

Supporting Information

Imidazolium-based 1,1'-bi-2-naphthol fluorescent probe for ratiometric and selective detection of DNA in water

Ming-Qi Wang, Kun Li^{*}, Hao-Ran Xu, Xiao-Qi Yu^{*}

Table of contents

1. Fig. S1. Plot of the fluorescent intensity ratios as a function of DNA	S2
2. Fig. S2. Absorption spectra of R-1	S2
3. Fig. S3. Electrophoretic mobility shift assay of R-1	S3
4. Fig. S4. Ionic strength effects on the fluorescence intensity of R-1 with DNA	S3
5. Fig. S5. Fluorescent changes of R-1 with NaCl	S4
6. Scheme S1. Synthesis of compound S-1 .	S4
7. Scheme S2. Synthesis of compound R-g1 .	S5
8. Fig. S6. Fluorescent changes of S-1 with various anions	S5
9. Fig. S7. Fluorescence titration of R-1 towards DNA	S6
10. Fig. S8. Fluorescent changes of R-g1 with various anions	S6
11. Fig. S9. Fluorescence titration of R-g1 towards CT-DNA	S7
12. Fig. S10. Emission spectra of EB bound to CT-DNA at increasing amounts of R-1	S7
13. Fig. S11. Emission spectra of EB bound to CT-DNA at increasing amounts of S-1	S8
14. Fig. S12. Emission spectra of EB bound to CT-DNA at increasing amounts of R-g1	S8
15. Fig. S13. Binding constants of the three compounds	S9
16. Fig. S14. Fluorescence titration of R-1 towards CT-ssDNA	S9
17. Fig. S15. Fluorescent response of R-1 towards synthetic double and single stranded DNAs	S10
18. Fig. S16. Sequence selectivity of R-1	S10
19. Fig. S17. Absorption spectra of R-1 with ssDNA	S10
20. Experimental section of S-2 , S-1 , R-g2 and R-g1 .	S11-S25

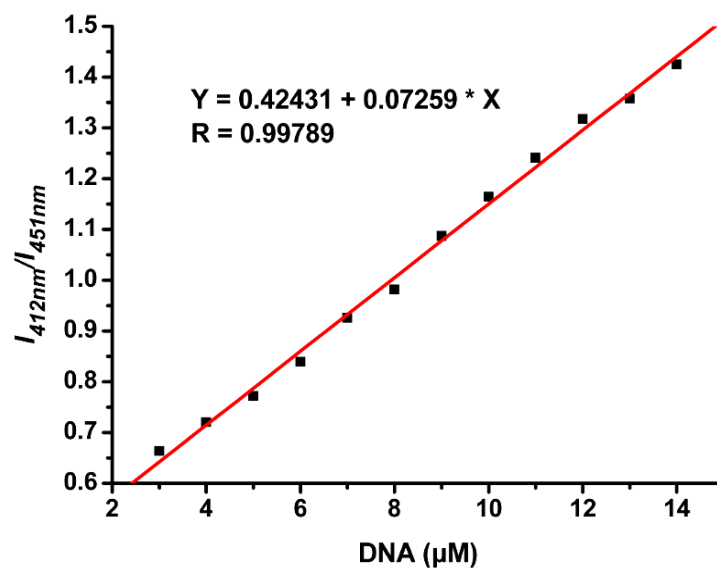


Fig. S1. Plot of the fluorescent intensity ratios at 412 and 451 nm as a function of the CT-DNA concentrations.

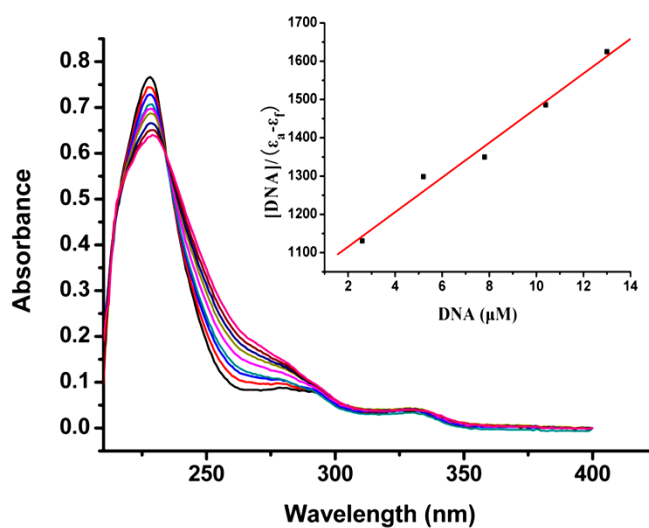


Fig. S2. Absorption spectra of **R-1** (10 μM) with increasing concentrations of CT-DNA (0-30 μM) in HEPES buffer (pH 7.4, 10 mM). Inset: corresponding half-reciprocal plot of **R-1**. (R = 0.9907)

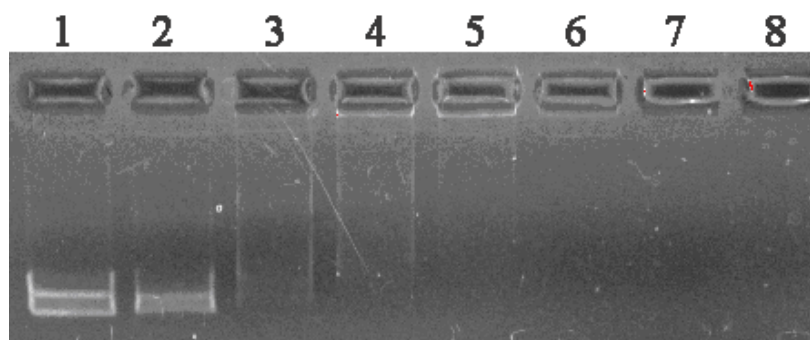


Fig. S3. Electrophoretic mobility shift assay of **R-1** in the presence of pUC 19 (7 $\mu\text{g/mL}$) in HEPES buffered (pH 7.4, 10 mM) water. Lanes 1–8, [**R-1**]: 0, 0.033, 0.067, 0.167, 0.333, 0.667, 1.334, 3.334 mM.

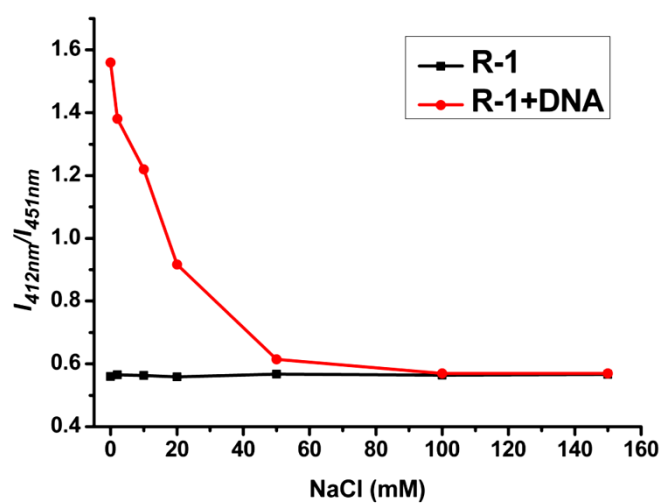


Fig. S4. Fluorescence intensity ratio (I_{412}/I_{451}) of **R-1** (10 μM , square) and **R-1**+2 equiv DNA (circle) in HEPES buffer (pH 7.4, 10 mM) with increasing addition of NaCl. [NaCl]: 0–150 mM.

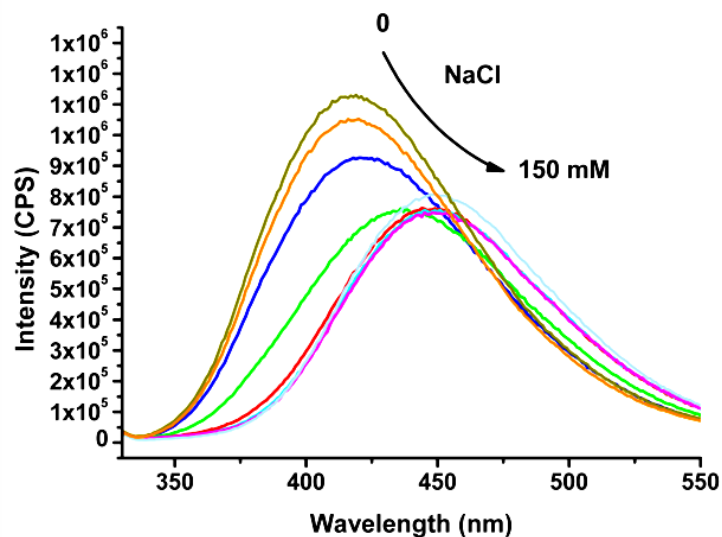
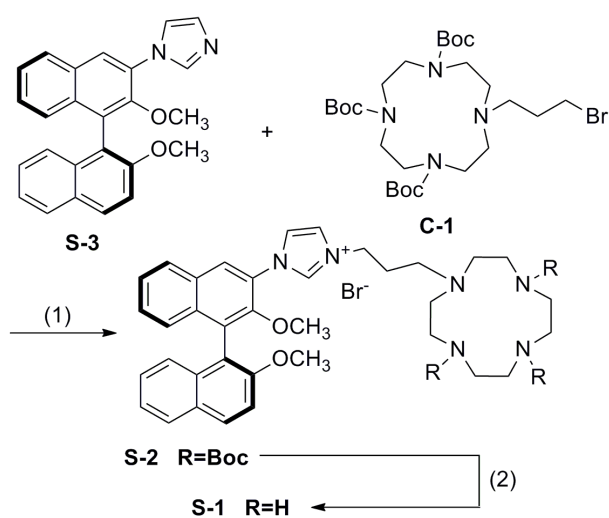
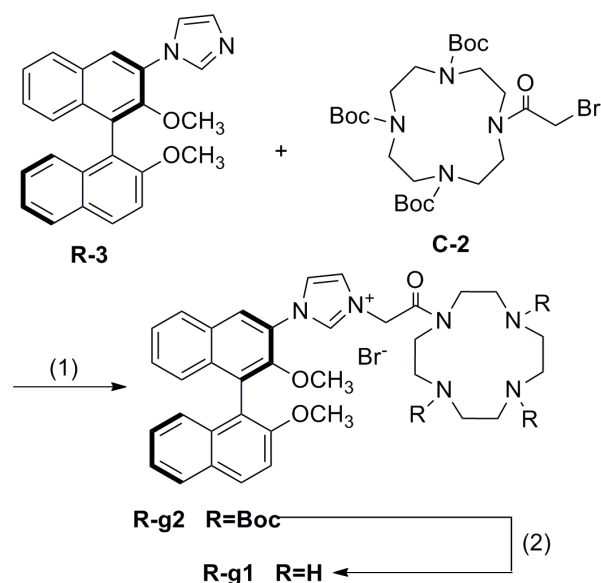


Fig. S5. Fluorescence changes of **R-1** (10 μ M) and **R-1**+2 *equiv* DNA (circle) in HEPES buffered (pH 7.4, 10 mM) water (excitation at 291 nm) with increasing addition of NaCl. [NaCl]: 0-150 mM.



Scheme S1. Synthesis of compound **S-1**. Conditions: (1) CH₃CN, 80 °C, 72 h; (2) MeOH/HCl, rt, 12 h.



Scheme S2. Synthesis of compound **R-g1**. Conditions: (1) CH₃CN, 80 °C, 72 h; (2) MeOH/HCl, rt, 12 h.

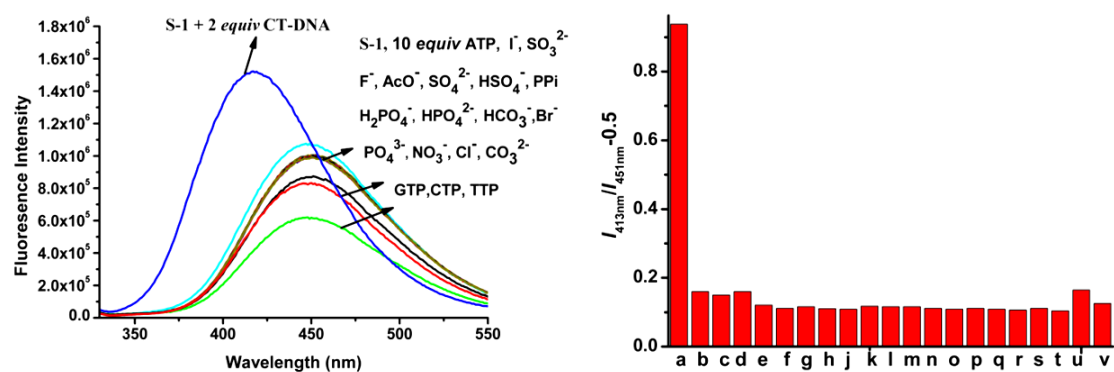


Fig. S6. Fluorescent changes of **S-1** (10 μ M) with various anions (10 equiv) and CT-DNA (2 equiv) in HEPES buffered (pH 7.4, 10 mM) water (excitation at 291 nm). a. CT-DNA, b. ATP, c. TTP, d. GTP, e. CTP, f. PO₄³⁻, g. NO₃⁻, h. H₂PO₄⁻, j. HPO₄²⁻, k. Br⁻, l. I⁻, m. PPI, n. AcO⁻, o. SO₃²⁻, p. HCO₃⁻, q. F⁻, r. SO₄²⁻, s. CO₃²⁻, t. HSO₄⁻, u. Cl⁻, v. **S-1**.

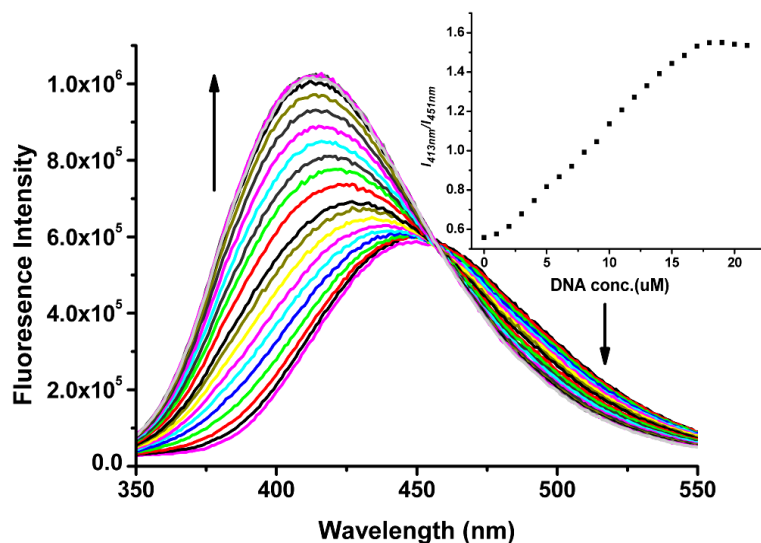


Fig. S7. Fluorescent emission spectra of **S-1** (10 μ M) in the presence of different concentrations of CT-DNA (0-20 μ M) in HEPES buffered (pH 7.4, 10 mM) water. Excitation wavelength was 291 nm, and emission was at 451 and 412 nm. Inset: Ratiometric calibration curve I_{412}/I_{451} as a function of CT-DNA concentration.

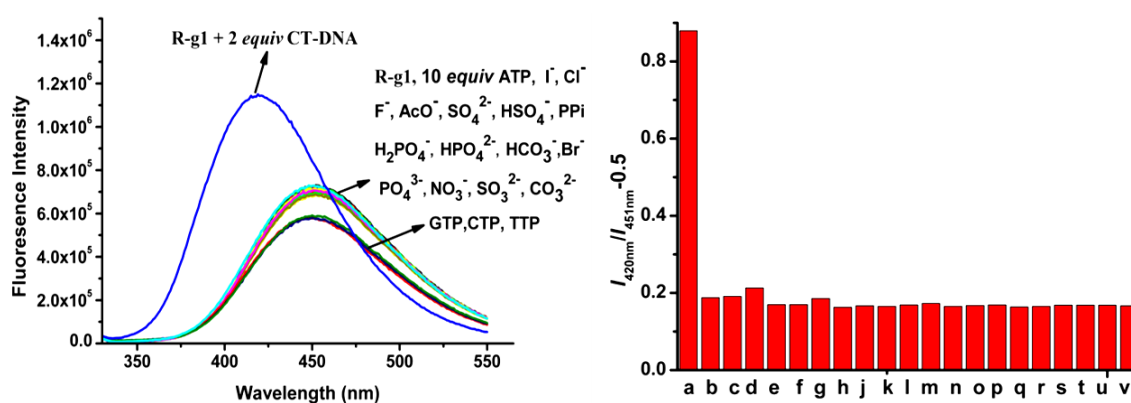


Fig. S8. Fluorescent changes of **R-g1** (10 μ M) with various anions (10 equiv) and CT-DNA (2 equiv) in HEPES buffered (pH 7.4, 10 mM) water (excitation at 291 nm). a. CT-DNA, b. CTP, c. TTP, d. GTP, e. HSO_4^- , f. AcO^- , g. ATP, h. H_2PO_4^- , j. HPO_4^{2-} , k. Br^- , l. I^- , m. PPi, n. PO_4^{3-} , o. SO_3^{2-} , p. HCO_3^- , q. F^- , r. SO_4^{2-} , s. CO_3^{2-} , t. NO_3^- , u. Cl^- , v. **S-1**.

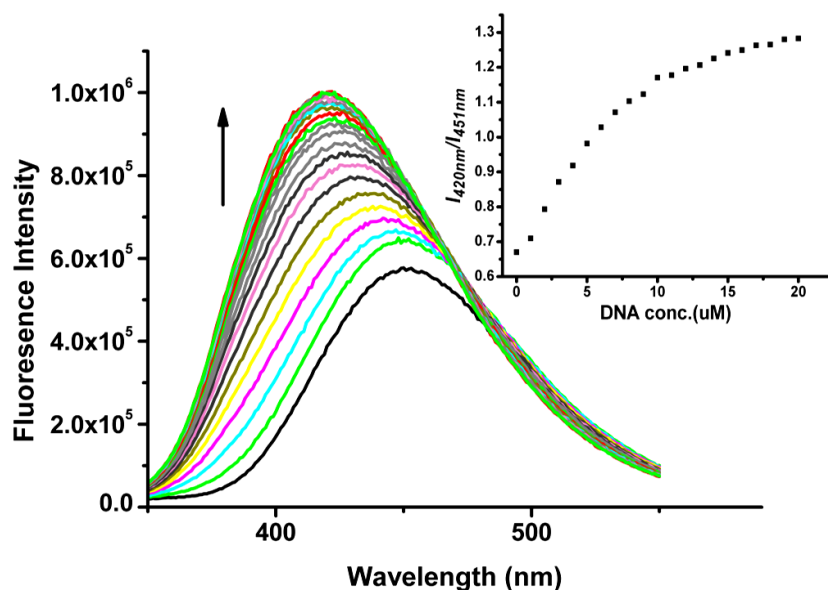


Fig. S9. Fluorescent emission spectra of **R-g1** (10 μM) in the presence of different concentrations of CT-DNA (0-20 μM) in HEPES buffered (pH 7.4, 10 mM) water. Excitation wavelength was 291 nm, and emission was at 451 and 412 nm. Inset: Ratiometric calibration curve I_{412}/I_{451} as a function of CT-DNA concentration.

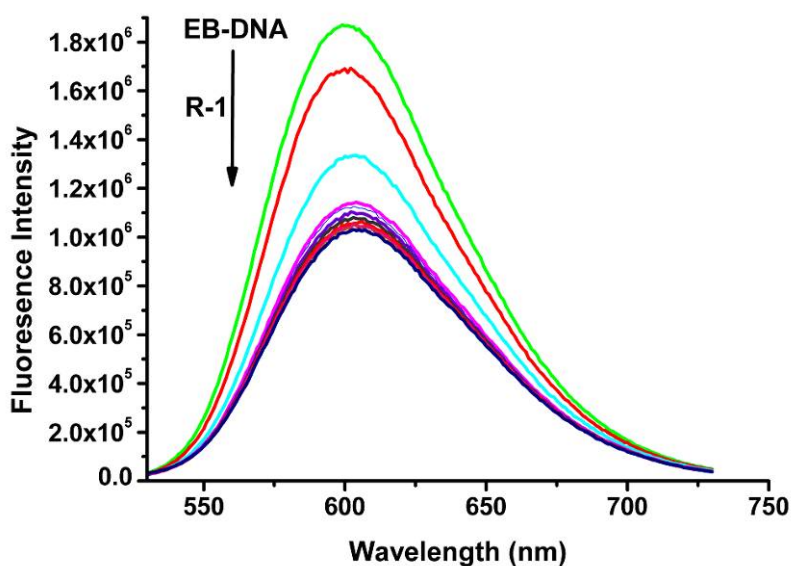


Fig. S10. Emission spectra of EB bound to CT-DNA at increasing amounts (0-10 μM) of **R-1** in HEPES buffered (pH 7.4, 10 mM) water.

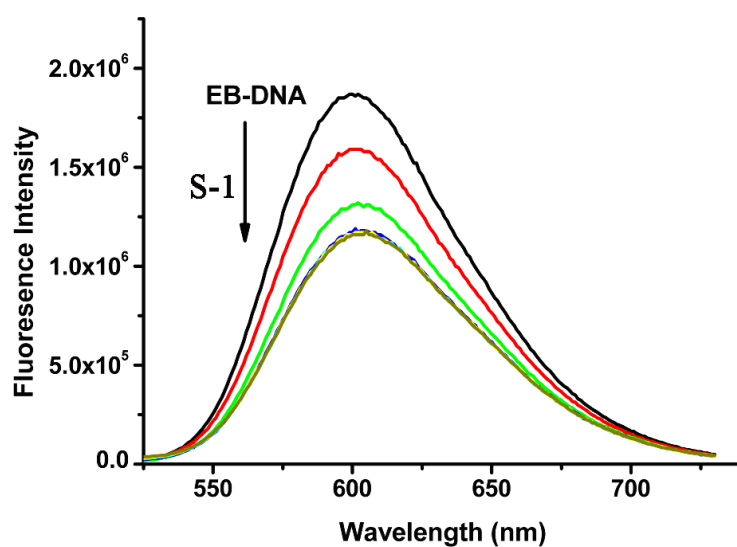


Fig. S11. Emission spectra of EB bound to CT-DNA at increasing amounts (0-10 μM) of **S-1** in HEPES buffered (pH 7.4, 10 mM) water.

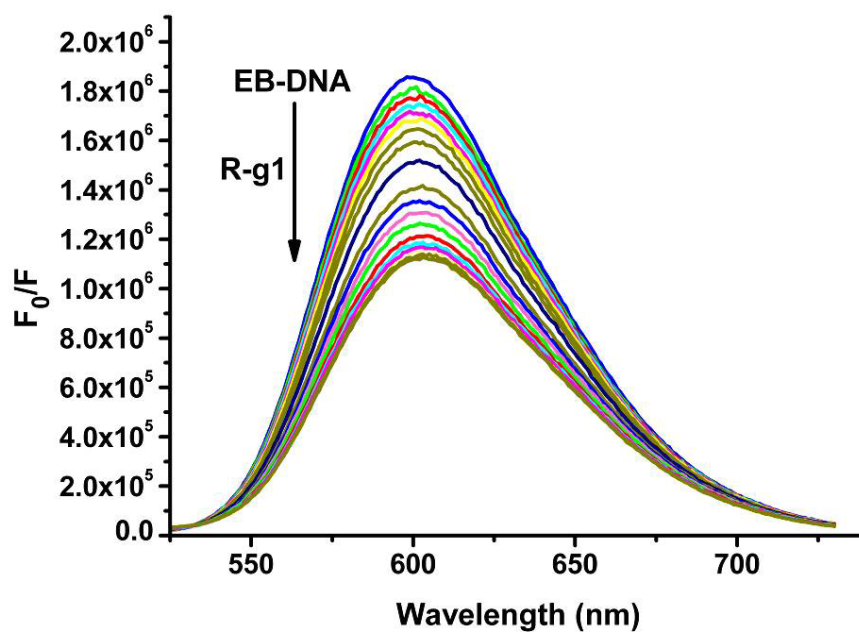


Fig. S12. Emission spectra of EB bound to CT-DNA at increasing amounts (0-20 μM) of **R-g1** in HEPES buffered (pH 7.4, 10 mM) water.

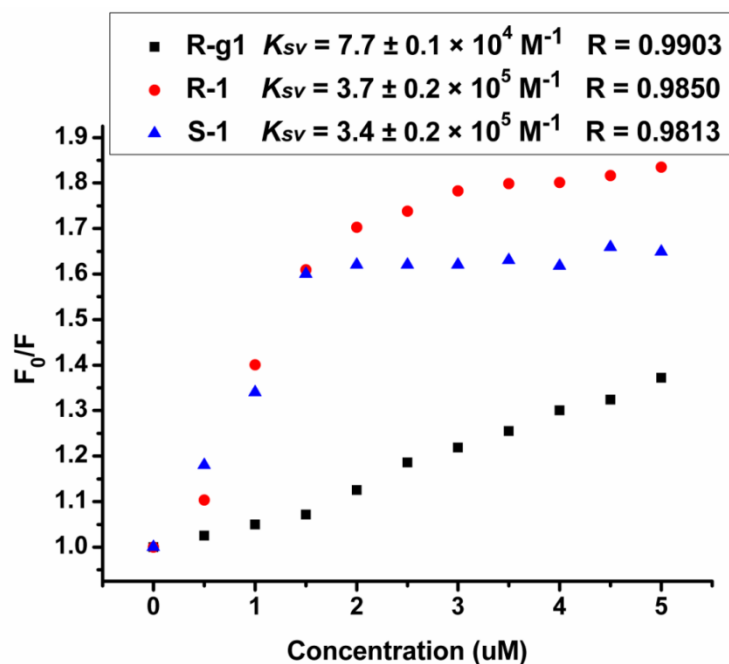


Fig. S13. Binding constants of three compounds **R-1**, **S-1** and **R-g1**.

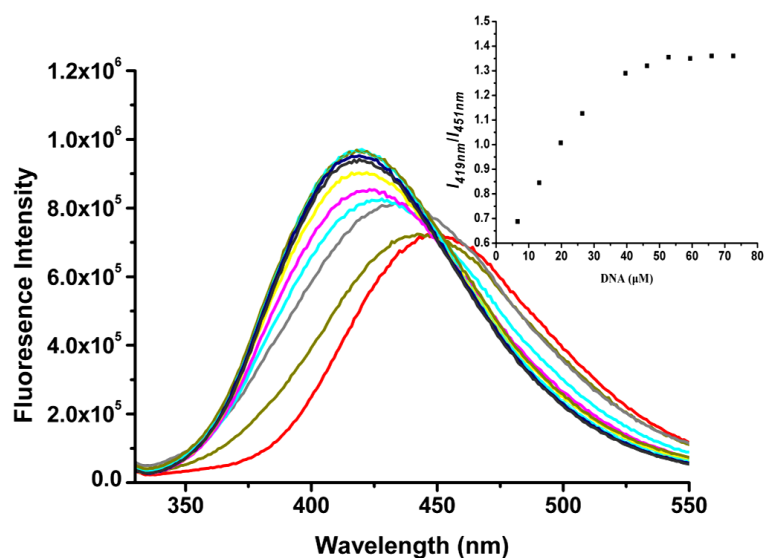


Fig. S14. Fluorescent emission spectra of **R-1** (10 μM) in the presence of different concentrations of CT-ssDNA (0-80 μM) in HEPES buffered (pH 7.4, 10 mM) water. Excitation wavelength was 291 nm, and emission was at 451 nm and 419 nm. Inset: Ratiometric calibration curve I_{419}/I_{451} as a function of CT-ssDNA concentration.

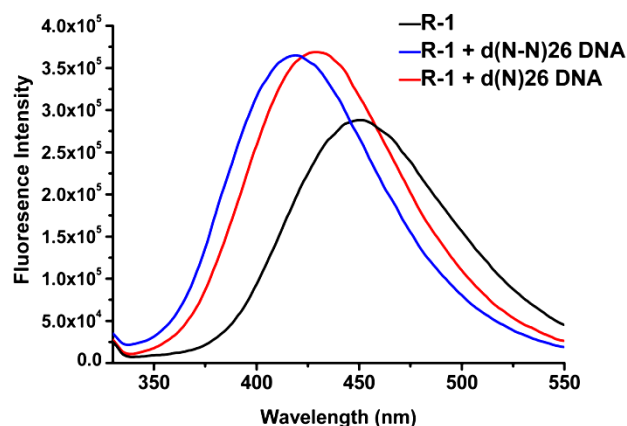


Fig. S15. Fluorescent emission spectra of **R-1** (10 μ M) in the presence of synthetic double and single stranded DNAs (0-80 μ M) in HEPES buffered (pH 7.4, 10 mM) water. Excitation wavelength was 291 nm, and emission was at 451 and 419 nm. Inset: Ratiometric calibration curve I_{419}/I_{451} as a function of CT-DNA concentration. d(N)26: 5'-TCCTGTGCTGAAGTCTGCCGTTAGTG-3'. d(N')26: 3'-CACTAACGGCAGACTTCAGCACAGGA-5'

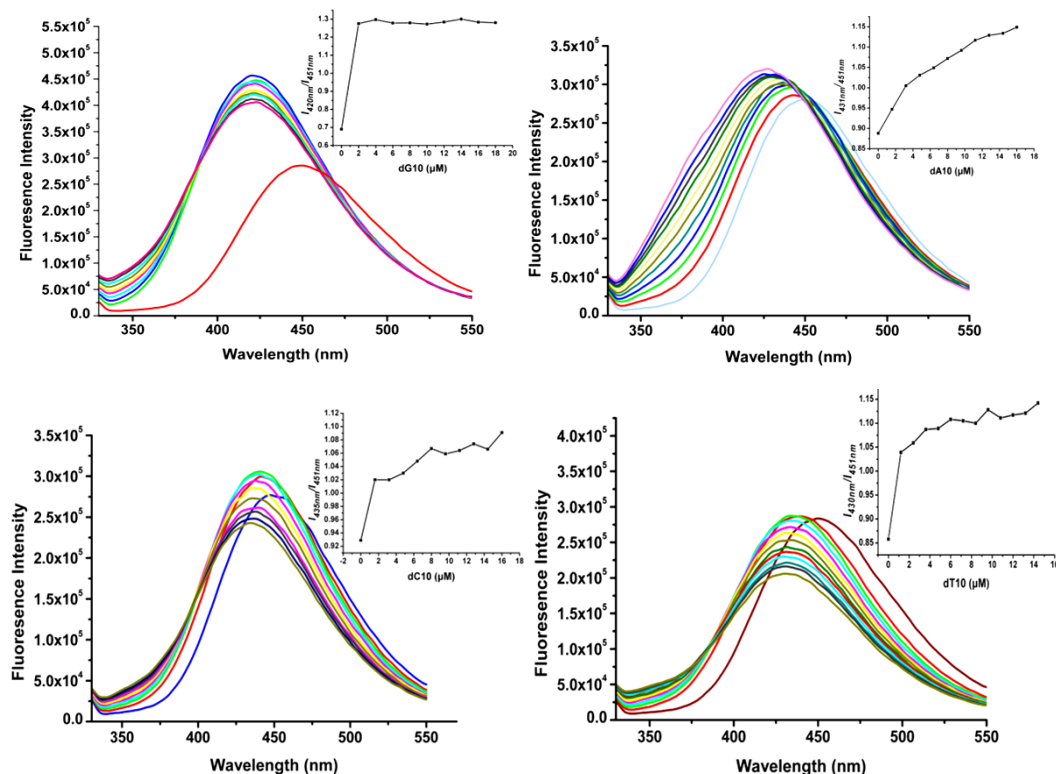


Fig. S16. Fluorescent emission spectra of **R-1** (4 μ M) in the presence of different concentrations of ssDNA(0-30 μ M) in HEPES buffered (pH 7.4, 10 mM) water. Excitation wavelength was 291 nm, and emission was at 451 and 419 nm. Inset: Ratiometric calibration curve as a function of ssDNA concentrations. d(A)10: 5'-AAAAAAAAAA-3'; d(T)10: 5'-TTTTTTTTTT-3'; d(C)10: 5'-CCCCCCCCC-3'; d(G)10: 5'-GGGGGGGGG-3';

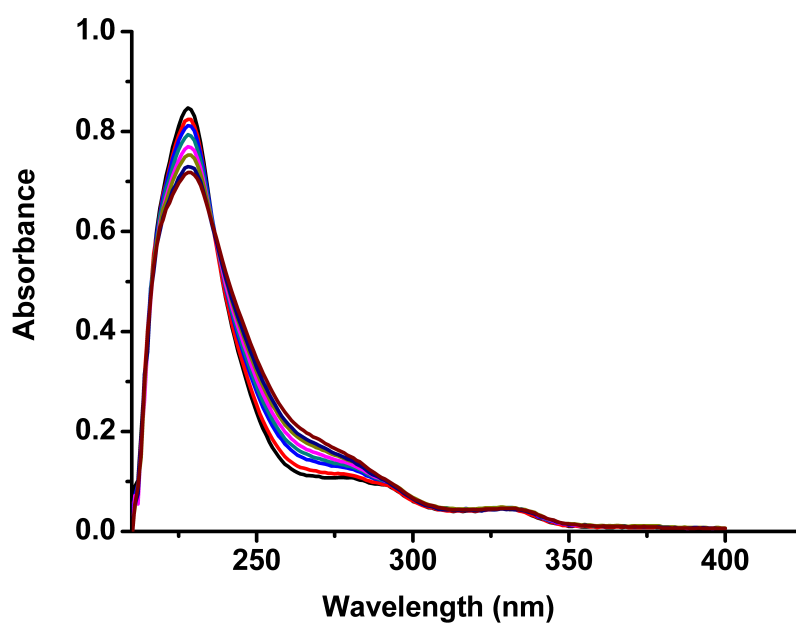


Fig. S17. Absorption spectra of **R-1** (10 μM) with increasing concentrations of CT-ssDNA (0-20 μM) in HEPES buffer (pH 7.4, 10 mM).

Experimental Section

Chemicals and materials

(*R*)- and (*S*)-BINOL were purchased from aladdin (Shanghai, China). 1,4,7,10-tetraazacyclododecane were purchased from GuideChem. The ONDs (in a PAGE purified), ATP, GTP, CTP, TTP, PPi and HEPES were purchased from Sangon Biotech (Shanghai), Co.,Ltd. Plasmid DNA (pUC 19) were purchased from Takara Biotechnology Company. All reagents and chemicals KF, KBr, KCl, KI, NaH₂PO₄, Na₂HPO₄, Na₃PO₄, NaOAc, NaNO₃, Na₂SO₃, Na₂CO₃, NaHCO₃ and KHSO₄ were of analytical grade and used without further purifications. CT-DNA (stored at 4 °C and used for not more than 4 days) which was purchased from Sigma Aldrich was directly dissolved in water at a concentration of 1 mg/mL. Its concentration was determined according to absorption intensity at 260 nm with a molar extinction coefficient value of 6600 M⁻¹cm⁻¹. Water used in all experiments was purified by a milli-Q system.

Instrumentation

High Resolution Mass Spectrometer (HRMS) and MALDI-TOF-MS spectra data were respectively recorded on a Bruker Daltonics Bio TOF mass spectrometer and a Voyager-DE PRO mass spectrometer. The ¹H NMR spectra measured on a Bruker AV II spectrometer and the δ scale in ppm referenced to residual solvent peaks or internal tetramethylsilane (400 MHz for ¹H and 100 MHz for ¹³C). Absorption spectra performed on a Hitachi U1900 at 298K between 215-400 nm. Fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer with a 1-cm pathlength cuvette at 298 K. Electrophoresis were analyzed by 1% agarose gel containing 1.0 g/ml ethidium bromide at 40 V for 30 min in TAE buffer. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a gel documentation system.

Determination of Equilibrium Constants

The equilibrium binding constant K_b can be determined from a double-reciprocal plot of the change in the apparent extinction coefficient of the ligand vs. DNA concentration. The equation representing this relationship is

$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b (\epsilon_b - \epsilon_f)$, where ϵ_a , ϵ_f and ϵ_b are the apparent, free, and bound ligand extinctions, respectively. Fit the plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$, the K_b was obtained

from the ratio of the slope to the Y intercept.

Stern-Volmer equation

The relative binding propensity of the compound to CT-DNA was established by the classical Stern–Volmer equation: $F_0/F = 1 + K_{sv}[Q]$, where F_0 and F are the fluorescence intensities in the absence and presence of the complex, respectively, K_{sv} is a linear Stern-Volmer quenching constant, $[Q]$ is the concentration of complex.

Synthesis of S-2. A solution of **S-3** (0.76, 2.0 mmol) and **C-1** (1.47 g, 2.5 mmol) in anhydrous acetonitrile (60 mL) was heated at 80 °C for 72 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel eluting with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (20:1, v/v) to afford **S-2** (0.87 g, 48.8 %) as a pale yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 10.63 (s, 1 H), 8.43 (s, 1 H), 8.02 (s, 2 H), δ 7.86-7.82 (m, 2 H), δ 7.75 (s, 1 H), δ 7.44 (s, 2 H), δ 7.31-7.26 (m, 3 H), δ 7.14 (s, 1 H), δ 7.07 (s, 1 H), δ 4.72 (m, 2 H), δ 3.79 (t, 3 H, $J = 4.0$ Hz), δ 3.49-3.19 (br, 14 H), δ 2.76-2.65 (m, 5 H), δ 2.21 (m, 2 H), δ 1.42-1.22 (m, 29 H); ^{13}C NMR (100 MHz, CDCl_3) δ 154.9, 148.1, 133.6, 130.91, 129.0, 128.2, 127.3, 127.0, 125.6, 125.5, 124.7, 124.0, 123.7, 117.8, 113.3, 61.3, 56.5, 48.9, 28.7, 28.5; HRMS: (ESI) m/z calcd for $\text{C}_{51}\text{H}_{69}\text{N}_6\text{O}_8$ $[\text{M} - \text{Br}]^+$ 893.5171, found 893.5172.

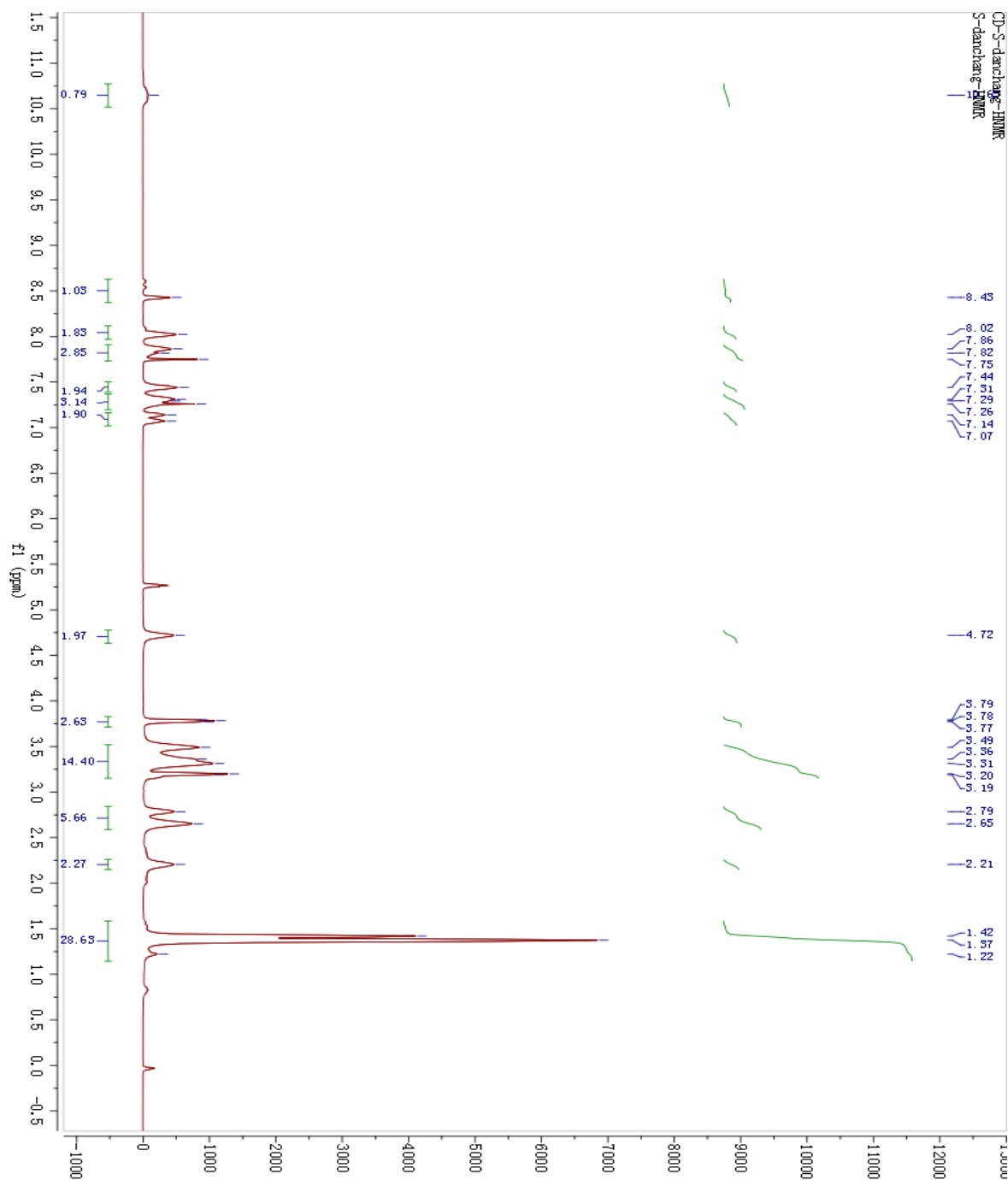
Synthesis of S-1. An excess amount of saturated HCl-methanol solution was added to **R-2** (0.87 g, 1 mmol). The mixture was stirred for 4 hours and monitored by TLC. After completion of the reaction, the solvent was removed under vacuum. Then the solid was dissolved in deionized water (5 mL) and pH was adjusted to alkalinity by saturated aqueous NaHCO_3 . The crude product was extracted with hot CHCl_3 (3×100 mL) and the organic layer was dried with anhydrous Na_2SO_4 . The residue was purified by flash column chromatography on aluminum oxide eluting with $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ (10:1, v/v) to afford **S-1** (0.42 g, 70.8 %) as a yellow solid. ^1H NMR (400 MHz, CDCl_3) δ 10.71 (s, 1 H), δ 8.41 (s, 1 H), δ 8.22 (s, 1 H), δ 8.00 (t, 2H, $J = 1.2$ Hz), δ 7.86 (d, 1 H, $J = 8$ Hz), δ 7.67 (s, 1 H), δ 7.42-7.25 (m, 5 H), δ 7.13 (dd, 2 H, $J_1 = 8$ Hz, $J_2 = 24$ Hz), δ 4.72 (t, 2 H $J = 8$ Hz), δ 3.77 (s, 3 H), δ 3.18 (s, 3 H), δ 2.75-2.61 (br, 18 H), δ 2.28-2.25 (m, 2 H), δ 1.22 (m, 2 H); ^{13}C NMR (100 MHz, D_2O) δ 154.9, 148.1, 133.6, 130.8, 130.0, 128.9, 128.3, 128.2, 127.2, 126.5, 125.5, 124.7, 117.8, 113.2, 61.1, 56.5, 52.4, 51.5, 48.8, 47.6, 45.8, 45.7, 29.1; HRMS: (ESI) m/z calcd for $\text{C}_{36}\text{H}_{45}\text{N}_6\text{O}_2$ $[\text{M} -$

Br]⁺ 593.3599, found 593.3568.

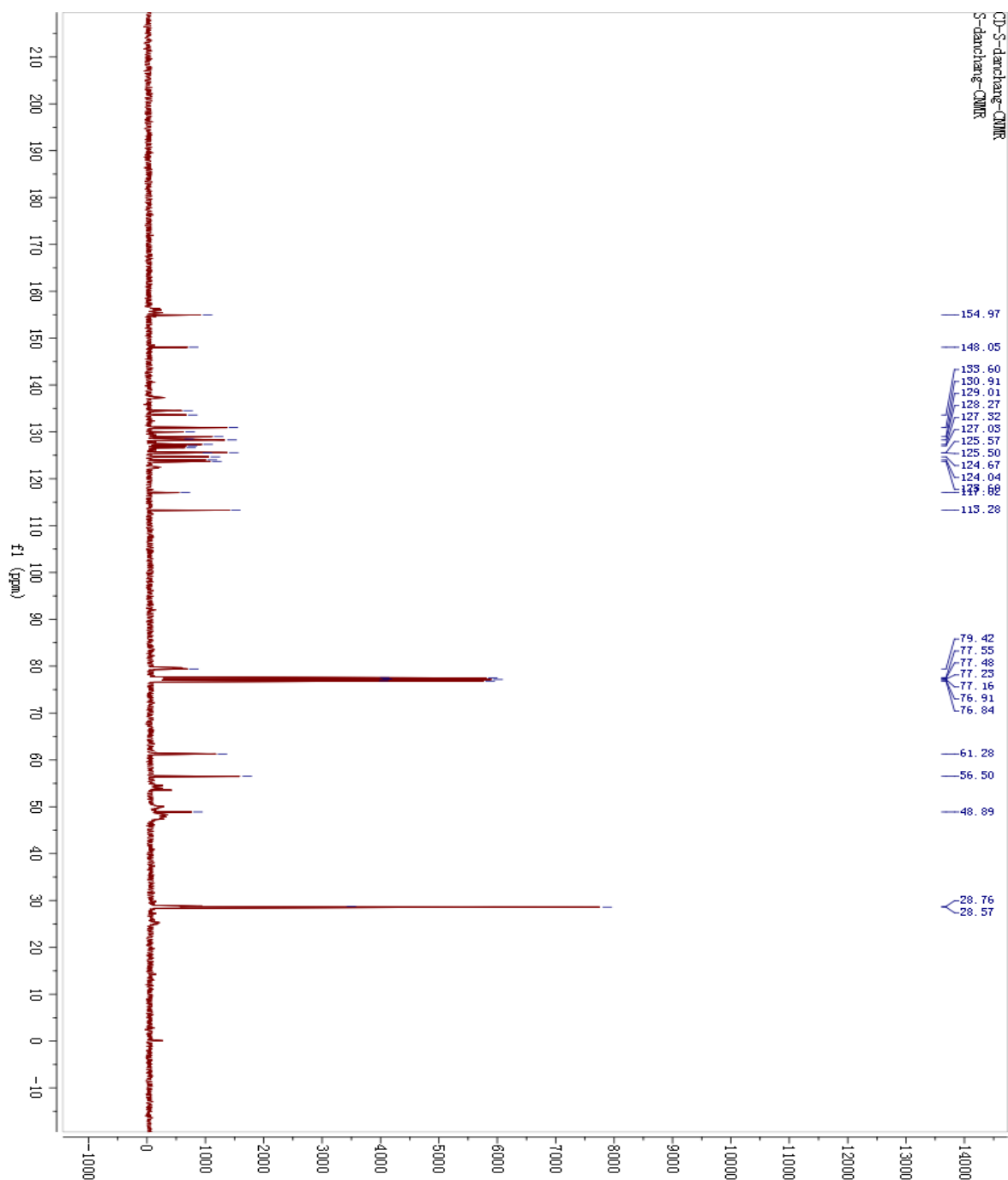
Synthesis of R-g2. A solution of **R-3** (0.5 g, 1.3 mmol) and **C-2** (0.93 g, 1.9 mmol) in anhydrous acetonitrile (60 mL) was heated at 80 °C for 72 h. After cooling to room temperature, the solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography on silica gel eluting with CH₂Cl₂/CH₃OH (15:1, v/v) to afford **R-2** (0.4 g, 34.5 %) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 10.32 (s, 1 H), δ 8.25 (s, 1 H), δ 8.04 (d, 1H, *J*₁ = 8.0 Hz), δ 7.98 (d, 1H, *J*₁ = 8.0 Hz), δ 7.88-7.84 (m, 2 H), δ 7.67 (s, 1 H), δ 7.45 (d, 2 H, *J* = 8.0 Hz), δ 7.33-7.26 (m, 3 H), δ 7.16 (d, 1 H, *J* = 8.0 Hz), δ 7.08 (d, 1 H, *J* = 8.0 Hz), δ 5.95 (s, 2 H), δ 3.79-3.21 (br, 22 H), δ 1.46-1.43 (m, 27H); ¹³C NMR (100 MHz, CDCl₃) δ 165.1, 157.5, 154.9, 148.1, 130.8, 129.8, 128.8, 128.2, 127.3, 125.6, 125.1, 124.7, 124.2, 124.0, 122.4, 116.9, 113.2, 80.8, 80.3, 61.3, 56.4, 51.1, 50.9, 49.9, 49.8, 28.6; HRMS: (ESI) *m/z* calcd for C₅₀H₆₅N₆O₉ [M - Br]⁺ 893.4808, found 893.4806.

Synthesis of R-g1. Saturated HCl-methanol solution was added to **R-2** (0.4 , 0.45 mol) and monitored by TLC. After stirring for 6 h, the solvent was removed under vacuum to afford a pale yellow solid. Then the solid was dissolved in deionized water (5 mL) and pH was adjusted to alkalinity by saturated aqueous NaHCO₃. The crude product was extracted with hot CHCl₃ (3×100 mL) and the organic layer was dried with anhydrous Na₂SO₄. The residue was purified by flash column chromatography on aluminum oxide eluting with CH₂Cl₂/CH₃OH (10:1, v/v) to afford **R-g1** (0.23 g, 86 %) as a yellow solid. ¹H NMR (400 MHz, CDCl₃) δ 10.28 (s, 1H), δ 8.19 (s, 1H), δ 8.02 (t, 2H, *J* = 8.0 Hz), δ 7.96 (d, 1H, *J* = 12.0 Hz), δ 7.87 (d, 1H, *J* = 8.0 Hz), δ 7.63 (s, 1H), δ 7.47-7.42 (m, 2H), δ 7.34-7.25 (m, 3H), δ 7.16 (d, 1H, *J* = 8.0 Hz), δ 7.07 (d, 1H, *J* = 8.0 Hz), δ 6.19 (m, 2H), δ 3.78 (s, 6H), δ 3.21 (s, 4H), δ 3.00-2.65 (s, 12 H); ¹³C NMR (100 MHz, D₂O) δ 166.7, 154.9, 148.2, 130.8, 129.8, 129, 128.7, 128.4, 128.2, 127.3, 126.6, 125.7, 124.6, 116.9, 113.2, 61.2, 56.4 51.5, 49.8, 49.1, 48.9, 47.1, 46.8, 45.6, 45.1; HRMS: (ESI) *m/z* calcd for C₃₅H₄₁N₆O₃ [M - Br]⁺ 593.3235, found 593.3239.

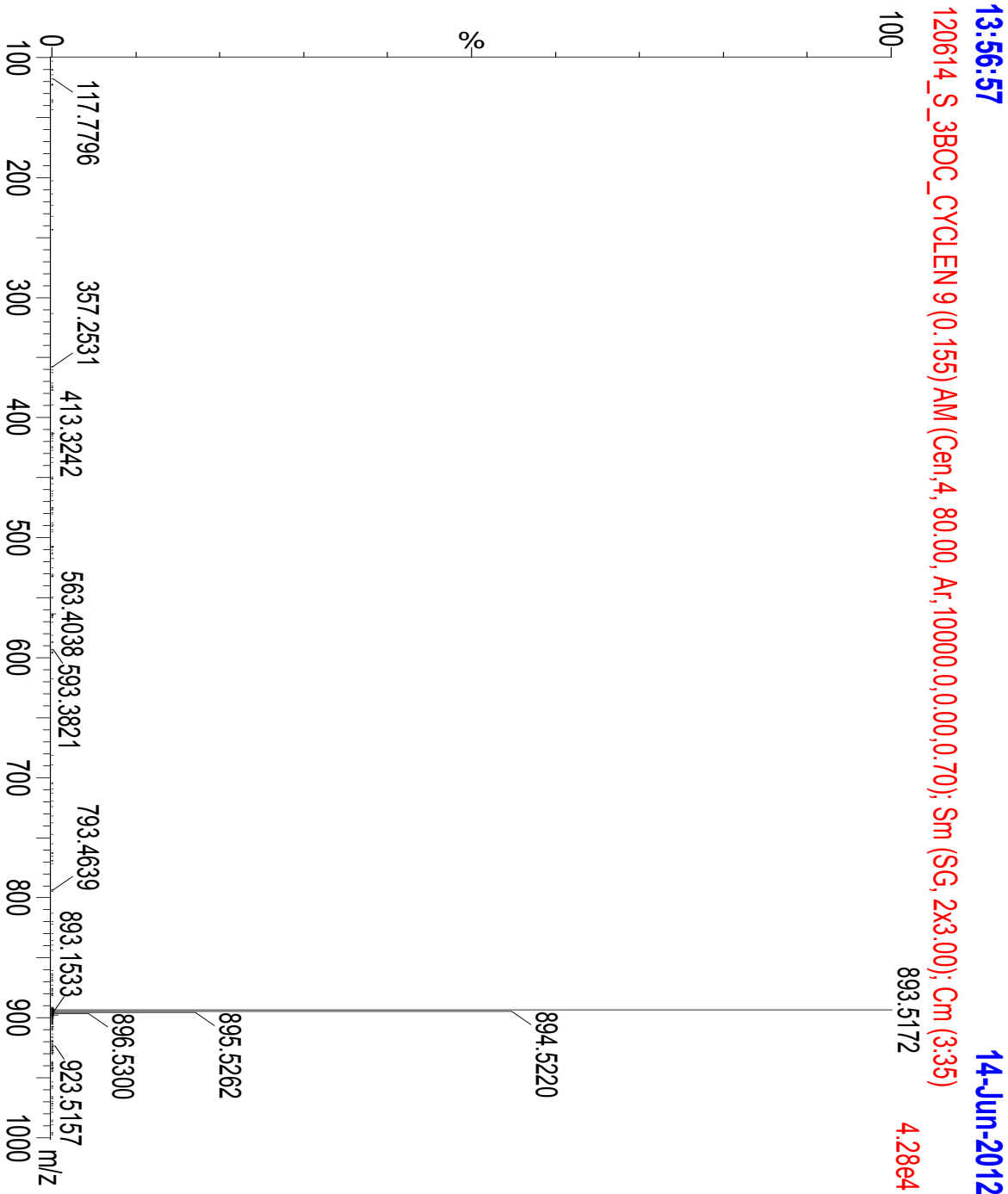
¹H NMR spectrum of compound S-2 in CDCl₃ (400 MHz).



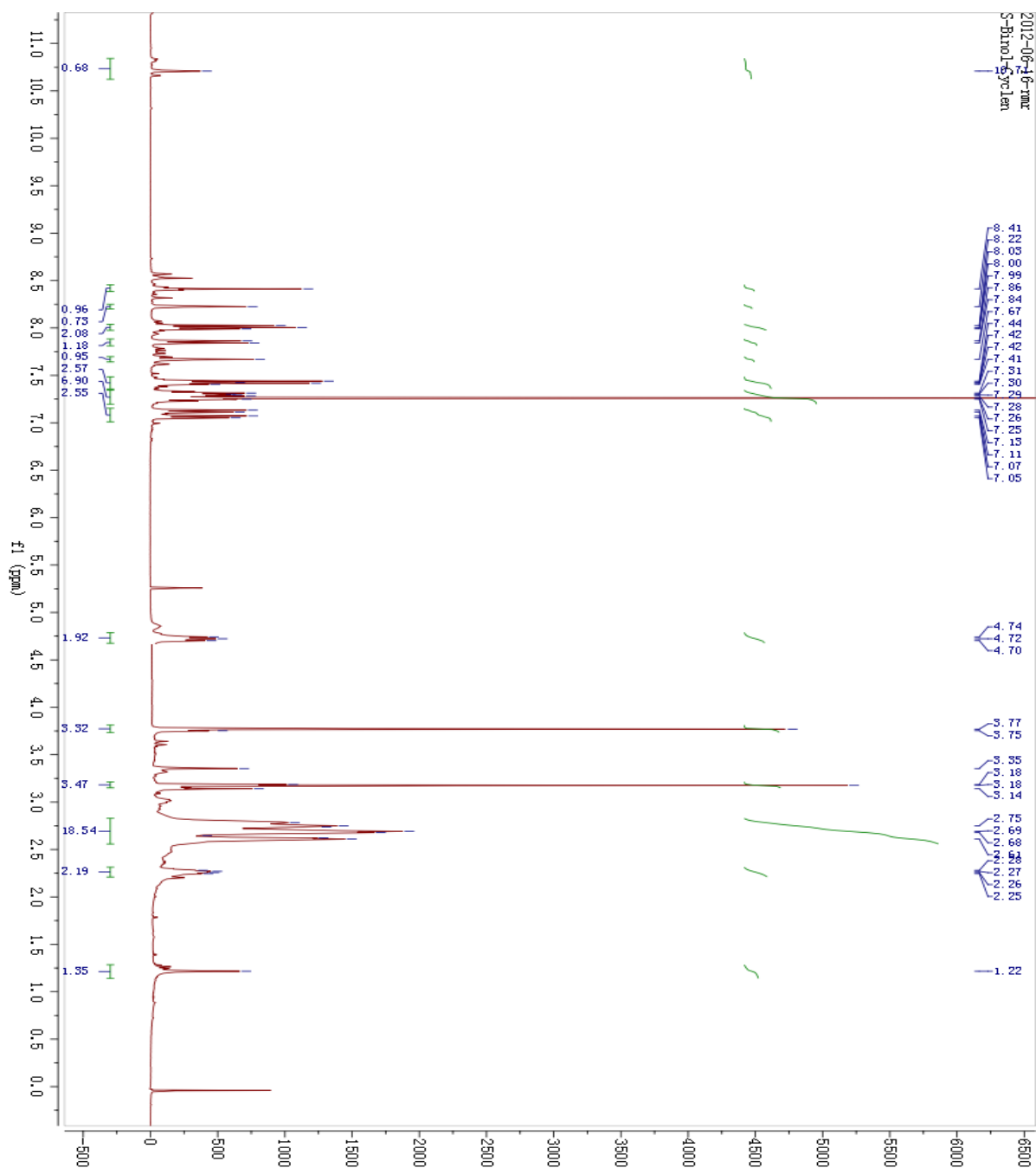
¹³C NMR spectrum of compound S-2 in CDCl₃ (100 MHz).



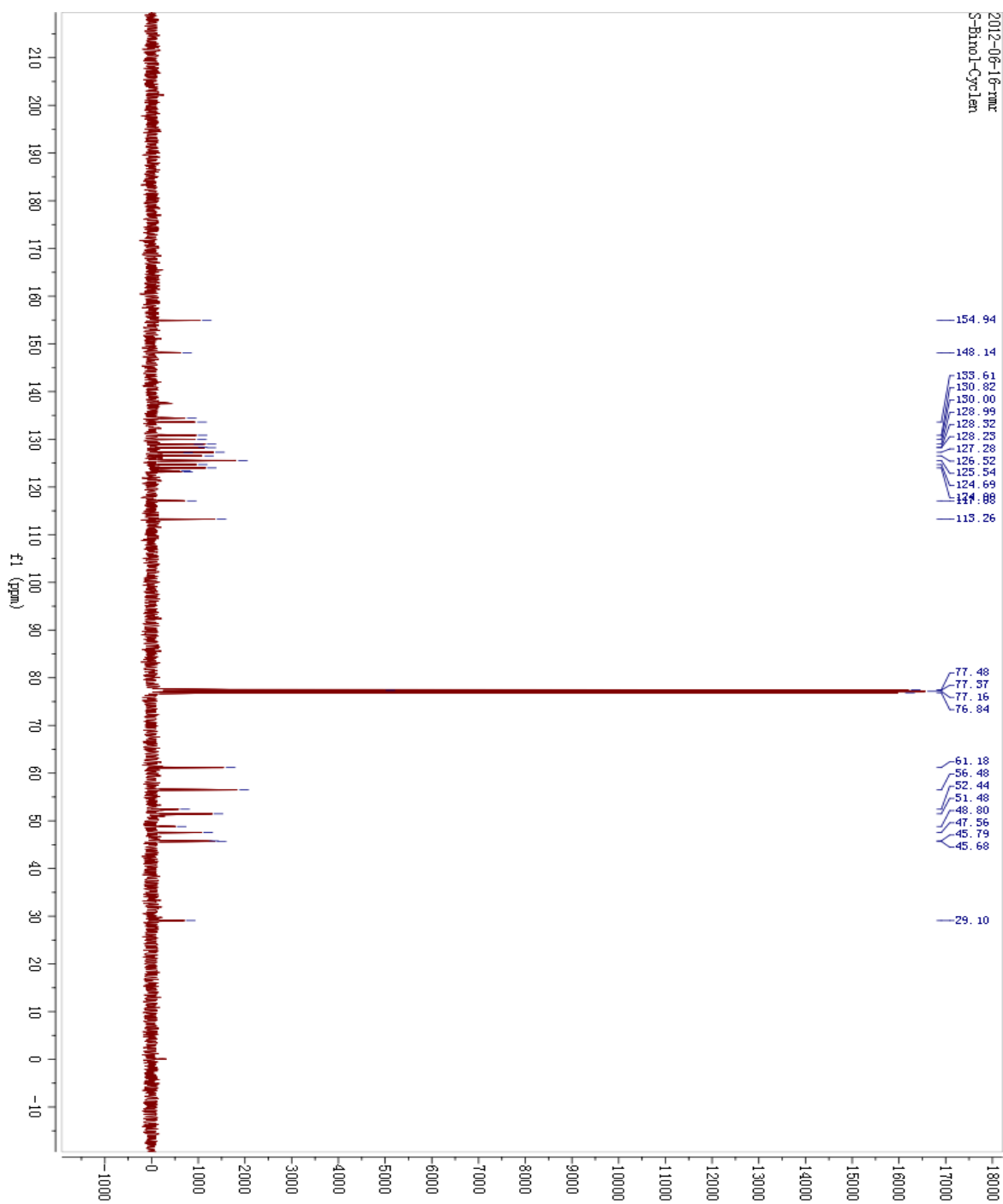
HRMS spectrum of compound S-2.



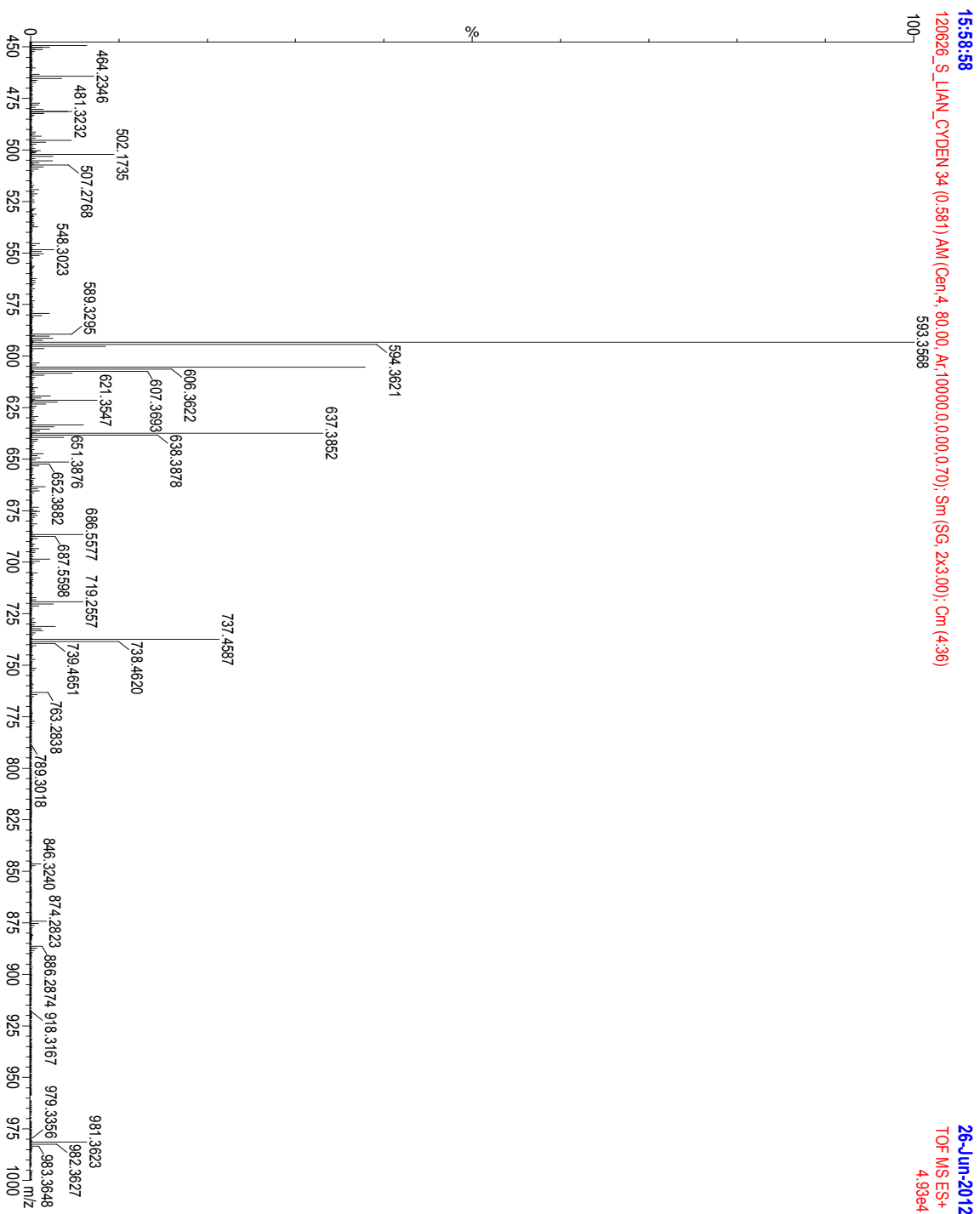
¹H NMR spectrum of compound S-1 in CDCl₃ (400 MHz).



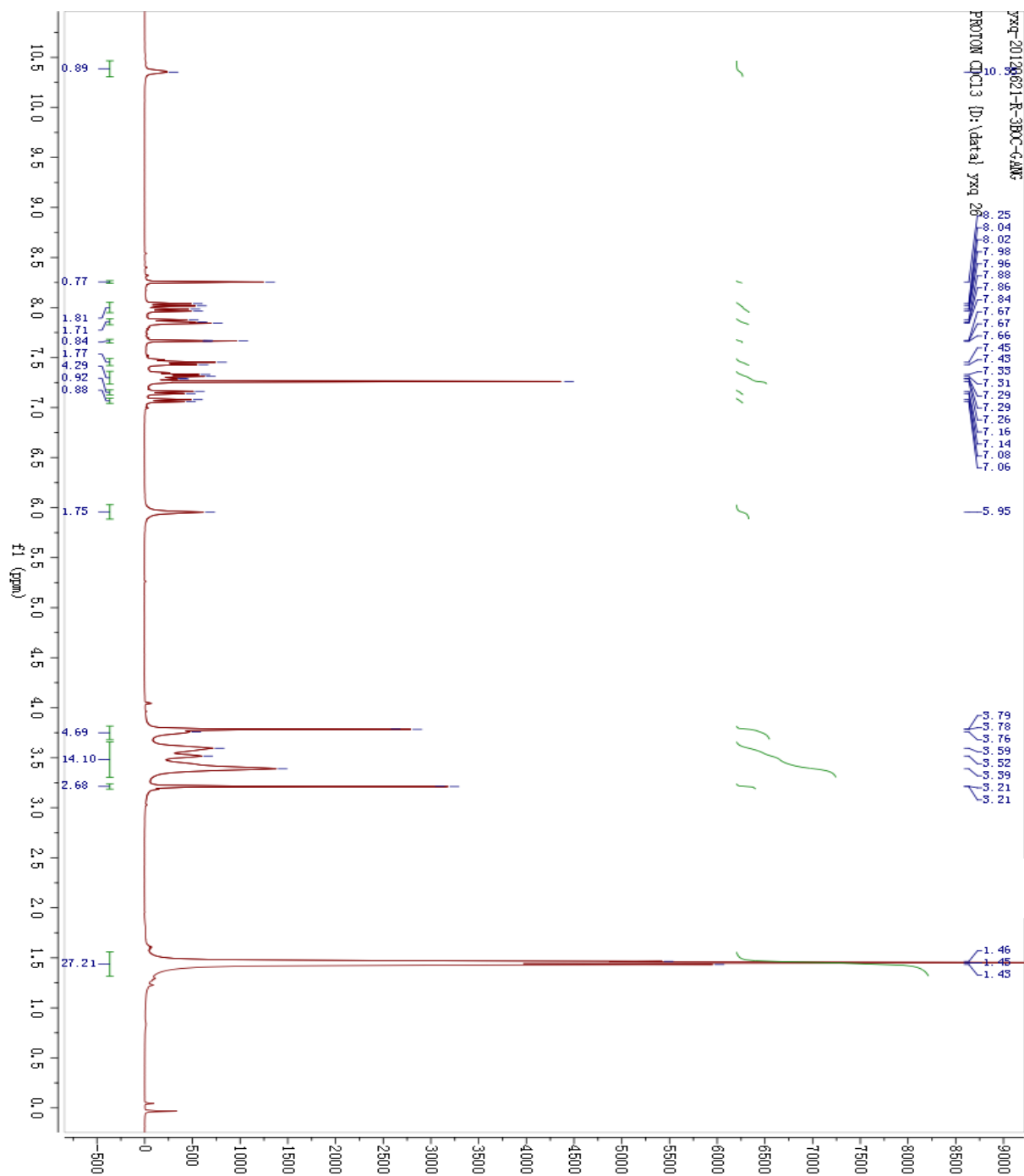
¹³C NMR spectrum of compound S-1 in CDCl₃ (100 MHz).



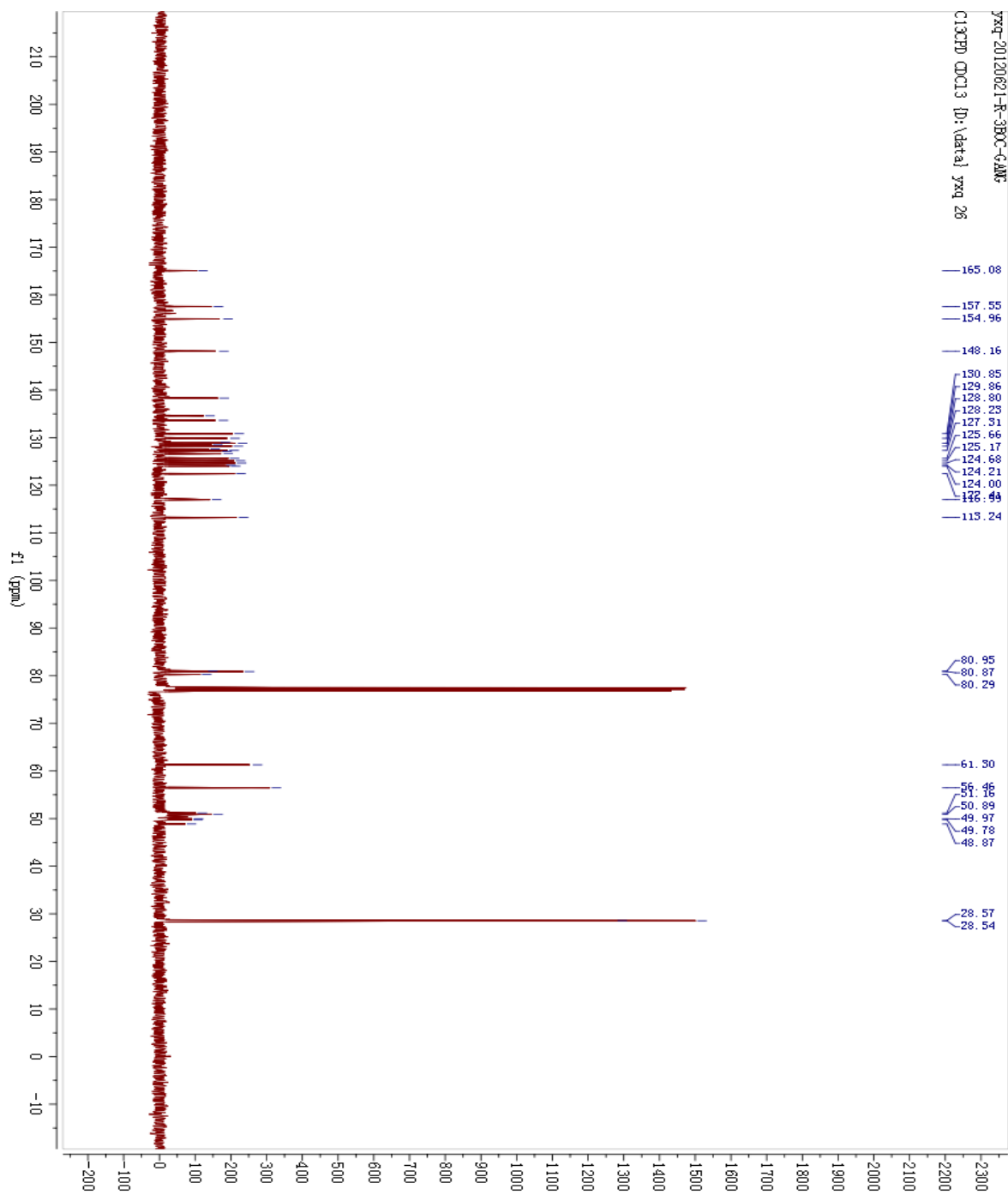
HRMS spectrum of compound S-1.



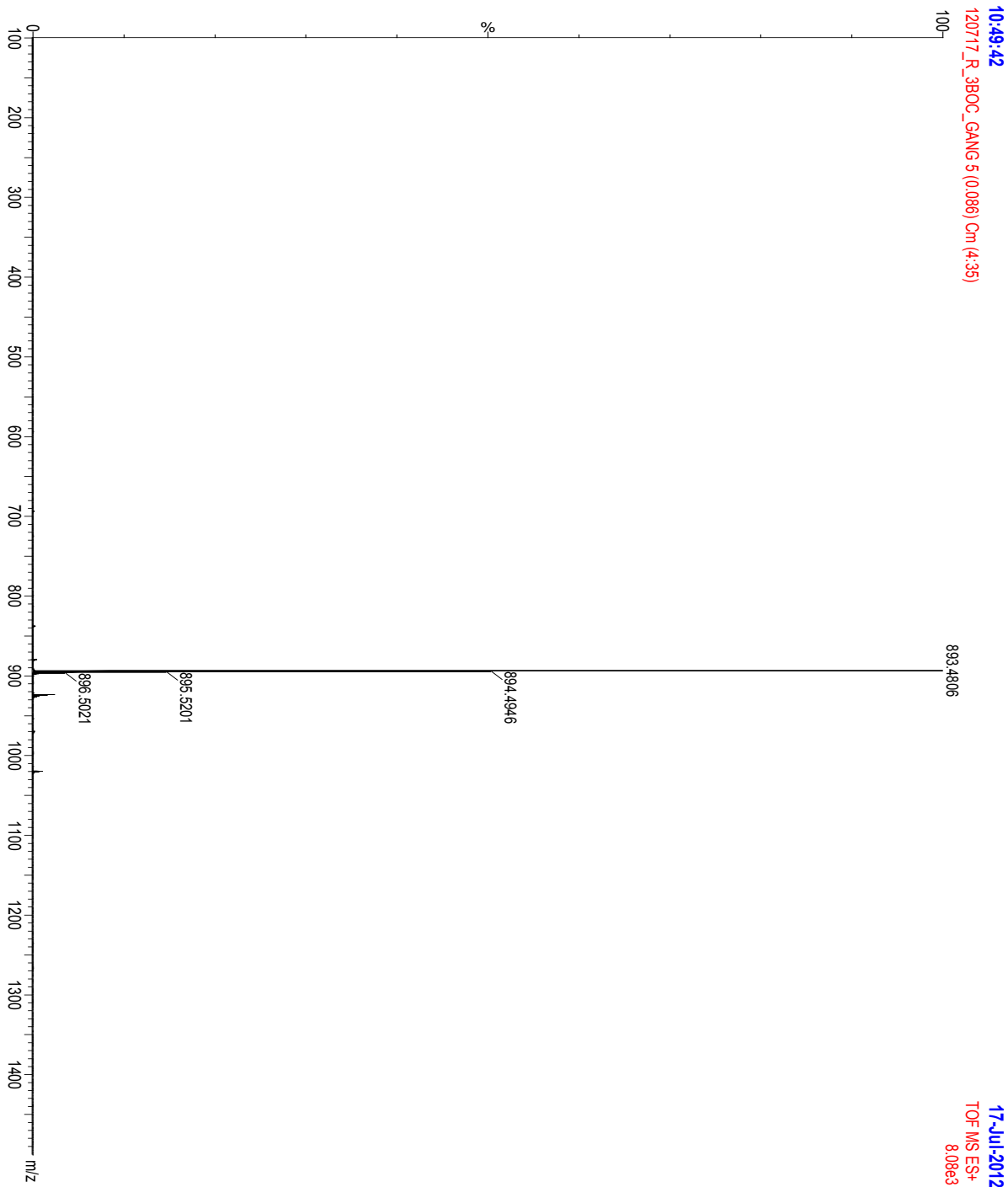
¹H NMR spectrum of compound R-g2 in CDCl₃ (400 MHz).



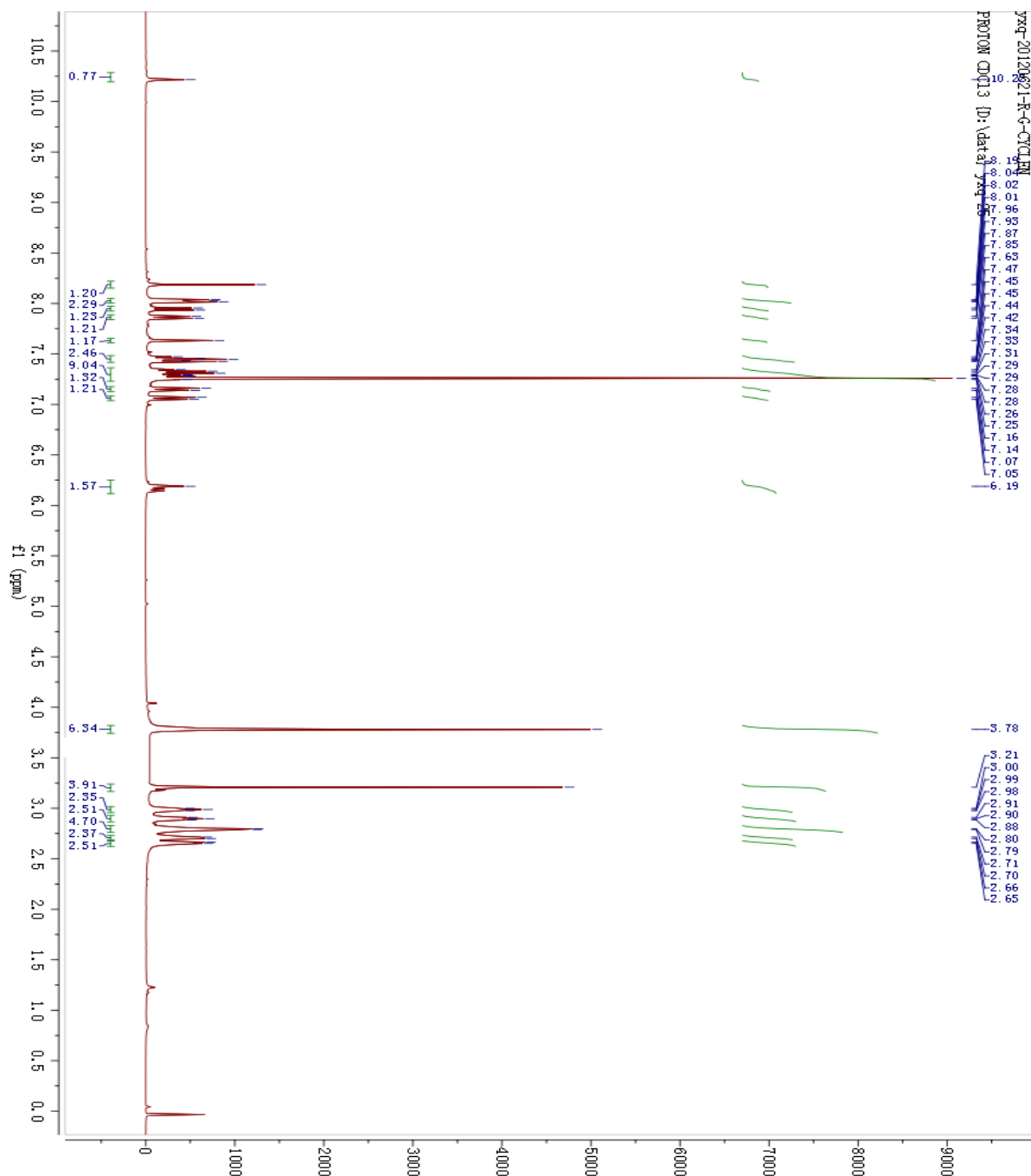
¹³C NMR spectrum of compound R-g2 in CDCl₃ (100 MHz).



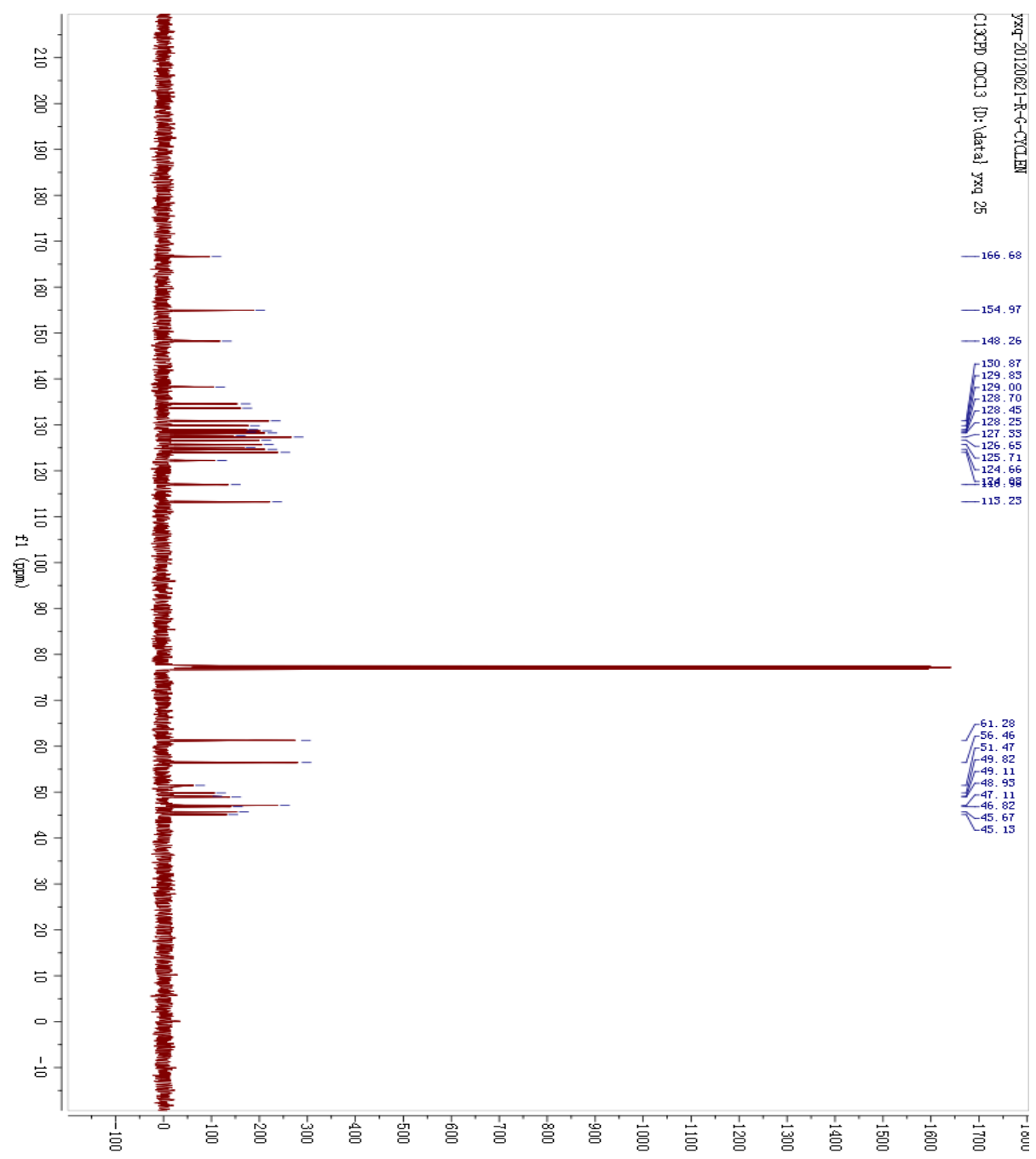
HRMS spectrum of compound R-g2.



¹H NMR spectrum of compound R-g1 in CDCl₃ (400 MHz).



¹³C NMR spectrum of compound R-g1 in CDCl₃ (100 MHz).



HRMS spectrum of compound R-g1.

