RESEARCH ARTICLE

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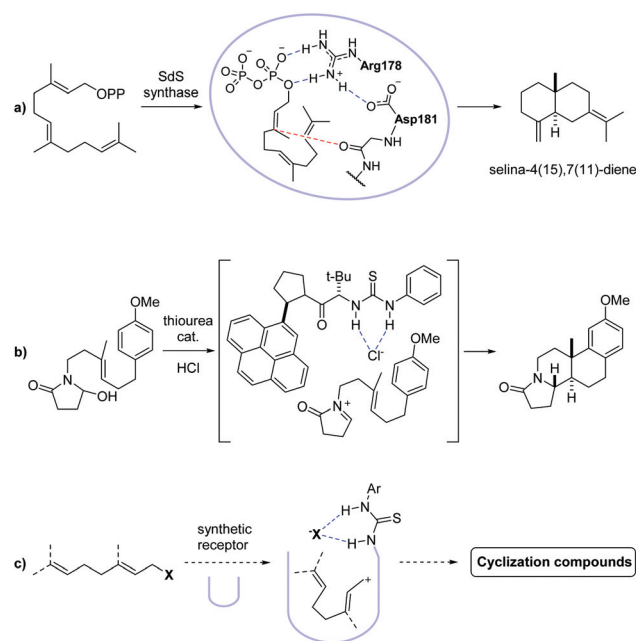# Binding of ion pairs in a thiourea-functionalized self-folding cavitand†

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We report the synthesis of a new synthetic cavitand receptor with an embedded thiourea moiety directed at effective molecular recognition of ion pairs. Initial prospects towards the use of cavitands in biomimetic carbocationic cyclizations have been carried out, employing ionic guests as stable surrogates for putative substrates and intermediates. The cavitand displays tight binding with tetramethylammonium salts, with unforeseen slow exchange kinetics of both cation and anion components.

Introduction

Supramolecular catalysis constitutes a promising approach for streamlining the synthesis of complex molecules by promoting selective transformations of organic substrates.[1] This concept is inspired mostly by nature, and hence the most genuine examples are arguably those which rely on the encapsulation of substrates in confined spaces akin to buried enzymatic pockets.[2] Imitation of the natural terpene cyclization machinery (Scheme 1a) is one of the most sought goals in this area, and a variety of encapsulation strategies have been described to carry out analogous carbocationic cyclizations in the confined spaces of π-rich synthetic receptors.[3] However, none of the previously reported examples feature specific activation of the cyclization precursors by purpose-built functionalities. Given the existing parallels with anion binding catalysis,[4] developed mainly by the group of Jacobsen (Scheme 1b), we envisaged melding the two concepts by installing a thiourea activating handle to a supramolecular receptor, with the aim of encapsulating, isolating and modulating the reactivity of carbocationic intermediates derived from linear polyene precursors (Scheme 1c).[5] As long term goals of this research, we envisage promoting non-stop propagation of carbocationic cascades and overriding the innate selectivity of cyclizations carried in the bulk.

We describe herein our latest efforts in this area, including the preparation of a new thiourea-functionalized self-folding

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†Electronic supplementary information (ESI) available: ¹H and ¹³C NMR spectra of new compounds, HRMS spectrum of **1**, ¹H NMR spectra showing thiourea–alkyl sulphate interactions, ¹⁹F NMR titration data for **1** and **6**, synthetic schemes not detailed in the main text, molecular model of **1** with geranyl sulphate. See DOI: 10.1039/c7qo00191f

Scheme 1 Carbocationic cyclization processes (a) in the buried active site of a terpene cyclase, triggered by specific interactions with neighboring residues, (b) through small molecule anion-binding with a thiourea catalyst, and (c) proposed bioinspired strategy with a thiourea-appended synthetic receptor.

cavitand,[6] the study of its binding abilities and initial attempts to bind substrates relevant to enzymatic carbocationic cyclization processes.

Results and discussion

Inspiration for this work came from a recent report describing a hitherto unknown cyclization mechanism in bacterial sesqui-

terpene cyclases, whereby ionization of farnesyl pyrophosphate is triggered by the synchronized effect of a guanidinium group hydrogen bonding to the pyrophosphate anionic head, and a neighbouring carbonyl providing nucleophilic anchimeric assistance (Scheme 1a).[7]

Capitalizing on our experience, we envisaged the preparation of a resorcin[4]arene derived, amide stabilized deep cavitand 1,[8] bearing an attached thiourea group (Fig. 1). We sought to use a 3,5-bis(trifluoromethyl)phenyl thiourea to provide a receptor with improved anion binding ability with respect to our previously reported octa(ureido) cavitand 3.[9] Central to our design is the ability of resorcin[4]arene-derived cavitands to stabilize positively charged species through cation–π interactions,[10] adding up to the thiourea motif to provide an effective ion-pair receptor.[11] Preliminary modelling studies revealed that a *para*-substituted phenyl spacer between

the thiourea group and the customary benzimidazole attaching moiety sufficed to prevent interference with the stabilizing amide hydrogen bond seam, while providing enough space to bind a monoterpene precursor such as a geraniol derivative, its polar end interacting with the thiourea's NHs and the aliphatic portion being buried in the deep aromatic pocket (Fig. S1†).

Cavitand 1 was prepared from the known diamino platform[10] which has been used for the construction of other monofunctionalized self-folding cavitands (Scheme 2).[12] After some experimentation, we found that reduction of dinitro cavitand 4a followed by FeCl$_3$ catalysed oxidative condensation with aldehyde 5 delivered the desired thiourea-appended cavitand in satisfactory yields.[13] We prepared model compound 6 in an analogous manner, while 5 was obtained in two steps from commercial materials (Schemes S1 and S2†).

Cavitand 1 was characterized by ^{1}H NMR, ^{13}C NMR and HRMS. The ^{1}H NMR spectra of 1 in acetone-d_6 or CDCl$_3$ (Fig. 2) display the distinct features of hexaamide-benzimidazole cavitands, namely, a far downfield shift at δ 11.8 ppm corresponding to the hydrogen-bonded benzimidazole NH, six separate resonances for the amide NH's indicative of slow rotation around the aryl-N bond relative to the NMR timescale, and four overlapping triplets around δ 5.65 ppm diagnostic for the cavitand adopting a folded, chiral "vase" conformation.[14]

Effective substrate binding is a requisite for supramolecular catalysis and we hypothesized that species already bearing an anionic portion such as polyisoprene pyrophosphates would benefit from enhanced binding in 1, with respect to analogues bearing neutral leaving groups (*e.g.* halides, acetates). The preparation of organic pyrophosphates is cumbersome, requiring tedious purifications which limit its practicability.[15] We reasoned that analogous alkyl sulphates, which can be easily prepared by reaction of an alcohol with sulphur trioxide,[16] would serve our purpose just as well. Eventually, we found that reaction of the requisite alcohol with SO$_3$·pyridine, followed by

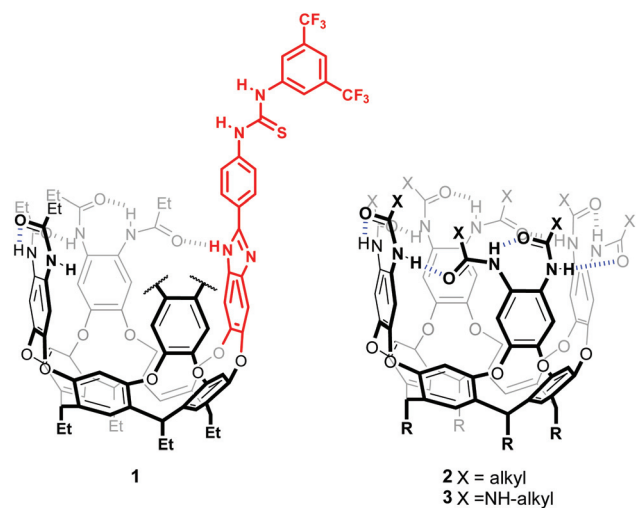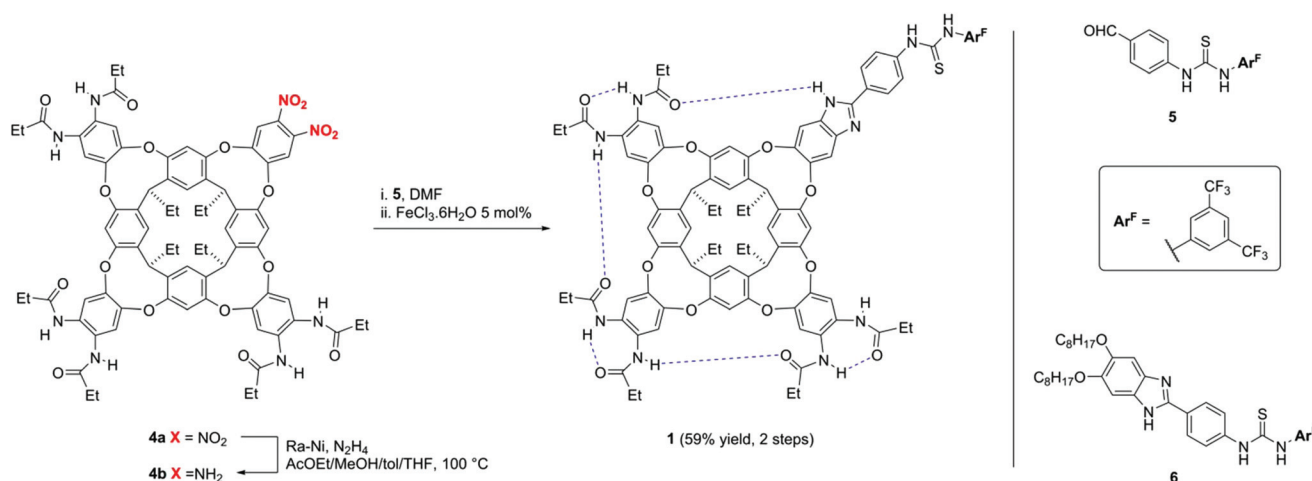**Fig. 1** Structure of hexaamide-thiourea self-folding cavitand 1, and previously reported octa(amido)- and octa(ureido)- cavitands, 2 and 3.

Scheme 2 Synthesis of hexaamide–thiourea cavitand 1, and model compound 6.

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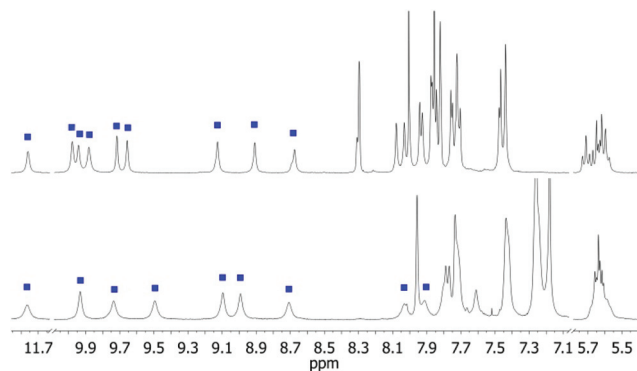*Org. Chem. Front.*, 2017, **4**, 1244–1249 | **1245**

Fig. 2 Downfield regions of the ^{1}H NMR spectra (400 MHz) of **1** in acetone-d_6 (top) and CDCl$_3$ (bottom). Blue squares indicate the NH resonances.

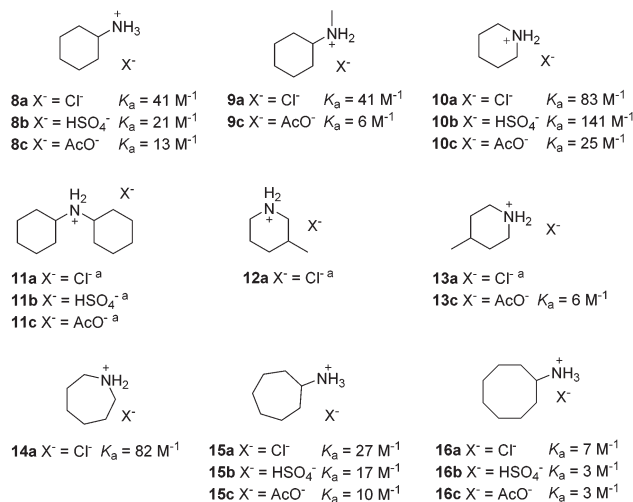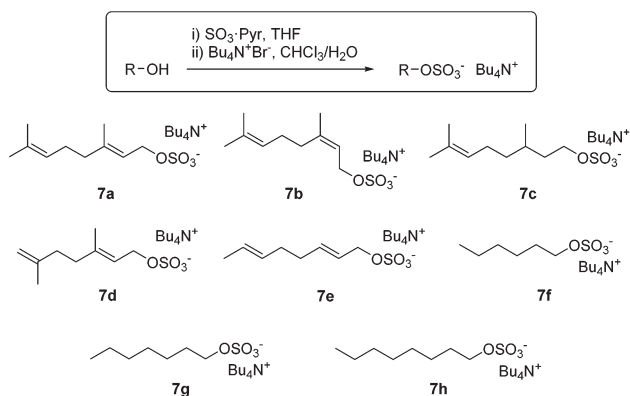|		
8a X$^-$ = Cl$^-$ K_a = 41 M^{-1}	**9a** X$^-$ = Cl$^-$ K_a = 41 M^{-1}	**10a** X$^-$ = Cl$^-$ K_a = 83 M^{-1}
8b X$^-$ = HSO$_4^-$ K_a = 21 M^{-1}	**9c** X$^-$ = AcO$^-$ K_a = 6 M^{-1}	**10b** X$^-$ = HSO$_4^-$ K_a = 141 M^{-1}
8c X$^-$ = AcO$^-$ K_a = 13 M^{-1}		**10c** X$^-$ = AcO$^-$ K_a = 25 M^{-1}
11a X$^-$ = Cl$^-$ ^{a}	**12a** X$^-$ = Cl$^-$ ^{a}	**13a** X$^-$ = Cl$^-$ ^{a}
11b X$^-$ = HSO$_4^-$ ^{a}		**13c** X$^-$ = AcO$^-$ K_a = 6 M^{-1}
11c X$^-$ = AcO$^-$ ^{a}		
14a X$^-$ = Cl$^-$ K_a = 82 M^{-1}	**15a** X$^-$ = Cl$^-$ K_a = 27 M^{-1}	**16a** X$^-$ = Cl$^-$ K_a = 7 M^{-1}
	15b X$^-$ = HSO$_4^-$ K_a = 17 M^{-1}	**16b** X$^-$ = HSO$_4^-$ K_a = 3 M^{-1}
	15c X$^-$ = AcO$^-$ K_a = 10 M^{-1}	**16c** X$^-$ = AcO$^-$ K_a = 3 M^{-1}

Fig. 3 Ammonium guests used in this study and binding constants with **1** (K_a) estimated by ^{1}H NMR. ^{a} Binding is too weak to accurately integrate the bound guest's resonances.

Scheme 3 Synthesis of tetrabutylammonium monoterpene sulphates and non-biological analogues.

cation exchange with tetra(n-butylammonium) bromide furnished the corresponding tetra(n-butylammonium) sulphates **7a–h** in multigram quantities and excellent yields, without the need for chromatographic purification (Scheme 3). Most conveniently, the salts prepared herein are soluble in both water and polar organic solvents. Very much like polyisoprenyl pyrophosphates, allylic sulphates **7a–b** hydrolyse in neutral or mildly acidic aqueous solution, making them promising ionization precursors for bioinspired carbocationic cyclizations.

We then proceeded to test the binding of **7a–h** with **1** in CDCl$_3$ solution. Host–guest complexes of amide-stabilized self-folding cavitands are kinetically stable in the NMR time scale and molecules buried in the cavity experience strong upfield shifts in the ^{1}H NMR spectra caused by the anisotropic shielding of the multiple aromatic rings.[17] For guests with aliphatic portions, this feature facilitates the monitoring of complex formation by the appearance of the bound guest's resonances in the far upfield region of the spectrum (δ 0 to −4 ppm) which is devoid of any interference from other species present in solution. Much to our dismay, no upfield resonances were observed at all upon treatment of **1** with excess quantities of

monoterpene sulphates **7a–c**. Smaller terpene analogues (**7d–e**) or n-alkyl sulphates (**7f–h**) – with increased conformational flexibility – did not provide evidence of internalization in the receptor's cavity either. In spite of this, a downfield shift of the urea's NHs is apparent in all these cases (Fig. S2†), suggesting that a significant interaction between the thiourea and the sulphate moiety is taking place. This is corroborated by the downfield shift of the singlet observed in the ^{19}F NMR spectra of **1** in CDCl$_3$ upon titration with increasing amounts of **7c**. Nonlinear fit analysis of the obtained binding isotherm revealed a binding constant K_a of 1.04×10^4 M^{-1}. The presence of a single resonance throughout the titration indicates a binding event in the fast exchange regime. We also tested the analogue acetates of **7a–h** as potential neutral substrates with hydrogen bonding leaving groups, but no binding was observed either. These results illustrate the challenge of displacing highly competitive solvents – such as CDCl$_3$ – by attempting to couple the effect of separate binding motifs in a cooperative manner.‡

We next examined the binding of ionic substrates which could mimic carbocationic intermediates along a putative cyclization process inside **1**. We chose to use ammonium cations, which are typical inhibitors of terpene cyclases and work as static surrogates of a reacting carbocationic species.[18] Cognizant of the challenge of binding linear aliphatic substrates, we set out to explore the binding of cyclic ammonium salts as surrogates for carbocationic intermediates folded in compact but energetically disfavoured contorted conformations. Different ring sizes and counter anions were assayed with the aim of probing the binding space of **1** (Fig. 3). Gratifyingly, upon addition of various 6-, 7- and 8-membered

‡ Analysis in less competitive solvents such as mesitylene or tetrachloromethane was prevented by the low solubility of **1** and the ionic guests in these compounds.

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cyclic ammonium salts to **1**, the appearance of new resonances in the far upfield region confirmed the formation of new encapsulation species –now in the slow exchange regime (Fig. 4). Binding constants were estimated by direct integration of the NMR spectra (Fig. 3). The low values extracted from our data prevent an accurate quantitative analysis but some trends can be inferred. The cyclooctylammonium ion seems to be close to the upper size limit for the number of atoms that can be accommodated in the aromatic pocket of **1**. Interestingly, while cyclohexylammonium ion pairs (**8**) display a reasonable affinity, the addition of an extra cyclohexyl group to the ammonium moiety (**11**) virtually depletes binding, despite the fact that it can protrude out of the cavity through the upper rim. We interpret this as a result of the second cyclohexyl ring interfering with the anion binding site. For cyclic ammonium salts, no general trends can be observed upon variation of the counter-anion. Again, the low magnitude of the binding constants precludes any analysis invoking subtle effects of basi-

Table 1 Association constants of tetramethylammonium salts with cavitands **1**, **2a**, and model compound **6**[a]

Host	Guest (Me$_4$N$^+$X$^-$)	X$^-$	K_a[a] (M^{-1})
1	17a	F$^-$	>14000[b]
1	17b	Cl$^-$	>16000[b]
1	17c	Br$^-$	2400[b]
1	17d	HSO$_4^-$	>5000[b]
1	17e	AcO$^-$	>11000[b]
2a	17e	AcO$^-$	750[b]
6	17a	F$^-$	255[c]
6	17b	Cl$^-$	106[c]
6	17e	AcO$^-$	486[c]

[a] In CDCl$_3$/CD$_3$OD 95 : 5, [Host] = 2.15 mM approx. [b] Determined by ^{1}H NMR. [c] Determined by ^{19}F NMR titrations.

city, size, and shape.[19] The signature of the upfield peaks in the ^{1}H NMR spectra reveals that these species bind with the ammonium group close to the polar rim defined by the hydrogen bonded amide groups, and therefore charge–dipole interactions with the amide carbonyl groups must significantly contribute to the overall binding stabilization.

Finally, much increased binding was observed when we employed tetramethylammonium salts **17a–e** as guests.§ Even with sub-stoichiometric amounts of the guest, tetramethylammonium fluoride, chloride, acetate or hydrogensulphate were totally encapsulated within **1** as ascertained by ^{1}H and ^{19}F NMR (*vide infra*). Only traces of the free ammonium species can hardly be made out in the ^{1}H NMR spectra – beyond the integration threshold. For these ion pairs the magnitude of the association constant is thus too large to be measured by NMR techniques. On the other hand, weaker binding is observed for tetramethylammonium bromide, in good agreement with the well-established lower affinity of thioureas for anions with larger polarizability.[20] Since previously reported cavitands devoid of specific anion binding motifs bind tetraalkylammonium salts with reasonable affinities,[21] we set out to clarify the role of the thiourea moiety with additional experiments. First, the parent octaamide cavitand **2a** (Fig. 1, X = R = Et) showed an association constant for Me$_4$N$^+$AcO$^-$ (**17e**, K_a = 7.5 × 10^2 M^{-1}) which is at least one order of magnitude weaker than that with **1**. Model compound **6**, lacking a specific anchor for the tetramethylammonium cation, displays also much reduced affinity for tetramethylammonium ion pairs (including acetate **17e**, K_a = 4.9 × 10^2 M^{-1}), as ascertained by non-linear fit analysis of ^{19}F NMR titration data (Table 1). Taken together, these data support a cooperative effect of both binding motifs in **1**. Most interestingly, ^{19}F NMR titration experiments with **6** always show a fast exchange scenario, while titration of **1** with tetramethylammonium salts reveals slow exchange dynamics (Fig. 5). While this is expected when looking at the bound ammonium ion resonances in the ^{1}H NMR spectra, it is not immediately
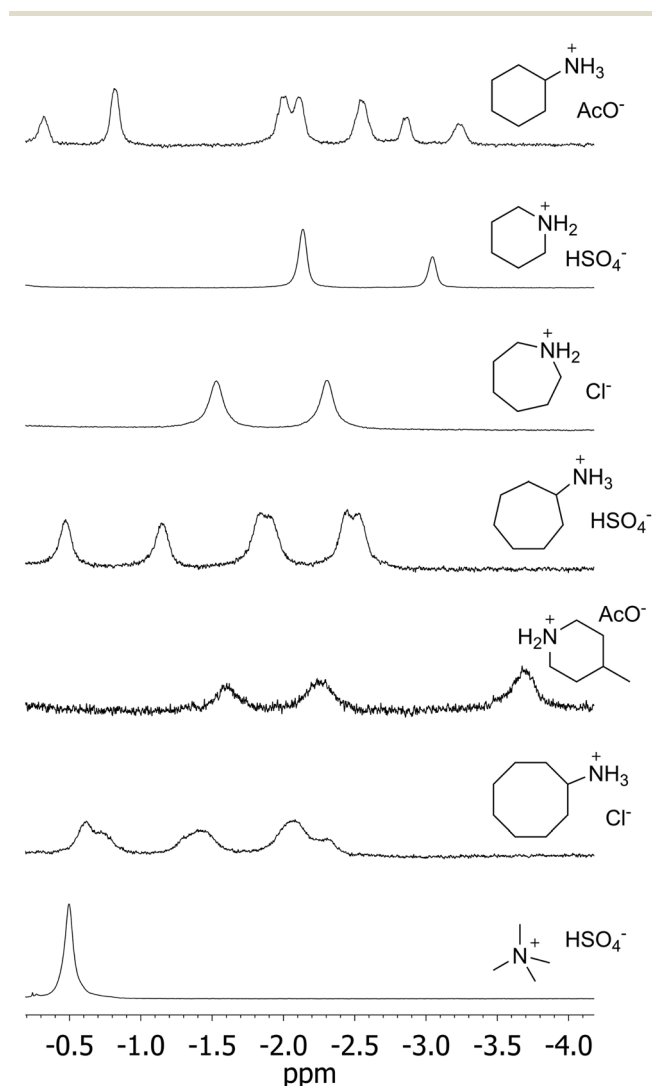

Fig. 4 Far upfield region of the ^{1}H NMR spectra of **1** (CDCl$_3$) with excess of various ammonium salts, showing the resonances of aliphatic portions of the guests buried deep in the cavity.

§ CDCl$_3$/CD$_3$OD 95 : 5 was used as a solvent to ensure full solubility of the salts. The vase conformation of **1** remains unaltered in this solvent mixture.
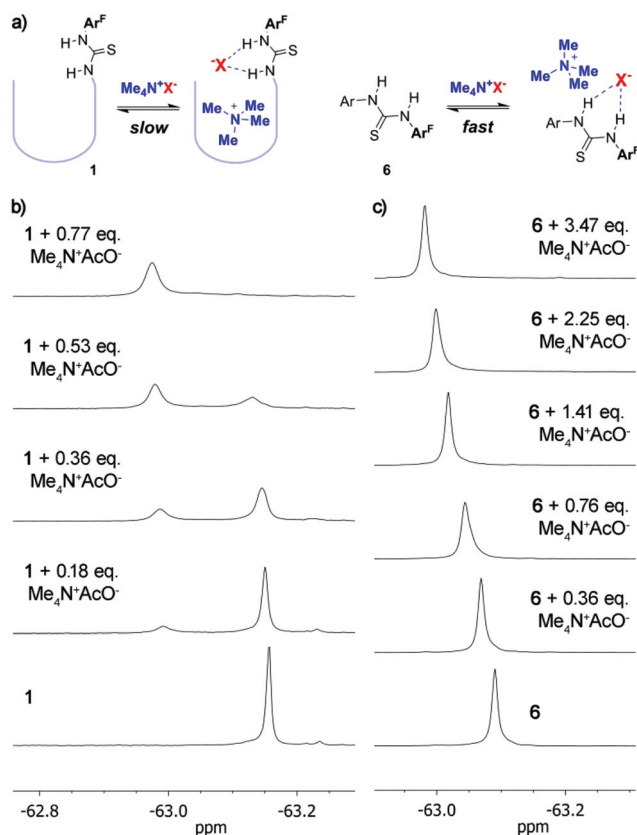
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Fig. 5 (a) Different exchange regimes with tetramethylammonium salts and evolution of the ^{19}F NMR spectra (CDCl$_3$/CD$_3$OD 95 : 5) upon titration with Me$_4$N$^+$AcO$^-$ of (b) **1** and (c) **6**.

apparent that the anion binding site – which is exposed to the solvent – would behave similarly. This is a promising feature for future developments in catalysis because the combination of both anion and cation specific binding sites in the cavitand enables not only improved binding but also effective confinement of the cation from bulk medium by temporally blocking the cavitand's opening with the anion.

Despite the reluctance of **1** to form kinetically stable complexes with linear substrates buried in the hydrophobic pocket, we hypothesized whether ionization of allylic sulphates could take place by specific activation with the thiourea function outside the cavity, followed by rapid internalization of the nascent carbocation in the aromatic pocket. We therefore monitored solutions of **7a–b**/**7d–e** and **1** in CDCl$_3$ over time to check for the formation of new compounds. As ascertained by ^{1}H NMR, the solutions remain basically unaltered at room temperature. When heated to 40 °C the substrates remain stable and no trace of new compounds is observed, but decomposition of **1** is quite apparent.

Conclusions

In summary, we have prepared a new self-folding cavitand receptor with two complementary binding sites: an electron-

rich hydrophobic pocket – suitable for binding of hydrocarbons and cationic species – and a thiourea motif directed to anion binding. The two sites work in concert to tightly bind tetramethylammonium ion pairs in CDCl$_3$, resulting in slow exchange of both components relative to the NMR time scale. Salts of cyclic ammonium ions – used as stable models for reactive carbocationic species – were also bound in **1** albeit with much reduced affinity. The lack of binding for linear substrates prevented the use of **1** in developing a biomimetic carbocationic cyclization of terpene-like polyene substrates. Future developments in this area will address the main limitations of the current design, namely (a) the reduced buried space in **1**, which makes it unlikely to bind precursors and/or reactive intermediates of 8 carbon atoms or more, and (b) the low binding affinities resulting from the use of a competitive solvent. For the latter, the preparation of water soluble equivalents of **1** is underway in order to exploit hydrophobic effects, which should facilitate folding of linear substrates into energetically disfavoured more compact conformations.[22]

Acknowledgements

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