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Anion recognition by cyclic peptides

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Anion binding selectivity can often be controlled by judicious arrangement of recognition moieties around an anion of interest. Indeed, nature takes advantage of large peptides/proteins to provide highly efficient and selective anion receptors using a small number of amino acid building blocks placed in a precise arrangement. Cyclic peptides are ideal synthetic scaffolds to position binding residues in a similarly preorganised manner as their synthetic versatility and rigidified structure allows precise control over their size and shape. This review summarises the recent use of such cyclic peptide scaffolds as receptors for various anionic species.

Introduction

The ubiquitous role of anionic species in biology, medicine, catalysis and the environment has led to the establishment of anion binding, sensing and transport as a burgeoning area of research particularly in the field of supramolecular chemistry.[1–5] Molecules with the ability to selectively bind to specific anionic targets have numerous potential applications in these areas.

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In natural systems, highly efficient and selective anion recognition is achieved through the construction of large peptides/proteins that take advantage of the numerous H-bonding interactions available from various amino acids (the OH groups of serine, threonine and tyrosine, the NH group in the indole moiety of tryptophan, and the guanidinium group of arginine) with additional contributions from NH groups along the protein backbone. In fact arginine, which provides electrostatic interactions in addition to hydrogen bond donors to interact with anions, is the most prevalent amino acid present in naturally occurring enzymes; testament to the fact that two-thirds of all known enzymes either act on anionic substrates or require anionic coenzymes.[6] Similarly, metal ions bound to proteins often provide electrostatic interactions for selective anion binding

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Kate Jolliffe received her BSc (Hons 1) in 1993 and PhD in 1997 from the University of New South Wales. She then held positions at Twente University, the Netherlands; the University of Nottingham, UK and the Australian National University before taking up an Australian Research Council QEII research fellowship at The University of Sydney in 2002. In 2007 she became a Senior lecturer at the same institution and was promoted to Associate Professor in 2008 and to full Professor in 2009. Her research interests encompass elements of synthetic organic chemistry, supramolecular chemistry and peptide chemistry.

that are crucial to their biological function (*e.g.* annexin binding to phospholipid membranes occurs through Ca^{2+} ions).[7]

Perhaps nature's most elegant solution to the issue of anion binding and transport is the sulphate binding protein (SBP) in which exquisite selectivity and affinity are achieved solely through the use of charge neutral hydrogen bond donation from the peptide. The SBP relies on a network of seven hydrogen bonds most of which are formed between the oxygens of the sulphate anion and NH groups of the protein backbone. Pflugrath and Quiocho demonstrated that sulfate is bound in a cavity formed by the intersection of two protein globular domains where it is inaccessible to solvents.[8] Interestingly, the binding pocket does not contain any positively charged guanidinium residues, cations or water molecules that interact with the buried sulfate and hence the SBP relies primarily on hydrogen bond formation. Alternatively, in the phosphate binding protein (PBP), two electrostatic interactions to a positively charged guanidinium group in addition to 9 hydrogen bonds between the oxygens of phosphate and hydrogen bond donor groups of the protein (five hydrogen bonds to backbone NH groups, four to serine or threonine OH groups) ensure that the anion is tightly bound in the protein centre.[9] An additional hydrogen bond between the proton of hydrogenphosphate and a carboxylate hydrogen bond acceptor is an important factor for crucial substrate selectivity. The precise arrangement of hydrogen bonds in the PBP–phosphate complex has been shown to be the most important factor for substrate binding affinity (and selectivity) and the contribution from this preorganisation is significantly more important than the electrostatic interaction between the anion and the guanidinium group.[10]

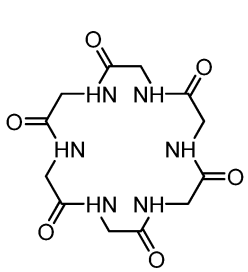
An area of intense focus within supramolecular chemistry in recent years has been the development of artificial anion receptors which mimic natural systems in their ability to bind selectively to a target anion. A large body of research has built up around the development of synthetic receptors that selectively bind their target anion but in general these structures differ markedly from those utilised by nature. Receptors based on macrocyclic and interlocked structures,[11,12] tripodal scaffolds,[13–15] and metal ions[16–19] have all been used with varying success. Although such synthetic receptors generally rely on the same type of interactions used by nature for anion binding, many use structurally unrelated recognition motifs based on amides, ureas, thioureas and, more recently, squaramides to provide hydrogen-bond donor sites.[20–26]

However, the elegant manner in which nature uses complementary H-bond interactions and preorganisation has inspired research into development of synthetic receptors for various guests based on amino acid containing structures that combine both natural and non-natural binding motifs.[27,28] While the deliberate use of peptide based building blocks potentially leads to biomimetic systems, the preorganisation afforded by large protein structures can also be mimicked by cyclic peptides which are of particular interest in this respect. Rigid structures that present multiple functional groups in the same direction are particularly advantageous in molecular recognition and further preorganisation achieved using backbone modifications or aromatic spacers within the peptide backbone provides for efficient and selective binding of guests based on their size and shape. Indeed, several groups have focussed their attention on the development of cyclic peptide based scaffolds as supramolecular receptors for anions and the following review will focus on the use of cyclic peptide based anion receptors and their development to date. The report is divided into two broad sections; the first describes those cyclic peptides that bind anions directly through interactions with the peptide backbone while the second focuses on the use of side chain functional groups and recognition moieties to enhance the binding affinity and selectivity of these receptors for anions.

Anion binding to peptide backbones

Simple cyclic peptides

In early research in this area, gas phase *ab initio* calculations were performed to evaluate a cyclohexapeptide comprised of six glycine molecules (**1**). This cyclohexaglycyl molecule was calculated to have strong affinities for both cations and anions in the gas phase, and was termed an amphi-ionophore.[29] The results implied that such structures should be able to bind either cations (Li^+, Na^+, Be^{2+}, Mg^{2+}) or anions (F^-, Cl^-) where the flexible receptor could orient itself such that the carbonyls point towards the centre of the macrocycle to allow for cation binding, or alternatively the NH groups are directed inwards for anion coordination. In a subsequent report, molecular dynamics and molecular mechanics calculations were used to investigate the similar cyclohexaalanyl (**2**), a cyclohexapeptide composed of six alanine molecules in the aqueous phase.[30] These calculations suggested that **2** was capable of binding either a cation or an anion in the presence of water molecules. In a similar manner to **1**, it was suggested that **2** would bind to cations through the carbonyl groups and to anions using the NH groups.



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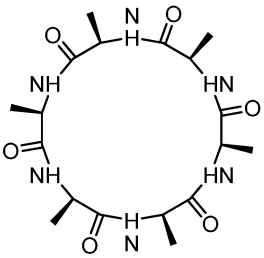

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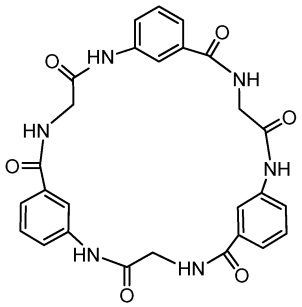
Praveena and Kolandaivel have reported a theoretical study on α–γ hybrid cyclic hexapeptide **3**, which is more rigid than **1** and **2** as a result of the incorporation of aromatic residues (3-aminobenzoic acid; Aba) into the macrocyclic peptide backbone.[31] Although no experimental results were reported, density functional theory (DFT) calculations suggested that F^-, Cl^-, and Br^- were capable of binding inside the cavity of this cyclic peptide. Both NH and CH bonds were found to take part in anion binding with the NH groups pointing towards the internal cavity to facilitate anion binding and resulting in significant distortion

of the cyclic structure. Overall binding energies were predicted to be in the order $F^- > Cl^- > Br^-$.

In experimental studies, Ishida and co-workers have prepared a number of cyclic peptides containing the same unnatural amino acid (Aba),[32] which was employed to reduce receptor flexibility and induce a macrocycle conformation amenable to anion binding.[33–35] UV-vis titration experiments in DMSO indicated that the disodium salt of 4-nitrophenyl phosphate forms 1 : 1 complexes with the receptors [$K_a = 1.2 \times 10^6$ M^{-1} for cyclo-(Ala-Aba)$_3$ (**4**)]. Additional evidence for the binding mode was obtained by ^{1}H NMR spectroscopy, which indicates that **4** has C_3-symmetry that is maintained on binding the phosphoester. On addition of 4-nitrophenyl phosphate, the signals attributable to the amide protons undergo large downfield shifts, consistent with the formation of hydrogen bonds. Changing the side chains of the α-amino acid did not significantly affect the binding affinity. However, increasing the size of the macrocycle to the analogous octapeptide resulted in a decrease in binding affinity of two orders of magnitude.[33]



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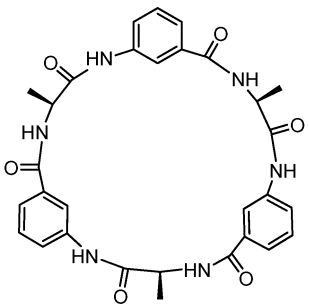

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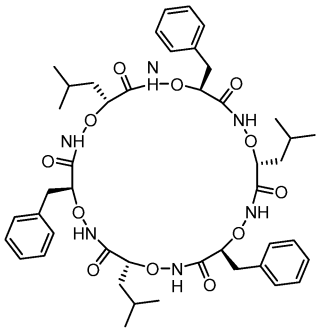
Yang *et al.* have described a cyclic hexapseudopeptide, **5** composed of alternating D,L-α-aminoxy acids that adopts a well defined C_3 symmetric 'bracelet-like' conformation in $CDCl_3$.[36] HF/6-31G* optimized lowest-energy conformational analysis of **5** determined that its secondary structure is stabilised by intramolecular C═O–H–N hydrogen bonds and results in the α-protons of the aminoxy acid residues pointing towards the inside of the macrocycle. Furthermore, **5** was shown to selectively bind Cl^- over other halide anions in $CDCl_3$ where the association constants for the chloride and the fluoride complexes were determined to be 11 880 M^{-1} and 30 M^{-1}, respectively. The selectivity of **5** for Cl^- was suggested to be determined by the better size complementarity between the anion and the cavity of the macrocycle as opposed to its hydrogen-bonding strength. In a subsequent report cyclic hexapeptide **6**, comprising alternating D-α-amino and D-α-aminoxy acids, was shown to function as a more effective anion receptor than **5**.[37] Macrocycle **6**, which has fewer aminoxy amide NH units than compound **5**, together with a smaller ring size, displayed enhanced binding toward anions, with association constants for the chloride, bromide and iodide complexes of 15 000 M^{-1}, 910 M^{-1} and 51 M^{-1}, respectively whilst maintaining good selectivity towards Cl^-. Moreover, **6** was capable of extracting anions from aqueous solution into organic phases where ^{1}H NMR spectroscopic analysis revealed that Cl^- was extracted with 77% efficiency while NO_3^- ions were extracted with just 35% efficiency despite being more lipophilic than Cl^- ions.



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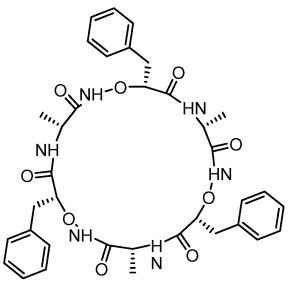

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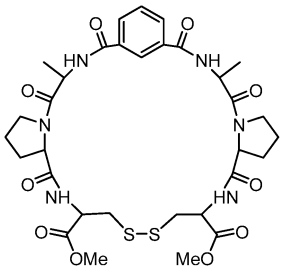
Cystine bridged structures

Cheng and co-workers have developed a number of conformationally constrained cystine based cyclic peptide anion receptors such as **7**, an amphi-receptor that binds to both cations and anions.[38] However, anion affinity of **7** was moderate in comparison to metal binding with the highest anion binding affinity being attributed to F^- with $K = 4.18 \times 10^2$ M^{-1} in CD_3CN.



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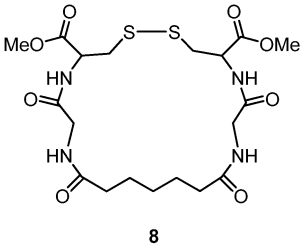
The similar polymethylene-bridged cystine–glycine-containing pseudo-cyclopeptides **8** and **9** have been developed by the same group.[39] ^{1}H NMR binding studies with halide ions showed that although the smaller macrocycle **8** showed moderate affinity for F^-, Cl^- and Br^-, ($K = 4.44 \times 10^2$ M^{-1}, 9.91×10^2 M^{-1} and 1.91×10^2 M^{-1}) macrocycle **9** failed to bind any halide ions in $CDCl_3$, presumably as a result of its high flexibility.



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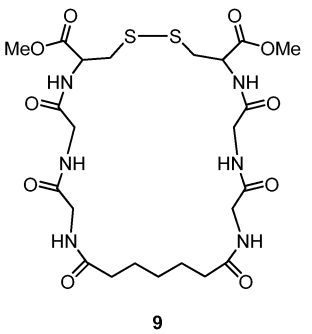

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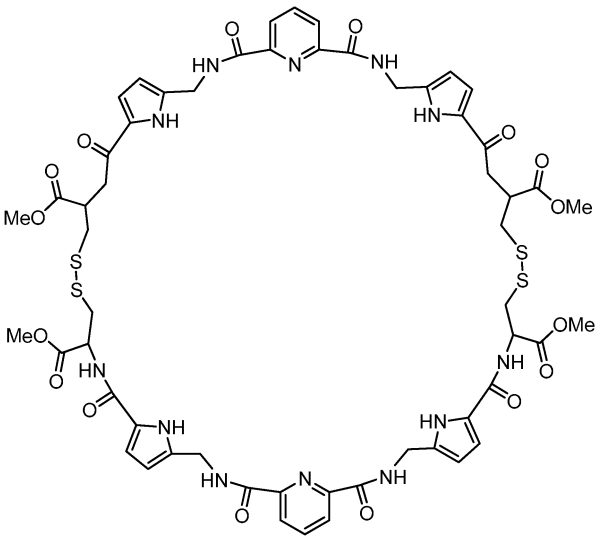
In a subsequent report two additional conformationally constrained pseudo-cyclopeptides **10** and **11** consisting of pyrrole-, pyridine-, and cystine-moieties were also shown to act as neutral receptors for anions.[40] This UV-vis study revealed

that the [2+2] receptor (**10**) gave 1 : 2 complexes with F^- and AcO^- ions whereas the [1+1] receptor (**11**) formed 1 : 1 complexes. In addition, it was concluded that the additional pyrrole moieties in the macrocyclic backbone not only further constrained the receptor's conformational freedom, but also enabled more effective anion binding through the pyrrole–NH hydrogen bonds as evidenced from preliminary ^{1}H NMR measurements. This resulted in F^- and AcO^- affinities of K = 1.43×10^7 M^{-1}, and 6.87×10^6 M^{-1} respectively for **11** and F^- and AcO^- affinities of $K = 7.84 \times 10^9$ M^{-2}, and 1.06×10^{10} M^{-2} respectively for **10** in CH_3CN; considerably higher than those reported for analogue **7** which lacks the additional pyrrole H-bond donors.

Two L-cystine-based cyclic oligourea receptors **13** and **14** were reported by the same authors where the multiple urea moieties were incorporated as part of the cyclic framework.[42] The anion binding properties of these receptors, synthesised by a one-step condensation of L-cystine dimethyl ester with triphosgene were investigated using NMR spectroscopy and mass spectrometry. The 27-membered [3+3] macrocycle **13** was found to bind chloride and bromide *via* hydrogen bond formation to



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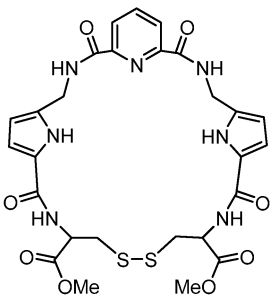

11

Ranganathan *et al.* found that the aromatic-bridged cystine-containing pseudo-cyclopeptide **12**, a member of the cystinophane family, was an effective receptor for l,ω-alkane dicarboxylates.[41] A combination of disulfide bridges and rigid aromatic units introduced conformational constraints into the cyclopeptide backbone. The authors proposed a bis-bidentate or tetrahydrogen bonded structure (Fig. 1) for the glutarate complex which was supported by a maximum NH shift at a mole ratio of 1 : 1 (receptor : anion) when examined by ^{1}H NMR titrations in $CDCl_3$. The 26-membered macrocycle, with all four amide NHs pointing into the center of the cavity exhibited effective binding to 1,ω-alkane dicarboxylic acids with maximum affinity (K = 3.69×10^2 M^{-1}) and selectivity for glutaric acid.

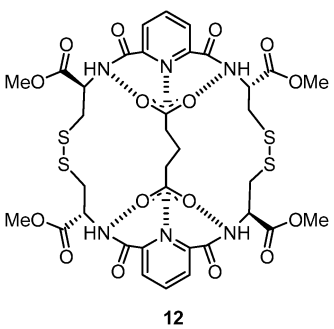

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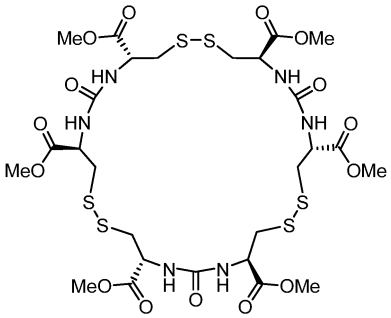
Fig. 1 The proposed tetrahydrogen-bonded structure for the molecular recognition of **12** and glutaric acid.

the urea NH groups but did not bind iodide, which was deemed too large to fit inside the macrocyclic cavity. The trigonal planar nitrate anion, which has matching symmetry and a complementary fit with **13** showed moderate binding in $CDCl_3$ (K = 5.2×10^2 M^{-1}). The larger 36-membered [4+4] tetraurea **14** exhibited binding to the larger squarate anion with an association constant of 3.21×10^2 M^{-1}.

Backbone modified cyclic peptides

Kubik and co-workers have prepared a number of Aba containing cyclic peptides and analogues thereof in which the Aba residue is replaced by Apa (Apa; 3-aminopicolinic acid). Their initial experiments indicated that cyclo[Glu(iPr)-Aba]$_3$ **15** forms host:guest complexes with cations, *via* cation–π interactions, when mixed with *n*-butyltrimethylammonium iodide ($BTMA^+I^-$). The stoichiometry of the complex was confirmed as 1 : 1 by Job's plot analysis and a moderate binding affinity [K_a ($BTMA^+$) = 300 M^{-1} in $CDCl_3$] was determined by ^{1}H NMR using the upfield shift of the guest protons, which are shielded by the aromatic Aba residues. Interestingly, when the iodide was replaced by tosylate, a different complex was observed in which both the anion and cation are bound simultaneously [K_a (TsO^-) *ca.* 10^9 M^{-1};



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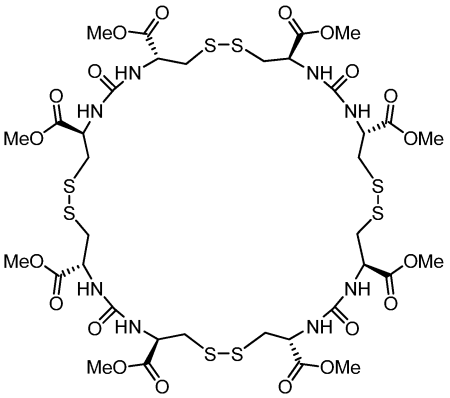

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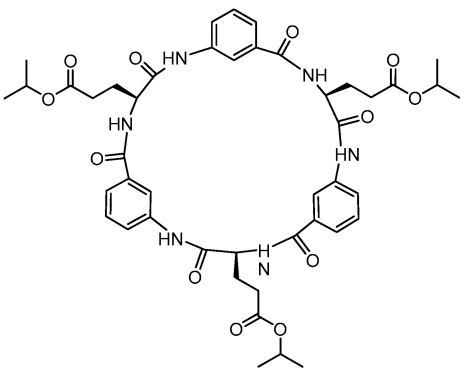
K_a ($BTMA^+$) 3.88×10^6 M^{-1}]. The increased binding affinity for $BTMA^+$ compared to that observed in the absence of a binding anion is attributed to preorganisation of the cyclic peptide where anion binding stabilises a conformation suited for cation binding and also allows the cation to interact with the simultaneously bound anion in a ternary complex. Molecular modeling showed that the optimal conformation for anion complexation is one in which all of the NH groups converge towards the center of the peptide ring.[43]



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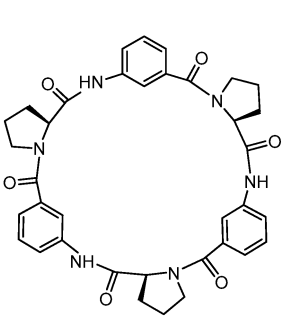
Replacement of the Glu(iPr) residues in **15** with proline results in further rigidification of the cyclic peptide **16** and increased binding constants for both anions and cations.[44] Substitution of the 4-positions of the Aba residues, such as in receptor **17** prevents a conformation in which the NH groups point toward the center of the macrocyclic cavity and brings about a total loss of anion affinity. However, the increased conformational control brings about preorganisation for cation binding and leads to increased cation affinity.[45,46]

Conversely, replacing the Aba residues in receptor **16** with 6-aminopicolinic acid (Apa) residues yields cyclic hexapeptides such as **18** in which the ring nitrogens of the aromatic subunits induce a converging arrangement of the NH binding sites, resulting in an increase in anion affinity.[47] ^{1}H NMR experiments indicated that while the affinity of **16** for iodide in DMSO is extremely low, the association constant of the iodide complex of **18** amounts to 150 M^{-1} in the same solvent.[27] Interestingly, **18** was shown to associate with anions in highly competitive



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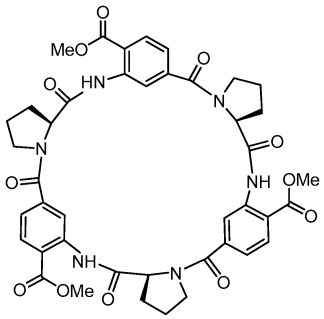

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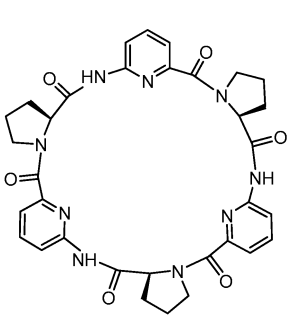
protic solvent mixtures such as 80% D_2O–CD_3OD where 2 : 1 sandwich-type complexes with Cl^-, Br^-, I^- and SO_4^{2-} were formed as evidenced by Job's plot, mass spectrometric and X-ray crystal structure analysis (Fig. 2).[47] As a result of the particular binding mechanism of **18**, the anion loses its solvent shell during formation of the complex and is shielded from the surrounding solvent molecules resulting in the completely desolvated anion being bound by six hydrogen bonds in a cavity between two peptide rings. Subtle changes to the cyclic peptide structure, such as replacement of the Pro residues with 4*R*-Hyp (Hyp; hydroxyproline) results in altered binding behavior where the hydroxyl groups present on receptor **19** prevent the formation of 2 : 1 complexes. In contrast to **18**, **19a** and **19b** form 1 : 1 complexes with anions with moderate binding affinities in the range of 1 to 100 M^{-1} in 80% D_2O–CD_3OD.[48]



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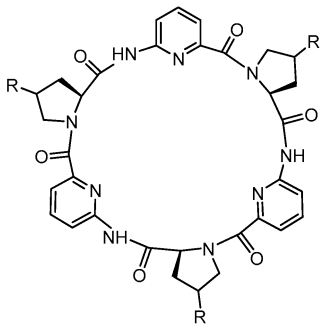

19a: R = (*R*)-OH
19b: R = (*S*)-OH

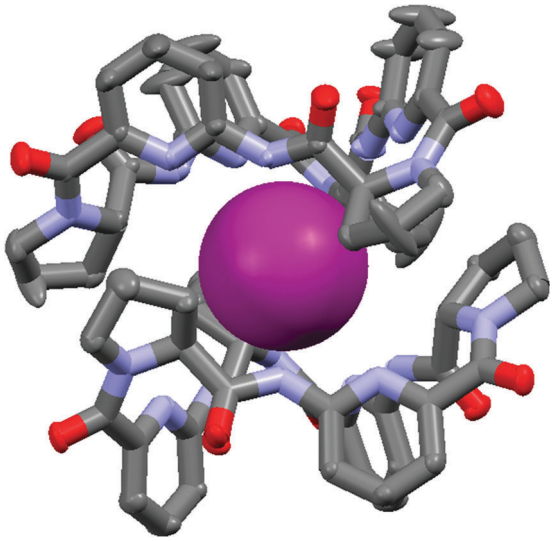

Fig. 2 X-ray crystal structure of the 2 : 1 'sandwich complex' formed between **18** and iodide. Figure generated from X-ray diffraction data originally published in ref. 47.

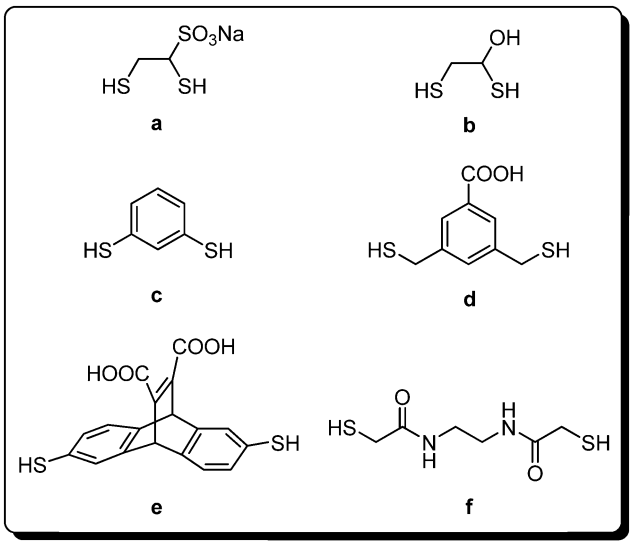

Fig. 4 The six dithiols of varying length and rigidity used to optimise the linking unit between two Apa-based cyclic peptides.

In a subsequent report receptor **20**, in which two cyclopeptide units are covalently linked with an adipinic acid spacer, stabilises the sandwich-type complexes with halides, sulfate and nitrate, which now exhibit 1 : 1 binding stoichiometry (Fig. 3).[49] Stability constants of these complexes, determined by ^{1}H NMR titrations and isothermal titration microcalorimetry, are in the range of 10^5–10^2 M^{-1} in 50% water–methanol and decrease in the order $SO_4^{2-} > I^- > Br^- > Cl^- > NO_3^-$. This order was rationalised in terms of anion size, with larger anions having a better fit with the host cavity. The higher stability of the sulfate complex is attributed to the ability of this oxo-anion to form stronger hydrogen bonds with the NH groups of the receptor. Comparison of the binding constants determined for **18** and **20** showed that anion binding to the bis(cyclopeptide) is considerably more efficient than that of its monotopic relative.[50]

In a more recent study by Otto and Kubik, Dynamic Combinatorial Libraries (DCLs) have been used to optimise the linking unit between two Apa-based cyclic peptides to yield superior synthetic hosts that can bind sulfate and iodide in aqueous solution.[51] The strategy involved variation of the linker using reversible disulfide chemistry in an equilibration controlled mixture of bis(cyclopeptide) disulfide **21** with six dithiols (a–f) of varying structural length and rigidity (Fig. 4). Templation with K_2SO_4 and KI resulted in an amplification of two bis(cyclopeptides) **22** and **23** which exhibited association constants of 5.4×10^6 M^{-1} and 6.8×10^6 M^{-1} respectively, with sulfate and 2.9×10^4 M^{-1} and 5.6×10^4 M^{-1} respectively, with iodide in 2 : 1 (v/v) CH_3CN/H_2O. An X-ray crystal structure of the sulfate complex of **23** revealed that the anion is bound between the peptide rings of the biscyclopeptide in a manner similar to the 2 : 1 sandwich complex between **18** and iodide (Fig. 5). Subsequent studies on **22** and **23** examined the effect of solvent composition on anion binding affinity, where it was observed that increasing the water content of a solvent mixture serves to favour the formation of the 2 : 1 macrocycle : anion complex in more competitive media. Through both X-ray crystal structure and microcalorimetric analysis it was shown that intramolecular contacts between the nonpolar surfaces of the individual cyclic peptide scaffolds bring about hydrophobic interactions within the receptor that do not directly involve the guest but contribute to the overall stability of the complex.[52] This was confirmed by the observation that 2 : 1 complex formation is not favorable in DMSO where no hydrophobic interactions

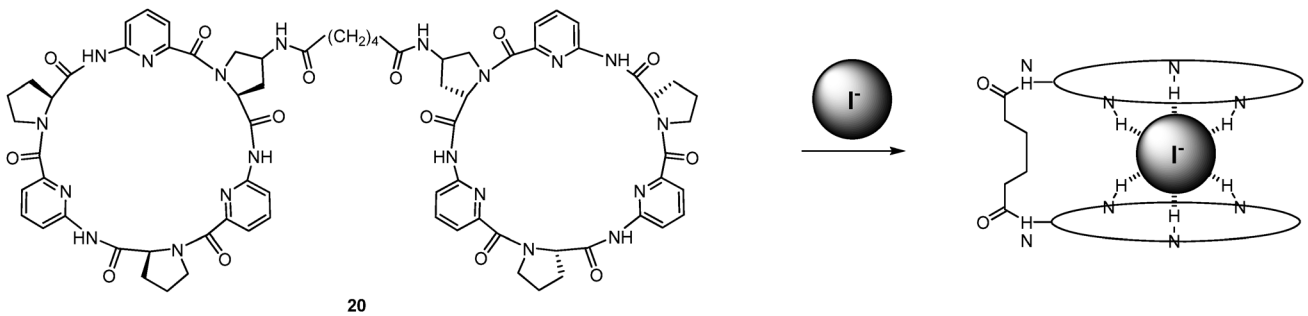

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Fig. 3 Complexation of iodide by a molecular 'oyster'.

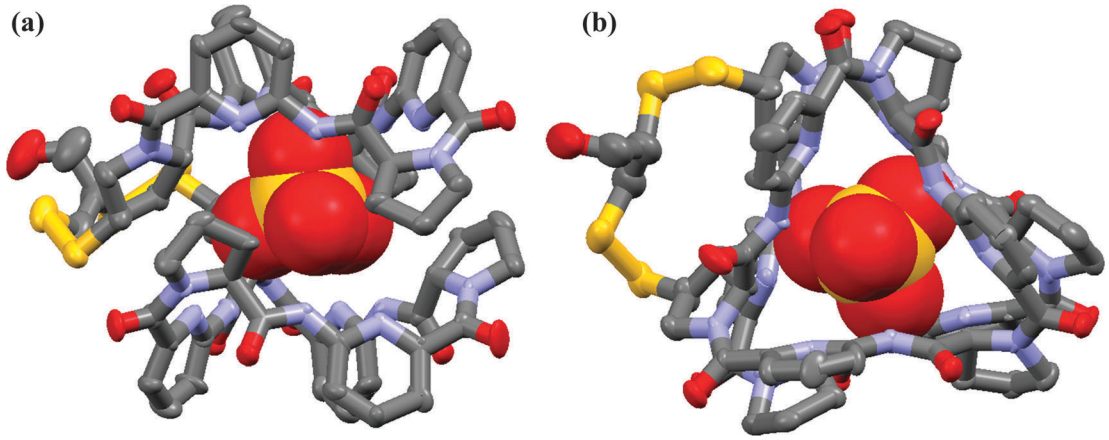
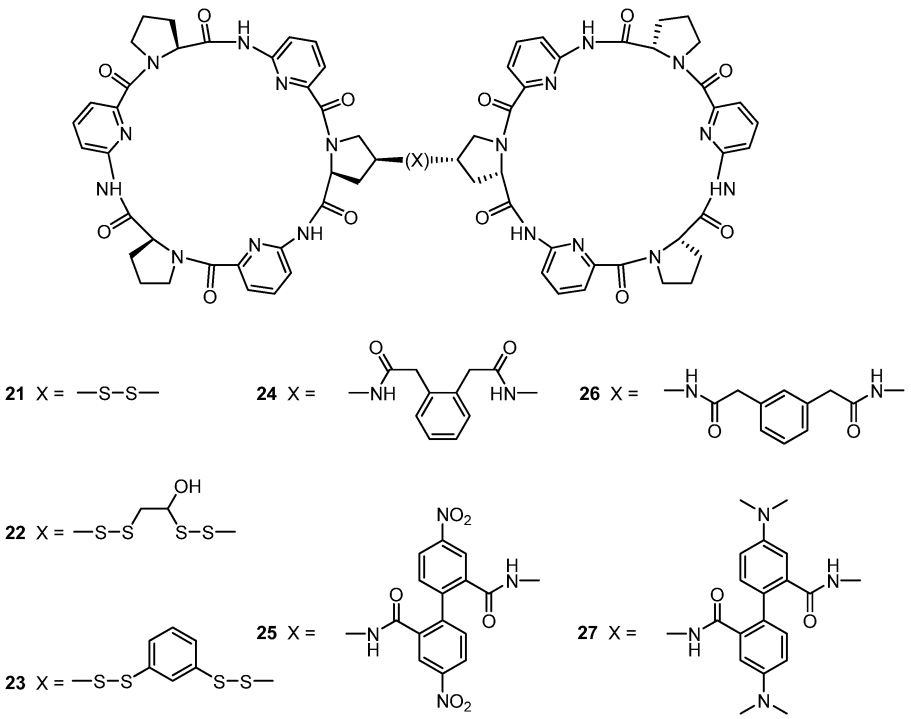


Fig. 5 X-ray crystal structure of the 1 : 1 'sandwich complex' formed between **23** and sulfate (a) viewed from the side and (b) viewed from above. Figure generated from X-ray diffraction data originally published in ref. 52.

take place and the fact that receptor **19**, which displays hydrophilic hydroxy groups around its periphery, does not form 2 : 1 complexes even in pure water.

A slight structural modification of **25** led to fluorescent bis(cyclopeptide) **27**.[54] This highly selective fluorescent sensor is capable of optical detection of sulfate in water–methanol mixtures

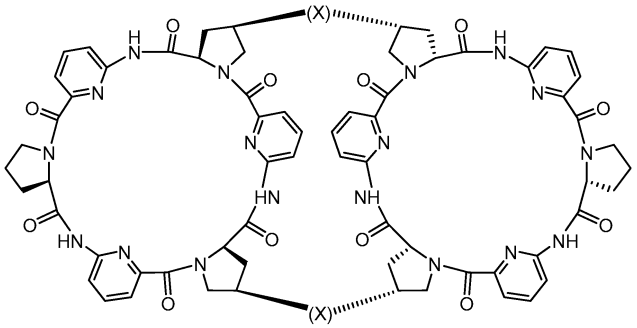


In a more recent study, a systematic evaluation of the influence of the linking unit between two cyclopeptide rings on their anion affinity in aqueous solvent mixtures was undertaken.[53] A series of receptors, **24–26** in which the linkers become progressively more rigid were synthesised. Although, large differences in the anion affinity and selectivity of **24–26** were not observed, the thermodynamics of anion complexation were shown to be profoundly different as measured by isothermal calorimetric analysis. Enthalpic and entropic contributions determined the similar overall binding affinities of **24–26** towards anions but simultaneously showed significant variation as the linking structure was changed.

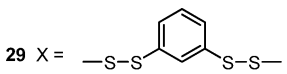
(1 : 1; v/v) in the presence of excess chloride. Incorporating a 4,4′-bis(dimethylamino)-substituted biphenyl linker, the fluorescence of **27** is selectively quenched in the presence of sulfate whereas other anions such as iodide, bromide, chloride, nitrate, perrhenate, perchlorate and hydrogenphosphate do not show any significant quenching. Interestingly, the sulfate affinities measured by isothermal calorimetry showed that **27** ($\log K = 3.87$ in 50% H_2O–CH_3OH) was a significantly weaker receptor than **25** ($\log K = 5.32$ in 50% H_2O–CH_3OH). This behaviour was attributed to the different electronic effects of the substituents in the linkers of **25** and **27** where **27** requires the linker

to adopt an energetically unfavourable conformation upon sulfate complexation.

Recently the dynamic combinatorial approach has also been extended to macrobicyclic receptors where two cyclopeptide rings are connected *via* two linkers. **28** and **29** were expected to exhibit enhanced anion complex formation by increasing receptor pre-organisation.[55] ITC studies indicated that the nature of the linkers between the cyclopeptide rings has a pronounced effect on anion binding where **28**, with flexible aliphatic linkers, and **29** with rigid aromatic linkers were shown to differ in sulfate binding affinity by over an order of magnitude. This large difference in anion affinities between **28** ($\log K$ = 8.67 in 33% H_2O–CH_3CN (v/v)) and **29** ($\log K$ = 7.59 in 33% H_2O–CH_3CN (v/v)) was rationalised in terms of the affinity being dependant on a delicate balance between preorganisation and flexibility. With nanomolar affinity, **28** maintains the highest known affinity for sulfate by neutral receptors in aqueous solution to date.



28 X = —S–S S–S—

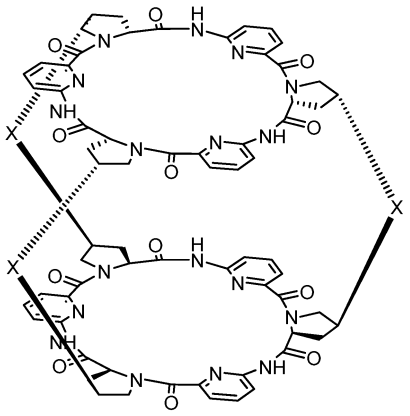


29 X = —S–S S–S—

30 and **31** represent an alternative approach used by Kubik *et al*. where the triply- and mono- linked bis-cyclopeptides are formed by covalently attaching alkyne and azide derivatives of **18** through copper-catalyzed azide–alkyne cycloaddition.[56] Interestingly, the sulfate affinity of **30** ($\log K$ = 5.70 in 50% H_2O–CH_3OH (v/v)) is not significantly higher than that of **31** ($\log K$ = 4.96 in 50% H_2O–CH_3OH (v/v)) despite the increased preorganisation that having three linkers affords. ITC data revealed that such behaviour is likely to be due to the switch in the binding enthalpy from exothermic to endothermic upon increasing the number of linkers. Similarly, ^{1}H NMR spectroscopy and X-ray crystallography both suggest that **30** and **31** adopt similar sulphate:receptor conformations where the overall sulfate affinity of both bis-cyclopeptides is in the same range but with differing contributions from entropic and enthalpic terms.

In a further development, attempts to synthesise **32** using regioselective triazole formation resulted in the cyclodimerisation of an α,ω-difunctionalised precursor *via* thermal azide–alkyne 1,3-dipolar cycloaddition yielding a cyclic pseudotetrapeptide **33**.[57] Introduction of the triazole ring into the peptide structure retained the overall conformation of the macrocycle in which the NH groups, important for substrate binding, remain directed to the inside of the macrocyclic cavity.

The synthesis of the C_3 symmetric cyclic pseudohexapeptide **32** was achieved using ruthenium(II)-catalyzed azide-alkyne-cycloadditions to give the desired 1,5-disubstitued 1,2,3-triazole rings. Detailed conformational studies and anion binding analysis were also reported.[58] Conformational analysis confirmed that **32** is structurally similar to cyclic hexapeptide **18** where retention of converging NH groups pointing inside the macrocyclic cavity indicated a favourable preorganisation for anion binding. However, subtle differences in the structures of both receptors (Fig. 6) yielded characteristic variations in their properties. While **32** exhibits intrinsically higher anion affinities than **18** in competitive solvents (*e.g.* $K = 1.6 \times 10^4$ M^{-1} for **31** while $K = 5.8 \times 10^2$ M^{-1} for **18** with Br^- in CH_3OH), its propensity to form sandwich-type 2 : 1 complexes with two macrocycles surrounding one anion is significantly lower than that of the



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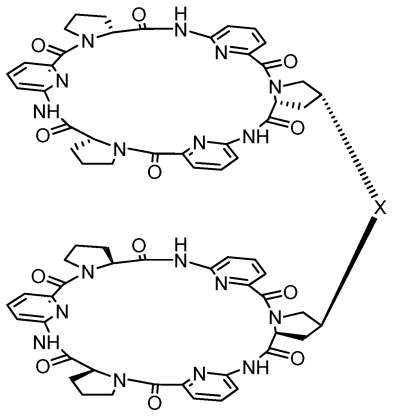

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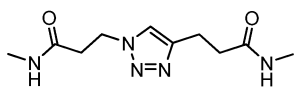
X =



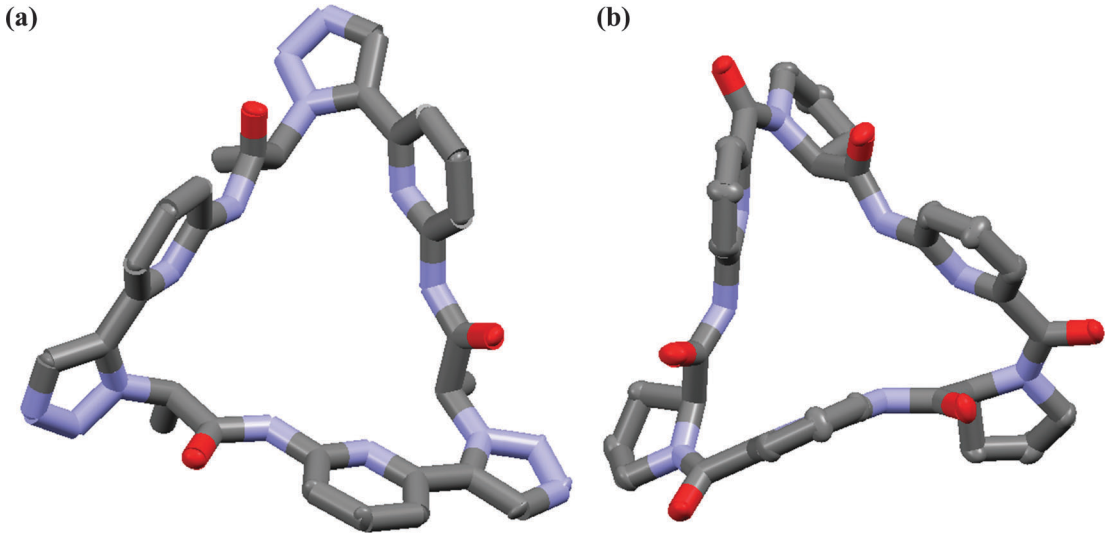
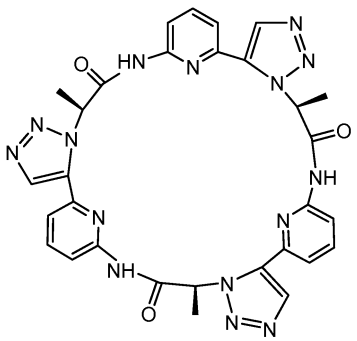


Fig. 6 Comparison of the X-ray crystal structures of (a) **32** and (b) **18**. Figure generated from X-ray diffraction data originally published in ref. 58 and 47.

cyclopeptide. This is thought to be due to a reduction in hydrophobic effects around the binding site of pseudopeptide **32** which results in a reduction of cooperative complex formation in methanol. Increasing water content however, has a strongly stabilising influence on the second binding step, where formation of the 2 : 1 complexes with sulfate in 2 : 1 CH_3OH : H_2O (v/v) clearly demonstrates that hydrophobic interactions are still important for these systems.



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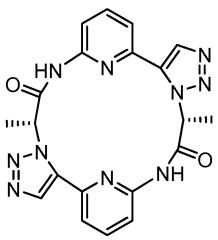

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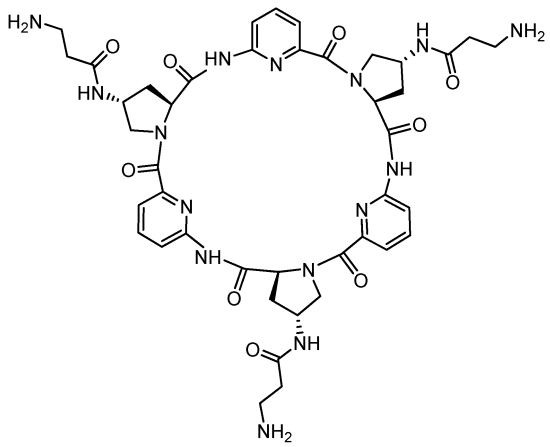
Selective sulfate recognition in water has also recently been reported by the same group using a cyclopeptide containing alternating Apa residues alternating with (4*R*)-4-aminoproline subunits with appended β-alanine residues **34**.[59] **34** shows high affinity and selectivity for sulfate in aqueous solution even in the presence of AcO^-, Cl^-, and HPO_4^{2-} or $H_2PO_4^-$ ($\log K$ = 4.20 for SO_4^{2-} in acetate buffer at pH = 4.8). Mass spectrometry, ^{1}H NMR spectroscopy and ITC analyses suggest that the binding behavior of **34** results from a combination of electrostatic interactions with the β-alanine arms of **34** which wrap around a sulfate anion and the hydrogen bond donor sites converging towards the guest.

Recently, Alfonso and Luis have reported the synthesis of a number of pseudopeptidic macrocycles and cages where they



34

found small pseudopeptidic cages of the general structure depicted in Fig. 7 could be efficiently assembled by a triple nucleophilic substitution macrobicyclization reaction.[60–62] The success of the macrobicyclization was strongly dependant on the central scaffold, where tris(2-aminoethyl)amine (tren) was shown to direct correct preorganization for the closure of macrobicyclic scaffolds. The binding of chloride by the protonated forms of the

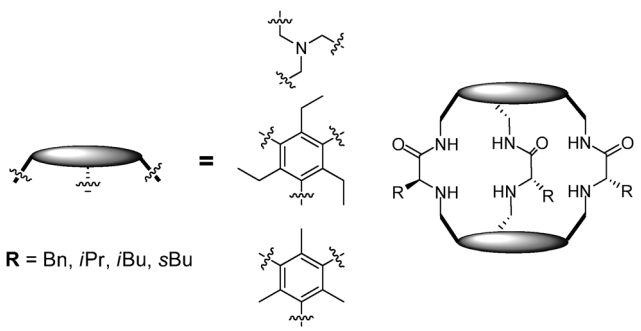
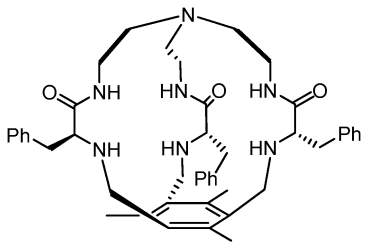


Fig. 7 General structure of the pseudopeptidic tripodal cages.

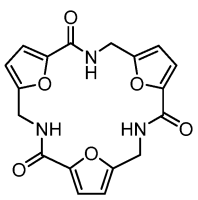
cages were studied by X-ray diffraction as well as by NMR spectroscopy and mass spectrometry. Although the binding pockets of the different cages are similar in terms of size and shape, the chloride recognition abilities of these receptor was shown to be greatly affected by the peripheral substitution of the peptide residues with reported 1 : 1 (receptor : anion) log β values ranging from 2.35 to 4.35 M^{-1} in aqueous acetonitrile solution (CD_3CN/H_2O 95 : 5). X ray crystallography analysis revealed that the HCl salts of several cages exist with chloride either partially or completely caged within the cavity of the macrobicycle. Interestingly, the aliphatic/aromatic substitution seemed to change the mode of binding where Phe cages were seen to completely encapsulate the chloride ion by electrostatic ammonium NH$\cdots$$Cl^-$ hydrogen bonds, whereas the aliphatic cages were shown to bind two chloride ions through ammonium and amide NH groups, leading to partial encapsulation. The ability of selected cages to transport chloride through lipid bilayers was also demonstrated where, again, stark contrasts were observed in transport rates of the different cages. This effect was thought to be directly related to lipophillicity where the most lipophilic derivative was found to be the most active transporter while the most hydrophilic derivative displayed little transport activity.



35

A subsequent report described the interaction of one of these receptors, **35**, with different inorganic anions.[63] The fully protonated form of this macrobicycle was studied by ^{1}H NMR and ESI-MS and showed that **35** bound chloride two orders of magnitude more strongly than any other halide. X-ray crystal diffraction studies of the corresponding HX salts of **35** (X = F, Cl, and Br) showed that the fluoride ion was too small to fit tightly within the cavity and lead to the inclusion of a water molecule inside the cavity instead. Conversely, in the presence of the bromide anion, a large geometrical distortion of the structure was observed suggesting that bromide is too large to fit completely inside the cage cavity, and the receptor must twist and distort in order to interact with this larger anion. The high chloride selectivity was found to be a result of perfect size complementarity of chloride within the cavity where the inclusion of the anion induces a slight expansion of the cavity and implies an extremely tight fitting receptor:anion complex; the inner binding site being defined by a symmetric array of electrostatic H-bonding interactions (Fig. 8).

Chakraborty and co-workers have prepared a cyclic trimer of 5-(aminomethyl)-2-furan carboxylic acid **36** as a receptor for carboxylate anions. ^{1}H NMR titrations indicated that **36** binds acetate ions in a 1 : 1 ratio with an association constant of 8.64×10^3 M^{-1} in CD_3CN.[64] Another furyl cyclopeptide **37** has recently been developed by Robina and co-workers, the ability of which to bind cyanide, acetate and chloride anions was determined by ^{1}H NMR titrations in CD_3CN.[65] This "bowl-like" structure was found to form 1 : 1 anion : receptor complexes with cyanide, acetate and chloride anions where chloride was found to have the tightest association with receptor **37** exhibiting a binding constant of $K = 1.63 \times 10^4$ M^{-1}.



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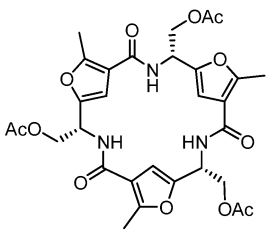

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A cyclic tetrapeptide **38** has also been prepared from alternating (*S*)-C-linked carbo-β-amino acid and *R*-aminoxy acid.[66] NMR and mass spectral analysis demonstrated that this symmetric cyclic peptide exhibited a halide binding affinity trend

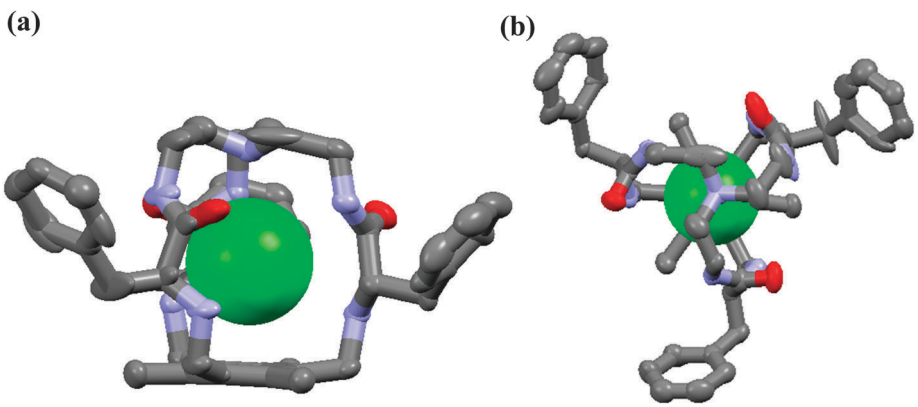
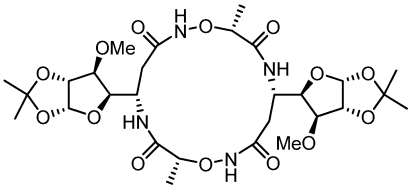


Fig. 8 Side (a) and upper (b) views of the crystal structure of the [**35**·4Cl^-] salt with the chloride anion clearly shown binding inside the macrocyclic cavity. Externally bound chloride anions, crystallization water molecules and hydrogens have been omitted for clarity. Figure generated from X-ray diffraction data originally published in ref. 63.

where Cl^- ($K = 513\ M^{-1}$) > Br^- ($K = 111\ M^{-1}$) > I^- (too weak to be determined) in CH_3OH. Moreover, its linear tetrapeptide analogue **39** did not display any anion binding capability indicating the importance of the preorganised cyclic structure for anion binding.



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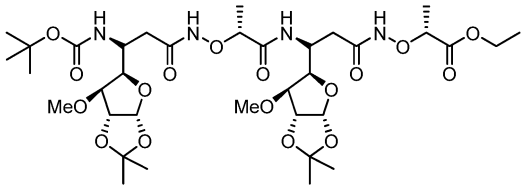
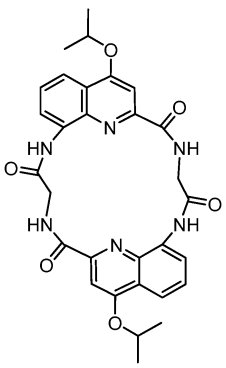


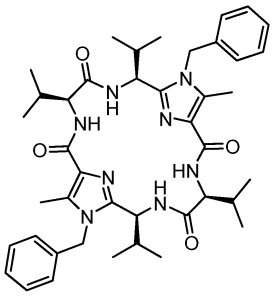
39

A cyclic tetrapeptide **40** composed of alternating glycine and 8-amino-4-iso-butoxyquinoline-2-carboxylic acid has been prepared by Chen and co-workers.[67] This fluorescent chemosensor was evaluated as a selective fluoride receptor in CH_3CN by ^1H NMR and fluorescence spectroscopy. Fluorescence quenching in the presence of fluoride was ascribed to a photoinduced electron transfer from the anion to a quinoline unit present in the macrocycle. Association constants of $K_a = 4.8 \times 10^9\ M^{-1}$, $2.34 \times 10^4\ M^{-1}$, $1.59 \times 10^3\ M^{-1}$ and $3.0 \times 10^4\ M^{-1}$ for F^-, AcO^-, $H_2PO_4^-$, Cl^-, and Br^- respectively were estimated from the titration data.



40

The Haberhauer group has synthesized a series of cyclic peptide scaffolds that are structurally related to the *Lissoclinum* family of natural hexapeptides, and have demonstrated their potential for use in molecular recognition and combinatorial chemistry.[68–71] The series of C_2-symmetric azole-containing macrocyclic peptides **41–44** were reported in 2009 and their ability to bind anions was studied in detail.[72] ^1H NMR titrations in DMSO d_6/5% $CDCl_3$ were employed to measure the binding capability of these macrocycles with dihydrogen phosphate, acetate, fluoride, hydrogen sulfate, toluene sulfonate, methyl sulfonate, chloride, nitrate, bromide, iodide, and perchlorate. Job's plot analyses indicated that all anions were bound in a 1 : 1 fashion and dihydrogen phosphate was found to be the most strongly bound anion in all cases. The thiazole receptor **43** bound most strongly to $H_2PO_4^-$ ($K_a = 3 \times 10^4\ M^{-1}$) followed by the oxazole receptor **42** ($K_a = 2.47 \times 10^4\ M^{-1}$) and the imidazole receptor **41** ($K_a = 2.64 \times 10^3\ M^{-1}$). The lowest binding affinities were generally observed with receptor **44** which was attributed to the increased size of the macrocyclic interior. Interestingly, while the highest binding affinities were observed for thiazole **43** the best selectivity was exhibited by the more basic imidazole receptor **41** (10 fold selectivity for $H_2PO_4^-$ over AcO^-), an effect attributed to the higher basicity of the azole nitrogen atom thus allowing the protons of the $H_2PO_4^-$ ion to form additional hydrogen bonds.



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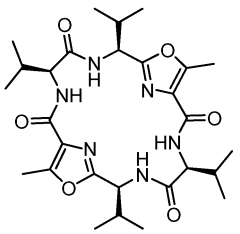

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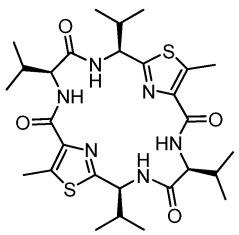

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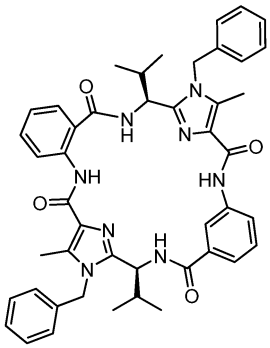


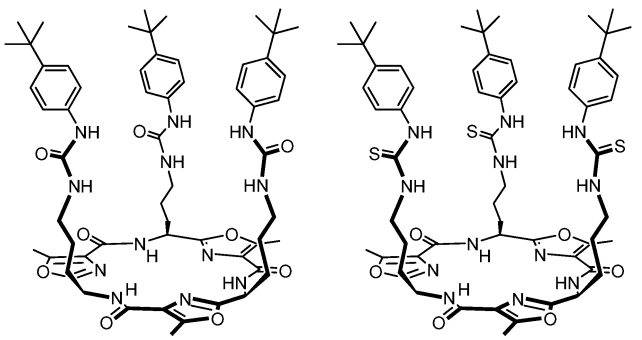
44

Binding through side chain functional groups

As demonstrated above, the *Lissoclinum* family of cyclic peptides, in which amino acids alternate with azole heterocycles in a macrocyclic ring, offers a useful scaffold for the construction of molecular receptors.[73,74] The peptide backbone is rigidified in a similar manner to those of the Apa containing peptides described above, with a network of bifurcated hydrogen bonds between the azole nitrogen atoms and the amide protons providing additional rigidity and directing all of the backbone hydrogen bond donors into the centre of the macrocycle. This conformation results in a structure in which, if all amino acid side chains are of the same configuration, they project from one face of the scaffold providing the ability to add additional

convergent binding sites to one face of the cyclic peptide. This class of scaffold is readily synthesised using standard solution or solid phase peptide synthesis techniques, and a wide range of functional groups can be appended *via* the amino acid side chains to provide additional binding sites for anionic guests.[75–77]

In initial studies, we investigated the potential of these scaffolds by functionalising the side chains with thio(urea) moieties to provide additional hydrogen bond donor sites to those provided by the peptide backbone. Tripodal urea **45** and thiourea **46** were readily prepared by functionalisation of an ornithine derived *Lissoclinum*-type scaffold.[78] It was demonstrated using ^{1}H NMR spectroscopy that the tris-urea **45** self-associated in $CDCl_3$ whereas the thiourea **46** did not, while neither compound showed self-association in a more polar mixture of 10% v/v DMSO-d_6/$CDCl_3$. Both compounds were found to bind strongly and selectively to sulfate in $CDCl_3$ and 10% v/v DMSO-d_6/$CDCl_3$ ($K_a > 10^4$ M^{-1}) and this selectivity was attributed to a binding mode, supported by ^{1}H NMR spectroscopy, in which sulphate forms nine hydrogen bonds to the receptors, with three of these coming from the amide protons of the cyclic peptide and remaining interactions attributed to hydrogen bond donation from the urea/thiourea sidearms.



45 46

In an effort to increase the binding affinity and selectivity of tripodal cyclic peptide based receptors, a subsequent study of receptors **46–51** indicated that reducing the distance between the thiourea and cyclic peptide backbone hydrogen bond donors results in increased affinity for a range of anions, with **48** exhibiting significantly higher binding affinities for a range of anions than **46** and **47** (Table 1). Notably, in 9 : 1 mixtures of water and DMSO-d_6 all six compounds exhibited remarkably high selectivity and affinity for sulfate ions with apparent stability constants still too high to quantify by ^{1}H NMR ($K_a > 10^4$ M^{-1}). This was attributed to a good fit between the host and sulfate ions, together with effective shielding of this anion from the solvent (Fig. 9). For compounds **50** and **51**, which were soluble in aqueous DMSO-d_6 containing up to 25% water, affinites for sulfate in this solvent remained high (**50**: K_a = 221 M^{-1}; **51**: $K_a > 10^4$ M^{-1}) and the trend for increased binding affinity on bringing the thiourea sites closer to the cyclic peptide scaffold was apparent.

Further studies on thiourea based receptors led to the design of two cryptands, **52** and **53** formed by reacting tripodal

Table 1 Apparent stability constants (K_a, M^{-1}) of **46–48** towards various anions in $CDCl_3$[a,b]

	K_a (M^{-1})		
Anion[c]	**46**	**47**	**48**
Cl^-	298	900	$>10^4$
Br^-	104	150	$>10^4$
I^-	36	75	2100
NO_3^-	74	980	$>10^4$
HSO_4^-	1700	—[d]	2680
TsO^-	189	540	$>10^4$
SO_4^{2-}	$>10^4$ [e]	$>10^4$ [e]	$>10^4$ [e]

[a] Determined at 300 K. Data was fitted to a 1 : 1 binding model. K_a values are an average obtained from monitoring both thiourea NH signals. Errors < 15%. [b] Addition of acetate and hydrogen phosphate resulted in deprotonation of the thiourea groups. [c] Anions added as their tetrabutylammonium salts. [d] Peak broadening prevented an association constant from being determined. [e] Titration displayed slow exchange on the NMR timescale.

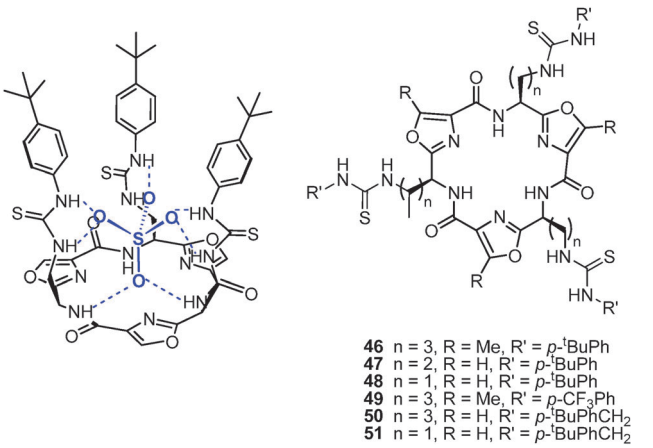
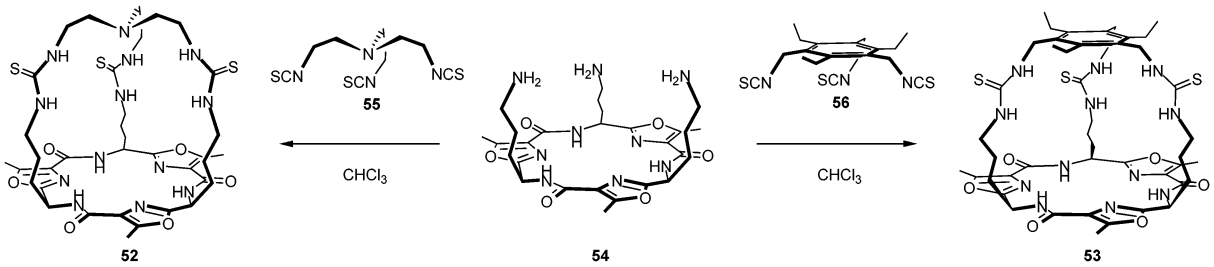


Fig. 9 Proposed binding mode between **48** and sulfate and structures of compounds **46–51**.

cyclic peptide amine **54** with tris-isothiocyanates **55** and **56** under dilute conditions (Scheme 1). These trivalent capping units exhibit complementary geometries for the cyclic peptide containing three oxazole units and the resulting cryptand-like systems were shown to be excellent anion receptors.[79] X-ray crystal structure analysis revealed that in the solid state the thiourea groups of **52** point outside of the cryptand cavity while those of **53** were arranged such that their H-atoms are directed inside the cavity and hydrogen bond to solvent guest molecules (Fig. 10). Preliminary anion binding studies conducted by titrating **52** and **53** with the tetrabutylammonium salts of a range of monovalent anions in 0.5% v/v H_2O/DMSO-d_6 indicated that **52** bound to fluoride, chloride, bromide and acetate with high affinities ($K_a > 10^4$ M^{-1}) while cryptand **53** showed enhanced selectivity towards acetate anions only ($K_a > 10^4$ M^{-1}). This behaviour was thought to be due to the increased flexibility of the tren-capped cryptand **52** compared to that of the rigid 1,3,5-triethylbenzene capped **53**. A subsequent more detailed study reported further anion binding behaviour of **52** and **53** including the binding of sulphate anions and titrations conducted in mixed water–DMSO solutions.[80] **52** and **53** were again found to



Scheme 1 General synthetic approach to cyclic peptide based cryptands **52** and **53**.

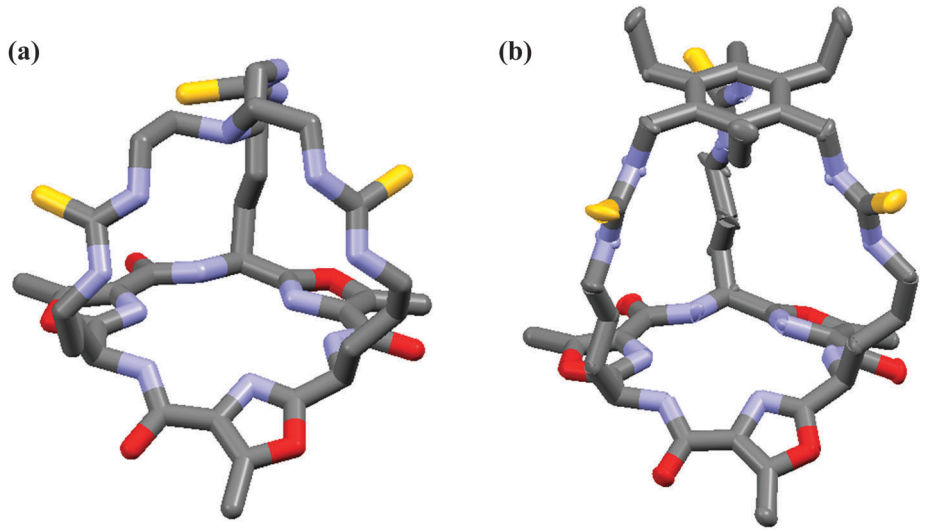


Fig. 10 X-ray crystal structures of (a) **52** and (b) **53**. Solvent molecules have been removed for clarity. Figure generated from X-ray diffraction data originally published in ref. 79.

bind selected anions in a 1 : 1 manner in 0.5% H_2O/DMSO-d_6 with both receptors having $K_a > 10^4$ M^{-1} for acetate and sulphate in this solvent. Increasing the competitiveness of the solvent by addition of 10% H_2O in DMSO-d_6 enabled the selectivity of **52** to be determined as $SO_4^{2-} > Cl^- > AcO^- > Br^-$. This selectivity was attributed to a hydrogen bond network formed *via* nine hydrogen bonds to SO_4^{2-}; six from the thioureas and three from the cyclic peptide amide backbone. In contrast, ^{1}H NMR evidence suggested that chloride and acetate anions did not benefit from such interactions and were bound through thiourea hydrogen-bonding interactions only. Somewhat surprisingly, the affinity of the preorganised cryptand **52** for sulfate ions in the more competitive 25% v/v H_2O/DMSO-d_6 mixture was observed to be significantly lower (K_a for SO_4^{2-} = 141 M^{-1}) than that of the tripodal analogue **49** (K_a for $SO_4^{2-} > 10^4$ M^{-1}). This was suggested to be due to better shielding of the anionic guest from the solvent by the tripodal **49** as a result of hydrophobic interactions between the aromatic substituents, as indicated by the significant and complex changes in the ^{1}H NMR chemical shifts of the aromatic protons of these receptors, which are observed upon addition of SO_4^{2-}. These interactions are not possible for the tren-capped cryptand **52**.

The high sulfate selectivity and affinity observed for receptors **46**, **52** and **53** and their apparent ability to shield this anion from the environment led to an examination of their ability to function as transmembrane anion transporters.[81] This study used a new technique to monitor sulfate transport, using ^{33}S-labelled sulfate and paramagnetic agents such as Mn^{2+} and Fe^{3+} to discriminate between intra- and extravesicular sulfate by ^{33}S NMR experiments. Cryptand **53** was the only cyclic peptide based receptor that was found to function as an anion transporter and this was attributed to its cage-like structure that offers nine hydrogen bonds to its anionic guest and screens the anion from the membrane environment. Furthermore, **53** was also found to function *via* a Mn^{2+}/SO_4^{2-} symport mechanism, a process thought to be driven by the overall neutral charge of the complex and the many metal coordinating N and O atoms in the cyclopeptide ring.

The contribution of both amide and thiourea hydrogen bond donors from these cyclic peptides to the binding of sulfate but not to other anions suggested that simpler (non-cyclic) peptidic structures might also be capable of sulfate binding. A family of small linear peptides **57–62** was therefore investigated and it was found that these also show the same impressive selectivity for sulphate.[82] Receptors **57–62** with two hydrogen bond donor sites based on either thiourea or squaramide binding sites showed significantly higher affinity for SO_4^{2-} in 20% v/v H_2O–DMSO-d_6 than for various other anions. Moreover, the selectivity for sulphate appears to arise from a synergistic interaction between

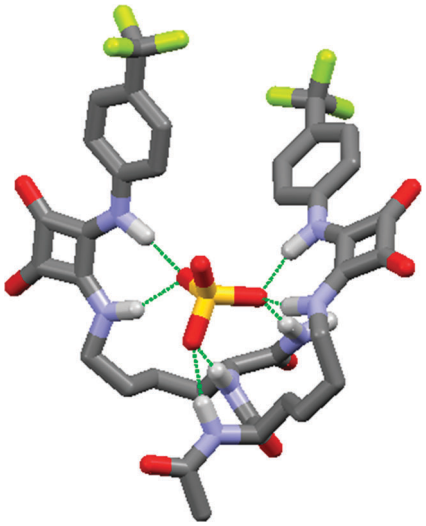
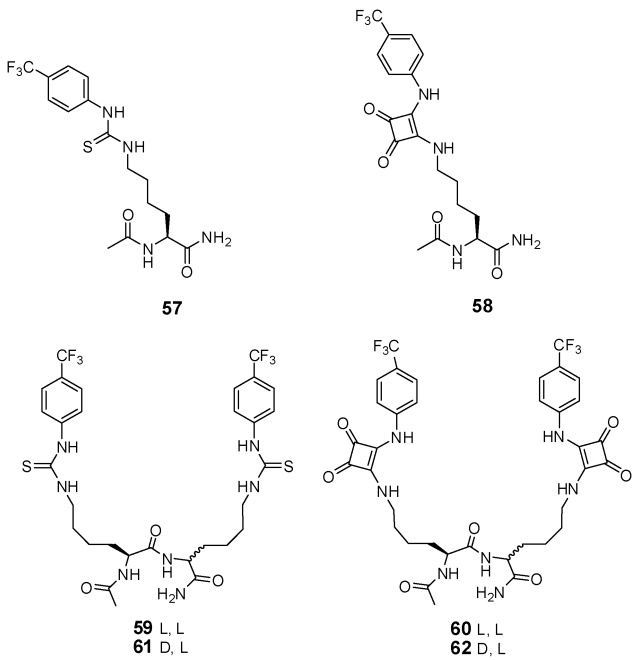


Fig. 11 DFT calculated structure of the SO_4^{2-} complex of **60** showing sulfate inserted into the binding cavity stabilised by 7 N–H···O hydrogen-bond interactions.

both the amide backbone and the thiourea/squaramide NH protons in a similar manner to that observed for the cyclic peptide analogues; behaviour that was not observed with AcO^-, BzO^-, or Cl. Additional evidence from DFT calculations afforded evidence for a possible host–guest orientation where such peptide based receptors were shown to wrap around the sulphate ion, binding through seven hydrogen bonds in a manner similar to the binding of SO_4^{2-} to the SBP (Fig. 11). Although non-cyclic structures, this result perfectly exemplifies the remarkable selectivity afforded by complementary interactions between side chain binding motifs and the inherent ability of the backbone amides to participate in anion binding in aqueous solutions.

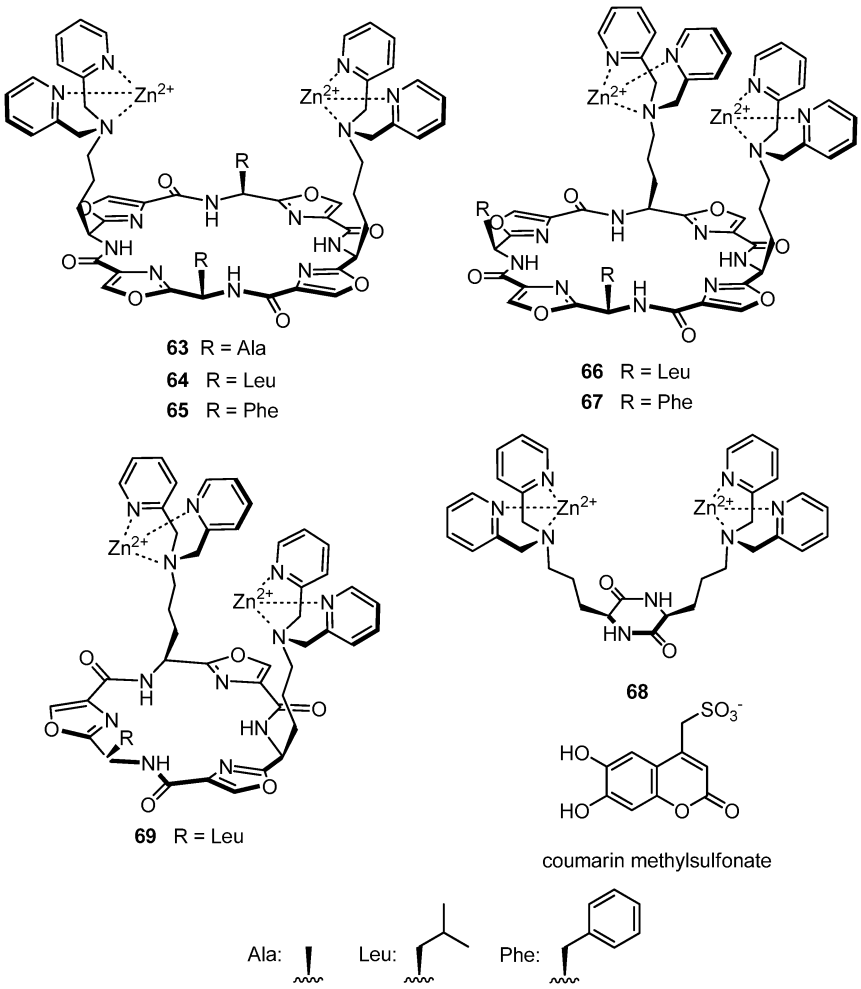


57 **58**

59 L, L
61 D, L

60 L, L
62 D, L

In contemporaneous studies, we explored anion receptors based on a *Lissoclinum*-type cyclic peptide scaffold with two pendant dipicolylamino (DPA) arms complexed to zinc(II). Receptors **63–67** possess common structural features, having the same relatively flat macrocyclic scaffold constructed from an oxazole modified cyclic peptide. The cyclic octapeptide **63** was initially chosen as a scaffold to position two Zn(II)–DPA groups at an appropriate distance to complement the size and geometry of the pyrophosphate anion ($P_2O_7^{4-}$; PPi) to improve selectivity over other phosphate oxoanions (*e.g.*, HPO_4^{2-}, ATP and ADP). In combination with coumarin methylsulfonate, a fluorescent indicator, **63** exhibited high sensitivity ($\log K_a$ = 8.0) for PPi anions in indicator displacement assays (IDAs) under mimicked physiological conditions (pH 7.2, 5 mM HEPES, 145 mM NaCl). In addition, **63** showed complete selectivity for PPi over monophosphate derivatives, including HPO_4^{2-} which showed no indicator displacement, and significant selectivity (2 orders of magnitude) for PPi over ATP and ADP.[83] When a smaller peptide scaffold was used, such as the diketopiperazine in receptor **68**, both binding affinity and selectivity for PPi dropped significantly ($\log K_a$ = 6.0 for PPi and 5.3 for ATP), suggesting that the larger scaffold is required for the design of a PPi-selective sensor.[84] In a more comprehensive investigation of the binding properties of **63–67** and **69**, the non-binding side-chain steric bulk, the relative position of binding sites, and the scaffold size were all found to affect the ability of these receptors to discriminate between polyphosphate ions.[76,85] Significant differences in binding affinity and selectivity were observed for **63–65**, in which the steric bulk of the non-binding side chains was altered, indicating that while the hydrophobic cleft provided by the Leu side chains of **64** results in similar affinity for all three anions ($\log K_a$ 7.4–7.6), the smaller Ala substituents of **63** provide enhanced selectivity for PPi over ATP and ADP. In contrast, for **65** in which the non-binding substituents are replaced by Phe side chains, binding was in the order ATP ≈ PPi > ADP. Similarly, moving the Zn(II)–DPA binding sites closer together on the peptidic scaffold resulted in increased selectivity for PPi over ATP and ADP in both the Phe and Leu series. Notably, the affinity of **66** (with proximal Zn(II)–DPA side chains) for PPi ($\log K_a$ 8.8) is more than an order of magnitude higher than that of **64** ($\log K_a$ 7.4), in which the DPA side chains are distal, while for **67** affinity for all three anions dropped slightly in comparison to that of **65**. Surprisingly, changing the size of the cyclic peptide scaffold while maintaining the same distance between the binding sites had a significant effect on binding affinity with **69** binding PPi ($\log K_a$ 9.8) an order of magnitude more strongly than **66** ($\log K_a$ 8.8) and with increased selectivity over both ATP and ADP. While most of the receptors showed some selectivity for pyrophosphate over ATP and ADP in water and saline, this selectivity was significantly enhanced in the biologically relevant Krebs buffer giving chemosensing ensembles capable of selective recognition of pyrophosphate in the presence of excess ATP. In particular, the ensemble formed between coumarin methylsulfonate and **69** showed a remarkable ability to discriminate between PPi and ATP/ADP in Krebs buffer. Only PPi was able to completely displace the indicator from the receptor under these conditions, as demonstrated by full recovery of the fluorescence signal (Fig. 12).



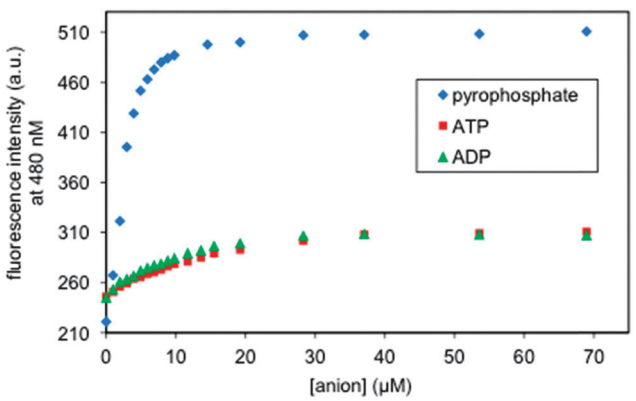
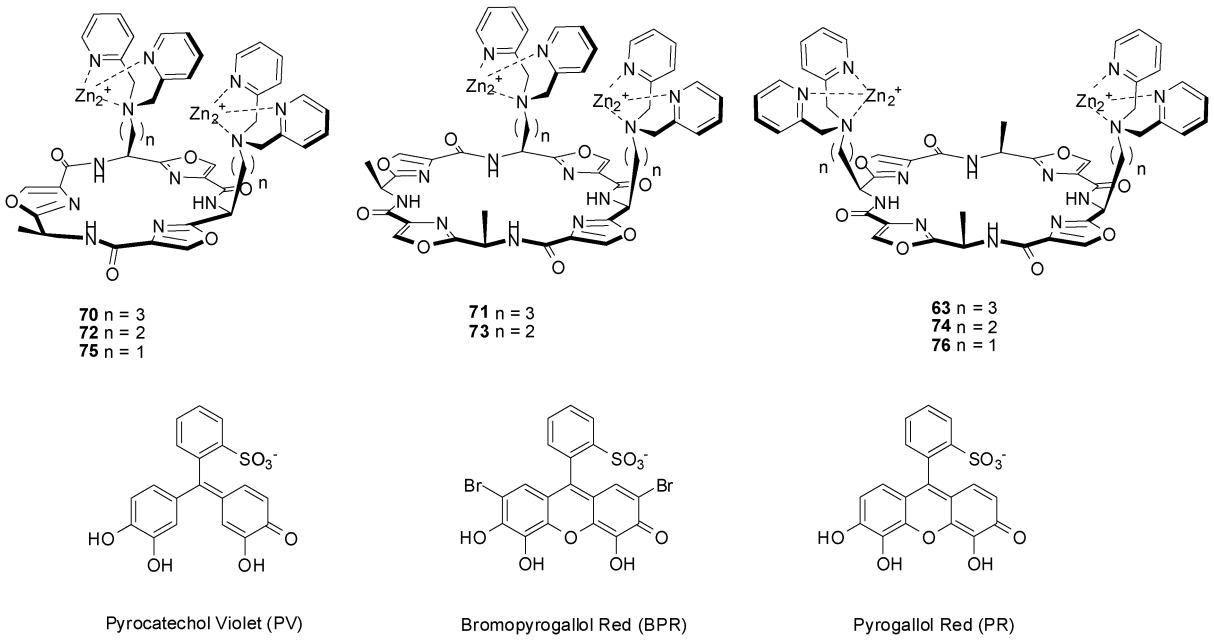


Fig. 12 A comparison of the changes in fluorescence emission observed for the complex formed between a coumarin methylsulfonate indicator and **69** (10 mm) upon the addition of various anions in Krebs buffer solution.

We envisaged that by screening libraries of both receptors and indicators, it would be possible to obtain chemosensing ensembles with improved selectivity for PPi over other polyphosphates. In an effort to tune these IDAs for naked-eye sensing of PPi in aqueous media we studied small libraries of both anion receptors and colourimetric indicators. We discovered that the selection of a suitable indicator with the optimal macrocyclic scaffold affords near complete selectivity for PPi over other anions under biologically relevant concentrations with changes clearly visible to the naked eye (Fig. 13).[86] In this study a new family of cyclic peptide receptors **70–76** were synthesised in order to probe the optimal size and geometry of the receptors as well as studying the effect of shortening the distance between the anion binding sidechains and the cyclic peptide scaffold. The best discrimination between the anions studied was observed for receptors **73** and **74** in which the Zn(II)–DPA binding sites were appended to a tetraoxazole scaffold with spacers of two methylene units between the scaffold and the anion binding sites. These particular receptors provided a compromise between the flexibility required for induced fit binding of the PPi guest and the steric blocking of other anions by the scaffold. Importantly, the selectivity of the chemosensing ensembles could be further tuned by changing the indicator, with pyrogallol red and bromopyrogallol red both providing enhanced discrimination between polyphosphate ions when compared to pyrocatechol violet. In addition, we established that these receptors are even more selective for PPi in Krebs saline solution and through the use of calibration experiments we demonstrated that such systems could be exploited to measure low levels (< 2 μM) of PPi even in the presence of >125 times excess of ATP.



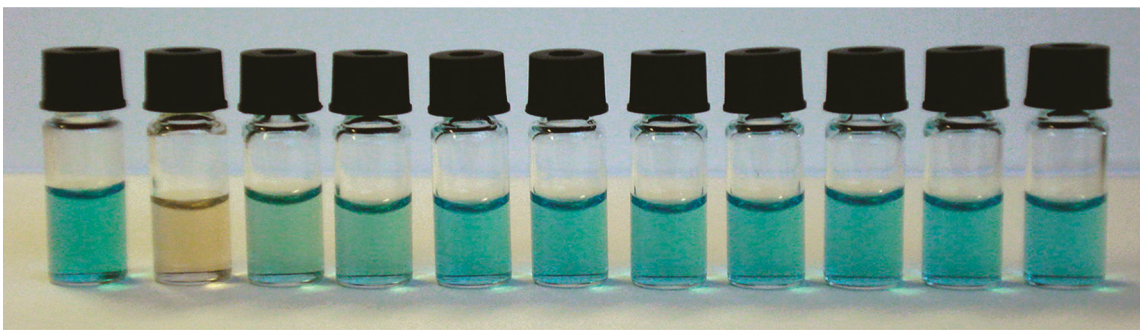


Fig. 13 The colours of the 1 : 1 mixtures of **71** : PV (20 μM each) with and without anions (sodium salts) from left to right: no anion, PPi, ATP, ADP, AMP, c-AMP, phosphothreonine, phosphoserine, phosphotyrosine, HPO_4^{2-} and citrate (5 equiv. each).

Interestingly, in a more recent report we have observed that a library of bis(Zn(II)–DPA) functionalised linear peptides, synthesised using an efficient solid phase peptide synthesis strategy, also functioned as chemosensors for anions in water with high affinity and selectivity for PPi over ATP and ADP.[87] It was demonstrated that additional aromatic side chains provided enhanced discrimination between PPi and ATP. The significant selectivity for PPi over ATP and ADP observed under mimicked physiological conditions with such simple peptidic scaffolds paves the way for numerous potential uses of peptide based IDAs.

Conclusions

This Feature Article summarises recent work on the design and development of cyclic peptides capable of binding to anionic guests. Cyclic peptides have been shown to be highly effective scaffolds for use in anion recognition. Their constrained conformation provides preorganisation for both backbone amide hydrogen bond donors and side chain functional groups for this purpose. The ability to readily incorporate both natural and novel amino acids into these structures provides an efficient method by which to modify and tune the anion recognition properties of these receptors for specific anionic guests and also provides a means through which to isolate the anion from a competitive solvent environment. The introduction of backbone rigidity and/or sidechains bearing anion recognition motifs has been found to provide improved anion affinities and selectivities. As a result of the ease of synthesis of such receptors using standard peptide synthesis techniques, families of related receptors can be prepared thereby enabling the factors affecting anion binding by these systems to be elucidated. This information is leading to the design and synthesis of more selective receptors capable of operating in highly competitive conditions (*e.g.* physiological conditions). This will allow the future exploration of the wide range of potential applications for peptide based anion receptors in a variety of fields.

Acknowledgements

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