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Thomas Schrader

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Thomas Schrader. Recognition with Large Calixarene Dimers and Varying Spacers. *Supramolecular Chemistry*, 2008, 20 (01-02), pp.109-115. 10.1080/10610270701747040 . hal-00513520

**HAL Id: hal-00513520**

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## Recognition with Large Calixarene Dimers and Varying Spacers

Journal:	<i>Supramolecular Chemistry</i>
Manuscript ID:	GSCH-2007-0110.R1
Manuscript Type:	Special Issue Paper
Date Submitted by the Author:	09-Oct-2007
Complete List of Authors:	Schrader, Thomas; Universitätsstr. 5
Keywords:	calixarene
<p>Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.</p> <p>Figure 1a and 1b b.cdx          Figure 1a and 1b.cdx          Figure 2 and 3 b.cdx          Figure 2 and 3.cdx          Figure 4 and 5 b.cdx          Figure 4 and 5.cdx</p>	



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**Supramolecular Chemistry**

Dr. Vladimir Sidorov  
Assistant Professor  
Department of Chemistry  
Virginia Commonwealth University  
1001 West Main Street  
Richmond, VA 23284-2006  
U.S.A.  
e-mail: [vasidorov@vcu.edu](mailto:vasidorov@vcu.edu)

Prof. Dr. Thomas Schrader  
Fachbereich Chemie  
Universitätsstr. 5  
45117 Essen  
Tel.: int. +49-201-183-3081  
Fax: int. +49-201-183-4252  
e-mail: [Thomas.Schrader@uni-due.de](mailto:Thomas.Schrader@uni-due.de)  
Essen, Oct. 7, 2007

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**Manuscript GSCH-2007-0110: DNA Recognition with Large Calixarene Dimers and Varying Spacers**

Dear Dr. Sidorov,  
thank you very much for your positive evaluation of our above-mentioned manuscript. We have now revised the paper (and its Supporting Information) according to the reviewers' comments and highlighted all respective changes in yellow. We adhered to almost every suggestion from the referees and explain below which appropriate changes we have made:

**Reviewer 1:** We included Ungaro's important contribution and cited the JACS paper. We corrected redundant references. We changed entry 4 in Table 1 as well as corrected several misspellings found by the meticulous referee – thank you!

**Reviewer 2:** We inserted a statement about additional experimental evidence for major groove binding into the first paragraph of the "Results and Discussion" chapter. We also clearly stated the protonation state of all species at neutral pH (page 4 and 5). Likewise we specifically defined series I and II (page 4 and 5). We also corrected the isolated species of both series to be hexa- or octacations. On the top of page 8, we inserted a longer paragraph detailing the melting behaviour of **2-6**. Here we also tried to explain the origin of the magnified inverted melting curves by complexation accompanied with direct noncovalent interactions between the ligand and host chromophores. We apologize for a wrong cuvette diameter (which is 2mm instead of 0.2 mm, explaining the "extraordinary" extinction coefficient). However, we can confirm, that all solutions remained clear and showed no turbidity due to potential aggregation of the complexes throughout the entire temperature range.

We corrected inconsistencies between numbering schemes in the main text and the SI. In addition, we made a major change, suggested by reviewer 2, in that the synthetic procedure of a representative compound from the new series II was incorporated into a new Experimental Part in the main text, together with the general description of fluorescence titrations, ethidium bromide displacement assays, UV-melting curve determinations, and CD spectra acquisitions. The Supporting Information was stripped of all passages and Figures already found in the main text, and now contains the synthetic procedures for compounds **2-5**, and the fluorescence titrations. We also corrected some missing NMR signals for the tri-Boc-protected calixarene and several misspellings in the SI. I trust that the manuscript is now ready for printing.

Kind regards,                      Thomas Schrader

## DNA Recognition with Large Calixarene Dimers and Varying Spacers

Caroline J. Breitkreuz, Reza Zadmard, T. Schrader\*

**Introduction.** DNA-binding molecules constitute a large part of all anticancer drugs, emphasizing their paramount medicinal significance.<sup>1</sup> 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 basepairs, or occupy the minor or major groove. Natural highly charged polyamines, such as spermine and spermidine, which are present in cell nuclei in mM concentrations, nonspecifically attack the phosphodiester backbone and form multiple salt bridges.<sup>2</sup> This is also the domain of numerous unspecific cationic DNA binders: thus, amphiphiles, dendrimers and polymers have been developed as transfection agents.<sup>3</sup> Polythiophenes which also recognize the DNA/RNA phosphate backbone, have recently presented as color sensors for the hybridization of ss DNA.<sup>4</sup> Small molecules usually intercalate or insert into the minor groove.<sup>5</sup> Flat aromatic cations such as aminoacridines and actinomycin or bisintercalators such as sandramycin seek  $\pi$ -stacking stabilization by maximal base pair overlap and intercalate preferentially into pyrimidine-purine sites, with the general consequence of DNA distortion.<sup>6</sup> 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, optimizing van-der-Waals interactions. AT-rich regions are narrow (3-4 Å), GC-rich tracts much wider ( $\leq 8$  Å). Importantly, it was found, that groove-binders generally do not significantly change the DNA-conformation. Unraveled tunable sequence-selectivity and binding efficiency was later achieved with hairpin polyamides presented by the Dervan group.<sup>7</sup> 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.<sup>8</sup> 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.<sup>9</sup> Interestingly enough, the zinc finger<sup>10</sup> and the leucine zipper motif,<sup>11</sup> two typical

(1) Cragg, G.M.; Newman, D.J.; Snader, K.M. *J. Nat. Prod.* **1997**, *60*, 52-60.

(2) Thomas, G.Jr. et al., *Nucl.Acids Res.* **2000**, *28*, 3379-3385.

(3) Reviews: Miller, A.D. *Angew. Chem.* **1998**, *110*, 1862-1880 (liposomes); Stiriba, S.E.; Frey, H.; Haag, R. *Angew. Chem.* **2002**, *114*, 1385-1390 (dendrimers); Kakizawa, Y.; Kataoka, K. *Adv. Drug Deliv. Rev.* **2002**, *54*, 203-222 (polymers).

(4) Ho, H.-A.; Béra-Abérem, M.; Leclerc, M. *Chem. Eur. J.* **2005**, *11*, 1718-1724.

(5) Tse, W.C.; Boger, D.L. *Chemistry & Biology* **2004**, *11*, 1607-1617.

(6) For a review on DNA intercalators, see: Wilson, W.D. in *Comprehensive Natural Products Chemistry* (Eds.: Nakanishi, K.; Barton, D.), Elsevier, New York **1999**, 427-476.

(7) Dervan, P.B. *Bioorg. Med. Chem.* **2001**, *9*, 2215-2235; Neidle, S. *Nat. Prod. Rep.* **2001**, *18*, 291-309.

(8) For DNA binding agents, see: Johnson, D.S.; Boger, D.L. in *Comprehensive Supramolecular Chemistry*, (Ed.: Murakami, Y.), Elsevier, Oxford **1996**, 73-176.

(9) Reviews: Sundquist, W.I.; Lippard, S.J. *Coord. Chem. Rev.* **1990**, *100*, 293-322; Lawley, P.D.; Philipps, D.H. *Mutat. Res.* **1996**, *355*, 13-40.

(10) Klug, A.; Rhodes, D. *Trends Biochem. Sci.* **1988**, *12*, 465-469; Elrod-Erickson, M.; Benson, T.E.; Pabo, C.O. *Structure* **1998**, *6*, 451-464.

protein domains which fit snugly into DNA's major groove, have similar dimensions (averaged  $\alpha$ -helix diameters of  $\sim 1.3$  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  $\sim 2 \times 1$  nm.<sup>12</sup> This tetracationic  $[\text{Fe}_2\text{L}_3]^{4+}$  ligand binds tightly to the floor of the major groove and induces DNA bending and intramolecular coiling. Later, the same group found, that this molecule also stabilizes three-way junctions in DNA strands.<sup>13</sup>

**Results and Discussion.** We have recently discovered that dimeric calixarenes<sup>14</sup> with 6 anilino groups at their upper rims bind tightly to double-stranded DNA molecules, irrespective of their number of base-pairs.<sup>15</sup> Binding occurs without significant (de)stabilization 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 strand's phosphodiester backbone, by utilizing multiple electrostatic interactions, as evidenced by AFM techniques.<sup>16</sup> 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.<sup>17</sup> Specifically,  $pK_a$  titrations on the ammonium calix[4]arenes revealed decreasing  $pK_a$  values for each single  $\text{NH}_3^+$  group starting from  $pK_1 = 5.0$  and  $pK_2 = 4.5$  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 the calixarene ligand.<sup>18</sup> 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: a) in order to distinguish between gene promoters, a base-sequence of at least 5-6 base-pairs must be spanned and specifically

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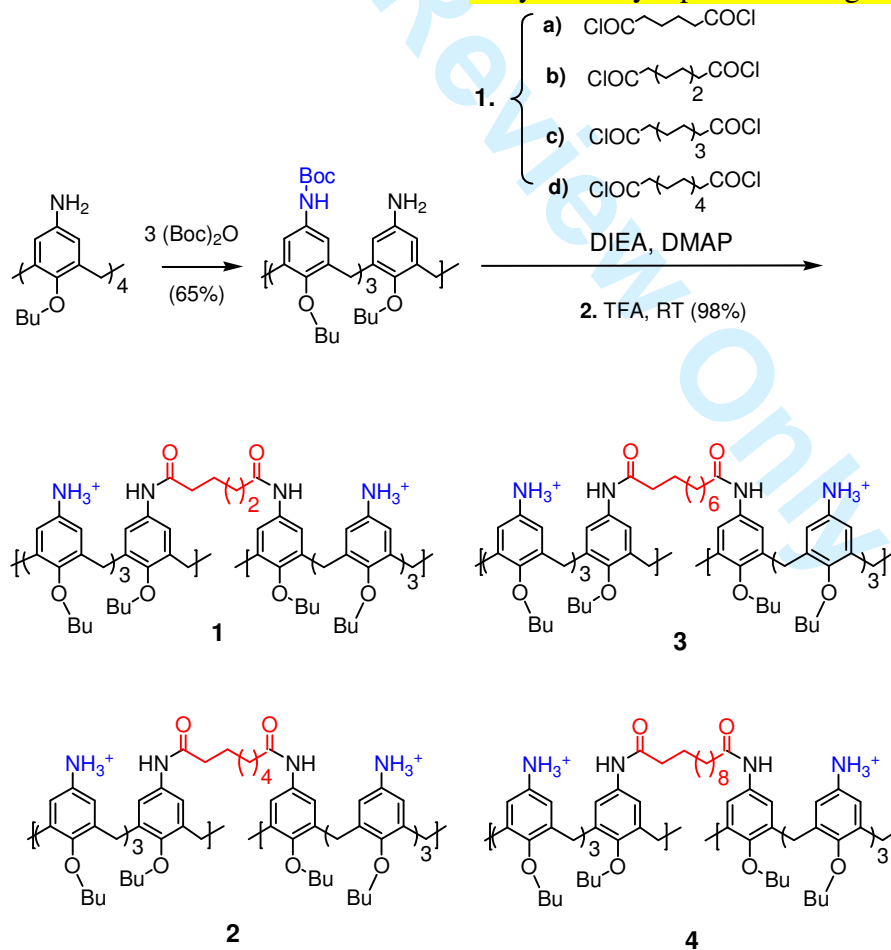
DNA intercalators. In *Comprehensive Natural Products Chemistry*, K. Nakanishi and D. Barton, eds. (New York: Elsevier), **1999**, 427-476.

(18) RNA's minor groove is much shallower, but has the same diameter as its major groove.

1  
2  
3 recognized. However, if the simple alkyl spacer is elongated from 4 to 10 atoms in the bridge,  
4 will this lead to a significant loss of affinity for DNA's major groove? b) in order to compete  
5 with regulatory proteins, affinities must simultaneously be optimized up to the nanomolar level.  
6 Additional hydrogen bonds between polar groups in the bridge and the nucleic bases at the floor  
7 of DNA's major groove may not suffice and should be further complemented by favorable strong  
8 noncovalent interactions. Will, e. g., additional positive charges introduced into the bridging unit  
9 tighten the complex between DNA and its calixarene ligand?  
10  
11

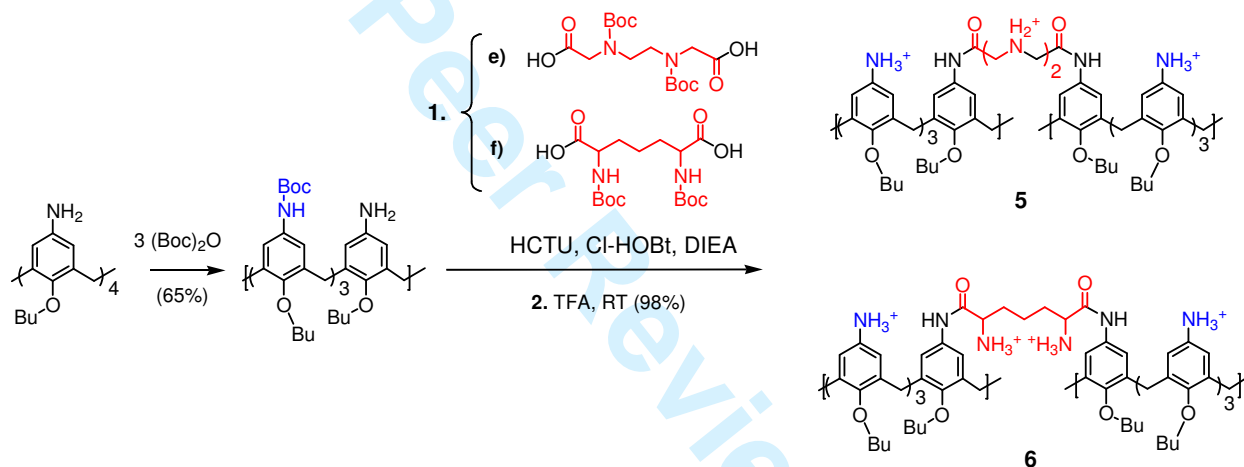
12 After the prototype molecule **1**, which inserted into the major groove of 12 bp ds DNA with 10  
13 micromolar **affinities**, we have now synthesized a whole series of related compounds with varied  
14 bridging units. In one series, the alkyl bridge between both calixarenes was varied in successive  
15 steps of C-2 ethylene units from 4 over 6 and 8 up to 10 carbon atoms. The longest derivative  
16 should be able to span 7 base-pairs of the major groove. In a second series, we introduced two  
17 full positive charges directly into the bridging alkyl chain or alternatively into its close vicinity.  
18 From these derivatives we hoped to receive the answers on the above-detailed questions.  
19

20 The synthetic routes followed the main strategic path, already outlined in our preliminary  
21 communication: Construction of a tri-Boc-protected tetraamino-calix-[4]-arene was followed by  
22 double amide coupling with doubly activated diacids. A final deprotection of all six urethanes  
23 with TFA yielded the target molecules **1-4** as **hexa**(trifluoroacetate) salts. Thus the first series was  
24 prepared from diacid dichlorides with central C<sub>4</sub>, C<sub>6</sub>, C<sub>8</sub> and C<sub>10</sub> carbon chains (Figure 1a).  
25 In spite of their increasing lipophilicity, these compounds could all be dissolved completely in  
26 aqueous Hepes buffer with 50% added methanol. **They all carry 2 positive charges at neutral pH.**  
27  
28  
29



**Figure 1a.** Synthesis of calixarene dimers with alkyl spacers of varying length (1-4).

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<sub>6</sub> spacer. Attachment of the cations in the close vicinity of the spacer's main chain was realized by introducing a double branch into the amino acid molecule – as in pimelic acid. Both bis(amino acids) were activated with HCTU/CI-HOBt and doubly coupled to the triprotected calixarene.<sup>19</sup> 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** (Figure 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 1b.** Synthesis of calixarene dimers with varying cationic bridging units (5,6).

**Fluorescence titrations** of **1-6** were carried out with a Fluorescein-labeled 12 bp DNA.<sup>20</sup> 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.<sup>21</sup> Very smooth curves were obtained in all cases, which could be nicely fitted to a standard nonlinear algorithm (Figure 2). The respective association constants were normalized to a 1:1 complexation event, assuming a noncooperative process; they

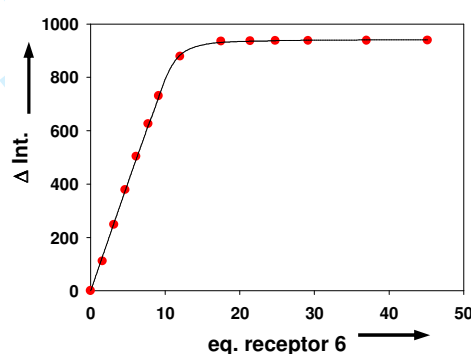
(19) Davis, A.M.; Teague, S.J. *Angew. Chem. Int. Ed.* **1999**, *38*, 737; Sabatino, G.; Mulinacci, B.; Alcaro, M.C.; Chelli, M.; Rovero, P.; Papini, A.M. *Lett. Pept. Sc.* **2002**, *9*, 119; Marder, O.; Shvo, Y.; Albericio, F.; *Chim. Oggi-Chem. Today*, **2002**, *20*, 37.

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are summarized in Table 1. At first glance it becomes obvious, that elongation of the central alkyl spacer chain from C<sub>4</sub> to C<sub>10</sub> 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<sub>4</sub> to C<sub>10</sub> does not lower the calixarene dimer's affinity towards DNA – an important prerequisite for the construction of ligands suitable for spanning 5-6 base pairs.

FI-DNA (double strand) with 12 base pairs: 5`-[FI]GTG ACG AAC CTC-3`  
5`-GAG GTT CGT CAC-3`



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

**Table 1.** Stoichiometries and **association** constants obtained from fluorescence-titration experiments between Fluorescein-labeled ds-12bp-DNA and calixarene dimers **1-6**.

Entry	Receptor	DNA	$K_a \text{ (M}^{-1}\text{)}$	stoich.
1	Dimer <b>1</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$8e+5 (\pm 23\%)^2$	17:1 <sup>3</sup>
2	Dimer <b>2</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$7e+5 (\pm 25\%)^2$	24:1 <sup>3</sup>
3	Dimer <b>3</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$8e+5 (\pm 24\%)^2$	18:1 <sup>3</sup>
4	Dimer <b>4</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$9e+5 (\pm 20\%)^2$	21:1 <sup>3</sup>
5	Dimer <b>5</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$2e+6 (\pm 22\%)^2$	18:1 <sup>3</sup>
6	Dimer <b>6</b>	ds FI-DNA (12 bp, 1 mM Hepes <sup>1</sup> )	$6e+6 (\pm 22\%)^2$	12:1 <sup>3</sup>

<sup>1</sup> Hepes buffer in methanol / water (1:1). <sup>2</sup> statistical error from curve fitting. <sup>3</sup> From Job plots: ratios are given in receptor : DNA.

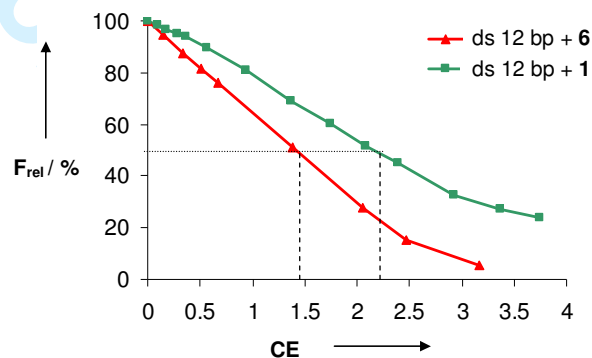
Compounds **5** and **6** clearly demonstrate the expected affinity increase due to an elevated charge state. However, this effect is much more pronounced in the branched pimelic acid derivative **6** than in the linear diglycine **5**. We tentatively explain this difference with an optimal placement of the additional positive charges within DNA's major groove.<sup>22</sup> It is well known, that minor groove

(22) 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) Edwards, K.J.; Jenkins, T.C.; Neidle, S.



binders are drawn towards the center of negative charge density which is located just above the nucleic bases.<sup>23</sup> 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 (Figure 3).<sup>24</sup> Both series display moderate  $C_{50}$  values, but especially series II is characterized by extremely low  $CE_{50}$  values, 500-1000 times lower than those of spermine (Table 2). These findings underline again the importance of hydrophobic forces for the inclusion process and rule out any significant DNA backbone recognition via ion pairs.<sup>25</sup>



**Figure 3.** Ethidium bromide displacement from ds 12 bp DNA ( $c = 1\mu\text{M}$ ) by dimer **1** and **6** ( $\text{H}_2\text{O}/\text{MeOH}$  1:1, 9.4 mM NaCl, 2 mM Hepes, pH 7.1).  $F_{\text{rel}}$  = relative fluorescence emission intensity; CE = charge excess ratio.

**Table 2.** Ethidium bromide displacement assay for ds 12 bp DNA and **1** and **6**.

Ligand / DNA	[NaCl] [mM]	Nominal charge	$C_{50}$ [ $\mu\text{M}$ ]	$CE_{50}$
<b>1</b> + 12 bp	9.4	+2	28	2.3
<b>6</b> + 12 bp	9.4	+4	9	1.5

Absorption intensities in **UV-vis spectra** were again drastically shifted towards higher values for complexes with ds DNA, whereas much lower extinction coefficients were recorded for ss DNA complexes. This behaviour is generally reflected in inverted and enlarged melting curves (Figure

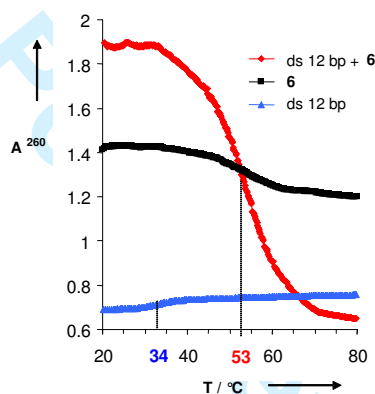
*Biochemistry* **1992**, *31*, 7104-7109; b) Brown, D.G.; Sanderson, M.R.; Garman, E.; Neidle, S. *J. Mol. Biol.* **1992**, *226*, 481-490; c) Laughton, C.A.; Tanious, F.; Nunn, C.M.; Boykin, D.W.; Wildon, W.D.; Neidle, S. *Biochemistry* **1996**, *35*, 5655-5661.

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4). However, contrary to our original observations with **1** and a 20 bp ds-DNA, all new calixarenes derivatives significantly stabilize the short 12 bp DNA duplex (DNA:  $T_m = 34^\circ\text{C}$ ; DNA-complexes:  $T_m = 52\text{-}58^\circ\text{C}$ ). Since now even **1** shifts the melting temperature from  $34^\circ\text{C}$  to  $53^\circ\text{C}$ , the stabilization seems to depend mainly on the total DNA length rather on the dimer structure. We tentatively assume, 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 stabilize the duplex by electrostatic crosslinking, similar to spermine and spermidine.<sup>26</sup>



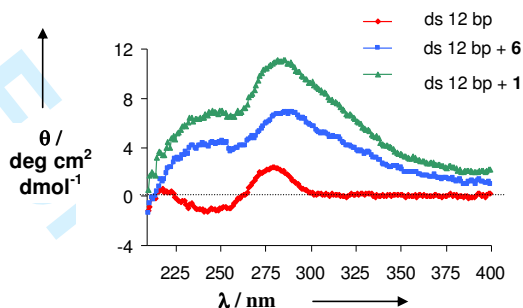
**Figure 4.** UV-vis-melting curve for pimelic acid derivative **6** in its complex with ds-12bp DNA.

**CD curves** were measured for **1** and **6** in their complexes to ds DNA at micromolar concentrations (Figure 5). 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.<sup>27</sup> Clearly, the inclusion of the (aggregated) DNA ligand induced a conformational twist in the DNA molecule in order to optimize supramolecular contacts. At this point, we can only speculate, if the contraction of DNA's major groove and the elimination of the minor groove are pointing to an ideal conformation for inclusion of the monomeric ligand in a cleft of just 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.<sup>12</sup> Finally, this result is another indication for major groove binding of our artificial calixarene dimers. Most polymerases and many endonucleases

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lead to a similar change if they bind to DNA's major groove.<sup>28</sup> Further implications of this induced conformational change must be elucidated in the future.



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

In the future, we will try to calculate the structure of our new DNA complexes with standard modeling 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 12bp-ds DNA. Absolute molar masses referenced against pullulan standards, are expected to exceed by far the expected values for monomeric species and pointed to tetra- or pentameric oligomers. Their structure remains to be elucidated, but involves most likely van-der-Waal contacts between the butoxy tails and the extended alkyl spacers. Finally, nondenaturing gel-electrophoresis will be tried on the complex between 12 bp-ds DNA 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 stabilize 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:

(28) a) Beabealashvily, R.S.; Ivanov, V.I.; Minchenkova, L.E.; Savotchkina, L.P. *Biochim. Biophys. Acta*, **259**, 35–40; 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*, 327–333.

**2,6-Bis[(*tert*-butoxycarbonyl)-amino]-pimelic acid bis-[5,11,17-tris[(*tert*-butoxy-carbonyl)-amino]-25,26,27,28-tetrabutoxy-calix[4]aren-23 amide].** 2,6-Bis[(*tert*-butoxy-carbonyl)-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  $\mu$ L (58 mg, 0.45 mmol) of *N*-ethyl-diisopropylamine 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-tetrabutoxy-calix[4]arene (150 mg, 0.15 mmol), dissolved in 4 mL dichloromethane /DMF (3:1), was added dropwise to the solution. After stirred for 24 h at an ambient temperature the reaction mixture was washed three times with distilled water, dried over Na<sub>2</sub>SO<sub>4</sub> and subsequently evaporated to dryness. The resulting residue was purified by chromatography over silica gel (ethylacetate/ cyclohexane 1:2), *R<sub>f</sub>* = 0.57. Yield: 141 mg (0.059 mmol; 80%). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  = 1.00 (m, 24 H); 1.29 (m, 16 H); 1.49 (s, 18 H); 1.50 (s, 18 H); 1.59 (s, 36 H); 1.71 (m, 2 H); 1.85 (m, 16 H); 1.97 (m, 4 H); 3.08 (m, 8 H); 3.68 (m, 2 H); 3.80 (m, 8 H); 3.89 (m, 8 H); 4.39 (m, 8 H); 6.37-6.90 (m, 24 H). <sup>13</sup>C NMR (125.6 MHz, CDCl<sub>3</sub>):  $\delta$  = 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): calc. for C<sub>135</sub>H<sub>194</sub>N<sub>10</sub>O<sub>26</sub>+Na<sup>+</sup>: *m/z* = 2395.4090, found: *m/z* = 2395.4026.

**2,6-Diaminopimelic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxycalix[4]aren-23-amide}-octakis-(trifluoroacetate) 6.** A suspension of 0.27 mg of the octa-Boc protected diaminopimelic acid-bridged dimer (11  $\mu$ mol) was dissolved in 5 mL of abs. dichloromethane. To this solution was added 5 mL trifluoroacetic acid. The resulting solution was stirred for 22 h at ambient temperature and subsequently evaporated to dryness. Yield: 28 mg (10  $\mu$ mol; 95%). <sup>1</sup>H NMR (500 MHz, *d*<sub>4</sub>-MeOH):  $\delta$  = 1.02 (m, 24 H); 1.36 (m, 8 H); 1.63 (m, 8 H); 1.74 (m, 2 H); 1.88 (m, 16 H); 3.15 (m, 4 H); 3.24 (d, 4 H, *J* = 13.5 Hz); 3.75 (m, 8 H); 3.93 (t, 2 H, *J* = 7.5 Hz); 4.01 (m, 4 H); 4.09 (m, 4 H); 4.48 (m, 8 H); 6.10 (m, 8 H); 7.05 (s, 2 H); 7.08 (s, 2 H); 7.31, 7.33, 7.37, 7.46 (s, 4 H). <sup>13</sup>C NMR (176 MHz, *d*<sub>4</sub>-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): calc. for [C<sub>95</sub>H<sub>194</sub>N<sub>10</sub>O<sub>26</sub>+Na<sup>+</sup>]<sup>+</sup>: *m/z* = 1594.9895, found: *m/z* = 1593.4728.

#### Oligonucleotide structure, which was studied with these experiments:

Fl-DNA (double strand) with 12 base pairs: 5`-[Fl]GTG ACG AAC CTC-3`  
5`-GAG GTT CGT CAC-3`

**Fluorescence titration experiments:** Fluorescence titrations were performed with 12 bp-ds DNA in Hepes-buffered solutions (1 mM, pH = 7.1) in concentrations as stated below. For convenience, the host compounds **1-6** were diluted in the DNA solutions, so that there was no change in the DNA concentration throughout the entire titration. 700  $\mu$ L of these solutions were filled into cuvettes and up to 500  $\mu$ L 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  $\mu\text{M}$  concentration (per base pair). After stirring for 5 min and standing for 1 min the fluorescence was measured. CE values were calculated as ratio of positive charges in the receptor vs. negative charges of DNA in the respective complex mixture. CE<sub>50</sub> values are defined as the CE at 50 % of the original fluorescence emission intensity gained by intercalated ethidium bromide.

**UV-melting curves with 12 bp ds DNA 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  $\mu\text{M}$  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°C to 80°C and the temperature was recorded every minute (= 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  $\mu\text{M}$ ) or its complex with calixarene dimer **1** or **6** (30  $\mu\text{M}$ ) were scanned from 200 nm 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.

**Acknowledgments.** 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.

**Supporting Information.** Full experimental details on synthesis and characterization of the calixarene dimers and the fluorescence titrations.

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**Correspondence Author Address:**

Prof. T. Schrader, Fachbereich Chemie der Universität Duisburg-Essen,  
Universitätsstr. 5, 45117 Essen (Germany)  
Fax: (+49) 201-183-3081  
E-mail: Thomas.Schrader@uni-due.de

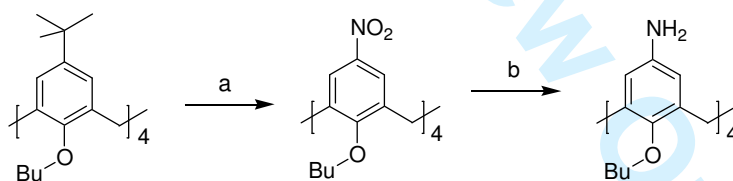
# DNA Recognition by Large Calixarene Dimers with Varying Spacers

Caroline J. Breitzkreuz, Reza Zadmand, Thomas Schrader\*

## Supporting Information:

**General Remarks.** All reagents were purchased at highest commercial grade and used as supplied. All solvents were dried over molecular sieve. Anhydrous solvents were distilled from the following drying agents: acetonitril (calcium hydride), dichloromethane (calcium hydride) and methanol (magnesium). Reactions were monitored by thin layer chromatography (TLC) with *Merck* silica gel 60 F<sub>254</sub> plates. Silica gel 60 for flash chromatography (particle size 230-400 mesh) was supplied by *Merck*. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise noted. Melting points were determined on a Kofler apparatus Thermophan from *Reichert* and are uncorrected. <sup>1</sup>H and <sup>13</sup>C spectra were recorded at 300 K on a *Bruker* Avance DMX 300 or DRX 500 spectrometer. Chemical shifts are reported as  $\delta$  values in ppm and calibrated on the particular solvent signal; multiplicities are indicated by s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad). <sup>13</sup>C spectra are broadband decoupled and calibrated on the particular solvent signal. High-resolution electron spray ionisation (ESI) mass spectra were recorded on a MAT 95 S *Finnigan* spectrometer. Samples (20  $\mu$ L) were introduced as 10<sup>-7</sup> M solutions in HPLC-grade methanol at flow rates of 20  $\mu$ L·min<sup>-1</sup>. Heated capillary temperature: 150°C. Ion spray potential: 3.5 kV (positive ESI), 3.0 kV (negative ESI). About 20-30 scans were averaged to improve the signal to noise ratio. UV-vis experiments were performed on a V-550 spectrometer from *Jasco* with a *Haake* water temperating unit. *Helma* cuvettes with 2 mm inner diameter were used for the experiments.

**Synthesis of the calix[4]arene monomer.** The synthesis of the tetraamino calixarene halfsphere starts with ipsonitration of the parent calixarene in its *p*-position, followed by catalytic hydrogenation.



**Figure S1.** Synthetic route to the calix[4]arene tetraaniline half-sphere : a) HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, 65%. b) H<sub>2</sub>, Pd/C, 89%.

**5,11,17,23-Tetraamino-25,26,27,28-tetrabutoxy-calix[4]arene.** 5,11,17,23-Tetranitro-25,26,27,28-tetrabutoxy-calix[4]arene (4.48 g, 5.41 mmol) and Pd/C (0.504 g, 9 wt%) were dissolved in a 1:1 mixture of toluene and ethanol (150 mL), and hydrogenated. When the hydrogen-uptake subsided, the solution was filtered over celite and evaporated to dryness. Yield: 3.82 g (5.40 mmol; 100 %). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  = 0.96 (t, 12 H, *J* = 7 Hz), 1.42 (m, 8 H, *J* = 7 Hz), 1.84 (m, 8 H, *J* = 7Hz), 2.88 (d, 4 H, *J* = 13Hz), 3.22 (s, 8H, NH<sub>2</sub>); 3.76 (t, 8 H, *J* = 7 Hz), 4.27 (d, 4 H, *J* = 13Hz), 6.06 (s, 8 H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>):  $\delta$  = 14.8, 20.8, 31.9, 33.8, 77.1, 124.8, 126.3, 138.1, 158.4. MS (FD): *m/z* = 708 (M<sup>+</sup>), 731 (M+Na)<sup>+</sup>.

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3 **5,11,17-Tris[(*tert*-butoxycarbonyl)-amino]-23-amino-25,26,27,28-tetrabutoxy-**  
4 **calix[4]arene.** 5,11,17,23-Tetraamino-25,26,27,28-tetrabutoxycalix[4]arene (2.0 g, 2.6 mmol)  
5 was dissolved in abs. dichloromethane (150 mL). Di-*tert*-butylcarbonate (1.73 g, 7.84 mmol)  
6 was dissolved in 20 mL abs. dichloromethane and was added during 30 min. The mixture was  
7 stirred for 18 h at ambient temperature. Subsequently, dichloromethane was removed in  
8 vacuo, and the resulting residue was purified by chromatography over silica gel (ethylacetate/  
9 *n*-hexane 1:3),  $R_f = 0.10$ . Yield: 1.35 g (4.81 mmol; 50 %).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  
10  $\delta = 0.94$  (m, 12 H), 1.25 (m, 4 H,  $J = 7$  Hz), 1.42 (s, 9 H, Boc), 1.53 (s, 18 H, Boc), 1.53 (m, 4  
11 H), 1.78 (m, 8 H,  $J = 7$  Hz), 3.04 (2d, 4 H,  $J = 13$  Hz), 3.63 (m, 4 H); 3.89 (m, 4 H), 4.35 (d, 4  
12 H,  $J = 13$  Hz), 5.68, 6.04, 6.34, 6.81, 7.11, 7.51 (5s, 10 H). Mp 128°C; MS (FD):  $m/z$  (%) =  
13 1008 (M ; 100%).  
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17 **Adipic acid bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetrabutoxy-**  
18 **calix[4]aren-23 amide].** The monomeric amine 5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-  
19 23-amino-25,26,27,28-tetrabutoxy-calix[4]arene (0.10 g, 0.099 mmol) was dissolved in  
20 40 mL of abs. dichloromethane under an argon atmosphere and subsequently treated with  
21 25  $\mu\text{L}$  of *N*-ethyl-diisopropylamine and a catalytic amount of 4-(dimethylamino)pyridine (one  
22 crystal). To this suspension was added adipic acid dichloride (9.04 mg, 0.049 mmol) in 2 mL  
23 of abs. dichloromethane very slowly. The reaction mixture was stirred for 24 h at ambient  
24 temperature and subsequently evaporated to dryness. The resulting residue was purified by  
25 chromatography over silica gel (ethylacetate/ *n*-hexane 1:2),  $R_f = 0.06$ . Yield: 0.08 g  
26 (0.04 mmol; 40 %).  $^1\text{H NMR}$  (300 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.96$  (m, 24 H); 1.29 (m, 16 H); 1.44 (s,  
27 18 H); 1.50 (s, 36 H); 1.80 (m, 20 H); 2.28 (t, 4 H,  $J = 7$  Hz); 3.01 (d, 4 H,  $J = 13.5$  Hz); 3.07  
28 (d, 4 H,  $J = 13.5$  Hz); 3.71 (t, 8 H,  $J = 7.5$  Hz); 3.86 (t, 8 H,  $J = 7.5$  Hz); 4.30 (d, 8 H,  $J = 13.5$   
29 Hz); 6.31 (s, 4 H); 6.41 (b, 6 H); 6.47 (sb, 4 H); 6.76 (s, 4 H); 6.80 (s, 4 H); 7.33 (sb, 2 H).  $^{13}\text{C}$   
30 NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.1, 14.2, 19.3, 19.6, 25.1, 28.2, 28.3, 28.5, 28.6, 31.2, 32.1,$   
31  $32.4, 36.9, 74.9, 80.1, 119.9, 120.9, 131.7, 132.0, 132.1, 134.6, 136.2, 152.6, 153.2, 153.4,$   
32  $153.5, 153.8, 171.3$ . Mp 192-194°C; MS (ESI pos., MeOH):  $m/z$  2151  $[\text{M}+\text{Na}]^+$ . HRMS  
33 (ESI-pos., MeOH): calc. for  $\text{C}_{124}\text{H}_{174}\text{N}_8\text{O}_{22}+\text{Na}^+$ :  $m/z = 2150.2743$ , found:  $m/z = 2150.3278$ .  
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39 **Adipic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxycalix[4]aren-23-amide}-**  
40 **hexakis-(trifluoroacetate) 1.** A suspension of 0.58 mg of the hexa-Boc protected adipoyl-  
41 bridged dimer (28  $\mu\text{mol}$ ) was dissolved in 5 mL of abs. dichloromethane. To this solution was  
42 added 5 mL trifluoroacetic acid. The resulting solution was stirred for 16 h at ambient  
43 temperature and subsequently evaporated to dryness. Yield: 60 mg (27  $\mu\text{mol}$ ; 98 %).  $^1\text{H NMR}$   
44 (500 MHz,  $d_4$ -MeOH):  $\delta = 0.85$  (m, 24 H); 1.18 (m, 8 H); 1.39 (m, 8 H); 1.69 (m, 20 H); 2.2  
45 (t, 4H,  $J = 6.5$  Hz); 2.96, 3.05 (two d, je 4 H,  $J = 13.5$  Hz); 3.63 (t, 8 H,  $J = 7.5$  Hz); 3.82 (m,  
46 8 H,  $J = 7.5$  Hz); 4.23, 4.30 (two d, je 4 H,  $J = 13.5$  Hz); 6.23, 6.30 (dd, je 4 H,  $J = 3.5$  Hz);  
47 6.78 (s, 4 H); 6.94 (s, 4 H).  $^{13}\text{C}$  NMR (100 MHz, MeOD):  $\delta = 14.17, 14.23, 19.42, 19.55,$   
48  $28.53, 28.60, 31.33, 32.19, 32.41, 75.03, 80.19, 119.82, 120.03, 120.18, 121.35, 127.65,$   
49  $132.18, 155.24, 153.44$ .  $^{19}\text{F}$  NMR (188 MHz, MeOD):  $\delta -73.19$  (s). mp: >350°C. HRMS  
50 (ESI-pos., MeOH): calc. for  $[\text{C}_{94}\text{H}_{132}\text{N}_8\text{O}_{10}\cdot 5\text{H}^+]^+$ :  $m/z = 1527.966$ , found:  $m/z = 1527.9194$ .  
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55 **Suberic acid bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetrabutoxy-**  
56 **calix[4]aren-23 amide].** The monomeric amine 5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-  
57 23-amino-25,26,27,28-tetrabutoxy-calix[4]arene (0.15 g, 0.15 mmol) was dissolved in 40 mL  
58 of abs. dichloromethane under an argon atmosphere and subsequently treated with 38  $\mu\text{L}$  of  
59 *N*-ethyl-diisopropylamine and a catalytic amount of 4-(dimethylamino)pyridine. To this  
60 suspension was added suberoyl chloride (15.62 mg, 0.074 mmol) in 3 mL of abs. dichloro-  
methane very slowly. The reaction mixture was stirred for 36 h at ambient temperature and  
subsequently evaporated to dryness. The resulting residue was purified by chromatography

over silica gel (ethylacetate/ cyclohexane 1:2),  $R_f = 0.37$ . Yield: 0.13 g (0.06 mmol; 80 %).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.96$  (m, 24 H); 1.32 (m, 16 H); 1.44 (s, 18 H); 1.51 (s, 36 H); 1.64 (m, 4 H); 1.73 (m, 4 H); 1.82 (m, 16 H); 2.25 (t, 4H,  $J = 7$  Hz); 3.05 (dd, 8 H); 3.71 (t, 8 H,  $J = 7.5$  Hz); 3.88 (t, 8 H,  $J = 7.5$  Hz); 4.35 (d, 8 H,  $J = 13.5$  Hz); 6.16 (s, 2 H); 6.29 (s, 4 H); 6.41 (s, 4 H); 6.47 (s, 4 H); 6.83 (s, 8 H); 7.05 (s, 2 H).  $^{13}\text{C NMR}$  (125.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.13, 14.24, 19.31, 19.60, 22.83, 25.59, 28.61, 31.25, 32.10, 32.44, 62.01, 72.45, 74.93, 74.97, 80.17, 119.80, 119.90, 120.01, 120.91, 131.68, 131.96, 132.14, 134.58, 136.26, 136.31, 152.62, 153.24, 153.43, 153.49, 153.74, 171.51$ . HRMS (ESI-pos., MeOH): calc. for  $\text{C}_{126}\text{H}_{178}\text{N}_8\text{O}_{22}+\text{Na}^+$ :  $m/z = 2179.2981$ , found:  $m/z = 2179.3002$ .

**Suberic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxycalix[4]aren-23-amide}-hexakis-(trifluoroacetate) 2.** A suspension of 0.58 mg of the hexa-Boc protected suberoyl-bridged dimer (28  $\mu\text{mol}$ ) was dissolved in 5 mL of abs. dichloromethane. To this solution was added 5 mL trifluoroacetic acid. The resulting solution was stirred for 20 h at ambient temperature and subsequently evaporated to dryness. Yield: 60 mg (27  $\mu\text{mol}$ ; 96 %).  $^1\text{H NMR}$  (500 MHz,  $d_4$ -MeOH):  $\delta = 1.04$  (m, 24 H); 1.43 (m, 12 H); 1.37 (m, 12 H); 1.62 (m, 8 H); 1.74 (m, 4 H); 1.90 (m, 16 H); 2.39 (t, 4H,  $J = 6.5$  Hz); 3.17, 3.26 (two d, je 4 H,  $J = 13.5$  Hz); 3.80 (m, 8 H); 4.01 (t, 4 H,  $J = 7.5$  Hz); 4.08 (t, 4 H); 4.47, 4.51 (two d, je 4 H,  $J = 13.5$  Hz); 6.17 (s, 4 H); 6.26 (s, 4 H); 7.01 (s, 4 H); 7.22 (s, 4 H).  $^{13}\text{C NMR}$  (176 MHz,  $d_4$ -MeOH):  $\delta = 14.51, 14.67, 14.72, 20.37, 20.44, 20.91, 23.89, 27.00, 28.13, 30.35, 30.90, 32.02, 32.17, 62.42, 73.69, 76.50, 76.55, 121.32, 121.78, 122.82, 123.81, 134.26, 136.54, 137.40, 139.47, 155.54, 155.63, 158.52, 162.71, 162.90, 174.71$ . HRMS (ESI-pos., MeOH): calc. for  $[\text{C}_{96}\text{H}_{130}\text{N}_8\text{O}_{10}+\text{Na}^+]^+$ :  $m/z = 1578.9834$ , found:  $m/z = 1578.9491$ .

**Sebacic acid bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetrabutoxycalix[4]aren-23 amide].** The monomeric amine 5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-23-amino-25,26,27,28-tetrabutoxy-calix[4]arene (0.15 g, 0.15 mmol) was dissolved in 40 mL of abs. dichloromethane under an argon atmosphere and subsequently treated with 38  $\mu\text{L}$  (29.72 mg, 0.23 mmol) of *N*-ethyldiisopropylamine and a catalytic amount of 4-(dimethylamino)pyridine. To this suspension was added sebacyl chloride (17.70 mg, 0.074 mmol) in 3 mL of abs. dichloromethane very slowly. The reaction mixture was stirred for 36 h at ambient temperature and subsequently evaporated to dryness. The resulting residue was purified by chromatography over silica gel (ethylacetate/ cyclohexane 1:2),  $R_f = 0.53$ . Yield: 0.122 g (0.056 mmol; 74 %).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.96$  (m, 24 H); 1.29 (m, 16 H); 1.44 (s, 18 H); 1.48 (m, 8 H); 1.51 (s, 36 H); 1.63 (m, 4 H); 1.82 (m, 16 H); 2.24 (t, 4H,  $J = 7$  Hz); 3.05, 3.07 (two d, je 4 H,  $J = 13.5$  Hz); 3.71 (dt, 8 H); 3.88 (t, 8 H,  $J = 7.5$  Hz); 4.35 (d, 8 H,  $J = 13.5$  Hz); 6.11 (s, 2 H); 6.30 (s, 4 H); 6.38 (s, 4 H); 6.49 (s, 4 H); 6.83 (s, 8 H); 7.00 (s, 2 H).  $^{13}\text{C NMR}$  (125.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.10, 14.21, 19.30, 19.58, 22.81, 25.67, 28.58, 31.24, 32.08, 32.41, 61.91, 72.46, 74.46, 74.91, 74.98, 80.10, 80.16, 119.77, 119.87, 120.06, 120.56, 131.80, 131.91, 132.10, 134.57, 136.21, 136.28, 152.63, 153.11, 153.41, 153.48, 153.70, 171.42$ . HRMS (ESI-pos., MeOH): calc. for  $\text{C}_{128}\text{H}_{182}\text{N}_8\text{O}_{22}+\text{Na}^+$ :  $m/z = 2207.3294$ , found:  $m/z = 2207.3245$ .

**Sebacic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxycalix[4]aren-23-amide}-hexakis-(trifluoroacetate) 3.** A suspension of 0.62 mg of the hexa-Boc protected sebacyl-bridged dimer (28  $\mu\text{mol}$ ) was dissolved in 5 mL of abs. dichloromethane. To this solution was added 5 mL trifluoroacetic acid. The resulting solution was stirred for 22 h at ambient temperature and subsequently evaporated to dryness. Yield: 62 mg (27  $\mu\text{mol}$ ; 98 %).  $^1\text{H NMR}$  (500 MHz,  $d_4$ -MeOH):  $\delta = 1.05$  (m, 24 H); 1.43 (m, 16 H); 1.61 (m, 8 H); 1.71 (tb, 4 H); 1.94 (m, 16 H); 2.37 (t, 4H,  $J = 6.5$  Hz); 3.22, 3.31 (two d, je 4 H,  $J = 13.5$  Hz); 3.87 (m, 8 H); 4.02, 4.11 (two t, je 4 H,  $J = 7.5$  Hz); 4.53(dd, 8 H,  $J = 13.5$  Hz); 6.44 (s, 4 H); 6.51 (s, 4 H);



7.00 (s, 4 H); 7.15 (s, 4 H).  $^{13}\text{C}$  NMR (125.6 MHz,  $d_4$ -MeOH):  $\delta = 14.52, 14.63, 14.69, 20.42, 20.49, 20.81, 23.88, 27.03, 30.52, 30.55, 31.92, 32.08, 33.44, 33.53, 33.75, 38.01, 62.39, 73.64, 76.55, 76.64, 76.74, 122.69, 122.88, 123.11, 124.28, 134.32, 136.89, 137.83, 138.94, 155.06, 156.90, 158.61, 174.83$ . HRMS (ESI-pos., MeOH): calc. for  $[\text{C}_{98}\text{H}_{134}\text{N}_8\text{O}_{10}+\text{Na}^+]^+$ :  $m/z = 1607.0147$ , found:  $m/z = 1607.0122$ .

**Dodecanedioic acid bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetra-butoxy-calix[4]aren-23 amide].** The monomeric amine 5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-23-amino-25,26,27,28-tetrabutoxy-calix[4]arene (0.15 g, 0.15 mmol) was dissolved in 50 mL of abs. dichloromethane under an argon atmosphere and subsequently treated with 38  $\mu\text{L}$  (29.72 mg, 0.23 mmol) of *N*-ethyl-diisopropylamine and a catalytic amount of 4-(dimethyl-amino)pyridine. To this suspension was added dodecanedioyl dichloride (19.77 mg, 0.074 mmol) in 3 mL of abs. dichloromethane very slowly. The reaction mixture was stirred for 36 h at ambient temperature and subsequently evaporated to dryness. The resulting residue was purified by chromatography over silica gel (ethylacetate/ cyclohexane 1:2),  $R_f = 0.57$ . Yield: 0.123 g (0.056 mmol; 74 %).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.96$  (m, 24 H); 1.30 (m, 16 H); 1.44 (s, 18 H); 1.51 (s, 36 H); 1.48 (m, 12 H); 1.63 (m, 4 H); 1.82 (m, 16 H); 2.24 (t, 4H,  $J = 7$  Hz); 3.05, 3.08 (two d, je 4 H,  $J = 13.5$  Hz); 3.71 (m, 8 H); 3.89 (t, 8 H,  $J = 7.5$  Hz); 4.36 (d, 8 H,  $J = 13.5$  Hz); 6.16 (s, 2 H); 6.30 (s, 4 H); 6.42 (s, 4 H); 6.50 (s, 4 H); 6.84 (s, 8 H); 7.06 (s, 2 H).  $^{13}\text{C}$  NMR (125.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.08, 14.19, 14.21, 19.28, 19.56, 22.79, 25.73, 28.57, 31.21, 32.03, 32.07, 32.40, 61.84, 72.48, 74.89, 74.96, 80.06, 80.13, 119.79, 119.89, 120.01, 120.45, 131.84, 131.93, 132.09, 134.53, 136.20, 136.26, 152.58, 153.04, 153.40, 153.48, 153.70, 171.45$ . HRMS (ESI-pos., MeOH): calc. for  $\text{C}_{130}\text{H}_{186}\text{N}_8\text{O}_{22}+\text{Na}^+$ :  $m/z = 2235.3607$ , found:  $m/z = 2235.3608$ .

**Dodecanedioic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxycalix[4]aren-23-amide}-hexakis-(trifluoroacetate) 4.** A suspension of 0.62 mg of the hexa-Boc protected dodecanedioyl-bridged dimer (28  $\mu\text{mol}$ ) was dissolved in 5 mL of abs. dichloromethane. To this solution was added 5 mL trifluoroacetic acid. The resulting solution was stirred for 22 h at ambient temperature and subsequently evaporated to dryness. Yield: 59 mg (26  $\mu\text{mol}$ ; 92 %).  $^1\text{H}$  NMR (500 MHz,  $d_4$ -MeOH):  $\delta = 1.01$  (m, 24 H); 1.38 (m, 20 H); 1.59 (m, 8 H); 1.68 (m, 4 H); 1.88 (m, 16 H); 2.34 (t, 4H,  $J = 6.5$  Hz); 3.16, 3.25 (two d, je 4 H,  $J = 13.5$  Hz); 3.80 (m, 8 H,  $J = 7.5$  Hz); 4.02, 4.08 (zwei t, je 4 H,  $J = 7.5$  Hz); 4.46, 4.50 (two d, je 4 H,  $J = 13.5$  Hz); 6.23, (s, 4 H); 6.32 (s, 4 H); 6.98 (s, 4 H); 7.16 (s, 4 H).  $^{13}\text{C}$  NMR (176 MHz,  $d_4$ -MeOH):  $\delta = 14.08, 14.42, 14.51, 14.57, 20.25, 20.32, 20.49, 20.74, 23.74, 27.00, 30.50, 30.56, 30.67, 30.76, 31.86, 32.00, 33.08, 33.32, 33.41, 33.49, 33.64, 37.92, 62.28, 73.54, 76.38, 76.43, 76.50, 121.51, 121.96, 122.72, 123.82, 134.11, 136.51, 137.28, 137.40, 139.21, 155.28, 155.80, 158.49, 162.68, 162.47, 174.70$ . HRMS (ESI-pos., MeOH): calc. for  $[\text{C}_{100}\text{H}_{139}\text{N}_8\text{O}_{10}+\text{H}^+]^+$ :  $m/z = 1613.0605$ , found:  $m/z = 1613.0677$ .

**Ethylene-bis[(*tert*-butoxycarbonyl)amino]-*N,N'*-diacetic acid bis-[5,11,17-tris[(*tert*-butoxycarbonyl)-amino]-25,26,27,28-tetrabutoxy-calix[4]aren-23 amide].** Ethylene-bis[(*tert*-butoxycarbonyl)amino]-*N,N'*-diacetic acid (28 mg, 0.074 mmol) was dissolved in 50 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  $\mu\text{L}$  (58 mg, 0.45 mmol) of *N*-ethyl-diisopropylamine 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-tetra-butoxycalix[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  $\text{Na}_2\text{SO}_4$  and subsequently evaporated to dryness. The resulting residue was purified by chromatography over silica gel

(ethylacetate/ cyclohexane 1:2),  $R_f = 0.49$ . Yield: 134 mg (0.057 mmol; 77 %).  $^1\text{H NMR}$  (500 MHz,  $\text{CDCl}_3$ ):  $\delta = 1.01$  (m, 24 H); 1.29 (m, 16 H); 1.49 (m, 72 H); 1.86 (m, 16 H); 3.11 (dd, 8 H); 3.51 (m, 4 H); 3.76 (m, 8 H); 3.87 (m, 8 H); 4.00 (m, 4 H); 4.40 (m, 8 H); 6.25, 6.41, 6.48, 6.58, 6.68, 6.82, 6.96 (sb, 24 H).  $^{13}\text{C NMR}$  (125.6 MHz,  $\text{CDCl}_3$ ):  $\delta = 14.15, 14.21, 14.25, 19.37, 19.55, 22.84, 23.33, 26.85, 27.05, 28.51, 28.61, 29.85, 31.30, 32.09, 32.19, 32.39, 62.02, 72.48, 74.92, 80.07, 119.96, 120.15, 132.06, 153.47$ . HRMS (ESI-pos., MeOH): calc. for  $\text{C}_{134}\text{H}_{192}\text{N}_{10}\text{O}_{26}+\text{Na}^+$ :  $m/z = 2381.3830$ , found:  $m/z = 2381.3934$ .

**Ethylenediamine- $N,N'$ -diacetic acid bis-{5,11,17-tri-amino-25,26,27,28-tetrabutoxy-calix[4]aren-23-amide}-octakis-(trifluoroacetate) 5.** A suspension of 0.54 mg of the octa-Boc protected ethylene diamine diacetic acid-bridged dimer (23  $\mu\text{mol}$ ) was dissolved in 5 mL of abs. dichloromethane. To this solution was added 5 mL trifluoroacetic acid. The resulting solution was stirred for 22 h at ambient temperature and subsequently evaporated to dryness. Yield: 50 mg (22  $\mu\text{mol}$ ; 97 %).  $^1\text{H NMR}$  (500 MHz,  $d_4$ -MeOH):  $\delta = 1.02$  (m, 24 H); 1.34 (m, 8 H); 1.62 (m, 8 H); 1.89 (m, 16 H); 3.16 (d, 4 H,  $J = 13.5$  Hz); 3.25 (d, 4 H,  $J = 13.5$  Hz); 3.39 (s, 4 H); 3.77 (t, 8 H,  $J = 7.5$  Hz); 3.95 (s, 4 H); 4.03 (t, 4 H); 4.09 (t, 4 H); 4.48 (dd, 8 H,  $J = 13.5$  Hz); 6.16 (s, 4 H); 6.23 (s, 4 H); 7.05 (s, 4 H); 7.32 (s, 4 H).  $^{13}\text{C NMR}$  (176 MHz,  $d_4$ -MeOH):  $\delta = 14.50, 14.57, 14.67, 14.72, 20.34, 20.41, 20.63, 20.93, 23.89, 28.13, 30.63, 30.91, 31.99, 32.15, 33.23, 33.45, 33.49, 33.64, 33.83, 62.42, 73.68, 76.47, 76.57, 121.62, 122.02, 122.30, 123.99, 133.66, 136.45, 137.22, 137.86, 139.49, 155.73, 158.61, 162.80, 163.00$ . HRMS (ESI-pos., MeOH): calc. for  $[\text{C}_{94}\text{H}_{129}\text{N}_{10}\text{O}_{10}+\text{Na}^+]^+$ :  $m/z = 1558.9919$ , found:  $m/z = 1558.9880$ .

## Fluorescence titrations

FI-DNA (single strand, 12 base pairs) vs. host **1** in 1 mM Hepes in water/ methanol (1:1):

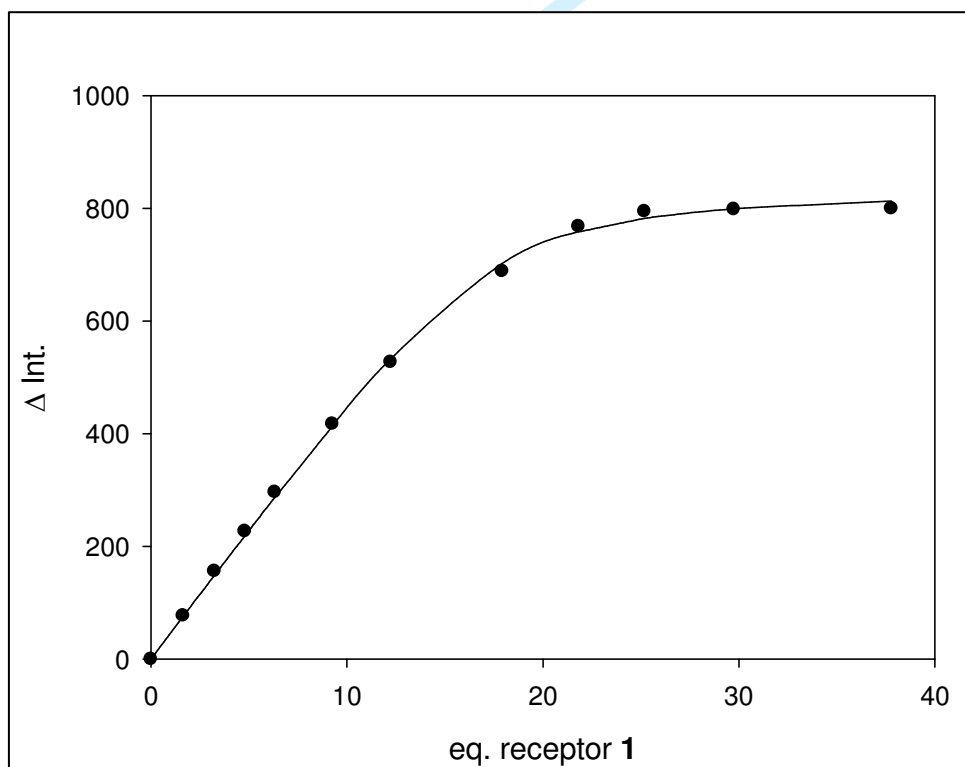
$$C_{\text{FI-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 1}} = 2.65 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor <b>1</b> $\mu\text{L}$	Total volume $\mu\text{L}$	$C_{\text{FI-DNA}}$ $10^{-6} \text{ mol/l}$	$C_{\text{recep.1}}$ $\text{mol/l}$	ratio $C_{\text{FI-DNA}} / C_{\text{recep.1}}$	fluorescence emission	relative emission difference
0	700	2.00	0		819.860	0
10	710	2.00	3.2753E-06	1.6377	742.895	76.965
20	720	2.00	6.4707E-06	3.2353	664.051	155.809
30	730	2.00	9.5889E-06	4.7944	592.951	226.909
40	740	2.00	1.2633E-05	6.3164	523.692	296.168
60	760	2.00	1.8508E-05	9.2539	402.452	417.408
80	780	2.00	2.4457E-05	12.2287	292.837	527.023
120	820	2.00	3.5845E-05	17.9223	131.206	688.654
150	850	2.00	4.3629E-05	21.8147	52.1227	767.7373
180	880	2.00	5.0360E-05	25.1799	25.5241	794.3359
220	920	2.00	5.9472E-05	29.7361	21.5599	798.3001
300	1000	2.00	7.5557E-05	37.7783	20.0421	799.8179

Graphical representation of the titration curve:

$$K_a = 8.0 \cdot 10^5 \text{ M}^{-1} \text{ (23\%)}$$

Stoichiometry: 17:1 (receptor to DNA)



FI-DNA (single strand, 12 base pairs) vs. host **2** in 1 mM Hepes in water/ methanol (1:1):

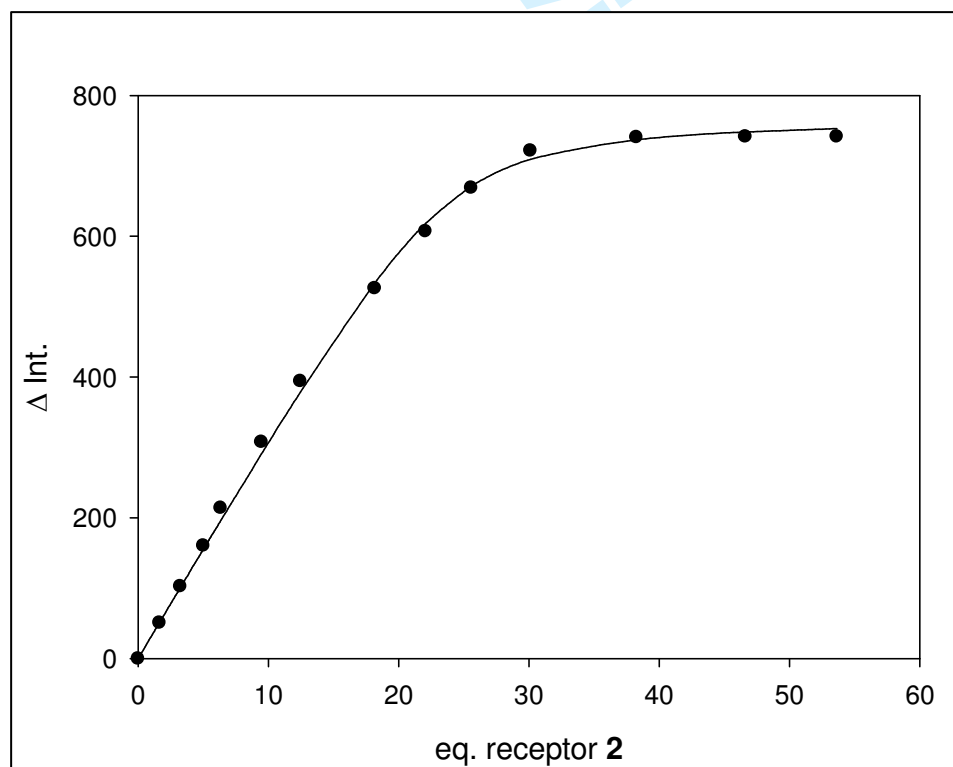
$$C_{\text{FI-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 2}} = 2.65 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor 2 $\mu\text{L}$	Total volume $\mu\text{L}$	$C_{\text{FI-DNA}}$ $10^{-6} \text{ mol/l}$	$C_{\text{recep2}}$ $\text{mol/l}$	ratio $C_{\text{FI-DNA}}/C_{\text{recep.2}}$	fluorescence emission	Relative emission difference
0	700	2.00	0		760.837	0
10	710	2.00	3.2937E-06	1.6469	709.855	50.982
20	720	2.00	6.5067E-06	3.2533	658.14	102.697
30	730	2.00	1.0028E-05	5.0141	600.412	160.425
40	740	2.00	1.2702E-05	6.3508	546.815	214.022
60	760	2.00	1.8967E-05	9.4833	453.174	307.663
80	780	2.00	2.4928E-05	12.4642	366.738	394.099
120	820	2.00	3.6336E-05	18.1681	234.812	526.025
150	850	2.00	4.4133E-05	22.0666	153.784	607.053
180	880	2.00	5.1145E-05	25.5725	92.1361	668.7009
220	920	2.00	6.0246E-05	30.1230	39.3851	721.4519
300	1000	2.00	7.6519E-05	38.2594	20.3079	740.5291
400	1100	2.00	9.3247E-05	46.6236	19.4369	741.4001
500	1200	2.00	1.0725E-04	53.6525	19.1599	741.6771

Graphical representation of the titration curve:

$$K_a = 7.2 \cdot 10^5 \text{ M}^{-1} \text{ (25\%)}$$

Stoichiometry: 24:1 (receptor to DNA)



FI-DNA (single strand, 12 base pairs) vs. host **3** in 1 mM Hepes in water/ methanol (1:1):

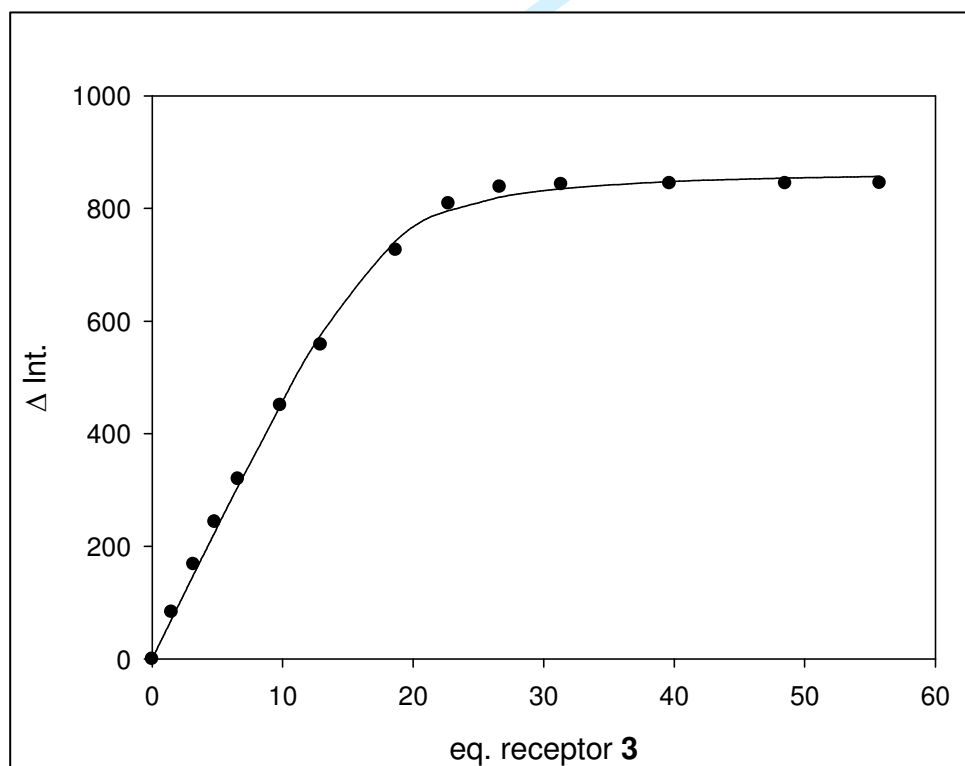
$$C_{\text{FI-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 3}} = 2.75 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor <b>3</b>	Total volume	$C_{\text{FI-DNA}}$	$C_{\text{recep3}}$	ratio	fluorescence emission	Relative emission difference
$\mu\text{L}$	$\mu\text{L}$	$10^{-6} \text{ mol/l}$	$\text{mol/l}$	$C_{\text{FI-DNA}}/C_{\text{recep.3}}$		
0	700	2.00	0		864.066	0
10	710	2.00	2.9901E-06	1.4951	780.547	83.5190
20	720	2.00	6.3288E-06	3.1644	695.78	168.2860
30	730	2.00	9.5866E-06	4.7933	620.731	243.3350
40	740	2.00	1.3158E-05	6.5792	544.302	319.7640
60	760	2.00	1.9650E-05	9.8248	413.505	450.5610
80	780	2.00	2.5827E-05	12.9133	306.021	558.0450
120	820	2.00	3.7326E-05	18.6631	138.120	725.9460
150	850	2.00	4.5428E-05	22.7141	55.3715	808.6945
180	880	2.00	5.3278E-05	26.6388	26.0097	838.0563
220	920	2.00	6.2688E-05	31.3440	21.006	843.0600
300	1000	2.00	7.9301E-05	39.6503	19.8587	844.2073
400	1100	2.00	9.7010E-05	48.5048	19.4939	844.5721
500	1200	2.00	1.1148E-04	55.7379	19.9968	845.0692

Graphical representation of the titration curve:

$$K_a = 8.2 \cdot 10^5 \text{ M}^{-1} \text{ (24\%)}$$

Stoichiometry: 18:1 (receptor to DNA)



**Fl-DNA (single strand, 12 base pairs) vs. host 4 in 1 mM Hepes in water/ methanol (1:1):**

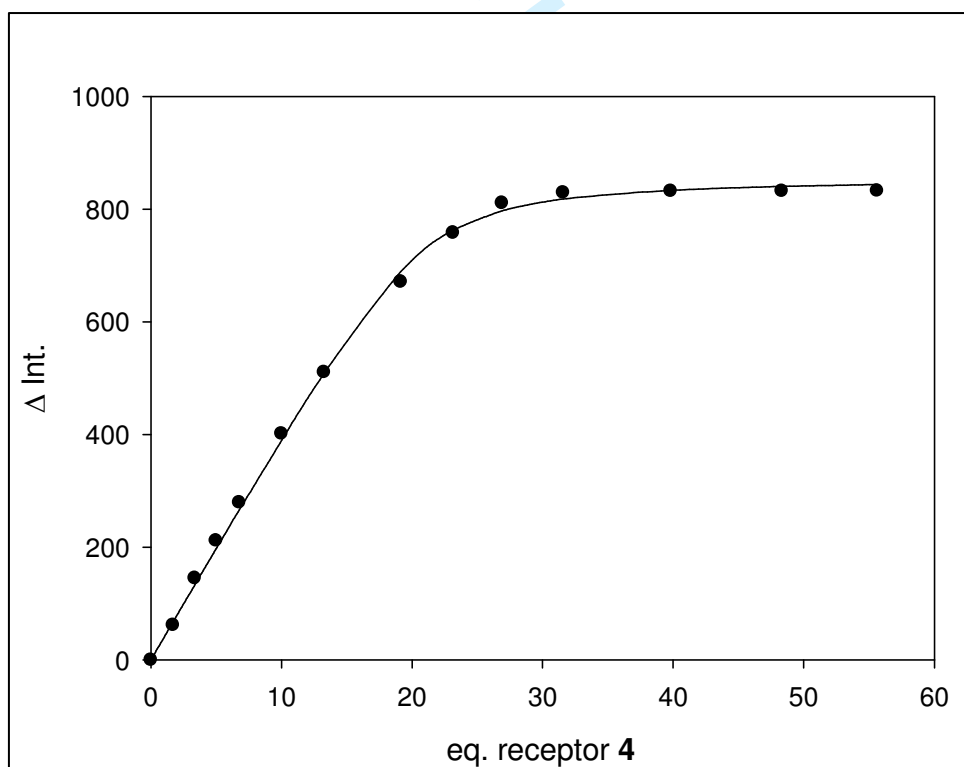
$$C_{\text{Fl-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 4}} = 2.73 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor 4 $\mu\text{L}$	Total volume $\mu\text{L}$	$C_{\text{Fl-DNA}}$ $10^{-6} \text{ mol/l}$	$C_{\text{recep.4}}$ $\text{mol/l}$	ratio $C_{\text{Fl-DNA}}/C_{\text{recep.4}}$	fluorescence emission	Relative emission difference
0	700	2.00	0		851.171	0
10	710	2.00	3.4131E-06	1.7065	789.367	61.8040
20	720	2.00	6.7420E-06	3.3710	706.213	144.9580
30	730	2.00	9.9899E-06	4.9950	639.606	211.5650
40	740	2.00	1.3551E-05	6.7753	517.768	279.4030
60	760	2.00	2.0020E-05	10.0100	449.634	401.5370
80	780	2.00	2.6528E-05	13.2641	340.787	510.3840
120	820	2.00	3.8271E-05	19.1357	179.995	671.1760
150	850	2.00	4.6297E-05	23.1484	93.2459	757.9251
180	880	2.00	5.3792E-05	26.8962	40.2843	810.8867
220	920	2.00	6.3135E-05	31.5675	21.7135	829.4575
300	1000	2.00	7.9623E-05	39.8114	19.0927	832.0783
400	1100	2.00	9.6652E-05	48.3258	18.9398	832.2312
500	1200	2.00	1.1123E-04	55.6175	18.7336	832.4374

Graphical representation of the titration curve:

$$K_a = 9.1 \cdot 10^5 \text{ M}^{-1} \text{ (20\%)}$$

Stoichiometry: 21:1 (receptor to DNA)



**Fl-DNA (single strand, 12 base pairs) vs. host 5 in 1 mM Hepes in water/ methanol (1:1):**

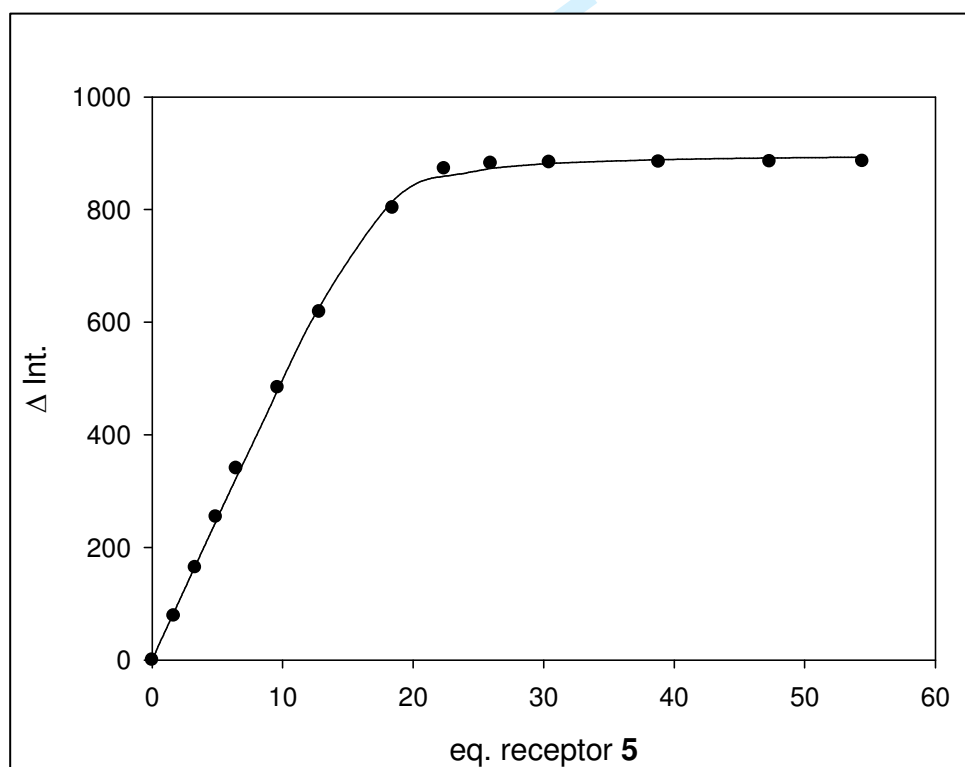
$$C_{\text{Fl-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 5}} = 2.70 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor 5 $\mu\text{L}$	Total volume $\mu\text{L}$	$C_{\text{Fl-DNA}}$ $10^{-6} \text{ mol/l}$	$C_{\text{recep.5}}$ $\text{mol/l}$	ratio $C_{\text{Fl-DNA}}/C_{\text{recep.5}}$	fluorescence emission	Relative emission difference
0	700	2.00	0		904.641	0
10	710	2.00	3.3387E-06	1.6694	826.241	78.4000
20	720	2.00	6.5958E-06	3.2979	740.549	164.0920
30	730	2.00	9.7741E-06	4.8871	650.541	254.1000
40	740	2.00	1.2877E-05	6.4383	564.533	340.1080
60	760	2.00	1.9229E-05	9.6147	420.815	483.8260
80	780	2.00	2.5622E-05	12.8109	286.412	618.2290
120	820	2.00	3.6847E-05	18.4235	101.474	803.1670
150	850	2.00	4.4758E-05	22.3788	31.7145	872.9265
180	880	2.00	5.1873E-05	25.9365	22.469	882.1720
220	920	2.00	6.0857E-05	30.4285	20.815	883.8260
300	1000	2.00	7.7631E-05	38.8156	19.8649	884.7761
400	1100	2.00	9.4622E-05	47.3108	19.3026	885.3384
500	1200	2.00	1.0885E-04	54.4245	18.8576	885.7834

Graphical representation of the titration curve:

$$K_a = 1.9 \cdot 10^6 \text{ M}^{-1} \text{ (22\%)}$$

Stoichiometry: 18:1 (receptor to DNA)



FI-DNA (single strand, 12 base pairs) vs. host **6** in 1 mM Hepes in water/ methanol (1:1):

$$C_{\text{FI-DNA}} = 2.00 \cdot 10^{-6} \text{ mol/l}, C_{\text{recep. 6}} = 2.73 \cdot 10^{-4} \text{ mol/l}$$

Addition receptor <b>6</b>	Total volume	$C_{\text{FI-DNA}}$	$C_{\text{recep.6}}$	ratio	fluorescence emission	Relative emission difference
$\mu\text{L}$	$\mu\text{L}$	$10^{-6} \text{ mol/l}$	$\text{mol/l}$	$C_{\text{FI-DNA}}/C_{\text{recep.6}}$		
0	700	2.00	0		960.269	0
10	710	2.00	3.1659E-06	1.5829	849.24	111.0290
20	720	2.00	6.2550E-06	3.1275	712.456	247.8130
30	730	2.00	9.2701E-06	4.6351	582.037	378.2320
40	740	2.00	1.2214E-05	6.1069	456.775	503.4940
50	750	2.00	1.5443E-05	7.7217	334.531	625.7380
60	760	2.00	1.8244E-05	9.1219	229.597	730.6720
80	780	2.00	2.3985E-05	11.9924	81.8646	878.4044
120	820	2.00	3.4980E-05	17.4898	24.8944	935.3746
150	850	2.00	4.2780E-05	21.3901	23.5997	936.6693
180	880	2.00	4.9532E-05	24.7659	23.0383	937.2307
220	920	2.00	5.8302E-05	29.1511	22.7367	937.5323
300	1000	2.00	7.4005E-05	37.0026	22.1398	938.1292
400	1100	2.00	9.0345E-04	45.1727	21.4867	938.7823

Graphical representation of the titration curve:

$$K_a = 6.2 \cdot 10^6 \text{ M}^{-1} \text{ (22\%)}$$

Stoichiometry: 12:1 (receptor to DNA)

