CHAPTER III

EXPERIMENTAL

3.1 General procedures

3.1.1 Analytical measurements and materials

All materials and solvents chemicals were purchased from Aldrich, Fluka, Merck and TCI as standard analytical grade and were used without further purification. Commercial grade solvents such as dichloromethane, hexane, methanol and ethyl acetate were purified by distillation prior to use. Anhydrous solvents such as dichloromethane was dried via distillation over CaH_2 under nitrogen atmosphere. Column chromatography was carried out on silica gel (Kieselgel 60, 0.063-0.200 mm, Merck). Thin layer chromatography (TLC) was performed on silica gel plates (Kieselgel 60, F_{254}, 1 mm, Merk). Dimethyl sulfoxide as AR grade used in UV-visible and fluorescence measurement was used without drying.

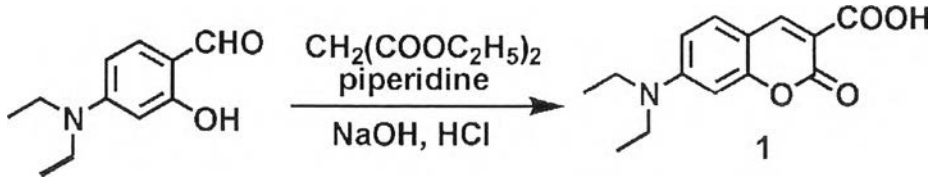
^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 and Bruker DRX 400 MHz nuclear resonance spectrometer. All chemical shifts were given in part per million (ppm) using the residual proton or carbon signal in deuterated solvents as internal references. MALDI-TOF mass spectra were carried out on Bruker Daltonics MALDI-TOF using α-hydroxy cyanocinnamic acid (CCA) as matrix. Elemental analysis was carried out on CHNS/O analyzer by ignition combustion gas chromatography separated by frontal analysis and qualitative detected by thermal conductivity detector. Thin-layer chromatography (TLC) was performed on silica gel plates supplied by Merck (Kieselgel 60 F254, 1mm). Absorption spectra were measured by a Varian Cary 50 UV-vis spectrophotometer. Fluorescence spectra were

performed on a Varian eclipse spectrofluorometer by personal computer data processing unit. The light source is Cary Eclipse a pulsed xenon lamp and a detector is a photomultiplier tube.

3.2 Experimental procedure

3.2.1 Synthesis of coumarin based sensor

3.2.1.1 Preparation of 7-diethylamino-2-oxo-2H-chromen-3-carboxylic chloride (1). [89]



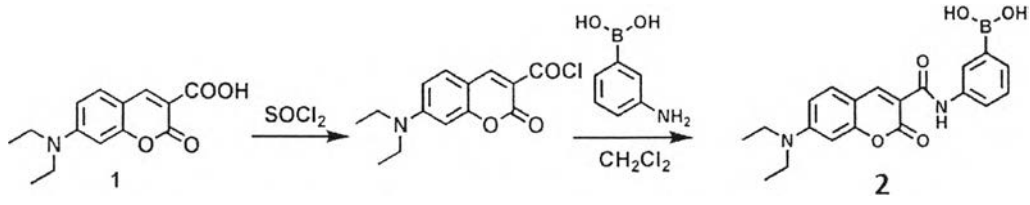
Into a 50 ml of round-bottomed flask with a magnetic bar and condenser, 4-diethylaminosalicylaldehyde (0.38 g, 2 mmol), diethylmalonate (0.61g, 4 mmol) and piperidine (0.2 ml, 2 mmol) was dissolved in absolute ethanol. The reaction mixture was heated under reflux with stirring for 6 hrs. Then 10% NaOH was added in the portion. After that, the reaction mixture was stirred for 15 min at 80 ^{o}C and cooled to room temperature. Then acidification to pH 2 with HCl under ice bath gave precipitate which was collected by suction filtration. The percipitate was washed with water and recrystallized with ethanol to obtain orange solid in 80% yield.

Characterization data for 1

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 12.35 (s, 1H), 8.66 (s, 1H), 7.45 (d, J = 4.0 Hz, 1H), 6.70 (m, 1H), 6.53 (s, 1H), 3.48 (t, J = 7.2 Hz, 4H), 1.59 (s, 6H).

3.2.1.2 Preparation of 3-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido) phenylboronic acid (2) (Cum_B).



Compound **1** (0.19g, 0.72 mmol) was mixed with dry $SOCl_2$ under N_2 atmoshere. After stirring for 3 hrs, the solution of 3-aminophenylboronic acid (0.1 g, 0.72 mmol) in CH_2Cl_2 was transferred dropwise, using a canula, into dry compound **1**. The reaction was stirred at room temperature for 24 hrs. The yellow precipitate in 76% yield was collected by suction filtration and washed with CH_2Cl_2.

Characterization data for 2

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 10.75 (m, 1H), 8.76 (s, 1H), 8.09 (s, 1H), 7.97 (m, 1H), 7.81 (d, *J* = 4.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.32 (t, *J* = 8.0 Hz, 1H), 6.84 (m, 1H), 6.67 (d, *J* = 4.0 Hz, 1H), 3.43 (t, *J* = 6.8 Hz, 4H), 1.14 (t, *J* = 7.2 Hz, 6H).

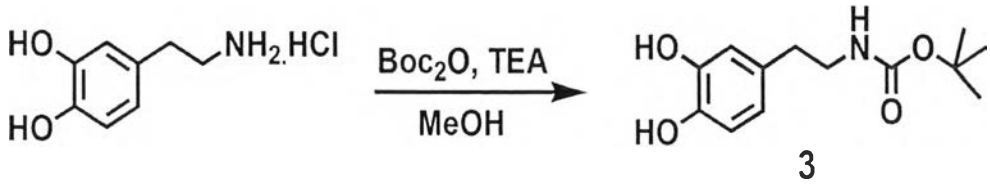
^{13}C-NMR (400 MHz, $CDCl_3$) δ (in ppm):

δ = 155.8, 137.4, 137.3, 132.4, 128.4, 127.8, 127.7, 127.4, 127.3, 121.7, 116.0, 115.5, 77.3, 77.0, 76.6, 71.5, 71.4, 35.7, 28.4

MALDI-TOF mass: Anal. Calcd. for $C_{20}H_{21}BN_2O_5$ m/z = 380.15, found 380.37

3.2.2 Synthesis of naphthalimide based sensor

3.2.2.1 Preparation of N-tert-Butoxycarbonyl-3,4-dihydroxiphenylethylamine (3). [90]



3

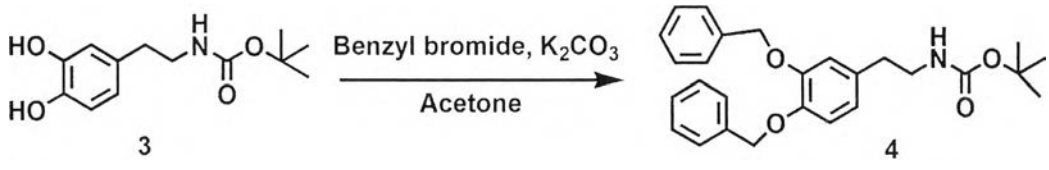
Into a 50 ml of two-neck bottom flask equipped with a magnetic bar and condenser, a solution of dopamine hydrochloride (0.50 g, 2.60 mmol) was stirred for 5 minutes in MeOH at room temperature under nitrogen atmosphere until homogeneous. A portion of triethanolamine (0.39 ml, 2.60 mmol) and *tert*-butyl dicarbonate (0.62 g, 2.86 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 30 minutes. The solvent was removed under vacuum. The crude reaction was dissolved in CH_2Cl_2 and washed with 3M HCl and brine. The organic layer was dried with sodium sulfate and concentrated by reduced pressure to obtain a white solid **3** in 95% yield.

Characterization data for 3

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 8.72 (s, 1H), 8.61 (s, 1H), 6.79 (t, J = 5.2 Hz, 1H), 6.57 (t, J = 8.0 Hz, 1H), 6.38 (d, J = 8 Hz, 1H), 2.99 (t, J = 7.6 Hz, 2H), 2.46 (t, J = 7.2 Hz, 2H), 1.35 (s, 9H).

3.2.2.2 Preparation of N-tert-Butoxycarbonyl-3,4-dibenzyloxyphenylethylamine (4). [90]



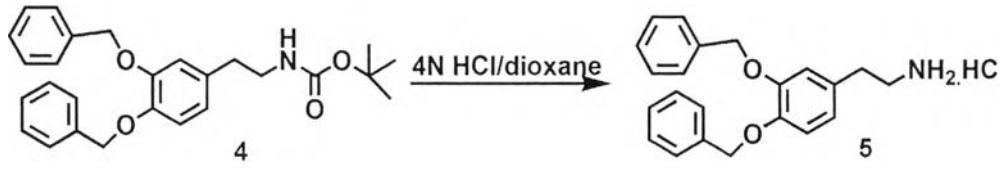
Compound **3** (0.49 g, 1.93 mmol) in 10 ml acetone was stirred at room temperature under nitrogen atmosphere until homogeneous. After 5 minutes, benzyl bromide (2.29 ml, 19.3 mmol) and K_2CO_3 (2.67 g, 19.3 mmol) were added into the solution of compound **3**. The bottom flask was wrapped with aluminium foil and the reaction mixture was stirred for 16 hrs. The acetone solution was concentrated by a rotary evaporation. The crude product was purified by column chromatography on silica with 20% hexane/dichloromethane to obtain a white solid **4** in 85% yield.

Characterization data for 4

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 7.44-7.28 (m, 10H), 6.90 (q, J = 8.4, 2H), 6.67 (d, J = 8.0 Hz, 1H), 5.07 (d, J = 4.0 Hz, 4H), 3.07 (q, J = 4.0 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 1.34 (s, 9H).

3.2.2.3 Preparation of 2-(3,4-Bis-benzyloxy-phenyl)-ethylamine (5). [90]



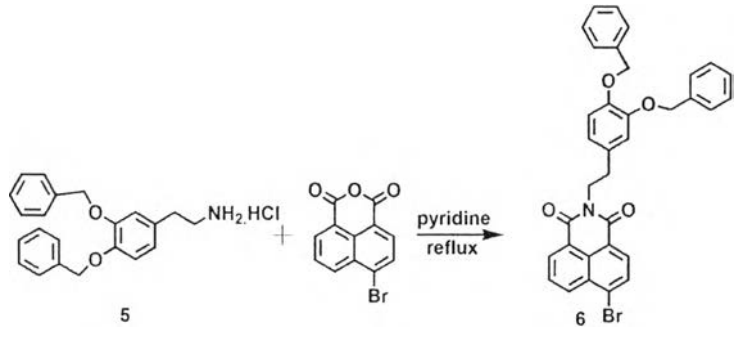
Compound **4** (0.30 g, 0.69 mmol) was added into 4N HCl/ dioxane. Then, the reaction mixture was stirred at room temperature for 30 minutes under nitrogen atmosphere. The solvent was removed under vacuum to obtain a white solid **5** give 100% yield.

Characterization data for 5

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 7.44-7.33 (m, 10H), 6.98 (t, J = 4.0 Hz, 2H), 6.74 (d, J = 8.0 Hz, 1H), 5.09 (d, J = 8.0 Hz, 4H), 2.97 (m, 2H), 2.75 (t, J = 7.2 Hz, 2H).

3.2.2.4 Preparation of compound 6.



Into a 50 ml two-neck bottom flask equipped with a magnetic bar and a Dean-Stark equipment to remove water, the corresponding 4-bromo-1,8-naphthalenedicarboxylic acid anyhydride (0.46 g, 1.38 mmol), compound **5** (0.3 g, 1.38 mmol), molecular sieves, 10 mg of zinc acetate were stirred in 15 ml of pyridine under nitrogen atmosphere. The reaction mixture was refluxed for 12 hrs. After that, pyridine was removed under reduced pressure and extracted with CH_2Cl_2. The organic layer was dried with $NaSO_4$ and evaporated by vacuum. The residue was purified by chromatography on silica gel using 95:5 ethyl acetate/ dichloromethane to give a light green- yellow solid (60% yield).

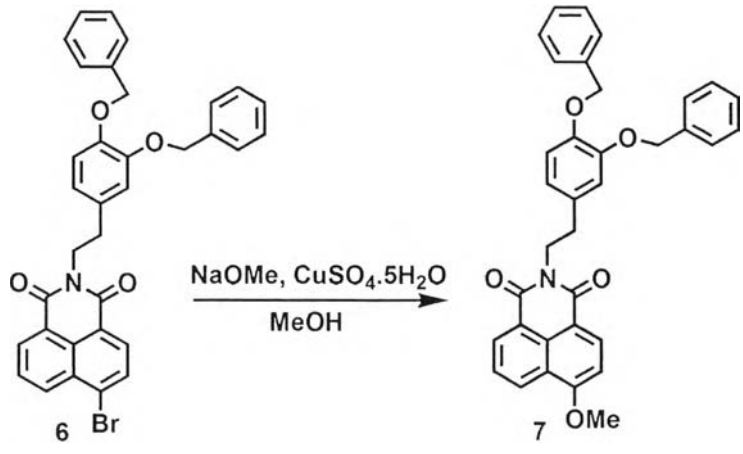
^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 8.52 (m, 2H), 8.30-8.18 (dd, *J* = 8.0, Hz 2H), 7.97 (t, *J* = 8.4 Hz, 1H), 7.34 (m, 10H), 6.95 (d, 2H), 6.76 (d, 1H), 5.02 (d, *J* = 4.0 Hz, 4H), 4.18 (q, *J* = 12 Hz, 2H), 2.82 (t, *J* = 7.6 Hz, 2H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (in ppm):

δ = 140.2, 137.5, 137.3, 133.3, 132.0, 131.2, 131.1, 129.0, 128.4, 128.1, 127.7, 127.7, 127.4, 127.3, 121.9, 115.9, 115.5, 77.3, 77.0, 76.7, 71.5, 71.3, 41.9, 33.7

3.2.2.5 Preparation of compound 7.



A mixture of compound **6** (0.5 g, 0.84 mmol), 12 mg of $CuSO_4.5H_2O$ and a 10:1 stoichiometric ratio of sodium methoxide in methanol was heated under reflux with stirring for 18 hrs. After that, methanol was removed under reduced pressure and extracted with CH_2Cl_2. The organic layer was dried with $NaSO_4$ and the solvent removed under vacuum to give light-green solid in 40% yield.

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

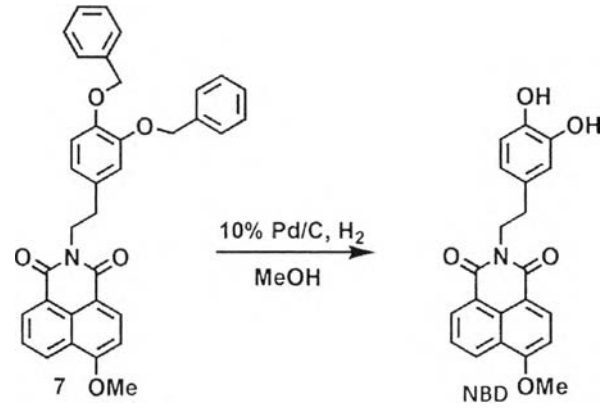
δ = 8.49 (m, 3H), 7.82 (t, *J* = 8.0 Hz, 1H), 7.35 (m, 10H), 6.94 (t, *J* = 8.4 Hz, 2H), 6.76 (t, *J* = 8.0 Hz, 1H), 5.01 (d, *J* = 4.0 Hz, 4H), 4.12 (q, *J* = 8.0 Hz, 2H), 4.10 (s, 3H), 2.81 (q, *J* = 8.0 Hz, 2H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (in ppm):

δ = 160.8, 133.4, 131.5, 131.2, 129.4, 128.7, 128.7, 128.5, 128.4, 127.6, 127.4, 127.5, 125.9, 121.9, 116.0, 115.6, 105.2, 77.3, 77.0, 76.6, 71.6, 71.3, 56.2, 41.7, 33.8

MALDI-TOF mass: Anal. Calcd. for $C_{35}H_{29}NO_5$ m/z = 543.20, Found: 543.42

3.2.2.6 Preparation of compound NBD.



A solution of compound **7** (0.2 g, 0.37 mmol) in methanol was stirred at room temperature for 5 min. 10% Pd /C was added into the mixture solution under H_2 atmosphere. The reaction was stirred vigorously at room temperature for 24 hrs. After the reaction mixture was filtered, the percipitate was washed with CH_2Cl_2. The filtrate was purified by recrystallization in methanol to give a light green solid in 15% yield.

^{1}H-NMR (400 MHz, DMSO-d_6) δ (in ppm):

δ = 8.83 (s, 1H), 8.67 (s, 1H), 8.51 (m, 3H), 7.82 (t, J = 16 Hz, 1H), 7.34 (t, J = 8.0 Hz, 1H), 6.53 (q, J = 12 Hz, 2H), 6.47 (m, 1H), 4.15 (q, J = 4.0 Hz, 2H), 4.12 (s, 3H), 2.70 (m, 2H).

^{13}C-NMR (400 MHz, $CDCl_3$) δ (in ppm):

δ = 160.4, 162.8, 160.3, 145.1, 143.6, 133.2, 131.0, 129.5, 128.2, 126.4, 121.9, 119.2, 115.9, 115.5, 106.3, 56.6, 41.2, 40.2, 39.9, 39.7, 39.7, 39.5, 39.3, 39.4, 38.2, 33.0

Elemental Analysis: Anal.Calcd. for $C_{21}H_{17}NO_5$: C,69.41; H, 4.72; N, 3.85

Found: C, 69.35; H, 4.63; N, 3.82

3.2.3 Synthesis of gold nanoparticles

Hydrogen tetrachloroaurate (20 ml, 0.25 mM) was dissolved in 80 ml of water and sodium citrate (20 ml, 2.5 mM) was added to the portion. The mixture solution was stirred vigorously for 30 minutes. Immediately, the reaction was turned to red and kept stirred for 15 minutes to ensure the complete reaction.

3.3 The complexation studies of sensor Cum_B by fluorescent spectrophotometry

3.3.1 Complexation studies of sensor Cum_B with various saccharides

Firstly, a stock solution of $1x10^{-3}$ M of sensor **Cum_B** was prepared in DMSO. A stock solution of $1x10^{-2}$ M of various saccharides including fructose, glucose, galactose, ribose, lactose and maltose in 0.1 M phosphate buffer pH 7.4 were prepared in volumetric flasks. The solution of various saccharides was added to 2 ml of $5x10^{-5}$ M of sensor **Cum_B** in a 1- cm quartz cuvette by micropipette and stirred for 30 minutes at room temperature before monitoring the fluorescence spectra under the following conditions:

Start: 420 nm

End: 800 nm

Excitation: 430 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

3.3.2 Determination of the stoichiometry of sensor Cum_B with saccharides complexes by Job's method

Typically, the stock solution of sensor **Cum_B** and sugars were prepared in 5% DMSO: 0.1 M phosphate buffer pH 7.4 at concentration of $5x10^{-5}$ M in volumetric flask. The portion of **Cum_B** was mixed with portions of the stock solution of saccharides to give the final mode fraction of saccharides and **Cum_B** according to Table 3.1. The solution mixture was stirred for 30 minutes at room temperature and was pipetted to a 1-cm quartz cuvette to provide the total volume to 2 ml. The emission spectrum of complex was measured after stirring.

Table 3.1 Mole fraction and volume of sensor **Cum_B** ($5x10^{-5}$ M) and saccharide in the stock solution ($5x10^{-5}$ M) for Job's method.

point	Mole fraction of Cum_B	mole fraction of saccharide	volume of Cum _B (ml)	volume of saccharide (ml)
1	1.0	0.0	2.00	0.00
2	0.9	0.1	1.80	0.20
3	0.8	0.2	1.60	0.40
4	0.7	0.3	1.40	0.60
5	0.6	0.4	1.20	0.80
6	0.5	0.5	1.00	1.00
7	0.4	0.6	0.80	1.20
8	0.3	0.7	0.60	1.40
9	0.2	0.8	0.40	1.60
10	0.1	0.9	0.20	1.80
11	0.0	1.0	0.00	2.00

3.3.3 Determination of detection limit of sensor Cum_B

Typically, a solution of $5x10^{-5}$ M of sensor **Cum_B** in 5% DMSO: 0.1 M phosphate buffer pH 7.4 was prepared by adding 0.5 ml of stock solution of **Cum_B** ($1x10^{-3}$ M) in 10 ml volumetric flask. Fluorescence spectra of **Cum_B** were recorded for 10 times at room temperature under the following condition:

Start: 420 nm

End: 800 nm

Excitation: 430 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

3.3.4 Complexation studies of sensor Cum_B with saccharide by fluorescence titration

A stock solutions of sensor **Cum_B** ($1x10^{-3}$ M) was prepared in DMSO and stock solution of $1x10^{-2}$ M of saccharides in 5 % DMSO with 0.1 M phosphate buffer pH 7.4 was prepared in volumetric flask. The solution of saccharides was added to 2 ml of the sensor solution in various amounts of saccharide as listed in table 3.2 and the mixture solution was stirred for 30 minutes at room temperature. Fluorescence spectra were measured under the following condition:

Start: 420 nm

End: 800 nm

Excitation: 430 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

Table 3.2 The concentration of saccharide used in complexation studies with sensor Cum_B

Point	[Cum_B] (M)	[sugar] (M)	Vol. of sugar (ml)	Vol.total (ml)
1	5.00×10^{-5}	0.00	0.000	2.000
2	2.50×10^{-6}	2.49×10^{-5}	0.005	2.005
3	2.49×10^{-6}	4.98×10^{-5}	0.010	2.010
4	2.48×10^{-6}	9.90×10^{-5}	0.020	2.020
5	2.46×10^{-6}	1.48×10^{-4}	0.030	2.030
6	2.45×10^{-6}	1.96×10^{-4}	0.040	2.040
7	2.44×10^{-6}	2.44×10^{-4}	0.050	2.050
8	2.43×10^{-6}	2.91×10^{-4}	0.060	2.060
9	2.40×10^{-6}	3.85×10^{-4}	0.080	2.080
10	2.38×10^{-6}	4.76×10^{-4}	0.100	2.100
11	2.36×10^{-6}	5.66×10^{-4}	0.120	2.120
12	2.33×10^{-6}	6.98×10^{-4}	0.150	2.150
13	2.29×10^{-6}	8.26×10^{-4}	0.180	2.180
14	2.26×10^{-6}	9.50×10^{-4}	0.210	2.210
15	2.23×10^{-6}	1.07×10^{-3}	0.240	2.240
16	2.20×10^{-6}	1.19×10^{-3}	0.270	2.270

Point	[Cum_B] (M)	[sugar] (M)	Vol. of sugar (ml)	Vol.total (ml)
17	2.17×10^{-6}	1.30×10^{-3}	0.300	2.300
18	2.15×10^{-6}	1.42×10^{3}	0.330	2.330
19	2.12×10^{-6}	1.53×10^{-3}	0.360	2.360
20	2.09×10^{-6}	1.63×10^{-3}	0.390	2.390
21	2.04×10^{-6}	1.84×10^{-3}	0.450	2.450
22	2.00×10^{-6}	2.00×10^{-3}	0.500	2.500

3.4 The complexation studies of sensor NBDB by fluorescent spectrophotometry

3.4.1 Complexation studies of sensor NBDB

The stock solution of $1x10^{-3}$ M of **NBD** was prepared in DMSO. $1x10^{-3}$M. The stock solution of 3-aminophenylboronic acid ($1x10^{-3}$M) was prepared in 0.1 M phosphate buffer pH 7.4. The solution of **NBD** was mixed with the solution of 3-aminophenylboronic acid to give final concentration of **NBD** and 3-aminophenylboronic acid according to Table 3.3. After volume adjustment with DMSO and 0.1 M phosphate buffer pH 7.4, the mixture was stirred for 30 minutes at room temperature and placed in a 1- cm quartz cuvette. Fluorescence spectra were measured under the following condition:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

Table 3.3 Volume of **NBD** with 3-aminophenylboronia acid stock solution ($1x10^{-3}$ M) and final concentration of **NBD** with 3-aminophenylboronic acid (*m*-BA)

Vol. of NBD stock solution (µl)	[NBD] (M)	Vol. of stock *m*-BA solution (µl)	[*m*-BA] (M)
200	$1x10^{-4}$	20	$1x10^{-5}$
180	$9x10^{-5}$	18	$9x10^{-6}$
160	$8x10^{-5}$	16	$8x10^{-6}$
140	$7x10^{-5}$	14	$7x10^{-6}$
120	$6x10^{-5}$	12	$6x10^{-6}$
100	$5x10^{-5}$	10	$5x10^{-6}$
80	$4x10^{-5}$	8	$4x10^{-6}$
60	$3x10^{-5}$	6	$3x10^{-6}$
40	$2x10^{-5}$	4	$2x10^{-6}$
20	$1x10^{-5}$	2	$1x10^{-6}$

3.4.2 Complexation studies of sensor NBDB with various saccharides

Initially, the stock solution of $1x10^{-3}$ M of **NBD** was prepared in DMSO and $1x10^{-3}$ M of 3-aminophenylboronic acid was prepared in 0.1 M phosphate buffer pH 7.4. A stock solution of various saccharides ($1x10^{-2}$M) including fructose, glucose, galactose, ribose, lactose and maltose in 0.1 M phosphate buffer pH 7.4 were

prepared in volumetric flasks. The mixture solution of $1x10^{-4}$ M **NBD** and $1x10^{-5}$ M 3-aminophenylboronic acid was stirred for 30 minutes at room temperature to give sensor **NBDB**. Next, the solution of sugar was added directly to 2 ml of sensor **NBDB** in a 1- cm quartz cuvette by micropipette and stirred for 30 minutes at room temperature prior to measurement of fluorescence spectra under the following conditions:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

3.4.3 Study on the concentration effect of modified gold nanoparticles by NBDB

Into a 2.0 ml quartz cuvette, 0.2 ml of sensor **NBDB** in 10 % DMSO: 0.1 M phosphate buffer pH 7.4 was mixed with $1.4x10^{-4}$ M of gold nanoparticles in various volume of gold nanoparticles according to table 3.4. Aqueous solution of gold nanoparticles was added to a portion of **NBDB** in 10% DMSO: 0.1 M phosphate buffer pH 7.4 and the mixture were stirred vigorously for 20 minutes. **NBDB** was transferred to the aqueous phase and the red colour of the solution did not change. Next, the

mixture solution was centrifuge (10000 rpm, 20 min) at 25 ^{o}C 2 times. Fluorescence spectra were measured under the following condition:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

Table 3.4 Volume of the stock solution of gold nanoparticles ($1.4x10^{-4}$ M) and the final concentration of gold nanoparticles in 2 ml

Volume of gold nanoparticles stock solution (ml)	Final concentration of gold nanoparticles (μM)
0	0
0.2	14
0.4	28
0.6	42
0.8	56
1.0	70

3.4.4 Complexation studies of modified of gold nanoparticles by NBDB (NBDB_AuNPs) with various saccharides

The stock solutions of sensor **NBDB_AuNPs** were prepared in 10%DMSO: 0.1 M phosphate buffer pH 7.4. The stock solution of various saccharides ($1x10^{-2}$ M) including fructose, glucose, galactose, ribose, lactose and maltose in 0.1 M phosphate buffer pH 7.4 were prepared in volumetric flasks. The solution of various saccharides was added to 2 ml of sensor **NBDB_AuNPs** in a 1- cm quartz cuvette by micropipette and stirred for 30 minutes at room temperature before monitoring the fluorescence spectra under the following conditions:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

3.4.5 Determination of detection limit of sensor NBDB_AuNPs

Typically, the solution of sensor **NBDB_AuNPs** in 10% DMSO: 0.1 M phosphate buffer pH 7.4 was prepared by adding 1.0 ml of stock solution of **NBDB_AuNPs** in 10 ml volumetric flask. Fluorescence spectra of **NBDB_AuNPs** were recorded for 10 times at room temperature under the following condition:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

3.4.6 Complexation studies of sensor NBDB_AuNPs with saccharide by fluorescence titration

The stock solutions of sensor **NBDB_AuNPs** was prepared in 10% DMSO with 0.1 M phosphate buffer pH 7.4 and the stock solution of $1x10^{-2}$ M saccharides in 10 % DMSO with 0.1 M phosphate buffer pH 7.4 was prepared in volumetric flask. The solution of saccharides was added directly to 2 ml of the sensor solution in various amounts of saccharide as listed in table 3.5 and the mixture solution was stirred for 30 minutes at room temperature. Fluorescence spectra were measured under the following condition:

Start: 375 nm

End: 800 nm

Excitation: 385 nm

Excitation Slit: 5.0

Emission Slit: 5.0

Scan rate and the PMT voltage: 680

Table 3.5 The concentration of saccharide used in complexation studies with sensor NBDB_AuNPs

Point	[NBDB_AuNPs] (M)	[sugar] (M)	Vol. of sugar (ml)	Vol.total (ml)
1	1.00×10^{-5}	0.00	0.000	2.000
2	9.99×10^{-6}	1.00×10^{-5}	0.002	2.002
3	9.98×10^{-6}	2.49×10^{-5}	0.005	2.005
4	9.96×10^{-6}	3.98×10^{-5}	0.008	2.008
5	9.95×10^{-6}	5.47×10^{-5}	0.011	2.011
6	9.93×10^{-6}	6.95×10^{-5}	0.014	2.014
7	9.92×10^{-6}	8.43×10^{-5}	0.017	2.017
8	9.90×10^{-6}	9.09×10^{-5}	0.020	2.020
9	9.88×10^{-6}	1.23×10^{-4}	0.025	2.025
10	9.85×10^{-6}	1.48×10^{-4}	0.030	2.030
11	9.80×10^{-6}	1.96×10^{-4}	0.040	2.040
12	9.76×10^{-6}	2.44×10^{-4}	0.050	2.050
13	9.66×10^{-6}	3.38×10^{-4}	0.070	2.070
14	9.57×10^{-6}	4.31×10^{-4}	0.090	2.090
15	9.43×10^{-6}	5.66×10^{-4}	0.120	2.120
16	9.22×10^{-6}	7.83×10^{-4}	0.170	2.170
17	9.00×10^{-6}	9.91×10^{-4}	0.220	2.220
18	8.70×10^{-6}	1.30×10^{-3}	0.300	2.300
19	8.33×10^{-6}	1.67×10^{-3}	0.400	2.400
20	8.00×10^{-6}	2.00×10^{-3}	0.500	2.500
21	7.41×10^{-6}	2.59×10^{-3}	0.700	2.700

Point	[NBDB_AuNPs] (M)	[sugar] (M)	Vol. of sugar (ml)	Vol.total (ml)
22	6.67×10^{-6}	3.33×10^{-3}	1.000	3.000

3.4.7 Determinations of loading efficiency of NBDB capped gold nanoparticles [91]

The sensor **NBDB** incorporated gold nanoparticles (AuNPs) was prepared according to the topic of 3.4.3. To determine the loading efficiency of **NBDB** capped gold nanoparticles, the remaining free **NBDB** was separated from aqueous solution by centrifugation at 10000 rpm at 25 ^{o}C for 20 minutes. The purple supernatant was collected and quantified by UV-Vis spectrophotometry. Loading efficiency of samples was calculated following equation:

Loading efficiency (%) =

$$\frac{\text{(Total amount of NBDB incorporated AuNPs - free NBDB)}}{\text{(Total amount of NBDB incorporated AuNPs)}} \times 100$$