Studies on the Interactions of the New 2,6-Bis[2-(heteroaryl)vinyl]-1-methylpyridinium Cations with the Decamer d(CGTACGTACG)$_2$

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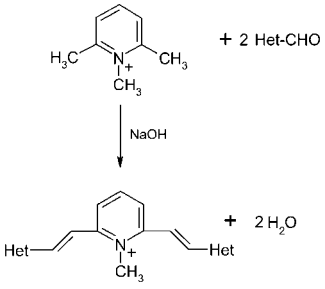
The synthesis and spectroscopic characterization of 2,6-bis[2-(heteroaryl)vinyl]-1-methylpyridinium iodides (heteroaryl = 1-methylpyrrol-2-yl, furan-2-yl, thiazol-2-yl, imidazol-2-yl, 5-bromofuran-2-yl) is reported. Interactions of furan and pyrrole derivatives with the decamer duplex d(CGTACGTACG)$_2$ were clearly detectable by UV/Vis, CD, and ^{1}H NMR spectroscopy, and attention is drawn to the complementarity of the above techniques in such studies. The NMR evidence is consistent with literature data ascribed to intercalation.

Introduction

Well-known DNA binding agents such as berenil, distamycin, and pentamidine contain functionalized aromatic and heteroaromatic moieties linked by carbon and/or heteroatom linkers. Replacement of the central linkers in pentamidine and berenil with furan rings resulted in stable drug−DNA complexes characterizable by crystal structure determination,[1] highlighting the importance of the linker, not necessarily involved in the DNA binding, in determining the binding selectivity. We have recently reported the synthesis of new potential antitumour agents, 2,6-bis[2-(heteroaryl)vinyl]pyridines,[2] in which three heteroaromatic rings are linked by two ethylenic double bonds performing a "spacing" function. Circular dichroism provided evidence for the binding of the furyl and thiazolyl derivatives to selected oligonucleotide sequences.[3] Preliminary in vitro antitumour tests pointed to the furyl derivative, with a logIC_{50} = −6.54 for in vitro inhibition against breast carcinoma cells, as the most active compound, confirming the key role of the furan moieties, which may be regarded as a "lead" for structure optimization, in the above series. In this context we report here the synthesis of water-soluble 2,6-bis[2-(heteroaryl)vinyl]-1-methylpyridinium cations **1**−**5** and spectroscopic (UV/Vis, CD, NMR) studies on their interactions with the decamer d(CGTACGTACG)$_2$. The aim of this work is to test the DNA binding ability of the above new compounds by spectroscopic methods with a view to possible future applications as antitumour agents (Scheme 1).



	Het
1	1-Methylpyrrol-2-yl
2	Furan-2-yl
3	Thiazol-2-yl
4	Imidazol-2-yl
5	5-Bromofuran-2-yl

Scheme 1

Results and Discussion

The synthesis of 2,6-bis[2-(heteroaryl)vinyl]-1-methylpyridinium cations **1**−**5** was achieved by condensation of 1,2,6-trimethylpyridinium iodide with heteroaromatic aldehydes in ethanol (see Exp. Sect.). As expected, the pyridinium α-methyl protons were more reactive then the pyridine ones, due to the greater electron-withdrawing effect of the positively charged ring nitrogen atom. Yields were therefore better than those obtained for the synthesis of the analogous 2,6-bis[2-(heteroaryl)vinyl]pyridines,[2] and reaction conditions were milder.

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Under appropriate experimental conditions, outlined in the Exp. Sect., pure *trans-trans* isomers were obtained in all cases, as evidenced by the ethylenic protons' *J* coupling constants in the NMR spectra. Compounds **1** and **2** posses two strongly electron-donating (pyrrole and furan) moieties linked by vinyl linkers to the strongly electron-withdrawing pyridinium ring, while in compounds **3**−**5** the terminal ring electron-donating effect is strongly reduced. Derivatives with such extended conjugation would be expected to exhibit significant solvatochromic shifts. The absorption maxima in protic and aprotic solvents of various polarity are reported in Table 1.

Table 1. Solvatochromic shifts for 2,6-bis[2-(heteroaryl)vinyl]-1-methy-pyridinium cations **1**−**5**

Compound	Solvent[a]			
	$CHCl_3$	EtOH	MeOH	H_2O
1	475.5	464.2	466.2	450.4
	(4.48)	(4.52)	(4.63)	(4.35)
2	421.1	401.3	405.7	400.4
	(4.52)	(4.35)	(4.52)	(4.33)
3	386.2	374.9	−	371.0
	(4.27)	(4.28)		(4.27)
4	−	424.8	400.1	390.3
		(3.68)	(3.90)	(3.90)
5	420.5	403.3	402.3	406.6
	(3.85)	(3.88)	(3.85)	(3.97)

[a] log ε in parentheses.

Water/chloroform solvatochromic shifts for cations **3**−**5** are in the range 13−15 nm, while pyrrole and furan derivatives **1** and **2** exhibit shifts of 25.08 and 20.61 nm, respectively. This finding is consistent with the high donor capability of furan and pyrrole rings and the high acceptor capability of the pyridinium ring in such push-pull-push (donor-acceptor-donor, D-A-D) systems. Applications of such push-pull conjugated molecules as possible candidate components in nonlinear optics, documented for push-pull ethenes such as 4-dimethylamino-4′-nitrostilbene (DANS)[4] and heterocyclic analogues with π-excessive and π-deficient heteroaromatic compounds[5] (D-A-D systems), are outside the scope of this paper.

Investigation of the binding of small ligands to DNA is of great importance in medicinal chemistry, due to the key role of DNA in the regulation of biological processes. Recent advances in human genome research have provided impetus to design and synthesize small molecules specifically targeted at DNA sequences. Excellent contributions in this area have been made by Dervan and co-workers,[6−9] who synthesized heteroaromatic polyamides able to effect molecular recognition of predetermined DNA sequences. In this context, the DNA binding ability of cations **1**−**5** was tested with the self-complementary decamer $d(CGTACGTACG)_2$, the double strand of which has been shown by UV/Vis absorption and induced circular dichroism[10] to possess a high affinity for dystamycin through a minor groove binding mode elucidated by NMR spectroscopy.[11] All the derivatives **1**−**5** exhibited satisfactory water solubility, allowing their interactions with the decamer $d(CGTACGTACG)_2$ to be studied in dilute aqueous solution at various ligand/duplex ratios. The interactions of cations **1**−**5** with the decamer $d(CGTACGTACG)_2$ were first investigated by UV/Vis difference spectra at constant duplex concentration, with increasing ligand concentration.

In order to rule out ligand−ligand interactions such as aggregation etc., the linearity of the Lambert−Beer law in the concentration range examined was verified for all ligands **1**−**5**. In the absence of interaction with the decamer, difference spectra should exhibit no absorbance in the ligand's characteristic absorption wavelength range. Such behaviour was verified for ligands **3**−**5**, while ligands **1** and **2** showed systematic spectral variations. As an example, Figure 1 shows the difference spectra registered at various concentrations of 1-methyl-2,6-bis[2-(1-methylpyrrol-2-yl)vinyl-]pyridinium iodide (**1**) at constant double helix concentration. Figure 1 shows that, on increasing the ligand/duplex ratio from 0.24 to 4.73, progressive increases in a negative band at 442 nm and in a positive band at 505 nm are observed. An isosbestic point at 478 nm indicates the presence of an equilibrium between two species.[12] The same absorption trend observed on addition of the furan derivative **2** denotes a clear interaction with the DNA double helix.

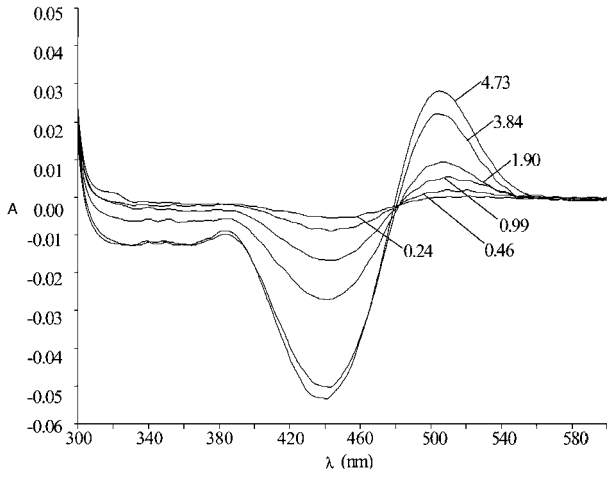

Figure 1. Difference spectra at 25 °C (Tris 0.02 M, NaCl 0.2 M, pH = 7.4) of 1-methyl-2,6-bis[2-(pyrrol-2-yl)vinyl]pyridinium iodide, ligand **1**, and $d(CGTACGTACG)_2$ ($9.17 \cdot 10^{-6}$ M) at increasing ligand/duplex ratios

The interactions of ligands **1**, **2**, and **5** with $d(CGTACGTACG)_2$ were also investigated by circular dichroism (CD). CD measurements have been widely used to provide evidence of DNA conformational changes. Upon binding of drug molecules, by intercalation, for example, alteration of the DNA secondary structure or distortion of the helix usually occurs, producing changes in DNA CD bands. The study of DNA conformational changes is an area of great interest, since drug-induced distortions of DNA structure play an important role in biological processes, by interfering with repair, replication and transcription systems. Figure 2 shows the CD spectra at 25 °C (Tris 0.02 M, NaCl 0.2 M, pH = 7.4) of 2,6-bis[2-(furan-2-yl)vi-

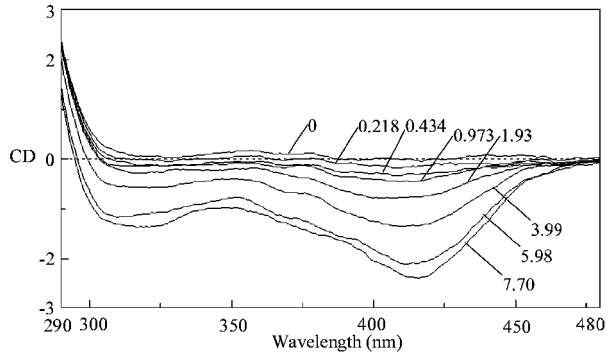

Figure 2. CD spectra at 25 °C (Tris 0.02 M, NaCl 0.2 M, pH = 7.4) of 2,6-bis[2-(furan-2-yl)vinyl]-1-methylpyridinium iodide, ligand **2**, and d(CGTACGTACG)$_2$ ($9.17\cdot10^{-6}$ M) at increasing ligand/duplex ratios

nyl]-1-methylpyridinium iodide (ligand **2**), and d(CGTACGTACG)$_2$ at increasing ligand to duplex ratios.

Ligands **1**−**5** are nonchiral molecules and their solutions do not exhibit any CD signals, unless a chiral interacting agent is added. Addition of ligand **2** to the decamer d(CGTACGTACG)$_2$ induced minor changes in the CD spectral bands in the 200−300 nm range, denoting that the B conformation was retained. The appearance of an induced CD band in the absorption region of cation **2** (300−480 nm) indicated a clear interaction with the DNA duplex (Figure 2). Upon increasing the ligand/duplex ratio, the negative band with a maximum at 417 nm increased in intensity up to ligand/duplex ratio of 5.98, at which a maximum at 315 nm became evident. At higher ligand/duplex ratios, the intensities of both negative bands varied slightly. In analogy with ligand **2**, the DNA B conformation was retained for ligand **1** and the appearance of an induced dichroic band at 448 nm confirmed its interaction with decamer d(CGTACGTACG)$_2$.

On addition of the 5-bromofuran derivative **5**, minor modifications observed in the ligand characteristic absorption range appeared somewhat embedded in the spectrum noise. CD measurement did therefore not provide clear evidence for interaction of the above molecule with the duplex.

The interactions of ligands **2** and **5** with the decamer d(CGTACGTACG)$_2$ were lastly investigated by ^{1}H NMR spectroscopy. This technique requires higher sample concentrations than are needed for UV and CD spectroscopy. In this case the duplex concentration was set at $1.25\cdot10^{-4}$ M, which gave a sufficient signal/noise ratio, and the proton chemical shift variations upon addition of increasing quantities of ligand were studied. Its low solubility at the above concentrations prevented the study of cation **1**.

Figure 3 shows the partial ^{1}H NMR spectrum (δ = 8.40−4.77) of the DNA double strand when titrated with **2**. The assignments for the decamer duplex[13] are reported in the bottom trace. The two strands of the duplex remained magnetically equivalent throughout the titrations, and no splitting of resonances was observed at any stage, thus indicating that the complex was short-lived on the NMR timescale. Almost all resonances underwent upfield chemical shift changes (Δδ), which increased with increasing ligand/duplex ratio. Duplex signals exhibiting traceable variation upon ligand addition are listed in Table 2. Terminal nucleotides showed larger changes, with a Δδ value of 0.14 ppm for H5 of C1 and a Δδ value of 0.12 ppm for H1′ of G10 at the final ratio of 8:1. Three signals (H2 of A8, H2 of A4 and H1′ of C5) underwent modest downfield

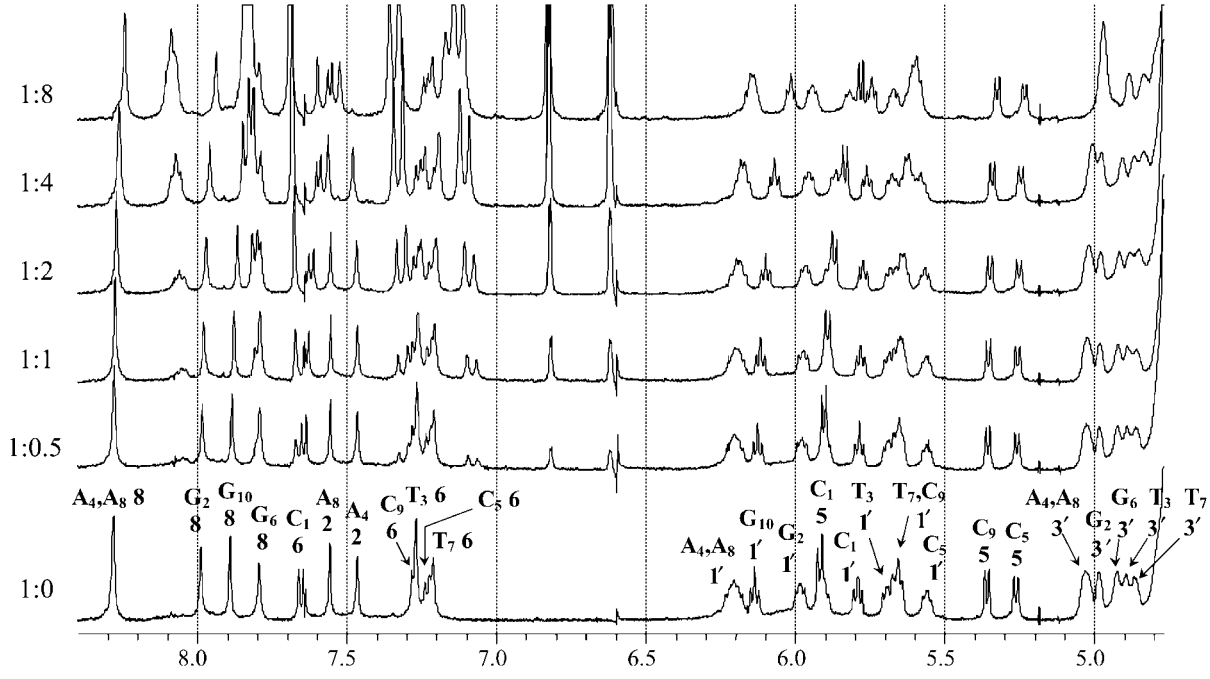

Figure 3. ^{1}H NMR spectra at 25 °C (Tris 0.02 M, NaCl 0.2 M, pH = 7.4) of 2,6-bis[2-(furan-2-yl)vinyl]-1-methylpyridinium iodide, ligand **2**, and d(CGTACGTACG)$_2$ ($1.25\cdot10^{-4}$ M) at increasing ligand/duplex ratios

Table 2. ^{1}H NMR shifts of resolved d(CGTACGTACG)$_2$ signals at increasing ratios of ligand **2**

		[**2**]/[d(CGTACGTACG)$_2$]					
		0	0.5	1	2	4	8
C1	H6	7.663	7.653	7.644	7.628	7.603	7.565
	H5	5.927	5.913	5.900	5.878	5.841	5.789
	H1'	5.791	5.786	5.783	5.773	5.761	5.745
G2	H8	7.991	7.986	7.981	7.973	7.959	7.939
	H1'	5.983	5.977	5.972	5.966	5.957	5.942
	H3'	4.984	4.984	4.983	4.979	4.976	4.970
	H4'	4.372	4.370	4.370	4.370	4.370	4.370
T3	H6	7.270	7.268	7.265	7.254	7.239	7.215
	CH_3	1.515	1.513	1.509	1.504	1.495	1.481
	H1'	5.691	5.687	5.686	5.680	5.678	5.670
	H3'	4.892	4.892	4.891	4.880	4.867	4.832
	H4'	4.207	4.201	4.199	4.188		
A4	H8	8.283	8.281	8.278	8.272	8.263	8.245
	H2	7.467	7.467	7.467	7.468	7.480	7.525
	II3'	5.031	5.027	5.023	5.021	5.004	4.970
	H4'	4.431	4.427	4.420	4.409		
C5	H6	7.240	7.237	7.232	7.226	7.210	
	II5	5.269	5.267	5.264	5.260	5.252	5.241
	H1'	5.562	5.563	5.564	5.566	5.581	
G6	H8	7.794	7.793	7.793	7.789	7.788	7.794
	H3'	4.924	4.923	4.921	4.916	4.906	4.884
	H4'	4.335	4.331	4.326	4.313	4.279	
T7	H6	7.213	7.211	7.209	7.203	7.193	
	CH_3	1.454	1.451	1.447	1.443	1.429	1.401
	H3'	4.868	4.863	4.859	4.852	4.833	
A8	H8	8.283	8.281	8.278	8.272	8.263	8.245
	H2	7.559	7.558	7.556	7.556	7.563	7.600
	H3'	5.031	5.027	5.023	5.021	5.004	4.970
	H4'	4.403	4.401	4.397			
C9	H6	7.284	7.284	7.282	7.278	7.269	7.244
	H5	5.367	5.365	5.362	5.357	5.348	5.332
	H2'	1.850	1.851	1.849	1.848		
G10	H8	7.893	7.887	7.880	7.868	7.848	
	H1'	6.137	6.127	6.117	6.101	6.070	6.016
	H3'	4.651	4.646	4.639	4.627	4.610	4.583

changes. When mixed with the DNA duplex, the resonances of ligand **2** showed significant upfield shifts, which varied slightly on increasing the ligand/duplex ratio. The most affected signals were those of H4′ ($\Delta\delta$ = 0.161 ppm at the 8:1 ratio), H3′ ($\Delta\delta$ = 0.158 ppm) and the N−CH_3 group ($\Delta\delta$ = 0.123 ppm) in the pyridine ring, and of the olefinic spacer ($\Delta\delta$ = 0.112 ppm for Hb and 0.142 for Ha). The furan ring proton signals were comparatively less shifted, with $\Delta\delta$ values of 0.050, 0.030, and 0.014 ppm at the 8:1 ratio for H3, H4, and H5, respectively. The above data support information gained from UV difference spectroscopy and CD, clearly indicating an interaction between **2** and the duplex, and suggest that ligand binding occurs through intercalation. ^{1}H NMR aromatic ligand proton shifts on DNA addition have been used as a sensitive direct method for distinguishing intercalation from the groove binding mode.[14−15] Minor groove binding has been reported to cause slight downfield shifts in DAPI (4′,6-diamidino-2-phenylindole) aromatic protons,[16] while more relevant upfield shifts were attributed to intercalation effects.

Table 3. ^{1}H NMR shifts of resolved d(CGTACGTACG)$_2$ signals at increasing ratios of ligand **5**

		[**5**]/[d(CGTACGTACG)$_2$]					
		0	0.5	1	2	4	8
C1	H6	7.663	7.660	7.659	7.647	7.631	7.608
	H5	5.927	5.922	5.914	5.900	5.876	5.839
	H1'	5.791	5.787	5.782	5.777	5.765	5.749
G2	H8	7.991	7.991	7.988	7.983	7.974	7.960
	H1'	5.983	5.982	5.977	5.972	5.959	5.940
	H3'	4.984	4.985	4.984	4.984	4.979	4.974
	H4'	4.372	4.374	4.373	4.373	4.369	4.367
T3	H6	7.270	7.271	7.271	7.263	7.263	-
	CH_3	1.515	1.514	1.512	1.508	1.502	1.492
	H1'	5.691	-	5.684	-	-	-
	H3'	4.892	-	4.895	-	-	-
	H4'	4.207	4.206	4.202	4.193	-	-
A4	H8	8.283	8.284	8.283	8.281	8.277	8.273
	H2	7.467	7.465	7.465	7.467	7.469	7.476
	H1'	6.206	6.208	6.200	6.195	6.185	6.170
	H3'	5.031	5.030	5.029	5.022	5.019	5.014
	H4'	4.431	4.431	4.427	4.424	4.416	-
C5	H6	7.240	7.241	-	-	7.242	-
	H5	5.269	5.269	5.268	5.267	5.265	5.262
	H1'	5.562	5.559	5.563	5.564	5.568	5.582
G6	H8	7.794	7.798	7.798	7.800	7.816	7.838
	H1'	5.912	5.907	5.899	5.886	5.861	5.824
	H3'	4.924	4.925	4.924	4.925	4.920	4.914
	H4'	4.335	4.336	4.333	4.328	4.220	4.303
	H2'	2.579	2.580	2.577	2.574	2.567	2.560
T7	H6	7.213	7.216	7.215	7.213	7.212	7.208
	H1'	5.658	5.653	5.652	5.649	5.644	5.637
	CH_3	1.454	1.453	1.451	1.448	1.444	1.444
	H3'	4.868	-	4.862	4.859	4.862	4.852
A8	H8	8.283	8.284	8.283	8.281	8.277	8.273
	H2	7.559	7.556	7.556	7.555	7.555	7.557
	H1'	6.206	6.208	6.200	6.195	6.185	6.170
	H3'	5.031	5.030	5.029	5.022	5.019	5.014
	H4'	4.403	4.406	4.403	4.400	4.396	-
C9	H6	7.284	7.287	7.286	7.286	7.285	7.284
	H5	5.367	5.366	5.364	5.362	5.357	5.349
	H1'	5.658	5.653	5.652	5.649	5.644	5.637
G10	H8	7.893	7.893	7.890	7.885	7.877	7.863
	H1'	6.137	6.133	6.127	6.117	6.097	6.067
	H3'	4.651	4.652	4.649	4.645	4.640	4.630
	H2''	2.579	2.580	2.577	2.574	2.567	2.560

The results of similar ^{1}H NMR experiments performed with compound **5** are summarized in Table 3. The spectra showed a trend similar to that seen with **2**, but with smaller chemical shift variations. This indicated a weaker interaction than found with **2**, not clearly evidenced by UV difference spectroscopy and circular dichroism. In addition, the ligand signals exhibiting major changes in this case were also those of the pyridine ring and of the olefinic spacer. These results suggest a common nature for the mechanisms of ligand/duplex interaction for cations **2** and **5**, and attest to the complementarity of the three spectroscopic tech-

niques used in determining ligand/duplex binding interactions.

In conclusion, this study, providing clear evidence for the DNA binding ability of some of the title water-soluble cations, encourages us to carry out further investigations to test their possible application as antitumor agents.

Experimental Section

General: Heteroaromatic carbaldehydes were Aldrich commercial products. UV/Vis difference spectra were recorded with a Perkin−Elmer Lambda 2S spectrometer. CD spectra were recorded with a Jasco J-600 spectropolarimeter. All UV and CD measurements for binding experiments were performed in 0.2 M Tris-HCl (pH = 7.4) and 0.2 M NaCl at 22 °C at constant DNA concentration ($9.17\cdot10^{-6}$ M) and increasing ligand concentration ($2\cdot10^{-6}$ to $7.06\cdot10^{-5}$ M). Decamer d(CGTACGTACG)$_2$, purchased from GENSET, was HPLC grade pure. The single-strand concentration was determined spectrophotometrically in water, using the extinction coefficient $\varepsilon_{260} = 1.05\cdot10^{-5}$. The DNA was then lyophilized and dissolved in 0.02 M Tris-HCl (pH = 7.4) and 0.2 M NaCl to obtain the double helix. The optical melting curve was registered at 260 nm with a temperature increase of 0.5 °C/min, starting at 8 °C. The melting transition started above 30 °C; under the experimental conditions the duplex state is thus prevalent. DNA concentrations are expressed throughout as molar duplex concentrations. The ^{1}H NMR spectra of compounds **1**−**5** were recorded with a Varian Inova® spectrometer operating at 500 MHz, at 25 °C in $CDCl_3$, $(CD_3)_2SO$, or D_2O with TMS or acetone (δ = 2.225) as internal standards. The spectral width was set to 5,000 Hz, with an excitation pulse of 60°, an acquisition time of 3.5 s and a digital resolution of 0.15 Hz/pt after zero-filling. Duplex/ligand mixtures were dissolved in 0.02 M Tris-HCl (pH =7.4) and 0.2 M NaCl, exchanged with 99.9% D_2O by lyophilization and finally dissolved in 99.96% D_2O. The duplex concentration was $1.25\cdot10^{-4}$ M, while the ligand concentrations varied from $0.625\cdot10^{-4}$ M to 10^{-3} M. Fast Atom Bombardment (FAB) mass spectra were recorded with a double focusing Kratos MS 50 mass spectrometer equipped with the standard FAB source and the Maspec2 data acquisition and processing system (Mass Spectrometry Services Ltd.). Mass resolution was 3,000 and the matrix was 3-nitrobenzyl alcohol. The electron impact (EI) mass spectra were recorded at 70 eV with a QMD 1000 GC-MS system (Carlo Erba Instruments) equipped with the solid sample direct insertion probe. Spectra were acquired at 3 s/scan maintaining the source temperature at 200 °C.

1-Methyl-2,6-bis[2-(1-methylpyrrol-2-yl)vinyl]pyridinium Iodide (1): 1,2,6-trimethylpyridinium iodide[17] (0.20 g, $8\cdot10^{-4}$ mol) was dissolved in H_2O (18 mL) and mixed with 1-methylpyrrole-2-carbaldehyde (0.33 mL, $3.2\cdot10^{-3}$ mol). NaOH (20%, 4.6 mL) was added with stirring over 30 minutes. After 200 min, the solution, now containing a red precipitate, was extracted four times with 20 mL of ether. The precipitate, filtered from the aqueous solution, was recrystallized from aqueous ethanol. Yield: 0.035 g. 10%, red needles, m.p.: >210 °C, ^{1}H NMR ($CDCl_3$): δ = 3.87 (s, 6 H, H_1), 4.28 (s, 3 H, $H_{1'}$), 6.24 (dd, J_{3-4} = 4 Hz, J_{4-5} = 3 Hz, 2 H, H_4), 6.82 (m, 2 H, H_5), 6.90 (dd, J_{3-5} = 1 Hz, 2 H, H_3), 6.96 (d, $J_{a\text{-}b}$ = 15.5 Hz, 2 H, H_a), 7.53 (d, 2 H, H_b), 8.06 (d, 2 H, $J_{3'\text{-}4'}$ = 7.5 Hz, $H_{3'}$), 8.13 (t, 1 H, $H_{4'}$), MS: positive FAB: M^+=304, EI *m*/*z*: 290, 142.

1-Methyl-2,6-bis[2-(furan-2-yl)vinyl]pyridinium Iodide (2): 1,2,6-Trimethylpyridinium iodide[17] (0.20 g, $8\cdot10^{-4}$ mol) was dissolved in H_2O (18 mL) and mixed with furan-2-carbaldehyde (0.62 g, $6.4\cdot10^{-3}$ mol, 4:1 ratio). NaOH (20%, 4.6 mL) was then added with stirring over 30 min. After 1 h, a precipitate was filtered off, washed with cold water and recrystallized from H_2O. Yield: 0.028 g. 65%, yellow microcrystals, m.p. 214−215 °C. ^{1}H NMR ($CDCl_3$): δ = 4.36 (s, 3 H, $H_{1'}$), 6.54 (dd, J_{3-4} = 3.5 Hz, J_{4-5} = 1.5 Hz, 2 H, H_4), 6.93 (d, 2 H, H_3), 7.22 (d, $J_{a\text{-}b}$ = 15.5 Hz, 2 H, H_a), 7.52 (d, 2 H, H_b), 7.56 (broad s, 2 H, H_5), 8.09 (d, $J_{3'\text{-}4'}$ = 8.0 Hz, 2 H, $H_{3'}$), 8.27 (t, 1 H, $H_{4'}$). MS (positive FAB): *m*/*z* = 278 [M^+].

1-Methyl-2,6-bis[2-(thiazol-2-yl)vinyl]pyrdinium Iodide (3): 1,2,6-Trimethylpyridinium iodide[17] (0.1 g, $4\cdot10^{-4}$ mol) was dissolved in H_2O (1 mL) and mixed with thiazole-2-carbaldehyde (70.5 μL, $8\cdot10^{-4}$ mol). NaOH (20%, 32 μL) was then added in two 16-μL portions. The solution became cloudy due to the presence of a yellow precipitate, which was recrystallized from H_2O. Yield: 0.144 g. 82%, yellow prisms, m.p. 210−212 °C. ^{1}H NMR (DMSO): δ = 4.31 (s, 3 H, $H_{1'}$), 7.85 (d, $J_{a\text{-}b}$ = 15.5 Hz, 2 H, H_a), 8.01 (d, 2 H, H_b), 8.03 (d, J_{3-4} = 3 Hz, 2 H, H_5), 8.09 (d, 2 H, H_4), 8.41 (d, $J_{3'\text{-}4'}$ = 8 Hz, 2 H, $H_{3'}$), 8.55 (t, 1 H, $H_{4'}$). MS (positive FAB): *m*/*z* = 312 [M^+].

2,6-Bis[2-(imidazol-2-yl)vinyl]-1-methylpyridinium Iodide (4): Imidazole-2-carbaldehyde (0.1543 g, $1.6\cdot10^{-3}$mol) was dissolved in warm H_2O (1.3 mL). 1,2,6-Trimethylpyridinium iodide[17] (0.2 g, $8\cdot10^{-4}$ mol) and NaOH (20%, 0.257 mL) were then added. After 1 h, a red precipitate (very hygroscopic) was filtered off and recrystallized from EtOH. Yield: 0.098 g. 30%, red microcrystals, m.p. > 300 °C. ^{1}H NMR (D_2O): δ = 4.09 (s, 3 H, $H_{1'}$), 7.27 (d, $J_{a\text{-}b}$ = 16 Hz, 2 H, H_a), 7.15 (s, 4 H, H_4−H_5), 7.40 (d, 2 H, H_b), 7.95 (d, $J_{3'\text{-}4'}$ = 8 Hz, 2 H, $H_{3'}$), 8.19 (t, 1 H, $H_{4'}$), MS (positive FAB): *m*/*z* = 278 [M^+].

2,6-Bis[2-(5-bromofuran-2-yl)vinyl]-1-methylpyridinium Iodide (5): 1,2,6-Trimethylpyridinium iodide[17] (0.052 g, $2.1\cdot10^{-4}$ mol) and 5-bromofuran-2-carbaldehyde (0.073 g, $4.2\cdot10^{-4}$ mol) were dissolved in warm EtOH (3 mL). After the addition of a drop of 20% NaOH, the solution became dark and no aldehyde was observed by TLC (ethyl acetate). No precipitation was noticed, the solution was concentrated in a rotary evaporator and an excess of ether was then added. The resulting yellow-green precipitate was filtered and recrystallized from aqueous ethanol. Yield: 0.063 g. 53%, yellow-green prisms, m.p. 183−185 °C. ^{1}H NMR ([D_6]DMSO): δ = 4.32 (s, 3 H, $H_{1'}$), 6.96 (d, J_{3-4} = 3.5 Hz, 2 H, H_4), 7.17 (d, 2 H, H_3), 7.38 (d, $J_{a\text{-}b}$ = 16 Hz, 2 H, H_a), 7.70 (d, 2 H, H_b), 8.30 (d, $J_{3'\text{-}4'}$ = 8.5 Hz, 2 H, $H_{3'}$), 8.49 (t, 1 H, $H_{4'}$). MS (positive FAB): *m*/*z* = 438 [M + ^{81}Br,^{81}Br], 436 [M + ^{79}Br,^{81}Br], 434 [M + ^{79}Br,^{79}Br].

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