Supporting Information for"Naphthoimidazolium-cholesterol derivative as a ratiometric fluorescence based chemosensor for the chiral recognition of carboxylates"

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Experimental

Unless otherwise noted, materials were obtained from commercial suppliers, and were used without further purification. Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates of 0.25 mm thickness. Preparative TLC was performed using Merck 60 F_{254} plates of 1 mm thickness. ^1H NMR and ^{13}C NMR spectra were recorded using Bruker (300 and 75) MHz. Chemical shifts are expressed in ppm and coupling constants (J) in Hz. Melting points were measured using a Büchi 530 melting point apparatus. Fluorescence emission spectra were obtained by FS-2 fluorescence spectrometry (Scinco) at room temperature.

Synthesis of compound 3: 4.0 g of cholesteryl chloroformate (8.9 mmol) and 2-iodoethanol (1.58 g, 9.2 mmol) were dissolved in 30 ml of anhydrous dichloromethane. 1.42 ml of pyridine was then added at 0 °C. The mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, hexane/dichloromethane = 1:1, v/v). 4.2 g of white solid was obtained, yield: 80.8 %. M.p. = 105.3-107.1 °C. ^1H NMR (300 MHz, CDCl$_3$) δ5.42 (s, 1H, He), 4.45-4.56 (m, 1H, Hc), 4.38 (t, 2H, J = 6.9 Hz, Hb), 3.33 (t, 2H, J = 6.9 Hz, Ha), 2.41 (d, 2H, J = 6.6 Hz, Hd), 1.88-2.05 (m, 5H,Cholesteryl Hs), 0.87-1.70 (m, 33H, Cholesteryl Hs), 0.69 (s, 3H,Cholesteryl-CH$_3$).^{13}C NMR (75MHz, CDCl$_3$) δ 139.6, 123.5, 78.8, 67.9, 57.1, 56.5, 50.4, 42.7, 40.1, 39.9, 38.4, 37.2, 36.9, 36.6, 36.2, 32.2, 28.6, 28.4, 28.1, 24.7, 24.2, 23.3, 23.0, 21.5, 19.7, 19.1, 12.3. FAB-HRMS C$_{30}$H$_{49}$IO$_3$ ([M+Na]$^+$) calcd 607.2624, found 607.2624.

Synthesis of compound 4: 61 mg of **2** (0.33 mmol) and 193 mg of **3** (0.33 m mol) were added into 5 ml of acetonitrile. The mixture stirred at reflux under ni trogen atmosphere for 24 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was washed with methanol and d ried to give 205 mg of **4** as a white solid, yield: 81.1 %. M.p. = 215.9-217.4 °C. ^1H NMR (300 MHz, CDCl$_3$) δ11.35 (s, 1H, Ha), 8.26 (s, 1H, Hb), 8.16 (s, 1H, Hc), 8.09-8.14 (m, 2H, Hd and Hg), 7.66-7.72 (m, 2H, He and Hg), 5.26 (s, 1H, Hl), 5.13 (t, 2H, J = 5.4 Hz, Hi), 4.80 (t, 2H, J = 5.4 Hz, Hj), 4.34 (s, 3H, H h), 4.19-4.30 (m, 1H, Hk), 0.87-2.15 (m, 40H, Cholesteryl Hs), 0.67 (s, 3H, Cho lesteryl-CH$_3$). ^{13}C NMR (75 MHz, CDCl$_3$) δ 153.8, 146.2, 138.9, 133.8, 131.9, 1 31.7, 130.7, 128.4, 128.3, 127.5, 123.1, 110.9, 110.3, 100.0, 78.8, 56.6, 56.1, 49. 9, 42.3, 39.5, 36.4, 31.8, 28.0, 23.8, 22.8, 22.6, 19.2, 18.7, 11.8. FAB-HRMS C$_4$$_2H_{59}IN_2O_3$ ([M+Na]$^+$) calcd 789.3468, found 789.3469.

Synthesis of compound 1 (NI-chol 1): 470 mg of compound **4** was dissolved in 8 ml of DMF, then aqueous saturated KFP_6 was added dropwise to give a white precipitate. The residue was filtered under reduced pressure and washed with water several times and dried in a vaccum oven at 40 °C for 24 h. 480 mg of **1** as a white solid was obtained, yield: 98.6 %. M.p. = 235.7-237.4 °C. ^{1}H NMR (300 MHz, CD_3CN) δ 9.18 (s, 1H, Ha), 8.43 (s, 1H, Hb), 8.40 (s, 1H, Hc), 8.14-8.21 (m, 2H, Hd and Hf), 7.67-7.72 (m, 2H, He and Hg), 5.27 (d, 1H, J = 4.5 Hz, Hm), 4.84 (t, 2H, J = 5.1 Hz, Hi), 4.64 (t, 2H, J = 5.1 Hz, Hj), 4.16 (s, 3H, Hh), 4.14-4.24 (m, 1H, Hk), 2.91 and 2.79 (dd, 1H, J = 0.3 Hz and 0.6 Hz, Hl), 2.11-2.15 (m, 2H, Cholesteryl Hs) 2.02-2.04 (m, 1H, Hl), 1.76-1.93 (m, 3H, Cholesteryl Hs), 1.37-1.60 (m, 11H, Cholesteryl Hs), 1.01-1.20 (m, 8H, Cholesteryl Hs), 0.87-0.97 (m, 14H, Cholesteryl Hs), 0.69 (s, 3H, Cholesteryl-CH_3). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 153.8, 147.6, 139.4, 131.4, 128.7, 127.1, 122.8, 111.5, 78.0, 65.5, 56.5, 56.0, 49.7, 46.4, 42.3, 36.6, 36.4, 36.2, 36.1, 35.7, 33.9, 31.7, 27.9, 27.4, 24.3, 23.7, 23.1, 22.8, 21.0, 19.2, 19.0, 21.1. FAB-HRMS $C_{42}H_{59}F_6N_2O_3P$ ($[M-PF_6]^+$) calcd 639.4526, found 639.4256.

Quantum chemical calculations The optimized structures were obtained using the DFT method (ωB97XD/ 6-31+G(d) or APFD/6-31+G(d)) as implemented in the Gaussian 16 package. The absorption spectra of optimized structures were calculated using TD-DFT method. The vibronic emission spectra of protonated and deprotonated **NI** were calculated with the bandwidth of 1200 cm^{-1} for individual vibronic peaks.
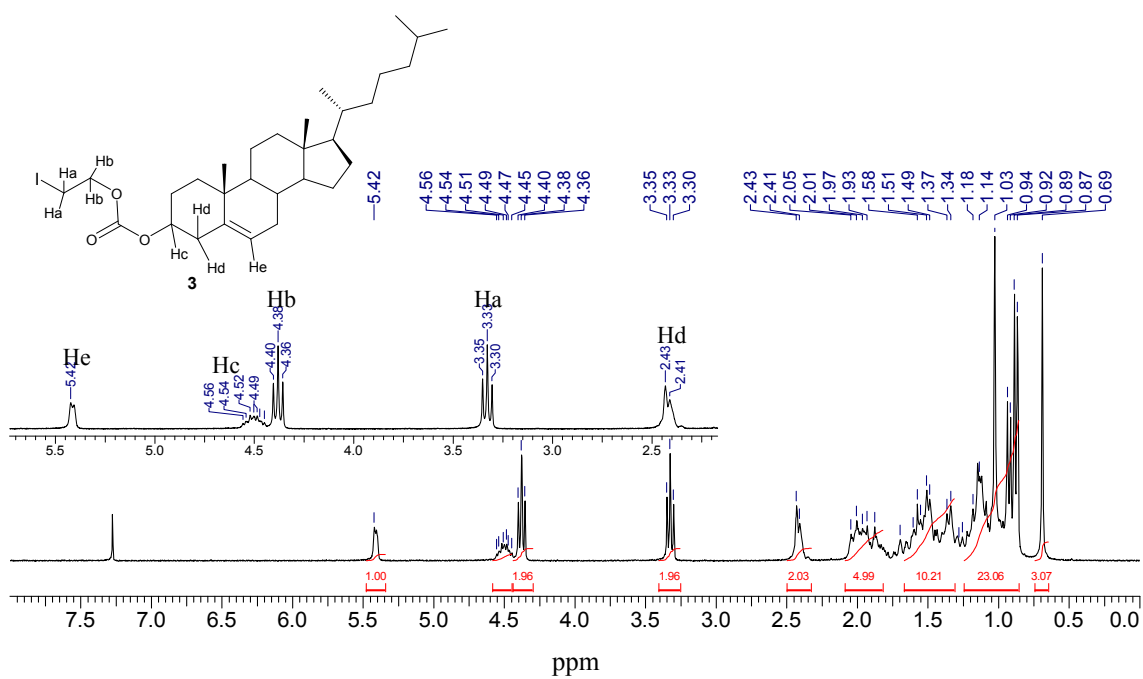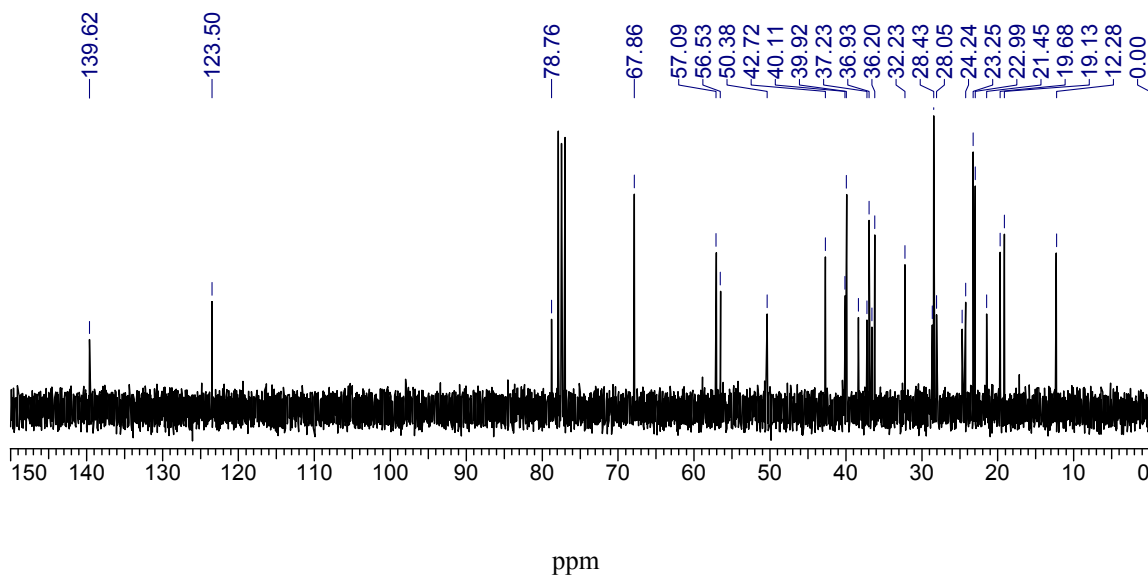
Figure S1 ^{1}H NMR of **3** (CDCl$_3$, 300 MHz).

Figure S2 ^{13}C NMR of **3** (CDCl$_3$, 75 MHz).
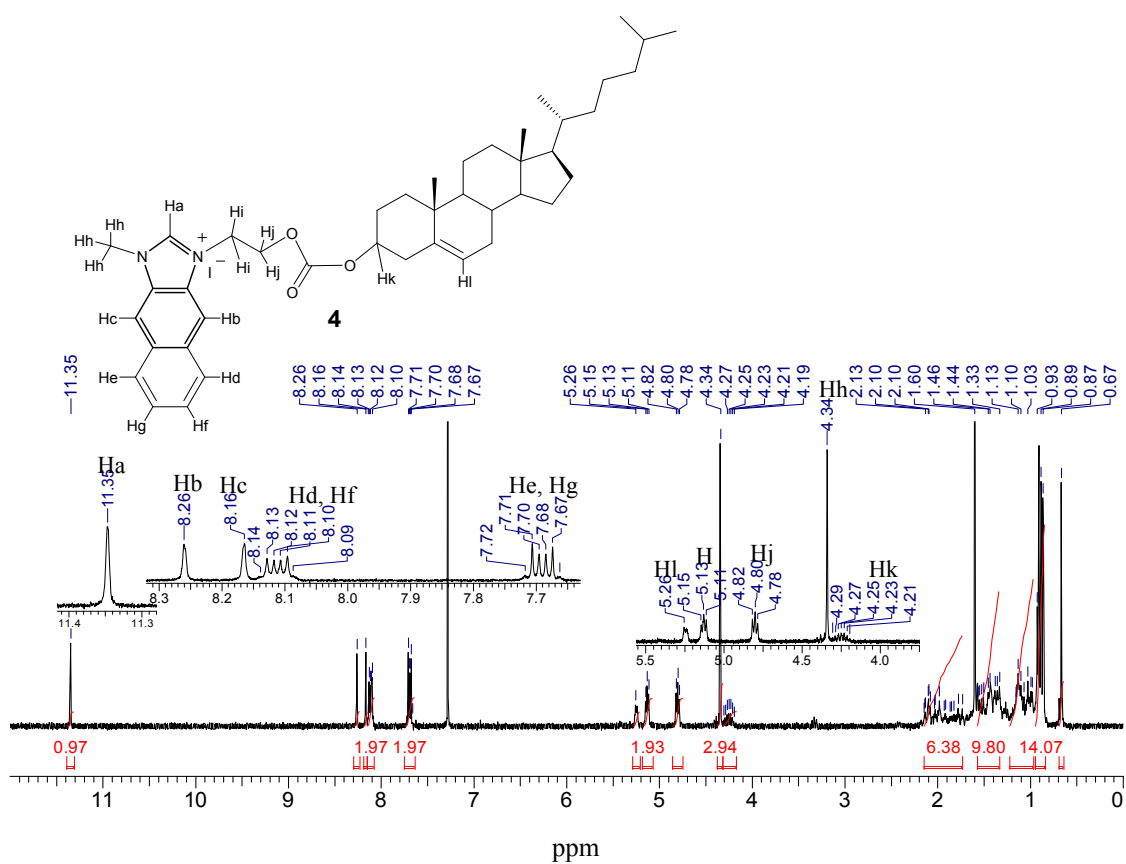
Figure S3 ^{1}H NMR of **4** (CDCl$_3$, 300 MHz).
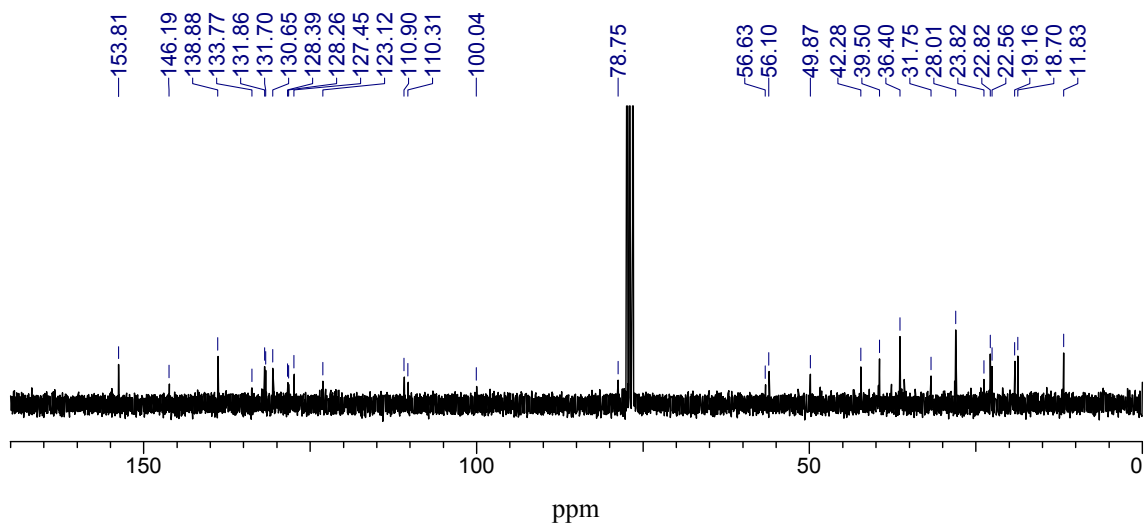
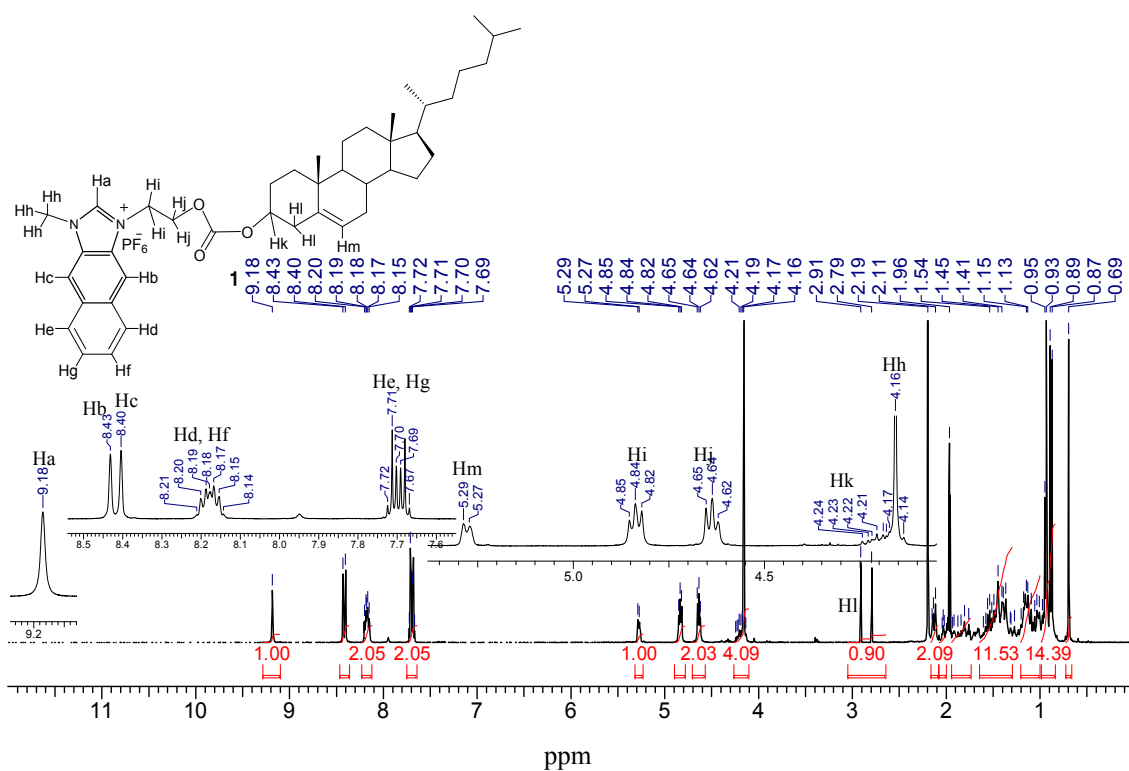**Figure S4** ^{13}C NMR of **4** (CDCl$_3$, 75 MHz).

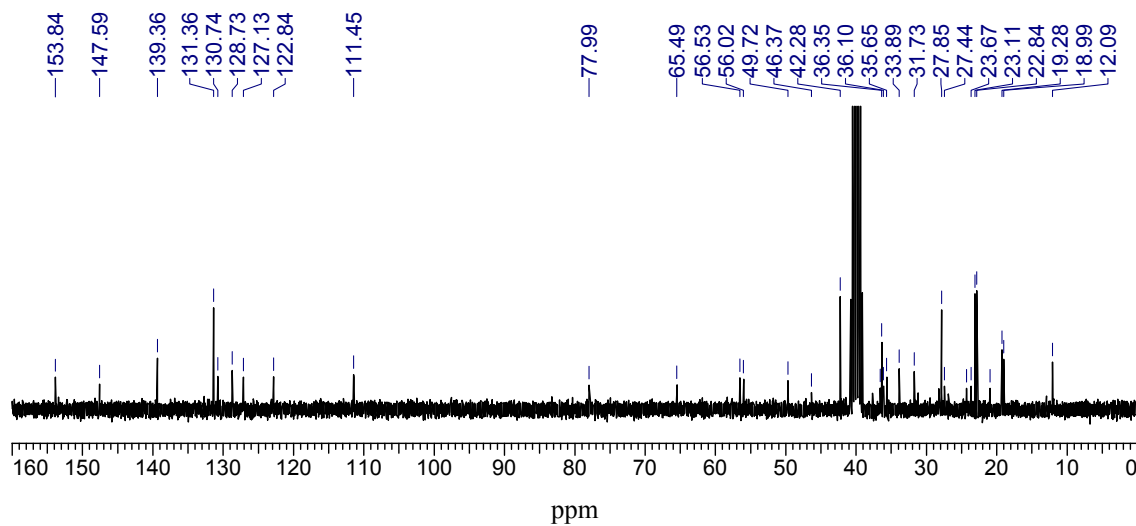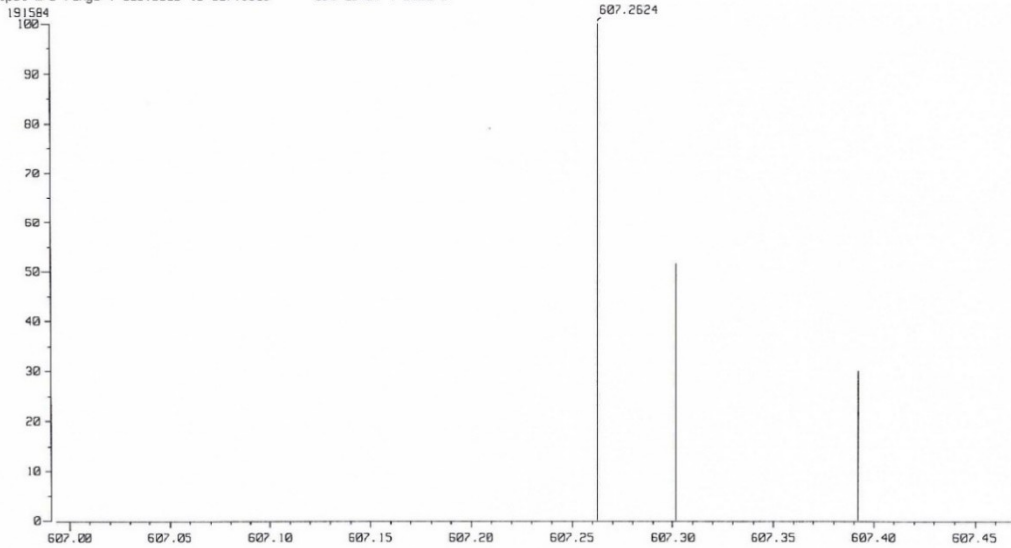**Figure S5** ^{1}H NMR of **1** (CD$_3$CN, 300 MHz).

Figure S6 ^{13}C NMR of **1** (CDCl$_3$, 75 MHz).

```
[ Mass Spectrum ]
Data : HFAB-POS-140211008        Date : 11-Feb-2014 18:03
Sample: 4
Note : with NBA + Na
Inlet : Direct                   Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.09 min        Scan# : 26
BP : m/z 607.2624    Int. : 18.27
Output m/z range : 606.9933 to 607.5309    Cut Level : 0.00 %
```

```
[ Elemental Composition ]                                  Page: 1
Data   : HFAB-POS-140211008           Date : 11-Feb-2014 18:03
Sample: 4
Note : with NBA + Na
Inlet : Direct                        Ion Mode : FAB+
RT : 2.09 min                         Scan#: 26
Elements : C 30/0, H 50/0, O 3/0, Na 1/0, I 1/0
Mass Tolerance      : 10mmu
Unsaturation (U.S.) : -1.0 - 100.0


Observed m/z    Int%
  607.2624     100.0
Estimated m/z   Error[ppm]  U.S.    C     H     O     Na    I
  607.2624       +0.0       5.5     30    49    3     1     1


[ Theoretical Ion Distribution ]                           Page: 1
Molecular Formula : C30 H49 O3 Na I
                (m/z   607.2624, MW   607.6115, U.S.  5.5)
Base Peak :    607.2624, Averaged MW :   607.6141(a),   607.6147(w)

   m/z      INT.
 607.2624 100.0000 ********************************************************
 608.2658  34.2164 ********************
 609.2690   6.2696 ****
 610.2720   0.8111
 611.2750   0.0821
 612.2779   0.0068
 613.2807   0.0005
```

Figure S7 HRMS FAB spectrum of **3**.

```
[ Mass Spectrum ]
Data  : HFAB-POS-140211009      Date : 11-Feb-2014 18:14
Sample: 5
Note : with NBA + Na
Inlet : Direct                  Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 2.17 min        Scan# : 27
BP : m/z 789.3469    Int. : 8.56
Output m/z range : 789.1355 to 789.5662    Cut Level : 0.00 %
```
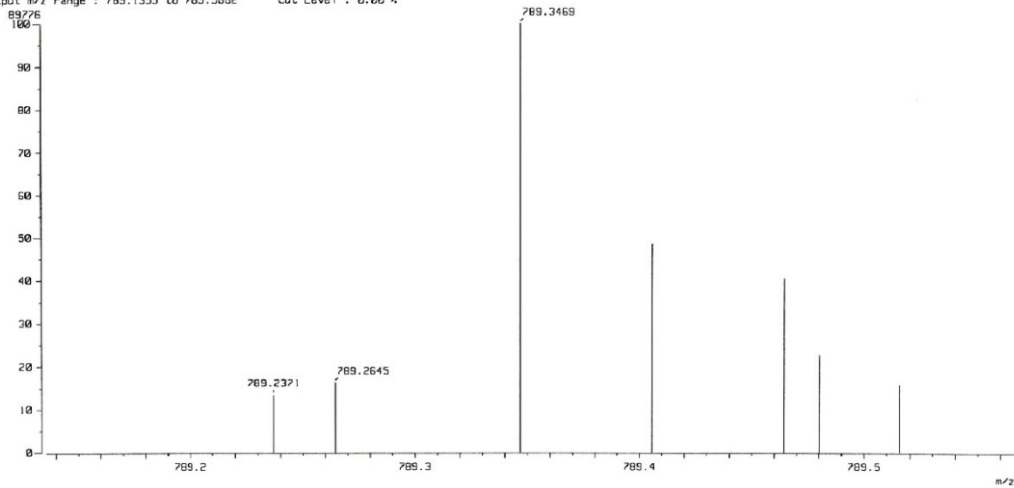
```
[ Elemental Composition ]                          Page: 1
Data  : HFAB-POS-140211009          Date : 11-Feb-2014 18:14
Sample: 5
Note : with NBA + Na
Inlet : Direct                      Ion Mode : FAB+
RT : 2.17 min                       Scan#: 27
Elements : C 42/0, H 59/0, O 3/0, N 2/0, Na 1/0, I 1/0
Mass Tolerance        : 10mmu
Unsaturation (U.S.) : -1.0 - 100.0

Observed m/z   Int%
  789.3469     100.0
Estimated m/z  Error[ppm]  U.S.    C     H     O    N    Na    I
  789.3468      +0.1       13.5    42    59    3    2    1     1


[ Theoretical Ion Distribution ]                   Page: 1
Molecular Formula : C42 H59 I N2 O3 Na
                   (m/z   789.3468, MW   789.8363, U.S. 13.5)
Base Peak :   789.3468, Averaged MW :   789.8397(a),   789.8404(w)

    m/z       INT.
  789.3468 100.0000 ********************************************************
  790.3501  48.4479 ***************************
  791.3533  12.0762 *******
  792.3564   2.0618 *
  793.3594   0.2703
  794.3624   0.0289
  795.3653   0.0026
  796.3683   0.0002
```
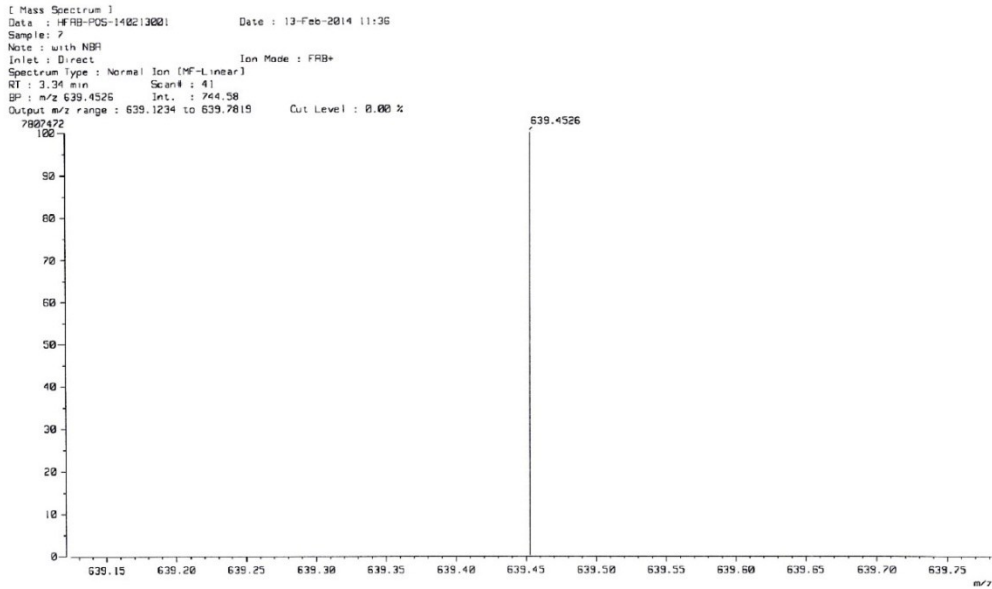
Figure S8 HRMS FAB spectrum of **4**.

[Mass Spectrum]
Data : HFAB-POS-140213001   Date : 13-Feb-2014 11:36
Sample: 7
Note : with NBA
Inlet : Direct   Ion Mode : FAB+
Spectrum Type : Normal Ion [MF-Linear]
RT : 3.34 min   Scan# : 41
BP : m/z 639.4526   Int. : 744.58
Output m/z range : 639.1234 to 639.7819   Cut Level : 0.00 %

[Elemental Composition]   Page: 1
Data : HFAB-POS-140213001   Date : 13-Feb-2014 11:36
Sample: 7
Note : with NBA
Inlet : Direct   Ion Mode : FAB+
RT : 3.34 min   Scan#: 41
Elements : C 42/0, H 59/0, O 3/0, N 2/0
Mass Tolerance   : 10mmu
Unsaturation (U.S.) : -1.0 - 100.0

Observed m/z   Int%
  639.4526   100.0
Estimated m/z Error[ppm] U.S.   C   H   O   N
  639.4526   +0.0   14.5   42   59   3   2

[Theoretical Ion Distribution]   Page: 1
Molecular Formula : C42 H59 O3 N2
                   (m/z 639.4526, MW 639.9421, U.S. 14.5)
Base Peak :   639.4526, Averaged MW :   639.9455(a), 639.9463(w)

    m/z   INT.
  639.4526 100.0000 **
  640.4559 48.4479 ***************************
  641.4591 12.0762 *******
  642.4622   2.0618 *
  643.4652   0.2703
  644.4682   0.0289
  645.4711   0.0026
  646.4740   0.0002

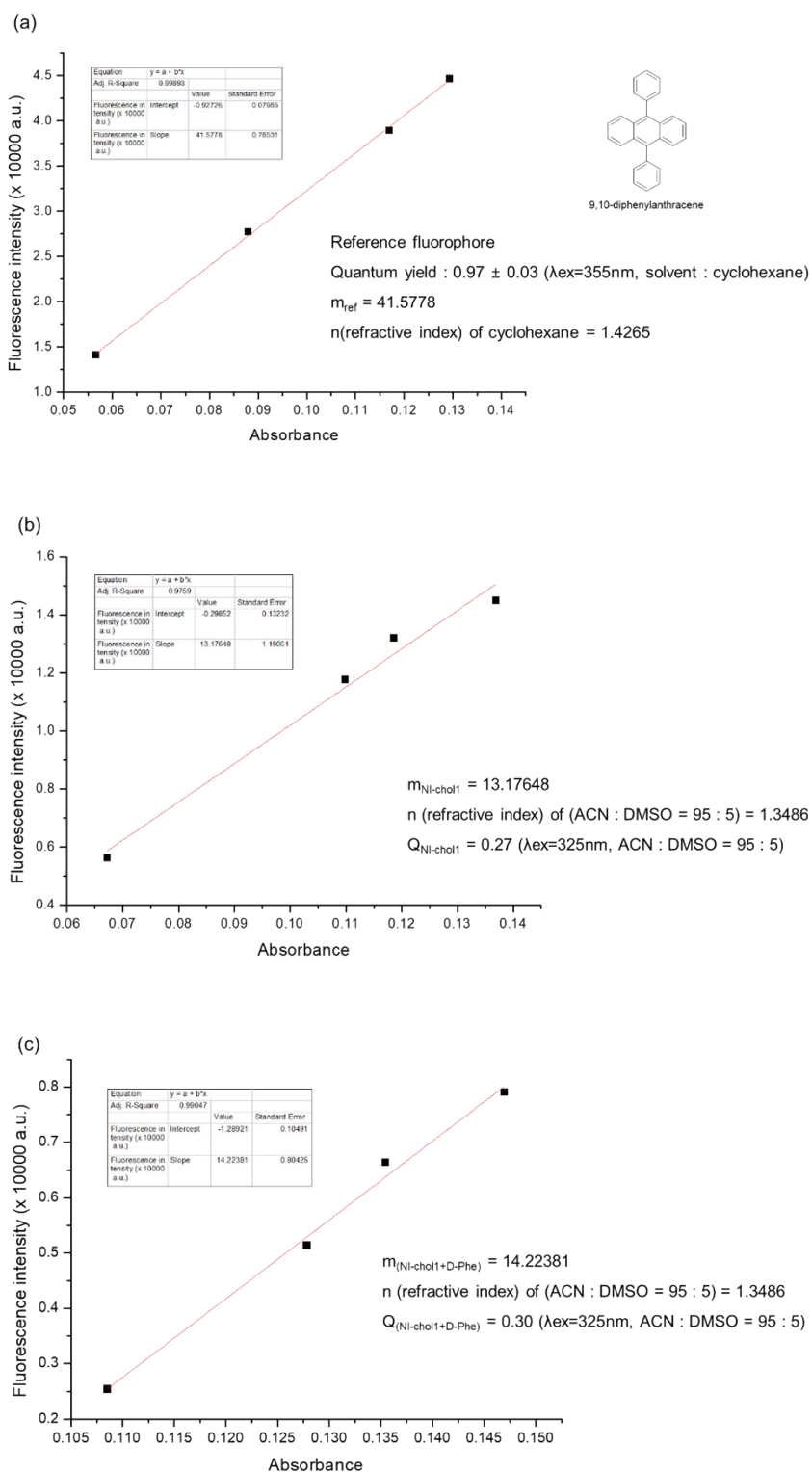**Figure S9** HRMS FAB spectrum of **1**.

Equation	y = a + b*x		
Adj. R-Square	0.99893		
		Value	Standard Error
Fluorescence intensity (x 10000 a.u.)	Intercept	-0.92726	0.07985
Fluorescence intensity (x 10000 a.u.)	Slope	41.5778	0.78531

9,10-diphenylanthracene

Reference fluorophore

Quantum yield : 0.97 ± 0.03 (λex=355nm, solvent : cyclohexane)

m_{ref} = 41.5778

n(refractive index) of cyclohexane = 1.4265

(b)

Equation	y = a + b*x		
Adj. R-Square	0.9759		
		Value	Standard Error
Fluorescence intensity (x 10000 a.u.)	Intercept	-0.29852	0.13232
Fluorescence intensity (x 10000 a.u.)	Slope	13.17648	1.19061

$m_{NI\text{-}chol1}$ = 13.17648

n (refractive index) of (ACN : DMSO = 95 : 5) = 1.3486

$Q_{NI\text{-}chol1}$ = 0.27 (λex=325nm, ACN : DMSO = 95 : 5)

(c)

Equation	y = a + b*x		
Adj. R-Square	0.99047		
		Value	Standard Error
Fluorescence intensity (x 10000 a.u.)	Intercept	-1.28921	0.10491
Fluorescence intensity (x 10000 a.u.)	Slope	14.22381	0.80426

$m_{(NI\text{-}chol1+D\text{-}Phe)}$ = 14.22381

n (refractive index) of (ACN : DMSO = 95 : 5) = 1.3486

$Q_{(NI\text{-}chol1+D\text{-}Phe)}$ = 0.30 (λex=325nm, ACN : DMSO = 95 : 5)

Figure S10 Absorbance vs. area of fluorescence of (a) 9,10-diphenylanthracene standard, (b) **NI-chol 1** only and (c) **NI-chol 1** with Boc-D-Phe.
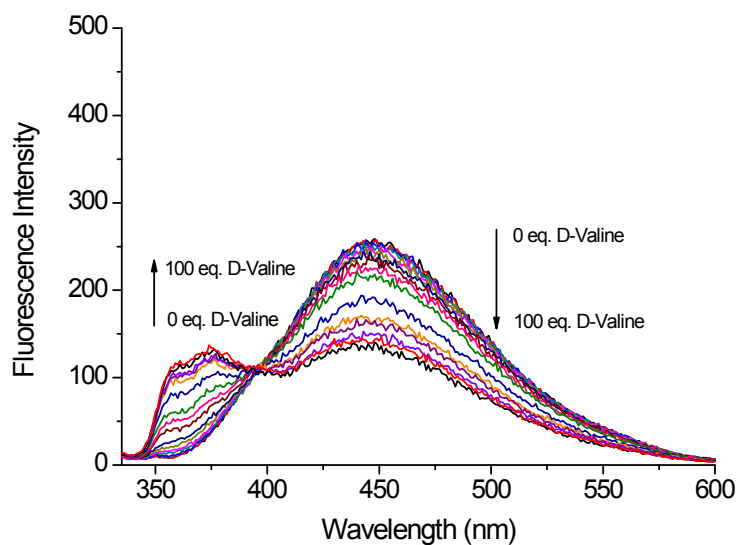
Figure S11 Fluorescent titrations of **NI-chol 1** (10 μM, ACN: DMSO=95∶5) with various equivalents of D-Val (slit 3×5).
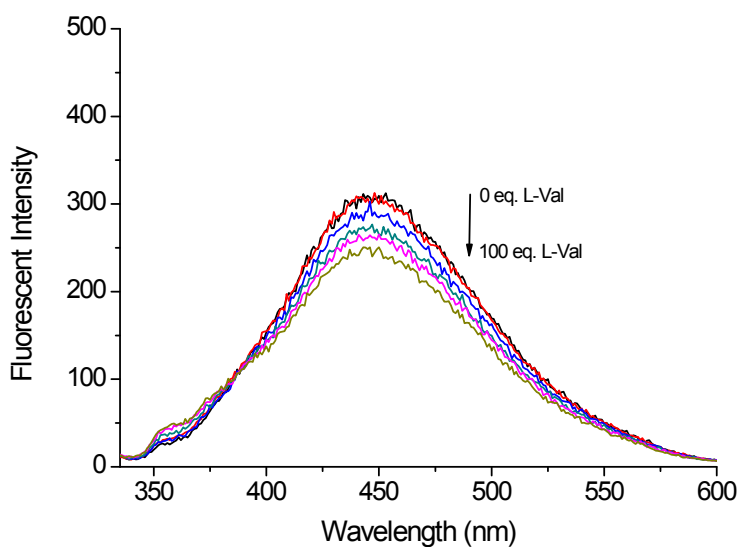

Figure S12 Fluorescent titrations of **NI-chol 1** (10 μM, ACN: DMSO=95∶5) with various equivalents of L-Val (slit 3×5).
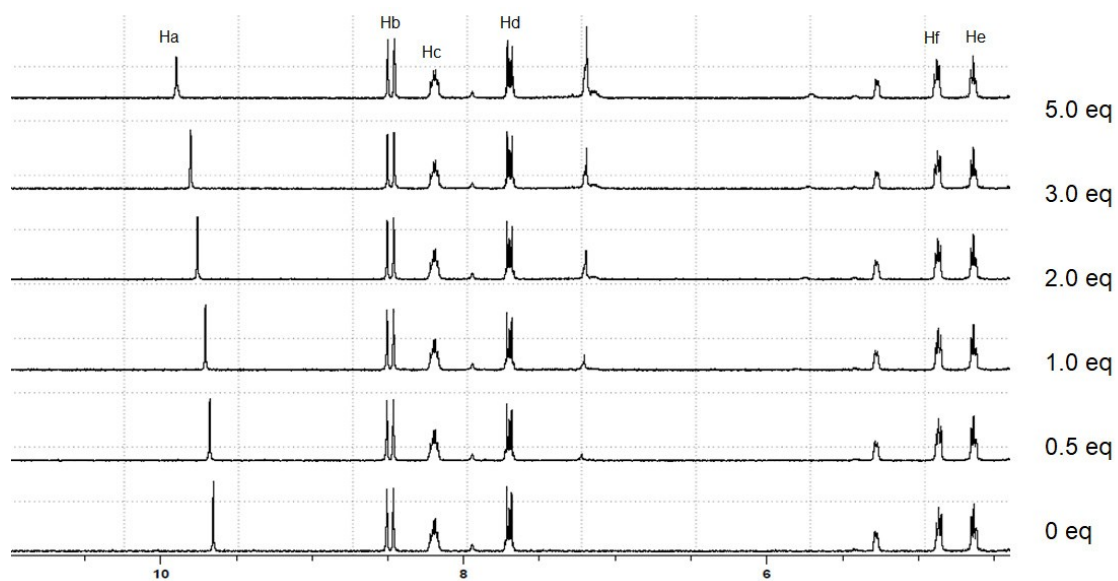
Figure S13 Partial ^{1}H NMR spectra (300 MHz) of **NI-chol 1** (5 mM) upon the addition of Boc-L-Phe (tetrabutylammonium salt) in CD$_3$CN-DMSO-d_6 (95:5, v/v).
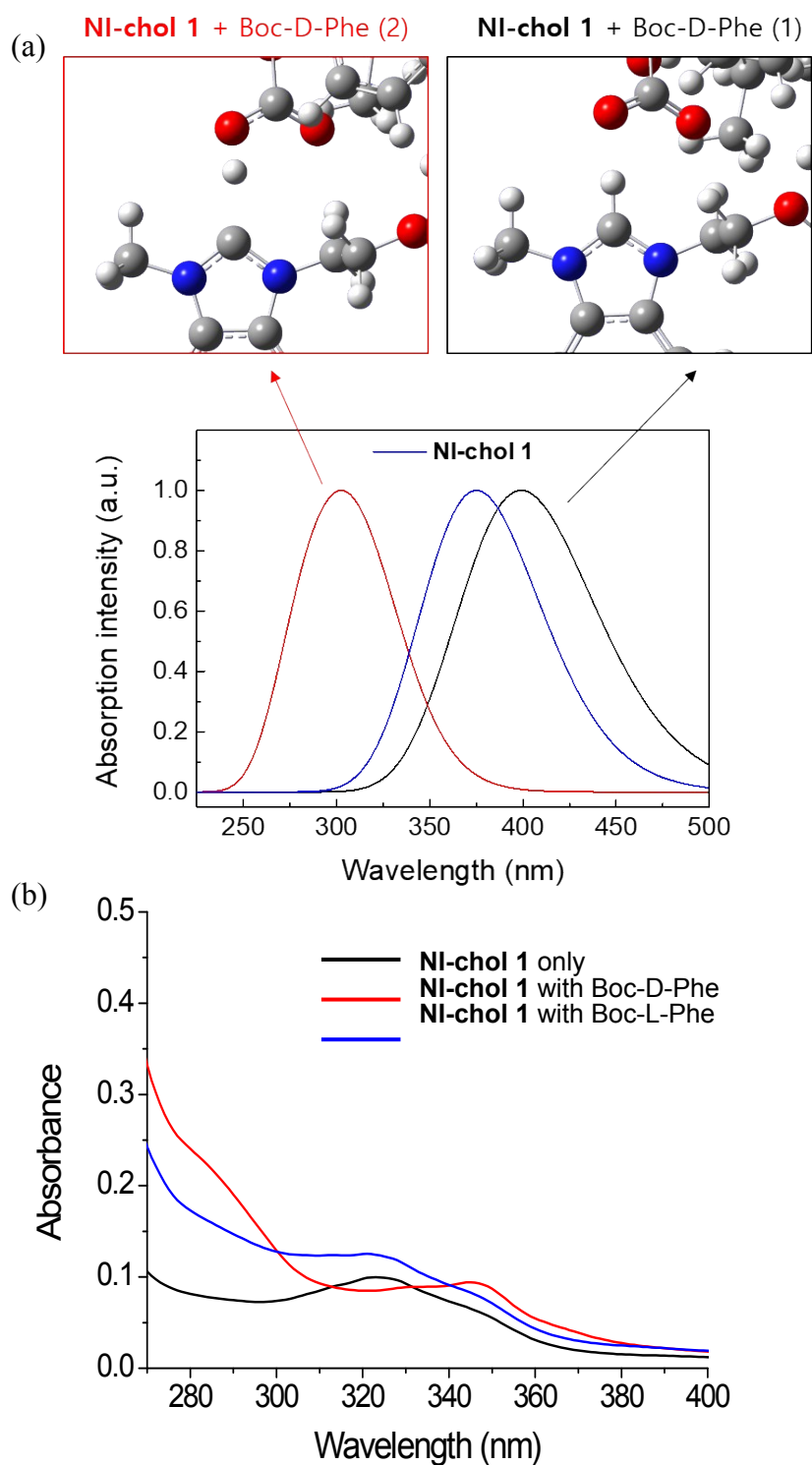
Figure S14 (a) The calculated absorption spectra of **NI-chol 1** and **NI-chol 1** with Boc-D-Phe. (b) UV/Vis spectra of **NI-chol 1** (10 μM) in CH₃CN-DMSO (95:5, v/v) with Boc-D-Phe and Boc-L-Phe (50 eq.).
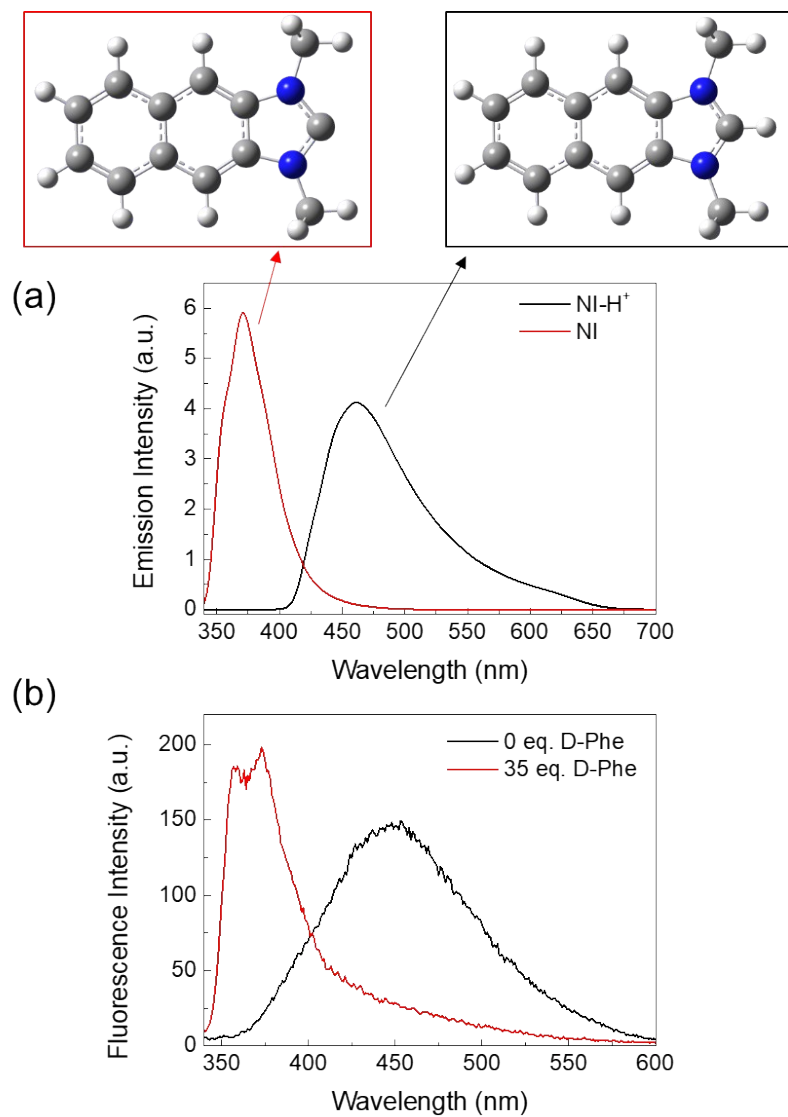
Figure S15 (a) The calculated (vibronic) emission spectra of protonated and deprotonated **NI**. (b) Fluorescence spectra of **NI-chol 1** (10 μM) with and without Boc-D-Phe in CH₃CN-DMSO (95:5, v/v).