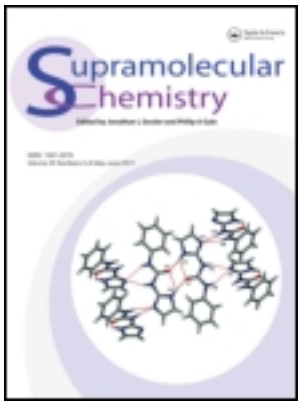

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# Synthesis of nucleobase-calix[4]arenes via click chemistry and evaluation of their complexation with alkali metal ions and molecular assembly

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# Synthesis of nucleobase-calix[4]arenes via click chemistry and evaluation of their complexation with alkali metal ions and molecular assembly

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In this study, calix[4]arene derivatives (**11**–**14**) bearing a single nucleobase (adenine, thymine, cytosine or guanine) were synthesised via click chemistry. The complexation ability of the synthesised derivatives with alkali metal ions was measured using MALDI-TOF mass spectrometry, and their molecular assembly in $CDCl_3$ was determined using $^1$H NMR. Calix[4]arene derivatives (**11**–**14**) formed 1:1 complexes with all alkali metal ions and the rank order for the complexation selectivity was $Rb^+ > Cs^+ > K^+ \cong Na^+ > Li^+$. The attachment of nucleobase at the upper rim of calix[4]arene had little effect on its complexation selectivity for alkali metal ions. Thymine-, adenine- and guanine-calix[4]arenes formed self-assembled structures in $CDCl_3$ via base–base interactions. In addition, adenine-calix[4]arene (**11**) bound to thymine-calix[4]arene (**12**) to form a discrete species via Hoogsteen hydrogen bonding.

**Keywords:** calixarene; nucleobase; click chemistry; complexation; molecular assembly



## Introduction

Calix[*n*]arenes (also known as calixarenes) are formed via phenol–formaldehyde condensation reactions, containing repeating phenolic units that are connected via methylene bridges (*1, 2*). The term '*n*' refers to the number of repeating phenolic units in the ring. The *n* values could range from 3 to 20, although tetramers ($n = 4$) and hexamers ($n = 6$) are most commonly seen in the literatures because of their availability and affordability. Calixarenes can have many conformational isomers because of the rotation of the phenolic unit (*3, 4*). For instance, calix[4]arenes can have up to four conformational isomers that are known as 'cone', 'partial-cone', '1,2-alternate' and '1,3-alternate'. The innate conformational flexibility of calixarenes provides a lipophilic cavity (binding pocket) that has been widely investigated for hosting versatile guest molecules ranging from metal ions (*5–7*) to small organic molecules (*8–10*). Calixarene derivatives have been used in nuclear waste treatment owing to their high selectivity for caesium and uranium ions (*11*). The selectivity of calixarenes for alkali, alkaline earth and transition metal ions has led to the development of cation-selective electrodes, optical sensors, artificial enzyme models and novel catalysts for organic reactions (*12, 13*). Chiral calixarenes (*14*) have also been developed for distinguishing between enantiomers – one of the most difficult tasks for molecular recognition.

Calixarenes containing DNA building units such as nucleotides, nucleosides and nucleobases have recently aroused much interest because of their versatile applications. Consoli and co-workers (*15*) reported water-soluble nucleotide-calixarene conjugates as potential DNA polymerase inhibitors. Cytosine-conjugated calix[4]pyrroles (a calixarene analogue) were constructed as neutral receptors for 5′-guanosine monophosphate (*16*). Kim and co-workers incorporated the calix[4]arene moiety into DNA oligonucleotide strands using phosphoramidite chemistry. They found that the calix[4]arene-oligonucleotide hybrids adopt a V-shaped conformation and can be used for constructing programmed oligonucleotide nanostructures (*17*). Many investigations of calixarenes containing DNA building units rely on the rationale that calixarenes can act as scaffolds to promote the intrinsic hydrogen bonding between nucleobases. For instance, self-assembled nanotubes were observed via G-quartet formation between 1,3-alternate-calix[4]arenes bearing guanosine units at the upper and the lower rim (*18*). We believe that calixarenes containing nucleobases are suitable model molecules for studying such molecular interactions. However, to our knowledge, previous reports on nucleobase-calixarenes are limited to the incorporation of thymine and adenine to calix[4]arenes via amide bond formation (*19*). In order to draw a complete picture, we herein report the facile synthesis of calix[4]arenes (**11**–**14**) functionalised with a single nucleobase (thymine, adenine, guanine or cytosine) at the upper rim via click chemistry. Their complexation with alkali metal ions was examined using MALDI-TOF mass spectrometry and their molecular interactions were determined using $^1$H NMR. All calix[4]arene derivatives show good complexation with

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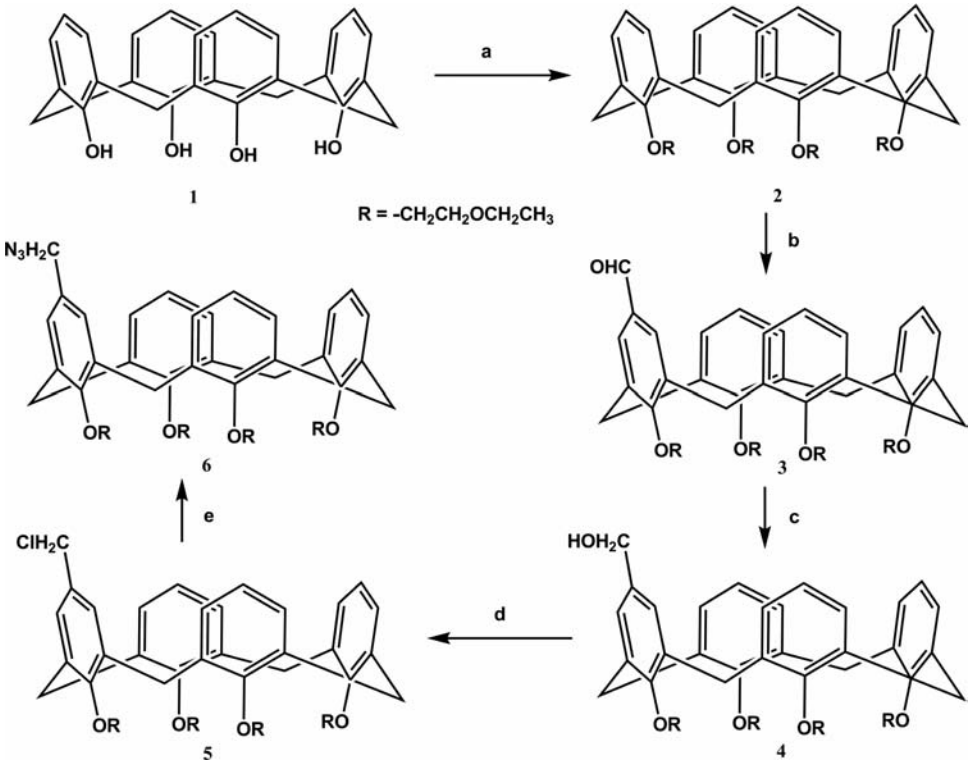



Scheme 1. Synthesis of **6**. Reagents and conditions: (a) NaH, 2-bromoethyl ethyl ether and DMF; (b) 1,1-dichloromethyl ether, $TiCl_4$ and $CHCl_3$; (c) $NaBH_4$ and EtOH; (d) $SOCl_2$ and DCM and (e) $NaN_3$ and DMF.

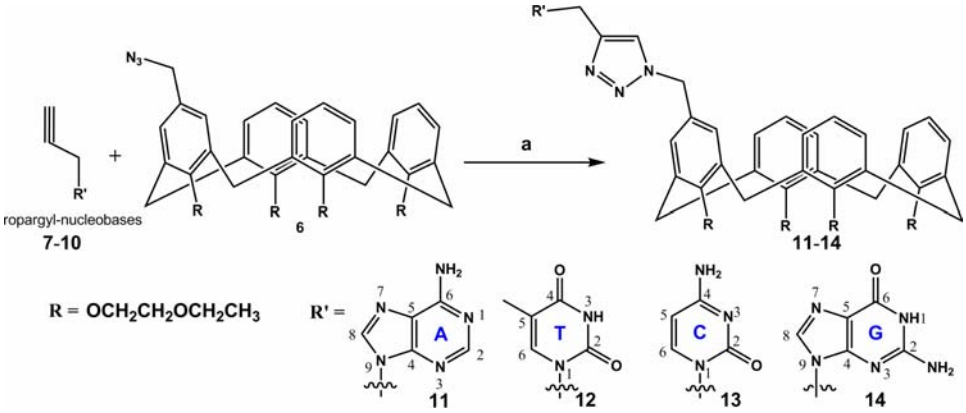
alkali metal ions with apparent selectivity. The results also reveal that nucleobase-calix[4]arenes are capable of self-association in $CDCl_3$ and calix[4]arenes bearing complementary nucleobases can bind to each other via base pairing.

## Results and discussion

### *Synthesis of calix[4]arenes containing a single nucleobase*

To simplify the study of the molecular interactions between nucleobase-calix[4]arenes, we sought to synthesise calix[4]arenes containing a single nucleobase. Numerous methods have been reported on modification of calix[4]arenes at the upper rim or the lower rim (*20, 21*). Substituents on either rim may affect the spatial arrangement of the molecules; hence, one can pre-organise a desired conformation by introducing substituents at proper positions. Calix[4]arene fixed in cone conformation contains a cavity with a calix shape that is known to fit for certain cations or organic molecules. To achieve the pre-organised cone conformation, all four hydroxyl groups at the lower rim can be alkylated using an ethoxyethyl group that is bulky enough to inhibit the rotation of phenolic unit (*22*). Tetra-*O*-alkylated calix[4]arene (**2**) was obtained by reacting calix[4]arene (**1**) with 1-bromo-2-ethoxyethane in the presence of NaH in good yield (Scheme 1) (*23*). This approach removes the interference of hydroxyl groups as hydrogen bond acceptors and donors, and, in addition, improves the solubility of the resulting calix[4]arene derivatives in organic media. Consequently, nucleobases need to be introduced to the upper rim. We chose click chemistry to prepare our molecules for its simplicity and mild reaction conditions. Moreover, the triazole moiety resulted from the click reaction may also provide unique complexation properties (*24*).

Nucleobases containing a propargyl group (**7**–**10** containing adenine, thymine, cytosine and guanine, respectively) and a 5-methylazidocalix[4]arene (**6**) were prepared separately for the click reaction. The syntheses of propargyl-nucleobases have been previously reported (*25, 26*). The detailed synthetic route for **6** is shown in Scheme 1. To introduce an azidomethyl group to the upper rim of calix[4]arene, compound **3** was obtained by mono-formylation of **2** using 1,1-dichlorodimethyl ether in the presence of $TiCl_4$ at $-10$°C in 77% yield. The control of temperature for this reaction is very important for obtaining a reasonable yield for monoformylated calix[4]-arene. At temperatures higher than $-10$°C, significant amounts of diformylated and triformylated products were obtained. Compound **4** was prepared by reduction of **3** using $NaBH_4$. Substitution reaction of **4** with $SOCl_2$




Scheme 2. Synthesis of **11**–**14**. Reagents and conditions: (a) $CuSO_4$, sodium ascorbate and 90–100°C.



afforded **5** (*27*). Reaction of **5** with $NaN_3$ yielded **6** in quantitative yield. The click reaction between propargyl-nucleobases (**7**–**10**) and **6** in the presence of catalytic amount of Cu(I) afforded the desired nucleobase-calix[4]arenes (**11**–**14**) in good yield (Scheme 2). Cu(I) warrants the formation of the 1,4-regioisomer of 1,2,3-triazole. All the synthesised nucleobase-calix[4]arenes (**11**–**14**) showed good solubility in common organic media.

### *Evaluation of complexation of nucleobase-calix[4]arenes with alkaline metal ions using MALDI-TOF mass spectrometry*

The attachment of long alkyl chains to the phenoxy groups ensures the immobilisation of nucleobase-calix[4]arenes into the cone conformation. Such a conformation can be easily determined by measuring the $^{13}C$ NMR chemical shifts of bridged methylene carbons (*28, 29*). A signature signal occurring at 30–31 ppm should be observed when neighbouring aromatic rings are oriented to the same side to yield a cone conformation. Following this simple rule, we conclude that all four synthesised calix[4]arenes (**11**–**14**) adopt a cone conformation (Supplementary Information, available online). The inner cavities of calixarenes with a pre-organised cone conformation allow them to accommodate molecules with matching sizes. In our cases, tethering a nucleobase to the upper rim of calixarenes further introduces supplementary hydrophobicity and steric effect, which may regulate their binding ability.

Calix[4]arenes are known to be strong chelators of alkali metal ions in the solution (*6, 30–32*), which has led to the development of sensor devices. In our experiments, MALDI-TOF mass spectrometry was used to determine the complexation of calixarenes with alkali metal ions. Without doping with alkali metal ions, compounds **11**–**14** yielded sodiated and potassiated species with high signal-to-noise ratio. For instance, the mass spectrum of adenine-calix[4]arene (**11**) showed its sodium adduct and potassium adduct at *m/z* 963.1 and 979.1, respectively (Figure 1). Such adducts are commonly observed contaminants in the MALDI mass spectra, especially

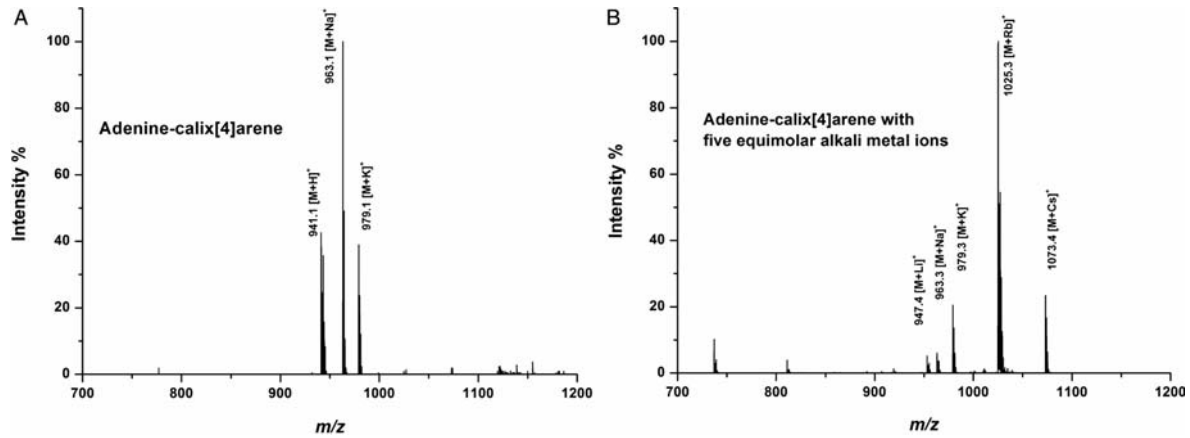



Figure 1. (A) Representative MALDI-TOF mass spectrum of **11**; (B) representative MALDI-TOF mass spectrum of **11** doped with equimolar $Li^+$, $Na^+$, $K^+$, $Rb^+$ and $Cs^+$.

Table 1. Relative intensities of adducts of **11**–**14** with alkali metal ions.

| Compound | $Li^+$ | $Na^+$ | $K^+$ | $Rb^+$ | $Cs^+$ |
|---|---|---|---|---|---|
| **11** | 9.6 ± 3.8 | 18.0 ± 4.8 | 22.7 ± 4.2 | 100 | 41.4 ± 14.3 |
| **12** | 14.2 ± 6.8 | 27.1 ± 11.8 | 25.5 ± 5.6 | 100 | 36.6 ± 9.9 |
| **13** | 10.7 ± 4.7 | 22.6 ± 6.5 | 27.8 ± 6.9 | 100 | 42.2 ± 12.1 |
| **14** | 13.1 ± 4.0 | 25.0 ± 9.6 | 25.3 ± 5.6 | 100 | 36.4 ± 7.5 |
| **2** | 29.6 ± 13.9 | 27.1 ± 9.3 | 22.6 ± 5.8 | 100 | 50.4 ± 12.5 |

Notes: In each experiment, the intensity of the $Rb^+$ adduct was set to 100 because it was the most significant peak.



when the compounds are polyoxygenated (*32*). The peaks representing the adducts of calixarene derivatives with other alkali metal ions such as $Li^+$, $Rb^+$ and $Cs^+$ with high signal-to-noise ratio could also be readily observed when they were doped with the corresponding alkali metal ions. These observations suggest that the attachment of nucleobase at the upper rim of calix[4]arene has little effect on its ability to chelate with alkali metal ions. To determine the selectivity of **11**–**14** for alkali metal ions, we measured their MALDI-TOF mass spectra when these compounds were doped with a solution mixture containing five equimolar alkali metal ions ($Li^+$, $Na^+$, $K^+$, $Rb^+$ and $Cs^+$). The intensities of the adduct peaks were affected by the laser power, the number of laser shots and the position where the laser beam focused on; therefore, for reliable measurements, 30 spectra were collected for each compound and the resulting data were subjected to statistical analysis (Student's *t*-test). The representative spectrum for **11** in the presence of five alkali metal ions is shown in Figure 1. In all the experiments, the adduct peaks with high signal-to-noise ratio were obtained. The relative selectivity can be expressed as a ratio of the intensity of one alkali metal ion adduct to that of another (Table 1). Regardless of the nucleobase, the rank order for the selectivity of **11**–**14** given by the relative intensity of the adduct peak relative to that of the $Rb^+$ adduct peak is $Rb^+ > Cs^+ > K^+ \cong Na^+ > Li^+$. The results showed that all compounds have the highest preference for complexation with $Rb^+$ over other four. For example, the $Rb^+/K^+$ selectivity for **12** is 3.9:1 and the $Rb^+/Cs^+$ selectivity for **12** is 2.7:1. Blanda and co-workers (*5*) reported calix[6]arene bis-crown-4 has predominant selectivity towards $Cs^+$. It is reasonable that calix[4]arenes with a smaller size of cavity prefer to complex with $Rb^+$. All compounds bind to $Cs^+$ more strongly than $Li^+$, $Na^+$ and $K^+$ in a statistically significant manner. The difference in selectivity for $Na^+$ and $K^+$ is not significant. The complexation of all compounds with $Li^+$ was fairly weak as compared to others. $Li^+$ that has a smaller size may not fit snugly into the cavity of calix[4]arene and could interact with the *O*-alkyl chains at the lower rim. In fact, the complexation of $Li^+$ and $Na^+$ with the methoxy groups at the lower rim of tetra-*O*-methyl-calix[4]arenes was determined by $^1H$ NMR (*33*). Similar results for compounds **1** and **2** were obtained except that no significant difference in selectivity was observed for complexation with $Li^+$, $Na^+$ and $K^+$. Taken together, we conclude that the attachment of nucleobase at the upper rim and at the lower rim of alkyl chains has minimal effect on the complexation ability of calix[4]arene with alkali metal ions.

### *Self-assembly of nucleobase-calix[4]arenes*

In principle, nucleobase-derived calixarenes are similar to the lipophilic guanosine analogues (*34*) that are known to exert the molecular self-assembly ability. For instance, Kotch and co-workers (*35*) reported that a guanosine-calixarene conjugate forms a discrete water-filled dimer that acts as a ditopic salt receptor. In some cases, molecular assembly can be identified by comparing the $^1H$ NMR spectra collected in non-polar $CDCl_3$ and polar DMSO-$d_6$, respectively. The hydrogen bonds are more stable in $CDCl_3$ than in DMSO-$d_6$ because the latter is a stronger hydrogen bond acceptor that disrupts H-bonding formation. In our study, ill-resolved spectra in the guanine proton regions of guanine-calix[4]arene **14** in $CDCl_3$ were observed (Figure 2), suggesting the formation of non-specific aggregation (*35*) or G-ribbons (*36*). The signal of the H8 of the guanine moiety that should appear at ~8 ppm was not noticeable. The hydrogen bonds between **14** were disrupted by the polar solvent DMSO-$d_6$ since a well-resolved spectrum was obtained. In DMSO-$d_6$, the H8 signal of unbound **14** was unambiguously observed at 7.8 ppm. The chemical shift of the amino N—H proton of **14** slightly shifted upfield ($\Delta\delta = 0.5$ ppm) in $CDCl_3$ relative to DMSO-$d_6$. In contrast, the imido N—H proton of the guanine moiety of **14** underwent a dramatic downfield shift ($\Delta\delta = 1.3$ ppm) in $CDCl_3$ relative to DMSO-$d_6$ (Figure 2). The imido N—H protons are usually shifted upfield in $CDCl_3$ compared to DMSO-$d_6$ (*35*). This unusual observation is evidence of the molecular interactions between guanine-calix[4]arenes. Molecular assembly of **11**–**13** by comparing their spectra in $CDCl_3$ and DMSO-$d_6$ is not conclusive. Unlike **14**, compounds **11**–**13** all yielded well-resolved spectra in $CDCl_3$ (Figure 2). The chemical shift of the imido-H of the thymine moiety of **12** underwent a significant upfield shift ($\Delta\delta = 2.7$ ppm) in $CDCl_3$ relative to DMSO-$d_6$.

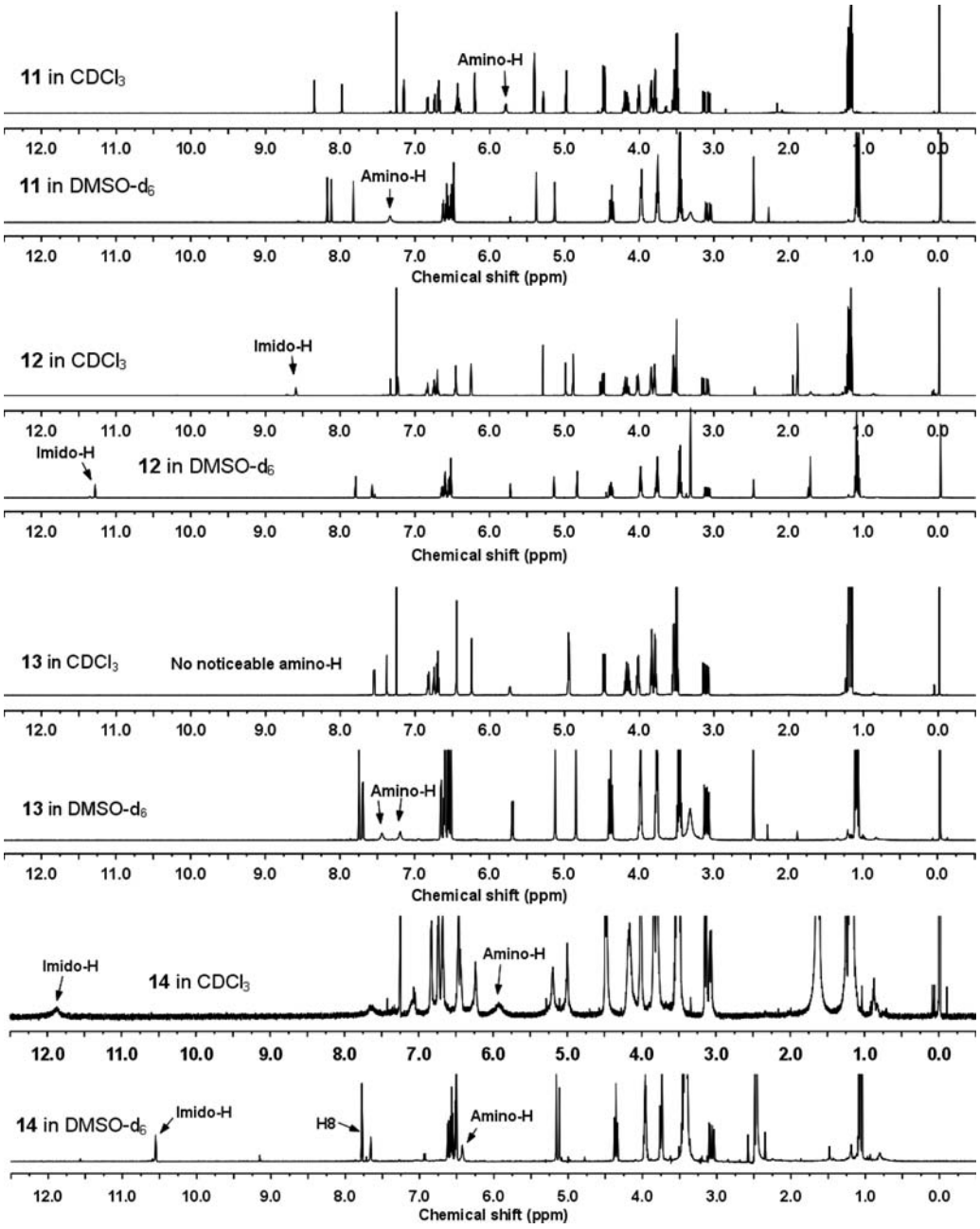



Figure 2. $^{1}$H NMR spectra of **11**–**14** in $CDCl_3$ and DMSO-$d_6$ at 25°C.

Adenine-calix[4]arene **11** in $CDCl_3$ also gave a upfield shift ($\Delta\delta = 1.5$ ppm) of the amino-H of the adenine moiety as compared to that in DMSO-$d_6$. The amino-H signal of the cytosine moiety of **13** could not be observed in $CDCl_3$ (Figure 2). Interestingly, two signals representing the amino-H of the cytosine moiety of **13** were found in DMSO-$d_6$, indicating that the chemical environment of the amino protons could be different.

Evidence for the molecular self-assembly of **11**, **12** and **14** came from the comparison of their $^{1}$H NMR spectra taken at various temperatures. The chemical shift of the imido-H of the thymine moiety of **12** is clearly temperature dependent (Figure 3). From 30 to −50°C, its chemical shift underwent a significant downfield shift from 8.5 to 10.4 ppm ($\Delta\delta = 1.9$ ppm), suggesting that the H-bonding between thymine-calix[4]arenes is stabilised as a function of decreasing temperature. At the lower temperatures, the signals of **12** became broader due to the increased viscosity of the solution. Similarly, the signals of the amino groups of the adenine moiety of **11** were also downfield shifted as a function of decreasing temperature. A chemical shift change of 0.8 ppm occurred

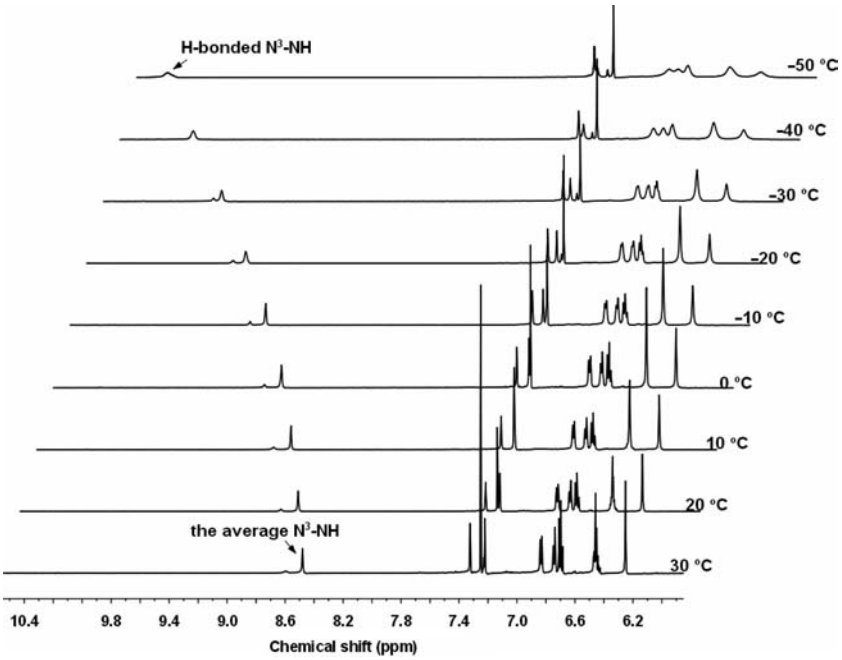



Figure 3. Representative $^1$H NMR spectra of **12** in $CDCl_3$ at various temperatures ranging from −50 to 30°C.

from 30 to −50°C (Supplementary Information, available online). These results are consistent with a previous report (*19*) although the structures of thymine- and adenine-calixarenes are different from ours. Both **11** and **12** in $CDCl_3$ at temperatures higher than 0°C afforded well-resolved spectra, indicating the formation of a discrete species instead of non-specific aggregation. It is noteworthy that except for the observed chemical shifts mentioned above, the chemical shifts of other proton signals were barely changed, suggesting that the molecular interactions most likely occurred between nucleobases. Plausible self-assembled dimeric structures of **11** and **12** are shown in Figure 4. The formation of thymine–thymine base pairs on the mononucleotide and oligonucleotide levels has been reported, and such base pairs are particularly stable when nucleotides are neutral (*37*). Adenine–adenine base pairs (e.g. Hoogsteen and reverse Hoogsteen) are commonly seen in DNA triplex helical structures (*38*). Several stable adenine–adenine structures in the presence of metal ions were observed using IR–UV double-resonance spectroscopy (*39*) and computational calculations (*39, 40*). The observation of adenine–adenine interactions here is remarkable, and it may lead to a useful approach to study base pairing using calixarenes as scaffolds. Ill-resolved $^1$H NMR spectra of **14** were obtained at all of the tested temperatures ranging from 30 to −50°C (Supplementary Information, available online). The broad signals of **14** at higher temperatures indicate the existence of strong H-bonding between **14**. Consistent with the solvent study, the broad signals of **14** also suggest that it may self-associate into oligomeric types of structures such as G-ribbons or G-quartets (*36, 41*). The self-assembly of cytosine-calix[4]arene (**13**) could not be clearly identified due to the lack of amino proton signals even at lower temperatures.

Further support for the self-assembly of **11**, **12** and **14** resulted from the concentration-dependent study. Calixarenes at higher concentrations have less solvation effect; as a consequence, the stabilisation of H-bonding could be observed. In our study, when the concentration of the nucleobase-calixarene increased from 5 to 25 mM in $CDCl_3$, similar signal downfield shifts (Figure 5) relative to those observed in the temperature-dependent studies were observed using $^1$H NMR. The concentration dependence of the NMR signals indicates that the self-assembled structures of **11**, **12** and **14** are formed via strong intermolecular H-bonding. Ill-resolved $^1$H NMR spectra in the guanine proton region were obtained at all three concentrations of **14**, revealing that the non-specific aggregation or oligomeric structures of **14** was predominant in the solution. The main contribution to this structure probably is the amino group of the guanine moiety as a hydrogen bond donor. The imido-H seems not to involve in the intermolecular interaction because its chemical shift ($\delta = 11.8$ ppm) is not concentration dependant.

### *Intermolecular assembly of nucleobase-calix[4]arenes*

Nucleobases recognise one another based on the known base-pairing pattern, such as Watson–Crick and Hoogsteen. The complete set of nucleobase (adenine, thymine, cytosine and guanine)-calix[4]arene conjugates enables us

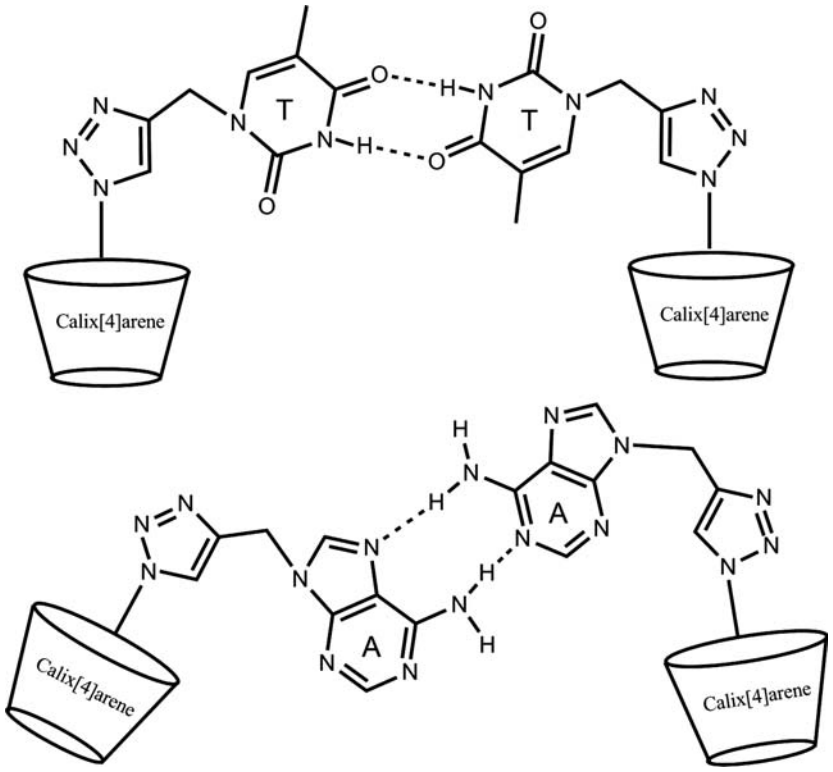



Figure 4. Plausible self-assembled structures of **11** (top) and **12** (bottom).

to investigate the intermolecular interactions between complementary bases. When cytosine-calix[4]arene (**13**) was mixed with guanine-calix[4]arene (**14**) in a 1:1 molar ratio in $CDCl_3$, the protons of purine and pyrimidine were ill-resolved (Figure 6). The H8 of the guanine moiety of **14** remained unnoticeable and the H5 and H6 signals of the cytosine moiety of **13** significantly diminished. These ill-resolved signals suggest that no discrete species were formed in the solution. The cytosine moiety of **13** could interact with the guanine moiety of **14** via non-specific aggregation to yield oligomeric structures. It is reasonable to conclude that the molecular interactions between the guanine moieties of **14** are very strong. The cytosine moiety of **13** could not effectively shift the guanine–guanine base-pairing equilibrium to the well-defined Watson–Crick cytosine–guanine interaction.

Mixing adenine-calix[4]arene (**11**) with thymine-calix[4]arene (**12**, 1:1) in $CDCl_3$ afforded a well-resolved spectrum (Figure 6), indicating the formation of one discrete species. All the signals of the mixture can be clearly assigned with respect to the spectra of **11** and **12**. The signals of the amino-H of the adenine moiety of **11** and the imido-H of the thymine moiety of **12** shifted downfield with a value of 0.6 and 1.6 ppm, respectively (Figure 6). The changes in the chemical shifts of the calix[4]arene region were barely noticeable, suggesting the molecular interactions indeed were between two nucleobases. The identities of the protons (H8 and H2) of the adenine moiety of **11** were unambiguously assigned with three-bond correlations in HMBC experiments (Figure 7). In the HMBC spectrum, the H8 of adenine showed two signals, representing the correlations with C4 and C5, respectively. Surprisingly, the correlation between H8 and the carbon of the methylene group that connects adenine and triazole was not noticeable. The H2 of adenine showed two signals, representing the correlations with C4 and C6, respectively. In addition, the correlations of the methylene protons with C5′, C8, C4′ and C4 were clearly observed. In the spectrum of the mixture (Figure 6), the signal representing the H8 of the adenine moiety of **11** shifted downfield with a value of 0.065 ppm while the chemical shift of the H2 signal was barely changed. The molecular recognition between **11** and **12** most likely involves the Hoogsteen base-pairing mode (*42, 43*), in which the chemical environment of the H8 is more drastically affected by proximate **12** than that of the H2. The latter is further apart from **12**, as shown in the proposed structure in Figure 8. It is necessary to point it out that the proposed interaction between **11** and **12** via the Hoogsteen base pairs in our study is different from the previously published data (*19*). Huang and co-workers suggested the formation of an assembled structure via the Watson–Crick base pairs when mixing adenine-calixarene and thymine-calixarene derivative (1:1). Their structure was proposed based on the assignments of the H8 and the H2 of the adenine moiety, which were exactly inverted as

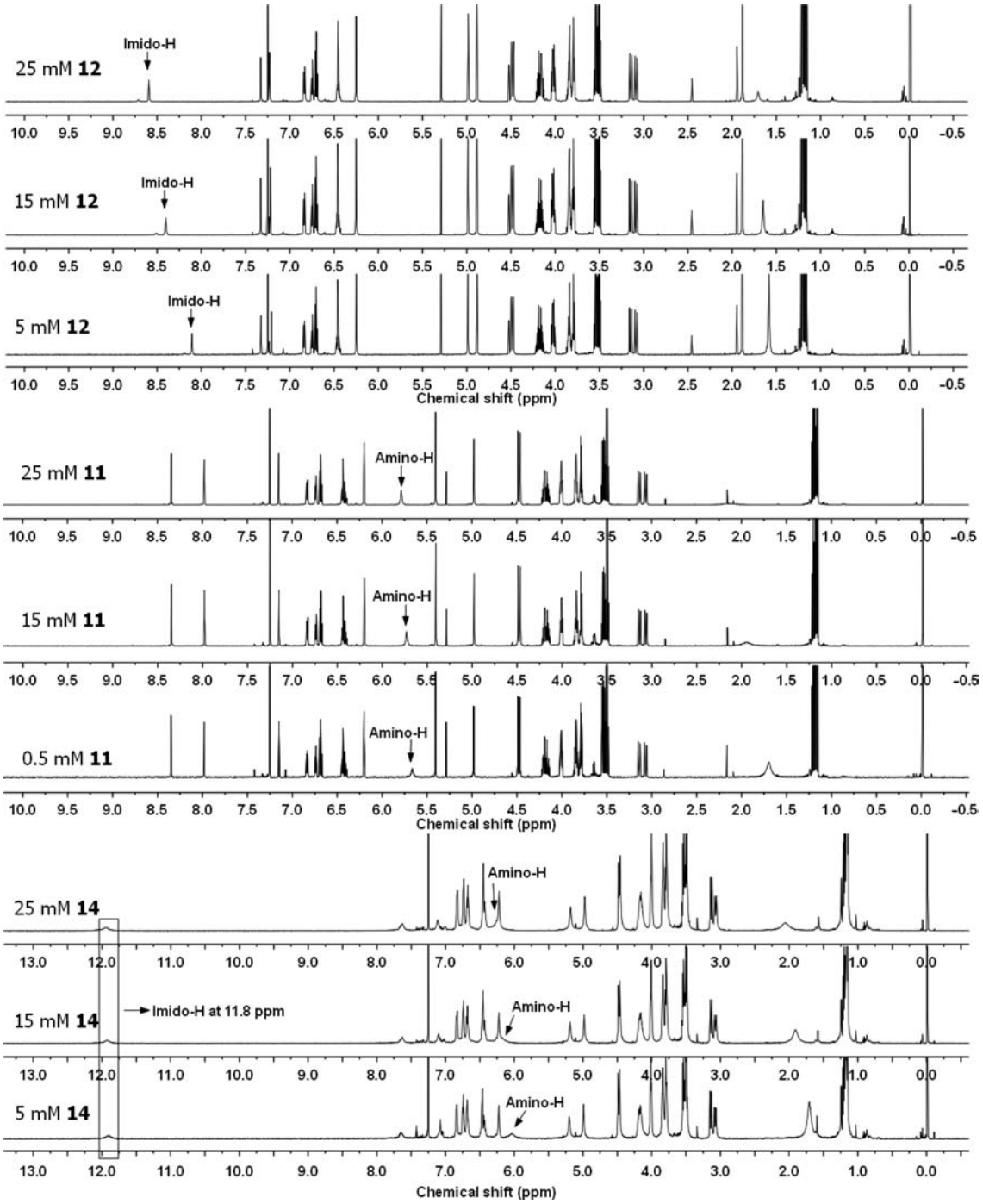



Figure 5. $^{1}$H NMR spectra of **11**, **12** and **14** in $CDCl_3$ at different concentrations (5, 15 and 25 mM) at 25°C.

compared to our results. The reason for the formation of the predominant structure via the Hoogsteen base pairs between **11** and **12** is unclear, probably because such an orientation of the complex has less steric hindrance. Further attempts to confirm the Hoogsteen base pairs by measuring the hydrogen-bonded protons using the NOE and HMBC techniques were inconclusive at this point.

## Conclusions

In summary, we use click chemistry to synthesise nucleobase-calix[4]arene derivatives (**11**–**14**) that cover a complete set of DNA nucleobases for study. All four calix[4]arene derivatives can efficiently complex with alkali metal ions with a reasonable rank order of selectivity. The presence of nucleobase at the upper rim

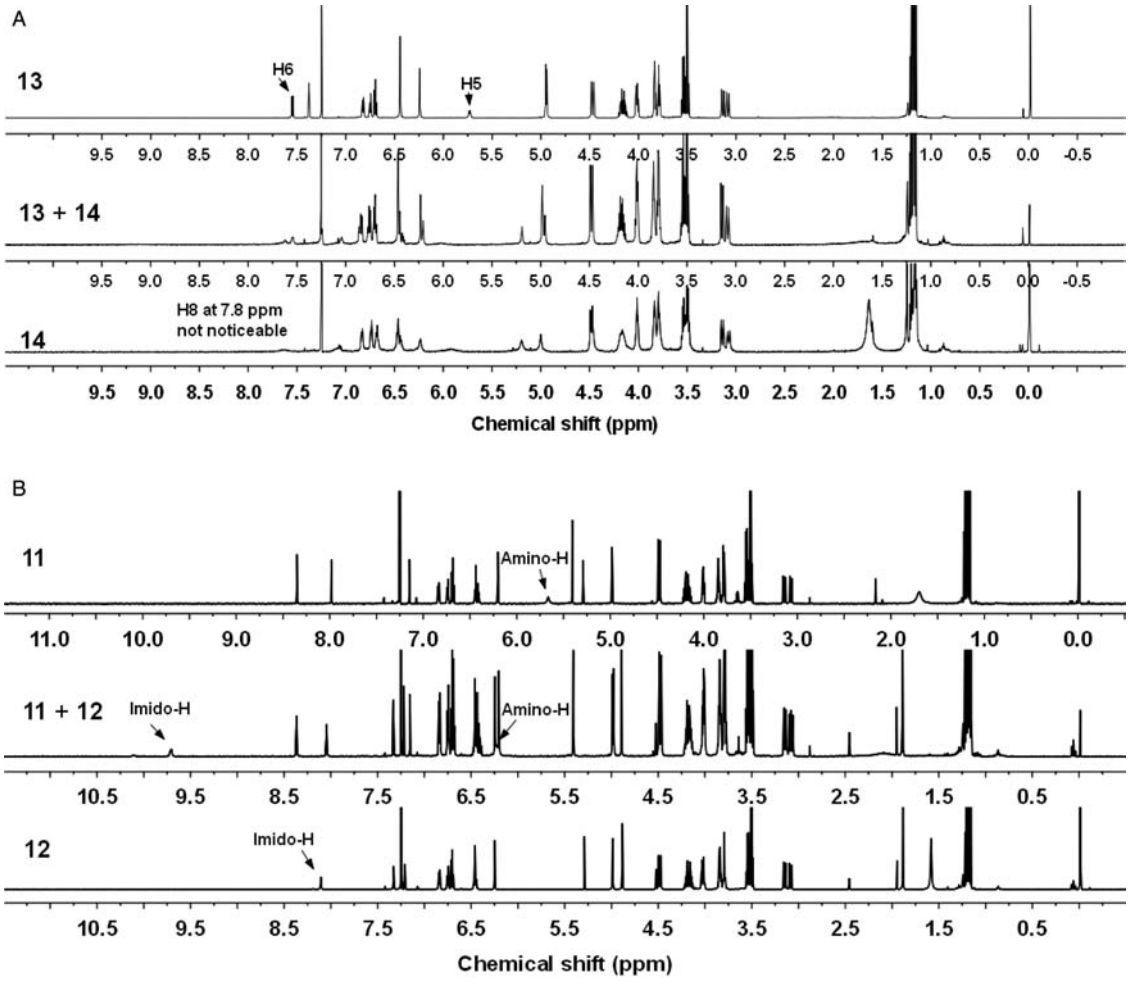



Figure 6. (A) $^{1}$H NMR spectra of **13**, **14** and **13** + **14** (equivalent molar ratio) in $CDCl_3$ and (B) $^{1}$H NMR spectra of **11**, **12** and **11** + **12** (equivalent molar ratio) in $CDCl_3$.



does not alter the selectivity of calix[4]arene towards alkali metal ions. As predicted, the recognition ability of nucleobases via base pairing leads to the assembly of calixarene derivatives in $CDCl_3$. Investigation of such molecules could provide deeper understanding on nucleobase pairing in organic media and construction of high-ordered molecular structures. The effect of metal ions on the assembly of **11**–**14** will be investigated in due course.

## Experimental

Unless otherwise specified, chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Fisher Scientific and used without further purification. Amino acids were purchased from Research Plus, Inc. (Denville, NJ, USA) and Sigma-Aldrich. Solvents were of HPLC grade. Reactions were carried out under argon using dry solvent, unless otherwise noted. $^{1}$H, $^{13}$C and HMBC spectra were collected on a JEOL ECA 600 MHz FT-NMR spectrometer (Peabody, MA, USA). MS (DART-TOF) spectra were collected on a JEOL Accu TOF LC$^{TM}$ time-of-flight mass spectrometer (Peabody, MA, USA) equipped with a DART$^{TM}$ source (Ionsense, Saugus, USA). The MALDI-TOF mass spectra were recorded using a Shimadzu AXIMA-CFT MALDI-TOF instrument (Shimadzu, Columbia, MD, USA) with a 337-nm nitrogen laser.

### *Mass spectrometric analysis (MALDI-TOF)*

2,5-Dihydroxybenzoic acid (DHB, 5 mg/100 μL in ethanol) was used as the matrix in all experiments. Nucleobase-calixarenes were dissolved in chloroform to make a 50 μM solution. The alkali metal ions were dissolved in distilled water to make the final concentration of each ion as 10 mM. The alkali metal ions solution was mixed with equal volume of matrix solution (DHB). The resulting solution (1 μl) was applied to the MALDI probe, followed by the calix[4]arene derivative solution (1 μl). The samples were crystallised

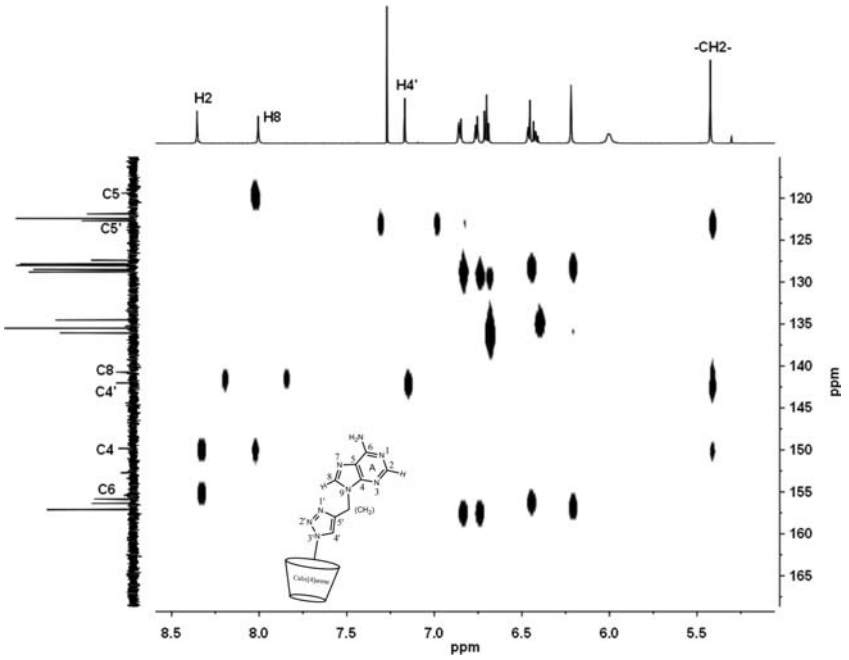



Figure 7. Representative HMBC spectrum of compound **11** in $CDCl_3$.



under a stream of air and subjected to mass spectral analysis. The total acceleration voltage was 20 kV. For laser desorption, a $N_2$ laser operated at 337 nm was employed. Experiments were carried out in a reflectron positive ion mode.

### *NMR studies*

In the temperature-dependent experiments, $^1$H NMR spectra of nucleobase-calix[4]arene conjugates (15 mM) in $CDCl_3$ were collected at various temperatures ranging from $-50$ to 30°C. In the concentration-dependent experiments, three concentrations (5, 15 and 25 mM) of nucleobase-calix[4]arene conjugates were used and the corresponding $^1$H NMR spectra were collected at 25°C. $^1$H NMR spectra of **11** mixed with equimolar **12** (7.5 mM each) and **13** mixed equimolar **14** (7.5 mM each) were collected in $CDCl_3$ at 25°C, respectively.

### *Statistical analysis*

One-tailed unpaired *t*-test was performed to show statistically significant ($p < 0.01$) and insignificant ($p > 0.01$) data.

### *5-Formyl-25,26,27,28-tetraethoxyethylcalix[4]arene (3)*

1,1-Dichloromethyl ether (113 mg, 0.098 mM) and 25,26,27,28-tetraethoxyethylcalix[4]arene (57 mg, 0.08 mM) were dissolved in chloroform (10 ml) and cooled at $-10$°C. To the solution, titanium tetrachloride (185 mg, 0.098 mM) was added. The reaction mixture was stirred at $-10$°C for 40 min and then treated with 20 ml of water. The organic layer was washed twice with water (10 ml), dried with anhydrous $Na_2SO_4$ and concentrated. Flash chromatography (hexane:EtOAc 3:2) of the residue yielded **3** (46 mg, 77%) as a colourless sticky oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 9.61 (s, 1H), 7.10 (s, 2H),

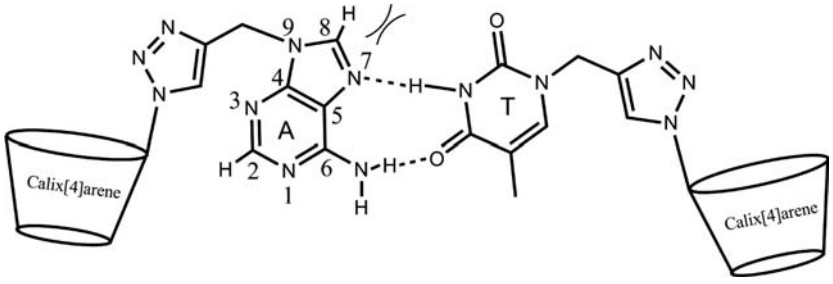



Figure 8. A plausible assembled structure between **11** and **12**.

6.70–6.63 (m, 4H), 6.63–6.58 (m, 2H), 6.54 (m, 2H), 6.50–6.45 (m, 1H), 4.52 (dd, $J = 51.4$ and 13.5 Hz, 4H), 4.21–4.05 (m, 8H), 3.89–3.75 (m, 8H), 3.53 (pd, $J = 7.0$ and 3.3 Hz, 8H), 3.18 (dd, $J = 45.1$ and 13.6 Hz, 4H), 1.22–1.16 (m, 12H); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 191.88, 191.84, 156.46, 156.28, 136.39, 135.39, 135.01, 134.42, 131.13, 130.22, 130.13, 128.77, 128.30, 128.21, 122.57, 122.23, 73.68, 73.33, 69.90, 69.82, 69.75, 69.72, 66.51, 66.47, 66.44,66.40, 30.95, 30.90, 15.42, 15.40, 15.38 and 15.36. HRMS (DART-TOF) calcd for $C_{45}H_{56}O_9$ ($M + H^+$) 741.3997, found 741.3901.

### *5-Hydroxyl-25,26,27,28-tetraethoxyethylcalix[4]arene (4)*

Sodium borohydride (50 mg, 1.32 mmol) was added into an ethanol solution (20 ml) of **3** (190 mg, 0.256 mmol) and stirred at room temperature for 2 h. The reaction mixture was quenched with saturated ammonium chloride (100 ml) and was subsequently extracted with ethyl acetate (200 ml). The organic layer was washed twice with water (200 ml), dried with anhydrous $Na_2SO_4$ and concentrated to yield **4** (quantitative yield) as a yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.70–6.65 (m, 4H), 6.64–6.59 (m, 2H), 6.54 (d, $J = 7.1$ Hz, 2H), 6.50 (d, $J = 6.4$ Hz, 1H), 6.46 (s, 2H), 4.49 (dd, $J = 13.4$, 10.0 Hz, 4H), 4.17–4.04 (m, 8H), 3.93 (s, 2H), 3.83 (m, 8H), 3.59–3.49 (m, 8H), 3.14 (d, $J = 13.5$ Hz, 4H), 1.22–1.17 (m, 12H); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 156.63, 156.40, 135.48, 135.38, 135.04, 134.94, 128.83, 128.48, 128.32, 128.21, 128.16, 122.36, 121.98, 73.33, 73.31, 73.14, 69.78, 69.76, 69.75, 66.47, 66.46, 66.42, 54.66, 30.93, 30.92, 15.40, 15.39 and 15.38. HRMS (DART-TOF) calcd for $C_{45}H_{58}O_9$ ($M + H^+$) 743.4154, found 743.4088.

### *5-Chloromethyl-25,26,27,28-tetraethoxyethylcalix[4]arene (5)*

Thionyl chloride (119 mg, 1.00 mmol) was added into a chloroform solution (10 ml) of **4** (90 mg, 0.121 mmol) and stirred at room temperature for 2 h. The reaction mixture was quenched with water (10 ml) and then extracted with chloroform (10 ml). The organic layer was washed twice with water (10 ml), dried with anhydrous $Na_2SO_4$ and concentrated. Flash chromatography (hexane:EtOAc 3:2) of the residue yielded **5** (85 mg, 92.4%) as a yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.80–6.32 (m, 11H), 4.49 (d, $J = 13.3$ Hz, 4H), 4.24 (s, 2H), 4.14 (t, $J = 5.8$ Hz, 4H), 4.09 (t, $J = 5.6$ Hz, 4H), 3.86–3.79 (m, 8H), 3.57–3.51 (m, 8H), 3.14 (d, $J = 13.4$ Hz, 4H) and 1.22–1.18 (m, 12H); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 156.61, 156.45, 135.38, 134.95, 130.99, 128.55, 128.44, 122.57, 77.37, 77.16, 76.94, 73.46, 73.17, 69.74, 66.48, 66.47, 66.46, 66.43, 46.74, 30.89, 15.41 and 15.38. HRMS (DART-TOF) calcd for $C_{45}H_{57}$ Cl $O_8$ ($M + H^+$) 761.3815, found 761.3741.

### *5-Azidomethyl-25,26,27,28-tetraethoxyethylcalix[4]arene (6)*

Sodium azide (20 mg, 0.308 mmol) was added into a DMF solution (10 ml) of **5** (85 mg, 0.121 mmol) and stirred at 80–90°C overnight. The reaction mixture was concentrated. Flash chromatography (hexane:EtOAc 4:1) of the residue yielded **6** (70 mg, 91.2%) as a pale white solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 6.69 (d, $J = 7.3$ Hz, 4H), 6.63 (t, $J = 7.4$ Hz, 2H), 6.56 (d, $J = 7.5$ Hz, 2H), 6.52 (d, $J = 6.9$ Hz, 1H), 6.47 (s, 2H), 4.51 (dd, $J = 13.2$ and 10.3 Hz, 4H), 4.18–4.06 (m, 8H), 3.94 (s, 2H), 3.89–3.81 (m, 8H), 3.59–3.51 (m, 8H), 3.15 (d, $J = 13.5$ Hz, 4H) and 1.25–1.18 (m, 12H); $^{13}$C NMR (150 MHz, $CDCl_3$): $\delta$ 156.65, 156.41, 156.30, 135.50, 135.39, 135.06, 134.96, 128.85, 128.50, 128.33, 128.25, 128.20, 128.18, 122.39, 122.37, 121.99, 73.36, 73.33, 73.27, 73.22, 73.17, 73.05, 69.91, 69.81, 69.78, 69.66, 66.55, 66.49, 66.48, 66.44, 66.39, 54.79, 54.66, 30.99, 30.89, 15.50, 15.49, 15.47, 15.45, 15.44, 15.43, 15.40, 15.39, 15.38, 15.35 and 15.33. HRMS (DART-TOF) calcd for $C_{45}H_{57}N_3O_8$ ($M + H^+$) 768.4218, found 768.4120.

### *General procedures for 11–14*

Sodium ascorbate (22 mg, 0.111 mmol), copper sulphate (3 mg, 0.0120 mmol) and 9-propargyl nucleobase **7**–**10** (0.116 mmol) were added into a dry DMF solution (1 ml) of **6** (70 mg, 0.091 mmol) and stirred at 90–100°C for 30 min under Ar. The reaction progress was monitored by thin layer chromatography (5% MeOH in dichoromethane (DCM). The reaction mixture was diluted with ethyl acetate (2.5 ml) and washed with water (3 × 2.5 ml). The organic layer was dried with anhydrous $Na_2SO_4$ and concentrated. Flash chromatography (5–10% MeOH in DCM) of the residue yielded the desired products **11**–**14**.

### *Compound 11*

Light yellow crystals (70 mg, 81.6%). $^1$H NMR ($CDCl_3$): $\delta$ 8.35 (s, 1H), 7.97 (s, 1H), 7.15 (s, 1H), 6.83 (d, $J = 7.3$ Hz, 2H), 6.74 (d, $J = 6.4$ Hz, 2H), 6.68 (t, $J = 7.4$ Hz, 2H), 6.39–6.45 (dt, $J = 8.5$ and 6.5 Hz, 3H), 6.20 (s, 2H), 5.73 (s, 2H), 5.40 (s, 2H), 4.98 (s, 2H), 4.48 (d, $J = 13.4$ Hz, 4H), 4.27–4.11 (m, 4H), 4.01 (dd, $J = 8.9$ and 5.2 Hz, 4H), 3.90–3.81 (m, 4H), 3.81–3.74 (m, 4H), 3.59–3.44 (m, 8H), 3.10 (dd, $J = 41.9$ and 13.4 Hz, 4H) and 1.18 (m, 12H); $^{13}$C NMR ($CDCl_3$): $\delta$ 157.08, 156.34, 155.80, 152.69, 149.78, 141.98, 140.69, 136.02, 135.45, 134.48,

128.76, 128.45, 127.97, 127.79, 127.36, 122.65, 122.37, 121.84, 119.37, 73.75, 73.66, 72.89, 69.76, 69.74, 69.72, 69.71, 66.54, 66.52, 66.36, 66.35, 66.33, 66.32, 54.10, 38.57, 37.72, 30.91, 30.86, 15.42, 15.40, 15.39, 15.38, 15.36 and 15.34. HRMS (DART-TOF) calcd for $C_{53}H_{64}N_8O_8$ ($M + H^+$) 941.4920, found 941.4905.

### *Compound 12*

Light yellow solid (69 mg, 82.0%). $^1$H NMR ($CDCl_3$): $\delta$ 9.15 (s, 1H), 7.33 (d, $J = 1.2$ Hz, 1H), 7.26 (s, 1H), 6.83 (dd, $J = 7.4$ and 1.5 Hz, 2H), 6.74 (dd, $J = 7.5$ and 1.5 Hz, 2H), 6.69 (t, $J = 7.4$ Hz, 2H), 6.50–6.42 (m, 3H), 6.25 (s, 2H), 4.98 (s, 2H), 4.89 (s, 2H), 4.48 (dd, $J = 13.4$ and 2.3 Hz, 4H), 4.25–4.09 (m, 4H), 4.02 (dt, $J = 9.5$ and 5.3 Hz, 4H), 3.90–3.81 (m, 4H), 3.79 (td, $J = 5.3$ and 2.5 Hz, 4H), 3.59–3.46 (m, 8H), 3.11 (dd, $J = 33.8$ and 13.5 Hz, 4H), 1.23–1.12 (m, 12H); $^{13}$C NMR ($CDCl_3$): $\delta$ 164.31, 157.05, 156.45, 155.86, 150.88, 141.99, 140.32, 140.24, 135.95, 135.53, 135.40, 134.53, 128.75, 128.46, 128.09, 128.00, 127.39, 123.29, 122.38, 121.88, 111.19, 73.68, 73.59, 72.93, 69.75, 66.52, 66.36, 54.19, 42.61, 36.70, 30.89, 29.77, 15.39 and 12.41. HRMS (DART-TOF) calcd for $C_{53}H_{65}N_5O_{10}$ ($M + H^+$) 932.4804, found 932.4826.

### *Compound 13*

Light yellow solid (71 m, 85.2%). $^1$H NMR ($CDCl_3$): $\delta$ 7.55 (d, $J = 9.1$ Hz, 1H), 7.39 (d, $J = 16.7$ Hz, 1H), 6.88–6.78 (m, 2H), 6.78–6.72 (m, 2H), 6.69 (t, $J = 7.4$ Hz, 2H), 6.45 (s, 3H), 6.25 (s, 2H), 5.74 (d, $J = 9.1$ Hz, 1H), 4.96 (s, 2H), 4.95 (s, 2H), 4.48 (dd, $J = 13.4$ and 1.9 Hz, 4H), 4.25–4.09 (m, 4H), 4.09–3.95 (m, 4H), 3.90–3.81 (m, 4H), 3.81–3.74 (m, 4H), 3.58–3.42 (m, 8H), 3.11 (dd, $J = 30.5$ and 13.5 Hz, 4H) and 1.22–1.11 (m, 12H); $^{13}$C NMR ($CDCl_3$): $\delta$ 157.02, 156.52, 155.90, 135.90, 135.56, 135.38, 134.57, 128.73, 128.52, 128.13, 128.01, 122.43, 121.89, 100.00, 73.70, 73.59, 72.95, 69.77, 69.74, 69.72, 66.53, 66.51, 66.36, 66.35, 30.91, 30.87, 15.41 and 15.36. HRMS (DART-TOF) calcd for $C_{52}H_{64}N_6O_9$ ($M + H^+$) 917.4808, found 917.4832.

### *Compound 14*

Light yellow solid (54 mg, 66.6% yield). $^1$H NMR ($CDCl_3$): $\delta$ 11.94 (s, 1H), 7.63 (s, 1H), 7.10 (s, 1H), 6.85 (m, 2H), 6.74 (m, 2H), 6.65 (m, 2H), 6.42 (m, 3H), 6.24 (d, $J = 23.4$ Hz, 2H), 5.20 (s, 2H), 5.00 (s, 1H), 4.46 (m, 4H), 4.14 (m, 4H), 4.02 (dd, $J = 22.0$ and 16.8 Hz, 4H), 3.91–3.69 (m, 8H), 3.61–3.38 (m, 8H), 3.18–2.98 (dd, $J = 30.5$ and 13.5 Hz, 4H) and 1.29–1.07 (m, 12H); $^{13}$C NMR ($CDCl_3$): $\delta$ 156.97, 156.39, 155.91, 135.86, 135.46, 135.38, 134.61, 128.68, 128.50, 128.03, 127.92, 122.42, 77.32, 73.68, 73.58, 72.94, 69.73, 68.24, 66.50, 66.35, 38.81, 30.91, 30.84, 30.44, 29.79, 29.01, 23.82, 23.08, 15.41, 15.37, 15.35, 14.15 and 11.05. HRMS (DART-TOF) calcd for $C_{53}H_{64}N_8O_9$ ($M + H^+$) 957.4875, found 957.4768.

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