SUPPORTING INFORMATION FOR:

Charting the chemical space of acrylamide-based inhibitors of zDHHC20

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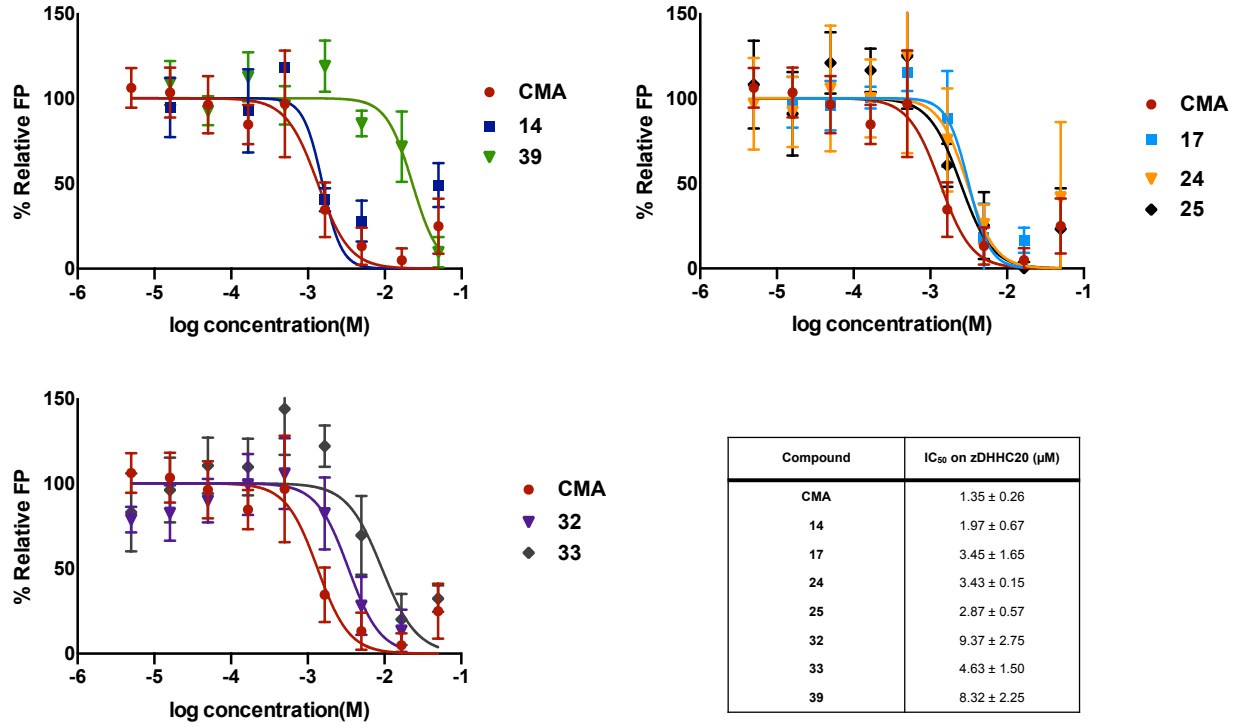


Compound	IC_{50} on zDHHC20 (μM)
CMA	1.35 ± 0.26
14	1.97 ± 0.67
17	3.45 ± 1.65
24	3.43 ± 0.15
25	2.87 ± 0.57
32	9.37 ± 2.75
33	4.63 ± 1.50
39	8.32 ± 2.25

Figure S1. Inhibition of zDHHC20 *in vitro* with 1 hour preincubation. Dose-response curves of **CMA** (red), **14** (blue), **39** (green), **17** (light blue), **24** (orange), **25** (black), **32** (purple) and **33** (gray) on zDHHC20 measured by the FP assay. Data represent mean ± standard deviation (*n=3*). The IC_{50} values were calculated from a four-parameter dose-response curve. The final concentrations of the FAM-NRas peptide (peptide-01) and palmitoyl-CoA are 4 μM and 1.25 μM, respectively. A detailed protocol can be found in the Material and Methods section.

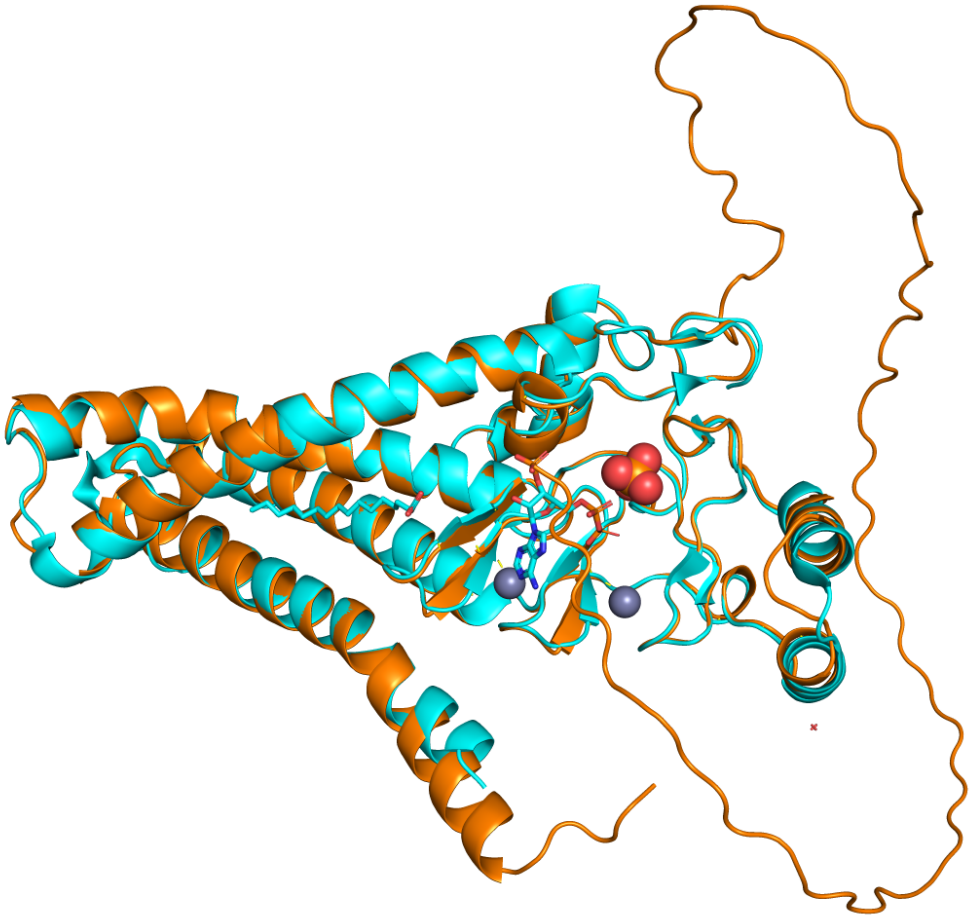


Figure S2. Overlay of the structure of human zDHHC20 (PDB: 6BML; cyan) with human zDHHC2 (AlphaFold: AF-Q9UIJ5-F1; orange)[1,2]. An RMSD of 0.4 was found between the two structures with Pymol.

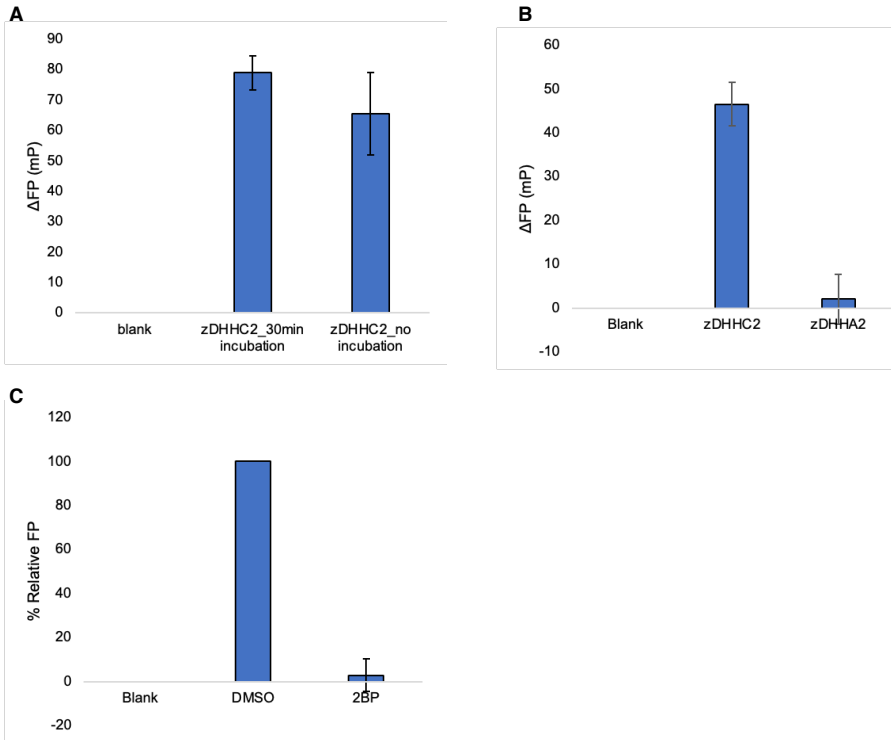
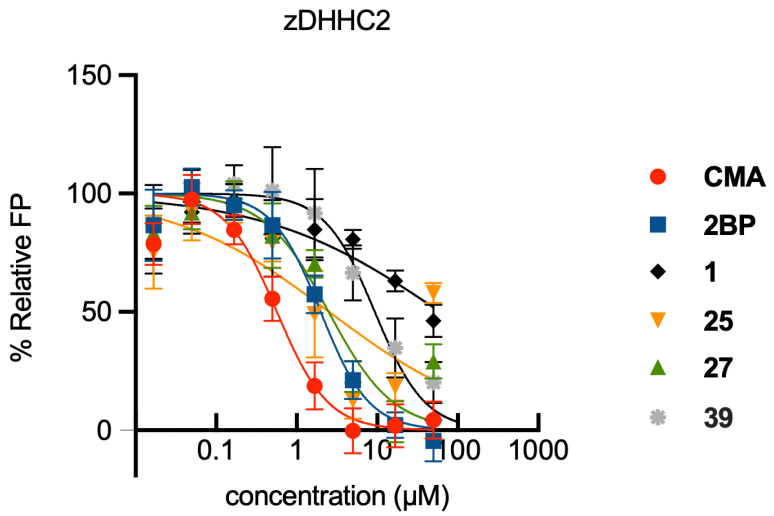


Figure S3. Validation of the zDHHC2 fluorescence polarization (FP) *in-vitro* assay. **A.** Endpoint FP of peptide-01 at t=30 min with buffer only, zDHHC2 with or without 30 min preincubation at 37℃ in reaction buffer. Data represent the mean ± standard deviation (n=2). **B.** Endpoint FP of peptide-01 at t=30 min with buffer only, wild-type (WT) zDHHC2, or mutant zDHHA2. Data represent the mean ± standard deviation (n=2). **C.** Endpoint FP of peptide-01 at t=30 min with buffer only, WT zDHHC2 treated with DMSO, or WT zDHHC2 treated with 20 μM 2BP. Data represent the mean ± standard deviation (n=3).



Compound	IC_{50} on zDHHC2 (μM]
2BP	2.02 ± 0.39
CMA	0.59 ± 0.12
1	59.9 ± 12.1
25	2.66 ± 1.25
27	2.02 ± 0.39
39	8.42 ± 0.70

Figure S4. Inhibition of zDHHC2 *in vitro* with 1 hour preincubation. Dose-response curves of **CMA** (red), **2BP** (blue), **1** (black), **25** (yellow), **27** (green) and **39** (light gray) on zDHHC2 measured by the FP assay. Data represent mean ± standard deviation (*n*=*3*). The IC_{50} values were calculated from a four-parameter dose-response curve. The final concentrations of the FAM-NRas peptide (peptide-01) and palmitoyl-CoA are 4 μM and 1.25 μM, respectively. A detailed protocol can be found in the Material and Methods section.

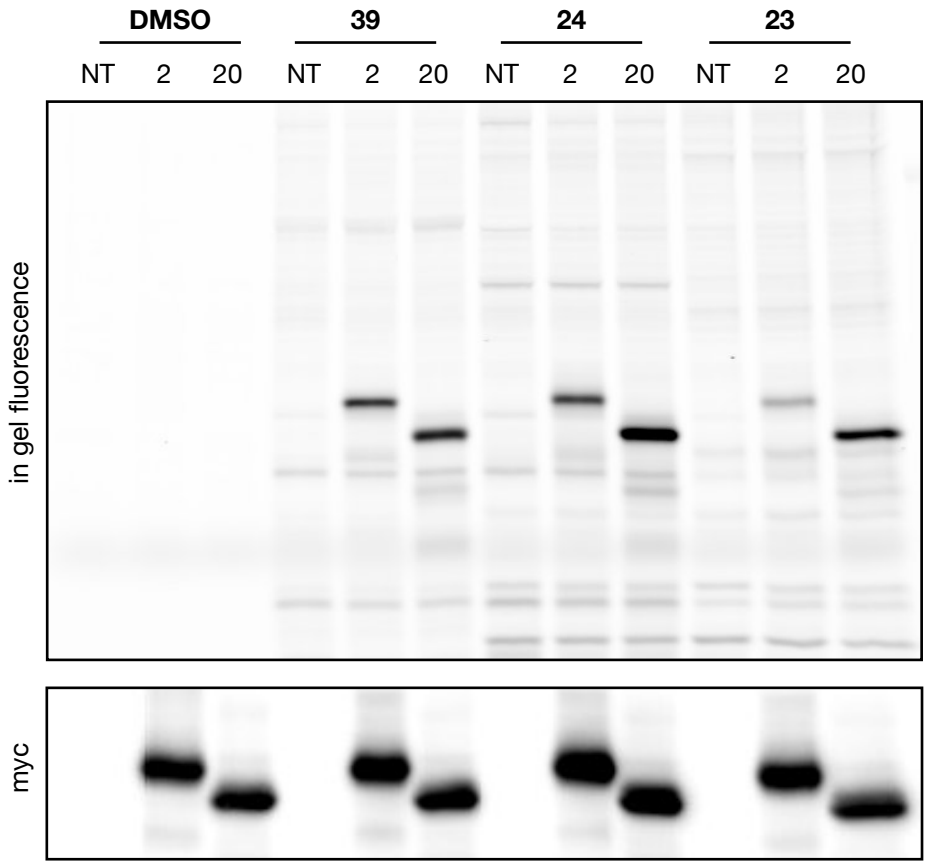


Figure S5. In-gel fluorescence analysis of **23**, **24** and **39** labeling of human zDHHC2 and zDHHC20 in serum-free media, following TAMRA-azide conjugation to **23**, **24** and **39** labeled species via Cu-AAC click reaction HEK293T cells overexpressing a DHHC protein were treated with either **23**, **24** or **39** (1 μM, 1 hours). α-HA Western blotting was used to verify the expression and size of DHHC proteins (*n=1*).

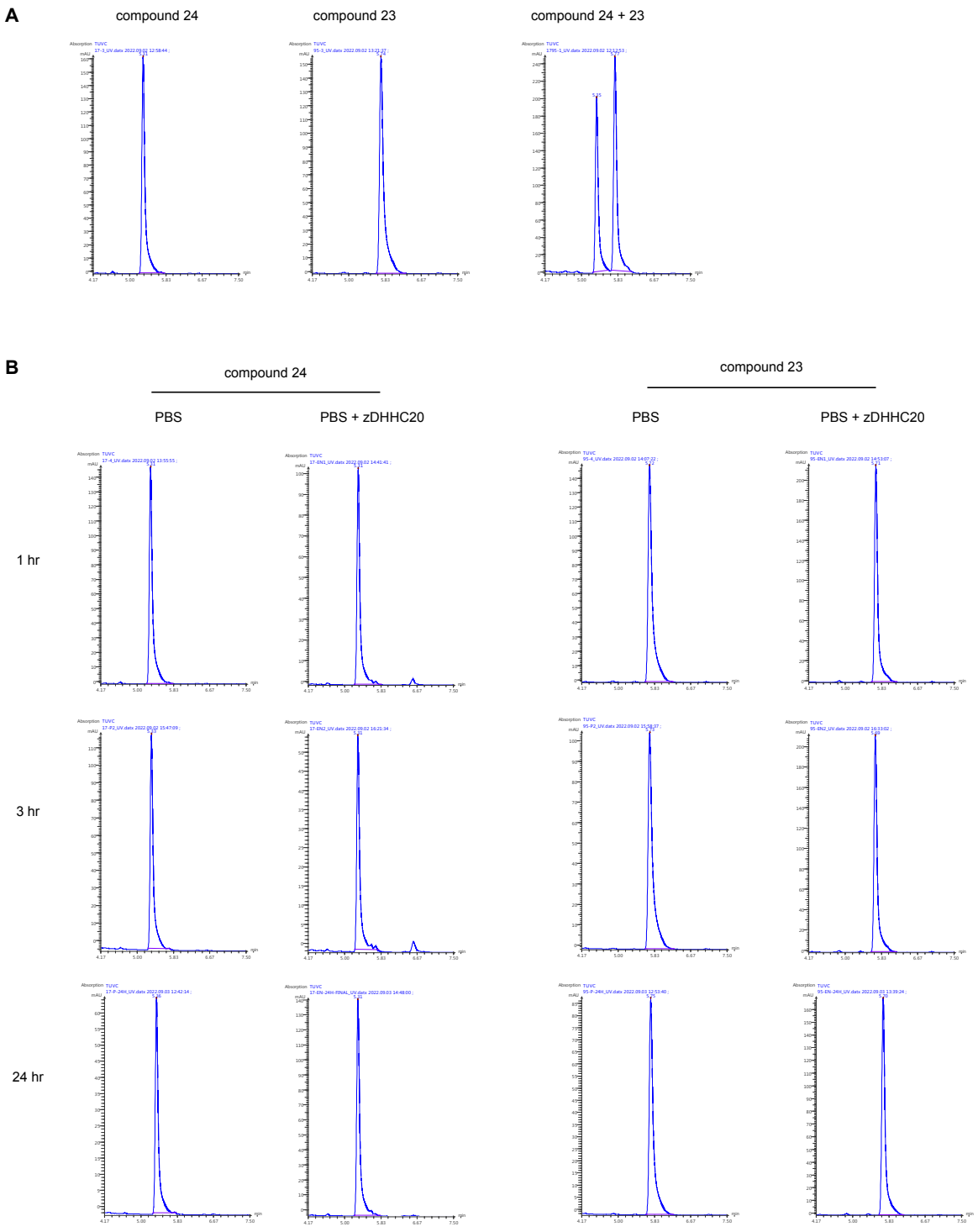
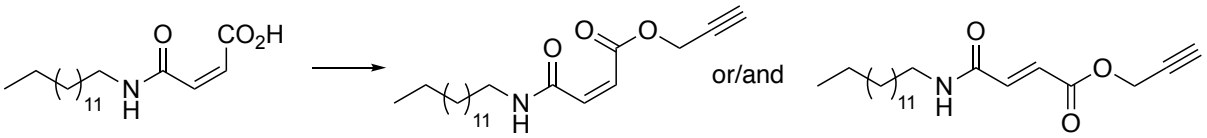


Figure S6. Isomer stability studies of **24** and **23**. a) UV traces of **24** (rt = 5.3), **23** (rt = 5.8) and **24** and **23** visualized via LCMS; and b) Incubation of **24** and **23** in PBS with and without zDHHC20 for 1, 3, and 24 hours with no isomerization observed via UV-LCMS.



20 **24** **23**

Base	θ	Time	Others	Product	Yield (%)	Scale (mg)
K_2CO_3 (3 eq.)	RT	1h	dark	E	-	≈ 150
K_2CO_3 (3 eq.)	RT	6h	light	E	-	≈ 200
None	RT	22h	light	E	-	≈ 80
NaH (2 eq.)	RT	18h	dark	Z:E (83/17)	65	≈ 250
NaH (1 eq.)	4°C	4 days	light	Z	16	≈ 300
NaH (0.5 eq.)	RT	2 days	dark	Z	34	≈ 100
NaH (1 eq.)	RT	4 days	dark	Z:E (93/7)	46	≈ 600

Table S1. Screening of the reaction conditions for the synthesis of compound **20.**

Materials and Methods

Safety Statement

No unexpected or unusually high safety hazards were encountered.

Log P Determination

Compounds Log P were determined using the Log P prediction tools from Marvin View.

Expression of human zDHHC20 and zDHHC2 in HEK293T cells

HEK293T cells (~3,000,000 cells per dish) were plated in a 15 cm dish (Corning). After 24 hours, at ~70-80% confluency, cells were transfected with 22 μg of pCE-puro myc-His_6-human zDHHC2 or zDHHC20 using PEI. Two days post transfection, Cells were harvested by centrifugation at 3000 x g and then washed with DPBS and flash frozen until purification.

Expression of human zDHHC2 in ExpiSf9 cells for IC_{50} determination

Recombinant baculovirus encoding myc-His_6-strep-human zDHHC2 was generated by cloning myc-His_6-strep-human zDHHC2 into a pFastBac1 vector and obtained after transfection in Sf9 cells (Novagen) followed by amplification in ExpiSf9 cells (ThermoFisher) according to manufactor's user manual. After treated with ExpiSf Enhancer for 18-20 hours, 200-ml ExpiSf9 insect cells were infected at 5.5-6 x 10^6 cells/mL with the zDHHC2 baculovirus. Infected cells were cultured at 28 °C shaker for 72 h. Cells were harvested by centrifugation at 4000 rpm and then washed with DPBS and flash frozen until purification.

Purification of human zDHHC2 and 20

On the day of purification, Cells were then lysed with the lysis buffer (50 mM Tris, 150 ml NaCl, 1 mM EDTA, 10% glycerol, 1 mM TCEP, pH 7.4 with 20 mM DDM and a protease inhibitor cocktail) and rotated end-over-end in 4 ˚C for 2 hours. After centrifugation at 12,000 xg at 4℃ for 20 min, cell debris was discarded, and the clear cell lysate was diluted with the same volume of DDM-free lysis buffer. Then 50% Takara His60 Ni Superflow Resin slurry (1 mL for 2x 15cm dishes HEK293T cells and 3 mL for ExpiSf9 cells from 200-mL culture) was used for incubation via end-over-end rotation at 4℃ for 2 hours. The Ni resin was then washed twice with 5x column volume of W1 buffer (20 mM imidazole, 50 mM Tris, 1 M NaCl, 20% glycerol, 1 mM TCEP, 0.3 mM DDM, pH 7.5) and W2 buffer (40 mM imidazole, 50 mM Tris, 1 M NaCl, 20% glycerol, 1 mM TCEP, 0.3 mM DDM, pH 7.5), respectively. Then the His-tagged proteins were eluted three times with 1x or 1.5x column volume of elution buffer (300 mM imidazole, 50 mM Tris, 1 M NaCl, 20% glycerol, 1

mM TCEP, 0.3 mM DDM, pH 7.5). After checking the presence of zDHHC proteins with Western blot, the fractions with zDHHC proteins were combined and concentrated (200-300 µl for protein from HEK293T cells, 3 mL for protein from ExpiSf9 cells) with Amicon Ultra-4 Centrifugal Filter Unit, 10 kDa. The concentration of zDHHC20 was determined initially by Bradford assay (Bio-Rad) and for subsequent batches, by fluorescence polarization (FP) assay. The concentration of zDHHC2 was determined by Coumassie Blue. Purified proteins were stored at -20℃.

In vitro fluorescence polarization assay of zDHHC20 and zDHHC2 for molecule screening and IC_{50} determination

The assay protocol is adapted from the 20-µl protocol from the previous paper[3]. Purified zDHHC20 (64 nM in 5 µL) or zDHHC2 (1 µM in 5 µL) in reaction buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 2 mM TCEP, 2 mM DDM, pH 7.0) or reaction buffer with protein storage buffer was added to a 384-well optical bottom plate (ThermoFisher). 5 µL of the small molecule stock in reaction buffer were added, resulting in a 10 µL mixture of enzyme and small molecule, and the mixtures were incubated in 37℃ for 1 hour (for zDHHC20) or in room temperature for 30 min (for zDHHC2). The MasterMix II (10 µL), containing 8 µM palmitoyl-CoA and 2.5 µM peptide 01(5-FAM-GTQGCMGLPCVVM-COOH) in reaction buffer, was added to initiate the reaction, resulting in 4 µM and 1.25 µM as the final concentration of palmitoyl-CoA and peptide 01, respectively. Fluorescence measurements were recorded on Synergy Neo2 Hybrid Multi-Mode Reader (BioTek Instruments, Inc.) with Dual FP Green filter cube. Fluorescence polarization (λ_{ex} = 485/20 nm, λ_{em} = 528/20 nm, gain=35, read from the top with height 7.5 mm, and filter switching method) was measured at 1-min time intervals for 2 hours at 37℃. Assay data were exported in Microsoft Excel 2016. For all of the molecule screening and IC_{50} determination, we performed the assay in kinetics mode, and data points at t=118 min (for zDHHC20) or 30 min (for zDHHC2) were utilized as readout of enzyme activities of different samples for comparison. Compounds were assessed at a final concentration of 10 µM for screening and through a serial dilution from 100 to 0.01 µM. All of data points are from 3 replicates. Dose-response curves and IC_{50} values were obtained using GraphPad Prism 9.

In cellulo labeling assay

HEK293T cells (~ 21’500 cells per well) were plated in 12-well plates (Fisher). After 20−24 h, cells were transfected with 0.6 µg of plasmids with Lipofectamine 2000. At 24 h post-transfection, cells were incubated with serum-free DMEM complemented with 1 µM of the desired compounds for 1h. Cells then were washed with DPBS twice and lysed with 80 µL of RIPA lysis buffer

supplemented with a protease inhibitor cocktail. After being spun down, the final cell lysate was collected and normalized to 1 mg. mL^{-1} in 30 µL before Cu-AAC conjugation. For Cu-AAC conjugation, Master mix (4 µL) made from equal volumes of 5 mM TAMRA-azide (click chemistry tools), 5 mM TBTA (Cayman), 50 mM CuSO4, and 50 mM TCEP was added into 30 µL of cell lysate. The resulting solution was incubated for 1 h at RT in darkness; then, the reaction was quenched with 6× Laemelli sample buffer containing 9% β-mercaptoethanol before running the SDS-PAGE gels. Proteins were resolved with 12% SDS-PAGE and visualized with FluoroChem R (Proteinsimple) using the MultiFluo-Green channel. Western blots of anti-HA were used as a control to indicate the molecular sizes of DHHC proteins and the amount of DHHC proteins. Cells were labeled in serum-free media without further notice.

Western blot

After SDS–PAGE, proteins were transferred onto methanol-preactivated Immobilon-P PVDF membranes (pore size 0.45 µm; Millipore) using a semi-dry transfer cell (Bio-Rad). After transfer, membranes were treated in accordance with standard Western blotting procedures, using a solution of 3% BSA (ThermoFisher) in either TBST (20 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween-20) or PBST (PBS, Fisher, with 0.1% Tween-20) wash buffer and mouse HA-tag (F7) antibodies (Cat. No. SC-7392, Santa Cruz). Membranes were visualized using SuperSignal West Pico PLUS chemiluminescent substrate (ThermoFisher) and recorded on a chemiluminescent western blot imaging system (Azure Biosystems C300).

Synthetic methods

General materials

For chemical synthesis, reagents and solvents were purchased from commercial sources and used without further purification. Silica gel P60 (SiliCycle, 40–63 μm, 230–400 mesh) was used for column chromatography. Analytical thin layer chromatography was performed using SiliCycle 60 F254 silica gel (pre-coated sheets, 0.25 mm thick) with detection at 214 nm.

Spectrometry

Low-resolution-mass spectral analysis and liquid chromatography analysis were carried out on an Advion Expression-L mass spectrometer (Ithaca, NY) with electron spray ionization (ESI) in the positive mode coupled to an Agilent 1220 Infinity LC System with an Agilent Poroshell 120 column (Santa Clara, CA). Two methods were performed as follows: (A) Gradient from 1:9 water:methanol/0.1% formic acid (FA) to 9:1 water:methanol/0.1% FA (3 min.); 9:1 water:methanol/0.1% FA (6 minutes) isocratic; gradient from 9:1 water:methanol/0.1% FA to 1:9 water:methanol/0.1% FA (1 min); 1:9 water:methanol/0.1% FA isocratic (1 min.); (B) Gradient from 5:5 water:methanol/0.1% FA to methanol (3 min.); gradient from methanol to 1:9 water:methanol/0.1% FA (8 min.). Automated flash column chromatography purification was carried out on a Biotage system Isolera One using SNAP Biotage columns. Compounds were analyzed by UPLC-MS and purity was assigned by analytical RP-UPLC. NMR spectra were recorded on the BRUKER Ascend 400 at the Department of Chemistry NMR Facility, University of Chicago, for ^{1}H-400 MHz and ^{13}C-101 MHz measurements. Chemical shifts are given in parts per million (δ) referenced to TMS (δ = 0.00 ppm ^{1}H-, ^{13}C-NMR). Coupling constants are given in Hertz. High resolution mass spectra measurements were performed on an Agilent 6224 Tof. using a combination of atmospheric pressure chemical ionization and electrospray ionization at the Department of Chemistry Mass Spectrometry Facility, University of Chicago.

Compounds **CMA**, **1**, **2**, **17**, **20** & **35** to **42** are already known and their synthesis can be found in [3] (compounds number 1 to 12).

General method A for the synthesis of secondary amine.

To a solution of the amine (1.0 eq.) in DMF (2-5 mL) was added potassium carbonate (2.0 eq.), followed by the desired bromo compound (0.75 eq.). The reaction mixture was stirred at 85°C until the reaction was complete (8-12 hours). Then, the reaction mixture was cooled down to r.t. and diluted with a saturated aqueous solution of sodium bicarbonate and the aqueous phase was

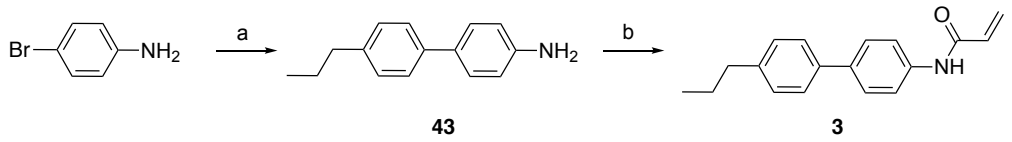
extracted with ethyl acetate (3x50 mL). The combined organics were then dried over Na_2SO_4 and concentrated. The obtained crude product was then purified by automated flash chromatography (2-10% MeOH in DCM) to afford the pure product.

General method B for the synthesis of acrylamide.

To a solution of the amine (1.0 eq.) in DMF (2-5 mL) was added potassium carbonate (3.0 eq.) and after 15 minutes of stirring, acryloyl chloride (2.0 eq.). The reaction mixture was stirred at r.t. for 2 hours or until the reaction was complete. The reaction mixture was then diluted with water (20 mL) and extracted with ethyl acetate (3x20 mL). The combined organics were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The obtained crude product was then purified by automated flash chromatography (20-80% ethyl acetate in hexanes) to afford the pure product.

General method C for the synthesis of acrylamide.

To a solution of the amine (1.0 eq.) in DCM (2-5 mL) was added triethylamine (3.0 eq.) and after 15 minutes of stirring, acryloyl chloride (2.0 eq.). The reaction mixture was stirred at r.t. for 2 hours or until the reaction was complete. The reaction mixture was then diluted with water (20 mL) and extracted with ethyl acetate (3x20 mL). The combined organics were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The obtained crude product was then purified by filtration on a pad if silica with 50% ethyl acetate in hexanes to afford the pure product.



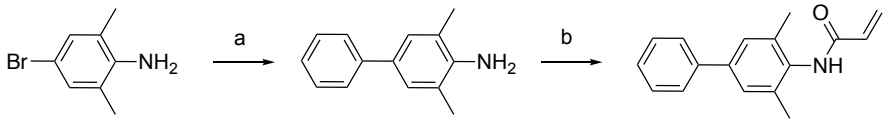
43 **3**

Scheme S1. Synthetic route used for the synthesis of **3**. Conditions: a) 4-*tert*-butylphenylboronic acid, K_2CO_3, $Pd(PPh)_3$, dioxane/water/MeOH (4:1:10), 80°C, 3h30, 52%. b) acryloyl chloride, NEt_3, DCM, r.t., 2h, 95%.

4'-propyl-[1,1'-biphenyl]-4-amine (43). 4-bromoaniline (0.58 mmol, 0.10 g), 4-*tert*-butylphenylboronic acid (0.87 mmol, 0.14 g), potassium carbonate (1.74 mmol, 0.24 g) and palladium tetrakis (0.03 mmol, 0.03 g) were dissolved into a mixture of dioxane/water/methanol (3 mL, 4:1:10) degassed under nitrogen. The reaction mixture was stirred at 80°C for 3 hour 30 minutes. Then, the reaction mixture was concentrated and taken back in water and diethyl ether. The organic phase was washed with brine, dried with Na_2SO_4 and concentrated. The crude

product was purified via automated flash column chromatography (20-100% EtOAc in hexanes) to afford **43** as a yellow solid (0.06 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 – 7.43 (m, 2H), 7.43 – 7.38 (m, 2H), 7.23 – 7.18 (m, 2H), 6.78 – 6.72 (m, 2H), 2.64 – 2.57 (m, 2H), 1.67 (dq, *J* = 14.8, 7.4 Hz, 3H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.65, 140.91, 138.66, 131.79, 128.91, 127.98, 126.36, 115.54, 37.80, 24.75, 14.06.

***N*-(4'-propyl-[1,1'-biphenyl]-4-yl)acrylamide (3).** Following general procedure C, **3** was obtained from **43** (0.28 mmol, 0.06 g) as a yellow solid (0.07 g, 95%). ^{1}H NMR (400 MHz, CDCl3) δ 7.65 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.32 (s, 1H), 7.23 (s, 1H), 6.50 – 6.42 (m, 1H), 6.27 (dd, *J* = 16.9, 10.2 Hz, 1H), 5.82 – 5.76 (m, 1H), 2.67 – 2.57 (m, 2H), 1.67 (dq, *J* = 14.8, 7.4 Hz, 3H), 0.97 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 141.95, 137.90, 137.56, 136.82, 131.25, 129.07, 128.07, 127.63, 126.80, 120.33, 37.82, 29.86, 14.28, 14.03. HRMS m/z [M] calcd for $C_{18}H_{19}NO$ 265.1467, found: 265.1478. UPLC (method A): t_R = 4.29 min., purity: 97%.

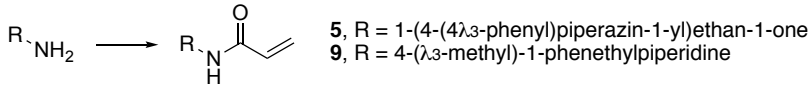


44 **4**

Scheme S2. Synthetic route used for the synthesis of **4**. Conditions: a) phenylboronic acid, K_2CO_3, $Pd(PPh)_3$, dioxane/water/MeOH (4:1:10), 80°C, 3h30, 41%. b) acryloyl chloride, K_2CO_3, THF, r.t., 2h30, 20%.

3,5-dimethyl-[1,1'-biphenyl]-4-amine (44). 4-bromo-2,6-dimethylaniline (0.50 mmol, 0.10 g), phenylboronic acid (0.75 mmol, 0.09 g), potassium carbonate (1.50 mmol, 0.21 g) and palladium tetrakis (0.03 mmol, 0.03 g) were dissolved into a mixture of dioxane/water/methanol (3 mL, 4:1:10) degassed under nitrogen. The reaction mixture was stirred at 80°C for 3 hour 30 minutes. Then, the reaction mixture was concentrated and taken back in water and diethyl ether. The organic phase was washed with brine, dried with Na_2SO_4 and concentrated. The crude product was purified via automated flash column chromatography (20-100% EtOAc in hexanes) to afford **44** as a brown oil (0.04 g, 41%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.57 (m, 2H), 7.43 (t, *J* = 7.7 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 1H), 7.26 (s, 2H), 3.67 (s, 2H), 2.29 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.40, 141.61, 131.14, 128.69, 127.14, 126.62, 126.18, 122.11, 17.92.

***N*-(3,5-dimethyl-[1,1'-biphenyl]-4-yl)acrylamide (4).** Following general procedure B with THF as solvent, **4** was obtained from **44** (0.21 mmol, 0.04 g) as a white solid (0.01 g, 20%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 – 7.52 (m, 2H), 7.45 – 7.39 (m, 2H), 7.34 (dd, *J* = 5.1, 2.1 Hz, 1H), 7.30 (s, 2H), 6.43 (dd, *J* = 17.0, 2.1 Hz, 1H), 6.36 (dd, *J* = 17.0, 9.5 Hz, 1H), 5.79 (dd, *J* = 9.5, 2.1 Hz, 1H), 2.30 (d, *J* = 6.3 Hz, 6H). NH hidden under the baseline. ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.16, 140.89, 140.51, 135.89, 132.90, 130.68, 129.97, 128.95, 128.81, 127.75, 127.68, 127.39, 127.28, 127.22, 18.99, 18.79. HRMS m/z [M] calcd for $C_{17}H_{17}NO$ 251.1310, found: 251.1311. UPLC (method A): t_R = 3.67 min., purity: 99%.



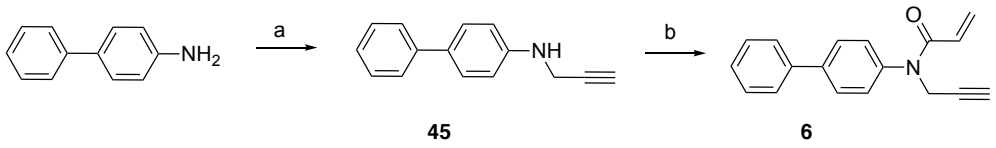
5, R = 1-(4-(4λ3-phenyl)piperazin-1-yl)ethan-1-one
9, R = 4-(λ3-methyl)-1-phenethylpiperidine

Scheme S3. Synthetic route used for the synthesis of **5 & 9**. Condition: acryloyl chloride, K_2CO_3, DMF, r.t., 1h, **5**: 41%, **9**: 65%.

***N*-(4-(4-acetylpiperazin-1-yl)phenyl)acrylamide (5).** 1-acetyl-4(4-aminophenyl) piperazine (0.10 g, 0.46 mmol, 1 eq.) was dissolved in dry DMF (5 mL) and potassium carbonate (0.31 g, 2.28 mmol, 5 eq.) was added. The suspension was stirred for 15 minutes at room temperature, and then acryloyl chloride (110 µL, 1.37 mmol, 3 eq.) was added. After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-8% MeOH in DCM) gave **5** (0.05 g, 41%) as a pink-tinged solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.51 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 6.40 (dd, J = 16.9, 1.6 Hz, 1H), 6.26 (dd, J = 16.9, 10.1 Hz, 1H), 5.71 (dd, J = 10.1, 1.6 Hz, 1H), 3.76 (t, J = 5.2 Hz, 2H), 3.62 (t, J = 5.1 Hz, 2H), 3.20 – 3.03 (m, 4H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.09, 163.54, 147.63, 131.29, 127.27, 121.41, 117.33, 77.26, 50.20, 49.83, 46.17, 41.30, 21.36. HRMS-ESI(+) m/z [M+] calculated for $C_{15}H_{19}N_3O_2$ 273.1477, found 273.1478. UPLC (method A): t_R = 3.95 min., purity: ≥99%.

***N*-((1-phenethylpiperidin-4-yl)methyl)acrylamide (9).** 1-[1-(2-Phenylethyl)piperidin-4-yl]methanamine (0.20 g, 0.92 mmol, 1 eq.) was dissolved in dry DMF (5 mL) and potassium carbonate (0.63 g, 4.58 mmol, 5 eq.) was added. The suspension was stirred for 15 minutes at room temperature, and then acryloyl chloride (220 µL, 2.75 mmol, 3 eq.) was added. After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and

concentrated. Purification via automated column chromatography (0-8% MeOH in DCM) gave **13** (0.16 g, 65%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 – 7.06 (m, 5H), 6.21 (dd, J = 17.0, 1.6 Hz, 1H), 6.04 (dd, J = 16.9, 10.2 Hz, 1H), 5.85 (d, J = 6.8 Hz, 1H), 5.56 (dd, J = 10.2, 1.6 Hz, 1H), 3.18 (t, J = 6.4 Hz, 2H), 2.98 (dt, J = 11.9, 3.4 Hz, 2H), 2.84 – 2.71 (m, 2H), 2.62 – 2.49 (m, 2H), 2.07 – 1.92 (m, 2H), 1.75 – 1.64 (m, 2H), 1.63 – 1.48 (m, 1H), 1.32 (qd, J = 12.1, 3.9 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.73, 140.09, 130.87, 128.69, 128.44, 126.41, 126.13, 60.69, 53.34, 44.96, 35.82, 33.49, 29.71. HRMS-ESI(+) m/z [M+] calculated for $C_{17}H_{24}N_2O$ 272.1889, found 272.1887. UPLC (method A): t_R = 0.41 min., purity: 91%.

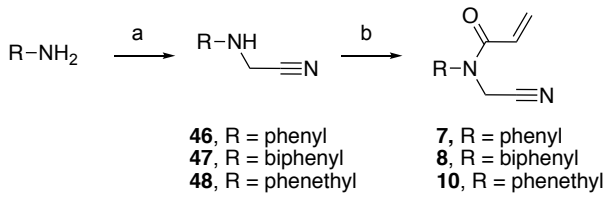


45 **6**

Scheme S4. Synthetic route used for the synthesis of **6**. Conditions: a) propargylbromide, K_2CO_3, DMF, 85°C, 8-15h, 65%. b) acryloyl chloride, K_2CO_3, DMF, r.t., 2h30, 82%.

***N*-(prop-2-yn-1-yl)-[1,1'-biphenyl]-4-amine (45).** Following general procedure A, 4-aminobiphenyl (0.60 mmol, 0.10 g) and propargylbromide (0.45 mmol, 50.0 µL) were reacted until completion of the reaction. The obtained crude was then purified by automated flash chromatography (20-100% EtOAc in hexanes) to afford **45** as a yellow solid (0.06 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 8.3, 1.2 Hz, 2H), 7.54 – 7.48 (m, 2H), 7.46 – 7.39 (m, 2H), 7.30 (tt, *J* = 6.8, 1.2 Hz, 1H), 6.81 – 6.76 (m, 2H), 3.99 (s, 3H), 2.27 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.34, 141.20, 131.66, 128.79, 128.06, 126.53, 126.39, 113.89, 81.02, 71.53, 33.75.

***N*-([1,1'-biphenyl]-4-yl)-*N*-(prop-2-yn-1-yl)acrylamide (6).** Following general procedure B, **6** was obtained from **45** (0.30 mmol, 0.06 g) as a yellow solid (0.06 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 – 7.60 (m, 2H), 7.60 – 7.56 (m, 2H), 7.44 (td, *J* = 6.8, 6.4, 1.6 Hz, 2H), 7.39 – 7.34 (m, 1H), 7.34 – 7.30 (m, 2H), 6.42 (dd, *J* = 16.8, 1.9 Hz, 1H), 6.09 (dd, *J* = 16.7, 10.3 Hz, 1H), 5.57 (dd, *J* = 10.3, 1.9 Hz, 1H), 4.57 (d, *J* = 2.5 Hz, 2H), 2.23 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.25, 141.28, 140.26, 139.89, 128.97, 128.67, 128.53, 128.26, 128.15, 127.84, 127.12, 78.96, 72.30, 38.72. HRMS m/z [M] calcd for $C_{18}H_{15}NO$ 261.1154, found: 261.1154. UPLC (method A): t_R = 5.01 min., purity: ≥ 99%.



Scheme S5. Synthetic route used for the synthesis of **7, 8 & 10**. Conditions: a) RBr, K_2CO_3, DMF, 85°C, 8-15h, **46**: 31%, **47**: 35%, **48**: 9.7%. b) acryloyl chloride, K2CO3, DMF, r.t., 2h30, **7**: 97%, **8**: 88%, **10**: 75%.

2-(phenylamino)acetonitrile (46). Following general procedure A, aniline (1.1 mmol, 0.10 g) and bromoacetonitrile (0.80 mmol, 56.0 µL) were reacted until completion of the reaction. The obtained crude was then purified by automated flash chromatography (0-100% EtOAc in hexanes) to afford **46** as a yellow solid (0.05 g, 31%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 6.90 (t, *J* = 7.4 Hz, 1H), 6.71 (d, *J* = 7.8 Hz, 2H), 4.06 (d, *J* = 5.2 Hz, 2H), 4.03 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.12, 129.64, 120.05, 117.20, 113.66, 32.64.

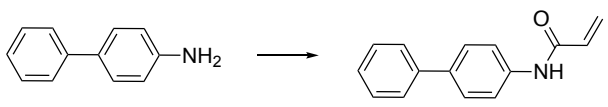
***N*-(cyanomethyl)-*N*-phenylacrylamide (7).** Following general procedure B, **7** was obtained from **46** (0.30 mmol, 0.05 g) as a yellow oil (0.05 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.41 (m, 3H), 7.31 – 7.24 (m, 2H), 6.46 (dd, *J* = 16.8, 1.8 Hz, 1H), 6.01 (dd, *J* = 16.8, 10.3 Hz, 1H), 5.63 (dd, *J* = 10.3, 1.8 Hz, 1H), 4.65 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.59, 140.14, 130.38, 130.20, 129.36, 128.03, 126.93, 115.40, 37.32. HRMS m/z [M] calcd for $C_{11}H_{10}N_2O$ 186.0793, found: 186.0795. UPLC (method A): t_R = 4.27 min., purity: 97%.

2-([1,1'-biphenyl]-4-ylamino)acetonitrile (47). 4-aminobiphenyl (0.25 g, 1.50 mmol, 1 eq.) was dissolved in DMF (8 mL) and bromoacetonitrile (83 µL, 1.20 mmol, 0.8 eq.) was added. The reaction mixture was heated to 80˚C and stirred overnight. The reaction mixture was then diluted with water and DCM and the aqueous phase extracted with DCM (3x 15 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-40% EtOAc in hexanes) gave **47** (0.11 g, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 – 7.51 (m, 4H), 7.45 – 7.38 (m, 3H), 7.33 – 7.27 (m, 1H), 6.83 – 6.78 (m, 2H), 4.17 (d, *J* = 6.8 Hz, 2H), 4.07 – 4.01 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.44, 140.84, 133.33, 128.89, 128.80, 128.42, 128.15, 126.77, 126.70, 126.54, 126.40, 115.52, 114.10, 32.87.

***N*-([1,1'-biphenyl]-4-yl)-*N*-(cyanomethyl)acrylamide (8). 47** (0.06 g, 0.26 mmol, 1 eq.) was then dissolved in dry DMF (3 mL) and cooled to 0˚C. Potassium carbonate (0.19 g, 1.32 mmol, 5 eq.) was added, and after the reaction was stirred for 15 minutes, it was followed by acryloyl chloride (64 µL, 0.79 mmol, 3 eq.). After 30 minutes, the reaction mixture was diluted with water and DCM and the aqueous phase extracted with DCM (3x 15 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-30% EtOAc in hexanes) gave **8** (0.06 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 – 7.65 (m, 2H), 7.64 – 7.56 (m, 2H), 7.53 – 7.44 (m, 2H), 7.44 – 7.37 (m, 1H), 7.37 – 7.31 (m, 2H), 6.50 (dd, *J* = 16.8, 1.8 Hz, 1H), 6.11 (dd, *J* = 16.8, 10.3 Hz, 1H), 5.68 (dd, *J* = 10.4, 1.8 Hz, 1H), 4.70 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.65, 142.39, 139.62, 139.16, 130.34, 129.13, 128.98, 128.35, 128.19, 127.26, 127.01, 115.46, 37.35. HRMS-ESI(+) m/z [M+] calculated for $C_{17}H_{14}N_2O$ 262.1106, found 262.1109. UPLC (method A): t_R = 3.60 min., purity: ≥ 99%.

2-((4-phenethylphenyl)amino)acetonitrile (48). Phenylaniline•HCl (0.15 g, 0.74 mmol, 1 eq.) was added to acetonitrile (5 mL), and DIPEA (130 µL, 0.74 mmol, 1 eq.) added slowly until the substrate dissolved. Bromoacetonitrile (47 µL, 0.67 mmol, 0.9 eq.) was added dropwise, and the reaction was then heated to 70˚C and stirred overnight. The reaction was then cooled, diluted with DCM, and washed with water, saturated sodium bicarbonate, and brine. Purification via automated column chromatography (0-30% EtOAc in hexanes) gave **48** (0.02 g, 9.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.24 (m, 2H), 7.19 (tt, *J* = 8.0, 1.4 Hz, 3H), 7.12 – 7.05 (m, 2H), 6.69 – 6.63 (m, 2H), 4.09 (d, *J* = 7.1 Hz, 2H), 3.86 (t, *J* = 7.1 Hz, 1H), 2.94 – 2.79 (m, 4H).

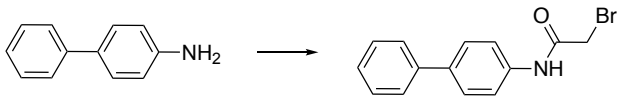
***N*-(cyanomethyl)-*N*-(4-phenethylphenyl)acrylamide (10). 48** (0.02 g, 0.07 mmol, 1 eq.) was dissolved in DMF (1 mL) and potassium carbonate (0.05 g, 0.36 mmol, 5 eq.) was added, followed by acryloyl chloride (17.5 µL, 0.22 mmol, 3 eq.). After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 10 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-50% EtOAc in hexanes) gave **10** (0.02 g, 75%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 – 7.26 (m, 4H), 7.24 – 7.15 (m, 5H), 6.46 (dd, *J* = 16.8, 1.8 Hz, 1H), 6.02 (dd, *J* = 16.8, 10.4 Hz, 1H), 5.64 (dd, *J* = 10.3, 1.8 Hz, 1H), 4.65 (s, 2H), 3.07 – 2.89 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.62, 143.21, 141.05, 137.86, 130.30, 129.97, 128.46, 128.44, 127.81, 126.90, 126.20, 115.35, 37.60, 37.43, 37.25. HRMS-ESI(+) m/z [M+] calculated for $C_{19}H_{18}N_2O$ 290.1419, found 290.1419. UPLC (method A): t_R = 2.84 min., purity: 98%.



11

Scheme S6. Synthetic route used for the synthesis of **11**. Condition: acryloyl chloride, NEt_3, DCM, r.t., 2h, 88%.

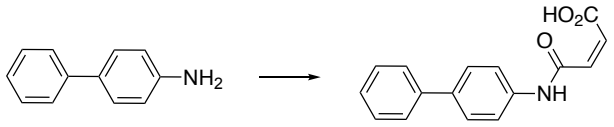
***N*-([1,1'-biphenyl]-4-yl)acrylamide (11).** Following general procedure C, **11** was obtained from 4-aminophenyl (0.59 mmol, 0.10 g) as a yellow solid (0.12 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.4 Hz, 2H), 7.61 – 7.56 (m, 4H), 7.47 – 7.40 (m, 2H), 7.33 (tt, *J* = 8.1, 2.0 Hz, 1H), 6.47 (dd, *J* = 16.8, 1.2 Hz, 1H), 6.27 (dd, *J* = 16.8, 10.2 Hz, 1H), 5.80 (dd, *J* = 10.2, 1.2 Hz, 1H). NH hidden under the baseline. ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.67, 140.54, 137.54, 137.12, 131.24, 128.93, 128.12, 127.80, 127.31, 126.98, 120.42. HRMS m/z [M] calcd for $C_{15}H_{13}NO$ 223.0997, found: 223.1002. UPLC (method A): t_R = 3.64 min., purity: 98%.



12

Scheme S7. Synthetic route used for the synthesis of **12**. Condition: bromoacetyl bromide, NEt_3, DCM, 0°C to r.t., 41h, 68%.

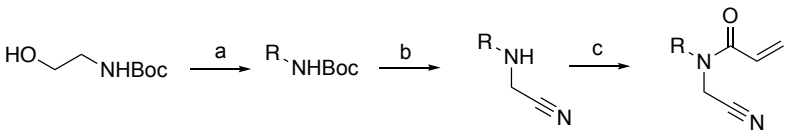
***N*-([1,1'-biphenyl]-4-yl)-2-bromoacetamide (12).** To a solution of 4-aminobiphenyl (0.60 mmol, 0.10 g) and NEt_3 (0.70 mmol, 98.0 µL) in dry DCM (3 mL) under nitrogen, was added dropwise bromoacetyl bromide (0.66 mmol, 57.0 µL) at 0°C. The reaction mixture is allowed to warm up at r.t. and left to stir at r.t. for 41h. Then the reaction mixture is concentrated and taken back in hexane and EtOAc. The obtained solution is filtered, and the filtrate is then filtered again through a pad of celite with EtOAc. The obtained solid is finally taken back in toluene twice and concentrated to afford **12** as a brown solid (0.12 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 7.61 (d, *J* = 3.7 Hz, 3H), 7.58 (d, *J* = 7.3 Hz, 3H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.3 Hz, 1H), 4.06 (s, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.51, 140.39, 138.24, 136.27, 128.96, 127.87, 127.45, 127.03, 120.48, 29.69. HRMS m/z [M] calcd for $C_{14}H_{12}BrNO$ 289.0102, found: 289.0109. UPLC (method A): t_R = 3.67 min., purity: 96%. (PMID: 25148591)



13

Scheme S8. Synthetic route used for the synthesis of **13**. Condition: maleic anhydride, toluene, 60°C, 2h15, quant.

(*Z*)-4-([1,1'-biphenyl]-4-ylamino)-4-oxobut-2-enoic acid (13). To a solution of maleic anhydride (1.18 mmol, 0.20 g) in toluene (3 mL) was added 4-aminobiphenyl (1.18 mmol, 0.12 g). The reaction mixture was stirred at 60°C for 2 hour 15 minutes. After full conversion, the reaction mixture was cooled down to r.t. then diluted with an aqueous solution of 1M HCl. The formed precipitate was filtered, washed with 1 M HCl, and then left to dry on high vacuum to afford **13** as a yellow solid (0.40 g, quant.). ^{1}H NMR (400 MHz, DMSO) δ 10.52 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 5H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 6.9 Hz, 1H), 6.49 (d, *J* = 12.0 Hz, 1H), 6.32 (d, *J* = 12.0 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 167.00, 163.33, 139.65, 138.09, 135.57, 131.69, 130.49, 129.00, 127.21, 127.08, 126.36, 119.94. HRMS m/z [M] calcd for $C_{16}H_{13}NO_3$ 267.0895, found: 267.0887. UPLC (method A): t_R = 3.68 min., purity: 98%.



49 —> 50 —> 14, R = 1-(2λ3-ethoxy)undecane
51 —> 52 —> 15, R = 1-(2λ3-ethoxy)octane

Scheme S9. Synthetic route used for the synthesis of **14 & 15**. Conditions: a) RI, NaH, THF, 0°C to r.t., 11h, **49**: 8%, **51**: 5%. b) 20% TFA in DCM then bromoacetonitrile, K_2CO_3, ACN, 85°C, 4h, **50**: 75%, **52**: 88%. c) acryloyl chloride, K2CO3, DMF, r.t., 30 minutes, **14**: 49%, **15**: 49%.

***tert*-butyl (2-(undecyloxy)ethyl)carbamate (49).** N-Boc ethanolamine (480 µL, 3.10 mmol, 1 eq.) was dissolved in dry THF (10 mL) and cooled to 0˚C under nitrogen. and NaH (60% immersion in oil, 0.15 g, 3.70 mmol 1.2 eq.) was added portionwise. After bubbling ceased, 1-iodoundecane (717 µL, 2.64 mmol, 1 eq.) was added and, after stirring at 0˚C for 30 minutes, the reaction was slowly warmed to room temperature and stirred for 10 hours. The reaction was then diluted with DCM (30 mL) and washed with water, saturated sodium bicarbonate, and brine. Concentration via rotary evaporation and purification via automated column chromatography (0-

40% EtOAc in hexanes) gave **49** (0.08 g, 8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.89 (s, 1H), 3.45 (t, *J* = 5.2 Hz, 2H), 3.40 (t, *J* = 6.7 Hz, 2H), 3.28 (q, *J* = 5.4 Hz, 2H), 1.53 (q, *J* = 6.9 Hz, 2H), 1.43 (s, 9H), 1.26 (d, *J* = 13.9 Hz, 16H), 0.86 (t, *J* = 6.7 Hz, 3H).

2-((2-(undecyloxy)ethyl)amino)acetonitrile (50). 49 (0.08 g, 0.24 mmol, 1 eq.) was dissolved in 20% TFA in DCM and stirred for 30 min. The reaction was then concentrated and washed three times with DCM. The crude product and potassium carbonate (0.04 g, 0.29 mmol, 1.2 eq.) were next stirred in acetonitrile (5 mL) for 15 minutes. Bromoacetonitrile (16 µL, 0.22 mmol, 0.9 eq.) was added, and the reaction was heated to 85 ˚C. After three hours, the reaction mixture was cooled and diluted with water and EtOAc. The organic layer was extracted with EtOAc (3x 30 mL), washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-10% MeOH in DCM) gave **50** as a pale yellow oil (0.08 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 (s, 2H), 3.58 – 3.50 (m, 2H), 3.42 (t, *J* = 6.7 Hz, 2H), 2.89 (t, *J* = 5.0 Hz, 2H), 1.73 (s, 1H), 1.56 (p, *J* = 6.7 Hz, 2H), 1.37 – 1.20 (m, 16H), 0.92 – 0.83 (m, 3H).

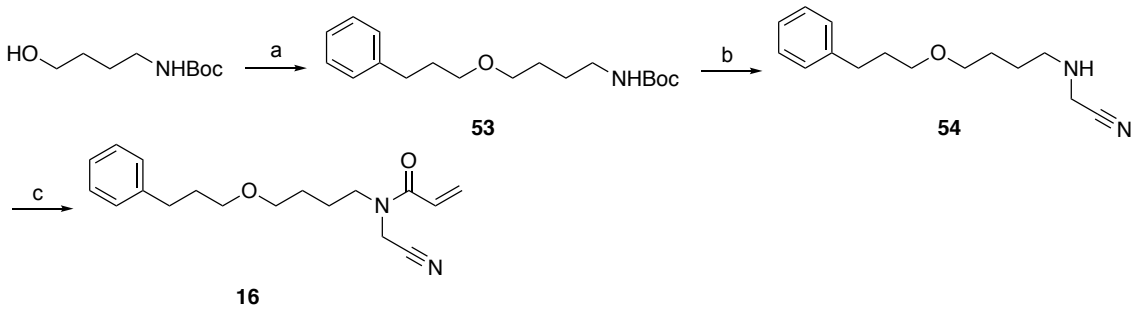
***N*-(cyanomethyl)-*N*-(2-(undecyloxy)ethyl)acrylamide (14). 50** (0.04 g, 0.17 mmol, 1 eq.) was dissolved in dry DMF (3 mL) under nitrogen and potassium carbonate (0.11 g, 0.81 mmol, 5 eq.) was added. The suspension was stirred for 15 minutes at room temperature, and then acryloyl chloride (39 µL, 0.48 mmol, 3 eq.) was added. After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (20-80% EtOAc in hexanes) gave **14** (0.029 g, 49%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.57 (dd, *J* = 16.7, 10.3 Hz, 1H), 5.78 (d, *J* = 10.2 Hz, 1H), 4.45 (s, 2H), 3.66 (t, *J* = 4.8 Hz, 2H), 3.59 (t, *J* = 4.8 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 1.53 (q, *J* = 6.5 Hz, 2H), 1.25 (s, 16H), 0.92 – 0.83 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.73, 130.22, 126.53, 115.94, 71.95, 69.49, 48.40, 35.17, 32.02, 29.73, 29.73, 29.68, 29.54, 26.20, 22.80, 14.24. HRMS-ESI(+) m/z [M+] calculated for $C_{18}H_{32}N_2O_2$ 308.2464, found 308.2475. UPLC (method A): t_R = 3.52 min., purity: ≥ 99%.

***tert*-butyl (2-(octyloxy)ethyl)carbamate (51).** N-Boc ethanolamine (480 µL, 3.10 mmol, 1 eq.) was dissolved in dry THF (10 mL) and cooled to 0˚C under nitrogen. and NaH (60% immersion in oil, 0.15 g, 3.70 mmol 1.2 eq.) was added portionwise. After bubbling ceased, 1-iodooctane (560 µL, 2.64 mmol, 1 eq.) was added and, after stirring at 0˚C for 30 minutes, the reaction was slowly warmed to room temperature and stirred for 10 hours. The reaction was then diluted with DCM

(30 mL) and washed with water, saturated sodium bicarbonate, and brine. Concentration via rotary evaporation and purification via automated column chromatography (0-40% EtOAc in hexanes) gave **51** (0.045 g, 5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.90 (s, 1H), 3.48 (t, *J* = 5.2 Hz, 2H), 3.43 (t, *J* = 6.7 Hz, 2H), 3.31 (q, *J* = 5.4 Hz, 2H), 1.62 – 1.53 (m, 2H), 1.46 (s, 9H), 1.37 – 1.25 (m, 10H), 0.93 – 0.86 (m, 3H).

2-((2-(octyloxy)ethyl)amino)acetonitrile (52). 51 (0.042 g, 0.16 mmol, 1 eq.) was dissolved in 20% TFA in DCM and stirred for 30 min. The reaction was then concentrated and washed three times with DCM. The crude product and potassium carbonate (0.04 g, 0.31 mmol, 2 eq.) were next stirred in acetonitrile (5 mL) for 15 minutes. Bromoacetonitrile (10 µL, 0.14 mmol, 0.9 eq.) was added, and the reaction was heated to 85 ˚C. After three hours, the reaction mixture was cooled and diluted with water and EtOAc. The organic layer was extracted with EtOAc (3x 30 mL), washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-10% MeOH in DCM) gave **52** as a pale tinged oil (0.03 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (d, *J* = 3.8 Hz, 2H), 3.56 (t, *J* = 5.0 Hz, 2H), 3.43 (t, *J* = 6.7 Hz, 2H), 2.91 (s, 1H), 1.93 (s, 1H), 1.53 (s, 2H), 1.38 – 1.20 (m, 10H), 0.93 – 0.84 (m, 3H).

***N*-(cyanomethyl)-*N*-(2-(octyloxy)ethyl)acrylamide (15). 52** (0.03 g, 0.14 mmol, 1 eq.) was dissolved in dry DMF (3 mL) under nitrogen and potassium carbonate (0.098 g, 0.71 mmol, 5 eq.) was added. The suspension was stirred for 15 minutes at room temperature, and then acryloyl chloride (34 µL, 0.42 mmol, 3 eq.) was added. After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (20-80% EtOAc in hexanes) gave **15** (0.019 g, 52%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.58 (dd, *J* = 16.7, 10.3 Hz, 1H), 6.42 (dd, *J* = 16.7, 1.9 Hz, 1H), 5.79 (d, *J* = 10.3 Hz, 1H), 4.45 (s, 2H), 3.67 (t, *J* = 5.2 Hz, 2H), 3.60 (t, *J* = 5.0 Hz, 2H), 3.42 (t, *J* = 6.6 Hz, 2H), 1.55 (t, *J* = 6.9 Hz, 2H), 1.33 – 1.22 (m, 10H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.63, 130.11, 126.42, 115.80, 71.86, 69.38, 48.27, 35.03, 31.81, 29.57, 29.38, 29.23, 26.10, 22.65, 14.10. HRMS-ESI(+) m/z [M+] calculated for $C_{15}H_{26}N_2O_2$ 266.1994, found 266.2002. UPLC (method A): t_R = 3.03 min., purity: 97%.

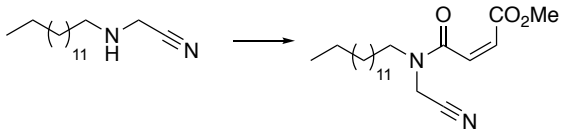


Scheme S10. Synthetic route used for the synthesis of **16**. Conditions: a) 1-bromo-3-phenylpropane, NaI, NaH, THF, 65°C, 8h, 14%. b) 20% TFA in DCM then bromoacetonitrile, K_2CO_3, ACN, 80°C, 8h, 46%. c) acryloyl chloride, K_2CO_3, DMF, r.t., 30 minutes, 70%.

***tert*-butyl (4-(3-phenylpropoxy)butyl)carbamate (53).** Boc-butanolamine (0.50 g, 2.64 mmol, 1 eq.) was dissolved in dry THF (6 mL) under nitrogen, and NaH (60% immersion in oil, 0.21 g, 3.17 mmol 1.2 eq.) was added portionwise. After bubbling ceased, 1-bromo-3-phenylpropane (402 µL, 2.64 mmol, 3 eq.) was added, along with a catalytic amount of NaI. The reaction mixture was then heated to 65 ˚C and stirred for 8 hours. It was then cooled and diluted with water and EtOAc, and the organic layer was extracted with EtOAc (3x 30 mL), washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (10-100% EtOAc in hexanes) gave **53** (0.11 g, 14%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 – 7.17 (m, 2H), 7.13 – 7.08 (m, 3H), 3.34 (td, *J* = 6.2, 1.6 Hz, 4H), 3.07 (t, *J* = 6.5 Hz, 2H), 2.61 (dd, *J* = 8.6, 6.8 Hz, 2H), 1.87 – 1.77 (m, 2H), 1.51 (dddd, *J* = 14.7, 8.1, 4.4, 1.8 Hz, 4H), 1.37 (s, 9H).

2-((4-(3-phenylpropoxy)butyl)amino)acetonitrile (54). 53 (0.05 g, 0.18 mmol, 1 eq.) was dissolved in 20% TFA in DCM and stirred for 30 min. The reaction was then concentrated and washed three times with DCM. The crude product and potassium carbonate (0.03 g, 0.21 mmol, 1.2 eq.) were next stirred in acetonitrile (5 mL) for 15 minutes. Bromoacetonitrile (11 µL, 0.15 mmol, 0.9 eq.) was added, and the reaction was heated to 80 ˚C. After 8 hours, it was cooled and diluted with water and EtOAc. The organic layer was extracted with EtOAc (3x 30 mL), washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-10% MeOH in DCM) gave **54** (0.02 g, 46%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.18 (m, 2H), 7.15 – 7.08 (m, 3H), 3.53 (s, 2H), 3.42 – 3.29 (m, 4H), 2.73 – 2.56 (m, 3H), 1.88 – 1.76 (m, 2H), 1.67 – 1.47 (m, 4H).

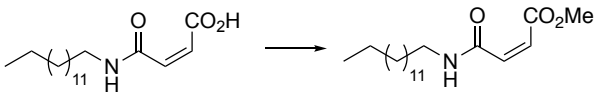
***N*-(cyanomethyl)-*N*-(4-(3-phenylpropoxy)butyl)acrylamide (16). 54** (0.04 g, 0.16 mmol, 1 eq.) was dissolved in DMF (3 mL) and potassium carbonate (0.11 g, 0.81 mmol, 5 eq.) was added, followed by acryloyl chloride (39 µL, 0.49 mmol, 3 eq.). After 30 minutes, the reaction mixture was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-50% EtOAc in hexanes) gave **16** (0.03 g, 70%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 – 7.26 (m, 2H), 7.21 (td, *J* = 5.3, 2.3 Hz, 3H), 6.64 – 6.42 (m, 2H), 5.82 (dd, *J* = 10.2, 2.1 Hz, 1H), 4.39 (s, 2H), 3.61 – 3.52 (m, 2H), 3.46 (dt, *J* = 8.5, 6.2 Hz, 4H), 2.71 (dd, *J* = 8.6, 6.8 Hz, 2H), 1.99 – 1.87 (m, 2H), 1.79 (p, *J* = 7.5 Hz, 2H), 1.71 – 1.58 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.34, 141.84, 130.45, 128.43, 128.37, 126.04, 125.85, 115.61, 70.26, 70.22, 70.11, 48.46, 36.04, 33.95, 32.38, 32.36, 31.25, 26.64, 26.14, 24.45. HRMS-ESI(+) m/z [M+] calculated for $C_{18}H_{24}N_2O_2$ 300.1838, found 300.1838. UPLC (method A): t_R = 3.60 min., purity: 97%.



18

Scheme S11. Synthetic route used for the synthesis of **18**. Condition: maleic anhydride, MeOH, conc. HCl, dry diethyl ether, r.t., on, 26%.

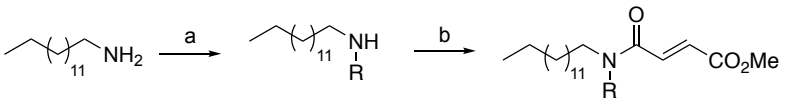
methyl (*Z*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (18). To a solution of maleic anhydride (0.64 mmol, 0.06 g) in dry diethyl ether (5 mL) was added 2-(tetradecylamino)acetonitrile (0.61 mmol, 0.15 g). The reaction mixture was stirred at room temperature for 2 hours. After full conversion, the solvent was evaporated via rotavapor. The remaining solid was redissolved in 5 mL MeOH, and 2-3 drops of conc. HCl was added. The reaction mixture was stirred at room temperature for overnight. After full conversion, the solvent was evaporated via rotavapor and the crude product was purified by automated flash chromatography (50-100% ethyl ether in hexanes) to afford **18** as white solid (0.058 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, J = 11.8 Hz, 1H), 6.10 (d, J = 11.9 Hz, 1H), 4.12 (s, 2H), 3.75 (s, 3H), 3.33 – 3.24 (m, 2H), 1.58 – 1.46 (m, 2H), 1.24 (d, J = 2.8 Hz, 22H), 0.86 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.33, 167.24, 165.89, 138.90, 122.41, 77.37, 77.25, 77.05, 76.73, 52.43, 49.35, 48.13, 31.91, 29.67, 29.64, 29.60, 29.51, 29.46, 29.35, 29.32, 29.18, 28.17, 26.63, 22.68, 14.12. HRMS m/z [M] calcd for $C_{21}H_{36}N_2O_3$ 364.2726, found: 364.2719. UPLC (method B): t_R = 3.83 min., purity: 99%.



20 **19**

Scheme S12. Synthetic route used for the synthesis of **19**. Condition: H_2SO_4, MeOH, 40°C, 1h30, 48%. For the synthesis of **20** see [3].

methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (19). To a solution of **20** (0.19 mmol, 0.06 g) in dry methanol (0.5 mL) was added concentrated sulfuric acid (0.76 mmol, 41.0 µL). The reaction mixture was stirred at 40°C for 1h 30 minutes. Then, the reaction mixture was extracted three times with EtOAc. The combined organics were washed with brine, dried with Na_2SO_4 and concentrated. The obtained crude was then purified by automated flash chromatography (20-50% EtOAc in hexanes) to afford **19** as a white solid (0.03 g, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 6.33 (d, *J* = 13.1 Hz, 1H), 6.12 (d, *J* = 13.1 Hz, 1H), 3.79 (s, 3H), 3.32 (q, *J* = 7.1 Hz, 2H), 1.57 (dd, *J* = 14.0, 6.3 Hz, 4H), 1.25 (s, 20H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.90, 163.94, 139.26, 124.65, 52.53, 39.95, 32.07, 29.84, 29.83, 29.83, 29.80, 29.73, 29.68, 29.50, 29.42, 29.32, 27.12, 22.84, 14.27. HRMS m/z [M] calcd for $C_{19}H_{35}NO_3$ 325.2617, found: 325.2616. UPLC (method B): t_R = 4.00 min., purity: 99%.



55, R = prop-2-yn-1-yl
56, R = cyanomethyl

21, R = prop-2-yn-1-yl
22, R = cyanomethyl

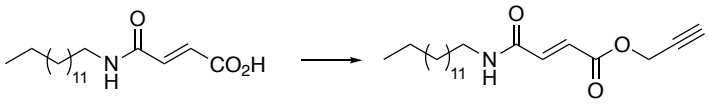
Scheme S13. Synthetic route used for the synthesis of **21 & 22**. Conditions: a) RBr, K_2CO_3, DMF, 85°C, 8-15h, **55**: 49%, **56**: 69%. b) monomethyl fumarate, Oxyma, DIC, DIPEA, DCM, r.t., 3h, **21**: 19%, **22**: 76%.

***N*-(prop-2-yn-1-yl)tetradecan-1-amine (55).** Following general procedure A, the product was obtained from tetradecylamine (0.70 mmol, 0.16 g) and propargyl bromide (1.70 mmol, 63.1 µL) as yellowish solid (0.14 g, 49%). ^{1}H NMR (400 MHz, CDCl3) δ 3.41 (d, *J* = 2.4 Hz, 2H), 2.67 (t, *J* = 7.2 Hz, 2H), 2.19 (t, *J* = 2.4 Hz, 1H), 1.46 (q, *J* = 7.3 Hz, 2H), 1.24 (s, 23H), 0.87 (t, *J* = 6.7 Hz, 3H). The spectrum is in accordance with the literature[3].

methyl (*E*)-4-oxo-4-(prop-2-yn-1-yl(tetradecyl)amino)but-2-enoate (21). 55 (0.08 g, 0.32 mmol, 1 eq.) was dissolved in DCM (3 mL) and cooled to 0˚C. In a separate round flask, monomethyl fumarate (0.13 g, 0.97 mmol, 3 eq.) was dissolved in DCM (3 mL), followed by DIPEA (84 µL, 0.48 mmol, 1.5 eq.), Oxyma (0.07 g, 0.48 mmol, 1.5 eq.), and then DIC (75 µL, 0.48 mmol, 1.5 eq.). After stirring for ten minutes, this mixture was added to the solution of **55**, which was then warmed to r.t. overnight. The reaction was then diluted with DCM and washed with water, saturated sodium bicarbonate, and brine. Concentration via rotary evaporation and purification via automated column chromatography (0-60% EtOAc in hexanes) gave **21** (0.02 g, 19%). HRMS-ESI(+) m/z [M+] calculated for $C_{22}H_{37}NO_3$ 363.2772, found 363.2772. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 24.1, 15.3 Hz, 1H), 6.82 (dd, *J* = 20.4, 15.3 Hz, 1H), 4.18 (dd, *J* = 68.8, 2.5 Hz, 2H), 3.79 (s, 3H), 3.53 – 3.44 (m, 2H), 2.22 (t, *J* = 2.5 Hz, 1H), 1.62 (dt, *J* = 13.7, 6.7 Hz, 2H), 1.26 (d, *J* = 18.6 Hz, 22H), 0.86 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.07, 164.19, 134.05, 133.49, 131.60, 131.11, 78.46, 78.26, 73.31, 72.06, 52.19, 47.64, 46.87, 37.84, 35.01, 31.92, 29.65, 29.54, 29.49, 29.36, 29.23, 29.11, 27.39, 26.88, 26.62, 22.69, 14.13. UPLC (method A): t_R = 4.41 min., purity: ≥ 99%.

2-(tetradecylamino)acetonitrile (56). Following general procedure A, the product was obtained from tetradecylamine (2.30 mmol, 0.50 g) and bromoacetonitrile (1.70 mmol, 120 µL) as a yellow solid (0.40 g, 69%). ^{1}H NMR (400 MHz, CDCl3) δ 3.56 (s, 2H), 2.69 (t, *J* = 7.1 Hz, 2H), 1.46 (p, *J* = 7.0 Hz, 2H), 1.23 (s, 23H), 0.85 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 161.22, 117.97, 48.96, 38.21, 37.40, 31.97, 29.74, 29.71, 29.64, 29.61, 29.53, 29.49, 29.41, 27.15, 22.74, 14.16. The spectrum is in accordance with the literature[3].

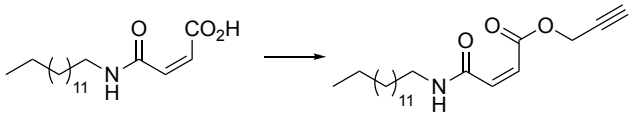
methyl (*E*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (22). To a solution of **56** (1.58 mmol, 0.40 g) in DCM (18 mL) was added a solution of monomethyl fumarate (4.74 mmol, 0.62 g), Oxyma (2.37 mmol, 0.34 g), DIC (2.37 mmol, 374 µL) and DIPEA (2.37 mmol, 414 µL). The reaction mixture was stirred at r.t. for 3 hours. After full conversion, the reaction mixture was concentrated. The obtained crude was then purified by automated flash chromatography (0-5% MeOH in DCM) to afford **22** as a yellow solid (0.44 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 15.2 Hz, 1H), 7.30 (d, *J* = 15.2 Hz, 1H), 4.77 (s, 2H), 4.21 (d, *J* = 4.0 Hz, 3H), 3.95 – 3.89 (m, 2H), 2.12 – 2.02 (m, 2H), 1.76 – 1.62 (m, 22H), 1.28 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.60, 164.71, 132.89, 131.91, 115.21, 52.30, 48.97, 34.11, 31.89, 29.64, 29.61, 29.56, 29.48, 29.41, 29.32, 29.13, 28.93, 26.44, 22.66, 14.09. HRMS m/z [M] calcd for $C_{21}H_{36}N_2O_3$ 364.2726, found: 364.2732. UPLC (method B): t_R = 6.67 min., purity: 98%.



35 **23**

Scheme S14. Synthetic route used for the synthesis of **23**. Condition: propargylbromide, K_2CO_3, DMF, r.t., 22h, 2%. For the synthesis of **35** see [3].

prop-2-yn-1-yl (*E*)-4-oxo-4-(tetradecylamino)but-2-enoate (23). To a solution of **35** (0.30 mmol, 0.09 g) in DMF (1 mL) was added potassium carbonate (0.90 mmol, 0.12 g) and propargylbromide (0.90 mmol, 80.0 µL). The reaction mixture was stirred at r.t. for 22 hours. After full conversion, the reaction mixture was extracted three times with diethyl ether. The combined organics were washed with brine, dried with Na_2SO_4 and concentrated. The obtained crude was then purified by automated flash chromatography (10-100% EtOAc in hexanes) to afford the pure product as a white solid (0.002 g, 2%). ^{1}H NMR (400 MHz, CDCl3) δ 6.92 (d, *J* = 15.3 Hz, 1H), 6.84 (d, *J* = 15.3 Hz, 1H), 5.74 (s, 1H), 4.79 (d, *J* = 2.5 Hz, 2H), 3.35 (q, *J* = 6.9 Hz, 2H), 2.50 (t, *J* = 2.5 Hz, 1H), 1.55 (dd, *J* = 13.1, 6.0 Hz, 3H), 1.25 (s, 21H), 0.88 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 173.82, 163.27, 137.57, 129.38, 75.47, 52.72, 46.98, 40.18, 32.07, 29.84, 29.81, 29.78, 29.72, 29.66, 29.61, 29.55, 29.51, 29.39, 27.04, 22.84, 14.27. HRMS m/z [M] calcd for $C_{21}H_{35}NO_3$ 349.2617, found: 349.2619. UPLC (method B): t_R = 4.10 min., purity: 96%.[4]

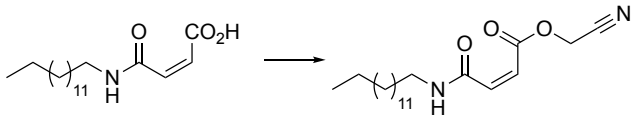


20 **24**

Scheme S15. Synthetic route used for the synthesis of **24**. Condition: propargylbromide, NaH, DMF, r.t., 4 days, 43% (93:7 Z/E ratio).

prop-2-yn-1-yl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (24). To a solution of **20** (2.14 mmol, 0.67 g) in DMF (14 mL) was added NaH (60% immersion in oil, 3.21 mmol, 0.13 g) and propargylbromide (10.7 mmol, 953 µL). The reaction mixture was stirred at r.t. for 4 days in the dark. Then, the reaction mixture was extracted three times with ethyl acetate. The combined organics were washed with brine, dried with Na_2SO_4 and concentrated. The obtained crude was then purified by automated flash chromatography (20-50% EtOAc in hexanes) to afford **24** as a yellow solid (0.32 g, 43%). ^{1}H NMR (400 MHz, CDCl3) δ 7.79 (s, 1H), 6.38 (d, *J* = 12.9 Hz, 1H),

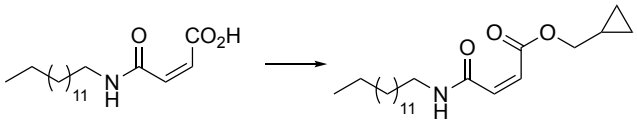
6.13 (d, *J* = 12.9 Hz, 1H), 4.77 (d, *J* = 2.5 Hz, 2H), 3.35 – 3.27 (m, 2H), 2.52 (t, *J* = 2.5 Hz, 1H), 1.56 (p, *J* = 7.3 Hz, 2H), 1.25 (s, 11H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 165.43, 163.81, 139.68, 123.95, 75.74, 52.94, 39.95, 32.06, 29.82, 29.79, 29.72, 29.67, 29.49, 29.41, 29.30, 27.09, 22.83, 14.26. HRMS m/z [M] calcd for $C_{21}H_{35}NO_3$ 349.2617, found: 349.2626. UPLC (method B): t_R = 4.05 min., purity: ≥ 99 %. A Z/E ratio of 93:7 is obtained from the reaction.



20 **25**

Scheme S16. Synthetic route used for the synthesis of **25**. Condition: bromoacetonitrile, Na_2CO_3, DMF, r.t., 5h, 74%.

cyanomethyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (25). 20 (0.05 g, 0.16 mmol, 1 eq.) was dissolved in DMF (5 mL), along with sodium carbonate (0.035 g, 0.32 mmol, 2 eq.) and bromoacetonitrile (23 µL, 0.23 mmol, 2 eq.). After stirring at room temperature for five hours, the reaction was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-60% EtOAc in hexanes) gave **25** (0.04 g, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.44 (d, *J* = 12.4 Hz, 1H), 6.15 (d, *J* = 12.5 Hz, 1H), 4.82 (s, 2H), 3.33 (td, *J* = 7.3, 5.7 Hz, 2H), 1.68 – 1.46 (m, 2H), 1.25 (s, 23H), 0.94 – 0.79 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.42, 163.38, 139.18, 123.18, 113.82, 48.89, 39.89, 39.76, 31.93, 29.70, 29.69, 29.66, 29.60, 29.54, 29.37, 29.26, 29.22, 26.94, 22.70, 14.13. HRMS-ESI(+) m/z [M+] calculated for $C_{20}H_{34}N_2O_3$ 350.2569, found 350.2576. UPLC (method A): t_R = 3.94 min., purity: ≥99%.

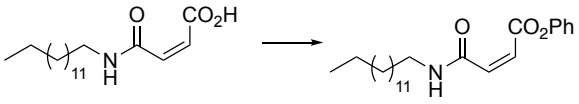


20 **26**

Scheme S17. Synthetic route used for the synthesis of **26**. Condition: cyclopropyl methyl bromide, Na_2CO_3, DMF, r.t., 5h, 74%.

cyclopropylmethyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (26). 20 (0.04 g, 0.128 mmol, 1 eq.) was dissolved in DMF (4 mL), along with sodium carbonate (0.027 g, 0.257 mmol, 2 eq.)

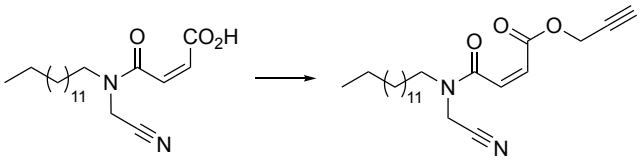
and cyclopropyl methyl bromide (25 µL, 0.257 mmol, 2 eq.). After stirring at room temperature for five hours, the reaction was diluted with water and EtOAc and the aqueous phase extracted with EtOAc (3x 20 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. Purification via automated column chromatography (0-60% EtOAc in hexanes) gave **26** (0.04 g, 74%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.35 (d, *J* = 16.4 Hz, 1H), 6.15 (d, *J* = 13.1 Hz, 1H), 4.01 (d, *J* = 7.4 Hz, 2H), 3.39 – 3.26 (m, 2H), 1.61 – 1.51 (m, 4H), 1.25 (s, 19H, presence of grease leading to overestimation of the integration), 1.19 – 1.11 (m, 1H), 0.88 (t, *J* = 6.8 Hz, 5H), 0.65 – 0.58 (m, 2H), 0.31 (q, *J* = 7.9, 6.3 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.76, 163.79, 139.16, 124.49, 52.39, 39.81, 31.93, 29.70, 29.66, 29.60, 29.55, 29.37, 29.28, 29.19, 26.98, 22.70, 14.12. HRMS-ESI(+) m/z [M+] calculated for $C_{22}H_{39}NO_3$ 365.93, found 365.2934. UPLC (method A): t_R = 4.10 min., purity: 97%.



20 **27**

Scheme S18. Synthetic route used for the synthesis of **27**. Condition: phenol, DCC, pyridine, DCM, r.t., 2h, 7%.

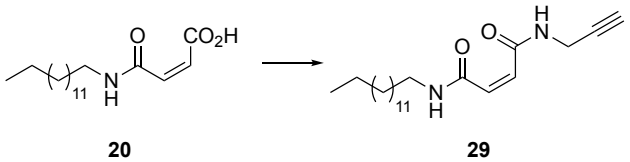
phenyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (27). DCC (0.21 mmol, 0.04 g) and a drop of pyridine were added to a solution of **20** (0.19 mmol, 0.06 g) and phenol (0.19 mmol, 17.0 µL) in DCM (1 mL). The reaction mixture was stirred at r.t. for 2 hours. Then the reaction mixture was diluted with water and extracted two times with EtOAc. The combined organics were washed two times with 0.2 M NaOH then two times with water, dried with Na_2SO_4 and concentrated. The obtained crude was then purified by automated flash chromatography (10-50% EtOAc in hexanes then 10-40% EtOAc in petroleum ether) to afford **27** as a white solid (0.005 g, 7%). ^{1}H NMR (400 MHz, CDCl3) δ 7.68 (s, 1H), 7.41 – 7.30 (m, 2H), 7.24 – 7.20 (m, 1H), 7.10 – 7.06 (m, 2H), 6.42 (d, *J* = 12.9 Hz, 1H), 6.28 (d, *J* = 12.9 Hz, 1H), 3.24 (td, *J* = 7.3, 5.7 Hz, 2H), 1.45 (q, *J* = 7.4 Hz, 2H), 1.30 – 1.07 (m, 22H), 0.81 (t, J = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.78, 150.20, 140.12, 129.75, 126.57, 124.47, 121.48, 40.04, 32.07, 29.84, 29.81, 29.79, 29.71, 29.65, 29.51, 29.39, 29.34, 27.10, 22.84, 14.27. HRMS m/z [M] calcd for $C_{24}H_{37}NO_3$ 387.2773, found: 387.278. UPLC (method B): t_R = 4.25 min., purity: 97%.[5]



17 **28**

Scheme S19. Synthetic route used for the synthesis of **28**. Condition: propargyl bromide, K_2CO_3, DMF, r.t., on, 56%. For the synthesis of **17** see [3].

prop-2-yn-1-yl (*Z*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (28). (*Z*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoic acid (0.212 g, 0.604 mmol, 1 eq.) was dissolved in DMF (5 mL) and then propargyl bromide (105 µL, 1.09 mmol, 1.5 eq) was added, followed by potassium carbonate (0.100 g, 0.725 mmol, 1.2 eq.). The reaction mixture was stirred overnight in the dark, diluted with DCM, and washed with saturated sodium bicarbonate and brine. After drying over Na_2SO_4 and concentration, purification via automated column chromatography (20-50% EtOAc in hexanes) gave **28** (0.132 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, *J* = 11.9 Hz, 1H), 6.12 (d, *J* = 11.9 Hz, 1H), 4.75 (d, *J* = 2.4 Hz, 2H), 4.39 (s, 2H), 3.42 – 3.34 (m, 2H), 2.49 (t, *J* = 2.5 Hz, 1H), 1.60 (q, *J* = 5.8 Hz, 2H), 1.37 – 1.21 (m, 22H), 0.93 – 0.83 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.82, 163.35, 137.13, 123.96, 115.02, 77.02, 75.45, 52.64, 48.95, 32.21, 31.92, 29.70, 29.68, 29.65, 29.61, 29.53, 29.46, 29.36, 29.15, 27.90, 26.56, 22.69, 14.13. HRMS m/z [M] calcd for $C_{23}H_{36}O_3N_2$ 388.2726, found: 388.273. UPLC (method B): t_R = 6.62 min., purity: 99%.

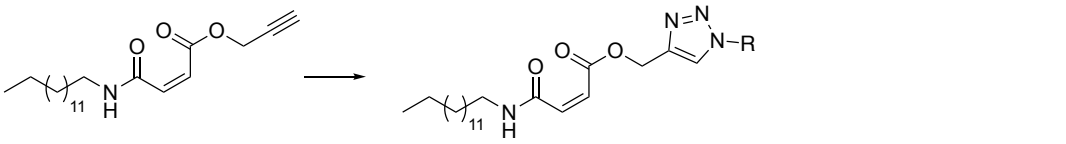


20 **29**

Scheme S20. Synthetic route used for the synthesis of **29**. Condition: propargylamine hydrochloride, Oxyma, DIC, DMF, r.t., 2h, 7%.

***N*^{1}-(prop-2-yn-1-yl)-*N*^{4}-tetradecylmaleamide (29).** To a solution of **20** (0.26 mmol, 0.08 g) in DMF (5 mL) was added propargylamine hydrochloride (0.78 mmol, 0.07 g), Oxyma (0.39 mmol, 0.06 g) and DIC (0.39 mmol, 61.5 µL). The reaction mixture was stirred at r.t. for 2 hours. After full conversion, water was added to the reaction mixture and the formed precipitate was filtered and washed with water. The obtained crude was then purified by automated flash chromatography (20-50% EtOAc in hexanes). The obtained residue was then taken back in MeOH, spined down

and the filtrate was separated from the precipitate and concentrated to afford **29** as a white solid (0.006 g, 7%). ^{1}H NMR (400 MHz, MeOD) δ 6.21 (d, J = 12.9 Hz, 1H), 6.18 – 6.13 (m, 1H), 4.03 (d, J = 2.6 Hz, 2H), 3.24 (t, J = 7.1 Hz, 2H), 2.61 (t, J = 2.6 Hz, 1H), 1.54 (q, J = 10.3, 8.5 Hz, 2H), 1.29 (s, 22H), 0.93 – 0.85 (m, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 167.07, 166.85, 133.42, 132.92, 132.07, 80.05, 72.53, 40.61, 33.08, 30.80, 30.78, 30.76, 30.70, 30.67, 30.48, 30.39, 30.08, 29.60, 28.05, 23.74, 14.43. HRMS m/z [M] calcd for $C_{21}H_{36}N_2O_2$ 348.2777, found: 348.2787. UPLC (method B): t_R = 4.24 min., purity: 95%.

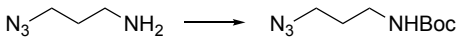


24

30, R = 2-methoxy-2-oxoethyl
31, R = *tert*-butoxyaminopropyl
32, R = 2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)propyl
33, R = PEG_4

Scheme S21. Synthetic route used for the synthesis of **30, 31, 32 & 33**. Condition: R-N_3, $CuSO_4.5H_2O$, sodium ascorbate, TBTA, THF/H_2O, r.t., 17-25h, **30**: 76%, **31**: 76%, **32**: 24%, **33**: 59%.

(1-(2-methoxy-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (30). To a solution of **24** (0.20 mmol, 0.07 g) and methyl azidoacetate (0.39 mmol, 38.0 µL) in THF/H2O (2 mL, 1:1) were added successively $CuSO_4.5H_2O$ (0.02 mmol, 0.005 g), sodium ascorbate (0.02 mmol, 0.004 g) and TBTA (0.004 mmol, 0.002 g). The reaction mixture was stirred at r.t. for 17 hours and 30 minutes. Then the reaction mixture was filtered through celite with DCM (30 mL) and concentrated. The obtained crude was then purified by automated flash chromatography (0-5% MeOH in DCM) to afford **30** as a white solid (0.07 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 1H), 7.72 (s, 1H), 6.33 (d, *J* = 12.8 Hz, 1H), 6.08 (d, *J* = 12.8 Hz, 1H), 5.33 (s, 2H), 5.17 (s, 2H), 3.80 (s, 3H), 3.34 – 3.22 (m, 2H), 1.53 (p, *J* = 7.4 Hz, 2H), 1.24 (s, 22H), 0.86 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.64, 165.87, 164.02, 142.65, 138.87, 125.36, 124.54, 58.44, 53.21, 50.82, 39.87, 32.01, 29.78, 29.75, 29.74, 29.70, 29.66, 29.44, 29.39, 29.30, 27.09, 22.77, 14.21. HRMS m/z [M] calcd for $C_{24}H_{40}N_4O_5$ 464.2999, found: 464.2993. UPLC (method B): t_R = 3.95 min., purity: 95%.

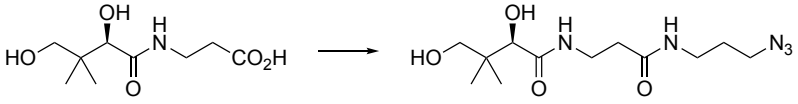


57

Scheme S22. Synthetic route used for the synthesis of **CD_118A**. Condition: 3-azido-1-propanamine, Boc_2O, triethylamine, DCM, 0°C to r.t., 23h30, 100%.

***tert*-butyl (3-azidopropyl)carbamate (57).** Triethylamine (0.80 mmol, 112 µL) was added to a solution of 3-azido-1-propanamine (0.80 mmol, 78.0 µL) in DCM (2 mL). The resulting solution was cooled down to 0°C and a solution of Boc_2O (0.88 mmol, 0.19 g) in 2 mL DCM was added dropwise to the reaction mixture, which is left to stir at 0°C for an hour and then at r.t. for 23 hours 30 minutes. The reaction mixture is then concentrated, and the obtained crude was then purified by automated flash chromatography (20-100% EtOAc in hexanes) to afford **57** as a transparent oil (0.16 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (s, 1H), 3.29 (t, *J* = 6.7 Hz, 2H), 3.18 – 3.09 (m, 2H), 1.70 (p, *J* = 6.7 Hz, 2H), 1.38 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.12, 85.25, 49.19, 29.37, 28.45, 27.46.[6]

(1-(3-((*tert*-butoxycarbonyl)amino)propyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (31). To a solution of **24** (0.20 mmol, 0.07 g) and **57** (0.40 mmol, 0.08 g) in THF/H2O (2 mL, 1:1) were added successively $CuSO_4.5H_2O$ (0.02 mmol, 0.005 g), sodium ascorbate (0.02 mmol, 0.004 g) and TBTA (0.004 mmol, 0.002 g). The reaction mixture was stirred at r.t. for 24 hours. Then the reaction mixture was filtered through celite with DCM (30 mL) and concentrated. The obtained crude was then purified by automated flash chromatography (0-5% MeOH in DCM) to afford **31** as a white solid (0.08 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (s, 2H), 6.33 (d, *J* = 12.9 Hz, 1H), 6.09 (d, *J* = 12.9 Hz, 1H), 5.31 (s, 2H), 4.84 (s, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 3.32 – 3.24 (m, 2H), 3.13 (q, *J* = 6.2 Hz, 2H), 2.09 (p, *J* = 6.7 Hz, 2H), 1.53 (q, *J* = 7.0 Hz, 2H), 1.43 (s, 9H), 1.24 (s, 22H), 0.90 – 0.82 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.95, 164.03, 156.28, 142.18, 138.73, 124.72, 124.37, 58.56, 47.77, 39.91, 32.03, 30.87, 29.80, 29.77, 29.76, 29.72, 29.68, 29.46, 29.41, 29.31, 28.49, 27.11, 22.80, 14.23. HRMS m/z [M] calcd for $C_{29}H_{51}N_5O_5$ 549.389, found: 549.3898. UPLC (method B): t_R = 4.12 min., purity: 96%.



58

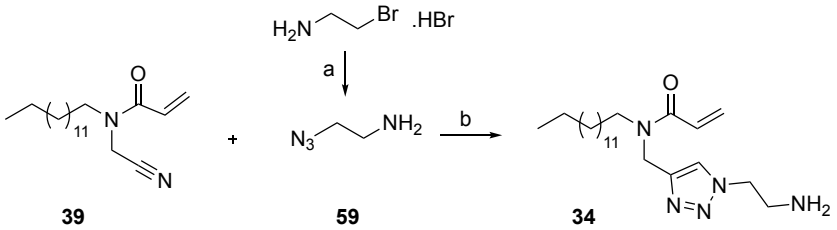
Scheme S23. Synthetic route used for the synthesis of **58**. Condition: D-pantothenic acid hemicalcium salt, 3-azido-1-propanamine, Oxyma, EDC, water, r.t., 2h, 9%.

***(R)-N*-(3-((3-azidopropyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide (58).** To a solution of D-pantothenic acid hemicalcium salt (0.84 mmol, 0.20 g), Oxyma (1.26 mmol, 0.18 g) and EDC (1.26 mmol, 0.20 g) in water (5 mL) was added 3-azido-1-propanamine (0.84 mmol, 82.0 µL) in water (5 mL). The reaction mixture was stirred at r.t. for 2 hours. After full conversion, the reaction mixture was concentrated. The obtained crude was then purified by automated flash chromatography (0-10% MeOH in DCM). The obtained residue was then purified by acid-base washing to afford **58** as a yellow oil (0.02 g, 9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (s, 1H), 6.42 (s, 1H), 3.99 (s, 1H), 3.75 – 3.63 (m, 2H), 3.56 (q, *J* = 6.1 Hz, 2H), 3.39 – 3.31 (m, 4H), 2.57 (d, *J* = 29.3 Hz, 2H), 2.48 – 2.40 (m, 2H), 1.78 (p, *J* = 6.7 Hz, 2H), 0.99 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.74, 171.73, 77.74, 71.05, 43.47, 39.44, 37.29, 36.02, 35.41, 28.82, 21.51, 21.11 – 20.17 (m).

(*R*)-(1-(3-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)propyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (32). To a solution of **24** (0.05 mmol, 0.02 g) and **58** (0.08 mmol, 0.02 g) in THF/H2O (1 mL, 1:1) were added successively $CuSO_4.5H_2O$ (0.005 mmol, 0.001 g), sodium ascorbate (0.005 mmol, 0.001 g) and TBTA (0.002 mmol, 0.001 g). The reaction mixture was stirred at r.t. for 19 hours. Then the reaction mixture was filtered through celite with DCM (20 mL) and concentrated. The obtained crude was then purified by automated flash chromatography (0-20% MeOH in DCM). The obtained residue was then triturated with *tert*-butylmethylester and filtrated to afford **32** as a yellow solid (0.008 g, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.36 (s, 1H), 6.59 (s, 1H), 6.33 (d, *J* = 12.5 Hz, 1H), 6.15 – 6.09 (m, 1H), 5.30 (d, *J* = 5.4 Hz, 2H), 4.42 (t, *J* = 6.5 Hz, 2H), 4.05 (s, 1H), 3.76 – 3.57 (m, 2H), 3.51 – 3.36 (m, 4H), 3.23 (ddd, *J* = 25.8, 12.8, 6.1 Hz, 3H), 2.40 – 2.27 (m, 2H), 2.15 (dd, *J* = 11.7, 5.8 Hz, 2H), 1.59 – 1.49 (m, 2H), 1.25 (s, 25H), 1.02 (s, 2H), 0.95 – 0.82 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.19, 171.69, 165.83, 164.55, 137.19, 125.53, 124.80, 77.88, 71.24, 58.44, 53.57, 48.61, 39.98, 39.47, 36.88, 36.08, 35.00, 32.06, 30.43, 29.87, 29.84, 29.83, 29.81, 29.80, 29.76, 29.72, 29.50, 29.45, 29.35, 27.14, 22.83, 21.74, 20.59, 14.27. HRMS m/z [M] calcd for $C_{33}H_{58}N_6O_7$ 650.4367, found: 650.4359. UPLC (method B): t_R = 3.84 min., purity: 86% (95% pure by NMR).

(1-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (33). To a solution of **24** (0.17 mmol, 0.06 g) and 1-azido-3,6,9-trioxaundecane-11-ol (0.34 mmol, 0.07 g) in THF/H2O (2 mL, 1:1) were added successively

$CuSO_4.5H_2O$ (0.02 mmol, 0.004 g), sodium ascorbate (0.02 mmol, 0.004 g) and TBTA (0.007 mmol, 0.004 g). The reaction mixture was stirred at r.t. for 24 hours 30 minutes. Then the reaction mixture was filtered through celite with DCM (30 mL) and concentrated. The obtained crude was then purified by automated flash chromatography (0-5% MeOH in DCM) to afford **33** as a white solid (0.06 g, 59%). ^{1}H NMR (400 MHz, CDCl3) δ 7.96 – 7.77 (m, 2H), 6.35 (dd, *J* = 12.8, 3.9 Hz, 1H), 6.10 (dd, *J* = 12.8, 8.5 Hz, 1H), 5.32 (d, *J* = 8.3 Hz, 2H), 4.55 (dt, *J* = 9.6, 5.0 Hz, 2H), 3.92 – 3.84 (m, 2H), 3.76 – 3.49 (m, 10H), 3.29 (q, *J* = 7.1 Hz, 2H), 2.60 (s, 2H), 1.54 (q, *J* = 6.8 Hz, 2H), 1.25 (s, 23H), 0.87 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (101 MHz, CDCl3) δ 165.92, 164.09, 139.07, 138.72, 124.68, 124.57, 72.58, 70.68, 70.63, 70.58, 70.54, 70.37, 69.48, 61.77, 58.55, 50.58, 39.95, 32.05, 29.83, 29.79, 29.75, 29.71, 29.49, 29.44, 29.35, 29.33, 27.14, 22.82, 14.26. HRMS m/z [M] calcd for $C_{29}H_{52}N_4O_7$ 568.3836, found: 568.3822. UPLC (method B): t_R = 3.84 min., purity: 95%.



Scheme S24. Synthetic route used for the synthesis of **34**. Conditions: a) NaN_3, K_2CO_3, DMF, 130°C, 30 minutes, quant. b) $CuSO_4.5H_2O$, sodium ascorbate, TBTA, THF/H_2O, r.t., 10h, 38%.

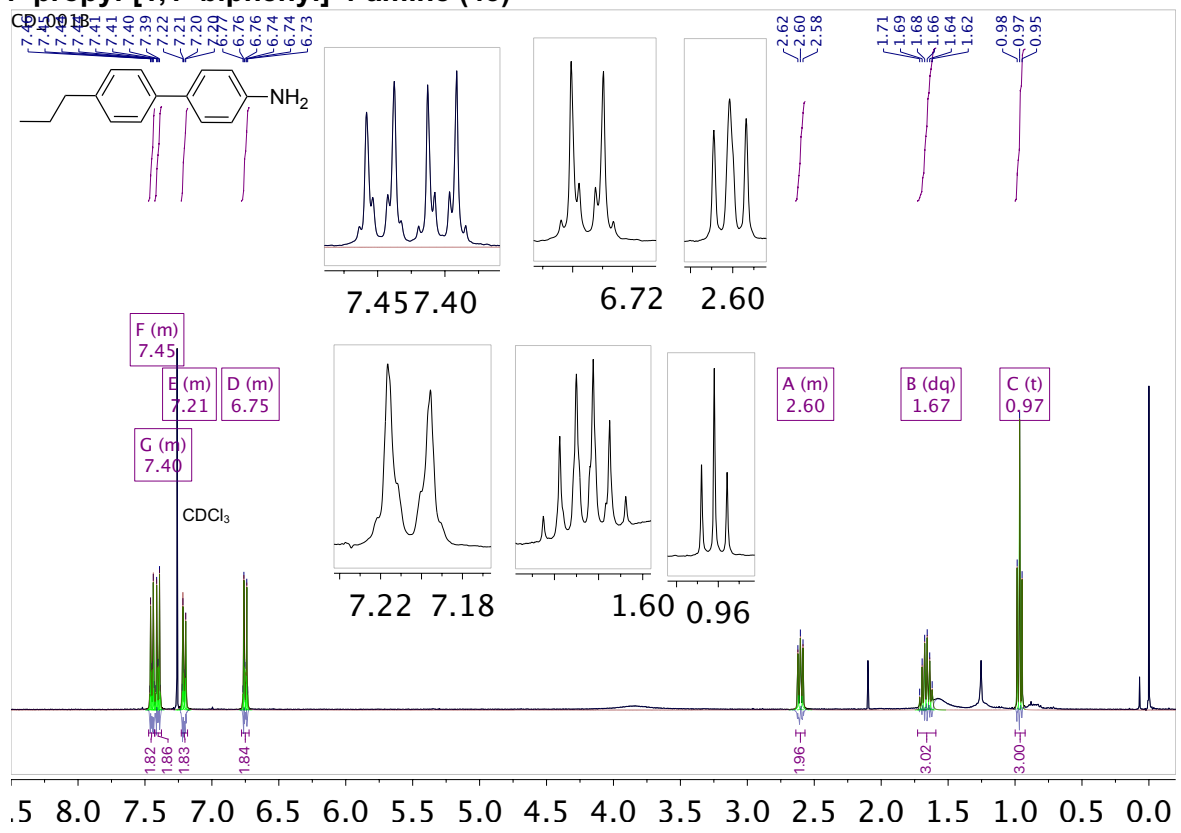
2-azidoethan-1-amine (59). K_2CO_3 (1.95 mmol, 0.27 g) and NaN_3 (5.85 mmol, 0.38g) was added successively to a solution of 2-bromoethylamine hydrobromide (1.95 mmol, 0.40 g) in DMF (2 mL). The reaction mixture was left to stir at 130°C for 30 minutes. After cooling down, the mixture was filtrated on celite with 100 mL DCM then concentrated to give **59** as a brown oil (0.33 g, quant.). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 – 3.30 (m, 2H), 2.93 – 2.84 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 54.84, 41.53.

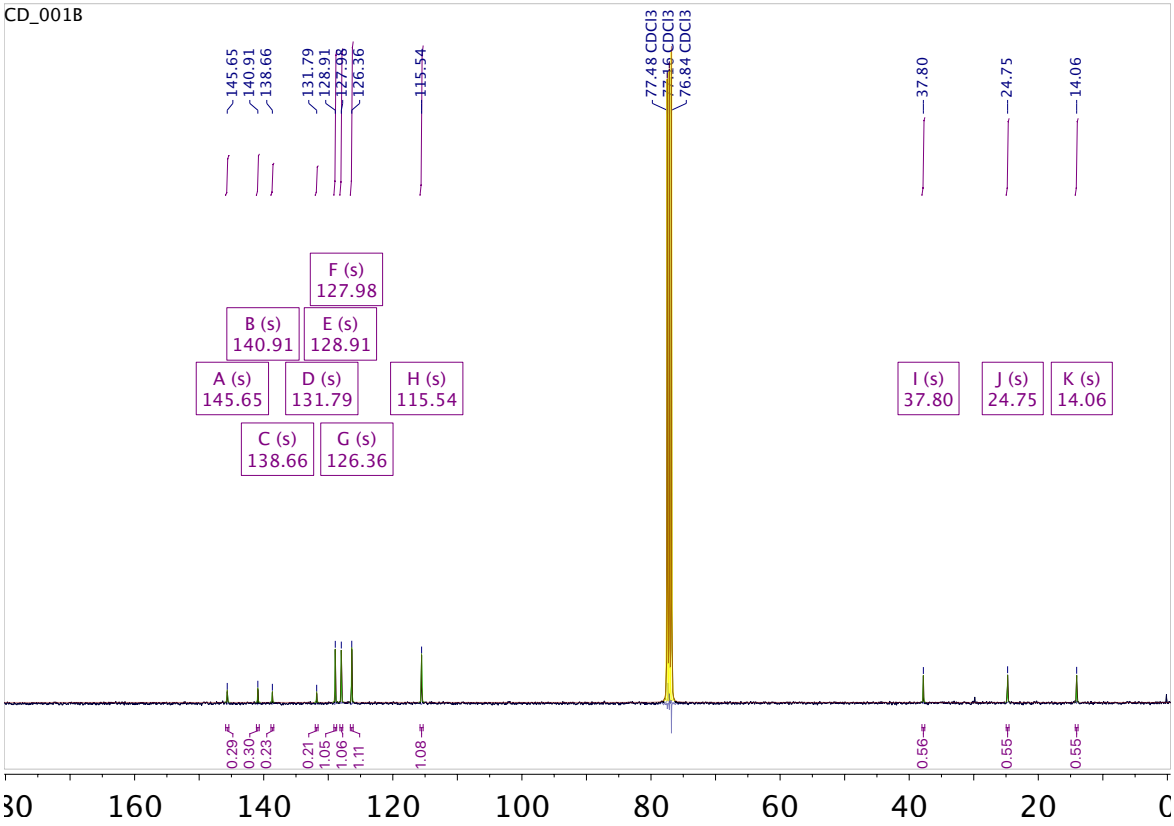
***N*-((1-(2-aminoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*-tetradecylacrylamide (34). 39** (0.087 g, 0.261 mmol, 1 eq.) was dissolved in a 1:1 mixture of THF:water (3 mL), followed by **59** (0.046 g, 0.522 mmol, 2 eq.), TBTA (0.004 g, 0.0051 mmol, 0.02 eq.), copper(II) sulfate (0.0065 g, 0.0261 mmol, 0.1 eq.) and sodium ascorbate (0.0056 g, 0.0261 mmol, 0.1 eq.). After stirring at room temperature for 10 hours, the reaction mixture was filter through celite and concentrated. Purification via automated column chromatography (0-10% MeOH in DCM) gave **34** (0.039 g,

38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 7.80 – 7.64 (m, 1H), 6.51 (td, *J* = 14.5, 8.8 Hz, 2H), 6.34 (dd, *J* = 16.7, 2.0 Hz, 1H), 5.70 (dt, *J* = 10.3, 2.6 Hz, 1H), 4.69 – 4.39 (m, 4H), 3.81 (q, *J* = 5.4 Hz, 2H), 3.41 (t, *J* = 7.9 Hz, 2H), 1.62 (q, *J* = 7.3 Hz, 2H), 1.24 (s, 23H), 0.90 – 0.80 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.27, 161.58, 129.23, 128.50, 127.72, 127.34, 49.63, 48.62, 46.65, 41.76, 37.84, 31.92, 29.68, 29.65, 29.62, 29.55, 29.35, 29.31, 27.62, 27.00, 26.74, 22.69, 14.12. HRMS-ESI(+) m/z [M+] calculated for $C_{22}H_{41}N_5O$ 391.3311, found 391.333. UPLC (method A): t_R = 3.94 min., purity: ≥ 99%.

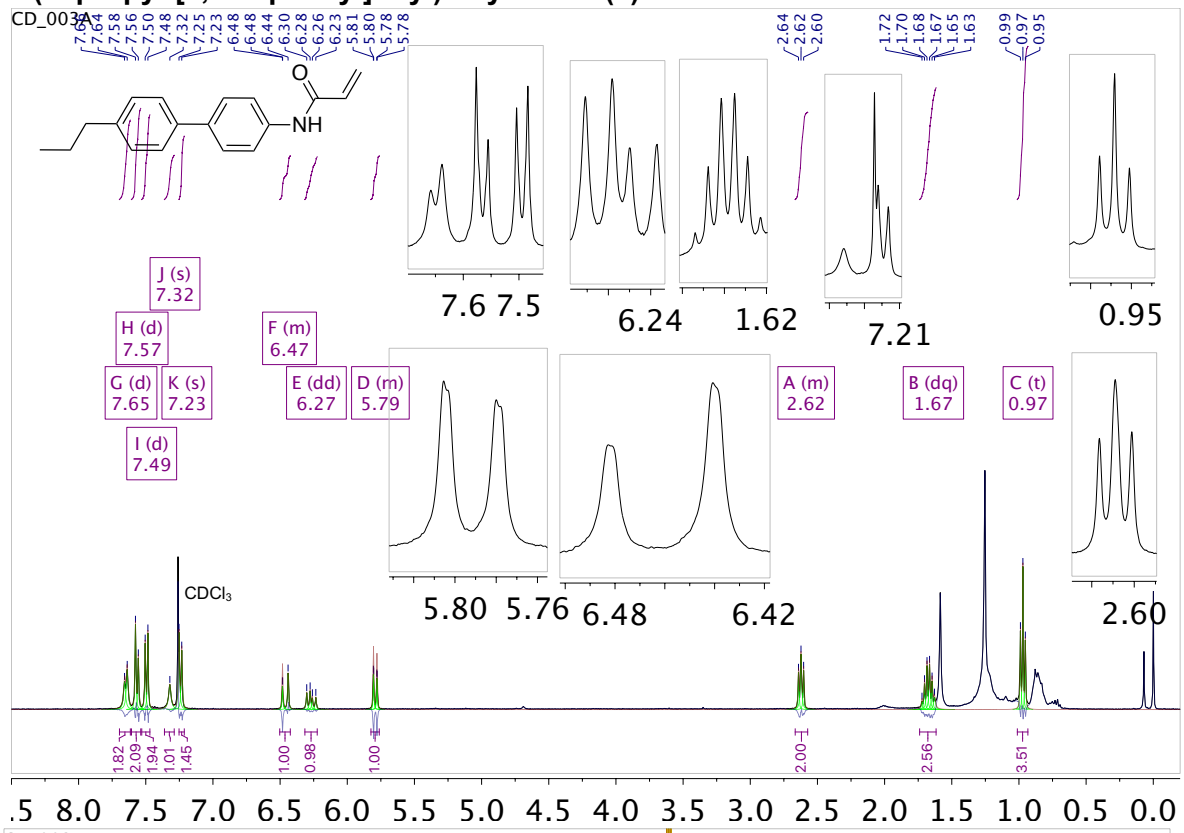
Spectra

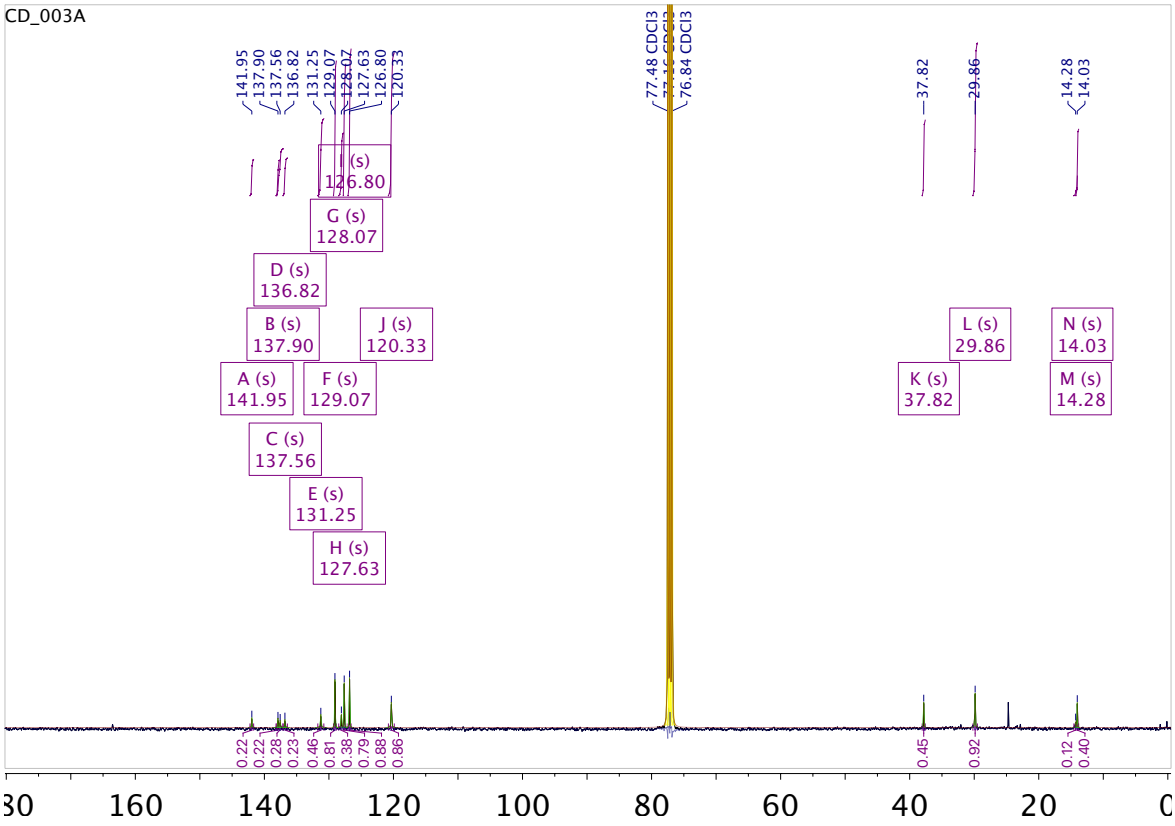
4'-propyl-[1,1'-biphenyl]-4-amine (43)



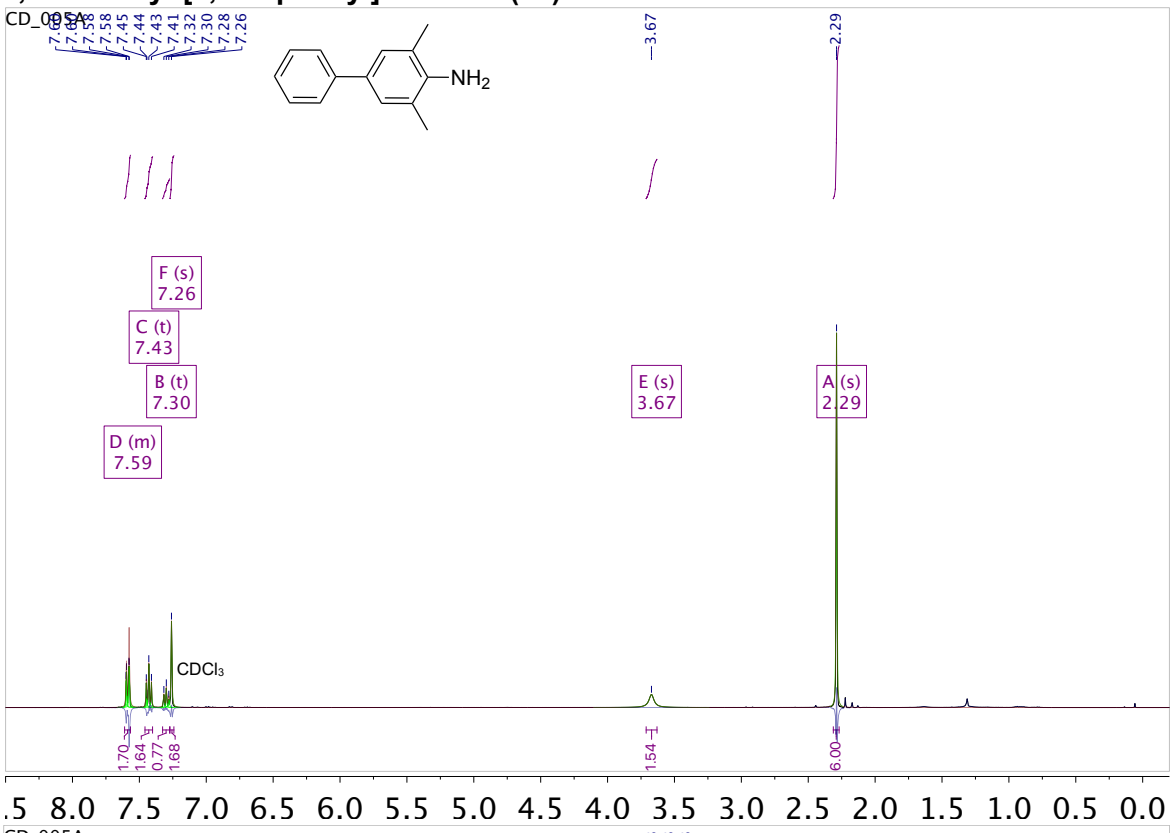


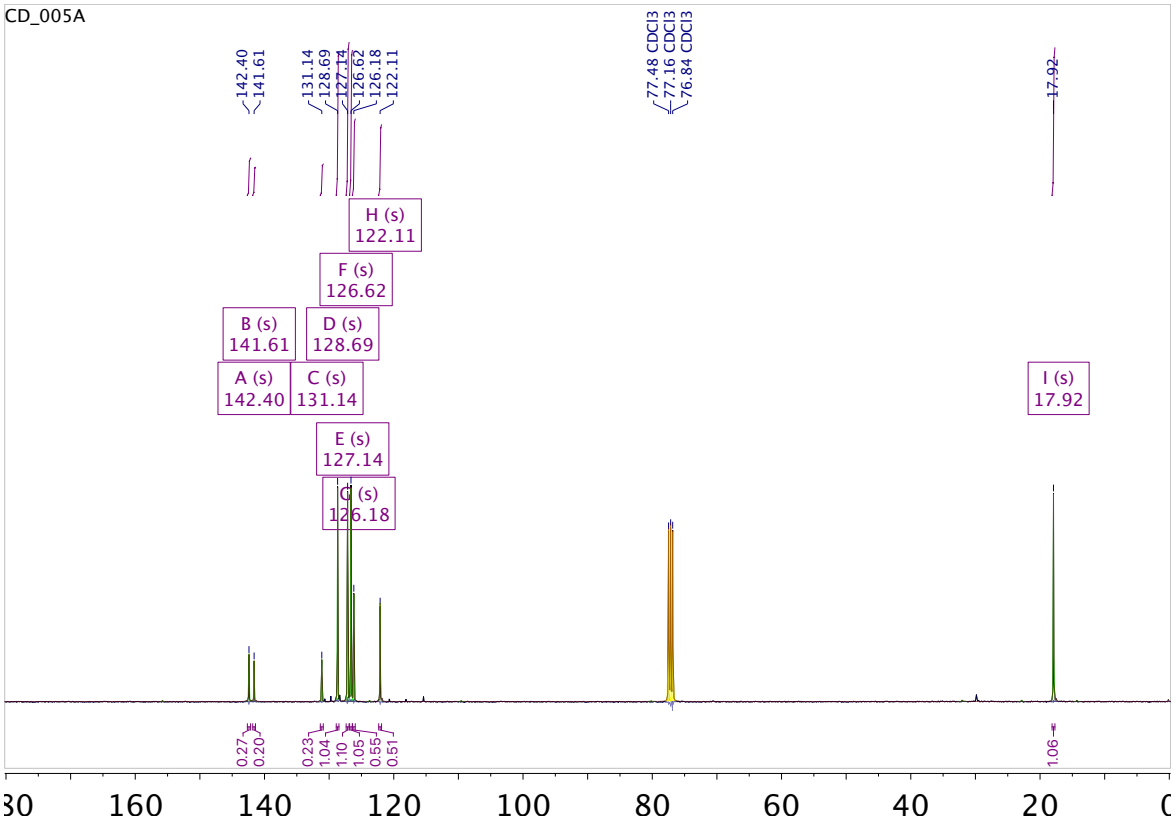
***N*-(4'-propyl-[1,1'-biphenyl]-4-yl)acrylamide (3)**



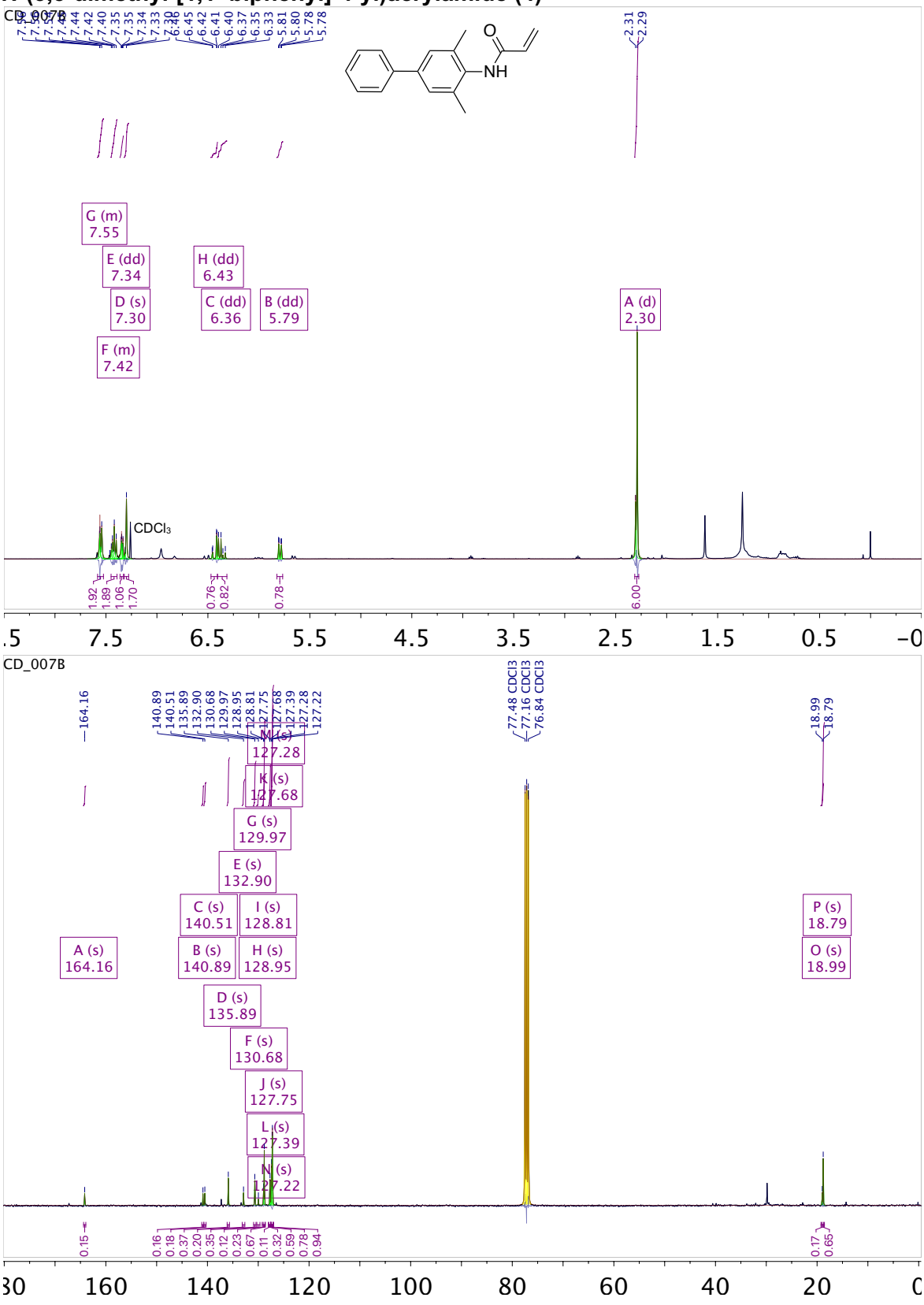


3,5-dimethyl-[1,1'-biphenyl]-4-amine (44)

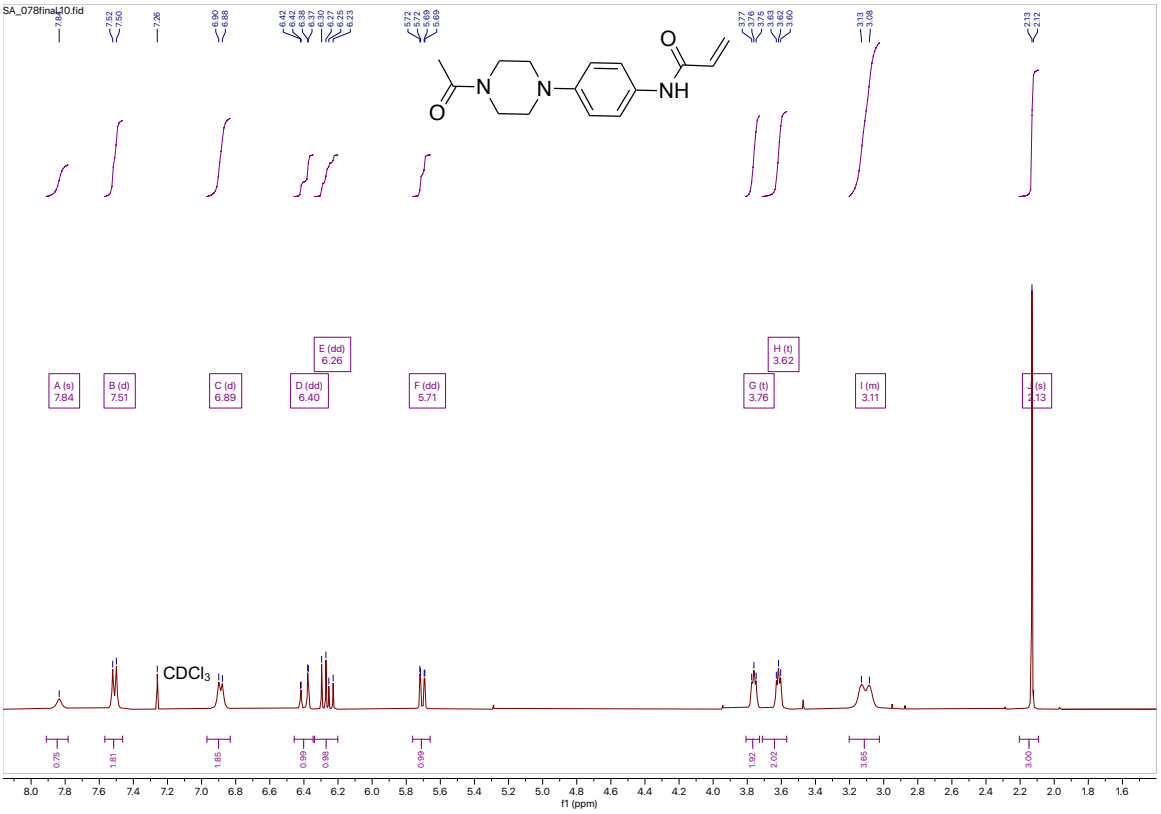


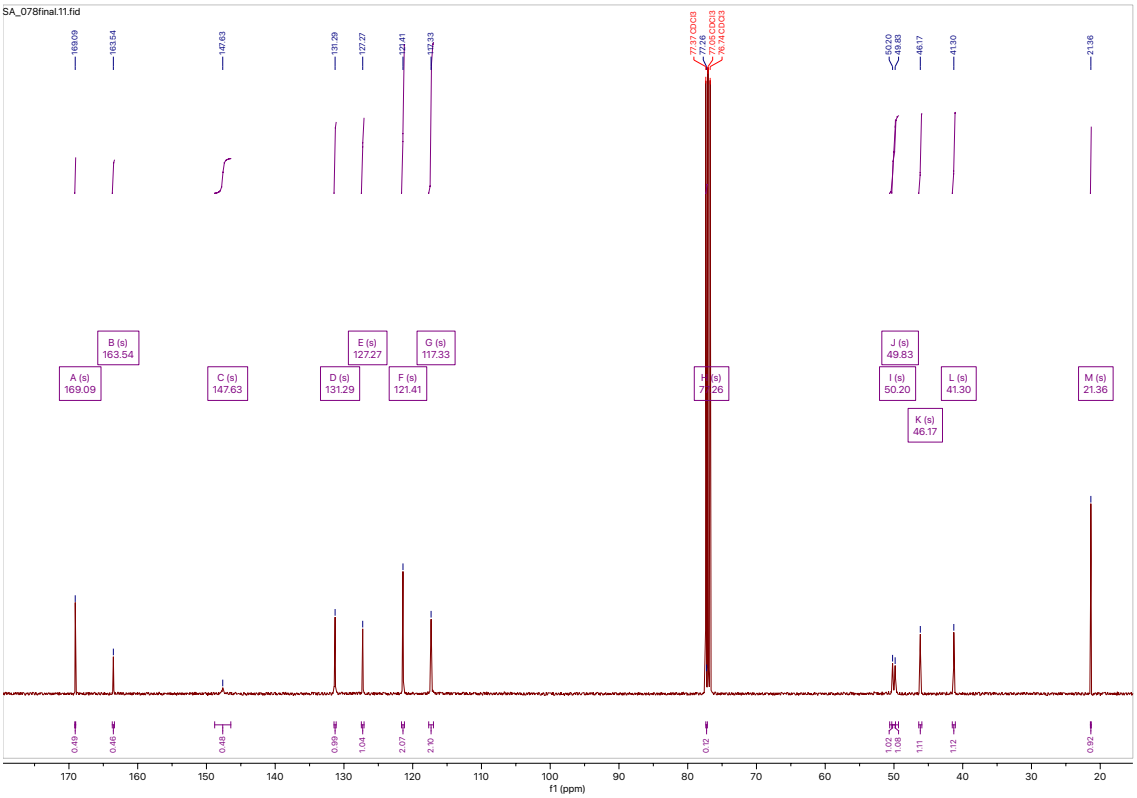


***N*-(3,5-dimethyl-[1,1'-biphenyl]-4-yl)acrylamide (4)**

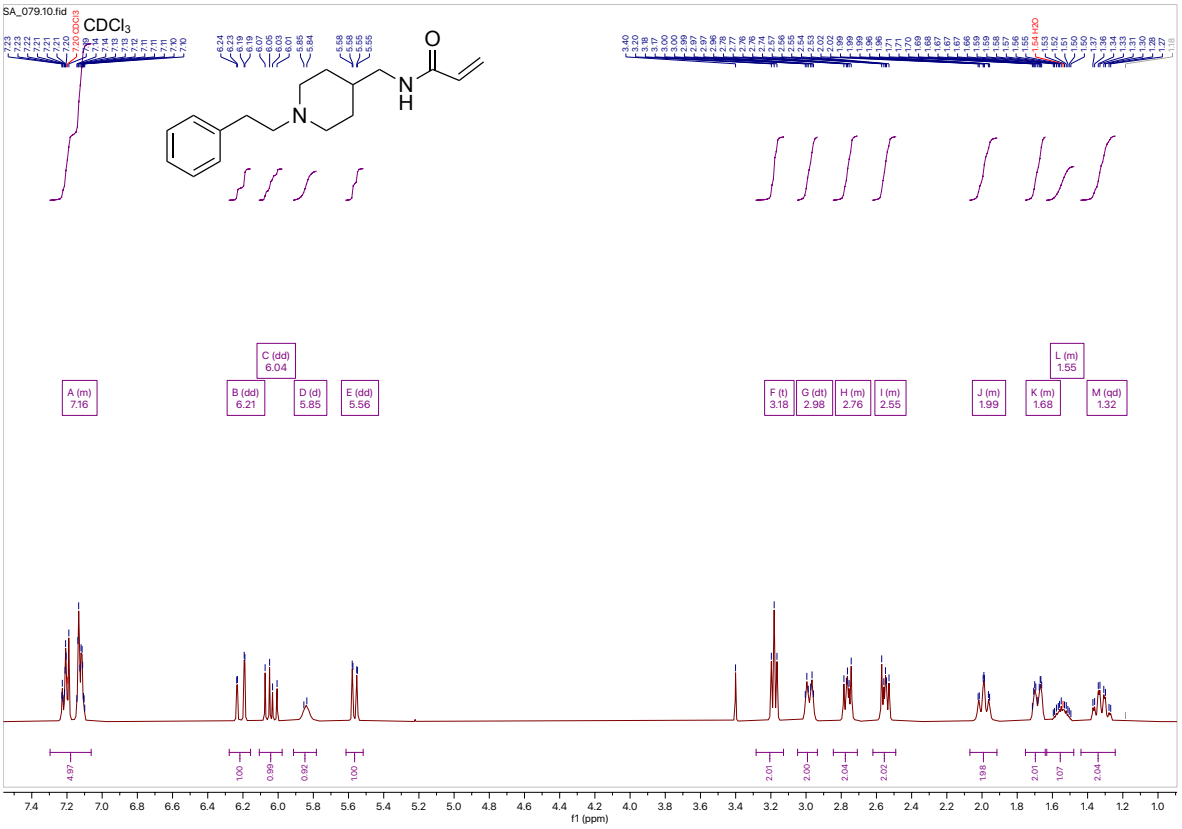


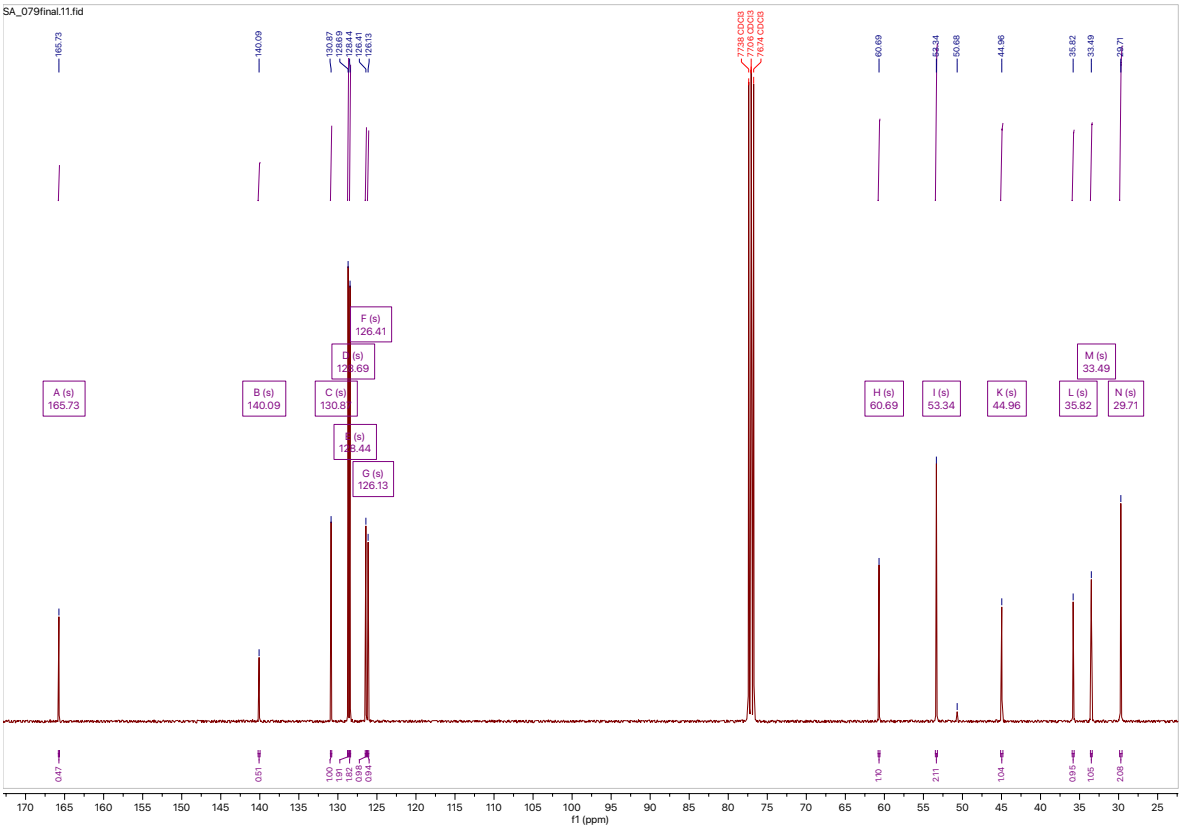
N-(4-(4-acetylpiperazin-1-yl)phenyl)acrylamide (5)



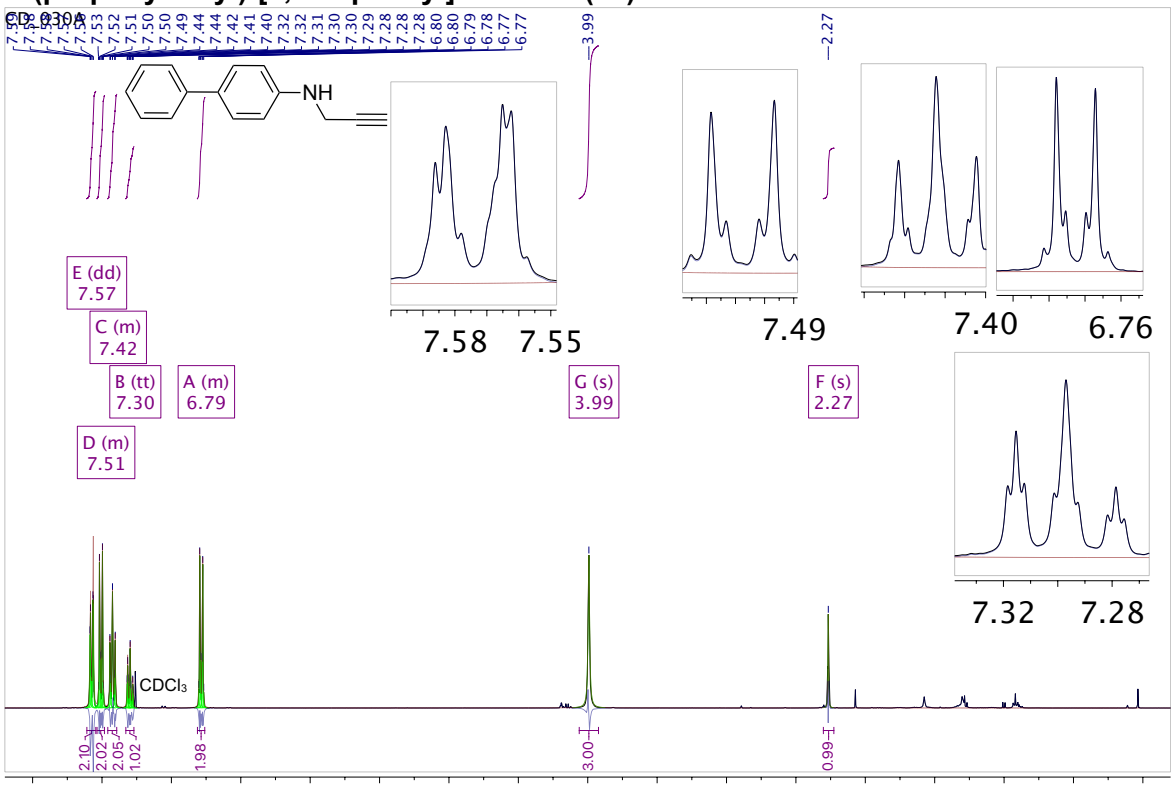


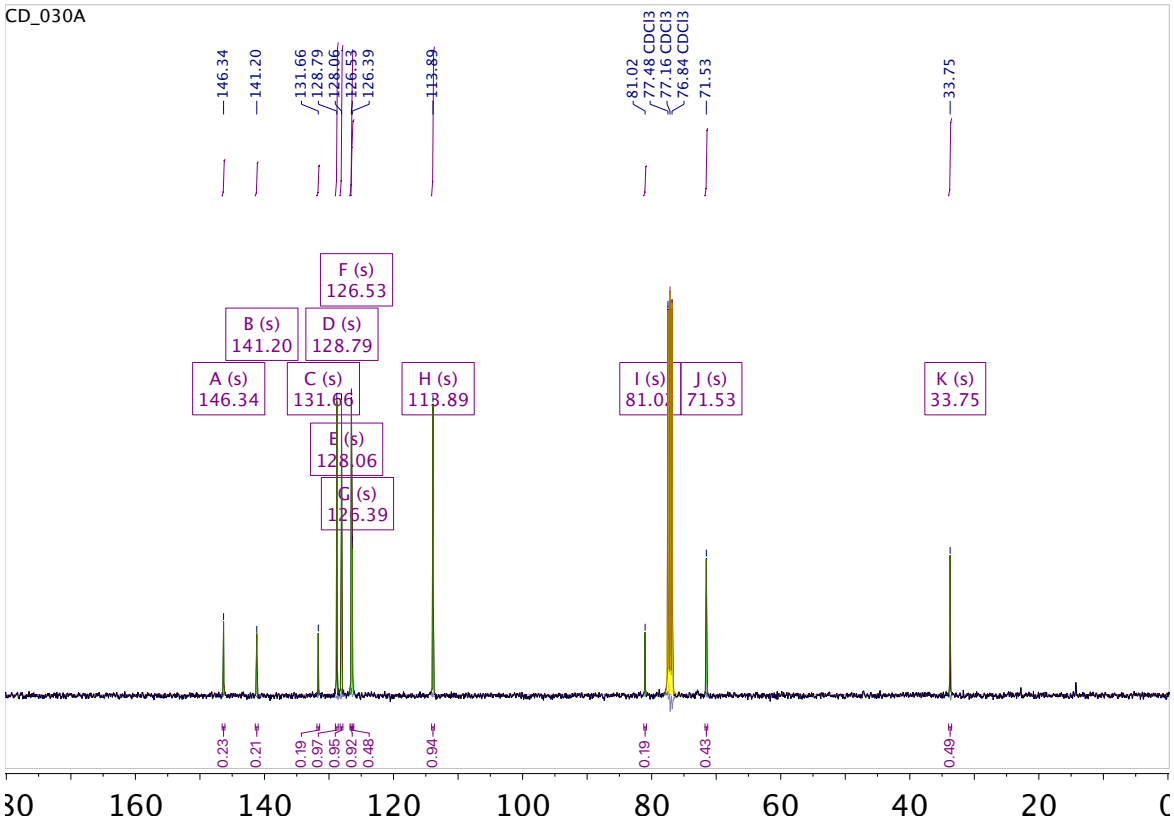
N-((1-phenethylpiperidin-4-yl)methyl)acrylamide (9)



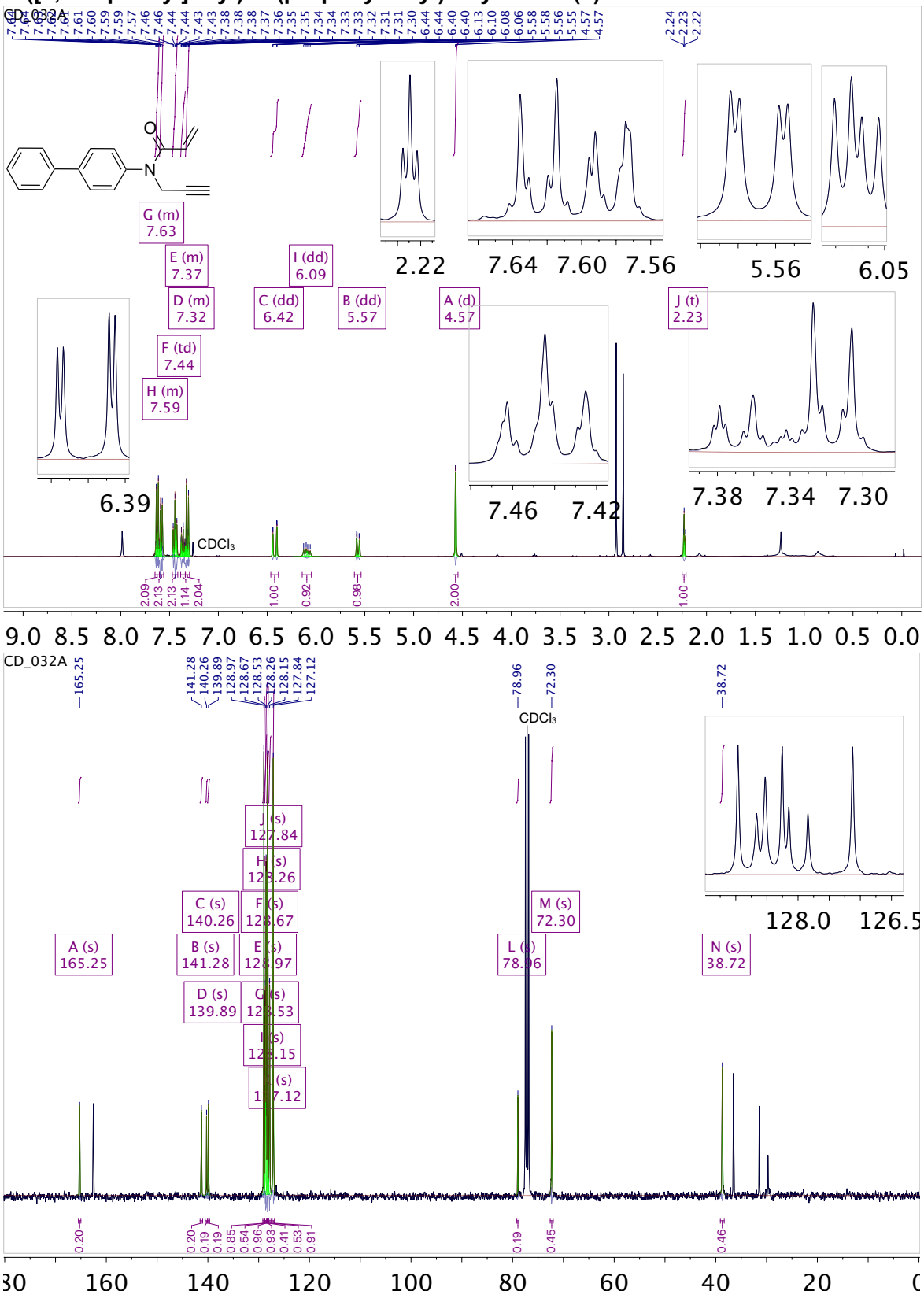


***N*-(prop-2-yn-1-yl)-[1,1'-biphenyl]-4-amine (45)**

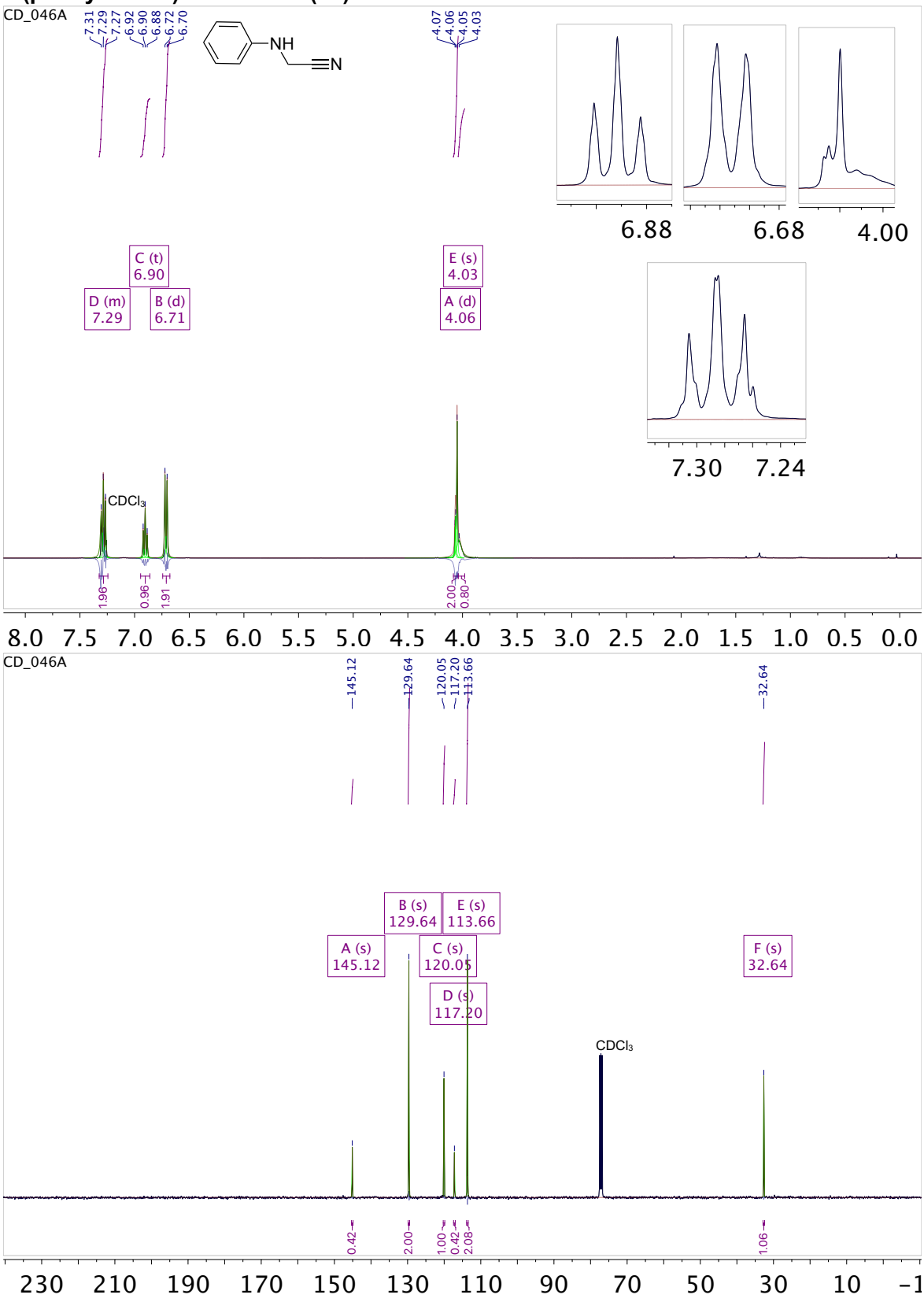




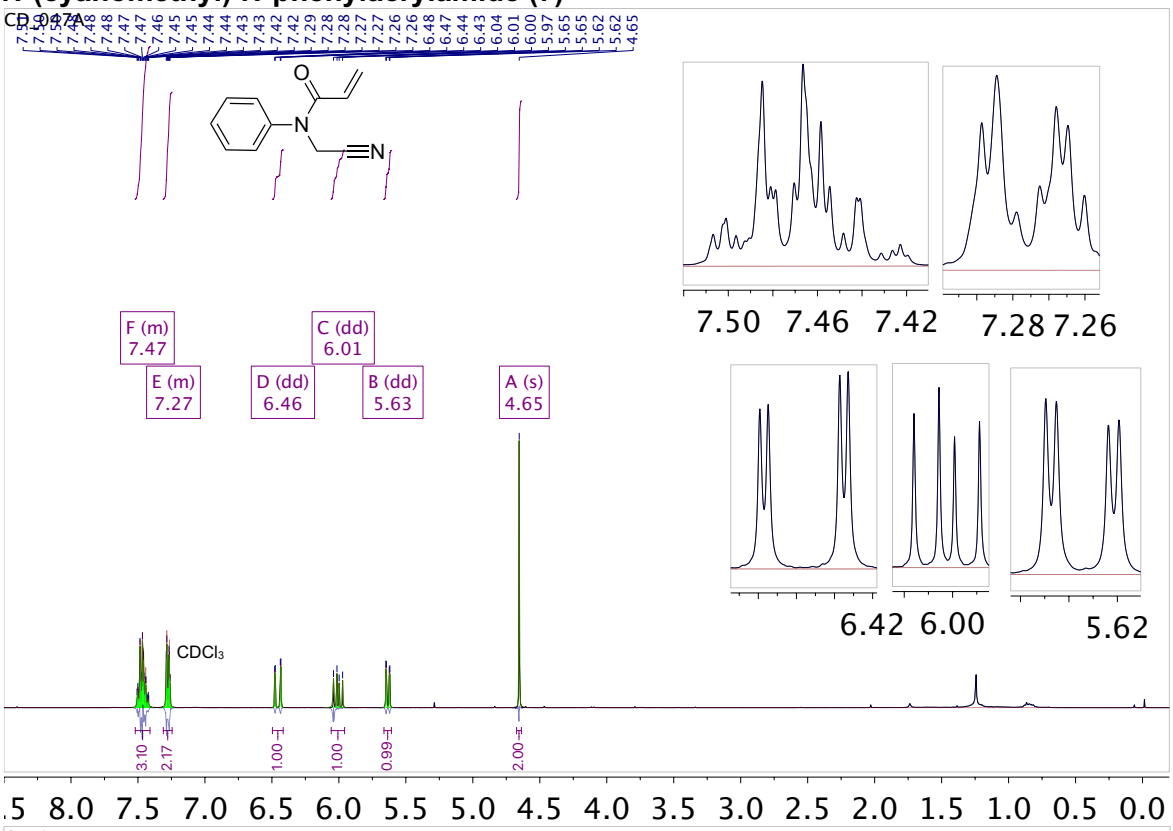
_N_-([1,1'-biphenyl]-4-yl)-_N_-(prop-2-yn-1-yl)acrylamide (6)

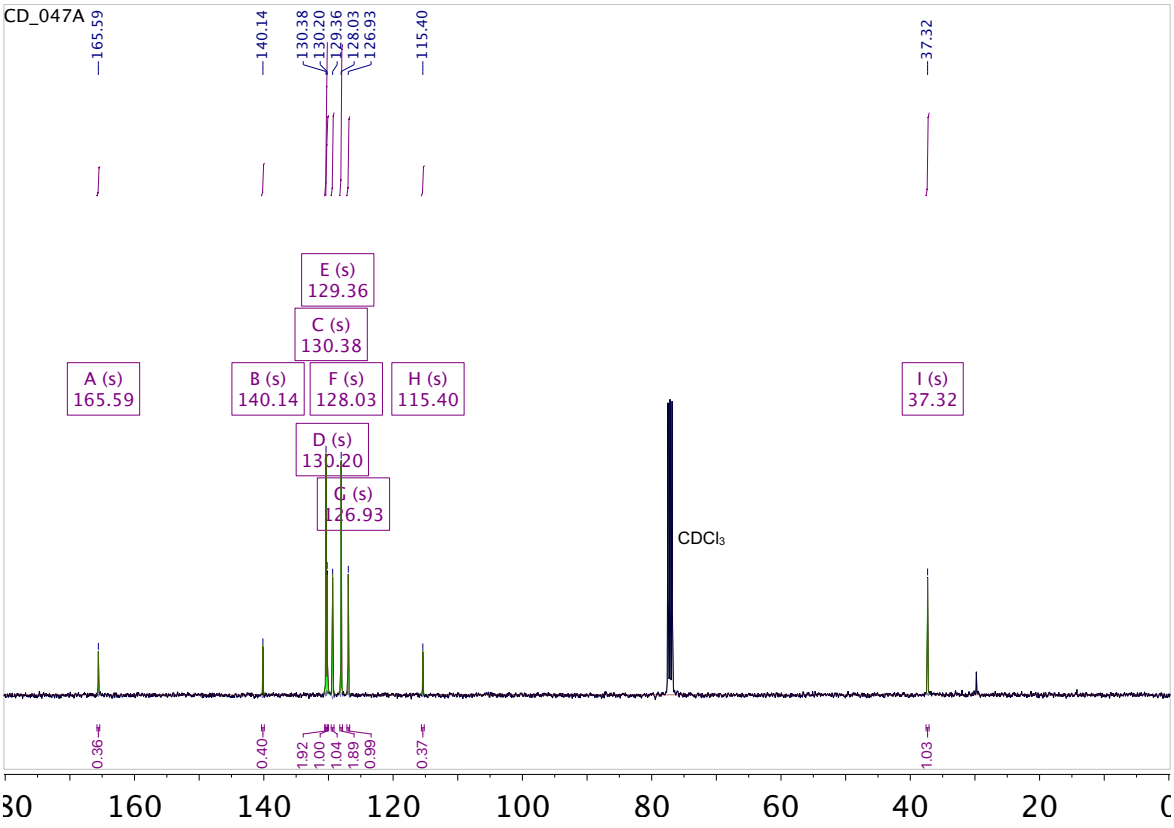


2-(phenylamino)acetonitrile (46)

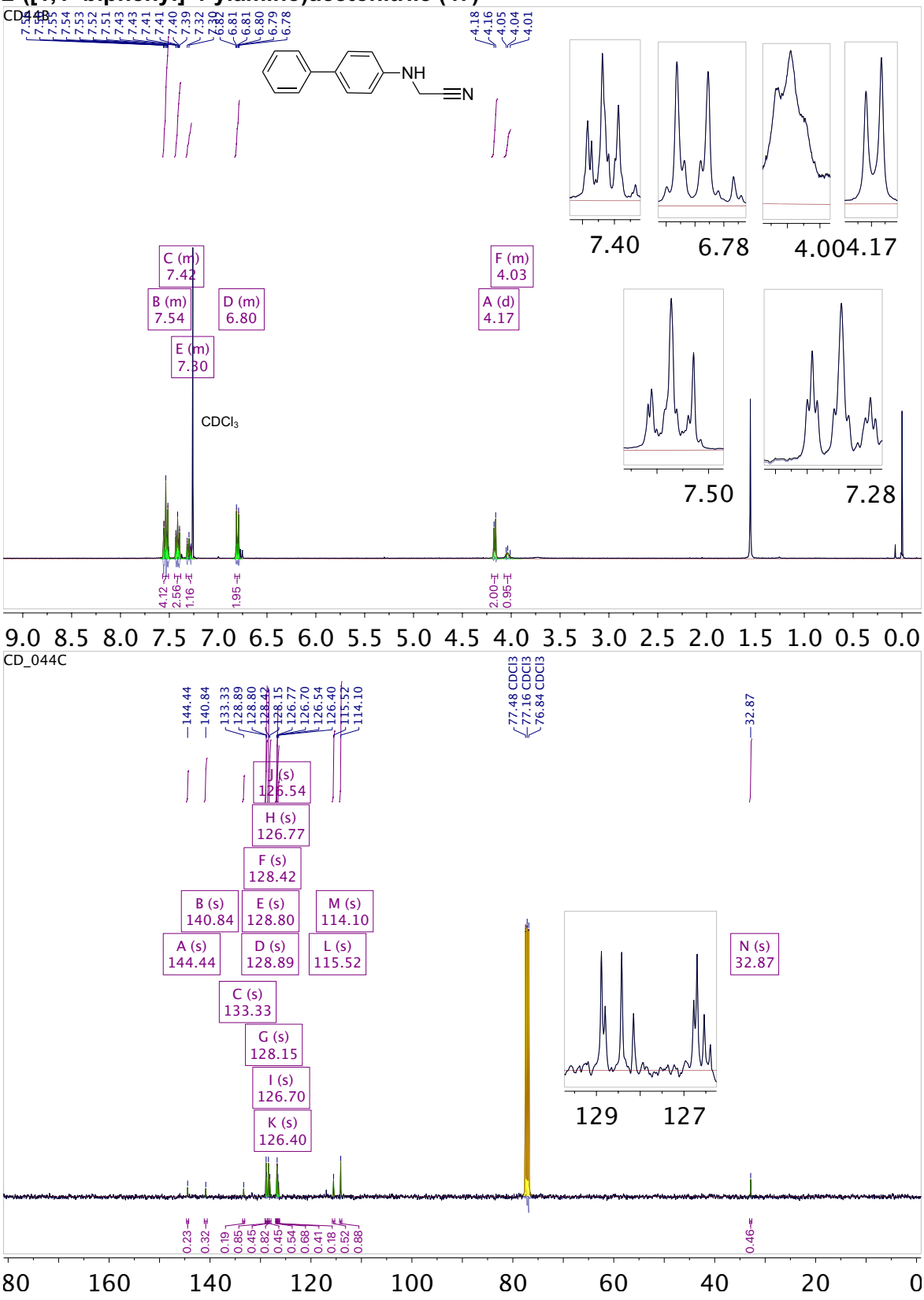


_N_-(cyanomethyl)-_N_-phenylacrylamide (7)

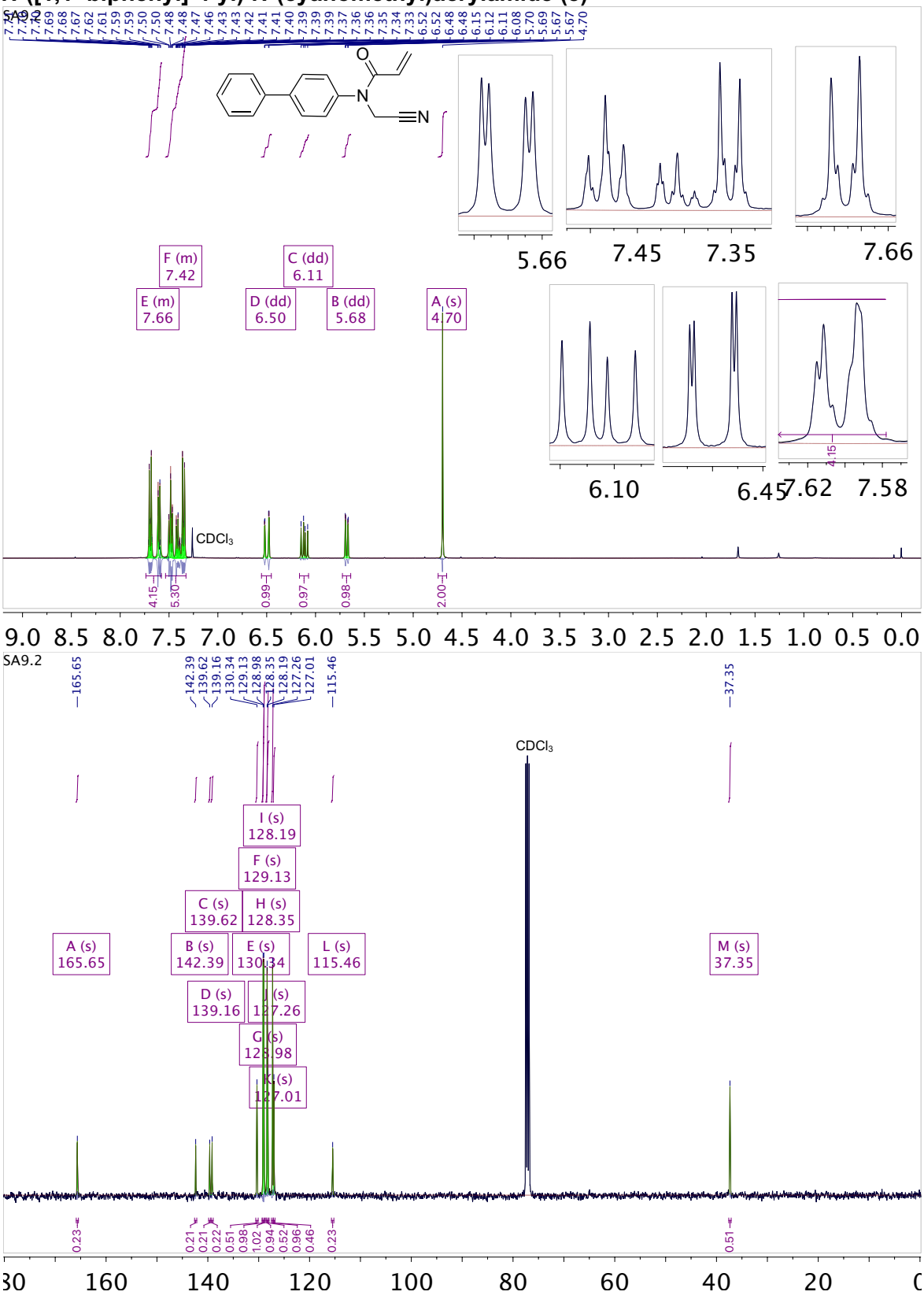




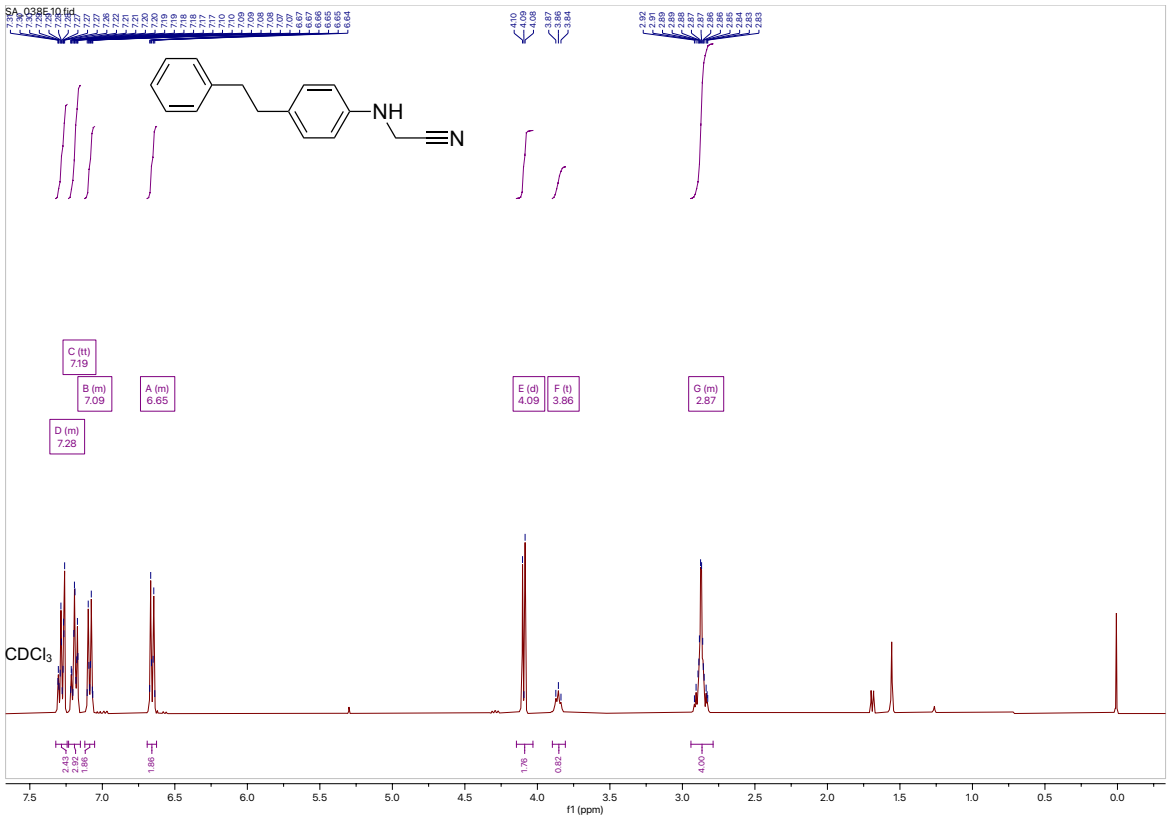
2-([1,1'-biphenyl]-4-ylamino)acetonitrile (47)



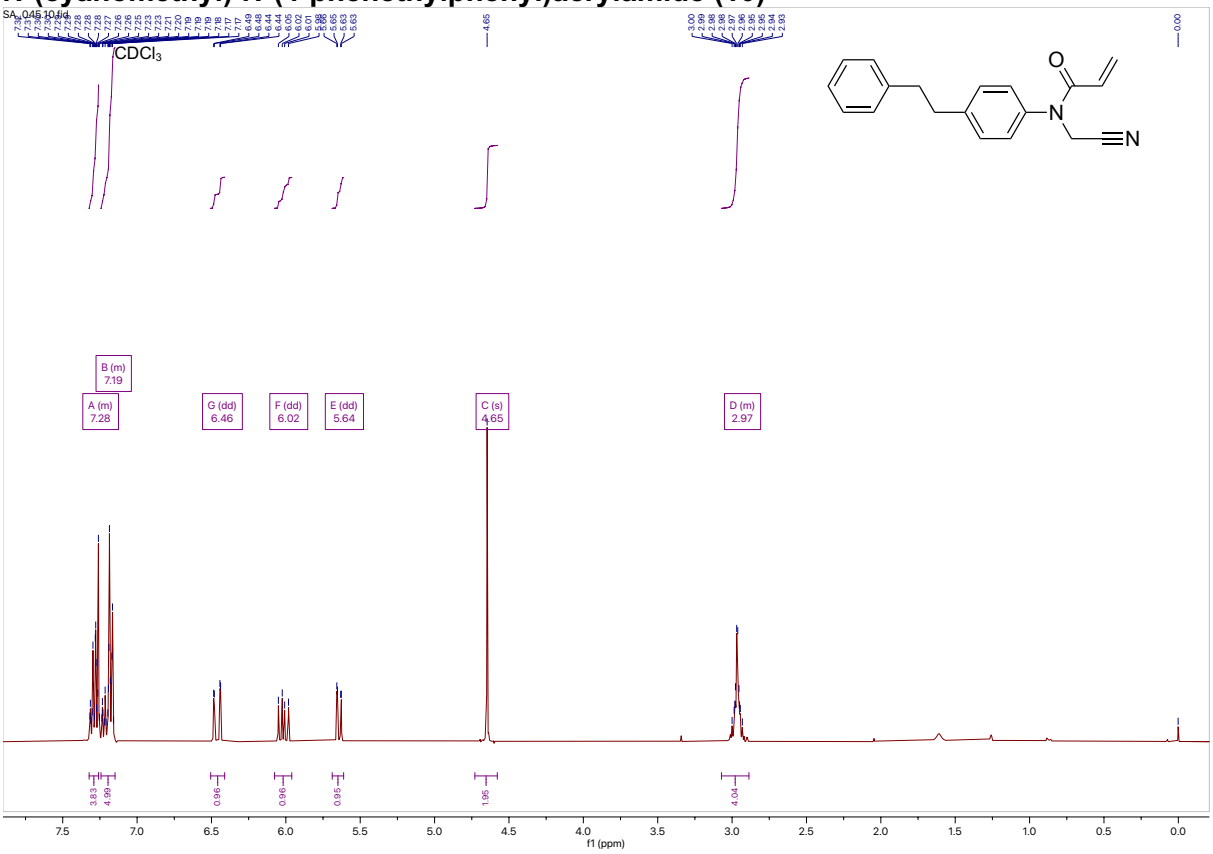
***N*-([1,1'-biphenyl]-4-yl)-*N*-(cyanomethyl)acrylamide (8)**

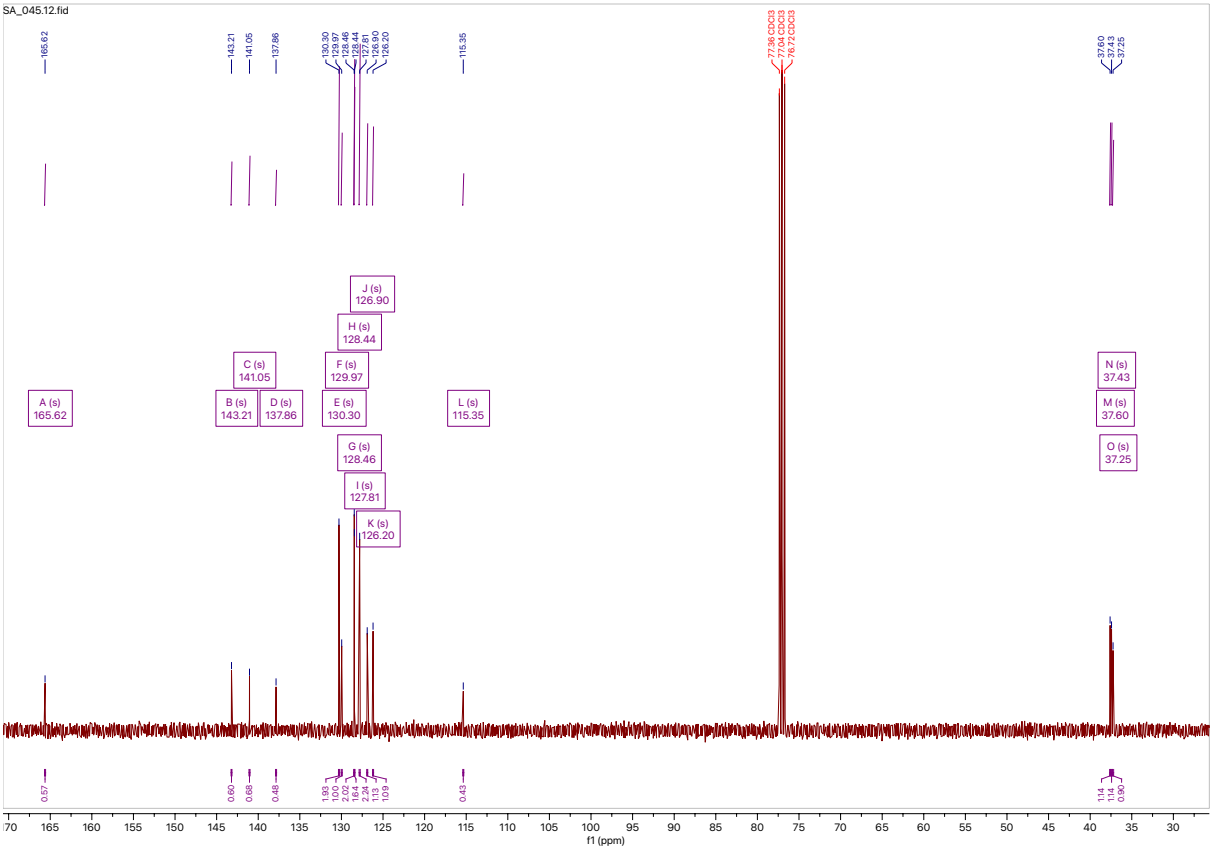


2-((4-phenethylphenyl)amino)acetonitrile (48)

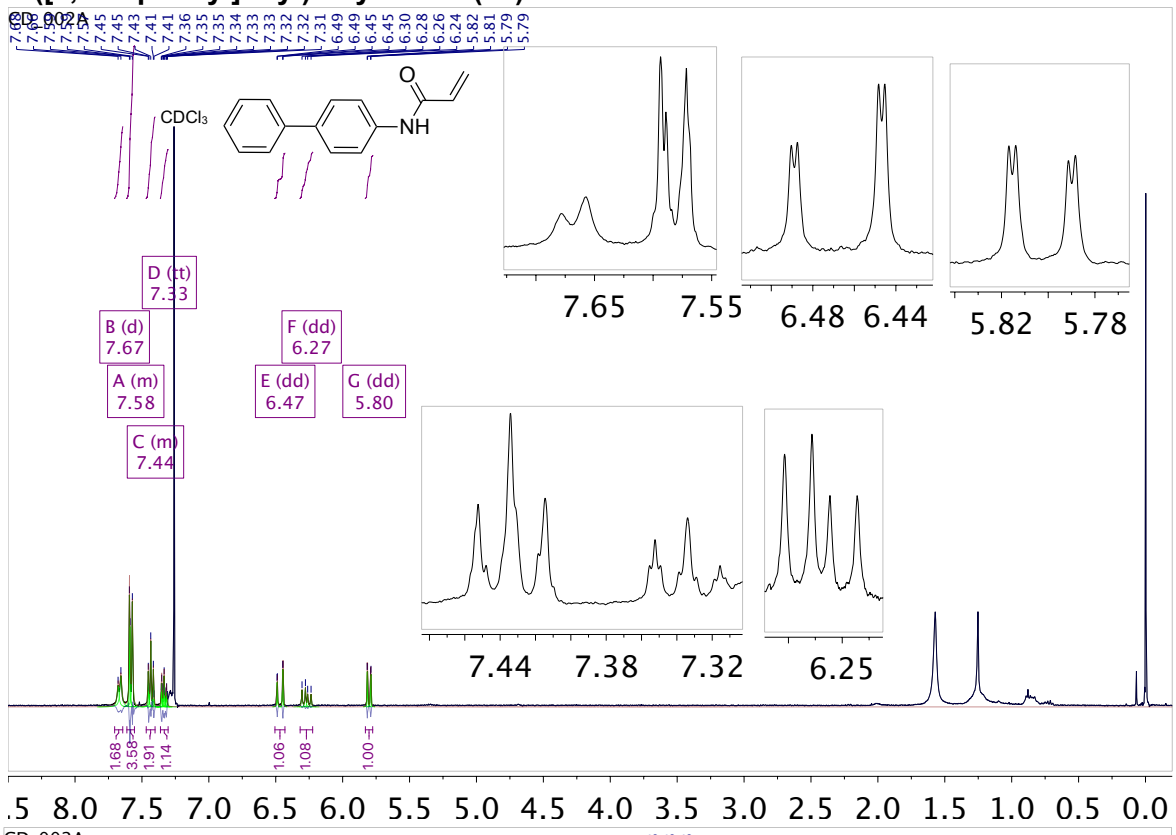


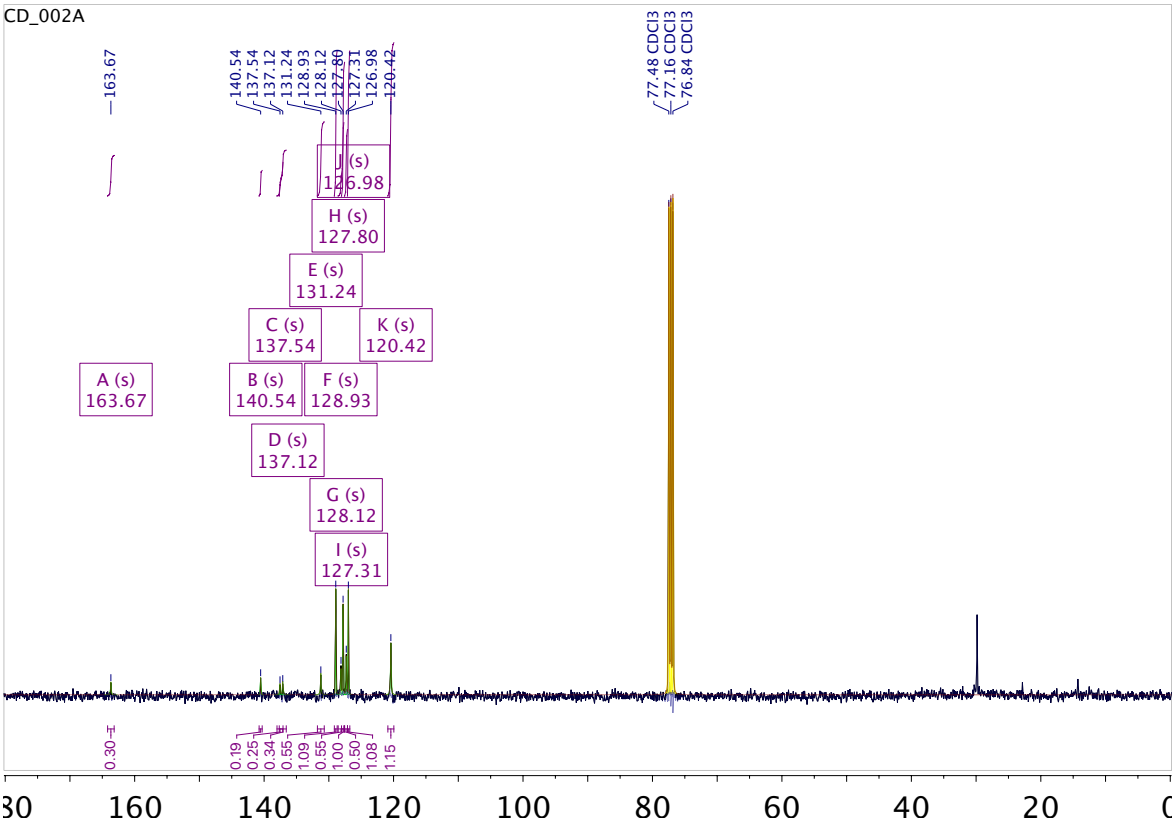
N-(cyanomethyl)-*N*-(4-phenethylphenyl)acrylamide (10)



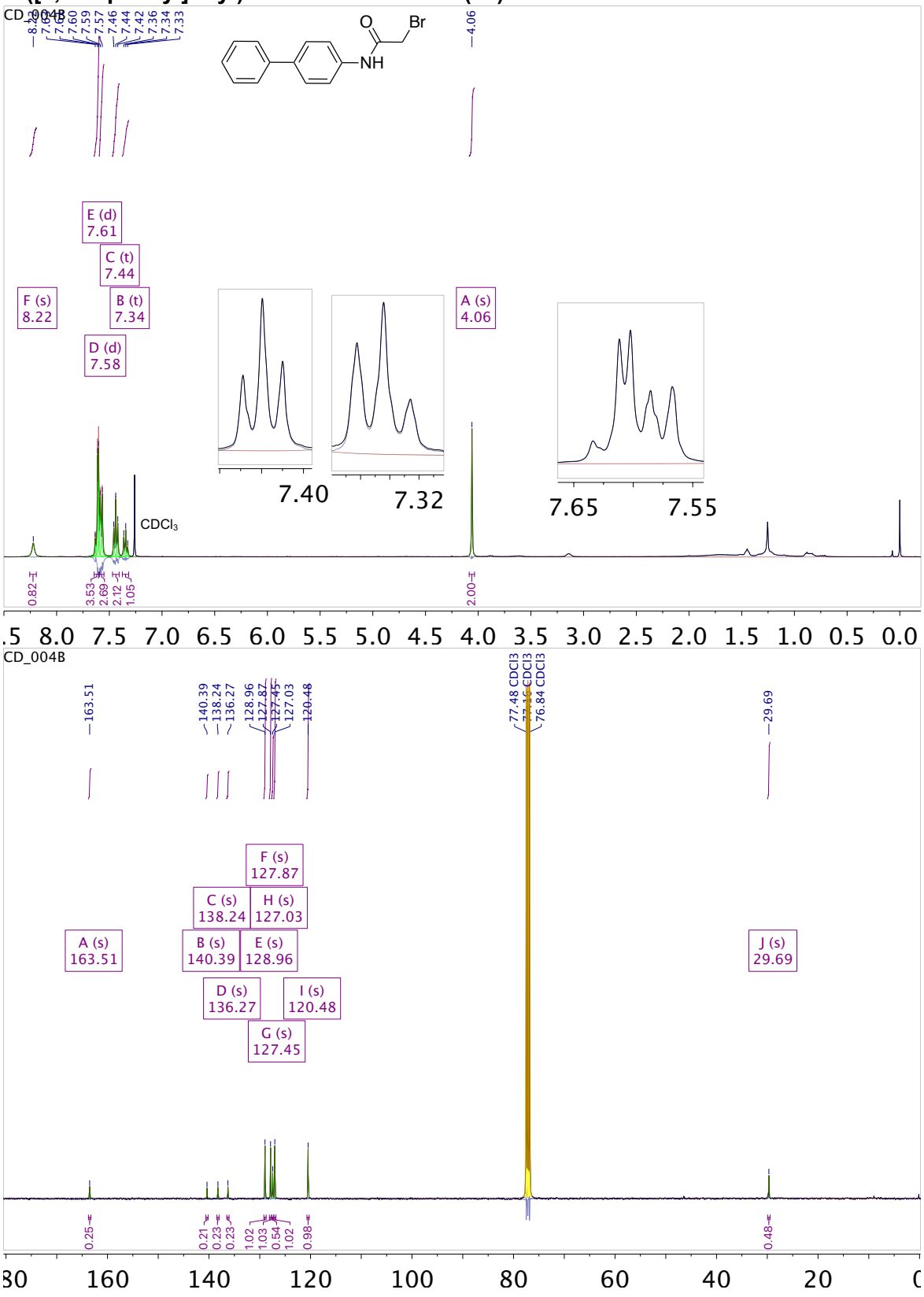


_N_-([1,1'-biphenyl]-4-yl)acrylamide (11)

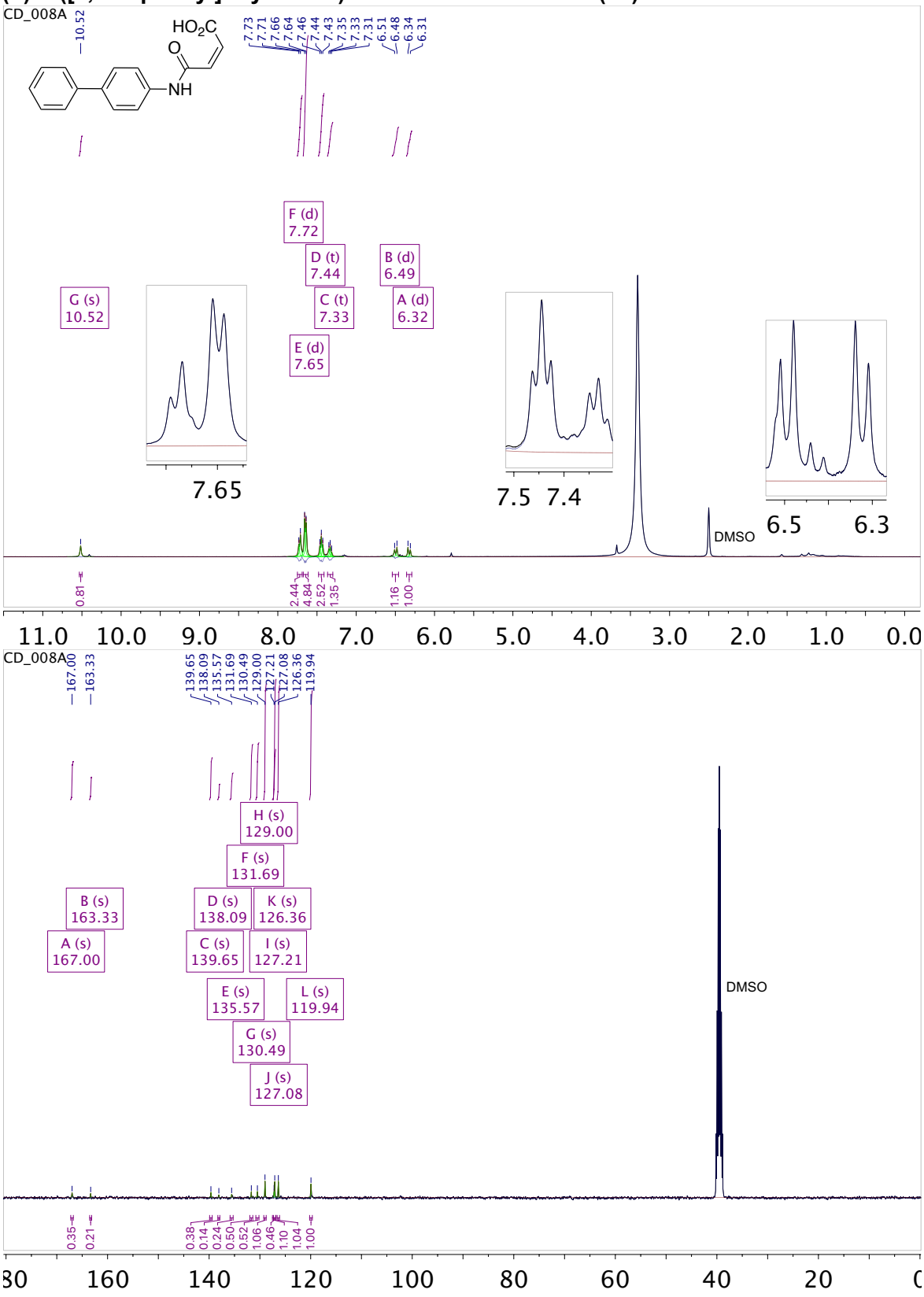




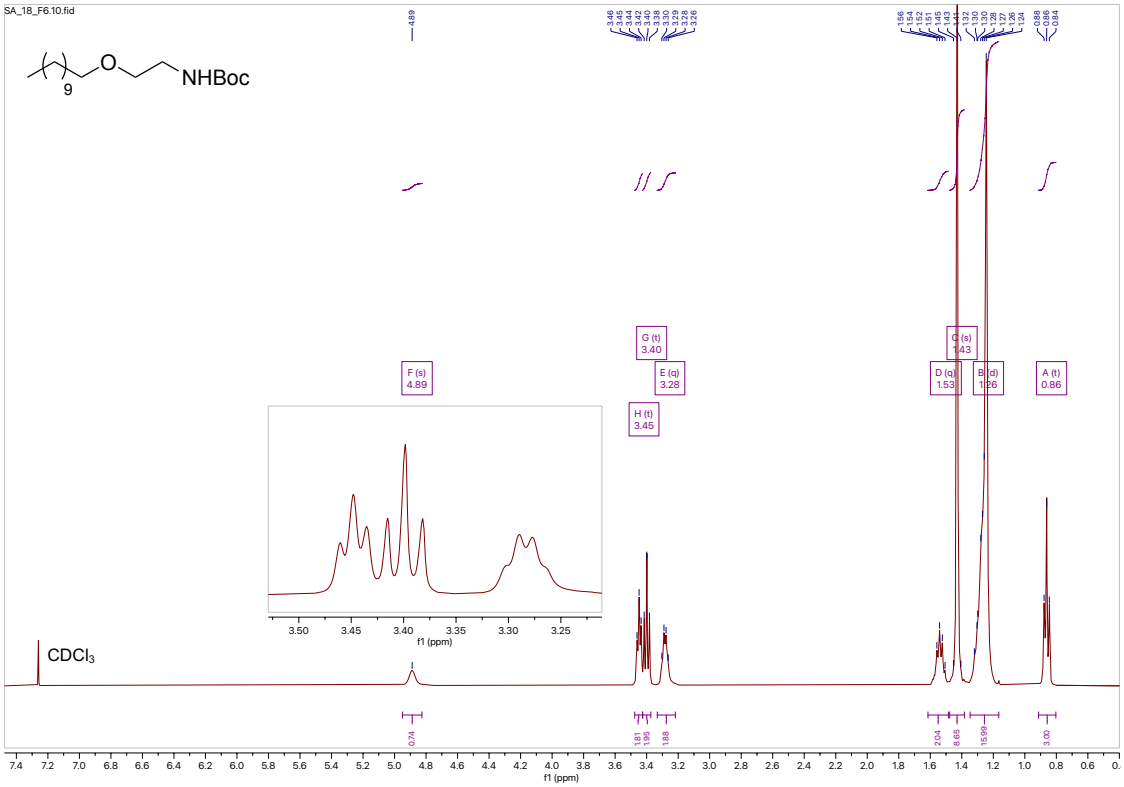
***N*-([1,1'-biphenyl]-4-yl)-2-bromoacetamide (12)**



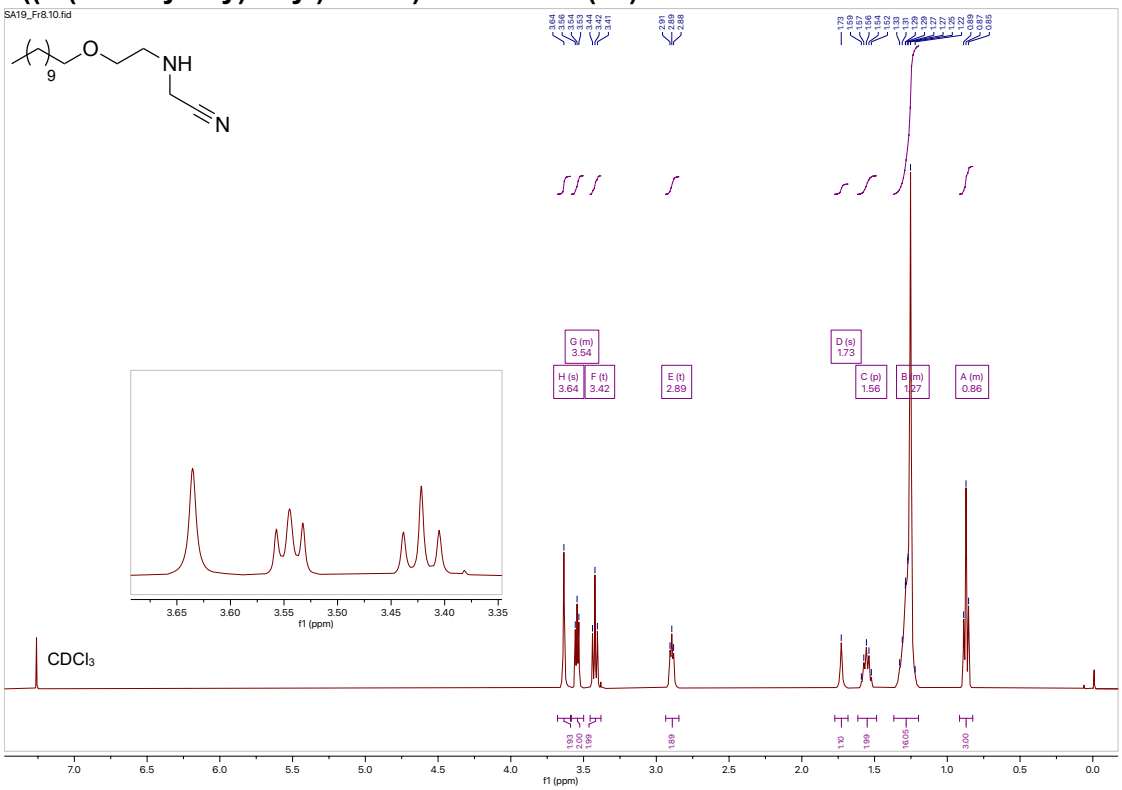
(*Z*)-4-([1,1'-biphenyl]-4-ylamino)-4-oxobut-2-enoic acid (13)



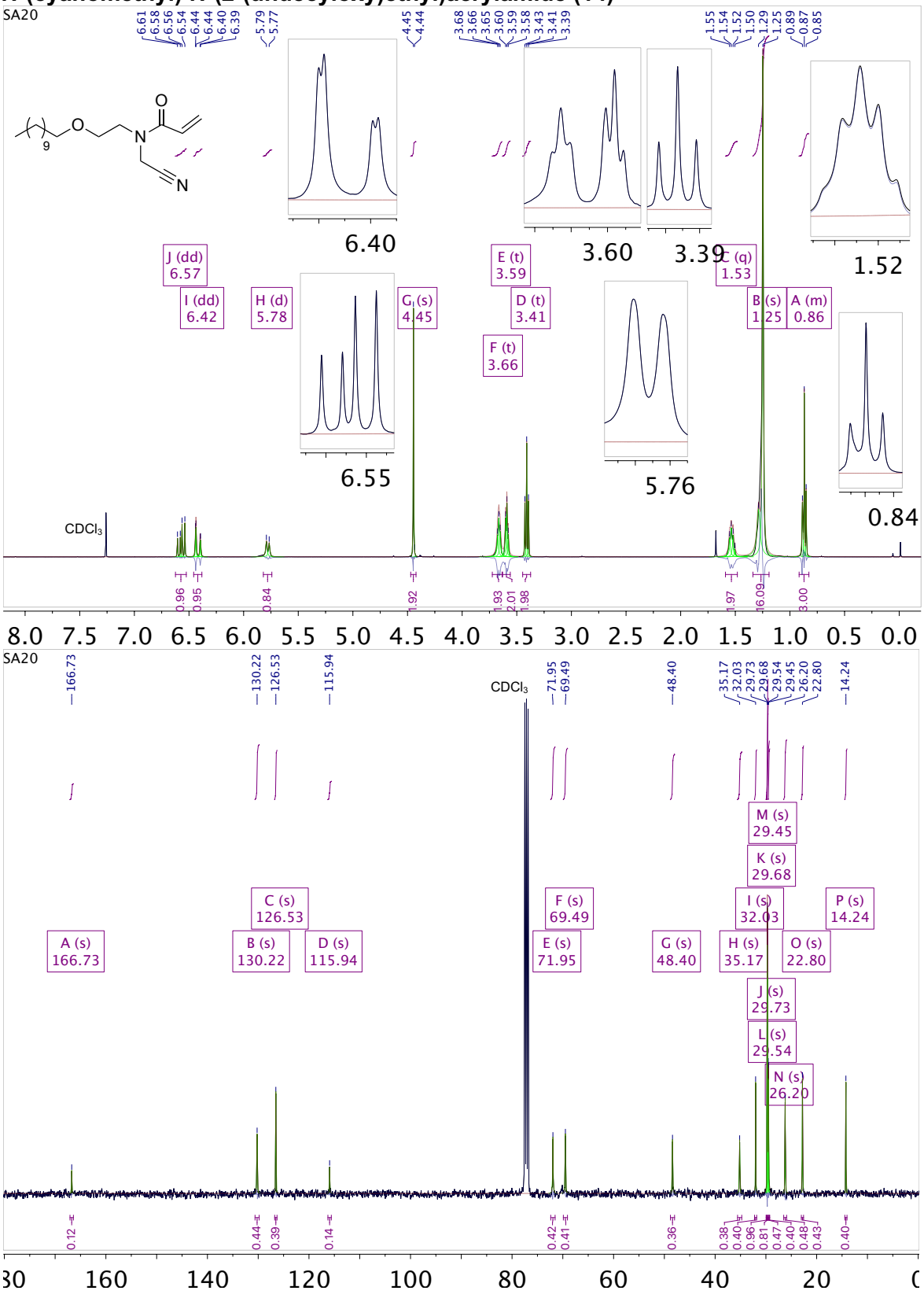
tert-butyl (2-(undecyloxy)ethyl)carbamate (49)



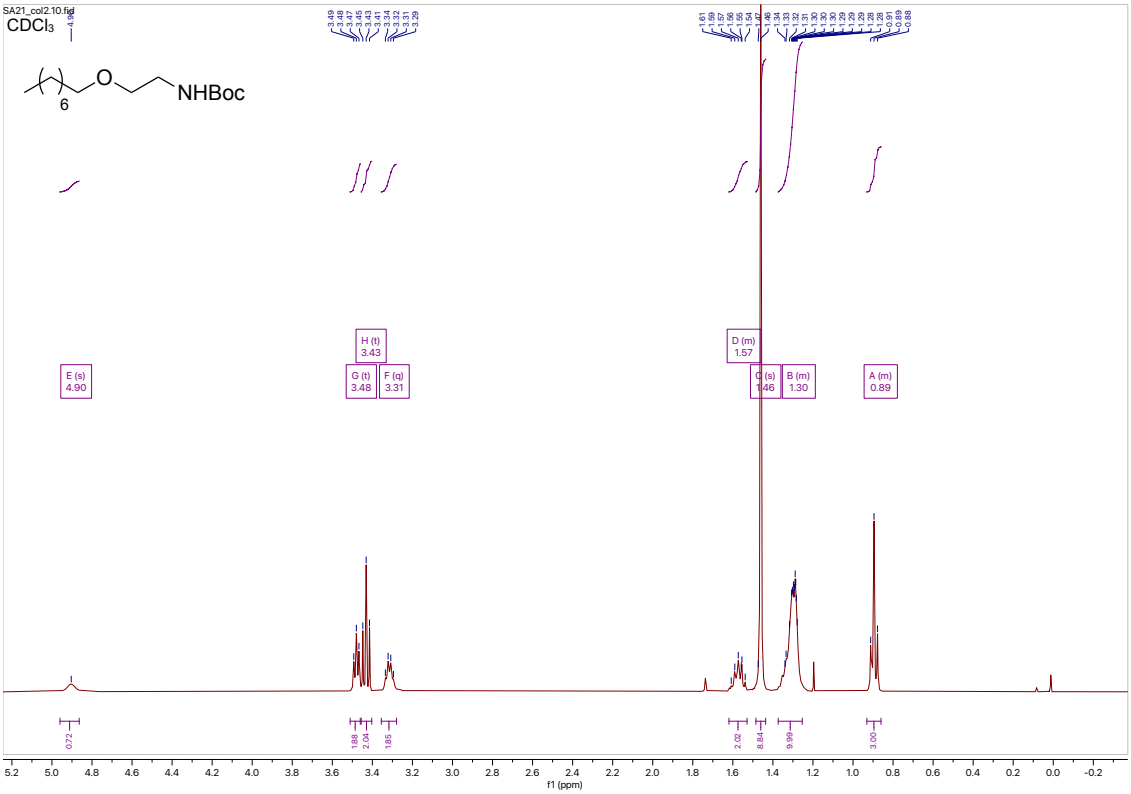
2-((2-(undecyloxy)ethyl)amino)acetonitrile (50)



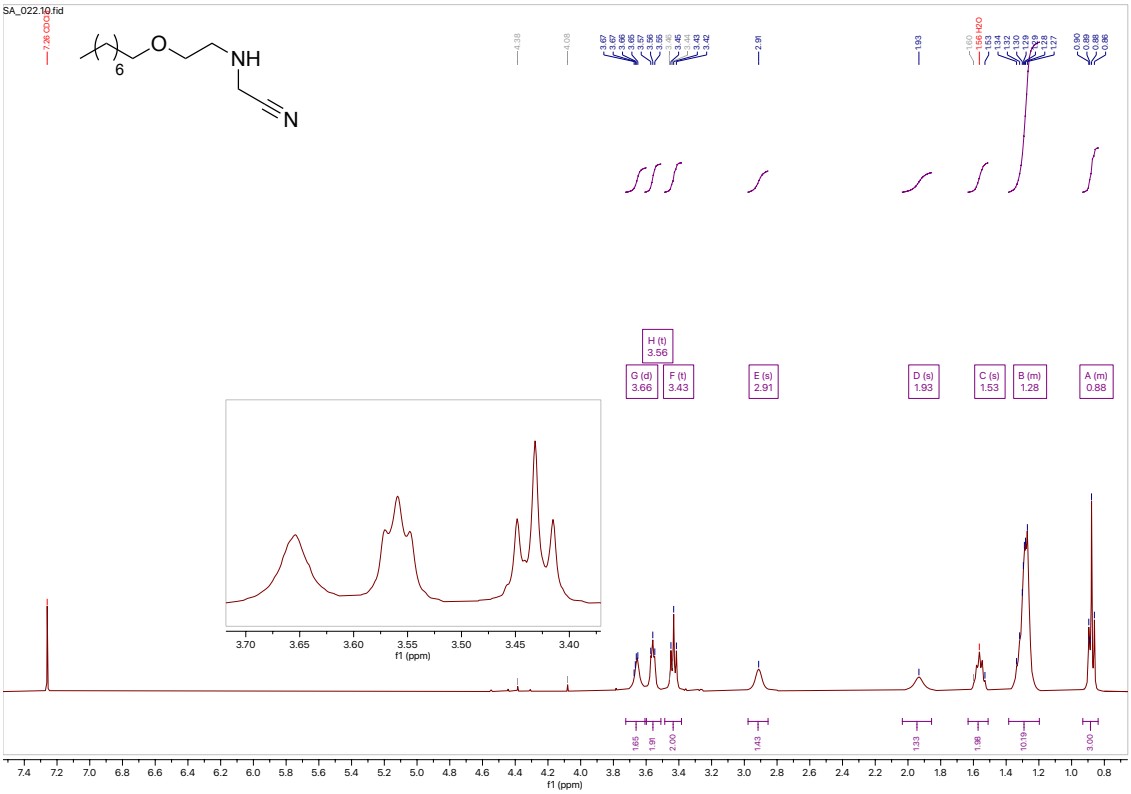
_N_-(cyanomethyl)-_N_-(2-(undecyloxy)ethyl)acrylamide (14)



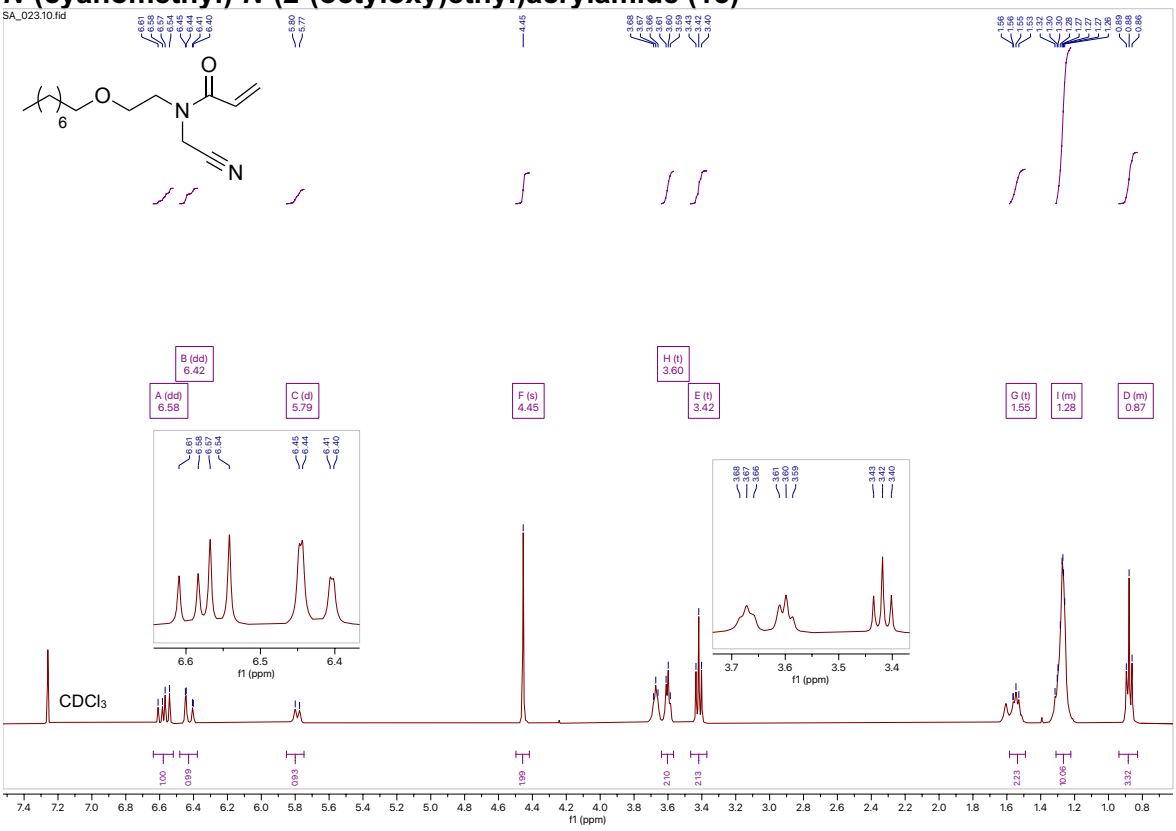
tert-butyl (2-(octyloxy)ethyl)carbamate (51)

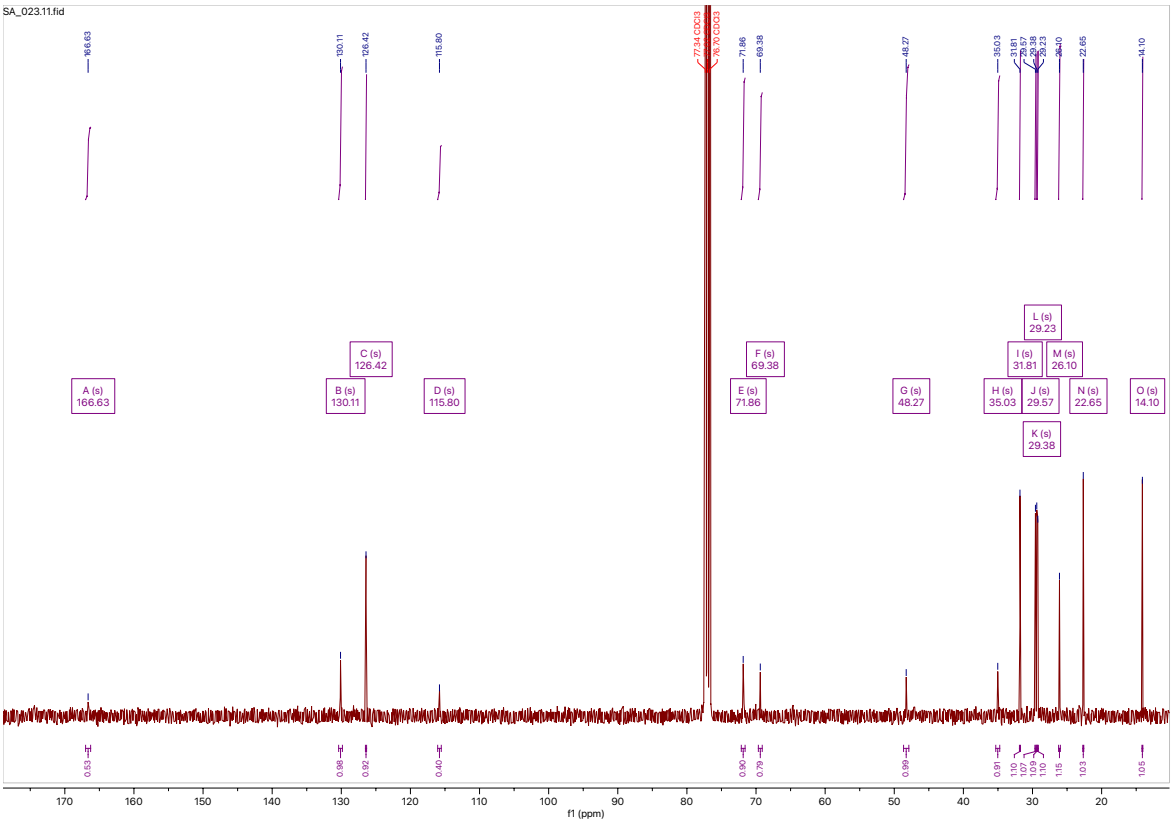


2-((2-(octyloxy)ethyl)amino)acetonitrile (52)

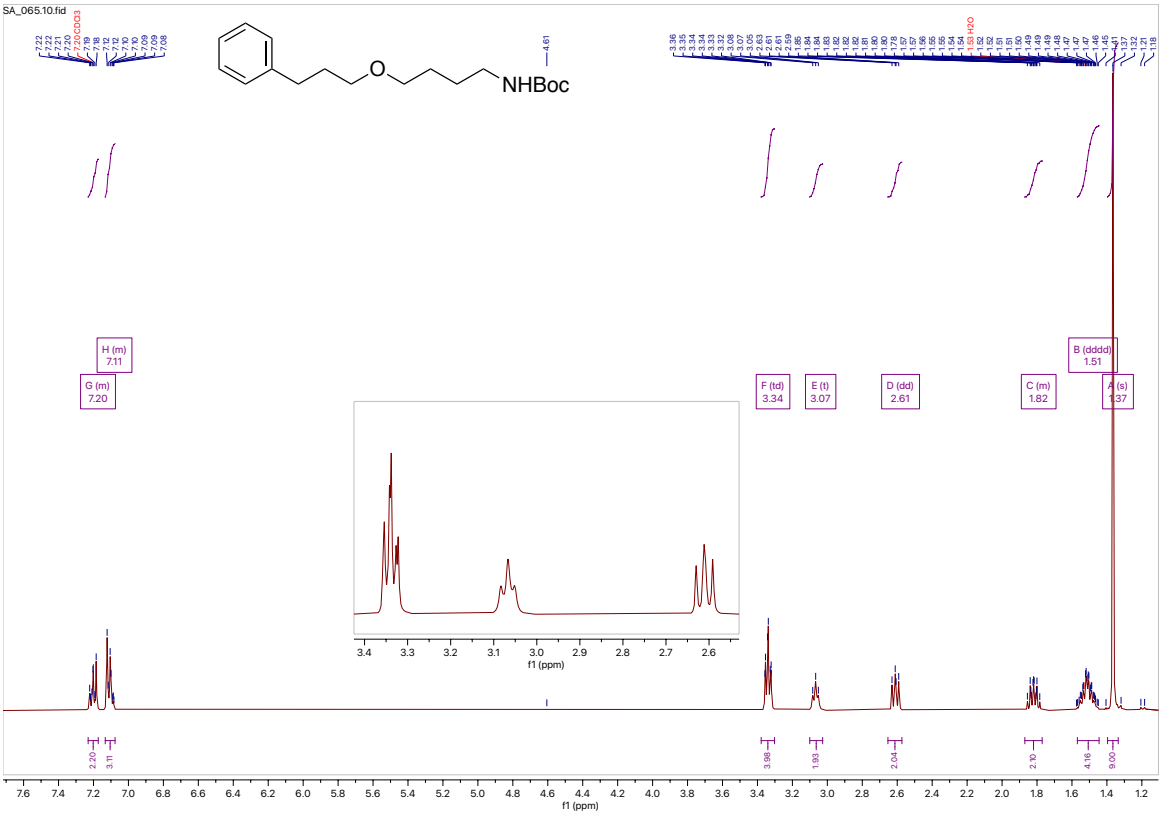


N-(cyanomethyl)-*N*-(2-(octyloxy)ethyl)acrylamide (15)

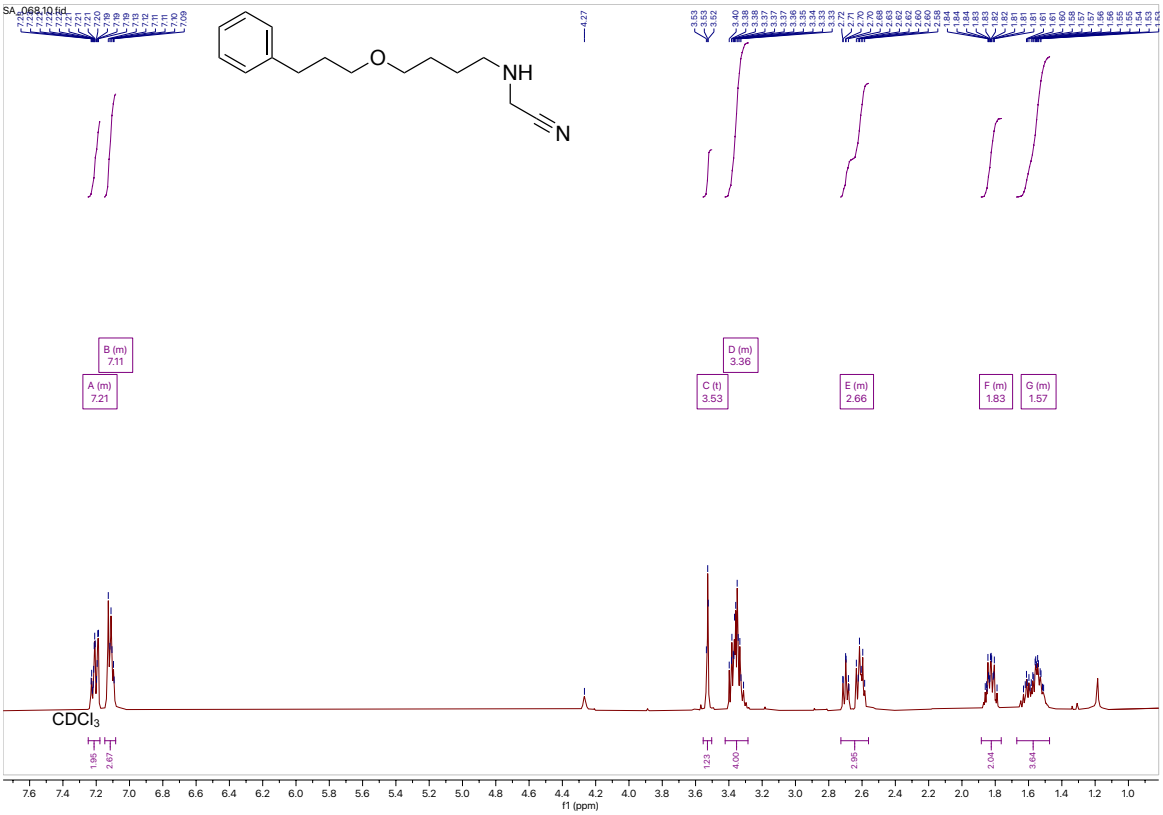




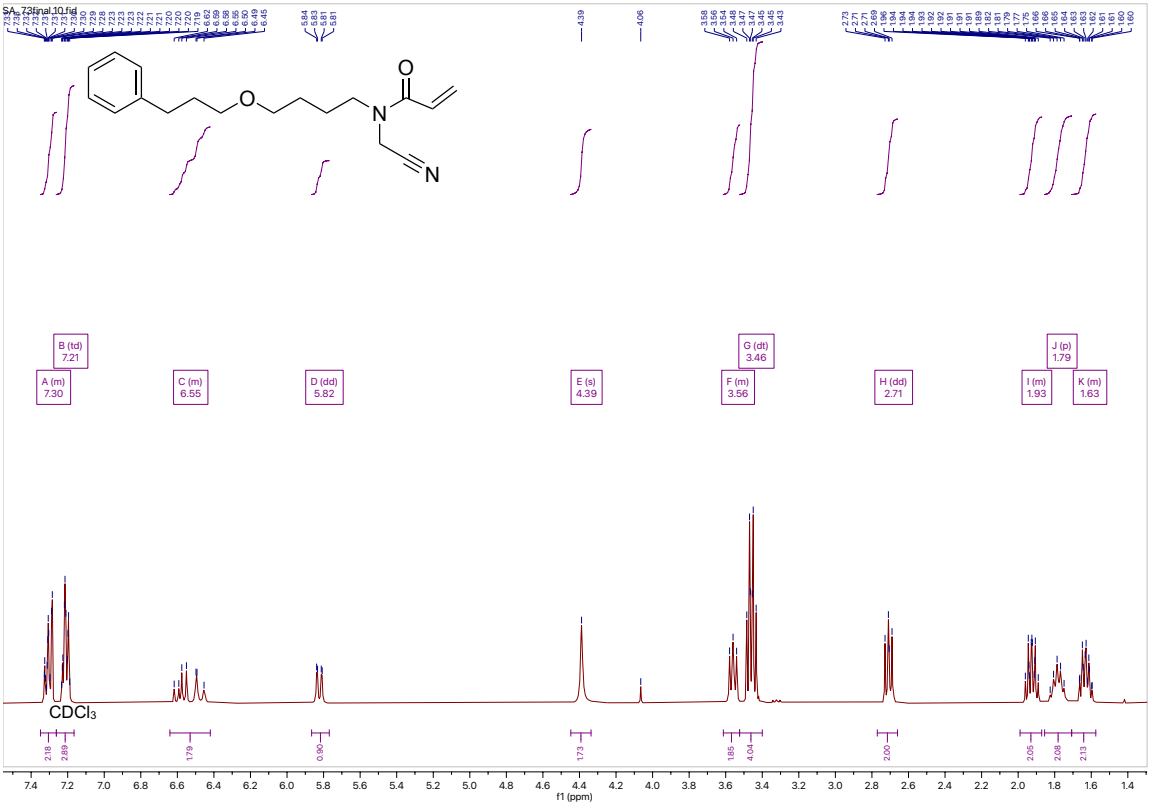
tert-butyl (4-(3-phenylpropoxy)butyl)carbamate (53)

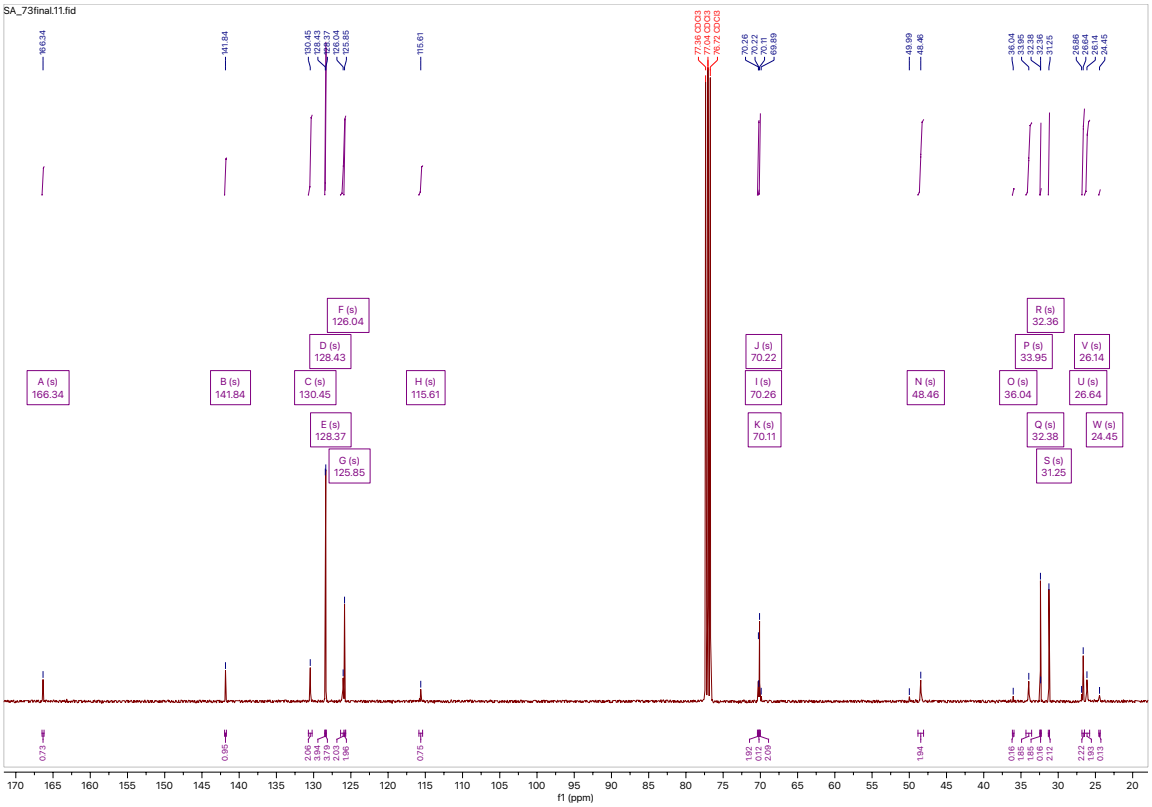


2-((4-(3-phenylpropoxy)butyl)amino)acetonitrile (54)

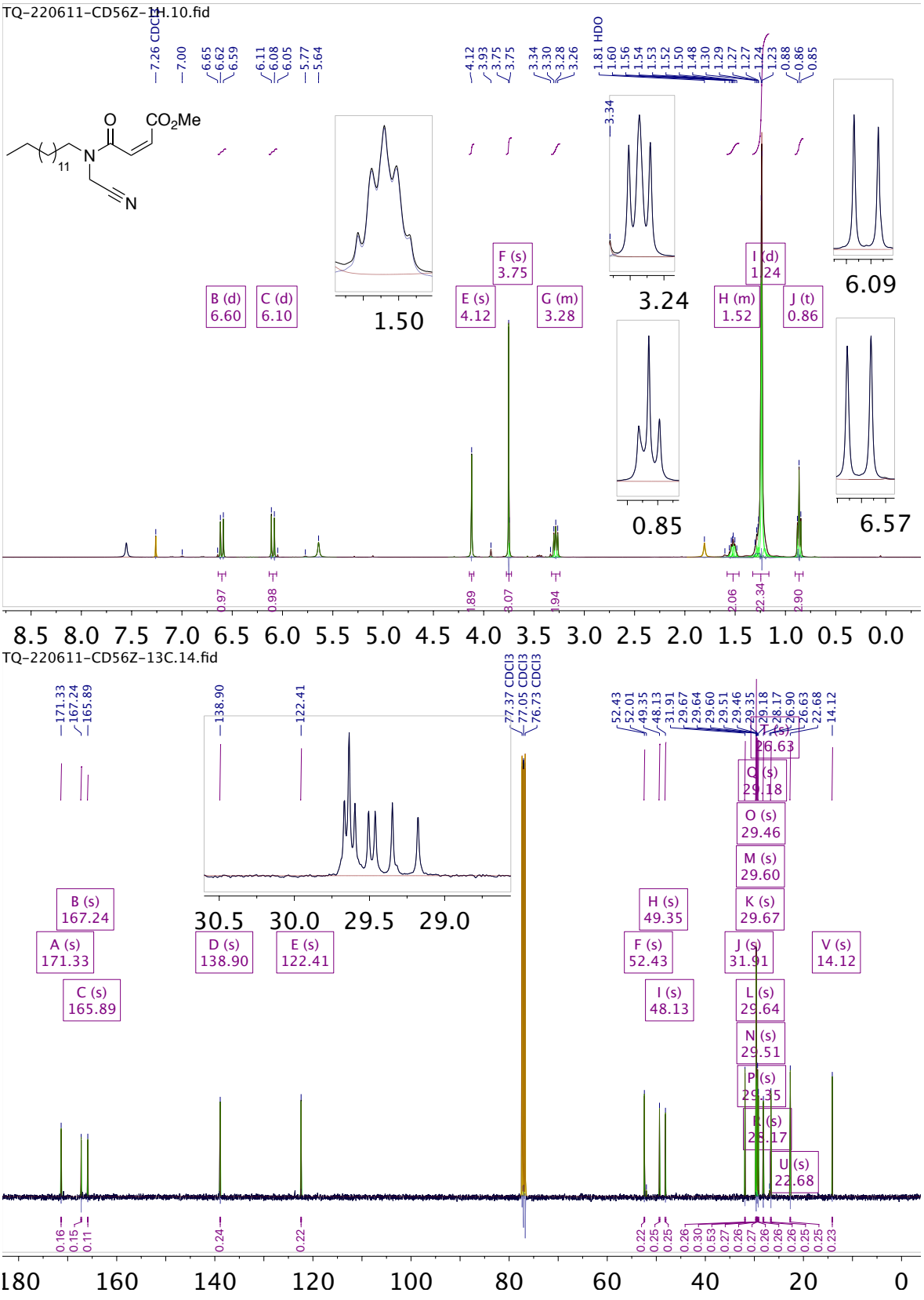


N-(cyanomethyl)-*N*-(4-(3-phenylpropoxy)butyl)acrylamide (16)

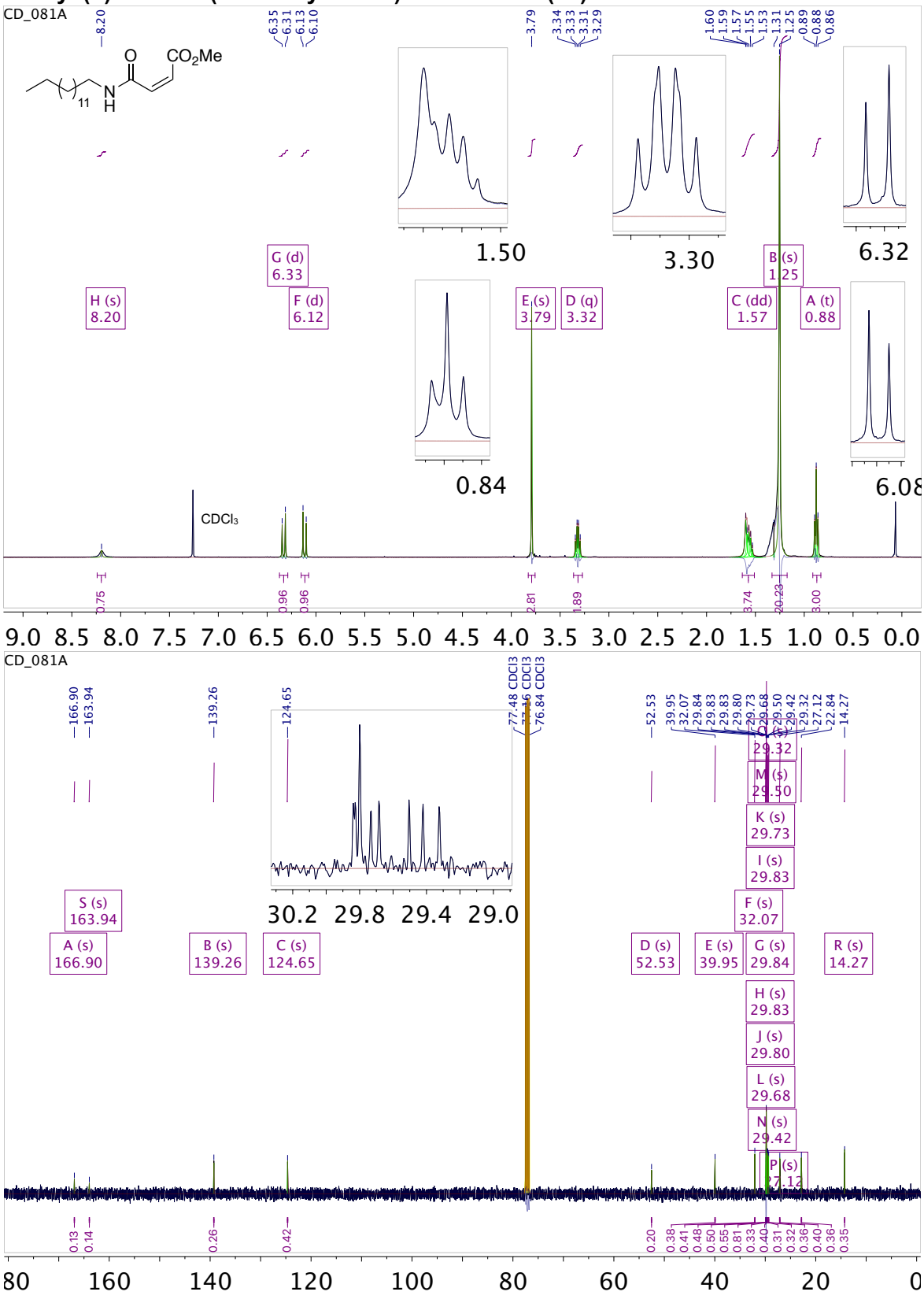




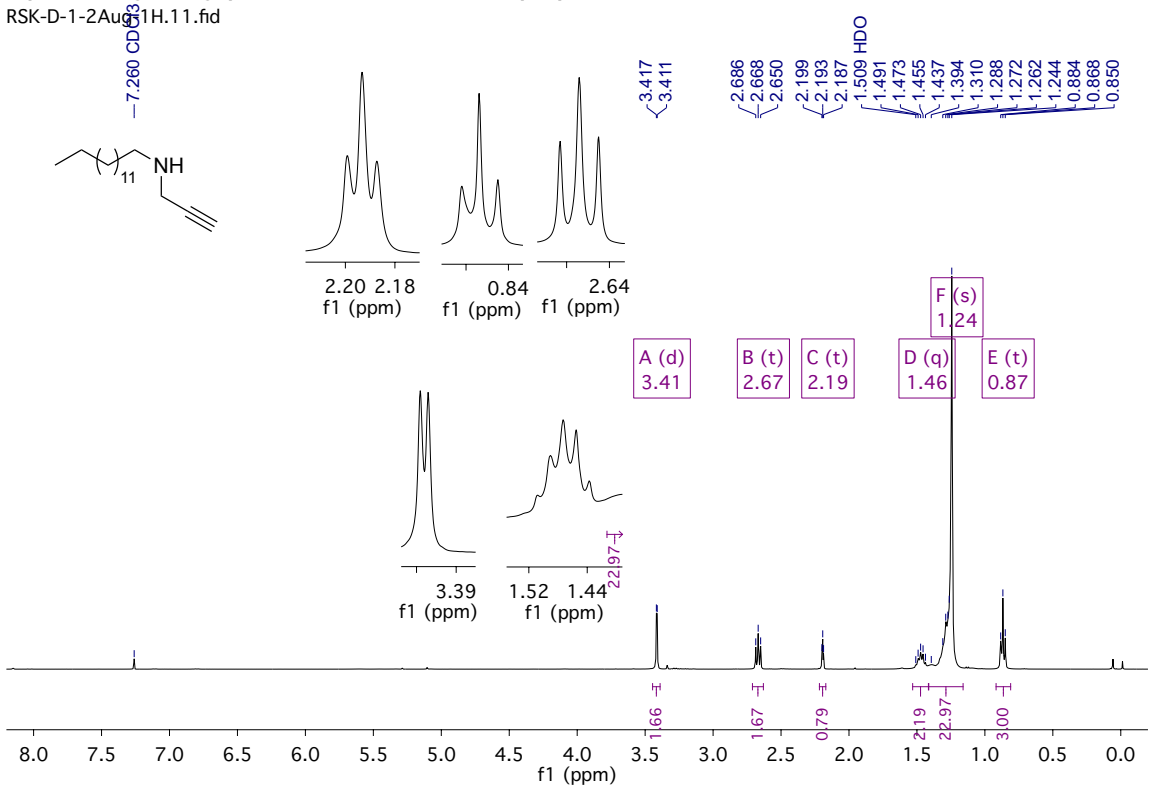
methyl (*Z*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (18)



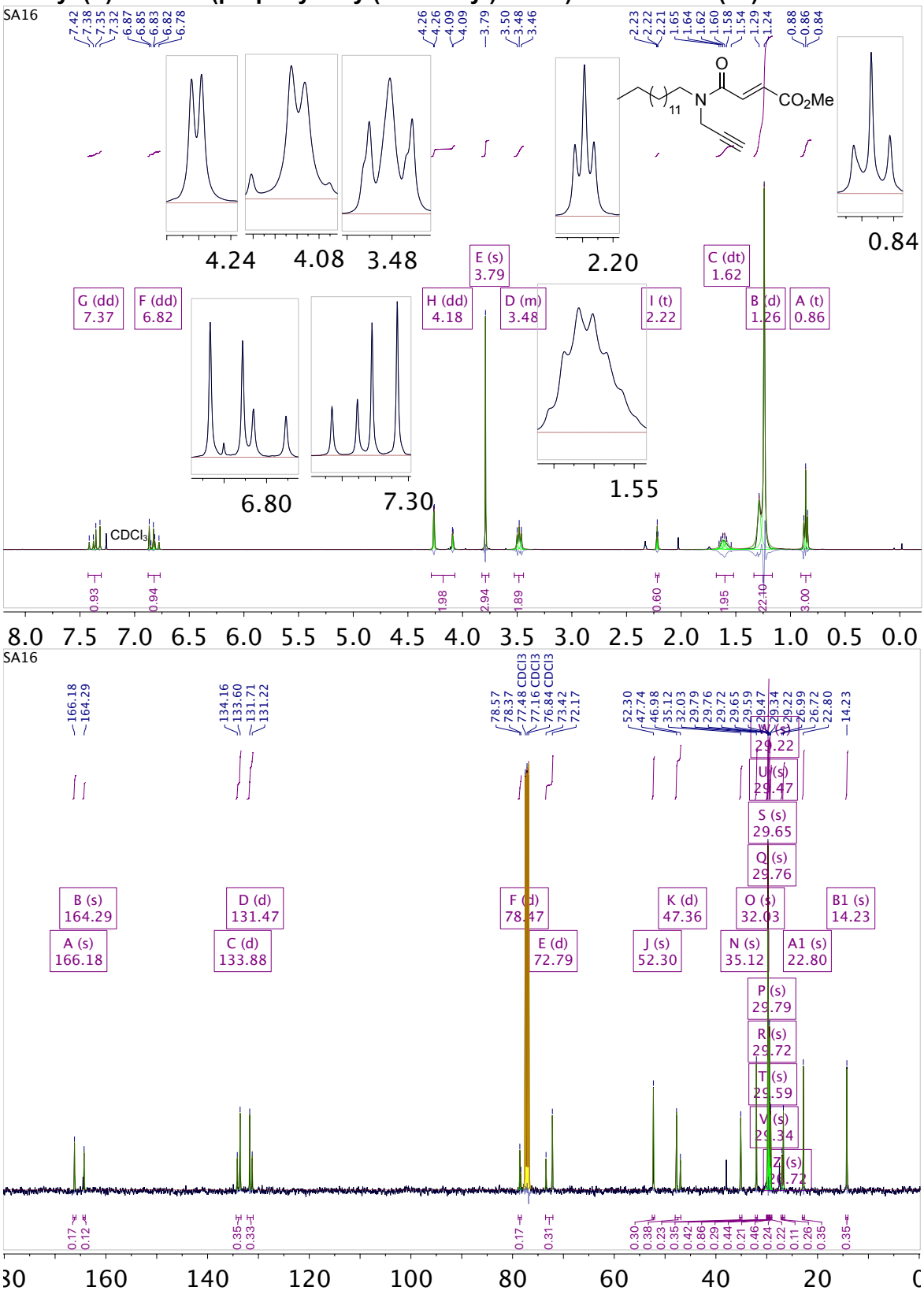
methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (19)



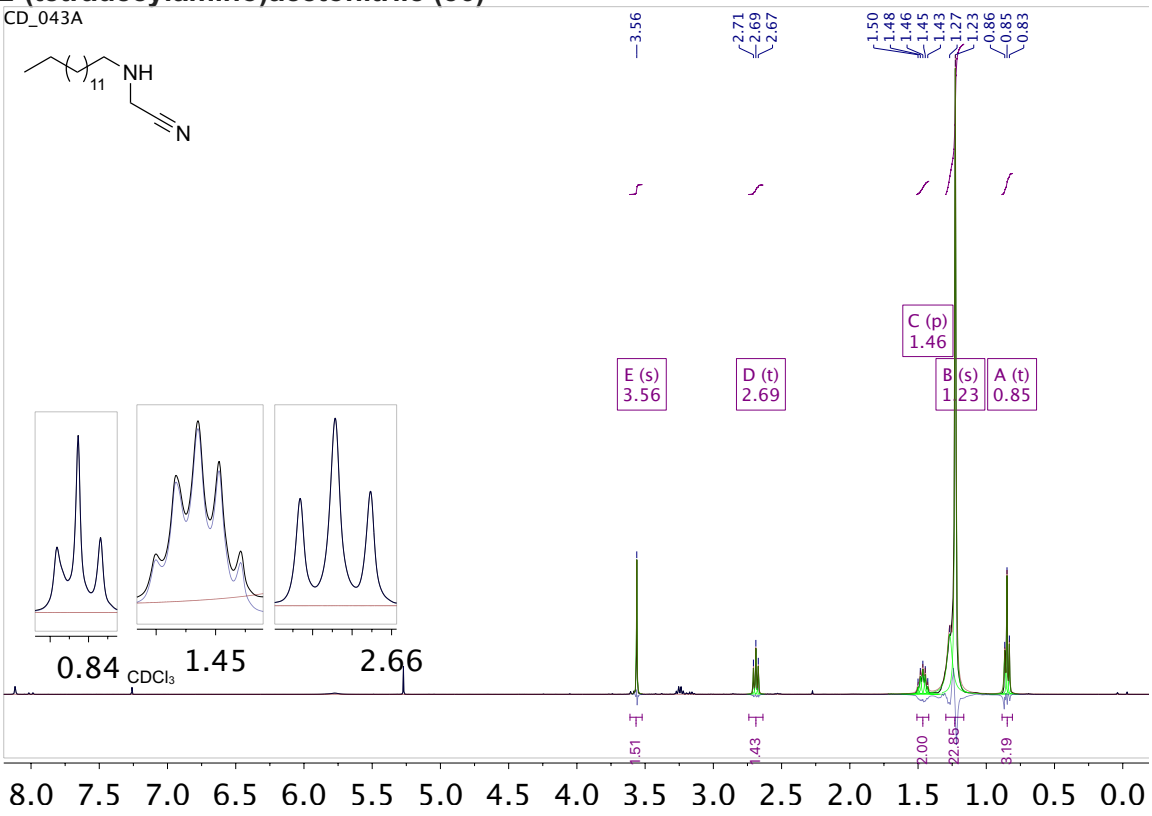
N-(prop-2-yn-1-yl)tetradecan-1-amine (55)



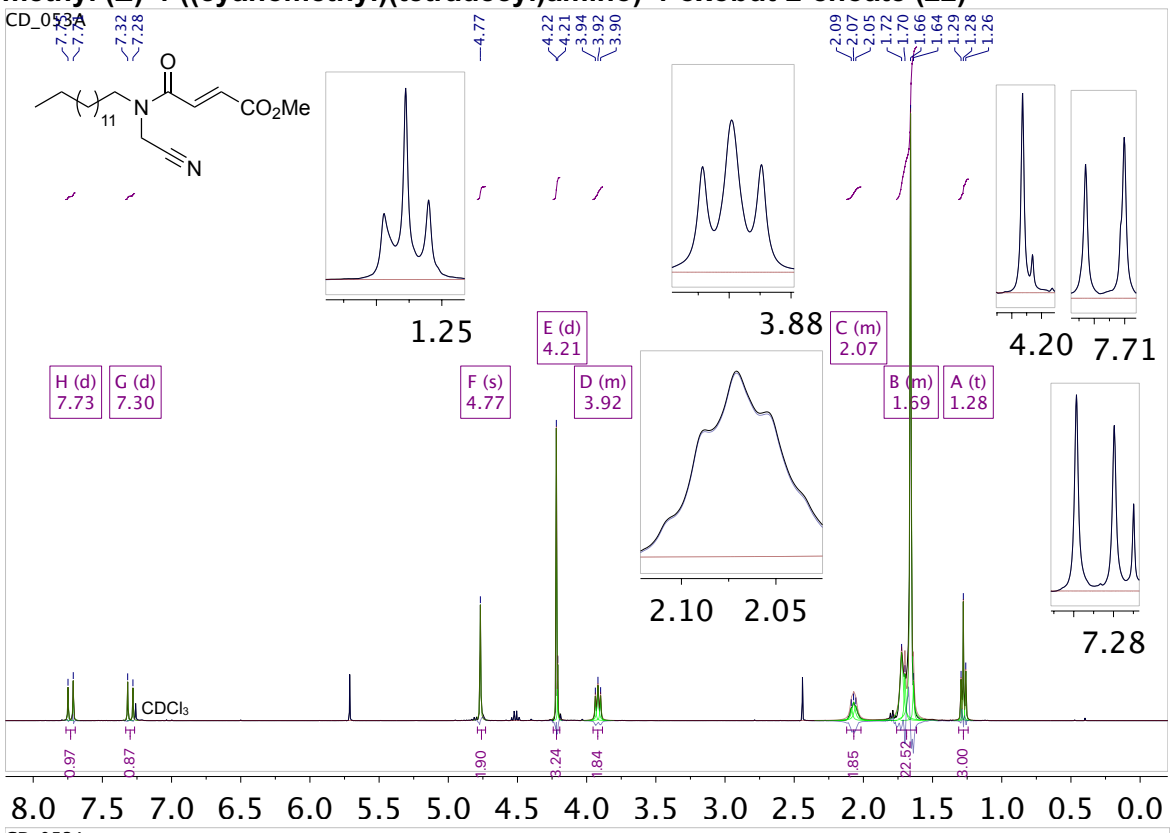
methyl (*E*)-4-oxo-4-(prop-2-yn-1-yl(tetradecyl)amino)but-2-enoate (21)

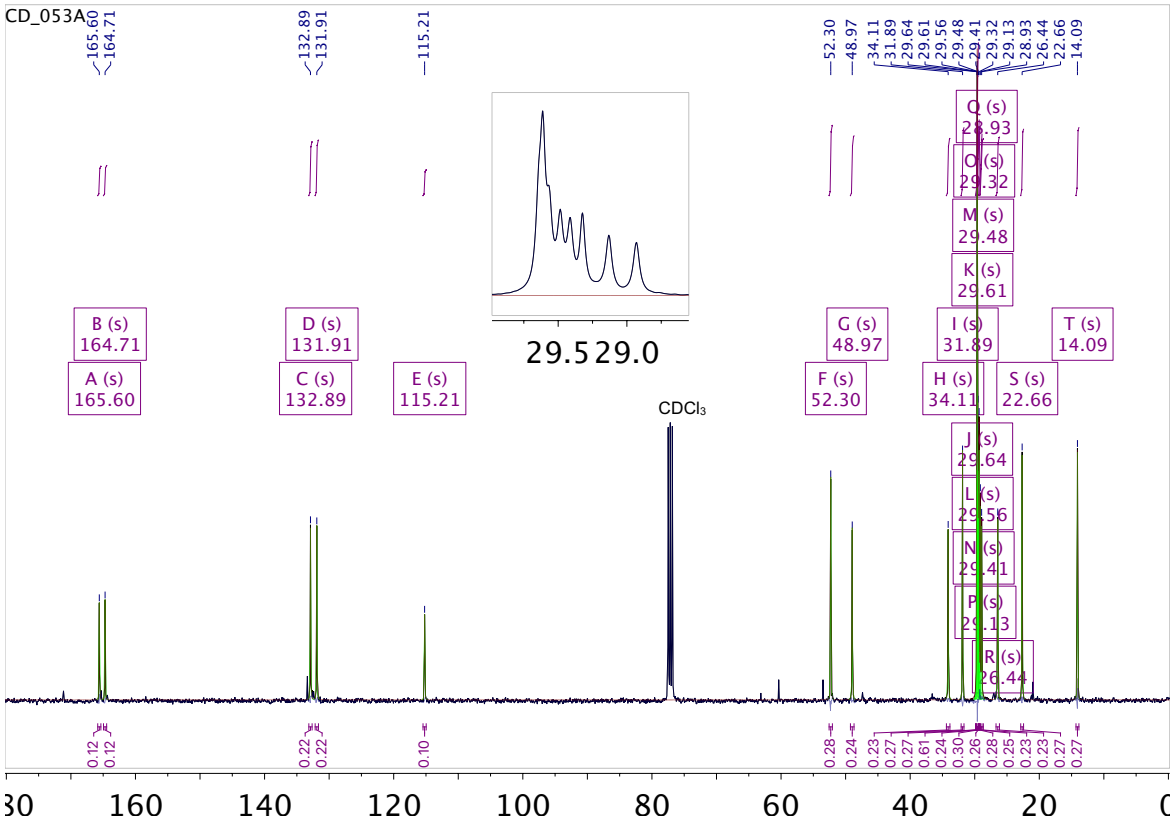


2-(tetradecylamino)acetonitrile (56)

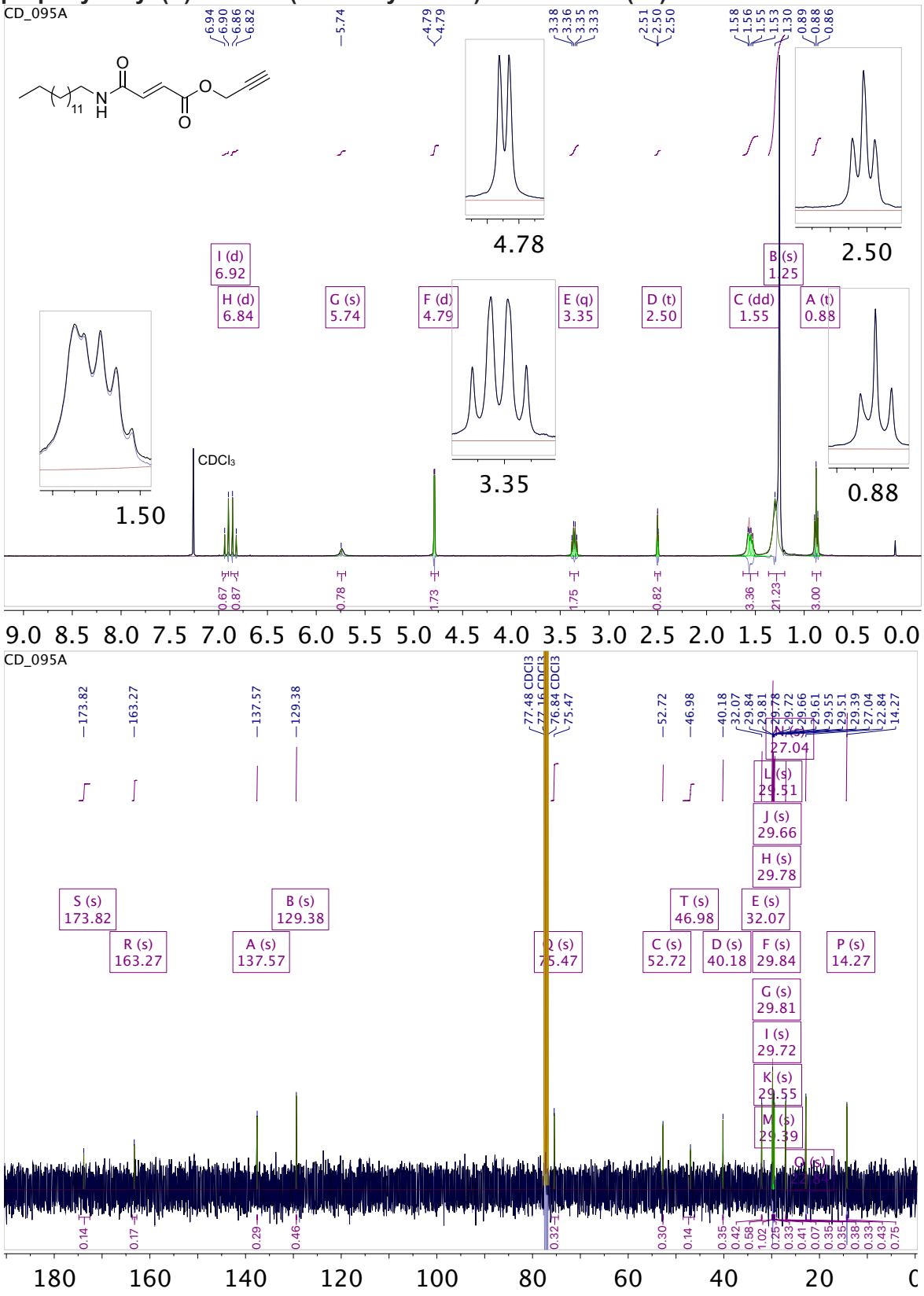


methyl (*E*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (22)

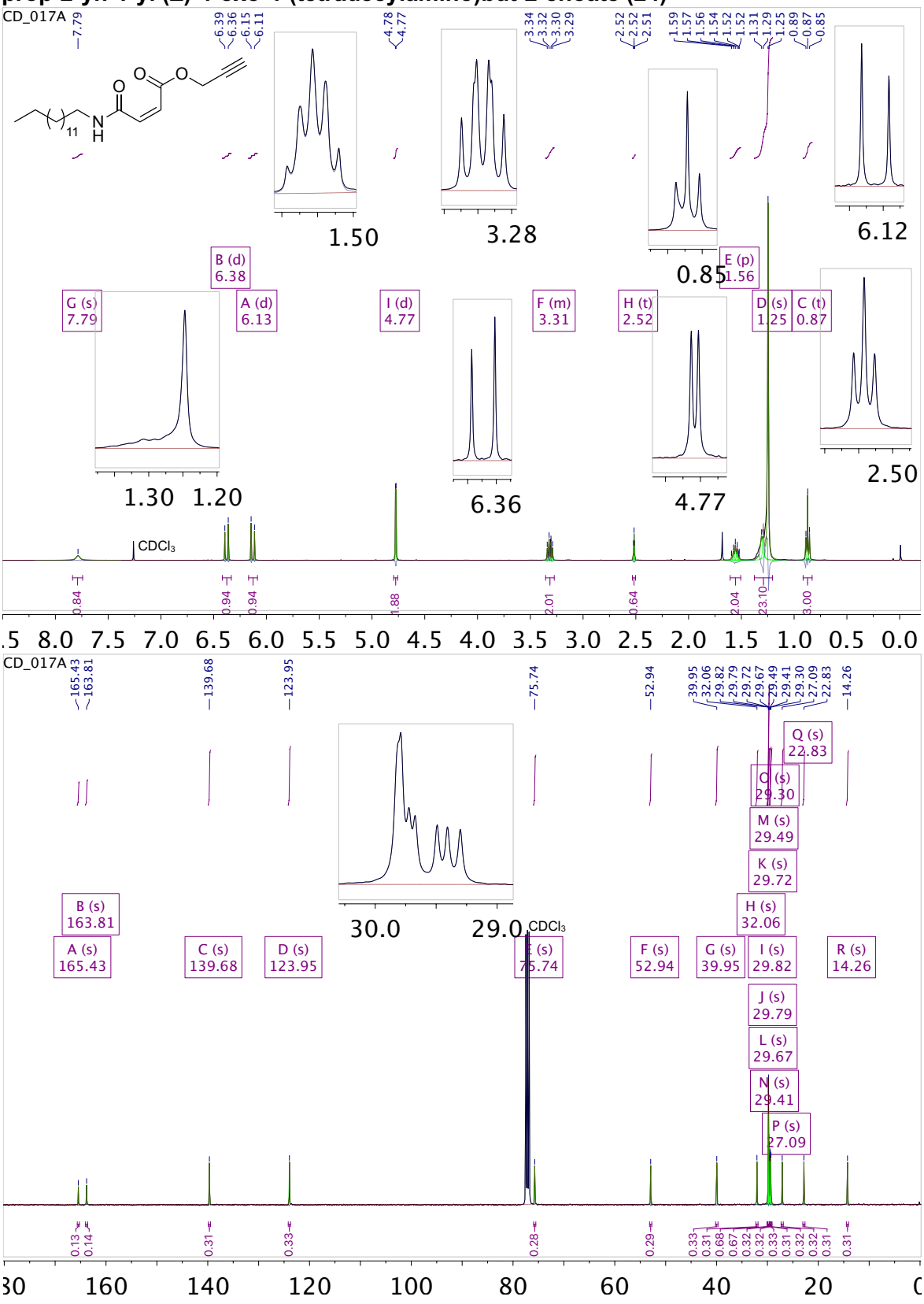




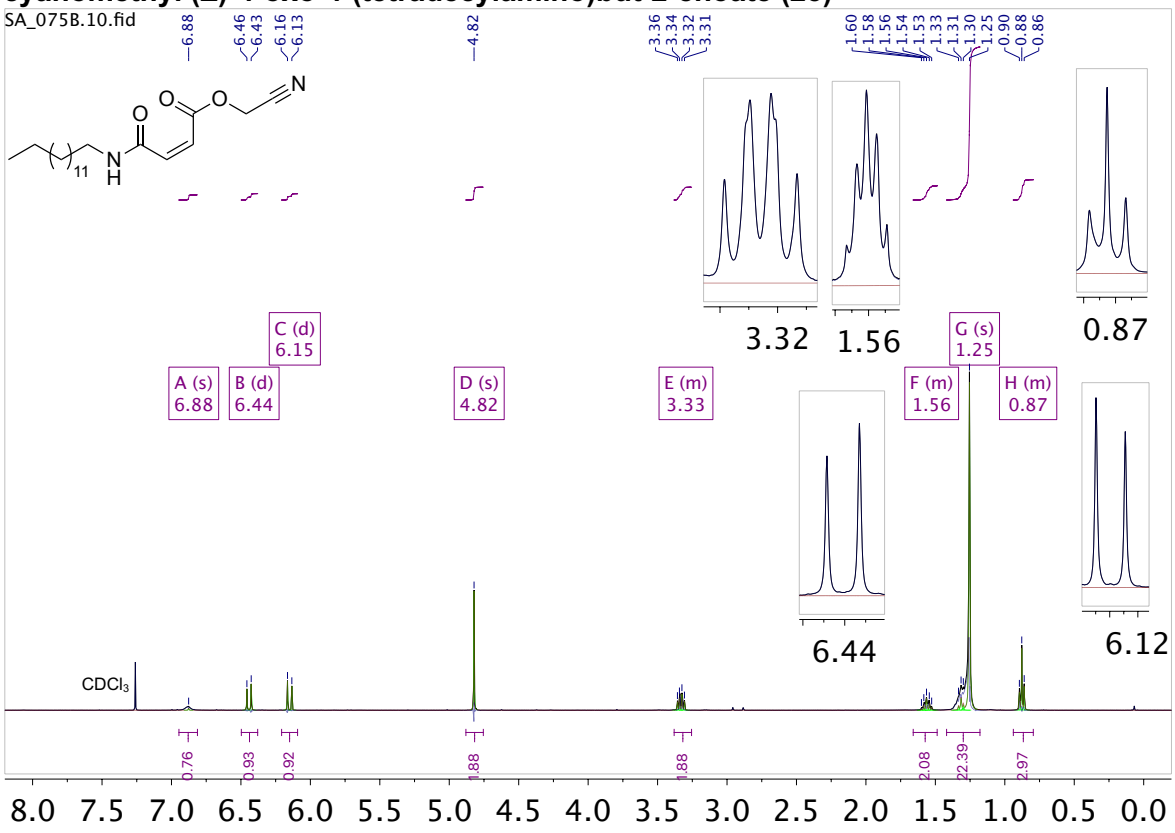
prop-2-yn-1-yl (*E*)-4-oxo-4-(tetradecylamino)but-2-enoate (23)

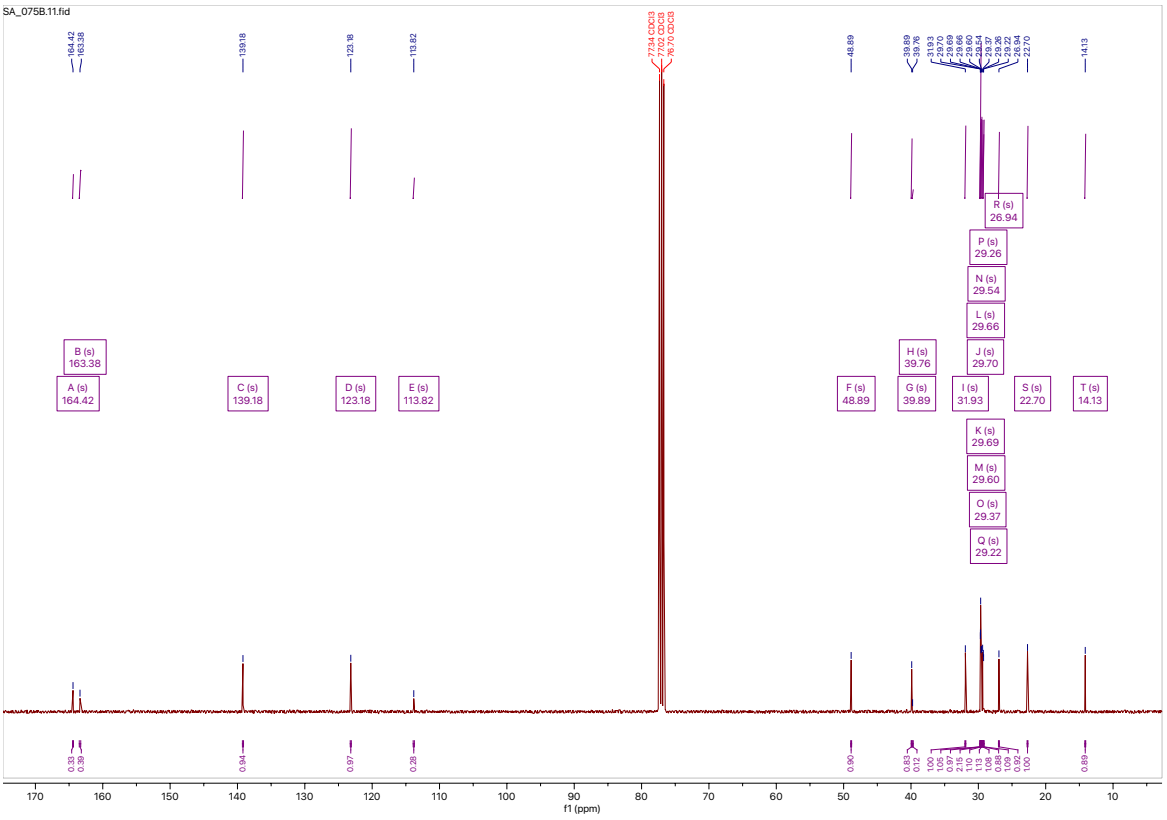


prop-2-yn-1-yl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (24)

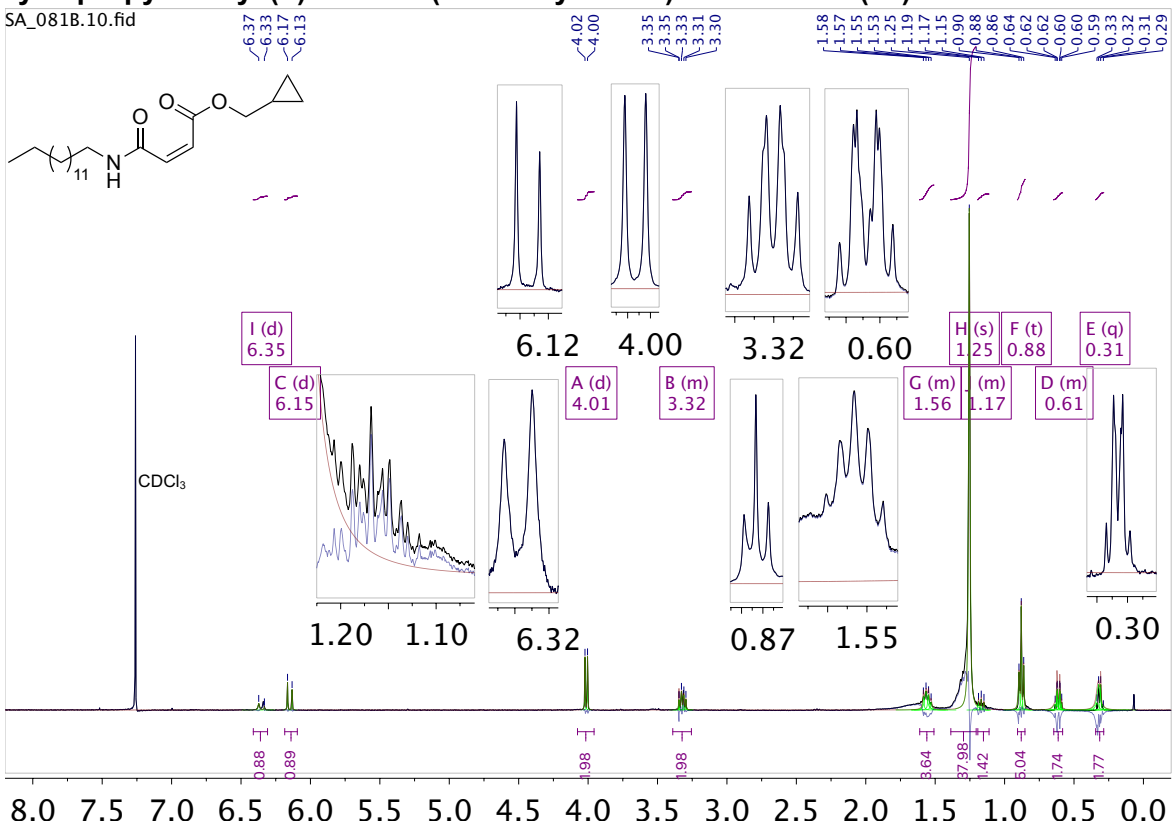


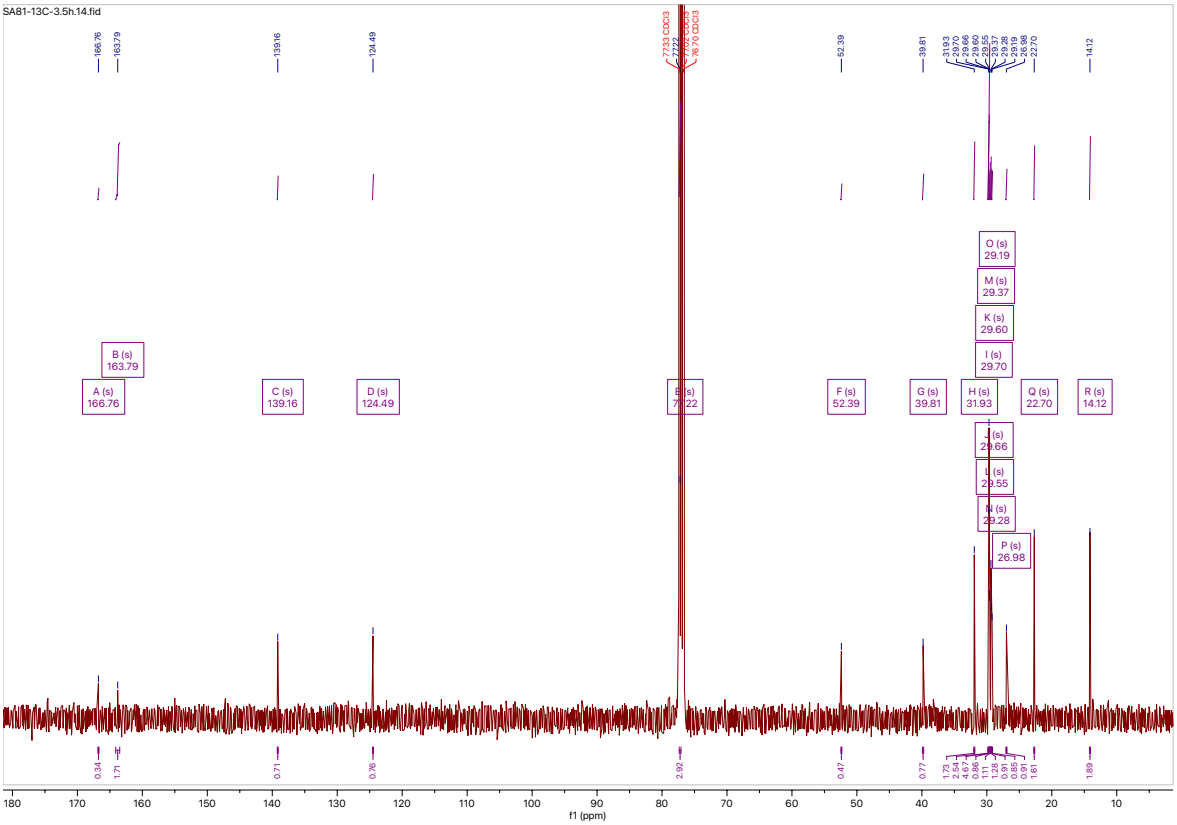
cyanomethyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (25)



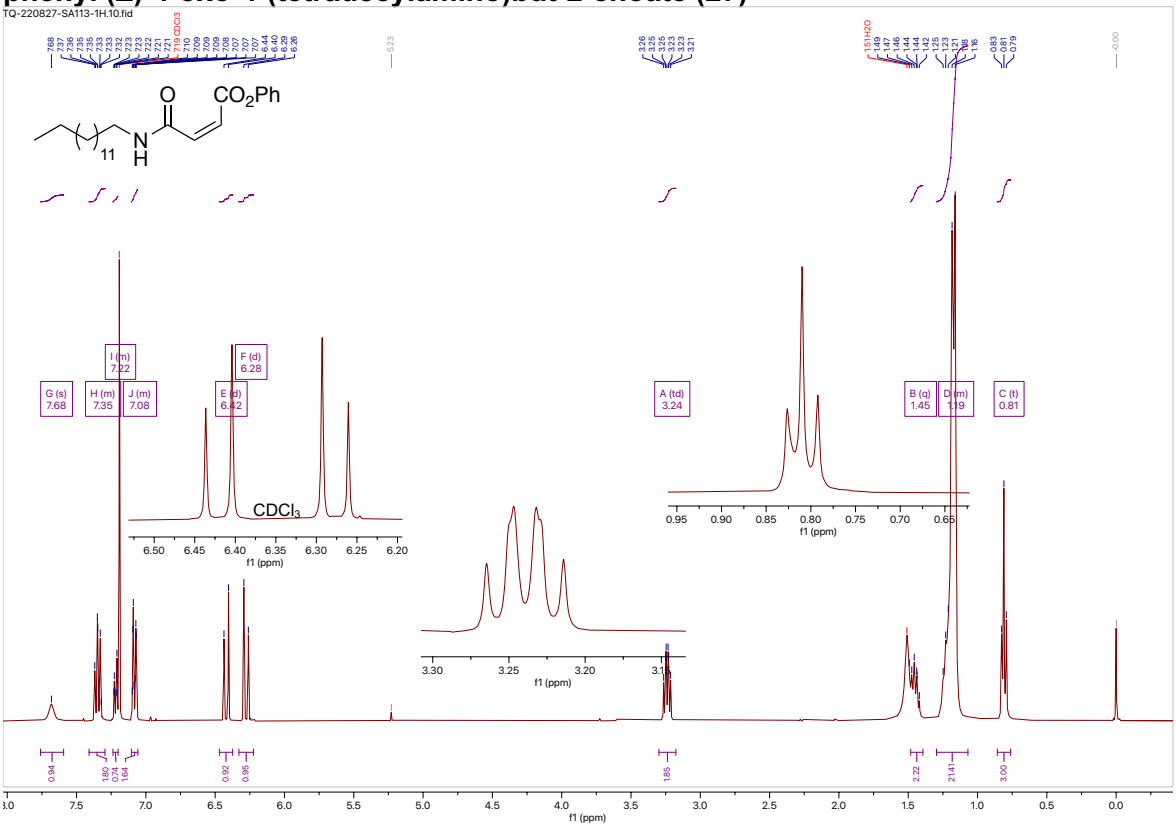


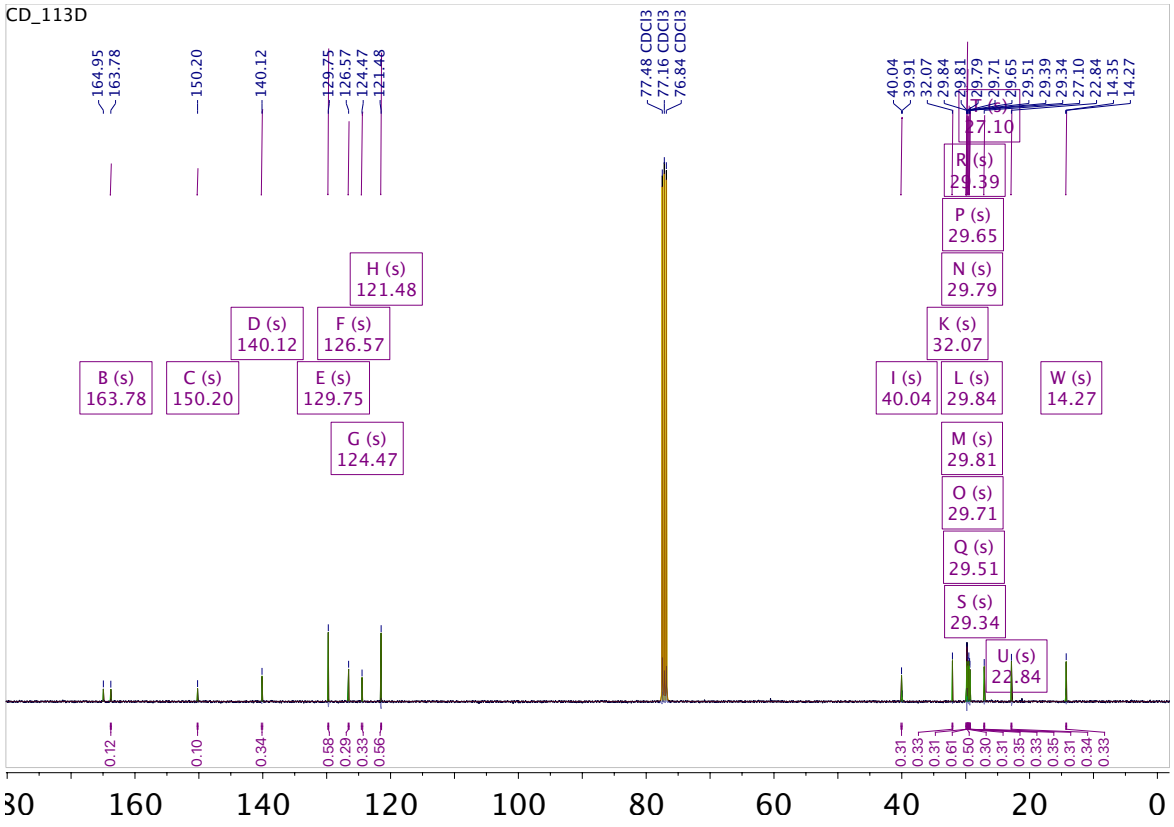
cyclopropylmethyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (26)



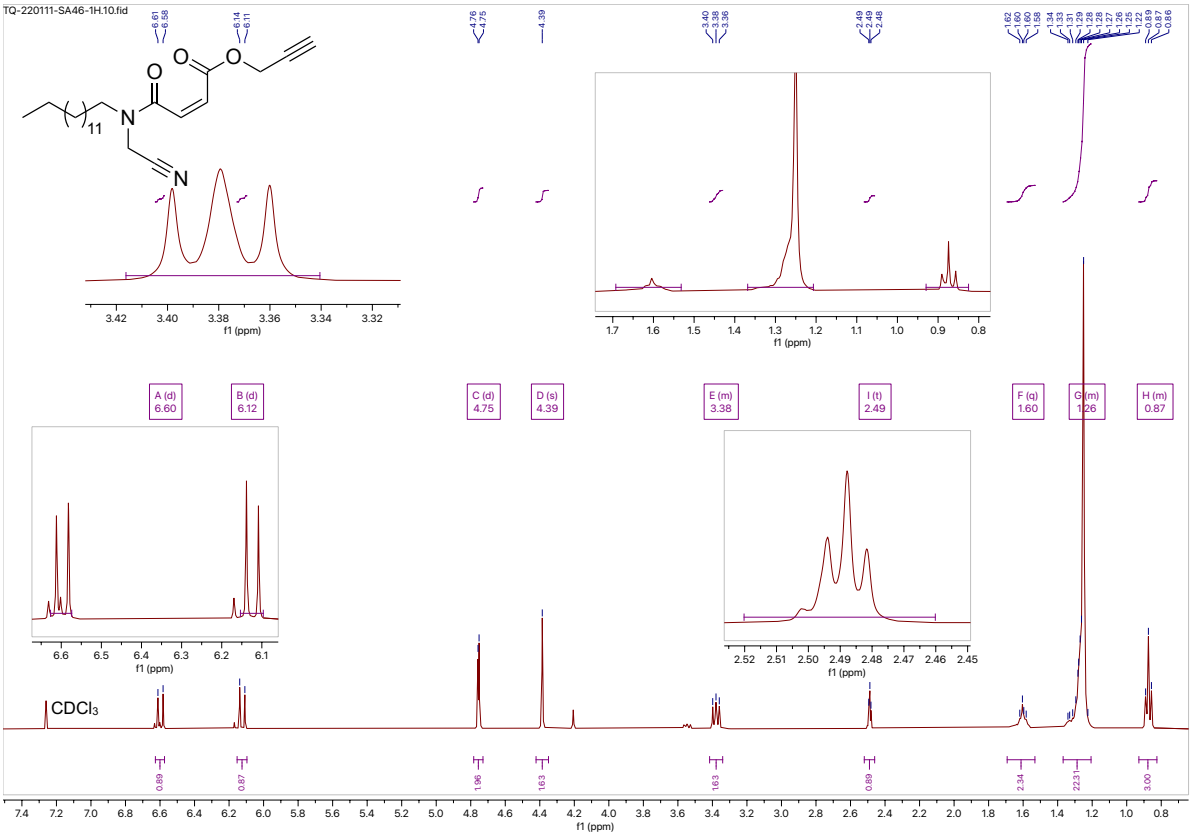


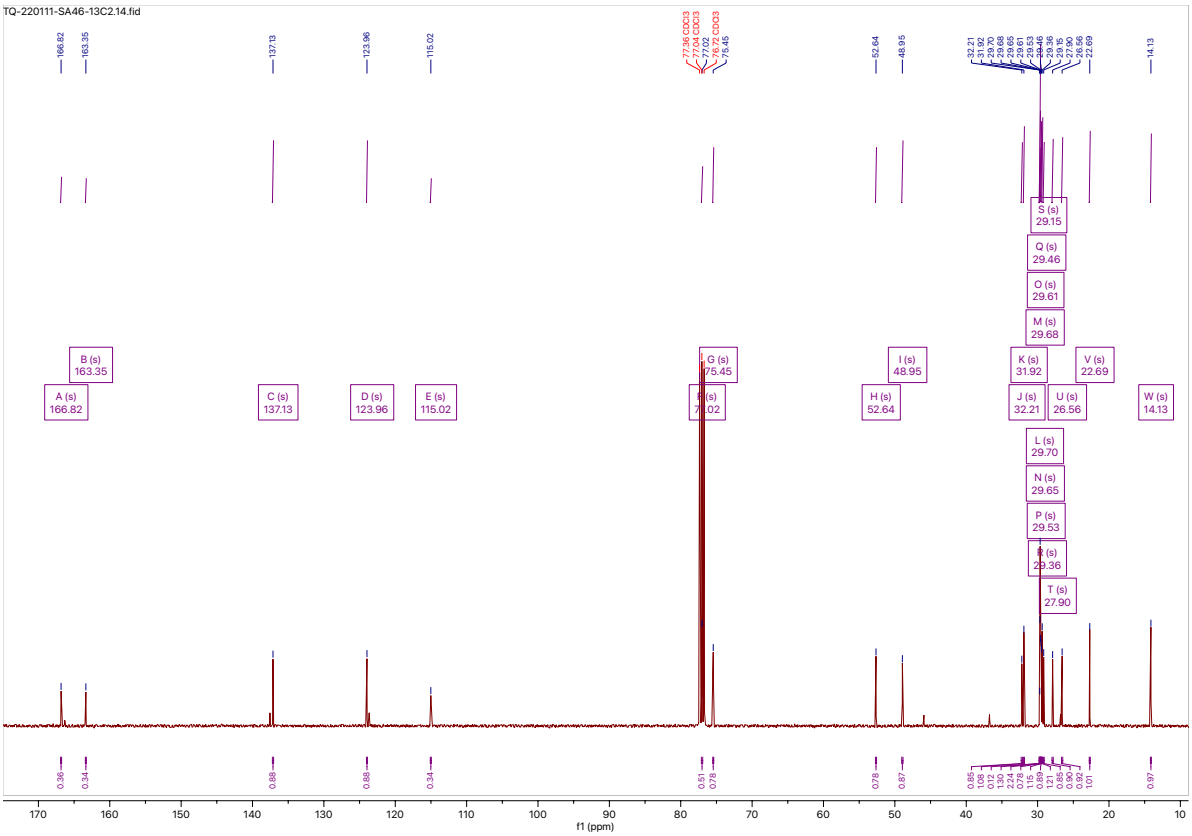
phenyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (27)



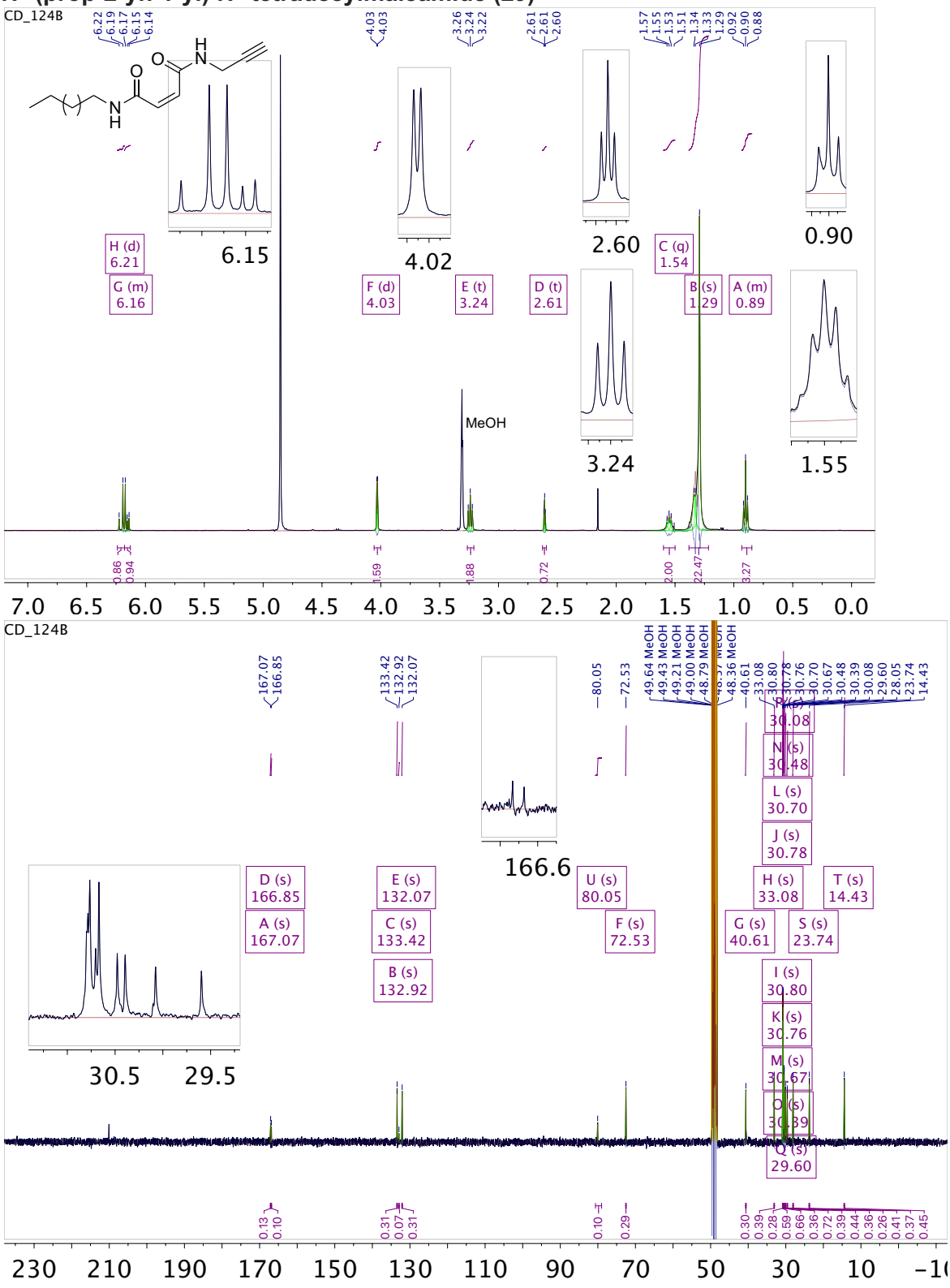


prop-2-yn-1-yl (*Z*)-4-((cyanomethyl)(tetradecyl)amino)-4-oxobut-2-enoate (28)

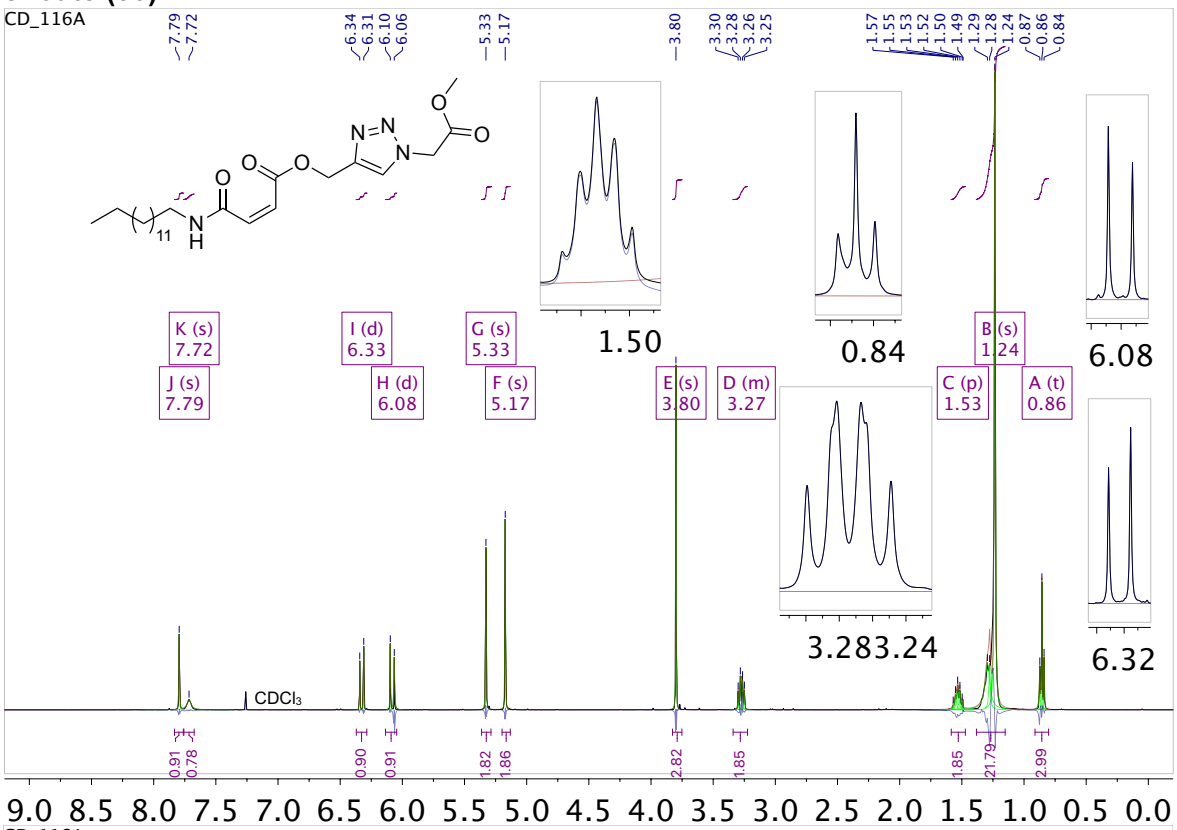


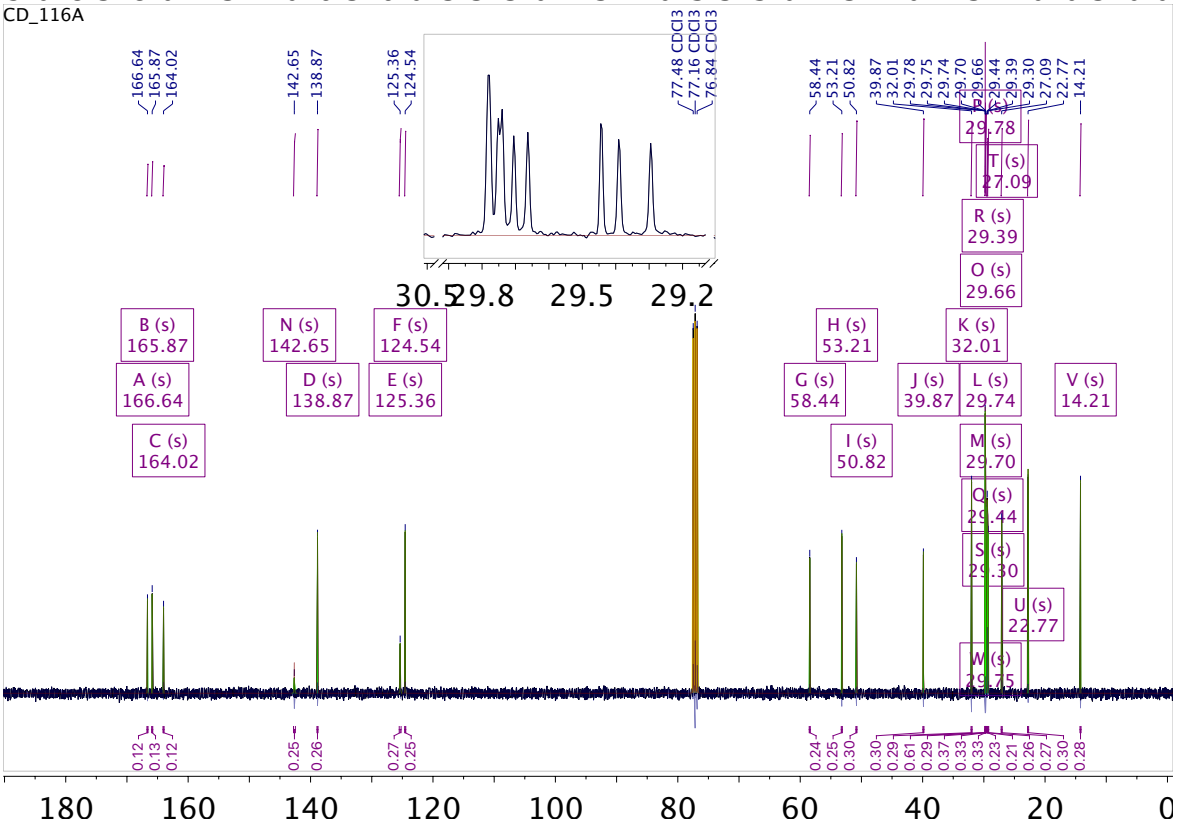


N^1-(prop-2-yn-1-yl)-N^4-tetradecylmaleamide (29)

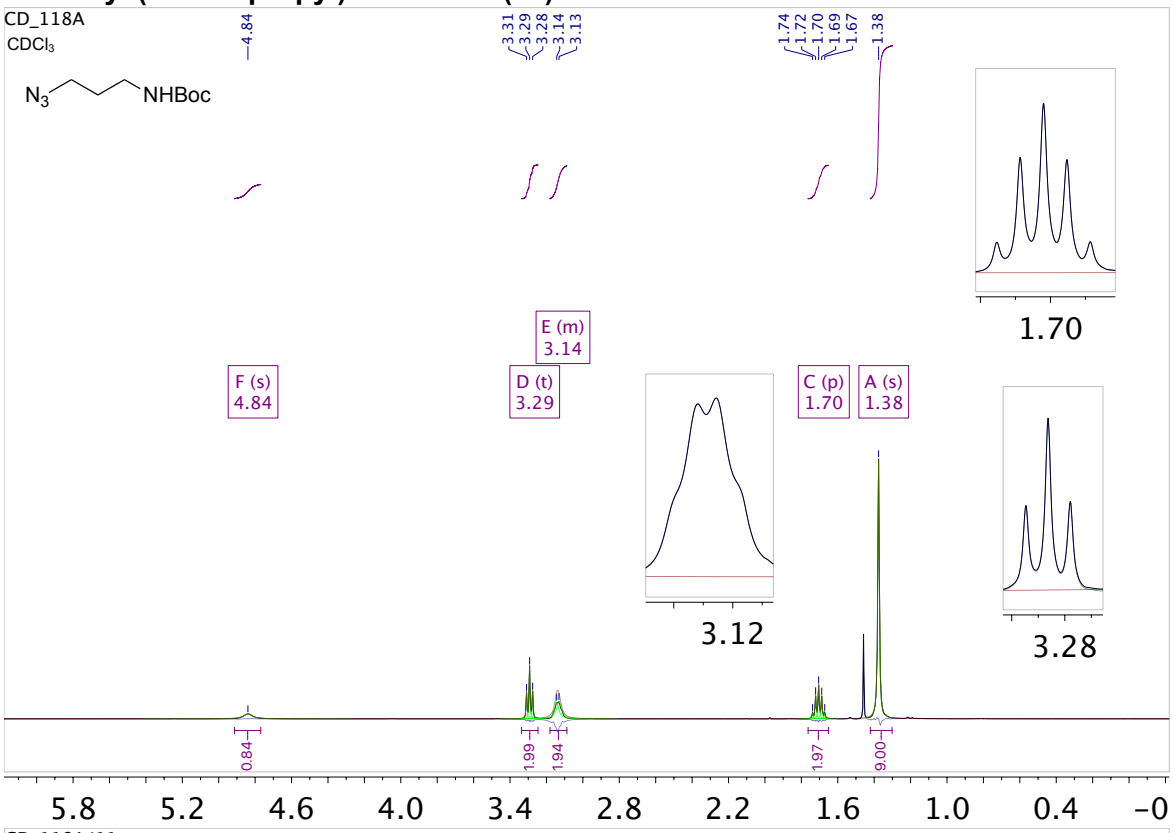


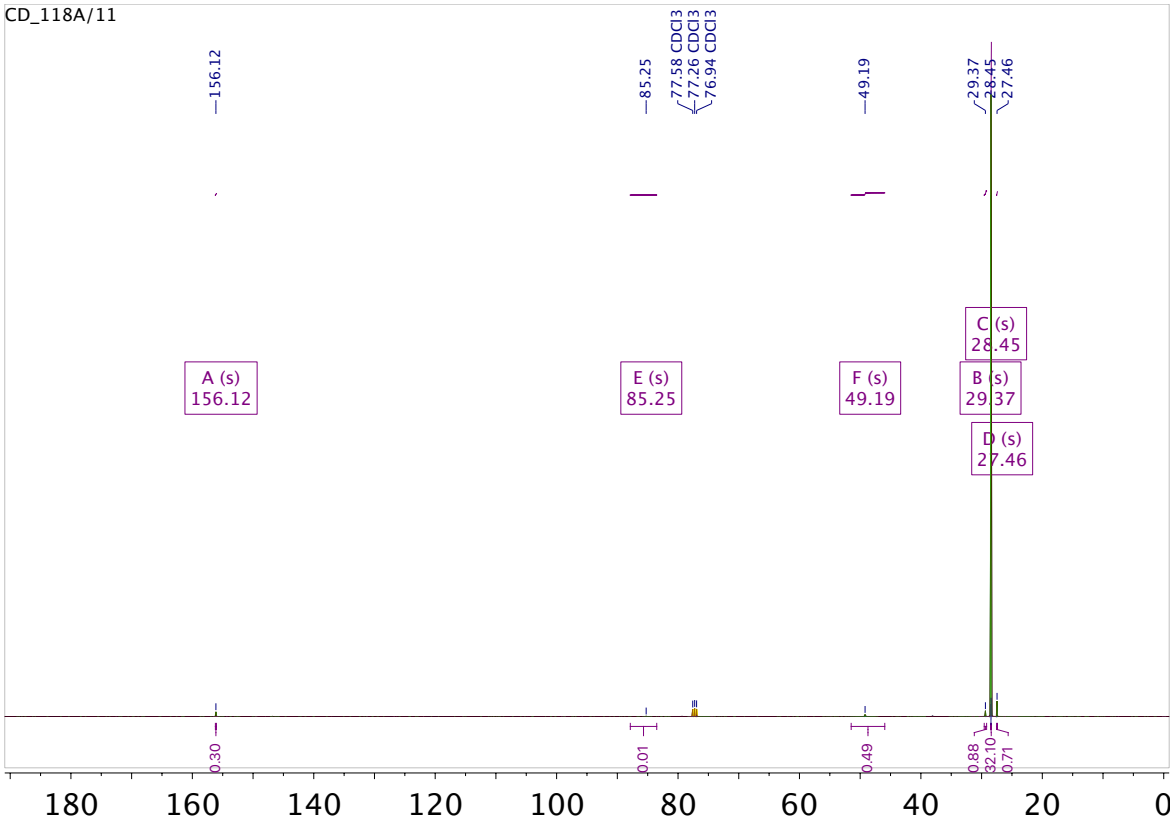
(1-(2-methoxy-2-oxoethyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (30)



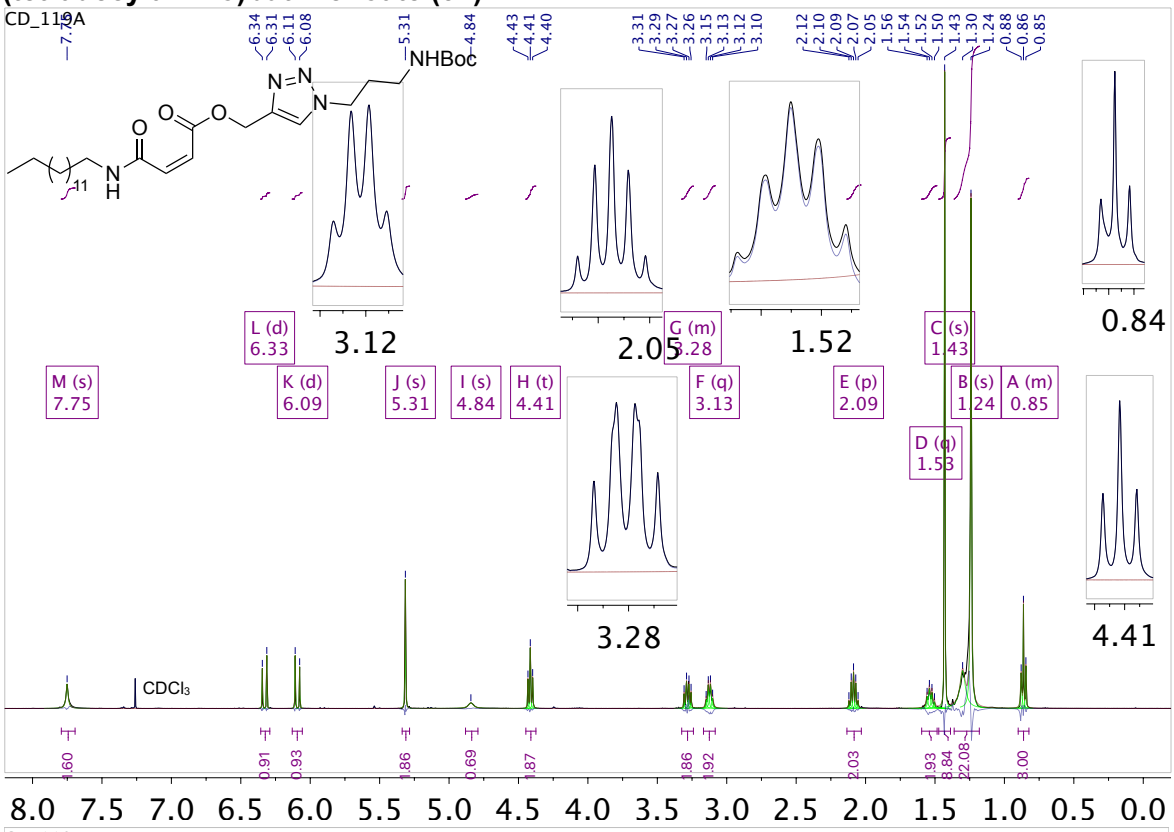


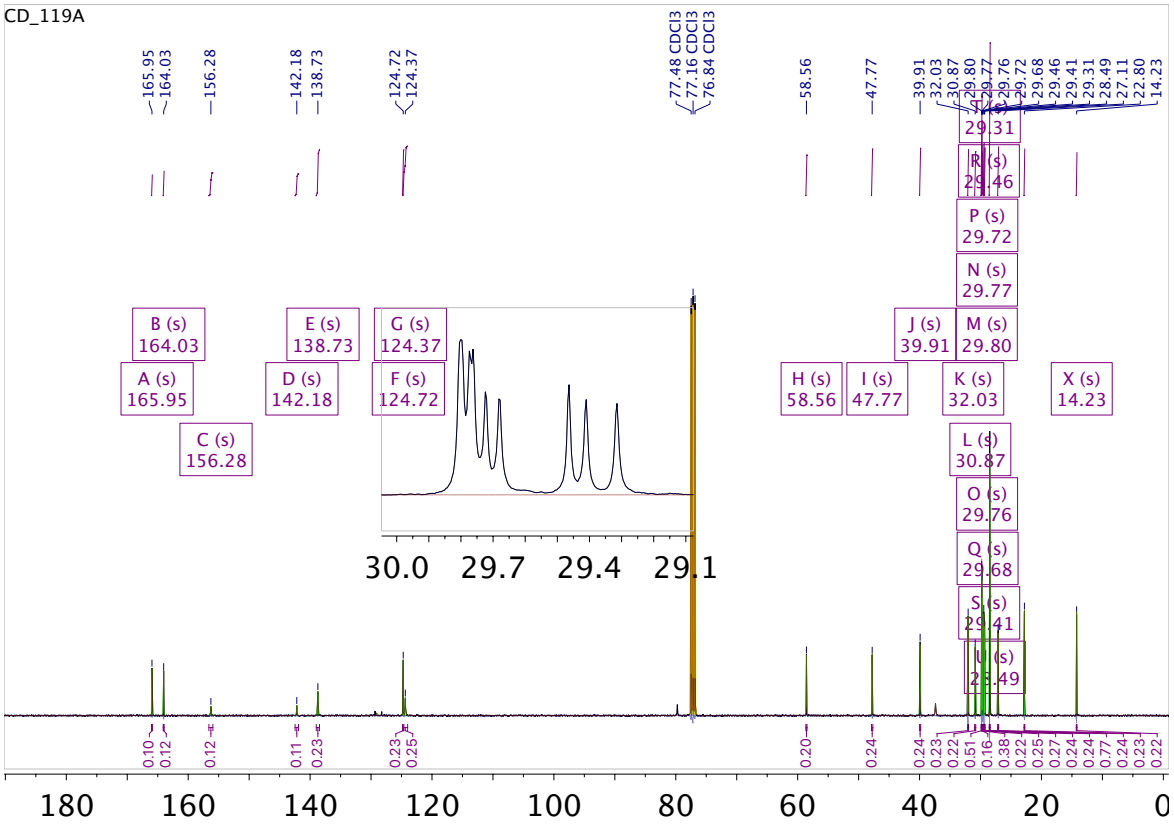
tert-butyl (3-azidopropyl)carbamate (57)



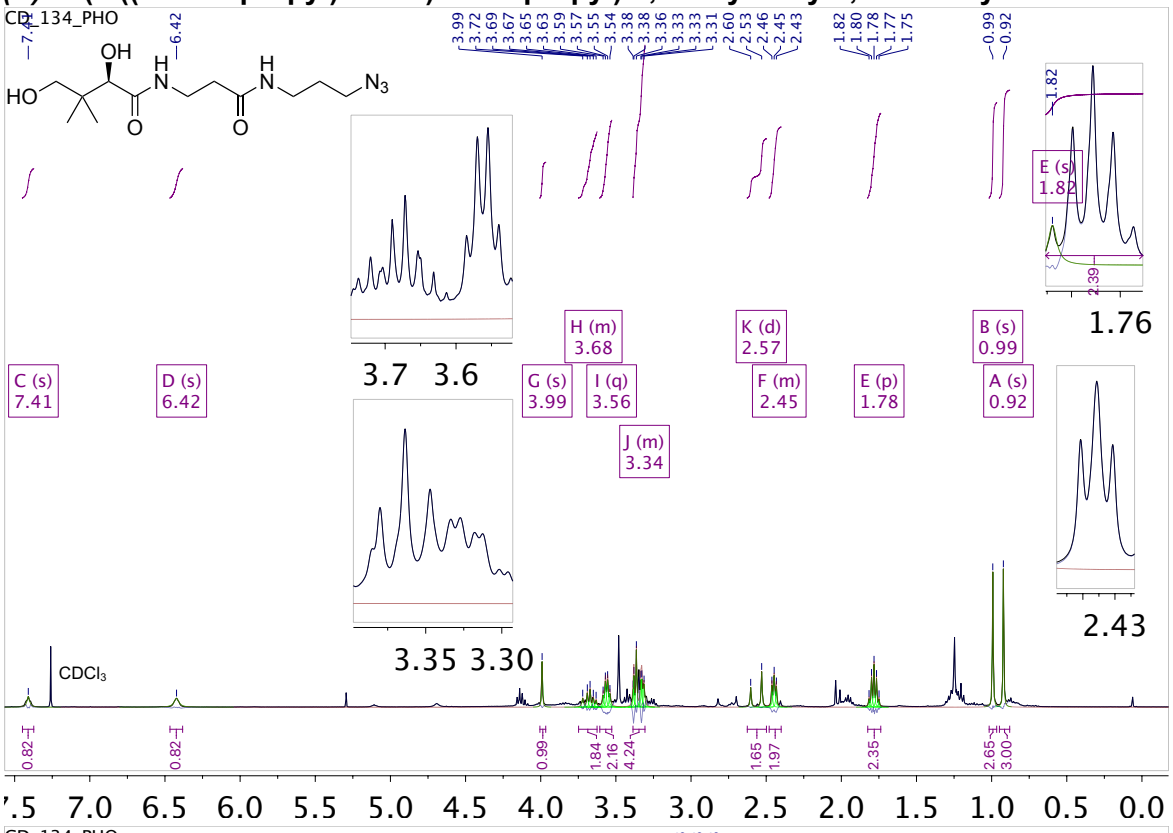


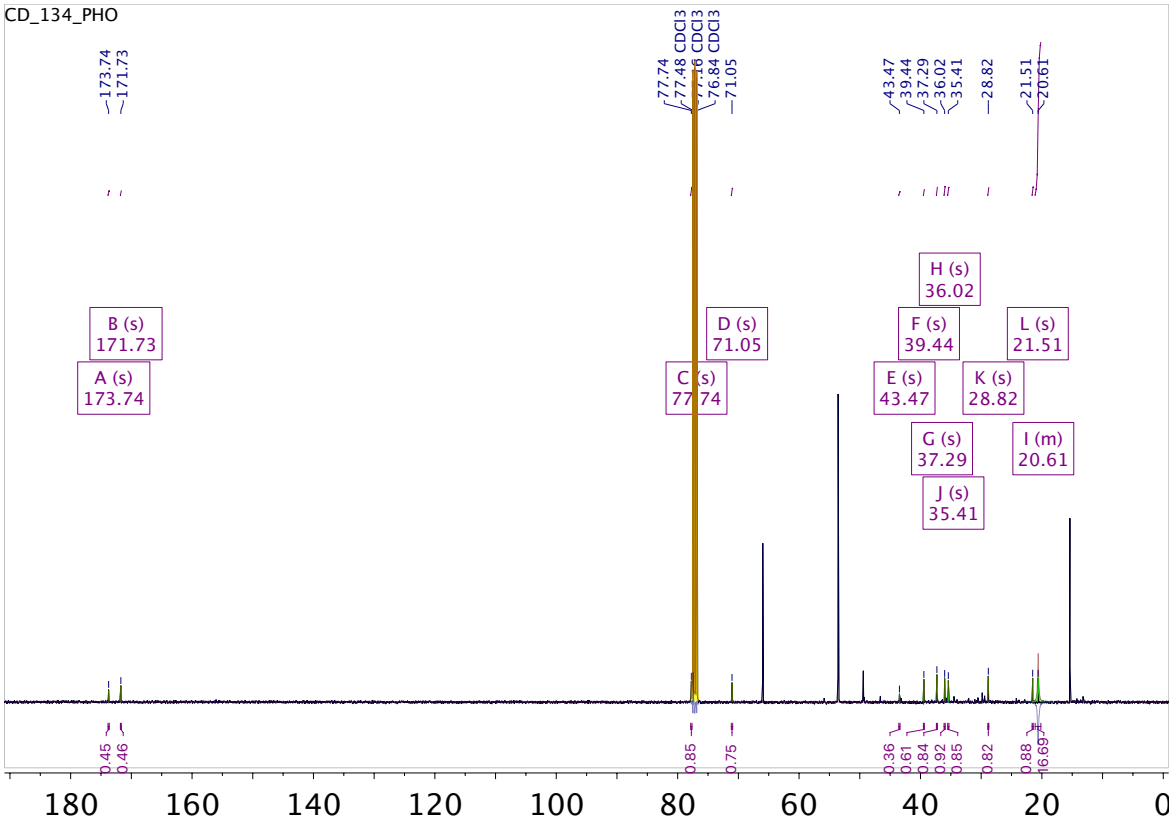
(1-(3-((*tert*-butoxycarbonyl)amino)propyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (31)



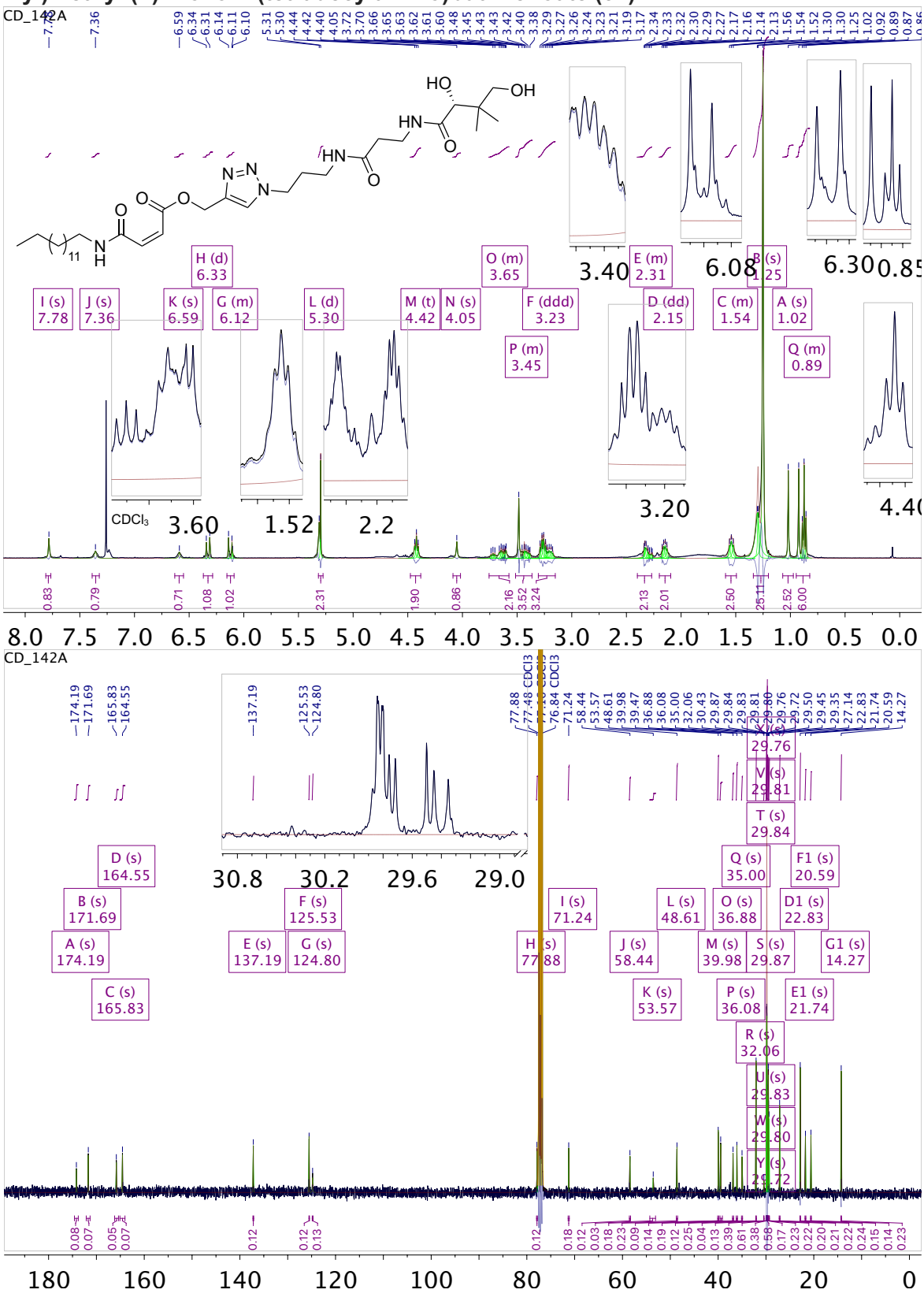


***(R)-N*-(3-((3-azidopropyl)amino)-3-oxopropyl)-2,4-dihydroxy-3,3-dimethylbutanamide (58)**

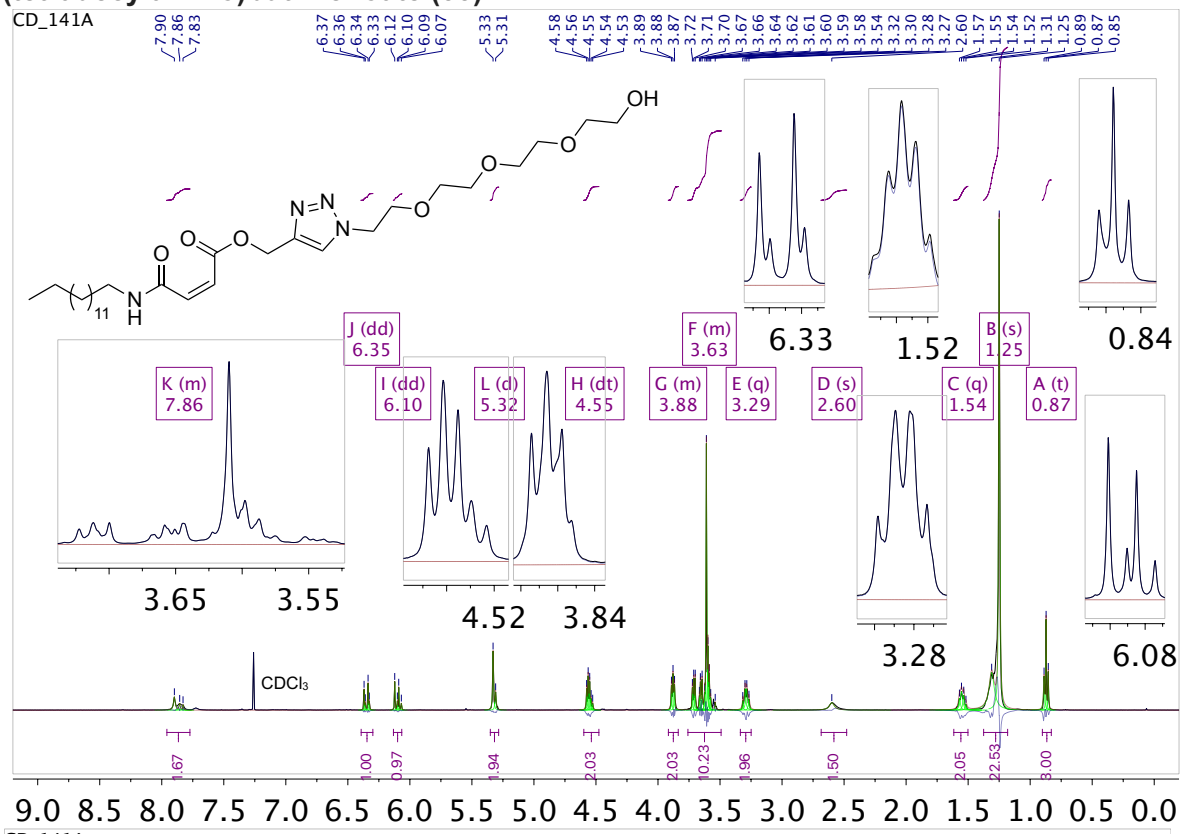


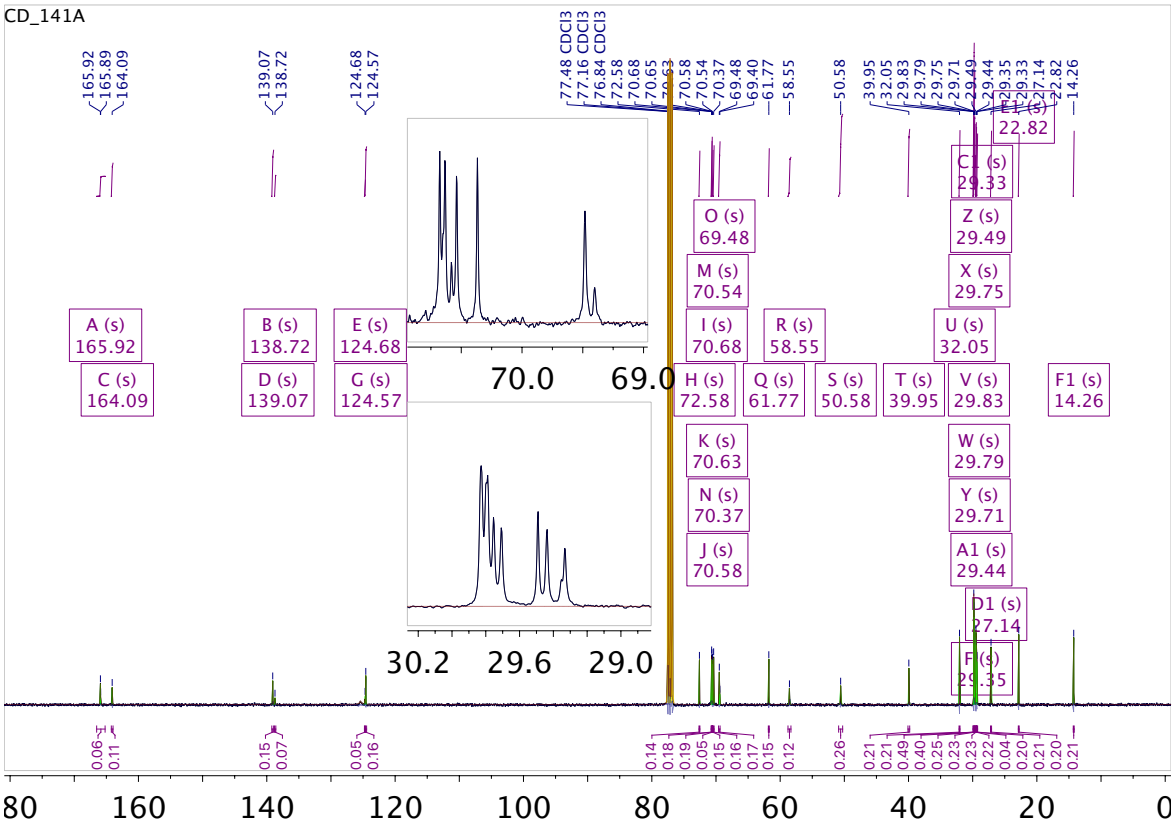


(*R*)-(1-(3-(3-(2,4-dihydroxy-3,3-dimethylbutanamido)propanamido)propyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (32)

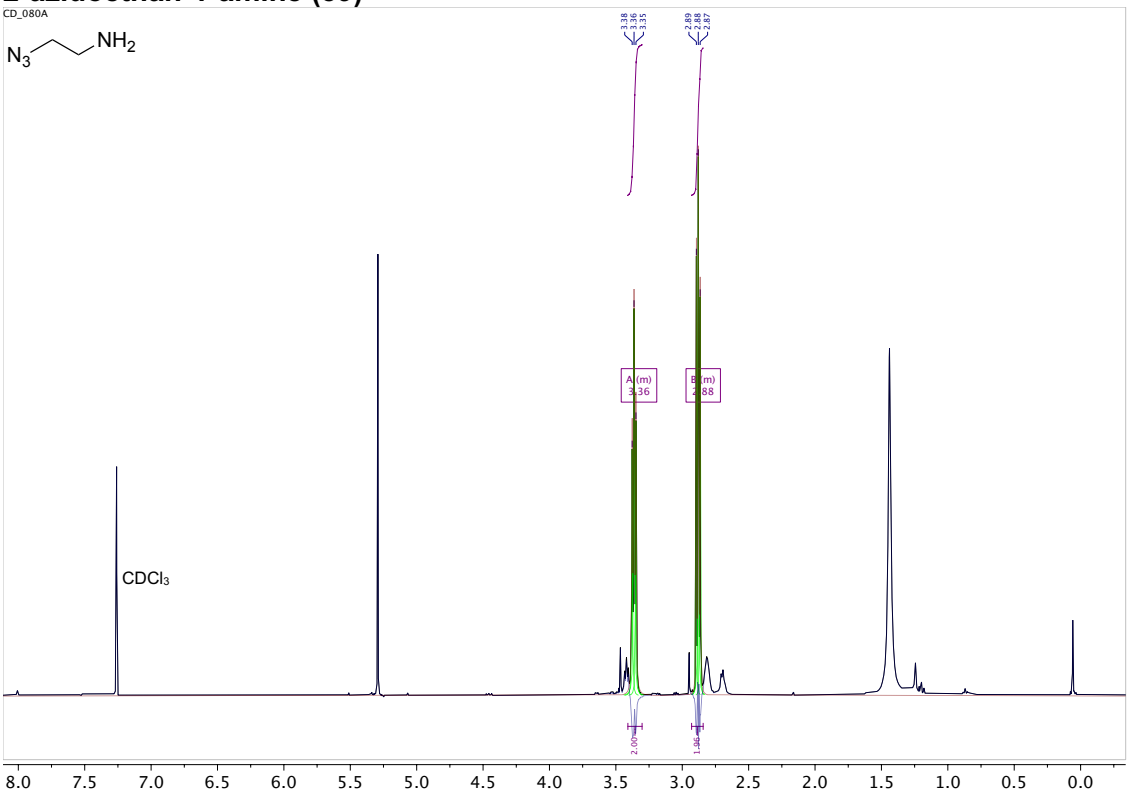


(1-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-1*H*-1,2,3-triazol-4-yl)methyl (*Z*)-4-oxo-4-(tetradecylamino)but-2-enoate (33)

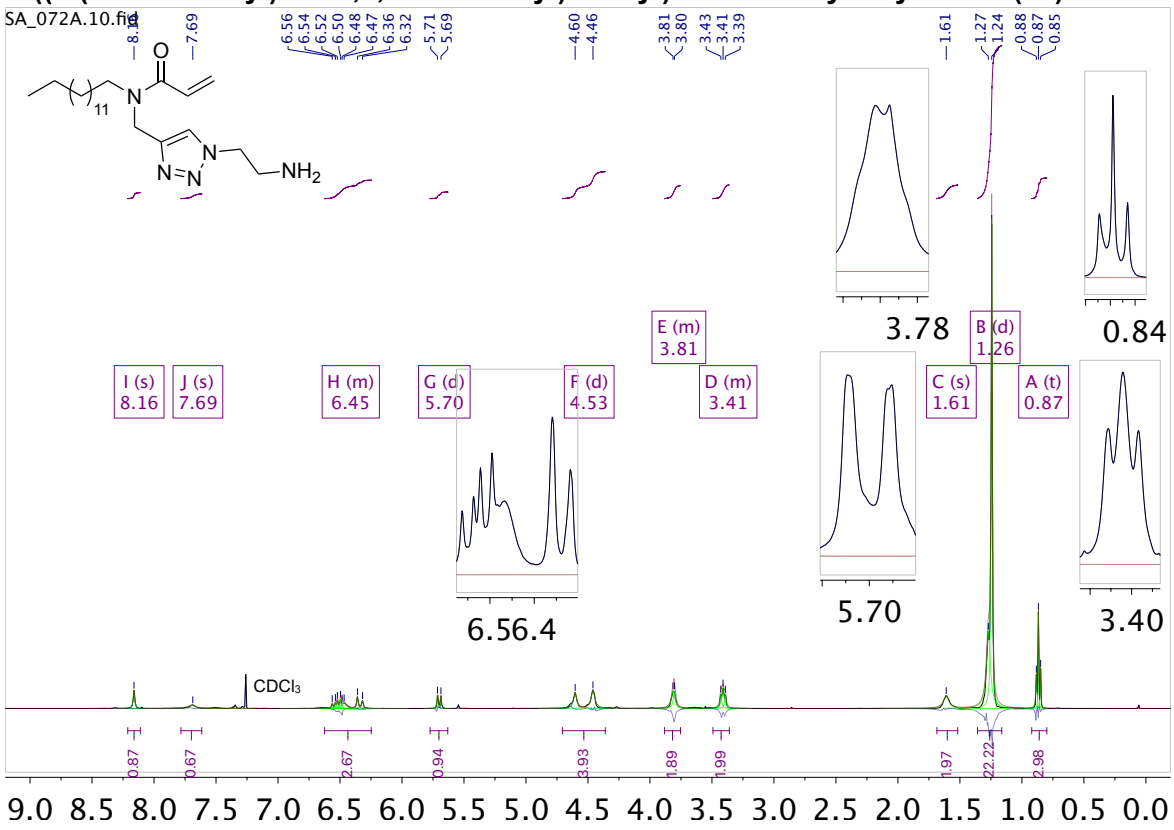




2-azidoethan-1-amine (59)



***N*-((1-(2-aminoethyl)-1*H*-1,2,3-triazol-4-yl)methyl)-*N*-tetradecylacrylamide (34)**



References

(1) Jumper, J.; Evans, R.; Pritzel, A.; Green, T.; Figurnov, M.; Ronneberger, O.; Tunyasuvunakool, K.; Bates, R.; Žídek, A.; Potapenko, A.; et al. Highly Accurate Protein Structure Prediction with AlphaFold. *Nature* **2021**, *596* (7873), 583–589.

(2) Varadi, M.; Anyango, S.; Deshpande, M.; Nair, S.; Natassia, C.; Yordanova, G.; Yuan, D.; Stroe, O.; Wood, G.; Laydon, A.; et al. AlphaFold Protein Structure Database: Massively Expanding the Structural Coverage of Protein-Sequence Space with High-Accuracy Models. *Nucleic Acids Res.* **2022**, *50* (D1), D439–D444.

(3) Azizi, S.-A.; Lan, T.; Delalande, C.; Kathayat, R. S.; Banales Mejia, F.; Qin, A.; Brookes, N.; Sandoval, P. J.; Dickinson, B. C. Development of an Acrylamide-Based Inhibitor of Protein S -Acylation . *ACS Chem. Biol.* **2021**, *16* (8), 1546–1556.

(4) Fasan, R. WO2015153761A2, 2015.

(5) Zaitceva, O.; Bénéteau, V.; Ryabukhin, D. S.; Eliseev, I. I.; Kinzhalov, M. A.; Louis, B.; Vasilyev, A. V.; Pale, P. Cyclization of Aryl 3-Aryl Propynoates into 4-Arylcoumarins Catalyzed by Cyclometalated Platinum(II) Complexes. *Tetrahedron* **2020**, *76* (14), 131029.

(6) Fabre, B.; Pícha, J.; Vaněk, V.; Selicharová, I.; Chrudinová, M.; Collinsová, M.; Žáková, L.; Buděšínský, M.; Jiráček, J. Synthesis and Evaluation of a Library of Trifunctional Scaffold-Derived Compounds as Modulators of the Insulin Receptor. *ACS Comb. Sci.* **2016**, *18* (12), 710–722.