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Examples of Regioselective Anion Recognition among a Family of Two-, Three-, and Four-"Armed" Bis-, Tris-, and Tetrakis(thioureido) [*n*]Polynorbornane hosts

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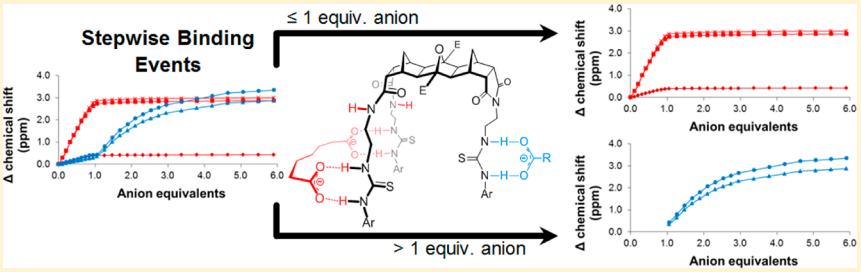
Supporting Information



ABSTRACT: A family of conformationally preorganized, [*n*]polynorbornane-based anion hosts **1a,b**–**6a,b** have been synthesized. The series includes receptors with 4, 8, and 12 H-bond donors. Using ^{1}H NMR titration techniques, evaluation of the new hosts against a series of alkyl and aryl dicarboxylates as well as a range of phosphoanionic species has revealed that the tris(thioureido) hosts (in particular **3a**) are capable of regioselectively binding dicarboxylates and pyrophosphate (H_2PPi^{2-}).

INTRODUCTION

Interest in supramolecular anion recognition chemistry has intensified over the last 10 years,[1,2] and this attention is not surprising, given the key roles that anions occupy in a number of biochemical processes. Phosphoanionic species of note[3] include Lipid A (a pyretic endotoxin),[4,5] ADP and ATP (crucial for cellular energy),[6,7] and inorganic phosphate (valuable as a fertilizer but also a cause of waterway eutrophication).[8] Dicarboxylate species also serve important roles in nature; for example, succinate (a key intermediate in the Krebs cycle).[6,9] As such, the recognition of these species is a well-justified focus for supramolecular chemists.[3,5,7,9]

Charge-neutral, conformationally preorganized, multiarmed receptors (such as those based on calix and cholic acid frameworks) are well suited to anion recognition studies.[10,11] Among these, the [*n*]polynorbornane class of scaffold is particularly attractive, as these scaffolds can be tailored to a range of predictable geometries.[12,13] Furthermore, they can be purposely functionalized at a variety of locations, including "edges" and "ends".[14] As a result of these properties, they have been exploited by a number of groups in the field of supramolecular chemistry.[15,16]

To further investigate the utility of these frameworks, thiourea-functionalized fused [3]- and [5]polynorbornane hosts **1**–**6** (Figure 1), with cleft dimensions of 6.6 and 10.4 Å, respectively, were designed as anion hosts with excellent potential to selectively bind larger/longer anions. Furthermore, by constructing a family of hosts that have the same cleft/cavity dimensions and only vary in the number of available H-bond donors, insight into how multiple H-bond donors participate in recognition events can be gleaned. Such well-ordered anion hosts also have potential for binding guests regioselectively: i.e., the controlled positioning of one (or multiple) guest(s) in a known region(s) of the host. Interactions of this nature are a critical requirement for certain enzymes,[17] but to the best of our knowledge this topic has not been widely explored in the field of anion recognition.[18] Synthetic hosts capable of performing such controlled multiple guest recognition would be valuable tools in the development of more sophisticated biomimetic organocatalysts/synthetic enzymes.[19]

In this paper the binding of a family of thiourea-functionalized, fused [*n*]polynorbornane hosts (**1**–**6**) to a number of alkyl and aryl dicarboxylates as well as phosphoanionic species ($H_2PO_4^-$, H_2PPi^{2-}, and ADP^{2-}) is evaluated (by means of ^{1}H NMR spectroscopy).

SYNTHESIS

Synthesis of both the *p*-fluorophenyl (**a** series) and *p*-nitrophenyl (**b** series) variants of the bis-, tris-, and tetrakis-(thioureido) hosts **1**–**6** (Figure 1) was completed as described previously[20] using the 1,3-dipolar cycloaddition of an alkene with a cyclobutene epoxide (e.g., hosts **6a,b**; Scheme 1).[12e] In brief, the strategy involved synthesizing the desired Boc-protected norbornene unit, assembling the protected [3]- or [5]polynorbornane framework through established protocols

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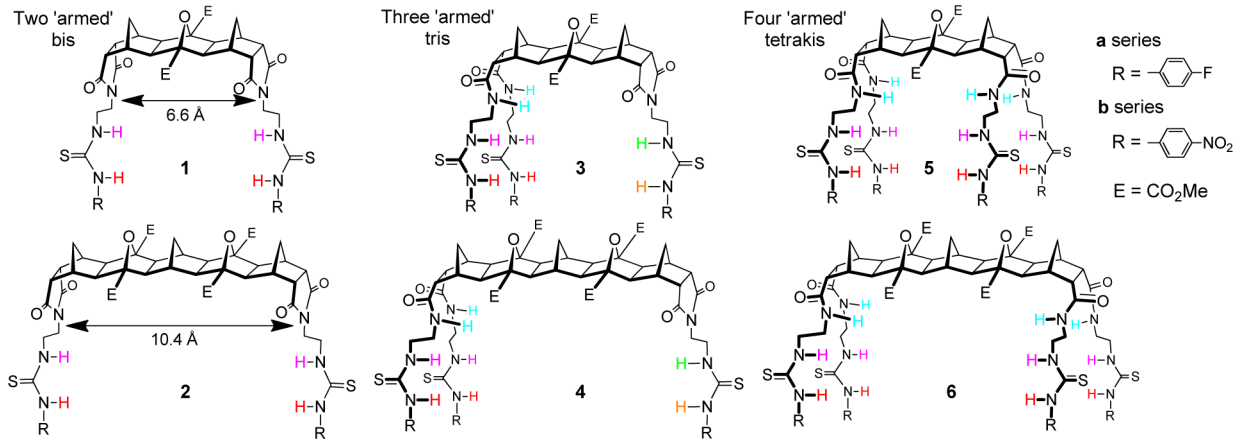
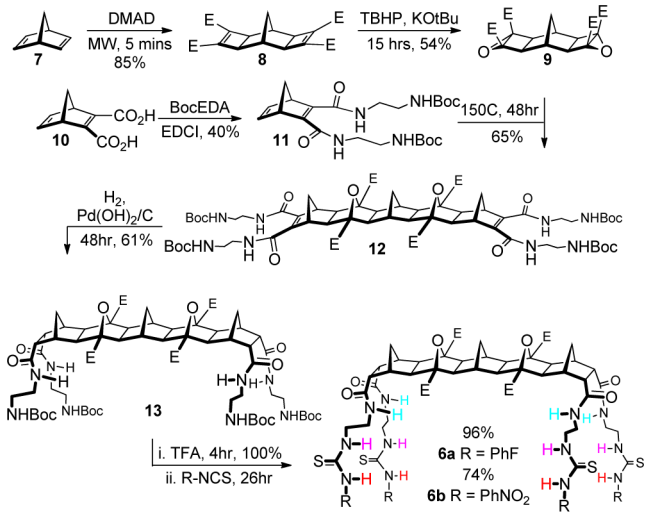


Figure 1. Multiarmed [*n*]polynorbornane hosts **1**–**6** synthesized and evaluated in the current study.

Scheme 1. Synthesis of [5]Polynorbornane Hosts 6a,b



(Mitsudo reaction, epoxidation, and 1,3-dipolar cycloaddition),[12b−e] and then deprotecting, followed last by reacting with an appropriate isothiocyanate (*p*-fluorophenyl, **a** series; *p*-nitrophenyl, **b** series) to afford the requisite thiourea-functionalized polynorbornane framework[20] (e.g., hosts **6a,b**; Scheme 1).

Hosts **1**, **2**, **5**, and **6** are remarkably symmetric (both C_{2v} and σ); indeed, hosts **5** and **6** contain eight urea N−H protons yet only two N−H signals were observed in the ^{1}H NMR spectrum of these compounds (see the Supporting Information). This level of symmetry does not exist for hosts **3** and **4**; hence, both "ends" of the framework could be monitored independently for binding.

RESULTS AND DISCUSSION

The interaction of the hosts with anionic species was evaluated by means of ^{1}H NMR titration techniques using DMSO-d_6 as solvent and a standard host concentration of 1.3×10^{-2} M for hosts **1** and **2** and 2.5×10^{-3} M for hosts **3**–**6**. For a summary of titration results including maximum $\Delta\delta$ and binding constants (log *K* values: calculated using winEQNMR[21]) see Tables 1 and 2 (for dicarboxylates and phosphoanions, respectively).

The binding for the *p*-nitrophenylurea (**b**) series was consistently stronger than that of the *p*-fluorophenylurea (**a**) series, and as such the discussion for the most part will be generalized: i.e. host **1** will be mentioned, not hosts **1a,b**. In many titrations deprotonation was observed for the *p*-nitrophenylurea (**b**) series (indicated by D in Tables 1 and 2), and this trend was attributed to the strong electron-withdrawing power of the nitro group ,which leads to increased ArN−H acidity.

A. Dicarboxylates. The range of anions included acetate, a series of flexible dicarboxylates $^-O_2C(CH_2)_nCO_2^-$ (n = 1–6) and terephthalate. All carboxylate anions were used as their tetrabutylammonium (n-Bu_4N^+) salts, and the results of the titration experiments are presented in Table 1.

1. Flexible Dicarboxylates and Hosts 1–4. Hosts **1** and **2**, with only four H-bond donors, were the simplest of the series, and it was envisaged that the shorter length dicarboxylates would complement the shorter cleft width of [3]-polynorbornane **1**. Nevertheless, titrations for **1** and the longer [5]polynorbornane **2** against the alkyl dicarboxylates were remarkably similar; strong but nonselective binding was observed (Figure 2). These results can be rationalized by the following: (i) the [3]polynorbornane host **1** can readily bind a longer alkyl dicarboxylate (such as suberate, n = 6) as the flexible hydrocarbon segment of the *guest*[22] can be oriented away from the host (Figure 2) and (ii) the [5]polynorbornane **2** can accommodate a short alkyl dicarboxylate due to the degree of "induced fit" incorporated into the *host* through the ethyl spacer (Figure 2). This seemingly small amount of host flexibility was enough to render size discrimination among the alkyl dicarboxylates virtually impossible.

For the three-armed hosts **3** and **4** the titration curves were in many instances remarkable—particularly for host **3a**. For example, the titration of **3a** against pimelate ($^-O_2C(CH_2)_5CO_2^-$, Figure 3) revealed neither a 1:1 H:G arrangement nor a typical 1:2 arrangement. Two clearly distinct binding events occurred that can only be rationalized if a stepwise, regioselective, association process was taking place. Initially (<1 equiv of pimelate) the dicarboxylate was strongly bound almost exclusively at the two-armed end, as evidenced by the rapid downfield shift in the corresponding urea protons. Indeed, when 1.0 equiv of pimelate had been added, there was an impressive 2.4 ppm difference between urea protons at either "end" of the host with the single-end urea protons having shifted only 0.5 ppm. With the two-armed end saturated, additional pimelate (>1.0 equiv) had no option but to bind to the single-arm end, and a distinct jump in the titration curve

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Table 1. Titration Results: Maximum Observed Chemical Shift for ArN−H, Binding Stoichiometry, and Binding Constants for Hosts 1−6 against Acetate and Dicarboxylates[a]

anion	param	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b
acetate	max $\Delta\delta$	3.4	3.5	3.5	3.6	2.8	3.5	2.7	3.3	2.1	2.7	2.0	2.9
	H:G	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2	1:2
	log K_1	2.8	3.2	2.9	3.1	2.9	3.2	2.9	3.1	2.8	2.9	2.7	3.0
	log K_2	2.3	3.0	2.3	3.0	2.5	2.5	2.4	2.6	2.4	2.7	2.5	2.6
malonate (n = 1)	max $\Delta\delta$	3.7	0.66	4.0	0.28	2.4	1.0	2.5	0.35	2.5	0.88	2.4	1.5
	H:G	1:1	D[d]	1:1	D	1:1P	D	1:1P	D	1:2	D	1:2	D
	log K_1	3.9		4.3		4.8		4.2		5.0		5.0	
	log K_2					D		D		4.3		4.3	
succinate (n = 2)	max $\Delta\delta$	3.8	3.6	4.1	4.1	3.6	3.0	2.9	1.0	2.9	3.0	2.9	3.0
	H:G	1:1	D	1:1	1:1	1:2sw[e]	D	1:1	D	1:2	1:2	1:2	1:2
	log K_1	4.5		4.8	5.0	4.9		4.0		5.0	~4.6[c]	5.0	4.6
	log K_2					3.0				4.9	5.3	4.8	5.3
glutarate (n = 3)	max $\Delta\delta$	3.8	4.0	4.0	4.0	4.1	3.1	3.7	2.8	2.9	3.0	2.8	3.1
	H:G	1:1	1:1	1:1	1:1	1:2sw	1:1	1:1	1:1	1:2	1:2	1:2	1:2
	log K_1	5.0	5.0	4.9	5.0	4.1	5.1	4.1	5.0	5.0	~4.6[c]	5.1	4.8
	log K_2					3.0				4.9	5.3	4.9	5.2
adipate (n = 4)	max $\Delta\delta$	3.7	3.9	4.0	4.1	3.5	3.2	3.6	3.1	3.0	3.1	3.0	3.1
	H:G	1:1	1:1	1:1	1:1	1.2sw	1:1P[f]	1:2sw	1:1	1:2	1:2	1:2	1:2
	log K_1	5.0[b]	5.2[b]	5.1[b]	5.3[b]	4.8	4.6	4.4	5.1	~4.9[c]	~4.9[c]	5.1	5.3
	log K_2					2.8	D	2.8		5.0	5.0	5.0	5.1
pimelate (n = 5)	max $\Delta\delta$	3.6	3.8	3.9	4.0	3.5	3.2	3.4	3.2	3.1	3.2	3.0	3.2
	H:G	1:1	1:1	1:1	1:1	1:2sw	1:1P	1:2sw	1:1P	1:2	1:2	1:2	1:2
	log K_1	5.0[b]	5.2[b]	5.1[b]	5.4[b]	4.8	4.3	4.5	3.8	~5.0[c]	~5.1[c]	5.1	5.0
	log K_2					2.8	D	2.9	D	4.6	4.8	4.9	5.0
suberate (n = 6)	max $\Delta\delta$	3.7	3.9	3.9	4.0	3.3	4.1	3.3	3.2	3.1	3.2	3.1	3.2
	H:G	1:1	1:1	1:1	1:1	1:2sw	1:2sw	1:2sw	1:1P	1:2	1:2	1:2	1:2
	log K_1	4.8[b]	5.2[b]	5.0[b]	5.3[b]	4.8	4.5	5.2	4.3	~4.5[c]	~4.0[c]	5.0	5.0
	log K_2					2.6	3.6	2.7	D	4.9	5.0	5.0	4.8
terephthalate	max $\Delta\delta$	3.4	3.5	3.6	3.7	3.1	3.5	3.6	3.8	2.1	2.8	3.4	3.7
	H:G	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:2	1:2
	log K_1	3.5[b]	3.8[b]	4.4[b]	>5.5[b]	3.2	3.6	4.4	4.9	2.9	3.1	2.9	3.0
	log K_2											3.0	4.5

[a]Unless indicated, a standard concentration of 1.3×10^{-2} M was used for hosts **1** and **2** and 2.5×10^{-3} M was used for the remainder (**3**−**6**). Errors ≤15% (see the Supporting Information for an analysis of errors). [b]For this anion the error associated with the binding constant was >20% at the standard concentration (1.3×10^{-2}); therefore, the titration was repeated at a lower concentration (1.0×10^{-3}), and these are the values reported. [c]For these isotherms the first binding constant was not easily defined—possibly as a result of a change in binding arrangement between the 1:1 and 1:2 H:G complexes (see Discussion and Figure 5). As such, an approximate value is reported. [d]In some instances either thiourea N−H could not be followed or the binding constant was unreliable due to deprotonation. In these instances $\Delta\delta$ is the value prior to the disappearance of the signal, no binding constant is reported, and D is given to designate deprotonation. [e]The term "sw" indicates a stepwise binding process where the second binding event only occurred after 1 equiv of the anion had been added. The binding constant for the second step was calculated by setting the chemical shift at 1 equiv as the origin (see the Supporting Information for an example). [f]For the three-armed hosts the titration data clearly indicated a binding event at one "end" of the framework, but it was impossible to accurately assign a second event due to deprotonation of the relevant N−H groups shortly after 1 equiv had been added. As such, P indicates possible (or partial) regioselective binding.

was observed. This jump was noticeable to the extent that if the titration curve at 1 equiv of anion was reset to 0, a binding constant for the second binding event (corresponding to 1:1 binding) could be determined. This second binding event was not as strong as the first, presumably due to electrostatic repulsion and the fact that only one urea was present to effect binding. Regioselective binding of pimelate was also observed for the [5]polynorbornane **4a** but was not as clear-cut for **3b** and **4b** (see the Supporting Information for all titration isotherms), where broadening of the relevant N−H made definitive judgment impossible. While the other hosts displayed a tendency for the stepwise binding of the alkyl dicarboxylates, it was host **3a** that provided the clearest examples of the regioselective binding with all dicarboxylates except malonate (see Table 1, where stepwise binding is indicated by "sw"). While the exact cause for this unusual binding preference is unknown (all attempts at crystallizing the host in the presence of anion have failed), molecular modeling (H-F/3-21G*) indicated that, even for suberate, the proposed binding mode can be accommodated with minimal distortion to either the host or the alkyl dicarboxlate if the two *endo* arms adopt a one carbonyl out and one carbonyl under conformation (see Figure 3 and the Supporting Information).

*2. Two Anions (Pimelate + Acetate) and Host **3a**.* To further probe the nature of this unusual stepwise binding, additional 'split' titrations were performed on hosts **3a** (see the Experimental Section and the Supporting Information for full details and also results for **3b** and **4b**). To a solution of **3a** in DMSO-d_6 was added 1 equiv of pimelate (n = 5), followed by an excess of acetate (Figure 4). At the 1 equiv point and beyond no change in the chemical shifts of the thiourea protons at the two-armed end were observed, indicating that acetate was

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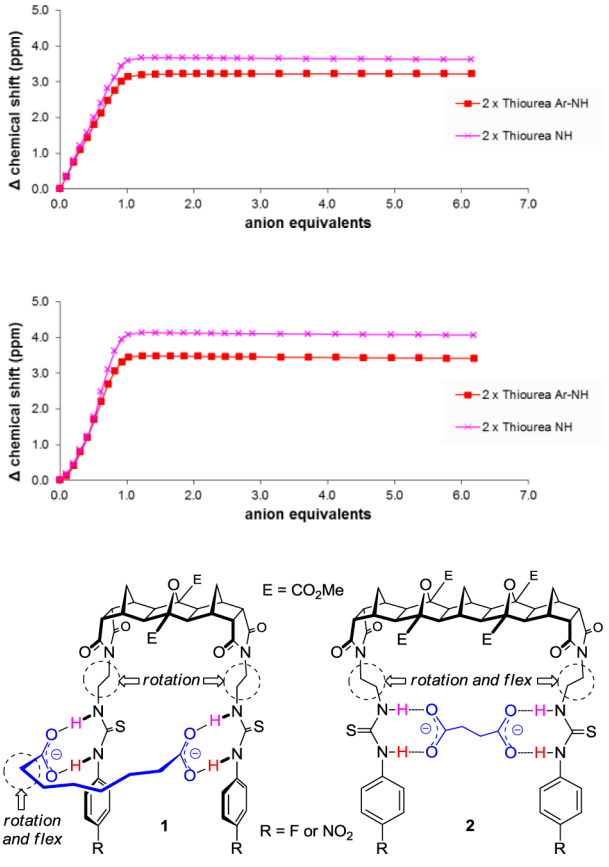

Figure 2. Binding isotherms: (top) titration of host **1a** with suberate ([H] = 1.3 × 10^{-2} M, log K = 4.8); (middle) titration of host **2a** with succinate ([H] = 1.3 × 10^{-2} M, log K = 4.3). (bottom) Postulated binding arrangement of **1** with suberate (left) and of **2** with succinate (right), showing strong binding regardless of dicarboxylate length or cleft width.

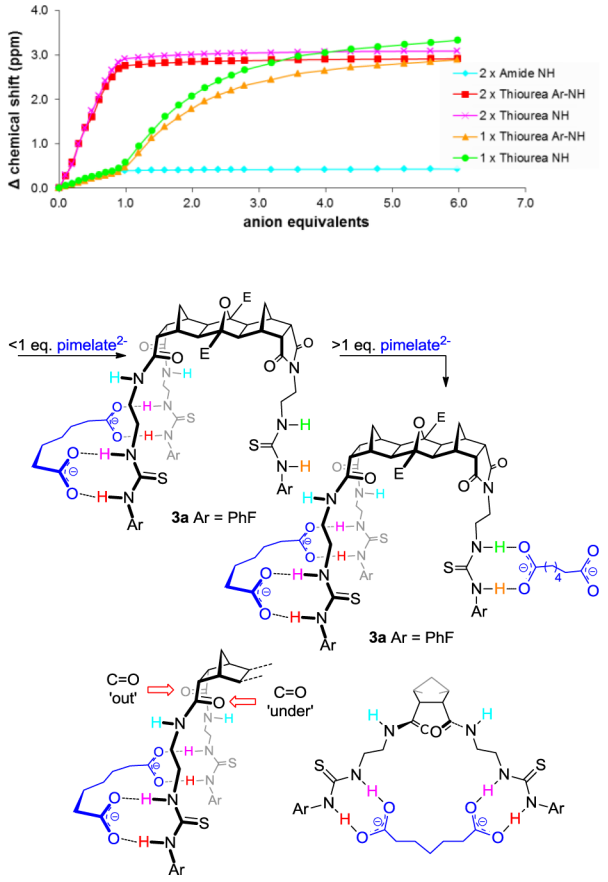

Figure 3. (top) Binding isotherm from the titration of host **3a** with pimelate (n = 5). (bottom) Illustration of the regioselective binding that underpins the observed titration results.

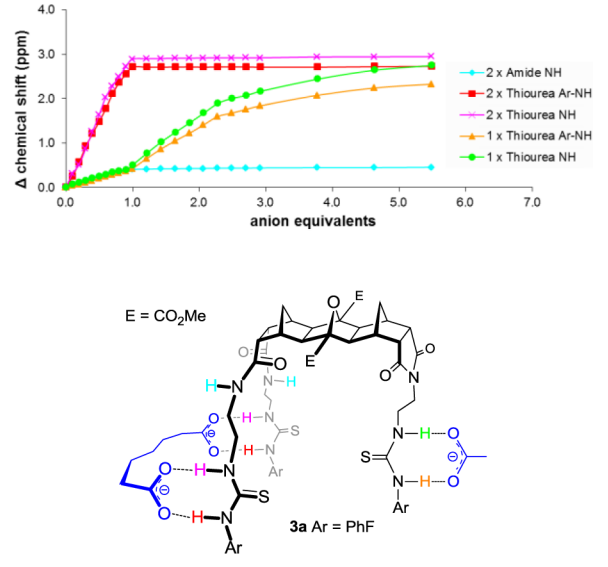

Figure 4. (top) Binding isotherm for titration of **3a** against pimelate (0–1.0 equiv) and acetate (1.0–7.0 equiv) ([H] = 2.5 × 10^{-3} M). (bottom) Proposed binding arrangement.

not capable of outcompeting the dicarboxylate at the two-armed end (acetate has a lower affinity for the two-armed end of these polynorbornane hosts; for **3a** log K_1(acetate) = 2.9, whereas log K_1(alkyl dicarboxylates) range from 4.1 to 4.9; see Table 1). The smaller $\Delta\delta$ associated with the second binding event in this instance further indicated that it is acetate alone (not displaced pimelate) binding to the single-arm end, as the isotherm matches that when acetate alone was used (see the Supporting Information). Thus for **3a**, pimelate and acetate could be assembled in a controlled fashion such that the dicarboxylate bound at the two-armed end and the acetate at the single-arm end.

To further explore the regioselective recognition process, the anion order was reversed and 1.0 equiv of acetate was added followed by an excess of pimelate (see the Supporting Information for isotherms). For **3a** the addition of acetate proceeded as expected (a bias to the two-armed end but with clear interaction to the single-arm end). Upon the addition of pimelate a distinct jump in the titration curve was seen for the thiourea protons at the two-armed end, indicating that acetate was being displaced by the more strongly binding pimelate. A small jump in the titration curve was also seen for the single-arm end. The region from 1 to 2 equiv was very similar to that seen when acetate alone was the titrant. The changes in the isotherm suggest that the displaced acetate binds with the single-arm urea group. After an excess (>2 equiv) of pimelate had been added, the whole isotherm began to resemble that of a pure pimelate titration, an outcome that is not unexpected, as both log K_1 and log K_2 of pimelate with **3a** are greater than that of acetate and with an excess of pimelate complete displacement of acetate may indeed be effected.

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This reverse titration (plus additional acetate/malonate titrations—see the Supporting Information) confirmed the remarkable nature of host **3a** and further supported the notion of a regioselective binding event. Such a two-step controlled binding event is significant, given that on inspection of the hosts all three urea groups appear to be equally accessible.

*3. Flexible Dicarboxylates and Hosts **5** and **6**.* With the split titrations complete for the three-armed hosts investigations continued with the dicarboxylates and the four-armed tetrathiourea hosts **5** and **6**. The binding of all dicarboxylates with these hosts was 1:2: not surprising, given the predisposition of urea and thiourea for carboxylates. It was possible that each dicarboxylate bound at each end of the framework rather than spanned the cleft; however, it is possible that both modes occurred depending on host and guest (Figure 5). The titration curves for adipate were the most interesting of

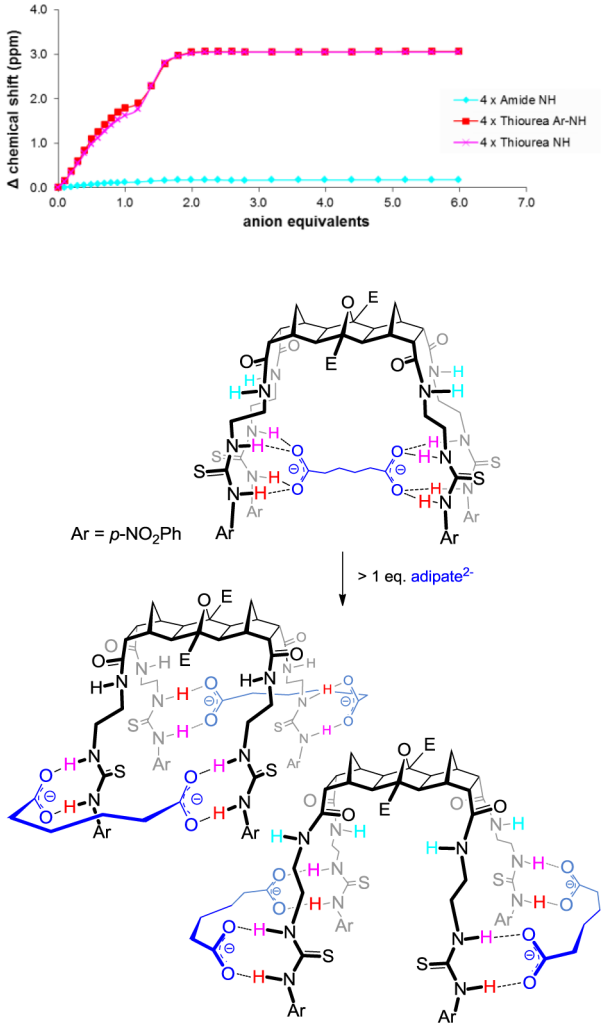

Figure 5. (top) Binding isotherm for the titration of host **5b** with adipate (n = 4) ([H] = 2.5 × 10^{-3} M). (bottom) Possible binding arrangements.

the series, and for each of the hosts **5** and **6** a bump appeared in the plot, but when the adipate concentration was increased above 1.2 equiv, the chemical shift quickly changed until 2.0 equiv of anion had been added. It is possible in these instances that initially all four urea groups were cooperating in the 1:1 binding of the anion, and then the 1:2 arrangement dominated (Figure 5).

*4. Terephthalate and Hosts **1**–**6**.* Titrations using terephthalate provided the most predictable results from the entire dicarboxylate series. The fixed length and rigidity of this bis-anionic species genuinely ensured that the cleft width of the host was a key factor determining binding strength. Indeed, for the two-armed hosts **1** and **2** both the titration curves and the resulting binding constants clearly illustrated that the [5]-polynorbornane host **2** bound terephthalate 2 orders of magnitude more strongly than the [3]polynorbornane **1** (for **2a**, log $K \approx 6.0$; for **1a**, log $K = 3.8$), as it possesses a cleft width of more appropriate dimensions.[20b]

Increasing the number of H-bond donors did little to affect this trend, as can be seen with hosts **3**–**5**. These hosts also bound terephthalate in a 1:1 fashion, where each urea N–H was involved in the binding event. Indeed, it was quite refreshing to see that host **3a** (so remarkable in its binding of alkyl dicarboxylates) bound this rigid dicarboxylate in a perfectly predictable 1:1 fashion in which all urea H-bond donors were involved.

The only deviation from this trend was seen for the four-armed host **6**, which bound two terephthalate guests.[20a] Electrostatic repulsion was likely mitigated by positioning the arms as far away as possible (Figure 6). It is unlikely, given the anion rigidity, that each end binds a single anion: hence, the proposed sawhorse arrangement of ethylurea groups around the [5]polynorbornane framework (Figure 5). Similar to that

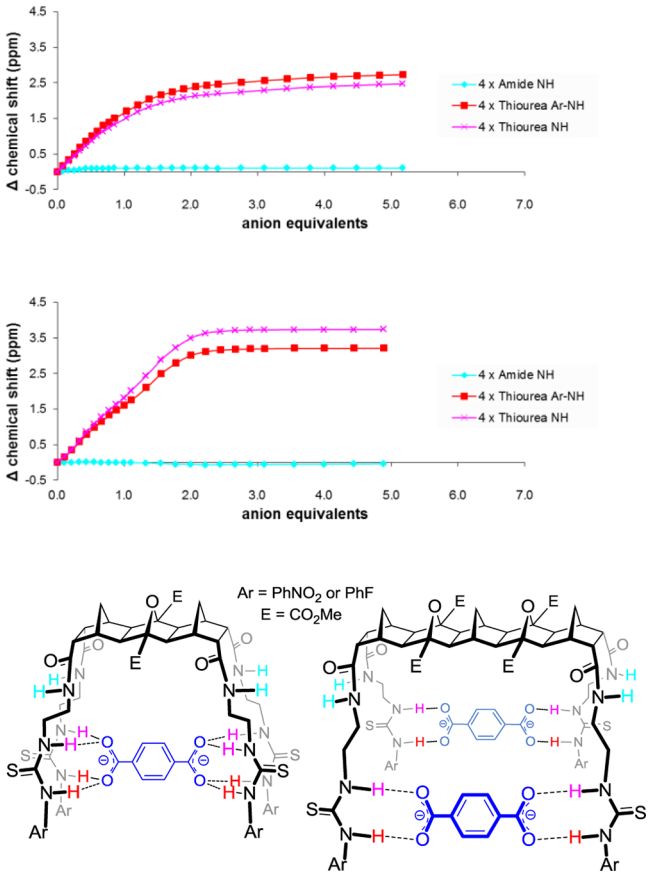

Figure 6. Binding isotherms of the titrations of hosts **5a** (top) and **6a** (middle) with terephthalate^{2-} ([H] = 2.5 × 10^{-3} M). (bottom) Proposed binding arrangements for hosts **5** and **6** with this anion.

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Table 2. Maximum Observed Chemical Shift for ArN−H, Host:Guest Stoichiometry, and Binding Constants for Phosphoanionic Species[a]

anion	param	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b
H_2PO^{4-}	max $\Delta\delta$	2.0	1.9	2.5	2.4	1.9	2.3	1.8	1.60	1.8	1.8	1.6	2.1
	H:G	1:1	1:1	1:2	1:2	1:2sw[c]	1:2sw	1:2sw	A[d]	1:2	1:2	1:2	1:2
	log K_1	2.6	2.9	2.7	3.5	3.5	3.2	3.3		2.7	2.8	2.6	2.8
	log K_2			2.5	3.0	<1	<1	<1		2.6	2.5	2.6	2.4
H_2PPi^{2-}	max $\Delta\delta$	0.53	1.3	0.40	1.1	1.4	1.8	1.3	1.7	1.1	1.7	1.1	1.7
	H:G	1:1	1:1	1:1	1:1	1:1 R[e]	1:1 R	1:1 R	A	1:2	1:2	1:2	1:2
	log K_1	1.8	3.3	1.5	2.8	3.8	4.7	3.4		3.0	3.0	4.2	3.5
	log K_2									2.2	3.0	2.5	3.2
$HPPi^{3-}$	max $\Delta\delta$	1.2	0.61	2.2	1.8	0.70	0.68	0.70	1.7	0.60	0.87	0.45	0.93
	H:G	D[b]	D	D	D	D	D	D	A, D	D	D	D	D
	log K_1												
	log K_2												
ADP^{2-}	max $\Delta\delta$	NP[f]	NP	NP	NP	1.2	1.6	0.94	1.5	0.75	1.3	0.60	1.5
	H:G					1:1	1:1	1:1	1:1	1:2	1:2	1:2	1:2
	log K_1					4.4	4.6	3.8	3.9	3.1	3.4	3.1	3.4
	log K_2									2.5	2.9	2.8	3.0

[a]A standard concentration of 1.3×10^{-2} M was used for hosts **1** and **2** and 2.5×10^{-3} M was used for the remainder (**3**−**6**). Errors ≤15% (see the Supporting Information for analysis of errors). [b]In some instances the urea thiourea N−H could not be followed or the binding constant was unreliable due to deprotonation. In these instances $\Delta\delta$ is the value prior to the disappearance of the signal, no binding constant is reported, and D is given to designate deprotonation. [c]The term "sw" indicates a stepwise binding process where the titration curve indicated that only after 1 equiv of the anion had been added did the second binding event occur. [d]The term "A" indicates a high degree of aggregation was noted, making assessment of the titration data (for log *K* and H:G stoichiometry) impossible. [e]The term "R" indicates that binding occurred at one end of the framework only. [f]The term "NP" indicates that the titration was not performed for these hosts.

seen for host **5** and adipate, the titration curve has a distinct inflection (up until ∼1.0 equiv the curve follows a trend similar to that for the other hosts, in which a 1:1 binding arrangement was favored), and then a critical concentration of anion was reached, whereupon the 1:2 arrangement dominated.

B. Phosphoanionic Species. The range of anions included dihydrogenphosphate ($H_2PO_4^-$) dihydrogenpyrophosphate (H_2PPi^{2-}), hydrogenpyrophosphate ($HPPi_3^-$), and adenosine-diphosphate (ADP^{2-}). While $H_2PO_4^-$ and $HPPi_3^-$ were employed as its tetrabutylammonium (n-Bu_4N^+) salt, the commercially available tributylammonium (n-Bu_3NH^+) salt of H_2PPi^{2-} and the DMSO-soluble disodium salt of the bis-anionic ADP^{2-} were used. The results of the titration experiments are presented in Table 2.

*1. Dihydrogenphosphate and Hosts **1**−**6**.* The interaction of $H_2PO_4^-$ with **1** was of moderate strength (log K_1 = 2.6 (**1a**) and 2.9 (**1b**)) and occurred in a 1:1 fashion. For **2** the longer cleft width precluded cooperative binding and a 1:2 H:G arrangement was noted (Table 2). The second binding constant was again smaller than the first, presumably due to electrostatic (anion:anion) repulsion.

For three-armed hosts **3** and **4** binding was determined to be 1:2 H:G, but again not in a typical fashion. Indeed, the two-armed end bound the first equivalent of $H_2PO_4^-$ almost exclusively (using both the urea N−H and the amide N−H protons; Figure 7) and it was not until >1 equiv of anion had been added that the other end of the framework began to bind weakly. It is interesting to note that the two arms of the identically functionalized bis-*endo* norbornane **14** do not cooperate in the binding of a single $H_2PO_4^-$ anion.[23]

For the four-armed hosts **5** and **6** the binding to $H_2PO_4^-$ was 1:2 H:G and both ends of the framework functioned in an identical fashion. Such a result was predictable for the larger host **6**, but as the *two*-armed [3]polynorbornane-based host **1** could form a 1:1 complex with $H_2PO_4^-$ using the thiourea from each end, it was thought the *four*-armed [3]polynorbornane

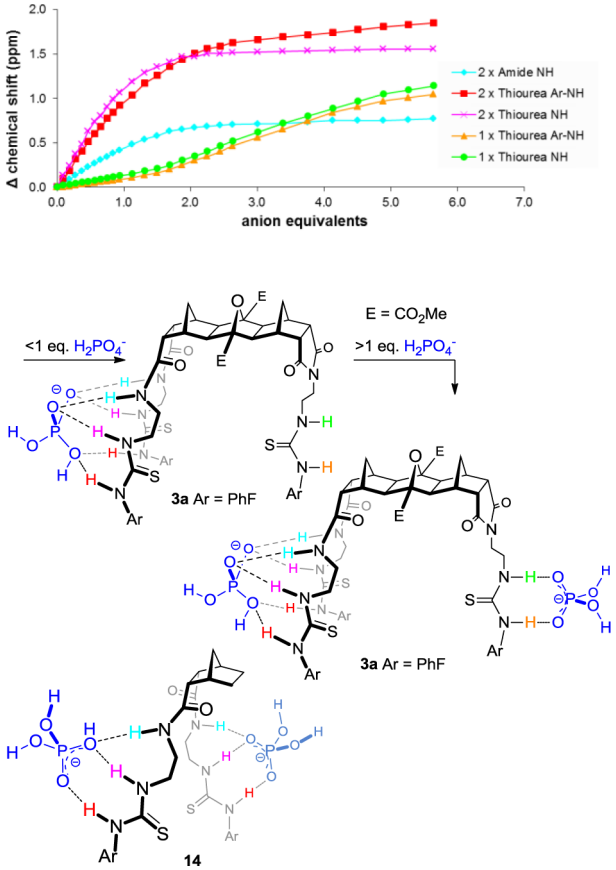

Figure 7. (top) Binding isotherm of the titrations of host **3a** with $H_2PO_4^-$ ([H] = 2.5×10^{-3} M). (middle) Proposed two-step binding mode. (bottom) 1:2 binding arrangement found in a previous study using simple *endo*/*endo* norbornane **14**.[23]

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host **5** would also behave in this fashion. It is likely that the 12 H-bond donors cannot surround a single $H_2PO_4^-$ anion in a low-energy complementary conformation; hence, both ends act independently.

*2. Dihydrogenpyrophosphate and Hosts **1**–**4**.* In a previous report from our group an unusual 2:1 host:guest stoichiometry was clearly identified for a two-armed [3]-polynorbornyl host with $(NBu_4)_2H_2PPi$.[20d] Such a 2:1 H:G arrangement has also been noted by Klarner[15a] and also Johnston[24] for a related fused polynorbornyl system. Unfortunately, in the current study the tetrabutylammonium salt was not available,[25] and when titrations with $(HNBu_3)_2H_2PPi$ were performed against **1** and **2** a clear 1:1 host:guest binding arrangement was evident. While the exact cause of the stoichiometry change was not precisely determined, it is likely due to the influence of the counter-cation. The influence of the counteraction on anion binding has also been noted in a study involving calixpyrrole anion hosts.[26] In the current study it is possible that the increased H-bonding ability and decreased steric bulk of the $HNBu_3$ cation results in better anion:cation association in solution and formation of the fully encapsulated 2:1 host:guest arrangement was disfavored.

Remarkable results were again obtained for titrations of H_2PPi^{2-} against the three-armed hosts **3** and **4**. In this instance, just as for hosts **1** and **2**, a 1:1 H:G binding stoichiometry was determined; however, unlike that observed for **1** and **2**, binding of the anion was not shared between both ends of the framework. Indeed, the resonances of the lone urea group barely moved during the *entire* course of the titration (Figure 8), suggesting very little or no participation in the binding

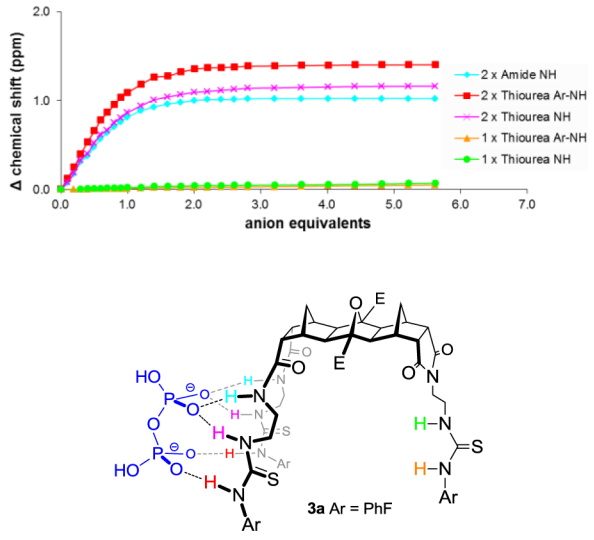

Figure 8. Titration curve (top) and proposed binding mode (bottom) of host **3a** with H_2PPi^{2-} ([H] = 2.5 × 10^{-3} M).

event. In contrast to that observed for the two-armed hosts **1** and **2**, the relatively large H_2PPi^{2-} guest did not appear to span the binding cleft; instead, the dianion was bound (in a similar fashion to the dicarboxylates) exclusively by the six H-bond donors of the two-armed end of the host (Figure 8). As the H_2PPi^{2-} anion was not spanning the binding cavity, the width of the cleft (either [3]- or [5]polynorbornane) had little or no effect on the strength of the 1:1 H:G complex formed, as evidenced by log K_1 for **3** and **4** (ranging from 3.4 for **4a** ([5]polynorbornane) to 3.8 for **3a** ([3]polynorbornane); Table 2).

For both hosts **3** and **4** the contribution from the amide protons was significant, with the magnitude of the observed changes in chemical shift approaching that of the thiourea protons (Figure 8), further supporting the one-sided binding mode.

*3. Two Anions (H_2PPi^{2-} and $H_2PO_4^-$) and Host **3**.* Again a series of additional titrations involving two anions were performed with hosts **3a**,**b** and **4a**. Remarkably, after either 1.0 equiv of H_2PPi^{2-} had been added to **3a**, the addition of $H_2PO_4^-$ resulted in no changes at the single-arm end until >4 equiv of additional $H_2PO_4^-$ had been added (Figure 9). This result again clearly reinforced the idea that the three-armed hosts preferentially recognize anions at the two-armed end, at the expense of what is an apparently perfectly accessible single urea group.

When the addition order was reversed, the preferential binding of H_2PPi^{2-} at the two-armed end was striking. Addition of up to 1 equiv of $H_2PO_4^-$ proceeded as expected; again with preference toward the two-armed end and with involvement from the amide N–H donors (Figure 9). The addition of only 0.5 equiv of H_2PPi^{2-} to the 1:1 **3a**:$H_2PO_4^-$ complex completely morphed the isotherm to that typically observed for H_2PPi^{2-}. Due to comparable pK_a values for the guest species (0.85, 1.96, 6.60, and 9.41 for each ionization of pyrophosphoric acid and 2.15, 7.20, and 12.33 for each ionization of phosphoric acid[27a]), any guest–guest acid–base interactions can be discounted. Acid–base chemistry between the tributylammonium counter-cation (pK_a = 10.89[27b]) and $H_2PO_4^-$ is also disfavored, again due to similar pK_a values. Ruling out guest–guest or guest–countercation interactions as the cause of the extraordinary changes in the binding isotherm, only the formation of a stable 1:1 host:guest complex between **3a** and H_2PPi^{2-} could elicit such a result.

Both the dramatic change in the binding isotherm and the stability of the H:G complex to excess $H_2PO_4^-$ indicate that not only was a stable 1:1 complex formed it was also an H:G arrangement where the single-arm end became *unavailable* for hydrogen bonding. Again the exact structure of the remarkable pyrophosphate complex with **3a** is unknown but is the subject of ongoing investigations.[28]

In order to test the robustness of this interaction, similar two-anion titration experiments were conducted using H_2PPi^{2-} and acetate (see the Supporting Information for isotherms). Results similar to those seen for H_2PPi^{2-} and $H_2PO_4^-$ were noted, but due to acetate basicity a small amount of guest–guest or guest–countercation acid–base chemistry cannot be ruled out. Nevertheless, the results reinforce the stability of the H:G complex between H_2PPi^{2-} and host **3a**.

*4. Pyrophosphate and Hosts **5** and **6**.* With the split titrations complete for the three-armed hosts, investigations continued with pyrophosphate and the four-armed tetrathiourea hosts **5** and **6**. Both the shorter [3]polynorbornanes **5** and the longer [5]polynorbornanes **6** bound H_2PPi^{2-} in a 1:2 fashion (Figure 10). As was seen for hosts **3** and **4**, the amide protons played a considerable role in the binding, with the change in chemical shift observed for these H-bond donors approaching 1.0 ppm. As both log K_1 and log K_2 were large, the binding conformation is likely to have the anions separated by a distance such that charge–charge repulsion is minimized (shown in Figure 10).

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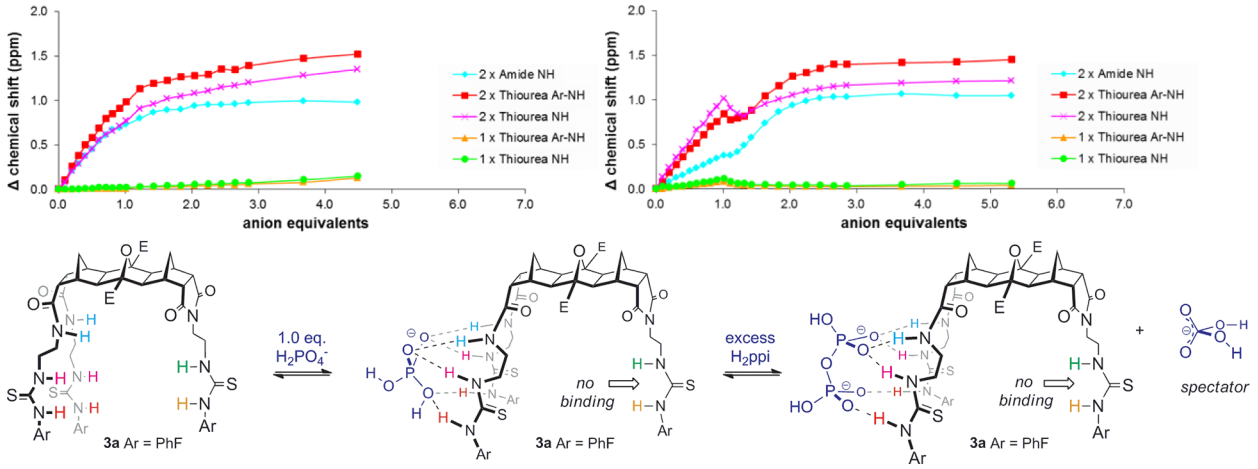

Figure 9. Mixed anion titration binding isotherms for titration of (top left) **3a** against H_2PPi^{2-} (0−1.0 equiv) and $H_2PO_4^-$ (1.0−5.5 equiv) and (top right) titration of **3a** against $H_2PO_4^-$ (0−1.0 equiv) and H_2PPi^{2-} (1.0−5.5 equiv). Also shown (bottom) is the proposed displacement of $H_2PO_4^-$ by H_2PPi^{2-} during the second titration.

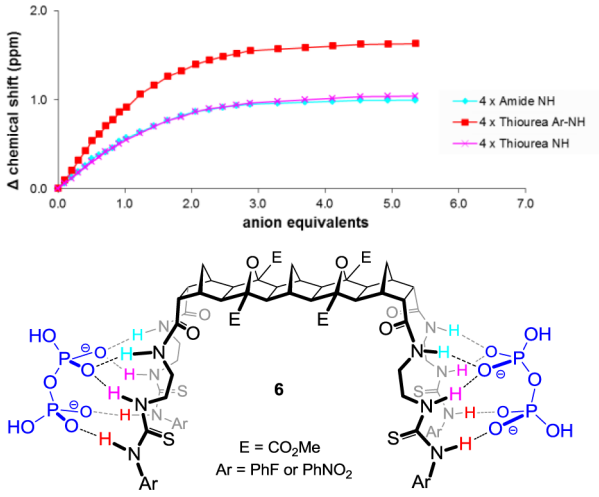

Figure 10. Titration isotherm and proposed binding mode of the four-armed [5]polynorbornane host **6** with H_2PPi^{2-}.

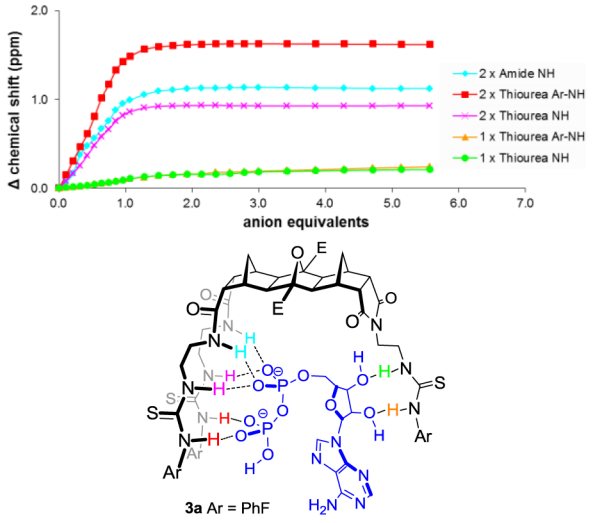

Figure 11. Titration isotherm (top) and proposed binding arrangement (bottom) of **3** with ADP^{2-}.

Unfortunately all titrations using the highly basic $HPPi^{3-}$ anion resulted in loss of the relevant N−H signals (rapid deprotonation) and no binding isotherms could be constructed. As such, no information about the binding, if any, of hosts **1**−**6** to $HPPi^{3-}$ can be inferred.

*5. Adenosine Diphosphate and Hosts **3**−**6**.* Only hosts **3**−**6** were evaluated against this anion, and the binding to **3** and **4** was very similar to that observed for pyrophosphate. The ADP^{2-} anion bound to the two-armed end of the host almost exclusively, and interestingly, the amide N−H protons played a larger role than those of the alkyl urea. The shorter [3]polynorbornane hosts bound the anion more strongly (log K_1 = 4.4 (**3a**), 4.6 (**3b**)) than the [5]polynorbornanes (log K_1 = 3.8 (**4a**), 3.9 (**4b**)), a result that suggests that cleft width plays a role and the urea protons at the one-armed end play a role in binding ($\Delta\delta$ = 0.3 ppm; Figure 11). Unfortunately, the exact site on ADP^{2-} for the additional interaction could not be determined but is likely to be either N3 or N7 of the adenine heterocycle or an O from the ribose ring. Not only does the additional interaction suggest that the anion binds within the cleft it also reinforces the notion that a correctly sized binding cleft will bind with greater strength.

The ^{1}H NMR titrations of **5** and **6** against ADP also indicated a 1:1 H:G binding event was taking place and the resonances for the equivalent H-bond donors at each end moved in unison. This result was strongly indicative of cooperative binding from both ends involving the thiourea and amide groups. Again it was of interest to note that the amide groups participated to the same extent as the aliphatic urea N−H atoms.

■ CONCLUSIONS

Regioselective binding of certain anions was observed for the three-armed hosts, particularly for [3]polynorbornane **3a**, where binding clearly favored the two-armed end. Indeed the pyrophosphate anion bound *exclusively* at this region of the host regardless of anion concentration. The ability of **3a** to perform regioselective recognition was exploited in the controlled stepwise addition of two anions to a single host (e.g., pimelate and acetate).

Another interesting feature of the titration curves was the inflection or bump that appeared in the isotherms of the hosts with 12 H-bond donors (e.g., **5b** against adipate and **6b** against

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terephthalate). Such examples indicate that the presence of multiple H-bond donors does not guarantee cooperative binding of the guest. In fact these examples clearly indicate that while 1:1 H:G is likely at low guest concentration a change to a stable 1:2 arrangement can occur when >1 equiv of guest is present.

Due to the results ranging from perfectly predictable to completely unexpected, a succinct picture of the anion binding properties of the [*n*]polynorbornane hosts is not easy to provide. Trends certainly exist, and a correlation with cleft width, anion size, and log K_1 was observed (e.g., **1** and **2** with terephthalate).

Such an interesting collection of results reinforce the principles that a delicate interplay exists between the dimensions of the host, the number of H-bond donors (host), and the number of H-bond acceptors (guest).

In conclusion, the evaluation of 12 new hosts against a series of phosphoanionic species was completed. Of the new hosts, the most remarkable results were obtained for the three-armed hosts—particularly **3a**—in which the anionic guests completely ignored a urea group when binding. Such controlled binding may have ramifications in the design and development of new organocatalysts/artificial enzymes. The exact causes of the unusual regioselectivity are currently being investigated, and developments on these fronts will be reported in due course.

EXPERIMENTAL SECTION

General Experimental Considerations. All reagents were obtained commercially and used without purification. NMR spectra were collected on a 270, 300, or 400 MHz FT-NMR spectrometer as indicated. Samples were dissolved in $CDCl_3$ or DMSO-d_6 (~1 mL) and reported relative to TMS (0.00 ppm). A 270 MHz FT-NMR spectrometer was employed to conduct the ^{1}H NMR spectroscopy titration experiments. Stimulated echo diffusion experiments were performed using a 500 MHz FT-NMR spectrometer.

Synthesis. The synthesis of all compounds has been described previously.[20]

General Procedure for Alkene + Cyclobutene Epoxide (ACE) 1,3-Dipolar Cycloadditions. A screw-cap pressure vessel was charged with equimolar amounts of the desired epoxide and norbornene unit (2.0 equiv in the case of bis-epoxides), the minimum amount of DCM or THF (depending on the solubility of the reactants) was added to dissolve the reactants, and then the vessel was sealed and heated with stirring at 140−150 °C for up to 72 h. Following this time the vessel was cooled to room temperature and opened, the solvent was removed under reduced pressure, and the crude product was purified by recrystallization or column chromatography.

General Procedure for Boc Removal and Thiourea Formation. The Boc-protected framework was stirred at room temperature in a solution of 20% TFA/DCM for 2−4 h before TLC analysis indicated complete consumption of starting material. Excess TFA and DCM were removed under reduced pressure before the remaining yellowish crude product was redissolved in $CHCl_3$ and then evaporated to dryness (twice) to ensure complete removal of TFA. Products were obtained as off-white solids, which were used directly in the following step.

The deprotected framework was dissolved in a solution of dry DIPEA and $CHCl_3$ before the desired isothiocyanate was added and the reaction mixture stirred at room temperature under a N_2 atmosphere for 18−46 h. Upon completion, as monitored by TLC, the reaction mixture was concentrated to dryness under reduced pressure, and then the desired compound was isolated by recrystallization or column chromatography.

General Procedure for nBu_4N^+ Salt Formation. A literature procedure[30] was employed in which the required dicarboxylic acid (1.0 equiv) was stirred in a nBu$_4$NOH/MeOH solution (1.0 M, 2.0 equiv) for 48 h. The solvent was removed under reduced pressure and complete dryness obtained by heating (70 °C) the crude solids under vacuum for 24 h to afford quantitative yields of the desired salts as white powders. The purity of the salts was confirmed by ^{1}H NMR spectroscopic analysis.

NMR Titration. *Single-Anion Titration.* In general a stock solution of each host was made up to 2.5×10^{-3} M in DMSO-d_6, and then 600 μL of this solution was then transferred to a 5 mm NMR tube and the spectrum collected. The chemical shifts (ppm) of the resonances corresponding to the amide and both thiourea H-bond donors, as well as the internal C−H protons, were recorded. A aliquot of the stock guest solution (5.0 μL of a 3.0×10^{-2} M in DMSO-d_6 solution, 0.1 equiv of guest) was then added to the host solution in the NMR tube by autopipette. The NMR tube was recapped, the solution was mixed, and then the ^{1}H NMR spectrum was collected. Again, the chemical shifts of the resonances corresponding to the amide and both thiourea H-bond donors, as well as the internal C−H protons, were recorded; this process was repeated until 2.0 equiv of guest had been added. The aliquot was then increased (10 μL, 0.2 equiv of guest), and the procedure was repeated until a total of 4.0 equiv of guest had been added. The final additions were made using larger aliquots (20 μL, 0.4 equiv of guest) until a total of 6.0 equiv of guest had been added. At this point an additional 150 μL aliquot (3.0 equiv) was added. The data were then plotted as a titration isotherm, and the association constant was determined through a nonlinear regression analysis using WinEQNMR.[21a] In cases where the resonances broadened, either a smoothing function was utilized or line broadening increased to more accurately determine the center of the peak.

Two-Anion Titration. The general titration procedure was as stated for the single-anion titrations. Anion solutions were switched at intervals specific to each titration: in the case of host **3a** with pimelate 0−1 equiv and with acetate 1−9 equiv. Pimelate was titrated with 10 × 5 μL aliquots (0.1 equiv each) of solution. The anion solution was then changed to TBA acetate for the remainder of the titration.

Stepwise Binding Analysis. For the stepwise binding exhibited by hosts **3** and **4**, a modified WinEQNMR protocol was used to determine log K_1 and log K_2. Each of the proton resonances responsible for the discrete binding events were separated into individual binding isotherms. The first binding event (K_1) between the two-armed end was analyzed without modification. In order to analyze the second binding event (K_2), the isotherm was modified to reset the values at 1.0 equiv of anion to 0 equiv (see Figure S1 in the Supporting Information). As interaction is insignificant before the 1.0 equiv mark (in comparison to the strong binding that occurs after 1.0 equiv), this provides an accurate representation of the overall binding strength of the second interaction.

Diffusion NMR. Stimulated echo (STE) experiments were performed using a diffusion time of 60 μs, a gradient time of 4 μs, and a maximum gradient value of 52 G cm^{-1} on a 500 MHz spectrometer. Each experiment consisted of 16 gradient steps, with 64 scans each. Diffusion coefficients were calculated for host **3a** at both the titration concentration (2.5 mM) and a dilute concentration (1.25 mM), and host **3a** and $HNBu_3H_2PPi$ (1:1) with the concentration of **3a** at both the titration concentration (2.5 mM) and a dilute concentration (1.25 mM).

Molecular Modeling. The equilibrium geometry of **3a** and suberate^{2-} was calculated using Spartan '08 or Spartan '10 (Wavefunction Inc.).[31] Initial minimization was conducted from the proposed geometry using MMFF at the ground state. The optimized structure was further refined by Hartree−Fock calculations. Images were generated using Accelrys[32] and POV-ray.[33]

Hartree−Fock: 3-21G(*) under vacuum. Total charge: dianion, subject to symmetry. Start from: MMFF. CPU time: 8:08:40.09, Energy = −5 199.143 693 7 E_h.

ASSOCIATED CONTENT

Supporting Information

Text, figures, and tables giving ^{1}H and ^{13}C NMR spectra of compounds **1**−**6**, binding isotherms, titration fit plots, a table of errors, molecular models, Job's plots, and an example of how

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stepwise binding was analyzed. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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