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DNA Sequence-Specific Ligands: XII.[1] Synthesis and Cytological Studies of Dimeric Hoechst 33258 Molecules

A. V. Gromyko*, K. V. Popov*, A. P. Mosoleva*, S. A. Streltsov*, S. L. Grokhovsky, V. A. Oleinikov***, and A. L. Zhuze*,[2]**

** Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul. Vavilova 32, Moscow, 119991 Russia*

*** Moscow Center of Medical Research of Oslo University, ul. Vavilova 34/5, Moscow, 119991 Russia*

**** Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, ul. Miklukho-Maklaya 16/10, Moscow, 117997 Russia*

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Abstract—We synthesized dimeric Hoechst dye molecules composed of two moieties of Hoechst 33258 fluorescent dye with the phenolic hydroxy groups tethered via pentamethylene, heptamethylene, or triethylene oxide linkers. A characteristic pattern of differential staining of chromosome preparations from human HL60 premonocytic leukemia cells was observed for all the three fluorescent dyes. The most contrasting pattern was obtained for the bisHoechst analogue with the heptamethylene linker; its quality was comparable with the picture obtained in the case of chromosome staining with 4',6-diamidino-2-phenylindole. The ability to penetrate into live human fibroblasts was studied for the three bisHoechst compounds. The fluorescence intensity of nuclei of live and fixed cells stained with the penta- and heptamethylene-linked bisHoechst analogues was found to differ only slightly, whereas the fluorescence of the nuclei of live cells stained with triethylene oxide-linked bisHoechst was considerably weaker than that of the fixed cells. The bisHoechst molecules are new promising fluorescent dyes that can both differentially stain chromosome preparations and penetrate through cell and nuclear membranes and effectively stain cell nuclei.

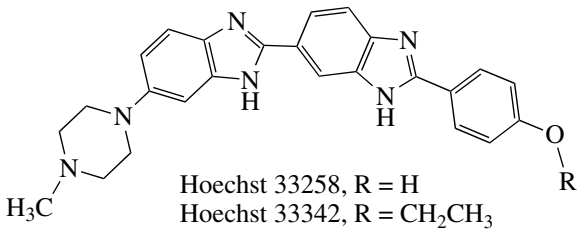
Key words: chromosome, dimeric Hoechst 33258 dye, DNA, fluorescent dye, minor groove ligand, synthesis

INTRODUCTION

The design of medicines that can site-specifically bind to DNA is an important pharmacological task, since the therapeutic activity of most of the currently used antitumor drugs depends on the potency and selectivity of their interaction with DNA.

There is now a tendency in anticancer and antiviral chemotherapy to replace the standard drugs that disrupt DNA in a nonselective manner by new agents that noncovalently interact with DNA and affect its replication and transcription. Therefore, the design of low-molecular ligands capable of noncovalent and site-specific binding to the DNA minor groove is of a great interest. These site-specific compounds would play an important role in the direction of them to the certain genes of genome and for the use in chemotherapy. Such compounds could serve as both the tools for studies and the therapeutic agents capable of controlling the gene expression in cells.

Previously, we had used a molecular model suggested by Zasedatelev *et al.* [2] for the DNA complexes with the AT-specific antibiotics netropsine and distamycin A and designed and synthesized dimeric ligands, bis-netropsines [3–6]. They are based on the pyrrolecarboxamide structural motif and proved to be the first class of ligands that can noncovalently and site-specifically be bound by DNA. The general principle of the design of these compounds consists in the linking one or several pyrrolecarboxamide blocks via various spacers. This structure enables the orientation and fixing these A·T-recognizing pyrrolecarboxamide blocks in the complex with DNA [5, 6]. The study of physicochemical [4, 6] and biochemical [1, 6–10] properties of these DNA complexes with dimeric ligands showed that the netropsine fragments are specifically bound by two A·T base pair clusters. Later, this approach was



Hoechst 33258, R = H
Hoechst 33342, R = CH_2CH_3

[1] For communication XI, see [1].

[2] Corresponding author: phone: +7 (095) 135-9718; fax: +7 (095) 135-1405; e-mail: zhuze@imb.ac.ru

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successfully used for the design of other dimeric sequence-specific compounds based on various combinations of pyrrolecarboxamide and imidazolecarboxamide blocks [11–15].

In this work, we chose the dye Hoechst 33258, which is widely used in cytology as the DNA-specific fluorescent label [16], as the starting compound for the design of a new class of dimeric site-specific ligands. It is known that Hoechst 33258 inhibits the interaction of the TATA-box-binding protein with DNA [17], is an efficient inhibitor of DNA topoisomerase I [18] and DNA helicases [19], and is a radioprotector [20].

Hoechst 33258 is capable of noncovalent binding to the minor groove of DNA B form. It preferentially interacts with the clusters composed of three to four A·T pairs (with the stoichiometry of one ligand molecule per binding site) and covers a halfturn of the double DNA helix [21–24]. It was found that, among all the A·T triplets, Hoechst 33258 prefers the (5')-AA(A/T) sequence and, in the case of A·T tetraplets, the (5')-AATT sequence [25].

The A·T specificity of Hoechst 33258 is determined by the backbone of the dye molecule consisting of two benzimidazole fragments linked to each other according to the head-to-tail principle. While interacting with DNA, each benzimidazole fragment forms a bifurcated (three-centered) hydrogen bond with the thymine O(2) atom and/or adenine N(3) atom of two adjacent A·T pairs and covers a region of about 1.5 bp in length [23, 26]. The Hoechst 33258 binding is also stabilized by electrostatic and strong van der Waals interactions with the minor groove walls.

We synthesized three dimeric bis(benzimidazole) molecules, namely, bisHoechsts (**Va**)–(**Vc**) composed of two dimeric Hoechst 33258 molecules whose phenyl hydroxyl groups are linked via pentamethylene, heptamethylene, or triethylene glycol bridges, respectively. Our goal was to design more specific ligands capable of recognizing two sites comprising three or four A·T pairs, *viz.*, $(A/T)_n$–NN–$(A/T)_n$ (where n = 3 or 4), and covering one turn of the DNA double helix. The choice of these bridges was based on both speculative models and the analysis of the oligonucleotide complexes with known bis(benzimidazole) ligands [27, 28].

RESULTS AND DISCUSSION

The choice of Hoechst 33258 as the basic compound for the design of new site-specific ligands was due to both its AT-specificity and the ability of its analogue, Hoechst 33342, to penetrate through cell membranes while retaining its fluorescent properties. We believed that the preservation of fluorescent properties would permit a reliable detection of even small amounts of the new ligands, which would make the designed bisHoechsts (**Va**)–(**Vc**) superior to the bisnetropsines, we previously synthesized, by enabling their localization in cells.

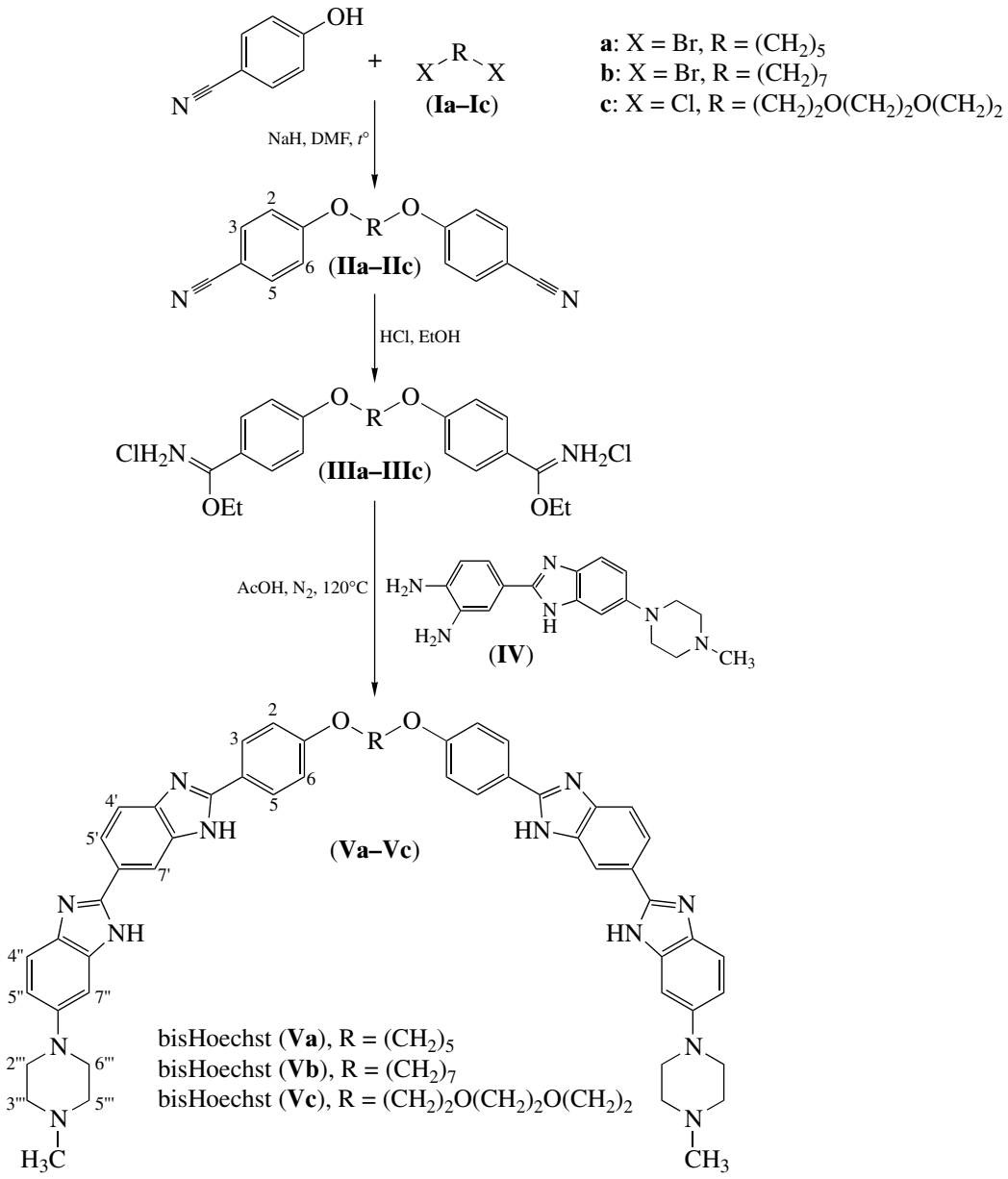
Three methods were tested for the synthesis of dimeric bis(benzimidazoles), with bisHoechst (**Va**) being an example. The coupling of 2-amino-4-[6-(4-methylpiperazine-1-yl)-1*H*-benzimidazole-2-yl]phenylamine (**IV**) [29] with ethyl 4-[(5-{4-[ethoxy(imino)methyl]phenoxy}pentyl)oxy]-1-phenylimidoate (**IIIa**) (reflux in glacial AcOH for 20 min under nitrogen) resulted in 29.4% yield of (**Va**) and proved to be the most efficient method (Scheme 1). Diimidate (**IIIa**) was synthesized by the Pinner reaction from 4-{[5-(4-cyanophenoxy)pentyl]oxy}benzonitrile (**IIa**), which, in its turn, was prepared by the alkylation of 4-hydroxybenzonitrile alcoholate with 1,5-dibromopentane in DMF.

The second pathway involved the synthesis of dialdehydes (**VIa**)–(**VId**) by the alkylation of *p*-hydroxybenzaldehyde alcoholate with α,ω-dibromoalkanes in order to couple them further with diamine (**IV**) and obtain the corresponding bis(benzimidazoles). However, it was found that a prolonged heating of diamine (**IV**) in ethanol with 4-[5-(4-formylphenoxy)pentyloxy]benzaldehyde (**VIa**) in the presence of *p*-benzoquinone in inert atmosphere did not result in the target (**Va**) because of considerable resinification of the reaction mixture (Scheme 2). Therefore, (**VIb**)–(**VId**) were not used further for the preparation of the corresponding bisHoechsts.

In the third pathway, the coupling of diamine (**IV**) with methyl 4-{5-[4-(methoxycarbonyl)phenoxy]pentyloxy}benzoate (**VII**) in the presence of P_2O_5 in methanesulfonic acid [30] began to form (**V**) after the first 15 min. However, this later degraded in the course of reaction to give the Hoechst 33258 mesyl derivative (according to MALDI TOF mass spectrometry). Therefore, this approach also has not been developed.

These results made us to synthesize (**Vb**) and (**Vc**) in 24.8 and 23.3% yields by coupling diamine (**IV**) with diimidates (**IIIb**) and (**IIIc**), respectively (Scheme 1).

An alteration of absorption spectrum of (**Va**) in the presence of calf thymus DNA indicated the formation of their complex [31]. Chromosomes are identified in most of cytogenetic studies by the pattern of differential staining. A large number of procedures have been developed for the specific staining of pairs of homologous chromosomes [32]. For the procedures based on the fluorescence *in situ* hybridization (FISH) of DNA sequences, various types of the so-called Q-staining are used. Its basis is the staining with the AT-specific fluorochromes due to uneven distribution of AT/GC pairs along chromosomes. DAPI (4',6-diamidino-2-phenylindol) is the dye that is most often used for these purposes, since its spectral characteristics are compatible with those of the dye used for DNA labeling [33]. In addition to DAPI, Hoechst 33258 is also used for the differential chromosome staining in a number of cases [34]. We tested each of the synthesized fluorochromic dyes (**Va**)–(**Vc**) and found that all of them provide the differential staining. The most contrast pattern was



Scheme 1. The scheme of synthesis of bisHoechsts (**Va**)–(**Vc**).

obtained with Hoechst (**Vb**) (see the figure); its pattern was comparable with that obtained with DAPI.

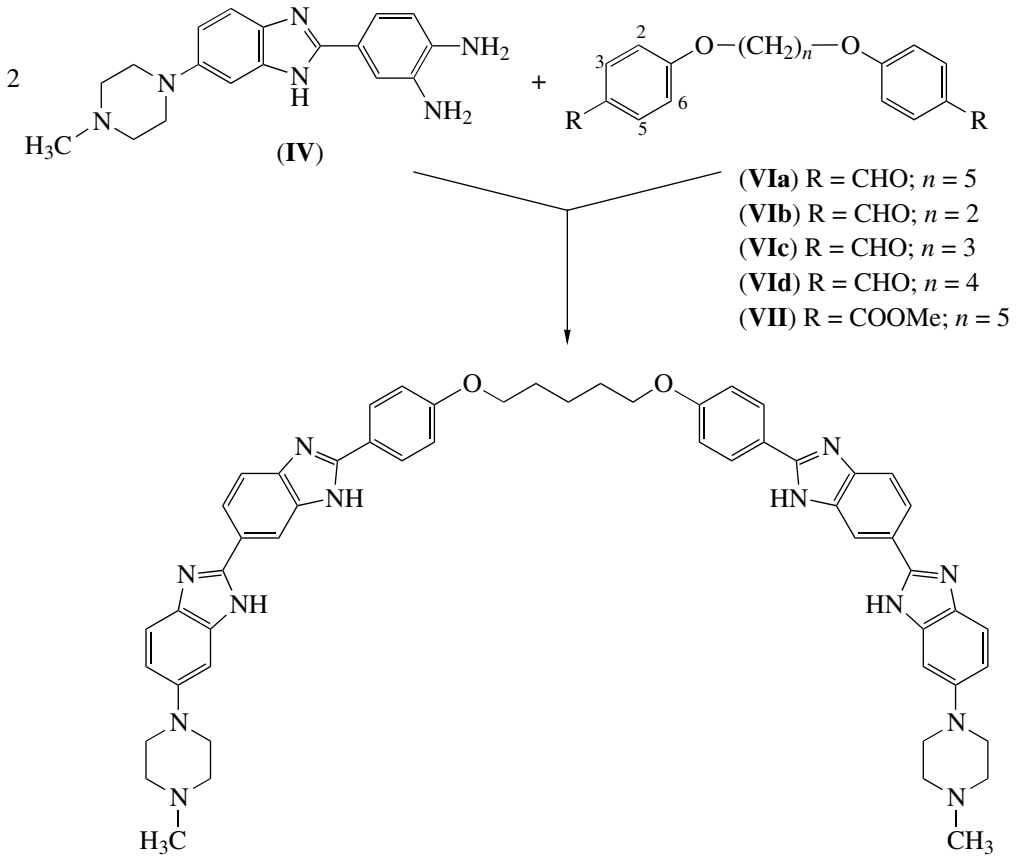
It is known that Hoechst 33258 can penetrate through the intact cell membranes [35]. The study of the ability of bisHoechsts (**Va**)–(**Vc**) to pass into live human fibroblast cells demonstrated that the fluorescence brightness of live and fixed cells stained with bisHoechsts (**Va**) and (**Vb**) only slightly differed from one another. The fluorescence of nuclei of live cells stained with bisHoechst (**Vc**) was considerably lower than that of fixed cells. However, we did not study the time-dependent dynamics of dye penetration into cells.

To summarize, the synthesized compounds, bisHoechsts (**Va**)–(**Vc**) are new promising fluorescent dyes capable of penetration through the cell and nuclear membranes and effectively staining the cell nuclei.

In our future work, we plan to study the site specificity of bisHoechst analogues using the footprinting with DNase I and evaluate the ability of these compounds to inhibit the catalytic activity of human DNA topoisomerase I.

EXPERIMENTAL

4-Hydroxybenzaldehyde, 1,5-dibromopentane, 1,7-dibromoheptane, and sodium hydride (80% suspension in Vaseline oil) were from Fluka (Switzerland); 4-hydroxybenzonitrile was from Aldrich (United States);

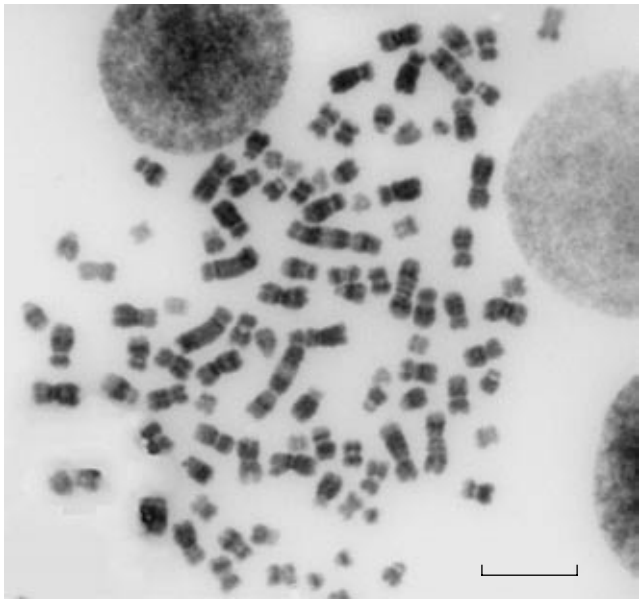


Scheme 2. The alternative scheme of synthesis of bisHoechst (**Va**).

and 1,2-bis(2-chloroethoxy)ethane was from Merck (Germany).

Solutions of compounds in organic solvents were dried with Na_2SO_4. As a rule, the solvents were evaporated on a rotor evaporator at 50°C in a vacuum of a water-jet pump. The compounds were dried in a vacuum over P_2O_5 and NaOH. Melting points were determined on a Boethius hot plate (Germany) and were uncorrected. Hydrogenation was carried our in the presence of the Adams catalyst [36] at atmospheric pressure and room temperature until the hydrogen absorption was ceased. The homogeneity of the compounds synthesized was monitored by TLC on the Kieselgel 60 F_{254} precoated plates (Merck, Germany) in system A, 50 : 1 methanol–Et_3N, and system B, 100 : 1 $CHCl_3$–isopropanol. The compounds on chromatograms were detected in UV light according to the absorption at 254 nm or fluorescence excited at 365 nm. The absorption spectra of bisHoechsts (**Va**)–(**Vc**) were registered in 1 mM cacodylate buffer (pH 6.8) in the presence of 10 vol % DMSO. The ^{1}H NMR spectrum was registered on an AMX-400 spectrometer (Bruker, Germany) (working frequency of 400 MHz) in DMSO-d_6 at 23°C (if not stated otherwise); the residual DMSO resonance was an internal standard. The numerations of hydrogen atoms in benzimidazole and piperazine cycles are given in Scheme 1. The assignment of ^{1}H NMR signals of (**Va**)–(**Vc**) was done using the information in [37]. The bisHoechst mass spectra were registered on a time-of-flight mass spectrometer Vision-2000 (ThermoBioanalysis, UK) using a linear mode of positive ion registration; 2,5-dihydroxybenzoic acid as a matrix ionization by N_2 laser (337 nm) were used. Mass spectra of dinitriles (**IIa**)–(**IIc**), dialdehydes (**VIa**)–(**VId**), and (**VII**) were obtained by electron impact ionization on a Polaris CQ 230 (Finnigan, United States) and a MS-890 (Kratos, UK) at 160–200°C using the direct inlet and the electron energy of 70 eV; the temperature of ionization chamber was 250°C.

A general procedure for the synthesis of dinitriles (IIa)–(IIc). 4-Hydroxybenzonitrile (2.4 mmol) was added to a suspension of NaH (2.4 mmol) in DMF (3.0 ml) at 0°C, the mixture was heated at 80°C under stirring until the complete dissolution of NaH, and α,ω-dihalogenoalkane (**Ia**)–(**Ic**) (1.0 mmol) was added. The solution was heated at 80°C for 1 h [for (**Ia**) and (**Ib**)] or at 110–120°C for 1 h in the presence of NaI (0.1 mmol) [in the case of 1,2-bis(2-chloroethoxy)ethane (**Ic**)], cooled, and water (10 ml) was added.



A negative image of metaphase chromosomes of the HL-60 cells stained with bisHoechst (**Vb**). A scale is 10 μm.

The precipitate was filtered, washed with water, dried, and the resulting dinitrile (**II**) was twice recrystallized from ethanol.

4-{[5-(4-Cyanophenoxy)pentyl]oxy}benzonitrile (IIa); yield 95.7%; R_f 0.34 (B); mp 109–110°C; ^{1}H NMR: 1.56 (2 H, m, $OCH_2CH_2CH_2$), 1.79 (4 H, m, OCH_2CH_2), 4.07 (4 H, t, *J* 6.23, OCH_2), 7.09 (4 H, d, *J* 9.03, H2 + H6), and 7.75 (4 H, d, *J* 9.03, H3 + H5); MS (Polaris CQ 230), *m*/*z*: 307.1 $[M + H]^{+\cdot}$, calc. for $C_{19}H_{18}N_2O_2$: *M* 306.3.

4-{[7-(4-Cyanophenoxy)heptyl]oxy}benzonitrile (IIb); yield 85.7%; R_f 0.38 (B); mp 112°C; ^{1}H NMR: 1.39 [6 H, m, $OCH_2CH_2(CH_2)_3$], 1.72 (4 H, m, OCH_2CH_2), 4.04 (4 H, t, *J* 6.54, OCH_2), 7.08 (4 H, d, *J* 8.72, H2 + H6), 7.74 (4 H, d, *J* 8.72, H3 + H5); MS (Polaris CQ 230), *m*/*z*: 333.9 $[M + H]^+$, calc. for $C_{21}H_{22}N_2O_2$: *M* 334.4.

4-(2-{2-[2-(4-Cyanophenoxy)ethoxy]ethoxy}ethoxy)benzonitrile (IIc); yield 71.0%; R_f 0.06 (B); mp 77–80°C; ^{1}H NMR: 3.60 (4 H, s, $EtOCH_2CH_2OEt$), 3.75 (4 H, t, *J* 4.05, $PhOCH_2CH_2$), 4.17 (4 H, t, *J* 4.05, $PhOCH_2$), 7.10 (4 H, d, *J* 8.40, H2 + H6), 7.75 (4 H, d, *J* 8.40, H3 + H5); MS (Polaris CQ 230), *m*/*z*: 352.9 $[M + H]^+$, calc. for $C_{20}H_{20}N_2O_4$: *M* 352.4.

A general procedure for the synthesis of diimido esters dihydrochlorides (IIIa)–(IIIc). A suspension of dinitriles (**IIa**)–(**IIc**) (1 mmol) in absolute ethanol (5 ml) was saturated with dry hydrogen chloride at 0°C for 15 min, until the starting compounds were completely dissolved. The reaction mixture was kept at room temperature for 4 days in a sealed flask, evaporated in a vacuum to dryness, and the solid residues of (**IIIa**)–(**IIIc**) were dried over NaOH and P_2O_5 in a vacuum and used in further reactions without purification.

BisHoechst (Va). A solution of ethyl 4-[(5-{4-[ethoxy(imino)methyl]phenoxy}pentyloxy]-1-phenylimidate dihydrochloride (**IIIa**) (0.313 g, 0.665 mmol) and 2-amino-4-[6-(4-methylpiperazin-1-yl)-1*H*-benzimidazol-2-yl]phenylamine (**IV**) [29] (0.429 g, 1.330 mmol) in AcOH (5 ml) was refluxed for 20 min under nitrogen, cooled, the precipitate of bright yellow crystals was filtered, washed with AcOH and benzene, the filtrate was evaporated in a vacuum, and the residue was dissolved in AcOH (3 ml). A thick suspension formed after the addition of three drops of concentrate HCl was refluxed until the complete dissolution of all crystals. Bright yellow crystals precipitated after cooling the mixture were filtered and washed with AcOH and benzene. Both portions of crystals were combined and recrystallized from a mixture of methanol (15 ml) and Et_3N (1 ml). The precipitate was washed on filter with methanol, dissolved in a 10 : 2 methanol–AcOH mixture (12 ml), and refluxed with activated charcoal. The charcoal was removed, the solution was evaporated in a vacuum, and the residue was recrystallized from a 10 : 1 methanol–Et_3N mixture (11 ml). The formed precipitate was washed on filter with methanol, dissolved in AcOH (3 ml), and two drops of concentrated HCl were added. The solution was evaporated in a vacuum, the residue was suspended in boiling methanol (4 ml), and a solution of conc. HCl (five drops) in methanol (3 ml) was added. The resulting suspension was kept overnight in a refrigerator, the yellow crystalline product was separated, washed twice with methanol, and dried over NaOH and P_2O_5 in a vacuum to give 0.222 g (29.4%) of (**Va**) hexahydrochloride; mp > 260°C; R_f 0.14 (A); λ_{max} 341 nm (ε_{341} 70000 M^{-1} cm^{-1}). BisHoechst (**Va**) exhibits a yellow–green fluorescence in solution. For more accurate assignment of resonances within the ranges of 3.0–4.0 and 7.0–8.5 ppm, its ^{1}H NMR spectrum was registered at 380 K: 1.69 (2 H, m, $OCH_2CH_2CH_2$), 1.91 (4 H, m, OCH_2CH_2), 2.85 (6 H, s, NCH_3), 3.37 (8 H, m, H3''' + H5'''), 3.57 (8 H, m, H2''' + H6'''), 4.19 (4 H, t, *J* 6.30, OCH_2), 7.14 (4H, d, *J* 8.72, H2 + H6), 7.15 (2 H, dd, *J* 9.03, 1.87, H5''), 7.23 (2 H, d, *J* 1.87, 2 x H7''), 7.62 (2 H, d, *J* 9.03, H4''), 7.76 (2 H, d, *J* 8.41, H4'), 8.12 (2 H, dd, *J* 8.41, 1.56, H5'), 8.22 (4 H, d, *J* 8.72, H3 +,H5), 8.51 (2 H, d, *J* 1.56, H7'), MS (Vision-2000), *m*/*z*: 918.6 $[M + H]^+$, calc. for $C_{55}H_{56}N_{12}O_2$: *M* 917.1.

BisHoechst (Vb). A solution of ethyl 4-[(5-{4-[ethoxy(imino)methyl]phenoxy}heptyloxy]-1-phenylimidate dihydrochloride (**IIIb**) (0.323 g, 0.65 mmol) and 2-amino-4-[6-(4-methylpiperazin-1-yl)-1*H*-benzimidazol-2-yl]phenylamine (**IV**) (0.425 g, 1.32 mmol) in AcOH (4 ml) was refluxed for 30 min under nitrogen, cooled to room temperature, the resulting gel-like mass was separated, washed with benzene, dissolved in

methanol (10 ml), reprecipitated with a 1 : 1 mixture of isopropanol–concentrated ammonia, and the precipitate was recrystallized from methanol (5 ml). The methanolic filtrate was evaporated in a vacuum, and the residue was crystallized from methanol (10 ml). The mother liquids were combined, evaporated, and recrystallized several times from a 2 : 3 mixture of methanol–isopropanol. All the crystals were combined and dissolved in a methanol–AcOH mixture, the solvents were removed in a vacuum, and the residue was recrystallized from a 10 : 1 methanol–Et_3N mixture (11 ml). The resulting precipitate was dissolved in a 5 : 1 methanol–AcOH mixture (12 ml) and treated three times with activated charcoal, until the solution became light yellow. After the solvent removal in a vacuum, the residue was recrystallized from a 10 : 1 methanol–Et_3N mixture (5.5 ml) and kept overnight in a refrigerator. The resulting precipitate was separated, washed with methanol, and dissolved in a 5 : 1 methanol–AcOH mixture (12 ml). The organic solvents were removed in a vacuum, and the residue was recrystallized from a 8 : 1 methanol–AcOH mixture (9 ml). Concentrate HCl (0.5 ml) was added to the solution obtained after the treatment of the crude product with a 10 : 1 methanol–AcOH mixture (11 ml), and the resulting suspension was kept overnight in a refrigerator to give a yellow compound, which was separated, washed twice with methanol (5 ml) on filter, and dried in a vacuum over NaOH and P_2O_5 to give 0.188 g (24.85%) of (**Vb**) hexahydrochloride, mp >260°C; R_f 0.16 (A); λ_{max} 342 nm (ε_{342} 82 700 M^{-1} cm^{-1}). BisHoechst (**Vb**) exhibits a yellow–green fluorescence in solution; ^{1}H NMR: 1.46 (6 H, m, $OCH_2CH_2(CH_2)_3$), 1.78 (4 H, m, OCH_2CH_2), 2.85 (6 H, s, NCH_3), 3.21 (8 H, m, H3''' + H5'''), 3.54 (4 H, m, H2''' + H6'''), 3.89 (4 H, m, H2''' + H6'''), 4.10 (4 H, t, *J* 6.23, OCH_2), 7.16 (4 H, d, *J* 8.72, H2 + H6), 7.17 (2 H, s, H7''), 7.30 (2 H, dd, *J* 9.03, *J* 1.25, H5''), 7.68 (2 H, d, *J* 9.03, H4''), 7.87 (2 H, d, *J* 8.72, H4'), 8.19 (2 H, d, *J* 8.72 + 1.25, H5'), 8.25 (4 H, d, *J* 8.72, H3 + H5), 8.59 (2 H, s, H7'), and 10.95 (4 H, s, NH); MS (Vision-2000), *m/z*: 946.4 [*M* + H]$^+$, calc. for $C_{57}H_{60}N_{12}O_2$: *M* 945.1.

BisHoechst (Vc). A solution of diamine (**IV**) (0.603 g, 1.87 mmol) and diimidoester dihydrochloride (**IIIc**) ((0.485 g, 0.935 mmol) was refluxed in AcOH (5 ml) for 30 min under nitrogen, and evaporated. The residue was dissolved in hot water and treated with concentrate ammonia to precipitate the product. After the solution was decanted, the oil-like product was washed with water, dissolved in methanol, and refluxed with activated charcoal. The filtrate was evaporated; the residue was dissolved in ethanol (3 ml) and reprecipitated with a mixture of water (3 ml) and concentrate ammonia (0.4 ml). The product was repeatedly reprecipitated from ethanol (3 ml) with water (1 ml) followed by a thrice reprecipitation from ethanol (3 ml) with acetone (7 ml). The resulting compound was refluxed with ethanol (3 ml), cooled, and filtered. The precipitate was crystallized from methanol (6 ml) and suspended in a mixture of DMF (0.6 ml) and isopropanol (3 ml) under heating. The addition of water (2 ml) first resulted in the dissolution of suspension, while the addition of another 4 ml of water led to crystallization. The precipitate was separated, washed with isopropanol and water, and dried in a vacuum to give 0.229 g (25.4%) of (**Vc**). The base (**Vc**) was converted to the protonated form by dissolution in a methanol–AcOH mixture, evaporation of the solvents, dissolution of the residue in methanol (4 ml), and treatment of the solution with a mixture of methanol (3 ml) and concentrate HCl (1 ml). The yellow hexahydrochloride of (**Vc**) was precipitated in acetone (12 ml), separated, washed with acetone, and dried in a vacuum to give 0.258 g (23.34%) of (**Vc**); mp >260°C; R_f 0.12 (A); λ_{max} 340 nm (ε_{340} 70 800 M^{-1} cm^{-1}). BisHoechst (**Vc**) exhibited a yellow–green fluorescence in solution. For more accurate assignment of resonances within the ranges of 3.0–4.0 and 7.0–8.5 ppm, the ^{1}H NMR spectrum was registered at 380 K: 2.85 (6 H, s, NCH_3), 3.37 (8 H, m, H3''' + H5'''), 3.59 (8 H, m, H2''' + H6'''), 3.71 (4 H, s, $EtOCH_2CH_2OEt$), 3.85 (4 H, t, *J* 4.83, $PhOCH_2CH_2O$), 4.26 (4 H, t, *J* 4.83, $PhOCH_2$), 7.14 (4 H, d, *J* 8.72, H2 + H6), 7.18 (2 H, dd, *J* 9.03 and 1.87, H5''), 7.24 (2 H, d, *J* 1.87, H7''), 7.64 (2 H, d, *J* 9.03, H4''), 7.77 (2 H, d, *J* 8.72, H4'), 8.13 (2 H, dd, *J* 8.72 and 1.25, H5'), 8.22 (4 H, d, *J* 8.72, H3 + H5), and 8.54 (2 H, s, H7'); MS (Vision-2000), *m/z*: 963.7 [*M* + H]$^+$, calc. for $C_{56}H_{58}N_{12}O_4$: *M* 963.0.

A general procedure for the synthesis of dialdehydes (VIa)–(VId). Dialdehydes were obtained by the procedure of the synthesis of dinitriles (**II**) using the corresponding α,ω-dibromoalkanes: namely, 1,5-dibromopentane for (**VIa**); 1,2-dibromoethane for (**VIb**); 1,3-dibromopropane for (**VIc**), and 1,4-dibromobutane for (**VId**); 4-hydroxybenzaldehyde was used instead of 4-hydroxybenzonitrile. The reaction proceeded for 1 h at 80°C. After the reaction was over, the target products were precipitated with water, separated, washed with water, dried, and used in further reactions without additional purification.

Analytical samples of the dialdehydes were obtained after recrystallization from benzene–hexane.

4-[5-(4-Formylphenoxy)pentyloxy]benzaldehyde (VIa); yield 94.6%; R_f 0.15 (A); mp 80–82°C; ^{1}H NMR: 1.58 (2 H, m, $OCH_2CH_2CH_2$), 1.82 (4 H, m, OCH_2CH_2), 4.11 (4 H, t, *J* 6.54, OCH_2), 7.12 (4 H, d, *J* 8.72, H2 + H6), 7.85 (4 H, d, *J* 8.72, H3 + H5), and 9.86 (2 H, s, CHO); MS (MS-890), *m/z*: 312.0 [*M*]$^{+\cdot}$, calc. for $C_{19}H_{20}O_4$: *M* 312.36.

4-[2-(4-Formylphenoxy)ethoxy]benzaldehyde (VIb); yield 23.0%; R_f 0.14 (A); mp 118–120°C; ^{1}H NMR: 4.49 (4 H, s, CH_2CH_2), 7.19 (4 H, d, *J* 8.50, H2 + H6), 7.88 (4 H, d, *J* 8.5, H3 + H5), 9.88 (2 H, s, CHO). MS (MS-890), *m/z*: 270.0 [*M*]$^{+\cdot}$; calc. for $C_{16}H_{14}O_4$ *M* 270.28.

4-[3-(4-Formylphenoxy)propoxy]benzaldehyde (VIc); yield 88.1%; R_f 0.16 (A); mp 125–126°C; ^{1}H NMR: 2.25 (2 H, m, $CH_2CH_2CH_2$), 4.27(4 H, t, *J* 6.23, OCH_2), 7.15 (4 H, d, *J* 8.50, H2 + H6), 7.86 (4 H, d, *J* 8.50, H3 + H5), 9.84 (2 H, s, CHO); MS (MS-890), *m*/*z*: 284.0 $[M]^{+\cdot}$, calc. for $C_{17}H_{16}O_4$: *M* 284.30.

4-[4-(4-Formylphenoxy)butoxy]benzaldehyde (VId); yield 90.18%; R_f 0.14 (A); mp 100–102°C; ^{1}H NMR: 1.91 (4 H, m, OCH_2CH_2), 4.17 (4 H, m, OCH_2), 7.13 (4 H, d, *J* 8.72, H2 + H6), 7.86 (4 H, d, *J* 8.72, H3 + H5), 9.86 (2 H, s, CHO); MS (MS-890), *m*/*z*: 298.0 $[M]^{+\cdot}$, calc. for $C_{18}H_{18}O_4$: *M* 298.33.

Methyl 4-{5-[4-(methoxycarbonyl)phenoxy]pentyloxy}benzoate (VII). Dimethyl ester (**VII**) was obtained as described for the preparation of dinitriles (**IIa**)–(**IIc**) using 1,5-dibromopentane and methyl 4-hydroxybenzoate instead of 4-hydroxybenzonitrile. The reaction proceeded at 80°C for 2 h. After the reaction was over, the target product was precipitated with water, separated, washed with water, dried, and used in further reactions without additional purification. Its yield was 81.8%; R_f 0.26 (A); mp 91–93°C; ^{1}H NMR: 1.57 (2 H, m, $OCH_2CH_2CH_2$), 1.80 (4 H, m, OCH_2CH_2), 3.80 (6 H, s, CH_3), 4.07 (4 H, t, *J* 6.54, OCH_2), 7.02 (4 H, d, *J* 8.72, H2 + H6), 7.89 (4 H, d, *J* 8.72, H3 + H5); MS (Polaris CQ 230), *m*/*z*: 372.0 $[M]^{+\cdot}$, calc. for $C_{21}H_{24}O_6$: *M* 372.41.

The study of the ability of dyes to differentially stain chromosomes. Chromosome preparations were obtained from human promonocytic leukemia HL-60 cells (ATCC, United States). The cells were cultured in the RPMI 1640 medium (PanEco, Russia) containing 40 μg/ml gentamycin (Akrikhin, Russia) and 10% embryonic calf serum (Hyclone, United States) at 37°C, 5% CO_2, and 95% air humidity. The preparations were prepared using the standard protocol [38] without colchicine-induced accumulation of the cells at the mitosis stage. The cells at the stage of logarithmic growth were subjected to osmotic shock in 75 mM NaCl solution at 37°C for 30 min and fixed in a 3 : 1 methanol–AcOH mixture. The resulting suspension was dropped onto wet glass plates. The plates were dried and stored at room temperature. The preparations were stained with 1 μM dye solutions in the buffer containing 10 mM Tris–HCl (pH 7.5) and 0.1 M NaCl for 30 min and were analyzed in the same solution. The image was registered with the CoolSnap chamber (Roper Scientific, United States) fixed up on a fluorescent microscope Leitz using a PlanApo64x NA1.4 objective and a filter combination for DAPI (Ex 358/Em 461). The image was edited by the ImageJ 1.3 program (National Institute of Health, United States, http://rsb.info.hih.gov/ij).

The evaluation of the ability of the dyes to penetrate into live cells. Normal human fibroblasts were used for the evaluation of the ability of the dyes to penetrate into live cells. The cells were cultured in the DMEM medium (PanEco, Russia) containing 40 μg/ml gentamycin (Akrikhin, Russia) and 10% embryonic calf serum (Hyclone, United States) at 37°C, 5% CO_2, and 95% air humidity. The cells were transferred onto cover-glasses placed in separate Petri dishes and analyzed after two days. Two glasses were used for each dye. One cover-glass of each pair of Petri dishes was fixed in 70% ethanol, stained with 1 μM dye solution in the phosphate buffered saline (PBS) for 30 min, washed with PBS three times, and kept in PBS. The dyes were added in the cultural medium of the second dish up to the concentration of 1 μM, the cells were washed out from the dye after 2 h, kept in PBS, and were immediately analyzed using the same microscope and the same combination of filters as used for the study of differential staining of chromosome preparations. The fluorescence brightness of fixed and live cell nuclei was compared visually.

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