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Supporting Information

A colorimetric and ratiometric fluorescent pH probe based on ring opening/closing approach and its applications in monitoring cellular pH change

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Experimental section

1. General information and methods

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance-III 400 MHz Spectrometer (at 400 and 100 MHz, respectively) using tetramethylsilane (TMS) as an internal standard. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High-resolution mass spectra (HRMS) were performed on a Bruker Autoflex mass spectrometer (MALDI-TOF). Fluorescence and absorption spectra were collected on a PE LS50B and a Cary UV-300 spectrometer, respectively. The melting point was determined with a MEL-TEMPII melting point apparatus (uncorrected). X-ray intensity data were measured at room temperature (298 K) on a Bruker AXS Kappa ApexII Duo diffractometer using frames of oscillation range 0.3º, with $2^{o} < \theta < 28^{o}$. The structures were solved by the direct method and refined by full-matrix least-squares on F2 using the SHELXTL program package. The pH measurements were performed on an Orion 420A pH mV temperature meter with a combined glass-calomel electrode. Double-distilled (DI) water was used throughout. Excitation wavelength is set at 460 nm. Excitation/emission slit = 3/3 nm.

All reagents for synthesis were obtained commercially and were used without further purification. Solvents such as acetonitrile (ACN), ethanol (EtOH), methanol (MeOH) and 1,4-dioxane were purchased from commercial sources and were the highest grade, dry *N,N*-dimethylformamide (DMF) was distilled in calcium hydride. Silica gel (200 – 300 mesh, MACHEREY-NAGEL GmbH & Co. KG) was used for column chromatography. Analytical thin-layer chromatography was performed using TLC silica gel 60 F254 (aluminum sheets, Merck KGaA). Ag^{+}, Li^{+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and Fe^{3+} were purchased as perchlorates, K^{+}, Na^{+} and Mg^{2+} were purchased as chlorides. These inorganic salts were stored in a vacuum desiccator.

2. Sample preparation

The probes were dissolved in ACN as a stock solution (1 mM). In the interference experiment, inorganic salts and other inference species were dissolved in DI water as a stock solution (10 mM). Britton-Robinson buffer solution was prepared by dissolving acetic acid, boric acid and phosphoric acid in DI water (40 mM). Slight variations in the pH of the solution were achieved by adding the minimum volumes of NaOH or HCl.

3. Absorption and fluorescence analysis

Absorption spectra and fluorescence spectra were collected with 1.0 cm quartz cells. The detection procedures were as following: 10 μL of solution of probe in ACN is added to Britton-Robinson buffer solution (40 mM, containing 50% ACN), then the mixture equilibrates for 2 min before measurement. Excitation wavelength is set at 460 nm. Excitation and emission slits are set to 3.0 nm and 3.0 nm, respectively.

4. Cell culture

HK-1 nasopharyngeal carcinoma cell was cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum and 5% heat-inactivated new born calf serum (Gibco) and antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin, Gibco) at 37^{0}C in a humidified incubator with 5% CO_2.

5. Confocal microscopy and Cell Calibration

A pH calibration curve was generated using K^+ ionophore-treated HK-1 cells. The cells were initially treated with probe **1** (20 μM) for 2 hours. After incubation, the probe was removed by washing the cells with PBS. The washed cells were then incubated with 5 μM negericin. After 20 minutes of incubation, the cells were washed, and further incubated with calibration buffer (125 mM KCl, 20 mM NaCl, 0.5 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 25 mM buffer; acetate for pH 5.0; MES for pH 6.0; HEPES for pH 7.0)[1-4] for 20 minutes. A laser scanning confocal microscope (Olympus Fluoview 1000) with fluorescence and a differential interference contrast (DIC) system was used to study the fluorescent signals. The probe was excited with multi-line argon laser at a wavelength of 488 nm. The emission was collected at 496 – 536 nm (I_{green}) and 630–700 nm (I_{red}). An oil immersion objective with a magnification of 60x was used for image capturing. The pH calibration curve was obtained by the plots of I_{green}/I_{red} versus pH value.

6. Cytotoxicity study

MTT assay was performed to determine cell viability. HK-1 cells were seeded at a density of 1 x 10^4 per well in 96-well plates. After 24 hours incubation, the medium in the wells was replaced with different concentrations of probe **1** (5 μM, 10 μM, 20 μM, 50 μM). After 24 hours incubation, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (250 μg/mL) was added to each well (100 μL/well). After 3 hours incubation, 70 μL of the medium was removed and formazan crystals were dissolved with 100 μL DMSO for 10 min on a shaker. The absorbance of each sample was measured by a micro-plate reader at wavelengths of 540 nm and reference at 690 nm. The relative cell viability (%) for each sample was calculated.

7. References

1. M. Lee, N. G. Gubernator, D. Sulzer and D. Sames, *J. Am. Chem. Soc.* 2010, **132**, 8828.
2. H. J. Kim, C. H. Heo and H. M. Kim, *J. Am. Chem. Soc.* 2013, **135**, 17969.
3. J. Llopis, J. M. McCaffery, A. Miyawaki, M. G. Farquhar and R. Y. Tsien, *Proc. Natl. Acad. Sci. U.S.A.* 1998, **95**, 6803.
4. J. M. Holopainen, J. Saarikoski, P. K. J. Kinnunen and I. Järvelä, *Eur. J. Biochem.* 2001, **268**, 5851.

8. Synthesis

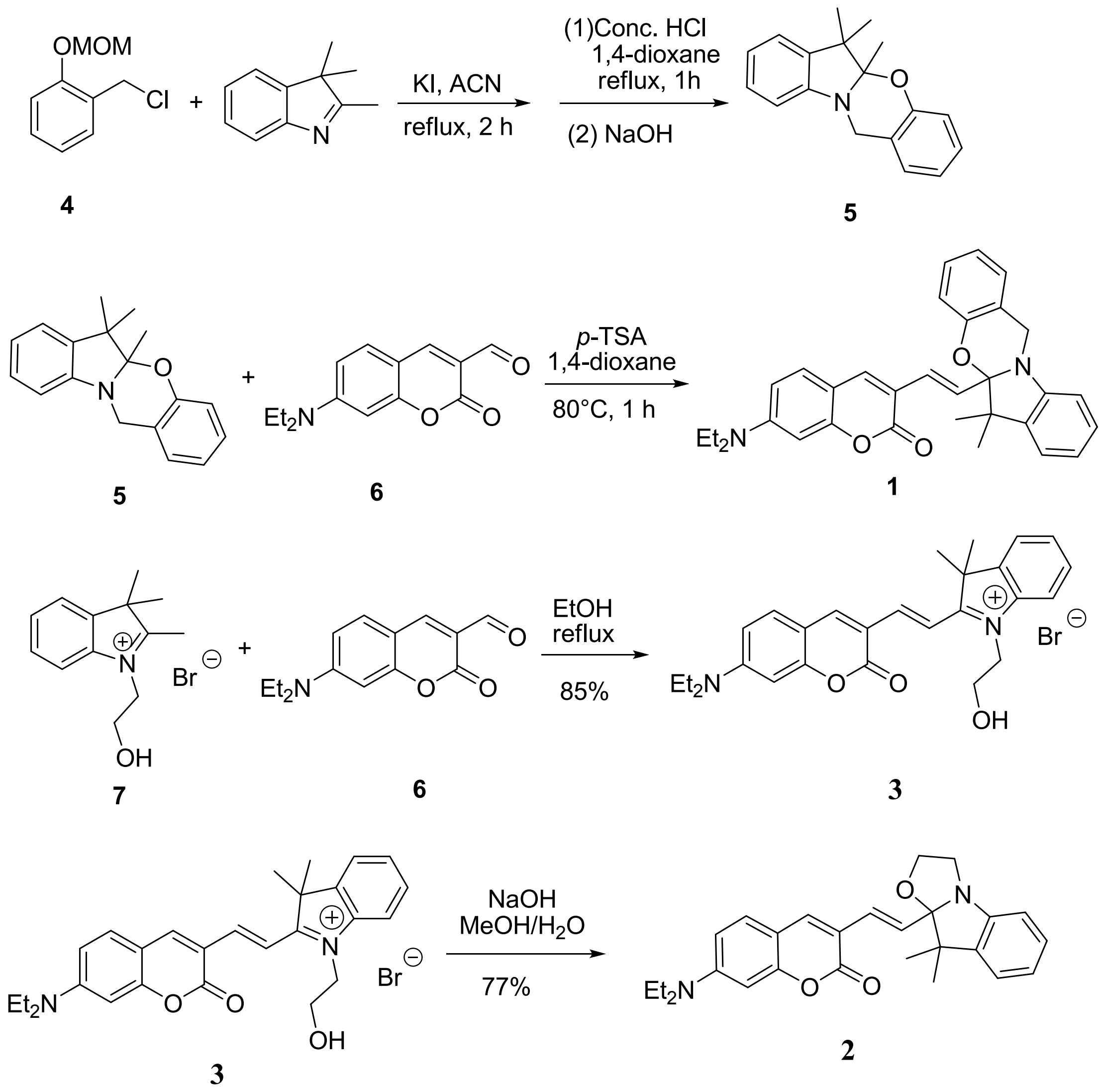

Fig. S1 Synthesis of probes **1**, **2** and **3**.

5*a*,6,6-Trimethyl-6,12-dihydro-5*aH*-benzo[5,6][1,3]oxazino[3,2-*a*]indole (5)

A mixture of **4** (0.4 g, 2.1 mmol), 2,3,3-trimethyl-3*H*-indole (0.37 g, 2.4 mmol) and KI (0.7 g, 4.2 mmol) in ACN was heated to reflux overnight. After cooling, solvent was evaporated and the residue was purified by column chromatography on silica gel (PE : EA = 1 : 1 and then DCM : MeOH = 20 : 1) to afford a red solid. Then this solid was dissolved in 1,4-dioxane (10 mL) and several drops of conc. HCl was introduced. The mixture was refluxed for 1 hour. After cooling, solvent was evaporated and the residue was fractionated in EA (10 mL) and water (10 mL). The aqueous phase was separated and basified with 1 M NaOH and then extracted with EA (2 × 20 mL). The organic phases were combined and washed with brine (20 mL), dried over anhydrous $MgSO_4$. After removal of solvent, the residue was purified by column chromatography on silica gel (PE : EA = 30 : 1) to afford **5** as a white solid (0.42 g, 75% yield).

m.p.: 126–128 °C.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18-7.08 (4H, m), 6.90-6.82 (2H, m), 6.72 (1H, dd, *J* = 8.2 Hz, *J'* = 0.9 Hz), 6.61 (1H, d, *J* = 7.8 Hz), 4.60 (2H, s), 1.60 (3H, s), 1.59 (3H, s), 1.23 (3H, s) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 153.2, 147.6, 138.5, 127.8, 127.4, 126.7, 122.1, 119.9, 119.7, 118.8, 117.8, 108.2, 100.2, 47.8, 40.3, 26.0, 19.1, 16.2 ppm.

HRMS (MALDI-TOF): m/z calcd for $C_{18}H_{20}NO$ [M + H^+] 266.1539, found, 266.1550.

(*E*)-7-(Diethylamino)-3-(2-(6,6-dimethyl-6,12-dihydro-5*aH*-benzo[5,6][1,3]oxazino[3,2-*a*]indol-5*a*-yl)vinyl)-2*H*-chromen-2-one (1)

To a solution of **5** (0.265 g, 1.0 mmol) and **6** (0.245 g, 1.0 mmol) in 1,4-dioxane (5 mL) was added *para*-toluenesulfonic acid monohydrate (20 mg, 0.1 mmol) and the mixture was heated to 80 °C for 1 hour. After cooling, solvent was removed under reduced pressure and the residue was dissolved in EA (20 mL). The organic phase was basified with 0.1 M NaOH, washed with water (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$. After removal of solvent, the residue was purified by column chromatography on silica gel (PE : EA = 10 : 1) to afford **1** as a light green solid (g, 65% yield).

m.p.: > 211 °C decomposed.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (1H, s), 7.22 (1H, d, *J* = 8.9 Hz), 7.12-7.01 (4H, m), 6.88 (1H, d, *J* = 16.0 Hz), 6.82-6.77 (2H, m), 6.73 (1H, d, *J* = 15.9 Hz), 6.60 (1H, d, *J* = 7.8 Hz), 6.56 (1H, dd, *J* = 8.8 Hz, *J'* = 2.5 Hz), 6.47 (1H, d, *J* = 2.4 Hz), 4.57 (H, d, *J* = 17.1 Hz), 4.49 (H, d, *J* = 17.1 Hz), 3.41 (4H, q, *J* = 7.1 Hz), 1.53 (3H, s), 1.23-1.19 (9H, m) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 160.9, 155.8, 153.6, 150.7, 147.5, 140.4, 138.7, 130.0, 128.9, 127.6, 127.4, 127.3, 126.6, 122.0, 120.0, 119.9, 119.6, 117.2, 116.4, 109.0, 108.7, 108.4, 101.8, 97.0, 49.9, 44.8, 41.0, 26.7, 18.7, 12.5 ppm.

HRMS (MALDI-TOF): m/z calcd for $C_{32}H_{33}N_2O_3$ [M + H^+] 493.2485, found, 493.2491.

(*E*)-2-(2-(7-(Diethylamino)-2-oxo-2*H*-chromen-3-yl)vinyl)-1-(2-hydroxyethyl)-3,3-dimethyl-3*H*-indol-1-ium bromide (**3**)

A mixture of **7** (118 mg, 0.42 mmol) and **6** (102 mg, 0.42 mmol) in absolute EtOH (10 mL) was refluxed overnight under N_2 atmosphere. After cooling, solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (PE : EA = 2 : 1 and then DCM : MeOH = 30 : 1) to afford **3** as a dark blue solid (182 mg, 85% yield).

m.p.: > 250 °C decomposed.

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.22 (1H, s), 8.52 (1H, d, *J* = 16.2 Hz), 8.14 (1H, d, *J* = 13.1 Hz), 8.11 (1H, d, *J* = 6.1 Hz), 7.54-7.46 (3H, m), 7.42-7.39 (1H, m), 6.68 (1H, dd, *J* = 9.1 Hz, *J'* = 2.4 Hz), 6.45 (1H, d, *J* = 2.3 Hz), 5.25 (1H, t, *J* = 7.8 Hz), 4.85 (2H, t, *J* = 5.3 Hz), 4.20-4.15 (2H, m), 3.50 (4H, q, *J* = 7.1 Hz), 1.81 (6H, s), 1.27 (6H, t, *J* = 7.1 Hz) ppm.

^{13}C NMR (100 MHz, $CDCl_3$) δ 182.0, 161.5, 158.8, 154.4, 149.6, 147.6, 143.0, 140.7, 134.6, 129.3, 128.6, 122.6, 113.3, 112.8, 111.2, 110.9, 109.6, 96.8, 59.1, 51.7, 49.5, 45.6, 28.0, 12.6 ppm.

HRMS (MALDI-TOF): m/z calcd for $C_{27}H_{31}N_2O_3^+$ [M^+] 431.2329, found, 431.2338.

(*E*)-7-(Diethylamino)-3-(2-(9,9-dimethyl-2,3,9,9a-tetrahydrooxazolo[3,2-*a*]indol-9a-yl)vinyl)-2*H*-chromen-2-one (**2**)

To a solution of **3** (120 mg, 0.23 mmol) in MeOH (5 mL) was added aqueous 1.0 M NaOH (5mL) and the mixture was stirred at room temperature for 1hour. MeOH was removed under reduced pressure and the aqueous phase was extracted with Et_2O (3 × 10 mL). The organic phases were combined and washed with water (10 mL), dried over anhydrous $MgSO_4$. After removal of solvent, the residue was purified by column chromatography on silica gel (PE : EA : TEA = 100 : 10 : 1) to afford **2** as a green semisolid (78 mg, 77% yield).

[1]H NMR (400 MHz, $CDCl_3$) δ 7.59 (1H, s), 7.26 (1H, d, *J* = 8.8 Hz), 7.16 (1H, dt, *J* = 7.6 Hz, *J'* = 1.3 Hz), 7.07 (1H, dd, *J* = 7.4 Hz, *J'* = 0.9 Hz), 6.93 (1H, dt, *J* = 7.4 Hz, *J'* = 0.9 Hz), 6.81 (1H, dd, *J* = 15.8 Hz, *J* = 0.4 Hz), 6.79 (1H, d, *J* = 7.7 Hz), 6.67 (1H, d, *J* = 15.8 Hz), 6.58 (1H, dd, *J* = 8.8 Hz, *J* = 2.5 Hz), 6.50 (1H, d, *J* = 2.4 Hz), 3.81-3.77 (1H, m), 3.69-3.60 (2H, m), 3.49-3.40 (5H, m), 1.44 (3H, s), 1.22 (6H, t, *J* = 7.1 Hz), 1.17 (3H, s) ppm.

[13]C NMR (100 MHz, $CDCl_3$) δ 161.2, 155.8, 150.8, 150.6, 139.8, 139.5, 128.9, 128.0, 127.5, 126.8, 122.4, 121.5, 116.9, 112.0, 110.1, 109.0, 108.8, 97.1, 63.5, 50.2, 47.9, 44.8, 28.6, 20.4, 12.5 ppm.

HRMS (MALDI-TOF): m/z calcd for $C_{27}H_{31}N_2O_3$ [M + H^+] 431.2329, found, 431.2297.

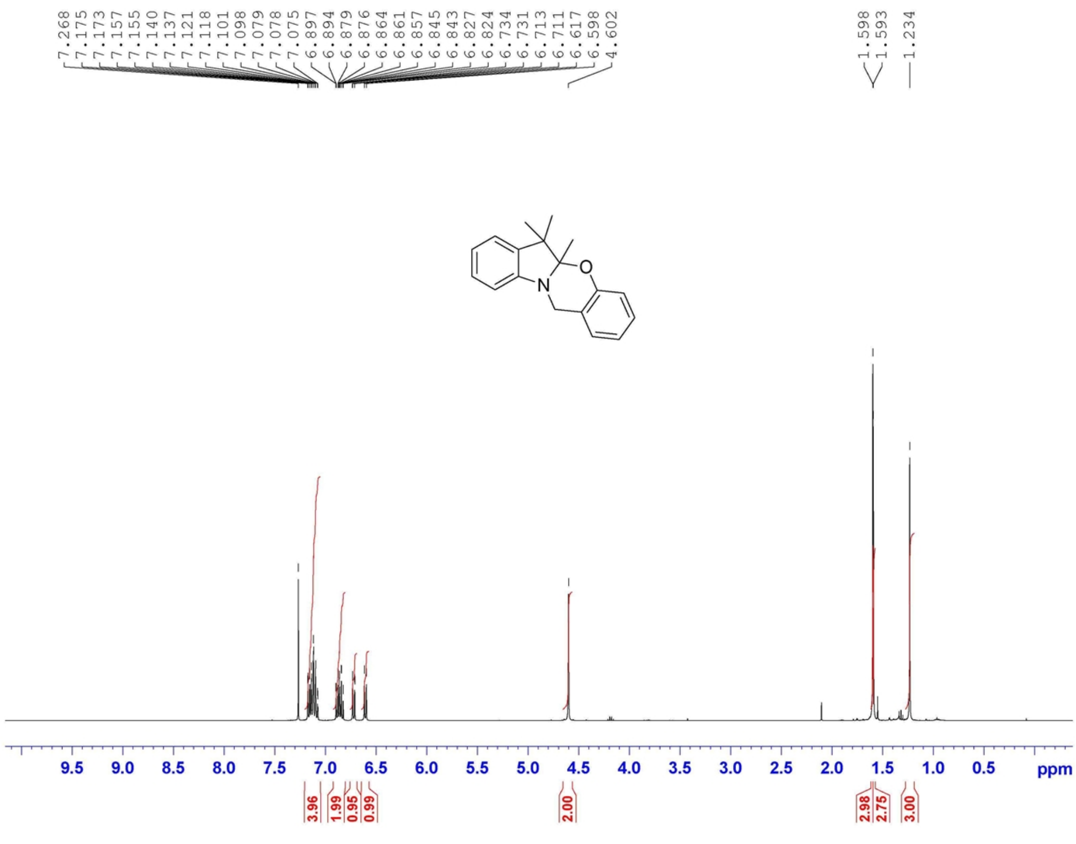

Fig. S2 [1]H NMR spectrum of probe **5** ($CDCl_3$, 400 MHz).

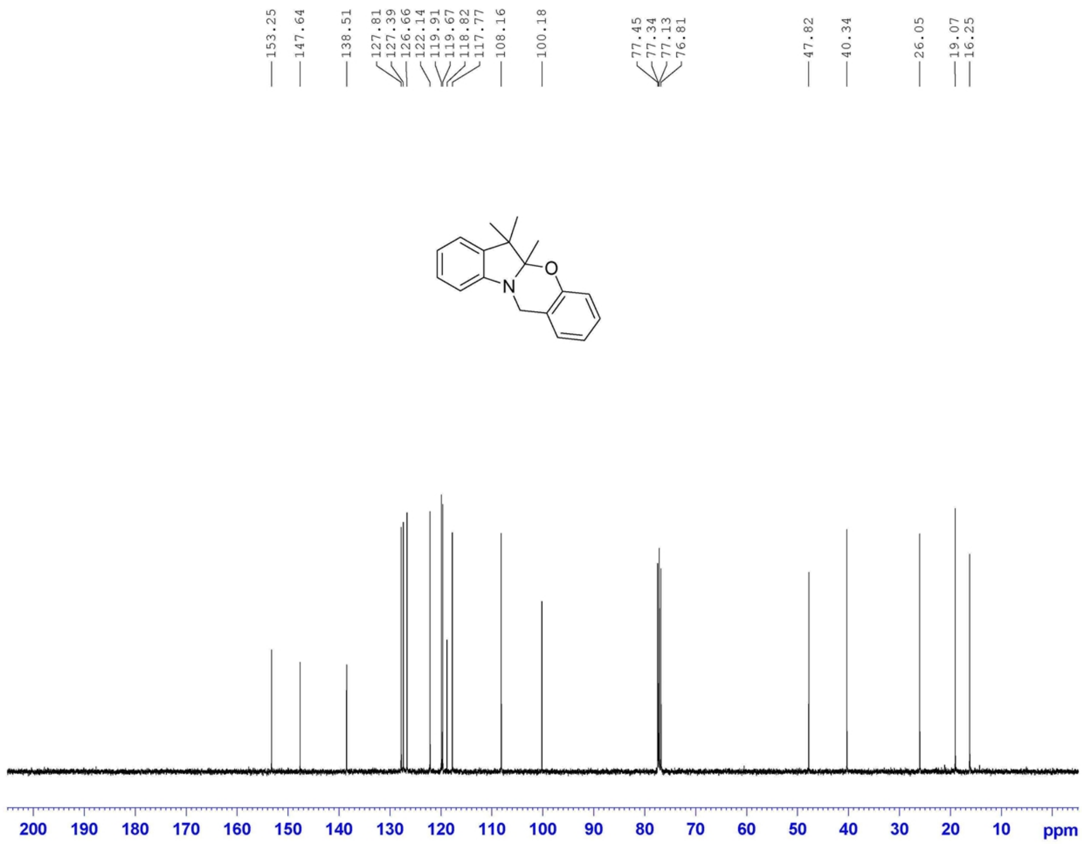

Fig. S3 ^{13}C NMR spectrum of probe **5** ($CDCl_3$, 100 MHz).

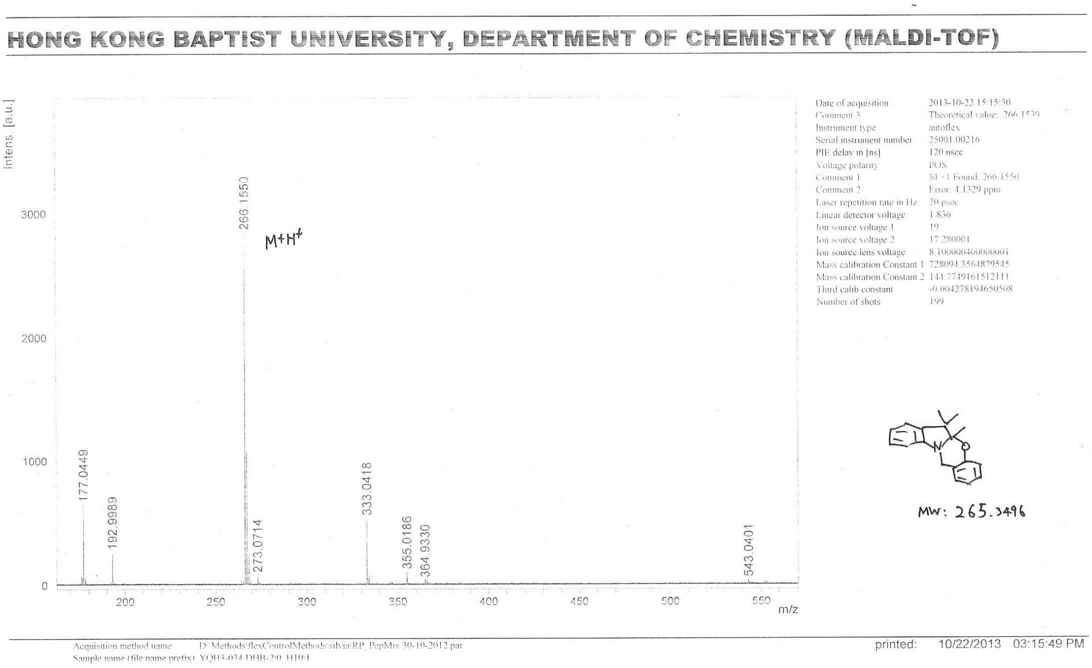

Fig. S4 MALDI-TOF HRMS spectrum of probe **5**.

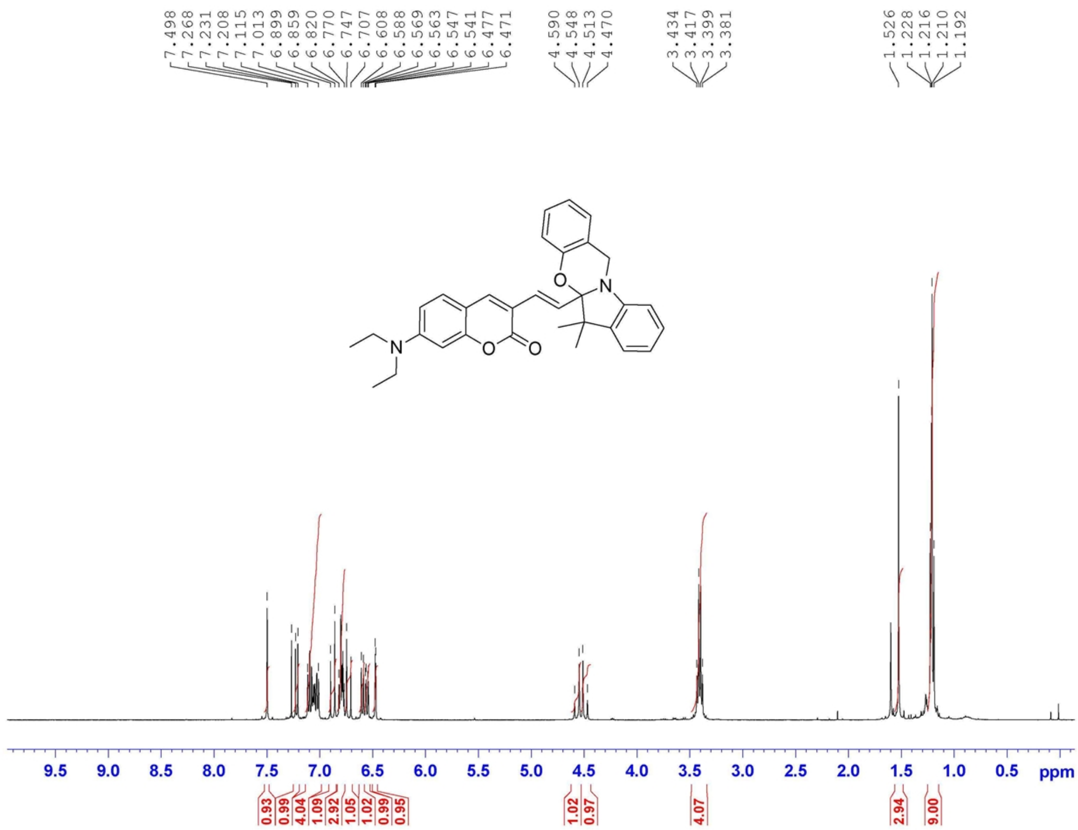

Fig. S5 ^{1}H NMR spectrum of probe **1** ($CDCl_3$, 400 MHz).

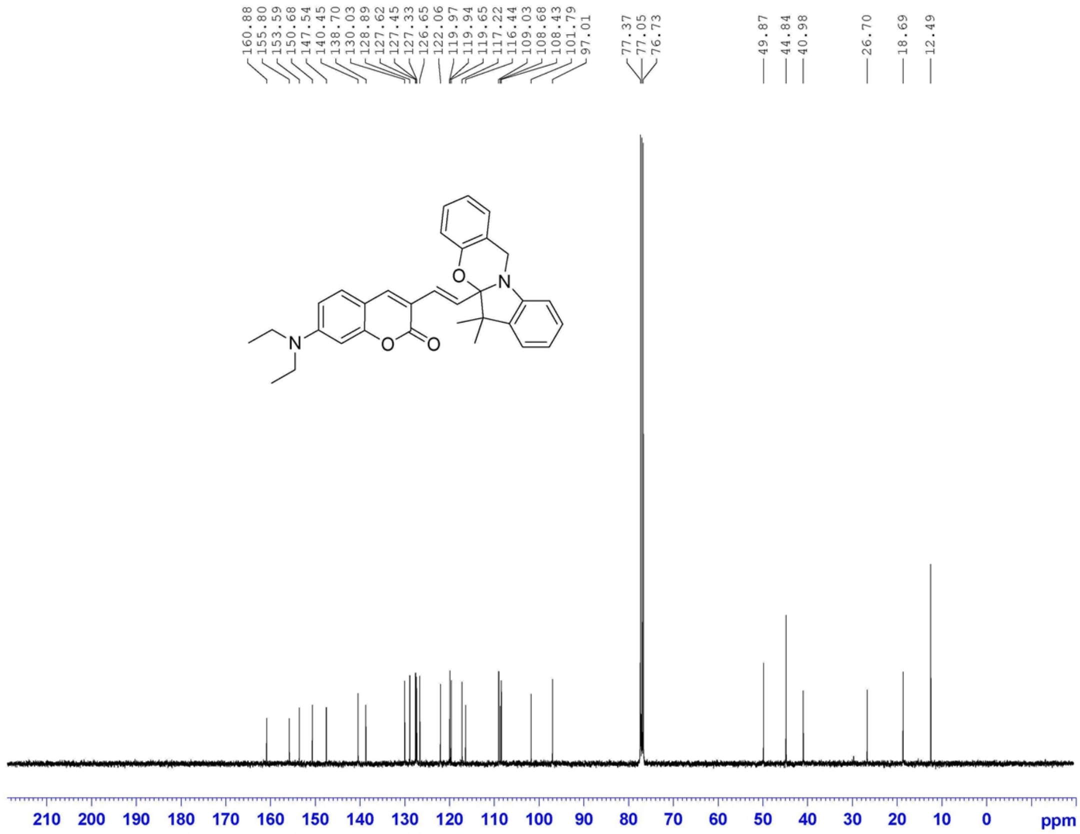

Fig. S6 ^{13}C NMR spectrum of probe **1** ($CDCl_3$, 100 MHz).

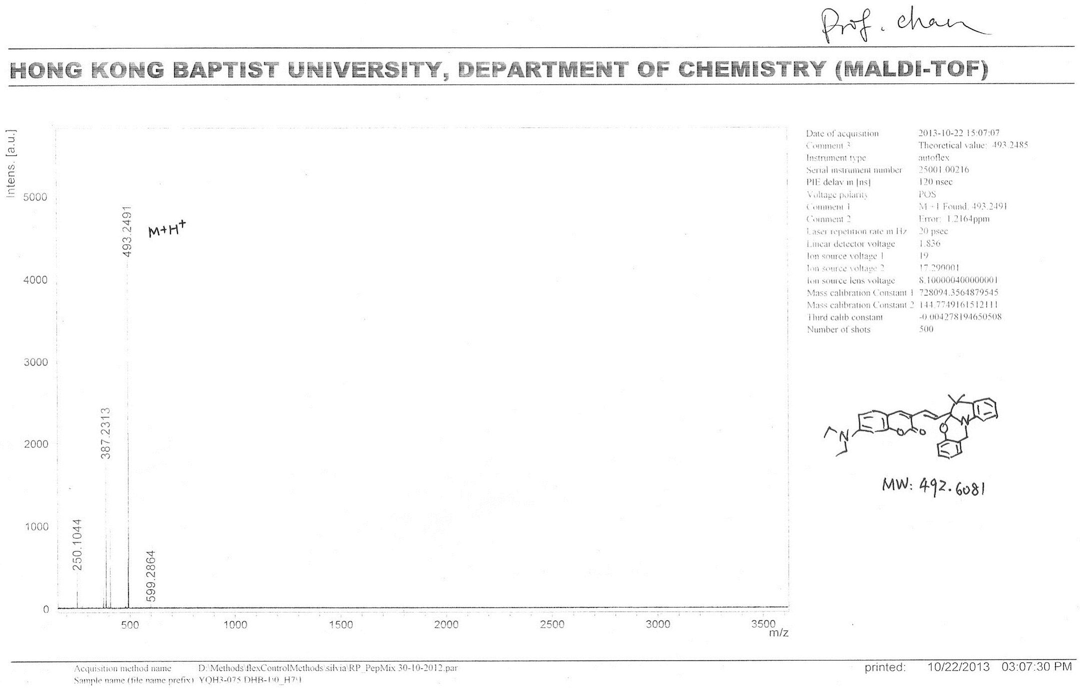

Fig. S7 MALDI-TOF HRMS spectrum of probe **1**.

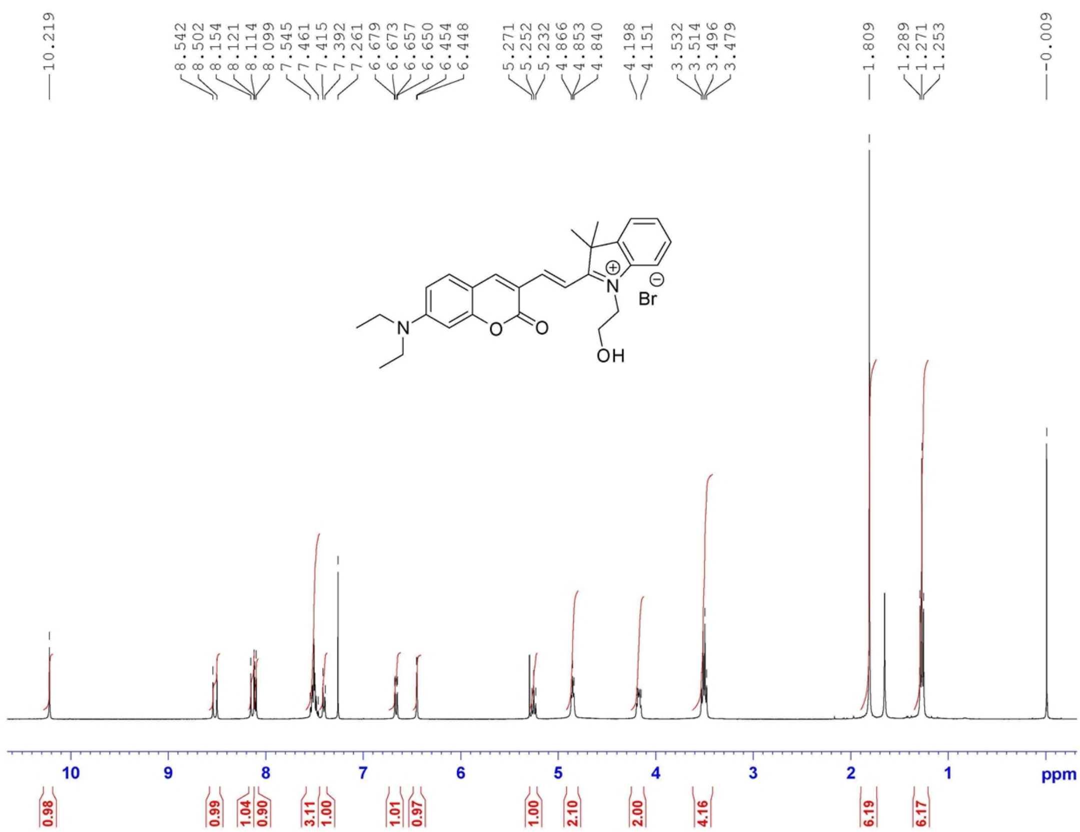

Fig. S8 ^{1}H NMR spectrum of probe **3** ($CDCl_3$, 400 MHz).

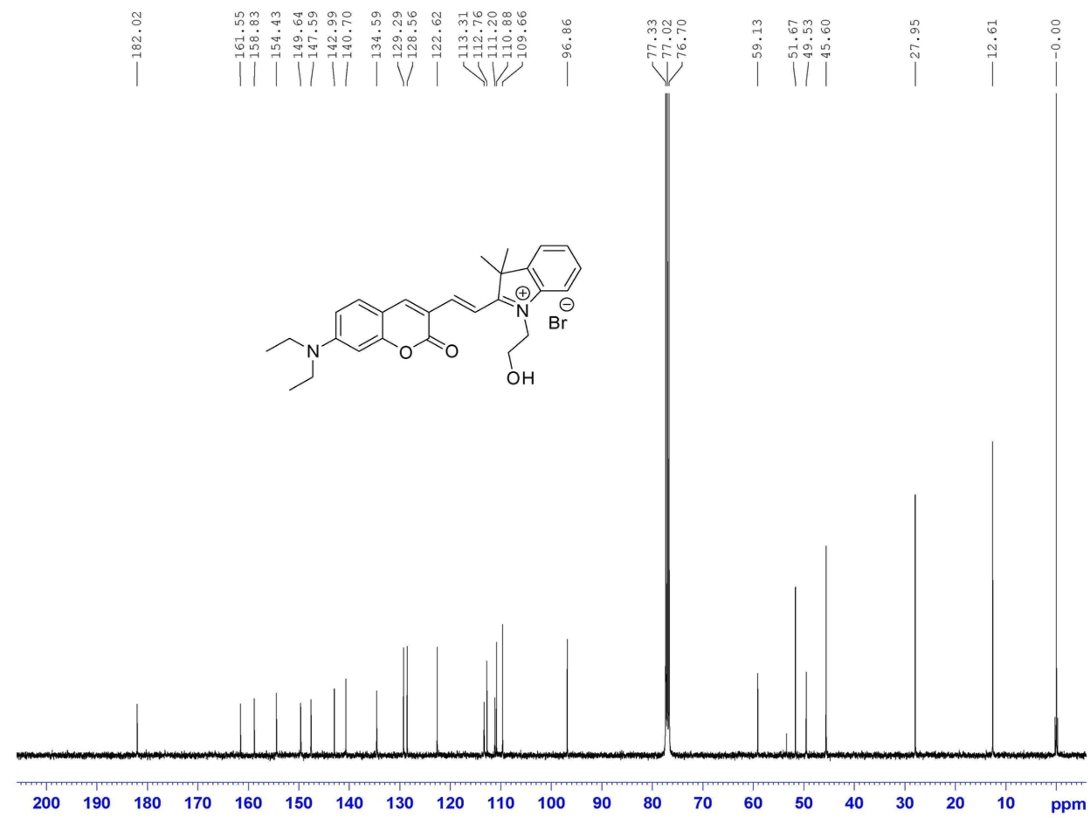

Fig. S9 [13]C NMR spectrum of probe **3** ($CDCl_3$, 100 MHz).

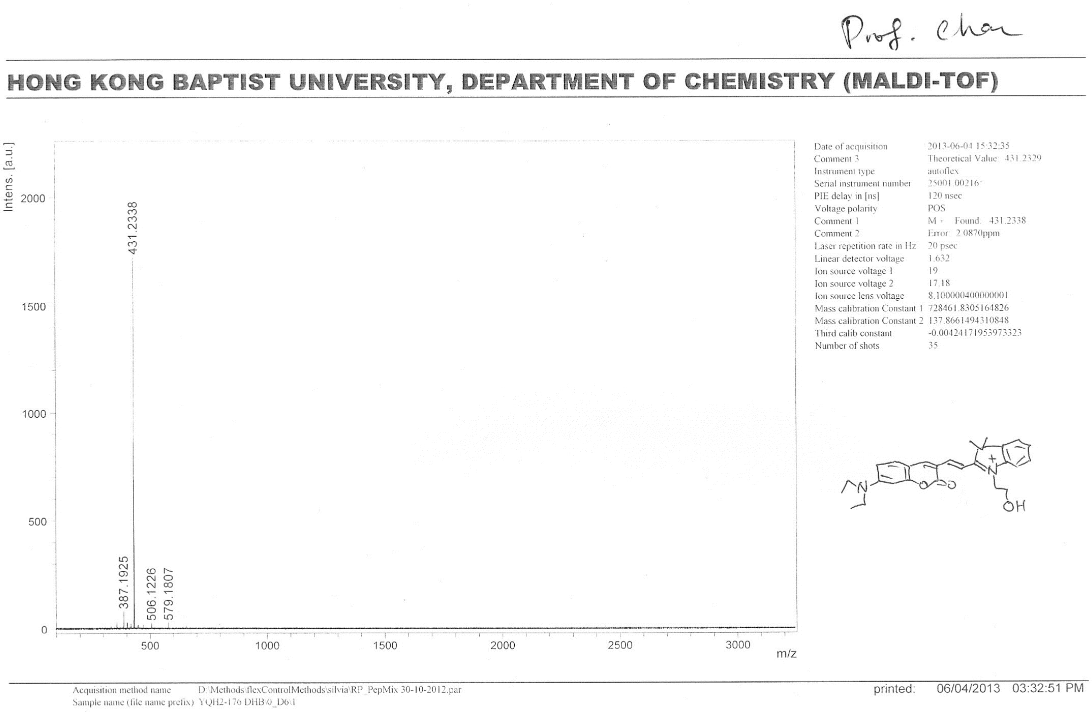

Fig. S10 MALDI-TOF HRMS spectrum of probe **3**.

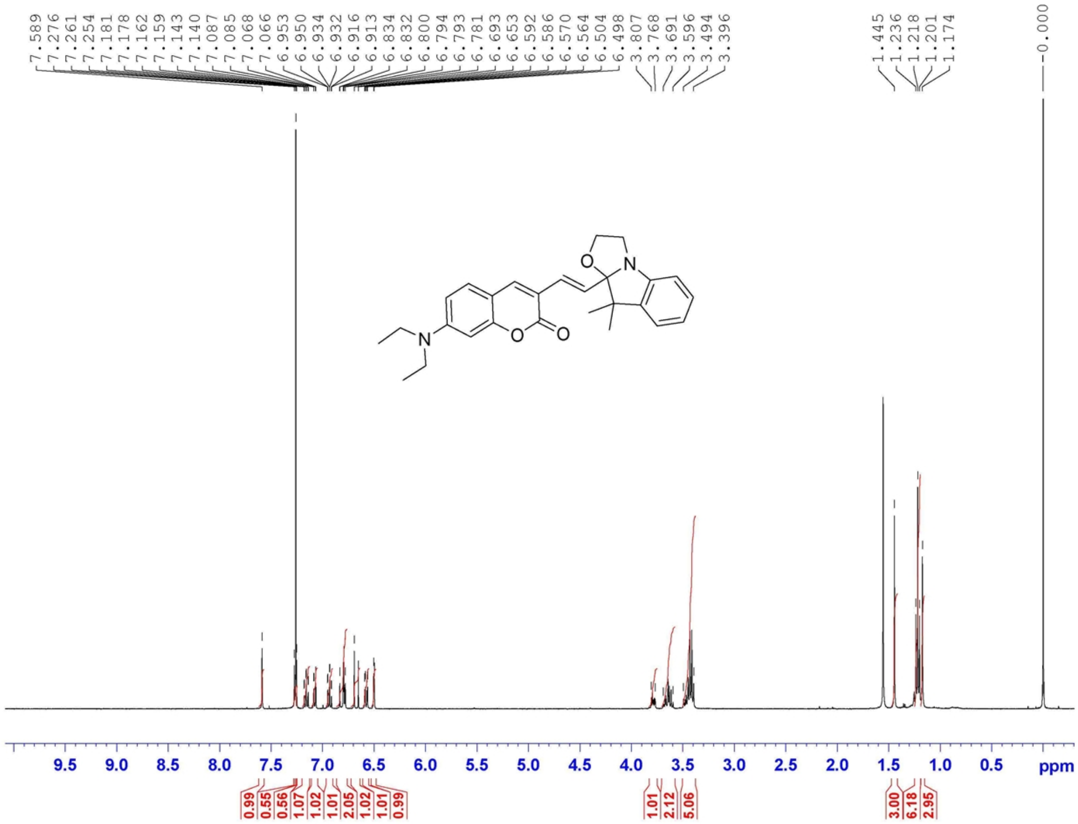

Fig. S11 ^{1}H NMR spectrum of probe **2** ($CDCl_3$, 400 MHz).

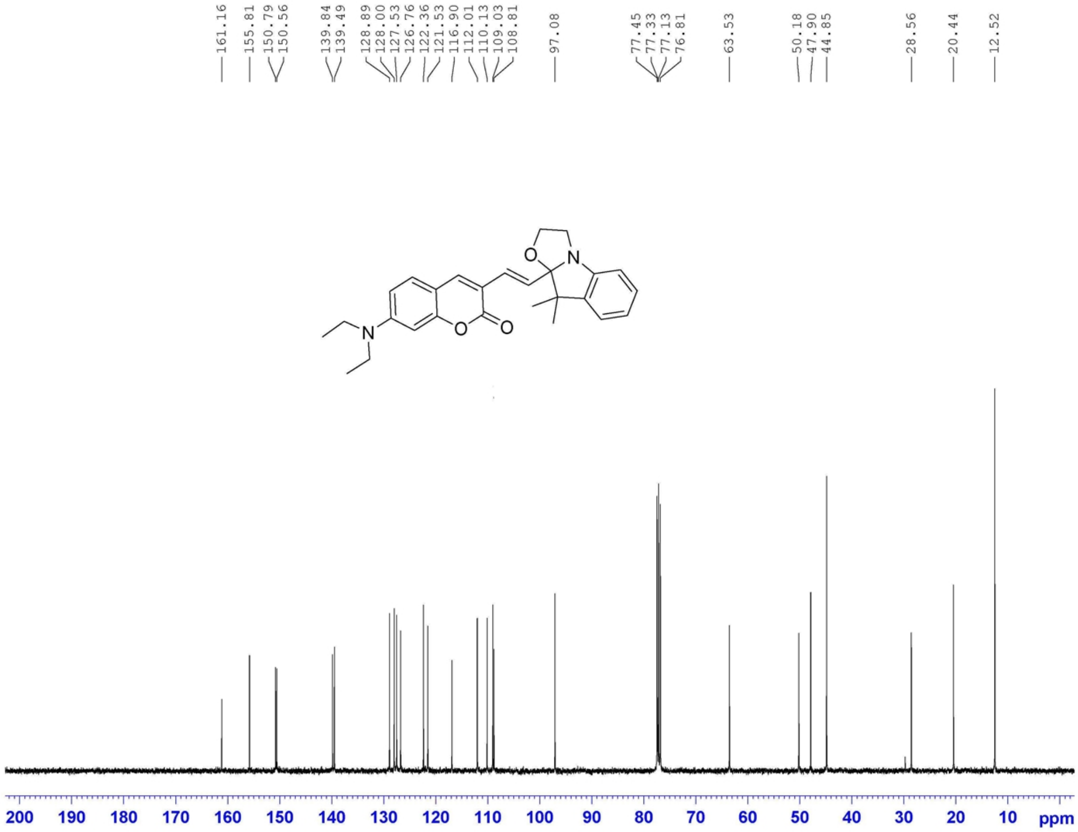

Fig. S12 ^{13}C NMR spectrum of probe **2** ($CDCl_3$, 100 MHz).

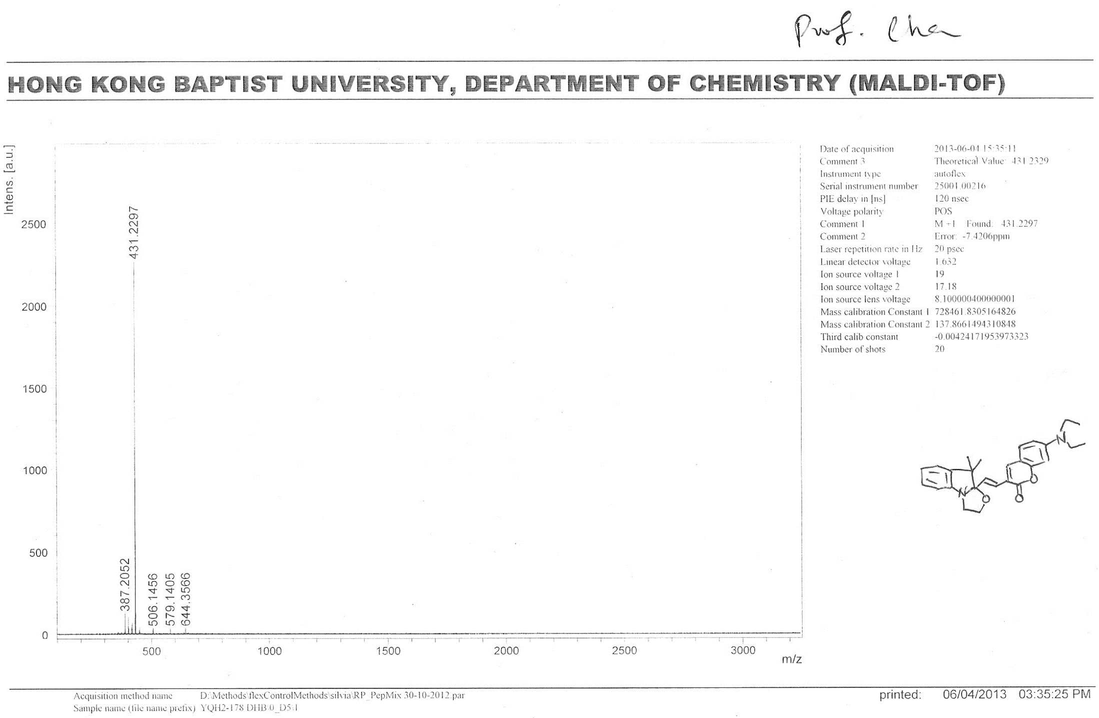

Fig. S13 MALDI-TOF HRMS spectrum of probe **2**.

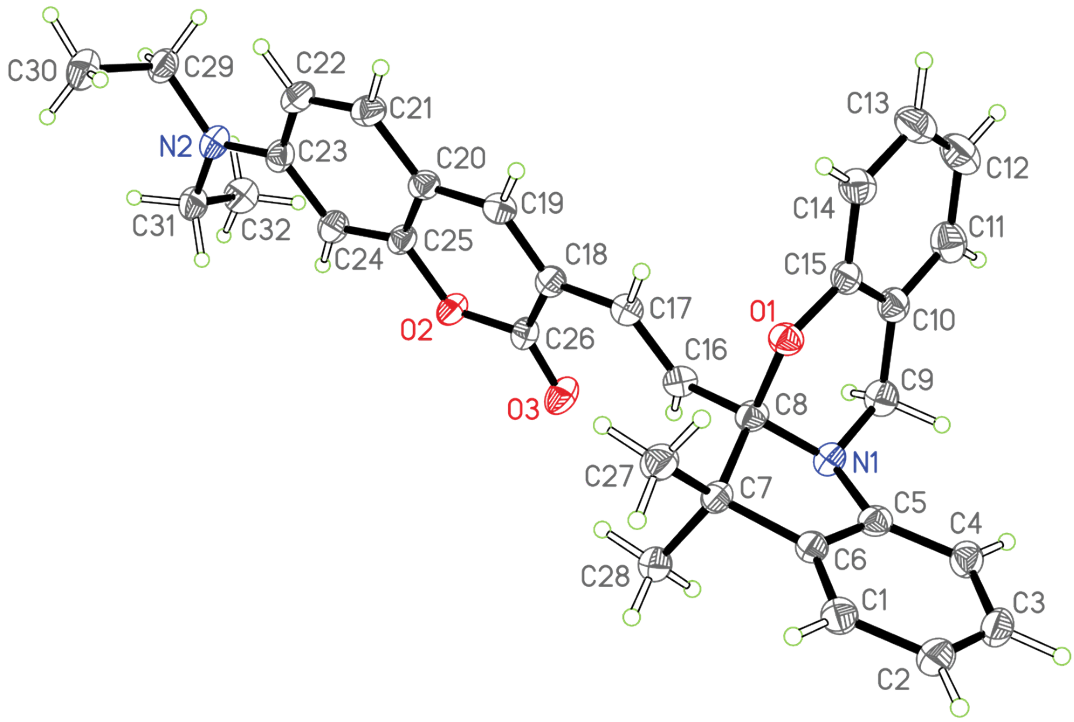

Fig. S14 X-ray crystal structure of **1**. All hydrogen atoms were omitted for clarity (50% probability level for the thermal ellipsoids).

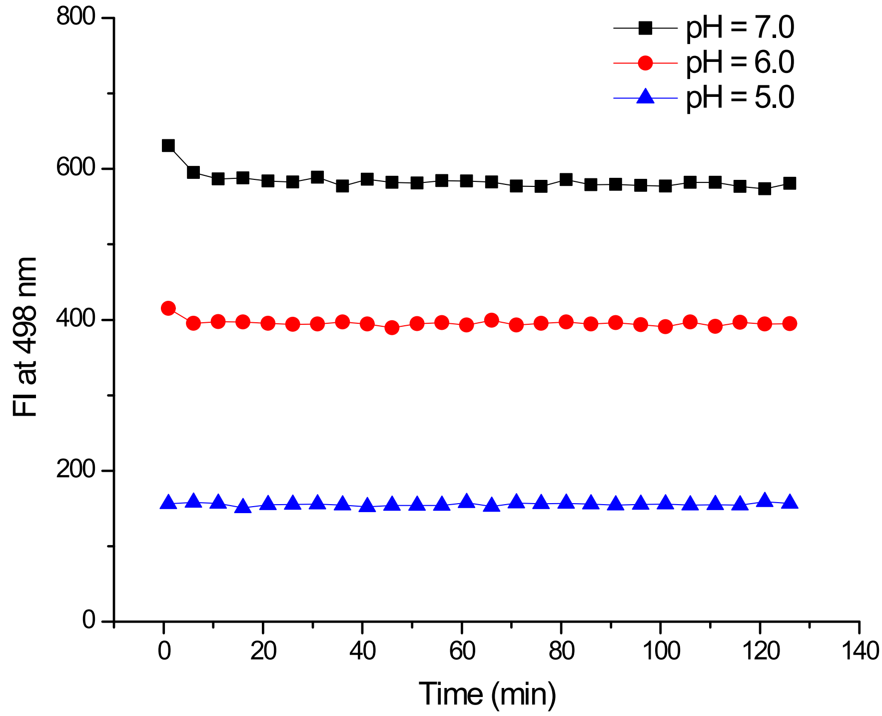

Fig. S15 Time-dependent profiles of probe **1** (5 μM) in Britton-Robinson buffer (40 mM, containing 50% ACN) at pH 5.0, 6.0 and 7.0, respectively.

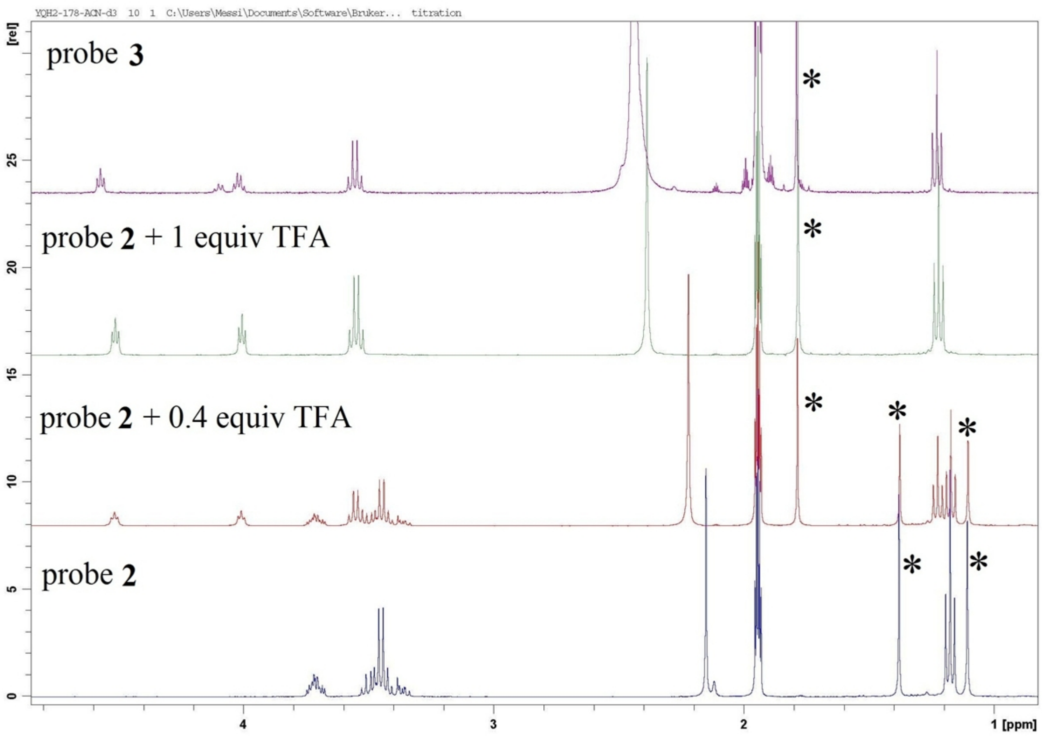

Fig. S16 Partial ^{1}H NMR titration spectra (only δ 0.8 – 5.0 region shown) of probe **2** in ACN-d_3 upon addition of TFA.

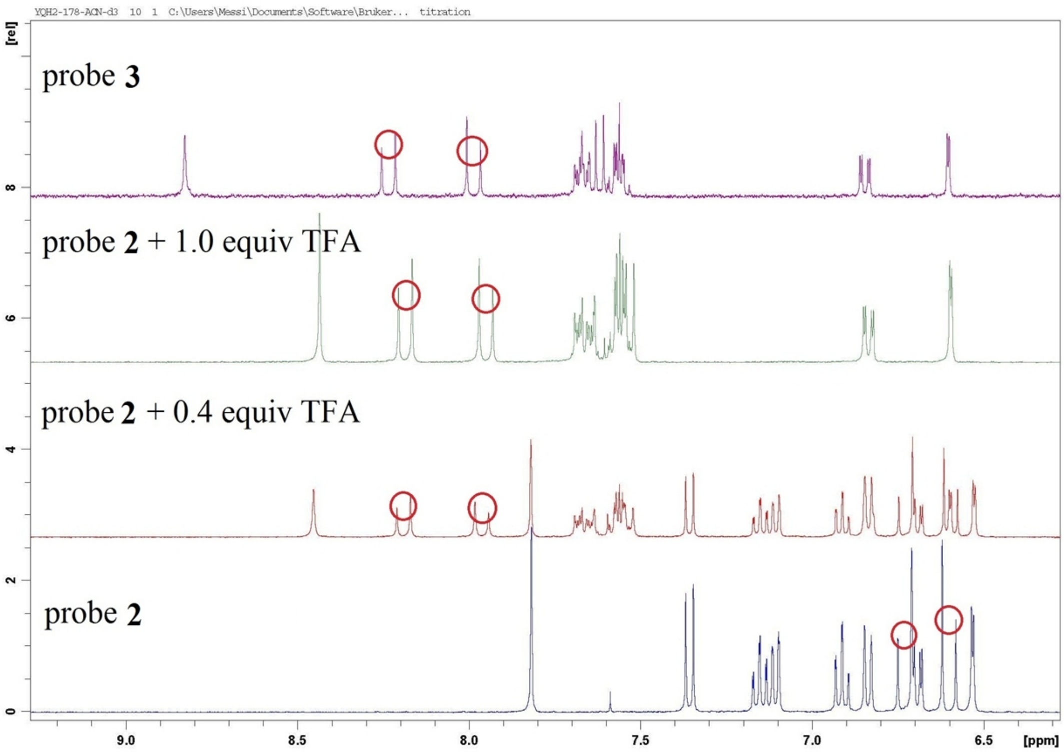

Fig. S17 Partial ^{1}H NMR titration spectra (only δ 6.3 – 9.6 region shown) of probe **2** in ACN-d_3 upon addition of TFA.

Table S1. Crystal data and structure refinement for probe **1**.

Compound reference	**1**
Chemical formula	$C_{32}H_{32}N_2O_3$
Formula Mass	492.60
Crystal system	Monoclinic
a/Å	11.5661(8)
b/Å	17.7172(12)
c/Å	14.3741(10)
α/°	90.00
β/°	105.941(2)
γ/°	90.00
Unit cell volume/Å^3	2832.3(3)
Temperature/K	133(2)

Space group	*P*21/*n*
No. of formula units per unit cell, Z	4
Radiation type	
Absorption coefficient, μ/mm^{-1}	0.074
No. of reflections measured	27644
No. of independent reflections	5136
Final R_1 values ($I > 2\sigma(I)$)	0.0425
Final w$R(F^2)$ values ($I > 2\sigma(I)$)	0.0981
Final R_1 values (all data)	0.0627
Final w$R(F^2)$ values (all data)	0.1038
Goodness of fit on F^2	1.053
Flack parameter	
Rogers parameter	
CCDC number	

Table S2. Bond lengths [A] and angles [deg] for probe **1**.

O(1)-C(15)	1.3881(17)
O(1)-C(8)	1.4545(18)
O(2)-C(26)	1.3711(18)
O(2)-C(25)	1.3741(18)
O(3)-C(26)	1.2137(19)
N(1)-C(5)	1.407(2)
N(1)-C(8)	1.4550(19)
N(1)-C(9)	1.4599(19)
N(2)-C(23)	1.364(2)
N(2)-C(29)	1.457(2)
N(2)-C(31)	1.461(2)
C(1)-C(6)	1.376(2)
C(1)-C(2)	1.390(2)
C(1)-H(1A)	0.9500
C(2)-C(3)	1.380(2)
C(2)-H(2A)	0.9500
C(3)-C(4)	1.390(2)

C(3)-H(3A)	0.9500
C(4)-C(5)	1.385(2)
C(4)-H(4A)	0.9500
C(5)-C(6)	1.391(2)
C(6)-C(7)	1.505(2)
C(7)-C(27)	1.519(2)
C(7)-C(28)	1.543(2)
C(7)-C(8)	1.557(2)
C(8)-C(16)	1.495(2)
C(9)-C(10)	1.496(2)
C(9)-H(9A)	0.9900
C(9)-H(9B)	0.9900
C(10)-C(15)	1.387(2)
C(10)-C(11)	1.391(2)
C(11)-C(12)	1.379(3)
C(11)-H(11A)	0.9500
C(12)-C(13)	1.380(3)
C(12)-H(12A)	0.9500
C(13)-C(14)	1.377(2)
C(13)-H(13A)	0.9500
C(14)-C(15)	1.382(2)
C(14)-H(14A)	0.9500
C(16)-C(17)	1.330(2)
C(16)-H(16A)	0.9500
C(17)-C(18)	1.453(2)
C(17)-H(17A)	0.9500
C(18)-C(19)	1.362(2)
C(18)-C(26)	1.456(2)
C(19)-C(20)	1.412(2)
C(19)-H(19A)	0.9500
C(20)-C(25)	1.393(2)
C(20)-C(21)	1.405(2)
C(21)-C(22)	1.370(2)
C(21)-H(21A)	0.9500
C(22)-C(23)	1.418(2)
C(22)-H(22A)	0.9500
C(23)-C(24)	1.400(2)
C(24)-C(25)	1.370(2)
C(24)-H(24A)	0.9500
C(27)-H(27A)	0.9800
C(27)-H(27B)	0.9800
C(27)-H(27C)	0.9800
C(28)-H(28A)	0.9800
C(28)-H(28B)	0.9800

C(28)-H(28C)	0.9800
C(29)-C(30)	1.516(2)
C(29)-H(29A)	0.9900
C(29)-H(29B)	0.9900
C(30)-H(30A)	0.9800
C(30)-H(30B)	0.9800
C(30)-H(30C)	0.9800
C(31)-C(32)	1.513(2)
C(31)-H(31A)	0.9900
C(31)-H(31B)	0.9900
C(32)-H(32A)	0.9800
C(32)-H(32B)	0.9800
C(32)-H(32C)	0.9800
C(15)-O(1)-C(8)	114.49(11)
C(26)-O(2)-C(25)	123.42(12)
C(5)-N(1)-C(8)	106.73(12)
C(5)-N(1)-C(9)	121.25(12)
C(8)-N(1)-C(9)	114.93(12)
C(23)-N(2)-C(29)	122.39(14)
C(23)-N(2)-C(31)	121.49(14)
C(29)-N(2)-C(31)	116.12(13)
C(6)-C(1)-C(2)	118.85(15)
C(6)-C(1)-H(1A)	120.6
C(2)-C(1)-H(1A)	120.6
C(3)-C(2)-C(1)	120.09(16)
C(3)-C(2)-H(2A)	120.0
C(1)-C(2)-H(2A)	120.0
C(2)-C(3)-C(4)	121.78(15)
C(2)-C(3)-H(3A)	119.1
C(4)-C(3)-H(3A)	119.1
C(5)-C(4)-C(3)	117.44(15)
C(5)-C(4)-H(4A)	121.3
C(3)-C(4)-H(4A)	121.3
C(4)-C(5)-C(6)	121.20(15)
C(4)-C(5)-N(1)	129.43(14)
C(6)-C(5)-N(1)	109.32(13)
C(1)-C(6)-C(5)	120.63(15)
C(1)-C(6)-C(7)	130.32(14)
C(5)-C(6)-C(7)	108.96(13)
C(6)-C(7)-C(27)	115.10(13)
C(6)-C(7)-C(28)	108.56(13)
C(27)-C(7)-C(28)	109.11(12)
C(6)-C(7)-C(8)	100.00(11)
C(27)-C(7)-C(8)	113.86(13)

C(28)-C(7)-C(8)	109.81(13)
O(1)-C(8)-N(1)	109.93(11)
O(1)-C(8)-C(16)	110.56(12)
N(1)-C(8)-C(16)	113.18(13)
O(1)-C(8)-C(7)	106.37(11)
N(1)-C(8)-C(7)	102.36(12)
C(16)-C(8)-C(7)	113.96(12)
N(1)-C(9)-C(10)	111.75(13)
N(1)-C(9)-H(9A)	109.3
C(10)-C(9)-H(9A)	109.3
N(1)-C(9)-H(9B)	109.3
C(10)-C(9)-H(9B)	109.3
H(9A)-C(9)-H(9B)	107.9
C(15)-C(10)-C(11)	118.32(15)
C(15)-C(10)-C(9)	120.76(14)
C(11)-C(10)-C(9)	120.91(14)
C(12)-C(11)-C(10)	121.03(16)
C(12)-C(11)-H(11A)	119.5
C(10)-C(11)-H(11A)	119.5
C(11)-C(12)-C(13)	119.57(16)
C(11)-C(12)-H(12A)	120.2
C(13)-C(12)-H(12A)	120.2
C(14)-C(13)-C(12)	120.45(16)
C(14)-C(13)-H(13A)	119.8
C(12)-C(13)-H(13A)	119.8
C(13)-C(14)-C(15)	119.66(16)
C(13)-C(14)-H(14A)	120.2
C(15)-C(14)-H(14A)	120.2
C(14)-C(15)-C(10)	120.96(14)
C(14)-C(15)-O(1)	117.00(14)
C(10)-C(15)-O(1)	122.03(14)
C(17)-C(16)-C(8)	123.55(14)
C(17)-C(16)-H(16A)	118.2
C(8)-C(16)-H(16A)	118.2
C(16)-C(17)-C(18)	128.31(15)
C(16)-C(17)-H(17A)	115.8
C(18)-C(17)-H(17A)	115.8
C(19)-C(18)-C(17)	121.13(14)
C(19)-C(18)-C(26)	118.15(14)
C(17)-C(18)-C(26)	120.54(14)
C(18)-C(19)-C(20)	122.96(15)
C(18)-C(19)-H(19A)	118.5
C(20)-C(19)-H(19A)	118.5
C(25)-C(20)-C(21)	115.89(14)

C(25)-C(20)-C(19)	118.01(14)
C(21)-C(20)-C(19)	126.08(15)
C(22)-C(21)-C(20)	121.54(15)
C(22)-C(21)-H(21A)	119.2
C(20)-C(21)-H(21A)	119.2
C(21)-C(22)-C(23)	121.37(15)
C(21)-C(22)-H(22A)	119.3
C(23)-C(22)-H(22A)	119.3
N(2)-C(23)-C(24)	121.02(15)
N(2)-C(23)-C(22)	121.48(15)
C(24)-C(23)-C(22)	117.50(15)
C(25)-C(24)-C(23)	119.54(15)
C(25)-C(24)-H(24A)	120.2
C(23)-C(24)-H(24A)	120.2
C(24)-C(25)-O(2)	116.21(14)
C(24)-C(25)-C(20)	124.14(15)
O(2)-C(25)-C(20)	119.66(14)
O(3)-C(26)-O(2)	115.38(14)
O(3)-C(26)-C(18)	126.89(14)
O(2)-C(26)-C(18)	117.72(13)
C(7)-C(27)-H(27A)	109.5
C(7)-C(27)-H(27B)	109.5
H(27A)-C(27)-H(27B)	109.5
C(7)-C(27)-H(27C)	109.5
H(27A)-C(27)-H(27C)	109.5
H(27B)-C(27)-H(27C)	109.5
C(7)-C(28)-H(28A)	109.5
C(7)-C(28)-H(28B)	109.5
H(28A)-C(28)-H(28B)	109.5
C(7)-C(28)-H(28C)	109.5
H(28A)-C(28)-H(28C)	109.5
H(28B)-C(28)-H(28C)	109.5
N(2)-C(29)-C(30)	113.16(14)
N(2)-C(29)-H(29A)	108.9
C(30)-C(29)-H(29A)	108.9
N(2)-C(29)-H(29B)	108.9
C(30)-C(29)-H(29B)	108.9
H(29A)-C(29)-H(29B)	107.8
C(29)-C(30)-H(30A)	109.5
C(29)-C(30)-H(30B)	109.5
H(30A)-C(30)-H(30B)	109.5
C(29)-C(30)-H(30C)	109.5
H(30A)-C(30)-H(30C)	109.5
H(30B)-C(30)-H(30C)	109.5

N(2)-C(31)-C(32)	114.28(14)
N(2)-C(31)-H(31A)	108.7
C(32)-C(31)-H(31A)	108.7
N(2)-C(31)-H(31B)	108.7
C(32)-C(31)-H(31B)	108.7
H(31A)-C(31)-H(31B)	107.6
C(31)-C(32)-H(32A)	109.5
C(31)-C(32)-H(32B)	109.5
H(32A)-C(32)-H(32B)	109.5
C(31)-C(32)-H(32C)	109.5
H(32A)-C(32)-H(32C)	109.5
H(32B)-C(32)-H(32C)	109.5