New Porphyrin-Nucleobase Hybrid Compounds and Their Interaction with Nucleosides and Nucleic Acids

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Four new porphyrin derivatives are described: one (**1**) with a methoxyphenyl group and three pyridinium units (for comparison only), and three others with either adenine (**2**, **3**) or thymine (**4**) bases. The synthetic strategies vary from known literature procedures in that the methylation of pyridinium subunits takes place *before* the formation of the hybrid molecule, beginning with simultaneous condensation of pyridinealdehyde and 3-(propionyloxy)benzaldehyde, with subsequent chromatography under improved conditions. The thymine derivative **4** is obtained from trimethylated tetrapyridylporphyrin **T4PyP** by treatment with a suitable thymine-alkyl bromide. Dilution experiments in water show the absence of intermolecular porphyrin associations under the experimental conditions used. UV and NMR spectroscopic data indicate strong intramolecular self-stacking between the porphyrin moiety and the covalently attached nucleobases. This results in smaller affinities of porphyrin-nucleobase conjugates towards added nucleosides than observed with the reference compounds tris- or tetrakis-pyridinium porphyrin (**1** and **TMPyP**, respectively). Compounds **2**–**4** displayed no significant discrimination between A and T based on complementarity of nucleobases, which is explained by the strong competition from bulk water with Watson–Crick hydrogen bonds. Interactions of ligands **1**–**4** with ct DNA and ss-RNA polynucleotides were studied by fluorimetric and UV/Vis titration. In most cases the fluorescence of **1**–**4** was first quenched by addition of polynucleotide at porphyrin/polynucleotide ratios (r) close to saturation of intercalation binding sites, and then enhanced at ratios $r < 0.1$. The changes at $r > 0.1$ were attributed to intermolecular association of porphyrins along the polynucleotide groove, while the changes observed at large excess of intercalation binding sites ($r << 0.1$) indicate intercalation of porphyrin into the polynucleotide. Scatchard analyses at $r << 0.1$ are in most cases in line with the formation of only 1:1 complexes. With poly U the non-complementary compounds **1** and **4** showed only increased fluorescence, while the complementary compounds **2** and **3** showed decreases as well as increases. Addition of poly U to **2** and **3** induced strong bathochromic shifts of the Soret bands, characteristic of intercalation. Although no affinity difference between complementary and non-complementary complexes of **2**–**4** and ss-polynucleotides was observed, significant differences in emission changes in poly U titrations suggest specific interactions of the adenine conjugates **2** and **3**.

Introduction

Interactions between porphyrins and nucleic acids are of longstanding interest, not least in view of possible medicinal applications.[1] Supramolecular complexes of water-soluble porphyrins with nucleotides or nucleosides have been characterized by high affinities, but show little selectivity with respect to the nucleobase.[2] To date, there are very few artificial receptors for nucleosides and nucleotides that possess the capability for strong discrimination between the four nucleobases.[3] One usually observes only preference for the larger purine in comparison to the pyrimidine bases,[2] with a significant, but until recently overlooked, dependence on the buffer used in the experiments.[4] In addition, only a few small molecules that display specific interactions with one nucleobase in polynucleotides are so far known. Amongst these, a number of intercalator–nucleobase conjugates have been designed and prepared as potential intercalative complexes, with which the target polynucleotide would be additionally stabilized by Watson–Crick hydrogen bonds between a covalently attached nucleobase and complementary bases in the polymer.[5] In this work we describe new water-soluble porphyrins (**1** to **4**, Scheme 1) with covalently attached nucleobases, which were capable of base selectivity with nucleosides through Watson–Crick base pairing, and of binding modes with nucleic acids differing from those previously studied.

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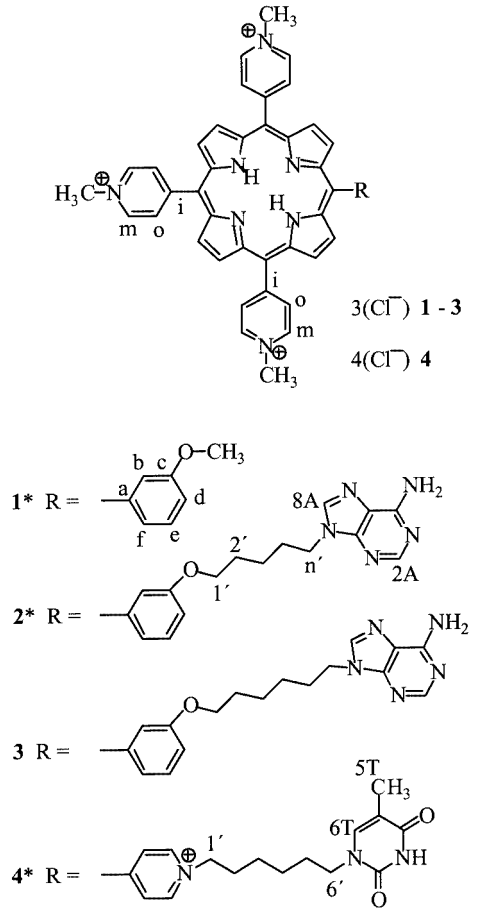


Scheme 1. Compounds **1**−**4**, with atom numbering for NMR signals porphyrins **1**−**4** and starting materials

Results

Syntheses

Over the last ten years, several approaches towards hybrid molecules of tricationic or tetracationic porphyrins have been explored.[6,7] The most widely adopted technique is a mixed-aldehyde synthesis of pyridyl porphyrin, followed by the synthesis of the hybrid molecule and the subsequent quaternization of pyridyl fragments.[7] Generally, however, the yield of the final product does not exceed 5−7% if the "mixed aldehyde" procedure involving Little's adaptation[8] of Adler−Longo[9] conditions is used at the first step. A better approach has recently been devised by Meunier et al.,[10] the symmetrical T4PyP being monoalkylated with the linker and converted into the hybrid molecule, with subsequent quaternization of the remaining pyridyl fragments, giving fairly high yields based on commercially available T4PyP.

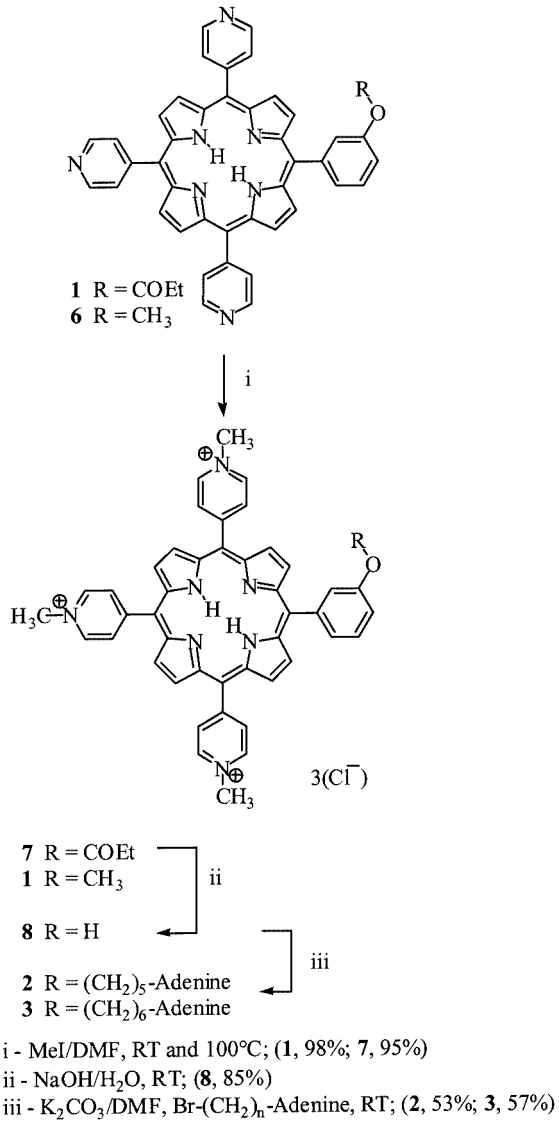
In addition to the low yields of desired product, the existing synthetic procedures afford unwanted by-products in which the adenine residue has been methylated. The formation of the water-soluble derivative takes place in the last step, through methylation of pyridyl fragments,[7,10,12] but substitution at the *N*-1 adenine position also takes place under these conditions (MeI/DMF, room temp.).[11] This problem cannot be overcome by changing the reaction conditions, but only through a change of the synthetic strategy. For thymine derivatives, treatment of 5,10,15-(4-pyridyl)-20-(*o*-, *m*-, or *p*-hydroxyphenyl)porphyrin with halogen- or tosyl-alkyl derivatives under basic conditions[7a,12] is not applicable since alkylation at the *N*-3 position of thymine also occurs under the same conditions (DMF/K_2CO_3, Alk-Hal, room temp. or heating),[13] which is probably one reason for the low yield of hybrid porphyrin-thymine observed previously.[14]

Taking these complications into account, we have developed two new approaches, which differ from the methods described above in that the methylation step takes place *before* the formation of the hybrid molecule. The general strategy consists of treatment of nucleotide bromoalkyl derivatives with *tricationic* porphyrins containing a free hydroxyl group in one case, or a free pyridinium group in another.

The porphyrin precursors **5** and **6** were obtained by cross-condensation of appropriate aldehyde mixtures with pyrrole in propionic acid by the Adler−Longo method. It is known that purification of heteroarylporphyrins on silica gel is often accompanied by loss of much of the product; this problem and hydrolysis of the acetyl group were observed for compound **5**, as with other esterified phenoxyporphyrins.[15] Deactivation of silica gel by triethylamine, however, totally inhibited the strong absorption of pyridyl porphyrins and the hydrolysis of the propionyl group of porphyrin **5**. Methylation of the pyridylporphyrins **5** and **6** under standard conditions (DMF/MeI)[6] afforded the water-soluble derivatives **7** and **1**, respectively (Scheme 2). The corresponding water-soluble porphyrin **8**, with the free phenol group, was easily obtained by saponification of **7**.

Synthesis of cationic porphyrin hybrid molecules from bromoalkyl derivatives and hydroxyphenyl organic-soluble pyridyl porphyrins with further quaternization was used earlier.[7,12] It was noticed, however, that efforts to use water-soluble derivatives of hydroxyphenyl pyridyl porphyrins and bromoalkyl derivatives did not provide the desired hybrid molecules.[7a] We have now found conditions (K_2CO_3/DMSO, room temp.) under which the reaction between water-soluble derivative of hydroxyporphyrin **8** and bromoalkyl derivatives of adenine Br-$(CH_2)_n$-adenine (n = 5, 6) gave the cationic porphyrin-adenine derivatives **2** and **3** with yields of at least 50% after chromatographic separation. The course of the reaction was easily observable on silica TLC.[16] Purification was possible by the general approach described by Miskelly for the separation of mixed (*N*-methyl-2-pyridiniumyl)porphyrins[17] by preparative TLC with MeCN/H_2O/KNO_3 eluent systems.[16]

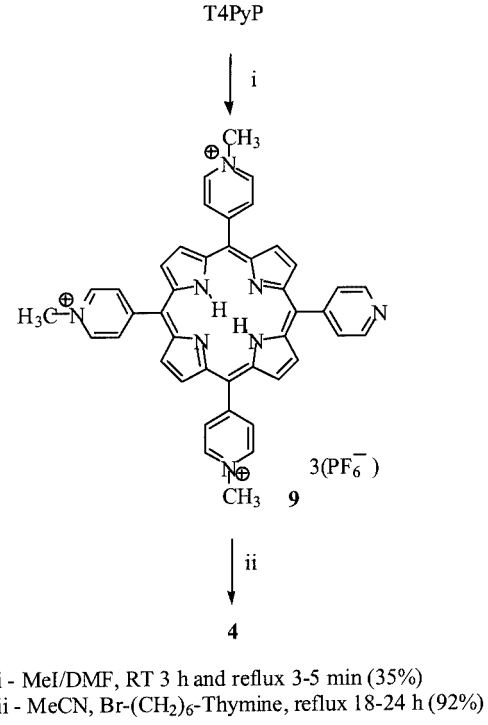
Treatment of **8** with 1-(6-bromohexyl)thymine did not give the desired result. The NMR spectroscopic data of the purified product indicated a mixture of up to three compounds, evidently formed by *N*-3 alkylation of thymine under these basic conditions (vide supra);[13] these products were indistinguishable by chromatography, and all separation attempts were unsuccessful. Attempts to obtain the water-soluble thymine derivative by monoalkylation of



Scheme 2. Synthetic pathways via the mono-phenoxy porphyrin derivative **1**

T4PyP with Br-$(CH_2)_6$-thymine under conditions as given in ref.[10] and further methylation were also unsuccessful. It is known that alkylation of the pyridyl group in **T4PyP** by iodo- or bromoalkyl derivatives is a clean reaction in various solvents (DMF, DMSO, MeCN, $MeNO_2$, etc).[6] On the other hand, *N-3* alkylation of thymine by bromoalkyl derivatives does *not* occur in absence of base if acetonitrile is used as a solvent.[18] We therefore tried to proceed via the trimethylated **T4PyP** to further alkylation of the free pyridyl substituent with bromoalkyl derivatives. This approach may be considered as a complementary method to the Meunier technique mentioned above.[10]

Several conditions for preparation of fully alkylated pyridylporphyrins have been reported,[6] but the partially methylated **T4PyP** had not been described until now,[19] although there are a few reports on other partially alkylated derivatives.[20] We examined several systems (methyl *p*-toluenesulfonate (MTS)/DMF; MTS/$MeNO_2$; MeI/DMF) and have found conditions under which 5,10,15-tris(1-methylpyridinium-4-yl)-20-pyridin-4-ylporphyrin **9** [Tri(MPy)PyP] could be obtained with yields of about 35% (Scheme 3).[21] The tri(MPy)PyP porphyrin, as its PF_6^- salt, is perfectly soluble in acetonitrile. Treatment of tricationic precursor **9** with the bromoalkyl derivative of thymine in this solvent gave the hybrid molecule **4** in almost quantitative yield.



Scheme 3. Synthesis of porphyrin derivative **4**

Spectroscopic Properties

The ligands **2** and **3** can be viewed as analogues of reference compound **1**, while compound **4** is an analogue of the well known porphyrin derivative **TMPyP**.[6] Intermolecular association of **1**−**4** within the concentration range ($0.05-1 \times 10^{-5}$ mol·dm^{-3}) was excluded by dilution experiments[22] by both UV/Vis and fluorescence spectroscopy, in full agreement with recently reported general data concerning the absence of intermolecular aggregation for tetracationic and tricationic porphyrin derivatives.[23]

In their UV/Vis spectra (Table 1, Figure 1) the Soret bands of adenine derivatives **2** and **3** exhibit small but significant red shifts and a pronounced hypochromic effect relative to **1**; a similar hypochromic effect was observed on comparison of **4** and **TMPyP**. The same effects are present, although less pronounced, for the Q band (for **2** no hypochromicity is observed). The differences in the UV/Vis spectra strongly suggest an intramolecular stacked conformation of nucleobase−porphyrin derivatives (especially for compounds **2** and **3** with *meta*-orientation of linker) with significant interactions between the nucleobase and the porphyrin core;[2a,22] this is also supported by the NMR spectroscopic data discussed below [Table S1, Supporting Information (see footnote on the first page of this article) entries 2,

Table 1. Spectroscopic properties of **1−4** in aqueous media

	UV/Vis[a]				Fluorescence[b]		
	Soret band		Q band				
	λ /nm	ε /м$^{-1}$ cm^{-1}	λ /nm	ε /м$^{-1}$ cm^{-1}	$\lambda_{exc.}$ /nm	$\lambda_{em.}$ /nm	Relative intensity
1	424	$1.84\cdot10^5$	520	$1.16\cdot10^4$	424	652	55.5
2	427	$1.63\cdot10^5$	522	$1.17\cdot10^4$	427	653	63.5
3	428	$1.58\cdot10^5$	522	$1.09\cdot10^4$	428	654	74.8
4	423	$1.54\cdot10^5$	520	$0.92\cdot10^4$	423	652	21.0
TMPyP	423	$2.06\cdot10^5$	520	$1.41\cdot10^4$	423	652	32.8

[a] PIPES buffer (pH = 7, $c = 1\cdot10^{-3}$ mol·dm^{-3}). [b] $c = 2\cdot10^{-6}$ mol·dm^{-3} (pH = 7, Na cacodilate buffer, $c = 1\cdot10^{-3}$ mol·dm^{-3}).

5, and 8; for atom numbering see Scheme 1]. The fluorescence spectra of **2** and **3**, in comparison to **1**, are characterized by enhancement and slightly red-shifted maxima (Table 1, Figure 2). In contrast, the stacking interaction with thymine, as found for derivative **4**, quenches the fluorescence of **TMPyP**. It is interesting to note that the small differences in structure between **1** and **TMPyP** give rise to opposite effects upon intramolecular stacking with nucleobases (under the presumption that thymine and adenine do not induce opposite emission changes of porphyrin system).[24] The changes in Soret band and fluorescence in the order **1 − 2 − 3** point to the importance of spacer length on intramolecular stacking, and therefore spectroscopic properties, of conjugates.

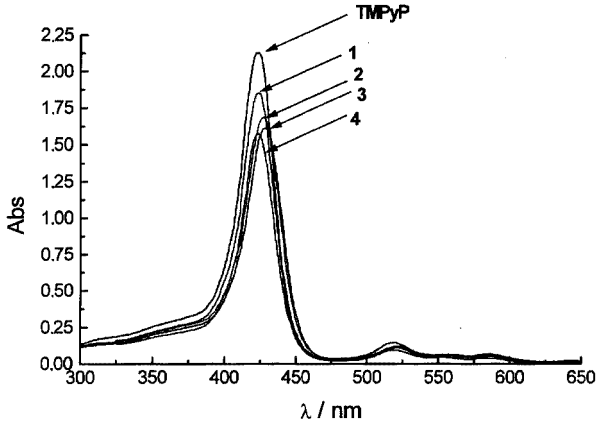


Figure 1. UV/Vis spectra of **1−4** ($c = 1.03\cdot10^{-5}$ mol·dm^{-3}) and the reference **TMPyP**

Interactions with Nucleosides

Interactions of **1−4** with nucleosides were studied by UV/Vis and fluorimetric titrations, the former being more reliable due to its larger spectroscopic changes and therefore used for determination of binding constants (*Ks*) and stoichiometries of the complexes. For most UV/Vis titrations (e.g., Figure 3), the best fit was obtained for 1:1 porphyrin-nucleoside (P:N) complex stoichiometry. Formation of 1:2 complexes was in some cases observed at the end of UV/Vis titration (molar ratio P:N > 0.001), with less than 20% of the 1:2 complex formed (Table 2, estimated values in brackets). Formation of 1:2 complexes was confirmed by fluorimetric titrations (e.g., opposite emission changes, Table 3).

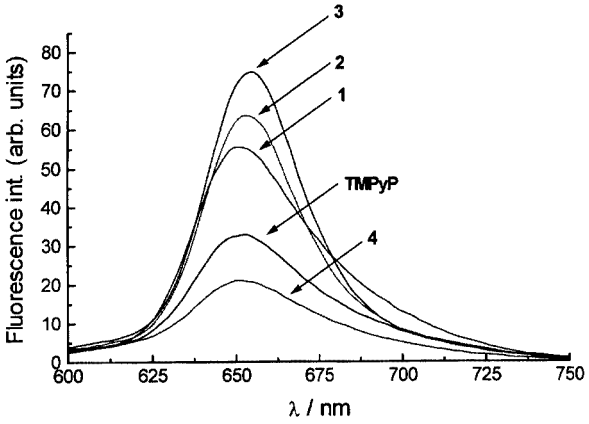


Figure 2. Fluorescence spectra of **1−4** ($c = 2\cdot10^{-6}$ mol·dm^{-3}) and the reference **TMPyP**

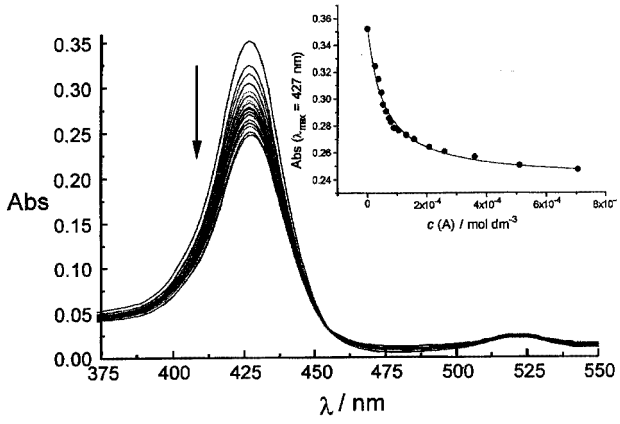


Figure 3. UV/Vis titration of **3** ($c = 2\cdot10^{-6}$ mol·dm^{-3}) with adenosine, pH = 7.0 (PIPES buffer, $c = 1\cdot10^{-3}$ mol·dm^{-3}); best fitting curve obtained for 1:1 stoichiometry (● experimental values; − calculated values)

The calculated *Ks* values for all combinations of **1−4** with nucleosides are the same within the error of the method, showing no preference between complementary porphyrin−nucleobase conjugates and nucleosides. The binding constants, of around $Ks \approx 10^4$ mol^{-1}·dm^3 (Table 2), for 1:1 complexes of **1** are 10−30 times larger than reported for its close analogue **TMPyP**.[2a] For **TMPyP**, however, it was noted that *Ks* values are strongly dependent on ionic strength.[2a] The 100 times lower ionic strength present in titrations of **1−4**, as compared to those

Table 2. Binding constants $\log K_{11}$ ($\log K_{12}$)[a] of **1−4** with nucleosides, PIPES buffer ($c = 1{\cdot}10^{-3}$ mol·dm^{-3}, pH = 7 (UV/Vis titrations)

	A[a]	T	*G*[b]	*C*[b]
1	4.16±0.03	4.51±0.05	3.9±0.06	4.7±0.1
2	4.25±0.04 (2.3±0.03)	4.12±0.05	4.1±0.09	4.4±0.3
3	4.24±0.03	4.18±0.05	4.2±0.1	4.1±0.12
4	4.21±0.05 (2.4±0.2)	4.01±0.05	3.61±0.13	4.16±0.13

[a] *K11* and *K12* refer to the corresponding equilibria P + N $\rightarrow_{\leftarrow}$ PN and PN + N $\rightarrow_{\leftarrow}$ PN_2, respectively, where P is porphyrin compound, N is nucleoside; K_{12} values were only estimated (as far as possible), due to the low percentages of 1:2 complex formed. [b] Because of the poor solubility of nucleosides, stability constants were calculated from the experimental data in the range of 10−50% of 1:1 complex formed.

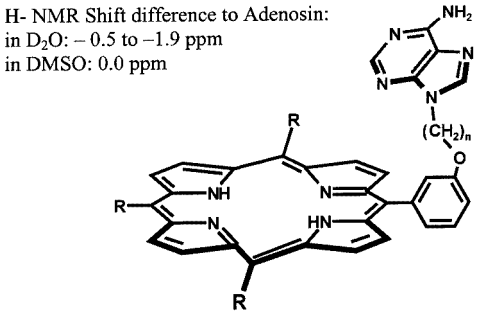
Table 3. Changes in spectroscopic properties of **1−4** induced by binding of nucleosides

		Δλ[a]/nm			
		A	T	*G*	C
	1	0.9 (36)	1.2 (60)	1 (20)	0.5 (24)
UV/Vis (% H)[b]					
2	1.0 (38)	0.4 (29)	3 (31)	0.4 (23)	
3	0.3 (30)	0.1 (32)	0.3 (4)	0 (5)	
4	1.5 (33)	0.6 (17)	2 (18)	0.4 (3)	
	1	4 (+47)	1 (+90)	[d]	[d]
Fluorescence (% *I*)[c]					
2	< 1 (−10,+2)	0 (−12,+10)	[d]	[d]	
3	< 1 (−6)	0 (−6)	[d]	[d]	
4	0 (+38)	0 (+47)	[d]	[d]	

[a] $\Delta\lambda = \lambda - \lambda_0$, difference between maxima wavelengths of free and complexed porphyrin, respectively. [b] H is percentage of Soret band hypochromicity ($Abs_0 - Abs_{obs.}/Abs_0 \times 100$) induced by complex formation. [c] *I* is percentage of emission intensity change ($I_{obsd.} - I_0/I_0 \times 100$, maxima at 653 nm) induced by complex formation. [d] Not measured due to more suitable changes for calculation of binding constants obtained in UV/Vis experiments.

used for **TMPyP** in earlier work,[2a] is probably the cause of the noticeable differences in *Ks* values. The spectroscopic properties of the complexes **1−4** with the different nucleosides (Table 3) reveal minor differences. Both fluorescence and UV/Vis spectral changes are more pronounced in all titrations for **2** than those observed for **3**. This is in agreement with the previously noted correlation between spacer length and the strength of intramolecular stacking (see Spectroscopic Properties). The longer spacer (**3**) allows more efficient intramolecular stacking; consequently adenine has a stronger influence on the spectral properties of porphyrin than in the analogue with a shorter spacer (**2**). Therefore, additional complexation of nucleoside by **3** induces less pronounced spectral changes of porphyrin than found with **2**.

The absence of any significant Watson−Crick type of hydrogen bonding selectivity can be explained by the dominance of intramolecular stacking interactions, which make any other binding contribution marginal. Similar observations have been made with some porphyrin and, for example, diazapyrenium complexes, which can even show no influence of ion-ion interactions, although in water these are much stronger than hydrogen bonds.[25] The lower *Ks* values obtained for **2−4** than for reference porphyrin **1** can be explained by partial covering of the porphyrin entity with the covalently attached nucleobase and is experimentally supported by the solvent effect on the NMR shifts. Only in water do the adenine protons (8A, 2A) and adenine spacer protons [at C(5) and C(4) for **2**, and at C(6), C(5) for **3**] show upfield shifts [$\Delta\delta = 0.99-1.63$ ppm (for 8A); $\Delta\delta = 1.84-2.28$ ppm (for 2A) and $\Delta\delta$ = up to 2.89 ppm for adenine spacer protons] induced by the ring current of the porphyrin (Scheme 4). These upfield shifts disappear in DMSO as the consequence of disrupted intramolecular stacking (Table S1, Supporting Information, entries 2, 5, and 8; for atom numbering see Scheme 1).[26]



Scheme 4. NMR shift differences between the hybrid porphyrin-adenine derivative **3** and adenosine

Interactions with ct DNA and ss-RNA Polynucleotides

Interactions of **1−4** with nucleic acids were studied by fluorimetric and UV/Vis titration. In most titration experiments the fluorescence of **1−4** was quenched by addition of polynucleotide at ratios porphyrin/polynucleotide (*r*) close to saturation of intercalation binding sites, followed by fluorescence increase at ratios $r > 0.1$ (Table 4, Figure 4). In some cases similar behavior was also observed in UV/Vis spectra (Figures 4, 5), but in general absorbances of **1−4** were much less sensitive to different types of complexes. Therefore, the affinities of **1−4** toward polynucleotides were studied by fluorimetric titrations and, where possible, checked by UV/Vis titration. As noted for **1−4**, opposite fluorescence changes as a function of ratios *r* had previously been reported for **TMPyP** analogues and their complexes with ds- and ss-polynucleotides and had been explained in terms of the coexistence of two binding modes of porphyrin.[23,27] Spectroscopic changes at $r > 0.1$ were attributed to intermolecular association of porphyrins along the polynucleotide polyanion, while the changes of emission observed at large excess of intercalation binding sites ($r < 0.1$) were explained by the intercalation of porphyrin into polynucleotide.[24] We therefore processed fluorescence titration data collected at large excesses of binding sites ($r < 0.1$) by the Scatchard equation according to the

Table 4. Binding constants *Ks*, porphyrin/RNA ratios (*n*) of **1**−**4** with polynucleotides obtained by fluorimetric titration, and spectroscopic properties of the complexes

[a][b]		**1**	**2**	**3**	**4**
ct DNA	n	0.09	0.05	0.1	0.12
	log *Ks*	5.84	6.32	5.95	5.96
	[c]I	−34 %, +125 %	−54 %, +87 %	−56 %, +84 %	−50 %, +108 %
	Δλ/nm	+10, −7	+6, −5	+5, −4	+3, −3
poly U	n	0.14			0.21
	log *Ks*	6.04	a	a	6.01
	[c]I	+143 %	−56 %, +86 %	−64 %, +81 %	+160 %
	Δλ/nm	+6	+9, −4	+8, −3	+1
poly A	n	0.07	0.07	0.02	0.1
	log *Ks*	6.64	6.08	5.5	5.75
	[c]I	−56 %, +106 %	−56 %, +26 %	-64 %, +63 %	−27 %, +69 %
	Δλ/nm	+12, −4	+8, −2	+7, −2	+11, −4
poly G	n	0.03	0.05	0.02	b
	log *Ks*	6.69	5.69	6.14	b
	[c]I	−77 %, +34 %	−80 %, +23 %	−84 %, +18 %	−76 %, +4 %
	Δλ/nm	+10, +2	+7, +3	+6, +2	+8, +2

[a]Binding constants could not be calculated, due to the coexistence of two binding modes. [b] The emission changes at excess of polynucleotide were too small for accurate calculation of binding constants. [c] *I* is percentage of emission intensity change (maxima at 653 nm) induced by complex formation.

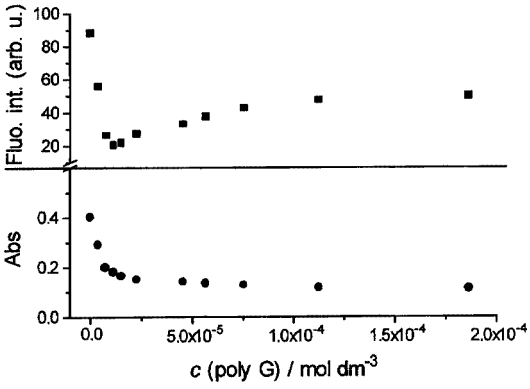


Figure 4. UV/Vis (●, λ_{max} = 424 nm) and fluorimetric (■, λ_{exc} = 424 nm, λ_{em} = 651 nm) titration of **1** ($c = 2\cdot10^{-6}$ mol·dm^{-3}) with poly G, pH = 7 (Na cacodilate buffer, c = 0.02 mol·dm^{-3})

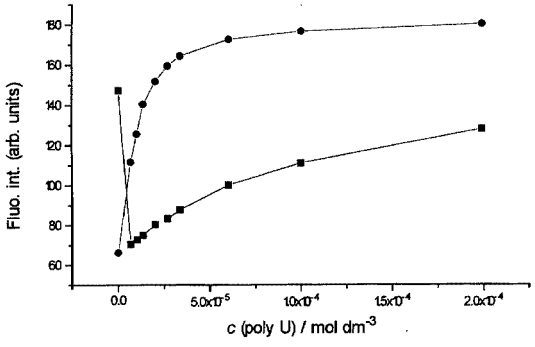


Figure 5. Fluorimetric titration of **2** (■, complementary compound) and **4** (●, non-complementary compound) with **poly U**, *c* (**2** and **4**) = $2\cdot10^{-6}$ mol·dm^{-3}, pH = 7 (Na cacodilate buffer, c = 0.02 mol·dm^{-3})

model proposed in ref.[27] and the results are presented in Table 4.

For comparison, fluorimetric titration of **TMPyP** with *ct* (calf thymus) DNA was conducted under the same experimental conditions as used for **1**−**4**. The values obtained from titration data collected at excesses of *ct* DNA (log*Ks* = 6.28 for n = 0.1) are in good agreement with previously reported data[2a,28] and of the same order of magnitude as values found for **1**−**4** (Table 4). The applied experimental conditions [low ionic strength, low porphyrin/polynucleotide ratios (*r*)] should promote intercalation as the binding mode,[22] taking into account that the studied compounds **1**−**4** are not sterically hindered to any great extent (in comparison with analogous porphyrin−nucleobase conjugates that do intercalate)[14]. According to the results in Table 4, it seems plausible to propose intercalation as the dominant binding mode for both ss- and ds-polynucleotides. However, there remains a question as to how the binding affinities of **1**−**4** toward ds- and ss-polynucleotides can be the same, while most classical intercalators bind more strongly to ds- than to ss-polynucleotides.[29] The presence of two binding modes dependent on the ratio *r* points to an important influence of other types of interactions besides intercalation.[24,27] It was also noted that higher flexibility and hydrophobicity of ss-polynucleotides than of ds-polynucleotides is responsible for observed ss-selectivity of the porphyrin macrocycle.[27] Taking all this into account and according to the proposed model for calculation of *Ks* and *n* values,[27] one can conclude that intercalation of **1**−**4** into poly A and poly G is additionally stabilized by electrostatic and hydrophobic interactions of the porphyrin moiety to a greater extent than upon binding to *ct* (calf thymus) DNA. The sum of these interactions gives rise to a binding of **1**−**4** fairly indifferent towards differences in polynucleotide structure (ds- vs. ss-), Watson−Crick complementarity (**2**,

3 vs. **4** with poly A and poly G, respectively), and type of substituents (**2**−**4** vs. the reference compounds **1** and **TMPyP**).

Interestingly, fluorimetric titrations of compounds **1** and **4** with poly U showed *only increases* in fluorescence, while titrations of compounds **2** and **3** (porphyrin-adenine conjugates) with poly U showed both *decreases and increases* (Figure 5). Addition of poly U to both **2** and **3** induced strong bathochromic shifts of the Soret bands, characteristic of intercalation, in their UV/Vis spectra (10 nm). Changes in UV/Vis spectra (Figure 6) pointed to affinities similar to those observed by fluorescence titration, suggesting two binding modes at excesses of intercalation binding sites. It seems that **2** and **3** form at least three different complexes with poly U, one at high ratio r and two or more at values of $r < 0.1$.

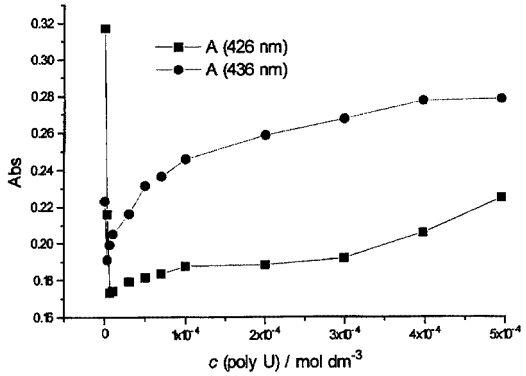


Figure 6. UV/Vis titration of **2** ($c = 2 \cdot 10^{-6}$ mol·dm^{-3}) with poly U, pH = 7 (Na cacodilate buffer, $c = 0.02$ mol·dm^{-3}); absorption maxima of free **2** at 426 nm and of the **2** − polyU complex at 436 nm

Such differences in the results obtained from titration with poly U than with the other ss-polynucleotides points to a significantly different structure of the former in aqueous media. Poly U, in contrast to other ss-polynucleotides, does not form an organized helical structure in water under these experimental conditions, due to weak stacking interactions between the bases.[30] One may therefore assume that interactions of self-aggregated **2** and **3**[24,27] with poly U at high ratios r would not take place; due to this there will be no quenching of fluorescence as observed for **1** and **4**. The observed different titration results upon binding of **2** and **3** are obviously due to the attached adenosine substituents.

Conclusions

The synthesis of nucleobase-porphyrin hybrids targeting nucleic acids requires new synthetic strategies. We have shown how new systems can be obtained from tricationic porphyrin precursors bearing free hydroxyl groups or free pyridinium groups.

Such nucleobase-porphyrin hybrids can exhibit unusual binding properties towards nucleic acids, evident from UV/Vis, fluorescence, and ^{1}H NMR spectra. The binding properties of the hybrid derivatives **1**−**4** are strongly influenced by self-stacking between porphyrin and the attached nucleobase; NMR shifts in combination with solvent-induced changes give clear evidence of this. The lack of significant base selectivity in complexes with different nucleosides emphasizes the problems involved in the development of selective receptors based on hydrogen bonds in water as medium. Selectivity in aqueous solution can be expected only for hosts with a hydrophobic cavity, containing a hydrogen bond recognition unit inside, protected from competition of bulk water. Opposite fluorimetric changes of **1**−**4** upon addition of *ct* DNA, poly G, and poly A as a function of the ligand/nucleic acids ratio r reveal a variety of interaction modes. Promising selectivities could be observed by titrations with poly U, which gave different results with the complementary compounds (**2**, **3**) than with non-complementary ones (**1**, **4**, **TMPyP**), due to the covalently attached adenosine.

Experimental Section

Synthetic Procedures: All reagents and solvents were reagent grade and used as received. Anhydrous DMF was purchased from Aldrich or Merck. Dichloromethane was heated under reflux with phosphorus pentoxide and distilled. TLC was performed on Merck silica gel 60 F254 plates. Gravity column chromatography was carried out with Merck silica gel type 60 (70−230 mesh). Preparative scale TLC was performed on silica gel plates (Merck, 60, layer thickness 1000 μm). Dowex 1-8-200 anion exchange resin, chloride form (Acros chemicals) was used for ion exchange. All solution of porphyrins were filtered through 0.5 μm membrane filters (Gelman Science) before crystallization.

Elemental analysis with porphyrins is often not satisfactory and so was not used, as the material can retain significant amount of solvent and sometimes solid material such as KNO_3 or NH_4PF_6 used to precipitate cationic porphyrins from water solutions; in particular, pyridiniumporphyrins as polyamine chlorides are very hygroscopic.[31] The structures and purities of all new compounds (**1**−**9**) were checked by laser desorption mass spectrometry and confirmed by ^{1}H NMR (often including ^{13}C NMR, COSY, and NoeSY experiments). UV/Vis data were also collected. All NMR spectroscopic data are collected in Table 5.

The porphyrin compounds were analyzed by MALDI-TOF-MS (Matrix-assisted laser desorption/ionization time of flight mass spectrometry) with cyano-4-hydroxycinnamic acid matrix.[7h] UV/Vis spectra were recorded on a Cary 3 spectrophotometer in 1 cm cells. ^{1}H NMR and ^{13}C NMR were recorded on Bruker DRX 500 or AM 400 systems.

5,10,15,20-Tetrakis(pyridin-4-yl)porphyrin (T4PyP) was synthesized by a known procedure.[32] 9-(5-Bromopentyl)adenine[33] and 9-(6-bromohexyl)adenine[34] were prepared by alkylation of adenine by the appropriate dibromoalkanes under conditions similar to those reported earlier for 9-(2-bromoethyl)adenine,[35] and these compounds were recrystallized from methanol/water mixtures. The 1-(6-bromohexyl)thymine was synthesized as previously described.[36]

General Procedure for Separation of Neutral Pyridyl Porphyrins: A column ($d = 45$ mm, $l = 650$ mm) was packed with SiO_2 (300 g)

Table 5. ^{1}H NMR chemical shifts of substituted pyridinoporphyrins; solvents: S = [D$_6$]DMSO, D = D$_2$O, concentration $c = 1\cdot10^{-3}$ M, unless stated otherwise; the number of the corresponding equivalent protons is given in brackets

Proton/ Compd.	Pyridine-*ortho*	Pyridine-*meta*	b	c/ d	e/ f	NCH_3	Porph.-NH	Pyrrole	1′	2′−*x*′	*n*′	8A/ 6T	2A/ 5T	Base NH/NH_2
1 (S)	9.01(4)	9.51(4)	7.66	7.31	7.63(2)	4.72(6)	−3.03	9.13(4)	3.33					
	8.99(2)	9.52(2)				4.73(3)		9.05(4)						
2[a] (S)	9.01(4)	9.54(4)	7.78	7.45	7.75	4.75	−3.02	9.15(4)	4.18	1.86−2′	4.18(5′)	8.22	8.13	7.45
	9.00(2)	9.55(2)			7.78			9.04(4)		1.46−3′				
										1.91−4′				
2 (D)	9.00(2)	9.35(2)	7.62	6.96	7.52e	4.83(3)		≈9 br	3.71	1.14	2.02(5′)	6.53	6.34	
$2\cdot10^{-3}$	8.82(4)	9.18(4)			7.77f	4.71(6)				0.75				
										0.65*				
2 (D)	9.03(2)	9.34(2)	7.80	7.31	7.74e			≈9 br	4.21	1.89;1.72;1.46;1.29[b]		7.23	6.29	
$1\cdot10^{-4}$	8.96(4)	9.30(4)			7.92f									
3 (D)	8.99(2)	9.33(2)	7.64	6.97	7.49e	4.81(3)		≈9 br	3.51	1.48;0.91;0.53; 0.36(2)[b]		5.96	6.17	
$3\cdot10^{-3}$	8.74(4)	9.1(4)			7.75f	4.65(6)								
3 (D)	8.96(4)	9.35(2)	7.92	7.43	7.77			≈9.1 br	4.32	2.35;1.89;1.74;1.29; 1.04[b]		6.59	5.85	
$2\cdot10^{-4}$		9.27(4)			7.90									
4[c] (S)	8.99(6)	9.50(6)				4.74	−3.09	9.24(2)	4.95	2.29−2′	3.73−6′	7.62	1.80	11.23
	9.01(2)	9.57(2)						9.18(6)		1.64−3′				
										1.49−4′				
										1.74−5′				
5 (S)	8.27	9.05	8.03	7.64	7.88		−3.03	8.94(2)	2.70	1.18				
					8.13			8.91(6)						
6 (S)	8.27	9.05	8.03	7.65	7.88		−3.04	8.93(2)	3.65					
					8.13			8.91(6)						
7 (S)	8.99(4)	9.49(4)	8.04	7.69	7.92	4.72	−3.04	9.14(4)	2.71	1.18				
	8.98(2)	9.49(2)			8.13			9.07(4)						
8 (S)	9.01(4)	9.51(4)	7.66	7.31	7.63	4.73(6)	−3.03	9.15(4)						
	8.99(2)	9.52(2)			(2)	4.73(3)		9.05(4)						
9 (S)	8.99(4)	9.47(6)				4.71	−3.06	9.14(4)						
	8.98(2)	9.10(2)						9.05(4)						
	8.26(2)													
TMPyP-4PF_6 (S)	8.97	9.47				4.72	−3.11	9.17						
TMPyP-4Cl (D, 10^{-4})	9.06	9.38				4.86		≈9.22						

[a] ^{13}C NMR δ = 157.08 (5c), 156.50 (10i,20i), 156.43 (15i), 154.73 (6A), 150.80 (2A), 149.28 (4A), 144.17 (10m,15m,20m), 141.54 (5a), 141.42 (8A), 141.38 (1,4,6,9,11,14,16,19), 132.05 (10o,15o,20o), ≈ 132 br (2,3,8,7,12,13,17,18), 128.09 (5e), 127.17 (5f), 122.52 (5), 120.77 (5b), 118.49 (5A), 115.23 (10,20), 114.82 (5d), 114.54 (15), 67.53 (1′), 47.79 (NMe), 42.87 (5′), 29.04 (4′), 28.08 (2′), 22.57 (3′) ppm. [b] Broad signals that give no cross-peak connectivity information in the corresponding COSY and T-ROESY spectra. [c] ^{13}C NMR δ = 164.29 (4T), 156.36 (5i), 156.15 (10i,15i,20i), 150.92 (2T), 144.23 (5m,10m,15m,20m), 143.40 (6T), 141.43 (1,4,6,9,11,14,16,19), 132.44 (5o), 132.04 (10o,15o,20o), ≈ 132 br (2,3,8,7,12,13,17,18), 115.78 (5,10,15,20), 108.44 (5T), 60.67 (1′), 47.86 (NMe), 46.91 (6′), 30.82 (2′), 28.31 (5′), 25.39 (3′), 25.29 (4′), 11.89 (T-Me) ppm.

and a CH_2Cl_2/MeOH/Et_3N (400:12:1; v:v, 700 mL) mixture. The porphyrin mixture (1.5 g) was dissolved in CH_2Cl_2/MeOH (100:3; v:v, 100 mL) and was loaded onto the column. A mixture of CH_2Cl_2/MeOH (50:1; v:v) and then CH_2Cl_2/MeOH (100:3; v:v) was used as eluent (speed: about 100 cm^3/h) to obtain sufficient separation of the porphyrins.

General Procedure for Separation of Cationic Derivatives of Pyridiniumyl Porphyrins: A small amount of Dowex 1-8-200 Cl^- was added to a mixture of cationic derivatives (either hybrid molecule or partially methylated T4PyP; ≈ 100 mg) with methanol (3 mL) and water (0.5 mL). After 15 min the porphyrin solution was loaded onto a preparative TLC sheet (20 cm × 20 cm; 1000 μm) and eluted with MeCN/H_2O/KNO_3sat [(80 mL):(10 mL):(10 mL)]. The fraction containing porphyrin-adenine hybrid molecule or trimethylated T4PyP was ground with Celite (1−2 g) and placed in a small column (d = 10 mm, l = 250 mm). The porphyrin was eluted with 30−40 mL of MeCN/H_2O/NH_4PF_6 (10 mL:10 mL:0.15 g) mixture. The porphyrin solution was allowed to evaporate until the porphyrin crystallized as the PF_6^- salt. The exchange of PF_6^- for Cl^- was performed with an acetone/methanol mixture (1:3) on Dowex 1-8-200 Cl^-. The cationic porphyrins with chloride counterions were precipitated by addition of diethyl ether or acetone.

Compound 5: A mixture of 3-hydroxybenzaldehyde (4.27 g, 35 mmol), propionic acid (50 mL), and propionic anhydride (10 mL) was stirred at 100 °C for 40 min (until full dissolution of the aldehyde). After the mixture had cooled to 60−70 °C, 4-pyridinecarboxaldehyde (8.50 mL, 90 mmol) was added. This mixture of aldehydes and pyrrole (8.33 mL, 120 mmol) were then added separately from dropping funnels to a refluxing mixture of propionic acid (500 mL) and propionic anhydride (15 mL). After one hour and subsequent solvent evaporation, a purple crude material was obtained. This was cooled to 30−40 °C and DMF (100 mL) was then added to achieve overnight precipitation of the porphyrin material. The purple solid was filtered off and washed with methanol, yielding 3.84 g of mixed porphyrins. After chromatography (see procedure for neutral pyridyl porphyrins) the monosubstituted porphyrin 5 was obtained (1.35 g, 6.5%). UV/Vis (CH_2Cl_2): λ_{max} (lgε) = 417 (5.61), 513 (4.18), 547 (3.79), 589 (3.80), 646 (3.31) nm.

^{1}H NMR (DMSO): see Table S1 (Supporting Information). LD-MS: *m*/*z* (calcd. exact mass for M = $C_{44}H_{31}N_7O_2$, 689.25), obsd. 688.9 [M] (100%), 632.7 [M − COEt] (28).

Compound 6: This compound was prepared by treatment of *m*-anisaldehyde (1.36 g, 10 mmol), 4-pyridinecarboxaldehyde (2.84 mL, 30 mmol), and pyrrole (2.80 mL, 40 mmol) in propionic acid by the Adler−Longo procedure.[9] Separation and purification were as for porphyrins 5, (0.448 g, 6.9%). UV/Vis (CH_2Cl_2): λ_{max} (lgε) = 417 (5.54), 512 (4.26), 546 (3.92), 587 (3.93), 646 (3.71) nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). LD-MS: *m*/*z* (calcd. exact mass for M = $C_{42}H_{29}N_7O$, 647.24), obsd 646.9 [M] (100%).

Compound 7: Porphyrin 5 (0.405 g, 0.59 mmol) was dissolved in DMF (60 mL), and methyl iodide (1800 μL, 29.0 mmol) was added. The mixture was stirred at room temperature for 2 h and then at 100 °C for 5 min. After the mixture had cooled to room temperature, the product was precipitated by addition of diethyl ether. The desire product (0.469 g, 95%) was obtained after treatment with anion-exchange resin Dowex 1−8 (chloride form) in methanol solution and recrystallization from a methanol/acetone mixture. UV/Vis (H_2O): λ_{max} (lgε) =422 (5.32), 520 (4.17), 558 (3.80), 584 (3.84), 641 (3.36) nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{47}H_{40}Cl_3N_7O_2$, 839.23 and for M′ = [M − 3 Cl^-] = $C_{47}H_{40}N_7O_2$, 734.32), obsd 734.1 [M − 3 Cl] (100%), 677.0 [M − 3 Cl, − COEt] (10).

M′ − formula and exact mass are calculated without the counterions, as noted by Meunier at all, see MS data for cationic porphyrins Tables 1 and 2, ref.[7g] and Table 2 in ref.[10]

Compound 1: Methoxypyridylporphyrin 6 (203 mg, 0.31 mmol) was treated with methyl iodide (950 μL, 15 mmol) by the procedure described for 7, to give the cationic porphyrin 1 [243 mg, 98%). UV/Vis (H_2O)]: λ_{max} (lgε) =422 (5.30), 520 (4.10), 558 (3.72), 585 (3.76), 641 (3.25) nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{45}H_{38}Cl_3N_7O$, 797.22 and for M′ = [M − 3 Cl^-] = $C_{45}H_{38}N_7O$, 692.31), obsd 691.0 [M − 3 Cl] (100%), 677.0 [M − 3 Cl, − COEt] (10); PF_6^- counterion form: MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{45}H_{38}F_{18}N_7OP_3$, 1127.21 and for M′ = [M − 3 PF_6^-] = $C_{45}H_{38}N_7O$, 692.31), obsd 835.0 [M − 2 PF_6] (30%), 691.0 [M − 3 PF_6] (100), 677.0 [M − 3 PF_6, − Me] (5).

Compound 8: Sodium hydroxide (0.124 g, 3.10 mmol) was added to a solution of 7 (0.261 g, 0.31 mmol) in water (40 mL) and the mixture was stirred at room temperature for 20 min (the color of the solution changed from red to dark brown). The pH of the reaction mixture was then adjusted to 6.5 by addition of HCl. The product was precipitated by addition of acetone (250 mL). Recrystallization from methanol solution, again by addition of acetone, afforded a red, amorphous solid, (0.208 g, 85%). UV/Vis (H_2O): λ_{max} (lgε) = 424 (5.26), 519 (4.06), 561 (3.89), 581 (3.86), 639 (3.43) nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{44}H_{36}Cl_3N_7O$, 783.20 and for M′ = [M − 3 Cl^-] = $C_{44}H_{36}N_7O$, 678.30), obsd 676.1 [M − 3 Cl] (100%).

Compound 2: Hydroxyporphyrin 8 (81 mg, 0.10 mmol) was stirred in dry DMF (30 mL) under nitrogen at 60 °C for 40 min to dissolve the porphyrin. Heating was then removed, and after 25 min K_2CO_3 (69 mg, 0.50 mmol) was added at room temperature. 9-(5-bromopentyl)adenine (142 mg, 0.50 mmol) was added after 25 min, and the mixture was stirred at room temperature for one hour. The material was then filtered off and the solution was adjusted to pH = 6.8 with 0.1 M HCl. The crude product was precipitated by addition of a acetone/diethyl ether mixture (1:1, 60 mL).

The mixture of porphyrins was separated on preparative silica gel thin layer chromatography plate (20 cm×20 cm; 1000 μm) as described in the general procedure for separation, to give hybrid molecule **2**, (52 mg, 53%). UV/Vis (H_2O): λ_{max} (lgε) = 426, 522, 559, 589, 645 nm. ^{1}H NMR (DMSO) and ^{13}C NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{54}H_{49}Cl_3N_{12}O$, 986.32 and for M′ = [M − 3 Cl^-] = $C_{54}H_{49}N_{12}O$, 881.42), obsd 881.1 [M − 3 Cl] (100%).

Compound 3: This compound was prepared by the procedure described for **2**, by treatment of hydroxyporphyrin **8** (79 mg, 0.10 mmol) with 9-(6-bromohexyl)adenine (149 mg, 0.50 mmol); (58 mg, 57%). UV/Vis (MeCN, for PF_6^- counter-ion form): λ_{max} (lgε) = 425, 518, 554, 592, 647 nm. UV/Vis (H_2O): λ_{max} (lgε) = 427, 522, 559, 589, 645 nm. ^{1}H NMR (H_2O): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{55}H_{51}Cl_3N_{12}O$, 1000.34 and for M′ = [M − 3 Cl^-] = $C_{55}H_{51}N_{12}O$, 895.43), obsd 894.7 [M − 3 Cl] (100%).

Compound 9: Methyl iodide (250 μL, 4.02 mmol) was added to a 1000 mL, one-necked, round-bottomed flask containing T4PyP (502 mg, 0.81 mmol) and DMF (400 mL). The mixture was stirred at room temperature for 3 h and was then heated under reflux for 3−5 min. After the mixture had cooled, acetone/diethyl ether (1:1, 400 mL) was slowly added; the precipitate formed was isolated by filtration and washed with diethyl ether. Yield: 550 mg (mixture of porphyrins; after anion exchange, Cl^- form).

TLC analysis [silica, MeCN/H_2O/KNO_3 satd., (8:1:1)] of the precipitate confirmed the presence of TMPyP, of the desired major component, and of mono-, and isomeric dimethyl derivatives as minor components. 98.6 mg of the mixture was separated by preparative TLC (20 cm×20 cm; 1000μm, one TLC plate), following the general procedure for cationic porphyrins to afford Tri(MPy)-PyP; (56.8 mg, 35%) (as PF_6^- salt). UV/Vis (MeCN): λ_{max} (lgε) = 422, 516, 550, 589, 644 nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{43}H_{35}F_{18}N_8P_3$, 1098.19 and for M′ = [M − 3 PF_6^-] = $C_{43}H_{35}N_8$, 663.30), obsd. 953.9 [M − PF_6] (5%), 808.6 [M − 2 PF_6] (48), 663.4 [M − 3 PF_6] (100), 649.3 [M − 3 PF_6, − Me] (11).

5,10,15,20-Tetrakis(1-methylpyridinium-4-yl)porphyrin[6] Tetrakis(hexafluorophosphate) (TMPyP·4PF6): The TMPyP was prepared as described above for compound 9, except that 10 equiv. excess of methyl iodide (500 μL, 8.03 mmol) was used for methylation of T4PyP (502 mg, 0.81 mmol). TLC [silica, MeCN/H_2O/KNO_3 sat, (8:1:1)] showed complete methylation of pyridinium groups. Diethyl ether (300 mL) was added to precipitate the TMPyP as the iodide. After anion exchange to Cl^- (Dowex 1-8-200, Cl^-) the TMPyP[6] tetrachloride (650 mg, 98%) was obtained. After anion exchange with NH_4PF_6, the TMPyP in hexafluorophosphate form was obtained. UV/Vis (MeCN): λ_{max} (lgε) = 422, 516, 550, 589, 644 nm. ^{1}H NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{44}H_{38}F_{24}N_8P_4$, 1258.18 and for M′ = [M − 4 PF_6^-] = $C_{44}H_{38}N_8$, 678.32), obsd. 1111.3 [M − PF_6] (8%), 966.1 [M − 2 PF_6] (44), 821.7 [M − 3 PF_6] (89), 806.7 [M − 3 PF_6, − Me] (32), 677.8 [M − 4 PF_6] (100), 662.7 [M − 4 PF_6, − Me] (32).

Compound 4: A sample of trimethylated derivative **9** in PF_6^- form (20.1 mg, 0.018 mmol) was added to acetonitrile (20 mL) in a 50 mL round-bottomed flask. The bromoalkylthymine (Br-$(CH_2)_6$-thymine, 210 mg, 0.73 mmol) was then added and the mixture was stirred at reflux. The reaction progress was monitored by TLC [sil-

ica, MeCN/H_2O/KNO_3 satd., (8:1:1)]. After the reaction was complete (18−24 h) the solvent volume was reduced to 5 mL under reduced pressure. The crude product was precipitated with diethyl ether (to remove any excess of bromoalkylthymine), followed by treatment of the solid with anion-exchange resin (Dowex 1-8-200, Cl^-) in methanol solution. The product in Cl^- form was precipitated from methanol by acetone/diethyl ether (1:1); (17.0 mg, 92%) as chloride. UV/Vis (H_2O): λ_{max} (lgε) = 423, 560, 585, 641 nm. ^1H NMR (DMSO): see Table S1 (Supporting Information; see also footnote on the first page of this article). ^{13}C NMR (DMSO): see Table S1 (Supporting Information). MALDI-MS: *m*/*z* (calcd. exact mass for M = $C_{54}H_{52}Cl_4N_{10}O_2$, 1012.30 and for M′ = [M − 4 Cl^-] = $C_{54}H_{52}N_{10}O_2$, 872.43), obsd. 872.0 [M − 4 Cl] (100%), 857.0 [M − 4 Cl, − Me] (8), 691.9 [M − 4 Cl, − Thy-$(CH_2)_4$] (8), 675.9 [M − 4 Cl, − Thy-$(CH_2)_5$] (15).

Spectroscopic Investigations: Samples of analyzed compounds were dissolved in bidistilled water, $c = 1{\cdot}10^{-4}$ mol·dm^{-3} and diluted with Na cacodilate buffer or PIPES buffer ($c = 1{\cdot}10^{-3}$ mol·dm^{-3}, pH = 7.0) to give (0.05 to 1.00) × 10^{-5} mol·dm^{-3} solutions. Adsorption of porphyrin compounds on the walls of quartz cuvettes was observed, with experimental problems similar to those observed with some acridinium compounds.[29] All spectroscopic experiments were therefore conducted in 3 mL acrylic cuvettes, in which adsorption was negligible.

Interactions with Nucleosides: Both UV/Vis and fluorimetric titrations were carried out by addition of a concentrated stock solution of nucleoside (ca $1.5{\cdot}10^{-2}$ mol·dm^{-3}) containing ca. $2{\cdot}10^{-6}$ mol·dm^{-3} of porphyrin to an equally concentrated solution of porphyrin in a 3 mL acrylic cuvette. In this manner, the concentration of the porphyrin receptor was constant, while the concentration of nucleoside varied. Incubation time was 60 s. Titrations were made in a range of about 20−80% of 1:1 complex formed. Fluorimetric titrations were performed in Na cacodilate buffer solution (pH = 7.0, $c = 1{\cdot}10^{-3}$ mol·dm^{-3}, while UV/Vis titrations were performed in PIPES buffer solution (pH = 7.0, $c = 1{\cdot}10^{-3}$ mol·dm^{-3}). The data were analyzed with the SPECFIT[37] program and the results are given in Tables 1 and 2. Results obtained from UV/Vis and fluorescence titrations are of the same order of magnitude, varying by max. 10%.

Interactions with Polynucleotides: Polynucleotides were purchased as noted − poly A, poly C, and poly U (Pharmacia); poly G (Sigma), calf thymus (ct) DNA (Aldrich) − and dissolved in Na cacodilate buffer ($c = 1{\cdot}10^{-3}$ mol·dm^{-3}, pH = 7.0); the concentration of phosphates was determined spectroscopically.

Fluorescence titrations were used to determine the binding affinity toward polynucleotides. Maxima of Soret bands were used for excitation, and changes of fluorescence emission were monitored at their respective maxima. Titrations were carried out by addition of a concentrated stock solution of polynucleotide (ca. $1{\cdot}10^{-2}$ mol·dm^{-3}) to a solution of porphyrin (ca $2{\cdot}10^{-6}$ mol·dm^{-3}) in a 3 mL acrylic cuvette, with an incubation time of about 90 s. Processing the titration data at large excess of intercalation sites ($r < 0.1$) according to the Scatchard equation[25,38] and the model proposed by Slama-Schwok and Lehn[27] gave binding constants (*Ks*) and [bound intercalator]/[polynucleotide phosphate] ratios (*n*). The values for *Ks* and *n* given in Table 4 had satisfactory correlation coefficients (> 0.999).

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