Supporting Information

Understanding the structural requirements for activators of the Kef bacterial potassium efflux system

Jessica Healy[1+], Silvia Ekkerman[2+], Christos Pliotas[2], Morgiane Richard[2], Wendy Bartlett[2], Samuel C. Grayer[1], Garrett Morris[3], Samantha Miller[2], Ian R. Booth[2], Stuart J. Conway[1†], Tim Rasmussen[2†]

[1]From the Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, United Kingdom

[2]School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom

[3]Crysalin, Ltd, Cherwell Innovation Center, 77 Heyford Park, Upper Heyford, Oxfordshire, OX25 5HD, United Kingdom

[+]J. Healy and S. Ekkerman contributed equally to the work

[†]To whom correspondence should be addressed: Tim Rasmussen, School of Medical Sciences, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, AB25 2ZD, United Kingdom, Tel.: 0044-1224-437540; Fax: 0044-1224-437465; E-mail: t.rasmussen@abdn.ac.uk or Stuart J. Conway, Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, United Kingdom, Tel.: 0044-1865-285109; Fax: 0044-1865-285102; E-mail: stuart.conway@chem.ox.ac.uk

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Table S1: Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or description	reference
Strains		
MJF276	F- *ΔkdpABC5 thi rha lacI lacZ trkD1kefB157 kefC::Tn10*	1
MJF335	MJF276 *gshA*::Tn*10*(Kan)	2
MJF373	MJF276 *ΔkefFC::kan, Δcrp kefB::Tn10*	3
Plasmids		
pTrcEcKefFCH_6	KefFC from *Escherichia coli* with C-term His_6 on KefC	3
pTrcSdKefH_6	Kef from *Shewanella denitrificans* OS217 with C-term H_6	3
pTrcSdKefQCTDH_6	Soluble domain of S.d. Kef starting at K391and fused loop	3

Table S2: Homology of SdKef to Kef systems from *E. coli*. Shown are the percentages identity (bold) and similarity (italic) of the SdKef amino acid sequence to the sequences of KefC and KefB from *E. coli*. These numbers where obtained by a BLAST search (http://blast.ncbi.nlm.nih.gov).

% identity or *%similarity* of SdKef:	to EcKefC	to EcKefB
full length	**45%** *64%*	**44%** *65%*
cytosolic domain*	**41%** *60%*	**39%** *62%*

* for residue after the Q-linker from residue V402 (EcKefC numbering)

Analysis of ligand binding by fluorescence spectroscopy.

The data were fitted using a standard saturation isotherm; where the fluorescent DNGSH ligand is L and SdKefQCTD is M and with M + L ⇔ ML.

$$K_d = \frac{[M][L]}{[ML]} \quad (1)$$

The conservation of mass for SdKefQCTD is given by $n[M]_0 = [M] + [ML]$ where the total concentration of SdKefQCTD subunits is $[M]_0$, n is the number of active binding sites per M, $[L]_0$ is the total concentration of ligand and the concentration of free ligand is [L]. Accordingly, for DNGSH, $[L]_0 = [L] + [ML]$. If these expressions are substituted into equation 1, a quadratic equation for the protein-ligand complex [ML] is obtained that can be solved for [ML] and expressed as the fraction of bound ligand, f_B (equation 2). This equation considers the depletion of L upon binding and was used for direct titrations of the ligand with $[L]_0$ as independent variable or for inverse titrations with $[M]_0$ as independent variable.

$$f_B = \frac{[ML]}{[L]_0} = \frac{1}{2[L]_0}\left\{\left(n[M]_0 + [L]_0 + Kd\right) - \right. \quad (2)$$
$$\left. - \sqrt{(n[M]_0 + [L]_0 + K_d)^2 - 4[L]_0 n[M]_0}\right\}$$

Two fluorescence parameters were used to obtain f_B from experimental data. The peak position in the emission spectrum, λ_{max}, was obtained by fitting a skewed Gaussian to the experimental spectra as described earlier (1). λ_{max} was used as it was less easily disturbed than fluorescence intensity. Secondly, steady state fluorescence anisotropy, r_{obs}, was used. However, using λ_{max} and r_{obs} requires the consideration of the quantum yield of the free ligand, Φ_L, relative to bound ligand, Φ_{ML}, in form of the ratio $Q = \Phi_{ML}/\Phi_L$ (2). λ_{max} and r_{obs} can be expressed as simple sums depending on the fluorescence intensity of the bound and free ligand, I_{ML} and I_L (equation 3 and 4).

$$\lambda_{\max} = \frac{\lambda_{ML} I_{ML} + \lambda_L I_L}{(I_{ML} + I_L)} \quad (3)$$

$$r_{obs} = \frac{r_{ML} I_{ML} + r_L I_L}{(I_{ML} + I_L)} \quad (4)$$

Intensities are defined with f_B and the quantum yields as $I_{ML} = \Phi_{ML} f_B$ and $I_L = \Phi_L (1-f_B)$, which can be substituted into equation 3 and solved for f_B:

$$f_B = \frac{1}{1 + Q\frac{(\lambda_{\max} - \lambda_{ML})}{(\lambda_L - \lambda_{\max})}} \quad (5)$$

This can be rearranged to solve for λ_{max} as shown in equation 6.

$$\lambda_{\max} = \frac{\frac{\lambda_L}{f_B Q} - \frac{\lambda_L}{Q} + \lambda_{ML}}{\left(1 + \frac{1}{f_B Q} - \frac{1}{Q}\right)} \qquad (6)$$

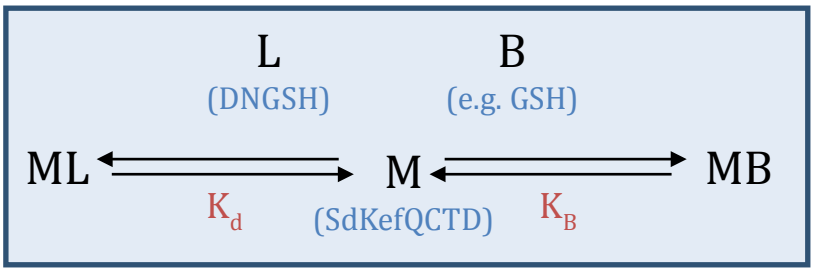
Similar equations to 5 and 6 can be obtained for the fluorescence anisotropy (not shown) using equation 4. Equation 6 substituted with equation 2 was used to fit the experimental data using the program Matlab2012a (Mathworks). λ_L = 572.5 nm was measured directly on DNGSH samples. Inverse titrations with high SdKefQCTD concentrations allowed a good estimation of λ_{ML} = 530 nm and Q = 4. These three parameters were kept fixed during the fitting while K_d and n were optimized. No corrections for non-specific binding were performed as binding experiments on the mutant Q419K indicated that levels of non-specific binding are low (see main text).

Rasmussen, T., Edwards, M. D., Black, S. S., Rasmussen, A., Miller, S., and Booth, I. R. (2010) Tryptophan in the pore of the mechanosensitive channel MscS: assessment of pore conformations by fluorescence spectroscopy. *J. Biol. Chem.* **285**, 5377–84

Jameson, D. M., and Seifried, S. E. (1999) Quantification of protein-protein interactions using fluorescence polarization. *Methods ((Amsterdam, Neth.)* **19**, 222–33

Analysis of fluorescence competition experiments

Competition experiments were analyzed according to Thrall *et al.* (4) considering depletion of detecting fluorescence ligand DNGSH (L) and competing ligand B on the basis of a single site binding model.



The dissociation constants are defined as:

$$K_d = \frac{[M][L]}{[ML]} \quad \text{and} \quad K_B = \frac{[M][B]}{[MB]}$$

Mass conservations are given as:

$[M]_0 = [M] + [ML] + [MB]$

$[L]_0 = [L] + [ML]$

$[B]_0 = [B] + [MB]$

where the indices “0” indicate the total concentrations of the species.

A cubic equation of [ML] is obtained by substitutions:

$[ML]^3 + a[ML]^2 + b[ML] + c = 0$

with the coefficients:

$a = \{[M]_0(K_B - K_d) + [L]_0(2K_B - K_d) + [B]_0K_d - K_d^2 + K_dK_B\}/(K_d - K_B)$

$b = \{[M]_0[L]_0(K_d - 2K_B) - [L]_0^2K_B - [L]_0K_d([B]_0 + K_B)\}/(K_d - K_B)$

$c = [M]_0[L]_0^2K_B/(K_d - K_B)$

The cubic equation is solved introducing T and R as:

$T = (a^2 - 3b)/9$

$R = (2a^3 - 9ab + 27c)/54$

If $T^3 - R^2 \geq 0$, there are three solutions of the cubic equation, one of them is meaningful:

$$\Theta = \arccos\left(\frac{R}{\sqrt{T^3}}\right)$$

$$[ML]_1 = -2\sqrt{T}cos\left(\frac{\Theta}{3}\right) - \frac{a}{3}$$

$$[ML]_2 = -2\sqrt{T}cos\left(\frac{\Theta + 2\pi}{3}\right) - \frac{a}{3}$$

$$[ML]_3 = -2\sqrt{T}cos\left(\frac{\Theta + 4\pi}{3}\right) - \frac{a}{3}$$

With $R^2 - T^3 > 0$ there is only one solution:

$$[ML] = -sign(R)\left[\left(\sqrt[3]{\sqrt{R^2 - T^3} + |R|} + \frac{T}{\sqrt[3]{\sqrt{R^2 - T^3} + |R|}}\right)\right] - \frac{a}{3}$$

[ML] was substituted as fB into the expression for λ_{max}:

$$\lambda_{\max} = \frac{\frac{\lambda_L}{f_B Q} - \frac{\lambda_L}{Q} + \lambda_{ML}}{\left(1 + \frac{1}{f_B Q} - \frac{1}{Q}\right)}$$

Fitting was performed with the known concentration of SdKefQCTD, $[M]_0$, and DNGSH, $[L]_0$, ratio of quantum yields ,Q = 4, peak position of the free ligand, λ_L = 572.5 nm, peak position of bound ligand, λ_{ML} = 530 nm using Matlab2012a (Mathworks). The number of active sites on QCTD, n, and dissociation constant for the competitor, K_B, were optimized.

Chemical Synthesis

General experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 MHz, 100 MHz) or Bruker Avance III (500 MHz, 125 MHz). The chemical shift data for each signal are given as δ_H in units of parts per million (ppm) relative to tetramethylsilane (TMS) where δ (TMS) = 0.00 ppm. The multiplicity of each signal is indicated by: s (singlet); br s (broad singlet); d (doublet); t (triplet); q (quartet); dd (doublet of doublets); ddd (doublet of doublet of doublets); dt (doublet of triplets) or m (multiplet). The number of protons (n) for a given resonance signal is indicated by nH. Coupling constants (*J*) are quoted in Hz and are recorded to the nearest 0.1 Hz. Identical proton coupling constants (*J*) are averaged in each spectrum and reported to the nearest 0.1 Hz. The coupling constants are determined by analysis using Bruker TopSpin software. ^{1}H and ^{13}C spectra were assigned using 2D NMR experiments including COSY, HSQC and HMBC.

Mass spectra. Electrospray ionization spectra were obtained on Micromass LCT; Micromass LCT Premier; and Bruker MicroTOF spectrometers operating in positive in positive or negative mode from solutions of methanol or water.

Melting points were obtained on a Kofler hotstage microscope, and are uncorrected. The solvent(s) from which the sample was crystallized is given in parentheses.

Microanalyses were obtained from the Elemental Analysis Service, London Metropolitan University, London.

Optical rotations were measured using Perkin Elmer Model 241 and 341 polarimeters, in cells with a path length of 1 dm. The light source was maintained at 589 nm. The concentration (*c*) is expressed in g/100 mL (equivalent to g/0.1 dm^3). Specific rotations are denoted $[\alpha]_D^T$ and are given in implied units of 10^{-1} deg cm^2 g^{-1} (T = ambient temperature in ˚C).

Infrared Spectra were obtained either as: **a** thin film on sodium chloride discs or **b** as a thin film or paste on PTFE cards (as indicated). The spectra were recorded on Perkin Elmer GX FT-IR or Bruker Tensor 27 spectrometers. Absorption maxima are reported in wavenumbers (cm^{-1}).

Lyophilisation refers to the removal of water by using a Christ Alpha 2-4 LD-2 Freeze Drier attached to a rotary-vane oil pump.

Anhydrous dichloromethane was obtained by passing through a column of activated alumina, according to the Grubbs procedure (5).

Chemicals were purchased from Sigma Aldrich or Alfa Aesar and were used without further purification. The UV light source was provided by a Philips HB175 Facial Solarium (UVA, 365 nm, P = 4 × 15 W). Reverse phase column chromatography was carried out on Fluka Ltd silica gel 100 C18-reversed phase, under a positive pressure of compressed air. Analytical TLC analysis was performed using Merck 60 RP-18 $F_{254}S$ aluminum-supported thin layer chromatography sheets and visualized

using UV light (λ_{max} 254 nm) or an ethanolic solution of ninhydrin followed by thermal development.

In vacuo refers to the use of a rotary evaporator attached to a diaphragm pump. Petroleum ether refers to the fraction boiling between 40-60 °C.

N-Allyl-5-(dimethylamino)naphthalene-1-sulfonamide (**2**). To allylamine (21 mg, 28 µL, 0.37 mmol, 1 eq), and diisopropylethylamine (239 mg, 1.85 mmol, 5 eq) in CH_2Cl_2 (5 mL) was added a solution of dansyl chloride (100 mg, 0.37 mmol, 1 eq) in CH_2Cl_2 (3 mL). The reaction was allowed to stir overnight at RT. After 18 h the reaction was adjudged to be complete by TLC analysis, was concentrated *in vacuo* and purified by silica gel column chromatography (20:80 ethyl acetate: petroleum ether), furnishing *N-allyl-5-(dimethylamino)naphthalene-1-sulfonamide* **2** (109 mg, 100%) as a fluorescent yellow crystalline solid: R_f 0.15 (ethyl acetate/petroleum ether 20:80); m.p. 62-66 °C (CH_2Cl_2); ν_{max} (thin film)/cm^{-1}; 1644 (s), 1316 (m); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (d, *J* = 8.6 Hz, 1H,), 8.30 (d, *J* = 8.6 Hz, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 7.58 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.53 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.2 (d, *J* = 7.6 Hz, 1H), 5.69-5.57 (m, 1H), 5.09 (dt, *J* = 17.0, 1.2 Hz, 1H), 5.01 (dt, *J* = 10.2, 1.2 Hz, 1H), 4.75 (t, *J* = 6.2 Hz, 1H), 3.54 (ddd, *J* = 12.2, 6.2, 1.2 Hz, 2H), 2.90 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 151.9, 134.7, 133.0, 130.5, 129.8, 129.6, 129.6, 128.4, 123.2, 118.8, 117.5, 115.3, 45.8, 45.4; HRMS *m/z* (ES$^+$) [Found; (M+Na)$^+$ 313.0891 $C_{15}H_{18}N_2NaO_2S$ requires M$^+$, 313.0987]; *m/z* 289.10 ([M-H]$^-$, 100%); Anal. Calcd. for $C_{15}H_{18}N_2O_2S$: C, 62.0; H, 6.2; N, 9.6. Found: C, 62.0; H, 6.3; N, 9.6.

S-((5-(Dimethylamino)naphthalen-1-yl)sulfonylaminopropyl) glutathione (**3**). *N*-Allyl-5-(dimethylamino)naphthalene-1-sulfonamide (100 mg, 0.34 mmol, 1 eq), L-glutathione (420 mg, 1.36 mmol, 4 eq), TCEP·HCl (194 mg, 0.68 mmol, 2 eq) and 2,2-dimethoxyphenyl acetophenone (17 mg, 0.07 mmol, 0.2 eq) were stirred at RT in THF/H_2O (1:2, 3 mL) in the presence of light (365 nm, 4 × 15 W) for 5 h. After which time the reaction was extracted with CH_2Cl_2 (2 × 5 mL). The aqueous layer was lyophilized and the crude material purified by RP C-18 silica gel column chromatography (MeOH/H_2O 0:100, 50:50), furnishing *S-((5-(dimethylamino)naphthalen-1-yl)sulfonylaminopropyl) glutathione* **3** (88 mg, 40%) as a hygroscopic yellow solid: R_f 0.35 (MeOH:H_2O 50:50); $[\alpha]_D^{20}$ −19.2 (*c* 0.25, H_2O); ν_{max} (PTFE card)/cm^{-1}; 3057 (w), 1719 (m), 1647 (m), 1527 (m), 1153 (m); ^{1}H NMR (500 MHz, D_2O): δ 8.35 (d, *J* = 8.5 Hz, 1H), 8.15 (d, *J* = 8.5 Hz, 1H), 8.11 (d, *J* = 7.5 Hz, 1H), 7.88-7.55 (m, 2H), 7.26 (d, *J* = 7.5 Hz, 1H), 4.20 (dd, *J* = 8.5, 5.1 Hz, 1H), 3.67-3.52 (m, 3H), 2.85 (t, *J* = 6.8 Hz, 2H), 2.73 (s, 6H), 2.43 (dd, *J* = 13.9, 5.1 Hz, 1H), 2.39-2.28 (m, 3H), 2.07 (t, *J* = 6.8 Hz, 2H), 2.04-1.96 (m, 2H), 1.34 (qn, *J* = 6.8 Hz, 2H); ^{13}C NMR (125 MHz; D_2O): δ 176.0, 174.7, 173.9, 171.6, 151.3, 133.9, 130.1, 129.9, 128.9, 128.8, 128.7, 128.3, 123.9, 119.0, 115.9, 54.1, 52.8, 44.8, 43.2, 40.7, 32.5, 31.4, 28.2, 27.6, 26.2; HRMS *m/z* (ES$^-$) [Found; (M-H)$^-$ 596.1852 $C_{25}H_{34}N_5O_8S_2$ requires M$^-$, 596.1854]; *m/z* (ES$^-$) 596.2 ([M-H]$^-$, 100%); Anal. Calcd for $C_{25}H_{35}N_5O_8S_2$: C, 50.2; H, 5.9; N, 11.7. Found 50.1; H, 5.7; N, 11.7.

General procedure for the synthesis of GSX via 1,4-addition. L-GSH (100 or 200 mg, 0.325 or 0.65 mmol, 1 eq) and NaOH (13 or 26 mg, 0.325 or 0.65 mmol, 1 eq) were stirred in 50:50 MeOH:H_2O (2.5 or 5 mL), to this solution an enone was added (0.325 or 0.65 mmol, 1 eq) and the resulting solution stirred at RT (20 min-48 h). The

reaction solution was concentrated *in vacuo* and purified by RP C-18 silica gel column chromatography (MeOH:H_2O, 0:100, 20:80 50:50) and lyophilized. In some cases, multiple RP C-18 columns were required to isolate the product in sufficient purity for biological testing.

*S-N-Ethylsuccinimido glutathione (**ESG**).* After 2 columns *S-N*-ethylsuccinimido glutathione (630 mg, 90%, mixture of diastereomers) was isolated as a colorless foamy solid: R_f 0.72 (H_2O); $[\alpha]_D^{20}$ −12.3 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz; D_2O): δ 4.60-4.52 (m, 1H), 3.94 (dd, *J* = 9.3, 4.1 Hz, 0.5H), 3.93 (dd, *J* = 9.3, 4.1 Hz, 0.5H), 3.68-3.64 (m, 3H), 3.42 (q, *J* = 7.2 Hz, 2H), 3.27-2.86 (m, 3H), 2.59 (dd, *J* = 8.1, 4.2 Hz, 0.5H), 2.55 (dd, *J* = 8.1, 4.2 Hz, 0.5H), 2.40-2.45 (m, 2H), 2.01-2.08 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 1.5H), 1.00 (t, *J* = 7.2 Hz, 1.5H); *m/z* (ES^+) 455 ($[M+Na]^+$, 100%), Anal. Calcd. for $C_{16}H_{24}N_4O_8S$: C, 44.4; H, 5.6; N, 13.0. Found C, 44.3; H, 5.4; N, 12.8 (6).

*S-N-Methylsuccinimido glutathione (**4**).* RP C-18 silica gel column furnished *S-N*-methylsuccinimido glutathione (**4**) (mixture of diastereoisomers) as a hygroscopic colorless solid (132 mg, 97%); R_f 0.7 (H_2O); $[\alpha]_D^{20}$ −13.7 (*c* 1.0, H_2O); ν_{max} (solid)/cm^{-1}; 3255 (m), 1693 (s), 1588 (s); ^{1}H NMR (500 MHz, D_2O): δ 4.57 (dd, *J* = 8.0, 5.0 Hz, 0.5H), 4.54 (dd, *J* = 9.1, 5.0 Hz, 0.5H), 3.64-3.60 (m, 3H), 3.27-3.10 (m, 2H), 3.09 (dd, *J* = 14.4, 8.3 Hz, 0.5H), 2.89 (dd, *J* = 14.4, 9.2 Hz, 0.5H), 2.84 (s, 3H), 2.55 (dd, J = 9.8, 4.3 Hz 0.5H), 2.60 (dd, J = 9.8, 4.3 Hz, 0.5H), 2.45-2.38 (m, 2H), 2.08-2.03 (m, 2H); ^{13}C NMR (125 MHz, D_2O): δ Diastereomer 1; 179.7, 178.5, 176.2, 175.0, 173.9, 171.5, 54.1, 53.1, 43.4, 40.8, 36.1, 32.9, 31.4, 26.2, 25.0; Diastereomer 2; 179.6, 178.5, 176.2, 174.9, 173.9, 171.5, 54.1, 52.8, 43.4, 40.1, 35.9, 32.8, 31.4, 26.1, 25.0; HRMS *m/z* (ES^-) [Found; $(M-H)^-$ 417.1089 $C_{15}H_{21}N_4O_8S$ requires M^+, 417.1080] *m/z* (ES^-) 417 ($[M-H]^-$, 100 %); Anal. Calcd for $C_{15}H_{22}N_4O_8S$: C, 43.0; H, 5.3; N, 13.4. Found: C, 42.9; H, 5.4; N, 13.0.

*S-N-tert-Butylsuccinimido glutathione (**5**)*. RP C-18 silica gel column furnished *S-N-tert*-butylsuccinimido glutathione (**5**) (mixture of diastereoisomers) as a colorless hygroscopic solid (110 mg, 75%); R_f 0.7 (H_2O); $[\alpha]_D^{20}$ −4.0 (*c* 0.5, H_2O); ν_{max} (solid)/cm^{-1}; 3271 (m), 2980 (w), 1698 (s), 1645 (s), 1596 (m); ^{1}H NMR (400 MHz, D_2O): δ 4.59 (dd, *J* = 4.7, 3.2 Hz, 0.5H), 4.57 (dd, *J* = 4.7 3.2 Hz, 0.5H), 3.82 (dd, *J* = 9.2, 4.3 Hz, 0.5H), 3.78 (dd, *J* = 9.2, 4.3 Hz, 0.5H), 3.72-3.66 (m, 3H), 3.26 (dd, *J* = 9.1, 4.7 Hz, 0.5H), 3.25 (dd, *J* = 9.1, 4.7 Hz, 0.5H), 3.19-3.01 (m, 2H), 2.90 (dd, *J* = 14.1, 9.1 Hz, 0.5H), 2.52-2.39 (m, 2.5H), 2.11-2.03 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, D_2O): δ Diastereomer 1: 180.5, 179.4, 176.4, 175.2, 174.2, 171.8, 53.6, 53.1, 43.7, 40.3, 36.3, 32.9, 31.8, 27.7, 25.5; Diastereomer 2: 180.5, 179.4, 176.4, 175.3, 174.2, 171.9, 54.5, 53.1, 43.7, 41.2, 36.6, 33.2, 31.8, 27.7, 25.6; HRMS *m/z* (ES^-) [Found; $(M-H)^-$ 459.1557. $C_{18}H_{27}N_4O_8S$ requires M^-, 459.1550.]; *m/z* (ES^-) 459 ($[M-H]^-$, 100 %).

*S-N-Benzylsuccinimido glutathione (**6**).* RP C-18 silica gel column furnished *S-N*-benzylsuccinimido glutathione (**6**) (mixture of diastereoisomers) as a colorless hygroscopic solid (136 mg, 86%); R_f 0.7 (H_2O); $[\alpha]_D^{20}$ −5.4 (*c* 0.5, H_2O); ν_{max} (solid)/cm^{-1}; 3277 (m), 1698 (s), 1643 (s), 1595 (s); ^{1}H NMR (400 MHz, D_2O): δ 7.30-7.19 (m, 5H), 4.57 (s, 2H), 4.53 (dd, *J* = 8.6, 5.1 Hz, 1H), 3.98 (dd, *J* = 9.2, 3.9 Hz,

0.5H), 3.95 (dd, *J* = 9.2, 3.9 Hz, 0.5H), 3.72-3.58 (m, 3H), 3.27-3.14 (m, 1.5H), 3.09 (dd, *J* = 13.8, 5.4 Hz, 0.5H), 2.99 (dd, *J* = 13.8, 8.4 Hz, 0.5H), 2.85 (dd, *J* = 13.8, 8.4 Hz, 0.5H), 2.63 (dd, *J* = 11.3, 4.1 Hz, 0.5H), 2.58 (dd, *J* = 11.3, 4.1 Hz, 0.5H), 2.45-2.38 (m, 2H), 2.08-2.01 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ Diastereoisomer 1; 193.8, 179.4, 178.2, 176.5, 175.1, 174.3, 174.7, 135.5, 129.3, 128.5, 128.1, 128.0, 53.5, 52.9, 43.1, 42.8, 40.9, 36.1, 32.9, 31.7, 26.4; Diastereoisomer 2; 193.8, 179.4, 178.3, 176.5, 175.2, 174.3, 174.8, 135.5, 129.3, 128.5, 128.1, 128.0, 54.5, 52.9, 43.1, 42.8, 40.2, 36.1, 33.1, 31.8, 26.5; HRMS *m/z* (ES^-) [Found; $(M-H)^-$ 493.1398 $C_{21}H_{25}N_4O_8S$ requires M^-, 493.1393.]; *m/z* (ES^+) 517 ([M+Na]$^+$, 100 %); Anal. Calcd for $C_{21}H_{26}N_4O_8S.2H_2O$: C, 47.5; H, 5.7; N, 10.5. Found: C, 47.4; H, 5.7; N, 10.7.

*S-N-Cyclohexylsuccinimido glutathione (**7**)*. RP C-18 silica gel column furnished *S-N*-cyclohexylsuccinimido glutathione (**7**) (1:1 mixture of diastereoisomers) as colorless hygroscopic solid (116 mg, 74%); R_f 0.65 (H_2O); $[\alpha]_D^{20}$ −7.0 (*c* 0.5, H_2O); ν_{max} (solid)/cm^{-1}; 3271 (m), 2933 (w), 1693 (s), 1643 (s); ^{1}H NMR (400 MHz, D_2O): δ 4.55 (dd, *J* = 8.3, 5.2 Hz, 0.5H), 4.53 (dd, *J* = 9.0, 4.9 Hz, 0.5H,), 3.89-3.76 (m, 2H), 3.71-3.58 (m, 3H), 3.22 (dd, *J* = 14.3, 5.2 Hz, 0.5H), 3.16-3.06 (m, 1.5H), 3.02 (dd, *J* = 14.3, 8.3 Hz, 0.5H), 2.87 (dd, *J* = 14.3, 9.0 Hz, 0.5H), 2.56-2.47 (m, 1H), 2.45-2.39 (m, 2H), 2.10-2.02 (m, 2H), 1.86 (q, *J* = 12.3 Hz, 2H), 1.73-1.65 (m, 2H), 1.55-1.46 (m, 4H), 1.24-1.11 (m, 2H); ^{13}C NMR (100 MHz, D_2O): δ Diastereomer 1; 179.9, 178.9, 176.5, 175.3, 174.2, 171.9, 54.4, 53.5, 52.9, 43.7, 40.8, 36.3, 31.8, 28.5, 28.5, 26.5, 25.7, 25.1; Diastereomer 2: 179.9, 178.9, 176.5, 175.2, 174.2, 171.8, 54.4, 53.1, 52.9, 43.7, 40.0, 36.0, 31.9, 28.5, 28.5, 26.6, 25.7, 25.1; HRMS *m/z* (ES^+) [Found; $(M+Na)^+$ 509.1662. $C_{20}H_{30}N_4NaO_8S$ requires M^+, 509.1677.]; *m/z* (ES^-) 485 ([M-H]$^-$, 100 %).

*S-Cyclopentan-2,4-dion-1-yl glutathione (**8**)*. Three RP C-18 silica gel columns (H_2O, 20:80 MeOH:H_2O, 50:50 MeOH:H_2O), furnished *S-cyclopentan-2,4-dion-1-yl glutathione* **8** (mixture of diastereomers, 98 mg, 37%) as a hygroscopic yellow solid: R_f 0.69 (MeOH/H_2O 50:50); ν_{max} (PTFE card)/cm^{-1}; 2964 (w), 1718 (s); ^{1}H NMR (400 MHz; D_2O): δ 4.48 (dd, *J* = 8.0, 5.0 Hz, 1H), 4.45 (dd, *J* = 8.0, 4.6 Hz, 1H), 3.72-3.54 (m, 4H), 2.97-2.90 (m, 1H), 2.89-2.75 (m, 4H), 2.52-2.36 (m, 2H), 2.28 (dd, *J* = 10.4, 2.4 Hz, 1H), 2.25 (dd, *J* = 10.4, 2.4 Hz, 1H), 2.12-2.02 (m, 2H); ^{13}C NMR (100 MHz; D_2O): δ diastereomer 1: 206.1, 203.7, 176.6, 175.2, 174.4, 172.2, 54.4, 53.6, 49.2, 46.3, 43.7, 42.2, 31.6, 30.9, 25.5; δ diastereomer 2: 206.1, 203.8, 176.6, 175.2, 174.4, 172.2, 54.4, 53.7, 49.2, 46.6, 43.7, 42.4, 31.6, 31.1, 25.5; HRMS *m/z* (ES^+) [Found; $(M+Na)^+$ 426.0941 $C_{15}H_{21}N_3NaO_8S$ requires M^+, 426.0947]; *m/z* (ES^-) 402 ([M-H]$^-$, 100 %).

*S-Cyclopentan-2-on-1-yl glutathione (**9**).* Two RP C-18 silica gel columns (H_2O, 20:80 MeOH: H_2O, 50:50 MeOH:H_2O), furnished S-cyclopentan-2-on-1-yl glutathione **9** (mixture of diastereomers, 45 mg, 18%) as a colorless hygroscopic solid: R_f 0.66 (RP MeOH/H_2O 50:50); ^{1}H NMR (400 MHz, D_2O): δ 4.55-4.49 (m, 1H), 3.71-3.64 (m, 3H), 3.51 (t, *J* = 6.1 Hz, 1H), 3.06 (dd, *J* = 14.5, 5.1 Hz, 1H), 3.04 (dd, *J* = 14.5, 5.1 Hz, 1H), 2.90-2.79 (m, 1H), 2.48-2.17 (m, 4H), 1.92-1.79 (m, 1H), 2.06 (dd, *J* = 14.0, 7.1 Hz, 2H); *m/z* (ES^+) 412 ([M+Na]$^+$, 100%). The data are in good agreement with the literature values (7).

*S-Pentan-3-on-1-yl glutathione (**10**)*. RP C-18 silica gel column furnished S-pentan-3-on-1-yl glutathione (**10**) as a colorless hygroscopic solid (123 mg, 96%); R_f 0.6 (MeOH/H_2O 50:50); $[\alpha]_D^{20}$ −25.2 (*c* 0.5 in H_2O); ^{1}H NMR (500 MHz, D_2O): δ 4.49 (dd, J = 9.0, 4.9 Hz, 1H), 373-3.63 (m, Gly-CH_2, 3H), 3.00 (J = 14.1, 4.9 Hz, 1H), 2.82-2.66 (m, 4H), 2.51-2.39 (m, 4H), 2.09-2.03 (m, 2H), 0.92 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, D_2O): δ 216.5, 176.1, 175.0, 174.2, 171.9, 54.1, 53.0, 43.3, 41.5, 35.9, 33.1, 31.4, 26.3, 25.4, 7.0; HRMS *m/z* (ES^+) [Found; $(M+Na)^+$ 414.1295. $C_{15}H_{25}N_3O_7SNa$ requires M^+, 414.1305.]; *m/z* $(ES)^-$ 390 ($[M-H]^-$, 100 %).

*S-Hexan-3-on-1-yl glutathione (**11**)*. RP C-18 silica gel column furnished S-hexan-3-on-1-yl glutathione (**11**) as hygroscopic colorless solid (120 mg, 93%); R_f 0.54 (MeOH/H_2O 50:50); $[\alpha]_D^{20}$ −23.0 (*c* 0.5 in H_2O); ^{1}H NMR (400 MHz, D_2O): δ 4.45 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.66 (d, *J* = 17.2 Hz, 1H), 3.65-3.62 (m, 1H), 3.61 (d, *J* = 17.2 Hz, 2H), 2.95 (dd, *J* = 14.2, 5.0 Hz, 1H,), 2.79-2.61 (m, 5H), 2.44-2.35 (m, 4H), 2.08-1.99 (m, 2H), 1.43 (sx, *J* = 7.4 Hz, 2H), 0.75 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, D_2O): δ 216.2, 176.2, 174.9, 174.0, 171.9, 54.1, 53.2, 44.6, 43.3, 41.9, 33.1, 31.4, 26.2, 25.4, 16.9, 12.9; HRMS *m/z* (ES^-) [Found; $(M-H)^-$ 404.1491 $C_{16}H_{27}N_3O_7S$ requires M^-, 404.1491]; *m/z* (ES^-) 404 ($[M-H]^-$, 100 %).

*S-Octan-3-on-1-yl glutathione (**12**).* RP C-18 silica gel column furnished *S-octan-3-on-1-yl glutathione* **12** (151 mg, 54%) as a hygroscopic yellow solid: R_f 0.13 (MeOH/H_2O 50:50); $[\alpha]_D^{20}$ −26.4 (*c* 0.25 in H_2O); ν_{max} (PTFE card)/cm^{-1}; 2961 (w), 1774 (w), 1735 (w); ^{1}H NMR (400 MHz, D_2O): δ 4.45 (dd, *J* = 9.0, 5.0 Hz, 1H), 3.66-3.61 (m, 3H), 2.95 (dd, *J* = 14.4, 5.0 Hz, 1H), 2.78-2.61 (m, 5H), 2.43-2.37 (m, 4H), 2.03 (qn, *J* = 6.8 Hz, 2H), 1.42 (qn *J* = 7.3 Hz, 2H), 1.20-1.07 (m, 4H), 0.73 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, D_2O): δ 216.8, 176.5, 175.2, 174.2, 172.2, 54.4, 53.4, 43.6, 42.8, 42.2, 33.5, 31.7, 30.9, 26.6, 25.8, 23.4, 22.1, 13.5; HRMS *m/z* (ES^+) [Found; $(M+H)^+$ 434.1954 $C_{18}H_{32}N_3O_7S$ requires M^+, 434.1961]; *m/z* (ES^+) 456.1 ($[M+Na]^+$, 100 %); Anal. Calcd for $C_{18}H_{31}N_3O_7S$: C, 49.9; H, 7.2; N, 9.7. Found: C, 49.8; H, 7.2; N, 9.6.

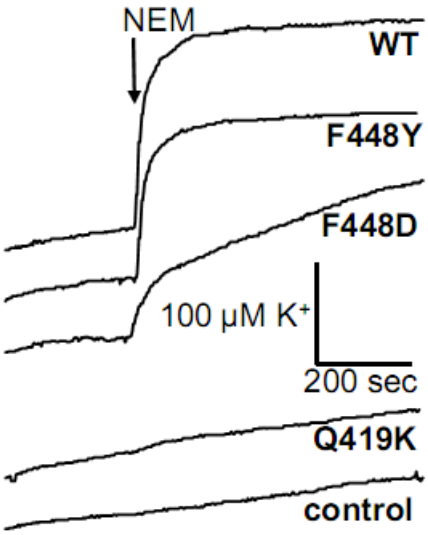


Figure S1: Potassium efflux experiments for SdKef mutants. Potassium efflux from *E. coli* strain MJF335 transformed with SdKef WT and the mutants F448Y, F448D, and Q419K after treatment with 0.5 mM NEM. Cells were grown in the presence of GSH. A control of the strain in the absence of Kef-plasmids is shown which was exposed to the same concentrations of electrophiles.

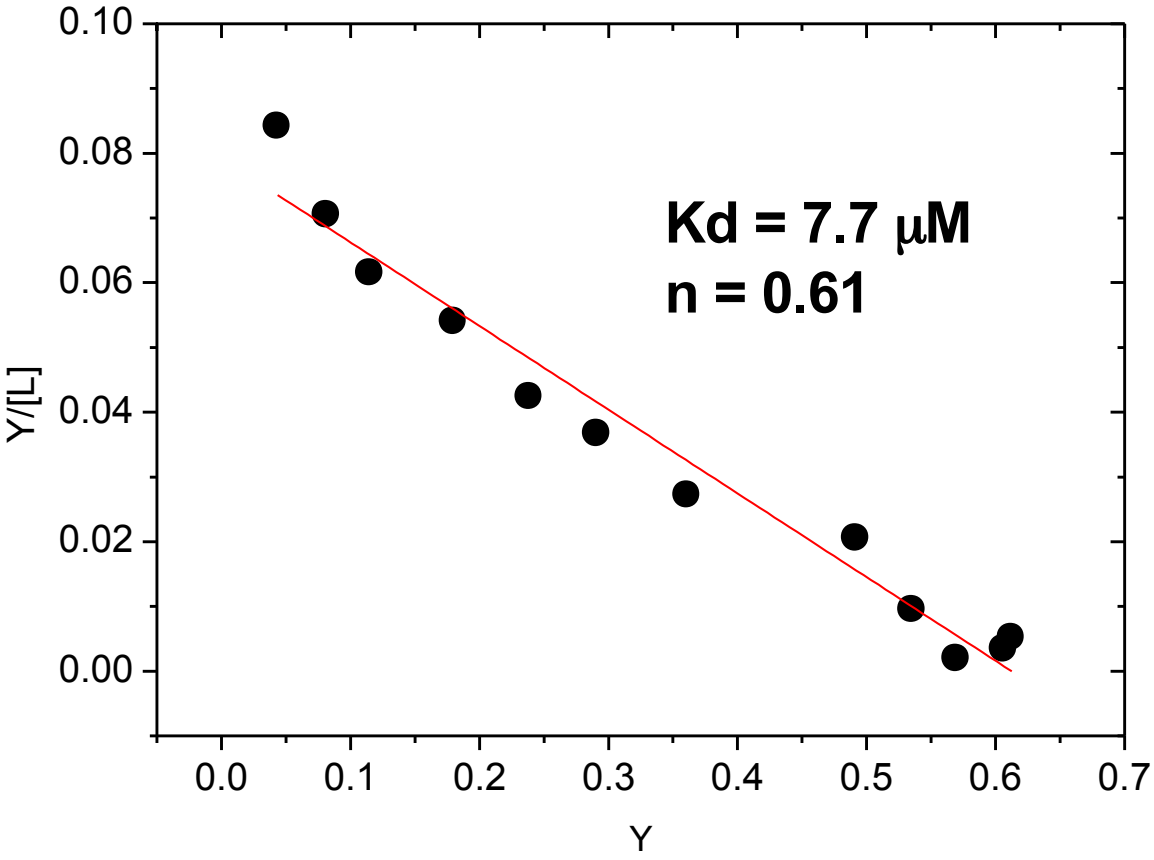


Figure S2: Scatchard plot for DNGSH binding. Shown is an example for DNGSH binding to SdKefQCTD. Linear fitting provide estimates for the dissociation constant, Kd, and binding stocheometry, n.

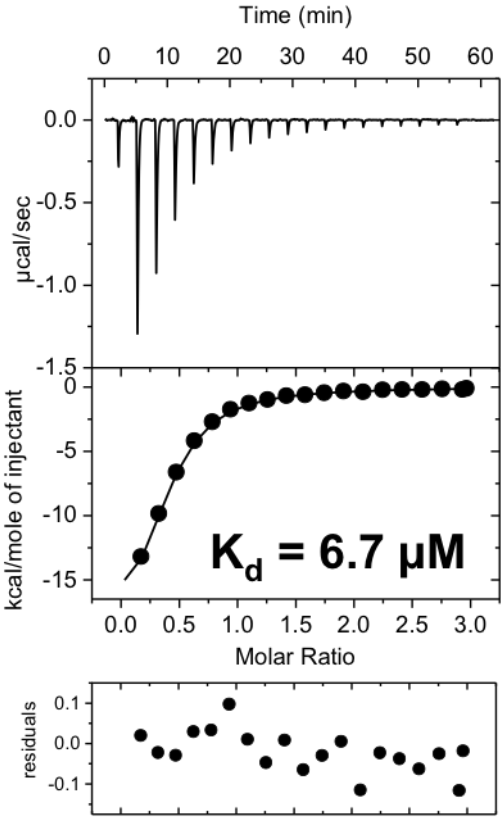


Figure S3: ITC binding experiments for the GSH adduct of *t*-butylmaleimide (5) with SdKefQCTD. Titration of **5** is shown with an SdKefQCTD concentration of 49 µM. Residuals are shown for a single-site binding model. A representative curve from a single experiment is shown.

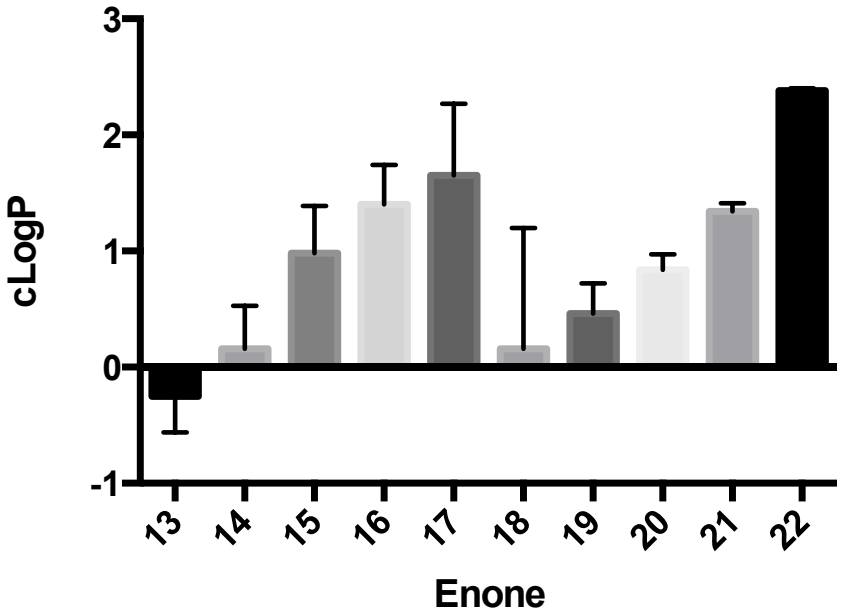


Figure S4: Bar graph representing cLogP of the enones employed in this study. LogP values were calculated using Virtual Computational Chemistry Laboratory (8).

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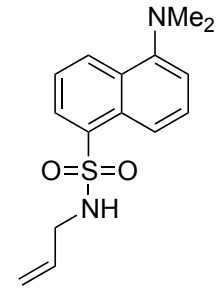
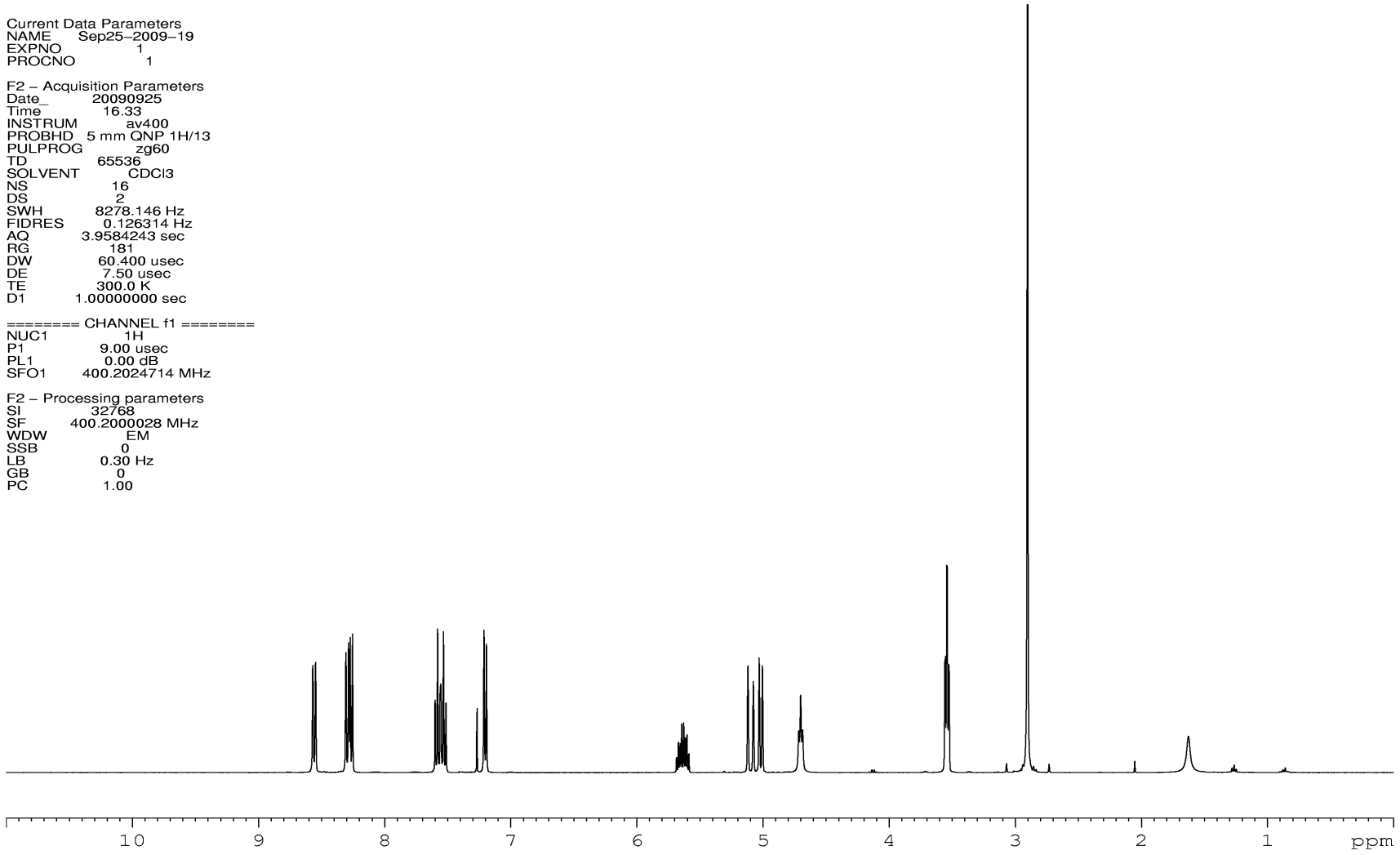
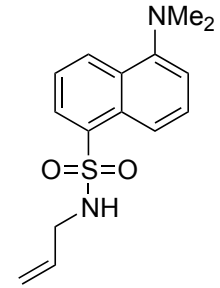


N-Allyl-5-(dimethylamino)naphthalene-1-sulfonamide (**2**)



N-Allyl-5-(dimethylamino)naphthalene-1-sulfonamide (**2**)



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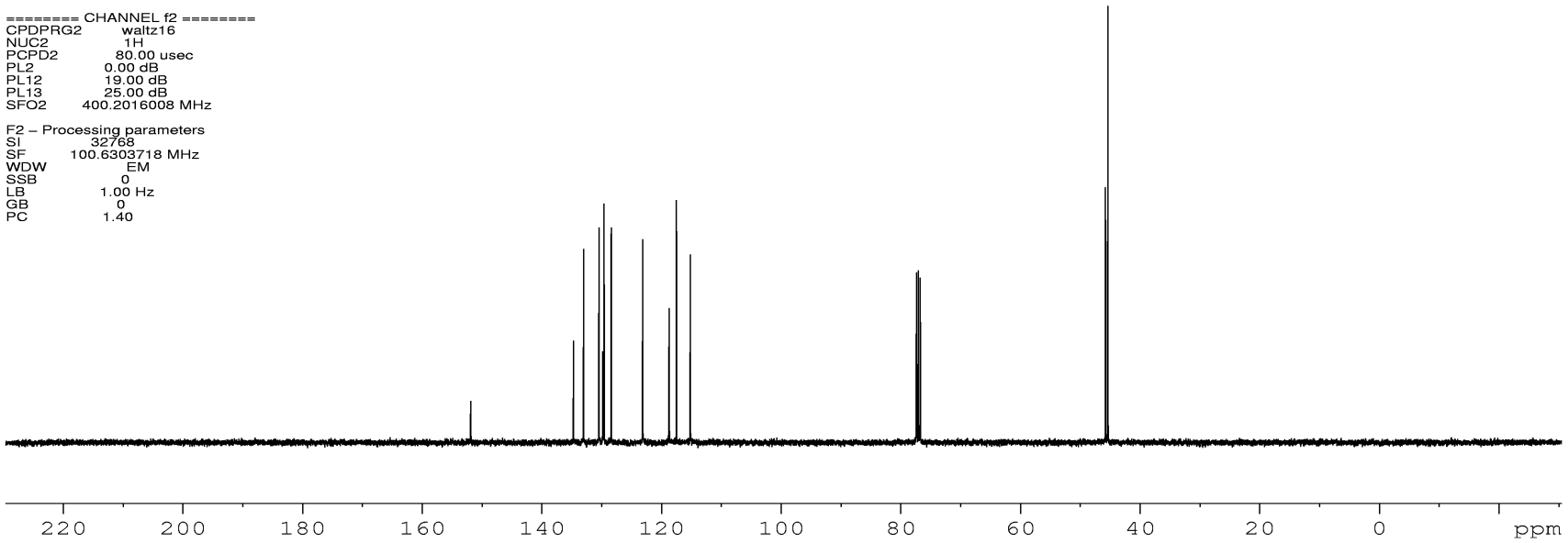

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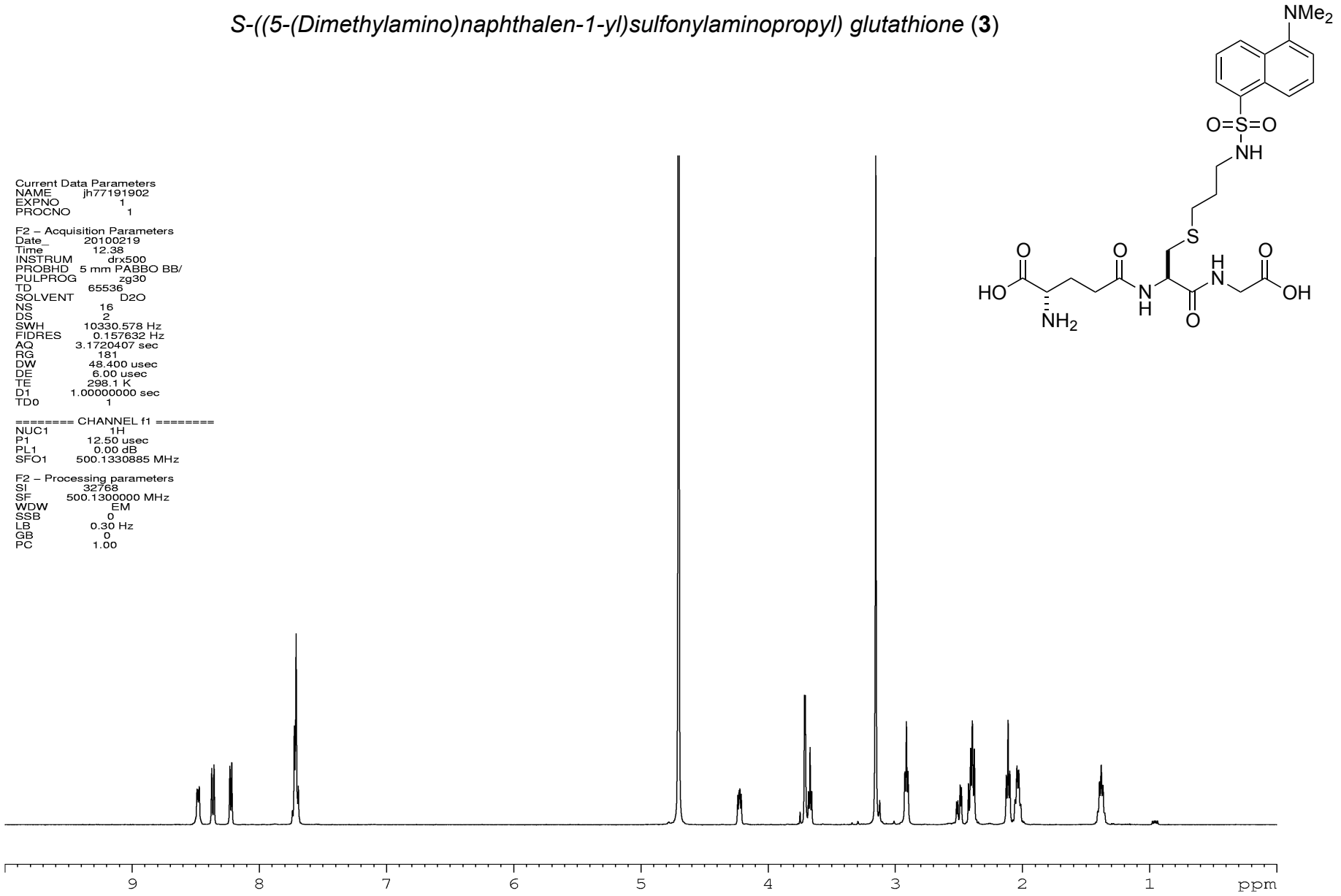
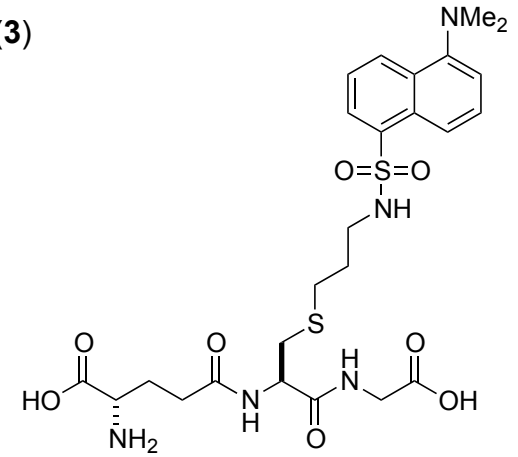
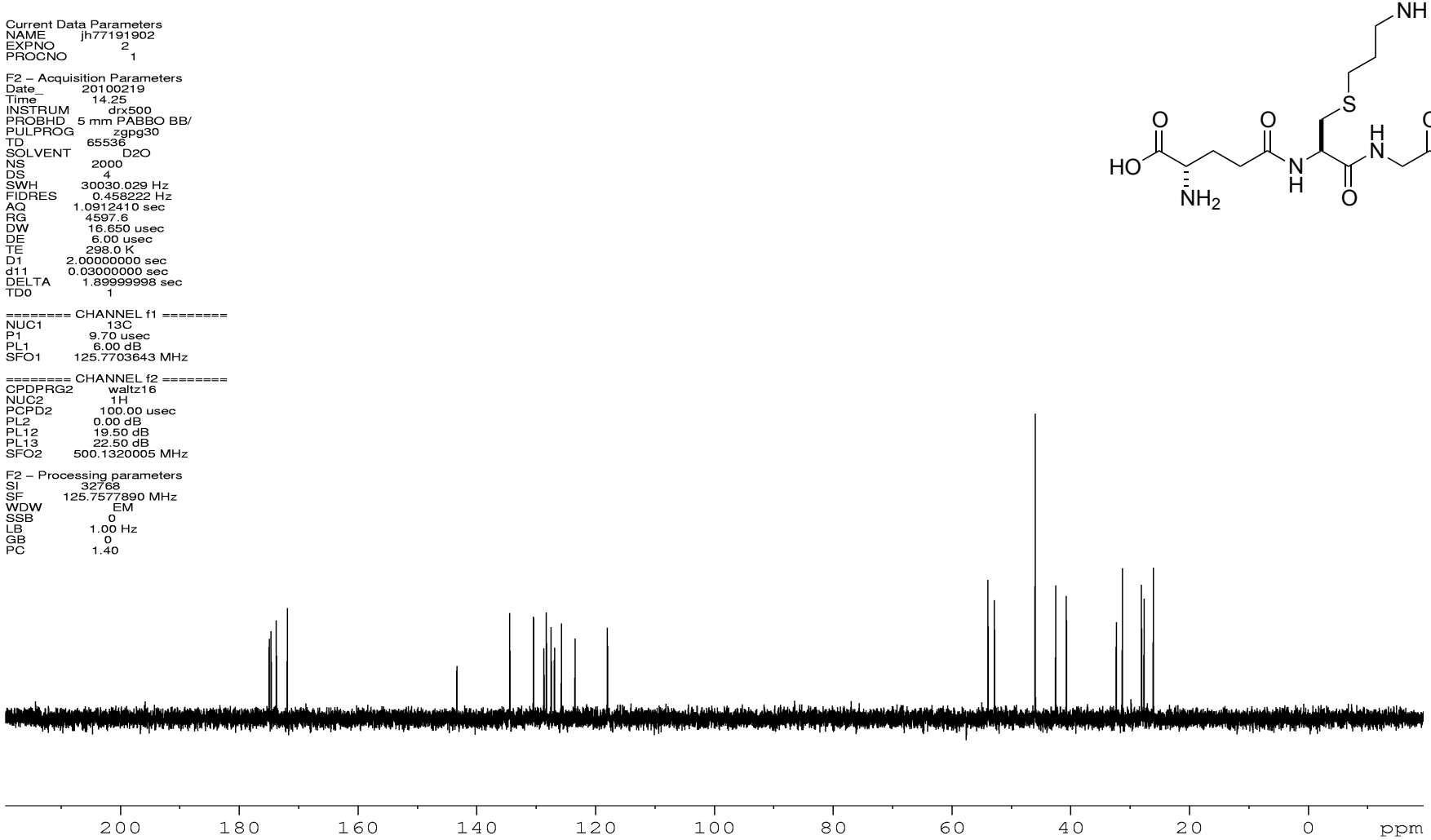


S-((5-(Dimethylamino)naphthalen-1-yl)sulfonylaminopropyl) glutathione (**3**)





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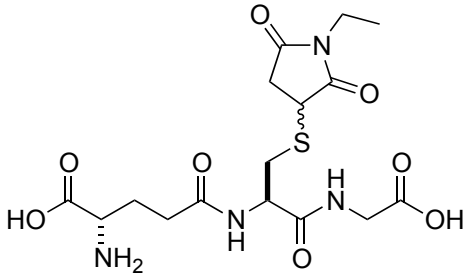


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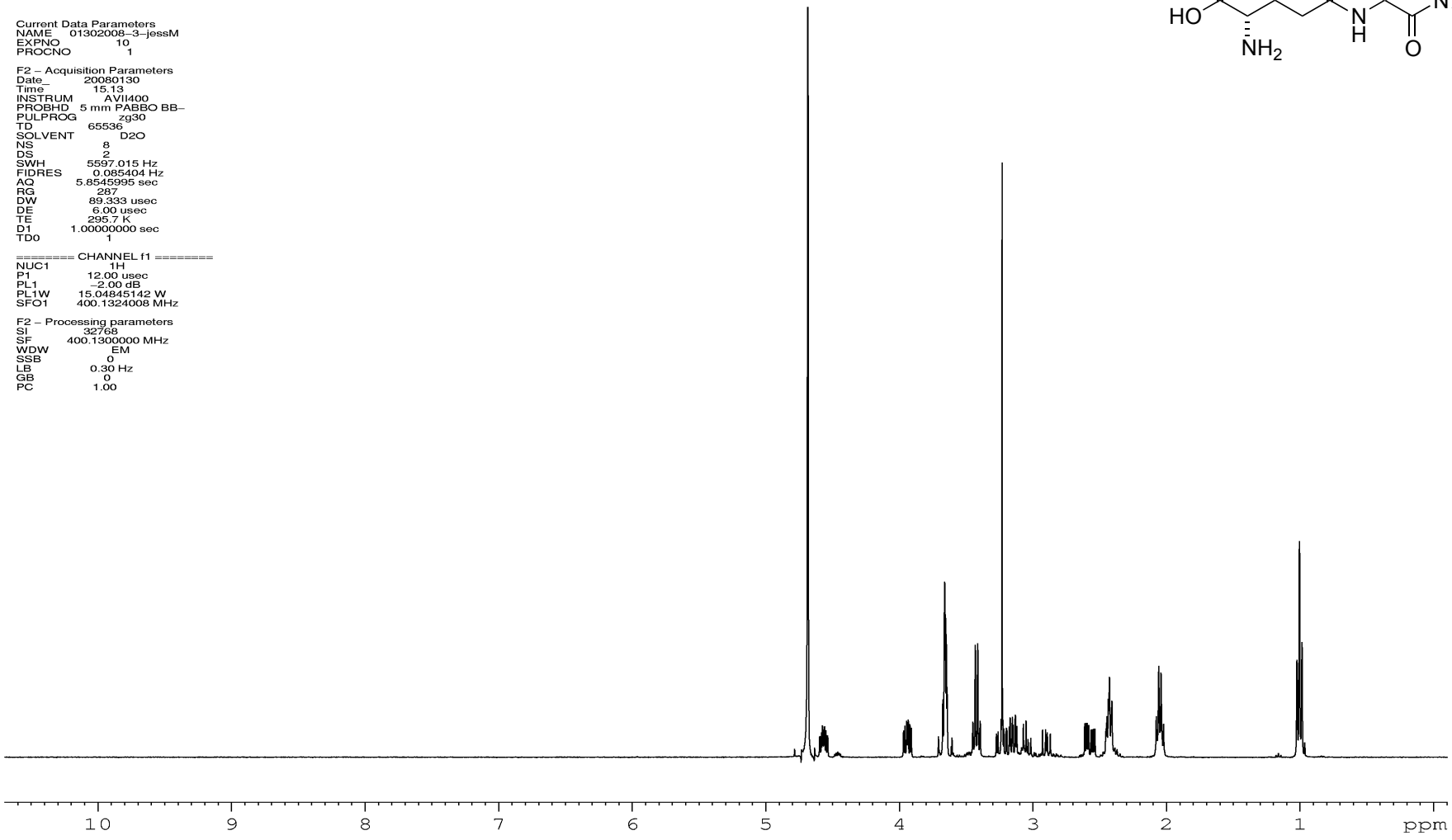

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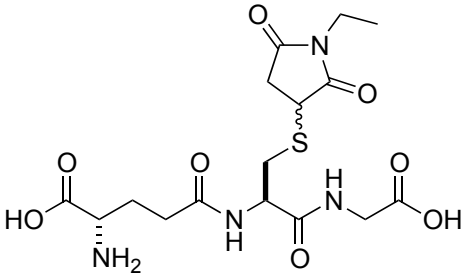

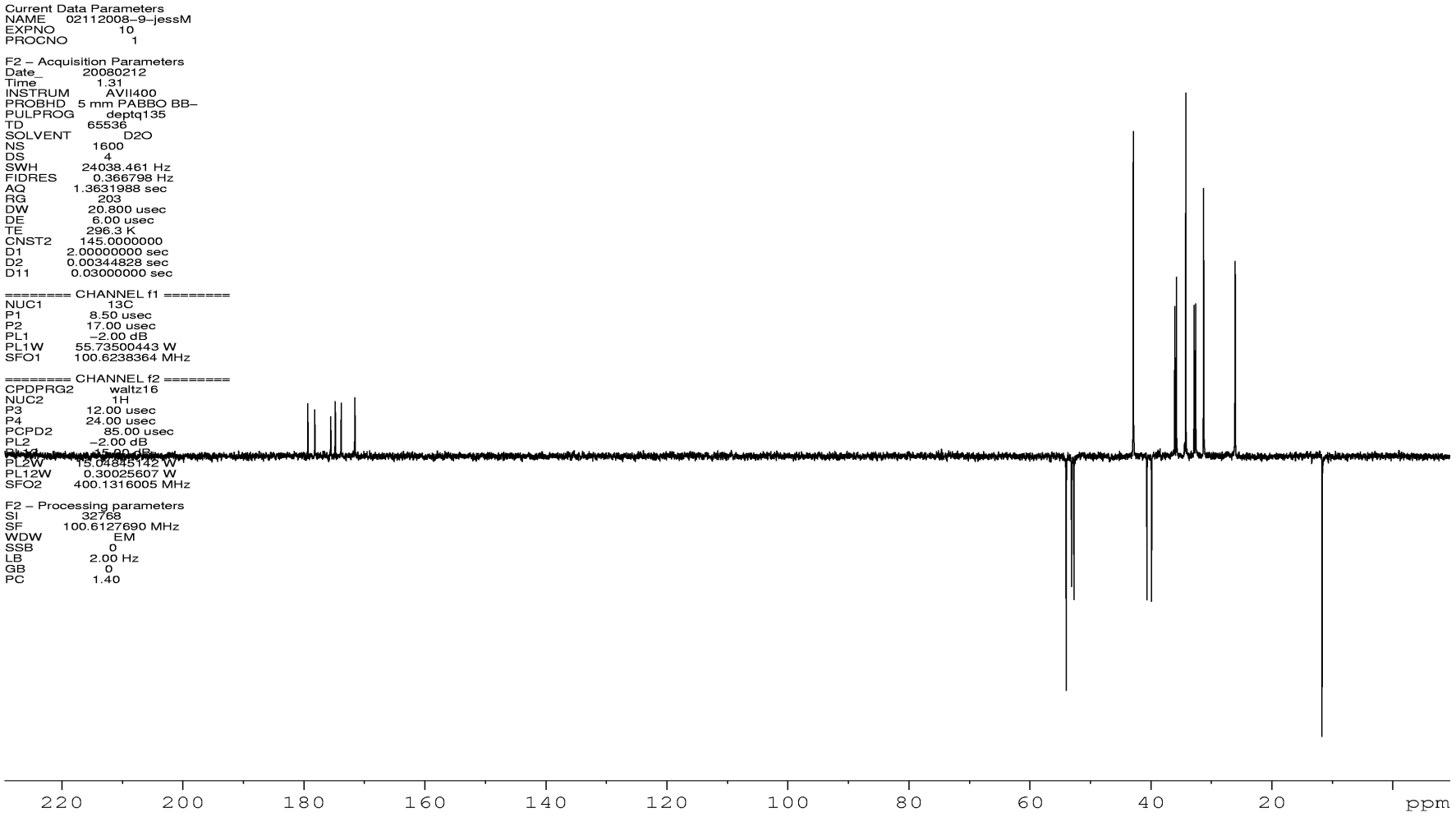

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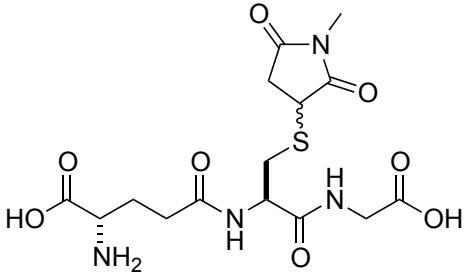
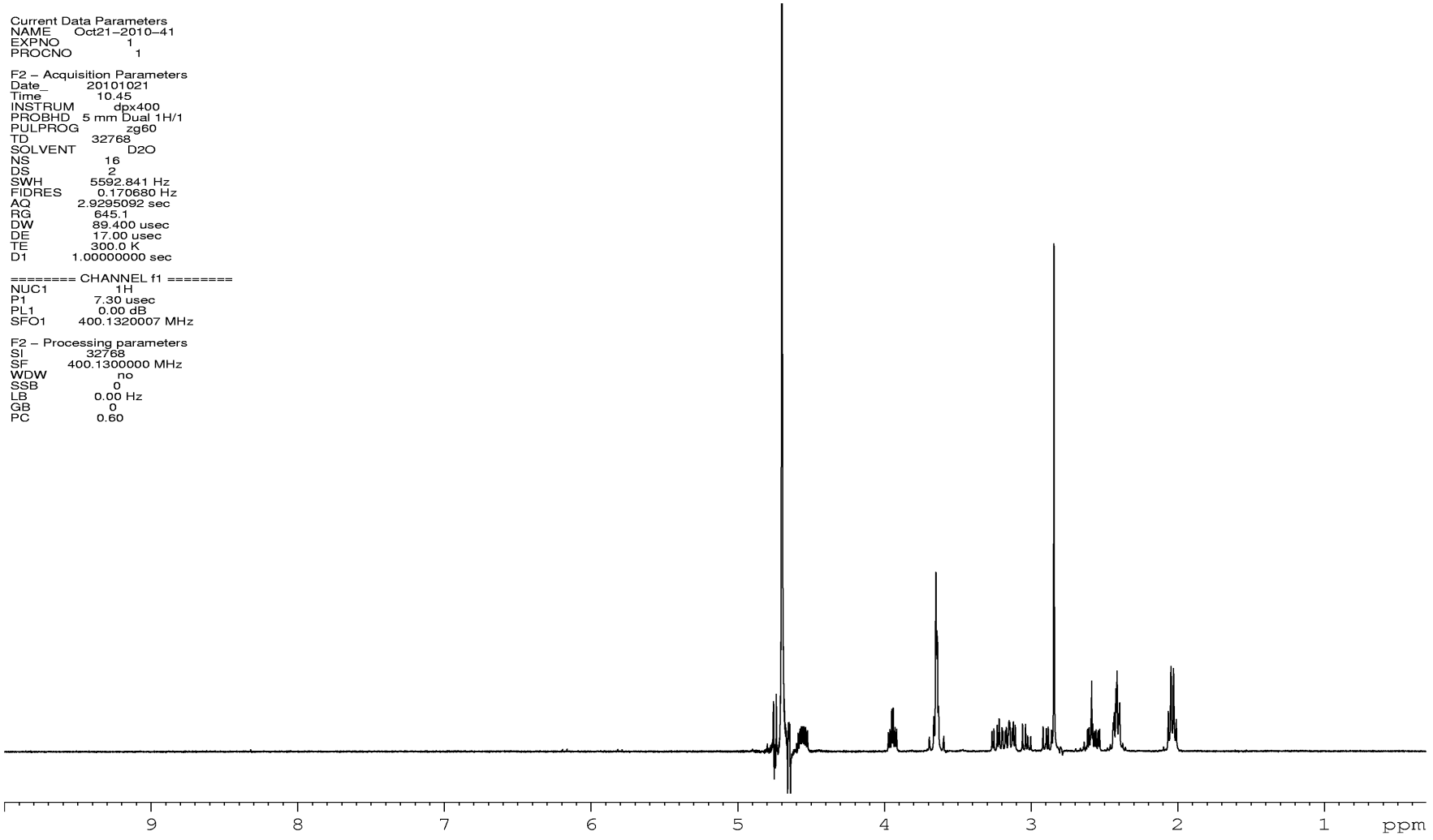
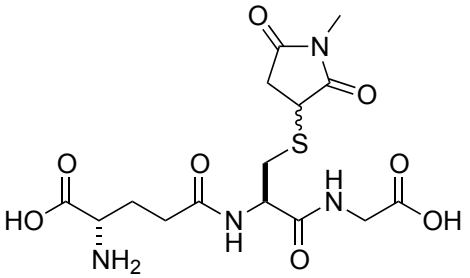




*S-N-Methylsuccinimido glutathione (**4**)*



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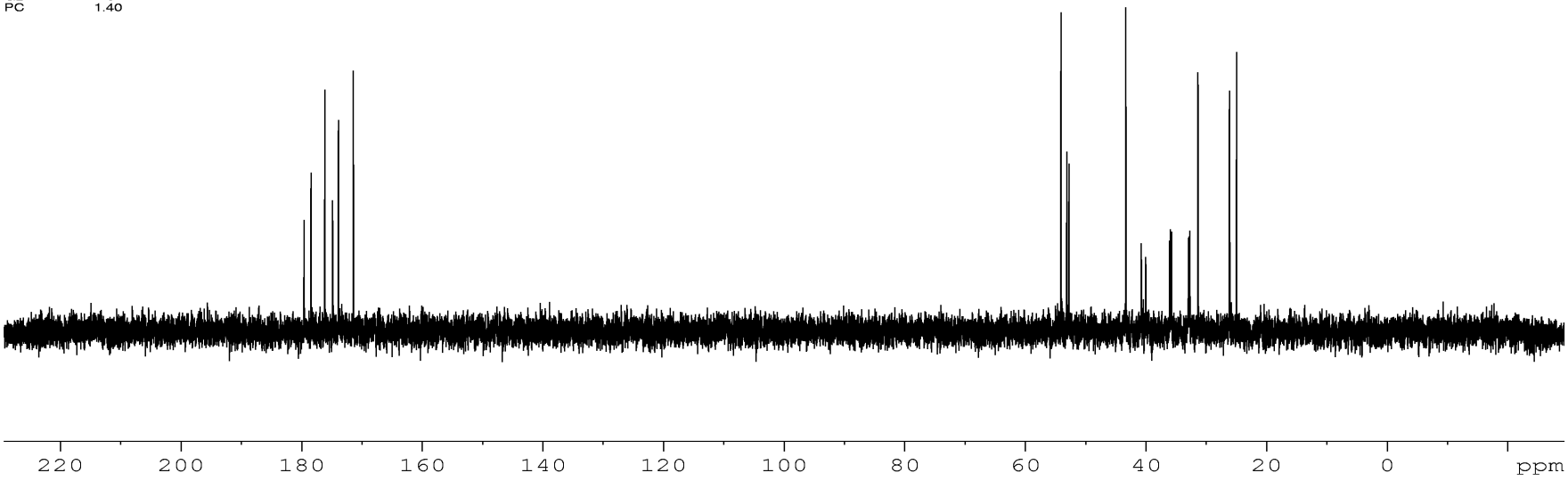
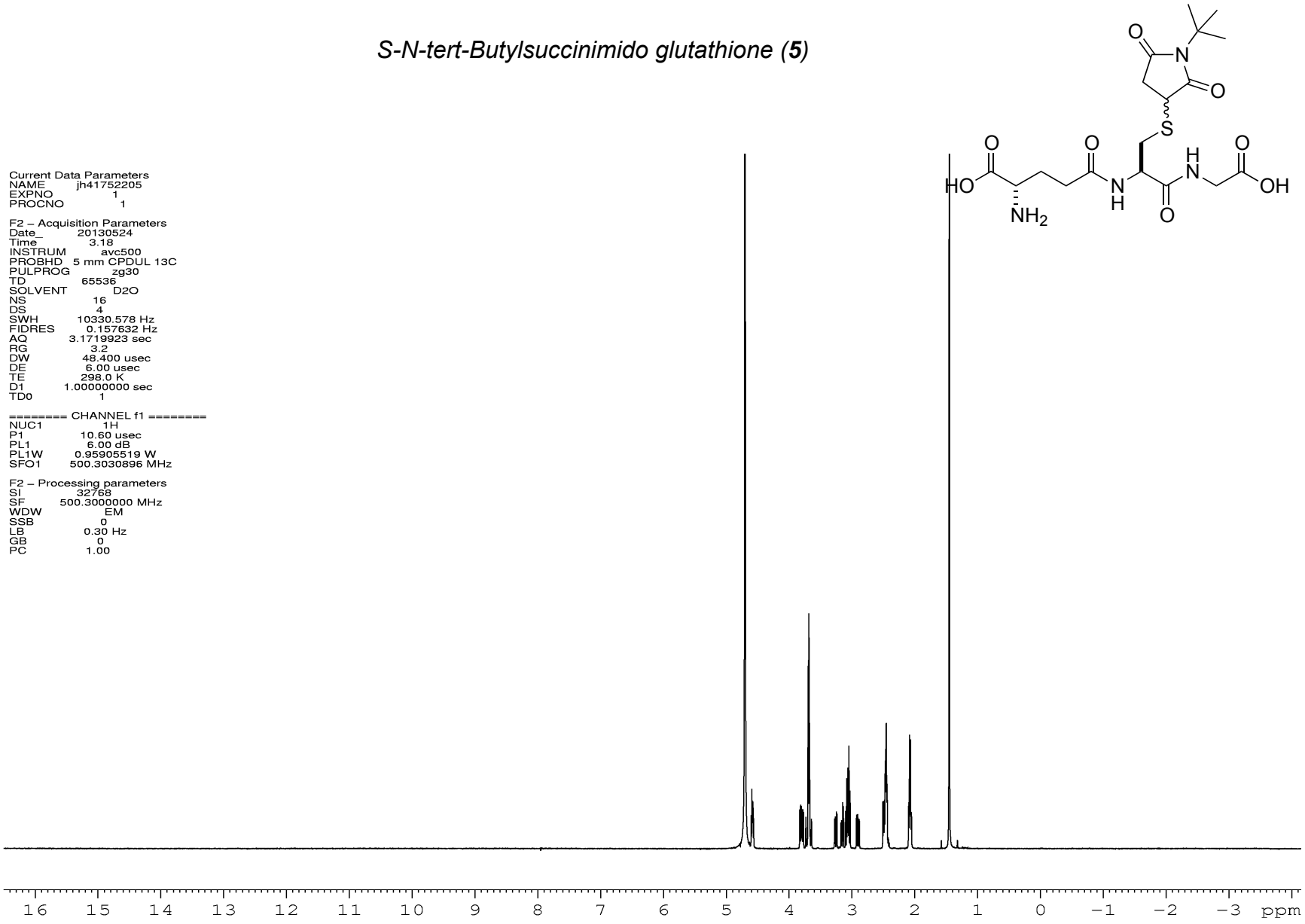
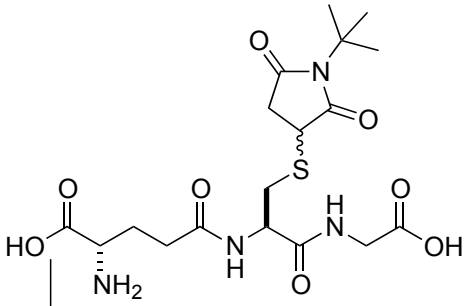


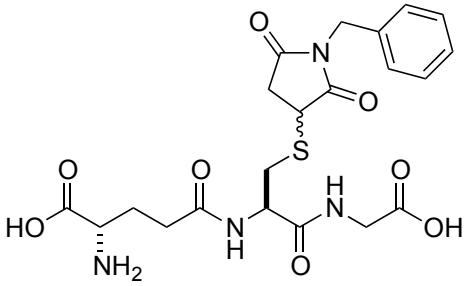
*S-N-tert-Butylsuccinimido glutathione (**5**)*

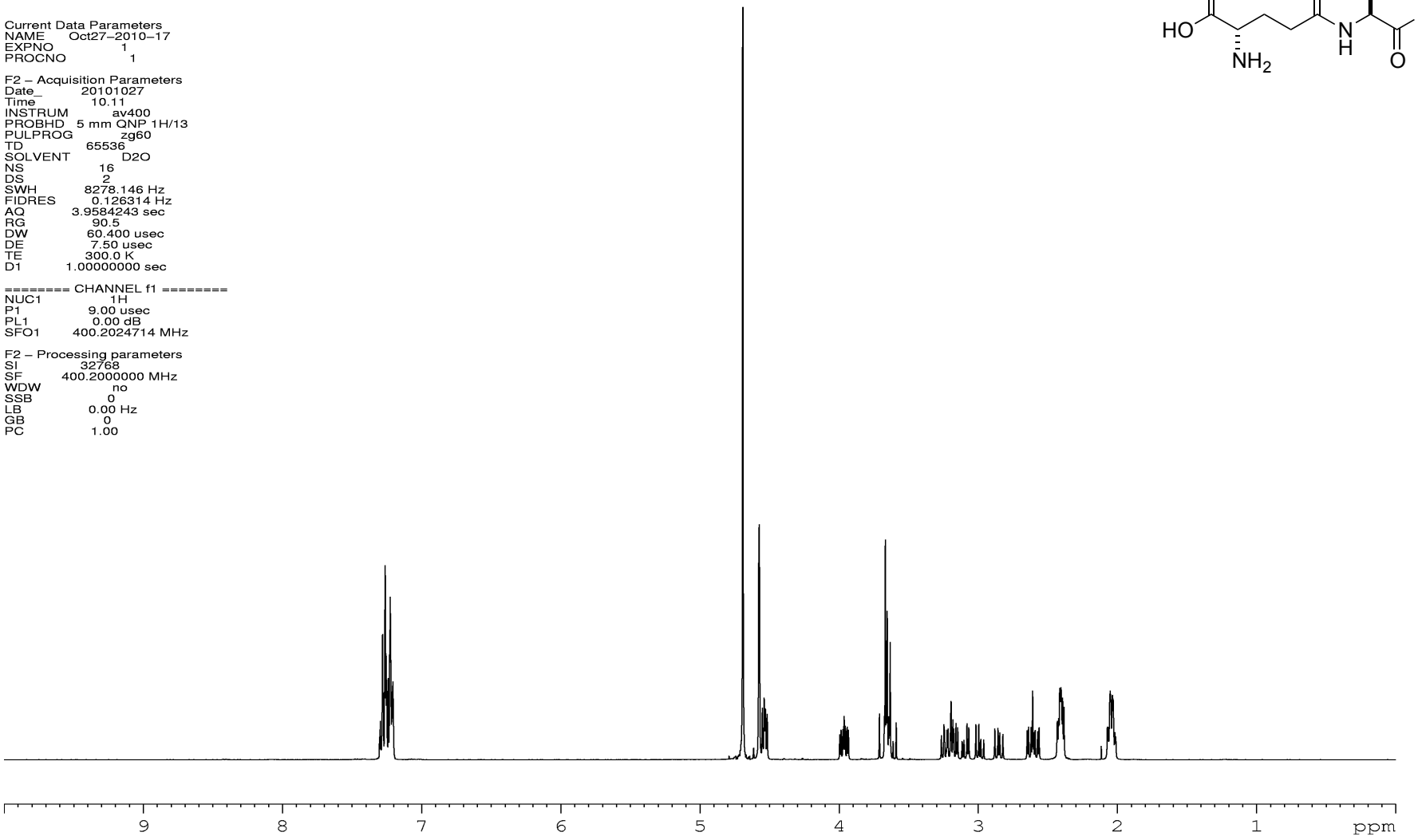


*S-N-tert-Butylsuccinimido glutathione (**5**)*

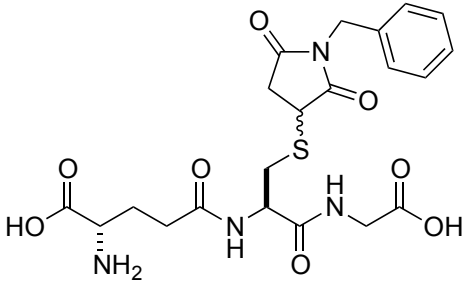


*S-N-Benzylsuccinimido glutathione (**6**)*





*S-N-Benzylsuccinimido glutathione (**6**)*



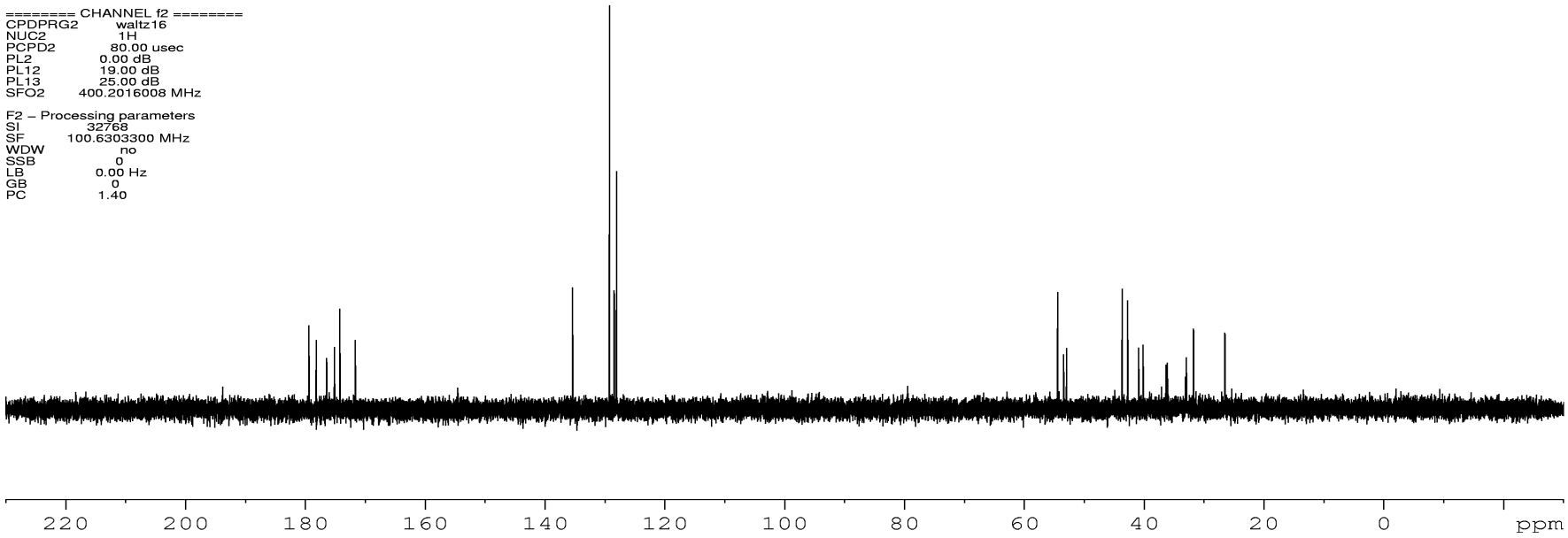
Current Data Parameters
NAME Oct27–2010–17
EXPNO 2
PROCNO 1

F2 – Acquisition Parameters
Date_ 20101027
Time 10.19
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 256
DS 4
SWH 26178.010 Hz
FIDRES 0.798889 Hz
AQ 0.6259188 sec
RG 32768
DW 19.100 usec
DE 7.50 usec
TE 300.0 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1

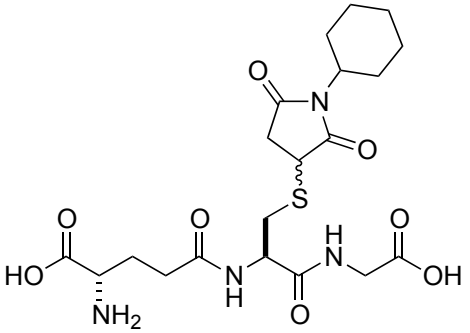
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 0.00 dB
SFO1 100.6403931 MHz

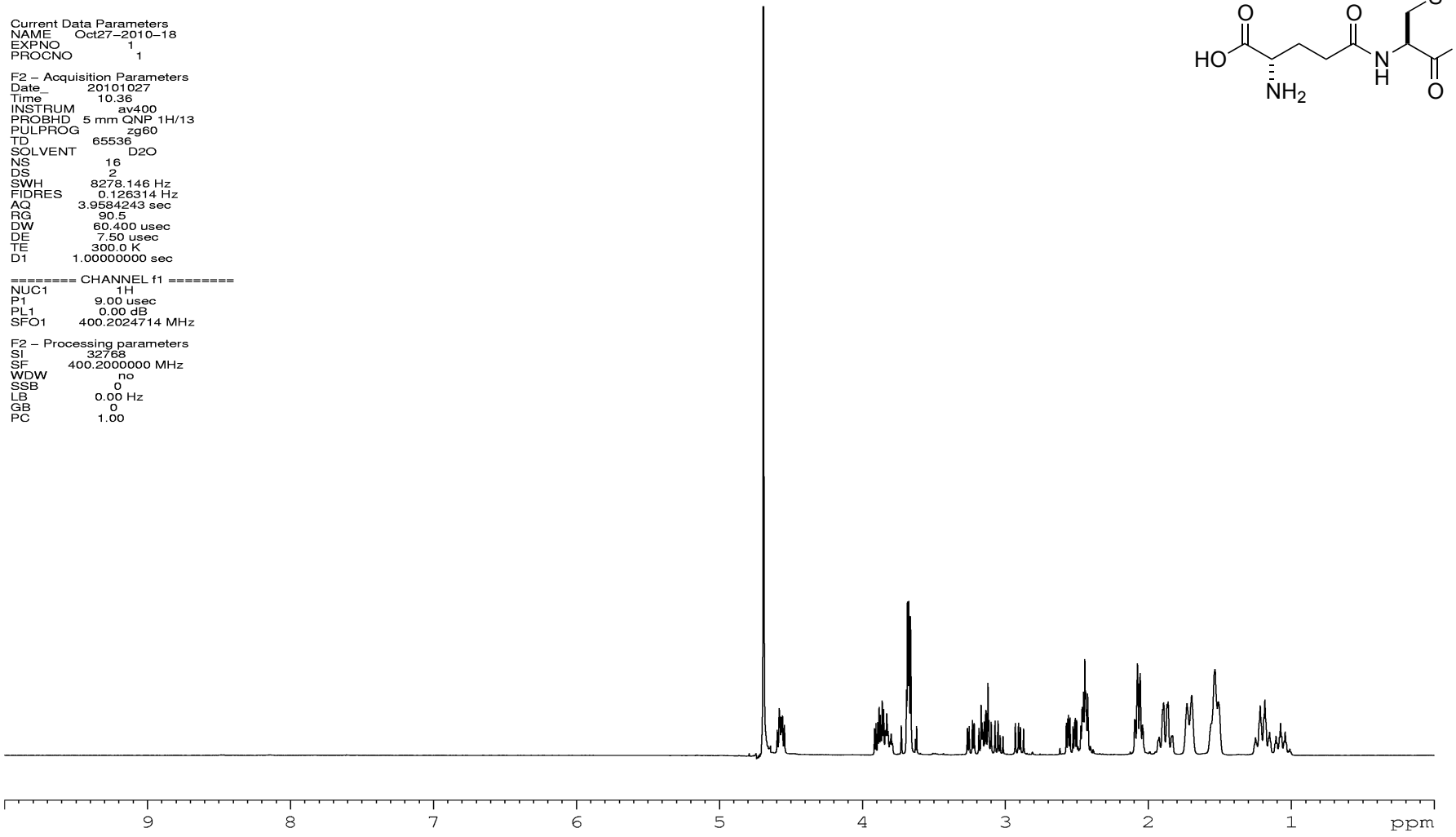
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 19.00 dB
PL13 25.00 dB
SFO2 400.2016008 MHz

F2 – Processing parameters
SI 32768
SF 100.6303300 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40

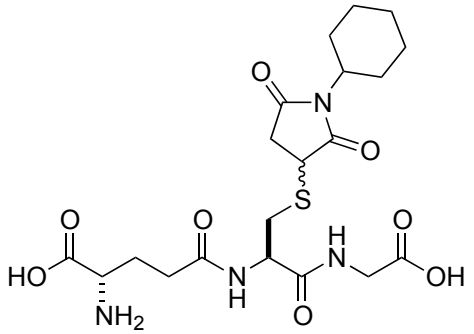


*S-N-Cyclohexylsuccinimido glutathione (**7**)*





*S-N-Cyclohexylsuccinimido glutathione (**7**)*



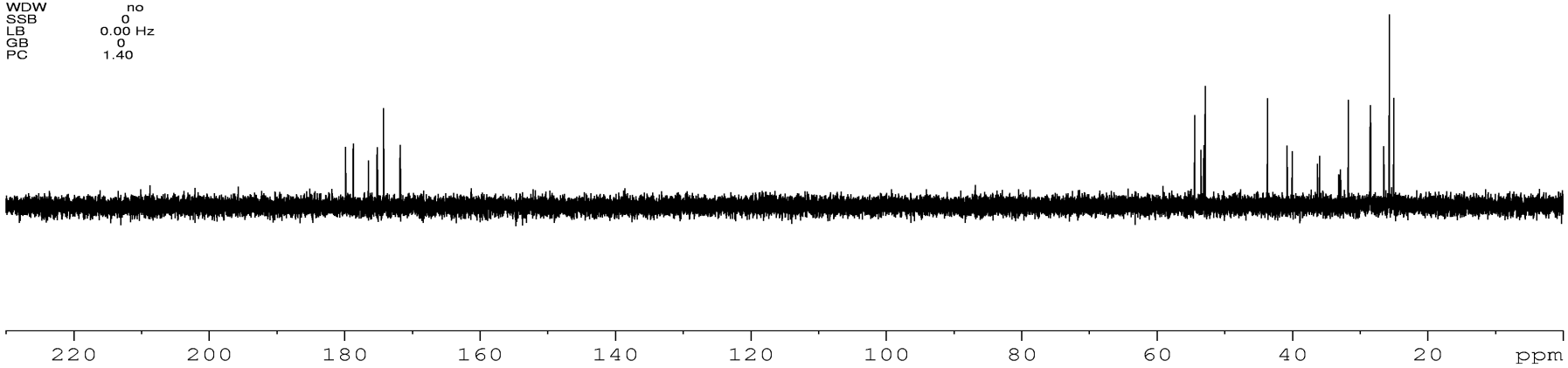
Current Data Parameters
NAME Oct27–2010–18
EXPNO 2
PROCNO 1

F2 – Acquisition Parameters
Date_ 20101027
Time 10.44
INSTRUM av400
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 32768
SOLVENT D2O
NS 256
DS 4
SWH 26178.010 Hz
FIDRES 0.798889 Hz
AQ 0.6259188 sec
RG 32768
DW 19.100 usec
DE 7.50 usec
TE 300.0 K
D1 1.00000000 sec
D11 0.03000000 sec
TD0 1

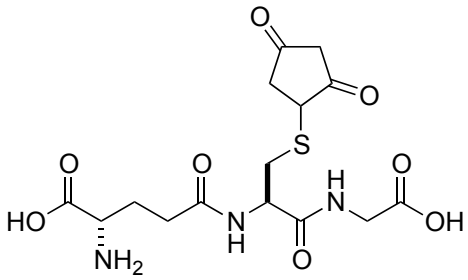
======== CHANNEL f1 ========
NUC1 13C
P1 9.50 usec
PL1 0.00 dB
SFO1 100.6403931 MHz

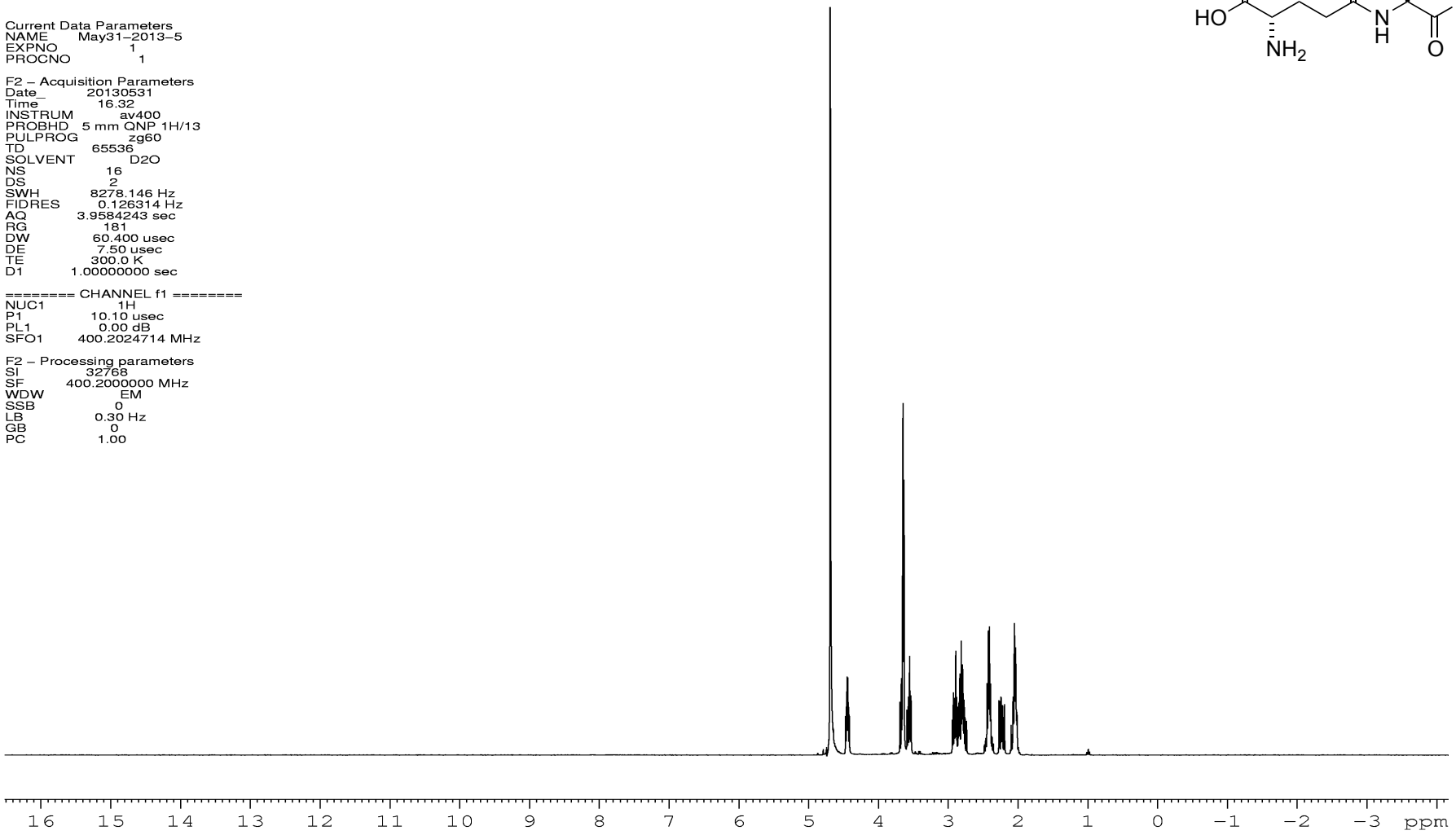
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 0.00 dB
PL12 19.00 dB
PL13 25.00 dB
SFO2 400.2016008 MHz

F2 – Processing parameters
SI 32768
SF 100.6303300 MHz
WDW no
SSB 0
LB 0.00 Hz
GB 0
PC 1.40

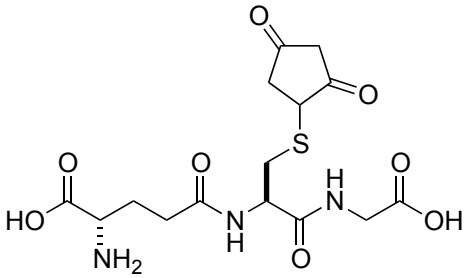


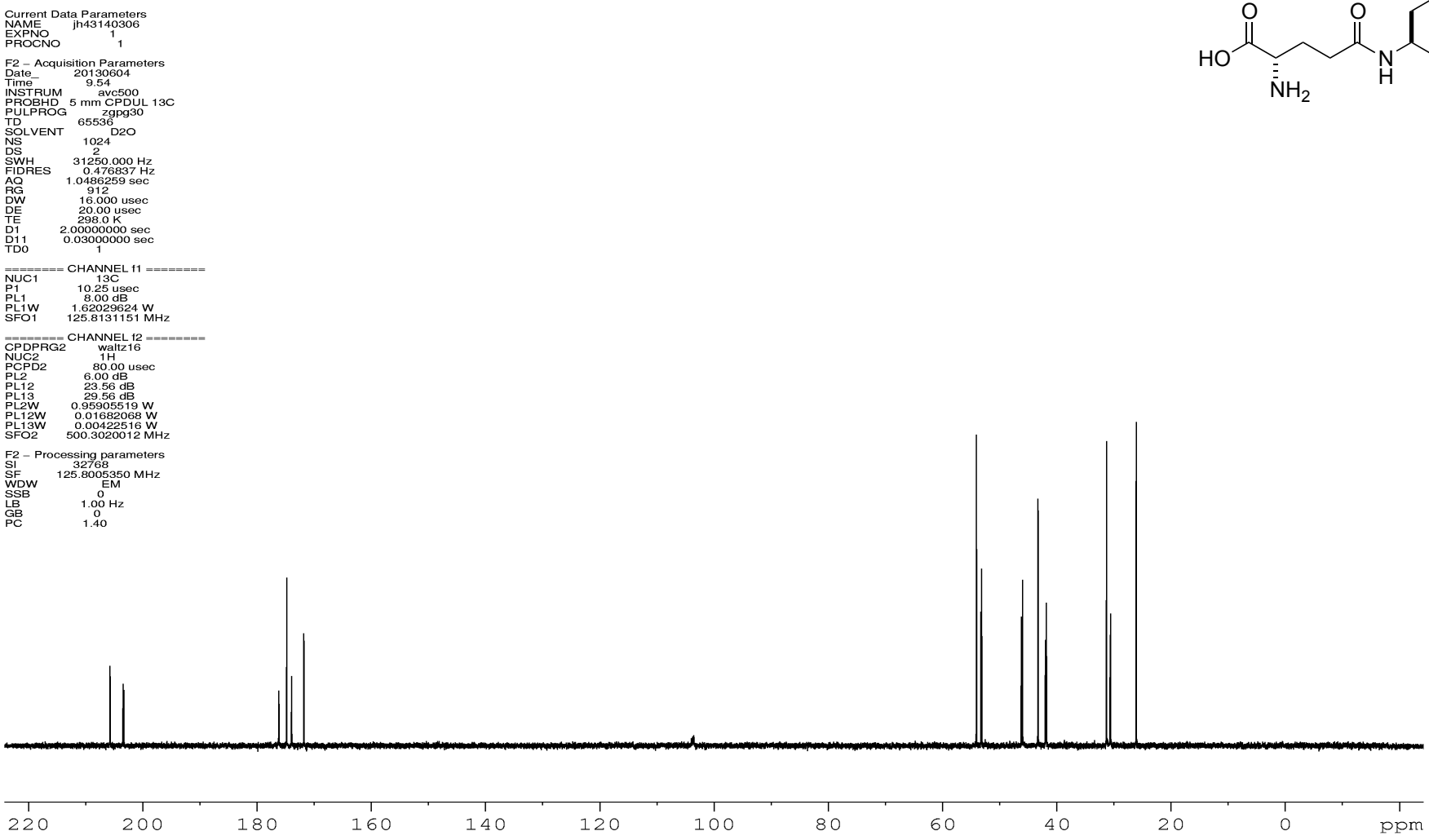
*S-Cyclopentan-2,4-dion-1-yl glutathione (**8**)*



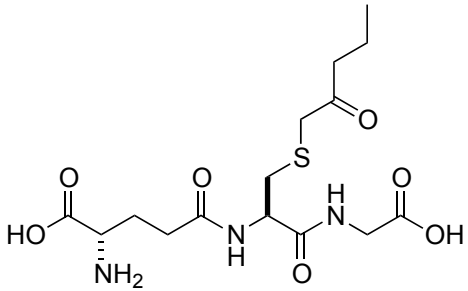


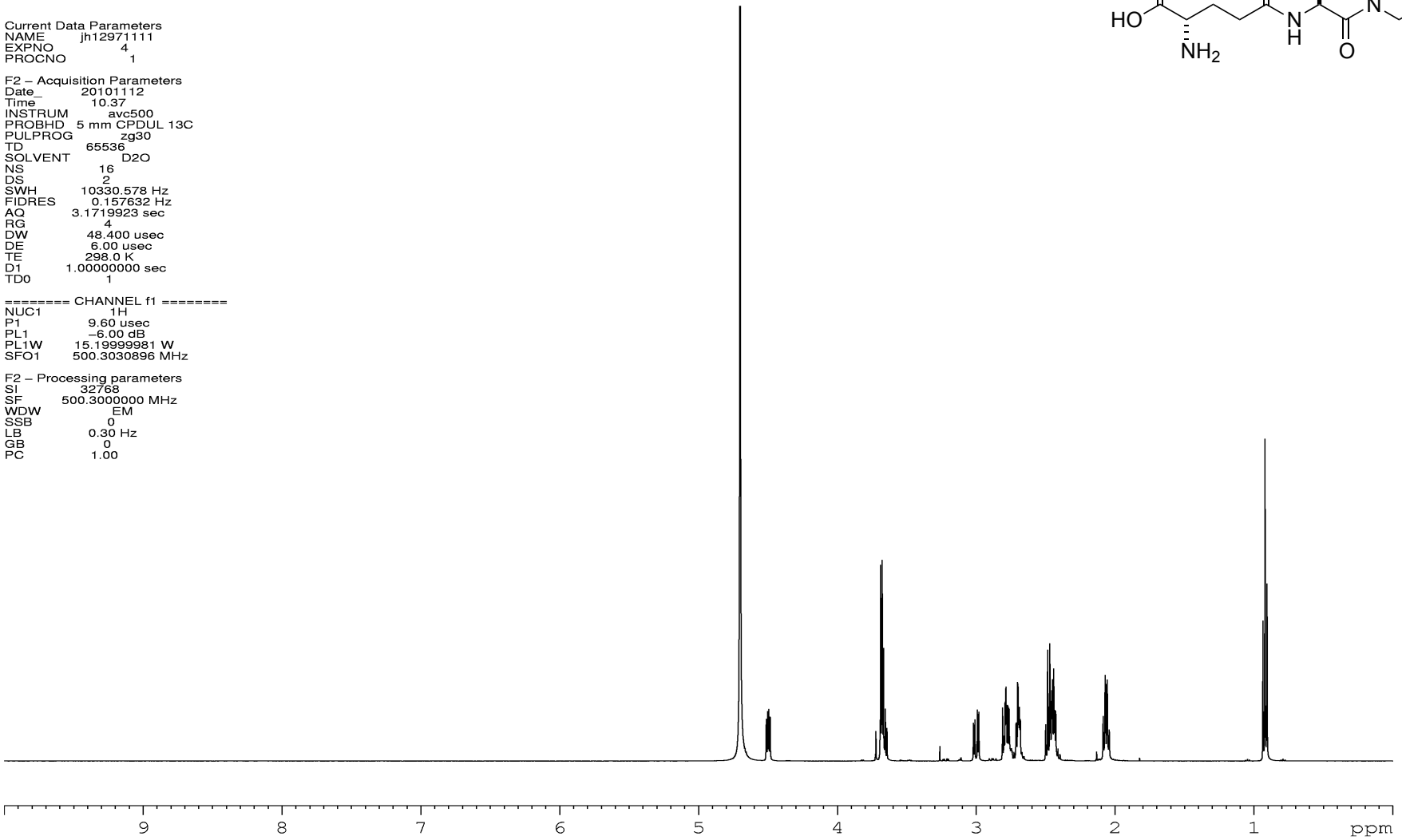
*S-Cyclopentan-2,4-dion-1-yl glutathione (**8**)*



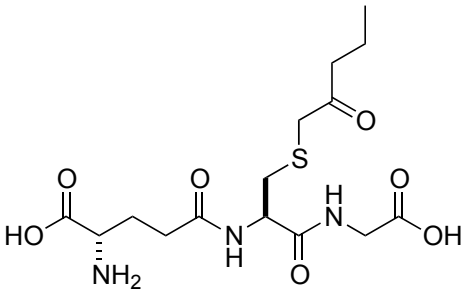


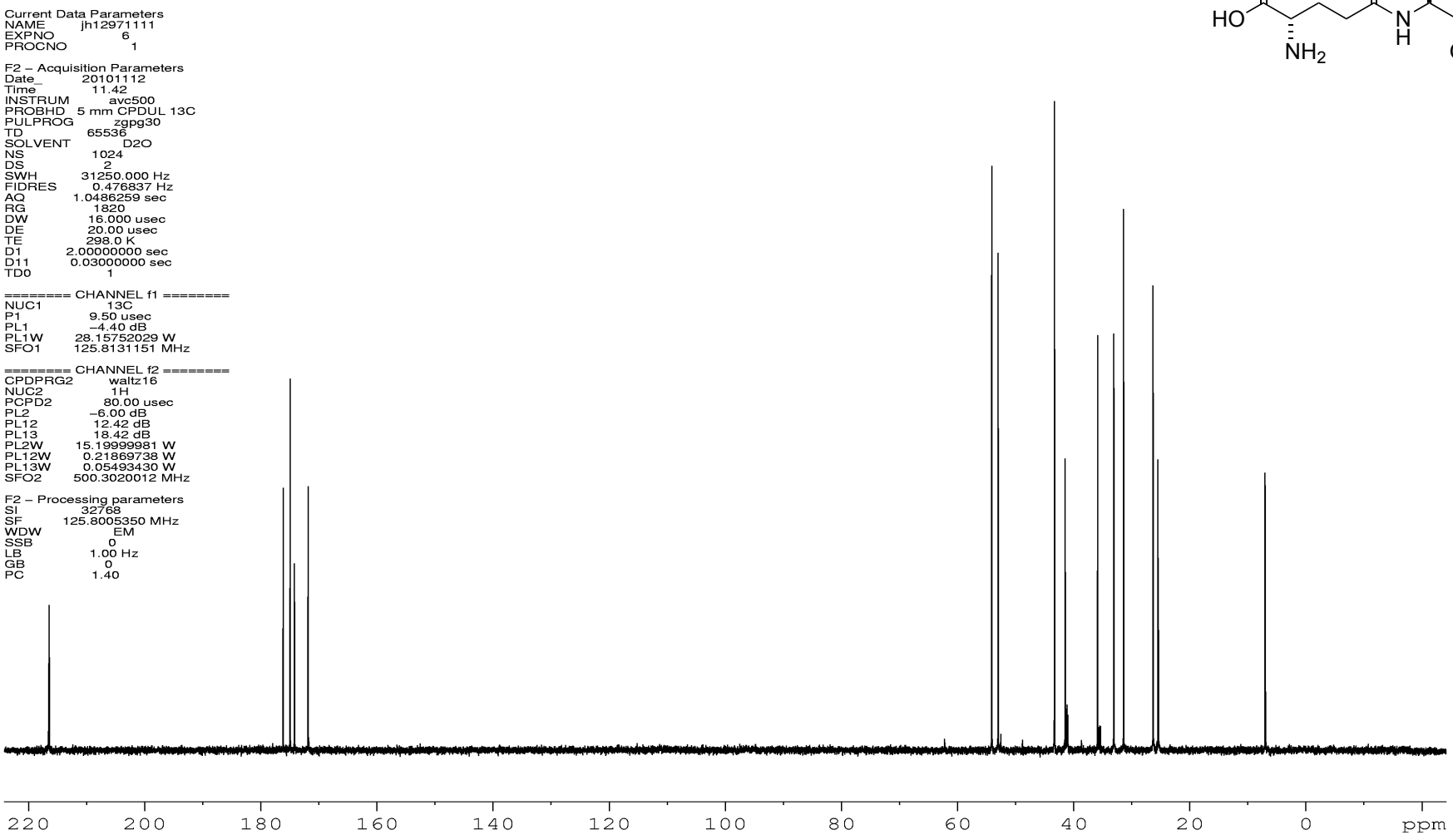
*S-Pentan-3-on-1-yl glutathione (**10**)*



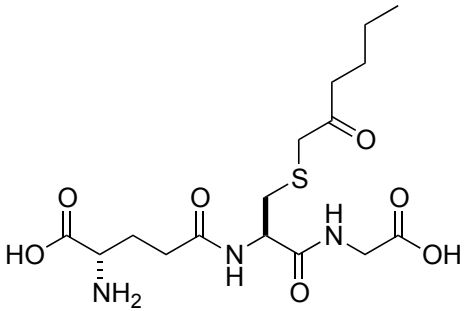


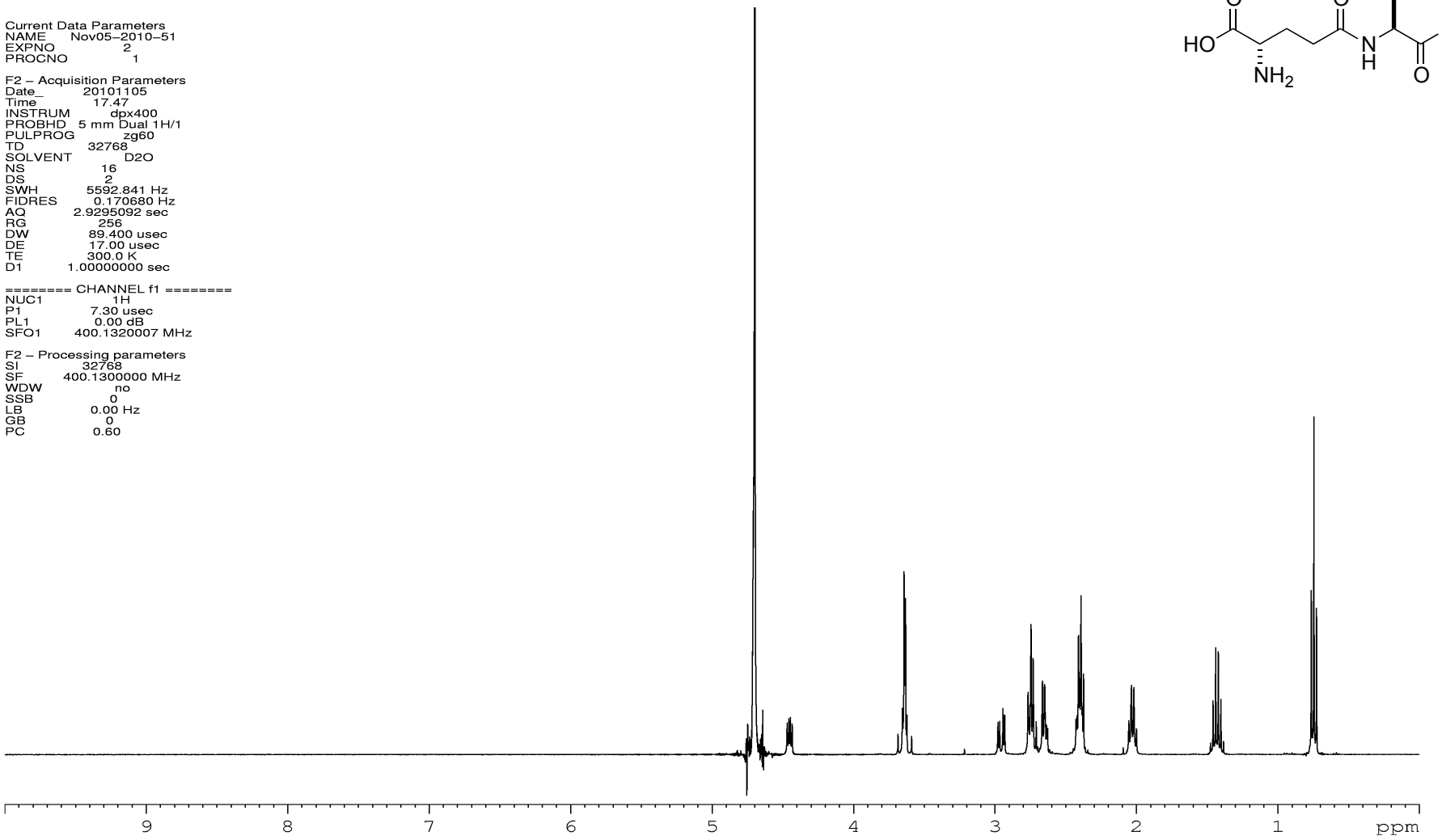
*S-Pentan-3-on-1-yl glutathione (**10**)*



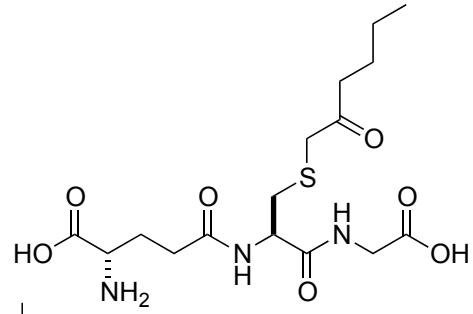


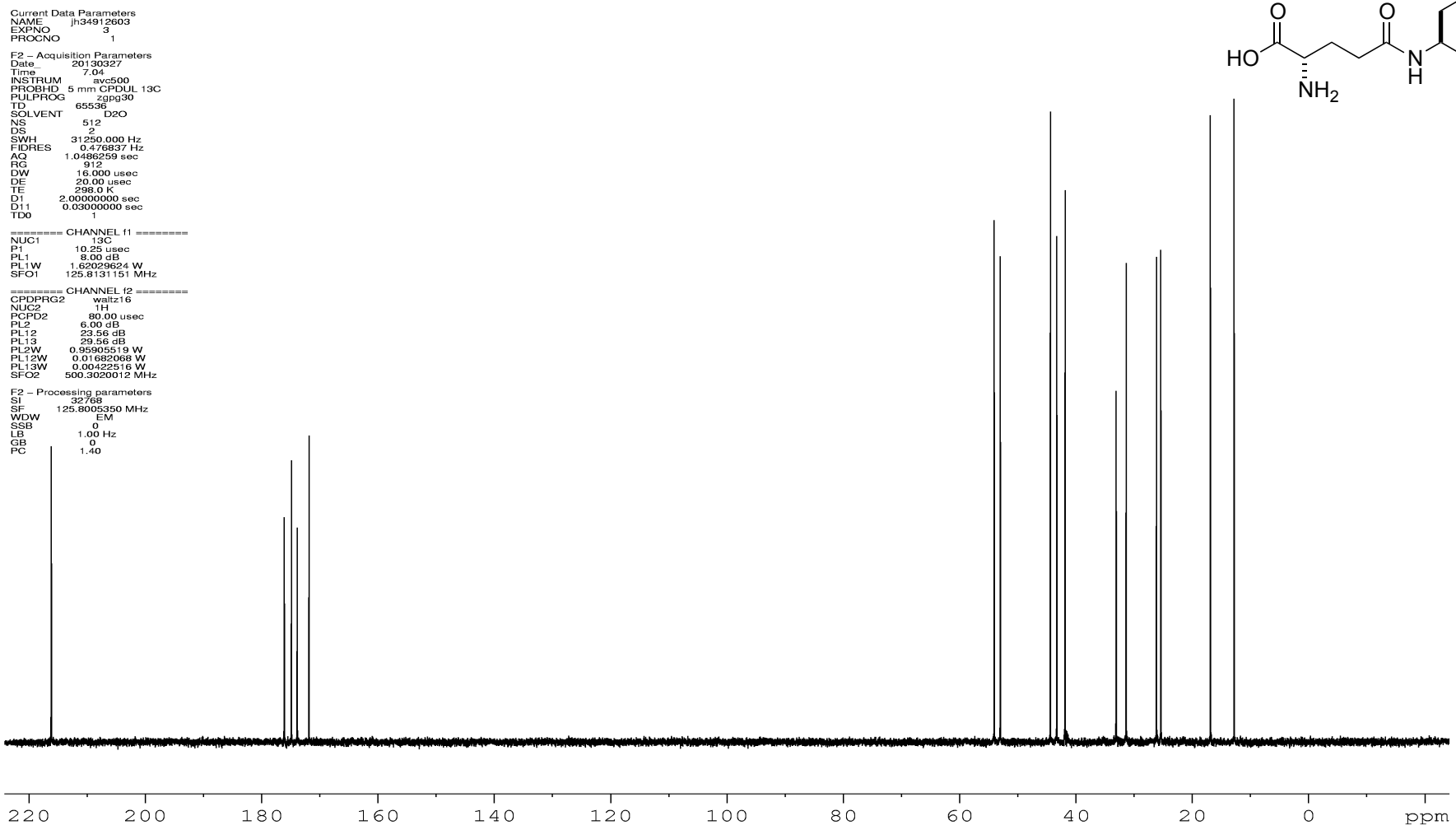
*S-Hexan-3-on-1-yl glutathione (**11**)*



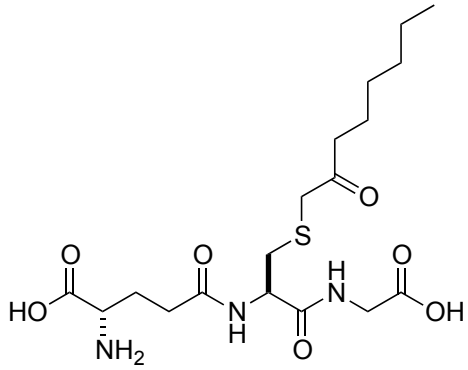


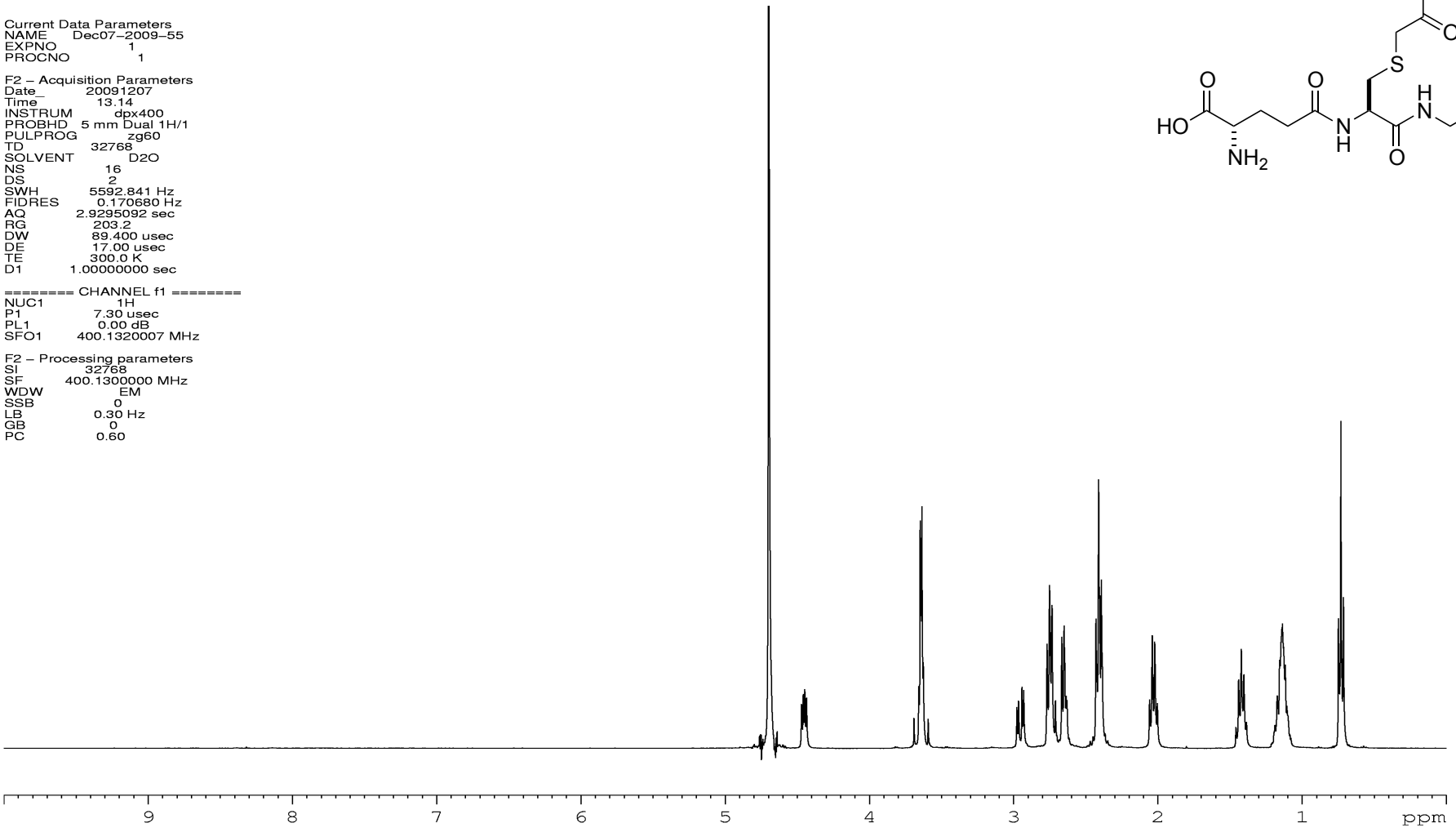
*S-Hexan-3-on-1-yl glutathione (**11**)*



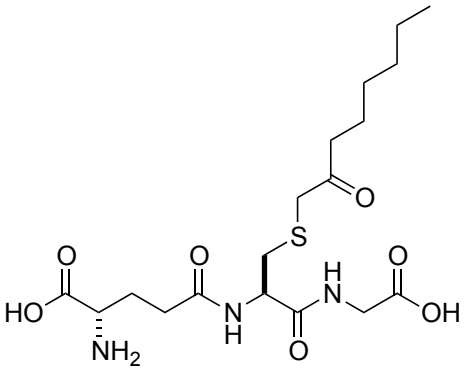


*S-Octan-3-on-1-yl glutathione (**12**)*





*S-Octan-3-on-1-yl glutathione (**12**)*



Current Data Parameters
NAME jh41762205
EXPNO 2
PROCNO 1

F2 – Acquisition Parameters
Date_ 20130524
Time 6.59
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0486259 sec
RG 912
DW 16.000 usec
DE 20.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 13C
P1 10.25 usec
PL1 8.00 dB
PL1W 1.62029624 W
SFO1 125.8131151 MHz

======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 6.00 dB
PL12 23.56 dB
PL13 29.56 dB
PL2W 0.95905519 W
PL12W 0.01682068 W
PL13W 0.00422516 W
SFO2 500.3020012 MHz

F2 – Processing parameters
SI 32768
SF 125.8005350 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40