In Vivo Monitoring of Carbonic Anhydrases Expression on Growth of Larval Zebrafish: A New Environment-Sensitive Fluorophore for Responsive Turn-On Fluorescence

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Supporting Information

Contents

1. General details

All chemical reactions were performed under an atmosphere of nitrogen and the workups were carried out in air. All the solvents used for the condition optimization were dried using reported procedures. Unless noted, all materials were purchased from commercial suppliers (Acros, Sigma-Aldrich, Alfa Aesar, SHOWA) and used as received. Tosylazide was prepared in house using conventional procedure.[S1] ^{1}H & ^{13}C NMR spectra were recorded on BrukerUltrasheild™ 300 & 75 MHz and JEOL 400 & 100 MHz spectrometer respectively. NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz). Solvent Residual peaks calibrations: for ^{1}H NMR: $CDCl_3$: 7.2600 ppm; MeOD: 3.3100. For ^{13}C NMR: $CDCl_3$: 77.23 ppm; MeOD: 49.15. Melting Points of the products were calculated in open capillary tubes using Fargo Melting Point Apparatus MP-2D. Infra-Red spectra were recorded using Perkin Elmer 100 FTIR Spectrometer. High Resolution Mass Spectra (HRMS) were performed on an Electronspray Ionization Time-of-Flight (ESI-TOF), Fast Atom Bombardment (FAB), Electron Ionization (EI), and Atmospheric-pressure chemical ionization Time-of-Flight (APCI-TOF) mass spectrometer. Flash chromatography was performed using silica gel (43-60 mm, Merck). Absorption spectra were recorded on JASCO V-630 spectrophotometer. Fluorescence spectra were recorded using Hitachi F-7000 fluorescence spectrophotometer. The fluorescence imaging of cells was carried out by using Laser Scanning Confocal Microscope (LSM 700, Zeiss, Germany). For the CFP, images were taken using a 405 nm excitation laser with an SP490 emission filter; SP490 indicates short pass 490 nm. For SA-DQI, images were taken using a 488 nm excitation laser with a BP490-580 emission filter; BP490-580 indicates band pass 490-580 nm. For HCS NuclearMask™ Red Stain, a 555 nm excitation laser with LP640 emission filter; LP640 indicates long pass 640 nm. The fluorescence imaging of zebrafish was carried out by using Lumar V12 stereomicroscope (Carl Zeiss). The fluorescence was observed by filtering through a 530/43 bandpass (λ_{ex} = 488 nm). MTT assay's absorptions were recorded by SpectraMax i3 (Molecular Devices). The pET51b-hCA, hCAII-PDGFR and CFP-hCAII-PDGFR plasmids are by courtesy of Prof. Kui-Thong Tan[S2] at Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan. The cervical cancer cell line HeLa was ordered from Food Industry Research and Development Institute (Taiwan). The lung cancer cell line A549 was ordered from ATCC (The U.S.A).The animal use protocol and experiment were approved by the National Sun Yat-sen University Animal Care Committee (approval reference #10231).

2. Computational Methods for the vector of dipole moment of 2,3-dihydroquinolin-4-imine (DQI)

The ground state DFT and excited state TD-DFT calculations were carried out by using PBE0 exchange-correlation functionals[S3] in combination with the 6-31+G* basis set. Empirical dispersion corrections of Grimme's D3 method[S4] were incorporated in the calculations. The solvent effects (dichloromethane, ε = 8.93) were taken into account by the SMD continuum solvation model.[S5] All the calculations were performed with Gaussian 09 package.[S6]

3. hCAII protein expression and purification

The pET51b-hCA vector encoded hCAII protein with C-terminal His-tag[S2] were transformed to E. coli strain BL21(DE3). The bacteria cell culture was incubated at 37 °C in LB broth medium with ampicillin (working concentration, 100 μg/mL). As the culture have reached OD600 at 0.8~1.0, hCAII expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. The culture were grown for 12 hours at 18 ℃ and harvested by centrifugation (12000 rcf, 5 min, 4 ℃). The cells were lysed by ultra-sonication. The insoluble protein and cell debris were removed by centrifugation (12000 rcf, 60 min, 4 ℃). The crude hCAII proteins were purified by FPLC with HisTrap™ HP column and used imidazole solution to elute. The purified protein was concentrated, dialysis against PBS buffer (pH 7.2) and stored at -20 °C. The concentration of protein was determined using Pierce™ BCA Protein Assay Kit. The purity of the protein was examined by SDS-PAGE with commercial Instant Blue staining solution.

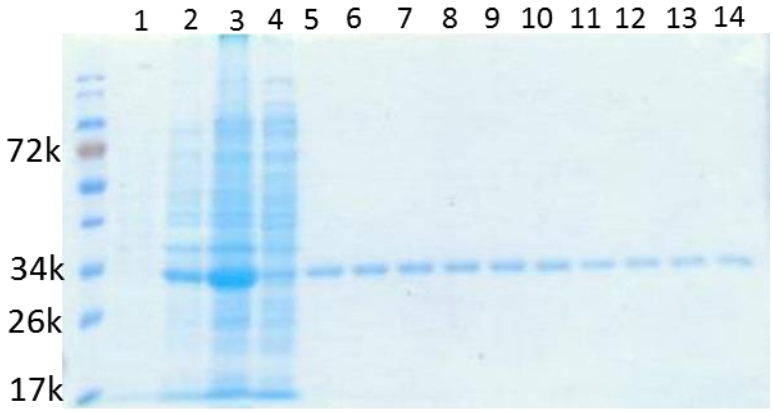


Figure S1. SDS-PAGE analysis of hCAII. **Lane 1** : Before induction; **Lane 2** : After induction; **Lane 3** : cell lysate; **Lane 4** : flow through after loading; **Lane 5-14** :

eluted hCAII. Protein marker was purchased from Bioman (Prestained Protein Ladder)

4. The dissociation constant of SA-DQI and SA-TEG-DQI

The dissociation constant of SA-conjugated DQIs for carbonic anhydrase have been determined and described as follows. First, Job's plot analysis has been performed to determine the binding of SA-DQI to hCAII as 1:1 molar ratio (Shown in Fig. S2).

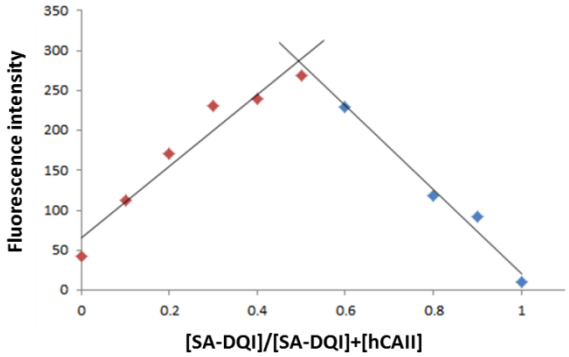


Figure S2. Job's plot of SA-DQI to hCAII

To calculate the dissociation constants (K_d) of SA-DQI and SA-TEG-DQI from enhancement of fluorescence, the change in fluorescence was normalized and plotted against the concentration of hCAII (Shown in Fig. S3).

For SA-DQI, the fluorescence titration curve was fitted on 1:1 binding isotherm model with R^2 = 0.99. I and I_0 denote the intensities in the presence and absence of hCAII and determined the K_d as 1.138 ± 0.04 μM

For SA-TEG-DQI, the fluorescence titration curve was fitted on 1:1 binding isotherm model with R^2 = 0.98. I and I_0 denote the intensities in the presence and absence of hCAII And determine the K_d as 0.456 ± 0.04 μM

The error bar was calculated from three independent experiments. The experiments were run in triplicate, and a one-site-specific binding model (GraphPad Prism 7.0) was used for curve fitting.

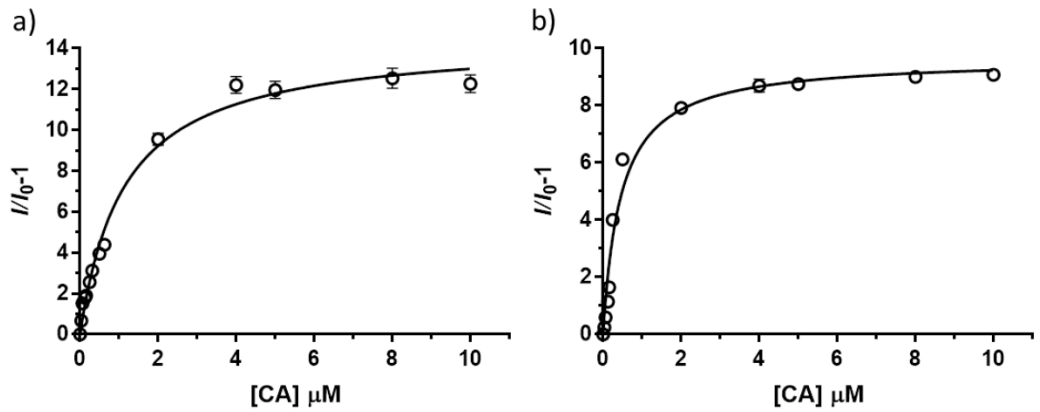


Figure S3. a) Titration curve of SA-DQI (1μM) with increasing concentration of hCAII (0-10 μM); b) Titration curve of SA-TEG-DQI (1μM) with increasing concentration of hCAII (0 -10 μM)

5. Transfection of HeLa cells.

The HeLa cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The HeLa cells were plated in two-well chamber slides at a density of 6 x 10^4 cells per well and were incubated at 37 ℃ in air with 5% CO_2 for 24 hours. The cells were transfected using X-treme GENE HP DNA transfection reagent (Roche Applied Science). Two micro-grams of CFP-hCAII-PDGFR DNA plasmid and 6 μL X-treme GENE HP Reagent in 200 μL Opti-MEM were used per well and incubated for 30 minutes at room temperature. The complexes were distributed evenly per well and incubated for 24 hours post transfection (Figure S4).

Figure S4 showed the results of CFP-hCAII-PDGFR transfected on the cell membrane. The CFP signal could be detected by 405 nm laser (Figure S4 (i)), but no significant fluorescence can be observed under 488 nm laser(Figure S4 (ii)).

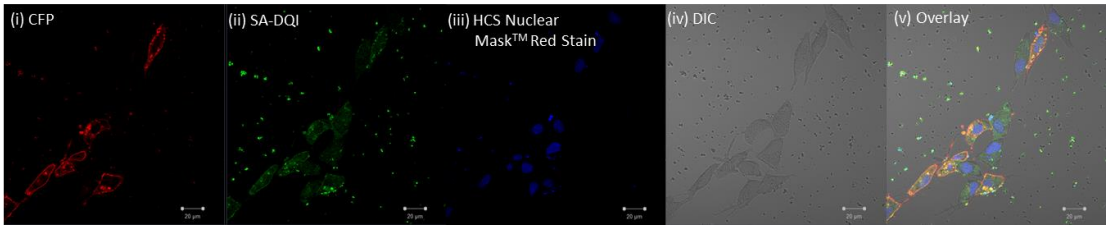


Figure S4. HeLa cells expressed the CFP–hCAII–PDGFR protein on the cell membrane. Scale bars represent 20 μm

6. Fluorescence imaging of transfected HeLa cells.

For the SA-DQI recognition experiment, the transfected cells were treated with 1 μM of SA-DQI prepared in Opti-MEM (1.0 % DMSO (v/v)). Without removing unbound SA-DQI or EZA, fluorescence imaging was carried out using a Laser Scanning Confocal Microscope (LSM 700, Zeiss, Germany). For the SA-DQI channel, the images were taken using a 488 nm laser and 490 - 580 nm emission filter. For observing cyan fluorescence protein (CFP), we used a 405 nm laser and SP490 emission filter (SP490 indicates short pass 490 nm).

For EZA competition experiments, the SA-DQI treated transfected HeLa cells were washed with Opti-MEM twice and treated with 300 μM of EZA solution, prepared in Opti-MEM (1.0 % DMSO (v/v)). The fluorescence imaging was carried out using a Laser Scanning Confocal Microscope (LSM 700, Zeiss, Germany). For the SA-DQI channel, the images were taken by using a 488 nm laser and 490 - 580 nm emission filter. For HCS NuclearMask^{TM} Red Stain (Thermo Fisher Scientific, USA), we used a 555 nm laser and LP640 emission filter (LP640 indicates long pass 640 nm). For CFP, we used a 405 nm laser and SP490 emission filter.

For nucleus and cell membrane staining, 24 hours after transfection, the cells were washed with Opti-MEM twice and stained with HCS NuclearMask^{TM} Red Stain. After 30 minutes incubation, excess HCS NuclearMask^{TM} Red Stain was removed. For HCS NuclearMask^{TM} Red Stain, we used a 555 nm laser and LP640 emission filter.

7. Colocalization analysis

In this study, we utilized CFP-hCAII-PDGFR plasmid to transfect the cyan-fluorescent protein and human carbonic anhydrase II on the cell membrane. According to the transfection mechanism, plasmid would combine with transfection reagent and penetrate cell membrane by endocytosis to cell nucleus. Afterward, CFP-hCAII-PDGFR would be expressed and release from the cell nucleus to the cell membrane because of the PDGFR which was a membrane-anchoring domain. Therefore, the granules were the protein of CFP-hCAII-PDGFR which generated from cell nucleus and moved to the cell membrane that went through cytoplasm. Since the CFP signal can locate the presence of hCAII, the co-localization of CFP and SA-DQI signals can support the enhanced SA-DQI fluorescence coming from the binding with hCAII.

For the quantitative analysis of the fluorescence image co-localization, Mander's overall overlap coefficient (MOC) has been performed to elucidate the co-localization between CFP-hCAII-PDGFR and SA-DQI. Mander's coefficient supplies a method

that determines the overlap of two fluorescence channels while taking into account pixel intensity which means the total amount of fluorophores overlap with each other. The equation of Mander's overall overlap coefficient is shown as follow.

$$MOC = \frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2} \sqrt{\sum_{i=1}^{n} y_i^2}}$$

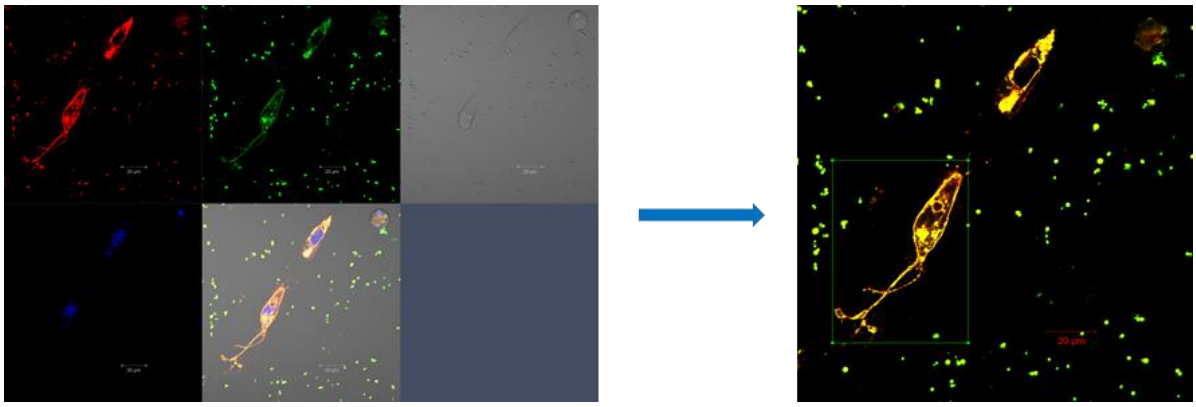


Figure S5. Using Mander's coefficient to analyze transfected HeLa cells treated with SA-DQI by ZEN software

The Mander's overlap coefficient of CFP-hCAII-PDGFR and SA-DQI in HeLa cell was acquired and analyzed by ZEN 2012 software which shown in figure S5 with the value as 0.70. Furthermore, we would like to verify the overlap coefficient of CFP-hCAII-PDGFR and SA-DQI again, therefore, we have already repeated the experiment which showed in figure S6. The overlap coefficient of CFP-hCAII-PDGFR and SA-DQI in HeLa cells are 0.82 (Fig.S6 (1)), 0.78 (Fig.S6 (2)), 0.64 (Fig.S6 (3)).

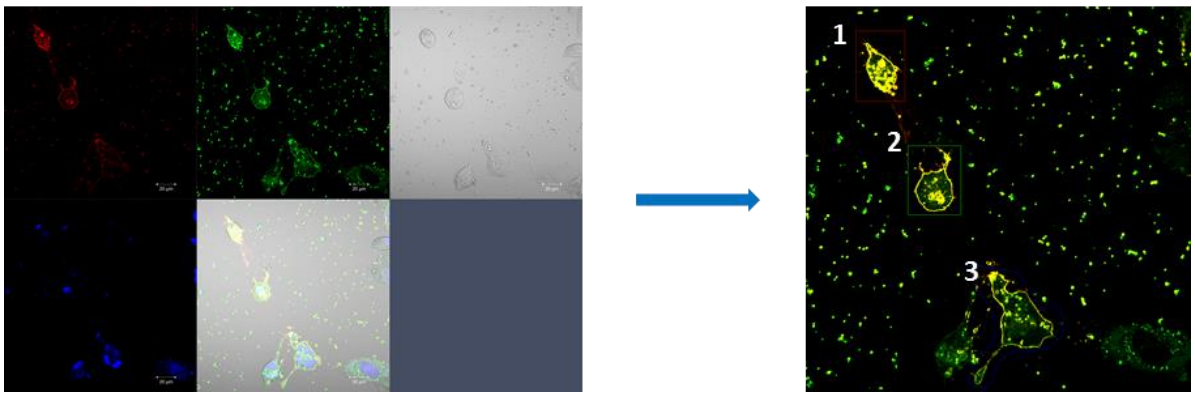


Figure S6. Using Mander's coefficient to analyze transfected HeLa cells treated with SA-DQI by ZEN 2012 software

8. CFP-hCAII-PDGFR transfected HeLa cell treated with SA-TEG-DQI

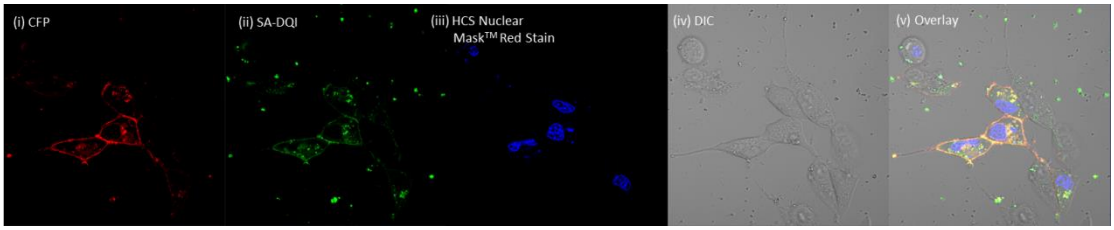


Figure S7. CFP-hCAII-PDGFR transfected HeLa cells treated with SA-TEG-DQI (5 μM)

9. Fluorescence imaging of transfected hCAII-PDGFR cells.

The 293T cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The HeLa cells were plated in two-well chamber slides at a density of 6 x 10^4 cells per well and were incubated at 37 ℃ in air with 5% CO_2 for 24 hours. The cells were transfected using X-treme GENE HP DNA transfection reagent (Roche Applied Science). Two micro-grams of hCAII-PDGFR DNA plasmid and 6 μL X-treme GENE HP Reagent in 200 μL Opti-MEM were used per well and incubated for 30 minutes at room temperature. The complexes were distributed evenly per well and incubated for 24 hours post transfection

The hCAII-PDGFR transfected cells were treated with SA-DQI (1 μM). The fluorescence signal can be observed on cell membranes without removing the unbound SA-DQI, as shown in Figure S8a. To check the selective recognition and competition, the inhibitor EZA (300 μM) was added to SA-DQI-treated on cell membrane. The competition causes the dissociation of SA-DQI from the membrane hCAII, thus suppressing the fluorescence from membrane bound SA-DQI (Figure S8b).

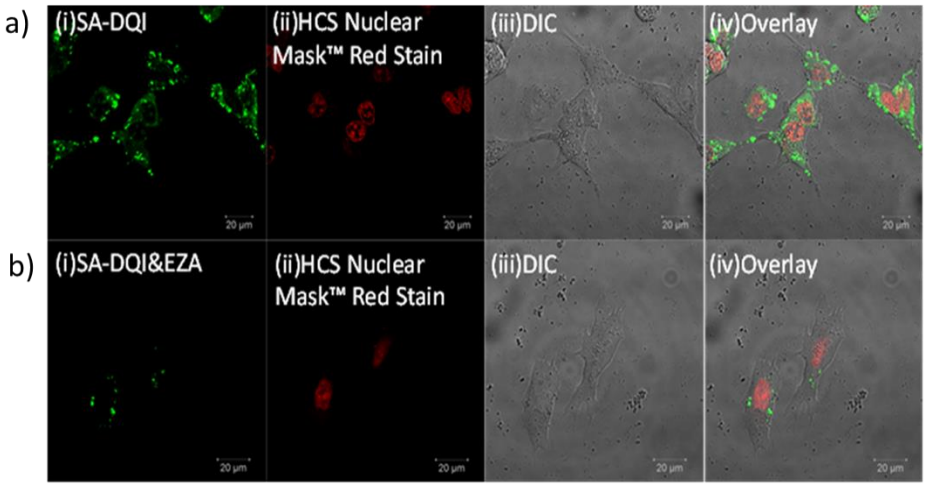


Figure S8. Cells imaging of 293T cells expressing the hCAII–PDGFR protein on the membrane a) 293T cells expressing the hCAII–PDGFR protein were treated with 1 μM SA-DQI. b) 293T cells expressing the hCAII–PDGFR protein were treated with 1 μM SA-DQI and then subjected to competition by the addition of 300 μM EZA. Scale bars represent 20 μm

10. A549 cell imaging under hypoxic mimic condition

The A549 cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The A549 cells were plated in two-well chamber slides at a density of 6 x 10^4 cells per well and were incubated at 37 ℃ in air with 5% CO_2 for 24 hours. The cells were added deferoxamine mesylate (DFO) (200 μM)) in DMEM and were incubated at 37 ℃ in air with 5% CO_2 for 24 hours.

The A549 cells were treated with SA-TEG-DQI (10 μM) which . The fluorescence signal can be observed on cell membranes without removing the unbound SA-TEG-DQI, as shown in Figure S9a.To check the selective recognition and competition, the inhibitor EZA (300 μM) was added to SA-TEG-DQI-treated on cell membrane.

We performed the A549 cell treated with SA-TEG-DQI under hypoxic-mimic (in the presence of DFO (200 μM)) or normoxic condition. The fluorescence intensity enhanced on A549 cell membrane when treated with SA-TEG-DQI (10 μM) under hypoxic mimic and non-wash condition (Figure S9(a)). The fluorescence intensity decreased upon addition of the strong inhibitor, EZA (300 μM) (Figure S9(b)).

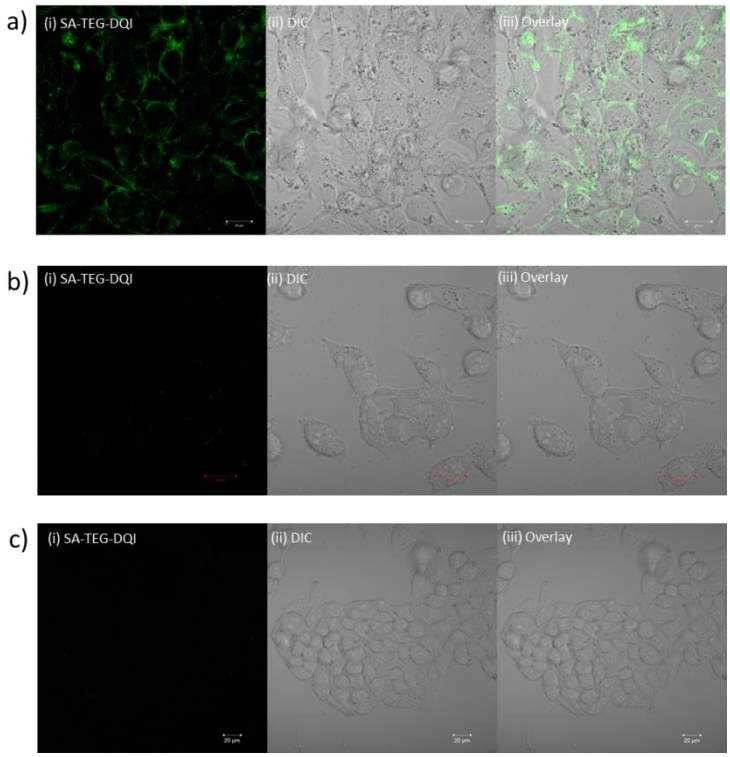


Figure S9. Transmembrane-type hCA imaging of A549 cells utilizing SA-TEG-DQI (10 μM); a) without EZA; b) with EZA ((300 μM) cultured under hypoxic conditions.;(c) Transmembrane-type hCA imaging of A549 cells utilizing SA-TEG-DQI (10 μM) cultured under normoxic conditions

11. CAII *In vivo* imaging on zebrafish.

Zebrafish (Danio rerio) wild-type AB strain were raised and maintained under standard laboratory conditions. Fertilized eggs were collected, dechorionated by pronase (1mg/mL, Sigma, Japan) and cultured in E3 media. Endogenous pigmentation was blocked by adding 1-phenyl-2-thiouria (PTU) (0.003%) to media at 6 hours post fertilization (hpf). Ten ng SA-TEG-DQI (1.25 mM in 2% DMSO) was injected at 3 days post fertilization (dpf) zebrafish. The injected zebrafish embryos were recovered for one hour and immobilized in 1.5% low melting point agarose (Invitrogen) and imaged using a Lumar V12 stereomicroscope (Carl Zeiss). The fluorescence was observed by filtering through a 530/43 bandpass (λ_{ex} = 488 nm). For control experiment, CA2a morpholino oligonucleotide (MO-CA2a), purchased from Gene-Tools (Philomath, OR) to knockdown CAII expression, and three ng were injected into embryos at 1–2 cell stage using a microinjector FemtoJet (Eppendorf, Germany).

12. Blue shift of emission for SA-DQI/hCAII interaction

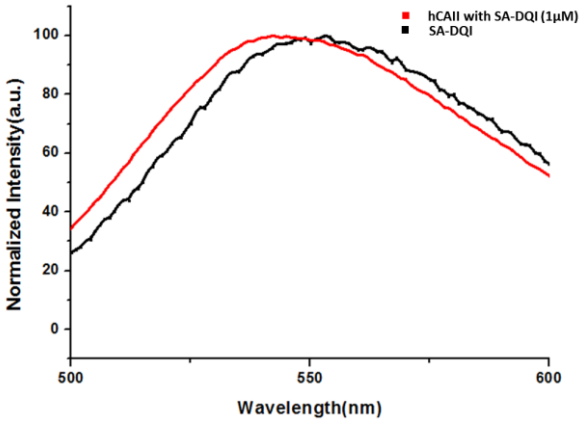


Figure S10. A blue emission wavelength shift after SA-DQI/hCAII interaction

13. MTT assay

The cytotoxic effect of SA-DQI was determined by incubating cells at a density of 1×10^3 per well of 96-well flat bottomed plates (Biofil) 24 hours before treatment. Cells were incubated with 150 µl/well of DMEM containing 10%FBS and increasing concentrations of SA-DQI (range 0–5 µM) for 24 hours. Next, 50 µl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Co.) solution was added to each well, followed by a 2 hours incubation at 37°C. The formazan crystals were dissolved using 100 µL of DMSO and absorbance was measured at 570 nm on a SpectraMax i3 multiwell plate reader (Molecular Devices). Results are presented as percentage of survival taking the control (untreated cells) as 100% survival.

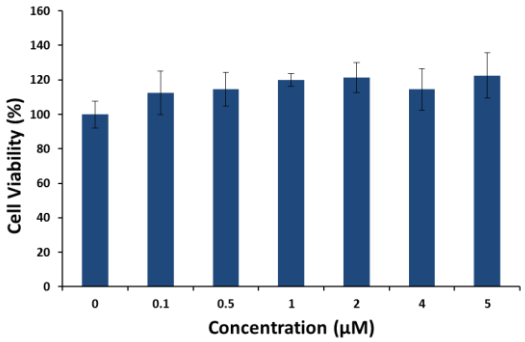


Figure S11. Viability of the cells after 24 h incubation with various concentrations of SA-DQI.

14. Selectivity of SA-DQI with hCAII test

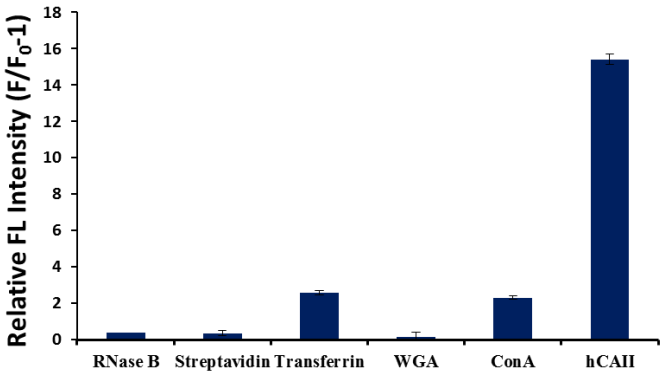
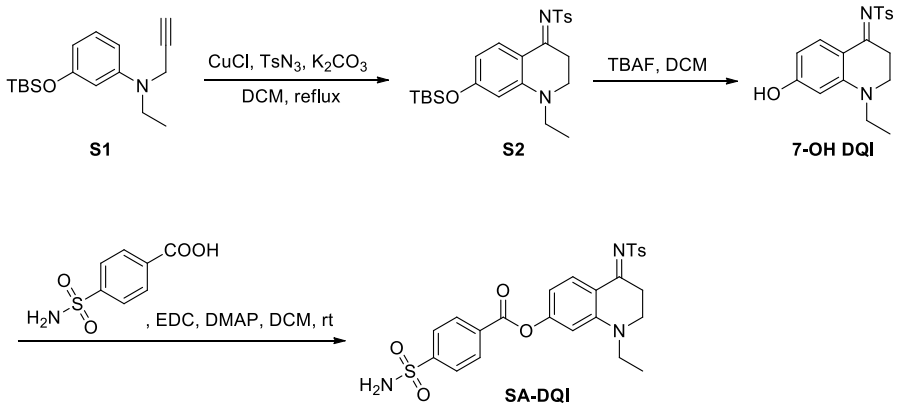


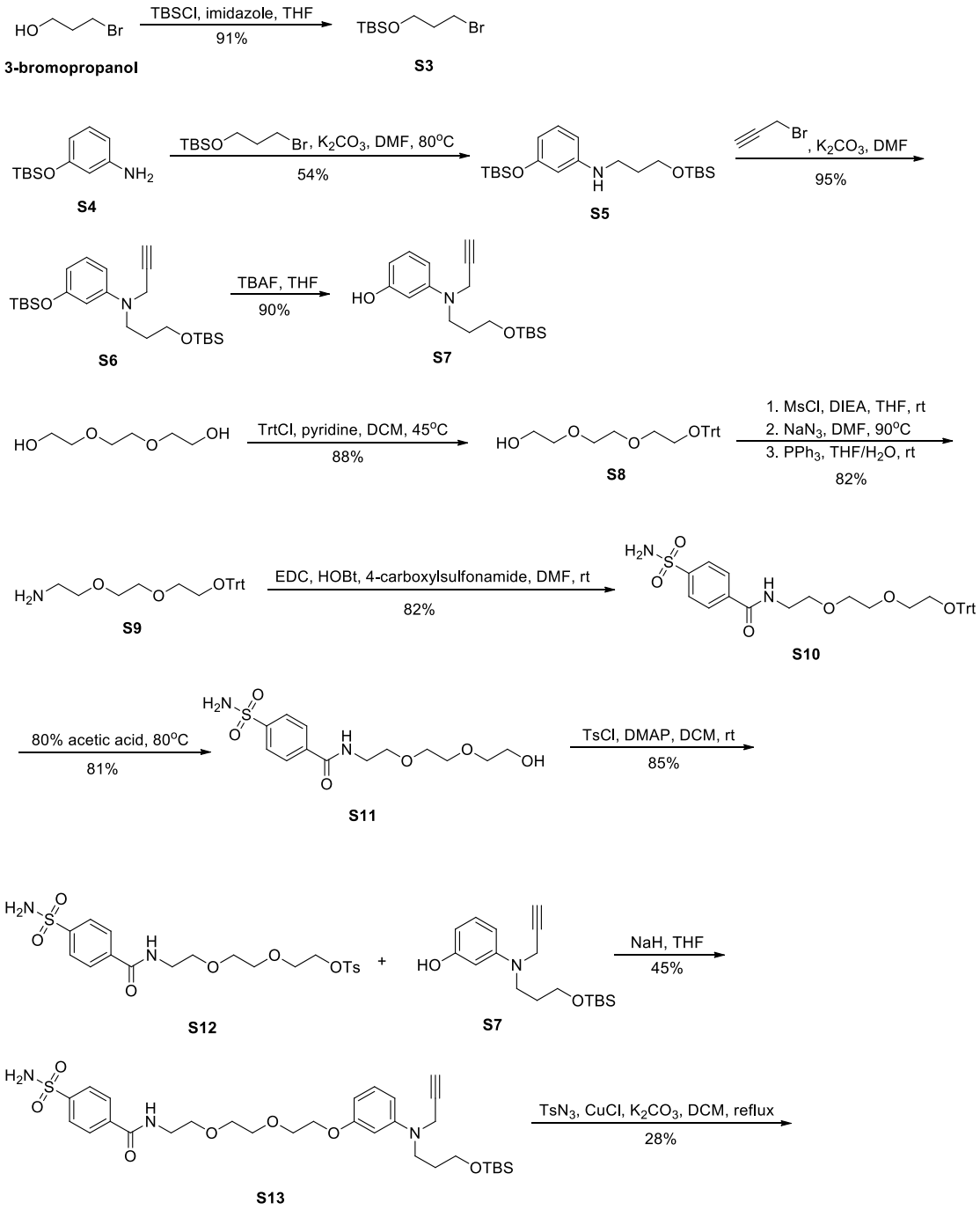
Figure S12. Selectivity of SA-DQI (1 μM) with CAII and five other non-targeted proteins.

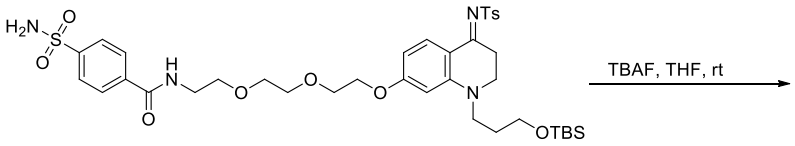
15. Procedure for the synthesis of SA-DQI[S7]



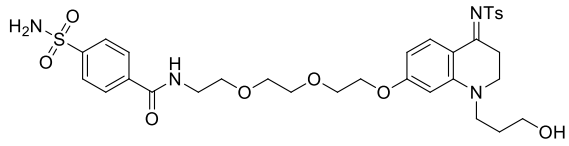
To a stirred solution of 4-sulfamoylbenzoic acid (44 mg, 0.218 mmol) in Dichloromethane (2 mL) was added 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (42 mg, 0.218 mmol), and 4-Dimethylaminopyridine (9 mg, 0.073 mmol) at room temperature under nitrogen atmosphere. After 20 minutes, added *N*-(1-ethyl-7-hydroxy-2,3-dihydroquinolin-4(1H)-ylidene)-4-methylbenzenesulfonamide (50mg, 0.145 mmol). After TLC confirmed the completion of the reaction, the reaction mixture was quenched with H_2O (2 ml), diluted with DCM (10 mL) and washed with H_2O (2 x 10 mL). The mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silicagel column chromatography to afford the desired product (**SA-DQI**) (47 mg, 62%) as a yellow solid.

16. Procedure for the synthesis of SA-TEG-DQI



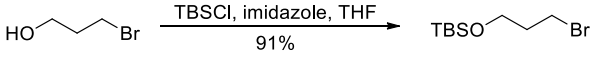


S14



SA-TEG-DQI

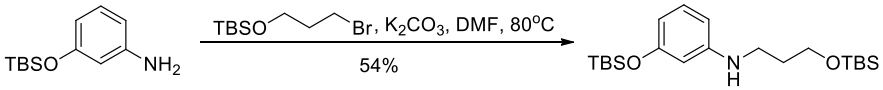
a) Synthesis of (3-bromopropoxy)(*tert*-butyl)dimethylsilane (S3)



3-bromopropanol **S3**

To a stirred solution of 3-bromopropanol (300 mg, 2.16 mmol) in THF (10 mL) was added *t*-butylmethylsilyl chloride (390 mg, 2.59 mmol) and imidazole (220 mg, 3.24 mmol) and the resultant mixture was stirred for 2h at rt. After TLC confirmed the completion of the reaction, the reaction mixture was quenched with H_2O (10 ml), diluted with EA (10 mL) and washed with H_2O (2 x 10 mL). The mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the desired product (496 mg, 91%) as a off oil.

b) Synthesis of 3-((*tert*-butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl) oxy)propyl)aniline (S5):

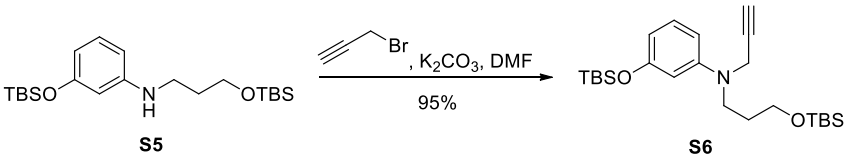


S4 **S5**

To a stirred solution of compound **S4** (970 mg, 4.35 mmol) in DMF (80 mL) was added (3-bromopropoxy)(*tert*-butyl)dimethylsilane (1424 mg, 5.65 mmol) and potassium carbonate (600 mg, 4.35 mmol) and the resultant mixture was stirred under nitrogen atmosphere and heated at 80℃ for 12 h. After TLC confirmed the completion of the reaction, the reaction mixture was quenched with H_2O (40 ml), diluted with EA(50 mL) and washed with chilled H_2O (2 x 200 mL). The mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced

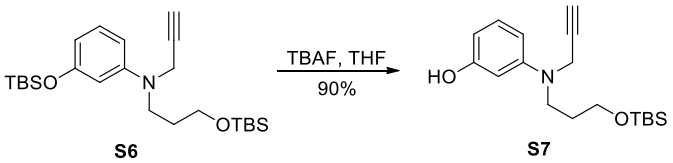
pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (931 mg, 54%) as a off oil.

c) Synthesis of 3-((*tert*-butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl) oxy)propyl)-*N*-(prop-2-yn-1-yl)aniline (S6):



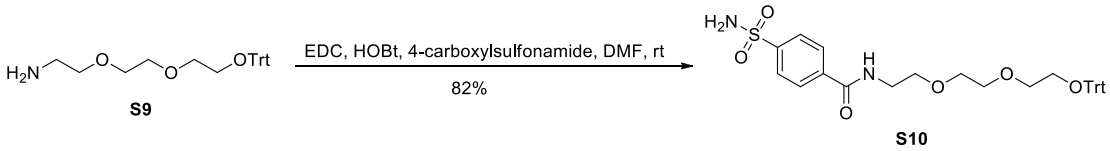
To a stirred solution of compound **S5** (250 mg, 0.63 mmol) in DMF (1.5 mL) was added potassium carbonate (87 mg, 0.63 mmol) followed by 80% propargylbromide in toluene (0.178 mL, 1.90 mmol) at room temperature and the resultant mixture was stirred for 14h. The reaction mixture was diluted with EA (10 mL), washed with chilled H_2O (2 x 50 mL), dried over anhydrous $MgSO_4$, filtered and concentrated to afford the desired product (260 mg, 95%) as a pale yellow oil.

d) Synthesis of 3-((3-((*tert*-butyldimethylsilyl)oxy)propyl)(prop-2-yn-1-yl)amino) phenol (S7)



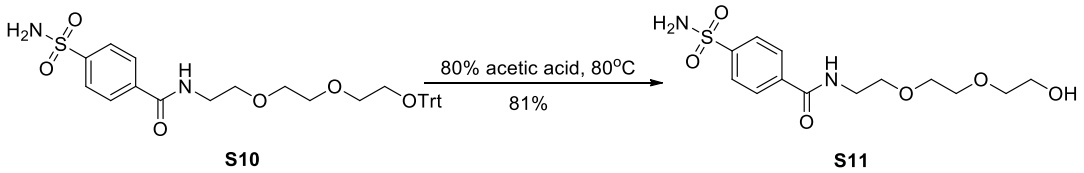
To a stirred solution of compound **S6** (330 mg, 0.63 mmol) in THF (8 mL) was added 1M TBAF in toluene (0.837 mL, 0.837 mmol) at room temperature and the resultant mixture was stirred for 30 min. The reaction mixture was diluted with EA (10 mL), washed with $NH_4Cl_{(aq.)}$ (2 x 10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (220 mg, 90%) as a brown oil.

e) Synthesis of 4-sulfamoyl-*N*-(2-(2-(2-(trityloxy)ethoxy)ethoxy)ethyl)benzamide (S10):



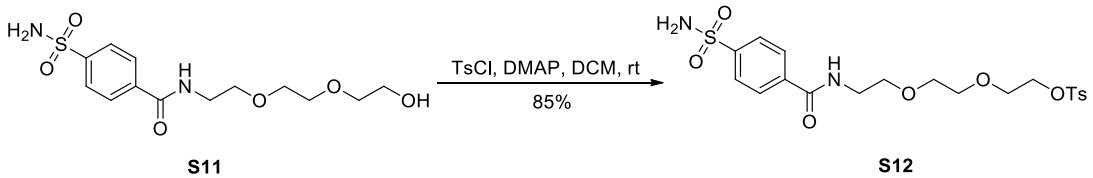
To a stirred solution of 4-carboxylsulfonamide (1368 mg, 6.80 mmole) in DMF (50 mL) was added HOBt (1895 mg, 12.37 mmole), and EDC (1920 mg, 12.37 mmole) to activated carboxylic acid at room temperature for 20 min. Then, the mixture was added DMF (10 mL) solution with compound **S9** (2420 mg, 6.19 mmol) and DIEA (3.24 mL, 18.55 mmole). After reacting for 3 hr at room tempeture, the reaction mixture was quenched with H_2O (50 mL), the crude mixture was extraction by EA/ chilled H_2O = 1/5 twice, the combined organic layers were dried over $MgSO_4$, filtered and concentrated to afford the desired product (2912 mg, 82%) as a white solid.

f) Synthesis of *N*-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S11):



To a stirred solution of compound **S10** (2410mg, 4.20 mmol) in 80% acetic acid (20 mL). The reaction mixture was heated at 80 ℃ for 1.5 hours under nitrogen atmosphere. Then, the reaction mixture was concentrated in vacuum and purified by column chromatography to obtain the desired product (1129 mg, 81%) as a off oil.

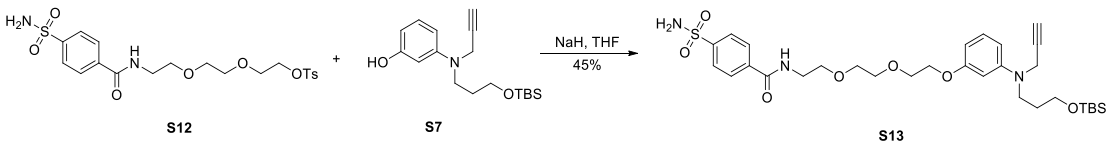
g) Synthesis of 2-(2-(2-(4-sulfamoylbenzamido)ethoxy)ethoxy)ethyl-4-methyl benzenesulfonate (S12):



To a stirred solution of compound **S11** (720 mg, 2.17 mmol) in THF (21 mL) was added TsCl (620 mg, 3.25 mmol), DMAP (132 mg, 1.08 mmol), and DIEA (1.14 mL, 6.50 mmol) at room temperature and the resultant mixture was stirred for 6 h. The

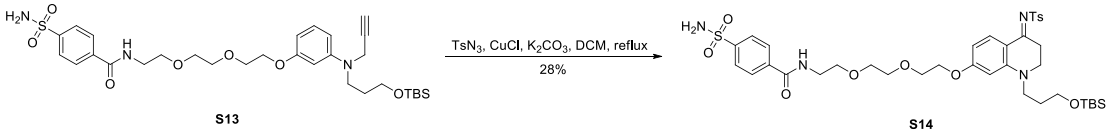
reaction mixture was diluted with EA (10 mL), washed with H_2O (2 x 30 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (897 mg, 85%) as a off oil.

g) Synthesis of *N*-(2-(2-(2-(3-((3-((*tert*-butyldimethylsilyl)oxy)propyl)(prop-2-yn-1-yl)amino)phenoxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S13):



To a stirred solution of compound **S7** (320 mg, 1.00 mmol) in dry THF (2 mL) was added 60% NaH (44 mg, 1.10 mmole) under 0 ^oC for 15 min and stirred at room temperature for 15 min. Then, the mixture was added dry THF (1 mL) solution with compound **S12** (97 mg, 0.20 mmol). The reaction mixture was heated at 60 ℃ for 6 hours under nitrogen atmosphere. The reaction mixture was quenched with $NH_4Cl_{(aq.)}$ (5 mL), the crude mixture was extraction by EA/ H_2O = 1/1 twice, the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (57mg, 45%) as a pale yellow solid.

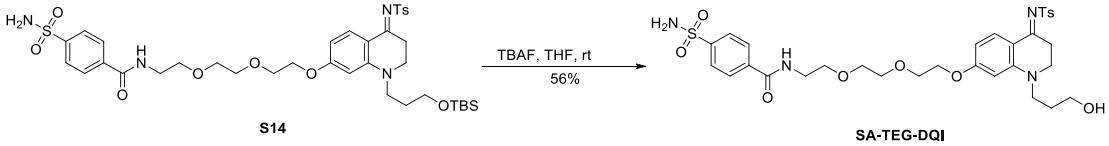
h) Synthesis of *N*-(2-(2-(2-((1-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4-(tosylimino)-1,2,3,4-tetrahydroquinolin-7-yl)oxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S14):



To a stirred solution of compound **S13** (50 mg, 0.09 mmol) in Dichloromethane (3 mL) was added tosylazide (20 mg, 0.10 mmol), potassium carbonate (60 mg, 0.43 mmol), and CuCl (0.86 mg, 0.009 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was refluxed for 1 h. Then, the reaction mixture was allowed to cool to the room temperature, quencehed with saturated NH_4Cl solution (5 ml), diluted with DCM (10 mL) and washed with H_2O (2 x 10 mL). The mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under

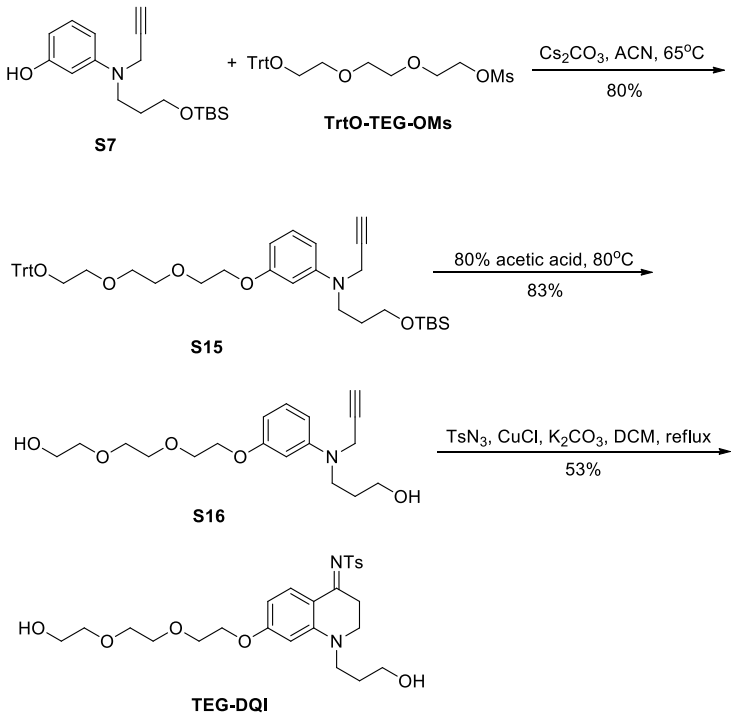
reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (20 mg, 28%) as a yellow oil.

i) Synthesis of *N*-(2-(2-(2-((1-(3-hydroxypropyl)-4-(tosylimino)-1,2,3,4-tetrahydro quinolin-7-yl)oxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (SA-TEG-DQI):

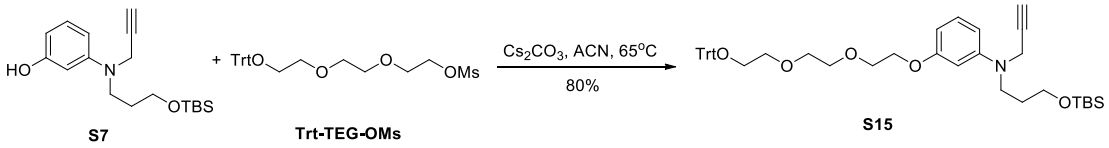


To a stirred solution of compound **S14** (27 mg, 0.034 mmol) in THF (0.6 mL) was added 1M TBAF in toluene (0.071 mL, 0.071 mmol) at room temperature and the resultant mixture was stirred for 5 h. The reaction mixture was diluted with EA (10 mL), washed with $NH_4Cl_{(aq.)}$ (2 x 10 mL), dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (13 mg, 56%) as a yellow oil.

17. Procedure for the synthesis of TEG-DQI



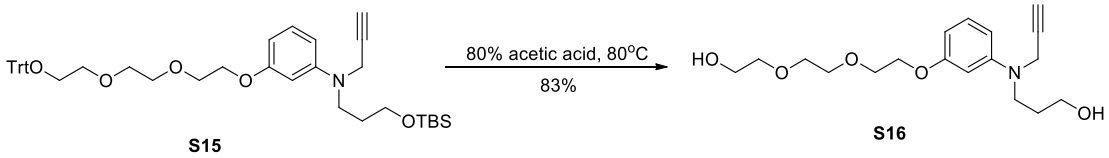
a) Synthesis of *N*-(3-((tert-butyldimethylsilyl)oxy)propyl)-N-(prop-2-yn-1-yl)-3-(2-(2-(2-(trityloxy)ethoxy)ethoxy)ethoxy)aniline (S15):



S7 Trt-TEG-OMs S15

To a stirred solution of compound **S7** (215 mg, 0.67 mmol) in dry ACN (1.5 mL) was added cesium carbonate (329 mg, 1.01 mmole) and Trt-TEG-OMs (316 mg, 0.67 mmol) under 65 ^{o}C for 12 hr under nitrogen atmosphere. The reaction mixture was quenched with H_2O (5 mL), the crude mixture was extraction by EA/ H_2O = 1/1 twice, the combined organic layers were dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (373mg, 80%) as a pale yellow oil.

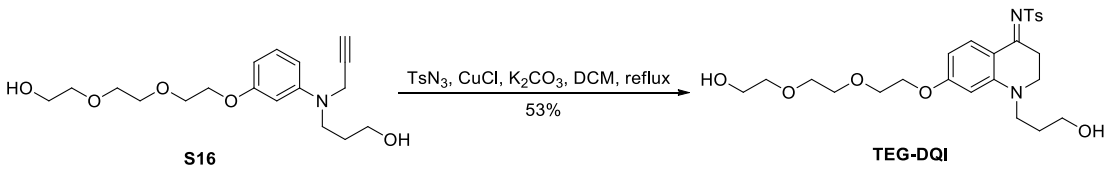
b) Synthesis of 3-((3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)phenyl)(prop-2-yn-1-yl) amino)propan-1-ol (S16):



S15 S16

To a stirred solution of compound **S15** (200mg, 0.29 mmol) in 80% acetic acid (10 mL). The reaction mixture was heated at 80 ℃ for 1 hr under nitrogen atmosphere. Then, the reaction mixture was concentrated in vacuum and purified by column chromatography to obtain the desired product (80 mg, 83%) as a pale yellow oil.

c) Synthesis of *N*-(7-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-1-(3-hydroxypropyl)-2,3-dihydroquinolin-4(1*H*)-ylidene)-4-methylbenzenesulfonamide (TEG-DQI):



S16 TEG-DQI

To a stirred solution of compound **S16** (50 mg, 0.15 mmol) in Dichloromethane (10 mL) was added tosylazide (44 mg, 0.22 mmol), potassium carbonate (103 mg, 0.74 mmol), and CuCl (1.47 mg, 0.015 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was refluxed for 3 h. Then, the reaction mixture was allowed to cool to the room temperature, quencehed with saturated NH_4Cl solution (10 ml), diluted with DCM (10 mL) and washed with H_2O (2 x 10 mL). The mixture was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the crude mixture and then purified by silica gel column chromatography to afford the desired product (40 mg, 53%) as a yellow oil.

18. Characterization of the final compounds and intermediates

3-((tert-butyldimethylsilyl)oxy)-*N*-ethyl-*N*-(prop-2-yn-1-yl)aniline (S1). Off oil (288 mg, 0.996 mmol, 87 %); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08 (t, *J* = 8.06 Hz, 1H), 6.45 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.34 (t, *J* = 2.28 Hz, 1H), 6.28 (dd, *J* = 7.8, 1.9 Hz, 1H), 3.99 (d, *J* = 2.4 Hz, 2H), 3.41 (q, *J* = 7.1 Hz, 2H), 2.18 (t, *J* = 2.3 Hz, 1H), 1.21 (t, *J* = 7.1 Hz, 3H), 0.99 (s, 9H) 0.21 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 149.5, 129.9, 109.8, 107.5, 106.2, 80.7, 71.8, 45.8, 39.9, 26.0, 18.5, 12.7, -4.1; IR (KBr) ν 3310, 2957, 2929, 2320, 1600, 1577, 1496, 1215 cm^{-1}; HRMS (HR-ESI) calcd for $C_{17}H_{28}NOSi$ $[M+H]^+$ 290.1940, found 290.1935.

***N*-(7-((tert-butyldimethylsilyl)oxy)-1-ethyl-2,3-dihydroquinolin-4(1*H*)-ylidene)-4-methylbenzenesulfonamide (S2)**. Off yellow solid (107.3 mg, 0.234 mmol, 68 %); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.94-7.84 (m, 3H), 7.3 (d, *J* = 8.1 Hz, 2H), 6.12 (dd, *J* = 8.8, 2.2 Hz, 1H), 6.08 (d, *J* = 2.0 Hz, 1H), 3.50-3.28 (m, 6H), 2.42 (s, 3H), 1.17 (t, *J* = 7.1 Hz, 3H), 0.97 (s, 9H), 0.22 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.7, 163.5, 153.2, 142.8, 140.0, 131.4, 129.4, 126.9, 113.3, 110.9, 102.9, 47.0, 45.9, 31.6, 25.8, 21.7, 18.4, 11.0, -4.1; IR (KBr) ν 2956, 2929, 2857, 1614, 1547, 1270, 1246, 1150, 1090 cm^{-1}; HRMS (HR-ESI) calcd for $C_{24}H_{34}N_2O_3SSiNa$ $[M+Na]^+$ 481.1957, found 481.1954.

***N*-(1-ethyl-7-hydroxy-2,3-dihydroquinolin-4(1*H*)-ylidene)-4-methylbenzenesulfonamide (7-OH DQI)**. Off yellow solid (158 mg, 0.459 mmol, quant.) mp 140-144 ^{o}C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92-7.83 (m, 3H), 7.3 (d, *J* = 8.1 Hz, 2H), 6.15-6.07 (m, 2H), 3.44-3.32 (m, 6H), 2.43 (s, 3H), 1.16 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.9, 163.9, 153.6, 143.1, 140.0, 132.1, 129.9, 126.9, 112.7, 107.2, 98.1, 47.0, 46.0, 31.5, 21.7, 11.1; IR (KBr) ν 3357, 2974, 2924, 1619, 1526, 1275, 1242,

1084 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{18}H_{20}N_2O_3NaS$ $[M+H]^+$ 367.1092, found 367.1095.

1-ethyl-4-(tosylimino)-1,2,3,4-tetrahydroquinolin-7-yl-4-sulfamoylbenzoate (SA-DQI): Off yellow solid (47 mg, 0.075 mmol, 62 %) mp 137-144 ℃; ^{1}H NMR (300 MHz, CH_3OD) δ 8.31 (d, *J* = 8.4 Hz, 2H), 8.07 (d, *J* = 8.4 Hz, 2H), 7.99 (d, *J* = 9.0 Hz, 1H), 7.86 (d, *J* = 8.1 Hz, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 6.80 (d, *J* = 1.8 Hz, 1H), 6.52 (dd, *J* = 9.0, 1.8 Hz, 1H), 3.60-3.43 (m, 4H), 3.35 (q, *J* = 7.5 Hz, 2H), 2.45 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, CH_3OD) δ 177.2, 164.9, 159.2, 154.1, 150.1, 145.1, 140.8, 133.8, 131.9, 131.6, 130.8, 128.0, 127.7, 117.5, 111.9, 107.3, 47.8, 46.9, 32.6, 21.7, 11.2; IR (KBr) ν 2925, 1738, 1622, 1549, 1342 cm^{-1}; HRMS (ESI-TOF) calcd for $C_{25}H_{24}N_3O_6S_2$ $[M-H]^-$ 526.1107; found 526.1105.

(3-bromopropoxy)(*tert*-butyl)dimethylsilane (S3): Off oil (496 mg, 1.97 mmol, 91 %); ^{1}H NMR (400 MHz, MeOD) δ 3.76 (t, *J* = 6.0 Hz, 2H), 3.53 (t, *J* = 6.4 Hz, 2H), 2.06-1.96 (m, 2H), 0.91 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, MeOD) δ 61.7, 36.9, 31.1, 26.5, 19.3, -5.1; IR (KBr) ν 2955, 2930, 1257, 1103, 836, 777 cm^{-1}; HRMS (HR-EI) calcd for $C_9H_{21}BrOSi$ $[M]^+$ 252.0545, found 252.0545.

3-((*tert*-butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)aniline (S5): Off oil (931 mg, 2.36 mmol, 54 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (t, *J* = 8.0 Hz, 1H), 6.28-6.18 (m, 2H), 6.13 (t, *J* = 2.4 Hz, 1H), 3.76 (t, *J* = 5.6 Hz, 2H), 3.20 (t, *J* = 6.4 Hz, 2H), 1.88-1.79 (m, 2H), 0.97 (s, 9H), 0.91 (s, 9H), 0.18 (s, 6H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 149.7, 129.9, 109.6, 106.8, 105.1, 62.3, 42.7, 31.9, 26.2, 25.9, 18.5, 18.4, -4.1, -5.2; IR (KBr) ν 2955, 2929, 1645, 1195, 836, 778 cm^{-1}; HRMS (HR-ESI) calcd for $C_{21}H_{42}NO_2Si_2$ $[M+H]^+$ 396.2754, found 396.2761.

3-((*tert*-butyldimethylsilyl)oxy)-*N*-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-*N*-(prop-2-yn-1-yl)aniline (S6): pale yellow oil (260 mg, 0.60 mmol, 95 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (t, *J* = 8.4 Hz, 1H), 6.47 (d, *J* = 8.0 Hz, 1H), 6.34 (s, 1H), 6.26 (d, *J* = 8.4 Hz, 1H), 3.99 (d, *J* = 2.4 Hz, 2H), 3.69 (t, *J* = 5.6 Hz, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.18 (t, *J* = 2.4 Hz, 1H), 1.88-1.78 (m, 2H), 0.98 (s, 9H), 0.91 (s, 9H), 0.20 (s, 6H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.8, 149.6, 129.8, 109.6, 107.3, 105.9, 80.4, 71.9, 60.7, 48.5, 40.5, 30.7, 26.2, 25.9, 18.5, 18.4, -4.1, -5.1; IR (KBr) ν 3312, 2067, 1643, 1603, 1578, 1255, 1193, 835, 779 cm^{-1}; HRMS (HR-ESI) calcd for $C_{24}H_{44}NO_2Si_2$ $[M+H]^+$ 434.2911, found 434.2917.

3-((3-((*tert*-butyldimethylsilyl)oxy)propyl)(prop-2-yn-1-yl)amino)phenol (S7): Brown oil (220 mg, 0.69 mmol, 90 %); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (t, *J* = 8.1 Hz, 1H), 6.44 (dd, *J* = 9.9, 1.8 Hz, 1H), 6.34 (s, 1H), 6.23 (dd, *J* = 7.8, 1.5 Hz, 1H), 4.01 (d, *J* = 2.1 Hz, 2H), 3.69 (t, *J* = 5.7 Hz, 2H), 3.46 (t, *J* = 7.2 Hz, 2H), 2.18 (t, *J* = 2.1 Hz, 1H), 1.89-1.75 (m, 2H), 0.92 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.8, 149.9, 130.3, 106.5, 104.6, 100.7, 80.4, 71.9, 60.6, 48.4, 40.4, 30.7, 26.2, 18.5, -5.1; IR (KBr) ν 3433, 2067, 1638, 1502, 1010, 832, 775 cm^{-1}; HRMS (HR-APCI) calcd for $C_{18}H_{30}NO_2Si$ $[M+H]^+$ 320.2046, found 320.2050.

4-sulfamoyl-*N*-(2-(2-(2-(trityloxy)ethoxy)ethoxy)ethyl)benzamide (S10): White solid (2912 mg, 5.07 mmol, 82 %), mp : 157-161 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94-7.85 (m, 4H), 7.48-7.41 (m, 6H), 7.32-7.25 (m, 6H), 7.24-7.17 (m, 3H), 3.73-3.64 (m, 8H), 3.59 (t, *J* = 5.6 Hz, 2H), 3.21 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0, 147.8, 145.6, 139.1, 130.0, 129.1, 128.9, 128.2, 127.4, 88.1, 72.0, 71.9, 71.6, 70.7, 64.6, 41.3; IR (KBr) ν 1651, 1645, 1568, 1338, 1154, 1095, 1012 cm^{-1}; HRMS (HR-ESI) calcd for $C_{32}H_{34}N_2O_6SNa$ $[M+Na]^+$ 597.2035, found 597.2040.

***N*-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S11):** Off oil (1129 mg, 3.40 mmol, 81 %); ^{1}H NMR (400 MHz, MeOD) δ 7.97 (s, 4H), 3.71-3.63 (m, 8H), 3.62-3.54 (m, 4H); ^{13}C NMR (100 MHz, MeOD) δ 169.1, 147.9, 139.2, 129.2, 127.5, 73.8, 71.6, 71.5, 70.6, 62.3, 41.2; IR (KBr) ν 3307, 1646, 1549, 1334, 1168, 1097 cm^{-1}; HRMS (HR-ESI) calcd for $C_{13}H_{20}N_2O_6SNa$ $[M+Na]^+$ 355.0940, found 355.0931.

2-(2-(2-(4-sulfamoylbenzamido)ethoxy)ethoxy)ethyl-4-methylbenzenesulfonate (S12): Off oil (897 mg, 1.85 mmol, 85 %); ^{1}H NMR (400 MHz, MeOD) δ 8.00-7.92 (m, 4H), 7.77 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 4,15-4.10 (m, 2H), 3.70-3.62 (m, 4H), 3.61-3.52 (m, 6H), 2.44 (s, 3H); ^{13}C NMR (100 MHz, MeOD) δ 169.0, 147.8, 146.7, 139.1, 134.5, 131.2, 129.2, 127.5, 71.7, 71.4, 70.9, 70.6, 69.9, 41.2, 21.7; IR (KBr) ν 3390, 1645, 1542, 1338, 1172, 1095 cm^{-1}; HRMS (HR-ESI) calcd for $C_{20}H_{26}N_2O_8S_2Na$ $[M+Na]^+$ 509.1028, found 509.1025.

***N*-(2-(2-(2-(3-((3-((*tert*-butyldimethylsilyl)oxy)propyl)(prop-2-yn-1-yl)amino)phenoxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S13):** Pale yellow solid (57 mg, 0.09 mmol, 45 %); mp : 122-127 ^{o}C; ^{1}H NMR (400 MHz, MeOD) δ 7.94 (s, 4H), 7.07 (t, *J* = 8.4 Hz, 1H), 6.47 (dd, *J* = 8.4, 2.4 Hz, 1H), 6.40 (t, *J* = 2.4 Hz, 1H), 6.29 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.09-4.05 (m, 2H), 4.02 (d, *J* = 2.4 Hz,

2H), 3.85,-3.80 (m, 2H), 3.74-3.65 (m, 8H), 3.59 (t, *J* = 5.2 Hz, 2H), 3.44 (t, *J* = 6.8 Hz, 2H), 2.53 (t, *J* = 2.8 Hz, 1H), 1.86-1.77 (m, 2H), 0.93 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, MeOD) δ 169.0, 161.3, 151.1, 147.8, 139.1, 130.9, 129.2, 127.4, 108.3, 104.2, 102.1, 81.5, 73.1, 71.9, 71.4, 71.1, 70.6, 68.5, 61.7, 41.3, 41.0, 31.8, 26.6, 19.3, -5.1; IR (KBr) ν 3281, 2508, 1645, 1599, 1447, 1337, 1164 cm^{-1}; HRMS (HR-ESI) calcd for $C_{31}H_{47}N_3O_7SSiNa$ $[M+Na]^+$ 656.2802, found 656.2805.

***N*-(2-(2-(2-((1-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-4-(tosylimino)-1,2,3,4-tetrahydroquinolin-7-yl)oxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (S14):**
Yellow oil (20 mg, 0.02 mmol, 28 %); ^{1}H NMR (400 MHz, MeOD) δ 7.92, (s, 4H), 7.90-7.81 (m, 3H), 7.39 (d, *J* = 8.4 Hz, 2H), 6.28-6.20 (m, 2H), 4.13 (t, *J* = 4.0 Hz, 2H), 3.82 (t, *J* = 4.4 Hz, 2H), 3.75-3.63 (m, 8H), 3.57 (t, *J* = 5.2 Hz, 2H), 3.49 (t, *J* = 6.8 Hz, 2H), 3.43 (t, *J* = 6.8 Hz, 2H), 3.23 (t, *J* = 7.2 Hz, 2H), 2.44 (s, 3H), 1.84-1.75 (m, 2H), 0.92 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, MeOD) δ 176.9, 168.9, 167.7, 155.3, 147.8, 144.8, 141.2, 139.0, 132.3, 130.7, 130.6, 129.2, 127.8, 127.4, 113.5, 107.3, 97.8, 71.9, 71.4, 70.7, 70.6, 68.9, 61.6, 41.3, 32.5, 30.5, 26.6, 21.7, 19.3, -5.1; IR (KBr) ν1645, 1542, 1338, 1172, 1095 cm^{-1}; HRMS (HR-FAB) calcd for $C_{38}H_{55}N_4O_9S_2Si$ $[M+H]^+$ 803.3180, found 803.3186.

***N*-(2-(2-(2-((1-(3-hydroxypropyl)-4-(tosylimino)-1,2,3,4-tetrahydroquinolin-7-yl)oxy)ethoxy)ethoxy)ethyl)-4-sulfamoylbenzamide (SA-TEG-DQI):** Yellow oil (13 mg, 0.019 mmol, 56 %); ^{1}H NMR (400 MHz, MeOD) δ 7.92 (s. 4H), 7.86-7.81 (m, 3H), 7.39 (d, *J* = 8.8 Hz, 2H), 6.29 (d, *J* = 2.4 Hz, 1H), 6.22 (dd, *J* = 9.2, 2.4 Hz, 1H), 4.19-4.13 (m, 2H), 3.88-3.81 (m, 2H), 3.74-3.61 (m, 8H), 3.57 (t, *J* = 5.6 Hz, 2H), 3.49 (t, *J* = 7.2 Hz, 2H), 3.45-3.39 (m, 2H), 3.27-3.21 (m, 2H), 2.44 (s, 3H), 1.86-1.77 (m, 2H); ^{13}C NMR (100 MHz, MeOD) δ 176.9, 169.0, 167.8, 155.5, 147.8, 144.8, 141.2, 139.1, 132.2, 130.7, 129.2, 127.8, 127.4, 113.5, 107.9, 97.5, 71.9, 71.4, 70.7, 70.6, 68.9, 60.3, 41.3, 32.4, 30.4, 21.6; IR (KBr) ν 3408, 1644, 1549, 1330, 1167, 1097 cm^{-1}; HRMS (HR-ESI) calcd for $C_{32}H_{40}N_4O_9S_2Na$ $[M+Na]^+$ 711.2134, found 711.2125.

***N*-(3-((tert-butyldimethylsilyl)oxy)propyl)-N-(prop-2-yn-1-yl)-3-(2-(2-(2-(trityloxy)ethoxy)ethoxy)ethoxy)aniline (S15): Pale** yellow oil (373 mg, 0.538 mmol, 80 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49-7.43 (m, 6H), 7.32-7.27 (m, 6H), 7.24-7.19 (m, 3H), 7.12 (t, *J* = 8.4 Hz, 1H), 6.47 (dd, *J* = 8.4, 2.0 Hz, 1H), 6.41 (t, *J* = 2.4 Hz, 1H), 6.31 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.11 (t, *J* = 4.8 Hz, 2H), 3.99 (d, *J* = 2.4 Hz, 2H), 3.87 (t, *J* = 4.8 Hz, 2H), 3.77-3.64 (m, 8H), 3.45 (t, *J* = 6.8 Hz, 2H), 3.24 (t, *J* = 5.2 Hz, 2H), 2.15 (t, *J* = 2.4 Hz, 1H), 1.86-1.77 (m, 2H), 0.91 (s, 9H), 0.06 (s,

6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 165.9, 153.4, 144.3, 142.9, 140.1, 129.5, 128.9, 127.9, 127.1, 112.8, 105.8, 97.0, 86.8, 77.5, 71.2, 71.0, 70.9, 63.5, 48.4, 48.1, 31.6, 29.6, 26.2, 21.7, 18.5, -5.1; IR (KBr) ν 2953, 2928, 2111, 1614, 1498, 1093 cm^{-1}; HRMS (HR-FAB) calcd for $C_{43}H_{55}NO_5Si$ $[M]^+$ 693.3850, found 693.3840.

3-((3-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)phenyl)(prop-2-yn-1-yl)amino) propan-1-ol (S16): Pale yellow oil (80 mg, 0.239 mmol, 83 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (t, *J* = 8.4 Hz, 1H), 6.52-6.44 (m, 2H), 6.33 (dd, *J* = 8.4, 1.8 Hz, 1H), 4.11 (t, *J* = 5.2 Hz, 2H), 3.99 (d, *J* = 2.4 Hz, 2H), 3.82 (t, *J* = 4.4 Hz, 2H), 3.75-3.64 (m, 8H), 3.61-3.56 (m, 2H), 3.46 (t, *J* = 6.8 Hz, 2H), 2.19 (t, *J* = 2.4 Hz, 1H), 1.89-1.80 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9, 149.7, 130.0, 107.3, 103.6, 101.6, 80.3, 72.7, 72.1, 70.9, 70.3, 69.9, 67.2, 61.8, 60.4, 48.4, 40.5, 30.2; IR (KBr) ν 3401, 2953, 2108, 1643, 1499, 1174, 1064 cm^{-1}; HRMS (HR-FAB) calcd for $C_{18}H_{28}NO_5$ $[M+H]^+$ 388.1967, found 388.1966.

***N*-(7-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)-1-(3-hydroxypropyl)-2,3-dihydroquinolin-4(1*H*)-ylidene)-4-methylbenzenesulfonamide (TEG-DQI):** Yellow oil (40 mg, 0.078 mmol, 53 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 9.2 Hz, 1H), 7.89 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 6.36 (d, *J* = 2.4 Hz, 1H), 6.20 (dd, *J* = 9.2, 2.4 Hz, 1H), 4.21-4.15 (m, 2H), 3.86-3.80 (m, 2H), 3.76-3.65 (m, 8H), 3.64-3.59 (m, 2H), 3.49 (t, *J* = 6.8 Hz, 2H), 3.44-3.34 (m, 4H), 2.42 (s, 3H), 1.86-1.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 165.7, 153.6, 142.9, 139.9, 131.4, 129.5, 126.9, 112.7, 106.0, 97.2, 72.7, 70.9, 70.2, 69.6, 67.3, 61.9, 59.6, 48.1, 47.7, 31.6, 29.4, 21.7; IR (KBr) ν 3436, 2927, 1641, 1543, 1244, 1142, 1089 cm^{-1}; HRMS (HR-FAB) calcd for $C_{25}H_{35}N_2O_7S$ $[M+H]^+$ 507.2165, found 507.2161.

19. Reference

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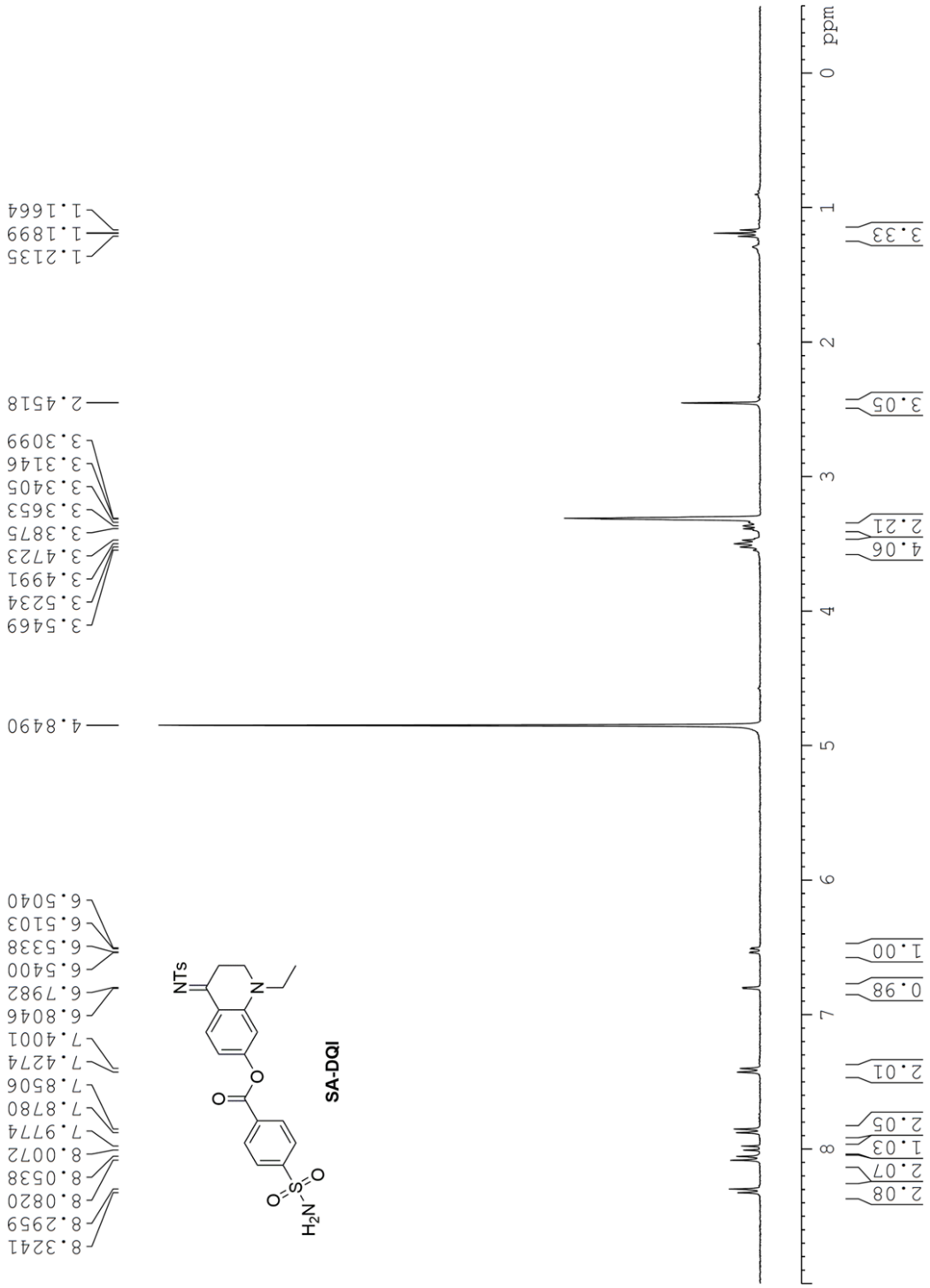
20. ^{1}H & ^{13}C NMR Spectra for the compounds



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O
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S
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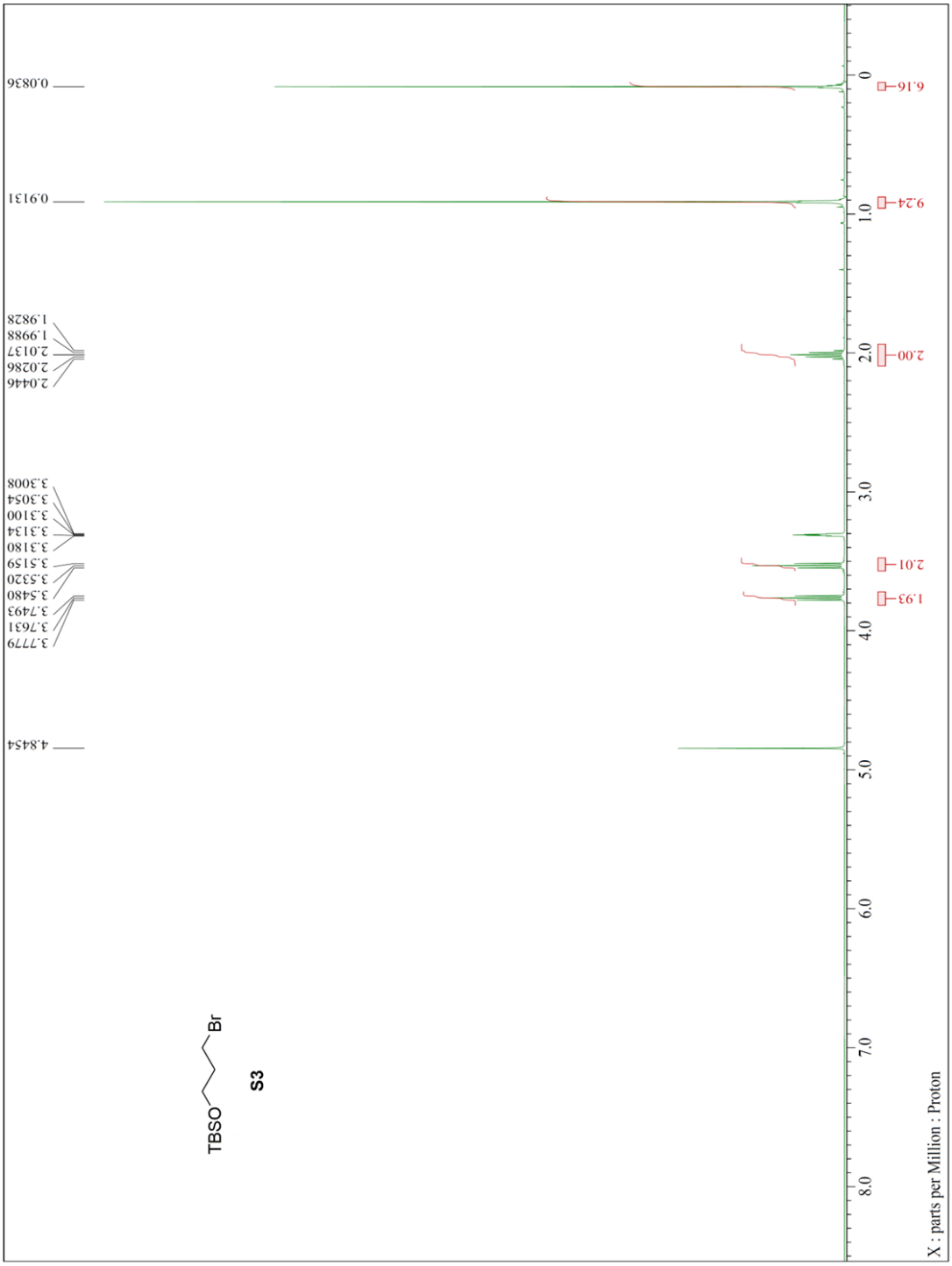
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2.00
2.01
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Br
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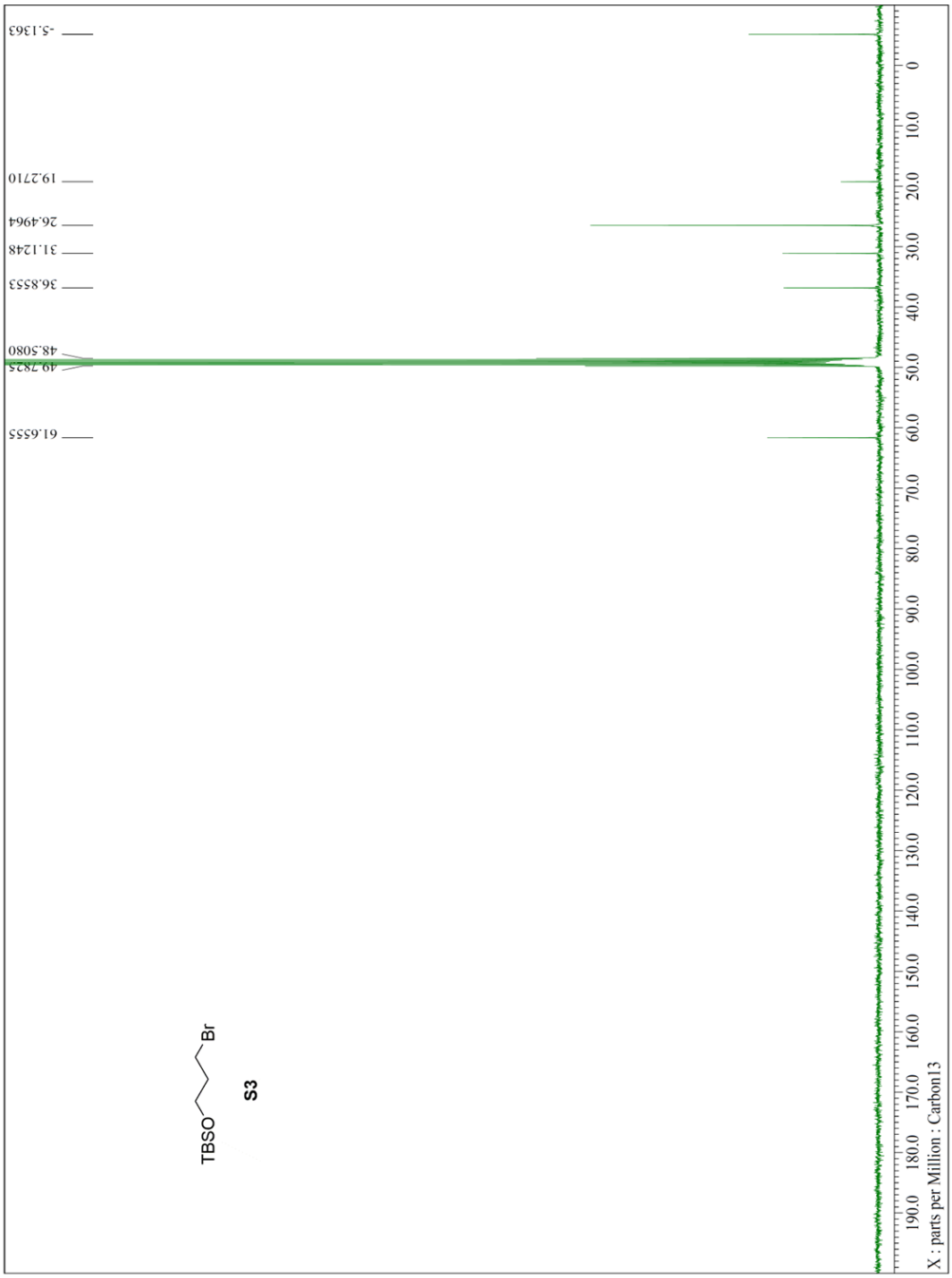
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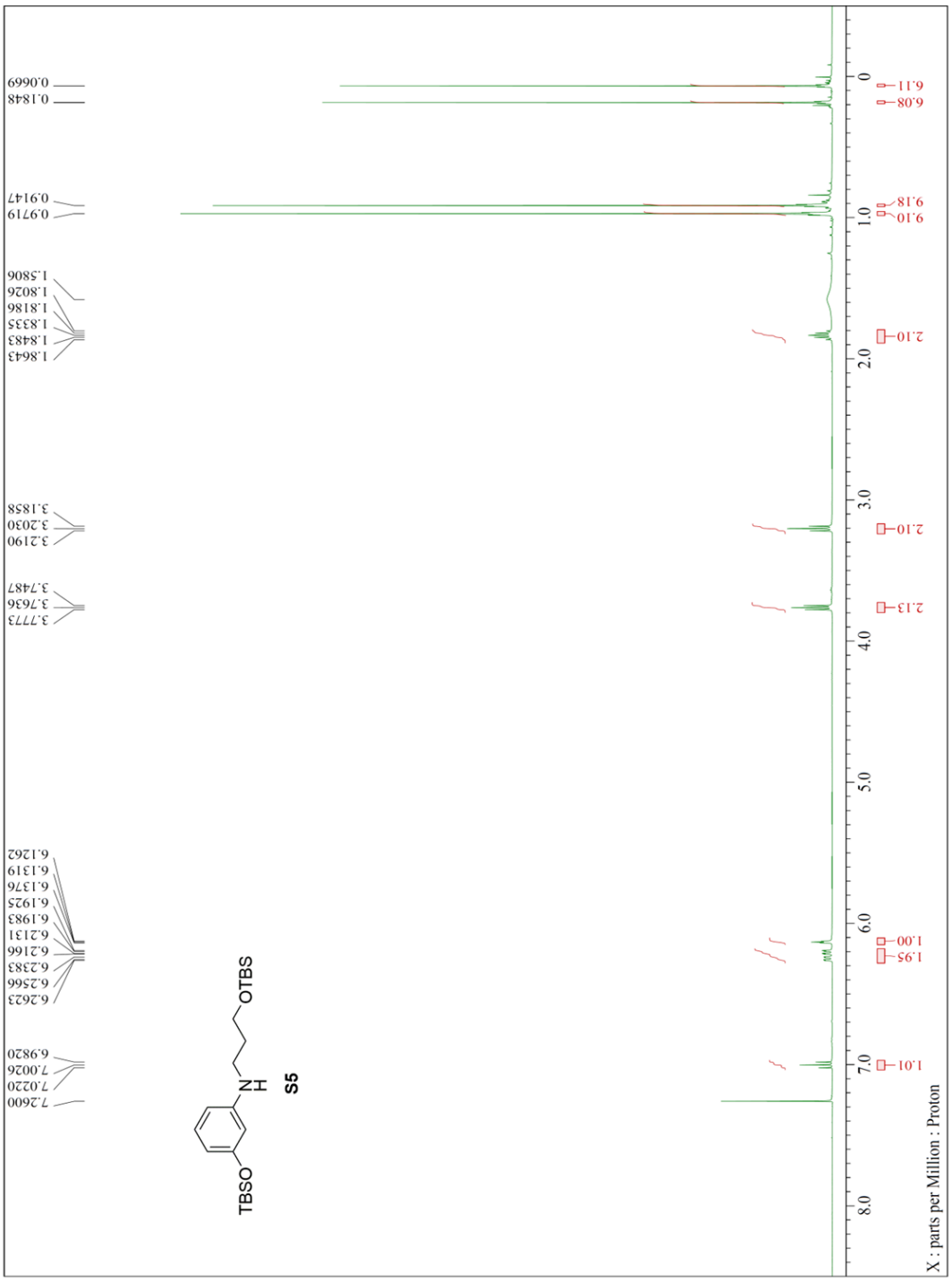
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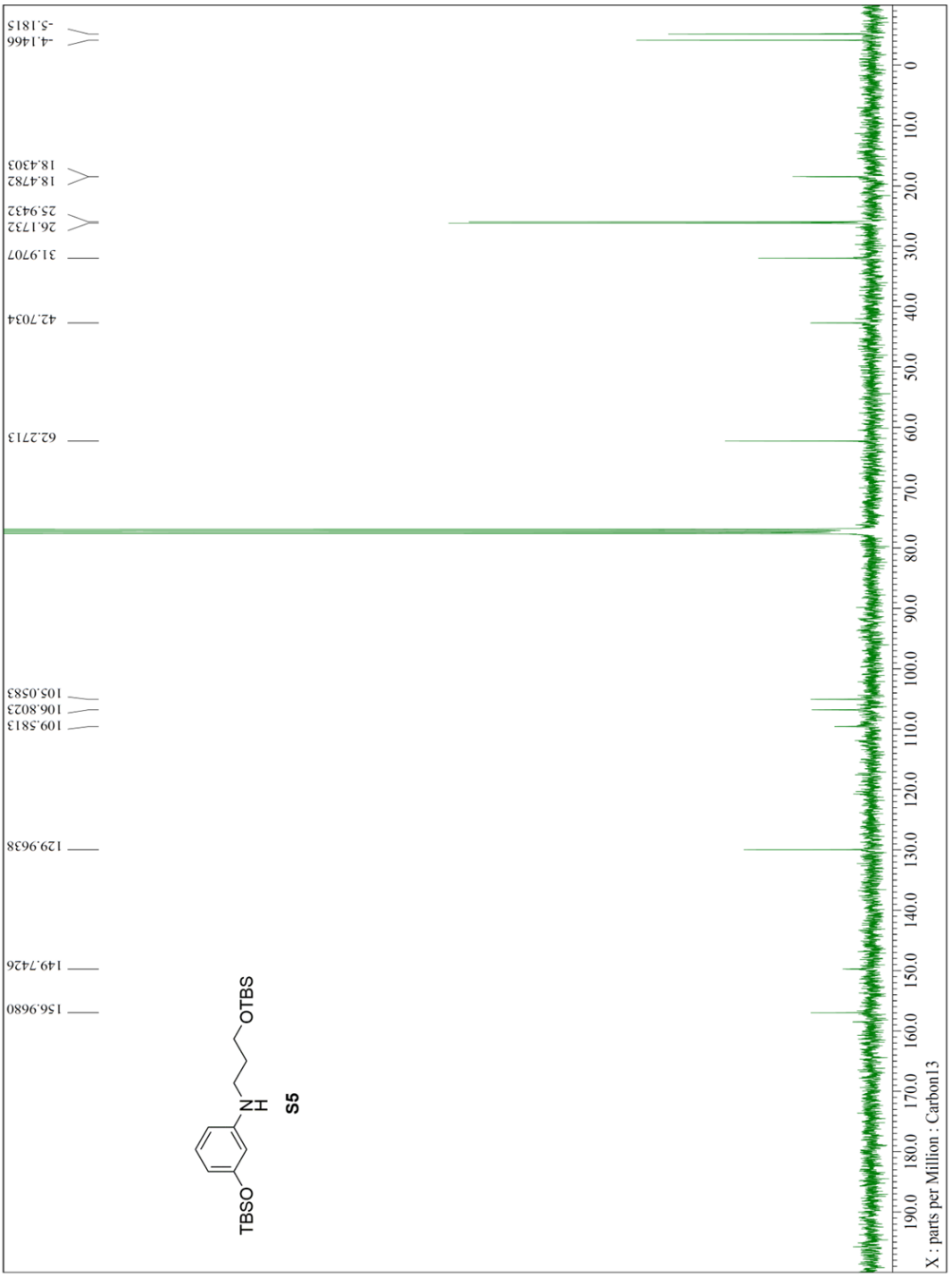
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H
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120.0
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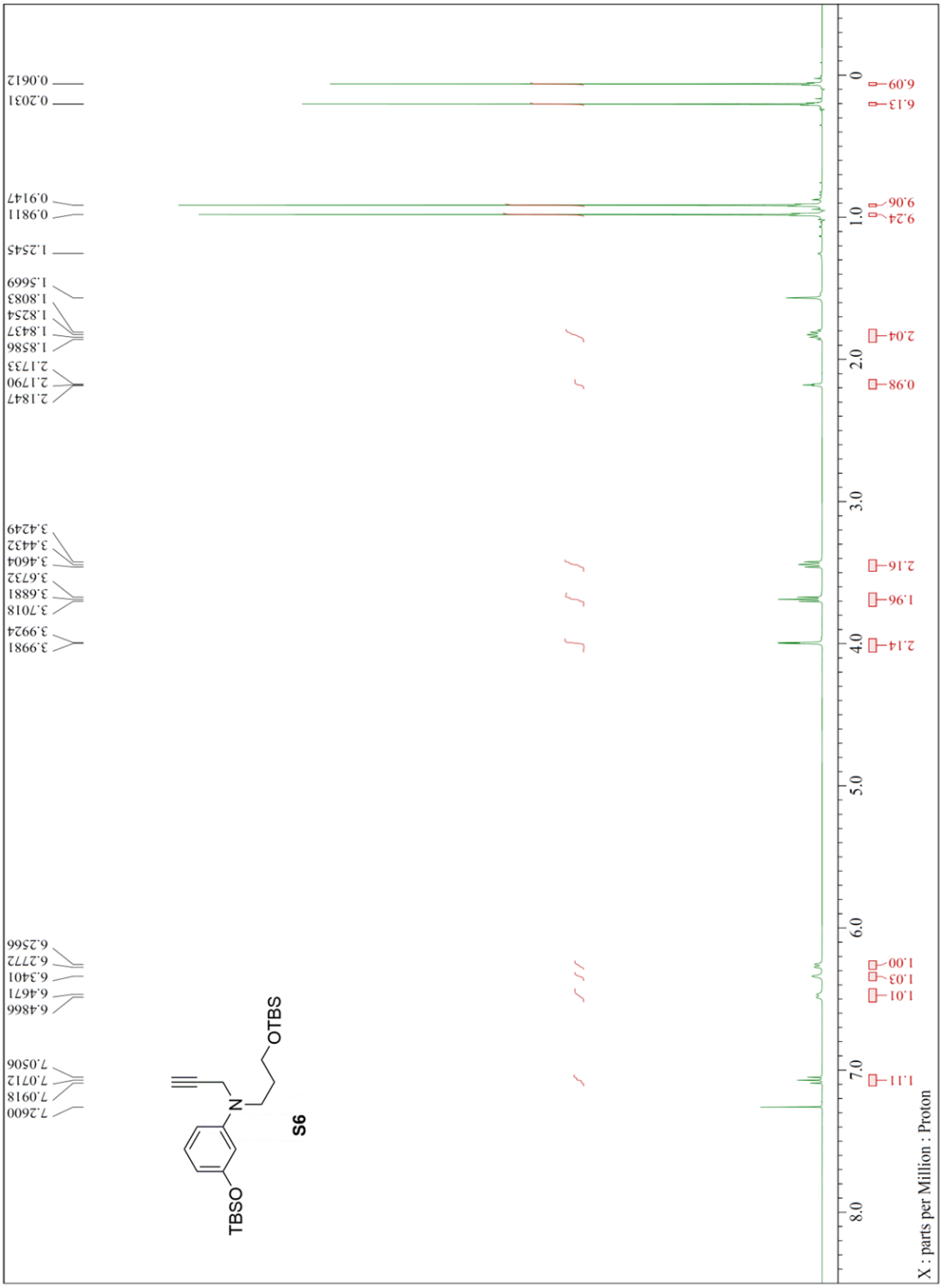
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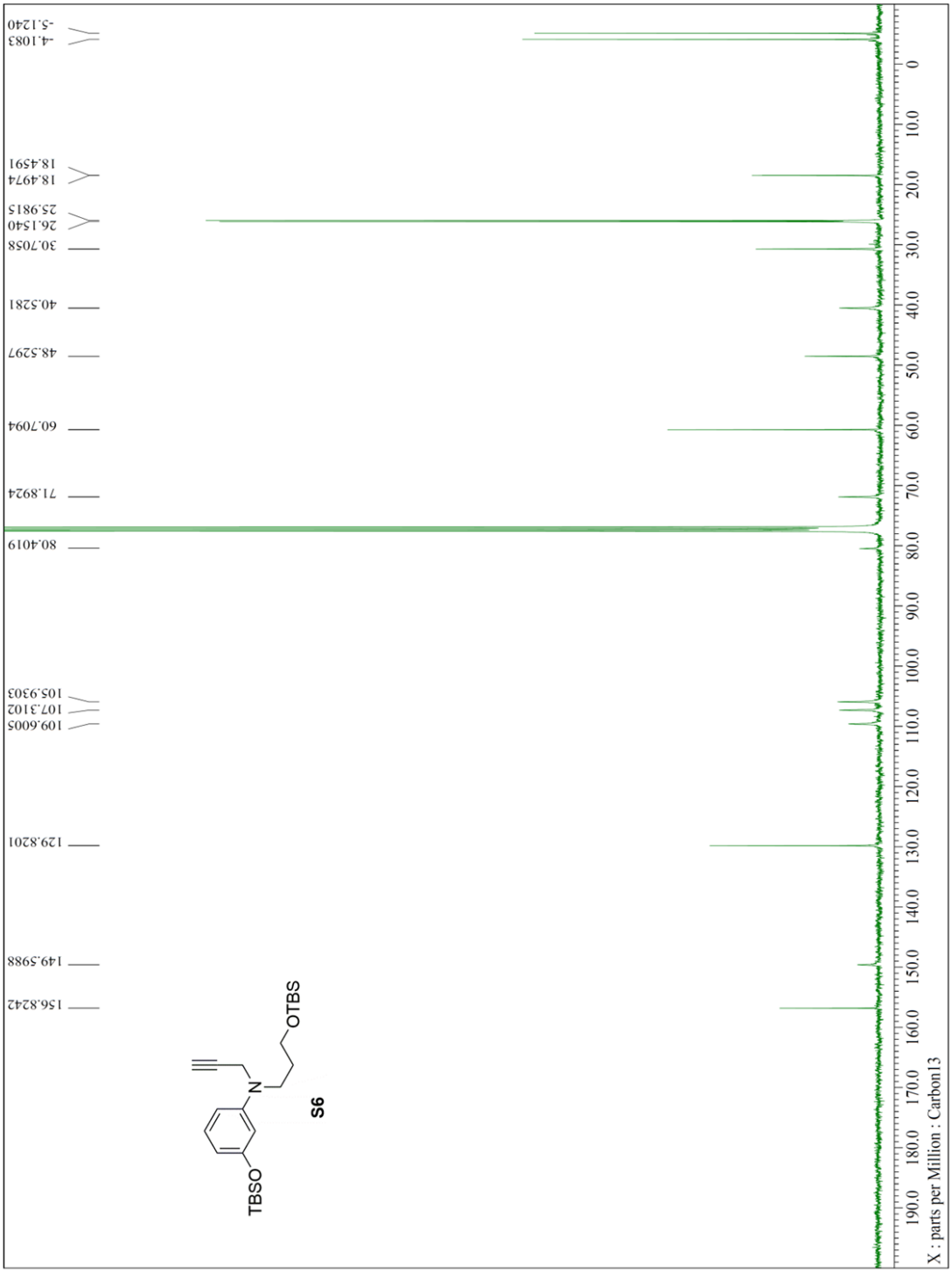
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S6
X : parts per Million : Carbon13

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6.4588
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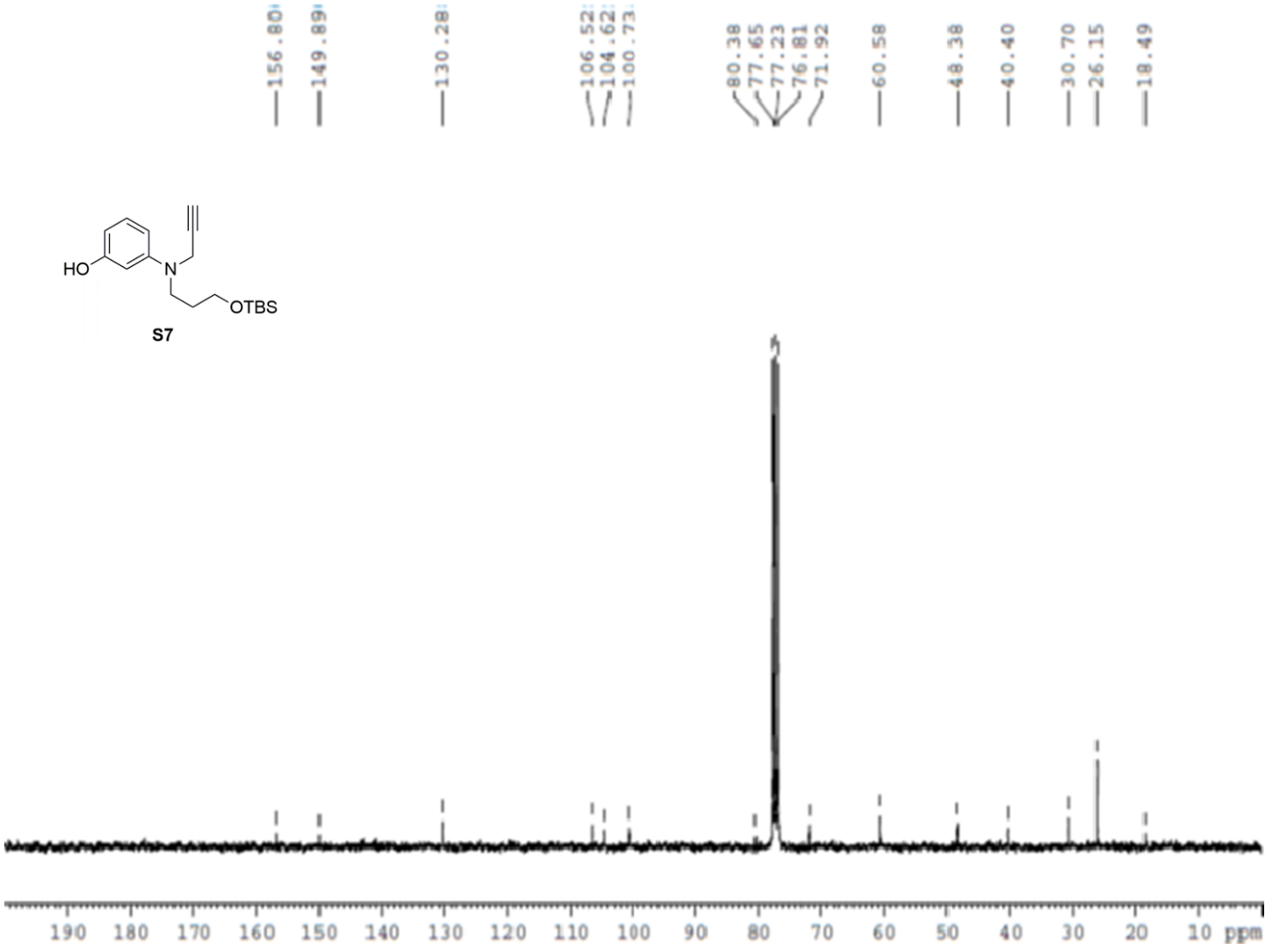
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S7
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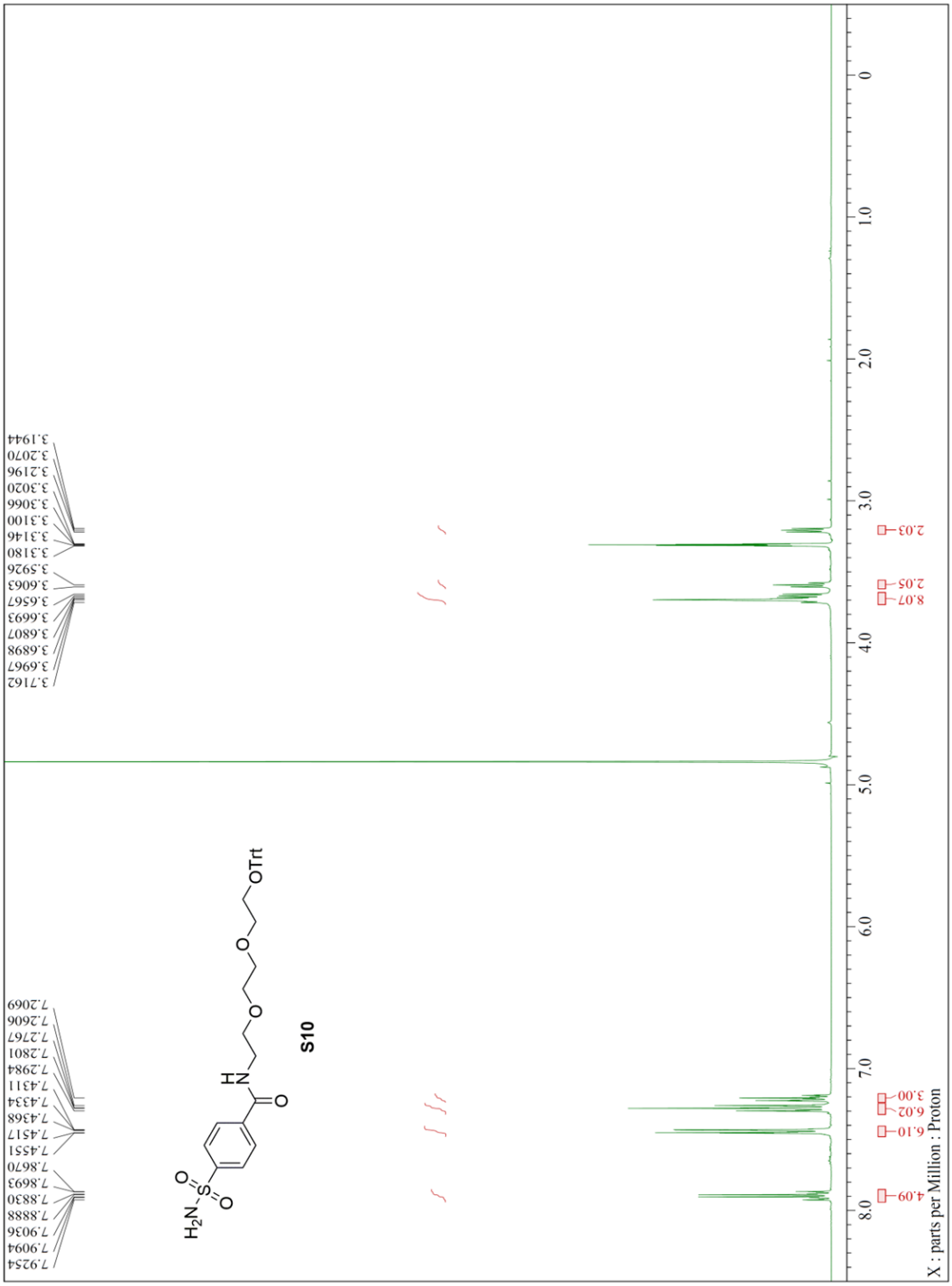
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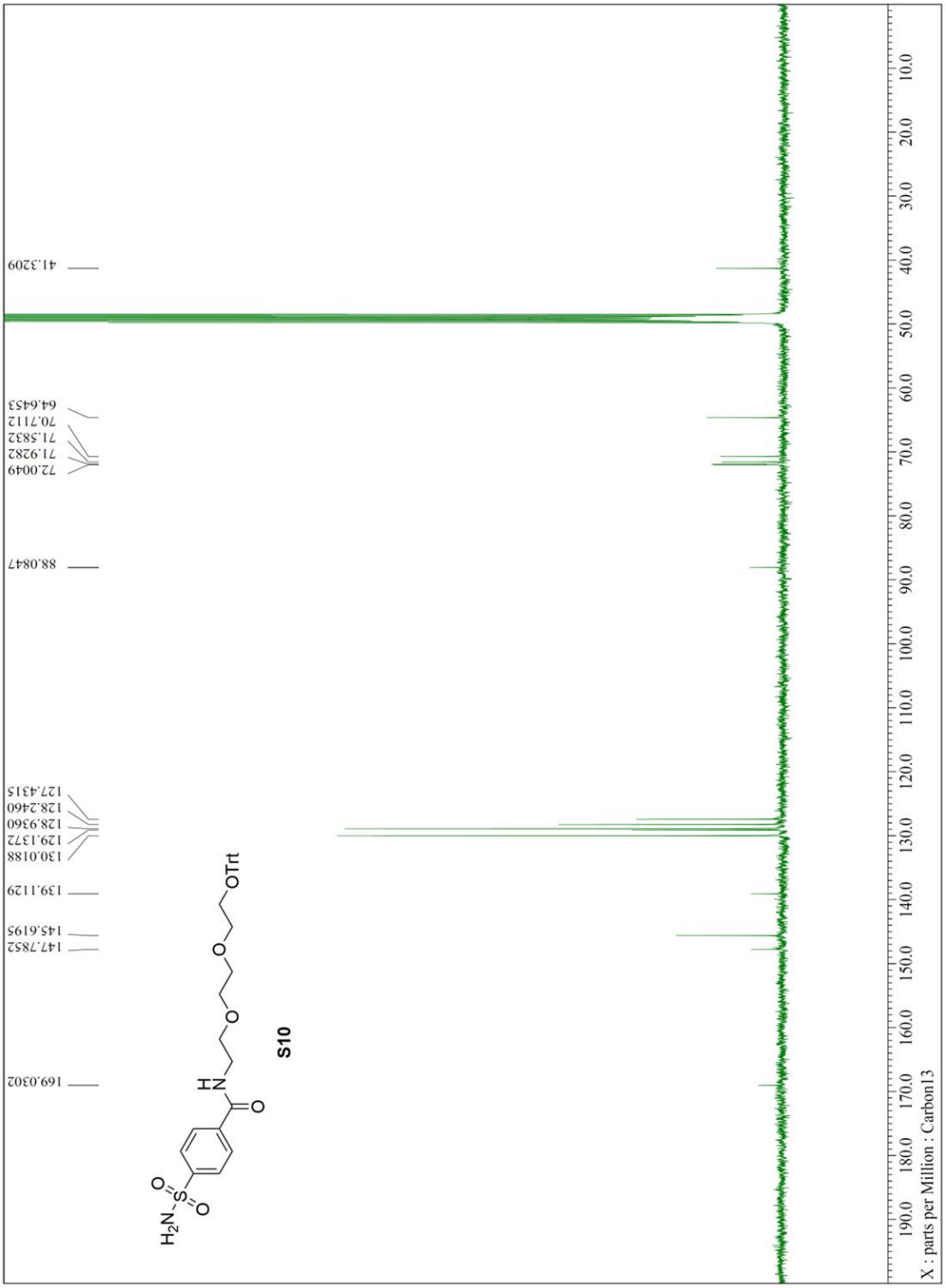
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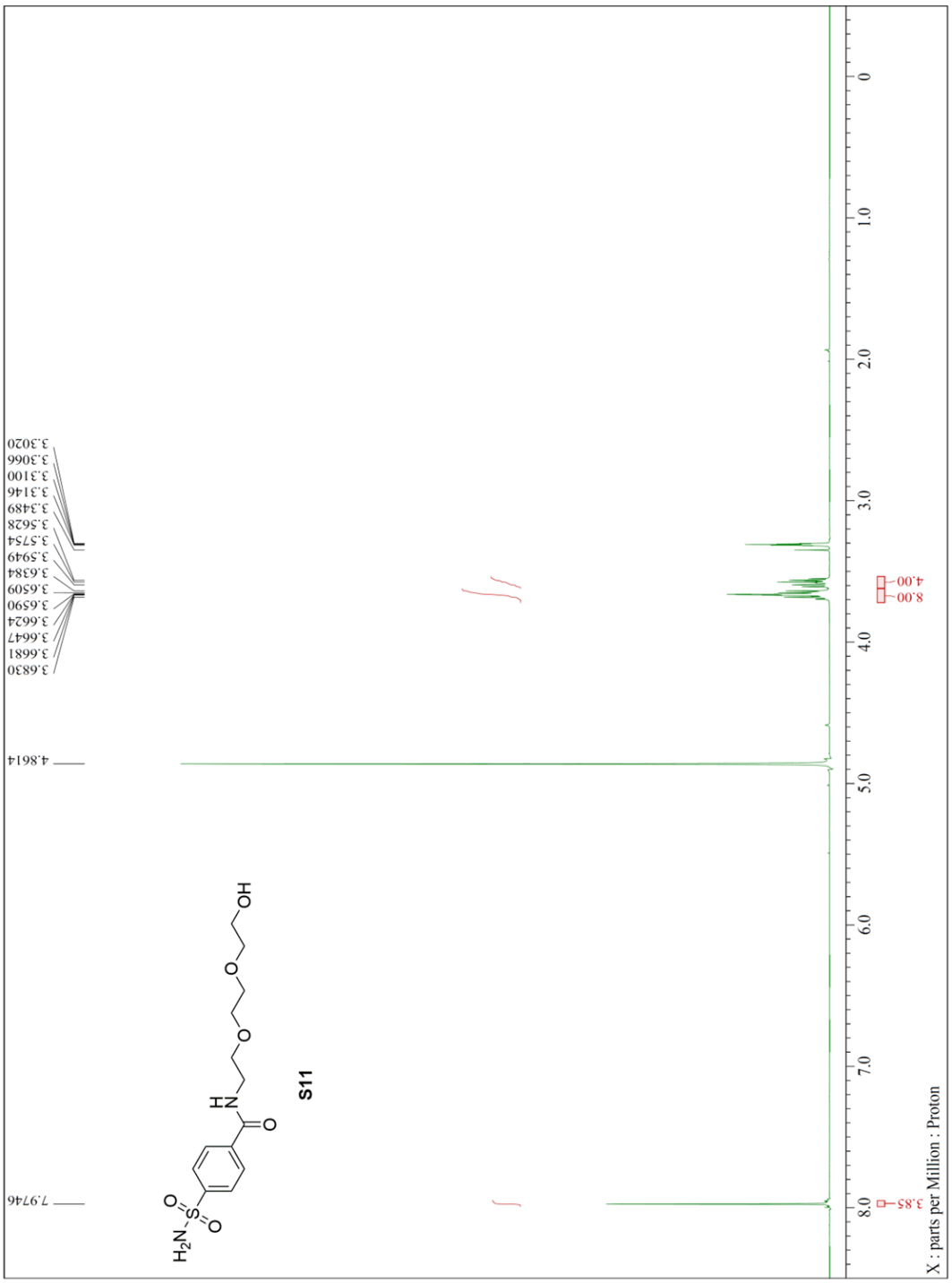
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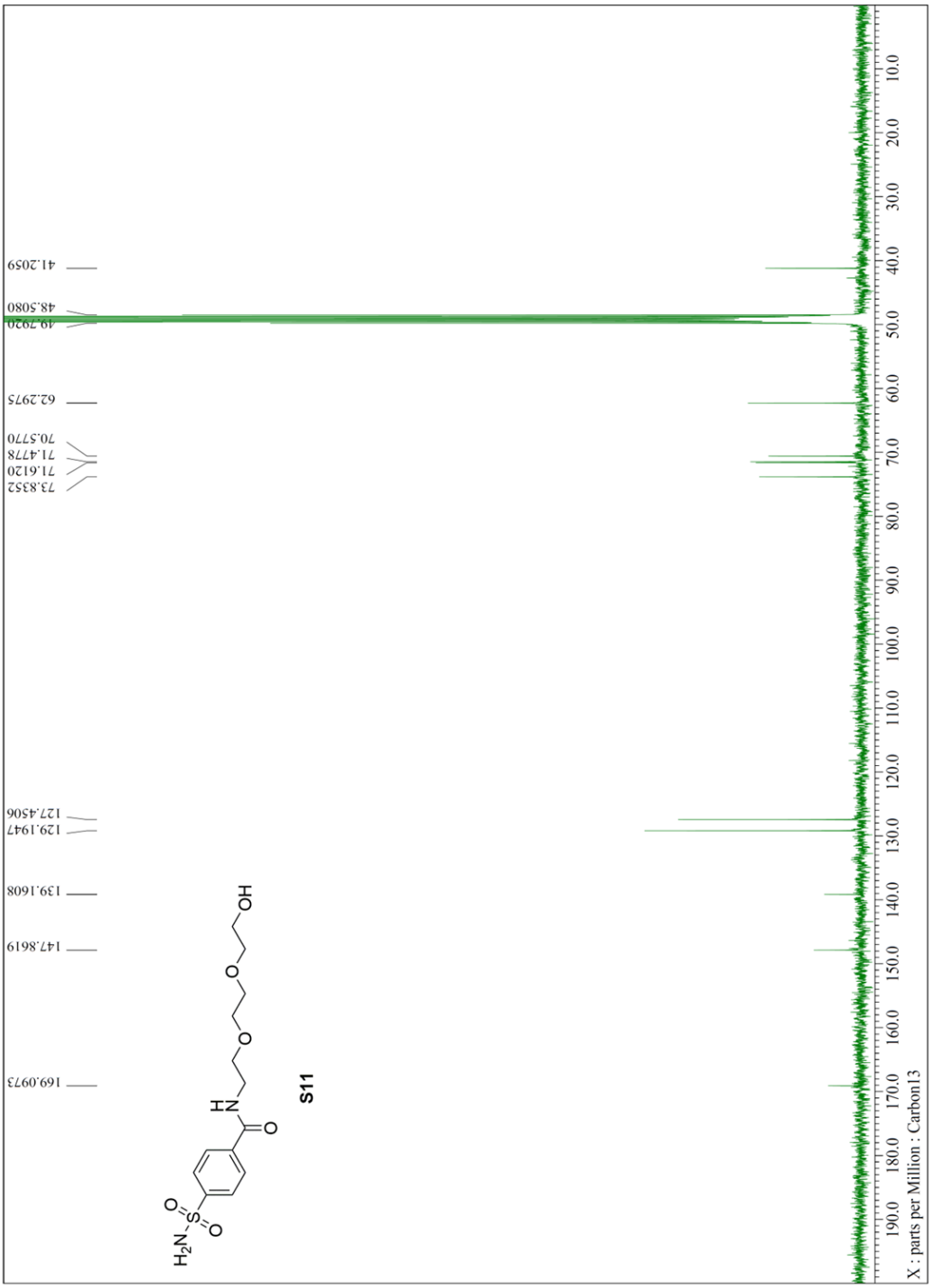
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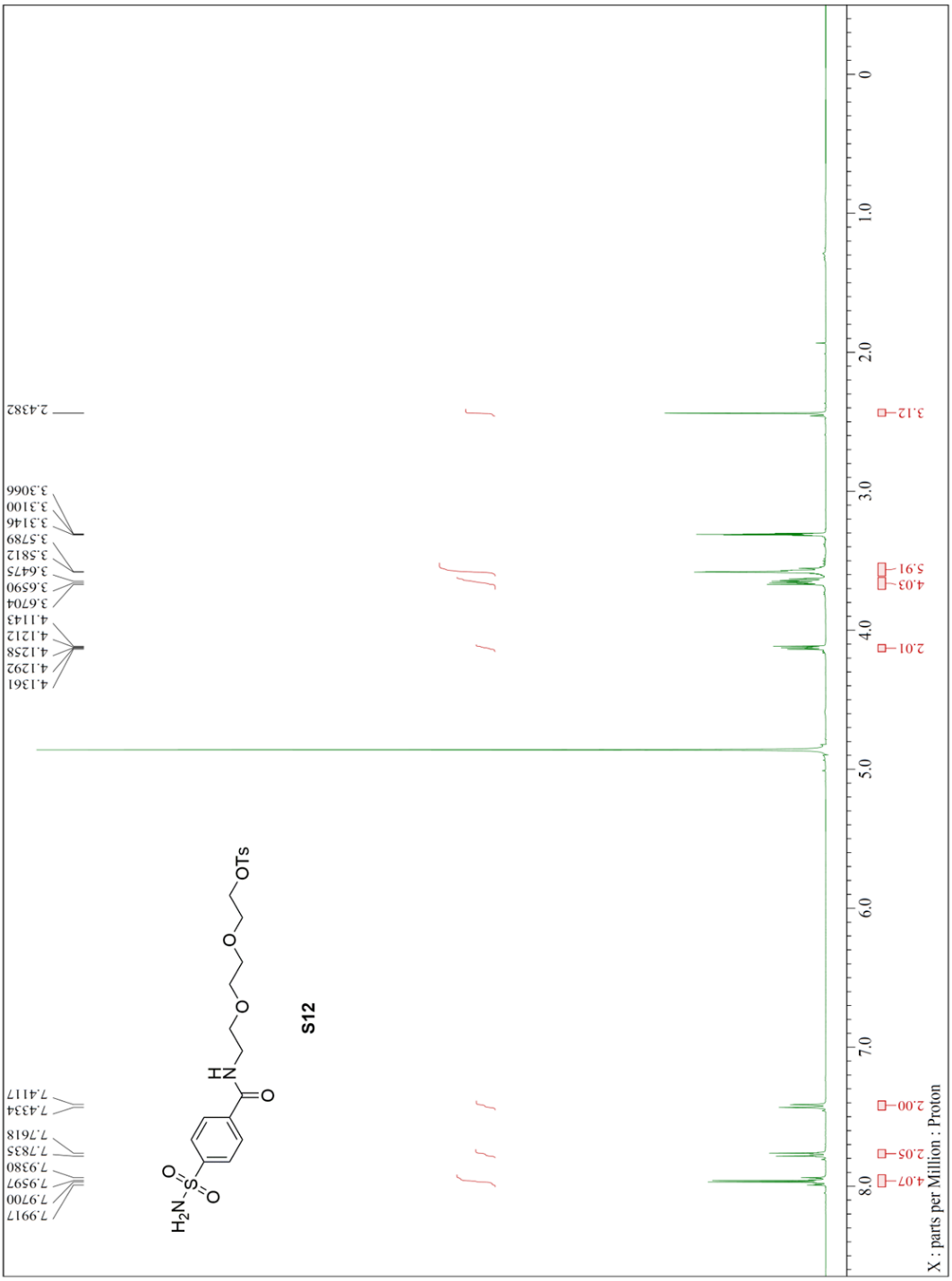
X : parts per Million : Carbon13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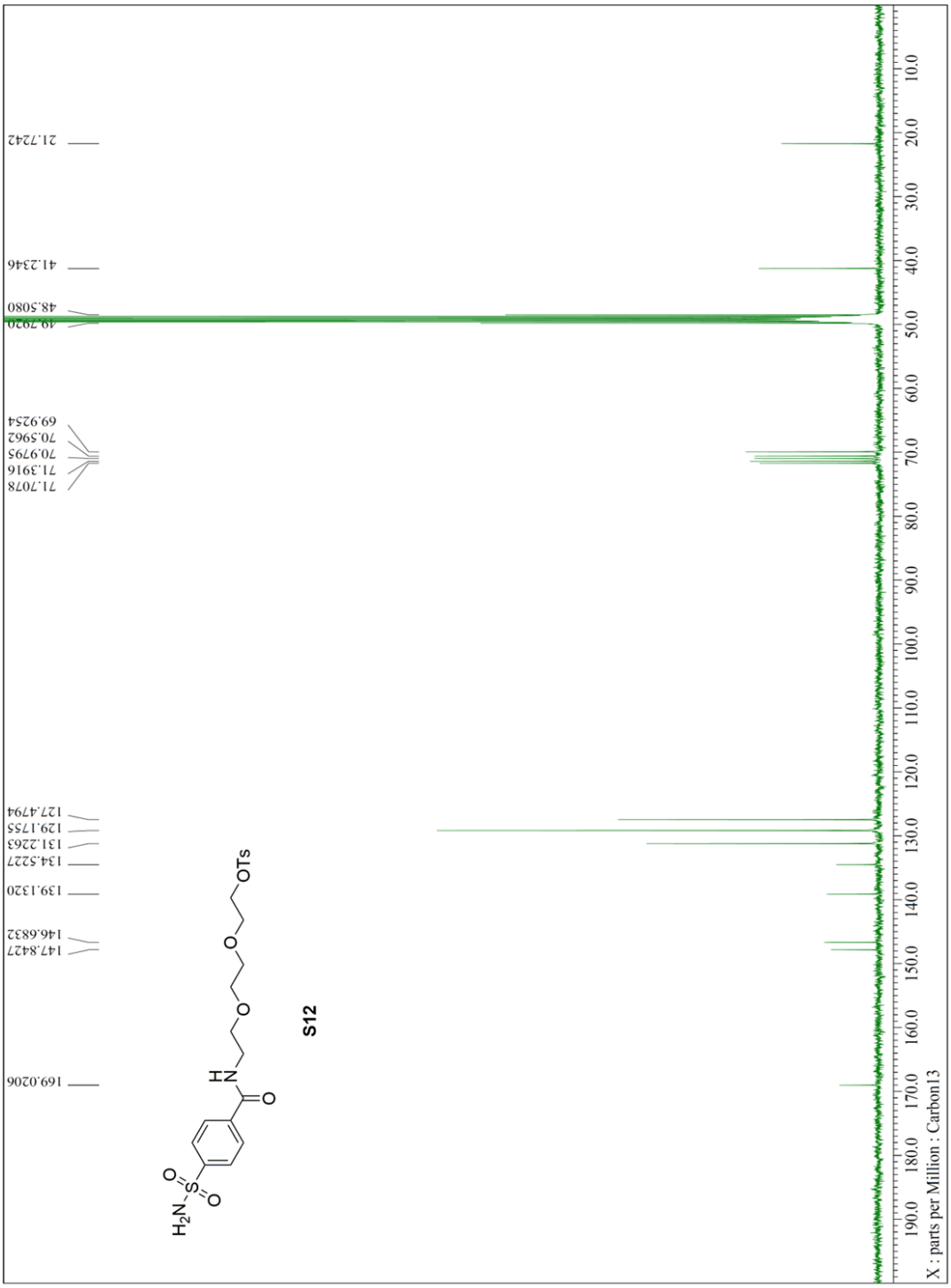
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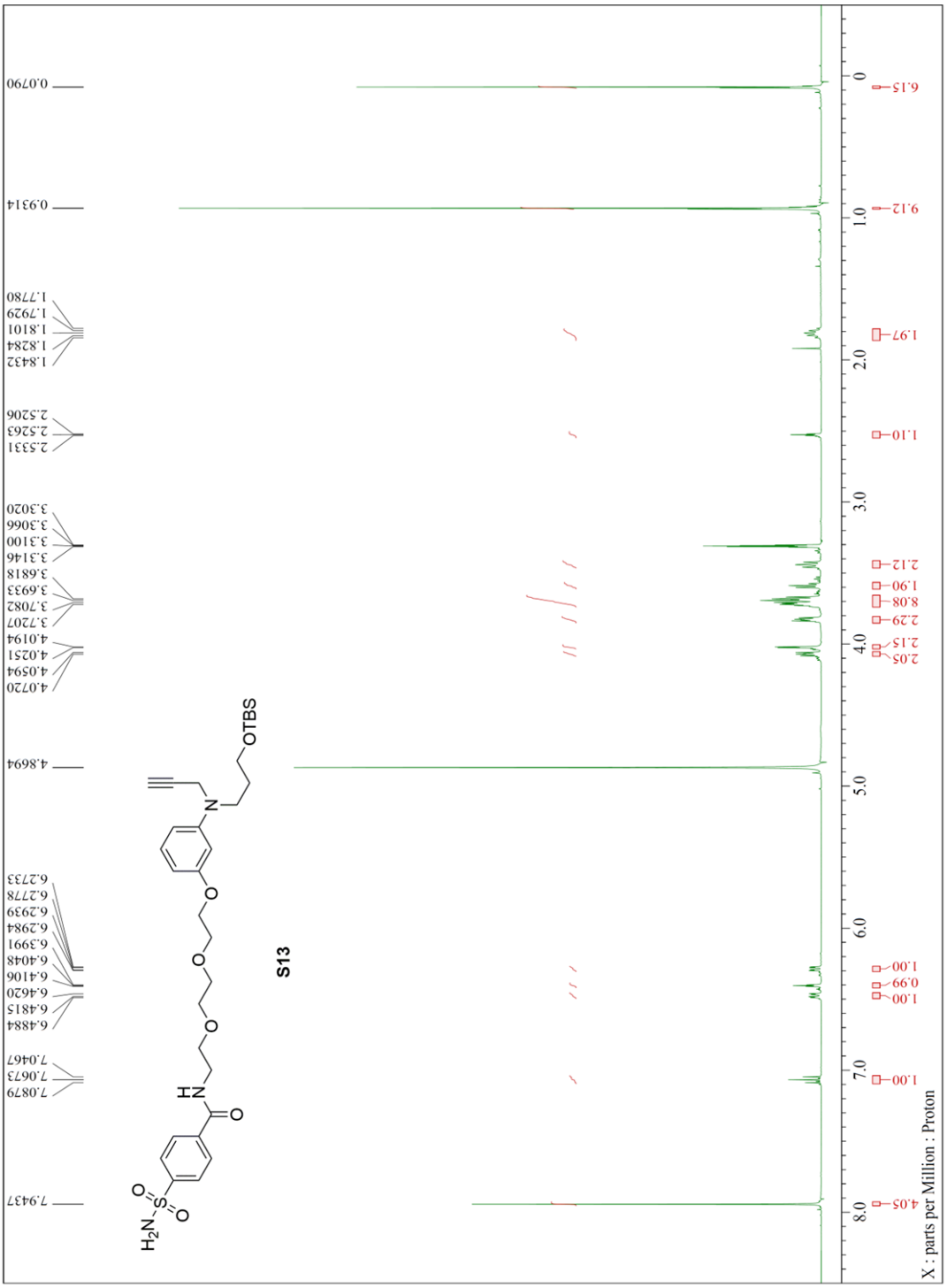
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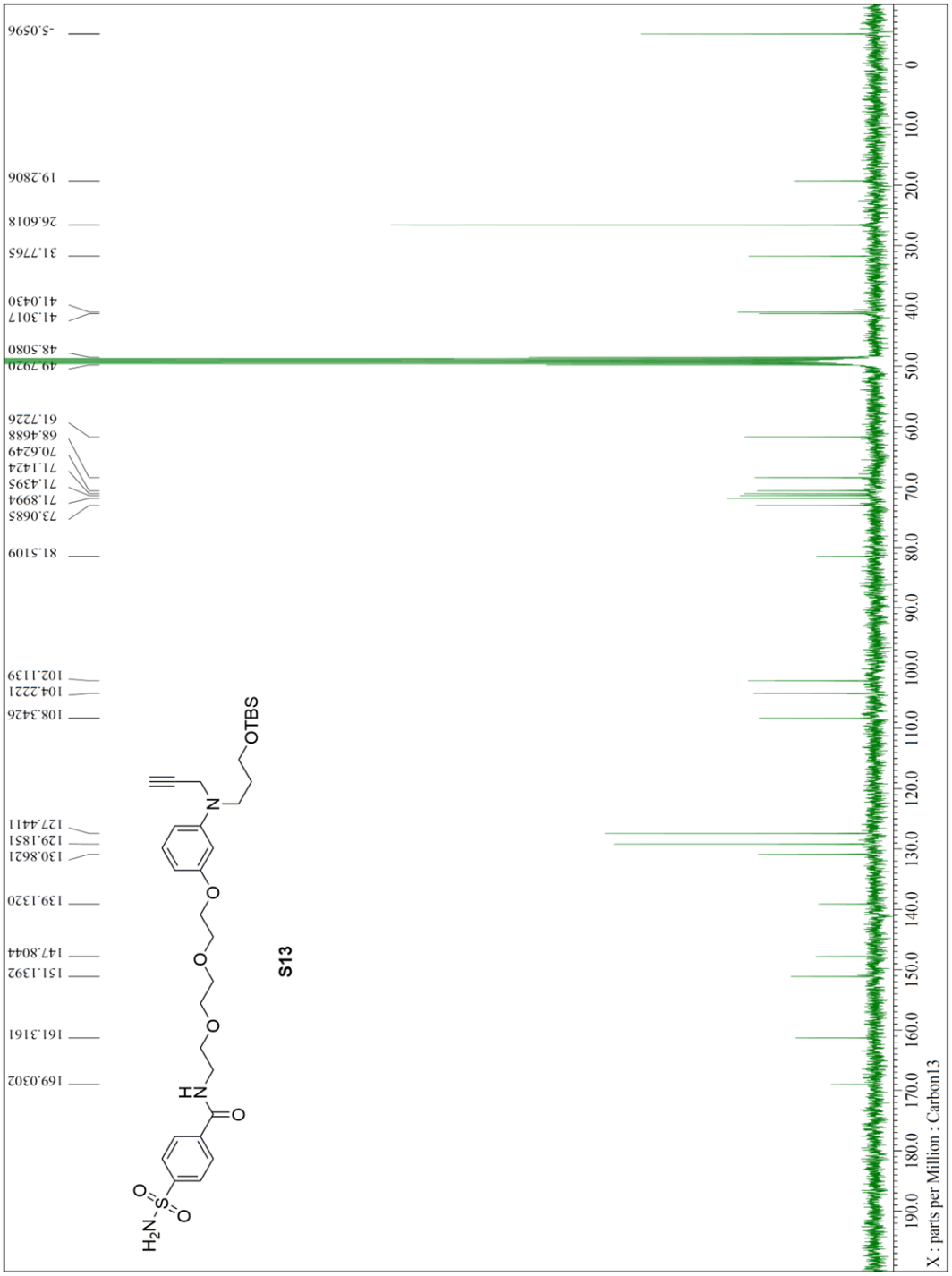
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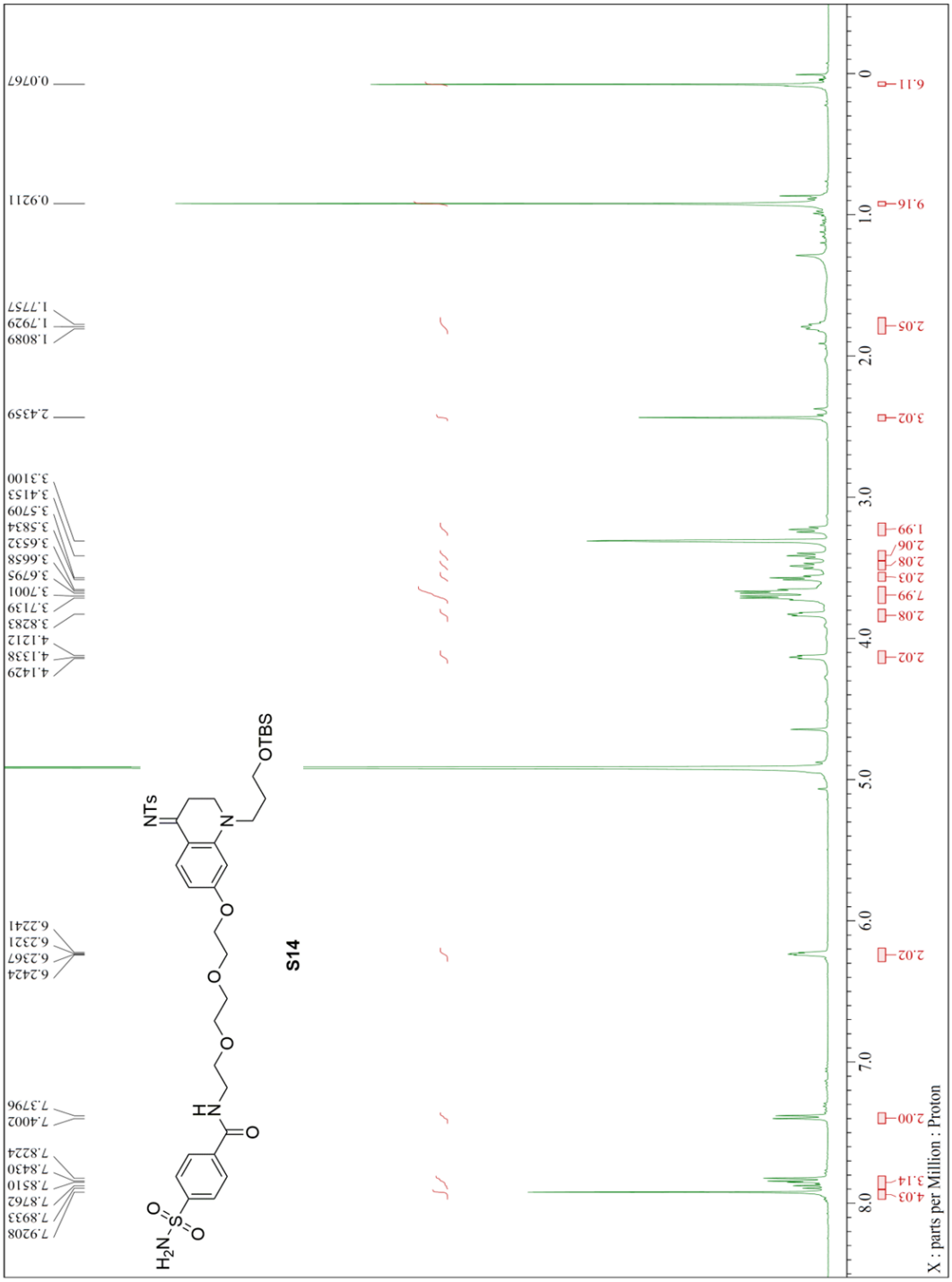
S13
X : parts per Million : Carbon13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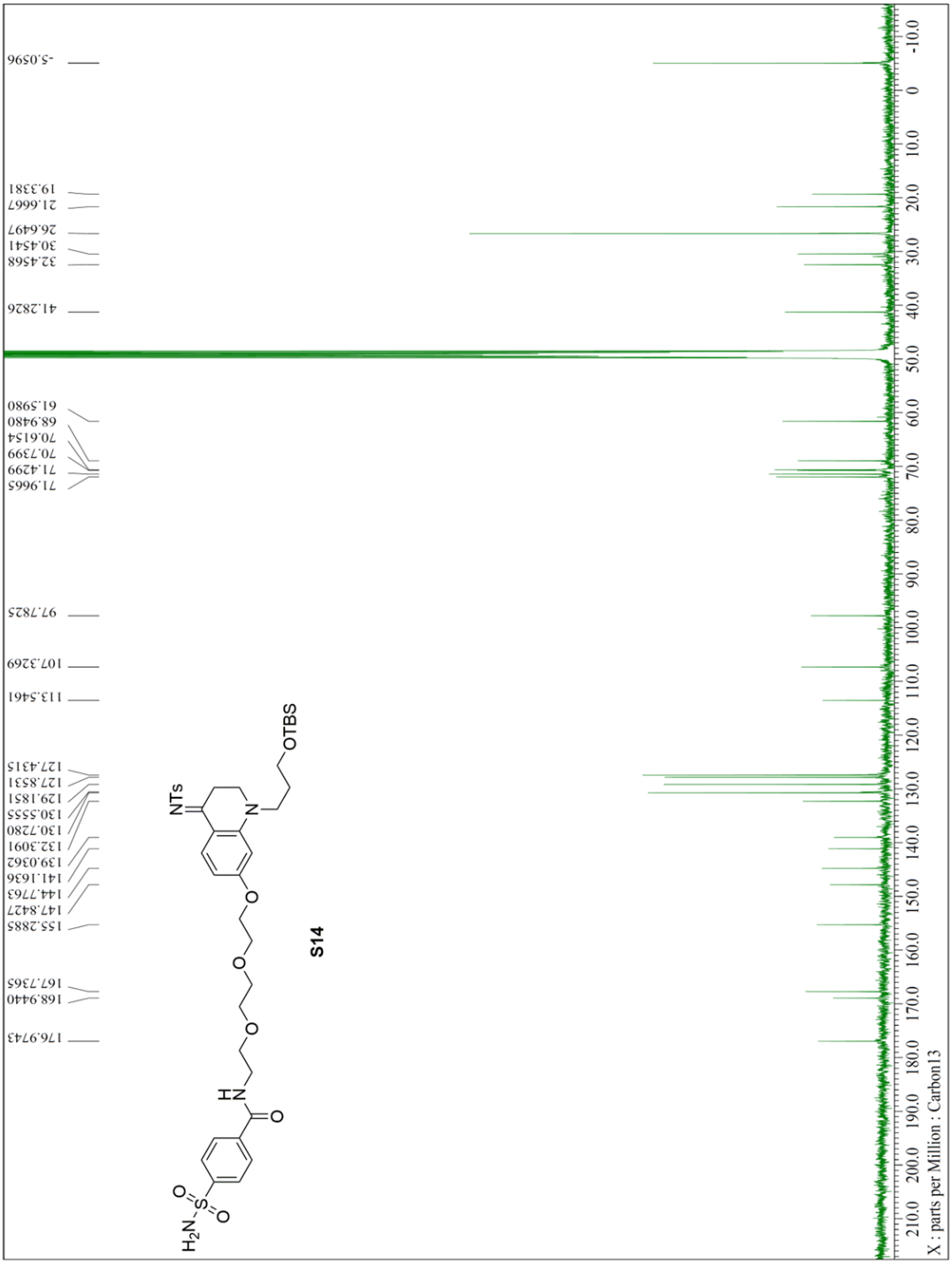
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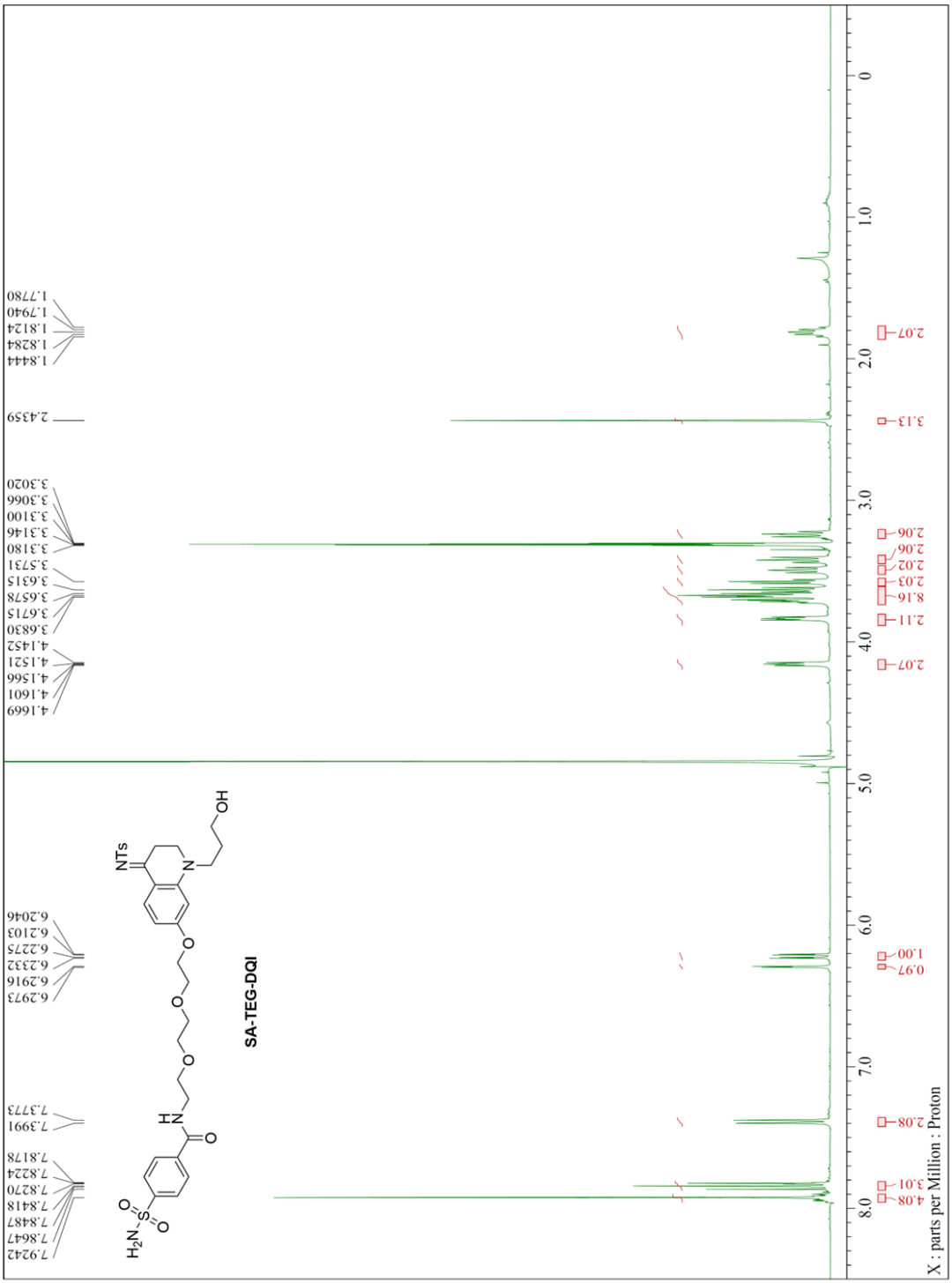
S14
X : parts per Million : Carbon13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6.2916
6.2332
6.2275
6.2103
6.2046
4.1669
4.1601
4.1566
4.1521
4.1452
3.6830
3.6715
3.6578
3.6315
3.5731
3.3180
3.3146
3.3100
3.3066
3.3020
2.4359
1.8444
1.8284
1.8124
1.7940
1.7780
2.07
3.13
2.06
2.06
2.02
2.03
8.16
2.11
2.07
0.97
1.00
2.08
3.01
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2.0
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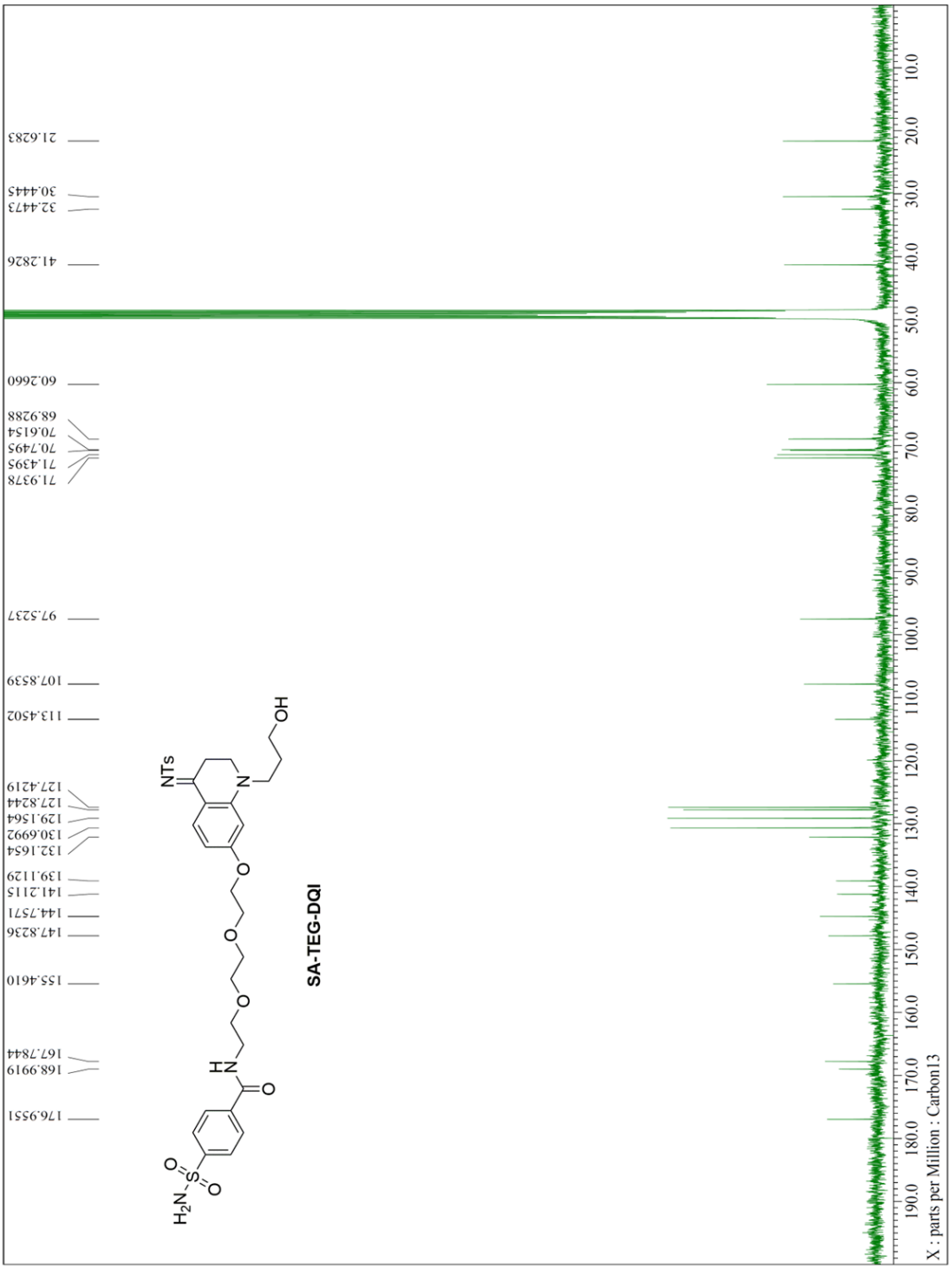
4.0
5.0
6.0
7.0
8.0
X : parts per Million : Proton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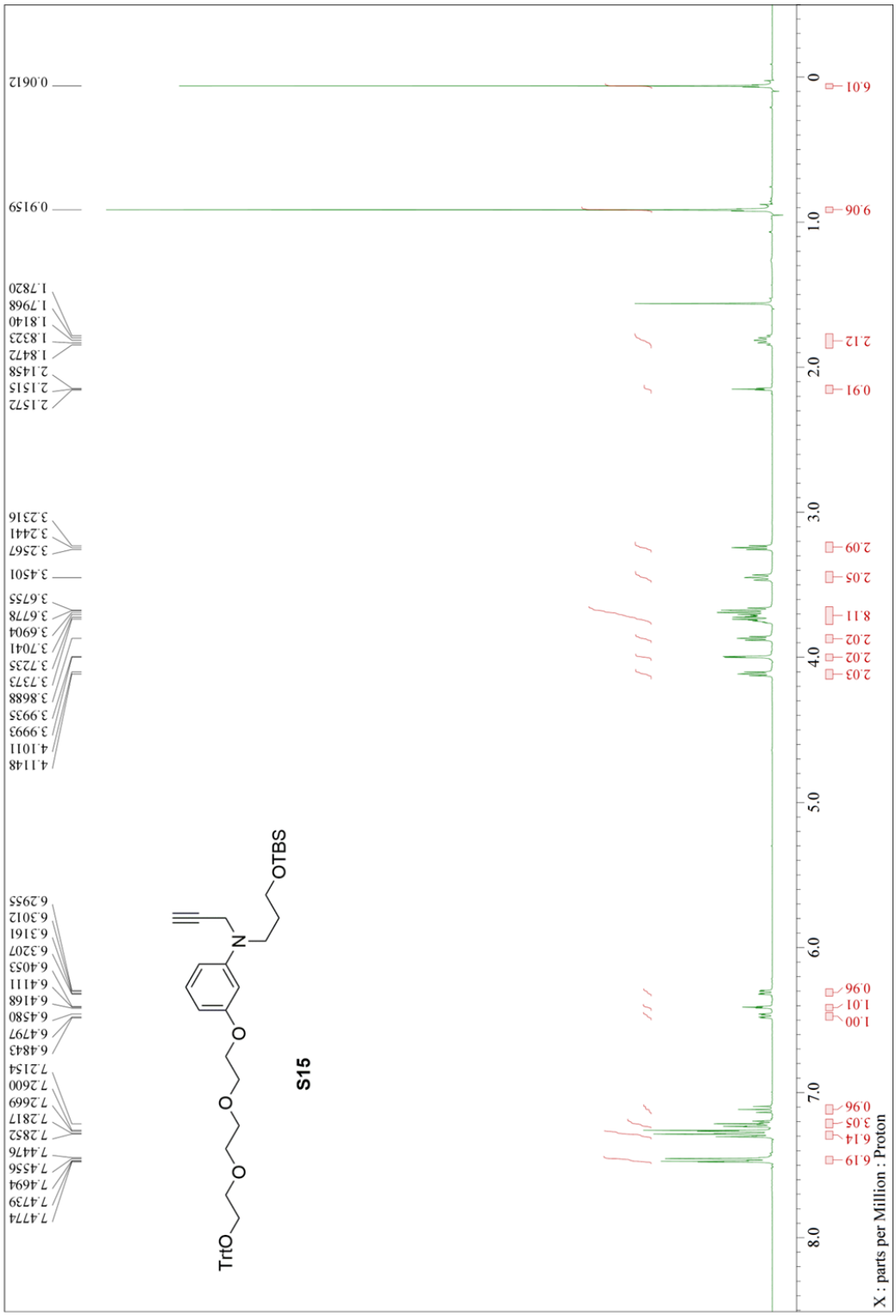
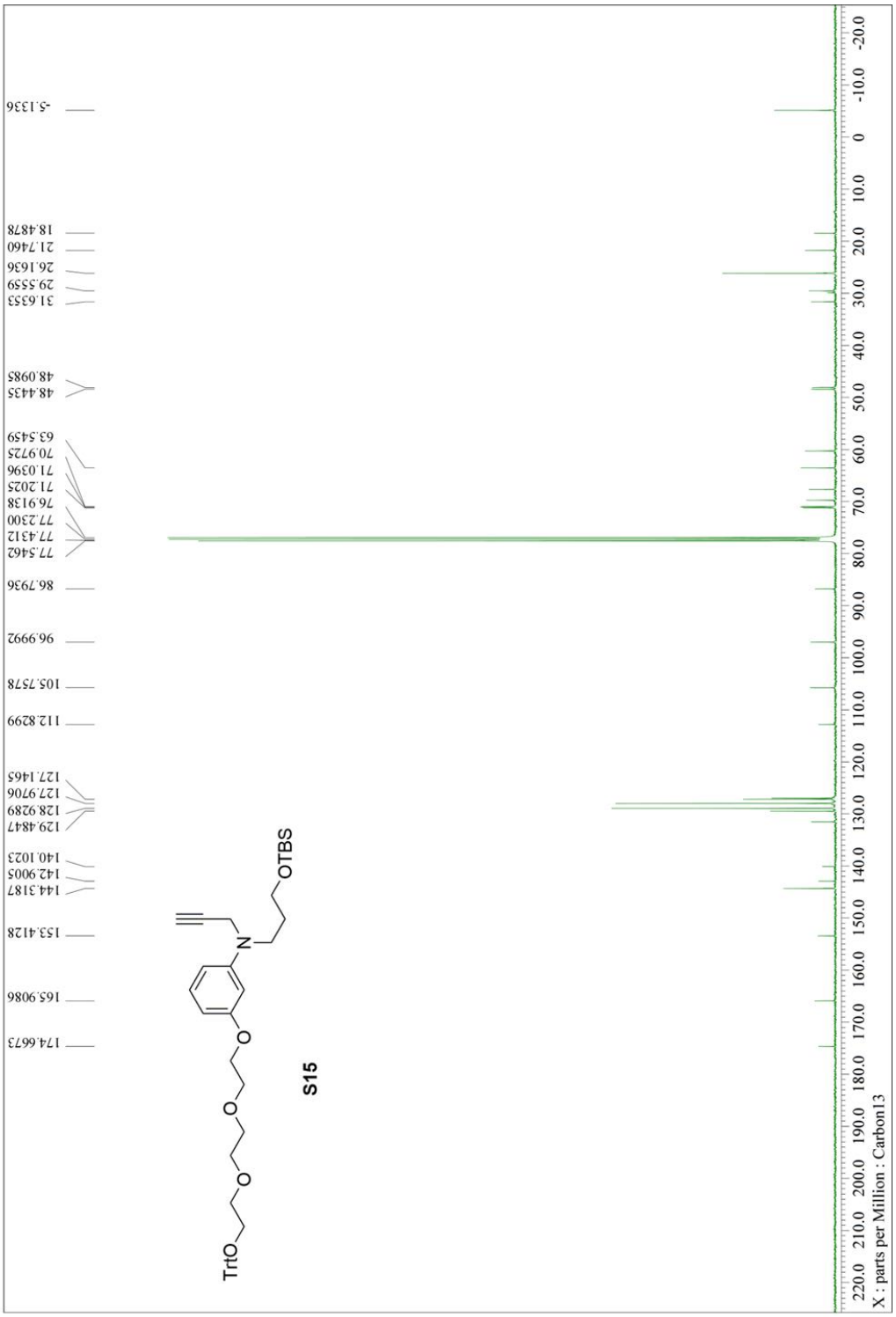
32.4473
41.2826
60.2660
68.9288
70.6154
70.7495
71.4395
71.9378
97.5237
107.8539
113.4502
127.4219
127.8244
129.1564
130.6992
132.1654
139.1129
141.2115
144.7571
147.8236
155.4610
167.7844
168.9919
176.9551
NTs
OH
SA-TEG-DQI
X : parts per Million : Carbon13


S15
OTBS
TrtO
N
O
X : parts per Million : Proton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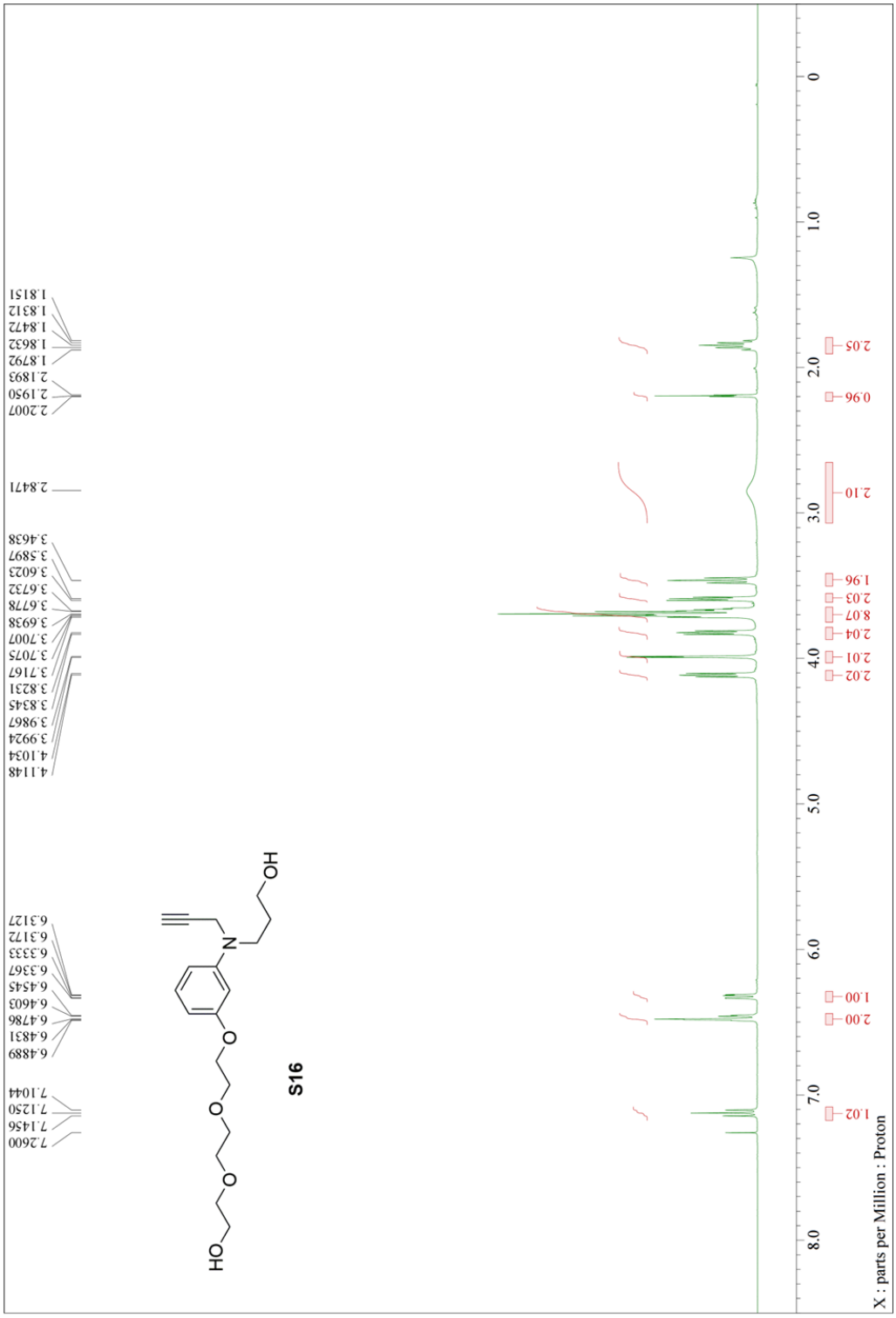
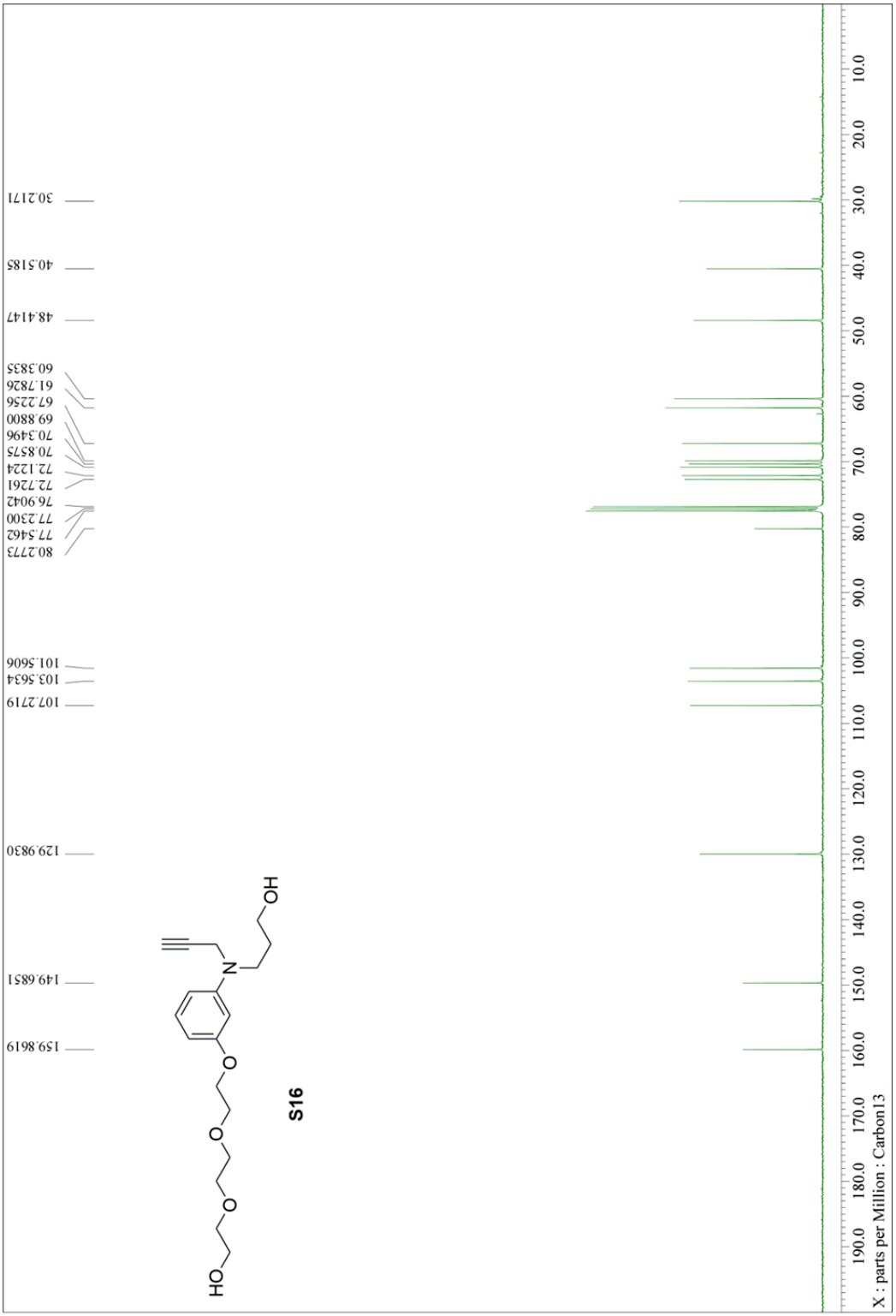
21.7460
26.1636
29.5559
31.6353
48.0985
48.4435
63.5459
70.9725
71.0396
71.2025
76.9138
77.2300
77.4312
77.5462
86.7936
96.9992
105.7578
112.8299
127.1465
127.9706
128.9289
129.4847
140.1023
142.9005
144.3187
153.4128
165.9086
174.6673
OTBS
N
O
O
O
O
TrtO
S15
220.0 210.0 200.0 190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0 -20.0
X : parts per Million : Carbon13


S16
X : parts per Million : Proton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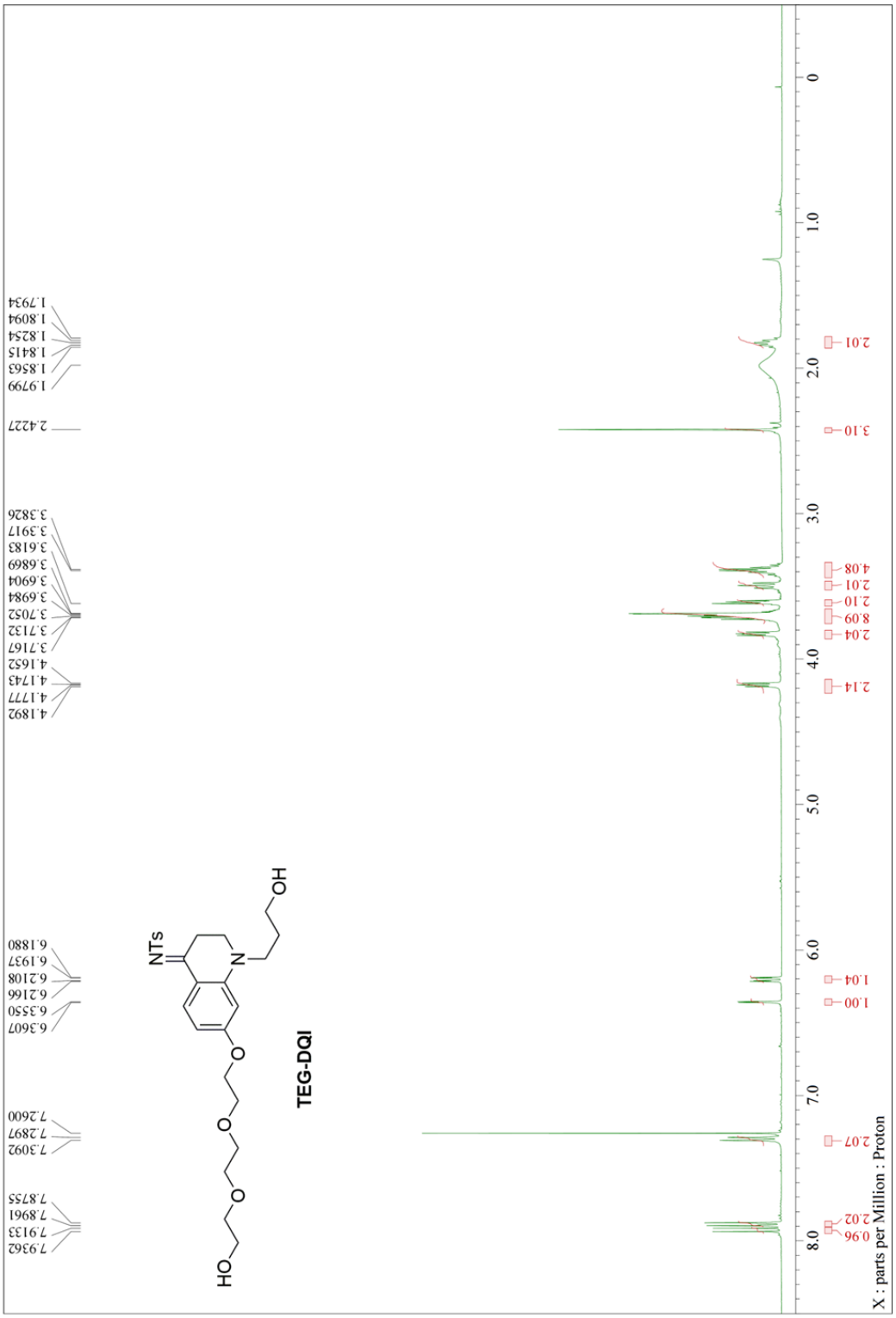
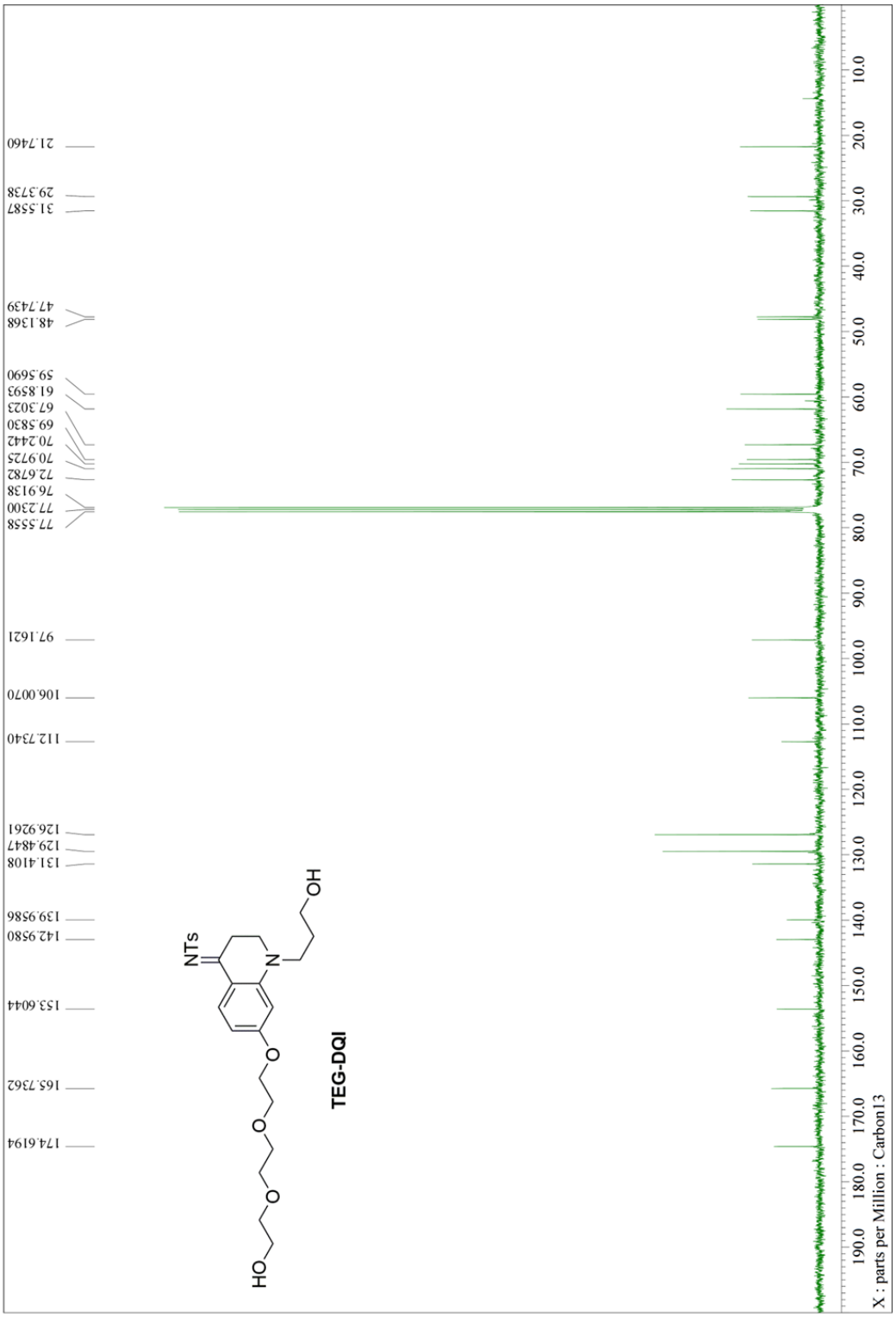
70.8575
72.1224
72.7261
76.9042
77.2300
77.5462
80.2773
101.5606
103.5634
107.2719
129.9830
149.6851
159.8619
OH
N
O
O
O
HO
S16
190.0
180.0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
X : parts per Million : Carbon13


TEG-DQI
NTs
HO
OH
X : parts per Million : Proton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31.5587
47.7439
48.1368
59.5690
61.8593
67.3023
69.5830
70.2442
70.9725
72.6782
76.9138
77.2300
77.5558
97.1621
106.0070
112.7340
126.9261
129.4847
131.4108
139.9586
142.9580
153.6044
165.7362
174.6194
NTs
N
OH
HO
O
O
O
TEG-DQI
190.0 180.0 170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0
X : parts per Million : Carbon13