Supporting Information

for

Development of a Cholesterol-conjugated Fluorescent Sensor for Site-specific Detection of Zinc Ion at the Plasma Membrane

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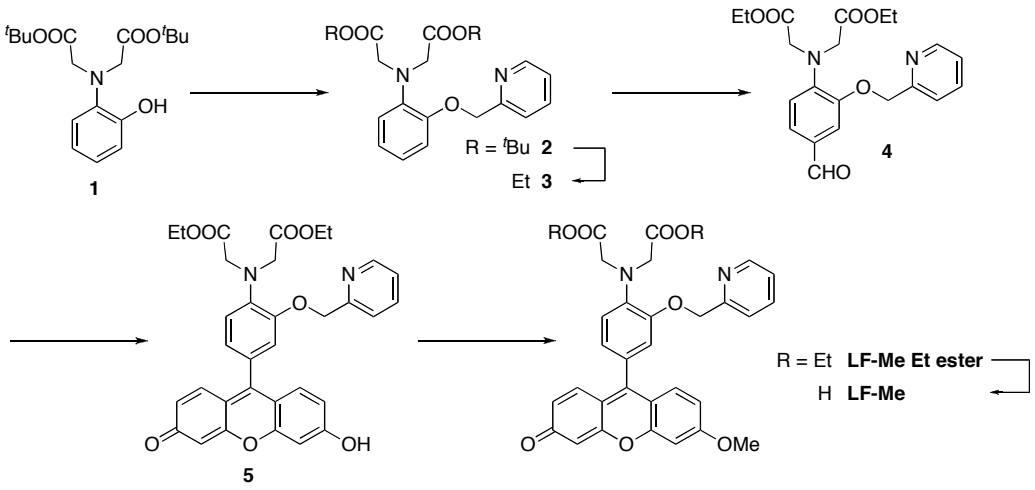
Contents

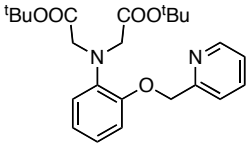
1. Synthesis

General. All chemicals used in this study were commercial products of the highest available purity and were further purified by the standard methods, if necessary.[S1] *N*,*N*-Bis(tert-butoxycarbonylmethyl)-2-hydroxyaniline (**1**), 8-(cholest-5-en-3β-yloxy)-3,6-dioxaoctan -1-ol (**6**), and **F-Me** were prepared according to the reported procedures.[S2-S4] NMR spectra were recorded on a JEOL JNM-EX-270 (at 270MHz to ^{1}H, 68 MHz to ^{13}C) or a JEOL JNM-ECX-500P (at 500 MHz to ^{1}H, 125 MHz to ^{13}C). Chemical shifts are given in ppm relative to tetramethylsilane (TMS). HRMS were recorded on a JEOL JMS-700 (FAB) or a Bruker micrOTOF II (ESI). TLC analyses were performed on Silica gel 60-F_{254} (Merck). Flash chromatography was performed on silica gel (Merck Silica Gel 60). ODS column chromatography was performed on a YFLC system (Yamazen Co., Osaka, Japan). Reverse phase HPLC was performed on Waters Delta 600 system (10 × 250 mm, XBridge^{TM}C$_{18}$).

Scheme S1. Synthesis of LF-Me

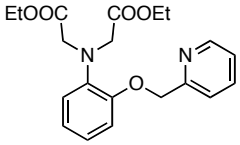


N,N-Bis(tert-butoxycarbonylmethyl)-2-(2-pyridylmethoxy)aniline, **2**.



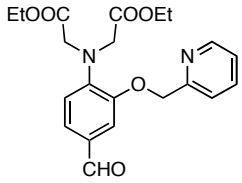
Compound **2** was synthesized according to the modified procedures.[S5] To a mixture of **1** (6.30 g, 18.7 mmol), KI (4.66 g, 28.1 mmol), and dry DMF (50 mL), was slowly added NaH (1.34 g, 56.0 mmol) at 0 °C. After stirring at room temperature for 30 min under argon atmosphere, 2-picoly chloride hydrochloride (4.60 g, 28.1 mmol) was added and the mixture was stirred at 80 °C overnight. After removal of the solvent by evaporation, brine (50 mL) was added to the residue and the mixture was extracted with AcOEt (50 mL × 3). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography ($CHCl_3$/hexane/AcOEt, 1:4:0.4) to give **2** as a pale yellow oil (3.56 g, 45 %). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.57 (1H, d, *J* = 5.0 Hz), 7.69 (1H, t, *J* = 7.5 Hz), 7.57 (1H, d, *J* = 7.5 Hz), 7.20 (1H, dd, *J* = 7.5, 5.0 Hz), 6.82-6.89 (4H, m), 5.26 (2H, s), 4.11 (4H, s), 1.43 (18H, s).

N,N-Bis(ethoxycarbonylmethyl)-2-(2-pyridylmethoxy)aniline, **3**.



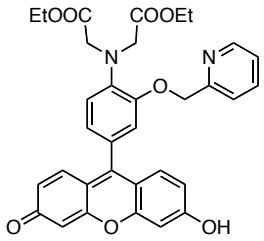
A solution of **2** (2.0 g, 4.67 mmol) in 50% TFA/CH_2Cl_2 (30 mL) was stirred at room temperature overnight. After evaporation of the solvent, the residue was dissolved in ethanol (100 mL) and a catalytic amount of concentrated H_2SO_4 was added. After refluxing overnight, ethanol was removed by evaporation and then saturated $NaHCO_3$ aqueous solution (50 mL) was added. The mixture was extracted with AcOEt (50 mL × 3). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (hexane/AcOEt, 2:1) to give **3** as a pale yellow oil (826 mg, 47 %). ^{1}H-NMR (270 MHz, $CDCl_3$) δ 8.58 (1H, d, *J* = 5.0 Hz), 7.71 (1H, t, *J* = 7.6 Hz), 7.56 (1H, d, *J* = 8.0 Hz), 7.22-7.20 (1H, m), 6.89-6.82 (4H, m), 5.24 (2H, s), 4.21 (4H, s), 4.13 (4H, q, *J* = 7.2 Hz), 1.22 (6H, t, *J* = 7.2 Hz); ^{13}C-NMR (68 MHz, $CDCl_3$) δ 171.36, 157.38, 150.27, 149.00, 139.25, 136.79, 122.49, 122.14, 121.60, 121.38, 119.82, 114.03, 71.44, 60.56, 53.81, 14.15; HRMS (ESI): *m/z* calcd for $C_{20}H_{24}N_2O_5Na$ ($[M+Na]^+$) 395.1583, found 395.1556.

4-(N,N-Bis(ethoxycarbonylmethyl)amino)-3-(2-pyridylmethoxy)benzaldehyde, **4**.



To a solution of **3** (826 mg, 2.22 mmol) in dry DMF (10 mL) was added dropwise $POCl_3$ (3.40 g, 22.2 mmol). After stirring at room temperature overnight under argon atmosphere, $CHCl_3$ (20 mL) was added to the solution and the mixture was poured into ice water (100 mL) to quench the excess $POCl_3$. The mixture was extracted with $CHCl_3$ (100 mL × 3) and the combined extracts were concentrated. The residue was then dissolved in AcOEt (50 mL) and the solution was washed with water (50 mL × 3), dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography (hexane/AcOEt, 3:1 to 1:1) to give **4** as a light brown oil (326 mg, 37 %). ^{1}H-NMR (270 MHz, $CDCl_3$) δ 9.75 (1H, s), 8.61 (1H, d, *J* = 4.6 Hz), 7.71 (1H, t, *J* = 7.5 Hz), 7.45-7.39 (3H, m), 7.27-7.23 (1H, m), 6.81 (1H, d, *J* = 8.4 Hz), 5.25 (2H, s), 4.26 (4H, s), 4.12 (4H, q, *J* = 7.2 Hz), 1.23 (6H, t, *J* = 7.2 Hz); ^{13}C-NMR (68 MHz, $CDCl_3$) δ 190.38, 170.63, 156.20, 149.32, 149.24, 145.14, 136.75, 129.96, 126.20, 122.79, 121.63, 117.01, 112.61, 71.79, 60.93, 53.98, 14.10; HRMS (ESI): *m/z* calcd for $C_{21}H_{24}N_2O_6Na$ ($[M+Na]^+$) 423.1532, found 423.1518.

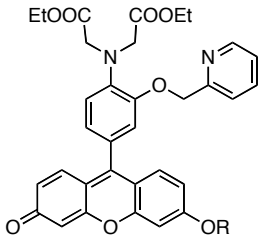
9-(4-(N,N-Bis(ethoxycarbonylmethyl)amino)-3-(2-pyridylmethoxy)phenyl)-6-hydroxy-3-oxo-3H-xanthe n, **5**.



A solution of **4** (411 mg, 1.03 mmol) and resorcinol (226 mg, 2.05 mmol) in $MeSO_3H$ (5 mL) was heated at 80 °C for 24 h. The reaction mixture was cooled to room temperature and poured into 40 mL of 3 M NaOAc solution. The resulting red solid was collected by filtration. The crude was dissolved in $CHCl_3$/MeOH 9:1 solution and the solution was dried over $MgSO_4$, and evaporated. The residue was purified with flash chromatography ($CHCl_3$/CH_3OH, 50:1) to give **5** as a red solid (84 mg, 14 %). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.20 (1H, brs), 8.48 (1H, s), 7.74 (1H, t, *J* = 7.5 Hz), 7.51 (1H, d, *J* = 7.5 Hz), 7.25-7.21 (1H, m), 7.16 (2H, d, *J* = 9.5 Hz), 6.95-6.93 (1H, m), 6.90-6.88 (2H, m), 6.80 (2H,

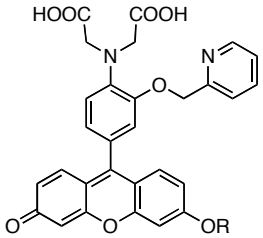
s), 6.74 (2H, d, *J* = 8.5 Hz), 5.22 (2H, s), 4.29 (4H, s), 4.18 (4H, q, *J* = 7.0 Hz), 1.27 (6H, t, *J* = 7.0 Hz); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 175.46, 171.31, 158.11, 156.36, 154.71, 149.38, 149.10, 140.95, 137.22, 131.50, 125.41, 123.72, 123.15, 122.04, 121.89, 118.34, 115.82, 114.64, 103.73, 71.58, 61.10, 54.09, 14.40; HRMS (ESI): *m/z* calcd for $C_{33}H_{31}N_2O_8$ ($[M+H]^+$) 583.2080, found 583.2066.

LF-Me Et ester.



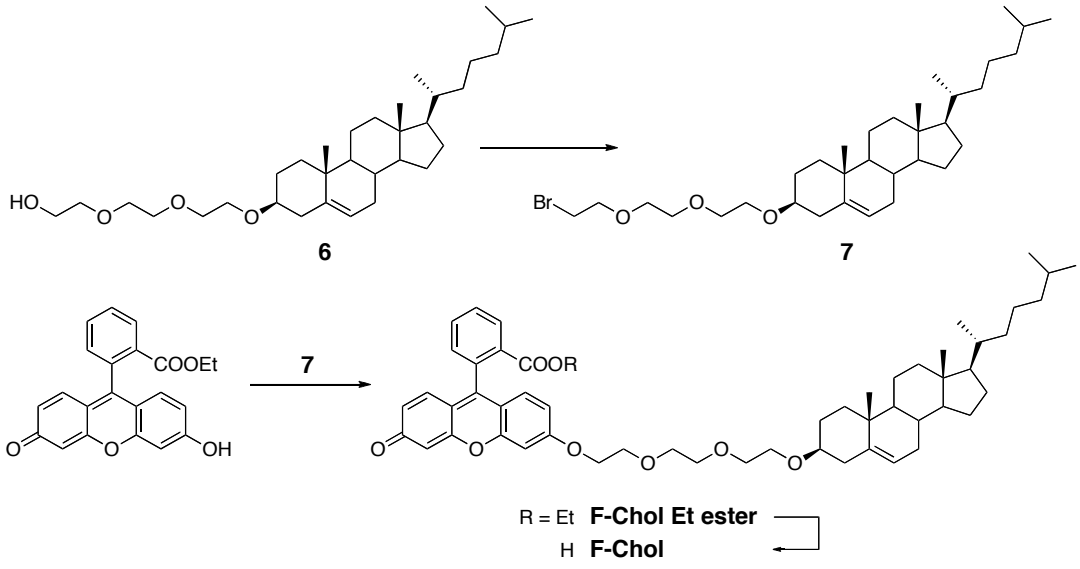
To a mixture of **5** (44 mg, 0.076 mmol) and K_2CO_3 (52 mg, 0.38 mmol) in DMF (5 mL) was added MeI (16 mg, 0.11 mmol) and the mixture was stirred overnight at 60 °C. After evaporation, water (30 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (30 mL × 3). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography ($CHCl_3$ only to $CHCl_3$/CH_3OH, 50:1) to give **LF-Methyl ethyl ester** as a dark red oil (23 mg, 51 %). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.52 (1H, d, *J* = 3.5 Hz), 7.74 (1H, t, *J* = 7.5 Hz), 7.51 (1H, d, *J* = 8.0 Hz), 7.26-7.22 (1H, m), 7.15 (1H, d, *J* = 9.0 Hz), 7.01 (1H, d, *J* = 9.5 Hz), 6.95 (1H, d, *J* = 9.5 Hz), 6.91 (1H, s), 6.87-6.85 (2H, m), 6.71(1H, d, *J* = 8.5 Hz), 6.48 (1H, d, *J* = 9.5 Hz), 6.41 (1H, s), 5.23 (2H, s), 4.29 (4H, s), 4.20 (4H, q, *J* = 7.0 Hz), 3.92 (3H, s), 1.28 (6H, t, *J* = 7.0 Hz); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 185.71, 171.37, 164.23, 159.13, 156.56, 154.88, 149.56, 149.48, 149.36, 140.69, 137.04, 131.05, 130.02, 129.80, 125.69, 123.46, 123.03, 121.89, 118.55, 117.90, 115.69, 114.59, 113.36, 105.78, 100.28, 71.76, 31.07, 56.12, 54.07, 14.41; HRMS (ESI): *m/z* calcd for $C_{34}H_{33}N_2O_8$ ($[M+H]^+$) 597.2237, found 597.2218.

LF-Me.

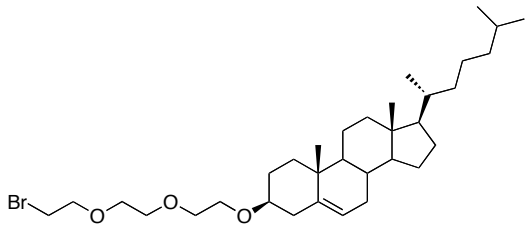


To a solution of **LF-Me Et ester** (18 mg, 0.030 mmol) in methanol (5 mL) was added $LiOH \cdot H_2O$ (70 mg, 1.67 mmol) and the mixture was stirred for 4 h at room temperature. After evaporation, the residue was dissolved in water (10 mL) and the aqueous solution was acidified by the addition of 0.1 M HCl. The mixture was extracted with $CHCl_3$ (10 mL × 3). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was purified by ODS column chromatography to give **LF-Me** as a brown solid (5 mg, 31 %), which was further purified by reverse phase HPLC. ^{1}H-NMR (500 MHz, CD_3OD) δ 8.59 (1H, d, J = 5.0 Hz), 8.06 (1H, t, J = 8.0 Hz), 7.83-7.80 (2H, m), 7.77(1H, d, J = 8.0 Hz), 7.58 (1H, d, J = 2.5 Hz), 7.56-7.54 (1H, m), 7.28-7.19 (4H, m), 7.17-7.13 (2H, m), 5.32 (2H, s), 4.36 (4H, s), 4.16 (3H, s); HRMS (ESI): m/z calcd for $C_{30}H_{23}N_2O_8$ ([M-H]$^-$) 539.1454, found 539.1476. The overall purity of this compound was confirmed by HPLC analysis.

Scheme S2. Synthesis of F-Chol

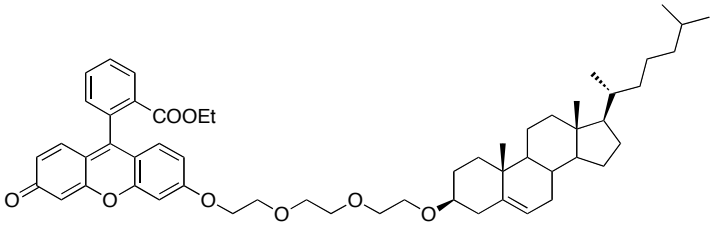


8-(Cholest-5-en-3β-yloxy)-3,6-dioxaoctan-1-bromide, **7**.



Cholesterol **7** was prepared by a different method reported previously.[S6] A solution of carbon tetrabromide (998 mg, 3.0 mmol) and triphenylphosphine (789 mg, 3.0 mmol) in THF (30 mL) were stirred at room temperature for 10 min under argon atmosphere. After the color of the solution turned into yellow, **6** (521 mg, 1.0 mmol) in THF (30 mL) was added to the solution. The mixture was further stirred at room temperature for 4 h under argon atmosphere. The reaction mixture was filtered through a pad of Celite and the filtrate was evaporated. The residue was purified by flash chromatography (hexane/AcOEt, 1:4) to give **7** as a yellow oil (271 mg, 46 %). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, br), 3.82 (2H, t, *J* = 6.4 Hz), 3.68-3.64 (8H, m), 3.47 (2H, t, *J* = 6.4 Hz), 3.24-3.13 (1H, m), 2.38-2.35 (1H,m), 2.23-2.18 (1H, m), 2.02-0.85 (38H, m), 0.67 (3H, s); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 140.9, 121.5, 79.5, 71.2, 71.0, 70.6, 70.5, 67.3, 56.8, 56.1, 50.2, 42.3, 39.8, 39.5, 39.1, 37.2, 36.8, 36.2, 35.8, 31.9, 31.9, 30.2, 28.3, 28.2, 28.0, 24.3, 23.8, 22.8, 22.5, 21.0, 19.4, 18.7, 11.8; HRMS (ESI): *m/z* calcd for $C_{33}H_{57}O_3{}^{79}BrNa$ ($[M+Na]^+$) 603.3389, found 603.3396.

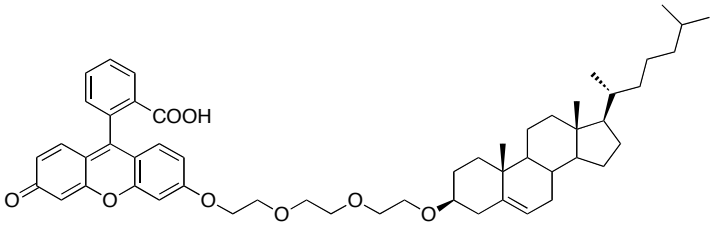
F-Chol Et ester.



F-Chol Et ester was obtained by the same procedure as described for **LF-Chol Et ester**, except that ethyl 2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoate (300 mg, 0.83 mmol) was used as the starting material instead of compound **5**. Yield: 516 mg, 72 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.25 (1H, d, *J* = 7.5 Hz), 7.74-7.65 (2H, m), 7.31 (1H, d, *J* = 7.5 Hz), 6.97 (1H, d, *J* = 2.0 Hz), 6.90-6.86 (2H, m), 6.77 (1H, dd, *J* = 9.0, 2.0 Hz), 6.54 (1H, dd, *J* = 10.0, 1.0 Hz), 6.45 (1H, d, *J* = 1.0 Hz), 5.33-5.32 (1H, m), 4.23 (2H, t, *J* = 4.5 Hz), 4.02 (2H, q, *J* = 7.2 Hz), 3.91 (2H, t, *J* = 4.5 Hz), 3.76-3.68 (4H, m), 3.64-3.63 (4H, m), 3.20-3.15 (1H, m), 2.37-0.85 (43H, m), 0.67 (3H, s); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 185.77,

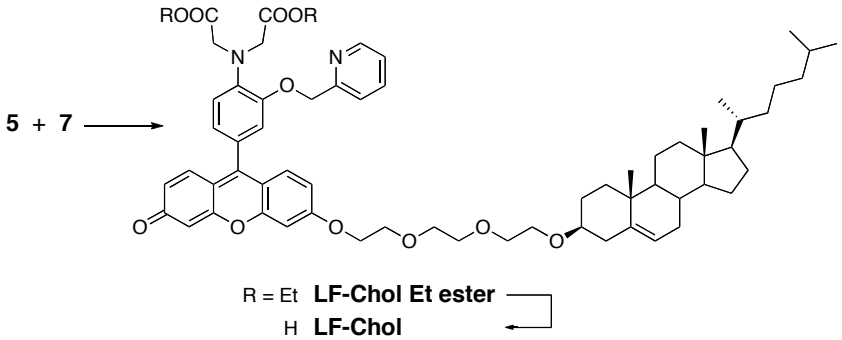
165.42, 163.35, 158.97, 154.22, 150.20, 141.01, 134.30, 132.58, 131.30, 130.87, 130.47, 130.34, 130.02, 129.70, 129.00, 121.65, 117.82, 115.11, 113.82, 105.86, 101.03, 79.58, 77.34, 71.05, 71.02, 70.75, 69.41, 68.32, 67.37, 61.42, 56.85, 56.25, 50.26, 42.40, 39.85, 39.58, 39.16, 37.30, 36.94, 36.26, 35.85, 32.02, 31.96, 28.44, 28.30, 28.08, 24.36, 23.90, 22.89, 22.63, 19.45, 18.80, 13.66, 11.93; HRMS (ESI): *m/z* calcd for $C_{55}H_{73}O_8$ ([M+H]$^+$) 861.5305, found 861.5237.

F-Chol.

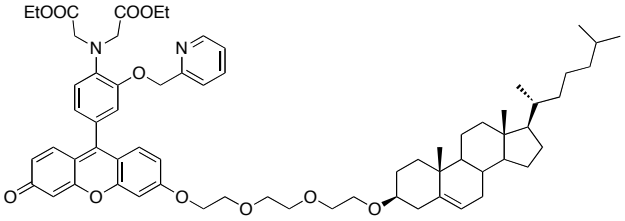


F-Chol was obtained by the same procedure as described for **LF-Me**, except that **F-Chol Et ester** (150 mg, 0.17 mmol) was used as starting material instead of **LF-Me Et ester**. Yield: 74 mg, 51 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.01 (1H, d, *J* = 7.0 Hz), 7.68-7.59 (2H, m), 7.16 (1H, d, *J* = 8.0 Hz), 6.77-6.75 (2H, m), 6.67 (2H, d, *J* = 9.0 Hz), 6.61-6.54 (3H, m), 5.33-5.32 (1H, m), 3.76-3.60 (12H, m), 3.22-3.17 (1H, m), 2.37-0.85 (40H, m), 0.67 (3H, s); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 169.92, 161.46, 158.74, 153.11, 152.70, 152.63, 140.85, 135.09, 129.75, 129.29, 129.13, 126.94, 125.11, 124.12, 121.77, 116.45, 112.66, 111.67, 111.30, 110.77, 103.18, 100.93, 79.74, 72.61, 70.88, 70.65, 70.33, 67.23, 61.79, 56.85, 56.24, 55.65, 50.25, 42.40, 39.86, 39.60, 38.99, 37.27, 36.92, 36.27, 35.86, 32.01, 31.95, 28.33, 28.09, 24.36, 23.91, 22.90, 22.64, 21.14, 19.43, 18.80, 11.93; HRMS (ESI): *m/z* calcd for $C_{53}H_{69}O_8$ ([M+H]$^+$) 833.4992, found 833.5222.

Scheme S3. Synthesis of LF-Chol

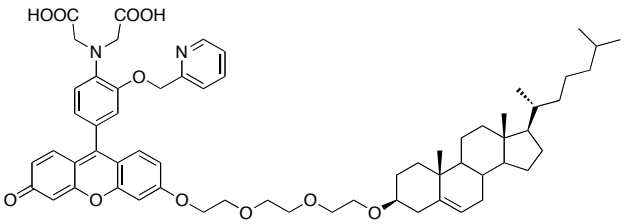


LF-Chol Et ester.



To a mixture of **5** (30 mg, 0.051 mmol) and K_2CO_3 (35 mg, 0.26 mmol) in DMF (2.5 mL) was added a solution of bromide **7** (39 mg, 0.067 mmol) in DMF (2.5 mL). The reaction mixture was stirred overnight at 60 °C. After evaporation, water (30 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (30 mL × 3). The combined extracts were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography ($CHCl_3$/MeOH, 50:1) to give the title compound, LF-Chol Et ester, as a red oil (23 mg, 41 %). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 8.53 (1H, d, *J* = 4.5 Hz), 7.73 (1H, t, *J* = 8.0 Hz), 7.50(1H, d, *J* = 7.5 Hz), 7.27-7.23 (1H, m), 7.14 (1H, d, *J* = 8.5 Hz), 7.01 (1H, d, *J* = 9.5 Hz), 6.95-6.91 (2H, m), 6.86-6.85 (2H, m), 6.74 (1H, dd, *J* = 9.0, 2.0 Hz), 6.48 (1H, d, *J* = 9.5 Hz), 6.41 (1H, s), 5.32-5.31 (1H, m), 5.23 (2H, s), 4.29 (4H, s), 4.23 (2H, t, *J* = 4.0 Hz), 4.20 (4H, q, *J* = 7.0 Hz), 3.92 (2H, t, *J* = 4.5 Hz), 3.76-3.70 (4H, m), 3.65-3.64 (4H, m), 3.20-3.15 (1H, m), 2.37-2.34 (1H, m), 2.22-2.17 (1H, m), 2.00-0.85 (44H, m), 0.66 (3H, s); ^{13}C-NMR (126 MHz, $CDCl_3$) δ 185.67, 171.38, 163.44, 159.11, 156.55, 154.75, 149.58, 149.44, 149.36, 141.05, 140.69, 137.03, 131.04, 129.98, 129.81, 128.51, 125.68, 123.46, 123.04, 121.90, 121.71, 118.54, 117.96, 115.69, 114.67, 113.75, 105.80, 100.95, 79.63, 71.77, 71.08, 70.82, 69.47, 68.35, 67.42, 61.08, 56.87, 56.26, 54.07, 50.28, 42.42, 39.88, 39.64, 39.20, 37.34, 36.98, 36.31, 35.92, 32.06, 31.99, 28.49, 28.36, 28.15, 24.41, 23.96, 22.96, 22.70, 21.18, 19.50, 18.84, 14.42, 11.98; HRMS (FAB): *m/z* calcd for $C_{66}H_{87}N_2O_{11}$ ($[M+H]^+$) 1083.6310, found 1083.6328.

LF-Chol.



LF-Chol was obtained by the same procedure as described for **LF-Me**, except that **LF-Chol Et ester** (20 mg, 0.018 mmol) was used as starting material instead of **LF-Me Et ester**. Yield: 5 mg, 26 %. For fluorescence imaging, **LF-Chol** was further purified by reverse phase HPLC (CH_3CN/H_2O

containing 0.1% TFA). Because available amount of this compound was too small for NMR analyses, mass analysis was performed to identify the product. HRMS (ESI): *m/z* calcd for $C_{62}H_{79}N_2O_{11}$ ($[M+H]^+$) 1027.5678, found 1027.5658. The overall purity of this compound was also confirmed by HPLC analysis.

2. Fluorescence Spectroscopy

Fluorescence spectra were recorded using a Hitachi F-2500 spectrometer with a slit width of 2.5 nm. The photomultiplier voltage was 700 V. To reduce fluctuations of the excitation intensity during measurement, the lamp was turned on for 1 h prior to the experiment. The path length was 1 cm with a cell volume of 3.0 mL. Quantum yields were determined by using fluorescein in 0.1 M NaOH (Φ = 0.95) as the fluorescence standard.[S7]

3. Determination of Apparent Dissociation Constant (K_d)

Dissociation constant was determined by the same procedure we reported before.[S5] The instensities at 517 nm were plotted and fitted to the following equation (Eq. 1).

$$I = (I_{min}\, K_d + I_{max}\, [Zn^{2+}]_{free})/(K_d + [Zn^{2+}]_{free}) \qquad \text{(Eq. 1)}$$

where I_{min} and I_{max} are the minimum and maximum fluorescence intensities observed in the absence and presence of 100 μM Zn^{2+}, respectively.

4. Cell Culture Experiments

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 10% fetal bovine serum (FBS, Gibco) and 1% Antibiotic-Antimycotic (Gibco) at 37 °C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (5×10^4) were passed on a glass-bottomed dish coated with poly-*L*-lysine (MW: 7,000-15,000) and incubated for 3 days. Immediately before the staining experiments, cells were washed twice with PBS buffer, and then the dish was filled with 1.5 mL of HBS. Finally, 0.75 μL of 5 mM probe solution in DMSO was added on the stage (final: 2.5 μM) and the cells were imaged with a FLUOVIEW confocal laser-scanning microscope (OLYMPUS) with Alexa Fluor 488 channel (488 nm excitation, 500-600 nm emission filter).

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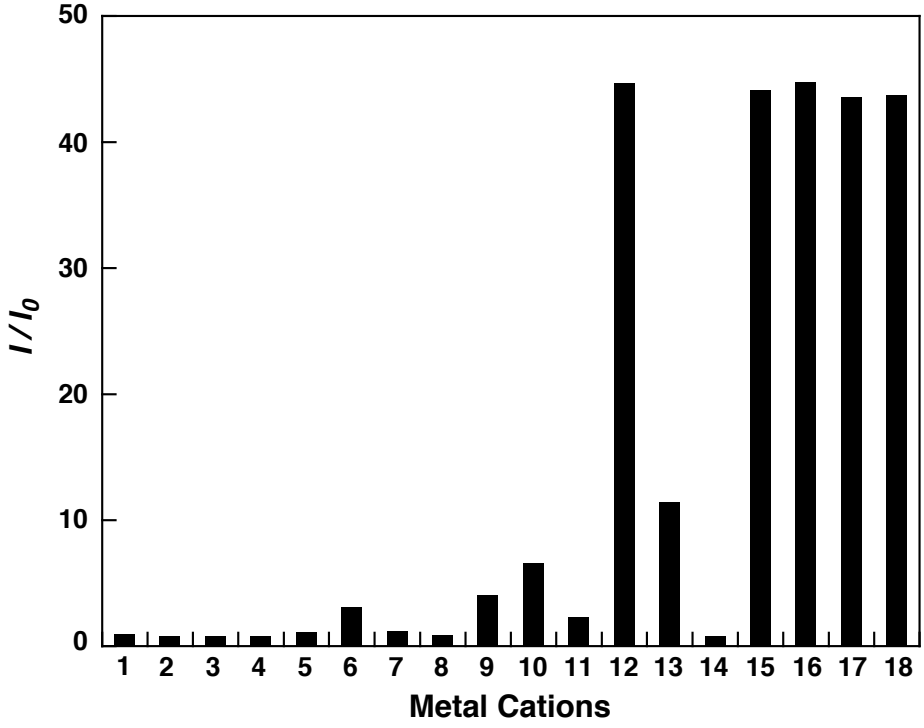

Figure S1. Fluorescence response of LF-Me (5 μM) at 517 nm as a function of various added metal cations (5 mM for Na^+, K^+, Ca^{2+}, and Mg^{2+}, 50 μM for all other cations) in 50 mM HEPES (pH 7.20, 0.1 M KNO_3). 1, no metal; 2, Na^+; 3, K^+; 4, Mg^{2+}; 5, Ca^{2+}; 6, Mn^{2+}; 7, Fe^{2+}; 8, Fe^{3+}; 9, Co^{2+}; 10, Ni^{2+}; 11, Cu^{2+}; 12, Zn^{2+}; 13, Cd^{2+}; 14, Hg^{2+}; 15, Zn^{2+} + Na^+; 16, Zn^{2+} + K^+; 17, Zn^{2+} + Mg^{2+}; 18, Zn^{2+} + Ca^{2+}.

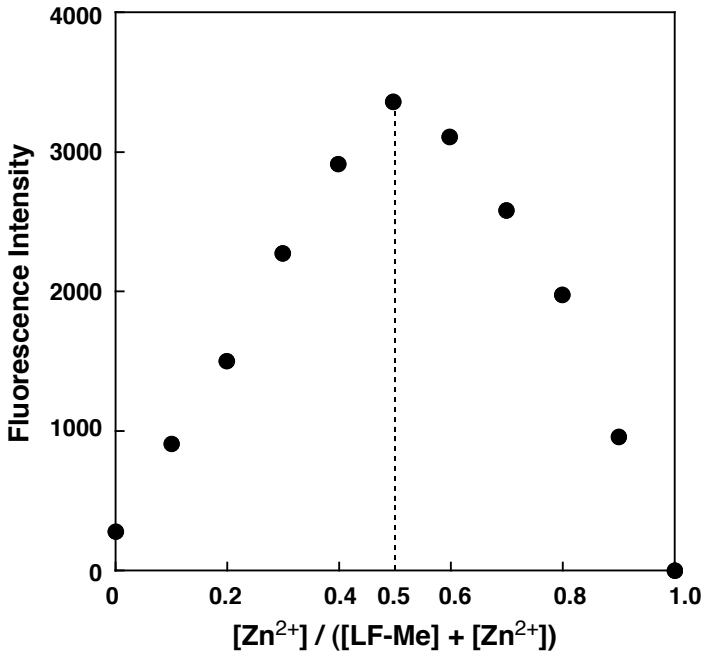

Figure S2. Job's plot of LF-Me in 50 mM HEPES buffer (pH 7.20, 0.1 M KNO_3). The sum of the concentration of LF-Me and Zn^{2+} is 10 μM.

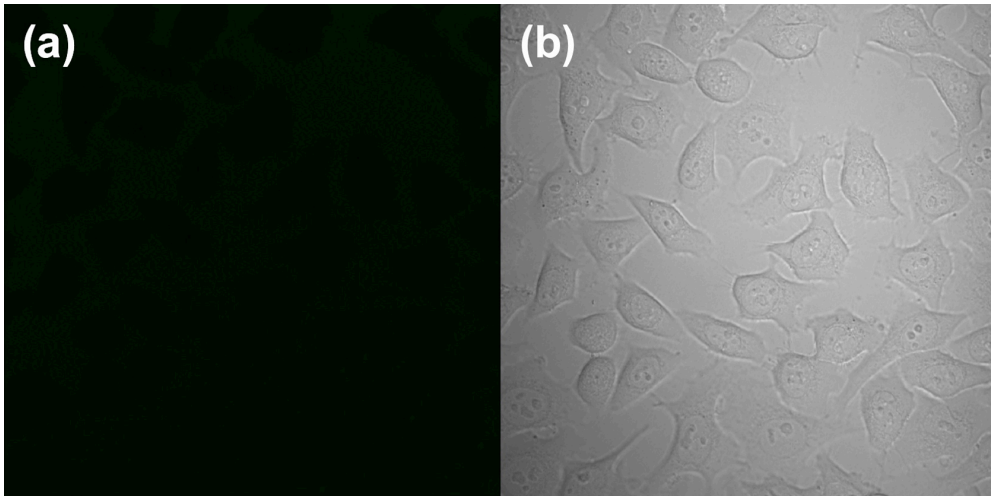

Figure S3. (a) Confocal fluorescence image of HeLa cells loaded 2.5 μM F-Me. (b) Bright-field transmission image of the cells shown in (a).

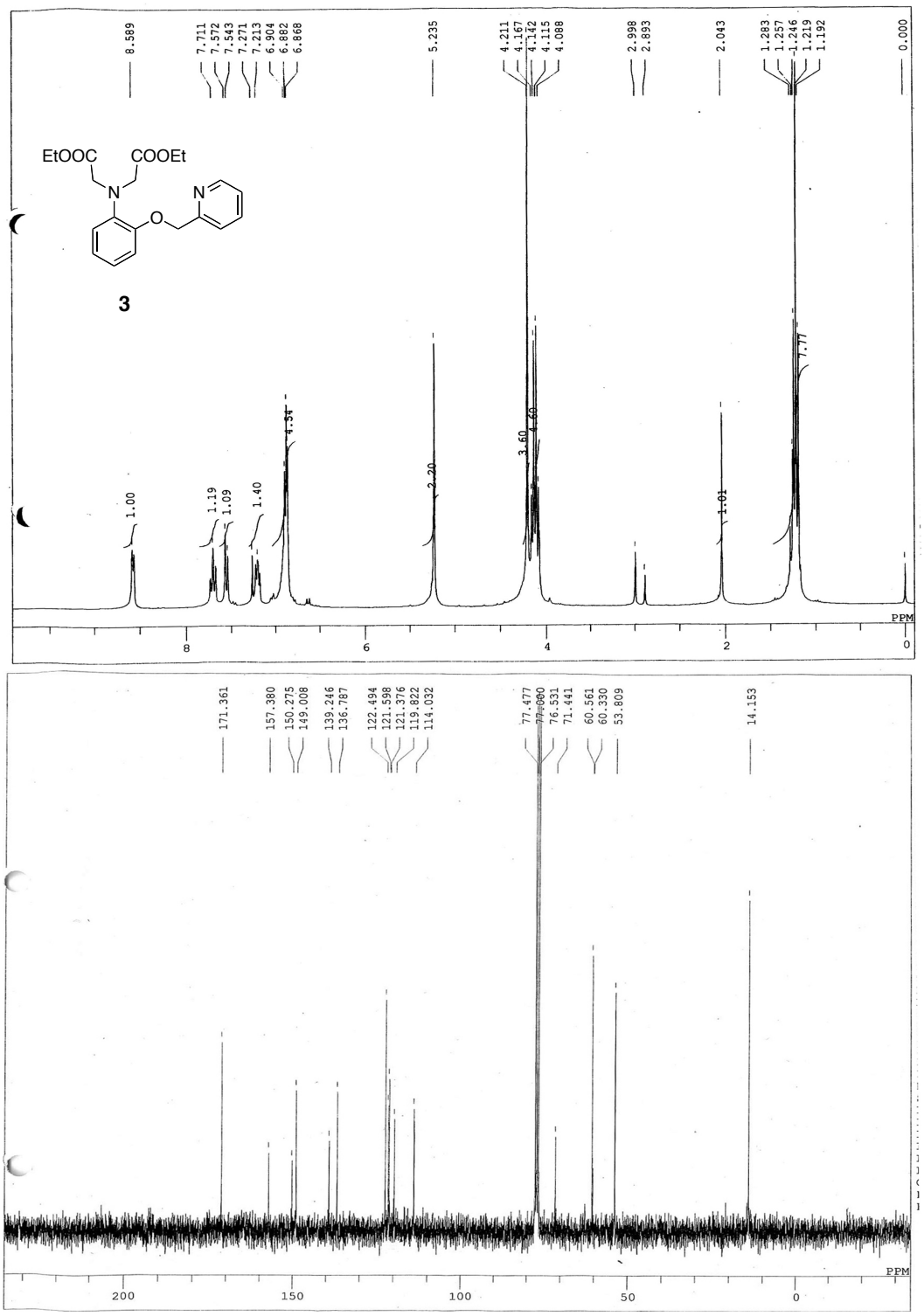

Figure S4. ^{1}H (270 MHz) and ^{13}C (68 MHz) NMR spectra of **3** in $CDCl_3$.

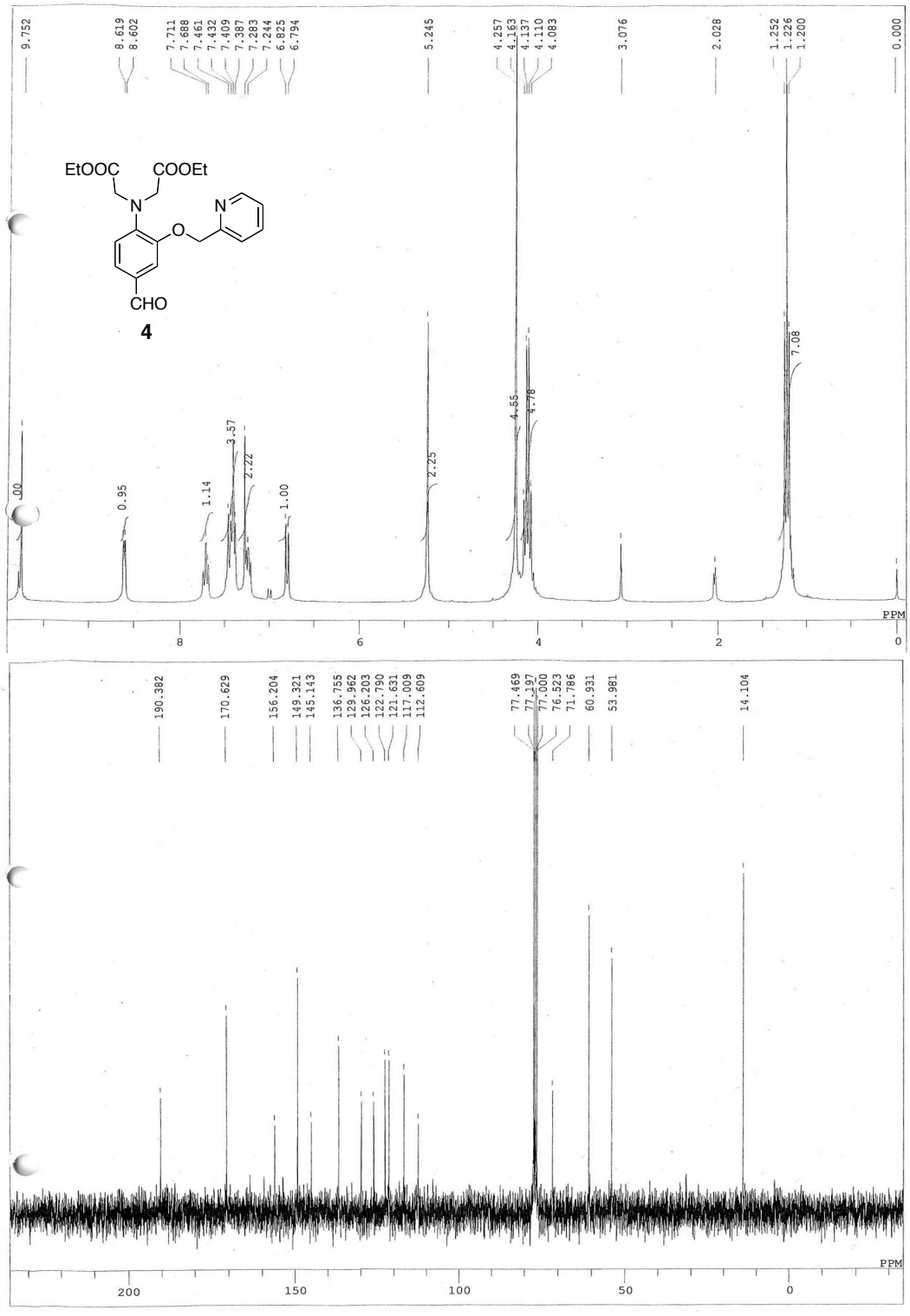

Figure S5. ^{1}H (270 MHz) and ^{13}C (68 MHz) NMR spectra of **4** in $CDCl_3$.

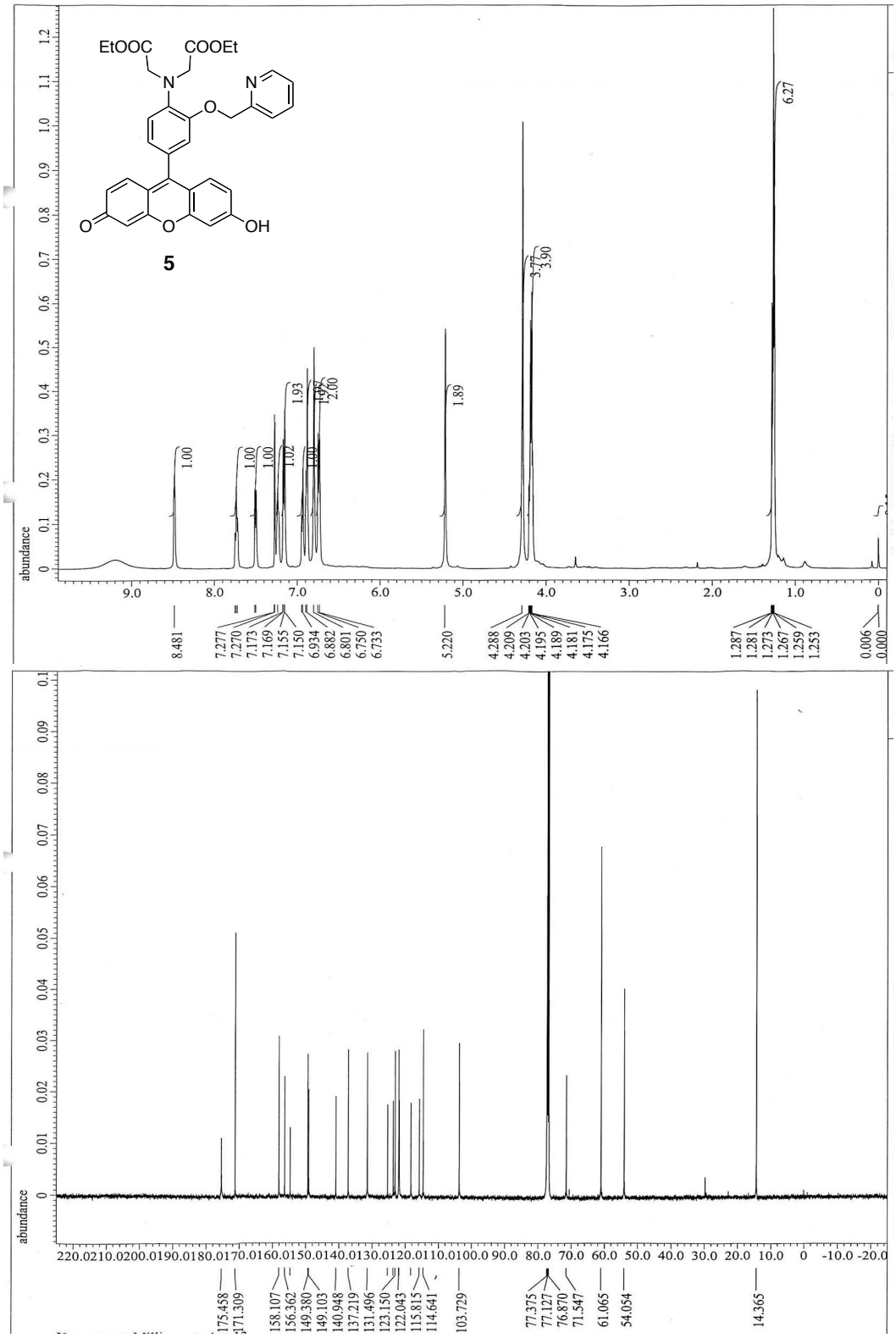

Figure S6. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra of **5** in $CDCl_3$.

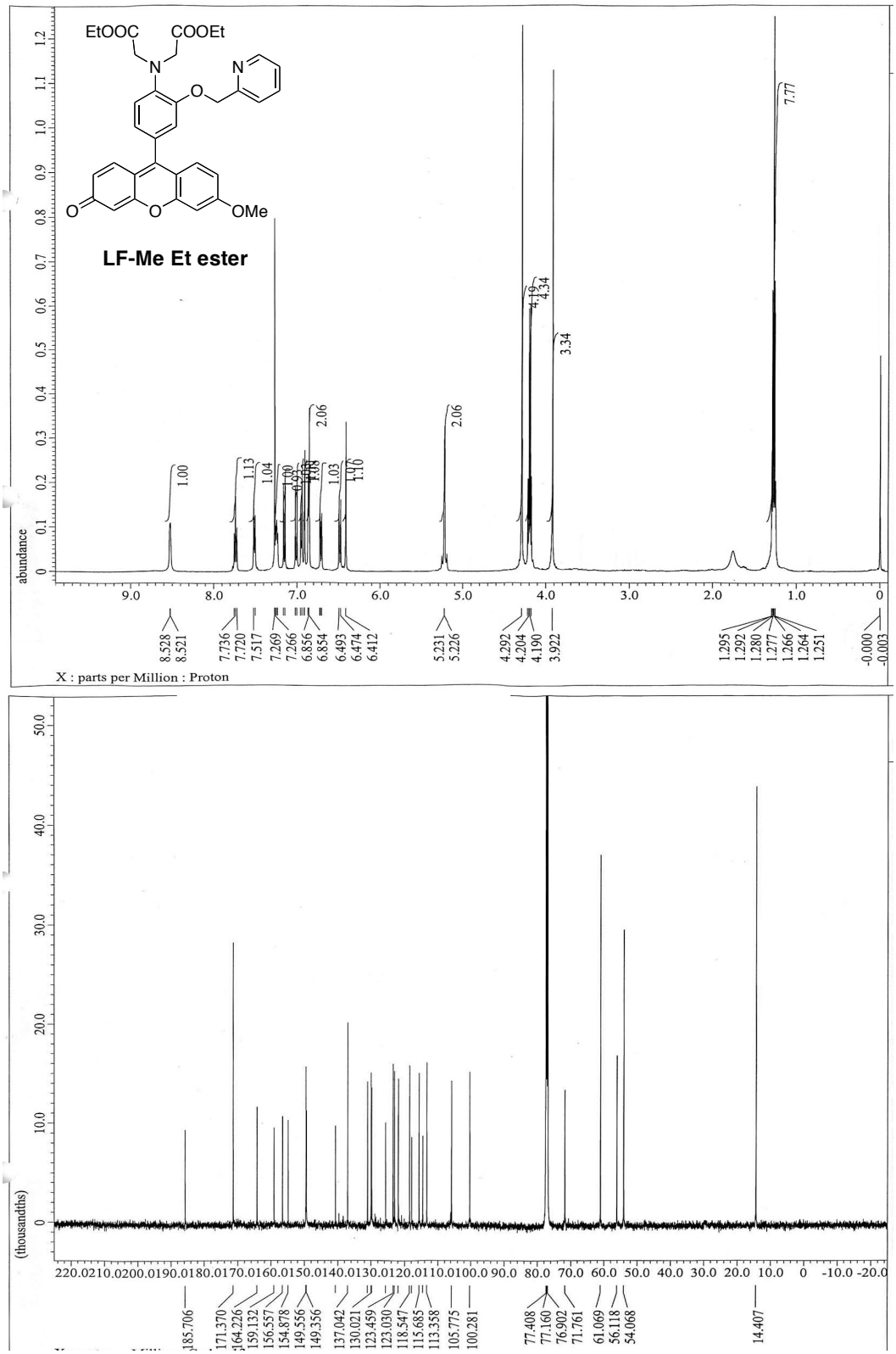

Figure S7. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra of **LF-Me Et ester** in $CDCl_3$.

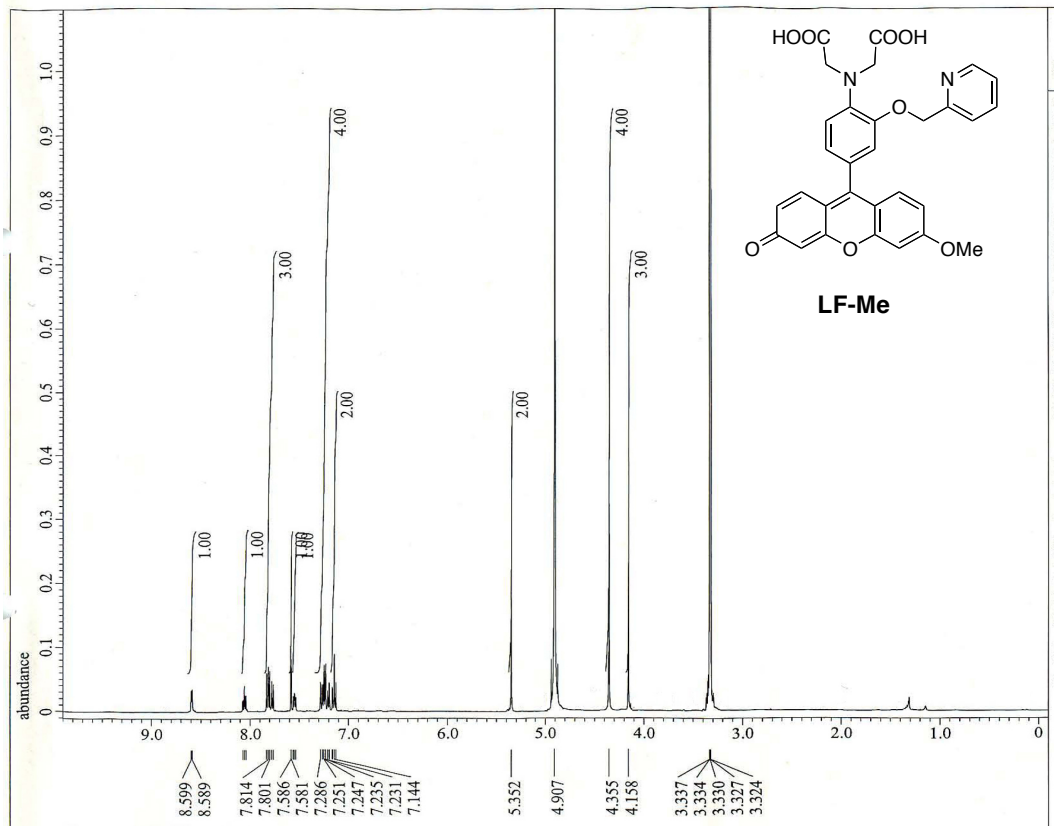

Figure S8. ^{1}H NMR spectra (500 MHz) of **LF-Me** in CD_3OD.

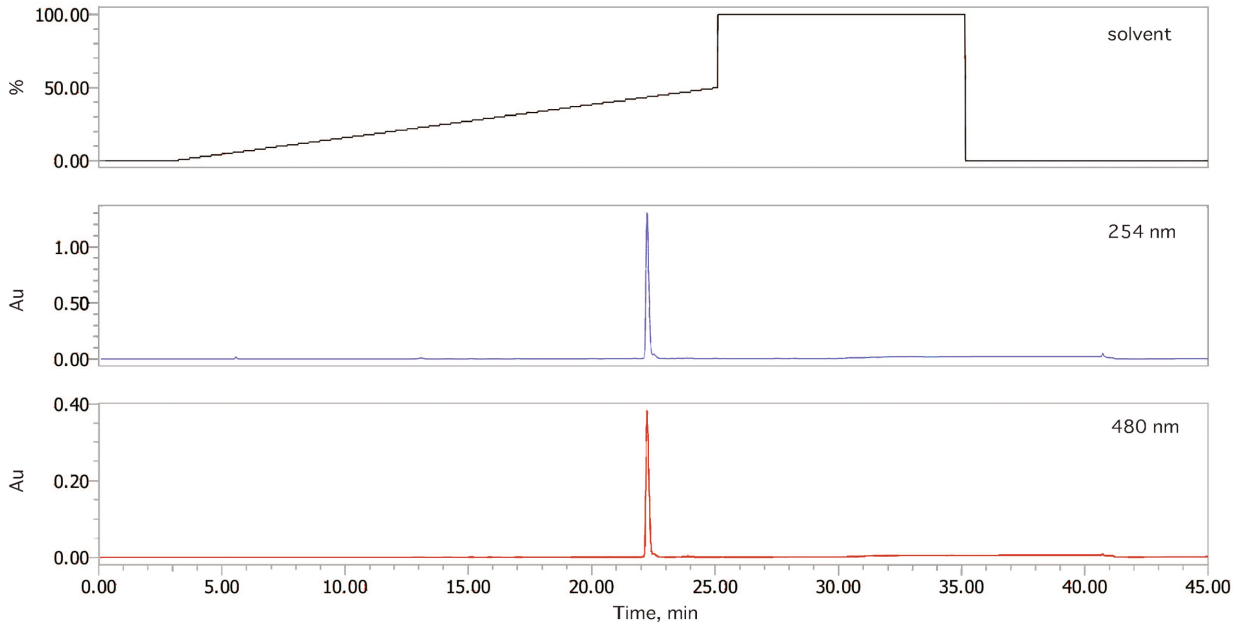

Figure S9. HPLC chart of **LF-Me** eluted with solutions A (water containing 0.1% TFA) and B (CH_3CN containing 0.1% TFA). Black line indicates the ratio of the solution B, blue and red lines indicate the absorbance at 254 and 480 nm, respectively.

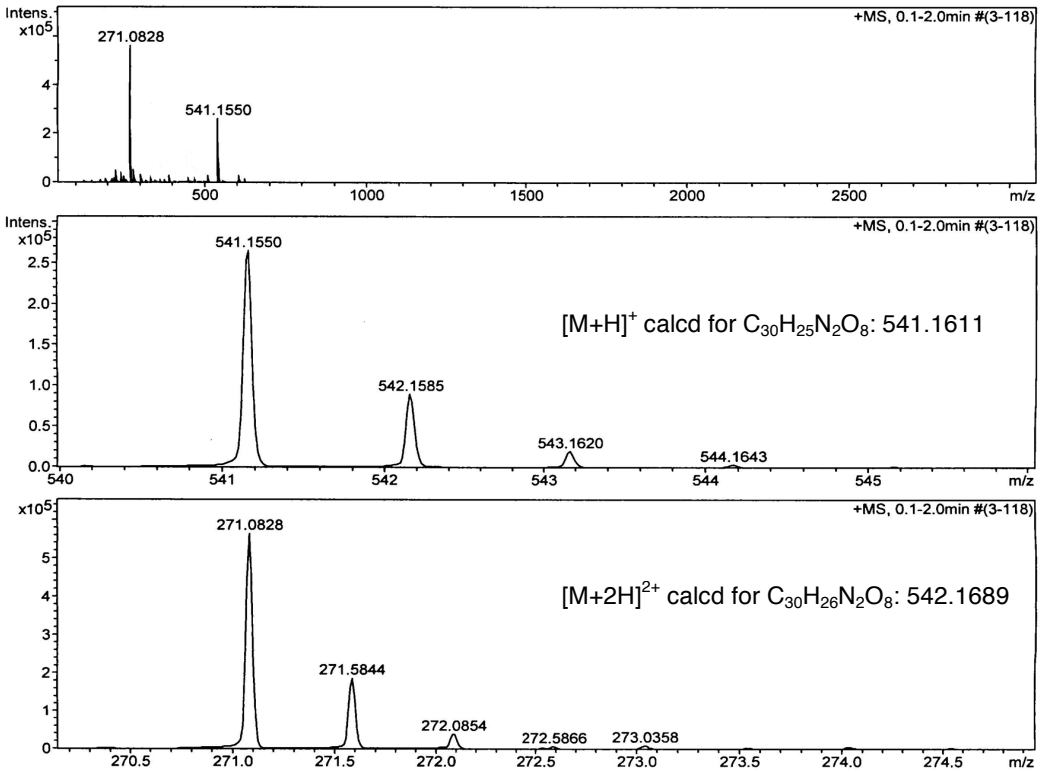

Figure S10. HR-ESI mass spectrum (pos.) of **LF-Me** after purification with semi-preparative HPLC.

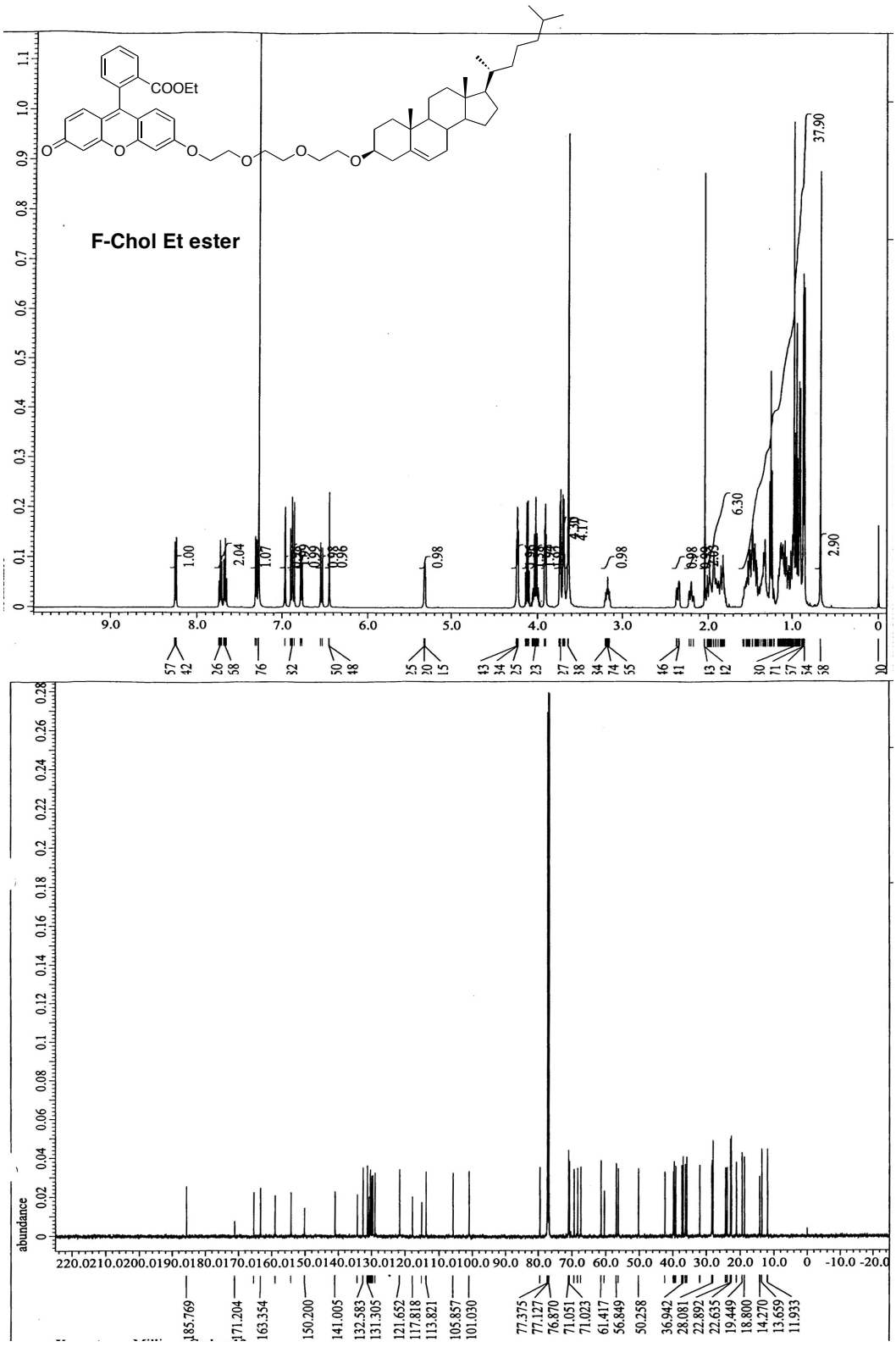

Figure S11. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra of **F-Chol Et ester** in $CDCl_3$.

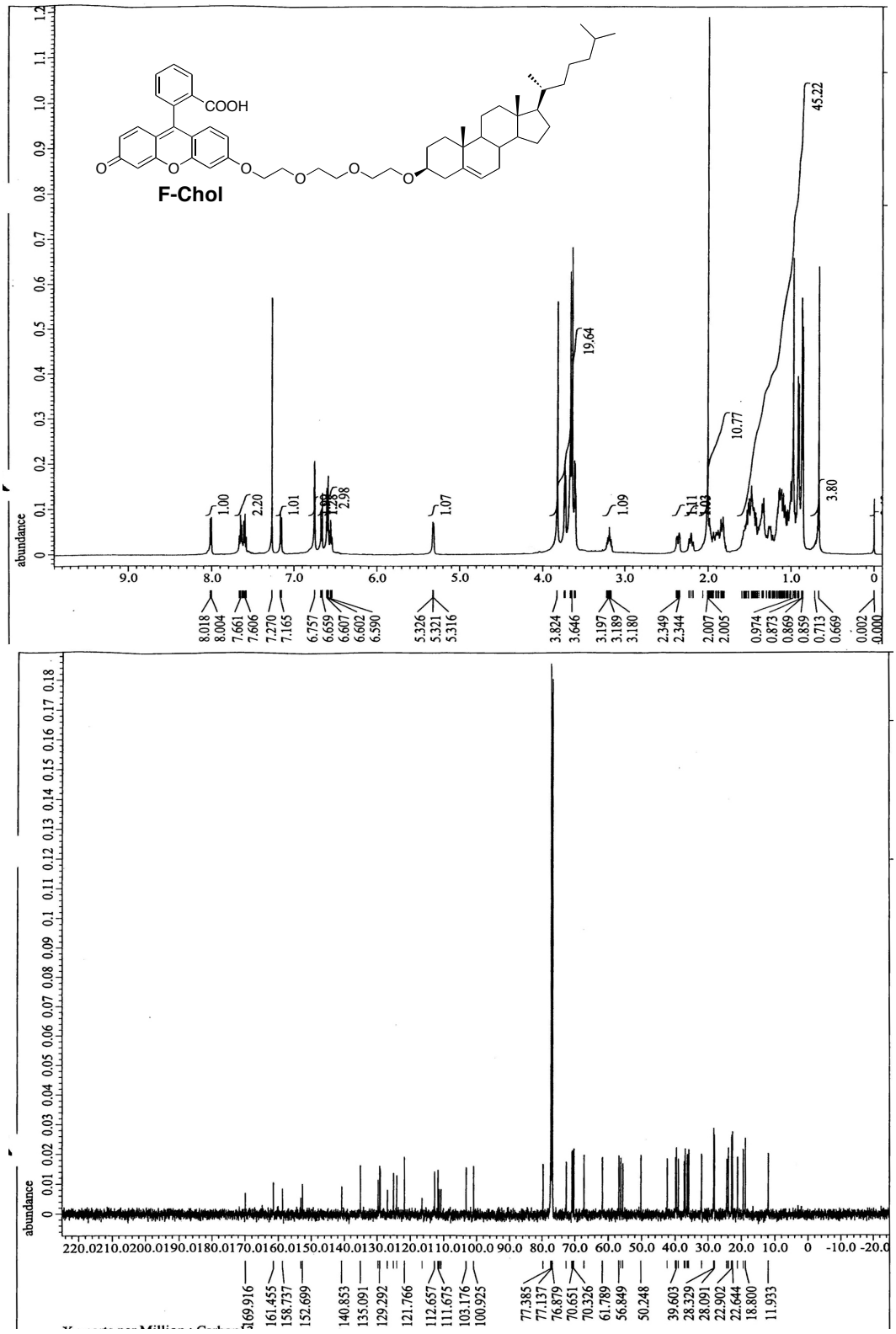

Figure S12. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra of **F-Chol** in $CDCl_3$.

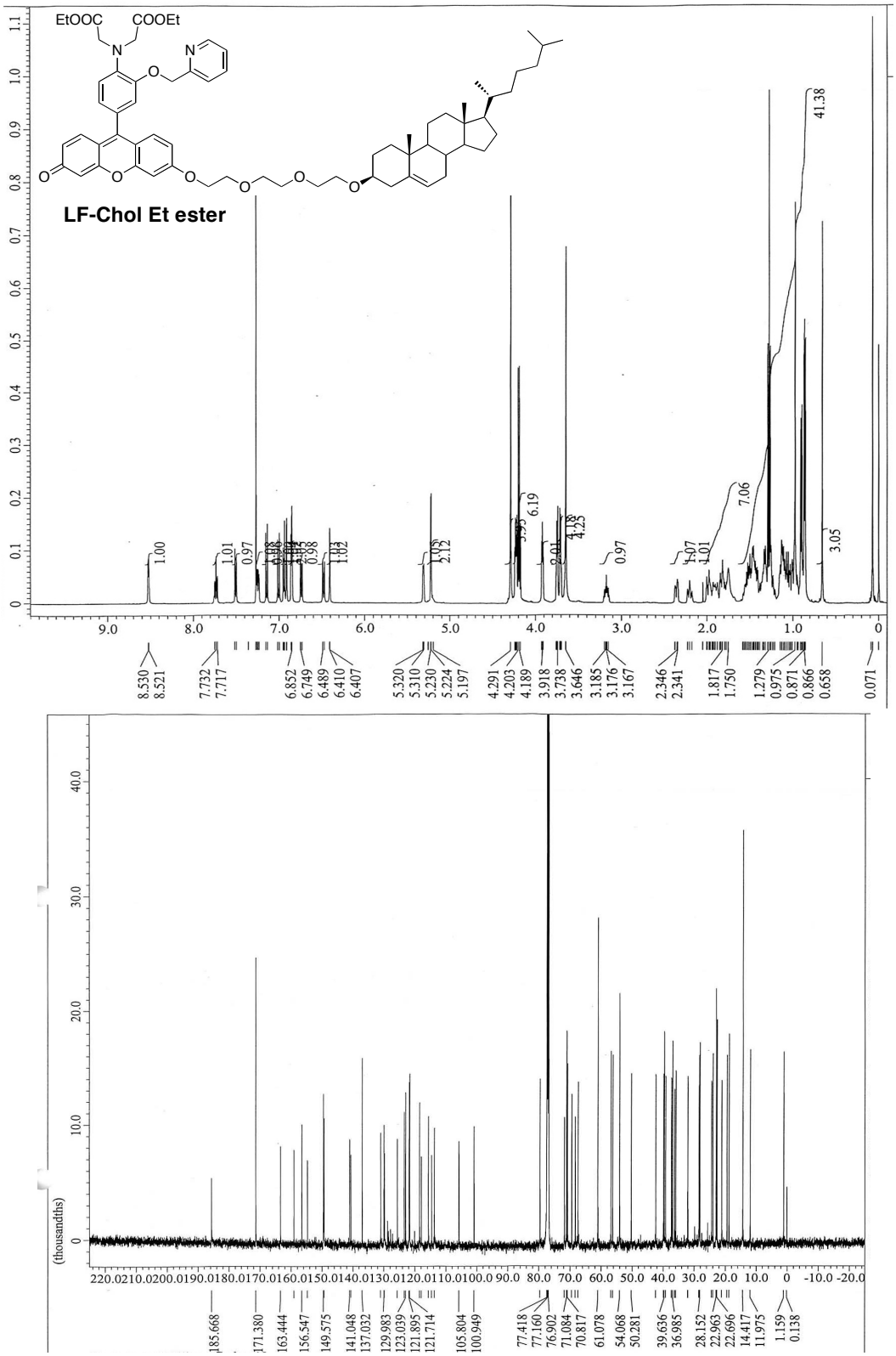

Figure S13. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR spectra of **LF-Chol Et ester** in $CDCl_3$.

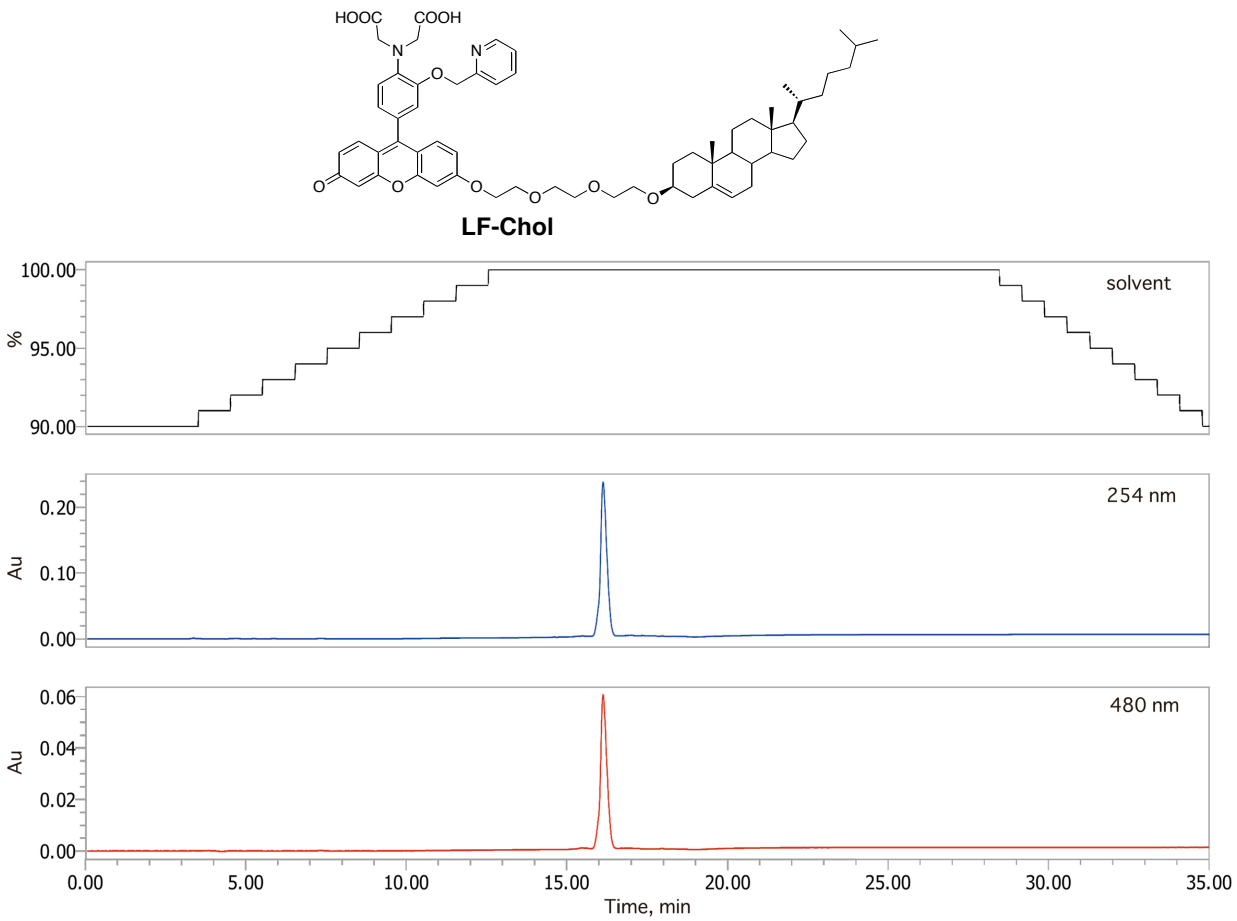

Figure S14. HPLC chart of **LF-Chol** eluted with solutions A (water containing 0.1% TFA) and B (CH_3CN containing 0.1% TFA). Black line indicates the ratio of the solution B, blue and red lines indicate the absorbance at 254 and 480 nm, respectively.

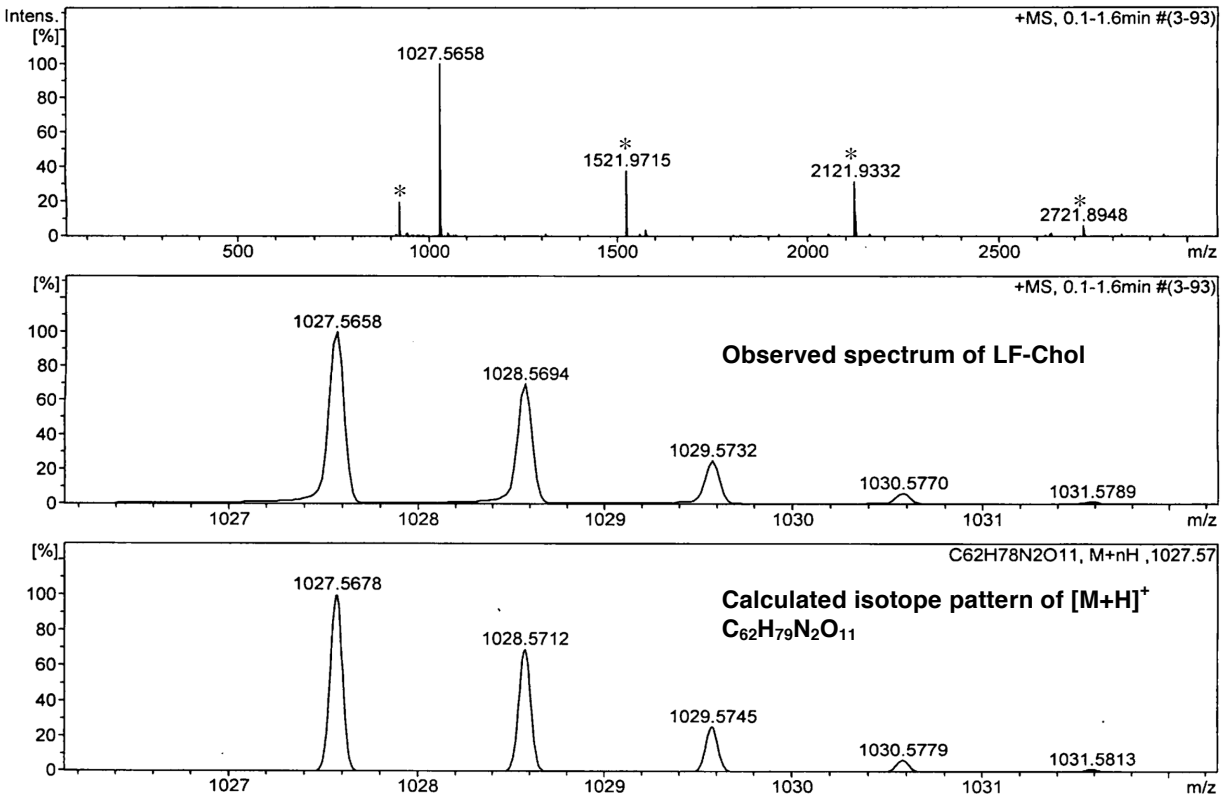

Figure S15. HR-ESI mass spectrum (pos.) of **LF-Chol** after purification with semi-preparative HPLC. Asterisks indicate internal calibration used (Tuning Mix, Agilent).