Electronic Supplementary Material (ESI) for RSC Advances.

Photo-Controlled Interactions of Azobenzene Micelle Derivatives with Antimicrobial Peptides

Matthew G. Roberson[a], Julia M. Duncan[b], Keveen J. Flieth[c], Laina M. Geary[e], Matthew J. Tucker[f]

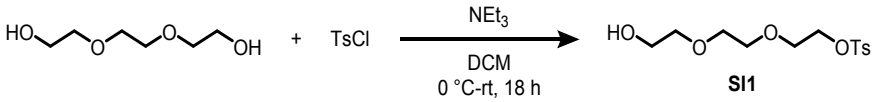
Experimental Section

General Information

All glassware were oven dried at 140 °C overnight and cooled under argon or nitrogen gas before use. Solvents were purified by Grubbs's method[1] using a commercial solvent purification system before use, unless otherwise noted. Reagents used in reactions were purchased from commercial suppliers and used as received, unless otherwise noted. Analytical thin-layer chromatography (TLC) was carried out using 0.25 mm commercial silica gel plates. Visualization was accomplished with UV light and/or potassium permanganate solution. Purification of reaction products was carried out by flash column chromatography using 40-63 µm silica gel. ^{1}H NMR (400 MHz or 500 MHz) and ^{13}C NMR (100 MHz or 125 MHz) were recorded in $CDCl_3$ solutions unless otherwise noted. ^{13}C NMR spectra were routinely run with broadband decoupling. Chemical shifts for ^{1}H and ^{13}C are reported in parts per million (ppm) down field from TMS, using residual $CDCl_3$ (7.26 ppm and triplet at 77.0 ppm, respectively) or tetramethylsilane as an internal standard. The following abbreviations are used: m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), etc.

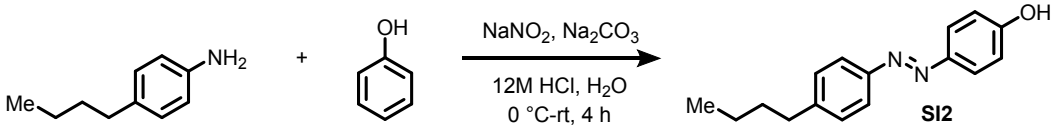
Synthetic Details and Characterization Data

2-[2-(2-Hydroxyethoxy)ethoxy]ethyl 4-methylbenzenesulfonate (SI1)



Triethylene glycol (33.5ml, 250mmol, 5 equiv) and dry DCM (75ml) were added to an oven-dried round bottom flask. The solution was cooled to 0°C, and then NEt_3 (10.5ml, 75mmol, 1.5 equiv) was added to the flask followed by the portion wise addition of *p*-toluenesulfonyl chloride (TsCl, 9.55g, 50mmol, 1 equiv). The solution was allowed to warm to rt and stirred for 18 hr. The solution was then washed with H_2O (3x75ml) and the aqueous layers were back extracted with DCM (75ml). The combined organic layers were washed with a 5% citric acid solution (3x33ml), dried over Na_2SO_4, decanted and the solvent was removed by vacuum and high vac. Compound **SI1** was obtained as a pale yellow oil (15.1 g, 99%). The structure was confirmed by ^{1}H and ^{13}C NMR.[2] ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.78 (d, J=8Hz, 2H), 7.33 (d, J=8Hz, 2H), 4.16-4.14 (m, 2H), 3.71-3.67 (m, 4H), 3.59 (s, 4H), 3.57-3.54 (m, 2), 2.43 (s, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 145.0, 133.0, 129.9, 128.1, 72.6, 70.9, 70.4, 69.3, 68.8, 61.8, 21.7 ppm.

4-[(4-Butylphenyl)azo]-phenol (SI2)

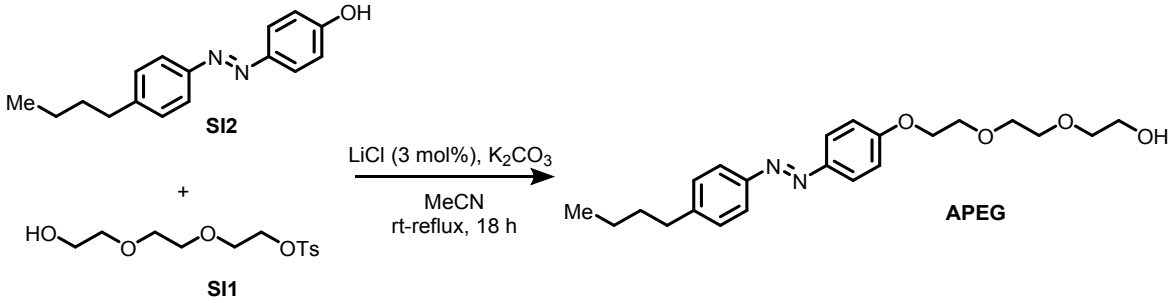


4-Butylaniline (8.0ml, 50mmol, 1 equiv) and water (75ml) were added to a round bottom flask. 12M HCl (22.5ml) was added to the solution, that was then stirred at rt for 1 hour. The solution was cooled to 0 °C, and a solution of $NaNO_2$ (3.45g, 50mmol, 1 equiv) in water (10.5ml) was slowly added to the round bottom flask, maintaining the internal temperature at 0 °C. The solution was stirred for 30 mins at 0 °C

[1] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518-1520.
[2] Moller, N.; Ruhling, A.; Lamping, S.; Hellwig, T.; Fallnich, C.; Ravoo, B. J.; Glorius, F. *Angew. Chem., Int. Ed.* **2017**, *56*, 4356-4360.

following complete addition. A solution of phenol (4.70g, 50mmol, 1 equiv) and Na_2CO_3 (15.85g, 150mmol, 3 equiv) in water (105ml) were then slowly added to the round bottom flask maintaining the internal temperature between 0-3 °C. Once all was added, the solution was stirred at that temperature of an hour. After 1 hour, 12M HCl was slowly added to the flask until pH=3 (solution turns orange, and bubbles). The mixture was extracted with EtOAc (3x225ml). The combined organic layers were dried with Na_2SO_4, decanted, and concentrated by vacuum. The red residue was purified by silica gel column chromatography (EtOAc:Hex 1:4, EtOAc:Hex 1:1 gradient). Compound **SI2** was obtained as an orange solid (10.8g, 85%), and the structure was confirmed by ^{1}H and ^{13}C NMR.[3] ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.89 (d, J=8.0Hz, 2H), 7.80 (d, J=8.0Hz, 2H), 7.30 (d, J=8.0, 2H), 6.93 (d, J=8.0Hz, 2H), 5.28 (Br s, 1H), 2.68 (t, J=8.0Hz, 2H), 1.64 (quin, J=8.0Hz, 2H), 1.38 (sex, J=8.0Hz, 2H), 0.94 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 158.1, 151.1, 147.4, 146.1, 129.2, 124.9, 122.7, 115.9, 35.7, 33.6, 22.50, 14.1 ppm.

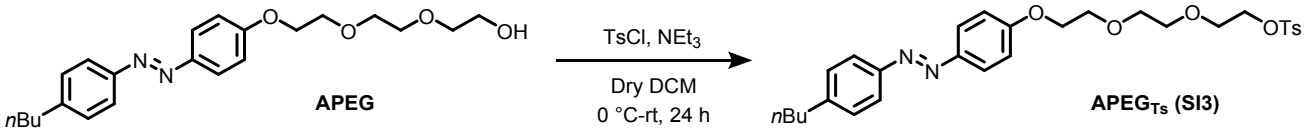
2-[2-[2-[4-[(1*E*)-2-(4-Butylphenyl)diazenyl]phenoxy]ethoxy]ethoxy]-ethanol (APEG)



LiCl (20mg, 0.3mmol, 0.03 equiv) and K_2CO_3 (6.9g, 50mmol, 5 equiv) were added to a flame-dried round bottom flask and purged with Ar. A solution of mono tosylated triethylene glycol SI**1** (3.04g, 10mmol, 1 equiv) in dry MeCN (100ml) was added to the flask. A solution of azo benzene SI**2** (2.79g, 11mmol, 1.1 equiv) in dry MeCN (35ml) was slowly added to the flask. The solution was heated to reflux and left to stir for 18 h. The solvent was then removed by vacuum and the residue was dissolved in DCM (125ml). The solution was washed with brine (3x50ml) and the aqueous layers were back extracted with DCM (50ml). The combined organic layers were dried over Na_2SO_4, decanted, and the solvent was removed by vacuum. The residue was purified by silica gel column chromatography (EtOAc:DCM 4:1, EtOAc:MeOH 49:1, and EOAc:MeOH 19:1 gradient). APEG was obtained as an orange solid (3.00g, 77%); spectral data were consistent with literature values.[4] m.p.= 63 °C. ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.89 (d, J=8.0Hz, 2H), 7.80 (d, J=8.0Hz, 2H), 7.30 (d, J=8.0Hz, 2H), 7.02 (d, J=8.0Hz, 2H), 4.21 (t, J=4.0Hz, 2H), 3.89 (t, J=4.0Hz, 2H), 3.75-3.69 (m, 6H), 3.62 (t, J=4.0Hz, 2H), 2.68 (t, J=8.0Hz, 2H), 2.43 (br s, 1H), 1.64 (quin, J=8.0Hz, 2H), 1.38 (sex, J=8.0Hz, 2H), 0.94 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 161.0, 151.1, 147.3, 146.0, 129.1, 124.6, 122.7, 114.9, 72.6, 70.99, 70.50, 69.8, 67.8, 61.9, 35.7, 33.6, 22.5, 14.1 ppm.

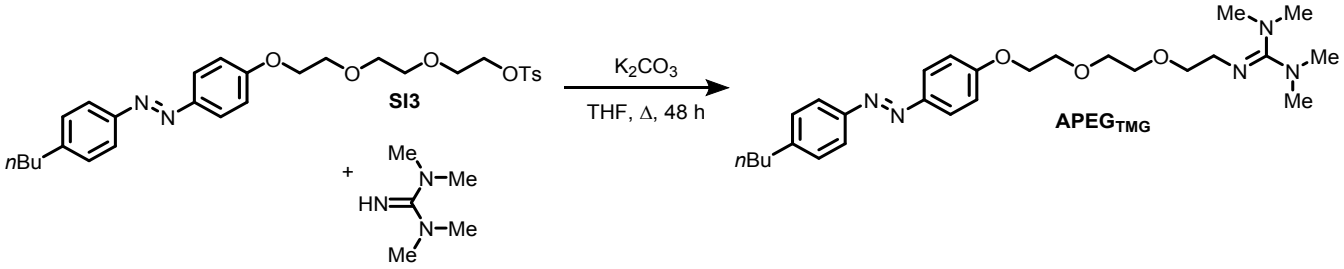
[3] Barclay, T. G.; Constantopoulos, K.; Zhang, W.;Fujiki, M.; Matisons, J. G. *Langmuir* **2012**, *28*, 14172-14179.
[4] Shang, T.; Smith, K. A.; Hatton, T. A. *Langmuir* **2003**, *19*, 10764-10773.

APEG$_{Ts}$ (**SI3**)



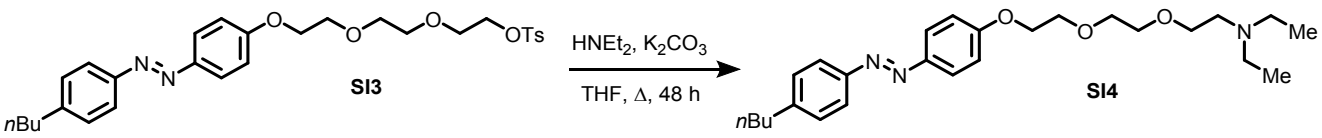
APEG (0.661g, 1.7mmol, 1 equiv) was added to and oven-dried round bottom flask and dissolved in dry DCM (7.5ml). The solution was cooled to 0 °C and NEt_3 (0.31ml, 2.2mmol, 1.3 equiv) was added to the flask. *p*-Toluenesulfonyl chloride (TsCl, 0.359g, 1.9mmol, 1.1 equiv) was added to the flask in portions. The cold bath was removed and the solution was brought to rt and stirred for 18 hr. At this time, the solution was heated to reflux and stirred for 4 hr. The solvent was removed by vacuum and the residue was purified by silica gel column chromatography (EtOAc:DCM 4:1). APEG$_{Ts}$ **2** was isolated as an orange gel (0.785g, 83%), and characterized by ^{1}H NMR and ^{13}C NMR before taking onto the next steps ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.65 (d, J=8.0Hz, 2H), 7.84 (d, J=8.0Hz, 2H), 7.79 (d, J=8.0Hz, 2H), 7.32 (d, J=8.0Hz, 2H), 7.30 (d, J=8.0Hz, 2H), 7.03 (d, J=8.0Hz, 2H), 4.20 (t, J=4.0Hz, 2H), 4.16 (t, J=4.0Hz, 2H), 3.86 (t, J=4.0Hz, 2H), 3.71-3.67 (m, 4H), 3.64-3.61 (m, 2H), 2.68 (t, J=8.0Hz, 2H), 2.42 (s, 3H), 1.64 (quin, J=8.0Hz, 2H), 1.38 (sex, J=8.0Hz, 2H), 0.94 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 162.0, 149.8, 146.7, 146.4, 145.0, 133.1, 129.9, 129.3, 128.1, 125.5, 122.8, 115.2, 70.9, 69.8, 69.4, 68.9, 68.0, 35.7, 33.5, 22.5, 21.8, 14.1 ppm.

APEG$_{TMG}$



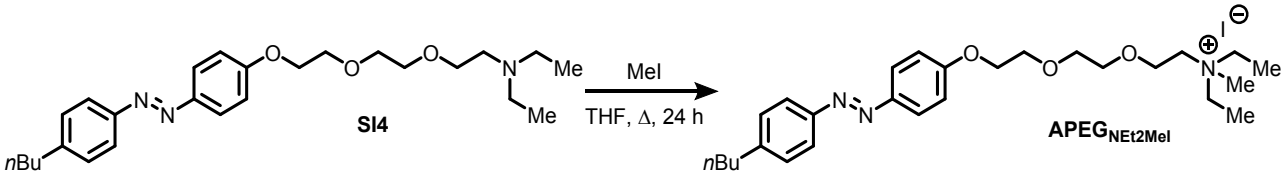
K_2CO_3 (350mg, 2.5mmol, 1eq) and a solution of APEG$_{Ts}$ **SI3** (1.360g, 2.5mmol, 1 equiv) in dry THF (10ml) were added to an oven-dried round bottom flask. Tetramethylguanidine (TMG; 1.25ml, 10mmol, 4 equiv) was added to the mixture. The mixture was heated to reflux and left to stir for 48 hr, monitored by TLC. The solvent was removed and the residue was purified by alumina gel column chromatography (EtOAc:DCM 4:1, EtOAc:MeOH 9:1). The solvent was removed and the residue was dissolved in EtOAc and cooled to form a precipitate. The solid was filtered off and the filtrate was dried under vacuum. APEG$_{TMG}$ was obtained as a dark red gel (1.11g, 91%). The structure was confirmed by ^{1}H, ^{13}C NMR, and HRMS. ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.87 (d, J=8.0Hz, 2H), 7.78 (d, J=8.0Hz, 2H), 7.28 (d, J=8.0Hz, 2H), 6.98 (d, J=8.0Hz, 2H), 4.17 (t, J=6.0Hz, 2H), 3.82 (t, J=6.0Hz, 2H), 3.76 (t, J=6.0Hz, 2H), 3.66 (s, 4H), 3.38 (t, J=6.0Hz, 2H), 3.02-2.85 (m, 6H), 2.66 (t, J=8.0Hz, 2H), 1.62 (quin, J=8.0Hz, 2H), 1.36 (sex, J=8.0Hz, 2H), 0.92 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 162.8, 160.9, 151.0, 147.3, 146.1, 129.1, 124.6, 122.6, 114.8, 70.7, 70.4, 69.9, 69.6, 67.9, 45.0, 40.3, 39.9, 35.6, 33.5, 22.4, 14.0 ppm. m/z $C_{27}H_{42}N_5O_3$ ($M+H^+$) calc. 484.3288; found 484.3295.

APEG$_{DMA}$ (**SI4**)



APEG$_{Ts}$ **SI3** (0.550g, 1mmol, 1 equiv), K_2CO_3 (0.138g, 1mmol, 1 equiv) and dry THF (5ml) were added to a round bottom flask. $HNEt_2$ (0.42ml, 4mmol, 4 equiv) was added to the mixture, heated to reflux and left to stir for 48 hr. The solvent was removed by vacuum and the residue was purified by alumina gel column chromatography (EtOAc:DCM 4:1, EtOAC:MeOH 9:1). Dimethyl APEG (APEG$_{DMA}$, **SI4**) was obtained as an orange gel was obtained (0.404g, 91%). The structure was verified by ^{1}H and ^{13}C NMR before going onto the next step. ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.88 (d, J=8.0Hz, 2H), 7.79 (d, J=8.0Hz, 2H), 7.29 (d, J=8.0Hz, 2H), 7.01 (d, J=8.0Hz, 2H), 4.20 (t, J=4.0Hz, 2H), 3.89 (t, J=4.0Hz, 2H), 3.74-3.64 (m, 4H), 3.59 (t, J=4.0Hz, 2H), 2.70-2.65 (m, 4H), 2.59 (q, J=8.0Hz, 4H), 1.64 (quin, J=8.0Hz, 2H), 1.37 (sex, J=8.0Hz, 2H), 1.03 (t, J=8.0Hz, 6H), 0.94 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 161.1, 151.1, 147.3, 145.9, 129.1, 124.6, 122.6, 114.9, 71.0, 70.6, 69.81, 69.75, 67.8, 52.3, 47.7, 35.7, 33.6, 22.5, 14.1, 11.7 ppm.

APEG$_{NEt2MeI}$



APEG$_{DMA}$ **SI4** (980mg, 2.2mmol, 1 equiv) was dissolved in dry THF (5ml) and added to an oven-dried round bottom flask. MeI (192µl, 3.0mmol, 1.4 equiv) was added to the solution. The solution was heated to reflux and left to stir for 24 hr and monitored by TLC. The solvent was removed by vacuum to obtain a solid. The solid was washed with cold Et_2O 4 times to remove starting materials. The solid was dried under vacuum, yielding diethylmethylammonium APEG iodide (APEG$_{Net2MeI}$) as an orange solid (1.09g, 85%). The structure was confirmed by ^{1}H, ^{13}C NMR, and HRMS. m.p.= 58-60 ^{o}C. ^{1}H NMR (400MHz, $CDCl_3$): δ= 7.87 (d, J=8.0Hz, 2H), 7.77 (d, J=8.0Hz, 2H), 7.28 (d, J=8.0Hz, 2H), 7.00 (d, J=8.0Hz, 2H), 4.18 (t, J=4.0Hz, 2H), 3.97 (t, J=4.0Hz, 2H), 3.84 (t, J=4.0Hz, 2H), 3.73 (t, J=8.0Hz, 2H), 3.39 (s, 4H), 3.56 (q, J=8.0Hz, 4H), 3.20 (s, 3H), 2.65 (t, J=8.0Hz, 2H), 1.61 (quin, J=8.0Hz, 2H), 1.39 (sex, J=8.0Hz, 2H), 1.31 (t, J=8.0Hz, 6H), 0.92 (t, J=8.0Hz, 3H) ppm. ^{13}C NMR (100MHz, $CDCl_3$): δ= 161.0, 151.0, 147.3, 146.1, 129.1, 124.7, 122.6, 122.3, 114.90, 114.87, 70.6, 70.5, 69.7, 67.9, 64.8, 57.9, 48.7, 48.2, 35.6, 33.5, 22.4, 14.0, 8.2 ppm. m/z $C_{27}H_{42}N_3O_3^+$ calc. 456.3221; found 456.3222.

APEG Photoisomerization

APEG Photoisomerization Reversibility. The photolizable azobenzene moiety of the APEG surfactants was utilized in all spectroscopy experiments. Samples were prepared by dissolving a known mass of surfactant in ultrapure water (18.2 MΩ*cm). Samples were kept in the dark until used. Initial measurements (i.e. prior to any direct irradiation) were made with the assumption that the surfactant population was purely *trans*. Isomerization to the *cis* isomer was achieved using 365 nm light. The first measurement following the initial irradiation was assumed to be 100% *cis*. Irradiation cycles (Figure S1) were then carried out by irradiating at 460 nm and 365 nm to obtain the *trans* and *cis* isomers, respectively. For each irradiation cycle, the total concentration of APEG surfactant remained constant. The OD of each cycle was collected at the π→π* transition maxima for the *trans* isomer (351 and 348 nm for $APEG_{NEt2MeI}$ and $APEG_{TMG}$, respectively).

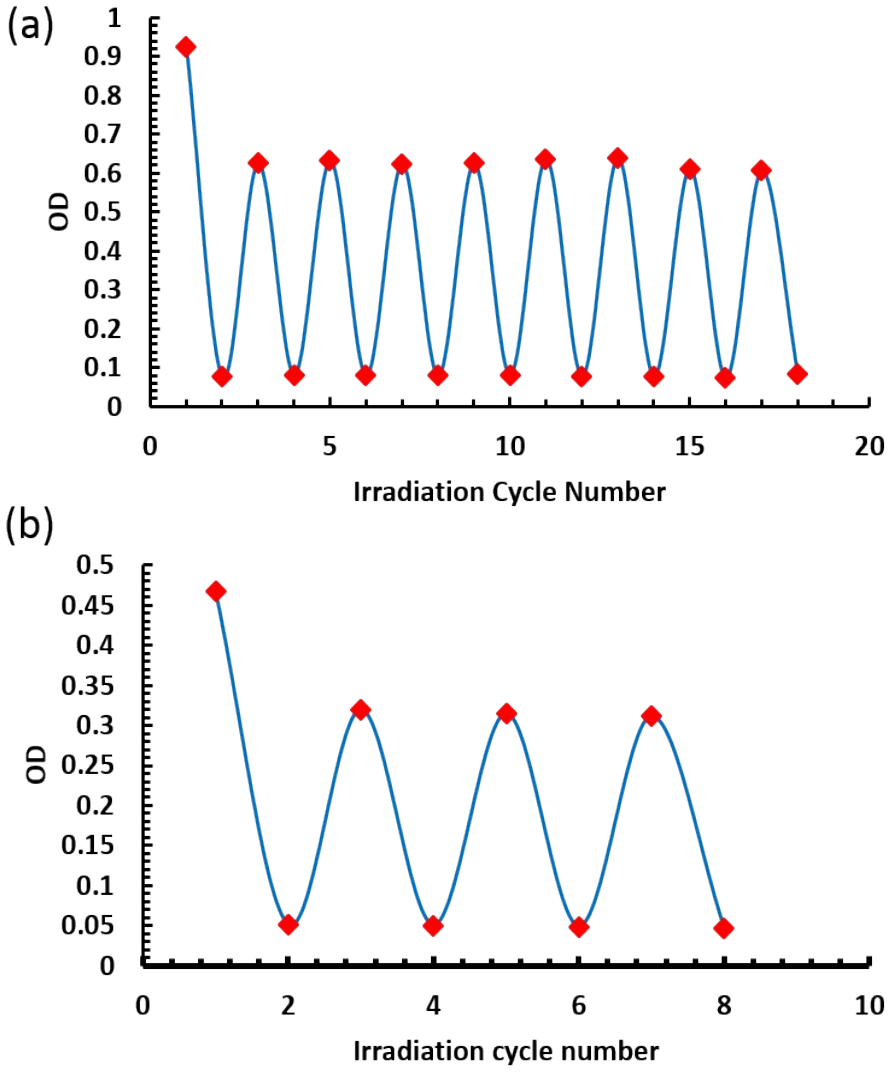


Figure S1. Irradiation cycles of (a) $APEG_{Net2MeI}$ and (b) $APEG_{TMG}$. Odd cycle integers represent primarily *trans* species, while even integer cycles represent predominately *cis* isomers. The OD of each cycle was collected at 351 and 348 nm for $APEG_{NEt2MeI}$ and $APEG_{TMG}$, respectively

Extinction Coefficient Determination

Extinction coefficients were obtained for isobestic points (226 nm) and π→π* transition maxima (348 and 351 nm for $APEG_{TMG}$ and $APEG_{NEt2MeI}$, respectively) of both the *trans* and *cis* isomers of the APEG surfactants using UV-Vis spectroscopy (Figure S2). Stock solutions were prepared by dissolving a known mass of surfactant in ultrapure water (18.2 MΩ*cm). Dilutions were then prepared from the stock solutions and subjected to UV-Vis analysis. Extinction coefficients for the *trans* species were collected prior to irradiation, based on the results of the irradiation cycles. The results of the extinction coefficient analysis are displayed in **Table S1**.

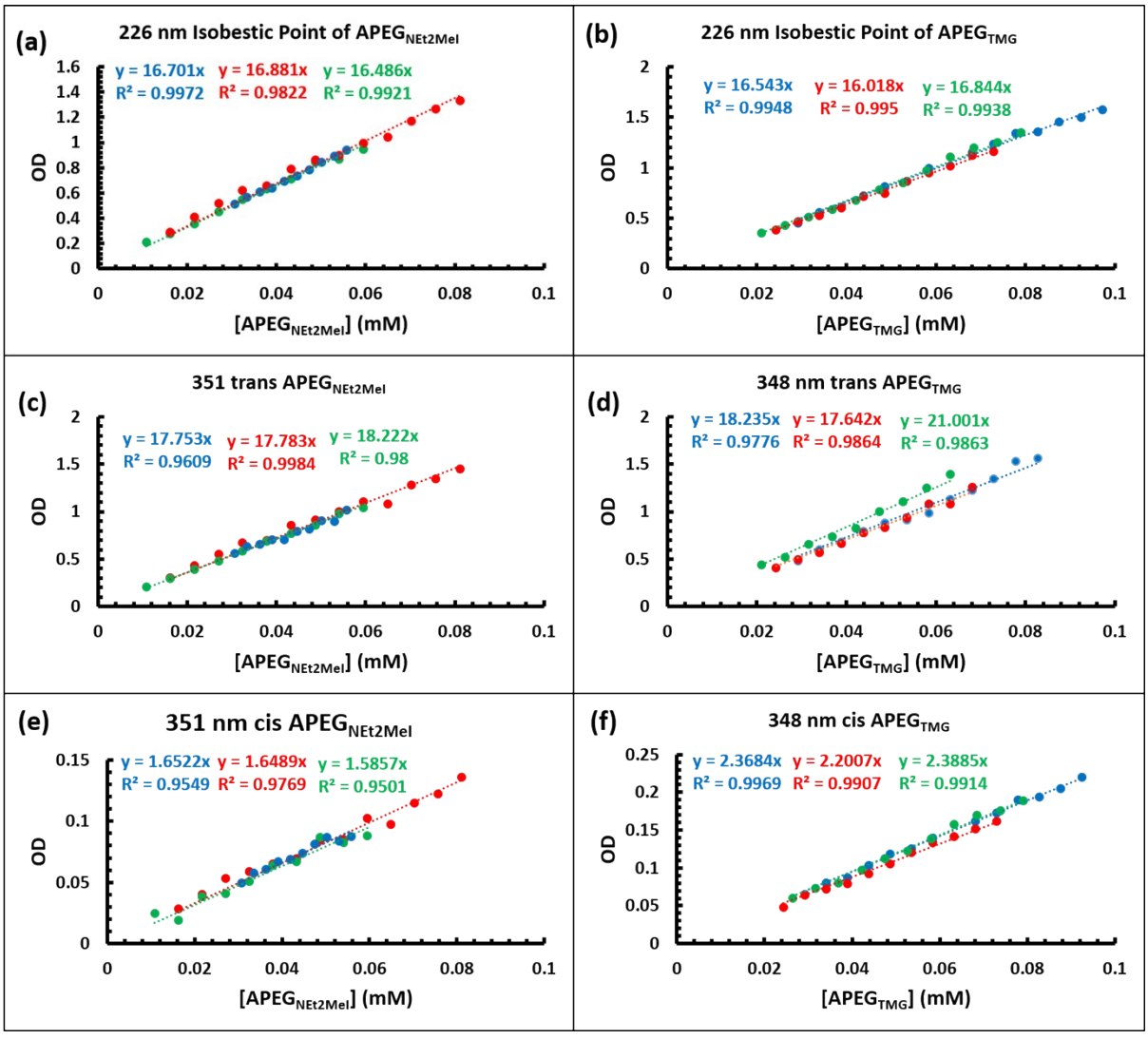


Figure S2. Calibration curves used to determine the extinction coefficients at the isobestic point (226 nm) (a & b), and the maxima at 351 and 348 nm for $APEG_{NEt2MeI}$ (c & e) and $APEG_{TMG}$ (d & f), respectively.

Table S1. Results of UV-Vis extinction coefficient analysis of the APEG surfactants.

Sample and Wavelength measured	**ε ($mM^{-1}*cm^{-1}$)**
$APEG_{TMG}$ (226 nm)	16.5 ± 0.4
Trans-$APEG_{TMG}$ (348 nm)	19.0 ± 1.8
Cis-$APEG_{TMG}$ (348 nm)	2.32 ± 0.10
$APEG_{NEt2MeI}$ (226 nm)	16.69 ± 0.20
Trans-$APEG_{NEt2MeI}$ (351 nm)	17.9 ± 0.3
Cis-$APEG_{NEt2MeI}$ (351 nm)	1.63 ± 0.04

CMC Determination by NMR

Sample Preparation Method: A stock solution of $APEG_{TMG}$ (45.1 mg, 0.09 mmol) in D_2O (5 ml) was prepared and sonicated for 5 minutes. After allowing the stock solution to stand undisturbed for 48 hours, samples of 0.18 – 18.6 mM were prepared by serial dilution. The residual solvent signal was referenced to 4.79 ppm for each sample.

Trans conformer: Samples were exposed to visible light for a 1 hour prior to collection of the ^1H NMR spectra.

Cis conformer: Samples were irradiated with 365 nm light for 30-60 minutes and kept in the dark directly prior to collection of the ^1H NMR spectra.

CMC Determination: Signals structurally assigned via 2D COSY NMR that displayed minimal overlap were selected for each sample spectrum. The chemical shift (δ, ppm) of each signal was plotted as a function of inverse concentration (1/C mM^{-1}).

Trans conformer: The CMC was calculated as the average value of the inverse of the horizontal intercept between two linear functions for each signal.

Cis conformer: The horizontal intercept between a cubic function and a linear function was approximated via Newton's method for each signal. The CMC was calculated the same way as the *trans* conformer.

FTIR Analysis

FTIR Setup. APEG samples were analyzed using a modified Nicolet 6700 FTIR spectrometer (Thermo Scientific, Waltham, MA). The sample cell used two calcium fluoride windows separated by a 50 µm

Teflon PTFE spacer allowing for collection of the sample and blank. Data collection was performed using OMNIC software. 32 scans were performed for each individual single beam spectrum obtained. A minimum of 64 individual single beam spectra were averaged to give the final single beam spectra of the sample and the blank. A sample's single beam spectrum was then reprocessed using the corresponding background single beam spectrum in order to give an absorbance spectrum. Absorbance spectra were baseline corrected using the OMNIC software. FTIR analysis was performed on both APEG surfactants in ultrapure water (18.2 MΩ*cm) (data not shown) and deuterium oxide, D_2O (Figure S3). Samples containing D_2O were first subjected to deuterium exchange by dissolving a known mass of surfactant in D_2O and then lyophilizing. Spectra for *trans* isomers were collected prior to irradiation. For the *cis* spectra, an irradiation source (365 nm) was incorporated into the FTIR setup so that the sample was constantly irradiated during data collection. For IR peak analysis, the solvent utilized (either D_2O or H_2O) was used as the blank.

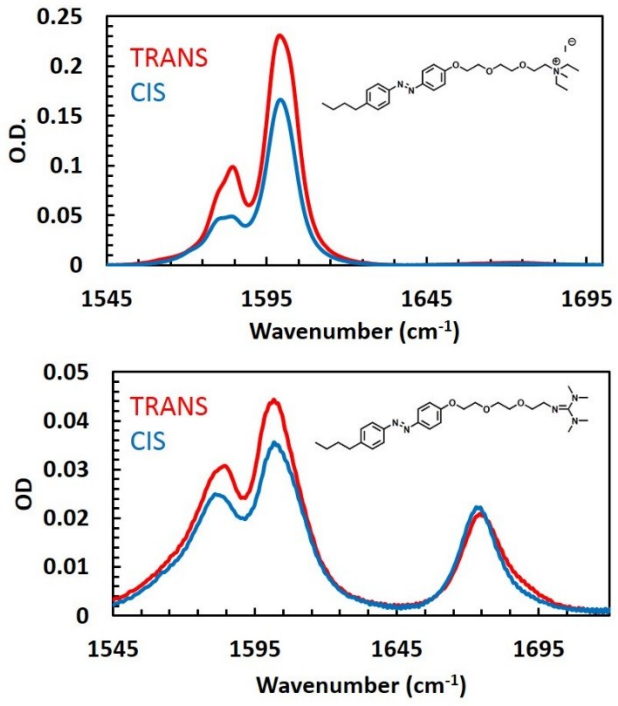


Figure S3. FTIR spectra of the amide I and II regions of $APEG_{Net2MeI}$ (Top) and $APEG_{TMG}$ (Bottom) in D_2O. Concentrations used were well above the CMC of each surfactant. The concentration of $APEG_{Net2MeI}$ and $APEG_{TMG}$ were 134 and 7 mM, respectively.

IR peak analysis of APEG Surfactants. Computational IR peak analysis was performed for the monomeric units of each isomer of $APEG_{TMG}$ and $APEG_{NEt2MeI}$ via standard geometrical optimization and frequency calculation in the gas pas phase via Gauss View. Calculated spectra were then compared to experimentally obtained FTIR spectra (Figure S3). Differences between computational and experimental results are (1) differences in phase and (2) concentration. Computations were ran in the gas phase while experimental IR spectra were collected in the presence of either H_2O or D_2O. The vibrational modes analyzed were for the monomers of each isomer of each surfactant. Experimentally, the vibrational extinction coefficients were found to be moderately weak for transitions occurring in both the amide I and II regions for both surfactants. Detection of the vibrational transitions of the APEG surfactants within the amide I and II region required concentrations exceeding the CMCs of the *cis* isomers of both surfactants. This then indicates that at appropriate concentrations, $APEG_{TMG}$ and $APEG_{NEt2MeI}$ should exhibit minimal interference in peptide analysis for this region in the IR (i.e. the signals of the transitions are weak enough that they can't be separated from the baseline at appropriate concentrations).

Analysis of the amide II region (1540-1600 cm^{-1}) revealed that $APEG_{TMG}$ and $APEG_{NEt2MeI}$ share a common set of peaks. These peaks are likely due to a combination of the azobenzene core structure (ring modes) as well as some coupled C-O stretching associated with the ethers of the glycol chain. Furthermore, both surfactants exhibit a dampening of the intensities of the amide II transitions upon

tans-cis isomerization. This may be attributed to a decrease in coupling between the intramolecular benzenes due to the loss of planarity upon *trans-cis* isomerization.

Analysis of the amide I region (1600-1700 cm^{-1}) revealed no peaks for $APEG_{NEt2MeI}$. $APEG_{TMG}$ however, exhibited a somewhat broad peak centered at 1675 cm^{-1} (Figure S3). This peak is attributed to the tetramethylguanidine head group of $APEG_{TMG}$. Minimal peak variability was observed between the different isomers. Again, the concentration of $APEG_{TMG}$ needed to observe this transition was far greater that the concentrations used for peptide analysis.

Synthesis of MP1. Standard protocol for solid phase peptide synthesis and purification was followed as outlined in the supporting information of a previous publication.[5] MP1 was subjected to HCl exchange post purification so as to remove any 2,2,2-Trifluoroacetic acid (TFA) which would otherwise absorb at 1673 cm^{-1}. Removal of TFA was critical for FTIR analysis. The HCl exchange procedure involved dissolving MP1 in 1% (v/v) HCl and then sonicating for 30-45 minutes. The sample was then cooled and lyophilized. This process was repeated twice in order to minimize the TFA bound to MP1.

FTIR analysis of MP1 in the presence of $APEG_{TMG}$. Deuterium exchange was performed on $APEG_{TMG}$ and MP1 as previously mentioned. An initial IR spectrum of MP1 (1.9 mM) in D_2O was obtained (Figure 3 blue) where D_2O was used as the blank. The MP1/D_2O solution was then used to dissolve $APEG_{TMG}$ (1.74 mM). An IR spectrum was obtained for this sample (Figure 3 red) where D_2O was again used as the blank. Using the blue and red spectra of Figure 3, the green difference spectrum of Figure 3 was generated.

In order to follow the structural changes induced upon micelle dissociation, an IR spectrum was then obtained post irradiation with 365 nm light (black dashed spectrum of Figure S4) was then used with the aforementioned MP1 *trans* $APEG_{TMG}$ spectrum (red spectrum of Figure S4) to generate the orange difference spectrum of Figure S4. Again, maxima correspond to features that became more dominate as a result of the associated change, while minima pertain to structures loss. Immediately, we note a loss of α-helical population as apparent by the minimum at 1651 cm^{-1}. Furthermore, potential maxima exist at 1610 cm^{-1}, from 1617 – 1635 cm^{-1}, at 1666 cm^{-1}, and at 1678 - 1700 cm^{-1}. Using the same assignment techniques as with the green difference spectrum, these maxima suggest that after micelle dissociation, an extent of the original structures (predominately random coil and β-content) were regained by MP1. The helical content originally induced by the presence of the micelles became diminished upon micelle dissociation, as noted by the minimum at 1651 cm^{-1} of the orange difference spectrum of Figure S4. Peak assignment of frequencies below 1603 cm^{-1} were disregarded as the contribution from the APEG vibrational modes interfered substantially here.

Evaluation of Change in pH upon Photoisomerization

To evaluate possible pH change in the sample upon photoisomerization, the pH was measured both prior to and after ~15 minutes of irradiation with 365 nm for the following samples:

(1) the individual $APEG_{TMG}$ surfactants in the absence of MP1
(2) MP1 in the absence of any $APEG_{TMG}$
(3) MP1 in the presence of $APEG_{TMG}$

[5] M. G. Roberson, D. K. Smith, S. M. White, I. S. Wallace and M. J. Tucker, *Biophysical Journal*, 2019, **116**, 1064-1074.

Fifteen minutes of irradiation was given to ensure full *trans-cis* isomerization of the $APEG_{TMG}$ micelles. Samples were also vortexed while being irradiated to further ensure complete isomerization. The results of the pH analysis are depicted in Table S2 below. Briefly, upon comparison of the pH before and after 365 nm irradiation, no significant change in pH was observed for any of the samples. The very slight changes in pH (0.1 - 0.2) upon photoisomerization of the APEG surfactants would not be significant enough to induce structural changes of the peptide (Table S2).

TABLE S2. Results from pH analysis due to photoisomerization of MP1 and APEG micelles.

	pH (no light)	pH (365nm)
$APEG_{TMG}$	7.82	8.02
MP1 without $APEG_{TMG}$	6.30	6.31
$APEG_{TMG}$ with MP1	6.94	7.19

FTIR analysis of MP1 with minimized $APEG_{TMG}$ transition contributions: In order to remove artifacts associated from the vibrational transitions of $APEG_{TMG}$, an altered experimental approach was performed. $APEG_{TMG}$ was weighed and dissolved in D_2O so as to give a concentration that was slightly greater than CMC of the *trans* species (1.45 ± 0.08 mM) but less than the CMC of the *cis* isomer (1.829 ± 0.019 mM). MP1 was then dissolved with the $APEG_{TMG}$ solution to give a peptide concentration of 4.8 mM. Using the CaF_2 cell and aforementioned protocol, FTIR analysis was performed using the pure $APEG_{TMG}$ solution as the blank.

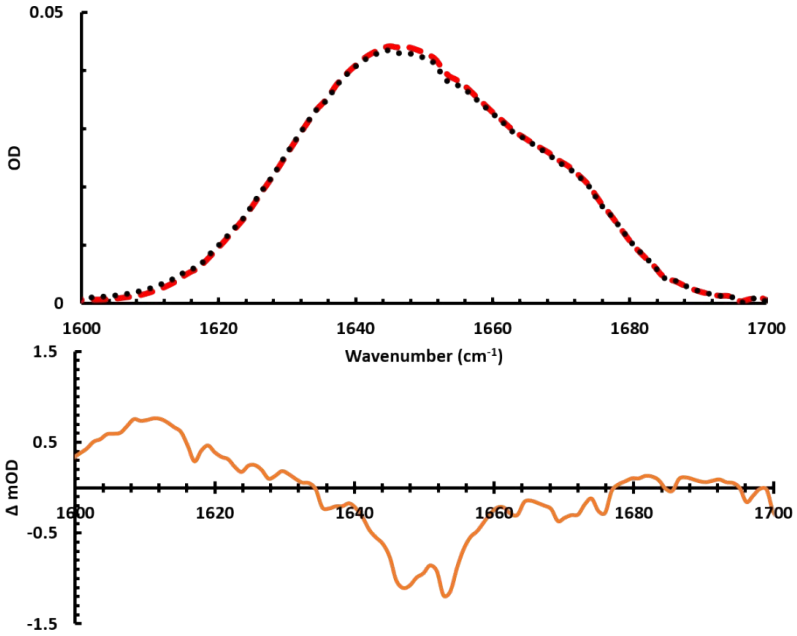


Figure S4. Linear IR spectra were obtained for MP1 in *trans* $APEG_{TMG}$ micelles (red) and in *cis* $APEG_{TMG}$ (black). The orange difference spectrum was then constructed by subtracting the red from the black spectrum. The difference spectrum depicts secondary structural changes of MP1 induced to the dissociation of $APEG_{TMG}$ micelles.

Monitoring MP1's ability to bind APEG$_{TMG}$ via Fluorescence

Environmental Sensitivity of Sulforhodomine-101 (Texas Red). Fluorescence measurements were proposed in order to assess binding of MP1 in the presence of APEG$_{TMG}$ micelles. Unfortunately, due to the high extinction coefficients of APEG and the large spectral region the electronic transitions of APEG encompass, the use naturally occurring fluorophores (tryptophan, tyrosine, or phenylalanine) for binding assessment was determined to be impractical. Instead, sulforhodamine-101 (Texas Red) (AnaSpec Inc., Fremont, CA) was covalently attached to a mutation of MP1 where a cysteine was added to the N-terminus (CMP1). Fluorescence spectra were obtained at room temperature using a Fluor-max 3 fluorimeter (Horiba Scientific, Kyoto, Japan).

To assess the environmental sensitivity of Texas Red, fluorescence measurements were performed on the free dye (no peptide) in various solvent environments, varying the dielectric. A stock solution of Texas Red in DMSO was prepared. From the stock solution, samples were further diluted with an equal volume of either H_2O, DMSO, or TFE in order to assure consistent concentration of the free dye between samples. Absorbance spectra were obtained (Figure S5) for each sample. Emission spectra were obtained by exciting at 587 nm. Emission and excitation slit widths were set to 2 nm. Emission spectra were then normalized to the corresponding absorbance of the sample at 587 nm (Figure S5).

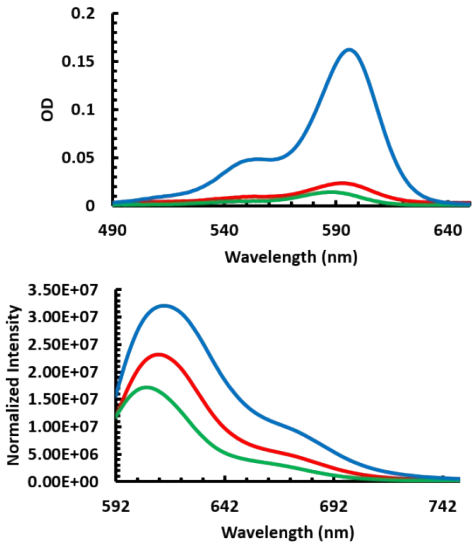


Figure S5. Absorbance (Top) and normalized emission spectra (Bottom) of the free sulforhodamine-101 (Texas Red) dye in the presence of DMSO/H_2O (blue spectra), DMSO (red spectra), and DMSO/TFE (green spectra). The concentration of Texas Red was consistent between all solvent mixtures. Emission spectra were normalized to the OD of each mixture at the excitation wavelength (587 nm).

As a control experiment, fluorescence measurements of the free Texas red dye were obtained in bulk water and in the presence of APEGTMG (Figure S6). No decrease in the fluorescence intensity and no blue shift were observed for the dye chromophore in the presence of the APEGTMG micelles. These results suggest that the presence of APEG molecules below the CMC is not significant enough to cause any significant spectral changes from bulk water.

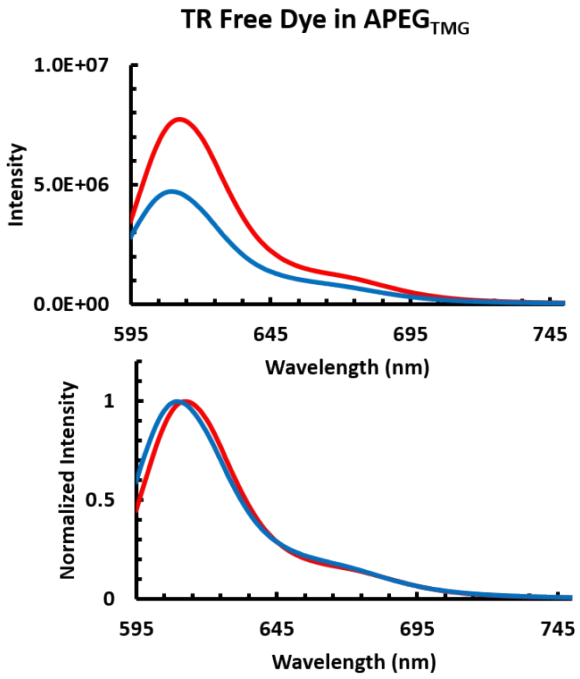


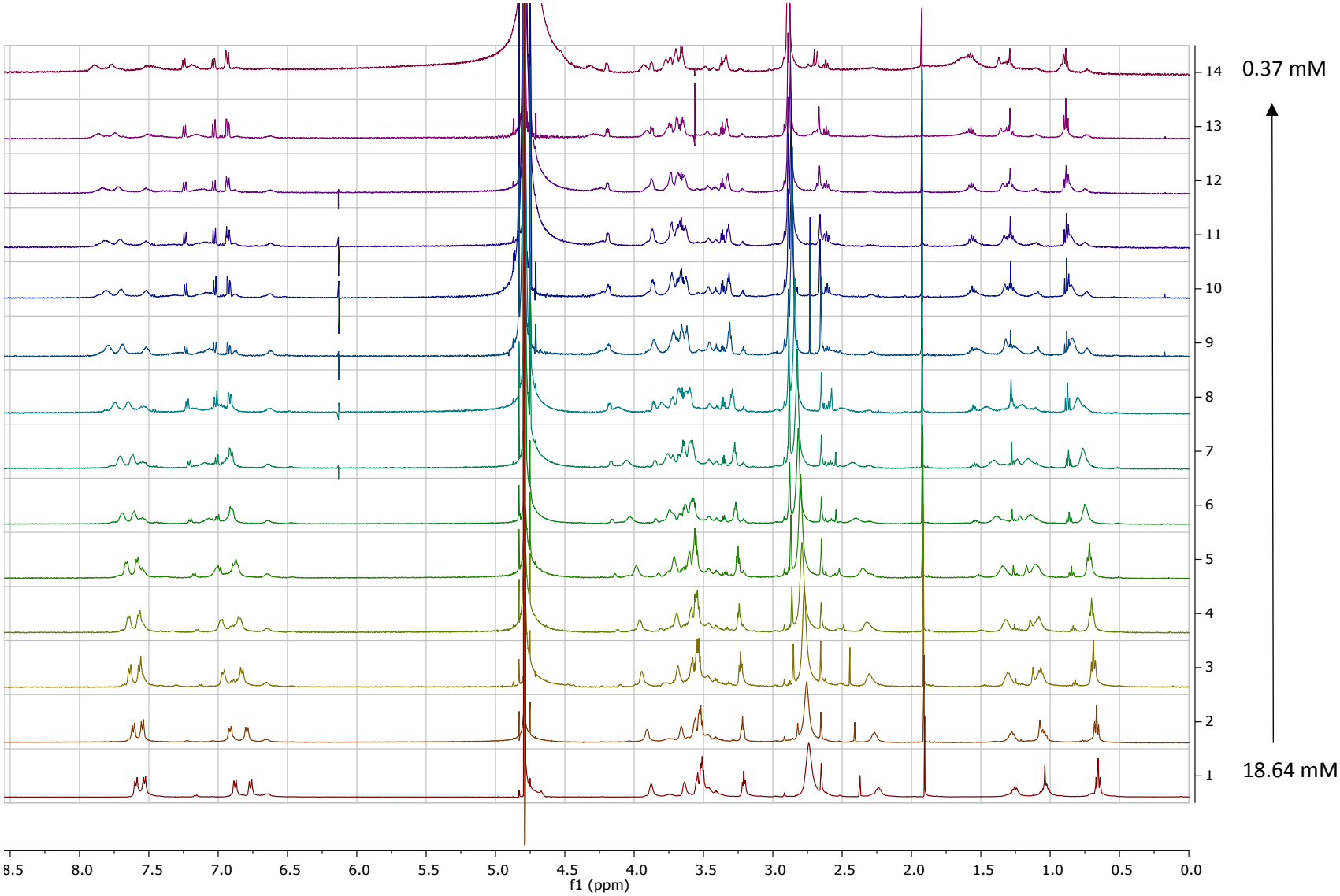
Figure S6. Fluorescence (top) and normalized fluorescence spectra (bottom) of Texas red free dye in the presence of water (blue spectra) and APEG$_{TMG}$ (red spectra). Emission spectra were normalized to the maximum intensity of the emission band.

Table S3. Maximum absorbance and emission wavelengths dependence of free sulforhodamine-101 based on solvent environment. Dielectrics[6] of the pure solvents are included for reference.

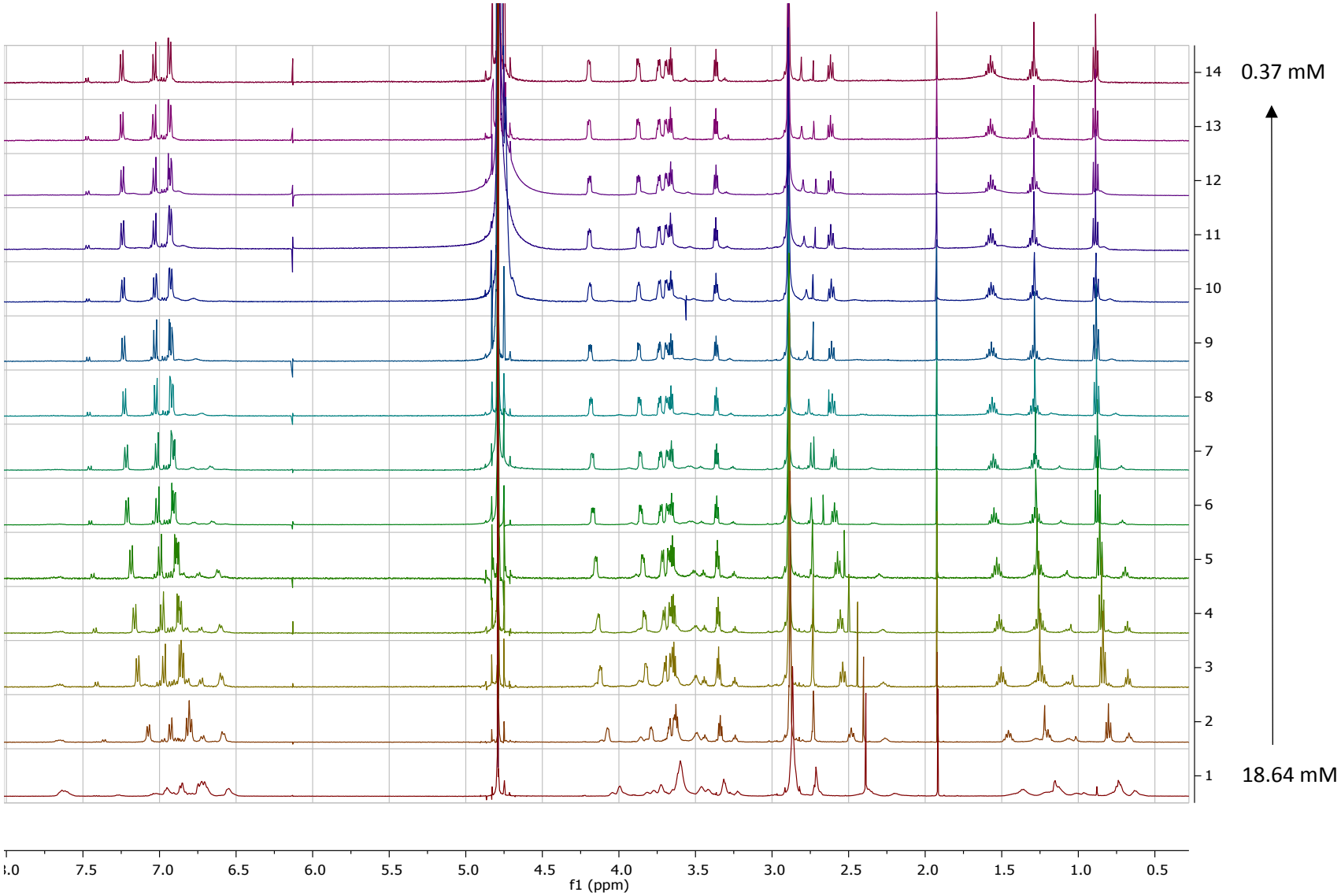
	DMSO/TFE	DMSO	DMSO/H2O
$\lambda_{max\ Absorbance}$ (nm)	593	588	596
$\lambda_{max\ Emission}$ (nm)	606	612	614
Dielectric of Pure Solvent[6]	8.55	46.7	80.1

[6] https://depts.washington.edu/eooptic/linkfiles/dielectric_chart%5B1%5D.pdf

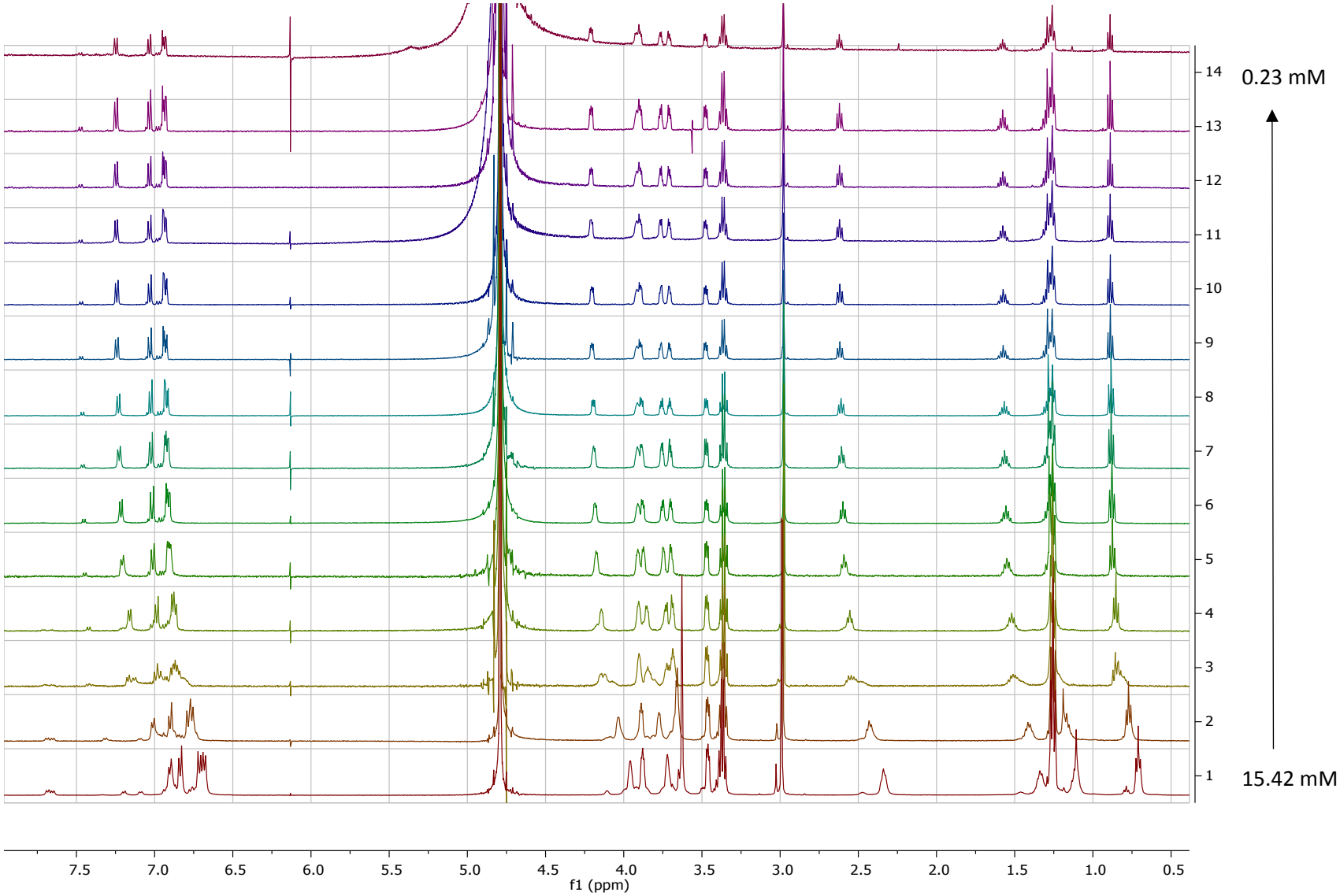
Stacked spectra of *trans*-APEG$_{TMG}$ for CMC determination



Stacked spectra of *cis*-APEG$_{TMG}$ for CMC determination

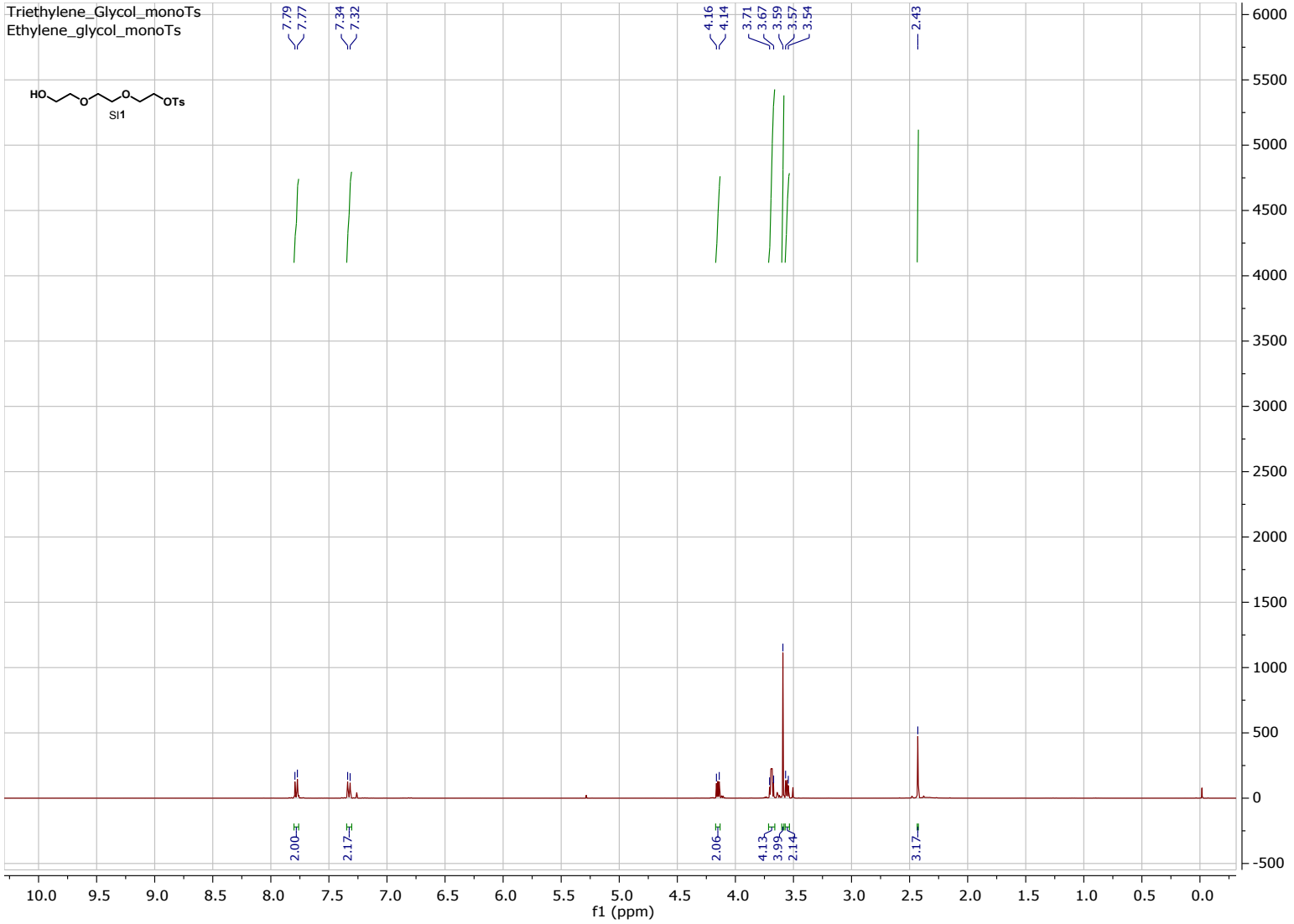


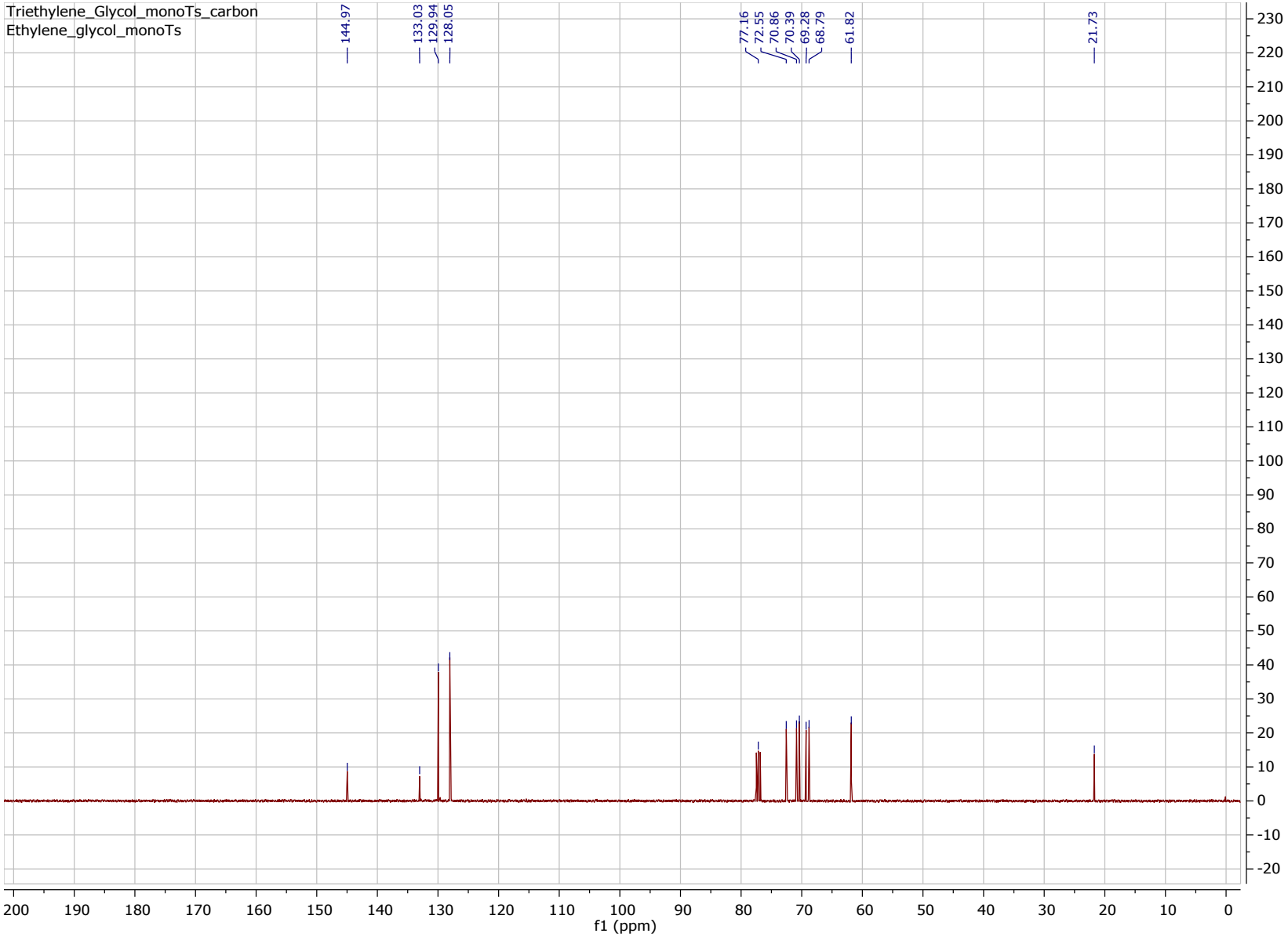
Stacked spectra of *cis*-APEG$_{NEt2Mel}$ for CMC determination



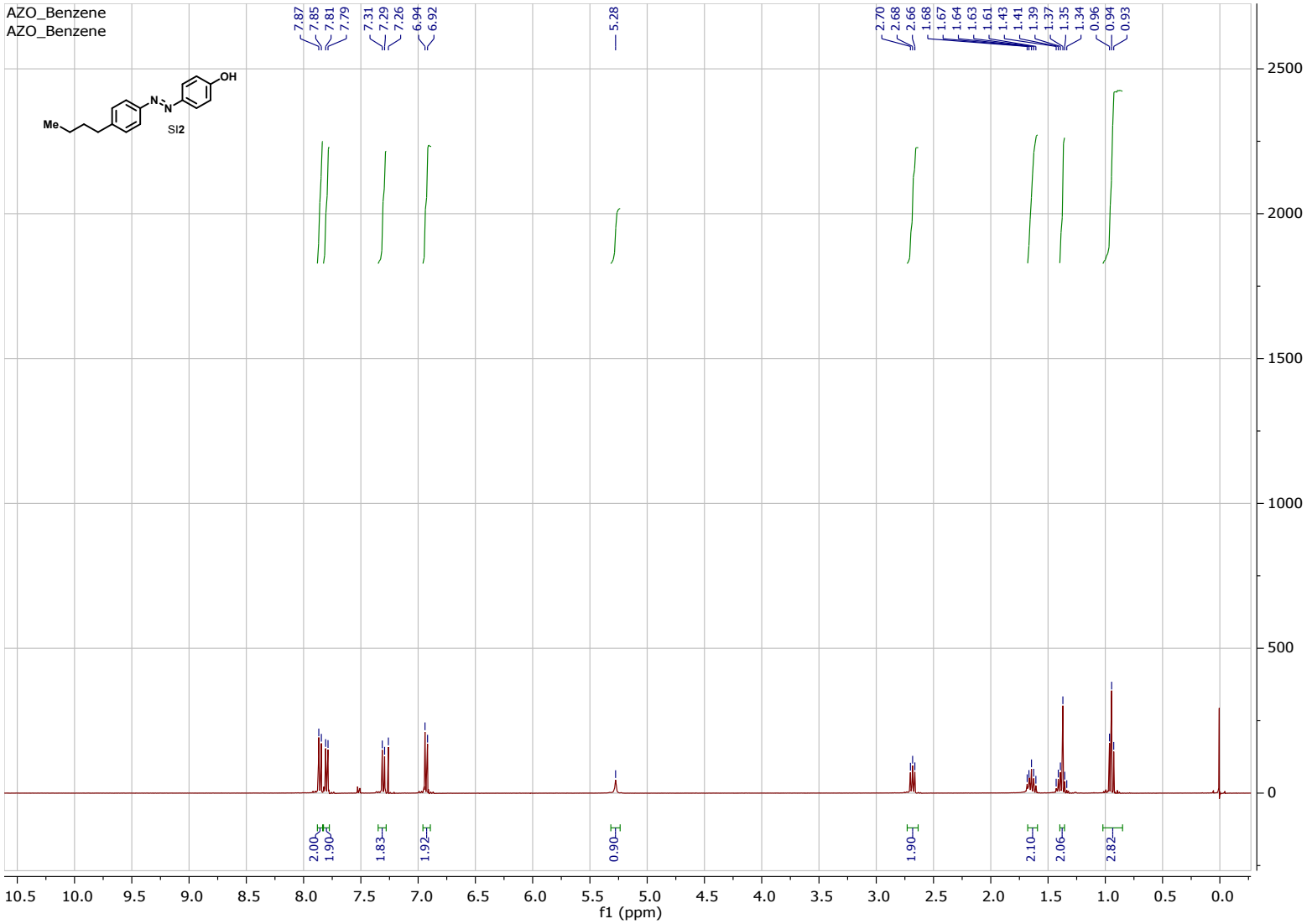
NMR Spectra

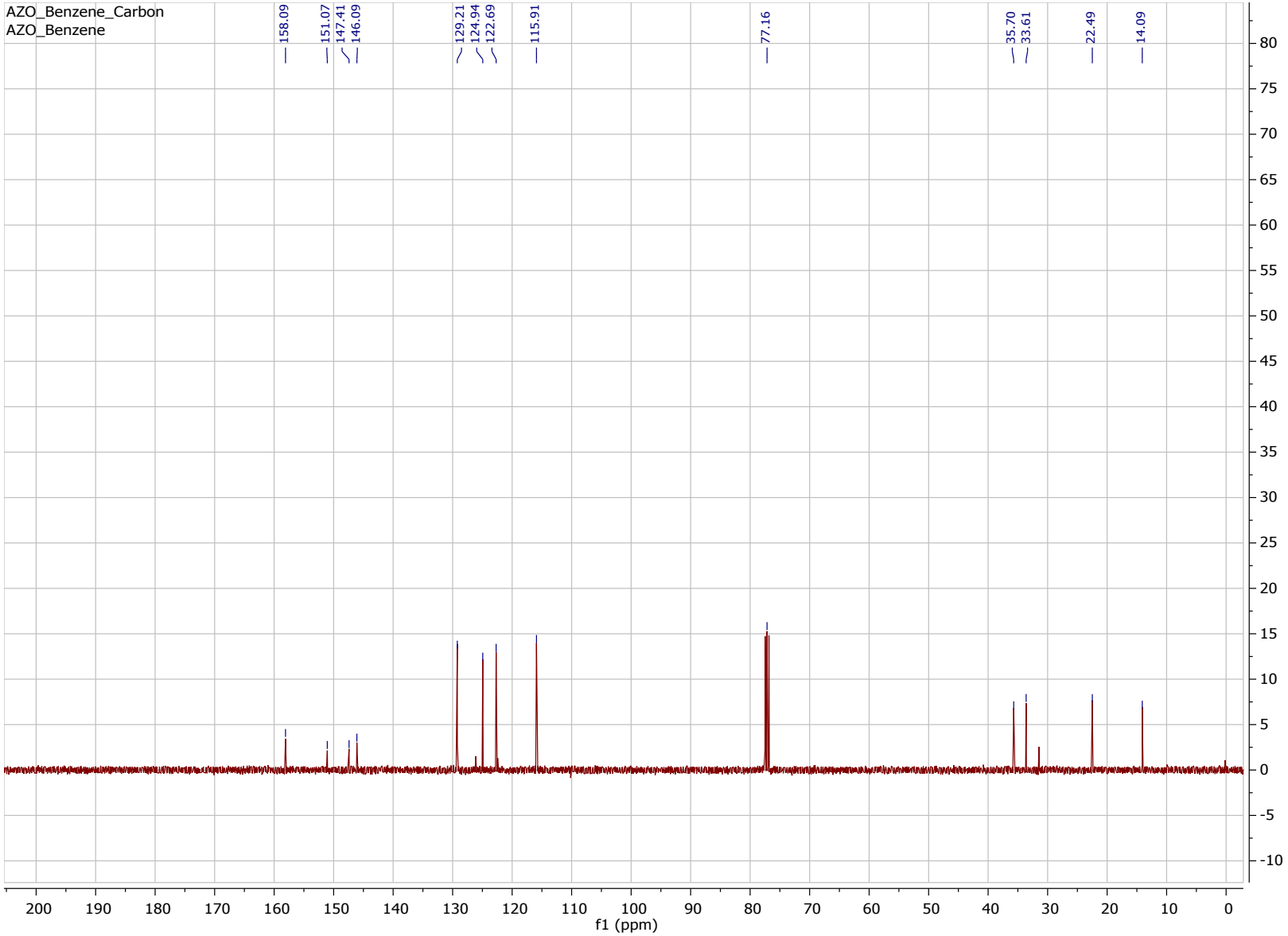
2-[2-(2-Hydroxyethoxy)ethoxy]ethyl 4-methylbenzenesulfonate (**SI1**)



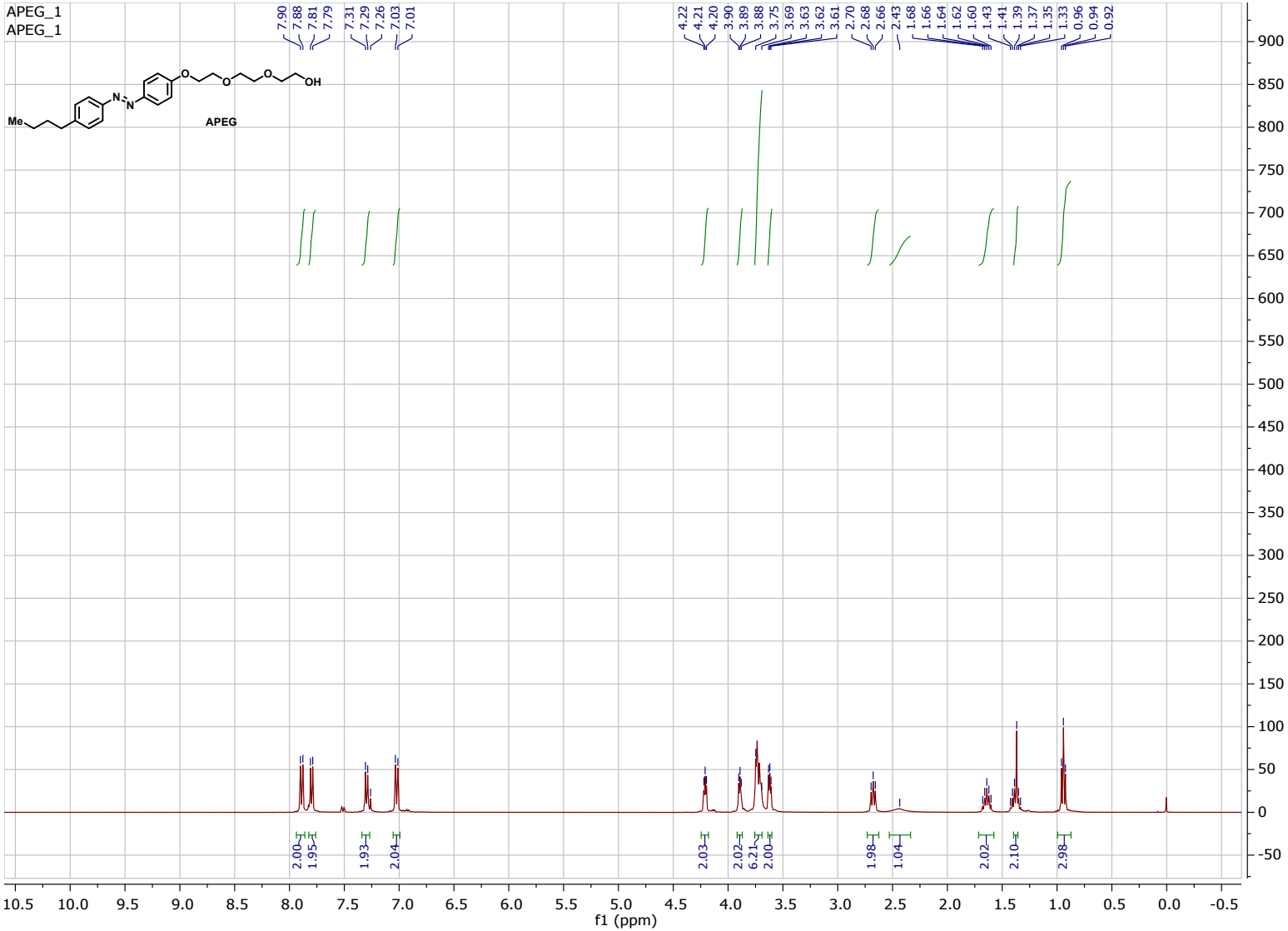

Triethylene_Glycol_monoTs_carbon
Ethylene_glycol_monoTs
144.97
133.03
129.94
128.05
77.16
72.55
70.86
70.39
69.28
68.79
61.82
21.73
f1 (ppm)

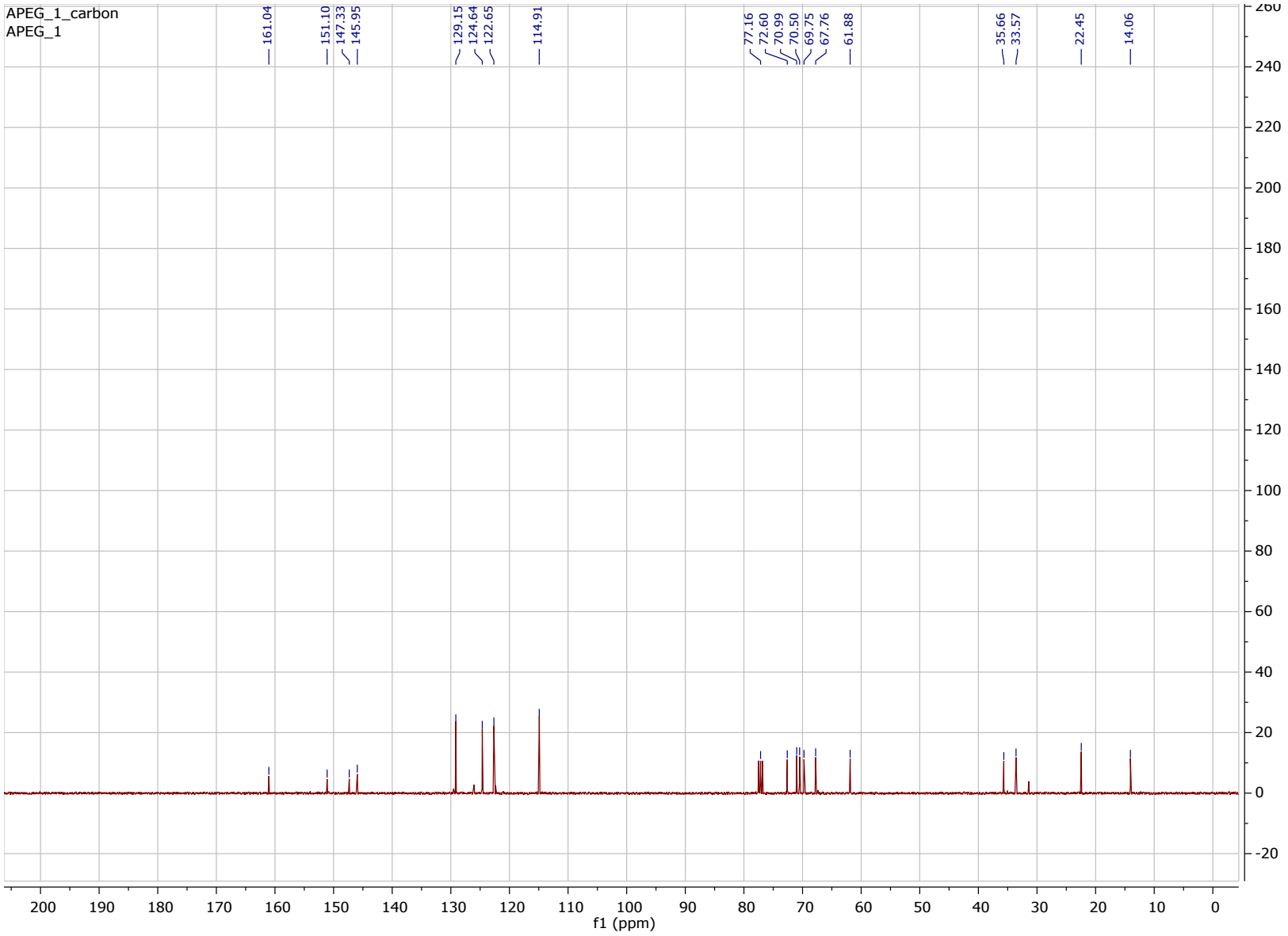
4-[(4-Butylphenyl)azo]-phenol (**SI2**)




AZO_Benzene_Carbon
AZO_Benzene
158.09
151.07
147.41
146.09
129.21
124.94
122.69
115.91
77.16
35.70
33.61
22.49
14.09
f1 (ppm)

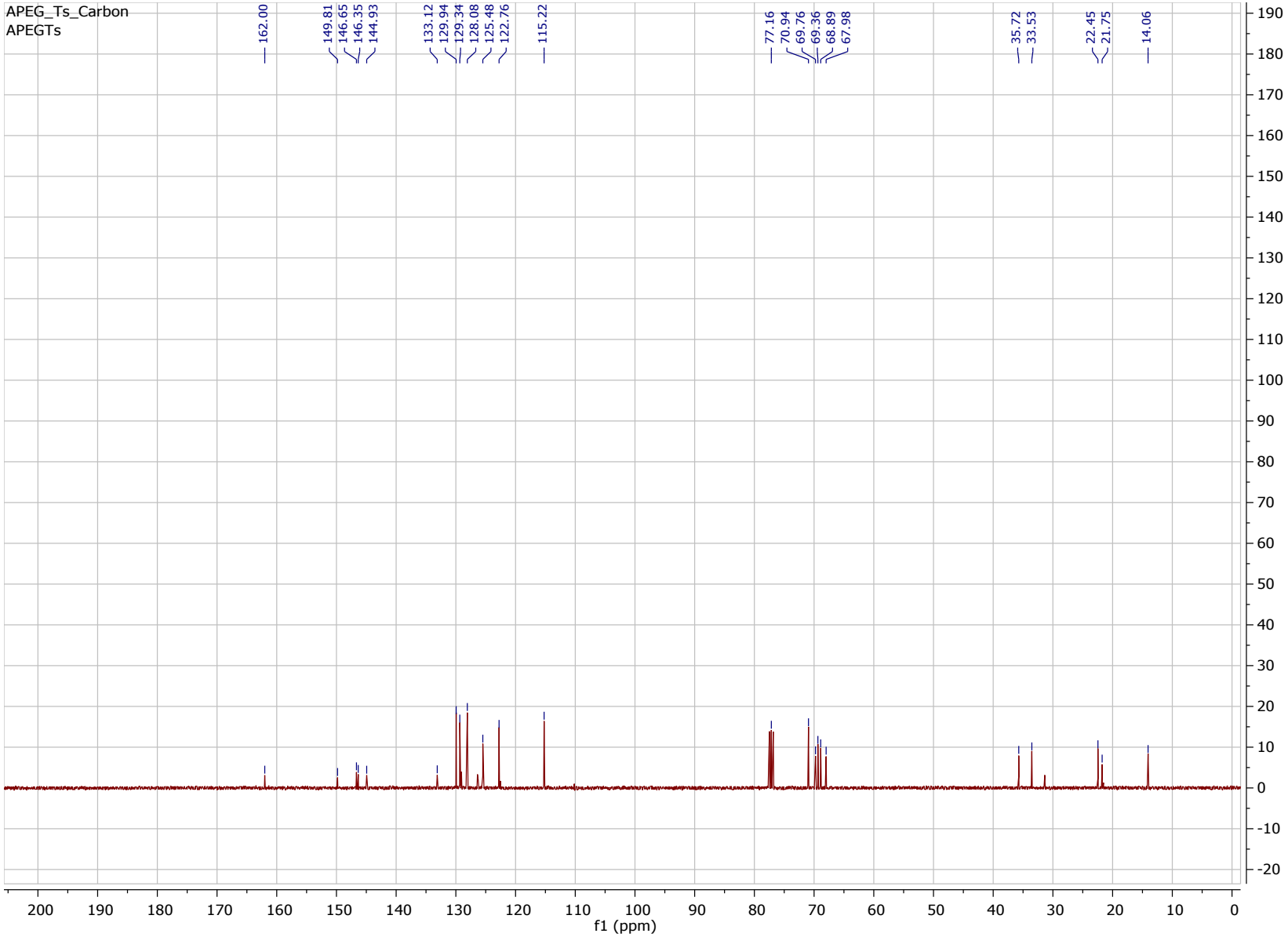
2-[2-[2-[4-[(1*E*)-2-(4-Butylphenyl)diazenyl]phenoxy]ethoxy]ethoxy]-ethanol (APEG)



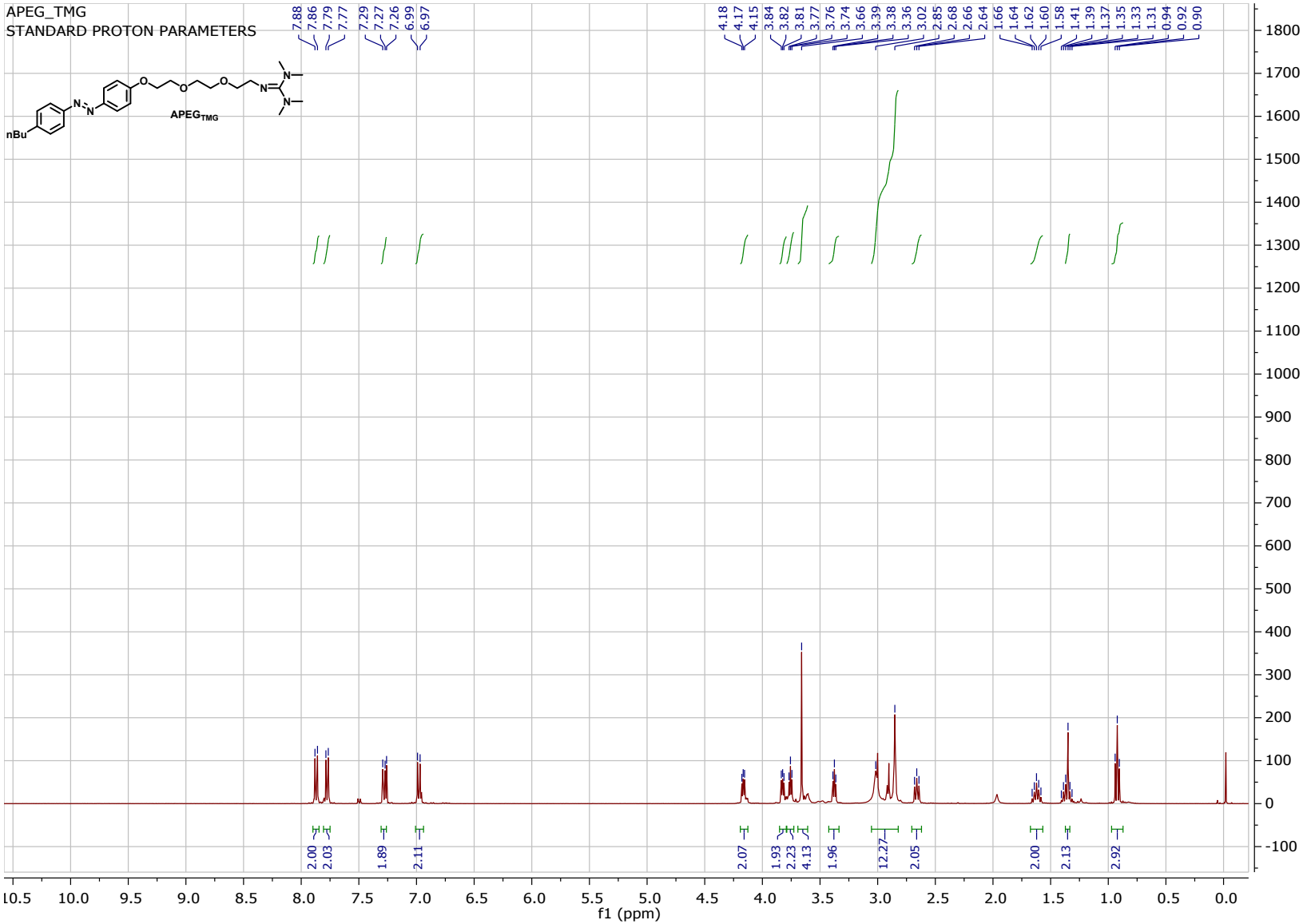

APEG_1_carbon
APEG_1
161.04
151.10
147.33
145.95
129.15
124.64
122.65
114.91
77.16
72.60
70.99
70.50
69.75
67.76
61.88
35.66
33.57
22.45
14.06
f1 (ppm)

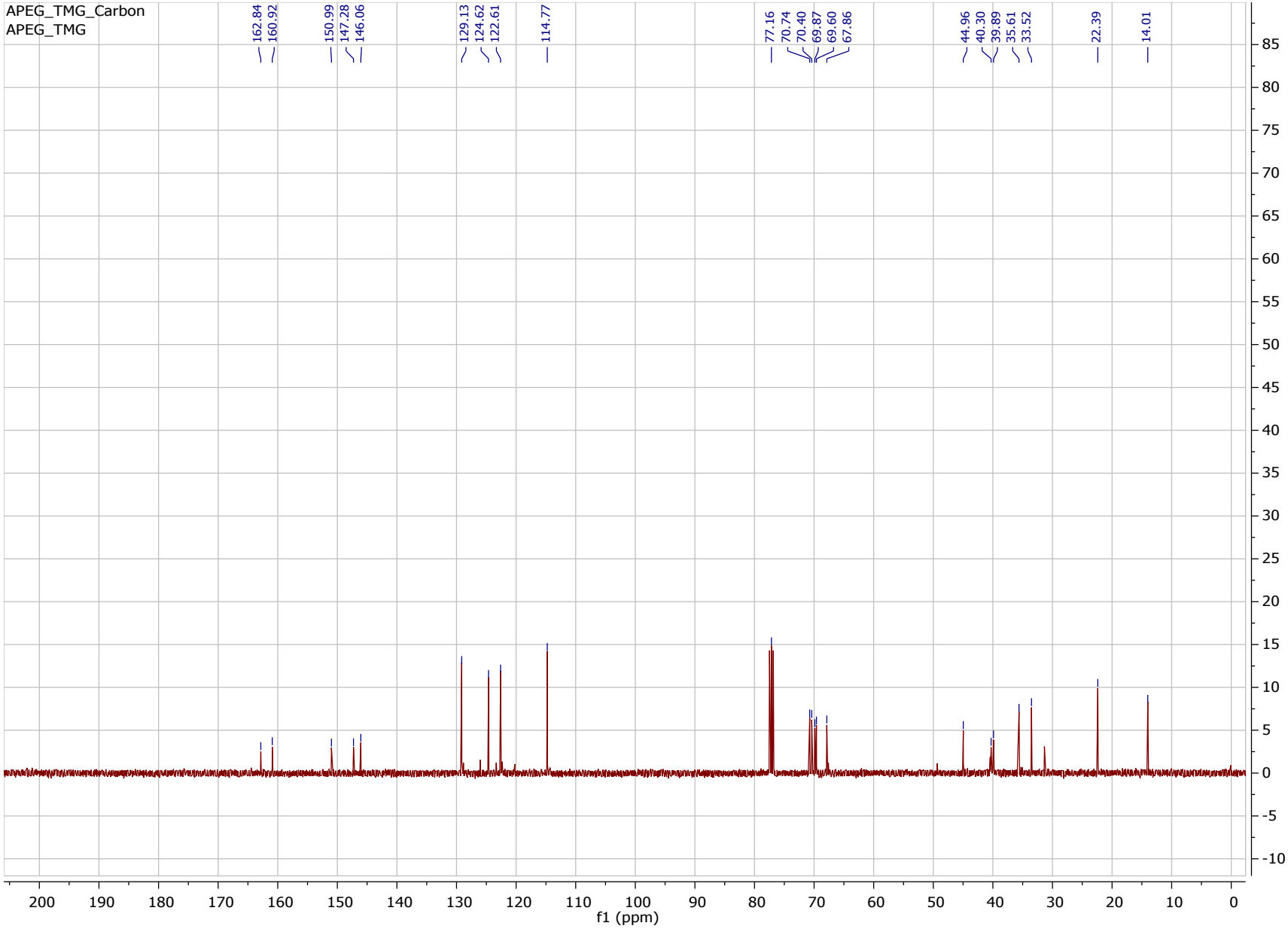
APEG$_{Ts}$ (**SI3**)


APEG_Ts
STANDARD PROTON PARAMETERS
nBu
OTs
APEG$_{Ts}$ (SI3)
f1 (ppm)

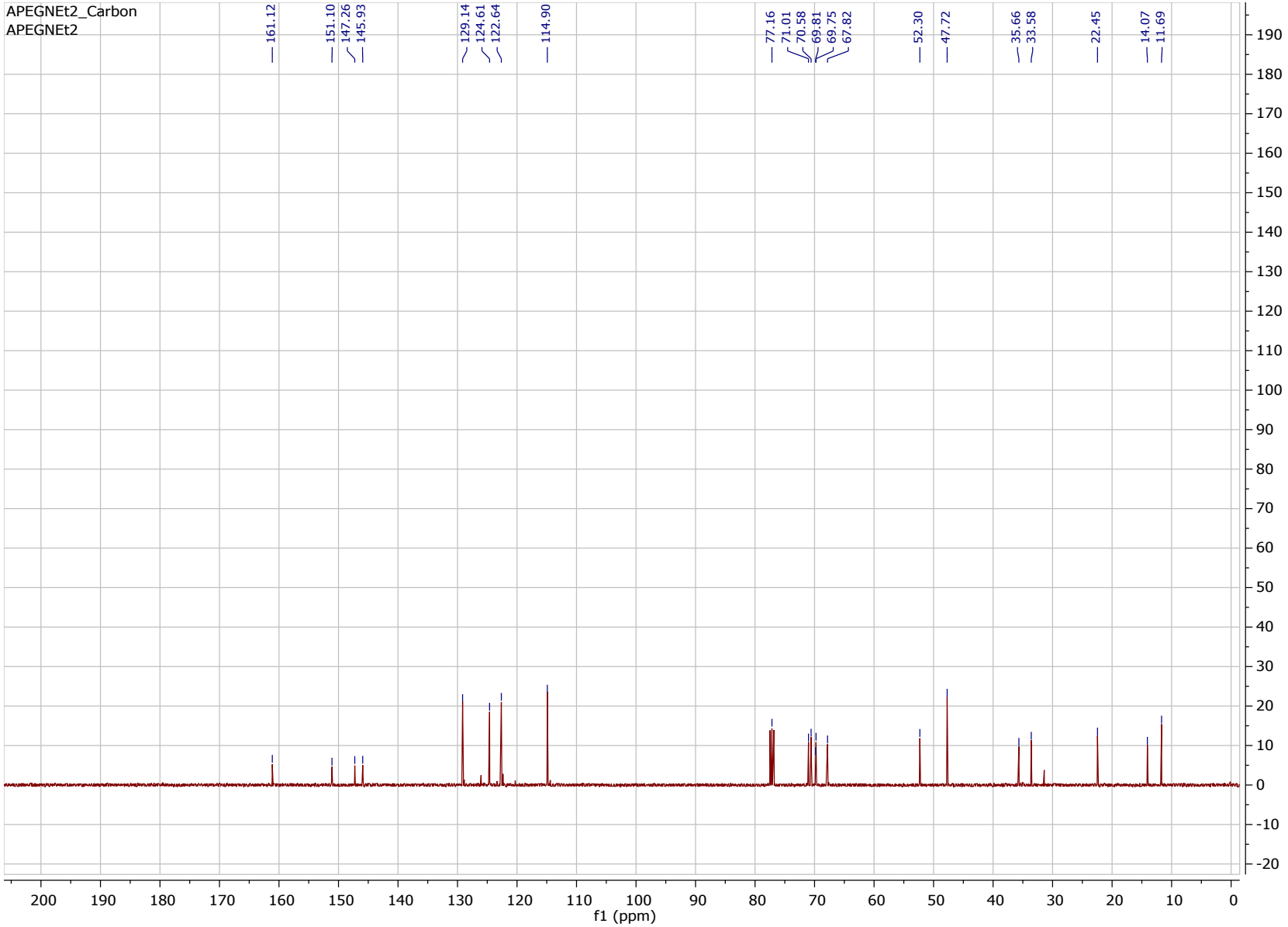

APEG_Ts_Carbon
APEGTs
162.00
149.81
146.65
146.35
144.93
133.12
129.94
129.34
128.08
125.48
122.76
115.22
77.16
70.94
69.76
69.36
68.89
67.98
35.72
33.53
22.45
21.75
14.06
f1 (ppm)

APEG$_{TMG}$

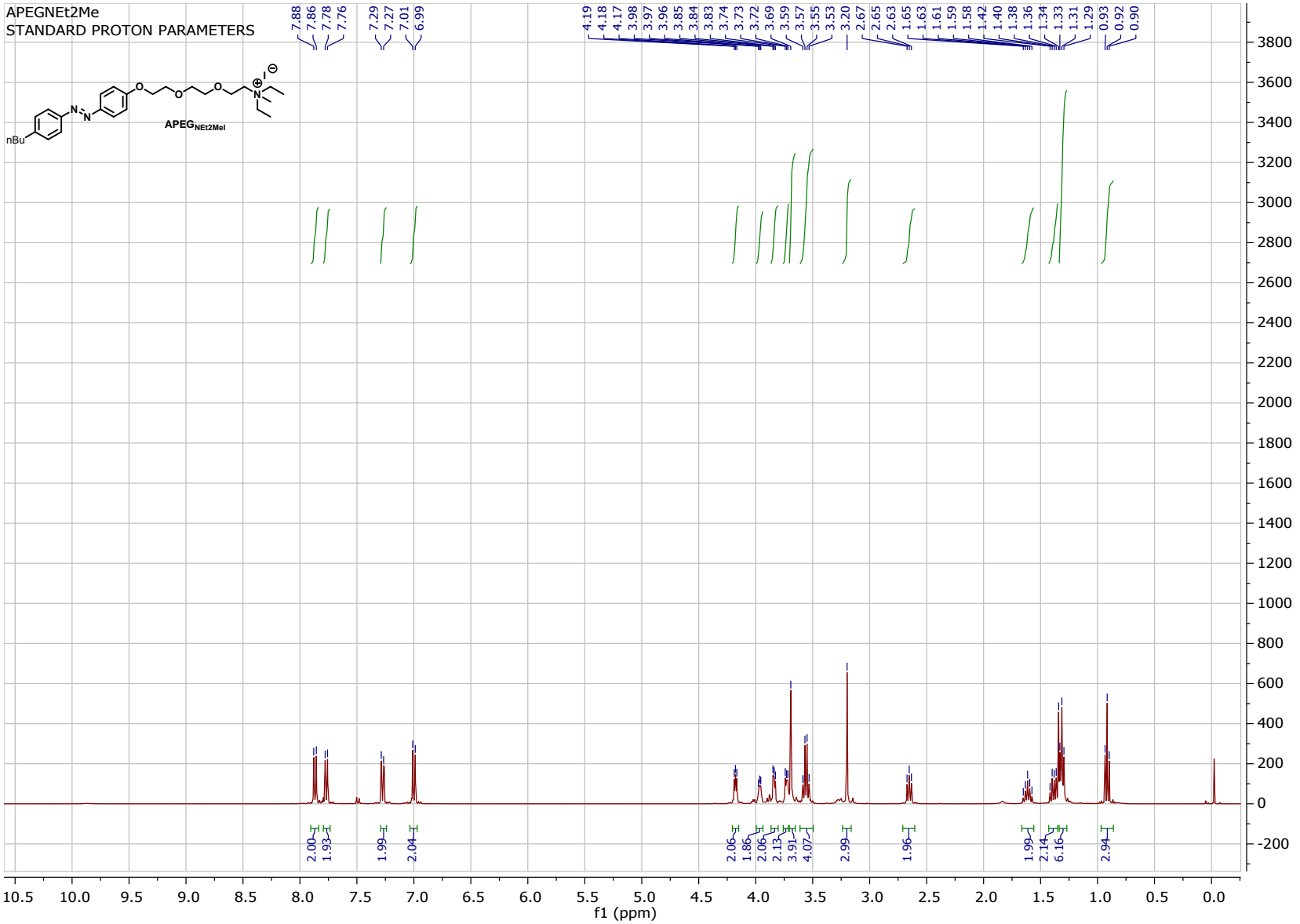


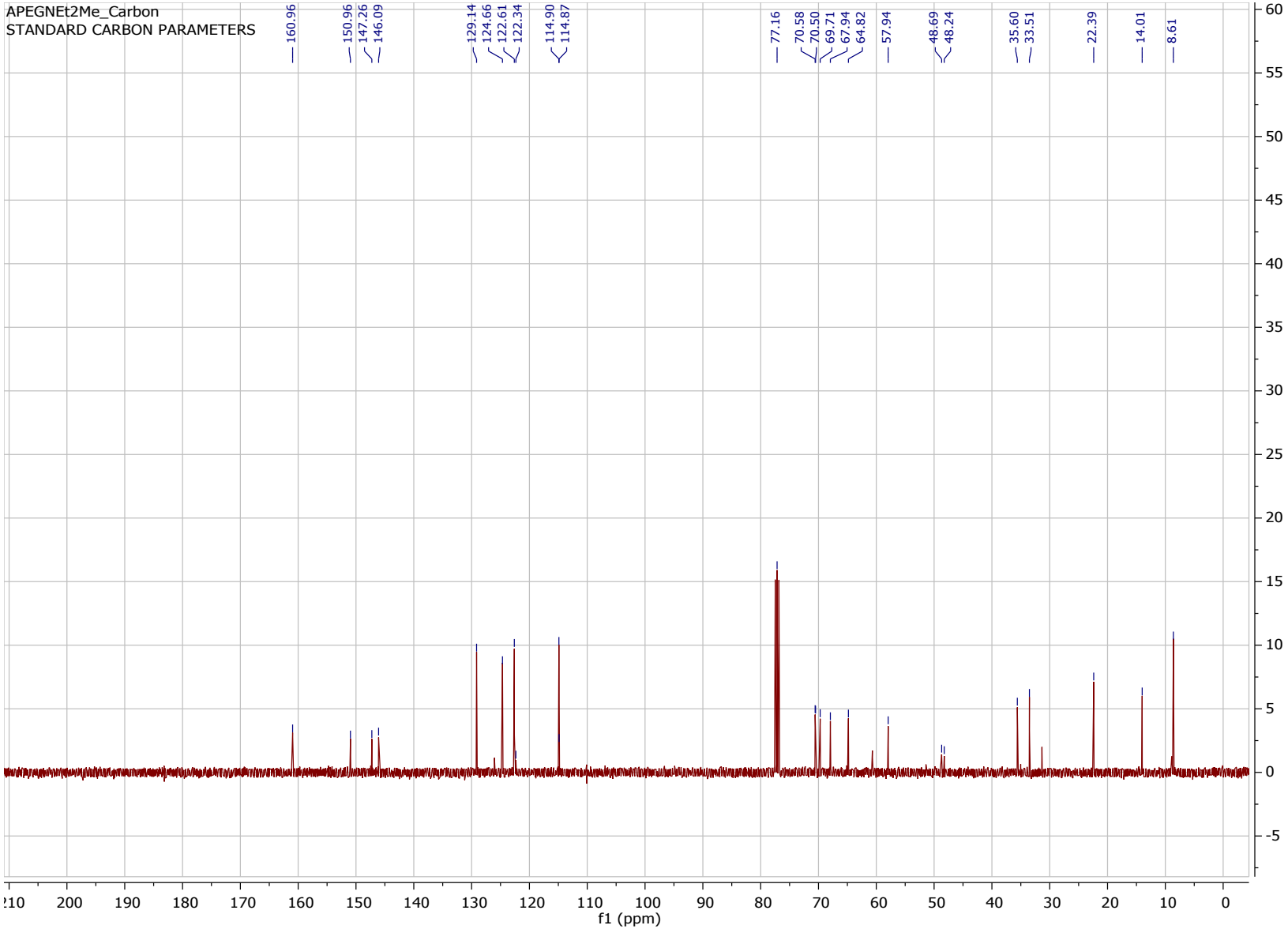

APEG_TMG_Carbon
APEG_TMG
162.84
160.92
150.99
147.28
146.06
129.13
124.62
122.61
114.77
77.16
70.74
70.40
69.87
69.60
67.86
44.96
40.30
39.89
35.61
33.52
22.39
14.01
f1 (ppm)

APEG$_{DMA}$ (**SI4**)


APEGNEt2_Carbon
APEGNEt2
161.12
151.10
147.26
145.93
129.14
124.61
122.64
114.90
77.16
71.01
70.58
69.81
69.75
67.82
52.30
47.72
35.66
33.58
22.45
14.07
11.69
f1 (ppm)

APEG$_{NEt2MeI}$




APEGNEt2Me_Carbon
STANDARD CARBON PARAMETERS
160.96
150.96
147.26
146.09
129.14
124.66
122.61
122.34
114.90
114.87
77.16
70.58
70.50
69.71
67.94
64.82
57.94
48.69
48.24
35.60
33.51
22.39
14.01
8.61
f1 (ppm)