Supporting Information

Activation of S-S Bond Cleavage Triggered by Hydrophobization and Lipophilization of Functionalized Dihydroasparagusic Acid

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Contents

General Information

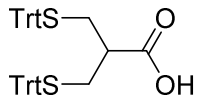
General.

The optical rotation was measured using a JASCO P-2200 polarimeter. Infrared spectra were measured with a Thermo Fisher Scientific NICOLET iS5(ATR) in neat. ^{1}H NMR spectra were measured with JNM-ECS400 or JNM-ECA600 spectrometers for samples in chloroform-*d* ($CDCl_3$) using either tetramethylsilane (for compound with a phenyl group) or $CHCl_3$ (7.26 ppm) as an internal reference. ^{13}C NMR spectra were measured with JNM-ECS400 or JNM-ECA600 spectrometers for samples in chloroform-*d* ($CDCl_3$) using $CDCl_3$ (77.0 ppm) as an internal reference. High-resolution mass spectra with JMS-T100TD (DART or ESI) mass spectrometers or JMS-700 (FAB) mass spectrometers.

LEAF^{TM} Purified Mouse $IgG2_a$, κ Isotype Ctrl Antibody (Bio Legend) was used for SDS-PAGE. The result of SDS-PAGE was measured by Odyssey Infrared Imaging System (M&S Techno Systems). Human CRP ELISA kit (protein tech) was used for ELISA. The result of ELISA was measured by Multi Midroplate Reader MTP-880lab (CORONA). Commercially available anhydrous DMF, CH_2Cl_2, THF and EtOH were employed for reactions. Commercially available 3-bromo-2-(bromomethyl)propionic acid **1** (Aldrich), sodium hydride (Wako Pure Chemical Industries), triphenylmethanethiol (Aldrich), TFA (Wako Pure Chemical Industries), triisopropylsilane (Tokyo Chemical Industry), butylamine (Tokyo Chemical Industry), EDCI (Tokyo Chemical Industry) and HOBt (Wako Pure Chemical Industries) are commercially available. The 2-(2-methoxyethoxy)ethan-1-amine[S1] and *N,N'*-bis[(*tert*-butyloxy)carbonyl]-$_L$-cystine dimethyl ester **5**[S2] are known compounds. Silica gel (Silica gel 60 N, 40-50 μm, Kanto Chemical) was used for chromatography. All reactions were carried out under N_2 or air atmosphere unless otherwise stated. Organic extracts were dried over Na_2SO_4.

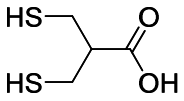
Experimental Section

3-(Tritylthio)-2-[(tritylthio)methyl]propanoic acid (2)



Triphenylmethanethiol (345.5 mg, 1.25 mmol) was slowly added to a cooled (0 °C) suspension of NaH (60.00 mg, 1.50 mmol) in dry DMF (7.5 mL). After stirring for 20 min, **1** (107.94 mg, 0.50 mmol) was added to the mixture. The mixture was heated at 70 °C and stirred for 5 h. Then, the reaction was quenched with H_2O, extracted with CH_2Cl_2, and concentrated to dryness. The residue was purified by column chromatography to afford **2** (234.2 mg, 74%) as a colorless solid. IR 3055, 2573, 1702 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$) δ 7.34-7.32 (m, 12H), 7.24-7.21 (m, 12H), 7.19-7.15 (m, 6H), 2.34 (dd, *J* = 12.4, 7.3 Hz, 2 H), 2.19 (dd, *J* = 12.4, 6.0 Hz, 2 H) 1.92-1.85 (m, 1 H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 178.3, 144.4, 129.5, 127.9, 126.7, 67.0, 44.4, 32.6; FAB HRMS calcd for $C_{42}H_{36}O_2S_2Na$ 659.2054, found 659.2072.

Dihydroasparagusic acid (DHAA)[S3]



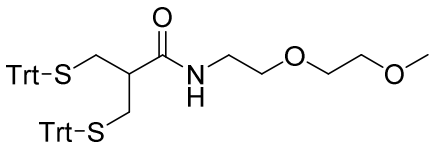
To a solution of the **2** (636.9 mg, 1.00 mmol) in CH_2Cl_2 (4 mL) was added TFA (1 mL) at room temperature. Then, triisopropylsilane (491μL, 2.40 mmol) was slowly added to the mixture. After stirring for 1 h, the solvent was removed under reduced pressure, quenched with sat. $NaHCO_3$ aq., and extracted with CH_2Cl_2. After the aqueous layer was added HCl aq. and extracted with AcOEt, the organic layer was washed with brine, dried, filtered, and concentrated. The crude product was purified by column chromatography giving pure **DHAA** (138.05 mg, 91%) as a white solid.

IR 2927, 2561, 1704 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 10.42 (brs, 1 H), 2.98-2.82 (m, 5 H), 1.53 (t, *J* = 8.6 Hz, 2 H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 178.2, 50.8, 24.1; DART HRMS calcd for $[C_4H_9O_2S_2]^+$ 153.0044, found 153.0036.

General procedure for condensation reaction of carboxylic acid 2 and amine

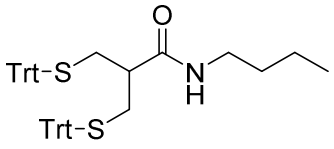
To a solution of the **2** (0.50 mmol) and amine (1.1 eq.) in DMF or CH_2Cl_2 (2.5 mL) was added HOBt and EDCI at 0 °C. The reaction mixture was then gradually warmed to room temperature. After stirring for 4-14 h at the same temperature, the reaction mixture was quenched by addition of water, and the mixture was extracted with CH_2Cl_2. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane-AcOEt to afford the corresponding amide.

***N*-[2-(2-Methoxyethoxy)ethyl]-3-(tritylthio)-2-[(tritylthio)methyl]propanamide (S1)**



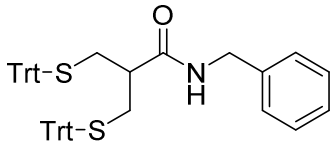
Compound **S1** (116.2 mg, 52%) was observed from **2** (210.0 mg, 0.33 mmol) as a colorless solid. IR 3323, 2920, 1674, 699 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34-7.32 (m, 12H), 7.26-7.23 (m, 12H), 7.20-7.17 (m, 6H), 5.57 (t, *J* = 5.5 Hz, 1H), 3.52-3.50 (m, 2H), 3.47 (t, *J* = 5.0 Hz, 2H), 3.43-3.40 (m, 2H), 3.38-3.35 (m, 2H), 3.34 (s, 3H), 2.38 (dd, *J* = 12.4, 8.2 Hz, 2H), 2.08 (dd, *J* = 12.3, 6.0 Hz, 2H), 1.21-1.14 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.2, 144.6, 129.6, 127.9, 126.7, 71.8, 70.3, 70.0, 66.9, 59.0, 46.3, 39.2, 33.7; FAB HRMS calcd for $C_{47}H_{47}NO_3S_2Na$ 760.2895, found 760.2876.

***N*-Butyl-3-(tritylthio)-2-[(tritylthio)methyl]propanamide (S2)**



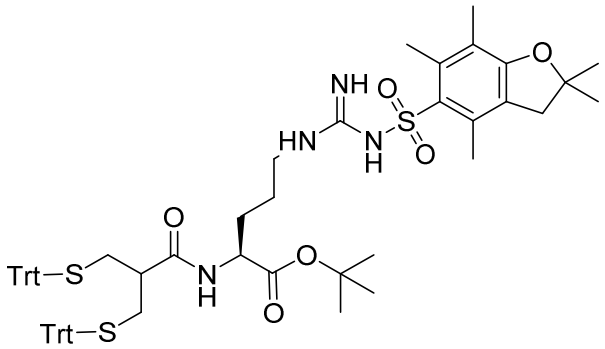
Compound **S2** (764.7 mg, 92%) was observed from **2** (764.2 mg, 1.20 mmol) as a colorless solid. IR 3256, 2924, 1633, 694 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.33-7.32 (m, 12H), 7.25-7.23 (m, 12H), 7.19-7.17 (m, 6H), 4.97 (t, *J* = 5.8 Hz, 1H), 3.14-3.11 (m, 2H), 2.39 (dd, *J* = 12.4, 7.9 Hz, 2H), 2.11 (dd, *J* = 12.4, 5.8 Hz, 2H), 1.42-1.37 (m, 2H), 1.32-1.26 (m, 3H), 0.88 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 151 MHz) δ 171.9, 144.6, 129.6, 127.9, 126.7, 67.0, 46.7, 39.2, 33.7, 31.5, 20.0, 13.7; FAB HRMS calcd for $C_{46}H_{45}NOS_2Na$ 714.2840, found 714.2873.

***N*-Benzyl-3-(tritylthio)-2-[(tritylthio)methyl]propanamide (S3)**



Compound **S3** (287.3 mg, 99%) was observed from **2** (254.8 mg, 0.40 mmol) as a colorless solid. IR 3406, 3295, 3056, 2920, 740 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32-7.31 (m, 12H), 7.24-7.21 (m, 17H), 7.19-7.17 (m, 6H), 5.23 (t, *J* = 5.5 Hz, 1H), 4.33 (d, *J* = 5.5 Hz, 2H), 2.43 (dd, *J* = 12.4, 8.2 Hz, 2H), 2.14 (dd, *J* = 12.3, 5.8 Hz, 2H), 1.30 -1.28 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.9, 144.5, 137.9, 129.7, 129.6, 128.5, 127.8, 127.4, 126.6, 66.9, 46.7, 43.5, 33.7; FAB HRMS calcd for $C_{49}H_{44}NOS_2$ 726.2864, found 726.2859.

***tert*-Butyl**
***N*-[(2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl]-*N*-{3-(tritylthio)-2-[(tritylthio)methyl]propanoyl}argininate (S4)**

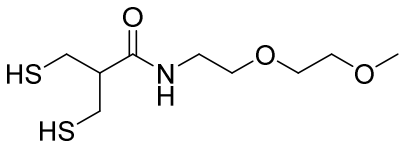


Compound **S4** (556.6 mg, 89%) was observed from **2** (275.5 mg, 0.57 mmol) as a colorless solid. IR 3329, 2973, 1549, 741 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ; 7.33-7.17 (m, 30H), 5.88 (d, *J* = 7.6 Hz, 1H), 5.79 (brs, 1H), 4.30 (dd, *J* = 12.4, 7.6 Hz, 1H), 3.20-3.18 (m, 1H), 3.09 (brs, 1H), 2.92 (s, 2H), 2.58 (s, 3H), 2.52 (s, 3H), 2.33 (dd, *J* = 12.7, 8.2 Hz, 1H), 2.28 (dd, *J* = 12.4, 9.6 Hz, 1H), 2.08 (s, 3H), 2.07-2.03 (m, 2H), 1.79-1.74 (m, 1H), 1.59-1.53 (m, 3H), 1.44 (d, *J* = 3.4 Hz, 6H), 1.41 (s, 9H), 1.14-1.10 (m, 1H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.7, 170.7, 158.5, 155.7, 144.4, 144.3, 138.3, 133.3, 132.2, 129.5, 129.4, 128.0, 127.9, 126.9, 126.7, 124.4, 117.3, 86.2, 82.7, 67.0, 66.9, 46.0, 43.2, 40.6, 33.7, 33.6, 30.8, 28.6, 27.9, 24.4, 19.3, 17.9, 12.5; FAB HRMS calcd for 1101.4692, found 1101.4701.

General procedure for deprotection of the trityl group

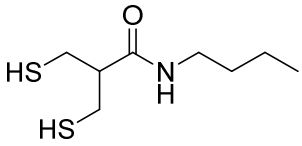
To a solution of the **S1** or **S2** (0.50 mmol) in CH_2Cl_2 (10 mL) were added TFA (1 mL) and triisopropylsilane (250 μL, 1.20 mmol) at room temperature. After stirring for 2.5 h, the solvent was removed under reduced pressure. The residual material was added into sat. $NaHCO_3$ *aq.*, and the aqueous layer was extracted with CH_2Cl_2. After the aqueous layer was added HCl aq. and extracted with AcOEt. The combined organic layers were washed with brine, dried, and concentrated to dryness. The crude product was purified by column chromatography to afford the desired dithiol.

***N*-2-Methoxyethyl-1,2-dithiolane-4-carboxamide (4a)**



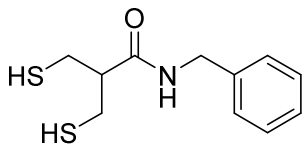
Compound **4a** (119.1 mg, 96%) was observed from **S1** (357.9 mg, 0.49 mmol) as a yellow oil.; IR 3305, 2928, 2360, 1650 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.40 (brs, 1H), 3.64-3.50 (m, 8H), 3.39 (s, 3H) 2.88-2.80 (m, 2H), 2.69-2.62 (m, 2H), 2.45-2.38 (m, 1H), 1.56 (dd, J = 7.8, 9.2 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.9, 71.8, 70.2, 69.8, 59.0, 54.6, 39.3, 25.9; DART HRMS calcd for $C_9H_{20}NO_3S_2$ 254.0885, found 254.0897.

***N*-Butyl-3-mercapto-2-(mercaptomethyl)propanamide (4b)**



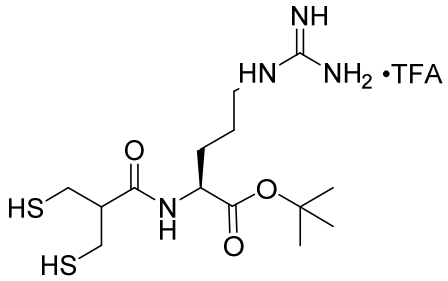
Compound **4b** (67.8 mg, 89%) was observed from **S2** (250.0 mg, 0.36 mmol) as a yellow oil. IR 3289, 2932, 2359, 1643 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.10 (brs, 1H), 3.30-3.25 (m, 2H), 2.84-2.77 (m, 2H), 2.67-2.59 (m, 2H), 2.42-2.35 (m, 1H), 1.51-1.46 (m, 4H), 1.34 (sext, J = 7.3 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.9, 71.0, 58.7, 54.6, 39.2, 25.9; DART HRMS calcd for $C_8H_{18}NOS_2$ 208.0830, found 208.0831.

***N*-Benzyl-3-mercapto-2-(mercaptomethyl)propanamide (4c)**



Compound **4c** (159.9 mg, 95%) was observed from **S3** (508.3 mg, 0.66 mmol) as a colorless solid. IR 3272, 2923, 2545, 1631, 694 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.32-7.24 (m, 5H), 6.79 (brs, 1H), 4.43 (s, 2H), 2.77-2.72 (m, 2H), 2.60-2.56 (m, 2H), 2.47-2.42 (m, 1H), 1.49 (t, J = 8.6 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.0, 137.8, 128.5, 127.5, 127.4, 54.2, 43.5, 25.8; DART HRMS calcd for $C_{11}H_{16}NOS_2$ 242.0673, found 242.0668.

(-)-*tert*-Butyl [3-mercapto-2-(mercaptomethyl)propanoyl]arginine trifluoroacetate (4d)



Compound of **4d** (223.0 mg, 97%) was obtained from **S4** (545.8 mg, 0.50 mmol) as a colorless solid.; $[\alpha]_D^{20}$ -64.9 (c 3.05, MeOH); IR 3167, 2960, 1643, 1163, 522 cm^{-1}; ^1H NMR (600 MHz, D_2O) δ 4.31-4.24 (m, 1H), 3.32-3.12 (m, 1H), 3.07-3.04 (m, 2H), 2.71-2.50 (m, 3H), 1.82-1.78 (m, 1H), 1.66-1.47 (m, 3H), 1.13 (s, 9H); FAB HRMS calcd for $C_{14}H_{29}N_4O_3S_2$ 365.1681, found 365.1683.

General procedure for aerial oxidation of dithiol

A solution of the dithiol (0.05 mmol) in 0.1 M Tris-HCl buffer (pH 7.0)/CH_3CN = 4:1, 1 mL) was stirred for 4-72 h under air. And then, the sampling (50 μL) was quenched with 10% HCl aq. (50 μL). The disappearance of dithiol was calculated by the result of ^1H NMR ($CDCl_3$ containing 0.05% TMS as an internal standard).

DHAA	0 h	1 h	4 h	9 h	12 h	24 h	30 h
Degree of disappearance (%)	0.0	1.2	11.9	34.1	40.1	87.8	96.6

$y = 3.402x$ ($R^2 = 0.9885$), $T_{1/2}$ = 14.7 h

4a-PEG	0 h	1 h	4 h	6 h	9 h	12 h
Degree of disappearance (%)	0.0	8.3	23.9	32.5	49.8	67.1

$y = 5.5871x$ ($R^2 = 0.9947$), $T_{1/2}$ = 8.9 h

4b-Bu	0 h	6 h	24 h	35 h	50 h
Degree of disappearance (%)	0.0	18.0	52.2	88.1	96.7

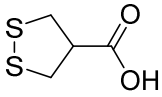
$y = 2.1369x$ ($R^2 = 0.9639$), $T_{1/2}$ = 23.4 h

4c-Bn	0 h	2 h	6 h	50 h	72 h
Degree of disappearance (%)	0.0	0.3	9.3	85.9	100.0

$y = 1.979x$ ($R^2 = 0.9182$), $T_{1/2}$ = 25.3 h

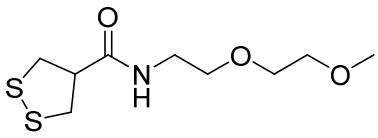
The oxidized disulfide products were also separated as follows. After the reaction was completed, the mixture was extracted with AcOEt. The extract was washed with water and brine, dried, and concentrated to dryness. The residue was chromatographed with hexane-AcOEt to afford the corresponding disulfide.

Asparagusic acid[S4]



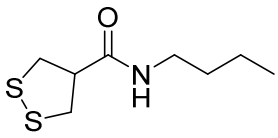
Asparagusic acid was a colorless oil. IR 2925, 1704, 1416 cm^{-1}, ^1H NMR (600 MHz, $CDCl_3$) δ 3.53-3.48 (m, 3H), 3.37-3.32 (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 178.2, 50.3, 41.2; DART HRMS calcd for $C_4H_7O_2S_2$ 150.9888, found 150.9881.

***N*-[2-(2-Methoxyethoxy)ethyl]-1,2-dithiolane-4-carboxamide (S5)**



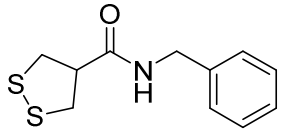
Compound **S5** was yellow oil. IR 3303, 2924, 1650 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 6.30 (brs, 1H), 3.64-3.62 (m, 2H), 3.58 (t, *J* = 5.2 Hz, 2H), 3.56-3.54 (m, 2H), 3.49 (q, *J* = 5.2 Hz, 2H), 3.40 (s, 3H), 3.37 (d, *J* = 7.2 Hz, 4H), 3.16 (quin, *J* = 7.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.4, 71.9, 70.2, 69.6, 59.0, 52.6, 42.6, 39.5; DART HRMS calcd for $C_9H_{18}O_3S_2$ 252.0728, found 252.0739.

***N*-Butyl-1,2-dithiolane-4-carboxamide (S6)**



Compound **S6** was a yellow oil. IR 3293, 2956, 1554 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 5.72 (brs, 1H), 3.36 (d, *J* = 6.9 Hz, 4H), 3.29-3.26 (m, 2H), 3.19 (quin, *J* = 6.9 Hz, 1H), 1.50 (quin, *J* = 7.6 Hz, 2H), 1.35 (sext, *J* = 7.6 Hz, 2H), 0.93 (t, *J* = 7.6 Hz, 3 H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.6, 52.6, 42.8, 39.6, 31.6, 20.0, 13.7; DART HRMS calcd for $C_8H_{16}OS_2$ 206.0673, found 206.0671.

***N*-Benzyl-1,2-dithiolane-4-carboxamide (S7)**

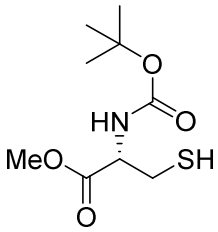


Compound **S7** was a colorless solid. IR 3282, 3029, 2931, 1628, 700 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.35-7.25 (m, 5H), 6.23 (brs, 1H), 4.44 (s, 2H), 3.39-3.34 (m, 4H), 3.22 (quin, J = 6.9 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.5, 137.7, 128.8, 127.7, 52.4, 43.8, 42.7; FAB HRMS calcd for $C_{11}H_{14}OS_2$ 240.0517, found 240.0522.

General Procedure for reduction of L-Cystine-derivative 5

To solution of **5** (234.29 mg, 0.50 mmol) in 0.1 M Tris-HCl buffer (pH 8.0)/acetonitrile (2:5, 1.75 mL) was added dithiol (0.05 mmol) at room temperature. After the mixture was stirred for 1.5 h under N_2, the solvent was removed under reduced pressure. The residual aqueous layer was extracted with hexane/AcOEt (1:1), and the organic layers was washed with water, brine, dried ($MgSO_4$), and concentrated to dryness. The crude product was purified by column chromatography giving pure **6** as a colorless solid. Chemical yield of **6** was calculated from the amount of dithiol (0.05 mmol). The chemical yields are summarized in Figure 3a.

***N*-[(*tert*-Butyloxy)carbonyl]-L-cysteine methyl ester (6)**[S5]



IR 3307, 2957, 1649 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.42 (d, J = 6.9 Hz, 2H), 4.62 (t, J = 3.7 Hz, 1H), 3.79 (s, 3H), 3.04-2.92 (m, 2H), 1.46 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.8, 155.1, 80.2, 54.8, 52.6, 28.2, 27.3; DART HRMS calcd for $C_9H_{18}NO_4S$ 236.0957, found 236.0937.

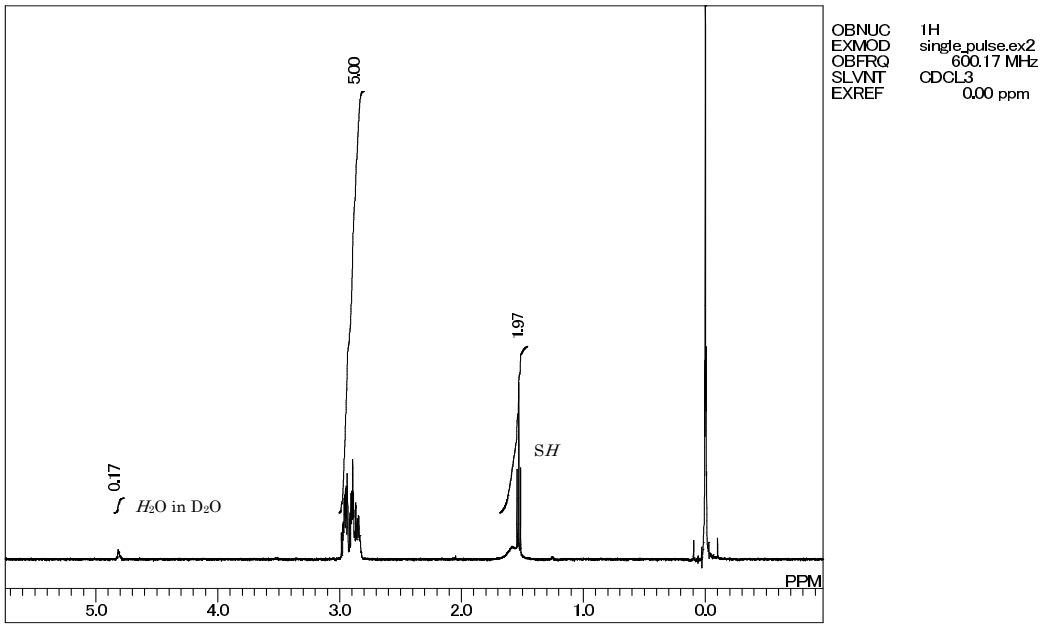
Procedure for H/D Interchange Examination of Dithiol in $CDCl_3$ and D_2O

To a solution of **DHAA** or **4c-Bn** in $CDCl_3$ (20 mM) was added 1 drop of D_2O (25 μl) under Ar. The relative integral value of S*H* in Figure 3 was calculated by ^1H NMR with time.

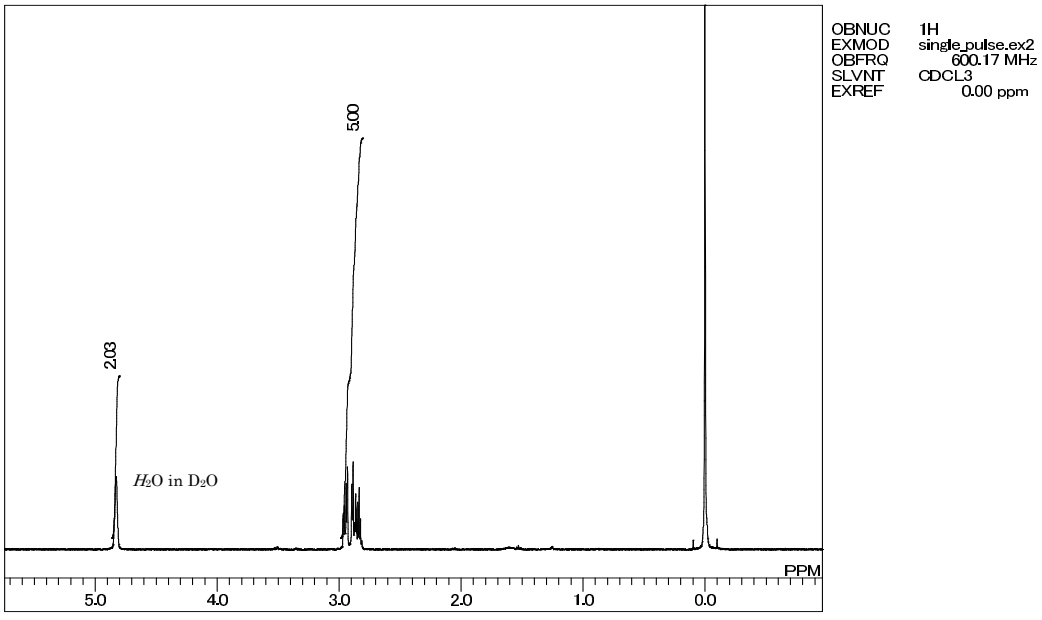
DHAA	**0 h**	**0.25 h**	**0.5 h**
S*H* integral	1.97	0.11	0.07

4c-Bn	**0 h**	**1 h**	**3 h**
S*H* integral	1.93	0.73	0.20

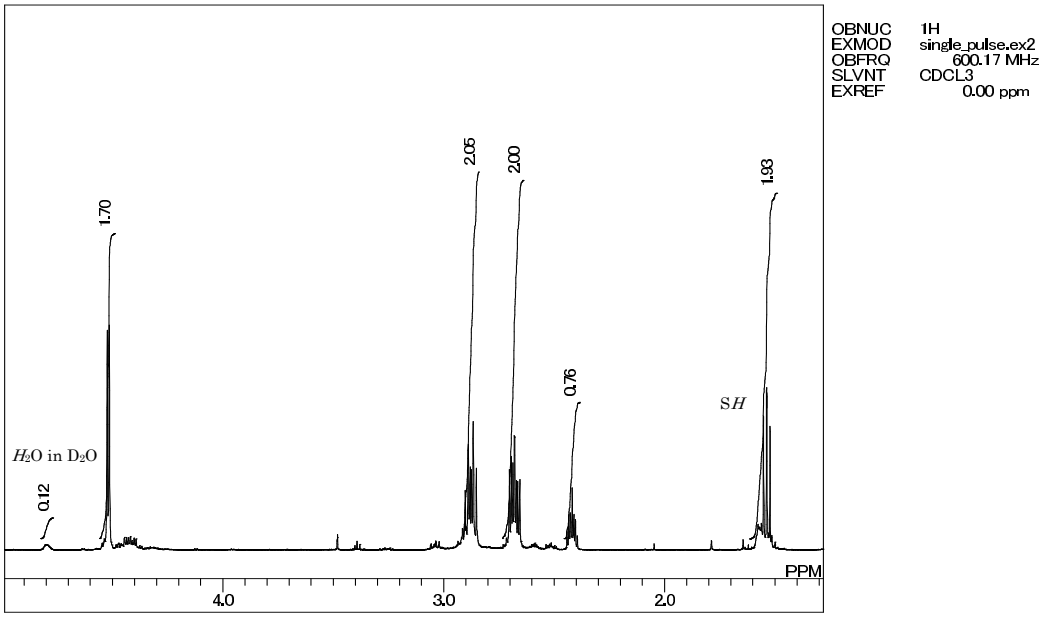
^1H-NMR of solution of DHAA (0 h, $CDCl_3$, D_2O)



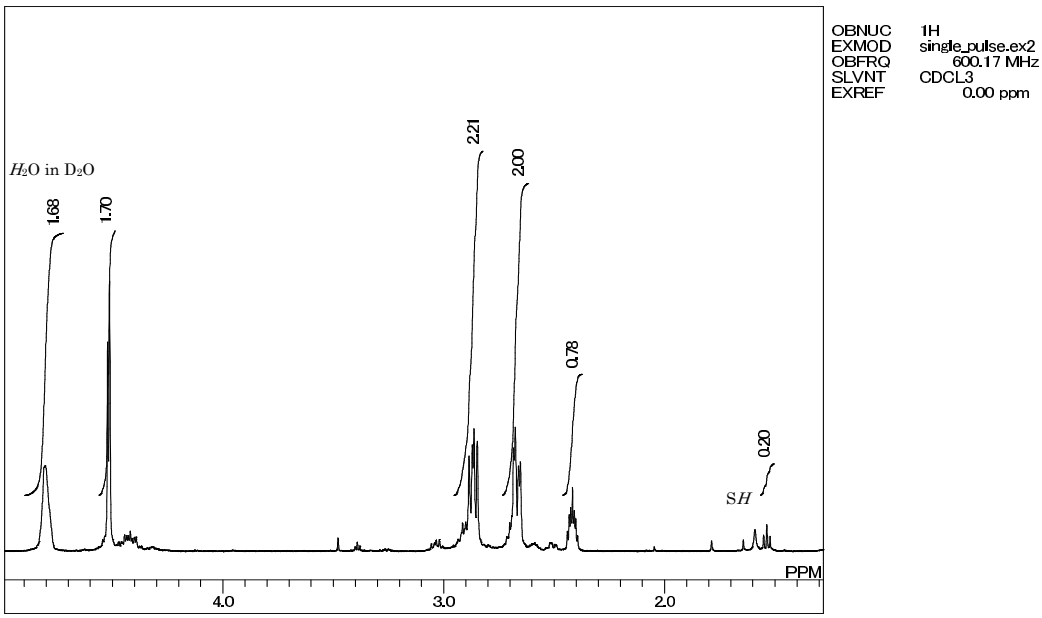
^1H -NMR of solution of DHAA (1 h, $CDCl_3$, D_2O)

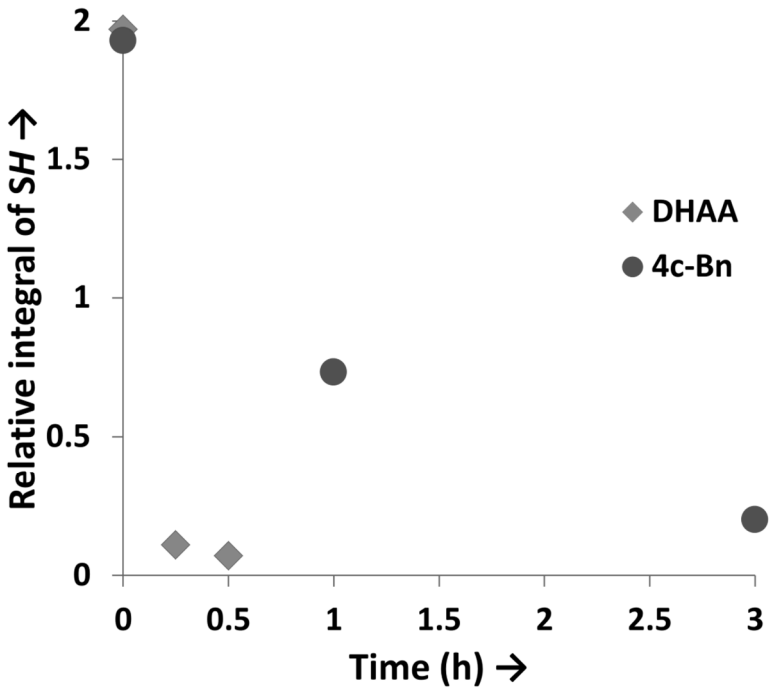


^{1}H -NMR of solution of **4c-Bn** (0 h, $CDCl_3$, D_2O)



^{1}H -NMR of solution of **4c-Bn** (3 h, $CDCl_3$, D_2O)




Relative integral of SH →
2
1.5
1
0.5
0
0
0.5
1
1.5
2
2.5
3
Time (h) →
DHAA
4c-Bn

Procedure for Monitoring Antibody Cleavage [S6] on SDS-PAGE

A mixture (10 μL) of 1.0 mM dithiol (10 nmol) and LEAF™ Purified Mouse IgG2$_{\alpha\kappa}$ Isotype Ctrl Antibody (Bio Legend) (2 μg) in 0.1 M Tris-Cl (pH 7.0)/acetonitrile (4 : 1), was incubated at room temperature for 15 min, and then cooled on ice. Iodoacetamide was added to each sample (final 15 mM) and incubated at room temperature for 60 min in the dark. After adding 2 x SDS sample buffer (0.125 M Tris-HCl (pH 7.0), 4% SDS, 20% sucrose, and 0.002% bromophenol blue) followed by boiling for 5 min, the mixture was analyzed on E-R10L e-PAGEL (ATTO) with AE-1410 EzRun (ATTO). The gel was stained with Coomassie Brilliant Blue and analyzed by a laser scanner Odyssey Classic Imaging System (LI-COR Biosciences).

fragment	reducing agent	ratio og IgG(%)			
		n=1	n=2	n=3	average
H_2L_2	DHAA	74.17	33.9	33.37	47.15
	4a-PEG	95.58	85.98	78.84	86.8
	4b-Bu	4.81	0.3	0.31	1.81
	4c-Bn	1.19	0.12	0.2	0.5
	4d-Arg	97.62	84.86	88.97	90.48
H	DHAA	0.83	6.93	9.24	5.67
	4a-PEG	0.04	0	0	0.01
	4b-Bu	18.3	41.71	55.61	38.54
	4c-Bn	41.69	50.08	66.78	52.85
	4d-Arg	0.24	0	0	0.08
L	DHAA	17.16	14.64	13.14	14.98
	4a-PEG	0	0	0	0
	4b-Bu	35.8	42.68	42.84	40.44
	4c-Bn	43.68	44.52	37.68	41.96
	4d-Arg	0.56	0	0.72	0.42

Procedure for Monitoring The Inhibitory Activity of Dithiol on ELISA

Reduction agent was dissolved in 0.1 M Tris-HCl buffer (pH 7.0)/ acetonitrile (4 : 1) and prepared to 0.1 M solution. Then, the 0.1 M solution (100 μL) containing reduction agent was added in antibody-coated microplates and reacted for 1 h at 37 ℃. After the reaction, the plates were washed (4 times). Then 100 μL of the human CRP standard (5 ng/mL) was added to each well and reacted for 2 h at 37 ℃. The plates were then washed again (4 times), 100 μL of the detection antibody solution was added to each well and reacted for 1 h at 37 ℃. The plates were then washed again (4 times), HRP conjugated antibody solution was added to each well and reacted for 40 minutes at 37 ℃. The plates were then washed again (4 times), 100 μL of the tetramethylbenzidine (TMB) substrate solution added to each well and reacted for 20 min at 37 ℃ in the dark (after dispense, the color turned to blue depending on the concentration). After completion of the reaction, the reaction was stopped with stop solution (after dispense, the color turned to yellow depending on the concentration), and the absorbance was measured at a wave length of 450 nm (reference wavelength, 630 nm) by a CORONA Multi Microplate Reader MTP-880Lab (CORONA ELECTRIC).

reducing agent	Abs.			
	n = 1	n = 2	n = 3	average
without reducing agent				* 0.461
DTT	0.126	0.216	0.327	0.223
DHAA	0.128	0.188	0.13	0.149
4a-PEG	0.149	0.13	0.111	0.130
4b-Bu	0.052	0.085	0.043	0.060
4c-Bn	0.046	0.045	0.045	0.045
4d-Arg	0.182	0.229	0.252	0.221
TCEP	0.144	0.19	0.184	0.173

*Absorbance adding no reducing agent was determined from the calibration curve ($y = 0.0821\ln(x) + 0.3288$).

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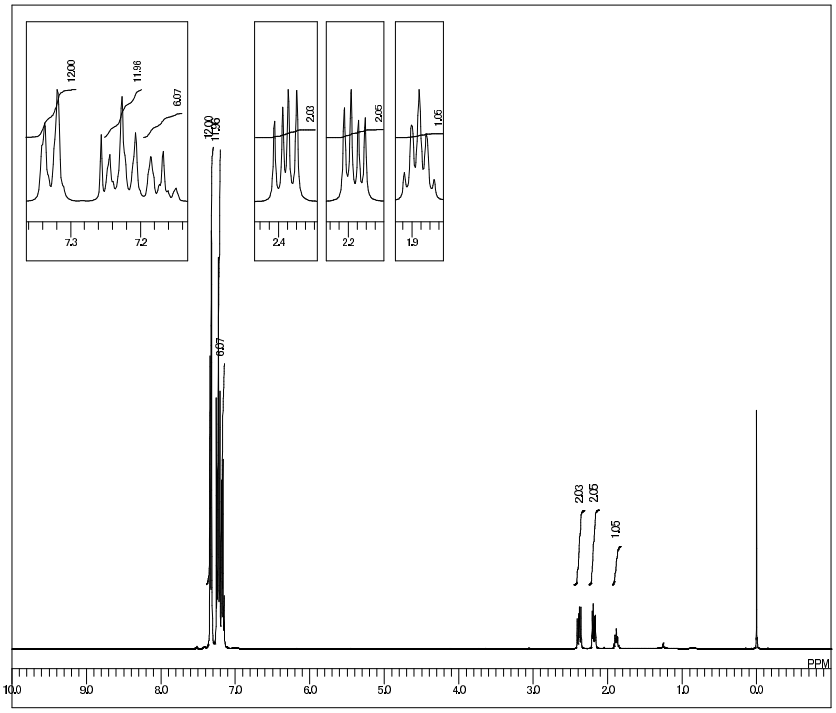
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2.03
2.05
1.05
6.07
2.03
2.05
1.05
PPM
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8.0
7.0
6.0
5.0
4.0
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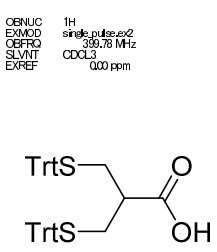
2.0
1.0
0.0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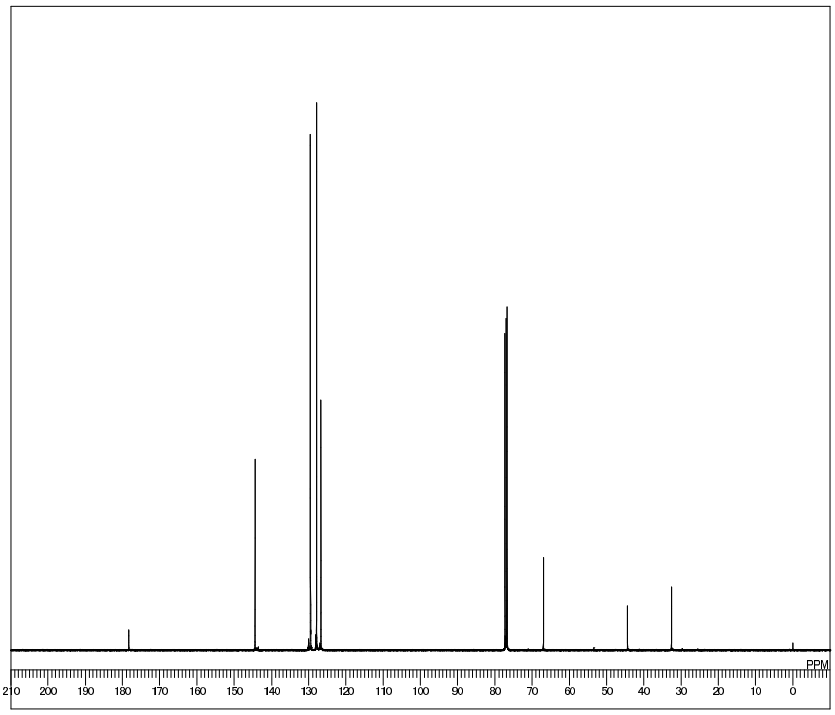
TrtS
O
OH

2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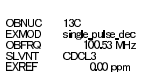
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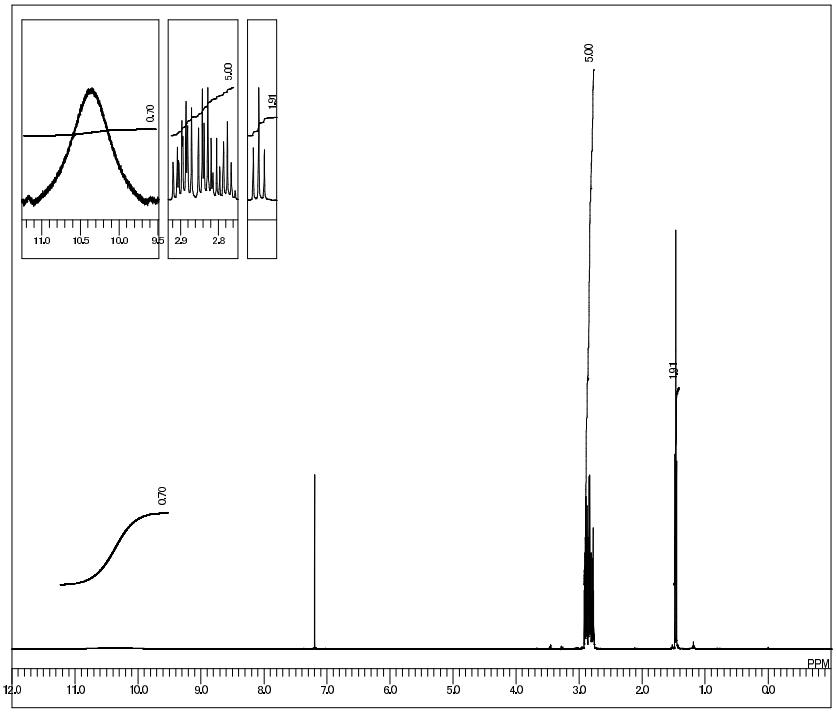

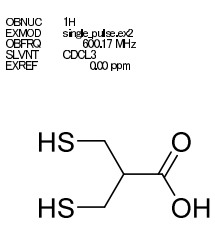

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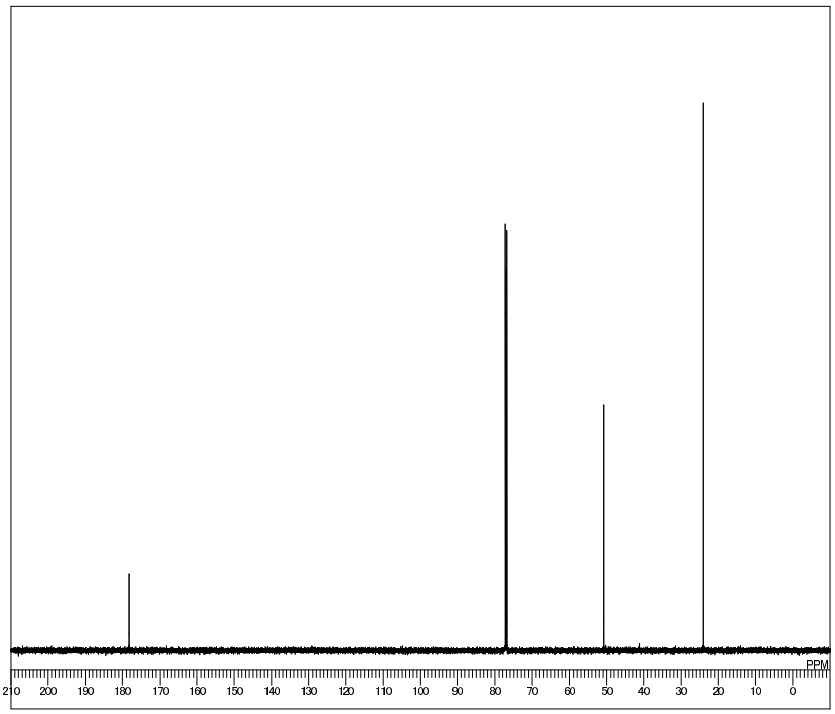

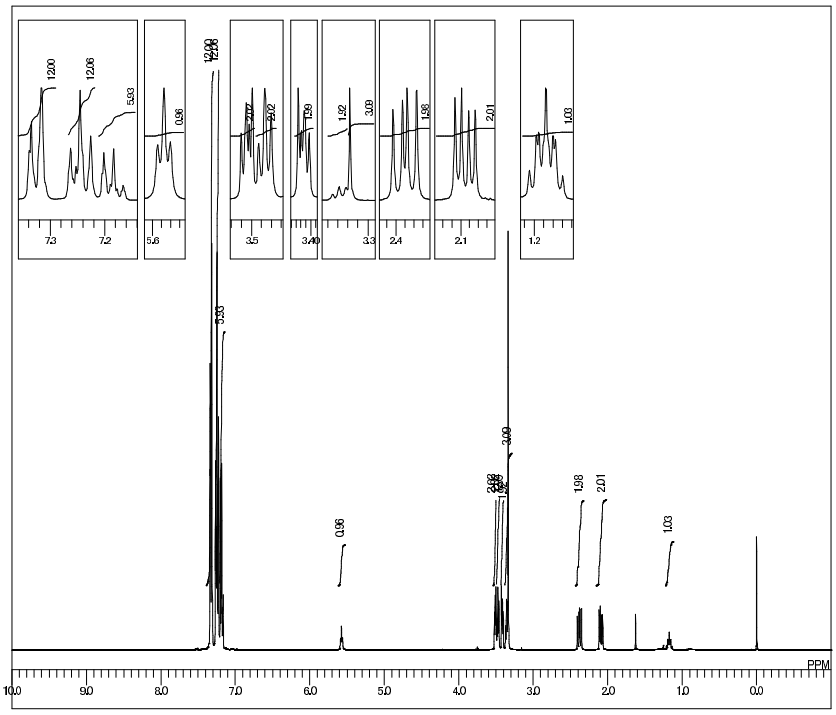
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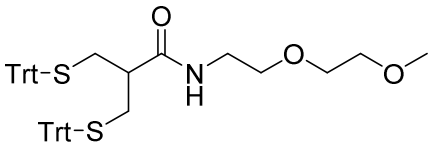
2.01
1.03
PPM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Trt-S
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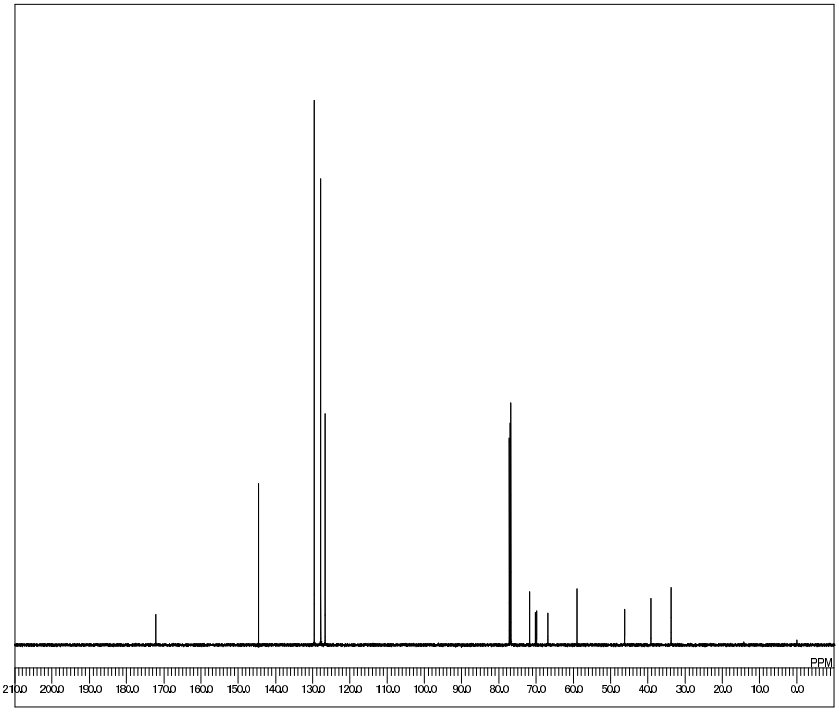
N
H
O
O

S1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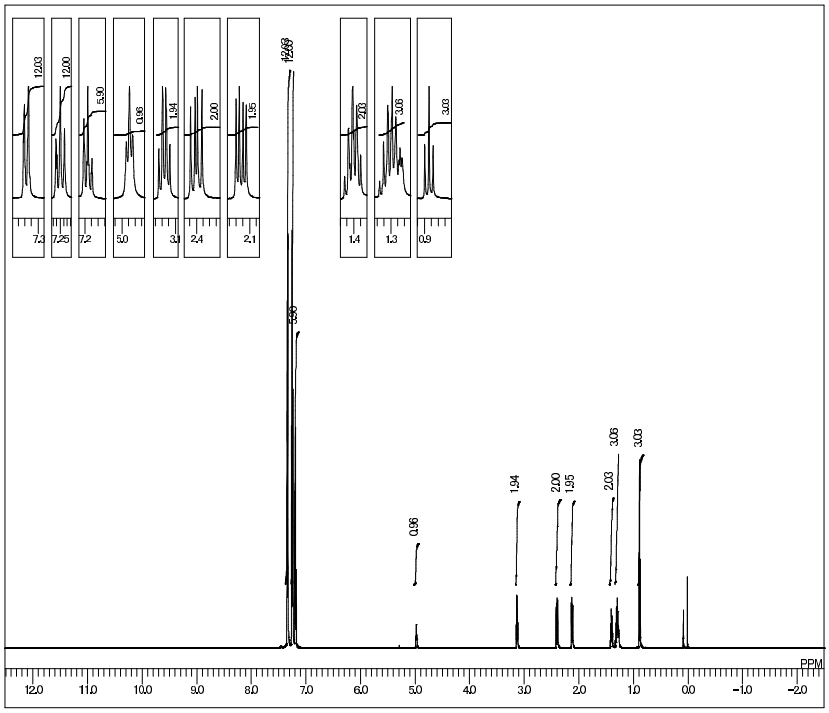
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1.95
2.03
3.06
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7.2
5.0
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2.1
1.4
1.3
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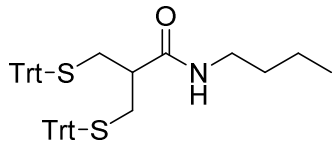
PPM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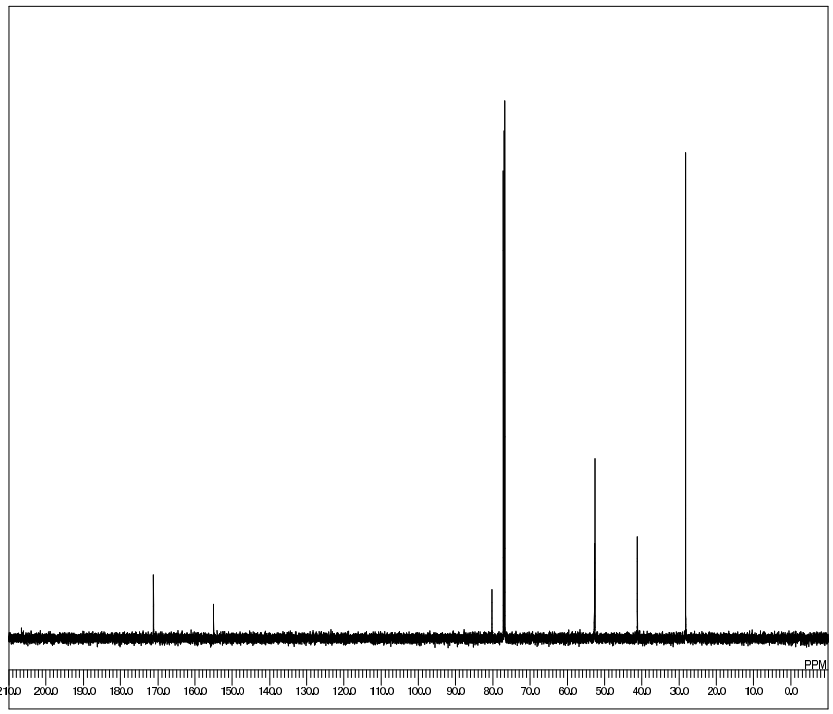
Trt-S
N
H
Trt-S

S2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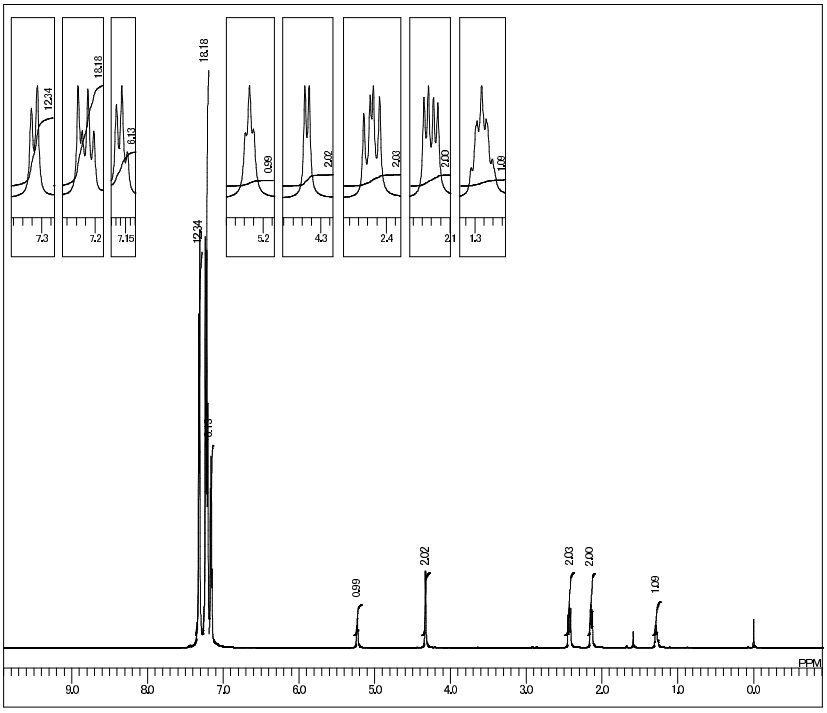
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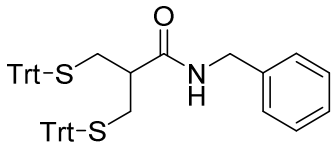
PPM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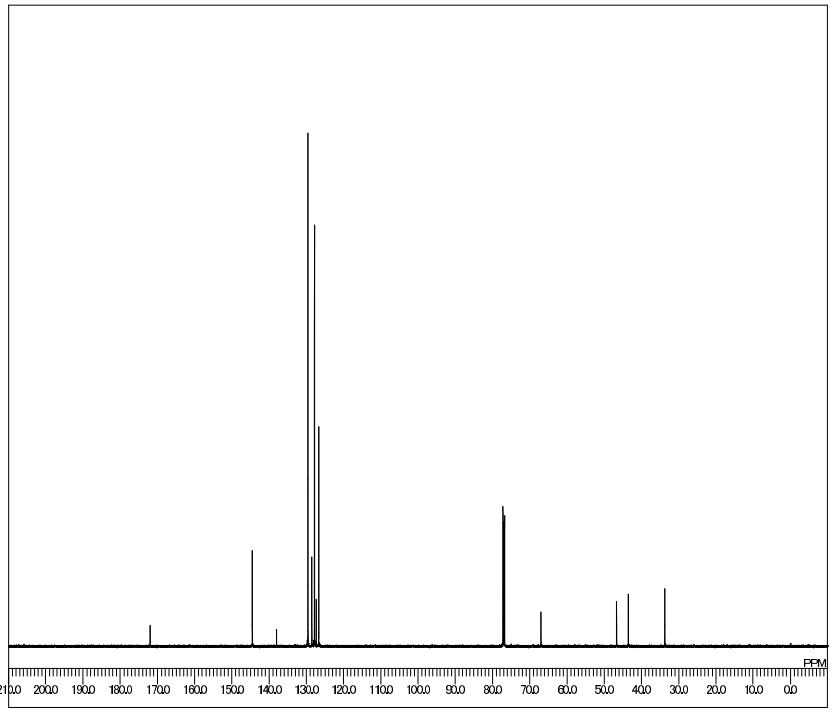
H
Trt-S

S3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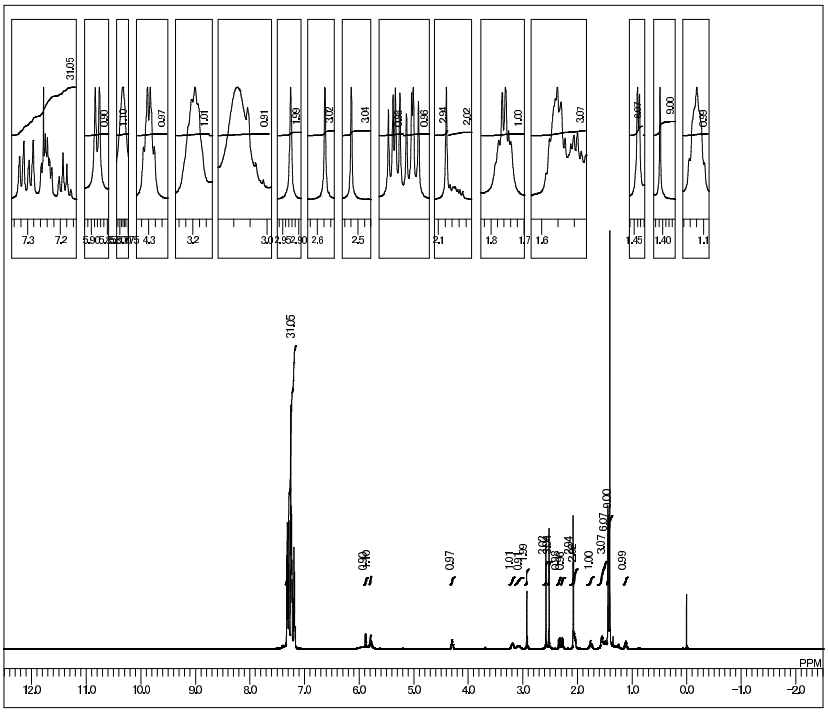
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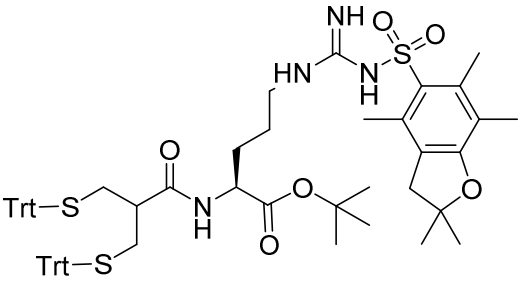
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H
O
O
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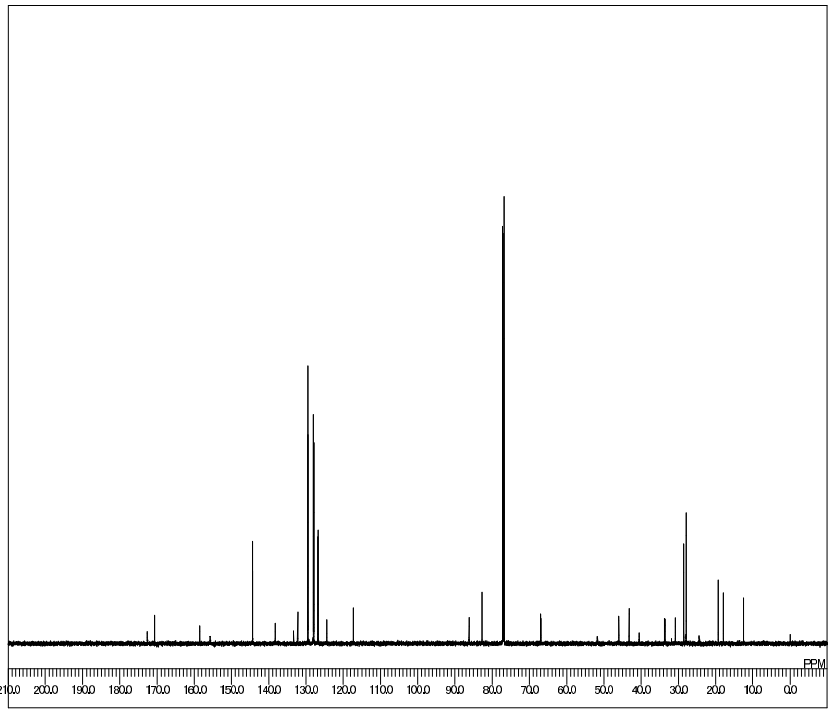
O

S4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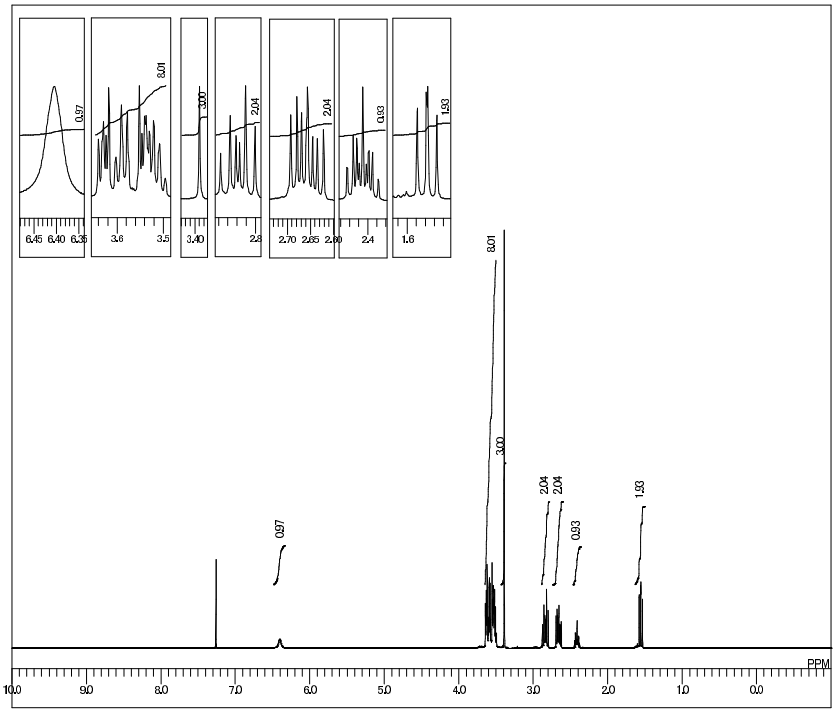

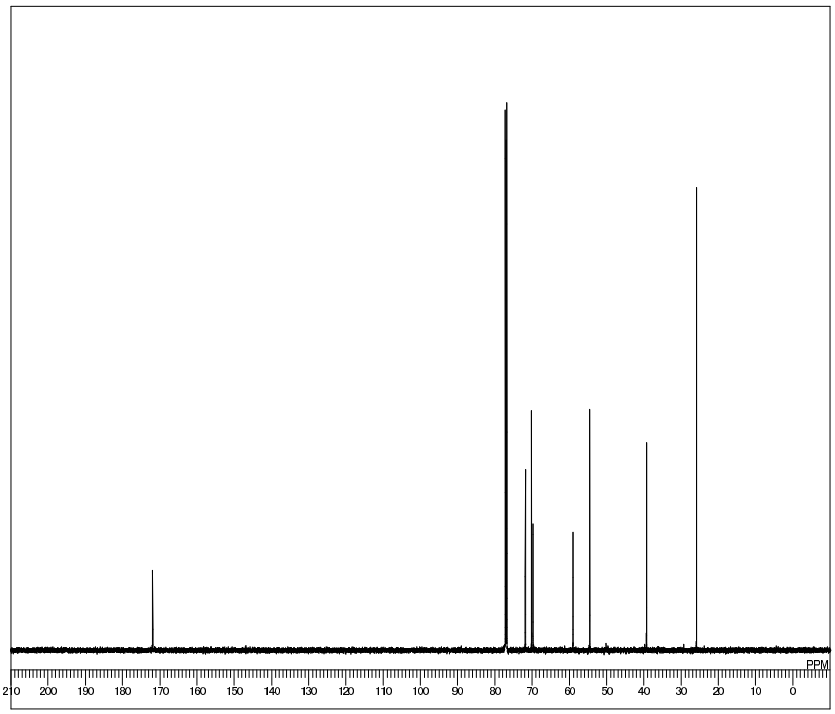

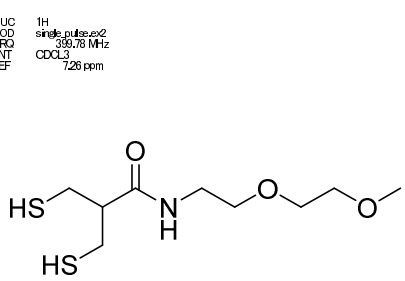

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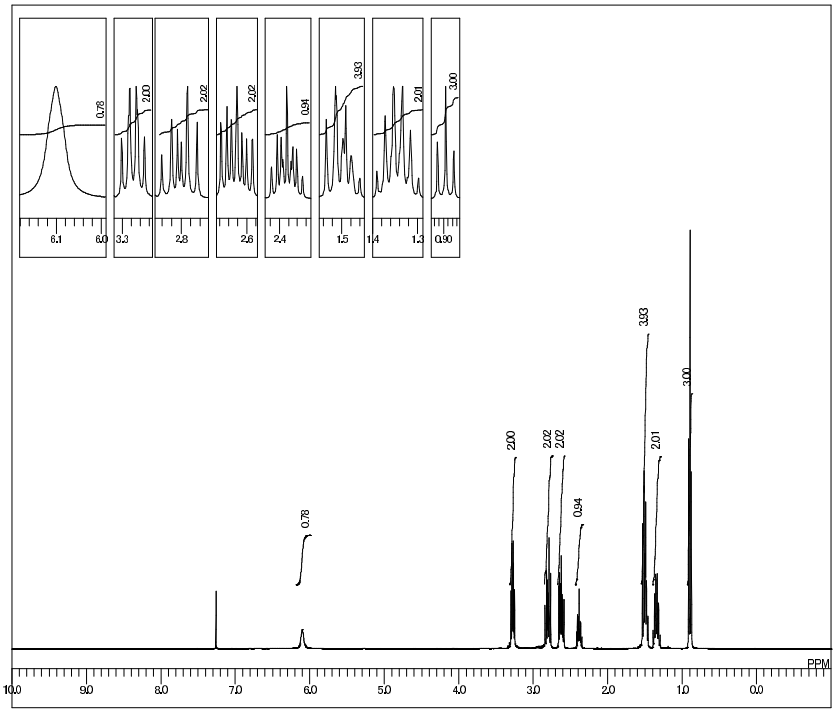
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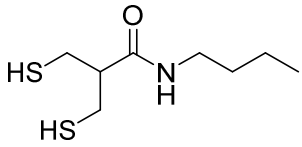
3.00
PPM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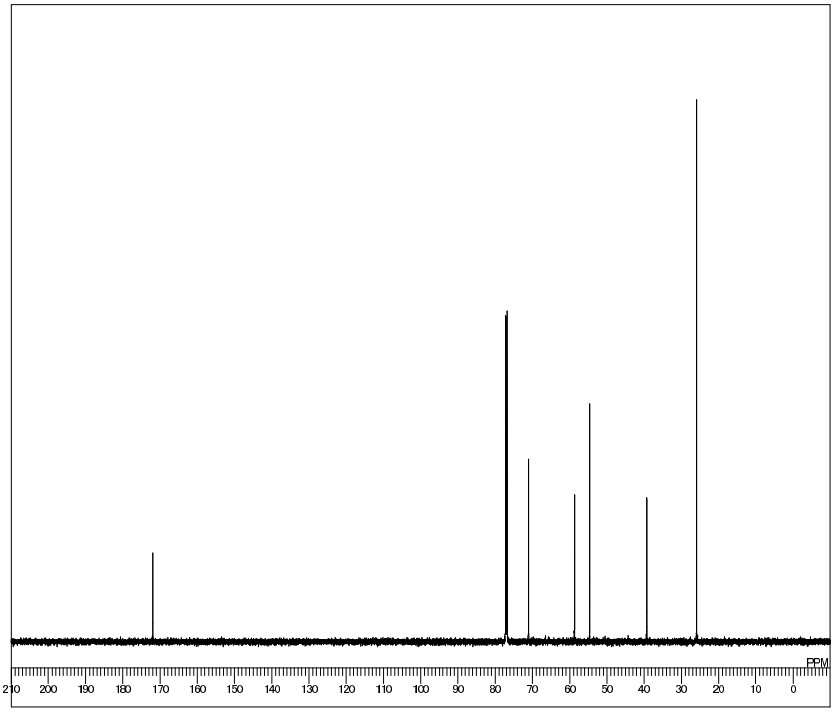
HS
N
H
HS

4b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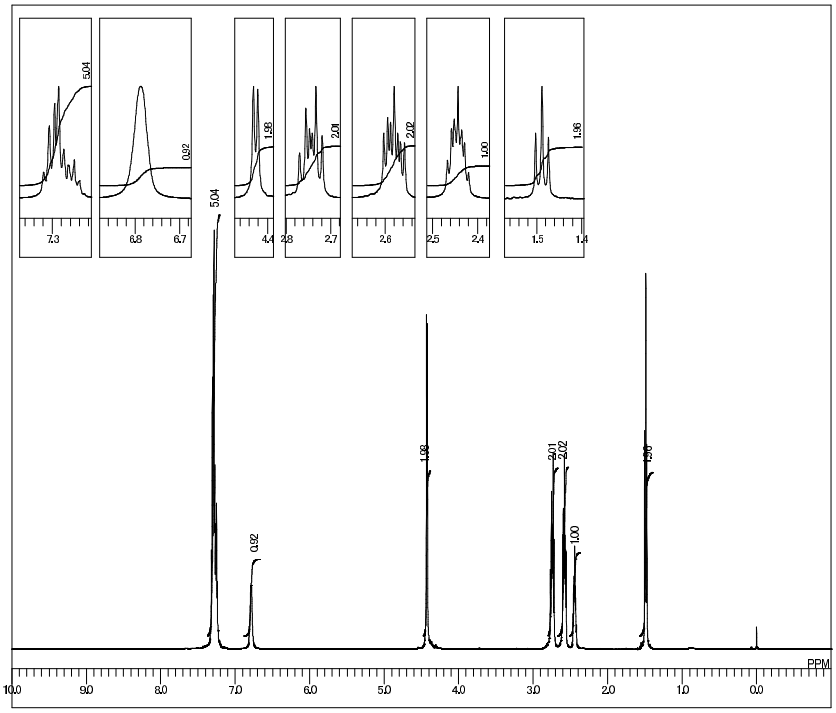
PPM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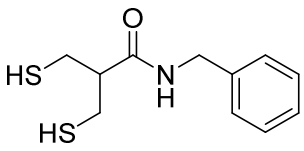
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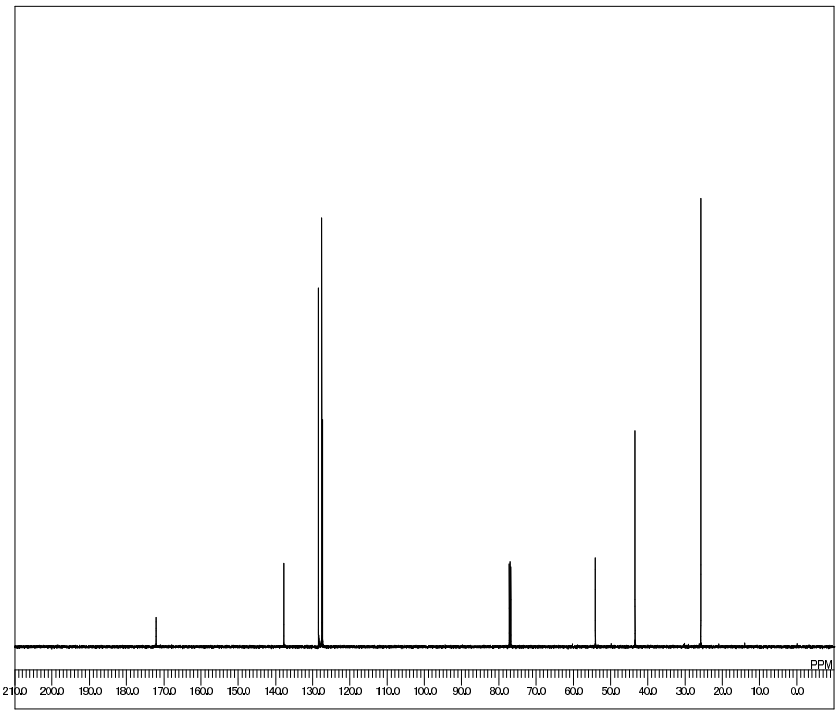
H

4c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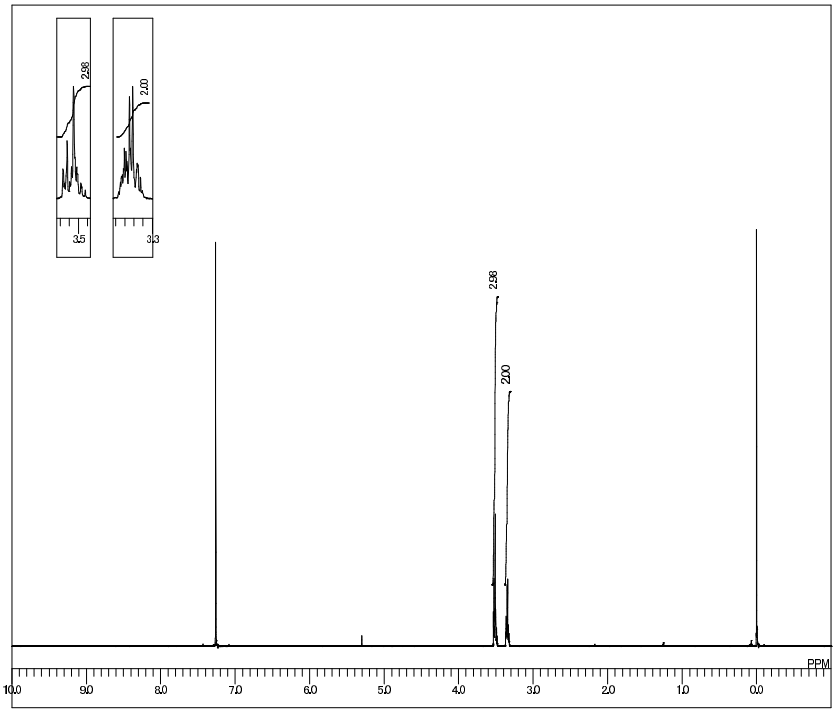

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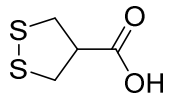
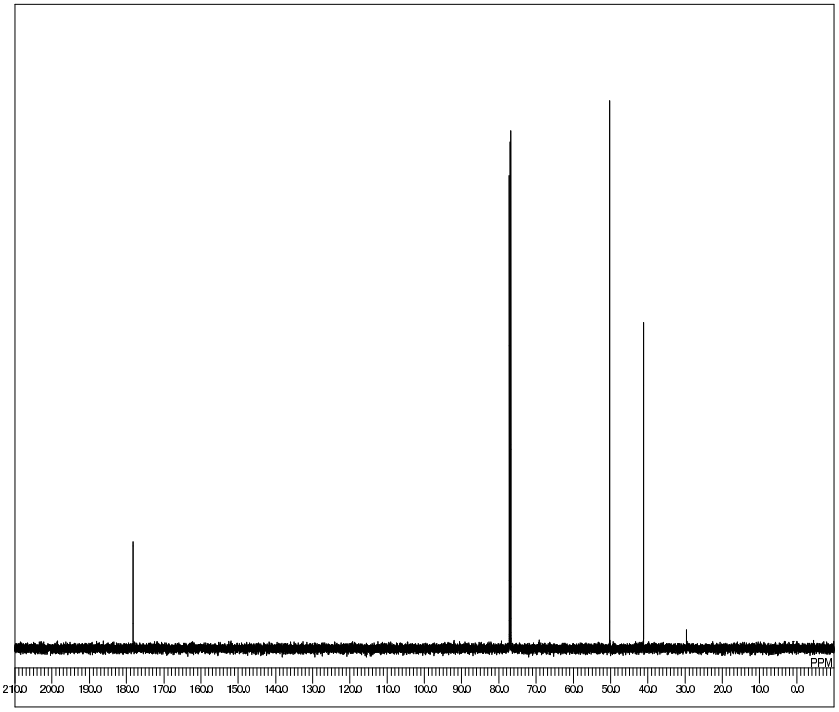
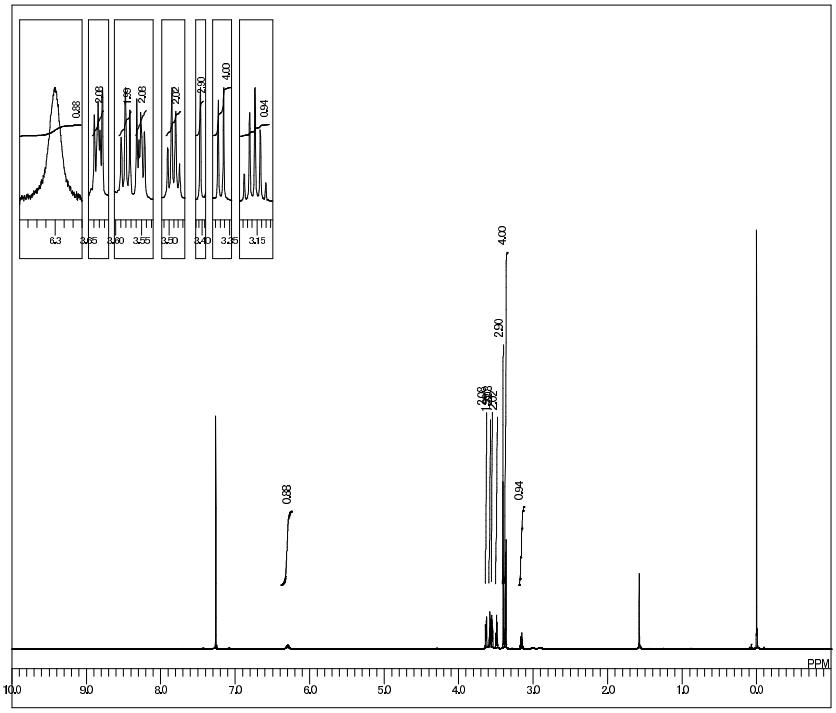
SLVNT CDCL3
EXREF 0.00 ppm

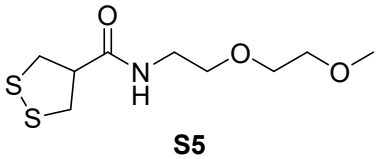


Asparagusic acid

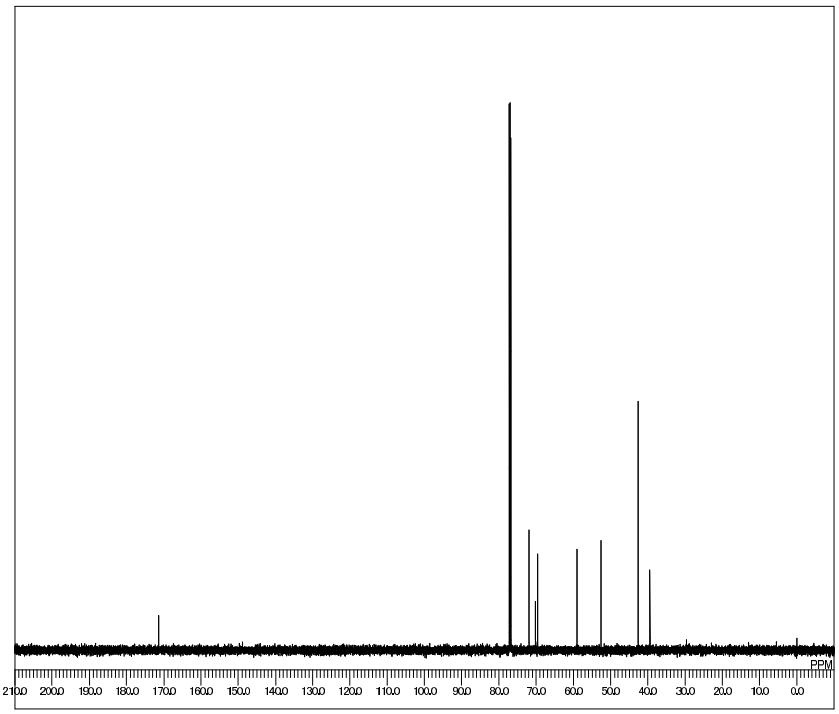


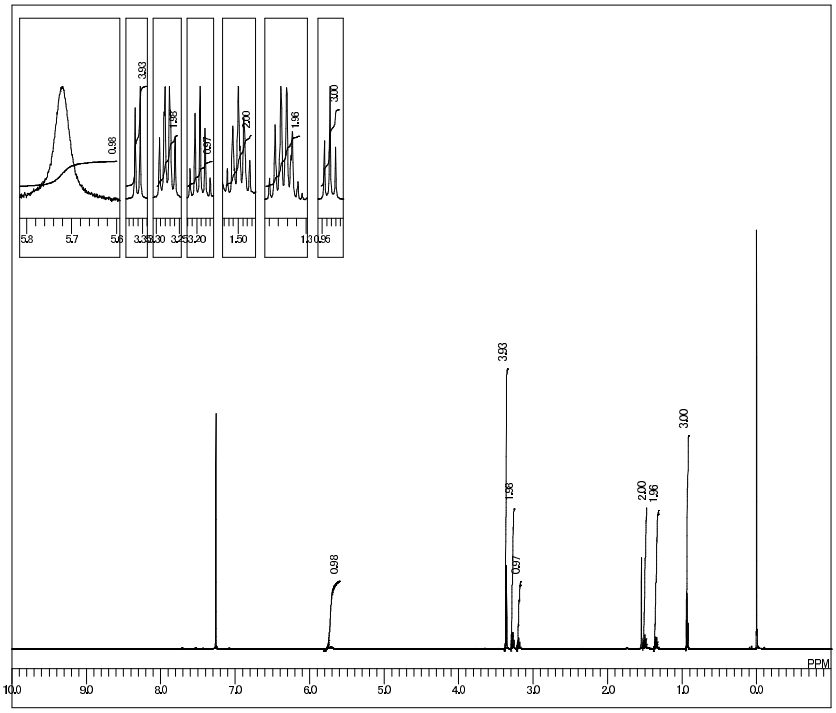
OBNUC 13C
EXMOD single_pulse_dec
OBFRQ 150.92 MHz
SLVNT CDCL3
EXREF 77.00 ppm

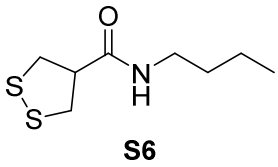

OBNUC 1H
EXMOD single_pulse.ex2
OBFRQ 600.17 MHz
SLVNT CDCL3
EXREF 0.00 ppm
0.88
2.03
1.99
2.08
2.02
2.90
4.00
0.94
4.00
2.90
0.88
0.94
PPM



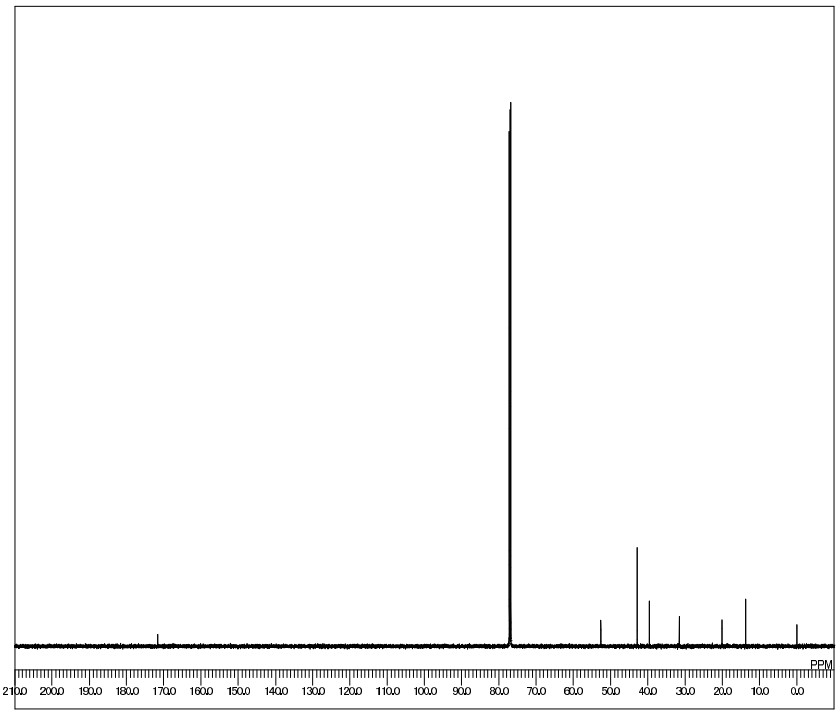
S5

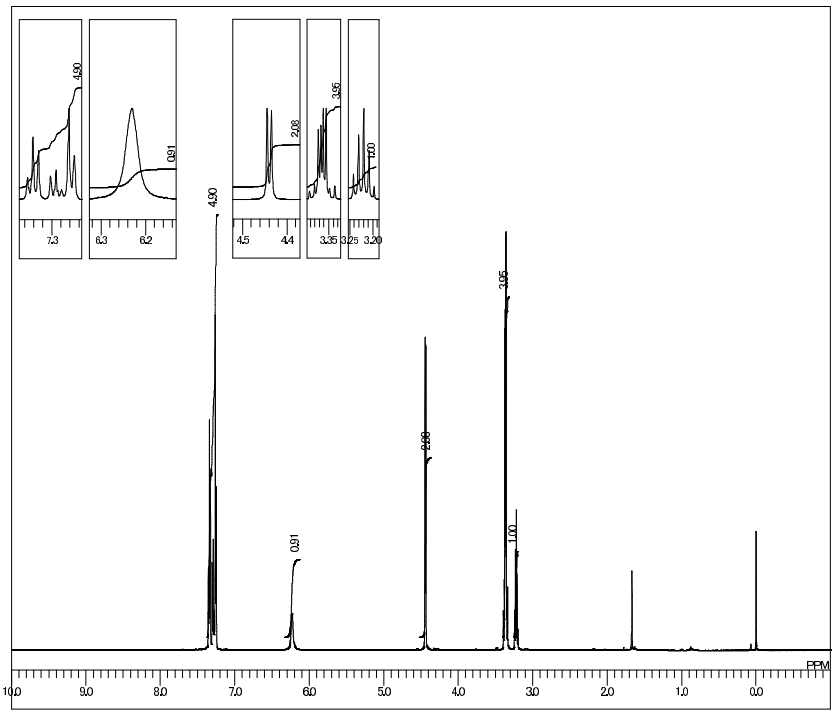

OBNUC 13C
EXMOD single_pulse_dec
OBFRQ 150.92 MHz
SLVNT CDCL3
EXREF 77.00 ppm
PPM

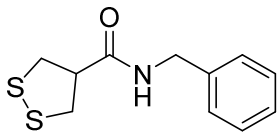

OBNUC 1H
EXMOD single_pulse.ex2
OBFRQ 600.17 MHz
SLVNT CDCL3
EXREF 0.00 ppm
0.98
3.93
1.36
0.97
2.00
1.95
3.00
PPM


O
N
H
S
S

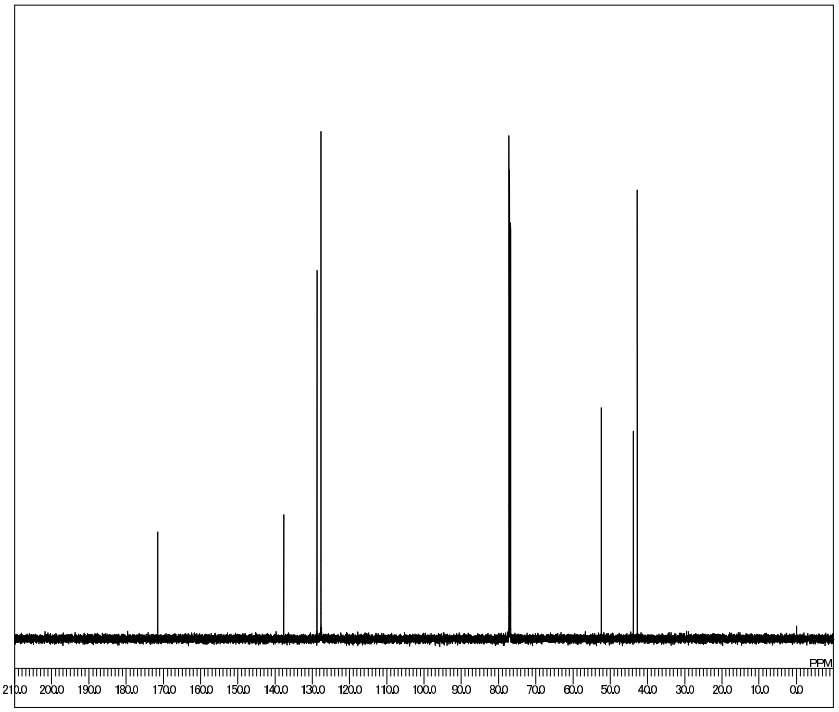
S6

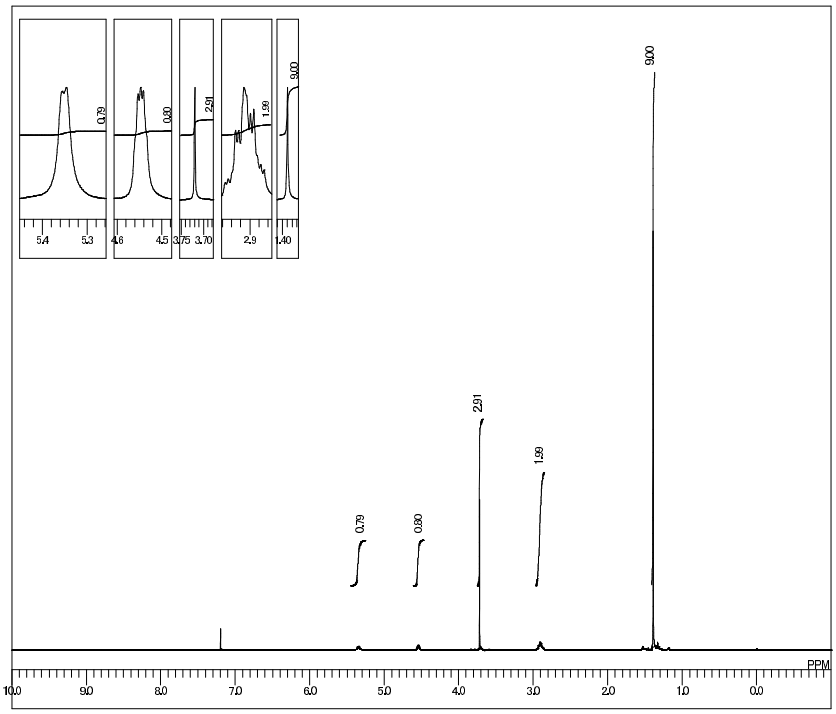

OBNUC 13C
EXMOD single_pulse_dec
OBFRQ 150.92 MHz
SLVNT CDCL3
EXREF 0.00 ppm
PPM

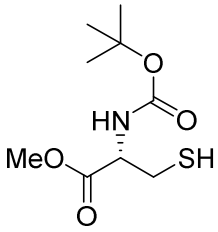

OBNUC 1H
EXMOD single_pulse.ex2
OBFRQ 600.17 MHz
SLVNT CDCL3
EXREF 0.00 ppm
4.90
0.91
2.08
0.95
2.89
3.05
1.00
PPM



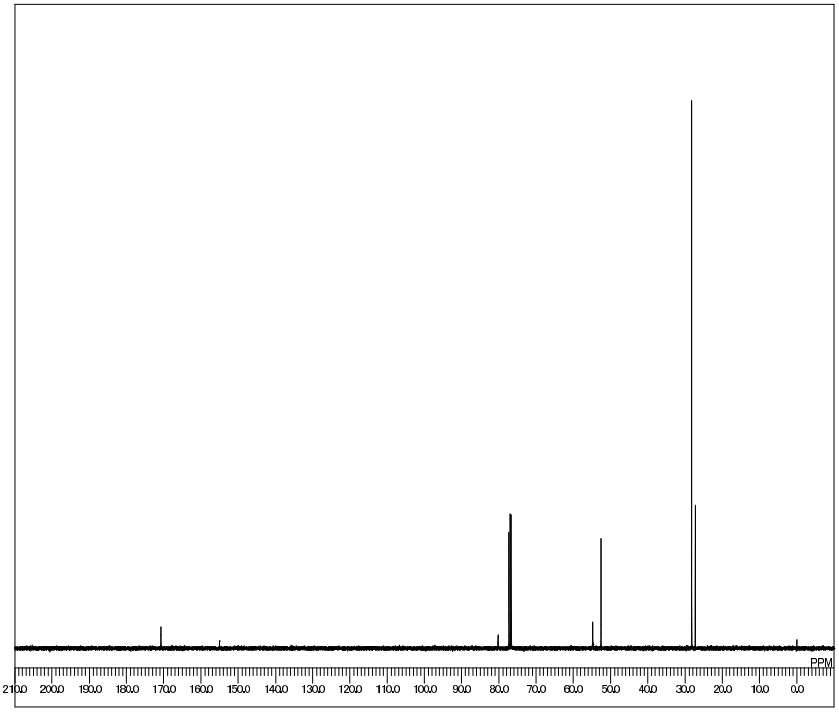
S7


OBNUC 13C
EXMOD single_pulse.dec
OBFRQ 150.92 MHz
SLVNT CDCL3
EXREF 77.00 ppm
PPM

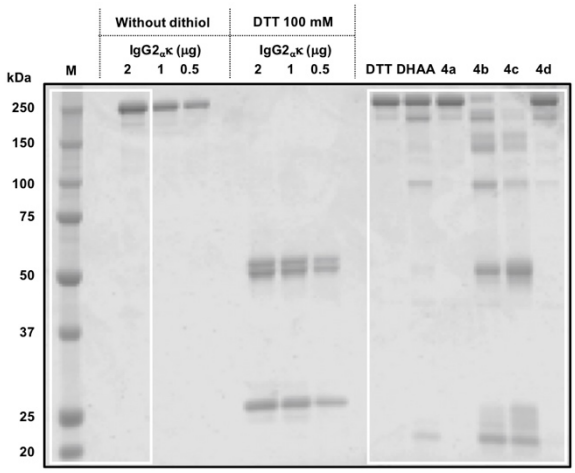

OBNUC 1H
EXMOD single_pulse.ex2
OBFRQ 600.17 MHz
SLVNT CDCL3
EXREF 0.00 ppm
0.79
0.80
2.91
1.99
9.00
PPM


O
HN
O
MeO
SH
O

6


OBNUC 13C
EXMOD single_pulse_dec
OBFRQ 100.53 MHz
SLVNT CDCL3
EXREF 77.00 ppm
PPM

The source data of Figure 3b



The quantitative data (Figure 3c) of the bands corresponding to H_2L_2

The ratio of IgG was calculated from the results of IgG2$_\alpha$K (2, 1, 0.5 μg) without dithiol.

The quantitative data (Figure 3c) of the bands corresponding to H, and L

The ratio of IgG was calculated from the results of IgG2$_\alpha$K (2, 1, 0.5 μg) with DTT (100 mM).