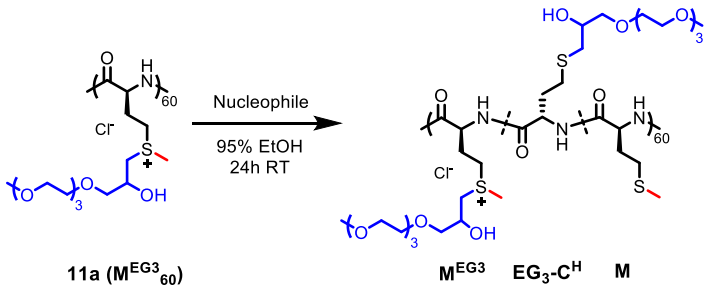
# Supplementary Information

## Chemoselective synthesis of functional homocysteine residues in polypeptides and peptides

Eric G. Gharakhanian and Timothy J. Deming*



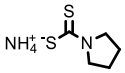
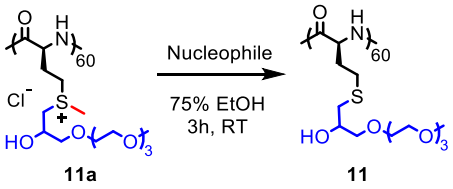

| Nucleophile | Product Selectivity (%) $M^{EG3}$ | $EG_3$-$C^H$ | M |
|---|---|---|---|
| None | 100 | 0 | 0 |
| KI | 100 | 0 | 0 |
|  | 93 | 0 | 7 |
|  | 100 | 0 | 0 |
|  | 0 | 100 | 0 |
|  | 0 | 100 | 0 |

**Figure S1.** Reaction of **11a** with various nucleophiles. Product selectivity indicates percent conversion to each type of product functional group. Conditions: **11a** and 5 eq nucleophile in sodium acetate-buffered 95% EtOH for 24h at 22 °C, followed by dialysis. Isolated yields ranged from 92 to 100%.



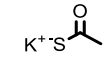
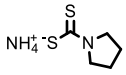
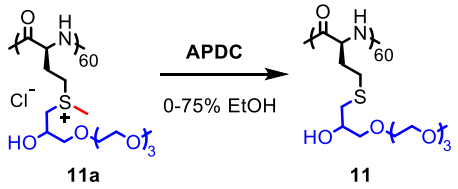

| Nucleophile | Conversion (%) |
|---|---|
|  | 20 |
|  | 80 |

**Figure S2.** Comparison of extent reaction conversion (i.e. percent of methionine sulfonium groups converted) using thioacetate or pyrrolidine dithiocarbamate (**APDC**) nucleophiles for the demethylation of **11a** at 3h time point. Conditions: **11a** and 5 eq nucleophile in 75% EtOH for 3h at 22 °C, quenched with $HCl_{(aq)}$, followed by dialysis.




| Mass Fraction EtOH (%) | Conversion (%) 3h | 24h |
|---|---|---|
| 0 | 10 | 58 |
| 50 | 33 | 99 |
| 75 | 80 | 100 |

**Figure S3**. Influence of EtOH/$H_2O$ solvent composition on demethylation rate. Conditions: **11a** and 5 eq **APDC** in 0-75% EtOH for 3-24h at 22 °C, quenched with $HCl_{(aq)}$, followed by dialysis. Conversion = percent of methionine sulfonium groups converted.

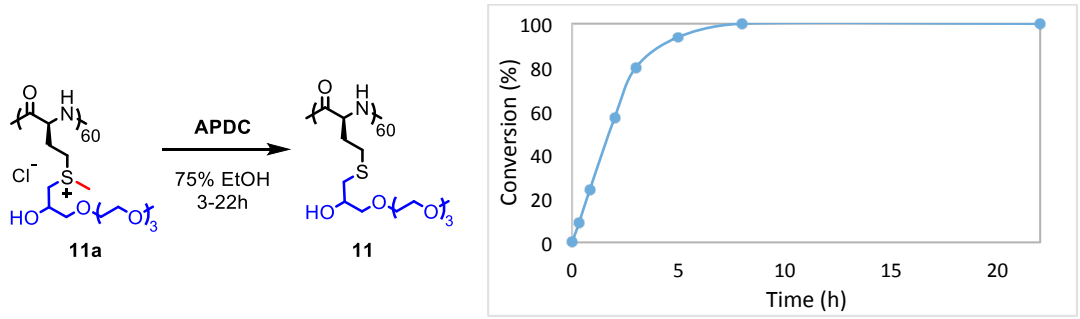



**Figure S4.** Conversion vs. time for demethylation of **11a** using **APDC**. Conditions: **11a** and 5 eq **APDC** in 75% EtOH for 0-22h at 22 °C, quenched with $HCl_{(aq)}$, followed by dialysis. Conversion = percent of methionine sulfonium groups converted.

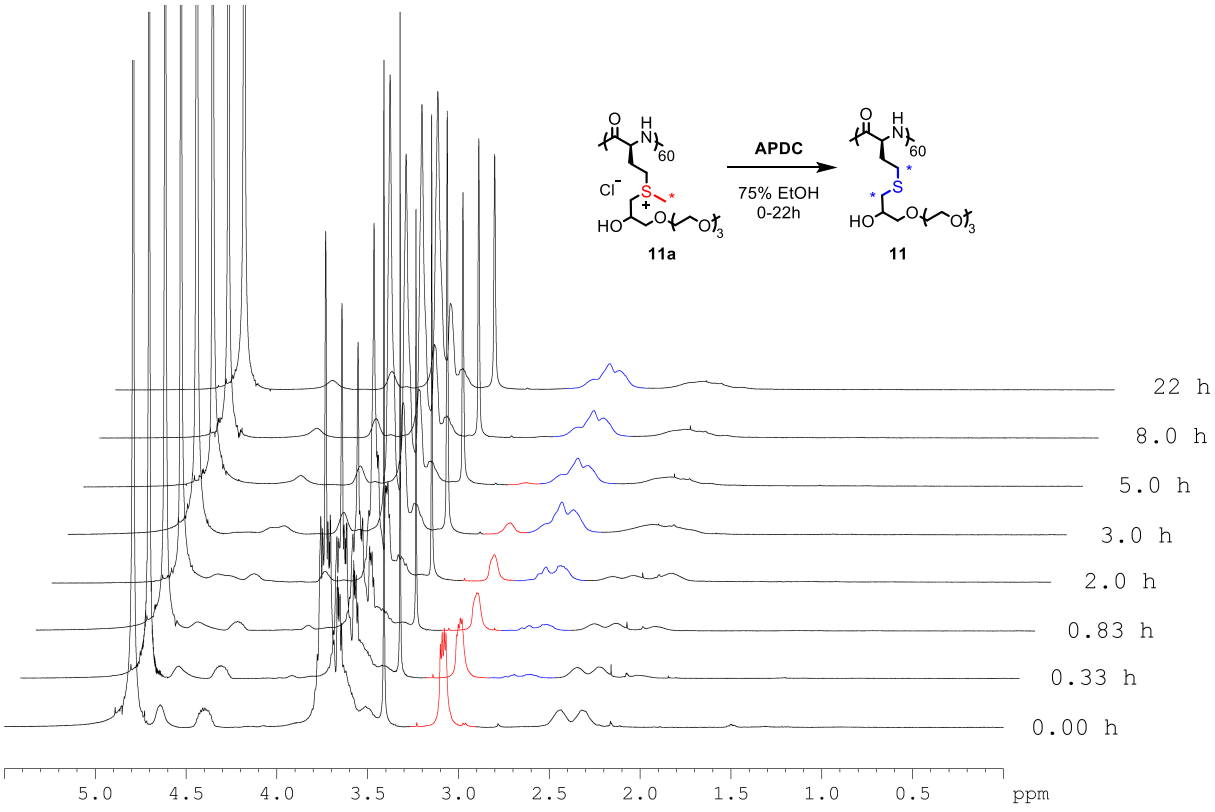



**Figure S5.** $^{1}$H NMR spectra at indicated time points for the reaction of **11a** with **APDC**. Resonances highlighted in red correspond to the methyl protons from **11a** residues (*); resonances highlighted in blue correspond to the of two sets of methylene protons from **11** residues (*).

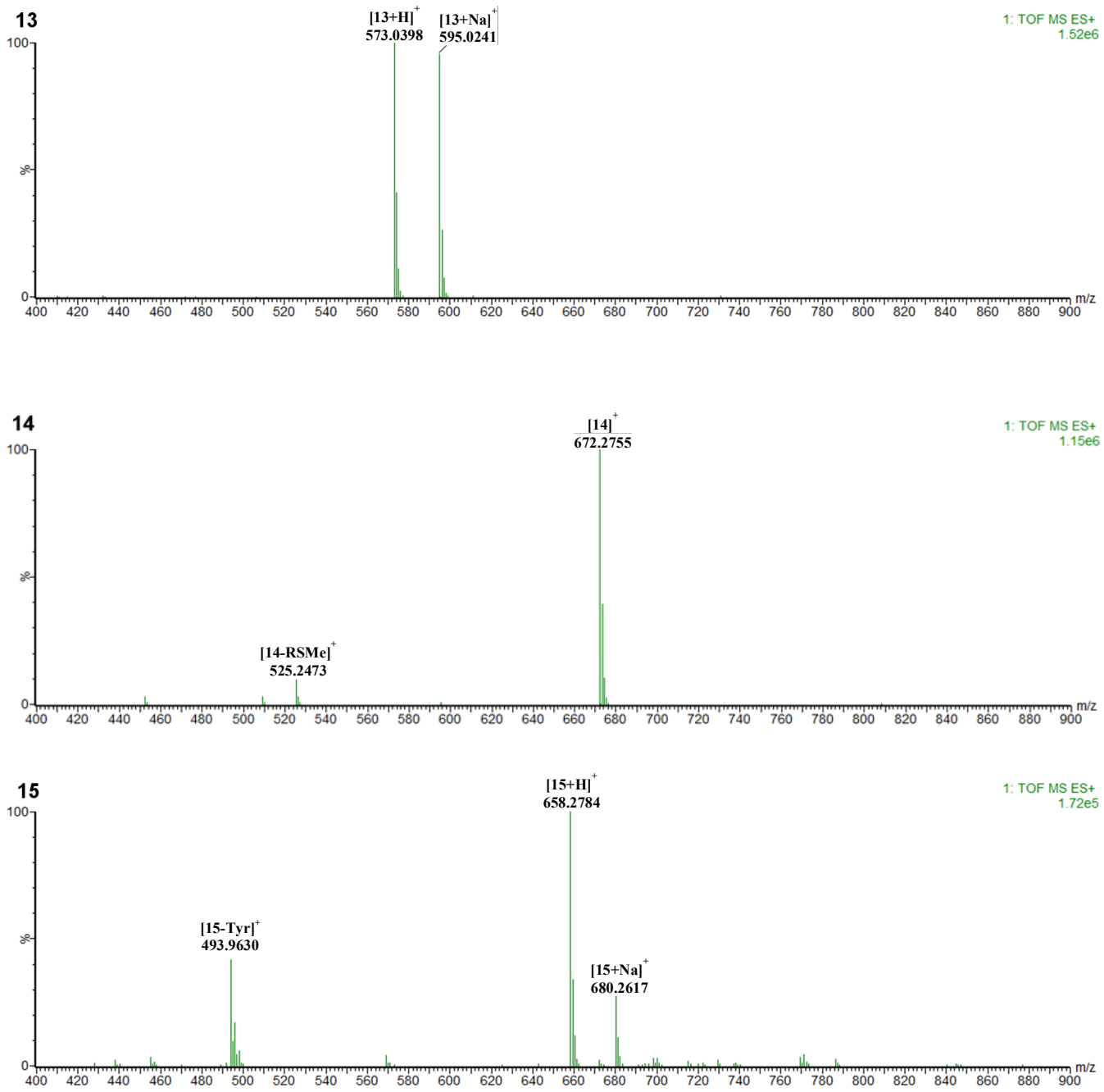



**Figure S6.** Top: Expanded range ESI-MS data for **13** with **[13+H]**$^+$ (573.0398 m/z) and **[13+Na]**$^+$ (595.0241 m/z) ions labeled. Middle: Expanded range ESI-MS data for **14** with [**14**]$^+$ (672.2755 m/z) and fragment **[14-RSMe]**$^+$ (525.2473 m/z) ions labeled. Bottom: Expanded range ESI-MS data for **15** with **[15+H]**$^+$ (658.2784 m/z), **[15+Na]**$^+$ (680.2617 m/z) and fragment **[15-Tyr]**$^+$ (493.9630 m/z) ions labeled.

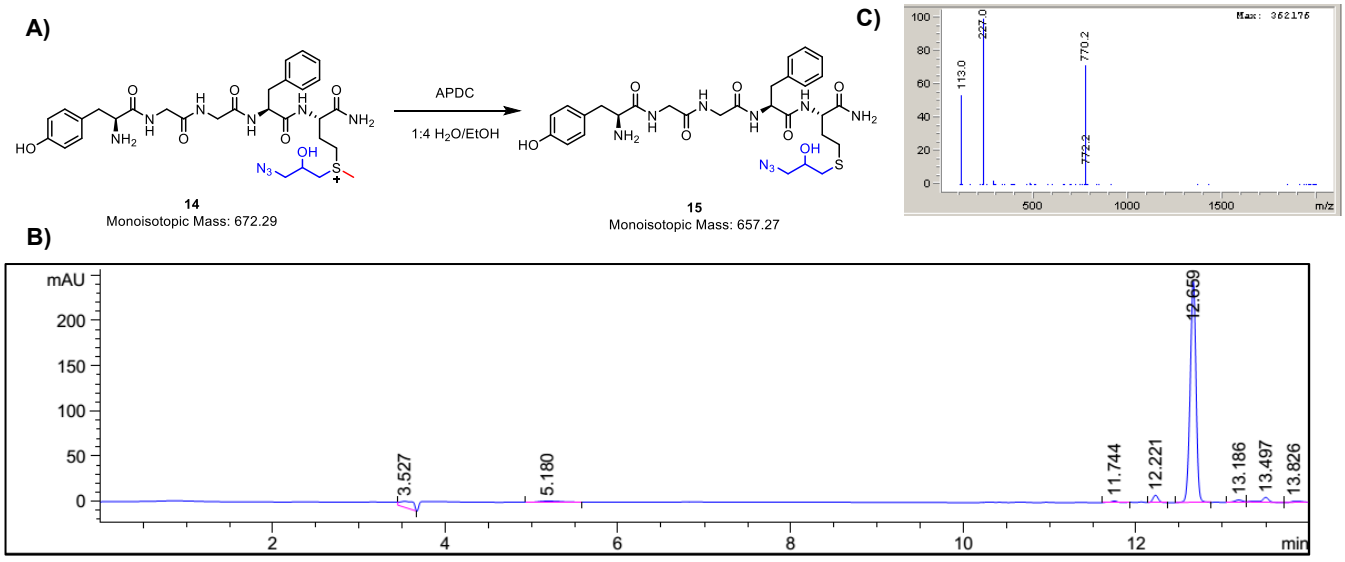



**Figure S7.** LC-MS data for **15**. Conditions: **14** (8.2 mM) and **APDC** (82 mM) in 75% EtOH at 22 °C for 26 h. Crude reaction mixture directly injected for LC-MS analysis. A) Scheme for synthesis of **15**. B) UV trace (λ = 280.4 nm) for LC of **15**. C) MS of LC peak at 12.659 min confirming identity of [**15**+TFA]$^-$ (770.2 m/z).

## 1) Materials and Methods

Unless otherwise stated, all polymer functionalization reactions were performed in glass vials, under ambient atmosphere. Small molecule reactions were performed under $N_2$ using oven dried glassware. All ethanol concentrations are reported in weight percent (w/w). Reactions at elevated temperature were controlled using a Corning PC 420D thermostated hotplate equipped with a thermocouple probe. $CH_2Cl_2$ was degassed by sparging with $N_2$ and dried by passing through alumina columns. Commercial anhydrous DMF and MeCN were used as received. All other reagents were used as received. Fisher ACS grade glacial AcOH was used as received. Met-Enkephalin amide (**14**) was obtained from GenScript and was reported >95% pure. Alkyl triflates were synthesized according to established procedures and used promptly.[1] Dialysis was performed using deionized water (18.2 MΩ-cm) prepared by passing in-house deionized water through a Millipore Milli-Q Biocel A10 unit. In all other cases, in-house reverse osmosis purified water was used. Thin-layer chromatography was performed with EMD gel 60 F254 plates (0.25 mm thickness) and visualized using a UV lamp or permanganate stain. Column chromatography was performed using Silicycle Siliaflash G60 silica (60-200 μm). Chromatography eluents are reported as volume percent (v/v). Dialysis was performed using regenerated cellulose dialysis tubing obtained from Spectrum Labs. CD Spectra were obtained with an Olis DSM 10 spectrophotometer using a 0.1 cm path length quartz cell. NMR spectra were recorded on a Bruker AV400 instrument with chemical shifts reported relative to residual solvent signal. Abbreviations of splitting pattern designations are listed in the abbreviation section. ESI-MS was performed using a Waters LCT Premier spectrometer. Small molecule samples were prepared in MeOH (1 mg/mL) and injected at a rate of 20 μL/min. Peptide samples (5 mM) were analyzed analogously using a 50% MeCN/$H_2O$ matrix. Analytical HPLC was performed using an Agilent 1100 HPLC system equipped with a Waters Sunfire™ C18, 4.6x250mm, 5μm column, an Agilent G1312A binary pump, a G1314A VWD and 6130 LC/MS

system equipped with an ESI source. Gradients of Solvent A (0.1% TFA/$H_2O$) and Solvent B (0.1% TFA/MeCN) were used as the mobile phase, operated with a 1.00 mL/min flow rate.

*Abbreviations:* Acetonitrile (MeCN), *N*-carboxyanhydride (NCA), degree of polymerization (DP), L-methionine (Met), L-methionine residue (**M**), L-Methionine sulfonium residue ($\mathbf{M^R}$), alkyl homocysteine residue ($\mathbf{R\text{-}C^H}$), glacial acetic acid (AcOH), electrospray ionization-mass spectrometry (ESI-MS), ethanol (EtOH), ethyl acetate (EtOAc), formic acid (HCOOH), diethyl ether ($Et_2O$), trifluoroacetic acid (TFA), trifluoroacetic anhydride (TFAA), *meta*-chloroperbenzoic acid (mCPBA), molecular weight cut-off (MWCO), room temperature (RT), equivalents (eq), methanol (MeOH), *N,N*-dimethylformamide (DMF), broad (br), doublet (d), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of multiplets (dm), doublet of quartets (dq), doublet of triplets (dt), pentet (p), quartet (q), septet (sep), sextet (sext) singlet (s), triplet (t), triplet of doublets (td), thin layer chromatography (TLC).

## 2) General Synthetic Procedures

**Poly(L-methionine)$_{60}$, $\mathbf{M_{60}}$**
Prepared by previously reported method.[2] Met NCA was polymerized with $Co(PMe_3)_4$ in THF under $N_2$ using a 20:1 monomer to initiator ratio. The DP was determined by endcapping a small aliquot from the polymerization mixture with 2 kDa PEG-isocyanate ($CH_3(OCH_2CH_2)_{45}N{=}C{=}O$) followed by $^1$H NMR analysis.[2] Found average DP = 58.

**$\mathbf{M_{60}}$ alkylation procedure A (Alkylation Procedure A)**
$\mathbf{M_{60}}$ was alkylated with an alkyl halide in $H_2O$ as previously reported.[3]

**$\mathbf{M_{60}}$ alkylation procedure B (Alkylation Procedure B)**
$\mathbf{M_{60}}$ was alkylated with an alkyl triflate in $CH_2Cl_2$/MeCN as previously reported.[3]

**$\mathbf{M_{60}}$ alkylation procedure C (Alkylation Procedure C)**
$\mathbf{M_{60}}$ was alkylated with an epoxide in AcOH as previously reported.[2]

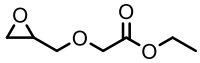
**$\mathbf{M^R}$ demethylation procedure A (Demethylation Procedure A)**
A solution of $\mathbf{M^R_{60}}$ in 75% $EtOH_{(aq)}$ (20 mM $\mathbf{M^R}$) was prepared in a vial and treated with **APDC** (5.0 eq per $\mathbf{M^R}$). The headspace of the vial was briefly flushed with a stream of $N_2$, then rapidly capped. The mixture was stirred vigorously at 22 °C. The initially homogenous solution became turbid with precipitate (polypeptide) over the course of minutes (products **5** & **6**) to hours (**1**-**4**). After 24h, the reaction mixture was centrifuged and the supernatant separated. The precipitate was triturated and then centrifuged 3x with MeOH, then 2x with $H_2O$ (both 40 µL per µmol $\mathbf{M^R}$ in substrate) and lyophilized.

**$\mathbf{M^R}$ demethylation procedure B (Demethylation Procedure B)**
A solution of $\mathbf{M^R_{60}}$ in 75% $EtOH_{(aq)}$ (20 mM $\mathbf{M^R}$) was prepared in a vial and was treated with **APDC** (5.0 eq per $\mathbf{M^R}$). The headspace of the vial was briefly flushed with a stream of $N_2$ and

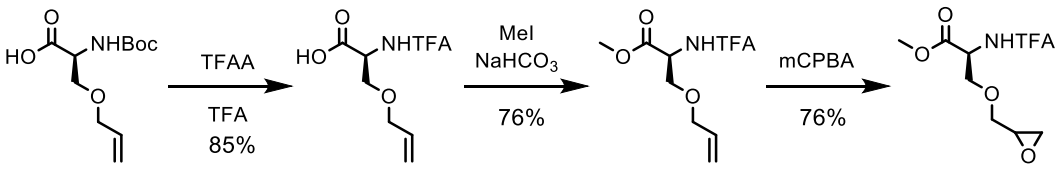
rapidly capped. The vial was vortexed until homogenous, then allowed to stand for 24h at 22 °C. The reaction mixture was directly treated with $K_2CO_3/H_2O$ to cleave the protecting group(s) (detailed deprotection conditions included in **Section 3**). The reaction mixture was transferred to a 2 kDa MWCO dialysis bag and dialyzed against 50% $MeOH_{(aq)}$ containing 3 mM HCl or 3 mM $NH_3$ (24h, 3 solvent changes) followed by $H_2O$ (8h, 3 $H_2O$ changes). The retentate was lyophilized to provide the functionalized polypeptide.

**$M^R$ demethylation procedure C (Demethylation Procedure C)**
A solution of **$M^R_{60}$** in 75% $EtOH_{(aq)}$ (20 mM **$M^R$**) was prepared in a vial and was treated with **APDC** (5.0 eq per **$M^R$**). The headspace of the vial was briefly flushed with a stream of $N_2$ and rapidly capped. The vial was vortexed until homogenous, then allowed to stand for 24h at 22 °C. The reaction mixture was transferred to a 2 kDa MWCO dialysis bag and dialyzed against 50% $MeOH_{(aq)}$ (24h, 3 solvent changes) followed by $H_2O$ (8h, 3 $H_2O$ changes). The retentate was lyophilized, to provide the functionalized polypeptide.

**3) Synthesis of Alkylating Agents**



**Ethyl 2-(oxiran-2-ylmethoxy)acetate, 9b**
Ethyl 2-(allyloxy)acetate[4] (0.95 g, 6.6 mmol, 1.0 eq) was dissolved in $CH_2Cl_2$ (25 mL). Commercial 70% mCPBA (2.4 g, 9.8 mmol, 1.5 eq) was added. The mixture was allowed to stir 2 days at 22 °C, then cooled on an ice bath. 10% $Na_2SO_{3(aq)}$ (12 mL) was added followed by 10% $Na_2CO_{3(aq)}$ (8.7 mL, 8.3 mmol, 1.3 eq) and EtOAc (60 mL). The solution was stirred for 10 min, then transferred to a separatory funnel using EtOAc (60 mL) and $H_2O$ (40 mL) to complete the transfer. The mixture was partitioned. The organic phase was washed with sat. $NaHCO_{3(aq)}$ (60 mL) and dried over $Na_2SO_4$. The extract was concentrated *in vacuo* and the residue was purified by flash chromatography (35% EtOAc/Hexanes). **9b** (0.73 g, 70% yield) was recovered as a colorless oil. $R_F$ = 0.61; 40% EtOAc/Hexanes.
$^1$H NMR (400 MHz, $CDCl_3$, 25 °C): δ 4.24 (q, *J* = 7.1 Hz, 2H), 4.15 (d, *J* = 16.4 Hz, 1H), 4.14 (d, *J* = 16.5 Hz, 1H), 3.90 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.49 (dd, *J* = 11.6, 5.9 Hz, 1H), 3.19 (m, 1H), 2.80 (dd, *J* = 4.8, 4.2 Hz, 1H), 2.62 (dd, *J* = 4.9, 2.7 Hz, 1H), 1.28 (t, *J* = 7.2 Hz, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$, 25 °C): δ 170.1, 72.1, 68.5, 60.9, 50.6, 44.0, 14.2. ESI-MS *m/z* = 182.9952 $[M+Na]^+$ (calcd 183.0633 for $C_7H_{12}O_4Na$).




**10d** **10c** **10b**

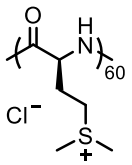
**Methyl *O*-(oxiran-2-ylmethyl)-*N*-(2,2,2-trifluoroacetyl)-(*S*)-serinate, 10b**
*O-allyl-N-(2,2,2-trifluoroacetyl)-(S)-serine,* ***10d***

*O*-allyl-*N*-(tert-butoxycarbonyl)-(*S*)-serine[5] (6.0 g, 25 mmol, 1.0 eq) was cooled on an ice bath. TFA (20 mL) was added. TFAA (4.1 mL, 29 mmol, 1.2 eq) was added dropwise over 5 minutes. The solution was stirred for 1h on the ice bath, then concentrated *in vacuo*. The residue was directly purified by flash chromatography (0:40:60 to 0.5:40:59.5 HCOOH:EtOAc:Hexanes). **10d** was isolated as an orange-red viscous oil (5.0 g, 85% yield).
$^1$H NMR (400 MHz, $CDCl_3$, 25 $^o$C): δ 7.75-7.29 (br s, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 5.84 (m, 1H), 5.26 (m, 2H), 4.78 (m, 1H), 4.04 (dt, *J* = 5.8, 1.3 Hz, 2H), 4.01 (dd, *J* = 7.0, 2.8 Hz, 1H), 3.75 (dd, *J* = 9.8, 3.4 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$, 25 $^o$C): δ 172.8, 157.6 (q, 38.5 Hz), 133.2, 118.4, 117.0 (q, *J* = 287.0 Hz), 72.5, 68.2, 52.9. $^{19}$F{$^1$H} NMR (376 MHz, $D_2O$, 25 $^o$C): δ -75.6. ESI-MS *m/z* = 240.0082 $[M-H]^-$ (calcd 240.0484 for $C_8H_9F_3NO_4$).

*Methyl O-allyl-N-(2,2,2-trifluoroacetyl)-(S)-serinate,* ***10c***
**10d** (1.3 g, 5.1 mmol, 1.0 eq) and $NaHCO_3$ (0.86 g, 10 mmol, 2.0 eq) were suspended in DMF (50 mL). Methyl iodide (1.6 mL, 26 mmol, 5.0 eq) was added. The suspension was stirred at 22 $^o$C overnight. The mixture was concentrated *in vacuo* and the residue was directly purified by flash chromatography (15% EtOAc/Hexanes). **10c** (1.0 g, 76% yield) was recovered as a pale yellow, mobile oil. $R_F$ = 0.30; 15% EtOAc/Hexanes.
$^1$H NMR (400 MHz, $CDCl_3$, 25 $^o$C): δ 7.13 (br s, 1H), 5.81 (m, 1H), 5.25 (dm, 13.9 Hz, 1H), 5.21 (dm, *J* = 6.9 Hz, 1H), 4.72 (dm, *J* = 8.2 Hz, 1H), 3.99 (dq, *J* = 5.7, 1.5 Hz, 2H), 3.94 (dd, *J* = 9.9, 3.0 Hz, 1H), 3.81 (s, 3H), 3.72 (dd, *J* = 9.8, 3.2 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$, 25 $^o$C): δ 169.1, 157.1 (q, *J* = 38.3 Hz), 133.5, 118.0, 117.1 (q, *J* = 288.6 Hz), 72.3, 68.5, 53.0, 53.0. $^{19}$F{$^1$H} NMR (376 MHz, $D_2O$, 25 $^o$C): δ -75.9. ESI-MS *m/z* = 254.0211 $[M-H]^-$ (calcd 254.0640 for $C_9H_{11}F_3NO_4$).

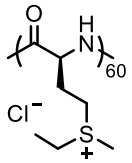
*Methyl O-(oxiran-2-ylmethyl)-N-(2,2,2-trifluoroacetyl)-(S)-serinate,* ***10b***
**10c** (0.90 g, 3.5 mmol, 1.0 eq), was dissolved in a 0.45 M mCPBA solution in $CH_2Cl_2$[2] (12 mL, 5.3 mmol, 1.5 eq). The mixture was allowed to stir 3 days at 22 $^o$C, then cooled on an ice bath. 10% $Na_2SO_{3(aq)}$ (7 mL) was added followed by 10% $Na_2CO_{3(aq)}$ (4.6 mL, 4.4 mmol, 1.3 eq) and EtOAc (60 mL). The solution was stirred for 10 min. $H_2O$ (20 mL) was added, then the mixture was partitioned. The organic phase was washed with sat. $NaHCO_{3(aq)}$ (30 mL) and dried over $Na_2SO_4$. The extract was concentrated *in vacuo* and the residue was purified by flash chromatography (35-40% EtOAc/Hexanes). **10b** (0.73 g, 76% yield) was recovered as a colorless oil. Epoxide *dr*: 2:1 ($^1$H NMR). $R_F$ = 0.25; 40% EtOAc/Hexanes. NMR data is for major diasteriomer.
$^1$H NMR (400 MHz, $CDCl_3$, 25 $^o$C): δ 4.69 (m, 1H), 4.10 (dd, *J* = 10.2, 3.3 Hz, 1H), 3.81 (m, 2H), 3.77 (s, 3H), 3.73 (dd, *J* = 10.1, 3.2 Hz, 1H), 3.43, (dd, *J* = 12.0, 5.4 Hz, 1H), 3.07 (m, 1H), 2.76 (t, *J* = 5.0 Hz, 1H), 2.59 (dd, *J* = 5.0, 2.8 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$, 25 $^o$C): δ 168.0, 156.9 (q, *J* = 37.1 Hz), 117.1 (q, *J* = 285.5 Hz), 71.6, 70.4, 53.2, 53.0, 50.7, 43.7. $^{19}$F{$^1$H} NMR (376 MHz, $D_2O$, 25 $^o$C): δ -75.9. ESI-MS *m/z* = 293.9496 $[M+Na]^+$ (calcd 294.0565 for $C_9H_{12}F_3NO_5Na$).

## 4) Synthesis of $M^R$ Polymers



**Poly(*S*-methyl-L-methionine sulfonium chloride), 1a**

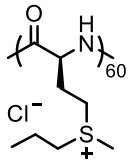
Prepared from $\mathbf{M_{60}}$ and methyl iodide using *Alkylation Procedure A*. Spectral data in agreement with those previously reported.[3]



**Poly(*S*-ethyl-L-methionine sulfonium chloride), 2a**

Prepared from $\mathbf{M_{60}}$ and ethyl triflate using *Alkylation Procedure B*. Yield: 99%.

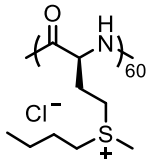
$^1$H NMR (400 MHz, $D_2O$, 25 $^o$C): δ 4.68-4.55 (br m, 1H), 3.60-3.30 (br m, 4H), 2.98 (d, $J$ = 5.2 Hz, 3H), 2.51-2.17 (br m, 2H), 1.50 (dt, $J$ = 7.4, 2.8 Hz, 3H).



**Poly(*S*-(n-propyl)-L-methionine sulfonium chloride), 3a**

Prepared from $\mathbf{M_{60}}$ and propyl triflate using *Alkylation Procedure B*. Yield: 97%.

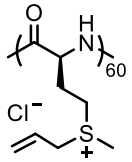
$^1$H NMR (400 MHz, $D_2O$, 25 $^o$C): δ 4.71-4.53 (br m, 1H), 3.74-3.23 (br m, 4H), 2.98 (d, $J$ = 5.2 Hz, 3H), 2.59-2.14 (br m, 2H), 2.05-1.79 (br m, 2H), 1.11 (t, $J$ = 7.3 Hz, 3H).



**Poly(*S*-(n-butyl)-L-methionine sulfonium chloride), 4a**

Prepared from $\mathbf{M_{60}}$ and butyl triflate using *Alkylation Procedure B*. Yield: 96%.

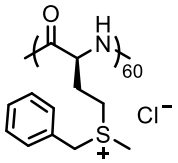
$^1$H NMR (400 MHz, $D_2O$, 25 $^o$C): δ 4.72-4.50 (br m, 1H), 3.62-3.29 (br m, 4H), 2.99 (d, $J$ = 5.0 Hz, 3H), 2.58-2.17 (br m, 2H), 1.85 (m, 2H), 1.54 (sext, $J$ = 7.3 Hz, 2H), 0.99 (t, $J$ = 7.3 Hz, 3H).



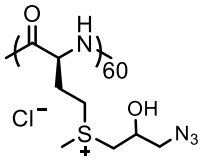
**Poly(*S*-allyl-L-methionine sulfonium chloride), 5a**

Prepared from $\mathbf{M_{60}}$ via a modified *Alkylation Procedure A*. $\mathbf{M_{60}}$ (16 mg, 0.122 mmol **M**, 1.0 eq) was suspended in AcOH. Allyl bromide (32 μL, 0.37 mmol, 3.0 eq) was added. The mixture was vigorously stirred at 37 $^o$C. After 24h, the limpid solution was transferred to a 2 kDa

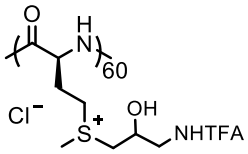
MWCO dialysis bag and dialyzed against 3 mM $HCl_{(aq)}$ (24h, 3 $H_2O$ changes). The retentate was lyophilized, to provide **5** (25 mg, 99% Yield).
$^1$H NMR (400 MHz, $D_2O$, 25 $^oC$): δ 6.07-5.89 (br m, 1H), 5.84-5.61 (br m, 2H), 4.66-4.55 (br m, 1H), 4.26-4.03 (br m, 2H), 3.57-3.32 (br m, 2H), 2.94 (t, $J$ = 6.0 Hz, 3H), 2.53-2.18 (br m, 2H).



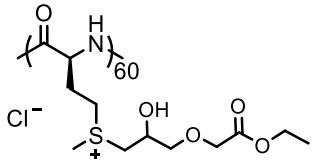
**Poly(*S*-benzyl-L-methionine sulfonium chloride), 6a**
Prepared from $\mathbf{M_{60}}$ and benzyl bromide using *Alkylation Procedure A*. Spectral data in agreement with those previously reported.[3]



**Poly(*S*-(3-azido-2-hydroxypropyl)-L-methionine sulfonium chloride), 7a**
Prepared from $\mathbf{M_{60}}$ and glycidyl azide using *Alkylation Procedure C*. Spectral data in agreement with those previously reported.[2]

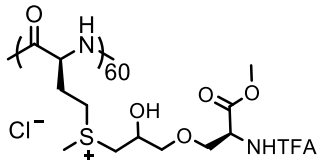


**Poly(*S*-(2-hydroxy-3-(2,2,2-trifluoroacetamido)propyl)-L-methionine sulfonium chloride), 8a**
Prepared from $\mathbf{M_{60}}$ and glycidyl trifluoroacetamide using *Alkylation Procedure C*. Spectral data in agreement with those previously reported.[2]

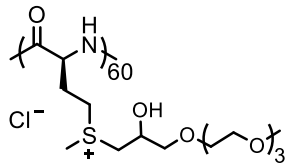


**Poly(*S*-(3-((1-ethoxy-1-oxoeth-2-yl)oxy)-2-hydroxypropyl)-L-methionine sulfonium chloride), 9a**
Prepared from $\mathbf{M_{60}}$ and **9b** using *Alkylation Procedure C*. Dialysis was conducted against 6 mM NaCl (24h, 3 $H_2O$ changes) then $H_2O$ (8h, 3 $H_2O$ changes) instead of $HCl_{(aq)}$, to reduce hydrolysis of the uncharacteristically labile ethyl ester. Recovered product showed 34% ethyl ester deprotection. Yield 96%.

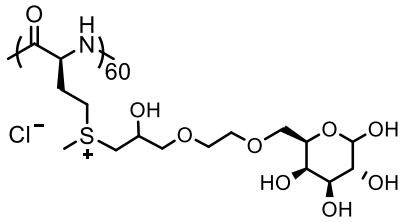
$^1$H NMR (400 MHz, $D_2O$, 25 $^oC$): δ 4.70-4.51 (br m, 1H), 4.51-4.35 (br m, 1H), 4.35-4.21 (br m, 2.6H), 4.01 (s, 0.6H), 3.97-3.40 (br m, 6H), 3.21-2.92 (br m, 3H), 2.55-2.18 (br m, 2H), 1.30 (t, *J* = 7.2 Hz, 2H).




**Poly(*S*-(3-(((*S*)-1-methoxy-1-oxo-2-(2,2,2-trifluoroacetamido)prop-3-yl)oxy)-2-hydroxypropyl)-L-methionine sulfonium chloride), 10a**
Prepared from **$M_{60}$** and **10b** using *Alkylation Procedure C*. Yield: 96%
$^1$H NMR (400 MHz, $D_2O$, 25 $^oC$): δ 4.98-4.89 (br m, 1H), 4.71-4.56 (br m, 1H), 4.44-4.32 (br m, 1H), 4.06-4.96 (br m, 2H), 3.83 (s, 3H), 3.79-3.34 (br m, 6H), 3.15-2.96 (br m, 3H), 2.58-2.17 (br m, 2H). $^{19}F\{^1H\}$ NMR (376 MHz, $D_2O$, 25 $^oC$): -75.1.




**Poly(*S*-(2-hydroxy-4,7,10,13-tetraoxatetradecyl)-L-methionine sulfonium chloride), 11a**
Prepared from **$M_{60}$** and 2-(2,5,8,11-tetraoxadodecyl)oxirane using *Alkylation Procedure C*. Spectral data in agreement with those previously reported.[2]




**Poly(S-((3-(2-(6-deoxy-D-galactopyranosid-6-yl)oxy)ethoxy)-2-hydroxypropyl)-L-methionine sulfonium chloride), 12a**
Prepared from **$M_{60}$** and 2-(2-((1,2:3,4-di-O-isopropylidene-6-deoxy-α-D-galactopyranosid-6-yl)oxy)ethoxymethyl)oxirane using *Alkylation Procedure C* followed by acid deprotection of the isopropylidene protecting groups. Spectral data in agreement with those previously reported.[2]

**5) Studies of Demethylation Reaction Conditions**

**Example reaction of $M^R_{60}$ with various nucleophiles**
A stock solution of **11a** (22 mg/mL, 55 mM **$M^R$**) in 95% EtOH was prepared. A buffered ethanol solution was prepared by mixing equal volumes of 0.27 M NaOAc in 95% EtOH with 0.27 M AcOH in 95% EtOH. **11a** stock (0.33 mL, 0.018 mmol **$M^R$**, 1.0 eq) was diluted with buffered ethanol (0.33 mL). Nucleophile (KI, 2-mercaptopyridine, potassium thioaceate or **APDC**) (0.090 mmol, 5.0 eq) was added if required. The reaction mixture was vortexed briefly

and allowed to stand at 22 $^{o}$C for 24h. The reaction mixture was transferred to a 2 kDa MWCO dialysis bag and dialyzed against $H_2O$ (36h, 5 $H_2O$ changes). The retentate was lyophilized and the reaction selectivity determined by $^{1}$H NMR.

For thioglycolate the procedure was as above, except a NaOAc solution was used instead of buffer. Therefore, **11a** stock (0.33 mL, 0.018 mmol $\mathbf{M^R}$, 1.0 eq) was diluted with 0.27 M NaOAc in 95% EtOH (0.33 mL). Thioglycolic Acid (0.090 mmol, 6.2 μL, 5.0 eq) was added. From there the procedure was as above.

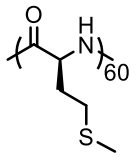
**Comparison of extent of reaction conversion**
An **11a** stock solution (7.8 mg/mL, 20 mM $\mathbf{M^R}$) in 75% $EtOH_{(aq)}$ was prepared. **11a** stock (0.65 mL, 0.013 mmol $\mathbf{M^R}$, 1.0 eq) was added to a vial containing an accurately weighed quantity of **APDC** (11 mg, 0.064 mmol, 5.0 eq) or potassium thioacetate (7.4 mg, 0.064 mmol, 5.0 eq). The headspace of the vial was briefly flushed with $N_2$ then rapidly capped. The reaction was stirred for 3.0h at 22 $^{o}$C. The reaction was then immediately quenched with 3 drops of con. $HCl_{(aq)}$, transferred to a 2 kDa MWCO dialysis bag and dialyzed against 3 mM $HCl_{(aq)}$ (4h, 2 $H_2O$ changes) followed by $H_2O$ (24h, 3 $H_2O$ changes). The retentate was lyophilized and extent of reaction conversion determined by $^{1}$H NMR.

**Influence of EtOH/$H_2O$ solvent composition on demethylation rate**
As above, using **11a** stock solutions in 75% $EtOH_{(aq)}$, 50% $EtOH_{(aq)}$ or 0% $EtOH_{(aq)}$. Aliquots were removed from the reaction and quenched at either 3h or 24h time points.

**Conversion vs. time study**
As preceding experiments, this study was performed using a stock solution of **11a** in 75% $EtOH_{(aq)}$. Aliquots were removed from the reaction mixture and quenched at 0.33, 0.83, 2.0, 3.0, 5.0, 8.0 and 22.0h time points.

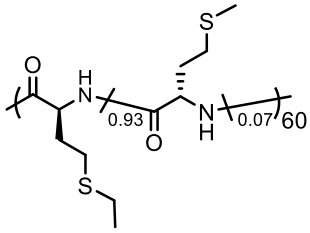
For the 0.00h time point a slight deviation was made. **11a** stock (0.65 mL, 0.013 mmol $\mathbf{M^R}$, 1.0 eq) was treated with 3 drops of con. $HCl_{(aq)}$. **APDC** (11 mg, 0.064 mmol, 5.0 eq) was added. The mixture was vortexed until homogenous and allowed to stand for 2 minutes. The mixture was transferred to dialysis and isolated as in preceding experiments.

## 6) Details for Synthesis of Specific R-$C^H$ Polymers




**Poly(L-Methionine), 1, 5-6**
Prepared from **1a**, **5a** or **6a** using *Demethylation Procedure A*. **5a** and **6a** became turbid with precipitate (polypeptide) in <10 min, while for **1a** precipitate began forming after ~6h.

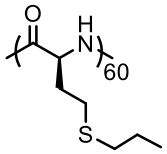
$^1$H NMR (400 MHz, D-TFA, 25 $^o$C): δ 4.93-4.70 (br m, 1H) 2.77-2.53 (br m, 2H) 2.29-1.94 (br m, 5H).



**Poly[(*S*-ethyl-L-homocysteine)$_{0.93}$-*stat*-(L-Methionine)$_{0.07}$], 2**

Prepared from **2a** using *Demethylation Procedure A*.

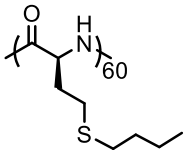
$^1$H NMR (400 MHz, D-TFA, 25 $^o$C): δ 4.98-4.82 (br m, 1.07H), 2.88-2.55 (br m, 4.14H), 2.38-2.03 (br m, 2.4H), 1.53-1.12 (t, $J$ = 7.6 Hz, 3H).



**Poly(*S*-propyl-L-homocysteine), 3**

Prepared from **3a** using *Demethylation Procedure A*.

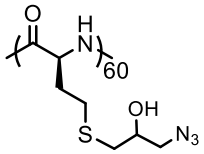
$^1$H NMR (400 MHz, D-TFA, 25 $^o$C): δ 4.93-4.77 (br m, 1H), 2.89-2.63 (br m, 2H), 2.59 (t, $J$ = 7.4 Hz, 2H), 2.27-2.07 (br m, 2 H), 1.65 (sext, $J$ = 7.4 Hz, 2H), 1.00 (t, $J$ = 7.4 Hz, 3H).



**Poly(*S*-Butyl-L-homocysteine), 4**

Prepared from **4a** using *Demethylation Procedure A*.

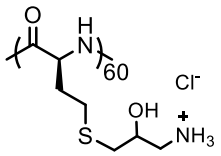
$^1$H NMR (400 MHz, D-TFA, 25 $^o$C): δ 5.52-5.13 (br m, 1H), 3.32-3.09 (br m, 2H), 3.05 (t, $J$ = 7.6 Hz, 2H), 2.73-2.52 (br m, 2H), 2.03 (p, $J$ = 7.6 Hz, 2H), 1.86 (sext, $J$ = 7.6 Hz, 2H), 1.35 (t, $J$ = 7.6 Hz, 3H).



**Poly(*S*-(3-azido-2-hydroxypropyl)-L-homocysteine), 7**

Prepared from **7a** using *Demethylation Procedure A*.

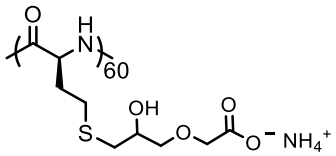
$^1$H NMR (400 MHz, D-TFA, 25 $^o$C): δ 5.26-4.68 (br m, 1H), 4.36-4.07 (br m, 1H), 3.89-3.43 (br m, 2H), 3.16-2.58 (br m, 4H), 2.43-2.04 (br m, 2H).



**Poly(*S*-(3-ammonio-2-hydroxypropyl)-L-homocysteine chloride), 8**

Prepared from **8a** using *Demethylation Procedure B*. Deprotection conditions: $H_2O$ (7.5 µL per µmol **R-C$^{H}$**) and $K_2CO_3$ (10 eq per **R-C$^{H}$**) were added. Allowed to stir vigorously at 40 $^{o}$C for 48h. Dialysis conditions: 50% $MeOH_{(aq)}$ containing 3 mM HCl (24h, 3 solvent changes) followed by $H_2O$ (8h, 3 $H_2O$ changes).

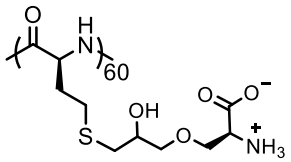
$^{1}$H NMR (400 MHz, $D_2O$, 25 $^{o}$C): δ 4.67-4.39 (br m, 1H), 4.18-3.97 (br m, 1H) 3.32 (d, *J* = 12.9 Hz, 1H), 3.04 (dd, *J* = 12.9, 9.6 Hz, 1H), 2.95-2.49 (br m, 4H), 2.23-2.00 (br m, 2H).



**Poly(ammonium *S*-(3-(carboxylatomethoxy)-2-hydroxypropyl)-L-homocysteine), 9**

Prepared from **9a** using *Demethylation Procedure B*. Deprotection conditions: $H_2O$ (5.5 µL per µmol **R-C$^{H}$**) and $K_2CO_3$ (6 eq per **R-C$^{H}$**) were added. The mixture was allowed to stir 18h at 37 $^{o}$C. Dialysis conditions: 50% $MeOH_{(aq)}$ containing 3 mM $NH_3$ (24h, 3 solvent changes) followed by $H_2O$ (8h, 3 $H_2O$ changes).

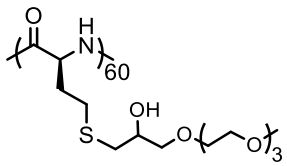
$^{1}$H NMR (400 MHz, $D_2O$, 25 $^{o}$C): δ 4.49-4.21 (br m, 1H), 4.22-3.86 (br m, 3H), 3.81-3.51 (br m, 2H), 3.27-3.55 (br m, 4H), 2.42-1.97 (br m, 2H).



**Poly(*S*-((*S*)-3-2-ammonio-2-carboxylatoethoxy)-2-hydroxypropyl)-L-homocysteine), 10**

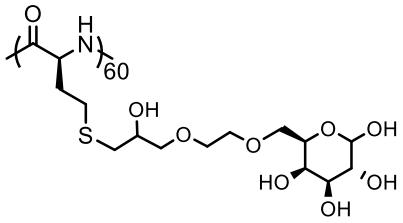
Prepared from **10a** using *Demethylation Procedure B*. Deprotection conditions: $H_2O$ (7.5 µL per µmol **R-C$^{H}$**) and $K_2CO_3$ (10 eq per **R-C$^{H}$**) were added. Allowed to stir vigorously at 40 $^{o}$C for 48h. Dialysis conditions: 50% $MeOH_{(aq)}$ containing 3 mM $NH_3$ (24h, 3 solvent changes) followed by $H_2O$ (8h, 3 $H_2O$ changes).

$^{1}$H NMR (400 MHz, $D_2O$, 25 $^{o}$C): δ *4.8-4.7 (1H)*$^{*}$, 4.61-4.16 (br m, 1H), 4.15-3.82 (br m, 3H), 3.82-3.45 (br m, 2H), 3.15-2.48 (br m 4H), 2.48-1.84 (br m, 2H). $^{*}$Obscured by solvent residual peak.



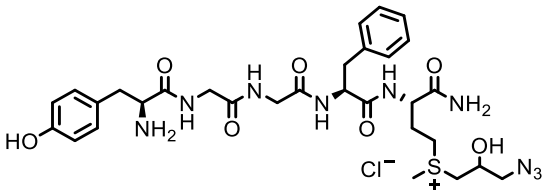
**Poly(*S*-(2-hydroxy-4,7,10,13-tetraoxatetradecyl)-L-homocysteine), 11**

Prepared from **11a** using *Demethylation Procedure C.*
$^1$H NMR (400 MHz, $D_2O$, 25 $^oC$): δ 4.50-4.15 (br m, 1H), 4.07-3.92 (br m, 1H), 3.85-3.51 (br m, 14H), 3.41 (s, 3H), 3.10-2.57 (br m, 4H), 2.57-1.96 (br m, 2H).

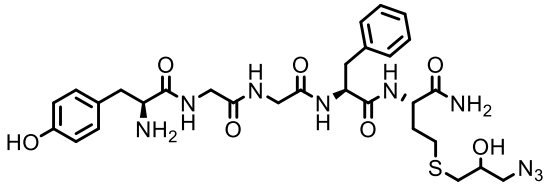


**Poly(*S*-((3-(2-(6-deoxy-D-galactopyranosid-6-yl)oxy)ethoxy)-2-hydroxypropyl)-L-homocysteine), 12**
Prepared from **12a** using *Demethylation Procedure C.* The product was found to contain a 1:2 ratio of α:β anomers ($^1$H NMR) in $D_2O$ at 25 $^oC$. Identification of anomers based on reported spectral assignments of D-galactose.[6]
$^1$H NMR (400 MHz, $D_2O$, 25 $^oC$): δ 5.29 (m, 0.34H), 4.62 (d, *J* = 7.8 Hz, 0.66H), 4.54-4.29 (br m, 1H), 4.29-3.41 (br m, 13H), 3.10-2.56 (br m, 4H), 2.56-1.84 (br m, 2H).

**7) Peptide Modifications**



**H-YGGF($M^{N3}$)-$NH_2$, 14**
A 35 mM solution of **13** in AcOH was prepared. A 150 mM solution of glycidyl azide in AcOH was prepared immediately before use. The solution of **13** (0.11 mL, 3.8 μmol, 1.0 eq) was treated with the glycidyl azide solution (0.25 mL, 38 μmol, 10 eq). The mixture was stirred on a 30 $^oC$ $H_2O$ bath for 24h. The volatiles were removed under high vacuum at 22 $^oC$. The residue was triturated with $Et_2O$ (2x 1.0 mL) then dissolved in 10 mM $HCl_{(aq)}$ (1 mL). The solution was lyophilized to provide **14** (2.4 mg, 88% yield) as a colorless amorphous solid.
ESI-MS *m/z* = 672.2780 $[M]^+$ (calcd 672.2927 for $C_{30}H_{42}N_9O_7S$).

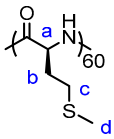


**H-YGGF($N_3$-$C^H$)-$NH_2$, 15**
**14** (2.2 mg, 3.3 μmol, 1.0 eq) was dissolved in an 82 mM **APDC** solution in 75% $EtOH_{(aq)}$ (0.40 mL, 33 μmol, 10 eq). The solution was stirred for 26h under $N_2$, then directly analyzed by HPLC-MS. Crude **15** was found to be 84% pure (% a/a) by UV (280.4 nm). ESI-MS concomitantly showed **[15+TFA]**$^-$ (calcd: 770.3 *m/z*, found: 770.2 *m/z*). The reaction mixture was also analyzed by high resolution ESI-MS.

ESI-MS *m/z* = 658.2784 $[M+H]^+$ (calcd 658.2771 for $C_{29}H_{40}N_9O_7S$).

**References**

(1) Vedejs, E.; Engler, D.A.; Mullins, M.J. *J. Org. Chem.,* **1977**, *42*, 3109-3113.
(2) Gharakhanian, E.G.; Deming, T.J. *Biomacromolecules*, **2015**, *16*, 1802-1806.
(3) Kramer, J.R.; Deming, T.J. *Biomacromolecules*, **2012**, *13*, 1719-1713.
(4) Molander, G.A.; Harris, C.R. *J. Org. Chem.,* **1997**, *62*, 2944-2956.
(5) Glenn, M.P.; Pattenden, L.K.; Reid, R.C.; Tyssen, D.P.; Tyndall, J.D.A. *J. Med. Chem.,* **2002**, *45*, 371-381.
(6) Spectral Database for Organic Compounds (SDBS); 1H NMR Spectra; SDBS No.: 1183; RN 10257-28-0; http://riodb01.ibase.aist.go.jp/sdbs/ (accessed December 1, 2015).




**1**

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DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
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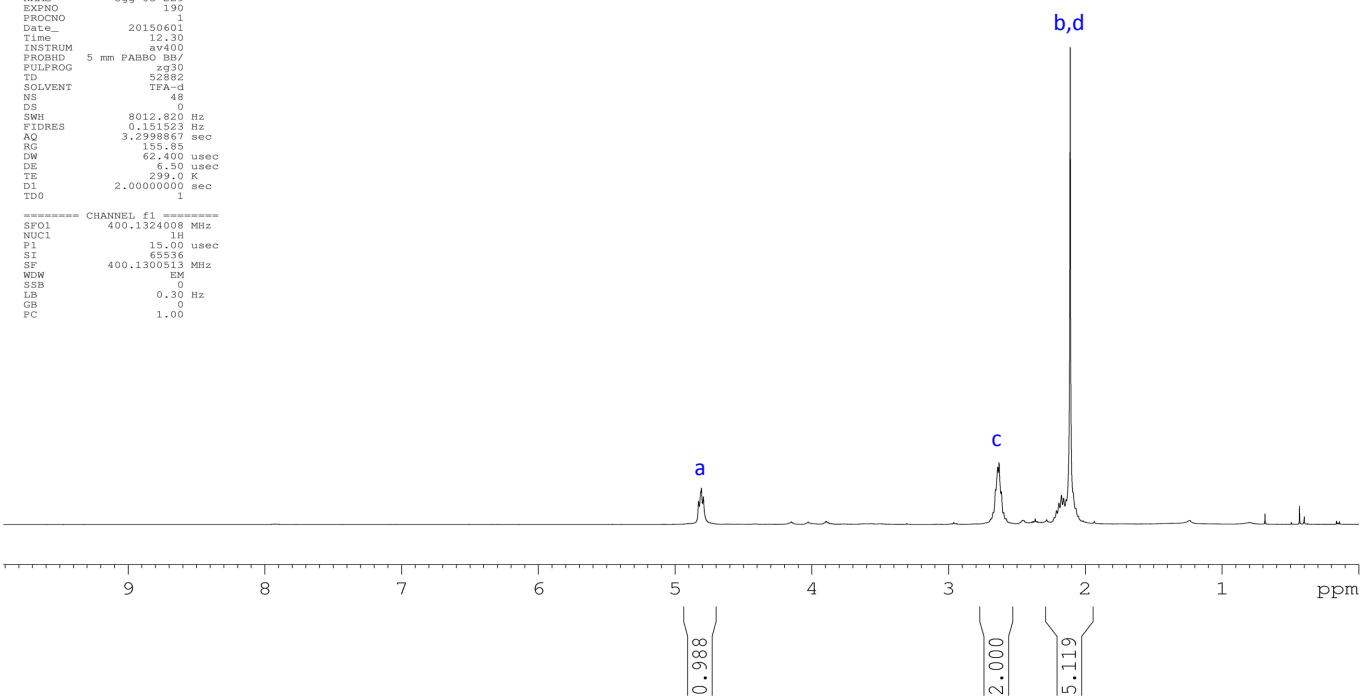


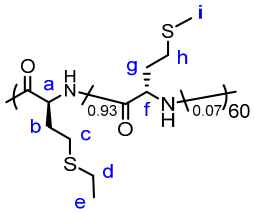


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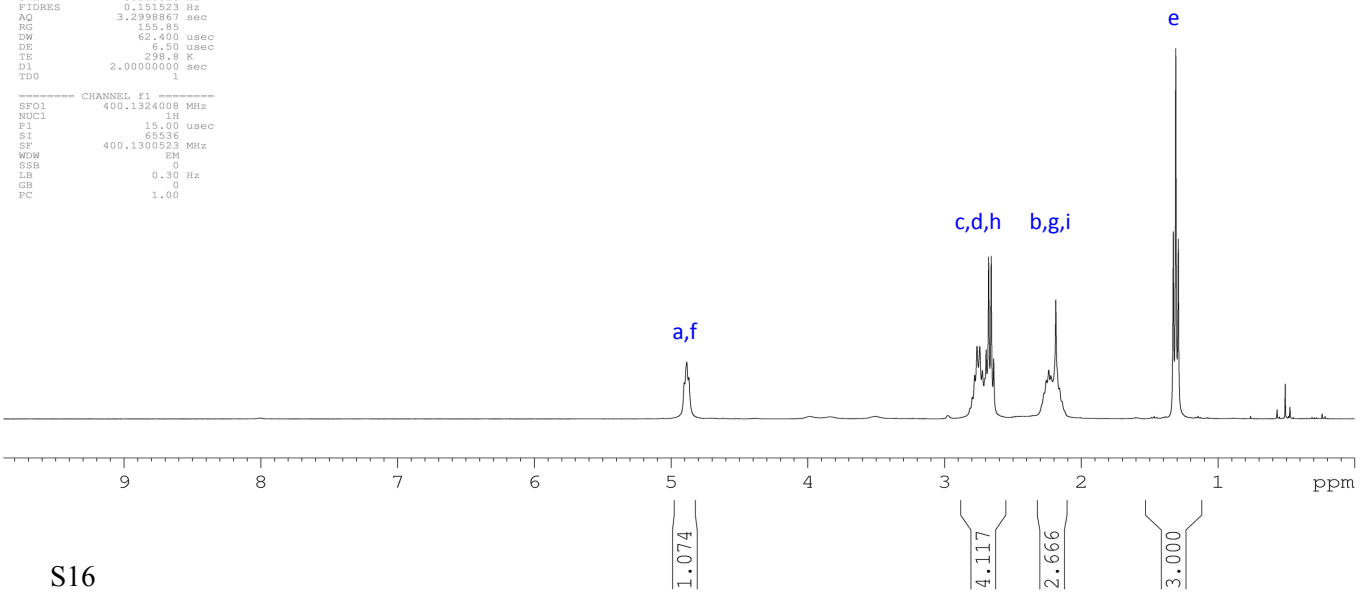
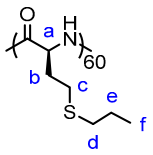
LB                    0.30 Hz
GB                       0
PC                    1.00
```

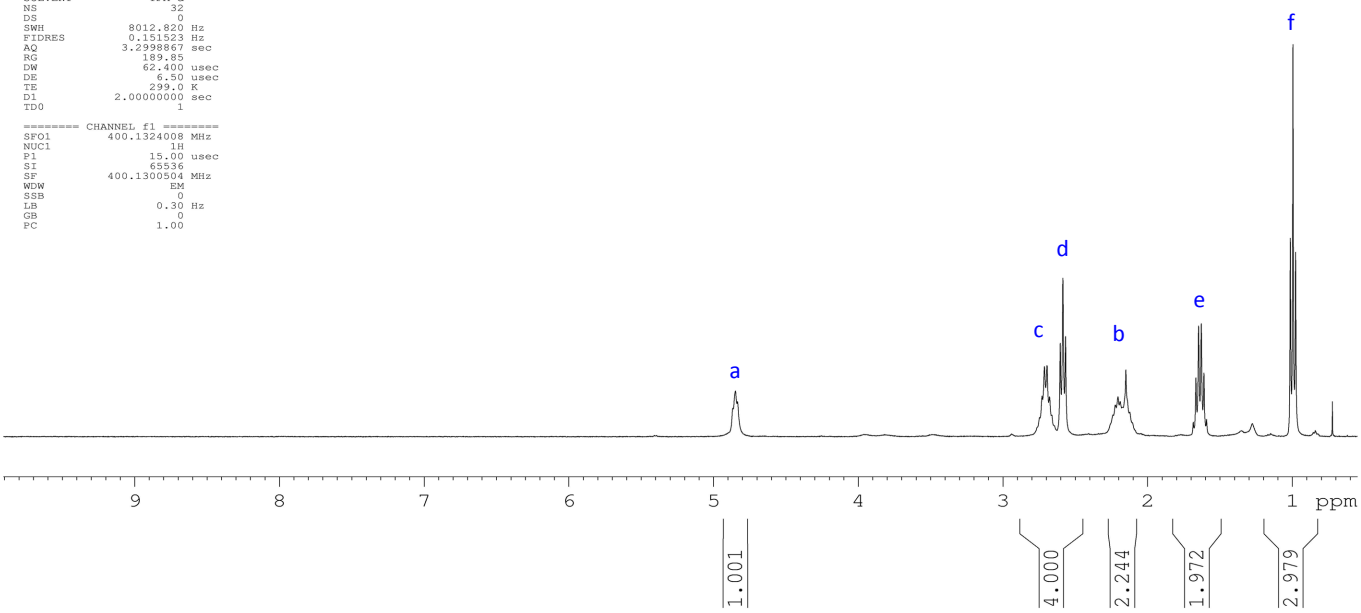


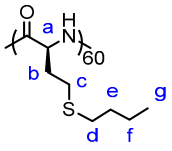



**3**

```
NAME            egg-04-007
EXPNO                  130
PROCNO                   1
Date_             20150727
Time                 12.16
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT              TFA-d
NS                      32
DS                       0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  189.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1300504 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

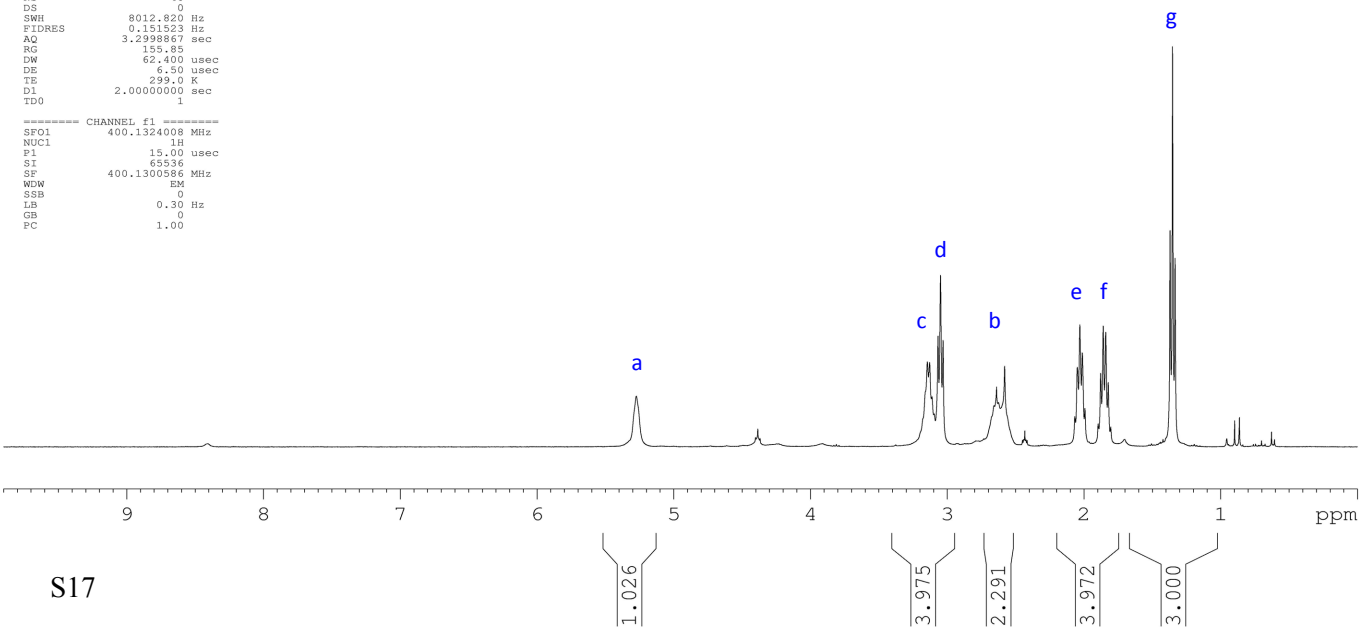


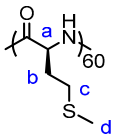




**4**

```
NAME            egg-03-277
EXPNO                  510
PROCNO                   1
Date_             20150626
Time                 21.20
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT              TFA-d
NS                      40
DS                       0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1300586 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

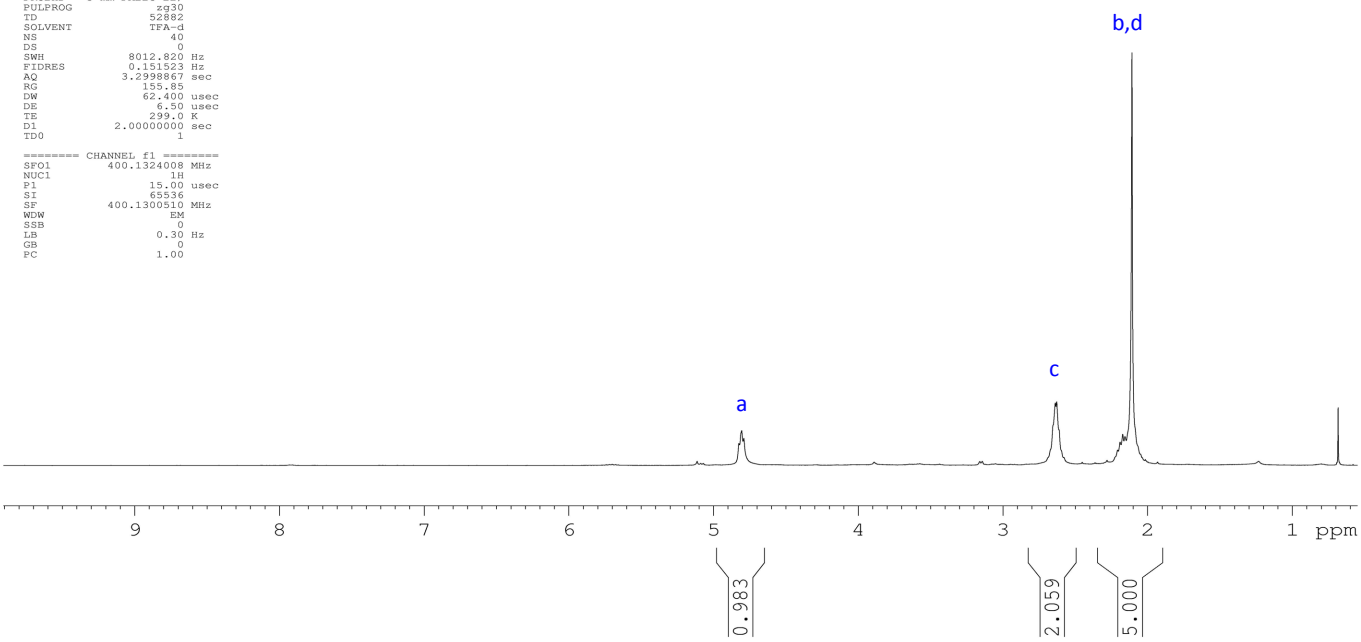


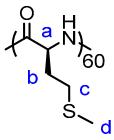



**5**

```
NAME            egg-03-235
EXPNO                  640
PROCNO                   1
Date_             20150602
Time                 21.17
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT              TFA-d
NS                      40
DS                       0
SWH               8012.820 Hz
FIDRES               0.151523 Hz
AQ                3.2998867 sec
RG                   155.85
DW                   62.400 usec
DE                     6.50 usec
TE                    299.0 K
D1                2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            400.1324008 MHz
NUC1                    1H
P1                    15.00 usec
SI                    65536
SF              400.1300510 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

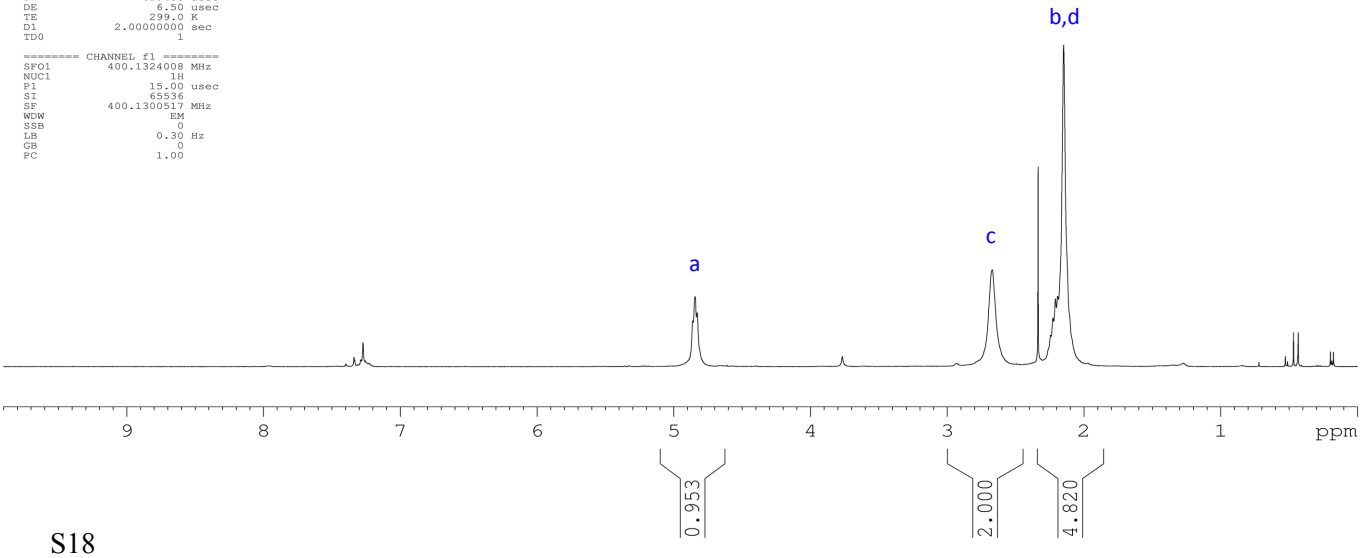


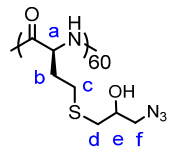




**6**

```
NAME            egg-03-284
EXPNO                  360
PROCNO                   1
Date_             20150630
Time                 16.31
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT              TFA-d
NS                      32
DS                       0
SWH               8012.820 Hz
FIDRES               0.151523 Hz
AQ                3.2998867 sec
RG                   155.85
DW                   62.400 usec
DE                     6.50 usec
TE                    299.0 K
D1                2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1            400.1324008 MHz
NUC1                    1H
P1                    15.00 usec
SI                    65536
SF              400.1300517 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

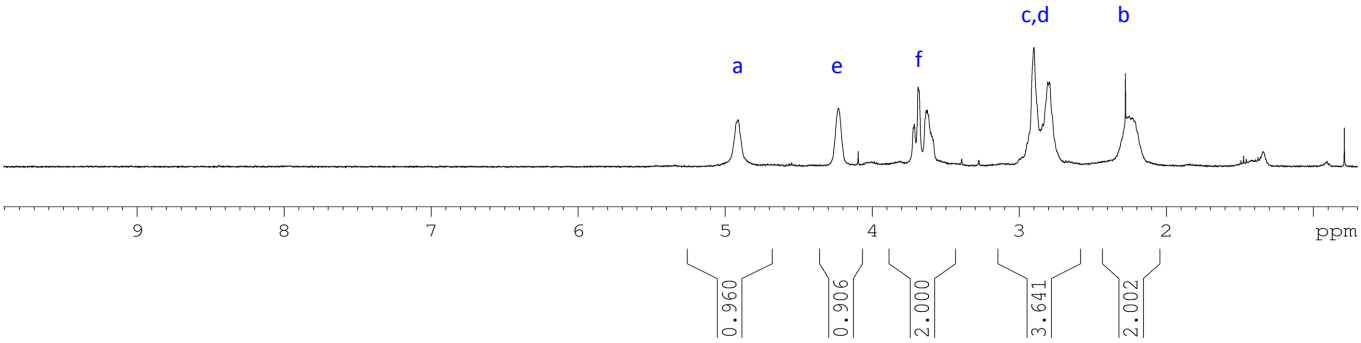


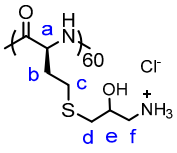



**7**

```
NAME            egg-04-054
EXPNO                  240
PROCNO                   1
Date_             20150826
Time                 15.15
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT              TFA-d
NS                      40
DS                       0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  189.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1300532 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

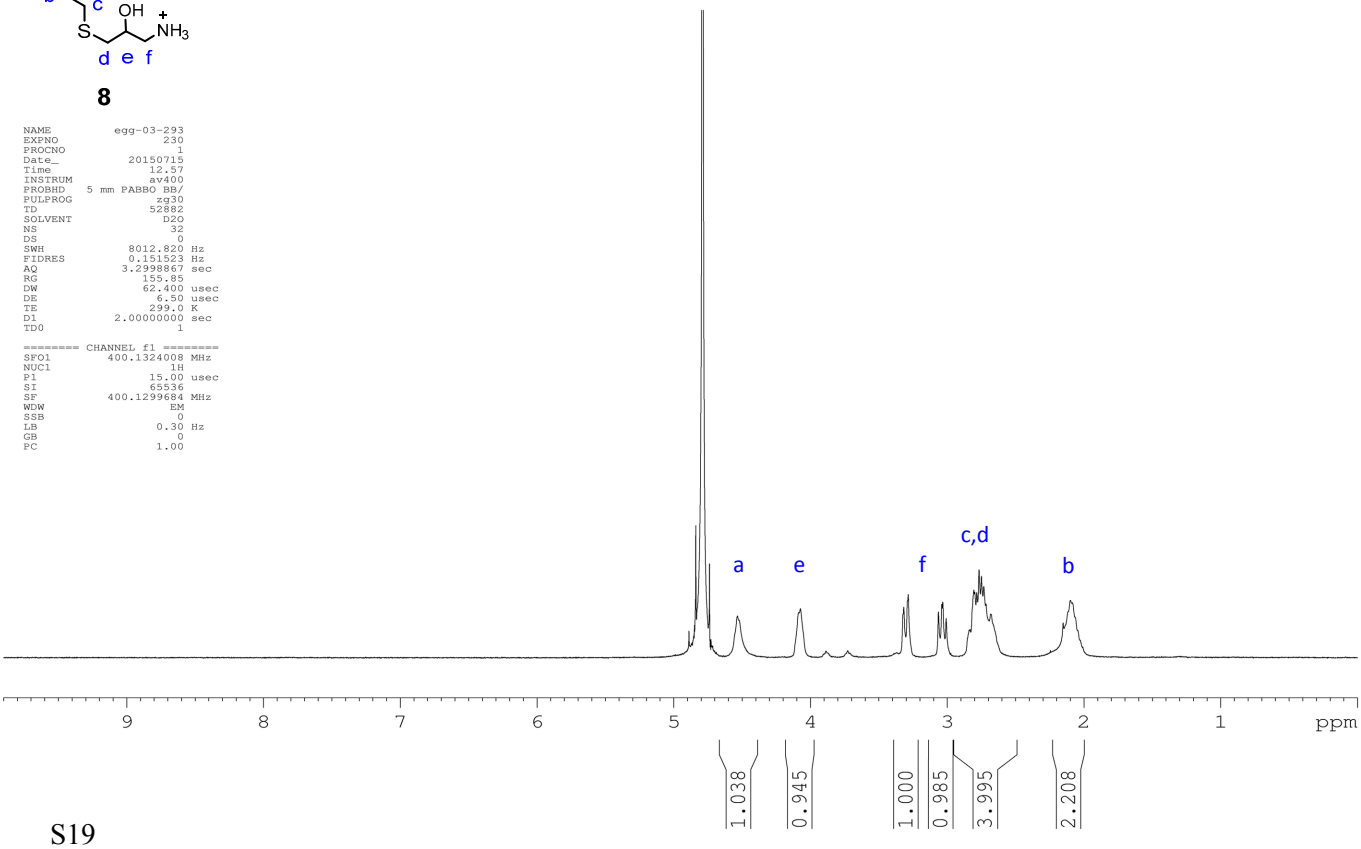


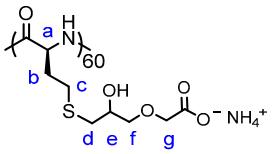




**8**

```
NAME            egg-03-293
EXPNO                  230
PROCNO                   1
Date_             20150715
Time                 12.57
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT                D2O
NS                      32
DS                       0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1299684 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

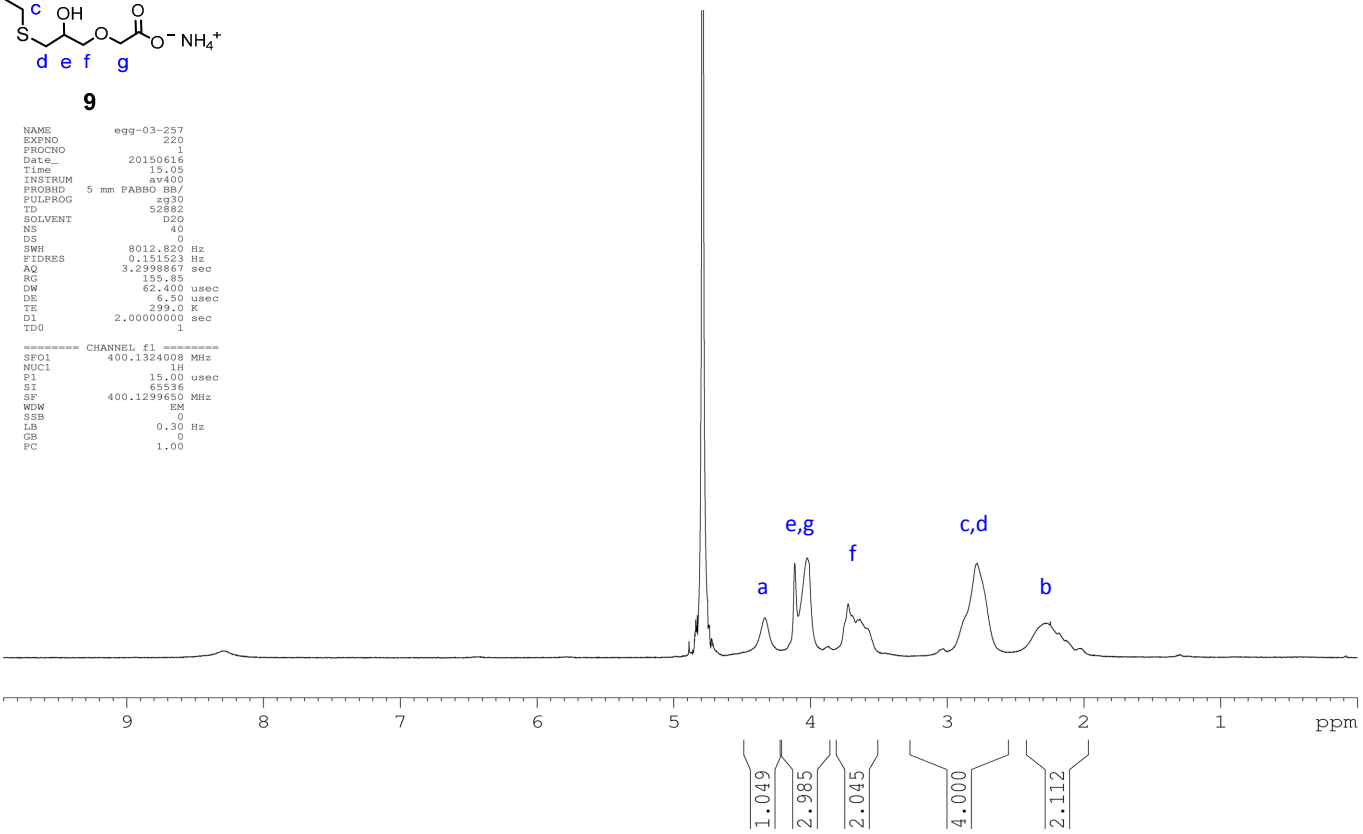


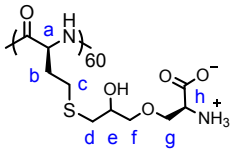



**9**

```
NAME            egg-03-257
EXPNO                  220
PROCNO                   1
Date_             20150616
Time                 15.05
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     40
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1              2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299650 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

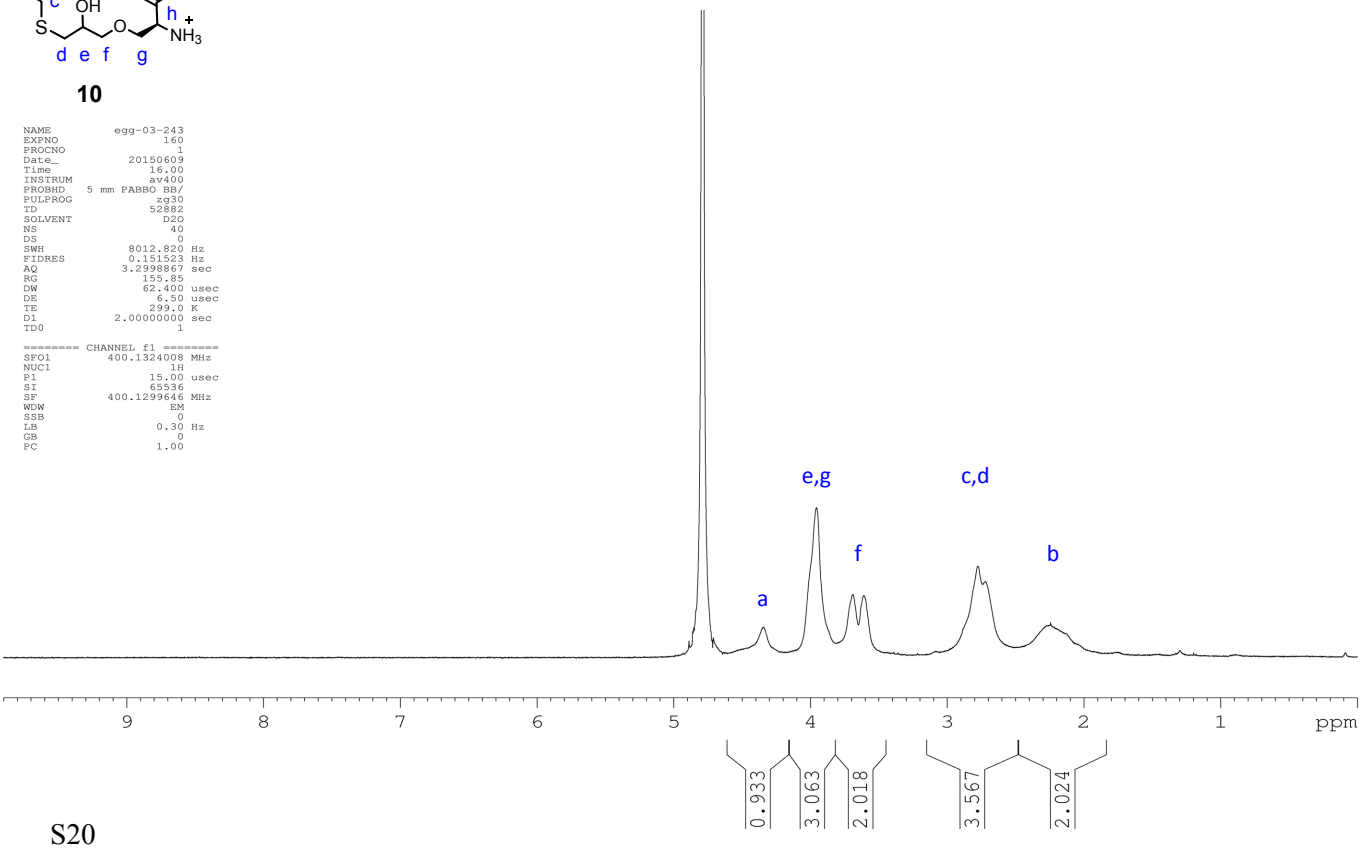


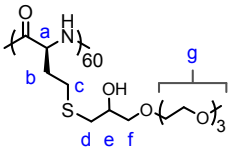




**10**

```
NAME            egg-03-243
EXPNO                  160
PROCNO                   1
Date_             20150609
Time                 16.00
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     40
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1              2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299646 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

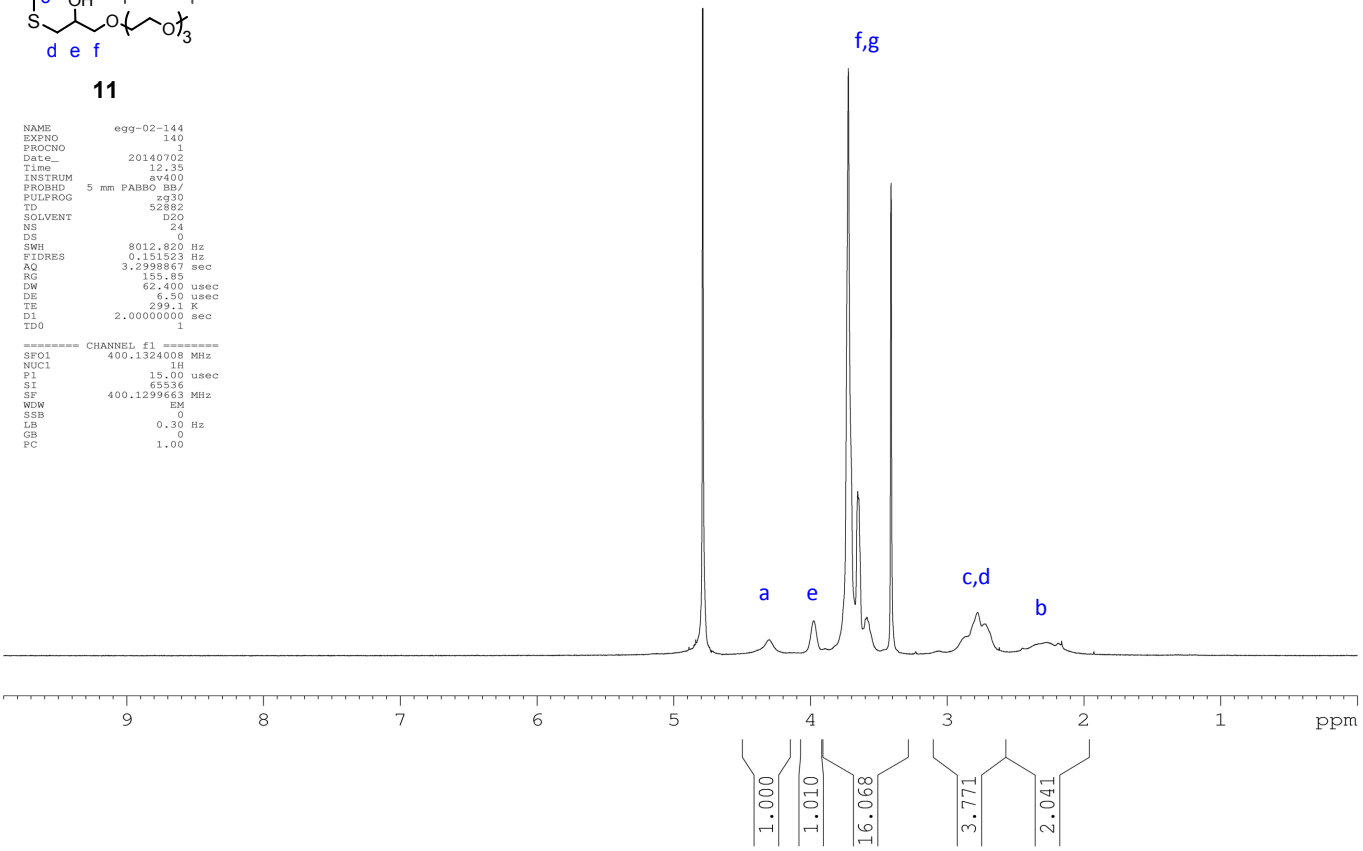


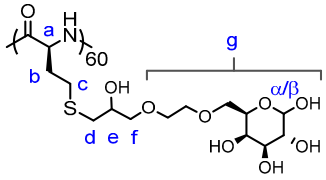



**11**

```
NAME            egg-02-144
EXPNO                  140
PROCNO                   1
Date_             20140702
Time                 12.35
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     24
DS                      0
SWH             8012.820 Hz
FIDRES          0.151523 Hz
AQ              3.2998867 sec
RG                 155.85
DW                 62.400 usec
DE                  6.50 usec
TE                 299.1 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299663 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

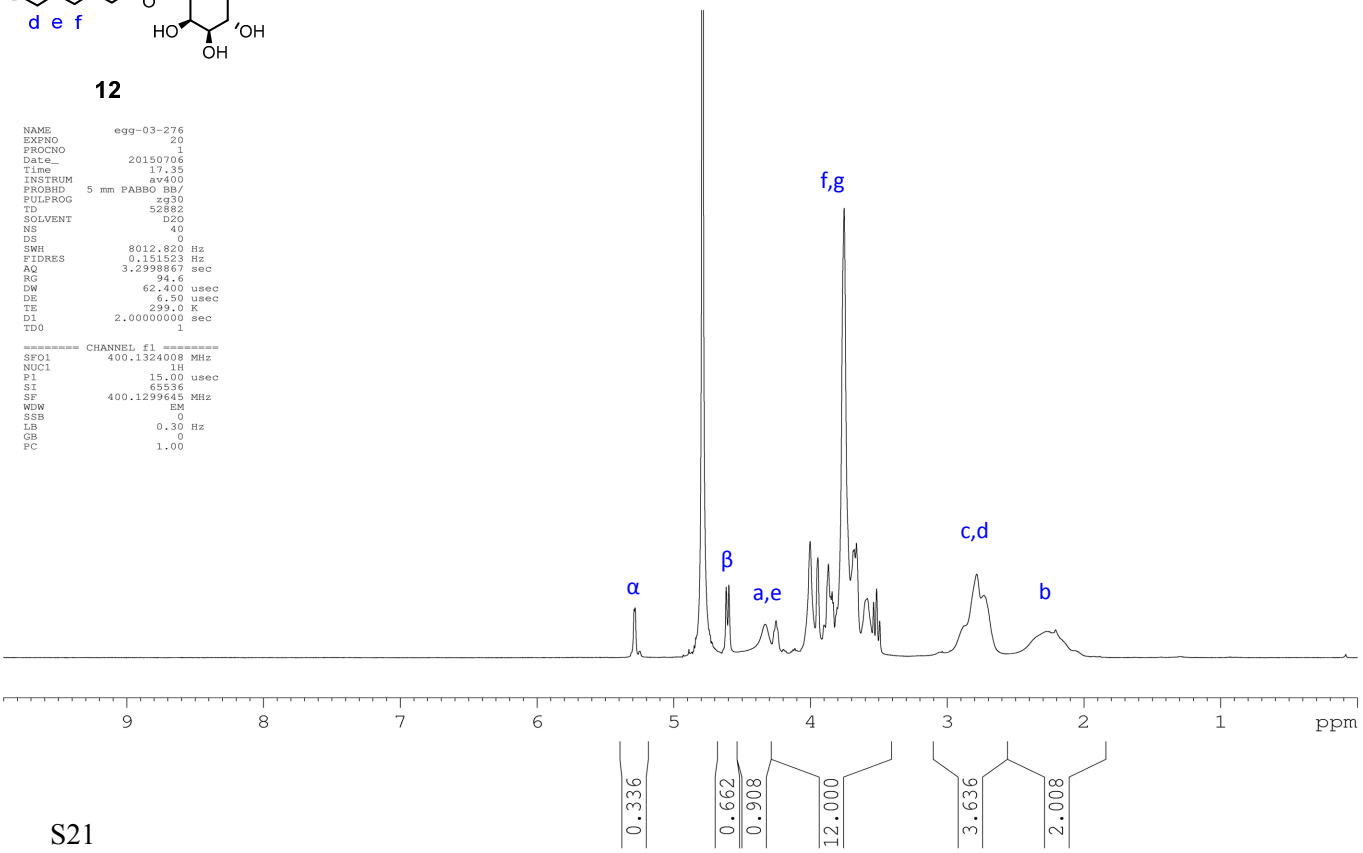


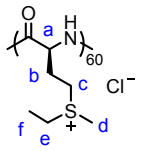




**12**

```
NAME            egg-03-276
EXPNO                   20
PROCNO                   1
Date_             20150706
Time                 17.35
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     40
DS                      0
SWH             8012.820 Hz
FIDRES          0.151523 Hz
AQ              3.2998867 sec
RG                  94.6
DW                 62.400 usec
DE                  6.50 usec
TE                 299.0 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299645 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

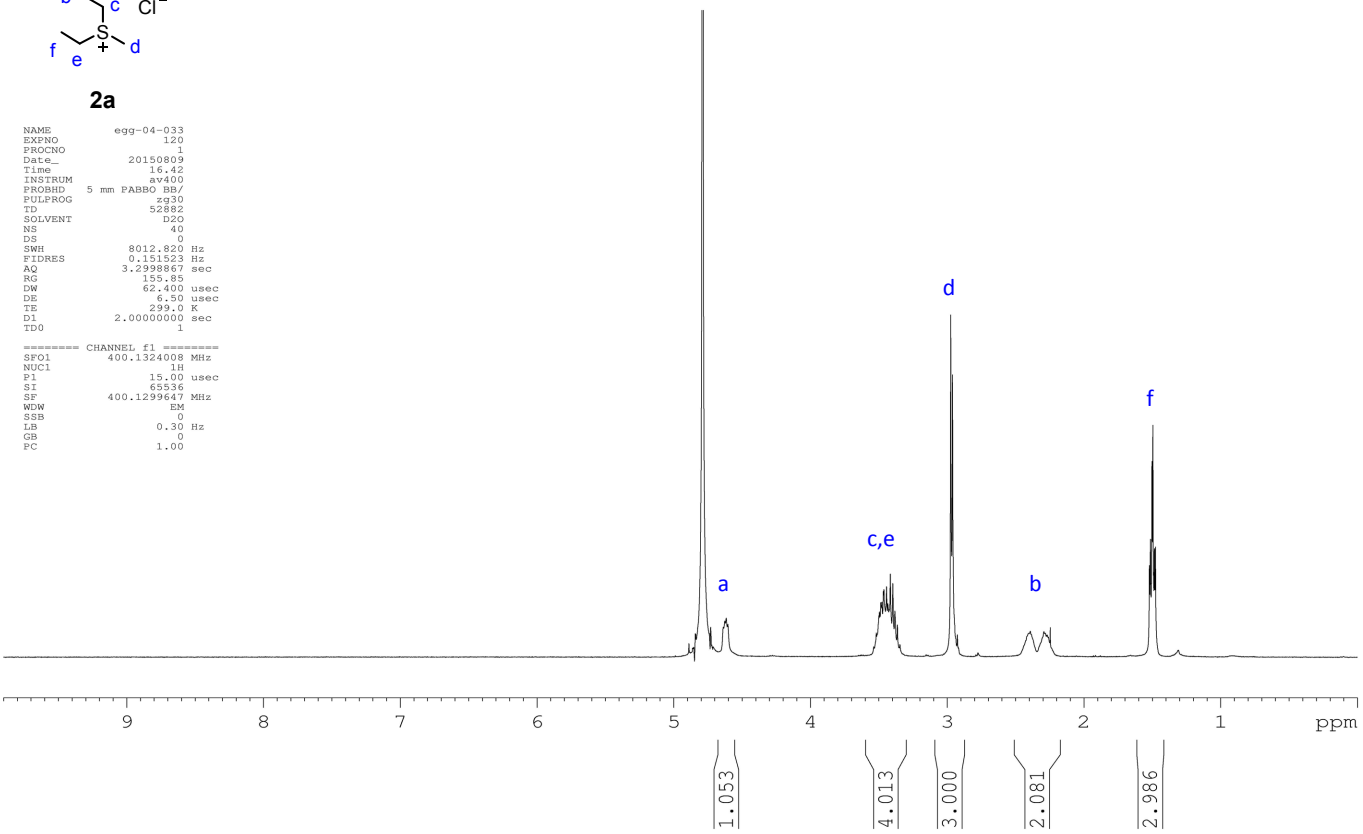


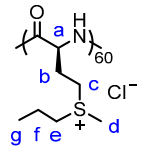



**2a**

```
NAME            egg-04-033
EXPNO                  120
PROCNO                   1
Date_             20150809
Time                 16.42
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     40
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299647 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

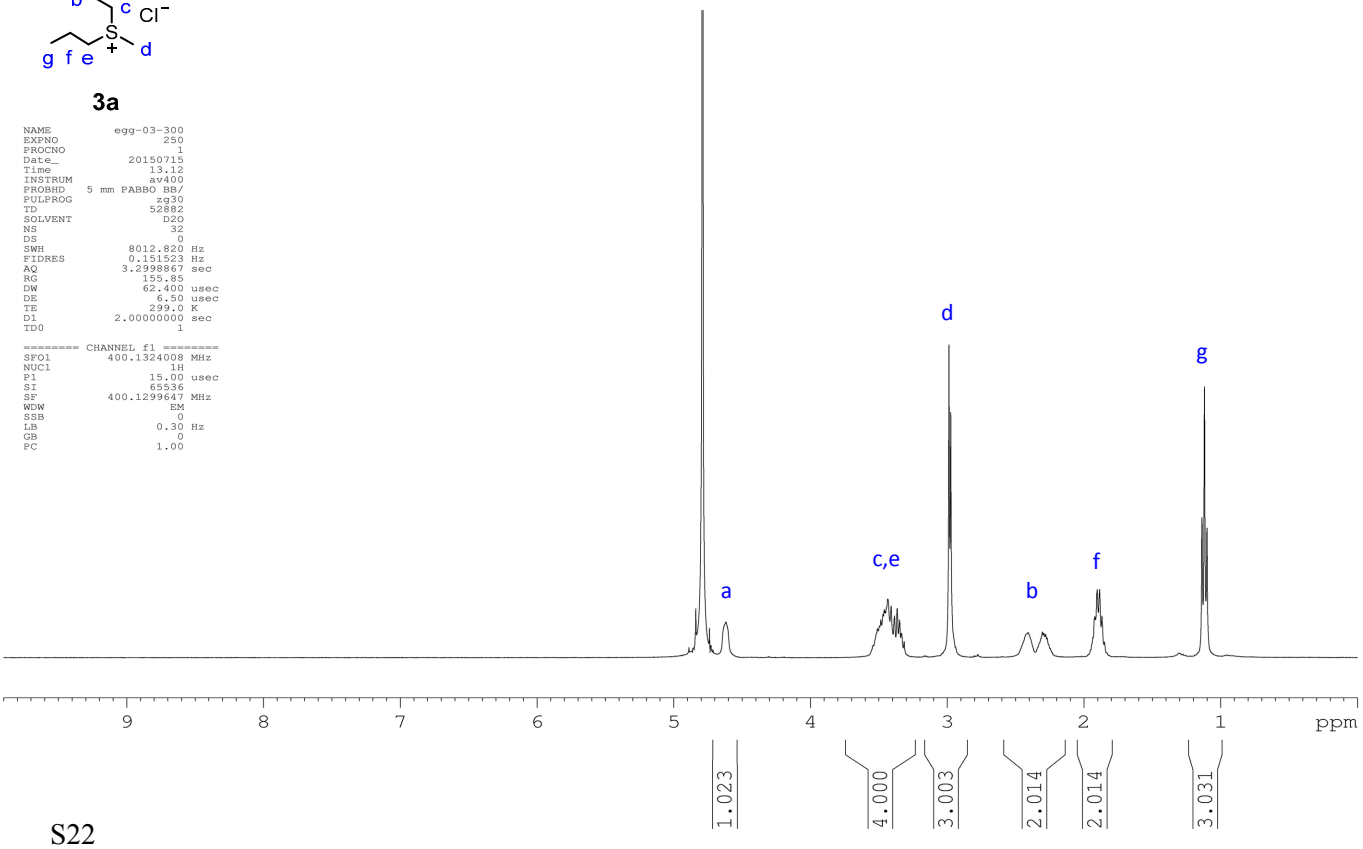


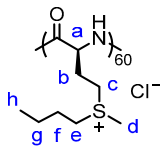




**3a**

```
NAME            egg-03-300
EXPNO                  250
PROCNO                   1
Date_             20150715
Time                 13.12
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     32
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299647 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

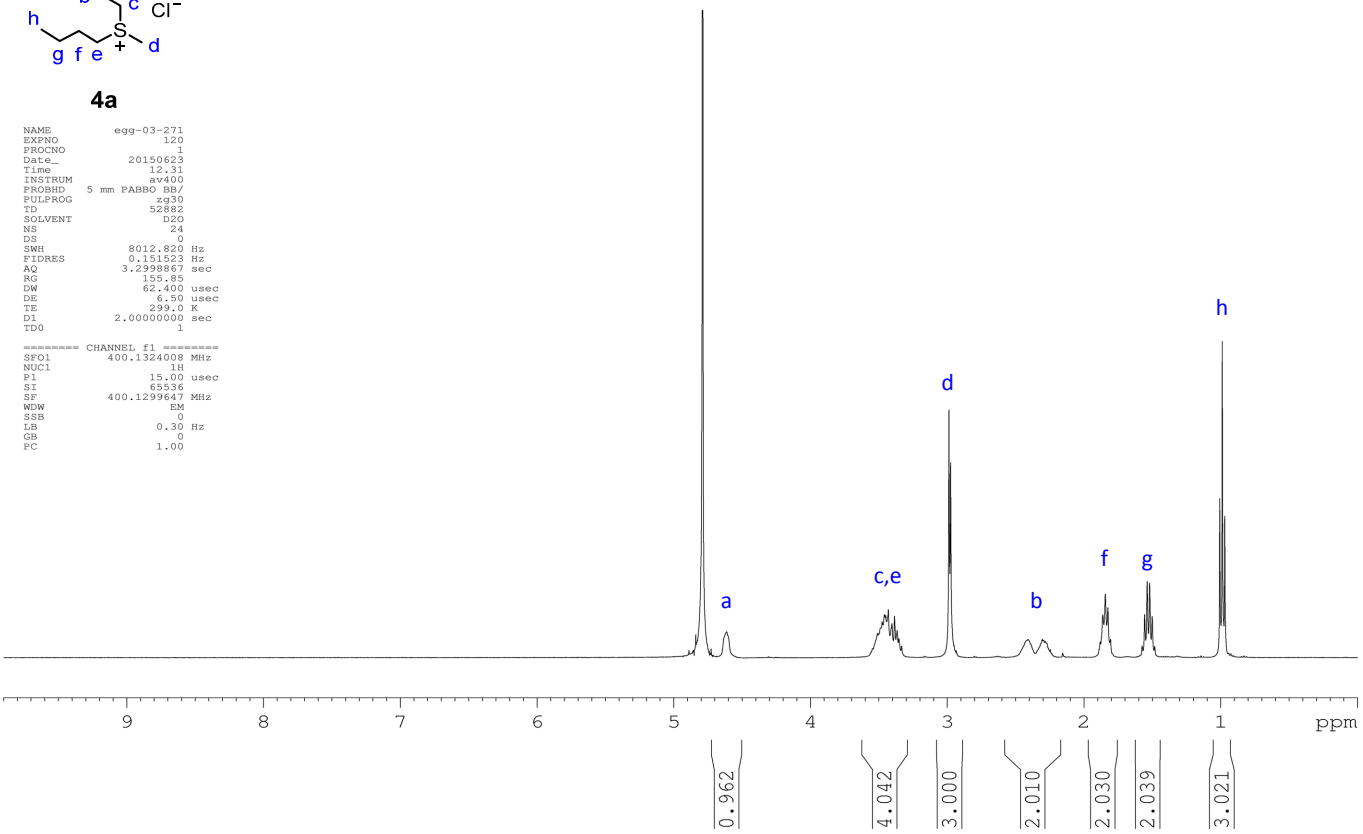


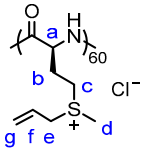



**4a**

```
NAME            egg-03-271
EXPNO                  120
PROCNO                   1
Date_             20150623
Time                 12.31
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     24
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  155.85
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1299647 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

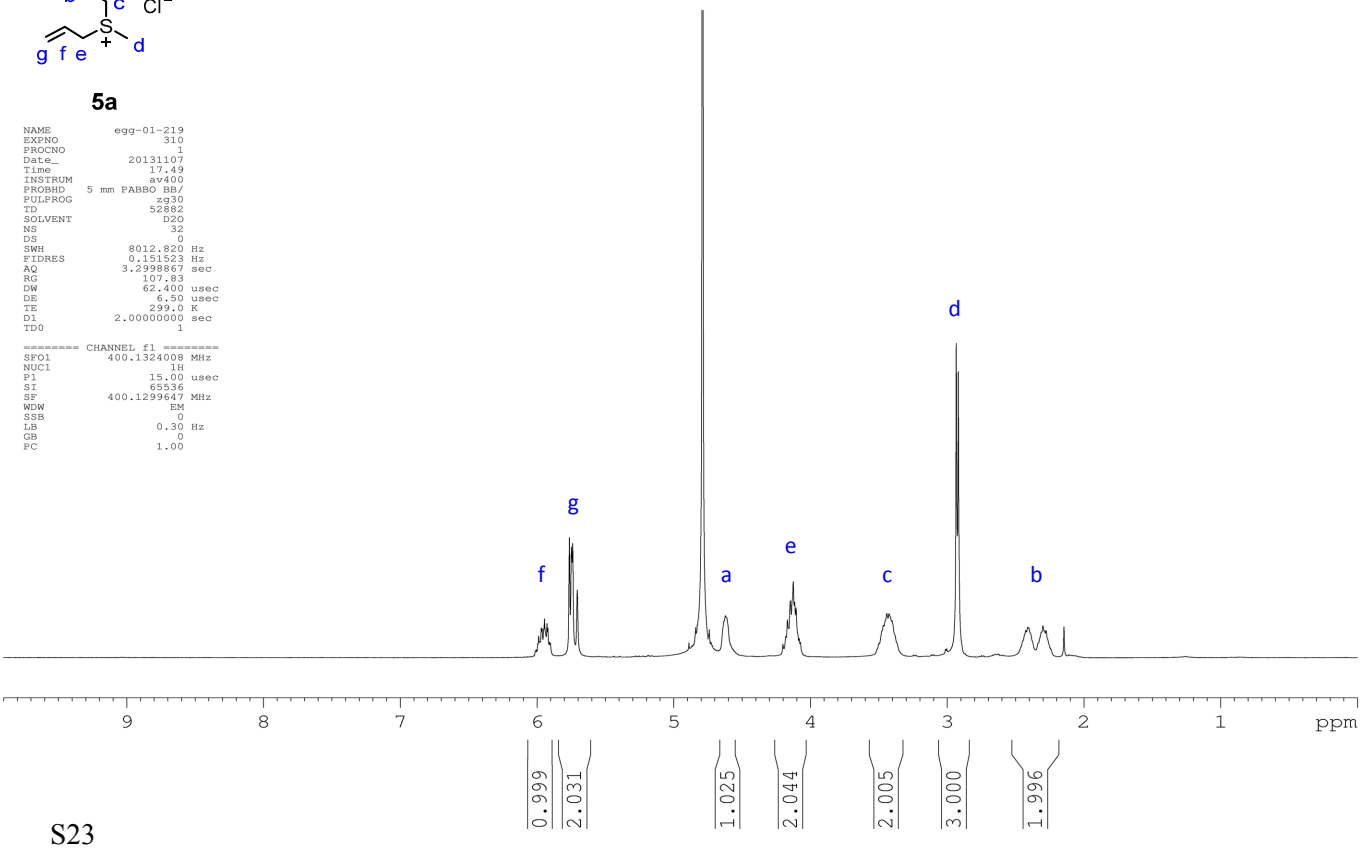


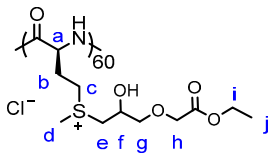




**5a**

```
NAME            egg-01-219
EXPNO                  310
PROCNO                   1
Date_             20131107
Time                 17.49
INSTRUM              av400
PROBHD   5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     32
DS                      0
SWH              8012.820 Hz
FIDRES           0.151523 Hz
AQ               3.2998867 sec
RG                  107.83
DW                  62.400 usec
DE                    6.50 usec
TE                  299.0 K
D1               2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                    1H
P1                   15.00 usec
SI                   65536
SF            400.1299647 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```

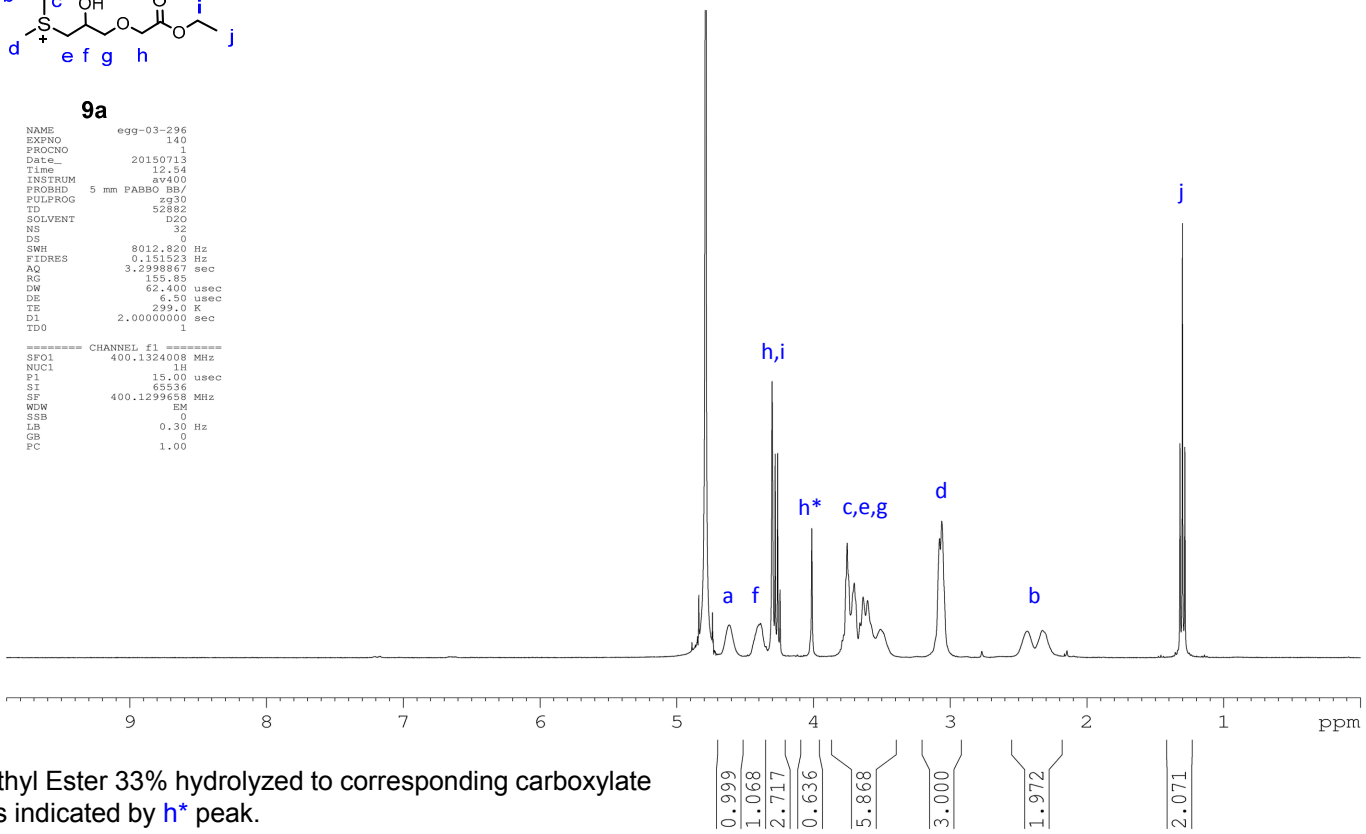




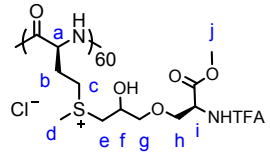

**9a**

```
NAME            egg-03-296
EXPNO                  140
PROCNO                   1
Date_             20150713
Time                 12.54
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     32
DS                      0
SWH             8012.820 Hz
FIDRES             0.151523 Hz
AQ              3.2998867 sec
RG                 155.85
DW                 62.400 usec
DE                   6.50 usec
TE                 299.0 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299658 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```



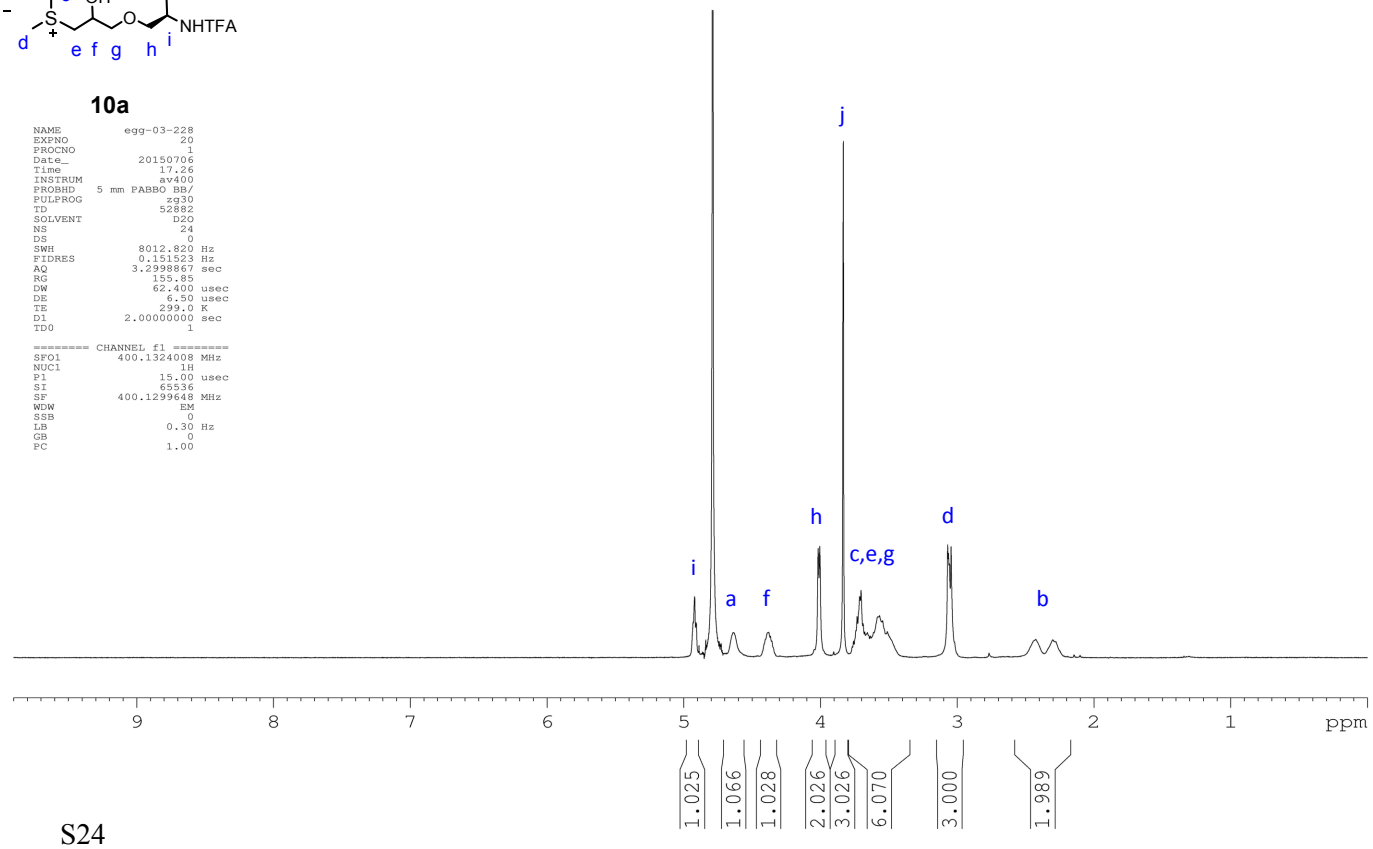

Ethyl Ester 33% hydrolyzed to corresponding carboxylate as indicated by h* peak.

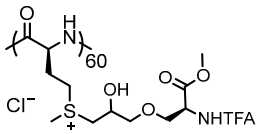



**10a**

```
NAME            egg-03-228
EXPNO                   20
PROCNO                   1
Date_             20150706
Time                 17.26
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG              zg30
TD                  52882
SOLVENT               D2O
NS                     24
DS                      0
SWH             8012.820 Hz
FIDRES             0.151523 Hz
AQ              3.2998867 sec
RG                 155.85
DW                 62.400 usec
DE                   6.50 usec
TE                 299.0 K
D1              2.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                   1H
P1                  15.00 usec
SI                  65536
SF            400.1299648 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```

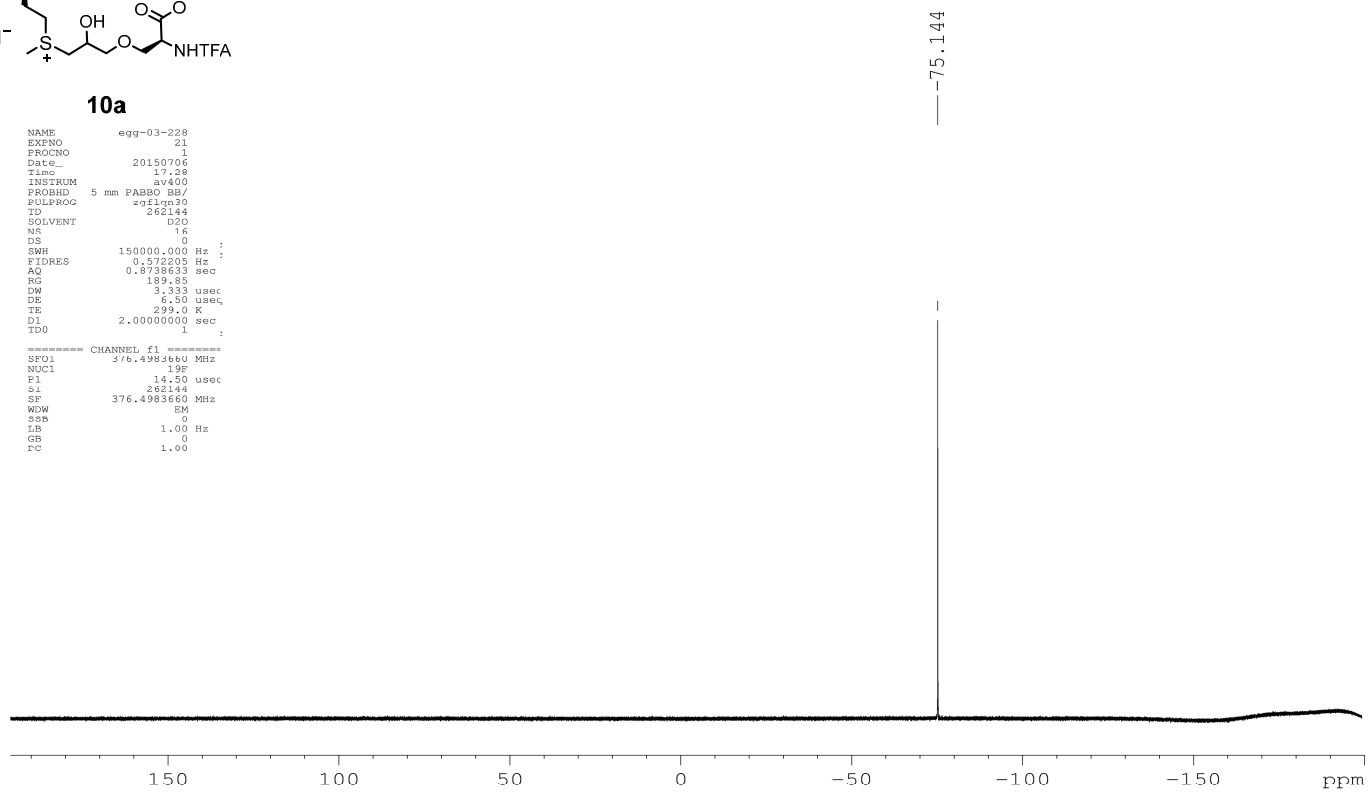


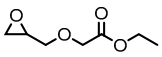


**10a**

```
NAME          egg-03-228
EXPNO                 21
PROCNO                 1
Date_           20150706
Time               17.28
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG        zgflqn30
TD               262144
SOLVENT             D2O
NS                   16
DS                    0
SWH          150000.000 Hz
FIDRES         0.572205 Hz
AQ            0.8738633 sec
RG               189.85
DW                3.333 usec
DE                6.50 usec
TE               299.0 K
D1            2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        376.4983660 MHz
NUC1               19F
P1               14.50 usec
SI             262144
SF          376.4983660 MHz
WDW                EM
SSB                 0
LB               1.00 Hz
GB                  0
PC               1.00
```

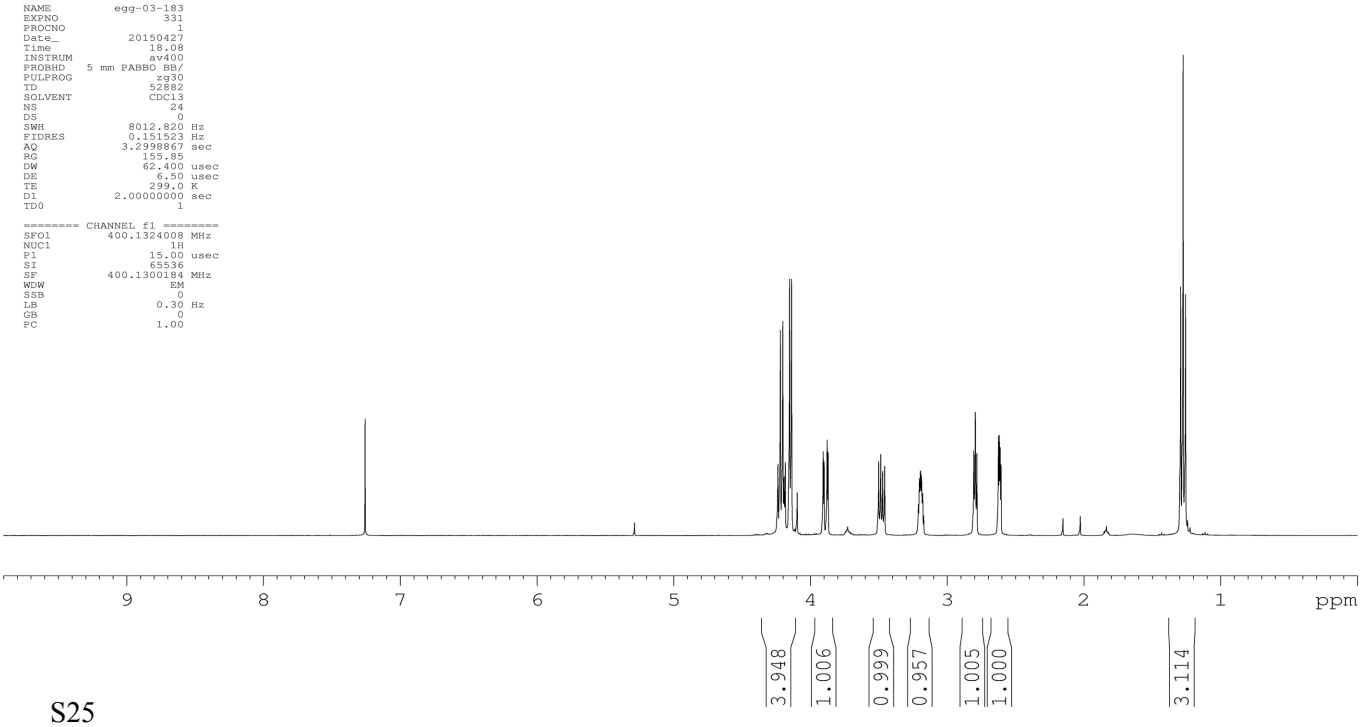


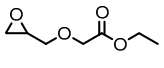



**9b**

```
NAME          egg-03-183
EXPNO                331
PROCNO                 1
Date_           20150427
Time               18.08
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD               52882
SOLVENT          CDCl3
NS                   24
DS                    0
SWH           8012.820 Hz
FIDRES        0.151523 Hz
AQ            3.2998867 sec
RG               155.85
DW               62.400 usec
DE                6.50 usec
TE               299.0 K
D1            2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        400.1324008 MHz
NUC1               1H
P1               15.00 usec
SI             65536
SF          400.1300184 MHz
WDW                EM
SSB                 0
LB               0.30 Hz
GB                  0
PC               1.00
```



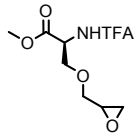


**9b**

```
NAME          egg-03-183
EXPNO                 50
PROCNO                 1
Date_           20151213
Time               15.57
INSTRUM            av400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  384
DS                    0
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976629 sec
RG                189.85
DW                19.800 usec
DE                 6.50 usec
TE                299.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1                13C
P1                10.00 usec
SI                65536
SF          100.6127701 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

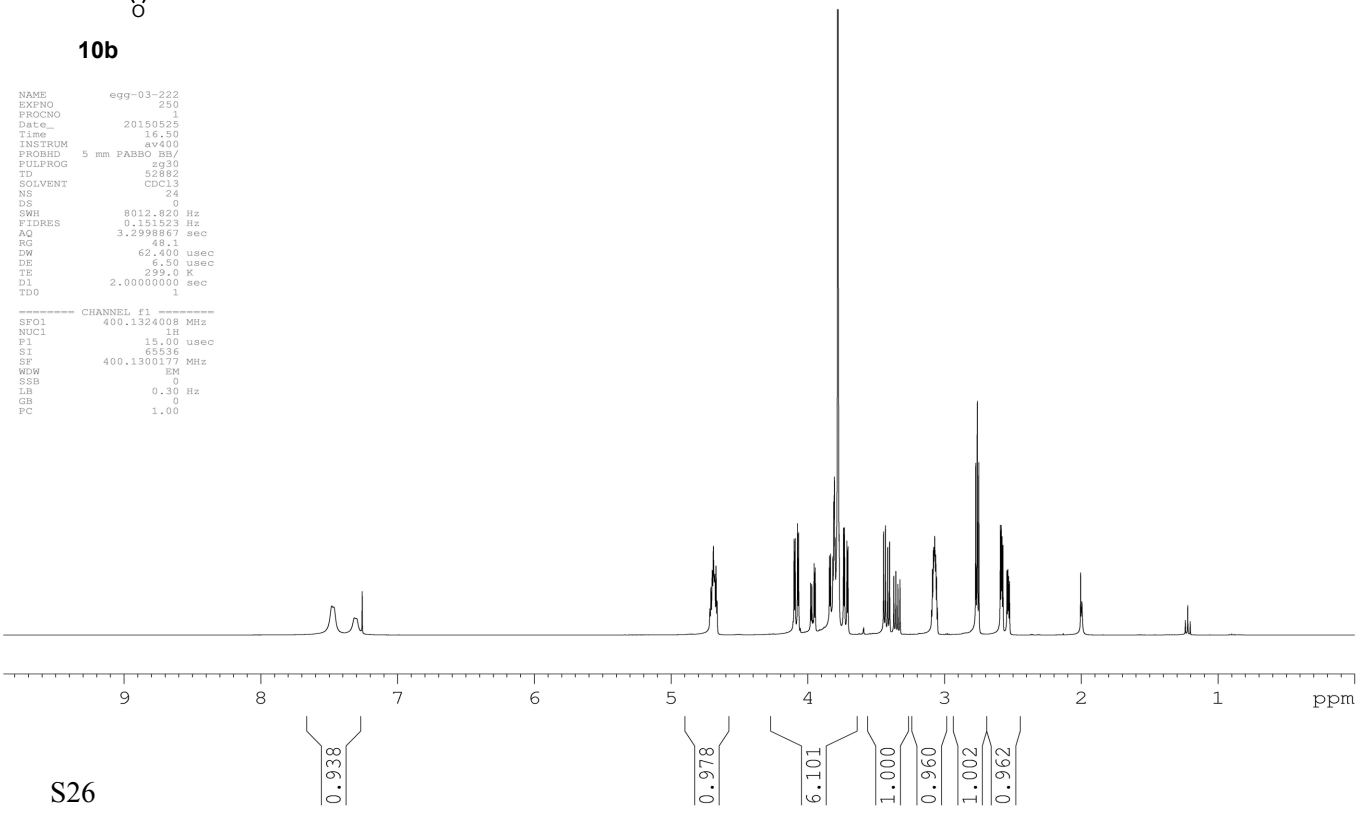


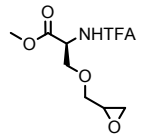




**10b**

```
NAME          egg-03-222
EXPNO                250
PROCNO                 1
Date_           20150525
Time               16.50
INSTRUM            av400
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 52882
SOLVENT           CDCl3
NS                    24
DS                    0
SWH            8012.820 Hz
FIDRES        0.151523 Hz
AQ            3.2998867 sec
RG                 48.1
DW                62.400 usec
DE                 6.50 usec
TE                299.0 K
D1           2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1        400.1324008 MHz
NUC1                 1H
P1                15.00 usec
SI                65536
SF          400.1300177 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```






**10b**

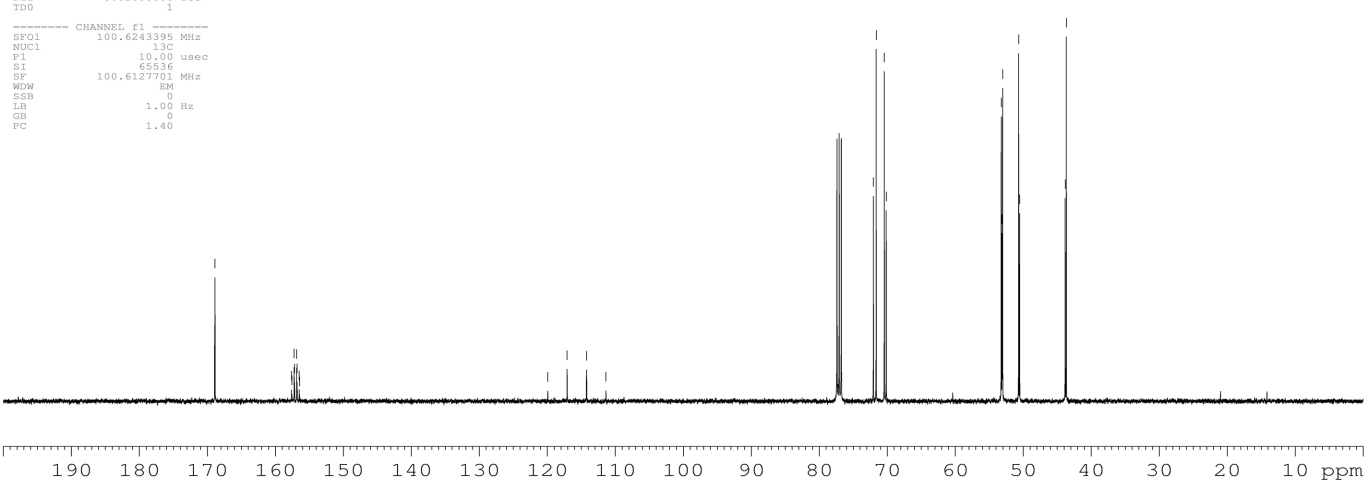
168.887
157.626
157.559
157.249
157.183
156.873
156.806
156.497
156.430

119.946
117.088
114.231
111.372

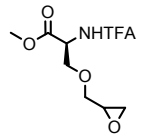
72.041
71.627
70.432
70.149
53.229
53.111
53.026
53.015
50.667
50.516
43.821
43.655

```
NAME          egg-03-222
EXPNO             252
PROCNO              1
Date_          20150525
Time              17.15
INSTRUM          av400
PROBHD   5 mm PABBO BB/
PULPROG        zgpg30
TD              65536
SOLVENT          CDCl3
NS                 400
DS                   0
SWH          25252.525 Hz
FIDRES           0.385323 Hz
AQ           1.2976629 sec
RG              189.85
DW              19.800 usec
DE               6.50 usec
TE              299.0 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1              13C
P1              10.00 usec
SI              65536
SF          100.6127701 MHz
WDW               EM
SSB                0
LB              1.00 Hz
GB                 0
PC              1.40
```



190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm




**10b**

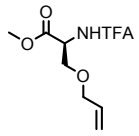
-75.871

```
NAME          egg-03-222
EXPNO             251
PROCNO              1
Date_          20150525
Time              16.52
INSTRUM          av400
PROBHD   5 mm PABBO BB/
PULPROG       zgfhigqn.2
TD             262144
SOLVENT          CDCl3
NS                  16
DS                   0
SWH          150000.000 Hz
FIDRES           0.572205 Hz
AQ           0.8738633 sec
RG              189.85
DW               3.333 usec
DE               6.50 usec
TE              299.0 K
D1           1.00000000 sec
D11          0.03000000 sec
D12          0.00002000 sec
TD0                 1

======== CHANNEL f1 ========
SFO1        376.4983660 MHz
NUC1              19F
P1              15.00 usec
SI             262144
SF          376.4983660 MHz
WDW               EM
SSB                0
LB              1.00 Hz
GB                 0
PC              1.00
```



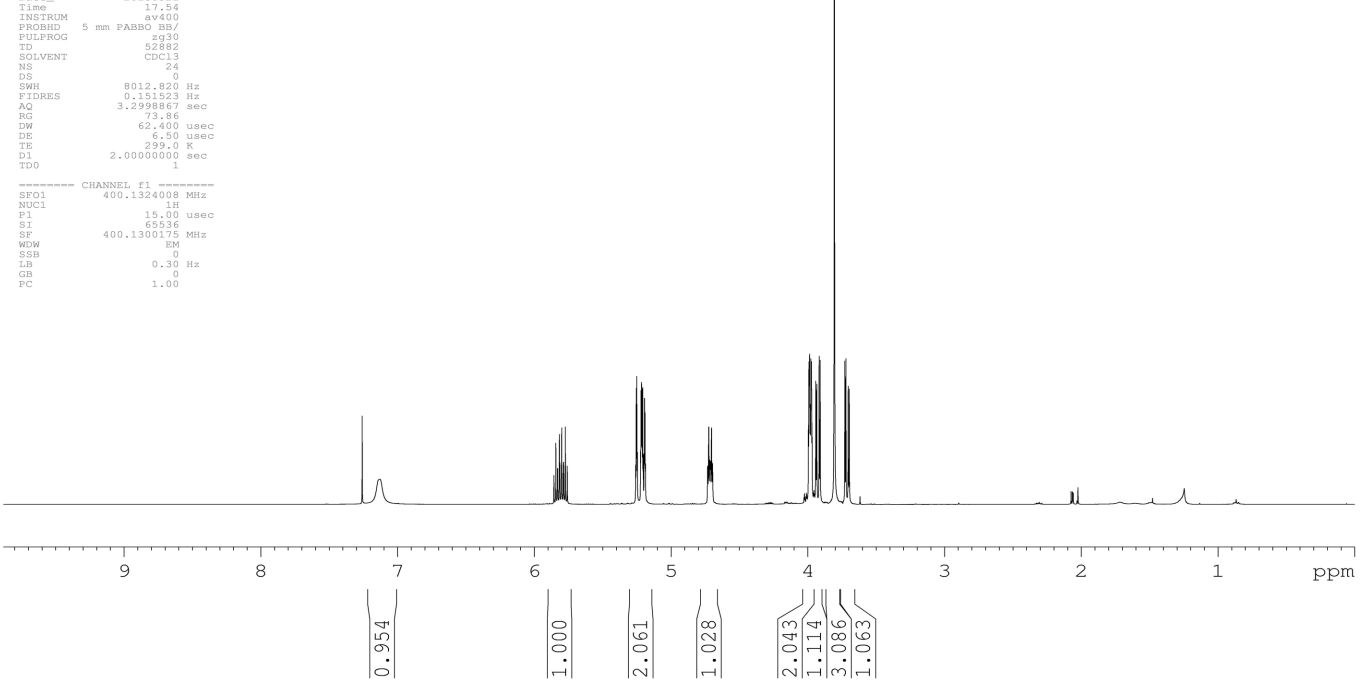
120 100 80 60 40 20 0 -20 -40 -60 -80 -100 ppm

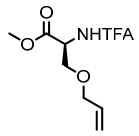



**10c**

```
NAME          egg-03-217
EXPNO                310
PROCNO                 1
Date_           20150521
Time               17.54
INSTRUM            av400
PROBHD   5 mm PABBO BB/
PULPROG             zg30
TD                 52882
SOLVENT            CDCl3
NS                    24
DS                     0
SWH             8012.820 Hz
FIDRES             0.151523 Hz
AQ              3.2998867 sec
RG                 73.86
DW                62.400 usec
DE                 6.50 usec
TE                299.0 K
D1             2.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          400.1324008 MHz
NUC1                  1H
P1                 15.00 usec
SI                 65536
SF            400.1300175 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```



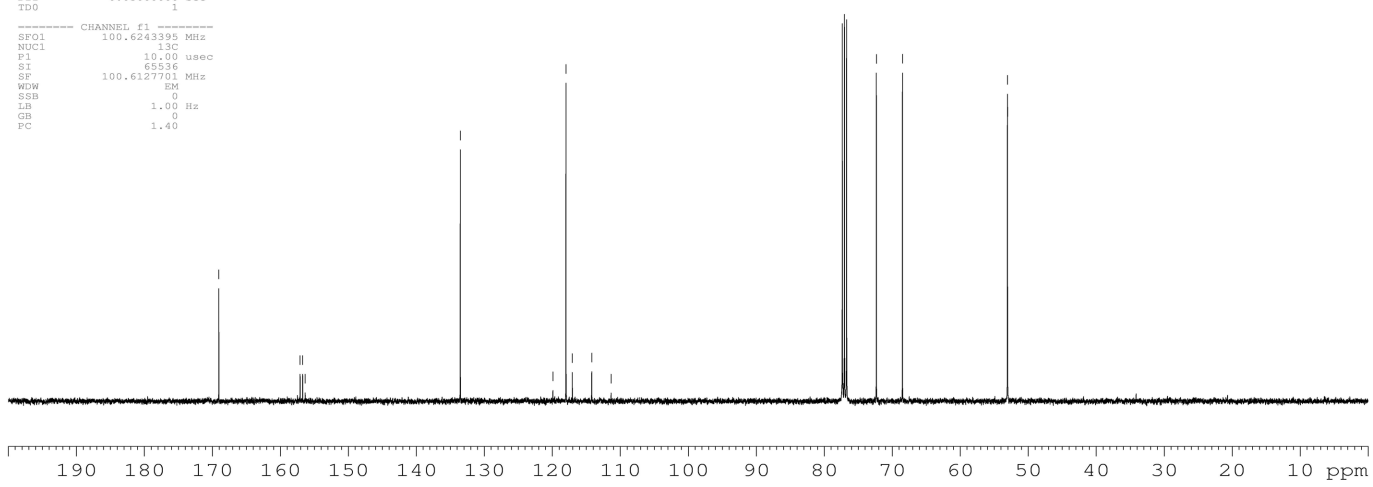



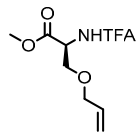

**10c**

169.060
157.107
156.731
156.356
133.535
119.922
117.993
117.065
114.207
111.350
72.338
68.502
53.060
53.038

```
NAME          egg-03-217
EXPNO                312
PROCNO                 1
Date_           20150521
Time               18.19
INSTRUM            av400
PROBHD   5 mm PABBO BB/
PULPROG           zgpg30
TD                 65536
SOLVENT            CDCl3
NS                   400
DS                     0
SWH            25252.525 Hz
FIDRES             0.385323 Hz
AQ              1.2976629 sec
RG                 189.85
DW                19.800 usec
DE                 6.50 usec
TE                299.0 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1          100.6243395 MHz
NUC1                  13C
P1                 10.00 usec
SI                 65536
SF            100.6127701 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```



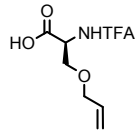



**10c**

```
NAME            egg-03-217
EXPNO                  311
PROCNO                   1
Date_             20150521
Time                 17.56
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG       zgfhigqn.2
TD                  262144
SOLVENT              CDCl3
NS                      16
DS                       0
SWH             150000.000 Hz
FIDRES            0.572205 Hz
AQ                0.8738633 sec
RG                   189.85
DW                    3.333 usec
DE                     6.50 usec
TE                    299.0 K
D1                1.00000000 sec
D11               0.03000000 sec
D12               0.00002000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           376.4983660 MHz
NUC1                     19F
P1                     15.00 usec
SI                    262144
SF             376.4983660 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.00
```

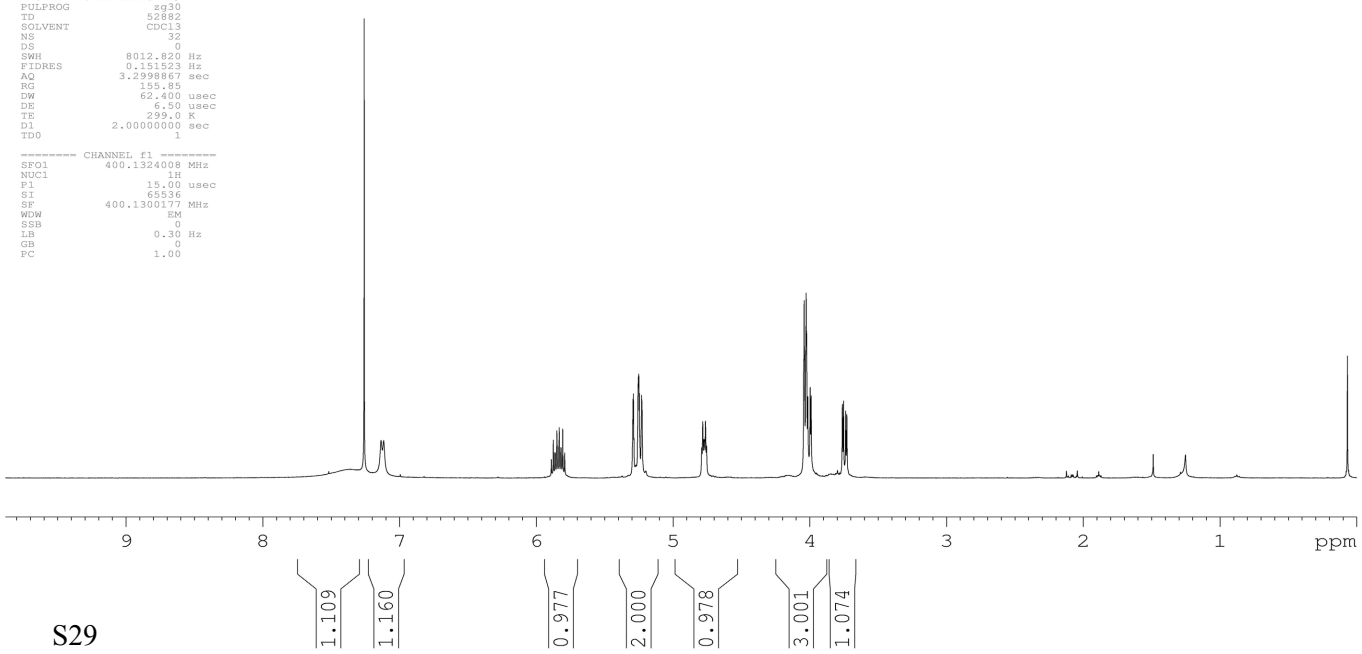


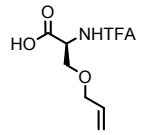




**10d**

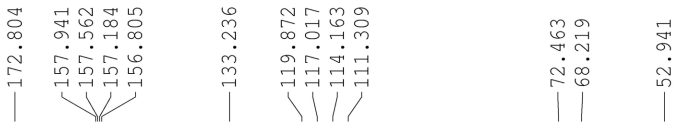
```
NAME            egg-03-125
EXPNO                   60
PROCNO                   1
Date_             20151213
Time                 16.04
INSTRUM              av400
PROBHD    5 mm PABBO BB/
PULPROG                zg30
TD                  52882
SOLVENT              CDCl3
NS                      32
DS                       0
SWH              8012.820 Hz
FIDRES            0.151523 Hz
AQ                3.2998867 sec
RG                   155.85
DW                   62.400 usec
DE                     6.50 usec
TE                    299.0 K
D1                2.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           400.1324008 MHz
NUC1                     1H
P1                     15.00 usec
SI                    65536
SF             400.1300177 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.00
```



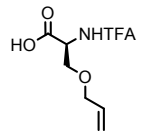



**10d**

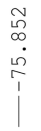



```
NAME          egg-03-125
EXPNO                240
PROCNO                 1
Date_           20151210
Time               15.58
INSTRUM           av400
PROBHD   5 mm PABBO BB/
PULPROG          zgpg30
TD                65536
SOLVENT           CDCl3
NS                  384
DS                    0
SWH           25252.525 Hz
FIDRES        0.385323 Hz
AQ            1.2976629 sec
RG               189.85
DW               19.800 usec
DE                6.50 usec
TE                299.0 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        100.6243395 MHz
NUC1                13C
P1                10.00 usec
SI                65536
SF          100.6127701 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```


**10d**




```
NAME          egg-03-125
EXPNO                  2
PROCNO                 1
Date_           20150223
Time               15.58
INSTRUM           av300
PROBHD   5 mm PABBO BB-
PULPROG          zgdc30
TD               131072
SOLVENT           CDCl3
NS                   21
DS                    0
SWH           75187.969 Hz
FIDRES        0.573639 Hz
AQ            0.8716788 sec
RG                 2048
DW                6.650 usec
DE                6.00 usec
TE                295.9 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 19F
P1                13.75 usec
PL1               -2.00 dB
PL1W        19.90535927 W
SFO1        282.4043550 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2               -2.00 dB
PL12              14.48 dB
PL2W        14.76977634 W
PL12W        0.33218035 W
SFO2        300.1318008 MHz
SI               131072
SF          282.4043550 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```