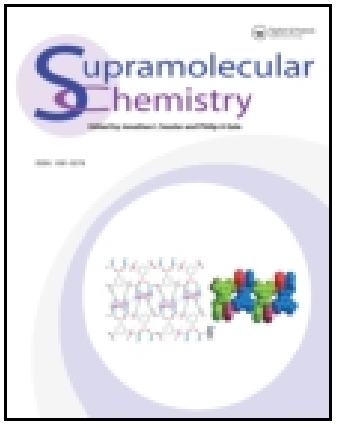

Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gsch20

A convenient and efficient one-pot method for the synthesis of novel acridine-calix[4]arene derivatives as new DNA binding agents via multicomponent reaction

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Published online: 26 Sep 2013.

Supramolecular Chemistry, 2014
Vol. 26, Nos. 5–6, 442–449, http://dx.doi.org/10.1080/10610278.2013.832763

A convenient and efficient one-pot method for the synthesis of novel acridine-calix[4]arene derivatives as new DNA binding agents via multicomponent reaction

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(*Received 27 May 2013; accepted 5 August 2013*)

A new approach is applied for the synthesis of novel acridine-calix[4]arene derivatives via a multicomponent reaction. These compounds have been characterised by ^{1}H NMR, ^{13}C NMR and HR-MS. Our binding studies between acridine-functionalised calix[4]arenes and calf thymus DNA (CT-DNA) via fluorescence titration show that these compounds have a good affinity to CT-DNA.

Keywords: DNA binding agent; multicomponent reaction; fluorescence titration; acridine-calix[4]arene derivatives

Introduction

Acridine derivatives are planar tricyclic aromatic molecules which possess the ability to intercalate tightly, but reversibly to the helical structure of DNA (*1*). Acridine derivatives are one of the oldest and most successful classes of bioactive agents and widely utilised as antimalarial, antiprotozoal, antibacterial and anticancer drugs (*2*). Moreover, acridine and its derivatives are a well-established class of DNA and RNA binding agents (*3*).

Although the intercalation of small molecules into DNA is a very important process, the mechanistic understanding of this process at the molecular level is lacking (*4*). Two common binding modes are proposed: intercalation or groove binding. Intercalation results from insertion of a planar aromatic substituent between DNA base pairs, with concomitant unwinding and lengthening of the DNA helix. Groove binding, in contrast, does not perturb the duplex structure to any great extent. Groove binders are typically crescent shaped, and fit into the minor groove with little distortion of the DNA structure (*5*).

Acridine and its derivatives are not toxic as compared with other DNA intercalators such as ethidium bromide (*6*).

Calixarenes are a family of synthetic macrocyclic receptors, which can be functionalised to provide selectivity towards targeted species. They have been extensively used in electroanalysis (*7*) and because of their dimensions exclude a potential intercalation or minor groove insertion (*8*).

In an earlier study by some of us, we have synthesised various types of dimeric calixarene derivatives, which can selectively bind double-stranded (ds) DNA and RNA with submicromolar K_f values in buffered aqueous solution (*9a*). Dimeric calixarenes can replace ethidium bromide, commonly used as intercalating agent, because of their efficient DNA binding (*8*, *9*).

Many synthetic methods for the synthesis of acridine derivatives have been reported (*10*). However, these methods are plagued by the limitation of prolonged reaction times, poor yields and side reactions of aldehydes. The application of heteropolyacids as catalytic materials is growing continuously in the catalytic field (*11*). In continuation of our investigations on the synthesis of 1,4-dihydropyridines (*12*) and dihydropyrimidines (*13–15*) via a multicomponent reaction (MCR), herein we describe a simple and efficient method for the preparation of acridine-functionalised calix[4]arene derivatives **8–10** using the heteropolyacid catalyst tungstophosphoric acid hydrate ($H_3[PW_{12}O_{40}]$). In this study, we introduce an efficient three-component reaction of amino-calix[4]arene, dimedone and 4-bromobenzaldehyde or 3-hydroxybenzaldehyde that provides an easy access to acridine-functionalised calix[4]arene derivatives. Furthermore, we studied the interaction of the synthesised calix[4]arene derivatives with calf thymus DNA (CT-DNA) via fluorescence titration experiments in aqueous solution and compared intercalation behaviour of acridine-functionalised calix[4]arene derivatives **8–10**.

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Results and discussion

p-tert-Butylcalix[4]arene **1** (*16*) is readily accessible in greater than 65% yield, which provides the starting point for the present investigation (Scheme 1). The main synthetic route starts from *p-tert*-butylcalix[4]arene **1** which is de-alkylated to give calix[4]arene **2** in 73% yield (*17*). The calix[4]arene **3** is obtained in 80% yield via *n*-butylation of calix[4]arene **2** with *n*-BuBr in the presence of NaH in dimethylformamide (DMF) (*18*). The calix[4] arenes **4** and **6** are synthesised by nitration of **3** via a modified method (*19*). We have investigated different conditions such as the concentration of nitric acid (65%) and glacial acetic acid, time and temperature of reaction for the synthesis of mono-nitrocalix[4]arene **4**. Our results indicated that 15 equivalents of nitric acid (65%) without glacial acetic acid are needed for this reaction. The reaction time was about 12 min. With this modified method, mono-nitrocalix[4]arene **4** was isolated in 60% yield. It should be noted that increasing the concentration of nitric acid (65%) above 30 equiv., the reaction time or using glacial acetic acid afforded a mixture of di-, tri- and tetra-nitrocalix[4]arenes. Calix[4]arene **6** was obtained in 30% yield by using the same conditions by increasing the reaction time to 23 min. Increasing the concentration of nitric acid to 30 equiv., while reducing the reaction time to 12 min gave a similar yield. It should be noted that using 15 equiv. of nitric acid in the presence of glacial acetic acid resulted in a mixture of mono, 1,2 alternate, 1,3 alternate, tri- and tetra-nitrocalix[4]arene derivatives.

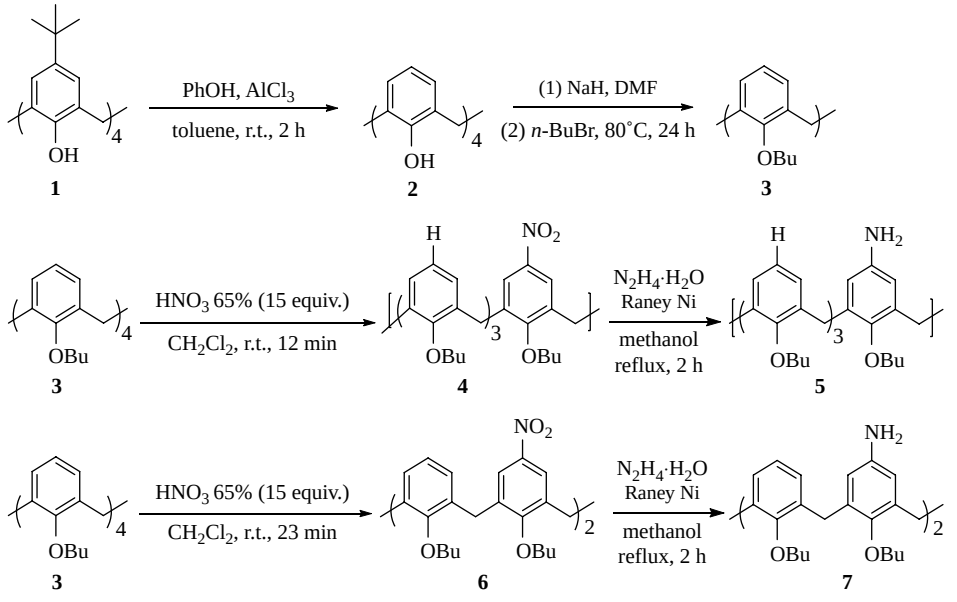
Reduction of calix[4]arenes **4** and **6** by Raney nickel as catalyst and hydrazine hydrate (65%) (*20*) afforded mono and di-aminocalix[4]arenes **5** and **7** in 95% and 90% yield, respectively (Scheme 1).

The MCR of mono-aminocalix[4]arene **5** with dimedone and 4-bromobenzaldehyde or 3-hydroxybenzaldehyde was conducted in the presence of $H_3[PW_{12}O_{40}]$ as the catalyst to yield novel acridine-calix[4]arene derivatives **8** and **9** in 46% and 50% yield, respectively (Scheme 2). To compare the influence of the acridine-calix[4]arene structure on the intercalation potential, we synthesised di-acridine-calix[4] arene **10** in 40% yield, using a similar MCR, from di-amine calix[4]arene **7** (1,3-alternate conformation) (Scheme 2). The acridine-calix[4]arenes **8**–**10** were characterised on the basis of their spectroscopic data such as ^1H and ^{13}C NMR and ESI-HR-MS.

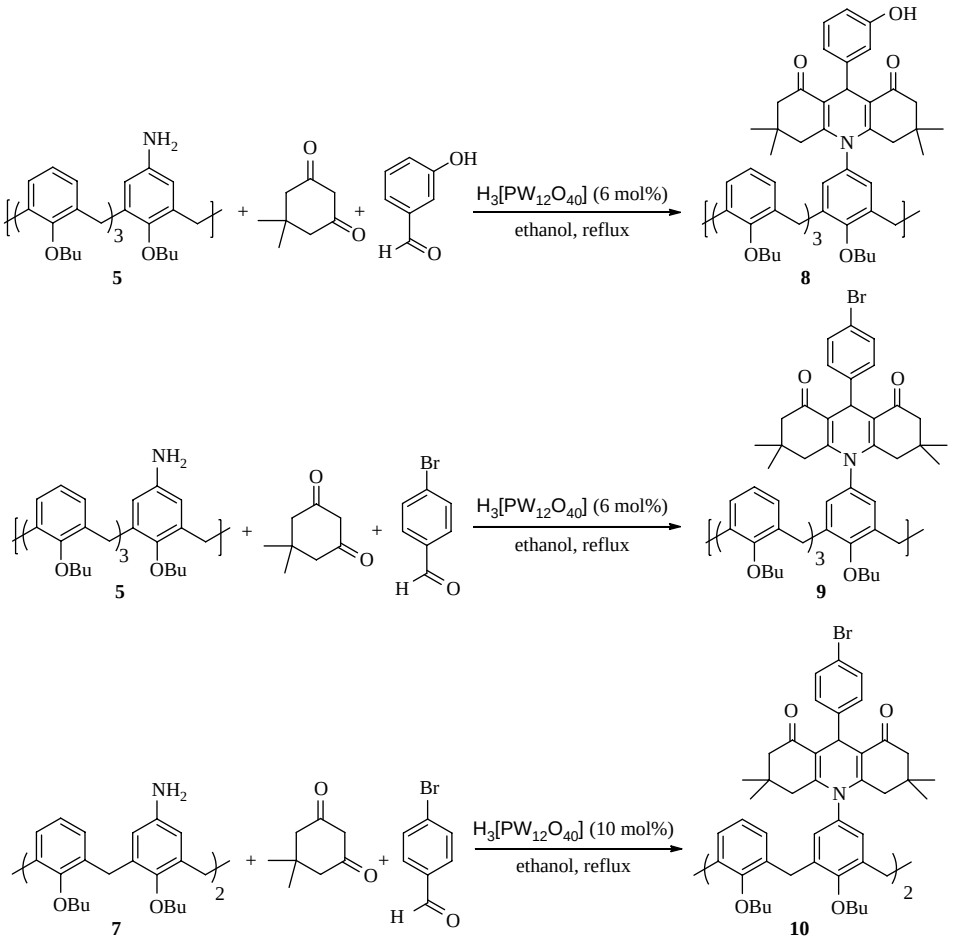
For comparison of binding of acridine-functionalised calix[4]arene derivatives **8**–**10** with an ordinary hydroxy-acridine derivative, we synthesised acridine **11** in 60% yield, from the reaction of aniline with dimedone and 3-hydroxybenzaldehyde in the presence of $H_3[PW_{12}O_{40}]$ as the catalyst (Scheme 3).

Fluorescence titration experiments

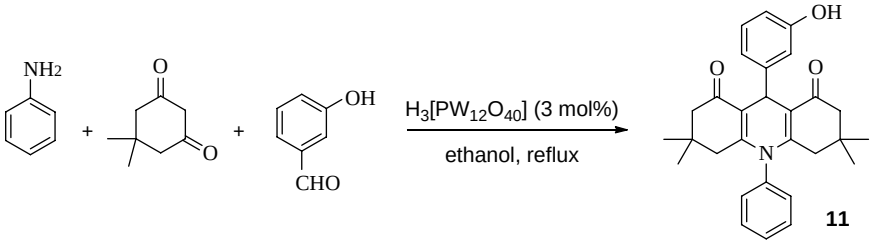
To further investigate the binding mode between calix[4] arene compounds **8**–**10** and CT-DNA, fluorescence titration experiments were carried out. Fluorescence titration is widely used, because of its sensitivity and the availability of the spectroscopic technique (*21*). Furthermore, this method requires micromolar concentrations while other methods such as NMR necessitate millimolar concentrations (*22*, *23*).



Scheme 1. Synthetic route to calix[4]arenes **1**–**7**.



Scheme 2. Synthetic route to acridine-calix[4]arene derivatives **8**–**10**.



Scheme 3. MCR for synthesis of 3-hydroxy-acridine derivative **11**.

The fluorescence titrations of calix[4]arene (1×10^{-6} M) **8** and **9** were carried out in Hepes-buffered (0.05 M, pH 7.1) and NaCl (0.05 M) aqueous solutions at 20°C. In the case of calix[4]arene **10**, fluorescence titrations were conducted under similar conditions, but in water: methanol (1:5) solution. For convenience, CT-DNA solution was diluted in each calix[4]arenes **8**, **9** or **10** derivative distinctly, so that there was no change in the CT-DNA concentration throughout the entire titration. For each compound, 1700 µl of the calix[4]arene solution (1×10^{-6} M) was filled into cuvettes and fluorescence intensity was measured (F_0), then up to 1600 µl of CT-DNA solution (1×10^{-7} M) was added stepwise. For each addition, after stirring for 30 s and standing for 2 min, the change in the emission intensity was measured (F_{obs}) as shown in Figures 1, 3 and 5. According to the fluorescence titration chart shown in Figures 1, 3 and 5, after each addition (CT-DNA) the intensity of fluorescence emission is decreased. The calculation of the binding constant (K_f) was carried out using Scatchard plots (Figures 2, 4 and 6) (*24*).

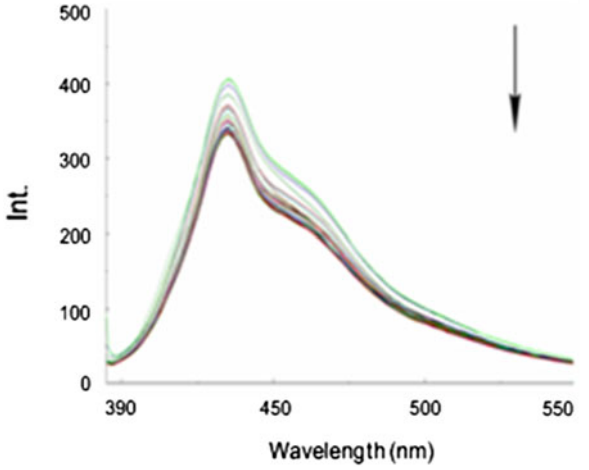


Figure 1. (Colour online) Decrease in the fluorescence intensity of 3-hydroxy-acridine-calix[4]arene **8**.

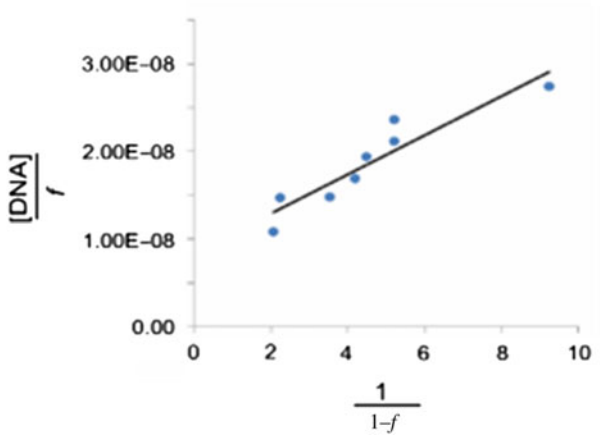


Figure 2. (Colour online) Scatchard plot of 3-hydroxy-acridine-calix[4]arene **8**.

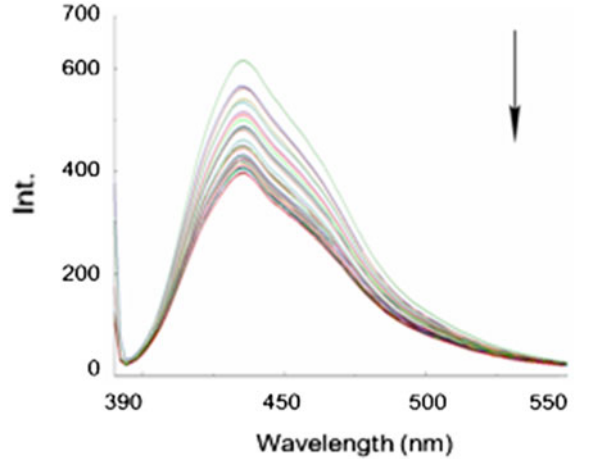


Figure 3. (Colour online) Decrease in the fluorescence intensity of 4-bromo-acridine-calix[4]arene **9**.

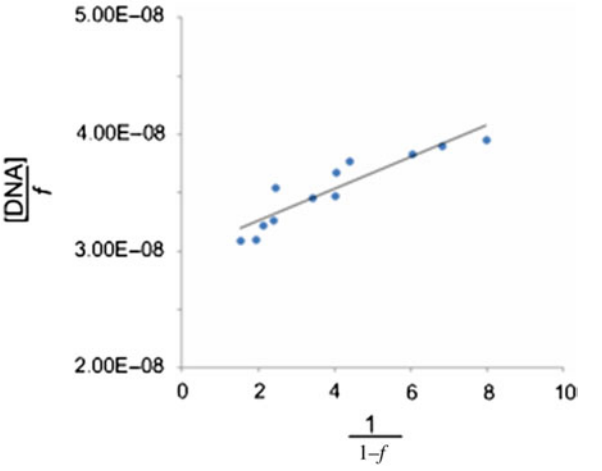


Figure 4. (Colour online) Scatchard plot of 4-bromo-acridine-calix[4]arene **9**.

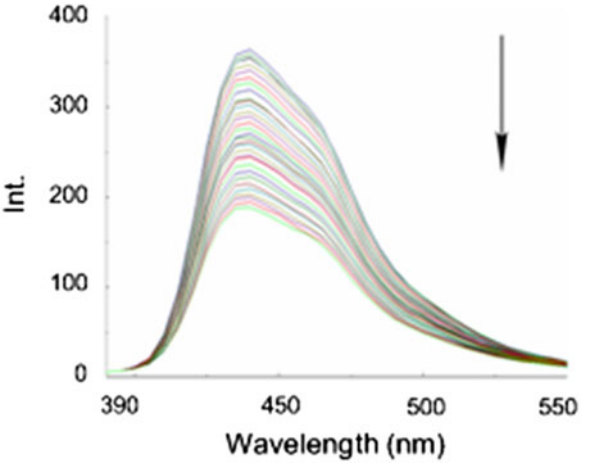


Figure 5. (Colour online) Decrease in the fluorescence intensity of di-4-bromo-acridine-calix[4]arene **10**.

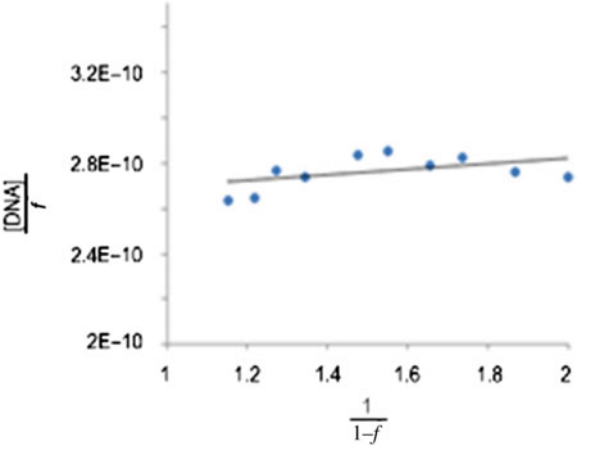


Figure 6. (Colour online) Scatchard plot of di-4-bromo-acridine-calix[4]arene **10**.

Table 1. Association constants between CT-DNA and acridine-calix[4]arene derivatives **8**–**10** and 3-hydroxy-acridine **11**.

Entry	Receptor	Guest	K_f (M^{-1})
1	3-Hydroxy-acridine-calix[4]arene **8**	CT-DNA	3.75×10^6
2	4-Bromo-acridine-calix[4]arene **9**	CT-DNA	2.18×10^7
3	Di-4-bromo-acridine-calix[4]arene **10**	CT-DNA	6.36×10^7
4	3-Hydroxy-acridine **11**	CT-DNA	1.34×10^8

Equations (1) and (2) were used to fit data to Scatchard plots. The results of binding constants are summarised in Table 1, and calculations and complete data are given in supplementary data.

$$\frac{[M]_{\text{total}}}{f} = \frac{1}{(N \cdot K_f(1-f))} + \frac{[L]_{\text{total}}}{N}, \quad (1)$$

$$f = \frac{F_0 - F_{\text{obs}}}{F_0 - F_{\text{max}}}, \quad (2)$$

where $[M]_{\text{total}}$ is the total volume of DNA after each addition, N is the number of binding sites per DNA molecule, K_f is the constant of complexation and $[L]_{\text{total}}$ is the total volume of ligand. Conversion to a linear plot using the term f, corresponding to the fraction of ligand bound during titration, was calculated using Equation (2). In this equation, F_0 is the fluorescence intensity of free ligand, F_{obs} is the fluorescence intensity after each addition and F_{max} is the fluorescence intensity when all possible ligand is bound to CT-DNA.

The association constants obtained for 3-hydroxy-acridine-calix[4]arene **8** and 3-hydroxy-acridine **11**, according to decreasing emission intensity (Figure 1) and Scatchard plot (Figure 2), are equal to 3.75×10^6 and $1.34 \times 10^8\,M^{-1}$, respectively (Table 1).

4-Bromo-acridine-calix[4]arene **9** has a strong emission (Figure 3) that was noticeably quenched during the titration process which yielded Scatchard plot in Figure 4 with an association constant of $2.18 \times 10^7\,M^{-1}$ (Table 1).

The influence of the number of binding sites on acridine-calix[4]arene in complexing with CT-DNA was investigated using the di-4-bromo-acridine-calix[4]arene **10** by measuring the changes in fluorescence intensity during titration with CT-DNA (Figure 5). 4-Bromo-di-acridine-calix[4]arene **10** Scatchard plot (Figure 6) allowed to determine an association constant of $6.36 \times 10^7\,M^{-1}$ (Table 1).

According to the fluorescence titration of acridine-calix[4]arenes **8**–**10** and 3-hydroxy-acridine **11**, we observed a high-binding constant for 3-hydroxy-acridine **11** (Table 1) that seems an unusual amount for a small and a non-ionic molecule; therefore, we carried out a Job plot to investigate the stoichiometry of this compound with ds-DNA with the following sequence:

12 base pair: 5′-GTG ACG AAC CTC-3′
5′-GAG CTT GGT AAC-3′

Crothers (25) previously reported that in intercalation process, each intercalator occupied at least 4 base pairs as the rule of nearest neighbour-exclusion. According to this rule, a 3:1 ratio was expected for 3-hydroxy-acridine **11** and ds-DNA, but the plot showed at least a 9:1 ratio between them. We also carried out a Job plot for 4-bromo-acridine-calix[4]arene **9** with ds-DNA to investigate the acridine-calix[4]arene stoichiometry with ds-DNA. Our result suggested a 2:1 ratio of acridine-calix[4]arene **9** and ds-DNA (Figure 7).

These results indicate that there is another interaction for 3-hydroxy-acridine **11** with ds-DNA. We assume that in the solution, the aggregation process of this compound is responsible for decreasing the intensity of the fluorescence. While in the case of 4-bromo-acridine-calix[4]arene **9**, just the interaction with ds-DNA is responsible of complexation, not aggregation or something else.

According to recent reports, DNA recognition efficiency of major groove binders can also be quantitatively characterised by ethidium bromide displacement assays (26). Hence, we carried out an ethidium bromide displacement assay for 3-hydroxy-calix[4]arene **8** and 4-bromo-acridine-calix[4]arene **9**. Unfortunately, as the binding constant of ethidium bromide for CT-DNA is $10^7\,M^{-1}$, our results show that these compounds cannot displace ethidium bromide.

In conclusion, we present an easy synthetic method of non-charged and large amphiphilic molecules, which selectively bind CT-DNA and ds-DNA with submicromolar K_f values in buffered aqueous solution.

Experimental

Materials

All reagents were purchased at highest commercial grade and used as supplied. All solvents were distilled. Reactions

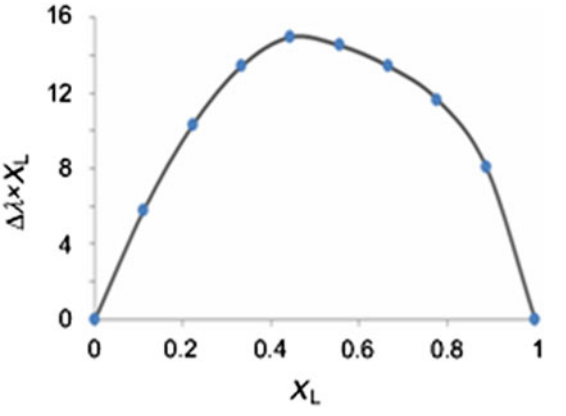


Figure 7. (Colour online) Job plot of 4-bromo-acridine-calix[4]arene **9** with 12 base-pair DNA.

were monitored by thin-layer chromatography (TLC) with Merck silica gel 60 F254 plates. Silica gel 60 for flash chromatography (particle size 230–400 mesh) was supplied by Merck, Darmstadt, Germany.

Instrumentation

Melting points were determined in evacuated capillaries with a Buchi B-545 apparatus. Mass spectra were obtained on a FISONS GC 8000/TRIO 1000 under 70 eV. ^{1}H or ^{13}C NMR spectra were recorded on a Bruker 80, 250, 300 and 500 or 300 and 500 MHz, respectively, in $CDCl_3$ or deuterated dimethyl sulfoxide (DMSO-d_6)/$CDCl_3$ using tetramethylsilane as internal standard. HR-MS was recorded with a Qstar ESI-q-TOF mass spectrometer (Applied Biosystems, Darmstadt, Germany). Fluorescence spectra were recorded using a Jasco FP-6500 device.

Calix[4]arenes **1**–**3** were synthesised according to the literature methods (*16*–*18*).

5-Nitro-26,27,28,29-tetrabutoxy calix[4]arene 4

To a solution of calix[4]arene **3** (0.50 g, 0.77 mmol) in CH_2Cl_2 (30 ml) was added 15 equiv. of 65% nitric acid (0.75 ml, 11.55 mmol) in one portion at room temperature. The solution turned to dark purple immediately and then to dark green when the reaction was finished by adding 25 ml water after 12 min. The organic layer was separated and washed with water (3 × 15 ml), saturated Na_2CO_3 (3 × 10 ml) and water again (3 × 15 ml), then dried over Na_2SO_4, and the solvent was evaporated. The product was isolated as yellow crystals by crystallisation in dichloromethane:methanol (3:2) in 60% yield. M.p.: 155°C; ^{1}H NMR ($CDCl_3$, 80 MHz): $\delta = 0.91$ (t, $J = 6.4$ Hz, 12H, $4CH_3$), 1.10–1.51 (m, 8H, $4CH_2$), 1.51–2.00 (m, 8H, $4CH_2$), 3.08 (d, $J = 13.6$ Hz, 2H, $ArCH_2Ar$), 3.12 (d, $J = 13.6$ Hz, 2H, $ArCH_2Ar$), 3.6–4.10 (m, 8H, $4OCH_2$), 4.35 (d, $J = 13.6$ Hz, 2H, $ArCH_2Ar$), 4.40 (d, $J = 13.6$ Hz, 2H, $ArCH_2Ar$), 6.16 (s, 3H, H_{Aryl}), 6.52–7.00 (m, 6H, H_{Aryl}), 7.05 (s, 2H, H_{Aryl}); MS (E.I.) (70 eV): *m/z* (%) 694 (3) $[M + 1]^+$, 693 (5) $[M]^+$, 664 (2), 636 (3), 580 (10), 524 (7), 468 (6), 149 (10), 119 (22), 57 (87), 29 (100); anal. calcd for $C_{44}H_{55}NO_6$ C, 76.16; H, 7.99; N, 2.02%. Found: C, 76.28; H, 8.07; N, 1.93%.

5-Amino-26,27,28,29-tetrabutyloxy calix[4]arene 5

To a mixture of calix[4]arene **4** (0.50 g, 0.72 mmol) in methanol (50 ml) was added 0.10 g Raney nickel and the mixture was refluxed for 30 min, then hydrazine hydrate 60% (2 ml, 0.04 mmol) was added dropwise. After completion of the reaction (2 h) as indicated by TLC, the solvent was evaporated and the residue was dissolved in dichloromethane, then washed with water (3 × 20 ml) and dried to obtain a white powder in 95% yield. ^{1}H NMR ($CDCl_3$, 80 MHz): $\delta = 1.16$ (t, $J = 6.8$ Hz, 12H, CH_3), 1.32–1.80 (m, 8H, CH_2), 1.80–2.40 (m, 8H, CH_2), 3.00–3.60 (m, 4H, $ArCH_2Ar$ and NH_2), 3.70–4.30 (m, 8H, $4OCH_2$), 4.56 (d, $J = 6.4$ Hz, 2H, $ArCH_2Ar$), 4.65 (d, $J = 6.4$ Hz, 2H, $ArCH_2Ar$), 6.08 (s, 2H, H_{Aryl}), 6.49–7.00 (s, 9H, H_{Aryl}).

5,11-Dinitro-26,27,28,29-tetrabutyloxy calix[4]arene 6

To a solution of **3** (0.50 g, 0.77 mmol) in CH_2Cl_2 (30 ml) was added 15 equivalents of 65% nitric acid (0.75 ml, 11.55 mmol) at once at room temperature under mild stirring. The reaction was stopped after 23 min by adding 25 ml of water. The solution was decanted and the organic layer was washed with water (3 × 25 ml), saturated Na_2CO_3 (3 × 10 ml) and water (3 × 25 ml) again, the solvent was dried over Na_2SO_4 and evaporated. The product was isolated as white crystals by crystallisation in dichloromethane:methanol (3:2) in 30% yield. M.p.: 182–187°C; ^{1}H NMR ($CDCl_3$, 80 MHz): $\delta = 0.92$ (t, $J = 2.4$ Hz, 12H, $4CH_3$), 1.1–1.6 (m, 8H, $4CH_2$), 1.6–2.06 (m, 8H, $4CH_2$), 3.16 (d, $J = 6.8$ Hz, 4H, $2ArCH_2Ar$), 3.68–4.1 (m, 8H, $4OCH_2$), 4.41 (d, $J = 6.8$ Hz, 4H, $2ArCH_2Ar$), 6.65 (s, 6H, H_{Aryl}), 7.36 (s, 4H, H_{Aryl}); MS (E.I.) (70 eV): *m/z* % 739 (2) $[M]^+$, 681 (3), 625 (5), 119 (11), 57 (87).

5,11-Diamine-26,27,28,29-tetrabutyloxy calix[4]arene 7

To a mixture of **6** (0.50 g, 0.67 mmol) in methanol (50 ml) was added 0.1 g Raney nickel and the mixture was refluxed for 30 min, then hydrazine hydrate 60% (4 ml, 0.08 mmol) was added dropwise. The reaction was completed after 2 h. The mixture was filtered on a sintered funnel and the solution was washed with water (3 × 20 ml) and dried to obtain a white powder in 95% yield. ^{1}H NMR: ($CDCl_3$, 500 MHz) $\delta = 0.99$–1.05 (m, 10H, CH_3 and CH_2), 1.38–1.49 (m, 4H, CH_2), 1.50–1.53 (m, 4H, CH_2), 1.86–1.93 (m, 10H, CH_2 and CH_3) 3.08 (d, $J = 10.0$ Hz, 4H, $ArCH_2Ar$), 3.7 (t, $J = 10.0$ Hz, 4H, OCH_2), 3.9 (t, $J = 10.0$ Hz, 4H, OCH_2), 4.3 (d, $J = 15.0$ Hz, 4H, $ArCH_2Ar$), 5.9 (s, 4H, H_{Aryl}), 6.71–6.83 (m, 2H, H_{Aryl}), 6.85 (d, $J = 10.0$ Hz, 4H, H_{Aryl}).

3-Hydroxy-acridine-calix[4]arene 8

To a mixture of calix[4]arene **5** (0.48 g, 0.72 mmol) in dry ethanol (5 ml) and $H_3[PW_{12}O_{40}]$ (0.12 g, 6 mol%) was added the first portion of dimedone (0.10 g, 0.72 mmol). The reaction mixture was heated at 80°C for 8 h. Then, 3-hydroxybenzaldehyde (0.88 g, 0.72 mmol) and the second portion of dimedone (0.10 g, 0.72 mmol) were added to this mixture. The reaction mixture was heated at 80°C for

16 h. After completion of the reaction, the crude product was purified by column chromatography with hexane:ethyl acetate (8:3) as eluent and crystallised in dichloromethane: methanol (2:1) to give **8** in 50% yield. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 0.92$ (s, 6H, $2CH_3$), 0.97–1.11 (m, 18H, $4CH_3$ and $2CH_3$), 1.26 1.5 (m, 4H, $2CH_2$), 1.57–1.78 (m, 4H, $2CH_2$), 1.83–2.15 (m, 12H, $6CH_2$), 2.18–2.34 (m, 5H, $2CH_2$ and OH), 3.21 (t, $J = 13.5$ Hz, 4H, $2ArCH_2Ar$), 3.66–3.84 (m, 4H, OCH_2), 4.11 (t, $J = 7.5$ Hz, 2H, OCH_2), 4.24 (t, $J = 7.5$ Hz, 2H, OCH_2), 4.47 (d, $J = 3.2$ Hz, 2H, $ArCH_2Ar$), 4.57 (d, $J = 3.2$ Hz, 2H, $ArCH_2Ar$), 5.31 (s, 1H, CH), 6.10–6.31 (m, 6H, H_{Aryl}), 6.62 (dd, $J = 3$ and 9 Hz, 1H, H_{Aryl}), 6.88–7.26 (m, 4H, NH_2 and H_{Aryl}), 7.08–7.31 (m, 4H, H_{Aryl}); ^{13}C NMR ($CDCl_3$, 300 MHz): $\delta = 13.97$, 14.14, 14.18, 19.01, 19.64, 26.78, 29.76, 30.73, 30.91, 31.93, 32.28, 32.36, 32,53, 32.71, 41.80, 42.47, 49.99, 74.74, 75.12, 75.54, 113.13, 114.05, 114.55, 116.12, 118.78, 121.87, 121.96, 125.24, 127.90, 128.17, 128.94, 129.02, 129.22, 130.09, 131.89, 131.89, 133.74, 136.91, 138.53, 139.05, 147.72, 150.49, 150.60, 155.22, 156.05, 157.72, 158.43, 196.21; HR-MS (ESI): m/z $[M]^+$ calcd for $C_{67}H_{81}NO_7$: 1011.6013; found: 1011.4021.

4-Bromo-acridine-calix[4]arene 9

To a solution of calix[4]arene **5** (0.48 g, 0.72 mmol) in dry ethanol (5 ml) and $H_3[PW_{12}O_{40}]$ (0.12 g, 6 mol%) was added the first portion of dimedone (0.10 g, 0.72 mmol). The reaction mixture was heated at 80°C for 8 h. Then (0.13 g, 0.72 mmol) of 4-bromobenzaldehyde and the second portion of dimedone (0.10 g, 0.72 mmol) were added to this mixture. The resulting mixture was heated at 80°C for 16 h. After completion of the reaction, the crude product was purified by column chromatography with hexane:ethyl acetate (8:3) as eluent and crystallised in dichloromethane:methanol (2:1) to give **9** in 46% yield. ^{1}H NMR ($CDCl_3$, 250 MHz): $\delta = 0.87$ (s, 6H, $2CH_3$), 0.96–1.08 (m, 18H, $4CH_3$ and $2CH_3$), 1.21–1.47 (m, 4H, $2CH_2$), 1.51–1.73 (m, 4H, $2CH_2$), 1.8–2.17 (m, 12H, $4CH_2$ and $2CH_2$), 2.19–2.31 (m, 4H, $2CH_2$), 3.14–3.3 (m, 4H, $2ArCH_2Ar$), 3.64–3.83 (m, 4H, $2OCH_2$), 4.07 (t, $J = 7.5$ Hz, 2H, OCH_2), 4.21 (t, $J = 17.5$ Hz, 2H, OCH_2), 4.44 (d, $J = 12.5$ Hz, 2H, $ArCH_2Ar$), 4.55 (d, $J = 12.5$ Hz, 2H, $ArCH_2Ar$), 5.24 (s, 1H, CH), 6.07–6.28 (m, 6H, H_{Aryl}), 6.91–7.00 (m, 3H, H_{Aryl}), 7.14 (d, $J = 7.5$ Hz, 2H, H_{Aryl}), 7.32–7.43 (m, 4H, H_{Aryl}); ^{13}C NMR ($CDCl_3$, 300 MHz): $\delta = 13.98$, 14.15, 14.19, 19.02, 19.66, 26.69, 29.84, 30.74, 30.93, 31.95, 32.30, 32.54, 32,68, 41.79, 42.47 50.17, 74.77, 75.15, 113.66, 114.16, 119.67, 121.87, 122.00, 126.67, 127.98, 128.97, 129.76, 130.06, 131.09, 131.75, 132.28, 133.84, 136.89, 138.89, 139.12, 145.49, 150.33, 155.25, 157.72, 158.52, 195.70; HR-MS (ESI): m/z $[M]^+$ calcd for $C_{67}H_{80}BrNO_6$: 1073.5169; found: 1073.5066.

Di-4-bromo-acridine-calix[4]arene 10

To a solution of calix[4]arene **7** (0.20 g, 0.29 mmol) in dry ethanol (5 ml), $H_3[PW_{12}O_{40}]$ (0.08 g, 10 mol%) was added the first portion of dimedone (0.82 g, 0.58 mmol). The reaction mixture was heated at 80°C for 8 h. Then, 4-bromobenzaldehyde (0.10 g, 0.58 mmol) and the second portion of dimedone (0.82 g, 0.58 mmol) were added to this mixture. The resulting mixture was heated at 80°C for 16 h. After completion of the reaction, the crude product was purified by column chromatography with hexane:ethyl acetate (8:3) as eluent and crystallised in dichloromethane: methanol (2:1) to give **10** in 40% yield. ^{1}H NMR ($CDCl_3$, 500 MHz) $\delta = 0.89$ (s, 6H, $2CH_3$), 0.91 (s, 6H, $2CH_3$), 0.95–1.20 (m, 24H, $8CH_3$), 1.43–1.48 (m, 4H, $2CH_2$), 1.66–1.72 (m, 4H, $2CH_2$), 1.91–1.95 (m, 4H, $2CH_2$), 1.99–2.06 (m, 8H, $4CH_2$), 2.13–2.19 (m, 4H, $2CH_2$), 2.21–2.28 (m, 8H, $4CH_2$), 3.28 (t, $J = 14.0$ Hz, 2H, CH_2), 3.78 (t, $J = 6.1$ Hz, 2H, OCH_2), 4.28 (t, $J = 8.2$ Hz, 2H, OCH_2), 4.60 (d, $J = 13.0$ Hz, 2H, CH_2), 5.27 (s, 2H, CH), 6.23 (m, 6H, H_{Aryl}), 7.03 (d, $J = 21.3$ Hz, 4H, H_{Aryl}), 7.37 (d, $J = 8.5$ Hz, 4H, H_{Aryl}), 7.41 (d, $J = 8.5$ Hz, 4H, H_{Aryl}); ^{13}C NMR ($CDCl_3$, 300 MHz): 13.99, 14.18, 18.99, 19.69, 26.58, 26.69, 29.66, 29.73, 29.90, 30.62, 30.74, 32.27, 32.44, 32.55, 32.70, 41.83, 42.54, 49.94, 50.20, 75.26, 75.52, 113.66, 114.27, 119.72, 121.65, 127.39, 127.49, 129.23, 129.75, 130.23, 131.10, 132.42, 132.57, 132.63, 138.64, 139.03, 145.41, 150.12, 150.22, 155.36, 158.33, 195.54, 195.70; HR-MS (ESI): m/z $[M]^+$ calcd for $C_{90}H_{104}Br_2N_2O_8$: 1498.6159; found: 1498.9059.

9-(3-Hydroxyphenyl)-3,3,6,6-tetramethyl-10-phenyl-3,4,6,7,9,10-hexahydroacridine-1,8(2H,5H)-dione 11

To a solution of aniline (0.13 g, 1.4 mmol) in 5 mL ethanol was added dimedone (0.14 g, 1 mmol) and heteropolyacid (HPA) (3%mol). The mixture was refluxed and the reaction was followed by TLC until the dimedone was entirely consumed. After 4 h, the second part of dimedone (0.14 g, 1 mmol) and 3-hydroxybenzaldehyde (0.12 g, 1 mmol) were added to the mixture. The reaction was refluxed for 4 h. The reaction was stopped by adding crushed ice to the mixture, filtered through sintered funnel and the residue was dissolved in warm ethanol. Then, the solvent was evaporated and the crude was crystallised in ethanol to give the product in 80% yield. M.p.: 250°C. ^{1}H NMR (DMSO-d_6/$CDCl_3$, 80 MHz): $\delta = 0.78$ (d, $J = 11.2$ Hz, 12H, $2CH_3$), 1.72–2.29 (m, 8H, $4CH_2$), 5.01 (s, 1H, CH), 6.42–7.71 (m, 9H, H_{Aryl}). MS (E.I.) (70 eV): m/z 441 (3) $[M]^+$, 348 (100), 272 (28), 93 (70), 77 (43).

References

(1) Di, G.C.; De, M.M.; Chiron, J.; Dehnas, F. *Bioorg. Med. Chem.* **2005**, *13*, 5560–5568.

(2) (a) Gamage, S.A.; Tepsiri, N.; Wilairat, P.; Wojcik, S.J.; Figgit, D.P.; Ralph, R.; Denny, W.A. *J. Med. Chem.* **1994**, *37*, 1486–1494; (b) Gamage, S.A.; Figgit, D.P.; Wojicik. S. J.; Ralph, R.; Ransijn, A.; Mauel, J. *J. Med. Chem.* **1997**, *40*, 2634–2642; (c) Mauel, J.; Denny, W.A.; Gamage, S.A.; Ransijn, A.; Wojcik, S.L.; Figgit, D.P.; Ralph, R. *Antimicrob. Agents Chemother.* **1993**, *37*, 991–996; (d) Rewcastle, G.W.; Atwell, G.J.; Chambers, D.; Baguley, B. C.; Denny, W.A. *J. Med. Chem.* **1986**, *29*, 472–477; (e) Atwell, G.J.; Rewcastle, G.W.; Baguley, B.C.; Denny, W.A. *J. Med. Chem.* **1987**, *30*, 664–669.

(3) Denny, W.A. *Curr. Med. Chem.* **2002**, *9*, 1655–1665.

(4) Mukherjee, A.; Lavery, R.; Bagchi, B.; Hynes, J.T. *J. Am. Chem. Soc.* **2008**, *130*, 9747–9755.

(5) Neto, B.A.D.; Lapis, A.A.M. *Molecules* **2009**, *14*, 1725–1746.

(6) Kivlehan, F.; Lefoix, M.; Moynihan, H.A.; Thompson, D.; Ogurtsov, V.I.; Herzog, G.; Arrigan, D.W.M. *Electrochim. Acta* **2010**, *55*, 3348–3354.

(7) O'Connor, K.M.; Arrigan, D.W.M.; Svehla, G. *Electroanalysis* **1995**, *7*, 205–215.

(8) Zadmard, R.; Schrader, T. *Angew. Chem. Int. Ed.* **2006**, *45*, 2703–2706.

(9) (a) Blecking, C.J.; Hu, W.; Zadmard, R.; Dasgupta, A.; Schrader, T. *Synthesis* **2011**, *8*, 1193–1204; (b) Zadmard, R.; Taghvaei-Ganjali, S.; Gorji, B.; Schrader, T. *Chem. Asian J.* **2009**, *4*, 1458–1464; (c) Breitkreuz, C.J.; Zadmard, R.; Schrader, T. *Supramol. Chem.* **2008**, *20*, 109–115.

(10) Das, B.; Thirupathi, P.; Mahender, I.; Reddy, V.S.; Rao, Y. K. *J. Mol. Catal. A Chem.* **2006**, *247*, 233–239.

(11) Mizuno, N.; Misono, M. *Chem. Rev.* **1998**, *98*, 199–218.

(12) Mirza-Aghayan, M.; Khoshkameh Langrodi, M.; Rahimifard, M.; Boukherroub, R. *Appl. Organomet. Chem.* **2009**, *23*, 267–271.

(13) Mirza-Aghayan, M.; Bolourtchian, M.; Hoseini, M. *Synth. Commun.* **2004**, *34*, 3335–3341.

(14) Mirza-Aghayan, M.; Moradi, A.; Bolourtchian, M.; Boukherroub, R. *Synth. Commun.* **2010**, *40*, 8–20.

(15) Mirza-Aghayan, M.; Moradi, A.; Bolourtchian, M. *J. Iran. Chem. Soc.* **2010**, *7*, 269–274.

(16) Gutsche, C.D.; Iqbal, M.; Stewart, D. *J. Org. Chem.* **1986**, *51*, 742–745.

(17) Chawla, H.M.; Srinivas, K. *Indian J. Chem.* **1993**, *32B*, 1162–1164.

(18) Verboom, W.; Durie, A.; Egberink, J.M.R.; Asfari, Z.; Reinhoudt, D.N. *J. Org. Chem.* **1992**, *57*, 1313–1316.

(19) Kelderman, E.; Verboom, W.; Engbersen, J.F.J.; Reinhoudt, D.N.; Heesink, G.J.T.; van Hulst, N.F.; Derhaeg, L.; Persoons, A. *Angew. Chem. Int. Ed.* **2003**, *31*, 1075–1077.

(20) Timmerman, P.; Boerrigter, H.; Verboom, W.; Reinhoudt, D.N. *Rec. Trav. Chim.* **1995**, *114*, 103–111.

(21) Marti, A.A.; Jockusch, S.; Stevens, N.; Ju, J.Y.; Turro, N.J. *Acc. Chem. Res.* **2007**, *40*, 402–409.

(22) Wheate, N.J.; Brodie, C.R.; Collins, J.G.; Kemp, S.; Aldrich-Wright, J.R. *Mini Rev. Med. Chem.* **2007**, *7*, 627–648.

(23) Ma, S.G.; Zhu, M.S. *Chem. Biol. Interact.* **2009**, *179*, 25–37.

(24) (a) Healy, E.F. *J. Chem. Educ.* **2007**, *84*, 1304–1307; (b) Williams, A.K.; Dasilva, S.C.; Bhatta, A.; Rawal, B.; Liu, M.; Korobkova, E.A. *Anal. Biochem.* **2012**, *422*, 66–73.

(25) Crothers, D.M. *Biopolymers* **1968**, *6*, 575–584.

(26) Shim, Y.-H.; Arimondo, P.B.; Laigle, A.; Garbesi, A.; Lavielle, S. *Org. Biomol. Chem.* **2004**, *2*, 915–921.