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Spectroscopic binding studies of novel fluorescent distamycin derivatives

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Abstract—Novel distamycin–porphyrin conjugates were synthesized and their interaction with calf thymus DNA was studied. Minor groove binding of the distamycin part of the molecule was confirmed. The porphyrin part of the conjugates exhibited intercalation and the non-specific electrostatic interaction with the phosphate groups of DNA.

The inhibition of DNA replication in rapidly growing cancer cells or viruses is often based on the interaction of small molecules with DNA. There are four possibilities for small molecules to interact reversibly with the B-form of DNA: (1) intercalation between base pairs, (2) electrostatic interaction with negatively charged phosphate groups, (3) minor groove binding and (4) major groove binding. Each type of interaction causes specific changes of the spectral properties of DNA and/or the interacting molecule. Whichever change occurs, the type of interaction can be confirmed.

Distamycin A containing three *N*-methylpyrrole units connected by peptide bonds is a naturally occurring antibiotic inhibiting transcription. It is a representative of minor groove binders[1] which are known to be specifically bonded to A–T rich sequences of DNA.[2] The preference for A–T rich sequences is explained by the steric hindrance to binding caused by NH_2 groups of guanine.[3] A positive electronic circular dichroism (ECD) signal in the distamycin range of absorption was induced if distamycin-containing ligand was bonded to the minor groove of DNA.[4] ECD is useful for confirming the specific interaction of ligands with proteins and polynucleotides. The secondary structure of the B-form of DNA reflected in the positive band at 275 nm and the negative band with slightly lower intensity at 245 nm is very sensitive to the interaction with ligands.[5] The toxicity of parent distamycin limits its use in medicine, therefore, new distamycin analogues are synthesized and investigated herein.

Our three newly synthesized derivatives contained a fluorescent label (simple fluorescein for comparison or porphyrin) to increase the methods for studying the interaction by fluorescence. Porphyrin shows a characteristic Soret absorption band at 450 nm. Intercalation of porphyrin with DNA is known to be connected with a large hypochromicity and a negative induced ECD signal in this region. The minor groove binding of porphyrin is accompanied by a small bathochromic shift of the Soret band, and a small hypo- or even hyperchromicity and a positive induced ECD band in the Soret region.[6] If the external binding to the phosphate backbone is accompanied by self-stacking of porphyrins, an exciton conservative ECD signal can be observed.[7] To study the influence of electrostatic binding, DNA can be substituted by simple nucleotides as model compounds. Unfortunately, all three distamycin derivatives synthesized possess low solubility in water which is sufficient for UV/vis, fluorescence and ECD measurements, but insufficient to obtain ^{1}H NMR spectra. The broadening of ^{1}H NMR signals for shorter more soluble distamycin analogues[8] was observed when DNA was added. To confirm the interaction by NMR, oligonucleotides were used as model compounds for DNA. Changes in their ^{1}H NMR spectra caused by additions of small amounts of distamycin derivatives were observed.

Simultaneous use of all these spectroscopic methods enabled us to identify the types of interactions between distamycin derivatives and DNA.

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Derivative **I** (Fig. 1) containing distamycin bonded to fluorescein was prepared[9] from nitrodistamycin.[10]

The synthetic protocol for the preparation of porphyrin–distamycin conjugates **II** and **III** (Fig. 2) was based on the quaternization reaction of the dimethylaminoalkyl group of the distamycin derivative with the corresponding tetrakis-(bromomethylphenyl)-porphyrin derivatives.[11]

UV/vis[12] and fluorimetry[13] were used to observe the changes in the spectra of individual derivatives caused by the addition of DNA.[14] During ECD measurements,[15] the induced ECD signal of the derivative and changes in the DNA structures were observed when the individual derivatives were added to a constant concentration of DNA.

Additions of DNA to ligand **I** caused small (<2%) hypsochromic changes of the fluorescein absorption band. Changes in the distamycin absorption band were more intense but hard to quantify because of overlapping with the absorption bands of DNA. The fact that the changes in the fluorescein band were so small was due to the long distance of the fluorescence label from the site of complexation. If the parent fluorescein salt was titrated with DNA, the absorption band of fluorescein did not change at all. This confirmed that the observed small changes of the fluorescein band were significant and caused by interaction of the distamycin part of the ligand with DNA. The addition of simple nucleotides to derivative **I** caused no significant changes in the UV/vis spectra.

Similarly, very small changes in the fluorescence spectrum were observed. The reason is the same as for the absorption spectra. If derivative **I** was added to DNA, the induction of a positive ECD band in the distamycin absorbance region at 340 nm and changes of the secondary structure of DNA were observed (Fig. 3). This corresponds to specific minor groove binding of the distamycin part of the ligand.[4]

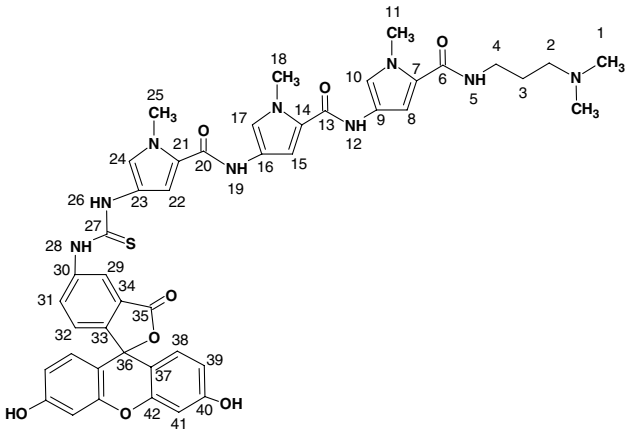

Figure 1. Fluorescein–distamycin conjugate **I**.

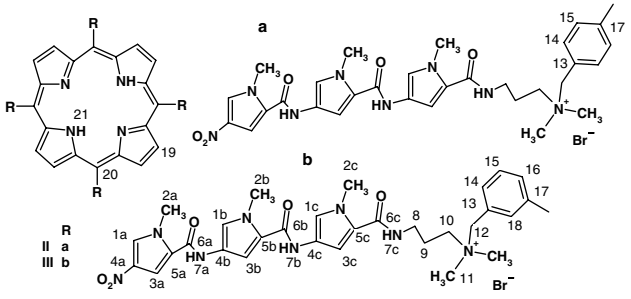

Figure 2. Porphyrin–distamycin conjugates **II** and **III**.

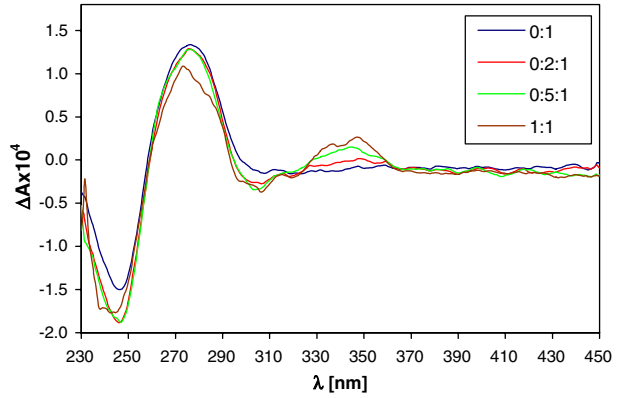

Figure 3. ECD titration of DNA by derivative **I** at different molar ratios $n(\mathbf{I})/n_{bp}$ (bp means base pairs of DNA).

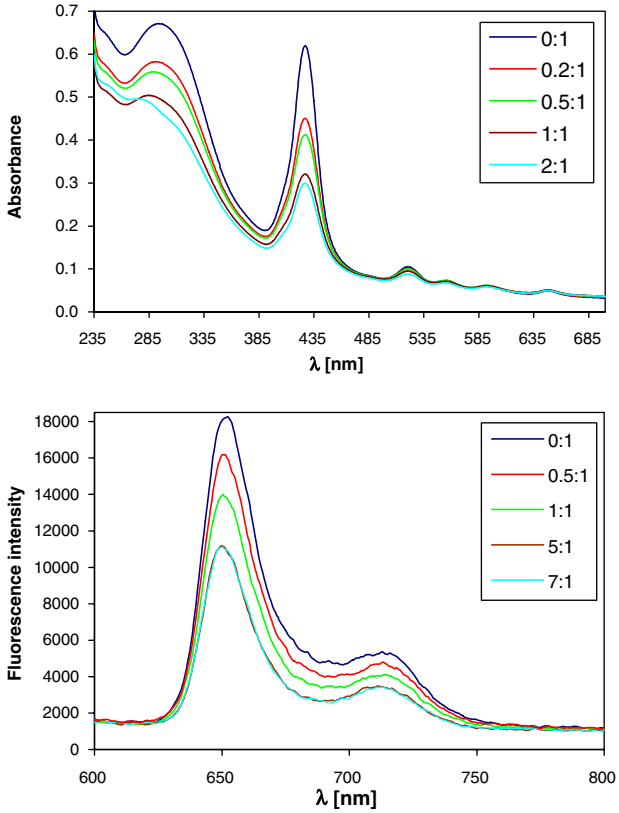

Figure 4. UV/vis (up) and fluorescence (down) spectra of the titrations of derivative **II** with DNA at different molar ratios $n_{bp}/n(\mathbf{II})$ (bp—base pairs of DNA).

Significant hypsochromic effects were observed both in the UV/vis and fluorescence spectra (Fig. 4) in the porphyrin region of derivative **II**.

A comparison with previous fluorescein–distamycin conjugate allowed the changes in the spectra corresponding to the distamycin and porphyrin parts of these derivatives to be distinguished. Thus, the observed intense changes in the UV/vis and fluorescence spectra could be caused by the presence of porphyrin which can intercalate into the DNA structure. The intercalation should be accompanied by a significant red shift of the absorption peak, but this effect was not observed. An electrostatic interaction between the phosphate backbone of DNA and the porphyrin part of the derivative is also possible because the porphyrin is positively charged due to the presence of four tetraalkylated nitrogens. If nucleotides[16] AMP, ADP, ATP and GMP as model compounds for studying the electrostatic interaction only were added to this derivative, qualitatively similar changes in UV/vis and fluorescence spectra were observed as if DNA had been added. This means that the electrostatic binding through phosphate bonds due to the presence of a positively charged porphyrin moiety (not present in derivative **I**) plays an important role and its influence cannot be distinguished from that of intercalation in the UV/vis and fluorescence spectra. A negative induced ECD signal at 450 nm for the porphyrin part of the molecule and a positive induced ECD signal at 330 nm for the distamycin part of the molecule (Fig. 5) comply with the assumption that the porphyrin part intercalates while the distamycin part binds to the minor groove. The structure of the parent DNA (negative and positive signals between 230 and 300 nm) is significantly influenced by additions of derivative **II**.

The spectral changes caused by derivative **III** were hard to distinguish from those of **II** in the UV/vis and fluorescence spectra. Also, changes in the UV/vis and fluorescence spectra caused by additions of simple nucleotides were very similar. The changes in ECD spectra were in general less pronounced for **III**, especially those corresponding to the distamycin signal. The ^{1}H NMR titrations[17] of oligonucleotides[18] with derivative **III** were performed. Broadening of the signals in the aromatic region of oligonucleotide $(dA–dT)_7$ was observed

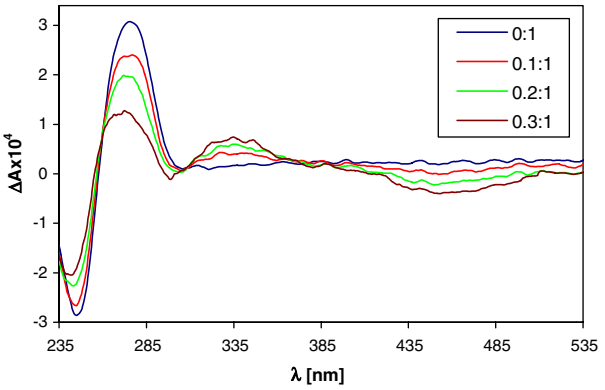

Figure 5. ECD titration of DNA with derivative **II** at different molar ratios n(**II**)/n_{bp} (bp—base pairs of DNA).

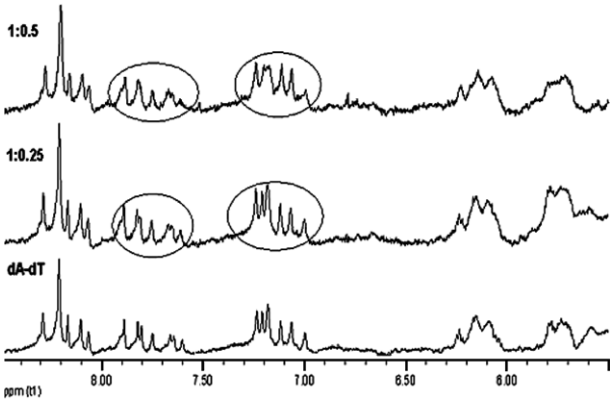

Figure 6. ^{1}H NMR titrations of oligonucleotide $(dA–dT)_7$ with **III** at different molar ratios n(dA–dT)/n(**III**).

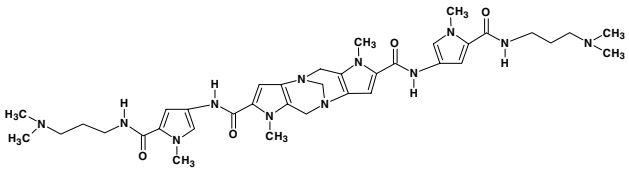

Figure 7. Bisdistamycin Tröger's base conjugate.

(Fig. 6). On the other hand, no changes in the ^{1}H NMR spectrum occurred for $(dG–dC)_7$. This corresponds with the preference for distamycin bonding to the A–T rich parts of DNA.

The same NMR experiments with oligonucleotides were repeated for comparison with the bisdistamycin Tröger's base conjugate (Fig. 7). For this derivative, the interaction with DNA through minor groove binding has already been described.[8] The same A–T preference was observed for the Tröger's base conjugate as for the porphyrin derivative **III**.

In conclusion, we have shown that distamycin–porphyrin conjugates interact with DNA. The distamycin part of the derivatives was found to bind to the minor groove of DNA with preference for A–T rich sequences. The porphyrin parts were found to exhibit two types of interactions, intercalation and non-specific electrostatic binding through DNA phosphate groups.

Acknowledgements

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References and notes

1. Atwood, J. L.; Gokel, G. W. In *Comprehensive Supramolecular Chemistry*; Pergamon: Oxford, 1996; Vol. 4, p 81–86.

2. For articles on distamycin–DNA binding, see: (a) Caesar, C.; Johnsson, R.; Ellervik, U.; Fox, K.; Lincoln, P.; Nordén, B. *Biophys. J.* **2006**, *91*, 904–911; (b) Mrksich, M.; Wade, W. S.; Dwyer, T. J.; Geierstanger, B. H.; Wemmer, D. E.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7586–7590; (c) Baliga, R.; Crothers, D. M. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 7814–7818; (d) Pang, Y.-P. *Bioorg. Med. Chem.* **2004**, *12*, 3063–3068; (e) Wilson, W. D.; Ratmeyer, L.; Zhao, M.; Strekowski, L.; Boykin, D. *Biochemistry* **1993**, *32*, 4098–4104.
3. Pelton, J. G.; Wemmer, D. E. *J. Am. Chem. Soc.* **1990**, *112*, 1393–1399.
4. Fasman, G. D. *Circular Dichroism and the Conformational Analysis of Biomolecules*; Plenum Press: New York, 1996.
5. Johnson, W. In Berova, N., Nakanishi, K., Eds.; Circular Dichroism: Principles and Applications; Wiley: NY, 2000; pp 703–718.
6. For articles on porphyrin–DNA binding, see: (a) Pasternak, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406; (b) Pasternak, R. F.; Antebi, A.; Ehrlich, B.; Sidney, D.; Gibbs, E. J.; Bassner, S. L.; Depoy, L. M. *J. Mol. Catal* **1984**, *23*, 235; (c) Rodriquez, M.; Bard, A. J. *Inorg. Chem.* **1992**, *31*, 1129–1135; (d) Nový, J.; Urbanová, M.; Volka, K. *J. Mol. Struct.* **2005**, *748*, 17–25.
7. Lee, S.; Jeon, S. H.; Kim, B. J.; Han, S. W.; Jang, H. G.; Kim, S. K. *Biophys. Chem.* **2001**, *92*, 35–45.
8. Valík, M.; Malina, J.; Palivec, L.; Foltýnová, J.; Tkadlecová, M.; Brabec, V.; Král, V. *Tetrahedron* **2006**, *62*, 8591–8600.
9. *Preparation of derivative* **I**: Nitrodistamycin (30 mg) was hydrogenated over Pd and then mixed with 25 mg of fluorescein-(5,6)-isothiocyanate (Fluka, 90%) in DMF and methanol solution (1:3). The reaction mixture was stirred at room temperature for 3 h. The precipitate formed was filtered, washed with water and dried. MS ES calc: 858, found $[M+H^+]$ 859. UV/vis (DMSO): fluorescein band at $\lambda_{max} = 495$ nm, $\varepsilon_{495} = 3.3 \times 10^4\,l\,mol^{-1}\,cm^{-1}$, distamycin band at 286 nm. Fluorescence band at $\lambda_{max} = 516$ nm. ^1H NMR (DMSO, 300 MHz, 298 K) δ: 2.17 (s, 6H, Nr. 1), 2.20 (t, $J = 7$ Hz, 2H, Nr. 2), 1.60 (quintet, $J = 7$ Hz, 2H, Nr. 3), 3.17 (q, $J = 7$ Hz, 2H, Nr. 4), 8.07 (t, $J = 5.7$ Hz, 1H, Nr. 5), 6.81 (s, 1H, Nr. 8), 7.17 (s, 1H, Nr. 10), 3.78 (s, 3H, Nr. 11), 7.02 (s, 1H, Nr. 15), 3.83 and 3.87 (s, 1H+1H, Nr. 18, 25), ^{13}C NMR δ: 45.0 (1), 56.9 (2), 27.0 (3), 37 (4), 161.2 (6), 123 (7), 104.0 (8), 122.8 (9), 117.7 (10), 35.9 (11), 36.4 and 36.1 (18, 25). Full assignment of the NMR signals of fluorescein part of molecule was not possible because of tautomeric forms present in solution and overlapping signals of the distamycin and fluorescein units. Numbering see Figure 1.
10. Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945–1952.
11. *Preparation of derivatives* **II** *and* **III**: Tetrakis(bromomethylphenyl)porphyrin (0.1 mmol) (*meta* and *para*) was dissolved in chloroform–acetonitrile mixture 10:1 (30 ml chloroform, 3 ml of acetonitrile) and 6 M equiv of aminodistamycin was added. The reaction mixture was refluxed for 24 h, and the precipitated product was filtered off, washed with dichloromethane and dried in vacuo. The yield was 76% for **II** and 89% for **III**. Both compounds are yellowish brown powders well soluble in DMSO. MS with FAB ionization: calcd 2974.89 g/mol ($C_{140}H_{154}Br_4N_{36}O_{20}$); found: 2976 ($MH^+$)—not observed for **II**, 2896 $(M-Br)^+$, 1409 $[(M-2Br)^{2+}/2]$, 913 $[(M-3Br)^{3+}/3]$, 665 $[(M-4\,Br)^{4+}/4]$. Elemental Anal. Calcd C, 56.42; H, 5.21; N, 16.92. Found: C, 56.19; H, 5.17; N, 16.73 for **II** and C, 56.21; H, 5.28; N, 16.79 for **III**. UV/vis (DMSO): four Q bands at 646 nm, 591 nm, 553 nm and 516 nm, the Soret band at 427 nm ($\varepsilon_{427} = 1.7 \times 10^5\,l\,mol^{-1}\,cm^{-1}$) and distamycin absorption band at 293 nm for **II**; the same Q bands, the Soret band at 425 nm ($\varepsilon_{425} = 3.3 \times 10^5\,l\,mol^{-1}\,cm^{-1}$) and distamycin absorption band at 286 nm for **III**. ^1H NMR (DMSO, 300 MHz, 298 K) for **II** (full assignment not possible, numbering see Fig. 2) δ: 8.20 (s, 4H, Nr. 1a), 6.93–7.28 (m, 4H+4H, Nr. 1b, 1c), 3.96 (s, 12H, Nr. 2a), 3.82–3.94 (s, 12H+12H, Nr. 2b, 2c), 7.59 (s, 4H, Nr. 3a), 6.93–7.28 (s, 4H+4H, Nr. 3b,3c), 10.28 (s, 4H, Nr. 7a), 9.95 (s, 4H, Nr. 7b), 8.18 (s, 4H, Nr. 7c), ^{13}C NMR for **II** (full assignment not possible, numbering see Fig. 2) δ: 37.5 (2a), 36.2 (2b), 36.0 (2c), 107.6 (3a), 104.6 (3b), 104.6 (3c), 133.7 (4a), 122.2 (4b), 121.4 (4c), 126.3 (5a), 123 (5b), 122.6 (5c), 156.9 (6a), 158.4 (5b), 161.6 (5c), 49.5 (11). ^1H NMR (DMSO, 300 MHz, 298 K) for **III** (numbering see Fig. 2) δ: 8.18 (s, 4H, Nr. 1a), 7.12 (s, 4H, Nr. 1b), 7.13 (s, 4H, Nr. 1c). 3.93 (s, 12H, Nr. 2a), 3.67 (s, 12H, Nr. 2b), 3.84 (s, 12H, Nr. 2c) 7.55 (s, 4H, Nr. 3a), 6.94 (s, 4H, Nr. 3b), 7.04 (s, 4H, Nr. 3c), 10.18 (s, 4H, Nr. 7a), 9.85 (s, 4H, Nr.7b), 8.29 (s, 4H, Nr. 7c), 3.42 (t, $J = 7$ Hz, 8H, Nr. 8), 2.26 (quintet, $J = 7$ Hz, 8H, Nr. 9), 3.62 (t, $J = 7$ Hz, 8H, Nr. 10), 3.31 (s, 24H, Nr. 11), 4.92 (s, 8H, Nr. 12), 8.03 (d, $J = 8.5$ Hz, 8H, Nr. 14), 8.35 (d, $J = 8.5$ Hz, 8H, Nr. 15), 8.91 (s, 8H, Nr. 19), −2.98 (s, 2H, Nr. 21). ^{13}C NMR for **III** (numbering see Fig. 2) δ: 127.8 (1a), 118.0 (1b), 118.4 (1c), 37.3 (2a), 35.9 (2b), 35.8 (2c), 107.5 (3a), 104.4 (3b), 104.5 (3c), 133.7 (4a), 122.1 (4b), 121.2 (4c), 126.1 (5a), 122.8 (5b), 122.6 (5c), 156.8 (6a), 158.2 (6b), 161.6 (6c), 35.7 (8), 23.3 (9), 62.0 (10), 49.5 (11), 68.0 (12), 118.0 (13), 131.4 (14), 134.6 (15), 142.9 (17), 127.8 (21), signal 19 not detected.
12. *UV/vis measurements*: spectrometer Varian Cary 400, spectral region 700–200 nm (600–200 nm for derivative **I**), 1 cm plastic cells (to decrease the adsorption of porphyrin derivatives on the walls of the cells), constant concentrations of derivatives (2×10^{-5} mol/l for **I** and 1×10^{-5} mol/l for **II** and **III**).
13. *Fluorescence measurements*: FluoroMax-2 (Instruments S.A., Inc, USA). 1 cm plastic cells to decrease the adsorption of porphyrin derivatives on the walls of the cells, excitation wavelength 428 nm, emission wavelength 600–800 nm, constant concentrations of derivatives (2×10^{-6} mol/l for **I** and 6×10^{-7} mol/l for **II** and **III**).
14. Calf thymus DNA from Sigma was dissolved in D_2O, lyophilized and then sequences around 800 base pairs were prepared by sonication. The amount of added DNA was expressed per base pairs having an average value of mass 600 g/mol.
15. *ECD measurements*: spectrophotometer Jasco J810, rate 100 nm/min, 2 accumulations, glass cells 1 cm, measured range 600–200 nm, constant concentration around 1×10^{-4} mol/l of calf thymus DNA.
16. Nucleotides (AMP, ADP, ATP, GMP) from Fluka or Sigma, purity over 99%.
17. *Binding studies with oligonucleotides*: Bruker NMR spectrometer Avance DRX (500 MHz), presaturation of water signal, 64 accumulations. A constant concentration of oligonucleotides (2.8×10^{-4} mol/l) was maintained during NMR titrations. Distamycin derivatives were added as high concentrated DMSO solutions because of their low solubility in water. The total amount of solution added was under 5%.
18. Oligonucleotides $(dA–dT)_7$ and $(dC–dG)_7$ (from Generi Biotech) were dissolved in cacodylate buffer (from Sigma) at pH = 7.4, concentration 0.02 mol/l and ionic strength 0.1 mol/l of NaCl in the mixture $H_2O–D_2O$ (9:1).