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New chromogenic and fluorogenic reagents and sensors for neutral and ionic analytes based on covalent bond formation—a review of recent developments

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Abstract To date, hydrogen bonding and Coulomb, van der Waals and hydrophobic interactions are the major contributors to non-covalent analyte recognition using ionophores, ligands, aptamers and chemosensors. However, this article describes recent developments in the use of (reversible) covalent bond formation to detect analyte molecules, with special focus on optical signal transduction. Several new indicator dyes for analytes such as amines and diamines, amino acids, cyanide, formaldehyde, hydrogen peroxide, organophosphates, nitrogen oxide and nitrite, peptides and proteins, as well as saccharides have become available. New means of converting analyte recognition into optical signals have also been introduced, such as colour changes of chiral nematic layers. This article gives an overview of recent developments and discusses response mechanisms, selectivity and sensitivity.

Keywords Chemosensor · Optical probe · Optical sensor · Biomolecules · Chemical warfare agents

Introduction

Selective ligands and ionophores for cations are well established in analytical chemistry and are mainly used in ion-selective electrodes. Similar ligands and ionophores have also been used to develop optical sensors, thereby taking advantage of the so-called mechanism of “co-extraction” or “ion-exchange”. However, the continuous optical detection of neutral analytes is mainly performed by enzyme-based electrodes or optodes, which are limited in operational stability and shelf life. In order to circumvent this limitation, new indicator dyes for neutral and ionic analytes have been developed that recognise the analyte through the formation of a covalent bond. This bond can be

a stable one—then a probe (chemodosimeter) for the analyte is obtained. However, covalent bond formation can also be a reversible process, and the chemosensor (chromoreactant) can be used for continuous monitoring of the analyte [1].

The colour changes necessary for optical transduction of the formation/breaking of the covalent bond can be achieved via several means: (a) the indicator dye reacts with the analyte via a specific functional group directly attached to the chromophore, and a change in electron acceptor/donor strength of this functional group changes electron delocalisation within the chromophore (internal charge transfer); (b) the interaction with the analyte does not affect the chromophore directly but causes photo-induced electron transfer from the receptor moiety to the fluorophore (connected to each other via a spacer), and (c) a cholesteryl-based receptor embedded in a chiral, nematic, liquid crystal film recognises the analyte via formation of a covalent bond and this recognition modulates the colouration of the liquid crystal film.

In the present paper recent developments in the field of indicator dyes and optical sensor layers for neutral and ionic analytes will be described, and future developments to obtain advanced indicator dyes tailored for practical applications will be discussed.

New indicator dyes for neutral and ionic analytes

There is a strong need for new indicator dyes to detect neutral and ionic analytes in order, e.g. to facilitate the measurement of drugs and metabolites in cells or to monitor amino acids and saccharides in the bioreactor. However, several important requirements have to be considered in order to obtain dyes that can be considered for practical application, namely (a) the indicator dyes have to provide significant and selective response to the analyte (preferably with a shift in absorbance/luminescence maximum to enable ratiometric measurements), (b) they should absorb and emit in the visible spectral range and should have a large Stokes' shift (to avoid cross-talk with

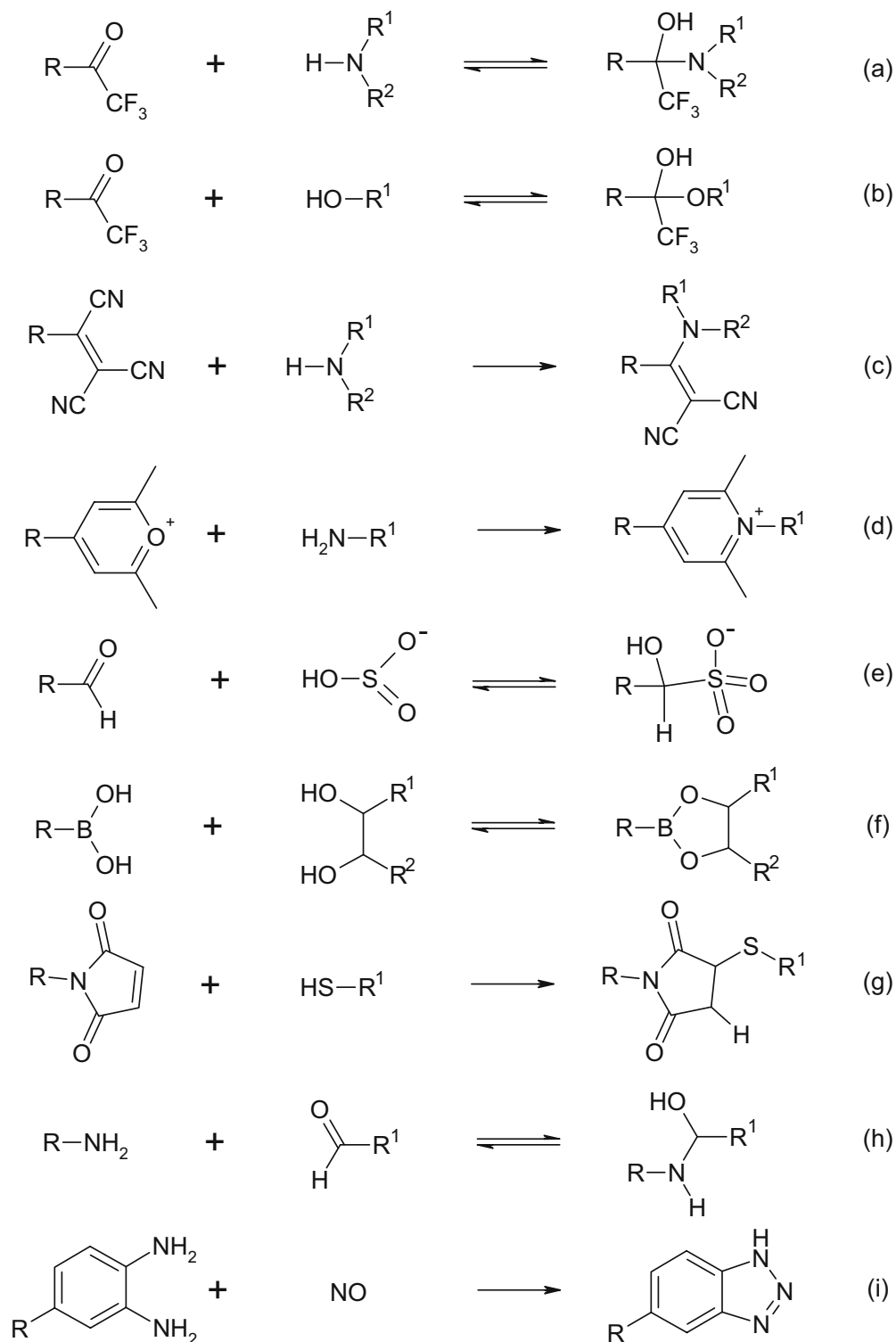
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autoluminescent samples and to simplify the signal evaluation), (c) they should be well water-soluble when applied in medical research (e.g. in cells), and (d) when applied for optical sensing, they should be polymer-soluble (i.e. highly lipophilic) or exhibit additional functional groups to link them covalently to the polymer support.

A large variety of indicator dyes and probes based on (reversible) covalent bond formation has become available

in the past decade, and the most prominent examples of chemical reactions used for optical evaluation are given in the following scheme (Fig. 1) [2]. Trifluoroacetyl groups (a,b) react with primary and secondary amines, to form hemi-aminals and, with tertiary amines, to form quaternary ammonium ion pairs. However, they are also capable of reacting with alcohols, thiols, phenols and thiophenols. In contrast, tricyanovinyl derivatives (c) only react with

Fig. 1 Receptor functions that can be used to recognise analyte molecules via the (reversible) formation of a covalent bond

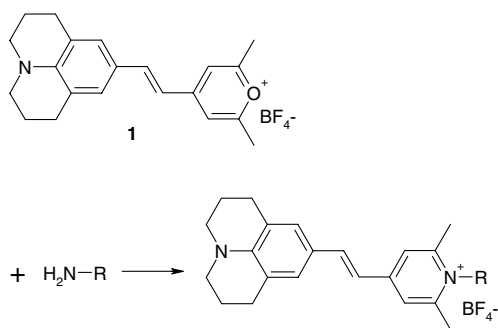


primary and secondary amines, to form 1-amino-2,2-dicyanovinyl derivatives. Pyrylium dyes (d) are even more selective and react with only primary amines, to form pyridinium derivatives. Nevertheless, under appropriate experimental conditions, they can also react with hydrogen sulphide and cyanide. Aldehyde functions (e) can perform the bisulphite addition reaction with hydrogen sulphite but can also react with hydrogen cyanide to form cyanohydrins, with hydrazine to form imines, and with amino acids to form iminium ions. Boronic acid functions (f) are well known for their interaction with saccharides and sugar acids to form cyclic boronic esters, but they also react with cyanide. Maleimides (g) perform the Michael addition reaction with thiols, and they form carbon-carbon bonds with ketones in the presence of basic catalysts. Primary amino groups (h) form Schiff bases with aldehydes and ketones and can form carbamic acid with carbon dioxide. Phenylene diamines (i) react with nitrogen monoxide to form the corresponding benzotriazole.

The present paper will introduce improved indicator dyes based on these chemical reactions and will also present some new approaches for the detection of electrically neutral and ionic analytes. This current review covers advances since the previous review written in 2004 and published in 2005 [2].

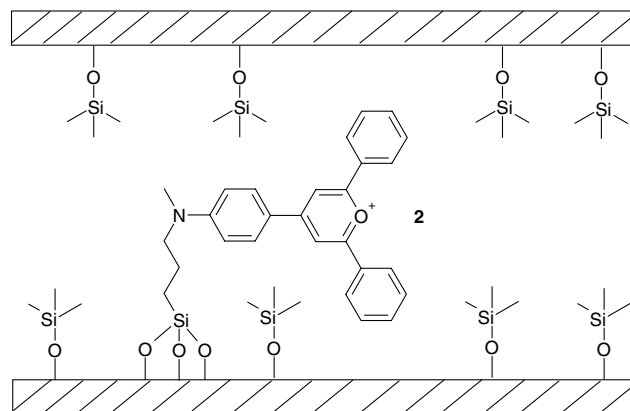
Detecting amines, diamines, amino acids and proteins

The group of O.S. Wolfbeis has recently introduced selective optical probes for primary aliphatic amines based on pyrylium dyes [3, 4]. These so-called “chameleon stains” were initially developed to label proteins and peptides but can also be used to probe simple primary aliphatic amines. Specifically, the 2,6-dimethylpyrylium probe **1** exhibited an excitation maximum at 611 nm and emission at 665 nm. Upon interaction with amines and subsequent internal charge transfer, the blue colour changed to red with excitation at 503 nm and emission at 602 nm. Since the main application of **1** is labelling of biomolecules it must react irreversibly, and, therefore, only a kinetic evaluation of sensor signals can be used to quantify amines. Furthermore, **1** does not show any preference for specific primary amines (e.g. of different

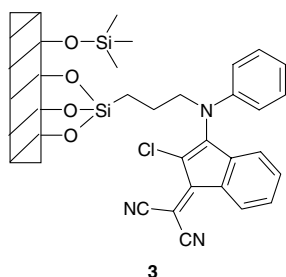


chain length), when evaluated in buffer of pH 9.0. However, this unselective reaction makes the dye especially suited for labelling applications.

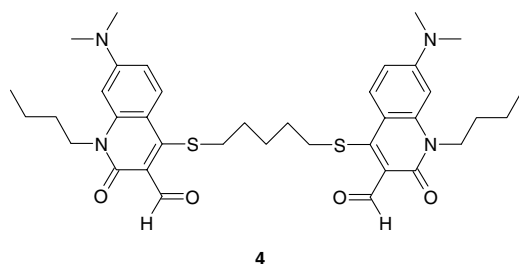
Maria Comes and co-workers used a similar chemical structure, e.g. 4-(4-aminophenyl)-2,6-diphenyl pyrylium perchlorate **2** for the detection of aliphatic amines in aqueous solution [5]. Again, the pyrylium derivative was easily converted into a pyridinium derivative when exposed to primary aliphatic amines in water:acetonitrile (4:1 v/v) causing the colour to change from magenta to yellow. In order to induce selectivity in the recognition process, they covalently attached the pyrylium derivative into the cavities of mesoporous silica material MCM-41. As a consequence, both the hydrophobicity of the inner surface and the pore system affected the selectivity in the analyte recognition. While the hydrophobic surface favoured the enrichment of large lipophilic guest molecules, the pore system favoured the entrance of smaller amines than that of amines with long alkyl chains. Consequently, colour changes were found for amines with “medium” chain length, such as *n*-heptyl-, *n*-octyl- and *n*-nonylamine, while no response to amines with longer or shorter alkyl chains was observed. The same indicator dye was also embedded into the cavities of zeolite Beta, again yielding coloured particles with enhanced selectivity for slim lipophilic aminoalkanes, while no response was observed for bulky amines (e.g. 1-methylaminopyrene) or more hydrophilic amines (e.g. 6-amino-1-hexanol) [6].



The same research group also synthesised a longer-wavelength absorbing probe for amines by incorporating the dicyanomethylene indene derivative **3** into mesoporous MCM-41 [7]. Again, the resulting colorimetric sensor particles exhibited enhanced selectivity for primary aliphatic amines with medium chain length. Colour changes from purple to pale orange were observed, with a concomitant shift in absorbance maximum from 554 nm to 445 nm when measurements were performed at a pH of 11.2. It is supposed that a nucleophilic attack of primary amines at the double bond of the dicyanomethylene moiety took place. In the present case, the sensor particles were more sensitive to amines with slightly longer alkyl chains, i.e. for *n*-nonylamine, *n*-decylamine and *n*-undecylamine, and showed no response to cyanide, hydroxide, or cations.

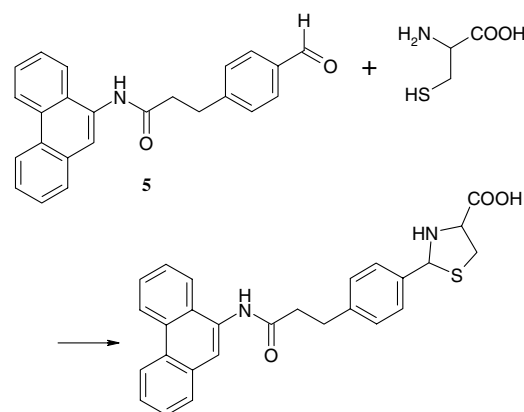


Secor and co-workers used a different approach to induce selectivity in the recognition of amines [8]. They synthesised a bifunctional receptor to specifically detect diaminoalkanes and the structurally related amino acids ornithine and lysine. When the dimer of quinolone aldehyde **4** was reacted with diaminopropane in methanol/water (1:1) buffered to pH 7.4, a shift in absorbance maximum from approximately 440 nm to 470 nm was observed, with a concomitant increase in fluorescence at 530 nm (when excited at 495 nm). Highest selectivity of **4** was observed for 1,3-diaminopropane ($K_{\text{assoc}}=6,700 \text{ M}^{-1}$), followed by lysine ($K_{\text{assoc}}=2,800 \text{ M}^{-1}$), ornithine ($K_{\text{assoc}}=2,400 \text{ M}^{-1}$), 1,5-diaminopentane ($K_{\text{assoc}}=2,200 \text{ M}^{-1}$) and 1,4-diaminobutane ($K_{\text{assoc}}=1,500 \text{ M}^{-1}$). NMR experiments confirmed 1:1 stoichiometry for the reaction of the aldehyde dimer **4** with diaminoalkanes to form bis-imines.

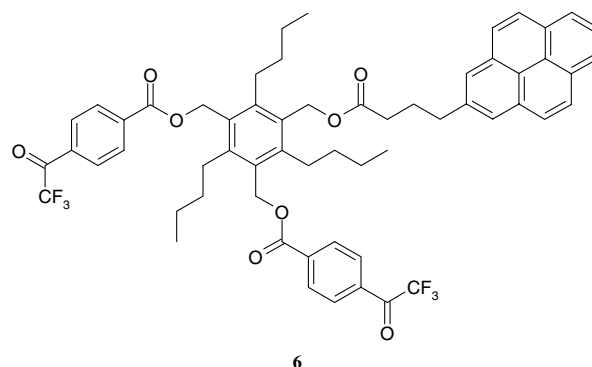


Tanaka and co-workers introduced a fluorogenic aldehyde for the detection of amino acids and, specifically, cysteine [9]. Their fluorescent phenanthrene derivative **5**, containing a reactive aldehyde function, reacted with cysteine to form a thiazolidine derivative. As a consequence of this specific chemical reaction, fluorescence increased significantly at 380 nm when excited at 250 nm. The probe was used for the detection of 100 μM –5 mM cysteine in sodium phosphate buffer of pH 7.0 containing 1% acetonitrile and 1% 2-propanol and was selective in that only glutathione showed small increase in fluorescence while the other amino acids investigated (e.g. methionine, serine, lysine, proline, histidine) did not show any response. From this selectivity pattern, and from the irreversible response, the authors concluded that thiazolidine rather than hemiaminal or iminium formation was responsible for the observed selectivity for cysteine. A kinetics evaluation was applied, making use of a microplate

spectrophotometer to detect fluorescence increases upon interaction with the analyte.

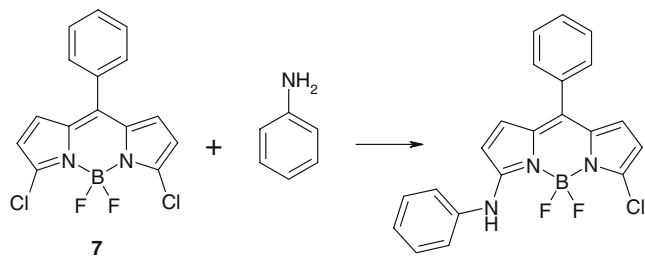


Sasaki and co-workers have used a different receptor group, i.e. the trifluoroacetyl function, to detect amino acids [10]. Similarly to Secor et al. (vide supra), they synthesised a bifunctional derivative **6**, where the first trifluoroacetyl group was suggested to form a hemi-aminal with the amino function, while the second trifluoroacetyl group reacted with the carboxylate function. The tripodal pyrene-based fluoroionophore **6** showed significant fluorescence enhancement at around 375 nm and 399 nm (excitation set to 342 nm) in acetonitrile solution upon exposure to glycine, cysteine and phenylalanine. A similar increase in fluorescence was observed when **6** was embedded in a thin layer of plasticised PVC and the resulting sensor layer was exposed to amino acids in a Tris- H_2SO_4 buffer of pH 8.6.

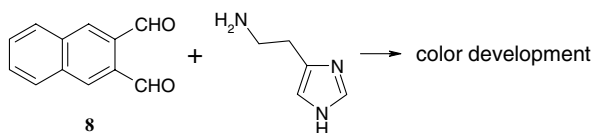


A possible new indicator dye for aromatic amines and nitrogen heterocycles has been presented by Rohand and co-workers. They synthesised 3,5-dichloro-BODIPY derivative **7** that showed significant reactivity towards amines in acetonitrile solution at room temperature [11]. Compound **7** exhibited absorbance and fluorescence maxima at 508 nm and 519 nm, respectively, when methanol was used as the solvent. Upon formation of a monosubstituted derivative with piperidine, the absorbance shifted to 479 nm and

fluorescence to 562 nm, while, in the case of aniline, the absorbance shifted to 498 nm and fluorescence to 566 nm. Therefore, the 3,5-dichloro-BODIPY derivative **7** may be of use for the detection of less nucleophilic amines.



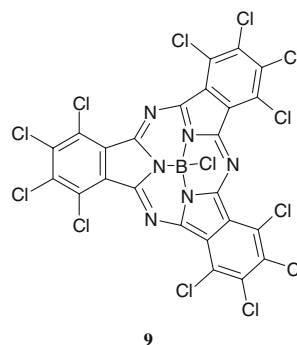
Oguri and co-workers found that the reagent 2,3-naphthalenedicarboxaldehyde **8** acted as a selective colour development reagent for histamine in the absence of any reductant, even in a weakly acidic buffer solution [12]. The reaction of histamine with **8** in a buffer of pH 6.0 caused an increase in absorbance at 552 nm within 10 min, and a linear calibration in the range 0.05–1.00 mM histamine was observed. No similar colour formation was observed for amino acids such as glycine, alanine, arginine, lysine, tryptophan or biogenic amines such as cadaverine, putrescine, spermidine or tyramine, while significant interference was observed from histidine and serotonin. The authors also developed a so-called histamine cartridge, where they used silica gel 60 in a column cartridge to enrich histamine from a real sample extract. They then washed the cartridge with buffer pH 6.0 and water, and, finally, they exposed the immobilised histamine to **8** in acetonitrile. In this case no interference from histidine and serotonin was observed, because these interfering species were eluted from the silica gel by being washed with buffer/water before exposure to 2,3-naphthalenedicarboxaldehyde solution.



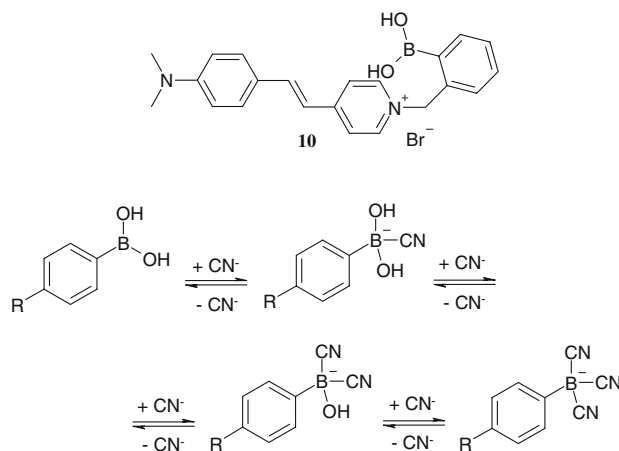
Detecting cyanide anion

Ros-Lis et al. synthesised subphthalocyanine fluorophore **9** with absorbance at 569 nm and an intensive emission at 581 nm (Φ_F in acetonitrile=0.26) [13]. This dye responded to nucleophilic anions by a complete decolouration, which was attributed to either a chemical reaction with the boron atom, with aromatic carbon atoms, or ring expansion upon interaction with the imine-type core. When pure acetonitrile was used as the solvent, compound **9** responded to fluoride, dihydrogen phosphate, acetate and cyanide. However, the authors found that by increasing the water content in acetonitrile from 1% to 5%, the selectivity in anion recognition increased significantly. With 1% of water in acetonitrile the dye responded to fluoride, acetate and

cyanide, while with 3% of water the dye responded to only fluoride and cyanide. With 5% of water the dye selectively responded to cyanide, and the binding constant was found to be $12,000 \text{ M}^{-1}$. Thus, by choosing the proper solvent, Ros-Lis et al. tailored the selectivity of the chemodosimeter **9** efficiently. The dye was also evaluated in acetonitrile/buffer (1:1) adjusted to pH 7.0 and pH 9.6, and higher sensitivity was observed at higher pH because predominantly nucleophilic cyanide was formed. Consequently, at pH 9.6 the limit of detection was 0.1 p.p.m., while it was 10 p.p.m. at pH 7.0.

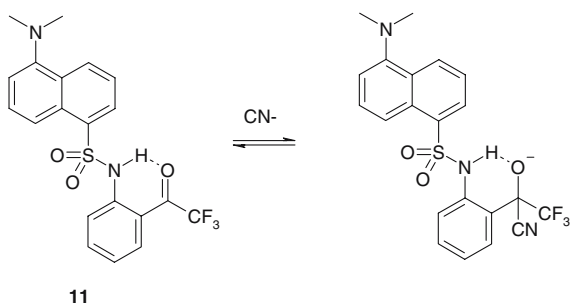


Badugu and co-workers have reported water-soluble hemicyanine dyes with boronic acid moieties that respond to cyanide [14, 15]. In this specific case, the interaction with cyanide caused a modulation of the electron-donating capabilities of the boronic acid function, which affected the internal charge transfer from the *N,N*-dimethylamino group to the electron-deficient quaternary ammonium ion. Consequently, 4-[4-(*N,N*-dimethylamino)styryl]-1-(2-boronobenzyl)pyridinium bromide **10** showed a significant increase in fluorescence at 600 nm (excitation at 475 nm), with highest sensitivity in the range of 1 μM to 30 μM cyanide. The indicator dye also operated well under physiological conditions, i.e. at high chloride background (up to 100 mM sodium chloride). Owing to a small but significant shift of the absorbance and fluorescence spectra to shorter wavelengths upon interaction with cyanide,

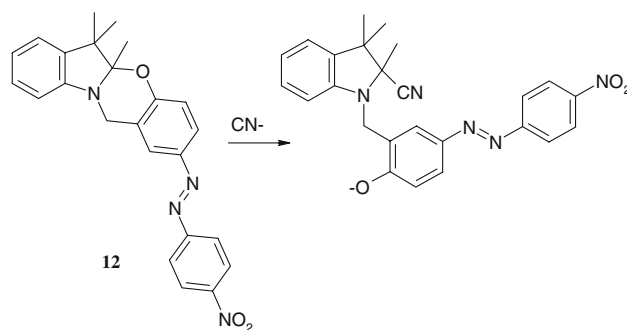


radiometric measurements became possible, thus eliminating signal drift caused by bleaching or leaching of **10**. The authors reported high selectivity for cyanide, and binding constants were found to be significantly smaller for glucose, fructose, fluoride and pH. However, in a parallel and independent investigation, Trupp et al. found that a structurally related dye showed sufficient sensitivity to enable saccharides detection in aqueous solution [16].

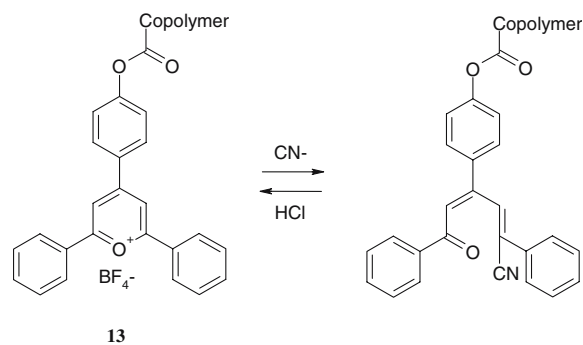
Chung et al. have applied the well-known chemical reactivity of fluorescent trifluoroacetyl derivatives for nucleophiles [17–19] to detect cyanide in acetonitrile solution [20]. The indicator 5-(dimethylamino)naphthalene-1-sulphonic acid [2-(2,2,2-trifluoroacetyl)phenyl]-amide **11** exhibited its absorbance maximum at 350 nm, and, when exposed to cyanide, acetate, fluoride or dihydrogen phosphate the fluorescence maximum at 530 nm shifted to 500 nm and increased up to fivefold. The authors enhanced the interaction with cyanide by stabilising the resulting adduct via an intramolecular hydrogen bond and found the association constant with tetrabutylammonium cyanide to be $3 \times 10^5 \text{ M}^{-1}$.



Tomasulo and co-workers have designed a chemodosimeter for cyanide anion based on an irreversible ring-opening reaction of an oxazine dye [21, 22]. In detail, a benzoxazine ring was fused into an indoline fragment giving 2-(4'-nitrophenylazo)-5a,6,6-trimethyl-5a,6-dihydro-12H-indolo[2, 1-b][1,3]benzoxazine **12**, which absorbed at around 380 nm. Upon reaction with cyanide the benzoxazine ring was opened and a 4-nitrophenylazophenolate chromophore was formed, with absorbance shifted to 580 nm. The dye molecule is soluble in organic solvents only, and when an acetonitrile solution was reacted with phosphate buffer of pH 7.6, sensitivity was in the millimolar range only. However, when **12** was dissolved in dichloroethane together with the phase transfer catalyst tetrabutylammonium chloride and shaken with aqueous solutions containing cyanide, the sensitivity for the colour change (of the dye in the organic phase) was in the micromolar concentration range, especially when the pH of the aqueous solution was as high as 9.0.



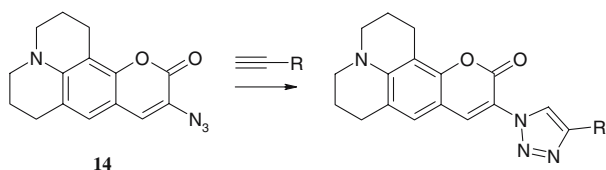
Garcia et al. have used well-known pyrylium probe chemistry (see detecting amines, diamines, amino acids and proteins) to measure the concentration of cyanide anion in aqueous solution [23]. Instead of incorporating their pyrylium dye **13** into a hydrophobic zeolite in order to protect it from nucleophilic anionic species (such as hydroxide), they polymerised a methacrylate derivative of the dye in the presence of hydrophilic methacrylate monomers (i.e. 2-ethoxyethyl methacrylate, 2,3-dihydroxypropyl methacrylate and the cross-linker ethylene glycol dimethacrylate) to form a thin polymer layer. Consequently, the pyrylium dye **13** became available for the reaction with aqueous cyanide and thus for the nucleophilic cleavage of the pyrylium moiety. When the co-polymer layer containing **13** was exposed to cyanide anion at pH 11.0, a colour change from yellow (absorbance maximum at 425 nm) to red (maximum at around 537 nm) was observed. The detection limit was found to be 4 mM cyanide, and no response for anions such as halogenides, nitrate, dihydrogen phosphate, sulphate or thiocyanate was found. Although chemical reactions of pyrylium dyes are considered to be irreversible, exposure of the dye to acidic solution (0.01 M hydrochloric acid) recovered the original colour of the pyrylium-containing layer and the colorimetric test strip could be repeatedly used (up to ten times) for cyanide detection.



Detecting terminal ethynyl derivatives

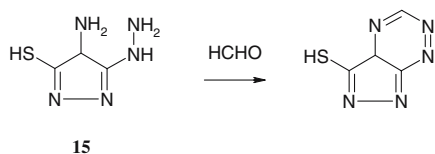
Although “click chemistry” is not relevant for analytical application at first sight, Sivakumar et al. presented a new approach to detecting ethynyl derivatives by using “click

chemistry” with azido-derivatives [24]. Initially non-fluorescent 3-azidocoumarins performed copper(I)-catalysed 1,3 dipolar cycloaddition reactions with terminal alkynes to give highly fluorescent 1,2,3-triazole products at room temperature in an ethanol/water (1:1) mixture. For example, 9-azido-2,3,5,6-tetrahydro-1H,4H-11-oxa-3a-aza-benzo[de]anthracen-10-one **14** reacted with various acetylenes yielding fluorescent triazoles with excitation maxima typically at 435 nm and emission maxima at around 508 nm. While this type of reaction was intended to generate a large library of pure fluorescent coumarine derivatives, this method might generally be used to detect the presence of acetylene derivatives.



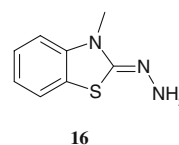
Detecting formaldehyde

Kawamura et al. developed a hand-held formaldehyde gas sensor for the detection of the so-called sick building syndrome [25]. For this, they dropped solutions of the reagent 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole **15**, together with aqueous potassium hydroxide, onto a glass filter, immediately prior to each measurement. When the filter containing this aqueous reagent mixture was exposed to gaseous formaldehyde at a flow rate of 400 ml/min, a purple colour with an absorbance maximum at around 540 nm developed. This colouration was monitored via an arrangement consisting of an LED as the light source and a photodiode for detecting the reflected light. The sensitivity of the device was in the range of 0.04–1 p.p.m. formaldehyde within a sampling time of 3 min. The sensor exhibited virtually no response to interfering species such as alcohols, ketones, acetic acid or ammonia at concentrations as high as 1,000 p.p.m., while a minor response was observed for acetaldehyde and glutaraldehyde. Furthermore, the signal readings were not affected by changing relative humidity in the range of 40% to 80% at 25°C.

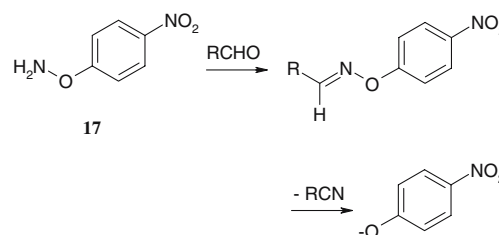


Toda and co-workers presented a portable system for continuous detection of gaseous formaldehyde, again based on a chromogenic reaction. They developed a stopped flow system using a diffusion scrubber unit to enrich formaldehyde and then formaldehyde was eluted with buffer to react with 3-methyl-2-benzothiazolone hydrazone **16** and FeCl₃, giving an intense colour at 629 nm. The limit of detection

was found to be 0.08 p.p.b.v and measurement time was in the range of 5 min [26].

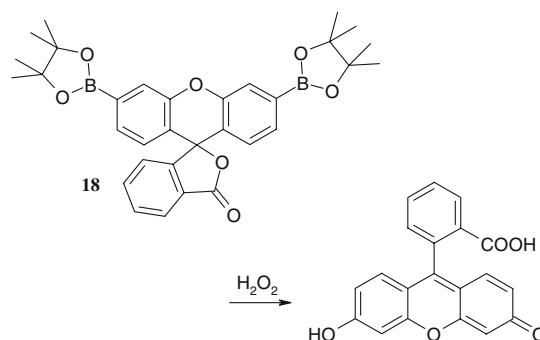


Salahuddin and co-workers have described a new chemical reaction for aldehyde detection based on oxime-bond fragmentation of chromogenic/fluorogenic structures that takes place at room temperature [27]. They synthesised oxyamines of nitrophenols and umbelliferones, which reacted with aliphatic and aromatic aldehydes at neutral pH to form oximes. These oximes underwent rapid fragmentation (β -elimination) reactions in the presence of the catalyst BSA to give the corresponding coloured or fluorescent phenolate anions. The authors evaluated the reaction of *O*-(4-nitrophenyl)-hydroxylamine **17** with formaldehyde in a microtiter-plate assay, using a buffer adjusted to pH 7.2. They measured the irreversible increase in absorbance at 405 nm and found sensitivity to be in the range of 1 μ M–4 mM formaldehyde.



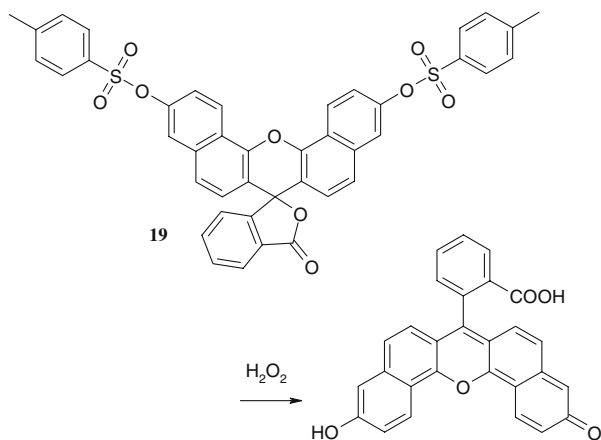
Detecting hydrogen peroxide

Chang and co-workers designed a cell-permeable optical probe for hydrogen peroxide using 3',6'-bis(pinacolatoboron)fluoran **18** [28]. This specific dye exhibited arylboronate groups, which were converted into phenols upon interaction with hydrogen peroxide.



Compound **18** was non-fluorescent under physiological conditions (pH 7.0) and did not absorb in the visible spectral range due to its lactone form. Exposure to hydrogen peroxide caused the hydrolytic deprotection of the boronate functions and generated the open coloured fluorescein, with concomitant fluorescence increase at around 510 nm when excited at 450 nm. The authors have evaluated the probe in living cells and confirmed that **18** is membrane-permeable and responds to hydrogen peroxide in the micromolar range. The probe exhibited a >500-times higher response for hydrogen peroxide over *tert*-butyl hydroperoxide, nitric oxide, or hypochloride.

The related naphthofluorescein disulphonate probe **19** was synthesised by Xu et al., where the emphasis was to shift the absorbance and fluorescence into the near infrared spectral range and to enhance sensitivity [29]. Again, the initial dye was a colourless lactone, and a hydrolytic deprotection of the naphthofluorescein disulphonate by hydrogen peroxide caused the formation of a coloured and fluorescent naphthofluorescein with a fluorescence maximum at 662 nm (excitation at 602 nm). The probe showed a linear calibration in the range of 6 nM–4 μM hydrogen peroxide and a detection limit of 81.5 pM in a mixture of DMSO/buffer (1:9) at pH 7.4. It exhibited good selectivity over interfering species such as ascorbic acid, glutathione, hypochloride or hydroxyl radical and could be applied for monitoring nanomolar concentrations of hydrogen peroxide in living cells.

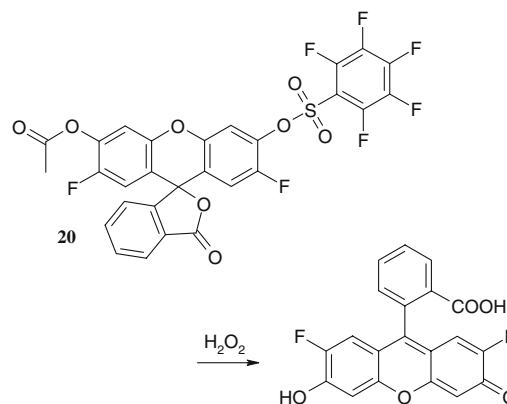


Maeda et al. have previously synthesised comparable fluorescein derivatives with chloro- and fluoro- substituents, e.g. compound **20**, and observed comparable selectivity and sensitivity (5 pmol to 90 nmol hydrogen peroxide), albeit with fluorescence increases at around 514 nm when excited at 492 nm [30].

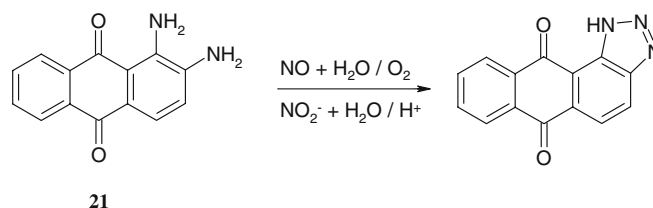
Detecting nitric oxide and nitrite anion

Recently, a review dedicated to the use of fluorescent probes for reactive nitrogen species has become available and covers the detection of nitric oxide, peroxyxynitrite anion

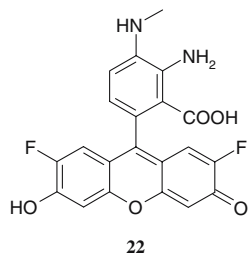
and some of its derivatives in biological and non-biological fluids [31].



Bru and co-workers doped cross-linked poly(2-hydroxyethyl methacrylate) films with 1,2-diaminoanthraquinone **21** in order to detect both nitric oxide and nitrite via formation of a colourless triazole [32]. They polymerised 2-hydroxyethyl methacrylate and ethyleneglycol dimethacrylate in the presence of 1,2-diaminoanthraquinone to give thin polymer layers with a red colour. Then, they exposed the layers to aqueous solutions of nitric oxide at pH 7.4 and observed that the absorbance maximum at around 520 nm disappeared and a new maximum at around 350 nm was formed. Although **21** was not covalently linked to the polymer matrix, no detectable leaching was observed when the layer was stored in buffered water (pH 7.4) for 6 months. A similar colour change was found for nitrite anion in 1% acetic acid solution, giving a linear correlation in a range from the detection limit (D.L.) to 120 μM. The same layer also responded directly to gaseous nitric oxide, which was related to the hydrophilic nature of the polymer film.

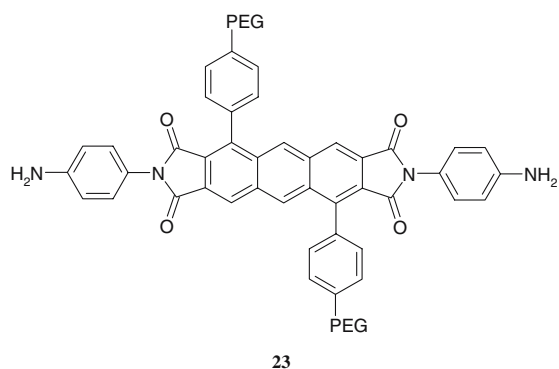


Zguris and Pishko presented fluorescent poly(ethylene glycol) hydrogel to detect nitric oxide in aqueous solution [33]. They embedded 4-amino-5-methylamino-2',7'-difluorofluorescein **22** in hydrogel microstructures using photolithography methods on glass substrates. The sensor layer showed a linear increase in fluorescence at around 515 nm (excitation at 480 nm) upon exposure to 0.5–8 μM nitric oxide in pH 7.4 phosphate buffer. The response of the layers was irreversible, and the authors reported significant leaching of **22** from the polymer matrix.



Detecting organophosphate warfare agents

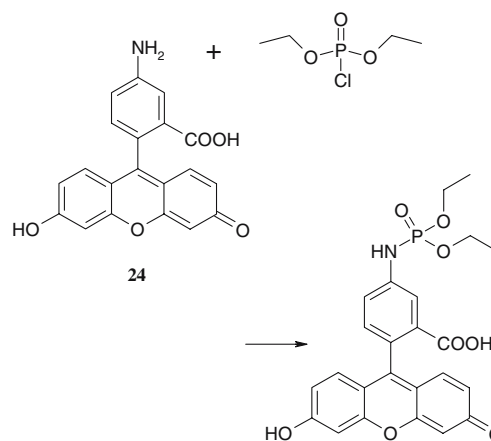
In an attempt to detect organophosphate warfare agents, Ilhan and co-workers developed an anthracene-type chemosensor molecule with enhanced solubility in polar solvents due to the introduction of tetraethyleneglycoxy groups into the chemical structure [34]. The resulting *N,N'*-bis(*p*-aminophenyl)-1,5-bis(*p*-(tetraethyleneglycoxy)phenyl)anthracene-2,3,6,7-tetracarboxyl bisimide **23** absorbed at 300 nm and 400 nm but showed virtually no fluorescence because of efficient PET from the amine donor to the anthracene acceptor. Reaction with thionyl chloride or acetyl chloride (mimics for organophosphate-based nerve gases) caused the formation of a broad green fluorescence (maximum at 499 nm with a shoulder at 530 nm), and the sensitivity for thionyl chloride was observed to be in the range of 10 μM –1 mM in anhydrous DMF as the solvent. The authors also immobilised **23** on silica and observed a similar increase in fluorescence.



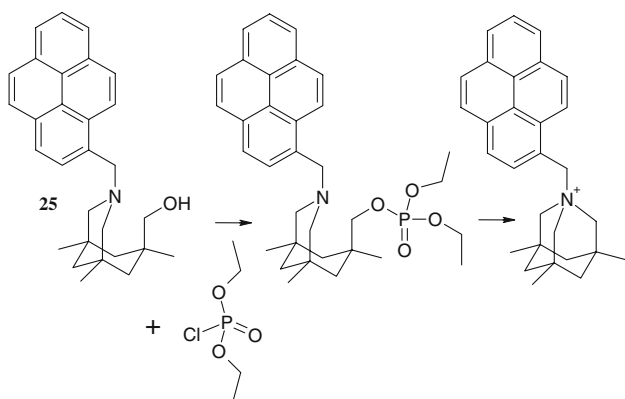
Finally, they also reacted **23** with dichlorothiophosphate, methylphosphonic dichloride and dimethylphosphinic chloride in DMF, all of which caused a significant enhancement of fluorescence, while less chemically-reactive dimethyl methylphosphonate showed no enhancement under similar experimental conditions.

The research group of David Walt presented an approach to the detection of reactive organophosphate chemical

warfare agent vapours by immobilising fluoresceinamine **24** on carboxylate-functionalised polymer microbeads covered with poly(2-vinylpyridine) [35]. The principle of operation was based on the fact that nerve agents possess reactive functions that inhibit acetylcholinesterase and that the same functions can also react with fluorescent indicator dyes. Fluoresceinamine exhibited weak fluorescence at around 515 nm (excitation set to 490 nm), because its fluorescence was quenched by the lone pair nitrogen of the amino group. Upon reaction with reactive acyl or phosphoryl groups, quenching was reduced significantly within fewer than 30 s, and the signal increase was as high as 200% for diethyl chlorophosphate. Non-reactive diisopropyl methylphosphonate and dimethyl methylphosphonate did not show any signal changes. The organophosphate-sensitive microbeads were evaluated in micro-wells etched into an optical fibre bundle and repeatedly (albeit irreversibly) responded to diethyl chlorophosphate in the 13–66 p.p.m. range, with subsecond response times. No response was observed for volatile organic vapours (e.g. ethanol, heptane or toluene), water or methyl salicylate (mustard gas simulant).

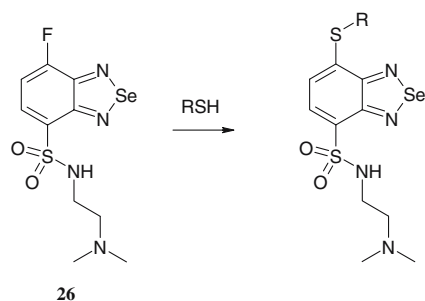


Dale and Rebek used a two-step chemical reaction to detect organophosphorus nerve agent mimics [36]. Again, the indicator dye 3-(pyren-1-ylmethyl)-1,5,7-trimethyl-3-azabicyclo[3.3.1]nonane-7-methanol **25** was essentially non-fluorescent due to PET from a lone pair nitrogen atom close to the pyrene moiety. Cyclisation of **25** to form the ammonium salt in the presence of reactive dialkyl chloro- and fluorophosphates was as fast as 10 s, and the fluorescence at 378 nm increased by 22-fold (excitation set to 340 nm). The authors also prepared a simple sensor layer by dispersing **25** on a filter paper and observed a strong and visible increase in fluorescence within 5 s exposure to 10 p.p.m. of diisopropyl fluorophosphate vapour.



Detecting peptides and proteins via thiol groups

Saimaru and co-workers synthesised the non-fluorescent probe 7-fluoro-*N*-[2-(dimethylamino)ethyl]-2,1,3-benzoselenadiazole-4-sulphonamide **26** for labelling peptides and proteins, based on its reactivity with thiols [37]. Upon interaction of **26** with proteins and peptides at pH 9.0 in the presence of the guanidine and 3-[(3-cholamidopropyl)dimethylammonio]propanesulphonic acid to facilitate the reaction, highly fluorescent derivatives were formed with excitation and emission maxima at around 426 nm and 540 nm, respectively. Subsequently, proteins and peptides such as BSA, α -lactalbumin, insulin, calcitonin, somatostatin, and oxytocin were isolated via HPLC, followed by enzymatic digestion and identification of the derivatised proteins using ESI-MS/MS.

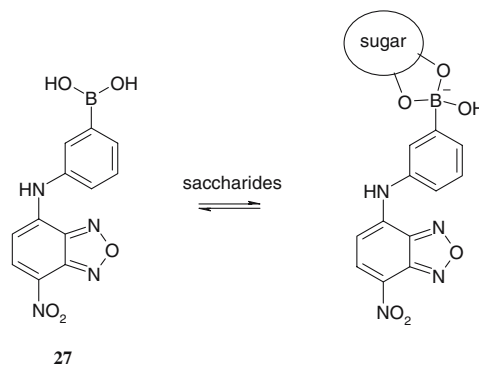


Detecting saccharides

Three comprehensive reviews on the progress in boronic acid-based fluorescent saccharide sensors have recently been published [38–40], so only the latest developments will be given. Generally, saccharide sensors make use of the reversible formation of a cyclic ester between the boronic acid receptor attached to the dye molecule and the diol function of saccharides. This ester formation affects the Lewis acidity of the boron atom and can cause changes

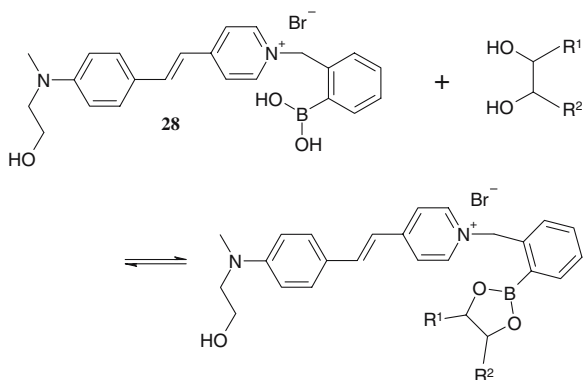
in internal charge transfer, in photo-induced electron transfer or in fluorescence resonance energy transfer.

Badugu et al. developed a wavelength ratiometric and colorimetric probe for glucose determination [41]. The presence of saccharides caused a ground-state binding interaction, influencing the intramolecular charge transfer within the dye molecule. Specifically, the probe 4-[*N*-(3-boronophenyl)]amino-7-nitrobenz-2-oxa-1,3-diazole **27** showed an absorbance maximum at around 490 nm in pH 7 phosphate buffer, which was shifted to 500 nm upon exposure to fructose, yielding a visible colour change from yellow to orange. The boronic acid function acted as an electron-withdrawing group and as a selective recognition moiety for saccharides. Interaction of the boronic acid function with saccharides caused formation of an anionic form that was no longer electron withdrawing. These changes in electron-acceptor strength at the boronic acid function directly affected the electron-donating capability of the amino group towards the nitro-group. Consequently, the presence of saccharides changed the probe's colouration. Sensitivity for fructose was in the 0.2–20 mM range, while it was in the range of 1–100 mM for glucose. The shift in absorbance maximum allowed for a ratiometric evaluation to provide signals that were less affected by bleaching of the dye or by light source instability.

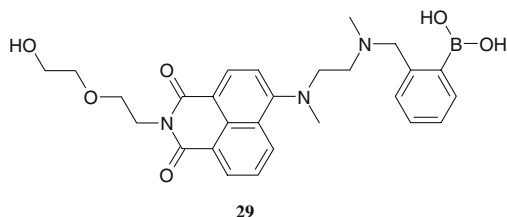


Trupp et al. have presented the hemicyanine dye 1-(2-dihydroxyborylbenzyl)-4-(2-{4-[(2-hydroxyethyl)-methylamino]-phenyl}-vinyl)-pyridinium-bromide **28** that exhibited its absorbance maxima at around 460 nm and emission at around 600 nm, showed significant increases in fluorescence upon exposure to saccharides and was used in aqueous solution at physiological pH [16]. The maximum signal increase of **28** upon exposure to D-fructose at a pH 7.13 was 86%, and the equilibrium constants were 280 M^{-1} for D-fructose, 20 M^{-1} for D-galactose, 4 M^{-1} for D-glucose, 0.4 M^{-1} for glycerol, and 0.3 M^{-1} for ethylene glycol. A similar recognition mechanism for saccharides has already been reported in the case of the structurally related 2-boronobenzylquinolinium derivatives, where the anionic saccharide bound form (boronate ester form) was stabilised by the cationic quaternary nitrogen heterocyclic

centre [42]. The *N*-hydroxyethyl function at the fluorophore not only enhances solubility in aqueous solution but may also be used to link the dye to polymer matrices (e.g. via a methacrylate group).

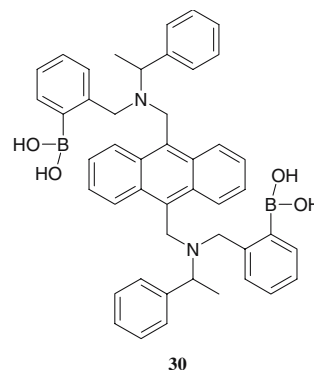


Trupp et al. also synthesised a structurally related naphthalimide derivative with similar reactivity for saccharides but based on a photo-induced energy transfer mechanism only. The dye 2-({[2-({[2-({2-(2-hydroxy-ethoxy)-ethyl]-1,3-dioxo-2,3-dihydro-1H-benzo[de]iso-quinolin-6-yl)-methyl-amino)-ethyl]-methyl-amino}-methyl)-boronic acid **29** showed a maximum fluorescence increase of 130% at 530 nm (excitation set to 410 nm) when exposed to D-fructose at pH 7.15 (Trupp et al., personal communication). Equilibrium constants of **29** were 170 M^{-1} for D-fructose, 8 M^{-1} for D-galactose, 2 M^{-1} for D-glucose, and 0.5 M^{-1} for ethylene glycol.



Zhao and co-workers developed an enantio-selective chemosensor for sugar acids by synthesising chiral fluorescent bisboronic acids **30** (denoted *R,R*-(-)-**1** and *S,S*-(+)-**1**) [43]. These anthracene-based dyes exhibited a significant increase in luminescence at 429 nm (excitation at 365 nm) when exposed to chiral sugar acids in a solution of 52.1% methanol in buffer. Specifically, D- and L-tartaric acid, D-glucaric acid, and D-gluconic acid were selectively detected. For example, at a pH of 7.0, the binding constants of *R,R*-**1** with D-tartaric acid and L-tartaric acid were found to be $\log K_D$ 4.79 and 2.07, respectively, while the binding constants of *S,S*-**1** for D-tartaric acid and L-tartaric acid were $\log K_D$ 2.09 and 4.81, respectively. Thus, the enantio-selectivity ($K_R:K_S$) was 490:1 for D-tartaric acid and 1:550

for L-tartaric acid. The binding constants with sugar acids were strongly pH-dependent, and it was found that selectivity was considerably improved when pH was adjusted accordingly. Thus, at pH 8.3 the chemosensor *R,R*-**1** exhibited a selectivity ratio of 7.2:1 for D-tartaric acid/D-sorbitol, while it was found to be 11,000:1 at pH 5.6. Similarly, the sensitivity was pH-dependent in that the binding constant of *R,R*-**1** with D-tartaric acid $\log K_D$ was 5.92 at pH 5.6, while $\log K_D$ was 2.79 at pH 8.3.

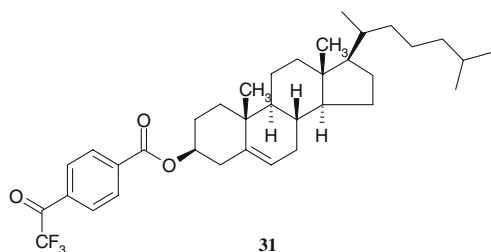


New detection principles for neutral analytes

Chiral nematic liquid crystals are systems that produce colouration without the need for chromogenic or fluorogenic structures. The colouration of the so-called cholesteric liquid crystals is based purely on the modulation in the reflectance of incident light [44]. Consequently, there is no need for complex chemical structures in order to shift absorbance into the visible spectral range, and the structurally simple molecules exhibit higher photostability than long-wavelength absorbing dye molecules.

In the cholesteric liquid crystal, the chiral molecules are gradually twisted against each other, so that they form a helical structure where the length of a 360° rotation is denoted the pitch. If the pitch length corresponds to the wavelength of light, then this light is reflected with different efficiency according to $\lambda = Pn$, where λ is the wavelength of the reflected light, P is the pitch length and n is the refractive index. The colouration depends on ambient temperature in that, with an increase of temperature, the length of the pitch rises and, accordingly, colour changes are observed. Consequently, the main application of cholesteric liquid crystals is temperature detection. The length of the pitch, however, may also be modified through the embedding of guest molecules. This approach has already been used to detect vapours of organic solvents (e.g. acetone, benzene, methanol or chloroform), albeit without selectivity in the recognition process [45, 46]. An alternative method to enhance selectivity in the recognition process is to develop cholesteric receptor molecules. Such a receptor molecule is 4-trifluoroacetylbenzoic acid cholesteryl ester **31**, which is sensitive to amine vapours [47]. For a stable sensor film, 7.2% **31** was combined with 12.7% cholesteryl chloride, 29.9% cholesteryl oleyl car-

bonate, 29.9% cholesteryl nonanoate and 20.3% poly (methyl methacrylate).



The addition of the polymer was necessary to guarantee mechanical stability, increasing the shelf life from several hours to up to 2 months. This polymer dispersed liquid crystal film showed a shift in reflectance maximum from

473 nm to 523 nm and a concomitant change in colour from blue to green when the concentration of gaseous 1-butylamine was as high as 3,000 p.p.m. A comparable spectral shift was observed upon exposure to gaseous diethylamine; however, only at concentrations higher than 10,000 p.p.m. This lesser sensitivity was due to the fact that primary amines, such as 1-butylamine, were less sterically hindered to interact with the trifluoroacetyl group of **31** to form a hemi-aminal than were secondary amines such as diethylamine. No signal changes were observed with concentrations as high as 18,000 p.p.m. of acetone, 8,000 p.p.m. of methanol or 7,500 p.p.m. of ethyl acetate.

While this approach was used for the detection of gaseous aliphatic amines, it is expected to be generic in that, by using other cholesteryl derivatives with appropriate receptor moieties, it may become possible to obtain liquid crystal sensor films for, e.g. biogenic aldehydes, alcohols or volatile toxic agents. Some characteristics of probes/sensors 1–31 are shown in Table 1.

Table 1 Figures of merit and spectral characteristics of probes/sensors 1–31 (*n.d.* not detected)

Molecule	Analytes	Sensitive range (or D.L.)	Change in absorbance maximum	Change in fluorescence maximum	Comments
1	Primary amines	<i>n.d.</i>	611 nm to 503 nm	665 nm to 602 nm	Label for proteins
2	Primary amines with medium chain length	<i>n.d.</i>	540 nm to 460 nm	<i>n.d.</i>	Receptor encaged in mesoporous materials
3	Primary amines with medium chain length	<i>n.d.</i>	554 nm to 445 nm	<i>n.d.</i>	Receptor encaged in mesoporous materials
4	Diaminoalkanes, ornithine, lysine	0.05–1.1 mM diaminopropane	440 nm to 470 nm	Increase at 530 nm (excitation 495 nm)	Bifunctional pre-organised receptor
5	Cysteine	100 μ M–5 mM cysteine	<i>n.d.</i>	Increase at 380 nm (excitation at 250 nm)	Evaluation via microplate spectrophotometer
6	Glycine, cysteine, phenylalanine	<i>n.d.</i>	<i>n.d.</i>	Increase at 399 nm (excitation at 342 nm)	Bifunctional pre-organised receptor
7	Anilines and cyclic aminoalkanes	<i>n.d.</i>	508 nm to 479 nm	519 nm to 562 nm	Detects less nucleophilic amines
8	Histamine	0.05 mM–1.00 mM histamine	Increase at 552 nm	<i>n.d.</i>	Flow injection system using pre-concentration cartridge
9	Cyanide, fluoride, dihydrogen phosphate, acetate	D.L. 0.1 ppm cyanide	Decrease at 569 nm	Decrease at 581 nm	Selectivity depends on water/acetonitrile ratio
10	Cyanide	1 μ M–30 μ M cyanide	460 nm to 450 nm	Increase at 600 nm (excitation at 475 nm)	Ratiometric indicator dye
11	Cyanide, fluoride, dihydrogen phosphate, acetate	<i>n.d.</i>	<i>n.d.</i>	530 nm to 500 nm and 5-fold increase in intensity	Measurements in organic solvent
12	Cyanide	μ M concentration range	380 nm to 580 nm	<i>n.d.</i>	Measurements in organic solvent
13	Cyanide	D.L. 4 mM cyanide	425 nm to 537 nm	<i>n.d.</i>	Sensor layer response can be regenerated

Table 1 (continued)

Molecule	Analytes	Sensitive range (or D.L.)	Change in absorbance maximum	Change in fluorescence maximum	Comments
14	Ethynyl derivatives	n.d.	n.d.	Increase at 508 nm (excitation at 435 nm)	Probe based on click chemistry
15	Formaldehyde	0.04 ppm–1 ppm formaldehyde	Increase at 540 nm	n.d.	Hand-held measurement device
16	Formaldehyde	D.L. 0.08 ppm formaldehyde	Increase at 629 nm	n.d.	Flow-system for discontinuous measurements
17	Formaldehyde, aliphatic and aromatic aldehydes	1 μ M–4 mM formaldehyde	Increase at 405 nm	n.d.	Microtitre-plate assay
18	Hydrogen peroxide	μ M concentration range	n.d.	Increase at 510 nm (excitation at 450 nm)	Probe for measurements in living cells
19	Hydrogen peroxide	6 nM–4 μ M hydrogen peroxide	n.d.	Increase at 662 nm (excitation at 602 nm)	Probe for measurements in living cells
20	Hydrogen peroxide	5 pmol–90 nmol hydrogen peroxide	n.d.	Increase at 514 nm (excitation at 492 nm)	Probe for measurements in living cells
21	Nitric oxide and nitrite	D.L.–120 μ M nitrite	520 nm to 350 nm	n.d.	Sensor layer for both gaseous and ionic species
22	Nitric oxide	0.5 μ M–8 μ M nitric oxide	n.d.	Increase at 515 nm (excitation at 480 nm)	Sensor layer
23	Organophosphate mimics, thionyl chloride, acetyl chloride	10 μ M–1 mM thionyl chloride	n.d.	Increase at 499 nm (excitation at 425 nm)	Measurements in organic solvent
24	Diethyl chlorophosphate	13 ppm–66 ppm diethyl chlorophosphate	n.d.	Increase at 515 nm (excitation at 490 nm)	Indicator microbeads evaluated on optical fibre array
25	Diethyl chlorophosphate, diisopropyl fluorophosphate	ppm range of diisopropyl fluorophosphate	n.d.	Increase at 378 nm (excitation at 340 nm)	Filter paper based indicator layer
26	Proteins and peptides	n.d.	n.d.	Increase at 540 nm (excitation at 426 nm)	Label for proteins and peptides
27	Saccharides	0.2 mM–20 mM fructose	490 nm to 500 nm	n.d.	Ratiometric colour indicator
28	Saccharides	0.5 mM–50 mM fructose	n.d.	Increase at 600 nm (excitation at 460 nm)	Functional probe for saccharides
29	Saccharides	1.0 mM–100 mM fructose	n.d.	Increase at 530 nm (excitation at 410 nm)	Functional probe for saccharides
30	Chiral tartaric acid, glucaric acid, gluconic acid	n.d.	n.d.	Increase at 429 nm (excitation at 365 nm)	Probe selectivity and sensitivity dependent on pH
31	Aliphatic amines	300 ppm–3000 ppm 1-butylamine	473 nm to 523 nm (reflectance change)	n.d.	Liquid crystalline (cholesteric) sensor film

Conclusions

Covalent bond formation is a viable means of providing selective interaction with, and sensitive recognition of, both neutral and ionic analytes. The combination of covalent bond formation with chromophores and fluorophores fosters the development of selective indicators for biologically relevant analytes, such as biogenic amines, amino acids, proteins, saccharides, and also for highly toxic warfare agents such as organophosphates or hydrogen cyanide. The new indicator dyes can be used to develop simple test strips for visual evaluation as well as optical sensors for continuous monitoring. In addition, colour changes caused by analyte incorporation into chiral nematic layers can also be used to generate significant colour changes. In this specific case, synthesis may be more facile because the receptor molecules do not have to be chromophores.

Generally, the trend is to design advanced functional dyes with build-in selectivity (e.g. via pre-organised receptor moieties) or to embed the dyes into mesoporous materials in order to modify selectivity in the recognition of the analyte.

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