Enantioselective lactate binding by chiral tripodal anion hosts derived from amino acids†‡

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Chiral, tripodal anion hosts derived from either *S*-phenylalanine or *S*-leucine bind D-lactate enantioselectively. The nature of the host–guest interaction has been probed by solution NMR methods and by DFT calculations. The calculations suggest that the D-lactate may form an additional hydrogen bond in the host–guest complex while the L-lactate complex contains an intramolecular hydrogen bond. Anion binding is in competition with host dimerisation, as demonstrated by DOSY and ^{1}H NMR spectroscopy, and DFT calculations.

Introduction

Anion sensing, and the binding or discriminating molecular recognition processes that accompany it, has become a highly topical field with potential applications in pollutant capture and detection, analytical and quality control chemistry, and in biological transport, imaging and monitoring.[1–7] Of particular importance are carboxylate anions because of their variety of structures, biochemical importance and high intrinsic basicity. While carboxylates generally bind strongly to a variety of hydrogen bond donor hosts their basicity can lead to host deprotonation and chemical degradation.[8–10] Carboxylate anions are frequently chiral and biological anion recognition is invariably highly enantioselective and since *ca.* 70% of enzyme substrates are anions,[11] chiral anion binding is clearly an interesting biomimetic target. One of the simplest chiral anions is lactate (2-hydroxy propanoate) of which L-(+)-lactate is important in biological metabolism. Compared to the rest of supramolecular anion coordination chemistry, enantioselective recognition is relatively underexplored,[12–16] particularly with regard to the enantioselective binding of lactate and its derivatives.[17,18] We now report the preparation and anion binding properties of two chiral tripodal hosts based on amino acid derivatives displaying enantioselective lactate binding.

Results and discussion

Hexasubstituted triethylbenzene or 'pinwheel' derivatives[19] have proved extremely useful anion binding scaffolds because of the host preorganisation imparted by the steric gearing about the central aromatic ring.[17,20–32] While a range of neutral derivatives have proved effective anions hosts, we have shown that cationic pyridinium species are also highly selective and versatile anion binding and sensing platforms.[33–37] By taking advantage of the modular synthesis[7,22] of this class of compound we sought to impart enantioselectivity by the use of amino acid derivatives in order to create a homochiral anion binding pocket as in tripodal hosts **3**, which were prepared as the *S*,*S*,*S*-enantiomers in a reasonably straightforward manner starting from the *S*-amino acid derivatives, as outlined in Scheme 1. The hosts are based on a 3-α-aminomethylpyridine anion binding motif which we have used before in ruthenium(II) based anion hosts[38,39] but not in pyridinium species. As a control we therefore also prepared the achiral tripodal hosts **4**.

The anion binding ability of hosts **3** and **4** was probed using ^{1}H NMR spectroscopic titrations in acetonitrile-d_3. The binding constants for halides, nitrate, acetate and lactate anions for both hosts obtained from HypNMR 2006[40,41] and the corresponding stoichiometry are shown in Table 1. The shape of the titration isotherms for hosts **3** in particular immediately suggests that the anion binding equilibria are more complex than a simple 1 : 1 stoichiometry. In compounds **3** it is only the doublet resonance

Table 1

Anion	Stoichiometry (H–G)	Log β_{HG}			
		3a	**3b**	**4a**	**4b**
Dimerisation constant	2 : 0	1.0[a]	2.3[a]	2.61(15)	2.82(4)
Cl^-	1 : 1	4.06(11)	4.17(3)	4.51(5)	3.74(6)
	1 : 2	6.64(11)	6.02(4)		
	2 : 1			8.14(3)	7.87(15)
Br^-	1 : 1	3.80(8)	3.92(1)	3.90(2)	3.61(3)
	1 : 2	6.29(8)	5.98(2)		
	2 : 1			7.51(4)	7.38(6)
I^-	1 : 1	3.27(6)	3.02(2)	—	—
	1 : 2	5.41(5)	5.85(5)		
NO_3^-	1 : 1	3.19(4)	—	3.12(4)	3.20(4)
	1 : 2	5.44(4)			
	2 : 1			5.69(13)	6.18(11)
$CH_3CO_2^-$	1 : 1	2.86(9)	3.23(1)	—	3.54(3)
	1 : 2	5.32(4)	4.86(1)		6.12(2)
L-$CH_3(OH)CO_2^-$	1 : 1	2.67(1)	2.82(3)	—	—
	1 : 2	4.57(1)	4.95(1)		
DL-$CH_3(OH)CO_2^-$	1 : 1	2.75(4)	3.25(1)	—	—
	1 : 2	5.02(1)	5.12(4)		

[a] Manual fit from concentration dependence data.

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‡ Electronic supplementary information (ESI) available: Plots and spectra for **3a**, **3b**, **4a** and **4b**. See DOI: 10.1039/b817889e

assigned to the pyridinium proton *para* to the nitrogen atom that undergoes a significant chemical shift change, with $\Delta\delta$ values up to *ca.* 0.9 ppm. All titration plots exhibit a marked sigmoidal shape (*e.g.* Fig. 1 which shows the isotherm obtained for Cl^- binding by **3a**) possibly suggesting cooperative binding or multiple equilibria. The anion binding stoichiometry was confirmed by Job plot for **3a** and acetate anion which indicated a 1 : 2 host–guest stoichiometry (see ESI‡). This model was then applied to fitting the titration data for hosts **3** with all of the anions studied. However, generally this model gave a very poor fit to the data particularly in the low anion concentration region. Accordingly we looked for additional possible equilibria. Examination of the concentration dependence of the ^{1}H NMR spectra of both **3a** and **3b** showed a small but consistent concentration effect (Fig. 2) allowing us to fit dimerisation constants, log $K_{dim} = 1.0$ and 2.3, respectively. A dimerisation process offers a convincing explanation for the apparent cooperativity in binding the two

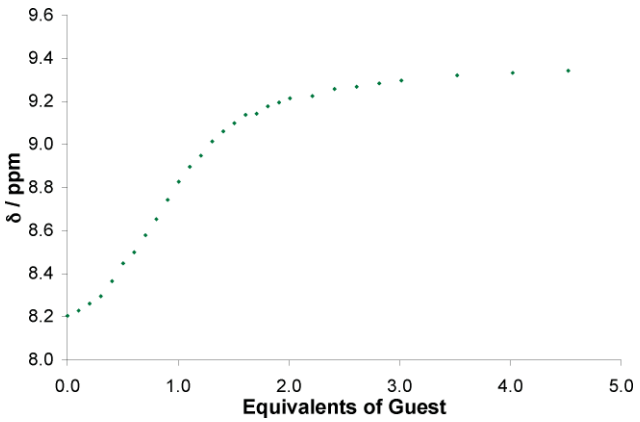


Fig. 1 Sigmoidal Cl^- binding isotherm for host **3a**.

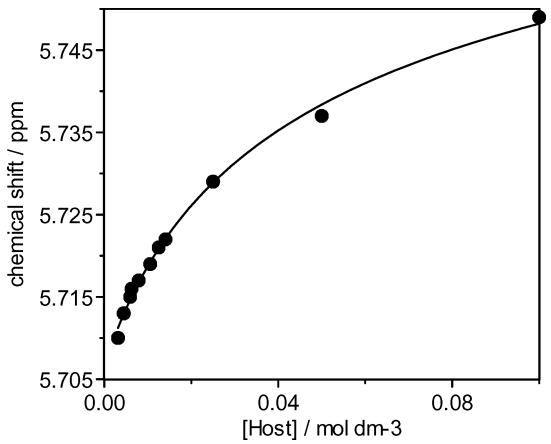
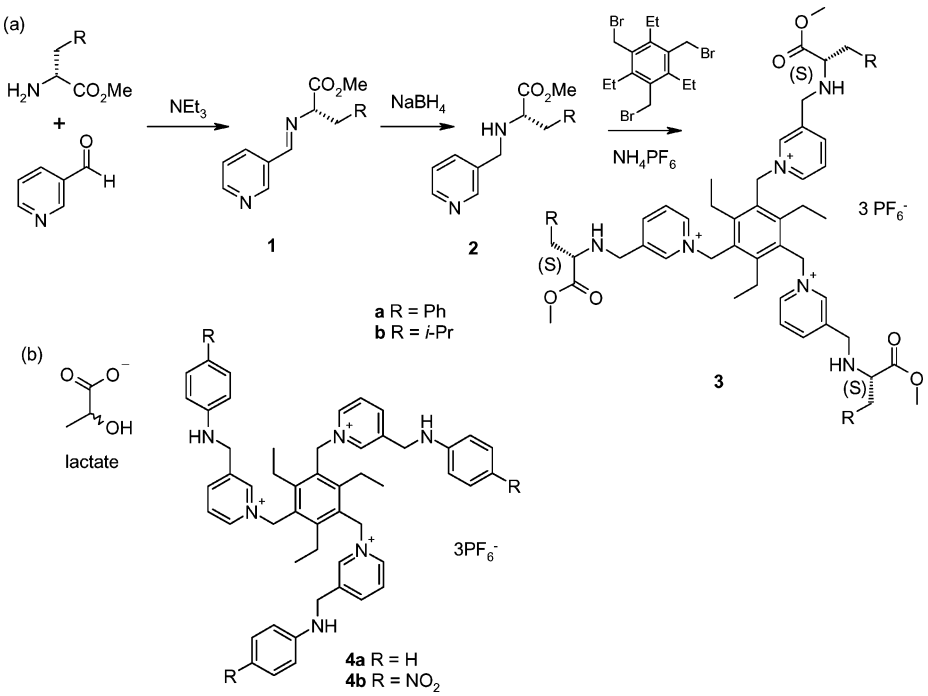


Fig. 2 Concentration dependence of the ^{1}H NMR spectrum of **3a**. Points are experimental data, the solid line is the calculated fit for log.

equivalents of anion guest with binding of the first anion in competition with dimer formation, while the 1 : 1 host–guest complex can more readily bind to a second anion. While we were unable to observe dimer formation by mass spectrometry, we sought confirmation of the anion-induced dissociation of a host dimer by DOSY NMR spectroscopy on **3a**.[42] The DOSY spectra indicated considerably faster diffusion (10×10^{-10} m^2 s^{-1}) for **3a** in the presence of 3 equivalents of racemic tetrabutylammonium lactate than where the predominant species is expected to be a monomeric lactate-bound host, than for the hexafluorophosphate salt which is expected to be a dimer, diffusion coefficient 7×10^{-10} m^2 s^{-1} (see ESI for DOSY spectra). Dimer formation has also been observed in related pyrene-based hosts.[43]

Adoption of a stoichiometry model incorporating a dimer of known, fixed dimerisation constant along with 1 : 1 and 1 : 2



Scheme 1 (a) Synthesis of chiral tripods **3**, (b) structures of the lactate anion and of compounds **4**.

host–guest complexes allowed a good fit to the titration data, giving the anion binding constants shown in Table 1 for **3a** and **3b**. The selectivity pattern for halide and nitrate anions is normal for this type of host,[44] with affinity decreasing modestly in the order $Cl^- > Br^- > I^- > NO_3^-$ more or less in accordance with the anions' charge density. Surprisingly, however, acetate and lactate are bound relatively weakly. The strong basicity of acetate usually results in very strong binding by hydrogen bond donor hosts in solvents such as acetontrile.[1,39,45] The comparative weakness of the carboxylate binding is also evidenced in the carboxylate $\Delta\delta$ values which are uncharacteristically slightly lower than for Cl^-. We surmise that this halide selectivity arises from steric effects that prevent the acetate and lactate CH_3^- groups from entering the tripodal cavity, and indeed the slightly bulkier lactate is bound more weakly than acetate.

Despite the fact that lactate binding is relatively weak with affinity more than an order of magnitude less than Cl^-, both hosts **3a** and **3b** show a significant and interesting preference for the lactate D-enantiomer, Fig. 3. We were unable to obtain a sample of D-lactic acid and hence titrations were carried out by comparing a commercial sample of the L-enantiomer (as its NBu_4^+ salt) with a racemic mixture. Fig. 3 shows that there is a marked difference in the response of the hosts to the two samples and this difference is reflected in the binding constants with both hosts proving selective by a factor of 1.5–3 comparing the observed β_{11} and β_{12} values. Given that we are dealing with a racemic mixture, this corresponds to a D/L selectivity of *ca.* 3–6, or an ee of up to *ca.* 70%. While not yet of the kind of selectivity required for resolution of chiral materials in a single step, this is a surprisingly positive result for such a flexible host system and augurs well for future refinement of the design. It is also interesting that the hosts are able to discriminate between lactate enantiomers even though they are halide-selective; indeed strong binding and effective discrimination are often mutually antagonistic.[44]

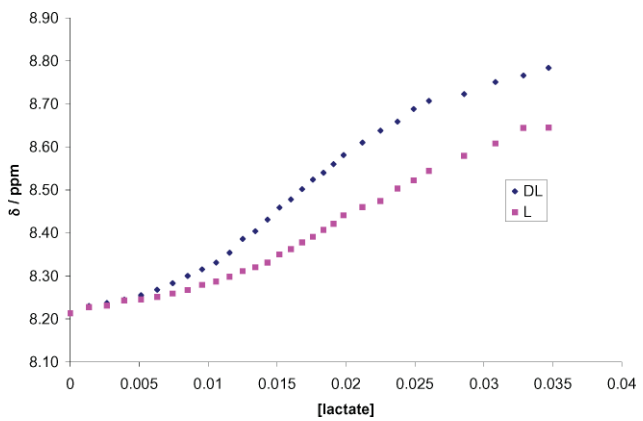


Fig. 3 Comparison of DL- and L-lactate binding by **3a**.

A final interesting feature of both hosts **3a** and **3b** upon carboxylate binding was the observation of a visual colour change from colourless or orange/red upon addition of an excess (up to *ca.* 17.5 equivalents) of acetate or lactate. The UV-Vis absorption spectrum of **3a** upon titration with acetate in acetonitrile solution is shown in Fig. 4. The red colour comes from the growth of a broad shoulder at around 350 nm extending into the visible region. This band is tentatively assigned to an anion–π* charge

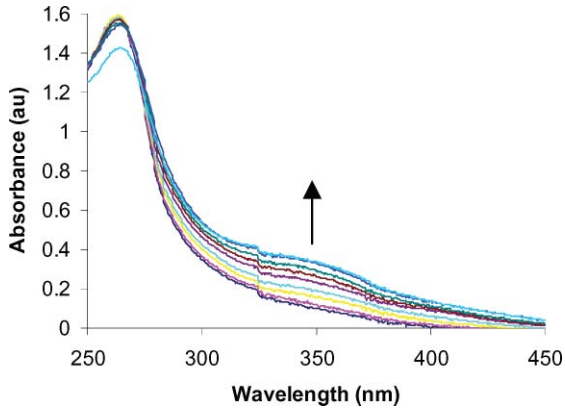


Fig. 4 Absorption spectra of **3a** in CH_3CN upon addition of up to 17.5 equivalents of $NBu_4^+CH_3CO_2^-$ resulting in an orange colouration.

transfer state involving the pyridinium acceptor.[37] This assignment is supported by TD-DFT calculations on **3b** which give a broad band around 400 nm (red shifts are quite common in TD-DFT calculations) that corresponds to several excited states, most of which have some amount of lactate–pyridinium π* charge-transfer character. Indeed the first three LUMOs are nearly degenerate and are pyridinium π* character (see ESI‡).

We sought to address both the molecular basis for the enantioselective lactate discrimination and the competing dimerisation process using DFT molecular models. We have found in the past that notionally gas phase DFT models can give excellent insights into possible conformational effects and binding modes in these kinds of systems when coupled with detailed solution phase information from titration and variable temperature NMR spectroscopic data.[35–37] The DFT calculated structure of the dimer $(\mathbf{3b})_2$ is shown in Fig. 5. In these flexible systems it is impossible to guarantee full sampling of conformational space and hence the structure in Fig. 5 represents one of a number of possible conformational minima which may well be in equilibrium in solution. It does, however, show the well-known alternating conformation around the hexasubstituted aromatic rings and a well-defined 10-membered ring hydrogen bonding motif involving

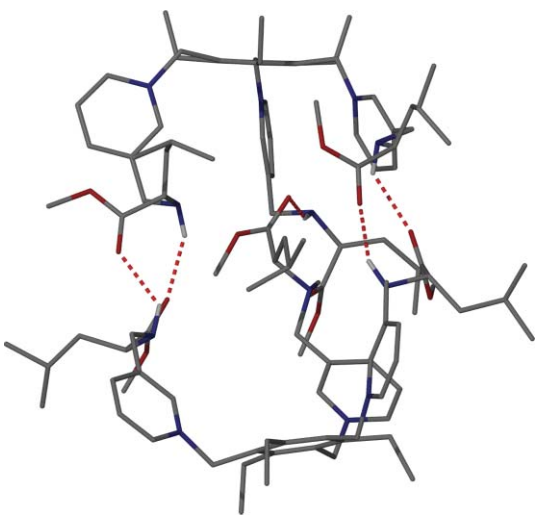


Fig. 5 DFT calculated structure of the proposed dimer $(\mathbf{3b})_2$ (host CH hydrogen atoms omitted for clarity).

the amine NH donor and ester oxygen atom acceptors. A CSD search[46,47] confirms that such a motif is common for esters joined to amide NH atoms (161 hits) and is also precedented for rarer amine esters (such as compounds **3**) in CSD refcode TICXIM (1,1′-bis(*p*-chlorophenyl)-6,7*a*-dimethyl-4,7*a*,1′,4′,5′,6′-hexahydro-pyrrolo(4,5-*a*)(1,2,4)triazole-5-spiro-6′-(1,2,4)triazine-3,3′-dicarboxylate).[48] The calculated structure also shows that it is feasible for the dimer to form in the absence of added anion by inclusion of one of the amino acid residue substituents within the dimer cavity. Such a structure is consistent with the observed dissociation of the dimer upon addition of anions to give 1 : 1 and 1 : 2 host–guest complexes since exposure of the hydrogen bonding functionality is a necessary feature of anion binding. Fig. 6 shows the DFT calculated structures of **3b** binding L- and D-lactate, respectively. The model of the D-lactate complex has an additional lactate NH···O interaction with the host compared to the L-lactate complex, in which the additional host–guest interaction is replaced by an intramolecular host NH···O interaction. This additional interaction is very much consistent with the stronger binding of the D-enantiomer observed in solution. However, these gas phase models must be treated tentatively since in solution solvation of the lactate OH and carboxylate groups in particular and compensating entropic effects[49] may well be highly significant. Clearly, however, 1 : 1 binding of both lactate enantiomers is feasible in these systems and competition between intramolecular and host–guest hydrogen bonding interactions may be a factor in the observed enantioselectivity.

As a further insight into the hosts' solution conformational behaviour we used variable temperature ^{1}H NMR spectroscopy in acetone-d_6 solution. At room temperature, in both acetone and acetonitrile solutions, hosts **3** display at least time-averaged C_3 symmetry. Lowering the temperature resulted in significant broadening of many of the host resonances, including, for example, the resonance at $\delta = 1.0$ ppm assigned to the CH_3 groups of the core aromatic ring ethyl substituents. At −70 °C this resonance splits into a major component at 1.3 ppm and a much smaller peak at 0.5 ppm. This latter upfield peak is indicative of a CH_3 group in the shielding region of one of the pyridinium rings and is consistent with some of the sample (but by no means all) being present in the '2-up, 1-down' conformation in which two pyridinium substituents occupy the opposite face of the hexasubstituted core to the other one.[34] The remainder of the spectrum becomes complex but is consistent with the presence of a number of different species or conformers and may well reflect a distribution of the sample into dimer and monomer, the latter existing as a '2-up, 1-down' conformer as well as a '3-up' isomer, as observed for related 3-aminopyridinium compounds,[34] Fig. 7. Re-recording the VT spectra in the presence of one equivalent of lactate resulted in modest qualitative changes to the chemical shifts of some resonances but a similar mixture of species was

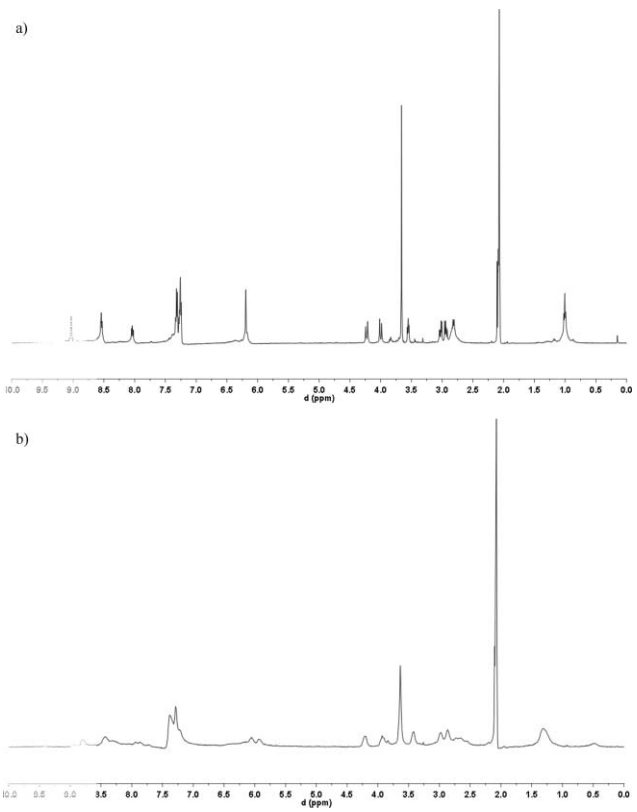


Fig. 7 Variable temperature ^{1}H NMR spectra of **3a** at (a) 20 °C and (b) −90 °C.

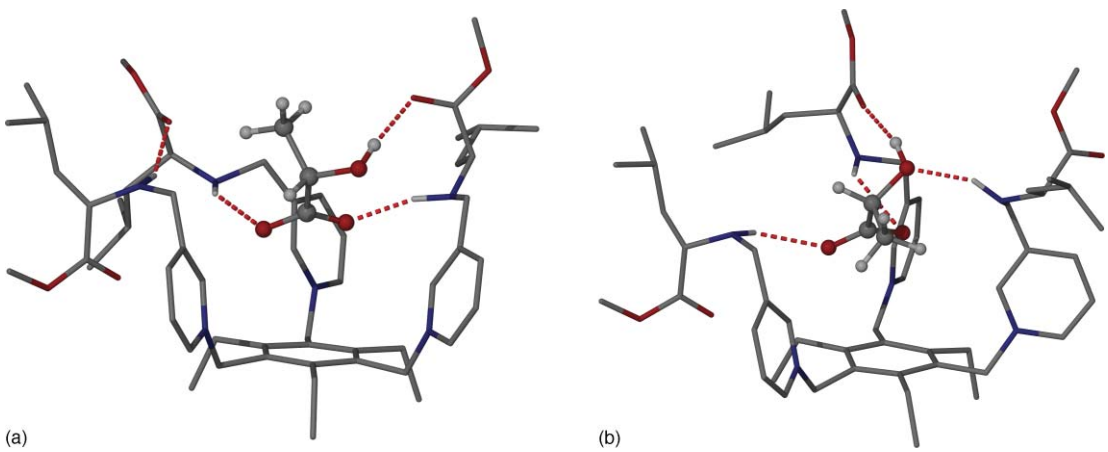


Fig. 6 DFT calculated structures of the 1 : 1 complexes of **3b** with (a) L-lactate and (b) D-lactate (lactate atoms shown as spheres, host CH hydrogen atoms omitted for clarity).

again observed at low temperature. Examination of the speciation plot for **3a** + L-lactate (see ESI‡) confirms that a mixture of all of the four possible species (1 : 0, 2 : 0, 1 : 1 and 1 : 2) is expected at this concentration.

Given the complex dimerisation and multiple anion binding behaviour of hosts **3**, we sought further insights into their conformational characteristics from the simplified model compounds **4**, which contain the 3-(aminomethyl)pyridinium binding group but without the chiral amino acid residues. The ^{1}H NMR titration isotherms for one of these compounds (**4b**) is shown in Fig. 8. Once again, the data exhibits significant sigmoidality, although the effect is far less pronounced than in compounds **3**. Also, interestingly, acetate appears to behave differently to the other anions with very significant chemical shift changes well beyond the addition of one equivalent of anion, while the plots for the other anions start to level out at around one equivalent, with a marked change in gradient in the case of Cl^- binding. The acetate data fitted well with the same model adopted for compounds **3**, with both a slightly enhanced acetate affinity and dimerisation constant, log $K_{dim} = 2.82$ (Table 1). Surprisingly, however, this model gave a very bad fit for all of the other anions. A simple 1 : 1 stoichiometry was also inappropriate given the sigmoidal nature of the isotherms and the fact that if dimerisation is present during the acetate titration, it must also be a competing process in all of the other experiments. We were, however, able to fit the data convincingly (see ESI‡) using a model involving the binding of a single anion by both monomer and dimer; *i.e.* our model incorporates four species of H–G stoichiometry 1 : 0, 2 : 0, 1 : 1 and 2 : 1. We rationalise this different stoichiometry by postulating that the host dimer for these less sterically hindered compounds can encapsulate halides and nitrate anions that do not have a pendant methyl substituent. Similar behaviour has been observed in related pyrene-derived compounds.[43] Such a postulate is consistent with the self-inclusion observed in the DFT model of **3b** (Fig. 5) which could disfavour the formation of such 2 : 1 complexes by that compound.

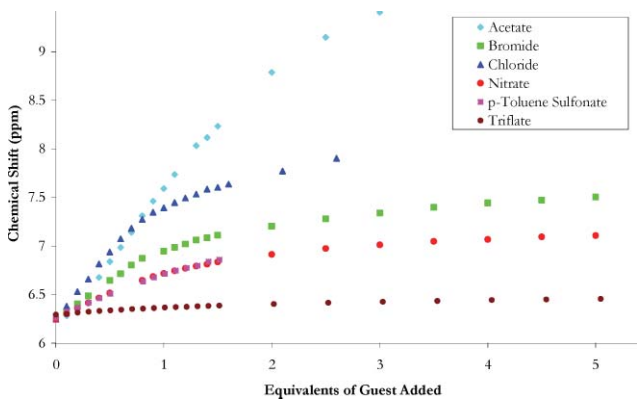


Fig. 8 Anion binding by **4b**.

Another surprising observation is the fact that the nitro compound **4b** actually binds anions more weakly than its phenyl analogue **4a**. This is in direct contrast to the usual effect of electron withdrawing substituents which tend to enhance amine/amide hydrogen bond donor ability and hence anion affinity.[15,39,50] In this case, the nitro substituents appear to enhance the dimerisation constant but it is **4a** that exhibits the highest chloride anion affinity. The origins of this effect are unclear and may be rooted in the complex nature of the equilibria and the fact that only limited data is available in the case of Cl^- due to precipitation at higher host–anion ratios.

Conclusion

In conclusion, we have designed a chiral tripodal anion host system displaying modest D-lactate selectivity amid the lactate enantiomers. The solution behaviour of this class of host system proved highly complex, however, with chloride binding being significantly favoured over all of the carboxylates studied. Moreover, anion binding is complicated by the formation of both 1 : 1 and 1 : 2 complexes, apparently in competition with a host dimerisation process as well as conformational equilibria. The combination of detailed NMR titration and variable temperature work coupled with DFT calculations and precedents from our previous work on related compounds allows some insight into the behaviour of the system, suggesting that the D-enantiomer is able to form more host–guest hydrogen bonding interactions. However, the solution speciation of these very flexible, sterically crowded systems does not allow for facile rationalisation of their behaviour. The apparent dimer formation in this case contrasts with the anion-induced 'zipping-up' of a unimolecular capsule that we have studied in a related compound.[36]

Experimental

All reagents were purchased from commercial sources and were used without further purification. 1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene was prepared as previously reported.[19] Tetrabutylammonium L-lactate was obtained from commercial sources. The D-enantiomer was not available. Racemic tetrabutylammonium lactate was prepared from the reaction of racemic lactic acid with one molar equivalent of tetrabutylammonium hydroxide in methanol in a Schlenk apparatus. After stirring for 2 hours, the methanol and the water were removed *via* a trap-to-trap vacuum distillation. ^{1}H, ^{13}C, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra were obtained from a Varian INOVA 500 spectrometer at a frequency of 500 MHz for ^{1}H and 125 MHz for ^{13}C or from a Bruker Avance 400 at a frequency of 400 MHz for ^{1}H and 100 MHz for ^{13}C. ^{1}H NMR spectroscopic titrations were performed on a Varian Mercury 400 BB spectrometer at a frequency of 400 MHz. Mass spectrometry data were obtained on a Thermo Finnigan LTQ spectrometer in ES+ and EI mode. C, H and N elemental analysis was performed on an Excitor Analytical Inc CE440 elemental analyser. Bromide analysis was performed on a DIONEX DX120 ion chromatograph. IR spectroscopy data was obtained from a Perkin Elmer Spectrum 100 FT-IR spectrometer. UV-Vis spectrophotometric titrations were performed on a UNICAM UV-Vis spectrometer (UV2-100) operated under PC-control using Vision software and the scan range was set to 220–450 nm.

Geometry optimizations were performed using the B3LYP functional in conjunction with the following basis: the 4-31G set on all carbon, nitrogen and hydrogen atoms, and the 6-31+G(d) set on all oxygen atoms. The basis was further augmented with two sets of diffuse sp functions on peripheral hydrogen-bonding hydrogen atoms. Minima were confirmed by analytical computation of the Hessian and noting positive definiteness. The time-dependent calculations were performed with an enlarged basis of 6-31G(d)

on all carbon, nitrogen and hydrogen atoms, plus the oxygen and peripheral hydrogen atoms as described above. Although the full conformational space was not sampled, preliminary computations were performed for each system to ensure the most sensible initial host–guest binding conformation. All computations were performed using the Gaussian03 program.[51]

Synthesis of methyl 3-phenyl-2-[(pyridin-3-ylmethyl)amino] propanoate (2a)

L-Phenylalanine methyl ester hydrochloride (10.0 g, 55.9 mmol) and one equivalent of triethylamine (5.65 g, 55.9 mmol) were dissolved in dry methanol (100 mL) and the mixture was stirred for 15 minutes. Two equivalents of 3-pyridinecarboxaldehyde (12.0 g, 111 mmol) were added along with magnesium sulfate. The mixture was heated to reflux for 24 hours under an inert atmosphere. The solution was then allowed to cool to room temperature at which point sodium borohydride (3.13 g, 82.7 mmol) was added slowly over a half hour period. The solution was then stirred for 2 hours under an inert atmosphere. The reaction was quenched to pH 3 with 1 M hydrochloric acid and then adjusted to pH 8 with 1 M sodium hydroxide. The product was extracted with dichloromethane and the residual solvent removed under reduced pressure. The product was purified by column chromatography using a 5% ethanol in dichloromethane solvent system resulting in the pure methyl 3-phenyl-2-[pyridin-3-ylmethyl]amino] propanoate (yield 4.56 g, 16.9 mmol, 30%). ^{1}H NMR (DMSO-d_6, δ/ppm, J/Hz): 8.55 (1H, s, Py C-*H*); 8.53 (1H, d, $J = 6.3$, Py C-*H*); 8.38 (1H, m, Ar C-*H*); 7.73 (1H, d, $J = 7.7$, Py C-*H*); 7.56 (1H, d, $J = 7.7$, Ar C-*H*); 7.39 (1H, t, $J = 3.9$, Py C-*H*); 7.20 (2H, m, Ar C-*H*); 3.6 (2H, dd, $J_d = 112$, $J_{dd} = 14$ CH_2NH); 3.38 (1H, t, $J = 7.0$, C*H*CO$_2$Me); 3.28 (3H, s, CO$_2$CH_3); 2.85 (2H, d, $J = 7.0$, CH_2Ph). ^{13}C{^{1}H} NMR (DMSO-d_6, δ/ppm): 174.7, 150.0, 149.7, 148.3, 139.0, 136.0, 134.7, 129.7, 128.3, 127.0, 124.0, 62.3, 53.7, 48.3, 39.6. EI-MS: m/z = 293 [M + Na]$^+$, 271 [M + H]$^+$. IR (ν/cm^{-1}): 3309, 2970, 1734, 1579, 1496, 1427, 1201, 1172, 1087, 1047, 1028, 746, 700, 634.

Synthesis of methyl 4-methyl-2-[(pyridin-3-ylmethyl)amino] pentanoate (2b)

L-Leucine methyl ester hydrochloride (10.0 g, 69.0 mmol) and one equivalent of triethylamine (6.97 g, 69.0 mmol) were dissolved in dry methanol (100 mL) and the mixture was stirred for 15 minutes. Two equivalents of 3-pyridinecarboxaldehyde (14.8 g, 138 mmol) were then added along with magnesium sulfate. The mixture was heated to reflux for 24 hours under an inert atmosphere. The solution was then allowed to cool to room temperature and two equivalents of sodium borohydride (5.24 g, 138 mmol) were added over a half hour period. The solution was stirred for 2 hours under an inert atmosphere. The reaction was quenched to pH 3 with 1 M hydrochloric acid and then adjusted to pH 8 with 1 M sodium hydroxide. The product was extracted with dichloromethane and the residual solvent removed under reduced pressure. The product was purified by column chromatography using a 15% ethanol in dichloromethane solvent system resulting in the pure methyl 4-methyl-2-[(pyridin-3-ylmethyl)amino] pentanoate (yield 6.37 g, 27.0 mmol, 39%). ^{1}H NMR ($CDCl_3$, δ/ppm, J/Hz): 8.52 (1H, s, Py C-*H*); 8.47 (1H, d, $J = 4.9$, Py C-*H*); 7.66 (1H, d, $J = 7.7$, Py C-*H*); 7.22 (1H, t, $J = 4.9$, Py C-*H*); 3.80 (2H, dd, $J_d = 161$, $J_{dd} = 13.3$, CH_2NH); 3.71 (3H, s, CO$_2$CH_3); 3.24 (1H, t, $J = 7.7$, C*H*CO$_2$CH$_3$); 1.75 (1H, m, CH$_2$C*H*(CH$_3$)$_2$); 1.44 (2H, t, $J = 7.7$, CH_2CH(CH$_3$)$_2$); 0.88 (3H, d, $J = 7.0$, CH$_2$CHCH_3); 0.82 (3H, d, $J = 6.3$, CH$_2$CHCH_3). ^{13}C{^{1}H} NMR ($CDCl_3$, δ/ppm): 176.2, 149.7, 148.5, 135.8, 135.0, 123.4, 59.4, 51.8, 49.5, 42.7, 25.0, 22.6, 22.0. EI-MS: m/z = 259.2 [M + Na]$^+$, 237.2 [M + H]$^+$. IR (ν/cm^{-1}): 2954, 1732, 1579, 1468, 1426, 1368, 1269, 1196, 1149, 1026, 990, 786, 713, 631.

Synthesis of phenylalanine-derived tripod (3a)

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (0.499 g, 2.12 mmol) and phenylalanine derivative **2a** (2.00 g, 7.41 mmol) were dissolved in dichloromethane (50 mL) and stirred at room temperature under a nitrogen atmosphere for 20 hours. The solvent was removed under reduced pressure to leave an orange oil which was washed with diethyl ether yielding the bromide salt as an orange solid (yield 1.18 g, 0.94 mmol, 44%). ^{1}H NMR (DMSO-d_6, δ/ppm, J/Hz): 9.08 (1H, s, py C-*H*); 8.99 (1H, d, $J =$ 6.0, py C-*H*); 8.45 (1H d, $J = 8.0$, py C-*H*); 8.08, 1H, t, $J = 6.8$, py C-*H*); 7.40 (5H, m, Ar CH); 6.03 (2H, 2,CH_2py); 4.13, 3.94 (2H, AB, $J = 15.6$, CH_2NH); 3.62 (2H, s, CO$_2$CH_3); 2.96 (2H, d, $J = 6.6$, PhCH_2CH$_3$); 2.65 (2H, d, $J = 6.8$, CHCH_2Ph); 0.88 (3H, t, $J = 6.6$, PhCH$_2$CH_3). ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz, δ/ppm): EI-MS: m/z = 546.4 [M – Br]$^{2+}$, 337.6 [M – 3Br]$^{3+}$. The bromide salt (1.18 g, 0.94 mmol) was dissolved in methanol and excess ammonium hexafluorophosphate (1.53 g, 9.4 mmol) was added and the mixture was stirred for 2 hours. The solution was filtered leaving the isolated PF_6 salt of the phenylalanine tripod (0.63 g, 0.44 mmol, 46% yield). Mp—decomposes 130 °C. ^{1}H NMR (CD_3CN, δ/ppm, J/Hz): 8.51 (1H, s, Py C-*H*); 8.32 (1H, d, $J = 8.0$, Py C-*H*); 8.23 (1H, d, $J = 5.6$, Py C-*H*); 7.87 (1H, t, $J = 6.4$, Py C-*H*); 7.20 (5H, m, Ar C-*H*); 5.74 (2H, s, CH_2Py); 4.13, 3.67 (2H, AB, $J = 16.0$, CH_2NH); 3.66 (3H, s, CO$_2$CH_3); 3.50 (1H, t, $J = 6.0$, C*H*CO$_2$CH$_3$); 2.95 (2H, m, PhCH_2CH$_3$); 2.52 (2H, d, $J = 7.2$, CHCH_2Ph); 0.91 (3H, t, $J = 7.2$, PhCH$_2$CH_3). ^{13}C{^{1}H} NMR (DMSO-d_6, δ/ppm): EI-MS: m/z = 337.6 [M – 3PF$_6$]$^{3+}$. IR (ν/cm^{-1}): 1732, 1497, 1455, 1202, 1141, 1034, 830. Anal. Calcd. for $C_{63}H_{75}F_{18}N_6O_6P_3 \cdot 2H_2O$: C 51.02, H 5.37, N 5.67. Found: C 51.12, H 4.92, N 5.67%.

Synthesis of leucine-derived tripod (3b)

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (2.40 g, 5.45mmol) and 3.5 equivalents of leucine ligand **2b** (4.50 g, 19.1 mmol) were dissolved in dichloromethane and the solution was left to stir for 20 hours under an inert atmosphere. The solvent was then removed under reduced pressure to leave the bromide salt as an orange solid. ^{1}H NMR ($CDCl_3$, δ/ppm, J/Hz): 9.77 (1H, s, Ph C-*H*); 9.46 (1H, d, $J = 5.6$, Py C-*H*); 8.35 (1H, d, $J = 8.4$, Py C-*H*); 8.12 (1H, t, $J = 7.2$, Py C-*H*), 6.19 (2H, s, CH_2Py); 4.13, 4.09 (2H, AB, $J = 15.2$, CH_2NH); 3.67 (3H, s, CO$_2$CH_3); 3.29 (1H, t, $J = 7.2$, C*H*CO$_2$CH$_3$); 2.53 (2H, q, $J = 7.0$, ArCH_2CH$_3$); 1.71 (1H, m, CH$_2$C*H*(CH$_3$)$_2$); 1.47 (2H, t, $J = 7.2$, CH_2CH(CH$_3$)$_2$), 1.01 (3H, t, $J = 7.0$, ArCH$_2$CH_3); 0.85 (6H, m, CH$_2$CH(CH_3)$_2$). ^{13}C{^{1}H} NMR ($CDCl_3$, δ/ppm): 175.5, 150.9, 144.4, 143.8, 143.1, 142.2, 129.1, 127.5, 59.8, 53.4, 51.7, 48.1, 42.4, 24.8, 22.8, 22.2, 15.9. EI-MS: m/z = 528 [M – 2Br]$^{2+}$, 304 [M – 3Br]$^{3+}$. The bromide

salt (7.40 g, 6.43 mmol) was dissolved in acetonitrile (50 mL) and an excess of ammonium hexafluorophosphate (10.5 g, 64.3 mmol) was then added and the mixture was heated to reflux overnight. The solvent was then removed under reduced pressure and the product redissolved in dichloromethane. The dichloromethane was removed under reduced pressure to leave the pure leucine tripod (2.63 g, 1.95 mmol, 36% yield). Mp—decomposes 95 °C. ^{1}H NMR (CD_3CN, δ/ppm, J/Hz): 8.67 (1H, s, Py C-*H*); 8.51 (1H, d, $J = 8.0$, Py C-*H*); 8.28 (1H, d, $J = 6.0$, Py C-*H*); 7.96 (1H, t, $J = 6.4$, Py C-*H*); 5.85 (2H, s, CH_2Py); 4.14, 3.66 (2H, AB, $J = 15.6$, CH_2NH); 3.67 (3H, s, CO_2CH_3); 3.29 (1H, t, $J = 7.6$, $CHCO_2CH_3$); 2.56 (2H, q, $J = 7.6$, $ArCH_2CH_3$); 1.77 (1H, m, $CH_2CH(CH_3)_2$); 1.47 (2H, t, $J = 7.2$, $CH_2CH(CH_3)_2$); 0.90 (6H, d, $J = 6.8$, $CH_2CH(CH_3)_2$); 0.86 (3H, t, $J = 2.8$, $ArCH_2CH_3$). ^{13}C NMR (DMSO-d_6, 100 MHz, δ/ppm): 175.1, 149.9, 145.2, 143.1, 142.3, 141.7, 128.2, 127.8, 58.7, 57.3, 51.5, 47.2, 41.7, 24.3, 23.5, 22.6, 22.0, 14.8. EI-MS: $m/z = 1345$ $[M]^+$, 528 $[M - 2PF_6]^{2+}$, 304 $[M - 3PF_6]^{3+}$. IR (ν/cm^{-1}): 2960, 1728, 1456, 1260, 1198, 1017, 828. Anal. Calcd. for $C_{54}H_{81}F_{18}N_6O_6P_3 \cdot 3H_2O$: C 46.35, H 6.27, N 6.01. Found: C 46.45, H 5.90, N 6.03%.

Tris(*N*-((pyridin-3-yl)methyl)benzeneamine) hexafluorophosphate (4a)

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (0.62 g, 1.4 mmol) and *N*-((pyridine-3-yl)methyl)benzeneamine[39] (1.5 g, 8.2 mmol) were dissolved in ethanol and refluxed for 120 hours. After this time, the solvent was removed under reduced pressure to yield the bromide salt as a yellow oil. Without purification, the bromide salt (0.32 g, 0.32 mmol) was dissolved in methanol (100 mL), and excess NH_4PF_6 (0.53 g, 3.2 mmol) was added. The solution was stirred at room temperature for 6 hours. During this time, a yellow precipitate formed. The solid was collected by filtration, and washed with methanol, and was found to be the desired product (yield 0.30 g, 0.25 mmol, 79%). ^{1}H NMR (CD_3CN, δ/ppm, J/Hz): 8.52 (3H, d, J= 6.0, Py-H); 8.49 (3H, s, py-H); 8.40 (3H, d, $J = 6.0$, Py-H); 8.01 (3H, t, $J = 6.0$, Py-H); 7.13 (6H, t, J= 8.5, Ar-H); 6.73 (3H, t, $J = 8.5$, Ar-H); 6.55 (6H, d, $J = 8.5$, Ar-H); 5.76 (6H, s, CH_2); 5.27 (3H, s, NH); 4.60 (6H, s, CH_2); 2.45 (6H, q, $J = 7.5$, CH_2); 0.77 (9H, t, $J = 7.5$, CH_3). ^{13}C{^{1}H}-NMR (CD_3CN, δ/ppm): 150.8; 147.2; 145.3; 143.3; 142.2; 129.6; 128.6; 128.0; 118.2; 117.6; 113.0; 58.1; 44.1; 24.1; 14.4. ES+ MS: 1043 $[M - PF_6]^+$, 449 $[M - 2PF_6]^{2+}$, 251 $[M - 3PF_6]^{3+}$. Anal: calcd. for $C_{51}H_{57}N_6(PF_6)_3$: C 51.52, H 4.83, N 7.07. Found: C 51.51, H 4.89, N 6.86%. IR: 3452 ν(NH).

Tris(4-nitro-*N*-((pyridin-3-yl)methyl)benzeneamine) hexafluorophosphate (4b)

1,3,5-Tris(bromomethyl)-2,4,6-triethylbenzene (0.64 g, 1.5 mmol) and 4-nitro-*N*-((pyridine-3-yl)methyl)benzeneamine[39] (1.0 g, 4.4 mmol) were dissolved in 1,2-dichloroethane and refluxed for 120 hours. After this time, the solvent was removed under reduced pressure to yield the bromide salt as a yellow oil. Without purification, the bromide salt (0.40 g, 0.29 mmol) was dissolved in methanol (100 mL), and excess NH_4PF_6 (2.3 g, 14.1 mmol) was added. The solution was stirred at room temperature for 6 hours. During this time, a yellow precipitate formed. The solid was collected by filtration, and washed with methanol, and was found to be the desired product (yield 0.33 g, 0.21 mmol, 65%). ^{1}H NMR (CD_3CN, δ/ppm, J/Hz): 8.57 (3H, s, Py-H); 8.51 (3H, d, $J = 8.4$, Py-H); 8.37 (3H, d, $J = 6.0$, Py-H); 8.00 (9H, m, Ar-H and Py-H); 6.65 (6H, m, Ar-H); 6.28 (3H, t, $J = 6.4$, NH); 5.80 (6H, s, CH_2); 4.71 (6H, d, $J = 6.4$, CH_2); 2.50 (6H, q, $J = 7.2$, CH_2); 0.80 (9H, t, $J = 7.2$, CH_3). ^{13}C{^{1}H}-NMR (CD_3CN, δ/ppm): 153.0; 151.0; 145.4; 142.4; 142.4; 141.6; 138.8; 128.8; 128.0; 126.2; 117.6; 112.1; 58.2; 43.6; 24.2; 14.5. ES+ MS: 1178 $[M - PF_6]^+$, 516 $[M - 2PF_6]^{2+}$, 296 $[M - 3PF_6]^{3+}$. Anal: calcd. for $C_{51}H_{54}N_6O_9(PF_6)_3$: C 46.26, H 4.08, N 9.52. Found: C 45.73, H 4.13, N 9.30%. IR: 3239 ν(NH).

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