Review

Israel Journal of Chemistry

DOI: 10.1002/ijch.200((full DOI will be filled in by the editorial staff))

Pillar[*n*]arenes at the Chemistry-Biology Interface

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Abstract: Macrocyclic chemistry has provided chemists with a wealth of molecular 'hosts'. Ever since resurgence in the field during the 1970s and 1980s these hosts' similarities to natural structures, such the active sites of enzymes, have been noted. Latterly there has been great interest in the recently reported pillar[*n*]arenes. As if to underline the importance of these compounds, exciting applications are starting to emerge, from electrochemical sensors to antimicrobial agents. Novel uses appear destined to have an impact on clinical conditions from Alzheimer's disease to cancer treatment. In order to demonstrate the impact of pillar[n]arenes across the chemistry-biology interface this review will cover the current state of the art from biomimicry and analyte-specific detection to emerging clinical applications. Examples include pillar[n]arene-based ion channels, enzyme-responsive compounds, imaging agents, biofilm inhibiting derivatives, drug complexing and drug releasing systems.

Keywords: Pillar[*n*]arenes • Macrocycles • Supramolecular chemistry • Receptors • Molecular recognition

1. Introduction

In the short time since the first paper on pillar[*n*]arenes was published[1], this new family of macrocycles has found diverse applications across the sciences. One area of interest is the interface between chemistry and biology from biomimicry to diagnostics and therapeutics. Many excellent reviews on pillar[*n*]arenes are available[2] but this review seeks to encompass those aspects of pillar[*n*]arene research which impact on biology, medicines and healthcare.

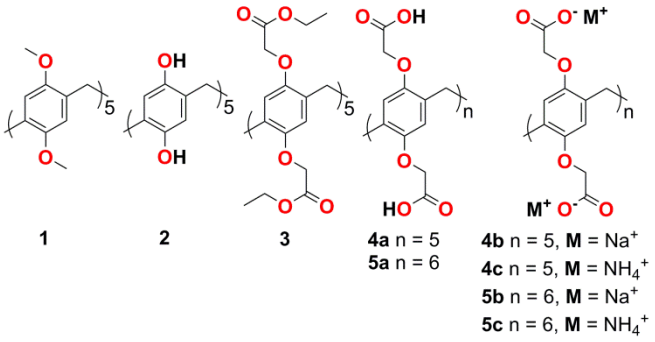

Figure 1. Key pillar[*n*]arene derivatives **1** - **5**.

Although many derivatives appear in the following pages, it is fortuitous that most key compounds can be accessed from Ogoshi's 1,4-dimethoxypillar[5]arene (**1**) via demethylation to the perhydroxylated species (**2**) and subsequent alkylation (e.g. **3**). Of particular note is the extensive use of carboxylatopillar[5]- and [6]arenes (**4a** and **5a**) together with their sodium (**4b** and **5b**) or ammonium (**4c** and **5c**) salts, as shown in Figure 1.

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2. Biomimetic pillar[n]arenes

2.1. Transmembrane channels

The phospholipid bilayers that form the exterior membranes of prokaryotic and eukaryotic cells are pierced by proteins and aggregates of small molecules to allow the passive and active transport of chemical species, from simple hydrated ions and metabolites to nucleic acids and proteins. While crystal structures of transmembrane proteins, electrophysiological techniques and computer simulations have advanced our knowledge of how these systems function, synthetic analogues have also been valuable in aiding our understanding of transmembrane transport phenomena.[3] With their well-defined macrocyclic annulus, pillar[*n*]arenes present rigid core which can be extended by *O*-alkylation to span phospholipid bilayers.[4]

The first indication that pillar[*n*]arenes had the potential to function as molecular channels came when Chen, Hou and colleagues published a trio of papers revealing how perhydroxypillar[5]arene (**2**), its ethyl ester (**3**) and other derivatives aligned themselves into tubes through which water molecules could pass.[5] These 'water wires' were able to conduct protons in a similar manner to electrons in a conventional metallic wire. The initial observation, backed up by X-ray crystallography, indicated that pillar[5]arene dimers could conduct protons by spanning an artificial phospholipid bilayer comprised of phosphatidylethanolamine, phosphatidylcholine (PC), phosphatidylserine (PS) and cholesterol. The proposed mechanism requires two pillar[5]arene molecules to align for charge to conduct protons in a manner similar to the gramicidin class of helical peptides. To give greater permanence to these channels, pairs of pillar[5]arenes were connected by alkyl chains of four to eight carbons through ester or amide links e.g. **6**. The optimum chain length for ester or amide dimers was found to be six carbons with the shortest and longest linkers imparting no channel activity. Conductivity occurred between pH 4.0 and 5.5 with the

current increasing with decreasing pH consistent with a proton transfer mechanism. Addition of alkali metal cations had no effect except for Na^+ which inhibited conductance through proposed complexation by the pillar[5]arene terminal ester groups. Figure 2 illustrates several examples of channel-forming derivatives.

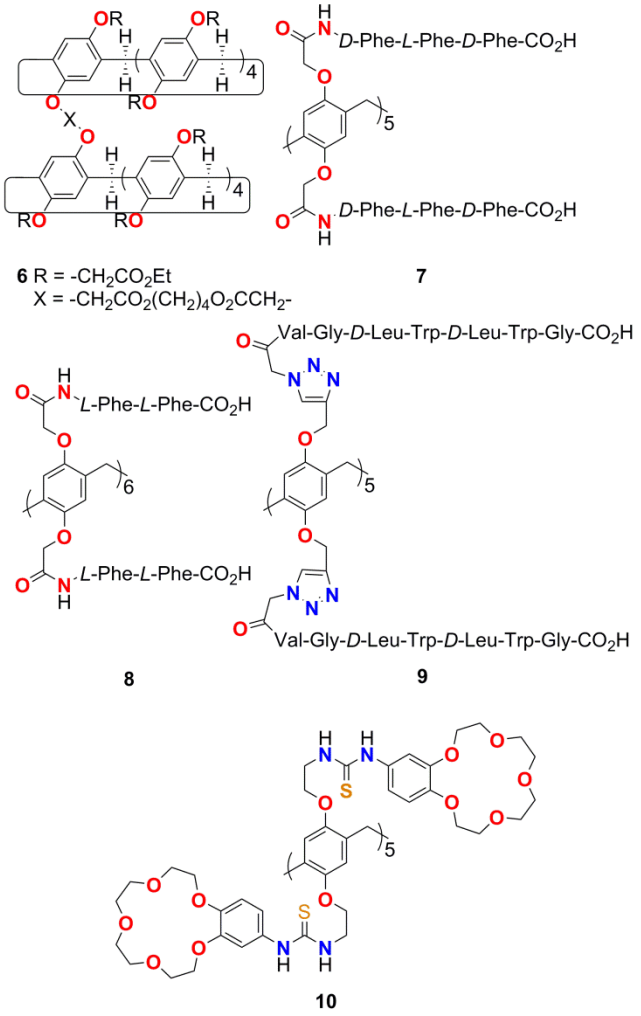

Figure 2. Transmembrane channel-forming pillar[*n*]arene derivatives **6** – **10**.

The Hou group then prepared a number of pillar[5]- and [6]arene amides with tripeptide substituents (e.g. **7** and **8**).[6] The amide links can form complementary hydrogen bonds, in a manner reminiscent of β-sheets found in proteins, and the substituents extend the cavity to about 3.5 nm which approaches the thickness of natural phospholipid bilayers. Pillar[*n*]arenes with sequences of *D*- and *L*-Phe moieties terminating in carboxylic acids or ethyl esters were inserted into large unilamellar vesicles made from egg yolk *L*-α-PC (EYPC-LUVs) and loaded with ^{13}C-labelled glycine. Both pillar[5]- and [6]arene with all-*L*-Phe substituents terminating in acid groups gave enhanced Gly transport through the vesicle membrane over passive diffusion. Similar experiments with *L*-Ala, *L*-Ser, *L*-Thr, *L*-Val, *L*-Leu and *L*-Phe demonstrated that all were transported by the pillar[6]arene derivatives, particularly those with the all-*L* sequence, whereas only Gly, *L*-Ala, *L*-Ser and *L*-Thr saw any enhanced transport by the smaller macrocycle. Transport activities were demonstrably higher for *L*-Phe over *D*-Phe when pillar[5]arene with all-*L*-Phe substituents was present in the vesicle.[6a] Pillar[5]arene **7** was shown by Kumar and Hou to increase water permeability through vesicles formed from PC and PS.[6b] Molecular dynamics suggested that arrays of macrocycles could aggregate and that this may change the morphology of the lipids by increasing the size of the vesicles until they became unstable and formed bilayers. As the pillar[n]arene derivatives could extend through the bilayer they can function as single channel conductors. This is evident from the current traces when the compounds were added to planar lipid bilayers which show clear 'open' and 'closed' states. Furthermore, transport rates were found to be similar to carbon nanotubes and only an order of magnitude lower than natural aquaporins. Other pillar[5]arenes with polypeptide substituents were found to have interesting biomimetic activity. The insertion of pillar[5]arenes with tripeptide substituents incorporating a single *D*- or *L*-Arg could be controlled by the voltage applied to EYPC-LUVs containing NaCl or KCl[6c] and pillar[5]arenes incorporating *L*-Trp had antimicrobial activity against Gram-positive bacteria *Staphylococcus epidermidis* but not against Gram-negative *Escherichia coli* or rat erythrocytes.[6d]

Xin, Dong and Chen appended two peptide sequences analogous to gramicidin A, along with shorter sequences such in **9**, to a pillar[5]arene through click chemistry.[7] In doing so they generated macrocycles capable of spanning a phospholipid bilayer. Experiments on EYPC-LUVs and planar bilayers comprised of 1,2-diphytanoyl-*sn*-*glycero*-3-phosphocholine indicated enhanced H^+ and K^+ transport, respectively. The compounds' biological activities were assessed against *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *S. epidermidis* and found to be less active against Gram-negative *E. coli* that the other Gram-negative bacteria. It was presumed that the mechanism of action was through membrane targeting and, for this reason, the compounds would have low haemolytic toxicity. Tests carried out on rat erythrocytes showed the compounds to have significantly lower haemolysis rates than gramicidin A. As with the gamicidins and the honeybee venom protein mellitin, these compounds undoubtedly form α-helices with the hydrophobic amino acid sidechains anchoring the pillar[5]arene in the phospholipid bilayer.

Feng and Barboiu appended benzo-15-crown-ether-5-ureido groups to a pillar[5]arene to give **10** with the intention that the crown ethers would complex and transport alkali metal cations.[8] NMR data suggested the formation of dimers at concentrations of 12 mM. When the compound was incorporated in EYPC-LUVs, transport data showed selectivity for K^+ and Rb^+ over the other cations tested. While the formation of some type of channel was proposed, the authors could not discount a shuttling mechanism in which two crown groups formed a sandwich with the cations.

Sun and Li approached the concept of ion-selective channels from a different angle. Having fabricated a nanopore in a polyimide membrane with an opening of 450 nm and a tip of 16 nm, they linked the ionic liquid 1-(3-aminopropyl)-3-methylimidazolium cation to the interior of the nanopore.[9] The effect of this was to allow anions to pass through the nanopore. Subsequently **4a** was added which bound to the imidazolium groups and reversed the selectivity towards cations. What made the system particularly interesting was that the effect could be reversed

by heating to 55ºC giving an analogue of a temperature sensitive ion channel.

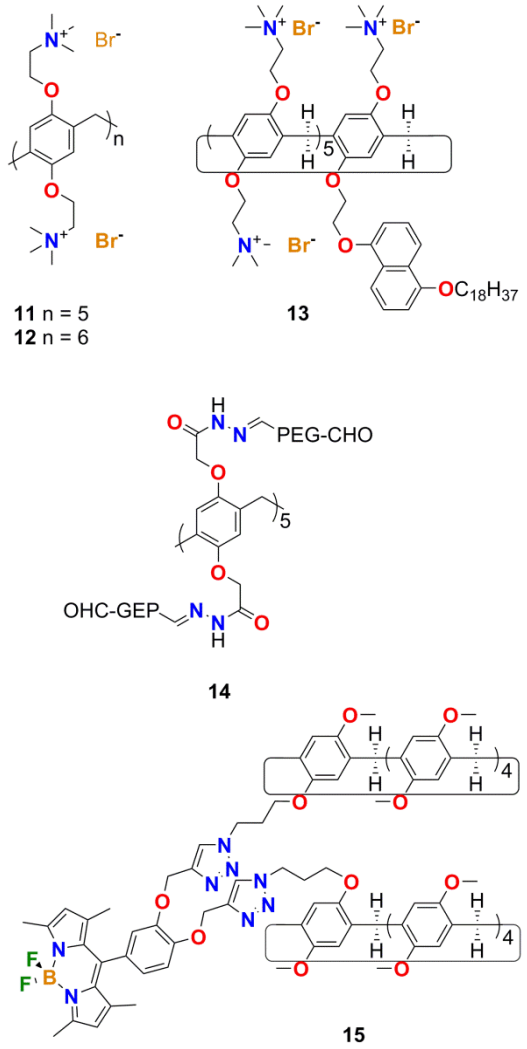

Figure 3. Enzyme-responsive and biomimetic pillar[*n*]arene derivatives **11** – **15**.

2.2. Enzyme-responsive and other biomimetic pillar[n]arenes

Water-soluble pillar[*n*]arenes make excellent capping agents during metal nanoparticle synthesis as noted by the groups of Pérez-Juste and Pastoriza-Santos which used **11** to prepare 120 nm gold nanoparticles (AuNPs).[10] Park, Moon, Kim and colleagues applied a similar method when using **5a** to generate AuNPs which, in the presence of silver cations, exhibited greater peroxidase-like activity than their citrate-stabilized analogues.[11] As with better known capping agents such as citrate, it is presumed that the pillar[6]arene's carboxylate groups spread out to cover the surface of the growing AuNPs until coverage is complete and growth ceases. Yu and colleagues prepared nanoparticles and nanosheets from **4a** and acetylcholine with an appended pyrene substituent.[12] Addition of acetylcholine esterase (AChE) cleaved the pyrene group leading to the formation of nanoribbons. Introduction of $HAuCl_4$ and $NaBH_4$ to the nanoparticles or nanosheets generated hybrid materials incorporating 6 nm AuNPs which grew on the addition of AChE. The hybrids were then used to catalyse the borohydride reduction of 4-nitroaniline to 1,4-diaminobenzene. In another example of enzyme responsive morphology, Zhou and Diao prepared micelles from **13**.[13] Their formation reflects the surfactant-like structure of the co-pillar[6]arene where the macrocycle is decorated with charged species and able to interact with water whereas the C_{18} substituent promotes micellar aggregation through hydrophobic effects. Hydrodynamic diameter measurements of 5 nm were consistent with two molecules meeting tail-to-tail in a micelle. Subsequent addition of adenosine triphosphate (ATP) transformed the micelles into vesicles which increased in diameter to 150 nm which was reversed on addition of alkaline phosphatase. In addition, the vesicles could be loaded with calcein which responded to alkaline phosphatase by triggering its release. The Liu group used an enzyme-responsive pillar[5]arene to form a self-healing polymer.[14] A pillar[5]arene ester coupled to an aldehyde-functionalized PEG_{4000}, **14**, was shown to form a crosslinked hydrogel but to introduce self-healing functionality glucose oxidase and catalase were added to the gel scaffold. Addition of glucose to a cut in the gel resulted in reaction with glucose oxidase and a reduction in pH which reformed the aldimine with the concomitant release of hydrogen peroxide ameliorated by catalase. Sun and Yen used the displacement of acridine orange from **5a** by choline to indicate the neurotransmitter's presence and showed that when choline oxidase was added it reacted to form glycine betaine and fluorescence was switched off.[15]

The Wang group has investigated another area of biomimicry: light harvesting.[16] A BODIPY-bridged pillar[5]arene dimer, **15**, and two BODIPY derivative guests combined to form highly stable supramolecular polymers that exhibited very strong absorption from 300 to 700 nm and, with energy transfer efficiencies up to 63%, represent one the best light harvesting models currently available.

Harnessing the enzyme-like and enzyme-responsive behaviour pillar[*n*]arene-based materials, such as those shown in Figure 3, will have clear impacts in areas such as stereoselective synthesis and sensor technology.

3. Pillar[*n*]arenes for detection

3.1. Imaging

The ability to functionalise pillar[*n*]arenes with a wide variety of substituents whilst keeping their inclusion properties almost unchanged was recognised by Stoddart as a route to molecular recognition and signalling.[17] Incorporating a single pyrene group to a methylated pillar[5]arene allowed **16** (Figure 4) to signal guest inclusion by quenching fluorescence through photoinduced electron transfer. The guests in question, alkylamines, alkanediamines and polyamines, could be detected at concentrations below 100 μM using 57 μM solutions of the pillar[5]arene in a 1:1 acetonitrile-water solution. Association constants of diaminoalkanes with three to eight carbon atoms were between 1.0×10^4 M^{-1} and 3.6×10^4 M^{-1} with ocylamine, spermine and spermidine all falling in the same range. Only *n*-hexylamine, with an association constant of 0.3×10^4 M^{-1} was significantly outside this range.

The Cohen group demonstrated that aqueous solutions of **4c** could encapsulate *n*-hexane diffused into the solution.[18] Subsequent bubbling of xenon gas led to the formation of an

inclusion complex involving one hexane molecule and one hydrophobic xenon atom as determined by ^{1}H and ^{129}Xe NMR. This suggests a route by which biomarkers containing xenon may be prepared.

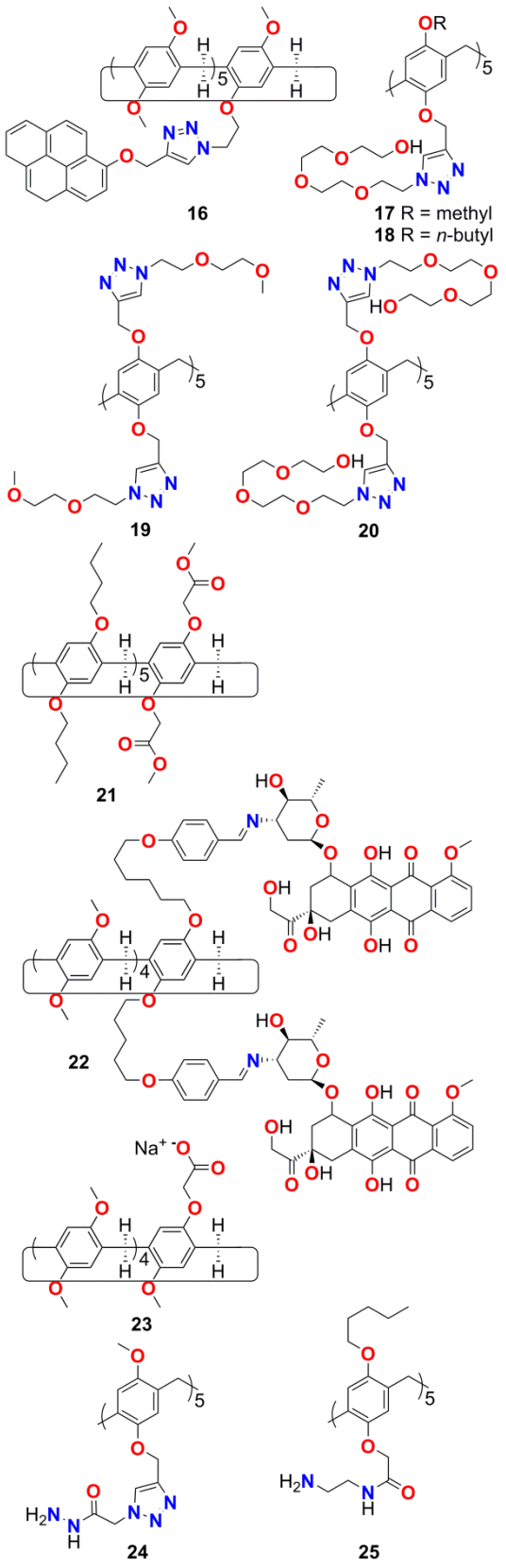

Figure 4. Pillar[*n*]arenes **16** – **25** for imaging.

Zhao and colleagues prepared vesicles from amphiphilic symmetric and asymmetric pillar[5]arenes **17** to **20**, shown in Figure 4.[19] Triethylene glycol or diethylene glycol monomethylether substituents, attached by click chemistry, facilitated vesicle formation in the presence or absence of rhodamine B, fluorescein isothiocyanate and a water-soluble viologen derivative. All the amphiphilic pillar[5]arenes were able to deliver rhodamine B but only asymmetric pillar[5]arenes could deliver the more hydrophobic fluorescein which enabled dual imaging of cells. Assays on immortalised cervical cancer HeLa cells indicated biocompatibility of the micelles formed from the pillar[5]arenes, however, they were also able to deliver doxorubicin (DOX) which was observed in both the cytoplasm and the nucleus.

The Xue group used **4a** as the capping agents of spherical silver nanoparticles (AgNPs) which were 18.7 ± 2.18 nm in diameter.[20] The AgNPs were stable and monodispersed in water so, given the pillar[5]arenes' propensity to bind amines and diamines, the effect of seven di- and polyamines was investigated. While some change in colour was observed for ursol, ethanediamine, 1,12-dodecylamine or 1,6-hexamethylenediamine, addition of the polyamines spermine, tetraethylenepentamine and triethylenetetramine resulted in a black precipitate. The authors proposed that this effect could be used to detect ultralow concentrations of polyamines by surface-enhanced Raman spectroscopy. The same group prepared AgNPs with water-soluble pillar[5]- or [6]arene with imidazolium substituents as capping agents.[21] Following the addition of aqueous amino acid 10^{-3} M solutions (Lys, Arg, His, Gly, Glu, Tyr, Asp, and Thr) only glutamatic acid elicited a red to yellow colorimetric response.

The Huang group prepared two cruciform species with either ethylpyridinium or naphthyl termini.[22] These stacked to form nanorods of charge transfer complexes, however, the cationic compound could act as the core of a supramolecule upon the addition of four equivalents of **21**. Administration of this complex together with the naphthyl species to MCF cells led to dethreading of the ethylpyridinium derivative and nanorod formation and blue fluorescence in the cytoplasm. Fluorescence was not observed in non-cancerous HEK293 cells due to their relatively higher pH. The group followed this work with the synthesis of pillar[5]arene-DOX conjugate **22**.[23] An asymmetric rotaxane thread with a triphenylphosphine stopper linked to a 4-(4-(1,2,2-triphenylvinyl)phenyl)pyridinium moiety by an alkyl spacer was linked through the pillar[5]arene to give a highly fluorescent rotaxane. The prodrug localised in the mitochondria of HeLa cells but not HEK293 cells, suggesting a method of delivering DOX to cancer cells whilst imaging drug delivery. The Yu group mixed 1-ethyl-4-(4-(1,2,2-triphenylvinyl)phenyl)pyridinium bromide with **23** to form a fluorescent complex.[24] The complex assembled into spherical particles with an average diameter of 43 nm which were administered to HeLa cells. No significant cytotoxicity was found and the highly fluorescent complex localised in the cytoplasm. These fluorescent pillar[*n*]arenes are shown in Figure 4.

The Li group prepared a hybrid material by grafting **24** (Figure 4) onto graphene.[25] Addition of the electron-deficient dye safranine T led to the formation of an inclusion

complex with the surface-bound pillar[5]arenes which quenched 95% of the dye's fluorescence through fluorescence resonance energy transfer with graphene. Safranine T could be displaced by paraquat, resulting in associated recovery of fluorescence, but not by 2,2'- or 4,4'-bipyridine. Paraquat analogues diallyl-4,4'-bipyridine and dibutyl-4,4'-bipyridine also had no effects. HeLa cells incubated with the graphene complex exhibited minimal fluorescence whereas safranine T alone or addition of paraquat to the complex, followed by extensive washing with phosphate buffered saline, fluoresced red. No acute cytotoxicity was found for the complex so it was administered subcutaneously to mice which were subsequently treated with paraquat or its two alkyl analogues. Fluorescence recovery was only observed in the first case making this a very selective *in vivo* test for paraquat.

Zhao, Tang, Li and colleagues grafted **25** (Figure 4) onto graphene oxide.[26] Treatment with acridine orange gave a complex in which the dye's fluorescence was quenched by 90%. It was unaffected by uric acid, dopamine, tyrosine, 4-nitrophenol, 4-aminophenol, hydroquinone, ascorbic acid, simple salts or sugars, with only acetaminophen able to regenerate fluorescence.

A displacement assay for ATP based on the **12**-Dapoxyl sodium sulfonate complex was developed by the Bitter group.[27] Enhanced fluorescence and a hypsochromic spectral shift was observed upon the addition of Dapoxyl sodium sulfonate which was ascribed to strong, yet partial, inclusion. Fluorescence quenching induced by ATP was an order of magnitude greater than that by its mono- and diphosphate homologues or guanosine-5′-triphosphate.

3.2. Electrochemical detection

The ability for guest inclusion to be monitored by electrochemical methods, particularly where that guest is a charged species, has long been utilised as a method of detection.[28] Use of the pillar[*n*]arenes as molecular receptors in electrochemical systems is starting to yield some interesting results.

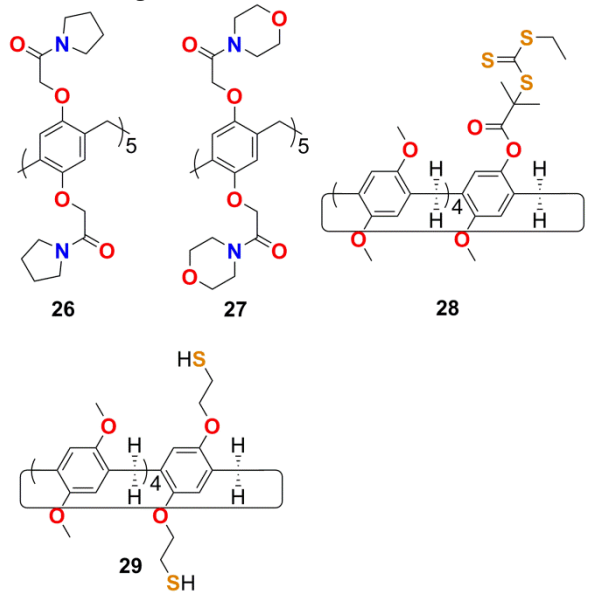

Figure 5. Pillar[*n*]arenes **26** – **29** for electrochemical detection.

Cragg and colleagues used Ogoshi's original dimethoxypillar[5]arene, **1**, as a modifier in carbon paste electrodes to detect alkali metal cations.[29] Thermodynamic simulations suggested that only Na^+ and K^+ would bind to the pillar[5]arene and give a response. Capacitive responses towards alkaline metal salts on composite sensors confirmed this selectivity. Na^+ could be monitored in the 4 x 10^{-2} to 0.2 M range and a limit of detection of 4.1 x 10^{-2} M. This was within the clinical range for the analyte whereas for K^+, while the sensitivity was slightly greater, the limit of detection was 2.28 x 10^{-3} M and above the lowest physiological concentration encountered. Similar selectivity was observed spectroscopically for pyrrolidino- and morpholinopillar[5]arene derivatives **26** and **27** (Figure 5) reported by Stoikov and colleagues, although greatest selectivity was for Li^+.[30] Following the discovery by Chen, Hou and colleagues that **3** could form 'water wires',[5] Cragg and colleagues incorporated the macrocycle in an ion-selective electrode and measured the change in potential from pH 1 to 4.[31] The response time after each addition was pH independent suggesting that the rate limiting step was due to the addition and loss of protons on the electrode.

Other groups investigated the effects on electrodes of surface modification by pillar[*n*]arenes. The Diao group prepared composite materials from **25** onto graphene oxide.[32] The modified graphene oxide was capped with AuNPs and solutions of the ternary composite deposited on glassy carbon electrodes (GCEs) which gave an excellent response to dopamine with a linear range of 1.5 × 10^{-8} to 1.9 × 10^{-5} M and detection limit of 1.2 × 10^{-8} M. Evtugyn and colleagues modified GCEs directly with **2** and demonstrated that the macrocycles achieve about 60% oxidation to the quinone and hydroquinone forms.[33] Addition of Ag^+ and Cu^{2+} ions generated AgNPs and Cu_2O particles, respectively. The group prepared an AChE biosensor from their GCE incorporating **2** by treating it with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide chloride followed by *N*-hydroxysuccinimide and finally AChE in phosphate buffered saline.[34] The addition of the pillar[5]arene gave an enhanced electrode response to sub-nanomolar concentrations of the anticholinesterase pesticides malaoxon, methylparaoxon, carbofuran and aldicarb over five orders of magnitude. A further example of a modified GCE from Evtugyn and colleagues involved deposition of carbon black onto the electrode surface prior to a coating of **2**.[35] Cyclic voltammetry was used to detect weak organic acids (oxalic, succinic, glycolic and acetic) and DNA from chicken erythrocytes, fish and salmon sperm. Interestingly, the method was able to differentiate between native DNA and that exposed to reactive oxygen species, sulfamethoxazole and DOX.

The groups of Hianik and Evtugyn developed aptasensors for cytochrome c from GCEs with layers of electropolymerized neutral red, **4a** terminated with neutral red and a DNA aptamer specific to cytochrome c.[36] The exact number of dye molecules attached by EDC/NHS coupling was not determined, however, cytochrome c was identifiable from 1 x 10^{-9} M to 1 x 10^{-3} M even in the presence of 1000-fold excesses of albumin, polyethylene glycol and lysozyme.

Liao, He and colleagues developed an electrochemical sensor to detect the breast cancer susceptibility gene

BRCA.[37] Pillar[5]arene **28** (Figure 5) was immobilised on a gold electrode before addition of DNA with an alkylamino-modified terminus that threaded through the macrocycles. Hybridization of BRCA target DNA with methylene blue-labelled signal DNA allowed it to bind, in turn, with the DNA-modified electrode surface. The differential pulse voltammetry signal decreased logarithmically in the range of 3.3×10^{-9} M to 3.3×10^{-5} M with a detection limit of 1×10^{-9} M making it of clinical utility.

Cragg and colleagues attached **29** (Figure 5) directly to gold electrodes through dithiol links and demonstrated selectivity for Li^+ over other alkali metals with binding decreasing with increasing ionic radii.[38] This differs from the group's observation of selectivity for Na^+ and K^+ with **1**, however, when immobilised in carbon paste that macrocycle is unable to rotate around its methylene bridges and cannot adopt a suitable coordination environment for Li^+. When attached to the gold surface through thiols on one ring the remaining rings can rotate to bind partially dehydrated Li^+ in a tetrahedral or octahedral environment in agreement with the findings of Stoikov.[30] The group used the same pillar[5]arene derivative to target the biogenic amines putrescine, spermidine and spermine.[39] The faradaic response depended on both the number of amine groups and the carbon chain length of the biogenic amine which allowed discrimination between the three.

3.3. DNA and protein recognition

The cavities of pillar[*n*]arenes are well known to bind small molecules with terminal amines which make them naturally complementary to many amino acid sidechains and the *N*-terminus of proteins. Similarly, it is possible to quaternarise pillar[*n*]arenes with ammonium, imidazolium or similar termini as shown in Figure 6, thus making them attractive to the anionic phosphate backbone of DNA or protein carboxylic acids.

The Li group used the threading properties of pillar[*n*]arenes to mask adipic acid linked to amino silane-modified silicon wafers.[40] Addition of **30** formed pseudorotaxanes with the adipic acid which could be observed by changes in surface contact angle upon changes in pH. Bovine serum albumin (BSA) bound to the surface when the pseudorotaxanes were present at pH 3 but not at pH 11 when the pillar[5]arenes no longer bound to adipic acid. It was hypothesised that hydrophobic effects were the driving forces that made BSA and the pillar[5]arenes interact and that, when the pillar[5]arene was no longer attracted to the functionalised silicon surface, BSA remained in solution.

The Bitter group developed a fluorescence indicator displacement assay based on non-fluorescent complexes formed between **4a** and 4-amino-1,8-naphthalimide derivatives with *N*-butylamine or *N*-(3-methylimidazol)ethyl substituents.[41] Of 19 common amino acids tested only lysine and arginine were able to elicit any fluorescence regeneration with the latter displacing more of the fluorescent dye. By contrast, the polyamine cadaverine was able to displace almost all of the dye. Another linear biogenic amine, putrescine, was ineffective which reflects the differences in binding affinities determined by Stoddart.[17]

Wei, Lin and colleagues used **31** as a highly selective and sensitive receptor for *L*-Trp with a limit of detection in the sub-micromolar region.[42] A 90% quenching of fluorescence was observed when five equivalents of *L*-Trp were added.

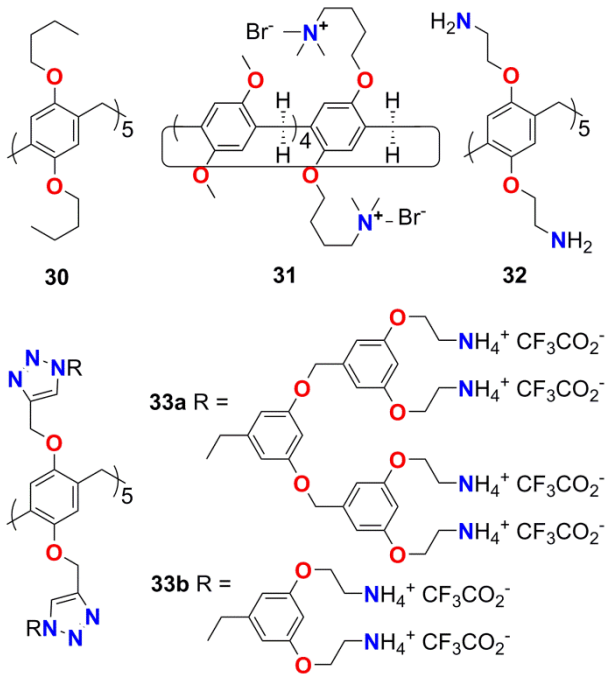

Figure 6. Pillar[*n*]arenes **30** – **33** for DNA and protein recognition.

Duan and Lu determined the association constants of complexes between **32** and 20 natural amino acids, several short chain dicarboxylic acids and glutathione.[43] Only glutathione, glutamic acid and aspartic acid exhibited strong binding, with K_a values of about 1×10^6 for all three, which was two orders of magnitude greater than the other analytes tested.

DNA transfection applications have been explored using cationic pillar[*n*]arenes. Nierengarten, Remy and colleagues prepared dendrimeric pillar[5]arenes **33a** and **33b** using click chemistry to attach the benzyloxycarbonyl-protected dendrons.[44] Treatment with excess trifluoroacetic acid gave the water soluble ammonium derivatives as their triflate salts which formed aggregates at concentrations above 3 nM. Both pillar[5]arenes were shown to bind plasmid DNA by gel electrophoresis and transfection experiments demonstrated that the complex can deliver DNA into HeLa cells. Transfection efficiencies, determined by a luciferase assay, were slightly lower than that of the standard transfection agent, polyethylenimine, but were also less cytotoxic.

The Junquera group adopted a different approach to transfection by incorporating **11** in lipoplexes composed of the sodium salt of 1,2-dioleoyl-*sn-glycero*-3-phospho-(1′-*rac*-glycerol)) and zwitterionic 1,2-dioleoyl-*sn-glycero*-3-phosphatidylethanolamine.[45] The cationic pillar[5]arenes facilitated the formation of lipoplexes from the anionic lipids and anionic plasmid DNA more successfully than simple metal cations. A DNA protection assay was used to demonstrate lipoplex stability against DNAse 1 degradation and experiments on monkey kidney (COS-7) cells showed

cell viability comparable to commercial transfection agents and 80 to 95% transfection.

4. Medical applications

4.1. Drug complexation

Macrocycles with hydrophobic cavities, particularly the cyclodextrins[46] and cucurbiturils[47], have been used to form inclusion complexes with numerous drug molecules to enhance their solubility and consequent bioavailability. The potential for pillar[*n*]arenes to act in this regard was first made clear from the X-ray structure of the **4a**·tetracaine complex published by Danylyuk and Sashuk.[48] Noting that **4a** had limited aqueous solubility, they dissolved it in an ethanol-water mixture. Inclusion of water, seen with **3**, was not observed in the crystal structure, as two ethanol molecules filled each macrocyclic cavity. Addition of the potent anaesthetic tetracaine to **4a** in ethanol-water solution resulted in crystals which revealed two binding modes. Each macrocycle held one tetracaine molecule with a second positioned between pairs of pillar[5]arenes to give a 2:3 host:guest stoichiometry (Figure 7). A range of drug molecules bound by pillar[*n*]arenes is shown in Figure 8.

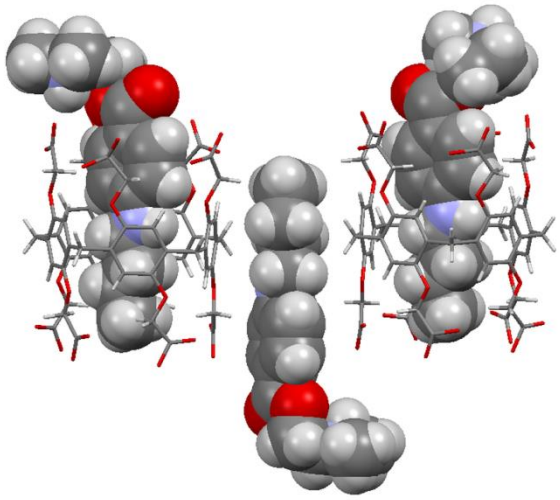

Figure 7. X-Ray structure of **4a**·tetracaine

Wheate and coworkers undertook a larger study of pillar[*n*]arene-drug complexation using NMR and molecular modelling to infer complexation.[49] Salts **4c**, **5c** and their pillar[7]arene analogue were used as excipients for memantine, used to treat Alzheimer's disease, the antiseptic chlorhexidine, the disinfectant proflavine and its derivative, acridine orange. In each case NMR evidence indicated inclusion of the drug molecule by the larger pillar[*n*]arenes although modelling indicated some binding affinity even by the pillar[5]arene. Other pharmaceutically important compounds such as the antiparasitic drug albendazole and *N*-acetyl-*L*-phenylalanine, found in the urine of patients with the genetic condition phenylketonuria, were found not to exhibit NMR shifts consistent with inclusion. This is despite both having the potential to bind though a combination of hydrogen bonding and hydrophobic effects and the known complexation of albendazole by cyclodextrins and cucurbiturils. Investigation of proflavine fluorescence quenching indicates endo- or exo-cavity complexation occurs with increasing concentrations of pillar[*n*]arene which supports inclusion and is also reminiscent of the crystal structure of the tetracaine complex. *In vitro* assays demonstrated that both the pillar[6]- and [7]arene derivatives were nontoxic but that **5a** inhibited proliferation of human embryonic kidney cells (HEK293) above 25 μM and human ovarian carcinoma cells (OVCAR3) above 250 μM. Growth of the latter were also inhibited by its pillar[7]arene analogue but at 100 μM.

Pisagatti and Notti reported that **4c** bound the powerful antibiotic amikacin disulfate.[50] To maximise the efficacy of the drug while minimising nephrotoxic and ototoxic side effects it needs to be directed at the microenvironment where it is required. At a 2:1 ratio of drug to carrier, inhibition of *S. aureus* growth was equal to that of the drug alone over 8 hours. Strong complexation (K_{ass} = 9.9 ± 1.28 × 10^3 M^{-1}) under physiological conditions suggests that the complex may be a route to better drug targeting.

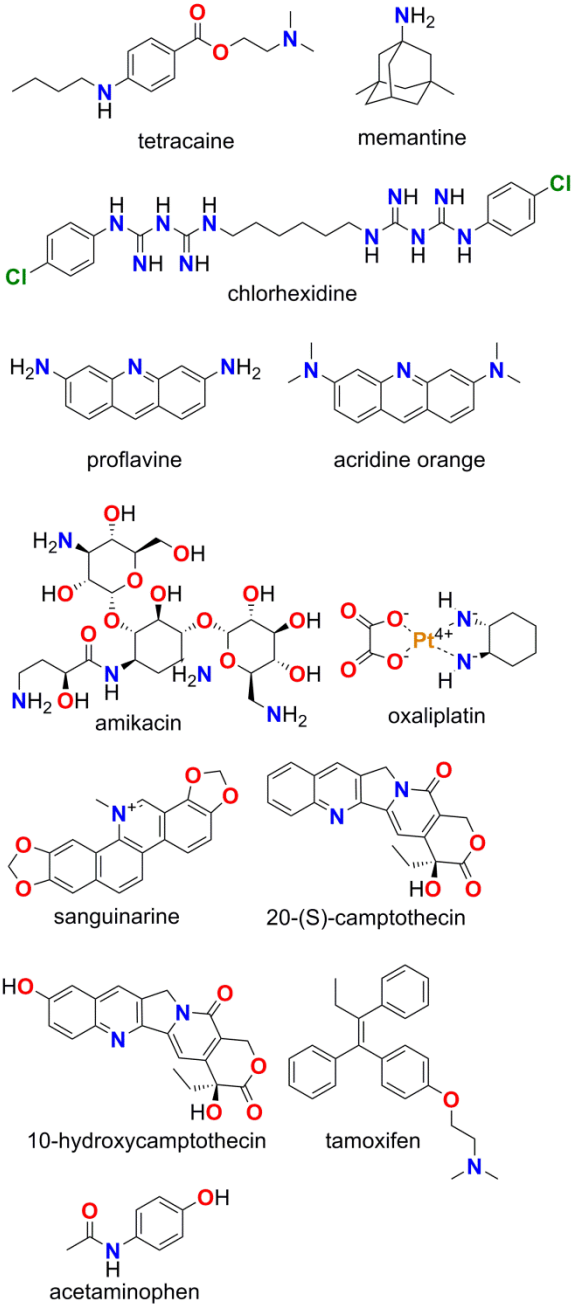

Figure 8. Drug molecules complexed by pillar[*n*]arenes.

Pillar[6]arene **5a** was found to bind the anticancer drug oxaliplatin effectively. A report from the groups of Sessler, Meng and Li detailed the pH-selective dissociation of the drug from the macrocycle with an association constant 24 times higher at pH 7.4 than at 5.4.[51] Complexation increased the drug's stability in plasma almost threefold and, significantly, did not reduce its efficacy against the HepG-2, MCF-7 and A549 cancer cell lines in i*n vitro* tests. *In vivo* effects on tumour growth were evaluated in xenograft Chinese Kun Ming (KM) mice bearing S180 (murine Sarcoma) tumours and showed that at doses of 35 $mgkg^{-1}$ the complex was more effective than the drug alone. Acute toxicity studies revealed that the complex was no more toxic than the drug itself and therefore encapsulation provides a safe way to enhance the anticancer properties of oxaliplatin.

Cui and coworkers found that the **5a** binds sanguinarine, a poorly water-soluble alkaloid with activity against bacteria and inflammation, and increases its aqueous solubility fourfold.[52] The complex formed at pH 7.4 and had a K_{ass} = $2.4 \pm 0.3 \times 10^5$ M^{-1}.The drug is effective against bacteria in its cationic form and complexation shifts its effective pKa from 8.3 to 10.3 thus increasing its activity. Antimicrobial assays using *S. aureus* and *E. coli* showed a twofold increase in activity against both the Gram-positive and Gram-negative bacteria in serial dilution experiments whereas no changes were observed for **5a** alone even when in tenfold excess.

Yu and Ma reported that the sparingly soluble, yet potent, anticancer agent 20-(*S*)-camptothecin (CPT) is also complexed by **5b**.[53] Both CPT and its derivative 10-hydroxycamptothecin (HCPT) were bound by the pillar[6]arene in what appear to be 2:1 host: guest complexes. Drug solubilities increased from low micromolar to 1.9 mM and 1.2 mM for CPT and HCPT, respectively, which made them soluble enough for intravenous injection. Tests on HeLa cells found that the complexes had lower IC_{50} values than for the drugs alone. These combined findings suggest that complexes of **5b** and **5c** with CPT or HCPT have the potential for intravenous administration at lower doses to deliver the same anticancer effects.

A different approach to CPT solublisation was adopted by Hu and colleagues.[54] Pillar[5]arene **34** was terminated with galactose substituents to bind CPT and impart water solubility. A prodrug was prepared from CPT linked via a disulfide bridge and triazole to an alkylammonium moiety. NMR indicated the formation of a 1:1 host:guest complex which aggregated into spheroids approximately 100 nm in diameter. Release of CPT from the nanoparticles was initiated by glutathione at concentrations between 2 and 10 mM with up to 87% of CPT being released with 200 minutes. Without glutathione, the nanoparticles were stable for at least a month under physiological conditions.

One of the most important anticancer agents of recent years is tamoxifen, however, it has poor water solubility and consequently low bioactivity. The Huang group formed a 1:1 inclusion complex between tamoxifen and **5c**.[55] Experiments on MCF-7 and HeLa cells comparing the host, tamoxifen and the complex found no adverse effects on cell viability over four hours but the complex reduced viability b 60 to 75% over the same period.

Venkataramanan and coworkers investigated the inclusion of the analgesic acetaminophen in **1** using computational and spectroscopic methods which indicate that a 1:1 complex is formed.[56] Complexation could have value in ameliorating the effects of acetaminophen overdose, however, the insolubility of the pillar[5]arene makes it unsuitable for such a purpose. The results point at a potential for water-soluble pillar[5]arenes to remove an excess of the drug.

4.2. Controlled drug release by micelles, vesicles and nanoparticles

Many methods exist to facilitate the controlled release of drugs by supramolecular systems. Macrocycles can aggregate into vesicles or micelles, encapsulating the molecules to be released, but they may also form simple host-guest complexes with release triggered by a change in environment. Finally, loaded macrocycles can form part of a hybrid material and function as nanovalves to release cargoes of drugs. Several pillar[n]arenes, such as those in Figure 9, have been used for drug release.

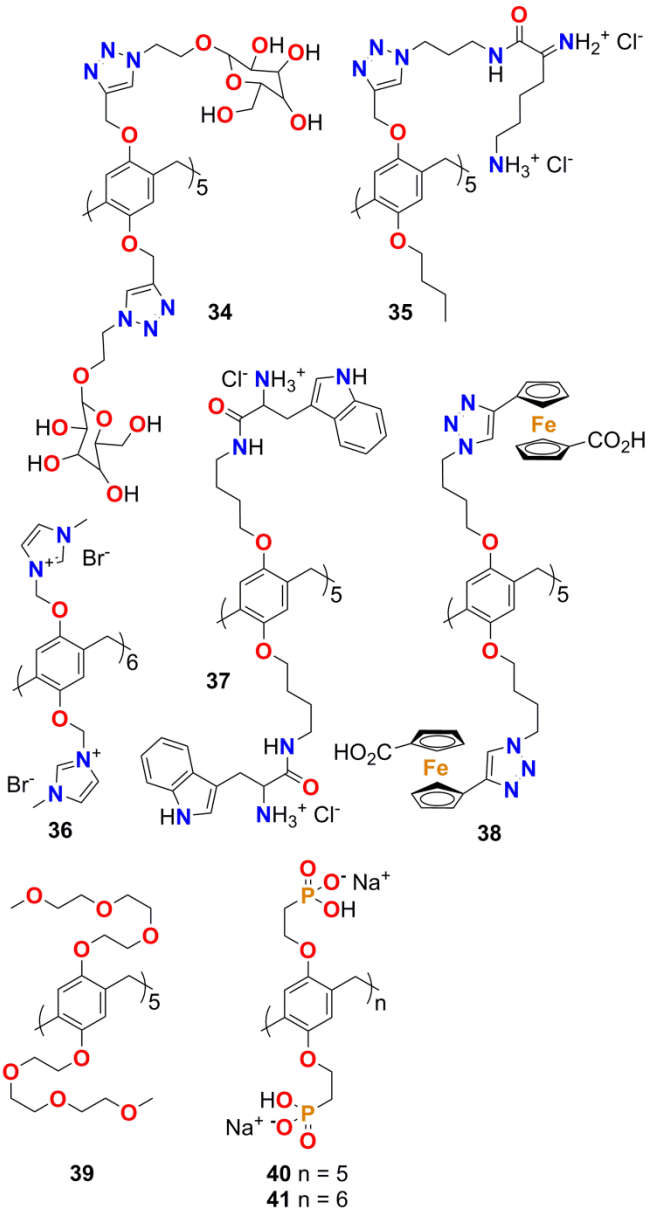

Figure 9. Pillar[*n*]arenes **30** – **33** for drug release.

Asymmetric pillar[*n*]arenes, in which the substituents in the *para* positions are non-identical, are predisposed to form structures such as bilayers, vesicles and micelles. In one of the earliest examples of micelle formation Sakurai and colleagues designed a pillar[5]arene with *n*-butyl and

propargyl substituents.[57] Isolation of the D_5 conformer and subsequent click reaction introduced *N*-propylaminolysine groups to give **35** which formed 4.5 nm micelles in water at pH 3. Based on small angle X-ray scattering and multi-angle light scattering experiments it was deduced that the pillar[5]arenes formed tail-to-tail dimers through hydrophobic forces and that the lysine groups wrap around this core to give the observed micelle-like properties. This example of stimulus-response aggregation would go on to feature in most drug delivery systems.

The Xue group prepared **36**, which formed micelles 85 nm in diameter upon the addition of sodium 4-(octadecyloxy)benzoate.[58] The vesicles collapsed at pH 4 providing a triggering mechanism for controlled release which was confirmed when the water-soluble fluorescent dye calcein, used as a drug model, could be detected when the pH of solutions containing calcein-containing vesicles was lowered. Hu and Wang reported a similar example of controlled release from vesicles using glutathione as the trigger.[59] A lysine derivative, 2,6-diamino-*N*-(2-mercaptoethyl)hexanamide, was linked to 9-(3,5-dimethylphenoxy)nonane-1-thiol by a disulfide bridge, to allow cleavage by glutathione, and included within **4a**. The complex formed spherical vesicles in the order of 100 nm in diameter which could be loaded with the broad spectrum anticancer drug metronidazole (MTZ). Addition of glutathione at pH 5 resulted in vesicle disaggregation and over 80% of the drug released within 45 minutes. A similar approach was adopted by the Wang group which found that the complex between **5a** and the anticancer drug DOX linked to a polyether- or alkyl-*N*-pyridinium groups by a hydrazone moiety formed nanospheres at pH 7.[60] When incubated in phosphate buffered saline at pH 7.4 release of DOX was less than 5% over nine hours whereas 85% of the drug was released over the same period at pH 5.5. Although free DOX was exhibited greater cytotoxicity than the complexes, the latter still displayed good dose responses to human ovarian cancer (SKOV3) cells, murine breast cancer (4T1) cells and HeLa cells. Delivery was not by the usual diffusion pathway but by nanoparticle endocytosis offering a route to administration which may circumvent drug resistance. Wang and colleagues also showed that **5a** could form binary vesicles when complexed with *N*-1-decyl-ferrocenylmethylamine.[61] Complexation, if 1:1, led to asymmetry which was confirmed by NMR and UV-visible spectroscopy. The effects of inclusion on the ferrocenyl moiety were observed by cyclic voltammetry. Addition of the complex to a solution of pyrene resulted in fluorescence quenching concomitant with aggregation into vesicles. The same experiment with the sodium salt of the pillar[6]arene's subunit, hydroquinone-*O*,*O'*-diacetic acid, induced no such changes. Dynamic light scattering experiments indicated the existence of structures 170 nm across which disassembled below pH 6. This suggested a potential therapeutic application as the microenvironment surrounding cancerous tumours tends to be below pH 7. MTZ was added to an aqueous solution of the pillar[6]arene complex to form vesicles about 700 nm in diameter which contained around 11% of the drug which was shown to be released upon acidification of the solution. To test the effects *in vitro*, human hepatocarcinoma (SMMC-7721) cells were incubated with MTZ-loaded vesicles. The effects on cell viability were slightly better over 96 hours than for MTZ alone, however, in tests on murine fibroblast (NIH3T3) cells the MTZ-containing vesicles had significantly lower chronic toxicity than MTZ alone. The same researchers also used complexes of **5a** with a pyridinium amphiphile to form vesicles which delivered DOX.[62] Results of drug inclusion were similar for the studies involving MTZ: lower toxicity towards NIH3T3 cells but similar cytotoxicity against cancer cells, in this case the MCF-7 breast cancer cell line.

Hu and colleagues used the same macrocycle to bind DOX attached to isonazide by a hydrazone bond.[63] Fluorescence microscopy of SKOV3 cells incubated with the complex indicated hydrolysis and co-location of DOX with the lysosomes and nuclei.

Xue, Shi and colleagues prepared a pseudorotaxane formed from **5a** and azobenzene-terminated 1,5-bis(hexyloxy)naphthalene.[64] Vesicles formed from this complex were used to encapsulate DOX which could be released either at low pH, with over 90% release at pH 2, or by irradiation at 365 nm for 40 minutes to give over 80% release. The same approach was adopted by Zhang, Hu and colleagues who used the smaller **4a** and a disubstituted naphthalimide with substituents incorporating disulfide bonds.[65] Vesicles containing DOX were able to release the drug in response to the presence of glutathione and were significantly more effective against both human alveolar adenocarcinoma (A549) cells and HeLa cells than DOX alone. Tong, Jiang and colleagues prepared vesicles from **5a** and poly(ε-caprolactone) with a terminal 4-((4-methoxyphenyl)diazenyl)phenoxy group with a quaternary ammonium linker.[66] The vesicles were loaded with DOX which was released in response to reducing the pH from 4.5 to 3 or from irradiation at 365 nm. The drug-free vesicles had negligible effects on liver hepatocellular (HepG2) cell viability at concentrations up to 250 μg ml^{-1} but over 24 hours the DOX-loaded vesicles reduced cell viability below 60% at drug concentrations in excess of 20 μg ml^{-1}.

Jin and Ji prepared micelles from **4a** and methyl viologen-functionalised DOX.[67] It was anticipated that the extracellular pH conditions would cleave the thiol link between the drug and methyl viologen substituent. The micelles were shown to be toxic towards HepG2 cells at pH 6 in a dose dependent manner with an IC_{50} value of 3.75 mg L^{-1}.

Pei and Pei synthesised **37** and prepared vesicles from its complex with a galactose derivative with an appended polyether and a pyridinium terminus.[68] HepG2 and DOX-resistant HepG2 (HepG2/ADR) cells were incubated with free DOX and DOX-loaded vesicles. Cell viability decreased significantly in the presence of both vesicle systems, however, DOX alone was much less cytotoxic towards the HepG2/ADR cells at all concentrations. The results indicate that the vesicles act through endocytosis and drug release and, by doing so, DOX avoids the pathway by which some HepG2 cells have become resistant. The same group used a combination of the functionalised galactose with **38** to prepare vesicles which disassembled only in the presence of both low pH and glutathione.[69] Inclusion of DOX within the vesicles was possible at up to 85% loading which is significantly higher than other systems. Incubation with MCF-7 cells and non-cancerous HEK293T cells showed significantly greater cytotoxicity over 72 hours against the former and significantly better protection for the latter.

Much of the controlled release research involving pillar[*n*]arenes has involved potential anticancer applications, however, the groups of Hu and Wang have developed a method to deliver insulin under hyperglycaemic

conditions.[70] Addition of 4-borono-1-hexadecylpyridin-1-ium bromide to **4a** formed vesicles. Electron microscopy showed that the 130 nm vesicles grew to 320 nm particles with rough surfaces when fluorescently labelled insulin was added, suggesting that insulin molecules bound to surface cavities. Addition of *D*-glucose at a normal physiological concentration led to 20% insulin release after an hour but at five times the concentration about 70% of the insulin was released over the same time period. Release at pH 7.0 was more than double that at pH 7.4 over an hour making the controlled release highly pH sensitive. The insulin-loaded vesicles also displayed good cytocompatibility when added to lung fibroblast (MRC-5) cells.

The Ji group developed a displacement assay for cysteine designed to work in the acidic microenvironment which surrounds tumours.[71] Pillar[5]arene **4a** formed a strong inclusion complex with a cysteine probe prepared from 1-(4-dimethylamino-phenyl)-ethanone and 4-pyridinecarboxaldehyde, and subsequently quaternarised with iodomethane. The probe could not be displaced from the yellow complex by cysteine until the pH dropped below 6 whereupon the probe was released and reacted with free cysteine to give a colourless solution. The group used an isomer of their cysteine probe, encapsulated within **39**, as a temperature sensitive cysteine detector.[72] When heated above its lower critical solution temperature decomplexation occurred leading to reaction between the probe and cysteine which was signalled by quenching the complexes orange colour. Tests on HeLa cells indicated the complex's low cytotoxicity over 24 hours.

The Yang group used the pillar[*n*]arenes' controlled release properties by adsorbing **4a** onto the surfaces of mesoporous silica nanoparticles (MSNs) previously modified to be covered with propylpyridinium groups.[73] When the MSNs were loaded with rhodamine 6G, before being capped with **4a**, the dye was released by acetylcholine proportional to the dose delivered. The group also investigated the properties of MSNs coated with a trimethylammonium-terminated propyl ester towards AuNPs capped with **4a**.[74] Filling the MSNs with rhodamine B prior to addition of the AuNPs allowed the system's controlled release to be monitored. Release was initiated by heat or displacement of AuNPs when diaminoethane was added thus providing two potential triggers for drug release. Short linear diamines are well known to bind to pillar[5]arenes and, in this context, affected the solubility of the AuNPs. Composite MSNs have also been prepared by the Fu group which appended 1,6-(1-pyridiniumhexyl)-hexanediamine groups from their surfaces and then formed pseudorotaxanes using **4a**.[75] The MSNs could be loaded with DOX prior to addition of the pillar[5]arenes and release of the drug triggered by low pH, high pH or high concentrations of Zn^{2+}. Tests against human breast adenocarcinoma (MCF7) cells showed that unloaded MSNs were non-toxic but when delivering DOX cell viability decreased below 20% after 48 hours at 9.5 μg mL^{-1}. This was slightly less cytotoxic than DOX alone but delivery was by an endocytosis pathway.

Hu, Zhu, Wang and colleagues prepared poly(γ-benzyl-*L*-glutamate)-based polymers which were then extended to give diaminobutyl termini.[76] Addition of **4a** led to the formation of pseudorotaxanes and aggregation into polymersomes which could be filled with MTZ. A copolymer which additionally incorporated biotin groups was designed to target biotin receptor-positive HeLa cells and degrade under the local low pH conditions to release MTZ. Tests on NIH3T3 cells showed lower cytotoxicity for both types of polymersome however MTZ toxicity towards HeLa cells was enhanced when delivered by the biotinylated polymersome.

The Du group prepared **40** which, as the polyanion, formed pseudorotaxanes on the surface of MSNs filled with DOX and coated with quaternary ammonium or pyridinium groups.[77] Cargo release was triggered by Zn^{2+}, acidic conditions or paraquat. MSNs were also formed around gold nanorods which allowed photothermal effects from near infra-red radiation at 808 nm to release the drug. Irradiation caused no adverse effects on A549 cells but addition of the gold nanorod MSNs caused apoptosis.

Wang and colleagues used **40** and **41** to construct binary micelles.[78] The pillar[*n*]arenes bound to the head group of an pyridinium-modified *N*-decylnaphthalimide to form structures about 200 nm in diameter. DOX- and MTZ-loaded micelles, which were much larger than the drug-free analogues, both performed slightly better than the drugs alone against both HeLa and human adenocarcinomic epithelial cell (A495) lines. Tests against L929 murine cells demonstrated the valuably familiar trait of reduced cytotoxicity of vesicle-included drugs over the free anticancer agents.

4.4. Glycoclusters

Bacterial adhesion to host cells is the first stage of infection and is associated with weak but highly specific interactions between the two. One key mechanism is for adhesin proteins to interact with monosaccharides found on cell surfaces.[79] To disrupt adhesion, and also to inhibit biofilm formation, compounds containing several sugars, often linked to rigid macrocycles, have been found to be more effective than those with a single sugar.[80] This multivalent approach has been applied to pillar[5]arenes (Figure 10), predominantly through click chemistry, to introduce five or ten sugar termini.

The Nierengarten group reported the attachment of ten acetylated mannose groups to pillar[5]arene by triazole linkers formed during the click reaction between the azido-functionalised pillar[5]arene and the acetylated mannose derivative.[81] The product was subsequently deacetylated to give **42**. Haemagglutination assays with the pillar[5]arene and an acyclic control showed that an almost sevenfold enhancement in inhibition due to the presence of the glycocluster. Later work on the same compound found that it showed some inhibition against a bacterial liposaccharide heptosyltransferase, WaaC, involved in lipopolysaccharide biosynthesis.[82] A month after Nierengarten's report, Huang and co-workers published the synthesis of galactose-appended **43** and asymmetric derivative **44**.[83] No cellular agglutination was observed for the symmetric pillar[5]arene, or an acyclic control, but the asymmetric derivative appeared to facilitate bacterial adhesion.

Imberty, Vidal and colleagues also investigated the effects of asymmetric **45** and **46** with galactose or fucose termini and **47** incorporating a triethylene glycol spacer between the triazole and galactose.[84] LecA, a bacterial lectin from *Pseudomonas aeuruginosa*, was treated with **45** and **47**; the longer chain derivative displayed a much lower IC_{50}, in the micromolar range, and minimum inhibitory concentration. This was shown to be due to the latter's ability to interact with five lectin monomers whereas the former was sterically crowded, due to short linkers between

the sugars and the macrocycle, and could only interact with three monomers. Nierengarten and colleagues investigated the effects of increasing the numbers of sugars by incorporating substituents with two monosaccharides.[85]

Fucose derivative **48** gave an IC_{50} value in the picomolar range against the lectin BambL from *Burkholderia ambifaria*. In an attempt to incorporate both fucose and galactose termini, the Nierengarten group prepared a number of rotaxanes from **49** and an alkyl thread terminating in either fucose or galactose.[86] The combination of ten galcatose units and a difucose thread was highly effective against both LecA and LecB as it contains complementary sugars to both lectins.

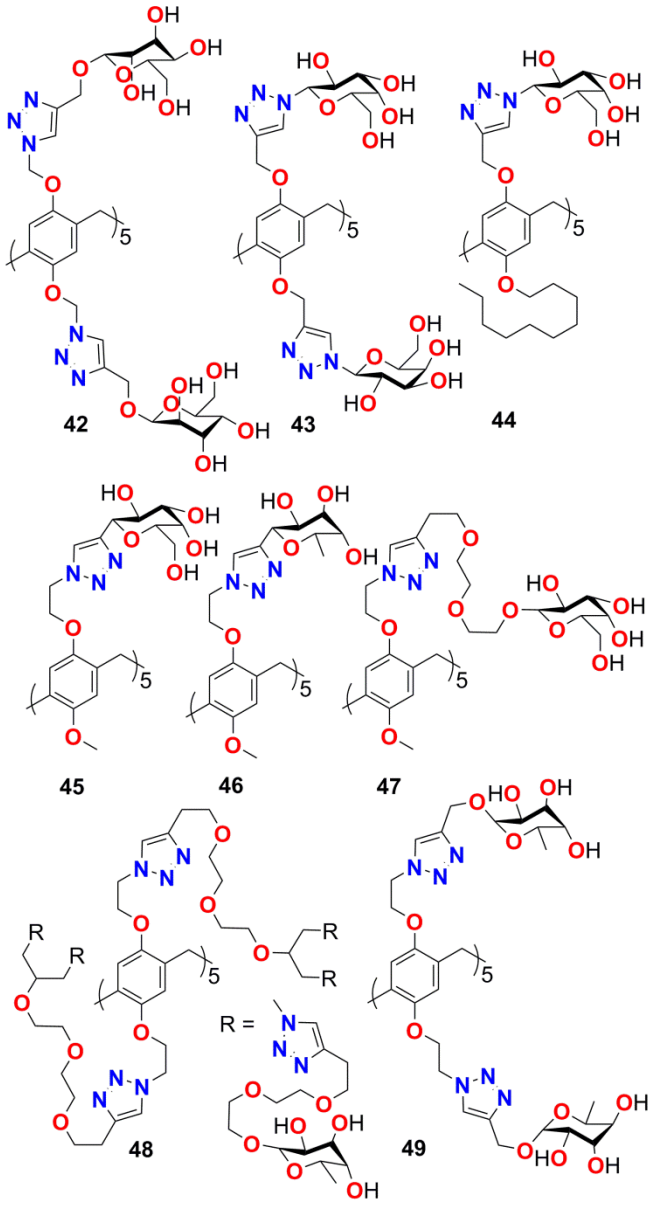

Figure 10. Pillar[*n*]arene glycoclusters **42** – **49**.

Pei and co-workers exploited the inclusion properties of **43** by threading the macrocycle onto propyldisulfonylpyridine groups affixed to cerium oxide nanorods.[87] To apply this material in a therapeutic mode, the nanorods were first immersed in a solution of DOX·HCl before the capping pillar[5]arenes were added. The nanorods were added to HepG2 whereupon endogenous glutathione cleaved the alkyldisulfonyl bond and released both the pillar[5]arenes and the drug. In the absence of the pillar[5]arene the cytotoxicity was lower due to the lack of targeting by the galactose termini.

4.5. Towards therapeutic pillar[5]arenes

It has already been shown how pillar[*n*]arenes can be used to bind and solubilise drugs or form nanospheres capable of releasing them in a controlled manner under conditions found in in the microenvironment around cancerous cells. Detection of key biomolecules, from simple amino acids through to proteins, has also been evidenced. Pillar[*n*]arene research is now starting to exploit the chemistry-biology interface to the point where its impact in medicine is now being felt as can be seen from the following examples.

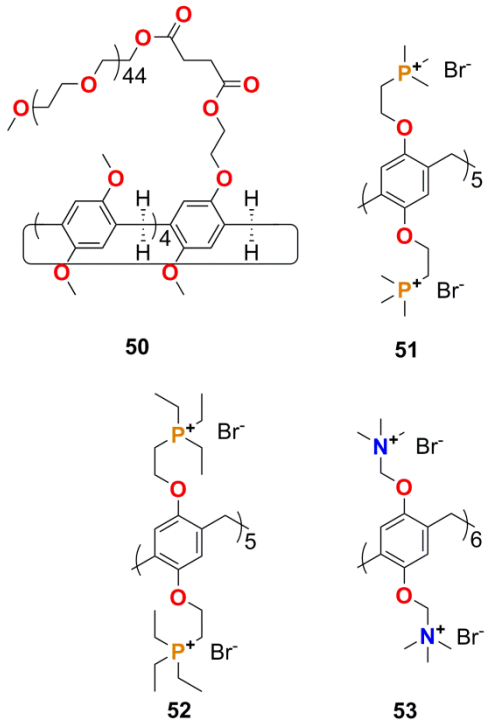

Figure 11. Therapeutic pillar[*n*]arenes **50** – **53**.

Yu and colleagues used **4c** to bind choline derivatives and found that it could inhibit acetylcholine hydrolysis.[88] As reduced acetylcholine levels are associated with Alzheimer's disease, pillar[*n*]arenes have the potential to deliver the neurotransmitter as a treatment for Alzheimer's-related memory loss.

The Huang group has shown that **12** binds ATP strongly in the presence of similar chemical species.[89] Delivering the pillar[6]arene as part of a micelle composed of a polyethylene glycol-folic acid co-polymer and filled with DOX to ovarian cancer (MCF-7/ADR) cells resulted in greater drug efficacy. The authors proposed that **12** removes ATP thus inhibiting the effect of the ATP-dependent efflux pump which would otherwise all ow the drug to escape and, in doing so, suggest a novel approach to tackling multidrug resistance.

Zhang and colleagues prepared **50** and a pyridinium-terminated porphyrin derivative bearing a disulfide bond which formed micelles in aqueous solution.[90] The presence of glutathione monoethyl ester in an acidic environment led to the release of the sensitising porphyrin. Irradiation at 420

nm for 10 minutes resulted in the viability of A549 cells dropping to 20% as a consequence of locally generated cytotoxic singlet oxygen. The group subsequently used a similar system to deliver a photosensitising porphyrin that accumulated in the mitochondria.[91] When mixed with **4a** the porphyrin's trimethyl ammonium terminus formed a pseudorotaxane with the macrocycle which self-assembled into micelles. Confocal laser scanning microscopy of A549 cells following treatment with the micelles revealed localisation in the mitochondria and irradiation induced oxidative damage to the mitochondria leading to cell death.

Tang, Huang and colleagues have applied results from controlled release systems to hollow mesoporous nanoparticles (HMNPs) through surface modification with diaminoalkanes which protonate below pH 7.[92] The silica HMNPs were of the order of 200 nm in diameter with pores of 3 nm and incorporated magnesium so that they biodegrade in days at pH 4.5 to avoid potentially harmful aggregation of silica particles. The surface modified HMNPs were loaded with DOX and **4c**. At low pH the pillar[5]arene was in the acid form and the surface of the HMNPs positively charged leading to disassembly and drug release. Solutions injected into mice with Lewis lung carcinoma tumours resulted in inhibition of tumour growth after 18 days, an outcome which was significantly better than DOX alone. Behavioural and blood tests on treated mice over 15 days revealed no adverse effects from the HMNPs .

The inhibition of biofilm formation is becoming an important goal in the fight against disease as these communities of microbes, held together by an extracellular matrix, have far greater antibiotic resistance than isolated cells. The disrupting abilities of charged pillar[*n*]arenes have been exploited by the Cohen group which produced a range of quaternary ammonium, imidazolium and phosphonium derivatives, e.g. **11**, **36**, **51** and **52**, and tested them on a wide range of bacterial biofilms.[93] Pillar[5]arenes with quaternary ammonium or imidazolium groups inhibited assembly of biofilms formed by several Gram-positive pathogens including methicillin-resistant *S. aureus* 33592 and *Enterococcus faecalis*.[94] Compounds **51** and **52** were found to have similar activities down to submicromolar concentrations and, in many cases, the compounds did not cause cell death, nor were they toxic to mammalian cells. By way of contrast the monomers were only inhibitory in the millimolar range. The most effective compound, however, was found to be pillar[6]arene, **53**.

5. Summary and outlook

The examples given above demonstrate the range of biomimetic and therapeutic possibilities for pillar[*n*]arenes. While artificial analogues of ion channels or enzymes are of interest, it would appear that detection and drug release will become the main areas for exploitation. To date pillar[*n*]arenes, particularly their carboxylate salts, can improve solubility and bioavailablility of drug molecules. Consequently, controlled release of drugs is an area of great promise. Although this has largely focused on doxorubicin it is clear that other drugs could be delivered in the same manner. The eventual move from research laboratory to the clinic should be substantially simplified due to the ease of synthesising **4a**, **5a** and their salts. Another area that is gaining in importance is the inhibition of biofilms which are responsible for both fouling of implants and increased bacterial resistance. Here too, pillar[*n*]arenes are having an impact. Finally, the molecular recognition properties of the pillar[*n*]arenes' cavities are being harnessed to detect a range of analytes, from metal cations to cytochromes.

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Received: ((will be filled in by the editorial staff))
Accepted: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff)

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Graphical abstract