Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Contents

S1. General.

Gramicidin A was obtained from Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were recorded at 400 MHz with a Mercury plus 400 spectrometer at 298 K. Chemical shifts were referenced to solvent residue. Mass spectra were recorded using a Bruker MicroTOF II spectrometer using the positive or negative mode. All cells were obtained from the National Infrastructure of Cell Line Resource (China) and Merck. Cell viability was measured using a BioTek Epoch2 Microplate reader. Flow cytometry was analyzed with a Gallios cytometer. The whole-cell patch clamp experiment was performed on a Nanion Port-a-Patch.

S2. Synthetic procedures and characterization data.

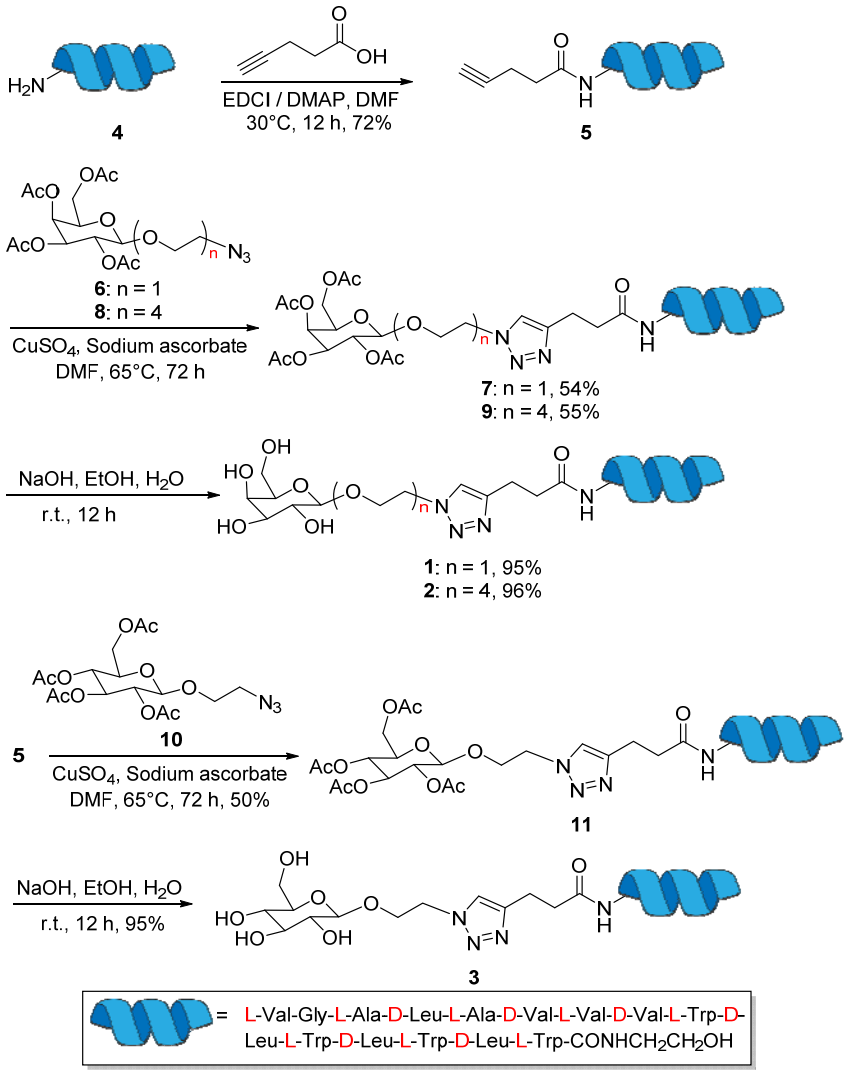


Figure S1. Synthetic protocols for **1-3**.

Preparation of 5:

To a solution of **4**[1] (0.05 mmol) in anhydrous DMF, 4-pentynoicacid (0.05 mmol), DMAP (0.08 mmol), and EDCI (0.08 mmol) were added. The mixture was stirred at 30°C for 12 h. Then, the mixture was concentrated under reduced pressure and poured into aqueous HCl solution (2%, 4 mL). The formed precipitate was collected by filtration and washed with water to give the crude product. This residue was subjected to purification by column chromatography using DCM /MeOH (10:1, v/v) as the eluent to yield **5** as a white solid. Yield: 72%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.72 (m, 2H), 7.54 (dd, J = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 4.71 (t, J = 5.6 Hz, 1H), 4.56-4.46 (m, 5H), 4.29-4.11 (m, 9H), 3.70 (d, J = 5.6 Hz, 2H), 3.43-3.39 (m, 2H), 3.21-3.09 (m, 6H), 2.91-2.87 (m, 4H), 2.74 (s, 1H), 2.43-2.35 (m, 4H), 2.02-1.97 (m, 4H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 172.3, 172.2, 172.0, 171.8, 171.7, 171.5, 171.3, 136.6, 127.6, 124.2, 121.2, 118.8, 118.7, 118.6, 111.6, 110.7, 110.2, 84.2, 71.7, 60.2, 58.4, 58.0, 54.2, 52.2, 52.0, 51.7, 49.0, 42.4, 42.2, 34.4, 31.2, 30.9, 30.7, 30.5, 28.3, 28.1, 24.7, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.7, 19.8, 19.7, 19.5, 18.8, 18.7, 18.6, 18.2, 18.0, 14.7. HR-MS (ESI-TOF): Calcd. For $C_{103}H_{144}N_{20}O_{17}$ $[M+2H]^+$: 968.0597. Found: 968.0590.

Preparation of 8:

To a solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethanol[2] (1.4 mmol) in anhydrous DCM, D-galactose pentaacetate (1.2 mmol), and Boron trifluoride etherate (2.5 mmol) were added. After stirring at 30°C for 12 h, the reaction mixture was diluted with DCM and washed with water. The organic phase was dried over Na2SO4 and then concentrated under reduced pressure. The residue was purified by column chromatography using PE/ethyl acetate (2:1 to 1:2, v/v) as the eluent to yield **8** as colorless oily liquid. Yield: 21%. ^{1}H NMR (400 MHz, $CDCl_3$-d): δ 5.39 (d, J = 3.4 Hz, 1H), 5.20 (dd, J = 10.3, 8.1 Hz, 1H), 5.01 (dd, J = 10.5, 3.4 Hz, 1H), 4.57 (d, J = 8.0 Hz, 1H), 4.15 (t, J = 7.3 Hz, 2H), 4.00 – 3.87 (m, 2H), 3.79-3.60 (m, 13H), 3.39 (t, J = 5.0 Hz, 2H), 2.15 (s, 3H), 2.05 (d, J = 4.3 Hz, 6H), 1.98 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$-d): δ 170.3, 170.2, 169.5, 101.3, 70.9, 70.7, 70.6, 70.3, 70.0, 69.0, 68.8, 67.1, 61.3, 50.6, 20.7, 20.6. HR-MS (ESI-TOF): Calcd. For $C_{22}H_{35}N_3O_{13}$ $[M+NH_4]^+$: 567.2508. Found: 567.2507.

General procedures for the preparation of 7, 9 and 11:

To a solution of **5** (0.04 mmol) in anhydrous DMF, **6**[3]**, 8 or 10**[4] (0.05 mmol), $CuSO_4 \cdot 5H_2O$ (0.02mmol), and sodium ascorbate (0.08 mmol) were added. After

stirring at 65°C for 72 h, the reaction mixture was concentrated under reduced pressure and poured into water. The formed precipitate was collected by filtration and washed with water to give the crude product. The obtained crude product was subjected to purification by column chromatography using DCM /MeOH (12:1, v/v) as the eluent to yield **7, 9, or 11** as a yellow solid.

7. Yield: 54%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.69 (m, 3H), 7.54 (dd, *J* = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 5.26 (s, 1H), 5.13 (m, 1H), 4.90 (t, *J* = 9.2 Hz, 1H), 4.71 (m, 2H), 4.56-4.46 (m, 5H), 4.30-4.04 (m, 15H), 3.88 (s, 1H), 3.70 (d, *J* = 5.6 Hz, 2H), 3.43-3.39 (m, 2H), 3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.11 (s, 3H), 2.02-1.95 (m, 7H), 1.91 (s, 6H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 172.2, 171.5, 170.4, 169.9, 169.5, 136.6, 127.6, 124.2, 121.2, 118.6, 111.6, 110.2, 100.1, 70.6, 70.5, 68.8, 67.8, 61.7, 60.2, 54.2, 49.0, 42.2, 34.4, 29.5, 29.2, 28.3, 24.7, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.7, 21.0, 20.8, 19.7, 19.6, 19.5, 18.8, 18.6, 18.5, 18.2, 18.0. HR-MS (ESI-TOF): Calcd. For $C_{119}H_{167}N_{23}O_{27}$ $[M+2H]^+$: 1176.6289. Found: 1176.6277.

9. Yield: 55%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.72 (m, 3H), 7.54 (dd, *J* = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 5.26 (d, *J* = 3.6 Hz, 1H), 5.15 (dd, *J* = 10.4, 3.5 Hz, 1H), 5.01-4.89 (m, 1H), 4.77-4.67 (m, 2H), 4.61 - 4.41 (m, 5H), 4.37-4.00 (m, 13H), 3.78 (t, *J* = 5.4 Hz, 3H), 3.71 (s, 2H), 3.56-3.39 (m, 14H), 3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.11 (s, 3H), 2.06-1.96 (m, 10H), 1.91 (s, 3H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2, 171.5, 170.4, 136.6, 127.6, 124.3, 122.7, 121.2, 118.8, 118.6, 111.6, 100.5, 70.7, 70.4, 70.3, 70.2, 70.1, 70.0, 69.9, 69.2, 69.1, 69.0, 67.8, 61.8, 60.2, 54.2, 52.0, 49.7, 49.0, 35.0, 30.3, 29.5, 28.3, 28.1, 24.7, 24.0, 23.5, 23.0, 22.1, 22.0, 21.7, 20.9, 20.8, 19.7, 19.6, 19.5, 18.7, 18.6, 18.5, 18.2, 18.0. HR-MS (ESI-TOF): Calcd. For $C_{125}H_{179}N_{23}O_{30}$ $[M+H]^+$: 2484.3291. Found: 2484.3282.

11. Yield: 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.69 (m, 3H), 7.54 (dd, *J* = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 5.24 (t, J = 9.6 Hz, 1H), 4.91 (t, *J* = 9.6 Hz, 1H), 4.82 (d, *J* = 8.0 Hz, 1H), 4.78 – 4.67 (m, 2H), 4.56-4.46 (m, 5H), 4.30-4.04 (m, 15H), 3.88 (s, 1H), 3.70 (d, *J* = 5.6 Hz, 2H), 3.43-3.39 (m, 2H),

3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.06-1.96 (m, 10H), 1.93 (s, 3H), 1.90 (s, 3H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.2, 171.5, 171.3, 136.6, 127.6, 124.2, 122.7, 121.2, 118.8, 118.6, 111.6, 110.8, 110.2, 99.7, 72.4, 71.2, 68.6, 68.0, 62.1, 60.2, 54.2, 49.6, 49.0, 42.5, 42.2, 35.0, 31.7, 31.2, 30.9, 30.3, 29.5, 29.2, 28.3, 28.1, 24.7, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.7, 21.0, 20.8, 20.7, 19.8, 19.6, 19.5, 18.8, 18.6, 18.5, 18.2, 18.0. HR-MS (ESI-TOF): Calcd. For $C_{119}H_{167}N_{23}O_{27}$ $[M+H]^+$: 2352.2505. Found: 2352.2508.

General procedure for the preparation of 1, 2 and 3:

To a solution of **7, 9 or 11** (0.01 mmol) in ethanol (2.5 mL), sodium hydroxide (0.06 mmol) dissolved in H_2O (1 mL) was added. After stirring at room temperature for 12 h, the reaction mixture was concentrated under reduced pressure and acidified with dilute HCl to pH = 7.0. The formed precipitate was collected by filtration and washed with water to yield **1, 2, or 3** as a yellow solid.

1. Yield: 95%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.69 (m, 3H), 7.54 (dd, J = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 4.95 (d, J = 4.2 Hz, 1H), 4.79-4.68 (m, 2H), 4.61-4.45 (m, 7H), 4.40 (d, J = 4.6 Hz, 1H), 4.34-3.97 (m, 14H), 3.90-3.81 (m, 1H), 3.71 (s, 2H), 3.64 (s, 1H), 3.51 (d, J = 6.4 Hz, 2H), 3.42 (d, J = 6.0 Hz, 3H), 3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.02-1.95 (m, 4H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ172.7, 172.5, 172.2, 172.0, 171.5, 171.3, 169.1, 136.6, 127.6, 124.2, 123.2, 121.2, 118.8, 118.7, 118.6, 111.6, 110.7, 110.2, 110.0, 104.0, 75.9, 73.8, 70.9, 68.6, 67.7, 60.9, 60.2, 59.0, 58.2, 58.0, 54.2, 52.2, 52.0, 51.8, 50.0, 49.0, 42.5, 42.2, 35.0, 31.2, 30.9, 30.7, 30.3, 29.5, 29.2, 28.3, 28.1, 24.7, 24.1, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.8, 21.7, 19.8, 19.7, 19.6, 19.5, 18.8, 18.7, 18.5, 18.2, 18.0. HR-MS (ESI-TOF): Calcd. For $C_{111}H_{159}N_{23}O_{23}$ $[M+H]^+$: 2184.2082 Found: 2184.2081.

2. Yield: 96%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.72 (m, 3H), 7.54 (dd, J = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 4.95 (d, J = 4.2 Hz, 1H), 4.79-4.68 (m, 2H), 4.61-4.45 (m, 7H), 4.40 (d, J = 4.6 Hz, 1H), 4.34-3.97 (m, 14H), 3.88-3.38 (m, 21H), 3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.02-1.95 (m, 4H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 172.2, 171.5, 136.6,

127.6, 124.2, 122.7, 121.2, 118.6, 111.6, 110.2, 104.1, 75.7, 74.0, 71.0, 70.2, 70.1, 70.0, 69.2, 68.6, 68.2, 60.9, 60.2, 54.3, 52.0, 51.8, 49.7, 49.0, 42.5, 42.2, 35.0, 30.3, 29.5, 29.1, 28.2, 28.0, 24.7, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.7, 19.7, 19.6, 19.5, 18.7, 18.4, 18.3, 18.1. HR-MS (ESI-TOF): Calcd. For $C_{117}H_{171}N_{23}O_{26}$ $[M+H]^+$: 2316.2869. Found: 2316.2856.

3. Yield: 95%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.76 (s, 3H), 10.71 (s, 1H), 8.32-8.30 (m, 2H), 8.22-7.93 (m, 12H), 7.80-7.72 (m, 3H), 7.54 (dd, J = 15.6 Hz, 7.8 Hz, 4H), 7.30-7.26 (m, 4H), 7.09-7.07 (m, 4H), 7.05-6.89 (m, 8H), 5.08 (d, J = 4.8 Hz, 1H), 4.96 (d, J = 4.7 Hz, 1H), 4.92 (d, J = 5.2 Hz, 1H), 4.68 (d, J = 5.8 Hz, 1H), 4.56 – 4.40 (m, 7H), 4.36-3.99 (m, 15H), 3.86 (s, 1H), 3.73-3.62 (m, 3H), 3.45-3.37 (m, 4H), 3.21-3.09 (m, 8H), 2.91-2.87 (m, 6H), 2.02-1.95 (m, 4H), 1.83-1.76 (m, 2H), 1.54-1.45 (m, 4H), 1.23-1.09 (m, 15H), 0.97-0.76 (m, 24H), 0.65-0.48 (m, 21H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 172.7, 172.2, 171.5, 171.3, 169.1, 136.6, 127.6, 124.2, 123.2, 121.2, 118.8, 118.7, 118.6, 111.6, 110.7, 110.2, 103.4, 77.5, 77.1, 73.8, 70.5, 67.8, 61.6, 60.2, 59.0, 58.5, 58.2, 58.0, 54.2, 52.2, 52.0, 51.8, 50.0, 49.0, 42.5, 42.2, 35.0, 31.2, 30.9, 30.3, 29.5, 28.3, 28.1, 24.7, 24.0, 23.5, 23.0, 22.3, 22.1, 22.0, 21.8, 21.7, 19.8, 19.7, 19.6, 19.5, 18.8, 18.7, 18.5, 18.2, 18.0. HR-MS (ESI-TOF): Calcd. For $C_{111}H_{159}N_{23}O_{23}$ $[M+H]^+$: 2184.2082. Found: 2184.2088.

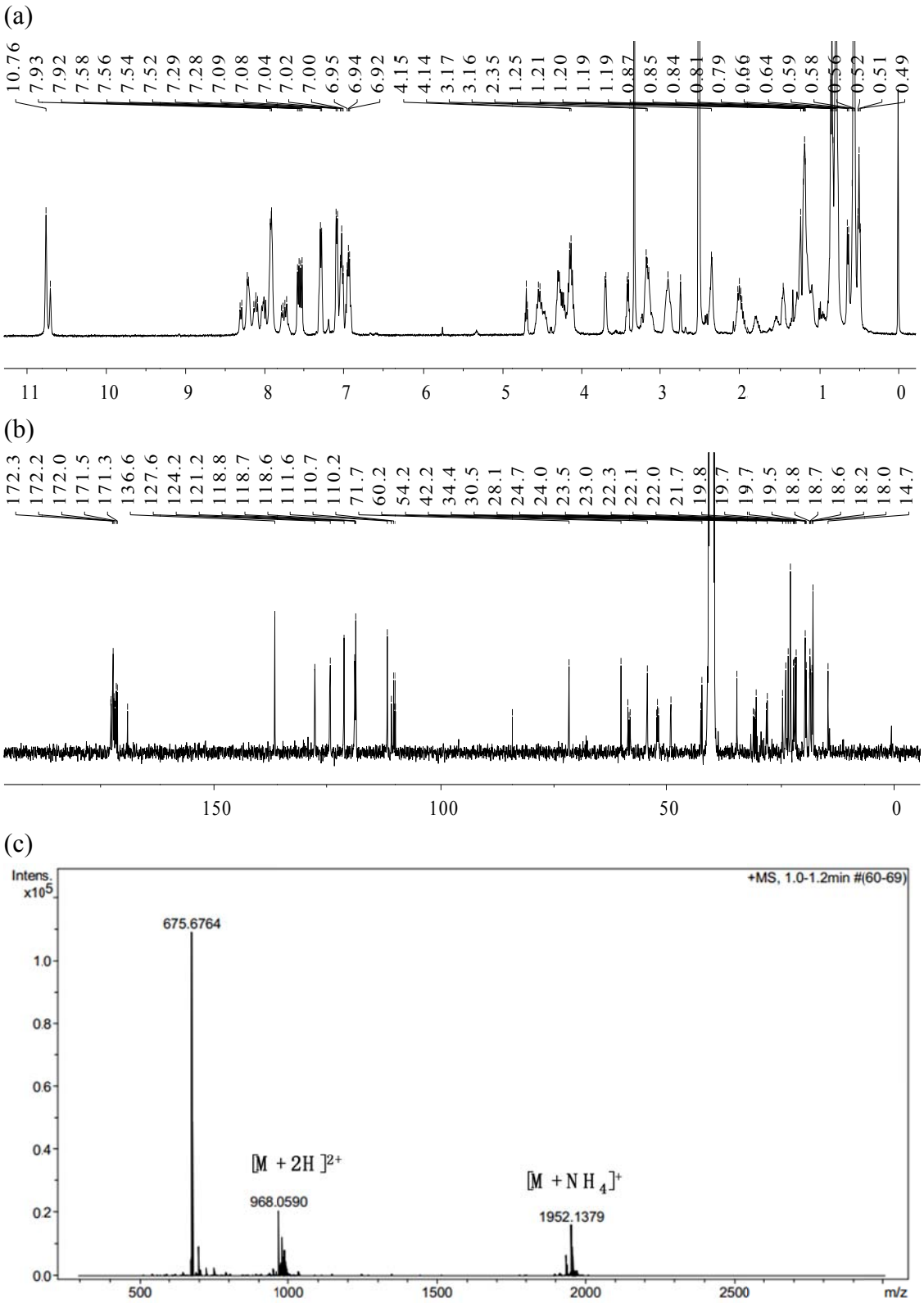


Figure S2. Structural characterization spectra for compound **5**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

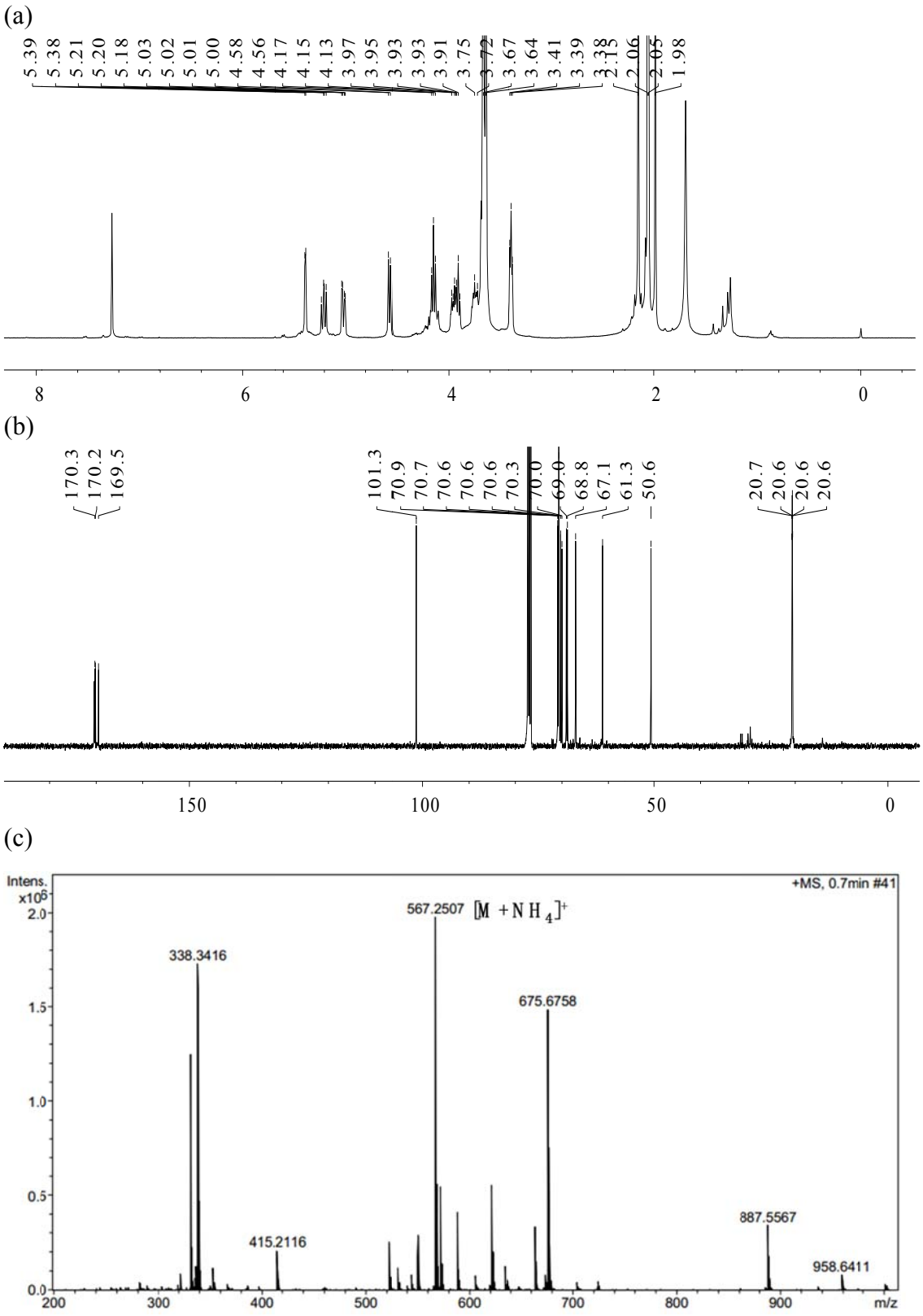


Figure S3. Structural characterization spectra for compound **8**. (a) ^{1}H NMR spectrum (400 MHz, $CDCl_3$-*d*); (b) ^{13}C NMR spectrum (100 MHz, $CDCl_3$-*d*); (c) Mass spectrum (ESI-TOF)

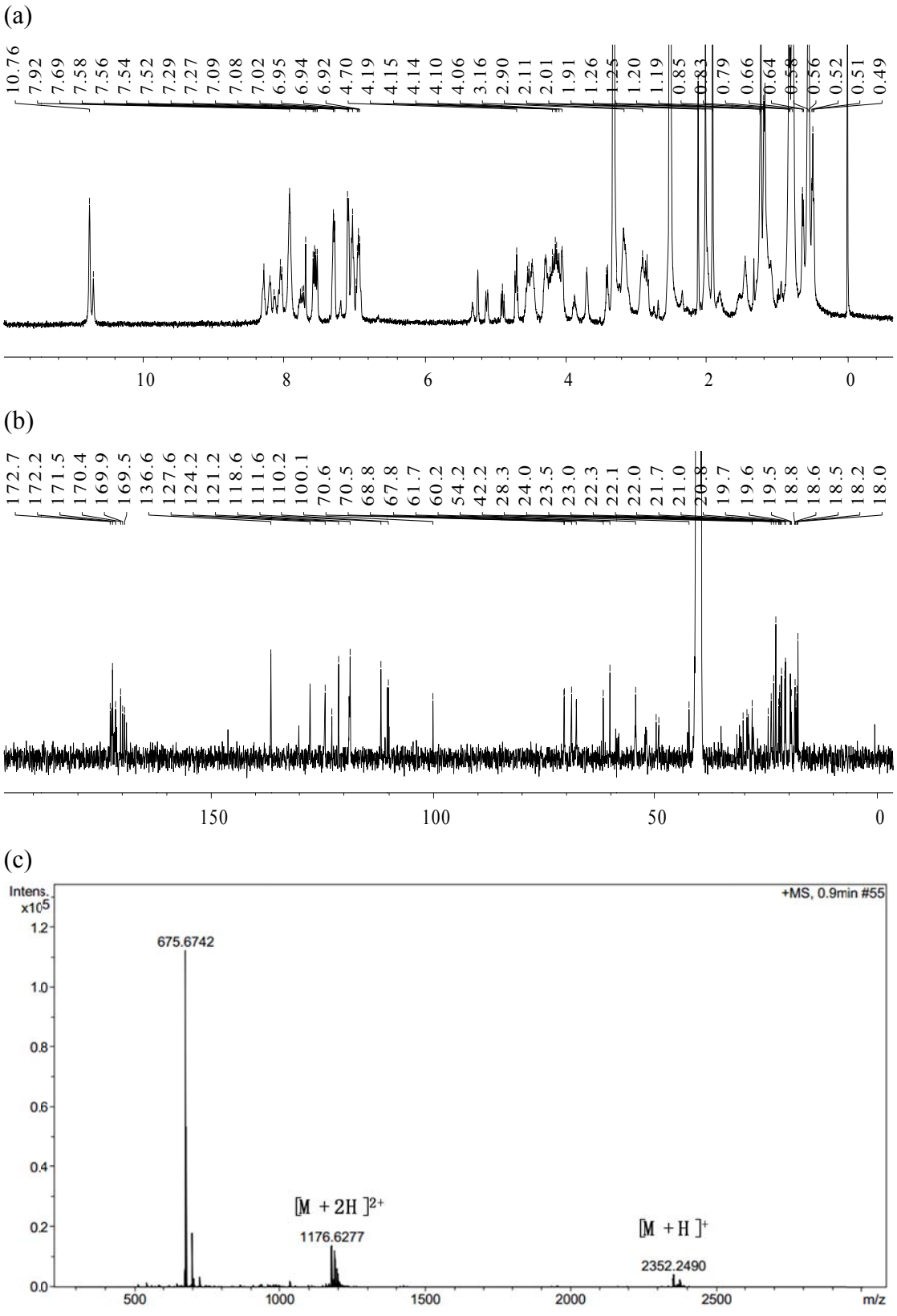
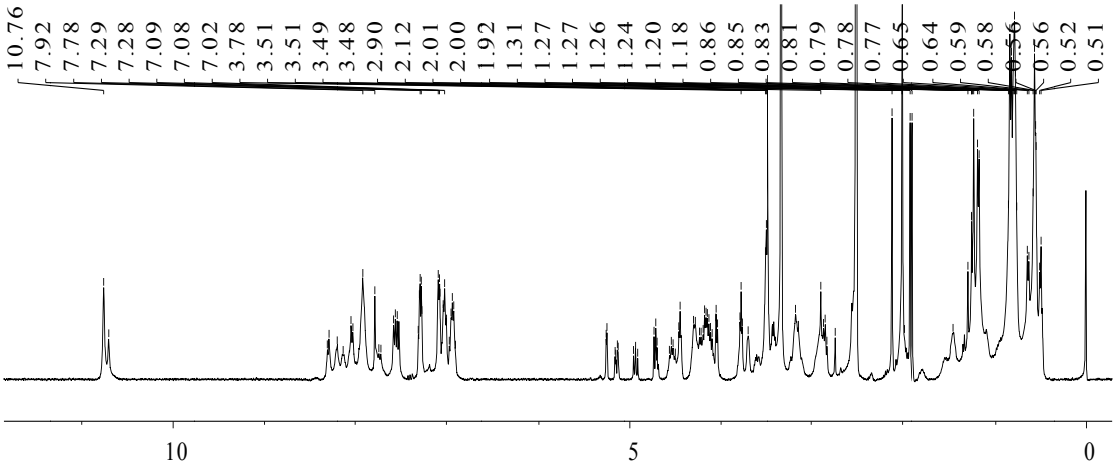
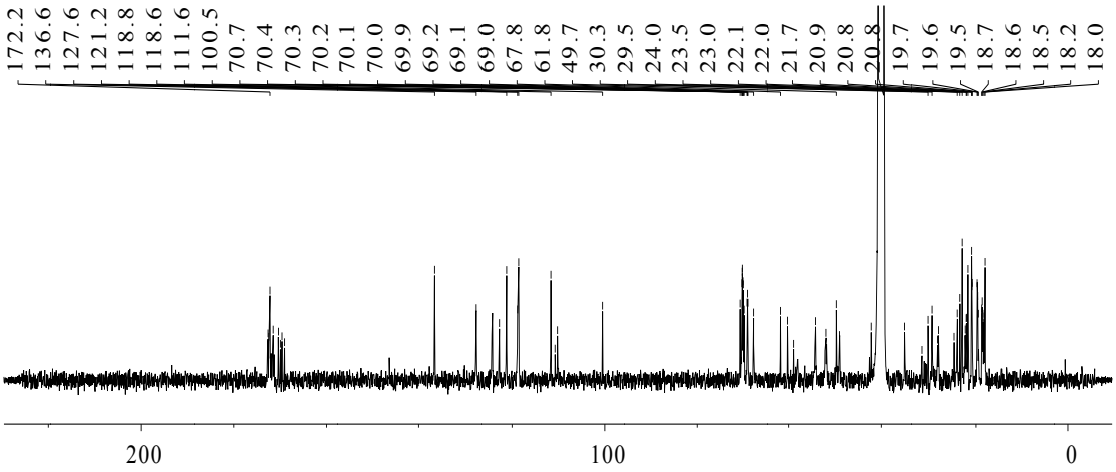


Figure S4. Structural characterization spectra for compound **7**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

(a)



(b)



(c)

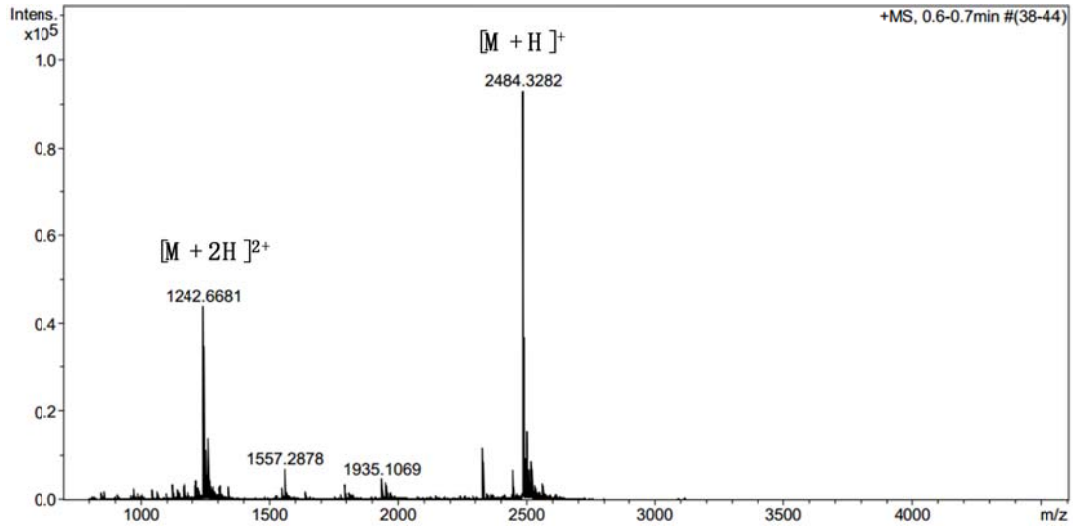


Figure S5. Structural characterization spectra for compound **9**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

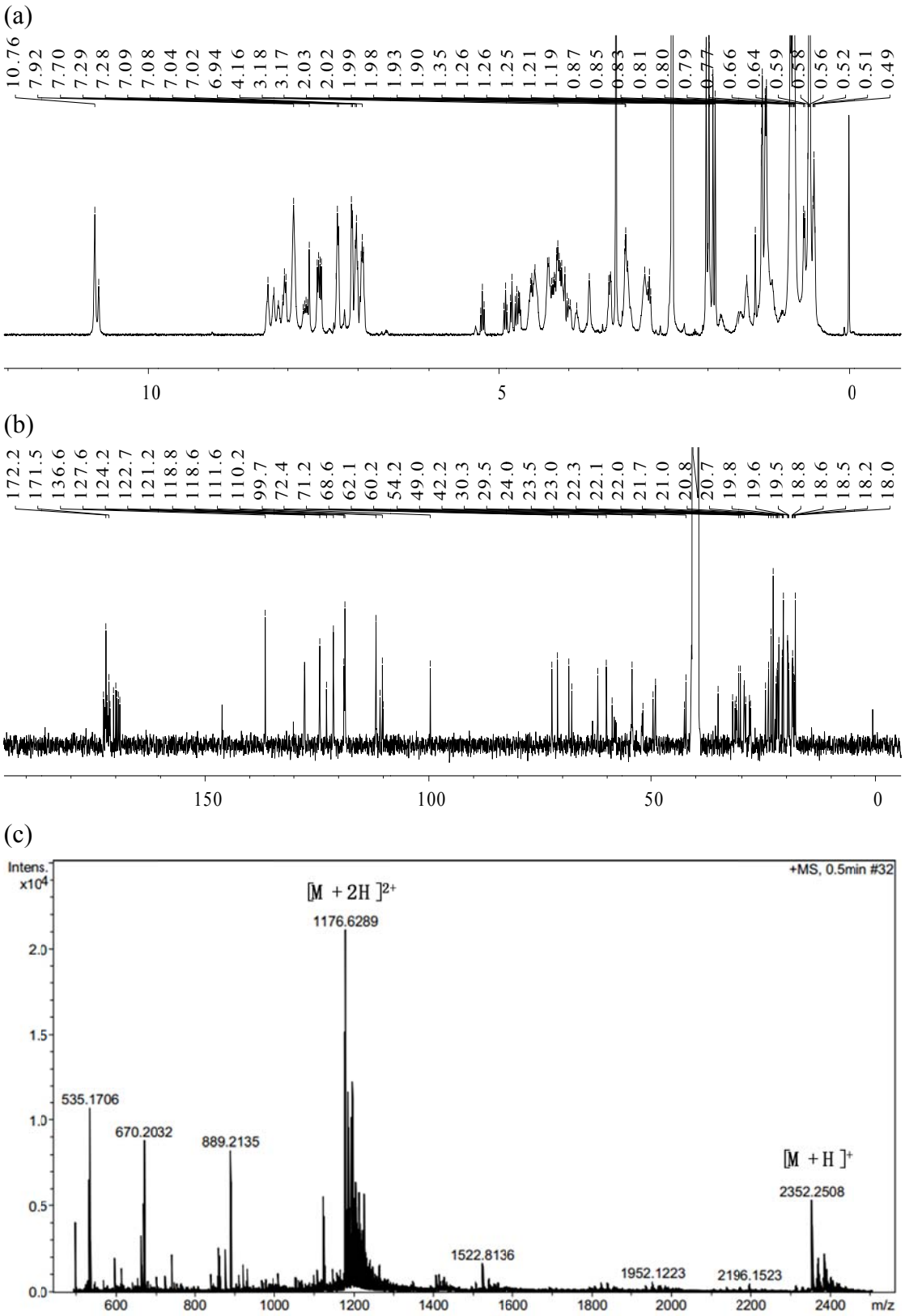


Figure S6. Structural characterization spectra for compound **11**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

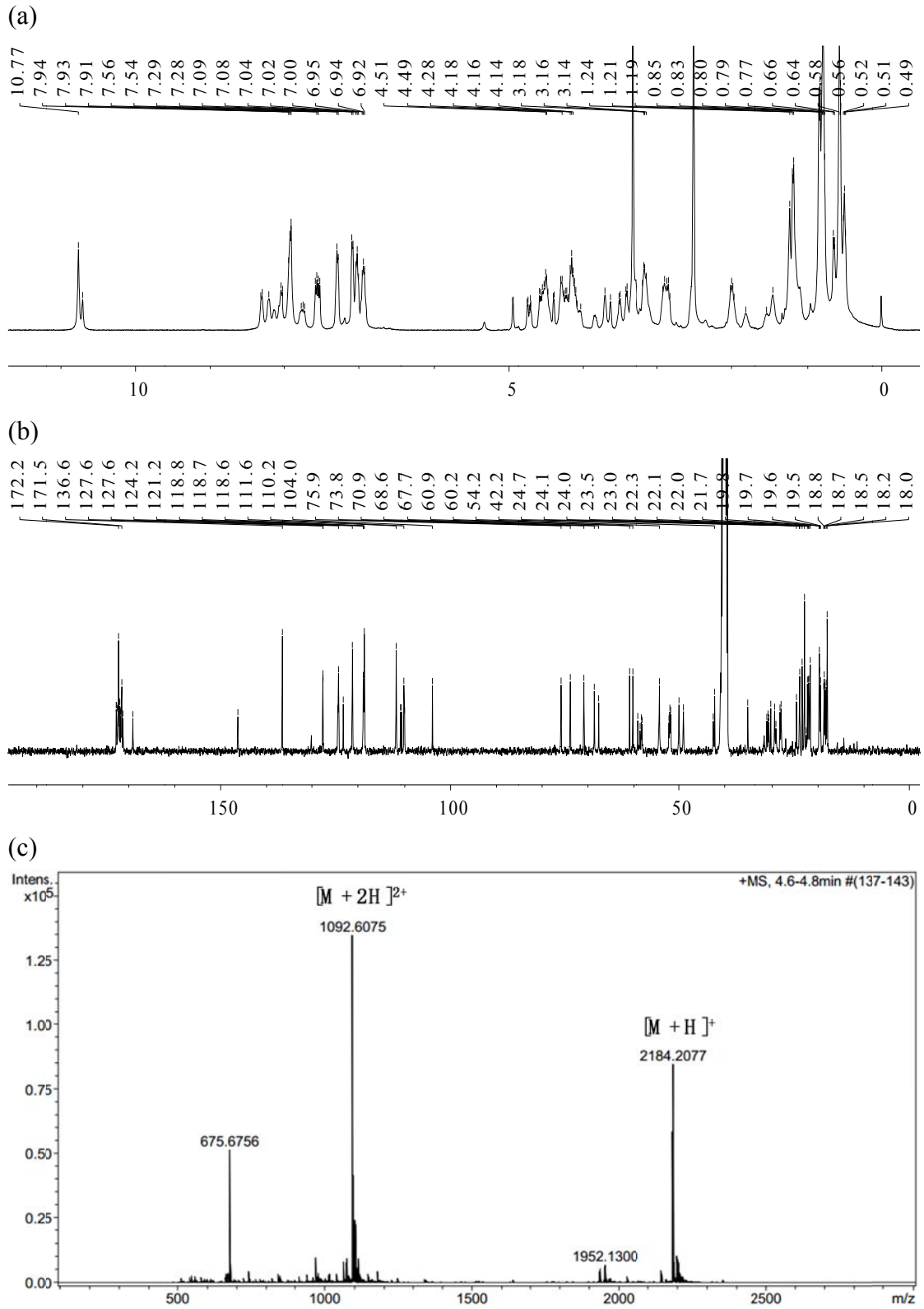


Figure S7. Structural characterization spectra for compound **1**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

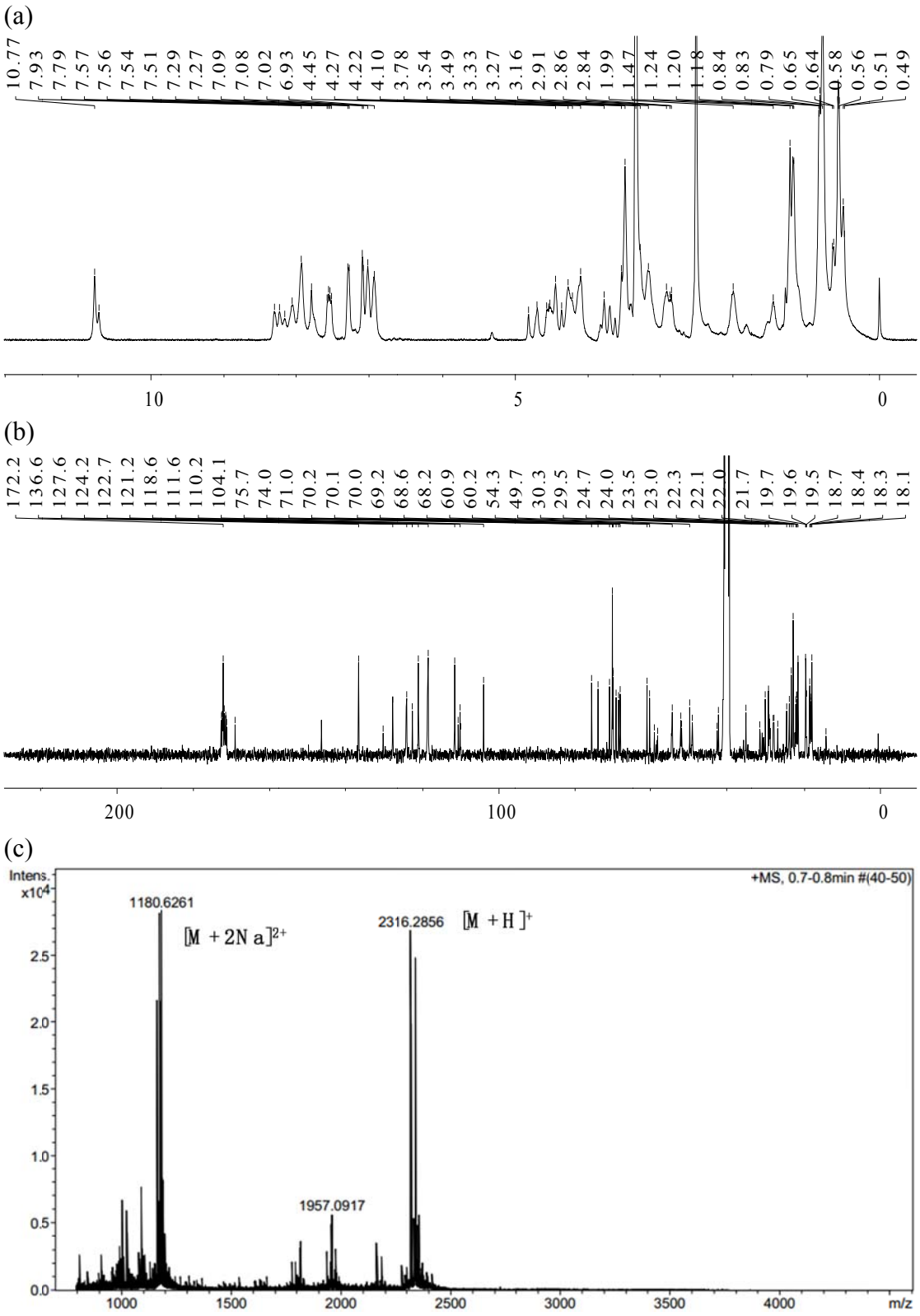


Figure S8. Structural characterization spectra for compound **2**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

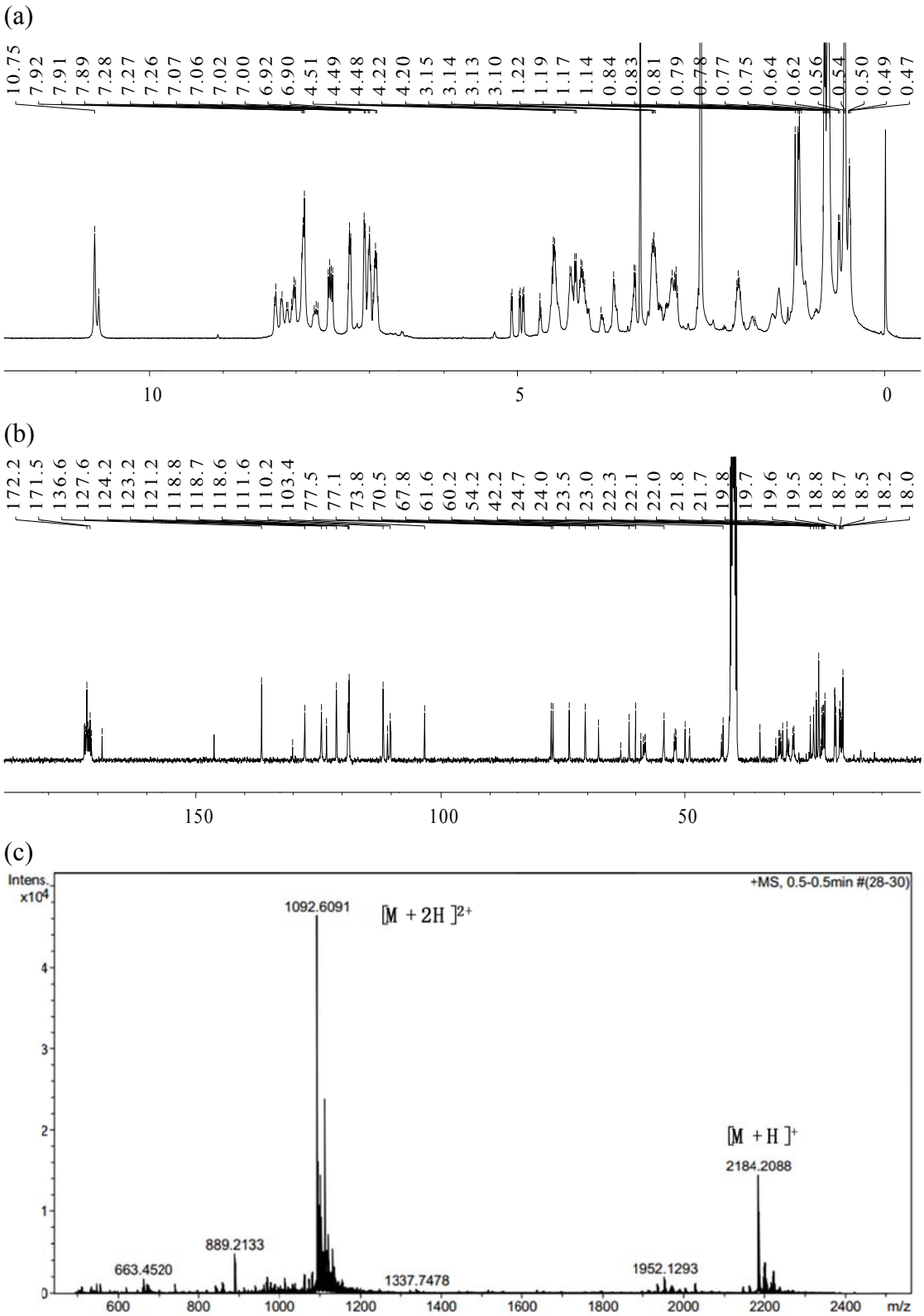


Figure S9. Structural characterization spectra for compound **3**. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) ^{13}C NMR spectrum (100 MHz, DMSO-d_6); (c) Mass spectrum (ESI-TOF).

S3. Procedures for conductance measurements with planar lipid bilayers:

The solution of diPhyPC in chloroform (10 mg/ml, 20 μL) was evaporated with nitrogen gas to form a thin film and re-dissolved in *n*-decane (5 μL). The lipid solution (0.5 μL) was injected on to the aperture (diameter = 200 μm) of the Delrin® cup (Warner Instruments, Hamden, CT) and then evaporated with nitrogen gas. In a typical experiment for measuring the channel conductance, the chamber (cis side) and the Delrin cup (trans side) were filled with an aqueous 1.0 M KCl solution. Ag-AgCl electrodes were applied directly to the two solutions, and the cis one was grounded. A planar lipid bilayer was formed by painting the lipid solution (1.0 μL) around the pretreated aperture and by a judgment of capacitance (80-120 pF). The solution of the tested compounds was added to the cis chamber and the solution was stirred for 5 min. Membrane currents were measured using a Warner BC-535D bilayer clamp amplifier. They were collected by PatchMaster (HEKA) with a sample interval at 5 kHz and then filtered with an 8-pole Bessel filter at 1 kHz (HEKA). The data were analyzed by FitMaster (HEKA) with a digital filter at 100 Hz.

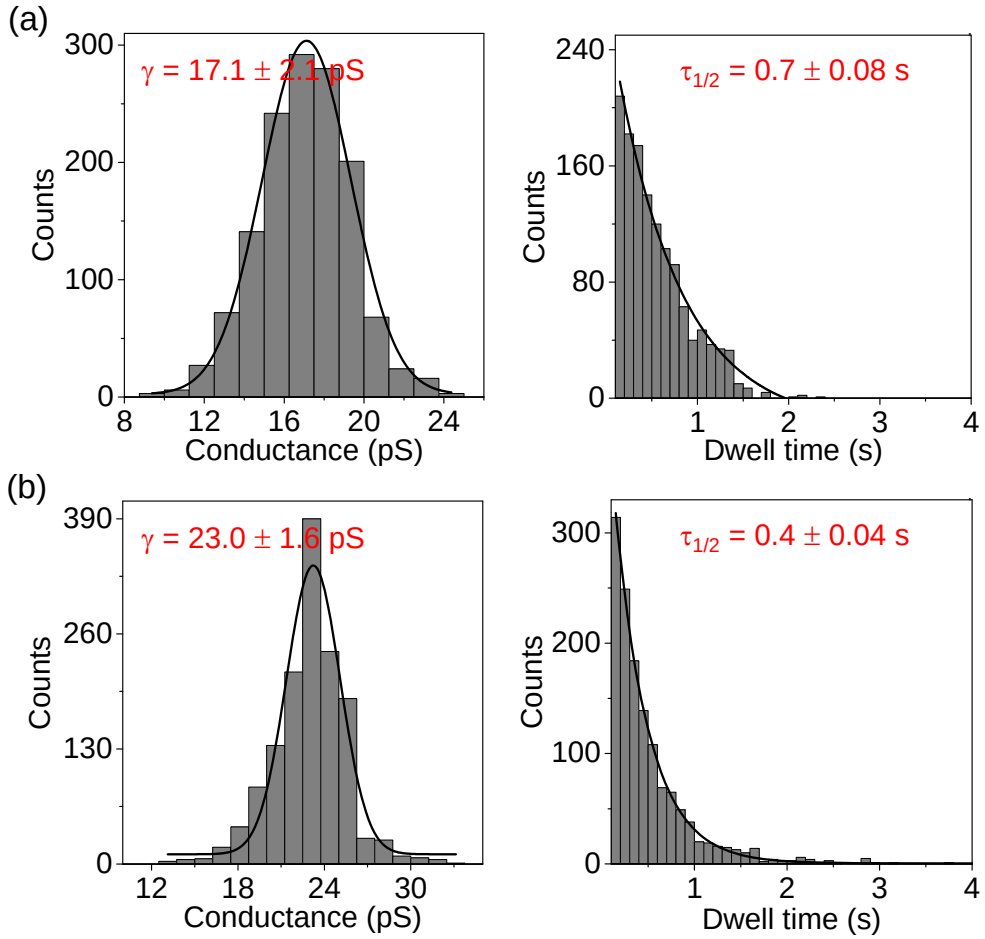


Figure S10. The event histograms of the conductance (left) and dwell time (right) by single-channel conductance measurements of (a) **1** and (b) gA.

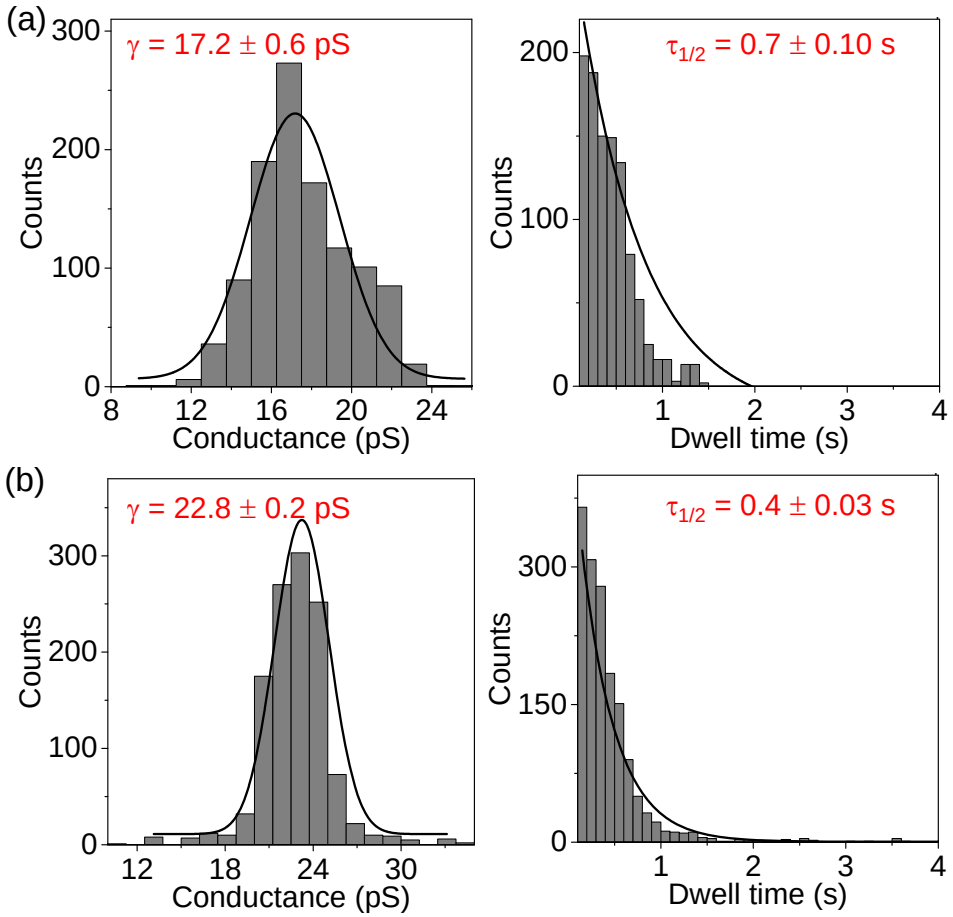


Figure S11. The event histograms of the conductance (left) and dwell time (right) by single-channel conductance measurements of (a) *N*-Cy5-gA and (b) *C*-Cy5-gA.

S4. Procedures for whole-cell patch-clamp experiments:

HepG2 cells were cultured and then incubated with 0.5 mL of trypsin-EDTA (0.05% trypsin, 0.02% EDTA) for 3 min at 37 °C. The collected cells were re-suspended in the patch clamp external recording solution. Whole-cell patch-clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch. The Port-a-Patch (Nanion Technologies) was used for cell capture, seal formation, whole-cell access, and programming of the experiment. EPC-10 (HEKA Germany) patch-clamp amplifiers with Patchmaster (HEKA) software were used for data acquisition. The data were analyzed using FitMaster (HEKA). Boroscillicate glass NPC-1 chips (Nanion Technologies) were used for automated patch-clamp recordings.

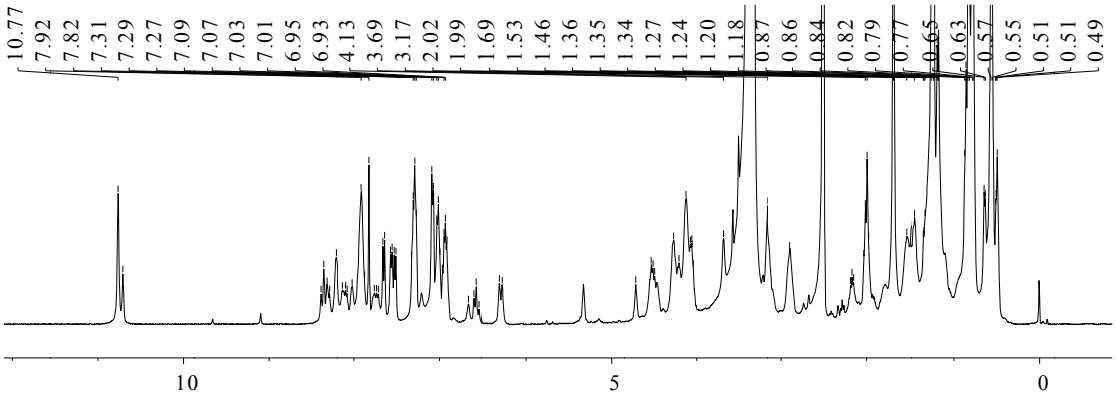
S5. Procedures for single-molecule fluorescence analysis:

(a) Channel labeling: To a solution of **4** or **gA** (0.006 mmol) in anhydrous DMF (2 mL) was added Cy5 (0.007 mmol), EDCI (0.012 mmol), and DMAP (0.012 mmol). The mixture was stirred at 40 ºC for 12 h. After removing the solvent under reduced pressure, the obtained residue was washed with aqueous HCl solution (2 %, 5 mL) and saturated aqueous NaCl. The crude product was then subjected to purification by reverse column chromatography using H_2O /MeOH (1:3, v/v) as the eluent to yield *N*-Cy5-gA or *C*-Cy5-gA as blue solid. The structures of both labeling products were confirmed by ^1H NMR and Mass spectra (Figure S11 and S12).

(b) Single-molecule fluorescence imaging: The imaging was performed on a home-built total internal reflection fluorescence microscope (TIRFM). Briefly, a single-mode fiber-coupled semiconductor laser (635 nm, SFOLT Co., Ltd, China) was mounted on a Nikon Ti-U inverted epi-fluorescence microscope (Japan). The expanded laser beam was focused on the back focal plane of a 100×, 1.49 NA TIRF objective. The signals from the sample were collected using an Andor iXon 897 EMCCD. The time dependent photobleaching trajectories from single molecules were extracted using ImageJ and analyzed by the Hidden Markov Model (HMM).

HepG2 cells were incubated with *N*-Cy5-gA or *C*-Cy5-gA (0.1 nM) for 5 min. Free molecules in the solution were then removed by washing the sample with the Dulbecco's Modified Eagle's Medium (DMEM) cell culture medium. The laser beam angle was adjusted to achieve highly inclined thin illumination to record the fluorescent signals of the molecules in the cell membranes. The intensity distribution was analyzed based on a cell membrane containing > 300 fluorescent spots.

(a)



(b)

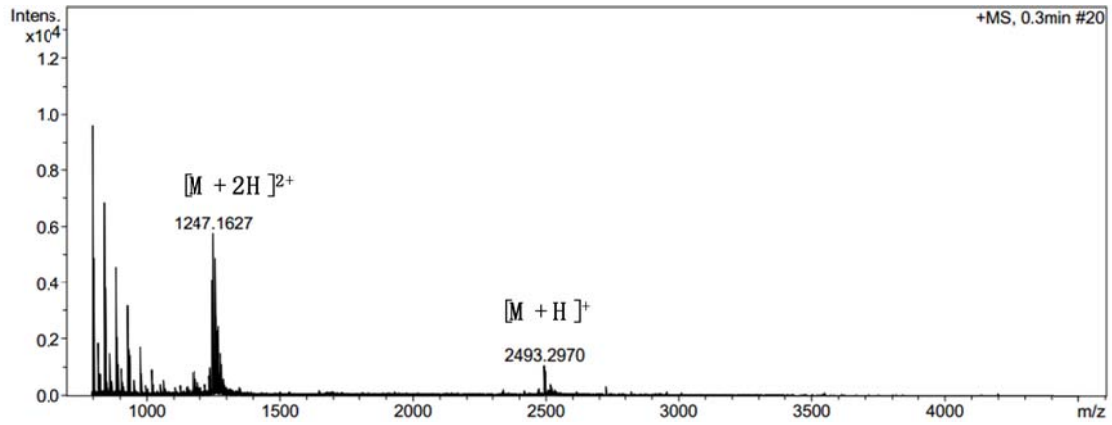
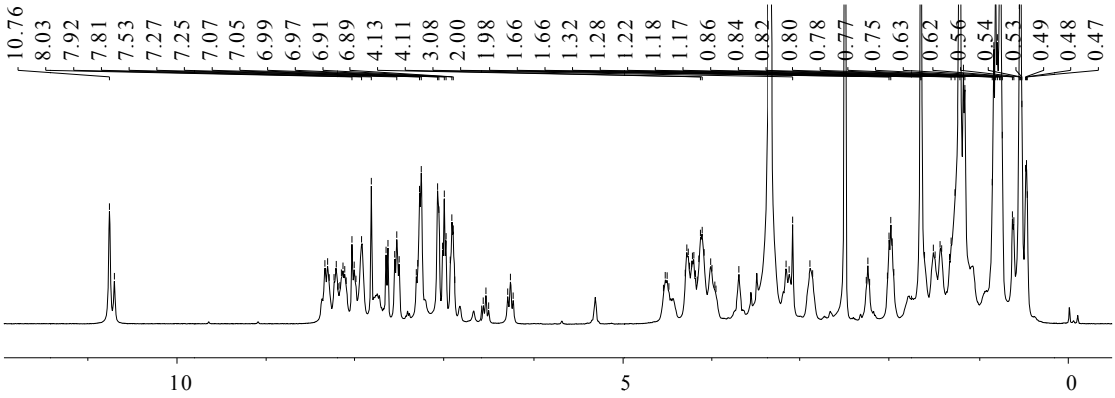


Figure S12. Structural characterization spectra for *N*-Cy5-gA. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) Mass spectrum (ESI-TOF). Calcd. For $C_{131}H_{178}N_{22}O_{23}S_2$ $[M+H]^+$: 2493.2980; Found: 2493.2970.

(a)



(b)

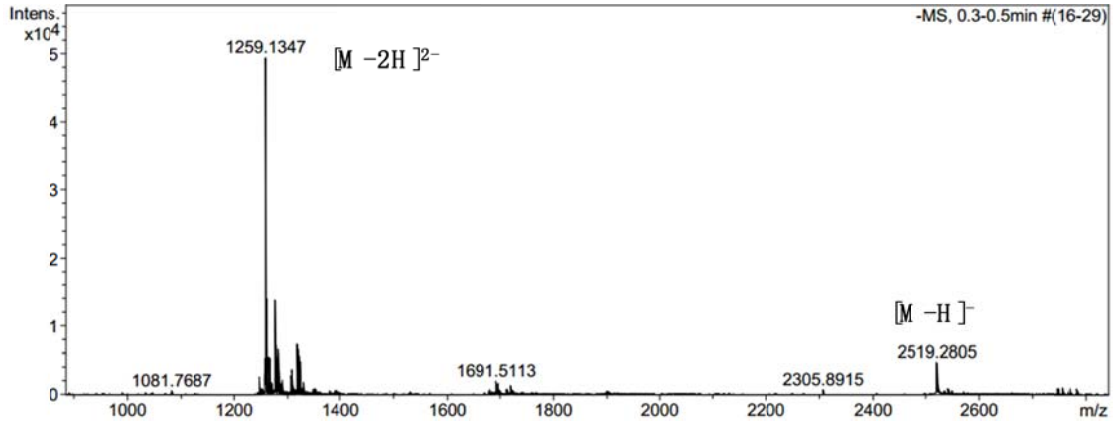


Figure S13. Structural characterization spectra for *C*-Cy5-gA. (a) ^{1}H NMR spectrum (400 MHz, DMSO-d_6); (b) Mass spectrum (ESI-TOF). Calcd. For $C_{132}H_{178}N_{22}O_{24}S_2$ $[M-2H]^{2-}$: 1259.1344; Found:1259.1347.

S6. Procedures for cell viability measurements:

(a) Cell culture: Hela cells (human cervical carcinoma cell line), HepG2 (human hepatocellular liver carcinoma cell line) cells, A549 (human pulmonary carcinoma cell line), MCF-7 (human breast cancer cell line) and LX-2 cells (normal human liver cell line) were used in this study. Cells were cultured in normal DMEM supplemented with 10% FBS, 50 units/mL penicillin and 50 units/mL streptomycin. Cells were maintained at 37^{o}C under a humidified atmosphere of 5% CO_2.

(b) General procedures: Cells were seeded in 96-well plates at a density of 1×10^5 cells per well in 100 μL of DMEM containing 10% FBS, 50 units/mL penicillin and 50 units/mL streptomycin, and cultured in 5% CO_2 at 37°C for 12 h. Then, the cells were incubated with various concentrations of compounds in culture media for 48 h or indicated time at 37°C. The cells were then treated with 10 μL of CCK-8 and incubated for 1-3 h. The absorbance was recorded at 450 nm using a BioTek Epoch2 Microplate reader. Cell viability was calculated using the formula ($A_{compound}$-A_{DMEM}) / ($A_{untreated}$-A_{DMEM}) ×100%.

(c) Cell viability measurements by pretreatment of the cells with galactose: Cells were seeded in 96-well plates at a density of 1×10^5 cells per well in 100 μL of DMEM containing 10% FBS, 50 units/mL penicillin and 50 units/mL streptomycin, and cultured in 5% CO_2 at 37 °C for 12 h. Before the addition of the tested compounds, galactose (160 mM) was added and incubated with HepG2 and HeLa cells for 1 h to block the asialoglycoprotein receptor (ASGPR) in the cell membranes. After washing with PBS buffer to remove free galactose, various concentrations of the compounds in culture media were added. Cell viability was measured with CCK-8 assays using the above standard procedures.

(d) Cell viability measurements by depleting of Na^+ or/and K^+ from the medium: HEPES-buffered solutions (HBSS) were prepared with the following compositions: 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose, 10 mM HEPES (pH 7.4). Na^+ or/and K^+ ions in buffer solutions were replaced with equimolar concentrations of respective NMDG (*N*-methyl-D-glucamine) salts to prepare Na^+-free or/and K^+-free HBSS solutions. Cells were incubated for 24 h with various concentrations of the tested compounds in HBSS solutions, or Na^+-free or K^+-free HBSS solutions at 37°C under a humidified atmosphere of 5% CO_2. Cell viability was measured with CCK-8 assays using the above standard procedures.

S7. Procedures for the hemolytic activity investigations:

Fresh Sprague Dawley rat blood was centrifuged at 3000 rpm and washed with PBS buffer until the supernatant was clear. The erythrocytes were then diluted to a final concentration of 1% (v/v) with PBS buffer. Aliquots of the erythrocyte suspension (200 μL) were placed into a sterile 96-well plate. 2.0 μL of serial solutions of channels in DMSO or DMSO alone was added to the wells in triplicate to reach the required concentration. Complete hemolysis was achieved by mixing the erythrocytes with 1% Triton X-100. The resulting mixture was gently shaken to mix well and incubated at 37 ºC for 2 h, followed by centrifugation at 3500 rpm for 10 min using a tabletop centrifuge. Aliquots (50 μL) of the supernatant were transferred into a new sterile 96-well plate containing 50 μL of PBS buffer in each well. The release of hemoglobin was monitored at 560 nm using a microtiter plate reader. Percentage hemolysis was calculated using the formula = ($A_{compound}$-A_{DMSO}) / ($A_{complete\ hemolysis}$-A_{DMSO}) ×100%. The channel concentration required to cause 50% hemolysis (HC_{50}) was read out directly from the graph.

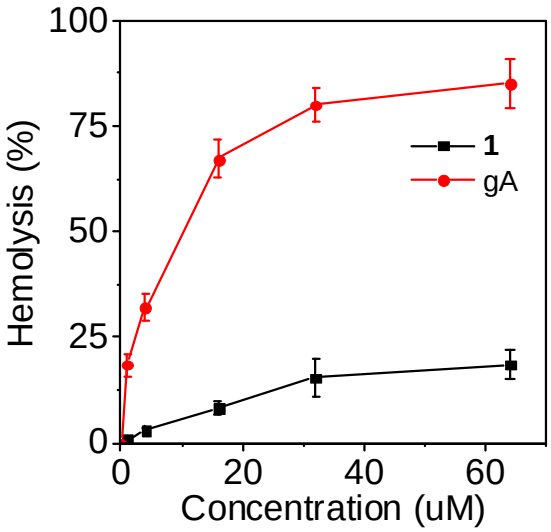


Figure S14. Dose-dependent hemolytic activity of compound **1** and **gA** against rat erythrocytes. The IC_{50} of **1** and gA were determined to be > 60 μM and 7 μM, respectively, suggesting that the decoration at *N*-terminus of gA could significantly decrease the hemolysis toxicity of the peptide.

S8. Procedures for flow cytometry investigations:

HepG2 and Hela cells were treated with the tested compound (5 μM) for the indicated time. Untreated cells were used as a negative control. After washing twice with PBS, the cells were incubated with 0.5 mL of 0.05% trypsin without EDTA for 5-10 min at 37°C and collected. Cells were re-suspended in the blending buffer (an apoptosis detection kit containing annexin V-FITC and PI dyes) to stain. The cells were incubated for another 15 min in the dark at 4°C. Then, they were analyzed by

flow cytometry equipped with a 488 nm argon laser light source, a 515 nm bandpass filter for FITC-fluorescence, and 623 nm bandpass filter for PI-fluorescence. The cell size was measured with a flow cytometer by exciting with a 488 nm argon laser and determining their distribution on a forward scatter versus side scatter dot plot. Light scattered in the forward direction is proportional to cell size, and light scattered at a 90° angle (side scatter) is proportional to cell density. A total of 10,000 events were acquired, and the cells were appropriately gated for analysis. Data were collected from three independent experiments.

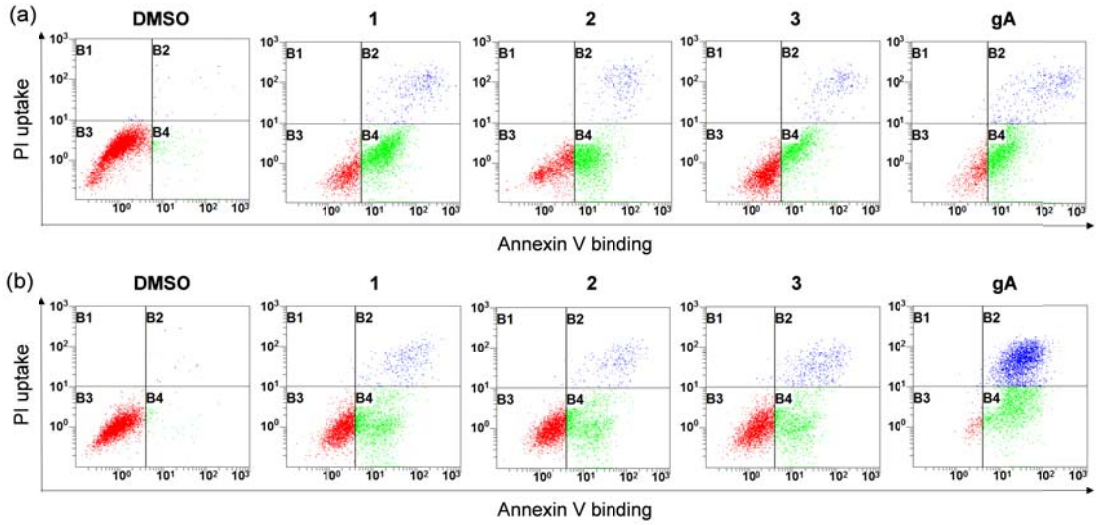


Figure S15. Flow cytometry analysis of (a) HepG2 and (b) Hela cells treated with **1-3** and gA (5 μM) for 48 h. The observation of annexin V^{pos}-PI^{neg} cells and annexin V^{pos}-PI^{pos} cells indicates the apoptosis of the cells in the presence of the peptides.

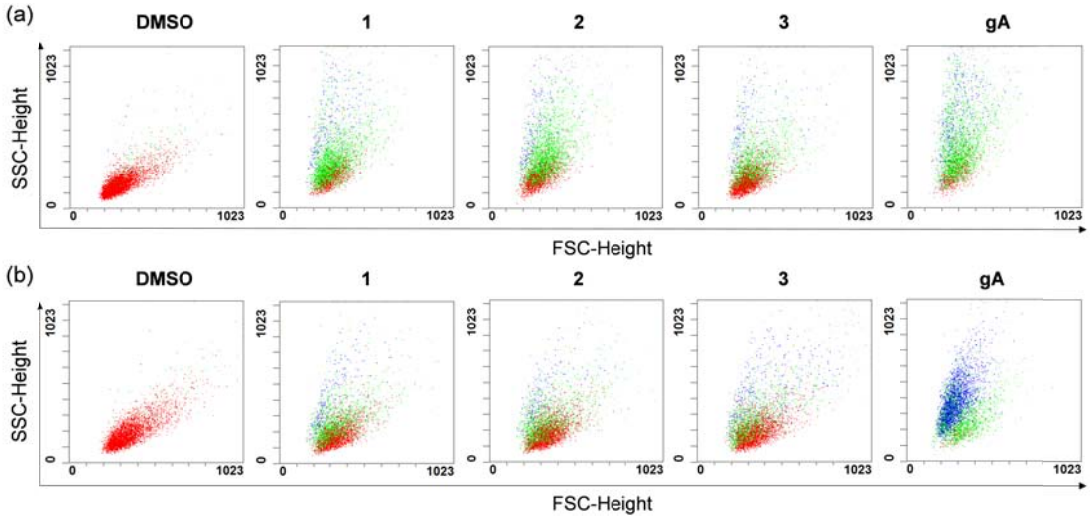


Figure S16. Cell size analysis of (a) HepG2 and (b) Hela cells treated with **1-3** and gA (5 μM) for 48 h.

S9. Procedures for Western blotting:

Proteins were separated by 10-15% SDS-PAGE. Rabbit PARP polyclonal (1:1000, Cell Signaling Technology), rabbit Caspase-3 monoclonal (1:1000, Abcam), rabbit Cleaved Caspase-3 monoclonal (1:1000, Abcam) and mouse β-actin (1:1000, Santa Cruz Biotechnology) antibodies were used as primary antibodies. Blots were performed using standard procedures.[5]

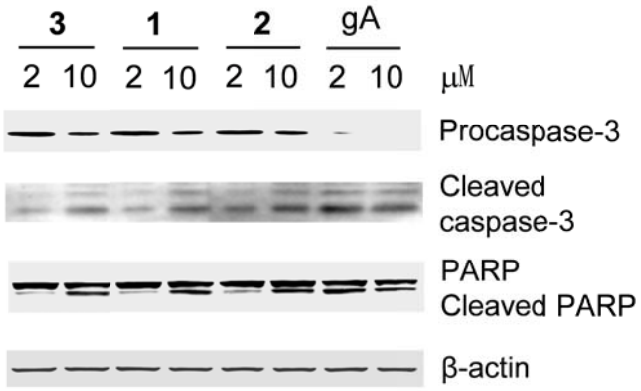


Figure S17. Immunobloting of Hella cells treated with the peptides for 48 h. The indicated proteins were immunoblotted with the corresponding antibodies.

S10. Procedures for cellular ion transport investigations:

HepG2 cells were incubated in culture media containing the corresponding fluorescent indicators of H^+ (1 μM), Na^+ (10 μM), K^+ (10 μM), Ca^{2+} (10 μM), and Cl^- (10 mM) and 0.04% Pluronic F-127 (for Na^+ and K^+ concentration measurements) at 37°C for 1.5 h. After washing with PBS to remove the free indicator, 100 μL HBSS solutions were added to the cells. The cells were incubated with the tested compound (5 μM) at 37°C for the indicated time. The fluorescence intensity of the ion indicators was measured using a fluorescence microplate reader. The fluorescent ion indicators used were: SBFI-AM (λ_{ex} = 340 nm, λ_{em} = 500 nm) for Na^+, PBFI-AM (λ_{ex} = 340 nm, λ_{em} = 500 nm) for K^+, BCECF-AM (λ_{ex} = 490/440 nm, λ_{em} = 530 nm) for H^+, Fluo-4 AM (λ_{ex} = 485 nm, λ_{em} = 538 nm) for Ca^{2+}, and MQAE (λ_{ex} = 350 nm, λ_{em} = 460 nm) for Cl^-.

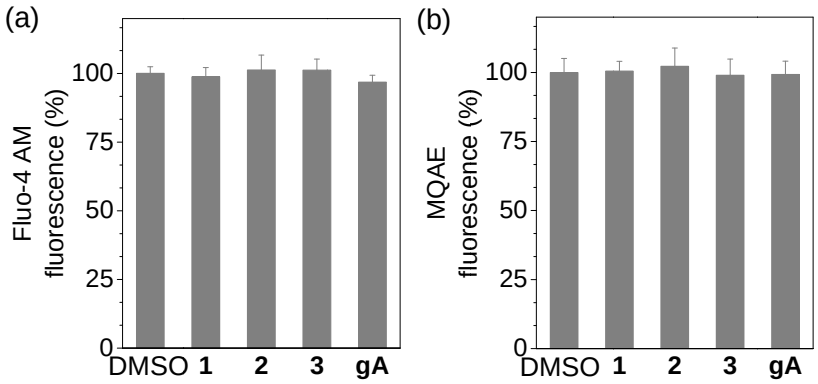


Figure S18. Cellular ion transport of **1-3** and gA (5.0 μM). The relative intracellular (a) Fluo-4 AM (Ca^{2+} probe) and (b) MQAE (Cl^- probe) fluorescence intensity after treatment of HepG2 cells with the **1-3** and gA (5 μM) for 8 h.

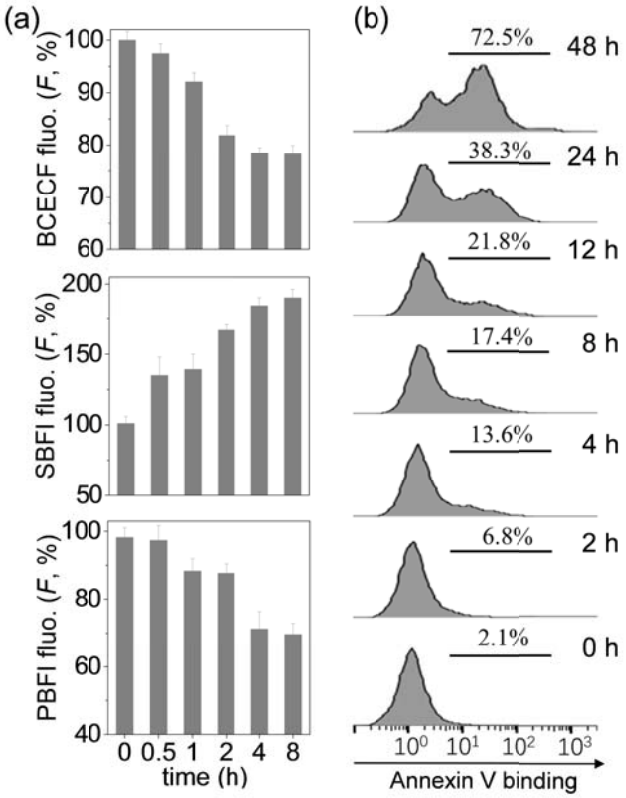


Figure S19. Investigation on the occurrence sequence of ion transport and apoptosis in the presence of **1** (5.0 μM). (a) Time-dependent intracellular BCECF (H^+ probe), SBFI (Na^+ probe), and PBFI (K^+ probe) fluorescence intensity measurements monitoring the ion concentration changes. (b) Time-dependent flow cytometry analysis monitoring the percentage changes of the annexin V-positive (apoptotic) cells.

S11. References:

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