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Quantification of Cooperativity in the Self-Assembly of H-bonded Rosettes

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Petr Motloch and Christopher A. Hunter*

The self-assembly of triaminopyrimidines with barbiturates and with cyanates was investigated in chloroform solution. Equimolar mixtures of two complementary components form stable macrocyclic 3:3 complexes (rosettes). The thermodynamics of self-assembly were quantified by using ^{1}H NMR titrations to measure the strength of pairwise H-bonding interactions between two rosette components (*K*), allosteric cooperativity associated with formation of a second H-bonding interaction with each component, and the effective molarity for cyclisation of the rosette motif (EM). Pyrimidine-cyanurate interactions are an order of magnitude more favourable than pyrimidine-barbiturate interactions, so the cyanurate rosettes are significantly more stable than barbiturate rosettes. There is no allosteric cooperativity associated with rosette formation, but the chelate cooperativity quantified by the product *K* EM is exceptionally high (10^2-10^4), indicating that there are no other species present that compete with rosette assembly. The values of EM for rosette formation are approximately 2 M for all four rosettes studied and are not affected by differences in peripheral substituents or intrinsic H-bond strength.

Introduction

Self-assembly of multivalent supramolecular systems is an important process in chemistry, biology and materials science. Although a large number of synthetic systems that form well-defined discrete self-assembled complexes have been reported, the factors that govern these processes are still poorly understood. For this reason, the design of new supramolecular motifs with predictable properties remains a challenge. The key to successful self-assembly is high chelate cooperativity, which can be quantified by the product *K* EM, where *K* is the association constant for the intermolecular interaction between two components of the assembly, and EM is the effective molarity for intramolecular interactions that give rise to (poly)macrocyclic closed structures.[1,2] The values of K are relatively straightforward to predict based on the chemical structures of the interacting partners,[3] but the relationship between EM and chemical structure is more problematic.[2,4-8] As part of a programme to quantify the values of EM in stable H-bonded assemblies, we describe here a detailed thermodynamic analysis of the rosette motif first reported by Whitesides (Figure 1).[9,10] Although many supramolecular assemblies based on the 3:3 complex formed by triaminotriazines and barbiturates have been described,[9-12] the parameters that govern self-assembly of these exceptionally stable supramolecular architectures have never been measured.

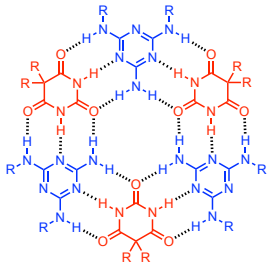

Figure 1. A H-bonded rosette self-assembled from a triaminotriazine and a barbiturate. R is a substituent.

Approach

As shown in Figure 1, triaminotriazines are commonly used for the self-assembly of H-bonded rosettes,[11,12] but replacement of this component by the corresponding pyrimidine offers some advantages that we exploit here. Pyrimidines do not suffer from the complications associated with the presence of multiple triaminotriazine rotamers,[13] and the pyrimidine aromatic proton provides a useful probe for studying supramolecular self-assembly using ^{1}H NMR spectroscopy. Figure 2 illustrates self-assembly of a H-bonded rosette via a sequence of intermolecular interactions to give linear oligomers, followed by intramolecular cyclisation of the 3:3 complex. The overall stability of the resulting assembly depends on the association constant for the intermolecular interactions (*K*) and the effective molarity for the intramolecular process (EM). The value of *K* can be estimated by measuring the association constant for a suitable reference system that can only form a 1:1 complex (K_{ref}). Here we measure K_{ref} using the complex illustrated in Figure 3, where one of the hydrogen-bonding sites

[a] *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK. E-mail: herchelsmith.orgchem@ch.cam.ac.uk*

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on each of the two rosette components is blocked by a substituent. Although this complex could further oligomerise, the intermolecular interactions would involve doubly H-bonded contacts which are relatively weak.[14] By measuring K_{ref} and the overall equilibrium constant for self-assembly of the rosette from the two components ($K_{rosette}$), the value of EM can be determined using Equation (1).

$$EM = \frac{K_{rosette}}{K_{\sigma} K_{ref}^{6}} \quad (1)$$

where the statistical factor $K_{\sigma} = {}^{32}/_{3}$ is determined by the symmetry of the system (see Figure S20).[15-18]

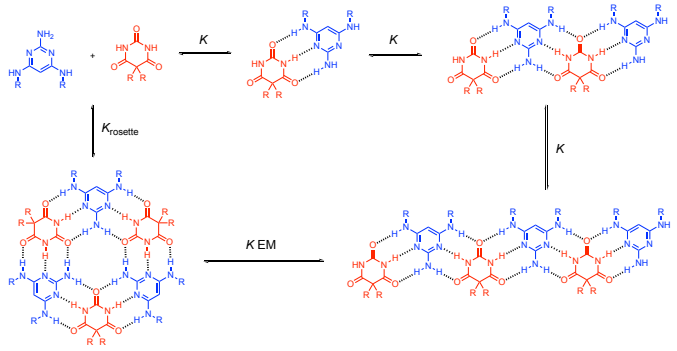


Figure 2. Self-assembly of a H-bonded rosette from a diaminopyrimidine and a barbiturate. Intermolecular triple H-bond interactions (*K*) lead to the assembly of linear oligomers, and the 3:3 complex can cyclise to form the rosette. EM is the effective molarity for the intramolecular interaction involved in cyclisation. R is a substituent.

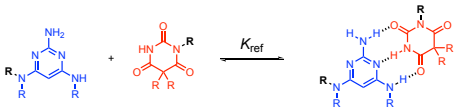


Figure 3. Complexes used to quantify the intermolecular H-bonding interactions between two rosette components (K_{ref}). R is a substituent.

However, there is a complication with the rosette structure, because some of the functional groups are involved in bifurcated H-bonding interactions, which may differ from the simple pairwise H-bonding interactions present in the reference complex shown in Figure 3. Therefore additional experiments are required to quantify the extent of any allosteric cooperativity associated with these interactions. The 1:2 complexes shown in Figure 4 allow direct measurement of the allosteric cooperativity associated with formation of multiple interactions with either the pyrimidine (Figure 4a) or the barbiturate component (Figure 4b). Comparison of the microscopic association constants for formation of the 1:1 (K_1) and the 1:2 (K_2) complexes shown in Figure 4 gives the allosteric cooperativity factor α (Equation (2)).

$$\alpha = \frac{K_2}{K_1} \quad (2)$$

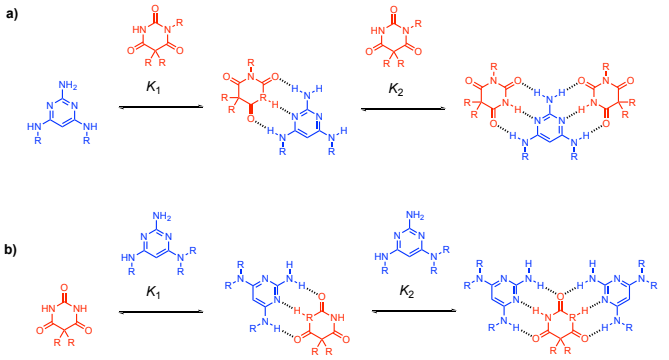
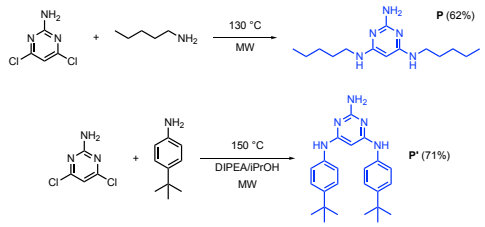


Figure 4. Complexes used to quantify allosteric cooperativity in self-assembly of H-bonded rosettes. (a) Allosteric cooperativity between the two binding sites on a pyrimidine is $\alpha = K_1 / K_2$. (b) Allosteric cooperativity between the two binding sites on a barbiturate is $\alpha = K_1 / K_2$. R is a substituent.

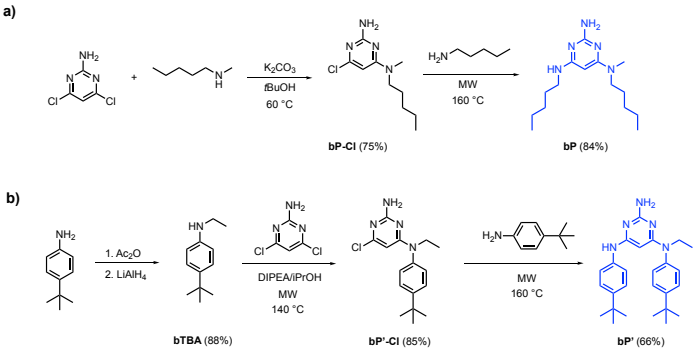
Results and Discussion

Synthesis

The triaminopyrimidines **P** and **P'** were each synthesised in one step (Scheme 1). Synthesis of the blocked-pyrimidines **bP** and **bP'** is shown in Scheme 2. Reacting 4,6-dichloropyrimidin-2-amine with *N*-methylpentylamine gave the singly substituted product **bP-Cl**, which was converted to **bP** by heating in neat *n*-pentylamine under microwave irradiation. For the synthesis of **bP'**, **bTBA** was prepared by a reaction of *tert*-butyl aniline with acetic anhydride followed by reduction using $LiAlH_4$. **bTBA** was then used for the synthesis of the singly substituted product **bP'-Cl**, which was converted to **bP'** by heating in neat *tert*-butyl aniline under microwave irradiation.



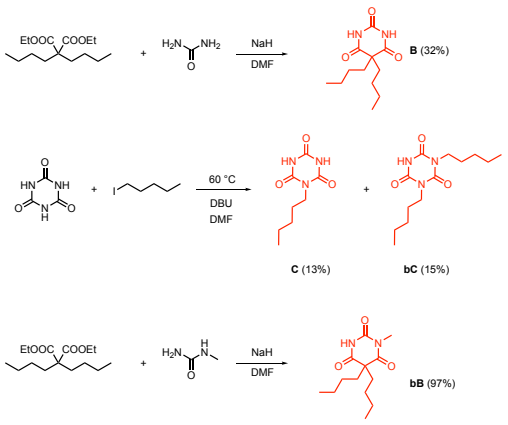
Scheme 1. Synthesis of pyrimidines.



Scheme 2. Synthesis of blocked pyrimidines.

Commercially available diethyl-barbiturate was not soluble enough in chloroform for NMR titrations, and therefore barbiturate **B** with longer alkyl chains was synthesised from

urea (Scheme 3). **bB** was synthesised by using *N*-methylurea instead of urea (Scheme 3). Cyanurate **C** was prepared from cyanuric acid and iodopentane, and **bC** was also obtained as a side product in this reaction (Scheme 3).



Scheme 3. Synthesis of cyanurates and barbiturates.

Measurement of K_{ref} in 1:1 complexes

To determine the values of K_{ref} for different combinations of rosette components, ^{1}H NMR titrations were carried out in $CDCl_3$ at 298 K using blocked-pyrimidines (**bP** or **bP'**) as hosts and blocked-barbiturate/cyanurate (**bB** or **bC**) as guests. ^{1}H NMR dilution experiments show that the pyrimidines do not self-associate to a significant extent at the concentrations used in these experiments ($K \approx 1$ M^{-1}, see Figures S5-S6). Similarly, the self-association constants for barbiturates and cyanurates in chloroform are too low to affect the titration experiments ($K <$ 10 M^{-1}).[11] The titration data fit well to a 1:1 binding isotherm in all cases (see Figure S7-S10), and the results are summarised in Table 1. The association constants measured with cyanurate **C** are approximately one order of magnitude higher than the corresponding values for barbiturate **B**.

Table 1. Association constants measured in $CDCl_3$ at 298 K by ^{1}H NMR titrations.[a]

Complex	K_{ref} [M^{-1}]
bP•bB	240 ± 5
bP'•bB	200 ± 10
bP•bC	3200 ± 40
bP'•bC	1200 ± 200

[a]Errors are quoted at the 95% confidence limit.

Measurement of allosteric cooperativity (α) in 1:2 complexes

To determine the values of α for different combinations of rosette components, ^{1}H NMR titrations were carried out in $CDCl_3$ at 298 K using the ditopic pyrimidines (**P** or **P'**) as hosts and blocked-barbiturate/cyanurate (b**B** or b**C**) as guests, or the ditopic barbiturate/cyanurate (**B** or **C**) as hosts and blocked-pyrimidines (**bP** or **bP'**) as guests. In order to assess whether there was any deviation of the titration data from a simple non-cooperative isotherm, the data were analysed in three different ways:

1. A non-cooperative binding isotherm, which assumes that a only two variables can be used to fit the data. The first variable is defined as K_{nc}, where $K_{nc} = K_1 = K_2$. The second variable was the change in chemical shift for formation of the 1:1 complex that was set to be identical to the subsequent change in chemical shift for formation of the 1:2 complex.
2. A non-cooperative binding isotherm assuming that $K_1 = K_2 = K_{ref}$, which was fixed at the value for the corresponding 1:1 complex in Table 1. The change in chemical shifts formation of the 1:1 complex and the subsequent change in chemical shift for formation of the 1:2 complex were fitted to the data as two independent variables.
3. A binding isotherm that allows for cooperativity with $K_1 = K_{ref}$, which was fixed at the value for the corresponding 1:1 complex in Table 1, and K_2 a variable that was fitted to the data. The change in chemical shifts formation of the 1:1 complex and the subsequent change in chemical shift for formation of the 1:2 complex were fitted to the data as two additional variables.

Table 2. Association constants (K_{nc} and K_2) and global errors (E) obtained by fitting ^{1}H NMR titration data measured in $CDCl_3$ at 298 K to different isotherms.[a]

	Isotherm 1		Isotherm 2		Isotherm 3	
Complex	K_{nc} [M^{-1}]	E [%]	K_{ref} [M^{-1}]	E [%]	K_2 [M^{-1}]	E [%]
P•bB$_2$	200 ± 10	0.7	242 ± 5	0.7	334 ± 5	0.1
P'•bB$_2$	220 ± 20	0.9	200 ± 10	0.9	320 ± 40	0.3
P•bC$_2$	3700 ± 200	2	3200 ± 40	1	1600 ± 10	0.5
P'•bC$_2$	690 ± 100	0.5	1200 ± 200	0.1	1200 ± 30	0.1
B•bP'$_2$	1400 ± 200	4	200 ± 10	1	200 ± 50	1

[a]Errors are quoted at the 95% confidence limit.

Table 2 summarises the results along with the global error E of the fit. Isotherm 3 uses an additional variable in the fit compared with isotherms 1 and 2, and so the value of E is always lower for the cooperative isotherm. When the ditopic pyrimidines were used as hosts, the titration data fit well to all three isotherms, and the values of K_{nc} and K_2 are similar to the corresponding values of K_{ref} measured for the simple 1:1 complexes. These results suggested that the value of α is approximately one in all cases, i.e. the allosteric cooperativity associated with self-assembly of H-bonded rosettes is negligible. When the ditopic barbiturate/cyanurate was used as hosts, the titration data generally did not fit well to any of the isotherms, which indicates that additional equilibria are present, so these systems cannot be used to measure α. However, the titration data for **B•bP'$_2$** did fit well to the non-cooperative isotherm 2 and to isotherm 3 giving a value of K_2 identical to K_1 and K_{ref}. The fit to isotherm 1 for this complex is poor, as judged by the large value of E, which implies that

chemical shift changes differ significantly for the first and second binding event. Since no allosteric cooperativity was observed in the formation of any of the 1:2 complexes, the values of K_{ref} from Table 1 were used to assess the chelate cooperativity associated with rosette self-assembly.

Measurement of chelate cooperativity (EM) in 3:3 rosettes

To establish rosette formation, millimolar solutions of 1:1 mixtures of two complementary components (namely **P**+**B**, **P'**+**B**, **P**+**C** or **P'**+**C**) were prepared in $CDCl_3$ and the ^{1}H NMR spectra were recorded at 298 K and 233 K (Figure 5). At 233 K, all of the mixtures showed the characteristic rosette signal at 14–15 ppm due to the H-bonded barbiturate/cyanurate NH group and two signals at 8–11 ppm due to the H-bonded pyrimidine NH and NH_2 groups.[9] At 298 K, the spectra were much broader. However, the H-bonded rosette signals were still observed for the cyanurate **C** mixtures, which indicates that these rosettes are kinetically more stable than the corresponding barbiturate **B** rosettes. This result is consistent with the higher value of K_{ref} measured for the cyanurate 1:1 complexes compared with the barbiturate 1:1 complexes (Table 1).

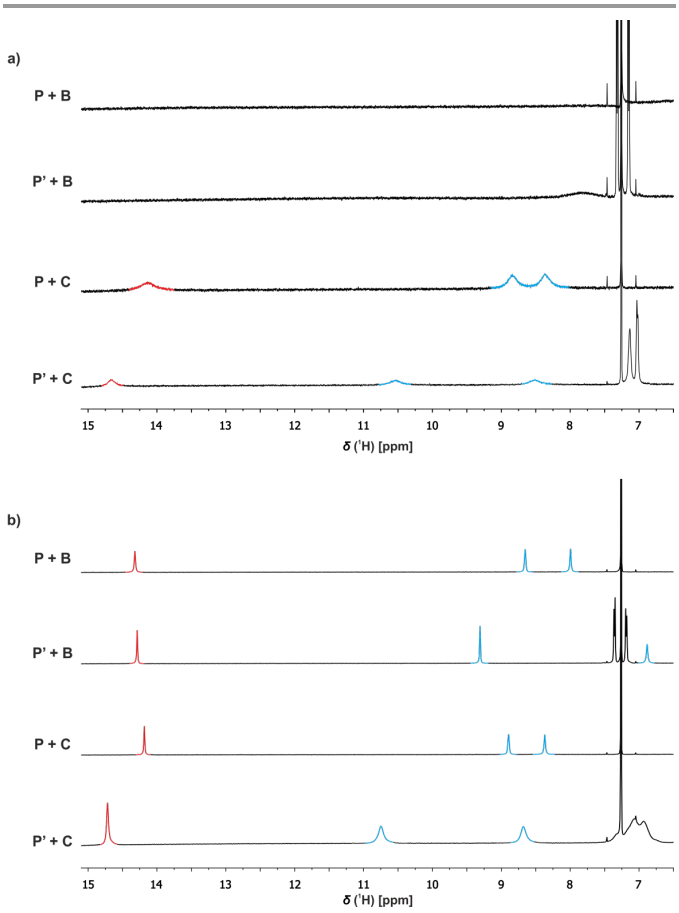


Figure 5. Partial ^{1}H NMR spectra (500 MHz, $CDCl_3$) of rosette assemblies at (a) 298 K and (b) 233 K. The concentration of each component was 3 mM. Barbiturate/cyanurate NH signal highlighted in red, and pyrimidine NH/NH_2 signals highlighted in blue.

Equilibrium constants for self-assembly of the H-bonded rosettes were determined by ^{1}H NMR titrations in $CDCl_3$ at 298 K using pyrimidines (**P** or **P'**) as hosts and barbiturate/cyanurate (**B** or **C**) as guests. The signal due to the aromatic proton of the pyrimidine stayed sharp for the whole titration, and the changes in chemical shift fit well to an all-or-nothing 3:3 isotherm in all cases (see Figures S16-S19). Fitting to an alternative isotherm that also allowed for opening of the rosette to give 1:2 complexes in the presence of excess guest did not change the result, because the 1:2 complexes are not significantly populated at the concentrations used. The values of $K_{rosette}$ are summarised in Table 3. Rosettes based on cyanurates are significantly more stable than rosettes based on barbiturates, because of the order of magnitude difference in the intrinsic H-bond strength, i.e. K_{ref}. However, the values of EM calculated using Equation (1) are practically identical (1-3 M) for all four rosettes. Whitesides used the peripheral crowding associated with *t*-butylphenyl substituents in **P'** to favour rosette formation in the crystalline state,[9] but the results in Table 3 show that in solution these bulky groups do not increase the EM for rosette formation compared with simple alkyl substituents. The value of EM for the H-bonded rosettes is at the high end of the values reported in the literature for supramolecular systems,[2] but it is substantially lower than the highest values of EM (10^2-10^3 M) found for H-bonded squares based on guanosine-cytosine base-pairing. The statistical correction K_σ lowers the value of EM measured for the rosettes by a factor of 10, but even without this correction, the EM would be orders of magnitude lower than the values reported for the González-Rodríguez systems.[19] The chelate cooperativity associated with rosette formation is quantified by the product K_{ref} EM, which is the equilibrium constant between the open and closed 3:3 complexes shown in Figure 2. The values of K_{ref} EM in Table 3 are 10^2-10^3 for the barbiturate rosettes and 10^3-10^4 for the cyanurate rosettes, which means that less than 1% of the species present are not in the form of fully assembled rosettes.

Table 3. Equilibrium constants and effective molarities for self-assembly of H-bonded rosettes in $CDCl_3$ at 298 K measured by ^{1}H NMR titrations.[a]

Complex	$K_{rosette}$ [M^{-5}]	K_{ref} [M^{-1}]	EM [M]	K_{ref} EM
P_3•B_3	(6.3 ± 0.1)×10^{15}	240 ± 5	3.1 ± 0.4	740 ± 80
P'_3•B_3	(8.4 ± 0.4)×10^{14}	200 ± 10	1.2 ± 0.4	250 ± 60
P_3•C_3	(2.7 ± 0.2)×10^{22}	3200 ± 40	2.4 ± 0.2	7500 ± 700
P'_3•C_3	(3.0 ± 0.1)×10^{19}	1200 ± 200	1.0 ± 0.9	1100 ± 900

[a]Errors are quoted at the 95% confidence limit.

Conclusions

Mixing stoichiometric quantities of a pyrimidine with a barbiturate or a cyanurate in non-polar solvents leads to quantitative formation of 3:3 rosette complexes, which are held

together by multiple H-bonding interactions. By using rosette components in which one of the H-bonding edges is blocked by a substituent, it is possible to dissect the free energy contributions that govern the self-assembly of H-bonded rosettes. ^{1}H NMR titrations were used to measure the equilibrium constants for formation of 1:1 complexes between two blocked components, 1:2 complexes between one rosette component and a blocked component, and 3:3 rosette complexes. The H-bonding interactions involved in pairwise interaction of two components are an order of magnitude stronger for cyanurates than for barbiturates and the rosette complexes are correspondingly more stable. The 1:2 complexes show that there is no allosteric cooperativity in H-bonded rosettes. The chelate cooperativity associated with the intramolecular interactions required to cyclise the rosette was quantified by the effective molarity, which is approximately 2 M for all four rosettes studied, and the product K_{ref} EM is 10^2-10^4 indicating that rosette self-assembly is highly cooperative with no other species populated to a significant extent in these systems.

Acknowledgements

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