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DNA Sequence-Specific Ligands: XVI. Series of the DBP(*n*) Fluorescent Dimeric Bisbenzimidazoles with 1,4-Piperazine-Containing Linkers[1]

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Abstract—A novel series of the **DBP(*n*)** fluorescent symmetric dimeric bisbenzimidazoles in which the bisbenzimidazole fragments were attached to an oligomeric linker with the 1,4-piperazine residue in its center were prepared. The **DBP(*n*)** molecules were distinguished by the number of methylene groups *n* (where ***n*** = 1, 2, 3, 4) in the linker. The **DBP(*n*)** synthesis was based on a condensation of the monomeric bisbenzimidazole (**MB)** with 1,4-piperazinedialkylcarbonic acids. The ability of the **DBP(*n*)** dimeric bisbenzimidazoles to form complexes with the double-stranded DNA was demonstrated by a complex of physicochemical methods, including spectroscopy in the visual UV-area, circular dichroism (CD), and fluorescence. The **DBP(1–4)** molecules were localized in the DNA minor groove by the CD method with the use of cholesteric liquid-crystalline dispersions (CLCD) of the double-stranded DNA. The **DBP(*n*)** dimeric bisbenzimidazoles were easily soluble in water, penetrated through cellular and nuclear membranes, and stained DNA in living cells distinct from the previously synthesized **DB(*n*)** series.

Keywords: dimeric bisbenzimidazole, DBP(*n*), complexes with the double-stranded DNA, DNA, minor-groove binder, fluorescence, circular dichroism

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INTRODUCTION

Preparation of low-molecular-weight compounds that site-specifically recognize nucleotide sequences in double-stranded DNAs is one of the urgent problems in bioorganic chemistry and molecular biology. In the long term, such molecular instruments which are specifically bound to definite nucleotide sequences of the genome could be used for an investigation and a control of an expression of concrete genes. Moreover, these target-directed compounds are of special interest for pharmacology, because a chemotherapeutic activity of the majority of the known antitumor agents depends on their affinity and selectivity of their interaction with a double-stranded DNA. From this point of view, low-molecular-weight compounds that noncovalently interact with the DNA minor groove are the most promising. These minor grove binders are free from many disadvantages of the traditional biologically active preparations on the basis of alkylating and intercalating agents. In particular, they do not damage DNA, do not significantly distort the DNA spatial structure, and are almost free from the side mutagenic effect.

Here, we continued our studies [1] of a creation of the **DBP(*n*)** novel series of the DNA site-specific ligands based on the Hoechst 33258 (Ht) dye (Fig. 1), which is widely used in cytology as a DNA-specific fluorescent label [2]. Ht is known to be noncovalently and AT-specifically bound to the DNA minor groove [3, 4]. It inhibits TATA box binding protein [5], effectively inhibits DNA-topoisomerase I [6], topo-II [7], and DNA-helicases [8], and exhibits the radioprotective effect [9]. The AT-specificity of Ht is determined by the backbone of the dye molecule that consists of the two covalently-bound benzimidazole fragments. Each of these fragments interacts with DNA, forms bifurcational (three-center) hydrogen bound with the O2 atom of thymine and/or with the N3 atom of adenine of the neighboring AT pairs, and covers a region of approximately one and a half base pairs [3]. The Ht

[1] The XVI series was published in [1].

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Abbreviations: dsDNA, the double-stranded DNA; X-ray; X-ray structural analysis; CD, circular dichroism; CLCD, the cholesteric liquid-crystalline dispersion; BOP, benzotriazolyl-1-oxy)tris(dimethylamino)-phosphonium hexafluorophosphate; DIPEA, *N*,*N*-diisopropylamine.

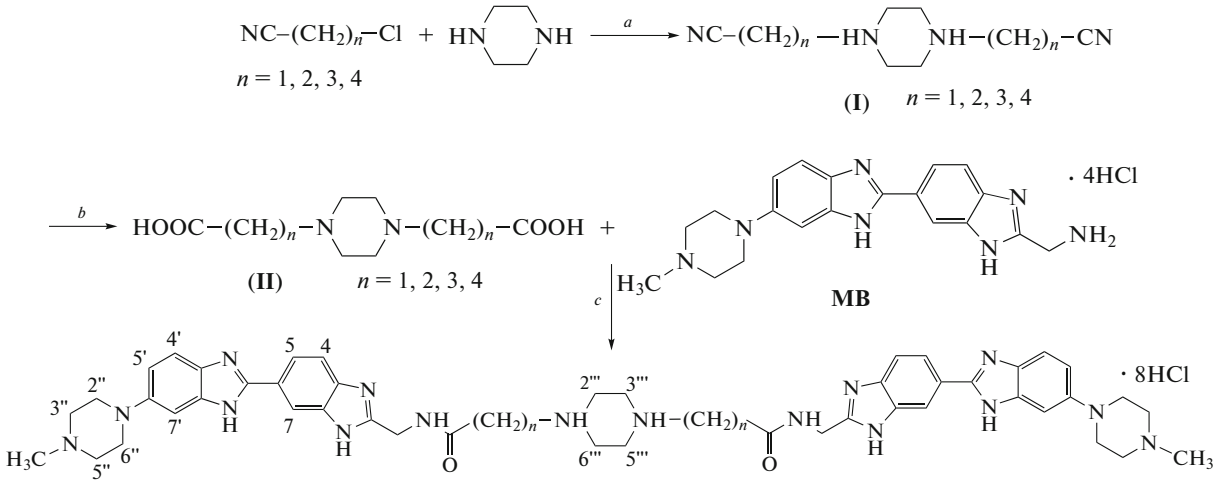
binding is also stabilized by electrostatic and intensive Van-der-Waals interactions with walls of the DNA minor groove.

Previously, we have synthesized the **DB(*n*)** dimeric analogues of Ht and studied their biochemical properties (Fig. 1) in order to create the compounds that would be noncovalently and site-specifically bound to the DNA minor groove [1, 11].

The benzimidazole fragments of the **DB(*n*)** molecules are attached "tail-to-tail' to oligomethylene linkers of different length (n = 1–12; Fig. 1), which allows them to form the structure that is isogeometrical to the DNA minor groove. Thus, these compounds act as bidentate ligands that are able to recognize nucleotide sequences with blocks from two AT-pairs which are located at a different distance from each other. **DB(*n*)** in low micromolecular concentrations prove to be inhibitors of a number of DNA-dependent enzymes: the eukaryotic DNA-topoisomerase I [12], the mouse DNA-methyltransferase Dnmt 3a [13], and helicase activity of the NS3 protein of the human hepatitis C virus [14]. The studies of **DB(*n*)** demonstrate that these compounds are novel promising fluorescent dyes that can penetrate through a cellular and nuclear membranes with an effective staining of the cell nuclei and differential staining of chromosomes [15]. However, these compounds are poorly soluble in water due to the aggregate formation.

The goal of our investigation is a creation and studies of the DNA interaction with new series of the **DBP(*n*)** dimeric bisbenzimidasoles (n = 1–4) (see Fig. 1) which do not have this disadvantage. The residue of 1,4-piperazine has been introduced into the structure of the oligomethylene linker of the **DB(*n*)** molecules in order to increase their solubility in aqueous solutions and affinity of the DNA complexes. Therefore, the novel compounds exist as tetracations at neutral pH values distinct from the dicationic structure of compounds of the **DB(*n*)** series, suggesting both the higher affinity to the double-stranded DNA due to the stronger electrostatic interactions and the better solubility in water.

A chemical synthesis of the **DBP(*n*)** dimeric bisbenzimidazoles was presented in the Scheme 1. 4-Piperazinedialkylcarbonic acids (**II**) were prepared by alkylation of 1,4-piperazine by nitriles of the corresponding ω-chloroalkylcarbonic acids with the formation of dinitriles of 1,4-piperazinedialkylcarbonic acids (**I**) and the subsequent hydrolysis with hydrochloric acid. The **DBP(*n*)** dimeric benzimidazoles were prepared by a condensation of the monomeric bisbenzimidazole (**MB**), which we synthesized previously [1], with 1,4-piperazinedialkylcarbonic acids (**II**) with the use of the BOP coupling reagent.



DBP(1) n = 1; **DBP(2)** n = 2; **DBP(3)** n = 3; **DBP(4)** n = 4

Scheme 1. Synthesis of **DBP(1,2,3,4)**. Reagents and conditions: **(***a***)**, DIPEA, 82°C, 40–45%; (*b*) concentrated HCl, 100°C, 2 h, 65–88%; (*c*) BOP, DIPEA, DMF, 0°C—room temperature 12 h, the yields 76–82%.

As in the case of the previous series of the **DB(*n*)** dimeric bisbenzimidazoles [1, 16], the formation of the complexes of these ligands with the double-stranded DNA and their characteristics were studied by physicochemical methods. The hypochromic effect and bathochromic shift in the absorption spectra of **DBP(*n*)** with an increase in the concentration of the double-stranded DNA pointed to the formation of the complexes (Fig. 2).

The fluorescent spectra also confirmed the interaction between the ligands and the double-stranded DNA, because they demonstrated the multifold

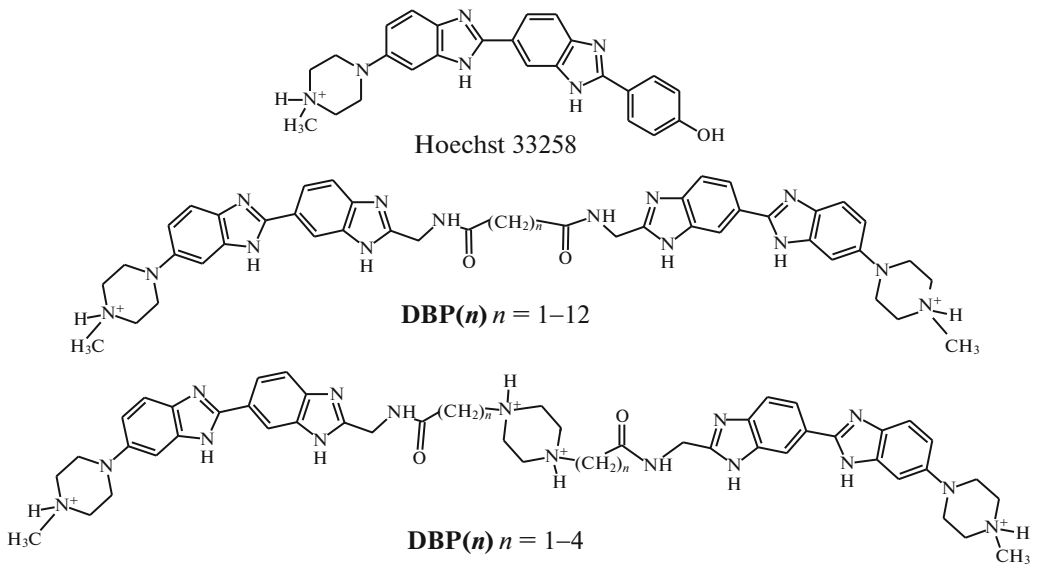


Fig. 1. Hoechst 333258 and the **DB(*n*)** and **DBP(*n*)** dimeric bisbenzimidazoles.

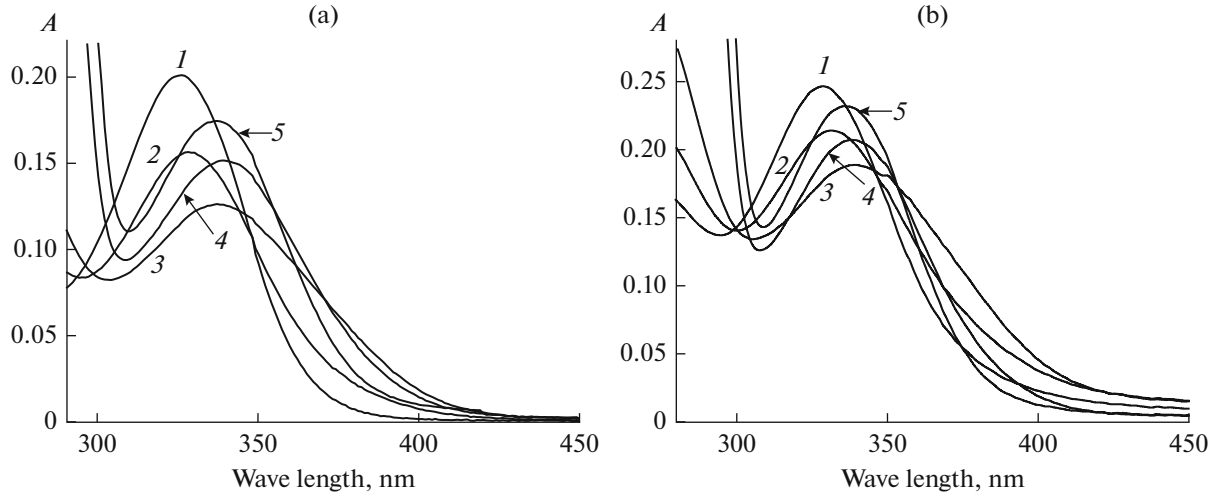


Fig. 2. The absorption spectra of (a) **DBP(2)** and (b) **DBP(4)** (curve *1*) in the absence and (curves *2*–*5*) in the presence of the double-stranded DNA: (a) [**DBP(2)**] = 5.42 μM; [DNA], 10^{-5} M of bp: 0.4.2 (*2*), 2.08 (*3*), 15.73 (*4*), 38.02 (*5*), (b) [**DBP(4)**] = 5.47 μM; [DNA], 10^{-5} M of bp: 0.42 (*2*), 2.08 (*3*), 19.91 (*4*), 38.02 (*5*). The buffer was 1 mM sodium cacodylate (pH 6.8). Hereinafter, the DNA molar concentration is given in M of bp (mol of bp/L).

increase in the **DBP(*n*)** fluorescence in the presence of the double-stranded DNA (Fig. 3).

The location of the synthesized compounds in the DNA groove was shown by the CD method with the use of the cholesteric liquid-crystalline dispersions (CD CLCDs) of the double-stranded DNA. The positive character of the new band in the CD CLCD spectrum of the ligands in the presence of DNA gave evidence of the **DBP(*n*)** binding in one of the grooves of the DNA double helix [1, 16] (Fig. 4).

An appearance of the intensive band (λ_{max} = 270 nm, $\Delta\varepsilon$ = 130–140 M^{-1} cm^{-1}) in the CD spectrum indicated of the formation of CLCDs of the DNA molecules.

Ht is bound to the double-stranded DNA in its minor groove according to the X-ray data [3, 17]. We believe that the **DBP(*n*)** compounds are also placed in the minor groove and form a complex with the double-stranded DNA, because they are the Ht dimers. The inhibitory activity of **DBP(1–4)** towards the eukaryotic DNA of topoisomerase I and the prokaryotic DNA-methyltransferase M.Sssl [18] in micromolar concentrations has been demonstrated in the biochemical experiments in vitro. Cytotoxicity of all the synthesized compounds was investigated in the MTT

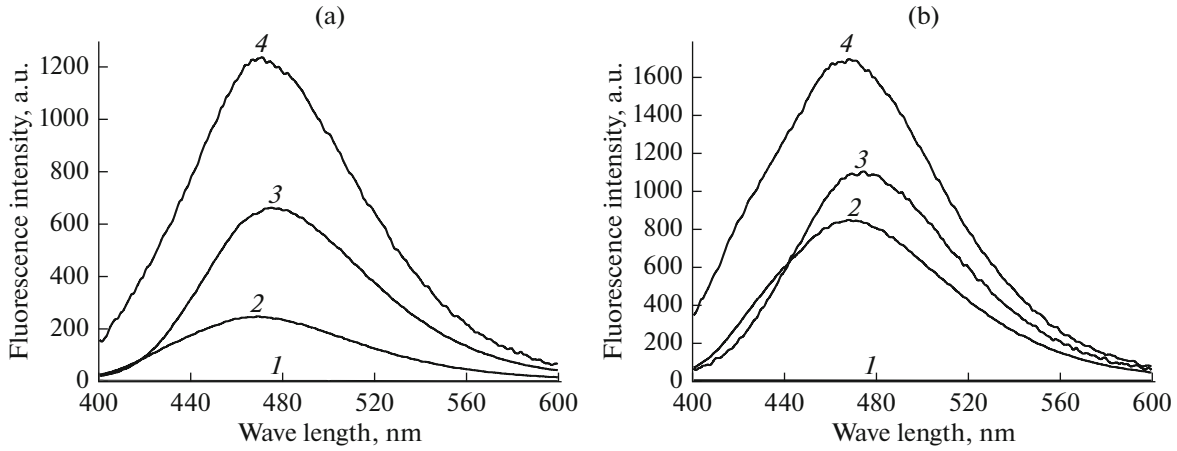


Fig. 3. The fluorescent spectra of (*1*) the buffer, (a) **DBP(2)**, and (b) **DBP(4)** (*2*) in the absence and (*3*, *4*) in the presence of the double-stranded DNA: [**DBP(2)**] = 5.42 × 10^{-6} M; [**DBP(4)**] = 5.47 × 10^{-6} M; [DNA], 10^{-6} M of bp: 20.8 (*3*); 62.02 (*4*). The buffer was 1 mM sodium cacodylate (pH 6,8). The excitation wavelength was 320 nm and the slit width was 5 nm.

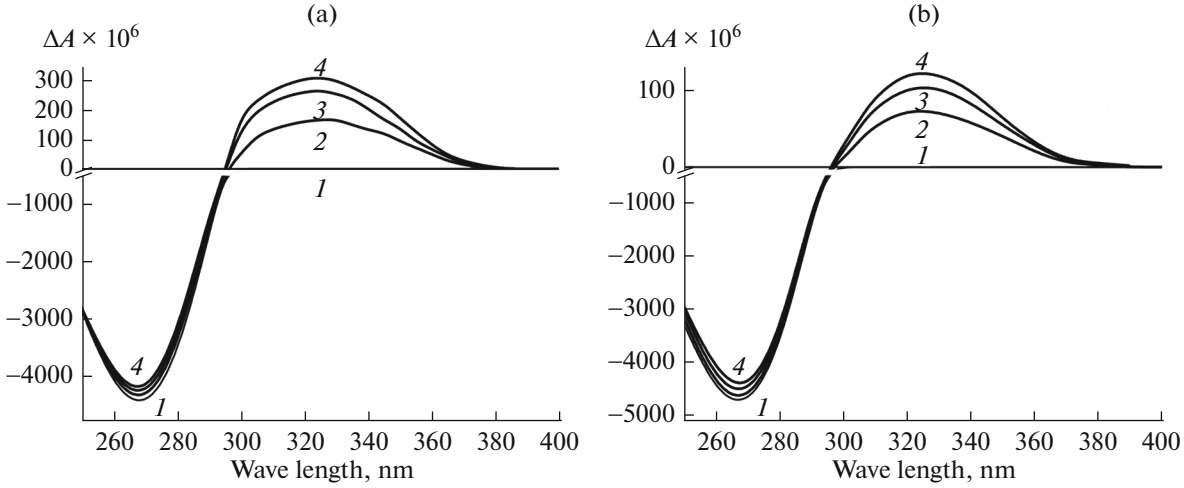


Fig. 4. The CD spectra of the cholesteric liquid-crystalline suspension of DNA from the calf thymus (curve *1*) in the absence and (curves *2*–*4*) in the presence of (a) **DBP(2)** and (b) **DBP(4)**. [**DBP(*n*)**], 10^{-6} M: 0 (*1*), 1.1 (*2*), 2.72 (*3*), 5.42 (*4*); [DNA] = 1.5 × 10^{-5} M of bp; the concentration of PEG-4000 was 170 mg/mL; 0.3 M NaCl + 2 mM sodium phosphate buffer, pH 6.85. The length of the optical path was 10 mm.

test towards the MCF-7 cellular lines of the breast cancer and the NKE-hTERT normal fibroblasts of the epithelium of the human kidney. The **DBP(1–4)** cytotoxicity was low (several dozens of micromoles) in all the cases [18].

EXPERIMENTAL

4-Aminobezonitrile, 4-methylmorpholin, isobutyl choroformate, DIPEA (*N, N*-diisopropylethylamine), BOP (benzotriazolyl-1-oxy)tris(dimethylamino)-phosphonium hexafluorophosphate), chloroacetonitrile, acrylonitrile, 5-chlorovaleronitrile (Alfa Aesar, United States), 1-methylpiperazine (Merck, Germany), 5-chlor-2-nitroaniline, piperazine, 4-chlorobutironitrile (Acros Organics, Belgium), sodium cacodylate, DMSO (Sigma, United States), PEG 4000 (Fluka, Switzerland), Boc-glycine, dioxane, DMF, AcOH, AcOEt, Ac_2O, EtOH, MeOH, Pr^iOH (Reakhim, Russia) were used in this study. Solutions of the substances in organic solvents were dried over Na_2SO_4. The solvents were evaporated on a rotary evaporator in a vacuum of a water-jet pump, usually, at 40–50°C. The substances were dried in vacuum over P_2O_5/NaOH. The boiling points were determined on a Boethius device (Germany) and were not corrected. A hydrogenation was performed using 10% Pd/C (Merck, Germany) at atmospheric pressure and room temperature to the end of the hydrogen absorption.

The homogeneity of the compounds were controlled by TLC on Kieselgel 60 F_{254} plates (Merck, Germany) in the mixture of Pr^iOH and concentrated NH_4OH (3 : 1). The substances were detected on the plates in UV-light at 254 nm and/or according to their fluorescence at 365 nm.

The ^{1}H NMR spectra were recorded on a AMX-400 spectrometer (Bruker, Germany, 400.0 MHz) in DMSO-d_6 (δ, ppm; *J*, Hz) at 32°C (unless otherwise indicated). A resonance from the residual protons of the solvent was used as an internal standard. The numeration of the hydrogen atoms of piperazine (Pip) and benzimidazole (Bim) cycles was given in the Scheme 1 of the **DBP(*n*)** synthesis. The resonances in the ^{1}H NMR spectra of **DBP(*n*)** were attributed on the basis of the published data [19].

The mass spectra were recorded by the MALDI-TOF method on a 4800 Plus mass spectrometer (AB Sciex, United States;) in the regime of a registration of positive ions with the use of a reflectrone (unless otherwise indicated). 2,5-Dihydroxybenzoic acid was used as a matrix. The Nd:YAG laser (355 nm) was applied.

The IR spectra were recorded on a 3100 FT-IR Excalibur Series spectrometer (Varian, United States).

*Spectral Studies of **DBP(n)***

The absorption spectra, the fluorescent spectra of solutions, and the CD spectra were recorded on a Cary100 spectrophotometer (Varian, United States), a Cary Exlipse spectrofluorimeter (Varian, United States), and an SKD-2 portable dichrometer (the Institute of Spectroscopy of the Russian Academy of Sciences, Troitsk), respectively. All the spectra were recorded in quartz cuvettes with the optical path length of 1 cm at 22°C.

The preparation of the calf thymus DNA (Sigma, United States) was dissolved in a water-salt solution of 0.3 M NaCl + 2 mM Na_3PO_4 (buffer A, pH 6.85) and depolymerized on an UZDN-2T ultrasound disperser. The DNA molecular mass after the ultrasound depolymerization was determined by an electrophoresis in the 1% agarose gel and proved to be (0.5–0.8) × 10^6 Da. The DNA concentration in solutions was determined spectrophotometrically using the molar extinction coefficient of ε_{260} = 13200 M^{-1} cm^{-1} (relative to one base pair).

The initial solutions of **DBP(2)** and **DBP(4)** with a concentration of ~1 mM were prepared by an addition of the ligand sample to DMSO. The DNA complexes with **DBP(2)** and **DBP(4)** were formed by the addition of the concentrated DNA solution (~4 mM of bp) to the ligand solution in 1 mM sodium cacodylate. The solutions were carefully stirred at room temperature and the absorption or fluorescent spectra were recorded.

The cholesteric liquid-crystalline dispersions of DNA (CD CLCDs) were prepared by mixing of equal volumes of the water-salt (0.3 M NaCl, pH 6.8) solutions of DNA and PEG (4000 Da, Fluka, Germany, 340 mg/mL). The CD spectrum was recorded one hour after the mixing. We observed an appearance of the intensive band (λ_{max} 270 nm, Δε 130–140 M^{-1} cm^{-1}) that suggested the formation of CLCDs of the DNA molecules. The ligand-DNA complexes in CLCDs were formed by the addition of the initial solution of **DBP(2)** or **DBP(4)** in portions from 2 to 5 μL to CLCD (2 mL) with intensive stirring. Then, the CD spectrum was recorded again in the area of absorption of DNA and the ligand (the interval of the wavelengths from 250 to 380 nm).

*The Synthesis of the **DBP(1–4)** Dimeric Bisbenzimidazoles*

The general method for the synthesis of 1,4-piperasinedialkylnitriles. The corresponding ω-chloroalkylnitrile (0.12 mol) was added dropwise within 40 min to the suspension of piperazine (5.00 g, 0.058 mmol) and DIPEA (0.1 mL, 0.57 mol) in anhydrous acetonitrile (30 mL) on stirring and ice-cooling. The reaction mixture was heated to 110°C for 2 h, cooled, and maintained at room temperature for 12 h. The solvent was evaporated, and the dry residue was dissolved in chloroform (40 mL) and water (30 mL). The organic layer was separated, and the aqueous layer was extracted with two portions of chloroform (10 mL each). The organic solvent was evaporated, and the dry residue was recrystallized from ethyl acetate (35 mL).

2,2'-(Piperazine-1,4-diyl)diacetonitrile: the yield 45.5%; mp 170–171°C; ^{1}H NMR: 2.56 (8 H, m, Pip), 3.67 (4 H, s, CH_2–CN).

3,3'-(Piperazine-1,4-diyl)dipropanonitrile: the yield 45.4%; mp 173–175°C; ^{1}H NMR: 2.50 (8 H, m, Pip), 2.55 (4 H, t, *J* 6.6, C**H**$_2$–CN), 2.65 (4 H, t, *J* 6.6, N–C**H**$_2$).

4,4'-(Piperazine-1,4-diyl)dibutanonitrile: the yield 45.7%; mp 101–102°C; ^{1}H NMR: 1.70 (4H, q, *J* 6.8, N-CH_2-C**H**$_2$), 2.33 (4H, t, *J* 7.0, C**H**$_2$–CN), 2.36 (8H, broadened s, Pip), 2.46 (4H, t, *J* 7.0, N–C**H**$_2$).

5,5'-(Piperazine-1,4-diyl)dipentanonitrile: the yield 36.8%; mp 183–185°C; ^{1}H NMR: 1.64 (4H, q, *J* 7.6, C**H**$_2$–CH_2–CN), 1.80 (4H, q, *J* 7.6, N–CH_2–C**H**$_2$), 2.56 (4H, t, *J* 7.6, C**H**$_2$–CN), 3.13 (4H, broadened s, N–C**H**$_2$), 3.33–3.80 (8H, m, Pip).

General method for the synthesis of 1,4-piperazinedialkylcarbonic acids. 1,4-Piperazinedialkylnitriles (5 mmol) were boiled in concentrated HCl (5 mL) for 2 h and remained to stay for a night at 4°C. The precipitate was filtered, washed with cool water, and dried in vacuum over P_2O_5/NaOH.

Dihydrochloride of 1,4-piperazinediacetic acid: the yield 65.3%; mp 250–252°C; ^{1}H NMR: 2.51 (8H, broadened s, Pip), 3.35 (4H, s, CH_2).

Dihydrochloride of 1,4-piperazinedipropanic acid: the yield 84.5%; mp 266–268°C; ^{1}H NMR (90°C): 2.79 (4H, t, *J* 7.4, C**H**$_2$–COOH), 3.25 (4H, t, *J* 7.4, N–C**H**$_2$), 3.40 (8H, broadened s, Pip).

Dihydrochloride of 1,4-piperazinedibutanic acid: the yield 78.5%; mp 234–235°C; ^{1}H NMR (90°C): 1.94 (4H, q, *J* 7.5, CO–CH$_2$–C**H**$_2$), 2.36 (4H, t, *J* 7.4, CO–C**H**$_2$), 3.05 (4H, t, *J* 7.4, N–C**H**$_2$), 3.44 (8H, broadened s, Pip).

Dihydrochloride of 1,4-piperazinedivaleric acid: the yield 88.3%; mp 249–251°C; ^{1}H NMR (90°C): 1.59 (4H, q, *J* 7.4, C**H**$_2$–CH$_2$–COOH), 1.74 (4H, q, *J* 7.5, N–CH$_2$–C**H**$_2$), 2.27 (4H, t, *J* 6.8, C**H**$_2$–COOH), 3.02 (4H, t, *J* 7.6, N–C**H**$_2$), 3.43 (8H, broadened s, Pip).

The general method for the synthesis of the DBP(1–4) dimeric bisbenzimidazoles. DIPEA (1.26 mL, 7.2 mmol) was added to the suspension of the monomeric bisbenzimidazole (**MB**, 405 mg, 0.8 mmol) [1] and the corresponding 1,4-piperazinedialkylcarbonic acid (0.4 mmol) in an anhydrous DMF (10 mL) with cooling on an ice-bath and stirring. The reaction mixture was stirred with cooling for 20 min, and BOP (400 mg, 0.9 mmol) was added. The reaction mixture was stirred for 15 min at 0°C and allowed to stay at room temperature for a night. The solvent was evaporated, and the oily dark precipitate was triturated with chloroform (10 mL) to the formation of beige crystals. The crystals were filtered, washed with chloroform (1 × 5 mL) and ethyl acetate (2 × 5 mL), and dried in air. After 30 min, the precipitate was solved in ethanol (10 mL), concentrated HCl (1 mL) was added, and the solution was evaporated. The solid residue was triturated with an anhydrous ethanol and filtered. The formed greenish yellow powder was dried in vacuum over P_2O_5/NaOH. The substances were homogenous according to TLC.

DBP(1) · 8 HCl: the yield 360 mg (76.2%); mp 294–295°C; R_f 0.70; λ_{max} 324 nm (ε_{324} 43500 M^{-1} cm^{-1}); ^{1}H NMR: 2.84 (6H, d, *J* 2.6, N–C**H**$_3$), 3.23 (8H, d, *J* 8.2, H(3", 5")), 3.45 (8H, broadened s, H(2"', 3"', 5"', 6"')), 3.54 (4H, d, *J* 8.2, H (2", 6")), 3.88 (4H, d, *J* 8.2, H (2", 6")), 3.97 (4 H, s, CO–C**H**$_2$). 4.81 (4H, d, *J* 5.1, C**H**$_2$–NH), 7.21 (2H, d, *J* 1.9, H7'), 7.35 (2H, dd, J_1 8.9, J_2 1.9, H5'), 7.72 (2H, d, *J* 8.9, H4'), 7.96 (2H, d, *J* 8.3, H4), 8.38 (2H, d, *J* 8.3, H5), 8.79 (2H, broadened s, H7), 9.43 (2H, broadened s, CH$_2$–N**H**), 11.21 (1H, broadened s, N**H**(Bim)); ^{13}C NMR (100 MHz, DMSO-d_6, 32°C, δ): 170.00; 155.08; 148,62; 133.22; 122.77; 117.07; 115.06; 114.38; 99.01; 57.04; 52.17; 49.89; 46.27; 41.95; 36.52; Mass spectrum, *m/z*: 889.57 $[M]^+$, calculated for $C_{48}H_{56}N_{16}O_2$ 889.06; IR (Varian 3100 FT-IR Excalibur Series), ν_{max} (KBr), cm^{-1}: 845, 963, 1149, 1210, 1253, 1402, 1462, 1639, 2931, 2961.

DBP(2) · 8 HCl: the yield 200 mg (41.4%); mp 287–288°C; R_f 0.68; λ_{max} 324 nm (ε_{324} 46000 M^{-1} cm^{-1}); ^{1}H NMR: 2.84 (6H, s, N–C**H**$_3$), 2.94 (4H, t, *J* 7.5, CO–C**H**$_2$), 3.23 (8H, d, *J* 8.2, H (3", 5")), 3.49 (4H, t, *J* 7.5, CH$_2$–N), 3.54 (4H, d, *J* 8.2, H(2", 6")), 3.65 (8H, broadened s, H(2"', 3"', 5"', 6"')), 3.86 (4H, d, *J* 8.2, H(2", 6")), 4.76 (4H, d, *J* 5.1, C**H**$_2$–NH), 7.20 (2H, d, *J* 1.9, H7'), 7.34 (2H, dd, J_1 8.9, J_2 1.9, H5'), 7.71 (2H, d, *J* 8.9, H4'), 7.97 (2H, d, *J* 8.3, H4), 8.39 (2H, d, *J* 8.3, H5), 8.79 (2H, s, H7), 9.18 (2H, t, *J* 5.1, CH$_2$–N**H**), 11.20 (2H, broadened s, N**H**(Bim)); ^{13}C NMR (100 MHz, DMSO-d_6, 32°C): 169.88; 155.37; 148.67; 147.96; 136.87; 134.86; 133.20; 126.27; 123.49; 118.81; 117.17; 115.30; 114.42; 98.93; 52.12; 51.07; 47.66; 46.21; 41.92; 36.49; 29.44; Mass spectrum *m/z*: 917.58 $[M]^+$, calculated for $C_{50}H_{60}N_{16}O_2$ 917.11; IR, ν_{max} (KBr), cm^{-1}: 846, 962, 1149, 1211, 1253, 1400, 1459, 1638, 2932, 2961.

DBP(3) · 8 HCl: the yield 410 mg (82.8%); mp 274–275°C; R_f 0.69; λ_{max} 324 nm (ε_{324} 50400 M^{-1} cm^{-1}); ^{1}H NMR (120°C): 2.02 (4H, q, *J* 7.6, CO–CH$_2$–C**H**$_2$), 2.43 (4H, t, *J* 7, CO–C**H**$_2$), 2.87 (6H, s, N–C**H**$_3$), 3.07 (4H, t, *J* 7.6, C**H**$_2$–N), 3.39 (16H, m, H(2", 3", 5", 6")), 3.59 (8H, broadened s, H(2"', 3"', 5"', 6"')), 4.66 (4H, broadened s, C**H**$_2$–NH), 7.23 (2H, dd, J_1 8.9, J_2 1.9, H5'), 7.25 (2H, s, H7'), 7.68 (2H, d, *J* 8.9, H4'), 7.81 (2H, d, *J* 8.9, H4), 8.22 (2H, d, *J* 8.2, H5), 8.40 (2H, broadened s, CH$_2$–N**H**); 8.64 (2H, s, H7); Mass spectrum, *m/z*: 945.43 $[M]^+$, calculated for $C_{52}H_{64}N_{16}O_2$ 945.17; IR, ν_{max} (KBr), cm^{-1}: 846, 962, 1149, 1211, 1253, 1400, 1459, 1638, 2932, 2961.

DBP(4) · 8 HCl: the yield 205 mg (81.0%); mp 271–272°C; R_f 0.72; λ_{max} 324 nm (ε_{324} 54800 M^{-1} cm^{-1}; ^{1}H NMR (90°C): 1.67 (4H, q, *J* 7, CO–CH$_2$–C**H**$_2$), 1.78 (4H, q, *J* 7.5, C**H**$_2$–CH$_2$–N), 2.33 (4H, t, *J* 7, CO–C**H**$_2$), 2.84 (6H, s, N–C**H**$_3$), 3.12 (4H, t, *J* 7.5, C**H**$_2$–N), 3.39 (8H, broadened s, H (2"', 3"', 5"', 6"')), 3.54 (16H, s, H (2", 3", 5", 6")), 4.66 (4H, d, *J* 5, C**H**$_2$–NH), 7.24 (2H, d, *J* 2.1, H7'), 7.28 (2H, dd, J_1 9, J_2 2.1, H5'), 7.70 (2H, d, *J* 9, H4'), 7.86 (2H, d, *J* 8.3, H4), 8.27 (2H, dd, J_1 8.3, J_2 1.2, H5), 8.49 (2H, t, *J* 5.1, N**H**–CH$_2$), 8.69 (2H, d, *J* 1.2, H7); Mass spectrum, *m/z*: 973.64 $[M]^+$, calculated for $C_{54}H_{68}N_{16}O_2$ 973.22; IR, ν_{max} (KBr), cm^{-1}: 846, 964, 1155, 1213, 1258, 1490, 1460, 1638, 2932, 2962.

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