J Fluoresc (2011) 21:1143–1154
DOI 10.1007/s10895-010-0791-1

ORIGINAL PAPER

Synthesis of Ethynylated Phenothiazine Based Fluorescent Boronic Acid Probes

Yubo Wu · Huimin Guo · Jingyin Shao · Xin Zhang · Shaomin Ji · Jianzhang Zhao

Received: 20 September 2010 / Accepted: 9 December 2010 / Published online: 7 January 2011

Abstract Ethynylated phenothiazine based fluorescent boronic acid probes were prepared. Sonogashira coupling reaction was used to introduce substituted phenylethynylene fragments to the phenothiazine fluorophore to extend the π-conjugation and to enhance the emission property. The photophysical properties and the binding properties of these probes with hydroxyl acids were investigated. We found that the probes with significant ICT effect show emissions which are sensitive to solvent polarity. The phenothiazine moiety is proved to be electron-donating. We found the substitution profile imparts significant effect on the photophysical properties of the probes. For example, one of the probes shows d-PeT effect, whereas the regioisomer probe with similar π-conjugation fragment but different substitution profile shows the a-PeT effect. The easy derivatization of phenothiazine fluorophore, the structure-photophysical property relation and the novel d-PeT fluorescence transduction profile of the phenothiazine based probes described herein may inspire more investigation into this fascinating research area.

Keywords Boronic acid · Fluorescent chemosensors · d-PeT · Phenothiazine

Electronic supplementary material The online version of this article (doi:10.1007/s10895-010-0791-1) contains supplementary material, which is available to authorized users.

Y. Wu · H. Guo (✉) · J. Shao · X. Zhang · S. Ji · J. Zhao (✉)
State Key Laboratory of Fine Chemicals, School of Chemical Engineering, Dalian University of Technology,
Dalian 116024, People's Republic of China
e-mail: guohm@dlut.edu.cn

J. Zhao
e-mail: zhaojzh@dlut.edu.cn

Introduction

Fluorescent probes attract much attention due to their versatile application in chemical, biological and environmental science. Binding units and fluorophores are the two critical components of the probes. Various sensing mechanisms have been employed for probes, such as photo-induced electron transfer (PeT) and intramolecular charge transfer (ICT) [1–12]. However, the binding motifs of the fluorescent probes are limited to hydrogen bonding or dative bonding. Probes based on hydrogen bonds are usually not effective in protic solvents, due to the strong competition of the solvents molecules. Concerning this aspect, the boronic acid probes are special due to their covalent bonding nature with saccharides or hydroxyl acids analyte [5, 9, 13–15]. Recognition of glucose or α-hydroxyl acids in aqueous solution have been achieved with fluorescent boronic acid probes [16–24].

We have been interested in study of boronic acid probes. For example, chiral recognition of tartaric acids has been observed with BINOL or anthracene based boronic acid probes [17–20]. For these PeT boronic acid probes (a-PeT, i.e. the fluorophore serves as the electron acceptor in the PeT process), usually the fluorescence emission is intensive at acidic pH, due to the protonation of the nitrogen atom and thus the inhibition of the PeT effect (quenching effect) [1–3]. However, the recognition of tartaric acid is not ideal at acidic pH, due to the strong background fluorescence and the minor fluorescence transduction upon analyte binding at acidic pH. We found that carbazole based boronic acid probe show the d-PeT effect, i.e. reverse PeT effect, with fluorophore as the electron donor and protonated amine/boronic acid group as the electron acceptor [21]. d-PeT probes show weak emission at acidic pH compared to that

at neutral or basic pH. This novel fluorescence transduction is beneficial for the fluorescent sensing of hydroxyl acids, for which the binding at acidic pH is much stronger than that at neutral pH.

Herein we extend the d-PeT fluorophore from carbazole to phenothiazine, which is a strong electron donating fluorophore, to construct d-PeT fluorescence boronic acid probes. As the phenothiazine itself shows very weak emission, therefore we prepared the ethynylated derivatives by Sonogashira coupling to extend the π-conjugation system and thus to enhance the fluorescence emission. We tuned the electronic feature of the phenothiazine by introducing electron donating and withdrawing groups and found out that the ICT effect imparts drastic influence on the emission profile (such as sensitivity toward solvent polarity) and the PeT effect of the probes. We found the substitution profile imparts significant effect on the photophysical properties of the probes. The regioisomer of probe **5**, i.e. probe **1**, with similar π-conjugation fragment but different substitution profile, shows the a-PeT effect. Furthermore, during the synthesis of the probes, we found a side reaction, i.e. the C≡C triple bonds of the modified fluorophore (with electron-deficient appendents) can be reduced by $NaBH_4$ to C=C double bonds, whereas $NaBH_3(CN)$ does not reduce the C≡C bonds. The feasible derivatization of phenothiazine makes these boronic acids interesting platform for further improvement of the selectivity and the photophysical properties. These findings may inspire new impetus in the area of fluorescent boronic acid probes and study of the photophysical properties of fluorescent probes.

Experimental

Materials and General Methods

All the chemicals are analytical pure and were used as received. NMR spectra were taken on a 400 MHz Varian Unity Inova spectrophotometer. Mass spectra were recorded with Q-TOF Micro MS spectrometer. UV–vis spectra were taken on a HP8453 UV-visible spectrophotometer. Fluorescence spectra were recorded on a JASCO FP-6500 or a Sanco 970 CRT spectrofluorometer. Fluorescence quantum yields were measured with quinine sulfate as the standard (Φ=0.54 in 0.05 M H_2SO_4). Fluorescence lifetimes were measured on a Horiba Jobin Yvon Fluoro Max-4 (TCSPC) instrument.

10-butyl-10H-phenothiazine (6)

To a stirred solution of phenothiazine (9.95 g, 50 mmol), CTAB (0.5 g) and NaOH (3.0 g) in 50 mL of acetone was added n-C_4H_9Br (8.2 g, 60 mmol). The mixture was refluxed for 6 h. Then the solvent was removed. The residue was extracted with dichloromethane (DCM) and washed with water. The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 1/3, V/V). 6.64 g of light green liquid was obtained, yield: 52.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.10–7.14 (m, 4 H), 6.86 (t, 2 H, *J*=7.2 Hz), 6.83 (d, 2 H, *J*=8.4 Hz), 3.80 (t, 2 H, *J*=7.2 Hz), 1.73–1.80 (m, 2 H), 1.38–1.48 (m, 2 H), 0.90 (t, 3 H, *J*=7.8 Hz).

10-butyl-10H-phenothiazine-3-carbaldehyde (7)

To stirred DMF (1.48 mL, 0.02 mol) was added dropwise $POCl_3$ (1.84 mL, 0.01 mol) of at 0 °C; when the solution turned to white solid, a solution of **6** (5.1 g, 0.02 mol) in chloroform (12 mL) was added. After the solid was dissolved, the mixture was refluxed for 16 h and then poured into ice water. The pH value of the mixture was brought to 8.0 by addition of sodium bicarbonate. After 30 min of stirring, the mixture was extracted with DCM. The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed and the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 2/1, V/V). 4.61 g of yellow oil was obtained, yield: 81.3%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 9.71 (s, 1 H), 7.51 (d, 1 H, *J*=8.0 Hz), 7.46 (s, 1 H), 7.08 (t, 1 H, *J*=8.0 Hz), 7.01 (d, 1 H, *J*=8.0 Hz), 6.87 (t, 1 H, *J*=8.0 Hz), 6.79 (d, 1 H, *J*=8.0 Hz), 6.76 (d, 1 H, *J*=8.0 Hz), 3.75 (t, 2 H, *J*=8.0 Hz), 1.67–1.74 (m, 2 H), 1.36–1.42 (m, 2 H), 0.87 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{17}H_{17}NOS([M + H]^+)$ 284.1, found 284.0.

7-bromo-10-butyl-10H-phenothiazine-3-carbaldehyde (8)

To a solution of **7** (4.0 g, 14.1 mmol) in 15 mL of glacial acetic acid was added dropwise a solution of bromine (0.72 mL, 14.1 mmol) in 5 mL of glacial acetic acid. The brown mixture was stirred at room temperature for 2 d. After addition of 150 mL of water and extracted with diethyl ether (3×100 mL), the organic layer was dried over $MgSO_4$. The solvents were removed and the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 3/1, V/V). 3.98 g of yellow solid was obtained, yield: 78.0%. Mp: 78–79 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) *δ* 9.75 (s, 1 H), 7.57 (d, 1 H, *J*=8.4 Hz), 7.48 (s, 1 H), 7.17 (d, 1 H, *J*=8.6 Hz), 7.11 (s, 1 H), 6.83 (d, 1 H, *J*=8.4 Hz), 6.64 (d, 1 H, *J*=8.6 Hz), 3.77 (t, 2 H, *J*=7.2 Hz), 1.69–1.76 (m, 2 H), 1.37–1.47 (m, 2 H), 0.90 (t, 3 H, *J*=7.6 Hz). APCI-mass: *m/z* calcd for $C_{17}H_{17}BrNOS$ ($[M + H]^+$) 362.0, found 362.0.

10-butyl-7-(2-phenylethynyl)-10H-phenothiazine-3-carbaldehyde (9)

To a degassed solution of **8** (500.0 mg, 1.38 mmol) in dry Et_3N (10 mL) and THF (3 mL) were successively added Pd $(OAc)_2$ (15.5 mg, 0.07 mmol), PPh_3 (14.5 mg, 0.06 mmol), CuI (10.5 mg, 0.06 mmol) and phenylacetylene (211.0 mg, 2.07 mmol). The reaction mixture was refluxed under nitrogen for 6 h. After removal of the solvent, the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 3/1, V/V). 270 mg of yellow solid was obtained, yield: 52.0%. Mp: 31–32 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 9.79 (s, 1 H), 7.62 (d, 1 H, *J*=8.4 Hz), 7.56 (s, 1 H), 7.49–7.51 (m, 2 H), 7.21–7.34 (m, 5 H), 6.88 (d, 1 H, *J*=8.4 Hz), 6.80 (d, 1 H, *J*=8.4 Hz), 3.86 (t, 2 H, *J*=7.2 Hz), 1.74–1.82 (m, 2 H), 1.42–1.51 (m, 2 H), 0.94 (t, 3 H, *J*=7.2 Hz). APCI-mass: m/z calcd for $C_{25}H_{21}NOS$ ($[M + H]^+$) 384.1, found 384.0.

10-butyl-7-{2-[4-(dimethylamino)phenyl]ethynyl}-10 H-phenothiazine-3-carbaldehyde (10)

10 was synthesized with similar procedure to that of **9**. Yield: 45.0%, yellow solid. Mp: 91–92 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 9.80 (s, 1 H), 7.63 (d, 1 H, *J*= 8.4 Hz), 7.58 (s, 1 H), 7.38 (d, 1 H, *J*=8.4 Hz), 7.27 (d, 1 H, *J*=9.2 Hz), 7.24 (s, 1 H), 6.89 (d, 1 H, *J*=8.4 Hz), 6.80 (d, 1 H, *J*=8.4 Hz), 6.67 (d, 1 H, *J*=8.4 Hz), 3.88 (t, 2 H, *J*= 7.6 Hz), 3.0 (s, 6 H), 1.77–1.84 (m, 2 H), 1.44–1.53 (m, 2 H), 0.95 (t, 3 H, *J*=7.6 Hz). APCI-mass: m/z calcd for $C_{27}H_{26}N_2OS$ ($[M + H]^+$) 427.2, found 427.1.

4-[2-(10-butyl-3-formyl-10H-phenothiazin-7-yl)ethynyl] benzonitrile (11)

11 was synthesized with similar procedure to that of **9**. Yellow solid was obtained, yield: 23.0%. Mp: 141–142 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 9.8 (s, 1 H), 7.55–7.65 (m, 6 H), 7.31 (d, 1 H, *J*=8.4 Hz), 7.26 (s, 1 H), 6.91 (d, 1 H, *J*=8.4 Hz), 3.88 (t, 2 H, *J*=8.0 Hz), 1.76–1.84 (m, 2 H), 1.43–1.52 (m, 2 H), 0.95 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{26}H_{20}N_2OS$ ($[M + H]^+$) 409.5, found 409.0.

N-{[10-butyl-3-(2-phenylethynyl)-10H-phenothiazin-7-yl] methyl}phenylmethanamine (12)

To a degassed solution of **9** (110.0 mg, 0.287 mmol) in dry THF (1.0 mL) and ethanol (5.0 mL) was added benzylamine (40.0 mg, 0.373 mmol). The reaction mixture was refluxed under nitrogen for 8 h. After the solution was cooled to room temperature, $NaBH_4$ (54.0 mg, 1.44 mmol) was added in several portions to the stirred solution and stirring was continued for 0.5 h. The resulting mixture was evaporated to dryness. The residue was extracted with water and dichloromethane. The organic layer was separated and dried over $MgSO_4$. After removing the solvent, the residual was purified by column chromatography (silica gel, DCM/MeOH, 40/1, V/V). 90.0 mg of yellow oil was obtained, yield: 66.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) *δ* 7.48 (d, 1 H, *J*=8.0 Hz), 7.25–7.34 (m, 10 H), 7.10 (s, 2 H), 6.76–6.87 (m, 2 H), 3.80 (t, 2 H, *J*=8.0 Hz), 3.79 (s, 2 H), 3.69 (s, 2 H), 1.73–1.8 (m, 2 H), 1.4–1.49 (m, 2 H), 0.92 (t, 3 H, *J*=7.2 Hz). TOF MS: ($C_{32}H_{30}N_2S$ + H^+) calcd 475.2, found 475.3.

Compound (13)

13 was synthesized with similar procedure to that of **12**. Yellow oil was obtained, yield: 73.4%, ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.24–7.38 (m, 10 H), 7.09 (s, 1 H), 6.78 (d, 1 H, *J*=8.0 Hz), 6.74 (d, 1 H, *J*=8.0 Hz), 6.83 (d, 1 H, *J*= 8.8 Hz), 3.79–3.83 (m, 4 H), 2.98 (s, 6 H), 1.73–1.80 (m, 2 H), 1.40–1.49 (m, 2 H), 0.92 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{34}H_{35}N_3S$ ($[M + H]^+$) 518.3, found 518.3.

Compound (14)

14 was synthesized with similar procedure to that of **12**. Yellow oil was obtained, yield: 76.6%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.59 (d, 2 H, *J*=8.0 Hz), 7.53 (d, 2 H, *J*= 8.0 Hz), 7.26 (s, 1 H), 7.23–7.33 (m, 7 H), 7.10 (d, 2 H, *J*= 12.0 Hz), 3.82 (t, 2 H, *J*=8.0 Hz), 3.79 (s, 2 H), 3.70 (s, 2 H), 1.74–1.81 (m, 2 H), 1.41–1.50 (m, 2 H), 0.92 (t, 3 H, *J*=8.0 Hz). TOF MS: ($C_{33}H_{29}N_3S + H^+$) calcd 500.2, found 500.0.

Probe (1)

12 (90.0 mg, 0.19 mmol), 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane (58.0 mg, 0.23 mmol) and K_2CO_3 (95.0 g, 0.69 mmol) were mixed in dry MeCN (5 mL), then the mixture was refluxed for 10 h under N_2. The reaction mixture was cooled to room temperature and diluted HCl was added, then the mixture was stirred for further 1 h. The solvent was removed under vacuum, and DCM was added to take up the residue. The organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified by column chromatography (silica gel, DCM/MeOH, 30/1, V/V). 40 mg of light yellow powder was obtained, yield: 34.5%. Mp: 110–111 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.79 (s, 1 H), 7.48 (d, 2 H, *J*=8.0 Hz), 7.27–7.34 (m, 14 H), 6.97 (s, 1 H), 6.80 (m, 2 H), 3.82 (t, 2 H, *J*= 7.2 Hz), 3.70 (s, 2 H), 3.59 (s, 2 H), 3.38 (s, 2 H), 1.75–

1.82 (m, 2 H), 1.42–1.51 (m, 2 H), 0.94 (t, 3 H, *J*=7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 145.1, 144.1, 141.6, 136.7, 136.0, 131.5, 131.3, 130.8, 130.3, 130.2, 130.1, 129.9, 129.0 128.9, 128.5, 128.3, 128.0, 127.7, 127.5, 127.4, 124.4, 123.9, 123.5, 117.0, 115.4, 114.5, 89.2, 88.9, 77.4, 77.1, 76.7, 61.3, 57.3, 56.4, 47.3, 28.9, 20.2, 13.8. ESI-HRMS ($C_{39}H_{37}BN_2O_2$ + H^+) calcd 609.2747, found 609.2745.

Probe (2)

13 (72.0 mg, 0.14 mmol), 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane (43.0 mg, 0.17 mmol) and K_2CO_3 (69.0 g, 0.5 mmol) were mixed in dry MeCN (5 mL), the mixture was refluxed for 10 h under N_2. The reaction mixture was cooled to room temperature and diluted HCl was added, then the mixture was stirred for further 1 h. The solvent was removed under vacuum, and the residue was taken up with DCM. The organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified with column chromatography (silica gel, DCM/MeOH, 30/1, V/V). 32 mg of yellow powder was obtained, yield: 32.8%. Mp: 137–138 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.73 (s, 1 H), 7.15–7.29 (m, 11 H), 7.08 (s, 1 H), 6.94 (d, 1 H, *J*=7.6 Hz), 6.88 (s, 1 H), 6.68 (d, 2 H, *J*=8.4 Hz), 6.56 (d, 1 H, *J*=8.4 Hz), 3.43–3.74 (m, 4 H), 2.89 (s, 6 H), 1.65–1.72 (m, 2 H), 1.32–1.41 (m, 2 H), 0.84 (t, 3 H, *J*=7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 150.1, 144.5, 144.4, 141.8, 136.8, 136.1, 132.7, 131.4, 130.5, 130.4, 130.0, 129.1, 128.7, 127.8, 127.8.6, 124.4, 124.1, 118.1, 115.5, 115.1, 112.0, 110.4, 90.5, 86.8, 61.5, 57.4, 56.5, 47.4, 40.4, 29.0, 20.3, 14.0. ESI-HRMS ($C_{41}H_{42}BN_3O_2S$ + H^+) calcd 652.3169, found 652.3169.

Probe (3)

14 (170.0 mg, 0.34 mmol), 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane (113.0 mg, 0.44 mmol) and K_2CO_3 (182.0 mg, 1.32 mmol) were mixed in dry MeCN (8.0 mL), and the mixture was refluxed for 10 h under N_2. The reaction mixture was cooled to room temperature and diluted HCl was added, then the mixture was stirred for further 1 h. The solvent was removed under vacuum, and DCM was added. The organic layer was washed with water and dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified with column chromatography (silica gel, DCM/MeOH, 30/1, V/V). 65 mg of yellow powder was obtained, yield: 30.2%. Mp: 127–128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.81 (d, 2 H, *J*=8.0 Hz), 7.60 (d, 2 H, *J*=8.0 Hz), 7.55 (d, 2 H, *J*=8.0 Hz), 7.27–7.35 (m, 9 H), 7.14 (d, 2 H, *J*=8.0 Hz), 7.03 (d, 2 H, *J*=8.0 Hz), 6.97 (s, 1 H), 6,80 (d, 2 H, *J*=8.0 Hz), 3.82 (t, 2 H, *J*=8.0 Hz), 3.69 (s, 2 H), 3.58 (s, 2 H), 3.5 (s, 2 H), 1.74–1.82 (m, 2 H), 1.42–1.51 (m, 2 H), 0.93 (t, 3 H, *J*=8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 145.9, 143.7, 141.5, 136.7, 135.9, 132.0, 131.8, 131.3, 131.1, 130.4, 129.9, 129.1, 128.9, 128.5, 128.4, 127.7, 127.5, 124.6, 123.7, 118.6, 115.8, 115.5 115.0, 111.1, 93.6, 87.8, 77.3, 77.0, 76.7, 61.4, 57.4, 56.4, 53.4, 47.4, 28.8, 20.1, 13.8. ESI-HRMS ($C_{40}H_{36}BN_3O_2S$ + H^+) calcd 634.2700, found 634.2722.

7-bromo-10-butyl-5-oxo-5,10-dihydro-phenothiazine-3-carbaldehyde (15)

To a stirred solution of (1.3 g, 3.6 mmol) of **8** in 18 mL of DCM, a solution of meta-chloroperoxybenzoic acid (3.6 mmol, 85%) in anhydrous DCM (15 mL) was added dropwise over 30 min at 0–5 °C. The reaction was completed within 2–4 h (monitored by TLC). The reaction mixture was washed with 10% potassium hydroxide solution (2×15 mL), 5% HCl solution (10 mL), and then saturated $NaHCO_3$ solution (10 mL) and the organic layer was dried over Na_2SO_4. After the solvent was removed, the residue was purified by chromatography (silica gel, DCM/MeOH, 50/1, V/V). 1.23 g of white solid was obtained, yield: 90.0%. Mp: 171–172 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 10.0 (s, 1 H), 8.42 (s, 1 H), 8.13 (d, 1 H, *J*=8.4 Hz), 8.08 (s, 1 H), 7.73 (d, 1 H, *J*=8.0 Hz), 7.51 (d, 1 H, *J*=8.8 Hz), 7.36 (d, 1 H, *J*=8.8 Hz), 4.24 (t, 2 H, *J*=6.4 Hz), 1.93–1.98 (m, 2 H), 1.52–1.62 (m, 2 H), 1.04 (t, 3 H, *J*=7.6 Hz). APCI-mass: m/z calcd for $C_{17}H_{16}BrNO_2S$ ($[M + H]^+$) 379.3, found 379.0.

4-[2-(10-butyl-3-formyl-5-oxo-5,10-dihydro-pheno-thiazin-7-yl)ethynyl]benzonitrile (16)

To a degassed solution of **15** (757.0 mg, 2.0 mmol) in dry Et_3N (15 mL) and THF (8 mL) were successively added $Pd(PPh_3)_4$ (69.0 mg, 0.06 mmol), PPh_3 (21.0 mg, 0.08 mmol), CuI (15.0 mg 0.08 mmol) and 4-ethynylbenzonitrile (254.0 mg 2.0 mmol). The reaction mixture was refluxed under nitrogen for 6 h. The resulting mixture was evaporated to dryness. The residue was extracted with taken up with DCM and washed with water. The organic layer was dried over $MgSO_4$. The solvent was removed and the residue was purified by chromatography with silica gel (acetone/petroleum ether, 1/1, V/V). 297 mg of yellow solid was obtained, yield: 35.0%. Mp: 201–202 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 10.02 (s, 1 H), 8.45 (s, 1 H), 8.15 (d, 2 H, *J*=8.0 Hz), 7.79 (d, 1 H, *J*=8.0 Hz), 7.65 (d, 2 H, *J*=8.0 Hz), 7.61 (d, 2 H, *J*=8.0 Hz), 7.54 (d, 2 H, *J*=8.0 Hz), 7.47 (d,

2 H, *J*=12.0 Hz), 4.28 (t, 2 H, *J*=8.0 Hz), 1.94–2.02 (m, 2 H), 1.55–1.65 (m, 2 H), 1.07 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{26}H_{20}N_2O_2S$ ($[M + H]^+$) 425.5, found 425.0.

4-[2-[3-[(benzylamino)methyl]-10-butyl-5-oxo-5, 10-dihydro-phenothia-zin-7-yl]ethynyl] benzonitrile (17)

To a degassed solution of **16** (8.0 mg, 0.66 mmol) in dry THF (2 mL) and ethanol (8 mL) was added benzylamine (92.0 mg, 0.86 mmol). The reaction mixture was refluxed under nitrogen for 8 h. After the solution was cooled to room temperature, (207 mg, 3.3 mmol) of $NaBH_4$ was added in portions to the stirred solution and the stirring was continued for 0.5 h. The resulting mixture was evaporated to dryness. The residue was taken up with DCM and washed with water. The organic layer was dried over $MgSO_4$. After removing the solvent, the residual was purified by column chromatography (silica gel, DCM/MeOH, 20/1, V/V). 130.0 mg of yellow solid was obtained, yield: 38.0%. Mp: 76–77 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 8.12 (s, 1 H), 7.94 (s, 1 H), 7.70 (d, 2 H, *J*=8.0 Hz), 7.60–7.66 (m, 4 H), 7.33–7.42 (m, 7 H), 4.20 (t, 2 H, *J*=8.0 Hz), 3.90 (s, 1 H), 3.85 (s, 1 H), 1.91–1.98 (m, 2 H), 1.53–1.62 (m, 2 H), 1.05 (t, 3 H, *J*=8.0 Hz). TOF MS: calcd for $C_{26}H_{20}N_2O_2S$ ($[M + H]^+$) 516.2, found 516.4.

Probe (4)

17 (100.0 mg, 0.19 mmol), 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane (63.0 mg, 0.24 mmol) and K_2CO_3 (100.0 g, 0.72 mmol) were mixed in dry THF (1 mL) and MeCN (5 mL) and refluxed for 10 h under N_2. The reaction mixture was cooled to room temperature and diluted HCl was added, then the mixture was stirred for further 1 h. The solvent was removed under vacuum, and DCM was added to take up the residue. The organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified by column chromatography (silica gel, DCM/MeOH, 20/1, V/V). 38.0 mg of yellow powder was obtained, yield: 30.0%. Mp: 151–152 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 8.14 (s, 1 H), 7.77–7.82 (m, 3 H), 7.62–7.68 (m, 4 H), 7.57 (d, 1 H, *J*=8.0 Hz), 7.42 (d, 2 H, *J*=8.0 Hz), 7.28–7.37 (m, 7 H), 7.18 (d, 2 H, *J*=8.0 Hz), 4.23 (t, 2 H, *J*=8.0 Hz), 3.63–3.77 (m, 6 H), 1.92–1.99 (m, 2 H), 1.55–1.64 (m, 2 H), 1.07 (t, 3 H, *J*=8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 141.1, 138.2, 137.2, 136.0, 135.8, 135.4, 134.9, 132.9, 132.1, 132.0, 131.2, 130.6, 130.1, 129.8, 128.6, 128.1, 127.8, 127.5, 123.4, 123.0, 118.5, 116.3, 116.1, 115.6, 111.4, 92.3, 88.6, 61.4, 57.4, 55.9, 49.6, 49.4, 49.1, 48.9, 48.7, 48.1, 28.4, 20.0, 13.7. ESI-HRMS ($C_{40}H_{36}BN_3O_3 + H^+$) calcd 650.2649, found 650.2629.

3-bromo-10-butyl-10H-phenothiazine (18)

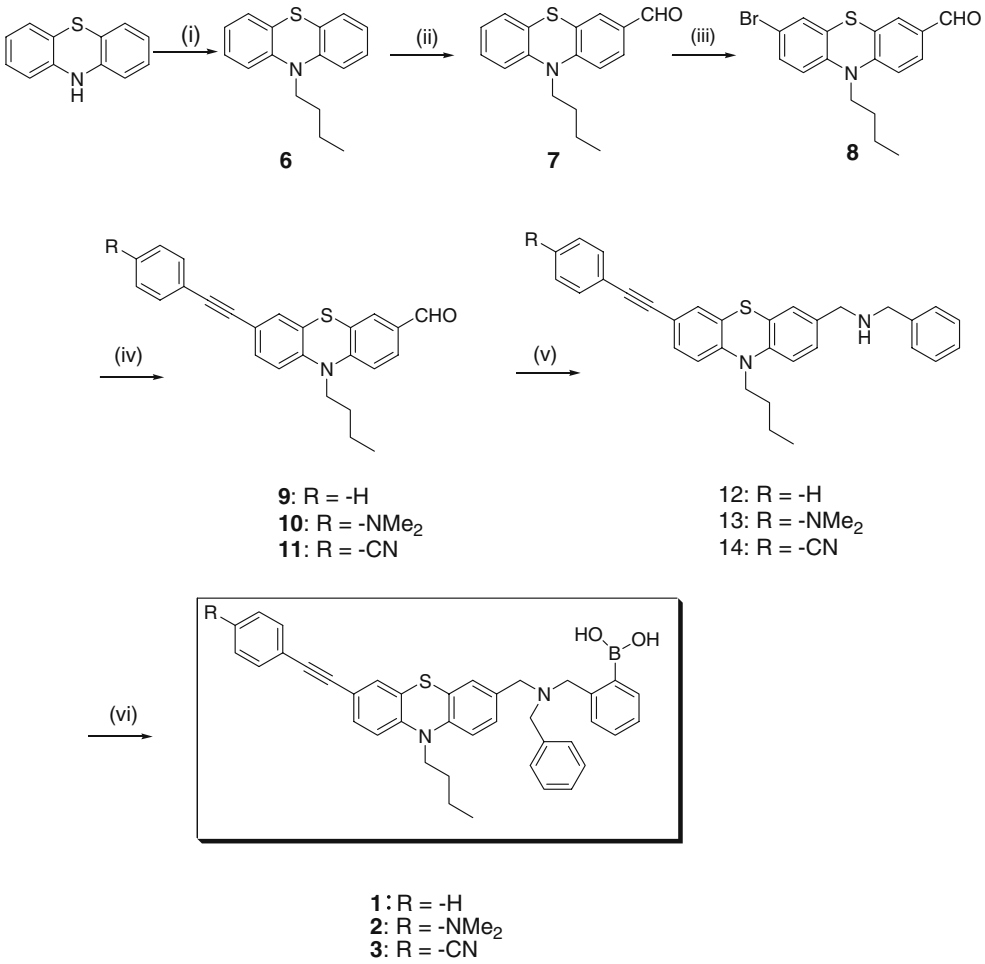
To a solution of **6** (1.4 g, 5.48 mmol) in chloroform (10 mL) was added a solution of NaOH (0.66 g, 6.44 mmol) in glacial acetic acid (40 mL), then to this solution was added dropwise a solution of bromine (0.28 mL, 5.48 mmol) in glacial acetic acid (6 mL) at 0 °C. The mixture was stirred at 0–5 °C for 1 h. The solvents were removed, after addition of 50 mL of water and 100 mL of dichloro-methane, the organic layer was dried with $MgSO_4$. The solvent was removed and the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 1/3, V/V). 1.28 g of light yellow liquid was obtained, yield: 70.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.17 (d, 2 H, *J*=8.0 Hz), 7.06–7.13 (m, 2 H), 6.86 (t, 1 H, *J*=8.0 Hz), 6.80 (d, 1 H, *J*=8.0 Hz), 6.60 (t, 1 H, *J*=8.0 Hz), 3.73 (t, 2 H, *J*=8.0 Hz), 1.66–1.75 (m, 2 H), 1.35–1.45 (m, 2 H), 0.88 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{16}H_{16}BrNS$ ($[M + H]^+$) 335.3, found 335.1.

10-butyl-3-ethynyl-10H-phenothiazine (19)

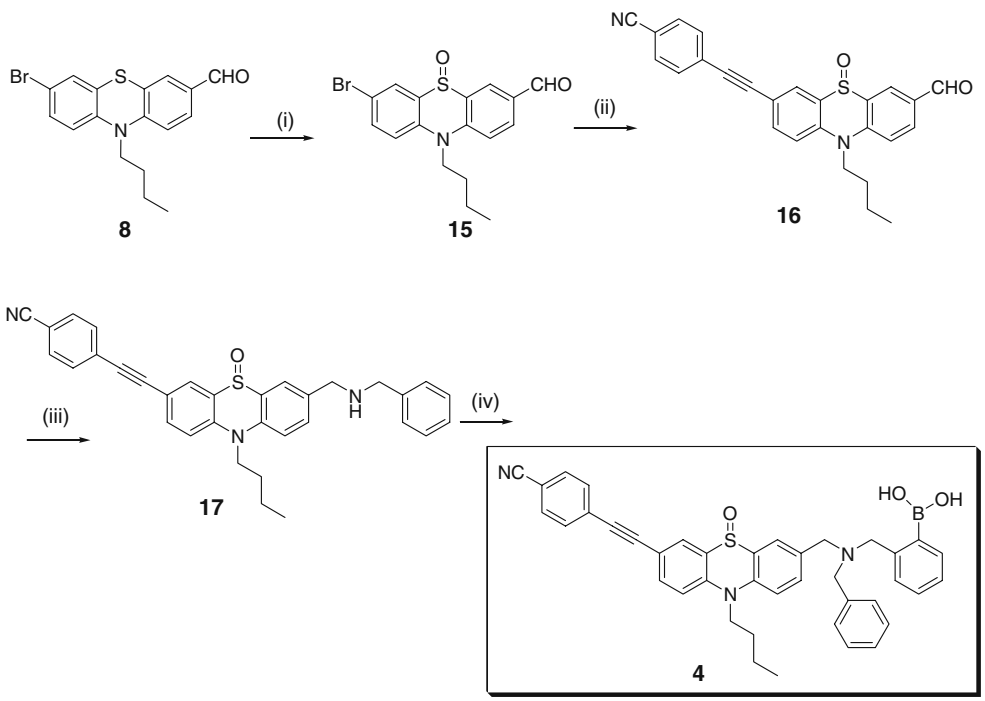
To a degassed solution of **18** (1.48 g, 4.43 mmol) in dry Et_3N (8 mL) and THF (5 mL) were successively added Pd $(PPh_3)_2Cl_2$ (124.0 mg, 0.17 mmol), PPh_3 (46.5 mg, 0.17 mmol), and CuI (33.7 mg, 0.17 mmol) and ethynyl-trimethylsilane (435.0 mg, 4.43 mmol). The reaction mixture was refluxed under nitrogen for 6 h. Then K_2CO_3 (1.65 g, 12 mmol) and methanol (5 mL) were added and the solution was stirred for 1 h at room temperature. The solvents were removed. The residue was taken up with DCM and washed with water. The organic layer was dried over $MgSO_4$. After the solvent was removed, the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 1/6, V/V). 1.04 g of yellow liquid was obtained, yield: 84.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.23 (m, 2 H), 7.09–7.16 (m, 2 H,), 6.90 (t, 1 H, *J*= 8.0 Hz), 6.84 (d, 1 H, *J*=8.0 Hz), 6.75 (d, 1 H, *J*=8.4 Hz), 3.81 (t, 2 H, *J*=7.2 Hz), 3.03 (s, 1 H), 1.73–1.80 (m, 2 H), 1.40–1.49 (m, 2 H), 0.92 (t, 3 H, *J*=8.4 Hz). APCI-mass: m/z calcd for $C_{18}H_{17}NS$($[M + H]^+$) 280.1, found 280.0.

3-[2-(10-butyl-10H-phenothiazin-7-yl)ethynyl] benzaldehyde (20)

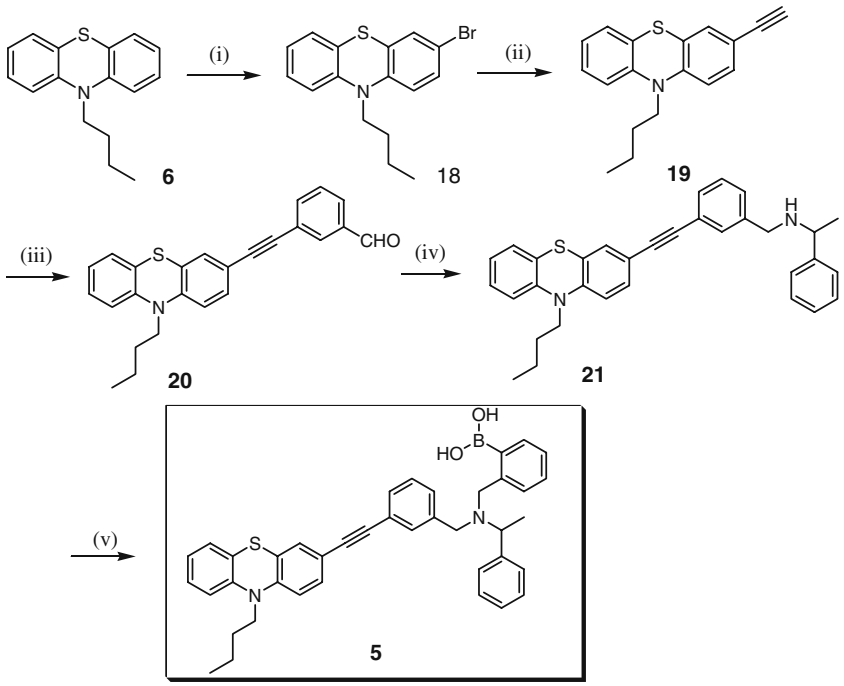
To a degassed solution of **19** (0.50 g, 1.79 mmol) in dry Et_3N (5 mL) and THF (3 mL) were successively added Pd $(PPh_3)_4$ (62.0 mg, 0.053 mmol), CuI (10.2 mg, 0.053 mmol) and 3-bromobenzaldehyde (397 mg, 4.43 mmol). The reaction mixture was refluxed under nitrogen for 6 h. The solvents were removed. The residue was taken up with DCM and washed with water. The organic layer was dried



Scheme 1 Synthesis of the 6-Ethynylated Phenothiazine Boronic acid Probes **1**–**3**. i) Phenothiazine, CTAB, NaOH, n-C_4H_9Br, acetone, reflux, 6 h, yield 60%; ii) $POCl_3$, DMF, $CHCl_3$, reflux, 16 h, 81%; iii) bromine, glacial acetic acid, room temperature for 24 h; iv) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, PPh_3, 4-ethynyl-N,N-dimethylaniline/ phenylacetylene/4-ethynylbenzonitrile, argon atmosphere, reflux, 6 h, 23–52%; v) benzylamine, ethanol, THF, reflux, 8 h, then $NaBH_4$ for **9**/**10**, $NaBH_3(CN)$ for **11**, 0 °C, 30 min; vi) acetonitrile, K_2CO_3, 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane, reflux, 10 h, 30–35%



Scheme 2 Synthesis of the sulfur-oxidized 6-ethynylated Phenothiazine Boronic acid Probes **4**. i) meta-chloroperoxybenzoic acid, dry DCM; ii) $Pd(PPh_3)_4$, CuI, Et_3N, 4-ethynylbenzonitrile, argon atmosphere, reflux, 6 h, 35%; iii) benzylamine, ethanol, THF, reflux, 8 h, then $NaBH_3(CN)$, 0 °C, 30 min, 38%; iv) acetonitrile, K_2CO_3, 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane, reflux, 10 h, 30%



Scheme 3 Synthesis of the 3-ethynylated Phenothiazine Boronic acid Probe **5.** i) bromine, glacial acetic acid, 0 °C, 1 h, 70%; ii) $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, PPh_3, ethynyltrimethylsilane argon atmosphere, reflux, 6 h, then K_2CO_3, MeOH, 1 h, 84%; iii) $Pd(PPh_3)_4$ CuI, Et_3N, 3-bromobenzaldehyde, nitrogen atmosphere, reflux, 6 h, 64%; iv) 8, benzylamine, ethanol, THF, reflux, 8 h, then $NaBH_3(CN)$, 0 °C, 30 min, v) acetonitrile, K_2CO_3, 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane, reflux, 10 h, 25%

over $MgSO_4$. After the solvents were removed, the residue was purified by column chromatography (silica gel, DCM/petroleum ether, 1/1, V/V). 0.44 g of yellow oil was obtained, yield: 64.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 10.0 (s, 1 H), 7.98 (s, 1 H), 7.79 (d, 1 H, *J*=8.0 Hz), 7.71 (d, 1 H, *J*=7.6 Hz), 7.47 (t, 1 H, *J*=7.6 Hz), 7.28 (t, 2 H, *J*=6.0 Hz), 7.10–7.17 (m, 2 H), 6.90 (t, 1 H, *J*=7.6 Hz), 6.84 (d, 1 H, *J*=8.0 Hz), 6.79 (d, 1 H, *J*=8.4 Hz), 3.83 (t, 2 H, *J*=7.2 Hz), 1.75–1.82 (m, 2 H), 1.41–1.57 (m, 2 H), 0.92 (t, 3 H, *J*=8.0 Hz). APCI-mass: m/z calcd for $C_{25}H_{21}NOS$ ($[M + H]^+$) 384.1, found 384.0.

N-{[3-[2-(10-butyl-10H-phenothiazin-7-yl)ethynyl] phenyl]methyl}-1-phenylethanamine (21)

To a solution of **20** (440.0 mg, 1.15 mmol) in dry THF (1 mL) and ethanol (5 mL) was added 1-phenylethanamine (209.0 mg, 1.72 mmol). The reaction mixture was refluxed under nitrogen for 8 h. After the solution was cooled to r.t., $NaBH_3(CN)$ (216.0 mg, 3.45 mmol) was added in several portions to the stirred solution and the stirring was continued for 0.5 h. The resulting mixture was evaporated to dryness. The residue was taken up with DCM and washed with water. The organic layer was dried over $MgSO_4$. After removing the solvent the residual was purified by column chromatography (silica gel, DCM/MeOH, 30/1, V/V). 199.0 mg of yellow oil was obtained, yield: 36.0%. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.44 (d, 1 H, *J*=8.0 Hz), 7.21–7.38 (m, 10 H), 7.10–7.16 (m, 2 H), 6.89 (t, 1 H, *J*=8.0 Hz), 6.84 (d, 1 H, *J*=8.0 Hz), 6.77 (d, 1 H, *J*=8.0 Hz), 3.78–3.86 (m, 3 H), 3.56–3.67 (m, 2 H), 1.74–1.81 (m, 2 H), 1.42–1.50 (m, 2 H), 1.37 (d, 3 H, *J*=6.8 Hz), 0.92 (t, 3 H, *J*=7.2 Hz). TOF MS: ($C_{33}H_{31}N_2S + H^+$) calcd 489.2, found: 489.1.

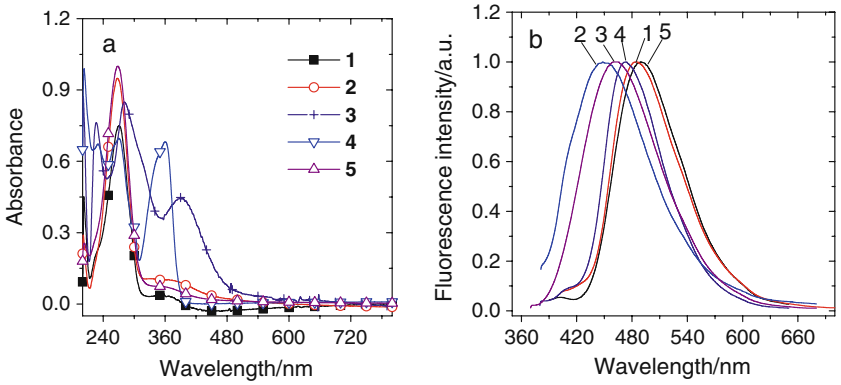


Fig. 1 UV–vis absorption spectra (**a**) and Fluorescence emission spectra (**b**) of probes **1**–**5** (1.0×10^{-5} mol dm^{-3}) in 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). pH 7.0, 25 °C

Table 1 Photophysical parameters of probes 1–5

	ε^a (M^{-1} cm^{-1})	λ_{abs} (nm)	λ_{em} (nm)	Stokes Shift (nm)	Φ^b (pH 4.0)	Φ^b (pH 7.0)	τ^c (ns)	$k_r{}^d$ (10^7 s^{-1})	$k_{nr}{}^e$ (10^7 s^{-1})
1	4.44×10^3	358	485	127	0.30	0.14	4.46	3.14	19.3
2	1.57×10^4	337	448	111	0.59	0.51	3.98	12.8	12.3
3	5.50×10^4	389	463	74	0.22	0.14	3.46	6.36	22.5
4	6.82×10^4	360	474	114	0.38	0.12	1.93	7.77	44.0
5	7.41×10^3	349	489	140	0.20	0.52	3.32	4.52	25.6

[a] In 5.0×10^{-2} mol dm^{-3} NaCl ionic buffer (52.1% methanol in water), pH 7.0; [b] Fluorescence quantum yields, with quinine sulfate as the standard (Φ= 0.54 in 0.05 M H_2SO_4); [c] Fluorescence lifetimes, with typical error of 0.01 ns. Concentrations of the probes are 3.0×10^{-5} mol dm^{-3}; [d] Radiative decay rate constants at pH 7.0, $k_r=\Phi/\tau$; [e] Non-radiative decay rate constants at pH 7.0, $k_{nr}=(1-\Phi)/\tau$

Probe (5)

21 (199.0 mg, 0.41 mmol), 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane (125.0 mg, 0.49 mmol) and K_2CO_3 (170.0 mg, 1.23 mmol) were mixed in dry MeCN (5 mL), refluxed for 10 h under N_2. The reaction mixture was cooled to room temperature and diluted HCl was added, then the mixture was stirred for further 1 h. The solvent was removed under vacuum, and DCM was added to take up the residue. The organic layer was washed with water and then dried over anhydrous $MgSO_4$. The solvent was removed under vacuum and the residue was purified by column chromatography (silica gel, DCM/MeOH, 40/1, V/V). 62.5 mg of light yellow powder was obtained, yield: 25.0%. Mp: 104–105 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, TMS) δ 7.79 (s, 1 H), 7.2–7.42 (m, 12 H), 7.1–7.17 (m, 14 H), 6.86–6.92 (m, 2 H), 6.80 (d, 1 H, *J*=8.0 Hz), 4.06 (d, 1 H, *J*=8.0 Hz), 3.85 (s, 2 H), 3.66 (s, 2 H), 3.32–3.41 (s, 2 H), 1.75–1.82 (m, 2 H), 1.57 (d, 3 H, *J*=8.0 Hz), 1.43–1.50 (m, 2 H), 0.92 (t, 3 H, *J*=8.0 Hz). ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 145.3, 144.6, 132.4, 131.2, 130.7, 130.2, 130.0, 129.9, 129.2, 129.0, 128.3, 128.2, 127.7, 127.4, 127.3, 127.2, 124.7, 124.1, 123.2, 122.6, 116.8, 115.5, 115.0, 89.0, 88.8, 58.2, 57.1, 53.4, 47.1, 41.8, 28.8, 20.0, 13.6. ESI-HRMS ($C_{39}H_{37}BN_2O_2S$ + MeOH-H_2O + H^+) calcd 623.2904, found 623.2895.

Results and Discussions

Synthesis Phenothiazine is a well-known electron donor and has been widely used in supramolecular photochemical

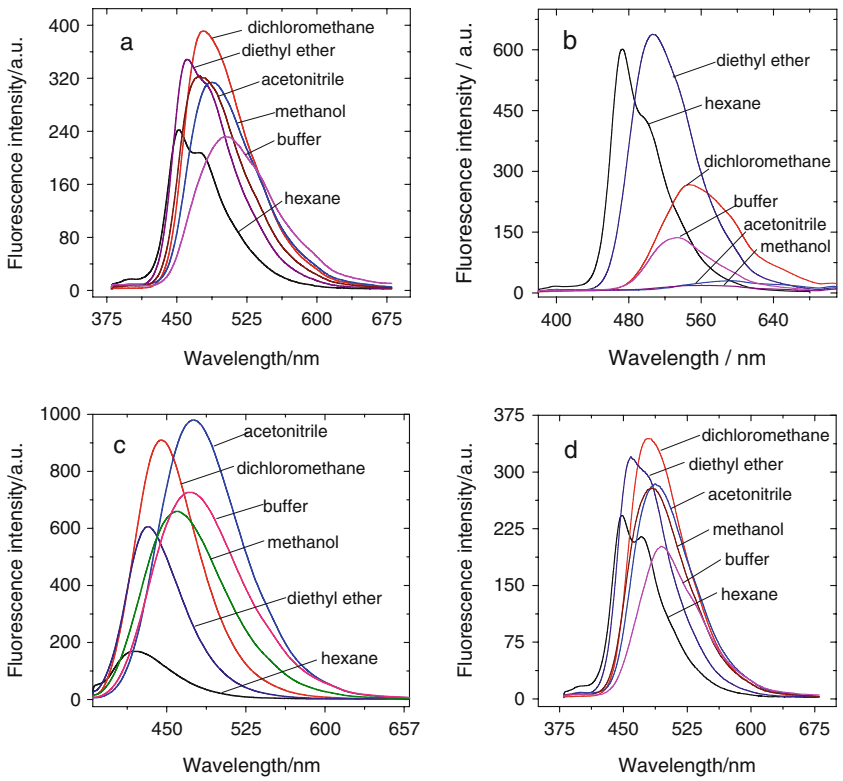


Fig. 2 Selected emission spectra of (**a**) **1** (**b**) **3** (**c**) **4** (**d**) **5** (1.0×10^{-6} mol dm^{-3}) in different solvents. λex=360 nm. 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). pH 7.0, 25 °C

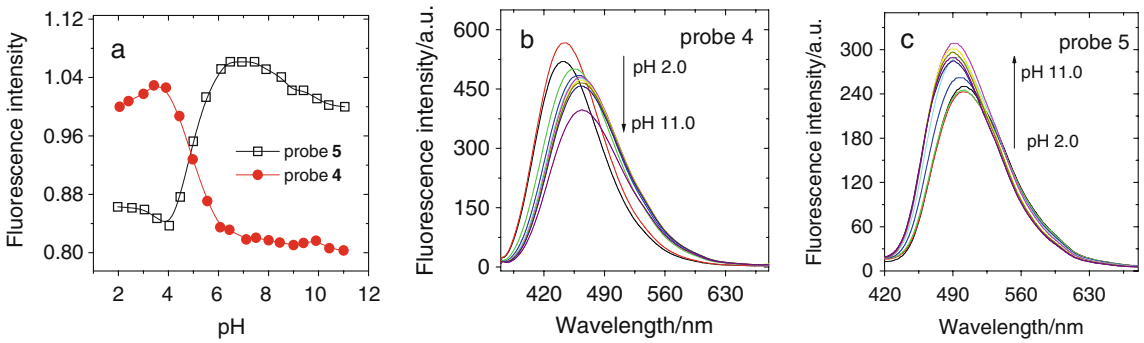


Fig. 3 **a** Normalized fluorescence intensity-pH profile of probes **4** and **5**, 1.0×10^{-6} mol dm^{-3} of probes in 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water); **b** Fluorescence spectra of probe **4** with variation of pH of the solution, λex=350 nm. **c** Fluorescence spectra of probe **5** with variation of pH of the solution, λex=360 nm. 25 °C

systems, in fluorescent dyes and electro optical materials and bioactive reagents [25–27]. However, very few examples are reported for fluorescent molecular probes. Herein we prepared new boronic acid probes with phenothiazine as the fluorophore, with the rational the d-PeT probes may be obtained.

The design rational of the boronic acid probes lies on the notion that the phenothiazine core is an electron donating fluorophore, therefore potential d-PeT effect is expected. In order to investigate the structure–property relationship of the probes, such as the effect of the electron donating or withdrawing ability of the substituents on the fluorescence transduction property, substituents such as NMe_2, CN, etc., were attached to the phenothiazine core. Phenothiazine was used as the starting material for synthesis (Scheme 1). First N-butylation was carried out with NaOH as the base. Then Vilsmeier reaction was used to induce a -CHO group to the 3-position of the N-butylphenothiazine. Monobromination was carried out to introduce bromo at the 6-position. Phenothiazine core does not show strong emission, thus π-conjugation extension is necessary to make the probe emissive. Sonogashira coupling was used to extend the π-conjugation of the fluorophore with introduce of substituted phenylethynylene groups. It should be pointed out that C≡C triplet bonds, instead of C=C double bonds, were used for extension of the π-conjugation because it is known that C=C may quench the fluorescence and the C≡C bonds, as good electron communication bridge, will not quench the fluorescence [28, 29]. Reductive amination was carried out to prepare the amine **12-14**, **17**, **21**. Interestingly, we found that the C≡C bond can be reduced to C=C double bond with $NaBH_4$ at ambient temperature, especially in the case of compound **11**. Therefore, a milder reagent of $NaBH_3(CN)$ was used for the reduction. Reaction of amine **12** and **14** with 2-(2-bromomethylphenyl)-1,3,2-dioxaborinane leads to the final boronic acid probe **1**–**3**. All the probes were obtained with satisfying yields.

In order to probe the effect of electron-donating ability of the fluorophore core on the photophysics of the probes, we designed probe 4 as a reference (Scheme 2) [30–34]. In probe **4**, the sulfur atom was oxidized to sulfoxide. We found drastic different photophysical properties for these two closely related probes (vie infra). The crucial step of synthesis of probe **4** is the selective oxidation of aldehyde **8** with m-chloroperoxybenzoic acid under mild condition. The synthesis procedures are similar to that of the probes **1**–**3** (Scheme 1).

Furthermore, in order to investigate the regioisomer of the probes **1**–**4**, a probe with the amine/boronic moiety attached on the phenyl end of the ethynylene bond, instead of the phenothiazine core, was designed (probe **5**, Scheme 3). The intermediate **20** was synthesized with acetylene **19** and 3-

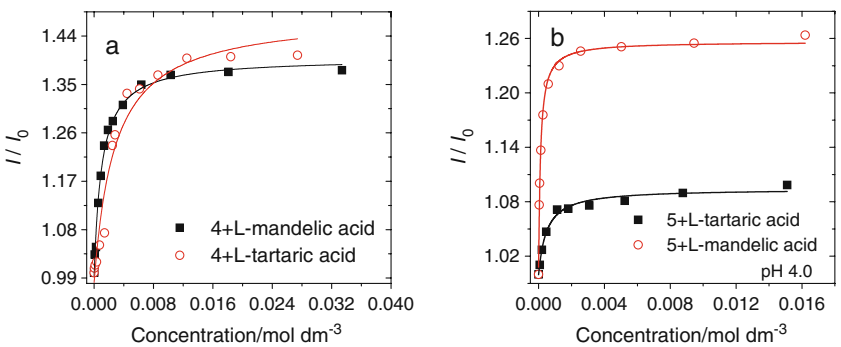


Fig. 4 Relative fluorescence intensity of probe **4** and **5** vs concentration of L-mandelic acid and L-tartaric acid. 1.0×10^{-7} mol dm^{-3} of probe **4** and **5** in 0.05 mol dm^{-3} NaCl ionic buffer (52.1% methanol in water). For probe **4**: λex=360 nm, λem=445 nm, pH=6.0. For probe **5**: λex=360 nm, λem=490 nm, pH=4.0. 25 °C

Table 2 Stability constants (M^{-1}) of probes 1–5 with tartaric acid and mandelic acid

Probes	L-tartaric acid	L-mandelic acid
1	$(1.64\pm0.32)\times10^2$ (pH 6.0)	$(2.67\pm0.55)\times10^2$ (pH 6.0)
2	$(3.64\pm0.47)\times10^2$ (pH 6.0)	$(1.42\pm0.22)\times10^3$ (pH 6.0)
3	$(0.63\pm0.06)\times10^2$ (pH 6.0)	$(1.62\pm0.56)\times10^2$ (pH 6.0)
4	$(9.77\pm0.79)\times10^2$ (pH 6.0)	$(3.62\pm0.87)\times10^2$ (pH 6.0)
5	$(2.08\pm0.40)\times10^3$ (pH 4.0)	$(8.67\pm0.89)\times10^3$ (pH 4.0)
	$(2.32\pm0.45)\times10^3$ (pH 7.0)	$(8.02\pm1.63)\times10^3$ (pH 7.0)

bromophenylaldehyde. The preparation of **21** was carried out by the reductive amination of **20** with $NaBH_3(CN)$.

UV–vis Absorption and Emission Spectra The excitation and emission spectra of the probes were investigated and the results of probes were presented in Fig. 1. The emission band is centered at 448–489 nm. A Stokes shift of 74–140 nm was observed. Large Stokes shift is beneficial for analytical purpose (inner filter effect can be eliminated) [1, 2]. Many known fluorophores are facing the limitation of small Stokes shifts, especially for the fused aryl fluorophores, such as BODIPY, fluorescein, xanthene dyes such as Rhodamine and carbazole, etc. we found electron-deficient fluorophore, such as **3**, gives the emission at blue-shifted region.

We found that the probes show moderate fluorescence quantum yields. Furthermore, the arrangement of the boronic acid group in the probes imparts significant effect on the photophysical properties of the probes. For example, probe **1** and **5** share similar π-conjugation framework, however, the fluorescence quantum yields are different, as well as the PeT effect. Probe **1** shows the normal a-PeT effect, whereas **5** shows the d-PeT effect.

The photophysical properties of the **1–5** were summarized in Table 1. Probe **3** shows a strong absorption band at 410 nm, which is due to the ICT effect (phenothiazine as electron donor and cyano groups as the electron acceptor) [1, 2, 35]. The sulfone derivative **4** shows a blue-shifted absorption at 360 nm, indicating the ICT effect is diminished. The absorption of **2** at blue-shifted wavelength indicates a weak ICT effect. These findings may prove useful for future design of phenothiazine-based fluorescent probes with red-shifted absorption at red-shifted wavelength range.

Solvent Polarity Dependence of the Emission of the Probes The ICT effect of the probes **1–5** was different, thus different solvent polarity sensitivities were expected for these probes. We investigated the emission of the ethynylated phenothiazine fluorophore in solvents with different polarity (Fig. 2). We found that the emission of probe **1** is not sensitive to the polarity of the solvents. For example, the probe gives similar emission wavelength and intensity in hexane and methanol. This result indicates that the ICT feature of the molecule is not significant [1]. Similar profile was observed for the regioisomer of probe **1**, i.e. probe **5**. Probe **2** shows solvent polarity-independent emission (see Supplementary Information). For probe **3**, however, the emission is highly sensitive to the solvent polarity. For example, the emission wavelength red-shifted from ca. 470 nm to ca. 600 nm with change the solvent from hexane to menthol, concomitantly the emission intensity decreased to 3.1% of the value in hexane. This high sensitivity of the emission intensity to the solvent polarity indicates that the ICT feature of the **3** is significant [1].

Interestingly, the reference of **3**, i.e. probe **4**, shows an emission which is not sensitive to the polarity of the solvents. For example, the emission of **4** in MeOH is stronger than that in hexane. The red-shift of the emission band is also much smaller. This result demonstrates that the ICT effect of probe **4** is smaller than that of probe **3**. This information will be helpful for design of new phenothiazine based fluorophores.

pH Titration a-PeT vs. d-PeT effect. The emission intensity-pH relation of the probes was studied. We found that probes **1**, **3** and **4** are normal a-PeT probes, i.e. the

Table 3 Standard deviations and limits of detection of probes 4 and 5 with tartaric acid and mandelic acid

Probe	Standard deviation[a]	Relative standard deviation[a]	analyte	limit of detection[b] (mol dm^{-3})
4 (pH 6.0)	0.5	0.20%	L-tartaric acid	1.15×10^{-5}
			L-mandelic acid	1.33×10^{-6}
5 (pH 4.0)	1.2	0.36%	L-tartaric acid	1.97×10^{-5}
			L-mandelic acid	5.33×10^{-6}

[a] Standard deviation of the fluorescence emission intensity of the probes alone. $c=1.0\times10^{-7}$ mol dm^{-3} in NaCl ionic buffer (52.1% methanol in water), $n=10$; [b] The limit of detection with different analytes is obtained with analytes concentration which gives the fluorescence response equal to three times the standard deviation

emission intensity at neutral and basic pH is diminished, while the emission is intensified at acidic pH. This is due to the protonation of the amine N atom at acidic pH and thus the inhibition of the photo-induced electron transfer from the N to fluorophore [3, 13].

Firstly the fluorescence emission intensity-pH profiles were investigated (Fig. 3). Normal a-PeT effect was observed for **4**, i.e. the emission intensity of the probes is higher at acidic pH than that at neutral or basic pH. This is due to the protonation of the N atom at acidic pH, thus the suppression of the PeT effect (quenching effect) [17–21]. Interestingly, for probe **5**, d-PeT effect was observed [21, 36, 37], i.e. the fluorescence emission intensity of the probe is intensified at neutral and basic pH, but reduced at acidic pH. We attribute the d-PeT effect to the photo-induced electron transfer from the fluorophore to the protonated amine/boronic acid groups. d-PeT probes are ideal for sensing hydroxyl acids at acidic pH with the boronic acid probes, due to the reduced background emission of the probes, higher binding constant compared to neutral pH and the improved fluorescence transduction efficiency [21, 36–39].

We noticed that the amine precursor of the probes (i.e. the amines) show much higher apparent pKa values than the probes (c.a. pKa=5.0, Fig. 4). For example, amine **14** and **17** show apparent pKa value of 7.28 and 7.43, respectively (see Supplementary information). The much lower pKa values of the probe are actually not due to the protonation of the alkyl amine moiety, rather it is due to the perturbation of the B-N interaction (either a direct B-N dative bond or solvent molecular inserted intramolecular hydrogen bond form) [5, 9, 14, 40, 41].

Typical binding curves of **4** and **5** with tartaric acid and mandelic acid are presented in Fig. 4. In the presence of mandelic acid or tartaric acid, fluorescence enhancement was observed for **4** and **5**. We found that with the d-PeT **5**, the fluorescent recognition of tartaric acid and mandelic acid with **5** were realized at pH 4.0. For the normal a-PeT probes, however, recognition of hydroxyl acids is impossible at pH 4.0, due to the strong background emission of the probes and the poor fluorescence modulation in the presence of analytes [18]. Herein we demonstrate the potential application of the d-PeT effect for the recognition of hydroxyl acids at acidic pH with fluorescent boronic acid probes. The binding constants were summarized in Table 2.

The dynamic range of probe **4** on mandelic acids and tartaric acids are 0–8 mM. The dynamic range of probe 5 on mandelic acids and tartaric acids are 0–0.5 mM. The dynamic ranges are useful for analysis of some real samples, such as the tartaric acid in red wine and tomato juice, for which the tartaric acid concentration is within the dynamic ranges of the probes [42, 43]. The standard deviations and limits of detection were summarized in Table 3.

In summary, we prepared new ethynylated phenothiazine based fluorescent boronic acid probes and the photophysical properties as well as the binding abilities of these probes with hydroxyl acids were studied. The ICT features of the probes were varied to study its effect on the photophysical properties. The probes show fluorescence emission in the range of 460–487 nm with Stokes shifts of 100–137 nm. d-PeT effect was observed for probe **5**, which shows diminished emission intensity at acidic pH but intensified emission at neutral pH. Fluorescent recognition of tartaric acid and mandelic acids were achieved at pH 4.0. d-PeT effect was observed for probe **5**, and normal a-PeT effects were observed for other probes. The easy derivatization of the phenothiazine core, the photophysical properties of the probes (large Stokes shift and high fluorescence quantum yields) and the systematic study of the photophysical properties will be useful for design of fluorescent probes.

Acknowledgements We thank the NSFC (20972024 and 21073028), the Fundamental Research Funds for the Central Universities (DUT10ZD212), Ministry of Education (SRFDP-200801410004 and NCET-08-0077), the Royal Society (UK) and NSFC (China-UK Cost-Share Science Networks, 21011130154), State Key Laboratory of Chemo/Biosensing and Chemometrics (2008009), the Education Department of Liaoning Province (2009 T015), State Key Laboratory of Fine Chemicals (KF0802) and Dalian University of Technology for financial support.

References

1. Lakowicz JR (1999) Principles of fluorescence spectroscopy. Kluwer, New York
2. Valeur B (2001) Molecular fluorescence: principles and applications. Wiley−VCH Verlag GmbH, New York
3. De Silva AP, Gunaratne HQN, Gunnlaugsson T, Huxley AJM, McCoy CP, Rademacher JT, Rice TE (1997) Signaling recognition events with fluorescent sensors and switches. Chem Rev 97:1516–1566
4. Pu L (2004) Fluorescence of organic molecules in chiral recognition. Chem Rev 104:1687–1716
5. James TD (2007) Saccharide-selective boronic acid based Photo-induced Electron Transfer (PET) fluorescent sensors. Top Curr Chem 277:107–152
6. Mohr GJ (2006) New chromogenic and fluorogenic reagents and sensors for neutral and ionic analytes based on covalent bond formation–a review of recent developments. Anal Bioanal Chem 386:1201–1214
7. Caltagirone C, Gale PA (2009) Anion receptor chemistry: highlights from 2007. Chem Soc Rev 38:520–563
8. Oshovsky GV, Reinhoudt DN, Verboom W (2007) Supramolecular chemistry in water. Angew Chem Int Ed 46:2366–2393
9. Wright AT, Anslyn EV (2006) Differential receptor arrays and assays for solution-based molecular recognition. Chem Soc Rev 35:14–28
10. Kim SK, Lee DH, Hong J, Yoon J (2009) Chemosensors for pyrophosphate. Acc Chem Res 42:23–31
11. Stefano C, Carmine C, Marco C, Claudia G, Giuseppe G, Dario P (2010) Shape selectivity in the synthesis of chiral macrocyclic amides. Tetrahedron 66:4206–4211

12. Rosario M, Arturo E, Alberto T, Pedro M (2010) A new bis (pyrenyl)azadiene-based probe for the colorimetric and fluorescent sensing of Cu(II) and Hg(II). Tetrahedron 66:3662–3667
13. James TD, Shinkai S (2002) Artificial receptors as chemosensors for carbohydrates. Top Curr Chem 218:159–200
14. Jin S, Wang J, Li M, Wang B (2008) Synthesis, evaluation, and computational studies of naphthalimide-based long-wavelength fluorescent boronic acid reporters. Chem Eur J 14:2795–2804
15. Xu Z, Kim SK, Han SJ, Lee C, Kociok-Kohn G, James TD, Yoon J (2009) Ratiometric fluorescence sensing of fluoride Ions by an asymmetric bidentate receptor containing a boronic acid and Imidazolium Group. Eur J Org Chem 18:3058–3065
16. Lavigne JJ, Anslyn EV (1999) Teaching old indicators new tricks: a colorimetric chemosensing ensemble for tartrate/Malate in Beverages. Angew Chem Int Ed 38:3666–3669
17. Zhao J, Fyles TM, James TD (2004) Chiral binol—bisboronic acid as fluorescence sensor for sugar acids. Angew Chem Int Ed 43:3461–3464
18. Zhao J, Davidson MG, Mahon MF, Kociok-Kohn G, James TD (2004) An enantioselective fluorescent sensor for sugar acids. J Am Chem Soc 126:16179–16186
19. Zhao J, James TD (2005) An enantioselective fluorescent sensor for sugar acids. J Mater Chem 15:2896–2901
20. Liang X, James TD, Zhao J (2008) 6, 6′-Bis-substituted BINOL boronic acids as enantiosele- ctive and chemselective fluorescent chemosensors for D-sorbitol. Tetrahedron 64:1309–1315
21. Han F, Chi L, Liang X, Ji S, Liu S, Zhou F, Wu Y, Han K, Zhao J, James TD (2009) 3, 6-Disubstituted Carbazole-Based bisboronic acids with unusual fluorescence transduction as enantioselective fluorescent chemosensors for tartaric acid. J Org Chem 74:1333–1336
22. Chi L, Zhao J, James TD (2008) Chiral mono boronic acid as fluorescent nantioselective sensor for mono α-hydroxyl carboxyl-ic acids. J Org Chem 73:84–4687
23. Zhang L, Kerszulis JA, Clark RJ, Ye T, Zhu L (2009) Catechol boronate formation and its electrochemical oxidation. Chem Commun 16:2151–2153
24. Leung D, Frantz FJ, Lynch VM, Anslyn EV (2008) Using enantioselective indicator displacement assays to determine the enantiomeric excess of α-amino acids. J Am Chem Soc 130:12318–12327
25. Kramer CS, Zeitler K, Muller TJ (2000) Synthesis of functional-ized ethynylphenothiazine fluorophores. Org Lett 2:3723–3726
26. Blazys G, Grigalevicius S, Grazulevicius JV, Gaidelis V, Jankauskas V, Kamparsc V (2005) Phenothiazinyl-containing aromatic amines as novel amorphous molecular materials for optoelectronics. J Photochem Photobiol A Chem 174:1–6
27. Lee C, Sung YW, Park JW (1999) Multiple equilibria of phenothiazine dyes in aqueous cyclodextrin solutions. J Phys Chem B 103:893–898
28. Zheng S, Lin N, Reid S, Wang B (2007) Effect of extended conjugation with a phenylethynyl group on the fluorescence properties of water-soluble arylboronic acids. Tetrahedron 63:5427–5436
29. Wang J, Jin S, Akol S, Wang B (2007) Design and synthesis of long-wavelength fluorescent boronic acid reporter compounds. Eur J Org Chem 2091–2099
30. Liu Y, Feng J, Ren A (2008) Theoretical study on photo-physical properties of bis-dipolar diphenylamino-endcapped oligoarylfluorenes as light-emitting materials. J Phys Chem A 112:3157–3164
31. Zhao G-J, Liu J-Y, Zhou J-C, Han K-L (2007) Site-selective photoinduced electron transfer from alcoholic solvents to the chromophore facilitated by hydrogen bonding: a new fluorescence quenching mechanism. J Phys Chem B 111:8940–8945
32. Li M-X, Zhang H-X, Zhou X, Pan Q-J, Fu H-G, Sun C-C (2007) Theoretical studies of the electronic structure and spectroscopic properties of $[Ru(Htcterpy)(NCS)_3]^{3-}$. Eur J Inorg Chem 2171–2180
33. Kavarnos G, Turro NJ (1986) Photosensitization by reversible electron transfer: theories, experimental evidence, and examples. Chem Rev 86:401–449
34. Cody J, Mandal S, Yang L, Fahrni CJ (2008) Differential tuning of the electron transfer parameters in 1, 3, 5-Triarylpyrazolines: a rational design approach for optimizing the contrast ratio of fluorescent probes. J Am Chem Soc 130:13023–13032
35. Ji S, Yang J, Yang Q, Liu S, Chen M, Zhao J (2009) Tuning the intramolecular charge transfer of alkynylpyrenes: effection photo-physical properties and its application in design of OFF-ON fluorescent thiol probes. J Org Chem 74:4855–4865
36. Ueno T, Urano Y, Setsukinai K, Takakusa H, Kojima H, Kikuchi K, Ohkubo K, Fukuzumi S, Nagano T (2004) Rational principles for modulating fluorescence properties of fluorescein. J Am Chem Soc 126:14079–14085
37. Matsumoto T, Urano Y, Shoda T, Kojima H, Nagano T (2007) A thiol-reactive fluorescence probe based on donor-excited photoin-duced electron transfer: key role of ortho substitution. Org Lett 9:3375–3377
38. McCarroll ME, Shi Y, Harris S, Puli S, Kimaru I, Xu R, Wang L, Dyer DJ (2006) Computational prediction and experimental evaluation of a photoinduced electron-transfer sensor. J Phys Chem B 110:22991–22994
39. Sunahara H, Urano Y, Kojima H, Nagano T (2007) Design and synthesis of a library of BODIPY-Based environmental polarity sensors utilizing photoinduced electron-transfer- con-trolled fluorescence ON/OFF switching. J Am Chem Soc 129: 5597–5604
40. Zhu L, Shabbir SH, Gray M, Lynch VM, Sorey S, Anslyn EV (2006) A structural investigation of the N-B interaction in an o-(N, N-Dialkylaminomethyl)arylboronate system. J Am Chem Soc 128:1222–1232
41. Collins BE, Sorey S, Hargrove AE, Shabbir SH, Lynch VM, Anslyn EV (2009) Probing intramolecular B-N interactions in ortho-aminomethyl arylboronic acids. J Org Chem 74:4055–4060
42. Xu J, Wang Y, Xian Y, Liu M, Jin L, Tanaka K (2003) Determination of electroinactive organic acids in red wine by ion-exclusion chromatography using a poly-o-phenylenediamine film modified electrode. Chromatographia 57:751–756
43. Beullens K, Kirsanov D, Irudayaraj J, Rudnitskaya A, Legin A, Nicolai BM, Lammertyn J (2006) The electronic tongue and ATR–FTIR for rapid detection of sugars and acids in tomatoes. Sens Actuators B 116:107–115