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Fluorescent Chemosensors for Carbohydrates: A Decade's Worth of Bright Spies for Saccharides in Review

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This review provides a chronological survey of over fifty fluorescent chemosensors for carbohydrates from the period between 1992 to the present. The survey contains only those sensors that are synthetic or chemosensory, utilize boronic acids and display a fluorescence response in the form of intensity changes or shifts in wavelength. With each compound listed, a description of the saccharide probe is given with regard to concentration, excitation and emission wavelengths, pH and solvent mixture proportions. In addition, the selectivity of each chemosensor is provided as well as the trends in binding constants. Where possible, a description of the fluorescence signaling mechanism is given as well as commentary on the probe's unique features within this class of sensors.

KEY WORDS: Fluorescent probes; saccharides; boronic acids; ICT; PET; FRET.

The last decade marks an important point in time for those whom have been involved in the development of fluorescent chemosensors for saccharides. In 1992 Czarnik and Yoon published a brief communication, regarded by many in the field to be the seminal work in fluorescent saccharide detection, on a simple probe which complexed fructose. Soon thereafter, Shinkai and James broke ground on developing these probes with a specificity for D-glucose. Their findings, published in the journal *Nature*- from which this review borrows part of its title, brought saccharide probes to the attention of a larger audience of biomedical and clinical researchers. The last 10 years have witnessed a considerable expansion of this work not only by Shinkai and James but also from many other organic, analytical, physical and medicinal chemists. In keeping with the progressive evolution of these "bright spies for saccharides," this review provides a chronological account of the many accomplishments made since 1992 to the present. While several reviews have been published on saccharide detection with boronic acids, we have not found one yet that focuses solely on the use of fluorescence as the limiting theme of its discussion nor completely covers the various contributions [1–3]. Given the focus of this journal as well as the numerous advantages that fluorescence detection offers in biomolecule detection, this review limits the number of examples to those chemosensory devices that are synthetic in nature, utilize boronic acids as a principle receptor component and display some type of fluorescence intensity change or wavelength shift in the presence of saccharides. As with any review, we begin with an apology for any examples and respective authors that we have overlooked in our compilation of chemosensory devices from the last 10 years. We have attempted to locate and access all of the fluorescent chemosensors relevant to mono and polysaccharides as well as many carbohydrate derivatives that were published in widely circulated chemical journals. We assume that the interested reader has already been introduced to this unique area of fluorimetric detection, so the interaction of boronic acids with saccharides as well as the explanation of binding constants or dissociation constants has been omitted. For more information on these relevant topics, the reader is referred to any one of several previously published reviews [4,5]. Our aim throughout the preparation of this review has been the generation of a useful and concise summary of the many

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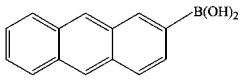
saccharide probes synthesized for those who are planning to use a previously reported sensor or are planning the next generation of carbohydrate probes.

As mentioned above, Yoon and Czarnik prepared a simple saccharide probe **1** in a single synthetic step to give anthracene boronic acid. This review begins as several others have done by introducing their chemosensor as the first published report of a fluorescent chemosensor for saccharides. When the concept of a chemosensor had few examples with which to reference, their probe exhibited the strongest binding to fructose in buffered pH solution of 7.4 with a fluorescence signal at 416 nm. Because this first paper focused on a comparison of polyol complexes, following fructose, the sensor was shown to bind 1,1,1-tris(hydroxymethyl)ethane followed by glucose and ethylene glycol [6].



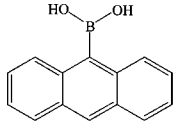
1

λ_{em} = *416 nm*

Measurement conditions: *receptor 7.5 × 10^{-5} M, 20mM phosphate buffer at pH 7.4, all solutions contain 1%(v/v) DMSO*

Selectivity*: Fructose > 1,1,1-Tris(hydroxymethyl)ethane > Glucose > Ethylene glycol*

This initial result was soon confirmed by two other monoboronic saccharide sensors. Sensor **2**, a positional isomer of 2-anthraceneboronic acid, showed similar behavior with saccharides by showing an identical trend in chelation enhanced fluorescence. Fructose displayed the greatest quenching of fluorescence at 400 nm followed by galactose and glucose. Despite these intial successes, the degree of quenching remained rather small and served as an indication that PET was not efficient although the boronate anion was directly bound to the chromophore [I(with saccharide)/I_0(without saccharide) ~0.7]. Catechol derivatives were found to bind with much higher affinity, consequently the PET response further increased the fluorescence quenching of the anthracene component [7].



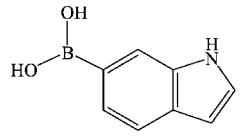
2

λ_{em} =*416 nm*

Measurement conditions: *receptor 7.5 × 10^{-5} M, 20mM phosphate buffer at pH 7.4, all solutions contain 1%(v/v) DMSO*

Selectivity*: Fructose*

5-Indolylboronic acid showed similar fluorescence quenching behavior with carbohydrates and was the first to be complexed with oligosaccharides. The fluorescence quenching response correlated with the inherent selectivity of monoboronic acid for sugars. Sensor **3**'s reponse to oligosaccharides was generally lower than predicted on this basis, although higher oligomers proved to be better at quenching fluorescence because of a secondary interaction with the indole N-H [8].



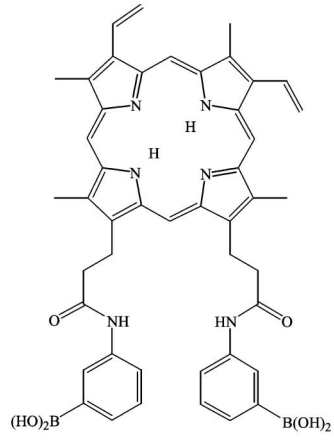
3

λ_{ex} = *290 nm* λ_{em} = *361 nm*

Measurement conditions: *receptor 5.0 × 10^{-5} M, 25 °C, at pH 9.0*

Selectivity *: D-fructose*

The first fluorescent bis-boronic acid appeared in 1993 when Shinkai *et al*. appended 3-aminophenylboronic acid to protoporhyrin system **4** shown below. This fluorescent probe displayed a remarkable 215 nm Stokes shift and the largest fluorescence enhancement to fructose at pH 10.5. One of the unique features from this system was the complete absence of fluorescence for this probe when no saccharide was present. This "Off-On" phenomena was attributed to an aggregation of porphyrin in aqueous solution that gave rise to a strong quenching effect. In the complexed form the saccharides conferred high solubility [9].



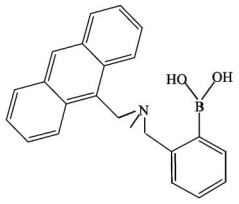
4

λ_{ex} = *415 nm* λ_{em} =*632 nm*

Measurement conditions: *receptor 1.0 × 10^{-5} M, 25 °C, DMSO:H_2O = 1:30, 0.067M carbonate buffer solutions at pH 10.5*

Selectivity *: D-fructose > D-arabinose > D-mannose*

The deployment of Wullf's benzylic amine spacer soon became a common feature used in many saccharide designs. The advantage of this additional spacer was found in the lower pKa at which several boronic acid receptor components could operate. Such a device was not overlooked by Shinkai and James as the first saccharide probe to utilize a benzylic amine was appended to anthracene in the form of sensor **5**. This probe, as well as many future designs, could provide fluorescence detection (420 nm) at pH conditions as low as 6.4. In addition, the photoinduced electron transfer, normally a quenching response with boronic acids had been reversed to become a chelation enhanced fluorescence response due to an interaction between amine lone pairs and boron. As with other monoboronic acid probes, this sensor displayed the greatest flourescence enhancement with fructose [10].



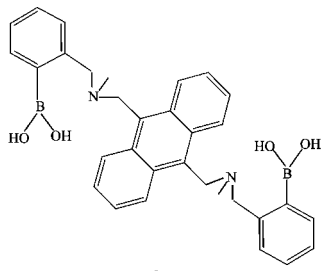
5

λ_{ex} = 370 nm λ_{em} = 420 nm

***Measurement conditions**: receptor 1.2 × 10^{-5} M, 0.01M NaCl solutions at 25 °C.*

***Selectivity** : D-fructose > D-glucose > Ethylene glycol*

The addition of a second benzylic amine group to their anthracene platform provided a dramatically altered selectivity as the pyranose form of glucose was shown to fit better than other monosaccharides into probe **6**'s pocket. Although one-third of the solvent composition required methanol for solvation of the probe, the placement and properties of the second receptor group ushered several new design approaches. Glucose, which had long been a target of clinical chemists looking to diagnose high blood sugar, could now be detected using simple chemosensory designs [11].



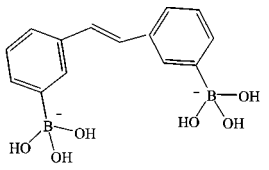
6

λ_{ex} = 370 nm λ_{em} =423 nm

***Measurement conditions**: receptor 1.0 × 10^{-5} M, 33.3% MeOH/H_2O buffer solutions at pH 7.77, 25 °C*

***Selectivity**: D-glucose > D-fructose > D-allose > D-galactose >Ethylene glycol*

In 1994 the photophysics of *cis-trans* isomerism found in boronic acid derivatives of *trans*- stilbene were investigated in the presence of disaccharides. Fluorescent probe **7** displayed a fluorescence enhancement of 358 nm via formation of a sugar-stilbene macrocycle at pH 10. The preferred *cis* geometry of **7** upon photoexcitation was stabilized in the presence of disaccharides and showed the greatest selectivity to melibiose [12].



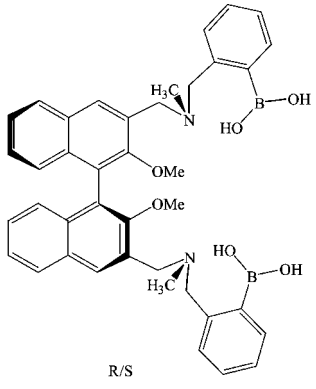
7

λ_{ex} = 310 nm λ_{em} = 358 nm

***Measurement conditions**: receptor 1.0 × 10^{-5} M, 0.01M Na_2CO_3-$NaHCO_3$ buffer solutions at pH 10.6.*

***Selectivity**: Melibiose > Gentiobiose > Maltotriose > Isomaltose > Trehalose, Saccharose, Maltose, Lactose*

Asymmetric fluorescent platforms were first introduced in 1995 in the form of *R*- and *S*- Binap ligands. This novel approach to chiral saccharide sensing provided an enhanced fluorescent signal (358 nm) for **8** in the presence of sugars by a combination of twist angle effects and a reduction in the quenching efficiency of the amino lone pairs. In the case of **8***R* at pH 7.7, the PET quenching efficiency was affected more strongly by D-fructose, D-glucose and L-galactose; the least by L-fructose, L-glucose and D-galactose. The opposite trend in fluorescence response was found in the case of **8***S* [13].



8

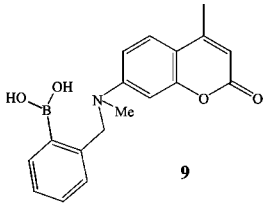
λ_{ex} = 289 nm λ_{em} = 358 nm

***Measurement conditions**: receptor 1.0 × 10^{-5} M, 33.3% MeOH/H_2O phosphate buffer solutions at pH 7.77, 25°C*

***Selectivity**: 8R:D-fructose, D-Glucose, D-mannose, L-Galactose*

8S:L-fructose, L-glucose

The search for higher quantum yields from the fluorescing reporter group yielded coumarin dyes as potential saccharide probe components. In 1:1 methanol/water solutions, a significant Stokes shift of 100 nm was found for sensor **9** in neutral pH. At 452 nm, this probe shows the largest chelation enhanced quenching response to fructose. Probe **9** may well have been the first to utilize internal charge transfer as an approach toward longer wavelength signals [14].

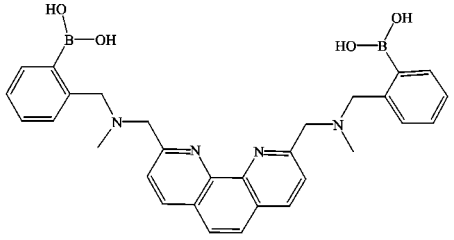


9

λ_{ex} = 360 nm λ_{em} = 452 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M, MeOH:H$_2$O= 1:1 (v/v),*
***Selectivity**: Fructose > Galactose >Arabinose > (Ethylene Glycol, Glucose, Glycerol) > Propane diol*

As the PET mechanism continued to play a dominant role in fluorescence detection of saccharides, investigations into the effects of positional isomers provided additional insight into this electron transfer process. The table below shows a series of related polcyclic aromatic hydrocarbons used as fluorescent platforms for the boronic acid moiety. Compounds **10–13** were examined in the presence of fructose from pH range of 2–11 to screen for optimal placement of boronic acid as well as fluorophore geometry. These pH titrations revealed that saccharide sensors **10b** and **13b** gave the largest PET response with the closest pH to physiologial conditions [15].

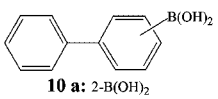
probes, the distance that separates boronic acids was investigated using phenanthroline. The spacer length provided by this fluorophore is considerably larger than the optimal distance for glucose inclusion. In this example, the fluorescence signal at 365 nm did not become apparent until large concentrations of saccharide were used in the assay. Sensor **14** was dubbed as a "sweet toothed" sensor since it was unable to form 1:1 complexes with monosaccharides, only 2:1 saccharide to sensor complexes [16].



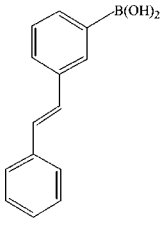
14

λ_{ex} = 273 nm λ_{em} = 365 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M, MeOH:H$_2$O= 1:2 (v/v),pH 7.77*
***Selectivity**: Glucose*

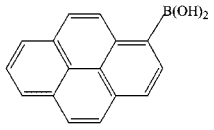
Eventually bifunctional receptor components were introduced onto the anthracene fluorescent platform in the form of a crown ether/boronic acid combination. **15** features a unique "glucose cleft" and "metal sandwich" system that produced an allosteric coupling effect when sugar and salt were dissolved together. In the case of small cations such as lithium, both crown ethers were involved in cation complexation and a 1:1 saccharide/sensor macrocycle was formed. However, larger cations such as



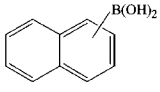
10 a: 2-B(OH)$_2$
10 b: 3-B(OH)$_2$
10 c: 4-B(OH)$_2$



11



12



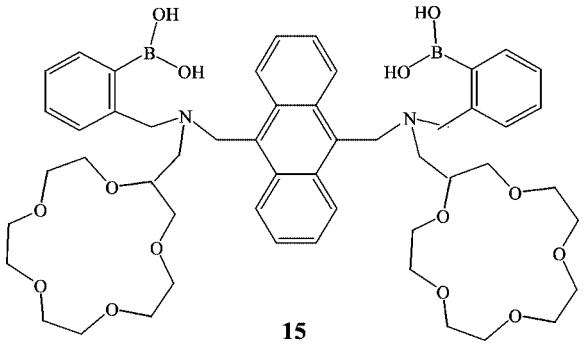
13 a: 1-B(OH)$_2$
13 b: 2-B(OH)$_2$

	10a	**10b**	**10c**	**11**	**12**	**13a**	**13b**
conc. (mol dm^{-3})	3.35 × 10^{-4}	3.35 × 10^{-6}	3.35 × 10^{-6}	3.35 × 10^{-5}	3.35 × 10^{-6}	3.35 × 10^{-6}	3.35 × 10^{-6}
λ_{ex} (nm)	230	246	246	296	338	268	268
λ_{em} (nm)	340	324	324	360	376	344	344

Fluorescence were measured in H$_2$O/DMSO = 99:1 at 25°C

As the binding of bis-boronic acid containing sensors to glucose became a common design feature in fluorescent

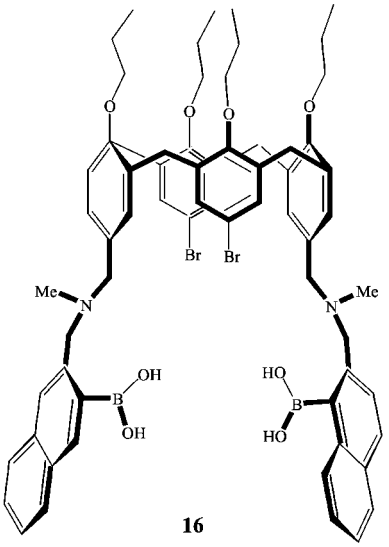
Na$^+$, K$^+$,Sr$^+$ disrupted this 1:1 complex and led to a non-fluorescent probe [17].



15

λ_{ex} = 370 nm λ_{em} = 423 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M, MeOH:H_2O = 1:2 (v/v), pH 7.77 at 25 °C*
Selectivity: *D-Glucose*

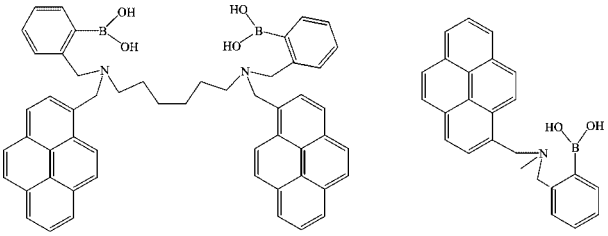
Efforts to develop a three-dimensional fluorescent chemosensor yielded a calixarene-based cavity appended to two naphthalene boronic acid groups. The so-called "sugar bowl" **16** displayed an optimal enhanced fluorescence emission for fructose at 337 nm. Mass spectral data confirmd the presence of a 1:1 binding complex with monosaccharides. Given the extent of long chain hydrocarbons attached to its lower rim, 100% methanol was required for solvation. An unexplored feature that this system presented lies in the availability of additional functionalization along the methylene bridges of the calixarene ring [18].



16

λ_{ex} = 279 nm λ_{em} = 337 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M in MeOH*
Selectivity: *D-fructose*

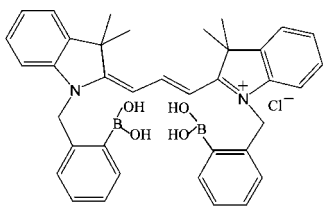
Sensor **17** provided a unique design to investigate the effect of saccharide binding on excimer fluorescence. Here, the rigidification imposed on a bis-pyrene system (separated by a hexamethylene spacer) competes with the hydrophobic effect that typically forces these two fluorophores to form excimers. The decrease in excimer emission (475 nm) occurs in a cooperative manner to increase monomer fluorescence at 376 nm. Given the spacer length and bis-boronic acid geometry, a preference for glucose was observed with the sensor. In order to confirm that this phenomenon is due entirely to decreased excimer states, a similar monoboronic/monopyrene system **18** was synthesized as a reference probe. Indeed, the anticipated monomer emission was the only fluorescence signal observed [19].



17 **18**

λ_{ex} = 343 nm λ_{em} = 376 nm and 475 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M in a MeOH: H_2O(3:1) mixture*
Selectivity: ***17*** *for glucose and* ***18*** *for fructose*

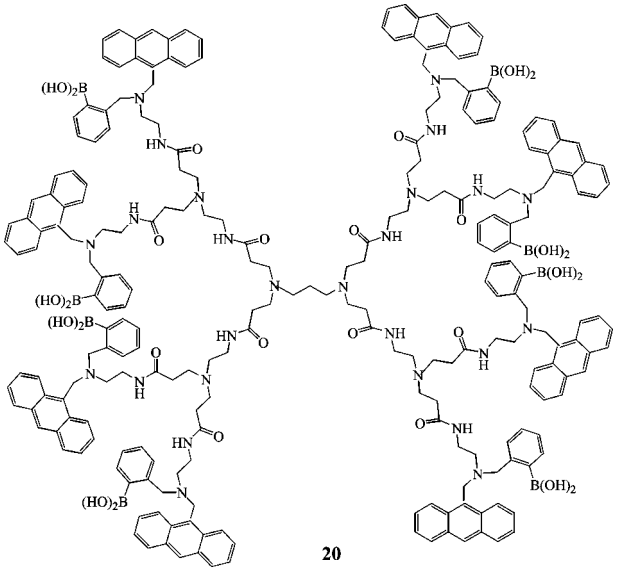
In addition to the use of PET as a fluorescence signaling device, suppression of rotational freedom or molecular "rigidification" has also been used effectively in saccharide detection. Cyanine, appended to two benzylboronic acid groups, displays fluorescence enhancement via the formation of a 1:1 complex with fructose at pH 10. Of the various sugars analyzed, arabinose gave the next highest fluorescence response. The increased fluorescence at 579 nm in the presence of sugar is attributed to a reduction in the rotational freedom in the ethylenic double bond [20].



19

λ_{ex} = 480 nm λ_{em} = 579 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M, MeOH:H_2O = 1:1 (v/v) 0.10 M carbonate buffer solutions at pH 10.0 at 25 °C*
Selectivity: *D-fructose > D- arabinose > D-mannose > D-xylose > D-glucose*

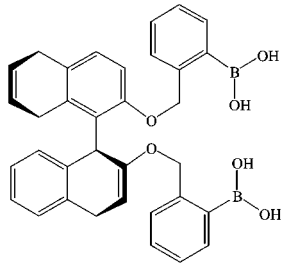
With the commercial availability of PAMAM starburst dendrimers, eight available amino groups were utilized to append the same number of anthracene fluorophores with their corresponding boronic acids. In example **20**, a dendritic saccharide "sponge" displayed high sensitivity to galactose and fructose at 423 nm in 100% methanol. Efforts to assign the binding mode as either 1:1 boronic acid:sugar or 2:1 boronic acids:sugar were hampered by the inherent complexity of the system and its large number of binding sites. The unusual sensitivity observed with galactose in this system was attributed to the apparent flexibility of this system relative to pre-organized clefts [21].



20

λ_{ex} = 370 nm λ_{em} = 423 nm
Measurement conditions: *receptor 3.18 × 10^{-6} M in MeOH at 25 °C*
Selectivity: *D-galactose > D-fructose > D-fucose > D-glucose > D-xylose > 1-methyl-D-galacto-pyranoside > Ethylene glyco*

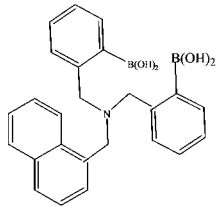
In 1997 a second chiral chemosensor derived from Binap appeared in the form of compound **21**. Whereas the earlier chiral sensor possessed phenylboronic acids at the 3,3′ positions, boronic acids were located at the 2,2′ sites of this second generation Binap system. This difference in positions not only affected the saccharide selectivity of the fluorescent probe, but also relied on a signaling mechanism other than PET. The (*R*)-2,2′-dihydroxy-1,1-binaphthyl platform exhibited the greatest chiral selection for L-xylose and D-talose, which resulted in a chiral discrimination ability of 8.7-fold and 2.0-fold over their enantiomers, respectively. The "rigidification effect" observed in sensor **21** was responsible for the increase in fluorescence intensity upon saccharide binding [22].



21

λ_{ex} = 238 nm λ_{em} = 412 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M, H_2O/MeOH (100:1) at 25°C*
Selectivity: *L-glucose*

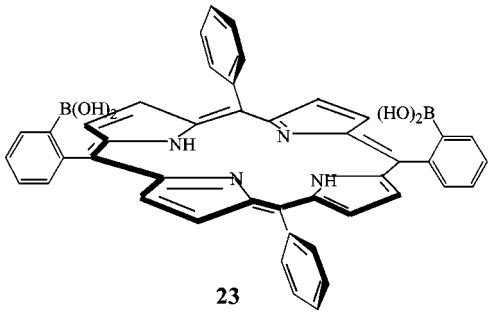
The effects of reduced cleft size were explored in the design of chemosensor **22**. On this probe a tertiary amine serves as the organizational node for two benzylboronic acids and a naphthyl group. The 1:1 binding motif was found to exclude "large" saccharides such as glucose, whereas "small" saccharides such as sorbitol were strongly complexed. By suppressing the effects of PET in the presence of a benzylic amine, fluorescence enhancement at 347 nm was observed upon saccharide binding. Fructose, dulcitol and xylitol followed sorbitol in small saccharide selectivity [23].



22

λ_{ex} = 296 nm λ_{em} = 347 nm
Measurement conditions: *receptor 1.0 × 10^{-5} M, H_2O/MeOH (300:1) pH 8.0 at 25°C*
Selectivity: *D-fructose*

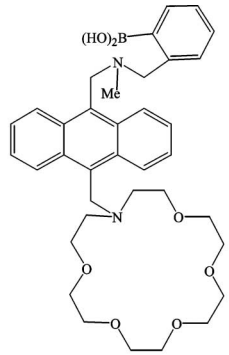
Cis-5,15-bis[2-(dihydroxyboronyl) phenyl]-10, 20-diphenyl-porphine (*cis*-DBBP) provided a rigid fluorescent platform for a bis-boronic acid cleft with a 9 Å distance. The fixed distance was used to assay for a series of disaccharides with an expected 1:1 binding isotherm. Among ten disaccharides tested, sensor **23** was found to respond only to D-lactulose with an increase in fluorescence. The porphyrin based probe displayed 650 nm enhanced emission in the presence of this disaccharide as long as the solution was maintained below 15°C. These conditions were required in order to prevent *cis-trans* isomerization from decomposing the desired cleft geometry [24].



23

λ_{ex} = 417 nm λ_{em} = 650 nm
Measurement conditions: *receptor 4.0 × 10^{-5} M, MeOH solutions at 15°C*
Selectivity: *D-lactulose*

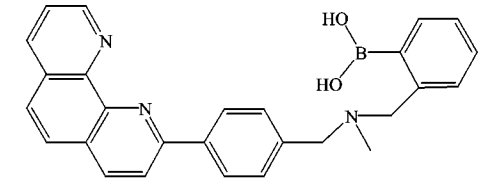
Carbohydrate derivatives, such as glucosamine, were eventually targeted for fluorescence detection with the use of bifunctional chemosensory designs. A monoaza-18-crown-6-ether served as the recognition component for the ammonium terminal of glucosamine hydrochloride. The efficacy of the crown ether for **24** was readily apparent when glucose failed to generate a fluorescence signal and glucosamine produced a fluorescence enhancement 425 nm of 2.2 fold at pH 7.2. The result demonstrated that for fluorescence output to occur, both a diol and ammonium group must be present in the guest [25].



24

λ_{ex} = 372 nm λ_{em} = 425 nm
Measurement conditions: *receptor 3.33×10^{-6} M, MeOH/H_2O (1:2) pH 7.2 at 25°C*
Selectivity: *D-glucosamine hydrochloride*

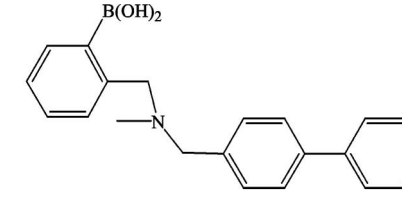
The problem of sensing uronic was addressed by utilizing a metal chelating component for carboxylate binding. Sensor **25** incorporated a phenanthroline ligand along with phenylboronic acid to give a design that is specific for metal chelation and diol complexation. Addition of Zn(II) in the form of $Zn(NO_3)_2 \cdot 6H_2O$ provided a cooperative effect for the carboxylates of several saccharides such as glucuronic, galacturonic and sialic acids. Among these three carbohydrates, the probe displayed the highest affinity for glucuronic acid at 375 nm in pH 8.0. Since saccharides interact with the receptor at two points to form a cyclic structure, this chiral complex was shown to be circular dichroism active [26].



25

λ_{ex} = 294 nm λ_{em} = 375 nm
Measurement conditions: *receptor1.0 ×10^{-5} M, MeOH/H_2O (2:1) pH 8.0 at 25°C*
Selectivity: *D-Uronic acid*

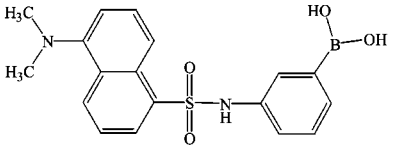
Sensor **26** was prepared for a comparative study with **4** to demonstrate that binding of monosaccharides in aqueous solutions preferentially occurs in the furanose form. The larger HOMO-LUMO gap of the biphenyl system in **26** was chosen to investigate the B-N interaction which is required to suppress PET from nitrogen to fluorophore. Since the 1,2-diols of furanose sugars have been shown to bind stronger than their corresponding pyranose forms, sugars that can not equilibrate between pyranoside and furanoside forms are expected to result in weaker B-N interactions (low fluorescence recovery). Control sensor **4** gave no detectable differences among sugars in the furanose or pyranose form. However, the biphenyl based **26** displayed low fluorescence recovery with sugars that exist in the pyranose form compared to those in their furanose form [27].



26

λ_{ex} = 270 nm λ_{em} = 318 nm
Measurement conditions: *MeOH/H_2O (1:2) pH 7.77*
Selectivity: *D-lactulose*

Incorporating the dansylamide dye into a fluorescent boronic acid led to chemosensor **27** for fructose. The chelation enhanced quenching of fluorescence at 508 nm was observed in the presence of sugars from this system and was described as a reverse photoinduced electron transfer process. Of the several unique features, the fluorescence Stokes shift (~200 nm) stands out as one of the largest reported for a saccharide sensor. The sensor proved to be directly applicable in the fructose concentration determination of jam, fruit juice and whole biscuit giving values quite similar to enzymatic test kits [28].



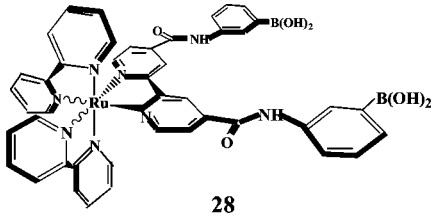
27

λ_{ex} = 324 nm λ_{em} = 508 nm

***Measurement conditions**: receptor 1.0 × 10 $^{-5}$ M, 1% v/v DMSO 0.1 M phosphate buffer solutions at pH 9.0, 20 ± 2 °C*

***Selectivity**: D-fructose*

The fluorescence detection of phosphorylated sugars was made possible with a heteroditopic ruthenium(II) bipyridyl receptor. This system provided adjacent binding sites via acidic NH amide groups as phosphate binding sites and phenylboronic acid as saccharide binding components. The ruthenium(II) bipyridyl derivative provided a MLCT emission band at 637 nm in water. Fructose-6-phosphate in its disodium salt displayed the highest affinity for this probe relative to glucose, galactose or gylcerol-phosphate analytes. A positive cooperative effect of the phosphate and diol functionalities with the dual receptor components of **28** resulted in a 1:1 binding stoichiometry [29].



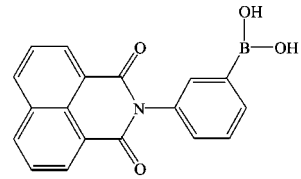
28

λ_{ex} = 470 nm λ_{em} = 637 nm

***Measurement conditions**: receptor 3.0 × 10 $^{-5}$ M, in water pH 7.3 at 25°C*

***Selectivity**: fructose-6-phosphate*

New designs in saccharide sensors appeared in 1999 with the use of the naphthalimides as the fluorescent component. Compound **29** was conveniently synthesized in a single step and represented the first fluor-spacer-receptor design to feature a C_0 spacer. This monoboronic acid system displayed chelation enhanced quenching of 400 nm fluorescence at pH 7.4. Similar to other monoboronic sensors, this probe exhibited the highest selectivity towards fructose followed by galactose and glucose. However, the extensive fluorescence quenching observed upon saccharide complexation was attributed to the C_0 design which is sensitive to conformational changes [30].



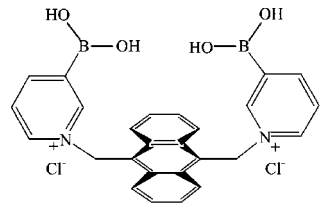
29

λ_{ex} = 354 nm λ_{em} = 400 nm

***Measurement conditions**: receptor 3.2 × 10 $^{-6}$ M, in phosphate buffer at pH 7.4 , 25 °C*

***Selectivity**: D-fructose > D-galactose > D-glucose*

Since water-soluble saccharide probes possessed only limited solubility at low concentrations, sensor **30** was developed with enhanced hydrophilic properties. The use of 3-pyridineboronic acid as receptor components for an anthracene platform was made possible with 9,10-bis(chloromethyl) anthracene. This fluroescent bisboronic acid probe, when converted to its pyrdinium hydrochloride salt, allowed for solubilities concentrated enough for ^{13}C NMR investigations. Selectivities for this probe were found to be similar to similar bisboronic acid sensors, however the coupling of NMR studies with fluorescence provided definitive proof that its glucose complex existed in an α-furanose conformation [31].



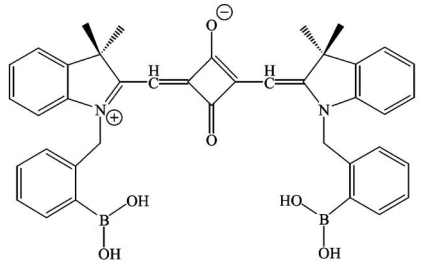
30

λ_{ex} = 377 nm λ_{em} = 427 nm

***Measurement conditions**: receptor 1.0 × 10 $^{-5}$ M, 0.05M phosphate buffer solutions at pH 7.4, 25°C*

***Selectivity**: D-glucose > D-galactose > D-fructose*

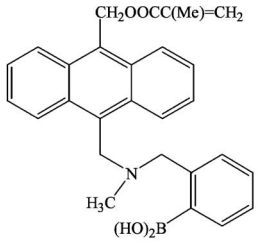
Perhaps the longest wavelength fluorescence signal reported for a carbohydrate probe can be found in squaraine derived sensor **31**. The emission maximum for this compound occurs at 645 nm with a γ-band shoulder at 695 nm. The so called "red to near IR" signaling displayed a 25% increase in fluorescence enhancement with fructose in ethanol/aqueous solution buffered at pH 10. Despite the bis-boronic cleft design, this probe exhibited a 1:2 squaraine/fructose stoichiometry. Comparable concentrations of galactose, gulcose and mannose gave only an 8% increase in emission intensity [32].



31

λ_{em} = 645 nm
***Measurement conditions**: In 0.05M EtOH/caronate buffer solutions at pH 10.0*
***Selectivity**: D-fructose>D-glucose, D-galactose, D-mannose*

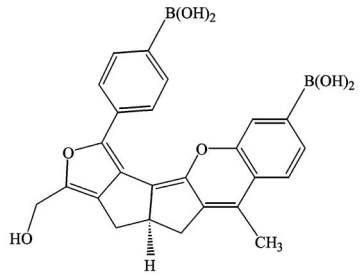
The first polymerizable chemosensor for carbohydrates featured a methylmethacrylate group appended to fluorescent anthracene platform. The fluorescent monomer **32** was polymerized using molecular imprinting methods in the presence of fructose. As with other boronic acids that utilize a benzylic amine as part of the receptor component, this probe design suppresses PET in the complexed state. Fluorescence enhancement at 426 nm indicated good selectivity for fructose. An additional feature of the polymer matrix was indicated by a comparison between monomer selectivity versus polymer where a greater selectivity for fructose is observed with the polymer [33].



32

λ_{ex} = 370 nm λ_{em} = 426 nm
***Measurement conditions**: receptor 2.5mg/mL , MeOH/H_2O (1:1 v/v) 0.05M phosphate buffer solutions (1:1) at pH 7.4*
***Selectivity**: D-fructose> D-glucose > D-mannose*

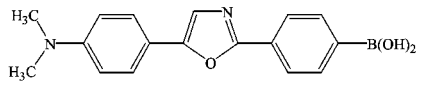
In addition to fluorophore development, new cleft designs were considered in 2001. A glucopyranose sensor **33** containing a pair of precisely positioned phenylboronic acid groups was developed using computer aided design. This fluorescent probe was designed to form cyclic boronates between the α-1,2 and 4,6-hydroxy groups of the pyranose form of glucose. Chelation enhance quenching of fluorescence at 447 nm occurs in the presence of monosaccharides at pH 7.5 in 30% methanol. In agreement with the computational predictions, this probe displayed the largest binding affinity for glucose. Compared to previously reported glucose probes, **33** exhibited a 400-fold greater affinity relative to other sugars [34].



33

λ_{ex} = 375 nm λ_{em} = 447 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M , 30% MeOH/H_2O phosphate buffer solutions at pH 7.5*
***Selectivity**:D-glucose > D-galactose > D-mannose >D-fructose*

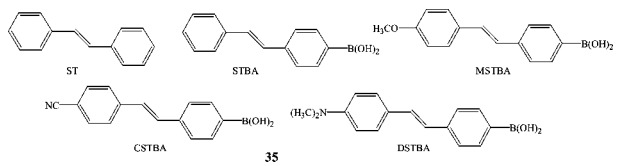
Efforts towards developing highly fluorescent probes resulted in the use of diphenyloxazole as reporter components. Donor-acceptor derivatives of these fluorophores are well known to show high fluorescence quantum yields, long wavelength emision and to be very sensitive to small variations that affect the ICT properties of the excited state. In the case of **34**, the ICT state is between the boronic acid, the electron withdrawing group, and the *N*,*N*-dimethylamino group, the electron donating component. In the presence of saccharides, the boronic acid group changes to its anionic form and the electron withdrawing properties of the boron are removed. The result is a blue shift in fluorescence from 557 nm in the uncomplexed form to 488 when complexed to saccharides [35].



34

λ_{ex} = 350 nm λ_{em} = 488 nm and 557 nm,
***Measurement conditions**: receptor 5.0 × 10^{-6} M , MeOH/H_2O (1:2 v/v) phosphate buffer solutions at pH 7.0, 25°C*
***Selectivity**:D-fructose> D-galactose > D-glucose*

Additional investigations into the charge-transfer effects associated with saccharide binding were conducted on *trans*-stilbene derivates of phenylboronic acid. By introducing electron donating and electron withdrawing groups onto the fluorescent scaffold five different stilbene derivatives were synthesized. Photophysical studies on these saccharide probes in the presence of fructose revealed that the emission spectrum of DSTBA displays a large solvent dependence which supports the effect that dimethylamino groups have on the excited state. Specifically, saccharide complexation induced a blue shift of about 50 nm and an increase of intensity because of the loss of electron-withdrawing properties for the anionic form of the boronic acid group. In contrast, a red shift of 35 nm and a decrease in intensity were observed for CSTBA as it undergoes transformation from neutral boronic acid to anionic boronate. These findings led to the conclusion that the anionic form of the boronic acid groups acts as an electron donor group and a CT state can be formed when an electron withdrawing group is present on the sensor [36].



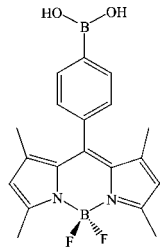
35

Spectral properties were measured in H_2O/MeOH (1:1 v/v) at room teperature

	ST	STBA	CSTBA	MSTBA	DSTBA
λ_{ex} (nm)	296	314	327	324	346
λ_{em} (nm)	348	358	385	391	485

All of these sensors display selectivity to D-fructose

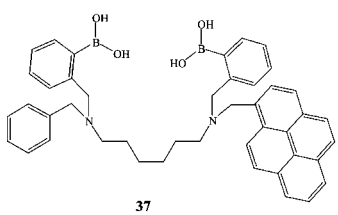
Boron-dipyrromethane dyes (better known as BODIPY^{TM}) present several advantages as fluorescent probes. They possess high extinction coefficients, high emission quantum yields and narrow emission bands. In addition, their building block synthesis allows for the development of many different analogs with emission ranges from 500 to 700 nm. Probe **36** provided a very narrow emission band with maxima at 510 nm in aqueous phosphate buffered to pH 7.5. In the presence of fructose, this monoboronic acid probe displayed an enhanced fluorescence response. These trends correlated well with fluorescent lifetime measurements reported at 4.1 ns for the bound complex compared to 3.5 ns in the absence of sugar [37].



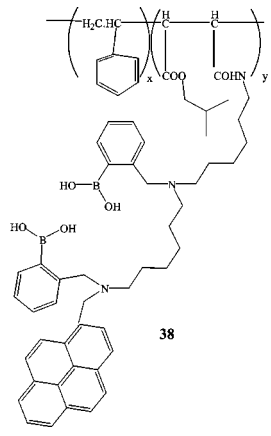
36

λ_{ex} = 495 nm λ_{em} = 510 nm
***Measurement conditions**: receptor 2.9 × 10^{-6} M in phosphate buffer solutions with pH 7.5 at room temperature*
***Selectivity**:D-fructose*

Attempts to investigate hexamethylene linkers as spacer groups for discrete bis-boronic acids probes as well as polymerizable systems resulted in the use of pyren-1-yl fluorophores. The advantage that sensor **37** has over bis-pyrenyl systems is the elimination of possible excimer emission due to stacking of two pyrene units. This effect was predicted to particularly occur when the bis-pyrenyl system undergo polymerization. The emission wavelength at 397 nm was monitored at pH 8.2 in methanol and supported the proof of concept design from monomer to polymer. Sensor **37** combined the spatial arrangements of the hexamethylene spacer along with the two aromatic groups shown below to produce a polymeric saccharide probe with high selectivity for glucose (**38**) [38].



37

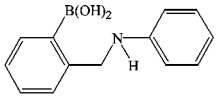


38

λ_{em} = 397 nm
***Measurement conditions**: receptor 1.0 × 10^{-7} M at MeOH/H_2O (52.1 wt%) phosphate buffer solutions at pH 8.21*
***Selectivity**:37 for D-glucose, 38 for D-fructose*

The use of ICT as an effective tool in ratiometric sensing was further elaborated with the synthesis of sensor **39**. In the trigonal form, boronic acid confers an electron withdrawing property onto the probe which forms an ICT

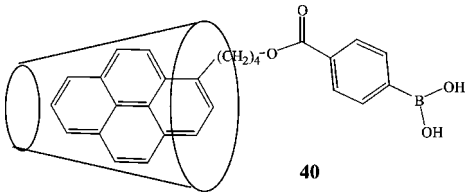
excited state. This emissive state exhibits fluorescence at 404 nm. In the anionic form, the tetrahedral boron becomes electron donating to the aromatic scaffold (as in the case with a complexed sugar) and the ICT state is diminished. The resulting fluorescence emission becomes blue shifted to 362 nm. Monoboronic acid probe **39** displayed the largest dynamic change in fluorescence at pH 8 in 50:50 methanol water with fructose [39].



39

λ_{ex} = 244 nm λ_{em} = 362 nm and 404 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M , EtOH/H_2O (1:2 v/v) phosphate buffer solutions at pH 8.21, 25°C*
***Selectivity**:D-fructose> D-galactose > D-glucose*

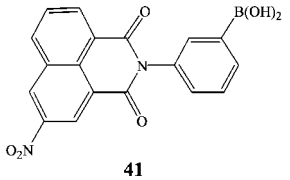
Inclusion complexes previously studied as hydrophobic effects between cyclodextrins and pyrene were aptly transformed into a fluorescence saccharide sensing system. In this example, a modest butyl chain appended to pyrene was esterified with 4-carboxyphenylboronic acid to give sensor **40**. The probe exhibited no fluorescence emission in water due to its aggregation; however the addition of β-CyD to this solution significantly changed its fluorescence properties. This "off-on" system displayed high fluorescence with two sharp peaks at 370 and 400 nm in 95% methanol:5% water (v:v). The binding selectivity of fructose > arabinose > galactose > glucose was consistent with monoboronic acids [40].



40

λ_{ex} = 328 nm λ_{em} = 370 nm and 400 nm
***Measurement conditions**: receptor 1.05 × 10^{-6} M in DMSO/H_2O (1:19 v/v) containing 5.0 mM β -CyD, 0.015M phosphate buffer solutions at pH 7.5*
***Selectivity**:D-fructose> L-arabinose > D-galactose > D-glucose*

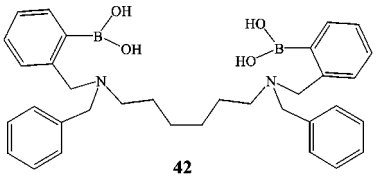
To promote charge-transfer excited states that display dual fluorescence, 3-nitro-1,8-naphthalic anhydride was coupled to 3-aminophenylboronic acid giving sensor **41**. In a manner similar to that of related *N*-phenylnaphthalimides, this sensor displays two emission bands (430/550 nm) from a single excitation wavelength while increasing the solvent polarity causes the longer wavelength to migrate toward the red. Although the nitro group significantly reduced the quantum yield of this system, the sensor displayed an anomalous sensitivity to glucose. Since dissociation constants reflected the expected trend for boronic acid:saccharide complexes, the photophysics of this system were deemed more sensitive to conformational changes associated with the binding of glucose relative to other common monosaccharides [41].



41

λ_{ex} = 337 nm λ_{em} = 430 nm and 550 nm
***Measurement conditions**: receptor 3.0 × 10^{-5} M in DMSO/ H_2O (1:99 v/v) 0.01M phosphate buffer solutions at pH 8.0, 25°C*
***Selectivity**:D-glucose >D-fructose > D-galactose*

A selective fluorescent assay for glucose was developed using Alizarin Red S (ARS) with a receptor specific for glucose. The glucose receptor component (**42**) was designed without a fluorescent signaling unit, so that ARS would serve as the only reporter. In this chemosensory ensemble, the ARS remains fluorescent at 464 nm so long as boronic acid is bound to its aromatic diol functionality. Upon addition of glucose, however, the sugar competes for the preorganized boronic acids of **51** in a 1:1 complex and a concomitant decrease of ARS fluorescence occurs [42].

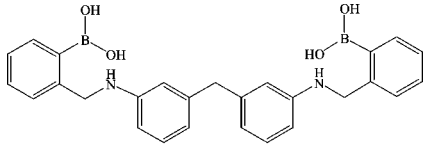


42

λ_{ex} = 464 nm and 495 nm λ_{em} = 570 nm for ARS
***Measurement conditions**: receptor 5.0 × 10^{-5} M in phosphate buffer solutions with 52.1 wt% MeOH at pH 8.21*
***Selectivity**:D-glucose >D-fructose*

Improving on the previous monoboronic acid design of **39**, sensor **43** utilizes two boronic acid and a similar ICT fluorescence to provide two wavelengths; one emission band occurring in the presence of sugar, another emission band in the absence of sugar. To confer sensor **43** with specificity for glucose, correct spacing of two boronic acid groups was achieved with a diphenylmethane platform. As with the earlier fructose ICT probe, the trigonal form of

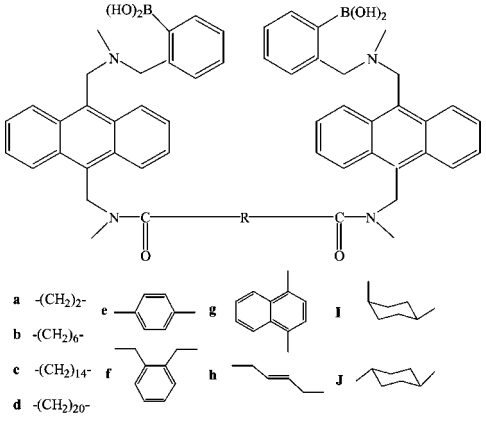
boronic acid promotes an ICT excited state. This emissive state exhibits fluorescence at 405 nm. In the anionic form, the tetrahedral boron becomes electron donating to the aromatic scaffold (as in the case with a complexed sugar) and the ICT state is diminished. The resulting fluorescence emission becomes blue shifted to 360 nm [43].



43

λ_{ex} = 265 nm λ_{em} = 360 nm and 405 nm
***Measurement conditions**: receptor 4.2 × 10^{-5} M in pH 8.21 phosphate buffer solutions with 52.1 wt% MeOH*
***Selectivity**: D-glucose >D-fructose > D-galactose*

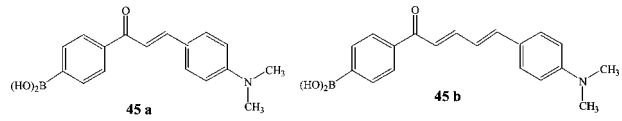
The enhanced glucose selectivity of saccharide sensor **44** resulted from a screening process which involved the evaluation of several aromatic spacers. An optimal spacer and corresponding glucose accomodating cleft was obtained from spacer **f**. The benzylic amine derived boronic acid provided the expected enhanced fluorescence signal at 426 nm in the presence of glucose via suppression of PET. In a related study, a fluorescent probe suitable for detection of Sialyl Lewis X utilized spacer **e** for optimal carbohydrate binding. Using cell cultures, **44 e** was shown to lable sLex-expressing HEPG2 cells at 1 μM while non-sLex-expressing cells were not labeled [44].



44

λ_{ex} = 370 nm λ_{em} = 426 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M in pH 7.4 phosphate buffer solutions with 50wt% MeOH (1:1 v/v)*
***Selectivity**: sensor e displays selectivity to sialyl lewis X*

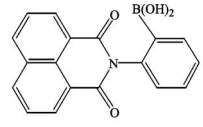
Advances in ICT based fluorescent probes for saccharides continued with the development of chalcone-analogue platforms. These compounds were conveniently prepared in a single step for the corresponding aldehydes and acetylphenylboronic acid. The concept of increased distance between donor/acceptor groups to promote and prolong the lifetime of charge transfer states was successfully applied to sensors **45a** and **45b**. Changes in hybridization of boronic acid from trigonal neutral to tetrahedral anion significantly affected the donor-group of *N*,*N*-dimethylamino substituents. In this example, polarization of the chemosensor is signficantly reduced as the boronic acid binds to fructose. An additional effect appears to be operating with these probes as the electron-withdrawing property of the carbonyl functionality is also dampened. The photophysical effect is observed as a blue-shift in fluorescence as the excited states no longer undergo solvent induced relaxation in polar media [45].



45 a **45 b**

*λ_{ex} = 438 nm, 445nm λ_{em} = 577 nm,663 nm for **a**, **b** respectively*
***Measurement conditions**: MeOH/phosphate buffer solutions (1:2 v/v) at pH 6.5*
***Selectivity**: D-fructose*

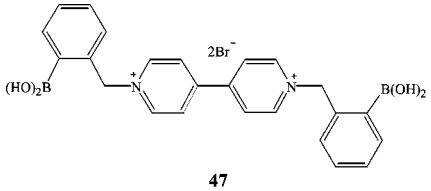
Photophysical effects of positional isomers involving phenylboronic acid were investigated with the use of sensors **29** and **46**. Given the sensitivity of C_0 or virtual spacers to conformational changes, the *ortho* and *meta* isomers were compared in the fluorescence response to sugars. Formation of the anionic form of the boronic acid induced a substantial decrease of the steady-state fluorescence of both compounds. Both compounds displayed a substantial decrease of their fluorescence intensity in the presence of sugars. A signficant increase of the fluorescence lifetime was observed for the *ortho*-derivative in the presence of sugar while a relatively small effect was obtained for the *meta*-derivative. The increase of the fluorescence was attributed to a more pronounced steric hindrance effect in the *ortho*-compound. These results implicated the potential use of the *ortho*-derivative as a sugar probe for fluorescence lifetime-based sensing [46].



46

λ_{ex} = 345 nm λ_{em} = 397 nm
***Measurement conditions**: In pH 7.5 phosphate buffer solutions at room temperature*
***Selectivity**: D-fructose > D-galactose > D-glucose*

A fluorescent chemosensing ensemble was developed from a two component system comprised of 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (pyranine) and *o*-BBV^{2+}. In this probe design, pyranine functions as the reporting group and its fluorescence is modulated through interaction with the boronic acid-substituted viologen. Compared to unimolecular saccharide probes, sensor **47** involves a discrete dye molecule and quencher molecule fitted with boronic acids. The probe utilizes the strong electron accepting properties of viologens to quench the fluorescence of an anionic dye, pyranine. In the presence of sugars, however, this quenching interaction is prevented by complexation of the *o*-BBV^{2+} with the diols of fructose [47]. The result of this interaction is observed as an enhanced fluorescence signal which appears at 510 nm.

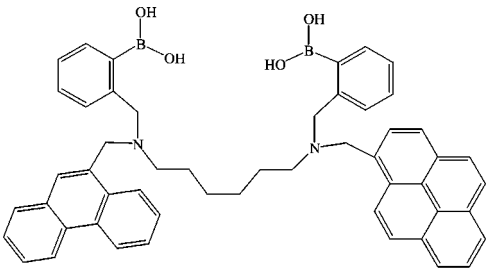


47

λ_{ex} = 470 nm λ_{em} = 510 nm (for pyranine)
***Measurement conditions**: In pH 7.4 phosphate buffer*
***Selectivity**: D-fructose > D-galactose > D-glucose*

Whereas fluorescence energy transfer had been applied to macromolecules such as protein and enzyme based assays, the technique had so far remained an unexplored fluorescence signaling mechanism in saccharide chemosensors. Utilizing the modular saccharide probe design of **37**, a novel FRET based sensor **48** was developed with phenanthrene as donor and pyrene as acceptor. Advantages of this new design included two fluorescence bands (369 nm from phenanthrene and 417 nm from pyrene) with which to conduct ratiometric detection. The FRET phenomenon was observed in the presence of saccharides as excitation at 299 nm (phenanthrene) resulted in no emission at 369 nm, but instead, sensor **48** displayed pyrene emission. Titration experiments with monosaccharides indicated that energy transfer for the phenanthrene donor to pyrene acceptor in a rigid 1:1 cyclic glucose complex is more efficient than in a flexible 2:1 acyclic fructose complex [48].

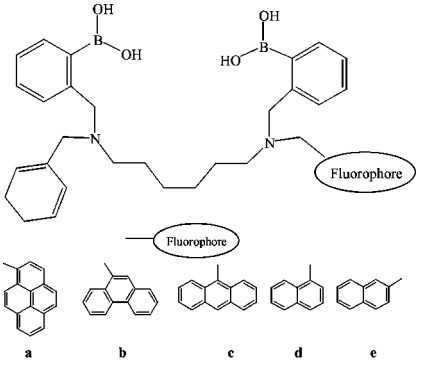
The next decade of research into fluorescent sensing of carbohydrates appears just as bright as the last. As the final year to be included in our review, 2003 ushered several new probes for saccharides and other carbohydrates. In sensors **49a–e**, combinations of various polycyclic aromatic hydrocarbons ranging from naphthalene to pyrene revealed sharp differences in saccharide selectivity. These



48

λ_{ex} = 299 nm λ_{em} = 369 nm and 417 nm
***Measurement conditions**: receptor 2.5 × 10^{-6} M in 0.1 M phosphate buffer solutions with 52.1 wt% MeOH at pH 8.21*
***Selectivity**: D-glucose*

modular sensors allowed for direct comparisons of saccharide selectivity since the receptor and spacer components remained constant throughout the study. The largest enhancements in fluorescence resulted from **49a** exhibiting glucose selectivity and **49e** showing enhanced galactose selectivity. Several factors were implicated in the preferences of one sugar over another such as size of the fluorophore-π surface (solvation) as well as the number of peri-hydrogens each fluorophore contains (steric crowding). Sensor **49a** contains a pyrene moiety which is the largest π-surface in the series and displayed for glucose over galactose. By reducing the size of the hydrophobic π-surface, as with **49e**, the selectivity switched from glucose to galactose [49].

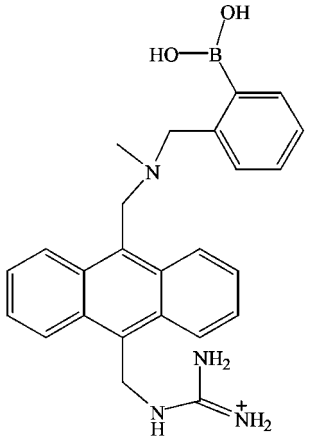


49

Fluorescence Measurements were Carried out in Aqueous Methanolic Phosphate Buffer Solutions (52.1 wt%) at pH 8.21

Fluorophore	Concentration	λ_{ex} (nm)	λ_{em} (nm)	Selectivity
a	1.0×10^{-6}	342	397	D-glucose
b	5.0×10^{-6}	299	369	D-glucose
c	1.0×10^{-6}	370	420	D-glucose
d	2.5×10^{-6}	275	335	D-galactose
e	2.5×10^{-6}	274	335	D-galactose

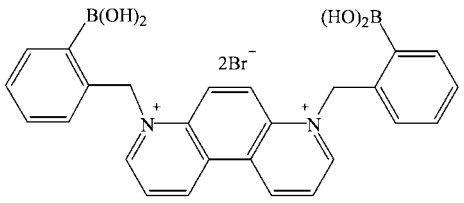
Because glucarate is a biologically active carbohydrate that exists in human serum, development of a fluorescent sensor for this analyte has potential applications in the chemopreventive treatment of certain cancers. Formation of a salt-bridge between the carboxylic acids of glucarate and a guanidinium recognition unit from the sensor were key design elements in sensor **50**. The remaining diol fixtures of the carbohydrate were addressed with a benzylic amine derived boronic acid. This strategy allowed for the suppression of the PET phenomena which gives rise to fluorescence enhancement upon glucarate complexation. The probe demonstrated good selectivity against similar carbohydrate derivatives such as gluconate, sorbitol, glucuronic acid and glucose with a fluorescence emission signal at 424 nm [50].



50

l_{ex} = 370 nm l_{em} = 424 nm
***Measurement conditions**: receptor 1.0 × 10^{-5} M, 50% MeOH/HEPES buffer solutions at pH 7.4*
***Selectivity**:D-glucarate*

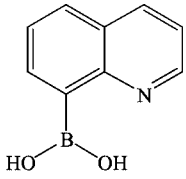
Further developments with fluorescent chemosensing ensemble designs were reported using 4,7-phenanthrolinium salts to give sensor **51**. The enhanced ability of *o*-PBBV^{2+} salts to accept electrons relative to the reduction potential of **47** led to improved quenching properties as well as greater sensitivity to and an unusual selectivity for glucose. In this example, the selectivity decreased in the order glucose > fructose > galactose. Since the chemical structure of the quencher can be modified to obtain glucose selectivity over fructose without having to modify the structure of the dye, this probe demonstrates the versatility of sensing ensemble designs relative to unimolecular saccharide probes [51].



51

λ_{ex} = 470 nm λ_{em} = 510 nm *(for pyranine)*
***Measurement conditions**: In pH 7.4 phosphate buffer*
***Selectivity**: D-glucose>D-fructose > D-galactose*

Use of a new fluorescent platform derived from quinoline resulted in 8-quinoline boronic acid (8-QBA) sensor **52**. The 8-QBA sensor is essentially non-fluorescent at pH above 5 and weakly fluorescent at lower pH in aqueous solution. Upon addition of fructose, the fluorescence intensity increases significantly to provide a simple "off-on" probe. Since the boron atom in 8-QBA exists primarily in a tetrahedral geometry and no B-N interaction can be readily formed with the quinoline nitrogen, sensor **52** appears to signal the presence of saccharides with a mechanism other than PET. A photophysical change that occurs with 8-QBA wherein the non-emissive $n\pi^*$ state is perturbed and gives rise to an emissive $\pi\pi^*$ excited state was attributed to this large fluorescence enhancement [52].

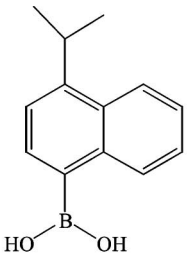


52

l_{ex} = 314 nm l_{em} = 417 nm
***Measurement conditions**: receptor 6.5 × 10^{-5} M in 0.1M phosphate buffer solutions at pH 7.4*
***Selectivity**:D-fructose*

Sensor **53** utilizes the ionization dynamics between trigonal boronic acid and tetrahedral boronate anion previously explored by Lakowicz and James to promote an ICT fluorescence response. For this system, a 4-(dimethylamino)naphthalene platform promotes an ICT mechanism to give an enhanced fluorescence response to fructose at 445 nm. In keeping with trends for monoboronic acid systems, the probe shows the largest

optical response to fructose, however its remarkable feature is a 41-fold emission enhancement in the presence of 50 mM of this sugar under neutral pH conditions. An additional advantage of this system is found in the one-step synthesis from commercially available starting material [53].



53

l_{ex} = 300 nm l_{em} = 445 nm

***Measurement conditions**: receptor 1.0 × 10^{-5} M in 0.1 M phosphate buffer solutions at pH 7.4*

***Selectivity**:D-fructose*

In retrospect, the last decade has witnessed an extensive display of fluorescent chemosensors for carbohydrates since Czarnik's first report. In addition to the application of PET as a viable signaling mechanism, a number of other photodynamic approaches such as ICT, MLCT, and FRET have been utilized in the search for longer wavelength emission, higher quantum yields and higher signal response. Furthermore, the field has greatly benefited from the work of many researchers whose field of expertise varies from molecular recognition, spectroscopy, organic synthesis, transition metal catalysis and medicinal chemistry. Their contributions have markedly expanded the field to include (1) the detection of not only monosaccharides, but carbohydrate derivatives of biological and medicinally relevent analytes, (2) the development of novel fluorescent dyes and signaling designs, (3) the use of time-dependent fluorescence as a detection tool, (4) the implementaion of computer-aided molecular design and finally the clinical application of these compounds to medical problems. Whereas many reviews can be regarded as a summarized conclusion to a particular field of research, we conclude this review of 10-plus years as a prelude to future contributions. It is our view that as the biological, medical and clinical importance of saccharides increases with each new discovery of their involvement in metabolism and disease-treatment, methods that provide an optical response to their activity will continue to gain importance.

REFERENCES

1. T. D. James, K. R. A. S. Sandanayake, and S. Shinkai (1995). Recognition of sugars and related compounds by " reading-out "-type interfaces. *Supramol. Chem.* **6**(1/2), 141–157.
2. T. D. James, K. R. A. S. Sandanayake, and S. Shinkai (1996). Saccharide sensing with molecular receptors based on boronic acid. *Angew. Chem., Int. Ed.* **35**(17), 1910–1922.
3. J. H. Hartley, T. D. Jame, and C. J. Ward (2000). Synthetic receptors. *J. Chem. Soc., Perkin Trans. 1* (19), 3155–3184.
4. W. Wang, X. Gao, and B. Wang (2002). Boronic acid-based sensors. *Current. Org. Chem.* **6** (14), 1285–1317.
5. T. D. James and S. Shinkai (2002). Host-Guest chemistry. *Top. Curr. Chem.* **218**, 159–200.
6. J. Yoon and A. W. Czarnik (1992). Fluorescent chemosensors of carbohydrates. A means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. *J. Am. Chem. Soc.* **114**(14), 5874–5875.
7. T. D. Jame, P. Linnane, and S. Shinkai (1996). Fluorescent saccharide receptors: A sweet solution to the design, assembly and evaluation of boronic acid derived PET sensors. *Chem. Commun.* (3), 281–288. (Original paper by Czarnik and Yoon cited in reference 7 could not be located, so review paper is listed above. Based on isomeric relation between **1** and **2**, conditions and saccharide binding for **2** were assumed to be similar to those of **1**.)
8. Y. Nagai, K. Kobayashi, H. Toi, and Y. Aoyama (1993). Stabilization of sugar-boronic esters of indolyboronic acid in water via sugar-indole interaction: A notable selectivity in oligosaccharides. *Bull. Chem. Soc. Jpn.* **66**(10), 2965–2971.
9. H. Murakami, T. Nagasaki, I. Hamachi, and S. Shinkai (1993). Sugar sensing utilizing aggregation properties of a boronic-acid-appended porphyrin. *Tetrahedron Lett.* **34**(39), 6273–6276.
10. T. D. Jame, K. R. A. S. Sandanayake, and S. Shinkai (1994). Novel photoinduced electron-transfer sensor for saccharides based on the interaction of boronic acid and amine. *J. Chem. Soc., Chem. Commun.* (4), 477–478.
11. T. D. Jame, K. R. A. S. Sandanayake, and S. Shinkai (1994). A glucose-selective molecular fluorescence sensor. *Angew. Chem. Int. Ed. Engl.* **33**(21), 2207–2209.
12. K. R. A. S. Sandanayake, K. Nakashima, and S. Shinkai (1994). Specific recognition of disaccharides by *trans*-3,3′-stilbenediboronic acid: Rigidification and fluorescence enhancement of the stilbene skeleton upon formation of a sugar-stilbene macrocycle. *J. Chem. Soc., Chem. Commun.* (14), 1621–1622.
13. T. D. Jame, K. R. A. S. Sandanayake, and S. Shinkai (1995). Chiral discrimination of monosaccharides using a fluorescent molecular sensor. *Nature (London)* **347**(23), 345–347.
14. K. R. A. S. Sandanayake, S. Imazu, T. D. Jame, M. Mikami, and S. Shinkai (1995). Molecular fluorescence sensor for saccharides based on amino coumarin. *Chem. Lett.* (2), 139–140.
15. H. Suenaga, M. Mikami, K. R. A. S. Sandanayake, S. Shinkai (1995). Screening of fluorescent boronic acids for sugar sensing which show a large fluorescence change. *Tetrahedron Lett.* **36**(27), 4825–4828.
16. P. Linnane, T. D. Jame, S. Imazu, and S. Shinkai (1995). A sweet toothed saccharide (PET) sensor. *Tetrahedron Lett.* **36**(48), 8833–8834.
17. T. D. Jame and S. Shinkai (1995). A diboronic acid "glucose cleft" and a biscrown ether "metal sandwich" are allosterically coupled. *J. Chem. Soc., Chem. Commun.* (14), 1483–1485.
18. P. Linnane, T. D. Jame, and S. Shinkai (1995). The synthesis and properties of a calixarene-based "sugar bowl." *J. Chem. Soc., Chem. Commun.* (19) 1997–1998.
19. K. R. A. S. Sandanayake, T. D. Jame, and S. Shinkai (1995). Two dimensional photoinduced electron transfer (PET) fluorescence sensor. *Chem. Lett.* (7), 503–504.
20. M. Takeuchi, T. Mizuno, H. Shinmori, M. Nakashima, and S. Shinkai (1996). Fluorescence and CD spectroscopic sugar sensing by a

cyanine-appended diboronic acid probe. *Tetrahedron* **52**(4), 1195–1204.
21. T. D. Jame, H. Shinmori, M. Takeuchi, and S. Shinkai (1996). A saccharide "sponge." Synthesis and properties of a dendritic boronic acid. *Chem. Commun*. (6), 705–706.
22. M. Takeuchi, S. Yoda, T. Imada, and S. Shinkai (1997). Chiral sugar recognition by a diboronic-acid-appended binaphthyl derivative through rigidification effect. *Tetrahedron* **53**(25), 8335–8348.
23. T. D. Jame, H. Shinmori, and S. Shinkai (1997). Novel fluorescence sensor for "small" saccharides. *Chem. Commun*. (1), 71–72.
24. H. Kijima, M. Takeuchi, and S. Shinkai (1998). Selective detection of D-lactulose by a porphyrin-based diboronic acid. *Chem. Lett*. (8), 781–782.
25. C. R. Cooper and T. D. Jame (1997). Selective D-glucosamine hydrochloride fluorescence signaling based on ammonium cation and diol recognition. *Chem. Commun*. (15), 1419–1420.
26. M. Takeuchi, M. Yamamoto, and S. Shinkai (1997). Fluorescent sensing of uronic acids based on a cooperative action of boronic acid and metal chelate. *Chem. Commun*. (18), 1731–1732.
27. C. R. Cooper and T. D. James (1998). Selective fluorescence signalling of saccharides in their furanose form. *Chem. Lett*. (9), 883–884.
28. G. P. Luis, M. Granda, R. Badia, and M. E. Díaz-García (1998). Selective fluorescent chemosensor for fructose. *Analyst* **123**(1), 155–158.
29. M. J. Deetz and B. D. Smith (1998). Heteroditopic ruthenium (II) bipyridyl receptor with adjacent saccharide and phosphate binding sites. *Tetrahedron Lett*. **39**(38), 6841–6844.
30. D. P. Adhikiri and M. D. Heagy (1999). Fluorescent chemosensor for carbohydrates which shows large change in chelation-enhanced quenching. *Tetrahedron Lett*. **40**(45)**,** 7893–7896.
31. H. Eggert, J. Frederiksen, C. Morin, and J. C. Norrild (1999). A new glucose-selective fluorescent Bisboronic acid. First report of strong α-furanose complexation in aqueous solution at physiological pH. *J. Org. Chem*. **64**(11), 3846–3852.
32. E. U. Akkaya and B. Kukrer (1999). Red to near IR fluorescent signaling of carbohydrates. *Tetrahedron Lett*. **40**(51), 9125–9128.
33. W. Wang, S. Gao, and B. Wang (1999). Building fluorescent sensors by template polymerization: The preparation of a fluorescent sensor for D-fructose. *Org. Lett*. **1**(8), 1209–1212.
34. W. Yang, Y. He, and D. G. Drueckhammer (2001). Computer-guided design in molecular recognition: Design and synthesis of a glucopyranose receptor. *Angew. Chem., Int. Ed*. **40**(9), 1714–1717.
35. N. DiCesare and J. R. Lakowicz (2001). A new highly fluorescent probe for monosaccharides based on a donor-acceptor diphenyloxazole. *Chem. Commun*. (19), 2022–2023.
36. N. DiCesare and J. R. Lakowicz (2001). Spectral properties of fluorophores combining the boronic acid group with electron donor or withdrawing groups. Implication in the development of fluorescence probes for saccharides. *J. Phys. Chem. A* **105**(28), 6834–6840.
37. N. DiCesare and J. R. Lakowicz (2001). Fluorescent probe for monosaccharides based on a functionalized boron-dipyrromethene with a boronic acid group. *Tetrahedron Lett*. **42**(52), 9105–9108.
38. S. Arimori, M. L. Bell, C. S. Oh, K.A. Frimat, and T. D. Jame (2001). Modular fluorescence sensors for saccharides. *Chem. Commun*. (18), 1836–1837.
39. S. Arimori, L. I. Bosch, C. J. Ward, and T. D. Jame (2001). Fluorescent internal charge transfer (ICT) saccharide sensor. *Tetrahedron Lett*. **42**(27), 4553–4555.
40. A. Tong, A. Yamauchi, T. Hayashita, Z. Zhang, B. D. Smith, and N. Teramae (2001). Boronic acid fluorophore/β-cyclodextrin complex sensors for selective sugar recognition in water. *Anal Chem*. **73**(7), 1530–1536.
41. H. Cao, D. I. Diaz, N. DiCesare, J. R. Lakowicz, and M. D. Heagy (2002). Monoboronic acid sensor that displays anomalous fluorescence sensitivity to glucose. *Org. Lett*. **4**(9), 1503-1505.
42. S. Arimori, C. J. Ward, and T. D. Jame (2002). A D-glucose selective fluorescent assay. *Tetrahedron Lett*. **43**(2), 303–305.
43. S. Arimori, L. I. Bosch, C. J. Ward, and T. D. Jame (2002). A D-glucose selective fluorescent internal charge transfer (ICT) sensor. *Tetrahedron Lett*. **43**(5), 911–913.
44. W. Yang, S. Gao, X. Gao, V. V. R. Karnati, W. Ni, B. Wang, W. B. Hook, J. Carson, and B. Weston (2002). Diboronic acids as fluorescent probes for cells expressing sialyl lewis x. *Bioorg. Med. Chem. Lett*. **12**(16), 2175–2177.
45. N. DiCesare and J. R. Lakowicz (2002). Chalcone-analogue fluorescent probes for saccharides signaling using the boronic acid group. *Tetrahedron Lett*. **43**(14), 2615–2618.
46. N. DiCesare, D. P. Adhikiri, J. J. Heynekamp, M. D. Heagy, and J. R. Lakowicz (2002). Spectroscopic and photophysical characterization of fluorescent chemosensors for monosaccharides based on *N*-phenylboronic acid derivatives of 1,8-naphthalimide. *J. Fluoresc*. **12**(2), 147–153.
47. J. N. Camara, J. T. Suri, F. E. Cappuccio, R. A. Wessling, and B. Singaram (2002). Boronic acid substituted viologen based optical sugar sensors: Modulated quenching with viologen as a method for monosaccharide detection. *Tetrahedron Lett*. **43**(7), 1139–1141.
48. S. Arimori, M. L. Bell, C. S. Oh, and T. D. Jame (2002). A modular fluorescence intramolecular energy transfer saccharide sensor. *Org. Lett*. **4**(24), 4249–4251.
49. S. Arimori, G. A. Consiglio, M. D. Philips, and T. D. Jame (2003). Tuning saccharide selectivity in modular fluorescent sensors. *Tetrahedron Lett*. **44**(25), 4789–4792.
50. W. Yang, J. Yan, H. Fang, and B. Wang (2003). The first fluorescent sensor for D-glucarate based on the cooperative action of boronic acid and guanidinium groups. *Chem. Commun*. (6), 792–793.
51. J. T. Suri, D. B. Cordes, F. E. Cappuccio, R. A. Wessling, and B. Singaram (2003). Monosaccharide detection with 4,7-phenanthrolinium salts: Charge-induced fluorescence sensing. *Langmuir* **19**(12), 5145–5152.
52. W. Yang, J. Yan, G. Springsteen, S. Deeter, W. Ni, and B. Wang (2003). A novel type of fluorescent boronic acid that shows large fluorescence intensity changes upon binding with a carbohydrate in aqueous solution at physiological pH. *Bioorg. Med. Chem. Lett*. **13**(6), 1019–1022.
53. X. Gao, Y. Zhang, and B. Wang (2003). New boronic acid fluorescent reporter compounds. 2. A naphthalene-based on–off sensor functional at physiological pH. *Org. Lett*. **5**(24), 4615–4618.