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Supporting Information

Directed Crosslinking of RNA by Glutathione-Triggered PNA-Quinone-Methide-Conjugates

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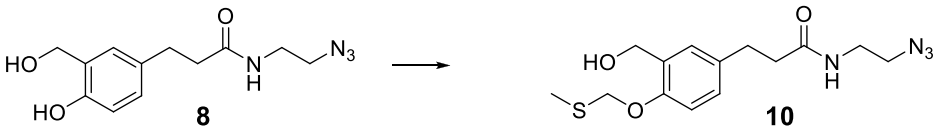
General

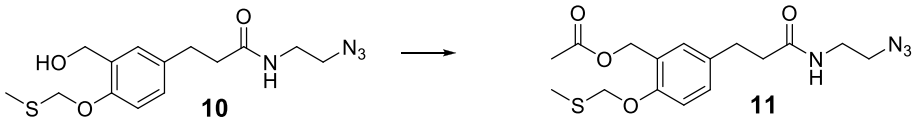
All chemicals were reagent grade and used as purchased. Reactions were performed under argon and monitored by TLC using silica gel 60 F-254 aluminum sheets (Macherey-Nagel 818333). Compounds were visualized by UV light (254 and 366 nm). Column chromatography was carried out on silica gel 60 (0.04 – 0.063 mm; Macherey-Nagel). Melting points (uncorrected) were recorded on a Kofler system. ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER DPX 250 or a BRUKER AV3 300 spectrometer at 300 K. Chemical shifts are expressed in parts per million (ppm) relative to the nondeuterated solvent signal DMSO-d_5 (δ_H = 2.50, δ_C = 39.51) or $CHCl_3$ (δ_H = 7.26, δ_C = 77.16) as an internal reference. ESI mass spectroscopy was performed on a ThermoFisher Surveyor MSQ. MALDI: PerSeptive Voyager-DE STR. High-resolution mass spectra (HRMS) were recorded on a Thermo Scientific MALDI LTQ Orbitrap XL.

N-(2-Azidoethyl)-3-(3-(hydroxymethyl)-4-((methylthio)methoxy)phenyl)propanamide 10



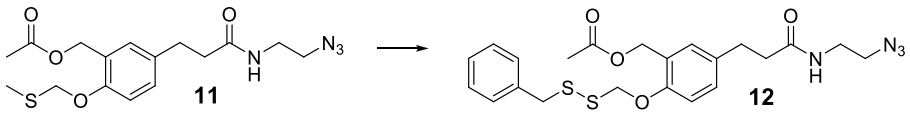
To a solution of compound **8** [1] (500 mg; 1.89 mmol; 1 eq) and NaI (310 mg; 2.07 mmol; 1.1 eq) in dry DMF (20 mL) were added a 1 M solution of potassium *tert*-butoxide in THF (2.08 mL; 2.08 mmol; 1.1 eq) dropwise at 0°C. The mixture was stirred for 10 min at 0°C. Then chloromethyl methyl sulfide **9** [2] (175 µL; 2.08 mmol; 1.1 eq) was added and the mixture was stirred for 18 h while being slowly brought to room temperature. Celite® 535 was added and the solvents were evaporated. The crude product was purified by flash column chromatography (cyclohexane/acetone 9:1 → 1:9 + 0.5% % Et_3N) to obtain compound **10** as a yellow oil (217 mg, 35 % yield). R_f = 0.58 (cyclohexane/acetone 1:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.09 (t, *J* = 5.4 Hz, 1 H), 7.23 (d, *J* = 2.1 Hz, 1 H), 7.02 (dd, *J* = 8.3, 2.2 Hz, 1 H), 6.91 (d, *J* = 8.3 Hz, 1 H), 5.24 (s, 2 H), 4.98 (t, *J* = 5.5 Hz, 1 H), 4.49 (d, *J* = 5.5 Hz, 2 H), 3.39–3.28 (m, 2 H), 3.28 - 3.17 (m, 2 H), 2.76 (t, *J* = 7.8 Hz, 2 H), 2.35 (dd, *J* = 8.9, 6.9 Hz, 2 H), 2.16 (s, 3 H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.8, 151.6, 133.7, 131.5, 127.2, 126.7, 113.3, 72.0, 57.9, 50.0, 38.2, 37.4, 30.5, 13.9 ppm. MS (ESI$^+$): *m/z* = 347.00 [M + Na$^+$]. HRMS (MALDI): *m/z* = 347.11493 [M + Na$^+$], calcd. for $C_{14}H_{20}N_4O_3S$+Na$^+$: 347.11483.

5-(3-((2-Azidoethyl)amino)-3-oxopropyl)-2-((methylthio)methoxy)benzyl acetate 11



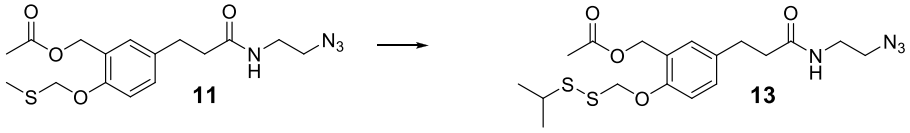
To a solution of compound **10** (207 mg; 0.64 mmol; 1 eq) in pyridine (1.5 mL) was added Ac_2O (0.5 ml). The mixture was stirred for 18 h at RT. Afterwards MeOH (10 mL) was added and after 30 min of stirring the solvents were evaporated. The crude product was purified by flash column chromatography (cyclohexane/acetone 9:1 → 1:2) to obtain compound **11** as a yellow oil (187 mg, 80 % yield). R_f = 0.64 (cyclohexane/acetone 1:1). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.07 (t, *J* = 5.4 Hz, 1 H), 7.20–7.09 (m, 2 H), 7.01 (dd, *J* = 7.0, 2.1 Hz, 1 H), 5.28 (s, 2 H), 5.03 (s, 2 H), 3.36–3.28 (m, 2 H), 3.28–3.17 (m, 2 H), 2.76 (t, *J* = 7.7 Hz, 2 H), 2.35 (t, *J* = 7.7 Hz, 2 H), 2.16 (s, 3 H), 2.05 (s, 3 H) ppm. ^{13}C NMR (75 MHz, DMSO-d_6) δ = 171.7, 170.2, 152.8, 133.9, 129.6, 128.9, 124.8, 113.8, 72.0, 61.1, 50.0, 38.1, 37.1, 30.1, 20.7, 13.7 ppm. MS (ESI$^+$): *m/z* = 389.03 [M + Na$^+$]. HRMS (MALDI): *m/z* = 389.12533 [M + Na$^+$], calcd. for $C_{16}H_{22}N_4O_4S$+Na$^+$: 389.12540.

5-(3-((2-Azidoethyl)amino)-3-oxopropyl)-2-((benzyldisulfanyl)methoxy)benzyl acetate 12



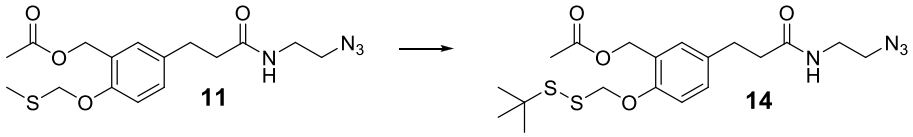
To a solution of compound **11** (60 mg; 0.16 mmol; 1 eq) in dry DCM (5 mL) were added NEt_3 (25 µL; 0.18 mmol; 1.1 eq) and SO_2Cl_2 (15 µL; 0.18 mmol; 1.1 eq) successively dropwise and the mixture was stirred for 1 h at rt. Then potassium thiotosylate (56 mg; 0.246 mmol; 1.5 eq) was added and the stirring continued for 1.5 h. Finally, benzyl mercaptan (43 µL; 0.32 mmol; 2 eq) was added. After 20 h of stirring the solvents were evaporated. The crude product was purified by flash column chromatography (cyclohexane/acetone 4:1 → 1:1) to obtain compound **12** as an off-white solid (55 mg, 71 % yield). R_f = 0.65 (cyclohexane/acetone 1:1). Mp. 63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.33–7.20 (m, 5 H), 7.19 (d, *J* = 2.3 Hz, 1 H), 7.13 (dd, *J* = 8.4, 2.3 Hz, 1 H), 6.80 (d, *J* = 8.4 Hz, 1 H), 5.72 (br. s, 1 H), 5.14 (s, 2 H), 5.09 (s, 2 H), 3.92 (s, 2 H), 3.40–3.34 (m, 4 H), 2.92 (t, *J* = 8.6 Hz, 2 H), 2.47 (dd, *J* = 8.4, 6.9 Hz, 2 H), 2.07 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.3, 171.0, 153.3, 137.1, 134.3, 130.2, 129.5, 129.3, 128.7, 127.7, 125.7, 113.5, 78.2, 61.8, 51.0, 44.2, 39.0, 38.5, 30.8, 21.2 ppm. MS (ESI$^+$): *m/z* = 497.03 [M + Na^+]. HRMS (MALDI): *m/z* = 497.12788 [M + Na^+], calcd. for $C_{22}H_{26}N_4O_4S_2$+Na^+: 497.12877.

5-(3-((2-Azidoethyl)amino)-3-oxopropyl)-2-((isopropyldisulfanyl)methoxy)benzyl acetate 13



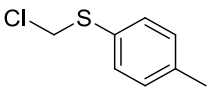
To a solution of compound **11** (60 mg; 0.16 mmol; 1 eq) in dry DCM (5 mL) were added Et_3N (25 µL; 0.18 mmol; 1.1 eq) and SO_2Cl_2 (15 µL; 0.18 mmol; 1.1 eq) successively dropwise and the mixture was stirred for 2 h at rt. Then potassium thiotosylate (56 mg; 0.246 mmol; 1.5 eq) was added and the stirring continued for 2 h. Finally, isopropyl mercaptan (30 µL; 0.32 mmol; 2 eq) was added. After 23 h of stirring the solvents were evaporated. The crude product was purified by flash column chromatography (cyclohexane/acetone 4:1 → 1:1) to obtain compound **13** as a yellow oil (58 mg, 83 % yield). R_f = 0.72 (cyclohexane/acetone 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.18 (d, *J* = 2.1 Hz, 1 H), 7.13 (dd, *J* = 8.3, 2.2 Hz, 1 H), 6.86 (d, *J* = 8.3 Hz, 1 H), 5.71 (br. s, 1 H), 5.31 (s, 2 H), 5.13 (s, 2 H), 3.43–3.35 (m, 4 H), 3.04 (septet, *J* = 6.7 Hz, 1 H), 2.93 (t, *J* = 7.6 Hz, 2 H), 2.47 (t, *J* = 7.7 Hz, 2 H), 2.11 (s, 3 H), 1.28 (d, *J* = 6.7 Hz, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.4, 171.1, 153.4, 134.2, 130.2, 129.3, 125.6, 113.4, 79.4, 61.8, 51.0, 41.6, 39.0, 38.5, 30.8, 22.7, 21.2 ppm. MS (ESI$^+$): *m/z* = 449.07 [M + Na^+]. HRMS (MALDI): *m/z* = 449.12824 [M + Na^+], calcd. for $C_{18}H_{26}N_4O_4S_2$+Na^+: 449.12877.

5-(3-((2-Azidoethyl)amino)-3-oxopropyl)-2-((*tert*-butyldisulfanyl)methoxy)benzyl acetate 14



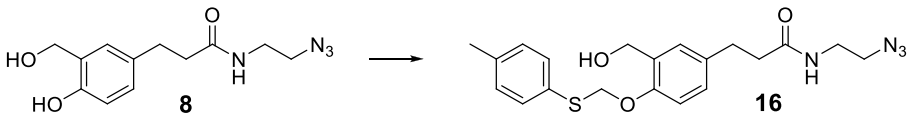
To a solution of compound **11** (100 mg; 0.273 mmol; 1 eq) in dry DCM (10 mL) were added NEt_3 (42 µL; 0.30 mmol; 1.1 eq) and SO_2Cl_2 (23 µL; 0.27 mmol; 1 eq) successively dropwise at 0°C. The mixture was stirred for 10 min at rt. Then potassium thiotosylate (93 mg; 0.41 mmol; 1.5 eq) was added and the stirring continued for 3 h. Finally, *tert*-butyl mercaptan (59 µL; 0.55 mmol; 2 eq) was added. After 16 h of stirring sat. aq. $NaHCO_3$ (40 mL) was added and the water phase was extracted with DCM (4 x 40 mL). The organic phase was washed with brine (40 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (cyclohexane/acetone 3:1 → 1:1) to obtain compound **14** as a yellow oil (40 mg, 33 % yield). R_f = 0.71 (cyclohexane/acetone 1:1). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.18 (d, *J* = 2.1 Hz, 1 H), 7.13 (dd, *J* = 8.3, 2.1 Hz, 1 H), 6.85 (d, *J* = 8.3 Hz, 1 H), 5.73 (br. s, 1 H), 5.30 (s, 2 H), 5.13 (s, 2 H), 3.46–3.27 (m, 4 H), 2.92 (t, *J* = 7.6 Hz, 2 H), 2.47 (t, *J* = 7.6 Hz, 2 H), 2.10 (s, 3 H), 1.33 (s, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ = 172.4, 171.1, 153.3, 134.2, 130.1, 129.2, 125.6, 113.4, 80.0, 61.8, 51.0, 47.6, 39.00, 38.5, 30.8, 30.0, 21.2 ppm. MS (ESI^+): *m/z* = 463.03 [M + Na^+]. HRMS (MALDI): *m/z* = 463.14390 [M + Na^+], calcd. for $C_{19}H_{28}N_4O_4S_2$+Na^+: 463.14442.

4-Chloromethylthiotoluene 15 [3]



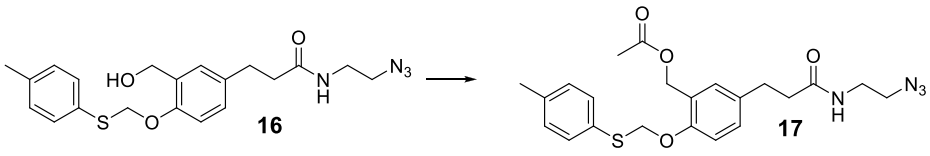
In an apparatus equipped with a reflux condenser and exhaust tube, paraformaldehyde (1.35 g; 45.5 mmol; 1.3 eq.) was dissolved in a mixture of conc. aqueous HCl (35 mL) and toluene (10 mL) and heated to 50 °C. Then, a solution of 4-thiocresol (4.35 g: 35 mmol; 1 eq.) in toluene (10 mL) was added at constant temperature within 40 min and stirred for 1 h at 50 °C and 2 h at RT. The apparatus was flushed with argon and the phases separated. The aqueous phase was extracted with toluene (2 x 20 mL) and the combined organic phases were dried over $NaSO_4$. The toluene was distilled at room pressure and the remaining crude product was fractionally distilled at reduced pressure (oil-sealed rotary vane pump) to obtain compound **15** as a colourless liquid (2.1 g, 35% yield). Bp. 85–86 °C at < 1 mbar (Ref. 3: 106–107 °C at 11 mm Hg). ^{1}H-NMR (250 MHz, $CDCl_3$) δ = 7.44 (d, *J* = 8.1 Hz, 2 H), 7.19 (d, *J* = 8.1 Hz, 2 H), 4.93 (s, 2 H), 2.36 (s, 3 H) ppm.

N-(2-Azidoethyl)-3-(3-(hydroxymethyl)-4-((p-tolylthio)methoxy)phenyl)propanamide 16



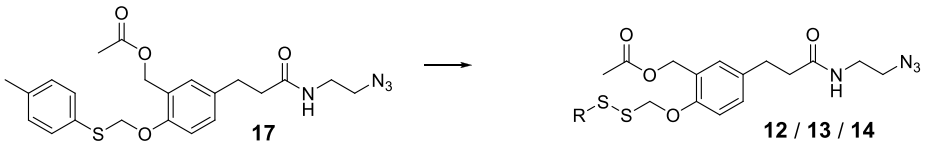
To a solution of compound **8** [1] (100 mg; 0.378 mmol; 1 eq.) in dry MeCN (5 mL) and DMF (5 mL) was added a 1 M solution of potassium *tert*-butanolate in THF (378 µL; 0.378 mmol; 1 eq.) at RT and the mixture was stirred for 10 min. Then, 4-chloromethylthiotoluene **15** (66 mg; 0.416 mmol; 1.1 eq.) was added and stirring continued for additional 16 h. The solution was poured into brine (60 mL) and extracted with ethyl acetate (4 x 40 mL). The combined organic phases were washed with brine (40 mL), dried over Na_2SO_4, and the solvent was removed in vacuo. The crude product was purified by flash column chromatography (cyclohexane/acetone 3:1 → 1:1) to obtain compound **16** as a yellow solid (120 mg, 79% yield). R_f = 0.36 (cyclohexane/acetone 4:1). Mp. 70 °C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.37 (d, *J* = 8.1 Hz, 2 H), 7.18–7.07 (m, 4 H), 6.85 (d, *J* = 8.3 Hz, 1 H), 5.72 (br. s, 1 H), 5.43 (s, 2 H), 4.59 (s, 2 H), 3.43–3.34 (m, 4 H), 2.92 (t, *J* = 7.6 Hz, 2 H), 2.47 (t, *J* = 7.6 Hz, 2 H), 2.33 (s, 3 H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 172.5, 153.2, 138.0, 134.2, 131.7, 130.8, 130.7, 130.1, 129.2, 128.6, 113.4, 73.9, 61.8, 51.0, 39.0, 38.5, 30.9, 21.2 ppm. MS (ESI$^+$): *m/z* = 423.23 [M + Na$^+$], calcd. for $C_{20}H_{24}N_4O_3S$+Na$^+$: 423.15.

5-(3-((2-Azidoethyl)amino)-3-oxopropyl)-2-((p-tolylthio)methoxy)benzyl acetate 17



Compound **16** (280 mg; 0.70 mmol; 1 eq.) was dissolved in a mixture of pyridine (2 mL), Ac_2O (1 mL), and DCM (10 mL) and stirred for 16 h at rt. MeOH (5 mL) was added and after 30 min the solvent was evaporated in vacuo. The crude product was purified by flash column chromatography (cyclohexane/acetone 9:1 → 1:1) to obtain compound **17** as a colourless solid (210 mg, 68% yield). Mp. 67 °C R_f = 0.47 (cyclohexane/acetone 4:1). ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.37 (d, *J* = 8.2 Hz, 2 H), 7.18 (d, *J* = 2.1 Hz, 1 H), 7.07 - 7.16 (m, 3 H), 6.87 (d, *J* = 8.3 Hz, 1 H), 5.71 (br. s, 1 H), 5.43 (s, 2 H), 5.08 (s, 2 H), 3.42–3.34 (m, 4 H), 2.92 (t, *J* = 7.6 Hz, 2 H), 2.47 (t, *J* = 7.7 Hz, 2 H), 2.33 (s, 3 H), 2.06 (s, 3 H) ppm. ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 172.4, 171.0, 153.3, 137.7, 134.0, 131.4, 131.3, 130.2, 130.0, 129.2, 125.7, 113.5, 73.9, 61.90, 51.0, 39.0, 38.5, 30.8, 21.19, 21.2 ppm. MS (ESI$^+$): *m/z* = 443.35 [M + H$^+$], calcd. for $C_{22}H_{26}N_4O_4S$+H$^+$: 443.18.

General procedure for the synthesis of disulphides 12-14 from *p*-tolylthiomethylether 17



To a solution of compound **17** (1 eq.) in dry DCM (5 mL) was added SO_2Cl_2 (1.01 eq.) and the mixture stirred for 30 min at room temperature. Cyclohexene (1.7 eq.) was then added and stirring was continued for another 20 min. After the addition of potassium thiotosylate (1.6 eq.), stirring was continued for another 25 min. Finally, the corresponding thiol was added (10 eq.). After 16 h, Et_3N (0.7 mL) was added and the solvent was removed in vacuo. Each crude product was purified by flash column chromatography (cyclohexane/acetone 9:1→ 1:1). ^{1}H NMR data of all three products were identical to those reported above.

	amount of 17	**yield (mass)**	**yield (%)**
Benzyl derivative **12**	70 mg / 0.16 mmol	21 mg	23 %
Isopropyl derivative **13**	31 mg / 0.07 mmol	21 mg	70 %
tert-Butyl derivative **14**	31 mg / 0.07 mmol	24 mg	78 %

Preparation of PNA conjugates 3-5

The conjugation of PNA and quinone methide precursors **12**-**14** was accomplished by CuAAC in solution. The crude alkyne-modified PNA [1] (typically 200 µL of a 1400 µM solution) was incubated with 5 eq of compounds **12**, **13**, or **14** in a DMSO:H_2O 1:1 solution containing 500 µM Cu(II)-TBTA complex and 5 mM sodium ascorbate for 2 h at 37 °C. (TBTA: Tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine). The products **3**-**5** were then purified by HPLC.

PNA conjugate 3

MS (MALDI) m/z = 3438.9 [M+H$^+$], calcd. for $C_{143}H_{184}N_{64}O_{36}S_2$+H$^+$: 3439.4

HPLC conditions:

analytical: Phenomenex Gemini C18, 150 x 4.6, 5 µm, 2 min hold 4 % MeCN, linear gradient of 4–35 % MeCN in 0.1 % TFA for 25 min, 1 mL/min, 50 °C, 260 nm, t_R = 27.0 min.

preparative: Phenomenex Gemini C18, 250 x 10, 10 µm, linear gradient of 0–50 % MeCN in 0.1 % TFA for 17 min, 4.0 mL/min, 50 °C, 260 nm, t_R = 17.1 min.

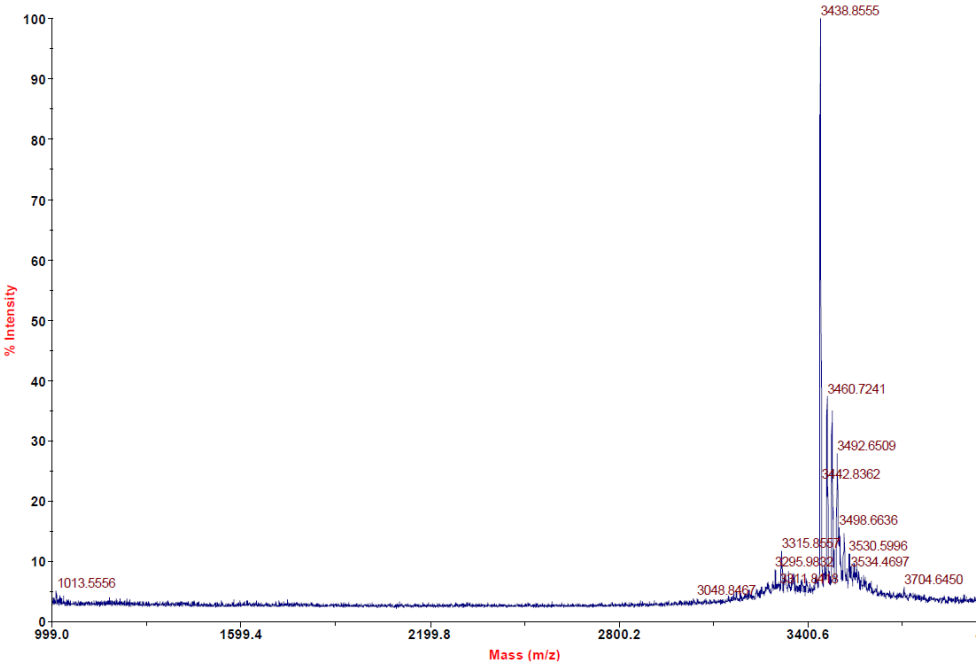


Figure S1: MALDI mass spectrum of conjugate **3**.

PNA conjugate 4

MS (MALDI) m/z = 3391.9 [M+H$^+$], calcd. for $C_{139}H_{184}N_{64}O_{36}S_2$+H$^+$: 3391.4

HPLC conditions:

analytical & preparative: Phenomenex Gemini C18, 150 x 4.6, 5 µm, 2 min hold 4 % MeCN, linear gradient of 4–35 % MeCN in 0.1 % TFA for 25 min, 1 mL/min, 50 °C, 260 nm, t_R = 25.5 min.

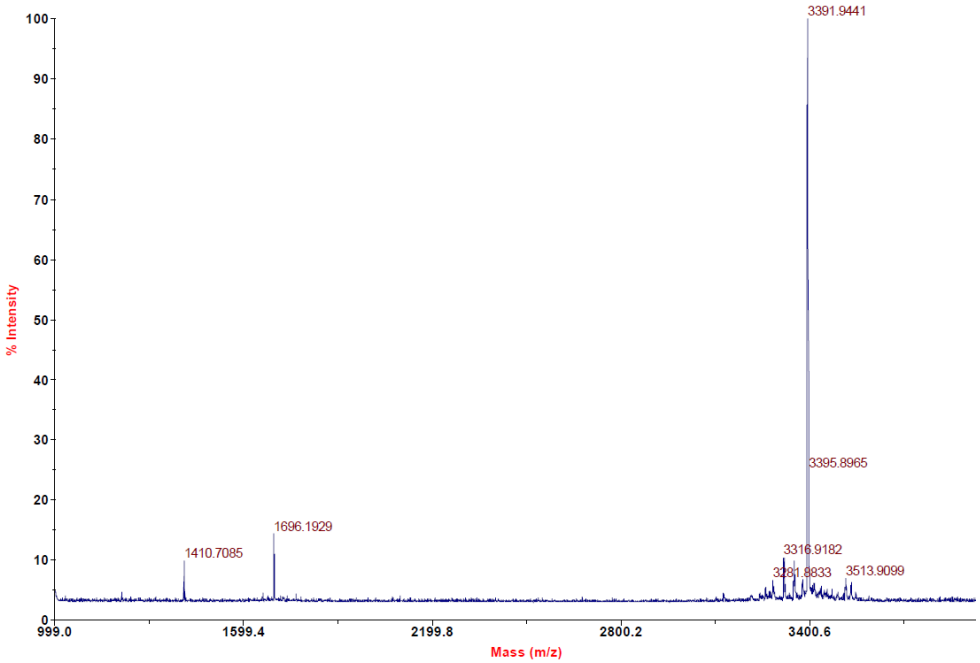


Figure S2: MALDI mass spectrum of conjugate **4**.

PNA conjugate 5

MS (MALDI) m/z = 3404.2 [M+H$^+$], calcd. for $C_{140}H_{186}N_{64}O_{36}S_2$+H$^+$: 3405.4

HPLC conditions:

analytical: Phenomenex Gemini C18, 150 x 4.6, 5 µm, 2 min hold 4 % MeCN, linear gradient of 4–35 % MeCN in 0.1 % TFA for 25 min, 1 mL/min, 50 °C, 260 nm, t_R = 25.1 min.

preparative: Phenomenex Gemini C18, 250 x 10, 10 µm, linear gradient of 0–50 % MeCN in 0.1 % TFA for 17 min, 4.0 mL/min, 50 °C, 260 nm, t_R = 17.3 min.

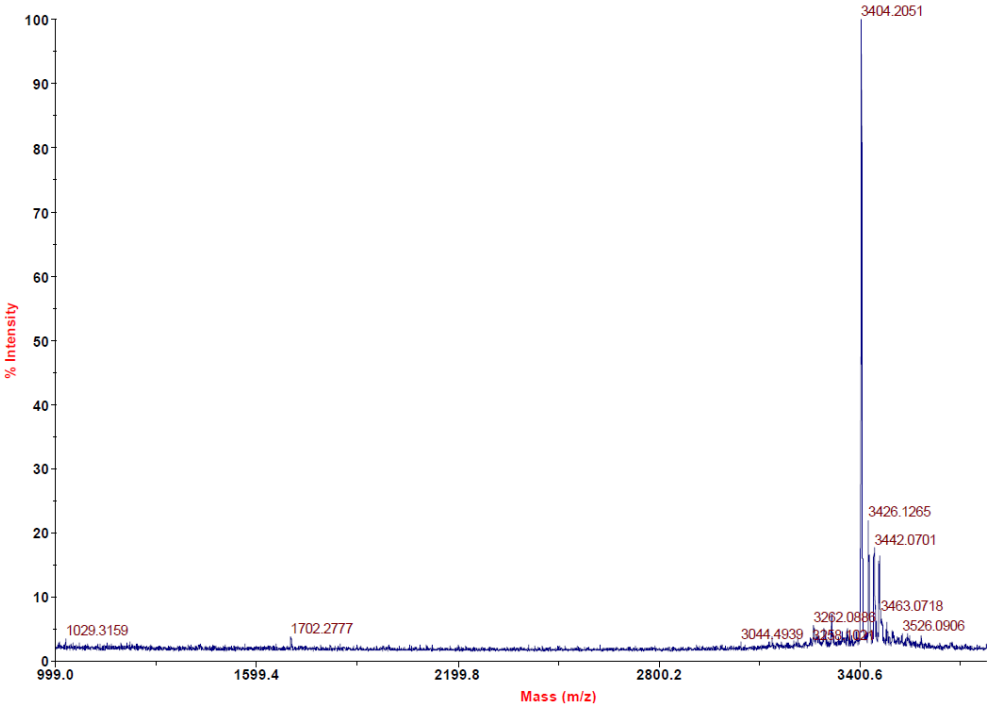


Figure S3: MALDI mass spectrum of conjugate **5**.

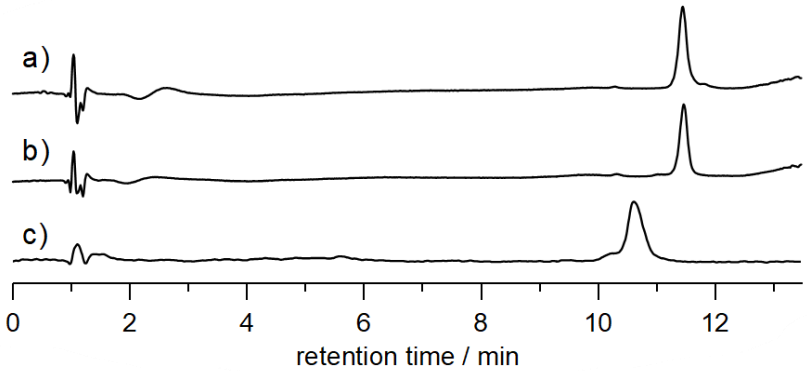


Figure S4: Stability of conjugates **3**-**5**. Incubation for 72 h in *absence* of GSH (20 µM of conjugate, 37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). a) Conjugate **5**. b) Conjugate **3**. c) Conjugate **4**. HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm.

Incubation with 0.5 mM GSH

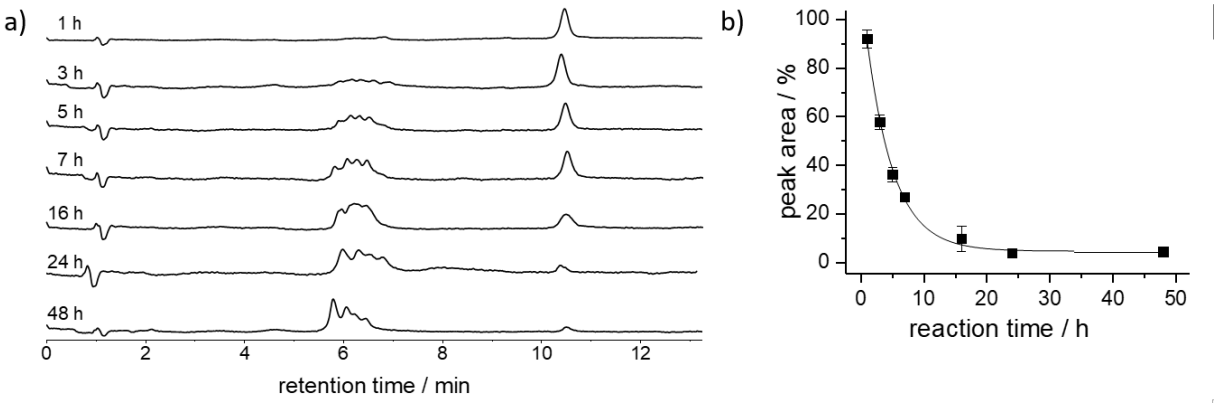


Figure S5: a) HPLC analysis of reductive activation of conjugate **4** (20 µM) in the presence 0.5 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm. b) Decay of [**4**] as a function of time and best fit to a first order rate model.

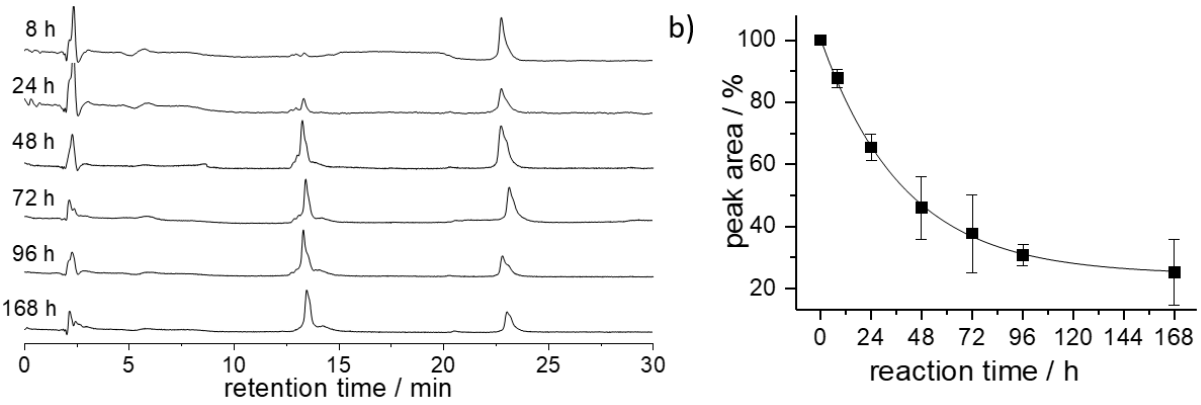


Figure S6: a) HPLC analysis of reductive activation of conjugate **5** (20 µM) in the presence 0.5 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, 2 min hold 4 % MeCN, linear gradient of 4–35 % MeCN in 0.1 % TFA for 25 min, 1 mL/min, 50 °C, 260 nm. b) Decay of [**5**] as a function of time and best fit to a first order rate model. Note that compared to Figures S4 and S5, a different HPLC gradient has been used.

Incubation with 10 mM GSH

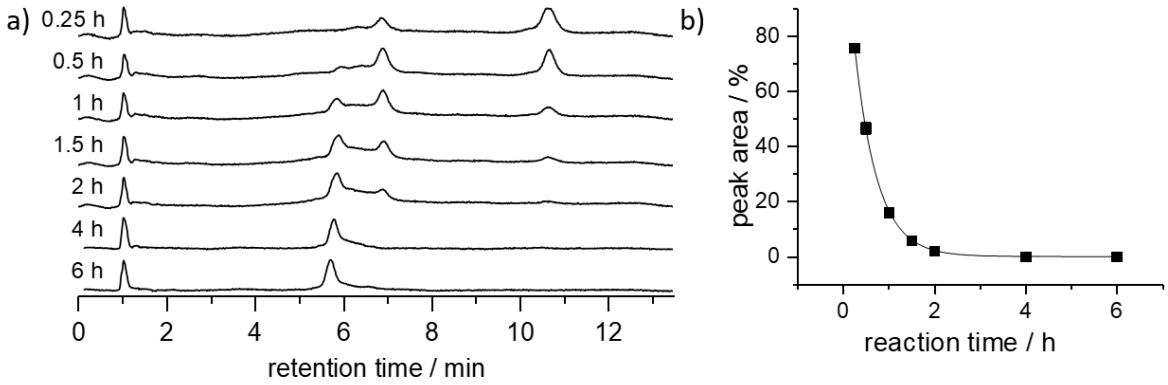


Figure S7: a) HPLC analysis of reductive activation of conjugate **4** (20 µM) in the presence 10 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm. b) Decay of [**4**] as a function of time and best fit to a first order rate model.

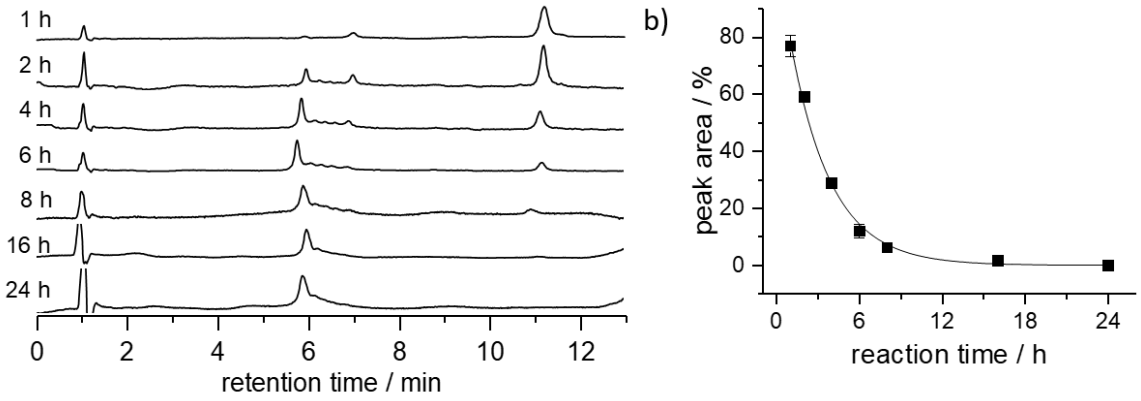


Figure S8: a) HPLC analysis of reductive activation of conjugate **5** (20 µM) in the presence 10 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm. b) Decay of [**5**] as a function of time and best fit to a first order rate model.

Isolation and characterization of the GSH-adduct of conjugate 3

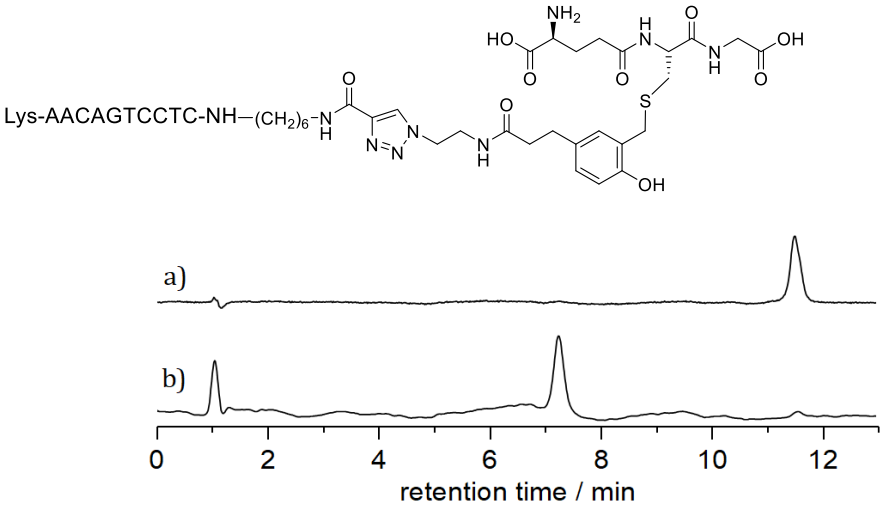


Figure S9: a) Conjugate **3** before incubation with GSH. b) Incubation with 10 mM GSH for 5 minutes (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm. The peak at 7.2 min was isolated and submitted to mass spectrometric analysis (see below).

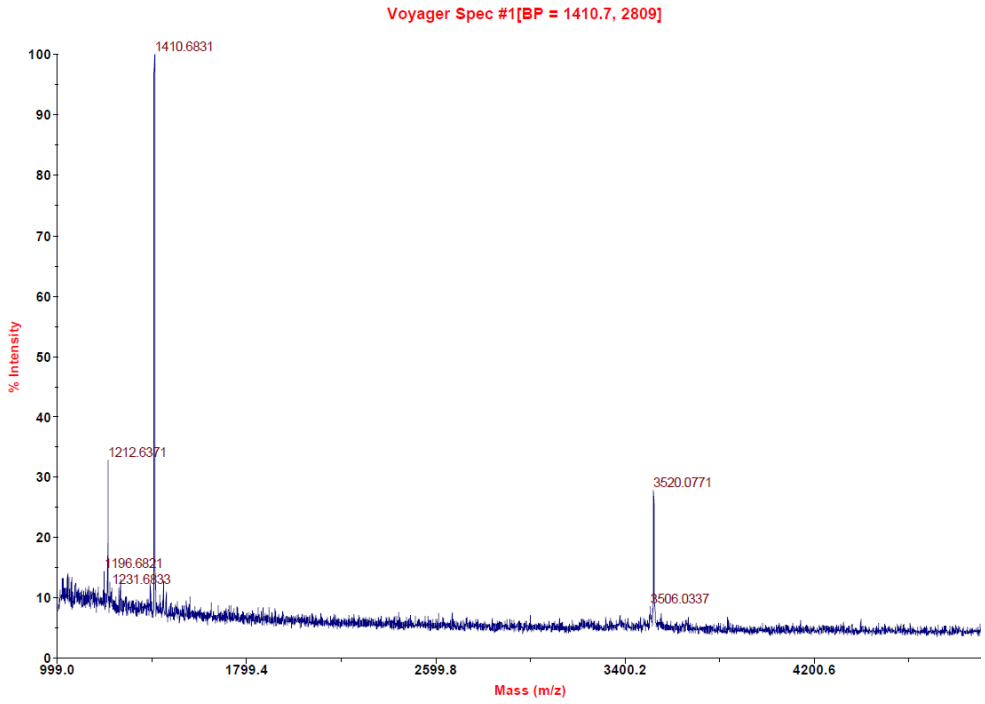


Figure S10: Mass spectrometric characterization of the GSH-adduct. MALDI: m/z = 3520.1 [M+H$^+$], calcd. for $C_{143}H_{189}N_{67}O_{40}S$+H$^+$: 3518.5

Kinetics of RNA alkylation after incubation with 10 mM GSH

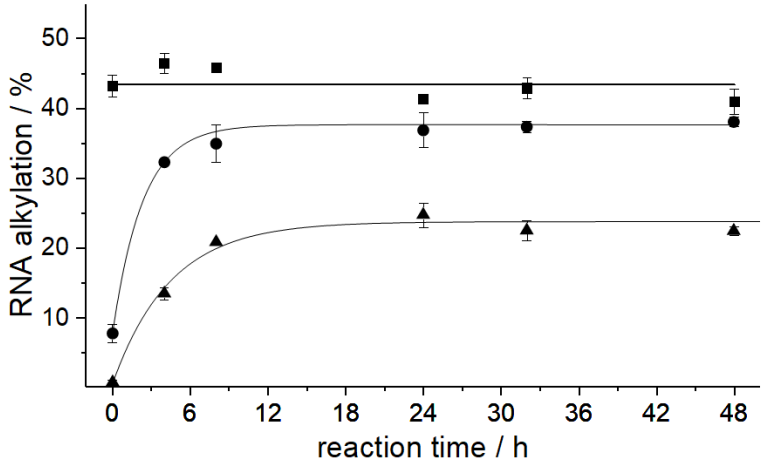


Figure S11: Comparison of RNA alkylation (3 µM) by conjugate **3** (6 µM, squares), by conjugate **4** (dots), and by conjugate **5** (triangles), each after activation with 10 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). Analysis by gel electrophoresis using the ALFexpress DNA sequencer. If possible, data points are fitted against a single exponential function.

Kinetics of RNA alkylation after incubation with *N*-acetyl cysteine

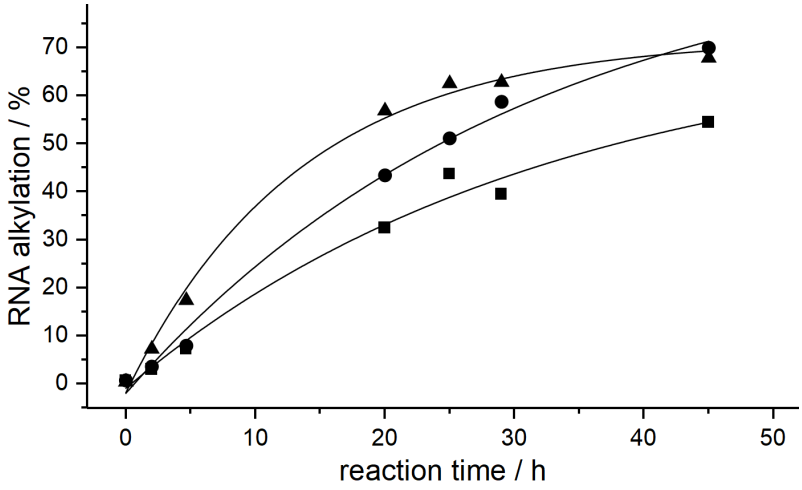
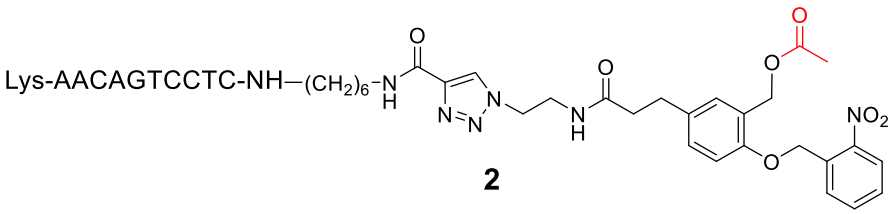


Figure S12: Alkylation of RNA (3 µM) by conjugate **3** (6 µM) in the presence of *N*-acetyl cysteine (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl): 50 µM (squares), 100 µM (dots), or 500 µM of *N*-acetyl cysteine. Analysis by gel electrophoresis using the ALFexpress DNA sequencer. Data points are fitted against a single exponential function. Compared to activation with GSH, slower alkylation is observed.

Stability of conjugate 2 at high GSH concentration: Deacetylation does not occur



2

Quinone methide **6** is the common intermediate in the reaction of all conjugates with the same RNA under identical conditions. It might be expected, therefore, that crosslinking kinetics are mainly determined by deprotection rates. In contrast, alkylation by conjugate **4** in particular was found considerably slower than deprotection. It is also not obvious to understand the low crosslinking yield of conjugate **5**. Once formed, the active electrophile **6** bound to its target RNA should obey first-order kinetics for each kind of reaction: Crosslinking, self-alkylation, quenching by GSH and by water. Product distribution should be independent from the different rates of deprotection for compound **3**-**5**. We therefore suspected that other hidden reactions might deactivate the quinone methide precursors. GSH as a powerful nucleophile might cleave off the acetate residue and prevent the formation of quinone methides. We checked this possibility by incubation of conjugate **2** with GSH (10 mM) for 48 h (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). In conjugate **2**, the redox labile alkyldithio methyl group is replaced by a light sensitive 2-nitrophenyl ether but the benzylic acetate ester should be as reactive as in conjugates **3**-**5**. HPLC analysis after incubation in the dark, however, only showed the unmodified conjugate **2** (black line in Figure S13). As a positive control, deacetylation was enforced by incubation with 2 M NaOH (room temperature, 2.5 h). A faster running new peak of the hydrolysis product now becomes visible (red line in Figure S13).

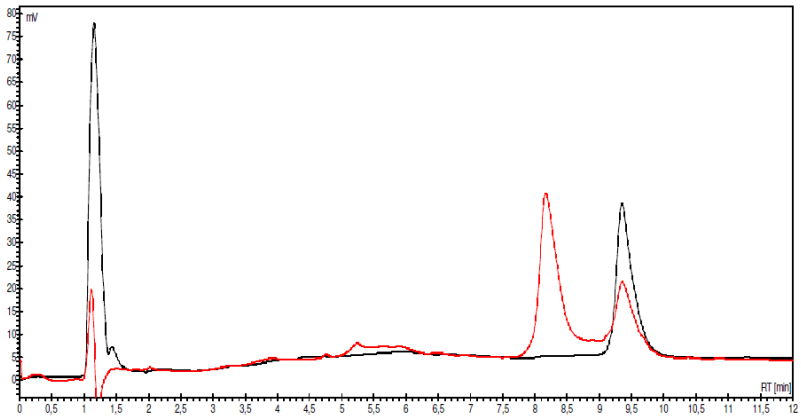


Figure S13: Stability of the benzylic acetyl group in conjugate **2** against GSH. Black: Chromatogram after incubation with GSH (10 mM, 37 °C, 48 h, 130 mM MES buffer pH 7.0, 130 mM NaCl). Red: Chromatogram after incubation with NaOH (2 M, room temperature, 2.5 h). HPLC conditions: Phenomenex Gemini C18, 150 x 4.6, 5 µm, linear gradient of 7–40 % MeCN in 0.1 M TEAA buffer (pH 7) for 10 min, 2 mL/min, 50 °C, 260 nm.

Alkylation critically depends on proper base pairing

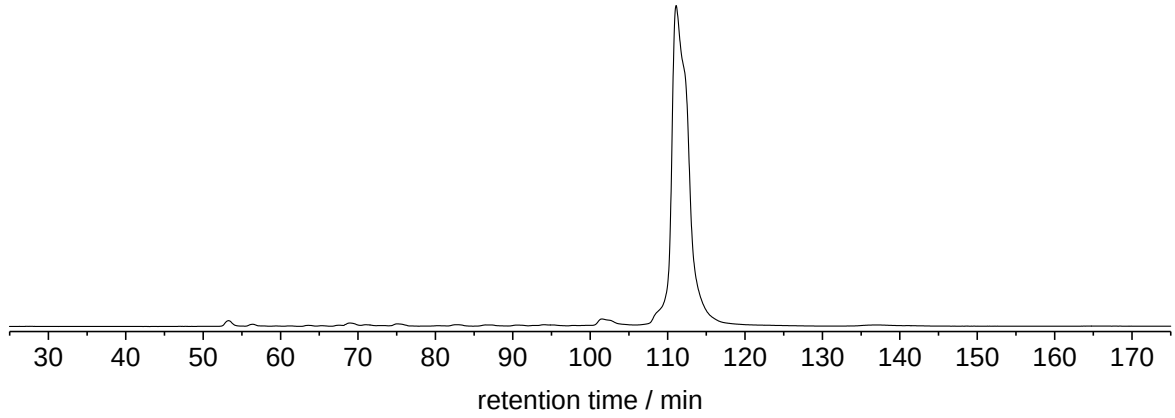


Figure S14: Incubation of a dye-labeled non-complementary RNA with conjugate **4** for 24 h in the presence of 0.5 mM GSH (37 °C, 130 mM MES buffer pH 7.0, 130 mM NaCl). The PNA-binding part UUGUCAGGAG of the RNA substrate has been replaced by UUGUGCGAGA (RNA sequence: $^{5'}$Cy5-T_{10}-AUACCUUGUGCGAGAAAGAGAGGCCGUUA-$T_4{}^{3'}$). Analysis by gel electrophoresis using the ALFexpress DNA sequencer (RNA substrate: 113 min; crosslinked RNA: 137 min). The lack of complementarity with conjugate **4** prevents alkylation of the RNA. For comparison: About 50 % of the complementary substrate is crosslinked under identical conditions (see Figures 2 and 4).

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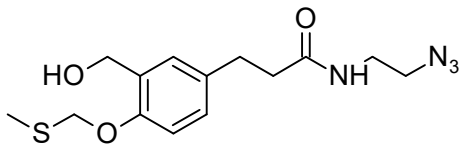

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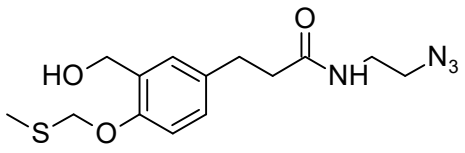

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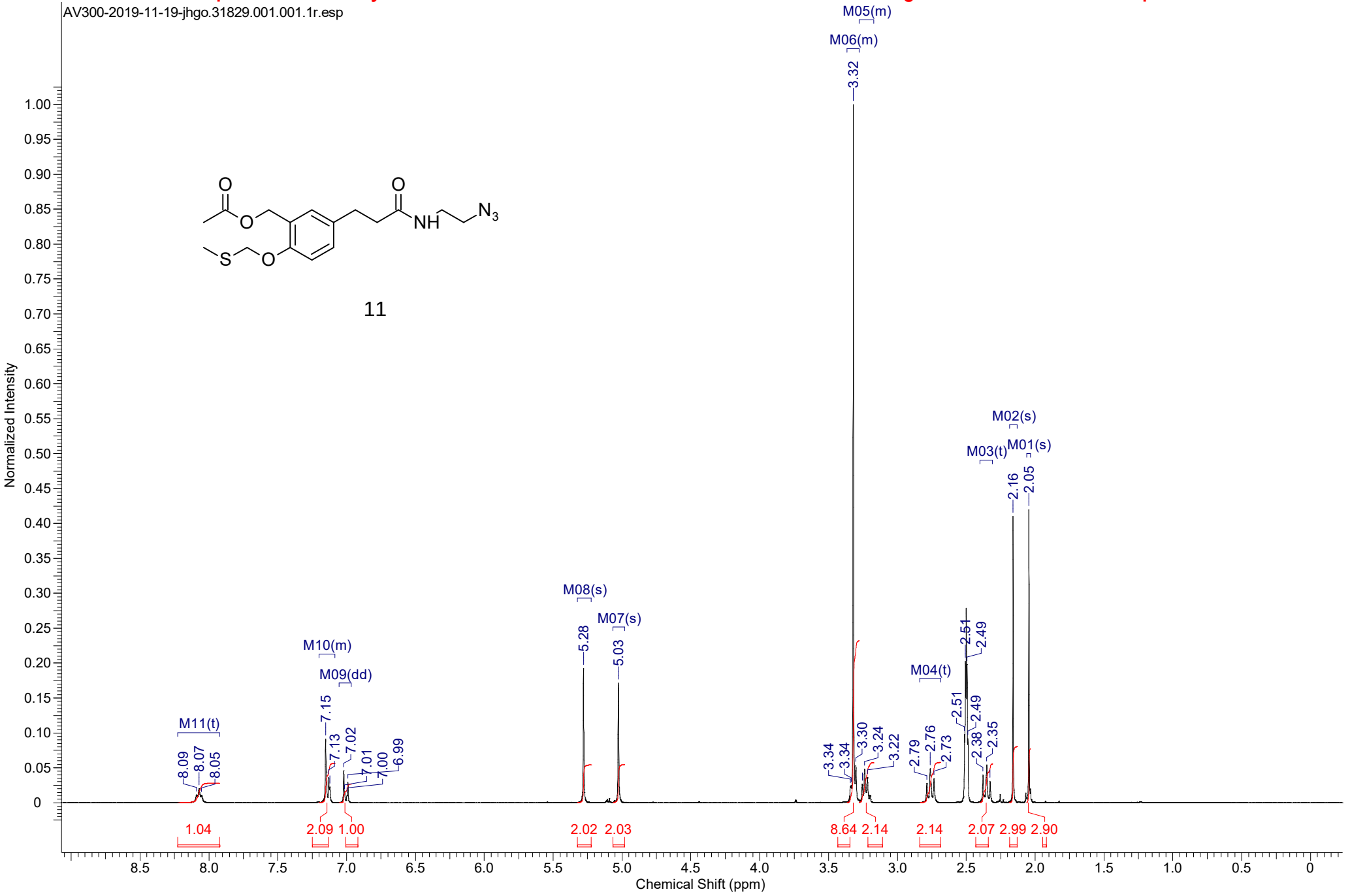

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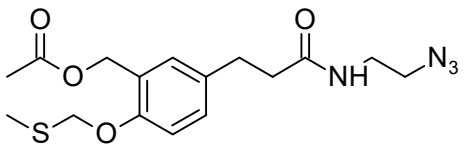

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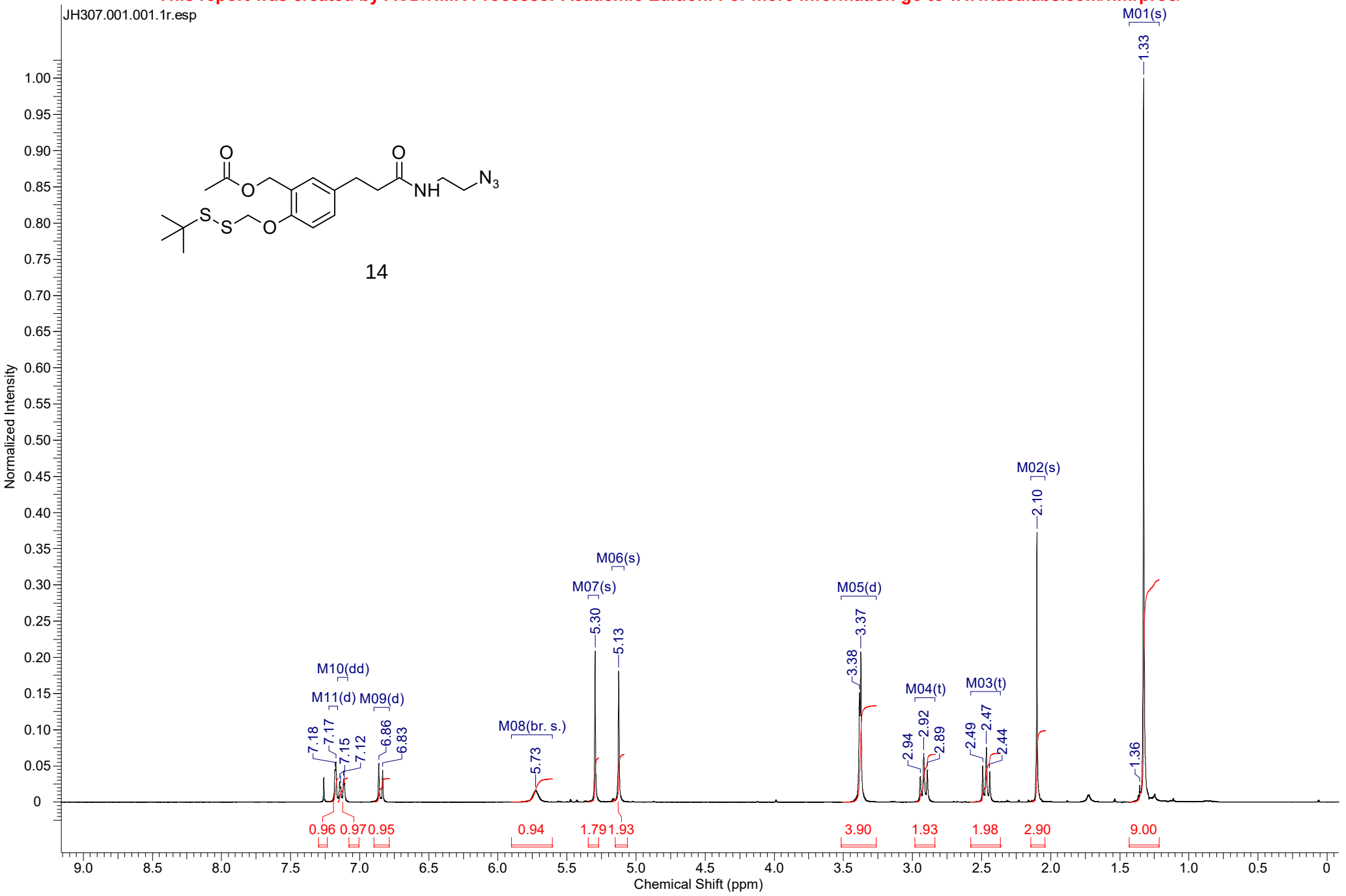

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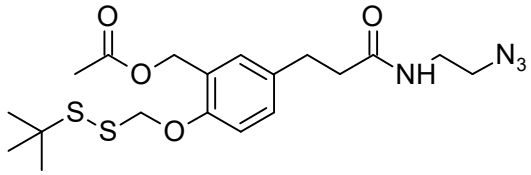

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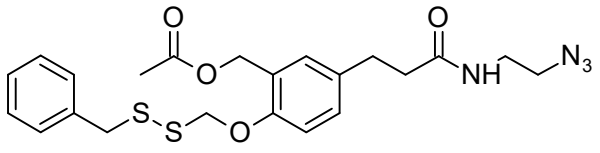

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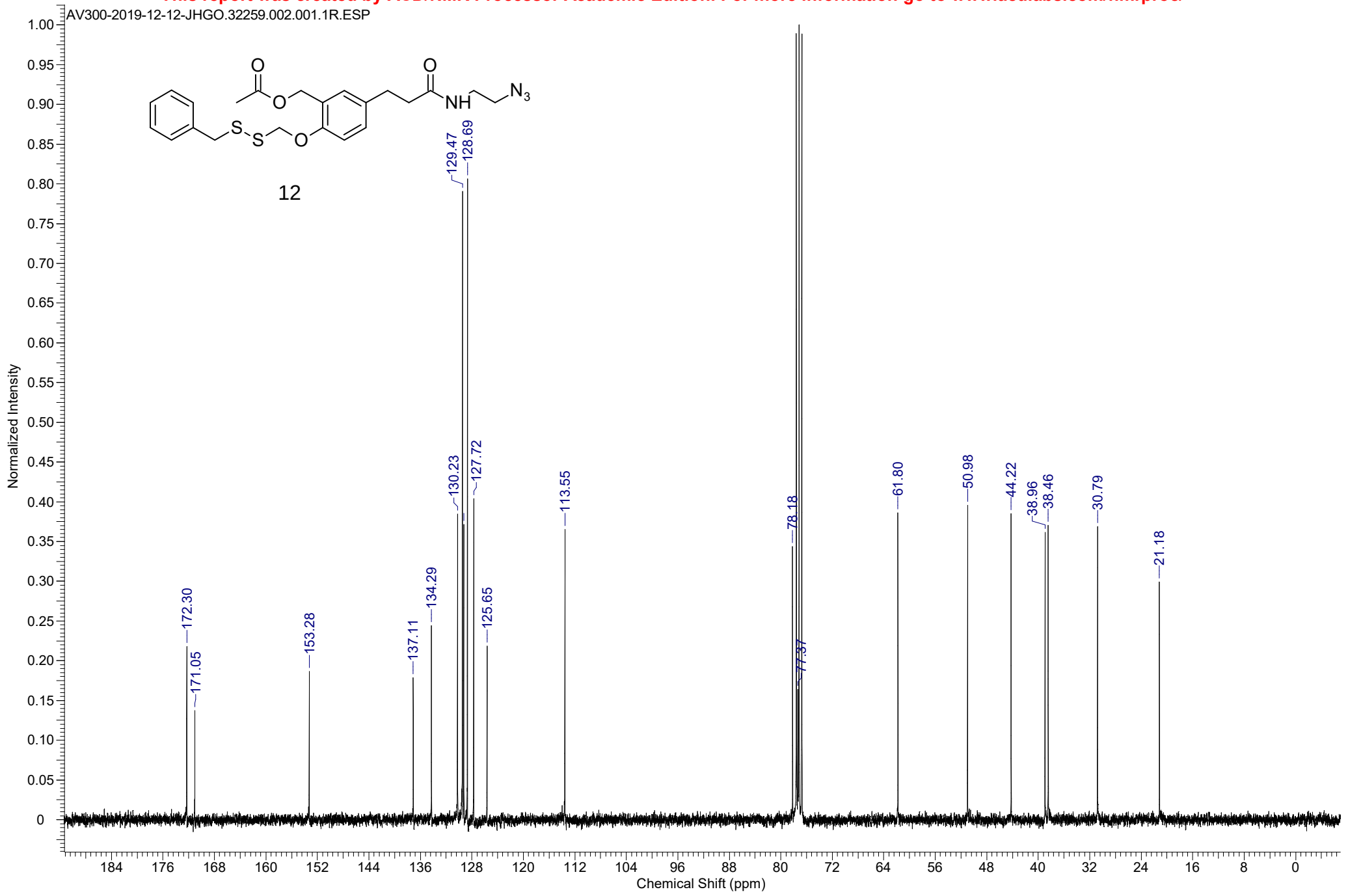

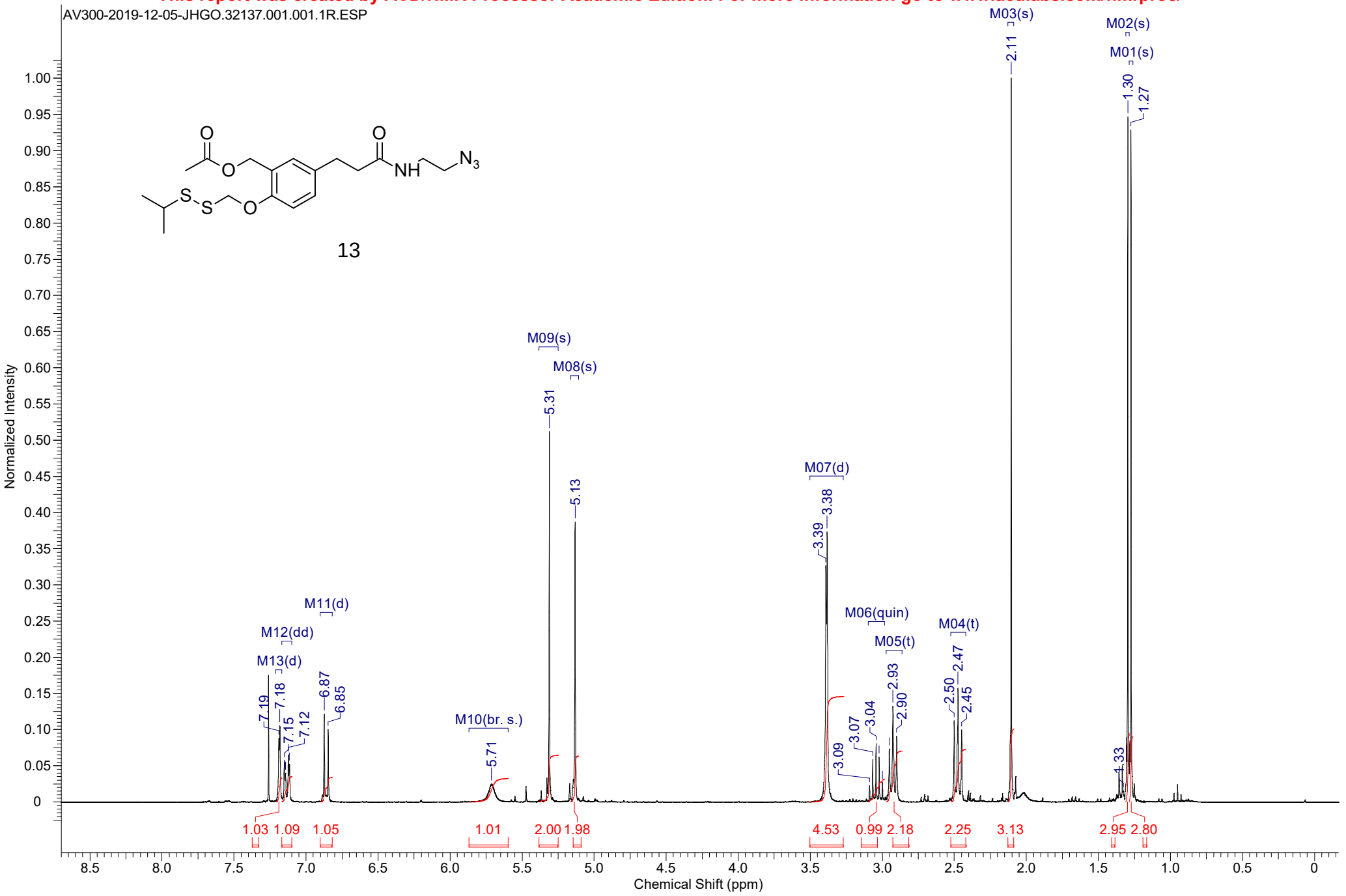

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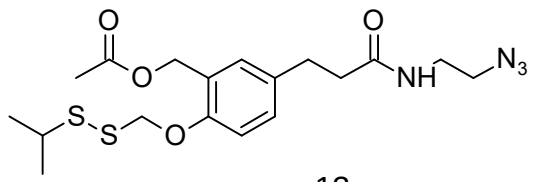

13

Normalized Intensity

Chemical Shift (ppm)

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M02(s)
M03(s)
M04(s)
M05(s)
M06(s)
M07(s)
M08(s)
M09(s)
M10(s)
M11(s)
M12(s)
M13(s)
M14(s)
M15(s)
M16(s)
M17(s)
172.40
171.08
153.44
134.15
130.17
129.27
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113.42
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51.01
41.62
38.99
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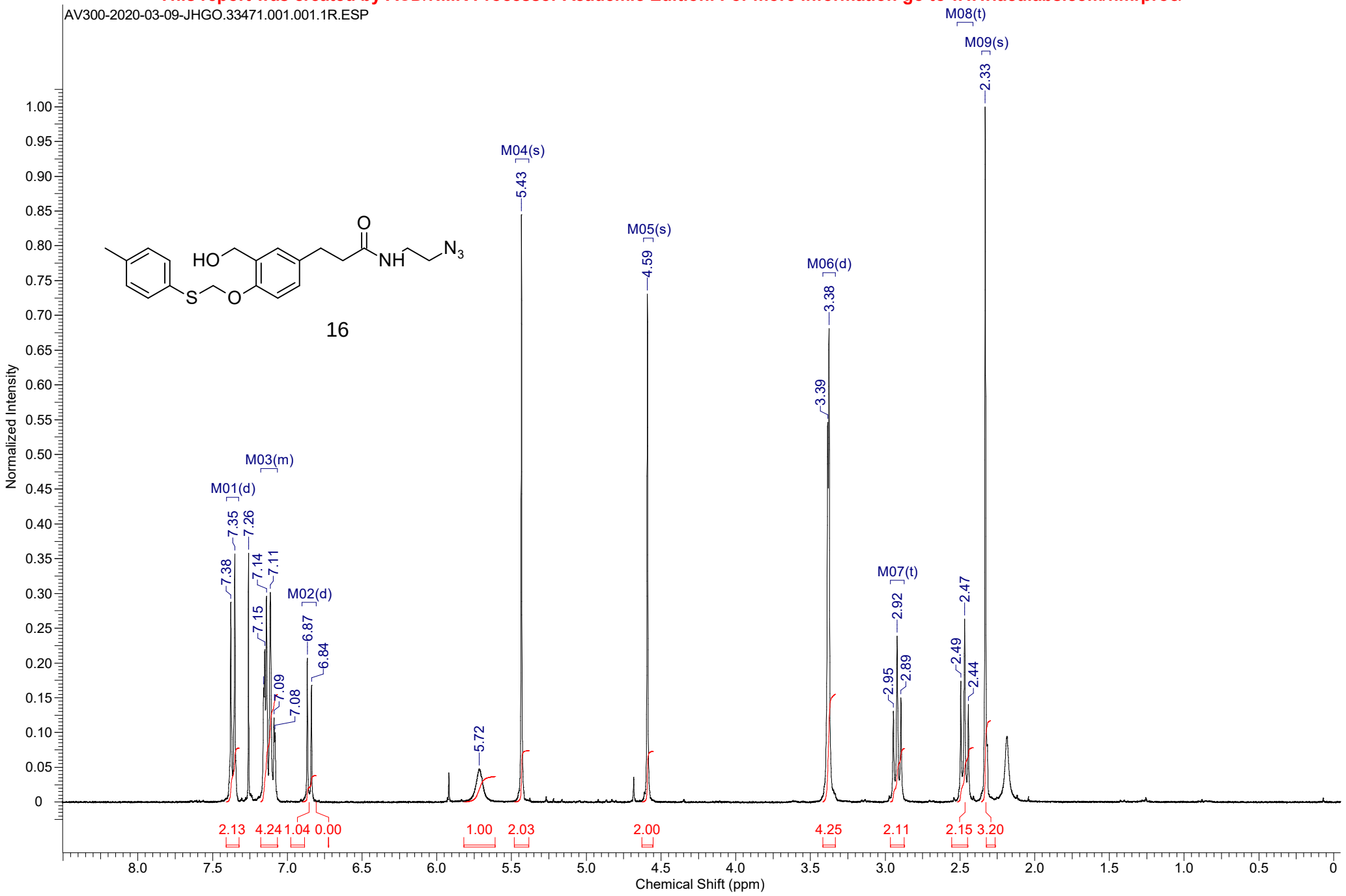
30.83
22.65
21.21



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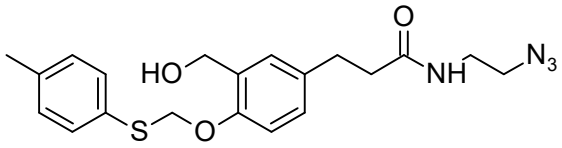

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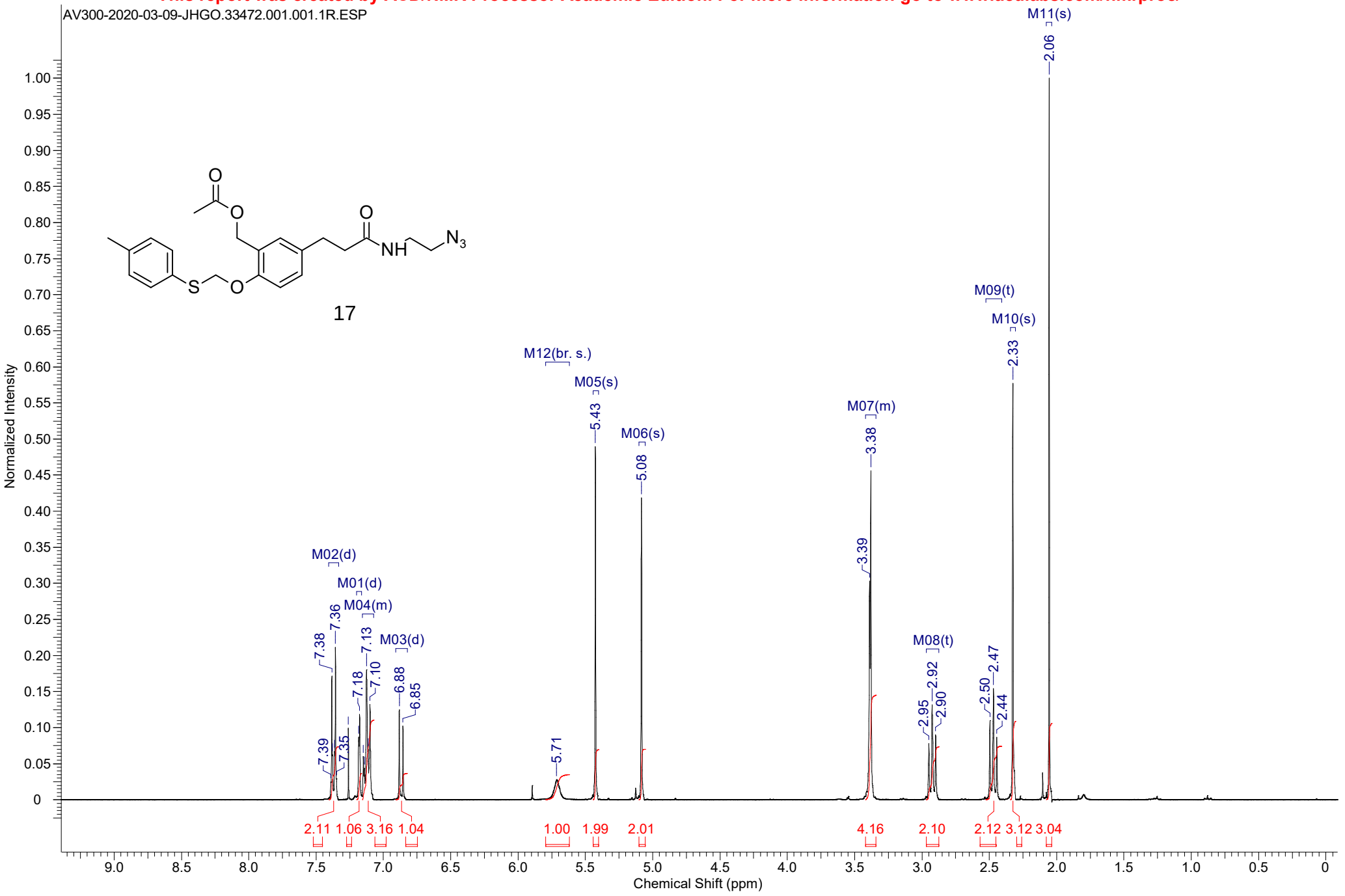

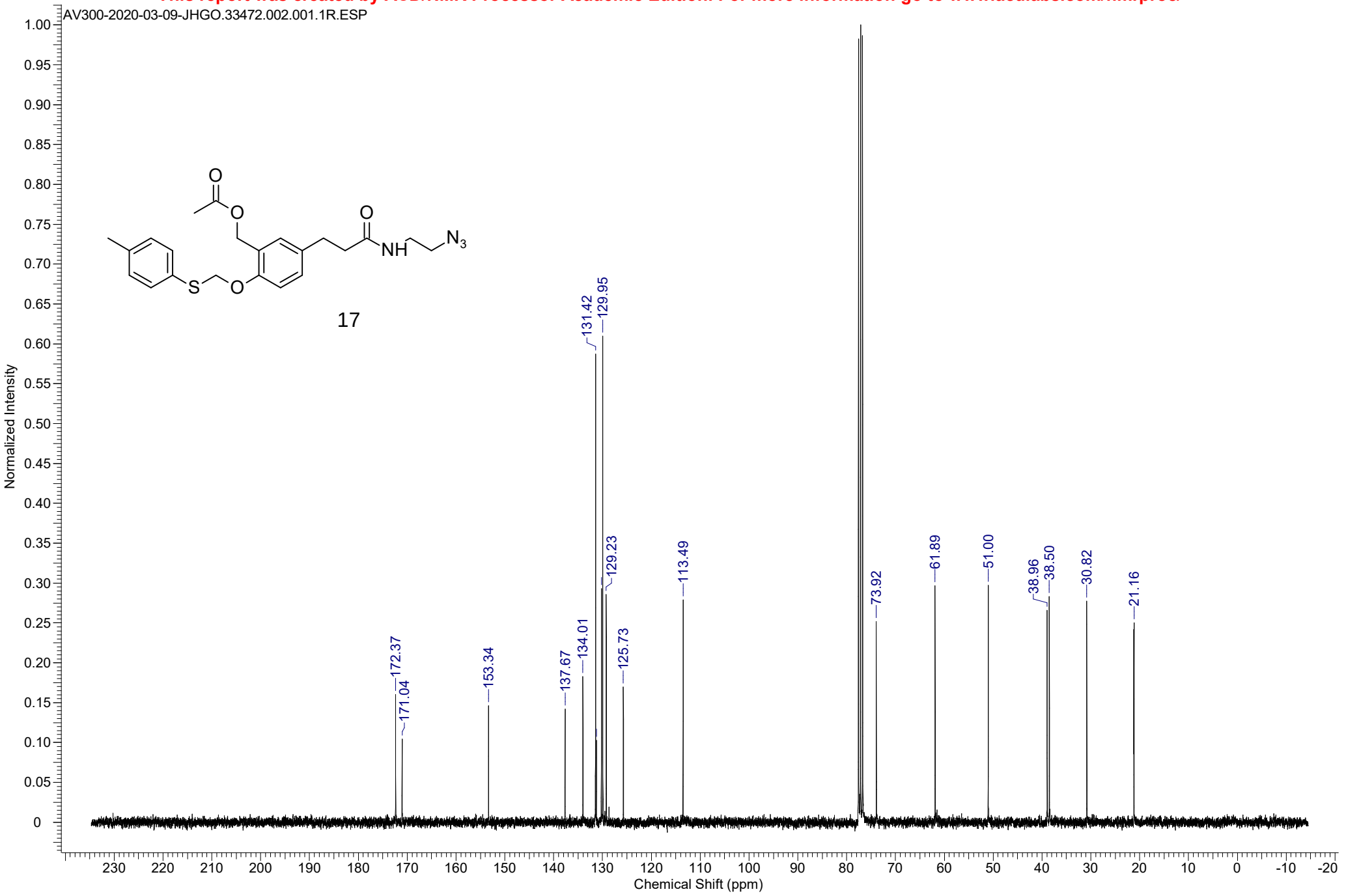

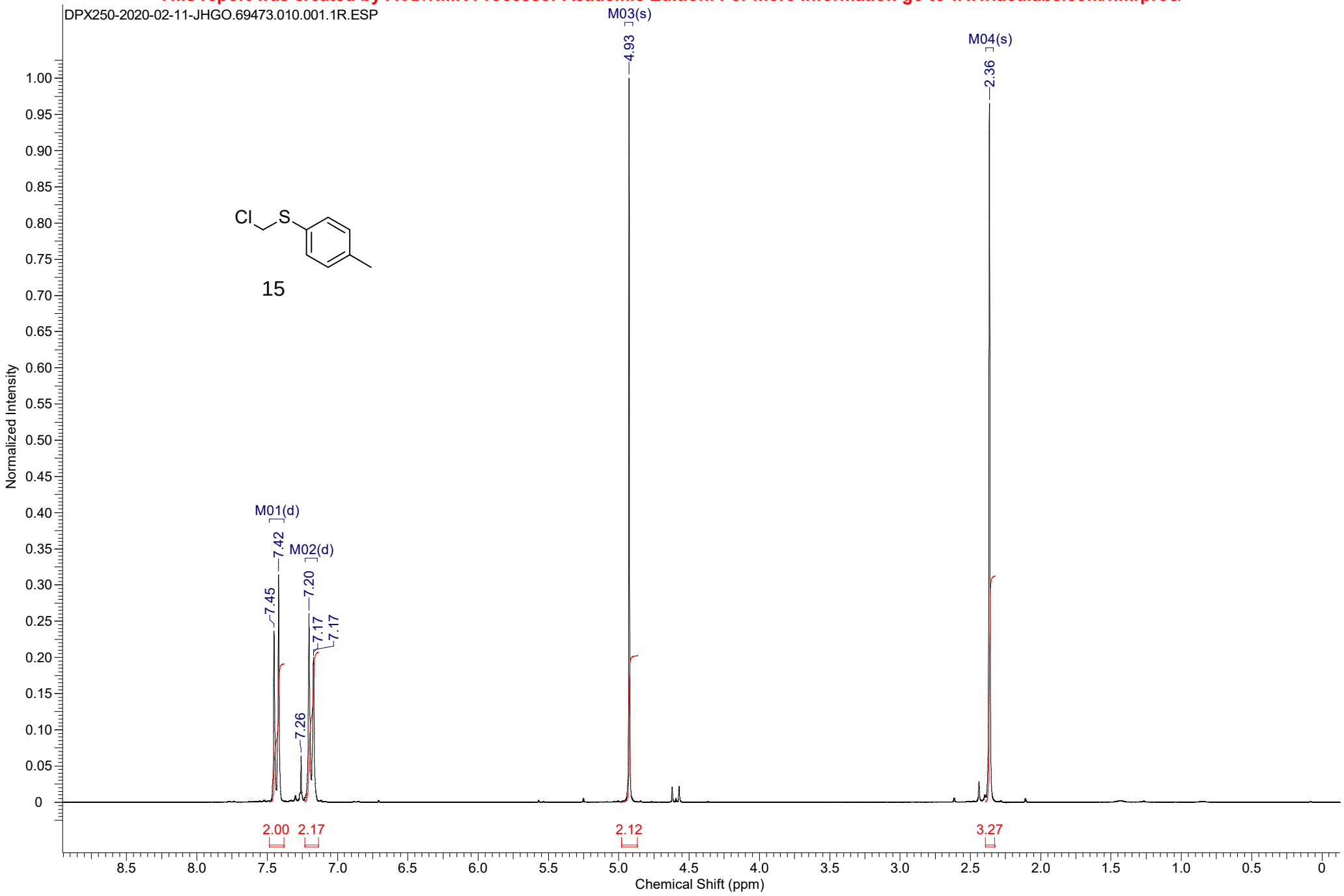

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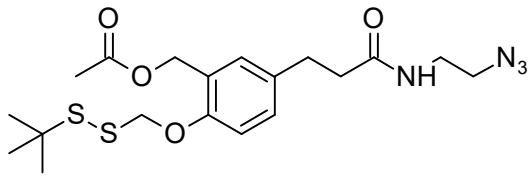

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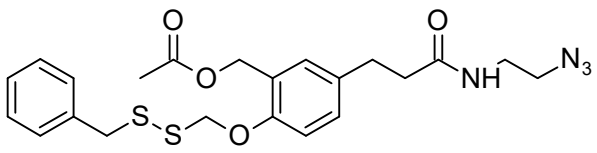

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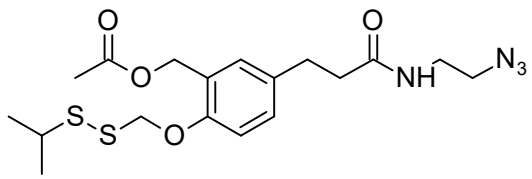

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