

SUPPLEMENTARY INFORMATION

Chemoproteomics Identifies Protein Ligands for Monoacylglycerol Lipids

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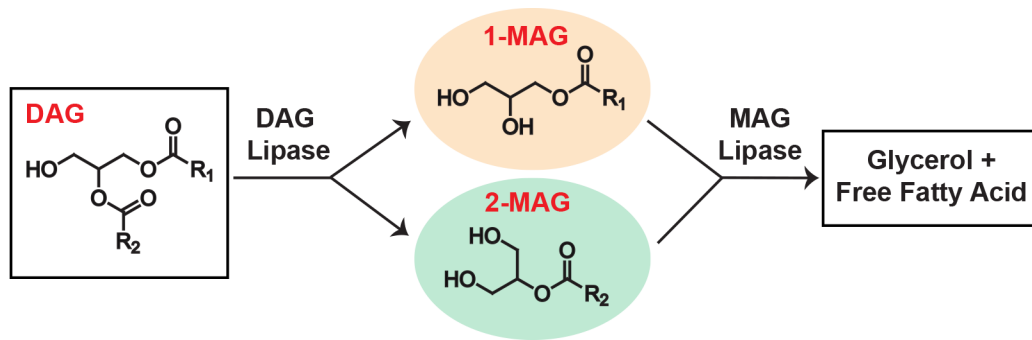
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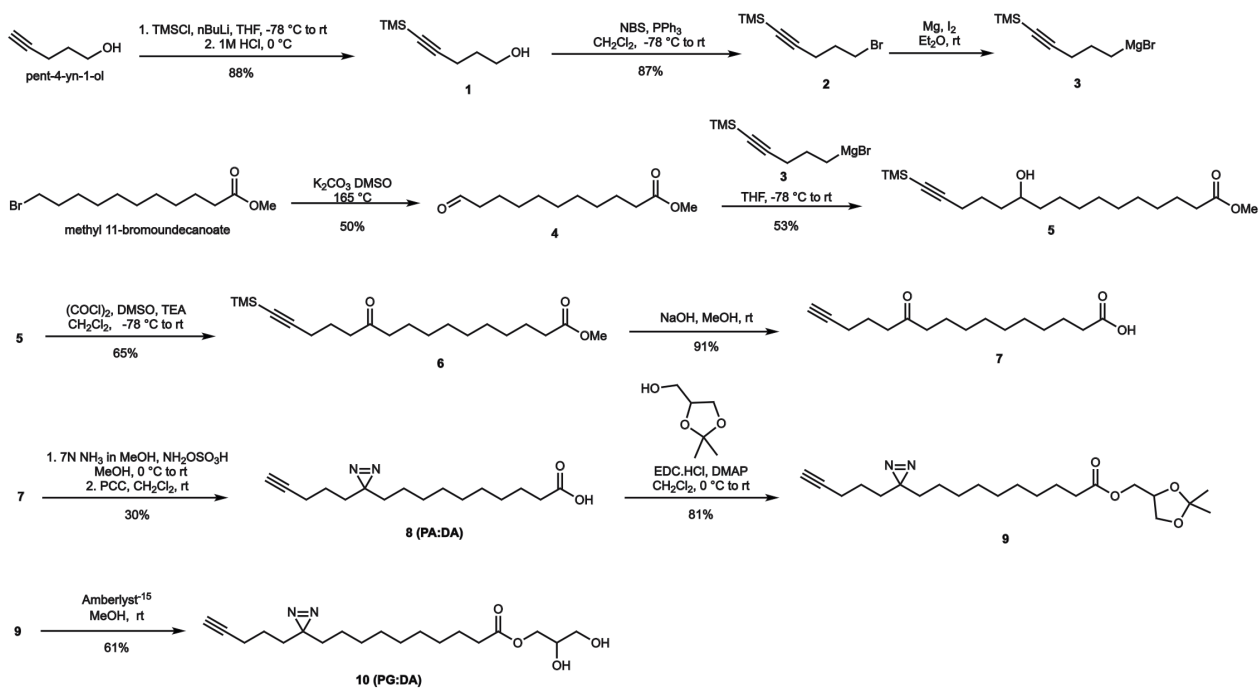
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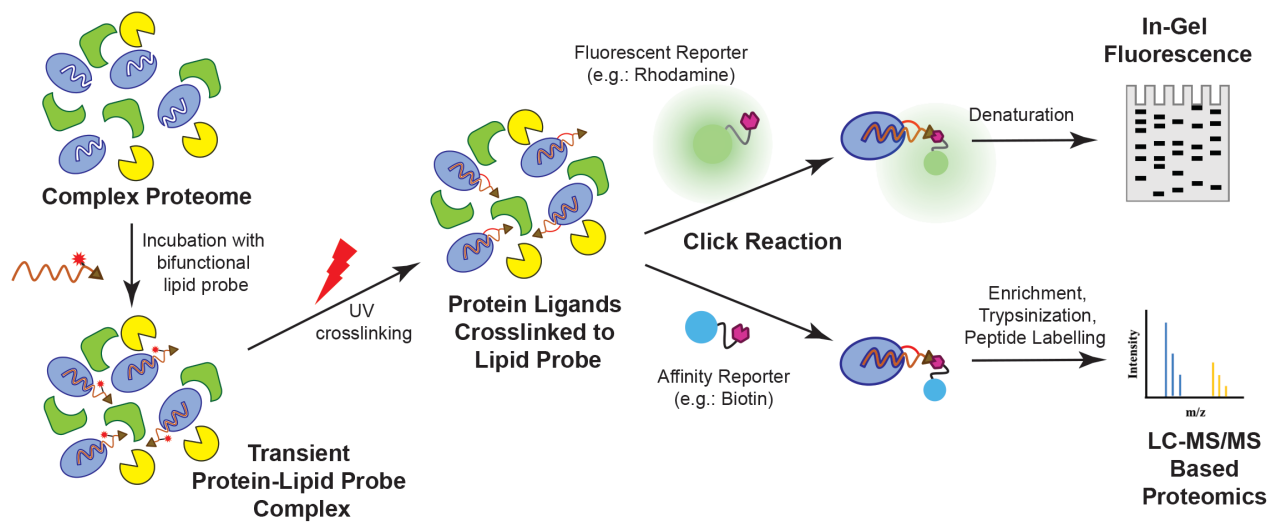
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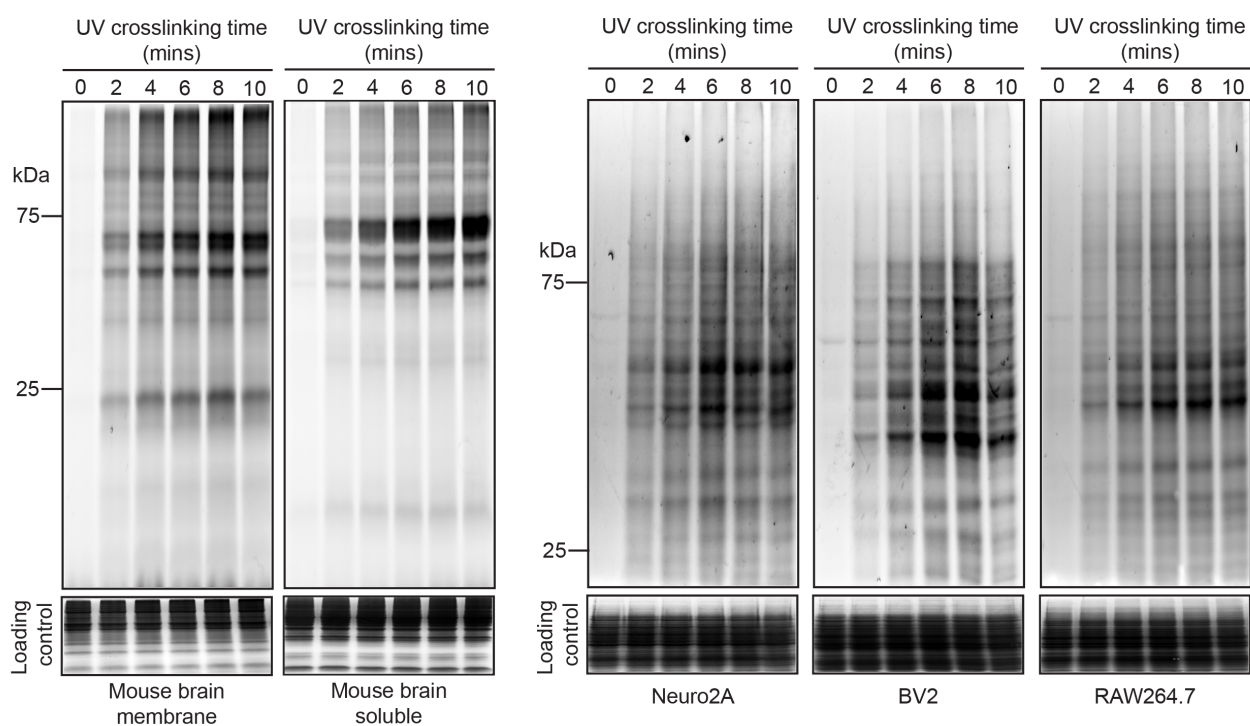
Supplementary Figure 1. Metabolism of MAG lipids. Monoacylglycerol (1-MAG and 2-MAG) lipids are biosynthesized from diacylglycerol (DAG) precursors by the action of dedicated DAG lipases. On the other hand, they are degraded by the action of MAG lipases to form glycerol and free fatty acids.



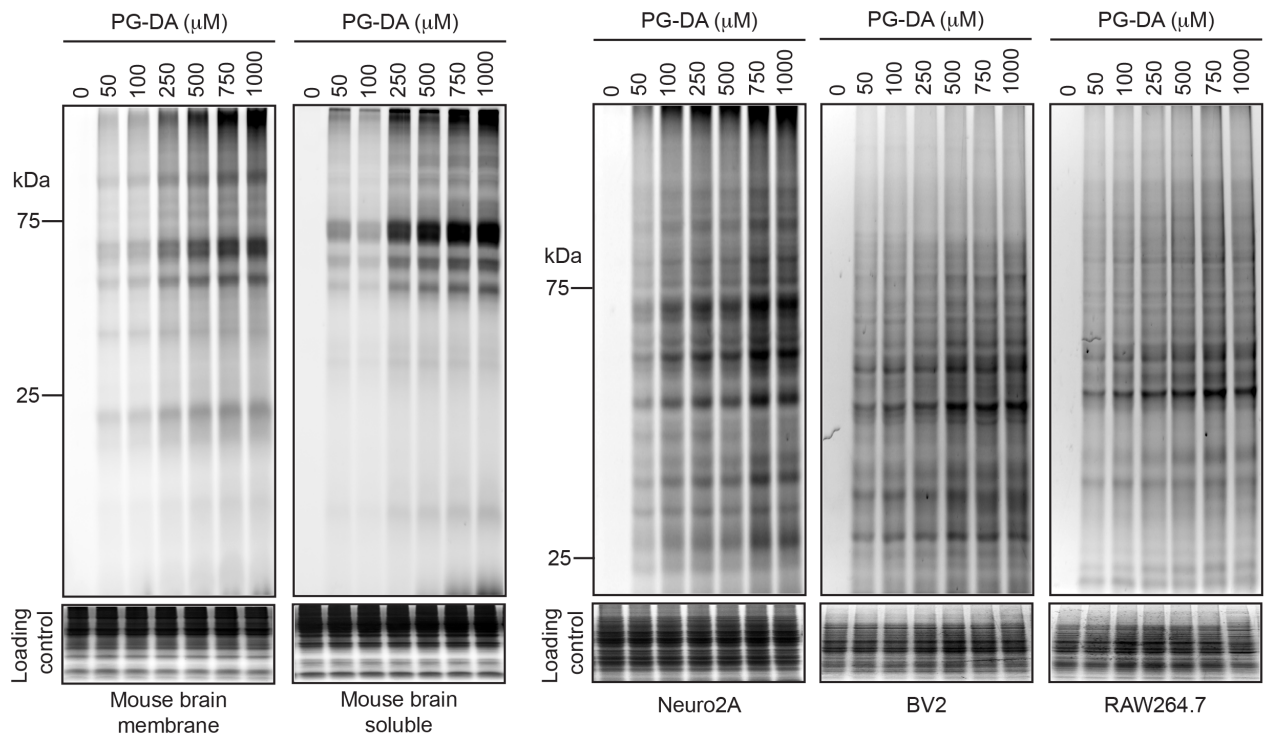
Supplementary Figure 2. The synthetic scheme used to generate the PG-DA probe. Complete details of all the synthesis and analytical characterization of the various intermediates can be found in the **Supplementary Synthetic Note**.



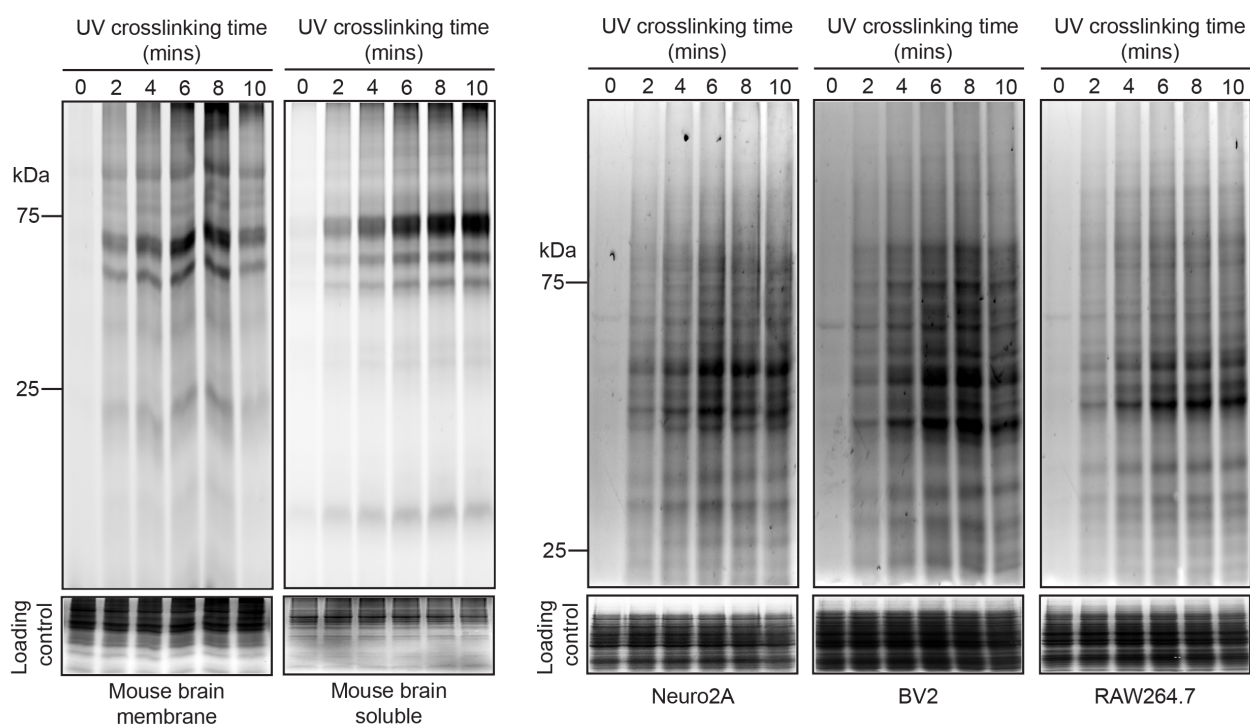
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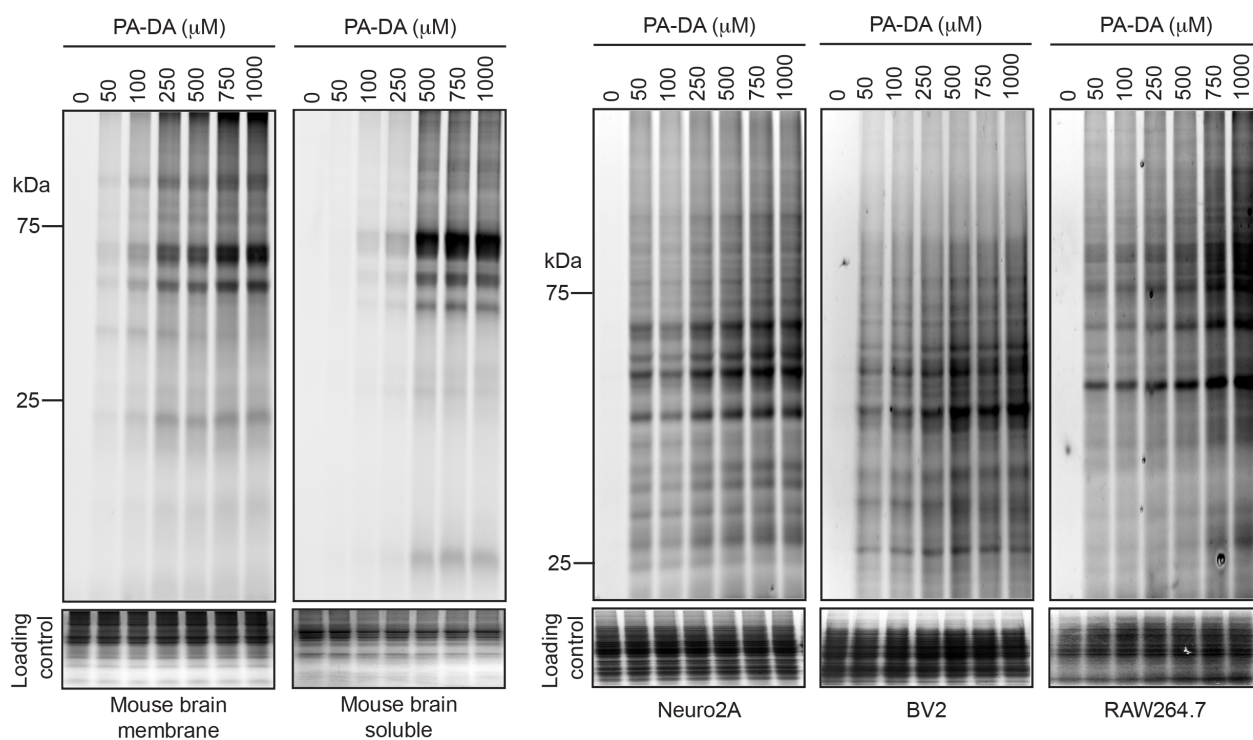
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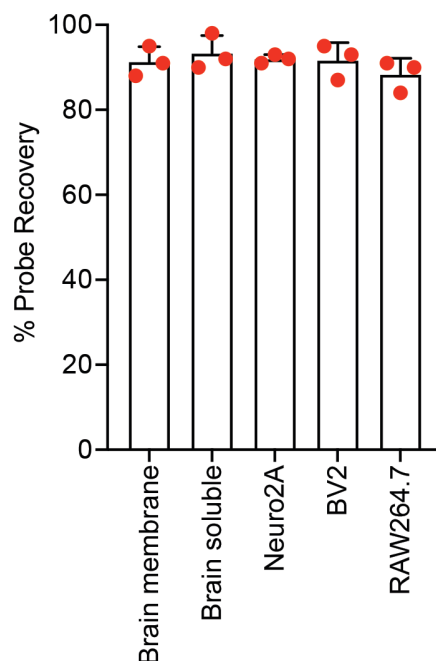
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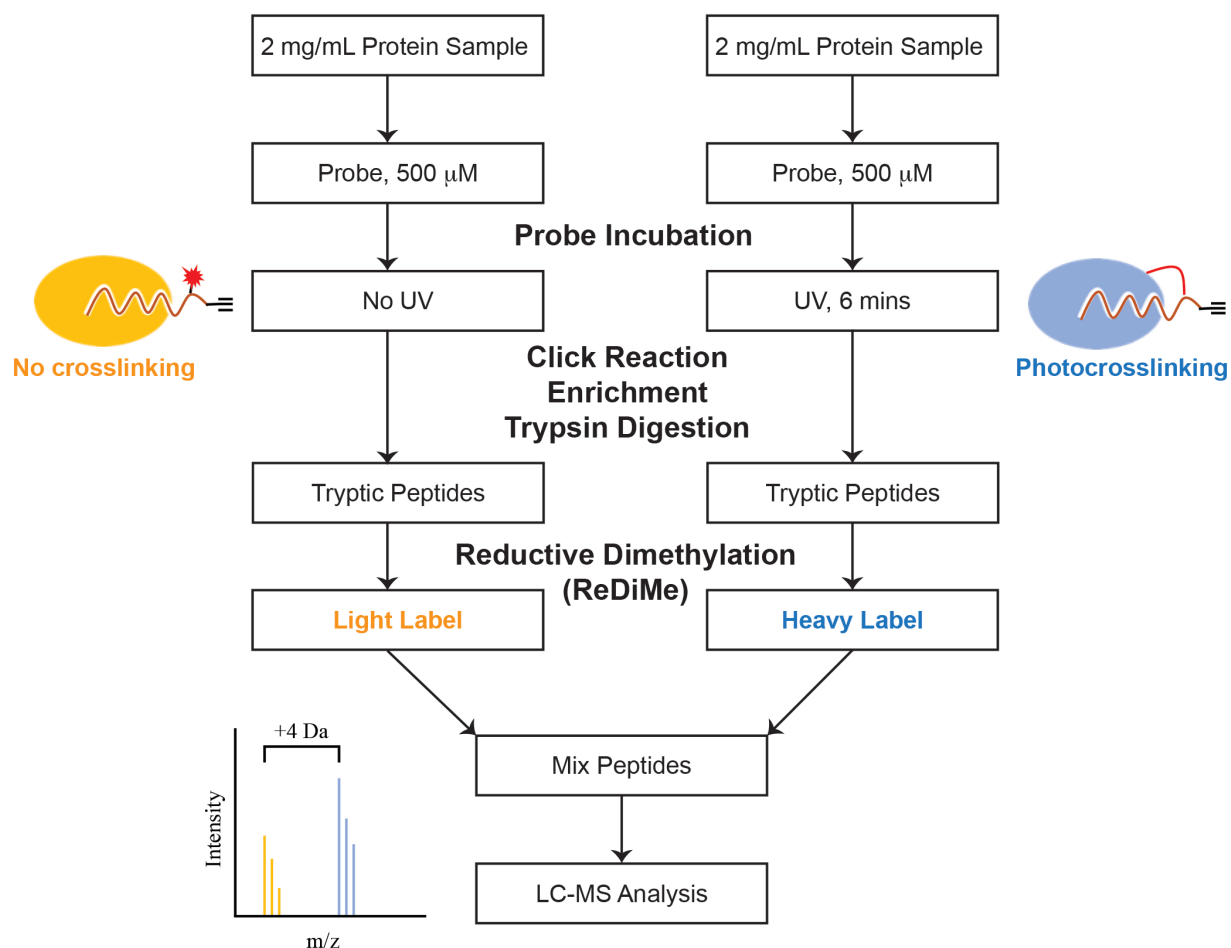
Supplementary Figure 6. UV-dependent crosslinking of the PA-DA probe (500 μ M) in various lysates. In this experiment, UV-crosslinking time was varied from 0 – 10 mins, and in all cases, 6 mins was found to be the optimal time for UV crosslinking. The Coomassie staining shows the loading control for all gels in this experiment. This experiment was done three times with reproducible results each time.



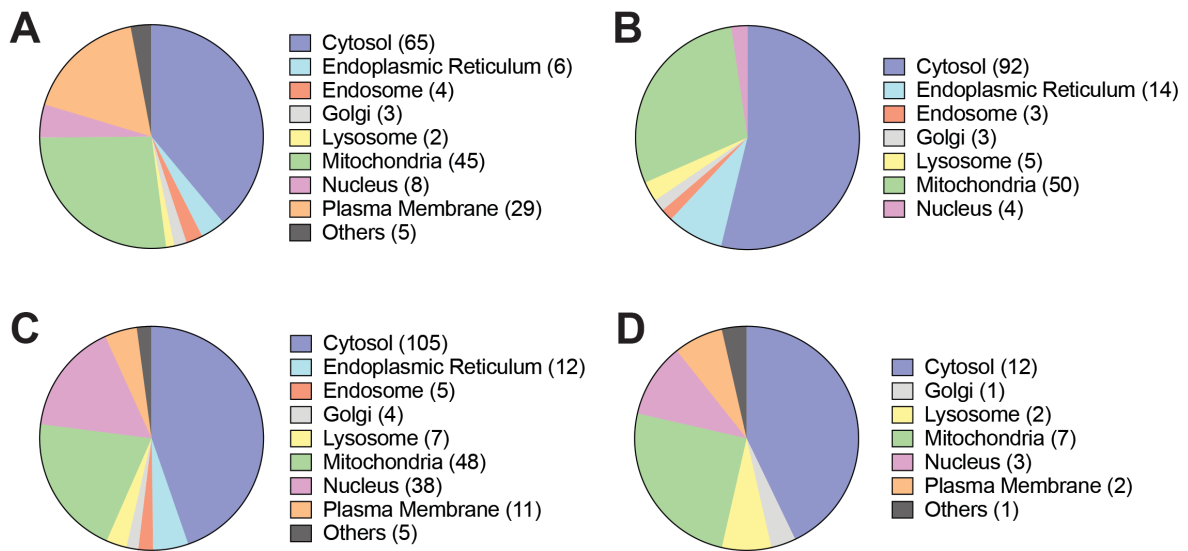
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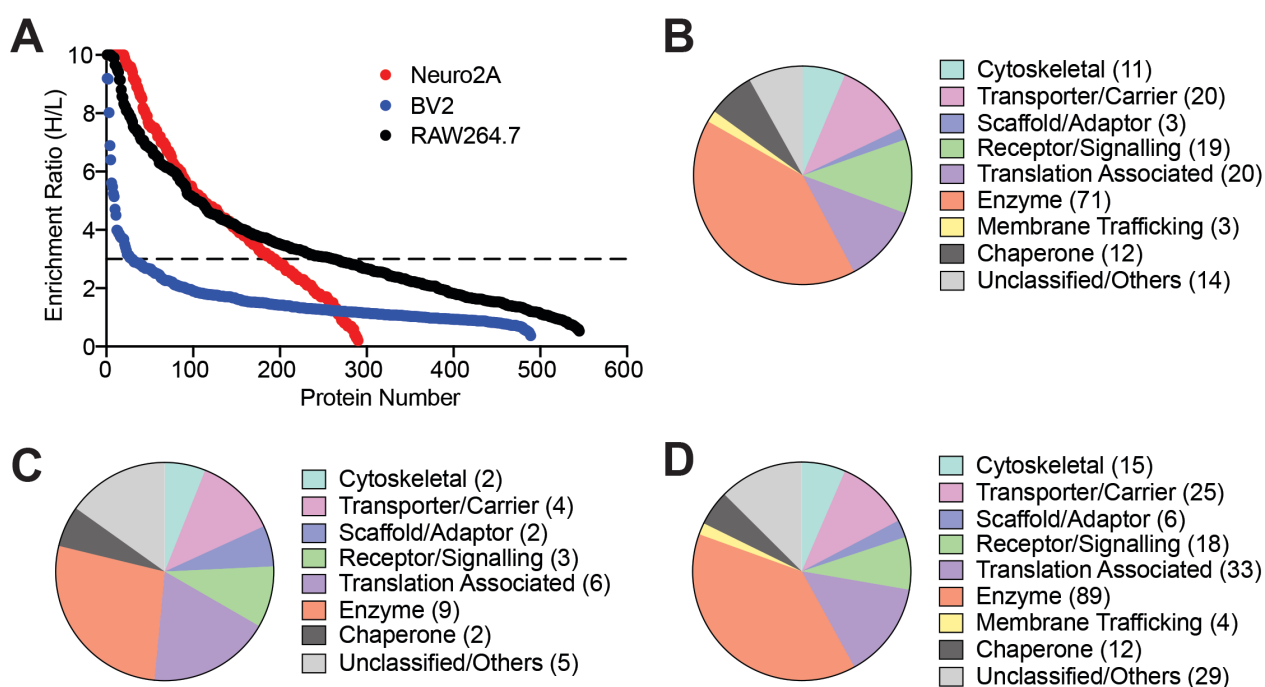
Supplementary Figure 8. Recovery of the PG-DA probe from various lysates. 50 nmol of the PG-DA probe was added to 1 mg of various lysates, and allowed to incubate under our experimental conditions (30 mins at 37 °C, 2 mM PMSF added for PG-DA experiments). Thereafter, the lysates were extracted using protocols reported previously¹⁻⁴, to enrich the intact PG-DA probe, and the molar concentration was estimated using LC-MS analysis previously reported by us^{3,4}. The PG-DA probe was assessed in the positive ion mode as the $(M+NH_4)^+$ species. For the PG-DA probe, under our experimental conditions, we found that a significant amount of the probe was intact (not degraded) in all the lysates.



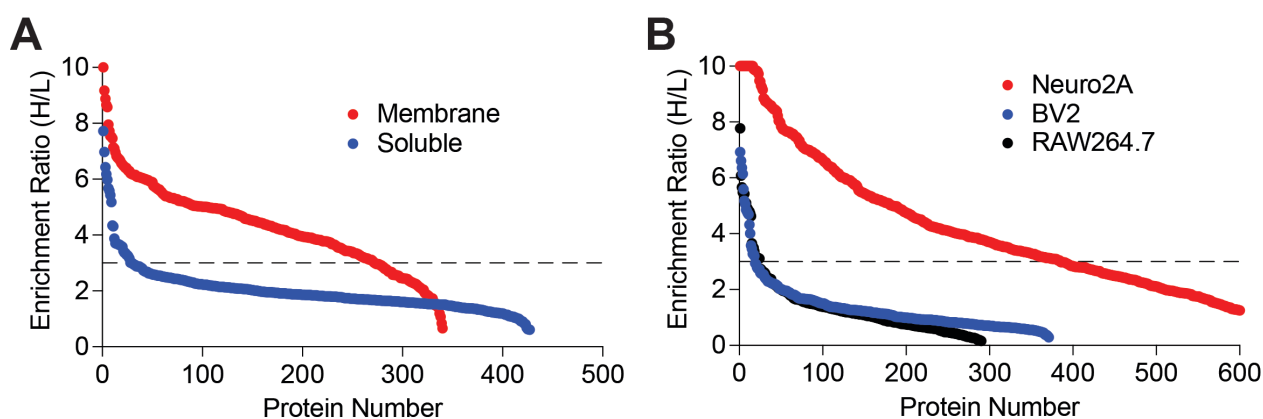
Supplementary Figure 9. A general workflow of the LC-MS/MS based quantitative chemoproteomics experiment for identifying total set of protein enriched by either the PG-DA or PA-DA probe in a UV-dependent manner from various mammalian lysates.



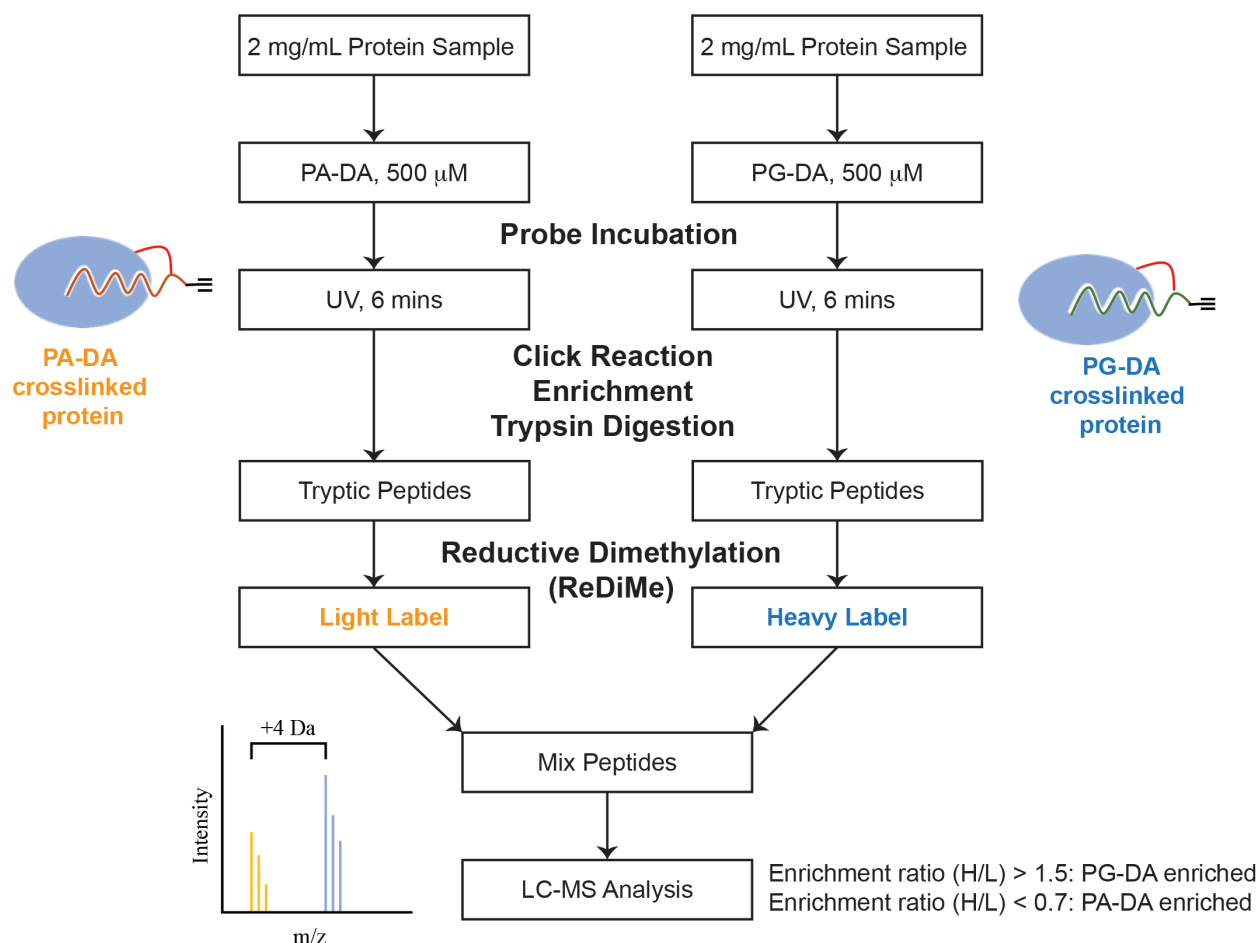
Supplementary Figure 10. Cellular localization of the proteins enriched by the PG-DA probe from the lysates of: **(A)** mouse brain, **(B)** Neuro2A cells, **(C)** RAW264.7 cells, and **(D)** BV2 cells, as per the UniProt database⁵.



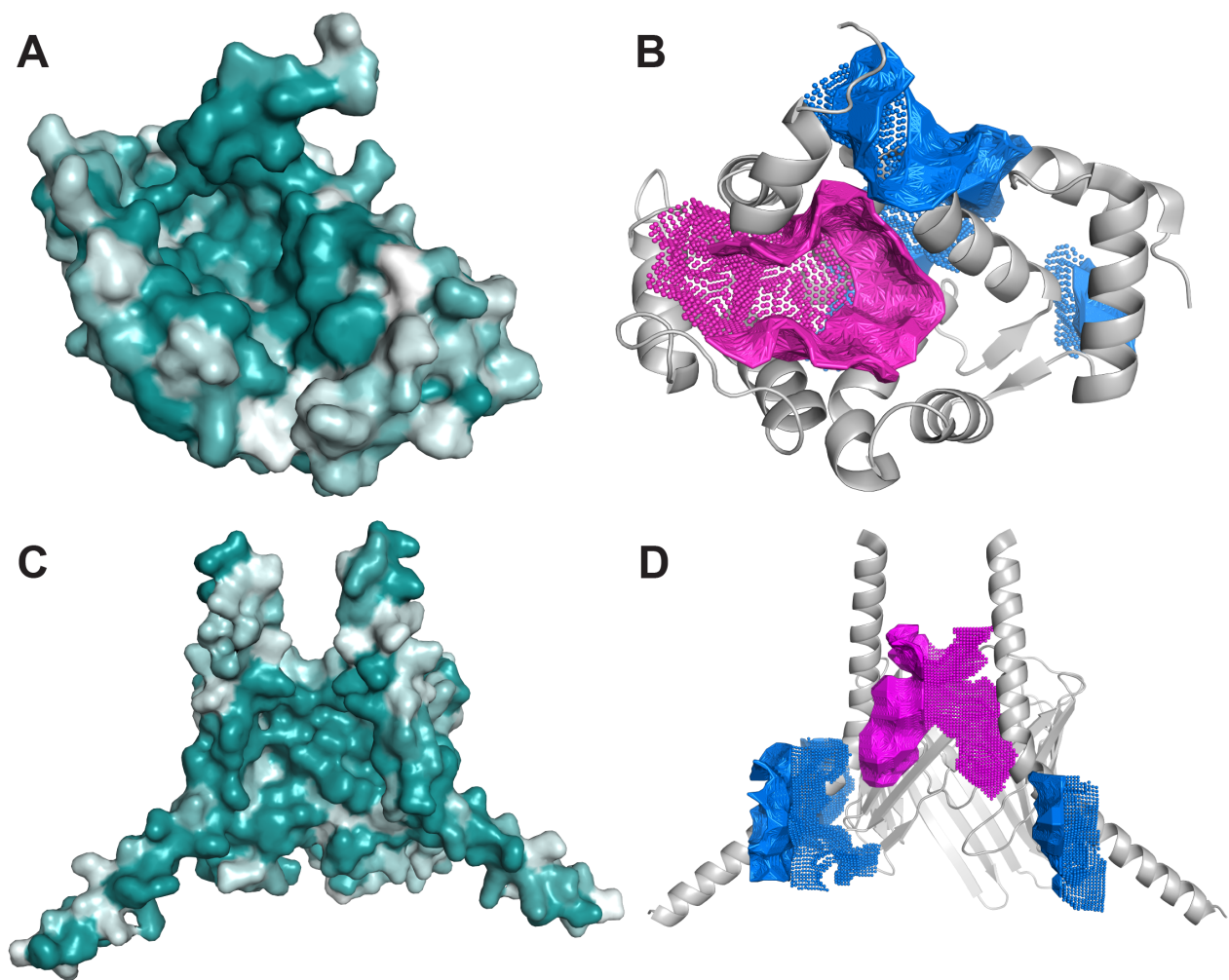
Supplementary Figure 11. (A) A LC-MS/MS based chemical proteomics experiment showing enrichment ratio (heavy:light; H:L) of the total proteins identified from the UV-dependent photocrosslinking of the PG-DA probe (500 μ M, 6 mins of UV exposure) from the lysates of various immortalized mammalian cell lines. Each data point represents the mean of the enrichment ratio obtained for the respective protein from two or three biological replicate for a particular proteomic fraction, based on the defined filtering criteria for this proteomics experiment. The horizontal dotted line denotes an enrichment ratio ≥ 3 , and proteins having an enrichment ratio above this threshold were considered enriched by the PG-DA probe, and taken forward for subsequent analysis. Complete details for all the proteins can be found in **Supplementary Data 1**. (B–D) Categorization of protein classes enriched by the PG-DA probe based on the Panther database annotation^{6,7} for: (B) Neuro2A; (C) BV2; and (D) RAW264.7 cells respectively.



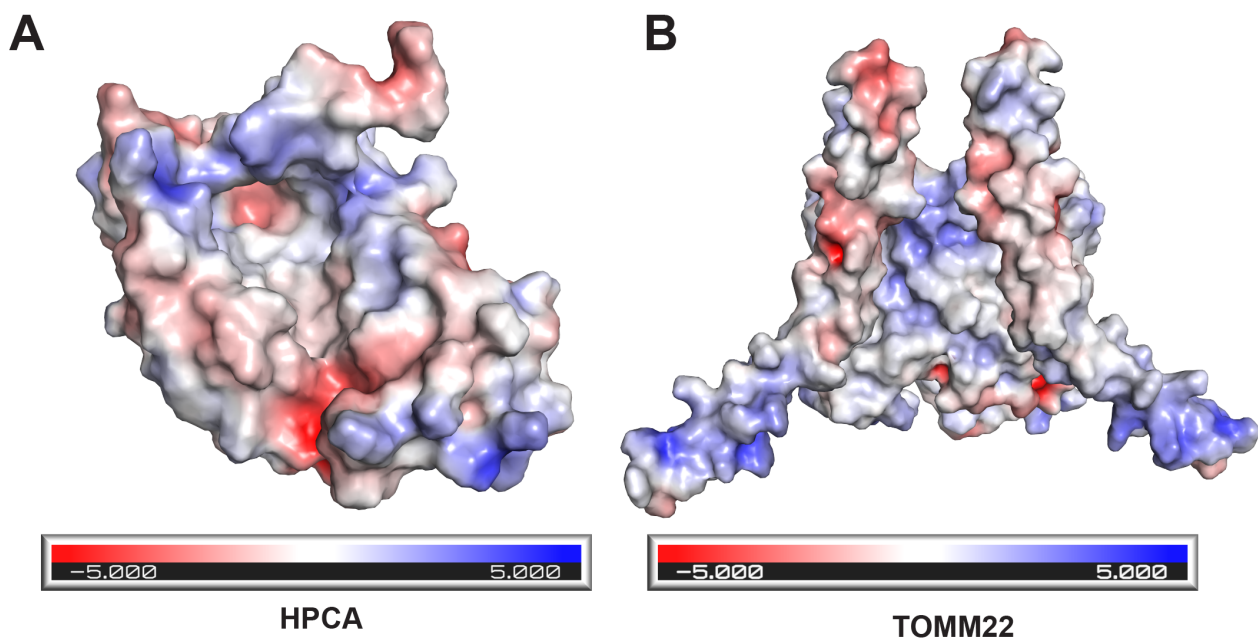
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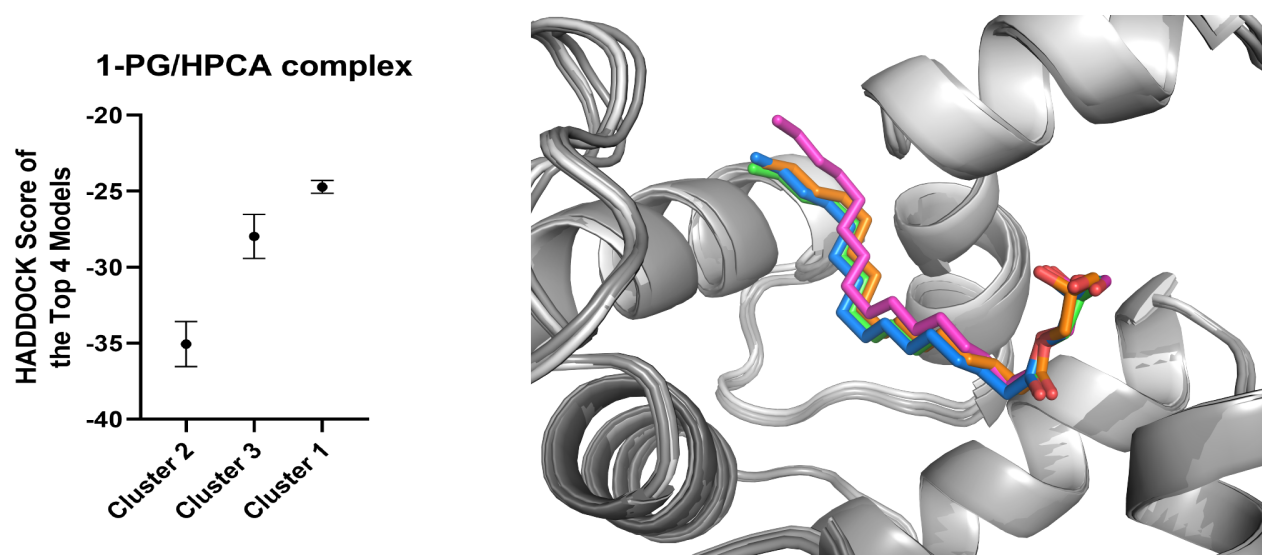
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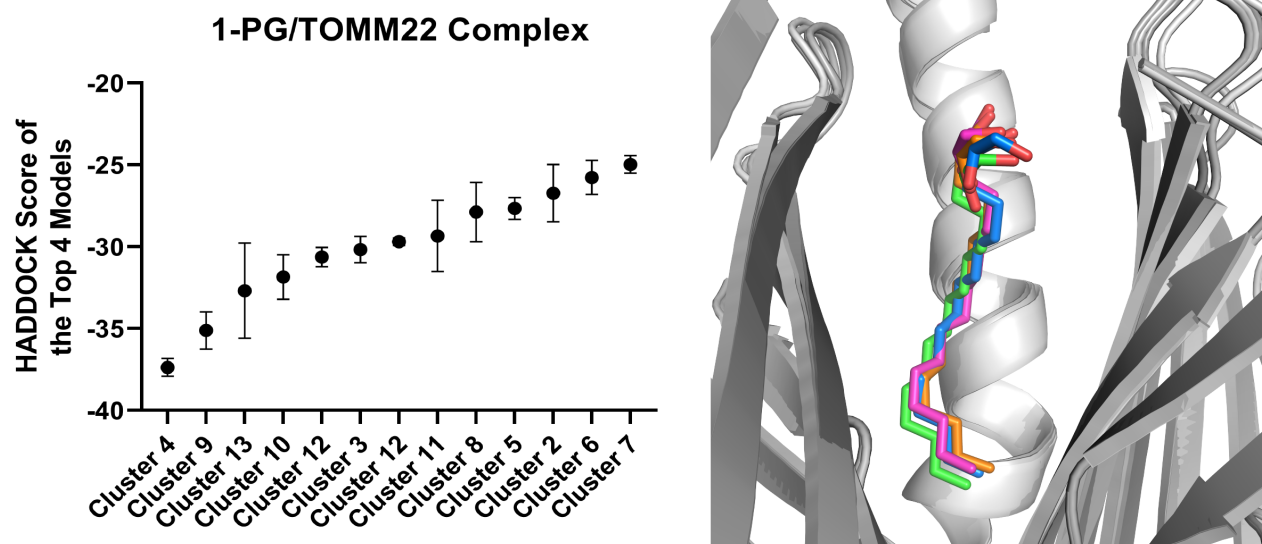
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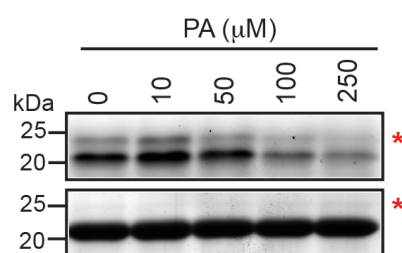
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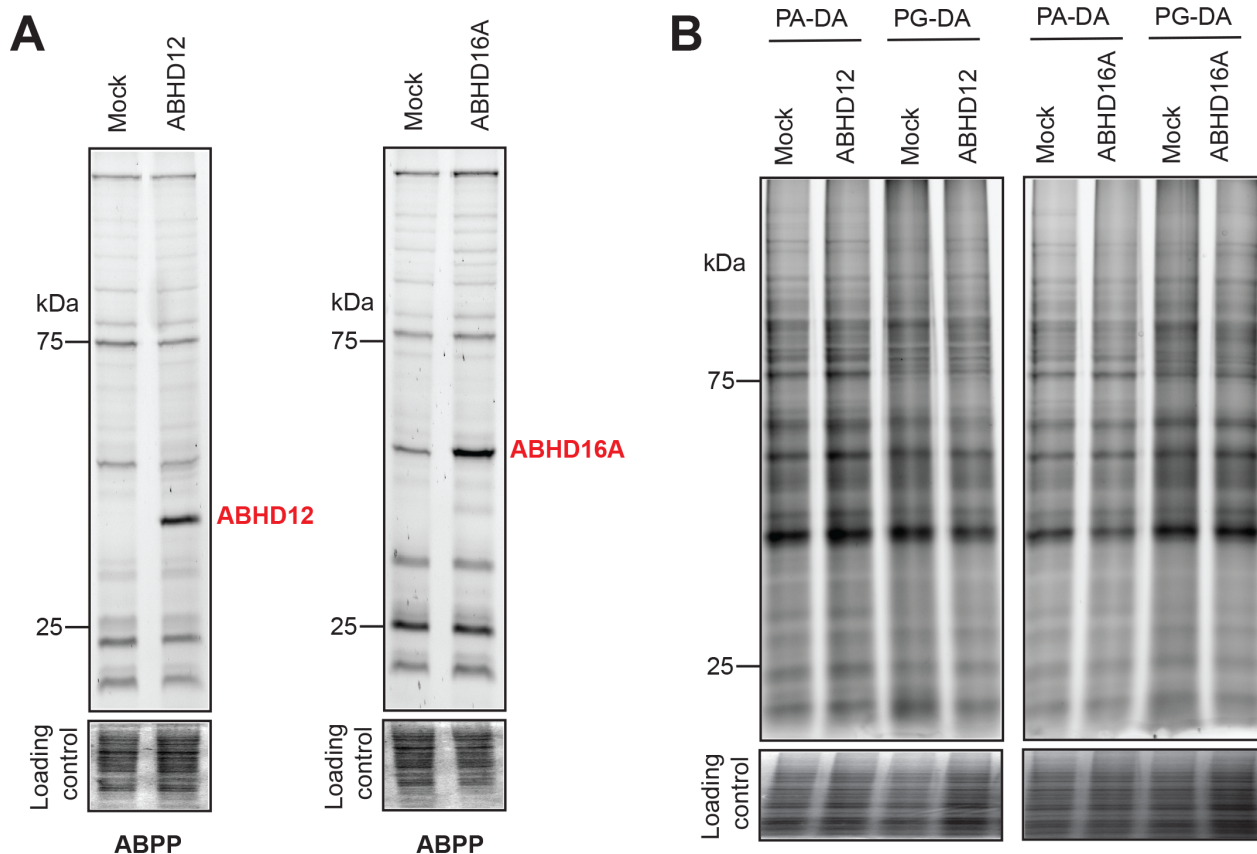
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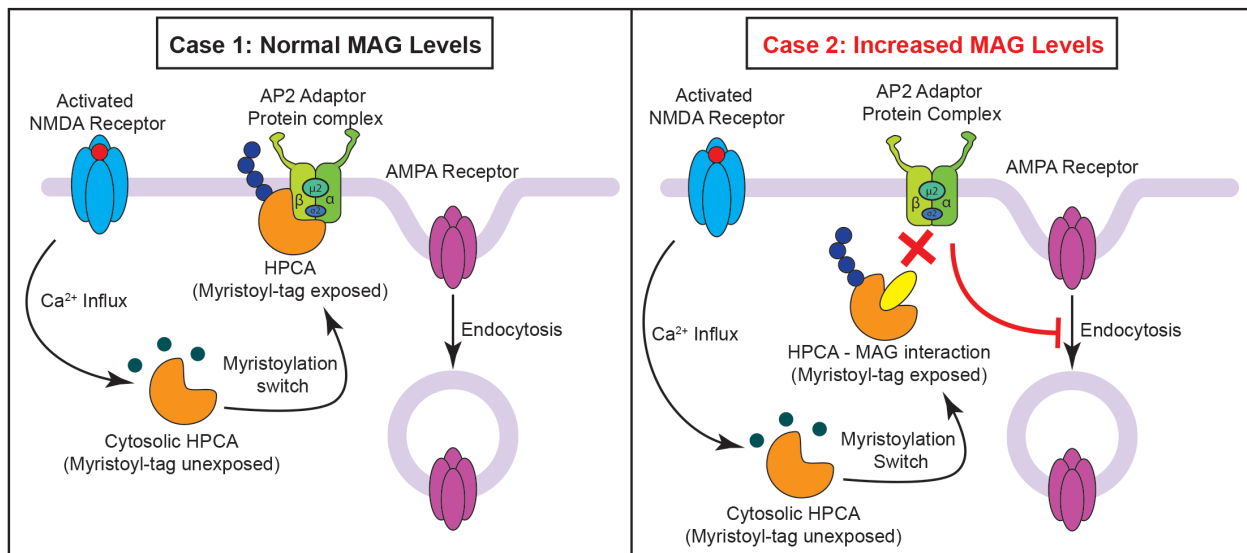
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Supplementary Figure 18. The effect of incubating increasing concentrations of palmitic acid (0 – 250 μM) on the binding of the PA-DA probe (10 μM) to recombinantly purified mouse HPCA (10 μM). The top panel shows the in-gel fluorescence, while the bottom panel is the Coomassie staining as the loading control for this respective gel. The red asterisk denotes the minor form of HPCA at 25-kDa in both gels. All other experimental conditions remain similar to those reported in Figure 6, and this particular experiment was done three times with reproducible results each time.



Supplementary Figure 19. (A) Representative activity-based protein profiling (ABPP)^{2,11,12} gels confirming the overexpression of ABHD12 and ABHD16A in cellular lysates of HEK293T cells relative to a mock transfection control. In this experiment, 100 μ g lysates were used per condition (100 μ L of 1 mg/mL), and the ABPP probe, FP-rhodamine was used at a final concentration of 2 μ M. The Coomassie staining shows the loading control for all gels in this experiment. This experiment was done two times with reproducible results each time. **(B)** A representative in-gel fluorescence experiment showing the no binding of the PG-DA or PA-DA probe, to either ABHD12 or ABHD16A in HEK293T cellular lysates overexpressing these lipases. In this experiment, 200 μ g lysates were used per condition (100 μ L of 2 mg/mL), and the probes were used at 50 μ M final concentration, similar to the conditions in Figure 7A. The Coomassie staining shows the loading control for all gels in this experiment. This experiment was done three times with reproducible results each time.



Supplementary Figure 20. A possible model for MAG's effect on the HPCA-calcium sensing. See *Discussion* section for details.

SUPPLEMENTARY DATA 1: A LC-MS/MS based chemoproteomic characterization of the PG-DA probe as a function of UV-crosslinking in different mammalian lysates.

In all cases of these chemical proteomics experiments involving post-tryptic reductive demethylation (ReDiMe)², the tryptic peptides from the UV-crosslinked sample was labelled with heavy formaldehyde, while the tryptic peptides from the no UV sample was labelled with light formaldehyde. An enrichment ratio (H/L) represent relative abundance of a peptide in the heavy labelled sample versus the light labelled sample. For a protein to be classified as enriched by the PG-DA probe upon UV-crosslinking, it needed to be identified in at least 2 out of 3 replicates, have ≥ 3 quantified peptides per replicate, and have an enrichment ratio ≥ 3 in all replicates.

Tab A: List of proteins enriched by the PG-DA probe in the mouse brain membrane lysates.

Tab B: List of proteins enriched by the PG-DA probe in the mouse brain soluble lysates.

Tab C: List of proteins enriched by the PG-DA probe in the lysates obtained from Neuro2A cells.

Tab D: List of proteins enriched by the PG-DA probe in the lysates obtained from RAW264.7 cells.

Tab E: List of proteins enriched by the PG-DA probe in the lysates obtained from BV2 cells.

SUPPLEMENTARY DATA 2: A LC-MS/MS based chemoproteomic characterization of the PA-DA probe as a function of UV-crosslinking in different mammalian lysates.

In all cases of these chemical proteomics experiments involving post-tryptic reductive demethylation (ReDiMe)², the tryptic peptides from the UV-crosslinked sample was labelled with heavy formaldehyde, while the tryptic peptides from the no UV sample was labelled with light formaldehyde. An enrichment ratio (H/L) represent relative abundance of a peptide in the heavy labelled sample versus the light labelled sample. For a protein to be classified as enriched by the PA-DA probe upon UV-crosslinking, it needed to be identified in at least 2 out of 3 replicates, have ≥ 3 quantified peptides per replicate, and have an enrichment ratio ≥ 3 in all replicates.

Tab A: List of proteins enriched by the PA-DA probe in the mouse brain membrane lysates.

Tab B: List of proteins enriched by the PA-DA probe in the mouse brain soluble lysates.

Tab C: List of proteins enriched by the PA-DA probe in the lysates obtained from Neuro2A cells.

Tab D: List of proteins enriched by the PA-DA probe in the lysates obtained from RAW264.7 cells.

Tab E: List of proteins enriched by the PA-DA probe in the lysates obtained from BV2 cells.

SUPPLEMENTARY DATA 3: A competitive LC-MS/MS based chemoproteomics experiments comparing the protein ligands of the PG-DA probe versus the PA-DA probe in different mammalian lysates.

In all cases of these chemical proteomics experiments involving post-tryptic reductive demethylation (ReDiMe)², the tryptic peptides from the PG-DA treated sample was labelled with heavy formaldehyde, while the tryptic peptides from the PA-DA treated sample was labelled with light formaldehyde. An enrichment ratio (H/L) represent relative abundance of a peptide in the heavy labelled sample versus the light labelled sample. For a protein to be considered for any analysis in this experiment, it needed to be identified in at least 2 out of 3 replicates, and have ≥ 3 quantified peptides per replicate. A protein was considered enriched by the PG-DA probe, if it had an enrichment ratio ≥ 1.5 in all the replicates it was identified, while an enrichment ratio ≤ 0.7 classified a protein to be enriched by the PA-DA probe.

Tab A: Complete list of proteins identified in this competitive probe versus probe (PG-DA vs PA-DA) chemical proteomics experiments performed in the mouse brain membrane lysates.

Tab B: Complete list of proteins identified in this competitive probe versus probe (PG-DA vs PA-DA) chemical proteomics experiments performed in the mouse brain soluble lysates.

Tab C: Complete list of proteins identified in this competitive probe versus probe (PG-DA vs PA-DA) chemical proteomics experiments performed in the lysates from Neuro2A cells.

Tab D: Complete list of proteins identified in this competitive probe versus probe (PG-DA vs PA-DA) chemical proteomics experiments performed in the lysates from BV2 cells.

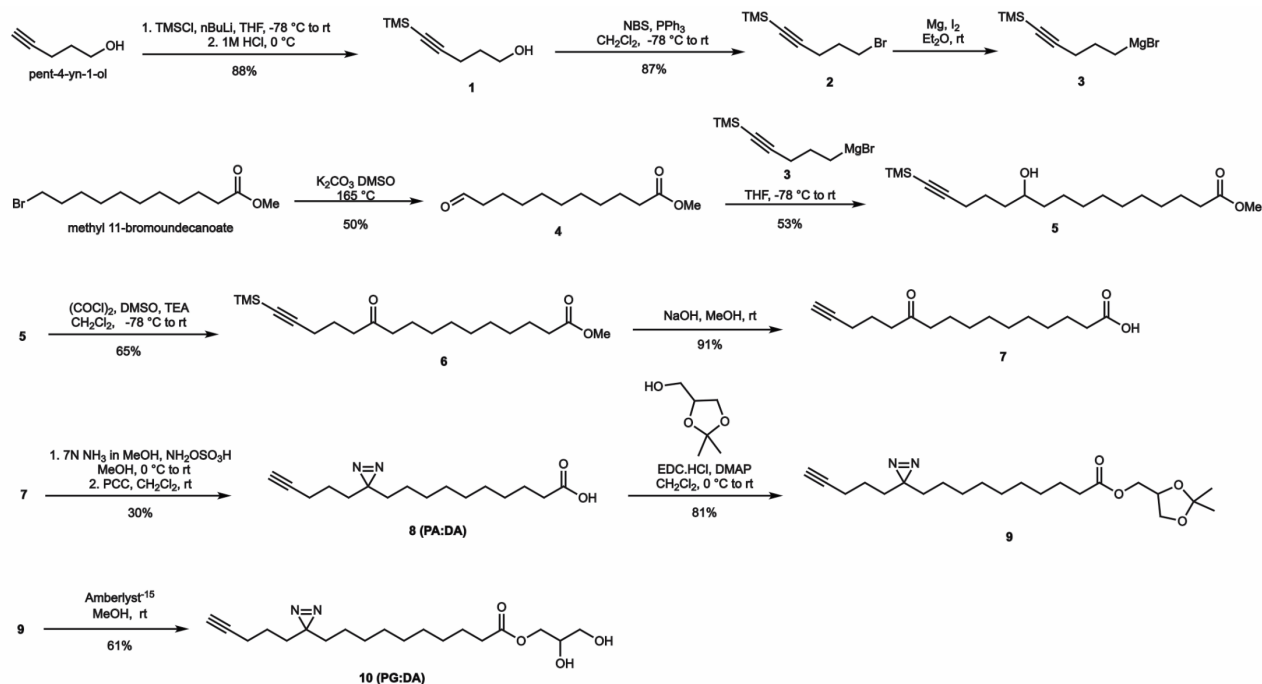
Tab E: Complete list of proteins identified in this competitive probe versus probe (PG-DA vs PA-DA) chemical proteomics experiments performed in the lysates from RAW264.7 cells.

SUPPLEMENTARY DATA 4. Datasets from the molecular docking of 1-PG into HPCA and TOMM22.

Tab A. Identification of the top 5 cavities by CavityPlus based on the DrugScore for HPCA and TOMM22.

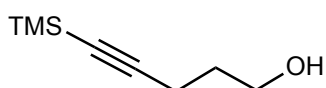
Tab B. Interaction energies for the 1-PG/HPCA and 1-PG/TOMM22 complexes for the top 4 models within the selected clusters.

SUPPLEMENTARY SYNTHETIC NOTE

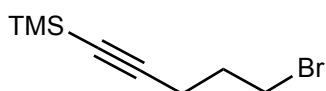


Synthesis: The bifunctional derivative, palmitoyl glycerol diazirine alkyne (PG-DA; compound **10**), features a palmitic acid chain with a terminal alkyne and an internal photoactive diazirine group (i.e., palmitic acid diazirine; PA-DA; compound **8**). A palmitic acid diazirine alkyne (**PA-DA**) was synthesized following previously established procedures with some modifications¹³. The reported synthesis of the aldehyde **4** used a lactone as a starting material, which is quite expensive and not readily commercially available. To address this shortcoming, we used a different approach. We started with an affordable and widely available alkyl halide, and converted it directly into the aldehyde using Kornblum oxidation¹⁴. Next, we encountered some difficulty with the earlier reported protocol¹³ for the Grignard reaction using (5-chloropent-1-yn-1-yl)trimethylsilane. Specifically, a substantial portion of the compound remained unreacted while attempting to prepare the Grignard reagent from the TMS-protected pentynyl chloride. Considering the superior reactivity of a bromide compared to a chloride with magnesium, we decided to prepare (5-bromopent-1-yn-1-yl)trimethylsilane and used this as a substrate for the Grignard reaction. First, TMS protection of the alkyne of pent-4-yn-1-ol was carried out to give **1** in 88% yield. An Appel-type reaction¹⁵ was used to convert the alcohol into the bromide **2** (87% yield). Treatment of a freshly prepared Grignard reagent **3** with aldehyde **4** afforded **5** in 53% yield. Subsequent steps were carried out using established protocols to produce **8 (PA-DA)**. For the synthesis of bifunctional MAG palmitic acid derivative, **PG-DA**, the following sequence of reactions was used. First, **8 (PA-DA)** was treated with 1,2-isopropylideneglycerol in the presence of 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDC.HCl) and the desired product **9** was isolated in 81% yield. Deprotection of the isopropylidene group of **9** using Amberlyst-15 gave **10 (PG-DA)** in 69% yield.

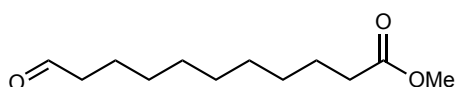
General. All chemicals were purchased from Sigma-Aldrich and TCl, unless otherwise mentioned and used as received. All reactions were carried out under an atmosphere of nitrogen (N₂) or argon (Ar). Glassware was oven- or flame-dried prior to use. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 pre-coated plates (0.25 mm thickness, Merck), and visualized with staining with potassium permanganate (KMnO₄) or phosphomolybdic acid (PMA) solutions. Column chromatography was performed on Rankem silica gel (100-200 mesh). Nuclear magnetic resonance (NMR) spectra were recorded using deuteriochloroform (CDCl₃), as the solvent. ¹H, ¹³C spectra were recorded on JEOL 400 MHz or Bruker 400 MHz (or 100 MHz for ¹³C) NMR spectrometers. The internal standards for the recorded NMR spectra were either residual solvent signals (Chloroform, δ_H = 7.26 ppm, δ_C = 77.2 ppm and or an internal standard tetramethylsilane (δ_H = 0.00 ppm, δ_C = 0.00 ppm). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz, multiplicities were reported by the following abbreviations: s (singlet), broad singlet (bs), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), m (multiplet). High-resolution mass spectra were obtained from high-resolution mass spectrometry (HRMS)–electrospray ionization–Q-TOF–LC–MS/MS (Sciex)



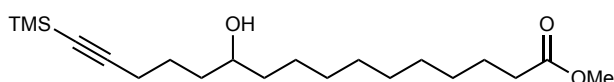
5-(Trimethylsilyl)pent-4-yn-1-ol (1). Compound **1** was synthesized according to a procedure reported previously (Harris et al. 2014) with some modifications¹⁶, and the analytical data collected by us is consistent with literature values. Briefly, in a flame-dried, two-necked round-bottom flask, containing 4-pentyn-1-ol (5.0 g, 59.44 mmol, 1.0 equiv.) and anhydrous THF (100 mL) were added, and the mixture was cooled to -78 °C. To this, *n*-butyllithium (52 mL, 2.5 M in hexanes, 130.76 mmol, 2.2 equiv.) was added dropwise over a period of 30 min and the temperature was maintained at -78 °C. Next, chlorotrimethylsilane (22.63 mL, 178.32 mmol, 3.0 equiv.) was added dropwise, and the reaction mixture was gradually warmed to room temperature and stirred for an additional 16 h. The reaction mixture was subsequently cooled to 0 °C and acidified with 1 M HCl (150 mL), followed by stirring for 1 h. The resulting mixture was then extracted with Et₂O (3 x 100 mL), and the combined organic layer was washed with water (250 mL), sat. NaHCO₃ (200 mL), and brine (200 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The obtained residue was purified by silica gel column chromatography (elution with gradient 10–25% EtOAc/ hexanes) to afford the compound **1** as a colourless oil (8.17 g, 88%). **TLC** *R*_f = 0.2 (10% EtOAc/ Hexanes; TLC stain, KMnO₄); **¹H NMR** (400 MHz, CDCl₃): δ 3.75 (t, *J* = 6.1 Hz, 2H), 2.34 (t, *J* = 6.9 Hz, 2H), 1.76 (quint, *J* = 6.6 Hz, 2H), 1.72 (bs, 1H), 0.14 (s, 9H); **HRMS** (*m/z*): [*M* + *H*]⁺ calcd. for C₈H₁₇OSi, 157.1048; found, 157.1051.



(5-Bromopent-1-yn-1-yl)trimethylsilane (2). Compound **2** was synthesized according to a procedure reported previously (Yao et al. 2002) with some modifications¹⁷, and the analytical data collected by us is consistent with literature values. To a solution of compound **1** (5.1 g, 32.63 mmol, 1 equiv.) in anhydrous CH₂Cl₂ (100 mL). Triphenylphosphine (10.27 g, 39.16 mmol, 1.2 equiv.) was added portion wise at –78 °C under N₂ atmosphere. *N*-Bromosuccinimide (6.39 g, 35.89 mmol, 1.1 equiv.) was then slowly added, and the reaction mixture was gradually warmed to room temperature and stirred for 6 h. After completion of reaction (TLC analysis), Et₂O (400 mL) was added to the reaction mixture and washed with sat. NaHCO₃ (2 x 200 mL), brine (200 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The resulting crude was purified by silica gel column chromatography (elution with 100% hexanes) to afford the **2** as a colourless oil (6.22 g, 87%). **TLC** *R*_f = 0.5 (100% hexanes; TLC stain, KMnO₄) **¹H NMR** (400 MHz, CDCl₃): δ 3.51 (t, *J* = 6.5 Hz, 2H), 2.41 (t, *J* = 6.8 Hz, 2H), 2.04 (quint, *J* = 6.6 Hz, 2H), 0.15 (s, 9H).



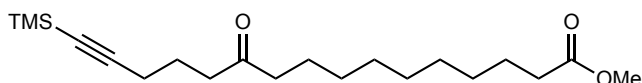
Methyl 11-oxoundecanoate (4). Compound **4** was synthesized according to a procedure reported previously (Ravi, S. et al. 2001) with some modifications¹⁴, and the analytical data collected by us is consistent with literature values. To a solution of methyl 11-bromoundecanoate (0.5 g, 1.791 mmol, 1 equiv.) in anhydrous DMSO (5 mL), NaHCO₃ (0.33 g, 3.94 mmol, 2.2 equiv.) was added. The reaction mixture was refluxed at 165 °C for 15 min and then cooled to room temperature, diluted with ice cold water (10 mL), and extracted with Et₂O (2 x 25 mL). The combined organic layer was washed with water (25 mL), sat. NaHCO₃ (20 mL), and brine (20 mL). The resulting organic layer was dried over Na₂SO₄, and concentrated *in vacuo*. The crude was purified by silica gel column chromatography (elution with gradient 10–25% EtOAc/ hexanes) to afford the compound **4** as a colourless oil (0.19 g, 50%). **TLC** *R*_f = 0.5 (10% EtOAc/ hexane; TLC stain, KMnO₄); **¹H NMR** (400 MHz, CDCl₃): δ 9.76 (t, *J* = 1.9 Hz, 1H), 3.66 (s, 3H), 2.41 (td, *J* = 77.4, 1.9 Hz, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.66–1.58 (m, 4H), 1.36–1.25 (m, 10H); **HRMS** (*m/z*): [*M* + *H*]⁺ calcd. for C₁₂H₂₃O₃, 215.1647; found, 215.1636.



Methyl 11-hydroxy-16-(trimethylsilyl)hexadec-15-ynoate (5). Preparation of Grignard reagent (3): The Grignard reagent was prepared based on the procedure reported by (Harris et al. 2014) with modifications¹⁶. Reaction flasks, glass syringes, needles and magnetic stir bars were dried at 110 °C in an oven for 2 h. Magnesium turnings were treated with a 10% aq. HCl solution, followed by subsequent rinsing with water and acetone. The magnesium turnings were then dried in the

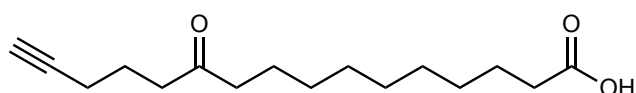
oven at 110 °C for 2 h. Before commencement of the reaction, all items in the oven were gradually cooled to room temperature in a desiccator. The solvents used for the reaction Et₂O and tetrahydrofuran (THF) were dried over sodium metal and then freshly distilled. Also freshly distilled bromide **2** (5-10 mm Hg (Torr), 85-90 °C; 70-80% yield) was used for the preparation for the Grignard reagent. A 100 mL Schlenk round-bottom flask was charged with the magnesium turnings (0.35 g, 14.45 mmol, 1.2 equiv.) and heated with a heat gun for 10 min under vacuum, and then cooled to room temperature under N₂ atmosphere. A magnetic stir bar was introduced into the flask, and anhydrous Et₂O (16 mL) was added, followed by the addition of a catalytic amount of iodine (8.6 mg, 0.068 mmol, 0.006 equiv.). The mixture was stirred at room temperature for 10 min. A solution of **2** (2.64 g, 12.04 mmol, 1.0 equiv.) in Et₂O (16 mL) was prepared and a small portion, approximately 20%, was added dropwise, and then reaction mixture was stirred at room temperature for 10-15 min until the colour of the solution transitioned from brown/red to colorless, signifying the initiation of the Grignard reagent. The remaining solution of **2** was then added dropwise over 15 min and the reaction mixture was stirred at room temperature for another 3 h. The Grignard reagent **3** was used immediately for the next step.

Grignard reaction: A solution of methyl 11-oxoundecanoate **4** (2.2 g, 10.27 mmol, 1.0 equiv.) in anhydrous THF (33 mL) was cooled to -78 °C under N₂ atmosphere. The Grignard solution **3** was added dropwise to the stirring solution over 15 min. The reaction mixture was then gently warmed to 0 °C and stirred for 1 h. Saturated NH₄Cl solution (250 mL) was used to quench the reaction. The product was extracted with EtOAc (3 x 100 mL), and the combined organic layers was dried with Na₂SO₄, and concentrated in *vacuo*. The resulting crude was purified by silica gel column chromatography (elution with gradient 10–20% EtOAc/ hexanes) to afford the compound **5** as a colorless oil (1.93 g, 53%). **TLC** *R*_f = 0.4 (20% EtOAc/ hexanes, stain with KMnO₄); The analytical data collected by us is consistent with reported literature (Hulce et al. 2013)¹³ values: **¹H NMR** (400 MHz, CDCl₃): δ 3.64 (s, 3H), 3.63–3.55 (m, 1H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.23 (t, *J* = 6.5 Hz, 2H), 1.63–1.38 (m, 8H), 1.27 (d, *J* = 4.6 Hz, 12H), 0.12 (s, 9H); **HRMS** (*m/z*): [M + H]⁺ calcd. for C₂₀H₃₉O₃Si, 355.2668; found, 355.2667.

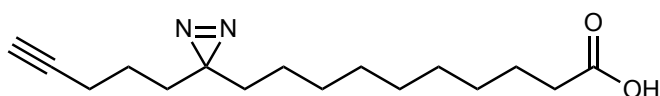


Methyl 11-oxo-16-(trimethylsilyl)hexadec-15-ynoate (6). Compound **6** was synthesized according to a procedure reported previously (Hulce et al. 2013)¹ with some modifications, and the analytical data collected by us is consistent with literature values. **Swern Oxidation:** To a stirring solution of Oxalyl chloride (0.87 mL, 10.16 mmol, 2.0 equiv.) in anhydrous CH₂Cl₂ (65 mL), DMSO (1.45 mL, 20.32 mmol, 4.0 equiv.) was added dropwise at -78 °C and the reaction mixture was stirred for 30 min. Subsequently, solution of compound **5** (1.8 g, 5.08 mmol, 1.0 equiv) in CH₂Cl₂ (5 mL) was added dropwise, and the reaction mixture was stirred for another 30 min at -78 °C. Lastly, triethylamine (2.84 mL, 20.32 mmol, 4 equiv.) was added, and after 30 min, the reaction mixture

was gently raised to 0 °C and stirred at this temperature for 1 h. The reaction mixture was then diluted with H₂O (300 mL) and extracted in CH₂Cl₂ (3 x 100 mL). The combined organic layer was washed with brine (150 mL) and dried over Na₂SO₄ and concentrated in *vacuo*. The crude residue was purified by silica gel column chromatography (elution with gradient 5–10% EtOAc/ hexanes) to afford the compound **6** as a white solid (1.17 g, 65% yield): **TLC** *R*_f = 0.4 (10% EtOAc/ hexanes; TLC stain, KMnO₄); **¹H NMR** (400 MHz, CDCl₃): δ 3.64 (s, 3H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.24 (t, *J* = 6.9 Hz, 2H), 1.75 (quintet, *J* = 7.0 Hz, 2H), 1.64–1.52 (m, 4H), 1.32–1.22 (m, 10H), 0.13 (s, 9H); **HRMS** (*m/z*): [M + H]⁺ calcd. for C₂₀H₃₇O₃Si [M + H]⁺ 353.2512, found, 353.2507.

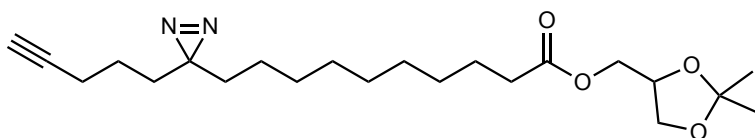


11-Oxohexadec-15-ynoic acid (7). Compound **7** was synthesized according to a procedure reported previously (Hulce et al. 2013) with some modifications¹³, and the analytical data collected by us is consistent with literature values. To a solution of **6** (1.16 g, 3.29 mmol, 1.0 equiv.) in MeOH (10 mL), NaOH (0.66 g, 16.45 mmol, 5 equiv.) in H₂O (10 mL) was added dropwise, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was acidified to pH 2–3 by treating with 10% aq. HCl at 0 °C, and extracted with Et₂O (3 x 200 mL). The combined organic layer was washed with brine (250 mL) and dried over Na₂SO₄, and concentrated in *vacuo*. The crude was purified by silica gel column chromatography (elution with gradient 15–25% EtOAc/hexanes, 1% HCO₂H) to afford the compound **7** as a white solid (798 mg, 91%). **TLC** *R*_f = 0.2 (20% EtOAc/ hexanes with 1% HCO₂H; TLC stain, PMA); **¹H NMR** (400 MHz, CDCl₃): δ 2.55 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.4 Hz, 2H), 2.34 (t, *J* = 7.5 Hz, 2H), 2.22 (td, *J* = 6.9, 2.8 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.78 (quintet, *J* = 7.1 Hz, 2H), 1.66–1.52 (m, 4H), 1.34–1.23 (m, 10H); **HRMS** (*m/z*): [M + Na]⁺ *m/z* calcd. for C₁₆H₂₆O₃Na, 289.1780; found 289.1788.

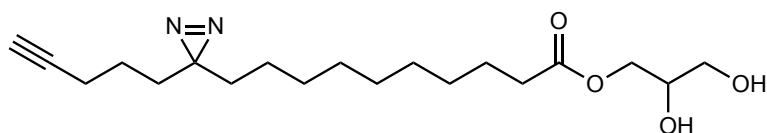


10-(3-(Pent-4-yn-1-yl)-3H-diazirin-3-yl)decanoic acid (PA-DA) (8) Compound **8 (PA-DA)** was synthesized according to a procedure reported previously (Hulce et al. 2013) with some modifications¹³, and the analytical data collected by us is consistent with literature values. The reaction and column chromatography were conducted in the dark due to the light sensitivity of the product. Briefly, in a sealed tube containing a stir bar, 11-oxohexadec-15-ynoic acid (200 mg, 0.75 mmol, 1.0 eq.) was added and cooled to 0 °C. 7 N NH₃ in MeOH (5.8 mL) was added dropwise and the resulting reaction mixture was stirred for 3 h, while maintaining the temperature at 0 °C. A solution of hydroxylamine-O-sulfonic acid (97 mg, 0.86 mmol, 1.15 equiv.) in MeOH (2.5 mL) was gradually added dropwise to the reaction mixture. The seal tube was covered with aluminium foil

and the reaction mixture was stirred at room temperature overnight, and then the solvent was evaporated under a stream of nitrogen gas (N₂). To the remaining residue, Et₂O (10.0 mL) was added, leading to the formation of a suspension containing insoluble salts, which were subsequently filtered off. The filtrate (Et₂O layer) was concentrated under reduced pressure, and the residue obtained was re-dissolved in anhydrous CH₂Cl₂ (12.0 mL) and pyridine (1.75 mL). To this mixture, pyridinium chlorochromate (PCC, 485 mg, 2.25 mmol, 3.0 equiv.) was then added, and the reaction mixture was stirred for 3 h at room temperature. Subsequently, the reaction mixture was passed through a silica pad using a solvent mixture of 70% EtOAc/ hexanes containing 1% formic acid (HCO₂H), and the resulting solution was concentrated *in vacuo*, and the crude was purified by silica gel column chromatography (elution with gradient 10–15% EtOAc/hexanes, 1% HCO₂H) to afford the compound **8** as a white solid (63 mg, 30%). **TLC** *R*_f = 0.4 (30% EtOAc/hexanes with 1% HCO₂H; TLC stain PMA) **¹H NMR** (400 MHz, CDCl₃): δ 2.33 (t, *J* = 7.5 Hz, 2H), 2.15 (td, *J* = 6.9, 2.6 Hz, 2H), 1.94 (t, *J* = 2.6 Hz, 1H), 1.61 (quintet, *J* = 7.5 Hz, 2H), 1.52–1.43 (m, 2H), 1.39–1.15 (m, 14H), 1.12–1.01 (m, 2H); **HRMS** (*m/z*): [M + H]⁺ calcd. for C₁₆H₂₇N₂O₂, 279.2073; found, 279.2071.



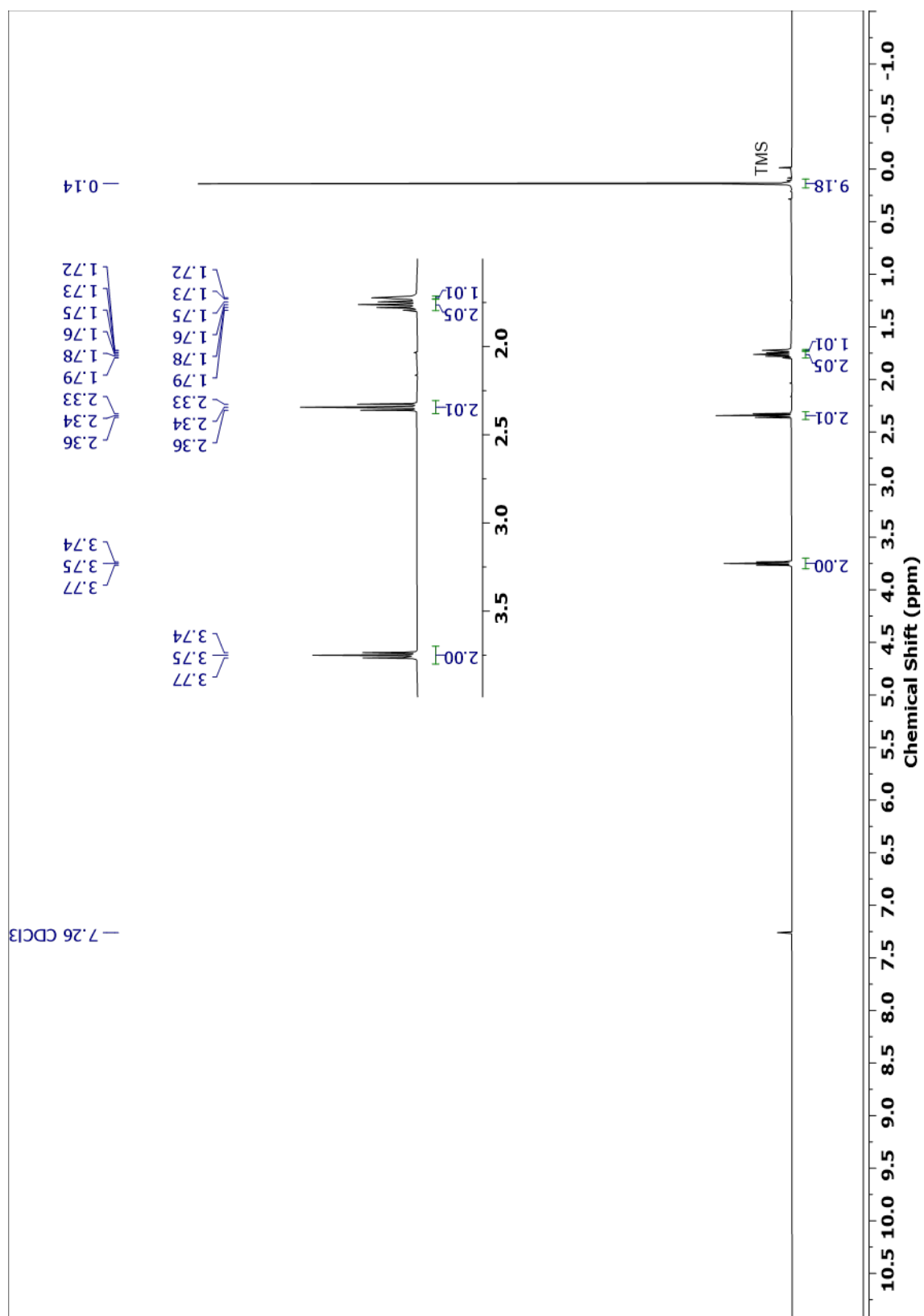
2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 10-(3-(pent-4-yn-1-yl)-3H-diazirin-3-yl)decanoate (9**)** To a solution of **8** (**PA-DA**, 48 mg, 0.17 mmol, 1.0 equiv.) and 1,2-isopropylideneglycerol (23 mg, 0.17 mmol, 1.0 equiv.) in anhydrous CH₂Cl₂ (4.8 mL), 4-dimethylaminopyridine (5 mg, 0.04 mmol, 0.25 equiv.) was added. The mixture was cooled to 0 °C and EDC.HCl (0.05 g, 0.26 mmol, 1.5 equiv.) was added in a single portion. The reaction mixture was gradually warmed to room temperature and stirred for 16 h. After completion of reaction (TLC analysis), the reaction was diluted with CH₂Cl₂ (50 mL) and washed with sat. NaHCO₃ (2 x 20 mL) and brine (50 mL). The combined organic layer was dried over Na₂SO₄, and concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (elution with gradient 5–10% EtOAc/ hexanes) to afford **9** as a colorless oil (55 mg, 81%). **TLC** *R*_f = 0.3 (10% EtOAc/ hexanes; TLC stain, PMA); **¹H NMR** (400 MHz, CDCl₃): δ 4.35–4.27 (m, 1H), 4.16 (dd, *J* = 11.5, 4.7 Hz, 1H), 4.12–4.04 (m, 2H), 3.73 (dd, *J* = 8.4, 6.2 Hz, 1H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.16 (td, *J* = 6.9, 2.6 Hz, 2H), 1.94 (t, *J* = 2.7 Hz, 1H), 1.65–1.56 (m, 2H), 1.51–1.45 (m, 2H), 1.43 (s, 3H), 1.38–1.18 (m, 17H), 1.12–1.01 (m, 2H); **¹³C NMR** (100 MHz, CDCl₃): δ 173.8, 110.0, 83.6, 73.8, 69.0, 66.5, 64.7, 34.2, 33.0, 32.0, 29.4, 29.4, 29.3, 29.3, 29.2, 28.6, 26.8, 25.5, 25.0, 23.9, 22.9, 18.1; **HRMS** (*m/z*): [M + H]⁺ calcd. for C₂₂H₃₇N₂O₄, 393.2753; found, 393.2752.



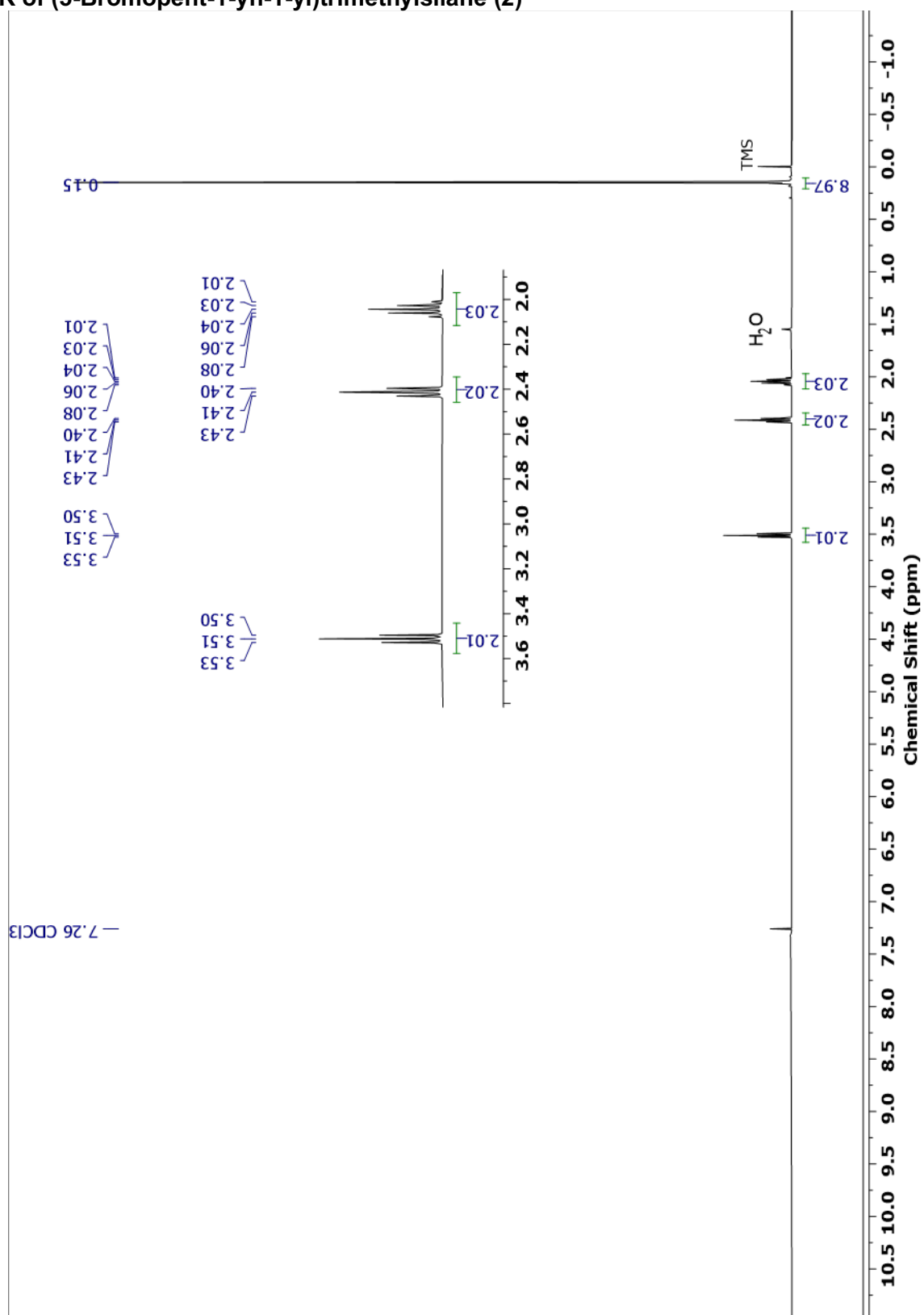
2,3-Dihydroxypropyl 10-(3-(pent-4-yn-1-yl)-3H-diazirin-3-yl)decanoate (PG-DA) (10).

Amberlyst-15 (H^+ form, 88 mg, 0.28 mmol, 2 equiv.) was added to a solution of **compound 9** (55 mg, 0.140 mmol, 1 equiv.) in MeOH (9.0 mL). The resulting reaction mixture was stirred for 18 h at room temperature. After completion of reaction (TLC analysis), Amberlyst-15 was filtered off, and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel column chromatography (elution with gradient 30–60% EtOAc/ hexanes) to afford the desired compound **10 (PG-DA)** as a colorless oil (30 mg, 61%). **TLC** R_f = 0.5 (60% EtOAc/hexanes; TLC stain, PMA); **^1H NMR** (400 MHz, CDCl_3): δ 4.21–4.07 (m, 2H), 3.95–3.87 (m, 1H), 3.68 (dd, J = 11.5, 3.9 Hz, 1H), 3.58 (dd, J = 11.5, 5.9 Hz, 1H), 2.91 (bs, 1H), 2.56 (bs, 1H), 2.33 (t, J = 7.6 Hz, 2H), 2.14 (td, J = 7.0, 2.6 Hz, 2H), 1.94 (t, J = 2.7 Hz, 1H), 1.67–1.54 (m, 2H), 1.51–1.42 (m, 2H), 1.38–1.15 (m, 14H), 1.11–1.00 (m, 2H); **^{13}C NMR** (100 MHz, CDCl_3) δ 174.5, 83.6, 70.4, 69.0, 65.2, 63.5, 34.2, 32.9, 31.9, 29.4, 29.3, 29.2, 29.2, 29.2, 28.6, 25.0, 23.9, 22.9, 18.1; **HRMS** (m/z): $[\text{M} + \text{H}]^+$ calcd. for $\text{C}_{19}\text{H}_{33}\text{N}_2\text{O}_4$, 353.2440; found 353.2437.

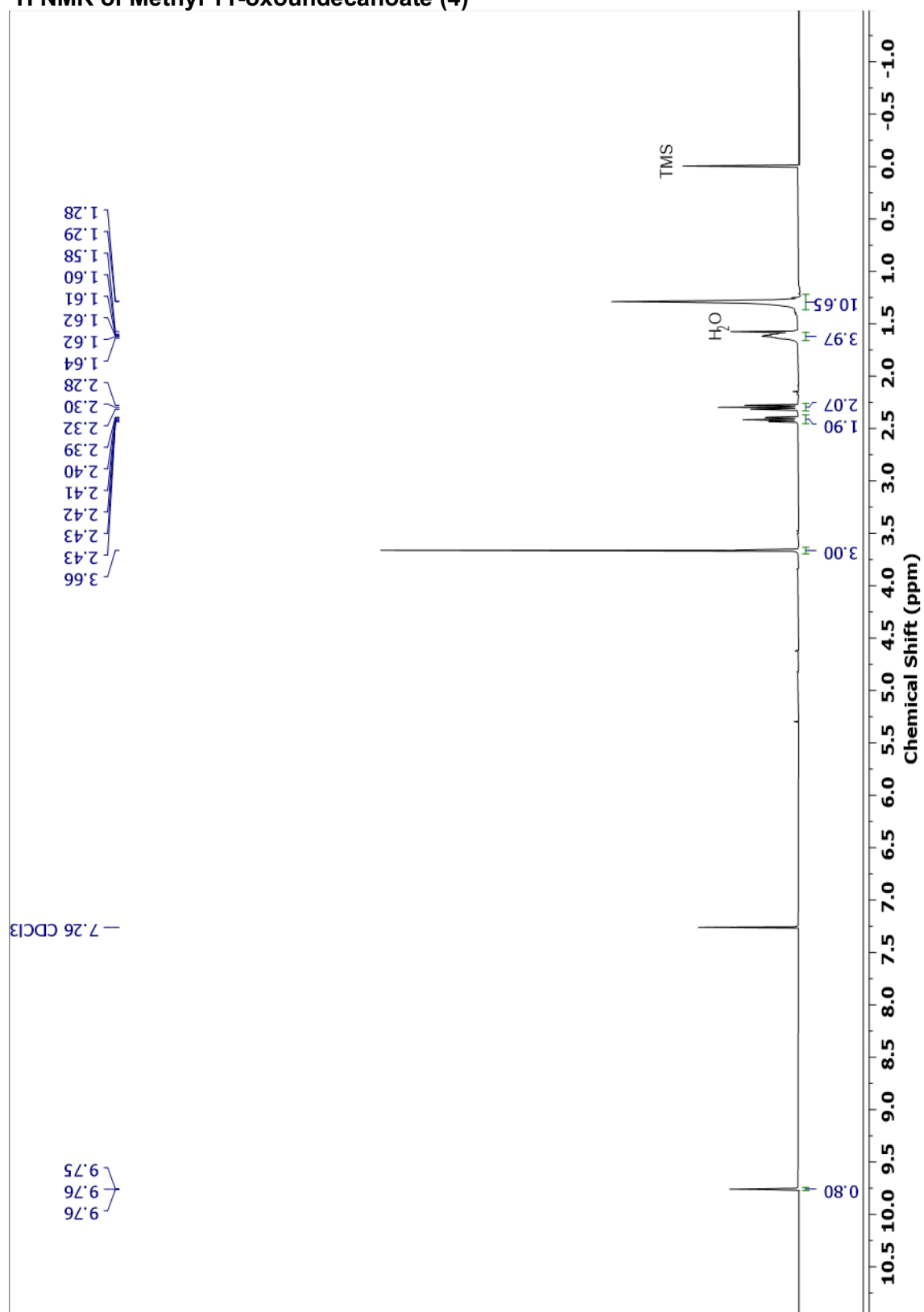
¹H NMR of 5-(Trimethylsilyl)pent-4-yn-1-ol (1)



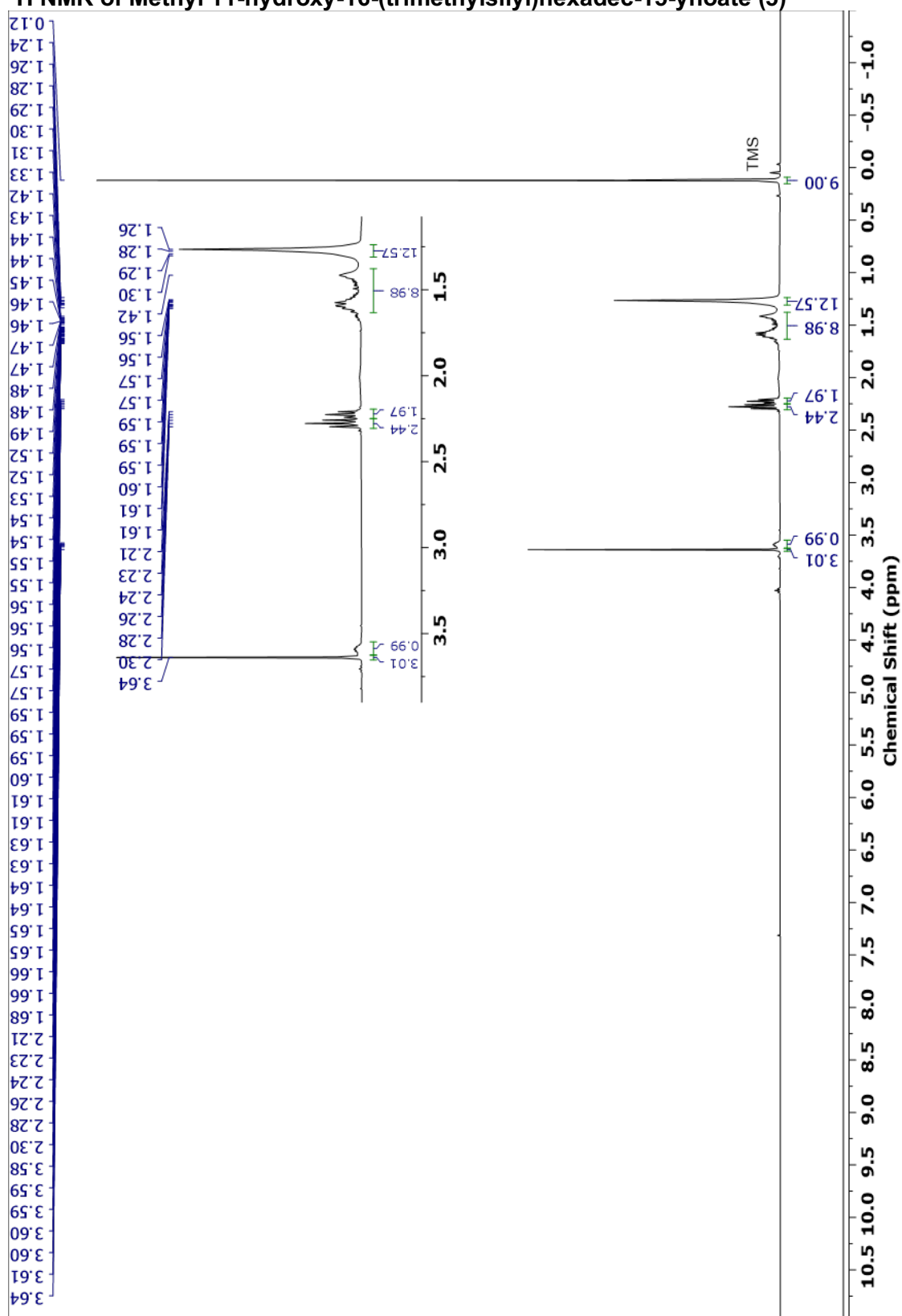
¹H NMR of (5-Bromopent-1-yn-1-yl)trimethylsilane (2)



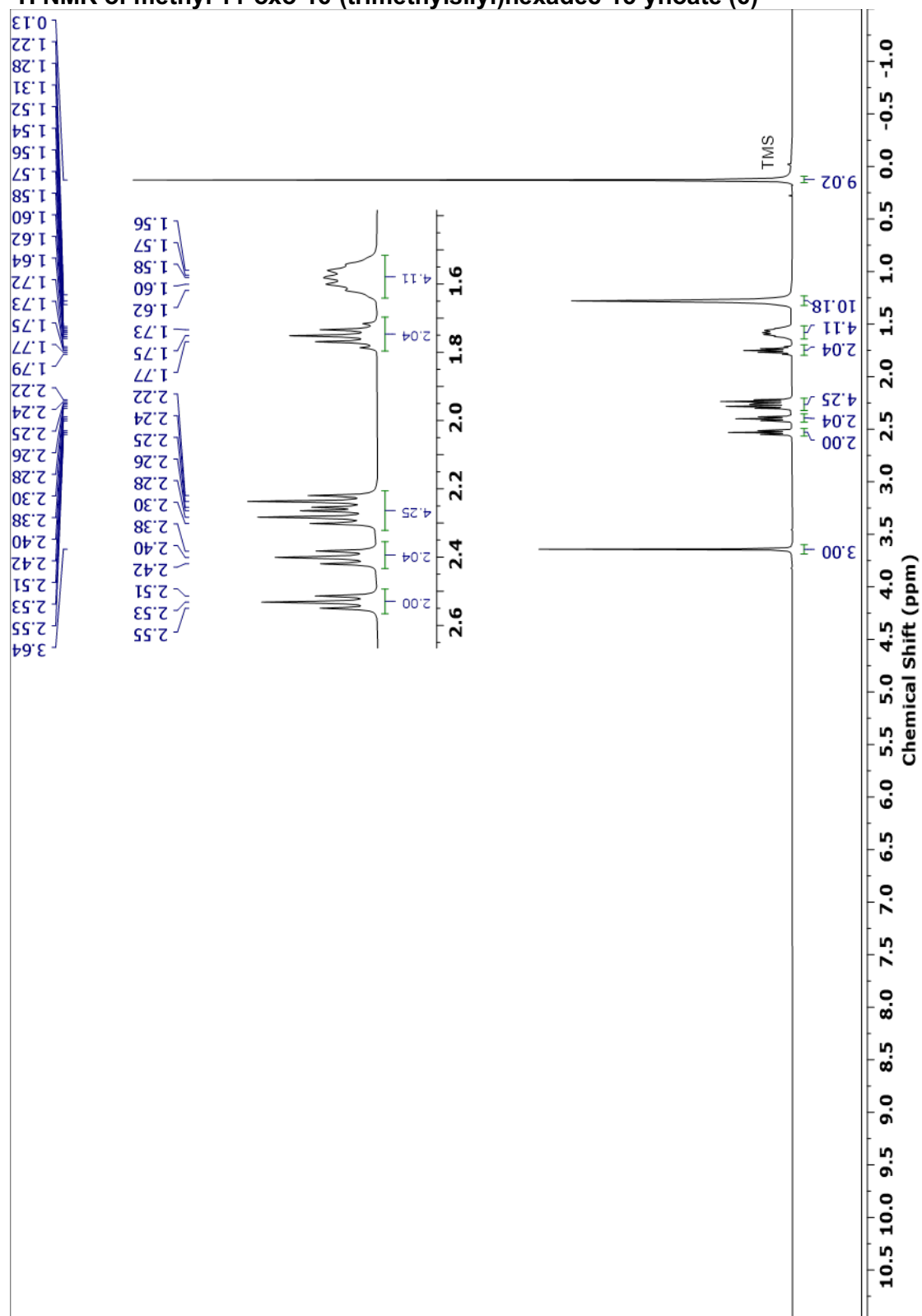
¹H NMR of Methyl 11-oxoundecanoate (4)



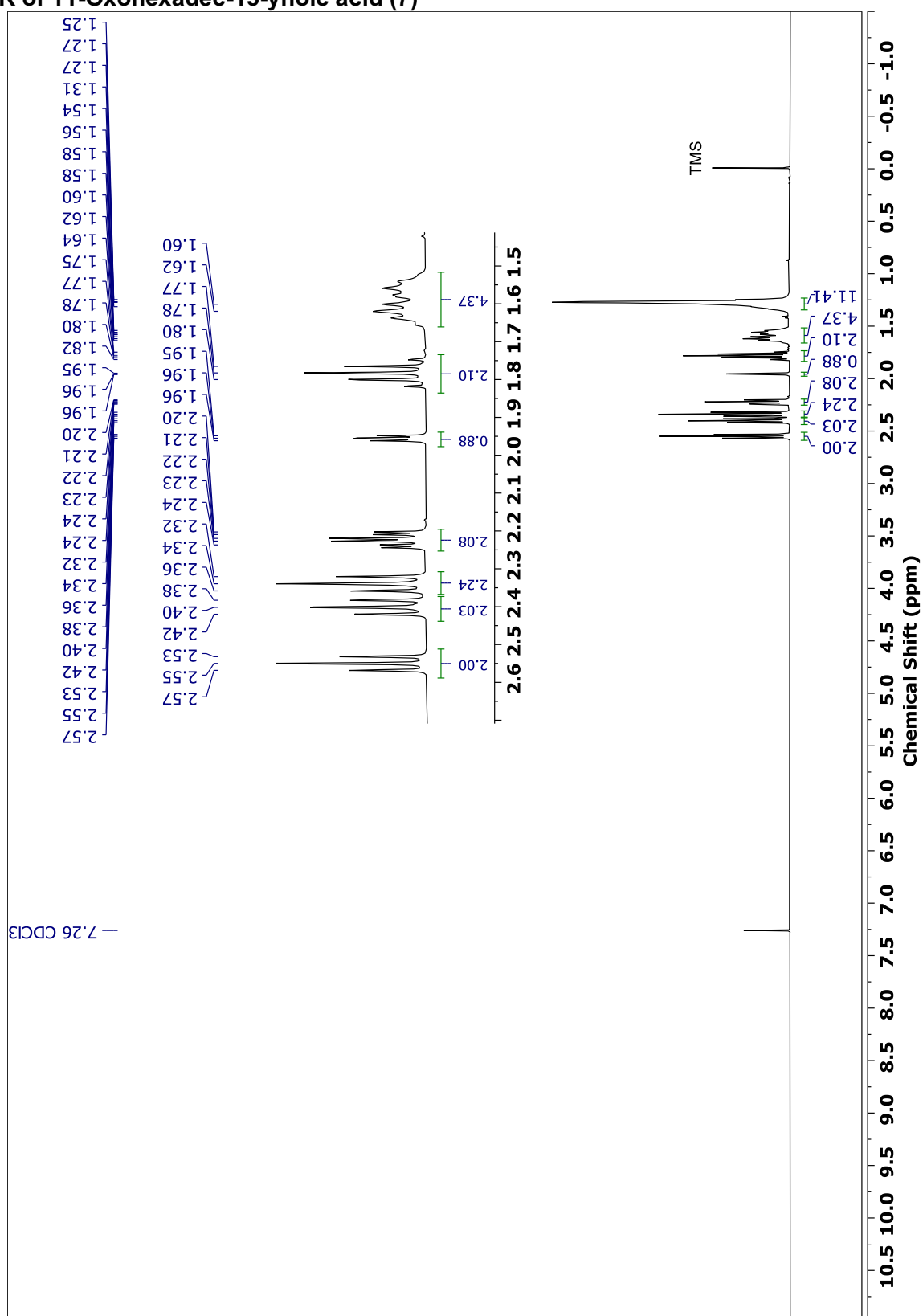
¹H NMR of Methyl 11-hydroxy-16-(trimethylsilyl)hexadec-15-ynoate (5)



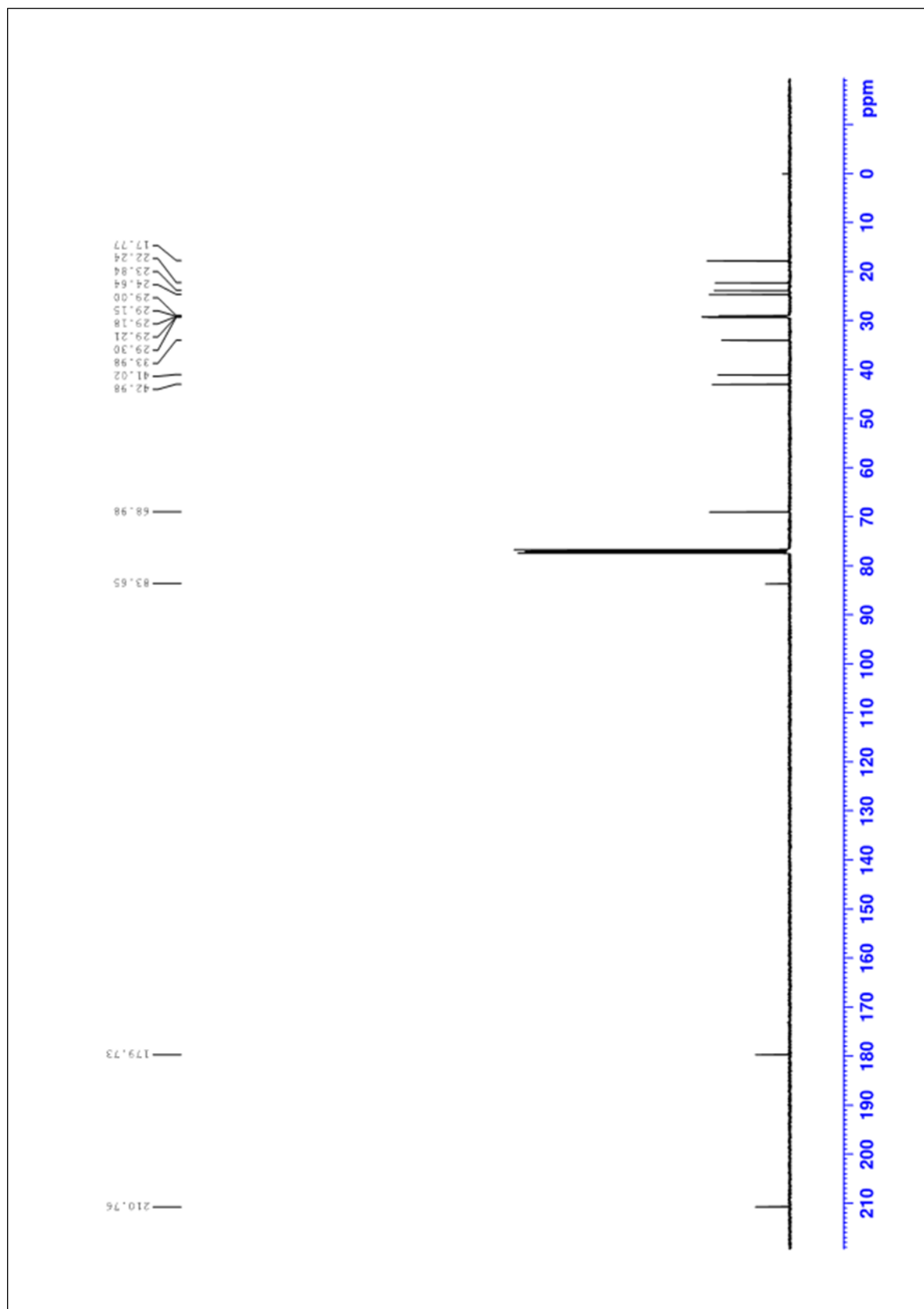
¹H NMR of methyl 11-oxo-16-(trimethylsilyl)hexadec-15-ynoate (6)



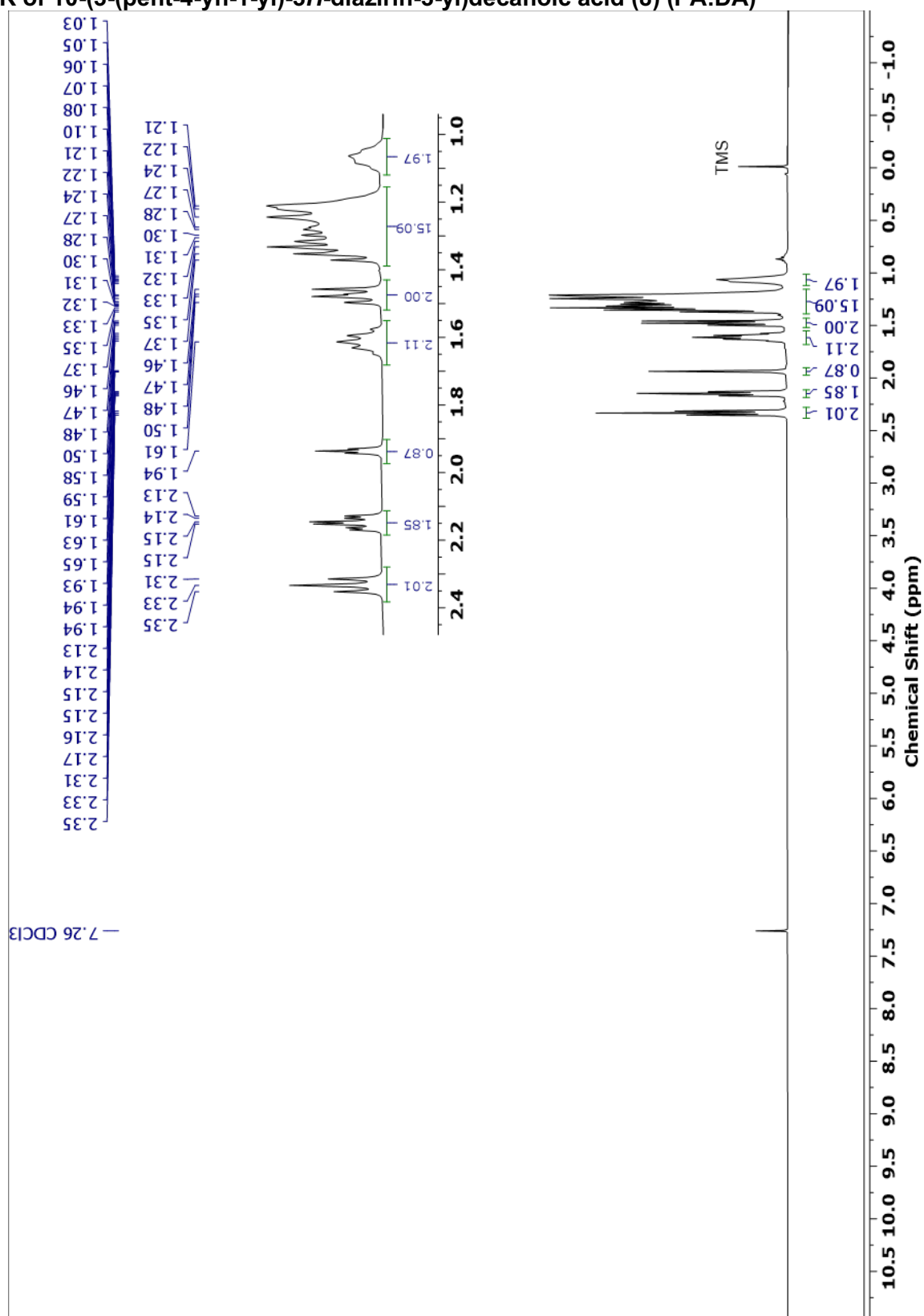
¹H NMR of 11-Oxohexadec-15-ynoic acid (7)



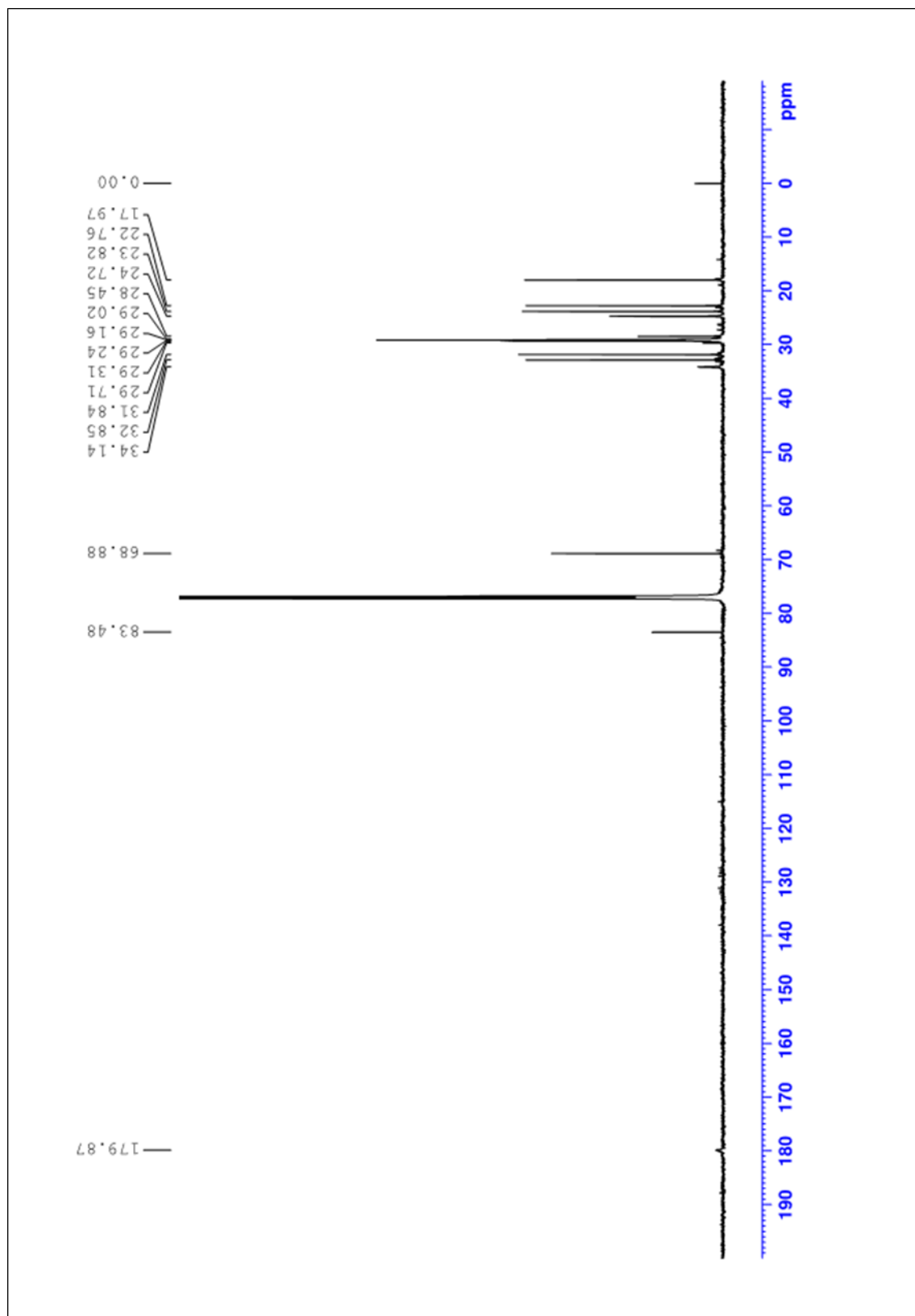
^{13}C NMR of 11-Oxohexadec-15-ynoic acid (7)



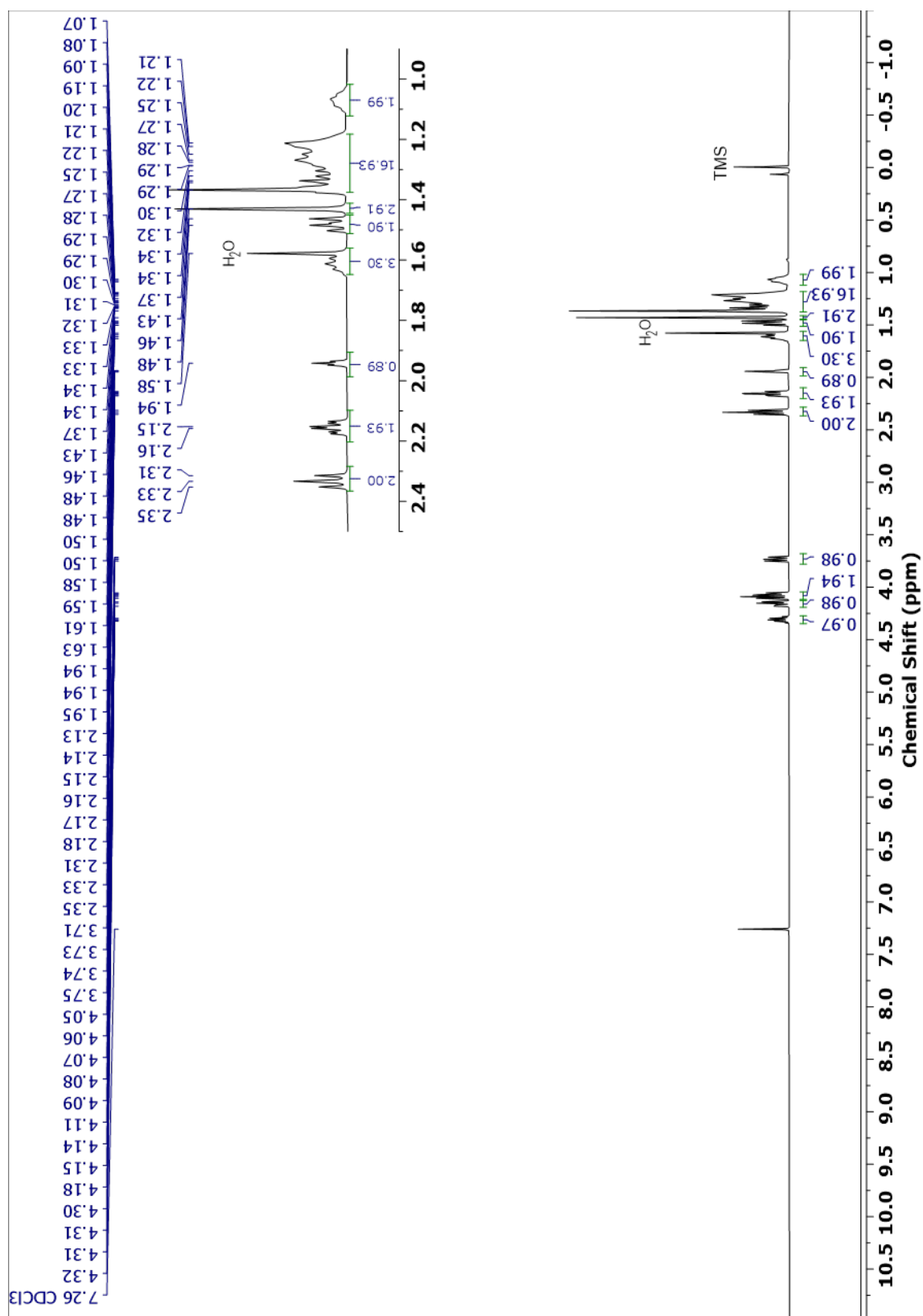
¹H NMR of 10-(3-(pent-4-yn-1-yl)-3*H*-diazirin-3-yl)decanoic acid (8) (PA:DA)



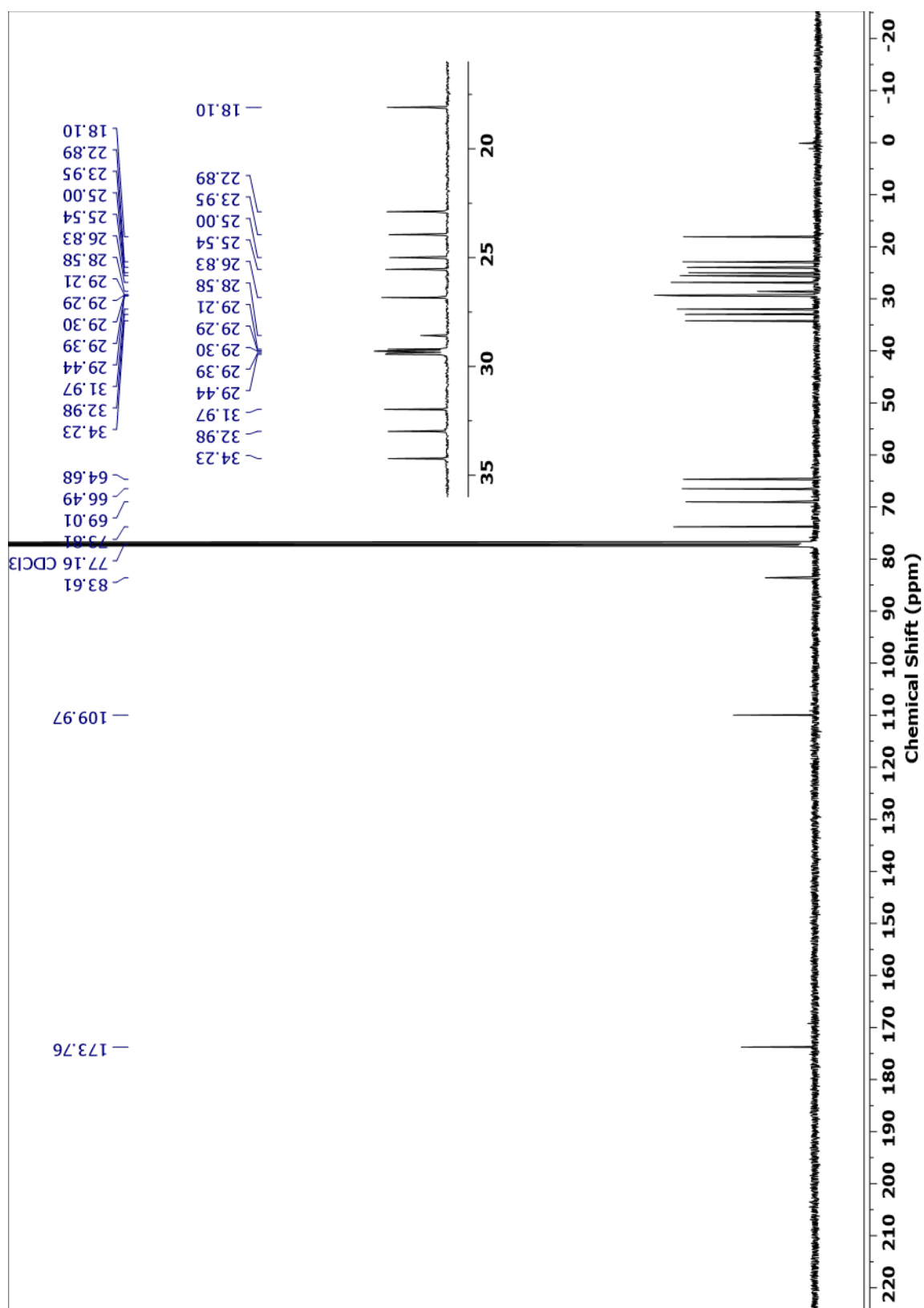
^{13}C NMR of 10-(3-(pent-4-yn-1-yl)-3*H*-diazirin-3-yl)decanoic acid (8) (PA:DA)



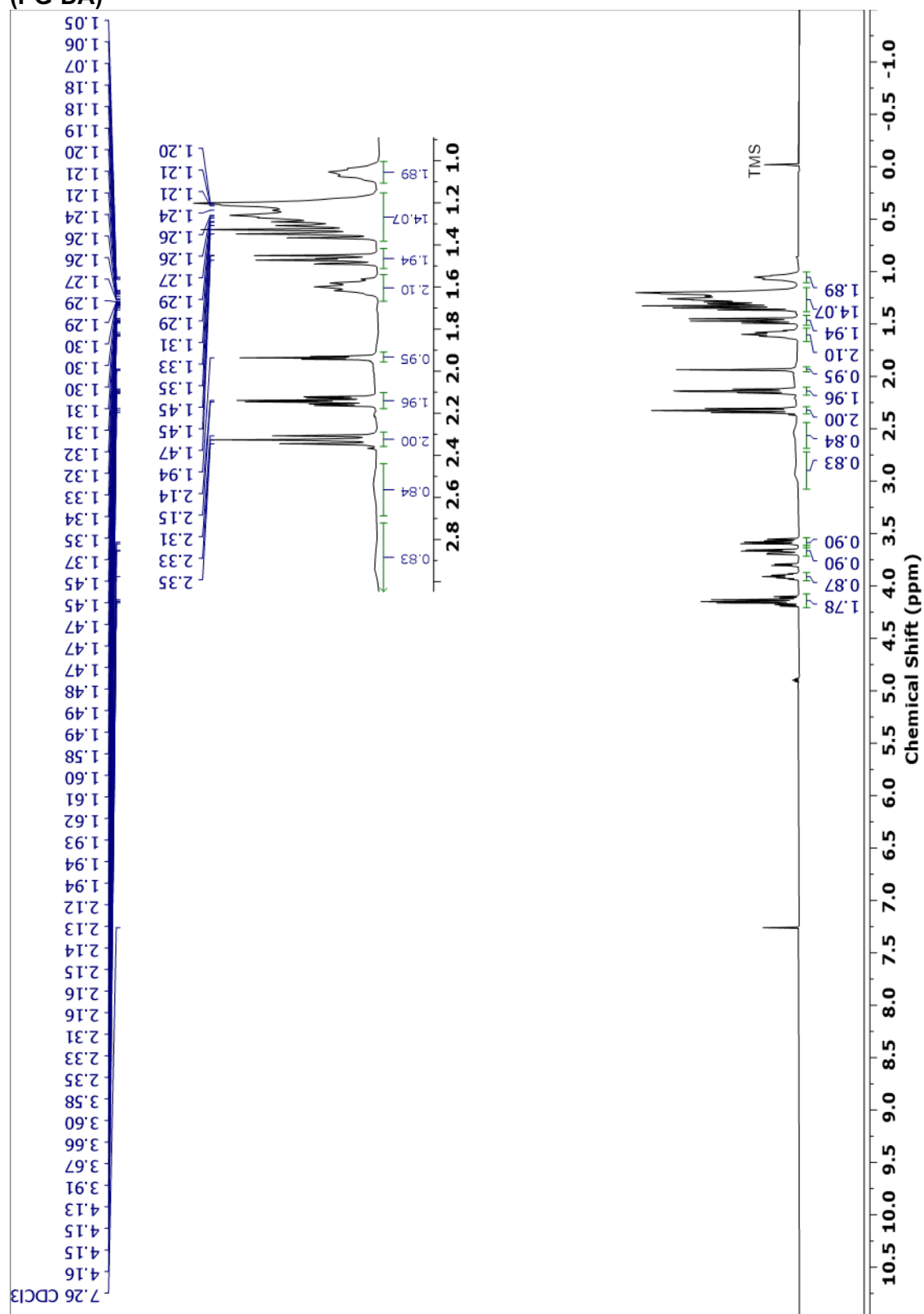
¹H NMR of (2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 10-(3-(pent-4-yn-1-yl)-3H-diazirin-3-yl)decanoate (9)



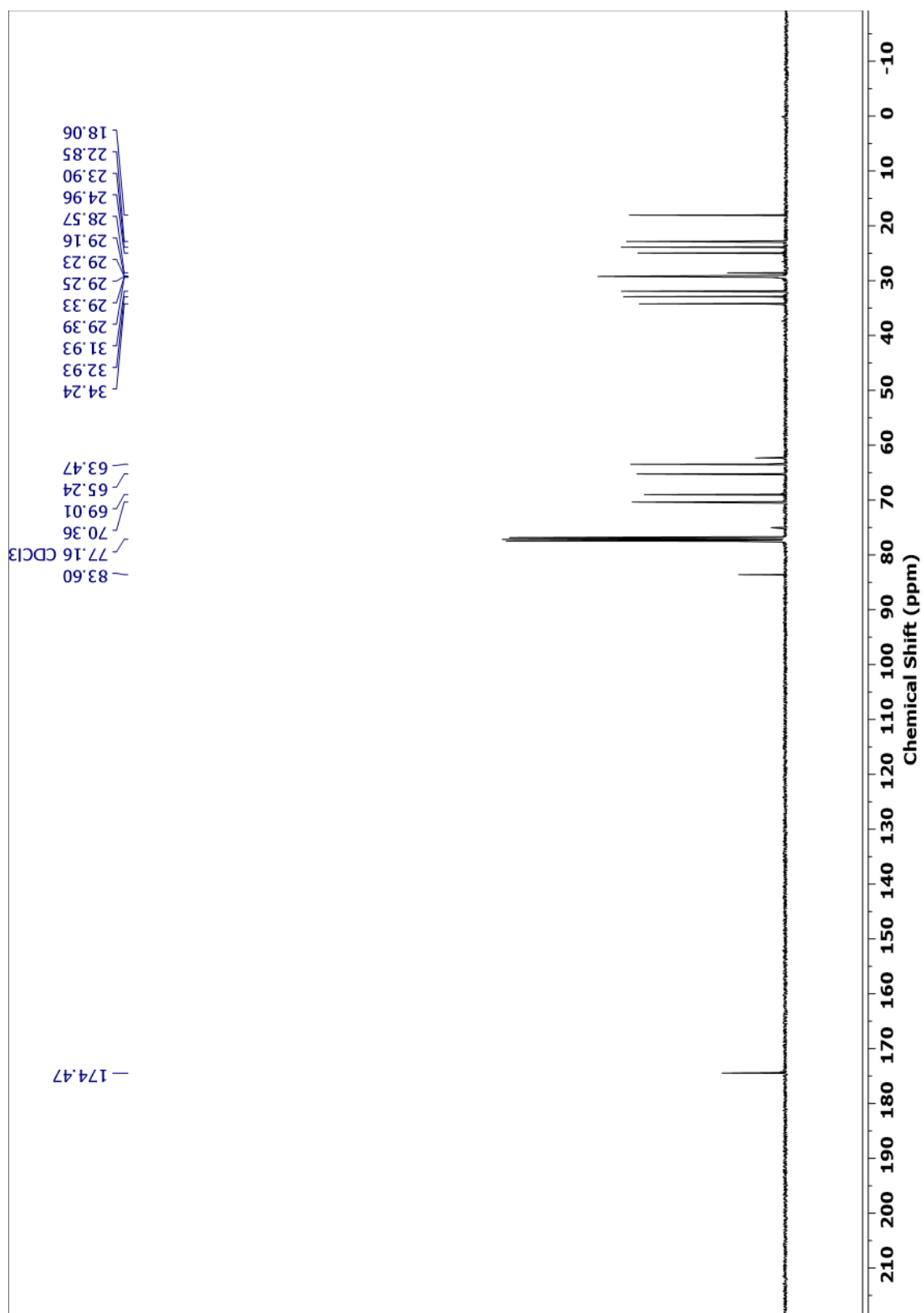
¹³C NMR of (2,2-Dimethyl-1,3-dioxolan-4-yl)methyl 10-(3-(pent-4-yn-1-yl)-3*H*-diazirin-3-yl)decanoate (9)



¹H NMR of 2,3-dihydroxypropyl 10-(3-(pent-4-yn-1-yl)-3H-diazirin-3-yl)decanoate (10) (PG-DA)



^{13}C NMR of 2,3-dihydroxypropyl 10-(3-(pent-4-yn-1-yl)-3*H*-diazirin-3-yl)decanoate (10) (PG-DA)



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