a reactive group and the optimization of the geometry of the reactive center. Previously we showed^{3,4} that the introduction of a phenazine fragment into an oligonucleotide (at 5'- or 3'-terminal phosphate), which carries an alkylating function on the opposite end of the chain, increased significantly the degree of modification of the oligonucleotide target, which is likely due to the increase in the melting points of duplexes carrying the phenazine fragments.⁵ The introduction of two phenazine fragments at both of the terminal phosphate groups increases the stability of a formed duplex to such an extent that a real possibility appears to create selective alkylating agents based on short oligonucleotides.⁵ The absence of methods for introduction of two or more functional substituents into the phenazine molecule under mild conditions prevents the appearance of these reagents. The goal of the present work was a search for such a method for the introduction of two different amino groups into the molecule of phenazinium salt.

The methods based on condensation of substituted anilines, nitrobenzene derivatives, *o*-quinones, and *o*-phenylenediamine are normally used to obtain polysubstituted phenazines.⁶ As a rule, these processes occur under drastic conditions and in low yields or

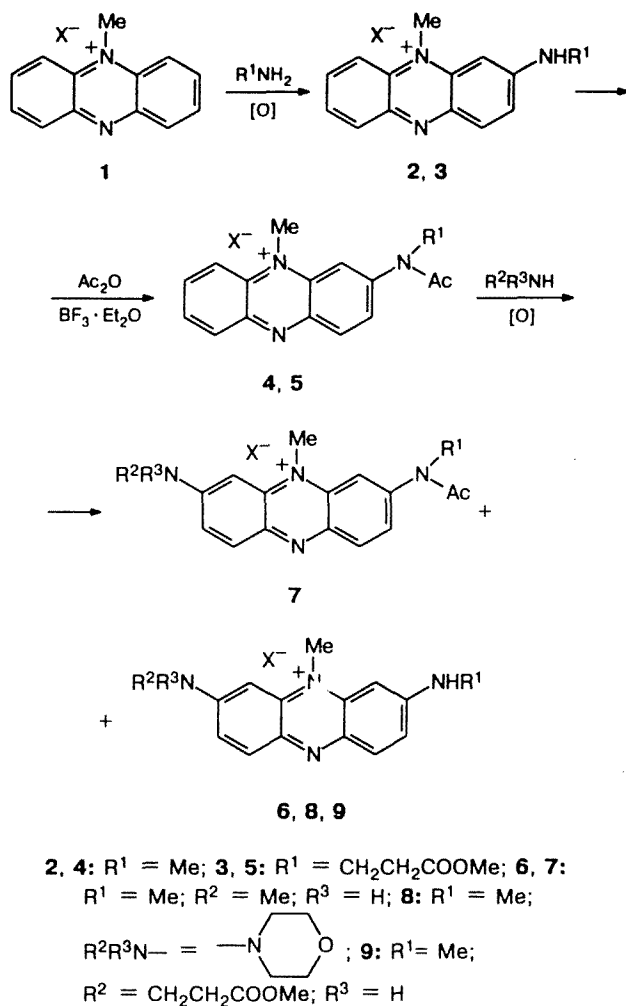
require starting compounds, which are synthesized in many stages. These methods are best suited to symmetrically substituted phenazines. Nucleophilic substitution of hydrogen in quaternary salts of azines, which usually occurs under mild conditions,⁷ is also used to synthesize substituted phenazines. However, the introduction of the first substituent into the ring deactivates immediately the heterocyclic system with regard to subsequent nucleophilic substitution of the hydrogen atom, which is quite convenient to obtain monosubstituted phenazinium salts⁸ but does not allow one to use this reaction directly in the synthesis of other derivatives. Nevertheless, in the study of some analogs of pyocyanin, a natural phenazine antibiotic, it was noted⁹ that *O*-methylation of 9-methylphenazin-2-one made it possible to perform repeated hydroxylation of quaternary phenazinium salt at position 7.

This approach (*i.e.*, the change in the electronic properties of the substituent) was chosen by us as the basis for the development of a method for the synthesis of 2,7-diaminophenazinium salts (Scheme 1). The interaction of 9-methylphenazinium methylsulfate (1) with methylamine and methyl ester of β -alanine easily gives 9-methyl-2-methylamino- (2) and 2-[2-(methoxycarbonyl)ethyl]amino-9-methylphenazinium (3) perchlorates in high yields.

The electronic spectra of compounds 2 and 3 are similar to those characteristic of 2-aminophenazinium salts (Table 1), and the ¹H NMR spectra have signals in the range of 6.5–6.7 ppm with a pronounced coupling constant ⁴*J* that is characteristic of aromatic protons in *meta*-position. As expected, acetylation of compounds 2 and 3 occurs with difficulty, and an acid catalyst is necessary to carry out the reaction. The best results were obtained with boron trifluoride etherate. 9-Methyl-2-*N*-methylacetamido- (4) and 2-*N*-[2-(methoxycarbonyl)ethyl]acetamido-9-methylphenazinium (5) perchlorates have spectral characteristics corresponding to the

* For part 7, see Ref. 1.

Scheme 1



structures suggested. For example, in their 1H NMR spectra, one can observe the downfield shift of the signals for the protons at position 1 by ~ 1.5 ppm as compared to compounds 2 and 3, which suggests an increase in the

Table 1. UV spectra of mono- and diamino derivatives of quaternary phenazinium salts (EtOH)

Compound	λ_{max}/nm (ϵ)
2	238 (42500), 290 (39500), 365 (8000), 515 (19000)
3	234 (32500), 290 (33000), 386 (11500), 530 (16500)
4	236 (32000), 247 (19800), 290 (27600), 385 (12000), 520 (14200)
5	214 (10200), 262 (25200), 410 (10600)
6	235 (5300), 270 (12800), 540 (15600)
7	240 (26500), 294 (27500), 385 (10000), 510 (16500)
8	233 (6670), 280 (23100), 543 (28200)
9	235 (5700), 271 (24800), 540 (27700)

acceptor character of the phenazine cycle. However, insufficient stability precludes the isolation of the analytical samples of these compounds, and they were used in subsequent syntheses without additional purification.

The reaction of acetamides 4 and 5 with methylamine, morpholine, and methyl ester of β -alanine were investigated. The reactivity of acetamides in these reactions was approximately one order of magnitude lower than that of the starting quaternary salt 1 (TLC). Nevertheless, acetamide 4 is completely consumed in the reaction with methylamine even after 6 h at room temperature, and 9-methyl-2,7-bis(methylamino)- (6) and -2-*N*-methylacetamido-7-methylaminophenazinium (7) perchlorates were isolated from the reaction mixture. Their spectral and analytical data correspond well to those expected. The formation of compound 6 in the course of the reaction attests that the product of direct nucleophilic substitution (7) undergoes partial deacylation under the conditions chosen, probably, in the reaction with an excess methylamine. This fact allows one to avoid an additional stage of deacetylation by merely increasing the reaction time, though this leads to a certain decrease in the yield of the target product, which was exemplified with the synthesis of 9-methyl-2-methylamino-7-morpholinophenazinium perchlorate (8) (procedures A and B). 7-[2-(Methoxycarbonyl)ethyl]amino-9-methyl-2-methylaminophenazinium perchlorate (9) was obtained by reaction of methyl ester of β -alanine with acetamide 4 or of methylamine with acetamide 5 in 51 and 46 % yields, respectively. As was demonstrated by the chromatographic analysis of the reaction mixture, the relatively low yield of diamine 9 is probably due to aminolysis of the ester group and side reactions, which occur upon prolonged storage of quaternary phenazinium salts with an excess of amines.

These directions of the reactions were studied in more detail with the interaction of compound 4 with morpholine as an example. Along with the target compound 8 isolated in 36 % yield (procedure B), a series of substituted phenazines was obtained, which were identified by mass spectrometry and 1H NMR spectroscopy (Table 2). As can be seen from the table, the main side processes, which occur in the nucleophilic substitution of hydrogen in quaternary phenazinium salts, are dequaternization of the phenazinium salt and substitution of the *N*-methylacetamido group. We observed similar reaction products, when monoamino derivatives of quaternary phenazinium salts were treated with bases.⁹ When substituents resistant to acid hydrolysis are introduced, the reduction in the reaction time is justified as well as the involvement of an additional stage of acid hydrolysis (procedure A for compound 8).

Experimental

UV spectra were recorded on a Specord M-40 spectrophotometer in EtOH and are listed in Table 1. 1H NMR spectra were recorded on a Bruker WR-200 spectrometer with HMDS

Table 2. The main by-products obtained in the reaction of 9-methyl-2-*N*-methylacetamidophenazinium perchlorate (**4**) with morpholine

Compound	Molecular formula	M_{calc}	M_{exp}	^1H NMR, δ , J/Hz	Solvent
Phenazine	$\text{C}_{12}\text{H}_8\text{N}_2$	180.06874	180.06850	7.82 (q, 4 H, H arom., $J = 3$); 8.24 (q, 4 H, H arom., $J = 3$)	CDCl_3
2-Morpholino-phenazine	$\text{C}_{16}\text{H}_{15}\text{N}_3\text{O}$	265.12150	265.12129	3.48 (t, 4 H, NCH_2 , $J = 4$); 3.92 (t, 4 H, OCH_2 , $J = 4$); 7.39 (s, 1 H, H(1)); 7.73 (m, 4 H, H arom.); 8.34 (m, 3 H, H arom.)	CDCl_3
2- <i>N</i> -Methyl-acetamido-7-morpholino-phenazine	$\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_2$	336.15862	336.15856	2.07 (s, 3 H, COMe); 3.43 (s, 3 H, NMe); 3.51 (t, 4 H, NCH_2 , $J = 4$); 3.92 (t, 4 H, OCH_2 , $J = 4$); 7.34–8.19 (m, 6 H, H arom.)	CDCl_3
2- <i>N</i> -Methyl-acetamido-phenazine	$\text{C}_{15}\text{H}_{13}\text{N}_3\text{O}$	251.10586	251.10603	2.07 (s, 3 H, COMe); 3.44 (s, 3 H, N^+Me); 7.86 (m, 2 H arom.); 8.03 (d, 1 H arom., $J = 3$); 8.25 (m, 2 H arom.)	CDCl_3
9-Methyl-phenazin-2-one	$\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}$	210.07931	210.07971	3.84 (s, 3 H, Me); 6.07 (d, 1 H arom., $J = 2$); 6.89 (dd, 1 H arom., $J = 2$); 7.48 (m, 2 H arom.); 7.85 (m, 3 H arom.)	$(\text{CD}_3)_2\text{CO}$

as the internal standard. Mass spectra were obtained with a Finnigan MAT-8200 spectrometer (electron impact, 70 eV). The course of the reactions and the purity of the compounds were monitored by TLC on Silufol UV-254 plates in the $\text{Bu}^i\text{OH}-\text{MeCOEt}-\text{HCOOH}-\text{H}_2\text{O}$ system (8 : 6 : 3 : 3). Preparative chromatography was performed on a Waters 600E chromatograph. Melting points were determined in sealed capillaries on a Kristall instrument, the rate of heating was 0.3 $^{\circ}\text{C min}^{-1}$ near the range of melting. Correct data of elemental analysis were obtained for compounds **2**, **3**, **6**–**9**.

9-Methyl-2-methylaminophenazinium perchlorate (2) was synthesized by the known procedure.³ M.p. 177–180 $^{\circ}\text{C}$, R_f 0.56. ^1H NMR (CD_3CN), δ : 3.18 (d, 3 H, NHCH_3 , $J = 5$ Hz); 4.28 (s, 3 H, ^+NMe); 6.56 (d, 1 H, H(1), $J = 2$ Hz); 7.50 (dd, 1 H, H(3), $J_{1,3} = 2$ Hz, $J_{3,4} = 10$ Hz); 7.65 (br.s, 1 H, NH); 7.84 (m, 1 H, H arom.); 7.92 (d, 1 H, H(4), $J = 10$ Hz); 8.12 (m, 3 H, H arom.). Found (%): C, 51.93; H, 4.33; N, 12.98. $\text{C}_{14}\text{H}_{14}\text{ClN}_3\text{O}_4$. Calculated (%): C, 51.89; H, 4.32; N, 12.97.

2-[2-(Methoxycarbonyl)ethyl]amino-9-methylphenazinium perchlorate (3) was obtained by the known procedure³ from 9-methylphenazinium methylsulfate (1 g, 3.3 mmol) and methyl ester of β -alanine hydrochloride (1.14 g, 8.2 mmol) in a yield of 0.8 g (62 %), m.p. > 220 $^{\circ}\text{C}$ (decomp.), R_f 0.64. ^1H NMR (CD_3CN), δ : 2.81 (t, 2 H, COCH_2CH_2 , $J = 6.5$ Hz); 3.68 (s, 3 H, OMe); 3.82 (m, 2 H, COCH_2CH_2); 4.29 (s, 3 H, ^+NMe); 6.68 (d, 1 H, H(1), $J = 2$ Hz); 7.54 (dd, 1 H, H(3), $J_{1,3} = 2$ Hz, $J_{3,4} = 10$ Hz); 7.63 (s, 1 H, NH); 7.88 (m, 2 H, H arom.); 8.15 (m, 3 H, H arom.). Found (%): C, 51.58; H, 4.65; N, 10.64. $\text{C}_{17}\text{H}_{18}\text{ClN}_3\text{O}_6$. Calculated (%): C, 51.54; H, 4.64; N, 10.61.

9-Methyl-2-*N*-methylacetamidophenazinium perchlorate (4). $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.5 mL, 5.4 mmol) was added to a solution of compound **2** (0.3 g, 0.9 mmol) in 5 mL of Ac_2O , and the reaction mixture was stirred at 20 $^{\circ}\text{C}$ for 1 day. Then Et_2O was added until precipitation began, and the reaction mixture was stirred for 2 h. The crystals that formed were filtered off and washed with a small amount of Ac_2O and then with Et_2O .

Compound **4** was further used without additional purification. Yield 0.25 g (62 %), m.p. > 200 $^{\circ}\text{C}$ (decomp.), R_f 0.25. ^1H NMR (CD_3CN), δ : 2.34 (s, 3 H, COMe); 3.63 (s, 3 H, NCH_3); 4.76 (s, 3 H, ^+NMe); 8.21 (m, 2 H, H arom.); 8.37 (d, 1 H, H(1), $J = 2$ Hz); 8.55 (m, 4 H, H arom.).

2-*N*-[2-(Methoxycarbonyl)ethyl]acetamido-9-methylphenazinium perchlorate (5). $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 mL, 21.7 mmol) was added to a suspension of compound **3** (1 g, 2.5 mmol) in 20 mL of Ac_2O , and the reaction mixture was stirred for 3 h. Then Et_2O was added until precipitation began, and the reaction mixture was stirred for 2 h. The sediment was filtered off and washed with Et_2O , and then reprecipitated with ether from acetone. Compound **5** was further used without additional purification. Yield 0.45 g (40 %), m.p. 133–136 $^{\circ}\text{C}$, R_f 0.25. ^1H NMR (CD_3CN), δ : 2.27 (s, 3 H, COMe); 2.70 (t, 2 H, COCH_2CH_2 , $J = 6.5$ Hz); 3.55 (s, 3 H, OMe); 4.28 (t, 2 H, $\text{COCH}_2\text{CH}_2\text{N}$, $J = 6.5$ Hz); 4.79 (s, 3 H, ^+NMe); 8.25 (m, 2 H, H arom.); 8.37 (d, 1 H, H(1), $J = 2$ Hz); 8.57 (m, 4 H, H arom.).

9-Methyl-2,7-bismethylaminophenazinium perchlorate (6) and 9-methyl-2-*N*-methylacetamido-7-methylaminophenazinium perchlorate (7). Gaseous MeNH_2 was passed through a suspension of compound **4** (0.2 g, 0.6 mmol) in 10 mL of dry MeOH for 6 h. The precipitate was filtered off and washed with hot EtOH. The mother liquor was concentrated, dissolved in the minimal amount of water, and applied onto a column (2×30 cm) with a polymeric dextran gel Molselect G-10. Compounds **7** and **6** were eluted with water in succession. The yield of compound **7** was 0.09 g (42 %), m.p. > 300 $^{\circ}\text{C}$ (decomp.), R_f 0.36. ^1H NMR (CD_3CN), δ : 2.18 (s, 3 H, COMe); 3.29 (s, 3 H, HNCH_3); 3.52 (s, 3 H, CONMe); 4.38 (s, 3 H, ^+NMe); 6.75 (d, 1 H, H(8), $J = 2$ Hz); 7.60 (dd, 1 H, H(6), $J_{6,8} = 2$ Hz, $J_{6,5} = 10$ Hz); 7.82 (dd, 1 H, H(3), $J_{1,3} = 2$ Hz, $J_{3,4} = 9$ Hz); 8.00 (d, 1 H, H(4), $J = 10$ Hz); 8.25 (d, 1 H, H(1), $J = 2$ Hz); 8.34 (d, 1 H, H(5), $J = 9$ Hz). Found (%): C, 51.58; H, 4.80; N, 14.24. $\text{C}_{17}\text{H}_{19}\text{ClN}_4\text{O}_5$. Calculated (%): C, 51.67; H, 4.81; N, 14.18.

The yield of compound **6** was 0.07 g (36 %), m.p. > 240 °C (decomp.), R_f 0.5. ^1H NMR (CD_3CN), δ : 3.13 (s, 6 H, NMe); 4.20 (s, 3 H, ^+NMe); 6.70 (d, 2 H, H(1) and H(8), $J = 2$ Hz); 7.35 (dd, 2 H, H(3) and H(6), $J_{3,1} = 2$ Hz, $J_{3,4} = 10$ Hz); 7.86 (d, 2 H, H(4) and H(5), $J = 10$ Hz). Found (%): C, 51.06; H, 4.82; N, 15.90. $\text{C}_{15}\text{H}_{17}\text{ClN}_4\text{O}_4$. Calculated (%): C, 51.02; H, 4.81; N, 15.87.

9-Methyl-2-methylamino-7-morpholinophenazinium perchlorate (8). A. Morpholine (0.1 mL, 1.2 mmol) was added to a suspension of compound **4** (0.1 g, 0.3 mmol) in 10 mL of dry MeOH, and the reaction mixture was stirred for 3 h. The precipitate was filtered off and washed with hot EtOH. The filtrate was concentrated, and the residue was dissolved in EtOH. A mixture of compound **8** and 9-methyl-2-*N*-methylacetamido-7-morpholinophenazinium perchlorate was precipitated with ether. The sediment was dissolved with heating in 5 mL of 0.1 *M* HClO_4 and refluxed for 2 h. On cooling, compound **8** (0.085 g, 71 %) precipitated, m.p. > 205 °C (decomp.), R_f 0.29. ^1H NMR ($\text{DCON}(\text{CD}_3)_2$), δ : 3.02 (d, 3 H, NHCH_3 , $J = 4$ Hz); 3.40 (t, 4 H, $\text{NCH}_2\text{CH}_2\text{O}$, $J = 5.5$ Hz); 3.69 (t, 4 H, $\text{NCH}_2\text{CH}_2\text{O}$, $J = 5.5$ Hz); 4.13 (s, 3 H, ^+NMe); 6.62 (d, 1 H, H(1), $J = 2$ Hz); 7.03 (d, 1 H, H(8), $J = 2$ Hz); 7.26 (dd, 1 H, H(3), $J_{1,3} = 2$ Hz, $J_{3,4} = 9$ Hz); 7.57 (dd, 1 H, H(6), $J_{8,6} = 2$ Hz, $J_{6,5} = 10$ Hz); 7.65 (br.s, 1 H, NH); 7.71 (d, 1 H, H(4), $J = 9$ Hz); 7.92 (d, 1 H, H(5), $J = 10$ Hz). Found (%): C, 52.90; H, 5.15; N, 13.71. $\text{C}_{18}\text{H}_{21}\text{ClN}_4\text{O}_5$. Calculated (%): C, 52.83; H, 5.19; N, 13.69.

B. Morpholine (0.1 mL, 1.2 mmol) was added to a suspension of compound **4** (0.1 g, 0.3 mmol) in 10 mL of dry MeOH, and the reaction mixture was stirred for 3 days. The precipitate was filtered off and washed with hot EtOH. The filtrate was concentrated, and the residue was dissolved in EtOH. Compound **8** was precipitated with ether. After crystallization from 0.1 *M* HClO_4 , the yield of compound **8** was 0.04 g (36 %). The mother liquor after precipitation with ether was concentrated, the residue was dissolved in a minimal amount of chloroform and chromatographed on a column (1×20 cm) with Silasorb-600 (15–20 mm, detection at 360 nm) using gradient elution $\text{CHCl}_3 \rightarrow \text{CHCl}_3\text{--MeOH}$, 5 : 1. The main fractions were concentrated, and phenazine, 2-morpholinophenazine, 2-*N*-methylacetamido-7-morpholinophenazine, 2-*N*-methylacetamidophenazine, and 9-methylphenazin-2-one were obtained in yields of 6–12 mg. The main characteristics of the compounds obtained are given in Table 2.

7-[2-(Methoxycarbonyl)ethyl]amino-9-*N*-methyl-2-methylaminophenazinium perchlorate (9). A. Methyl ester of β -alanine hydrochloride (0.74 g, 0.53 mmol) and Et_3N (100 μL , 0.7 mmol) were added to a suspension of compound **4** (0.1 g, 0.3 mmol) in 5 mL of dry MeOH, and the reaction mixture

was stirred for 3 days. The precipitate was filtered off and washed with hot EtOH, and the solvent was concentrated. The residue was dissolved in EtOH, and compound **9** was precipitated with ether. After crystallization from 0.1 *M* HClO_4 , the yield of compound **9** was 0.06 g (51 %), m.p. > 170 °C (decomp.), R_f 0.45. Found (%): C, 50.76; H, 4.95; N, 13.21. $\text{C}_{18}\text{H}_{21}\text{ClN}_4\text{O}_6$. Calculated (%): C, 50.84; H, 4.94; N, 13.18.

B. Gaseous MeNH_2 was passed through a suspension of compound **5** (0.1 g, 0.2 mmol) in 10 mL of anhydrous MeOH for 6 h. The reaction mixture was allowed to stand for 3 days and then processed as in procedure A. The yield of perchlorate **9** was 0.045 g (46 %), m.p. > 170 °C (decomp.), R_f 0.45. ^1H NMR ($\text{DCON}(\text{CD}_3)_2$), δ : 2.35 (t, 2 H, $\text{NCH}_2\text{CH}_2\text{CO}$, $J = 6.5$ Hz); 2.72 (m, 2 H, $\text{NCH}_2\text{CH}_2\text{CO}$); 3.16 (d, 3 H, HNMe , $J = 4$ Hz); 3.72 (s, 3 H, OMe); 4.32 (s, 3 H, ^+NMe); 6.56 (d, 1 H, H(1), $J = 2$ Hz); 6.76 (d, 1 H, H(8), $J = 2$ Hz); 7.36 (dd, 1 H, H(3), $J = 9$ Hz); 7.48 (dd, 1 H, H(3), $J = 10.2$ Hz); 7.92 (m, 2 H, H(4) and H(5)).

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References

1. M. I. Dobrikov, R. Yu. Dudko, A. S. Levina, L. M. Khalimskaya, and G. V. Shishkin, *Bioorg. Khim.*, 1996, in press [*Russ. J. Bioorg. Chem.*, 1996 (Engl. Transl.)].
2. M. A. Warpehoski and D. E. Harper, *J. Am. Chem. Soc.*, 1995, **117**, 2951.
3. V. F. Zarytova, I. V. Kutyavin, V. N. Sil'nikov, and G. V. Shishkin, *Bioorg. Khim.*, 1986, **12**, 911 [*Sov. J. Bioorg. Chem.*, 1986, **12** (Engl. Transl.)].
4. S. G. Lokhov, M. A. Podyminogin, D. S. Sergeev, V. N. Sil'nikov, I. V. Kutyavin, and G. V. Shishkin, *Bioconjugate Chem.*, 1992, **3**, 414.
5. S. G. Lokhov, A. A. Koshkin, I. V. Kutyavin, M. A. Podyminogin, and A. V. Lebedev, *Bioorg. Khim.*, 1995, **21**, 196 [*Russ. J. Bioorg. Chem.*, 1995, **21** (Engl. Transl.)].
6. A. Weissberger, *The Chemistry of Heterocyclic Compounds*, **11**, *Phenazines*, Interscience, New York, 1957.
7. O. N. Chupakhin and I. Ya. Postovskii, *Usp. Khim.*, 1976, **45**, 908 [*Russ. Chem. Rev.*, 1976, **45** (Engl. Transl.)].
8. H. McIlwain, *J. Chem. Soc.*, 1937, **59**, 1704.
9. A. I. Kiprianov and G. M. Prilutskii, *Ukr. Khim. Zh.* (Russ. Ed.), 1956, **22**, 359.
10. G. V. Shishkin and V. N. Sil'nikov, *Khim. Geterosikl. Soedin.*, 1986, 1658 [*Chem. Heterocycl. Compd.*, 1986 (Engl. Transl.)].

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