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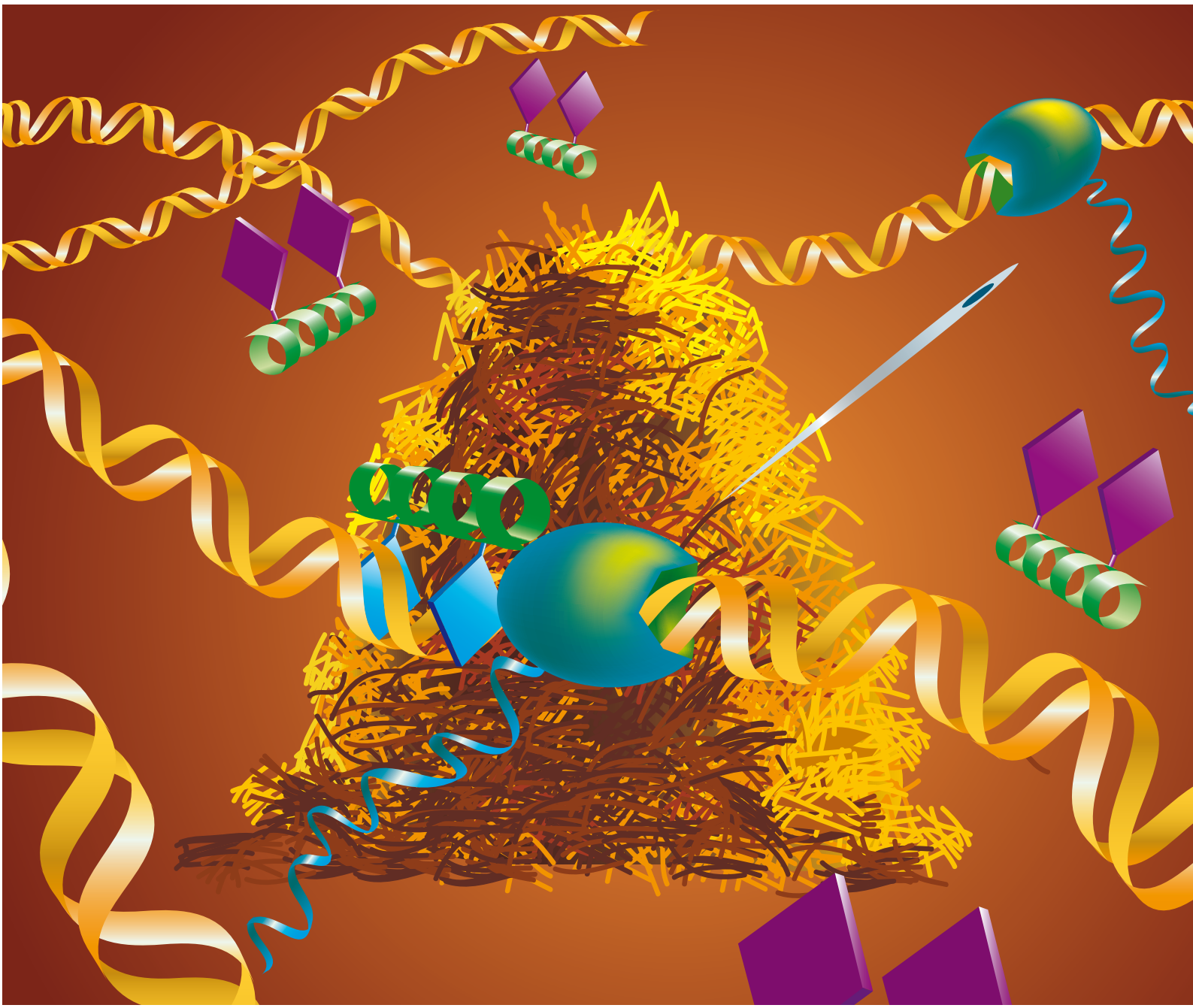

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FULL PAPER

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Towards sequence selective DNA binding: design, synthesis and DNA binding studies of novel bis-porphyrin peptidic nanostructures†

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A new series of peptidic nanostructures bearing two intercalating moieties was designed and synthesized to achieve selective recognition of DNA sequences. A cationic porphyrin was attached to a glutamic acid side chain and the latter introduced into a peptidic sequence by standard solid-phase peptide synthesis methodology. Conformation of the hydrosoluble peptidic structures bearing two cationic porphyrins was studied by circular dichroism. Using UV–visible spectroscopy and induced circular dichroism, we demonstrate that the compounds are fully intercalated upon binding to double-stranded DNA and that the compounds exhibit a tremendous preference for GC over AT sequences for intercalation.

Introduction

The development of nanoscale tools having specific applications is of great interest in many areas of science. One application of utmost importance is to develop molecular tools able to probe a specific nucleic acid sequence at the genome wide scale.[1,2] Such functional supramolecular tools have extremely interesting biomedical applications and could be used in biosensors, for diagnostic and as therapeutic agents.[1–3] Impressive advances in the design of sequence-selective DNA-binding agents have been achieved during the last decade. For example, pyrrole–imidazole polyamides that can be programmed to recognize specific DNA sequences with affinities and specificities comparable to DNA binding proteins have been developed.[2,4] Other successful approaches include peptide nucleic acids (PNAs),[5] Cu^{2+}-mediated assembly of bipyridine–Hoechst ligand molecules,[6] triple-helix-forming oligonucleotides,[7] modified zinc-finger proteins,[8] and other molecules such as other minor-groove binders and intercalators.[9]

Intercalation of small aromatic molecules in double-stranded DNA (dsDNA) is an extremely efficient binding mode.[10,11] Numerous compounds, including some natural products and some clinically used chemotherapeutic agents, interact with DNA by intercalating one or more aromatic groups between base pairs of the double helix.[10–12] Whereas initial studies focused on molecules with a single intercalating moiety,[11] the promise of improved sequence specificity has led researchers to investigate compounds that contain more than one intercalating group.[13,14] Bis-intercalating compounds showed higher DNA binding affinities and slower dissociation rates than the corresponding monomers.[10,15] Over the past two decades, a great number of dimeric forms of DNA intercalators, such as bis-acridinecarboxamides, bis-naphthalimides, bis-imidazoacridones has been developed as potential anticancer drugs.[13–16]

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† Electronic supplementary information (ESI) available: UV–vis spectra of DNA titrations and ICD spectra of peptides **6–12** with poly(dGdC)$_2$, poly(dAdT)$_2$ and CT-DNA. See DOI: 10.1039/b803281e

Our approach to achieve sequence selective DNA binding is to use engineerable peptidic frameworks to orient properly two DNA intercalating moieties. The distance between the intercalating units can be easily modified and this variation should provide sequence preferences among the synthesized bis-intercalator peptides. Among the available intercalating moieties, the cationic *meso*-tetrakis(4-*N*-methylpyridiniumyl)porphyrin (TMPyP) was chosen because of its high affinity for DNA and its well known DNA-binding properties.[17,18] We now report the solid-phase synthesis, conformational studies and DNA-binding properties of a new series of bis-porphyrin peptides.

Results and discussion

Design

The starting point for the design of a versatile peptidic scaffold was based on work previously reported, by our group[20] and others,[21] for the construction of engineerable peptidic frameworks. The general concept of this work was to use α-helical peptidic structures as scaffolds to orient multiple macrocyclic ligands on top of each other. One major advantage of using this strategy is the possibility to easily vary the distance between the intercalating moieties and their orientation simply by changing their position in the peptidic sequence. In this case, hydrosolubility of the peptidic framework is a very important prerequisite to allow DNA-binding in aqueous media and prevent aggregation. Unfortunately, most of the amino acids that induce an α-helix are hydrophobic (ala, leu, *etc.*). To avoid this inconvenience, the peptidic framework was also based on the stabilized hydrosoluble α-helices reported by Marqusee and Baldwin.[19] It was shown that a 17-mer alanine-based peptide **1** containing three glutamic–lysine residue pairs spaced by 4 residues readily forms an α-helix in water (Fig. 1). Under an α-helix structure, the Glu^{-}-(i + 4)Lys^{+} ion pairs or salt bridges stabilize the helix and confer the hydrosolubility.

On the other hand, the intercalating units are attached to the peptidic structure *via* an amide bond to glutamic acid side chains. This modified glutamic acid can be introduced into the peptide simply by replacing an alanine (Fig. 1). To do so,

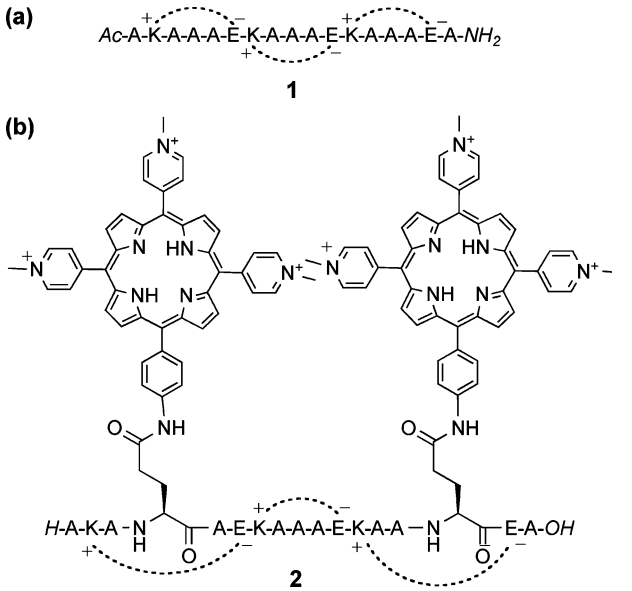


Fig. 1 Sequences of the alanine-based hydrosoluble α-helical peptides; (a) 17-mer peptide designed by Marqusee and Baldwin;[19] (b) peptide **1** bearing two porphyrins. The dashed lines represent ion pair formation under an α-helical structure. (Ac = acetate.)

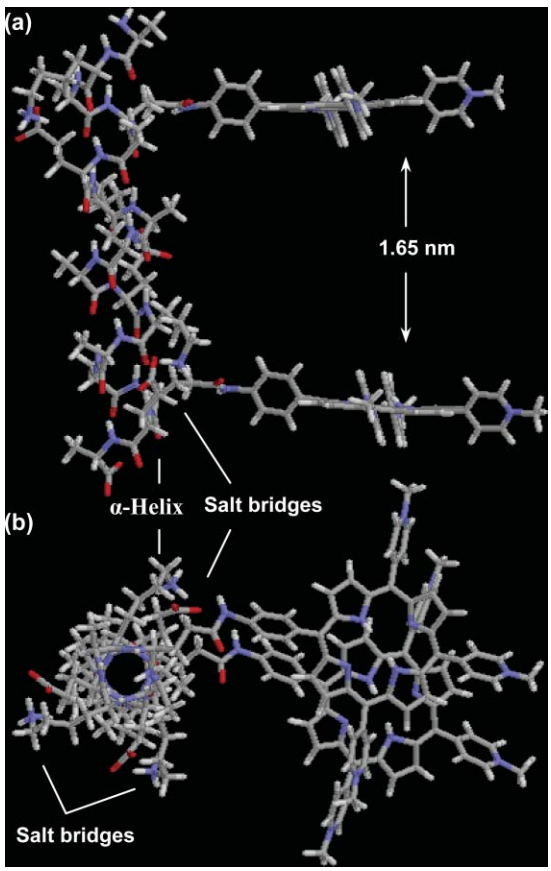


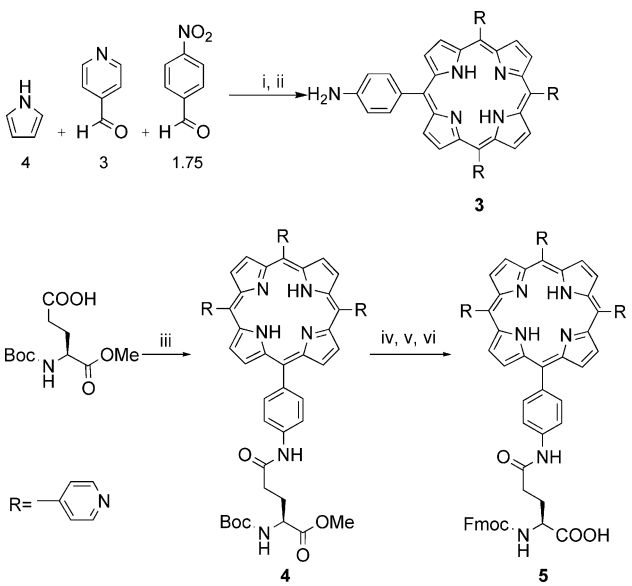
Fig. 2 17-Mer peptide **2** bearing two porphyrins; (a) side view; (b) top view.

porphyrin modified glutamic acids are synthesized beforehand and introduced into the peptide during solid-phase synthesis. Another important feature in the design of a bis-intercalator peptide is the positioning of the intercalating units into the peptidic sequence. We found that to orient the intercalating units on the same side of the α-helix, they would have to be introduced at positions 4 and 15 in a 17-mer peptide (Fig. 2) and at positions 2 and 16 in an 18-mer peptide. It can be easily observed that simply by changing the position of the intercalating units into the peptidic sequence, the distance between them is also modified. For example, if the peptidic framework adopts an α-helical conformation, the distance between the intercalating moieties is, when calculated with 0.15 nm per amino acid,[22] 1.65 nm for the 17-mer and 2.10 nm for the 18-mer.

The influence of the peptidic framework rigidity was also an interesting feature to investigate. More adaptable (flexible) peptidic frameworks composed only of glycine were designed. The distance between the intercalating units was varied simply by changing the number of glycines between them.

Synthesis

The first step of the synthesis was to prepare the porphyrin modified glutamic acids. *N*-Fmoc glutamic acids bearing a porphyrin were synthesized as previously described.[23] Briefly, 5-(4-nitrophenyl)-10,15,20-tris(4-pyridinyl)porphyrin was prepared using the classical Adler–Longo procedure by refluxing a mixture of 4-nitrobenzaldehyde (1.75 eq.), 4-pyridinecarboxaldehyde (3 eq.) and pyrrole (4 eq.) in the presence of acetic anhydride (Ac_2O) in propionic acid (Scheme 1).[24] After purification by silica gel chromatography, the nitro group was reduced by means of stannous chloride in 6 N HCl to yield quantitatively the 5-(4-aminophenyl)-10,15,20-tris(4-piridinyl)porphyrin **3**. The coupling reaction was achieved by activating the carboxylic function of *N*-Boc-glutamic acid methyl ester with ethylchloroformate in



Scheme 1 *Reagents, conditions and yields:* (i) $CH_3CH_2CO_2H$–Ac_2O reflux, 1.5 h; 8%; (ii) $SnCl_2$, 6 N HCl; 98%; (iii) (a) ClCOOEt, TEA, DCM, 0 °C, 30 min, (b) **3**, TEA, DCM, 0 °C, 2 h; 80%; (iv) 1 N NaOH, THF, 0 °C, 30 min; 90%; (v) 4M HCl–dioxane, 30 min; 99%; (vi) Fmoc-OSu, DIEA, MeCN–H_2O (9 : 1), 3 h; 80%.

dichloromethane in the presence of triethylamine (TEA), followed by addition of **3**.[23,24] The fully protected modified amino acid **4** was

obtained in 80% yield. Methyl ester cleavage on **4** was performed with 1 N NaOH in THF followed by Boc deprotection with 4 M HCl in 1,4-dioxane and reprotection using Fmoc-OSu to give **5** in very good yield after purification by short column silica gel chromatography.

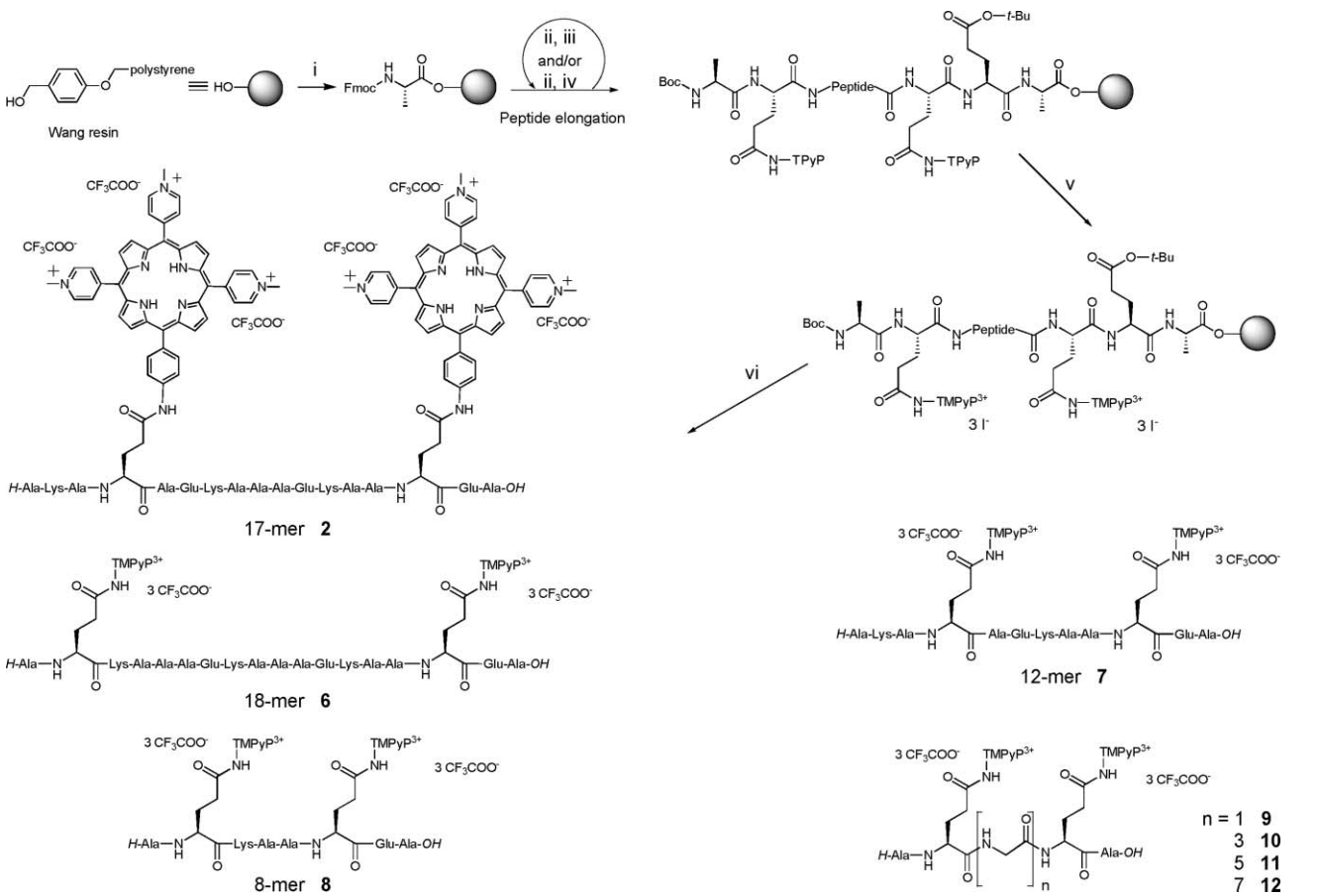
The bis-intercalator peptides were synthesized on Wang resin following standard Fmoc strategy solid-phase peptide synthesis (Scheme 2).[25] The first amino acid, Fmoc-Ala-OH, was attached to the resin *via* activation with diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) in the presence of diisopropylethylamine (DIEA) in DMF. Fmoc deprotection was performed twice using a 20% piperidine solution in DMF for 15 minutes. Each standard Fmoc-protected amino acid (Ala, Gly, Lys(Boc), and Glu(O*t*-Bu)) was coupled in a 5-fold excess by activating with DIC-HOBt-DIEA in DMF. The modified amino acid **5** was coupled in a reduced 1.5-fold excess to the amino free resin-bound peptide using *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) in the presence of DIEA in DMF for 3 h.[23] The final amino acid was *N*-Boc protected to generate free peptides after cleavage under acidic conditions. Most conveniently, *N*-methylation of the pyridyl groups was carried out directly on solid-phase with an excess of iodomethane in DMF.[23] Finally, methylated peptides were cleaved from the resin using a mixture of trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water (95 : 2.5 : 2.5). Synthesized peptides were obtained in good yields (25–38% overall yield) and in a highly pure form after purification. Peptide purity was determined by HPLC and ESI-MS was used for characterization.

Conformational studies

Conformational analyses of peptides **2**, **6–8** were performed using circular dichroism (CD) to demonstrate the helical structure of the peptidic frameworks. CD spectra were recorded for peptides **2**, **6–8** at 50 μM in a buffer solution (Tris·HCl 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0) (Fig. 3). Highly populated helical structures were observed for peptides **2** and **6** with negative maxima at 206 and 222 nm and a positive maximum at 191 nm, whereas peptide **7** showed weaker ellipticity and peptide **8** showed no ellipticity. These results suggest that only peptides **2** and **6** are long enough to form a stable α-helix structure in water. The same curves were observed for peptides in which the porphyrin modified amino acids have been replaced by alanines. This last experiment confirmed that the presence of porphyrin units does not affect the structure of the peptidic framework.

DNA binding studies

We have previously demonstrated that a cationic porphyrin attached to a peptide maintains its DNA binding properties.[23] DNA binding studies with peptides **2**, **6–12** were performed to



Scheme 2 Synthesis of bis-cationic porphyrin peptides **2**, **6–12**. *Reagents and conditions:* (i) Fmoc-Ala-OH, DIC, HOBt, DIEA, DMF, 24 h; (ii) 20% piperidine–DMF, 2 × 15 min; (iii) **5**, HATU, DIEA DMF, 3 h; (iv) Fmoc-Ala-OH or Fmoc-Glu(O*t*Bu)-OH or Fmoc-Lys(Boc)-OH or Boc-Ala-OH, DIC, HOBt, DIEA, DMF, 1 h; (v) CH_3I, DMF, 24 h; (vi) TFA–TIS–H_2O (95 : 2.5 : 2.5), 4 h.

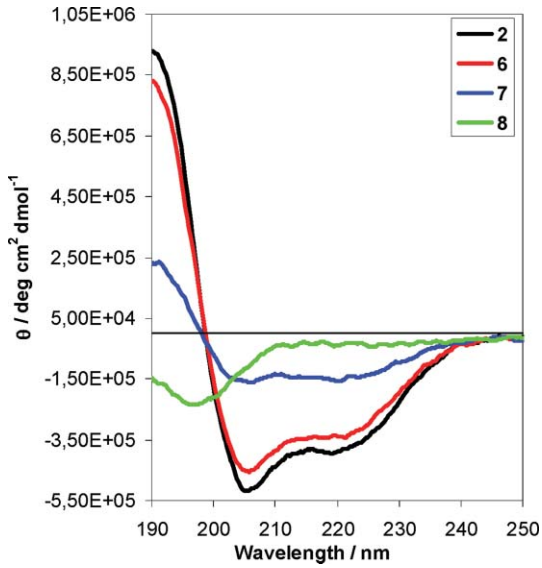


Fig. 3 CD spectra of compounds **2**, **6–8** (50 μM) in a buffer solution Tris·HCl 10 mM, NaCl 50 mM, EDTA 1mM, pH 7.0.

investigate the mode of interaction, the selectivity (GC *vs.* AT), and the impact of the distance between the intercalating units as well as the influence of the peptidic framework (rigidity *vs.* adaptability). The modes of interaction of porphyrins with DNA have been studied by several groups and their results established three types of binding modes: intercalative binding, groove binding, and outside binding.[26] Intercalative binding has been found to occur dominantly at GC-rich regions, groove binding at AT-rich regions, and outside binding at both GC-rich and AT-rich regions.[18,27] During spectrophotometric titration with DNA, the intercalated porphyrin species has the following characteristics: (i) a large red shift of the Soret band (≥15 nm),[28,29] (ii) substantial hypochromicity (≥35%),[28,29] and (iii) an induced negative CD band in the Soret region.[18,29,30] In contrast, the groove binding porphyrin species has the following characteristics: (i) a small red shift in the Soret band (≤8 nm),[28,29] (ii) little hypochromicity or hyperchromicity of the Soret maximum,[28,29] and (iii) an induced positive CD band in the Soret region.[18,28,29] On the other hand, the outside binding porphyrin species is characterized by an induced conserved Soret region.[30]

Interaction of peptides **2**, **6–12** with various types of DNA (calf thymus DNA (CT-DNA), poly(dAdT)$_2$ and poly(dGdC)$_2$) was examined by spectrophotometric titration in the Soret region (UV and CD). The extraordinary extinction coefficient of the Soret band for the cationic porphyrins allowed spectrophotometric detection of porphyrin–DNA interactions at very low concentration (5 μM). Visible spectra of compounds **2**, **6–12** were recorded in the presence of an increasing amount of DNA in a TE buffer (Tris·HCl 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0) at $1/R$ values up to 50, where R denotes input ratio of [porphyrin] : [base pairs]).[29,30]

It has been shown previously that during titration with GC rich DNA (CT-DNA and poly(dGdC)$_2$), an important bathochromic shift ($\Delta\lambda_{max}$) and hypochromicity (H) with one set of isosbestic points were observed for the Soret band of the cationic monoporphyrin H_2TMPyP (Fig. 4).[23] These results corresponded to an intercalative binding and because of the presence of an isosbestic point throughout the titration, the optical contribution certainly came from two distinct species, free and bound porphyrin chromophores.[28] In contrast, when poly(dAdT)$_2$ was used, the bathochromic shift and the hypochromicity were less important (Fig. 4). These results were characteristic of a groove binding mode.

Titration of compounds **2**, **6–12** with poly(dGdC)$_2$ showed also an important decrease of the Soret band with strong bathochromic shifts ($\Delta\lambda_{max}$ = 16–22 nm) and important hypochromicities (%H = 43.2–49.8) (Fig. 4 and Table 1). The hypochromicity was determined by the equation $H = (A_f - A_b)/(A_f) \times 100$, where A_f and A_b represent the Soret absorbances of the free and bound porphyrins, respectively. These results are characteristic of intercalative binding and demonstrate that both porphyrins intercalate into GC-rich DNA since the spectral changes are comparable to those of the monoporphyrin. No isosbestic points were observed for peptides **2**, **6–12**, suggesting that the binding process is more than two steps and can be explained in terms of a collaboration effect. In contrast, during titration with poly(dAdT)$_2$, the intensity of the Soret band decreased much less for each compound studied. Bathochromic shifts from 4 to 8 nm and hypochromicities from 3.6 to 23.1% were observed (Table 1). These spectral changes indicate that peptides **2**, **6–12** interact with AT-rich DNA by groove binding. On the other hand, during titration with CT-DNA, intermediate spectral changes with bathochromic shifts from 8 to 14 nm and hypochromicities from 11.4 to 30.9% were observed for compounds **2**, **6–12** (Table 1). The intensity of the Soret band of each bis-porphyrin peptide decreased together with a red shift at the initial step, and then the intensity of the red shifted peak increased with further DNA additions. These results propose that in most cases, two modes of interaction are observed and could be explained by the probability of the second porphyrin finding a nearby GC-rich site for intercalation. When no GC-rich site can be found for intercalation in the neighborhood, the second porphyrin interacts with AT-rich sites *via* groove binding or simply by outside binding. Once again, no isosbestic points were observed for peptides **2**, **6–12**, suggesting a collaboration effect. The obtained results demonstrate that the length and structural properties of the peptidic framework do not seem to play an important role in the mode of interaction with various DNA.

Induced circular dichroism (ICD) in the Soret region is very helpful for analysis of the binding mode of an achiral porphyrin to chiral DNA.[18,31] Peptides **2**, **6–12** did not show any ICD in the

Table 1 UV–Vis spectral changes of peptides **2**, **6–12** in the presence of DNA

	Poly(dGdC)$_2$		Poly(dAdT)$_2$		CT-DNA	
Peptide	$\Delta\lambda_{max}$	%H	$\Delta\lambda_{max}$	%H	$\Delta\lambda_{max}$	%H
H_2TMPyP	20 nm	51.5	8 nm	26.7	16 nm	43.5
2	20 nm	44.6	8 nm	3.6	8 nm	26.5
6	18 nm	45.5	6 nm	8.1	8 nm	25.3
7	16 nm	44.9	4 nm	10.0	8 nm	26.6
8	20 nm	47.3	8 nm	16.8	12 nm	11.4
9	22 nm	43.2	6 nm	23.1	10 nm	30.9
10	18 nm	44.5	8 nm	18.1	12 nm	28.8
11	18 nm	49.8	6 nm	14.9	10 nm	28.7
12	22 nm	49.0	8 nm	5.4	14 nm	28.2

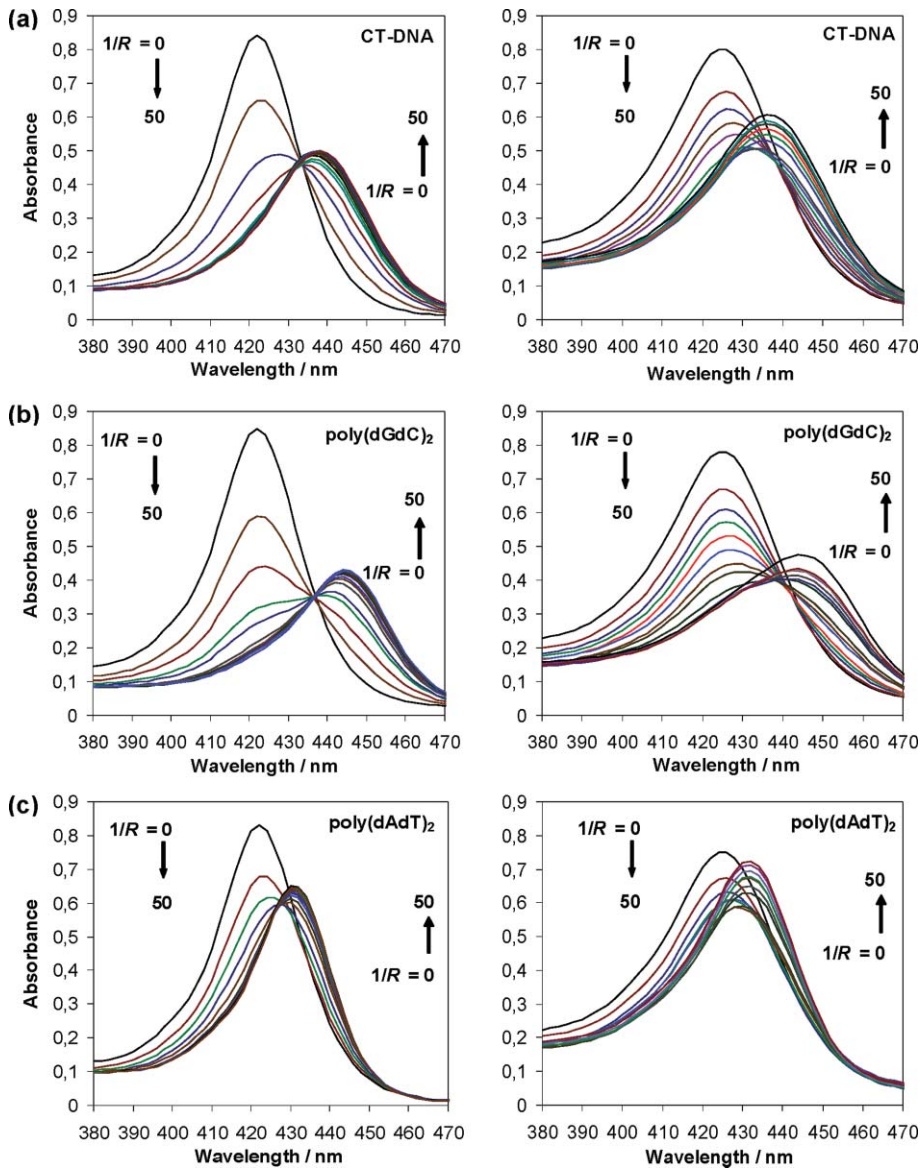


Fig. 4 Spectrophotometric titrations of H_2TMPyP (left) and **2** (right) with various DNAs; (a) with CT-DNA; (b) with poly(dGdC)$_2$; (c) with poly(dAdT)$_2$.

absence of duplex DNA, but characteristic spectra in the Soret region were induced with addition of DNA in buffer (Tris·HCl 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0). Fig. 5 shows the ICD spectra of the monoporphyrin H_2TMPyP and peptide **2** bound to CT-DNA, poly(dAdT)$_2$ and poly(dGdC)$_2$ at $1/R = 6$. In the presence of poly(dAdT)$_2$, the ICD spectrum for compound **2** comprised a small negative peak at 428 nm and a large positive peak at 444 nm corresponding to groove binding. In the presence of poly(dGdC)$_2$ and CT-DNA, a negative peak was induced at 447 and 442 nm, respectively, corresponding to intercalative binding. ICD spectra of compounds **6–12** bound to various DNAs showed similar profiles. The obtained results showed only one mode of interaction for peptides **2**, **6–12** in the presence of CT-DNA and do not support the groove binding mode theory for the second porphyrin. The data suggest instead an interaction by outside binding for some of the second porphyrins, since no spectral changes are observed for this mode of interaction compared to a positive peak for groove binding.

The binding studies with various DNAs clearly showed that intercalative binding occurs at GC-rich regions and groove binding at AT-rich regions.[18,29–32] The observed DNA binding properties for peptides **2**, **6–12** were all similar and suggest that the length and structural properties of the peptidic framework do not seem to play an important role for the mode of interaction with various DNA.

Conclusions

We have described a new class of bis-intercalating compounds composed of two cationic porphyrins attached to engineerable peptidic nanostructures. The design of the α-helical framework allows us to easily modify the orientation of the intercalating units and the distance between them. The solid-phase synthesis of the bis-porphyrin peptides allows a certain flexibility in the design, structure and intercalating units simply by replacing one or

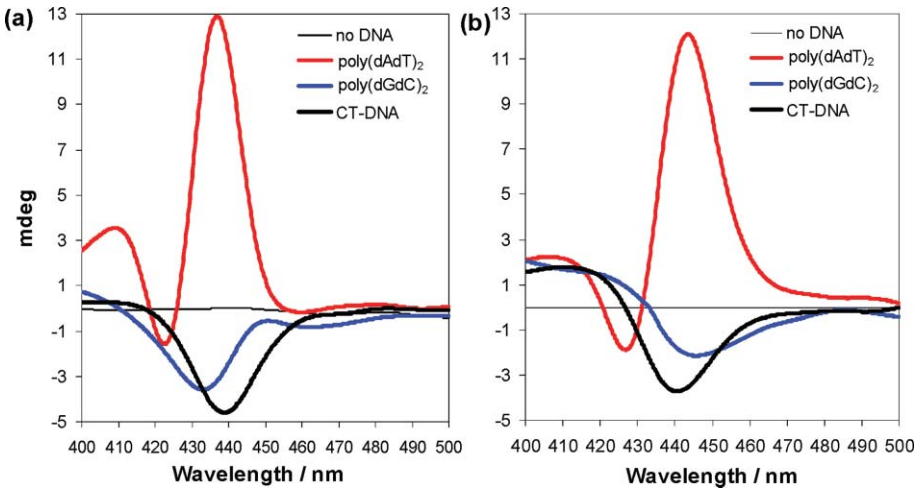


Fig. 5 Induced CD with CT-DNA, poly(dGdC)$_2$ and poly(dAdT)$_2$ at $1/R = 6$. (a) H_2TMPyP; (b) peptide **2**.

more amino acids in the sequence. The α-helical structure of the designed peptidic frameworks, observed by CD, shows that α-helical hydrosoluble peptides can be used as frameworks for the development of functional supramolecular devices. Using UV–visible titrations and induced CD for DNA binding studies with various DNA, we found that intercalative binding occurs at GC-rich regions and groove binding at AT-rich regions and that a collaboration effect (cooperativity) takes place. DNAse I foot-printing to determine the sequence specificity of the bis-porphyrin compounds and kinetic studies are currently in progress in our laboratory. Binding of the synthesized peptides to quadruplex DNA and inhibition of the telomerase are also being studied. Photonuclease activity of the compounds and their metal complex (Zn, Cu) derivatives will also be analyzed.

Experimental

Synthesis

5-*p*-Nitrophenyl-10-15-20-(tripyridin-4-yl)porphyrin. *p*-Nitrobenzaldehyde (85 mmol) and 4-pyridine carboxaldehyde (157 mmol) were added to a 1 L round bottom flask containing propionic acid (750 mL) and acetic anhydride (75 mL) at 110 °C. Pyrrole (208 mmol) was then added portionwise and the reaction mixture was refluxed for 1.5 h. The propionic acid and the acetic anhydride were removed by distillation until 75–100 mL was left in the flask. The remaining mixture was neutralized with a solution of 1 N NaOH on ice, filtered and washed with a solution of 1 N NaOH (3×) and with water (3×). The solid was dried overnight, dissolved in DCM and filtered. The DCM was removed under reduced pressure and the resulting purple solid was purified by silica gel chromatography (gradient of 2.5% to 10% ethanol–DCM) affording 2.8 g (4.22 mmol, 8%) of purple solid.^{1}H NMR (300 MHz; $CDCl_3$), $\delta = -2.7$ (s, 2H, NH pyrrole), 8.16–8.18 (m, 6H, 2,6 pyridine), 8.38–8.41 (d, 2H, H_{ortho} NO_2), 8.66–8.68 (d, 2H, H_{meta} NO_2), 8.77–8.92 (m, 8H, H pyrrole), 9.05–9.15 (m, 6H, 3,5 pyridine); EI-MS (70 eV): 662 (M^+); UV–visible ($CHCl_3$), λ/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 418 (353), 514 (18.2), 548 (6.3), 588 (5.8), 644 (1.9).

5-*p*-Aminophenyl-10-15-20-(tripyridin-4-yl)porphyrin (3). The nitro-porphyrine (4 mmol) was dissolved in a solution of 6 N HCl (400 mL) followed by addition of $SnCl_2$ (20 mmol) and the mixture was stirred at room temperature for 24 h. The reaction mixture was neutralized with a solution of 1 N NaOH and NaOH on ice. The aqueous basic solution was extracted with DCM and the resulting organic phase was washed with water (3×). The organic phase was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure affording 2.5 g (3.96 mmol, 98%) of purple solid. ^{1}H NMR (300 MHz; $CDCl_3$), $\delta = -2.7$ (s, 2H, NH pyrrole), 7.05–7.10 (d, 2H, $J = 5$ Hz, H_{ortho} NH_2), 7.95–8.00 (d, 2H, $J = 5$ Hz, H_{meta} NH_2), 8.16–8.20 (m, 6H, 2,6 pyridine), 8.80–8.88 (m, 6H, 3,5 pyridine), 9.05–9.12 (m, 8H, H pyrrole); EI-MS (70 eV): 632 (M^+); UV–visible ($CHCl_3$), λ/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 420 (255), 514 (15), 548 (7.7), 588 (5), 644 (6.4).

N^{α}-*t*-Butyloxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tripyridin-4-yl)porphyrin)-L-glutamic acid methyl ester (4). Boc-Glu-OMe **3** (3.8 mmol) was dissolved in dry DCM at 0 °C followed by addition of triethylamine (4.6 mmol) and ethyl chloroformate (3.8 mmol). The reaction mixture was stirred for 30 min under N_2 atmosphere and evaporated to dryness under reduced pressure. The resulting solid was redissolved in dry DCM at 0 °C followed by the addition of a solution of porphyrine **1** (1.3 mmol) in dry DCM and triethylamine (4.6 mmol). The reaction mixture was stirred for 1 h at 0 °C and 1 h at room temperature under N_2 atmosphere. The reaction mixture was washed with a solution of 5% $NaHCO_3$ (m/v) (3×) and water (3×). The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The resulting solid was purified by silica gel chromatography (2.5% to 10% ethanol–DCM) to afford 0.91 g (1.04 mmol, 80.3%) of purple solid.^{1}H NMR (300 MHz, DMSO-d_6), $\delta = -2.98$ (s, 2H, NH pyrrole), 1.45 (s, 9H, *t*-butyl), 1.95–2.05 (m, 1H, $H^{\beta}2$), 2.15–2.25 (m, 1H, $H^{\beta}1$), 2.59–2.68 (m, 2H, H^{γ}), 3.75 (s, 3H, OCH_3), 4.14–4.21 (m, 1H, H^{α}), 7.41–7.45 (d, 1H, $J = 6$ Hz, NH), 8.05–8.12 (d, 2H, $J = 8$ Hz, H_{ortho} NHCO), 8.14–8.18 (d, 2H, $J = 8$ Hz, H_{meta} NHCO), 8.22–8.30 (m, 6H, 2,6 pyridine), 8.87–8.98 (m, 8H, H pyrrole), 9.05–9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH_8); ESI-MS: m/z 876 = $(M + H)^+$;

UV–visible ($CHCl_3$), λ/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 420 (344), 516 (16.9), 552 (8.2), 590 (6.0), 642 (3.7).

N^{α}-*t*-Butyloxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tripyridin-4-yl)porphyrin)-L-glutamic acid. Boc-Glu(TPyP)-OMe **4** (0.8 mmol) was dissolved in THF at 0 °C followed by the addition of a solution of 1 N NaOH (3.2 mmol). The reaction mixture was stirred at room temperature until complete conversion was observed and the THF was evaporated under reduced pressure. The remaining aqueous phase was extracted with DCM (2×) combined with the addition of acetic acid. The acetic acid breaks the emulsion and pushes the N^{α}-protected amino acid in the organic phase. The colored organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining acetic acid was co-evaporated with toluene (4×) under reduced pressure or lyophilized. The resulting purple solid was purified by short column silica gel chromatography (DCM, 10% ethanol–DCM and finally MeOH–DCM (1 : 1)) to afford 620 mg (0.72 mmol, 90%) of purple solid. ^{1}H NMR (300 MHz, DMSO-d_6), $\delta = -2.98$ (s, 2H, NH pyrrole), 1.45 (s, 9H, *t*-butyl), 2.01–2.19 (m, 2H, H^{β}), 2.52–2.58 (m, 2H, H^{γ}), 3.85–3.91 (m, 1H, H^{α}), 6.38–6.42 (d, 1H, $J = 6$ Hz, NH Glu), 8.05–8.18 (m, 4H, H_{ortho} NH + H_{meta} NH), 8.22–8.30 (m, 6H, 2,6 pyridine), 8.87–8.98 (m, 8H, H pyrrole), 9.05–9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH^{δ} Glu); ESI-MS: m/z 862 = (M + H)$^+$; UV–visible (MeOH), λ/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 416 (265), 512 (12.9), 546 (5.9), 588 (4.0), 650 (4.9)

N^{α}-9-Fluorenylmethoxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tripyridin-4-yl)porphyrin)-L-glutamic acid (5). Boc-Glu(TPyP)-OH (0.7 mmol) was dissolved in a solution of 4 M HCl–dioxane (1.4 mmol) at 0 °C and stirred for 30 min at room temperature. The reaction mixture was evaporated to dryness and the resulting solid dried under vacuum. The N^{α}-deprotected amino acid was dissolved in H_2O–MeCN (1 : 9) at 0 °C followed by addition of DIEA (4.2 mmol) and Fmoc-OSu (0.77 mmol). The reaction mixture was stirred for 3 h at room temperature and the MeCN evaporated under reduced pressure. The resulting solution was extracted with DCM (2×) combined with the addition of acetic acid as described previously. The colored organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining acetic acid was co-evaporated with toluene (4×) under reduced pressure or lyophilized. The resulting purple solid was purified by short column silica gel chromatography (10% ethanol–DCM and MeOH–DCM (1 : 1)) to afford 550 mg (0.56 mmol, 80%) of purple solid. ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = -2.98$ (s, 2H, NH pyrrole), 2.05–2.35 (m, 2H, H^{β}), 2.54–2.60 (m, 2H, H^{γ}), 3.97–4.01 (m, 1H, H^{α}), 4.20–4.41 (m, 3H, H9 fluorenyl + OCH_2-fluorenyl), 6.90–6.95 (d, 1H, $J = 6$ Hz, NH Glu), 7.32–7.41 (m, 4H, H_{arom} fluorenyl), 7.70–7.78 (d, 2H, H_{arom} fluorenyl), 7.80–7.88 (d, 2H, H_{arom} fluorenyl), 8.05–8.26 (m, 10H, H_{ortho} NH + H_{meta} NH + 6H, 2,6 pyridine), 8.71–8.95 (m, 8H, H pyrrole), 9.00–9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH^{δ}); ESI-MS: m/z 985 = (M + H)$^+$; UV–visible (MeOH), λ/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 416 (181), 514 (10.7), 548 (6.7), 590 (5.3), 650 (5.0).

General procedure for the synthesis of peptides 2 and 6–12. All peptides were synthesized manually using standard solid-phase peptide chemistry with Fmoc-protected amino acids on Wang resin (0.7 mmol g^{-1}).[25] Coupling of Fmoc-protected amino acids (5 eq.) was achieved with DIC (5 eq.), HOBt (5 eq.) and DIEA (6 eq.) in DMF during 30 min at 0 °C. Fmoc-Glu(TPyP)-OH **5** (1.5 eq.) was coupled using HATU (1.5 eq.) and DIEA (2 eq.) in DMF for 3 h. Fmoc protecting groups were removed with 20% piperidine in DMF (2 × 15 min). After each coupling and deprotection, the resin was washed thoroughly with DMF (3×), MeOH (3×) and DMF (3×). *N*-Methylation of the porphyrin side chains on a solid support was achieved with a mixture of iodomethane and DMF (6 : 94) for 24 h followed by washing with DMF (5×). The peptides were cleaved from the resin with a mixture of trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water (95 : 2.5 : 2.5) for 4 h at room temperature. The resin was filtered and washed with DCM (2×) and MeOH (3×). The filtrate was evaporated under reduced pressure and the resulting mixture precipitated with diethyl ether. The solid was washed with diethyl ether (3×) and purified by exclusion chromatography using Sephadex® LH-20 in MeOH or semi-preparative RP-HPLC (C-18, MeCN 10–50% in 30 min).

H-Ala-Lys-Glu(TMPyP)-Ala-Ala-Glu-Lys-Ala-Ala-Ala-Glu-Lys-Ala-Ala-Glu(TMPyP)-Glu-Ala-OH (2). ESI-MS, m/z: calc. 3008.4 (M + H)$^+$, found 3008.5. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 422 (172). RP-HPLC $t_R = 9.6$ min (10–50%).

H-Ala-Glu(TMPyP)-Lys-Ala-Ala-Ala-Glu-Lys-Ala-Ala-Ala-Glu-Lys-Ala-Ala-Glu(TMPyP)-Glu-Ala-OH (6). ESI-MS, m/z: calc. 3079.5 (M + H)$^+$, found 3078.8. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 424 (121). RP-HPLC $t_R = 9.6$ min (10–50%).

H-Ala-Lys-Ala-Glu(TMPyP)-Ala-Glu-Lys-Ala-Ala-Glu(TMPyP)-Glu-Ala-OH (7). ESI-MS, m/z: calc. 2537.9 (M + H)$^+$, found 2537.6. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 424 (100). RP-HPLC $t_R = 9.5$ min (10–50%).

H-Ala-Glu(TMPyP)-Lys-Ala-Ala-Glu(TMPyP)-Glu-Ala-OH (8). ESI-MS, m/z: calc. 2138.4 (M + H)$^+$, found 2138.6. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 424 (121). RP-HPLC $t_R = 9.4$ min (10–50%).

H-Ala-Glu(TMPyP)-Gly-Glu(TMPyP)-Ala-OH (9). ESI-MS, m/z: calc. 1796.1 (M + H)$^+$, found 1797.0. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 422 (187). RP-HPLC $t_R = 6.6$ min (10–50%).

H-Ala-Glu(TMPyP)-Gly-Gly-Gly-Glu(TMPyP)-Ala-OH (10). ESI-MS, m/z: calc. 1910.2 (M + H)$^+$, found 1910.8. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 424 (124). RP-HPLC $t_R = 7.4$ min (10–50%).

H-Ala-Glu(TMPyP)-Gly-Gly-Gly-Gly-Gly-Glu(TMPyP)-Ala-OH (11). ESI-MS, m/z: calc. 2024.3 (M + H)$^+$, found 2023.1. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 422 (151). RP-HPLC t_R = 9.8 min (10–50%).

H-Ala-Glu(TMPyP)-Gly-Gly-Gly-Gly-Gly-Gly-Gly-Glu(TMPyP)-Ala-OH (12). ESI-MS, m/z: calc. 2138.4 (M + H)$^+$, found 2137.9. UV–visible (buffer TE 10 mM, NaCl 50 mM, EDTA

1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ M^{-1} cm^{-1}): 424 (259). RP-HPLC $t_R = 9.7$ min (10–50%).

Conformational studies by circular dichroism

Samples were prepared in a buffer Tris·HCl 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0 with a peptide concentration of 50 μM. CD spectra were recorded at 25 °C on a Jasco J-710 spectropolarimeter with at least 20 scans from 250 to 190 nm. Spectra are expressed in mean residue molar ellipticity [θ] (mdeg·cm^2/dmol) and are corrected for the background. The molecular weight used was 113 g mol^{-1}. The pathlength of the quartz cell was 0.05 cm.

DNA binding studies

Sample preparation. The concentrations of polynucleic acids for measurements were determined spectrophotometrically with $\varepsilon_{260} = 1.31 \times 10^4$ M^{-1} cm^{-1} (as base pair) for CT-DNA, $\varepsilon_{262} = 6.6 \times 10^3$ M^{-1} cm^{-1} (as base pair) for poly(dAdT)$_2$ and $\varepsilon_{256} = 8.4 \times 10^3$ M^{-1} cm^{-1} (as base pair) for poly(dGdC)$_2$.[31] Bis-porphyrin peptide concentrations for measurements were also determined spectrophotometrically using the ε determined during compound characterization. All measurements were carried out in a buffer Tris·HCl 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0.

UV–visible titration. Spectroscopic measurements were carried out on a Hewlett Packard HP 8452-A spectrophotometer. Absorbances were measured in a 1 mL, 10 mm pathlength quartz cuvette, using 0.5 mL of a 5 μM solution of porphyrin (2.5 μM of bis-porphyrin peptide) and adding successive aliquots of a solution containing the same concentration of porphyrin and a 100 fold excess of DNA (500 μM, $1/R = 100$).

Induced circular dichroism. CD measurements were performed with a 1.5 mL, 0.5 cm cylindrical quartz cuvette, using the same general procedure as above to analyse the mixture with different $1/R$ values. Aliquots of a 250 μM DNA solution were added successively to 0.75 mL of a 25 μM solution of porphyrin (12.5 μM of bis-porphyrin peptide). All the spectra were obtained by an average of 20 accumulations from 500 to 400 nm.

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