Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

DNA Recognition with Large Calixarene Dimers and Varying Spacers

Caroline J. Breitkreuz[a]; Reza Zadmard[a]; T. Schrader[a]
[a] Fachbereich Chemie der Universität Duisburg-Essen, Essen, Germany

To cite this Article Breitkreuz, Caroline J. , Zadmard, Reza and Schrader, T.(2008) 'DNA Recognition with Large Calixarene Dimers and Varying Spacers', Supramolecular Chemistry, 20: 1, 109 — 115
To link to this Article: DOI: 10.1080/10610270701747040
URL: http://dx.doi.org/10.1080/10610270701747040

Supramolecular Chemistry, January–March 2008 Vol. 20 (1–2), pp. 109–115

Taylor & Francis
Taylor & Francis Group

DNA Recognition with Large Calixarene Dimers and Varying Spacers

CAROLINE J. BREITKREUZ, REZA ZADMARD and T. SCHRADER*

Fachbereich Chemie der Universität Duisburg-Essen, Universitätsstr. 5, 45117 Essen, Germany

(Received 2 August 2007; Accepted 14 October 2007)

INTRODUCTION

DNA-binding molecules constitute a large part of all anticancer drugs, emphasising their paramount medicinal significance [1]. Very often, the intensive study of natural products with respect to their binding characteristics paved the way towards a systematic elaboration of more potent drugs, with the ultimate goal of achieving high sequence selectivity. DNA recognition in nature mainly follows four different paths: efficient binding agents either target the phosphodiester backbone, intercalate into the base pairs, or occupy the minor or major groove. Natural highly charged polyamines, such as spermine and spermidine, which are present in cell nuclei in micromolar concentrations, non-specifically attack the phosphodiester backbone and form multiple salt bridges [2]. This is also the domain of numerous unspecific cationic DNA binders; thus, amphiphiles, dendrimers and polymers have been developed as transfection agents [3]. Polythiophenes, which also recognise the DNA/RNA phosphate backbone, have recently been presented as colour sensors for the hybridisation of ssDNA [4]. Small molecules usually intercalate or insert into the minor groove [5]. Flat aromatic cations, such as aminoacridines and actinomycin, or bis-intercalators, such as sandramycin, seek π-stacking stabilisation by maximal base-pair overlap and intercalate preferentially into pyrimidine–purine sites, with the general consequence of DNA distortion [6]. Polyamides with repeating pyrrole units and terminal protonated nitrogens such as distamycin represent the prototype of minor groove binders; during their investigation, the origin of their marked AT preference was identified as shape selectivity for the narrow groove walls, optimising van der Waals interactions. AT-rich regions are narrow (3–4 Å), whereas GC-rich tracts are much wider (≤8 Å). Importantly, it was found that groove binders generally do not significantly change the DNA conformation. Unravelled tunable sequence selectivity and binding efficiency was later achieved with hairpin polyamides presented by the Dervan group [7]. Although most regulatory proteins dock onto the wider, shallower major groove, which offers much more hydrogen bonding sites and thus more information, very few natural products are known to bind selectively to this important region. In addition, most of them gain their free binding energy from intercalation and/or further alkylate DNA bases [8]. This is also the case with the very few artificial major groove binders known today, such as the crosslinking agents: nitrogen mustard, melphalan or cisplatin [9]. Interestingly enough, the zinc finger [10] and the leucine zipper motif [11], two typical protein domains which fit snuggly into DNA's major groove, have similar dimensions (averaged α-helix diameters of ~1.3 nm) and also a comparable overall topology of a large basic cylinder with a polar protic face reading the nucleic bases on the groove's floor. Recently, Hannon *et al.* presented the first example of an artificial noncovalent major groove binder, a tetracationic metallo-supramolecular cylinder with a size of ~2 × 1 nm [12]. This tetracationic $[Fe_2L_3]^{4+}$ ligand binds tightly to the floor of the major groove and induces DNA bending and intramolecular coiling. Later, the same group

*Corresponding author. Fax: + 49 201 183 3081. E-mail: Thomas.Schrader@uni-due.de

ISSN 1061-0278 print/ISSN 1029-0478 online © 2008 Taylor & Francis
DOI: 10.1080/10610270701747040

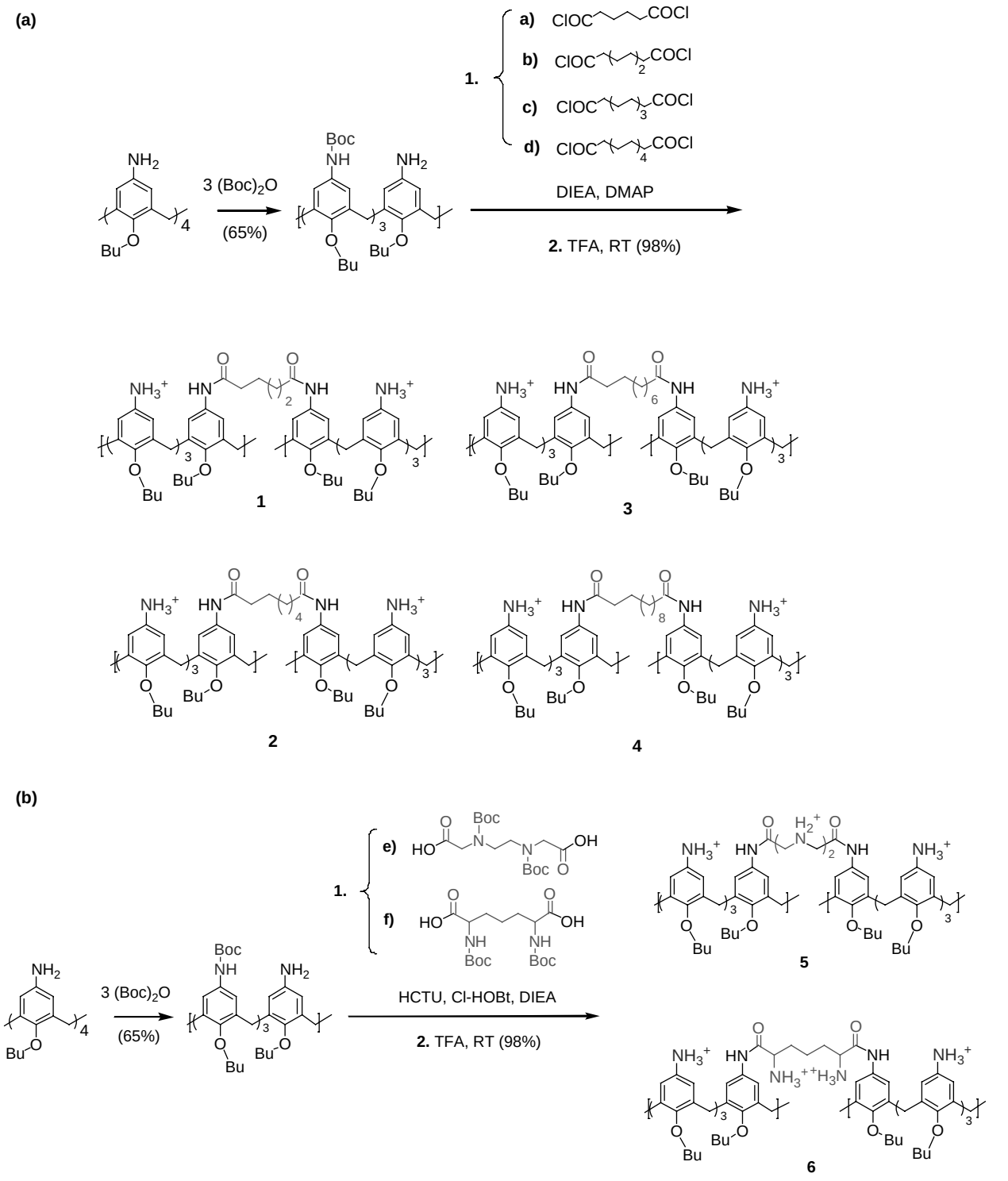

FIGURE 1 (a) Synthesis of calixarene dimers with alkyl spacers of varying length (**1**–**4**). (b) Synthesis of calixarene dimers with varying cationic bridging units (**5**, **6**).

found that this molecule also stabilises three-way junctions in DNA strands [13].

RESULTS AND DISCUSSION

We have recently discovered that dimeric calixarenes [14] with six anilino groups at their upper rims bind tightly to double-stranded DNA molecules, irrespective of their number of base pairs [15]. Binding occurs without significant (de)stabilisation or conformational distortion, as evidenced by unchanged melting curves and CD spectra. It is, however, accompanied with drastic changes in UV absorption intensities, indicating a specific interaction with the chromophores of the nucleic bases. Such inverted

and magnified UV absorbance is not observed with similar guanidinium-based calixarene monomers, recently reported by the Ungaro group. They achieved strong DNA binding and subsequent transfection with various guanidinium-decorated calixarene derivatives; these, however, most likely attach to the double-stranded phosphodiester backbone, by utilising multiple electrostatic interactions, as evidenced by AFM techniques [16]. Since calixarenes are too large for intercalation or minor groove insertion, and the anilino groups are hardly protonated at neutral pH, strong experimental evidence is produced for major groove binding [17]. Specifically, pK_a titrations on the ammonium calix[4]arenes revealed decreasing pK_a values for each single NH_3^+ group starting from p$K_1 = 5.0$ and p$K_2 = 4.5$ down to even lower basicities. From these, it was calculated that at pH 7 only one group of each calixarene in the dimer is protonated, resulting in a maximum net charge of +2. The assumed binding mechanism was further supported by complete displacement of intercalated ethidium bromide as well as a superior interaction with RNA, whose major and minor grooves have similar dimensions as that of the calixarene ligand [18]. It was reasoned that this new class of synthetic major groove binders has the potential of sequence-selective DNA recognition, with the prospect of deliberate specific interference with gene expression.

In this respect, two immediate questions must be answered. [1] In order to distinguish between gene promoters, a base sequence of at least 5–6 bp must be spanned and specifically recognised. However, if the simple alkyl spacer is elongated from 4 to 10 atoms in the bridge, will this lead to a significant loss of affinity for DNA's major groove? [2] In order to compete with regulatory proteins, affinities must simultaneously be optimised up to the nanomolar level. Additional hydrogen bonds between polar groups in the bridge and the nucleic bases at the floor of DNA's major groove may not suffice and should be further complemented by favourable strong noncovalent interactions. Will, for example, additional positive charges introduced into the bridging unit tighten the complex between DNA and its calixarene ligand?

After the prototype molecule **1**, which inserted into the major groove of 12 bp dsDNA with 10 μM affinities, we have now synthesised a whole series of related compounds with varied bridging units. In one series, the alkyl bridge between both calixarenes was varied in successive steps of C-2 ethylene units from 4 to 6 and 8 up to 10 carbon atoms. The longest derivative should be able to span 7 bp of the major groove. In a second series, we introduced two full positive charges directly into the bridging alkyl chain or alternatively into its close vicinity. From these derivatives, we hoped to receive the answers on the above-detailed questions.

The synthetic routes followed the main strategic path, already outlined in our preliminary communication. Construction of a tri-Boc-protected tetraamino-calix[4]arene was followed by double amide coupling with doubly activated diacids. A final deprotection of all six urethanes with TFA yielded the target molecules **1**–**4** as hexa(trifluoroacetate) salts. Thus, the first series was prepared from diacid dichlorides with central C_4, C_6, C_8 and C_{10} carbon chains (Fig. 1a). In spite of their increasing lipophilicity, all these compounds could be dissolved completely in aqueous Hepes buffer with 50% added methanol. They all carry two positive charges at neutral pH.

For a medium size spacer with internal cationic groups, we chose a linear no natural amino acid derivative, i.e. ethylene diamine diacetic acid which corresponds to a diaza-C_6 spacer. Attachment of the cations in the close vicinity of the spacer's main chain was achieved by introducing a double branch into the amino acid molecule—as in pimelic acid. Both bis(amino acids) were activated with HCTU/Cl-HOBt and doubly coupled to the triprotected calixarene. [19] Since the additional amino groups were likewise protected as Boc moieties, they were removed simultaneously with all the other calixarene Boc groups leading directly to the desired target molecules **5** and **6**. These carried two full additional positive charges, i.e. they were isolated as octacations (Fig. 1b). Unfortunately, the two additional charges did not suffice for rendering the whole calixarene dimers water soluble. Thus, for a better comparison, all consecutive DNA-binding experiments were carried out in the same 1:1 water/methanol mixture containing 1mM Hepes buffer. At neutral pH, the two representatives of series II thus carry four positive charges.

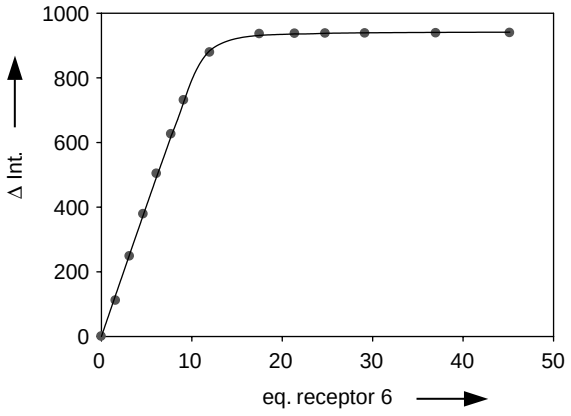

FIGURE 2 Binding isotherm for the complexation of 12 bp dsDNA with tetracationic pimelic acid-based calixarene dimer **6**. Note the sharp kink around the equivalence point indicating extremely strong binding ($K_a = 6 \times 10^6\,M^{-1}$).

TABLE I Stoichiometries and association constants obtained from fluorescence titration experiments between fluorescein-labelled 12 bp dsDNA and calixarene dimers **1**–**6**

Entry	Receptor	DNA	K_a (M^{-1})	Stoichiometry
1	Dimer **1**	ds Fl-DNA (12 bp, 1 mM Hepes*)	8×10^5 (±23%)†	17:1‡
2	Dimer **2**	ds Fl-DNA (12 bp, 1 mM Hepes*)	7×10^5 (±25%)†	24:1‡
3	Dimer **3**	ds Fl-DNA (12 bp, 1 mM Hepes*)	8×10^5 (±24%)†	18:1‡
4	Dimer **4**	ds Fl-DNA (12 bp, 1 mM Hepes*)	9×10^5 (±20%)†	21:1‡
5	Dimer **5**	ds Fl-DNA (12 bp, 1 mM Hepes*)	2×10^6 (±22%)†	18:1‡
6	Dimer **6**	ds Fl-DNA (12 bp, 1 mM Hepes*)	6×10^6 (±22%)†	12:1‡

*Hepes buffer in methanol/water (1:1). †Statistical error from curve fitting. ‡From Job plots: ratios are given in receptor:DNA.

Fluorescence titrations of **1**–**6** were carried out with a fluorescein-labelled 12 bp DNA [20]. In all cases, the strong fluorescence emission was fully quenched during the titration process, indicating a tight interaction with the calixarenes. From Job plots, relatively large stoichiometric ratios were determined, indicating again a substantial amount of ligand self-aggregation before or concomitant with major groove insertion [21]. Very smooth curves were obtained in all cases, which could be nicely fitted to a standard nonlinear algorithm (Fig. 2). The respective association constants were normalised to a 1:1 complexation event, assuming a non-cooperative process; they are summarised in Table I. At first glance, it becomes obvious that elongation of the central alkyl spacer chain from C_4 to C_{10} does not lower the affinity of these extended dimers for DNA's major groove. Although no clear trend is observed, the four corresponding K_d values are all close to micromolar, with small deviations. Interestingly, the stoichiometric factors even seem to increase with increasing overall spacer length of the ligands. This is counterintuitive and may indicate an enhanced self-aggregation of the extended calixarene dimers via their growing central alkyl chains. Thus, it can be concluded that even the significant chain extension in the bridging unit even from C_4 to C_{10} does not lower the calixarene dimer's affinity towards DNA—an important prerequisite for the construction of ligands suitable for spanning 5–6 bp.

Fl-DNA (double strand) with 12 bp: 5′-[Fl]GTG ACG AAC CTC-3′ 5′-GAG GTT CGT CAC-3′.

Compounds **5** and **6** clearly demonstrate the expected affinity increase due to an elevated charge state. However, this effect is much more pronounced in the branched pimelic acid derivative **6** than in the linear diglycine **5**. We tentatively explain this difference with an optimal placement of the additional positive charges within DNA's major groove [22]. It is well known that minor groove binders are drawn towards the centre of negative charge density, which is located just above the nucleic bases [23]. The affinity increase due to the additional two positive charges is not dramatic, but still amounts to roughly one order of magnitude (K_d (6) ~160 nM).

The high binding constants are reflected in *ethidium bromide* displacement experiments. Similar to the preliminary tests with **1**, all the other calixarene dimers in series I (**1**–**4**) and II (**5** and **6**) are able to completely expel the intercalated dye from DNA double strands (Fig. 3) [24]. Both series display moderate C_{50} values, but especially series II is characterised by extremely low CE_{50} values, 500–1000 times lower than those of spermine (Table II). These findings underline again the importance of hydrophobic forces for the inclusion process and rule out any significant DNA backbone recognition via ion pairs [25].

Absorption intensities in *UV–vis spectra* were again drastically shifted towards higher values for complexes with dsDNA, whereas much lower extinction coefficients were recorded for ssDNA complexes. This behaviour is generally reflected in inverted and enlarged melting curves (Fig. 4). However, contrary to our original observations with **1** and a 20 bp dsDNA, all new calixarenes derivatives significantly stabilise the short 12 bp DNA duplex (DNA: $T_m = 34$°C; DNA complexes: $T_m = 52$–58°C). Since now even **1** shifts the melting temperature from 34 to 53°C, the stabilisation seems to depend mainly on the total DNA length rather than on the dimer structure. We tentatively assume

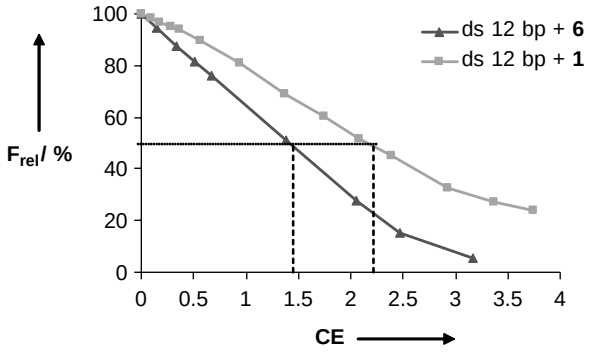

FIGURE 3 Ethidium bromide displacement from 12 bp dsDNA ($c = 1$ μM) by dimers **1** and **6** (H_2O/MeOH 1:1, 9.4 mM NaCl, 2 mM Hepes, pH 7.1). F_{rel}, relative fluorescence emission intensity; CE, charge excess ratio.

TABLE II Ethidium bromide displacement assay for 12 bp dsDNA and **1** and **6**

Ligand/DNA	[NaCl] (mM)	Nominal charge	C_{50} (μM)	CE_{50}
1 + 12 bp	9.4	+2	28	2.3
6 + 12 bp	9.4	+4	9	1.5

that incorporation of aggregated calixarenes into DNA's major groove clamps together the dangling ends of the short DNA duplex. Obviously, the new calixarene derivatives **2**–**6** in both series also interact with the nucleic base chromophores through insertion into the major groove. At this point, the unusual UV–vis extinctions of DNA complexed by our new calixarene dimers and the concomitant magnified melting curves can only be explained by a strong noncovalent interaction between ligand and host chromophores. No direct experimental evidence was found for **5** and **6** to stabilise the duplex by electrostatic crosslinking, similar to spermine and spermidine [26].

CD curves were measured for **1** and **6** in their complexes to dsDNA at micromolar concentrations (Fig. 5). A close inspection reveals that both ligands lead to a drastic conformational change towards a geometry of the A-DNA type. The most pronounced change occurs with **1**, while the complex with tetracationic **6** seems to be an intermediate on the way from B- to A-DNA [27]. Clearly, the inclusion of the (aggregated) DNA ligand induced a conformational twist in the DNA molecule in order to optimise supramolecular contacts. At this point, we can only speculate whether the contraction of DNA's major groove and the elimination of the minor groove are pointing to an ideal conformation for the inclusion of the monomeric ligand in a cleft of just

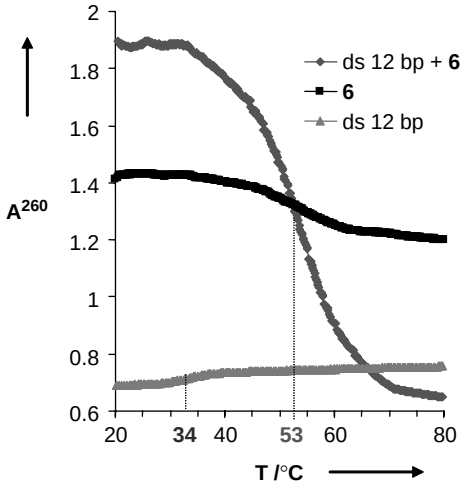

FIGURE 4 UV–vis-melting curve for pimelic acid derivative **6** in its complex with 12 bp dsDNA.

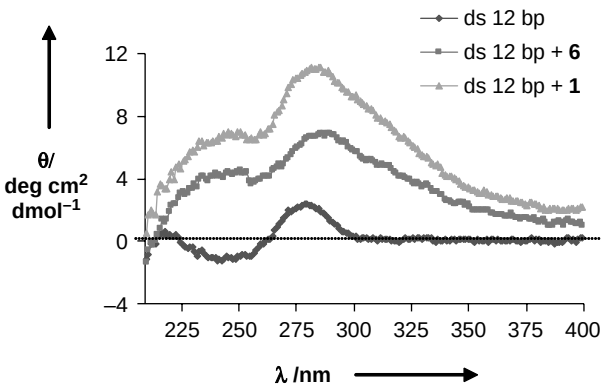

FIGURE 5 CD spectra for pure 12 bp-DNA (B-DNA) and its complexes with **1** and **6** (~ A-DNA).

the right size (similar to RNA clefts). Interestingly, the nature of the bridge allows a certain conformational control of the complexed DNA. This may become valuable for a rational design of sequence-selective DNA binders. A similar behaviour has already been observed by Hannon with his supramolecular Fe(II) cylinder [12]. Finally, this result is another indication for a major groove binding of our artificial calixarene dimers. Most polymerases and many endonucleases lead to a similar change if they bind to DNA's major groove [28]. Further implications of this induced conformational change must be elucidated in the future.

In the future, we will try to calculate the structure of our new DNA complexes with standard modelling software (MacroModel, SYBYL). Molecular mechanics calculations, Monte Carlo and MD simulations should reveal possible influences on DNA conformation while binding into the major groove. In order to find out more about a potential self-aggregate, we will conduct a full GPC analysis with **1**, **6** and 12 bp dsDNA. Absolute molar masses referenced against pullulan standards are expected to exceed by far the expected values for monomeric species and pointed to tetra- or penta-meric oligomers. Their structure remains to be elucidated, but involves most likely van der Waals contacts between the butoxy tails and the extended alkyl spacers. Finally, non-denaturing gel electrophoresis will be tried on the complex between 12 bp dsDNA and **1** or **6**. Thus, direct information can be gained on the mobility of various calixarene complexes with DNA in the electric field.

CONCLUSION AND OUTLOOK

In this investigation on a series of new dimeric aminocalixarenes as major groove binders, it could be shown that even a 10-atom alkyl bridge between both calixarenes does not weaken the complex formed with DNA. Additional positive charges

further stabilise the associate (~60-fold). Most importantly, the burial of the dimeric calixarenes into DNA's major groove is accompanied with a drastic conformational change towards an A-DNA geometry, which can to some extent be tuned by the nature of the bridging unit between both calixarenes. From this experimental basis, amino acids and heterocycles can now be introduced into the bridge in order to study their influence on DNA affinity and selectivity.

EXPERIMENTAL PART

Representative Example for the Synthesis of Cationic Calix[4]arene Dimers in Series II

*2,6-Bis[(*tert*-butoxycarbonyl)-amino]-pimelic Acid Bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetrabutoxycalix[4]arene-23 Amide]*

2,6-Bis[(*tert*-butoxycarbonyl)-amino]-pimelic acid (29 mg, 0.074 mmol) was dissolved in 40 ml of abs. dichloromethane/DMF (3:1) under an argon atmosphere. Cl-HOBt (63 mg, 0.37 mmol), HCTU (62 mg, 0.15 mmol) and 74 µl (58 mg, 0.45 mmol) of *N*-ethyldiisopropylamine were added to the solution and stirred for 10 min at 0°C. Then, the monomeric amine 5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-23-amino-25,26,27,28-tetrabutoxycalix[4]arene (150 mg, 0.15 mmol), dissolved in 4 ml dichloromethane/DMF (3:1), was added dropwise to the solution. After stirring for 24 h at an ambient temperature, the reaction mixture was washed three times with distilled water, dried over Na_2SO_4 and subsequently evaporated to dryness. The resulting residue was purified by chromatography over silica gel (ethylacetate/cyclohexane 1:2), $R_f = 0.57$. Yield: 141 mg (0.059 mmol; 80%). ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.00$ (m, 24H); 1.29 (m, 16H); 1.49 (s, 18H); 1.50 (s, 18H); 1.59 (s, 36H); 1.71 (m, 2H); 1.85 (m, 16H); 1.97 (m, 4H); 3.08 (m, 8H); 3.68 (m, 2H); 3.80 (m, 8H); 3.89 (m, 8H); 4.39 (m, 8H); 6.37–6.90 (m, 24H). ^{13}C NMR (125.6 MHz, $CDCl_3$): $\delta = 14.14$, 14.21, 14.25, 19.35, 19.55, 19.57, 22.83, 23.31, 26.84, 28.60, 29.84, 31.27, 32.06, 32.10, 32.16, 32.39, 61.99, 72.45, 74.94, 80.06, 80.25, 119.77, 120.10, 132.14, 153.26, 153.50. HRMS (ESI-pos., MeOH): calcd for $C_{135}H_{194}N_{10}O_{26} + Na^+$: $m/z = 2395.4090$, found: $m/z = 2395.4026$.

2,6-Diaminopimelic Acid Bis-{5,11,17-triamino-25,26,27,28-tetrabutoxycalix[4]arene-23-amide}-octakis-(trifluoroacetate) 6

A suspension of 0.27 mg of the octa-Boc-protected diaminopimelic acid-bridged dimer (11 µmol) was dissolved in 5 ml of abs. dichloromethane. To this solution, 5 ml of trifluoroacetic acid was added. The resulting solution was stirred for 22 h at an ambient temperature and subsequently evaporated to dryness. Yield: 28 mg (10 µmol; 95%). ^{1}H NMR (500 MHz, d_4-MeOH): $\delta = 1.02$ (m, 24H); 1.36 (m, 8H); 1.63 (m, 8H); 1.74 (m, 2H); 1.88 (m, 16H); 3.15 (m, 4H); 3.24 (d, 4H, $J = 13.5$ Hz); 3.75 (m, 8H); 3.93 (t, 2H, $J = 7.5$ Hz); 4.01 (m, 4H); 4.09 (m, 4H); 4.48 (m, 8H); 6.10 (m, 8H); 7.05 (s, 2H); 7.08 (s, 2H); 7.31, 7.33, 7.37, 7.46 (s, 4H). ^{13}C NMR (176 MHz, d_4-MeOH): $\delta = 14.49$, 14.57, 14.68, 14.74, 20.32, 20.39, 20.63, 20.94, 20.96, 23.89, 28.13, 30.92, 32.00, 32.17, 33.23, 33.47, 33.64, 33.84, 62.41, 73.68, 76.41, 76.48, 76.57, 117.44, 119.10, 122.44, 122.54, 122.62, 123.78, 133.37, 133.50, 136.34, 136.95, 138.06, 138.14, 139.52, 139.61, 156.07, 162.95, 163.14, 168.15. HRMS (ESI-pos., MeOH): calcd for $[C_{95}H_{194}N_{10}O_{26} + Na^+]^+$: $m/z = 1594.9895$, found: $m/z = 1593.4728$.

Oligonucleotide Structure, Which was Studied With These Experiments

Fl-DNA (double strand) with 12 bp: 5′-[Fl]GTG ACG AAC CTC-3′ 5′-GAG GTT CGT CAC-3′.

Fluorescence Titration Experiments

Fluorescence titrations were performed with 12 bp dsDNA in Hepes-buffered solutions (1 mM, pH 7.1) in concentrations as stated in the following. For convenience, the host compounds **1**–**6** were diluted in the DNA solutions, so that there was no change in the DNAconcentration throughout the entire titration. Seven hundred microlitres of these solutions were filled into cuvettes and up to 500 µl of the receptors solutions were added stepwise. The change of the emission intensity was measured as shown in the tables below. The binding isotherms were fitted by nonlinear regression methods, similar to the evaluation of NMR titrations.

Ethidium Bromide Displacement Assay

A Jasco FP-6500 spectrofluorimeter was used to record the data. Excitation of the sample was carried out in a 1.5 ml quartz cuvette with 546 nm excitation light and emission measured at 595 nm. The buffer solution contained 2 mmol of HEPES and 9.4 mmol of NaCl. The pH was adjusted to 7.1 with NaOH. Ethidium bromide was dissolved in the buffer to provide a 1.26 µM concentration (per base pair). After stirring for 5 min and standing for 1 min, the fluorescence was measured. CE_{50} values were calculated as a ratio of positive charges in the receptor versus negative charges of DNA in the respective complex mixture. The CE_{50} values are defined as the CE at 50% of the original fluorescence emission intensity gained by intercalated ethidium bromide.

UV–vis-melting Curves With 12 bp DsDNA and Dimer 6

The profiles of UV absorbance versus temperature were measured using a JASCO V-550 UV–vis spectrophotometer by monitoring the sample absorption at 260 nm. The sample cell was equipped with a water-circulated cell holder and the profiles of UV–vis absorption versus temperature were recorded. The sample cell was equipped with a Peltier type cell holder (ETC-273T). The concentration of the duplex DNA in each sample was 2 μM in 1 mM Hepes-buffered solution, pH 7.1. The experiments were carried out by increasing the temperature at a rate of 1.0°C/min from 20 to 80°C and the temperature was recorded every minute (equal to each additional 1.0°C).

Circular Dichroism Measurements

CD experiments were conducted in a quartz cuvette in 1 mM Hepes buffer (water/methanol 1:1; pH 7.1). The samples containing DNA (1 μM) or its complex with calixarene dimer **1** or **6** (30 μM) were scanned from 200 to 400 nm at a constant temperature of 20°C. Molar ellipticities were found to be consistent with pure B-DNA in its natural conformation and close to A-DNA for the complexes.

SUPPORTING INFORMATION

Full experimental details on synthesis and characterisation of the calixarene dimers and the fluorescence titrations.

Acknowledgements

We are indebted to the Haberhauer group (Essen) for measuring the CD spectra of micromolar DNA solutions with and without calixarene ligands. Funding by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

References

[1] Cragg, G. M.; Newman, D. J.; Snader, K. M. J. *J. Nat. Prod.* **1997**, *60*, 52.
[2] Thomas, Jr., G.;, et al., *Nucl. Acids Res.* **2000**, *28*, 3379.
[3] Miller, A. D. *Angew. Chem.* **2002**, *110*, 1862, Reviews(liposomes); Stiriba, S.E.; Frey, H.; Haag, R. *Angew. Chem.* **2002**, *114*, 1385 (dendrimers); Kakizawa, Y.; Kataoka, K. *Adv. Drug Deliv. Rev.* **2002**, *54*, 203 (polymers).
[4] Ho, H.-A.; Béra-Abérem, M.; Leclerc, M. *Chem. Eur. J.* **2005**, *11*, 1718.
[5] Tse, W. C.; Boger, D. L. *Chem. Biol.* **2004**, *11*, 1607.
[6] Wilson, W. D. In *Comprehensive Natural Products Chemistry*; Nakanishi, K., Barton, D., Eds.; Elsevier: New York, 1999;, For a review on DNA intercalators, see: pp 427.
[7] Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215, Neidle, S. *Nat. Prod. Rep.* **2001**, *18*, 291.
[8] Johnson, D. S.; Boger, D. L. In *Comprehensive Supramolecular Chemistry*; Murakami, Y., Ed.; Elsevier: Oxford, 1996;, For DNA binding agents, see: pp 73.
[9] Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1990**, *100*, 293, ReviewsLawley, P.D.; Philipps, D.H. *Mutat. Res.* **1996**, *355*, 13.
[10] Klug, A.; Rhodes, D. *Trends Biochem. Sci.* **1998**, *12*, 465, Elrod-Erickson, M., Benson, T.E., Pabo, C.O. *Structure* **1998**, *6*, 451.
[11] Landschultz, W. H.; Johnson, P. F.; McKnight, S. L. *Science* **1998**, *240*, 1759–1764, Fujii, Y., Toda, T., Yanagida, M., Hakoshima, T. *Nat. Struct. Biol.* **2000**, *7*, 889.
[12] Hannon, M. *Angew. Chem. Int. Ed.* **2002**, *99*, 5069, *Proc. Natl. Acad. Sci.* **2002**, *99*, 5069.
[13] Oleksi, A.; Blanco, A. G.; Boer, R.; Usón, I.; Aymamí, J.; Rodger, A.; Hannon, M. J.; Coll, M. *Angew. Chem. Int. Ed.* **2006**, *45*, 1227.
[14] Shinkai, S.; Ikeda, A. *Pure Appl. Chem.* **1999**, *72*, 275, Covalent unpolar calixarene dimers as C_{60} and C_{70} sensors:Haino, T.; Araki, H.; Fujiwara, Y.; Tanimoto, Y.; Fukazawa, Y. *Chem. Commun.* **2002**, 2148.
[15] Zadmard, R.; Schrader, T. *Angew. Chem. Int. Ed.* **2006**, *45*, 2703.
[16] Ungaro, R.; Casnati, A. *J. Am. Chem. Soc.* **2006**, *12*, 14529.
[17] Sundquist, W. I.; Lippard, S. J. *Coord. Chem. Rev.* **1999**, *100*, 293, Lawley, P.D.; Philipps, D.H. *Mutat. Res.* **1996**, *355*, 13. Review: Wilson, W.D. DNA Intercalators. In *Comprehensive Natural Products Chemistry*: Nakanishi, K., Barton, D., Eds.; Elsevier: New York, **1999**, pp. 427.
[18] RNA's minor groove is much shallower, but has the same diameter as its major groove.
[19] Davis, A. M.; Teague, S. J. *Angew. Chem. Int. Ed.* **1999**, *38*, 737, Sabatino, G.; Mulinacci, B.; Alcaro, M.C.; Chelli, M.; Rovero, P.; Papini, A.M. *Lett. Pept. Sci.* **2002**, *9*, 119; Marder, O.; Shvo, Y.; Albericio, F.; *Chim. Oggi-Chem. Today*, **2002**, *20*, 37.
[20] Schneider, H. J.; Kramer, R.; Simova, S.; Schneider, U. J. *Am. Chem. Soc.* **1988**, *110*, 6442, Wilcox, C.S. In *Frontiers in Supramolecular Chemistry*: Schneider, H.J.; Verlag Chemie: Weinheim, **1991**, p. 123.
[21] Job, P. *Ann. Chim.* **1928**, *9*, 113, Blanda, M.T.; Horner, J.H.; Newcomb, M.J. *Org. Chem.* **1989**, *54*, 4626.
[22] Edwards, K. J.; Jenkins, T. C.; Neidle, S. *Biochemistry* **1992**, *31*, 7104–7109, This is also exemplified by the non-intercalative binding mode of pentamidine and related drugs, which are drawn deeply into DNA's minor groove: (a)(b) Brown, D.G.; Sanderson, M.R.; Garman, E.; Neidle, S.J. *Mol. Biol.* **1992**, *226*, 481; (c) Laughton, C.A.; Tanious, F.; Nunn, C.M.; Boykin, D.W.; Wildon, W.D.; Neidle, S. *Biochemistry* **1996**, *35*, 5655.
[23] Hou, M. H.; Lin, S. -B.; Yuann, J. -M. P.; Lin, W. -C.; Wang, A. H. -J.; Kan, L. -S. *Nucleic Acids Res.* **2001**, *29*, 5121.
[24] Morgan, A. R.; Lee, J. S. *Nucleic Acids Res.* **1979**, *7*, 547–569, Shim, Y.-H., Arimondo, P.B., Laigle, A., Garbesi, A., Lavielle, S. *Org. Biomol. Chem.* **2004**, *2*, 915.
[25] Joester, D.; Losson, M.; Pugin, R.; Heinzelmann, H.; Walter, E.; Merkle, H. P.; Diederich, F. *Angew. Chem.* **2003**, *115*, 1524, Kostiainen, M.A., Hardy, J.G., Smith, D.K. *Angew. Chem.* **2005**, *117*, 2612.
[26] Tari, L. W.; Secco, A. S. *Nucleic Acids Res.* **1995**, *23*, 2065.
[27] Ivanov, V. I.; Minchenkova, L. E.; Minyat, E. E.; Frank-Kamenetskii, M. D.; Scholkina, A. K. *J. Mol. Biol.* **1974**, *87*, 817, Minyat, E.E., Ivanov, V.I., Kritzyn, A.M., Minchenkova, L.E., Scholkina, A.K. *J. Mol. Biol.* **1978**, *128*, 397.
[28] (a) Beabealashivily, R. S.; Ivanov, V. I.; Savotchkina, L. E.;, *Biochim. Biophys. Acta*, **259**, 35; (b) Frederick, C.A.; Grable, J.; Melia, M.; Samudzi, C.; Jen-Jacobson, L.; Wang, B.-C.; Greene, P.; Boyer, H.W.; Rosenberg, J.M. *Nature* **1984**, 309, 327