Supplementary data

Aminoglycoside-based Novel Probes for Bacterial Diagnostic and Therapeutic Applications

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Contents

Abbreviations

ATCC = American Type Culture Collection

BHI = Brain Heart Infusion Broth

Boc = tert-butyloxycarbonyl

Cfu = Colony-Forming Units

Cy = Cyanine Dyes

Cy 5-NHS = Cyanine 5-*N*-hydroxysuccinimide ester

DCM = Dichloromethane

DIPEA = Diisopropyl-ethyl amine

DMF = Dimethylformamide

DMEM = Dulbecco's modified Eagle's medium

DMSO = Dimethyl Sulfoxide

EC = Epithelial Cells

ESI-MS = Electrospray Ionization Mass Spectrometry

equiv. = equivalents

FBS = Fetal Bovine Serum

GFP = Green Fluorescent Protein

HRMS = High Resolution Mass Spectrometry

HPLC = High Performance Liquid Chromatography

LB = Luria-Bertani

LC-MS = Liquid Chromatography Mass Spectrometry

MIC = Minimum Inhibitory Concentration

MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

NB = Nutrient Broth

Neo = Neomycin B

NMR = Nuclear Magnetic Resonance

NHS = *N*-hydroxysuccinimide

OD = Optical Density

PBS = Phosphate Buffered Saline

Rpm = Revolutions Per Minute

R.T. = Room Temperature

TFA = Trifluoro-acetic Acid

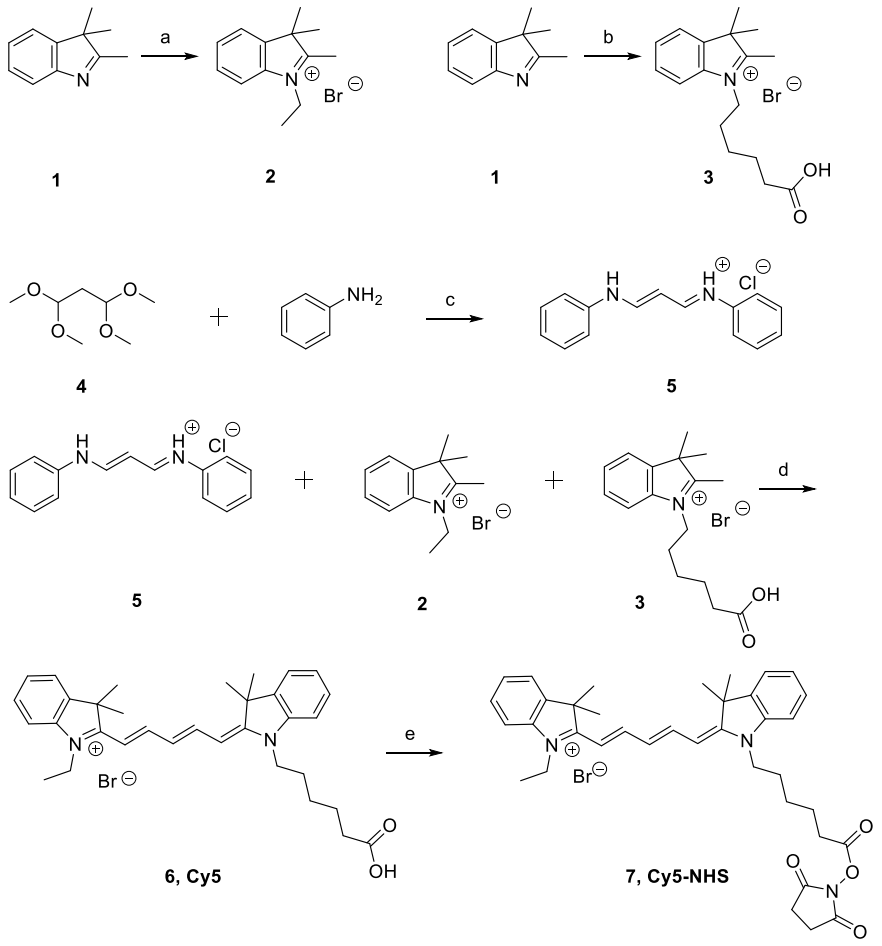
Ts = *p*-Toluenesulfonyl

TSB = Tryptic Soy Broth

General methods

All the chemicals were purchased from J&K. Commercially available reagents were used without further purification. Seven bacterial strains (*Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 12228), *Staphylococcus aureus* (*S. aureus*) (ATCC 29213), Methicillin resistant *Staphylococcus aureus* (MRSA) (ATCC 33592), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853), *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212), *Klebsiella pneumoniae* (*K. pneumoniae*) (ATCC700603), *Escherichia coli* (*E. coli*) (ATCC 25922)) were purchased from American Type Culture Collection (ATCC), USA. One emissive bacteria strain (green fluorescent protein (GFP)-expressed *Escherichia coli* (*E. coli*) DE3) was constructed following a previously reported method. [1] Tow yeast cells, *Pichia pastoris* GS115 and *Saccharomyces cerevisiae* INVSC1 cells, were obtained from Prof. Ping Zhu' Lab (Institute of Materia Medica, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China). Fluorescence emission spectra and full wavelength absorption spectra were performed on 2300 EnSpire TM macroplate reader. UV absorption spectra were recorded in a 10 mm path quartz cell on a Metash UV-5100B spectrometer. Scanning electron microscopy (SEM) images were performed on Nova NanoSEM 450 operated at 5 kV. OD values in MIC tests and MTS assays were also measured by 2300 EnSpire ^{TM} macroplate reader. Fluorescence microscopic imaging and confocal laser scanning microscopic imaging were conducted with ZEISS LSM 800 Confocal Microscope. All ^{1}H NMR spectra were recorded at 400 or 500 MHz, ^{13}C NMR spectra were recorded at 125 MHz respectively. Mass spectra (MS) were measured with Thermo LCQ Deca XP Max mass spectrometer for electrospray ionization mass spectra (ESI). The purity of probes was measured with Alltech HPLC 426.

Synthetic procedures and characterized data



Scheme S1. Synthesis of Cy 5 and Cy 5-NHS. Reagents and conditions: (a) bromoethane, acetonitrile, reflux, 24 h, for **2**; (b) 6-bromohexanoic acid, *o*-dichlorobenzene, 120 ℃, 24 h, for **3**; (c) hydrochloric acid, H_2O, 50 ℃, 2 h, for **5**; (d) **5** and **2**; acetic anhydride, reflux, 2 h, evaporate, then with **3**, anhydrous ethanol, anhydrous sodium acetate, reflux, 6 h, for **6**; (e) *N*,*N*′-disuccinimidyl carbonate, DIPEA, DMF, r.t., overnight, for **7**.

Compound **2**

Compound **1** (4.8 g) and bromoethane (4.9 g) were added to the acetonitrile (30 mL) and the mixture was refluxed for 24 hours. After cooling to room temperature, filtered and got pale pink solid, the solid was washed with ethyl acetate (30 mL), dried and got light pink solid 5.82 g, yield 72.0 %. ^{1}H NMR (400 MHz, DMSO-*d*6) δ 7.98 – 7.96 (m, 1H, -Ar), 7.85 – 7.83 (m, 1H, -Ar), 7.63 – 7.59 (m, 2H, -Ar), 4.52 – 4.47 (q, *J* = 7.3 Hz, 2H, -C*H*$_2$-), 2.84 (s, 3H, -C*H*$_3$), 1.52 (s, 6H, -C*H*$_3$), 1.45 – 1.41 (t, *J* =

7.3 Hz, 3H, -C*H*$_3$). HRMS (*m*/*z*) (M^+): calcd. for $C_{13}H_{18}N^+$ 188.1434, found 188.1436.

Compound **3**

Compound **1** (3.0 g) and 6-bromohexanoic acid (5.4 g) were added to the *o*-dichlorobenzene (20 mL) and the mixture was heated to 120 °C for 24 h. After cooling to r.t., filtered and was washed with ethyl acetate (30 mL). Dried and got gray white solid 4.09 g, yield 61.3 %. ^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.01 (dd, J_1 = 7.0, J_2 = 3.5 Hz, 1H, -Ar), 7.88 (dd, J_1 = 5.0, J_2 = 2.0 Hz, 1H, -Ar), 7.65 (p, J = 4.5 Hz, 2H, -Ar), 4.49 (t, J = 7.7 Hz, 2H, -C*H*$_2$-), 2.88 (s, 3H, -C*H*$_3$), 2.26 (t, J = 7.3 Hz, 2H, -C*H*$_2$-), 1.88 (p, J = 7.8 Hz, 2H, -C*H*$_2$-), 1.57 (s, 8H, -C*H*$_2$-, -C*H*$_3$), 1.46 (td, J_1 = 8.7, J_2 = 4.1 Hz, 2H, -C*H*$_2$-). HRMS (*m*/*z*) (M^+): calcd. for $C_{17}H_{24}O_2N^+$ 274.1802, found 274.1806.

Compound **5**

Compound **4** (4.9 g) and hydrochloric acid (4.25 mL) were added to distilled water (90 mL), and stirred at 50 °C. Then the solution of compound 4 (4.9 g), hydrochloric acid (4.25 mL) and distilled water (70 mL) was added dropwise to the reaction and continued to stir at 50 °C for 2 h. After cooling, filtered, dried and got solid 5.24 g, yield 67.9 %. ^{1}H NMR (500 MHz, Methanol-*d*4) δ 12.63 (s, 2H, -N*H*), 8.68 – 8.65 (d, J = 14.5 Hz, 2H, -Ar), 7.50 – 7.46 (t, J = 9.0, 4H, -Ar), 7.38 – 7.36 (d, J = 10.0, 4H, -Ar), 7.32 – 7.28 (m, 2H, -C*H*-), 6.27 – 6.21 (t, J = 14.5 Hz, 1H, -C*H*-). HRMS (*m*/*z*) (M^+): calcd. for $C_{15}H_{15}N_2^+$ 223.1230, found 223.1234.

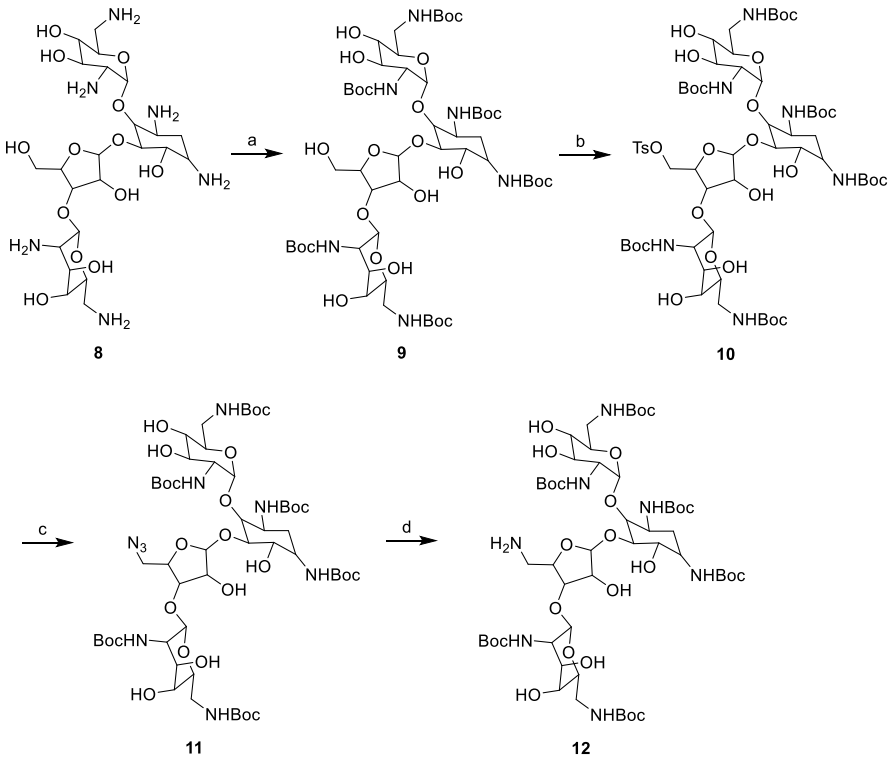
Compound **6** (Cy 5)

The compound **5** (1.16 g) and compound **2** (1.0 g) were added to the acetic anhydride (60 mL) and the mixture was stirred and refluxed for 2 h. The solution was cooled down to r.t. and the solvent was evaporated under reduced pressure. Then anhydrous ethanol (60 mL), compound **3** (1.3 g), and anhydrous sodium acetate (0.66 g) were added to the residue and stirred and refluxed for 6 h. After the reaction was complete, the product was purified by silica gel column chromatography (DCM:MeOH = 30:1) to give deep blue solid 1.18 g, yield 39.0 %, purity >95 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.04 (m, 2H, -Ar), 7.37 – 7.32 (m, 4H, -Ar), 7.22 – 7.16 (q, J = 8.4 Hz, 2H, -Ar), 7.10 – 7.06 (t, J = 8.0 Hz, 2H, -C*H*-), 6.85 (t, J = 12.4 Hz, 1H, -C*H*-), 6.44 – 6.41 (d, J = 13.6 Hz, 1H, -C*H*-), 6.32 – 6.29 (d, J = 12.8 Hz, 1H, -C*H*-), 4.16 – 4.00 (m, 4H, -C*H*$_2$-), 2.50 (t, J = 5.2 Hz, 2H, -C*H*$_2$-), 1.83 – 1.79 (m 2H, -C*H*$_2$-), 1.72 (m, 12H, -C*H*$_3$), 1.55 (t, J = 5.2 Hz, 2H, -C*H*$_2$-),1.40 (m, 2H, -C*H*$_2$-), 1.24 (s, 3H, -C*H*$_3$). HRMS (*m*/*z*) (M^+): calcd. for $C_{33}H_{41}O_2N_2^+$ 497.3163, found 497.3158.

Compound **7** (Cy 5-NHS)

The compound **6** (400 mg), *N*, *N*'-disuccinimidyl carbonate (266 mg) and DIPEA (268 mg) were

added to DMF (10 mL) and the mixture was stirred at room temperature overnight. After the reaction was complete, the product was purified by silica gel column chromatography (DCM:MeOH = 30:1) to give deep blue solid 350 mg, yield 75.0 %. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.24 (m, 2H, -Ar), 7.33 (m, 4H, -Ar), 7.22 – 7.17 (q, *J* = 6.8 Hz, 2H, -Ar), 7.07 (q, *J* = 9.2 Hz, 2H, -C*H*-), 6.80 (t, *J* = 13.2 Hz, 1H, -C*H*-), 6.38 (t, *J* = 13.2 Hz, 2H, -C*H*-), 4.16 (q, *J* = 7.2 Hz, 2H, -CH_2-), 4.08 (t, *J* = 6.8 Hz, 2H, -CH_2-), 2.85 (s, 4H, -CH_2-),2.64 (t, *J* = 7.2 Hz, 2H, -CH_2-), 1.84 (m, 4H, -CH_2-), 1.76 (s, 12H, -CH_3), 1.62 (t, *J* = 14.8 Hz, 2H, -CH_2-), 1.40 (t, *J* = 6.4 Hz, 2H, -CH_2-),1.24 (s, 3H, -CH_3). HRMS (*m*/*z*) (M^+): calcd. for $C_{37}H_{44}O_4N_3^+$ 594.3326, found 594.3314.[2]



Scheme S2. Synthesis of neomycin (C5")-amine. Reagents and conditions: (a) DMF, H_2O, triethylamine, $(Boc)_2O$, 60 ℃, 5 h, for **9**; (b) dry pyridine, *p*-toluenesulfonyl chloride, 0 ℃, overnight, for **10**; (c) Sodium azide, dry DMF, 80 ℃, 8 h, for **11**; (d) 10% $Pd(OH)_2/H_2/C$, methanol, r.t., overnight, for **12**.

Compound **9**

To the mixture of compound **8** (5.0 g) dissolved in DMF (60 mL) and H_2O (20 mL) was added triethylamine (10 mL) and $(Boc)_2O$ (10.8 g) and the mixture was heated to 60 °C for 5 h, then cooled down to room temperature. The reaction solution was extracted with water (40 mL) and ethyl acetate

(3 × 50 mL). The combined organic layer was dried over anhydrous Na_2SO_4, and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1) to give white solid 5.85 g, yield 59.1 %. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 6.67 (m, 1H, -C*H*-), 6.53 (s, 1H, -C*H*-), 5.29 (s, 1H, -C*H*-), 5.16 (s, 1H, -C*H*-), 4.90 (s, 1H, -C*H*-), 4.19 (s, 1H, -C*H*-), 3.97 (s, 1H, -C*H*-), 3.90 – 3.81 (m, 2H), 3.76 – 3.63 (m, 4H), 3.55 – 3.47 (m, 5H), 3.37 – 3.17 (m, 8H), 1.97 – 1.94 (m, 1H), 1.46-1.44 (m, 54H, -CH_3). HRMS (*m*/*z*) (M+H): calcd. for $C_{53}H_{95}O_{25}N_6$ 1215.6341, found 1215.6339.

Compound **10**

To a mixture of compound **9** (400 mg) dissolved in dry pyridine (10 mL) was added *p*-toluenesulfonyl chloride (367.7 mg) at ice bath and the mixture was stirred at room temperature overnight. The reaction solution was extracted with water (30 mL) and ethyl acetate (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4, and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1) to give white solid 410 mg, yield 90.9 %. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.14-8.12 (m, 1H), 7.90-7.72 (m, 2H, -Ar), 7.66 (m, 1H), 7.36 (m, 3H, -Ar, -N*H*), 6.11 (s, 1H), 5.74-5.47 (m, 2H), 5.29 (s, 2H), 5.21-5.11 (s, 1H), 4.94 (s, 2H), 4.87 (s, 1H), 4.73 (s, 1H), 4.40 (s, 1H), 4.33-4.11 (m, 4H), 3.90-3.81 (m, 4H), 3.73-3.39 (m, 8H), 3.15 (s, 2H), 2.68 (s, 2H, -CH_2-), 2.55-2.41 (m, 6H), 2.28-2.05 (m, 2H), 1.8-0.96 (m, 54H, -CH_3). HRMS (*m*/*z*) (M+H): calcd. for $C_{60}H_{101}O_{27}N_6S$ 1369.6430, found 1369.6423.

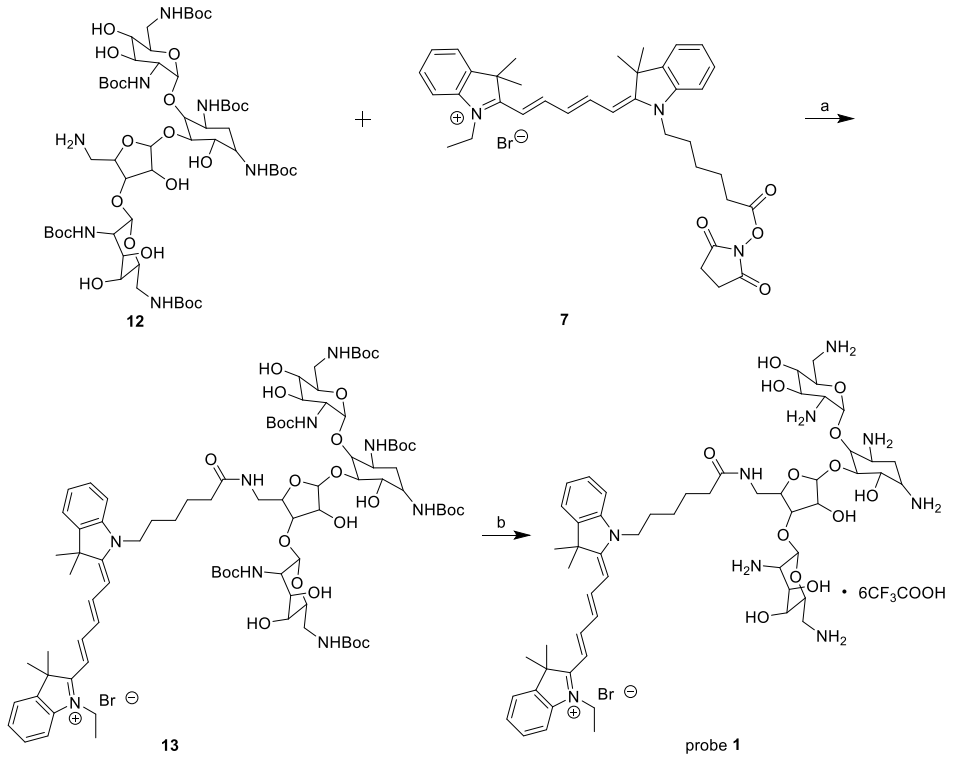
Compound **11**

Sodium azide (270 mg) was added to a solution of compound **10** (500 mg) in dry DMF (10 mL) and the mixture was stirred at 80 °C for 8 h. After cooling to room temperature the reaction mixture was then partitioned between water and ethyl acetate. The aqueous layer (30 mL) was separated and extracted with ethyl acetate (3 × 30 mL). The ethyl acetate layer was dried over Na_2SO_4, concentrated in vacuum. The mixture was used in next step directly. HRMS (*m*/*z*) (M+H): calcd. for $C_{53}H_{94}O_{24}N_9$ 1240.6406, found 1240.6383.

Compound **12**

The solution of compound **11** and 10% $Pd(OH)_2$/C (50 mg) in methanol (10 mL) was hydrogenated at room temperature and pressure for overnight and then filtered. Filtrate was concentrated and the residue was purified by silica gel column chromatography (DCM:MeOH = 20:1) to afford compound **12** (117 mg), as a white solid. The yield of the two step was 26.4%. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 6.93 (m, 1H, -C*H*-), 6.72 (d, *J* = 10.0 Hz,1H, -C*H*-), 5.59 (s, 1H, -C*H*-), 5.13 (s, 1H, -C*H*-), 4.91 (s, 1H, -C*H*-), 4.42 (s, 1H, -C*H*-), 4.16 (s, 1H, -C*H*-), 4.05 – 4.00 (m, 1H), 3.91 (s, 2H), 3.77 – 3.71

(m, 2H), 3.59 – 3.44 (m, 5H), 3.40 – 3.20 (m, 8H), 2.95 – 2.91 (m, 1H), 1.94 – 1.91 (m, 1H), 1.46-1.44 (m, 54H, -C*H*$_3$). HRMS (*m*/*z*) (M+H): calcd. for $C_{53}H_{96}O_{24}N_7$ 1214.6501, found 1214.6482.[3]



Scheme S3. Synthesis of probe **1**. Reagents and conditions: (a) dry DMF, DIPEA, r.t., 3 h, for **13**; (b) TFA, 0 ℃, 15min, for probe **1**.

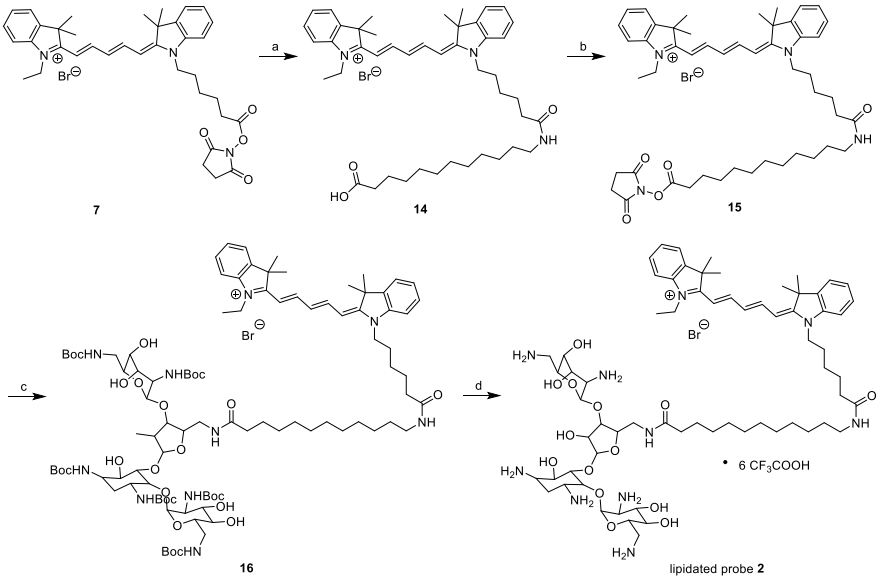
Compound **13**

To a solution of compound **12** (225 mg) in dry DMF, **7** (92 mg) and DIPEA (201 mg) were added and stirred at room temperature for 3 h. The reaction mixture was then partitioned between water and ethyl acetate. The aqueous layer (30 mL) was separated and extracted with ethyl acetate (3 × 30 mL). The ethyl acetate layer was dried over Na_2SO_4, concentrated in vacuum and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford compound **13** (108 mg, 32.9 %), as a blue solid. 1H NMR (400 MHz, Methanol-*d*4) δ 8.28 – 8.00 (m, 2H, -Ar), 7.50 – 7.40 (m, 2H, -Ar), 7.31 – 7.25 (m, 2H, -Ar), 7.05 (m, 2H, , -Ar), 6.67 – 6.61 (m, 2H, -C*H*-), 6.45 (s, 1H, -C*H*-), 6.33 – 6.26 (t, *J* = 12.0 Hz,1H, -C*H*-), 6.20 – 6.08 (m, 1H, -C*H*-), 4.78 – 4.59 (m, 6H), 4.31 – 3.90 (m, 7H), 3.76 – 3.72 (m, 2H), 3.57 (m, 4H), 3.39 – 3.31 (m, 8H), 3.25 – 3.00 (m, 8H), 2.37 – 2.35 (m, 2H), 1.95 – 1.83 (m, 2H), 1.73 (s, 8H, -C*H*$_3$), 1.42 (m, 38H, -C*H*$_3$), 1.35 – 1.14 (m, 23H, -C*H*$_3$). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 174.6, 173.0, 171.0, 157.1, 156.3, 156.1, 155.2, 153.6, 142.0, 141.9, 141.1,

140.9, 128.8, 128.6, 126.9, 125.2, 124.6, 122.1, 122.0, 111.1, 110.1, 103.2, 79.5, 79.4, 79.1, 73.9, 73.0, 72.3, 70.3, 58.4, 53.6, 53.4, 52.1, 52.0, 50.9, 49.4, 49.2, 48.9, 44.7, 41.9, 39.1, 36.0, 36.0, 31.9, 30.7, 30.3, 29.7, 29.6, 29.3, 28.6, 28.4, 28.0, 28.0, 27.2, 26.3, 25.5, 22.7, 18.6, 18.4, 17.6, 17.4, 14.1, 12.3, 12.0. HRMS (*m*/*z*) (M^+): calcd. for $C_{86}H_{134}O_{25}N_9^+$ 1692.9485, found 1692.9487.

Probe **1**

The TFA (3 mL) was added to compound **13** (100 mg) at 0 °C and the reaction mixture was stirred for 15min. Then the solvent was evaporated under reduced pressure and by adding ethyl acetate (5ml), blue solid was precipitated as probe **1** (70mg, 67.3%), purity >95 %. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.27 (m, 2H, -Ar), 7.52-7.40 (m, 4H, -Ar), 7.34 – 3.20 (m, 4H, -Ar, -C*H*-), 6.64 (d, *J* = 12.4 Hz, 1H, -C*H*-), 6.29 (t, *J* = 13.2 Hz, 2H, -C*H*-), 5.83 (s, 1H), 5.42 – 5.32 (m, 2H), 4.42 (s, 1H), 4.33-3.79 (m, 5H), 3.67 (d, *J* = 8.4 Hz, 4H), 3.33 (m, 15H), 2.45 (m, 1H), 2.30 (m, 2H), 2.07 (m, 2H), 1.84 (m, 2H), 1.74 (m, 12H, -CH_3), 1.61-1.26 (m, 7H). ^{13}C NMR (150 MHz, Methanol-d_4) δ 175.3, 173.1, 173.0, 161.8, 161.5, 154.4, 154.1, 142.2, 141.6, 141.4, 141.2, 128.4, 128.3, 125.2, 125.0, 124.8, 122.1, 117.9, 115.6, 110.5, 108.0, 102.7, 95.8, 95.6, 85.3, 82.1, 76.5, 75.2, 74.3, 727, 71.6, 70.7, 70.4, 68.0, 67.8, 60.2, 53.6, 51.4, 49.5, 49.2, 49.1, 48.8, 43.4, 40.4, 40.2, 38.6, 35.5, 28.0, 27.4, 26.9, 26.5, 26.4, 25.4, 19.5, 13.1, 11.2. HRMS (*m*/*z*) (M^+): calcd. for $C_{56}H_{86}O_{13}N_9^+$ 1092.6340, found 1092.6331.



Scheme S4. Synthesis of lipidated probe **2**. Reagents and conditions: (a) dry DMF, 12-amino dodecanoic acid, DIPEA, r.t., overnight, for **14**; (b) dry DMF, *N*, *N'*-disuccinimidyl carbonate, DIPEA, r.t., overnight, for 15; (c) compound **12**, dry DMF, DIPEA, r.t., 3 h, for **16**; (d) TFA, 0 ℃, 15 min, for lipidated probe **2**.

Compound **14**

To a solution of compound **7** (80mg) in dry DMF (10ml), 12-amino dodecanoic acid (75 mg) and DIPEA (154mg) were added and stirred at r.t. for overnight. The reaction mixture was then partitioned between water and ethyl acetate. The aqueous layer (30 mL) was separated and extracted with ethyl acetate (3 x 30 mL). The ethyl acetate layer was dried over Na_2SO_4, concentrated in vacuum and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford compound 14 (81 mg, 88.0 %), as a blue solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.02 – 7.98 (m, 2H, -Ar), 7.63 (m, 1H, -*NH*-), 7.39 – 7.35 (m, 4H, -Ar), 7.24 – 7.20 (m, 2H, -Ar), 7.14 (d, *J* = 8.0 Hz,1H, -C*H*-), 7.08 (d, *J* = 8.0 Hz,1H, -C*H*-), 6.96 (s, 1H, -N*H*), 6.53 (d, *J* = 13.5 Hz, 1H, -C*H*-), 6.33 (d, *J* = 13.0 Hz, 1H, -C*H*-), 4.11 (q, *J* = 7.5 Hz, 4H, -CH_2-), 3.22 (d, *J* = 10.5 Hz, 2H, -CH_2-), 2.43 – 2.35 (m, 4H, , -CH_2-), 1.84 (t, *J* = 7.5 Hz, 2H, -CH_2-), 1.73 (m, 12H, -CH_3), 1.63 – 1.55 (m, 4H, -CH_2-), 1.42 (m, 2H, -CH_2-), 1.28 – 1.23 (m, 13H, -CH_2-, -CH_3), 0.89 – 0.85 (m, 6H, -CH_2-). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 173.9, 173.0, 171.7, 153.6, 153.0, 141.9, 141.7, 141.2, 141.1, 128.7, 128.6, 126.6, 125.3, 124.9, 122.2, 122.1, 110.9, 110.1, 104.5, 103.2, 49.3, 49.1, 44.6, 39.6, 37.1, 34.3, 31.9, 30.0, 29.7, 29.6, 29.3, 29.0, 29.0, 28.9, 28.7, 28.1, 27.1, 26.8, 26.4, 25.3, 24.7, 22.7, 19.7, 14.1, 12.4. HRMS (*m*/*z*) (M^+): calcd. for $C_{45}H_{64}O_3N_3^+$ 694.4942, found 694.4933.

Compound **15**

To a solution of compound **14** (105 mg) in dry DMF (10 ml), *N*, *N*'-disuccinimidyl carbonate (68 mg) and DIPEA (69 mg) were added and stirred at r.t. for overnight. The reaction mixture was then partitioned between water and ethyl acetate. The aqueous layer (30 mL) was separated and extracted with ethyl acetate (3 × 30 mL). The ethyl acetate layer was dried over Na_2SO_4, concentrated in vacuum and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford compound **15** (93 mg, 78.7 %), as a blue solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.98 (m, 2H, -Ar), 7.37 (m, 4H, -Ar), 7.28 (m, 1H, -C*H*-), 7.23 (dd, J_1= 14.5 Hz, J_2 = 7.5 Hz, 2H, -Ar), 7.14-7.07 (m, 2H, -C*H*-), 6.97 (s, 1H, -N*H*), 6.58 (d, *J* = 14.0 Hz, 1H, -C*H*-), 6.35 (m, 1H, -C*H*-), 4.11 (m, 4H, -CH_2-), 3.23 – 3.19 (m, 2H, -CH_2-), 2.84 (m, 2H, -CH_2-), 2.65 (m, 2H, -CH_2-), 2.59 – 2.56 (m, 2H, -CH_2-), 2.39 – 2.35 (m, 2H, -CH_2-), 1.75 – 1.76 (m, 4H, , -CH_2-), 1.73 (m, 12H, -CH_3), 1.59 – 1.50 (m, 2H, -CH_2-), 1.43 – 1.37 (m, 2H, -CH_2-), 1.26 (m, 13H, -CH_2-, -CH_3) 0.86 (m, 6H, -CH_2-). ^{13}C NMR (150 MHz, Chloroform-*d*) δ 173.6, 173.1, 171.6, 171.1, 169.2, 168.7, 153.6, 152.9, 141.9, 141.7, 141.2, 141.1, 128.7, 128.6, 126.7, 125.2, 124.8, 122.2, 122.1, 110.9, 110.0, 104.6, 103.2, 60.4, 54.0, 49.1, 44.6, 39.6, 37.1, 36.1, 31.9, 30.9, 29.7, 29.5, 29.4, 29.3, 29.3, 28.7, 28.0, 27.1, 27.0, 26.4,

25.6, 24.5, 22.7, 21.0, 14.2, 14.1, 12.4. HRMS (*m*/*z*) (M^+): calcd. for $C_{49}H_{67}O_5N_4^+$ 791.5106, found 791.5105.

Compound **16**

To a solution of compound 12 (203 mg) in dry DMF (10 ml), compound 15 (83 mg) and DIPEA (75 mg) were added and stirred at room temperature for 3 h. The reaction mixture was then partitioned between water and ethyl acetate. The aqueous layer (30 mL) was separated and extracted with ethyl acetate (3 × 30 mL). The ethyl acetate layer was dried over Na_2SO_4, concentrated in vacuum and the residue was purified by silica gel column chromatography (DCM:MeOH = 30:1) to afford compound 16 (45 mg, 24.0 %), as a blue solid. HRMS (*m*/*z*) (M^+): calcd. for $C_{98}H_{157}O_{26}N_{10}^+$ 1890.1265, found 1890.1274.

Lipidated probe **2**

TFA (3 mL) was added to compound **16** (45 mg) at 0 °C. The reaction mixture was stirred at room temperature for 15 min. Then the solvent was evaporated under reduced pressure and by adding ethyl acetate (5 ml), blue solid was precipitated as lipidated probe **2** (30 mg, 64.1%), purity >95 %. ^{1}H NMR (500 MHz, Methanol-*d*4) δ 8.26 (m, 2H, -Ar), 7.51 (t, *J* = 6.4 Hz, 2H, -Ar), 7.43 (q, *J* = 7.7 Hz, 2H, -Ar), 7.29 (m, 4H, -Ar, -C*H*-), 6.64 (t, *J* = 12.4 Hz, 1H, -C*H*-), 6.30 (m, 2H, -C*H*-), 5.78 (s, 1H), 5.41 (d, *J* = 5.0 Hz, 1H), 5.32 (s, 1H), 4.43 (t, *J* = 4.5 Hz,1H), 4.31 (d, *J* = 6.8 Hz, 2H), 4.22 – 4.08 (m, 6H), 3.99 – 3.85 (m, 2H), 3.76 – 3.63 (m, 4H), 3.44 – 3.42 (m, 2H, -CH_2-), 3.36 – 3.24 (m, 9H), 3.14 – 3.11 (m, 2H, -CH_2-),2.28 – 2.20 (m, 4H, -CH_2-), 1.84 (t, *J* = 7.2 Hz, 2H), 1.74 (m, 10H, -CH_3), 1.61 (s, 2H), 1.47 – 1.46 (m, 6H), 1.42 – 1.38 (m, 4H), 1.30 (m, 12H), 1.00-0.87 (m, 4H). ^{13}C NMR (125 MHz, Methanol-d_4) δ 175.8, 174.3, 173.2, 172.9, 154.3, 154.1, 142.2, 141.7, 141.4, 141.2, 128.4, 128.3, 125.2, 124.9, 124.8, 122.1, 122.0, 110.6, 110.4, 108.0, 102.9, 102.6, 95.8, 95.5, 85.2, 82.0, 76.5, 75.0, 74.3, 72.6, 71.6, 70.7, 70.4, 68.0, 67.8, 60.2, 54.4, 53.6, 51.4, 49.2, 49.1, 43.3, 42.4, 40.4, 40.2, 39.0, 38.6, 35.8, 35.3, 29.4, 29.4, 29.3, 29.1, 29.1, 29.0, 26.8, 26.7, 26.5, 26.4, 26.0, 25.7, 25.2, 19.5, 17.3, 15.8, 13.1, 11.8, 11.2. HRMS (*m*/*z*) (M^+): calcd. for $C_{68}H_{109}O_{14}N_{10}^+$ 1289.8119, found 1289.8101.

UV-vis absorption and fluorescence spectra

The concentration of DMSO stock solution of probe **1**, lipidated probe **2**, Cy 5 or neomycin was diluted to 10 μM in Phosphate Buffered Saline (PBS). The UV-Visible spectra were recorded using a 2300 EnSpire TM macroplate reader. Wavelength interval: 1.0 nm. Fluorescence spectroscopic studies were also performed at the excitation wavelength of 640 nm.

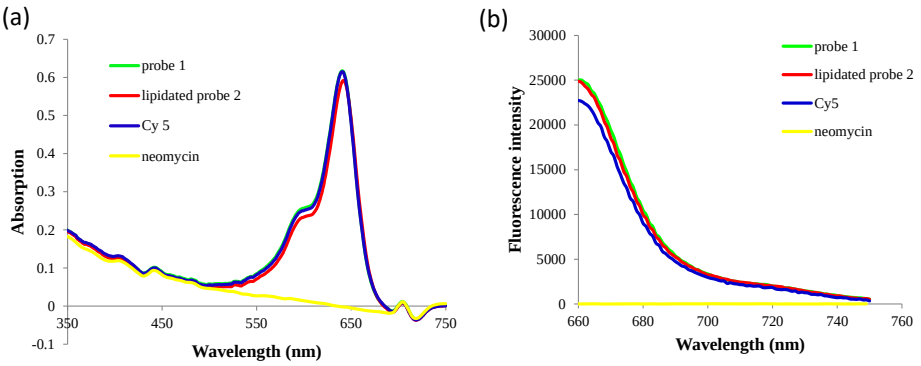


Fig. S1. Absorption and fluorescent emission spectra of probe **1**, lipidated probe **2**, Cy 5 or neomycin. (a) Absorption spectrum of probe **1**, lipidated probe **2**, Cy 5 and neomycin (10 μM in PBS). (b) Fluorescent emission spectrum of probe **1**, lipidated probe **2**, Cy 5 and neomycin (10 μM in PBS) excited at 640 nm. The UV-Visible and fluorescence spectra of probe **1**, lipidated probe **2**, Cy 5 and neomycin are shown with green, red, blue and yellow lines, respectively.

Bacteria and cell culture

Seven wild-type bacteria strains: *S. epidermidis*, methicillin sensitive and resistant *S. aureus*, *P. aeruginosa*, *E. faecalis*, *K. pneumoniae* and *E. coli* and one emissive bacteria strains: GFP-expressed *E. coli* (DE3) were used in this study. Luria-Bertani (LB) medium containing 100 μg/mL Ampicillin was used for culture of GFP-expressed *E. coli* (DE3). Tryptic Soy Broth (TSB) medium was used for culture of methicillin sensitive *S. aureus*, *P. aeruginosa* and *E. coli*. Nutrient broth (NB) was used for culture of methicillin resistant *S. aureus*, *K. pneumoniae* and *S. epidermidis*. Brain Heart Infusion Broth (BHI) was used for culture of *E. faecalis*. Single colony from the stock agar plate was added to 10 mL of liquid medium, then was grown at 37 °C on a shaker incubator (180 rpm) overnight followed by a subculture until an OD_{600} of approximately 0.5-0.7 was reached.

The Raw 264.7 cells, epithelial cells (EC) and rat fibroblast L6 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) under humidified atmosphere of 5 % CO_2 at 37 °C.

Minimum inhibitory concentration (MIC) test[4]

1.0 mL aliquots of bacterial strains cultured in respective solution were collected and centrifuged. The cell pellets were washed twice and resuspended in sterile phosphate-buffered saline (PBS pH 7.2) at OD_{600} of 0.5, then further diluted to OD_{600} of 5×10^{-4}. Aliquots of this suspension (100 μL) were placed into a 96-well plate. The probe **1**, lipidated probe **2** and Cy 5 were diluted in PBS and then added into the bacteria suspensions to give the desired concentration. The cultures were then added respective solutions and further incubated at 37 °C for 24 h. The wells containing the same number of cells but no compounds and the wells containing the same culture solution but without bacterial cells were set as control groups. The plate was then read using a 96-well plate reader at 600 nm. Each concentration had triplicate values, and the whole experiment was done at three times and the MIC value was determined by taking the average of triplicate OD_{600} values for each concentration and plotting it against concentration. The data was then subjected to sigmoidal fitting. The MIC value was determined, as the point in the curve where the OD_{600} is similar to that of control having no bacteria (Table S1).

Table S1. Representative Minimal Inhibitory Concentrations (MIC) in μM for Various Bacterial Strains.

Compd	MIC (μM)						
	S.aureus[a]	MRSA[b]	*S.epidermidis*[c]	*K.pneumoniae*[d]	*E.coli*[e]	*P.aeruginosa*[f]	*E. faecalis*[g]
Neo B	0.4	>50	0.2	12.5	3.125	12.5	>50
Cy5	25	12.5	25	>50	>50	>50	>50
Probe **1**	50	6.25	1.56	>50	50	50	>50
Probe **2**	6.25	3.125	1.56	12.5	12.5	12.5	50

[a]ATCC 29213. [b]Methicillin-resistant *S.aureus* (ATCC 33592). [c]ATCC 12228. [d]ATCC 27853. [e]ATCC 25922. [f]ATCC 700603. [g]ATCC 29212.

MTS assay

The Raw 264.7 cells, epithelial cells (EC) and rat fibroblast L6 cells were seeded on a 96-wells containing 7500 cells per well in 100μL DMEM media and incubated for overnight before adding probe **1**, lipidated probe **2** or Cy 5. Upon incubation with different concentration of probe **1**, lipidated probe **2** or Cy 5 at 37 °C for 24 h, then incubated with cell culture medium containing 20% MTS (3-(4,5-di-methylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium, inner salt). After 4 h incubation at 37 °C, the absorbance was measured at 590 nm using a 2300 EnSpire TM

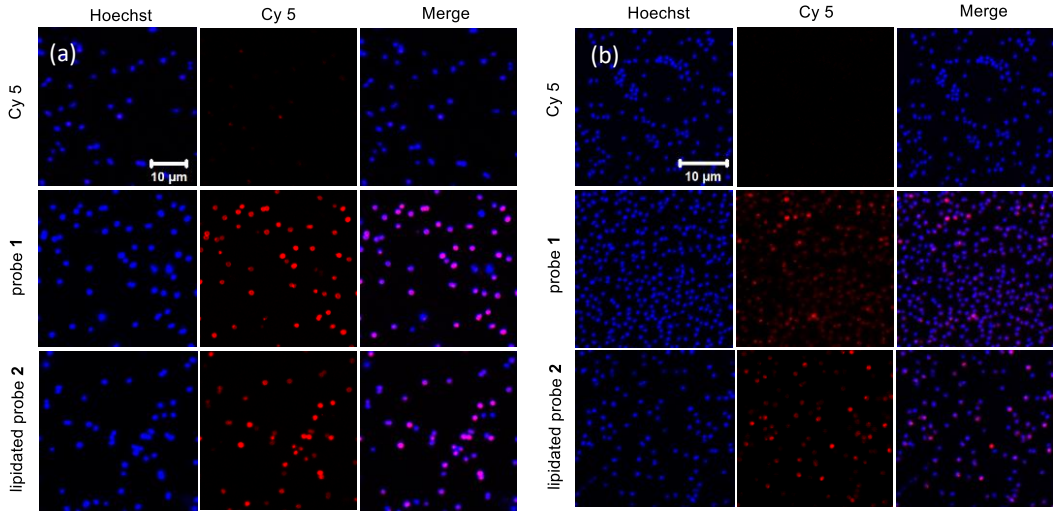
microplate reader. Cell viabilities at various concentrations were given as the percentage of control sample without probe. Each experiment was repeated three times.

Table S2. The cytotoxicity assays of probe **1**, lipidated probe **2** or Cy 5 in the Raw 264.7 cells, epithelial cells (EC) and rat fibroblast L6 cells

IC_{50}	probe **1** (μM)	lipidated probe **2** (μM)	Cy 5 (μM)
Raw 264.7 cells	>50	13.14	12.20
Epithelial cells	>50	25.11	12.57
Rat fibroblast L6 cells	>50	24.66	25.57

Confocal imaging of bacteria treated with probe 1, lipidated probe 2 or Cy 5

S. epidermidis, *MRSA*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* cells were cultured for 12 h in respective media at 37 °C. Bacterial strains cultured overnight in respective solution were harvested and washed twice with PBS (pH 7.4). The washed cells were resuspended in PBS with an OD600 of 0.5-0.7. Then 200 μL aliquots were treated with 5 μM of probe **1**, lipidated probe **2** or Cy 5 (Fig. S2). After incubation at 37 °C for 2 h, the cells were washed with PBS by centrifugation to remove the unbound reagents and treated with 20 μg/mL of Hoechst33258 at 37 °C for 30 min. Then a drop of the suspension was added into an 8-well chamber followed by covering with agarose pads.[5] Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope (Nikon Eclipse TE2000-E, CFI Plan-Apochromat VC 63 × oil immersedoptics), using a high pressure He-Ne lamp and diode laser for excitation (λ_{ex} = 640 ± 20 nm and λ_{em} = 660 ± 25 nm; λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm).



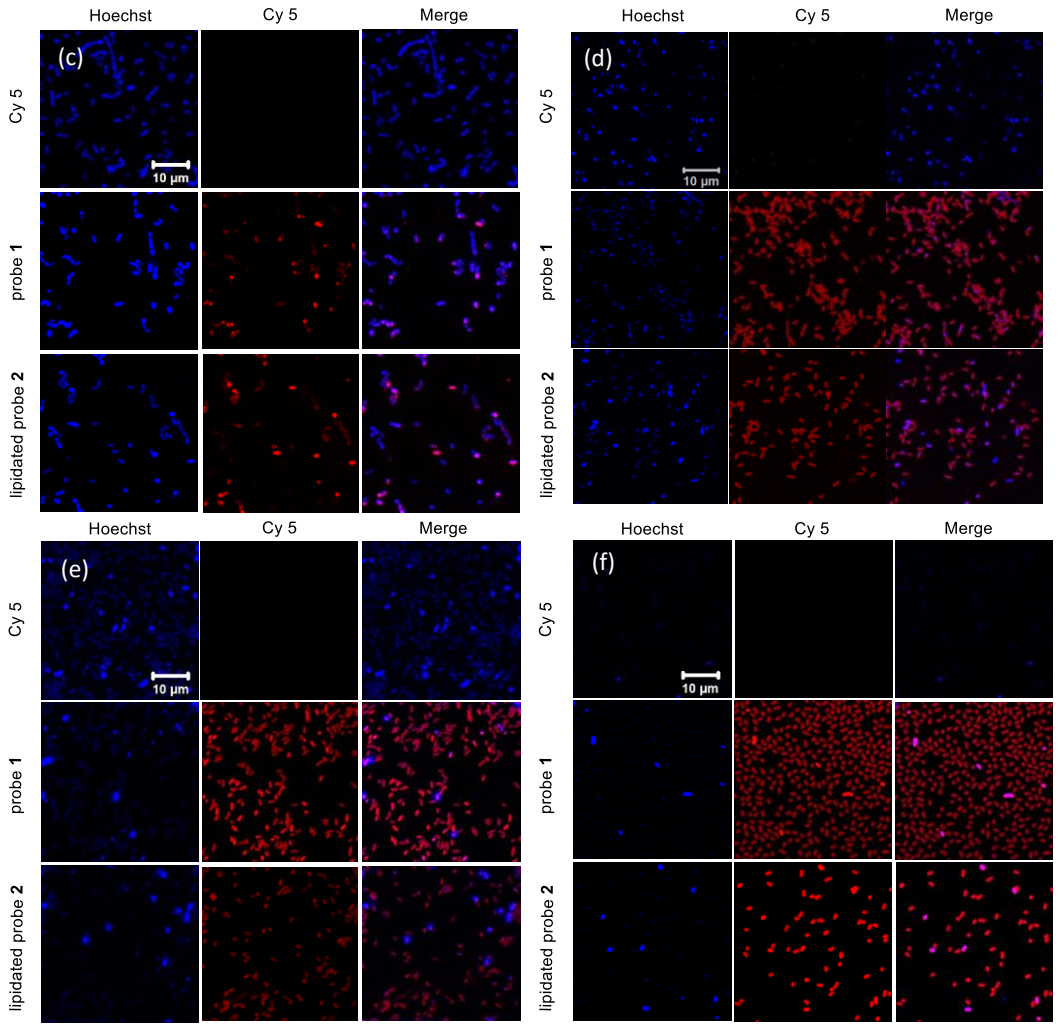


Fig. S2. Fluorescence images of *S. epidermidis* (a), MRSA (b), *E. faecalis* (c), *E. coli* (d), *K. pneumoniae* (e) and *P. aeruginosa* (f) cells staining with probe **1**, lipidated probe **2** or Cy 5. The bacterial cells were treated with 5 μM compounds at 37 °C for 2 h. λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm; λ_{ex} = 640 ± 20 nm and λ_{em} = 660 ± 25 nm. Scale bar = 10 μm.

Confocal imaging of mammalian cells treated with probe 1, lipidated probe 2 or Cy 5[6]

Upon reaching 80 % confluence, the Raw 264.7 cells or rat fibroblast L6 cells (0.3 mL, 1 × 10^5 cells/mL) were transferred into an 8-well chamber containing sterile coverslips at the bottom. The Raw 264.7 cells and rat fibroblast L6 cells were cultured for 24 h in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum (FBS) under humidified atmosphere of 5 % CO_2 at 37 °C. The cells were added 5 μM of probe **1**, lipidated probe **2** or Cy 5 and incubated at 37 °C for 2 h. After incubation, the adhesive Raw 264.7 or L6 cells were also washed with PBS at least for three

times to remove unbound probe **1**, lipidated probe **2** or Cy 5 molecules and treated with 2 μg/mL of Hoechst 33258 at 37 °C for 5 min. Later, cells attached on 8-well chamber were observed with ZEISS LSM 710 Confocal Microscope equipped with a 40 × immersion lens (Fig. S3).

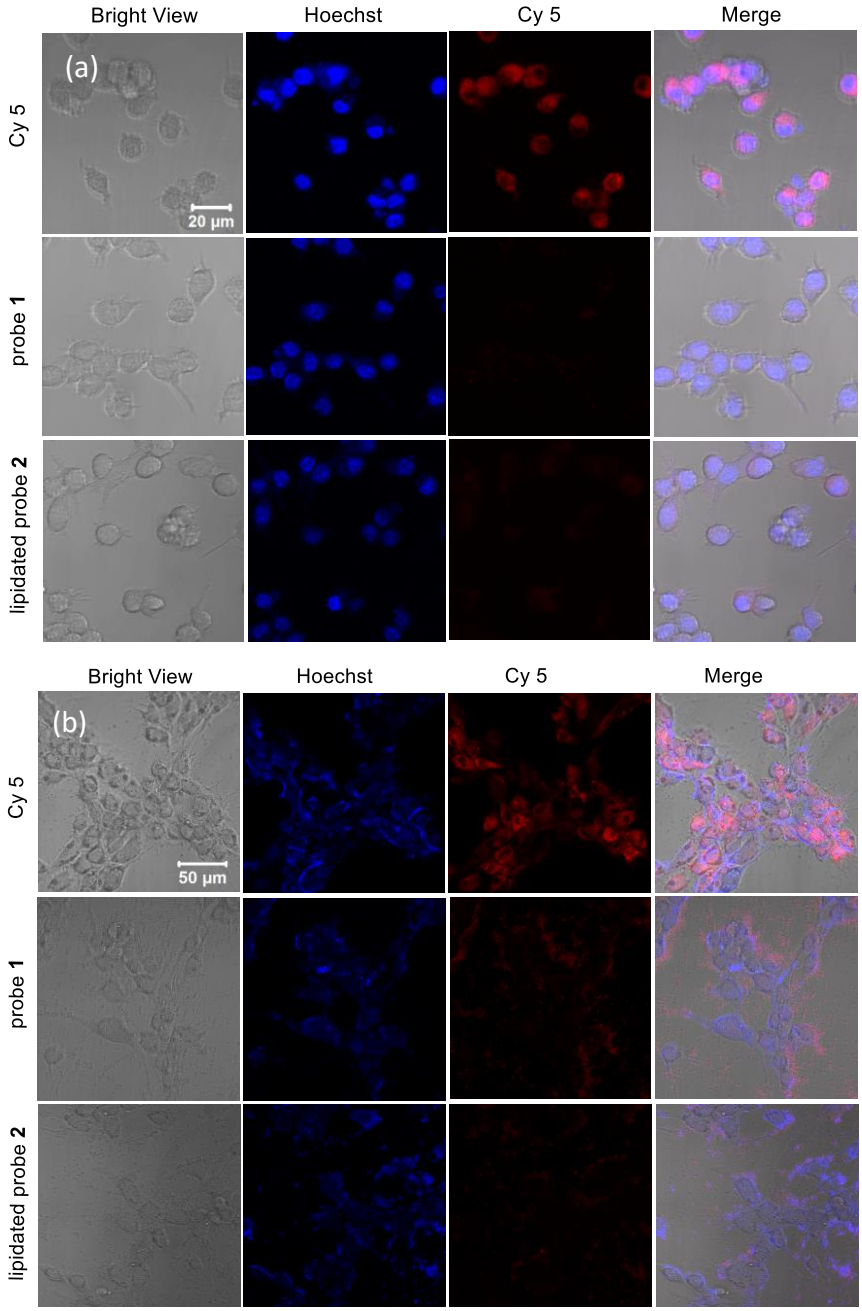
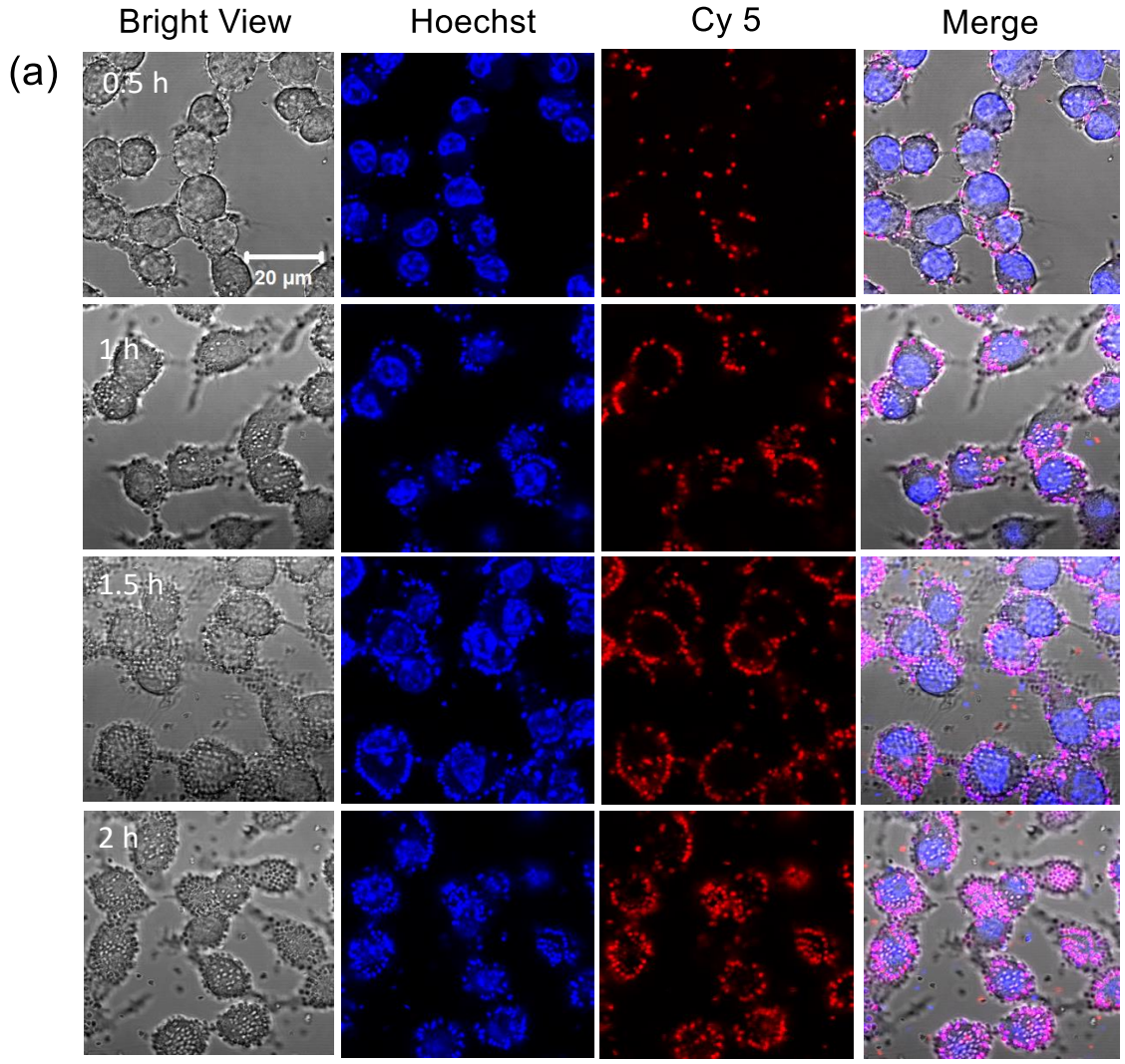


Fig. S3. Fluorescence images of the Raw 264.7 cells (a) or L6 cells (b) staining with probe **1**, lipidated probe **2** or Cy 5. The bacterial and mammalian cells were treated with 5 μM compounds at 37 °C for 2 h. λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm; λ_{ex} = 640 ± 20 nm and λ_{em} = 660 ± 25 nm; Scale bar = 20 μm; Scale bar = 50 μm.

Fluorescence microscopic imaging of mixture of bacterial and mammalian cells[7]

Upon reaching 80 % confluence, the Raw 264.7 (0.3 mL, 1 × 10^5 cells/mL) were transferred into an 8-well chamber containing sterile coverslips at the bottom. After overnight culture at 37 °C, the medium was replaced with 0.3 mL of *S. epidermidis*, methicillin resistant *S. aureus*, *P. aeruginosa* and *K. pneumoniae* suspended in PBS with OD_{600} of 0.5. Simultaneously, probe **1** was added into the wells containing the Raw 264.7 cells with a final concentration of 5 μM. After incubation at 37 °C for 0.5 h, 1 h, 1.5 h and 2 h, the bacteria in the suspension were isolated, followed by washing with PBS at least for three times to remove unbound probe **1** and treated with 2 μg/mL of Hoechst 33258 at 37 °C for 5 min. Finally, The Raw 264.7 cells with the bacterial attached on an 8-well chamber were observed with ZEISS LSM 710 Confocal Microscope equipped with 40 × immersion lens.



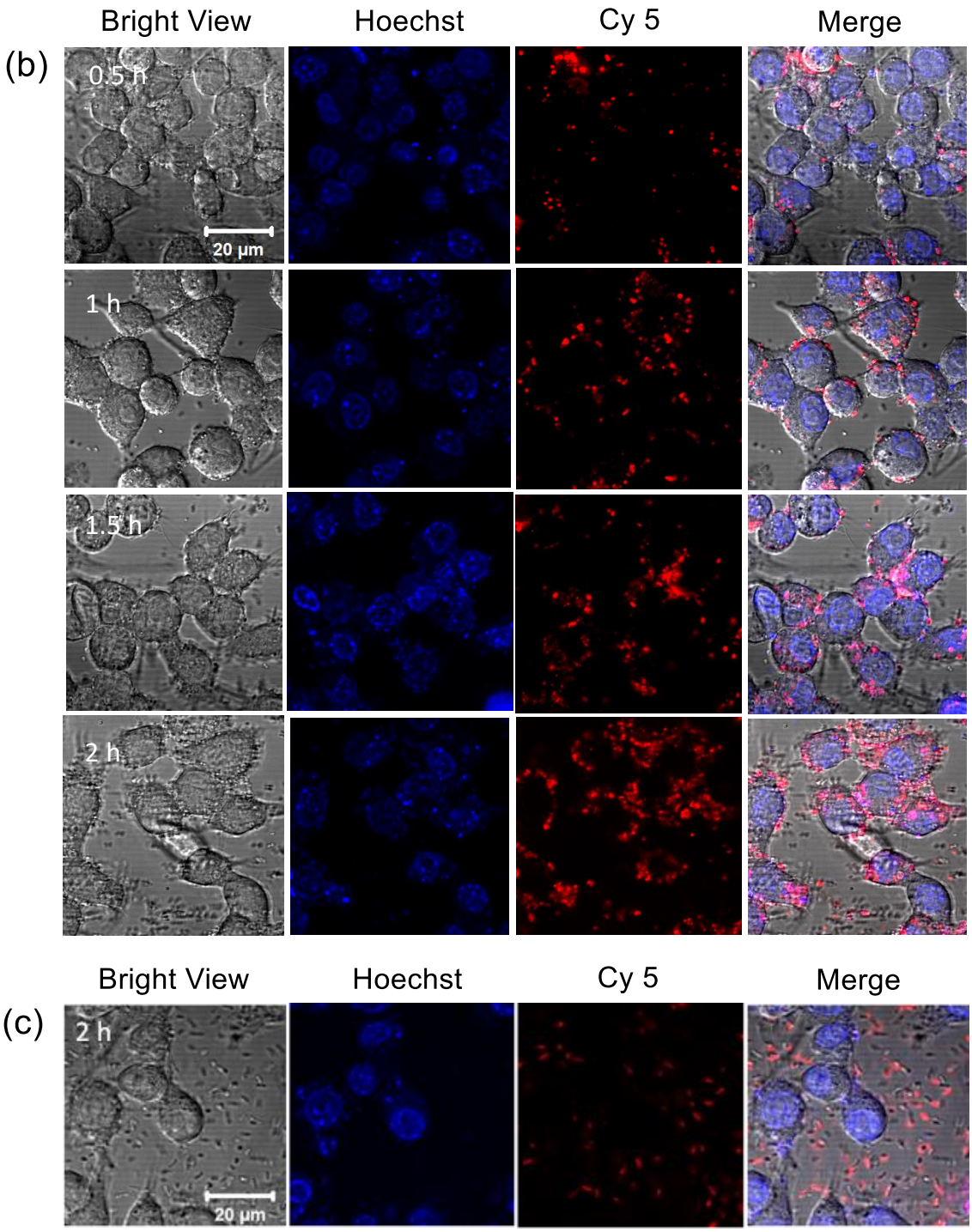


Fig. S4. Fluorescence images of the mixture of the Raw cells with *S. epidermidis* (a), methicillin resistant *S. aureus* (b) for 0.5 h, 0.5 h, 1 h, 1.5 h and 2 h, *P. aeruginosa* (c) for 2 h staining with probe **1**. The bacterial and mammalian cells were treated with 5 μM compounds at 37 °C for 2 h. λ_{ex} = 360 ± 20 nm and λ_{em} = 660 ± 25 nm; Scale bar = 20 μm.

Confocal imaging of GFP-expressed *E. coli* with Raw 264.7 cells

Upon reaching 80% confluence, the Raw 264.7 (0.3 mL, 1×10^5 cells/mL) were transferred into an 8-well chamber containing sterile coverslips at the bottom. After overnight culture at 37 °C, the medium was replaced with 0.3 mL of GFP-expressed *E. coli* suspended in PBS with OD_{600} of 0.5. Simultaneously, probe **1**, lipidated probe **2** or Cy 5 was added into the wells containing the Raw 264.7 cells with a final concentration of 5 μM. After incubation at 37 °C for 2 h, the bacteria in the suspension were isolated, followed by washing with PBS at least for three times to remove unbound probe **1**, lipidated probe **2** or Cy 5 molecules, and the adhesive Raw 264.7 were also washed with PBS. Then they are treated with 2 μg/mL of Hoechst 33258 at 37 °C for 5 min. Finally, Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope equipped with 40× immersion lens, using a 543 nm laser and 590 ± 60 nm filter for GFP-expressed *E. coli* (Fig. 3).

SEM measurement[8]

Methicillin resistant *S. aureus* (MRSA) cells were cultured for 12 h in NB media at 37 °C. The cells were centrifuged and resuspended in NB media. The suspension was added 5 μM of probe **1** or lipidated probe **2** and incubated at 37 °C for 2 h at 180 rpm shaking speed. After incubation, the cells were harvested by centrifugation at 5000 rpm and then unbound probe **1**, lipidated probe **2** or Cy 5 molecules were removed by washing with PBS at least for three times. The bacteria were then suspended and fixed by 2.5 % glutaraldehyde for 2-3 hours at room temperature. The glutaraldehyde was removed by centrifugation, and the bacteria pellets were re-suspended in sterile water, and then 10 μL of bacteria suspension was spotted on to the cover glasses. After natural drying in the air, the bacteria were dehydrated with a series of graded ethanol solution (30 %, 50 %, 70 %, 80 %, 90 %, and 100 % for 10 min). Later, 10 μL of dehydrated cells were dried at room temperature. Before SEM observation, the cells were pasted with the SEM conducting paste. Images were recorded by using Nova NanoSEM 450 operated at 5 kV.

Antibacterial assay[9]

Colony-forming unit (CFU) counting method was applied to study the antibacterial effects of probe **1**, lipidated probe **2**, Cy 5 or neomycin. Methicillin resistant *S. aureus* (MRSA) cells were cultured for 12 h in NB media at 37 °C. The cell pellets were resuspended in NB media at OD_{600} of 0.5, then further diluted to OD_{600} of 5×10^{-4}. The probe **1**, lipidated probe **2**, Cy 5 or neomycin was then added into the diluted bacterial suspension to achieve the final concentration of 5 μM. 200 μL portion of the

diluted bacterial suspension was spread on the solid NB agar plate and incubated at 37 °C for 16 h. The colonies formed.

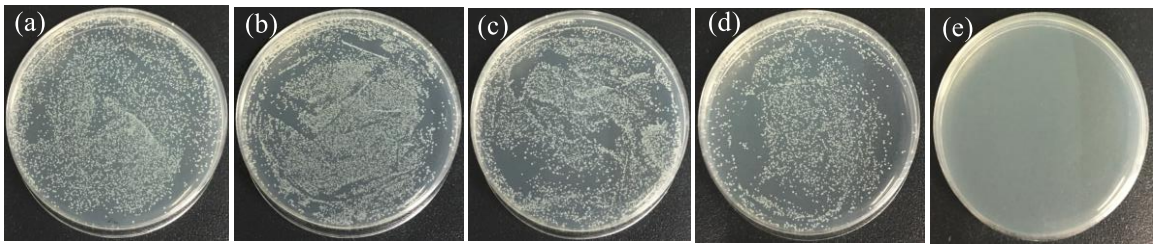
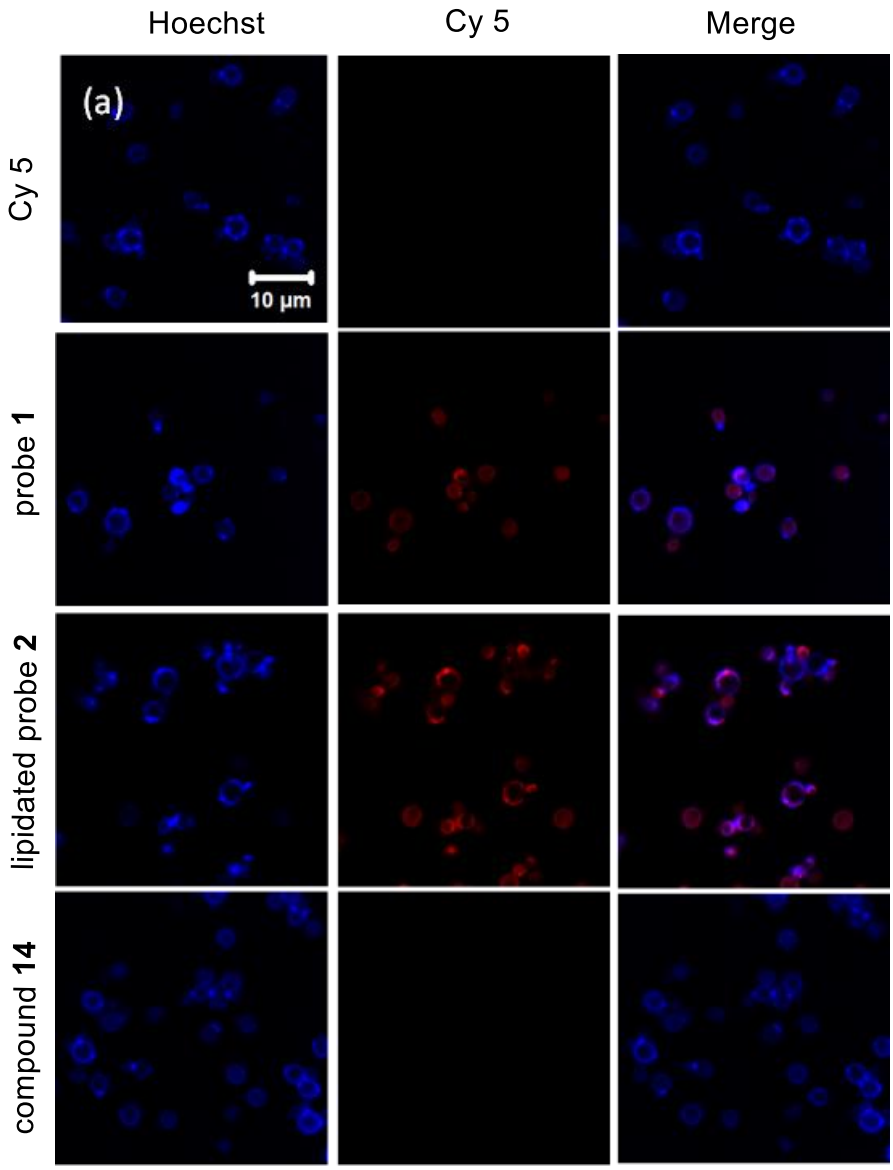


Fig. S5. Plate photographs for MRSA on NB agar plates supplemented with 5 μM Control (a), neomycin (b), Cy 5 (c), probe **1** (d) and lipidated probe **2** (e), then grew overnight.

Confocal imaging of fungi cells treated with probe 1, lipidated probe 2, Cy 5 or compound 14

Pichia pastoris GS115 and *Saccharomyces cerevisiae* INVSC1 cells were cultured for 12 h in respective media at 30 °C. Fungi cells cultured overnight in respective solution were harvested and washed twice with PBS (pH 7.4). The washed cells were resuspended in PBS with an OD_{600} of 0.5-0.7. Then 200 μL aliquots were treated with 5 μM of probe **1**, lipidated probe **2**, Cy 5 or compound **14** (Fig. S6). After incubation at 30 °C for 2 h, the cells were washed with PBS by centrifugation to remove the unbound reagents and treated with 20 μg/mL of Hoechst 33258 at 37 °C for 30 min. Then a drop of the suspension was added into an 8-well chamber followed by covering with agarose pads. Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope (Nikon Eclipse TE2000-E, CFI Plan-Apochromat VC 63 × oil immersed optics), using a high pressure He-Ne lamp and diode laser for excitation (λ_{ex} = 640 ± 20 nm and λ_{em} = 660± 25 nm; λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm).


Hoechst
Cy 5
Merge
(a)
10 µm
Cy 5
probe 1
lipidated probe 2
compound 14

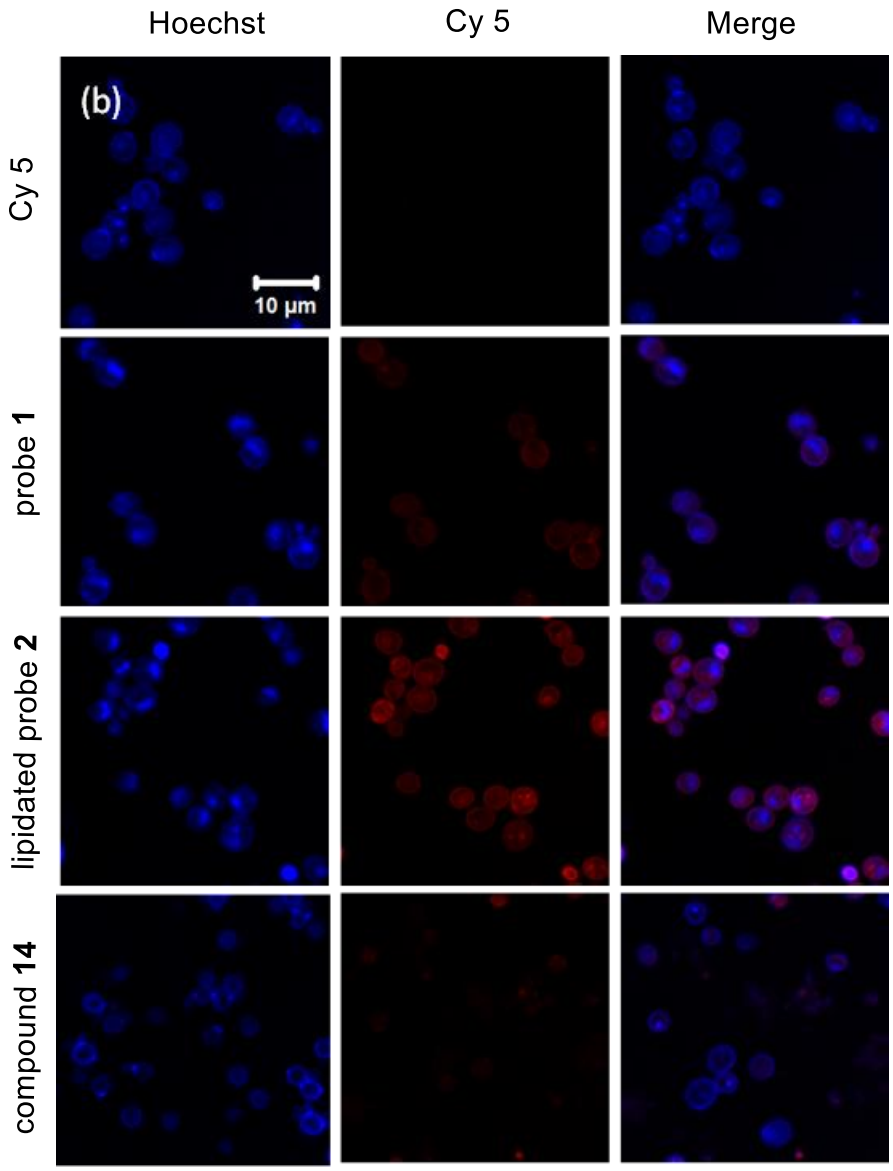
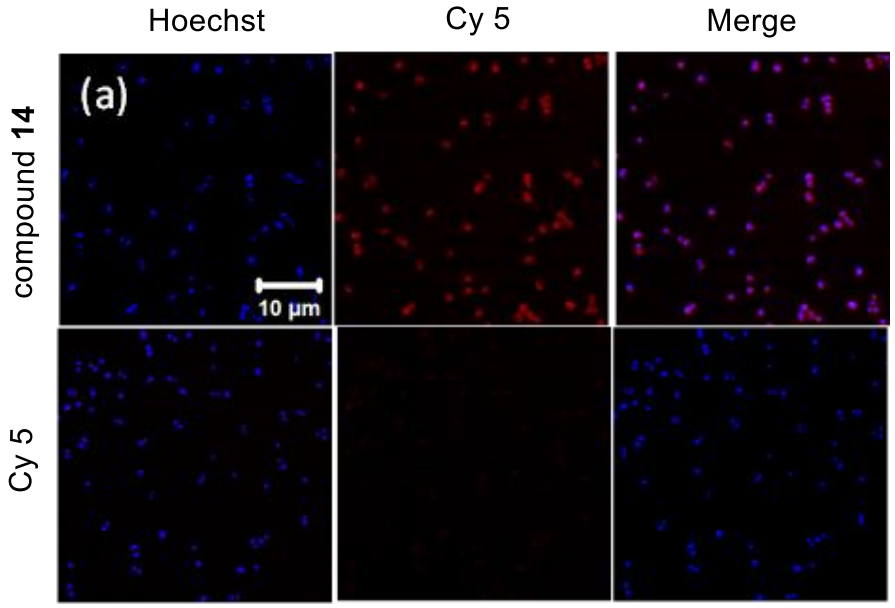


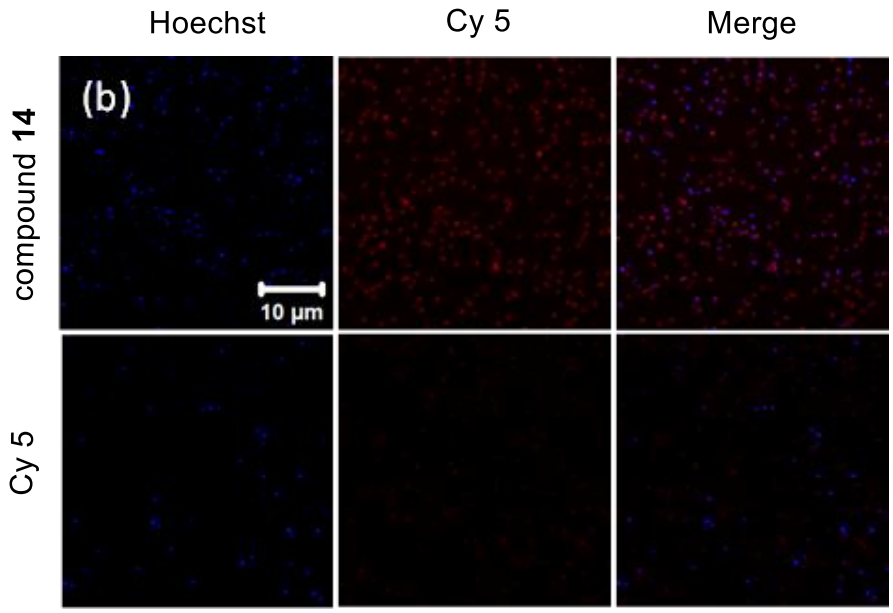
Fig. S6. Fluorescence images of *Pichia pastoris* GS115 (a), *Saccharomyces cerevisiae* INVSC1 (b) cells staining with probe **1**, lipidated probe **2**, Cy 5 or compound **14**. The fungi cells were treated with 5 μM compounds at 37 °C for 2 h. λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm; λ_{ex} = 640 ± 20 nm and λ_{em} = 660 ± 25 nm. Scale bar = 10 μm.

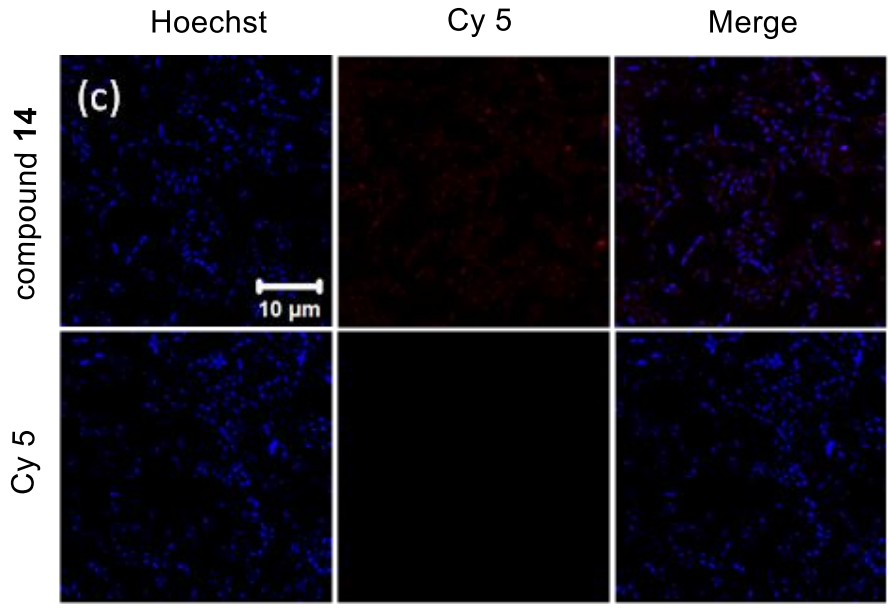
Confocal imaging of bacteria treated with Cy 5 or compound 14

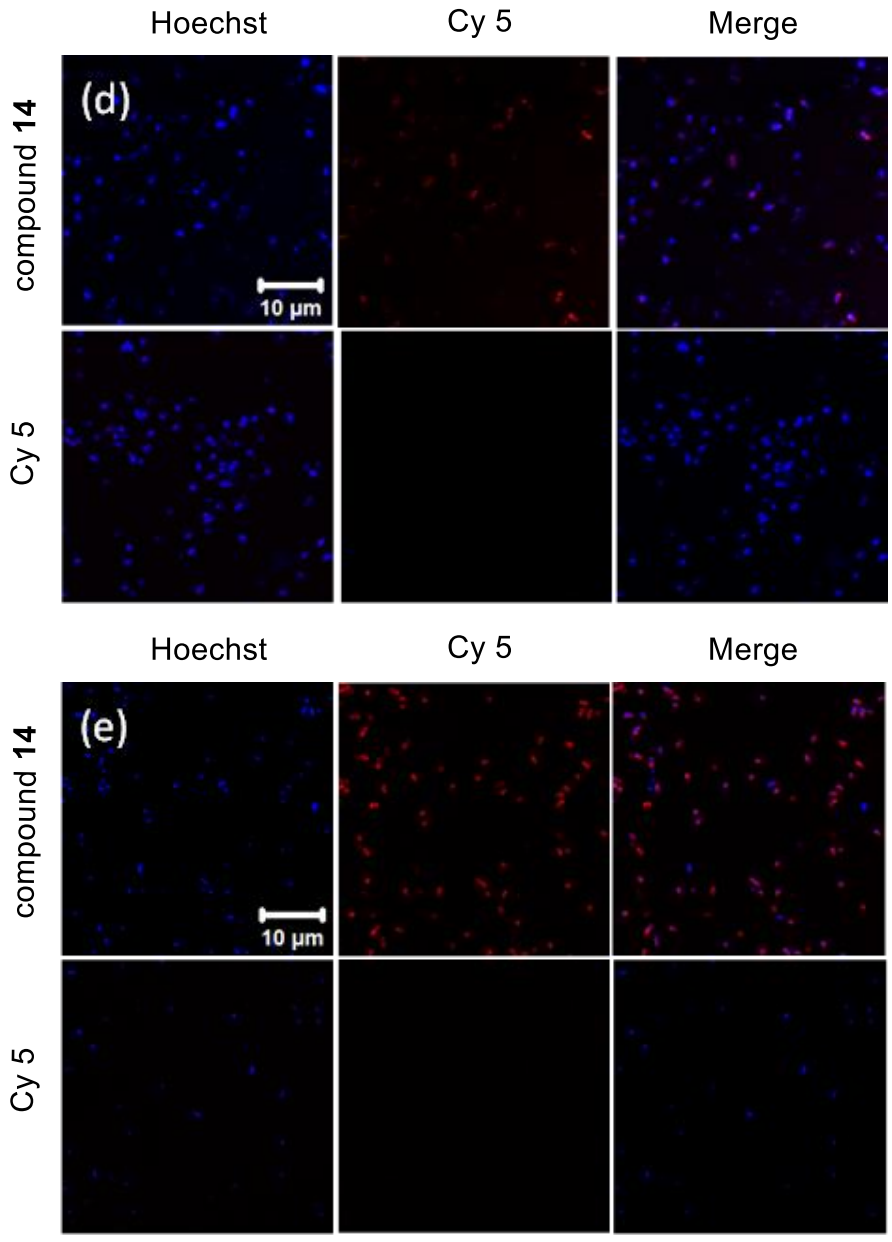
In order to clarify if the lipid linker could enhance membrane permeation, we further preformed confocal microscopic image to visualize the uptake of compound **14** (Cy 5-lipid linker) by *K. pneumoniae*, *E. coli*, *P. aeruginosa*, *MRSA*, *S. epidermidis* and *E. faecalis*, and compared with Cy5. As can be seen from Fig. S7 in Supplementary Information, the lipid linker enhanced membrane permeation of Cy 5, especially for *MRSA*, *K. pneumoniae* and *P. aeruginosa*.

S. epidermidis, MRSA, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* cells were cultured for 12 h in respective media at 37 ℃. Bacterial strains cultured overnight in respective solution were harvested and washed twice with PBS (pH 7.4). The washed cells were resuspended in PBS with an OD_{600} of 0.5-0.7. Then 200 μL aliquots were treated with 5 μM of Cy 5 or compound **14** (Fig. S7). After incubation at 37 ℃ for 2 h, the cells were washed with PBS by centrifugation to remove the unbound reagents and treated with 20 μg/mL of Hoechst 33258 at 37 ℃ for 30 min. Then a drop of the suspension was added into 8-well chamber followed by covering with agarose pads. Fluorescence images were acquired with ZEISS LSM 710 Confocal Microscope (Nikon Eclipse TE2000-E, CFI Plan-Apochromat VC 63 × oil immersed optics), using a high pressure He-Ne lamp and diode laser for excitation (λ_{ex} = 640 ± 20 nm and λ_{em} = 660± 25 nm; λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm).




Hoechst
Cy 5
Merge
(b)
compound 14
10 µm
Cy 5


Hoechst
Cy 5
Merge
(c)
compound 14
10 µm
Cy 5


Hoechst
Cy 5
Merge
(d)
compound 14
10 µm
Cy 5
Hoechst
Cy 5
Merge
(e)
compound 14
10 µm
Cy 5

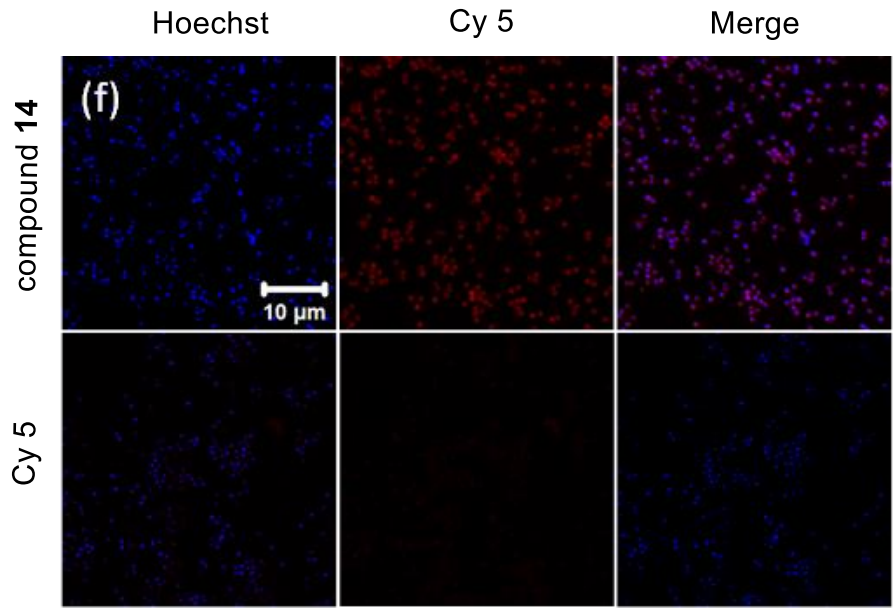


Fig. S7. Fluorescence images of *S. epidermidis* (a), MRSA (b), *E. faecalis* (c), *E. coli* (d), *K. pneumoniae* (e) and *P. aeruginosa* (f) cells staining with Cy 5 or compound **14**. The bacterial cells were treated with 5 μM compounds at 37 °C for 2 h. λ_{ex} = 360 ± 20 nm and λ_{em} = 460 ± 25 nm; λ_{ex} = 640 ± 20 nm and λ_{em} = 660 ± 25 nm. Scale bar = 10 μm.

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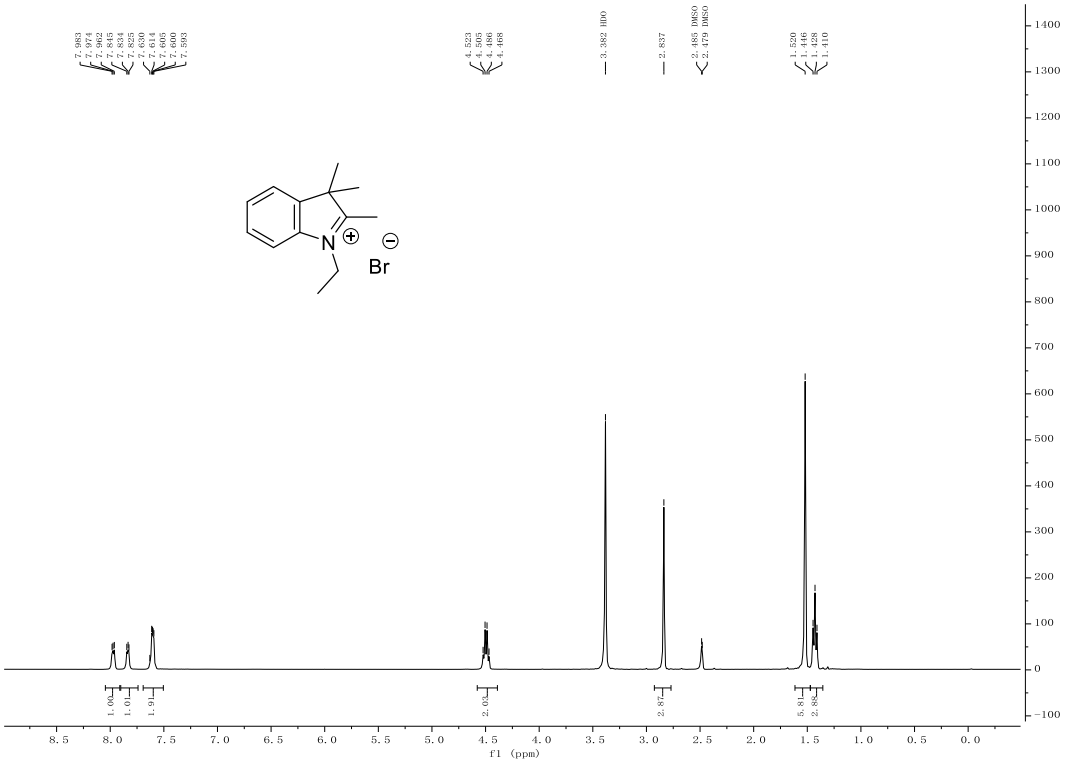
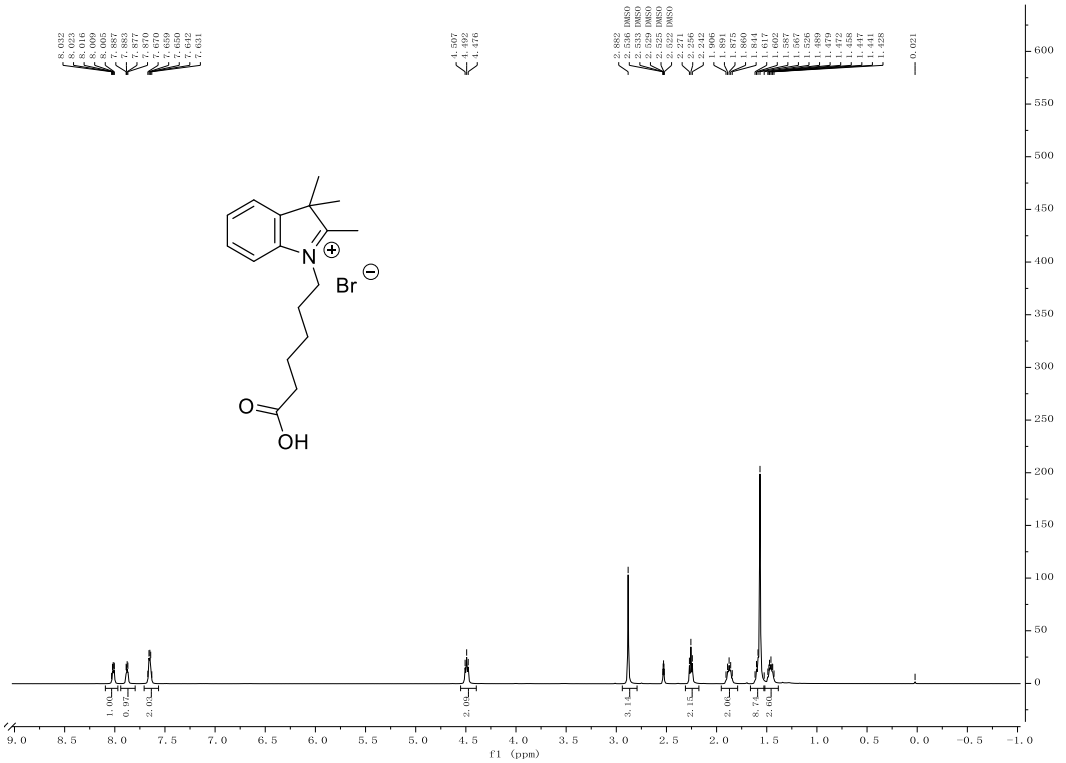
Copies of ^{1}H NMR and ^{13}C NMR spectrum of compounds

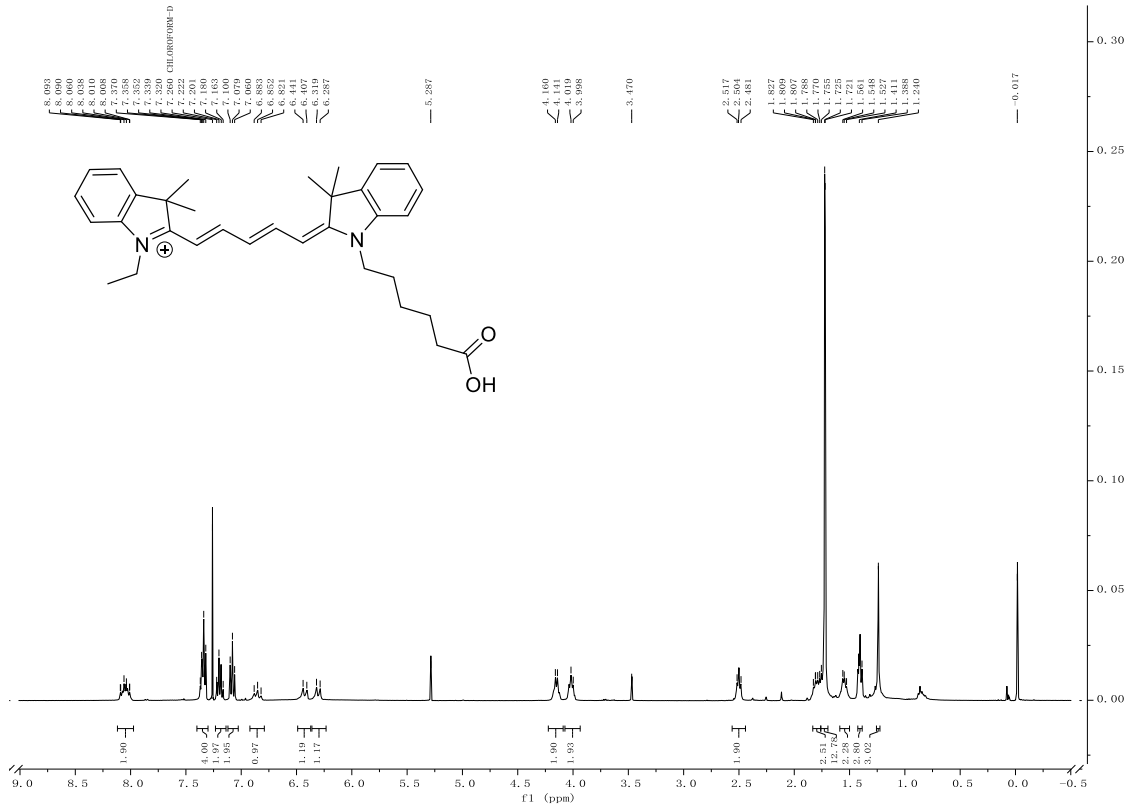


^{1}H NMR (400 MHz, DMSO-*d*6) of compound **2**

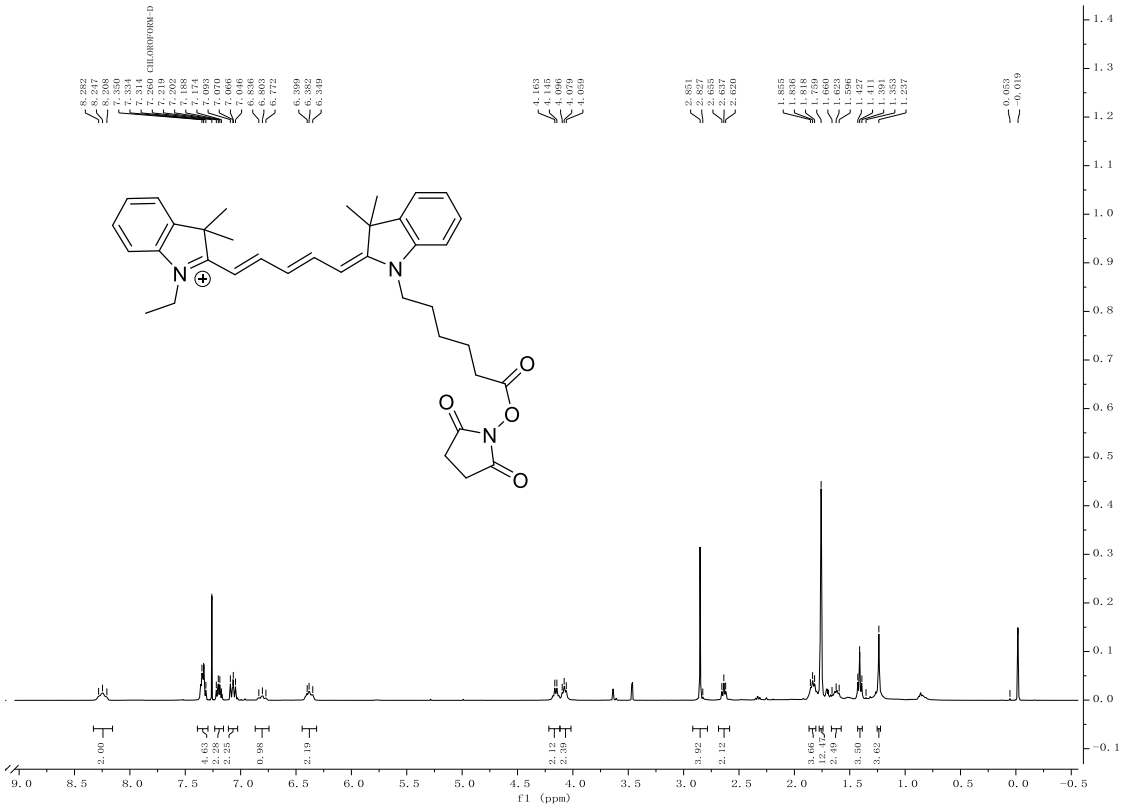


^{1}H NMR (500 MHz, DMSO-*d*6) of compound **3**

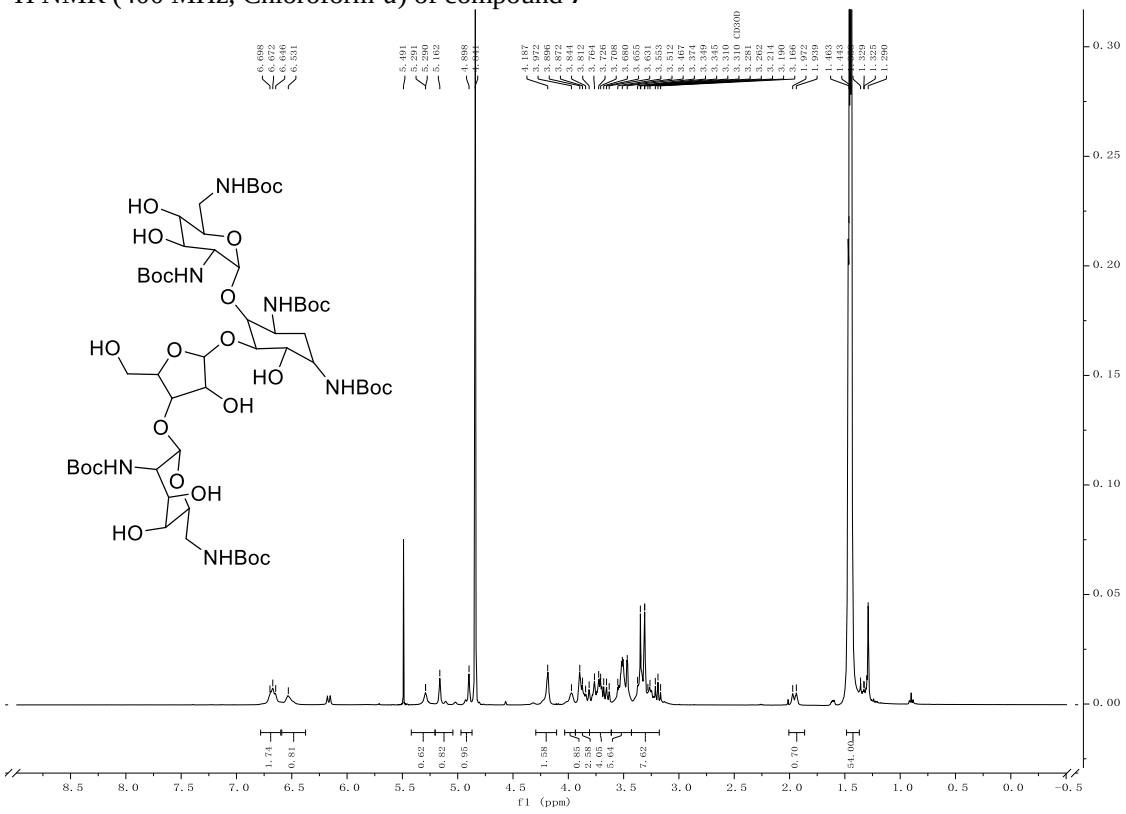
^{1}H NMR (500 MHz, Methanol-*d*4) of compound **5**



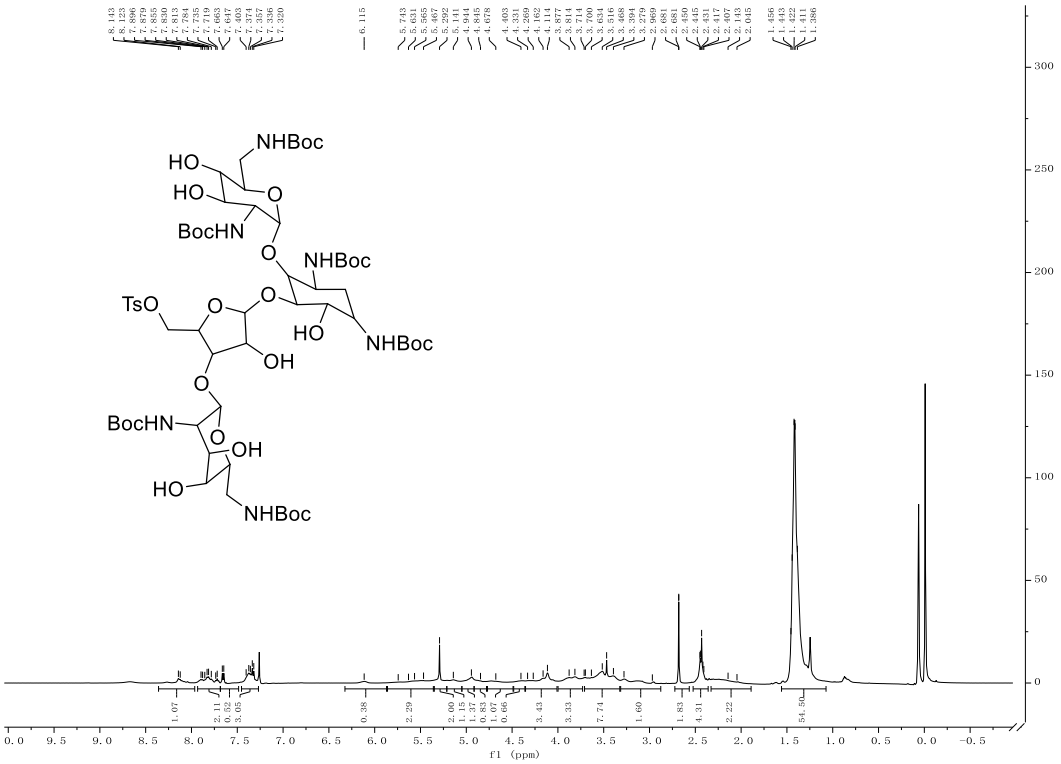
^{1}H NMR (400 MHz, Chloroform-*d*) of compound **6**



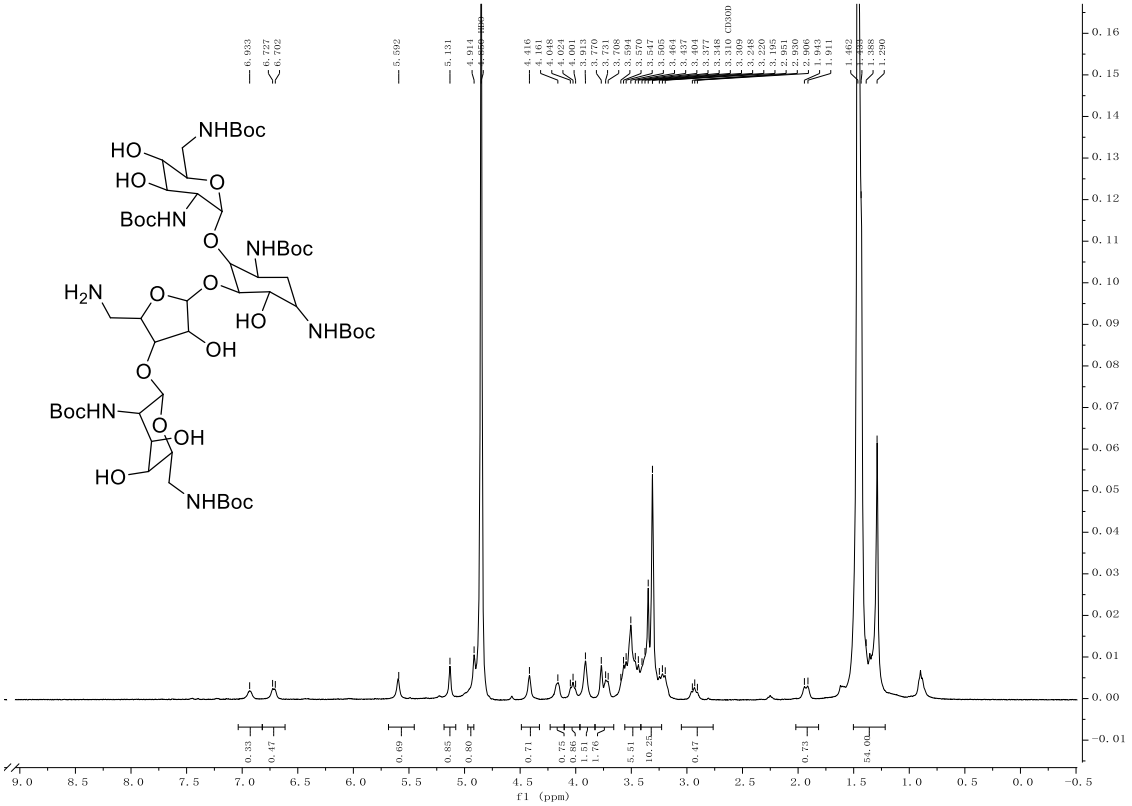
^{1}H NMR (400 MHz, Chloroform-*d*) of compound **7**



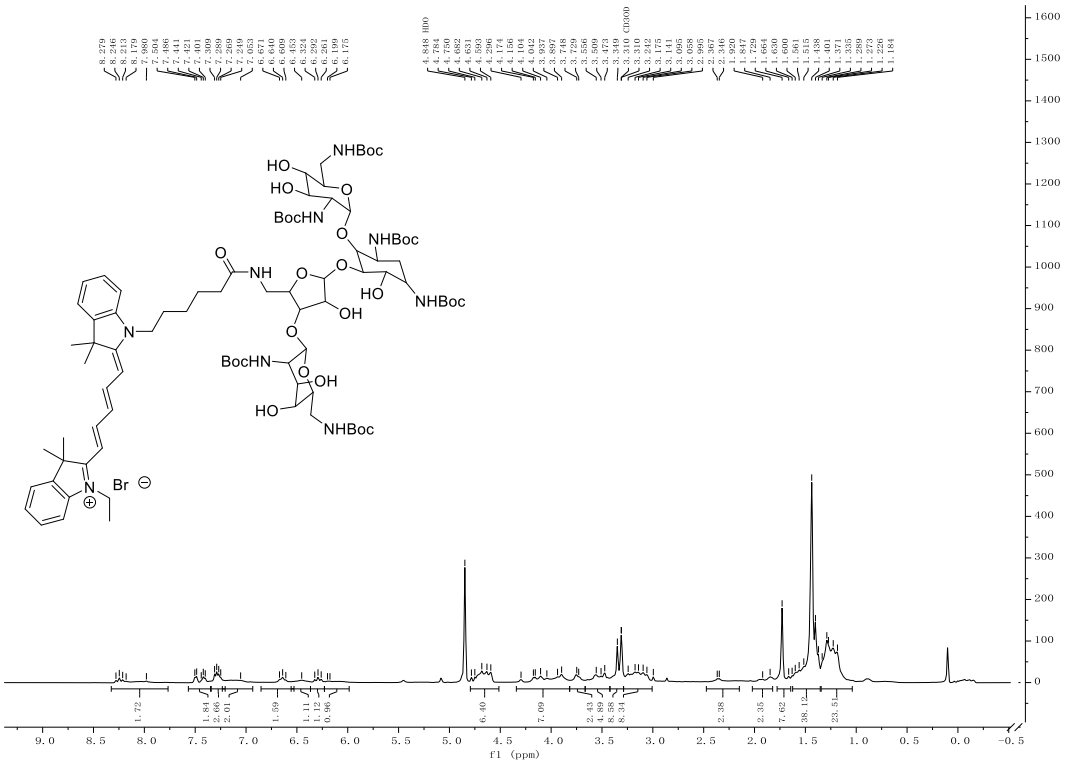
^{1}H NMR (400 MHz, Methanol-*d*4) of compound **9**



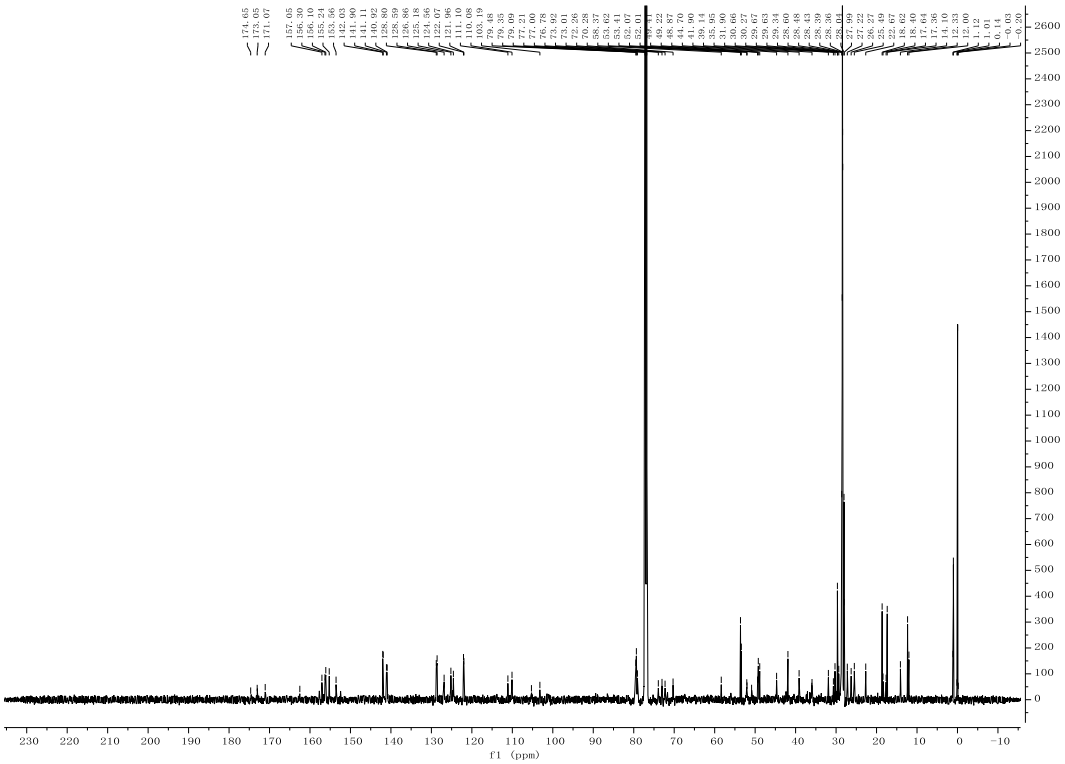
^{1}H NMR (500 MHz, Chloroform-*d*) of compound **10**



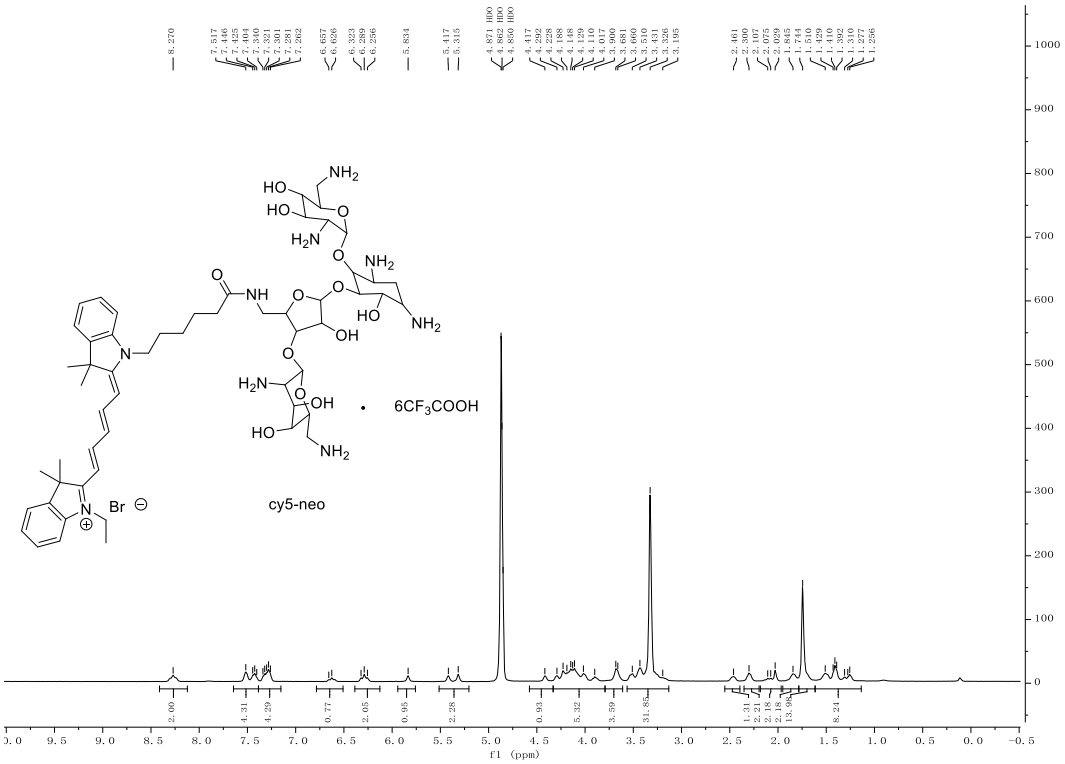
^{1}H NMR (400 MHz, Methanol-*d*4) of compound **12**



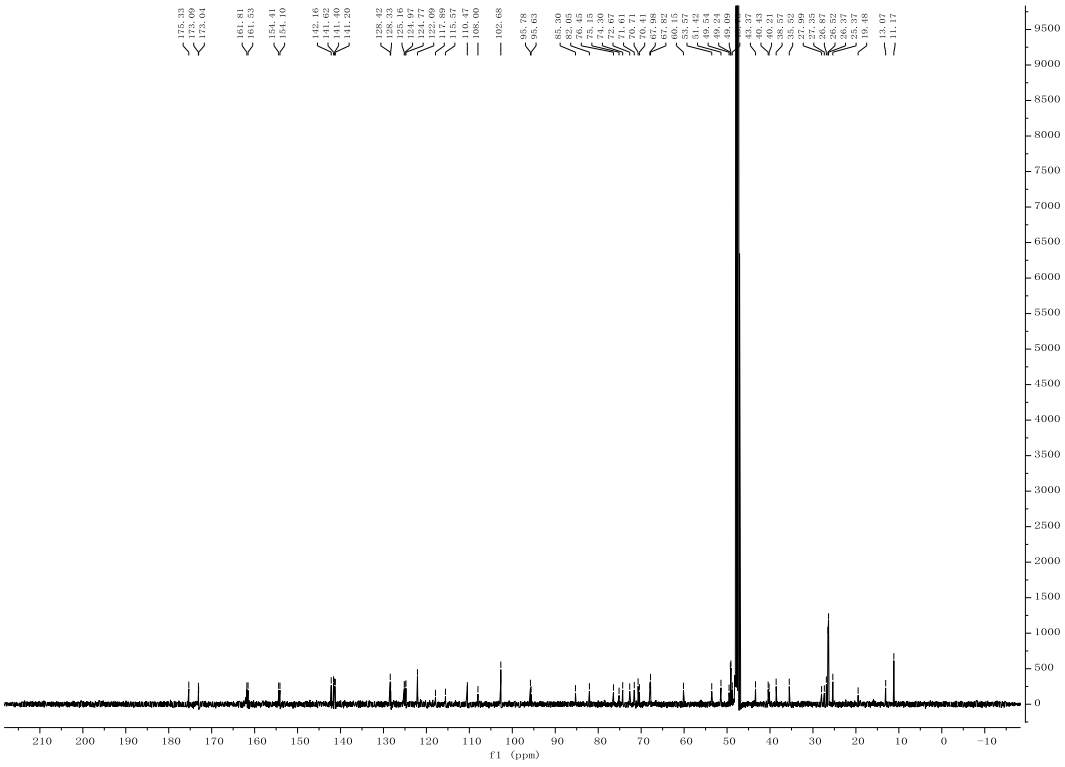
^{1}H NMR (400 MHz, Methanol-*d*4) of compound **13**



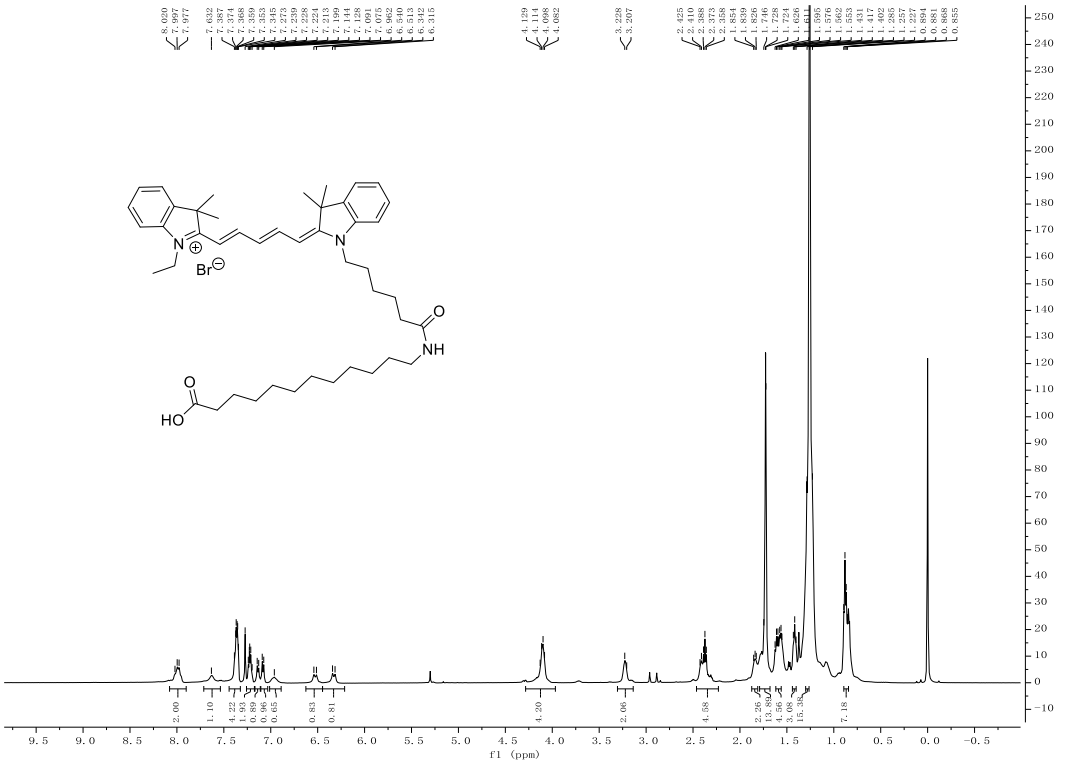
^{13}C NMR (150 MHz, Chloroform-*d*) of compound **13**



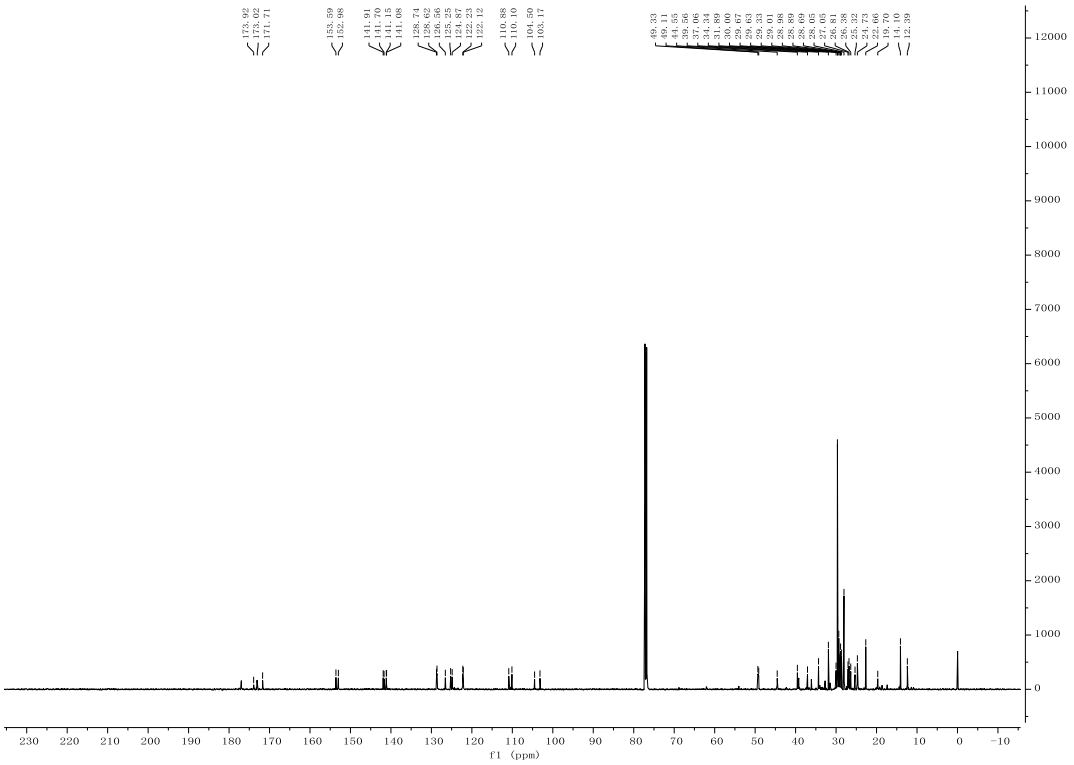
^{1}H NMR (400 MHz, Methanol-*d*4) of **probe 1**



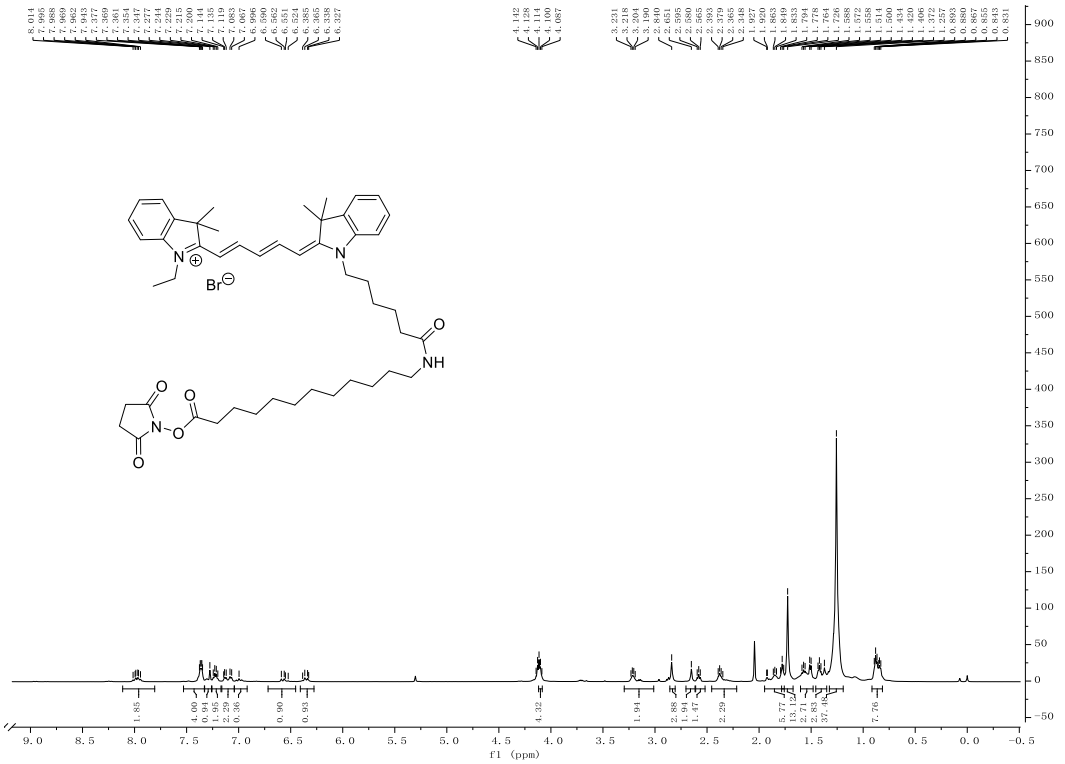
^{13}C NMR (150 MHz, Methanol-*d*4) of **probe 1**



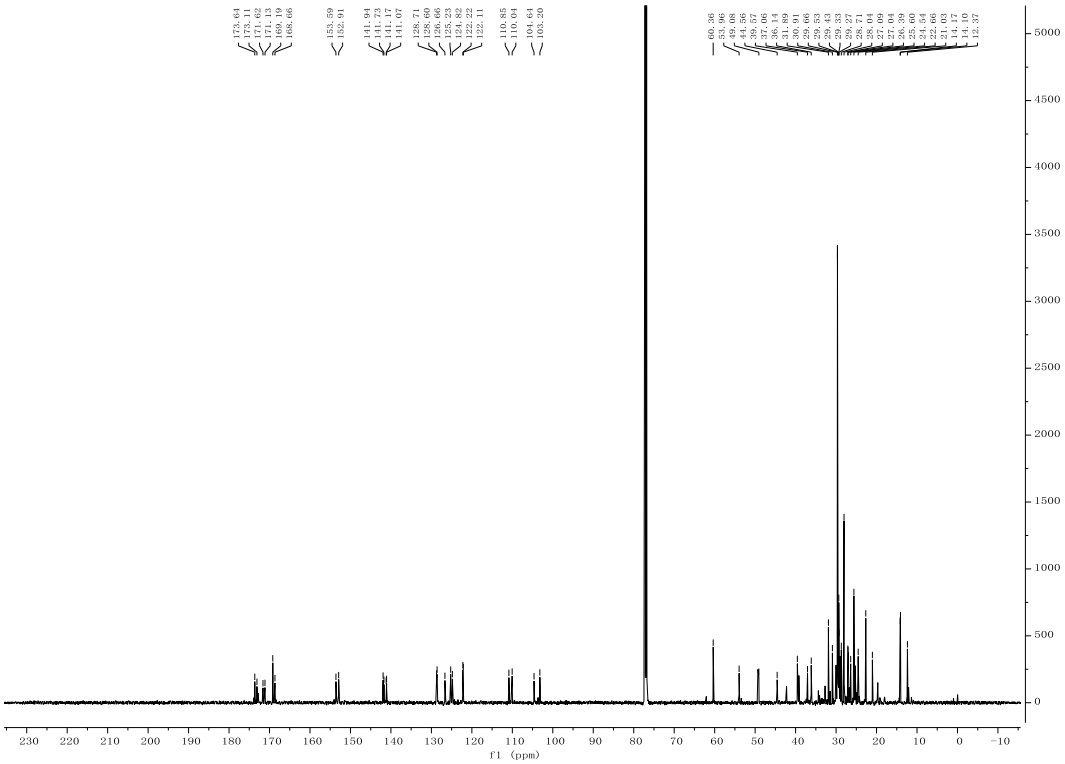
^{1}H NMR (500 MHz, Chloroform-*d*) of compound **14**



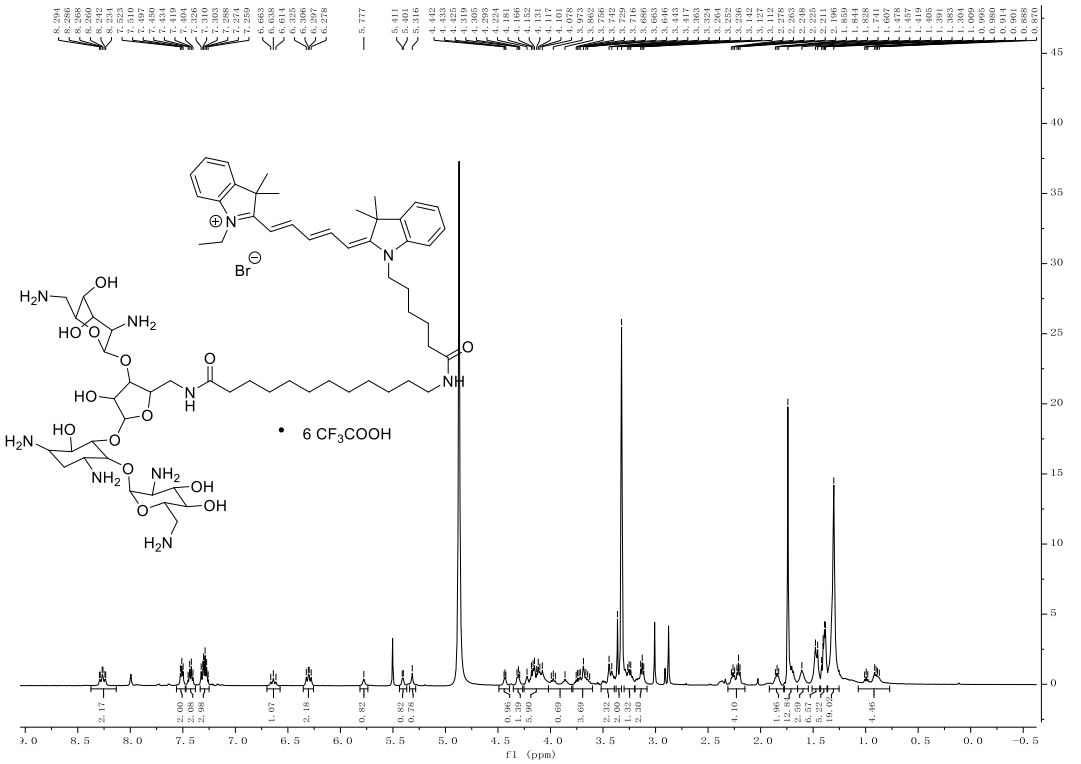
^{13}C NMR (150 MHz, Chloroform-*d*) of compound **14**



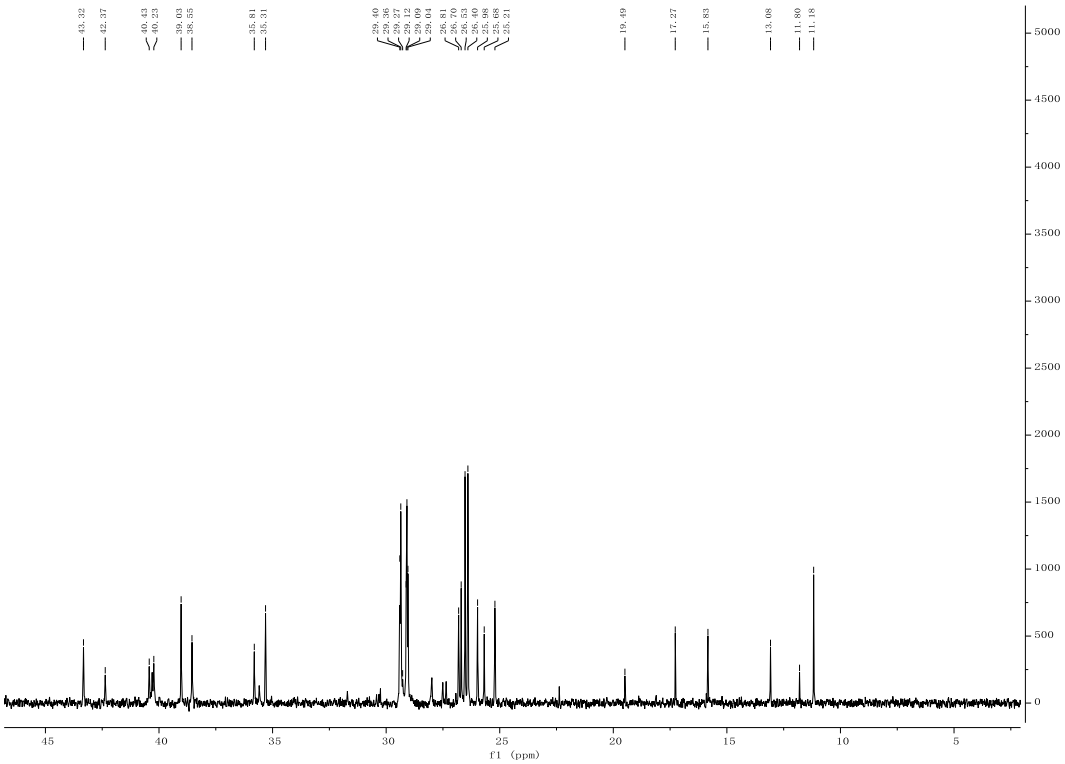
^{1}H NMR (500 MHz, Chloroform-*d*) of compound **15**



^{13}C NMR (150 MHz, Chloroform-*d*) of compound **15**

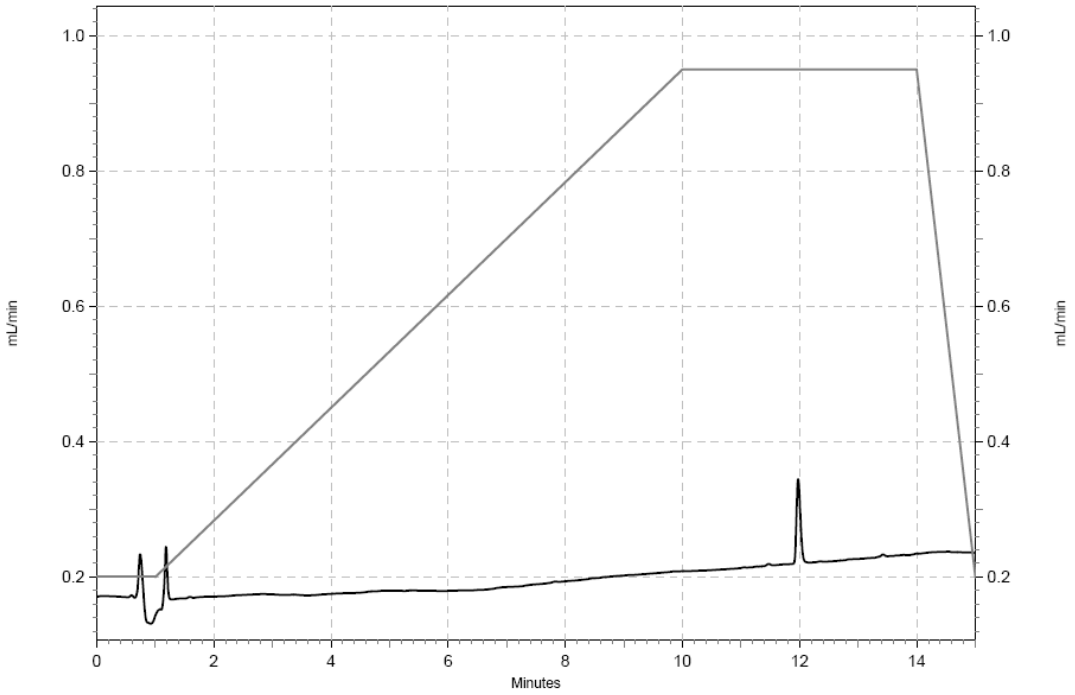


^{1}H NMR (500 MHz, Methanol-*d*4) of lipidated probe **2**

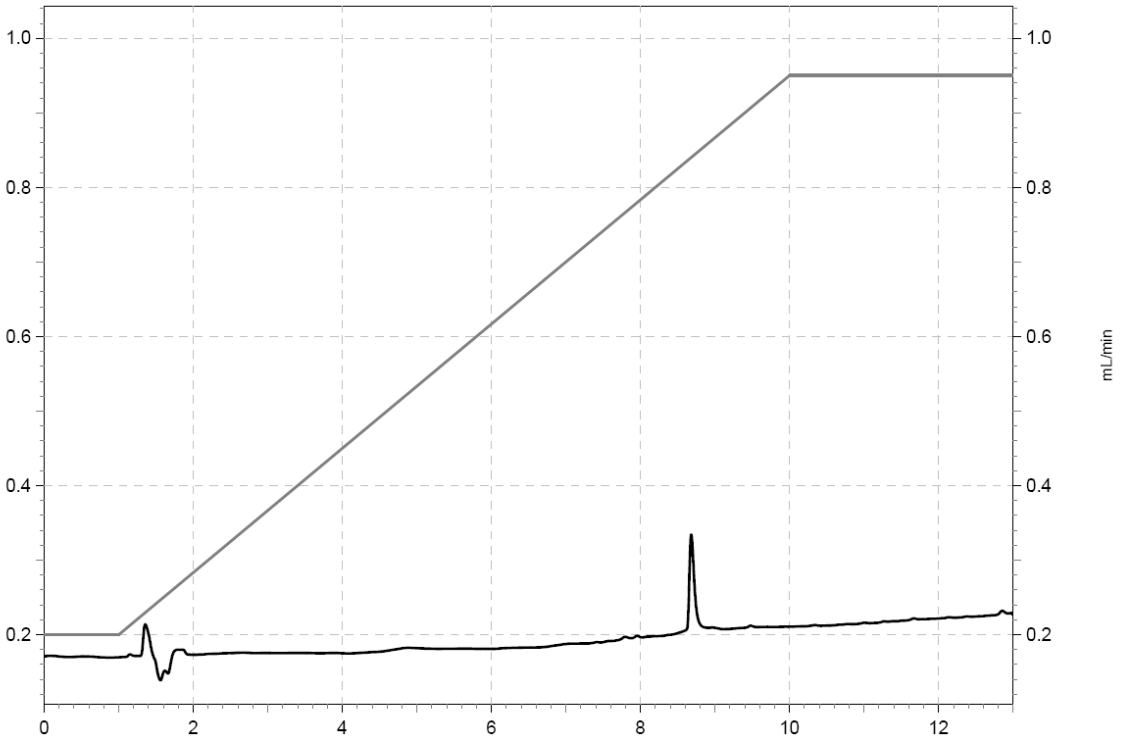


^{13}C NMR (150 MHz, Methanol-*d*4) of lipidated probe **2**

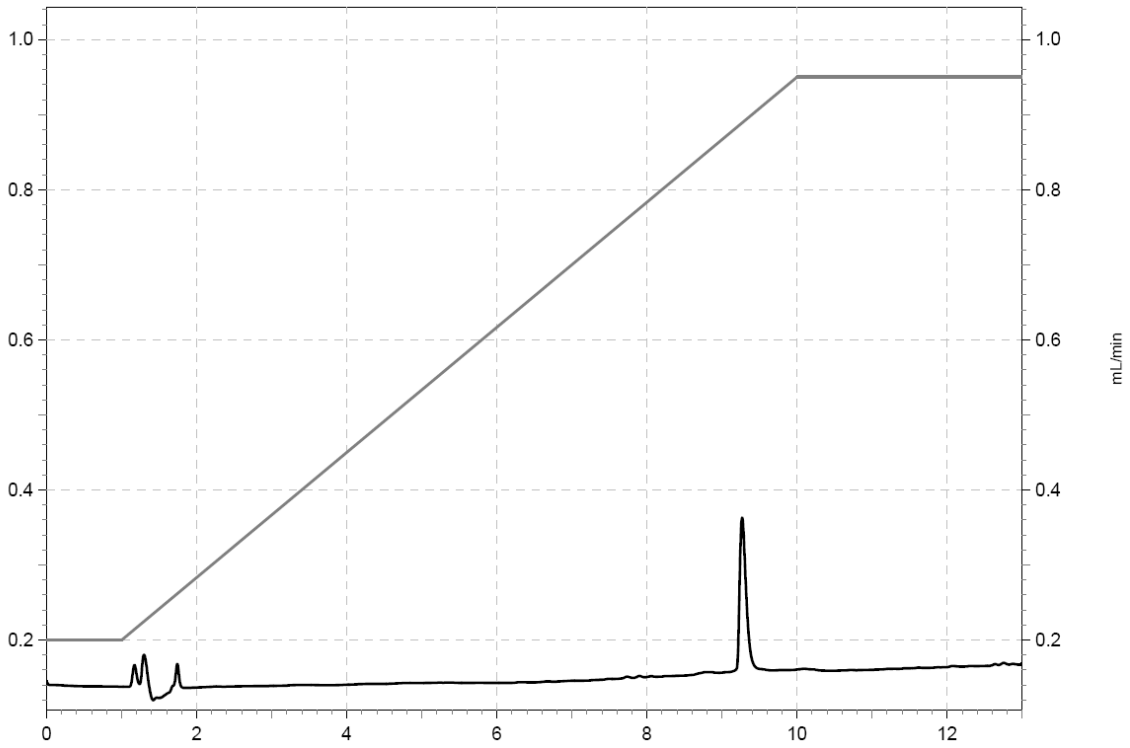
Purity measurement of compound 6, probe 1, lipidated probe 2 by HPLC



The purity of compound **6** (Cy **5**) by HPLC, > 98%.



The purity of **probe 1** by HPLC, > 98%.



The purity of lipidated probe **2** by HPLC, > 98%.