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Supplementary Information for

Live-Cell Epigenome Manipulation by Synthetic Histone Acetylation Catalyst System

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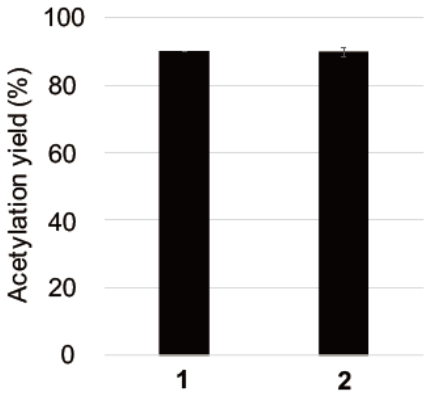


Figure S1. LC-MS/MS analysis of acetylation yield of H2BK120 on recombinant nucleosomes (0.37 μM) treated with **3** (10 mM) and TCEP (0.1 mM) with **1** or **2** (2 μM) at 37 ºC for 5 h. Error bars represent the data range of two independent experiments.

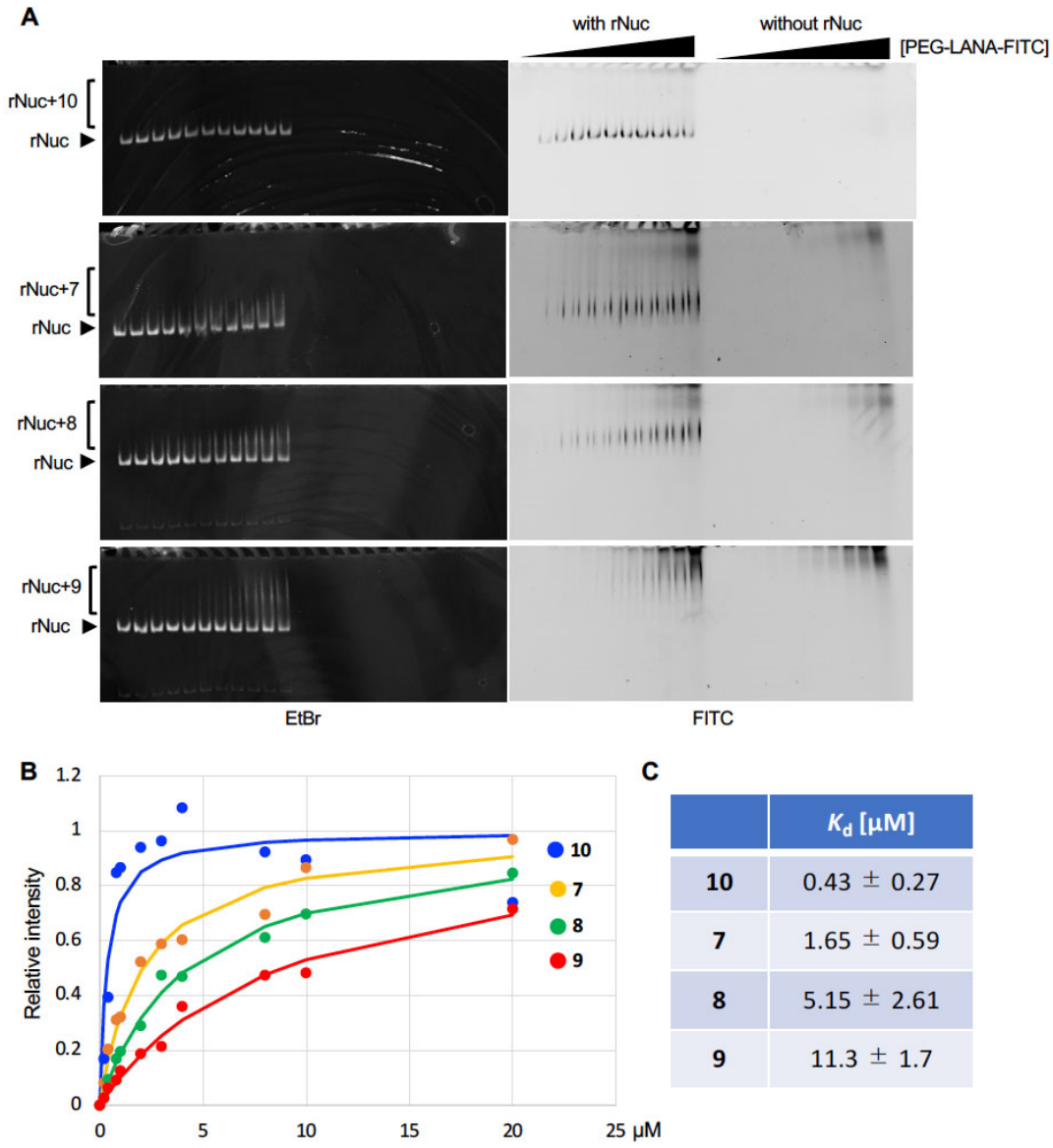
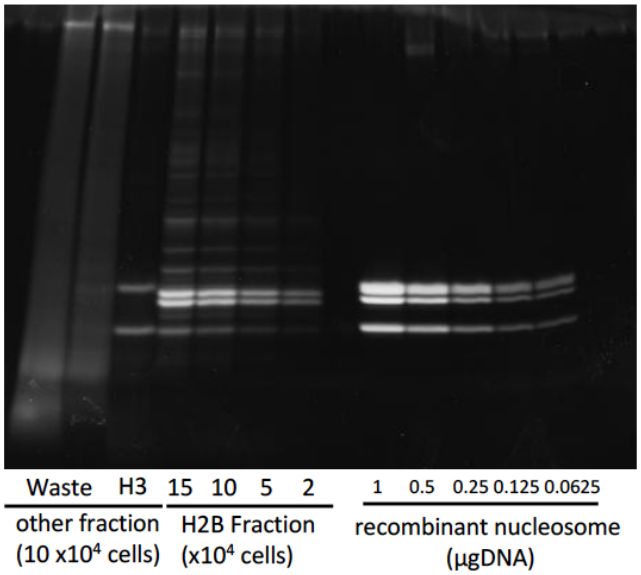


Figure S2. Electrophoretic mobility shift assay. (A) The indicated compounds (0, 0.2, 0.4, 0.8, 1, 2, 3, 4, 8, 10, and 20 μM) were incubated with or without recombinant nucleosomes (0.4 μM), in the presence of 150 mM KCl. The samples were then analyzed by 6% non-denaturing PAGE in 0.2 x TBE buffer. Recombinant nucleosomes were visualized by ethidium bromide staining (left). FITC signal of PEG-LANA-FITCs were detected (right). (B) Plot of relative FITC signals of each concentration of PEG-LANA-FITCs (μM) to those when all nucleosomes bound to PEG-LANA-FITC. Data were fit to a Hill slope model yielding K_d values. Fitted curves were described by lines of each color. (C) Calculated K_d values of **7**-**10** based on Michaelis-Menten equation. See "Electrophoresis Mobility Shift Assay" section for details. Error bars represent standard deviations of three independent experiments.



Unit conversion of 147 bp DNA	1 μg = 10.3 pmol
Nuclear radius	6 μm
Vol. of nucleus	905 pL/cell
Conc. of nucleosomes	~50 μM
Conc. of acidic patches	~100 μM

Figure S3. Estimation of histone concentration in cells. SDS-PAGE image of purified histones from cells and recombinant nucleosomes was shown. Histone was visualized by Oriole Fluorescent Gel Stain. The amount of nucleosome was calculated by calibration curve of H2A intensity of recombinant nucleosomes. Parameters used for calculation of the K_d value of PEG-LANA-FITC **7** in cells and the concentration of nucleosomes or acidic patches were shown in the table. See "Estimation of in-cell K_d of PEG-LANA-FITC" section for details.

Yield (%)	**11+ 3+**	**11– 3+**	**11– 3–**
H2A K5	0.55 (±0.06)	0.46 (±0.02)	N.D.
H2A K9	0.77 (±0.14)	0.23 (±0.02)	N.D.
H2B K116	10.39 (±0.63)	1.16 (±0.25)	N.D.
H2B K120	81.01 (±1.28)	N.D.	N.D.
H2B K125	4.94 (±2.18)	1.98 (±0.16)	N.D.
H3 K9	1.04 (±0.11)	0.73 (±0.06)	1.00 (±0.50)
H3 K14	1.05 (±0.18)	0.77 (±0.06)	0.50 (±0.01)
H3 K18	0.86 (±0.41)	0.82 (±0.01)	N.D.
H3 K23	1.09 (±0.50)	1.02 (±0.09)	0.39 (±0.05)
H3 K27Ac	0.91 (±0.33)	0.84 (±0.01)	N.D.
H3 K36Ac	0.55 (±0.52)	N.D.	N.D.
H3 K37Ac	0.54 (±0.16)	0.57 (±0.03)	N.D.
H4 K5	1.18 (±1.17)	1.61 (±0.01)	N.D.
H4 K8	N.D.	0.70 (±0.47)	N.D.
H4 K12	1.13 (±0.16)	1.15 (±0.59)	N.D.
H4 K16	2.56 (±0.24)	2.36 (±0.17)	0.25 (±0.15)
eDHFR K32	5.83 (±2.31)	4.37 (±0.84)	N.D.
eDHFR K38	2.65 (±0.97)	0.73 (±0.22)	0.32 (±0.24)
eDHFR K58	3.84 (±1.10)	2.02 (±0.58)	N.D.
eDHFR K76	N.D.	N.D.	N.D.
eDHFR K106	N.D.	N.D.	N.D.
eDHFR K109	N.D.	N.D.	N.D.

Figure S4. Regioselective histone acetylation in recombinant nucleosomes by PEG-LANA-DSSMe catalyst. LC-MS/MS analysis of acetylation yield of each lysine residue on nucleosomes (33 ng DNA/μL, 0.37 μM) and eDHFR-GFP (1 μM) treated with TCEP (0.1 mM), with or without **11** (10 μM) and **3** (30 mM) were shown. The average and error range are indicated (n = 2 independent experiments). N.D. not detected in either or both experiments.

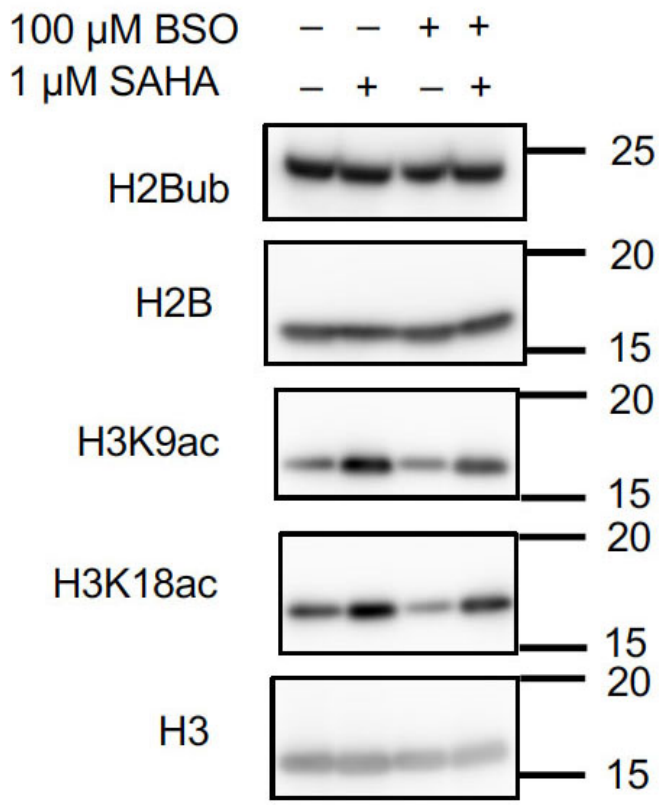


Figure S5. Analyses of histone acetylation and ubiquitination in BSO- or HDAC inhibitor-treated cells. HeLa S3 cells pretreated with or without BSO (100 μM) for 24 h were incubated with or without SAHA (1 μM) and BSO (100 μM) in DMEM at 37 ºC for 6 h. The level of H2BK120ac, H2BK120ub (H2Bub), and H3-tail acetylation (H3K9ac and H3K18ac) were examined by immunoblotting.

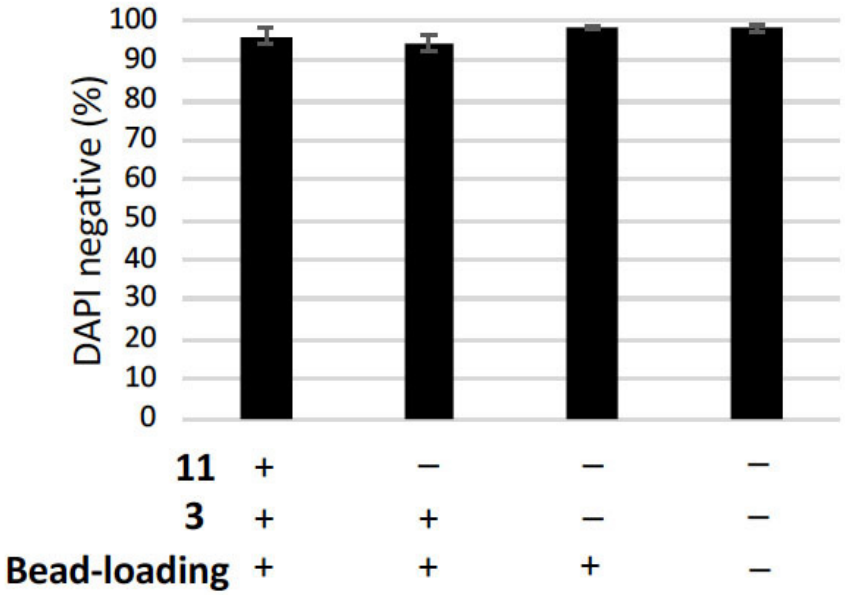
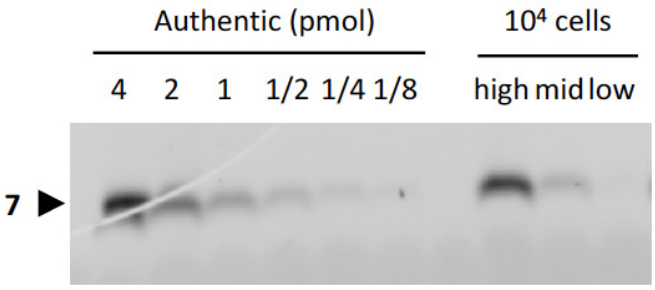


Figure S6. Examination of cell viability in in-cell reaction. The percentage of DAPI negative cells in Figure 3E were shown. The average and error range (bars) are indicated (n = 2 independent experiments).



	x10^{-16} mol / cell
high	3.2
mid	0.67
low	0.054

Figure S7. Estimation of concentration of **7** in cells in the three fractions ("high", "mid", and "low") sorted by flow cytometry. SDS-PAGE image of PEG_{550}-LANA-FITC **7** was shown. **7** recovered from 10^4 cells of "high", "mid", or "low" fraction was loaded. As reference, the indicated amount of **7** was loaded (Authentic). The amount of **7** in each fraction was calculated by calibration curve of authentic compounds.

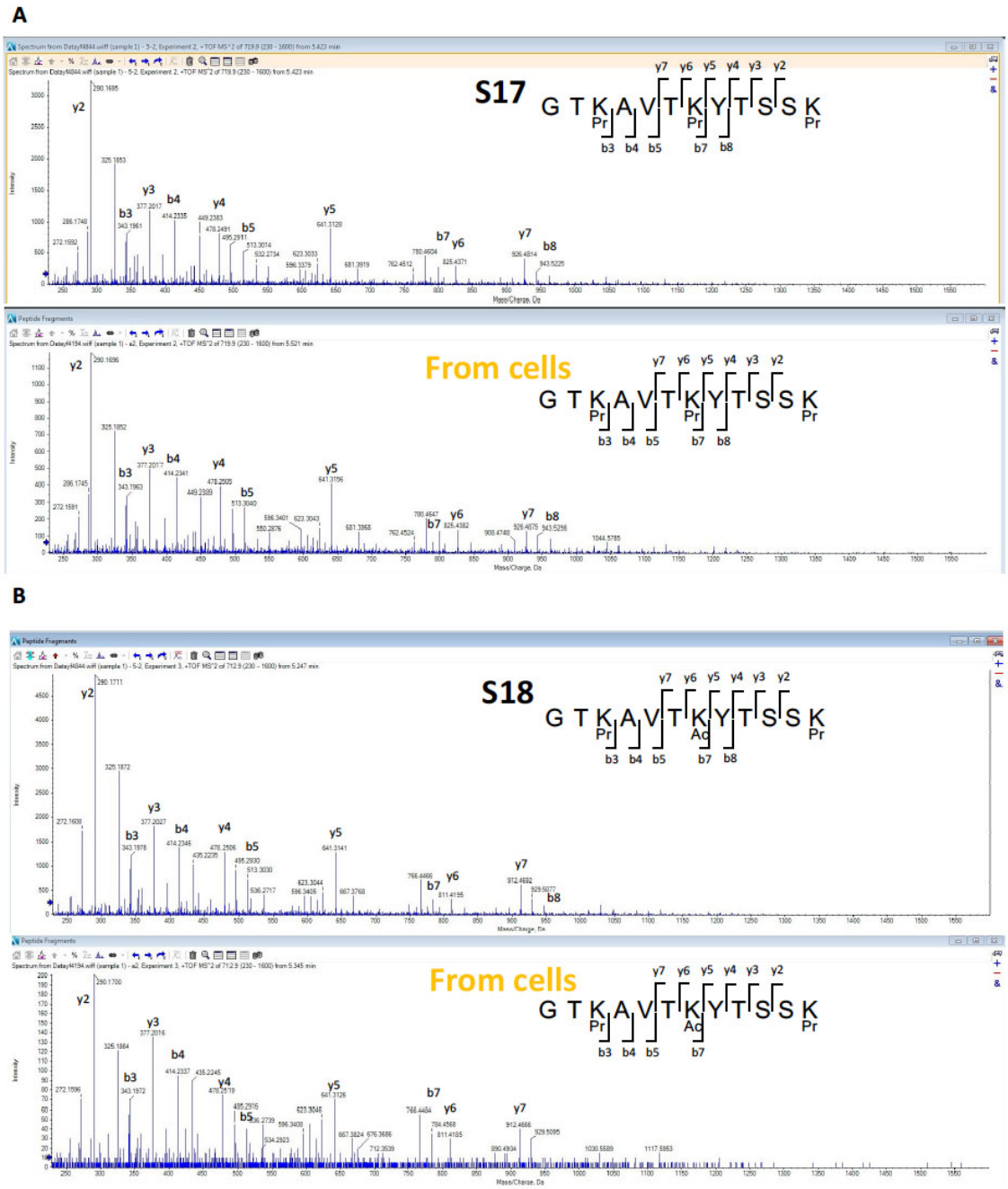


Figure S8. Characterization of H2B G114-K125 peptides identified by LC-MS/MS. LC-MS/MS spectra of the doubly charged, (A) H2BK120-propionylated peptides and (B) H2BK120-acetylated peptides were shown. It is noted that unmodified lysines were propionylated before LC-MS/MS analysis. Upper panel shows LC-MS/MS spectra of synthesized authentic peptide **S17** or **S18** (2 μM). Lower panel shows LC-MS/MS spectra of peptides recovered from cells after in-cell reaction.

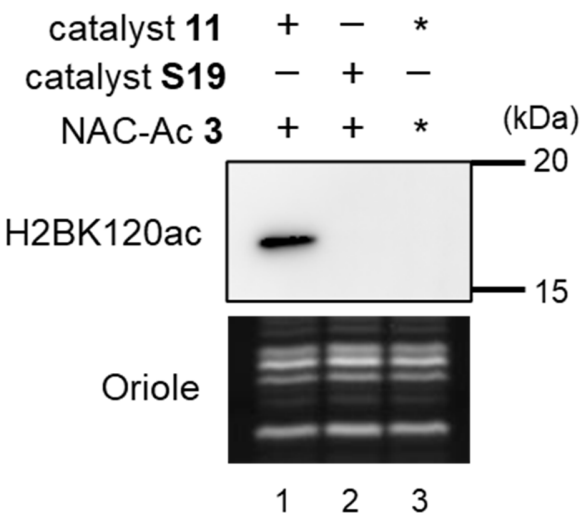


Figure S9. Additional data for the acetylation reaction by PEG-LANA-DSH in living cells. HeLa S3 cells pretreated with BSO (100 μM) were loaded with **11** or **S19** (500 μM), TCEP (2 mM), Dextran-Rhodamine (0.5 mg/mL), and **3** (30 mM), and incubated with growth medium containing **3** (30 mM) at 37 ºC for 8 h. After sorting living cells by flow cytometry, histones were extracted by acid extraction. For lane 3 (*), two populations of cells were prepared. In one population, HeLa S3 cells pretreated with BSO (100 μM) were loaded with **11** (500 μM), TCEP (2 mM), and Dextran-Rhodamine (0.5 mg/mL), and incubated with growth medium at 37 ºC for 8 h. In the other population, HeLa S3 cells pretreated with BSO (100 μM) were loaded with **3** (30 mM), TCEP (2 mM), and Dextran-Rhodamine (0.5 mg/mL), and incubated with growth medium containing **3** (30 mM) at 37 ºC for 8 h. After sorting living cells separately, two populations were mixed and histones were extracted. The level of H2BK120ac was examined by immunoblotting. Protein amount is indicated by Oriole staining. Representative data of two independent experiments are shown.

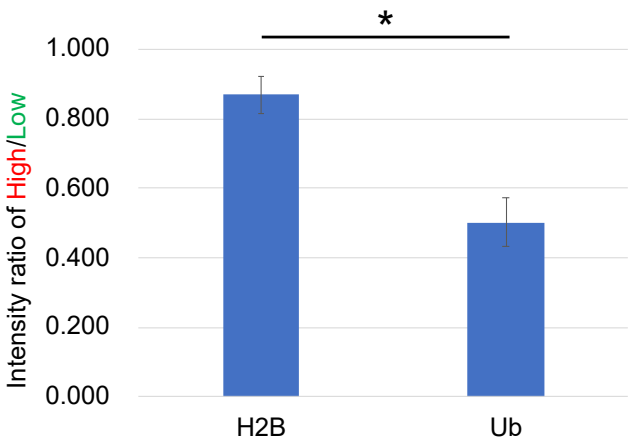


Figure S10. Additional data for Figure 4C.
The western blot data of H2B and H2BK120 ubiquitination in “High” or “Low” cell fraction obtained through sorting by flow cytometry was quantified by Fiji/ImageJ ver. 1.51h. The average intensity ratios of “High”/”Low” were shown. Error bars indicate SE (n=4 western blot data for two independent experiments). The asterisk indicates significant values for the two-tailed Student‘s t test: *$p<0.05$.

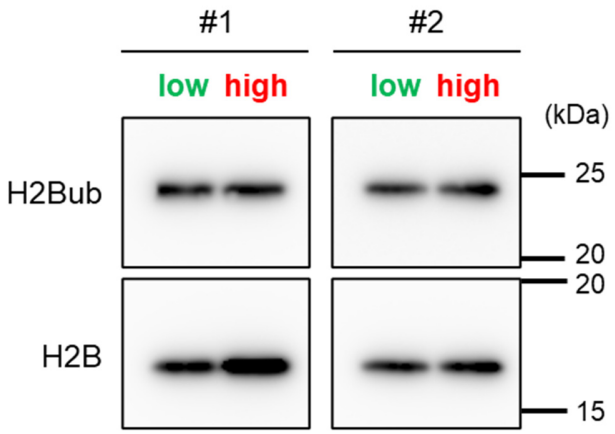


Figure S11. Effect of synthetic histone acetylation by PEG-LANA-DSH on H2B ubiquitination after 18 h of the reaction. HeLa S3 cells pretreated with BSO (100 μM) were loaded with **11** (500 μM), TCEP (2 mM), Dextran-Rhodamine (0.5 mg/mL), and **3** (30 mM), incubated with growth medium containing **3** (30 mM) at 37 ºC for 8 h and further incubated with growth medium in the absence of catalysts and donors at 37 ºC for 18 h. Acid-extracted histones from “high” or “low” cell fraction were analyzed by western blotting. Levels of H2BK120 ubiquitination and H2B in H2BK120-acetylated (high) or non-acetylated (low) cells were shown. Data from two independent experiments (#1 and #2) are shown. Decrease of H2B ubiquitination was not clearly observed in these cases, suggesting that turnover of H2B ubiquitination may not be sufficient for 18-h incubation. Note that we observed significant decrease of H2B ubiquitination after 36-h incubation (Figure 4C and S10).

Table S1. Targeted precursor ions and collision energies for H2BK120 containing peptides.

Peptide	Sequence	Precursor ion (m/z)	fragment ions	collision energy (V)	retention time (min)
H2B 114-125 (rNuc)	GTKAVT KYTSAK	711.90 (3Pr)	b_3, b_4, b_5, b_6 y_2, y_3, y_4, y_5	35	6.1-6.2
		704.89 (1Ac 2Pr)		35	6.0-6.1
		697.88 (2Ac 1Pr)		35	6.0-6.1
H2B 114-125 (in-cell)	GTKAVT KYTSSK	719.89 (3Pr)	b_3, b_4, b_5, b_6 y_2, y_3, y_4, y_5	40	5.4-5.7
		712.89 (1Ac 2Pr)		40	5.2-5.5
		705.88 (2Ac 1Pr)		40	4.7-4.9

Table S2. Targeted precursor ions and collision energies of eDHFR-GFP.

Peptide	Sequence	Precursor ion (m/z)	fragment ions	collision energy (V)	retention time (min)
18-33	NAMPWNLPADLAWFKR	993.51 (Pr)	b_7, y_9, y_{13}, y_{14}	43	8.2-8.4
		986.50 (Ac)		43	8.1-8.3
34-44	NTLNKPVIMGR	649.87 (Pr)	y_6, y_7, y_8, y_9	43	5.9-6.0
		642.86 (Ac)		43	5.8-5.9
58-71	KNIILSSQPGTDDR	800.42 (Pr)	b_3, y_6, y_9, y_{10}	43	5.7-5.9
		793.41 (Ac)		43	5.6-5.8
72-80	VTWVKSVDE	1118.57 (Pr)	b_5, b_7, b_8, y_5	43	6.3-6.6
		1104.56 (Ac)		43	n.d.
102-118	QFLPKAQKLYLTHIDAE	1064.08 (2Pr)	b_6, y_{10}, y_{11}, y_{12}	43	7.3-7.4
		1057.07 (1Ac 1Pr)		43	n.d.

n.d. not detected.

Experimental procedures

General

NMR spectra were recorded on JEOL ECX500 spectrometer, operating at 500 MHz for ^{1}H NMR and 124.51 MHz for ^{13}C NMR, or JEOL ECS400 spectrometer, operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Chemical shifts were reported in ppm on the δ scale relative to residual $CHCl_3$ (δ = 7.24 for ^{1}H NMR and δ = 77.0 for ^{13}C NMR), Acetone-d_6 (δ = 2.04 for ^{1}H NMR and δ = 29.8 for ^{13}C NMR), DMSO-d_6 (δ = 2.49 for ^{1}H NMR and δ = 39.5 for ^{13}C NMR) as an internal reference, respectively. Gel permeation chromatographies (GPC) were performed with JAI LC-920 NEXT. Preparative HPLC was conducted by using a JASCO HPLC system equipped with a UV-2075 spectrometer, PU-4086 pumps, a DG-4580 degasser, an MX-2080-32 mixer. Analytical HPLC was conducted by using a JASCO HPLC system equipped with a UV-2075 spectrometer, PU-4180 pumps, a DG-4580 degasser, and an MX-2080-32 mixer. ESI-MS spectra were measured on Agilent Technologies 6120 (for LRMS) or Bruker micrOTOF II spectrometer (for HRMS). MALDI-TOFMS was obtained with a Shimadzu Biotech Axima ToF^2 spectrometer. LC-MS/MS analyses were conducted using AB Sciex Triple TOF 4600 equipped with eksigent ekspert microLC 200, and data analysis was carried out on PeakView software (AB Sciex, version 1.2.0.3).

Materials

Poly(ethylene glycol) methyl ethers (M_n = 550, 750, and 2000) were purchased from Sigma-Aldrich (#202487, 202495, and 202509, respectively). All protected α-amino acids were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan) and Peptide Institute, Inc. (Osaka, Japan). NovaPEG Rink amide AM resin was purchased from Merck KGaA, Co. Thin layer chromatography (TLC) was performed on Merck Kieselgel 60 F254 (0.25 mm) plates. Column chromatography was performed with Kanto silica gel 60 (40-50 mesh). Other chemicals were used as received from commercial sources, unless otherwise stated.

Analytical HPLC

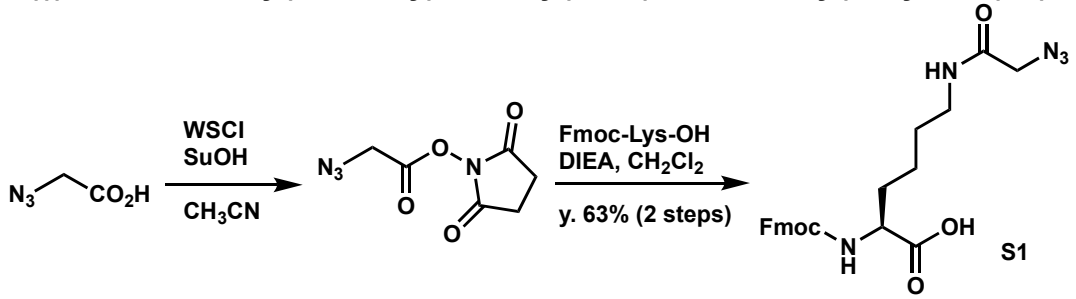
Analytical HPLC was performed using YMC-Triart C18 or YMC-Triart Phenyl (4.6 mm I.D. x 150 mm) column at 40 ºC with a gradient of acetonitrile in 0.1% aqueous TFA listed below at a flow rate of 1 mL/min.
Method A: YMC-Triart C18, 4.6 mm I.D. x 150 mm, linear gradient of 2-90% acetonitrile in 0.1% aqueous TFA over 3-15 min (UV: 254 nm)
Method B: YMC-Triart C18, 4.6 mm I.D. x 150 mm, linear gradient of 0-60% acetonitrile in 0.1% aqueous TFA over 5-25 min (UV: 254 nm)
Method C: YMC-Triart Phenyl C18, 4.6 mm I.D. x 150 mm, linear gradient of 2-90% acetonitrile in 0.1% aqueous TFA over 3-15 min (UV: 254 nm)
Method D: YMC-Triart Phenyl C18, 4.6 mm I.D. x 150 mm, linear gradient of 2-90% acetonitrile in 0.1% aqueous TFA over 3-15 min (UV: 230 nm)

Preparative HPLC

Preparative HPLC was performed using YMC-Triart C18 or YMC-Pack ODS-AM (20 mm I.D. x 250 mm or 10 mm I.D. x 250 mm) column at 40 ^{o}C with a gradient of acetonitrile in 0.1% aqueous TFA listed below at a flow rate of 10 or 3 mL/min. Method a: 2% acetonitrile for 3 min, followed by a linear gradient of 2–100% acetonitrile over 47 min with a flow rate of 3 mL/min. YMC-Triart C18, 230 nm. Method b: 2% acetonitrile for 3 min, followed by a linear gradient of 2–100% acetonitrile over 47 min with a flow rate of 10 mL/min. YMC-Triart C18, 230 nm. Method c: 25% acetonitrile for 5 min, followed by a linear gradient of 25–100% acetonitrile over 45 min with a flow rate of 10 mL/min. YMC-Triart C18, 254 nm. Method d: 50% acetonitrile for 5 min, followed by a linear gradient of 50-100% acetonitrile over 30 min with a flow rate of 3 mL/min. YMC-Triart C18, 254 nm. Method e: 25% acetonitrile for 5 min, followed by a linear gradient of 25-100% acetonitrile over 40 min with a flow rate of 3 mL/min. YMC-Triart C18, 254 nm. Method f: 25% acetonitrile for 5 min, followed by a linear gradient of 25-100% acetonitrile over 30 min with a flow rate of 3 mL/min. YMC-Triart C18, 254 nm. Method g: 0% acetonitrile for 5 min, followed by a linear gradient of 0-60% acetonitrile over 50 min with a flow rate of 10 mL/min. YMC-Triart C18, 230 nm. Method h: 0% acetonitrile for 5 min, followed by a linear gradient of 0-60% acetonitrile over 30 min with a flow rate of 10 mL/min. YMC-Triart C18, 254 nm. Method i: 0% acetonitrile for 5 min, followed by a linear gradient of 0-60% acetonitrile over 50 min with a flow rate of 10 mL/min. YMC-Triart C18, 254 nm. Method j; 0% acetonitrile for 5 min, followed by a linear gradient of 0-90% acetonitrile over 50 min with a flow rate of 10 mL/min. YMC-Triart C18, 254 nm. Method k; 0% acetonitrile for 5 min, followed by a linear gradient of 0-60% acetonitrile over 40 min with a flow rate of 10 mL/min. YMC-Triart C18, 254 nm. Method l; 10% acetonitrile for 10 min, followed by a linear gradient of 10-90% acetonitrile over 50 min with a flow rate of 10 mL/min. YMC-Triart C18, 230 nm.

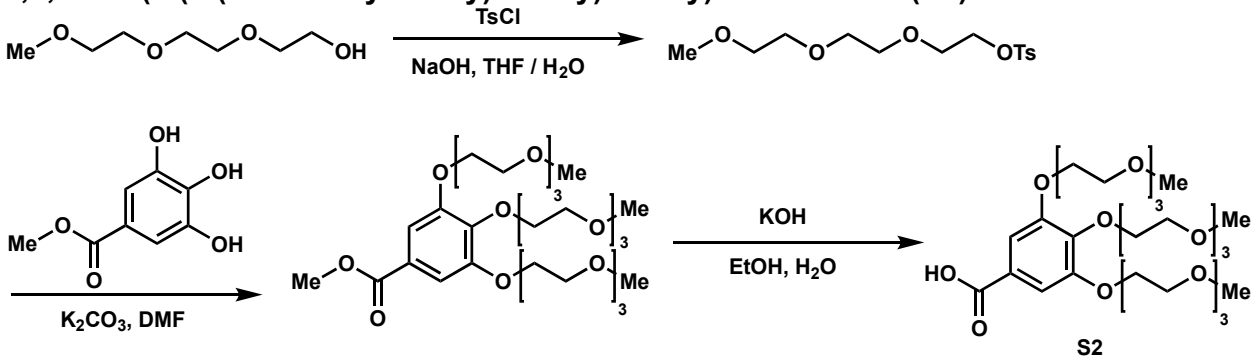
Synthesis

N^2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-N^6-(2-azidoacetyl)-L-lysine (S1)



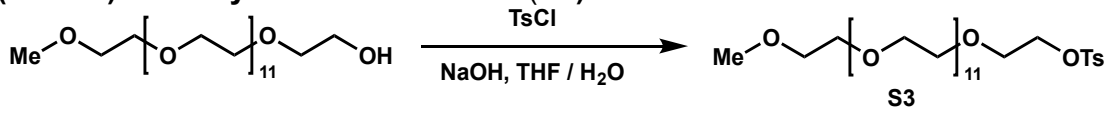
To a stirred solution of 2-azidoacetic acid (810 μL, 10.8 mmol) and *N*-hydroxysuccinimide (702 mg, 6.10 mmol) in acetonitrile (7.2 mL), WSCI•HCl (1.41 g, 7.35 mmol) was added at r.t., and the mixture was stirred for 3.5 h. After evaporation of acetonitrile, the residue was dissolved in 72 mL of CH_2Cl_2. To the solution, Fmoc-Lys-OH•AcOEt (3.00 g, 7.41 mmol) and *N,N*-diisopropylethylamine (3.81 mL, 28.2 mmol) were added at r.t. The mixture was stirred for 19 h, and 1 M HCl aq. was added. The mixture was extracted with CH_2Cl_2, and combined organic layers were concentrated. Purification with silica gel column chromatography (CH_2Cl_2/MeOH = 10/1) afforded **S1** (2.10 g, 4.65 mmol, y. 63%) as yellow solids. ^{1}H NMR (DMSO-d_6, 400 MHz) δ = 8.10 (brt, J = 5.5 Hz, 1H), 7.88 (d, J = 7.6 Hz, 2H), 7.72 (d, J = 7.6 Hz, 2H), 7.63 (d, J = 8.2 Hz, 1H), 7.41 (dd, J = 7.6 Hz, 7.6 Hz, 2H), 7.32 (dd, J = 7.6 Hz, 7.6 Hz, 2H), 4.28 (d, J = 6.9 Hz, 2H), 4.22 (t, J = 6.9 Hz, 1H), 3.94-3.89 (m, 1H), 3.78 (s, 2H), 3.10-3.04 (m, 2H), 1.71-1.58 (m, 2H), 1.41-1.29 (m, 4H).
There is an unidentified singlet peak at 3.16 ppm.
^{13}C NMR (DMSO-d_6, 100 MHz) δ = 174.1, 167.1, 156.2, 143.9, 140.8, 127.7, 127.1, 125.3, 120.2, 65.7, 53.8, 50.8, 48,7, 46.7, 30.4, 28.6, 23.1
ESI-HRMS: *m*/*z* Calcd for $C_{23}H_{25}N_5NaO_5$ $[M+Na]^+$: 474.1748, Found: 474.1737.

3,4,5-Tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzoic acid (S2)



Carboxylic acid **S2** was synthesized as reported in the literature (1).

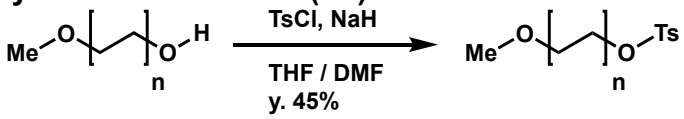
(PEG$_{550}$)-4-methylbenzenesulfonate (S3)



S3

Tosylate **S3** was synthesized as reported in the literature (2).

(PEG$_{750}$)-4-methylbenzenesulfonate (S4)



average n = 19 average n = 18

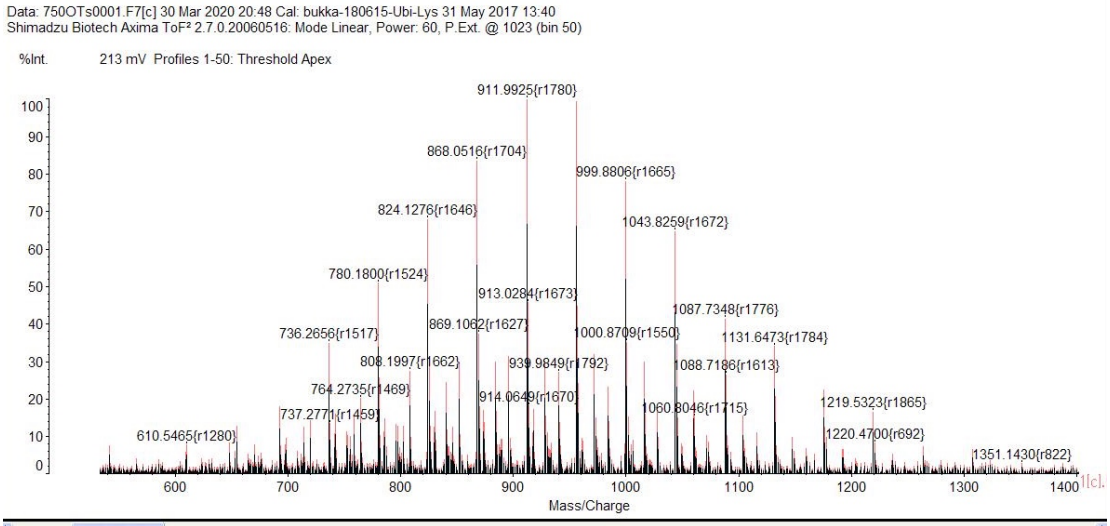
S4

Sodium hydride (60% dispersion in mineral oil) (1.00 g, 25.0 mmol) was dissolved in DMF (25 mL), and the solution of polyethylene glycol monomethyl ether (average Mn 750, 3.90 g, 5.20 mmol) in THF (25 mL) was added. After 4 h, *p*-toluenesulfonyl chloride (2.85 g, 15.0 mmol) was added portion-wise at 4 °C to the reaction mixture. After reaction at 80 ºC for 8 h, 25 mL H_2O was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with brine, and dried over Na_2SO_4. The organic layer was concentrated to give **S4** (2.28 g, 2.32 mmol, y. 45%) as colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ = 7.76, (d, *J* = 8.0 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 2H), 4.12 (t, *J* = 4.9 Hz, 2H), 3.66-3.50 (m, 70H), 3.34 (s, 3H), 2.41 (s, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ = 144.5, 132.7, 129.5, 127.6, 71.6, 70.4, 70.3, 70.2, 70.2, 70.2, 69.0, 68.3, 58.7, 21.3

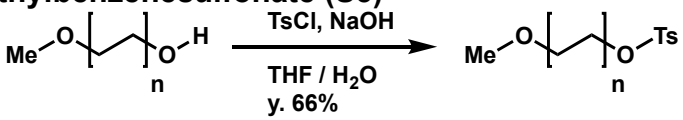
Based on the integration of the glycol protons, the average length of the PEG chain was n = 18.

MALDI-TOFMS spectrum of the product is shown below.



The peak of the highest intensity has *m*/*z* of 911.9, which corresponds to n = 16 ($C_{40}H_{74}NaO_{19}S$ $[M+Na]^+$: 913.4).

(PEG$_{2000}$)-4-methylbenzenesulfonate (S5)



average n = 43 average n = 43

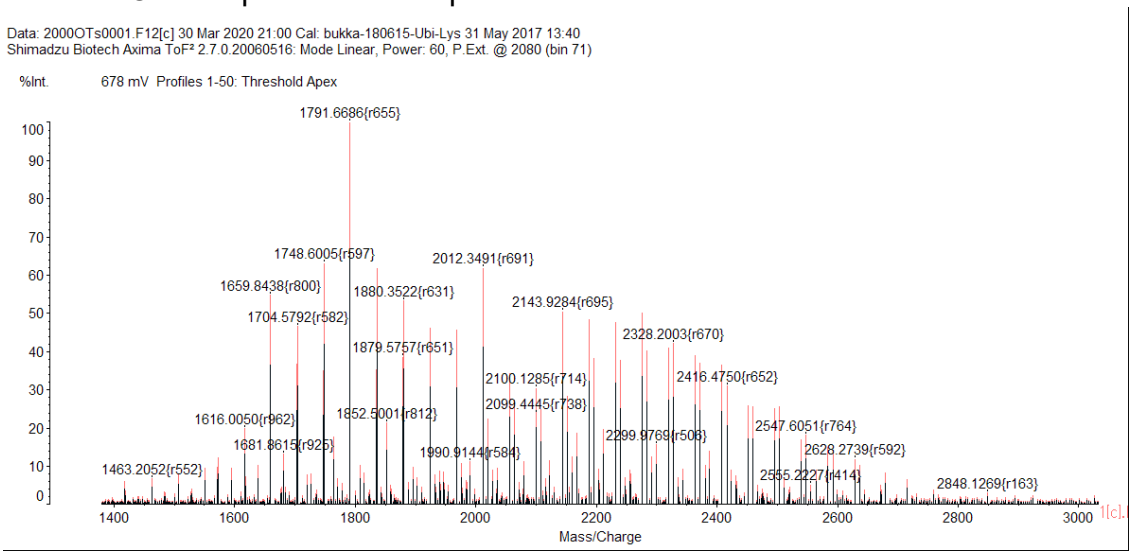
S5

Polyethylene glycol monomethyl ether (average Mn 2000, 1.0 g, 0.50 mmol) was dissolved in the mixture of 5 M NaOH aq. (10 mL) and THF (10 mL). To the reaction mixture, *p*-toluenesulfonyl chloride (475 mg, 2.50 mmol) was added portion-wise at 4 °C. After reaction at r.t. for 18 h, 75 mL H_2O was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with brine, and dried over Na_2SO_4. The organic layer was concentrated. The residue was purified with silica gel column chromatography (CH_2Cl_2:MeOH = 1:0 to 12.5:1) to give **S5** (688 mg, 330 μmol, y. 66%) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.72, (d, *J* =8.5 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 4.08 (t, *J* = 5.0 Hz, 2H), 3.76-3.38 (m, 170H), 3.30 (s, 3H), 2.38 (s, 3H).

^{13}C NMR ($CDCl_3$, 125 MHz) δ = 144.2, 132.5, 129.3, 127.4, 71.3, 70.1, 70.0, 69.9, 68.8, 68.1, 58.4, 21.1.

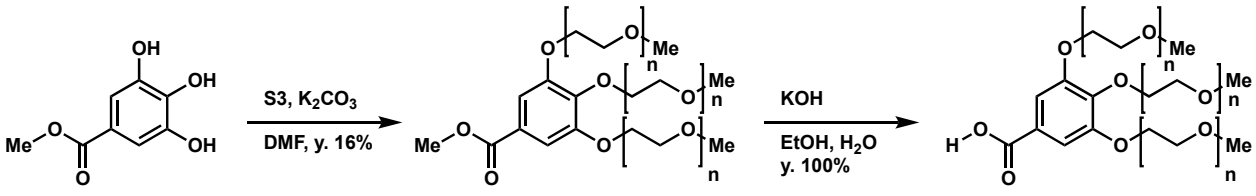
Based on the integration of the glycol protons, the average length of the PEG chain was n = 43.

MALDI-TOFMS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 2012.3, which corresponds to n = 41 ($C_{90}H_{174}NaO_{44}S$ $[M+Na]^+$: 2014.1).

3,4,5-Tris(PEG$_{550}$)-benzoic acid (S6)



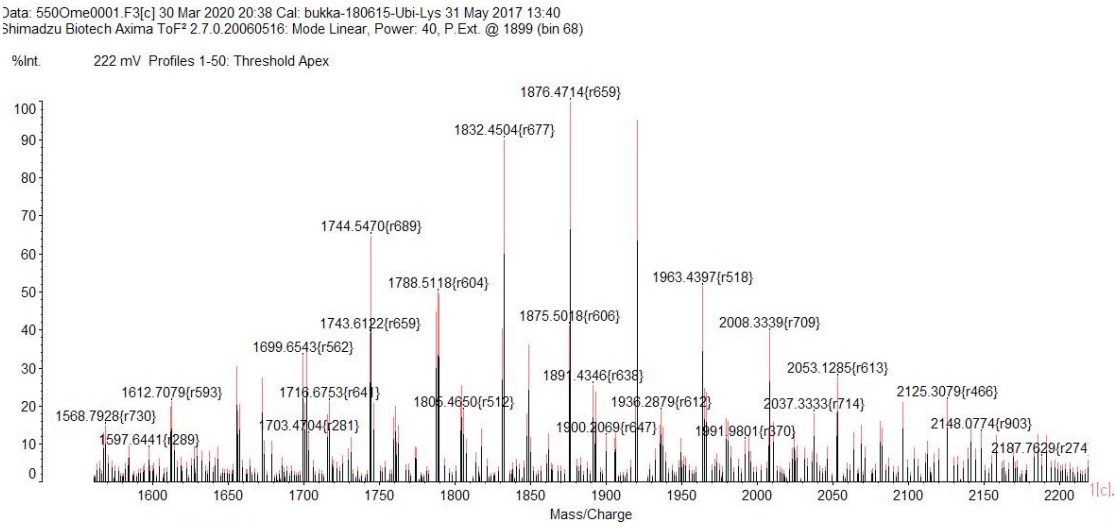
S6

Methyl gallate (180 mg, 0.978 mmol) and **S3** (3.03 g, 4.00 mmol) were dissolved in DMF (30 mL), and K_2CO_3 (1.40 g, 10.1 mmol) was added. The mixture was stirred at 80 ºC for 16 h, and the reaction mixture was filtered through Celite after cooling to room temperature. Water was added to the filtrate, and the aqueous layer was extracted with CH_2Cl_2. Combined organic layers were concentrated. Purification with silica gel column chromatography (CH_2Cl_2/MeOH = 40/1), followed by GPC, gave **Tris(PEG550)-gallate methyl ester** (320 mg, 0.154 mmol, 16%) as yellow oil. ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.20, (s, 2H), 4.13-4.09 (m, 6H), 3.76-3.74 (m, 7H), 3.68-3.66 (m, 3H), 3.59-3.40 (m, 144H), 3.37-3.33 (m, 7H), 3.16 (s, 9H).

^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166,8, 153.4, 143.7, 125.7, 109.6, 73.2, 72.6, 71.5, 71.4, 71.3, 71.3, 71.2, 71.2, 71.0, 70.3, 69.9, 58.8, 52.3.

Based on the integration of the glycol protons, the average length of the PEG chain was n = 14.

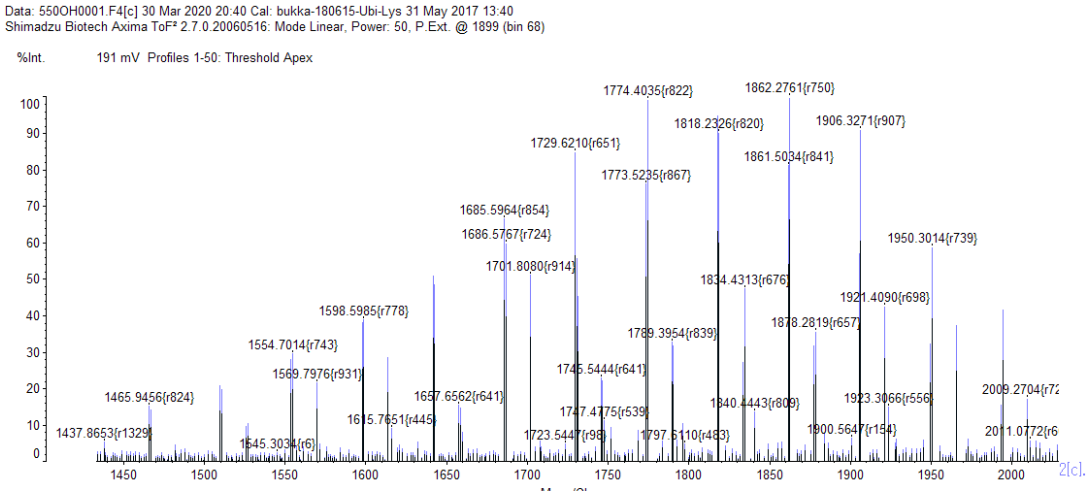
MALDI-TOFMS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 1832.4, which corresponds to n = 12 ($C_{83}H_{158}NaO_{41}$ $[M+Na]^+$: 1834.0).

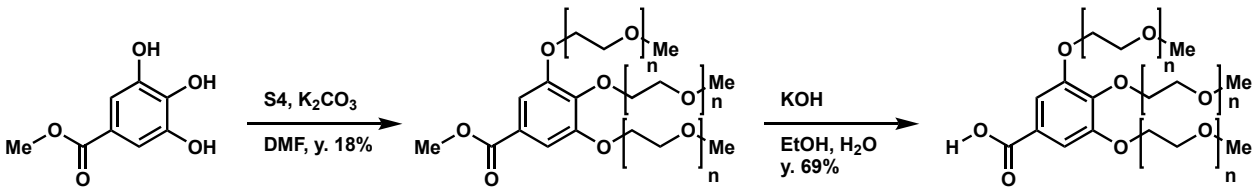
The ester was dissolved in EtOH (3 mL), and 1 M KOH aq. (320 µL, 0.320 mmol) was added. The mixture was stirred at 80 ºC for 15 h, and 1 M HCl aq. was added until the pH became 2. All the volatiles were removed under reduced pressure. The residue was extracted with CH_2Cl_2, and concentrated to afford **S6** (276 mg, 153

μmol, y. 100%) as yellow oil. ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.34, (s, 2H), 4.25-4.21 (m, 6H), 3.86 (t, *J* = 5.0 Hz, 4H), 3.79 (t, *J* = 5.5 Hz, 2H), 3.70-3.55 (m, 127H), 3.47-3.45 (m, 6H), 3.29 (s, 3H), 3.27 (s, 6H).
^{13}C NMR (Acetone-d6, 125 MHz) δ = 167,1, 153.2, 143.4, 126.1, 109.7, 73.4, 73.1, 72.5, 72.5, 71.7, 71.4, 71.3, 71.2, 71.2, 71.1, 71.1, 70.9, 70.9, 70.2, 69.7, 61.8, 58.7.
MALDI-TOFMS *m*/*z* Calcd for $C_{82}H_{156}NaO_{41}$ (n = 12) $[M+Na]^+$: 1820.0.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 12.
MALDI-TOFMS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 1818.23 which corresponds to n = 12 ($C_{82}H_{156}NaO_{41}$ $[M+Na]^+$: 1820.0).

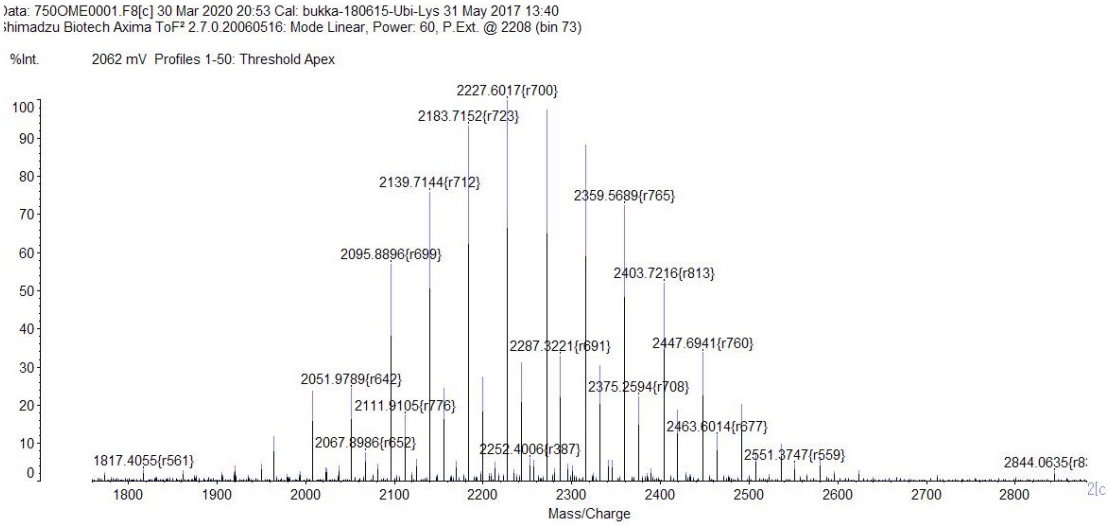
3,4,5-Tris(PEG$_{750}$)-benzoic acid (S7)



S7

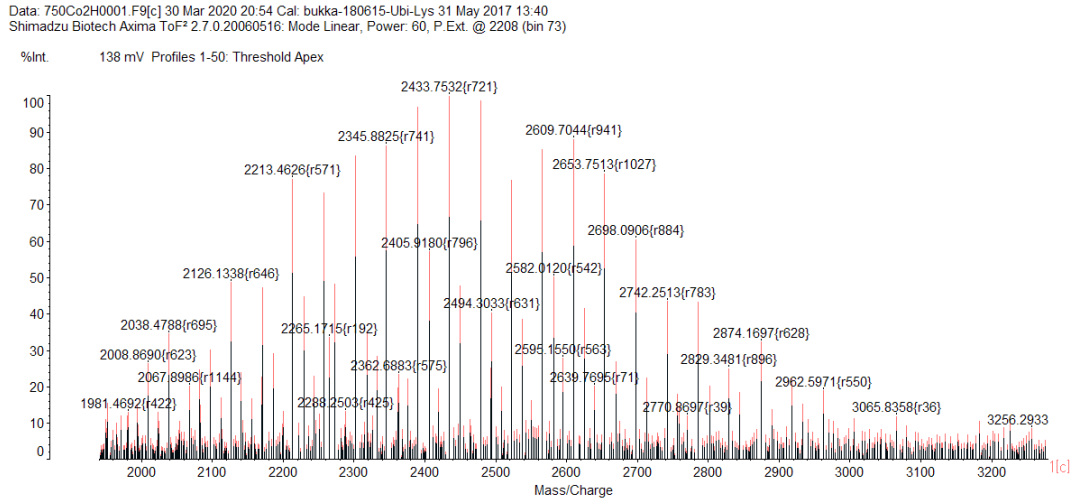
Methyl gallate (23 mg, 125 μmol) and **S4** (439 mg, 481 μmol) were dissolved in DMF (30 mL), and K_2CO_3 (173 mg, 1.25 mmol) was added. After the mixture was stirred at 80 ºC for 48 h, the reaction mixture was filtered through Celite. Water was added to the filtrate, and the aqueous layer was extracted with CH_2Cl_2. Combined organic layers were concentrated. Purification with silica gel column chromatography (CH_2Cl_2/MeOH = 1/0 to 8/1), followed by GPC, gave **Tris(PEG750)-gallate methyl ester** (61.0 mg, 22.3 μmol, y. 18%) as yellow oil. ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.20, (s, 2H), 4.13-4.09 (m, 6H), 3.76-3.74 (m, 7H), 3.67 (t, *J* = 5.0 Hz, 2H), 3.67-3.37 (m, 201H), 3.39-3.34 (m, 6H), 3.16 (s, 9H).

^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166.8, 153.4, 143.7, 125.7, 109.6, 73.2, 72.6, 71.4, 71.4, 71.3, 71.3, 71.3, 71.2, 71.0, 70.3, 69.9, 58.8, 52.4.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 19.
MALDI-TOFMS spectrum of the product is shown below.

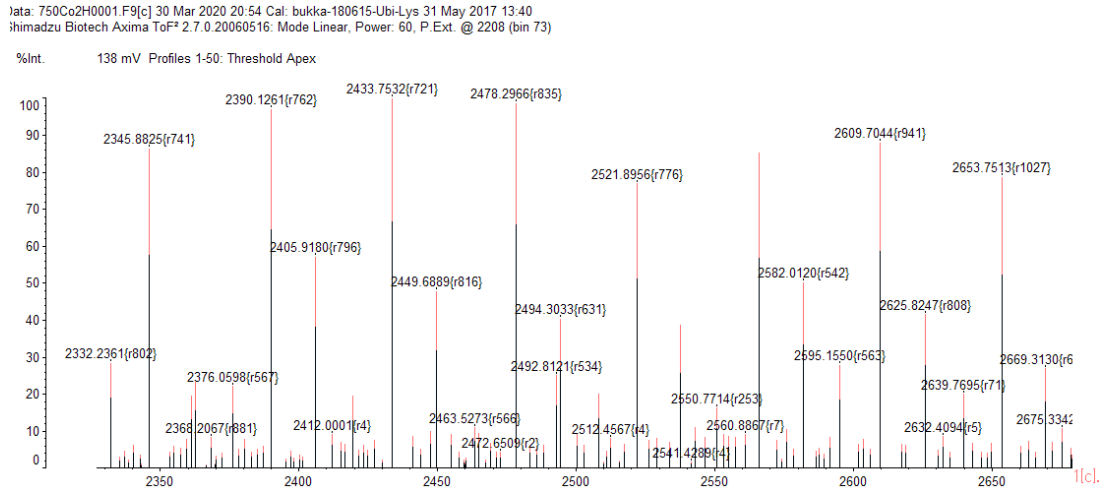


The peak of the highest intensity has *m*/*z* of 2227.6, which corresponds to n = 15 ($C_{101}H_{194}NaO_{50}$ $[M+Na]^+$: 2231.2).

The ester (343 mg, 0.125 mmol) was dissolved in EtOH (3 mL), and 1 M KOH aq. (250 µL, 0.250 mmol) was added. After the mixture was stirred at r.t. for 7 h, 1 M HCl aq was added until the pH became 1. All the volatiles were removed under reduced pressure. The residue was extracted by CH_2Cl_2, and concentrated to afford **S7** (247 mg, 86.5 µmol, y. 69%). ^{1}H NMR (Acetone-d_6, 500 MHz) δ = 7.22, (s, 2H), 4.13-4.09 (m, 6H), 3.75 (t, *J* = 4.6 Hz, 4H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.58-3.39 (m, 214H), 3.35-3.33 (m, 6H), 3.16 (s, 9H).
^{13}C NMR (Acetone-d_6, 125 MHz) δ = 167.1, 153.3, 143.4, 126.1, 109.7, 73.4, 73.1, 72.5, 71.4, 71.3, 71.2, 71.2, 71.2, 71.1, 71.0, 70.9, 70.2, 69.8, 61.8, 58.7.
MALDI-TOFMS *m*/*z* Calcd for $C_{100}H_{192}NaO_{50}$ (n = 15) $[M+Na]^+$: 2216.2.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 20.
MALDI-TOFMS spectrum of the product is shown below.

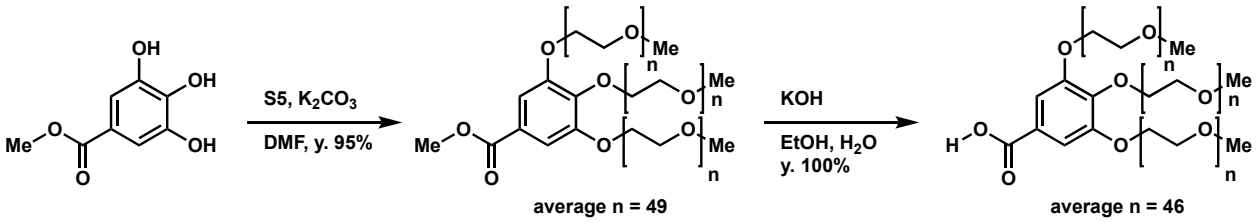


Zoomed view



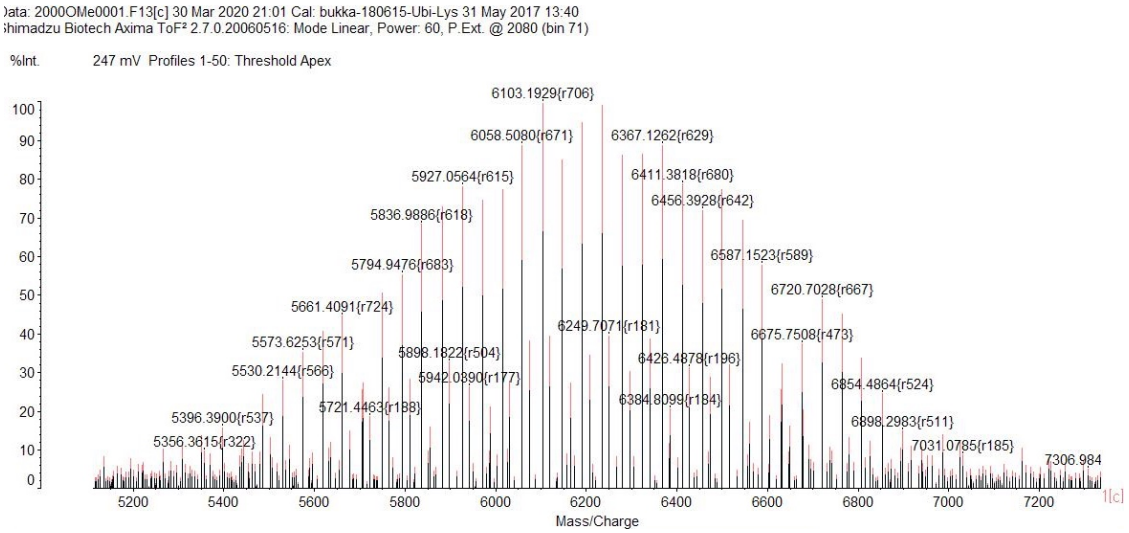
One of the peaks of the highest intensity has *m*/*z* of 2478.30 which corresponds to n = 17 ($C_{112}H_{216}NaO_{56}$ $[M+Na]^+$: 2481.4).

3,4,5-Tris(PEG2000)-benzoic acid (S8)



S8

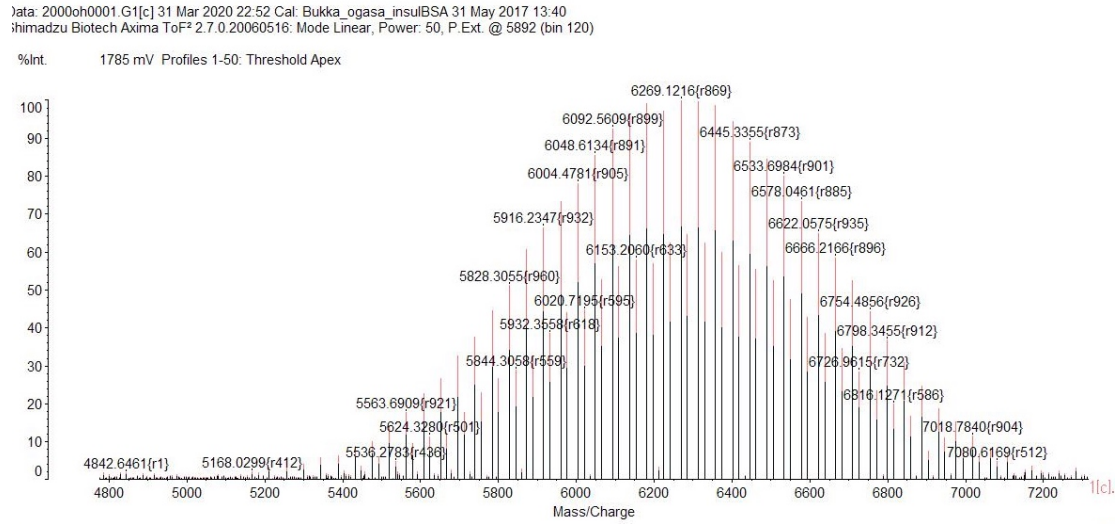
Methyl gallate (74 mg, 0.40 mmol) and **S5** (3.50 g, 1.68 mmol) were dissolved in DMF (12 mL), and K_2CO_3 (556 mg, 4.03 mmol) was added. After the mixture was stirred at 80 ºC for 58 h, the reaction mixture was filtered through Celite. Water was added to the filtrate. The aqueous layer was extracted with CH_2Cl_2, and concentrated. Purification with silica gel column chromatography (CH_2Cl_2/MeOH = 30/1 to 3/1) gave **Tris(PEG2000)-gallate methyl ester** (2.58 g, 0.38 mmol, y. 95%) as yellow solid. ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.21 (s, 2H), 4.14-4.10 (m, 6H), 3.76-3.74 (m, 7H), 3.68-3.67 (m, 2H), 3.66-3.63 (m, 4H), 3.59-3.38 (m, 562H), 3.36-3.34 (m, 8H), 3.29-3.27 (m,4H), 3.17 (s, 9H).
There is an unidentified singlet peak at 7.89 ppm.
^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166.8, 153.4, 143.6, 125.7, 109.5, 73.5, 73.2, 72.6, 71.8, 71.8, 71.7, 71.4, 71.4, 71.3, 71.2, 71.2, 71.2, 71.1, 71.0, 70.5, 70.5, 70.3, 69.9, 61.9, 58.7, 52.4.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 49.
MALDI-TOFMS spectrum of the product is shown below.



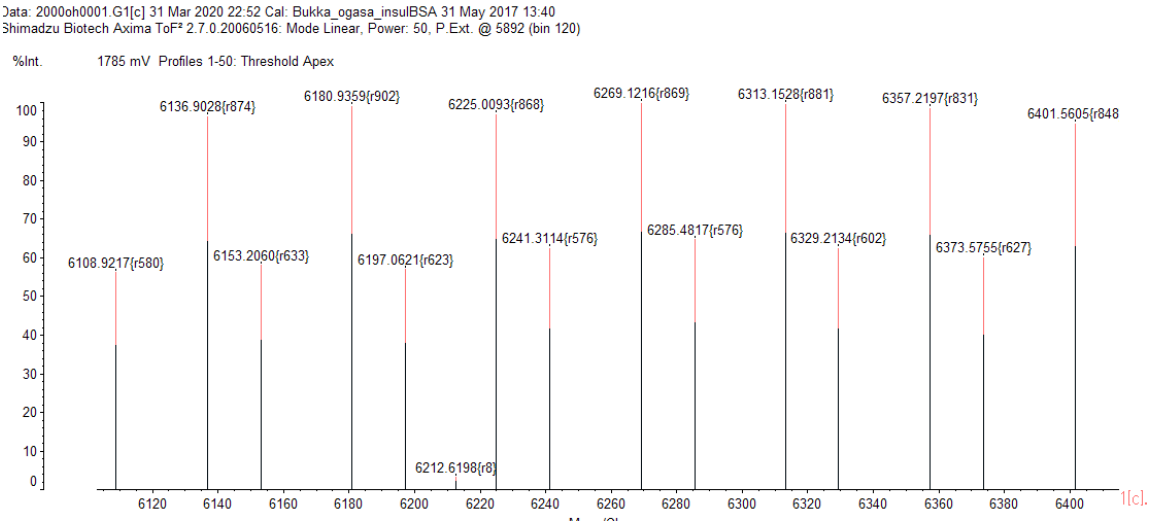
One of the peaks of the highest intensity has *m*/*z* of 6058.5, which corresponds to n = 44 ($C_{275}H_{542}NaO_{137}$ $[M+Na]^+$: 6062.5).

The ester (1.5 g, 0.22 mmol) was dissolved in EtOH (6.6 mL), and 1 M KOH aq. (440 µL, 0.440 mmol) was added. After the mixture was stirred at 80 ºC for 7 h, 1 M HCl aq was added until the pH became 1. All the volatiles were removed under

reduced pressure. The residue was extracted by CH_2Cl_2, and concentrated to afford **S8** (1.36 g, 0.22 mmol, y. 100%). ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.23 (s, 2H), 4.13-4.10 (m, 6H), 3.75 (t, *J* = 4.6 Hz, 4H), 3.68 (t, *J* = 5.5 Hz, 2H), 3.65-3.42 (m, 533H), 3.36-3.33 (m, 6H), 3.28 (t, *J* = 5.5 Hz, 2H), 3.17 (s, 9H).
^{13}C NMR (Acetone-d_6, 125 MHz) δ = 167,1, 153.3, 143.4, 126.1, 109.7, 73.4, 73.2, 72.6, 72.5, 71.8, 71.8, 71.6, 71.4, 71.3, 71.2, 71.2, 71.1, 71.0, 70.9, 70.5, 70.5, 70.3, 69.8, 61.8, 58.7.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 46.
MALDI-TOFMS spectrum of the product is shown below.

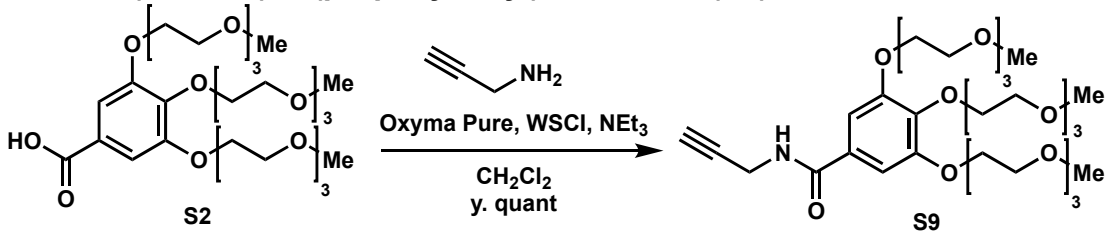


Zoomed view



One of the peak of the highest intensity has *m*/*z* of 6313.15, which corresponds to n = 46 ($C_{286}H_{564}NaO_{143}$ $[M+Na]^+$: 6312.6).

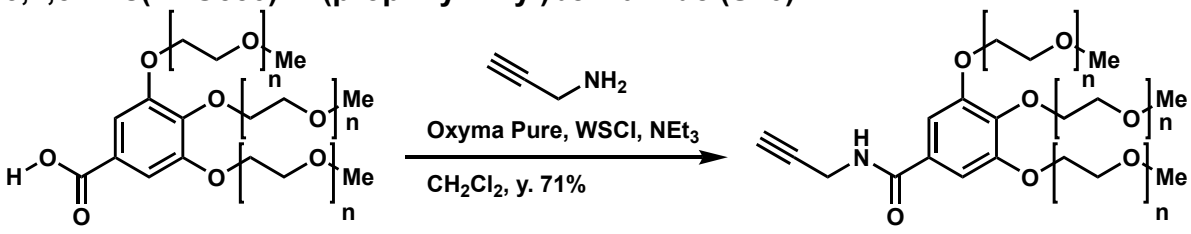
3,4,5-Tris(PEG150)-*N*-(prop-2-yn-1-yl)benzamide (S9)



S2 S9

Propargyl amine (11 mg, 0.20 mmol), **S2** (60.0 mg, 98.6 μmol), Oxyma Pure (7.10 mg, 50.0 μmol) and WSCI·HCl (38.0 mg, 198 μmol) were dissolved in CH_2Cl_2 (1 mL). To the solution, triethylamine (27.9 μL, 200 μmol) was added, and the mixture was stirred at r.t. for 22 h. To the mixture, 1 M HCl aq. was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq. and brine. The organic layer was concentrated to afford crude **S9**, which was purified with silica gel column chromatography (CH_2Cl_2/MeOH = 1/0 to 10/1) to afford **S9** (147 mg, 0.23 mmol, y. quant) as yellow oil. ^{1}H NMR (Acetone-d_6, 400 MHz) δ= 7.96 (brt, *J* = 5.0 Hz, 1H), 7.14 (s, 2H), 4.09-4.04 (m, 8H), 3.71 (t, *J* = 5.0 Hz, 4H), 3.65 (t, *J* = 5.0 Hz, 2H), 3.57-3.39 (m, 18H), 3.34 (t, *J* = 5.5 Hz, 6H), 3.15 (s, 9H), 2.55 (t, *J* = 2.3, 1H).
^{13}C NMR (Acetone-d_6, 100 MHz) δ = 166.4, 153.3, 142.0, 129.9, 107.5, 81.5, 73.1, 72.6, 71.9, 71.3, 71.2, 71.2, 71.1, 71.0, 70.3, 69.7, 58.7, 30.1.
ESI-HRMS: *m*/*z* Calcd for $C_{31}H_{52}NO_{13}$ $[M+H]^+$: 668.3253 Found: 668.3261.

3,4,5-Tris(PEG550)-*N*-(prop-2-yn-1-yl)benzamide (S10)

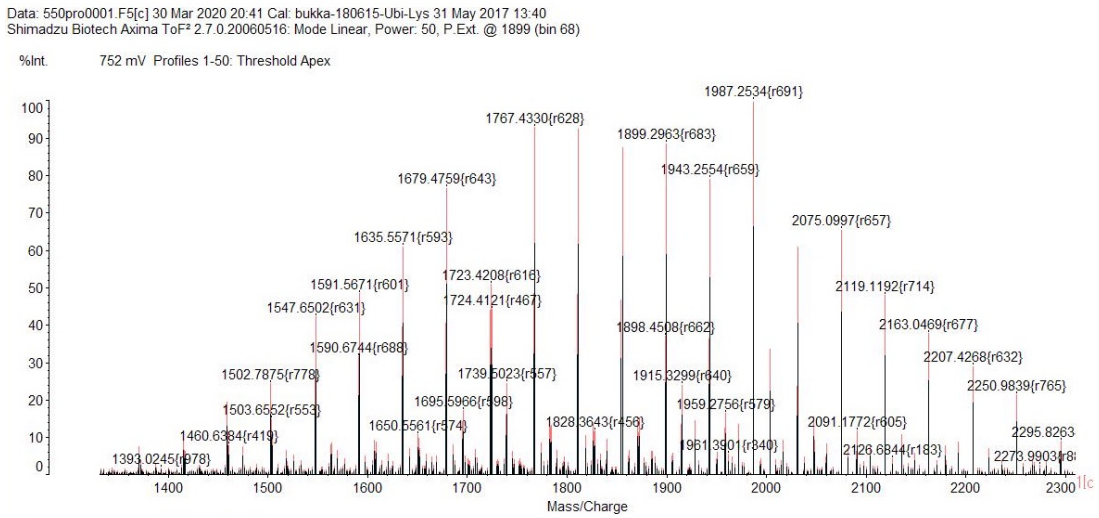


average n = 12 average n = 13

S6 S10

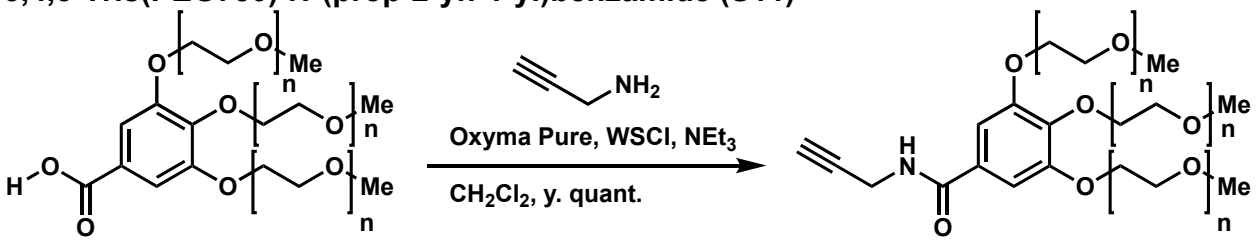
Propargyl amine (9.00 mg, 163 μmol), **S6** (100 mg, 55.6 μmol), Oxyma Pure (3.80 mg, 26.7 μmol) and WSCI·HCl (41.6 mg, 0.217 mmol) were dissolved in CH_2Cl_2 (543 μL). To the solution, triethylamine (30.0 μL, 21.4 μmol) was added, and the mixture was refluxed for 5 h. To the mixture, 1 M HCl aq. was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq., and brine. The organic layer was concentrated to afford crude **S10**, which was purified with preparative HPLC (Method c) to give **S10** (78.2 mg, 39.7 μmol, y. 71%) as yellow oil. ^{1}H NMR (Acetone-d_6, 500 MHz) δ = 8.09 (brt, *J* = 5.2 Hz, 1H), 7.28 (s, 2H), 4.23-4.16 (m, 8H), 3.85 (t, *J* = 5.2 Hz, 4H), 3.78 (t, *J* = 5.2 Hz, 2H), 3.73-3.41 (m, 140H), 3.28 (s, 9H), 2.67 (brs, 1H).
^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166.4, 153.3, 142.0, 129.9, 107.6, 81.7, 73.1, 72.6, 71.9, 71.4, 71.4, 71.3, 71.3, 71.2, 71.0, 70.3, 69.7, 58.8, 29.7.

Based on the integration of the glycol protons, the average length of the PEG chain was n = 13.
MALDI-TOFMS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 1987.2, which corresponds to n = 13 ($C_{91}H_{171}NNaO_{43}$ $[M+Na]^+$: 1989.1).

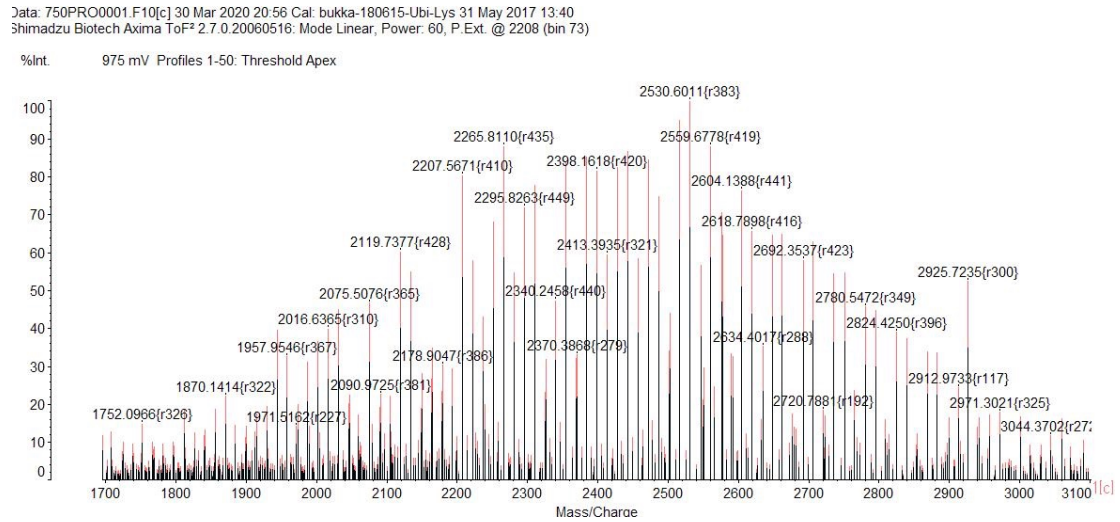
3,4,5-Tris(PEG750)-*N*-(prop-2-yn-1-yl)benzamide (S11)



average n = 20 **average n = 17**

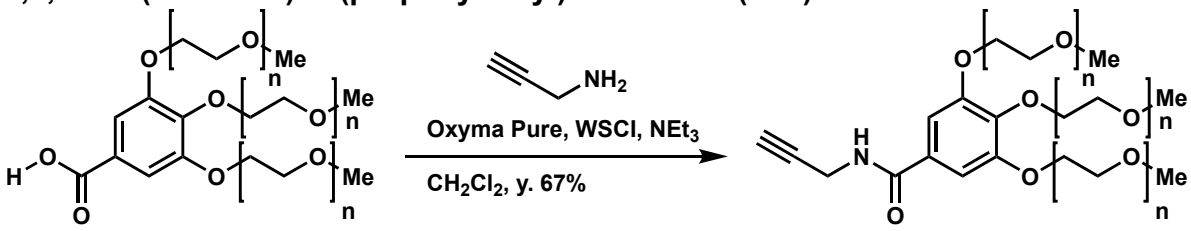
S7 **S11**

Propargyl amine (4.4 mg, 80 μmol), **S7** (108 mg, 38 μmol), Oxyma Pure (2.8 mg, 20 μmol) and WSCI·HCl (38 mg, 83 μmol) were dissolved in CH_2Cl_2 (0.5 mL). To the solution, triethylamine (22 μL, 0.16 mmol) was added, and the mixture was stirred at r.t. for 22 h. To the mixture, 1 M HCl aq. was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq., brine, and concentrated to afford crude **S11**, which was purified with silica gel column chromatography (CH_2Cl_2/MeOH = 1/0 to 10/1) to afford **S11** (118 mg, 45 μmol, y. quant) as yellow oil. ^{1}H NMR (Acetone-d6, 500 MHz) δ = 7.94 (brt, *J* = 5.2 Hz, 1H), 7.17 (s, 2H), 4.12-4.03 (m, 8H), 3.74 (t, *J* = 5.2 Hz, 4H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.58-3.40 (m, 186H), 3.35-3.31 (m, 7H), 3.17 (s, 9H), 2.56 (brs, 1H). ^{13}C NMR (Acetone-d6, 125 MHz) δ = 166.3, 153.3, 142.0, 130.0, 125.9, 107.6, 73.1, 72.6, 71.9, 71.4, 71.4, 71.3, 71.3, 71.2, 71.0, 70.3, 69.7, 58.8, 29.7.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 17.
MALDI-TOFMS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 2516.12 which corresponds to n = 17 ($C_{115}H_{219}NNaO_{55}$ $[M+Na]^+$: 2518.4).

3,4,5-Tris(PEG2000)-*N*-(prop-2-yn-1-yl)benzamide (S12)



average n = 46 **average n = 55**

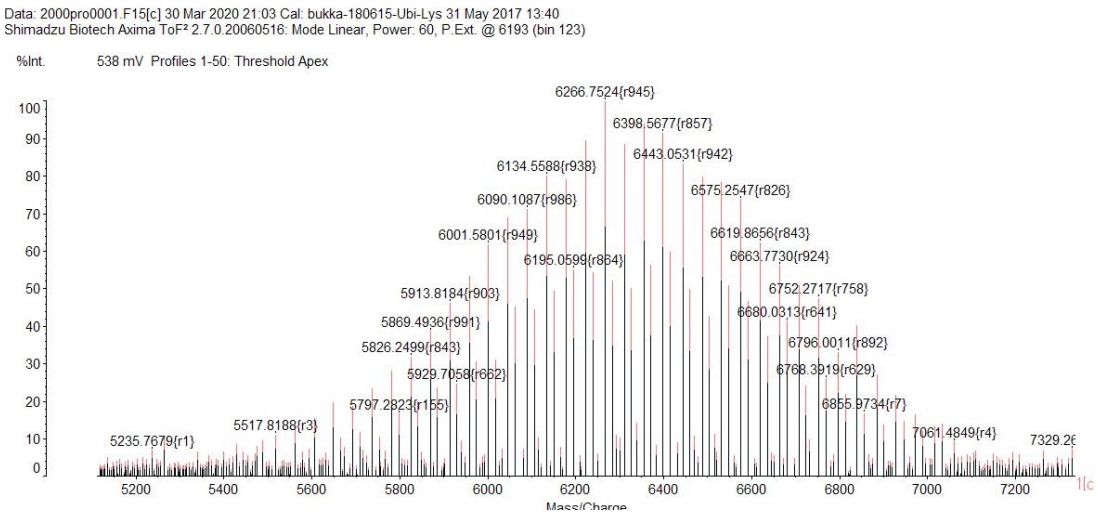
S8 **S12**

Propargyl amine (2.2 mg, 40 μmol), **S8** (88 mg, 14 μmol), Oxyma Pure (1.2 mg, 8.4 μmol) and WSCI·HCl (10 mg, 52 μmol) were dissolved in CH_2Cl_2 (0.15 mL). To the solution, triethylamine (5.0 μL, 36 μmol) was added, and the mixture was stirred at r.t. for 22 h. To the mixture, 1 M HCl aq. was added, and aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq. and brine. The organic layer was concentrated to afford **S12** (71 mg, 9.4 μmol, y. 67%) as yellow solid. ^{1}H NMR (Acetone-d_6, 500 MHz) δ = 8.06 (brt, *J* = 5.2 Hz, 1H), 7.30 (s, 2H), 4.24-4.17 (m, 8H), 3.86 (t, *J* = 5.2 Hz, 4H), 3.69 (t, *J* = 1.7 Hz, 2H), 3.66-3.51 (m, 636H), 3.47-3.42 (m, 8H), 3.28 (s, 9H), 2.68 (t, *J* = 2.3 Hz, 2H).

^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166.3, 153.3, 141.8, 130.0,107.6, 81.9, 73.1, 72.6, 71.9, 71.8, 71.6, 71.4, 71.3, 71.3, 71.2, 71.1, 71.0, 70.5, 70.4, 70.3, 69.7, 58.8, 29.5.

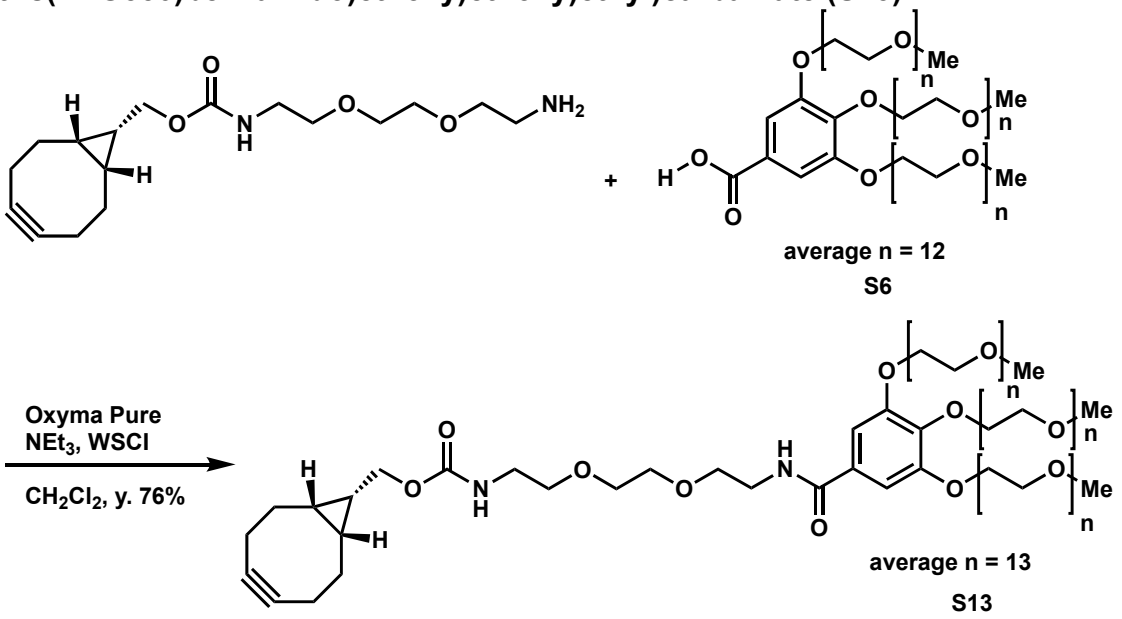
Based on the integration of the glycol protons, the average length of the PEG chain was n = 55.

MALDI-TOFMS spectrum of the product is shown below.



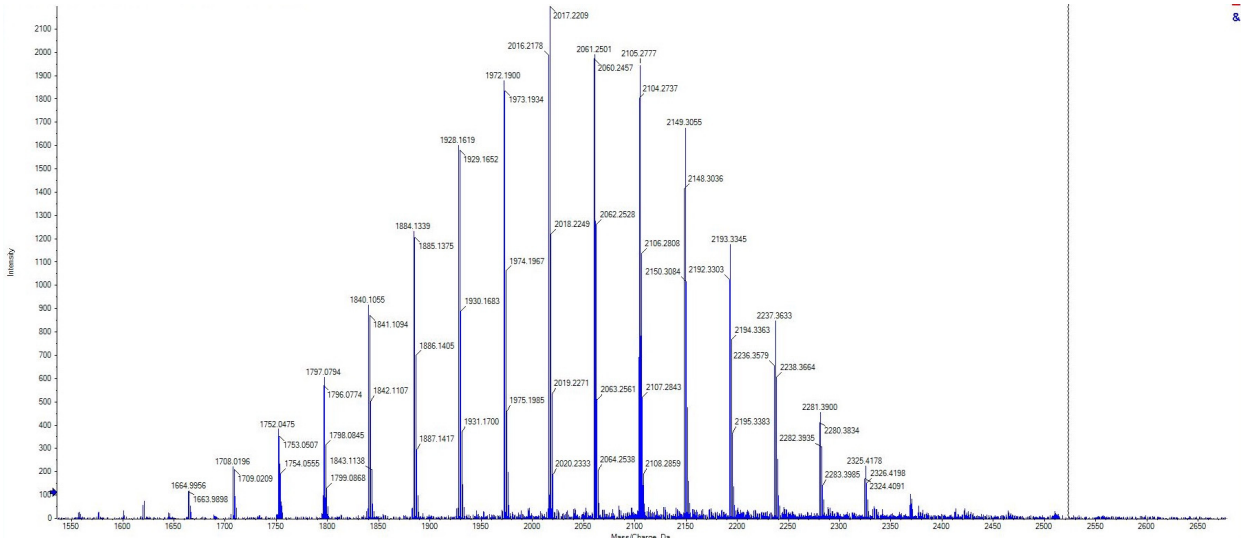
One of the peaks of the highest intensity has *m*/*z* of 6619.8, which corresponds to n = 48 ($C_{301}H_{591}NNaO_{148}$ $[M+Na]^+$: 6614.8).

((1*R*,8*S*,9*S*)-Bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-(3,4,5-tris(PEG550)benzamido)ethoxy)ethoxy)ethyl)carbamate (S13)



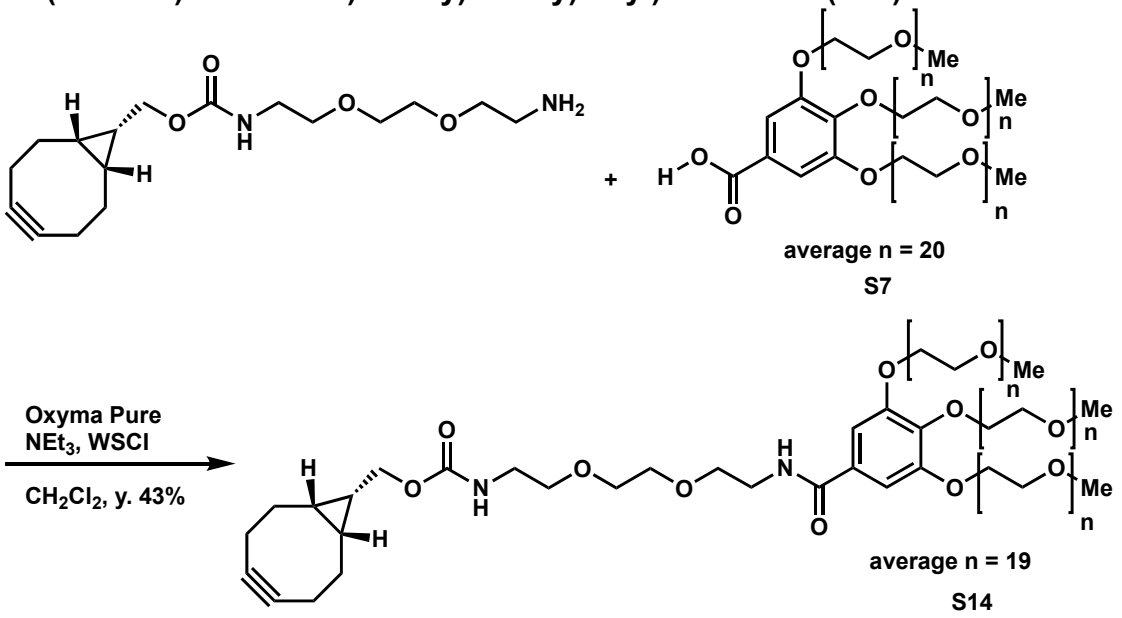
Bicyclo[6.1.0]non-4-yn-9-ylmethyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (15 mg, 45 μmol), **S6** (80 mg, 45 μmol), Oxyma Pure (3.5 mg, 23 μmol), and WSCI·HCl (17.3 mg, 90 μmol) were dissolved in CH_2Cl_2 (450 μL), and triethylamine (14 μL, 90 μmol) was added. The mixture was stirred at r.t. for 8 h. After addition of 1 M HCl aq. the mixture was extracted by CH_2Cl_2. The combined organic layers were washed by saturated $NaHCO_3$ aq., and concentrated. Purification with silica gel column chromatography (CH_2Cl_2/MeOH = 15/1 to 8/1) gave **S13** (75 mg, 34 μmol, y. 76%) as white solid. ^{1}H NMR (Acetone-d_6, 500 MHz) δ = 7.79 (brs, 1H), 7.26 (s, 2H), 6.19 (brs, 1H), 4.22-4.17 (m, 6H), 4.08 (d, *J* = 8.0 Hz, 2H), 3.85 (t, *J* = 4.6 Hz, 4H), 3.77 (t, *J* = 5.7 Hz, 2H), 3.70-3.41 (m,

152H), 3.29 (s, 9H), 2.25-2.12 (m, 6H), 1.57-1.55 (m, 2H), 1.33-1.28 (m, 1H), 0.92-0.88 (m, 2H).
Based on the integration of the glycol protons, the average length of the PEG chain was n = 13.
ESI-MS spectrum of the product is shown below.



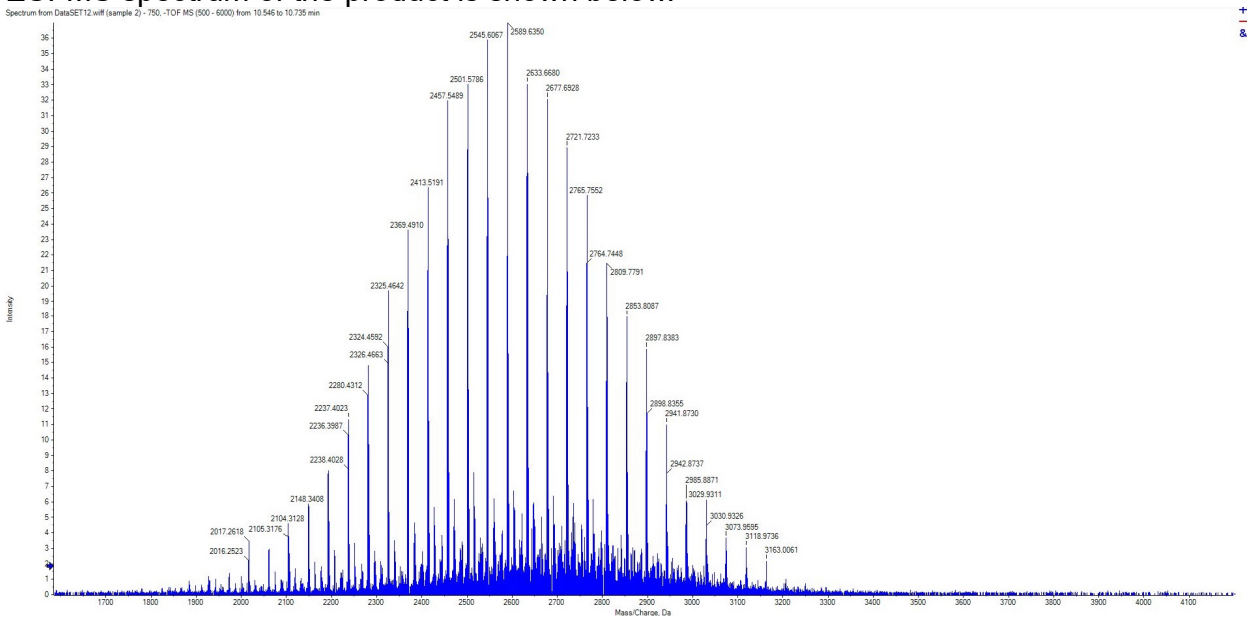
One of the peaks of the highest intensity has *m*/*z* of 2104.2, which corresponds to n = 12 ($C_{99}H_{181}N_2O_{44}$ [M-H]$^-$: 2103.2).

((1*R*,8*S*,9*S*)-Bicyclo[6.1.0]non-4-yn-9-yl)methyl (2-(2-(2-(3,4,5-tris(PEG750)benzamido)ethoxy)ethoxy)ethyl)carbamate (S14)



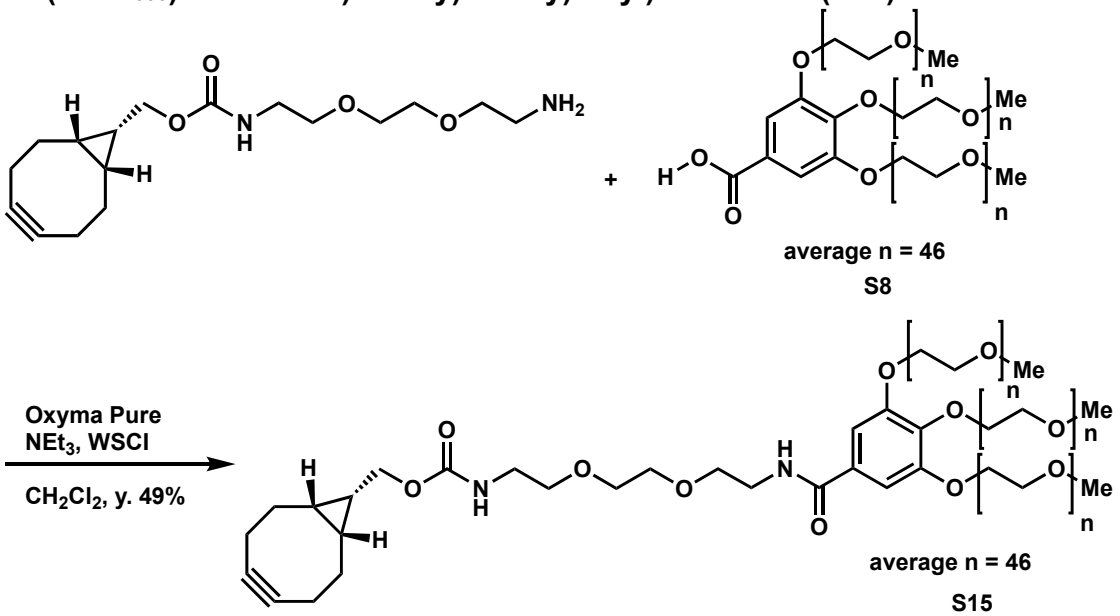
Bicyclo[6.1.0]non-4-yn-9-ylmethyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (77.6 mg, 28.3 μmol), **S7** (10.0 mg, 35.0 μmol), Oxyma Pure (2.5 mg, 18.6 μmol),

and WSCI·HCl (20.0 mg, 104 μmol) were dissolved in CH_2Cl_2 (500 μL), and triethylamine (10.0 μL, 71.7 μmol) was added. The mixture was stirred at r.t. for 6 h. To the mixture, 1 M HCl aq. was added, and the aqueous layer was extracted by CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, and the organic layer was concentrated. Purification with preparative HPLC (method c) afforded **S15** (46 mg, 15.2 μmol, y. 43%) as yellow oil. ^{1}H NMR (Acetone-d_6, 400 MHz) δ = 7.83 (brs, 1H), 7.23 (s, 2H), 6.21 (brs, 1H), 4.19-4.16 (m, 6H), 4.03 (d, J = 8.7 Hz, 2H), 3.82-3.80 (m, 4H), 3.74-3.72 (m, 2H), 3.65-3.40 (m, 219H), 3.23 (s, 9H), 2.14-2.02 (m, 6H), 1.55-1.48 (m, 2H), 1.31-1.28 (m, 1H), 0.86-0.79 (m, 2H).
Based on the integration of the glycol protons, the average length of the PEG chain was n = 19.
ESI-MS spectrum of the product is shown below.



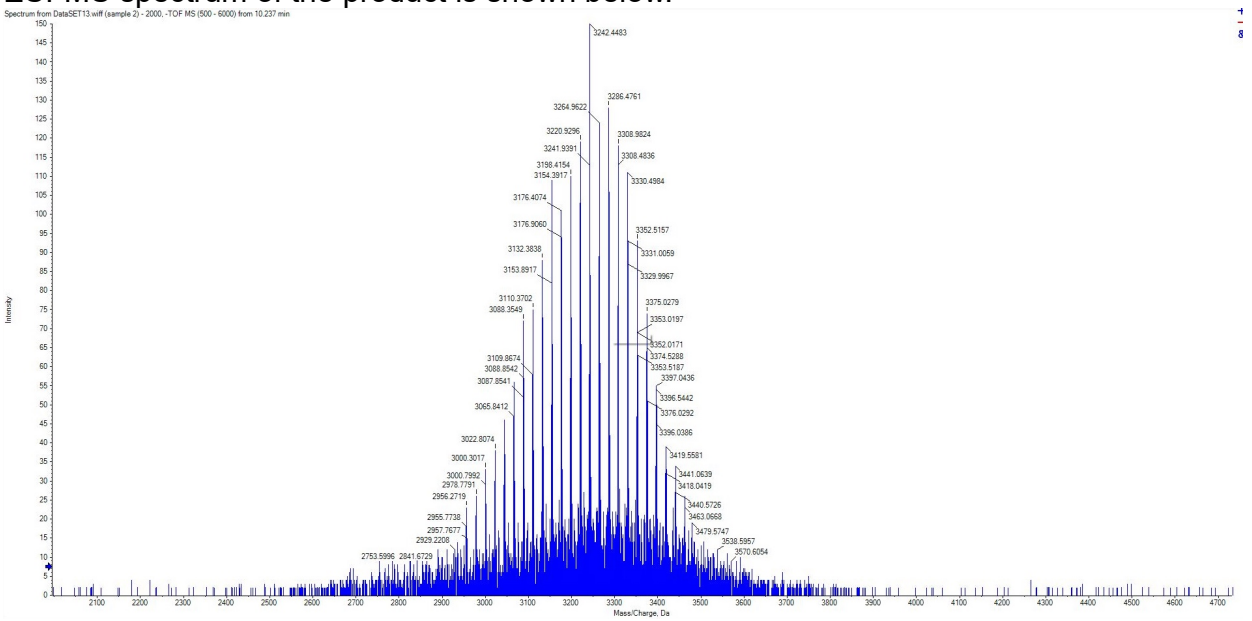
One of the peaks of the highest intensity has m/z of 2633.6, which corresponds to n = 16 ($C_{123}H_{229}N_2O_{56}$ $[M-H]^-$: 2631.5).

Bicyclo[6.1.0]non-4-yn-9-yl (2-(2-(2-(3,4,5-tris(PEG_{2000})benzamido)ethoxy)ethoxy)ethyl)carbamate (S15)



Bicyclo[6.1.0]non-4-yn-9-ylmethyl (2-(2-(2-aminoethoxy)ethoxy)ethyl)carbamate (5.0 mg, 15 μmol), **S8** (88 mg, 13.9 μmol), Oxyma Pure (1.2 mg, 8.4 μmol), and WSCl·HCl (12 mg, 62.5 μmol) were dissolved in CH_2Cl_2 (150 μL), and triethylamine (5 μL, 36 μmol) was added. The mixture was stirred at r.t. for 24 h. To the mixture, 1 M HCl aq. was added, and the aqueous layer was extracted with CH_2Cl_2. Combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, and the organic layer was concentrated. Purification with preparative HPLC (method d) afforded **S15** (45 mg, 6.8 μmol, y. 49%) as white solid. ^{1}H NMR (Acetone-d_6, 500 MHz) δ = 7.82 (brs, 1H), 7.27 (s, 2H), 6.23 (brs, 1H), 4.22 (t, *J* = 4.6 Hz, 4H), 4.20 (t, *J* = 4.6 Hz, 2H), 4.09 (d, *J* = 8.0 Hz, 2H), 3.86 (t, *J* = 4.6 Hz, 4H), 3.78 (t, *J* = 4.6 Hz, 2H), 3.47-3.51 (m, 543H), 3.47-3.42 (m, 10H), 3.28 (s, 9H), 2.23-2.12 (m, 6H), 1.57-1.55 (m, 2H), 1.33-1.30 (m, 1H), 0.91-0.87 (m, 2H).
^{13}C NMR (Acetone-d_6, 125 MHz) δ = 166.9, 157.5, 153.3, 141.8, 130.7, 107.6, 99.4, 73.2, 72.6, 71.8, 71.4, 71.3, 71.2, 71.0, 70.9, 70.6, 70.4, 70.3, 69.7, 62.5, 58.8, 41.4, 40.5, 21.7, 20.8, 19.8, 18.7.
Based on the integration of the glycol protons, the average length of the PEG chain was n = 46.

ESI-MS spectrum of the product is shown below.



One of the peak of the highest intensity has *m*/*z* of 3242.4, which corresponds to n = 45 ($C_{297}H_{575}N_2NaO_{143}$ $[M+Na-3H]^{2-}$: 3242.4).

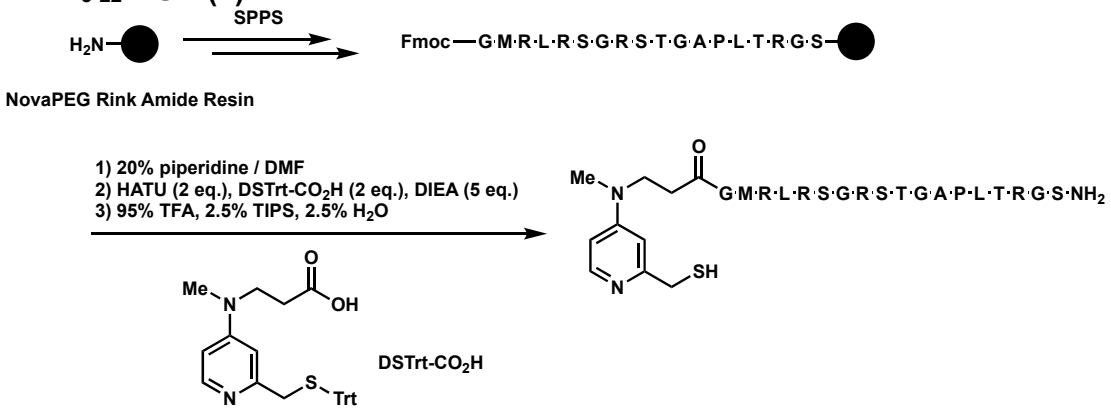
Synthesis of peptides

Peptides were synthesized on a solid phase using NovaPEG-Rink-amide-resin or Rink-Amide-AM-resin. Fmoc-amino acid was sequentially coupled using a DIC-HOBt method in DMF (3 equiv each) for 60 min at r.t. after removal of each Fmoc group with 20% piperidine-DMF for 10 min. DSH or DSSMe building block (1.5 equiv) was coupled using HATU-HOAt method (2 or 1.5 equiv each) with *N*,*N*-diisopropylethylamine (5 equiv) in DMF. *N*-Terminus was capped with 20% acetic anhydride in CH_2Cl_2 for 5 min, if necessary. The peptide was cleaved from the resin by treatment with TFA in the presence of TIPS and H_2O (95:2.5:2.5) for 90 min at r.t., concentrated under reduced pressure, and precipitated with ether to afford crude peptides, which were purified with preparative HPLC to afford product peptides as white solids after lyophilization.

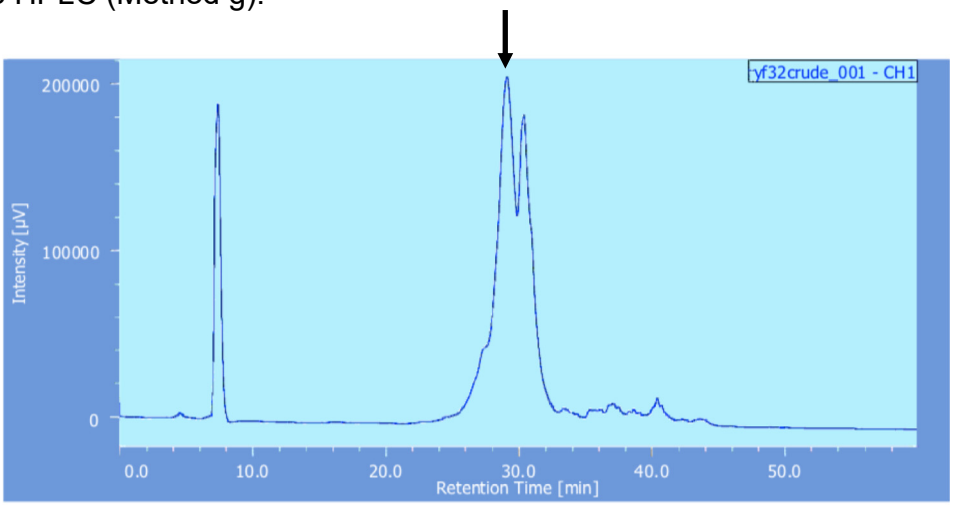
LANA$_{5\text{-}15}$-DSH (1)

Synthesized as reported in the literature (3).

LANA$_{5\text{-}22}$-DSH (2)

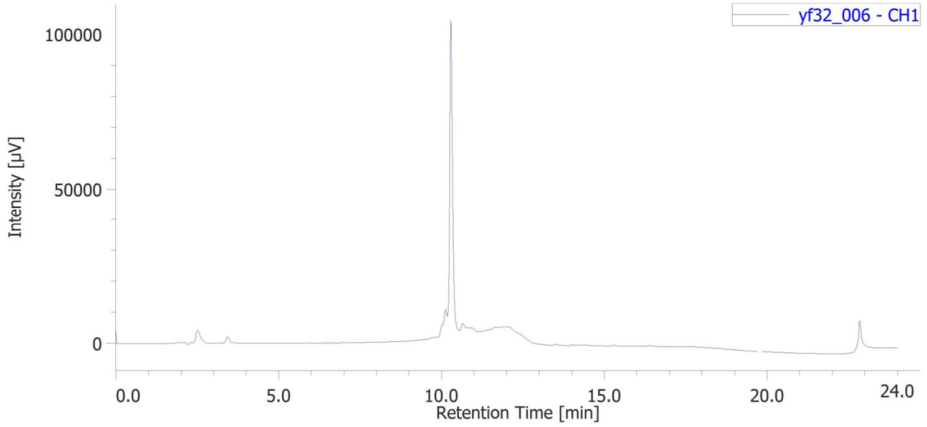


Crude HPLC (Method g):



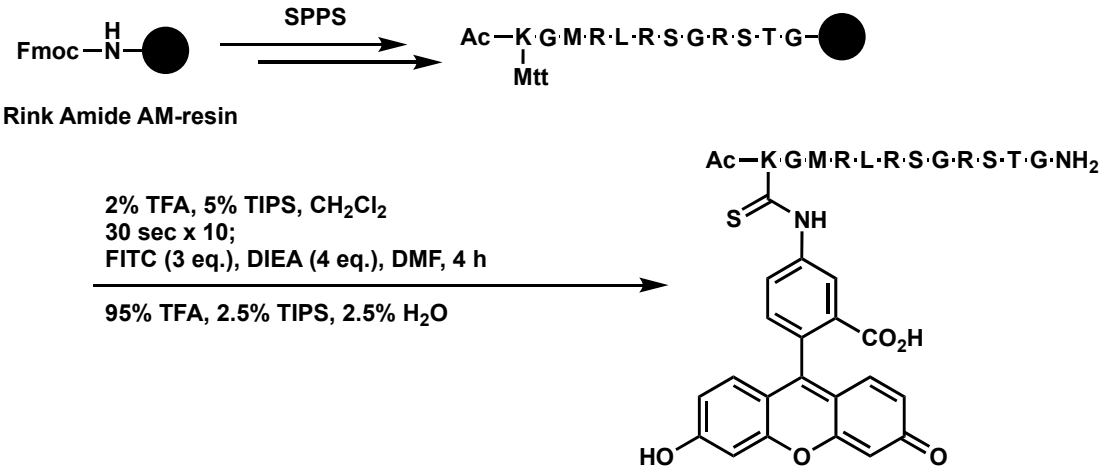
The black arrow indicates the position of the target peptide.

After HPLC separation (Method C) :

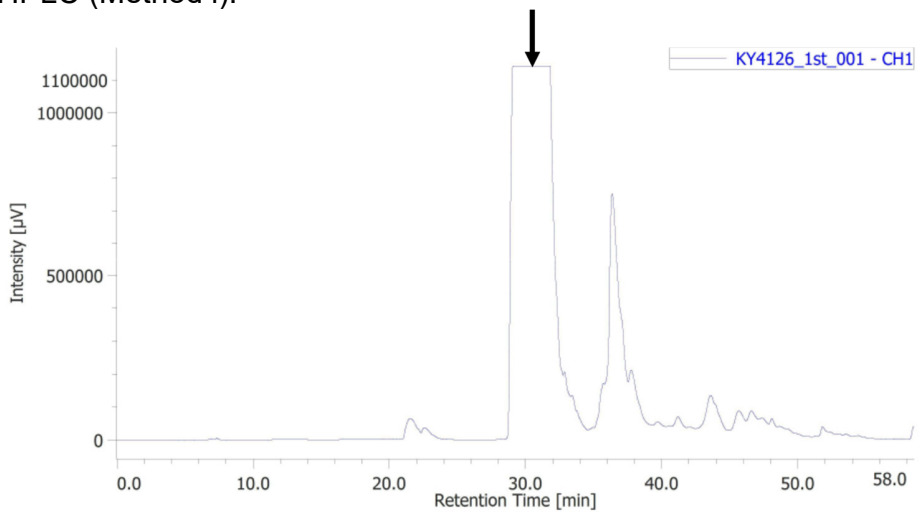


ESI-MS *m*/*z* Calcd: 689.7 $[M+3H]^{3+}$, Found : 689.9; Retention time: 10.3 min (Method C), 1.2 mg, y. 0.5 %.

LANA$_{5\text{-}15}$-FITC (4)

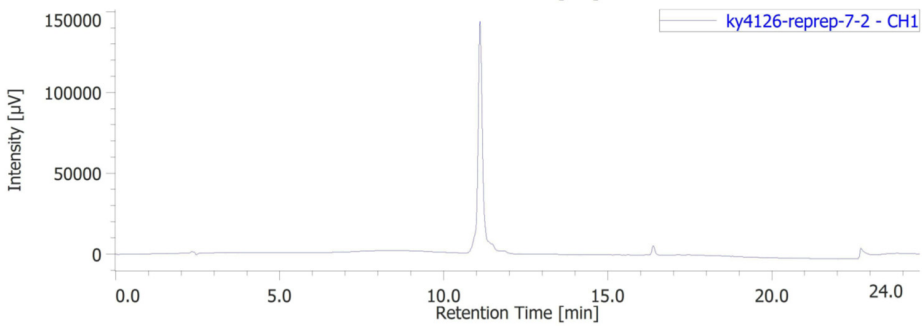


Crude HPLC (Method I):



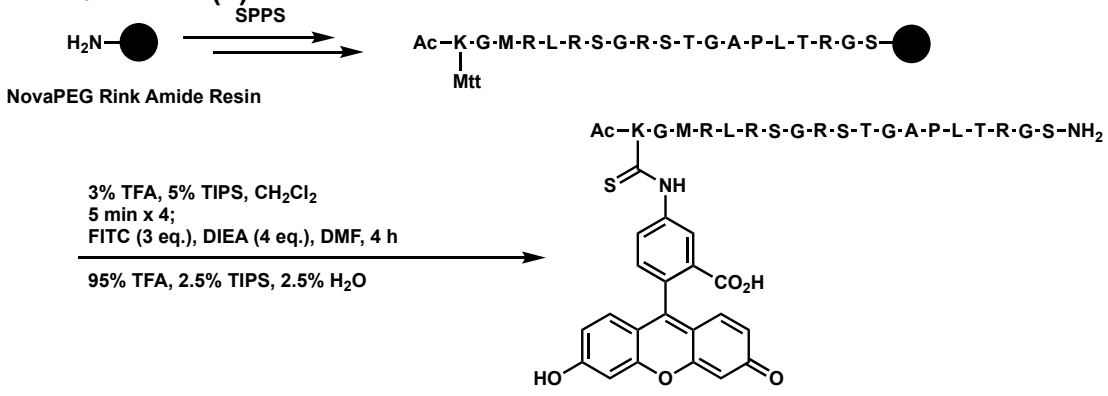
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):

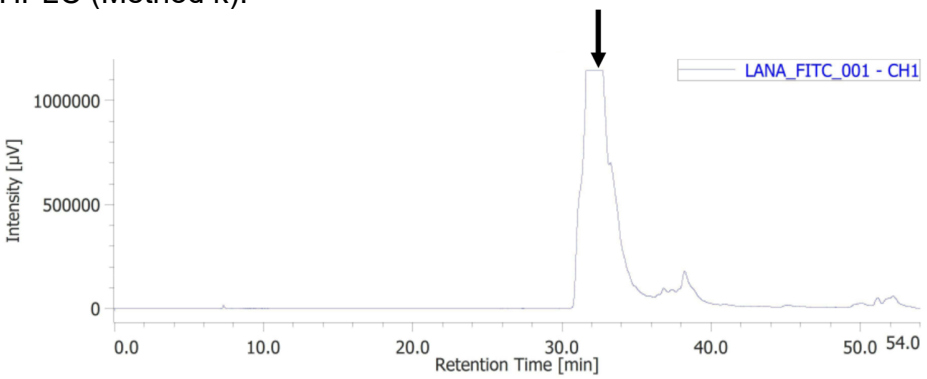


MALDI-TOFMS *m/z* Calcd: 1735.8 $[M+H]^+$, Found: 1735.4; Retention time: 11.2 min (Method A), 56.2 mg, y. 34%.

LANA$_{5\text{-}22}$-FITC (5)

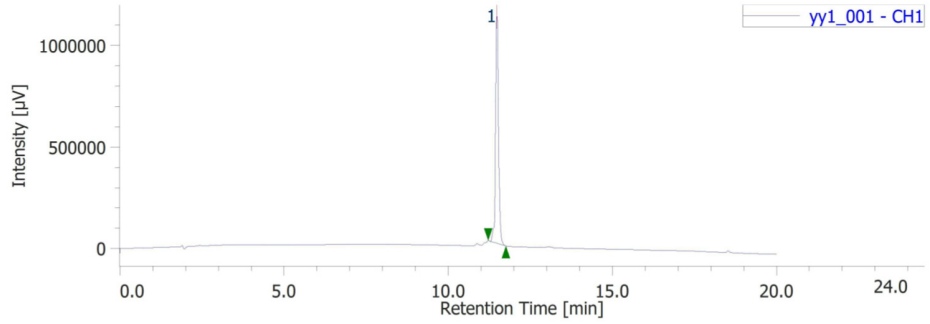


Crude HPLC (Method k):



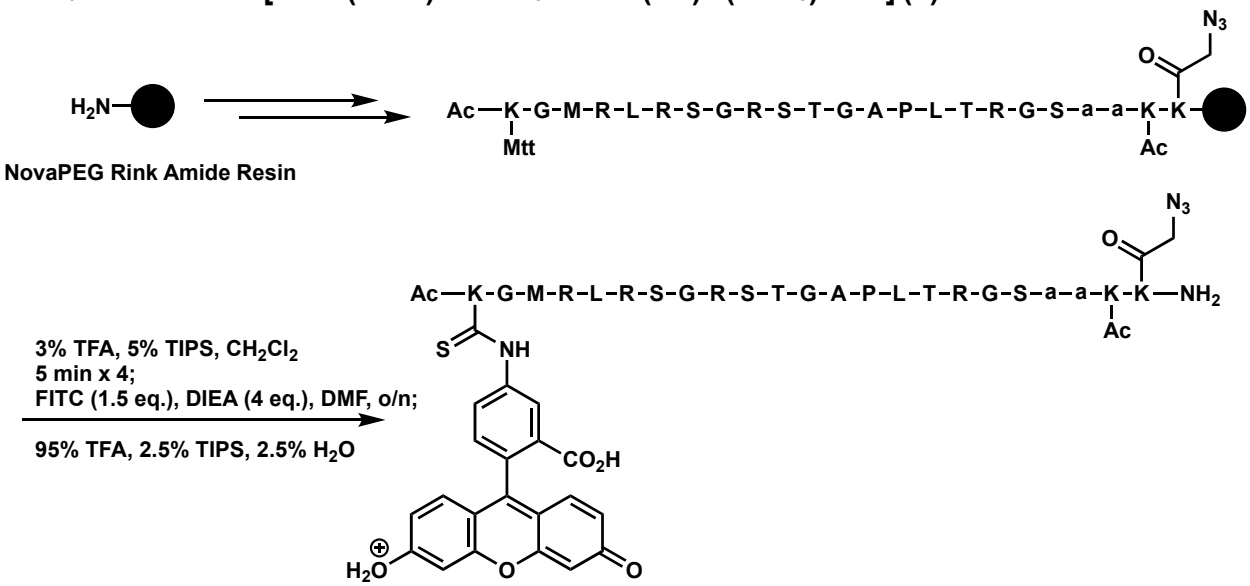
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):



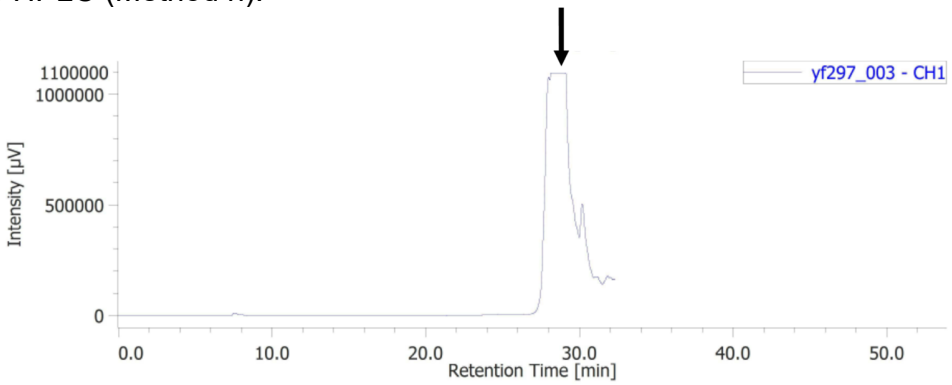
MALDI-TOFMS *m*/*z* Calcd: 2418.2 $[M+H]^+$, Found: 2418.8; Retention time: 11.5 min (Method A), 27.9 mg, y. 11.0%.

PEG_0-LANA-FITC [Ac-K(FITC)-$LANA_{5-22}$-aaK(Ac)K(AcN_3)-NH_2] (6)



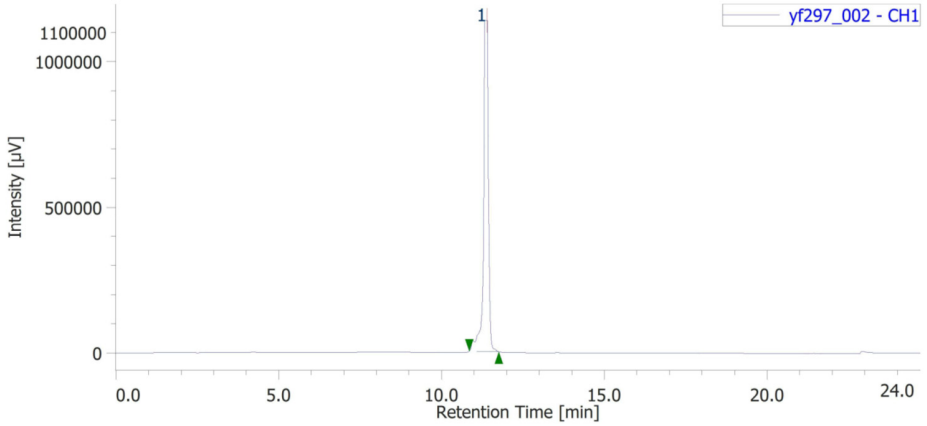
Small letter denotes D-amino acids.

Crude HPLC (Method h):



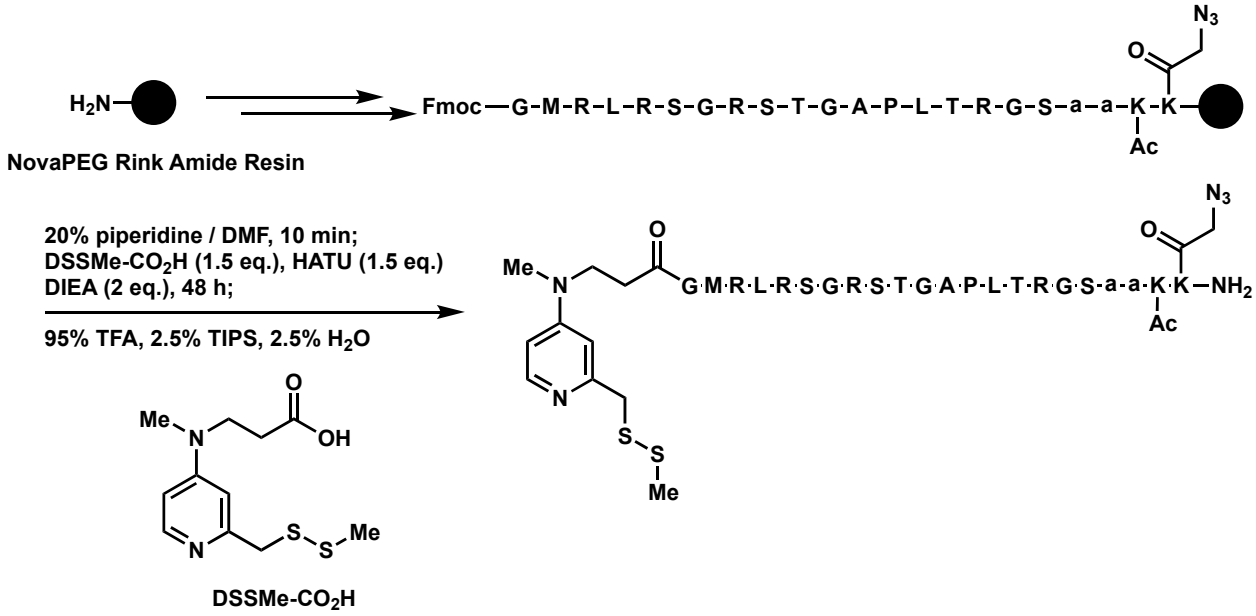
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):

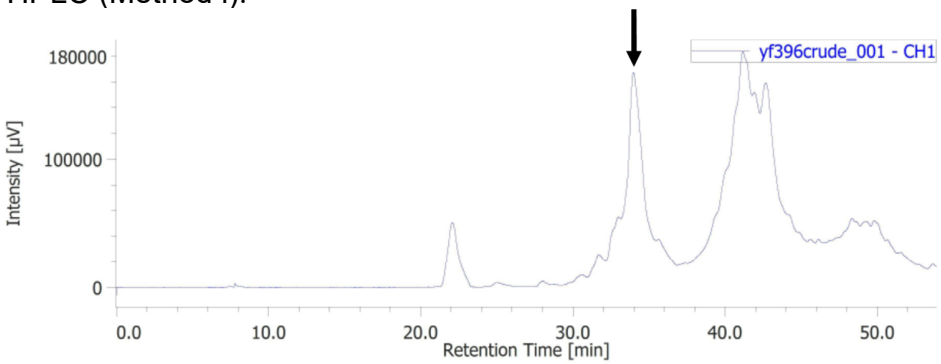


MALDI-TOFMS *m*/*z* Calcd: 2941.4 $[M+H]^+$, Found: 2941.2; Retention time: 11.4 min (Method A), 23.8 mg, y. 18.0%.

N_3-LANA-DSSMe [DSSMe- $LANA_{5-22}$-aaK(Ac)K(AcN_3)-NH_2] (S16)

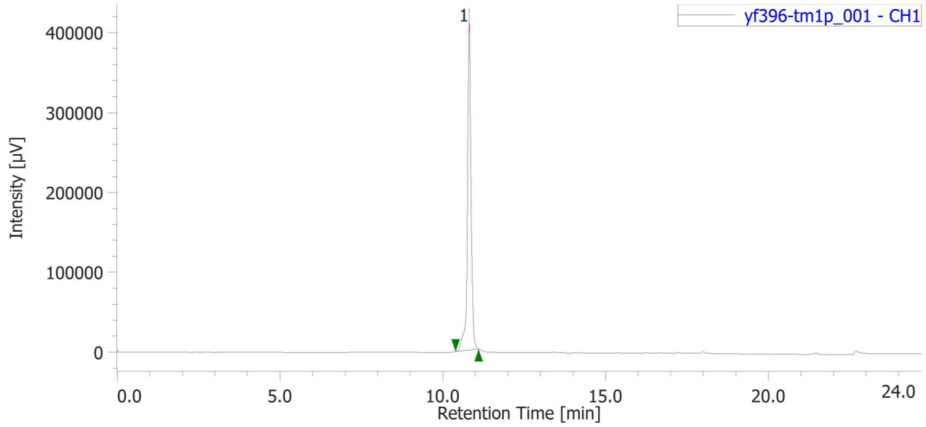


Crude HPLC (Method i):



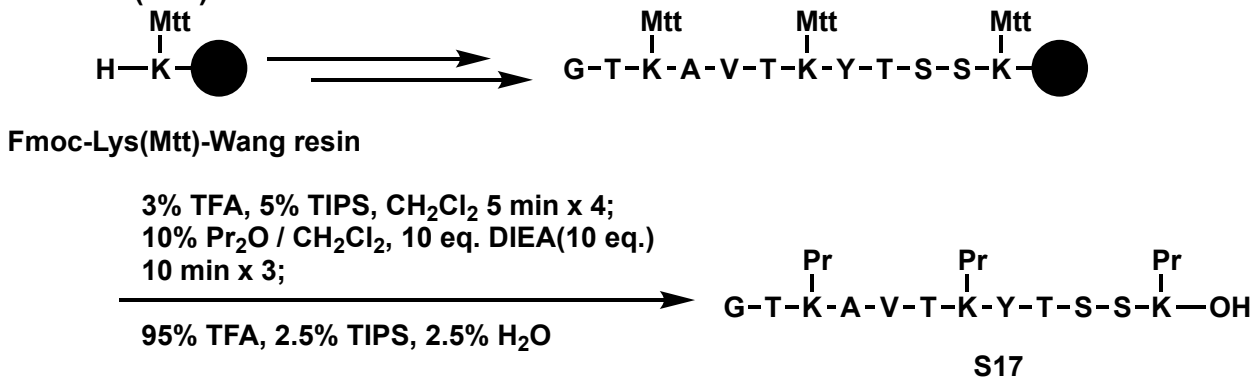
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):

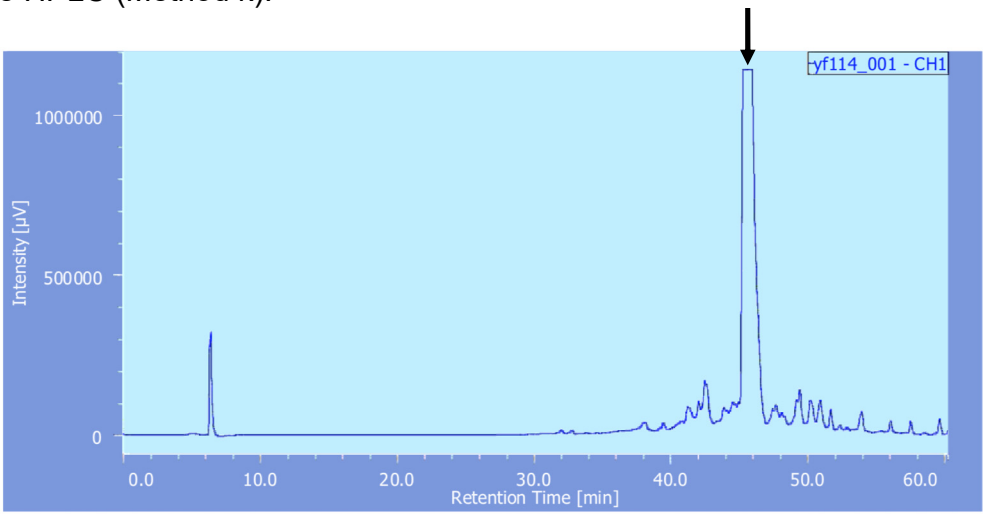


MALDI-TOFMS *m/z* Calcd: 2636.4 $[M+H]^+$, Found: 2635.3; Retention time: 10.8 min (Method A), 39.0 mg, y. 4.11 %.

Authentic peptide of H2B G114-K125 (lysine propionylated) for in-cell reaction (S17).

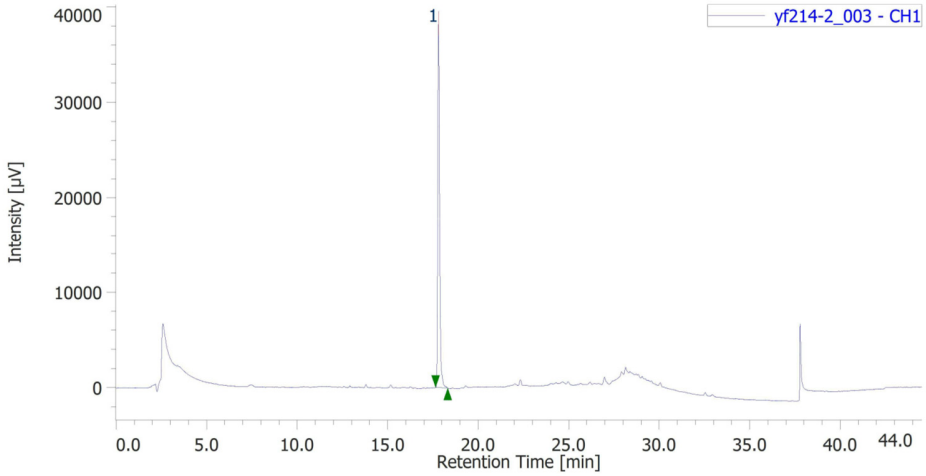


Crude HPLC (Method k):



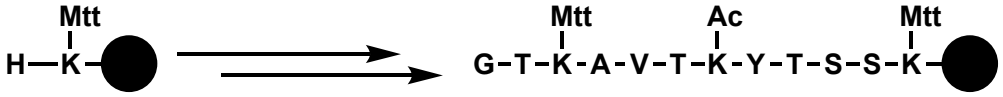
The black arrow indicates the position of the target peptide.

TM (Method B):



ESI-TOFMS *m*/*z* Calcd: 719.8 $[M+2H]^{2+}$, Found: 719.8; Retention time: 17.8 min (Method A), 1.65 mg, y. 5.17 %.

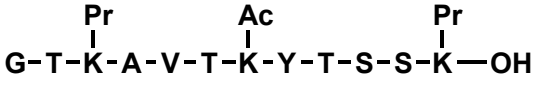
Authentic peptide of H2B G114-K125 (K120 acetylated) for in-cell reaction (S18).



Fmoc-Lys(Mtt)-Wang resin

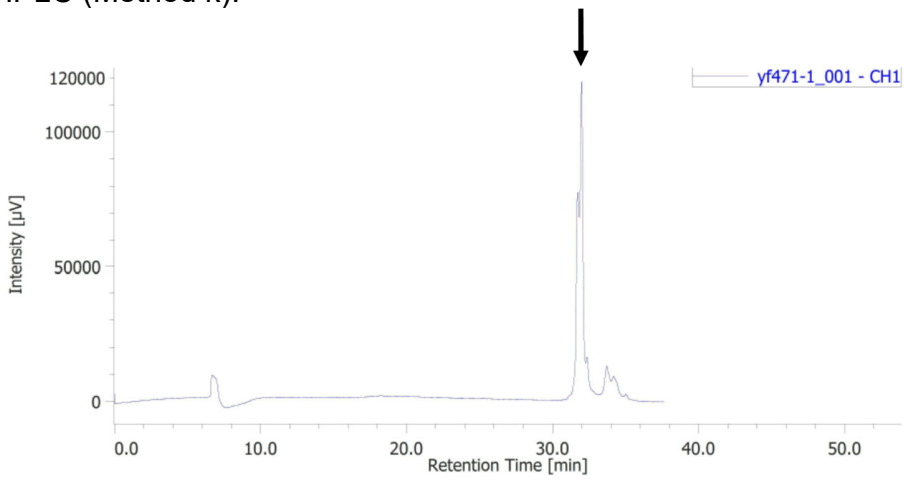
3% TFA, 5% TIPS, CH_2Cl_2 5 min x 4;
10% Pr_2O / CH_2Cl_2, 10 eq. DIEA(10 eq.)
10 min x 3;

95% TFA, 2.5% TIPS, 2.5% H_2O



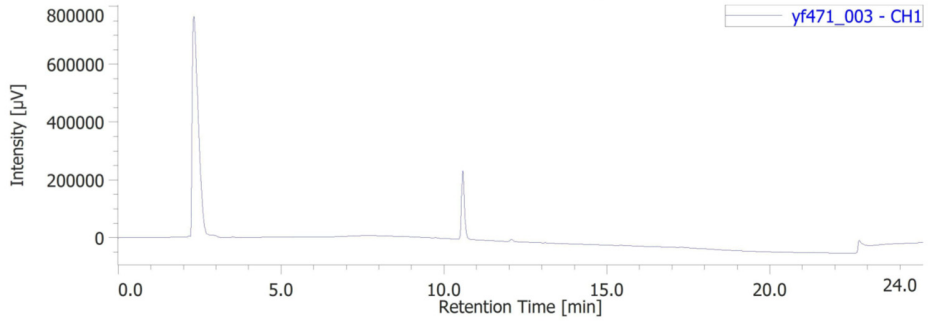
S18

Crude HPLC (Method k):



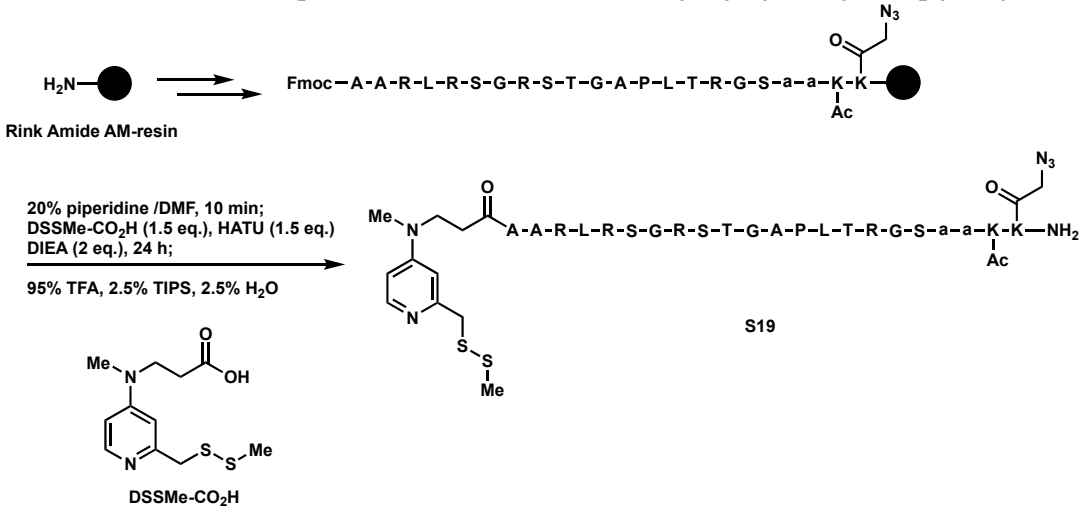
The black arrow indicates the position of the target peptide.

After HPLC separation (Method D):

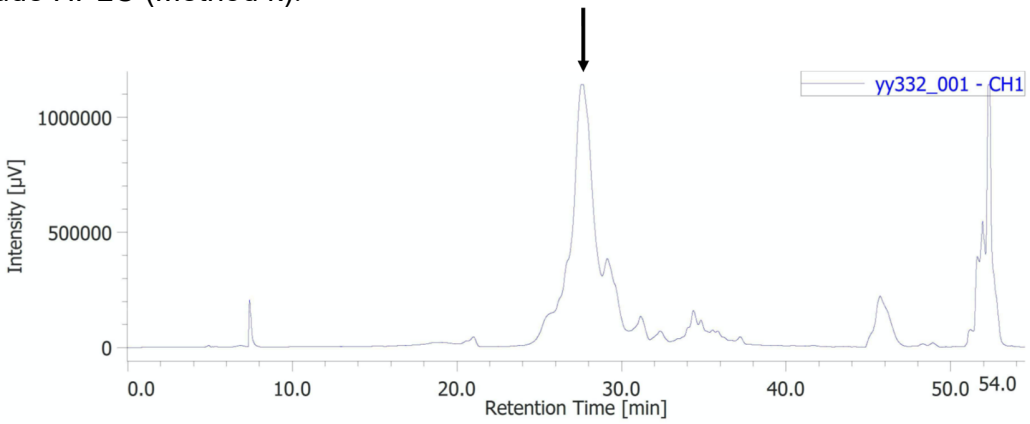


ESI-TOFMS *m*/*z* Calcd: 712.8 $[M+2H]^{2+}$, Found: 712.8; Retention time: 10.6 min (Method D), 1.77 mg, y. 10.4%.

N_3-mutLANA-DSSMe [DSSMe-AA-LANA$_{7-22}$-aaK(Ac)K(AcN_3)-NH_2] (S19)

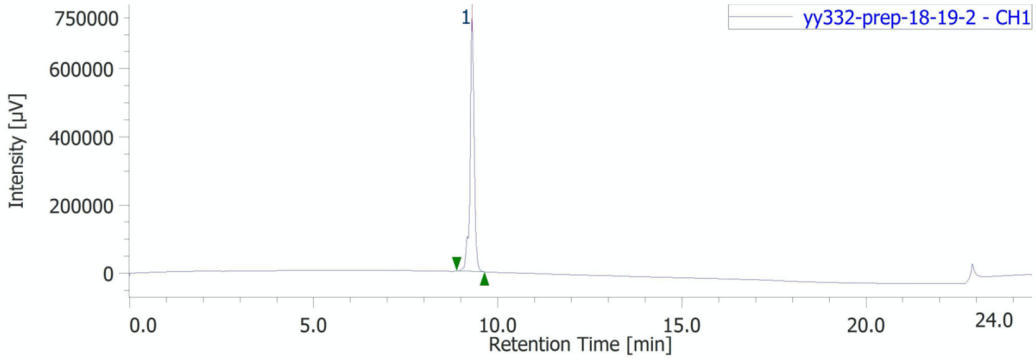


Crude HPLC (Method k):



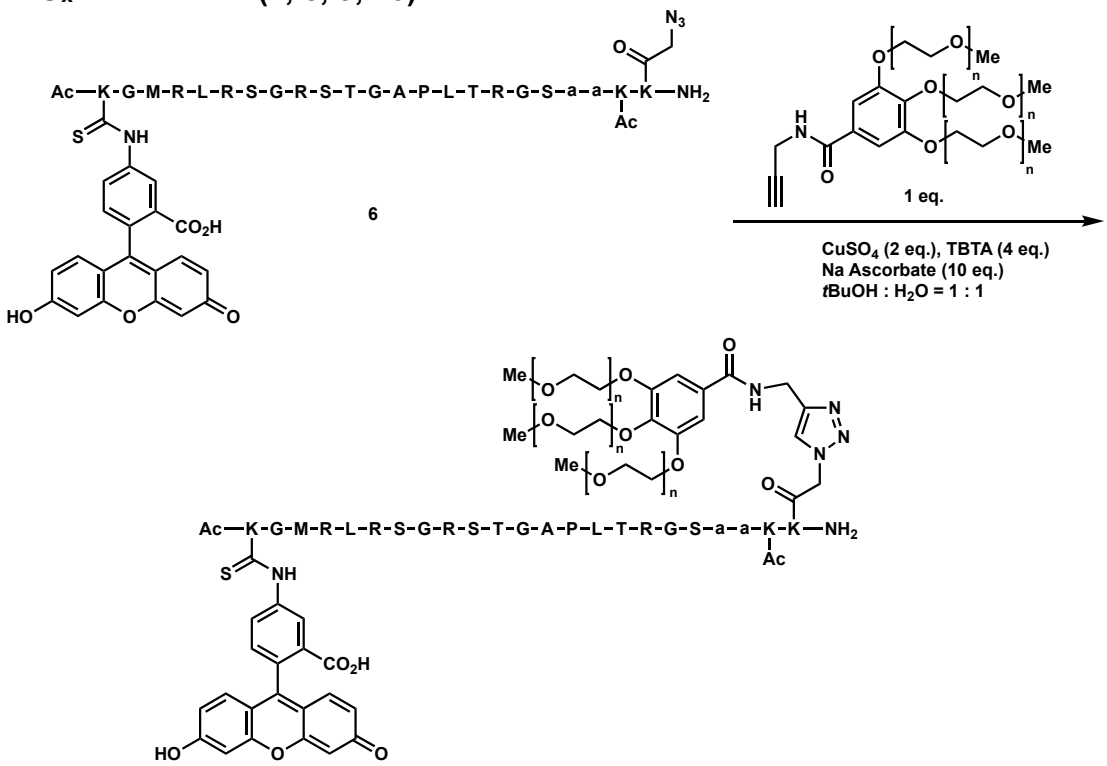
The black arrow indicates the position of the target peptide.

After HPLC separation (Method D):



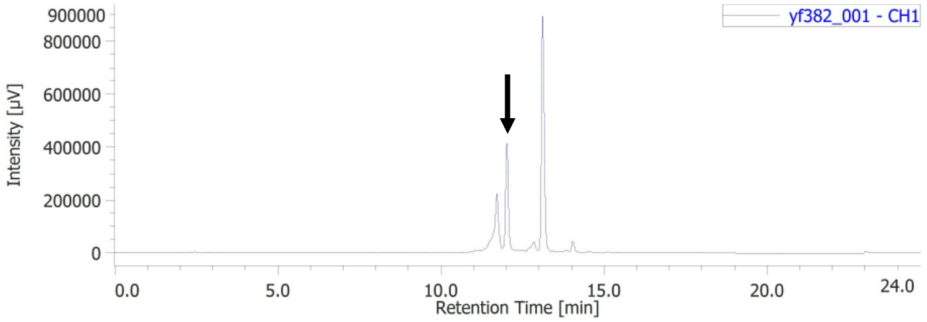
ESI-TOFMS *m*/*z* Calcd: 864.1 $[M+3H]^{3+}$, Found: 864.4; Retention time: 9.30 min (Method D), 35.0 mg, y. 19.3%.

Synthesis of PEG-conjugated peptides PEG$_x$-LANA-FITC (7, 8, 9, 10)



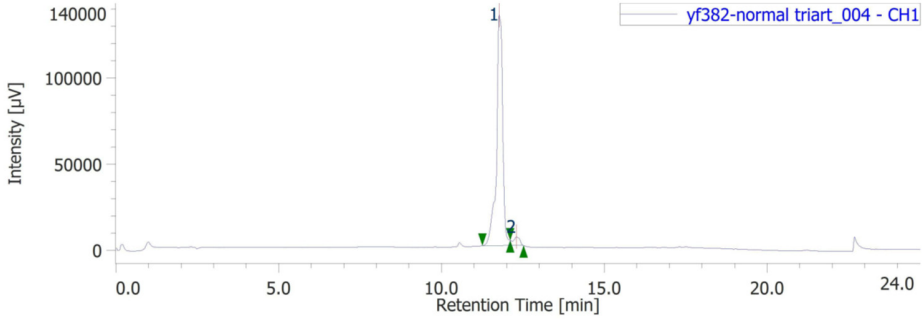
To a stirred solution of **6** (10 mM in water, 30 μL, 300 pmol) and alkyne **(S9-S12)** (10 mM in *t*BuOH, 30 uL, 0.30 μmol), Cu-TBTA solution in water:*t*BuOH (1:2, 75 μL; separately prepared by mixing 24 mM $CuSO_4$ solution in water and 24 mM TBTA solution in *t*BuOH) was added. Sodium ascorbate aqueous solution (100 mM, 30 uL, 3.0 umol) was lastly added, and the mixture was stirred at r.t. for 22 h. Insoluble materials were removed by filtration, and filtrate was purified with preparative HPLC (method e) to afford PEGx-LANA-FITC after lyophilization.

PEG$_{150}$-LANA-FITC (7)
Crude HPLC (Method A):



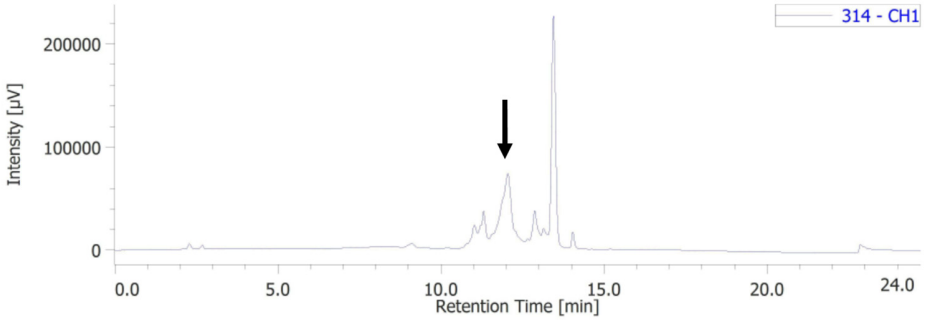
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):



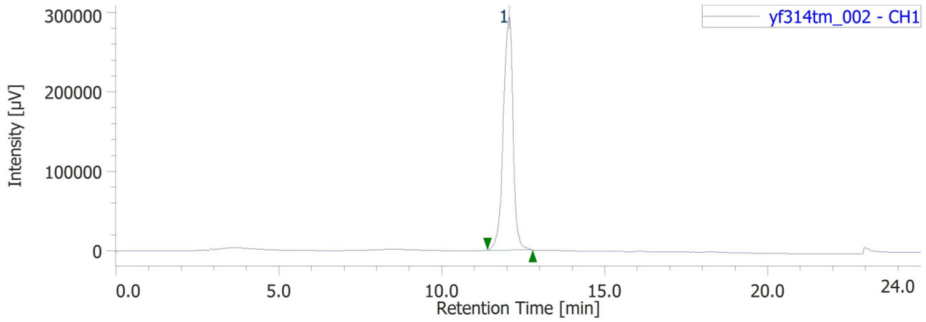
0.98 mg, 0.236 µmol, y. 79%, yellow powder
MALDI-TOFMS *m*/*z* Calcd: 3586.8 $[M+H]^+$, Found: 3587.2; Retention time: 11.8 min (Method A).

PEG$_{550}$-LANA-FITC (8)
Crude (Method A):



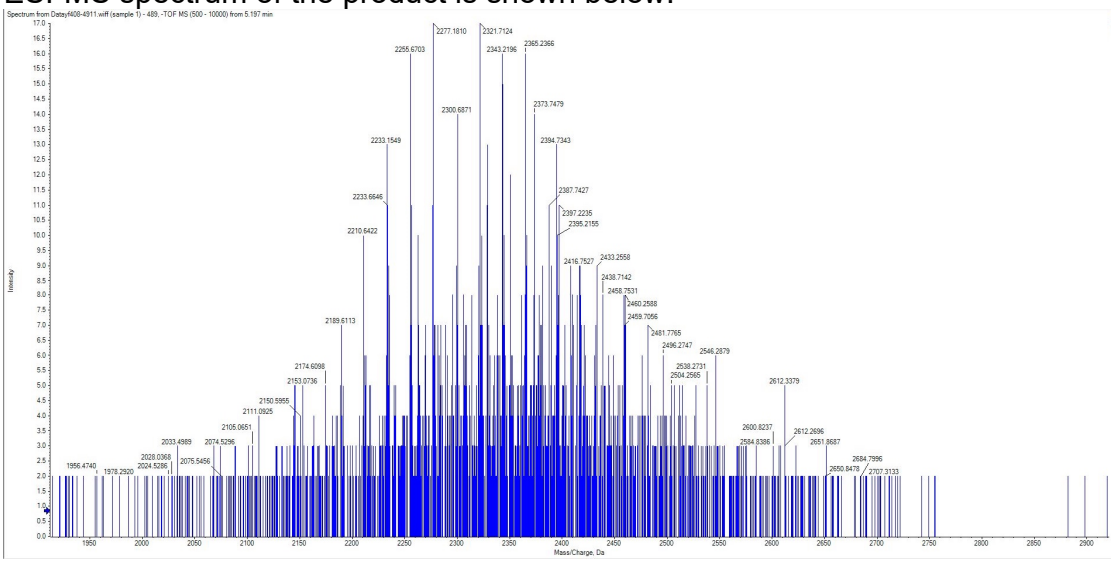
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):



1.27 mg, 0.344 µmol, y. quant., yellow powder. Retention time: 12.7 min (Method A).

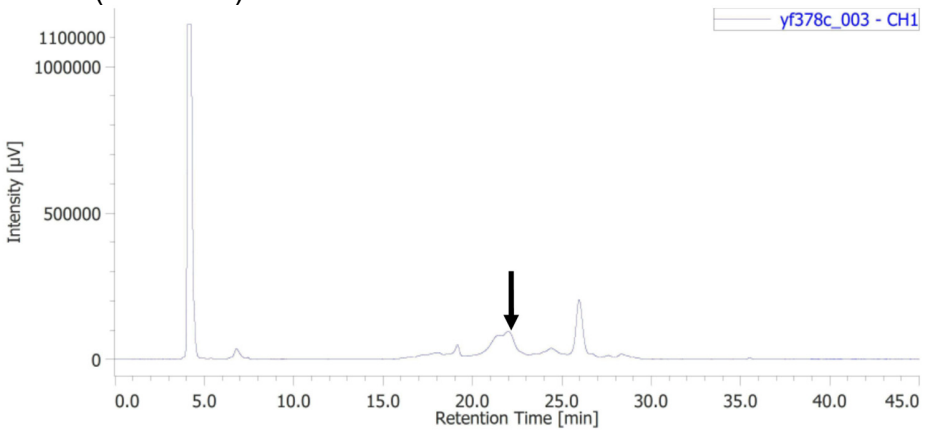
ESI-MS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 2321.7, which corresponds to n = 11 (Calcd: 2321.2 $[M-2H]^{2-}$).

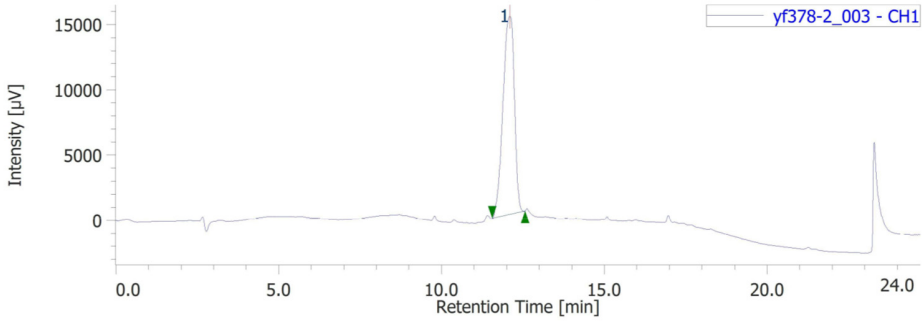
PEG_{750}-LANA-FITC (9)

Crude HPLC (Method e):



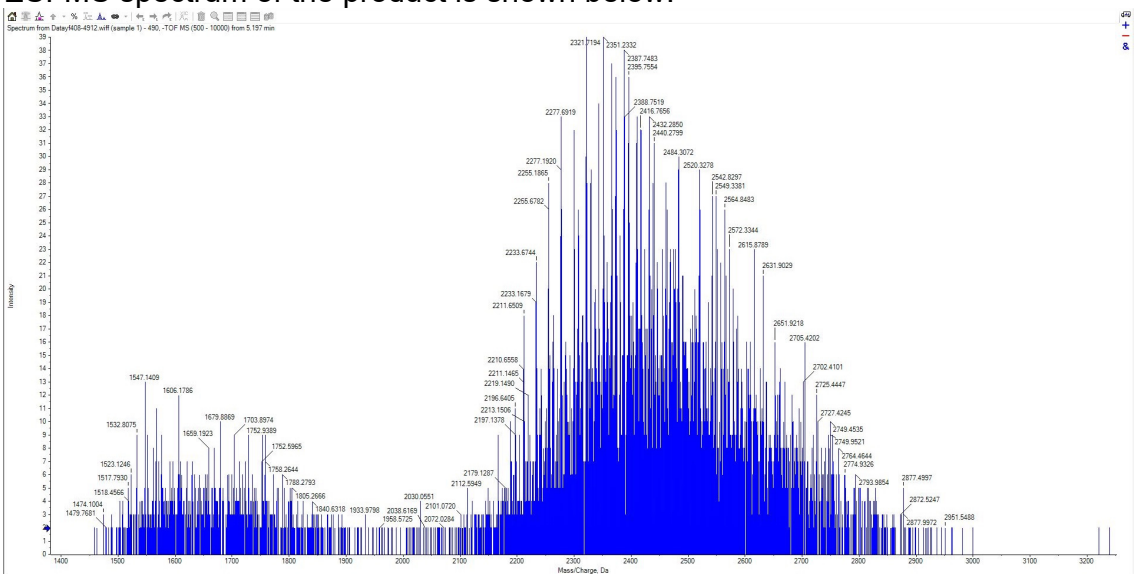
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):



1.52 mg, 0.247 µmol, y. 82%, yellow powder. Retention time: 12.1 min (Method A).

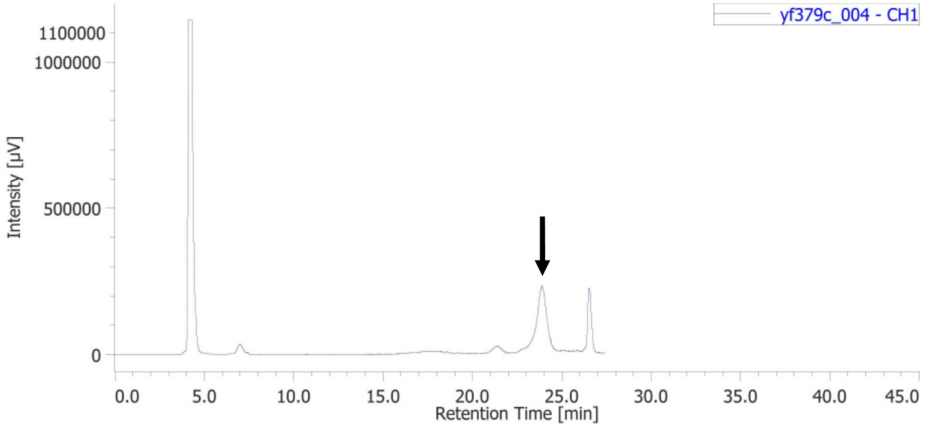
ESI-MS spectrum of the product is shown below.



Peaks in the range of 1500–1900 are those of product trivalent anions, and peaks in the range of 2200–2900 are those of product divalent anions. For example, one of the peaks of the divalent anions has *m*/*z* of 2520.3, which corresponds to n = 14 (Calcd: 2519.3 $[M-2H]^{2-}$).

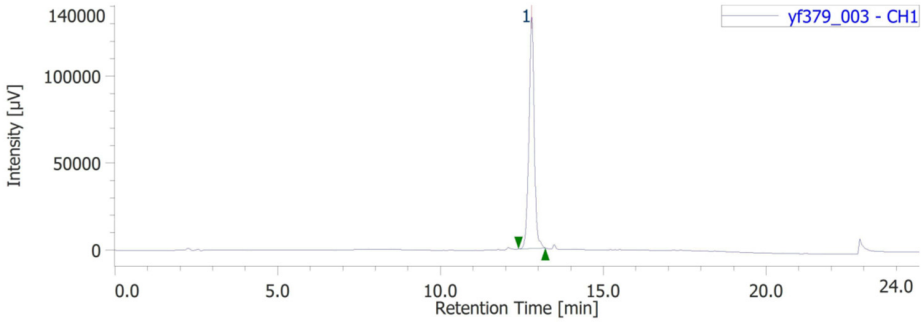
PEG_{2000}-LANA-FITC (10)

Crude HPLC (Method e):



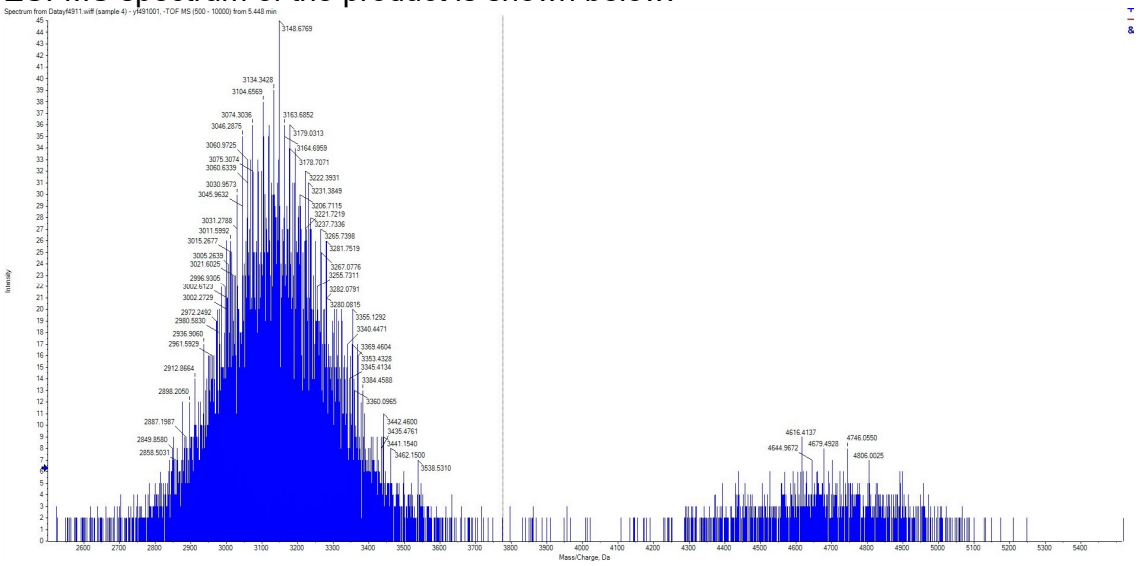
The black arrow indicates the position of the target peptide.

After HPLC separation (Method A):



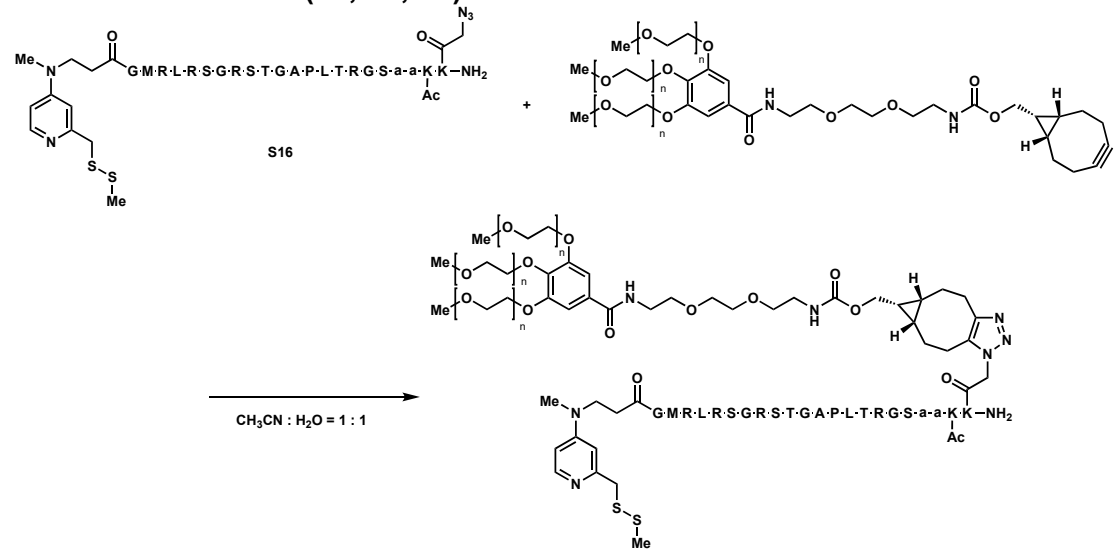
1.44 mg, 0.151 µmol, y. 50%, yellow powder. Retention time: 12.8 min (Method A).

ESI-MS spectrum of the product is shown below.



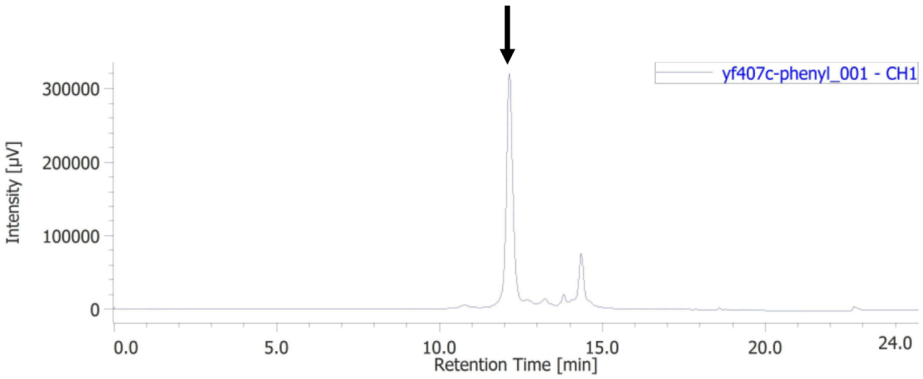
Peaks in the range of 2800–3600 are those of product trivalent anions, and peaks in the range of 4300–5000 are those of product divalent anions. For example, one of the peaks of the trivalent anions has *m*/*z* of 3134.3, which corresponds to n = 47 (Calcd: 3132.7 $[M-3H]^{3-}$).

PEG_x-LANA-DSSMe (11, 12, 13)



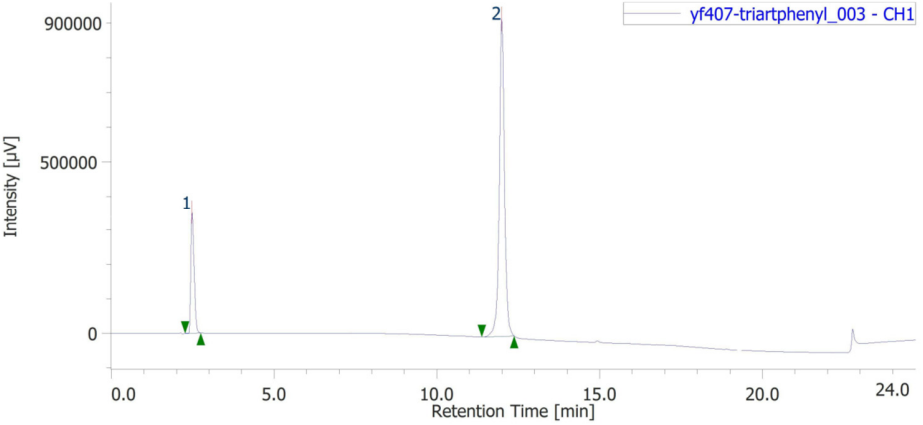
Peptide **S16** (10 mM in water, 800 µL, 8.0 µmol) and alkyne **S13**, **S14**, or **S15** (10 mM in MeCN, 800 µL, 8.0 µmol) were mixed in water:MeCN (1:1). The mixture was stirred at r.t for 24 h, and insoluble materials were removed by filtration. Filtrate was purified with preparative HPLC (Method f) to afford PEG_x-LANA-DSSMe as brown powder after lyophilization.

PEG_{550}-LANA-DSSMe (11)
Crude HPLC (Method C):



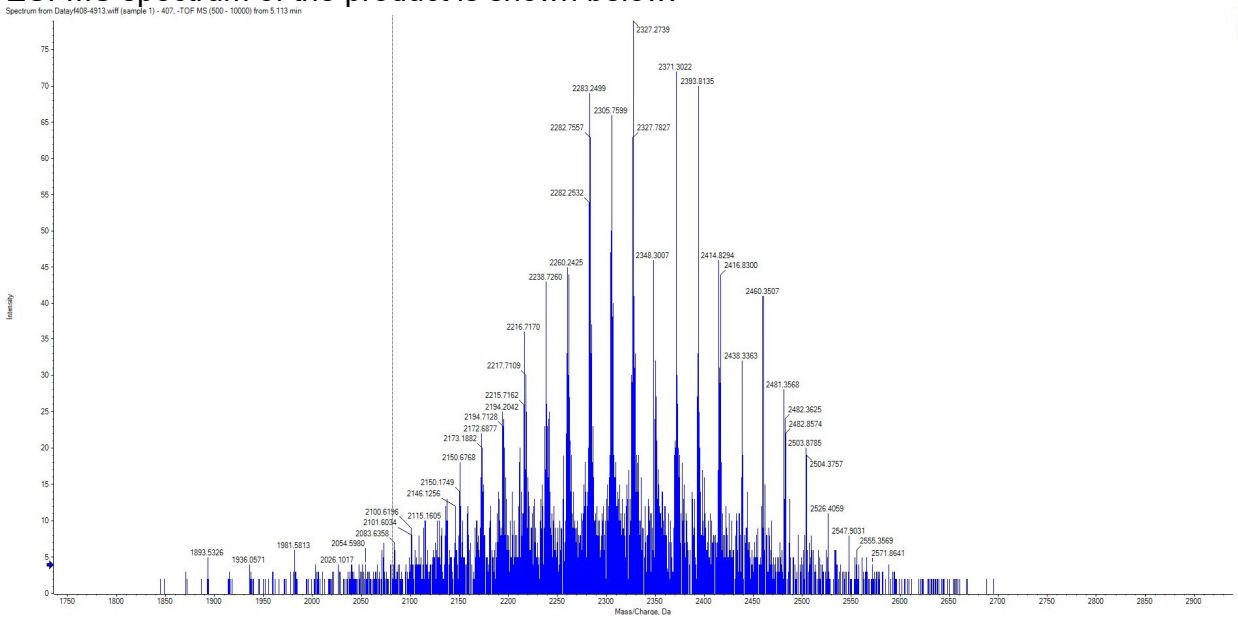
The black arrow indicates the position of the target peptide.

After HPLC separation (Method C):



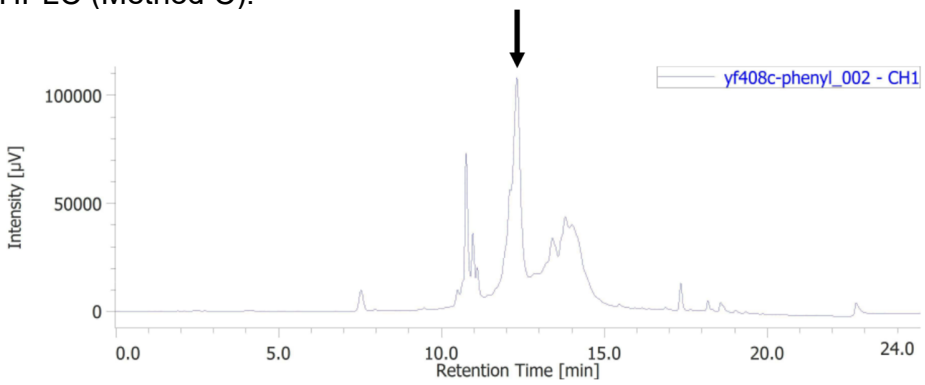
33.8 mg, 6.31 µmol, y. 79%. Retention time: 12.0 min (Method C).

ESI-MS spectrum of the product is shown below.



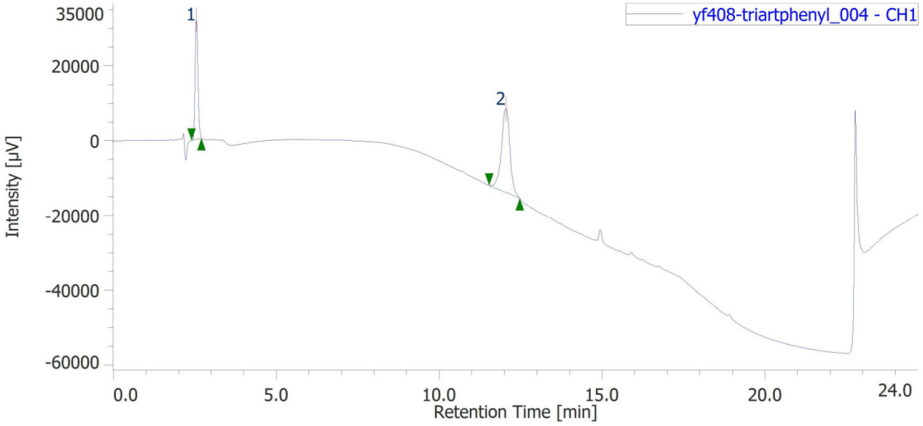
One of the peaks of the highest intensity has *m*/*z* of 2305.7, which corresponds to n = 11 (Calcd: 2303.2 $[M-2H]^{2-}$).

PEG_{750}-LANA-DSSMe (12)
Crude HPLC (Method C):



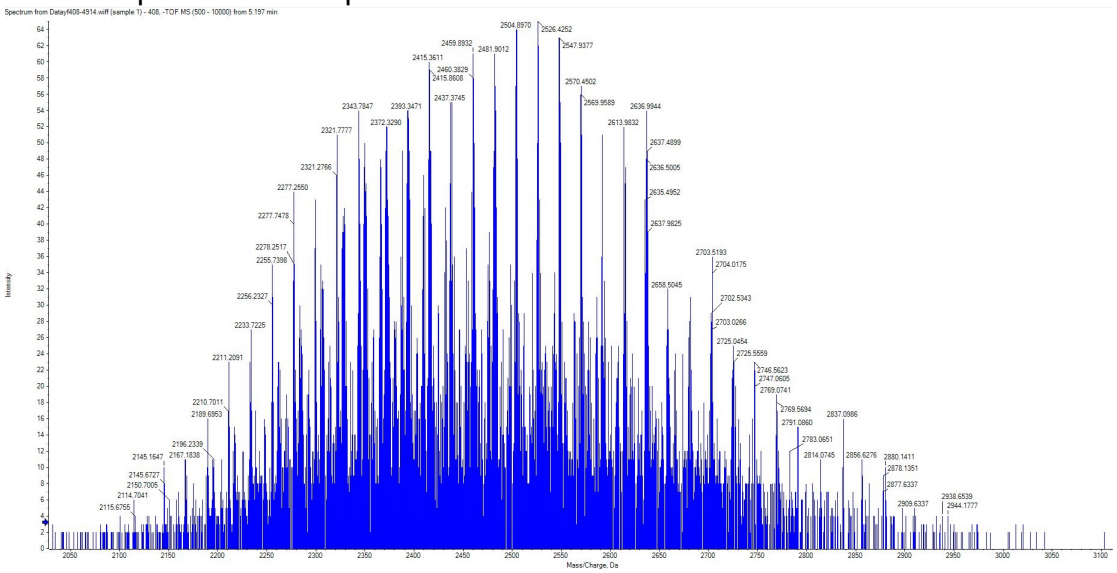
The black arrow indicates the position of the target peptide.

After HPLC separation (Method C):



3.33 mg, 0.588 µmol, y. 73%. Retention time: 12.1 min (Method C).

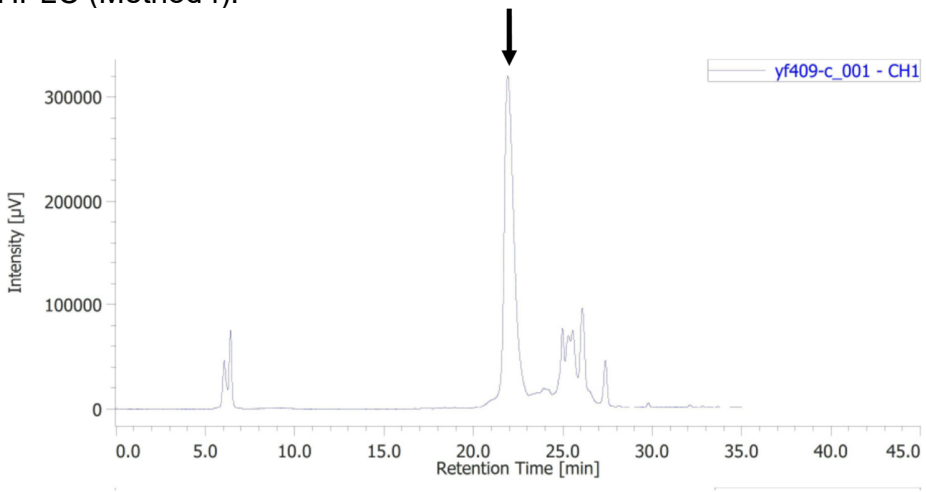
ESI-MS spectrum of the product is shown below.



One of the peaks of the highest intensity has *m*/*z* of 2504.8, which corresponds to n = 14 (Calcd: 2501.4 $[M-2H]^{2-}$).ESI-TOFMS *m*/*z* Calcd (n = 14): 2501.4 $[M-2H]^{2-}$;

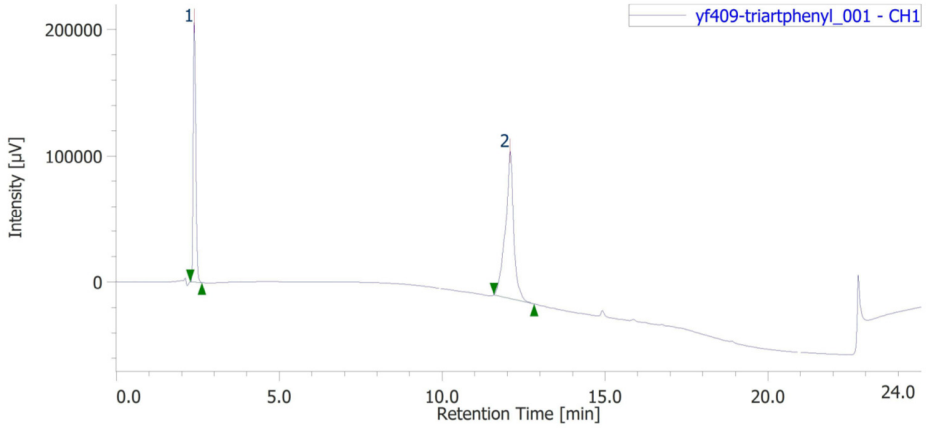
PEG_{2000}-LANA-DSSMe (13)

Crude HPLC (Method f):



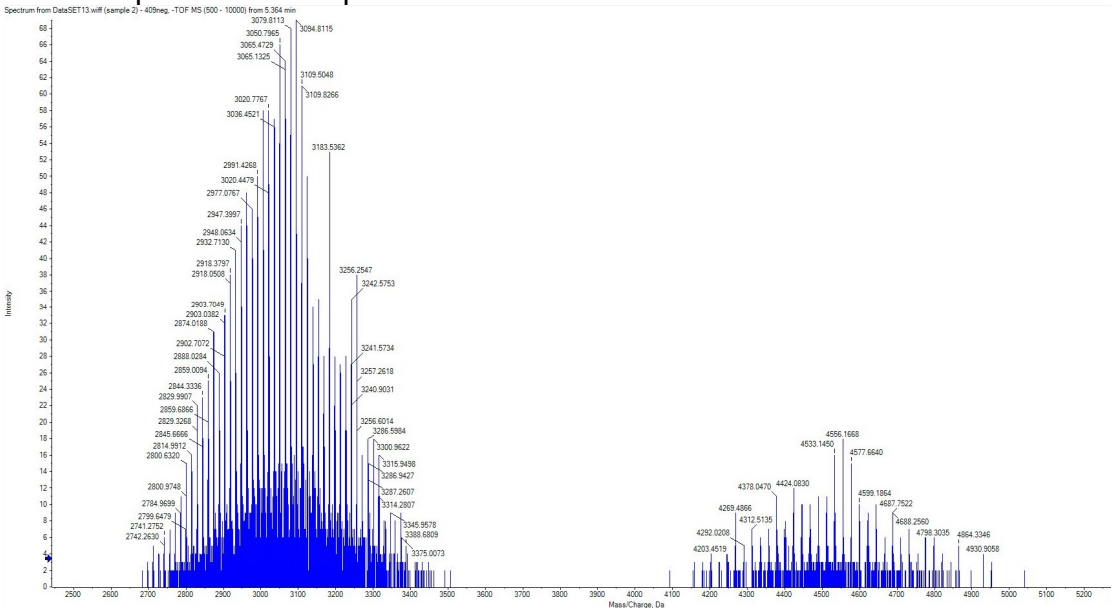
The black arrow indicates the position of the target peptide.

After HPLC separation (Method C):



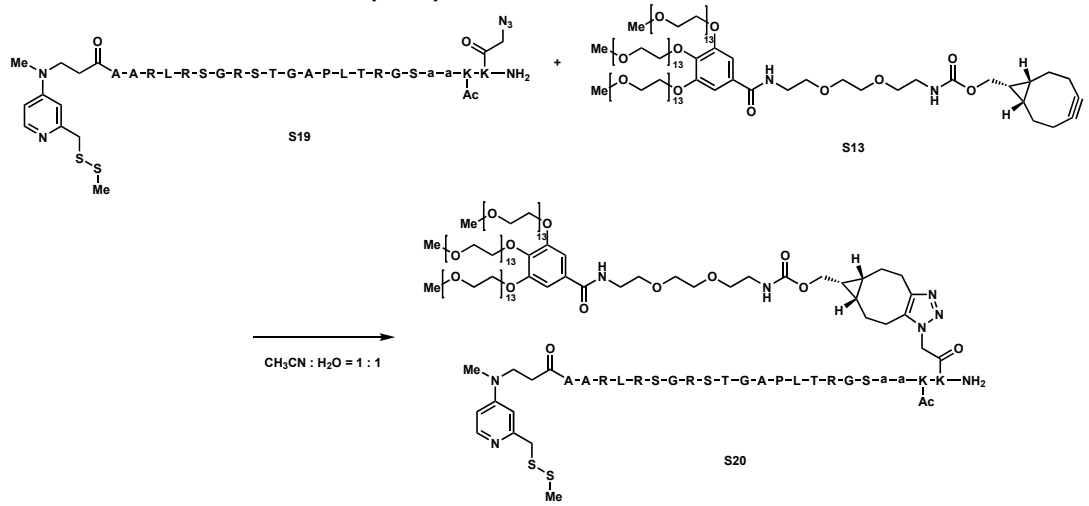
6.48 mg, 0.668 µmol, y. 84%. Retention time: 12.1 min (Method C).

ESI-MS spectrum of the product is shown below.



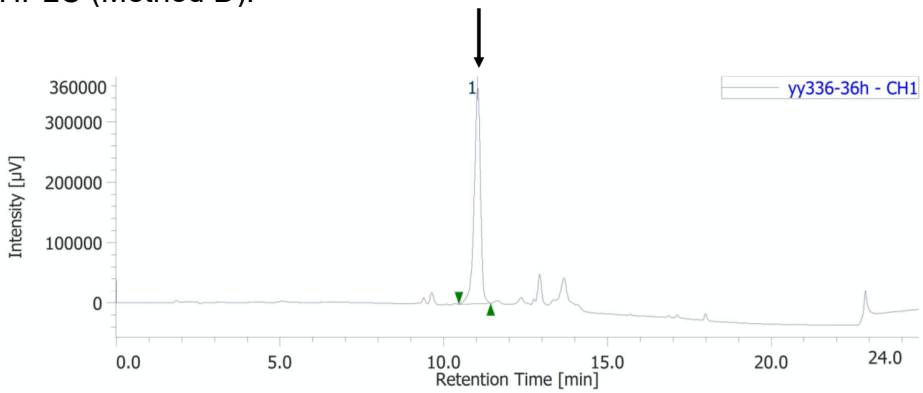
Peaks in the range of 2700–3500 are those of product trivalent anions, and peaks in the range of 4200–5000 are those of product divalent anions. For example, one of the peaks of the trivalent anions has *m*/*z* of 3079.8, which corresponds to n = 46 (Calcd: 3076.7 $[M-3H]^{3-}$).

PEG$_{550}$-mutLANA-DSSMe (S20)



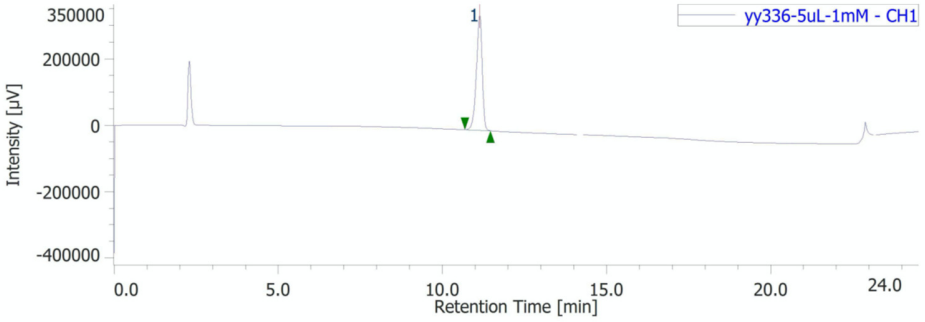
Peptide **S19** (10 mM in water, 77.2 µL, 0.772 µmol) and alkyne **S13** (50 mM in MeCN, 30.9 µL, 1.54 µmol) were mixed in water:MeCN (1:1). The mixture was stirred at r.t for 36 h, and insoluble materials were removed by filtration. Filtrate was purified with preparative HPLC (Method e) to afford **S20** (2.16 mg, 0.400 µmol, 51.9%) as white powder after lyophilization.

Crude HPLC (Method D):

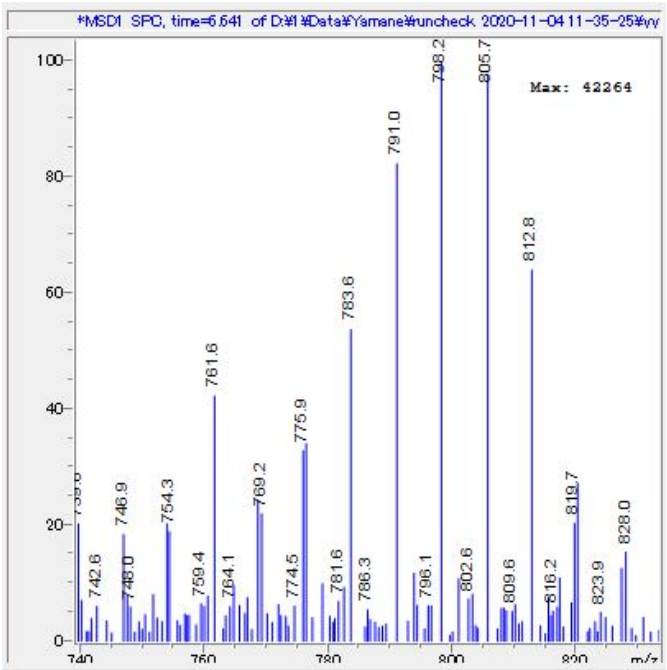


The black arrow indicates the position of the target peptide.

After HPLC separation (Method D):



Retention time: 11.1 (Method D)
ESI-TOFMS sprectrum of the product is shown below.



One of the peaks of the highest intensity has *m/z* of 805.7, which corresponds to n = 13 (Calcd: 805.3 $[M+6H]^{6+}$).

Method for biochemical experiments

Electrophoresis Mobility Shift Assay

Recombinant nucleosomes (0.4 μM for DNA/histone octamer concentration) were incubated with each concentration of PEG-LANA-FITC in 16 mM Tris-HCl (pH 7.5) buffer containing 0.8 mM DTT at 37 ºC for 1 h. The samples were analyzed by non-denaturing 6% PAGE in 0.2x Tris-borate EDTA (TBE) buffer (18 mM Tris base, 18 mM boric acid, and 0.4 mM EDTA). FITC signals were detected by ImageQuant LAS 4010 and DNA was visualized by ethidium bromide staining. The fluorescence intensities of nucleosome-bound PEG-LANA-FITC were measured using Fiji/ImageJ ver. 1.51h, and subtracted by background intensity from samples without nucleosomes. The intensities were fitted with the Michaelis-Menten equation [$I = A * x / (x + B)$]. I, the measured intensity of FITC signals (nucleosome-bound PEG-LANA-FITC); A, the intensity when all acidic patch bind to PEG-LANA-FITC; B, the dissociation constant (K_d); x, the concentration of the PEG-LANA-FITC.

Estimation of histone amount in living cells

2 x 10^6 HeLa S3 cells were harvested by trypsinization. Histone H2A/H2B were isolated with Histone Purification Kit (Active Motif) according to the manufacturer's instructions from the cells, and dissolved in 100 μL of 0.1 M Tris-HCl (pH7.5). 30 μL of 5x sample buffer and 20 μL of 1 M DTT were added. After boiling, the samples were separated by SDS-PAGE, and the gels were stained by oriole. Calibration curve was drawn by reconstituted nucleosome, and signals were measured using Fiji/ImageJ ver. 1.51h.

Estimation of in-cell K_d of PEG-LANA-FITC

In FRAP experiment, fluorescence recovery time depends on compound diffusion or binding/dissociation with histones. When the diffusion of the compound occurs negligibly fast, and the rate-determining step is the binding or dissociation of the PEG-LANA-FITC to nucleosomes, fluorescence at the bleached point, $f(t)$ was given in Eq.(1).

$$f(t) = I_{\mathrm{final}} \{1 - \left(\frac{k_{\mathrm{on}} [S_{\mathrm{eq}}]}{k_{\mathrm{on}} [S_{\mathrm{eq}}] + k_{\mathrm{off}}}\right) e^{(-kt)}\} \ldots (1)$$

I_{final}, the total recovery of fluorescence; k, the binding coefficient; t, time (s); k_{on}, the binding rate constant; k_{off}, the detachment rate constant; $[S_{\mathrm{eq}}]$, the concentration of free acidic patch. The dissociation constant (K_d) is defined as shown in Eq.(2),

$$K_{\mathrm{d}} = \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} \ldots (2)$$

Insert (2) into (1),

$$f(t) = I_{\mathrm{final}} \{1 - \left(\frac{[S_{\mathrm{eq}}]}{[S_{\mathrm{eq}}] + K_{\mathrm{d}}}\right) e^{(-kt)}\} \ldots (3).$$

From the FRAP experiment for PEG_{550}-LANA-FITC **7**, recovery curves were fitted as Eq.(4).

$$f(t) = 0.83\,(1 - 0.53\,e^{-2.5t}) \ldots (4)$$

Since the concentration of **7** should be negligibly lower than that of nucleosome in the experiments, [S_{eq}] can be approximated to acidic patch concentration. As the concentration of acidic patches was estimated to be about 0.1 mM (Figure S4), K_d value of **7** in living cells was estimated to be about 0.09 mM.

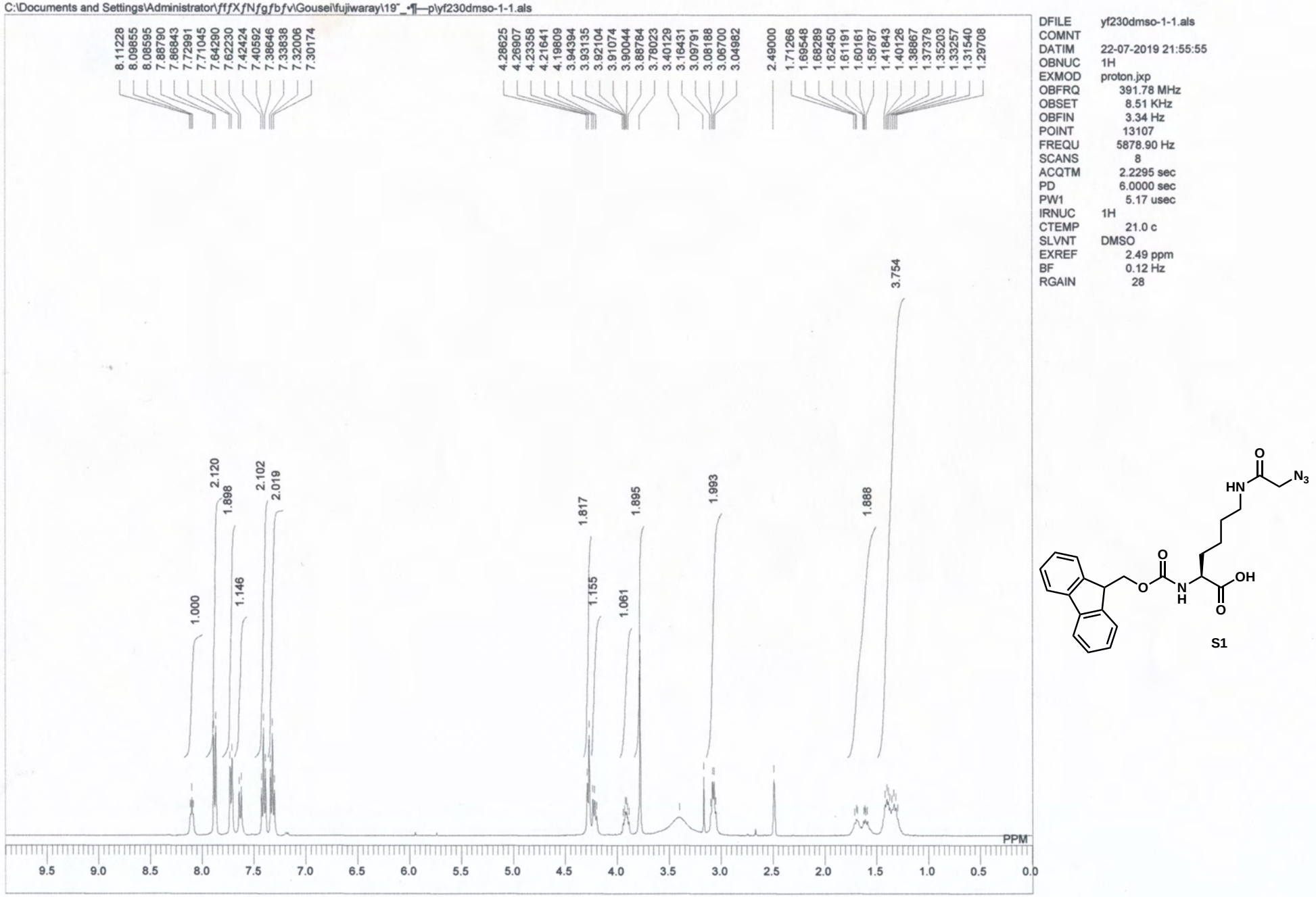
Quantification of the introduced PEG-LANA-FITCs into cells

0.5 mM PEG-LANA-FITC in PBS was incorporated into HeLa S3 cells by bead loading (4). Then the cells were harvested with accutase, and sorted into 3 groups (high, mid, low) as described above using FACS Arial II. 2 x 10^4 cells were collected, lysed in pre-chilled CRB buffer supplemented with 2 mM $MgCl_2$, 25 mU/μL Benzonase, protease cinhibitor cocktail (Sigma, P2714), and 1 mM PMSF on ice for 30 min. After centrifugation (15,000 rpm for 10 min at 4 ºC), the supernatants were separated by Novex™ 16% Tricine Protein Gels, and FITC signals were detected by ImageQuant LAS 4010.

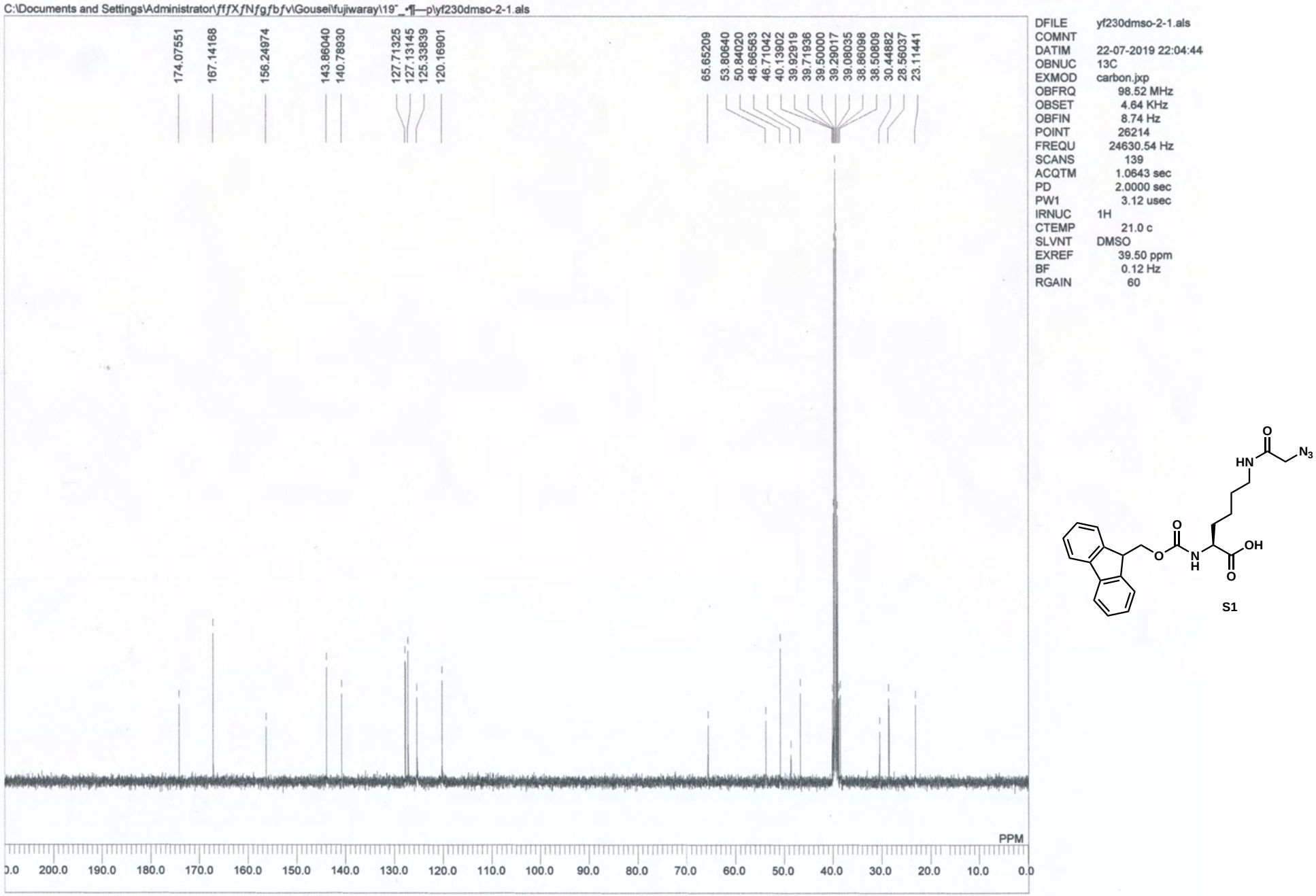
SI References

1. Shi, B., Yan, O., Tang, J., Xin, K., Zhang, J., Zhu, Y, Xu, G., Wang, R., Chen, J., Gao, W., et al. Hydrogen Sulfide-Activatable Second Near-Infrared Fluorescent Nanoassemblies for Targeted Photothermal Cancer Therapy. *Nano Lett.* **139**, 7568-7576 (2018).
2. Sui, B., Yue, X., Kim, B., and Belfield, K. D. Near-IR Two Photon Fluorescent Sensor for K+ Imaging in Living Cells. *ACS Appl. Mater. Interfaces* **7**, 17565-17568 (2015).
3. Amamoto, Y., Aoi, Y., Nagashima, N., Suto, H., Yoshidome, D., Arimura, Y., Osakabe, A., Kato, D., Kurumizaka, H., Kawashima, S. A., et al. Synthetic Posttranslational Modifications: Chemical Catalyst-Driven Regioselective Histone Acylation of Native Chromatin. *J. Am. Chem. Soc.* **139**, 7568-7576 (2017).
4. Sato, Y., Stasevich, T. J., and Kimura, H. Visualizing the Dynamics of Inactive X Chromosomes in Living Cells Using Antibody-Based Fluorescent Probes. *Methods Mol. Biol.* **1861**, 91-102 (2018).

Spectral data



S1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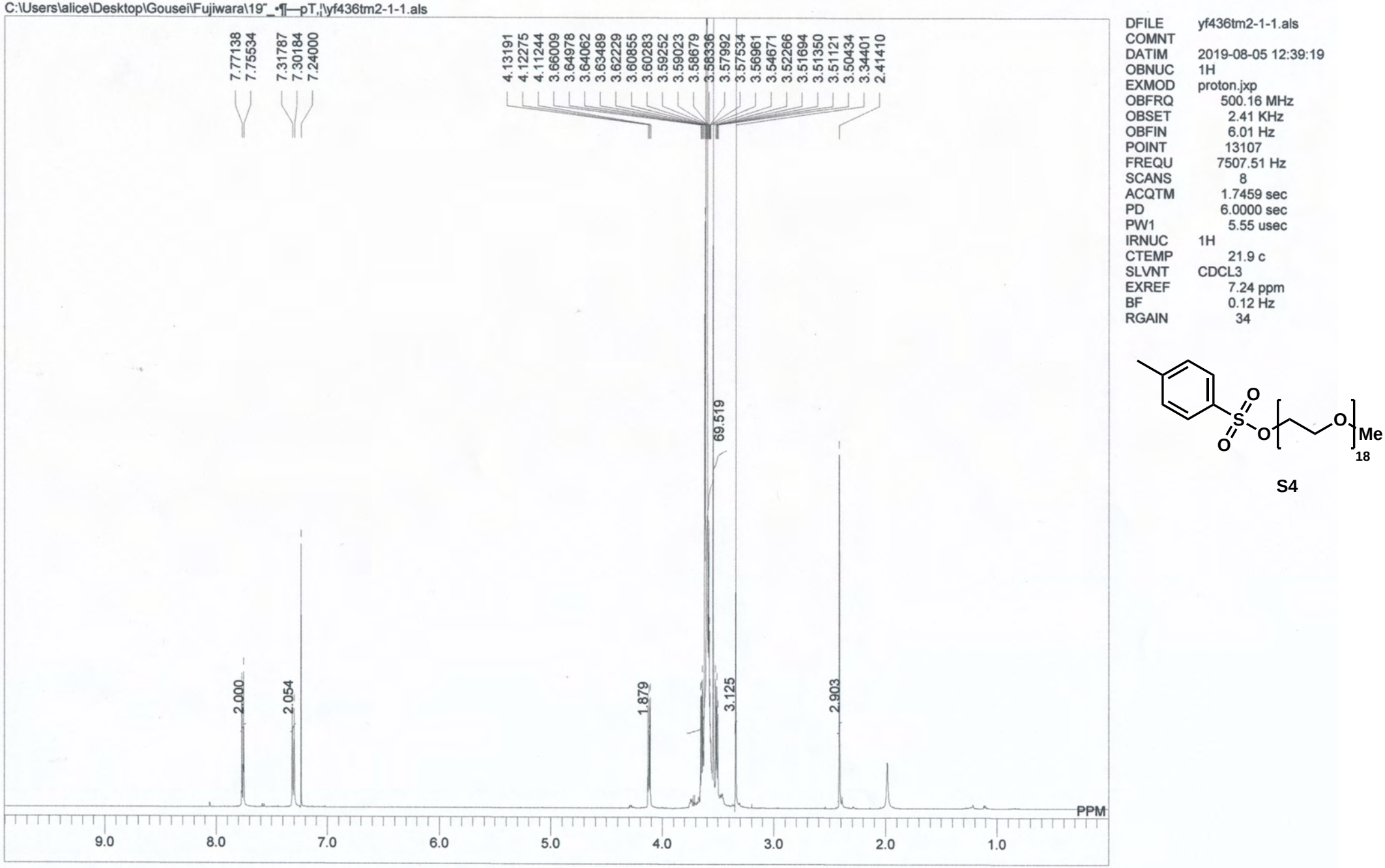
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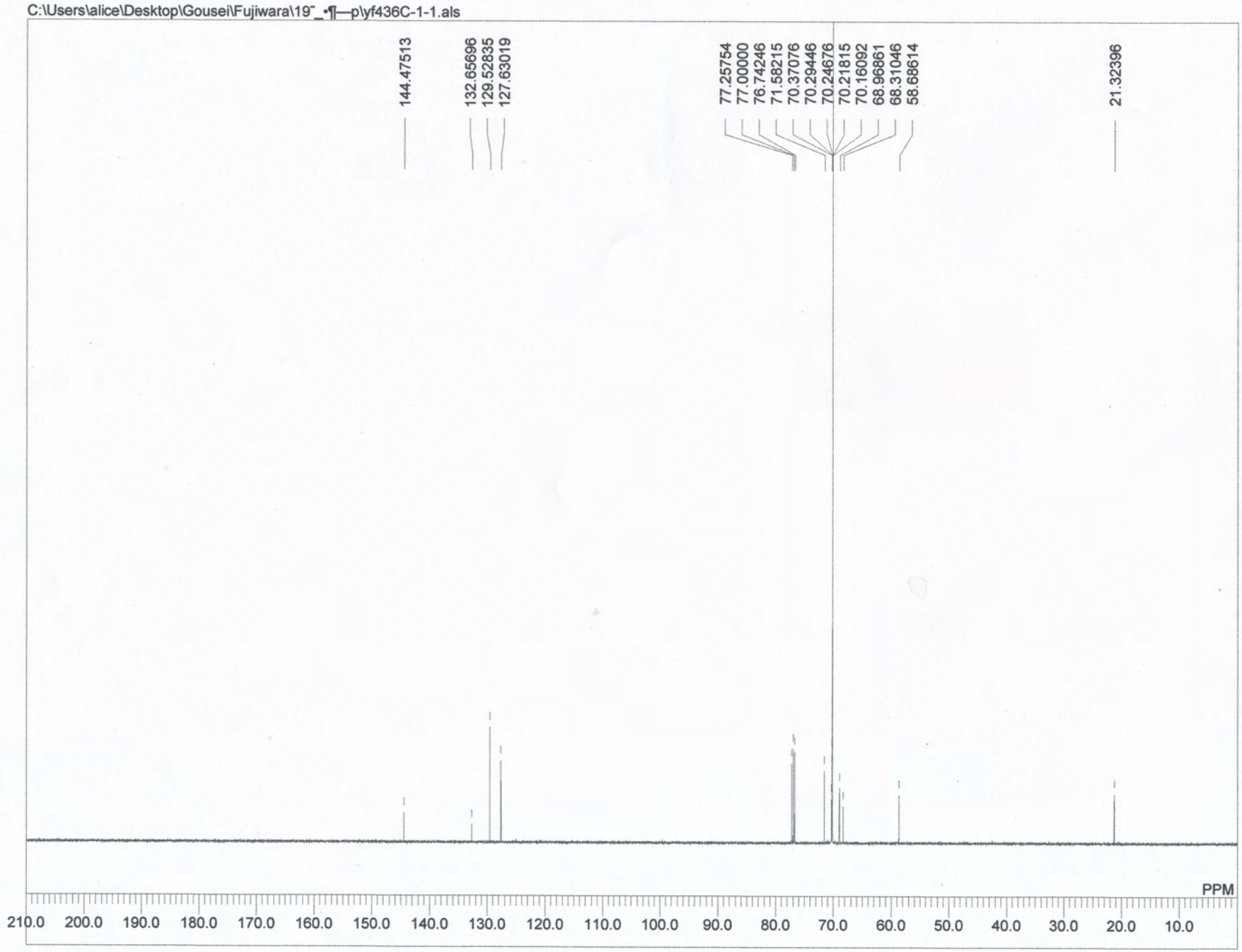
S4



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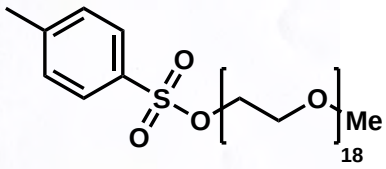
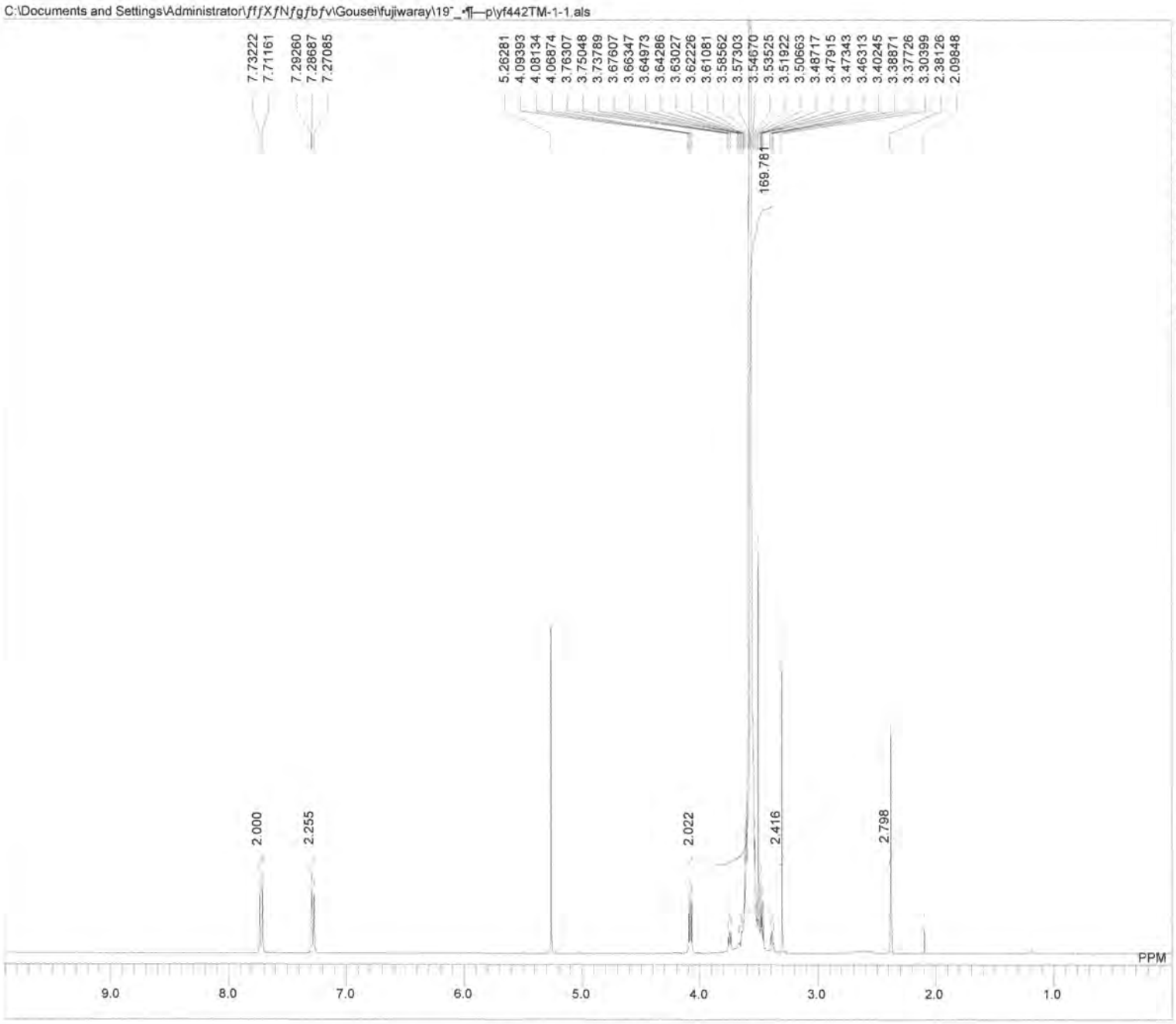
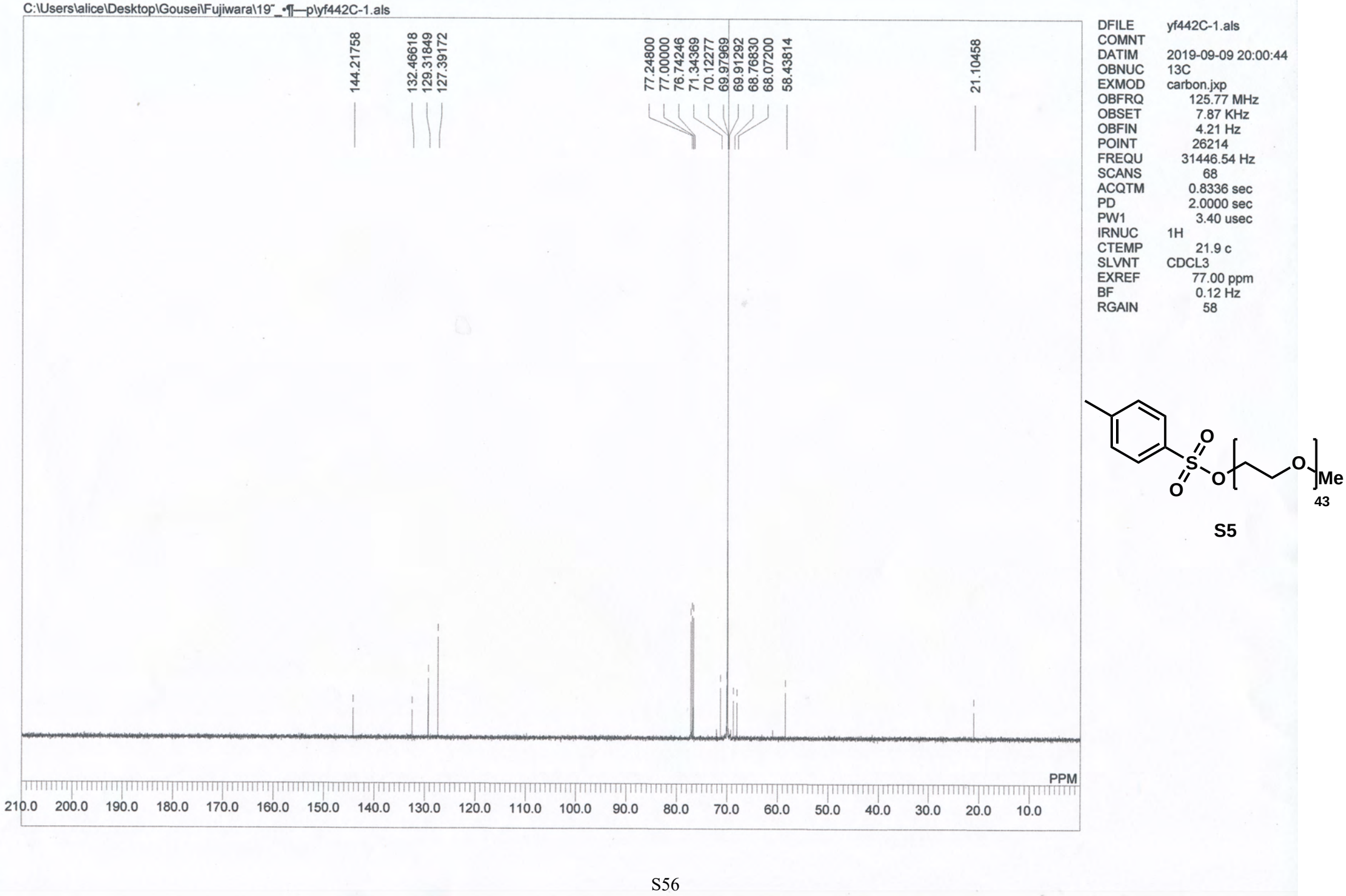
FREQU	31446.54 Hz
SCANS	69
ACQTM	0.8336 sec
PD	2.0000 sec
PW1	3.40 usec
IRNUC	1H
CTEMP	21.9 c
SLVNT	CDCL3
EXREF	77.00 ppm
BF	0.12 Hz
RGAIN	60



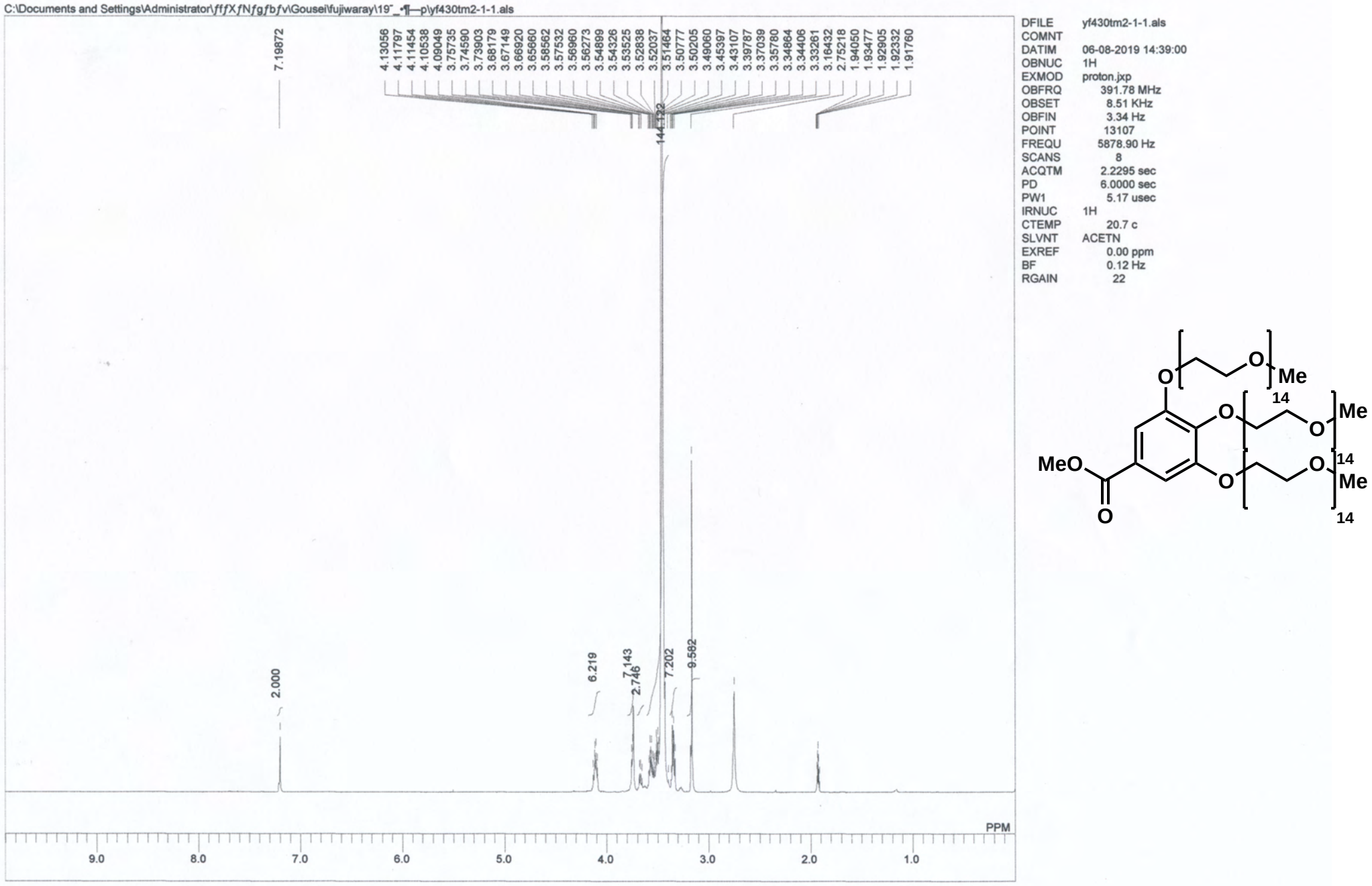
S4

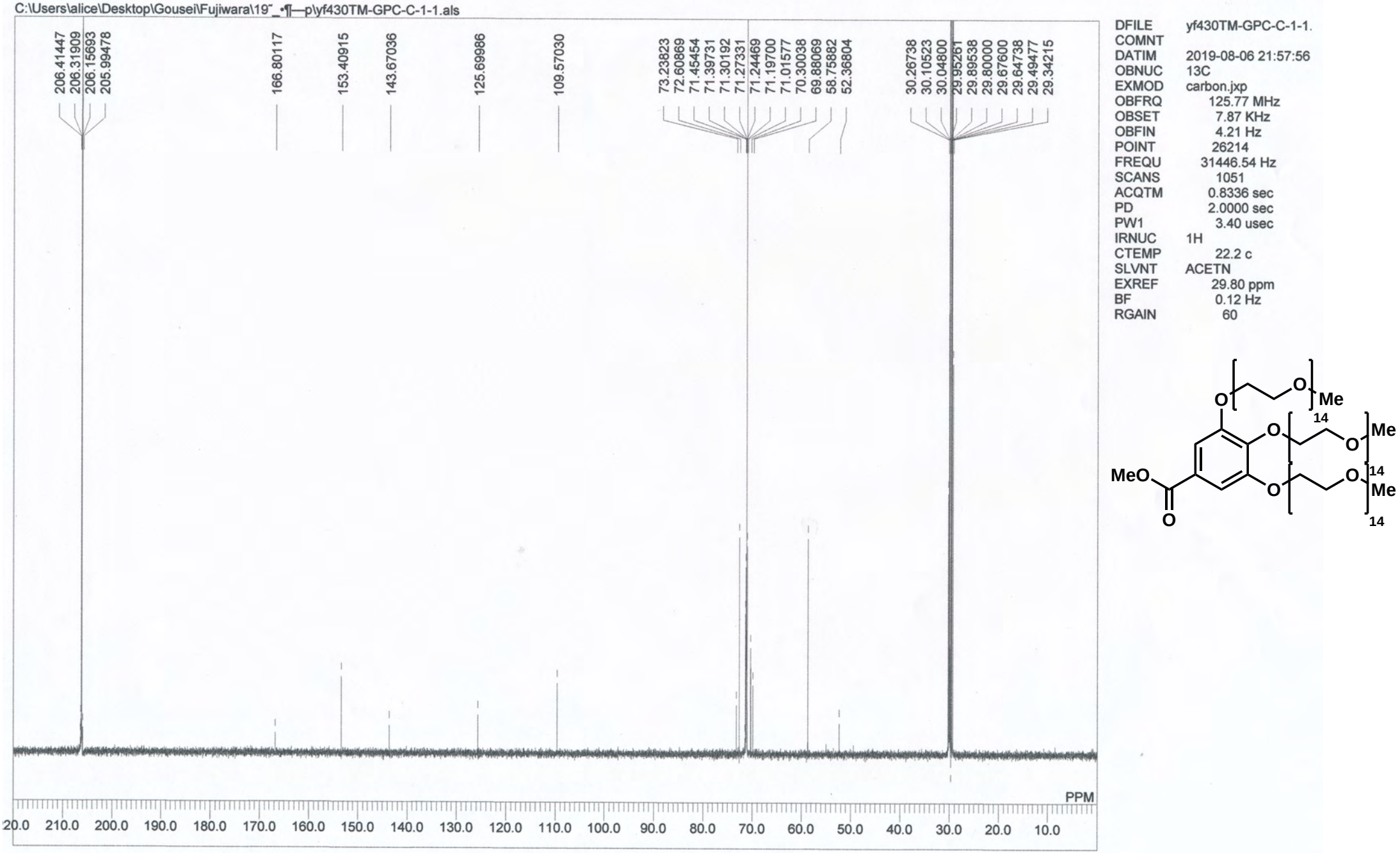

C:\Documents and Settings\Administrator\ƒfƒXƒNƒgƒbƒv\Gousei\fujiwaray\19¯_·¶—p\yf442TM-1-1.als
7.73222
7.71161
7.29260
7.28687
7.27085
5.26281
4.09393
4.08134
4.06874
3.76307
3.75048
3.73789
3.67607
3.66347
3.64973
3.64286
3.63027
3.62226
3.61081
3.58562
3.57303
3.54670
3.53525
3.51922
3.50663
3.48717
3.47915
3.47343
3.46313
3.40245
3.38871
3.37726
3.30399
2.38126
2.09848
169.781
2.000
2.255
2.022
2.416
2.798
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
DFILE yf442TM-1-1.als
COMNT
DATIM 10-08-2019 23:47:35
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.9 c
SLVNT CDCL3
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 18
O
S
O
O
O
Me
43

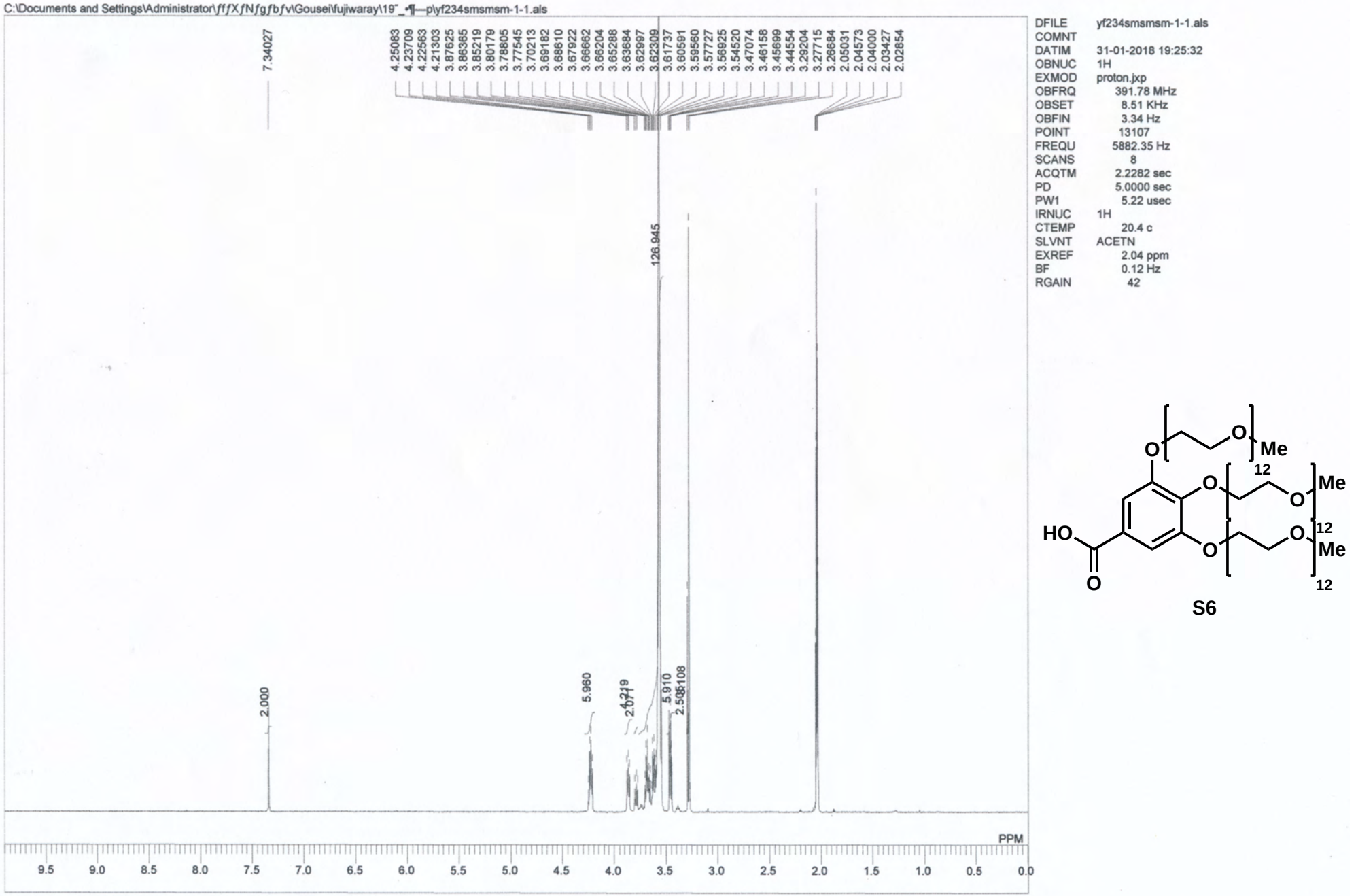
S5


C:\Users\alice\Desktop\Gousei\Fujiwara\19¯_•¶—p\yf442C-1.als
144.21758
132.46618
129.31849
127.39172
77.24800
77.00000
76.74246
71.34369
70.12277
69.97969
69.91292
68.76830
68.07200
58.43814
21.10458
DFILE yf442C-1.als
COMNT
DATIM 2019-09-09 20:00:44
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 68
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 21.9 c
SLVNT CDCL3
EXREF 77.00 ppm
BF 0.12 Hz
RGAIN 58
O
S
O
O
O
Me
43
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

S5

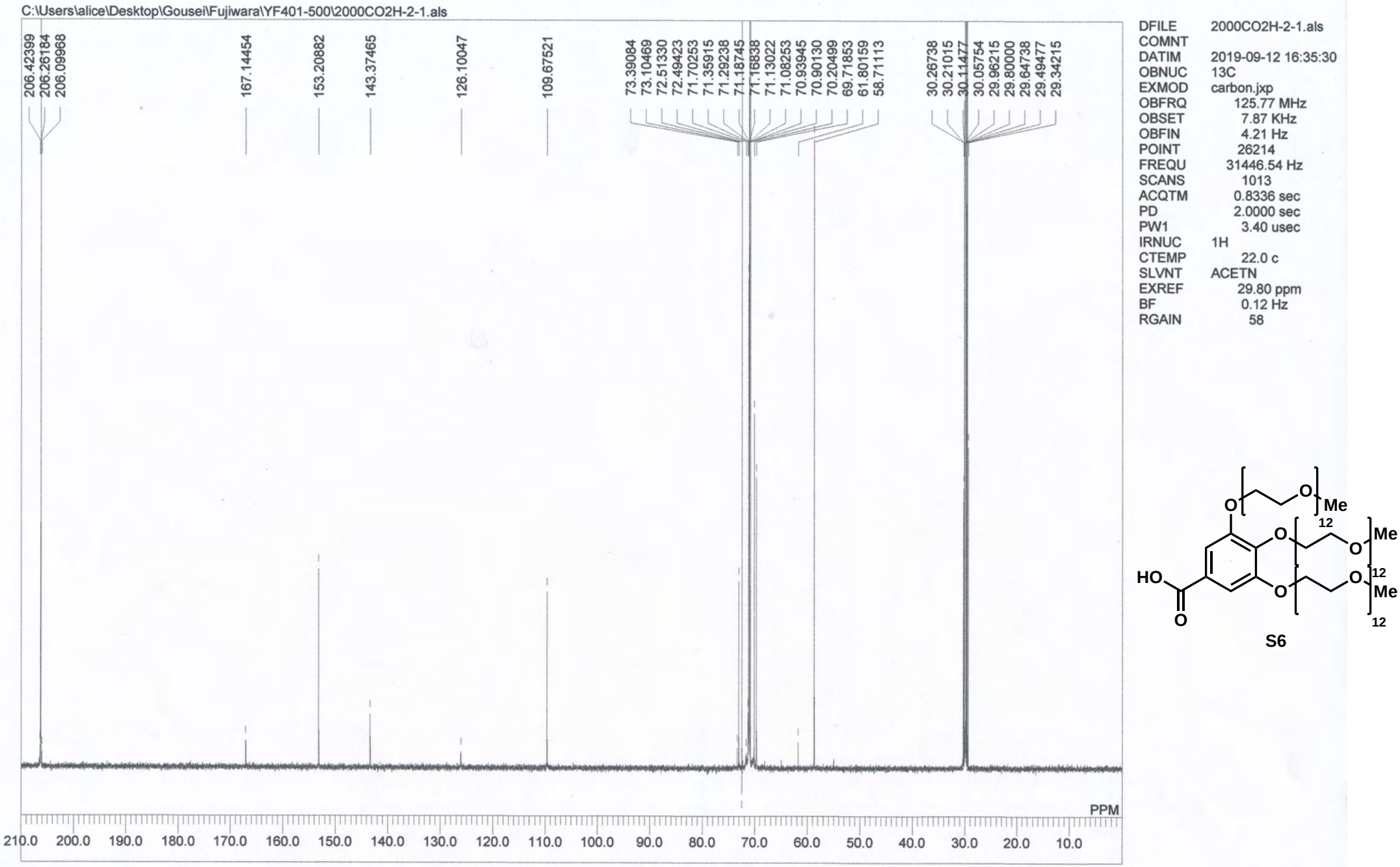

C:\Documents and Settings\Administrator\ƒfƒXƒNƒgƒbƒv\Gousei\fujiwaray\19¯_·¶—p\yf430tm2-1-1.als
7.19872
4.13056
4.11797
4.11454
4.10538
4.09049
3.75735
3.74590
3.73903
3.68179
3.67149
3.66920
3.65660
3.58562
3.57532
3.56960
3.56273
3.54899
3.54326
3.53525
3.52838
3.52037
3.51464
3.50777
3.50205
3.49060
3.45397
3.43107
3.39787
3.37039
3.35780
3.34864
3.34406
3.33261
3.16432
2.75218
1.94050
1.93477
1.92905
1.92332
1.91760
144.122
2.000
6.219
7.143
2.746
7.202
9.582
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
DFILE yf430tm2-1-1.als
COMNT
DATIM 06-08-2019 14:39:00
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.7 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 22
MeO
O
O
O
O
O
O
O
O
Me
14
Me
14
Me
14


C:\Users\alice\Desktop\Gousei\Fujiwara\19"_•¶—p\yf430TM-GPC-C-1-1.als
206.41447
206.31909
206.15693
205.99478
166.80117
153.40915
143.67036
125.69986
109.57030
73.23823
72.60869
71.45454
71.39731
71.30192
71.27331
71.24469
71.19700
71.01577
70.30038
69.88069
58.75882
52.36804
30.26738
30.10523
30.04800
29.95261
29.89538
29.80000
29.67600
29.64738
29.49477
29.34215
DFILE yf430TM-GPC-C-1-1.
COMNT
DATIM 2019-08-06 21:57:56
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 1051
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.2 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
MeO
O
O
Me
14
O
O
Me
14
O
O
Me
14
PPM
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0


C:\Documents and Settings\Administrator\ƒfƒXƒNƒgƒbƒv\Gousei\fujiwaray\19¯_·¶—plyf234smsmsm-1-1.als
7.34027
4.25083
4.23709
4.22563
4.21303
3.87625
3.86365
3.85219
3.80179
3.78805
3.77545
3.70213
3.69182
3.68810
3.67922
3.66662
3.66204
3.65288
3.63684
3.62997
3.62309
3.61737
3.60591
3.59560
3.57727
3.56925
3.54520
3.47074
3.46158
3.45699
3.44554
3.29204
3.27715
3.26684
2.05031
2.04573
2.04000
2.03427
2.02854
126.945
2.000
5.960
2.219
2.071
5.910
2.506
2.108
PPM
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
DFILE yf234smsmsm-1-1.als
COMNT
DATIM 31-01-2018 19:25:32
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5882.35 Hz
SCANS 8
ACQTM 2.2282 sec
PD 5.0000 sec
PW1 5.22 usec
IRNUC 1H
CTEMP 20.4 c
SLVNT ACETN
EXREF 2.04 ppm
BF 0.12 Hz
RGAIN 42

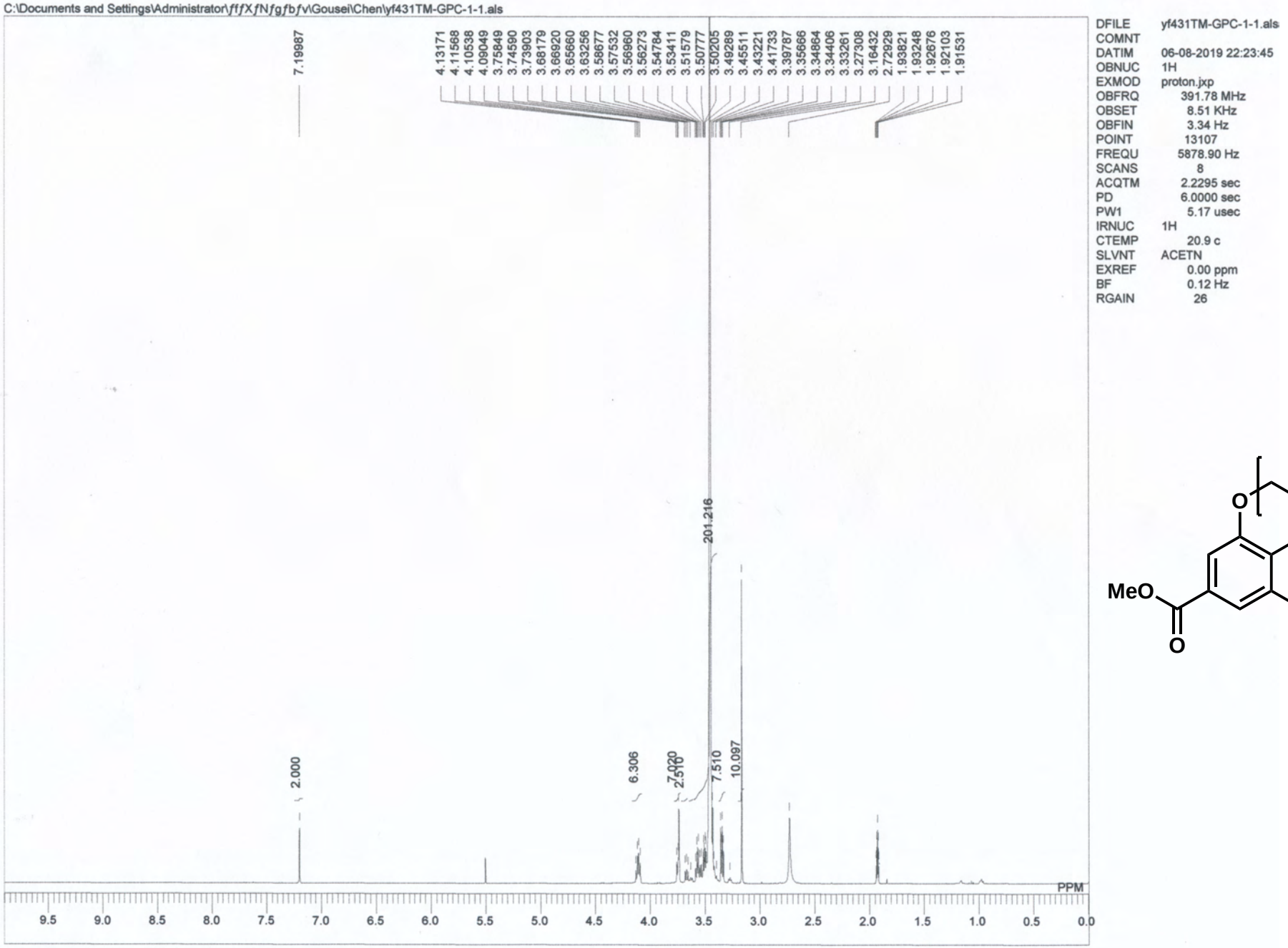
HO
O
O
O
Me
12
O
O
Me
12
O
O
12
Me
12

S6

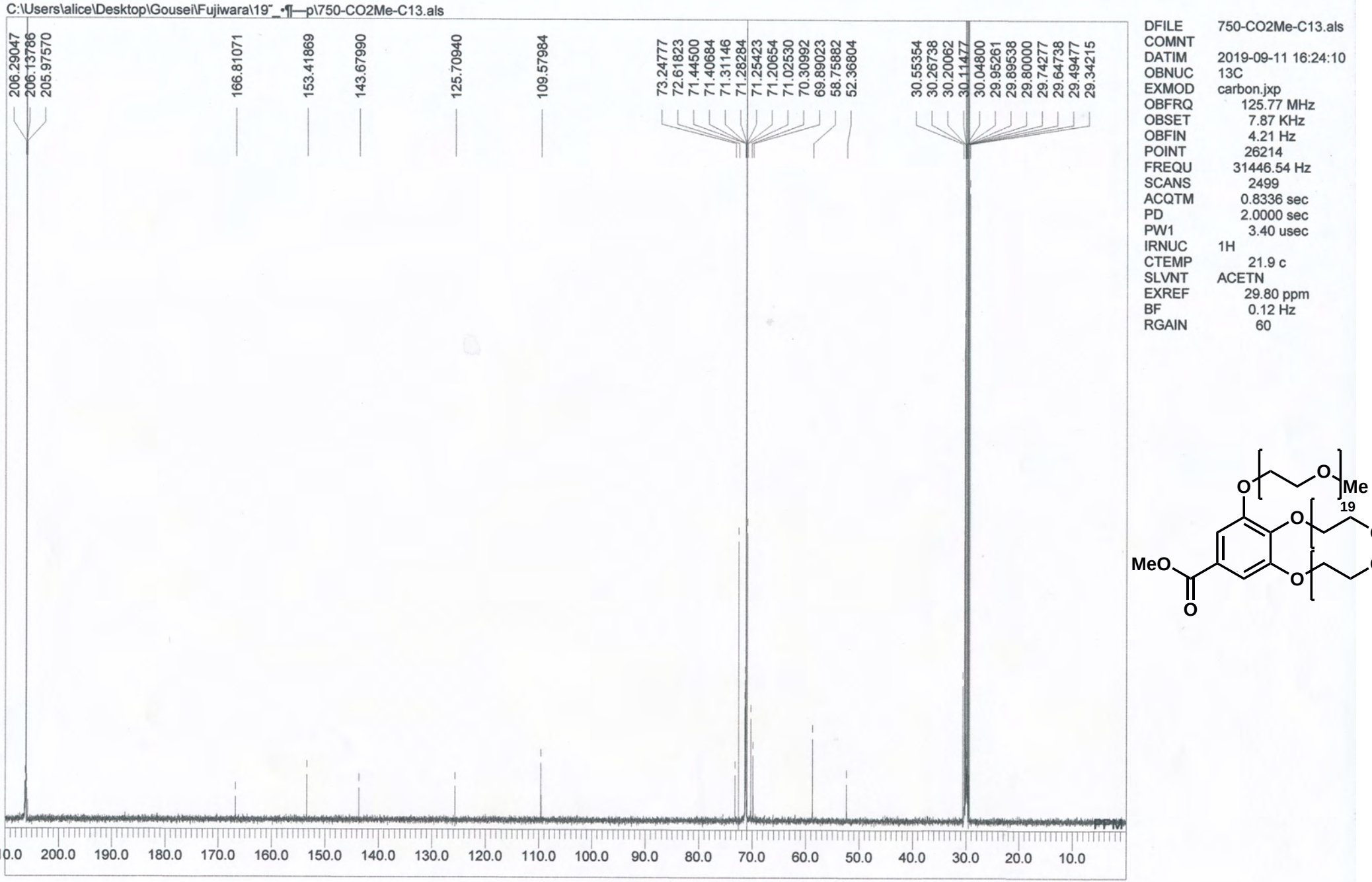

C:\Users\alice\Desktop\Gousei\Fujiwara\YF401-500\2000CO2H-2-1.als
206.42399
206.26184
206.09968
167.14454
153.20882
143.37465
126.10047
109.67521
73.39084
73.10469
72.51330
72.49423
71.70253
71.35915
71.29238
71.18745
71.16838
71.13022
71.08253
70.93945
70.90130
70.20499
69.71853
61.80159
58.71113
30.26738
30.21015
30.11477
30.05754
29.96215
29.80000
29.64738
29.49477
29.34215
DFILE 2000CO2H-2-1.als
COMNT
DATIM 2019-09-12 16:35:30
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 1013
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.0 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 58
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

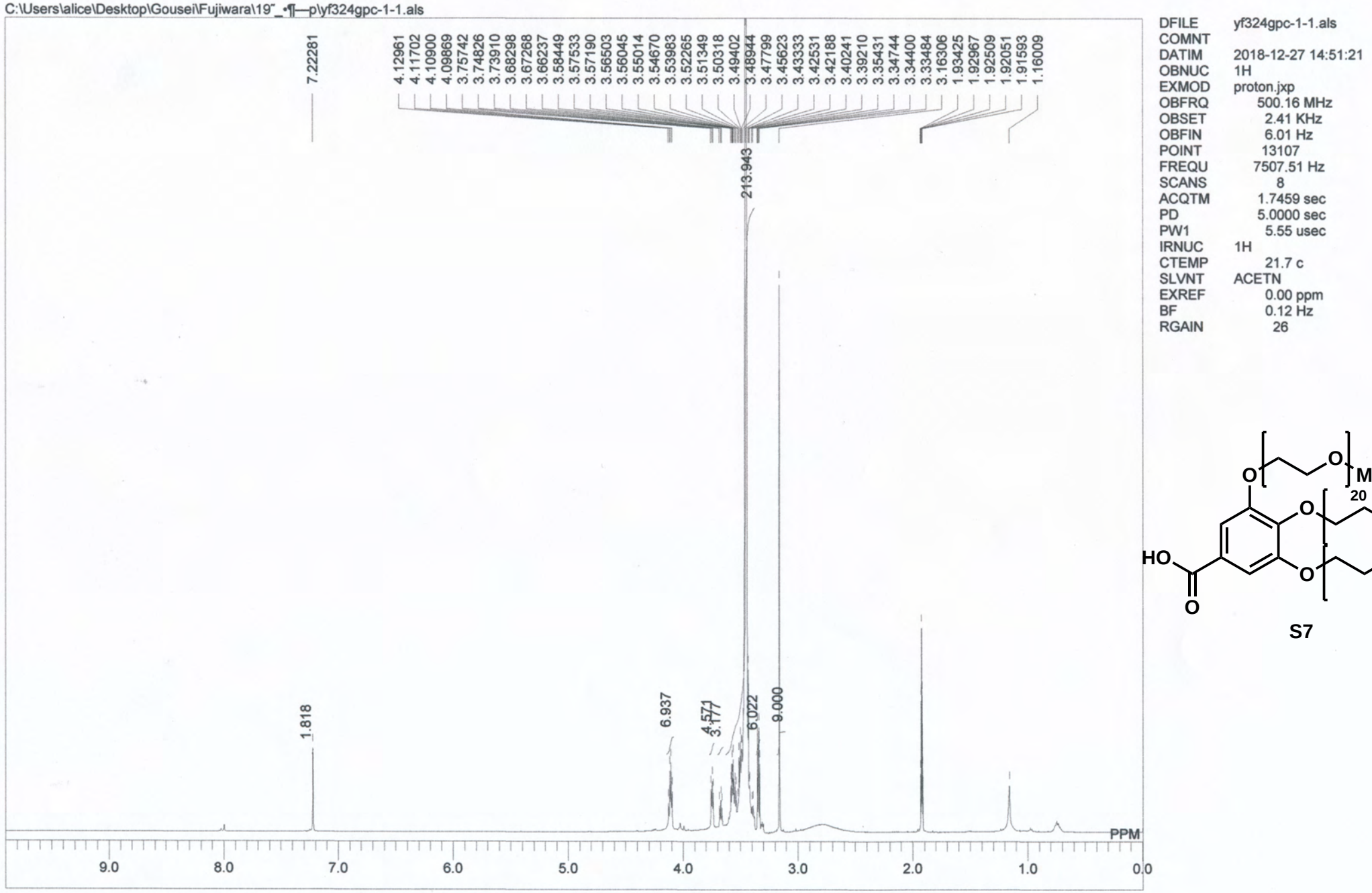
HO
O
O
O
Me
12
O
O
Me
12
O
O
Me
12

S6


C:\Documents and Settings\Administrator\ffXfNfgfbfv\Gousei\Chen\yf431TM-GPC-1-1.als
7.19987
4.13171
4.11568
4.10538
4.09049
3.75849
3.74590
3.73903
3.68179
3.66920
3.65660
3.63256
3.58677
3.57532
3.56960
3.56273
3.54784
3.53411
3.51579
3.50777
3.50205
3.49289
3.45511
3.43221
3.41733
3.39787
3.35666
3.34864
3.34406
3.33261
3.27308
3.16432
2.72929
1.93821
1.93248
1.92676
1.92103
1.91531
201.216
2.000
6.306
7.020
2.510
7.510
10.097
PPM
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
DFILE yf431TM-GPC-1-1.als
COMNT
DATIM 06-08-2019 22:23:45
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.9 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 26

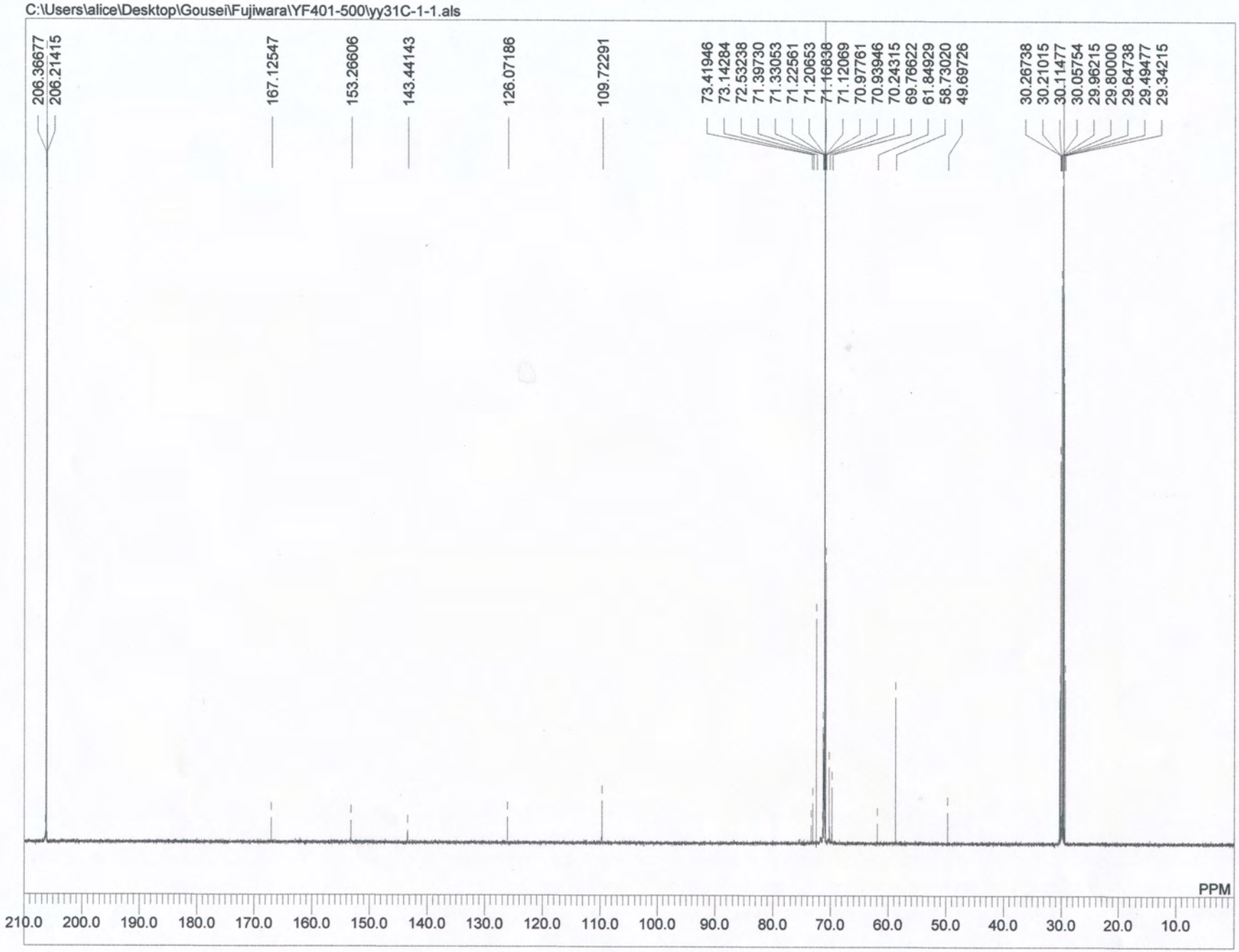
MeO
O
O
Me
19
O
O
Me
19
O
O
Me
19


C:\Users\alice\Desktop\Gousei\Fujiwara\19"_•¶—p\750-CO2Me-C13.als
206.29047
206.13786
205.97570
166.81071
153.41869
143.67990
125.70940
109.57984
73.24777
72.61823
71.44500
71.40684
71.31146
71.28284
71.25423
71.20654
71.02530
70.30992
69.89023
58.75882
52.36804
30.55354
30.26738
30.20062
30.11477
30.04800
29.95261
29.89538
29.80000
29.74277
29.64738
29.49477
29.34215
DFILE 750-CO2Me-C13.als
COMNT
DATIM 2019-09-11 16:24:10
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 2499
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 21.9 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
MeO
O
O
Me
19
Me
19
Me
19

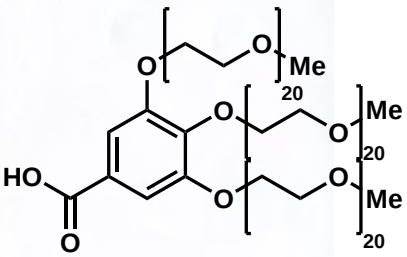


DFILE	yf324gpc-1-1.als
COMNT	
DATIM	2018-12-27 14:51:21
OBNUC	1H
EXMOD	proton.jxp
OBFRQ	500.16 MHz
OBSET	2.41 KHz
OBFIN	6.01 Hz
POINT	13107
FREQU	7507.51 Hz
SCANS	8
ACQTM	1.7459 sec
PD	5.0000 sec
PW1	5.55 usec
IRNUC	1H
CTEMP	21.7 c
SLVNT	ACETN
EXREF	0.00 ppm
BF	0.12 Hz
RGAIN	26

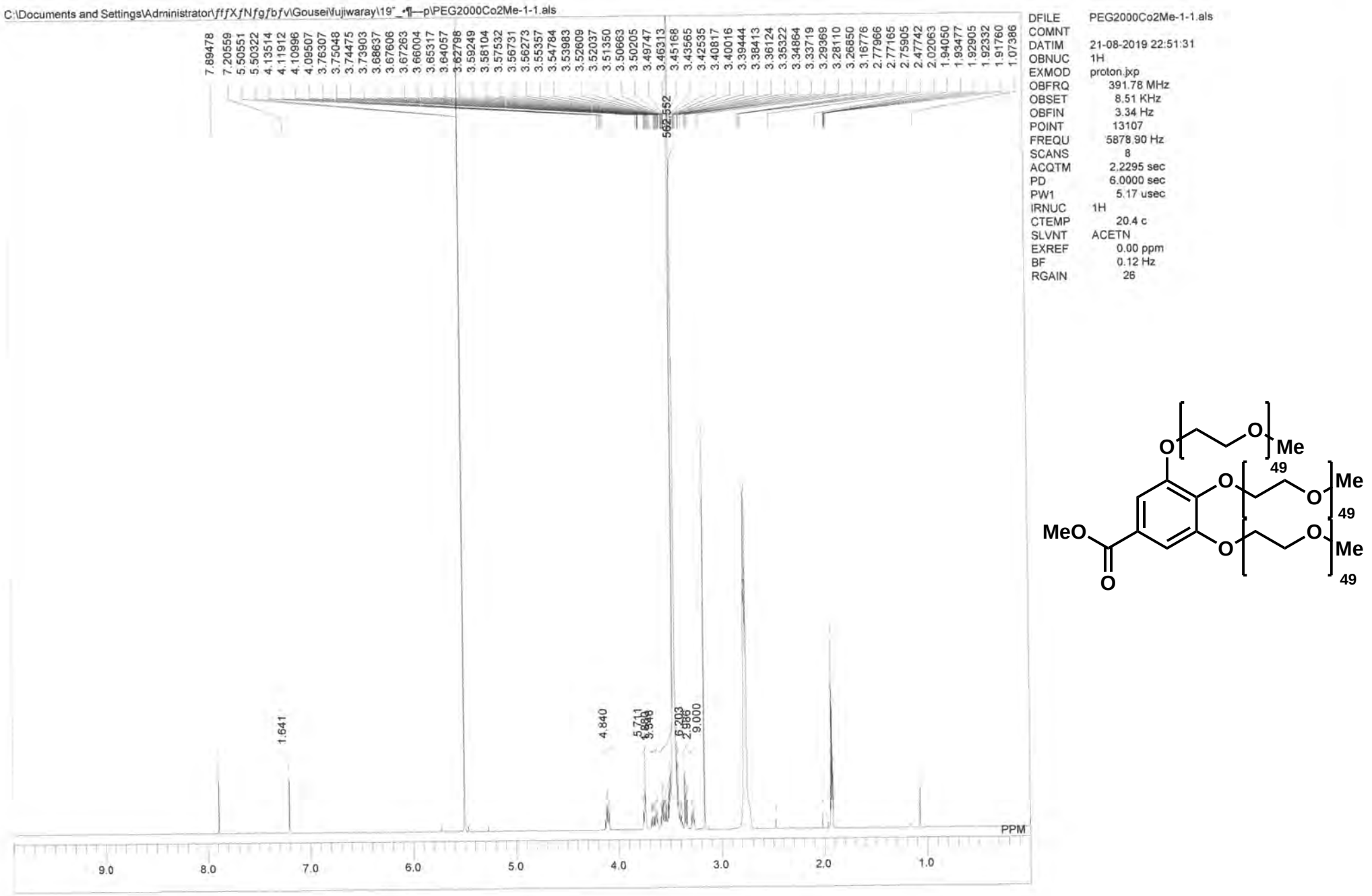
S7

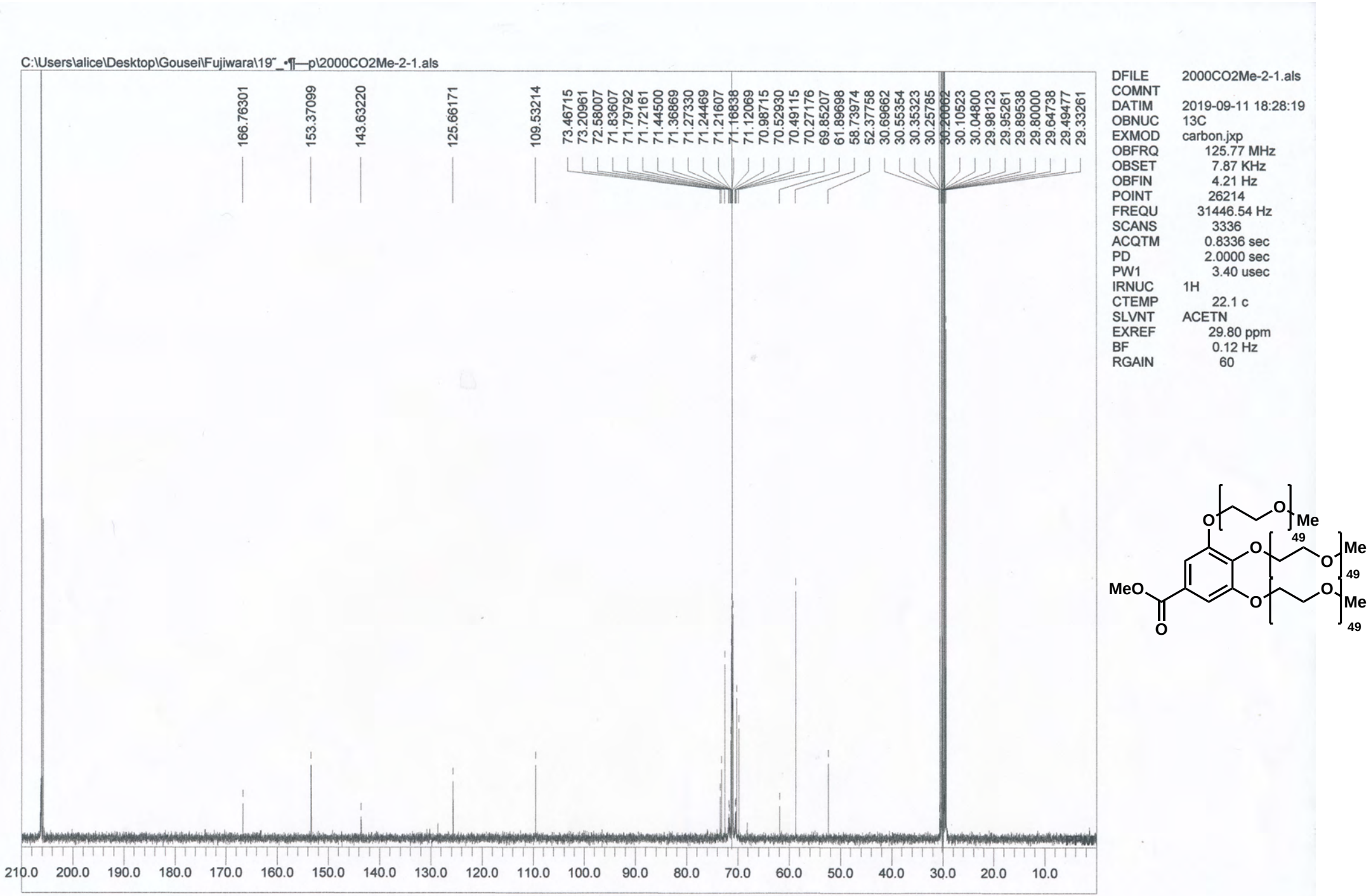


DFILE	yy31C-1-1.als
COMNT	
DATIM	2019-09-12 15:18:56
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	125.77 MHz
OBSET	7.87 KHz
OBFIN	4.21 Hz
POINT	26214
FREQU	31446.54 Hz
SCANS	682
ACQTM	0.8336 sec
PD	2.0000 sec
PW1	3.40 usec
IRNUC	1H
CTEMP	22.0 c
SLVNT	ACETN
EXREF	29.80 ppm
BF	0.12 Hz
RGAIN	60



S7


C:\Documents and Settings\Administrator\ffXfNfgfbfv\Gousei\ujiwaray\19"_•¶—p\PEG2000Co2Me-1-1.als
DFILE PEG2000Co2Me-1-1.als
COMNT
DATIM 21-08-2019 22:51:31
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.4 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 26
562.552
1.641
4.840
9.000
PPM
MeO
Me
49


C:\Users\alice\Desktop\Gousei\Fujiwara\19¯_•¶—p\2000CO2Me-2-1.als
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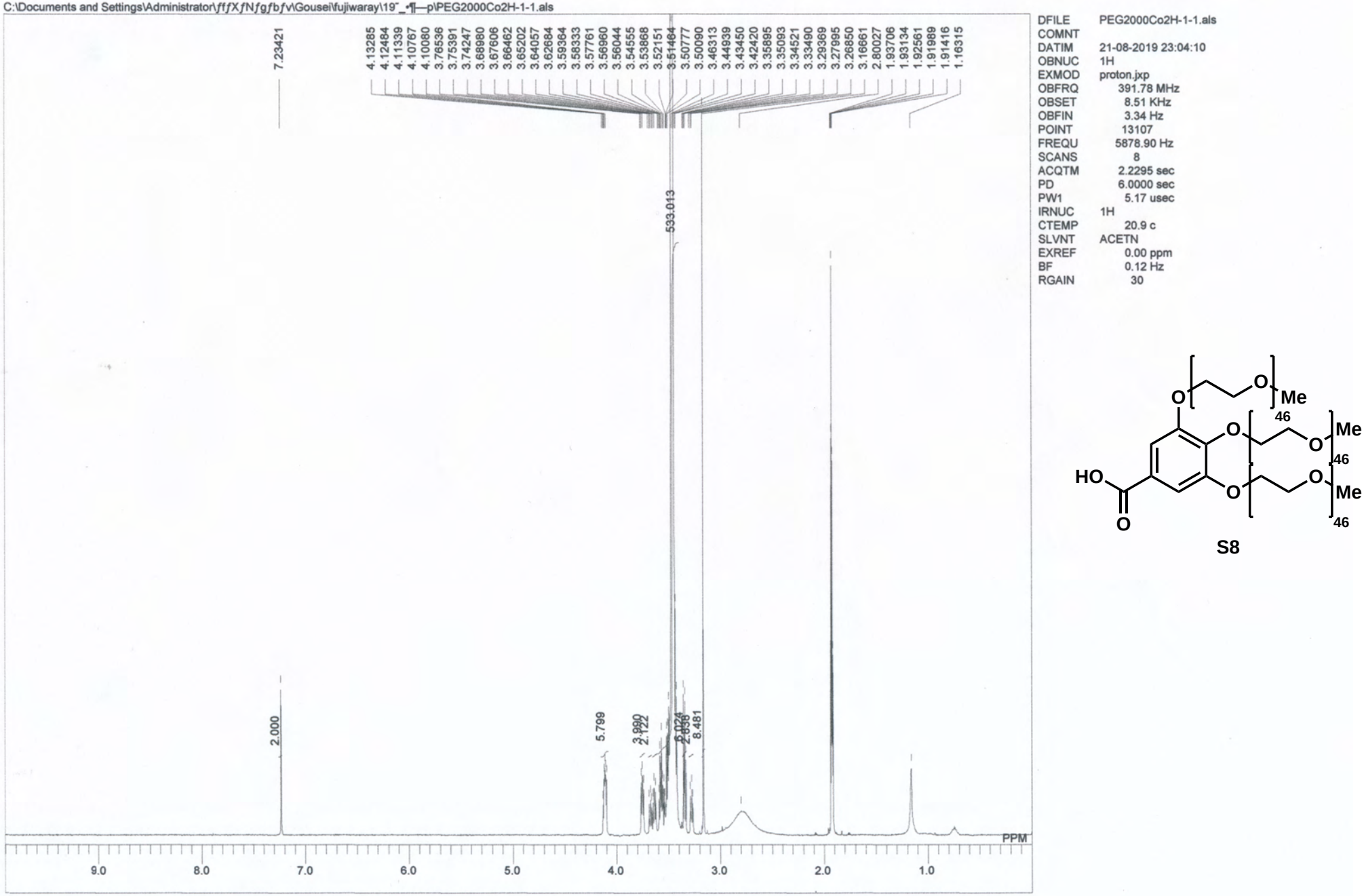
153.37099
143.63220
125.66171
109.53214
73.46715
73.20961
72.58007
71.83607
71.79792
71.72161
71.44500
71.36869
71.27330
71.24469
71.21607
71.16838
71.12069
70.98715
70.52930
70.49115
70.27176
69.85207
61.89698
58.73974
52.37758
30.69662
30.55354
30.35323
30.25785
30.20062
30.10523
30.04800
29.98123
29.95261
29.89538
29.80000
29.64738
29.49477
29.33261
DFILE 2000CO2Me-2-1.als
COMNT
DATIM 2019-09-11 18:28:19
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 3336
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.1 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
MeO
O
O
Me
49
O
O
Me
49
O
O
Me
49
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0


C:\Documents and Settings\Administrator\ƒƒƒXƒNƒgƒbƒv\Gousei\fujiwaray\19˜_•¶—p\PEG2000Co2H-1-1.als
DFILE PEG2000Co2H-1-1.als
COMNT
DATIM 21-08-2019 23:04:10
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.9 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 30
7.23421
4.13285
4.12484
4.11339
4.10767
4.10080
3.76536
3.75391
3.74247
3.68980
3.67606
3.66462
3.65202
3.64057
3.62684
3.59364
3.58333
3.57761
3.56960
3.56044
3.54555
3.53868
3.52151
3.51464
3.50777
3.50090
3.46313
3.44939
3.43450
3.42420
3.35895
3.35093
3.34521
3.33490
3.29369
3.27995
3.26850
3.16661
2.80027
1.93706
1.93134
1.92561
1.91989
1.91416
1.16315
533.013
2.000
5.799
3.990
2.122
5.074
2.038
8.481
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

HO
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46
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Me
46
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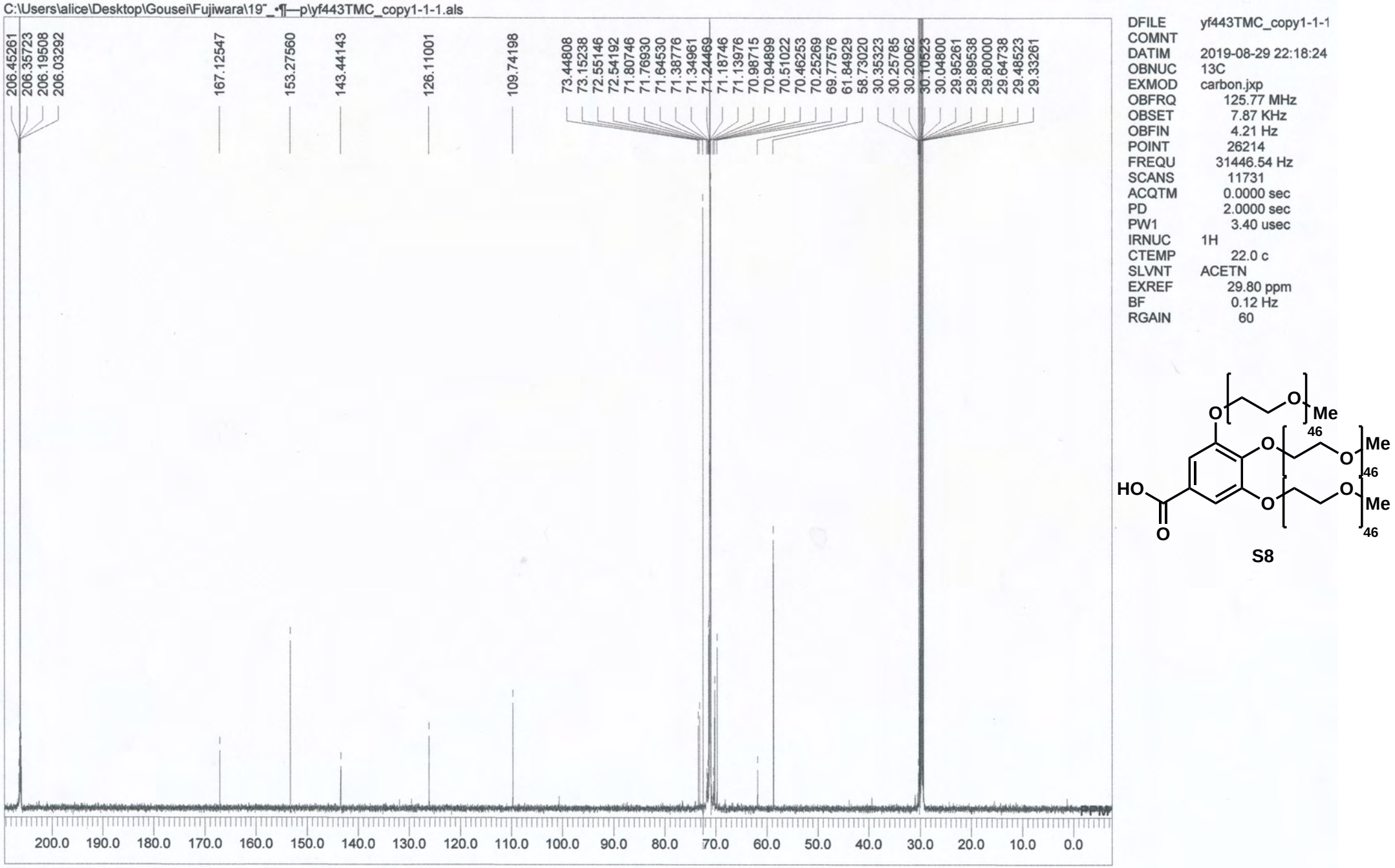
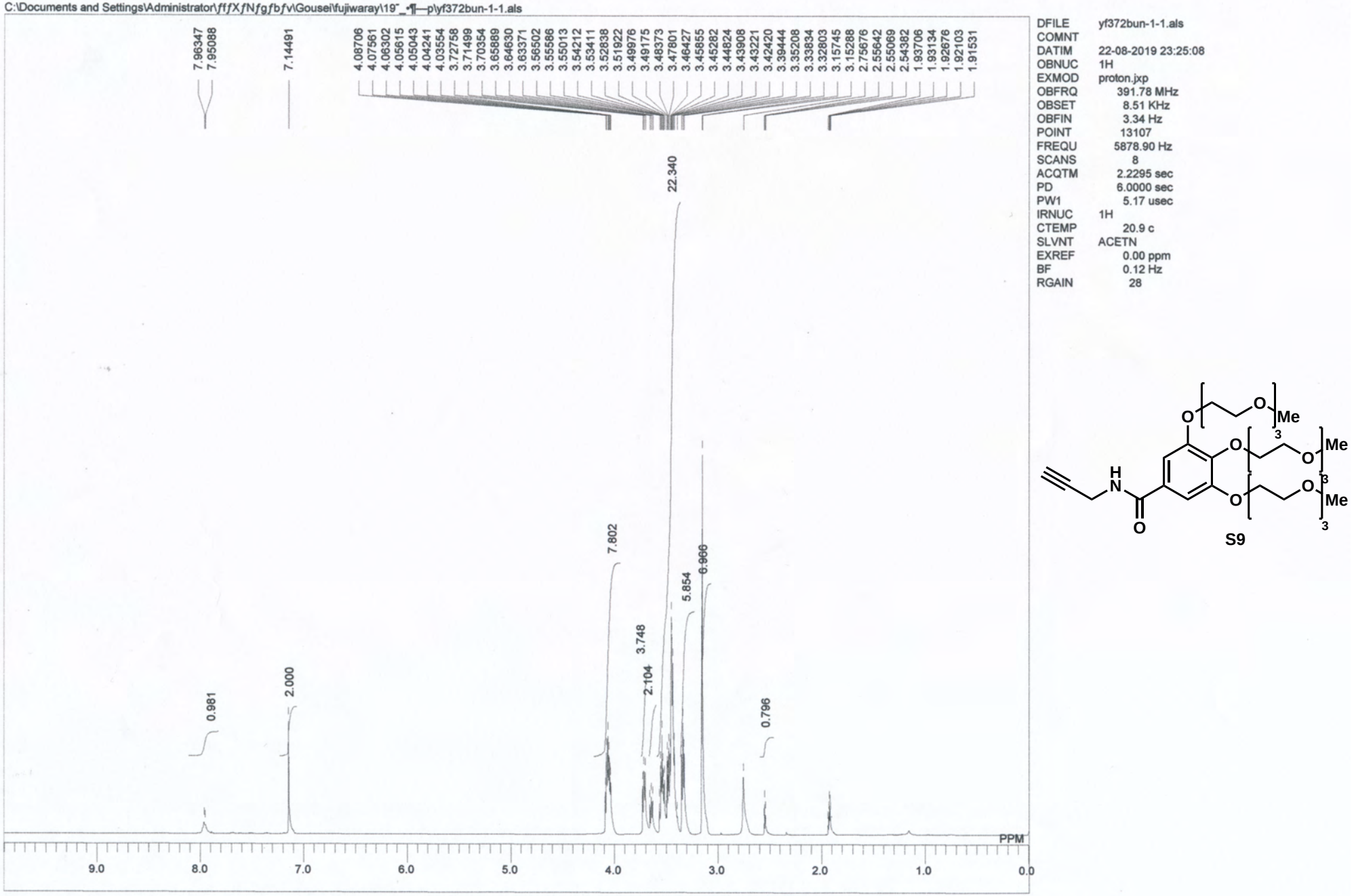
Me
46

S8



DFILE	yf443TMC_copy1-1-1
COMNT	
DATIM	2019-08-29 22:18:24
OBNUC	13C
EXMOD	carbon.jxp
OBFRQ	125.77 MHz
OBSET	7.87 KHz
OBFIN	4.21 Hz
POINT	26214
FREQU	31446.54 Hz
SCANS	11731
ACQTM	0.0000 sec
PD	2.0000 sec
PW1	3.40 usec
IRNUC	1H
CTEMP	22.0 c
SLVNT	ACETN
EXREF	29.80 ppm
BF	0.12 Hz
RGAIN	60

S8


C:\Documents and Settings\Administrator\ƒƒXƒNƒgƒbƒv\Gousei\fujiwaray\19¯_•¶—p\yf372bun-1-1.als
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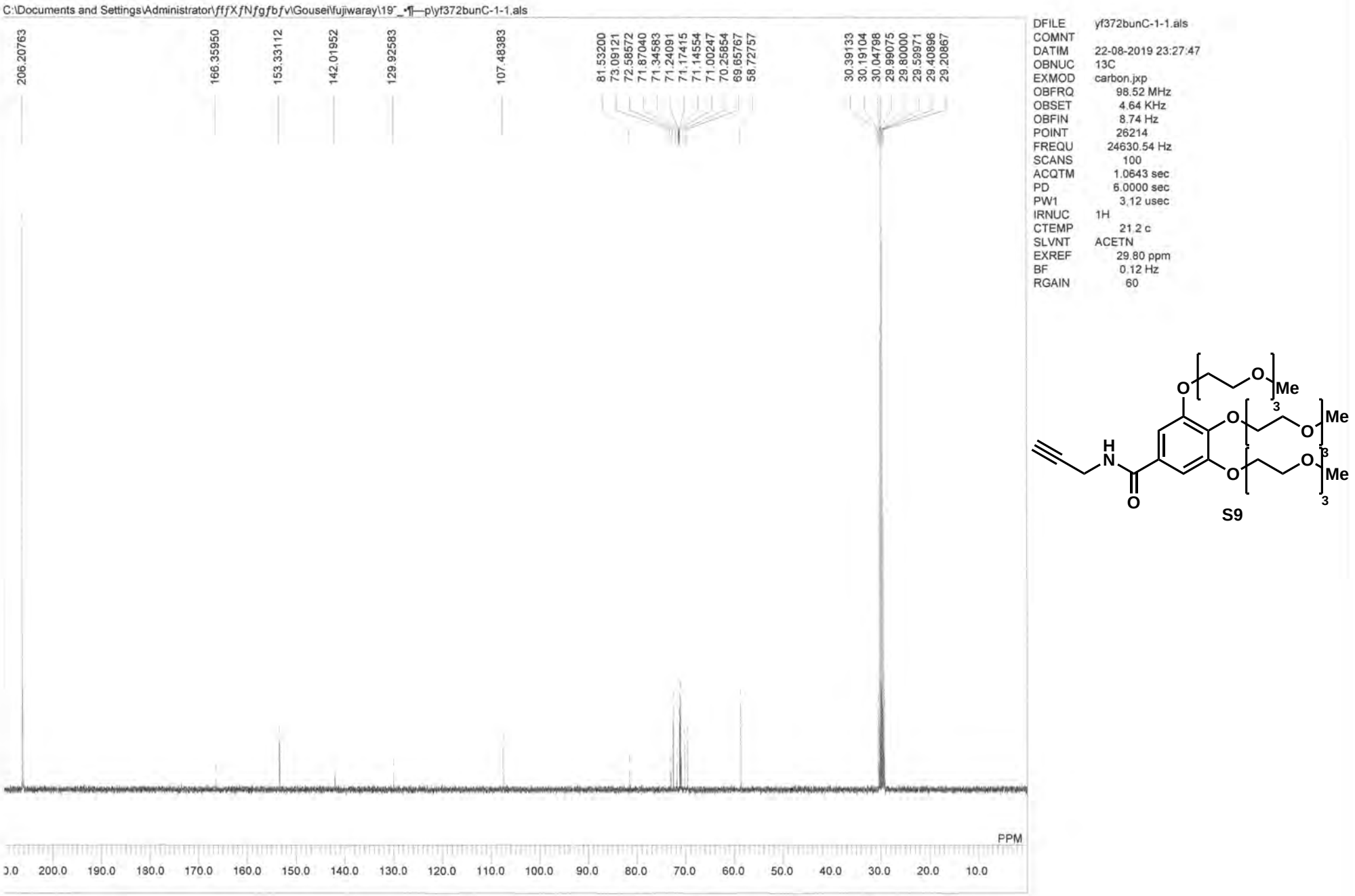
7.95088
7.14491
4.08706
4.07561
4.06302
4.05615
4.05043
4.04241
4.03554
3.72758
3.71499
3.70354
3.65889
3.64630
3.63371
3.56502
3.55586
3.55013
3.54212
3.53411
3.52838
3.51922
3.49976
3.49175
3.48373
3.47801
3.46427
3.45855
3.45282
3.44824
3.43908
3.43221
3.42420
3.39444
3.35208
3.33834
3.32803
3.15745
3.15288
2.75676
2.55642
2.55069
2.54382
1.93706
1.93134
1.92676
1.92103
1.91531
22.340
7.802
6.966
5.854
3.748
2.104
0.981
2.000
0.796
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
DFILE yf372bun-1-1.als
COMNT
DATIM 22-08-2019 23:25:08
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.9 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 28
O
Me
3
H
N
O
S9


C:\Documents and Settings\Administrator\ƒƒƒXƒNƒgƒbƒv\Gousei\fujiwaray\19¯_•¶—p\yf372bunC-1-1.als
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166.35950
153.33112
142.01952
129.92583
107.48383
81.53200
73.09121
72.58572
71.87040
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71.17415
71.14554
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70.25854
69.65767
58.72757
30.39133
30.19104
30.04798
29.99075
29.80000
29.59971
29.40896
29.20867
DFILE yf372bunC-1-1.als
COMNT
DATIM 22-08-2019 23:27:47
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 98.52 MHz
OBSET 4.64 KHz
OBFIN 8.74 Hz
POINT 26214
FREQU 24630.54 Hz
SCANS 100
ACQTM 1.0643 sec
PD 6.0000 sec
PW1 3.12 usec
IRNUC 1H
CTEMP 21.2 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
PPM
0.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

O
Me
3
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Me
O
3
H
N
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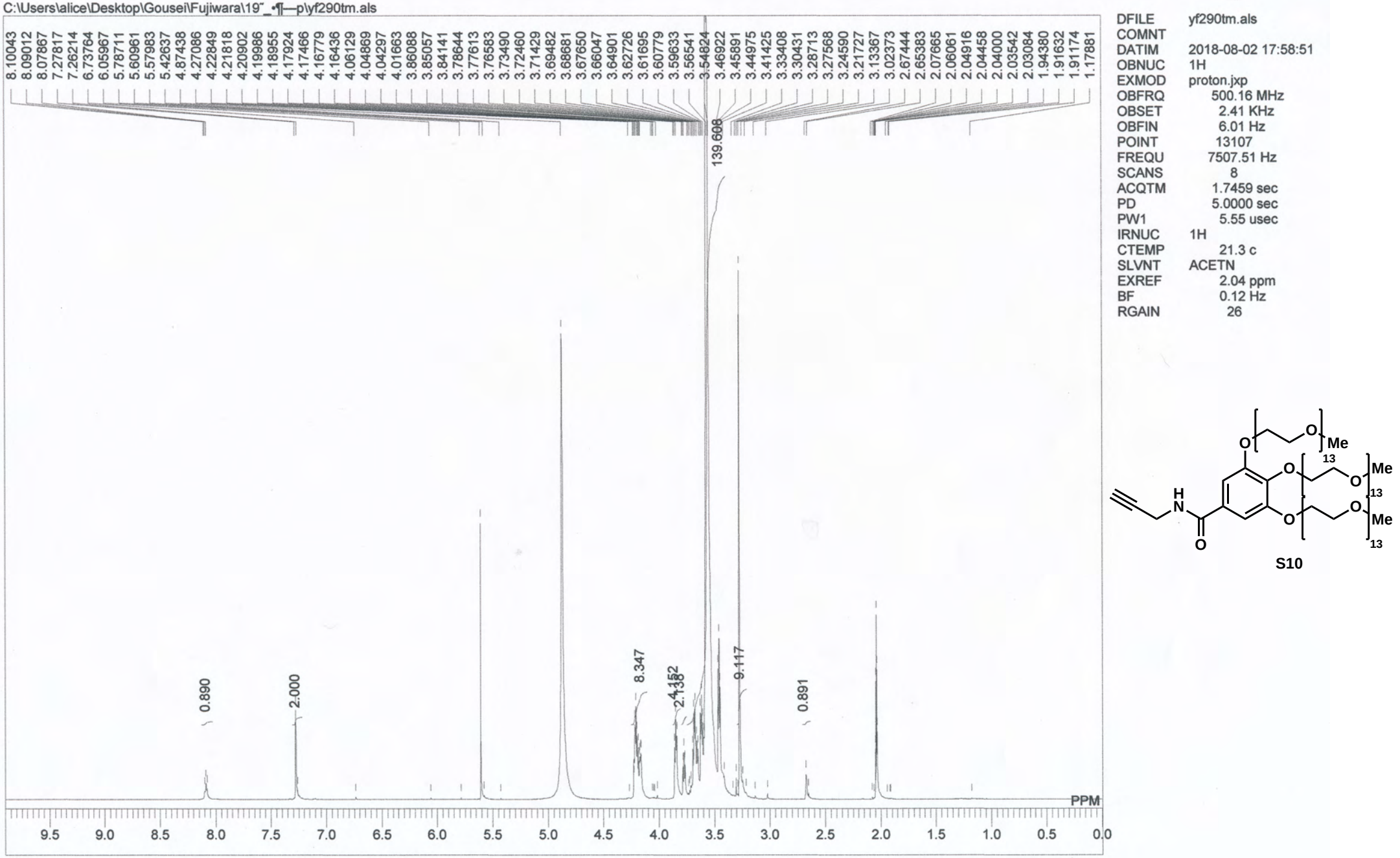
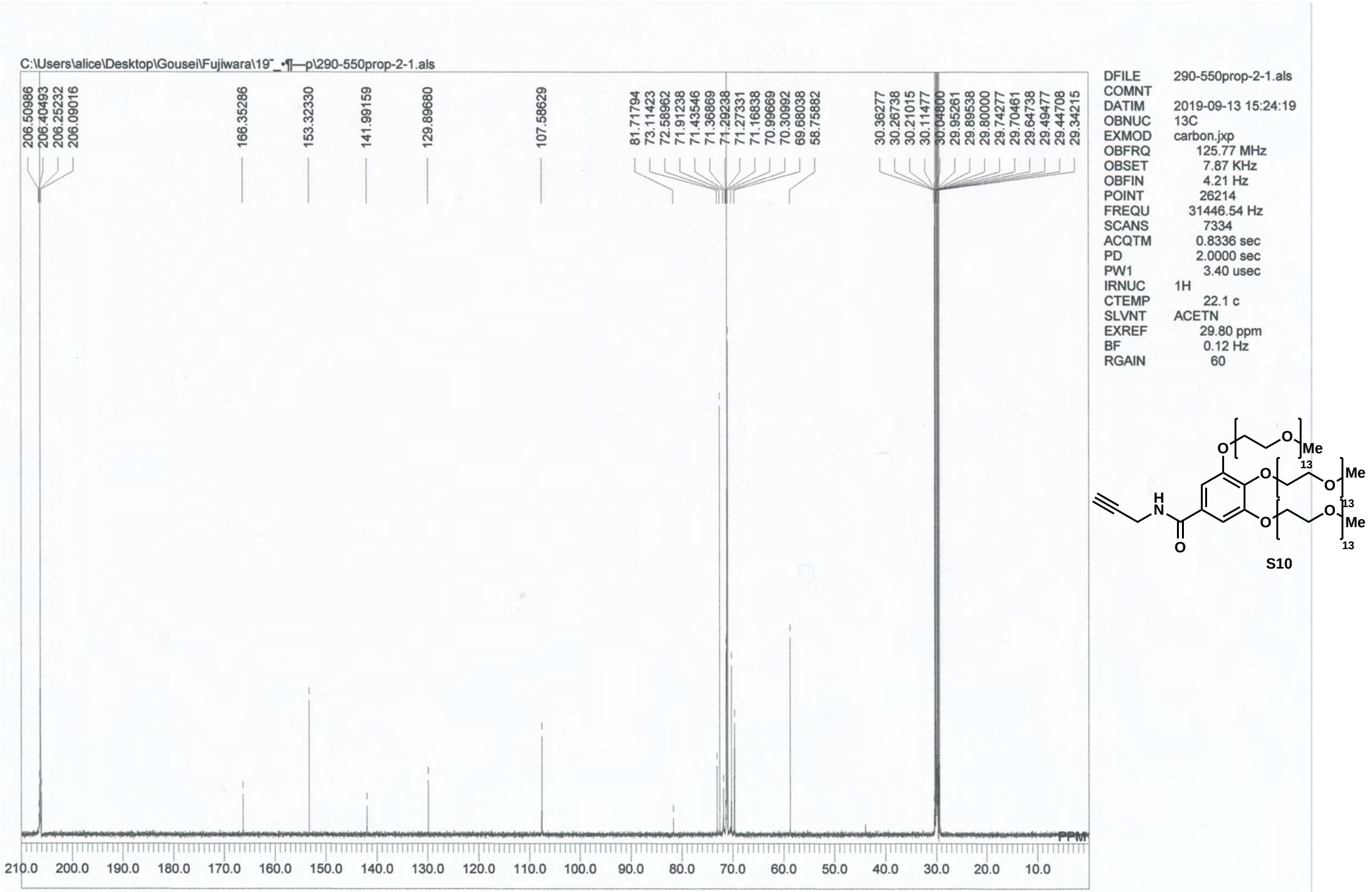
O
Me
3

S9


C:\Users\alice\Desktop\Gousei\Fujiwara\19˜_•¶—p\yf290tm.als
8.10043 8.09012 8.07867 7.27817 7.26214 6.73764 6.05967 5.78711 5.60961 5.57983 5.42637 4.87438 4.27086 4.22849 4.21818 4.20902 4.19986 4.18955 4.17924 4.17466 4.16779 4.16436 4.06129 4.04869 4.04297 4.01663 3.86088 3.85057 3.84141 3.78644 3.77613 3.76583 3.73490 3.72460 3.71429 3.69482 3.68681 3.67650 3.66047 3.64901 3.62726 3.61695 3.60779 3.59633 3.56541 3.54824 3.46922 3.45891 3.44975 3.41425 3.33408 3.30431 3.28713 3.27568 3.24590 3.21727 3.13367 3.02373 2.67444 2.65383 2.07665 2.06061 2.04916 2.04458 2.04000 2.03542 2.03084 1.94380 1.91632 1.91174 1.17881
139.608
0.890
2.000
8.347
4.152
2.138
9.117
0.891
PPM
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
DFILE yf290tm.als
COMNT
DATIM 2018-08-02 17:58:51
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 8
ACQTM 1.7459 sec
PD 5.0000 sec
PW1 5.55 usec
IRNUC 1H
CTEMP 21.3 c
SLVNT ACETN
EXREF 2.04 ppm
BF 0.12 Hz
RGAIN 26
O
Me
13
H
N
O

S10


C:\Users\alice\Desktop\Gousei\Fujiwara\19"_•¶—p\290-550prop-2-1.als
206.50986
206.40493
206.25232
206.09016
166.35286
153.32330
141.99159
129.89680
107.58629
81.71794
73.11423
72.58962
71.91238
71.43546
71.36869
71.29238
71.27331
71.16838
70.99669
70.30992
69.68038
58.75882
30.36277
30.26738
30.21015
30.11477
30.04800
29.95261
29.89538
29.80000
29.74277
29.70461
29.64738
29.49477
29.44708
29.34215
DFILE 290-550prop-2-1.als
COMNT
DATIM 2019-09-13 15:24:19
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 7334
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.1 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

O
O
Me
13
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O
Me
13
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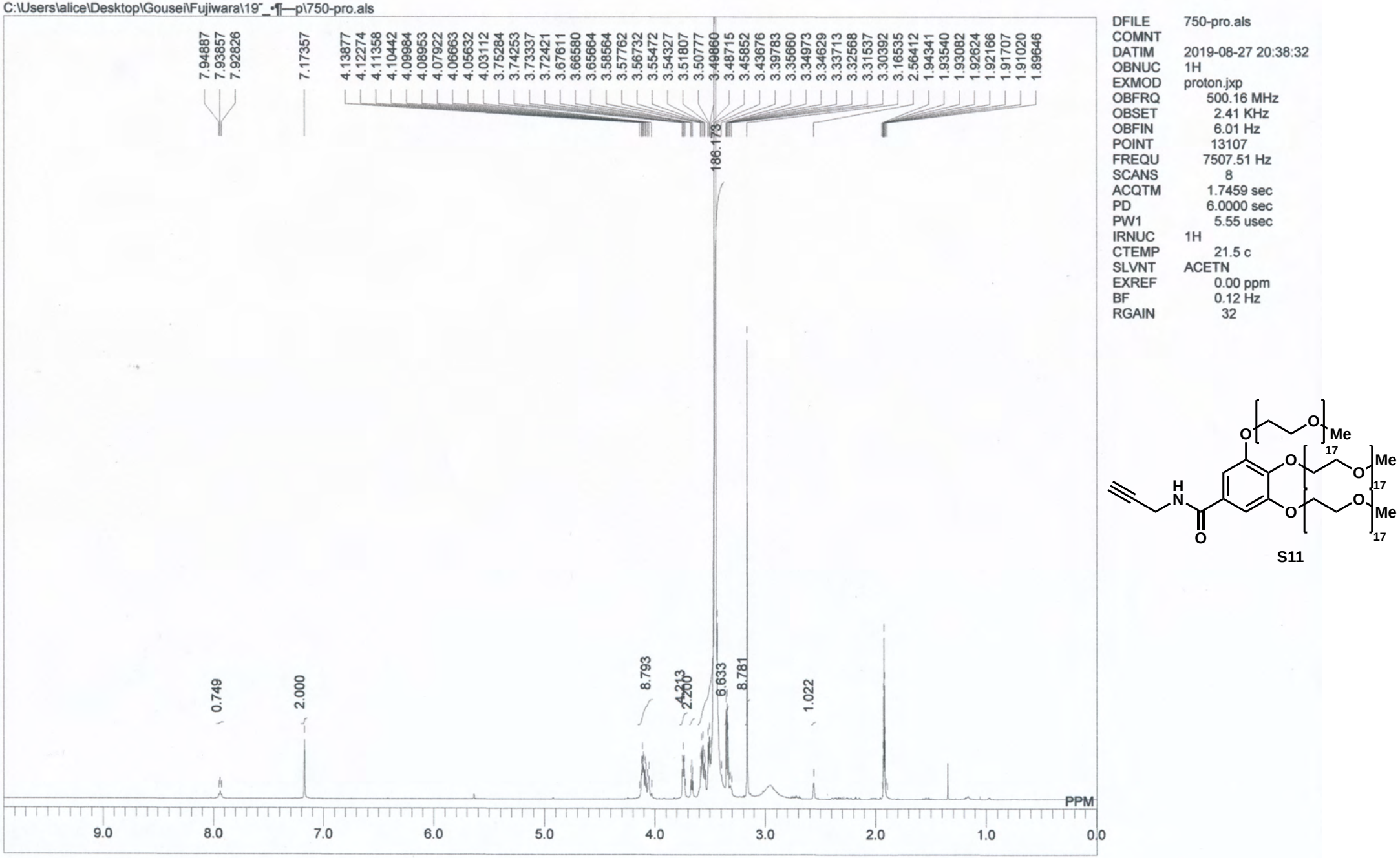
O
Me
13

S10


C:\Users\alice\Desktop\Gousei\Fujiwara\19¯_•¶—p\750-pro.als
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4.13877
4.12274
4.11358
4.10442
4.09984
4.08953
4.07922
4.06663
4.05632
4.03112
3.75284
3.74253
3.73337
3.72421
3.67611
3.66580
3.65664
3.58564
3.57762
3.56732
3.55472
3.54327
3.51807
3.50777
3.49860
3.48715
3.45852
3.43676
3.39783
3.35660
3.34973
3.34629
3.33713
3.32568
3.31537
3.30392
3.16535
2.56412
1.94341
1.93540
1.93082
1.92624
1.92166
1.91707
1.91020
1.89646
186.173
0.749
2.000
8.793
4.213
2.200
6.633
8.781
1.022
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
DFILE 750-pro.als
COMNT
DATIM 2019-08-27 20:38:32
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 8
ACQTM 1.7459 sec
PD 6.0000 sec
PW1 5.55 usec
IRNUC 1H
CTEMP 21.5 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 32
H
N
O
O
O
Me
17
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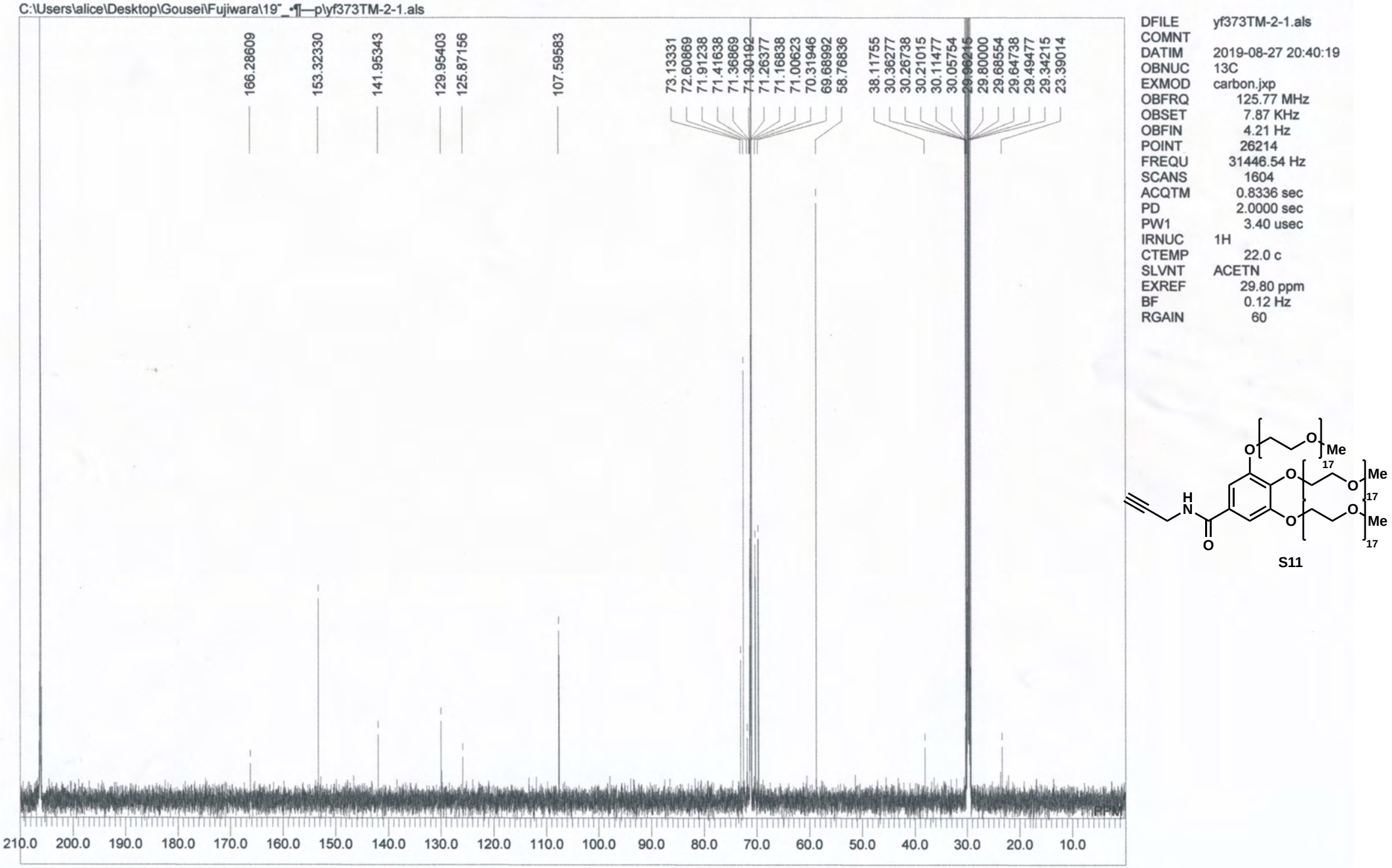
O
Me
17
O
O
Me
17

S11


C:\Users\alice\Desktop\Gousei\Fujiwara\19"_•¶—p\yf373TM-2-1.als
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141.95343
129.95403
125.87156
107.59583
73.13331
72.60869
71.91238
71.41638
71.36869
71.30192
71.26377
71.16838
71.00623
70.31946
69.68992
58.76836
38.11755
30.36277
30.26738
30.21015
30.11477
30.05754
29.80000
29.68554
29.64738
29.49477
29.34215
23.39014
DFILE yf373TM-2-1.als
COMNT
DATIM 2019-08-27 20:40:19
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 1604
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.0 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

O
O
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17
H
N
O
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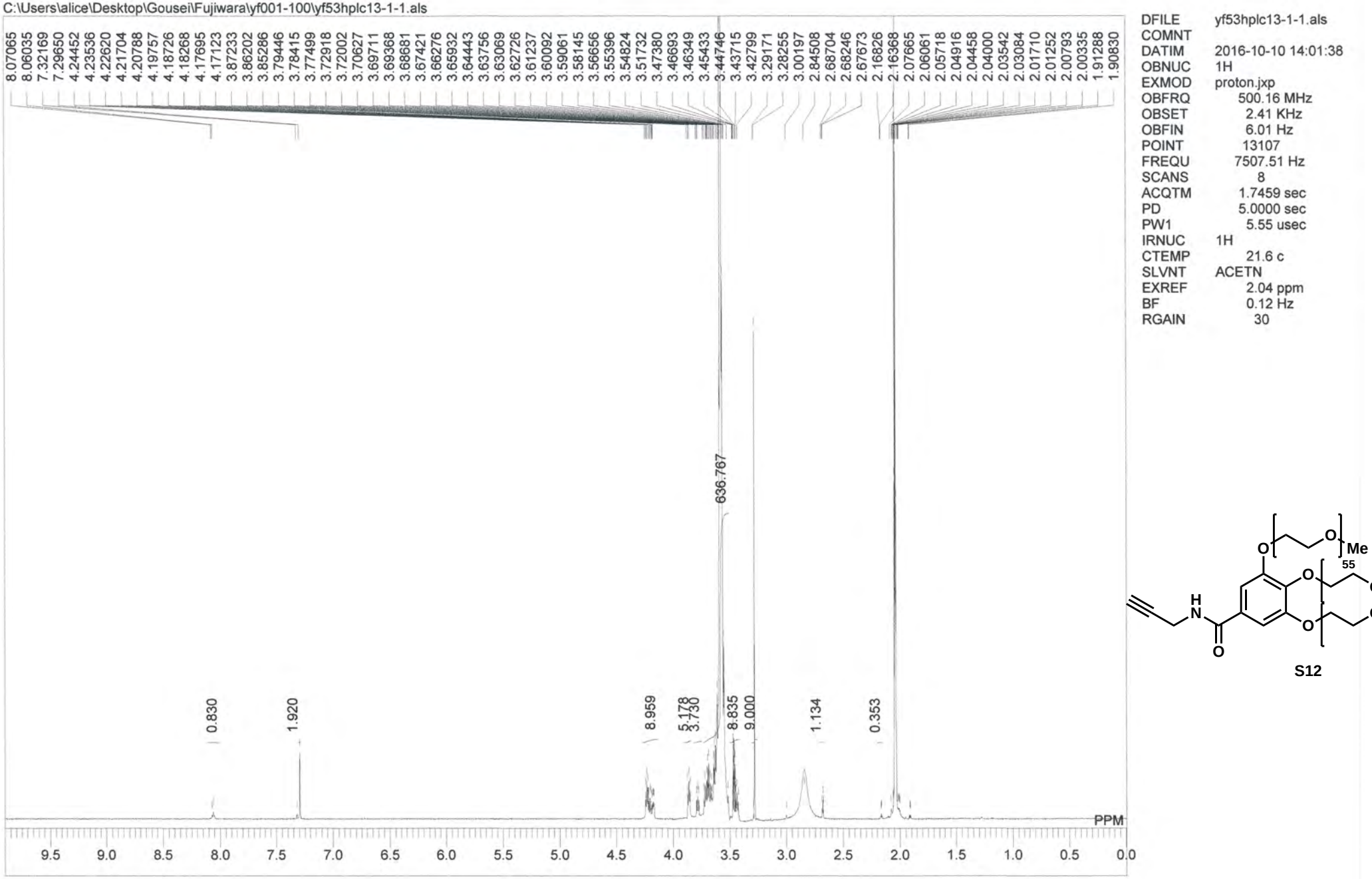
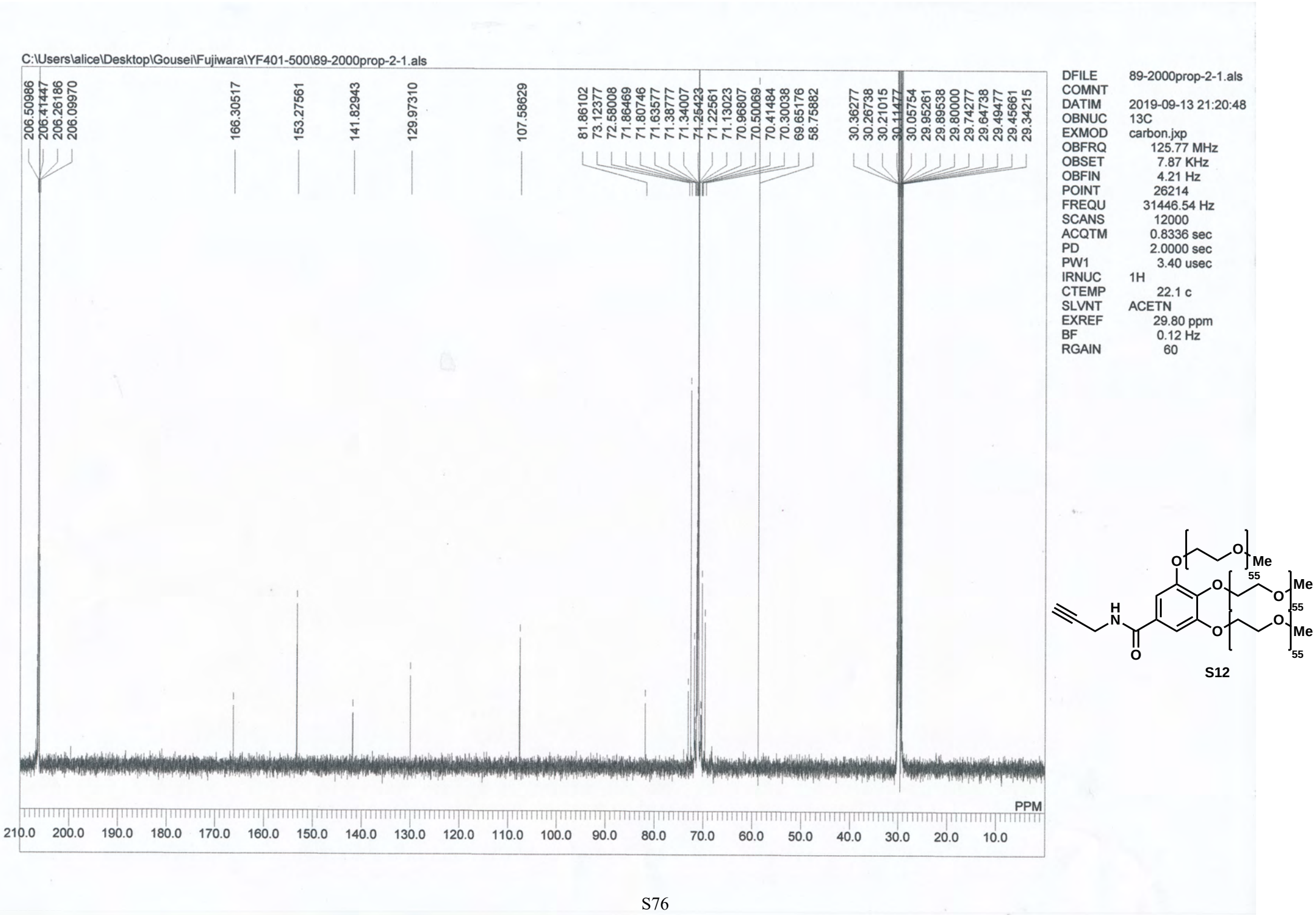
O
O
O
Me
17
Me
17
Me
17

S11


C:\Users\alice\Desktop\Gousei\Fujiwara\yf001-100\yf53hplc13-1-1.als
DFILE yf53hplc13-1-1.als
COMNT
DATIM 2016-10-10 14:01:38
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 8
ACQTM 1.7459 sec
PD 5.0000 sec
PW1 5.55 usec
IRNUC 1H
CTEMP 21.6 c
SLVNT ACETN
EXREF 2.04 ppm
BF 0.12 Hz
RGAIN 30
636.767
0.830
1.920
8.959
5.178
3.730
8.835
9.000
1.134
0.353
PPM
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

Me
55
H
N
O

S12


C:\Users\alice\Desktop\Gousei\Fujiwara\YF401-500\89-2000prop-2-1.als
206.50986
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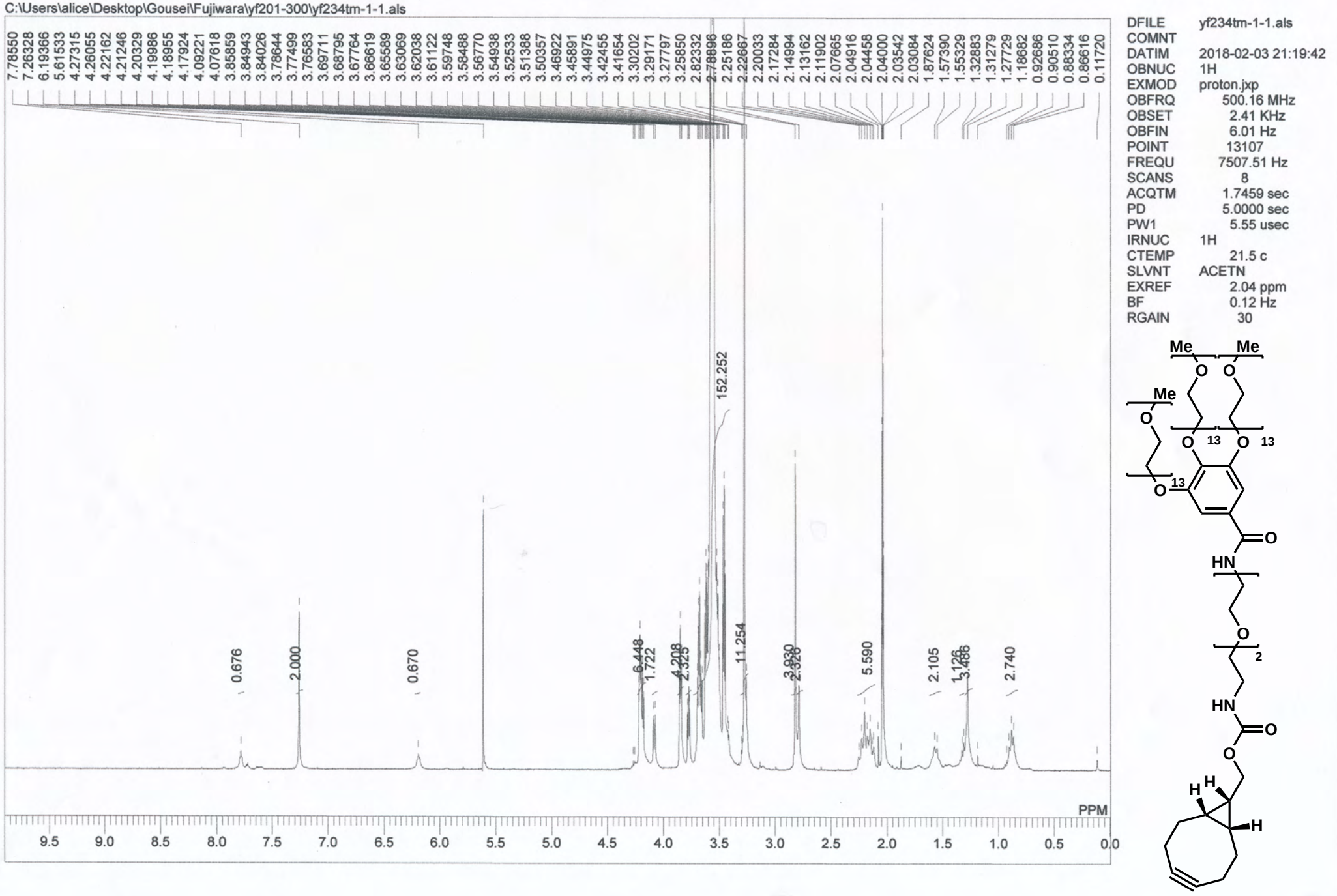
206.26186
206.09970
166.30517
153.27561
141.82943
129.97310
107.58629
81.86102
73.12377
72.58008
71.86469
71.80746
71.63577
71.38777
71.34007
71.25423
71.22561
71.13023
70.96807
70.50069
70.41484
70.30038
69.65176
58.75882
30.36277
30.26738
30.21015
30.11477
30.05754
29.95261
29.89538
29.80000
29.74277
29.64738
29.49477
29.45661
29.34215
DFILE 89-2000prop-2-1.als
COMNT
DATIM 2019-09-13 21:20:48
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 12000
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.1 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
PPM
210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0

O
Me
55
H
N
O

S12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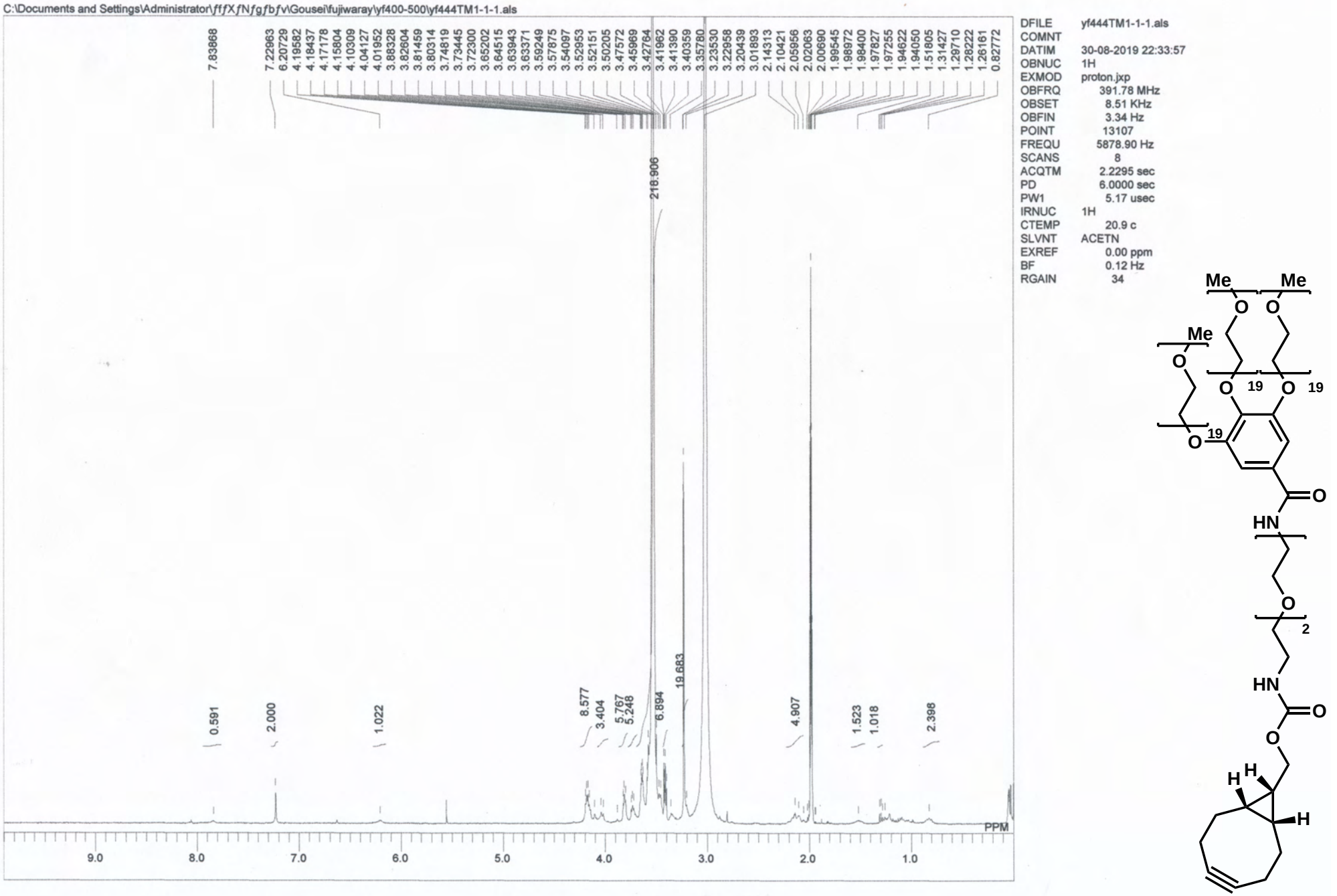
DFILE yf234tm-1-1.als
COMNT
DATIM 2018-02-03 21:19:42
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 8
ACQTM 1.7459 sec
PD 5.0000 sec
PW1 5.55 usec
IRNUC 1H
CTEMP 21.5 c
SLVNT ACETN
EXREF 2.04 ppm
BF 0.12 Hz
RGAIN 30
PPM

S77

S13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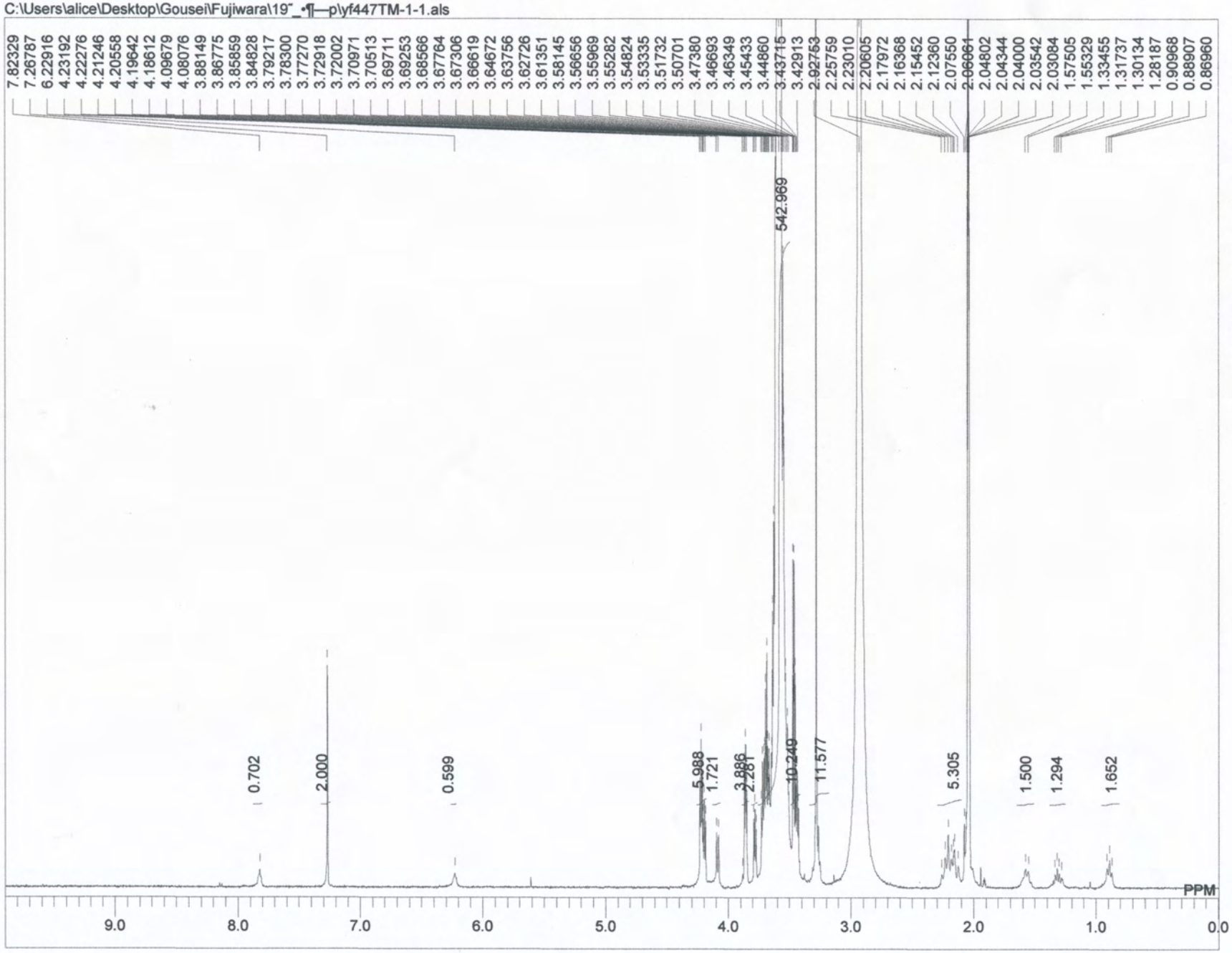
DFILE yf444TM1-1-1.als
COMNT
DATIM 30-08-2019 22:33:57
OBNUC 1H
EXMOD proton.jxp
OBFRQ 391.78 MHz
OBSET 8.51 KHz
OBFIN 3.34 Hz
POINT 13107
FREQU 5878.90 Hz
SCANS 8
ACQTM 2.2295 sec
PD 6.0000 sec
PW1 5.17 usec
IRNUC 1H
CTEMP 20.9 c
SLVNT ACETN
EXREF 0.00 ppm
BF 0.12 Hz
RGAIN 34
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0

Me
Me
Me
O
19
HN
2
HN
H
H
H

S14


C:\Users\alice\Desktop\Gousei\Fujiwara\19¯_•¶—p\yf447TM-1-1.als
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6.22916
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4.22276
4.21246
4.20558
4.19642
4.18612
4.09679
4.08076
3.88149
3.86775
3.85859
3.84828
3.79217
3.78300
3.77270
3.72918
3.72002
3.70971
3.70513
3.69711
3.69253
3.68566
3.67764
3.67306
3.66619
3.64672
3.63756
3.62726
3.61351
3.58145
3.56656
3.55969
3.55282
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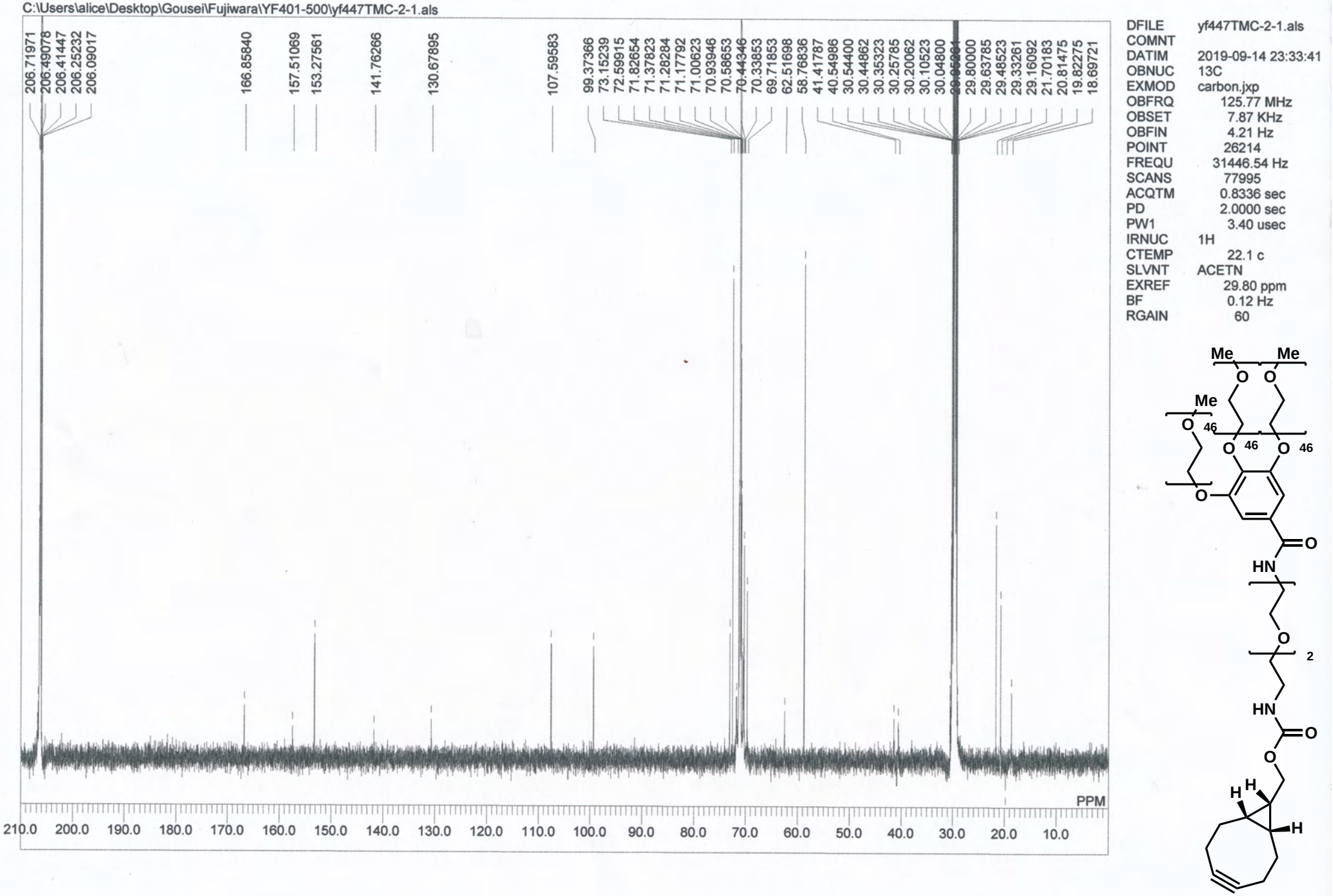
3.53335
3.51732
3.50701
3.47380
3.46693
3.46349
3.45433
3.44860
3.43715
3.42913
2.92753
2.25759
2.23010
2.20605
2.17972
2.16368
2.15452
2.12360
2.07550
2.06061
2.04802
2.04344
2.04000
2.03542
2.03084
1.57505
1.55329
1.33455
1.31737
1.30134
1.28187
0.90968
0.88907
0.86960
542.969
0.702
2.000
0.599
5.988
1.721
3.886
2.281
10.249
11.577
5.305
1.500
1.294
1.652
PPM
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
DFILE yf447TM-1-1.als
COMNT
DATIM 2019-09-07 15:12:56
OBNUC 1H
EXMOD proton.jxp
OBFRQ 500.16 MHz
OBSET 2.41 KHz
OBFIN 6.01 Hz
POINT 13107
FREQU 7507.51 Hz
SCANS 16
ACQTM 1.7459 sec
PD 6.0000 sec
PW1 5.55 usec
IRNUC 1H
CTEMP 21.7 c
SLVNT ACETN
EXREF 2.04 ppm
BF 0.12 Hz
RGAIN 34
Me
Me
Me
O
46
HN
HN
H
H
H

S15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206.25232
206.09017
166.85840
157.51069
153.27561
141.76266
130.67895
107.59583
99.37366
73.15239
72.59915
71.82654
71.37823
71.28284
71.17792
71.00623
70.93946
70.58653
70.44346
70.33853
69.71853
62.51698
58.76836
41.41787
40.54986
30.54400
30.44862
30.35323
30.25785
30.20062
30.10523
30.04800
29.80000
29.63785
29.48523
29.33261
29.16092
21.70183
20.81475
19.82275
18.69721
PPM
210.0
200.0
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
DFILE yf447TMC-2-1.als
COMNT
DATIM 2019-09-14 23:33:41
OBNUC 13C
EXMOD carbon.jxp
OBFRQ 125.77 MHz
OBSET 7.87 KHz
OBFIN 4.21 Hz
POINT 26214
FREQU 31446.54 Hz
SCANS 77995
ACQTM 0.8336 sec
PD 2.0000 sec
PW1 3.40 usec
IRNUC 1H
CTEMP 22.1 c
SLVNT ACETN
EXREF 29.80 ppm
BF 0.12 Hz
RGAIN 60
Me
Me
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