Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information

Mono-Acylation of a Polyamine-β-Cyclodextrin Based on Guest Mediated Acyl Migration

Rodolfo F. Gómez-Biagi, Mark Nitz*

Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, ON, Canada, M5S 3H6

Contents:

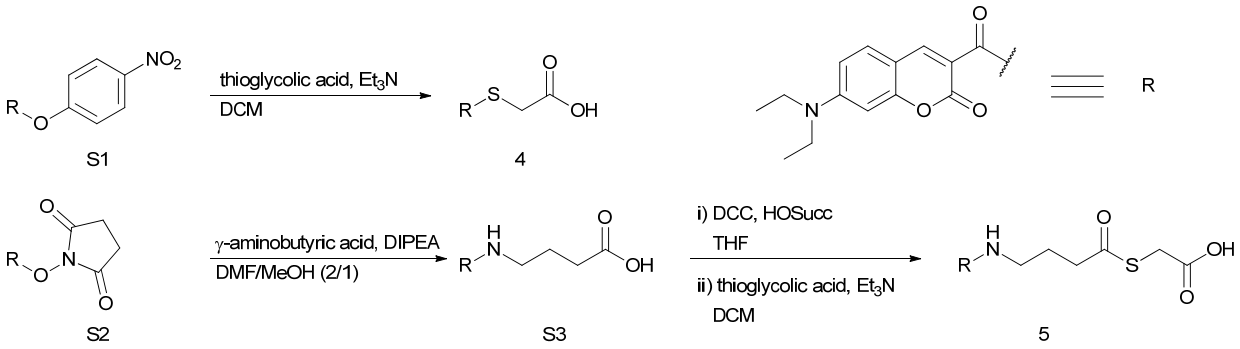
General Remarks

Unfractionated heparin was purchased from Celsus laboratories isolated from pig intestinal mucosa and was obtained as the sodium salt. Egg white lyzozyme was purchased from BioShop Canada Inc. All other chemicals were purchased from Sigma Aldrich. Solvents were purchased from Acros chemicals. All reactions were performed under a nitrogen-atmosphere and at room temperature, unless stated otherwise. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400, 500 or Bruker 400 MHz spectrometers at T = 297 K. Absorption spectra were measured on a Schimadzu UV-2401PC spectrophotometer at T = 296 K. Fluorescence studies were performed using a Perkin-Elmer LS-50BLuminescence Spectrophotometer. HPLC separations and analysis were performed on a Waters 1525 Binary HPLC pump and 2487 dual λ absorption detector using a Waters XBridge™ Prep BEH130 C18 5 μm (10 × 250 mm) reverse phase analytical column. Silica chromatography was performed with SiliCycle Silica-P Flash Silica Gel. High resolution mass spectra were obtained from an ABI/Sciex QStar mass spectrometer with an ESI source. The MALDI spectra were taken in a Waters® Micromass® MALDI micro MX™ (matrix-assisted laser desorption/ionization time-of-flight mass spectrometer [MALDI-ToF MS]).

Synthesis

Thioester guests 4 and 5

Compounds S1 and S2 were prepared according to a literature procedure.[1, 2]



S. Scheme 1: Synthesis of **4** and **5**

2-((7-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)thio)acetic acid (4). S1 (0.2 g, 0.52 mmol) was added to a solution of thioglycolic acid (0.054 mL, 0.78 mmol) and triethylamine (0.22 mL, 1.6 mmol) in DCM (15 mL). The reaction was stirred for approximately 16 h. After removal of the solvent under diminished pressure, the residue was taken up in a minimum amount of methanol (3 mL) and diluted with water (25 mL). The solution was cooled to 0°C in an ice bath, and then acidified using 1M HCl. The solution was allowed to stand for 1h at 0°C, the resulting solid was collected by filteration, and washed with water. The product was obtained as a dark yellow powder (TLC MeOH/DCM; 1/10, $R_f = 0.4$). Yield = 85% (0.146 g, 0.43 mmol). ^{1}H-NMR δ_H (400 MHz, d6-DMSO) 8.55 (s, J = 7.4 Hz, 1H), 7.70 (d, J= 9.1 Hz, 1H), 6.83 (dd, J= 9.1 Hz, 2.4 Hz, 1H), 6.61 (d, J= 2.2 Hz, 1H), 3.73 (s, 2H), 3.52 (q, J= 7.1 Hz, 4H), 1.16 (t, J =7.0, 6H). ^{13}C-NMR δ_C (100 MHz, d6-DMSO) 186.52, 170.89, 159.97, 158.70, 154.17, 147.54, 133.54, 112.81, 111.25, 108.18, 96.62, 45.23, 32.29, 13.03. HRMS-ESI m/z calcd for $C_{16}H_{18}NO_5S$ $[M+H]^+$ 336.0900, found 336.0910.

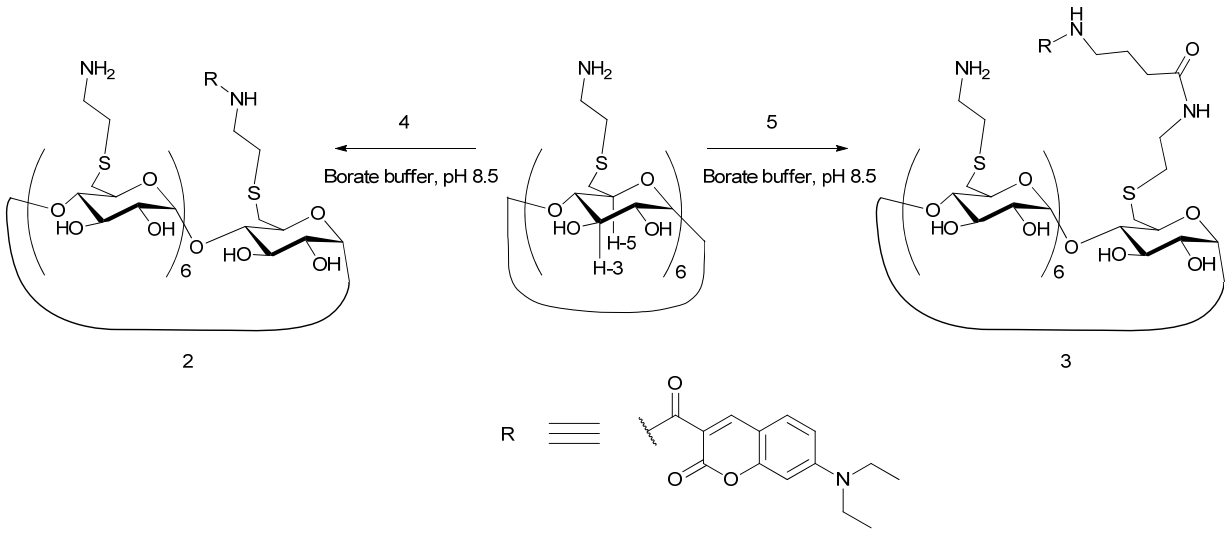
4-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)butanoic acid (S3). S2 (0.9 g, 2.5 mmol) was added to a solution of γ-aminobutyric acid (0.38 g, 3.68 mmol) and DIPEA (1.73 mL, 9.9 mmol) in DMF/MeOH (15 mL/7.5mL). The reaction was stirred for approximately 16 h. After removal of the solvent under diminished pressure, the residue was brought up in DCM, sonicated to dissolve, and washed with 1M HCl. The organic layer was dried over $MgSO_4$, filtered, and evaporated. The residue was brought up in a minimum amount of DCM and precipitated with pentane. The yellow precipitate was collected by filtration and washed with diethyl ether (TLC conditions: DCM/MeOH; 9/1, $R_f = 0.5$). Yield = 69% (0.59 g, 1.7 mmol). ^{1}H-NMR δ_H (400 MHz, d6-DMSO) 12.04 (br s, 1H), 8.71 – 8.56 (m, 2H), 7.67 – 7.56 (m, 1H), 6.76 (dd, *J*= 9.0 Hz, 2.4 Hz, 1H), 6.57 (d, *J*= 2.2 Hz, 1H), 3.44 (q, *J*= 7.04 Hz, 4H), 3.37 – 3.21 (m, 2H), 2.24 (t, *J* = 7.4 Hz, 2H), 1.72 (p, *J* = 7.2 Hz, 2H), 1.11 (t, *J* = 7.0 Hz, 6H). ^{13}C-NMR (100 MHz, d6-DMSO) δ 174.77, 162.91, 162.37, 157.85, 153.03, 148.27, 132.19, 110.74, 110.17, 108.32, 96.51, 44.99, 38.96, 31.85, 25.42, 12.98. HRMS-ESI m/z calcd for $C_{18}H_{23}N_2O_5$ $[M+H]^+$ 347.1601, found 347.1601.

2-((4-(7-(diethylamino)-2-oxo-2H-chromene-3-carboxamido)butanoyl)thio)acetic acid (5).(i) **S3** (0.57 g, 1.6 mmol) and *N*-hydroxysuccinimide (0.38 g, 3.3 mmol) were dissolved in a minimum amount of THF(15 mL). The solution was cooled in an ice bath. DCC (0.37 g, 1.8 mmol) was dissolved separately in 1 mL THF and subsequently added to the aforementioned solution. The reaction was allowed to warm to room temperature overnight. The reaction was filtered through a plug of silica, washed with EtOAc, and the filtrate was evaporated under diminished pressure. The residue was disolved in DCM, washed with a sodium bicarbonate solution (1 M), brine, dried over $MgSO_4$, filtered, and evaporated. The residue was brought up in a minimum amount of DCM, precipitated using pentane, and a canary yellow powder was collected by filtration (TLC conditions: EtOAc, $R_f = 0.4$). Yield = 69% (0.49 g, 1.1 mmol). This compound was used without further purification in the next step (ii). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.89 (t, *J* = 5.9 Hz, 1H), 8.68 (s, 1H), 7.41 (d, *J* = 9.0 Hz, 1H), 6.63 (dd, *J* = 9.0 Hz, 2.5 Hz, 1H), 6.48 (d, *J* = 2.3 Hz, 1H), 3.54 (dd, *J* = 13.0 Hz, 6.8 Hz, 2H), 3.44 (q, *J* = 7.1 Hz, 4H), 2.83 (br s, 4H), 2.76 – 2.64 (m, 2H), 2.06 (dt, *J* = 17.8 Hz, 7.1 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.23, 168.44, 163.66, 162.96, 157.87, 152.78, 148.38, 131.36,

110.33, 110.17, 108.58, 96.80, 45.29, 38.61, 28.84, 25.80, 25.08, 12.64. HRMS-ESI m/z calcd for $C_{22}H_{26}N_3O_7$ $[M+H]^+$ 444.1765, found 444.1777. (ii) The succinimidyl ester of S3 (0.47g, 1.05 mmol) was dissolved in a solution of DCM (20 mL) and triethylamine (0.44 mL, 3.2 mmol). Thioglycolic acid (0.12 mL, 1.7 mmol) was added to the aforementioned solution. The reaction was stirred for approximately 16 h. The solvent was removed under diminished pressure. The residue was taken up in a minimum amount of methanol (3 mL) and diluted with water (25 mL). After cooling the solution to 0°C using an ice bath, the product was precipitated by acidification using 1 M HCl. The solution was allowed to stand for 1 h at 0°C, filtered, and the solid washed with water. The product was obtained as a canary yellow powder (TLC conditions: MeOH/DCM; 1/9, R_f = 0.2). Yield = 56% (0.23 g, 0.55 mmol). ^{1}H NMR (400 MHz, d6-DMSO) δ 12.89 – 12.65 (br s, 1H), 8.64 (t, *J* = 5.9 Hz, 1H), 8.61 (s, *J* = 16.3 Hz, 1H), 7.64 (d, *J* = 9.0 Hz, 1H), 6.77 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.58 (d, *J* = 2.2 Hz, 1H), 3.66 (s, 2H), 3.45 (q, *J* = 7.0 Hz, 4H), 3.30 (dd, *J* = 13.0, 6.7 Hz, 2H), 2.65 (t, *J* = 7.5 Hz, 2H), 1.79 (p, *J* = 7.2 Hz, 2H), 1.12 (q, *J* = 7.1 Hz, 6H). ^{13}C NMR (100 MHz, dmso) δ 198.06, 170.23, 163.00, 162.34, 157.87, 153.06, 148.31, 132.22, 110.76, 110.15, 108.32, 96.53, 45.00, 41.23, 38.71, 31.84, 25.84, 12.99. HRMS-ESI m/z calcd for $C_{20}H_{25}N_2O_6S$ $[M+H]^+$ 421.1427, found 421.1421.

Monoacylated cyclodextrins 2 and 3

Compound **1** was prepared according to a literature procedure.[3]



S. Scheme 2: Synthesis of 2 and 3

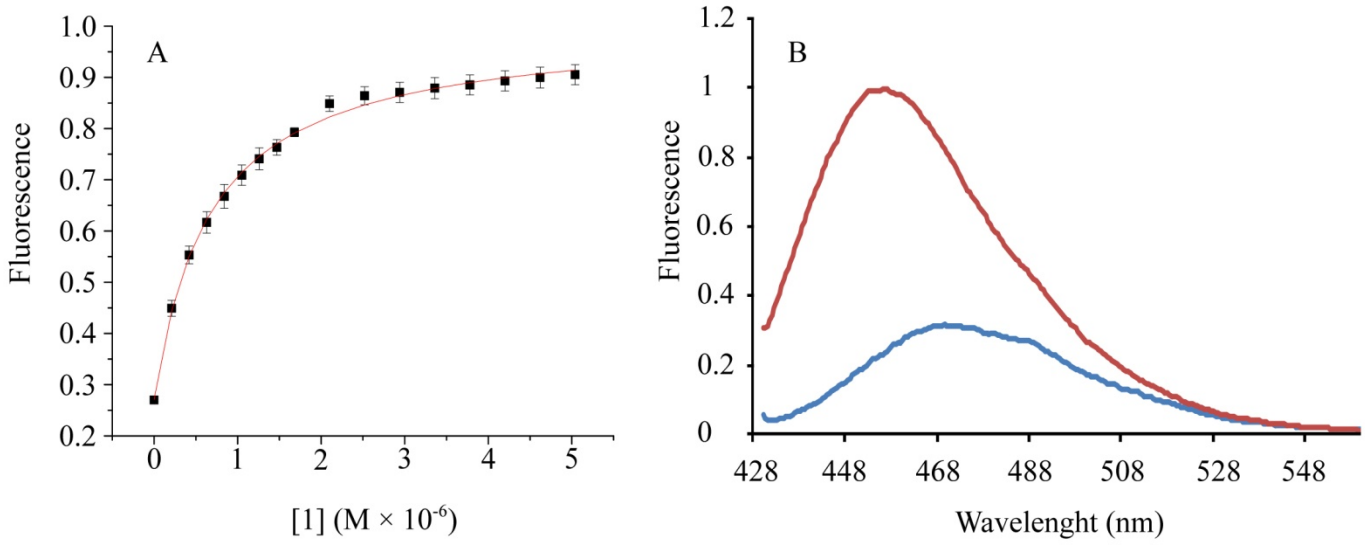
Mono-[6-deoxy-6-(7-(diethylamino)-N-(2-mercaptoethyl)-2-oxo-2H-chromene-3-carboxamide]-hexakis-[6-deoxy-6-(2-aminoethylsulfanyl)]-β-cyclodextrin (2).

1 (0.02 g, 12.8×10^{-3} mmol) and **4** (0.002 g, 6.4×10^{-3} mol) in 640 mL of borate buffer pH 8.5 (0.01 M) was stirred overnight. The solvent was removed under diminished pressure. The residue was taken up in a minimum amount of water and purified by RP-C_{18}-HPLC. The collected fractions were freeze dried. Yield (based on 1) = 45% (0.0051 g, 2.9×10^{-3} mmol). ^{1}H NMR (500 MHz, D_2O) δ 8.53 (s, 1H), 7.61 – 7.56 (m, 1H), 7.12 – 6.99 (m, 1H), 6.66 – 6.54 (m, 1H), 5.26 – 5.22 (m, 1H), 5.22 – 5.20 (m, 1H), 5.14 (m, 2H), 5.03 (s, 2H), 4.93 – 4.90 (m, 1H), 4.64 – 4.58 (m, 1H), 4.55 – 4.49 (m, 1H), 4.36 – 4.30 (m, 1H), 4.24 (s, 2H), 4.04 – 2.68 (m, 98H), 1.39 (m, 1H), 1.28 (m, 9H). HRMS-ESI m/z calcd for $C_{70}H_{120}N_8O_{31}S_7$ $[M+2H]^{2+}$ 896.3047, found 896.3046.

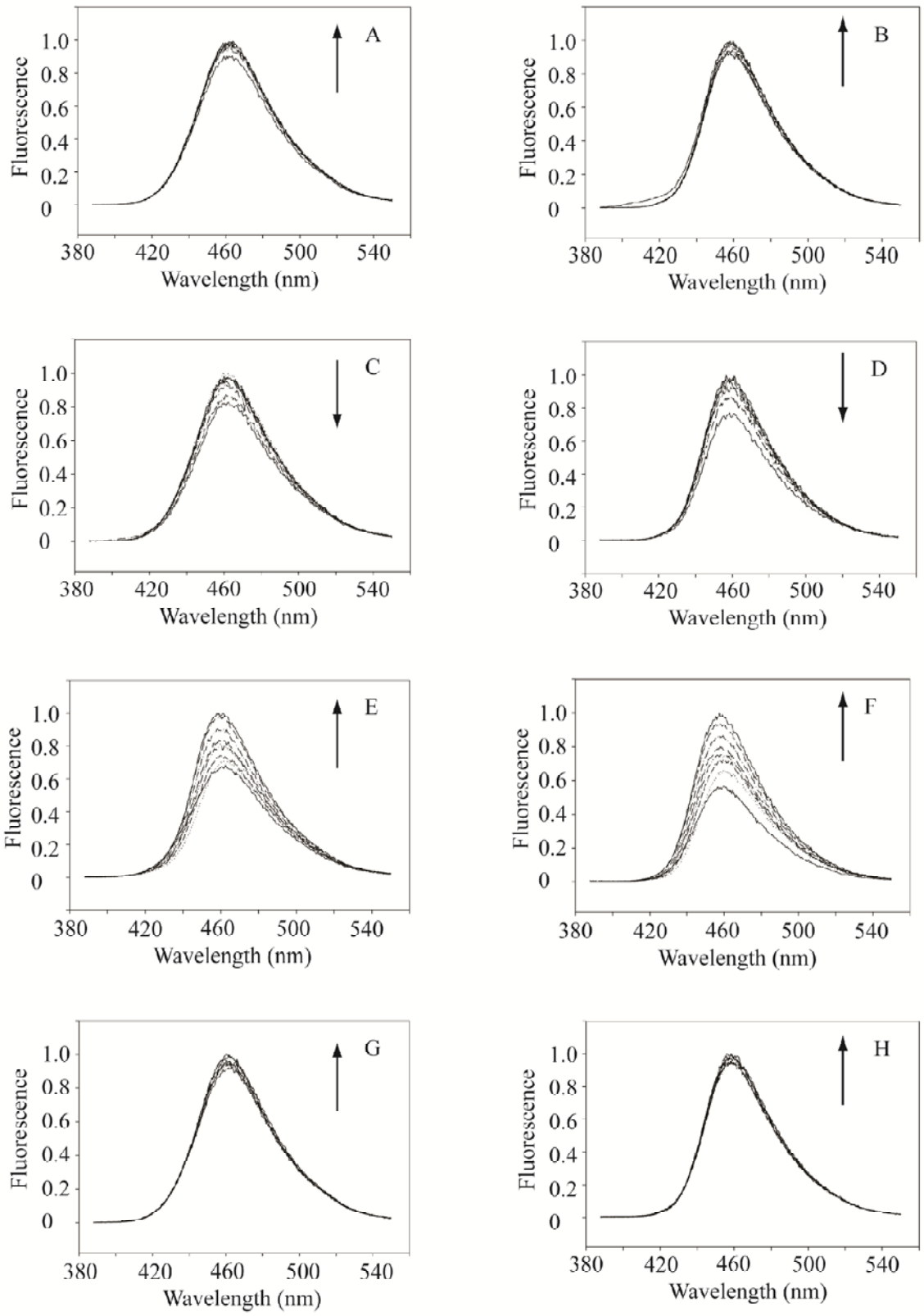
Mono-[6-deoxy-6-(7-(diethylamino)-N-(4-((2-mercaptoethyl)amino)-4-oxobutyl)-2-oxo-2H-chromene-3-carboxamide]-hexakis-[6-deoxy-6-(2-aminoethylsulfanyl)]-β-cyclodextrin (3). 1 (0.01 g, 6.4×10^{-3} mmol) and **5** (0.003g, 6.4×10^{-3} mmol) in 640 mL of borate buffer pH 8.5 (0.01 M) was stirred overnight. The solvent was removed under diminished pressure. The residue was taken up in a minimum amount of water and purified by RP-C_{18}-HPLC. The collected fractions were freeze dried. Yield (based on 1) = 78% (0.0095 g, 5.0×10^{-3} mmol). ^{1}H NMR (500 MHz, D_2O) δ 8.72 (s, 1H), 7.74 (d, J = 8.9 Hz, 1H), 6.74 (d, J = 8.7 Hz, 1H), 6.41 (s, 1H), 5.22 (s, 2H), 5.14 (s, 2H), 5.06 (s, 2H), 5.02 (s, 1H), 4.13 (t, J = 8.3 Hz, 1H), 4.08 (br s, 1H), 4.03 – 3.86 (m, 4H), 3.84 – 2.57 (m, 75H), 2.46 – 2.29 (m, 2H), 2.13 (br s, 1H), 1.96 (br s, 1H), 1.39 (t, J = 6.9 Hz, 6H). HRMS-ESI m/z calcd for $C_{74}H_{127}N_9O_{32}S_7$ $[M+2H]^{2+}$ 938.8310, found 938.8334.

Fluorescence studies

Due to the large increase in fluorescence emission observed upon complexation, the affinity of the coumarin **S3** could be measured directly. Borate buffer 100mM pH 8.5 was used. Plotting fluorescence versus the cyclodextrin concentration, the association constants (Ka) was determined to be $K_a = (1.5 \pm 0.08) \times 10^6$ M by fitting the data to a 1:1 binding isotherm. Association constants were calculated from the average of 3 independent titrations. The included graphs are a representative fit of the data (S. Figure 1).
The biomacromolecule stocks (heparin, chondroitin sulfate A, lysozyme, and glucose oxidase) were prepared by dissolving 50 mg of the analyte into 10 mL of phosphate buffer saline (PBS) pH 7.4 buffer. For these experiments the concentration of **2** or **3** was kept constant at 1.65×10^{-5} M. Each fluorescence spectra was taken from a separately prepared solution where the concentration of the biomacromolecule in question was increased for a total of eight spectra. The final concentrations of the biomacromolecules in question were 0 mg/mL, 0.05 mg/mL, 0.1 mg/mL, 0.15 mg/mL, 0.2 mg/mL, 0.33 mg/mL, 0.67 mg/mL and 1 mg/mL (S. Figure 2).



S. Figure 1: Fluorescence spectra of **1** and **S3**, and titration.
(A) Fluorescence titration of **1** with guest **S3** (4×10^{-9}M). (B) Fluorescence (λex = 368 nm) of 4×10^{-9}M **S3** in the absence (blue line) and presence (red line) of 4.6×10^{-6}M **1**.



S. Figure 2: Fluorescence spectra of **2** and **3** in the presence of biomacromolecules
(A) **2** and lysozyme. (B) **3** and lysozyme. (C) **2** and glucose oxidase. (D) **3** and glucose oxidase. (E) **2** and heparin. (F) **3** and heparin. (G) **2** and chondroitin sulfate A. (H) **3** and chondroitin sulfate A.

UV/Vis reaction studies

The S→N acyl transfer was followed by UV/Vis spectroscopy. During the aminolysis by **1** or ethylenediamine of **4** a blue shift (450 to 431 nm) in absorbance was observed. This change in absorbance was correlated to the disappearance of **4** as a function of time. The decrease in concentration of **4** was followed by observing the change in absorbance at a 455 nm. The observation of an isosbestic point indicates that no long lived intermediates were formed in the reaction. (SFigure 1 (A) and(B)). Thus the concentration of **4** could be calculation Beer's law and linear regression analysis using the following formula:

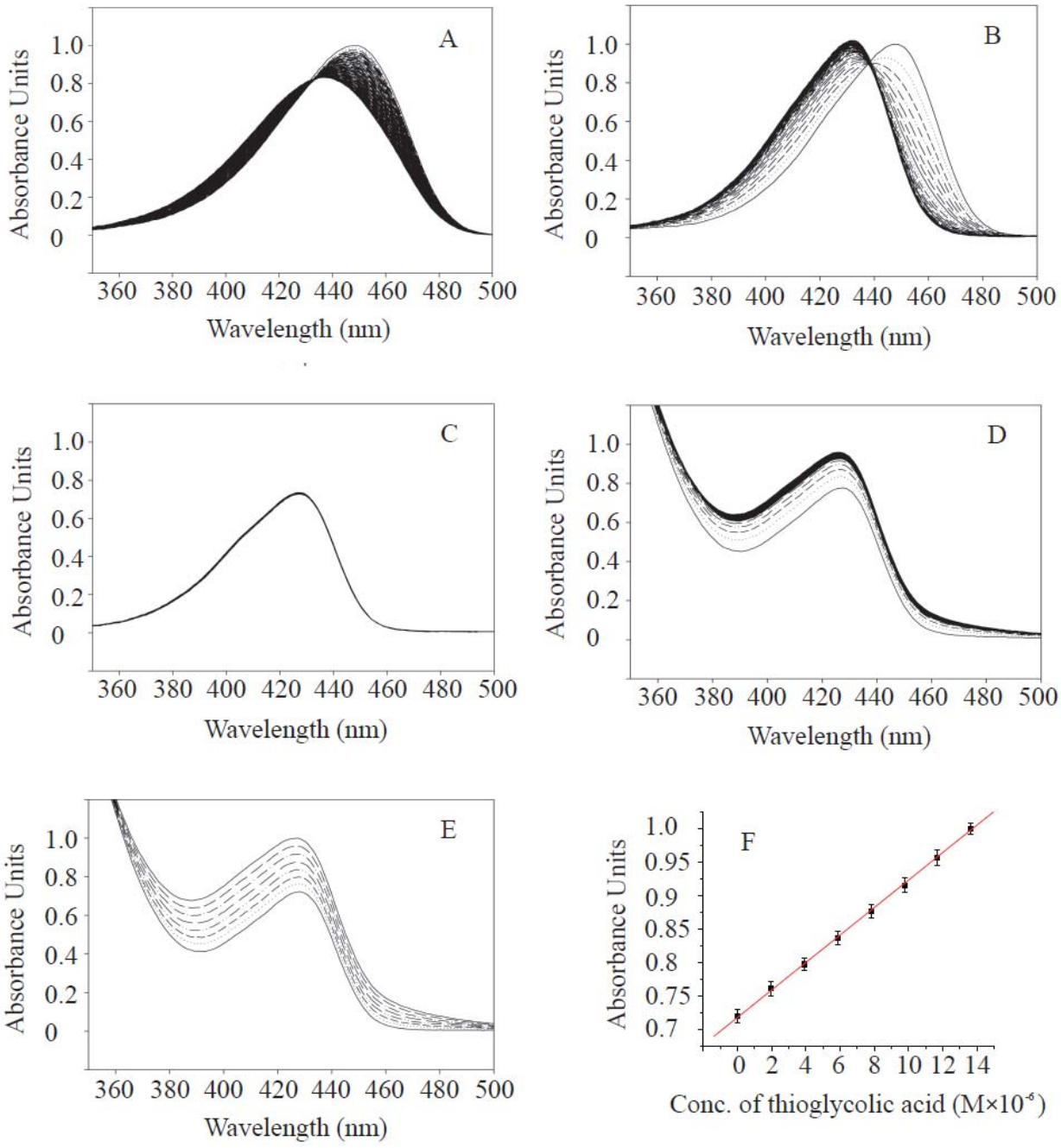
$$\frac{A - \varepsilon_a c_t^o}{\varepsilon_t - \varepsilon_a} = c_t$$

Where A is the absorbance at 455 nm, ε_a is the molar absorptivity of the amide product at 455 nm (13700 L mol^{-1} cm^{-1}), ε_t (54600 and 48400 L mol^{-1} cm^{-1}, outside and inside **1**'s cavity respectively) is the molar absorptivity of the thioester starting material at 455 nm, c_t^o is the initial concentration of the thioester, and c_t is the concentration of the thioester at a given time.

When **5** reacted with **1**, there was no change in the absorption spectrum. The formation of **3** was followed by adding Ellman's reagent to the reaction solution. As thioglycolic acid was formed during the reaction, it reduced Ellman's reagent to NTB^{-2}, which has an absorption maxima at 412 nm. The concentration of NTB^{-2} produced as the reaction proceeded was determined from a calibration curve using **S3** (final concentration of 9×10^{-4}M), and titrating in a known amount of thioglycolic acid. The aminolysis reaction using **5** was followed by observing the change in absorbance units starting at t=0 min. at a wavelength of 427 nm (why not 412?). The concentration of the product (3) was correlated to the formation of NTB^{-2} using the calibration curve shown in SFigure 1 (F).

Stocks were prepared as follows: **1** in borate buffer (100 mM, pH 8.5) and EDA in DMSO were prepared in 7.4×10^{-3}M and 3.4 M concentrations respectively. The thioester solutions of **4** and **5** in DMSO were prepared in 1.6×10^{-2} M and 5.9×10^{-2} M concentrations respectively. Ellmans reagent (5.1×10^{-2} M) was prepared in DMSO. For each of the reactions these stocks were diluted into a total volume of 1 mL borate buffer (100 mM, pH 8.5) at room temperature in a quartz cuvette. The final concentration of the components was; **1** 9×10^{-4}M, EDA 6.8×10^{-3}M, **4** and **5** 9.7×10^{-6}M, and DTNB 9×10^{-5}M. All of the reactions were carried out at 296K.

Control experiments for the hydrolysis of **4** and **5** were performed at a concentration of 9.7×10^{-6}M in borate buffer (100 mM, pH 8.5). No appreciable hydrolysis was observed after 24 h at room temperature (data not shown).



S. Figure 3: Reaction spectra of aminolysis and calibration curve.
(A) EDA (6.8×10^{-3}M) and **4** (9.7×10^{-6}M) in 1 mL borate buffer (100mM, pH 8.5), spectra were taken at 7.2 min intervals for atotal of 100 spectra. (B) **1** (9×10^{-4}M) and **4** (9.7×10^{-6}M) in 1 mL borate buffer (100 mM, pH 8.5), spectra were collected at 5 min intervals. 100 spectra were recorded. (C) **1** (9×10^{-4}M) and **5** (9.7×10^{-6}M) in 1 mL borate buffer (100 mM, pH 8.5), 85 spectra were recorded at 3 min intervals. . (D) **1** (9×10^{-4}M), **5** (9.7×10^{-6}M), and Ellmans reagent (5.1×10^{-2} M) in 1 mL borate buffer (100 mM, pH 8.5), 85 spectra were collected at 3 min intervals. (E) **1** (9×10^{-4}M), **S3** (9.7×10^{-6}M), and Ellmans reagent (5.1×10^{-2} M) in 1 mL borate buffer (100 mM, pH 8.5). After the first spectra subsequent spectra represents a titration of 2 µL of thioglycolic acid (7.9×10^{-4} M) in 1 mL borate buffer (100 mM, pH 8.5) for a total of 8 additions. (F) Calibration curve constructed from E at 427 nm.

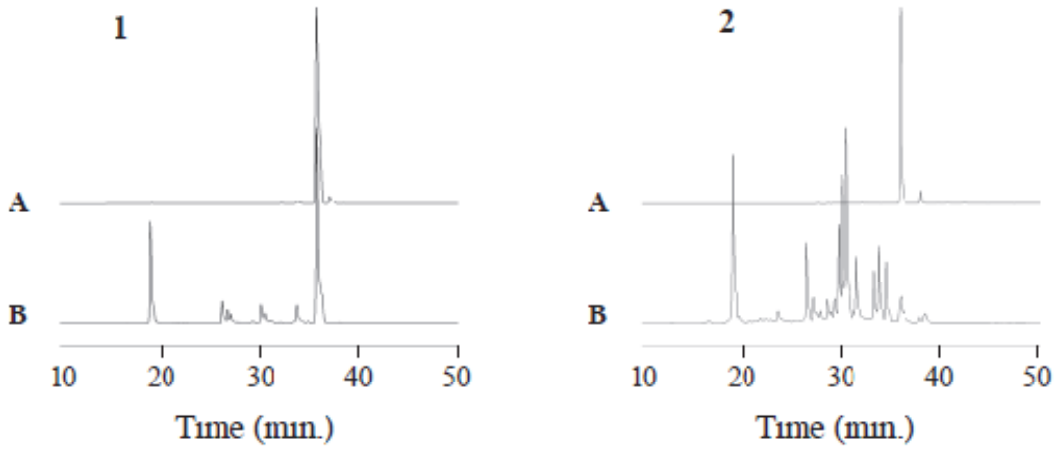
HPLC monitoring of reactions

HPLC elution method is displayed in S. Table 1 below.

S. Table 1: Ramping method for RP-HPLC

Min.	mL/min	Water (0.1%TFA) %	Acetonitrile (0.1%TFA) %
6	3.5	98	2
15	3.5	80	20
30	3.5	42.5	57.5
70	3.5	0	100

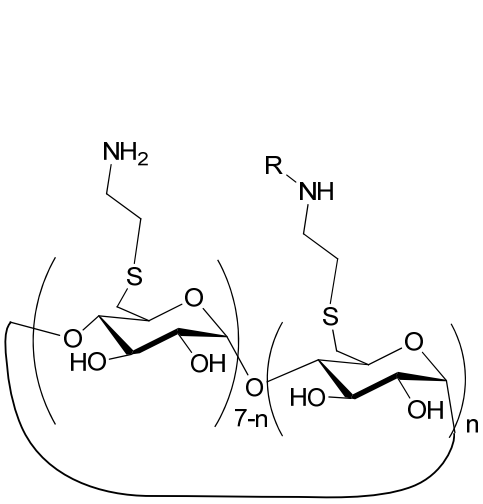
When **1** was reacted with **4** or **5**, with a 2 : 1 ratio of guest vs. host, better selectivity of monoacylation is observed with **5**. This can be seen in SFigure2. Aliquotes of the reaction between **1** and **4** with a 1 : 1 ratio and 2 : 1 ratio of guest vs. host were collected between 25 and 35 min. The mixture was analyzed by MALDI TOF.



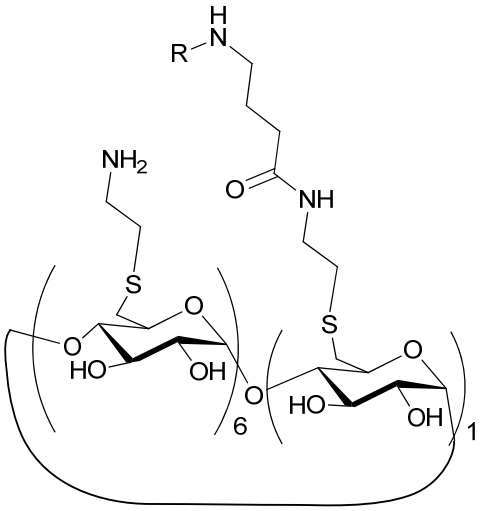
S. Figure 4: HPLC traces for the reaction of **1** with two equivalents of **4** or **5**. 1. (A) **5** (2×10^{-5}M). (B) Reaction of **5** (2×10^{-5}M) and **1** (1×10^{-5}M) in 6 mL borate buffer (10 mM, pH 8.5) overnight at room temperature. 2. (A) **4** (2×10^{-5}M). (B) Reaction of **4** (2×10^{-5}M) and **1** (1×10^{-5}M) in 6 mL borate buffer (10mM, pH 8.5) overnight at room temperature.

Maldi-TOF spectra

S. Table 2: Calculated MW of n-acylated **2** and **3**

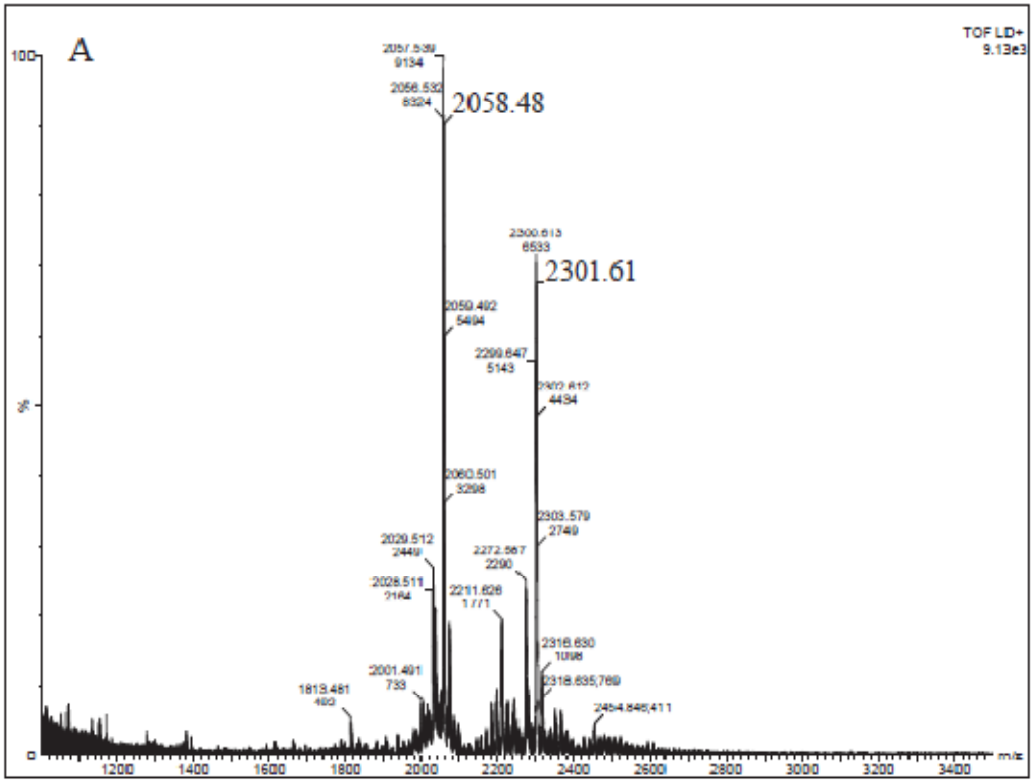


2



3

Compound	N	MW (g/mol) [M+H]+	MW (g/mol) [M+Na]+
2	0	1549.93	1571.91
	1	1793.18	1815.17
	2	2036.44	2058.42
	3	2279.70	2301.68
	4	2522.96	2544.94
3	N/A	1879.29	1900.27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2316.630
1098
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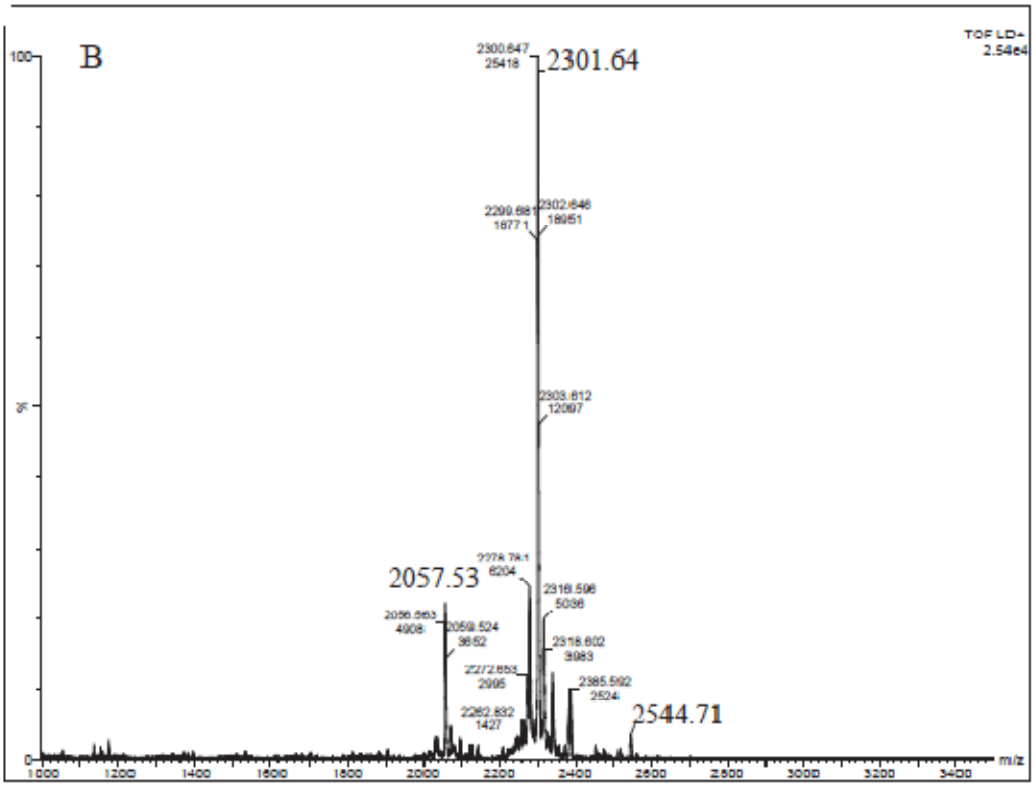
480
m/z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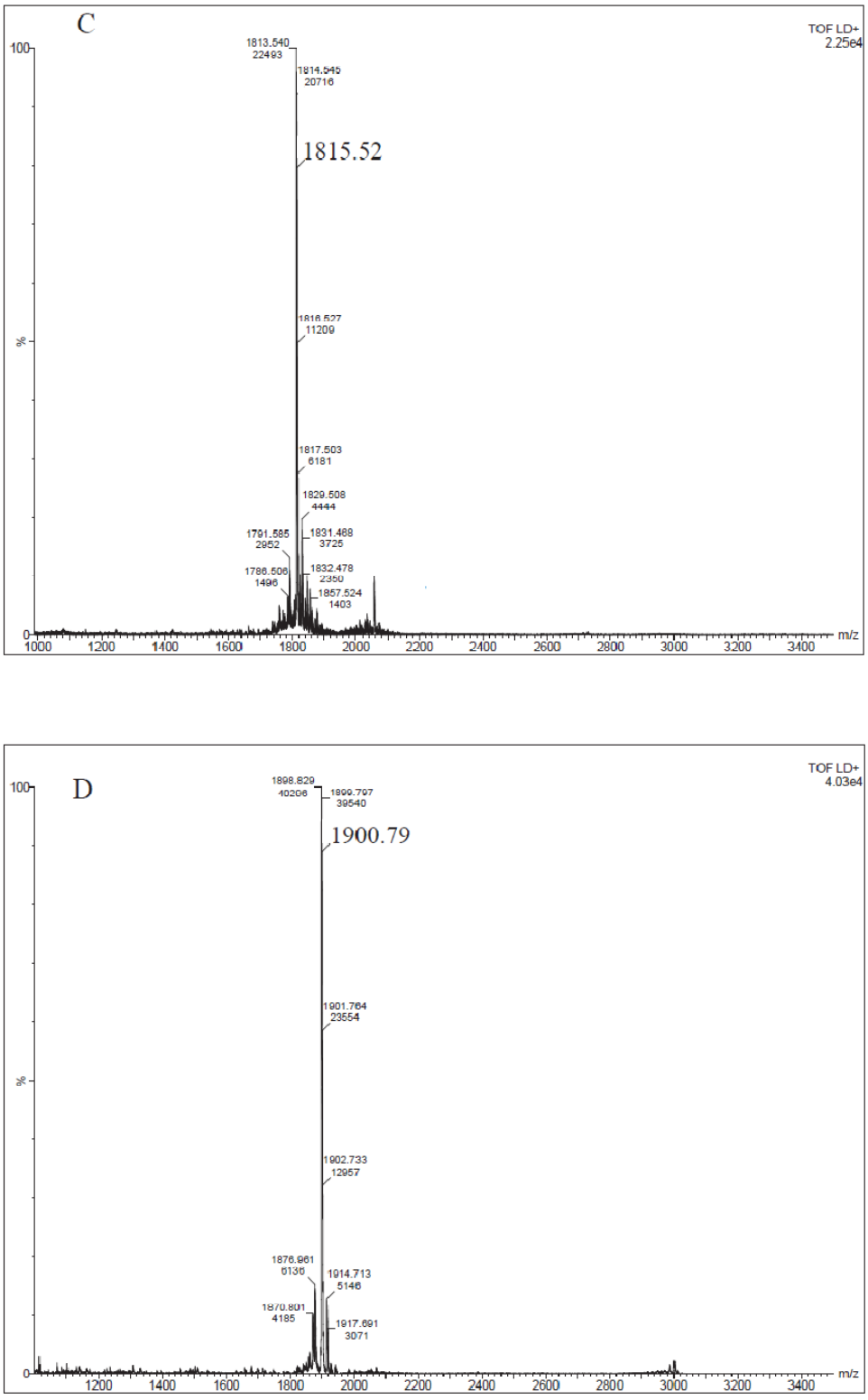


S. Figure 5: MALDI ToF MS spectra of HPLC traces. (A) Spectrum taken of compounds eluting between 25-40 min collected from the HPLC of the reaction between **1** and **4** at 1×10^{-5}M (Figure 3-C). (B) Spectrum taken of the compounds eluted between 25-40 min collected from the HPLC of the reaction between **1** at 1×10^{-5}M and **4** at 2×10^{-5}M (Figure S 2-2B). (C) Spectrum of 2. (D) Spectrum of 3.

NMR tables and spectra

2D TOCSY of **3** and **2**.

S. Table 3: Chemical shifts of ^{1}H-1 scalar couplings of **3** and **2**.

Compound (Concentration)	**2** (1.9±0.5 × 10^{-3}M)		**2** (4.3±0.5 × 10^{-3}M)		**3** (9.5±0.5 × 10^{-3}M)	
Position	δ (ppm)	2D-TOCSY	δ (ppm)	2D-TOCSY	δ (ppm)	2D-TOCSY
^{1}H-1	4.92	3.60 3.47 3.41 3.39			5.02	3.64 3.46 3.34 3.04 2.74
	5.03 & 5.04	3.71 3.68 3.65 3.53 3.5 3.44 2.73			5.04	3.61 3.34 2.80 2.63
	5.14	3.66 3.63 3.57 2.76 2.99			5.05	3.81 3.79 3.70 3.26 3.08
	5.16	4.24 3.83 5.16 4.51			5.12	3.99 3.73 3.27 3.1
	5.22	3.82 3.80 3.71 3.69	5.22	3.87 3.70	5.14	3.68 3.53 3.42 3.01 2.7
	5.24	4.24 3.80 3.77 3.23 3.31			5.21 & 5.22	4.08 & 3.95 3.89 & 3.77 3.74 & 3.40 3.25 & 3.09 3.18

gCOSY of **3** and **2**.

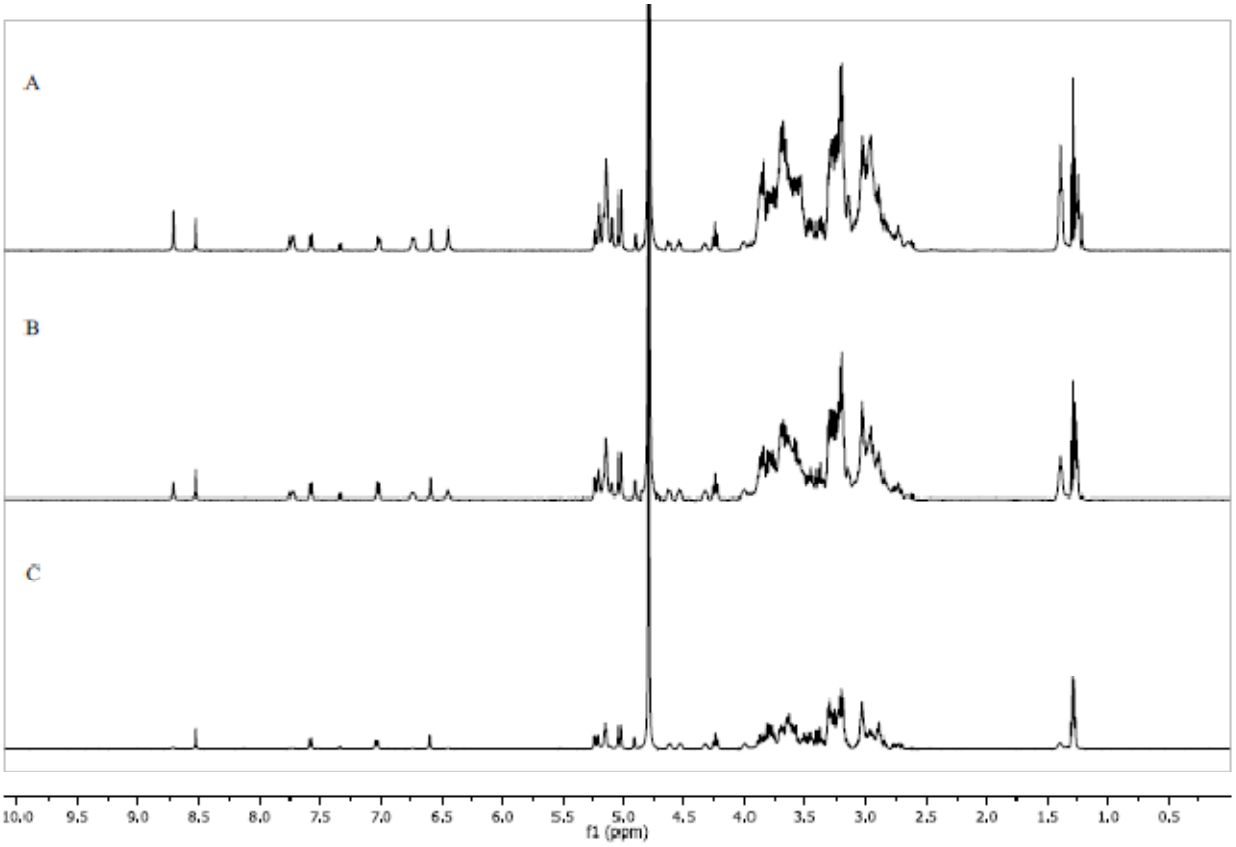
S. Table 4: Assignments of the scalar couplings of the ring sugar ^{1}H's of **3** and **2**.

Compound (Concentration	**2** (1.9±0.5 × 10^{-3}M)		**2** (4.3±0.5 × 10^{-3}M)		**3** (9.5±0.5 × 10^{-3}M)	
Position	δ (ppm)	COSY	δ (ppm)	COSY	δ (ppm)	COSY
^{1}H-1	4.92 5.03 & 5.04 5.14 5.16 5.22 5.24	^{1}H-2	5.22	^{1}H-2	5.02 5.04 5.05 5.12 5.14 5.21[a] & 5.22[a]	^{1}H-2
^{1}H-2	3.60 3.65 3.50 3.63 3.83 3.72 3.80	^{1}H-3	3.70	^{1}H-3	3.64 3.61 3.70 3.73[a] 3.68 3.74[a]	^{1}H-3
^{1}H-3	3.41 3.44 3.57 4.24 3.82 4.24	^{1}H-4[b]	3.87		3.46 3.34 3.98 3.42 3.89	^{1}H-4
^{1}H-4[b]	3.47 3.77	^{1}H-5			3.34 2.63 3.53	^{1}H-5
^{1}H-5	3.39				2.74 2.70	
[a] overlapped						

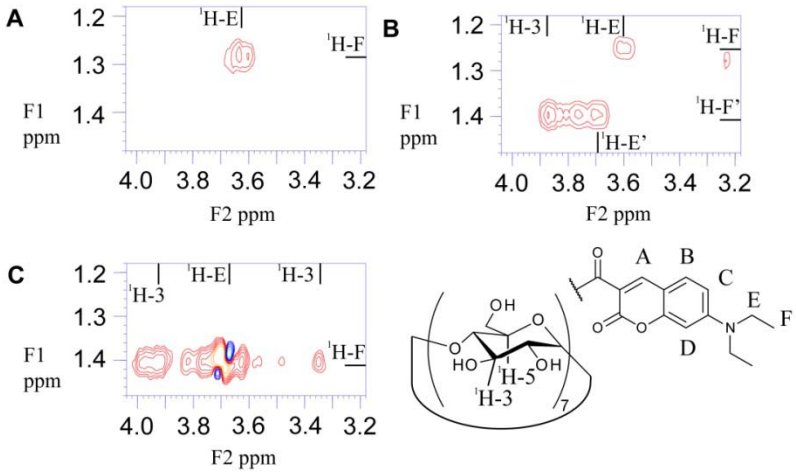
ROESY of **3** and **2**

S. Table 5: ROESY cross-peaks in the aromatic region of **3** and **2**.

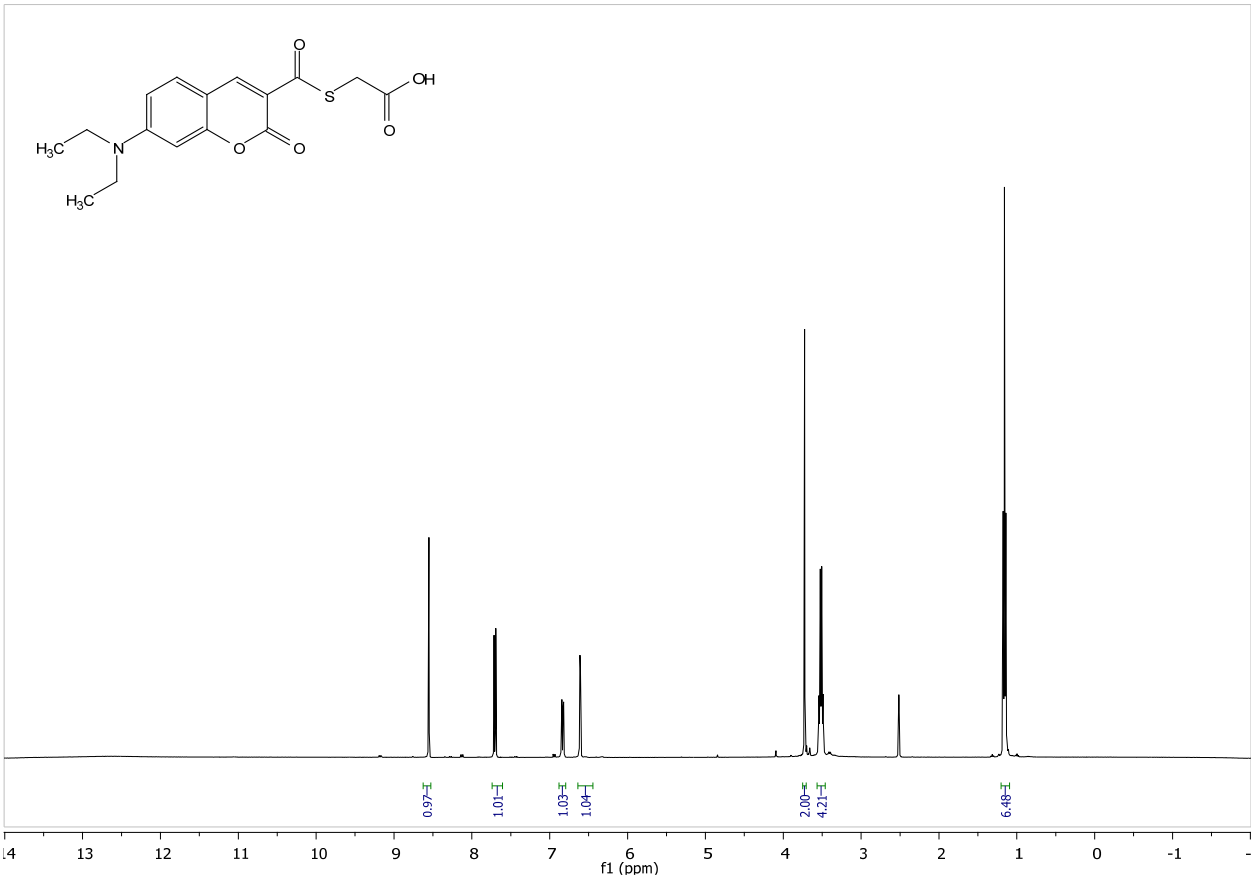
ROESY assignments of the aromatic region of **3** and **2**.	2 (1.9±0.5 × 10^{-3}M)		2 (4.3±0.5 × 10^{-3}M)		**3** (9.5±0.5 × 10^{-3}M)	
Position	δ (ppm)	ROE	δ (ppm)	ROE	δ (ppm)	ROE
^{1}H-A						
^{1}H-B						
^{1}H-C	7.04	^{1}H-E	7.03	^{1}H-E	6.75	^{1}H-3 (w) ^{1}H-E
^{1}H-D	6.61	^{1}H-E	6.6	^{1}H-E	6.42	^{1}H-3(w) ^{1}H-E (s)
^{1}H-A’						
^{1}H-B’						
^{1}H-C’			6.74	^{1}H-E’		
^{1}H-D’			6.46	^{1}H-E’		

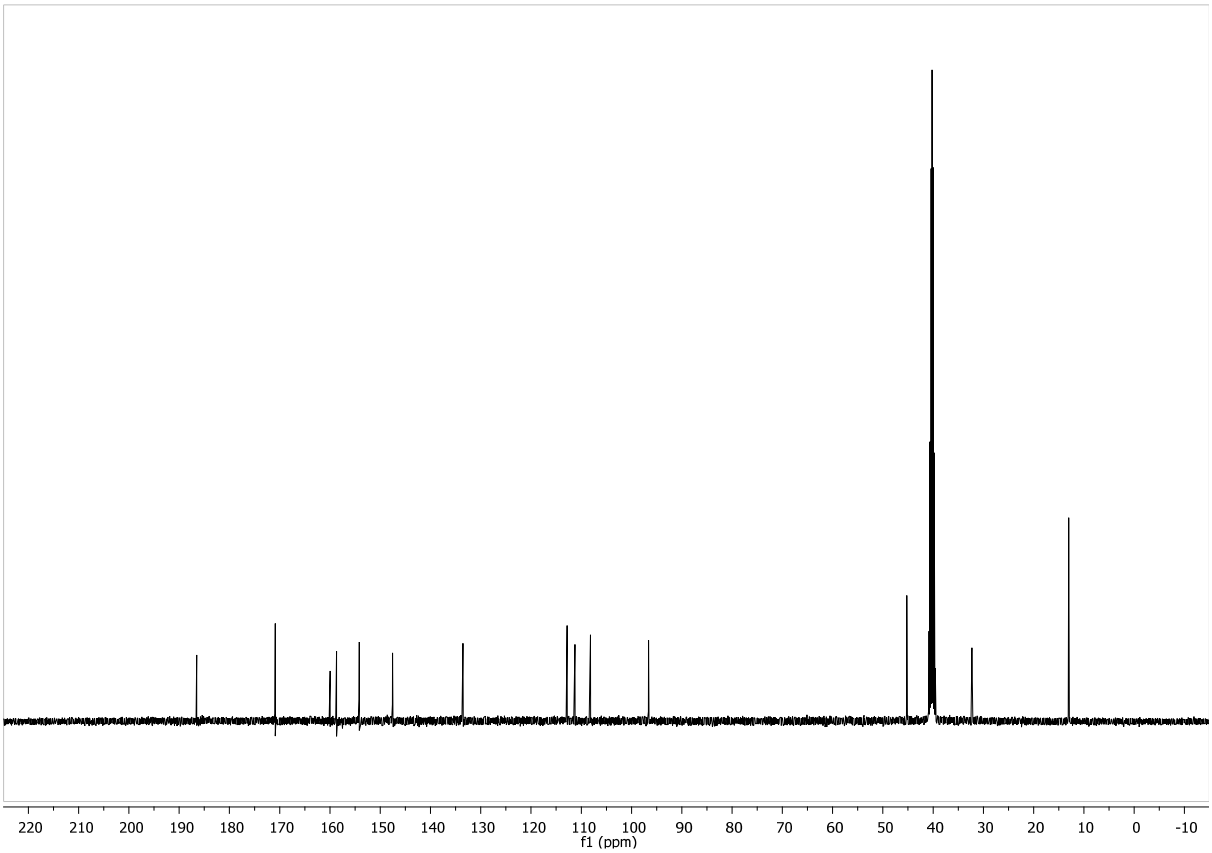


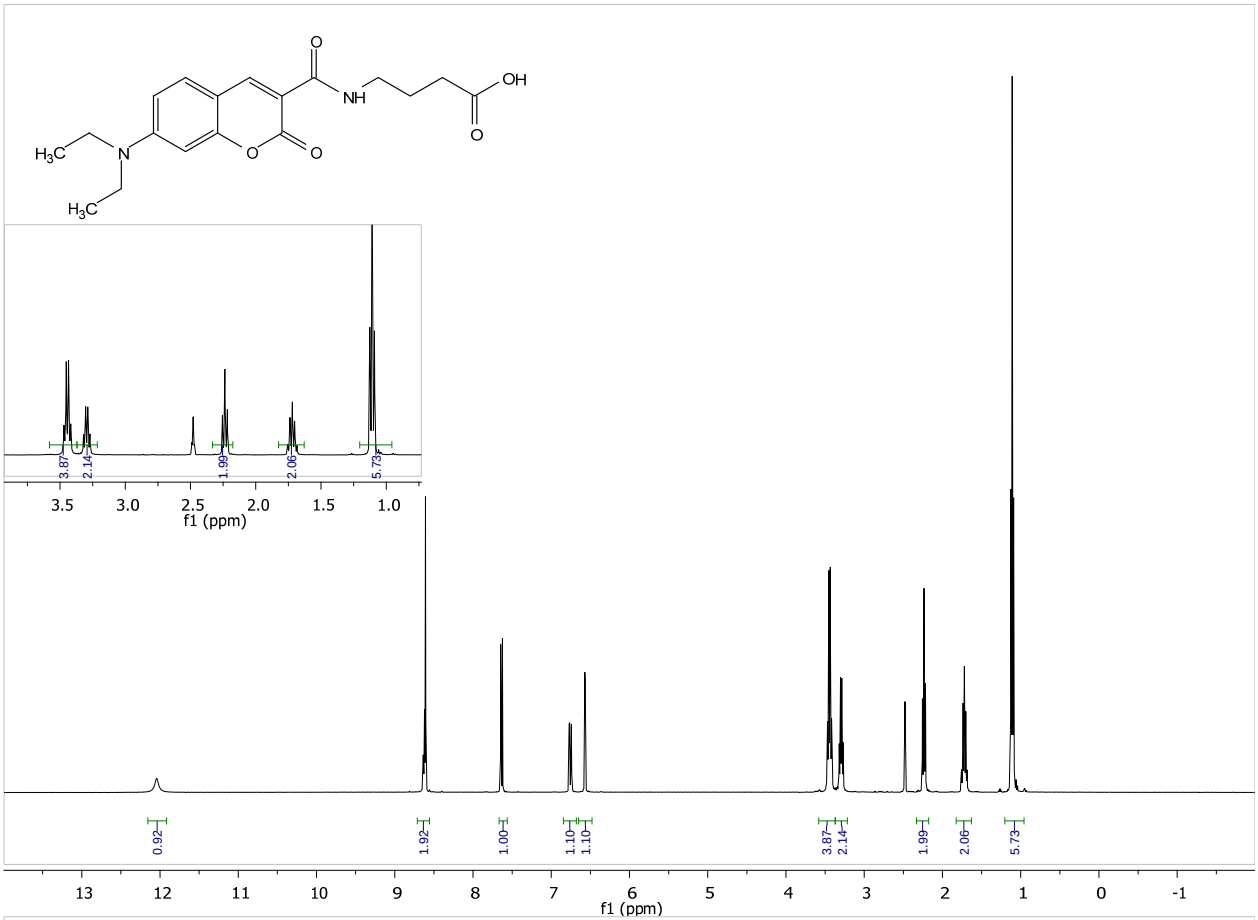
S. Figure 6: ^{1}HNMR Array of different concentrations of **2**. (A) 6.3±0.5 × 10^{-3}M. (B) 4.3±0.5 × 10^{-3}M. (C) 1.9±0.5 × 10^{-3}M.



S. Figure 7: Key ROESY cross-peaks for the structural elucidation of **2** and **3** in D_2O. Spectra were acquired in a 500 MHz at 298 K with a mixing time of 300 ms. A) **2** (1.9 ± 0.5 × 10^{-3} M). B) **2** (4.3 ± 0.5 × 10^{-3} M). C) **3** (9.5 ± 0.5 × 10^{-3} M).



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H3C
N
O
O
H3C
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2.06
5.73
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3.0
2.5
2.0
1.5
1.0
f1 (ppm)
0.92
1.92
1.00
1.10
1.10
3.87
2.14
1.99
2.06
5.73
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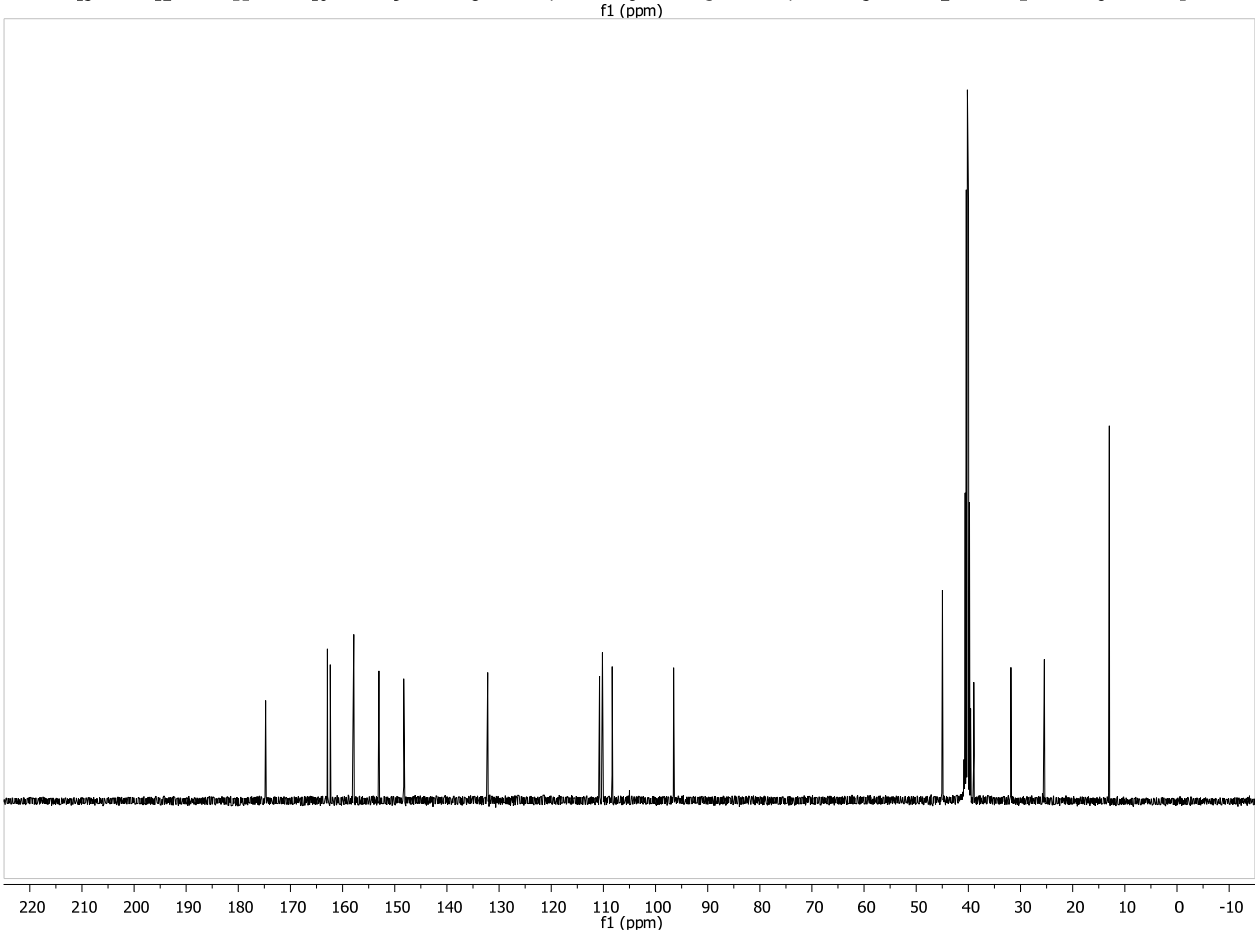
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)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200
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180
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120
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100
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80
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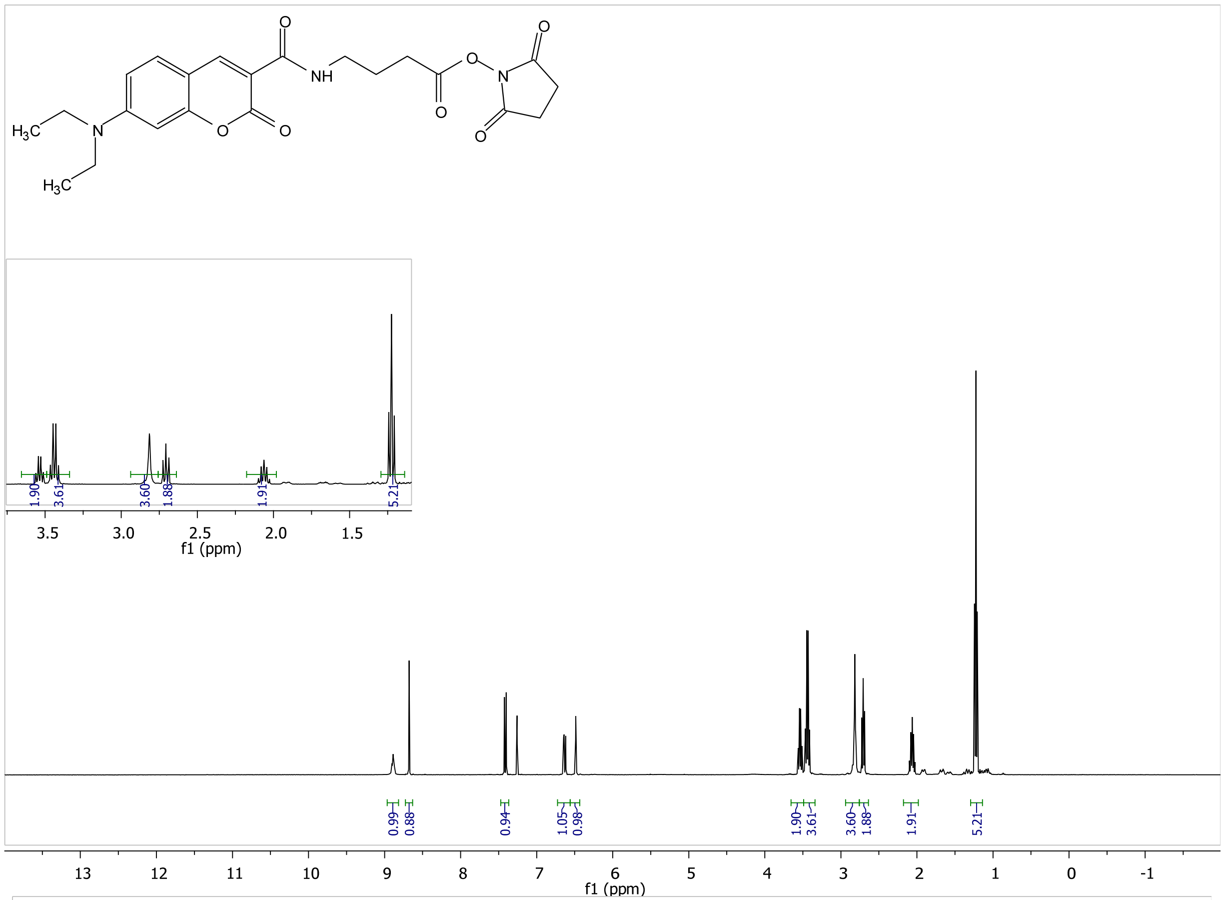
60
50
40
30
20
10
0
-10
f1 (ppm)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H3C
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2.0
1.5
f1 (ppm)
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0.88
0.94
1.05
0.98
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12
11
10
9
8
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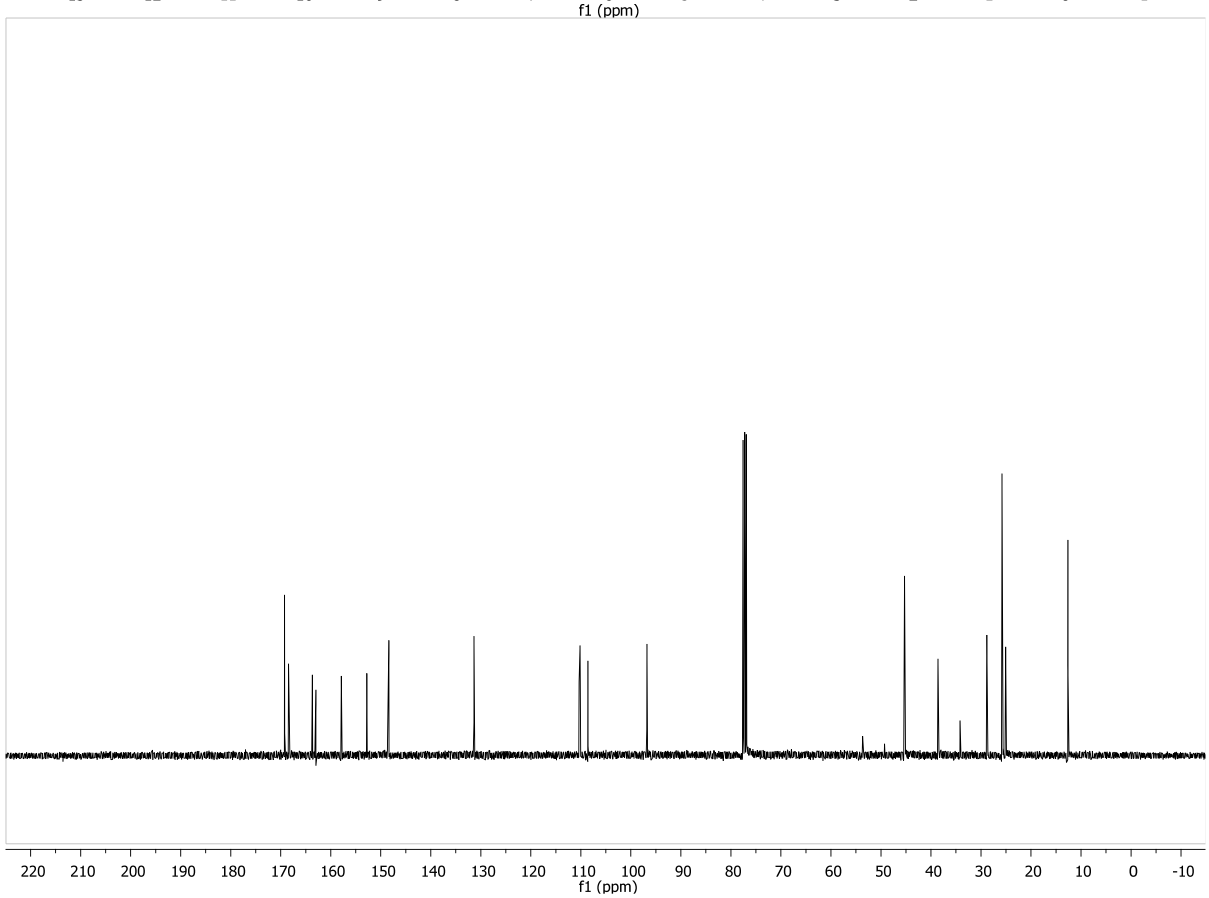
6
5
4
3
2
1
0
-1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20
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0
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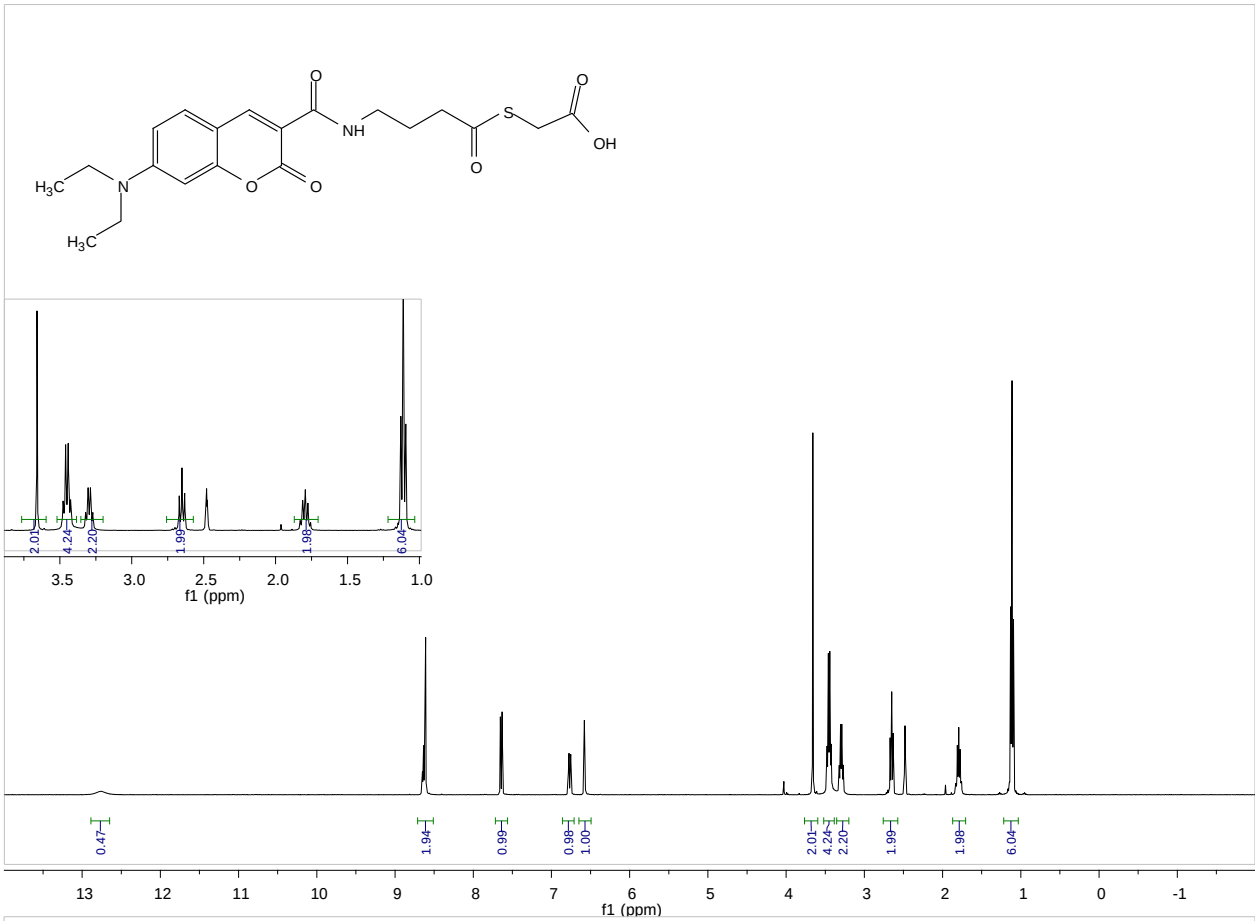
f1 (ppm)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NH
S
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OH
O
H3C
N
O
O
H3C
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4.24
2.20
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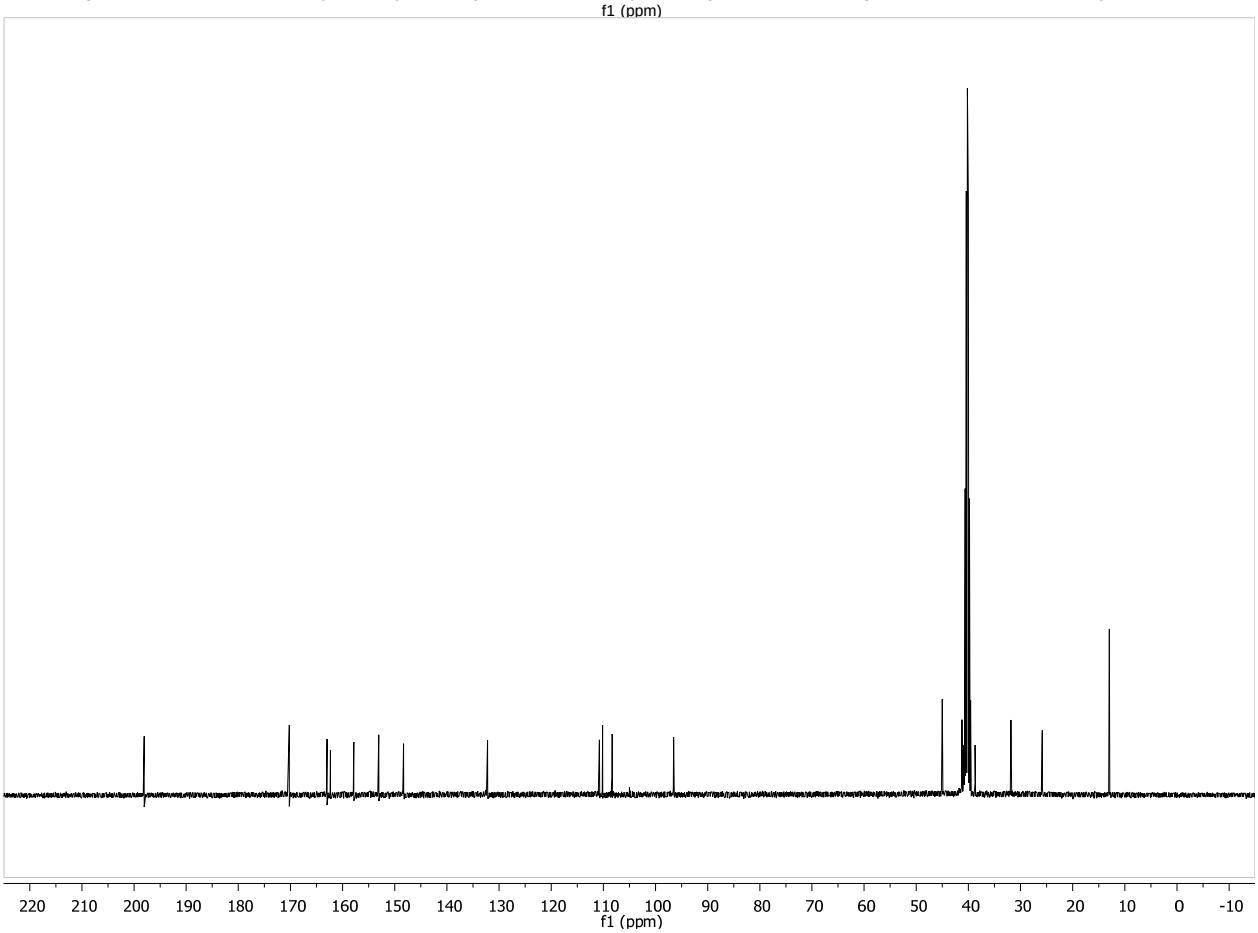
1.98
6.04
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)
0.47
1.94
0.99
0.98
1.00
2.01
4.24
2.20
1.99
1.98
6.04
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
f1 (ppm)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40
30
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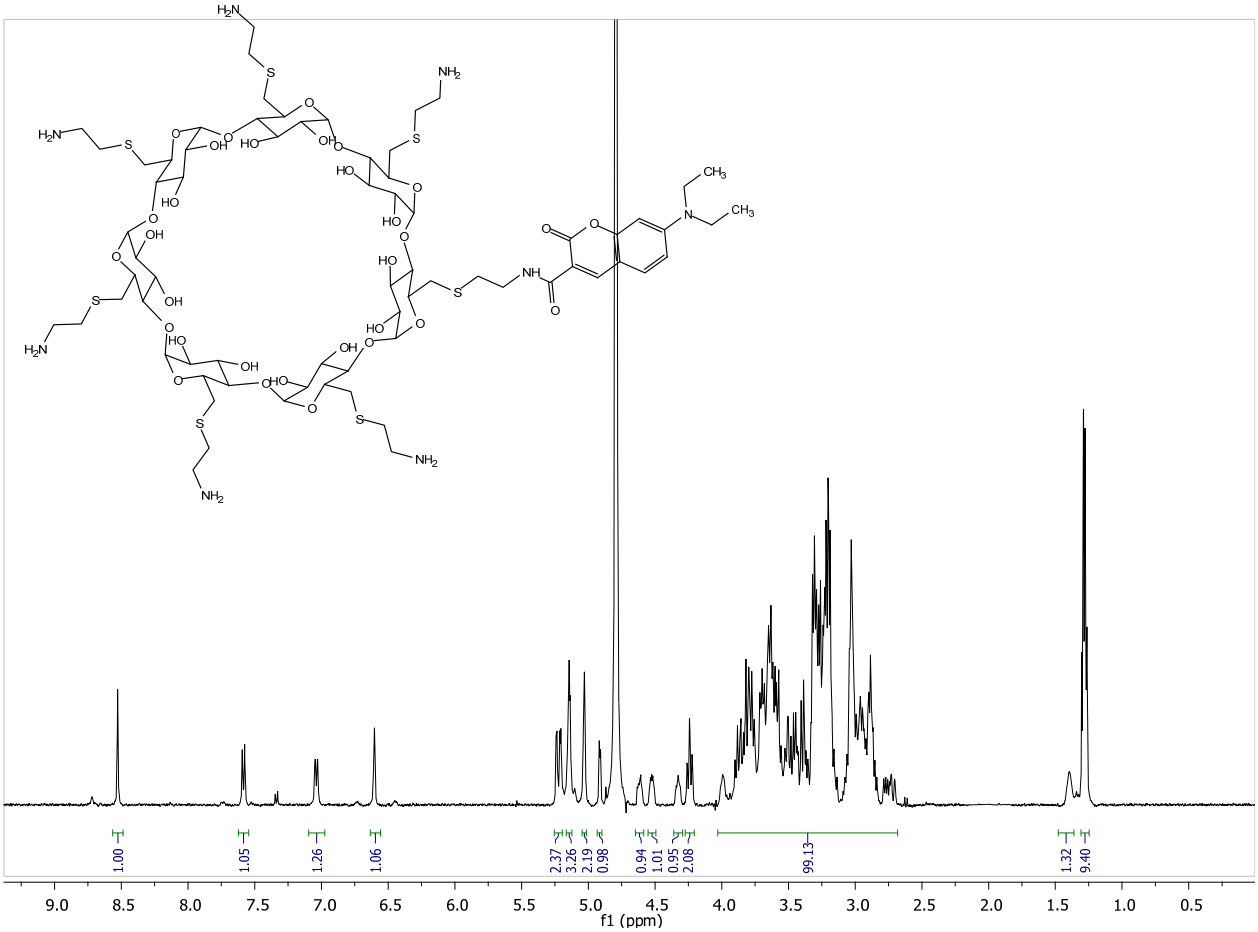
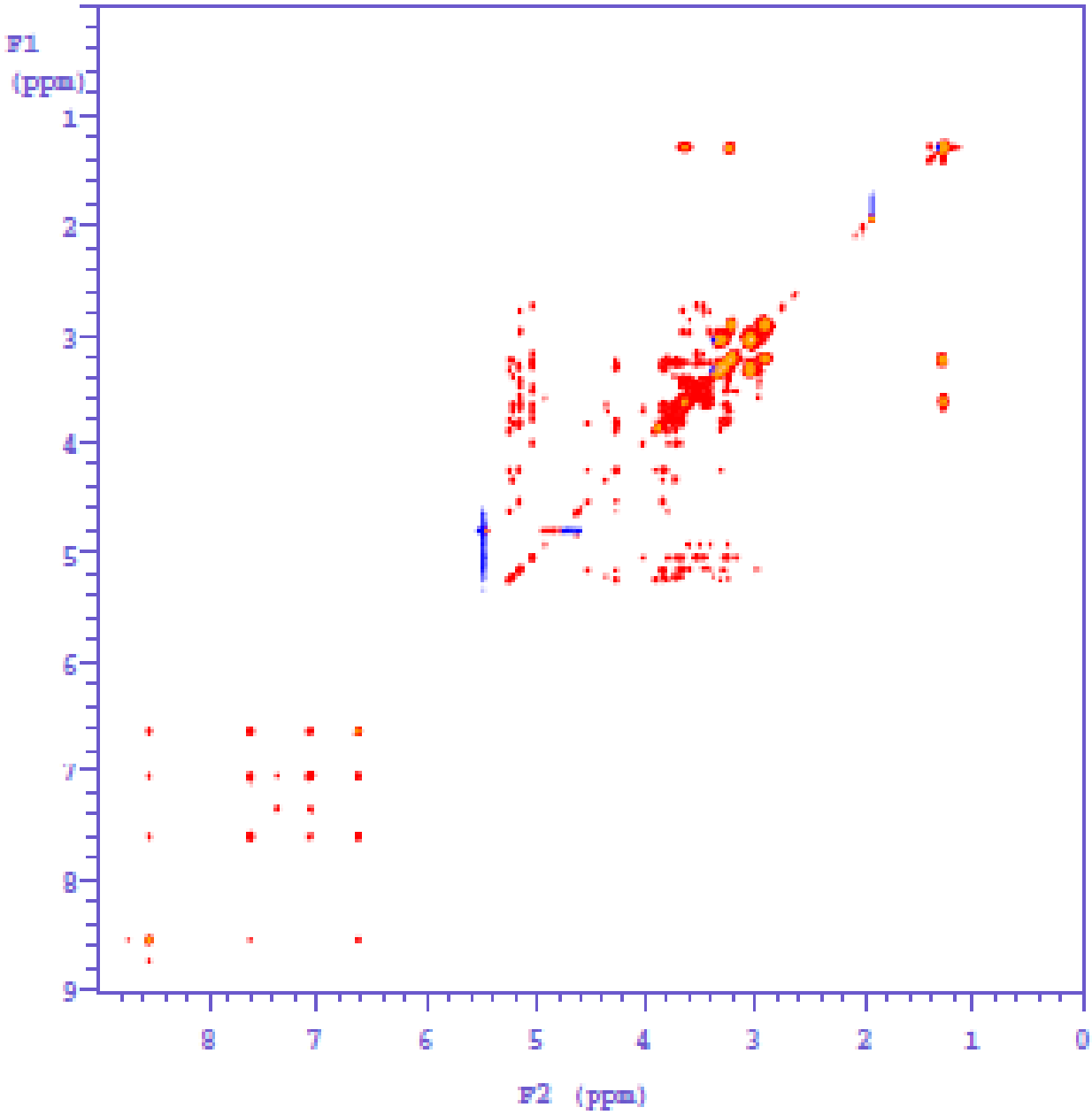
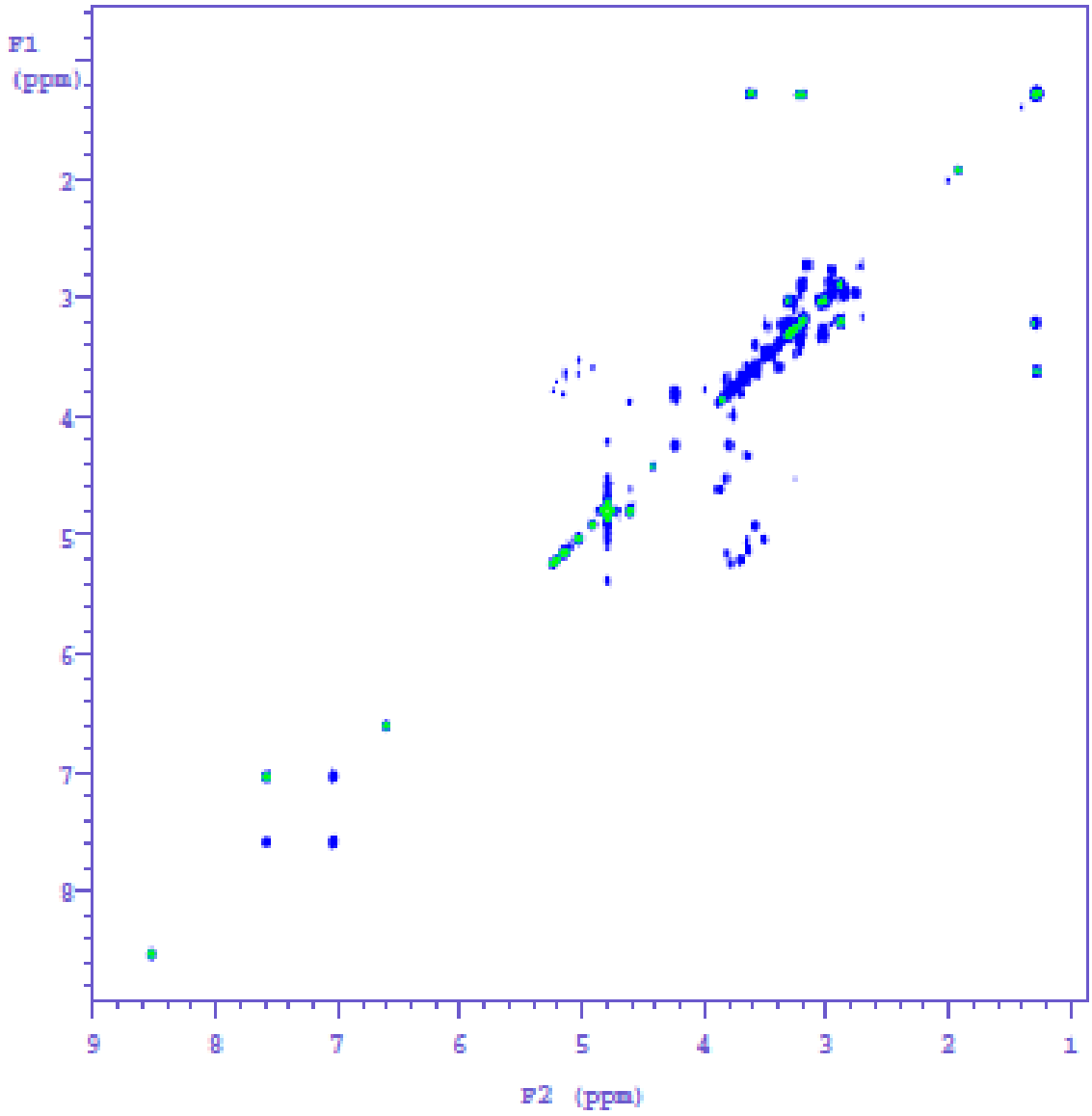
10
0
-10
f1 (ppm)

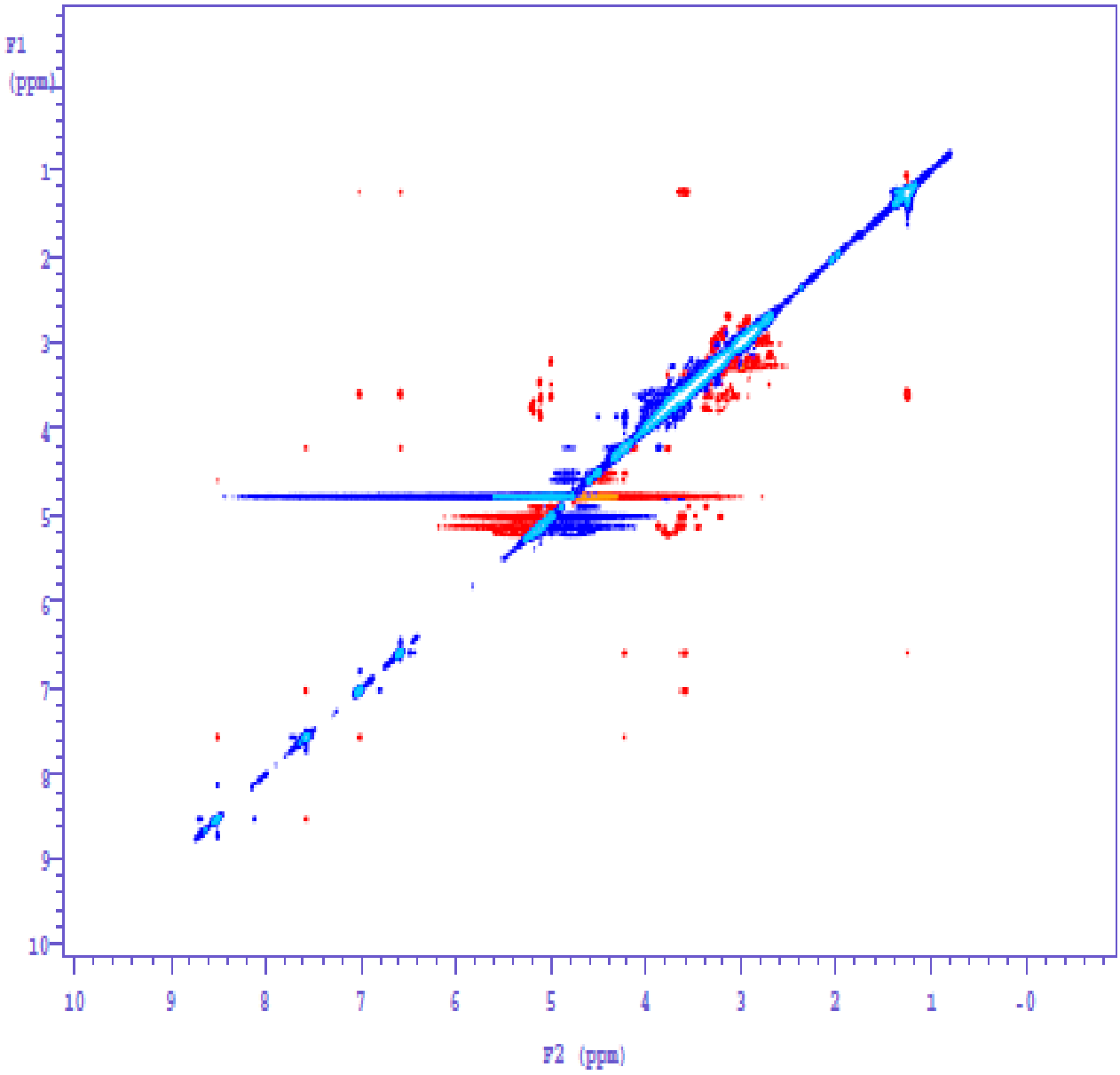




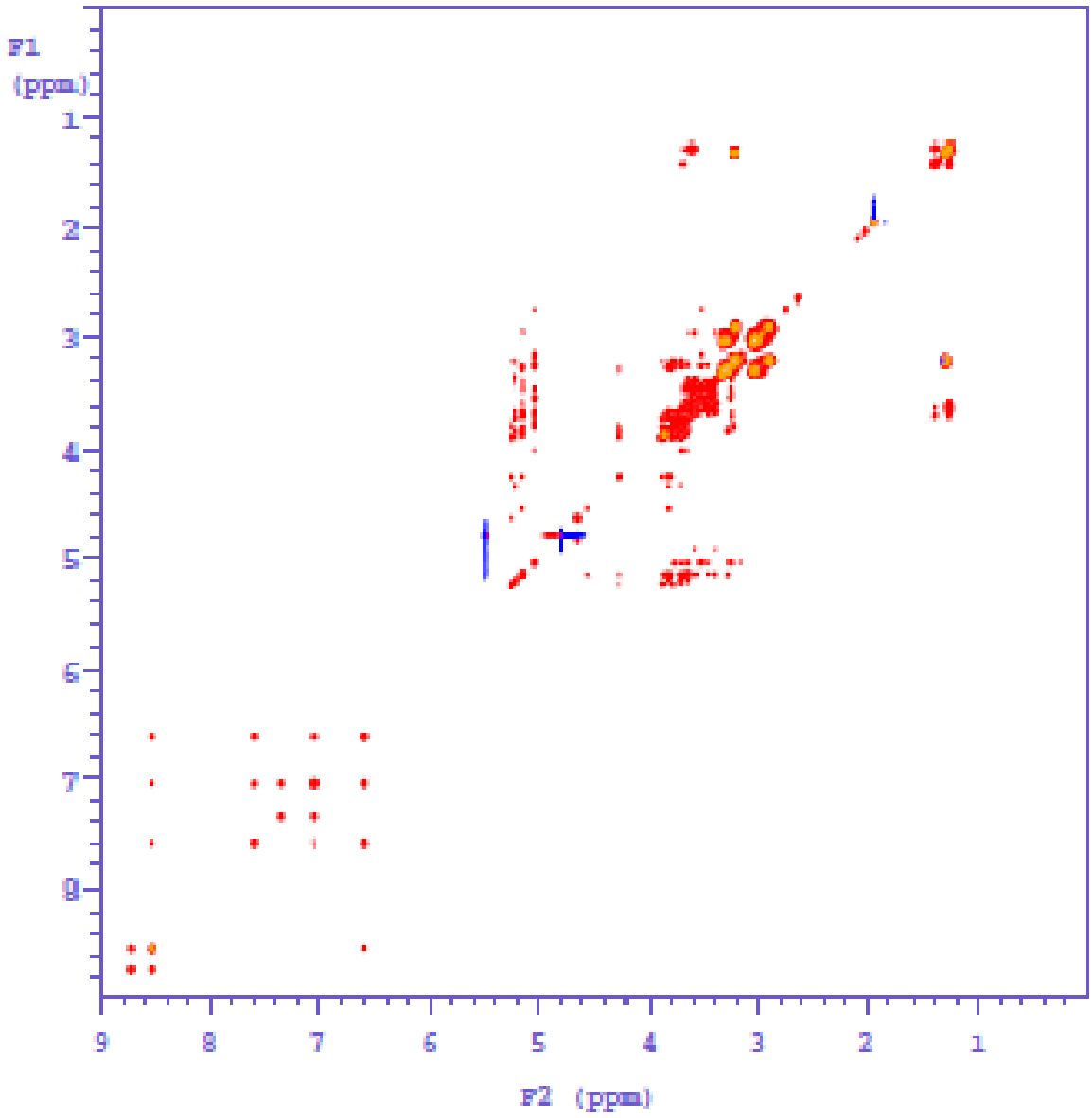
2D TOCSY of **2** ($1.9\pm0.5 \times 10^{-3}$M) 600ms mixing time.



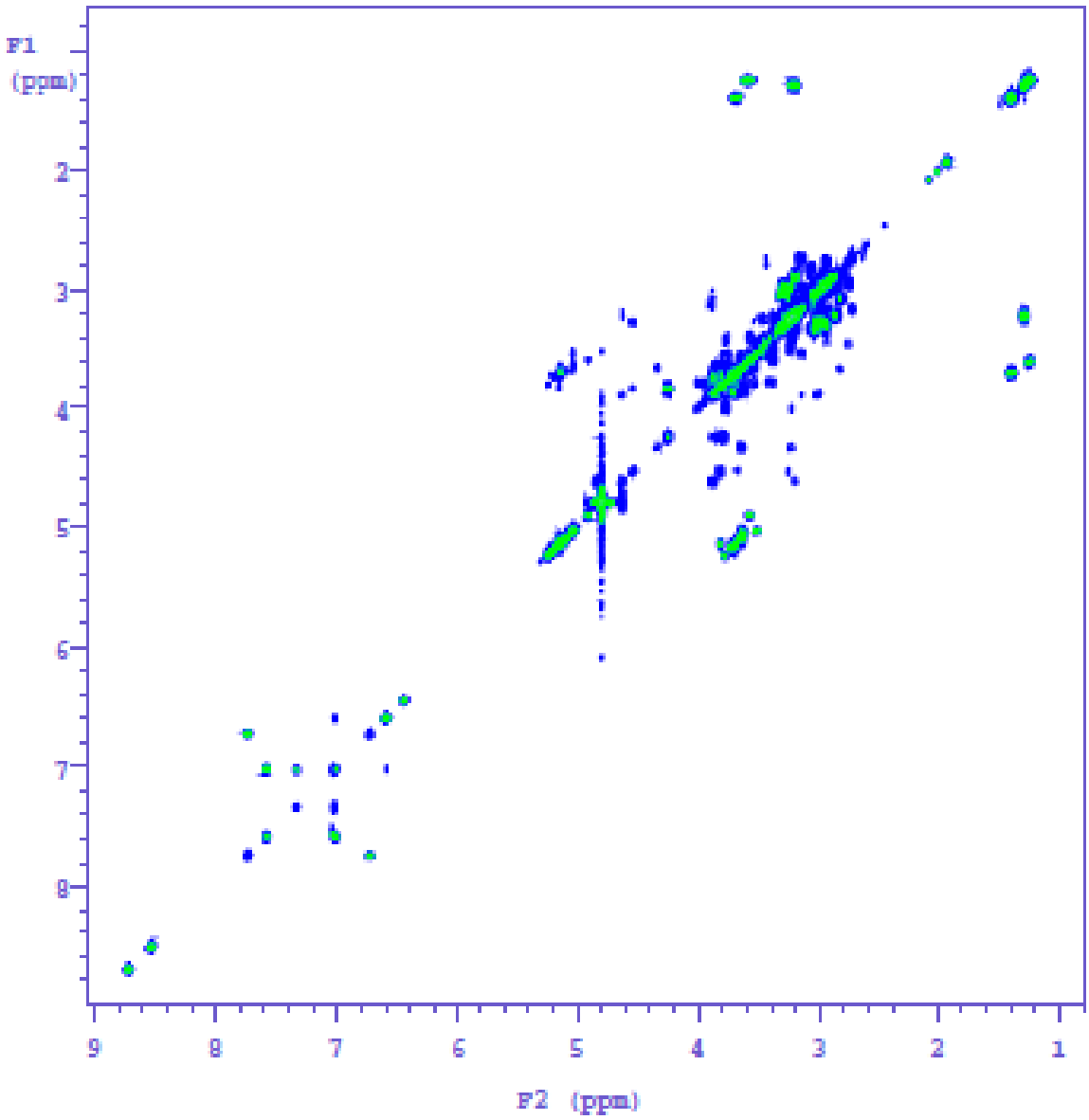
gCOSY of **2** ($1.9\pm0.5 \times 10^{-3}$M).



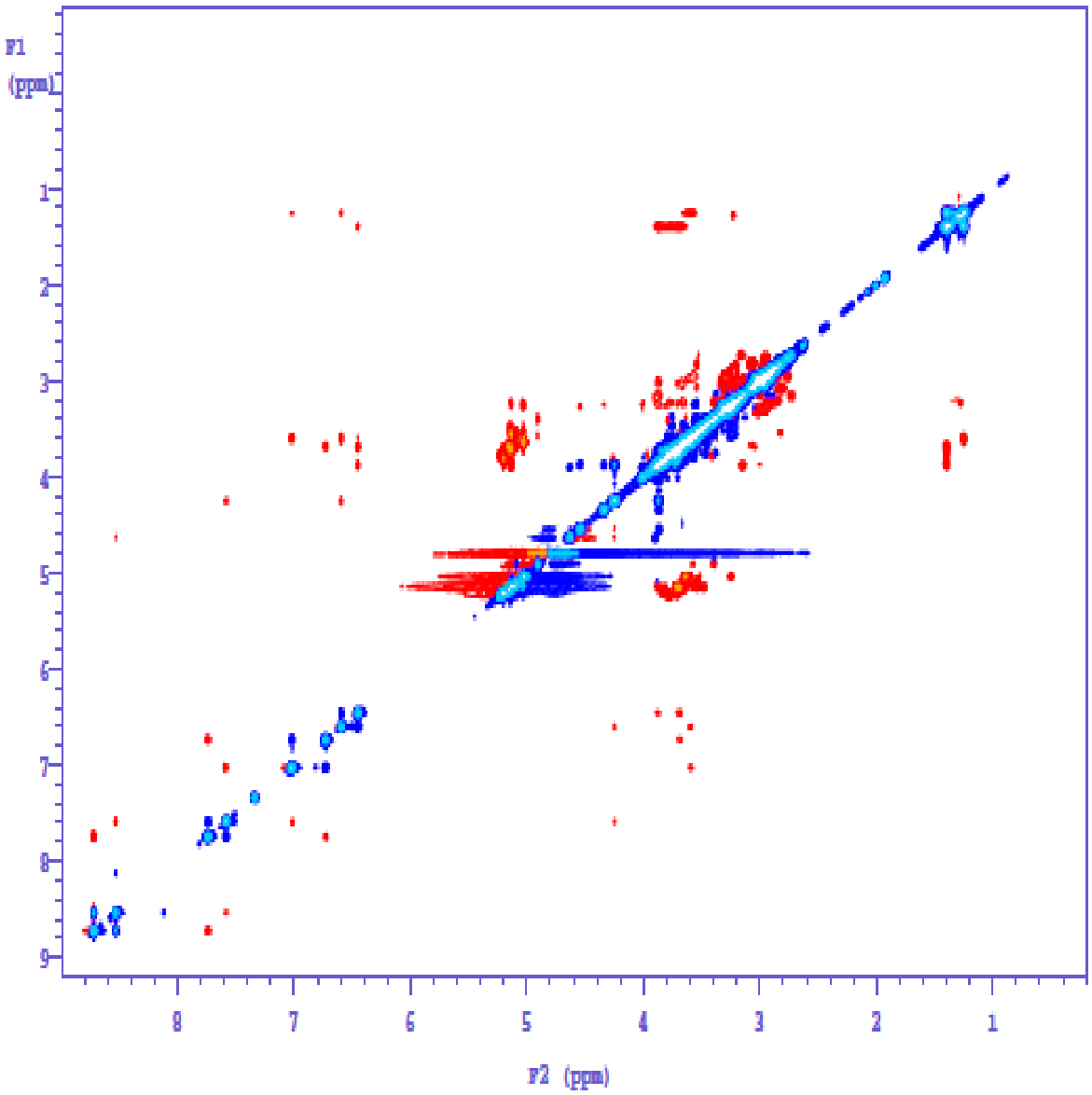
ROESY of **2** ($1.9\pm0.5 \times 10^{-3}$M) 300ms mixing time.



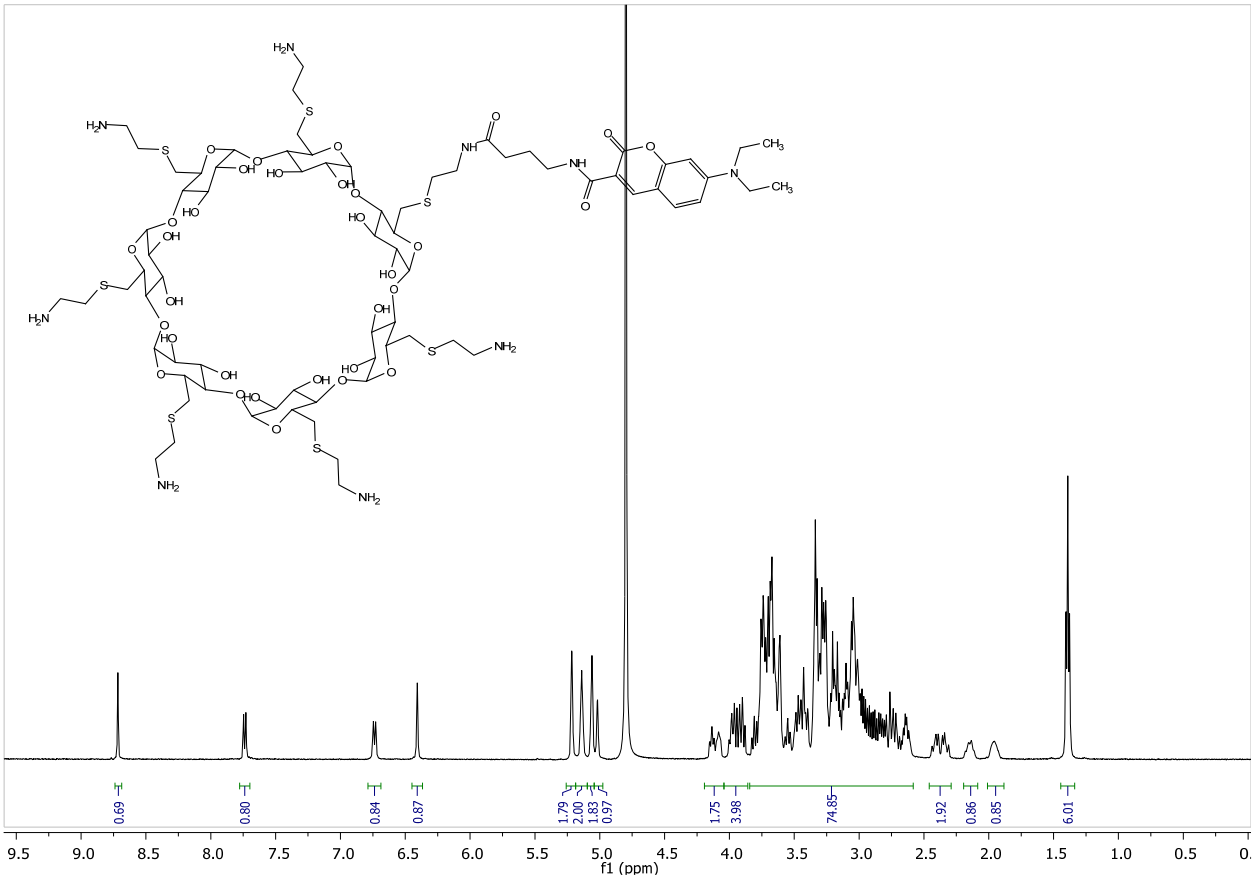
2D TOCSY of **2** ($4.3\pm0.5 \times 10^{-3}$M) 600ms mixing time.



gCOSY of **2** ($4.3\pm0.5 \times 10^{-3}$M).



ROESY of **2** (4.3±0.5 × 10^{-3}M) 300ms mixing time.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3.98
74.85
1.92
0.86
0.85
6.01
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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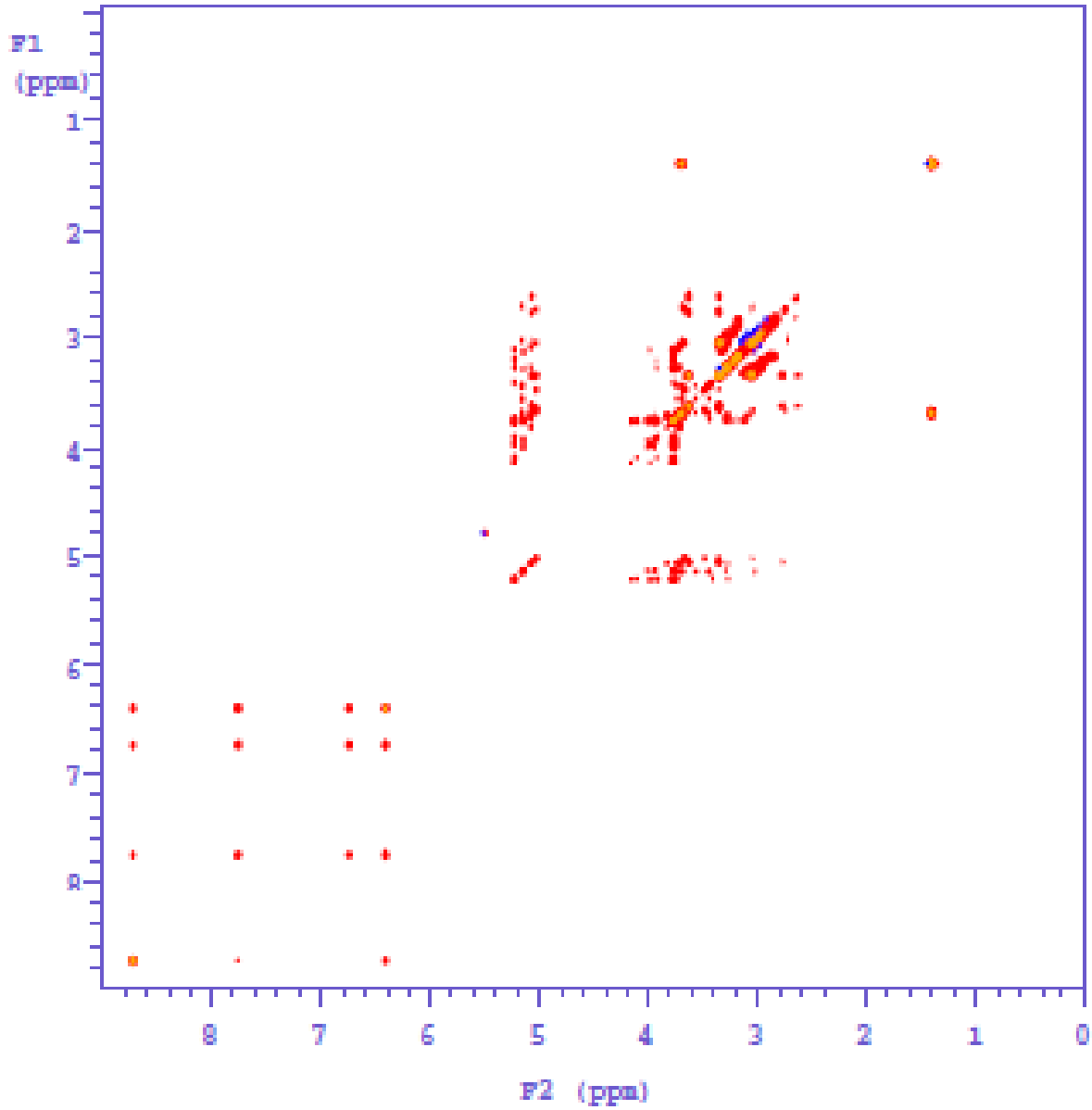
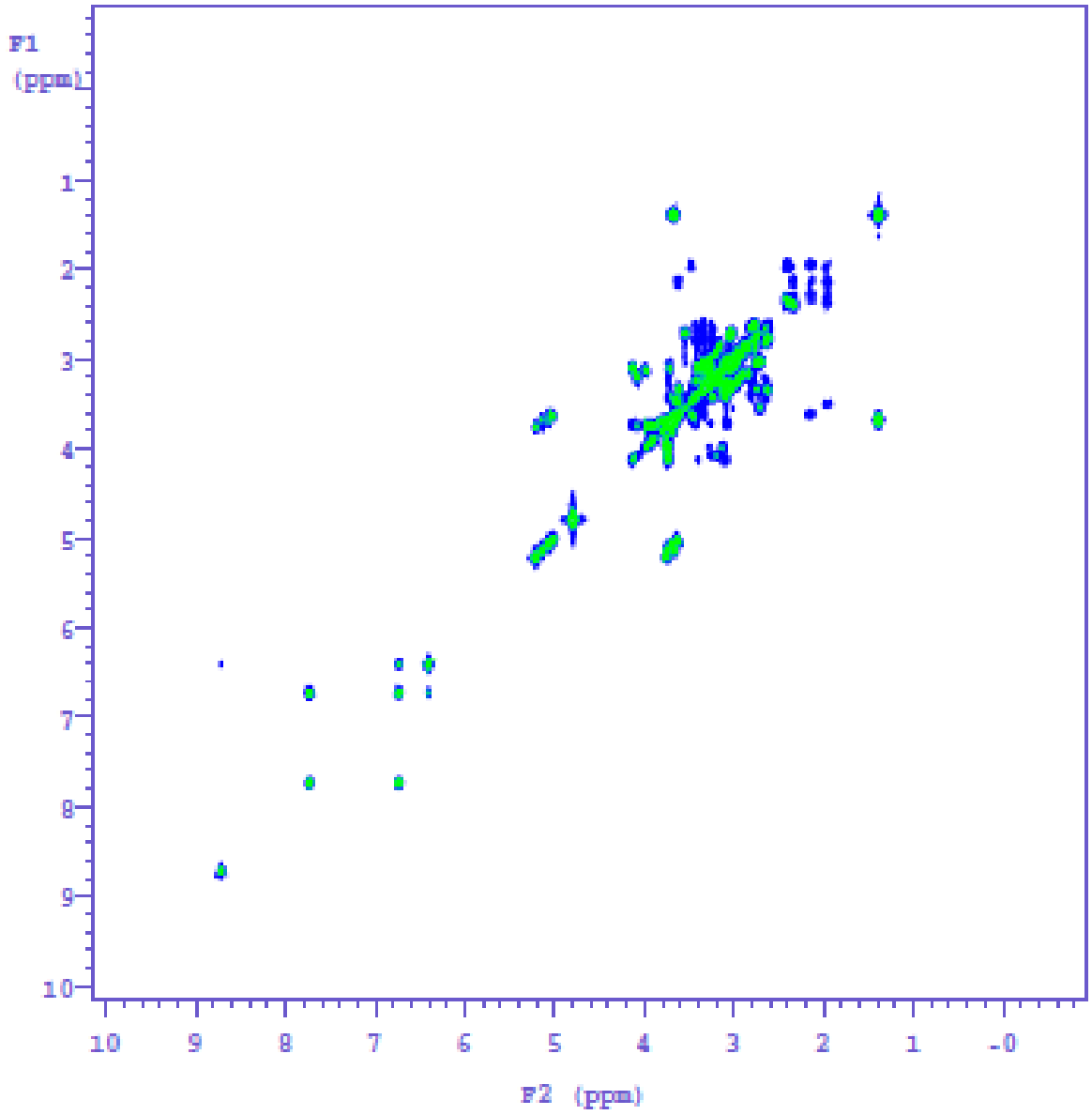
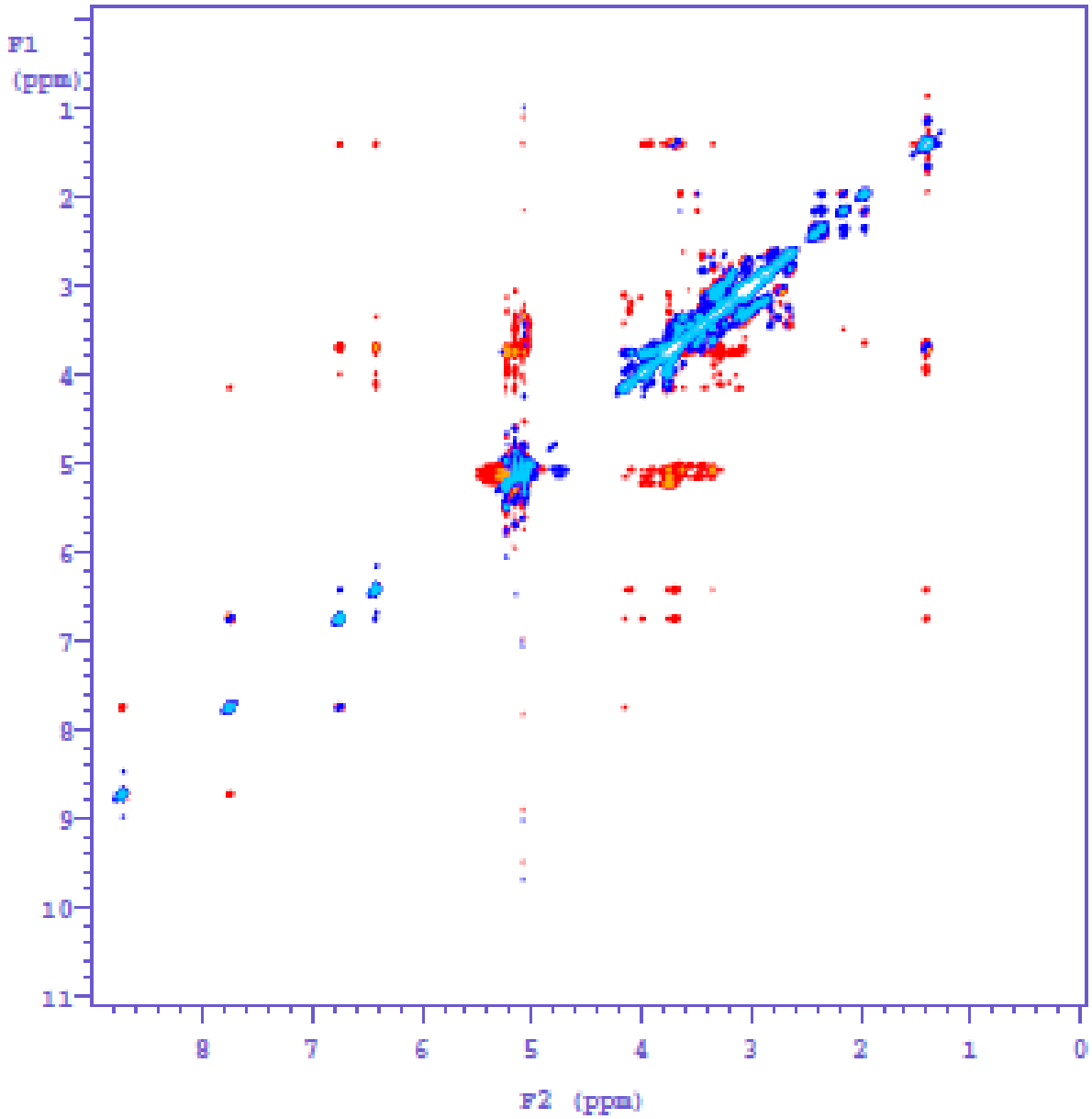
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)



2D TOCSY of **3** (9.5±0.5 × 10^{-3}M) 700ms mixing time.



gCOSY of **3** (9.5±0.5 × 10^{-3}M).



ROESY of **3** (9.5±0.5 × 10^{-3}M) 300ms mixing time.

References

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2. P. Gagnon, X. Huang, E. Therrien and J. W. Keillor, *Tetrahedron Letters*, 2002, **43**, 7717-7719.
3. R. B. C. Jagt, R. F. Gomez-Biagi and M. Nitz, *Angew. Chem., Int. Ed.*, 2009, **48**, 1995-1997.