TUTORIAL REVIEW

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Supramolecular optical chemosensors for organic analytes

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Supramolecular optical chemosensors are abiotic molecular devices that bind analytes by noncovalent interactions, producing a change in light absorption or fluorescence. This review summarizes recent progress in the development of such chemosensors for organic analytes based on artificial receptors. Important design considerations, such as analyte affinity, choice of chromophore or fluorophore, binding selectivity, and optical signaling mechanism are briefly discussed. Chemists have fashioned chemosensors from a wide range of molecular structures, including polyalcohols, crown ethers, calixarenes, helicenes, sterically geared tripods, metal complexes, pinwheels, porphyrins, and fused-ring heterocycles. Analytes of interest include amines, carboxylic acids, amino acids, hydroquinones, alkaloids, carbohydrates, peptides, urea and creatinine.

1 Introduction

Chemical sensors are generally understood to be *devices* that transform chemical information into analytically useful signals. The term *chemosensor* has been defined as a *molecule* of abiotic origin that signals the presence of matter or energy.[1] A key requirement of chemosensor function is that analyte binding must occur *reversibly*. This allows analyte concentration to be measured at equilibrium by optical detection of either the chemosensor-bound species or the analyte-free chemosensor. It also permits continuous measurements to be made with dynamic optical response to changing analyte concentrations. Chemodosimeters,[1] which can measure cumulative amounts of reactants, are based on irreversible chemical reactions. Such reactions are useful in single-measurement applications, as with timed-response uses of disposable sensors. Here we deal only with more broadly applicable phenomena involving reversible association between the chemosensor and its analyte. The chemosensors of interest bind their guest analytes by noncovalent interactions, hence they can be termed supramolecular chemosensors.

An optical chemosensor consists of a molecule incorporating a binding site, a chromophore or fluorophore, and a mechanism for communication between the two.[2] Analyte binding thus produces a change in chemosensor optical properties (absorption or fluorescence), as illustrated in Fig. 1. The chemosensor binding site by definition cannot be biological in origin (*e.g.*, an enzyme or antibody); it must be an artificial receptor. Biotic receptors in biosensors may have high affinity and selectivity for biological analytes, but artificial receptors have many potential advantages. Biomolecules are

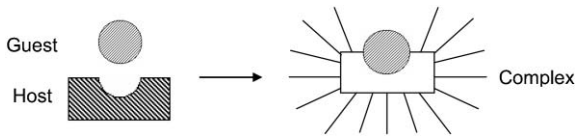
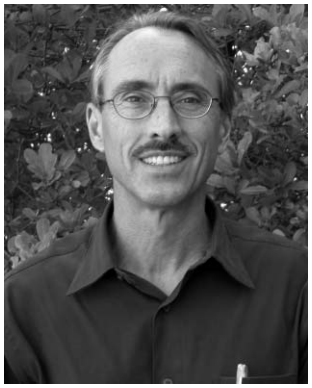


Fig. 1 Cartoon showing binding of an analyte (guest) by a chemosensor (host), producing a complex with altered optical properties, here an increase in fluorescence. (Reprinted from ref. 4 with permission. Copyright 2002 Elsevier.



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Thomas Bell received his PhD from University College, London in 1980, having conducted his thesis research with F. Sondheimer and D. J. Cram (UCLA). He worked with J. Meinwald as an NIH Postdoctoral Fellow at Cornell University, then joined the State University of New York at Stony Brook as an Assistant Professor in 1982. There, he reached the rank of Professor in 1991, then moved to his current position as Professor of Chemistry in 1995. He has been a Fellow of the American Association for the Advancement of Science since 1994; in 1990 and 1996 he was a Visiting Professor at Université Louis Pasteur in Strasbourg, France. His current interests include advanced materials, antiviral and immunomodulatory drugs, nanoscale molecular devices, and supramolecular chemistry, as well as hiking, mountain biking, skiing and snowboarding.

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sensitive to pH, oxidizing agents and heat, while abiotic receptors can be synthesized from more robust components. Artificial receptors can, in principle, be tailored for various analytes, and their physical properties can be adjusted to meet specific sensor requirements.

This review summarizes recent progress in the development of chemosensors for organic analytes based on artificial receptors. Carbohydrate chemosensors that form cyclic boronate esters with 1,2-diols have been reviewed relatively recently,[3] so our focus is on fluorescent or colored molecules that bind analytes by noncovalent, or supramolecular, interactions. Important aspects of optical chemosensor design include analyte affinity, choice of chromophore or fluorophore, binding selectivity, optical signaling mechanism, and immobilization method. Most of these issues have been described in some detail in other articles,[4–8] and they are introduced here briefly, prior to discussing recent progress in supramolecular chemosensors.

Analyte affinity is a matter of primary importance in chemosensor design. The usual challenge is to improve sensitivity by increasing binding strength, because abiotic hosts typically bind guests much more weakly than natural receptors. On the other hand, an analyte that is present in high concentration can saturate the chemosensor, so that fluctuations in guest concentration may not produce sufficiently large changes in the optical signal. A good rule of thumb for chemosensor design is that the target stability constant (K_s) should be approximately the inverse of the median guest concentration for the concentration range of interest.[4] This approach is especially good if it is not known whether the optical signal of the host or the complex will be most useful, but there are exceptions.[4]

Another important issue in chemosensor design is the choice of chromophore or fluorophore used to report analyte binding. In particular, the absorption wavelength must be compatible with the light-absorbing properties of the medium in which measurements are to be made and with the light source. For example, proteins absorb ultraviolet light, so optical chemosensors for analytes in biological fluids (*e.g.*, blood) should have λ_{max} values for absorption larger than *ca.* 400 nm.[8,9] Fortunately, Stokes' Law ensures that fluorescent chemosensors will emit light (λ_{em}) at longer wavelength than that used for excitation (λ_{ex}), but practical considerations also come into play here. For reasons of instrument configuration, sensor cost, and light scattering, it may be better to use optical filters to reduce excitation light reaching the detector, rather than to arrange the detector perpendicular to the incident light beam, as in conventional spectrofluorometers. Therefore, a large Stokes' shift is generally desired (*e.g.*, $\lambda_{em} - \lambda_{ex} > 50$ nm).

A chemosensor must quickly and specifically recognize its target analyte, unless an array of "differential receptors" is to be used in an "artificial nose" approach to recognize multiple analytes.[10,11] This selection process can result from selective binding or selective response, but in the latter case interfering substances will competitively inhibit optical response to the desired analyte. However, complete specificity for a single potential guest is not necessary because the chemosensor only needs to pick out its target from just the analytes typically present in the solution. Artificial receptors bind guest molecules by a combination of fundamental electrostatic, hydrogen bonding, and van der Waals interactions. These intermolecular forces can be controlled to effect molecular recognition by means of host–guest complementarity and host preorganization.[12]

While preorganization leads to stronger and more selective binding, it also increases rigidity. In chemosensors, rigidity can hinder access of the analyte to the binding site, slowing equilibration considerably. This can produce unacceptable delays in equilibrium measurements, simultaneously retarding kinetic measurements, as well. Therefore, chemosensor rigidity must be balanced with flexibility. A certain degree of flexibility may be desired in order to produce an optical response by an "induced fit" mechanism, which leads us to the next consideration in chemosensor design.

What about the mechanism coupling the binding event with signal transduction *via* the chromophore or fluorophore? It makes sense that the binding site and the optical reporter should be structurally integrated as much as possible in order to maximize this communication. In this context, it is useful to draw a distinction between intrinsic and extrinsic fluorophores or chromophores.[13,14] Intrinsic optical reporters are structurally integrated with the analyte binding site to maximize the influence of the bound guest on the optical properties of the chemosensor. Here, chemosensors have an advantage over biosensors. It is preferable to build a chromophore or fluorophore into a chemosensor binding site during its synthesis than to modify or introduce an optical reporter into the active site of an enzyme or the recognition site of an antibody. Such modifications of biological molecules usually damage their molecular recognition capabilities, so the optical reporter must be attached extrinsically to their binding sites.

Whether the optical reporter is intrinsic or extrinsic to the molecular recognition site, the mechanism for optical response should be considered during chemosensor design. While the mechanism of many known sensors, especially fluorescent chemosensors and biosensors, may not be well understood, Table 1 lists many mechanisms that have been identified and incorporated into chemosensor design.[4,8] Here an important distinction is made between guest binding effects on chromophores *vs.* fluorophores. Useful absorbance effects generally result from changes in molecular structure, including proton transfer, charge transfer, and isomerization. Fluorescence is much more sensitive to subtle changes in the geometry and electronic structure of the ground state, as well as the electronic excited state. It is uniquely responsive to physical processes affecting depopulation of the emissive excited state, such as conformational restriction occurring upon analyte complexation. As indicated in Table 1, fluorescent chemosensors can utilize several photophysical processes, in addition to all of the structural mechanisms available to chromophoric chemosensors.

Table 1 Optical response mechanisms involving supramolecular interactions between chemosensors and bound analytes

Proton transfer	Chromophore
Tautomerism	Chromophore
Skeletal isomerism	Chromophore
Charge transfer	Chromophore
Polarization	Chromophore
Solvent displacement	Fluorophore
Conformational restriction	Fluorophore
Quenching by guest	Fluorophore
Internal charge transfer (ICT)	Fluorophore
Twisted internal charge transfer (TICT)	Fluorophore
Resonance energy transfer (RET)	Fluorophore
Photoinduced electron transfer (PET)	Fluorophore

It is also useful to consider that more than one functional group of the analyte can influence the optical properties of an artificial receptor. For example, simultaneous binding of two

different groups can turn "ON" the optical signal, while either group alone has a negligible or distinguishable effect on the chemosensor. This "AND logic gate" effect can be used to enhance analyte specificity of the optical response.[15]

2 Examples of chemosensor types

The focus of this review is on supramolecular chemosensors that reversibly bind either neutral or charged organic molecules with an optical response, except for circular dichroism. Of particular interest are artificial receptors that recognize specific guests, so receptors for simple anionic moieties, such as carboxylate or phosphate groups of fatty acids, amino acids, nucleotides or phosphorylated peptides, are not included. Sensors based on polymers or other materials, sensing schemes involving guest displacement, and molecular probes for polymers or assemblies are also excluded.

The field of optical chemosensors is expanding rapidly, so space restrictions necessitate the omission of many interesting examples from this review. An attempt is made to classify recent and particularly noteworthy examples according to the structures of both chemosensors and analytes, though certain types of artificial receptors may be useful for a wide range of analytes. For example, chemosensors for numerous organic molecules are based on macrocyclic structures.[16]

2.1 Polyalcohol chemosensors for amines

Two research groups have approached the sensing of amines using functionalized calixarenes that can discriminate between enantiomers (Fig. 2). Kubo *et al.* synthesized receptor **1**, which gives different chromogenic responses to the enantiomers of phenylglycinol (K_s 66 M^{-1}) and phenylalaninol (K_s 159 M^{-1}) in ethanol. Significant optical response was only observed for one enantiomer.[17] Calixarene **1** works as a sensor because one of the two indophenol moieties undergoes deprotonation by the amine, producing an anion that binds the resulting ammonium group of the guest. It is believed that a hydrogen-bonding interaction occurs between the receptor and the hydroxy group of each substrate, as no discrimination/binding was observed for the enantiomers of 1-phenylethylamine. The overall effect is a bathochromic shift in the UV-visible absorption spectrum of receptor **1**. The Diamond research group synthesized calixarene **2**, which operates as a sensor *via* hydrogen bonding interactions between the hydroxy moiety and the amine guest in organic solvents.[18] This causes quenching of the fluorescence emission of the receptor, which shows some selectivity for (*R*)-1-phenylethylamine in chloroform (λ_{ex} 274 nm) and increased discrimination between enantiomers of phenylglycinol in methanol (λ_{ex} 230 nm).

Another molecule that has been shown to sense various chiral amines and amino alcohols in an organic solvent is the fluorescent helical diol **3**, reported by Reetz and Sostmann.[19] Chiral discrimination is detected by differences in the fluorescence quenching observed on binding the amine (λ_{ex} 318 nm, λ_{em} *ca.* 400–500 nm). In this case, it is believed that the hydroxy moieties of **3** form hydrogen bonds with the amino group of the analyte, and that proton transfer is not involved. The somewhat longer absorption wavelength of fluorosensor **3** is a potential advantage over **2**, but all three of these chemosensors apparently bind amines with relatively modest stability constants.

2.2 Azacrown chemosensors for protonated amines

Chemosensors for protonated diamines fashioned from anthracene-appended aza-18-crown-6 have been known for some time.[20] Anthracene crowns **4–6** in Fig. 3 represent more recent developments in this field. Two new bisazacrown anthracene derivatives were reported by Kim *et al.* to bind alkyldiammonium ions ($H_3N^+(CH_2)_nNH_3^+$) in either ethanol or methanol/chloroform (9 : 1).[21] In bis(aza-15-crown-5) analogue **4** (Fig. 3), the fluorescence of the anthracene moiety (λ_{ex} 367 nm, λ_{em} *ca.* 390–470 nm) is quenched by photoinduced electron transfer (PET) from the two nitrogens to the excited singlet state of the fluorophore. When both nitrogen electron pairs form hydrogen bonds with the diammonium ion, PET is inhibited, resulting in enhanced fluorescence. As might be expected, the selectivity of the chemosensor is dependent on the distance (chain length) between the two cations, with the following range of stability constants being observed: $n = 3$, K_s 4412 M^{-1}; $n = 4$, K_s 272 M^{-1}; $n = 6$, K_s 98 M^{-1}; $n = 5$, K_s 35 M^{-1}. The bis(aza-18-crown-6) analogue displayed similar binding selectivity.

Anthracene PET quenching is also used in azacrown **5** (Fig. 3), which was developed as a chemosensor for aminoacids by de Silva *et al.*[22] Again, selectivity was observed to be based on the length of the carbon chain between the two functional groups of the guest. For example, in MeOH–water (3 : 2) at pH 9.5 the following binding constants were measured: 5-aminopentanoic acid, K_s 84 M^{-1}; 3-aminopropanoic acid, K_s 17 M^{-1}. As observed for **4**, binding of the ammonium moiety to the azacrown turns off a PET process, so an enhancement of the fluorescence of the anthracene unit occurs. The carboxylate group of the guest apparently ion pairs and forms hydrogen bonds with the guanidinium moiety of the

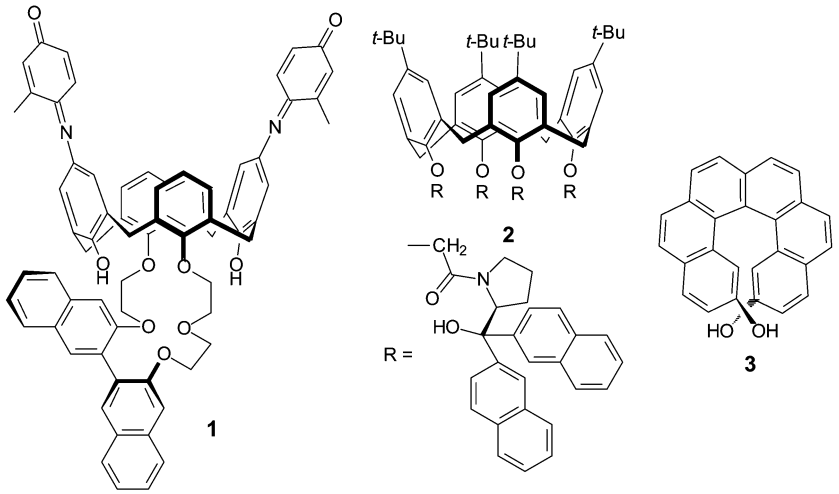

Fig. 2 Amine sensors based on calixarenes (**1** and **2**) and a helicene (**3**).

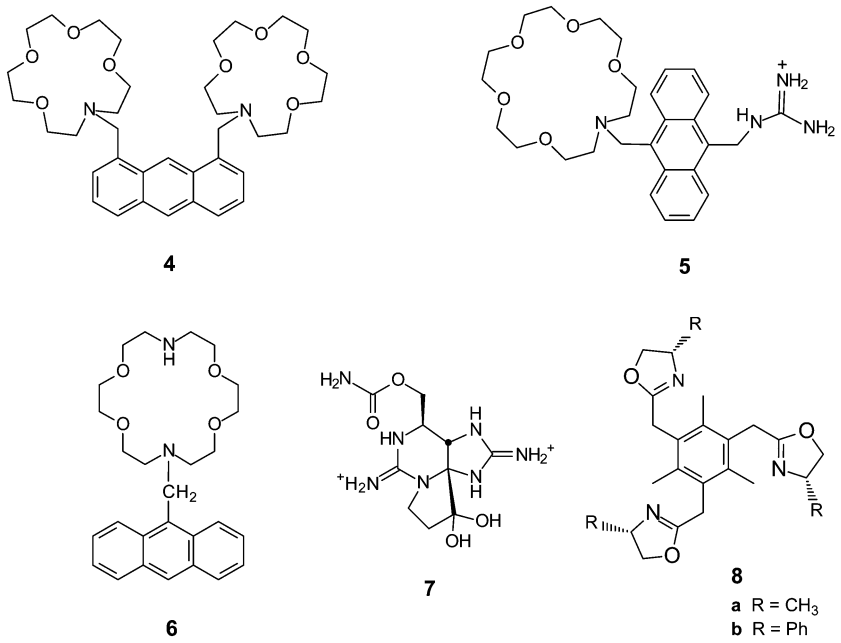

Fig. 3 Azacrown and tripodal chemosensors for alkyldiammonium ions (**4**), amino acids (**5**), saxitoxin (**6**), and alkylammonium ions (**8**).

host, but this does not significantly affect the fluorescence. Thus, similar fluorescence enhancement can also be seen with propylamine (K_s 79 M^{-1}). Therefore, **5** might be improved by replacing the guanidinium group with a PET-active carboxylate binding group to produce an AND gate, as has been accomplished in an analogue of **5** that functions as a fluorosensor for ion pairs.[15]

Gawley and Leblanc reported a range of azacrowns that bind saxitoxin (**7**), a potent marine toxin.[23] Receptor **6** was found to have the best binding constant in 4 : 1 ethanol–water: K_s 3.6 × 10^4 M^{-1}. In all cases, the binding was observed by an increase in the fluorescence of the receptor on addition of saxitoxin, and it was proposed that this was due to an inhibition of a PET process involving the benzylic nitrogen. However, the exact interaction was not established. Interestingly, the azacrowns gave no response on addition of simple organic molecules representing some of saxitoxin's functional groups (*e.g.*, adenine, arginine, and guanidinium).

Trisoxazolines **8a** and **8b** were synthesized by Ahn *et al.* as an alternative to azacrowns for binding and sensing ammonium and alkylammonium ions.[24] The flexible arms are conformationally organized by the formation of three hydrogen bonds between oxazoline nitrogens and acidic protons of the guest (RNH_3^+). This conformational restriction apparently decreases nonradiative decay of the benzene fluorophore. Disadvantages of this approach are the short, UV wavelengths for absorption and emission and the low absorptivity of the benzene fluorophore in **8a**, especially at the wavelength (272 nm) found to produce significant fluorescence in acetonitrile (λ_{em} *ca.* 300 nm). Tripodal chemosensor **8b** gives different fluorescent responses to (*R*)- and (*S*)-salts of 1-phenylethylamine, but binding constants for this and other alkylammonium guests were not reported. A chiral terpyridine/crown ether was recently reported by Wong *et al.* to bind α-phenylglycine methyl ester hydrochloride in dichloromethane enantioselectively ($K_s(S)$/$K_s(R)$ = 3.8).[25] As a fluorosensor, it also offers the potential advantage of longer wavelengths (λ_{ex} 316, λ_{em} 355 nm).

2.3 Amide, urea and guanidinium chemosensors for dicarboxylic acids

Several chemosensors have been developed using hydrogen bonding functional groups, such as amides, ureas and amidines, to bind carboxylic acids and carboxylates. The examples shown in Fig. 4 were selected to emphasize recognition of dicarboxylic acids. The first example (**9**) is a chromogenic sensor, while the remainder can act as fluorosensors. Amides **9–11** bind neutral carboxylic acids in nonpolar solvents, while ureas **12–14** and guanidinium **15** bind carboxylate anions in more polar solvents.

Cationic copper complex **9** was reported by Goodman, Hamilton and Weiss to bind dicarboxylic acids with some selectivity and color change from orange to red.[26] Binding constants, as measured by UV-visible spectroscopy in chloroform, are for example: glutaric acid (logK_s 4.9); *N*-benzyloxycarbonylglutamic acid (logK_s 4.6). It is proposed that bond rotations in the amide side arms, relative to the phenanthroline units of **9**, are required to favorably orient the carboxylic acid binding sites, and that this accounts for increased absorption at *ca.* 550 nm. As a result, different diacids produce not only different binding constants but also different spectral changes. Finally, it was suggested that complexation is caused by hydrogen bonding between the acid functionalities of the substrate and two acylaminopyridine moieties on two different ligands within **9**, and that a 2 : 1 analyte–chemosensor complex is formed.

Fluorescent chemosensor **10** (λ_{ex} 335 nm, λ_{em} 360–450 nm) was shown by Lustenberger *et al.* to bind *N*-(benzyloxy)carbonyl-protected aspartic acid (logK_s 4.8) and glutamic acid (logK_s 4.7) in dichloromethane.[27] The fluorescence response mechanism was not discussed, but conformational change can be expected when the guest carboxyl groups bind the amide side arms, which are structurally similar to those in **9**. A negative cooperativity effect was observed for the binding of a second guest.

Diamide cyclophane **11** was recently reported by Galindo *et al.* to bind Cbz-protected amino acids in dichloromethane with fluorescence response.[28] Recognition of dicarboxylic acids was not reported, but this chemosensor is included here because of its unusual response mechanism. Long wavelength emission (λ_{max} 390 nm) attributed to an "exciplex" between the naphthalene unit and neighboring amine groups is converted to typical naphthalene emission (λ_{max} 330 nm) upon protonation or association with a carboxylic acid. A small degree of enantiodifferentiation between L- and D-phenylalanine derivatives was observed.

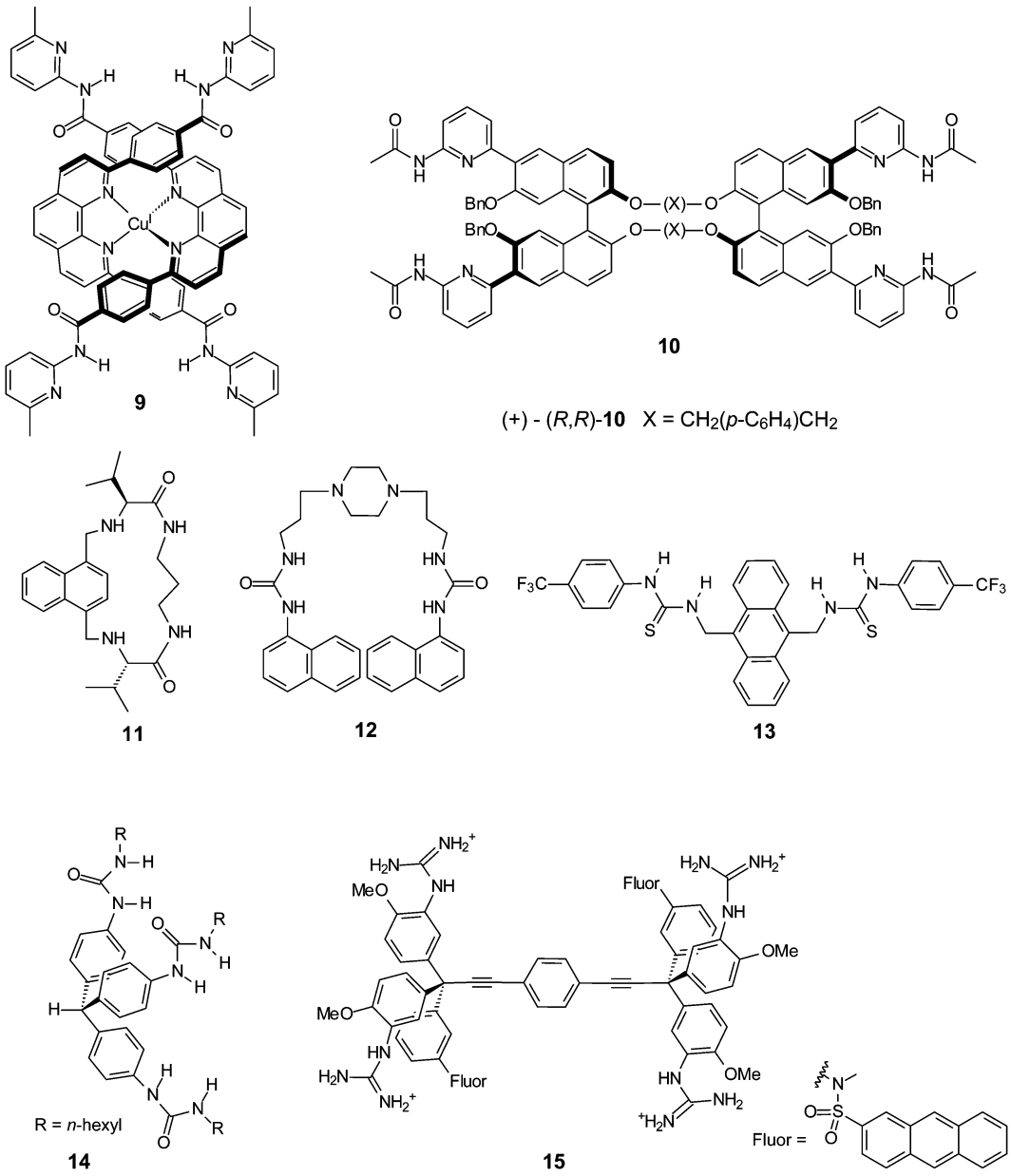

Fig. 4 Amide chemosensors for carboxylic acids (**9–11**) and urea/guanidinium chemosensors for dicarboxylates (**12–15**).

Urea-containing receptor **12** has been shown by Mei and Wu to give both chromogenic and fluorescence responses to α,ω-dicarboxylate anions ($^{-}O_2C(CH_2)_nCO_2^{-}$) in DMSO.[29] Observed selectivity is dependent on the chain length (n = 1, 3–6), with the greatest response being to pimelate (n = 5). It is believed that the sensing mechanism involves hydrogen bonding between the guest carboxylate groups and the urea hydrogens. This not only changes the longest wavelength absorption of the naphthalenes, but also produces a new fluorescence emission at longer wavelengths (*ca.* 500 nm). A similar approach has been used by Gunnslaugsson *et al.* with chemosensor **13**, which binds the following dianions in DMSO: glutarate (logK_s 3.7); malonate (logK_s 2.3).[30] In this case, the dicarboxylate forms hydrogen bonds with the thiourea moieties of the receptor. This switches on a PET process involving the thioureas and the anthracene unit, resulting in quenching of the anthracene fluorescence.

A third urea-type fluorosensor for dicarboxylates is triarylmethane derivative **14**, reported by Fan *et al.*[31] Binding of an aromatic dicarboxylic acid (terephthalate, K_s 2.3 $\times$ 10^4 M^{-1}) or tricarboxylic acid (trimesylate, K_s 2.1 $\times$ 10^4 M^{-1}) quenches fluorescence (λ_{ex} 268, λ_{em} 330 nm). No binding (by ^{1}H NMR spectroscopy) or fluorescence quenching was observed for halide, acetate, nitrate or nitrite ions, though benzoate was not compared. A tertiary amine bearing three 1-naphthylurea arms, as in **12**, was found to form weaker complexes than **14** with terephthalate and trimesylate. This effect was attributed to binding site preorganization afforded by the more rigid triarylmethane unit.

Partially preorganized triphenylmethane (trityl) "wheels" are also employed in "pinwheel receptor" **15**, reported by Raker and Glass.[32] In this case, cationic guanidinium groups enable binding of dicarboxylate anions in water. In the absence of a guest, the trityl groups in **15** can freely rotate about the *p*-phenylenediyne axis. Binding of small dicarboxylates can occur by hydrogen bonding to two guanidinium units on different trityl groups. Binding of two dicarboxylate guests then enforces an interaction between the pendant anthracene-sulfonamide fluorophores (λ_{ex} 376 nm, λ_{em} *ca.* 450 nm), with the result being a decrease in fluorescence intensity and a small bathochromic shift (23 nm) of the emission wavelength. The following affinities were observed in buffered (pH 7.5) aqueous media: malonate (logK_s 7.4); glutarate (logK_s 6.1); phthalate (logK_s 9.1). Of all the dicarboxylic acid chemosensors shown in

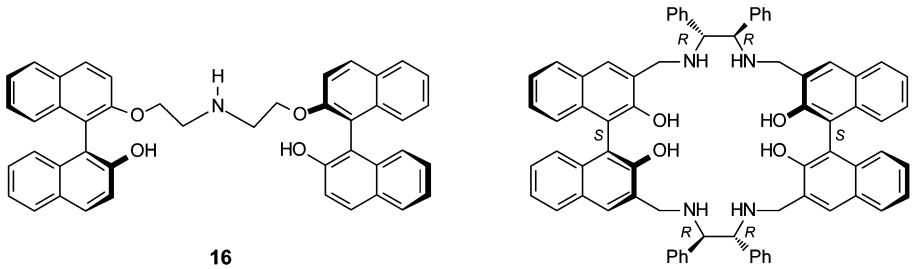

Fig. 5 Chemosensors for α-hydroxycarboxylic acids.

Fig. 5, **15** is perhaps the most attractive in terms of operating at relatively long wavelength in biologically relevant media.

2.4 Binaphthol/amine-containing chemosensors for α-hydroxycarboxylic acids

Pu and co-workers have been investigating chiral bisbinaphthyl-based fluorescent sensors for the enantioselective recognition of α-hydroxycarboxylic acids (Fig. 5). One example is **16** which, in benzene containing 2% DME, gave a greater fluorescence enhancement with (*S*)-mandelic acid (2.87 fold) when compared with (*R*)-mandelic acid (1.75 fold).[33] In addition, the binding constant of the complex with (*S*)-mandelic acid (348 M^{-1}) was found to be larger than that with the (*R*) enantiomer (163 M^{-1}). It is believed that the nitrogen atom of the sensor quenches the fluorescence of the binaphthyl chromophores (λ_{ex} 310, λ_{em} *ca.* 340–450 nm) by a PET process. Binding to an acid switches off this process, causing the fluorescence enhancement. Another example is **17**, which under similar conditions was found to give greatly enhanced fluorescence with (*S*)-mandelic acid, particularly at longer wavelength (424 nm), but only a small fluorescence enhancement was observed with (*R*)-mandelic acid.[34a]† Again it is believed that the nitrogen atoms of the sensor quench the fluorescence of the binaphthyl chromophores by a PET process. In this case, binding of tetramine **17** to four guest molecules switches off this process, causing fluorescence enhancement.

2.5 Porphyrin-based chemosensors for sugars and other small molecules

D'Souza has reported the use of suitably appended porphyrins to sense quinones and hydroquinones (Fig. 6). For example, porphyrin **18** was found to give a fluorescence response to hydroquinone in benzonitrile (λ_{em} 652, 720 nm).[35] The free receptor is weakly fluorescent, which is believed to be due to a PET process involving the excited singlet state of the porphyrin (donor) and the appended quinone (acceptor). However, the excited-state electron transfer process is inhibited when hydroquinone forms hydrogen bonds to the quinone, resulting in fluorescence enhancement. The binding constant was calculated from ^{1}H NMR data to be 13.1 M^{-1} in $CDCl_3$.

Neutral zinc porphyrin **19** was observed by both UV-visible and fluorescence spectroscopy to bind nicotine in toluene ($\log K_s$ 5.66).[36] The molecular recognition events are believed to involve the pyridyl nitrogen of nicotine axially ligating the zinc ion and the pyrrolidine nitrogen hydrogen bonding to the carboxylic acid group. The result is a bathochromic shift of the Soret and visible bands of **19** and a decrease in the intensity of the emission bands of the zinc porphyrin fluorophore at 605 and 650 nm (λ_{ex} 420 nm).

Král and coworkers have developed sensors for carbohydrates in aqueous environments, some based on appended porphyrins. One is cationic iron–porphyrin complex **20**, which in water/acetonitrile (1 : 1) gave UV-visible responses to a variety of carbohydrates, for example: D-glucose ($\log K_s$ 2.0); maltose ($\log K_s$ 2.4); maltotriose ($\log K_s$ 2.3).[37] Overall, the selectivity is relatively poor but a preference for disaccharides over monosaccharides is observed. Another example is **21**, which in water (containing 5% methanol) gave the following stability constants: D-glucose ($\log K_s$ 3.1); D-lactose ($\log K_s$ 4.5); maltotriose ($\log K_s$ 4.7).[38] Overall, receptor **21** shows some selectivity for the trisaccharide maltotriose, relative to monosaccharides. In both cases, sugar binding could be monitored by a decrease in the intensity of the Soret absorption bands (*ca.* 400–450 nm).

Tetraarylporphyrins of the type **22** bearing four urea side arms derived from amino acids, were reported as chemosensors for sugars by two research groups in 2002. Ladomenou and Bonar-Law observed a bathochromic shift of the Soret absorption band from 424 to 428 nm upon forming octyl or decyl pyranoside complexes in dichloromethane (K_s 8–50 × 10^4 M^{-1}).[39] Kim and Hong used fluoresence titrations to determine stability constants of octyl pyranoside complexes in chloroform (K_s 4–20 × 10^6 M^{-1}).[40] Both groups found that the metal-free porphine and zinc porphyrin formed hydrogen-bonded complexes of comparable strength, but Ladomenou and Bonar-Law reported that the zinc derivatives gave larger changes in UV-visible spectra.

2.6 Chemosensors derived from metal complexes for anion detection

Coordination of analyte donor atoms to metal sites can be exploited effectively for binding and signaling purposes in chemosensors.[41] Already discussed is example **19** (Fig. 6), in which coordination and hydrogen bonding sites cooperate to effect guest recognition. Three additional examples of metal-containing chemosensors are presented in Fig. 7. Building on earlier work, Fabbrizzi and coworkers used all-*cis*-2,4,6-triamino-1,3,5-trimethoxycyclohexane as a chemosensor scaffold by attaching a 9-anthrylmethyl fluorophore to one of the amino groups.[42] Resulting ligand **23** (ATMCA) forms a fluorescent complex with Zn^{II}, which binds various nucleotides and some carboxylate anions in water. Among simple carboxylic and dicarboxylic acids, only oxalate was detected ($\log K_s$ 4.3, 40% quenching). The anion of orotic acid (**24**) formed a stronger complex ($\log K_s$ 6.6) than various nucleotides and uniquely produced complete fluorescence quenching.

Research groups of Gunnlaugsson[43] and Parker[44] have been developing lanthanide complexes of cyclen triamides, such as **25**,[43] as luminescence probes of anionic biomolecules. For example, luminescence of **Tb(III)·25** (λ_{em} 491, 548 nm) is turned on by sensitization of the lanthanide ion excited state by a coordinated aromatic carboxylic acid upon displacement of metal-bound water molecules.[43] Thus, **Tb(III)·25** is an excellent chemosensor for salicylic acid (λ_{ex} 296 nm, $\log K_s$ *ca.* 4.5, H_2O) and shows no optical response to acetyl salicylate (Aspirin®).

† Ref. 34*b*, the full paper corresponding to the communication in ref. 34a, was added at proof.

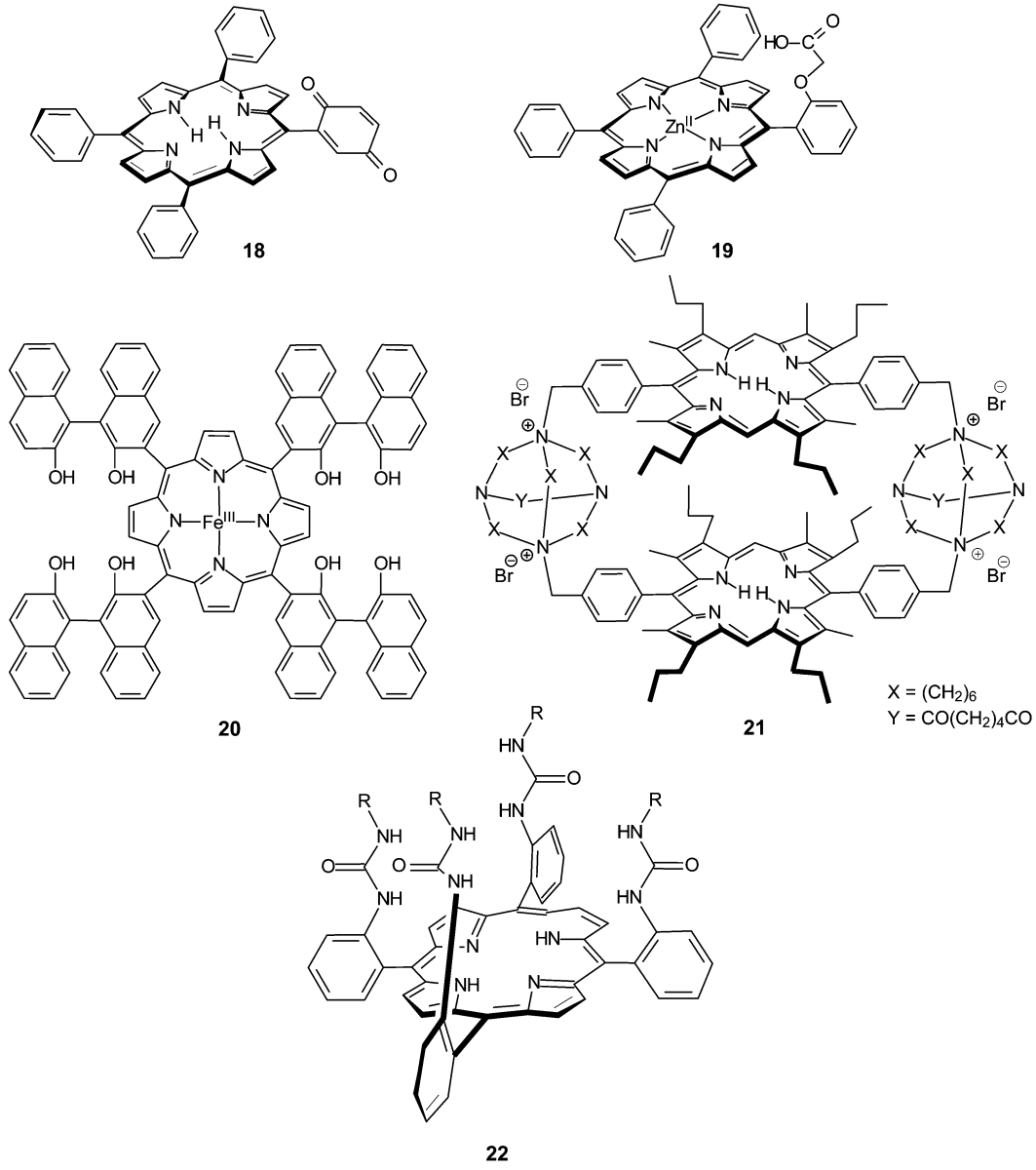

Fig. 6 Porphyrin chemosensors for hydroquinone (**18**), nicotine (**19**), and carbohydrates (**20–22**).

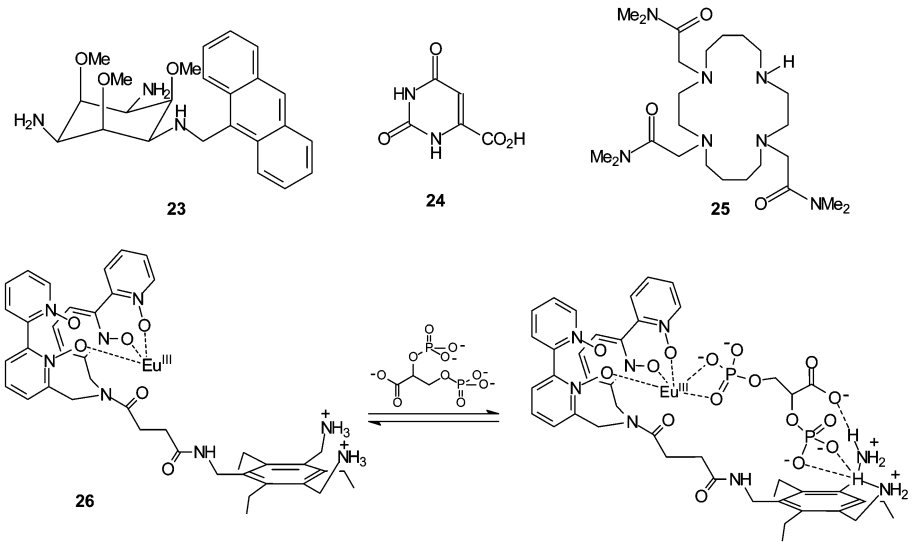

Fig. 7 Metal coordination approaches to chemosensors: Zn^{II} complex of **23** as a sensor for orotic acid (**24**); analyte sensitized luminescence of Tb^{III} complex of **25** upon binding salicylate; luminescence quenching of Eu^{III} complex **26** upon binding 2,3-bisphosphoglycerate.

Because the analyte acts as an optical "antenna," this method is highly specific. On the other hand, other anions may compete with analyte binding, and anions lacking chromophores could not be detected in the same manner.

Europium complex **26** was designed by the Anslyn group as a luminescence sensor for 2,3-bisphosphoglycerate (BPG).[45] Steric gearing in the tris-functionalized triethylbenzene scaffold favors the convergent conformation of the three functional side arms. The bis(2,2′-bipyridyl di-*N*-oxide)Eu^{III} luminophore of **26** (λ_{ex} 260 nm) is emissive only in solvents containing less than 5% water. In 50% methanol–acetonitrile, **26** binds BPG (K_s 6.7 × 10^5 M^{-1}) with quenching of the six-line Eu^{III} emission (λ_{max} 610 nm). Some selectivity was observed relative to 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate. Generally speaking, metal complexes have potential advantages stemming from strong binding, even in polar media, but the environment near the metal center needs to be carefully controlled to obtain selectivity.

2.7 Polyamide chemosensors for peptides

Still and co-workers have taken an alternative approach to chemosensors, whereby a potential receptor is synthesized and then tested against a bead-supported tripeptide library. For example, receptor **27** (Fig. 8) bound only two sequences of a solid-phase binding screen consisting of 3375 *N*-acetylated side chain-protected tripeptides, and was then shown to bind one of these tripeptides (as its *N*-acetyl, C-*n*-propylamide) in chloroform.[46] The free receptor is weakly fluorescent, indicative of highly effective fluorescence quenching of the fluorophore (**F**) by the quencher (**Q**). Binding of the tripeptide increases the **F**–**Q** separation, resulting in enhanced fluorescence. A binding constant of 2.6 × 10^5 M^{-1} was observed. Later, the reverse strategy was investigated, whereby an encoded combinatorial library of chemosensors was synthesized, similar in design to **27**, for screening of specific tripeptides.[47]

The latter approach was adopted by Hioki, who synthesized a 3375-membered library of peptido[4]calixarenes for testing against dye-labeled ^{5}Leu enkephalin derivative **28**.[48] Screening of the library with **28** gave fifteen potential peptidocalixarene sensors. One (**29**) was investigated in solution studies on a palmitoyl labeled ^{5}Leu enkephalin analog. Binding resulted in fluorescence enhancements of both the monomer and excimer emissions of the pyrene, though the response mechanism is unclear. In these examples, changes in the local environment (*e.g.*, polarity) of the pendant fluorophore, accompanying peptide binding in water, are apparently sufficient to produce useful fluorescent response.

2.8 Fused-ring heterocylic chemosensors for ureas, creatinine and sugars

Several chemosensors have been tailored to bind neutral organic molecules strictly *via* hydrogen-bond donor and acceptor groups. Fig. 9 shows some examples in which pyridine rings serve as hydrogen bond acceptors. Receptor **30** was designed by Bell *et al.* to bind urea *via* six hydrogen bonds.[49] Complexation in DMSO was accompanied by a 16 nm bathochromic shift in the UV-visible absorption of the receptor, from which a stability constant of 1.4 × 10^4 M^{-1} was calculated. The Thummel group pursued a different approach to complexing ureas, exemplified by receptor **31**.[50] Stability constants were determined by ^{1}H NMR spectroscopy in chloroform, as follows: imidazolidone (2.2 × 10^3 M^{-1}); barbital (1.4 × 10^4 M^{-1}). A related receptor in which the central pyridine faces outside the cavity gave stronger complexes.[50] A recent reinvestigation of **31** by Chou *et al.* found that the 420 nm emission band undergoes a bathochromic shift upon complexation of cyclic ureas, while binding of carboxylic acids quenches the emission.[51] The stability constant of **31**·imidazolidone in benzene was determined as 2.0 × 10^4 M^{-1} by fluorescence titration. The complexes of both **30** and **31** benefit from a high degree of host preorganization. While not specifically designed as optical chemosensors, both

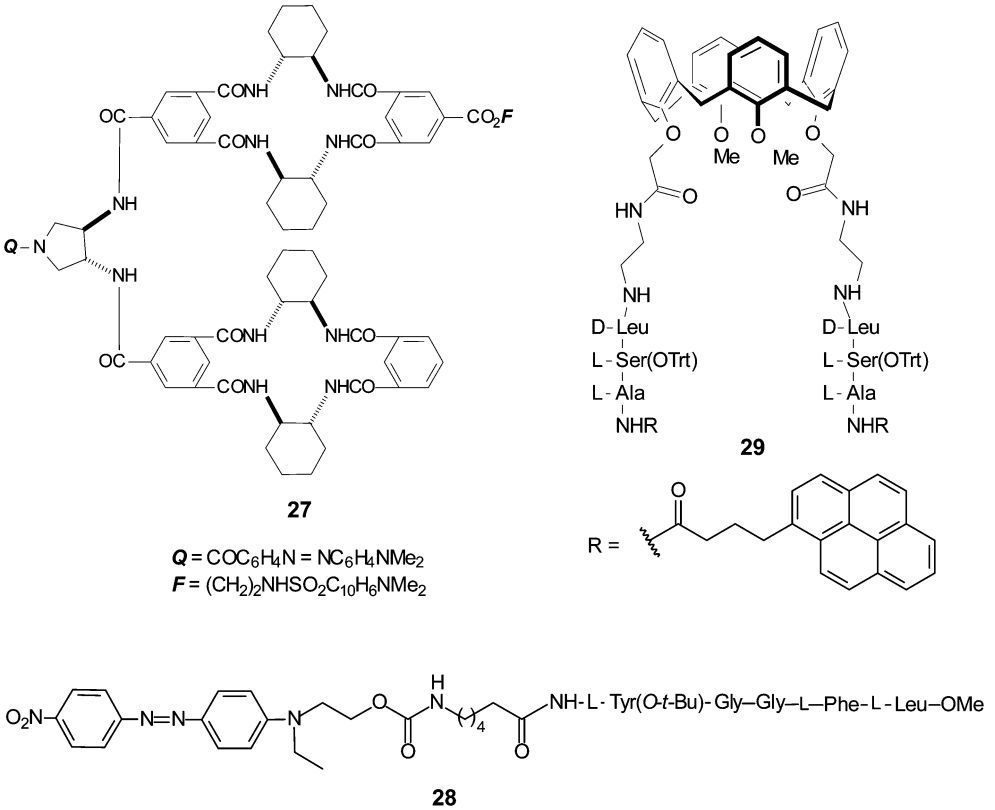

Fig. 8 Two chemosensors for peptides (**27** and **29**).

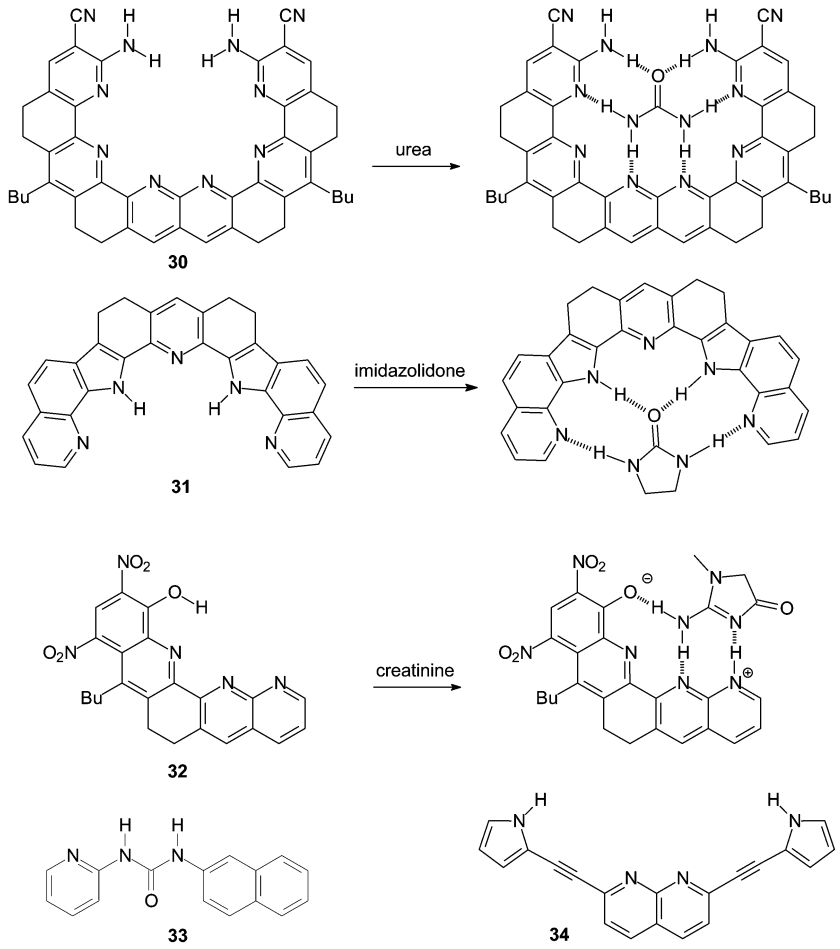

Fig. 9 Hydrogen-bonding chemosensors for ureas (**30** and **31**), creatinine (**32** and **33**) and monosaccharides (**34**).

have chromphores/fluorophores that are intrinsic to the binding site, so binding and optical signaling are naturally linked.

Receptor **32** was designed by Bell *et al.* to bind creatinine *via* three hydrogen bonds.[52] Creatinine is extracted from buffered water into a chloroform solution of **32**, producing a proton shift from the phenolic OH group to a naphthyridine nitrogen atom (see Fig. 9). This causes a large bathochromic shift of the longest wavelength absorption band, from which a K_s of 2×10^6 M^{-1} was calculated. This tautomerization illustrates how analyte binding can strongly influence an intrinsic chromophore to produce a large optical response. Fluorescent chemosensor **33**, which was also designed to bind creatinine *via* three hydrogen bonds, was reported by Mei and Wu[53] as an alternative to chromogenic system **32**. Extraction of creatinine from water into a chloroform solution of **28** enhances fluorescence of the naphthylurea moiety (λ_{ex} 300 nm, λ_{em} 370 nm), but the sensitivity of this chemosensor is unclear.

Receptor **34** was designed by Liao *et al.* to bind monosaccharides *via* two hydrogen-bond accepting nitrogens of the central 1,8-naphthyridine unit and two flanking hydrogen-bond donating pyrrole rings.[54] In dichloromethane, **34** binds octyl β-D-glucopyranoside with a shift of the 410 nm absorption band to *ca.* 435 nm (K_s 5×10^3 M^{-1}). The fluorescence band also undergoes a bathochromic shift from 475 to 535 nm, and the larger Stokes shift observed for the complex is attributed to stabilization of the ICT state by hydrogen bonding. Threefold binding selectivity was observed for octyl glucopyranoside *vs.* octyl galactopyranoside. The optical effects of sugar binding observed for **34** are potentially useful, but complexation is much weaker than for some chemosensors for sugars described earlier (Fig. 6).

3 Conclusions

Chemists have used many different approaches to design artificial receptors capable of selectively binding organic analytes with optical response. The result is a wide range of molecular structures constituting the binding and signal-generating components of chemosensors, whether the optical reporter is integrated with (intrinsic) or simply linked to the guest binding site (extrinsic). A variety of different host–guest interactions have been employed to stabilize the complex and varying degrees of preorganization have been used to enhance analyte selectivity. Most chemosensors reported to date are not sufficiently sensitive or selective for practical application, and they would usually require synthetic modification in order to tune wavelengths and to immobilize the chemosensor. Research to date in this field has been conducted almost entirely in the academic arena, and little attention has been paid to practical issues, such as incorporation of long wavelength fluorophores. Nevertheless, there has been substantial progress in developing chemosensors for organic analytes over the last decade. Over the next decade, combination of this knowledge with additional chemical ingenuity and enhanced funding from companies and governments should result in many practical sensors for organic analytes of biomedical and environmental interest.

References

1 A. W. Czarnik, in *Advances in Supramolecular Chemistry*, ed. G. W. Gokel, JAI Press, Greenwich, Connecticut, 1993, vol. 3, pp. 131–157.
2 A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302–308.
3 T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159–200.

4 T. W. Bell and N. M. Hext, in *Optical Biosensors: Present and Future*, eds. F. S. Ligler and C. Rowe Taitt, Elsevier Science, Amsterdam, The Netherlands, 2002, ch. 11, pp. 331–368.
5 V. A. Bren, *Russ. Chem. Rev.*, 2001, **70**, 1017–1036.
6 C. M. Rudzinski and D. G. Nocera, in *Optical Sensors and Switches*, ed. V. Ramamurthy and K. S. Schanze, Marcel Dekker, New York, 2001, ch. 1, pp. 1–91.
7 A. P. de Silva, D. B. Fox, T. S. Moody and S. M. Weir, in *Optical Sensors and Switches*, ed. V. Ramamurthy and K. S. Schanze, Marcel Dekker, New York, 2001, ch. 2, pp. 93–151; A. P. de Silva, D. B. Fox, T. S. Moody and S. M. Weir, *Pure Appl. Chem.*, 2001, **73**, 503–511.
8 J. K. Tusa and M. J. P. Leiner, *Ann. Biol. Clin.*, 2003, **61**, 183–191.
9 For examples of practical chemosensors for alkali metals in blood (λ_{ex} 470 nm), see: H. He, M. A. Mortellaro, M. J. P. Leiner, S. T. Young, R. J. Fraatz and J. K. Tusa, *Anal. Chem.*, 2003, **75**, 549–555; H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz and J. K. Tusa, *J. Am. Chem. Soc.*, 2003, **125**, 1468–1469.
10 K. J. Albert, D. R. Walt, D. S. Gill and T. C. Pearce, *Anal. Chem.*, 2001, **73**, 2501–2508.
11 J. J. Lavigne and E. V. Anslyn, *Angew. Chem. Int. Ed.*, 2001, **40**, 3118–3130; S. C. McCleskey, M. J. Griffin, S. E. Schneider, J. T. McDevitt and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 1114–1115.
12 D. J. Cram, *Science*, 1988, **240**, 760–767.
13 T. W. Bell, D. L. Beckles, P. J. Cragg, J. Liu, J. Maioriello, A. T. Papoulis and V. J. Santora, in *Fluorescent Chemosensors for Ion and Molecule Recognition*, ed. A. W. Czarnik, ACS Symposium Series 538, American Chemical Society, Washington, DC, 1993, ch. 7, pp. 85–103.
14 J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Edn., Kluwer/Plenum, New York, 1999.
15 For a recent example of an AND gate and references to chemosensors as logic gates, see: A. P. de Silva, G. D. McClean and S. Pagliari, *J. Chem. Soc., Chem. Commun.*, 2003, 2010–2011; A. P. de Silva and N. D. McClenaghan, *Chem. Eur. J.*, 2004, **10**, 574–586.
16 P. V. Bernhardt and E. G. Moore, *Aust. J. Chem.*, 2003, **56**, 239–258.
17 Y. Kubo, S. Maeda, S. Tokita and M. Kubo, *Nature*, 1996, **382**, 522–524; Y. Kubo, S. Maeda, S. Tokita and M. Kubo, *Enantiomer*, 1997, **2**, 287–292.
18 T. Grady, S. J. Harris, M. R. Smyth, D. Diamond and P. Hailey, *Anal. Chem.*, 1996, **68**, 3775–3782; T. Grady, T. Joyce, M. R. Smyth, S. J. Harris and D. Diamond, *Anal. Commun.*, 1998, **35**, 123–125.
19 M. T. Reetz and S. Sostmann, *Tetrahedron*, 2001, **57**, 2515–2520.
20 F. Fages, J.-P. Desvergne, H. Bouas-Laurent, J.-M. Lehn, J. P. Konopelski, P. Marsau and Y. Barrans, *J. Chem. Soc., Chem. Commun.*, 1990, 655–660; A. P. de Silva and K. R. A. Samankumara Sandanayake, *Angew. Chem. Int. Ed.*, 1990, **29**, 1173–117.
21 S. K. Kim, M. Y. Bang, S.-H. Lee, K. Nakamura, S.-W. Cho and J. Yoon, *J. Inclusion Phenom. Macrocycl. Chem.*, 2002, **43**, 71–75.
22 A. P. de Silva, H. Q. N. Gunaratne, C. McVeigh, G. E. M. Maguire, P. R. S. Maxwell and E. O'Hanlon, *Chem. Commun.*, 1996, 2191–2192.
23 R. E. Gawley, S. Pinet, C. M. Cardona, P. K. Datta, T. Ren, W. C. Guida, J. Nydick and R. M. Leblanc, *J. Am. Chem. Soc.*, 2002, **124**, 13448–13453.
24 K. H. Ahn, H.-Y. Ku, Y. Kim, S.-G. Kim, Y. K. Kim, H. S. Son and J. K. Ku, *Org. Lett.*, 2003, **5**, 1419–1422.
25 W.-L. Wong, K.-H. Huang, P.-F. Teng, C.-S. Lee and H.-L. Kwong, *Chem. Commun.*, 2004, 384–385.
26 M. S. Goodman, A. D. Hamilton and J. Weiss, *J. Am. Chem. Soc.*, 1995, **117**, 8447–8455.
27 P. Lustenberger, R. Welti and F. Diederich, *Helv. Chim. Acta*, 1998, **81**, 2190–2200.
28 F. Galindo, J. Becerril, M. I. Burguete, S. V. Luis and L. Vigara, *Tetrahedron Lett.*, 2004, **45**, 1659–1662.
29 M. Mei and S. Wu, *New J. Chem.*, 2001, **25**, 471–475.
30 T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Lett.*, 2002, **4**, 2449–2452.
31 A. Fan, H. K. Hong, S. Valiyaveettil and J. J. Vittal, *J. Supramolec. Chem.*, 2002, **2**, 247–254.
32 J. Raker and T. E. Glass, *J. Org. Chem.*, 2002, **67**, 6113–6116.
33 M.-H. Xu, J. Lin, Q.-S. Hu and L. Pu, *J. Am. Chem. Soc.*, 2002, **124**, 14239–14246.
34 (*a*) J. Lin, H.-C. Zhang and L. Pu, *Org. Lett.*, 2002, **4**, 3297–3300; (*b*) Z.-B. Li, J. Lin, H.-C. Zhang, M. Sabat, M. Hyacinth and L. Pu, *J. Org. Chem.*, 2004, **69**, 6284–6293.
35 F. D'Souza, G. R. Deviprasad and Y.-Y. Hsieh, *Chem. Commun.*, 1997, 533–534.
36 G. R. Deviprasad and F. D'Souza, *Chem. Commun.*, 2000, 1915–1916.
37 O. Rusin, K. Lang and V. Král, *Chem. Eur. J.*, 2002, **8**, 655–663.
38 V. Král, O. Rusin and F. P. Schmidtchen, *Org. Lett.*, 2001, **3**, 873–876.
39 K. Ladomenou and R. P. Bonar-Law, *Chem. Commun.*, 2002, 2108–2109.
40 Y.-H. Kim and J.-I. Hong, *Angew. Chem. Int. Ed.*, 2002, **41**, 2947–2950.
41 C. W. Rogers and M. O. Wolf, *Coord. Chem. Rev.*, 2002, 341–350.
42 L. Fabbrizzi, M. Licchelli, F. Mancin, M. Pizzeghello, G. Rabaioli, A. Taglietti, P. Tecilla and U. Tonellato, *Chem. Eur. J.*, 2002, **8**, 94–101.
43 T. Gunnlaugsson, A. J. Harte, J. P. Leonard and M. Nieuwenhuyzen, *Chem. Commun.*, 2002, 2134–2135.
44 R. S. Dickins, S. Aime, A. S. Batsanov, A. Beeby, M. Botta, J. I. Bruce, J. A. K. Howard, C. S. Love, D. Parker, R. D. Peacock and H. Puschmann, *J. Am. Chem. Soc.*, 2002, **124**, 12697–12705.
45 M. D. Best and E. V. Anslyn, *Chem. Eur. J.*, 2003, **9**, 51–57.
46 C.-T. Chen, H. Wagner and W. C. Still, *Science*, 1998, **279**, 851–854.
47 E. J. Iorio, Y. Shao, C.-T. Chen, H. Wagner and W. C. Still, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 1635–1638.
48 H. Hioki, M. Kubo, H. Yoshida, M. Bando, Y. Ohnishi and M. Kodama, *Tetrahedron Lett.*, 2002, **43**, 7949–7952.
49 T. W. Bell and Z. Hou, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1536–1538.
50 V. Hegde, C.-Y. Hung, P. Madhukar, R. Cunningham, T. Höpfner and R. P. Thummel, *J. Am. Chem. Soc.*, 1993, **115**, 872–878.
51 H.-C. Chou, C.-H. Hsu, Y.-M. Cheng, C.-C. Cheng, H.-W. Liu, S.-C. Pu and P.-T. Chou, *J. Am. Chem. Soc.*, 2004, **216**, 1650–1651.
52 T. W. Bell, Z. Hou, Y. Luo, M. G. B. Drew, E. Chapoteau, B. P. Czech and A. Kumar, *Science*, 1995, **269**, 671–674.
53 M.-H. Mei and S.-K. Wu, *Acta Chim. Sinica*, 2002, **60**, 866–869.
54 J.-H. Liao, C.-T. Chen, H.-C. Chou, C.-C. Cheng, P.-T. Chou, J.-M. Fang, Z. Slanina and T. J. Chow, *Org. Lett.*, 2002, **4**, 3107–3110.