Supplemental Information for:

Characterization of reactions between water soluble trialkylphosphines and thiol alkylating reagents: Implications for protein conjugation reactions

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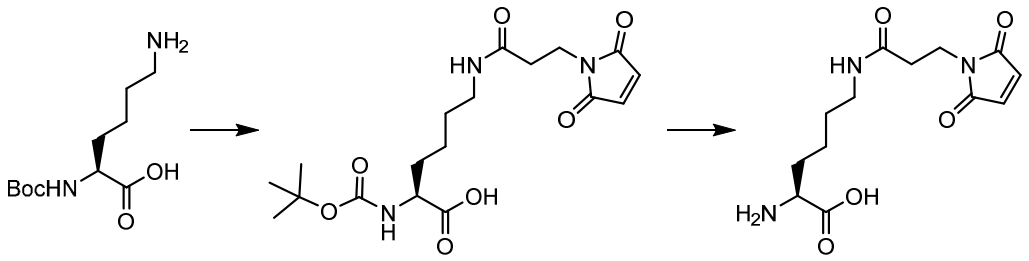
EXPERIMENTAL PROCEDURES

General

Chemical reagents were purchased from Sigma-Aldrich unless specifically stated. Anhydrous solvents were purchased from Sigma-Aldrich and used without further purification. All other solvents were purchased from Fisher Scientific. Yeast enolase protein was purchased from Sigma-Aldrich (Product; E6126). Pneumococcal capsular polysaccharide type 6B (Pn6B) was purchased from American Type Culture Collection (Rockville, Md.). Analytical thin layer chromatography (TLC) was carried out on Merck aluminium backed TLC plates silica gel 60 F254 (0.25 mm thickness), viewed using UV light of wavelength 254 nm or stained with potassium permanganate solution. Silica gel chromatography was performed on silica gel 60 Å (200-400 mesh) from Sigma-Aldrich. Standard work up is referring to diluting the reaction residue in appropriate organic solvent (mentioned), washing with an aqueous saturated sodium bicarbonate solution, followed by washing with brine. The organic phases were then combined and dried over anhydrous magnesium sulfate, filtered and the filtrate concentrated *in vacuo*. SDS-PAGE was performed using pre-cast 4-12% Bis-Tris NuPage gels (Invitrogen). Reverse phase chromatography (C-18) was performed using a VersaFlash hand held column (23 x 110 mm) from Supelco. Melting points were obtained using a Thermo Fisher IA9000 digital melting point apparatus. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded using Bruker Advance III (400 and 500 MHz) spectrometers with acquisition frequencies of 400 or 500 MHz for ^{1}H; 100 or 125 MHz for ^{13}C; and 162 MHz for ^{31}P. Deuterated solvents were purchased from Cambridge Isotope Laboratories. The NMR chemical shifts δ are recorded in parts per million (ppm) with reference to tetramethylsilane for ^{1}H and ^{13}C and phosphoric acid for ^{31}P NMR spectroscopy. The multiplicities are assigned as a singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of doublet of doublets (ddd), doublet of triplets (dt), triplet of doublets (td), broad (br) and multiplet (m). High resolution mass spectrometry was performed using a BrukerMicrOTOF electrospray ionisation mass spectrometer. Infrared spectra were recorder on a PerkinElmer Spectrum 65 FT-IR spectrometer. HPLC was performed on a Dionex Ultimate 3000 instrument equipped with a variable wavelength detector. SDS-PAGE was performed utilising an Invitrogen xCell

SureLock™ cell and a Biorad PowePac HV. Specific rotations were determined on an Optical Activity Ltd.: AA-10 automatic polarimeter.

Synthesis of *N^6-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-lysine* (**5**).



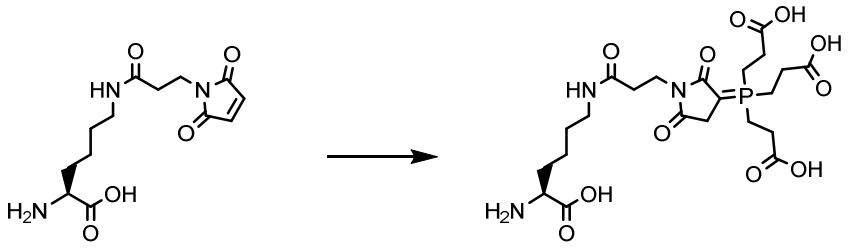
N^2-(tert-Butoxycarbonyl)-L-lysine (190 mg, 0.77 mmol) was dissolved in anhydrous DMF (5 mL). Triethylamine (108 µL, 0.77 mmol) and 3-maleimidopropionic acid N-hydroxysuccinimide ester (1.2 eq., 247 mg, 0.93 mmol) were added and the reaction was stirred at room temperature while under nitrogen gas for 18 hours. The solution was concentrated and purified by silica gel chromatography (5% MeOH/DCM → 20% MeOH/DCM) to yield N^2-(tert-butoxycarbonyl)-N^6-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-L-lysine (**S1**) as a white sticky solid (261 mg, 85 %). ^{1}H NMR, CD_3OD, 400 MHz: δ 1.37-1.55 (m, 13H, $CHCH_2CH_2CH_2CH_2$, 3 x CH_3), 1.60-1.84(m, 2H, $CHCH_2CH_2$), 2.45 (t, 2H, $COCH_2CH_2$, *J* = 7.0 Hz), 3.12 (m, 2H, $NHCH_2CH_2$), 3.77 (t, 2H, NCH_2, *J* = 7.0 Hz), 3.98 (m, 1H, $NHCHCO_2H$), 6.83 (s, 2H, $COCHCHCO$). ^{13}C NMR, CD_3OD, 100 MHz: δ 24.26 ($CHCH_2CH_2CH_2CH_2$), 28.78 (CCH_3), 29.91 ($NHCH_2CH_2CH_2$), 33.08 ($CHCH_2CH_2$), 35.48 (NCH_2CH_2), 35.81 ($NHCOCH_2CH_2$), 40.22 ($NHCH_2CH_2$), 56.08 ($NHCHCO_2H$), 80.28 ($OCCH_3$), 135.50 (2 x $COCH$), 158.03 ($NHCO_2C$), 172.13 (2 x $COCH$), 172.86 ($NHCOCH_2$), 179.14 ($CHCO_2H$). HRMS: Expected for $C_{18}H_{26}N_3O_7$ ($M\text{-}H^+$) = *m/z* 396.1776. Found: *m/z* 396.1809. Infrared (KBr): 3370, 1713, 1649 cm^{-1}. HPLC: column: Waters Symmetry Shield-RP8 (100 x 4.60 mm), gradient elution: (1.0 mL/min) 90 % water/MeCN containing 0.1 % TFA → 90 % MeCN/H_2O over 13 minutes, retention time, 6.15 mins., purity: 97%. Detection at 225 nm.

The Boc-protected derivative **S1** (80.0 mg, 0.20 mmol) was suspended in THF (1 mL) and cooled to 0 °C. TFA (4 mL) was slowly added to the solution which was left to warm to room

temperature and then stirred for a further 5 hours. The solution was concentrated and the residue was redissolved in 2 mL of water. The solution was neutralised with K_2CO_3 and subsequently purified by C-18 chromatography (100 % $H_2O \rightarrow$ 20 % MeOH/H_2O) to yield (**5**) as a transparent solid (43 mg, 71 %). ^{1}H NMR, D_2O, 400 MHz: δ 1.33 (m, 2H, $CHCH_2CH_2CH_2$), 1.45 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 1.85 (m, 2H, $CHCH_2CH_2CH_2$), 2.48 (t, 2H, $COCH_2CH_2$, *J* = 6.4 Hz), 3.12 (t, 2H, $NHCH_2CH_2$, *J* = 6.9 Hz), 3.72 (t, 1H, $NHCHCO_2H$, *J* = 5.9 Hz), 3.79 (t, 2H, NCH_2CH_2, *J* = 7.0 Hz), 6.87 (s, 2H, COCHCHCO). ^{13}C NMR, D_2O, 100 MHz: δ 21.76 ($CHCH_2CH_2CH_2CH_2$), 27.88 ($NHCH_2CH_2CH_2CH_2$), 30.02 ($CHCH_2CH_2CH_2$), 34.53 ($NCH_2CH_2CH_2$), 34.76 ($COCH_2CH_2CH_2$), 38.96 ($NHCH_2CH_2CH_2$), 54.62 ($NHCHCO_2H$), 134.45 (2 x NCOCH), 172.57 (2 x NCOCH), 173.37 ($NHCOCH_2CH_2$), 174.67 ($CHCO_2H$). HRMS: Expected for $C_{13}H_{18}N_3O_5$ ($M\text{-}H^+$) = *m/z* 296.1252. Found: *m/z* 296.1229. Infrared (KBr): 3325, 1714, 1630 cm^{-1}. HPLC: column: Phenomenex Luna-NH_2 (250 x 4.60 mm), gradient elution: (1.0 mL/min) 10 % MeCN in water containing 0.1 % TFA $\rightarrow$ 100 % MeCN over 20 minutes, retention time, 2.63 mins., purity: 100 %. Detection at 225 nm.

Reaction between TCEP and Maleimide:

Synthesis of *N^6-(3-{2,5-dioxo-3-[tris(2-carboxyethyl)-λ^5-phosphanylidene]pyrrolidin-1-yl}propanoyl)-L-lysine (**6**).*

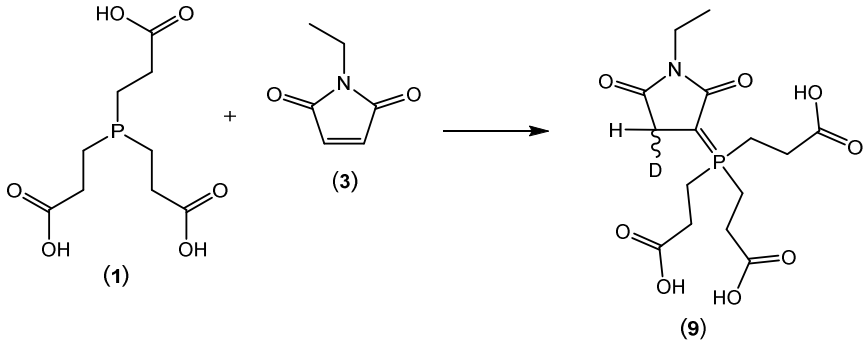


(**5**) (**6**)

Compound **5** (15 mg, 0.052 mmol) and TCEP (**1**, 0.9 eq., 13.4 mg, 0.0466 mmol) was dissolved in 2 mL of argon purged aqueous sodium phosphate (0.1 M, pH = 7). The reaction was stirred, under argon, and at room temperature for 1 hour. The reaction was subsequently purified by C-18 chromatography (100 % water). The product was lyophilised to give (**6**) as an amorphous white solid (18 mg, 70 %). ^{1}H NMR, D_2O, 400 MHz: δ 1.33-1.45 (m, 2H, $CHCH_2CH_2CH_2$), 1.47-1.55 (m, 2H, $NHCH_2CH_2CH_2$), 1.83-1.90 (m, 2H, $CHCH_2CH_2$), 2.51 (t, 2H, $NHCOCH_2$, *J* = 6.6 Hz), 2.74-2.79 (m, 12H, 3 x CH_2CH_2CO), 3.14 (t, 2H, $NHCH_2CH_2$, *J* =

6.9 Hz), 3.21-3.34 (m, 2H, CCH_2CO), 3.76 (t, 1H, NH_2CHCO_2H, *J* = 6.3 Hz), 3.80 (t, 2H, NCH_2CH_2, *J* = 6.6 Hz). ^{13}C NMR, D_2O, 100 MHz: δ 15.0 (d, PCH_2CH_2, *J* = 49.5 Hz), 21.7 ($CHCH_2CH_2CH_2$), 26.9 (d, 3 x $CH_2CH_2CO_2H$, *J* = 3.8 Hz), 27.8 ($NHCH_2CH_2CH_2$), 29.9 ($CHCH_2CH_2$), 29.5 ($COCH_2C$), 33.2 ($COCH_2CH_2$), 34.0 (m, $COCCH_2$), 36.4 (NCH CH_2), 39.0 ($NHCH_2CH_2$), 54.4 (NH_2CHCO_2H), 172.7 (d, NCOC, *J* = 3.4 Hz), 172.8 ($NHCOCH_2$), 174.5 ($CHCO_2H$), 175.5 (d, 3 x CH_2CO_2H, *J* = 11.8 Hz), 175.6 (d, $NCOCH_2$, *J* = 8.7 Hz). ^{31}P NMR, D_2O, 162 MHz: δ 38.9. HRMS: Expected for $C_{22}H_{33}N_3O_{11}P_1$ ($M\text{-}H^+$) = *m/z* 546.1858. Found: *m/z* 546.1864. Infrared (KBr): 3456, 1708, 1642 cm^{-1}. HPLC: column: Phenomenex Luna-C18 (250 x 4.60 mm), gradient elution: (0.7 mL/min) 100 % water containing 0.1 % TFA → 100 % MeCN over 16 minutes, retention time, 11.68 mins, purity, 87%). Detection at 225 nm.

Synthesis of *3,3',3''-[(1-ethyl-2,5-dioxopyrrolidin-3-ylidene, 4-deutero)-λ^5-phosphanetriyl]tripropanoic acid (**9**).*

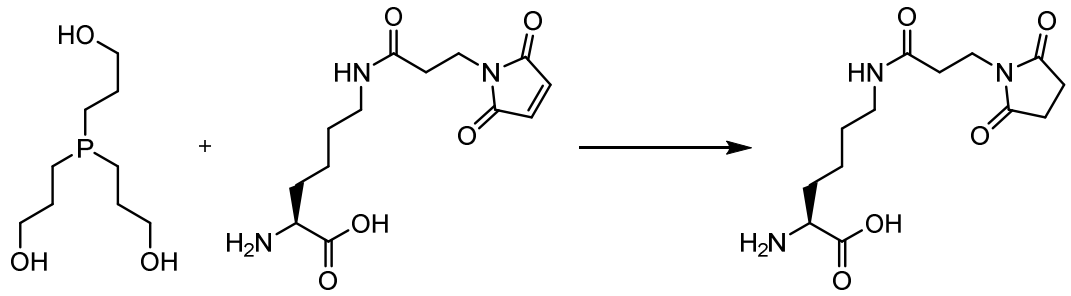


N-Ethyl maleimide (**3**, 10.0 mg, 0.0800 mmol) and TCEP (**1**, 0.9 eq., 20.6 mg, 0.0719 mmol) was dissolved in THF (1 mL) and argon purged deuterated aqueous sodium phosphate (0.1 M, pH = 7.0, 9 mL) and stirred under argon at room temperature for 1 hour. The reaction was concentrated *in vacuo* to 3 mL and then loaded onto a C-18 column for purification (100 % H_2O → 20 % MeCN/H_2O) to yield **9** as a pale yellow sticky solid (21.4 mg, 79 %). ^{1}H NMR, D_2O, 400 MHz: δ 1.10 (t, 3H, CH_2CH_3, *J* = 7.3 Hz), 2.77-2.89 (m, 12H, 3 x $CH_2CH_2CO_2H$), 3.26-3.14 (m, 1H, CCHDCO), 3.54 (q, 2H, NCH_2CH_3, *J* = 7.3 Hz). ^{13}C NMR, D_2O, 125 MHz: δ 11.5 (CH_2CH_3), 14.4 (d, 3 x $CH_2CH_2CO_2H$, *J* = 49.8 Hz), 25.8 (d, 3 x $CH_2CH_2CO_2H$, *J* = 4.0 Hz), 29.2 (m, COCHDC), 33.6 (m, COCCHD), 35.0 (NCH_2CH_3), 173.0 (d, NCOC, *J* = 3.5 Hz), 174.2 (d, CH_2CO_2H, *J* = 11.8 Hz), 176.0 (d, NCOCHD, *J* = 8.8 Hz). ^{31}P NMR, D_2O, 162 MHz: δ 39.2.

HRMS: Expected for $C_{15}H_{22}D_1N_1O_8P_1$ ($M+H^+$) = *m/z* 377.1219. Found: *m/z* 377.1222. Infrared (KBr): 3439, 1705 cm^{-1}. HPLC: column: Phenomenex Luna-C18 (250 x 4.60 mm), gradient elution (0.7 mL/min), water containing 0.1 % TFA → 100 % MeCN over 16 minutes, retention time, 12.73 mins, purity, 91 %. Detection at 225 nm.

Reaction between THPP and Maleimide:

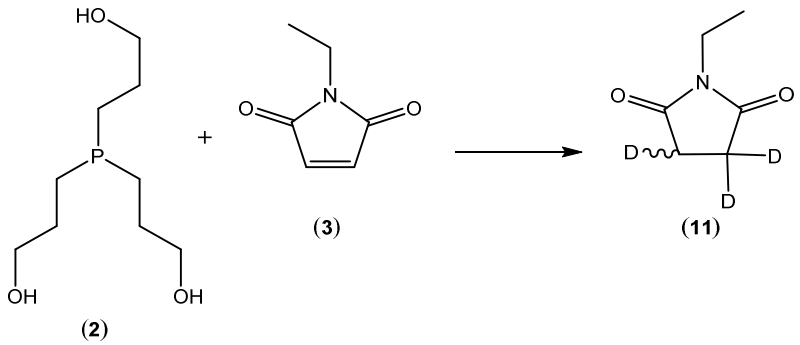
Synthesis of *N^6-[3-(2,5-dioxopyrrolidin-1-yl)propanoyl]-L-lysine (**8**).*



(**2**) (**5**) (**8**)

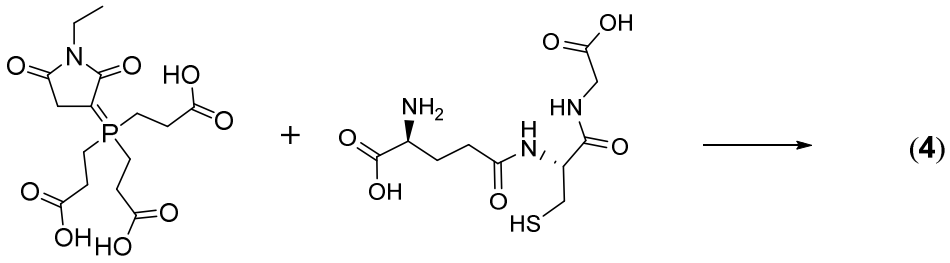
Compound **5** (10.0 mg, 0.034 mmol) and THPP (**2**, 0.9 eq., 6.3 mg, 0.03 mmol) was dissolved in 1 mL of argon purged aqueous sodium phosphate (0.1 M, pH = 7). The reaction was stirred, under argon, and at room temperature for 1 hour. The reaction was subsequently purified by C-18 chromatography (100 % water → 20 % MeCN/water). The product was lyophilised to give (**8**) as a white amorphous solid (5.35 mg, 59 %). ^{1}H NMR, D_2O, 400 MHz: δ 1.33-1.41 (m, 2H, $CHCH_2CH_2CH_2$), 1.43-1.55 (m, 2H, $NHCH_2CH_2CH_2$), 1.83 -1.91 (m, 2H, $CHCH_2CH_2$), 2.46 (t, 2H, $NHCOCH_2CH_2$, *J* = 6.8 Hz), 2.77 (s, 4H, $COCH_2CH_2CO$), 3.14 (t, 2H, $NHCH_2CH_2$, *J* = 6.8 Hz), 3.69-3.77 (m, 3H, NCH_2CH_2 & NH_2CHCO_2H). ^{13}C NMR, D_2O, 100 MHz: δ 21.73 ($CHCH_2CH_2CH_2$), 27.84 ($NHCH_2CH_2CH_2$), 27.98 (2 x $COCH$), 30.02 ($CHCH_2CH_2$), 33.79 ($COCH_2CH_2$), 35.47 (NCH_2CH_2CO), 38.99 ($NHCH_2CH_2$), 54.65 (NH_2CHCO_2H), 173.22 ($NHCOCH_2$), 174.72 ($CHCO_2H$), 181.11 (2 x $NCOCH$). Expected for $C_{13}H_{20}N_3O_5$ ($M-H^+$) = *m/z* 298.1408. Found: *m/z* 298.1412. Infrared (KBr): 3447, 1696 cm^{-1}. HPLC: column: Phenomenex Luna-NH_2 (250 x 4.60 mm), gradient elution: (1.0 mL/min) 10 % MeCN in water containing 0.1 % TFA → 100 % MeCN over 20 minutes, retention time, 2.77 mins., purity, 97%). Detection at 225 nm.

Synthesis of *1-ethyl-3-dideutero-(4R,S)-deutero-pyrrolidine-2,5-dione (**11**).*



A solution of THPP (**2**, 0.9 eq., 29.9 mg, 0.144 mmol) was prepared in THF (1 mL) and purged deuterated aqueous sodium phosphate (0.1 M, pH = 7.0, 9 ml). N-Ethyl maleimide (**3**, 20.0 mg, 0.16 mmol) was added slowly to the rapidly stirring solution of THPP. The reaction was left to stir for 30 minutes at room temperature. A further 0.1 eq. of THPP was added and left to stir for an additional 30 minutes. The reaction was diluted with 25 mL of diethyl ether and extracted with water (30 mL). The aqueous layer was extracted with diethyl ether (2 x 30 mL). The organic extraction layers were combined and dried using $MgSO_4$. The mixture was filtered and the organic solution was concentrated (550 mbar, 25 °C). The crude was purified by silica gel chromatography (0 $\rightarrow$ 2 % acetone/CH_2Cl_2) to yield **11** as a clear oil (10.8 mg, 52 %). ^{1}H NMR, $CDCl_3$, 400 MHz: δ 1.10 (t, 3H, CH_2CH_3, *J* = 7.2 Hz), 2.62 (br. s, 1H, $COCHDCD_2$), 3.50 (q, 2H, NCH_2CH_3, *J* = 7.2 Hz). ^{13}C NMR, $CDCl_3$, 100 MHz: δ 12.9 (CH_2CH_3), 27.7 (m, $COCHDCD_2CO$), 33.5 (NCH_2CH_3), 177.1 (2 x NCO). ESI-HRMS: Expected for $C_6H_6D_3N_1O_2$ ($M+Na^+$) = *m/z* 153.0714. Found: *m/z* 153.0704. Infrared (thin film): 1695 cm^{-1}. HPLC: column: Phenomenex Luna-NH_2 (250 x 4.60 mm), gradient elution: (1.0 mL/min) 10 % MeCN in water containing 0.1 % TFA $\rightarrow$ 100 % MeCN over 20 minutes, retention time, 2.90 mins, purity 99 %. Detection at 225 nm.

Exploring the reactivity of the TCEP-maleimide Ylene (4) towards thiol nucleophiles



(**4**)

A) pH 4.

N-Ethyl maleimide (**3**, 3.9 mg, 0.032 mmol) was dissolved in argon purged deuterated aqueous citric acid/sodium phosphate (0.5 mL, 0.1 M, pH = 4). TCEP (**1**, 1 eq., 9.0 mg, 0.032 mmol) was added and the reaction was incubated at room temperature for 1 hour. The TCEP-maleimide adduct (**4**) was evident by ^{31}P NMR (δ 38.9). Reduced glutathione (1.5 eq., 14.6 mg, 0.047 mmol) was added and the reaction was left at room temperature overnight. The reaction was monitored by ^{31}P NMR. The δ 38.90 ppm signal for **4** was still evident, with no appearance of new peaks in the spectrum.

B) pH 7.

N-Ethyl maleimide (**3**, 8.0 mg, 0.064 mmol) was dissolved in argon purged aqueous sodium phosphate (1.0 mL, 0.1 M, pH = 7.0). TCEP (**1**, 1 eq., 18.3 mg, 0.064 mmol) was added and the reaction was incubated at room temperature for 1 hour. Reduced glutathione (1.2 eq., 23.6 mg, 0.077 mmol) was added and the reaction was stirred at room temperature overnight. The reaction was monitored by HRMS. No glutathione-maleimide product was evident. The TCEP-maleimide adduct [**4**, *m/z* 374.1040 (M-H$^+$)] was still visible as well as unreacted glutathione [*m/z* 306.0787 (M-H$^+$)].

C) pH 8.

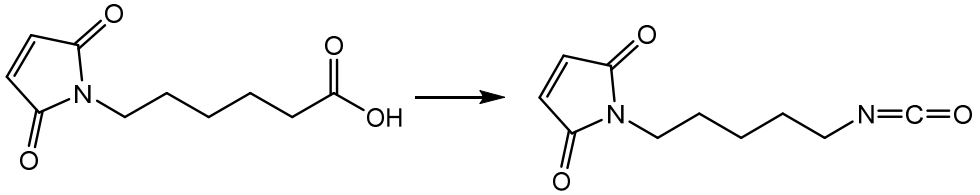
N-Ethyl maleimide (**3**, 4.0 mg, 0.032 mmol) was dissolved in argon purged, deuterated aqueous sodium phosphate (0.5 mL, 0.1 M, pH = 8.0). TCEP (**1**, 1 eq., 9.2 mg, 0.032 mmol) was added and the reaction was incubated at room temperature for 1 hour. Reduced

glutathione (1.5 eq., 14.7 mg, 0.048 mmol) was added and the reaction was left at room temperature overnight. The reaction was monitored by ^{31}P NMR. No change of the δ 38.90 ppm signal for **4** was evident.

The effect of not removing TCEP or THPP prior to maleimide PEGylation of a model protein.

Yeast enolase (1 mg/mL, Sigma Aldrich) was denatured in argon purged buffer (0.5 M Tris, pH = 7.2, 5 mM EDTA) containing 8 M urea at 85 °C for 15 minutes. The solution was allowed to cool to room temperature before aliquoting out 100 μL samples for the experiments. Varying concentrations of TCEP or THPP (1-10 mM) were added to aliquots of protein solution (11 μM) and incubated for 45 minutes at 25 °C. Maleimide-PEG2kDa (1 mM) was subsequently added and the reaction was incubated at 37 °C for 18 hours. Samples (15 μL) were taken from each of the reactions and added to Laemmli sample buffer (15 μL). Aliquots (9 μL) of these solutions were loaded into a precast gradient gel (4-12 % Bis-Tris, Invitrogen) along with a protein ladder (EZ-Run, Fisher Scientific) and resolved by SDS Page electrophoresis [MOPS running buffer (Invitrogen), 180 V, 60 mins]. The precast gels were stained by Coomassie solution and destained using a water/ethanol/acetic acid (16:3:1) solution.

Synthesis of N-(5-isocyanatopentyl)maleimide (**S3**).

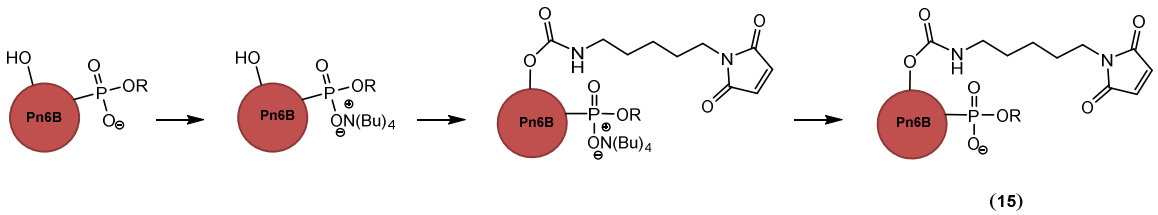


(**S2**) (**S3**)

6-Maleimidohexanoic acid **S2** (0.25 g, 1.18 mmol) was dissolved in anhydrous THF (4 mL) containing triethylamine (0.3 mL). Diphenylphosphoryl azide (0.38 mL, 1.76 mmol) was added and the reaction was stirred at room temperature for 2 hours. Anhydrous toluene (100 mL) was subsequently added and the solution was concentrated to a volume of 5 mL. The solution was heated at 70 °C for 2 hours and then allowed to cool back to room

temperature. The solution was loaded directly onto a silica gel column for purification: 100 % petroleum ether → 30 % EtOAc/petroleum ether to yield **S3** as a transparent oil (125.7 mg, 51 %). Spectral data was consistent with that reported in the literature.[1] ^{1}H NMR $CDCl_3$, 400 MHz: δ 1.37-1.42 (m, 2H, C*H*$_2$CH$_2$CH$_2$NCO), 1.58-1.66 (m, 4H, NCH$_2$C*H*$_2$ & CH$_2$C*H*$_2$CH$_2$NCO), 3.29 (t, 2H, CH$_2$C*H*$_2$NCO, *J* = 6.7 Hz), 3.52 (t, 2H, NC*H*$_2$CH$_2$, *J* = 7.2 Hz), 6.69 (s, 2H, COC*H*C*H*CO). ^{13}C NMR $CDCl_3$, 100 MHz: δ 23.62 (OCNCH$_2$CH$_2$*C*H$_2$), 27.82 & 30.57 (CHCONCH$_2$*C*H$_2$ & OCNCH$_2$*C*H$_2$CH$_2$), 37.44 (N*C*H$_2$CH$_2$), 42.69 (*C*H$_2$NCO), 121.89 (CH$_2$N*C*O), 134.05 (2 x CO*C*H), 170.78 (2 x *C*OCH). ESI-MS: Expected for $C_{10}H_{12}N_2Na_1O_3$ (M+Na$^+$) = *m/z* 231.0740. Found *m/z* 231.0739. Infrared (thin film): 2277, 1706 cm^{-1}.

Synthesis of Pn6B-maleimide (**15**).



Pn6B (10 mg) was added to 2 mL of water and stirred at room temperature until the carbohydrate dissolved. The viscous solution was then loaded onto an ion exchange column (Dowex 50W x 4, 200 mesh, tetrabutylammonium form) and incubated for 30 minutes before elution. The fractions containing carbohydrate were pooled and subsequently freeze dried to yield a white solid. The solid was added to 4 mL of anhydrous DMSO and stirred at 30 °C under N_2 overnight to dissolve the carbohydrate. An anhydrous DMSO solution (0.5 mL) of **I** (3 mg) was added to the carbohydrate solution and allowed to stir for 2 hours at room temperature. The solution was transferred to a dialysis bag (12-14 KDa cut-off) and dialysed against 5 litres of 0.1 M aqueous sodium phosphate (pH = 7), followed by 5 litres of 0.01 M aqueous sodium phosphate (pH = 7), and finally 5 litres of water. The solution within the dialysis bag was frozen and lyophilised to yield 6 mg of white solid (**15**). A portion of product (0.5 mg) was analysed by NMR spectroscopy to determine the maleimide/carbohydrate ratio. ^{31}P NMR, D_2O, 162 MHz: δ -0.08.

Synthesis of Pn6B-maleimide-TCEP adduct (**16**).

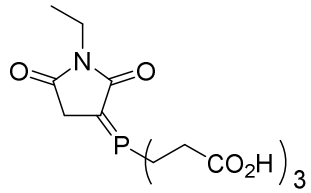
Maleimide functionalised Pn6B (**15**, 1.0 mg) was dissolved in water (1 mL) containing TCEP (2.0 mg). The solution was left to stand at room temperature (1 hour) then purified using a PD-10 desalting column. The fractions containing carbohydrate were pooled and lyophilised. The lyophilised powder was redissolved in D_2O and analysed by ^{31}P NMR spectroscopy. ^{31}P NMR, D_2O, 162 MHz: δ -0.08, 38.4.

Supplemental References

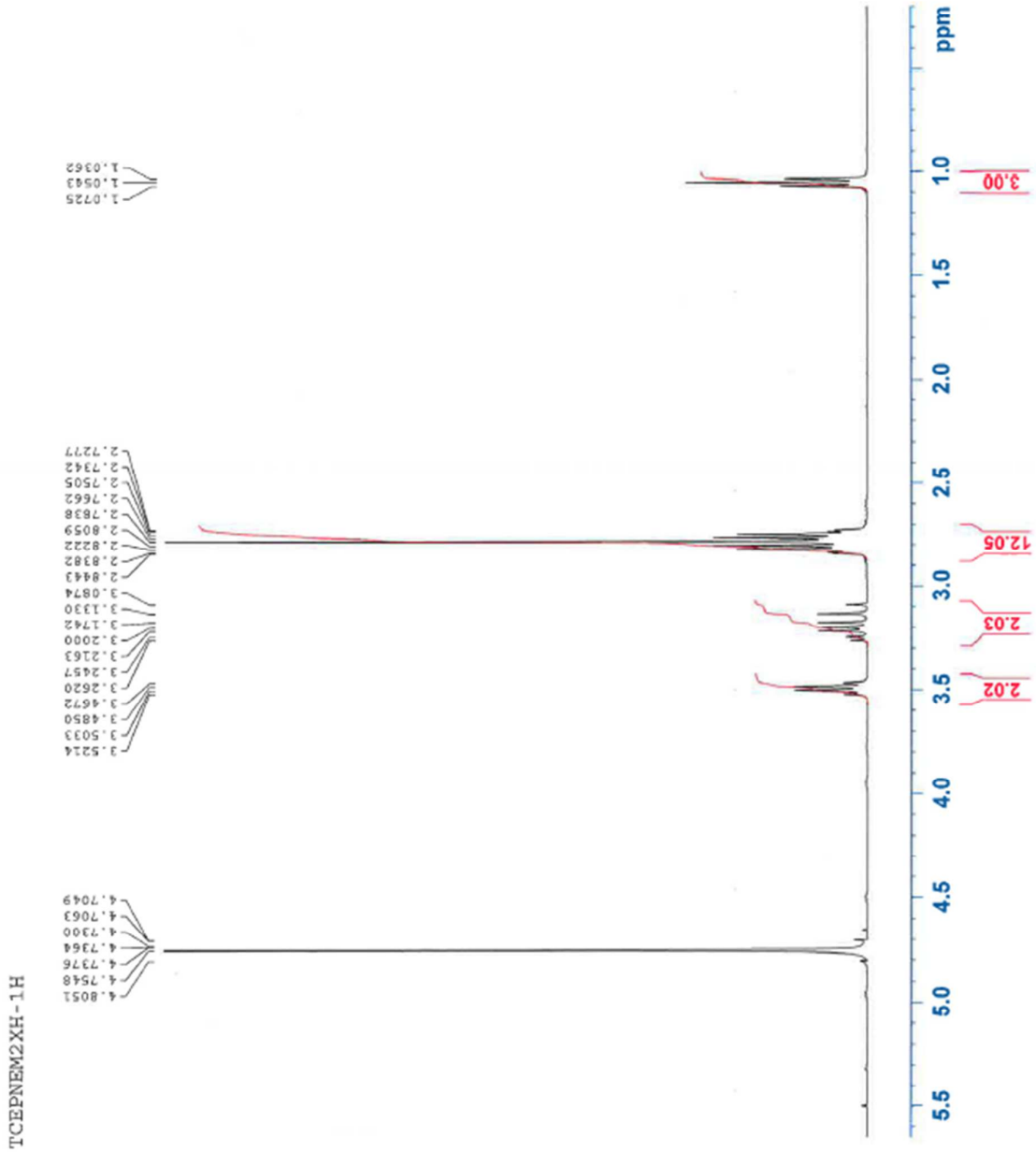
(1) Chudzik, S. J., Chinn, J. A., Swann, D. G., Burkstrand, M. J., *COATINGS FOR MEDICAL ARTICLES INCLUDING NATURAL BIODEGRADABLE POLYSACCHARIDES, US 2005/0255142 A l*. 17/11/2005: United States Patent Application Publication.

Analytical spectra

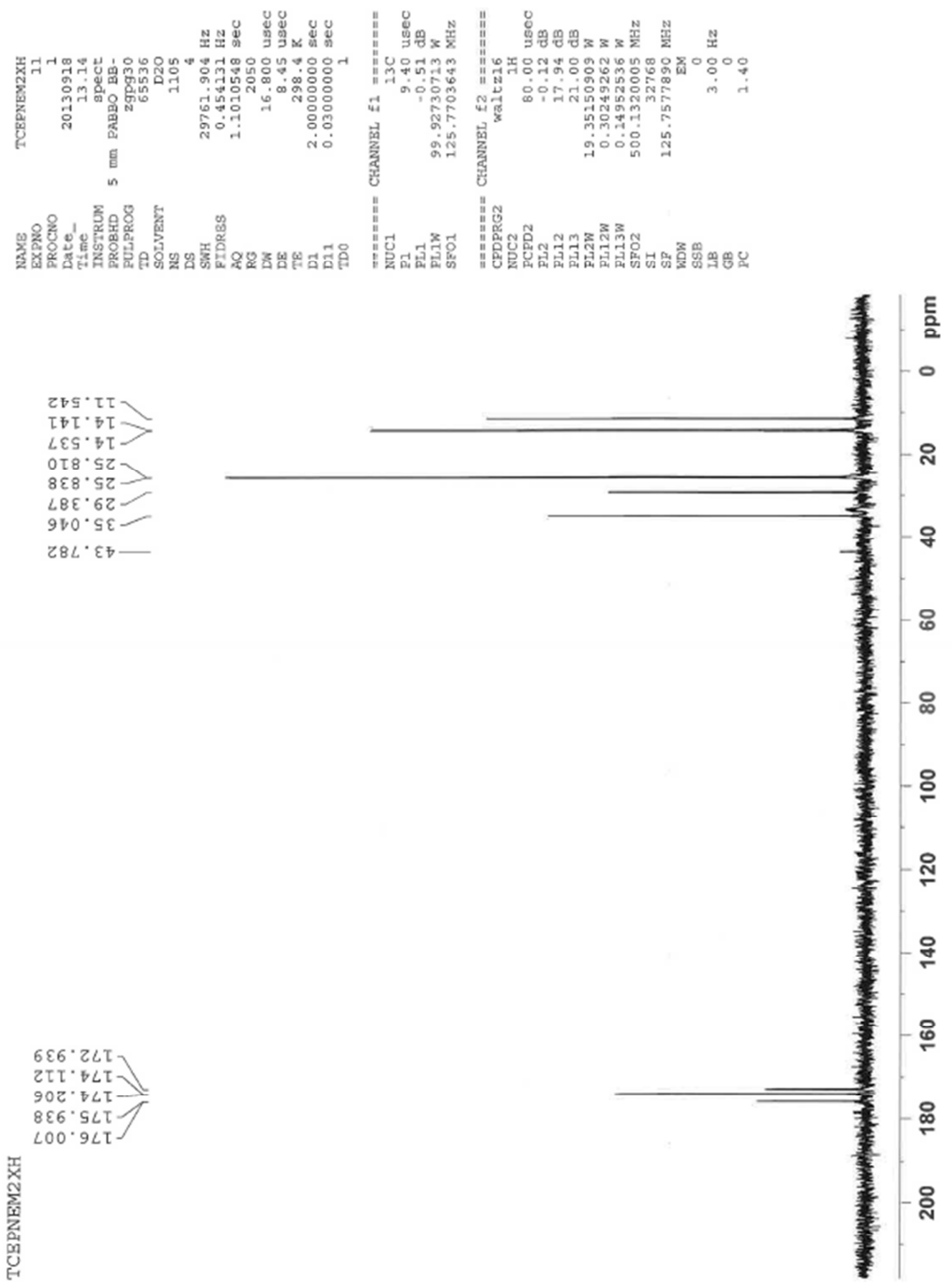
^{1}H NMR spectrum of compound (**4**)



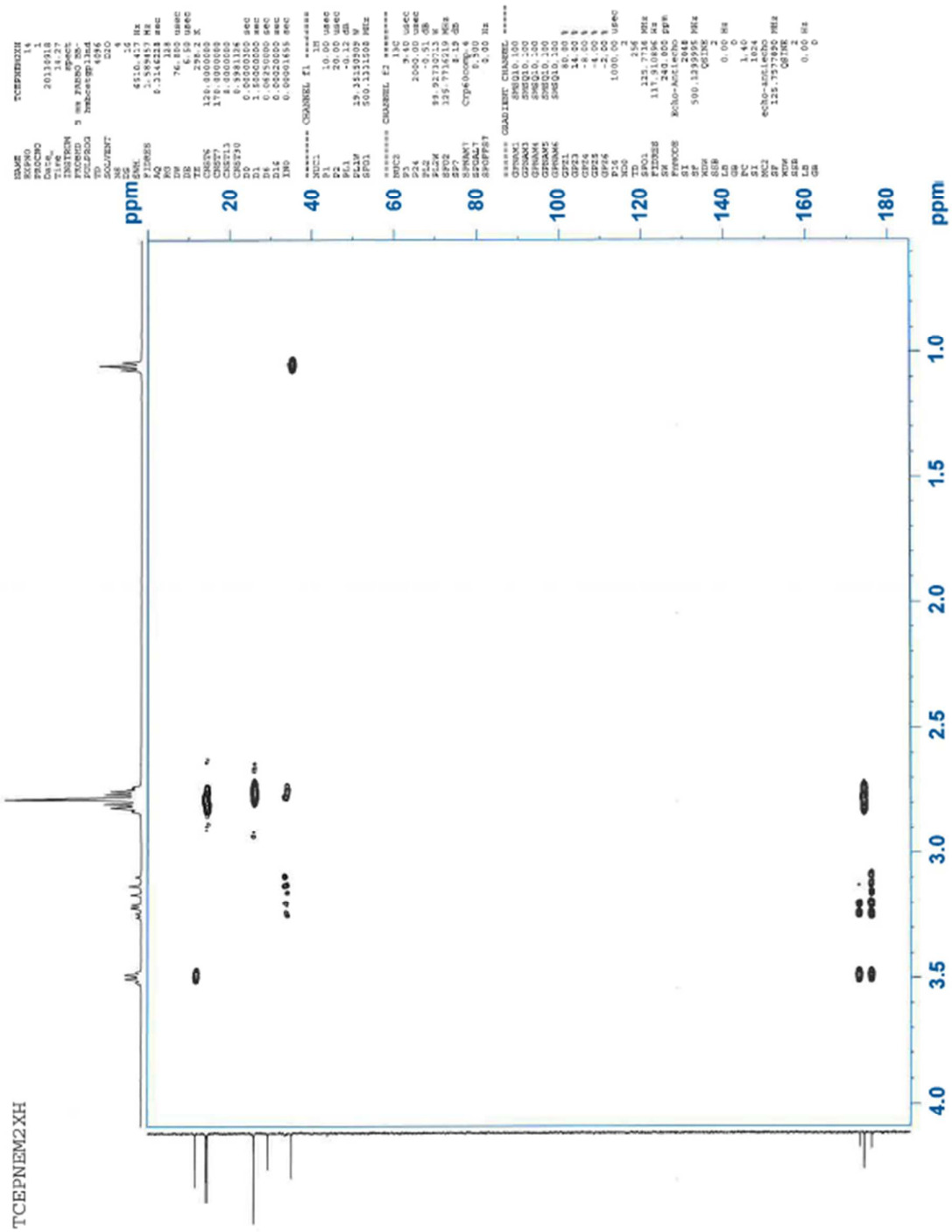
(**4**)



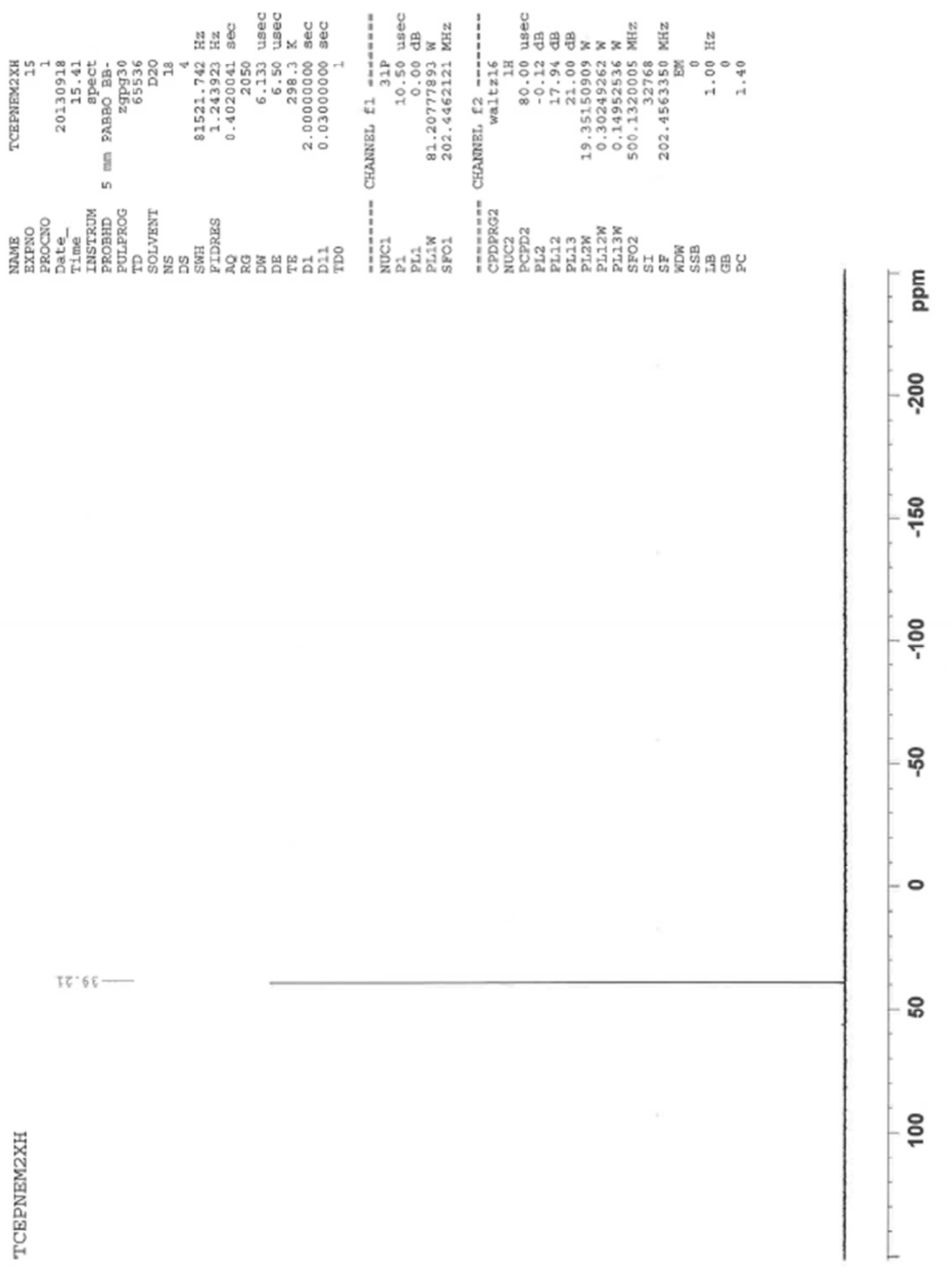
^{13}C NMR spectrum of compound (**4**)



HMBC spectrum for compound (**4**)



^{31}P NMR spectrum of compound (**4**)

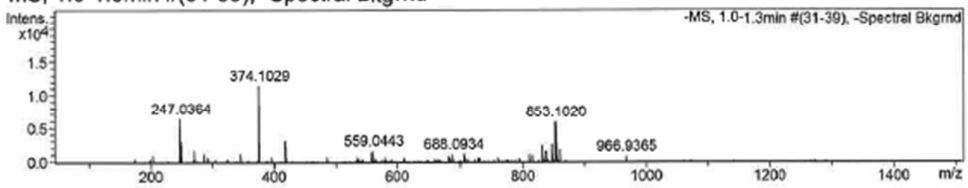


HRMS for compound (**4**)

Confirmation of Expected Formula

Sample-ID	wa_tk_tcepnem	Submitter	Terrence Kantner
Analysis Name	wa_tk_tcepnem_334404_12_01_37519.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to1500 loop inj.m	Acquisition Date	15/04/2013 11:21:53
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(31-39), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	247.0364	6470	56.7	222	1416.2
2	249.0509	3000	26.3	75	649.6
3	271.0310	1744	15.3	61	337.3
4	374.1029	11405	100.0	554	1323.9
5	418.0651	3139	27.5	157	319.8
6	831.1151	2345	20.6	230	107.9
7	847.0841	2491	21.8	265	108.1
8	853.1020	5905	51.8	642	251.0
9	854.1051	2322	20.4	209	98.3
10	859.1174	1883	16.5	190	78.4

Generate Molecular Formula Parameters

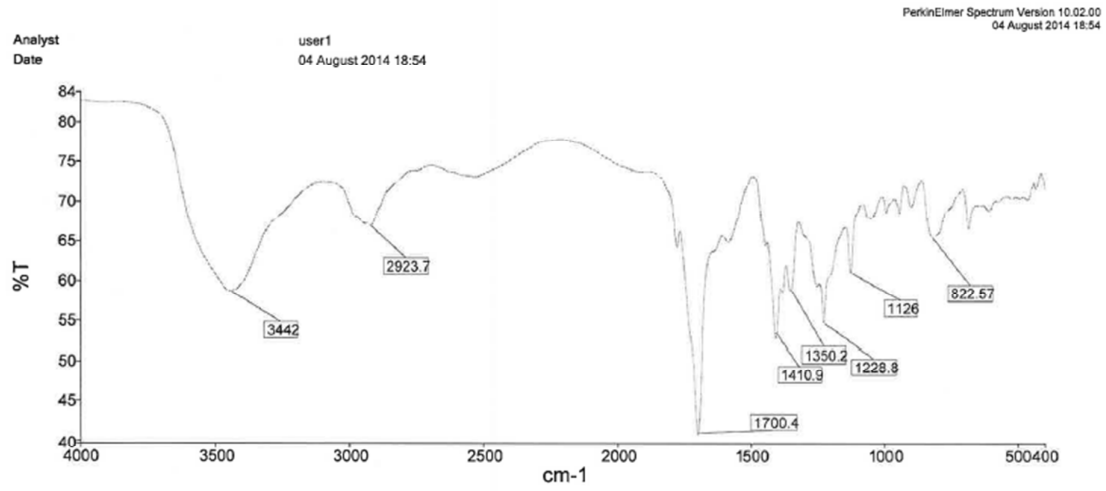
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C15 H22 N1 O8 P1 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	374.1029	374.100478	-4.90	0.0188	C 15 H 21 N 1 O 8 P 1

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

IR spectrum for compound (**4**)

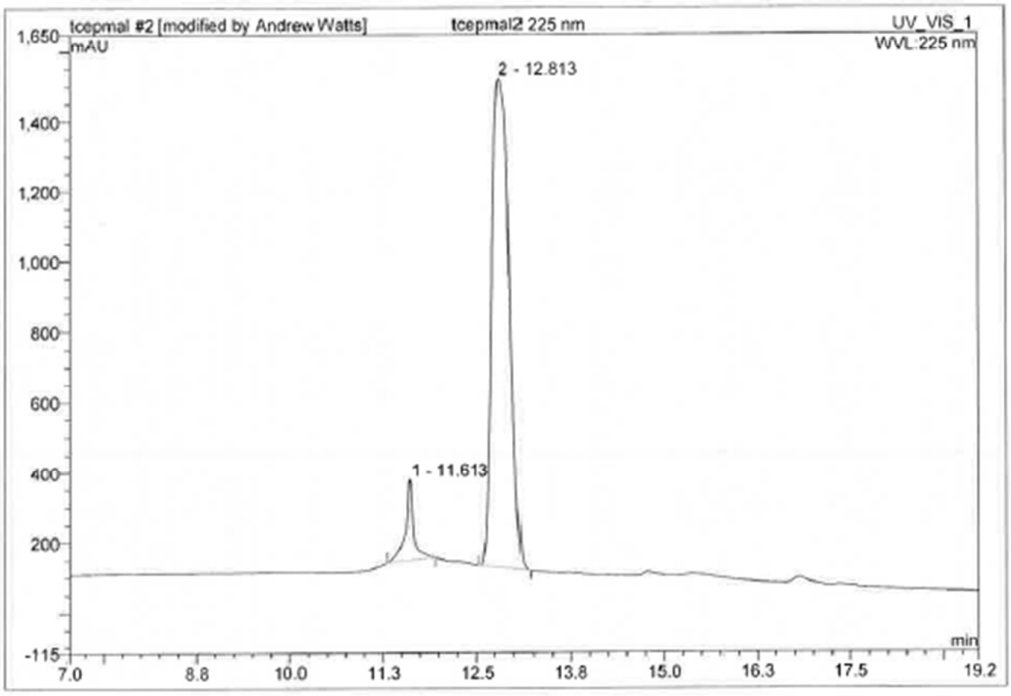


Sample Name	Description	Quality Checks
TCEPMal1KBr1KBr	Sample 005 By user1 Date Monday, August 04 2014	The Quality Checks do not report any warnings for the sample.

HPLC chromatogram for compound (**4**)

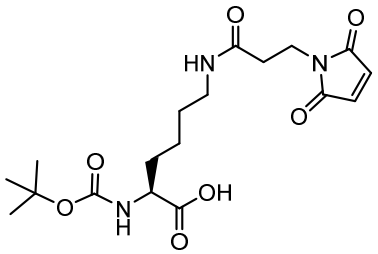
2 tcepmal2 225 nm

Sample Name:	**tcepmal2 225 nm**	*Injection Volume:*	**20.0**
Vial Number:	**2**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/4/2014 10:47**	*Sample Weight:*	**1.0000**
Run Time (min):	**25.46**	*Sample Amount:*	**1.0000**

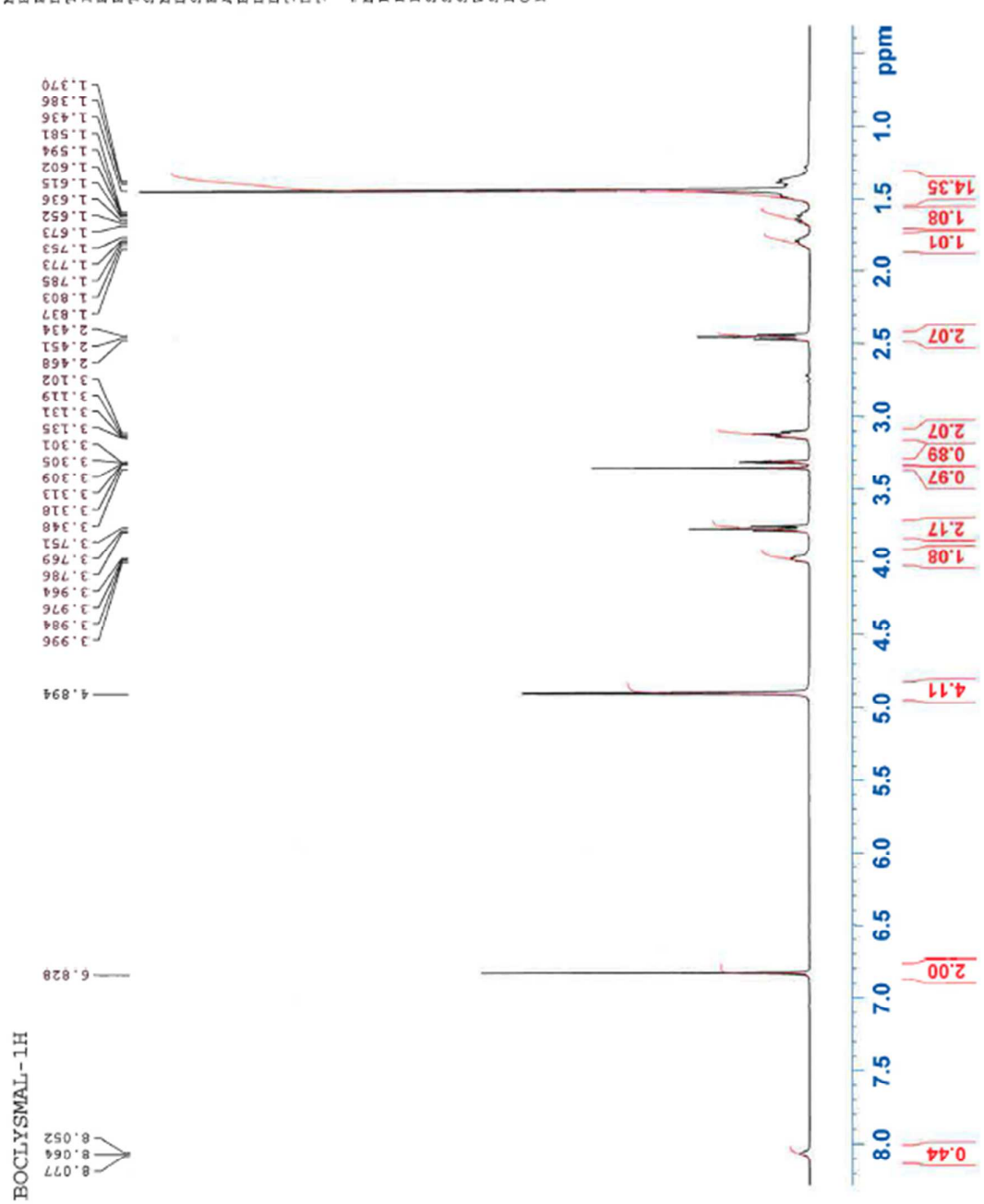


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.61	n.a.	230.949	27.871	6.96	n.a.	BMB*
2	12.81	n.a.	1392.828	372.825	93.04	n.a.	BMB*
Total:			1623.777	400.696	100.00	0.000	

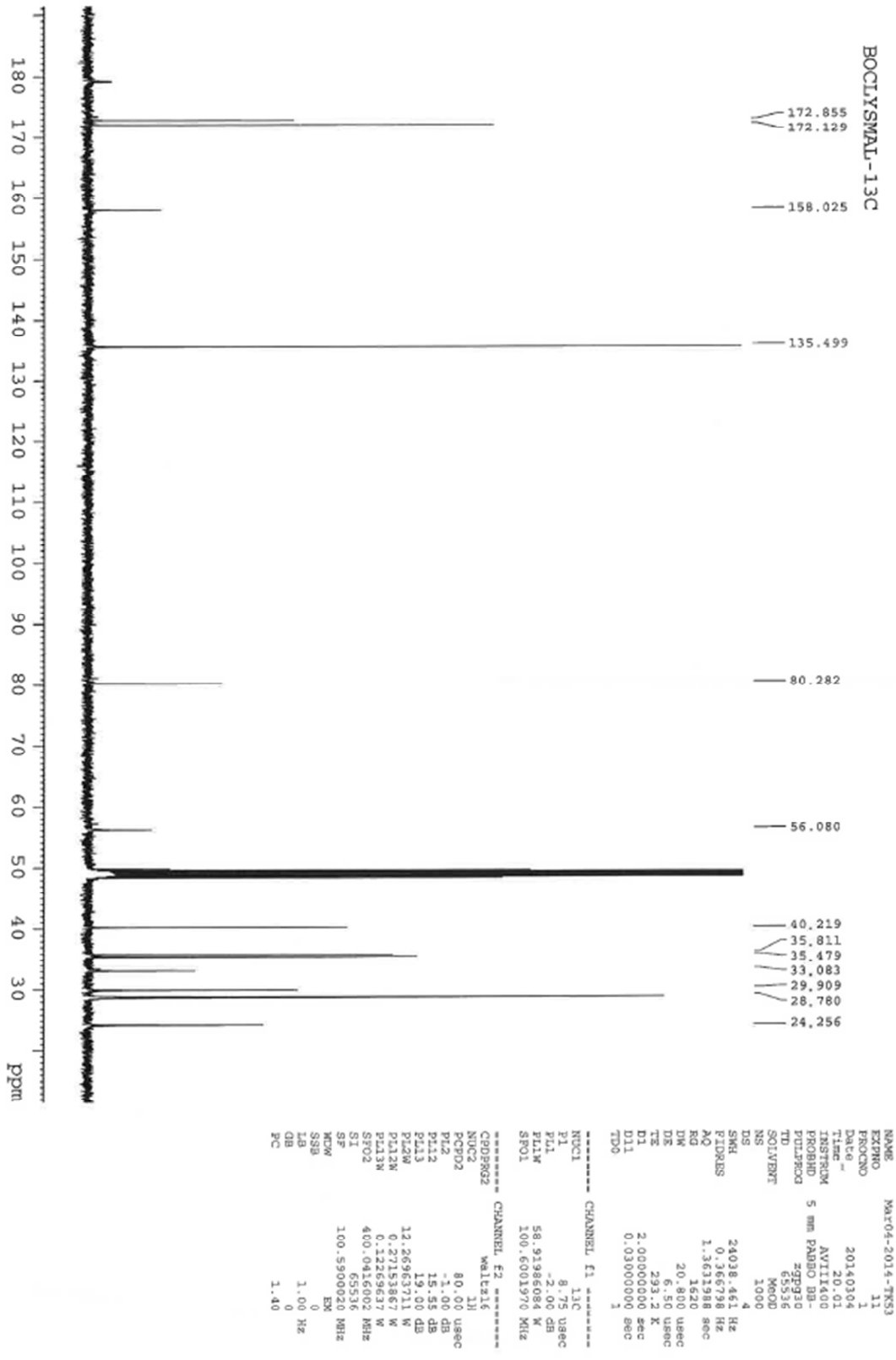
^{1}H NMR spectrum of compound (**S1**)



(**S1**)



^{13}C NMR spectrum of compound (**S1**)

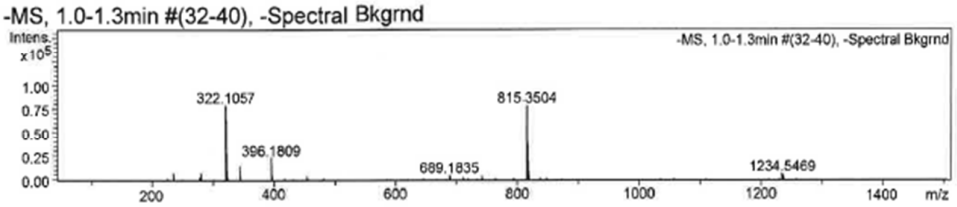


HRMS for compound (**S1**)

Confirmation of Expected Formula

Sample-ID	wa_tk_BocLysMalcooh	Submitter	Terrence Kantner
Analysis Name	wa_tk_BocLysMalcooh_340973_65_01_45148.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to1500 loop inj.m	Acquisition Date	19/08/2014 17:00:38
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(32-40), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	235.1090	7229	9.1	317	192.1
2	279.1000	7753	9.8	458	193.7
3	322.1057	79099	100.0	4289	2072.7
4	323.1082	13020	16.5	732	341.6
5	344.0886	15083	19.1	961	411.2
6	396.1809	22800	28.8	1482	663.0
7	815.3504	78501	99.2	9530	2690.7
8	816.3523	36338	45.9	4126	1260.1
9	817.3571	10329	13.1	1221	362.4
10	1234.5469	6486	8.2	1300	371.8

Generate Molecular Formula Parameters

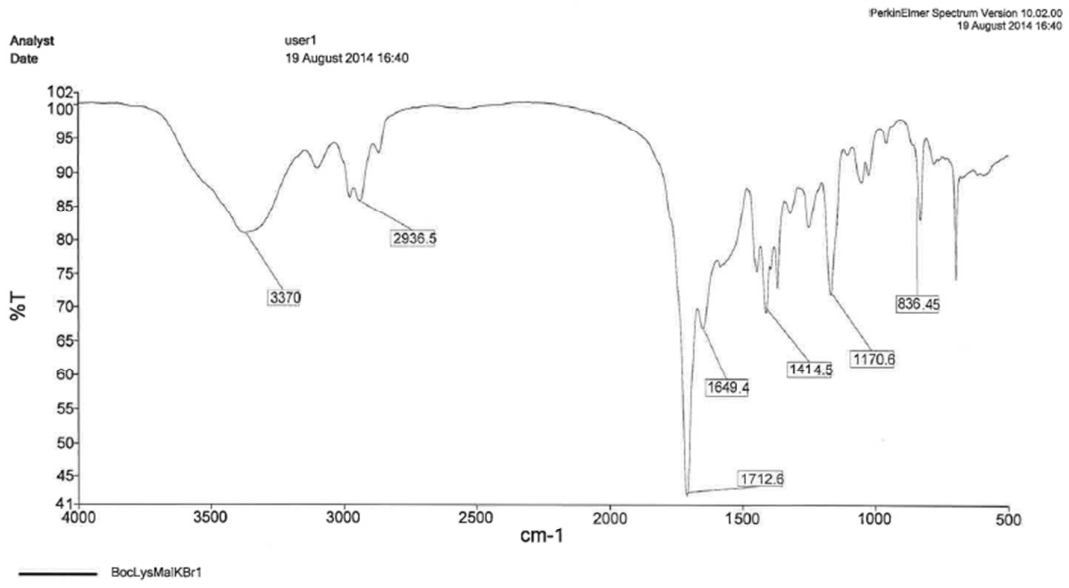
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C18 H27 N3 O7 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	396.1809	396.177075	-8.30	0.0082	C 18 H 26 N 3 O 7

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

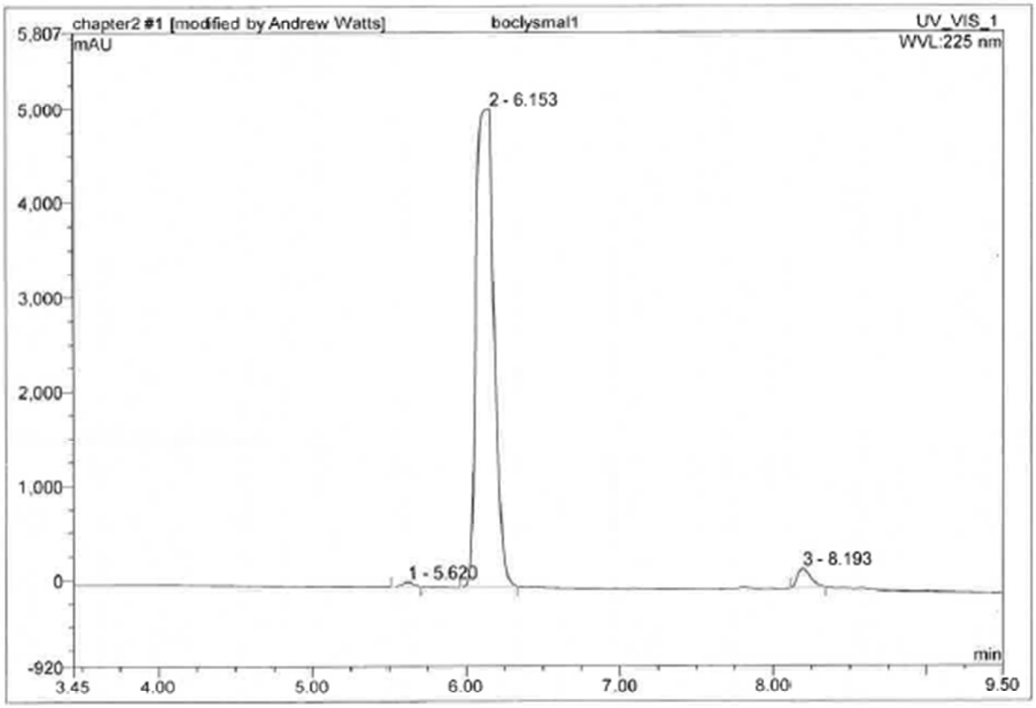
IR spectrum for compound (**S1**)



HPLC chromatogram for compound (**S1**)

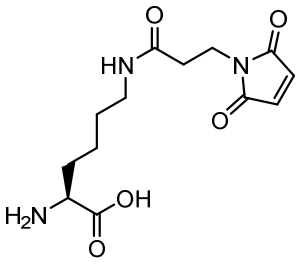
1 boclysmal1

Sample Name:	**boclysmal1**	*Injection Volume:*	**10.0**
Vial Number:	**1**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity c8 1**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/25/2014 11:34**	*Sample Weight:*	**1.0000**
Run Time (min):	**11.97**	*Sample Amount:*	**1.0000**

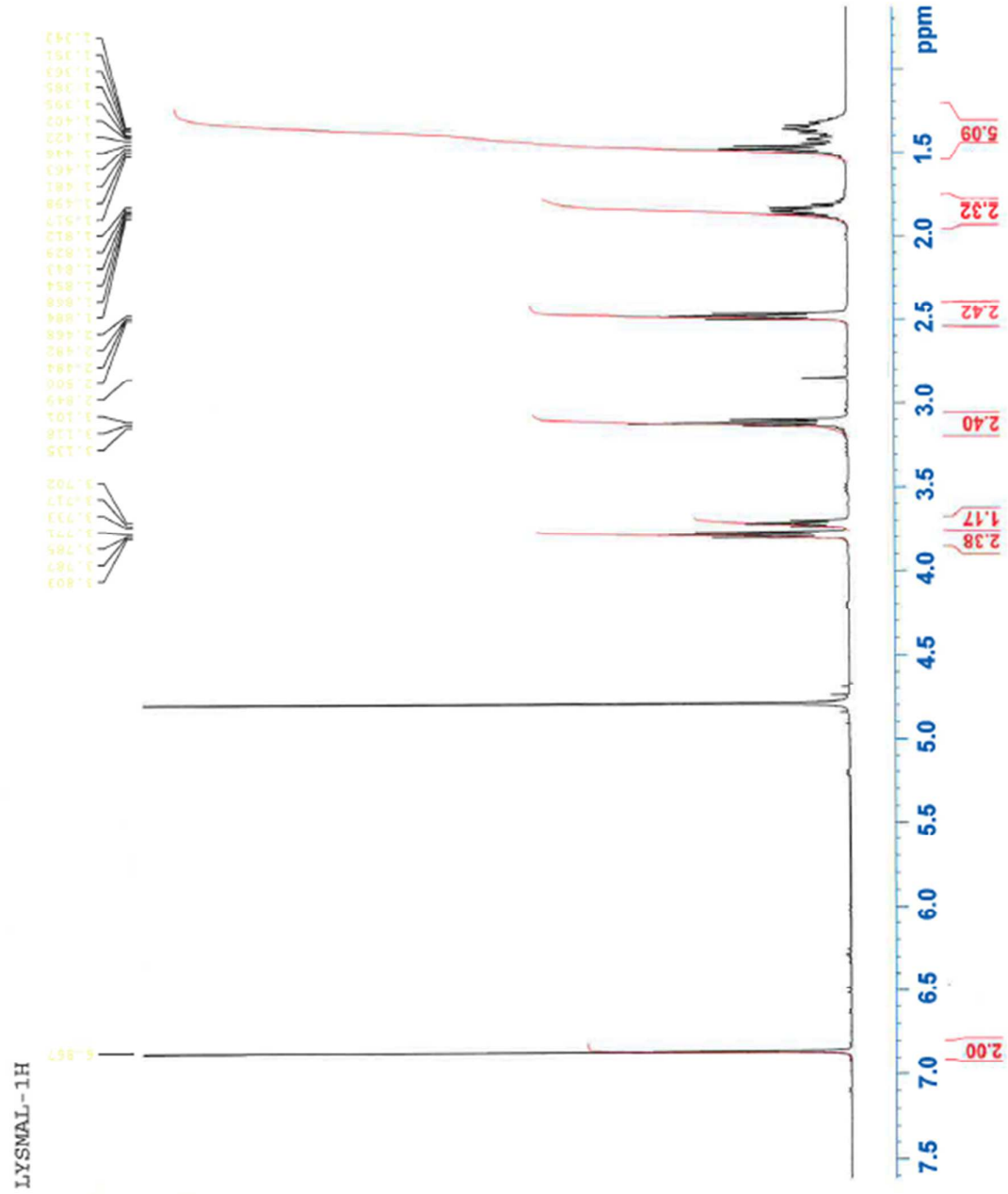


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	5.62	n.a.	46.276	4.299	0.60	n.a.	BMB*
2	6.15	n.a.	5073.021	687.347	96.62	n.a.	BMB*
3	8.19	n.a.	197.171	19.749	2.78	n.a.	BMB*
Total:			5316.468	711.395	100.00	0.000	

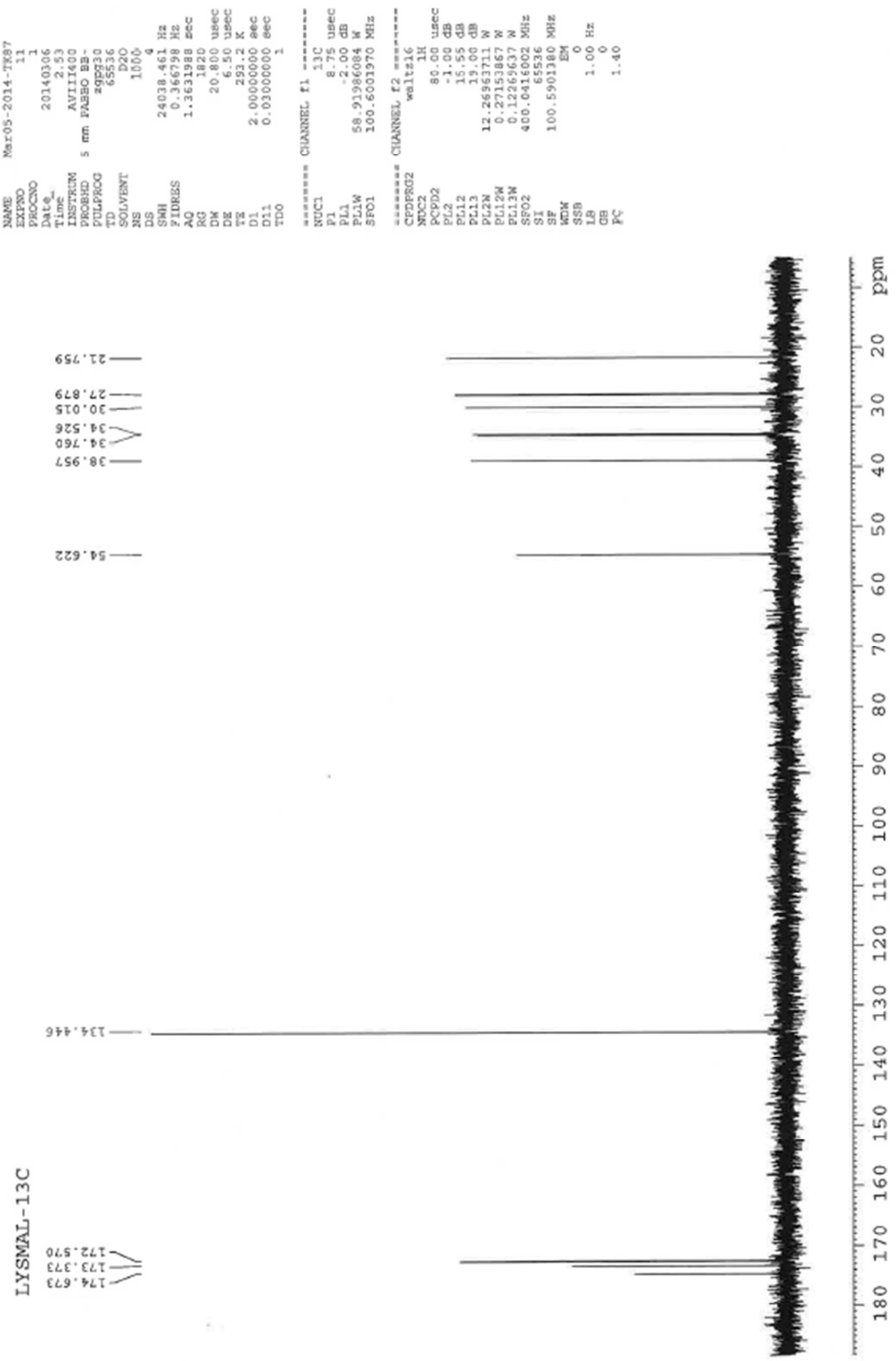
^{1}H NMR spectrum of compound (**5**)



(**5**)



^{13}C NMR spectrum of compound (**5**)

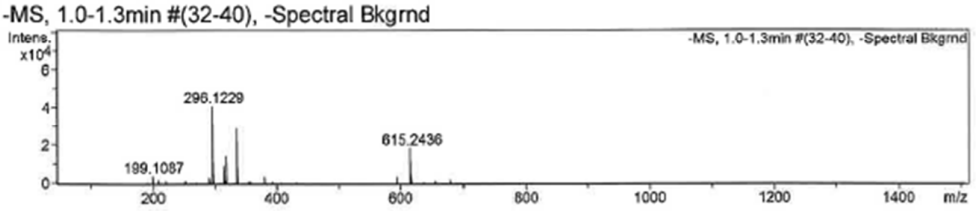


HRMS for compound (**5**)

Confirmation of Expected Formula

Sample-ID	wa_tk_lysmalazolac912	Submitter	Terrence Kantner
Analysis Name	wa_tk_lysmalazolac912_338864_87_01_42806.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to1500 loop inj.m	Acquisition Date	10/03/2014 16:35:29
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(32-40), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	199.1087	3835	9.4	105	180.9
2	296.1229	40586	100.0	2043	1899.8
3	297.1257	6843	16.9	300	323.4
4	314.1336	8851	21.8	494	499.2
5	318.1050	14401	35.5	681	850.6
6	336.1168	28613	70.5	1644	2151.3
7	337.1211	4377	10.8	232	334.2
8	381.1033	3395	8.4	200	802.4
9	615.2436	18329	45.2	1547	5226.5
10	616.2469	5445	13.4	498	1563.6

Generate Molecular Formula Parameters

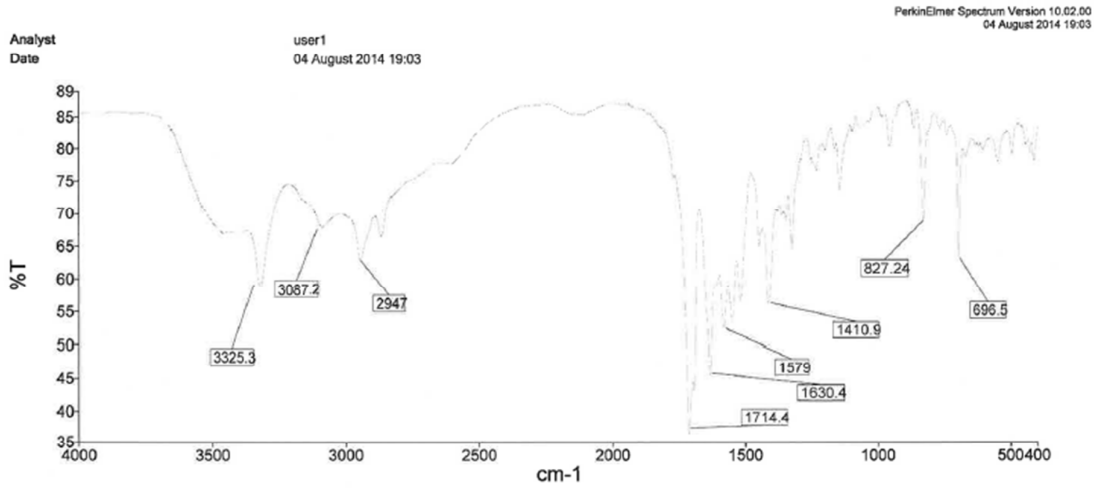
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C31 H52 N6 O18 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula

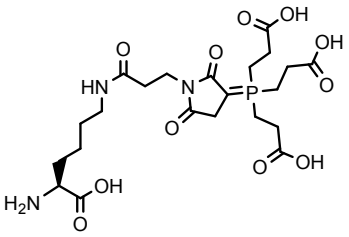
Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

IR spectrum for compound (**5**)

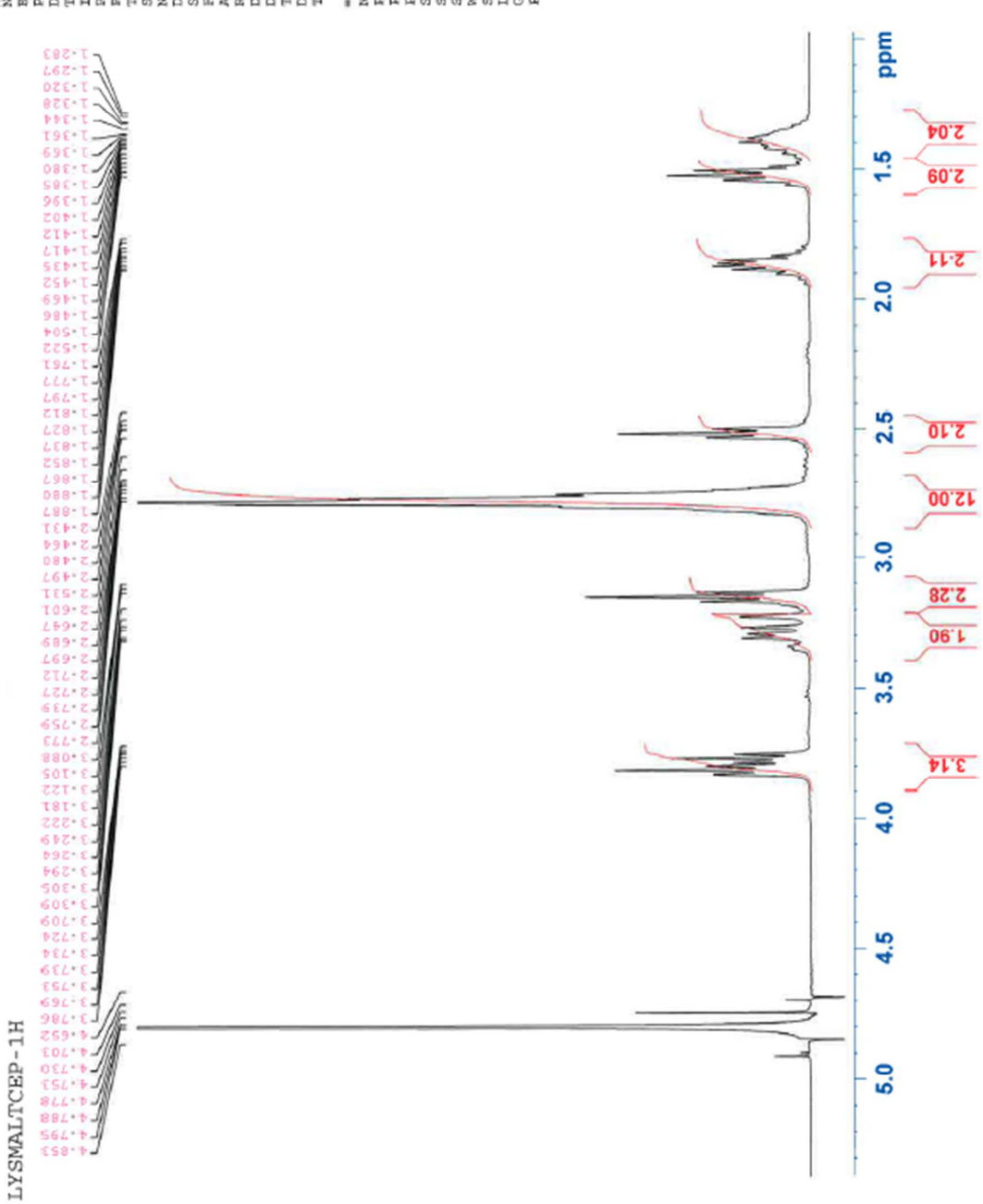


Sample Name	Description	Quality Checks
NH2MALLysCOOH1KBr	Sample 006 By user1 Date Monday, August 04 2014	The Quality Checks do not report any warnings for the sample.

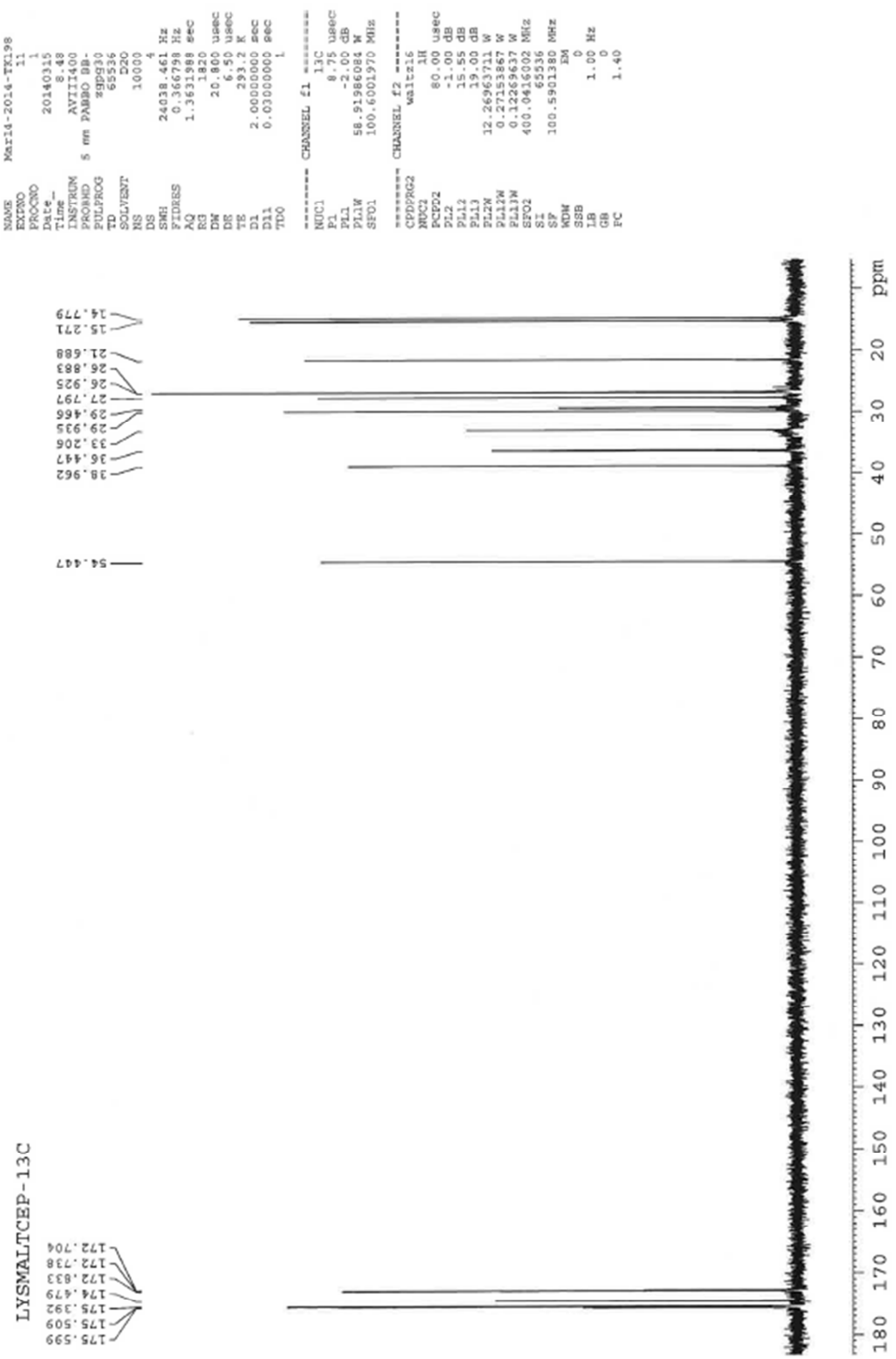
^{1}H NMR spectrum of compound (**6**)



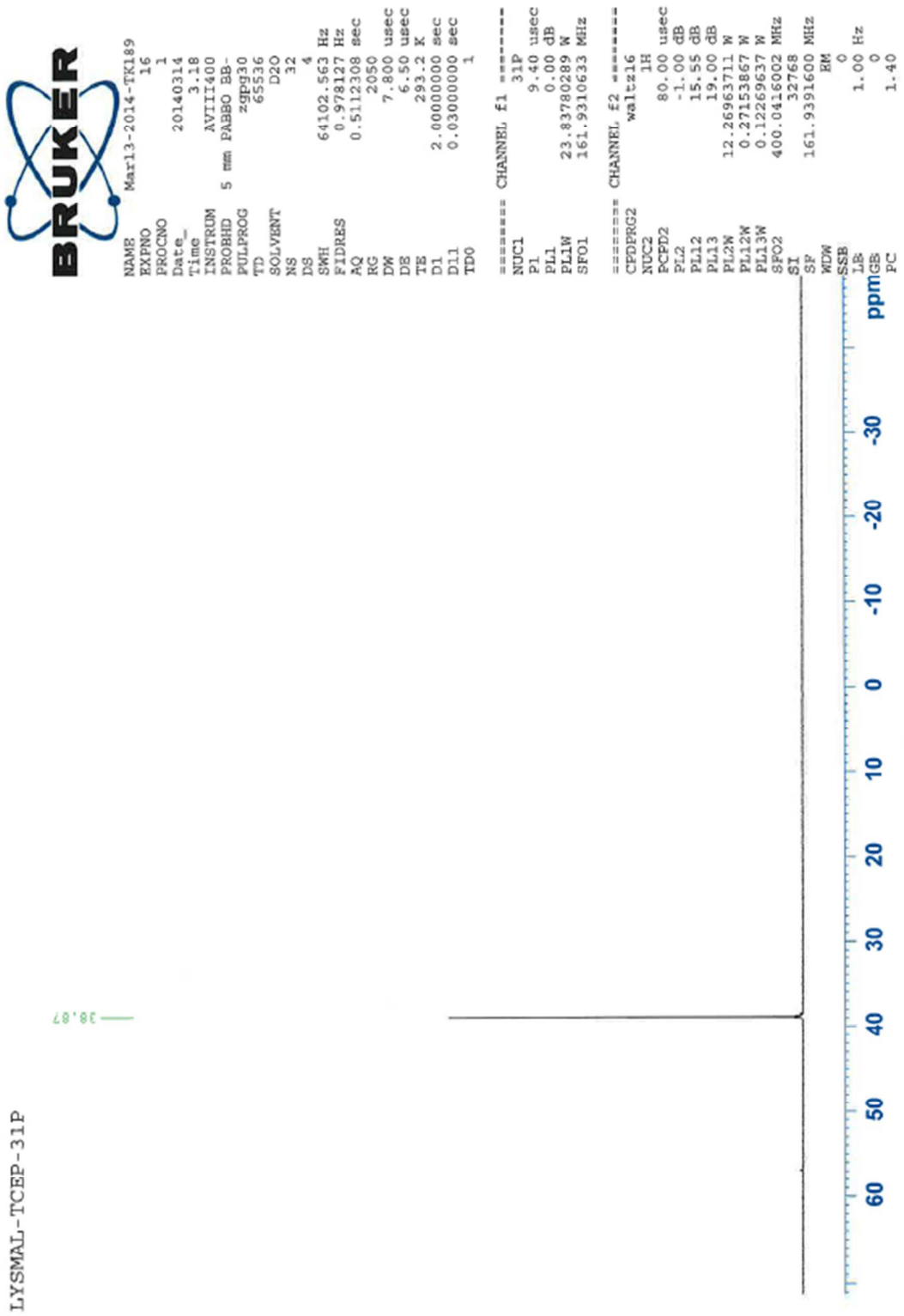
(**6**)



^{13}C NMR spectrum of compound (**6**)



^{31}P NMR spectrum of compound (**6**)

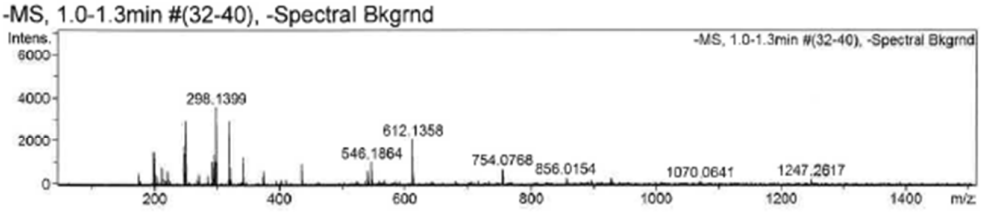


HRMS for compound (**6**)

Confirmation of Expected Formula

Sample-ID	wa_tk_lysmaltcep	Submitter	Terrence Kantner
Analysis Name	wa_tk_lysmaltcep_338873_98_01_42819.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to1500 loop inj.m	Acquisition Date	11/03/2014 12:57:53
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(32-40), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	199.1080	1486	41.8	40	848.0
2	247.0380	1764	49.6	65	677.5
3	249.0542	2965	83.3	122	1123.7
4	293.0157	1025	28.8	49	315.6
5	296.1246	1386	38.9	64	434.5
6	298.1399	3559	100.0	185	1129.3
7	320.1224	2963	83.3	162	1084.1
8	342.1075	1271	35.7	64	549.0
9	546.1864	1039	29.2	77	246.9
10	612.1358	2111	59.3	156	423.7

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C22 H34 N3 O11 P1 **Adduct(s):** H, Na

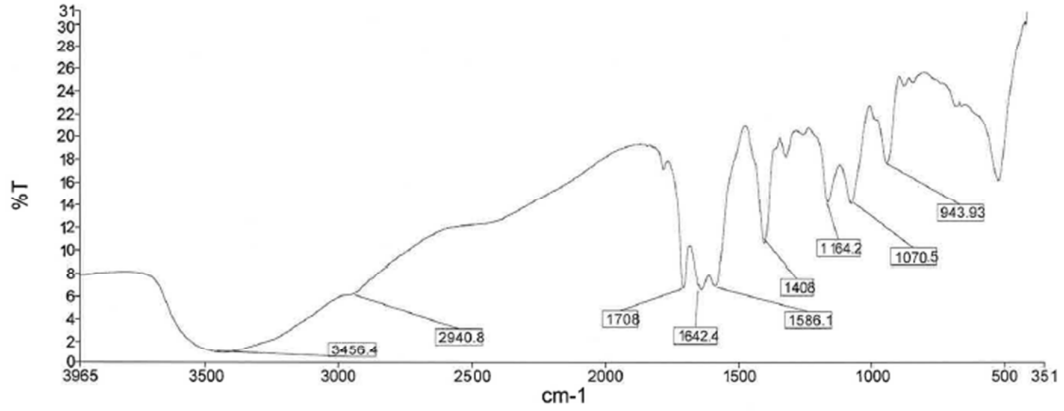
#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	546.1864	546.185270	-1.00	0.0288	C 22 H 33 N 3 O 11 P 1

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

IR spectra for compound (**6**)

PerkinElmer Spectrum Version 10.02.00
04 August 2014 18:31

Analyst user1
Date 04 August 2014 18:31

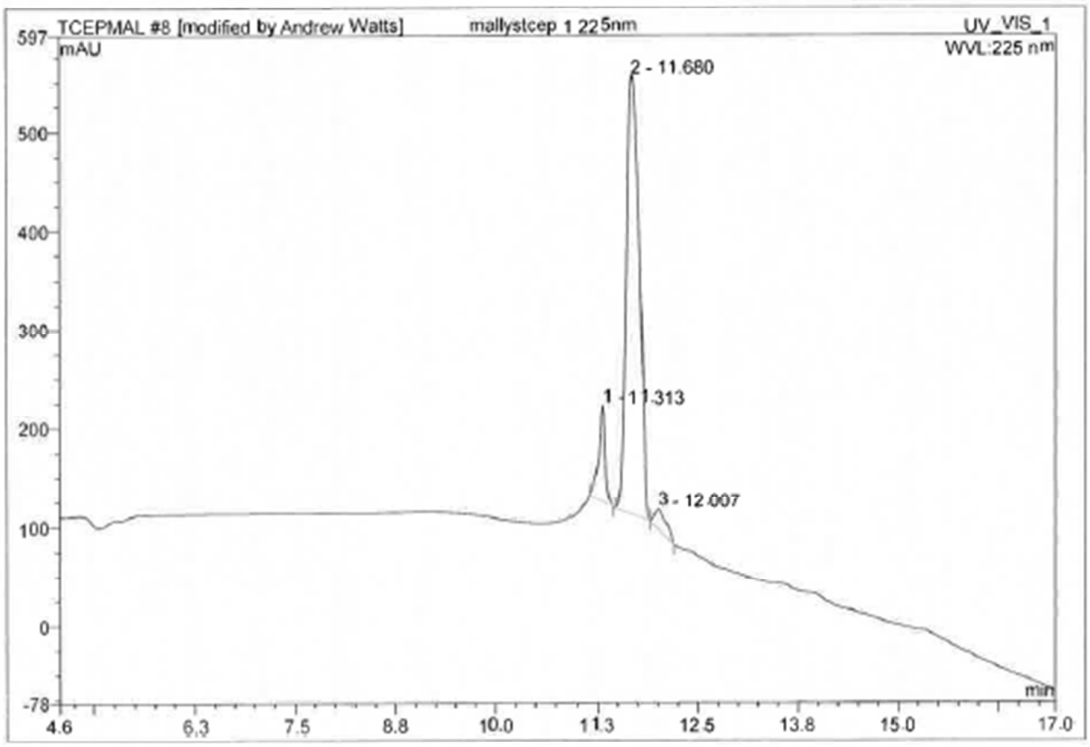


Sample Name	Description	Quality Checks
LysMalTCEP1KBr	Sample 003 By user1 Date Monday, August 04 2014	The Quality Checks do not report any warnings for the sample.

HPLC chromatogram for compound (**6**)

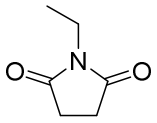
8 mallystcep 1 225nm

Sample Name:	**mallystcep 1 225nm**	*Injection Volume:*	**20.0**
Vial Number:	**8**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/25/2014 12:56**	*Sample Weight:*	**1.0000**
Run Time (min):	**17.27**	*Sample Amount:*	**1.0000**

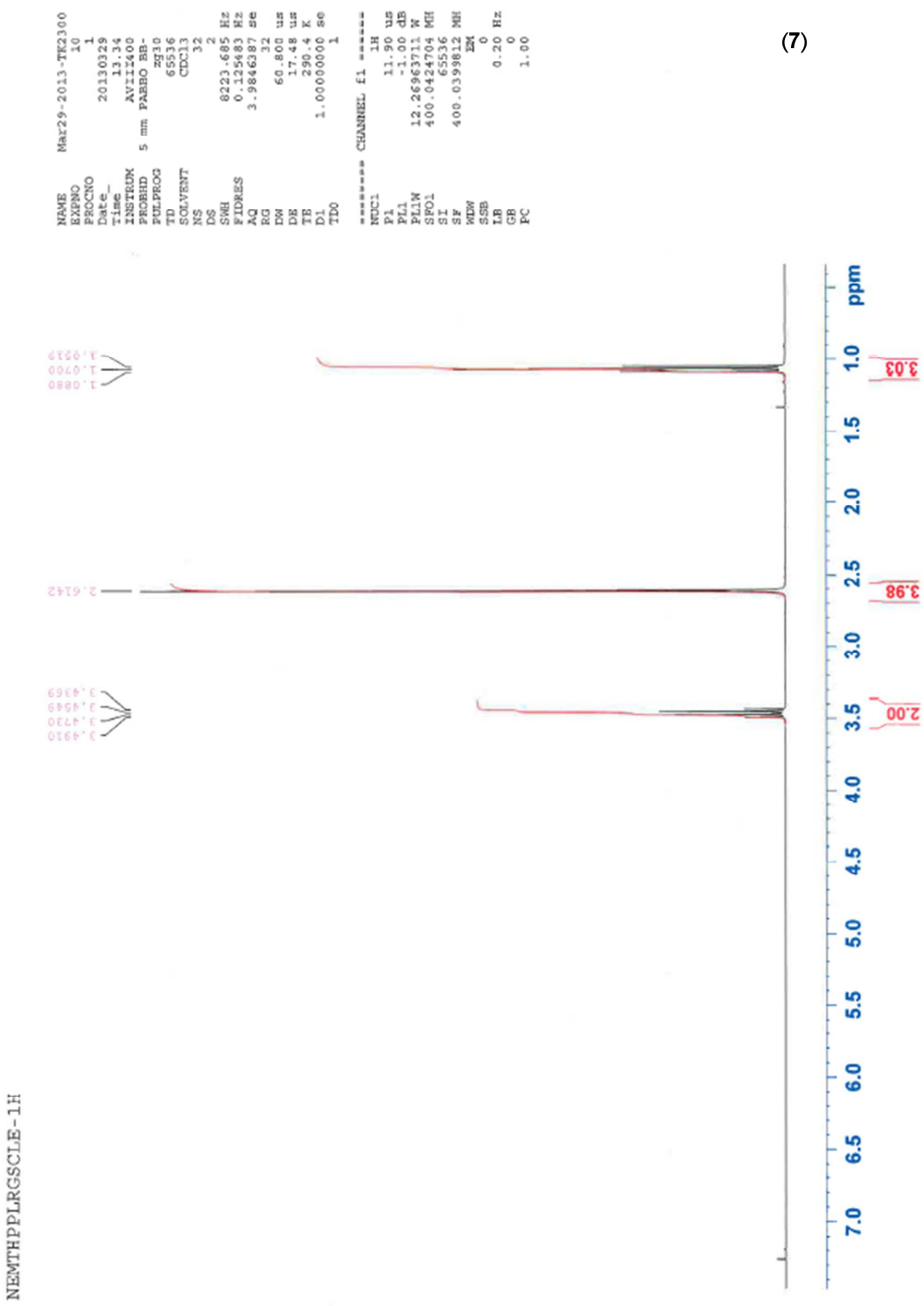


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.31	n.a.	96.660	9.160	9.70	n.a.	BMB*
2	11.68	n.a.	444.008	81.973	86.78	n.a.	BMb*
3	12.01	n.a.	19.603	3.332	3.53	n.a.	bMB*
Total:			560.271	94.465	100.00	0.000	

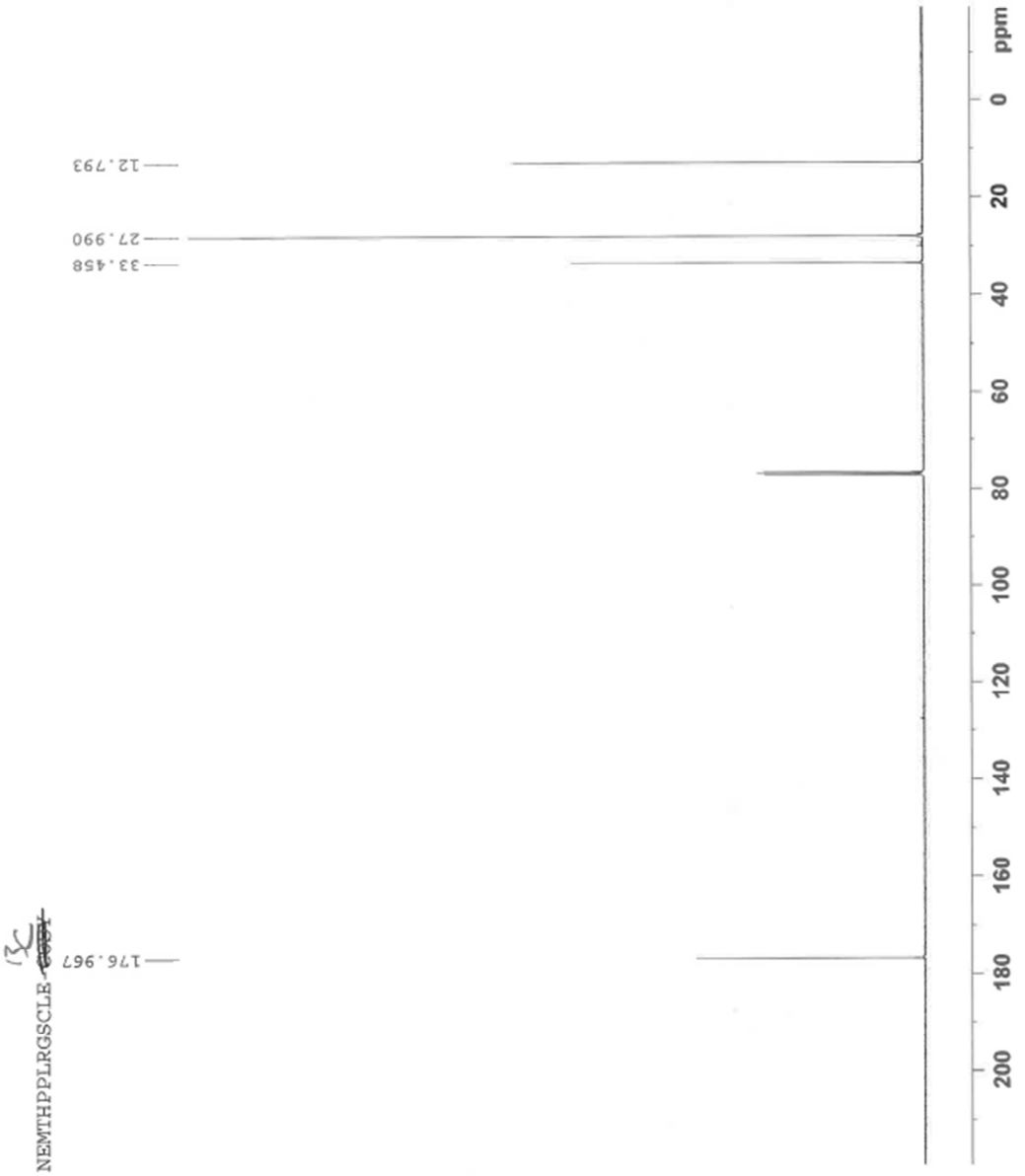
^{1}H NMR spectrum of compound (**7**)



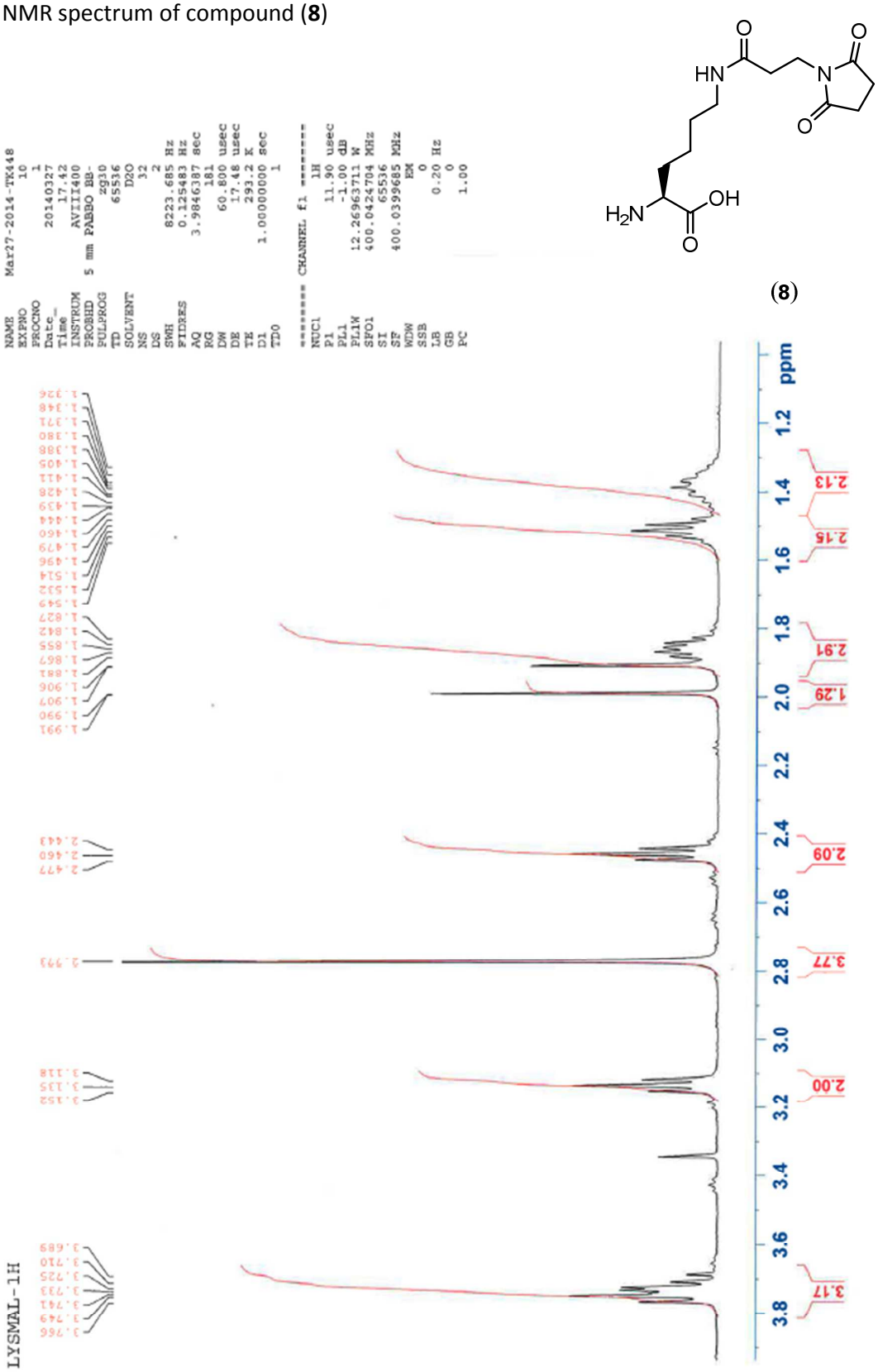
(**7**)



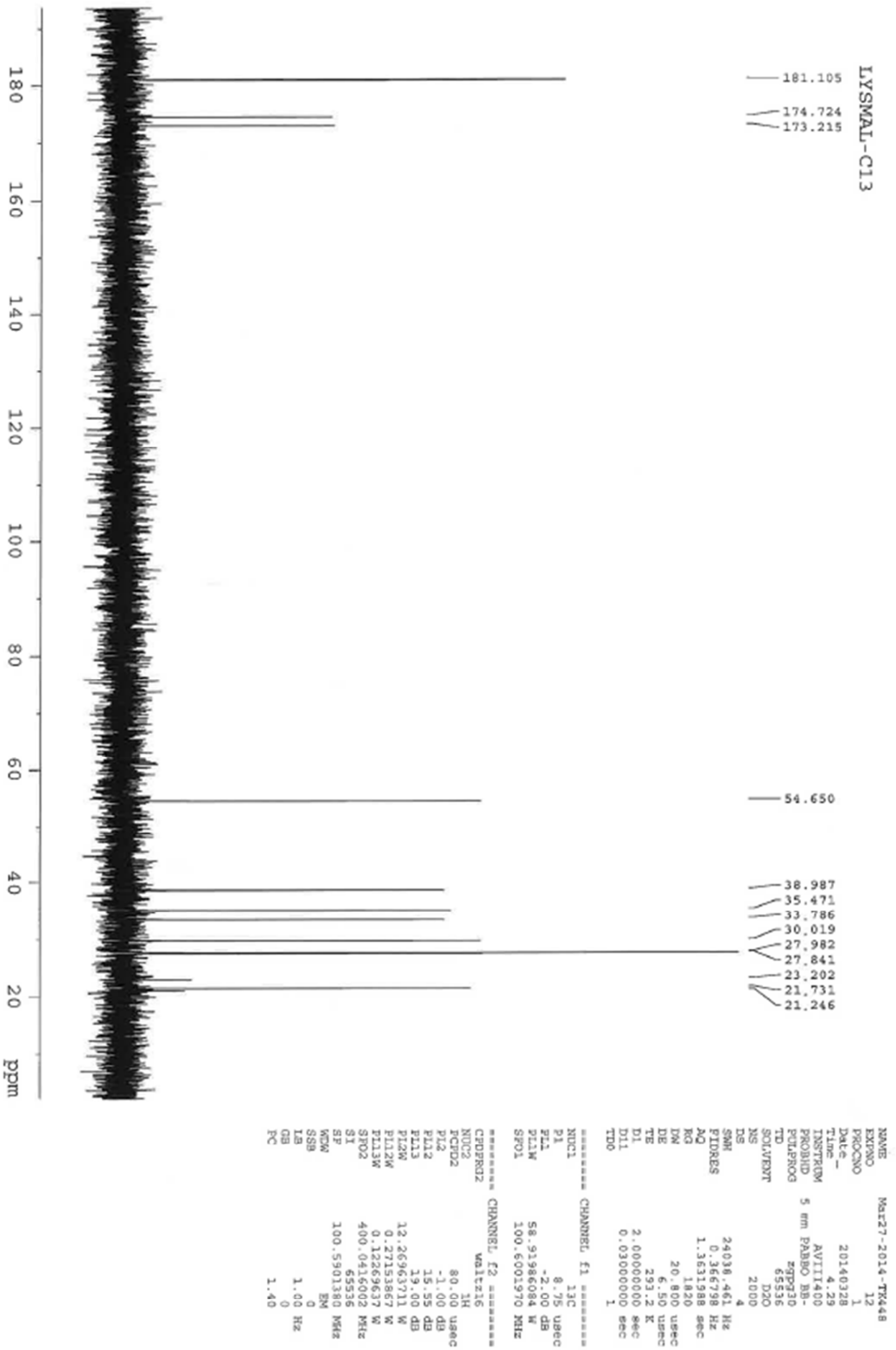
^{13}C NMR spectrum of compound (**7**)



^{1}H NMR spectrum of compound (**8**)



^{13}C NMR spectrum of compound (**8**)



NAME Mar27-2014-TK448
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PROCNO 1
Date_ 20140328
Time_ 4.29
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SOLVENT DZO
NS 2000
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631988 sec
RG 1820
DW 20.800 usec
DE 6.50 usec
TE 293.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
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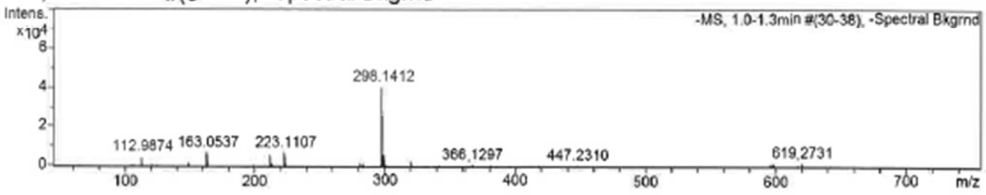
NUC1 13C
P1 8.75 usec
PL1 -2.00 dB
PL1W 58.91986084 W
SFO1 100.6001970 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -1.00 dB
PL12 15.55 dB
PL13 19.00 dB
PL2W 12.26963711 W
PL12W 0.27153867 W
PL13W 0.12269637 W
SFO2 400.0416002 MHz
SI 65536
SF 100.5901380 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

HRMS for compound (**8**)

Confirmation of Expected Formula

Sample-ID	wa_tk_lysmalthpp	Submitter	Terrence Kantner
Analysis Name	wa_tk_lysmalthpp_338942_60_01_42889.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to500 loop inj.m	Acquisition Date	17/03/2014 15:42:18
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(30-38), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	112.9874	4437	11.0	79	8320.9
2	148.9532	1877	4.7	50	2096.8
3	163.0537	7032	17.5	228	6793.9
4	212.0978	5586	13.9	60	2359.9
5	223.1107	6880	17.1	269	3355.7
6	298.1412	40198	100.0	2040	6686.8
7	299.1446	6298	15.7	308	1026.4
8	320.1249	2857	7.1	142	588.2
9	597.2918	1744	4.3	155	1238.6
10	619.2731	2072	5.2	184	1381.8

Generate Molecular Formula Parameters

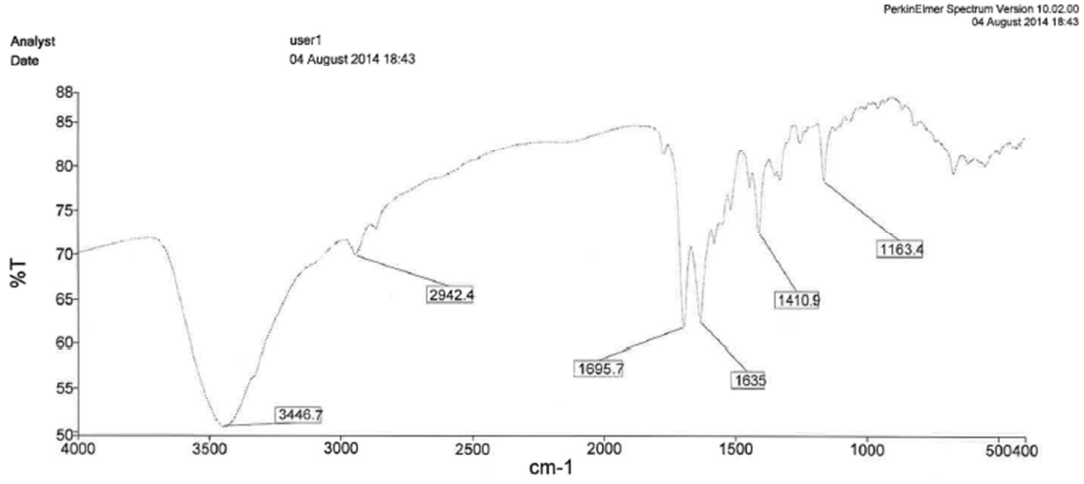
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C13 H21 N3 O5 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	298.1412	298.140296	-1.20	0.0029	C 13 H 20 N 3 O 5

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

IR spectrum for compound (**8**)

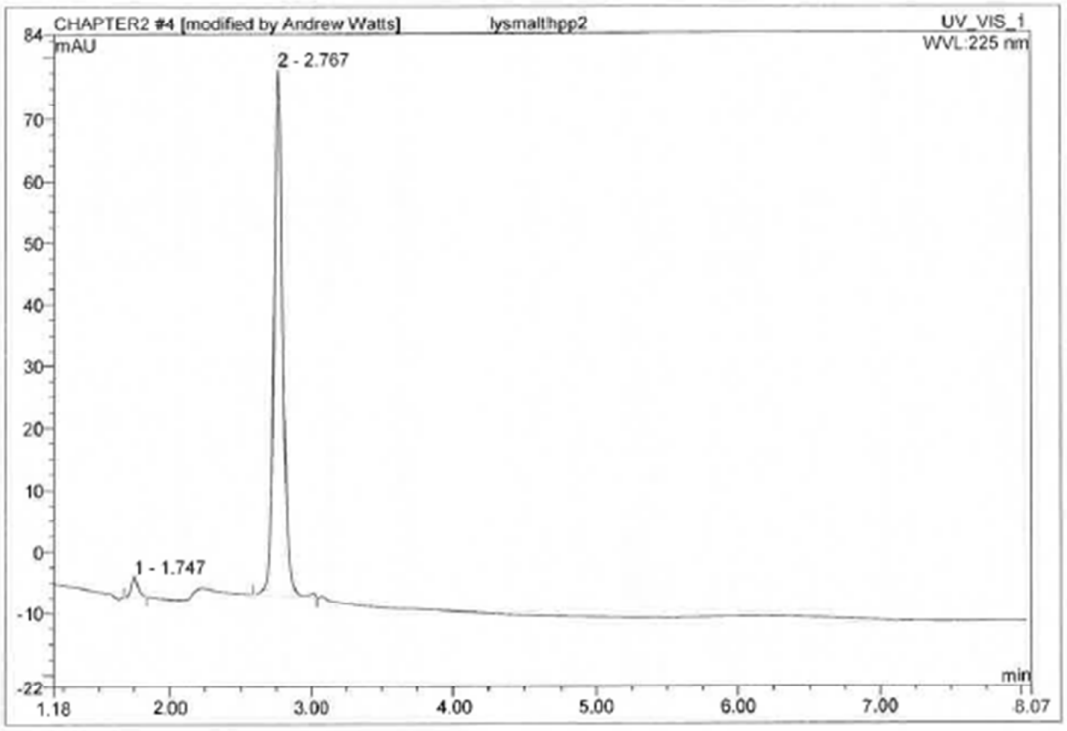


Sample Name	Description	Quality Checks
LysMalTHPP1KBr	Sample 004 By user1 Date Monday, August 04 2014	The Quality Checks do not report any warnings for the sample.

HPLC chromatogram for compound (**8**)

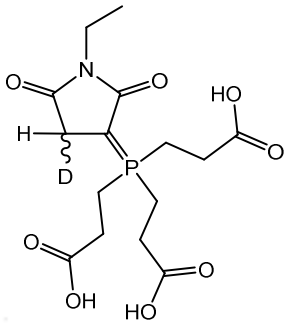
4 lysmalthpp2

Sample Name:	**lysmalthpp2**	*Injection Volume:*	**10.0**
Vial Number:	**4**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity c8 1**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/25/2014 14:05**	*Sample Weight:*	**1.0000**
Run Time (min):	**8.03**	*Sample Amount:*	**1.0000**

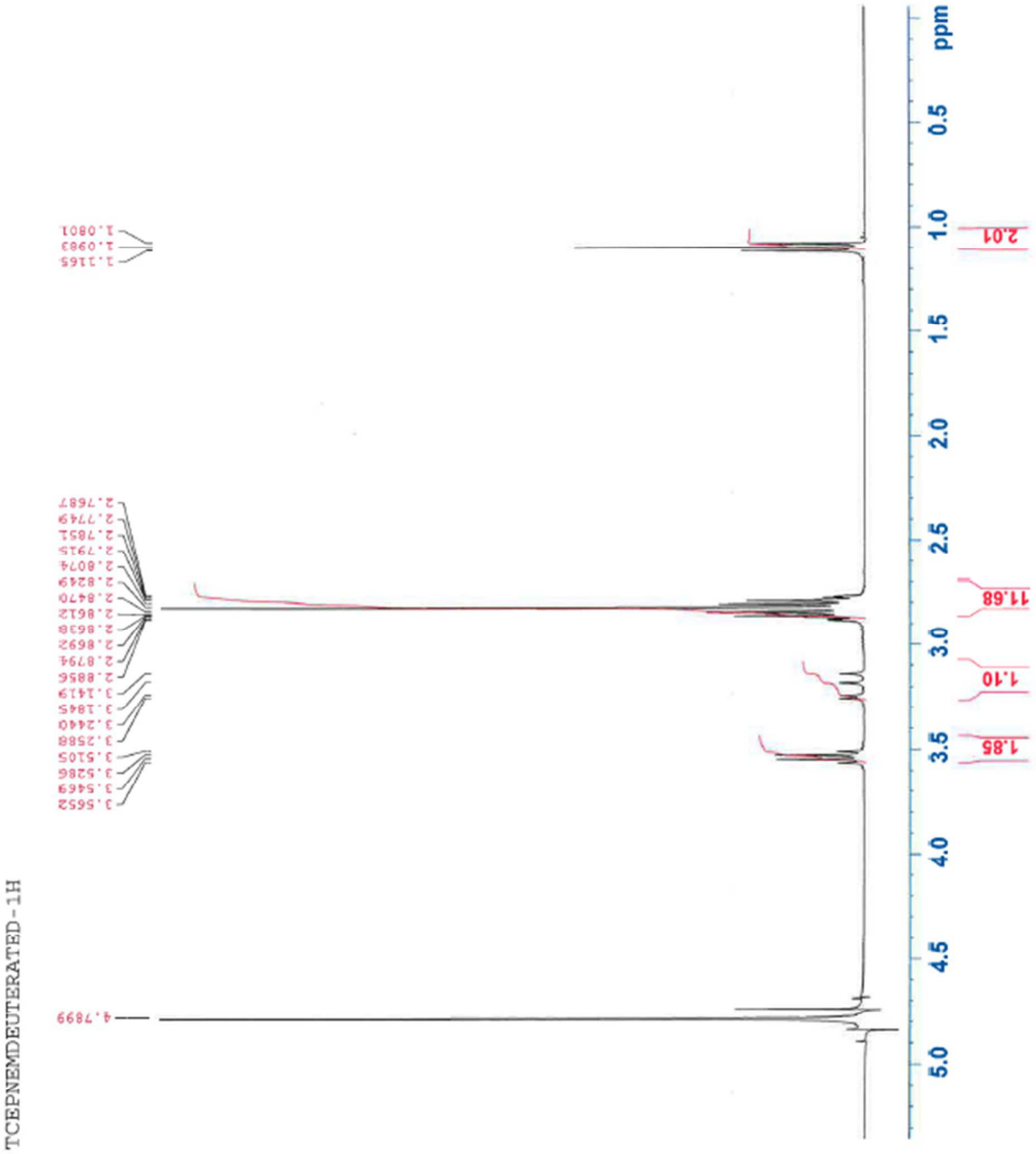


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.75	n.a.	3.415	0.190	2.94	n.a.	BMB*
2	2.77	n.a.	85.105	6.273	97.06	n.a.	BMB*
Total:			88.520	6.464	100.00	0.000	

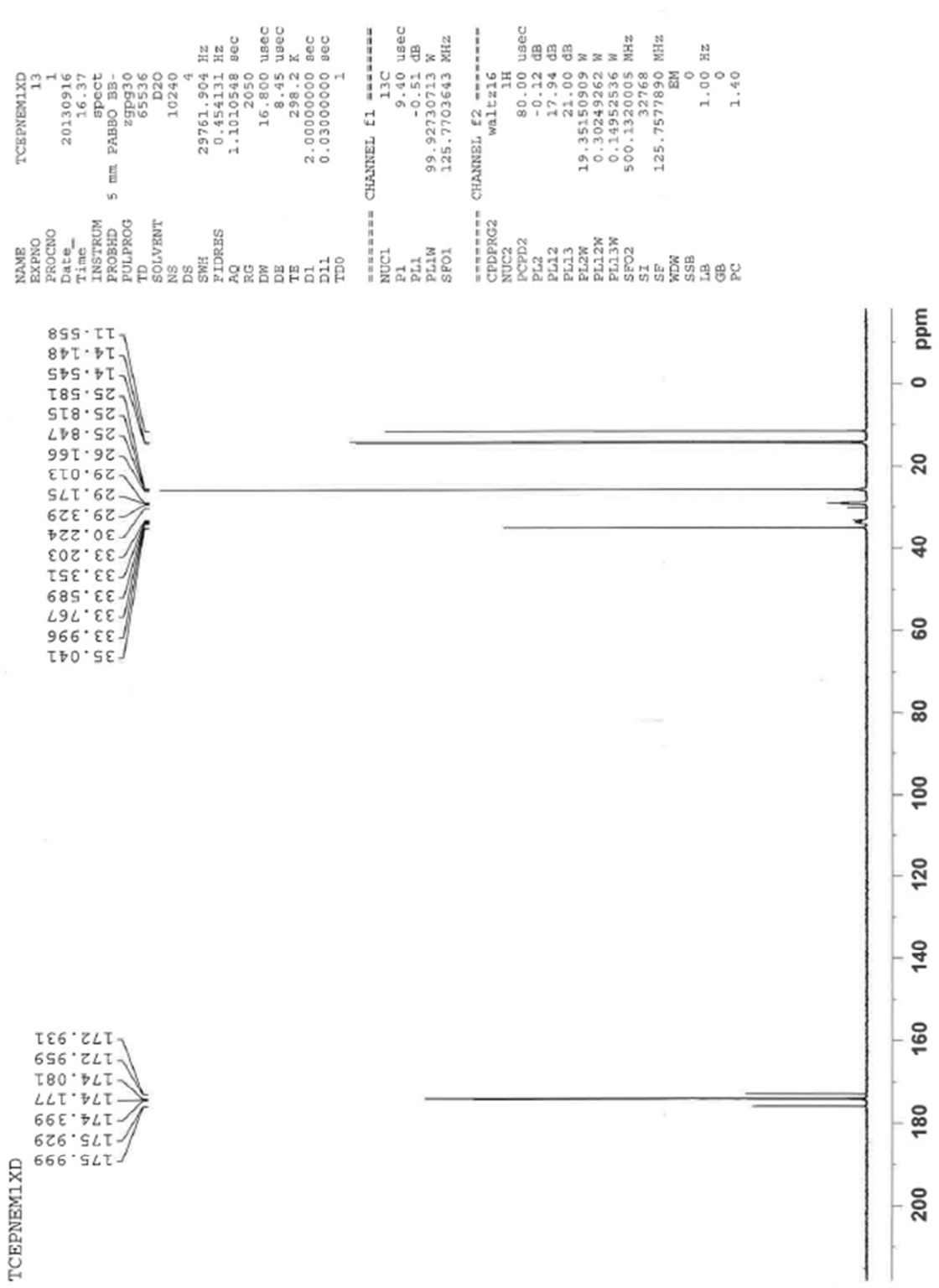
^{1}H NMR spectrum of compound (**9**)



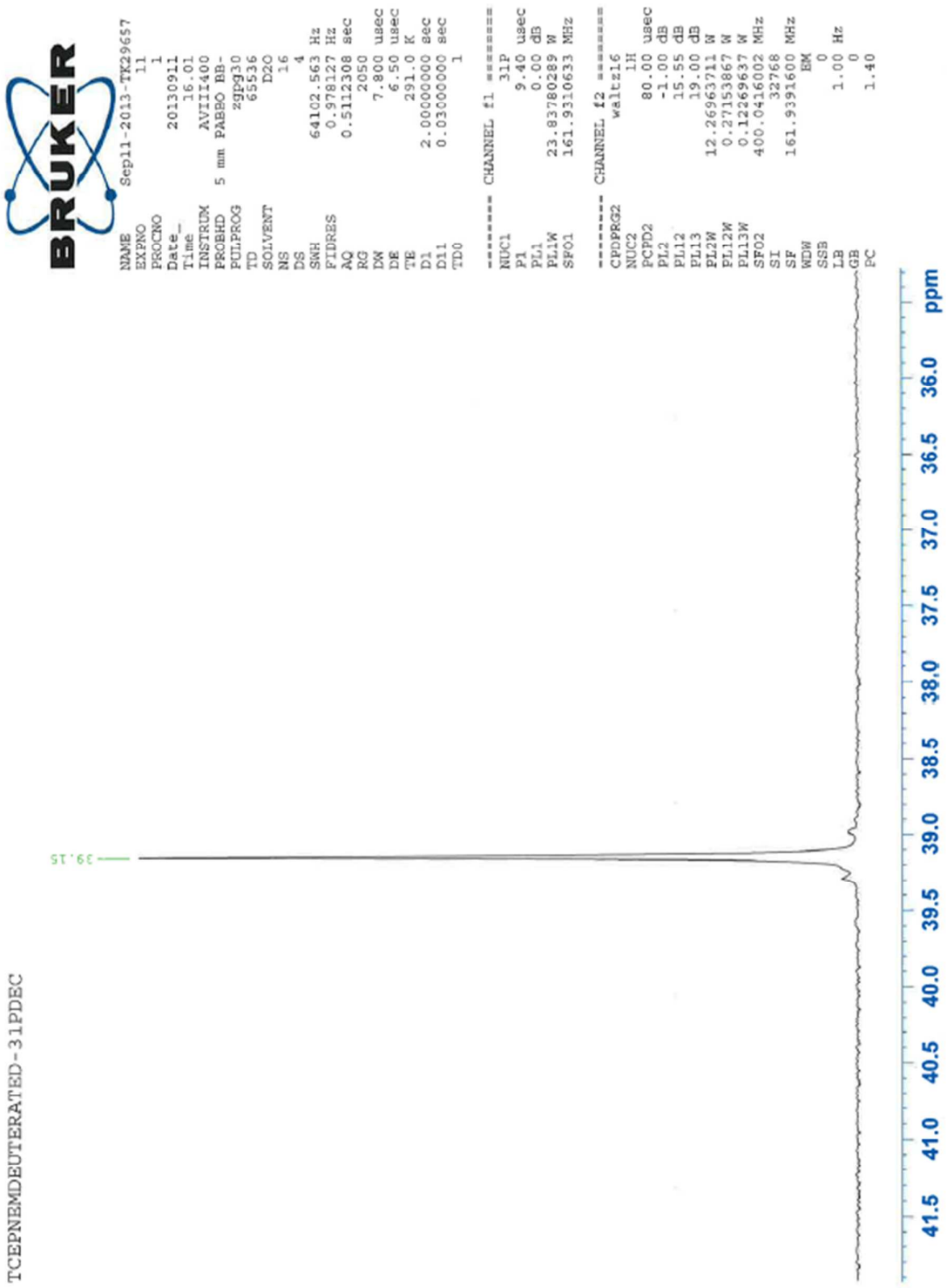
(**9**)



^{13}C NMR spectrum of compound (**9**)



^{31}P NMR spectrum of compound (**9**)

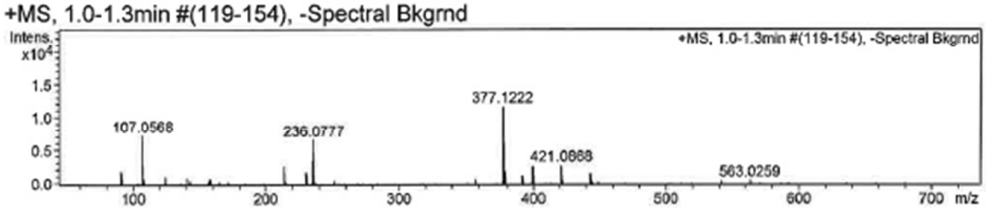


HRMS for compound (**9**)

Confirmation of Expected Formula

Sample-ID	wa_tk_tcepnemph4	Submitter	Terrence Kantner
Analysis Name	wa_tk_tcepnemph4_336560_91_01_40055.d	Supervisor	Andy Watts
Method used	Confirm Formula Positive 50to500 loop inj.m	Acquisition Date	10/09/2013 10:30:54
Ionisation Mode	positive electrospray (ESI)		

+MS, 1.0-1.3min #(119-154), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	91.0541	1999	17.1	24	3781.5
2	107.0568	7273	62.3	81	10173.3
3	214.0879	2601	22.3	50	1367.3
4	230.0772	1794	15.4	37	680.0
5	235.0773	3402	29.1	90	1185.8
6	236.0777	6640	56.9	95	2278.1
7	377.1222	11673	100.0	680	2427.5
8	378.1260	1902	16.3	123	389.2
9	399.1054	2546	21.8	169	459.7
10	421.0868	2794	23.9	206	579.2

Generate Molecular Formula Parameters

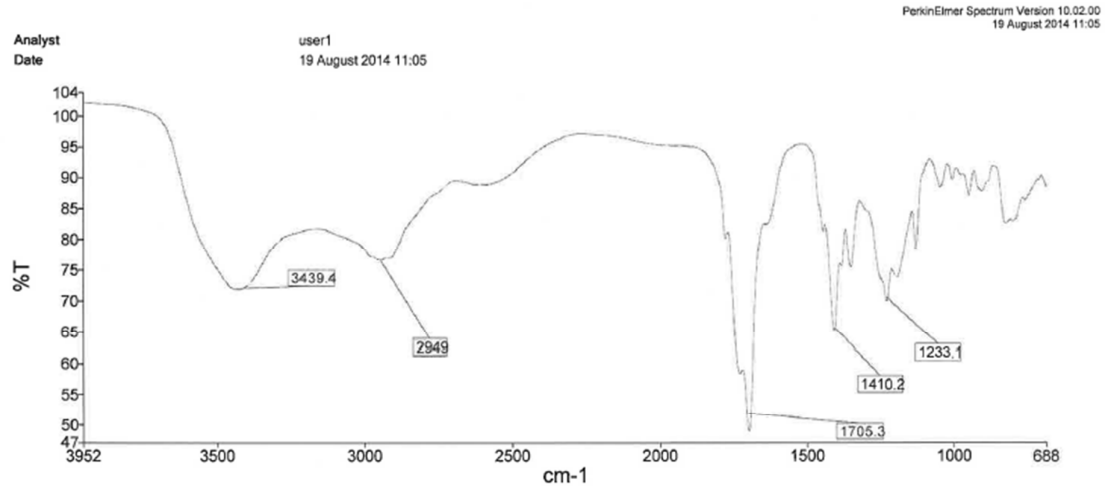
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C15 H22 N1 O8 P1 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

IR spectrum for compound (**9**)

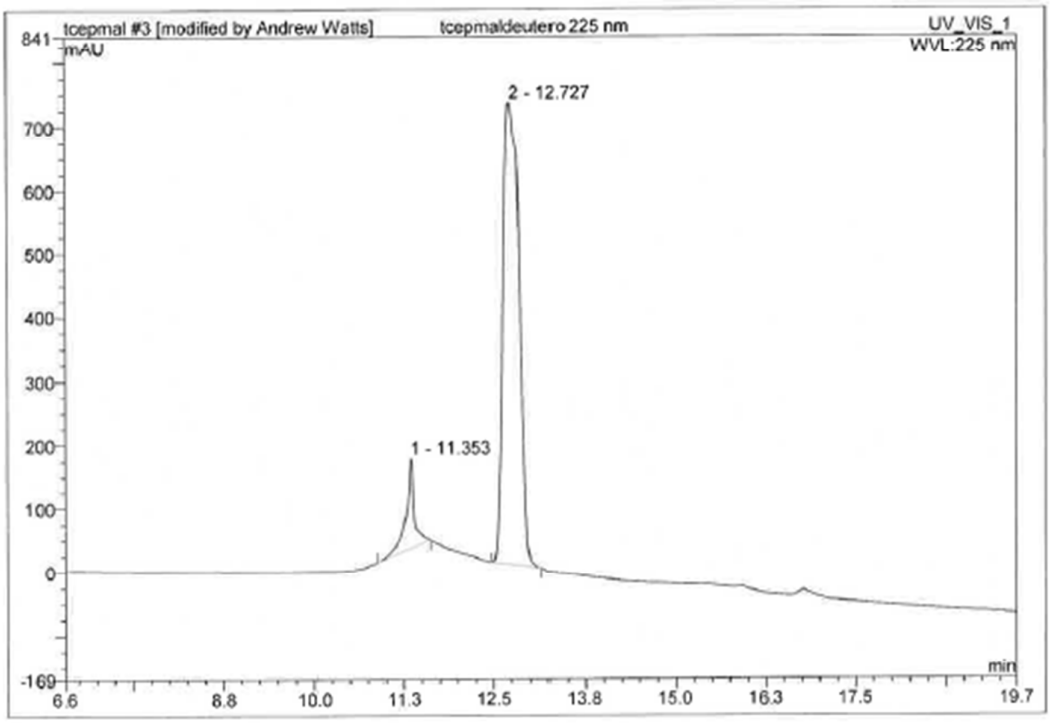


Sample Name	Description	Quality Checks
nemdeuterotcepKBr1	Sample 007 By user1 Date Tuesday, August 19 2014	The Quality Checks do not report any warnings for the sample.

HPLC chromatogram for compound (**9**)

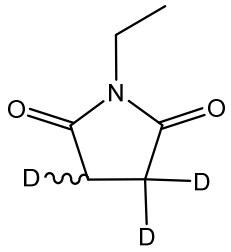
3 tcepmaldeutero 225 nm

Sample Name:	**tcepmaldeutero 225 nm**	*Injection Volume:*	**20.0**
Vial Number:	**3**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/4/2014 12:00**	*Sample Weight:*	**1.0000**
Run Time (min):	**25.05**	*Sample Amount:*	**1.0000**

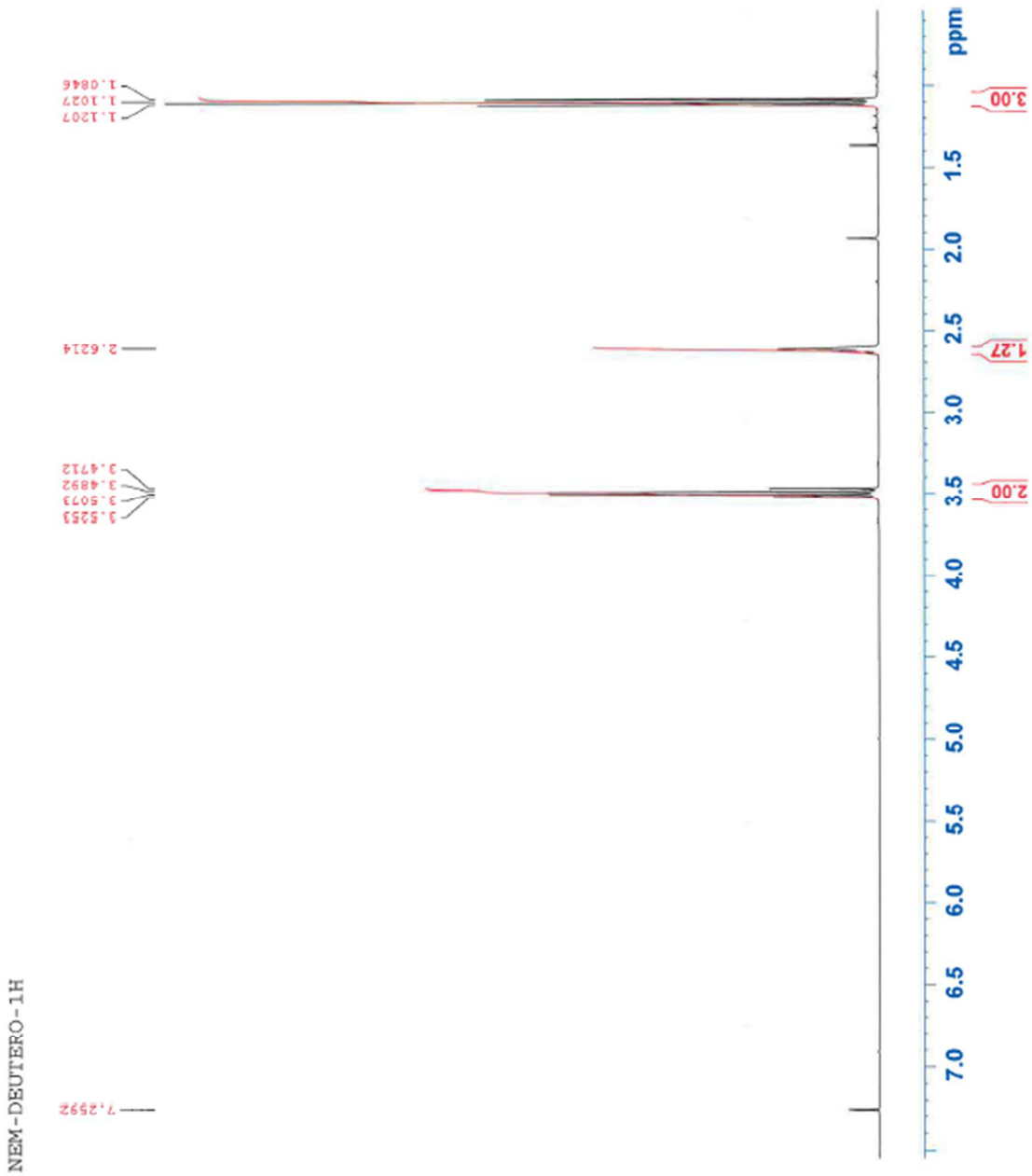


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11.35	n.a.	141.751	19.454	9.48	n.a.	BMB*
2	12.73	n.a.	726.486	185.819	90.52	n.a.	BMB*
Total:			868.237	205.273	100.00	0.000	

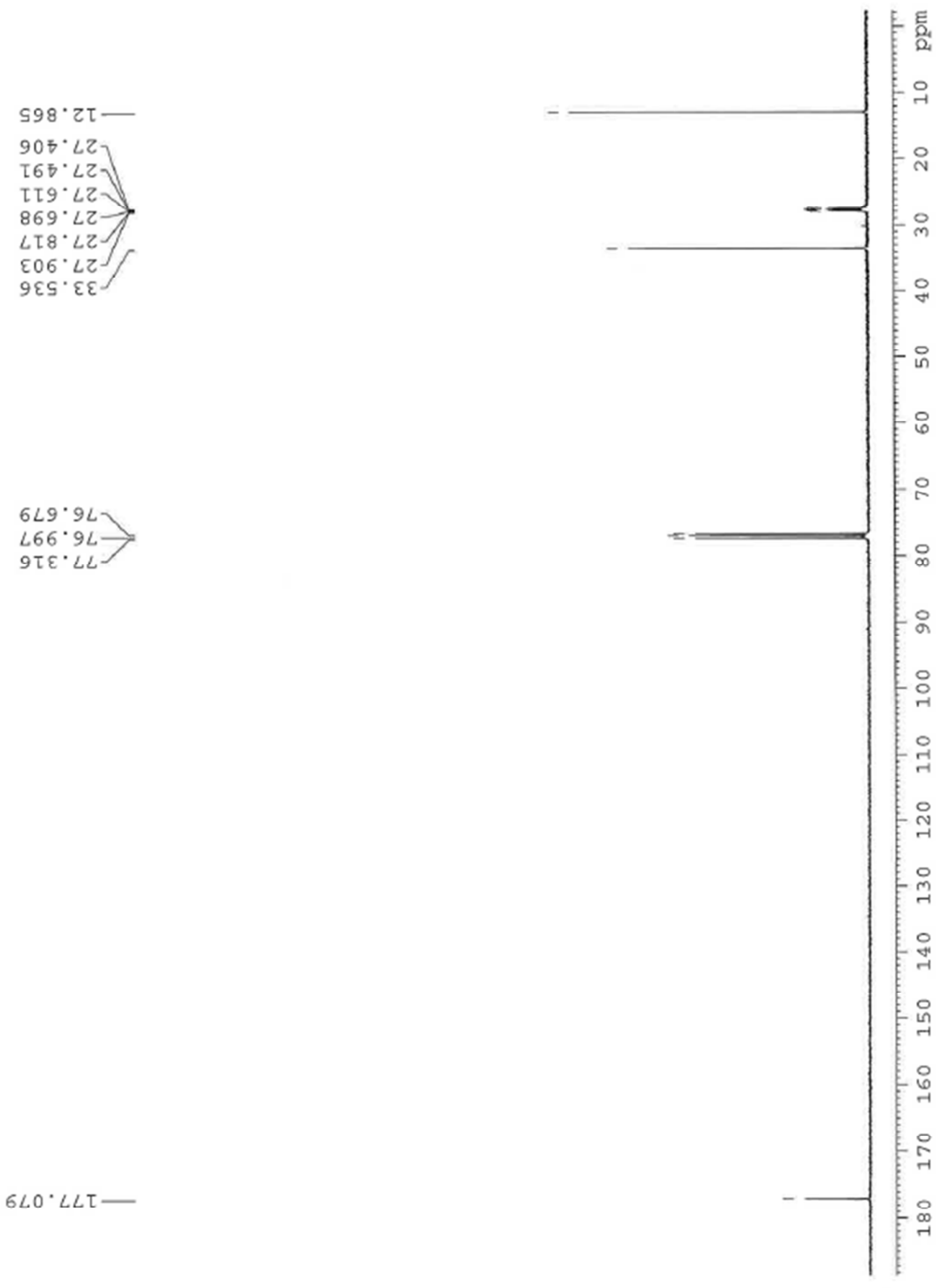
^{1}H NMR spectrum of compound (**11**)



(**11**)



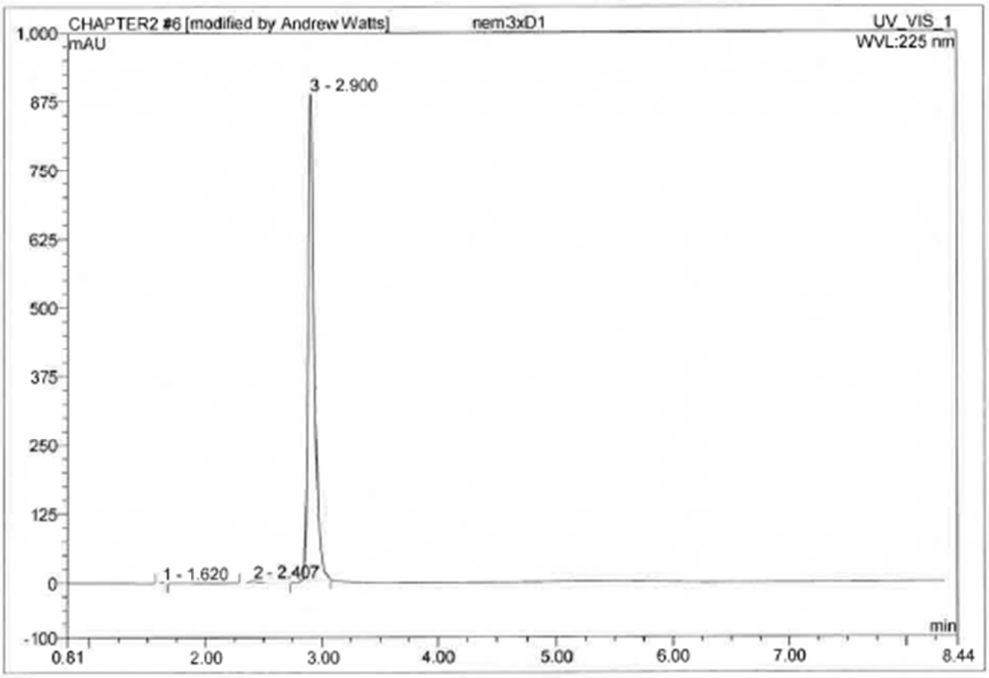
^{13}C NMR spectrum of compound (**11**)



HPLC chromatogram for compound (**11**)

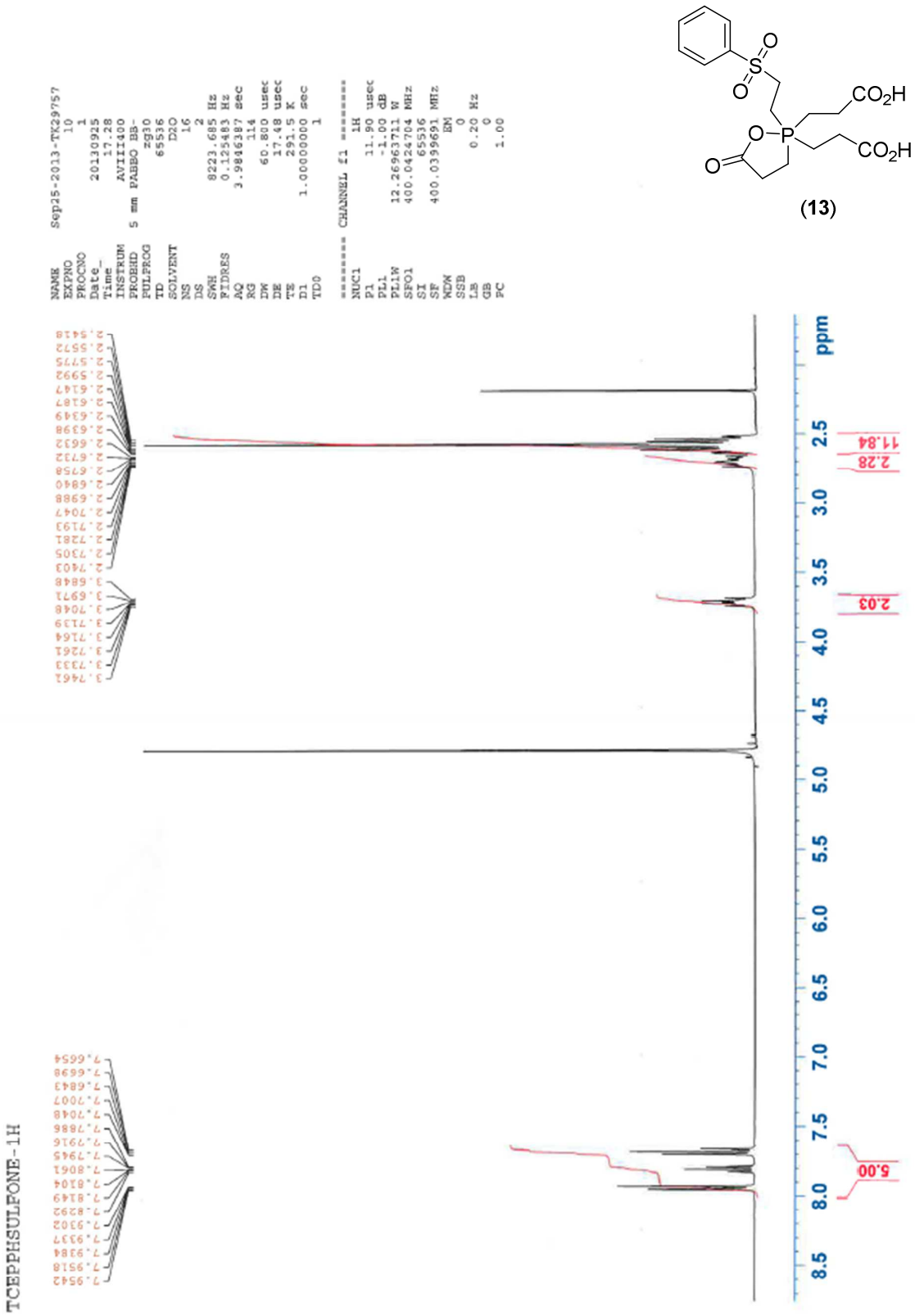
6 nem3xD1

Sample Name:	**nem3xD1**	*Injection Volume:*	**10.0**
Vial Number:	**6**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**225**
Control Program:	**thesis hplc purity c8 1**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/25/2014 15:05**	*Sample Weight:*	**1.0000**
Run Time (min):	**8.33**	*Sample Amount:*	**1.0000**



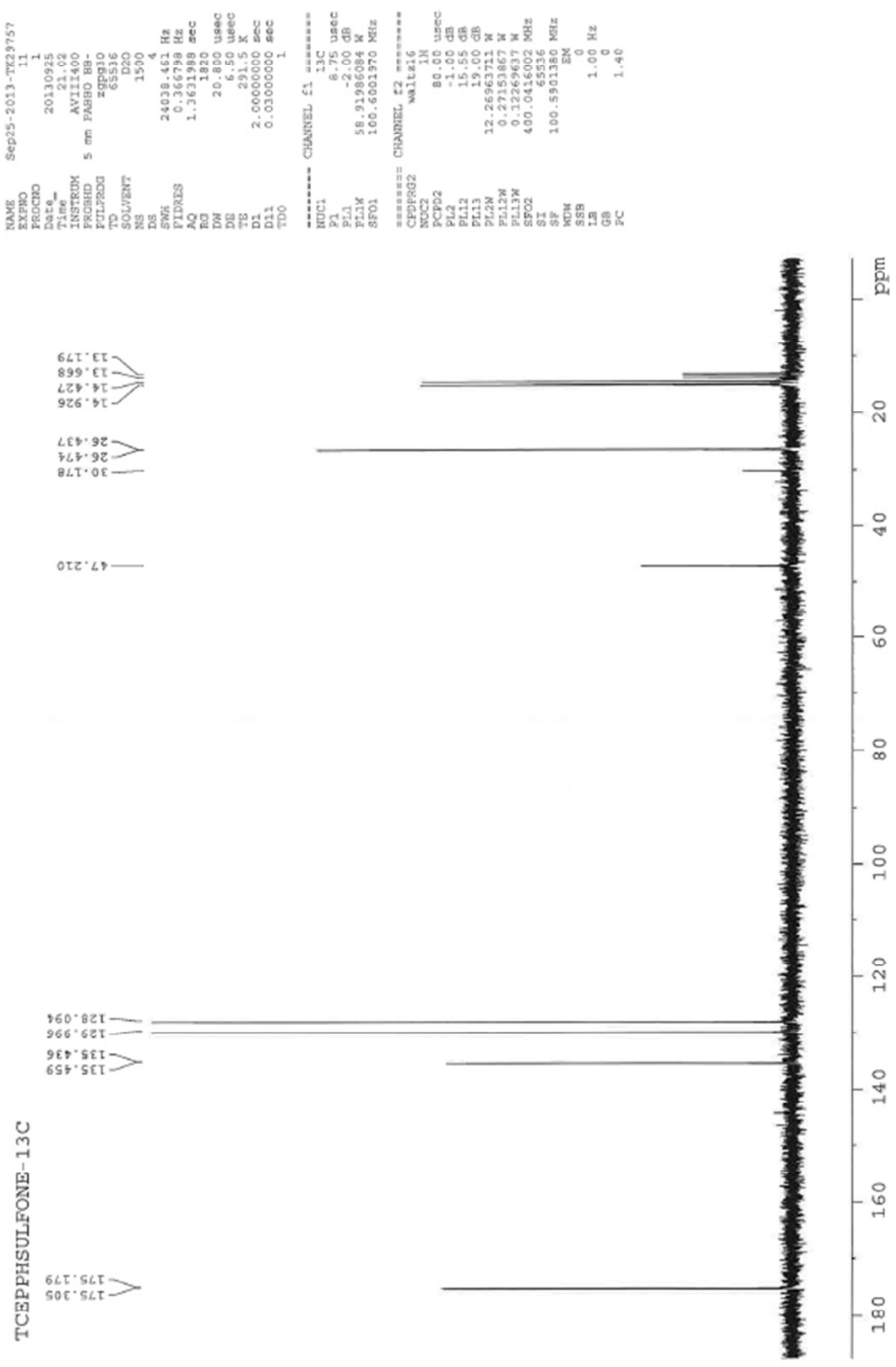
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	1.62	n.a.	1.339	0.067	0.12	n.a.	BMB*
2	2.41	n.a.	3.517	0.325	0.56	n.a.	BMB*
3	2.90	n.a.	884.590	57.453	99.32	n.a.	BMB*
Total:			889.446	57.845	100.00	0.000	

^{1}H NMR spectrum of compound (**13**)

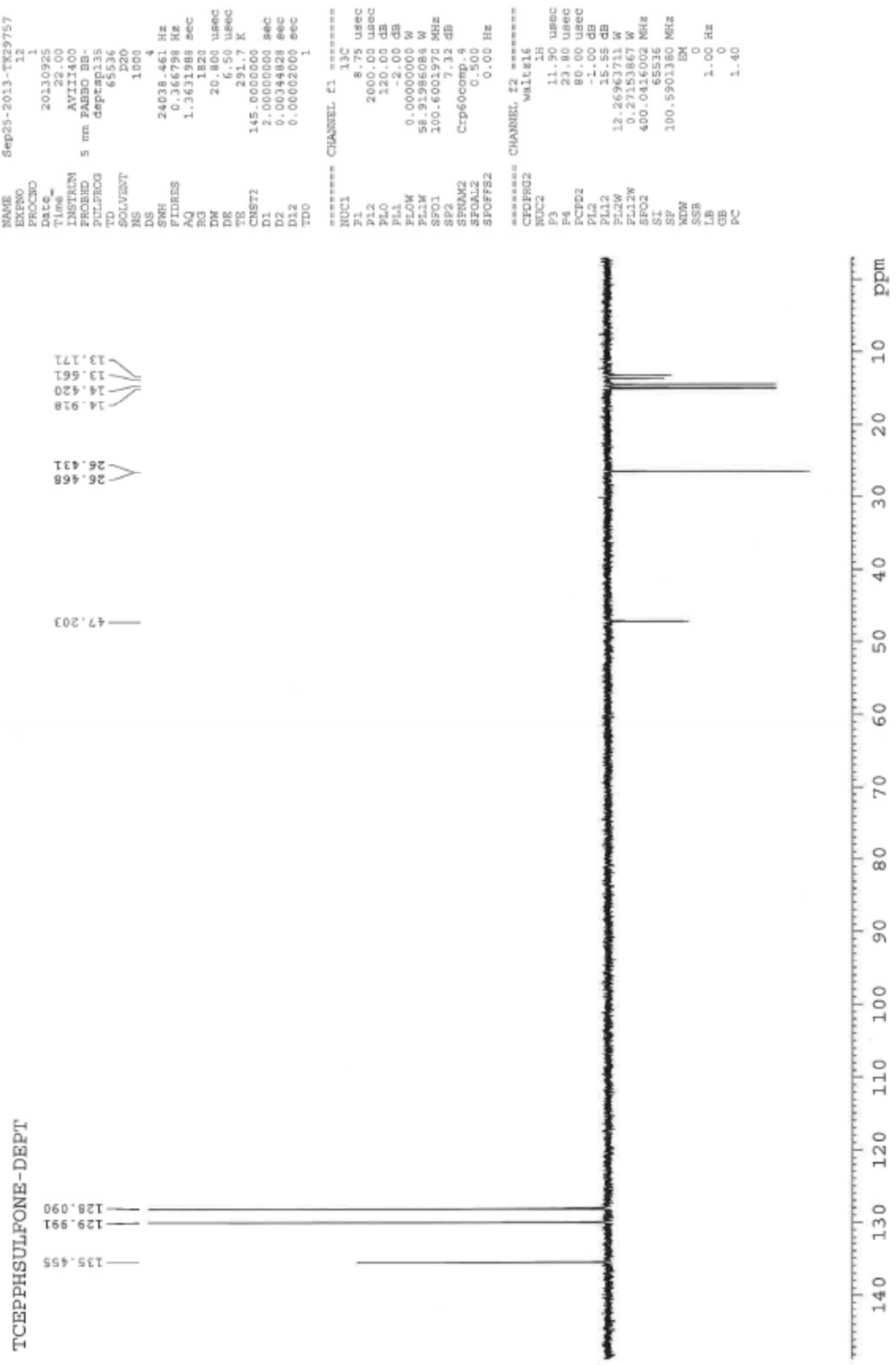


(**13**)

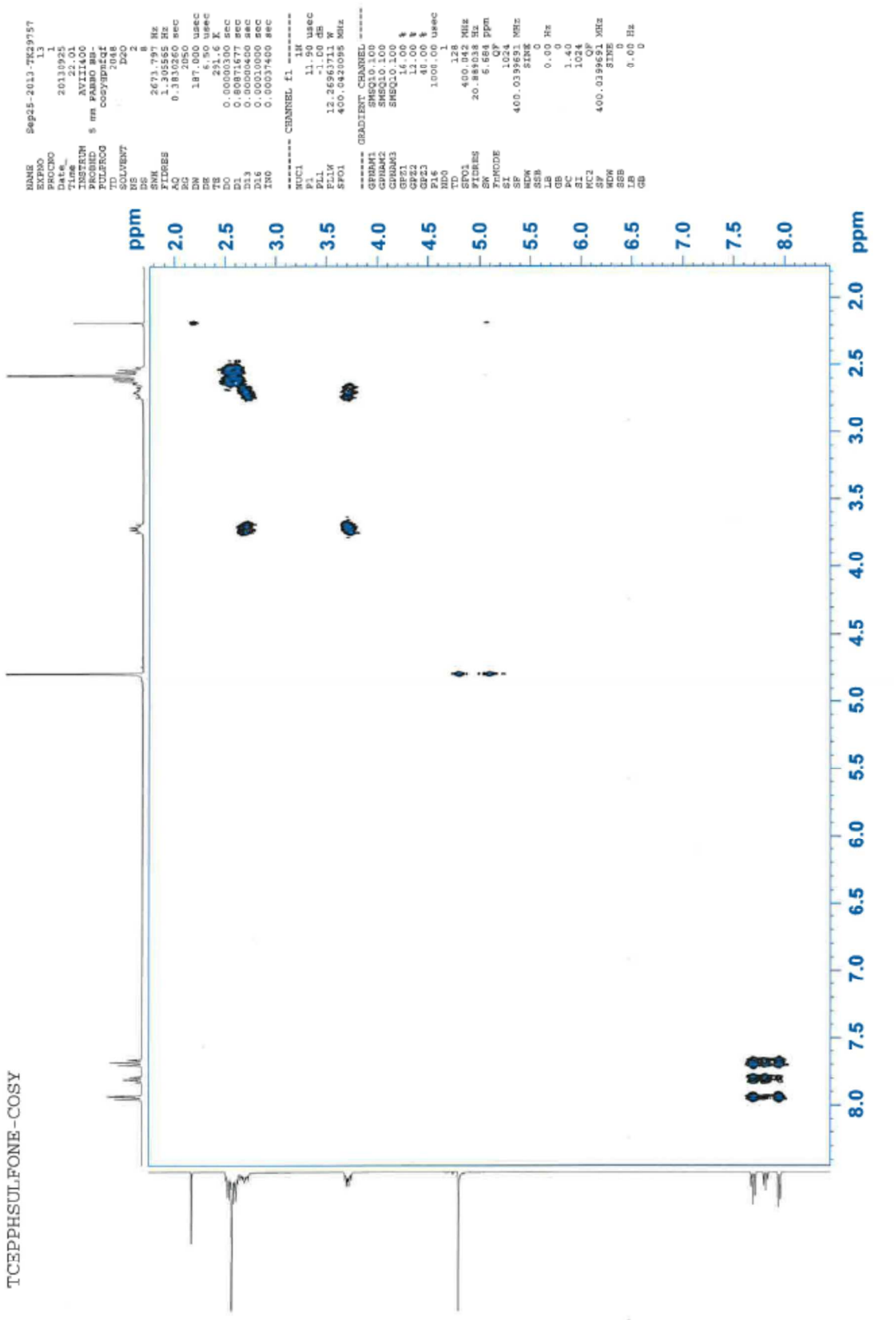
^{13}C NMR spectrum of compound (**13**)



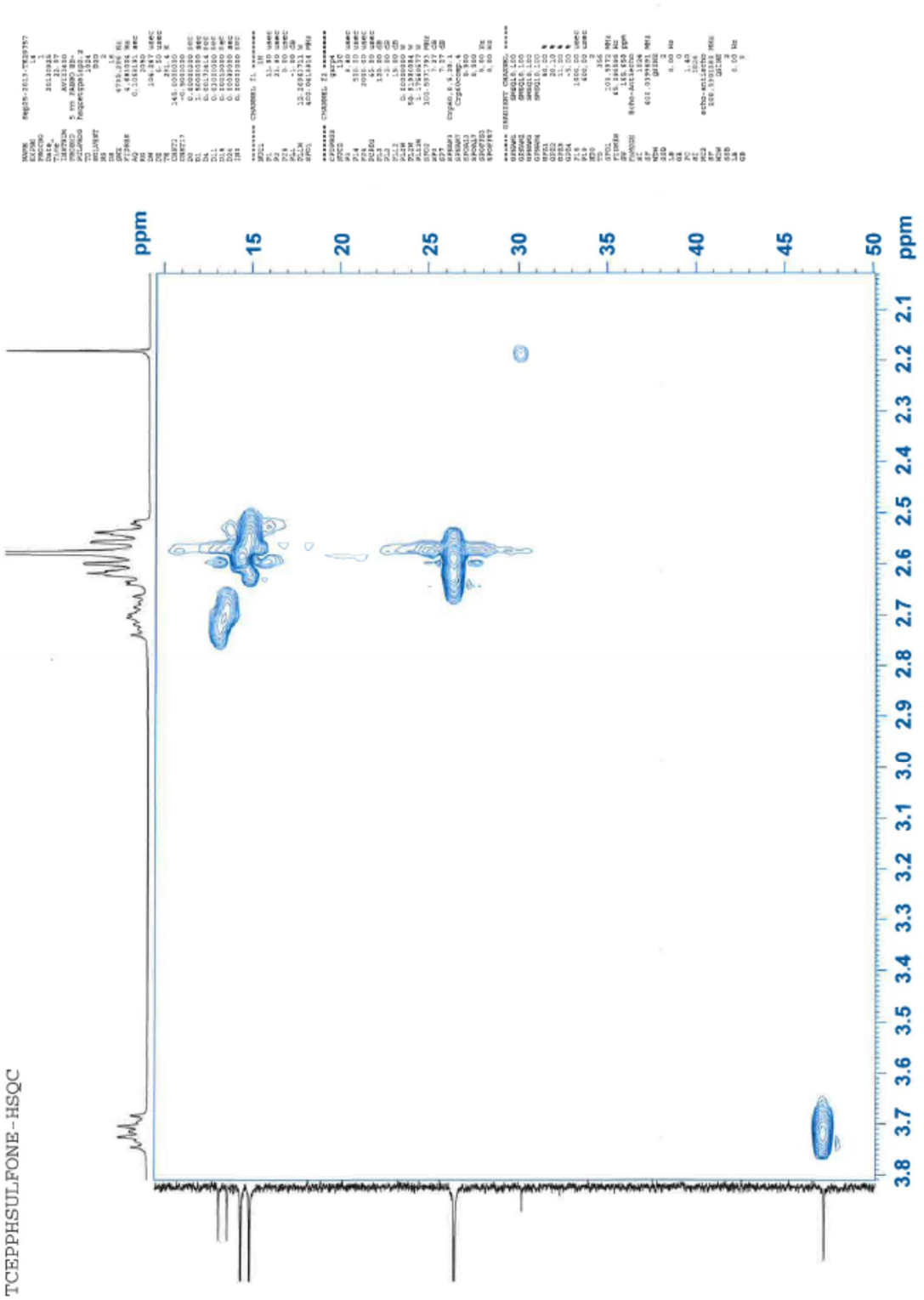
^{13}C DEPT spectrum of compound (**13**)



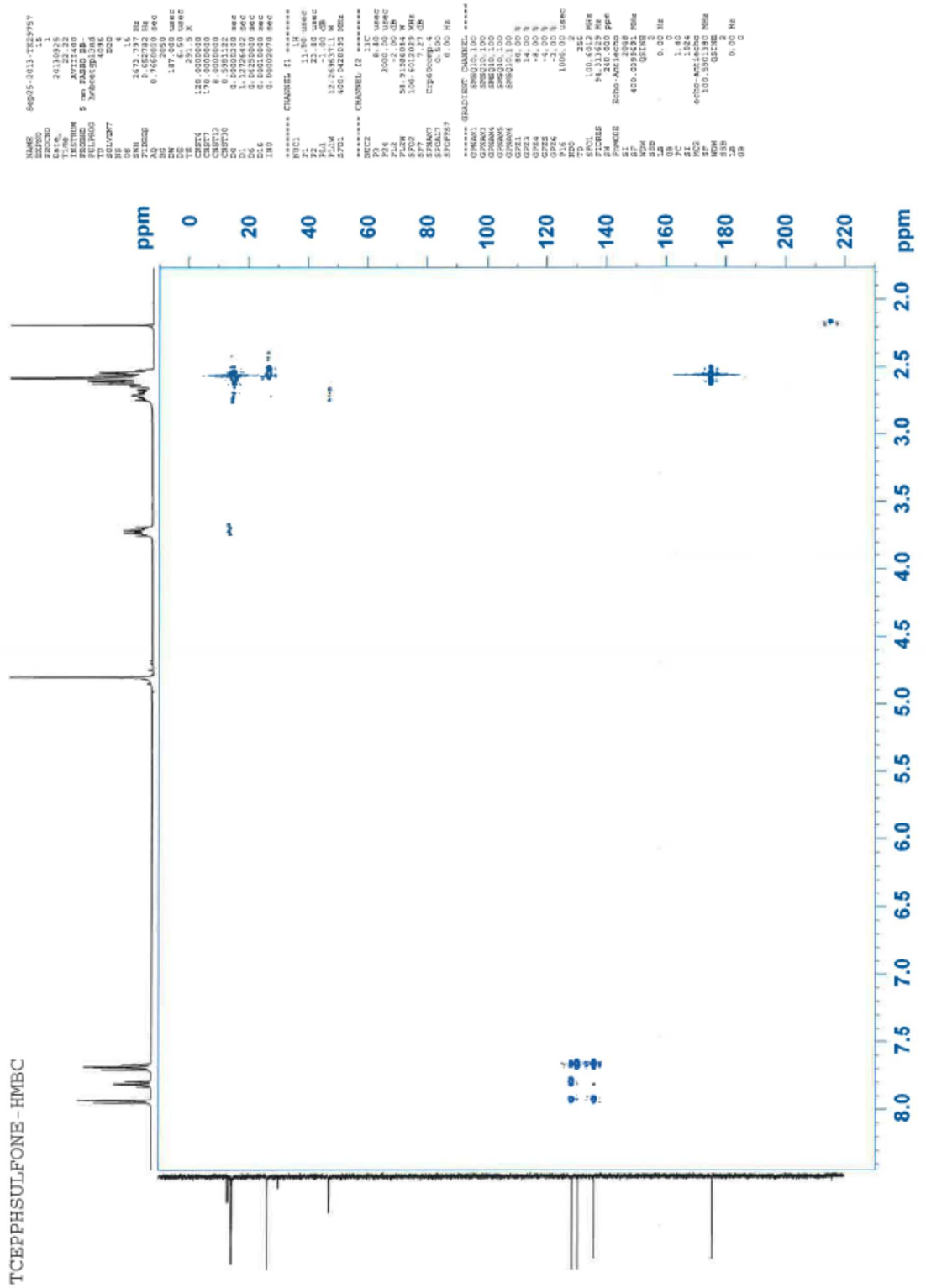
COSY NMR spectrum of compound (**13**)



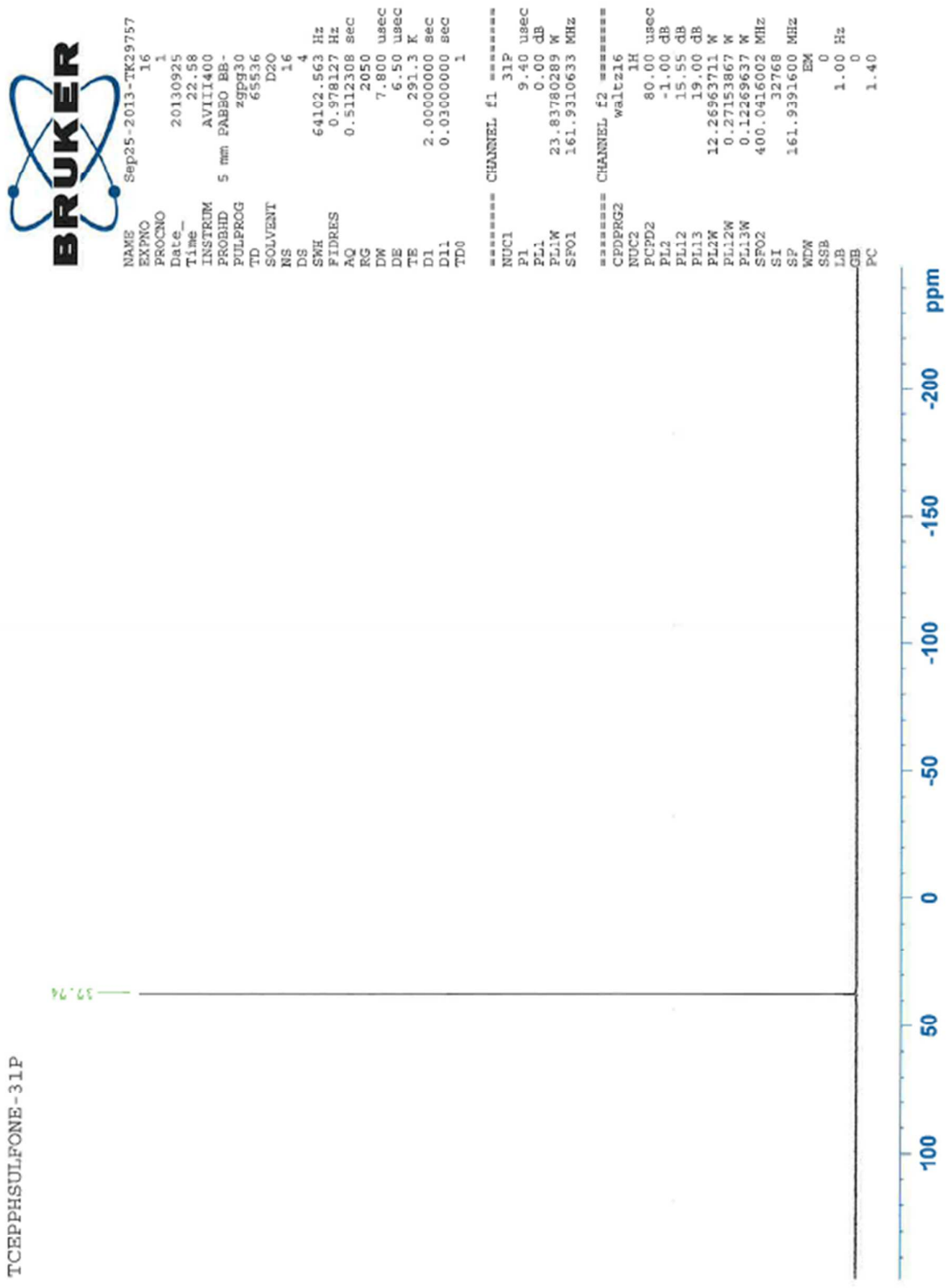
HSQC NMR spectrum of compound (**13**)



HMBC NMR spectrum of compound (**13**)



^{31}P NMR spectrum of compound (**13**)

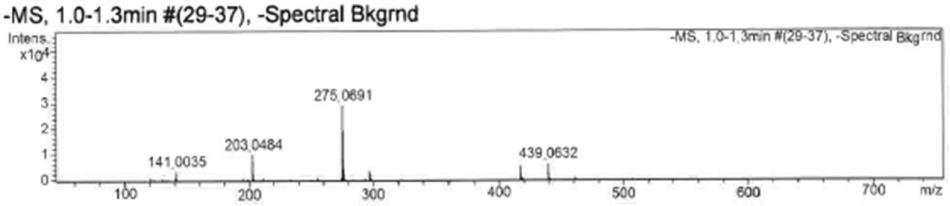


HRMS for compound (**13**)

Confirmation of Expected Formula

Sample-ID	wa_tk_tcepphsulfone	Submitter	Terrence Kantner
Analysis Name	wa_tk_tcepphsulfone_336797_31_01_40344.d	Supervisor	Andy Watts
Method used	Confirm Formula Negative 50to500 loop inj.m	Acquisition Date	24/09/2013 00:14:34
Ionisation Mode	negative electrospray (ESI)		

-MS, 1.0-1.3min #(29-37), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	121.0307	1234	4.3	20	403.7
2	141.0035	3545	12.2	85	1053.2
3	203.0484	9834	33.9	346	1575.1
4	212.0984	1250	4.3	12	183.5
5	274.9653	1294	4.5	93	181.5
6	275.0691	29025	100.0	1256	4084.2
7	276.0721	3627	12.5	160	519.3
8	297.0516	3361	11.6	162	768.8
9	417.0797	5456	18.8	365	1318.3
10	439.0632	6189	21.3	441	1495.8

Generate Molecular Formula Parameters

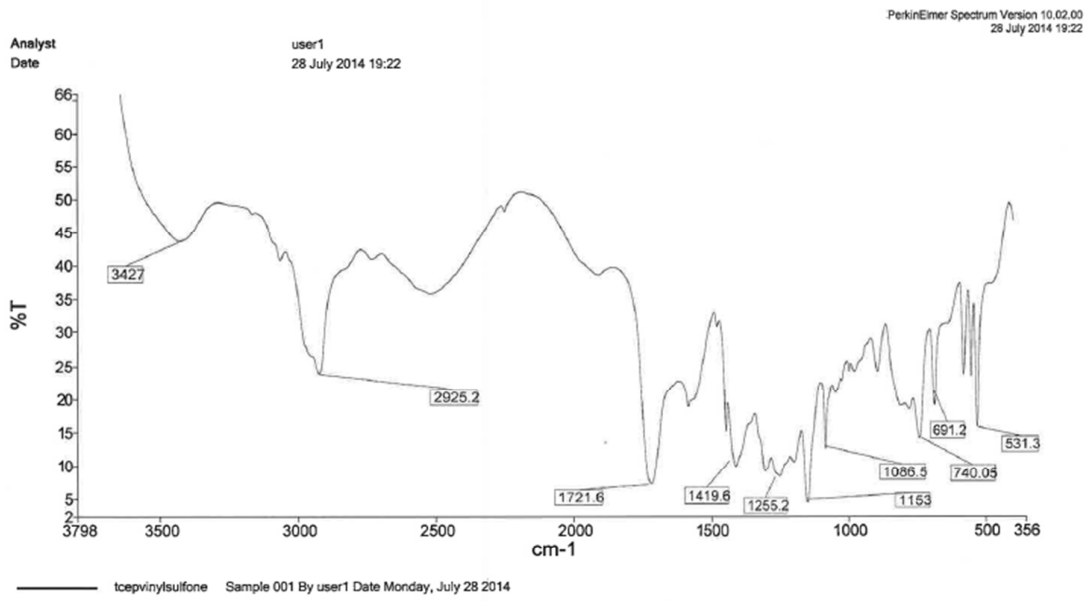
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
negative	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C17 H23 O8 P1 S1 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	417.0797	417.077300	-4.40	0.0068	C 17 H 22 O 8 P 1 S 1

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

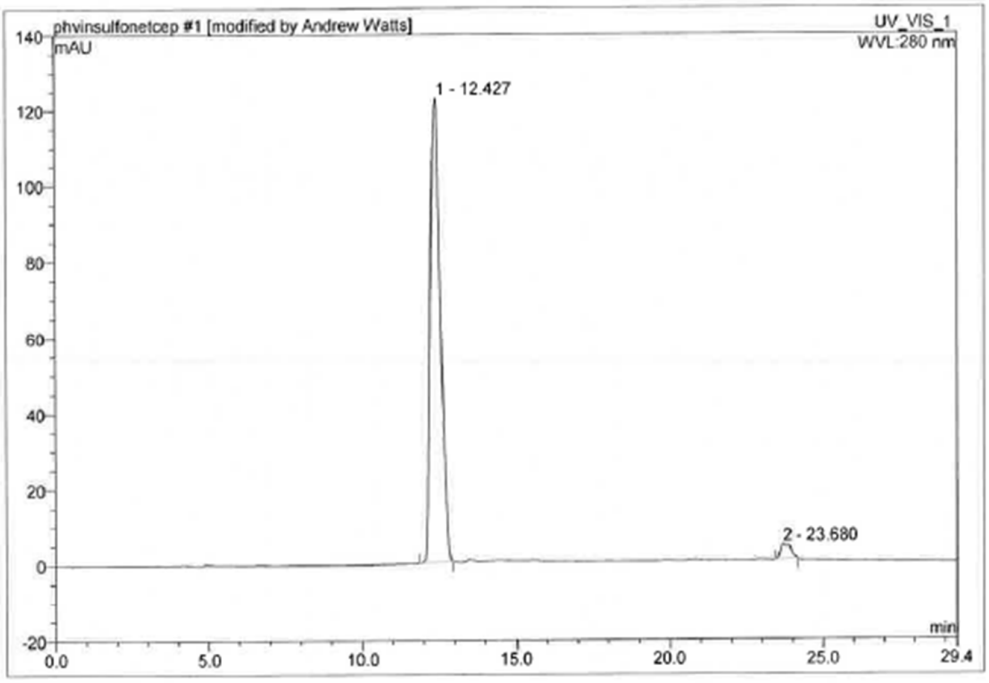
IR spectrum for compound (**13**)



HPLC chromatogram for compound (**13**)

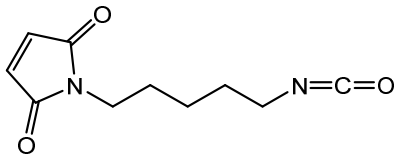
1 phvinsulfonetcep1

Sample Name:	**phvinsulfonetcep1**	*Injection Volume:*	**20.0**
Vial Number:	**1**	*Channel:*	**UV_VIS_1**
Sample Type:	**unknown**	*Wavelength:*	**280**
Control Program:	**thesis hplc purity2**	*Bandwidth:*	**n.a.**
Quantif. Method:	**U3000 test**	*Dilution Factor:*	**1.0000**
Recording Time:	**6/4/2014 13:49**	*Sample Weight:*	**1.0000**
Run Time (min):	**29.39**	*Sample Amount:*	**1.0000**

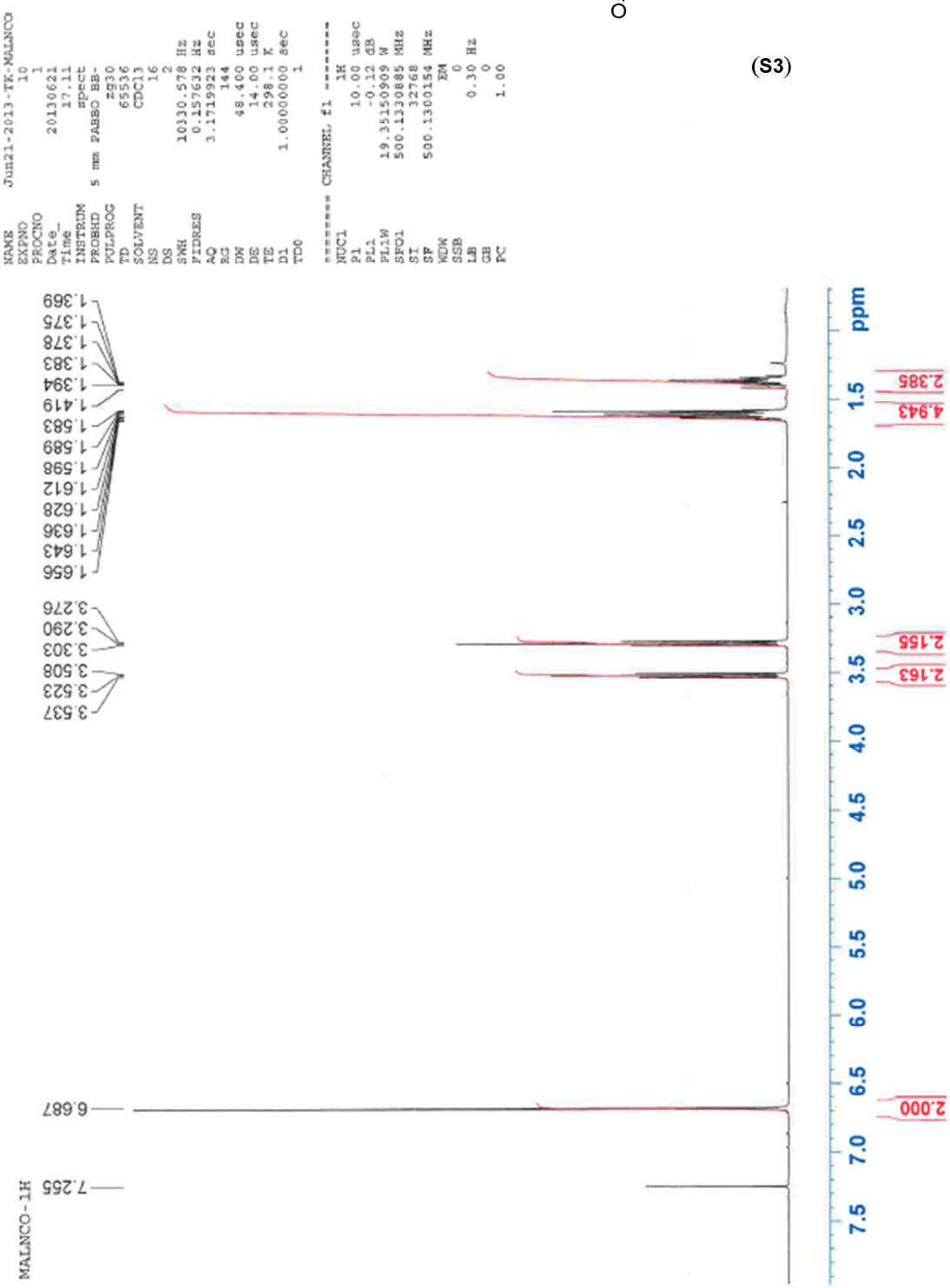


No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	12.43	n.a.	122.577	46.128	96.89	n.a.	BMB*
2	23.68	n.a.	3.860	1.482	3.11	n.a.	BMB*
Total:			126.437	47.610	100.00	0.000	

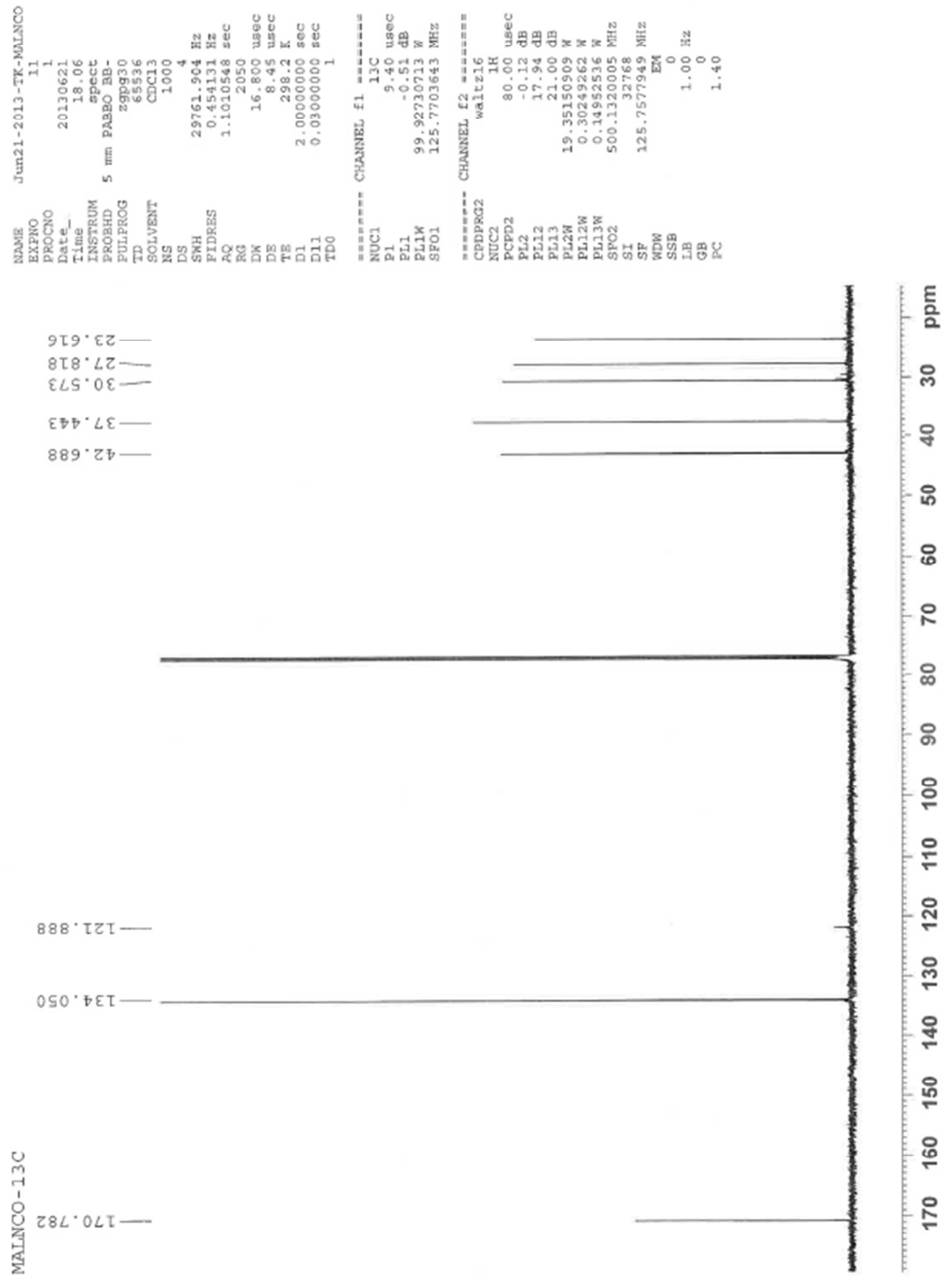
^{1}H NMR spectrum of compound (**S3**)



(**S3**)



^{13}C NMR spectrum of compound (**S3**)

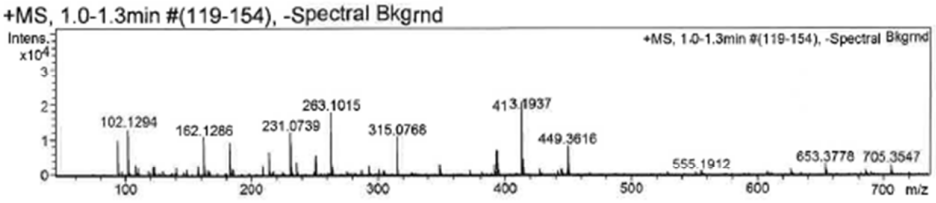


HRMS for compound (**S3**)

Confirmation of Expected Formula

Sample-ID	wa_tk_malNCOc	Submitter	Terrence Kantner
Analysis Name	wa_tk_malNCOc_335489_3_01_38767.d	Supervisor	Andy Watts
Method used	Confirm Formula Positive 50to500 loop inj.m	Acquisition Date	22/06/2013 13:45:15
Ionisation Mode	positive electrospray (ESI)		

+MS, 1.0-1.3min #(119-154), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	94.0656	9996	56.2	167	3392.2
2	102.1294	12882	72.4	235	3430.9
3	162.1286	10982	61.7	351	1760.6
4	183.1131	9328	52.4	281	1189.6
5	231.0739	12223	68.7	452	1049.4
6	263.1015	17789	100.0	850	1285.2
7	315.0768	11037	62.0	602	791.2
8	393.3003	7135	40.1	527	512.2
9	413.1937	17546	98.6	1469	919.0
10	449.3616	8433	47.4	723	390.9

Generate Molecular Formula Parameters

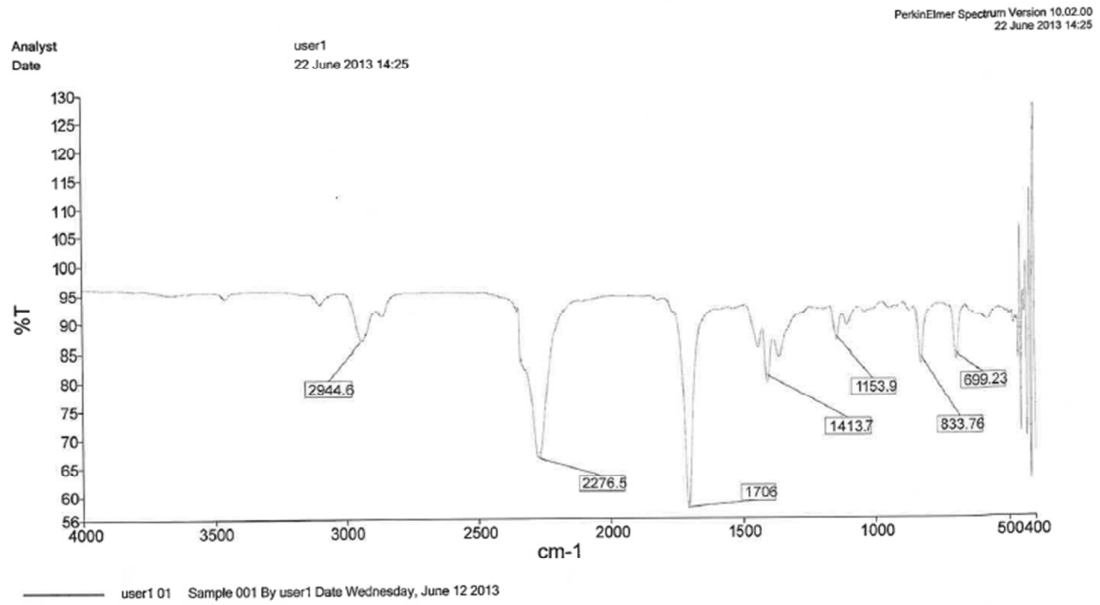
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	10 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C10 H12 N2 O3 **Adduct(s):** H, Na

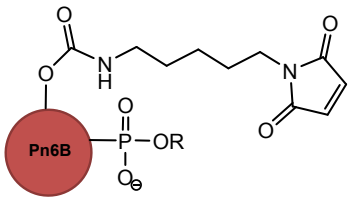
#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	231.0739	231.074562	0.60	0.0243	C 10 H 12 N 2 Na 1 O 3

Note: Sigma fits < 0.05 indicates high probability of correct MF, and mass accuracy of 5ppm or better is generally acceptable for publication

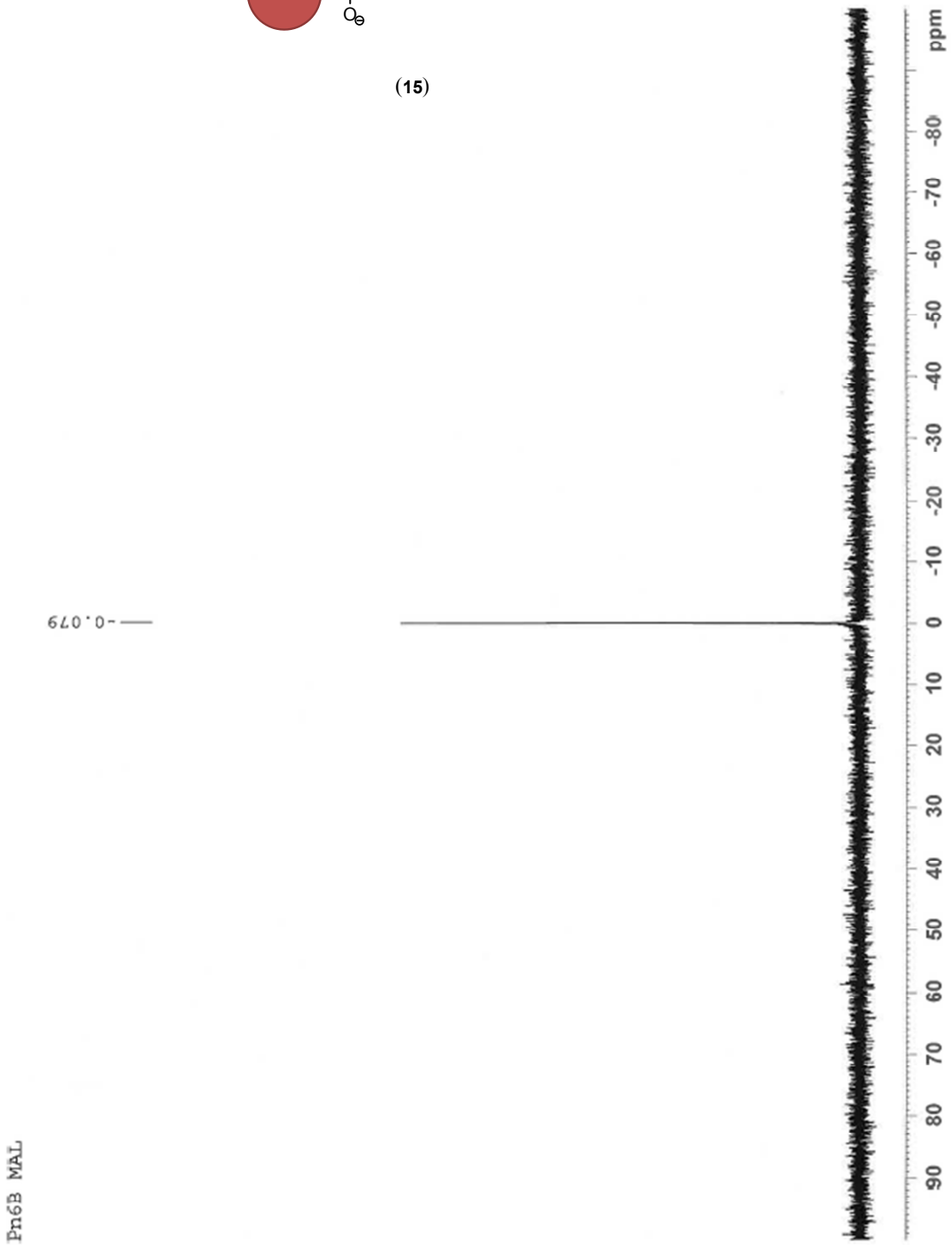
IR spectra for compound (**S3**)



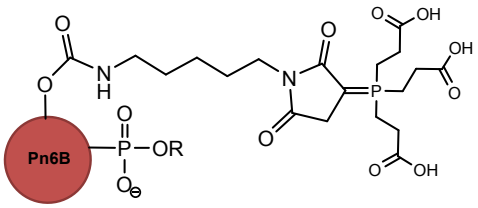
^{31}P NMR spectrum of compound (**15**)



(**15**)



^{31}P NMR spectrum of compound (**16**)



(**16**)