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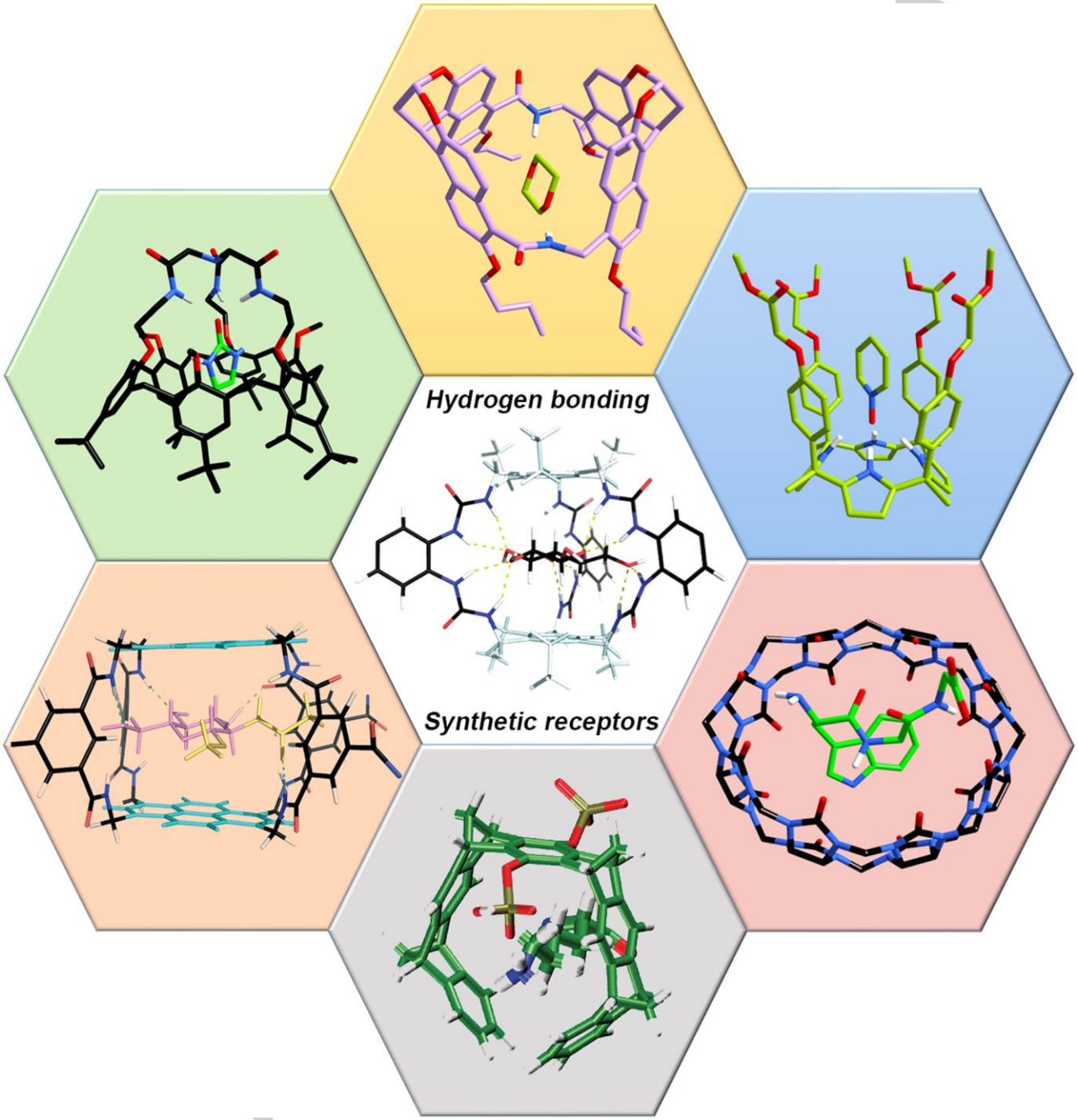
MINIREVIEW

Molecular Recognition Mediated by Hydrogen Bonding in Aqueous Media

Jinqiao Dong,[a] and Anthony P. Davis*[a]



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MINIREVIEW

Abstract: *Hydrogen bonding is a key governing force in molecular recognition, notably in biological systems. While it has been studied and exploited by supramolecular chemists for many years, most of this work has been conducted in organic solvents. Investigations in water, the biological solvent, have proceeded more slowly, largely because the interaction is weakened by solvation and less easy to detect. Recently it has become appreciated that the problems should be addressed, and work towards the deployment of H-bonding in water has accelerated. This minireview discusses a range of synthetic receptors designed to bind organic molecules in aqueous media by combining hydrogen bonding with hydrophobic interactions. Some of these systems are capable of high affinities and selectivities, raising the hope of biomedical applications in the near future.*

1. Introduction

Molecular recognition – the ability of molecules to bind others strongly and selectively – lies at the heart of biology. It is a complex process, difficult to understand fully, but at a practical level the principles are fairly clear. One molecule binds another by generating a complementary binding site or surface, where shape size and "supramolecular valence" are matched to the substrate. Of the intermolecular forces which drive recognition, hydrogen bonding is among the strongest and most influential. Not only does it operate directly between binding partners but in water, the biological solvent, it drives the hydrophobic effect by promoting solvent cohesion. By combining direct H-bonding with hydrophobic interactions, biology can achieve remarkable affinities and selectivities. Figure 1 shows two examples, a carbohydrate binding protein which binds glucose or galactose by deploying multiple H-bonds, along with hydrophobic/CH-π interactions,[1] and the binding of biotin by the protein avidin.[2] In the latter case the combination of H-bonding and hydrophobic effects yields affinities (K_a) of ~ 10^{15} M^{-1}, highlighting the potential of well-organised non-covalent interactions.

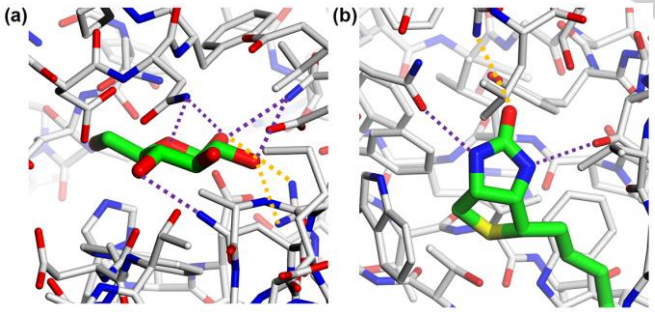

Figure 1. Examples of natural molecular recognition through hydrogen bonding. (a) X-Ray crystal structure of glucose complexed to the periplasmic galactose-binding protein from *Escherichia coli* (PDB: 2GBP).[1] (b) X-Ray crystal structure of avidin–biotin complex (PDB: 2AVI).[2]

Supramolecular chemists have sought to mimic biological molecular recognition, partly to enhance understanding but also with a view to biomedical applications. However, while water is the biological solvent, most studies involving hydrogen bonding have been performed in organic media. There are probably two main reasons. Firstly, the synthetic receptors used for this work are based on organic scaffolds which are much easier to handle in organic solvents. The design and synthesis of water-soluble systems is challenging and requires special effort. Secondly, that effort may not be rewarded because hydrogen bonds in water are generally weak. Both donor and acceptor are well-solvated, so water molecules must be displaced from both components and the free energy change on binding is usually small.

The problems are illustrated by a few early examples which appeared in the 1990s. For example, imides **1** (Figure 2a) were reported by the Rebek group in 1993 as receptors for adenine derivative **2** in water.[3] For large Ar groups K_a values rose as high as 70 M^{-1}, but for Ar = Ph the affinity of **1** for **2** was just 2 M^{-1}. Assuming that the Ph provides no hydrophobic interactions, this reflects the value of the two hydrogen bonds between host and guest. For analogous systems studied in $CDCl_3$, these H-bonds are worth at least 100 M^{-1}.[4] Receptor **3**, studied by Hunter and coworkers, was designed to bind cyclodipeptides such as **4** (Figures 2b-c). In this case the four intermolecular H-bonds generated a binding constant of 70 M^{-1} for **3** + **4**, compared to 10^6 M^{-1} for a similar system in $CDCl_3$.[5] Figure 2 also includes systems from more ambitious programs; Diederich's cyclophane **5** (Figure 2d)[6] was a first step towards a receptor for the D-Ala-D-Ala dipeptide targeted by the Vancomycin group of antibiotics,[7] while Still's macrotricycle **6** was screened for binding to a library of tripeptides.[8,9] Both systems gave encouraging results with negatively charged substrates although, given their polycationic nature, the role of H-bonding (as opposed to electrostatic or hydrophobic interactions) is difficult to determine.

Jinqiao Dong obtained his PhD degree under the supervision of Prof. Yong Cui at Shanghai Jiao Tong University in 2014. From 2015 to 2019, he worked with Prof. Dan Zhao at National University of Singapore as a postdoc. In March 2019, he joined the group of Prof. Anthony P. Davis at the University of Bristol as research associate. His research interests include supramolecular chemistry, porous materials, as well as functional peptides.

Tony Davis gained a BA in Chemistry from Oxford University in 1977, and a DPhil in 1979. After postdoctoral work in Oxford and ETH Zürich, he was appointed in 1982 as a Lecturer at Trinity College, Dublin. In September 2000 he moved to the University of Bristol, where he is Professor of Supramolecular Chemistry in the School of Chemistry. His research focuses on the development of supramolecular systems with potential for biological applications, especially carbohydrate receptors and transmembrane anion transporters. He has co-founded two companies to exploit discoveries in carbohydrate recognition and sensing; Ziylo, which was sold in 2018 to Novo Nordisk, and Carbometrics, which continues to work in the area.

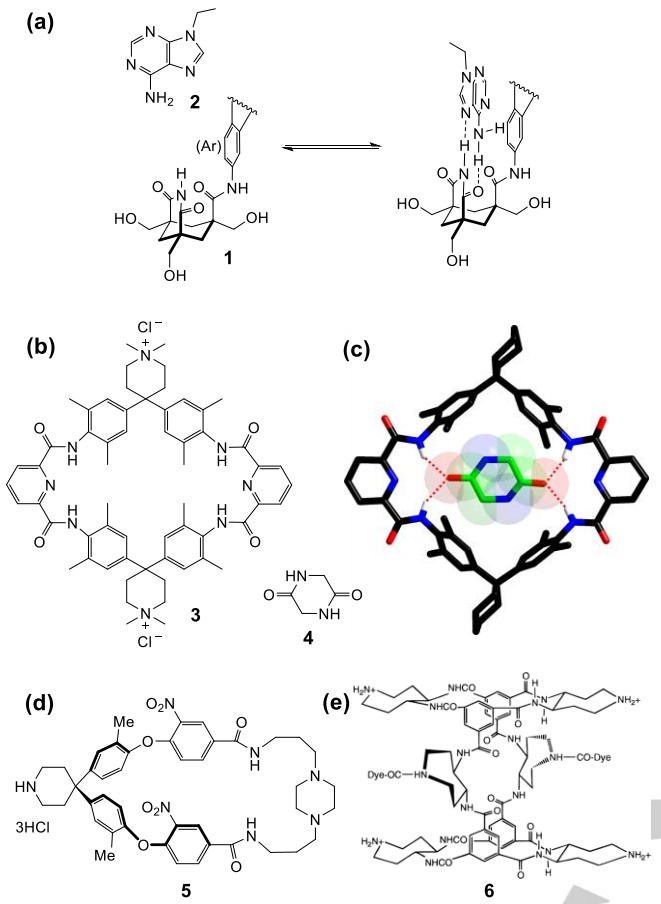

Figure 2. Early examples of water-soluble synthetic receptors with hydrogen-bond donors. (a) Rebek's receptor **1** for binding 9-ethyladenine **2**.[3] (b,c) Hunter's receptor **3** for binding cyclic dipeptides such as **4**;[5] (c) is the crystal structure of the complex between **4** and an organic soluble analogue of **3**.[10] (d) Diederich's receptor **5** for binding of N-protected α-amino acids.[6] (e) Still's receptor **6** for sequence-selective peptide binding.[8] Copyright 1996, American Chemical Society.

Since these early efforts progress has been made, slowly at first but accelerating as the need to study recognition in water has become more widely appreciated.[11-15] In this Minireview we summarize the recent advances in this area, considering design strategies, structural characterization, and binding properties in aqueous media. We restrict ourselves to organic molecular substrates with particular focus on molecules which are electrically neutral or lightly charged. For the binding of inorganic anions and highly charged substrates (e.g. nucleotides) in water, the reader is directed to other recent reviews.[16-18] We also focus on classical hydrogen bonds involving polar XH as donors. Non-classical CH···X bonds may also be important in water[19] but are not covered herein.

2. Design and synthesis of receptors for studies in water

2.1. General strategies

Receptors designed to bind polar molecules in water should meet a number of criteria. They must be water-soluble, and ideally they should be monomeric at reasonably high concentrations (otherwise binding studies will be difficult to perform). Most receptor architectures are fashioned from organic components and not naturally water-soluble, so special appendages are generally required. Externally-directed charged groups (positive or negative) are thus common features (Figure 3a). Dendrimeric units can be especially effective,[20] and have figured prominently in our own work.[21]

The receptor core will need to provide preorganised hydrogen bonding groups which complement those on the target, as precisely as possible. Generally these will be presented within a cleft or cavity, so that groups on all sides of the target may be addressed. Most targets will contain some hydrophobic surface, even if it is small, and this should also be complemented by apolar surfaces in the receptor (Figure 3). Contact between these surfaces will contribute to binding through the hydrophobic effect, enhanced by CH-π interactions where relevant. It is also possible that a further effect may come into play. It has been suggested that interspersing polar and apolar units may create surfaces which are difficult to hydrate, creating "high-energy water" which is easily displaced.[22,23] In this case, hydrogen bonding within amphiphilic cavities might be surprisingly effective.

Preorganising the binding elements, polar and apolar, is probably the main challenge in this work. Perfect rigidity is not desirable, as the receptor will need to adjust its shape to match its target, and to make room for entry and exit. On the other hand, too much flexibility lowers affinity through entropic effects and works against selectivity. It may also allow cavity collapse; holding the binding site open, so that water is forced into an unfriendly environment, may be the key to strong binding. Biology preorganises through the folding of flexible chains into specific arrangements, leaving gaps which happen to have everything in the right place. This strategy is less easy to implement for synthetic receptors, although impressive progress has been made.[24] It is more usual to design structures based on rigid components, often constrained by macrocyclic or macropolyclic topology. These frameworks must be synthetically accessible, and this has directed work towards particular structural types as summarised in the following sections.

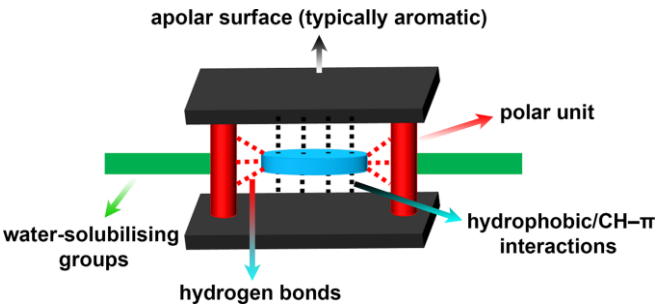

Figure 3. Design strategy for synthetic receptors exploiting hydrogen bonding in water.

2.2. Design options – synthetic cavities with hydrogen bonding capability

In the following sections, we highlight several types of molecular framework which have been used to deploy hydrogen bonding in water, paying attention to synthetic approaches and accessibility.

2.2.1. Macrocyclic lactams

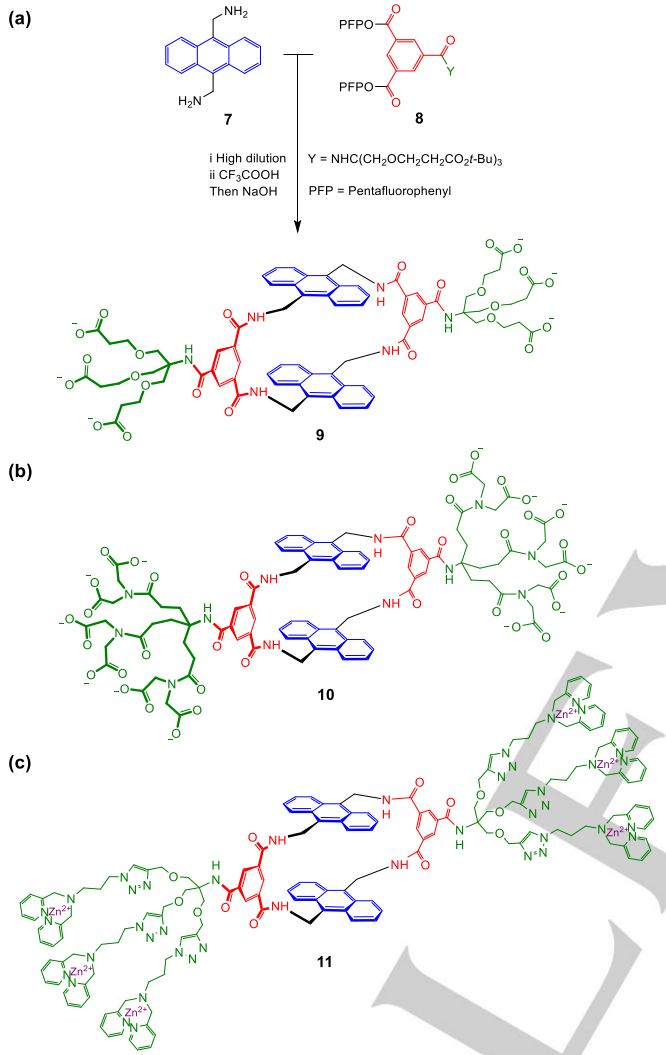

Figure 4. (a) Synthesis of tetralactam macrocyclic receptor **9**. Chemical structures of receptors **10** (b) and **11** (c) featuring different water-solubilising side chains. Receptor structures are colour-coded red = hydrogen bonding unit, blue = apolar surface, green = solubilising groups. This scheme is employed for the remainder of the article.

The amide linkage is one of easiest to form in organic synthesis, and is capable of acting as H-bond donor or acceptor. It is also structurally well-defined – in a C-CO-NH-C unit all atoms are coplanar and the central bond is generally *anti*, so that atomic positions are predictable. Thus, it is not surprising that macrocyclic amides (macrolactams) have featured prominently in receptor design. Macrocycles **3** and **5** (Figures 2b and 2d) provide early illustrations. More recently, bis-anthracenyl tetralactams such as **9**-**11** have proved to be versatile water-soluble receptors, and illustrate nicely the principles discussed in Section 2.1. They can be synthesised quite simply from the anthracenyl diamine **7** and isophthaloyl spacers **8** bearing a variety of dendrimeric solubilising groups as illustrated in Figure 4a. The rigid aromatic components ensure that the core macrocycle has limited conformational options and is unable to close such that the anthracene surfaces meet. Aromatic surfaces are thus exposed to water, which is released when the cavity is occupied by a guest. Though originally designed to bind carbohydrates (specifically glucose),[25,26] macrocycles **9** and **10** are more effective for polar aromatic systems (see Section 3.4)[27] and bind squaraine dyes with exceptionally high affinities (see Section 3.3).[28]

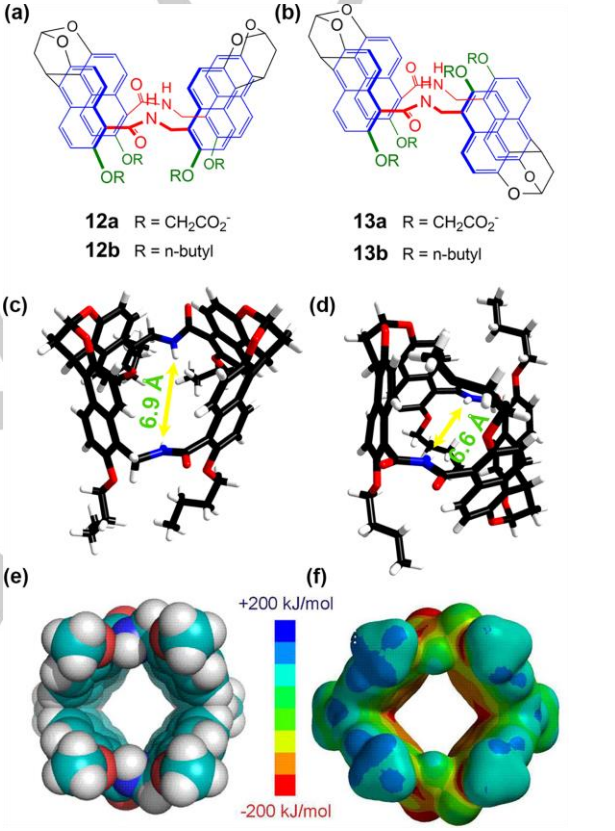

Figure 5. Chemical structures of *syn*-macrocyclic receptors **12** (a) and *anti*-macrocyclic receptors **13** (b). X-ray crystal structures of **12b** (c) and **13b** (d). Energy-minimized structure (e) and electrostatic potential surfaces (f) of the *syn*-isomer calculated at the PM06 level of theory.[30] Copyright 2016, American Chemical Society.

A second family of lactams, based on naphthalene dimers, is illustrated in Figure 5. As shown by Glass and coworkers,[29] the bis-naphthyl components are readily synthesised as diamines or bis-carboxylic acids, which are then cyclised together to give dilactams. The monomers are cleft-shaped, and this preorganises the dilactam structures so that open cavities are maintained. Cyclisation can give two geometries, **12** and **13**, both with potential for molecular recognition. The bis-naphthyl units possess peripheral oxygen atoms which can be used as linkage points for solubilising groups, e.g. the CH_2CO_2H in **12**/**13a**. In the original work of Glass **12**/**13a** were used to bind lipid molecules in

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water, presumably through hydrophobic interactions. However the Jiang group later recognised that the annular amides could be used for hydrogen bonding to substrates. In 2016 they reported the characterisation **12** and **13**, including X-ray crystal structures of **12**/**13b** (Figures 5 c/d). The NH groups were found to be angled inwards, and calculations highlighted the amphiphilic surface (Figures 5e/f). As discussed later (Section 3.4) binding studies showed that these "naphthotubes" were indeed capable of binding quite a wide range of polar molecules in water.[30]

2.2.2. Macropolycyclic lactams

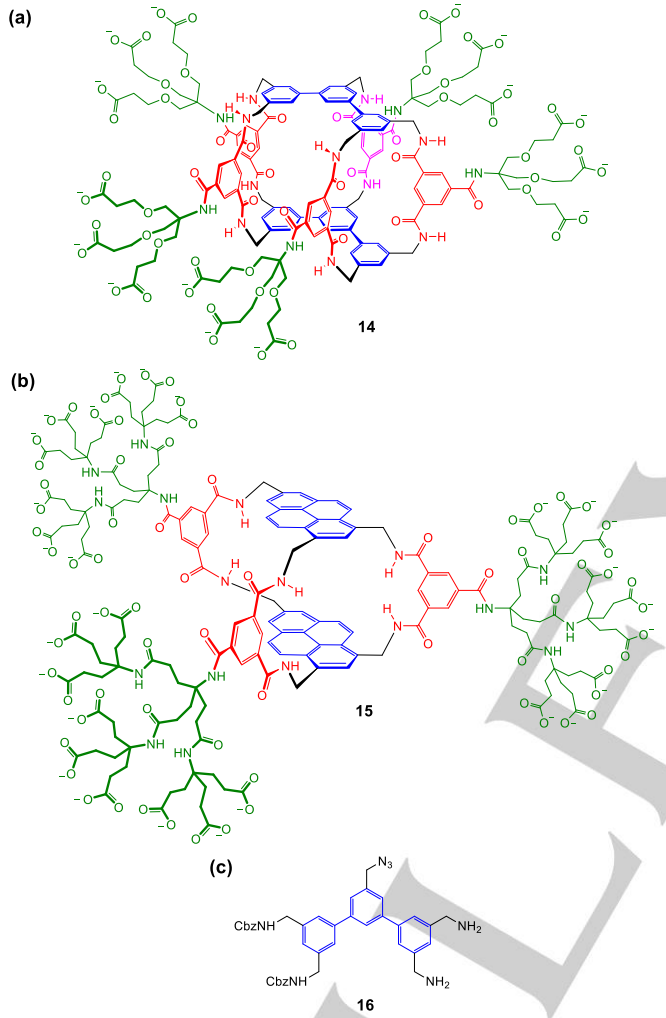

Figure 6. Chemical structures of macropolycyclic receptors **14**[31] and **15**,[32] and intermediate **16** used for the synthesis of **14**.

The ease of amide synthesis has encouraged the development of more complex polycyclic oligolactam architectures. Tricycle **6** is an early case,[8] and two further examples **14** and **15** are shown in Figure 6. Like **9**-**11** these structures incorporate isophthalamide spacers with externally-directed dendrimeric water-solubilising groups. Receptor **14**[31] is tetracyclic, featuring meta-terphenyl surfaces capable of hydrophobic/CH–π interactions, and ten annular amides capable of hydrogen bonding to guests. Receptor **15**[32] employs rigid pyrenyl units, potentially more effective than the flexible terphenyls, and a bicyclic architecture with six annular amides. In this case powerful nonacarboxylate solubilising groups were required to prevent aggregation in water. Both **14** and **15** were synthesised via controlled, stepwise procedures involving differentially protected amino intermediates (for example terphenyl **16** in the case of **14**).

Pyrene surfaces were also employed in receptors **17**-**19** (Figure 7). In these cases directed syntheses proved impractical and the cages were constructed from undifferentiated tetra-amine **20** via polycyclisations. Tricyclic **17** and **18** were formed together as a mixture, but separation was feasible.[33] Bicyclic **19** is chiral and was prepared with its enantiomer as a racemate.[34] The cages in Figures 6 and 7 were studied mainly as carbohydrate receptors and achieved some success, as described in Section 3.2.

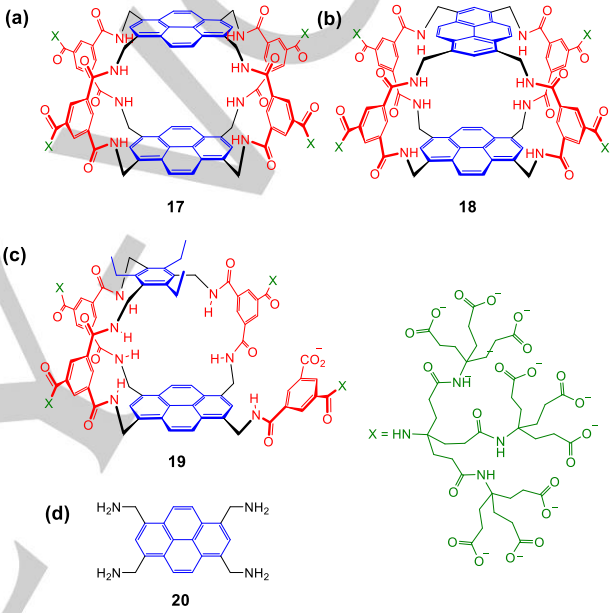

Figure 7. (a,b) Chemical structures of tricyclic pyrene-based receptors **17** ("eclipsed") and **18** ("staggered").[33] (c) Chiral receptor **19**.[34] (d) Tetra-amine intermediate **20** employed for synthesis of receptors **17**-**19**.

Finally receptor **21** from the Jabin group (Figure 8) represents a different type of polylactam architecture, based on a preformed hydrophobic cavity (a calix[6]arene) to which a polar roof has been added. The framework is decorated with neutral oligo(ethylene glycol) units,[35] which confer solubility in methanol/water mixtures. As discussed in Section 3.4 this system presents both H-bond donors and acceptors in its binding site and is complementary to a biotin model.

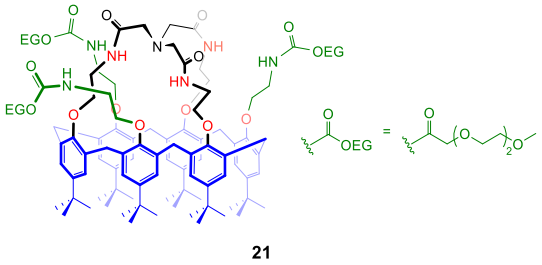

Figure 8. Chemical structure of tricyclic oligolactam receptor **21**.

2.2.3. Macropolycyclic ureas

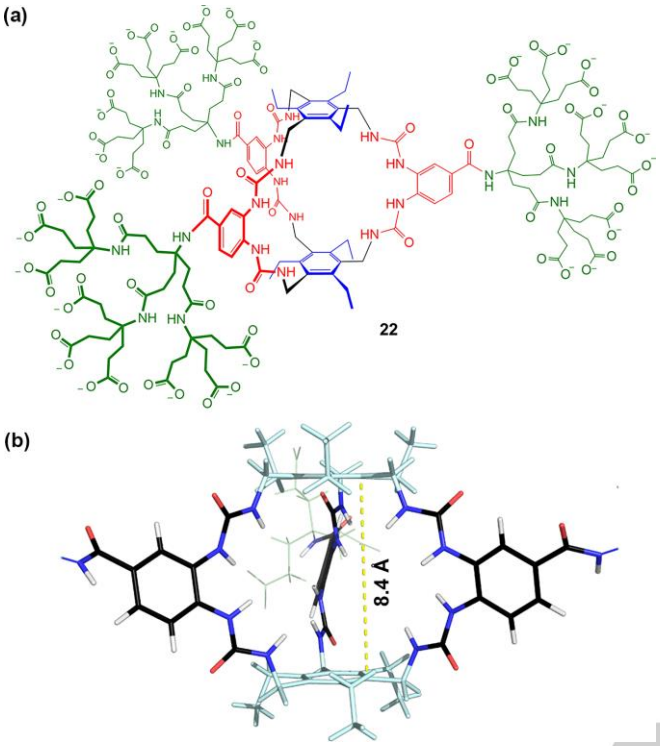

Figure 9. (a) Chemical structure of glucose receptor **22**. (b) Molecular modelling structure of **22** showing the hexaurea binding site (side-chains omitted for clarity).[36] Copyright 2019, Nature Research.

The urea group is less easy to synthesize and handle than the amide, and is more flexible due to the relatively small difference between anti- and syn-CO-NH. On the other hand, the close positioning of two NH groups has obvious potential for hydrogen bonding, so placing urea groups in macropolycyclic structures might seem a promising strategy. Thus far just one such receptor has been reported, the bicyclic hexaurea **22** (Figure 9).[36] As described in Section 3.2 this cage has proved remarkably successful for glucose, an especially important target, so more designs of this type may be explored in future.

2.2.4. Calix[4]pyrroles

Calix[4]pyrroles are tetrapyrrolic macrocycles wherein each pyrrole unit is connected covalently through disubstituted sp^3 hybridized *meso* carbon bridges. They are formed by condensation of pyrrole with ketones and are best known for binding anions in organic media, operating through four inward-directed NH groups.[37] However, employing the NH groups as H-bond donors fixes the calixpyrrole in a cone conformation, and if the *meso* substituents are large they can approach each other to form a hydrophobic pocket. Calix[4]pyrrole therefore offers a good opportunity to develop water-soluble synthetic receptors with amphiphilic interiors. This strategy was employed by the Ballester group, who reported the first water-soluble calix[4]pyrrole-based receptors in 2009.[38] Calixpyrrole **23a**, derived from pyrrole and 4-hydroxyacetophenone, was converted to tetra carboxylic acid **23b** and tetra-amine **23c** (Figure 10a), both soluble in water to 1 mM at pH = 7. ^{1}H NMR spectra indicated that they adopted a time-averaged cone conformation. The crystal structure of tetraester **23d** confirmed this conformation showing converging NH groups and a deep "four-wall" hydrophobic cavity (Figure 10b).

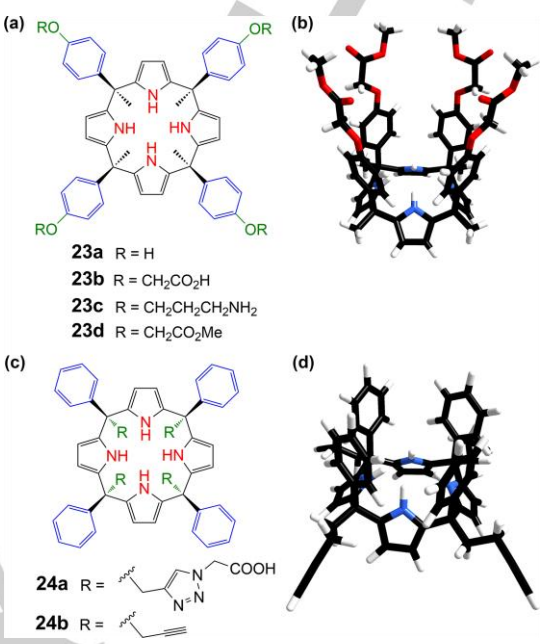

Figure 10. Chemical structures of calix[4]pyrrole-based receptors **23** (a) and **24** (c). X-ray crystal structures of **23d** (b) and **24b** (d).

Placing the solubilising groups at the upper rim may influence guest binding. To obtain full control of the binding site, the Ballester group changed the strategy for solubilisation, moving the polar substituents to the lower rim (Figure 10c).[39] The resulting aryl-extended calix[4]pyrrole **24a** with four carboxylic acid groups, was readily soluble in aqueous solutions at pH 7.4. A crystal structure of **24b** (an analogue of **24a**) confirmed the cone conformation with an aromatic cavity and an average N···N distance of 3.26 Å (Figure 10d). This design strategy also allowed receptor functionalization in both upper and lower rims. In receptors **25** (Figure 11), water-solubilising pyridinium groups are added at the lower rim while the upper rims are covalently bridged by phosphonate groups[40] forming isomers with different relative configurations of the two P=O groups (Figure 11a). A crystal structure of precursor **26** (P=O "out-out" isomer, Figure 11b) highlights the depth and potential tuneability of the cavity.

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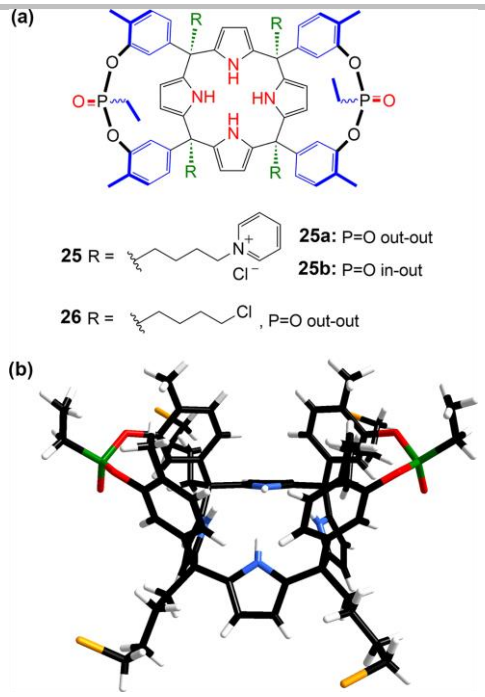

Figure 11. (a) Chemical structures of calix[4]pyrrole-based receptors **25** (water-soluble) and **26** (non-water-soluble). (b) X-ray crystal structure of **26** with P=O "out-out" configuration.

2.2.5. Molecular tweezers and clips

Although macrocyclic architectures are commonly used to preorganise binding sites, other strategies are possible. Klarner and Schrader have developed the "tweezer" and "clip" structures illustrated in Figure 12, using Diels-Alder reactions to build rigid curved frameworks.[41] Figure 12a shows a sequence leading to tweezer **30**; nine annulated six-membered rings are formed in the key cycloaddition between the dienophile centerpiece **28** and two diene walls **27**.[42] The rigid framework creates a preorganised hydrophobic interior lined with aromatic surfaces. Phosphonate groups are added to provide water-solubility and potential for hydrogen bonding, creating an amphiphilic binding site. Phosphate groups may also be used to enhance polar interactions, as in **31**. The synthetic strategy allows tuning of binding site geometry. Thus clip **32**, obtained using an alternative diene component, is more open and can accommodate more bulky substrates than **30** or **31**[41,43] Tweezers and clips have been used to moderate the behaviour of proteins as illustrated in Section 3.1.

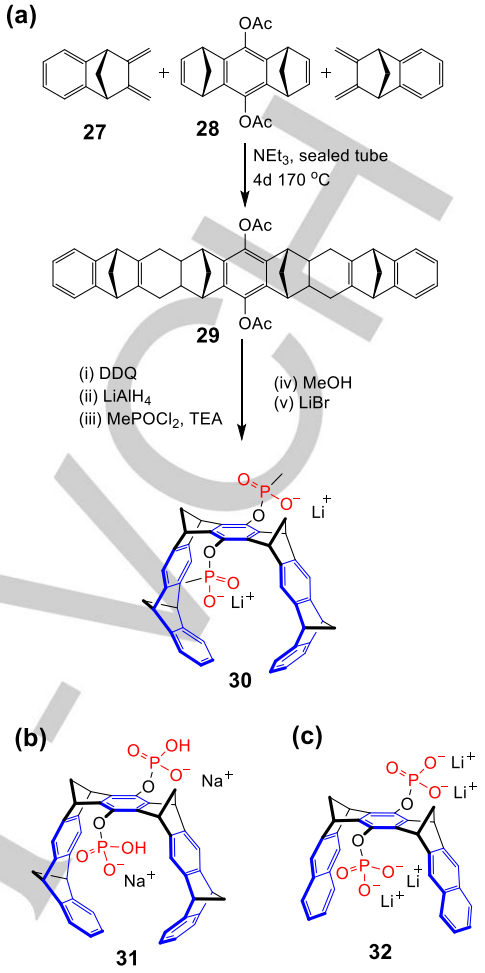

Figure 12. (a) Synthetic route to molecular bisphosphonate tweezer **30**. Chemical structure of phosphate tweezer **31** (b) and phosphate clip **32** (c).

2.2.6. Cucurbiturils

Finally, cucurbiturils such as **CB7** (Figure 13) are well-established as water-soluble receptors capable of exceptionally high affinities.[44] Although they operate mainly through hydrophobic interactions, their cavities are ringed with carbonyl oxygens angled inwards towards potential guests. These oxygens can act as H-bond acceptors, and the binding sites are thus amphiphilic. Hydrogen bonding can play a significant role in molecular recognition by cucurbiturils as discussed in Section 3.1. Various modifications are available, including acyclic analogues which are versatile and tuneable.[45]

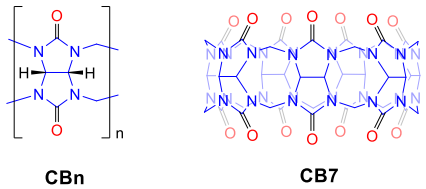

Figure 13. General structure of cucurbit[n]urils **CBn**, with **CB7** as an example.

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3. Host-guest binding by amphiphilic receptors in water.

In this Section we will show how the above structures have been used to demonstrate molecular recognition in water by combining hydrogen bonding with the hydrophobic effect. The discussion is organised according to guest type, considering biologically relevant substrates such as peptides, proteins and carbohydrates, as well as squaraine dyes and other small polar molecules (Figure 14).

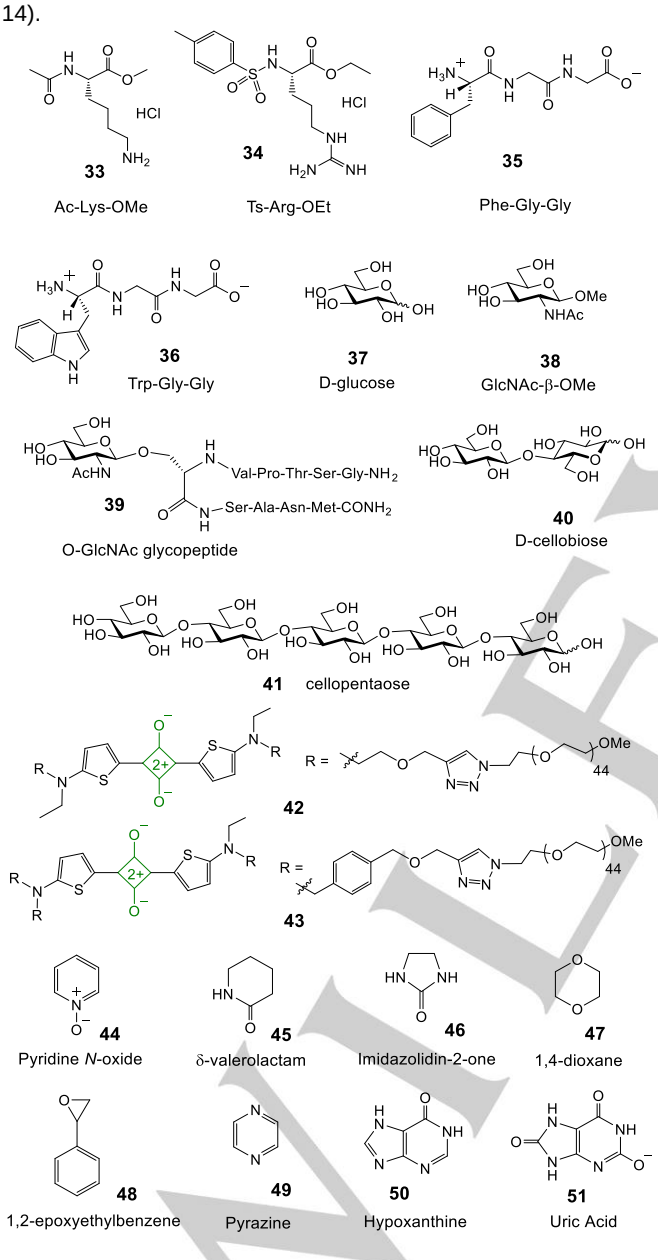

Figure 14. Chemical structures of guest molecules.

3.1. Peptides and proteins

Peptides and proteins are clearly important substrates; if any sequence could be targeted selectively, the biomedical applications would be many and varied. This is beyond our capabilities at present but, for example, selective binding to certain residues is a reasonable objective. Thus, Klarner and Schrader used molecular tweezer **30** to bind the lysine and arginine side chains in derivatives such as **33** and **34**.[42] As shown in Figures 15 a/b, the tweezer interior is complementary to the $(CH_2)_n$ portions of the side-chains, binding through hydrophobic and CH-π interactions. Meanwhile the ammonium and guanidinium termini can form hydrogen bonds with the phosphonate groups, which also H-bond to the sulfonamide NH in **34**. Tweezer **30** binds lysine derivative **33** with K_a ~23,000 M^{-1} in water, although salt effects reduce this affinity to 4400 M^{-1} in phosphate buffer. More recently the Waters group have investigated the binding of *N*-methylated arginine side-chains using receptors obtained via dynamic combinatorial chemistry. Arginine methylation is a post-translational modification with relevance to a number of diseases. Although the binding sites employed are largely apolar, H-bonding to unmodified NH appears to play a role in some case.[46]

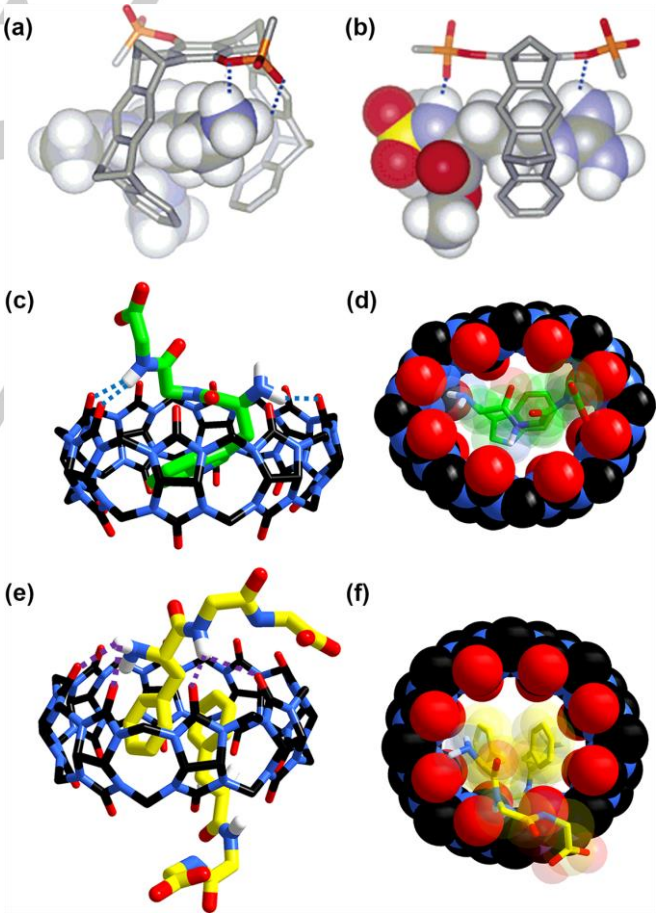

Figure 15. Monte Carlo simulations of the complexes between receptor **30** and Ac-Lys-OMe **33** (a) and Ts-Arg-OEt **34** (b). The results are consistent with NOESY data.[42] Copyright 2005, American Chemical Society. X-ray crystal structures of Trp-Gly-Gly **36**@**CB8** (c-d) and (Phe-Gly-Gly **35**)$_2$@**CB8** (e-f).

Cucurbiturils can also bind selectively to peptides. Given their hydrophobic interiors these toroidal structures might be expected to target aromatic side chains as in phenylalanine and tryptophan. Working with cucurbit[8]uril (**CB8**, *cf.* Figure 13) the group of Urbach showed that this is indeed the case. **CB8** is able to accommodate two aromatic units, and was found to bind and dimerise tripeptides Phe-Gly-Gly (**35**) and Trp-Gly-Gly (**36**) in aqueous solution with high affinity (ternary $K_a = 10^9 - 10^{11}$ M^{-2}).[47] Interestingly, other sequences such as Gly-Gly-Phe were bound very weakly if at all, suggesting that hydrophobic side-chain binding is not the whole story. An X-ray crystal structure of the Trp-Gly-Gly@**CB8** 1:1 complex highlights the role of polar interactions, revealing multiple hydrogen bonds between the NH groups of Gly residue and the carbonyl oxygens of **CB8** (Figures 15c-d). A crystal structure was also obtained of (Phe-Gly-Gly)$_2$@**CB8** (Figures 15e-f), and this again shows several hydrogen bonds between host and guest.

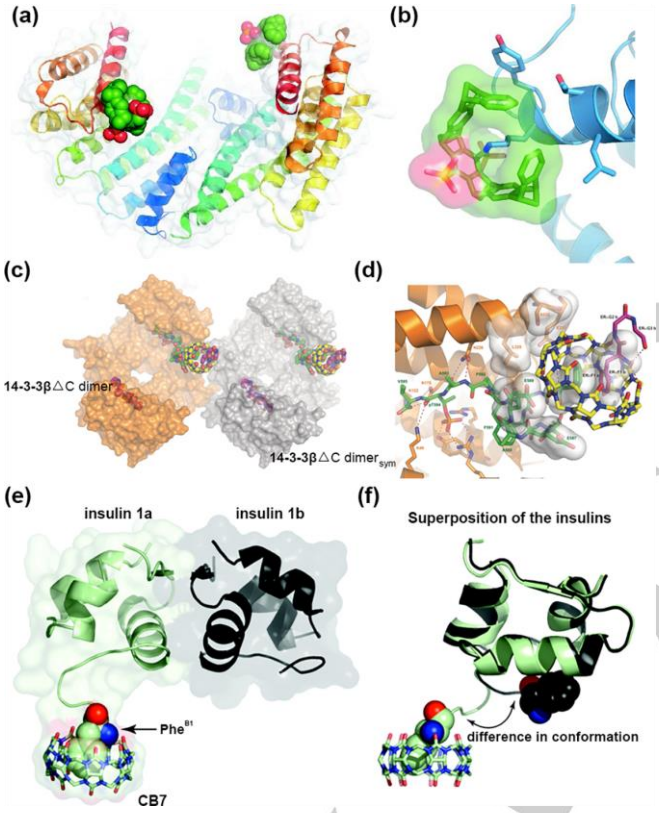

Figure 16. (a-b) Crystal structure of the 14-3-3σ protein@tweezer **31** complex.[48] Copyright 2013, Nature Research. (c-d) Crystal structure of the 14-3-3β/FGG-ER*α*/**CB8** complex (PDB: 5N10).[49] Copyright 2017, Wiley. (e-f) Crystal structure of the insulin@**CB7** complex.[50] Copyright 2011, American Chemical Society.

Both tweezers and CBs have been applied to proteins as well as peptides. The "14-3-3" proteins are targets of interest as they modulate various physiological process through protein-protein interactions. Tweezer **31** was found to bind one specific lysine side-chain of the protein 14-3-3σ as revealed by crystallography (Figures 16a,b).[48] The tweezer inhibited binding of the 14-3-3 protein to two partners, C-RafpS259 and ExoS. **CB8** was also used to modulate a 13-3-3 protein by dimerising two specially designed ligands (Figure 16c-d),[49] Meanwhile, **CB7** was shown to bind to the N-terminal phenylalanine (Phe) of human insulin with an equilibrium association constant of 1.5 × 10^6 M^{-1} (Figure 16e-f). Larger proteins without an N-terminal aromatic residue were bound 50~100 times more weakly, and insulin lacking terminal Phe was bound still less strongly.[50] These studies show that synthetic receptors can produce highly specific effects, even in complex biomolecules.

3.2. Carbohydrates

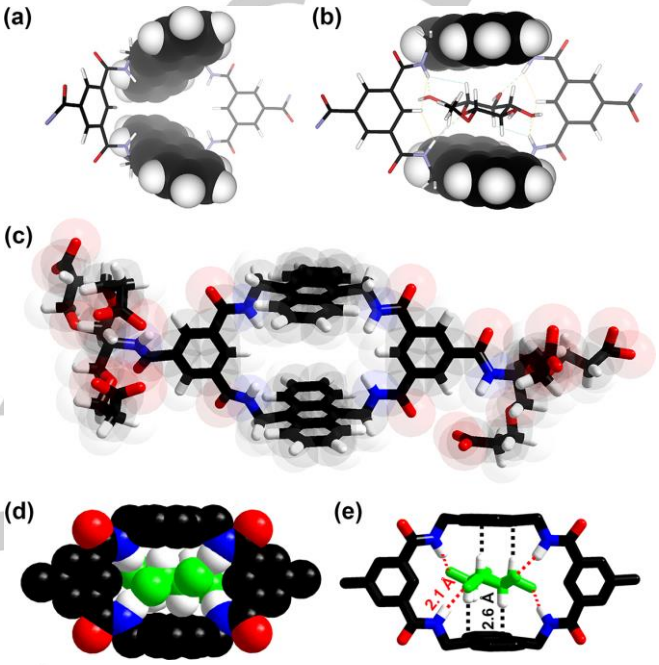

Figure 17. (a) The ground-state conformation of receptor **9** as predicted by Monte Carlo molecular mechanics calculations. (b) Computational model of the complex between methyl β-D-glucopyranoside and **9**.[25] Copyright 2012, Nature Research. (c-e) X-ray crystal structure of **9** complexed to β-D-glucopyranose.[51] (c) Structure of **9** in the complex, featuring four inward-directed NH groups at the center of the hydrophobic cavity. (d,e) Structure of the receptor core with carbohydrate guest, highlighting hydrogen bonding (~ 2.1 Å) and CH–π interactions (~ 2.6 Å).

Carbohydrates are especially challenging targets when dissolved in water, due to their hydrophilic nature and their general similarity to clusters of solvent.[21] Unlike peptides such as **33** and **35**, saccharides mostly lack extended hydrophobic surfaces which can be used to provide the main driving force for binding. Hydrogen bonding is expected to play an important role, but placing enough polar groups in the right positions is difficult. Even natural carbohydrate binding tends to be weak, and synthetic carbohydrate receptors have mostly shown very low affinities. The authors' group have pursued the binding of the "all-equatorial" family of carbohydrates, including glucose **37**, employing designs which sandwich the substrates between aromatic surfaces, complementary to axial CH groups, and H-bond to substrate OH using polar spacers. Most have been macrolactams (e.g. **9**, **10**) or macropolylactams (e.g. **14**, **15**, **17**, **18**), although the hexaurea **22** has played an important role as described below.

Glucose itself is an especially important substrate. Molecules which can bind and sense this target have potential for

application in the management of diabetes, one of the world's most widespread health issues. All the above macrolactams and polylactams bind glucose to some extent, but the most promising (at least initially) were the anthracene-based tetralactams typified by **9**. These systems combined ease of synthesis with moderate but useful affinity for glucose in water (e.g. 56 M^{-1} for **9** + glucose) and an optical response on binding (~3-fold increase in anthracene fluorescence).[25,26] Modelling studies indicated that these macrocycles should bind β-glucose and β-glucosides through a combination of hydrophobic/CH-π interactions and H-bonding (Figure 17a-b) and X-ray crystallography later confirmed this geometry (Figures 17 c-e).[51]

The later discovery that the bis-anthracenyl macrocycles also bind heterocycles such as uric acid **51** (see Section 3.4) has limited their use as glucose receptors, but fortunately the hexaurea **22** has provided a replacement.[36] This bicyclic cage is remarkably complementary to glucose. According to modelling, it is able to bind its target through formation of 10 H-bonds as well as CH-π interactions (see Figure 18a). The affinity of **22** for glucose, at ~18,600 M^{-1}, is far higher than any synthetic alternative and compares well with natural receptors. Perhaps more importantly, the hexaurea shows extreme selectivity for glucose and closely-related substrates, more typical of a biomolecule than a synthetic design. There is a very realistic prospect of employing **22** in medicine, and applications are under active investigation.

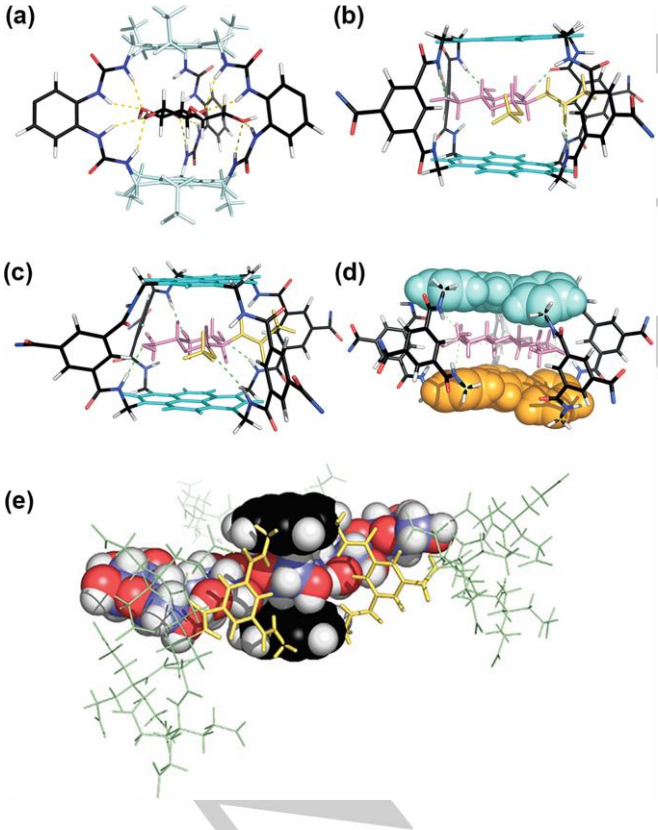

Figure 18. Computational models of complexes between carbohydrates and receptors: (a) D-glucose **37@22**,[36] Copyright 2019, Nature Research.; (b) GlcNAc-β-OMe **38@17**.; (c) GlcNAc-β-OMe **38@18**,[33] Copyright 2016, Wiley.; (d) D-cellobiose **40@14**,[31] Copyright 2007, AAAS.; (e) Cellopentaose **41@15**,[32] Copyright 2016, Nature Research.

Besides glucose, a particular target has been the β-*N*-acetylglucosaminyl (β-GlcNAc or O-GlcNAc) unit as in **38** and **39**. This moiety is a dynamic post translational modification of proteins that is involved in many cellular processes and has been linked to major diseases such as diabetes and Alzheimer's disease. Pyrene-based tricyclic receptors **17** and **18** were especially effective for O-GlcNAc.[33] "Staggered" receptor **18** bound GlcNAc-β-OMe (**38**) in water with an association constant of 18200 M^{-1}, 25 times larger than that of the natural lectin wheat germ agglutinin. "Eclipsed" receptor **17** was less effective than **18** for GlcNAc-β-OMe (2100 *vs* 18200 M^{-1}, Figure 18b-c) but showed even stronger binding to O-GlcNAc glycopeptide **39** (K_a ~ 70000 M^{-1}). For both **17** and **18** detailed NMR structures were obtained for complexes with **38**, revealing several intermolecular NH···O interactions. This work further demonstrates that biomimetic receptors can show affinities comparable to those of natural lectins.

Other systems have yielded good results with oligo- and poly-saccharides. In work published in 2007, tetracyclic receptor **14** was designed to encapsulate cellobiose **40**, an all-equatorial disaccharide (see Figure 18d). The binding results were excellent for the time, for example K_a = 600 M^{-1} for **14** + **40**, but just 11 M^{-1} for **14** + lactose (a disaccharide with just one axial OH).[31] The role of H-bonding was inferred from NMR-constrained molecular modelling. A later development was the bicyclic pyrene-based receptor **15**, featuring a much more open architecture. In this case oligo/polysaccharides are capable of threading through the cavity creating pseodorotaxanes (see Figure 18e). Affinities up to 12,000 M^{-1} were measured for cellodextrins such as **41**.[32] Again, H-bonding was inferred from NMR-based molecular modelling. Multiple threading on cellulose was also observed, suggesting potential for moderating the properties of this abundant natural polymer.

3.3. Squaraine dyes

Squaraine dyes such as **42**/**43** are substrates of special interest. They show intense fluorescence, typically in the red and near infrared region, making them very attractive for many types of biological imaging, sensing, and light harvesting applications. They also possess disadvantages, e.g. instability and a tendency to aggregate in water, but it turns out that these problems can be solved by complexation with water-soluble anthracenyl tetralactams such as **9**-**11**. Moreover, the combination of tetralactam and squaraine provides an outstanding example of amphiphilic complementarity (Figure 19a). The squaraine consists of a polar core with divergent oxygens, strong H-bond acceptors, flanked by hydrophobic aromatic groups. The anthracenyl tetralactams feature inward directed H-bond donors surrounded by hydrophobic aromatic surface, capable of sandwiching the squaraine guest. This leads to exceptionally high affinities, raising the possibility that the tetralactam-squaraine combination could be used as a "supramolecular glue" under biological conditions, complementing the widely-used biotin-avidin pairing.[52]

The potential of the squaraine-tetralactam system was first revealed in a study by the B. D. Smith and Davis groups, which showed that **9** binds **42** with $K_a = 1.1 \times 10^9$ M^{-1} in water, among the highest measured for synthetic systems.[53] The threading process was also notably fast, with $k_{on} \approx 10^6 - 10^7$ M^{-1} s^{-1}.

Usefully, the squaraine absorption and emission bands are red-shifted when the dye is encapsulated, providing visual signals that binding has taken place. Moreover, affinities could be further increased by appending *N*-benzyl groups to the squaraine core, as in **43**. After threading, the aromatic rings can fold back and make additional hydrophobic contacts with the receptor, leading to a picomolar affinity (K_a = 5.1 × 10^{10} M^{-1}) and very rapid threading (k_{on} = 7.9 × 10^7 M^{-1} s^{-1}) (Figure 19b).[54]

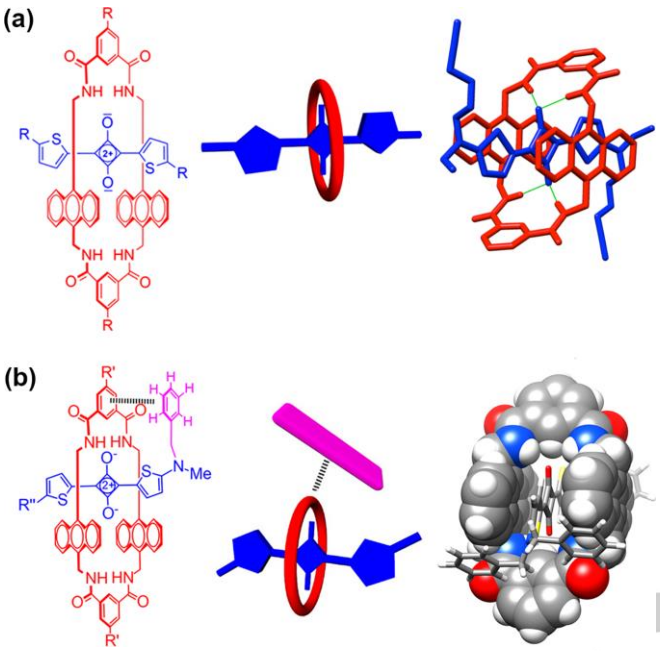

Figure 19. (a) Schematic illustration (left) and computational model (right) of squaraine-tetralactam system, showing intermolecular hydrogen bonds.[53] Copyright 2015, American Chemical Society. (b) Schematic illustration and computational model of enhanced host−guest binding through additional hydrophobic contacts between squaraine dye and receptor.[54] Copyright 2018, American Chemical Society.

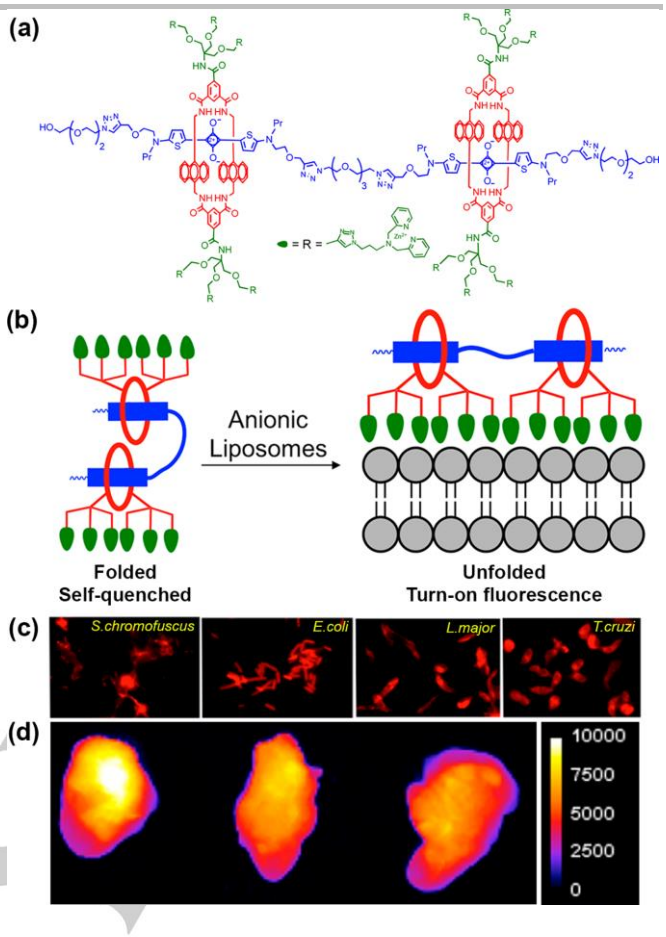

Figure 20. (a) A preassembled fluorescent probe for imaging anionic membranes. (b) The probe adopts an unfolded conformation, with turn-on fluorescence, upon association with anionic liposomes. (c) Fluorescence micrographs of *S. chromofuscus*, *E. coli*, *L. major*, and *T. cruzi*, stained with the probe. (d) Biodistribution of the probe in tumor-bearing rats at 24 h after dosing.[28] Copyright 2017, American Chemical Society.

This synthetic avidin ("synthavidin"[54]) system has been employed in self-assembly processes for biological applications. For example, the receptor **11** was bound to a linear scaffold with two squaraine docking stations to produce a dodecavalent fluorescent probe (Figure 20a).[28] The dodecavalent probe displayed turn-on fluorescence due to probe unfolding at negatively charged phospholipid membrane surfaces (Figure 20b). The probe was shown to have imaging selectivity in different types of microbial and mammalian cell culture as well as in a living rat tumor model (Figure 20c-d). This work highlights the potential of synthavidin technology for imaging applications, and many other uses can be envisaged.

3.4. Other substrates

There remain a variety of small polar substrate molecules which have been addressed by the receptors discussed in Section 2. Heterocycles have been a particular focus, given that many are water-soluble, most are capable of hydrogen bonding, and many are biologically relevant. Heterocycles also tend to have well-defined geometries so that structural predictions are more reliable. For example, pyridine *N*-oxide (**44**) is nicely complementary to the binding sites of the calix[4]pyrroles featured in Section 2.2.4, and has been used as a benchmark substrate for these systems. Thus, receptors **23b** and **23c** were shown to bind **44** with K_a = of 2.4 × 10^3 and 1.5 × 10^3 M^{-1} in water respectively.[38] The role of hydrogen bonding was supported by an X-ray crystal structure of **44** bound to methyl ester **23d** revealing a set of four NH···O^- H-bonds (Figure 21a). The capped structure **25a**, with inward directed ethyl groups, bound **44** considerably more strongly at K_a = 7 × 10^6 M^{-1}.[40] Presumably this reflects the increased hydrophobicity of the cavity. **25a** was also a good receptor for δ-valerolactam **45** (K_a = 2.3 × 10^5 M^{-1}). This substrate was bound eight times less strongly by in-out isomer **25b**, with its inward-directed P=O group and less hydrophobic binding site. The Jabin group's macropolylactam **21** is somewhat similar to the

calixpyrroles in possessing an array of inward-directed NH groups. It also features ether oxygens, which can act as H-bond acceptors, and this combination is complementary to imidazolidin-2-one (**46**) (a model for biotin). Tested in D_2O/CD_3OD (1:2), **21** bound only weakly to **46**, but a crystal structure of a model complex confirmed the expected binding mode with five intermolecular H-bonds (Figure 21b).[35]

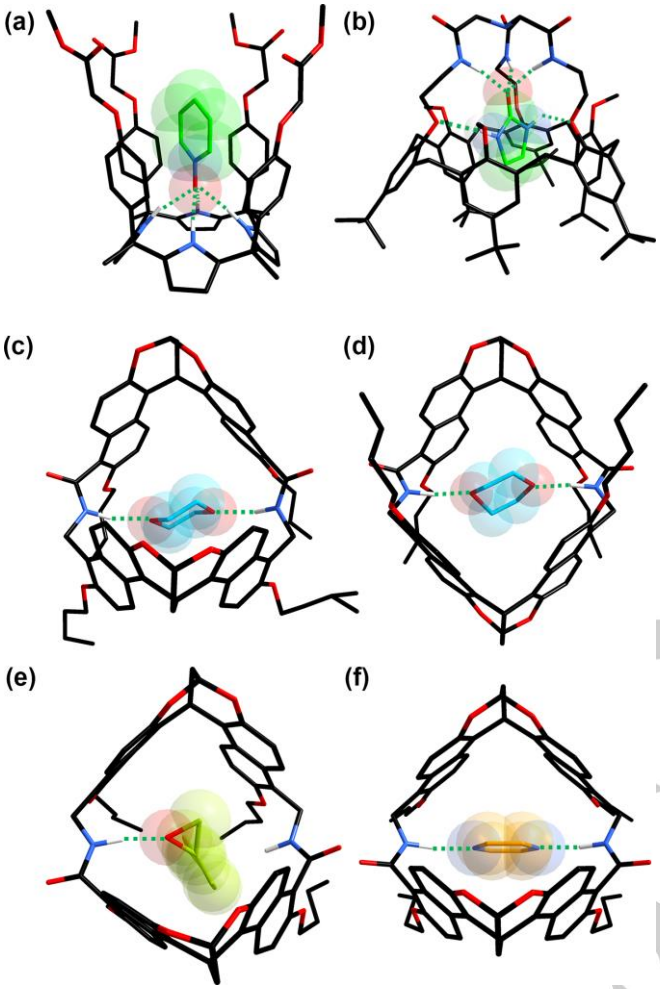

Figure 21. Crystal structures showing heterocycles in amphiphilic binding sites. The hosts are less polar analogues of water-soluble receptors: (a) Pyridine *N*-oxide **44**@**23d**; (b) Imidazolidin-2-one **46** in a variant of **21**; (c) 1,4-dioxane **47**@**12b**; (d) 1,4-dioxane **47**@**13b**; (e) 1,2-epoxyethylbenzene **48**@**12b**; (f) Pyrazine **49**@**12b**.

The bis-naphthalene-based macrolactams discussed in Section 2.2.1 (Figure 5) have been investigated with a wide range of small molecules. An early study identified 1,4-dioxane (**47**) as an especially good substrate, binding to *syn*-receptor **12a** with an association constant of 13500 M^{-1}, and *anti*-receptor **13a** with K_a = 3150 M^{-1}.[30] X-ray crystal structures of model complexes reveal that the 1,4-dioxane is stabilized by two hydrogen bonds in both *syn*- or *anti*-analogues (Figures 21c-d). It was later shown that the receptors can recognize epoxides, such as 1,2-epoxyethylbenzene (**48**).[55] As before, the association constant for *syn*-receptor **12a** (5.3×10^4 M^{-1}) was found to be higher than that for *anti*-receptor **13a** (2.4×10^4 M^{-1}). The role of hydrogen bonding in the epoxide complexes was also evidenced by X-ray crystal structures (Figure 21e). In a further development, N-heterocycles such as pyrazine (**49**) were added to the successful substrates.[56] Again, crystallography provided evidence for H-bonding in the complexes (Figure 21f).

Finally the bis-anthracenyl tetralactams such as **9**, also introduced in Section 2.2.1 (Figure 4), have already been discussed as receptors for carbohydrates (weak) and squaraines (very strong) (see Sections 3.2 and 3.3). Recently it has emerged that these systems are also effective for aromatic heterocycles, including a number of biogenic purine and pyrimidine bases. A study performed using dodeca-carboxylate receptor **10** recorded the unusually high affinity of ~10^7 M^{-1} for hypoxanthine **50**, and ~10^5 M^{-1} for other substrates such as adenine, caffeine, theobromine and uric acid **51**.[27] As with earlier substrates, binding presumably results from a combination of hydrophobic interactions, in this case with π-stacking, and NH···X hydrogen bonds (X = O or N; see Figure 22). The role of hydrogen bonding was confirmed by downfield movements of the N*H* NMR chemical shifts. The versatility of these tetralactams is hardly an advantage, but more a hindrance to the development of applications – as mentioned earlier, binding to uric acid prevented their use in glucose sensing. The history of this system perhaps serves as a warning – in practical terms, establishing binding is just the first step, rejection of other potential substrates is equally important and may be far more challenging.

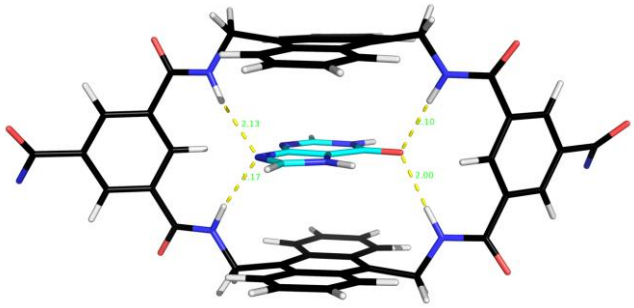

Figure 22. Computational model of the tetralactam core of **10** with hypoxanthine **50**.

4. Conclusions and Future Perspectives

Supramolecular chemistry has the potential for a wide variety of biomedical applications, provided sufficient selectivity can be achieved, and provided it can cope with complexities of binding in water. The deployment of hydrogen bonding in water is a key problem in this area and, as illustrated in this article, is attracting increasing attention. Although water attenuates H-bonding by competing for both donors and acceptors, it is becoming clear that amphiphilic binding sites providing full complementarity to substrate molecules can be remarkably effective. The highest affinity mentioned herein, for the binding of squaraine **43** to a tetralactam, is 5 x 10^{10} M^{-1} (see Section 3.3). Although it is sometimes argued that hydrophobic effects drive association in water while hydrogen bonding controls selectivity, it is difficult to believe that such strong binding results from hydrophobicity alone. However, it does seem likely that H-bonding is more effective when deployed within an amphiphilic cleft or cavity. Donors and acceptors surrounded by apolar surfaces may be poorly hydrated and, if positioned correctly, provide major contributions to binding free energy.

In practical terms, there are still few examples of systems which bind their targets with the affinity and selectivity required for applications, but recent developments are encouraging. For example, the hexaurea **22** shows real promise as a component of biocompatible glucose-responsive systems. In 2018, the rights to this receptor were sold to Novo Nordisk, a major pharmaceutical company specialising in diabetes, and a program to develop glucose-sensitive insulin is currently under way. Other applications are being pursued by Carbometrics, a start-up company in Bristol. Meanwhile, the squaraine-tetralactam system performs remarkably well as a high-affinity binding pair, and would seem to have genuine potential for controlled self-assembly and imaging.

Looking to the future, the design of preorganised binding sites with correct positioning of both polar and apolar components remains a difficult challenge. Routine modelling software is quite effective at predicting structures of rigid molecular frameworks, but not so useful for flexible architectures surrounded by water molecules. The prediction of binding modes and affinities is still less certain. This is probably the main reason why, as highlighted in this article, the range of receptor structures studied is still quite limited. Improved computational methodology might open up a wider range of options, still (probably) incorporating rigid components, but less exclusively so. In any case, much remains to be done in this area and we hope that efforts will continue to intensify. Supramolecular chemistry should aim to contribute to human health, and taming hydrogen bonding in water will be a key to success.

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Keywords: water-soluble receptors • hydrogen bonds • host-guest systems • molecular recognition

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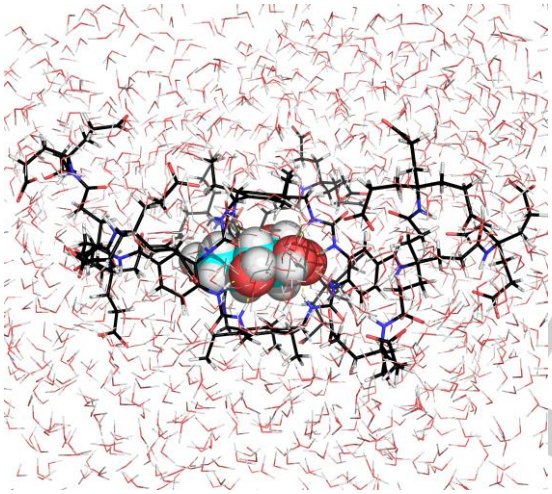
Entry for the Table of Contents



Hydrogen bonding is weakened by water, and thus hard to deploy under biological conditions. Nonetheless, there is increasing interest in solving the problems so that supramolecular chemistry can be exploited in medicine. This minireview highlights recent developments, including some with genuine promise for real-world applications.