Reagents for specific modification of biopolymers

10.* Activation of the phenazinium ring for nucleophilic substitution of hydrogen

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Direct nucleophilic substitution of primary and secondary amines for hydrogen in quaternary phenazinium salts containing an additional positive charge in the aliphatic part of the molecule was carried out. The substitution proceeds successively in positions 2 and 7, which allows selective introduction of different substituents into the heterocycle.

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

Introduction of fragments containing substituted phenazines into biopolymers endows the latter with new, unique properties that allow one to use such conjugates for specific modification of biopolymers. Thus the addition of phenazine at 3'- and/or 5'-terminal phosphates of oligonucleotides bearing alkylating groups significantly enhances the stability of oligonucleotide duplexes and hence makes alkylation of the target nucleotide more efficient.[2–4] Some phenazine derivatives are capable of generating hydroxyl radicals under mild conditions, which enables them to be used as reagents for site-directed DNA scission.[5,6] Several naturally occurring phenazine-containing antibiotics are known[7–10] to react effectively with biopolymers *in vivo*. However, these properties as well as the ability of phenazine derivatives to participate in electron-transfer reactions and formation of stable charge-transfer complexes,[11] have not been adequately used in construction of reagents for modification of biopolymers. These considerations stimulate the search for new ways of functionalization of the phenazine ring, especially under conditions where biopolymers retain their biological activity.

Despite a number of methods for the synthesis of various phenazine derivatives,[12] direct nucleophilic substitution of the hydrogen atom, which is characteristic of azine quaternary salts[13] and usually proceeds under mild conditions, is the most interesting. The reactivity of phenazine derivatives in this reaction can be estimated by comparing the charge electron density (kinetic control) and nucleophilic localization energies (thermodynamic control).[14] According to the literature data on the distribution of the electron density on an *N*-methylphenazinium salt,[15] substitution of the hydrogen atom should occur at position 2 or 4. However, the substitution at position 4 is thermodynamically unfavorable and hence only the hydrogen atom in position 2 should be substituted, which fully agrees with the experimental results.[12,16]

To estimate the relative reactivity of substituted quaternary phenazinium salts, we calculated the charge distribution for positions 2 and 7 in compounds **1—5**. These quantum-mechanical calculations (PM3) were performed for free cations *in vacuo* because, under real conditions of nucleophilic substitution of hydrogen, a nucleophile attacks either a solvated cation or an ionic pair separated by solvent molecules. The contribution of solvation to the charge distribution may be taken, in a first approximation, as the same for all quaternary phenazinium salts.

The calculations showed that the introduction of the electron-donor amino group into position 2 of a quaternary salt (compound **2**) significantly decreases the positive charge in the symmetrical position 7, thus precluding introduction of a second substituent. This suggests that the phenazine ring can be activated for nucleophilic substitution of hydrogen in 2-amino derivatives by acylating the amino group (compound **3**). This approach, based on a decrease in the donor effect of a substituent, has been realized by us experimentally,[17] though it imposes substantial limitations on the substituents to be introduced.

It should be noted that, according to calculations, a similar or even more pronounced effect of activation can be attained by introducing an additional positive charge into the substituent at the quaternary nitrogen atom. To check this assumption experimentally, we synthesized quaternary phenazinium salt (**4**), bearing an additional positive charge in the aliphatic fragment of the molecule, and studied its behavior in reactions with aliphatic amines.

Salt **4** was synthesized from 9-acetyl-10-(2-bromoethyl)-9,10-dihydrophenazine[18] (**6**) (Scheme 1). Treatment of compound **6** with an excess of

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* For Part 9, see Ref. 1.

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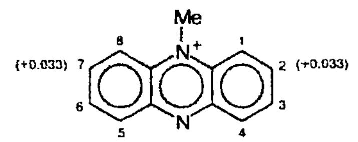

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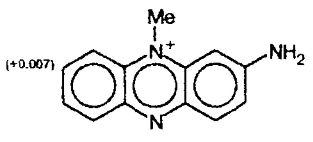

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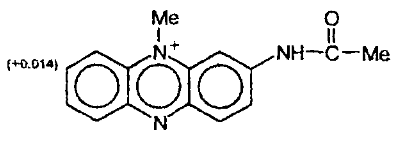

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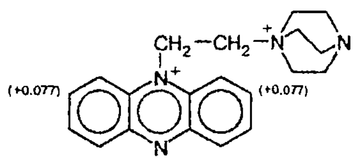

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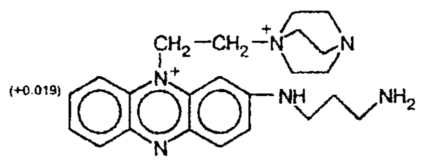

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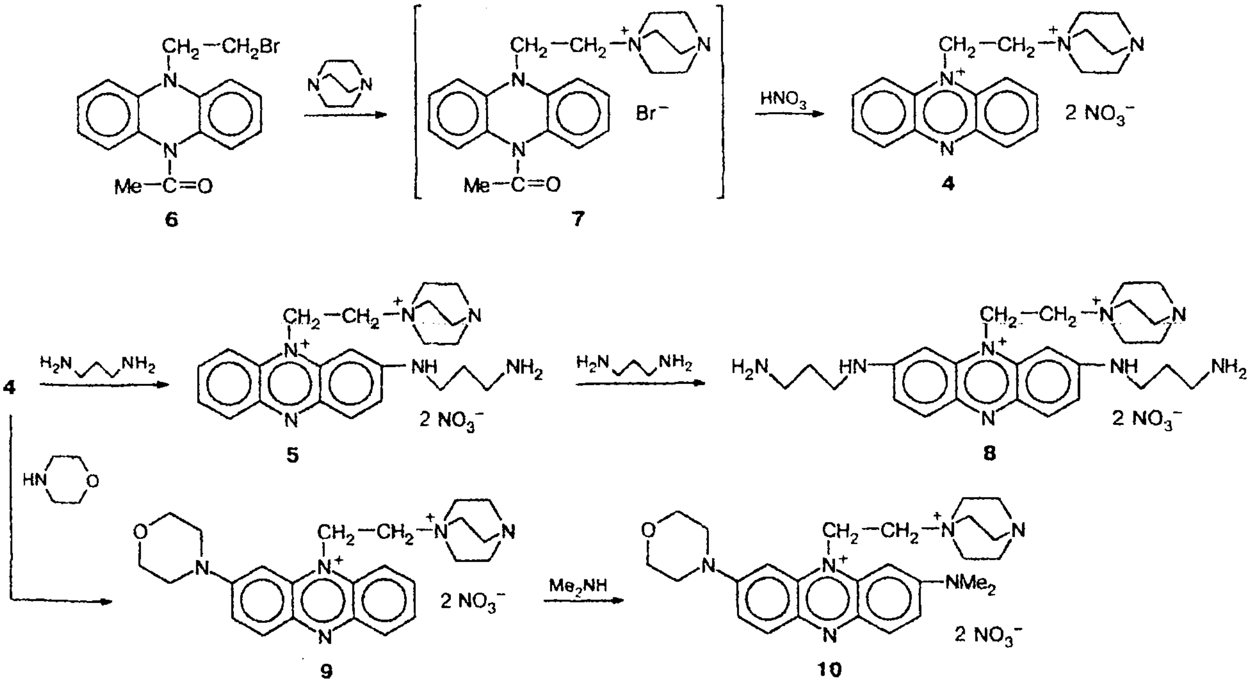
1,4-diazabicyclo[2.2.2]octane gives compound **7** in quantitative yield (TLC). The latter was treated *in situ* with conc. HNO_3 to give dinitrate **4** solvated with two HNO_3 molecules. Prolonged storage of this solvate in vacuum over alkali reduces the content of HNO_3 to 1.2—1.5 acid molecules per molecule of compound **4**. In the solid state, this compound is stable, but its aqueous and alcoholic solutions fast darken, especially in the light.

The reactions of bisquaternary salt **4** with nucleophiles were studied using propane-1,3-diamine, morpholine, and methylamine as examples. Reaction mixtures were analyzed by HPLC. Figure 1 shows kinetics of the reaction of compound **4** with a tenfold excess of propane-1,3-diamine. It can be seen that the content of a monosubstituted product (compound **5**) in the reaction mixture (~95%) is a maximum after 30 min. The reaction rate at position 7 of product **5** is much lower, so that the percentage of the disubstituted product (**8**) does not exceed 4—5% by that time. The reaction can be stopped in this stage to isolate the monosubstituted product (**5**) and introduce another nucleophile, which allows one to obtain a product containing different substituents in positions 2 and 7.

The possibility of synthesizing asymmetrically substituted phenazine derivatives was exemplified in successive introduction of the morpholine and dimethylamine residues (compounds **9** and **10**) (see Scheme 1).

Note that the substitution in quaternary phenazinium salts proceeds *via* an intermediate that is to be oxidized with oxygen. Thus, the preparative synthesis requires the

Scheme 1



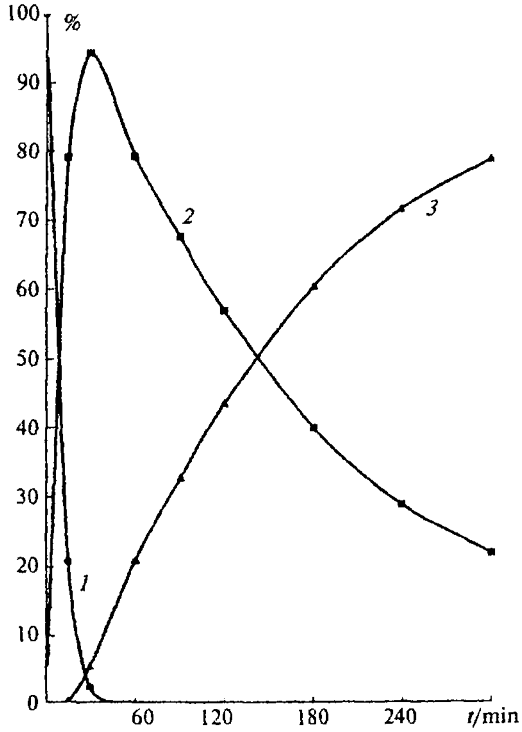


Fig. 1. The kinetics of reaction of bisquaternary salt **4** with propane-1,3-diamine: *1*, starting compound; *2*, monosubstituted product **5**; *3*, disubstituted product **8**.

use of low reactant concentrations and intense flow of air. Otherwise, a considerable amount of side products is formed. At present, 5'-phosphoramide derivatives of the oligonucleotide series have been prepared from compound **8**, and the influence of the introduced bisquaternary salt on the stability of duplexes of such oligonucleotide derivatives is being studied.

Experimental

UV spectra were recorded on a Specord M-40 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker WR-200 spectrometer with HMDS as the internal standard. The course of the reactions was monitored and the purity of compounds was checked by HPLC on a Milikhrom-4 analytical liquid chromatograph (Lichrosorb RP-18 column, gradient 0—80% MeOH, 0.1% CF_3COOH). Chromatograms were processed with CHROM software (A. S. Zenkov, Novosibirsk Institute of Bioorganic Chemistry, Siberian Branch of the Russian Academy of Sciences) by comparing peak areas at analytical wavelengths of 250 and 300 nm, with consideration of data obtained from chromatographing artificial mixtures of compounds under study. The following concentrations were used in kinetic experiments: 10^{-3} mol L^{-1} (derivatives of quaternary phenazinium salts) and 10^{-2} mol L^{-1} (propanediamine, methylamine, and morpholine) in MeOH. TLC was performed on (*A*) UV-254 Silufol plates in the system Bu^{t}OH—MeCOEt—HCOOH—H_2O = 8 : 6 : 3 : 3 and (*B*) DC-Fertigplatten RP-18 F_{254} (Merck) in the system 40% MeOH + 0.1% CF_3COOH. Melting points were measured in sealed capillaries on a Kristall setup, heating rate near the melting range 0.3 deg min^{-1}. Quantum-mechanical computations were performed for the ground state of cations by the PM3 method with geometry optimization (the Polak-Ribiere algorithm, RHF calculation) with the HYPERCHEM program (version 4.5).

9-(2-Quinuclidinioethyl)phenazinium dinitrate (4). 1,4-Diazabicyclo[2.2.2]octane (0.45 g, 4.0 mmol) was added to a solution of 9-acetyl-10-(2-bromoethyl)-9,10-dihydrophenazine (**6**) (0.13 g, 0.4 mmol) in 4 mL of DMF. The reaction mixture was stirred at 20 °C for one day. The solvent was evaporated, and the residue was washed with ether, dried, and suspended in 5 mL of water. Conc. HNO_3 (1 mL) was added, and the resulting solution was stirred at ~20 °C for 2 h. Then it was concentrated *in vacuo*, and the residue was dissolved in a minimum amount of water. Methanol was added until precipitation began. The crystals that formed were filtered off, washed with methanol, and dried *in vacuo* to give the solvate, **4**·$2HNO_3$ (0.20 g, 88%), m.p. >140 °C (decomp., from water). R_f = 0.20 (*A*). Found (%): C, 41.9; H, 4.74; N, 19.5. $C_{20}H_{24}N_6O_6 \cdot 2HNO_3$. Calculated (%): C, 42.1; H. 4.59; N, 19.6. UV (EtOH), λ_{max}/nm (ε): 210 (31000), 262 (34000), 392 (10800), 450 (2300). ^{1}H NMR (D_2O), δ: 3.66 (m, 6 H, $N(C\underline{H}_2—)_3$); 4.24 (m, 8 H, $—C\underline{H}_2—N^+(C\underline{H}_2—)_3$); 6.31 (m, 2 H, $—C\underline{H}_2—N^+_{Phen}$); 8.30 (m, 2 H, H-4,5); 8.69 (m, 4 H, H-2,3,6,7); 8.79 (m, 2 H, H-1,8).

2,7-Bis(3-aminopropyl)amino-9-(2-quinuclidinioethyl)phenazinium dinitrate (8). Propane-1,3-diamine (0.17 mL, 2.0 mmol) was added with vigorous stirring, ensuring saturation of the reaction mixture with air, to a suspension of compound **4**·$2HNO_3$ (0.11 g, ~0.2 mmol) in 10 mL of anhydrous MeOH, and the mixture was stirred at 20 °C for 18 h. The solvent was evaporated *in vacuo*, and the residue was dissolved in a minimum amount of methanol. Compound **8** was precipitated with ether. Threefold reprecipitation with ether from methanol was followed by dissolution in a minimum amount of H_2O—MeOH mixture (9 : 1) and chromatography (column 15×2 cm, Silasorb S-2, 15 μm, elution with aqueous methanol (a gradient from 0 to 80% MeOH)). The main fraction with R_f = 0.24 (*B*) was concentrated and reprecipitated with ether from methanol. Yield 0.08 g (69%), m.p. >217 °C (decomp., from methanol). UV (EtOH), λ_{max}/nm (ε): 251 (2160), 290 (25200), 543 (21400). ^{1}H NMR (D_2O), δ: 2.21 (t, 4 H, 2 $—CH_2—C\underline{H}_2—NH_2$, J = 8 Hz); 3.23 (m 4 H, 2 $—CH_2—C\underline{H}_2—CH_2—NH_2$); 3.84 (m, 10 H, 2 $NH—C\underline{H}_2—CH_2—$, $^+N—(C\underline{H}_2—)_3$); 4.01 (m, 8 H, $—C\underline{H}_2—N^+—(C\underline{H}_2—)_3$); 5.3 (m, 2 H, $—C\underline{H}_2—N^+_{Phen}$); 6.61 (s, 2 H, H-1,8); 7.83 (d, 2 H. H-3,6, $J_{3—4(6—5)}$ = 9 Hz); 7.88 (d, 2 H, H-4,5, $J_{4—3(5—6)}$ = 9 Hz).

2-Morpholino-9-(2-quinuclidinioethyl)phenazinium dinitrate (9). Morpholine (0.1 mL, 1.2 mmol) was added with vigorous stirring to a suspension of compound **4**·$2HNO_3$ (0.11 g, ~0.2 mmol) in 10 mL of anhydrous MeOH. Stirring was continued in air at 20 °C for 30 min. The reaction mixture was poured into ether. The precipitate that formed was twice reprecipitated with ether from methanol, dissolved in a minimum amount of H_2O—MeOH mixture (9 : 1), and chromatographed (column 15×2 cm, Silasorb S-2, 15 μm, elution with aqueous MeOH (a gradient from 0 to 80% MeOH)). The main fraction with R_f = 0.17 (*B*) was concentrated and reprecipitated with ether from methanol. Yield 0.06 g (56%), m.p. >170 °C (decomp., from methanol). R_f = 0.22 (*A*), 0.17 (*B*). UV (EtOH), λ_{max}/nm (ε): 236 (15800), 294 (13800), 401 (4450), 540 (8110). ^{1}H NMR (D_2O), δ: 3.61 (t, 4 H, $(—C\underline{H}_2—)_2O$, J = 8 Hz); 4.04 (m, 10 H,

$N(-C\underline{H}_2-)_2 + N(-C\underline{H}_2-)_3$); 4.16 (m, 8 H, $-CH_2-N^+-(C\underline{H}_2)_3-$); 5.38 (m, 2 H, $-C\underline{H}_2-N^+_{Phen}$); 6.87 (d, 1 H, H-1, J_{1-3} = 2 Hz); 8.19 (m, 6 H, H_{Phen}).

2-Dimethylamino-7-morpholino-9-(2-quinuclidinioethyl)phenazinium dinitrate (10). A 40% aqueous solution of dimethylamine (0.1 mL, ~0.8 mmol) was added with vigorous stirring in air to a solution of compound **9** (0.05 g, ~0.1 mmol) in 5 mL of MeOH. Stirring was continued at 20 °C for 18 h. The reaction mixture was concentrated *in vacuo*, and the solid residue was reprecipitated with ether from methanol, dissolved in a minimum amount of H_2O—MeOH mixture (9 : 1), and chromatographed (column 15×2 cm, Silasorb C-2, 15 μm, elution with aqueous MeOH (a gradient from 0 to 80% MeOH)). The main fraction with R_f = 0.36 (*B*) was concentrated and reprecipitated with ether from methanol. Yield 0.04 g, (70%), m.p. >156 °C (decomp., from methanol). R_f = 0.36 (*B*). UV (EtOH), λ_{max}/nm (ε): 231 (6170), 278 (23200), 543 (25400). ^1H NMR (D_2O), δ: 3.34 (s, 6 H, $-N(CH_3)_2$); 3.56 (t, 4 H, $(-C\underline{H}_2-)_2O$, J = 8 Hz); 3.90 (m, 10 H, $N(-C\underline{H}_2-)_2 + N(-C\underline{H}_2-)_3$); 4.27 (m, 8 H, $-C\underline{H}_2-N^+-(C\underline{H}_2)_3-$); 5.24 (m 2 H, $-C\underline{H}_2-N^+_{Phen}$); 6.53 (br.s, 2 H, H-1,8); 7.81 (d, 2 H, H-3,6, $J_{3-4(6-5)}$ = 9 Hz); 7.88 (d, 2 H, H-4,5, $J_{4-3(5-6)}$ = 9 Hz).

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References

1. E. D. Tenetova, M. I. Dobrikov, G. V. Shishkin, and V. D. Shteingarts, *Izv. Akad. Nauk, Ser. Khim.*, 1998, 328 [*Russ. Chem. Bull.*, 1998, **47**, 321 (Engl. Transl.)].
2. V. F. Zarytova, I. V. Kutyavin, V. N. Sil'nikov, and G. V. Shishkin, *Bioorg. Khim.*, 1986, 911 [*Sov. J. Bioorg. Chem.*, 1986 (Engl. Transl.)].
3. 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.
4. G. V. 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.)].
5. K. Nagai, B. J. Karter, J. Xu, and S. M. Hecht, *J. Am. Chem. Soc.*, 1991, **113**, 5099.
6. K. Nagai and S. M. Hecht, *J. Biol. Chem.*, 1991, **266**, 23994.
7. K. Takahashi, I. Takahashi, M. Morimoto, and F. Nomita, *J. Antibiot.*, 1986, **39**, 624.
8. W. Keller-Schierlein, A. Geiger, H. Zahner, and M. Brandl, *Helv. Chim. Acta*, 1988, **71**, 2058.
9. U. Hollstein and D. A. McCamey, *J. Org. Chem.*, 1973, **38**, 3415.
10. C. W. Van't Land, U. Mocek, and H. G. Floss, *J. Org. Chem.*, 1993, **58**, 6576.
11. R. Hasada and T. Yagi, *J. Biochem.*, 1977, **82**, 1469.
12. A. Weissberger, *The Chemistry of Heterocyclic Compounds*, **11**, *Phenazines*, Interscience Publishers, Inc., New York, 1957.
13. O. N. Chupakhin and I. Ya. Postovskii, *Usp. Khim.*, 1976, **45**, 908 [*Russ. Chem. Rev.*, 1976, **45** (Engl. Transl.)].
14. V. N. Charushin and O. N. Chupakhin, *Usp. Khim.*, 1984, **53**, 1648 [*Russ. Chem. Rev.*, 1984, **53** (Engl. Transl.)].
15. G. G. Dyadyusha and E. A. Panamareva, *Ukr. Khim. Zh.* [*Ukrainian Chemical Journal*], 1963, **29**, 1279 (in Russian).
16. S. B. Serebryannyi and P. A. Yufa, *Ukr. Khim. Zh.* [*Ukrainian Chemical Journal*], 1963, **29**, 322 (in Russian).
17. V. N. Sil'nikov, N. P. Luk'yanchuk, and G. V. Shishkin, *Izv. Akad. Nauk, Ser. Khim.*, 1996, 2059 [*Russ. Chem. Bull.*, 1996, **45**, 1955 (Engl. Transl.)].
18. G. V. Shishkin, *Khim. Heterotsikl. Soedin.*, 1984, 1407 [*Chem. Heterocycl. Compd.*, 1984 (Engl. Transl.)].

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