Supporting Information

Labelling and Natural Posttranslational Modification of Peptides and Proteins via Chemoselective Pd-Catalyzed Prenylation of Cysteine

Thomas Schlatzer,[a] Julia Kriegesmann,[b] Hilmar Schröder,[a] Melanie Trobe,[a]

Christian Lembacher-Fadum,[a] Simone Santner,[a] Alexander V. Kravchuk,[b]

Christian F. W. Becker*[b] and Rolf Breinbauer*[a]

[a] Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 9, A-8010 Graz, Austria
[b] Institute of Biological Chemistry, Faculty of Chemistry, University of Vienna, Währinger Strasse 38, A-1090 Vienna, Austria

*Correspondence to: christian.becker@univie.ac.at and breinbauer@tugraz.at

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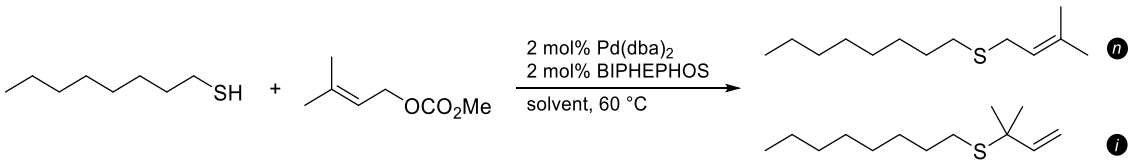
Reversibility of the Prenylation

Table S1. Time dependence of the *n*/*i* ratio during the Pd-catalyzed prenylation of 1-octanethiol.



Entry	Time [h]	CH_3CN		*tert*-BuOH/H_2O = 3/1	
		conv. [%][a]	*n*/*i* [a]	conv. [%][a]	*n*/*i* [a]
1	0.5	>99	89/11	>99	89/11
2	2	>99	94/6	>99	90/10
3	5	>99	99/1	n.d.	n.d.
4	18	n.d.	n.d.	>99	96/4
5	24	>99	>99/1	>99	98/2
6	48	n.d.	n.d.	>99	>99/1

[a] Conversions as well as *n*/*i* ratios were determined by GC-MS without internal standard. n.d. = not determined

Confirmation of *S*-Selectivity

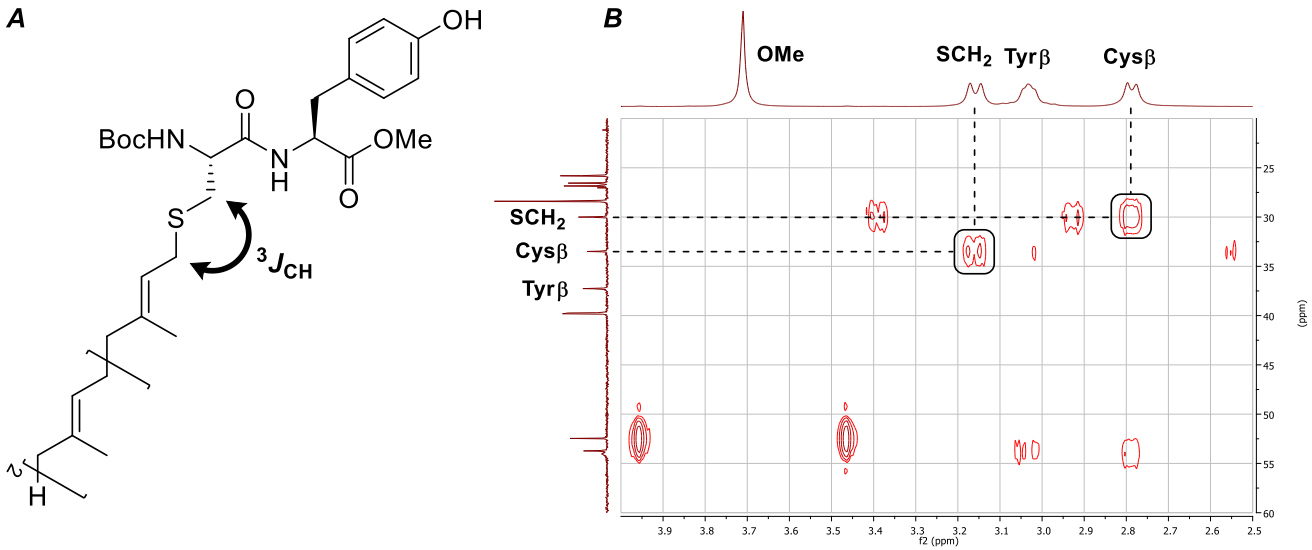
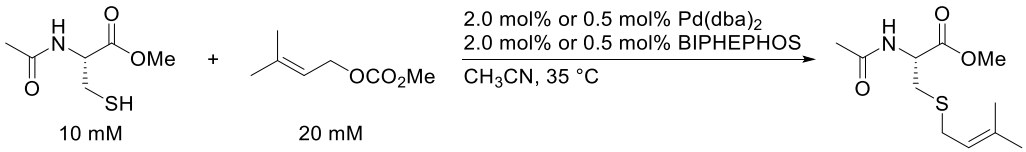


Figure S1. ***A*** Structure of farnesylated Boc-Cys-Tyr-OMe with observed $^3J_{CH}$ HMBC couplings unambiguously confirming the Cys-selectivity of the Pd-catalyzed farnesylation. ***B*** HMBC-spectrum (300 MHz, $CDCl_3$) with relevant crosspeaks highlighted.

Kinetics of the Pd-Catalyzed Prenylation



To ensure catalyst integrity during reaction monitoring the reactions were carried out in a glovebox.

In a 10 mL vial, equipped with a Teflon-coated magnetic stirring bar, an aliquot (● 400 µL / ■ 100 µL) of a stock solution of $Pd(dba)_2$ (4.6 mg, 8.0 µmol) and BIPHEPHOS (6.4 mg, 8.1 µmol) in 8.0 mL CH_3CN, preformed at 60 °C for 30 min, was diluted with CH_3CN (● 1200 µL / ■ 1500 µL). Then 200 µL of a 200 mM solution of prenyl methyl carbonate in CH_3CN and 200 µL of a 100 mM solution of AcCysOMe in CH_3CN were added. The reaction solution was stirred at 35 °C. HPLC-samples were diluted 1:5 using 15 mM 1-octanethiol (quenching agent) in CH_3CN and stored at -30 °C until the samples were removed from the glovebox for HPLC-analysis (HPLC-vials were purged with air immediately after removal from the glovebox).

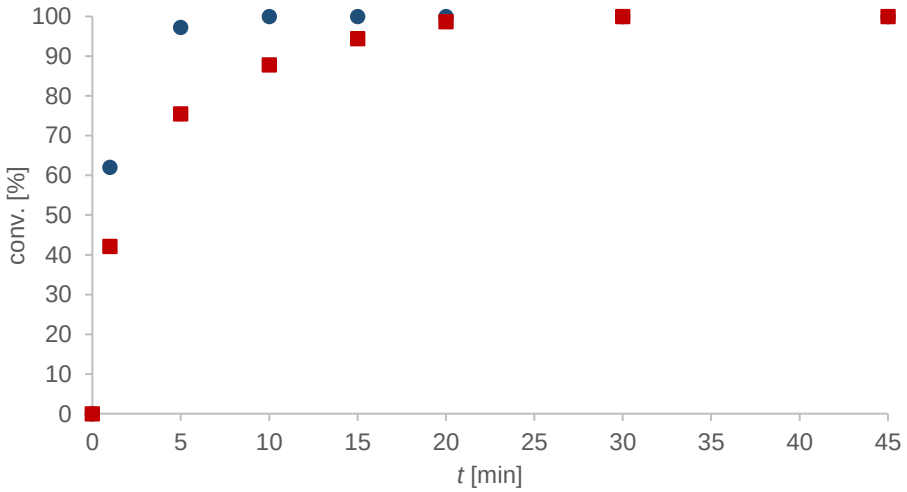


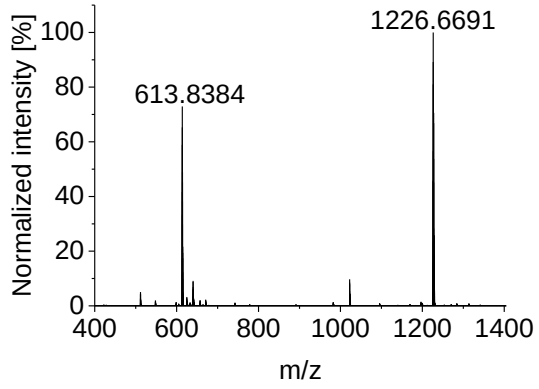
Figure S2. Kinetics of the Pd-catalyzed prenylation of AcCysOMe using 2.0 mol% (●) and 0.5 mol% (■) catalyst, respectively. The conversion was calculated from the ratio between integrals of the starting material and product at 210 nm.

Data Tryptic Digest

For the tryptic digest 0.1 mg of the modified peptides was dissolved in 30 µL 50 mM Tris pH 8.1 with 10 mM $CaCl_2$ and 1 µL trypsin (from porcine pancreas, proteomics grade, Sigma, 1 mg/mL in 1 mM HCl) was added. The digest was performed at 37 °C for 6 h at 300 rpm. The digested peptide samples were lyophilized and stored at -20 °C. LC-MS/MS was measured using a Dionex Ultimate 3000 nano HPLC-system coupled to a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific). The peptides were loaded on a µ-Precolumn (5 mm x 300 µm C4 PepMap300, 5 µm, 300 Å, Thermo Scientific) and then eluted to an analytical column (50 cm x 75 µm Accucore C4, 2.6 µm, 150 Å, Thermo Scientific). The runs were performed at 300 nL/min with 2 % ACN, 98 % ddH_2O, 0.1 % formic acid as buffer A and 80 % ACN, 20 % ddH_2O, 0.1 % formic acid as buffer B with the following gradient: 10 min 2 % B, 7-35 % B in 29 min, 35-40 % B in 2 min. MS scans were acquired in positive ion mode at a range of 400-1400 m/z at a resolution of 60000 (FWHM at 400 m/z). MS/MS scans of the 4 most abundant ions were performed using CID fragmentation at 35 % normalized collision energy.

GQRNREKTGESNSC(Farnesyl)VIL **calcd.: [M(Farnesyl)+H]$^+$: 1226.6701**

[M(Farnesyl)+2H]$^{2+}$: 613.8387



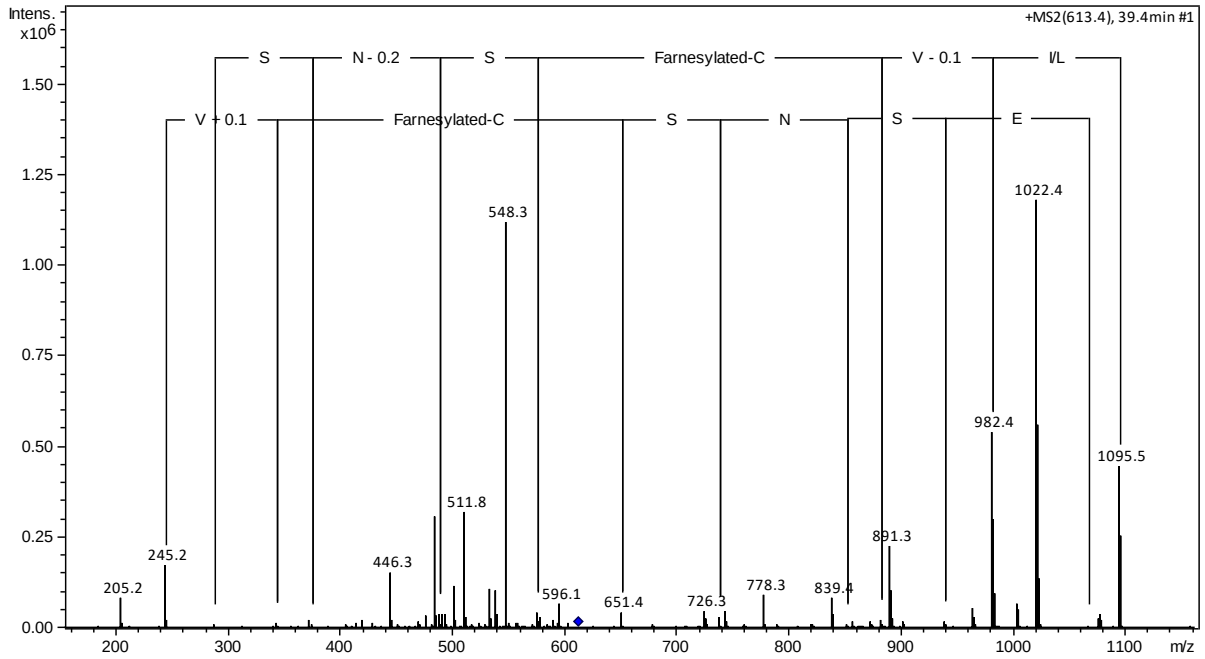
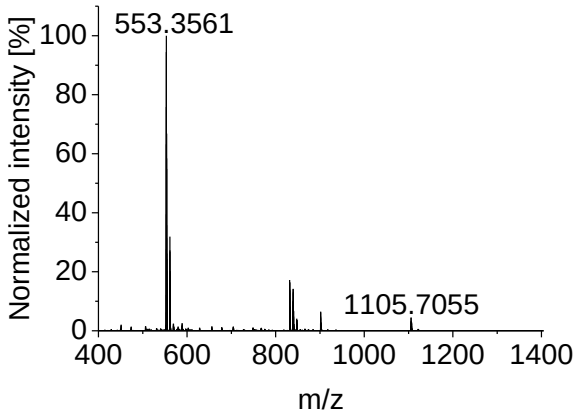


Figure S3. LC-MS/MS results (MS1 and MS2) of two farnesylated peptides. The analyzed fragment of the digested peptides is shown in red.

MGIINTLQKYYARVRGGRC(Farnesyl)AVLSALPKEEQIG **calcd.:** **[M(Farnesyl)+H]$^{+}$: 1105.7054**

[M(Farnesyl)+2H]$^{2+}$: 553.3563



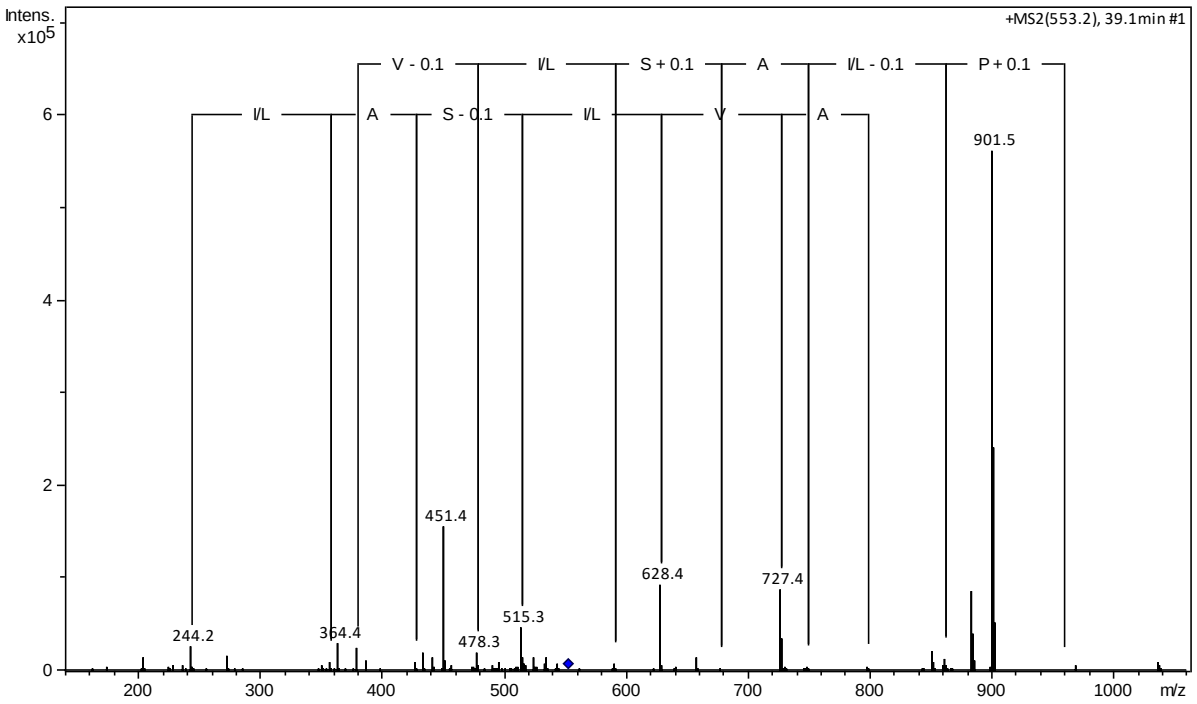


Figure S3 (cont'd). LC-MS/MS results (MS1 and MS2) of two farnesylated peptides. The analyzed fragment of the digested peptides is shown in red.

Circular Dichroism

For the circular dichroism measurements a Chirascan plus CD-spectrophotometer (Applied Photophysics) was used. The measurements were performed at 20-22 °C from 180 to 280 nm in 1 nm steps. Each spectrum was obtained by the average of three measurements and subtraction of the background. Protein concentrations between 0.1 and 0.6 mg/mL in 50 mM potassium phosphate buffer pH 7.0 were measured in a 1.0 mm micro cuvette. The raw data were exported as CSV files and processed using BeStSel and OriginPro.

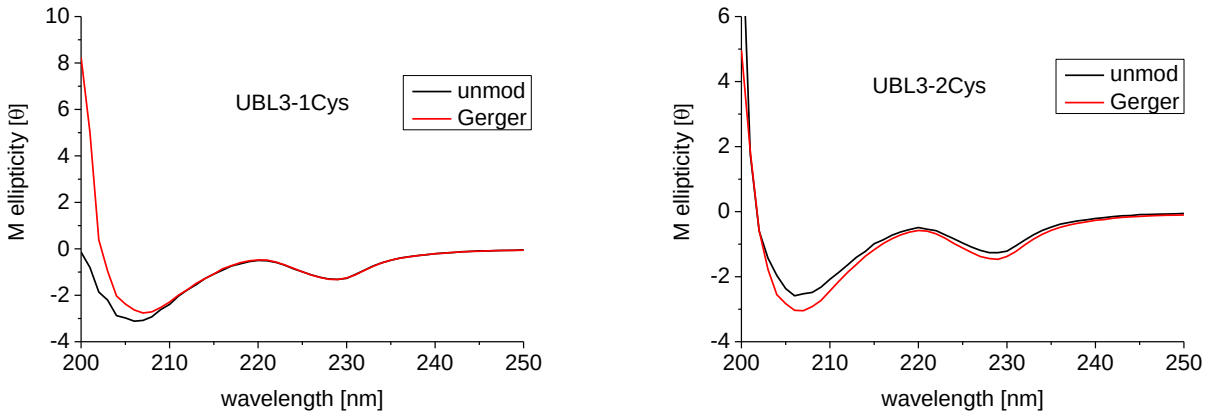


Figure S4A. CD measurements of unmodified and geranylgeranylated ("Gerger") UBL3. Protein concentrations: UBL3-1Cys-unmod: 0.4 mg/mL, UBL3-1Cys-Gerger: 0.6 mg/mL, UBL3-2Cys-unmod: 0.5 mg/mL, UBL3-2Cys-Gerger: 0.5 mg/mL.

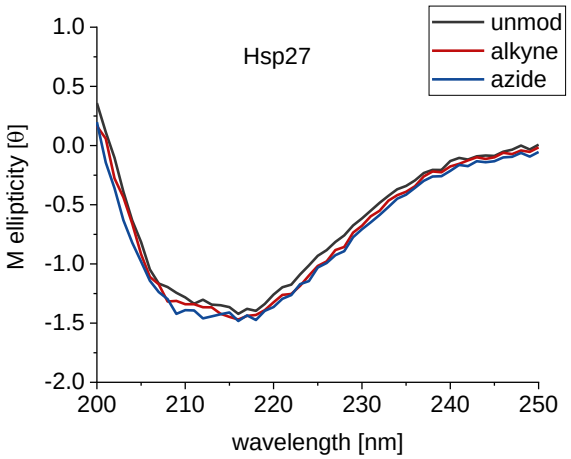


Figure S4B. CD measurements of unmodified and modified Hsp27. Protein concentrations: 0.1 mg/mL.

Farnesylation of Hsp27

Preparation of stock solutions: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) were suspended in 2.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution and cooled to rt. To this solution, **Rc** (0.67 mg, 2.4 µmol) was added (= **stock A**).

In an evacuated and argon-flushed 10 mL Schlenk flask, Hsp27 (5.7 mg, 0.25 µmol) was dissolved in 0.25 mL degassed ddH_2O (= **stock B**).

Reaction: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, 0.25 mL of **stock A** were heated to 40 °C before 0.25 mL of **stock B** were added. The resulting mixture was stirred at 40 °C (reaction monitoring via HPLC(-MS): 30-40 % conv. after 7 h).

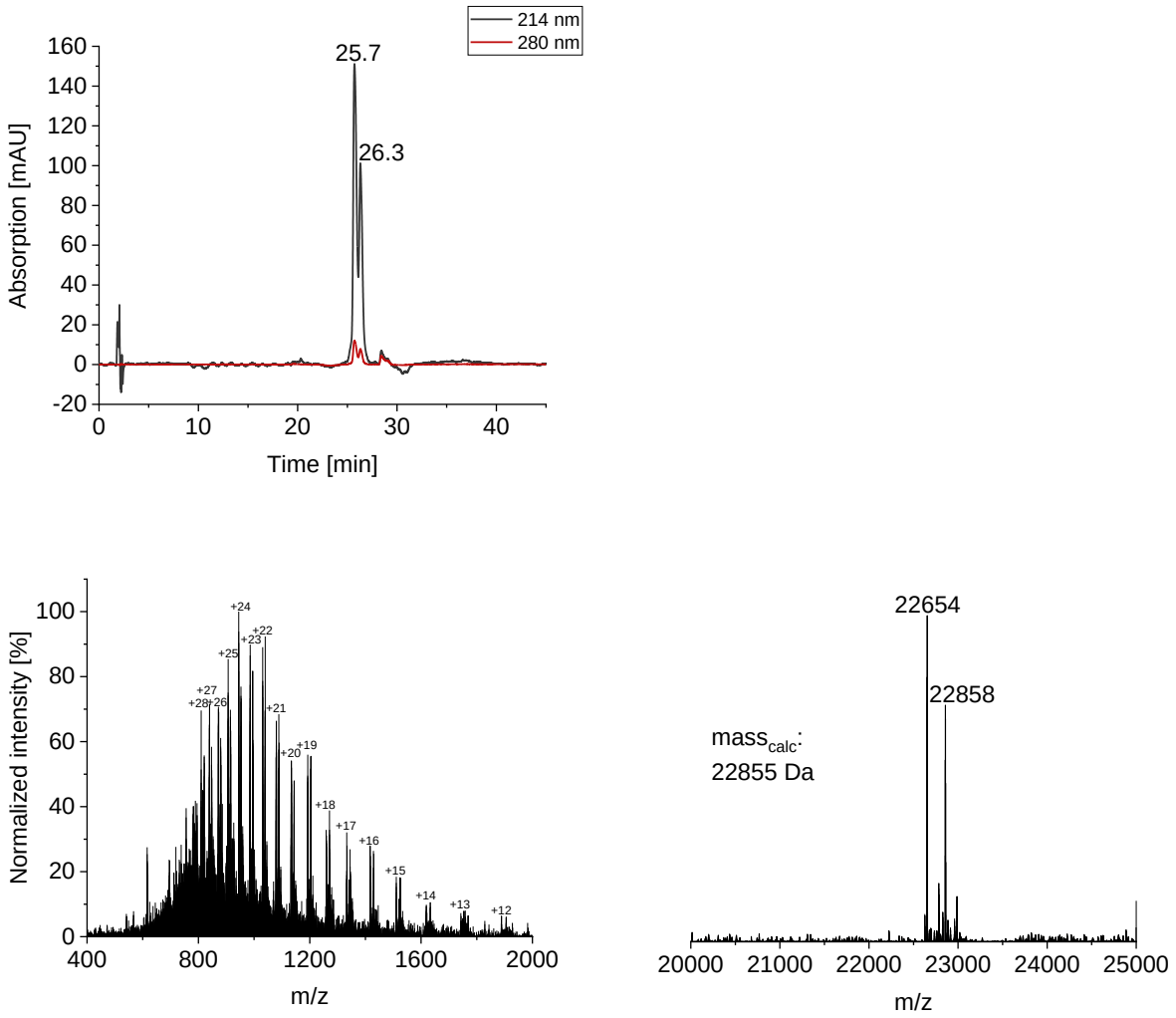


Figure S5. HPLC(-MS) data of farnesylated Hsp27 after 7 h.

General Information

If reactions were performed under inert conditions, e.g. exclusion of water, oxygen or both, all experiments were carried out using established Schlenk techniques or inside a Glovebox (MBraun UNIlab pro). Herein solvents were dried and/or degassed with common methods and afterwards stored under inert gas atmosphere (argon or nitrogen) over molecular sieves. In some cases, when explicitly mentioned, dry solvents were received from the mentioned suppliers. In general, when high vacuum (*in vacuo*) was stated in experimental procedures, typically a vacuum of 10^{-2}-10^{-3} mbar was applied. Degassing of solvents or reaction mixtures was performed by bubbling argon from a balloon via cannula through the solvent or the reaction mixture during ultrasonication for about 20 min. All reagents were added in a counterstream of inert gas to keep the inert atmosphere. All reactions were stirred with Teflon-coated magnetic stirring bars.

Molecular sieves (Sigma-Aldrich, beads with 8-12 mesh) were activated in a round-bottom flask with a gas inlet adapter by heating them carefully in a heating mantle at level 1 at least for 24 h under high vacuum until complete dryness was obtained. These activated molecular sieves were stored at rt under argon atmosphere.

Temperatures were measured externally if not otherwise stated. When working at a temperature of 0 °C, an ice-water bath served as the cooling medium. Lower temperatures were achieved by using an acetone/dry ice cooling bath. Reactions, which were carried out at higher temperatures than rt, were heated in a silicon oil bath on a heating plate (RCT basic IKAMAG® safety control, 0-1500 rpm) equipped with an external temperature controller.

Chemicals

All commercially available chemicals and solvents were purchased from Acros Organics, Alfa Aesar, Fisher, Fluka, Honeywell, Merck, Roth, Sigma-Aldrich, TCI, VWR and used without further purification, unless otherwise stated. Protected Fmoc-amino acids, resins and coupling reagents were purchased from Novabiochem and Iris. The medium and buffers for protein expression and purification were prepared with substances from Roth, Sigma-Aldrich and PanReac AppliChem.

Acetonitrile: Anhydrous acetonitrile was purchased from Alfa Aesar. It was transferred into an amber 1 L Schlenk bottle and stored over activated 3 Å MS under argon atmosphere.

Dichloromethane: Anhydrous dichloromethane was produced by pre-drying EtOH stabilized dichloromethane over P_4O_{10} and afterwards heating it under reflux over CaH_2 for 24 h under argon atmosphere. It was distilled into an amber 1 L Schlenk bottle over activated 4 Å MS and under argon atmosphere.

N,*N*-Dimethylformamide: *N*,*N*-Dimethylformamide was purchased in extra dry quality from Alfa Aesar. It was transferred into an amber 1 L Schlenk bottle and stored over activated 4 Å MS under argon atmosphere.

Methanol: Methanol was purchased from Fisher and heated under reflux over Mg and I_2 for 2 h. It was distilled into an amber 1 L Schlenk bottle and stored over activated 3 Å MS under argon atmosphere.

Solvents for peptide synthesis and chromatography were of “peptide synthesis grade” or “HPLC grade”.

Thin Layer Chromatography

Analytical thin layer chromatography (TLC) was carried out on Merck TLC silica gel aluminum sheets (silica gel 60, F254, 20 x 20 cm). All separated compounds were visualized by UV light (λ = 254 nm and/or λ = 366 nm) and by the listed staining reagents followed by the development in the heat.

$KMnO_4$: 3.0 g $KMnO_4$ as well as 20 g K_2CO_3 were dissolved in 300 mL H_2O and afterwards 5.0 mL 5 % aq. NaOH were added.

CAM: 50 g $(NH_4)_6Mo_7O_{24}$, 2.0 g $Ce(SO_4)_2$ and 50 mL conc. H_2SO_4 were dissolved in 400 mL water.

Ninhydrin: 1.5 g ninhydrin were dissolved in 100 mL *n*-butanol and then 3.0 mL AcOH were added.

DMACA: 0.1 % *p*-dimethylaminocinnamaldehyde (DMACA) were dissolved in EtOH/H_2SO_4 = 99/1.

Flash Column Chromatography

Flash column chromatography was performed on silica gel 60 from Acros Organics with particle sizes between 35 µm and 70 µm. Depending on the problem of separation, a 30 to 100 fold excess of silica gel was used with respect to the dry amount of crude material. The dimension of the column was adjusted to the required amount of silica gel and formed a pad between 10 cm and 30 cm. In general, the silica gel was mixed with the eluent and the column was equilibrated. Subsequently, the crude material was dissolved in the eluent and loaded onto the top of the silica gel and the mobile phase was forced through the column using a rubber bulb pump. The volume of each collected fraction was adjusted between 20 % and 40 % of the silica gel volume.

Gas Chromatography

GC-MS analyses were performed on an Agilent Technologies 7890A GC system equipped with a 5975C mass selective detector (inert MSD with Triple Axis Detector system) by electron-impact ionization (EI) with a potential of E = 70 eV. Herein, the samples were separated depending on their boiling point and polarity. The desired crude materials or pure compounds were dissolved and the solutions were injected by employing the autosampler 7683B in a split mode 1/20 (inlet temperature: 280 °C; injection volume: 0.2 µL). Separations were carried out on an Agilent Technologies J&W GC HP-5MS capillary column ((5 %-phenyl)methylpolysiloxane, 30 m x 0.2 mm x 0.25 µm) with a constant helium flow rate (He 5.0 (Air Liquide), 1.085 mL·min^{-1}, average velocity: 41.6 cm·s^{-1}). A general gradient temperature method was used:

50S: initial temperature: 50 °C for 1 min; linear increase to 300 °C (40 °C·min^{-1}); hold for 5 min; 1 min post-run at 300 °C; detecting range: 50.0-550.0 amu; solvent delay: 2.60 min.

High Performance Liquid Chromatography

Analytical HPLC-MS measurements were performed on a Shimadzu Nexera LCMS-2020 system (CBM-20A Prominence system controller, Nexera SIL-30AC autosampler, DGU-20A3 and DGU-20A5 on-line degassers, Nexera LC-30AD binary pump, FCV-20AH2 valve unit, CTO-20AC Prominence column oven, SPD-M20A Prominence photodiode array (PDA) detector (deuterium lamp, tungsten lamp, 190-800 nm)) equipped with single quadrupole ultra-fast LC/MS detector “LCMS-2020” or an Agilent Technologies 1200 Series system (G1379

Degasser, G1312 Binary Pump, G1367C HiP ALS SL Autosampler, G1330B FC/ALS Thermostat, G1316B TCC SL column compartment, G1365C MWD SL multiple wavelength detector (deuterium lamp, 190-400 nm)) equipped with a single quadrupole LCMS detector "6120 LC/MS" using electrospray ionization source (ESI in positive and negative mode). All separations were carried out on a reversed phase Agilent Poroshell 120 SB-C18 (100 x 3.0 mm, 2.7 µm) column equipped with a Merck LiChroCART® 4-4 pre-column, or a reversed phase Agilent Poroshell 120 EC-C18 (100 x 3.0 mm, 2.7 µm) column equipped with a Merck LiChroCART® 4-4 pre-column, or a Kromasil 300-5-C4 (150 x 4.6 mm, 5 µm) column. The following methods were used:

2-100 MeCN POROSHELL120SB-C18: 0.0 min: 98 % H_2O + 0.01 % HCOOH and 2 % CH_3CN; 0.0-6.0 min: linear gradient to 100 % CH_3CN; 6.0-8.0 min: 100 % CH_3CN; 8.0-8.5 min: linear gradient to 98 % H_2O + 0.01 % HCOOH and 2 % CH_3CN; 8.5-9.5 min: 98 % H_2O + 0.01 % HCOOH and 2 % CH_3CN; 0.700 mL·min^{-1}; 35 °C.

2-100 MeCN POROSHELL120EC-C18: 0.0 min: 98 % H_2O + 0.05 % TFA and 2 % CH_3CN; 0.0-6.0 min: linear gradient to 100 % CH_3CN; 6.0-6.5 min: 100 % CH_3CN; 6.5-6.51 min: linear gradient to 98 % H_2O + 0.05 % TFA and 2 % CH_3CN; 6.51-8.5 min: 98 % H_2O + 0.05 % TFA and 2 % CH_3CN; 0.700 mL·min^{-1}; 35 °C.

5-65-100 MeCN KROMASIL300-5-C4: 0.0 min: 95 % H_2O + 0.05 % TFA and 5 % CH_3CN; 0.0-10.0 min: linear gradient to 35 % H_2O + 0.05 % TFA and 65 % CH_3CN; 10.0-11.0 min: linear gradient to 100 % CH_3CN; 11.0-15.0 min: 100 % CH_3CN; 15.0-15.5 min: linear gradient to 95 % H_2O + 0.05 % TFA and 5 % CH_3CN; 1.000 mL·min^{-1}; 35 °C.

15-65-100 MeCN KROMASIL300-5-C4: 0.0 min: 85 % H_2O + 0.05 % TFA and 15 % CH_3CN; 0.0-10.0 min: linear gradient to 35 % H_2O + 0.05 % TFA and 65 % CH_3CN; 10.0-11.0 min: linear gradient to 100 % CH_3CN; 11.0-15.0 min: 100 % CH_3CN; 15.0-15.5 min: linear gradient to 85 % H_2O + 0.05 % TFA and 15 % CH_3CN; 1.000 mL·min^{-1}; 35 °C.

Nuclear Magnetic Resonance Spectroscopy

NMR spectra were recorded on a Bruker AVANCE III 300 spectrometer (^1H: 300.36 MHz; ^{13}C: 75.53 MHz) with autosampler. Chemical shifts δ are referenced to the residual proton and carbon signal of the deuterated solvent ($CDCl_3$: δ = 7.26 ppm (^1H), 77.16 ppm (^{13}C); DMSO-d_6: δ = 2.50 ppm (^1H), 39.52 ppm (^{13}C); CD_3OD: δ = 3.31 ppm (^1H), 49.00 ppm (^{13}C); D_2O: δ = 4.79 ppm (^1H)). Chemical shifts δ are given in ppm (parts per million) and coupling constants *J* in Hz (Hertz). If necessary, 1D spectra (APT and NOESY) as well as 2D spectra (H,H-COSY, HSQC, HMBC) were recorded for the identification and confirmation of the structure. Signal multiplicities are abbreviated as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet), dt (doublet of triplet), q (quadruplet), p (pentet) and m (multiplet). Deuterated solvents for nuclear resonance spectroscopy were purchased from euriso-top®.

High Resolution Mass Spectrometry

High-resolution mass spectra were recorded on a Waters Micromass GCT Premier system. Ionization was realized by an electron impact source (EI ionization) at a constant potential of 70 eV. Herein, individual samples were either inserted directly (direct inlet electron impact ionization; DI-EI) or prior to this gas chromatographically separated on

an Agilent 7890A system equipped with an Agilent Technologies J&W GC-column DB-5MS (length: 30 m; inner-diameter: 0.250 mm; film: 0.25 µm) at a constant helium flow. Molecule ions were analyzed by a time-of-flight (TOF) mass analyzer in the positive mode (TOF MS EI+).

Further high-resolution mass spectra were recorded using MALDI TOF on a Waters Micromass® MALDI micro MX Mass spectrometer. Dithranol (1,8-dihydroxy-9,10-dihydroanthracen-9-one) served as matrix and PEG as internal standard. Besides molecular formulas, calculated as well as determined *m/z* ratios of each molecule peak are denoted.

Further high-resolution mass spectra were recorded using a Bruker maXis UHR-TOF system (Qq-TOF instrument). The samples were injected directly, ionized via electrospray ionization (ESI) and analyzed in positive mode.

Determination of Melting Points

Melting points were determined on a Mel-Temp® melting point apparatus from Electrothermal with an integrated microscopical support. They were measured in open capillary tubes with a mercury-in-glass thermometer and were not corrected.

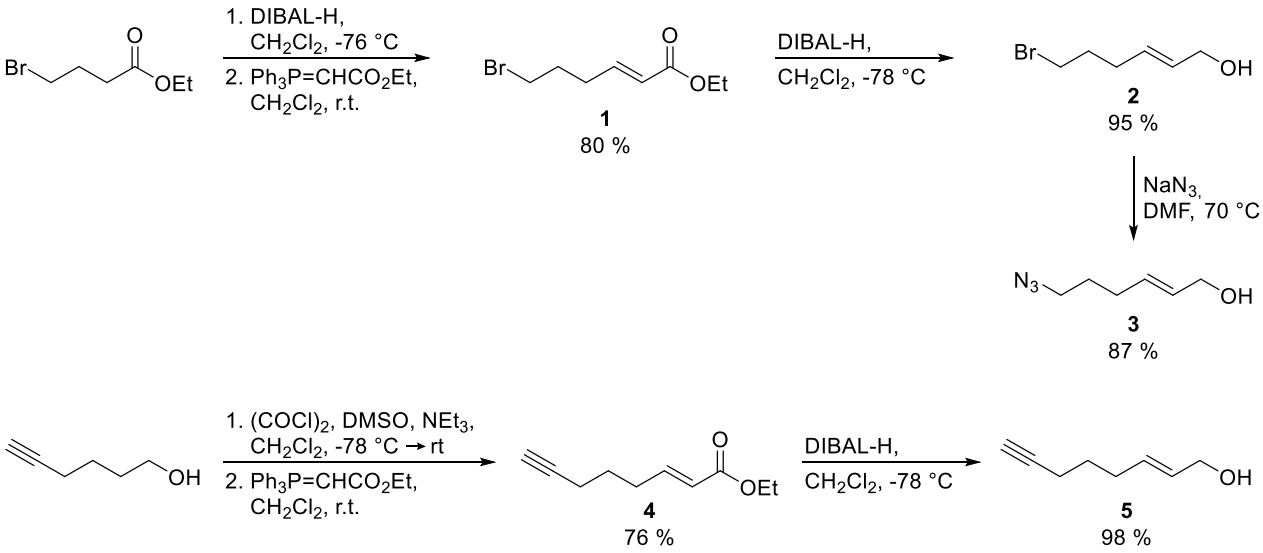
Determination of Optical Rotation

The specific optical rotation was determined on a Perkin Elmer Polarimeter 341 with an integrated sodium vapor lamp. All samples were measured at the D-line of the sodium light (λ = 589 nm) in a 10 cm cell. Concentrations are given in g/100 mL. Each optical rotation measurement was performed five times and the mean value is reported.

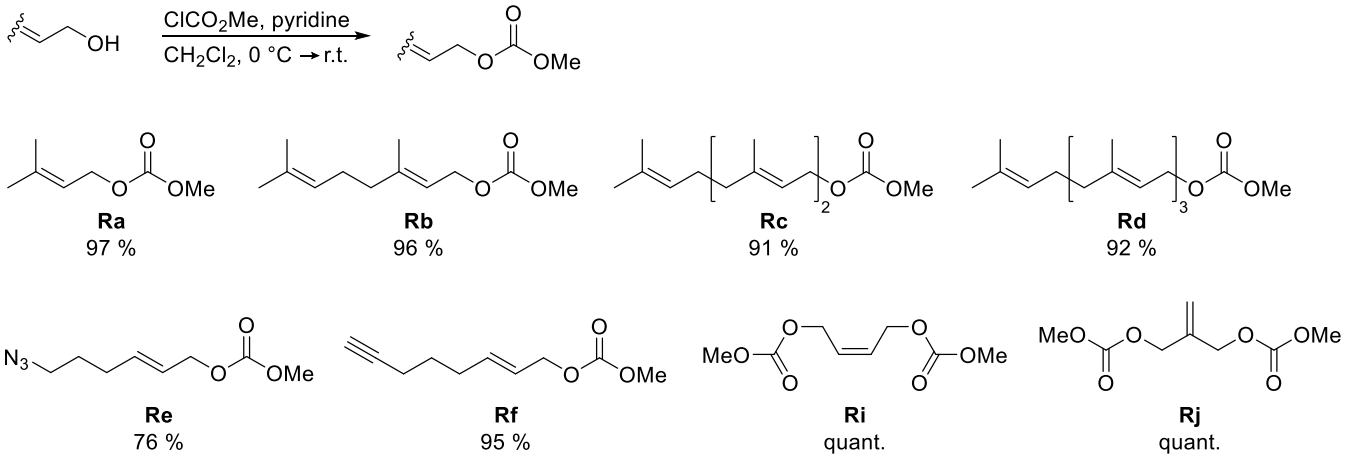
Experimental Procedures

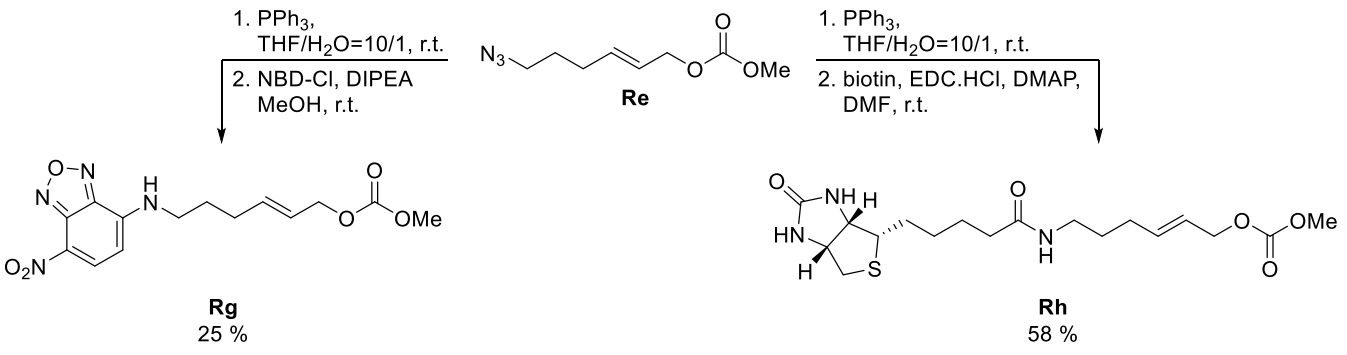
Overview

■ Synthesis of Allylic Alcohols

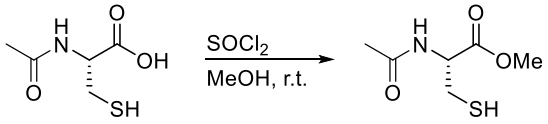


■ Synthesis of Allylic Carbonates



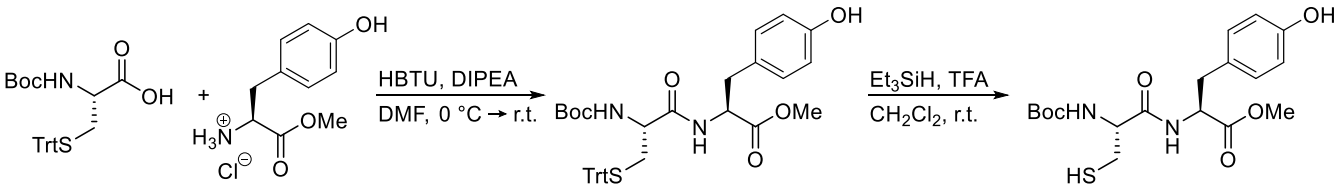


■ Synthesis of Substrate for Kinetic Measurements



6
63 %

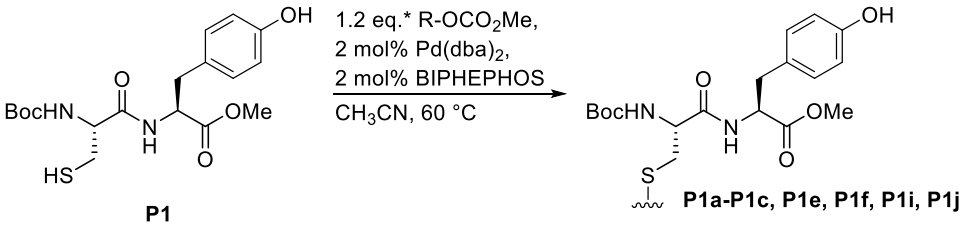
■ Synthesis of Dipeptidic Substrate



7
99 %

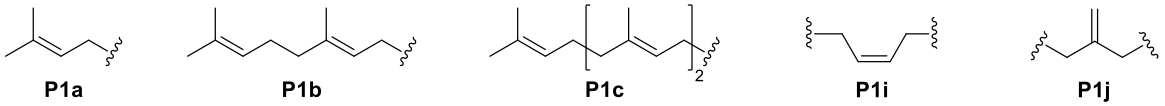
P1
70 %

■ Allylation of Dipeptidic Substrate and Glutathione



P1

P1a-P1c, P1e, P1f, P1i, P1j



P1a
88 %

P1b
84 %

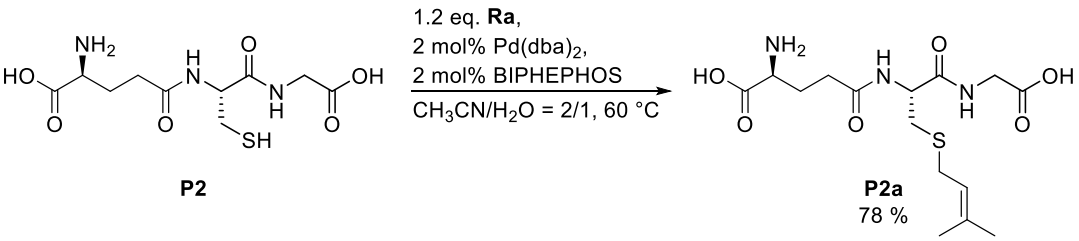
P1c
75 %

P1i
76 %
(* 0.5 eq.)

P1j
70 %
(* 0.5 eq.)

P1e
82 %

P1f
89 %



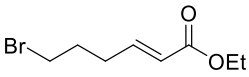
P2

P2a
78 %

Synthesis of Allylic Alcohols

Ethyl (*E*)-6-bromohex-2-enoate (1)

The intermediate (4-bromobutanal) was prepared according to a procedure described by Brown et al.[1]

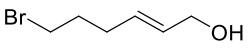


In a flame-dried and argon-flushed 1 L two-necked round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, ethyl 4-bromobutyrate (3.91 g, 20.0 mmol) was dissolved in anhydrous CH_2Cl_2 (300 mL) and cooled to -78 °C (dry ice/acetone). Subsequently, DIBAL-H (24.0 mL, 1.0 M soln. in CH_2Cl_2, 24.0 mmol) was added over 10 min and the mixture was stirred at -78 °C for 1 h. Upon complete consumption of the starting material (according to TLC), the reaction mixture was quenched by the addition of 200 mL 1 M HCl and slowly warmed to rt. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered and carefully concentrated under reduced pressure (700 mbar, 35 °C) to a final volume of approx. 30 mL.

The crude intermediate was quantitatively transferred into a 250 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, and then a solution of (carbethoxymethylene)triphenylphosphorane (8.37 g, 24.0 mmol) in CH_2Cl_2 (30 mL) was added over 10 min at rt. The reaction mixture was stirred overnight (reaction monitoring via TLC) and concentrated under reduced pressure. The crude product was adsorbed on 17.5 g SiO_2 and purified via flash column chromatography (500 g SiO_2, 20.0 x 8.0 cm, cyclohexane:EtOAc = 20:1 (v/v)) to give the desired compound as colorless oil (3.54 g, 80 % over two steps).

$C_8H_{13}BrO_2$ [221.09 g·mol^{-1}]
R_f = 0.61 (cyclohexane:EtOAc = 4:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 5.08 min; *m/z* (%) = 220 (23), 192 (47), 175 (100), 127 (64), 99 (84).
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.91 (dt, *J* = 15.7, 6.9 Hz, 1H), 5.87 (d, *J* = 15.6 Hz, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 3.41 (t, *J* = 6.5 Hz, 2H), 2.38 (q, *J* = 6.9 Hz, 2H), 2.03 (p, *J* = 6.8 Hz, 2H), 1.28 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 166.5, 146.8, 122.8, 60.4, 32.7, 31.0, 30.6, 14.4.
Analytical data is in accordance with the literature.[2]

(*E*)-6-Bromohex-2-en-1-ol (2)

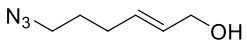


In a flame-dried and argon-flushed 250 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, ethyl (*E*)-6-bromohex-2-enoate (**1**) (3.42 g, 15.5 mmol) was dissolved in anhydrous CH_2Cl_2 (45 mL) and cooled to -78 °C (dry ice/acetone). Subsequently, DIBAL-H (32.5 mL, 1.0 M soln. in CH_2Cl_2, 32.5 mmol) was added over 15 min and the mixture was stirred at -78 °C for 30 min. During addition the colorless solution turns brightly yellow and decolorizes afterwards again. Upon complete consumption of the starting material (according to TLC), the reaction mixture was quenched by the addition of 45 mL 1 M HCl and slowly warmed to rt. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified via flash column

chromatography (75 g SiO_2, 10.0 x 4.5 cm, cyclohexane to cyclohexane:EtOAc = 2:1 (v/v)) to give the desired compound as colorless oil (2.64 g, 95 %).

$C_6H_{11}BrO$ [179.06 g·mol^{-1}]
R_f = 0.32 (cyclohexane:EtOAc = 2:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 4.48 min; *m/z* (%) = 160 (1), 134 (2), 107 (2), 81 (65), 57 (100).
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.77–5.59 (m, 2H), 4.10 (d, *J* = 3.8 Hz, 2H), 3.41 (t, *J* = 6.6 Hz, 2H), 2.28–2.13 (m, 2H), 1.95 (p, *J* = 6.8 Hz, 2H), 1.39 (br s, 1H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 130.8, 130.6, 63.7, 33.2, 32.1, 30.6.
Analytical data is in accordance with the literature.[3]

(*E*)-6-Azidohex-2-en-1-ol (3)

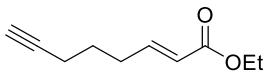


A 250 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar and air condenser, was charged with (*E*)-6-bromohex-2-en-1-ol (**2**) (2.62 g, 14.7 mmol), DMF (35 mL), NaN_3 (1.91 g, 29.4 mmol) and heated to 70 °C (oil bath) for 1 h. Upon complete consumption of the starting material (according to TLC), the reaction mixture was allowed to cool to rt and diluted with H_2O (100 mL). The resulting yellow solution was extracted with Et_2O (2 x 100 mL). The combined organic layers were washed with 1 M LiCl (3 x 30 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the desired compound as yellow oil (1.79 g, 87 %), which was used without further purification.

$C_6H_{11}N_3O$ [141.17 g·mol^{-1}]
R_f = 0.30 (cyclohexane:EtOAc = 2:1 (v/v), ninhydrin)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.76–5.60 (m, 2H), 4.11 (s, 2H), 3.29 (t, *J* = 6.8 Hz, 2H), 2.24–2.05 (m, 2H), 1.69 (p, *J* = 7.1 Hz, 2H), 1.36 (br s, 1H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 131.2, 130.4, 63.6, 50.9, 29.3, 28.4.
HRMS (ESI-TOF): calcd. for $C_6H_{12}NO^+$ $[M+H-N_2]^+$: 114.0919; found: 114.0912.

Ethyl (*E*)-oct-2-en-7-ynoate (4)

The intermediate (hex-5-ynal) was prepared according to a procedure described by Grafton et al.[4]



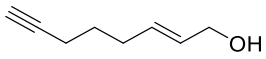
In a flame-dried and argon-flushed 250 mL three-necked round-bottom flask, equipped with a Teflon-coated magnetic stirring bar and dropping funnel, dimethyl sulfoxide (1.8 mL, 25 mmol) was added dropwise to a solution of oxalyl chloride (1.2 mL, 14 mmol) in anhydrous CH_2Cl_2 (50 mL) at -78 °C (dry ice/acetone) and stirred for 15 min. Then a solution of hex-5-yn-1-ol (0.997 g, 10.2 mmol) in anhydrous CH_2Cl_2 (12.5 mL) was slowly added. The resulting suspension was stirred for 15 min before triethylamine (7.1 mL, 51 mmol) was added. The reaction mixture was stirred for 30 min at -78 °C after which it was allowed to warm to rt (90 min, reaction monitoring via TLC).

Then (carbethoxymethylene)triphenylphosphorane (4.24 g, 12.2 mmol) was added in one portion and the light orange suspension was stirred at rt overnight (reaction monitoring via TLC) and concentrated under reduced pressure. The crude product was adsorbed on 15 g SiO_2 and purified via flash column chromatography (500 g SiO_2, 19.5 x 8.0 cm, cyclohexane:EtOAc = 15:1 (v/v)) to give the desired compound as yellowish oil (1.29 g, 76 % over two steps).

$C_{10}H_{14}O_2$ [166.22 g·mol^{-1}]
R_f = 0.32 (cyclohexane:EtOAc = 15:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 4.60 min; *m/z* (%) = 166 (2), 137 (37), 121 (58), 93 (86), 81 (100).
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.93 (dt, *J* = 15.7, 7.0 Hz, 1H), 5.84 (d, *J* = 15.6 Hz, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 2.32 (q, *J* = 7.1 Hz, 2H), 2.22 (td, *J* = 6.8, 2.2 Hz, 2H), 1.97 (t, *J* = 2.4 Hz, 1H), 1.68 (p, *J* = 7.1 Hz, 2H), 1.27 (t, *J* = 7.1 Hz, 3H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 166.7, 147.9, 122.2, 83.6, 69.1, 60.3, 31.0, 26.9, 18.0, 14.4.
Analytical data is in accordance with the literature.[4]

(*E*)-Oct-2-en-7-yn-1-ol (5)

This compound was prepared similar to a procedure described by Grafton et al.[4]



In an evacuated and argon-flushed 100 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, ethyl (*E*)-oct-2-en-7-ynoate (**4**) (1.26 g, 7.6 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) and cooled to -78 °C (dry ice/acetone). Subsequently, DIBAL-H (16 mL, 1.0 M soln. in CH_2Cl_2, 16 mmol) was added over 15 min and the mixture was stirred at -78 °C for 30 min. During addition the colorless solution turns brightly yellow and decolorizes afterwards again. Upon complete consumption of the starting material (according to TLC), the reaction mixture was quenched by the addition of 20 mL 1 M HCl and slowly warmed to rt. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic layers were washed with H_2O (10 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give the desired compound as slightly yellowish oil (0.928 g, 98 %), which was used without further purification.

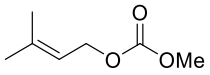
$C_8H_{12}O$ [124.18 g·mol^{-1}]
R_f = 0.30 (cyclohexane:EtOAc = 2:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 3.94 min; *m/z* (%) = 123 (5), 95 (79), 79 (81), 67 (100), 57 (71).
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.71–5.61 (m, 2H), 4.09 (br s, 2H), 2.26–2.10 (m, 4H), 1.95 (t, *J* = 2.4 Hz, 1H), 1.61 (p, *J* = 7.3 Hz, 2H), 1.44 (br s, 1H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 131.9, 130.1, 84.3, 68.6, 63.8, 31.2, 27.9, 17.9.
Analytical data is in accordance with the literature.[4]

Synthesis of Allylic Carbonates

General Procedure for the Synthesis of Allylic Carbonates

In a round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, methyl chloroformate was slowly added to an ice-cold solution of the corresponding allylic alcohol and pyridine in CH_2Cl_2. The resulting suspension was allowed to warm to rt. Upon complete consumption of the starting material (according to TLC), the reaction mixture was quenched by the addition of H_2O (1/3 of solvent volume) and stirred vigorously for at least 15 min. The organic layer was separated, washed twice with 1 M HCl (1/1 of solvent volume) and once with sat. $NaHCO_3$ (1/2 of the solvent volume), dried over Na_2SO_4, filtered and concentrated under reduced pressure (in case of volatile products a minimal pressure of 40 mbar at 35 °C was applied).
Allylic carbonates **Ra**-**Rc**, **Ri**, **Rj** were distilled to afford colorless products prior to use in allylation reactions, although crude products did not show any impurities according to ^{1}H-NMR-spectroscopy.

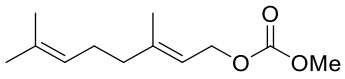
Methyl (3-methylbut-2-en-1-yl) carbonate (Ra)



Following the general procedure, 3-methylbut-2-en-1-ol (6.94 g, 80.6 mmol) was reacted with pyridine (20.0 mL, 247 mmol) and methyl chloroformate (19.0 mL, 246 mmol) in CH_2Cl_2 (180 mL). General work up afforded the desired compound as yellowish oil (11.3 g, 97 %).

$C_7H_{12}O_3$ [144.17 g·mol^{-1}]
R_f = 0.51 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 3.60 min; *m/z* (%) = 144 (1), 129 (1), 85 (42), 68 (100).
b.p. = 65-67 °C (19.5 mbar)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.36 (t, *J* = 7.2 Hz, 1H), 4.62 (d, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 1.75 (s, 3H), 1.71 (s, 3H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.0, 140.1, 118.2, 64.8, 54.7, 25.9, 18.1.
Analytical data is in accordance with the literature.[5]

(*E*)-3,7-Dimethylocta-2,6-dien-1-yl methyl carbonate (Rb)

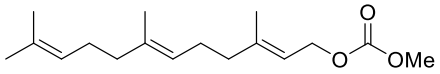


Following the general procedure, *trans*-geraniol (1.89 g, 12.2 mmol) was reacted with pyridine (3.2 mL, 40 mmol) and methyl chloroformate (3.0 mL, 39 mmol) in CH_2Cl_2 (30 mL). General work up afforded the desired compound as brownish yellow oil (2.51 g, 96 %).

$C_{12}H_{20}O_3$ [212.29 g·mol^{-1}]
R_f = 0.46 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
b.p. = approx. 100 °C (0.12 mbar)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.37 (t, *J* = 6.7 Hz, 1H), 5.12–5.03 (m, 1H), 4.65 (d, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 2.15–2.00 (m, 4H), 1.71 (s, 3H), 1.67 (s, 3H), 1.59 (s, 3H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.0, 143.3, 132.0, 123.8, 117.9, 64.9, 54.8, 39.6, 26.4, 25.8, 17.8, 16.6.
Analytical data is in accordance with the literature.[6]

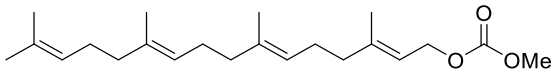
((2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl) methyl carbonate (Rc)



Following the general procedure, *trans,trans*-farnesol (0.968 g, 4.35 mmol) was reacted with pyridine (1.1 mL, 14 mmol) and methyl chloroformate (1.0 mL, 13 mmol) in CH_2Cl_2 (10 mL). General work up afforded the desired compound as yellow oil (1.11 g, 91 %).

$C_{17}H_{28}O_3$ [280.41 g·mol^{-1}]
R_f = 0.48 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
b.p. = approx. 145 °C (0.025 mbar)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.37 (t, *J* = 6.8 Hz, 1H), 5.13–5.04 (m, 2H), 4.65 (d, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 2.20–1.87 (m, 8H), 1.72 (s, 3H), 1.67 (s, 3H), 1.59 (s, 6H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.0, 143.4, 135.7, 131.5, 124.5, 123.7, 117.9, 64.9, 54.8, 39.8, 39.7, 26.9, 26.3, 25.8, 17.8, 16.7, 16.1.
HRMS (EI-DI): calcd. for $C_{17}H_{28}O_3^+$ $[M]^+$: 280.2039; found: 280.2043.

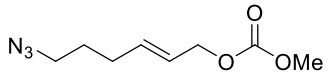
Methyl ((2*E*,6*E*,10*E*)-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraen-1-yl) carbonate (Rd)



Following the general procedure, *trans,trans,trans*-geranylgeraniol (101 mg, 0.348 mmol) was reacted with pyridine (59.2 µL, 0.73 mmol) and methyl chloroformate (49 µL, 0.63 mmol) in CH_2Cl_2 (1.0 mL). General work up afforded the desired compound as yellow oil (112 mg, 92 %).

$C_{22}H_{36}O_3$ [348.53 g·mol^{-1}]
R_f = 0.63 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.38 (t, *J* = 6.7 Hz, 1H), 5.17–5.02 (m, 3H), 4.65 (d, *J* = 7.1 Hz, 2H), 3.77 (s, 3H), 2.18–1.92 (m, 12H), 1.72 (s, 3H), 1.68 (s, 3H), 1.60 (s, 9H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.0, 143.4, 135.7, 135.1, 131.4, 124.5, 124.3, 123.7, 117.9, 64.9, 54.8, 39.9, 39.8, 39.7, 26.9, 26.8, 26.3, 25.8, 17.8, 16.7, 16.2, 16.1.
HRMS (EI-DI): calcd. for $C_{22}H_{36}O_3^+$ $[M]^+$: 348.2664; found: 348.2667.

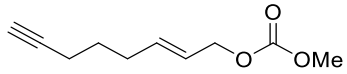
(*E*)-6-Azidohex-2-en-1-yl methyl carbonate (Re)



Following the general procedure, (*E*)-6-azidohex-2-en-1-ol (**3**) (0.908 g, 6.43 mmol) was reacted with pyridine (1.09 mL, 13.5 mmol) and methyl chloroformate (0.90 mL, 12 mmol) in CH_2Cl_2 (15 mL). The crude product afforded by the general work up was purified via flash column chromatography (50 g SiO_2, 15.0 x 3.0 cm, cyclohexane:EtOAc = 10:1 (v/v)) to give the desired compound as colorless oil (0.978 g, 76 %).

$C_8H_{13}N_3O_3$ [199.21 g·mol^{-1}]
R_f = 0.30 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.84–5.70 (m, 1H), 5.70–5.56 (m, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 3.77 (s, 3H), 3.27 (t, *J* = 6.7 Hz, 2H), 2.15 (q, *J* = 6.9 Hz, 2H), 1.68 (p, *J* = 7.2 Hz, 2H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.8, 135.3, 124.7, 68.4, 54.9, 50.8, 29.3, 28.1.
HRMS (ESI-TOF): calcd. for $C_8H_{13}N_3NaO_3^+$ [M+Na]$^+$: 222.0855; found: 222.0847.

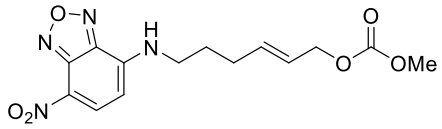
Methyl (*E*)-oct-2-en-7-yn-1-yl carbonate (Rf)



Following the general procedure, (*E*)-oct-2-en-7-yn-1-ol (**5**) (0.914 g, 7.36 mmol) was reacted with pyridine (1.25 mL, 15.5 mmol) and methyl chloroformate (1.0 mL, 13 mmol) in CH_2Cl_2 (15 mL). General work up afforded the desired compound as yellow oil (1.27 g, 95 %). Although NMR-analysis of the crude compound did not show any impurities, the material was purified via flash column chromatography (50 g SiO_2, 17.0 x 3.0 cm, cyclohexane: EtOAc = 10:1 (v/v)) prior to use in allylation reactions.

$C_{10}H_{14}O_3$ [182.22 g·mol^{-1}]
R_f = 0.32 (cyclohexane:EtOAc = 10:1 (v/v), $KMnO_4$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.85–5.68 (m, 1H), 5.68–5.51 (m, 1H), 4.54 (d, *J* = 6.2 Hz, 2H), 3.74 (s, 3H), 2.24–2.07 (m, 4H), 1.93 (t, *J* = 2.4 Hz, 1H), 1.59 (p, *J* = 7.2 Hz, 2H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.7, 135.9, 124.3, 84.0, 68.7, 68.5, 54.7, 31.1, 27.6, 17.8.
HRMS (ESI-TOF): calcd. for $C_{10}H_{14}NaO_3^+$ [M+Na]$^+$: 205.0841; found: 205.0833.

Methyl ((*E*)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)hex-2-en-1-yl) carbonate (Rg)



In a 5 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, PPh_3 (314 mg, 1.20 mmol) and H_2O (150 µL) were added to a solution of (*E*)-6-azidohex-2-en-1-yl methyl carbonate (**Re**) (119 mg, 0.596 mmol) in THF (1.5 mL). The yellow solution was stirred at rt until complete consumption of the starting material (according to TLC) was observed. After 3 h the reaction mixture was quantitatively transferred into a 10 mL round-bottom flask,

equipped with a Teflon-coated magnetic stirring bar, concentrated under reduced pressure and dried *in vacuo* for 30 min.

The colorless residue was suspended in anhydrous MeOH (3 mL) before DIPEA (0.52 mL, 3.0 mmol) was added. Then a solution of NBD-Cl (238 mg, 1.19 mmol) in anhydrous MeOH (6 mL) was added within 15 min whereupon the solution turned intensely green and the reaction mixture was stirred overnight at rt. Further NBD-Cl (119 mg, 0.595 mmol) was added to reach full conversion (according to TLC) after 2 h and the mixture was concentrated under reduced pressure. The crude product was adsorbed on 2 g SiO_2 and purified via flash column chromatography (100 g SiO_2, 35.0 x 3.0 cm, cyclohexane:EtOAc = 3:2 (v/v)) to give the desired compound as yellowish-green solid (51.0 mg, 25 % over two steps).

$C_{14}H_{16}N_4O_6$ [336.30 g·mol^{-1}]

R_f = 0.33 (cyclohexane:EtOAc = 3:2 (v/v), yellow fluorescence at 366 nm)

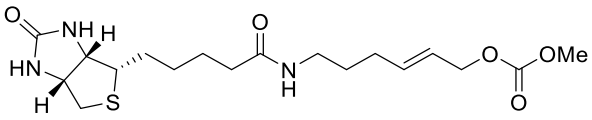
m.p. = 105-106 °C (decomp.)

^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.54 (br s, 1H), 8.50 (d, *J* = 8.7 Hz, 1H), 6.39 (d, *J* = 8.8 Hz, 1H), 5.94–5.77 (m, 1H), 5.68–5.53 (m, 1H), 4.52 (d, *J* = 6.1 Hz, 2H), 3.68 (s, 3H), 3.55–3.40 (m, 2H), 2.15 (q, *J* = 6.1 Hz, 2H), 1.77 (p, *J* = 7.0 Hz, 2H).

^{13}C NMR (76 MHz, DMSO-d_6): δ = 155.0, 145.1, 144.4, 144.2, 137.9, 135.2, 124.3, 120.6, 99.1, 67.8, 54.6, 42.8, 28.9, 26.8.

HRMS (MALDI-TOF): calcd. for $C_{14}H_{16}N_4O_6Na^+$ $[M+Na]^+$: 359.0967; found: 359.0968.

Methyl ((*E*)-6-(5-((3a*S*,4*S*,6a*R*)-2-oxohexahydro-1*H*-thieno[3,4-d]imidazol-4-yl)pentanamido)hex-2-en-1-yl) carbonate (Rh)



In a 5 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, PPh_3 (315 mg, 1.20 mmol) and H_2O (150 µL) were added to a solution of (*E*)-6-azidohex-2-en-1-yl methyl carbonate (**Re**) (119 mg, 0.598 mmol) in THF (1.5 mL). The yellow solution was stirred at rt until complete consumption of the starting material (according to TLC). After 3 h the reaction mixture was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude intermediate was quantitatively transferred into a 10 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, and dried *in vacuo* for 2.5 h. The colorless residue was dissolved in anhydrous DMF (3.0 mL) and biotin (177 mg, 0.726 mmol), DMAP (147 mg, 1.21 mmol) and EDC.HCl (231 mg, 1.20 mmol) were added to the yellow solution consecutively. The reaction mixture was stirred at rt overnight (reaction monitoring via TLC) and concentrated under reduced pressure. The orange residue was dissolved in CH_2Cl_2 and purified via flash column chromatography (90 g SiO_2, 27.5 x 3.0 cm, CH_2Cl_2:MeOH = 15:1 to 13:1 (v/v)) to give the desired compound as colorless solid (139 mg, 58 % over two steps).

$C_{18}H_{29}N_3O_5S$ [399.51 g·mol^{-1}]

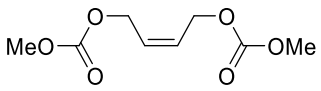
R_f = 0.32 (CH_2Cl_2:MeOH = 10:1 (v/v), 0.1 % DMACA in EtOH/H_2SO_4 = 99/1)

m.p. = 132 °C

$[\alpha]_D^{20}$ = + 49.4 (ρ = 0.51, MeOH)

^{1}H NMR (300 MHz, CD_3OD): δ = 5.92–5.75 (m, 1H), 5.72–5.54 (m, 1H), 4.55 (d, *J* = 6.1 Hz, 2H), 4.49 (dd, *J* = 7.6, 5.2 Hz, 1H), 4.30 (dd, *J* = 7.7, 4.4 Hz, 1H), 3.74 (s, 3H), 3.25–3.12 (m, 3H), 2.93 (dd, *J* = 12.7, 4.9 Hz, 1H), 2.71 (d, *J* = 12.6 Hz, 1H), 2.20 (t, *J* = 7.3 Hz, 2H), 2.11 (q, *J* = 6.9 Hz, 2H), 1.81–1.53 (m, 6H), 1.50–1.38 (m, 2H).
^{13}C NMR (76 MHz, CD_3OD): δ = 176.0, 166.1, 157.2, 136.9, 125.5, 69.4, 63.4, 61.6, 57.0, 55.2, 41.0, 39.8, 36.8, 30.6, 29.8, 29.7, 29.5, 26.9.
HRMS (MALDI-TOF): calcd. for $C_{18}H_{29}N_3O_5SNa^+$ $[M+Na]^+$: 422.1726; found: 422.1725.

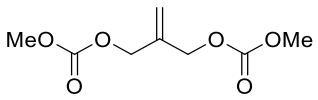
(*Z*)-But-2-ene-1,4-diyl dimethyl bis(carbonate) (Ri)



Following the general procedure, (*Z*)-but-2-ene-1,4-diol (2.00 g, 22.7 mmol) was reacted with pyridine (7.33 mL, 90.8 mmol) and methyl chloroformate (7.03 mL, 90.8 mmol) in CH_2Cl_2 (50 mL). General work up afforded the desired compound as yellowish oil (4.84 g, quant.).

$C_8H_{12}O_6$ [204.18 g·mol^{-1}]
R_f = 0.76 (cyclohexane:EtOAc = 1:1 (v/v), $KMnO_4$)
GC-MS (method: 50S): t_R = 5.04 min; *m/z* (%) = 128 (26), 85 (33), 69 (100), 59 (90).
b.p. = approx. 130 °C (0.10 mbar)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.80 (t, *J* = 3.9 Hz, 2H), 4.74 (d, *J* = 4.2 Hz, 4H), 3.78 (s, 6H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.7, 128.1, 63.3, 55.0.
Analytical data is in accordance with the literature.[7]

2-Methylenepropane-1,3-diyl dimethyl bis(carbonate) (Rj)



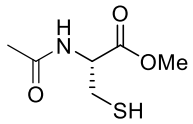
Following the general procedure, 2-methylenepropane-1,3-diol (0.989 g, 11.2 mmol) was reacted with pyridine (3.7 mL, 46 mmol) and methyl chloroformate (3.5 mL, 45 mmol) in CH_2Cl_2 (30 mL). General work up afforded the desired compound as yellow oil (2.41 g, quant.).

$C_8H_{12}O_6$ [204.18 g·mol^{-1}]
R_f = 0.57 (cyclohexane:EtOAc = 2:1 (v/v), $KMnO_4$)
b.p. = approx. 125 °C (0.095 mbar)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.34 (s, 2H), 4.66 (s, 4H), 3.78 (s, 6H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.6, 137.7, 118.3, 67.8, 55.0.
Analytical data is in accordance with the literature.[8]

Synthesis of Substrate for Kinetic Measurements

Ac-Cys-OMe (6)

This compound was prepared according to a procedure described by Bang et al.[9]



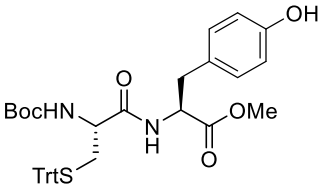
In a flame-dried and argon-flushed 250 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, *N*-Acetyl-L-cysteine (5.01 g, 30.7 mmol) was dissolved in anhydrous MeOH (100 mL). Then $SOCl_2$ (2.6 mL, 36 mmol) was added over a period of 10 min and the colorless solution was stirred at rt for 90 min. Upon complete consumption of the starting material (according to TLC), the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (80 mL), washed with brine (3 x 40 mL), after which the combined aqueous phases were back-extracted with EtOAc (3 x 40 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified via flash column chromatography (150 g SiO_2, 16.5 x 4.5 cm, CH_2Cl_2:MeOH = 80:1 to 40:1 (v/v)) to give the desired compound as colorless solid (3.41 g, 63 %).

$C_6H_{11}NO_3S$ [177.22 g·mol^{-1}]
R_f = 0.13 (CH_2Cl_2:MeOH = 80:1 (v/v), CAM)
HPLC-MS (method: 2-100 MeCN POROSHELL120EC-C18): t_R = 2.28 min; *m/z* (ESI+) = 178 $[M+H]^+$.
m.p. = 83-84 °C
$[\alpha]_D^{20}$ = + 70.3 (ρ = 0.98, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.42 (br s, 1H), 4.88 (dt, *J* = 7.7, 4.0 Hz, 1H), 3.79 (s, 3H), 3.00 (dd, *J* = 9.0, 3.9 Hz, 2H), 2.06 (s, 3H), 1.34 (t, *J* = 9.0 Hz, 1H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 170.8, 170.0, 53.7, 52.9, 27.0, 23.3.
Analytical data is in accordance with the literature.[9]

Synthesis of Dipeptidic Substrate

Boc-Cys(Trt)-Tyr-OMe (7)

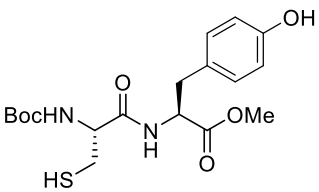
This compound was prepared similar to a procedure described by Ivkovic et al.[10]



In a flame-dried and argon-flushed 100 mL three-necked round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, Boc-Cys(Trt)-OH (2.00 g, 4.32 mmol) was dissolved in anhydrous DMF (25 mL) and DIPEA (2.9 mL, 17 mmol) was added. The solution was cooled to 0 °C (ice bath) before HBTU (1.96 g, 5.17 mmol) was added in one portion. After 5 min of activation time, H-Tyr-OMe.HCl (1.10 g, 4.77 mmol) was added and the reaction mixture was allowed to warm to rt and stirred for 1 h. Upon complete consumption of the starting material (according to TLC), the reaction mixture was quenched by the addition of brine (25 mL) and extracted with EtOAc (100 mL). The aqueous phase was separated and back-extracted with EtOAc (50 mL). The combined organic layers were washed with H_2O (2 x 25 mL) and brine (25 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified via flash column chromatography (500 g SiO_2, 21.5 x 8.0 cm, cyclohexane:EtOAc = 2:1 (v/v)) to give the desired compound as colorless powder (2.73 g, 99 %).

$C_{37}H_{40}N_2O_6S$ [640.80 g·mol^{-1}]
R_f = 0.34 (cyclohexane:EtOAc = 2:1 (v/v), CAM)
HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 6.30 min; *m/z* (ESI+) = 243 $[Trt]^+$.
m.p. = 56-59 °C
$[\alpha]_D^{20}$ = + 36.5 (ρ = 1.0, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40 (d, *J* = 7.3 Hz, 6H), 7.33–7.12 (m, 9H), 6.88 (d, *J* = 8.2 Hz, 2H), 6.62 (d, *J* = 8.3 Hz, 3H), 6.34 (br s, 1H), 4.84–4.65 (m, 2H), 3.80 (br s, 1H), 3.65 (s, 3H), 3.08–2.90 (m, 2H), 2.77-2.62 (m, 1H), 2.51 (dd, *J* = 12.4, 4.3 Hz, 1H), 1.40 (s, 9H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.6, 170.4, 155.5, 155.4, 144.5, 130.5, 129.7, 128.2, 127.1, 127.0, 115.6, 80.6, 67.4, 53.7, 53.6, 52.4, 37.2, 33.8, 28.4.
HRMS (MALDI-TOF): calcd. for $C_{37}H_{40}N_2O_6SNa^+$ $[M+Na]^+$: 663.2505; found: 663.2504.

Boc-Cys-Tyr-OMe (P1)



In an evacuated and argon-flushed 10 mL round-bottom flask, equipped with a Teflon-coated magnetic stirring bar, Boc-Cys(Trt)-Tyr-OMe (**7**) (169 mg, 264 µmol) was dissolved in anhydrous CH_2Cl_2 (2.7 mL) and Et_3SiH (51.0 µL, 319 µmol) was added. Subsequently, TFA (102 µL, 1.32 mmol) was added to the stirred solution at room

temperature whereupon a bright yellow color appeared. After 30 min the yellow color had disappeared and complete consumption of the starting material was indicated by TLC. The reaction mixture was quenched by the addition of sat. $NaHCO_3$ (3 mL). The aqueous phase was separated and back-extracted with CH_2Cl_2 (2 x 3 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified via flash column chromatography (17 g SiO_2, 20.0 x 1.5 cm, cyclohexane:EtOAc = 5:4 (v/v)) to give the desired compound as colorless powder (178 mg, 70 %).

$C_{18}H_{26}N_2O_6S$ [398.47 $g \cdot mol^{-1}$]

R_f = 0.32 (cyclohexane:EtOAc = 5:4 (v/v), CAM)

HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 4.52 min; *m/z* (ESI+) = 421 $[M+Na]^+$, *m/z* (ESI-) = 397 $[M-H]^-$.

m.p. = 93-95 °C

$[\alpha]_D^{20}$ = + 24.7 (ρ = 1.0, $CHCl_3$)

^1H NMR (300 MHz, $CDCl_3$): δ = 7.15 (br s, 1H), 6.94 (d, *J* = 7.7 Hz, 3H), 6.71 (d, *J* = 7.7 Hz, 2H), 5.52 (d, *J* = 7.3 Hz, 1H), 4.83 (q, *J* = 6.1 Hz, 1H), 4.31 (br s, 1H), 3.72 (s, 3H), 3.15–2.86 (m, 3H), 2.75–2.59 (m, 1H), 1.44 (s, 10H).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.8, 170.2, 155.7, 155.6, 130.5, 126.9, 115.8, 81.0, 55.8, 53.6, 52.6, 37.2, 28.4, 27.0.

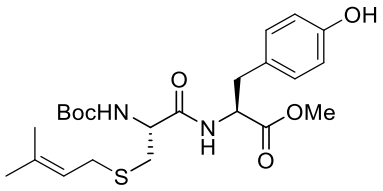
HRMS (MALDI TOF): calcd. for $C_{36}H_{50}N_4O_{12}S_2Na^+$ $[(2M-2H)+Na]^+$: 817.2764; found: 817.2769.

Allylation of Small Peptides

General Procedure for the Allylation of Small Peptides

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ and BIPHEPHOS were suspended in 1.0 mL anhydrous CH_3CN and stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Then allylic carbonate and Boc-Cys-Tyr-OMe (**P1**) were added and the resulting mixture was stirred at 60 °C for 2 h. The yellow-tan solution was cooled to rt and concentrated under reduced pressure. The crude product was purified via flash column chromatography to afford the desired compound.

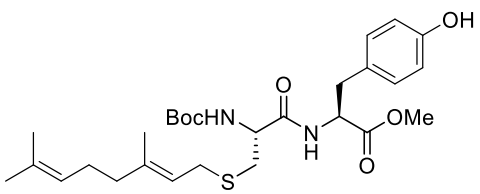
Boc-Cys(Pre)-Tyr-OMe (P1a)



Following the general procedure, methyl prenyl carbonate (**Ra**) (17.4 mg, 121 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.9 mg, 99.9 µmol) were reacted in the presence of $Pd(dba)_2$ (1.2 mg, 2.1 µmol) and BIPHEPHOS (1.5 mg, 1.9 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 16.5 x 1.0 cm, cyclohexane:EtOAc = 2:1 (v/v)) afforded the desired compound as colorless oil (41.1 mg, 88 %).

$C_{23}H_{34}N_2O_6S$ [466.59 g·mol^{-1}]
R_f = 0.23 (cyclohexane:EtOAc = 2:1 (v/v), CAM)
HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 5.43 min; *m/z* (ESI+) = 489 $[M+Na]^+$.
$[\alpha]_D^{20}$ = + 15.2 (ρ = 1.0, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.92 (d, *J* = 8.3 Hz, 3H), 6.68 (d, *J* = 8.0 Hz, 3H), 5.32 (br s, 1H), 5.19 (t, *J* = 7.2 Hz, 1H), 4.86–4.76 (m, 1H), 4.29–4.16 (m, 1H), 3.71 (s, 3H), 3.14 (d, *J* = 7.6 Hz, 2H), 3.08–2.94 (m, 2H), 2.79 (d, *J* = 6.2 Hz, 2H), 1.71 (s, 3H), 1.64 (s, 3H), 1.45 (s, 9H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.7, 170.7, 155.7, 155.5, 136.5, 130.5, 127.1, 120.0, 115.7, 80.8, 54.1, 53.7, 52.5, 37.3, 33.6, 30.1, 28.4, 25.8, 17.9.
HRMS (MALDI-TOF): calcd. for $C_{23}H_{34}N_2O_7SNa^+$ $[M+O+Na]^+$: 505.1985; found: 505.1997.

Boc-Cys(Ger)-Tyr-OMe (P1b)



Following the general procedure, geranyl methyl carbonate (**Rb**) (25.8 mg, 122 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.8 mg, 99.9 µmol) were reacted in the presence of $Pd(dba)_2$ (1.1 mg, 1.9 µmol) and BIPHEPHOS (1.6 mg,

2.0 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 16.0 x 1.0 cm, cyclohexane:EtOAc = 5:2 (v/v)) to give the desired compound as colorless oil (44.6 mg, 84 %).

$C_{28}H_{42}N_2O_6S$ [534.71 g·mol^{-1}]

R_f = 0.27 (cyclohexane:EtOAc = 2:1 (v/v), CAM)

HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 6.30 min; *m/z* (ESI+) = 435 $[M\text{-}Boc+2H]^+$.

$[\alpha]_D^{20}$ = + 13.9 (ρ = 1.0, $CHCl_3$)

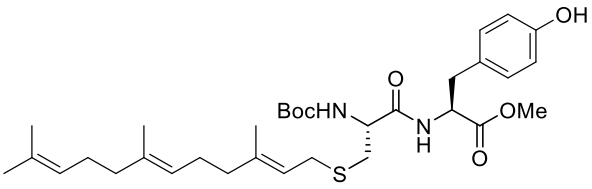
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.98 (br s, 1H), 6.91 (d, *J* = 8.2 Hz, 2H), 6.79 (s, 1H), 6.68 (d, *J* = 8.0 Hz, 2H), 5.40–5.28 (m, 1H), 5.20 (t, *J* = 7.3 Hz, 1H), 5.11–5.01 (m, 1H), 4.88–4.74 (m, 1H), 4.31–4.14 (m, 1H), 3.71 (s, 3H), 3.15 (d, *J* = 7.6 Hz, 2H), 3.10–2.94 (m, 2H), 2.78 (d, *J* = 6.2 Hz, 2H), 2.15–1.93 (m, 4H), 1.66 (s, 3H), 1.63 (s, 3H), 1.58 (s, 3H), 1.45 (s, 9H).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.7, 170.7, 155.7, 155.5, 140.1, 131.8, 130.5, 127.0, 124.0, 119.8, 115.7, 80.8, 54.0, 53.7, 52.5, 39.7, 37.3, 33.4, 30.0, 28.4, 26.6, 25.8, 17.8, 16.2.

HRMS (MALDI-TOF): calcd. for $C_{28}H_{42}N_2O_6SNa^+$ $[M+Na]^+$: 557.2661; found: 557.2661.

calcd. for $C_{28}H_{42}N_2O_7SNa^+$ $[M+O+Na]^+$: 573.2610; found: 573.2605.

Boc-Cys(Far)-Tyr-OMe (P1c)



Following the general procedure, farnesyl methyl carbonate (**Rc**) (34.4 mg, 123 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.8 mg, 99.9 µmol) were reacted in the presence of $Pd(dba)_2$ (1.1 mg, 1.9 µmol) and BIPHEPHOS (1.6 mg, 2.0 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 16.0 x 1.0 cm, cyclohexane:EtOAc = 5:2 (v/v)) to give the desired compound as colorless solid (45.4 mg, 75 %).

$C_{33}H_{50}N_2O_6S$ [602.83 g·mol^{-1}]

R_f = 0.32 (cyclohexane:EtOAc = 2:1 (v/v), CAM)

HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 7.02 min; *m/z* (ESI+) = 503 $[M\text{-}Boc+2H]^+$.

m.p. = 84-85 °C

$[\alpha]_D^{20}$ = + 18.6 (ρ = 1.0, $CHCl_3$)

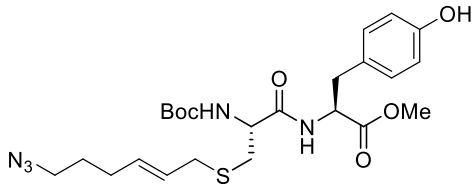
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.92 (d, *J* = 8.2 Hz, 3H), 6.68 (d, *J* = 8.1 Hz, 2H), 6.56 (s, 1H), 5.39–5.27 (m, 1H), 5.21 (t, *J* = 7.5 Hz, 1H), 5.15–5.01 (m, 2H), 4.89–4.75 (m, 1H), 4.29–4.14 (m, 1H), 3.71 (s, 3H), 3.16 (d, *J* = 7.6 Hz, 2H), 3.07–2.97 (m, 2H), 2.79 (d, *J* = 6.2 Hz, 2H), 2.15–1.92 (m, 8H), 1.67 (s, 3H), 1.64 (s, 3H), 1.59 (s, 6H), 1.45 (s, 9H).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.7, 170.7, 155.7, 155.4, 140.2, 135.5, 131.4, 130.5, 127.1, 124.5, 123.9, 119.7, 115.7, 80.8, 54.1, 53.7, 52.5, 39.8, 39.7, 37.3, 33.5, 30.0, 28.4, 26.9, 26.6, 25.8, 17.8, 16.3, 16.1.

HRMS (MALDI-TOF): calcd. for $C_{33}H_{50}N_2O_6SNa^+$ $[M+Na]^+$: 625.3287; found: 625.3290.

calcd. for $C_{33}H_{50}N_2O_7SNa^+$ $[M+O+Na]^+$: 641.3237; found: 641.3238.

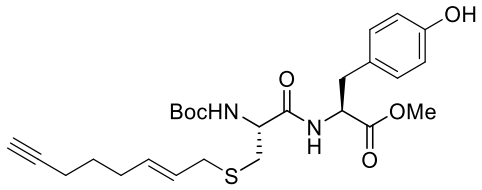
Boc-Cys(Azide)-Tyr-OMe (P1e)



Following the general procedure, allylic carbonate **Re** (24.4 mg, 122 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.7 mg, 99.6 µmol) were reacted in the presence of $Pd(dba)_2$ (1.2 mg, 2.1 µmol) and BIPHEPHOS (1.6 mg, 2.0 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 16.5 x 1.0 cm, cyclohexane:EtOAc = 2:1 (v/v)) to give the desired compound as colorless oil (42.8 mg, 82 %, HPLC-purity: 95 % (210 nm)).

$C_{24}H_{35}N_5O_6S$ [521.63 g·mol^{-1}]
R_f = 0.24 (cyclohexane:EtOAc = 2:1 (v/v), CAM)
HPLC-MS (method: 2-100 MeCN POROSHELL120EC-C18): t_R = 5.16 min; *m/z* (ESI+) = 544 $[M+Na]^+$.
$[\alpha]_D^{20}$ = +17.5 (ρ = 1.0, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.93 (d, *J* = 8.2 Hz, 3H), 6.69 (d, *J* = 8.2 Hz, 2H), 6.48 (br s, 1H), 5.63–5.48 (m, 1H), 5.48–5.26 (m, 2H), 4.87-4.74 (m, 1H), 4.28-4.14 (m, 1H), 3.72 (s, 3H), 3.25 (t, *J* = 6.7 Hz, 2H), 3.15–2.96 (m, 4H), 2.76 (d, *J* = 6.3 Hz, 2H), 2.10 (q, *J* = 6.9 Hz, 2H), 1.65 (p, *J* = 7.2 Hz, 2H), 1.46 (s, 9H).
^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.7, 170.6, 155.6, 155.4, 133.0, 130.5, 127.2, 126.7, 115.7, 80.8, 53.9, 53.7, 52.5, 50.9, 37.2, 34.1, 32.9, 29.4, 28.5, 28.4.
HRMS (MALDI-TOF): calcd. for $C_{24}H_{35}N_5O_7SNa^+$ $[M+O+Na]^+$: 560.2155; found: 560.2153.

Boc-Cys(Alkyne)-Tyr-OMe (P1f)



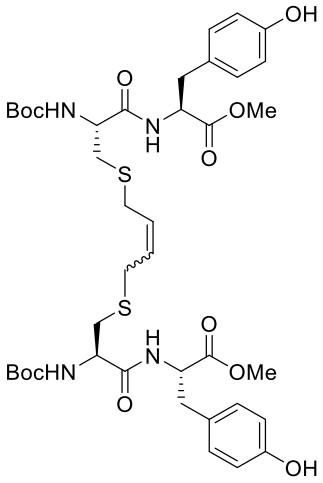
Following the general procedure, allylic carbonate **Rf** (21.8 mg, 120 µmol) and Boc-Cys-Tyr-OMe (**P1**) (40.6 mg, 102 µmol) were reacted in the presence of $Pd(dba)_2$ (1.2 mg, 2.1 µmol) and BIPHEPHOS (1.6 mg, 2.0 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 15.5 x 1.0 cm, cyclohexane:EtOAc = 2:1 (v/v)) to give the desired compound as colorless oil (45.6 mg, 89 %).

$C_{26}H_{36}N_2O_6S$ [504.64 g·mol^{-1}]
R_f = 0.25 (cyclohexane:EtOAc = 2:1 (v/v), CAM)
HPLC-MS (method: 2-100 MeCN POROSHELL120EC-C18): t_R = 5.09 min; *m/z* (ESI+) = 506 $[M+H]^+$.
$[\alpha]_D^{20}$ = + 24.5 (ρ = 1.0, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.91 (d, *J* = 8.1 Hz, 3H), 6.69 (d, *J* = 8.0 Hz, 2H), 5.63–5.47 (m, 1H), 5.47–5.28 (m, 2H), 4.86–4.73 (m, 1H), 4.31–4.12 (m, 1H), 3.71 (s, 3H), 3.13–2.97 (m, 4H), 2.75 (d, *J* = 6.2 Hz, 2H), 2.23–2.06 (m, 4H), 1.94 (s, 1H), 1.57 (p, *J* = 7.1 Hz, 2H), 1.45 (s, 9H).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.7, 170.7, 155.7, 155.5, 133.6, 130.5, 127.0, 126.3, 115.7, 84.3, 80.8, 68.7, 53.9, 53.7, 52.5, 37.2, 34.2, 32.8, 31.2, 28.4, 28.0, 17.9.

HRMS (MALDI-TOF): calcd. for $C_{26}H_{36}N_2O_7SNa^+$ $[M+O+Na]^+$: 543.2141; found: 543.2160.

Boc-Cys(*Z*-staple)-Tyr-OMe (P1i)



Following the general procedure, allylic carbonate **Ri** (10.5 mg, 51.4 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.8 mg, 99.9 µmol) were reacted in the presence of $Pd(dba)_2$ (1.3 mg, 2.3 µmol) and BIPHEPHOS (1.7 mg, 2.2 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 18.0 x 1.0 cm, cyclohexane:EtOAc = 1:1 (v/v)) to give the desired compound as colorless solid (32.2 mg, 76 %).

$C_{40}H_{56}N_4O_{12}S_2$ [849.02 g·mol^{-1}]

R_f = 0.23 (cyclohexane:EtOAc = 1:1 (v/v), CAM)

HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 5.41 min; *m/z* (ESI+) = 749 $[M\text{-Boc}+2H]^+$.

m.p. = 58-59 °C

$[\alpha]_D^{20}$ = + 13.1 (ρ = 0.97, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (br s, 2H), 7.06 (d, *J* = 7.6 Hz, 2H), 6.98 (d, *J* = 8.0 Hz, 4H), 6.75 (d, *J* = 8.0 Hz, 4H), 5.60–5.46 (m, 2H), 5.44–5.34 (m, 2H), 4.95–4.79 (m, 2H), 4.30–4.14 (m, 2H), 3.74 (s, 6H), 3.21–3.03 (m, 4H), 3.03–2.82 (m, 4H), 2.82–2.55 (m, 4H), 1.46 (s, 18H).

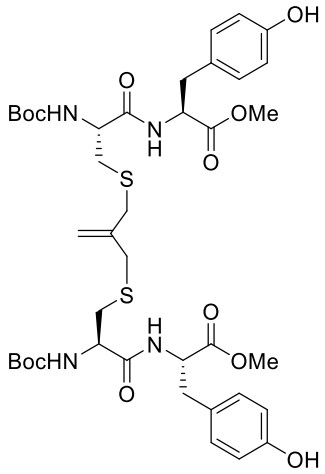
^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.8, 171.0, 156.0, 155.7, 130.5, 129.3, 126.7, 115.7, 81.1, 53.9, 53.5, 52.6, 37.4, 33.4, 33.2, 28.4.

HRMS (MALDI-TOF): calcd. for $C_{40}H_{56}N_4O_{12}S_2Na^+$ $[M+Na]^+$: 871.3234; found: 871.3121.

calcd. for $C_{40}H_{56}N_4O_{13}S_2Na^+$ $[M+O+Na]^+$: 887.3183; found: 887.3060.

calcd. for $C_{40}H_{56}N_4O_{14}S_2Na^+$ $[M+2O+Na]^+$: 903.3132; found: 903.2838.

Boc-Cys(*i*-staple)-Tyr-OMe (P1j)



Following the general procedure, allylic carbonate **Rj** (10.7 mg, 52.4 µmol) and Boc-Cys-Tyr-OMe (**P1**) (39.9 mg, 100 µmol) were reacted in the presence of $Pd(dba)_2$ (1.2 mg, 2.1 µmol) and BIPHEPHOS (1.6 mg, 2.0 µmol) in 1.0 mL anhydrous CH_3CN. Purification via flash column chromatography (6 g SiO_2, 18.0 x 1.0 cm, cyclohexane:EtOAc = 1:1 (v/v)) to give the desired compound as colorless solid (29.6 mg, 70 %).

$C_{40}H_{56}N_4O_{12}S_2$ [849.02 g·mol^{-1}]

R_f = 0.19 (cyclohexane:EtOAc = 1:1 (v/v), CAM)

HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 5.39 min; *m/z* (ESI+) = 749 $[M\text{-Boc}+2H]^+$.

m.p. = 67-69 °C

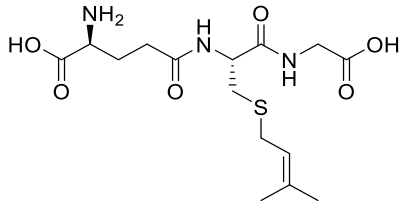
$[\alpha]_D^{20}$ = + 10.5 (ρ = 1.0, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.85 (br s, 2H), 7.01 (d, *J* = 8.0 Hz, 4H), 6.75 (d, *J* = 7.9 Hz, 6H), 5.43–5.27 (m, 2H), 4.96–4.79 (m, 2H), 4.67–4.53 (m, 2H), 4.23–4.08 (m, 2H), 3.77 (s, 6H), 3.25–2.90 (m, 8H), 2.72–2.53 (m, 4H), 1.47 (s, 18H).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 171.8, 170.7, 156.2, 155.9, 140.8, 130.7, 126.7, 117.5, 115.9, 81.2, 54.6, 53.3, 52.6, 37.3, 36.0, 34.1, 28.4.

HRMS (MALDI-TOF): calcd. for $C_{40}H_{56}N_4O_{12}S_2Na^+$ $[M+Na]^+$: 871.3234; found: 871.3249.
calcd. for $C_{40}H_{56}N_4O_{13}S_2Na^+$ $[M+O+Na]^+$: 887.3183; found: 887.3185.
calcd. for $C_{40}H_{56}N_4O_{14}S_2Na^+$ $[M+2O+Na]^+$: 903.3132; found: 903.3116.

H-γGlu-Cys(Pre)-Gly-OH (P2a)



In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (2.4 mg, 4.2 µmol) and BIPHEPHOS (3.4 mg, 4.3 µmol) were suspended in 2.0 mL CH_3CN and stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Then a solution of glutathione (**P2**) (61.4 mg, 200 µmol) in 1.0 mL H_2O and methyl prenyl carbonate (**Ra**) (34.4 mg, 239 µmol) were added and the

resulting mixture was stirred at 60 °C for 15 h. The solution was cooled to rt and concentrated under reduced pressure. The crude product was purified via reversed-phase flash column chromatography (SiO_2-C18, 9.0 x 3.0 cm, CH_3CN:H_2O = 2:98 to 15:85 (v/v)) to give the desired compound as slightly yellowish solid (58.4 mg, 78 %).

$C_{15}H_{25}N_3O_6S$ [375.44 g·mol^{-1}]
HPLC-MS (method: 2-100 MeCN POROSHELL120SB-C18): t_R = 3.10 min; *m/z* (ESI-) = 374 $[M-H]^-$.
m.p. = 210-211 °C
$[\alpha]_D^{20}$ = - 22 (ρ = 0.25, 0.1 M HCl)
^{1}H NMR (300 MHz, D_2O + DCl): δ = 4.94 (t, *J* = 7.6 Hz, 1H), 4.23 (dd, *J* = 8.5, 5.4 Hz, 1H), 3.84 (t, *J* = 6.4 Hz, 1H), 3.70 (s, 2H), 2.92 (d, *J* = 7.8 Hz, 2H), 2.70 (dd, *J* = 14.1, 5.3 Hz, 1H), 2.50 (dd, *J* = 14.0, 8.6 Hz, 1H), 2.39–2.22 (m, 2H), 2.04–1.85 (m, 2H), 1.42 (s, 3H), 1.35 (s, 3H).
^{13}C NMR (76 MHz, D_2O + DCl): δ = 174.1, 172.7, 172.6, 171.1, 137.7, 119.0, 53.1, 52.0, 41.0, 31.8, 30.8, 29.0, 25.3, 24.7, 16.9.
HRMS (MALDI-TOF): calcd. for $C_{15}H_{26}N_3O_6S^+$ $[M]^+$: 376.1542; found: 376.2619.

Peptide Synthesis

Peptides were synthesized by the Fmoc strategy and pre-loaded Wang and Tentagel resins were used as solid support. All equivalents are calculated based on the theoretical loading of the resin.

Manual Peptide Synthesis

All synthesis steps were performed in fritted syringes at rt on a rotator.

Before starting the synthesis, the resin was swollen in DMF for 1 h. Fmoc deprotection was performed with 20 % piperidine in DMF for 2 x 5 min and the resin was washed with DMF (3x). Fmoc amino acids (2.5 eq.) were dissolved in HBTU (0.5 M, 2.38 eq.) with DIPEA (5 eq.) and added to the resin for 2 x 30 min. After coupling, the resin was washed with DMF (3x) and at the end of the synthesis the resin was washed with DCM (3x) and dried.

For final cleavage the dry resin was treated with the cleavage solution (92.5 % TFA, 2.5 % EDT, 2.5 % TIPS, 2.5 % H_2O) for 3 h. The peptide was precipitated with cold diethyl ether and after centrifugation (4000 rpm, 10 min, 4 °C) the supernatant was removed. The crude peptide was dissolved in 6 M Gdn-HCl pH 4.7 and purified by preparative HPLC.

Automated Peptide Synthesis

The following peptides were synthesized with a microwave-based synthesizer (Liberty Blue, CEM). Fmoc was removed with 20 % piperidine in DMF for 80 s at 90 °C. Amino acids were coupled in DMF, Fmoc-Xaa-OH (5 eq., 0.2 M), Oxyma (5 eq., 1 M) and DIC (5 eq., 0.5 M) for 2 min at 90 °C. For some amino acids double or triple couplings were performed (see below). Histidine was coupled 8 min at 50 °C.

P3: GQRNREKTGESNSCVIL

P5: GQRNREKTGESNSCCVIL

P6: GQRNREKTGESNSCACVIL

P7: MGIINTLQKYYARVRGGRCAVLSALPKEEQIG

P8: EW**ACTAACK**FLAAHA

P9: EW**ACTACAK**FLAAHA

AA single coupling

AA double coupling

AA triple coupling

Purification and Analysis of Peptides by HPLC

Peptides were purified by preparative RP-HPLC on a C18 column (Kromasil 300-10C18 250x21.2 mm) on a Waters AutoPurification HPLC-MS system. The purification was performed at 20 mL/min with ddH_2O + 0.05 % TFA as solvent A and ACN + 0.05 % TFA as solvent B. Linear gradients either from 5-45 % or 2-30 % B in 40 min were used, depending on the peptide.

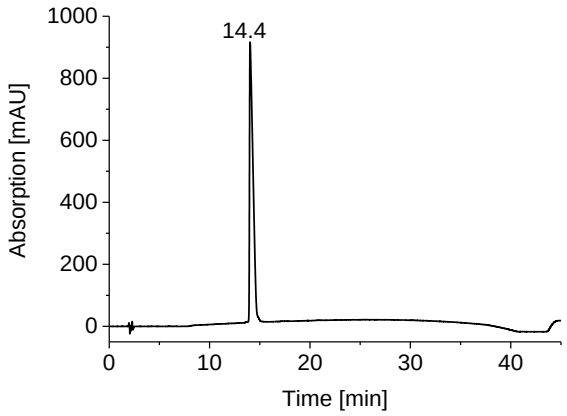
Peptide purity was investigated by ESI-MS on a Waters 3100 Mass Detector in positive ion mode and analytical RP-HPLC on a C4 column (Kromasil 300-5-C4 4.6x50 mm) on a Dionex Ultimate 3000 system. The runs were performed at 1 mL/min with ddH_2O + 0.1 % TFA as buffer A and ACN + 0.08 % TFA as buffer B and linear gradients either from 5-65 % or 2-20 % B in 30 min were used, depending on the peptide.

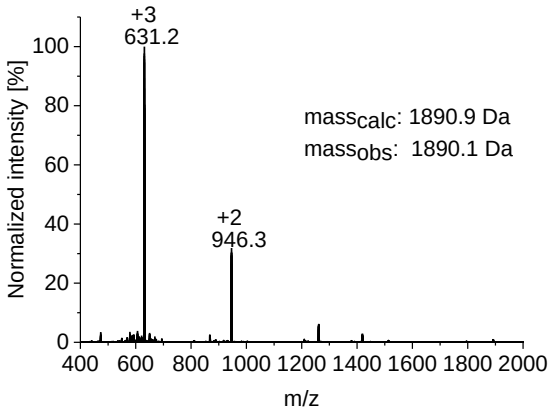
Table S2. Sequences and masses of all peptides.

Name	Sequence	$Mass_{calc}$:	$Mass_{obs}$:
P3	GQRNREKTGESNSCVIL	1890.9 Da	1890.1 Da
P4	GQRNREKTGESNSC	1478.7 Da	1478.0 Da
P5	GQRNREKTGESNSCCVIL	1906.9 Da	1906.6 Da
P6	GQRNREKTGESNSCACVIL	1978.0 Da	1977.4 Da
P7	MGIINTLQKYYARVRGGRCAVLSALPKEEQIG	3534.9 Da	3535.1 Da
P8	EWACTAACKFLAAHA	1592.7 Da	1592.4 Da
P9	EWACTACAKFLAAHA	1592.7 Da	1592.4 Da
P10	AGCKNFFWKTFTSC	1639.7 Da	1639.6 Da

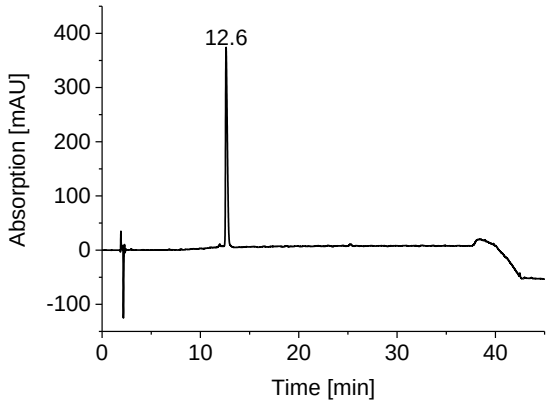
Analysis of the Peptides

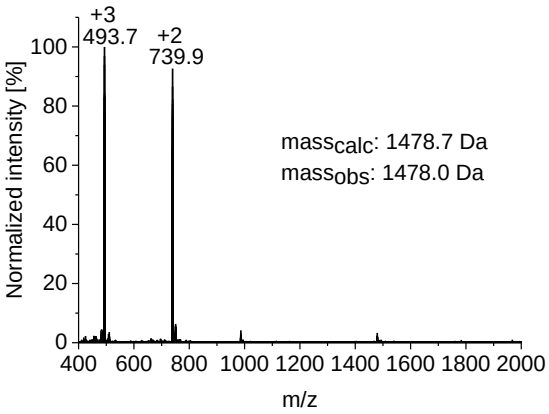
P3: GQRNREKTGESNSCVIL



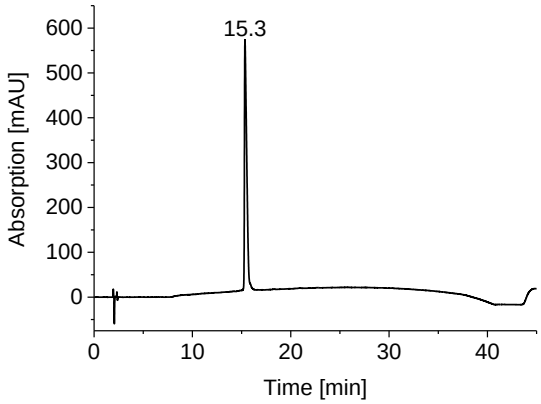


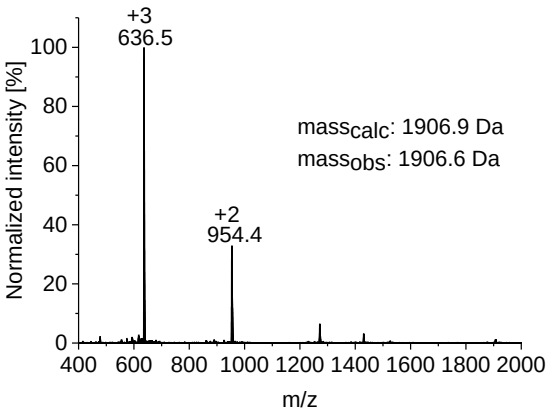
P4: GQRNREKTGESNSC



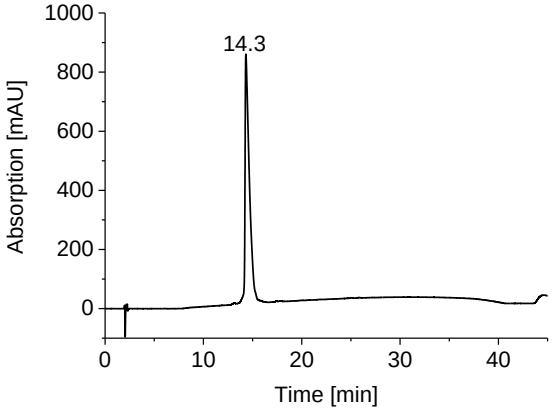


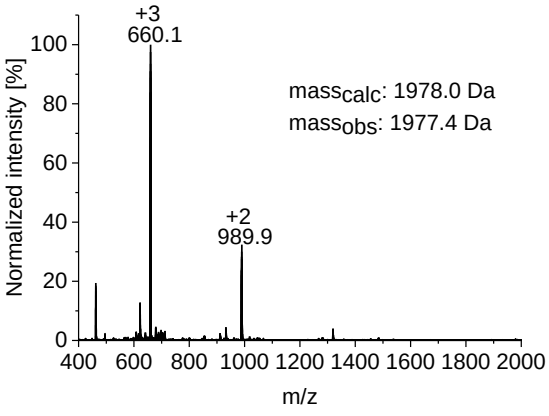
P5: GQRNREKTGESNSCCVIL



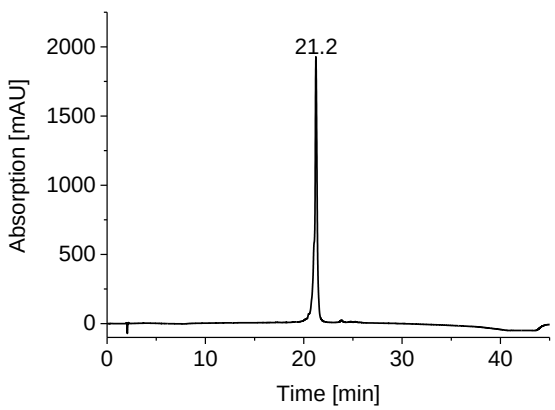


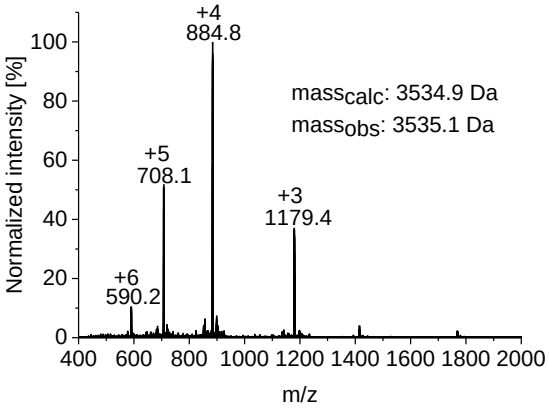
P6: GQRNREKTGESNSCACVIL



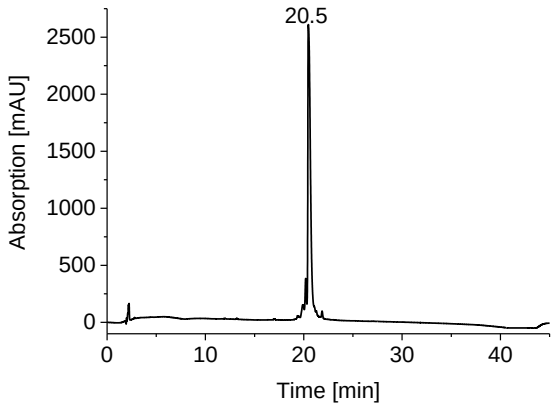


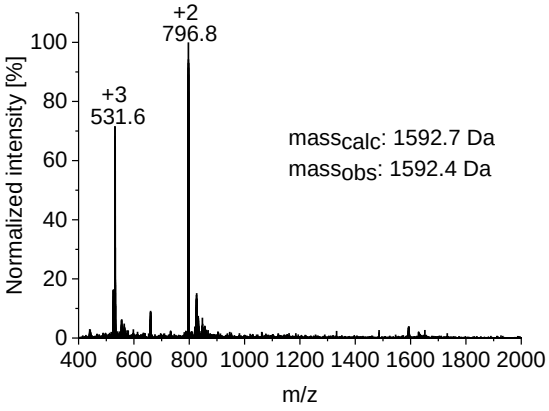
P7: MGIINTLQKYYARVRGGRCAVLSALPKEEQIG



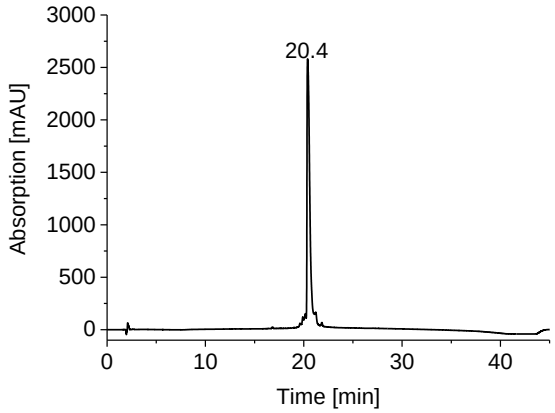


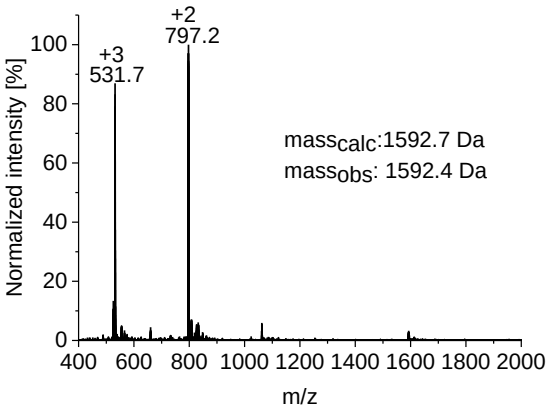
P8: EWACTAACKFLAAHA



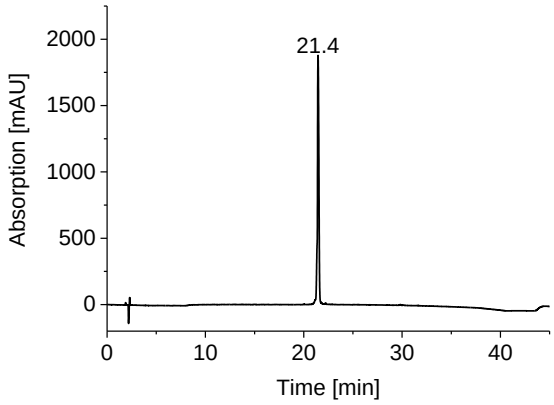


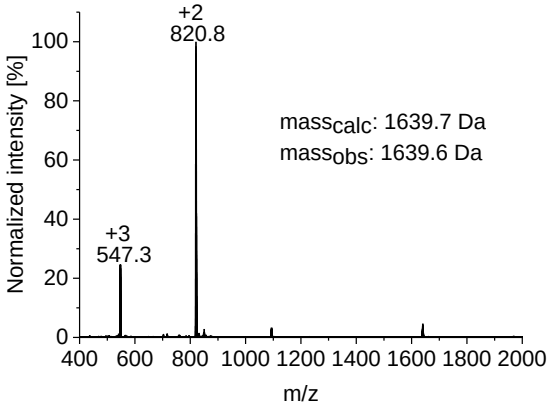
P9: EWACTACAKFLAAHA





P10: AGCKNFFWKTFTSC





Protein Expression and Purification

UBL3 Variants

The following plasmids were ordered from eurofins and delivered in a pEX-A2 vector with ampicillin resistance. They were cloned into a pET-28a(+) vector with a kanamycin resistance.

UBL3-1Cys:

CATATGGAGAACCTGTATTTCCAGGGTATGTCGAGCAATGTGCCAGCTGACATGATCAACTTACGCCTGATT
CTGGTATCTGGGAAAACGAAAGAGTTCCTGTTTTCACCGAACGATAGTGCGTCCGATATTGCCAAACACGTC
TATGACAACTGGCCGATGGATTGGGAGGAAGAACAGGTTAGCAGTCCGAACATTCTGCGCTTGATCTACCAA
GGTCGGTTTCTCCATGGCAATGTGACCTTAGGAGCGCTGAAACTGCCGTTTGGCAAAACCACAGTGATGCAT
TTGGTTGCACGTGAAACCCTTCCTGAACCCAATAGCCAAGGTCAGCGTAATCGCGAAAAGACTGGCGAATC
GAACTGCTAACTCGAG

UBL3-2Cys:

CATATGGAGAACCTGTATTTCCAGGGTATGTCGAGCAATGTGCCAGCTGACATGATCAACTTACGCCTGATT
CTGGTATCTGGGAAAACGAAAGAGTTCCTGTTTTCACCGAACGATAGTGCGTCCGATATTGCCAAACACGTC
TATGACAACTGGCCGATGGATTGGGAGGAAGAACAGGTTAGCAGTCCGAACATTCTGCGCTTGATCTACCAA
GGTCGGTTTCTCCATGGCAATGTGACCTTAGGAGCGCTGAAACTGCCGTTTGGCAAAACCACAGTGATGCAT
TTGGTTGCACGTGAAACCCTTCCTGAACCCAATAGCCAAGGTCAGCGTAATCGCGAAAAGACTGGCGAATC
GAACTGCTGCTAACTCGAG

Blue: NdeI restriction site

Green: XhoI restriction site

An overnight culture of *E. coli* BL21 DE3 transformed with the UBL3 plasmid was incubated in 100 mL LB medium containing kanamycin (30 µg/mL) at 37 °C. It was diluted with 1 L of fresh medium (30 µg/mL kanamycin) to an OD_{600} of 0.2-0.3 and incubated at 37 °C again. The cells were induced on reaching an OD_{600} of 0.6-0.8 with 1 mM IPTG and grown at 37 °C for 6 h. After harvesting by centrifugation (6000 rpm, 30 min, 4 °C), the pellet was resuspended in TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.5) and the cells were disrupted. After centrifugation (20000 rpm, 30 min, 4 °C) the supernatant was loaded on a HisTrap Ni-NTA column equilibrated with TBS buffer and it was eluted by using a linear imidazole gradient (0-300 mM imidazole in TBS). The pooled fractions were dialyzed against TBS buffer and the His_6 Tag was removed by a ratio of 1:30 of TEV protease. The TEV protease was removed by a HisTrap Ni-NTA column (linear gradient, 0-300 mM imidazole in TBS). Fractions containing the protein were pooled, 20 µL 100 µM TCEP were added and the protein was dialyzed against H_2O, lyophilized and stored at -20 °C. The protein was analyzed by HPLC and MS to confirm purity and molecular weight.

Hsp27 Protein

The following plasmid in pAK3038 vector was used for the expression of Hsp27:

ATGACCGAGCGCCGCGTCCCCTTCTCSCTCCTGNGGGGCCCCAGCTGGGACCCCTTCCGCGACTGGTACC
CGCATAGCCGCCTCTTCGACCAGGCCTTCGGGCTGCCCCGGCTGCCGGAGGAGTGGTCGCAGTGGTTAGG
CGGCAGCAGCTGGCCAGGCTACGTGCGCCCCCTGCCCCCCGCCGCCATCGAGAGCCCCGCAGTGGCCGC
GCCCGCCTACAGCCGCGCGCTCAGCCGGCAACTCAGCAGCGGGGTCTCGGAGATCCGGCACACTGCGGA
CCGCTGGCGCGTGTCCCTGGATGTCAACCACTTCGCCCCGGACGAGCTGACGGTCAAGACCAAGGATGGC
GTGGTGGAGATCACCGGCAAGCACGAGGAGCGGCAGGACGAGCATGGCTACATCTCCCGGTGCTTCACGC
GGAAATACACGCTGCCCCCCGGTGTGGACCCCACCCAAGTTTCCTCCTCCCTGTCCCCTGAGGGCACACTG
ACCGTGGAGGCCCCCATGCCCAAGCTAGCCACGCAGTCCAACGAGATCACCATCCCAGTCACCTTCGAGTC
GCGGGCCCAGCTTGGGGGCCCAGAAGCTGCAAAATCCGATGAGACTGCCGCCAAG

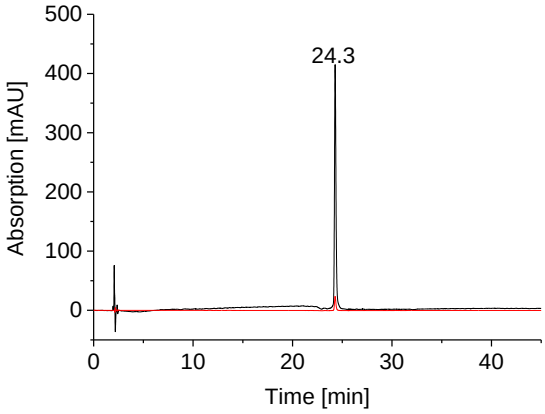
An overnight culture of *E. coli* BL21 DE3 RIL containing the Hsp27 plasmid was incubated in 200 mL LB medium containing ampicillin (100 µg/mL) at 37 °C. After diluting with 2 L of fresh medium (100 µg/mL ampicillin) to an OD_{600} of 0.2-0.3 and incubation at 37 °C again, the cells were induced on reaching an OD_{600} of 0.6-0.8 with 1 mM IPTG and grown at 37 °C for 4 h. The cells were harvested by centrifugation (6000 rpm, 30 min, 4 °C), the pellet was resuspended in TBS buffer (50 mM Tris, 150 mM NaCl, pH 7.5) and the cells were disrupted. After centrifugation (20000 rpm, 30 min, 4 °C), ammonium sulfate was added to the supernatant while stirring on ice until a saturation of 40 % was reached. The mixture was centrifuged (4000 rpm, 15 min, 4 °C), the pellet was dissolved in 20 mM Tris (pH 7.6), 10 mM $MgCl_2$, 30 mM NH_4Cl and 20 µL 100 µM TCEP were added. The protein was purified by HPLC and fractions containing the protein were pooled, lyophilized and stored at -20 °C. The protein was analyzed by HPLC and MS to confirm purity and molecular weight.

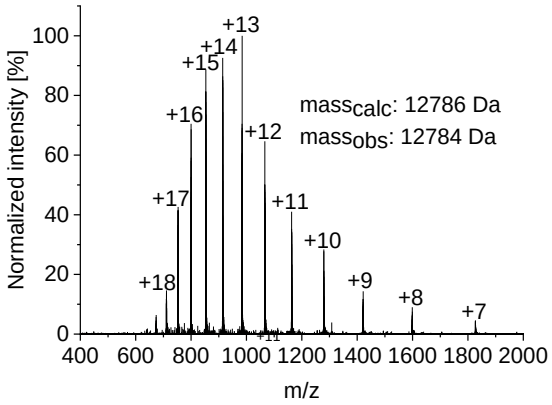
Table S3. Protein sequences and masses.

Name	**Sequence**	**$Mass_{calc}$:**	**$Mass_{obs}$:**
UBL3-1Cys	GMSSNVPADMINLRLILVSGKTKEFLFSPND SASDIAKHVYDNWPMDWEEEQVSSPNILR LIYQGRFLHGNVTLGALKLPFGKTTVMHLV ARETLPEPNSQGQRNREKTGESNC	12786 Da	12784 Da
UBL3-2Cys	GMSSNVPADMINLRLILVSGKTKEFLFSPND SASDIAKHVYDNWPMDWEEEQVSSPNILR LIYQGRFLHGNVTLGALKLPFGKTTVMHLV ARETLPEPNSQGQRNREKTGESNCC	12887 Da	12886 Da
Hsp27	(M)TERRVPFSLLRGPSWDPFRDWYPHSRL FDQAFGLPRLPEEWSQWLGGSSWPGYVR PLPPAAIESPAVAAPAYSRALSRQLSSGVS EIRHTADRWRVSLDVNHFAPDELTVKTKDG VVEITGKHEERQDEHGYISRCFTRKYTLPP GVDPTQVSSSLSPEGTLTVEAPMPKLATQS NEITIPVTFESRAQ LGGPEAAKSDETAAK	(22783 Da) 22651 Da	(22783 Da) 22653 Da

Analysis of the Proteins

UBL3-1Cys:





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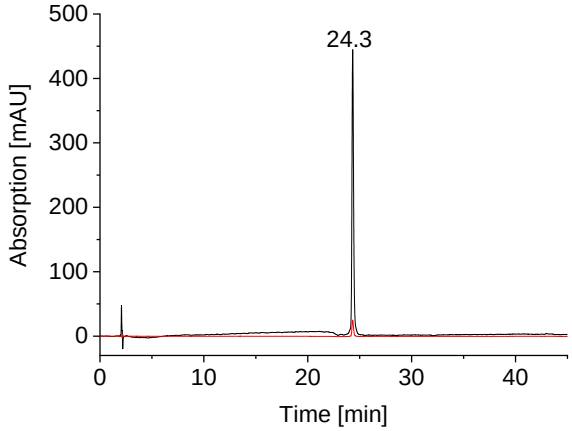
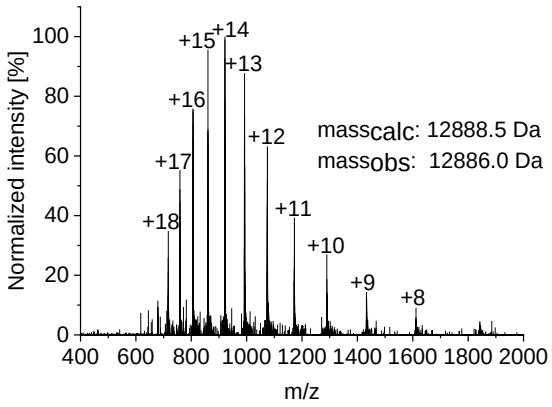
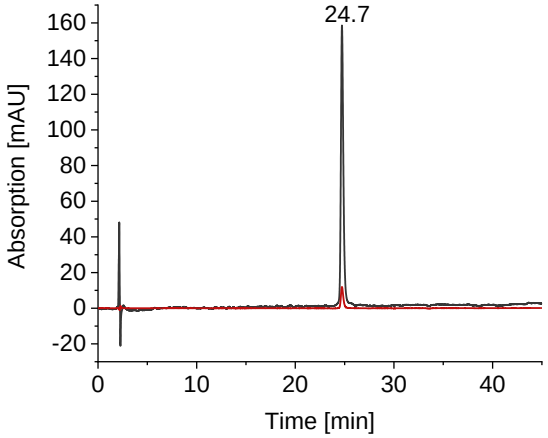
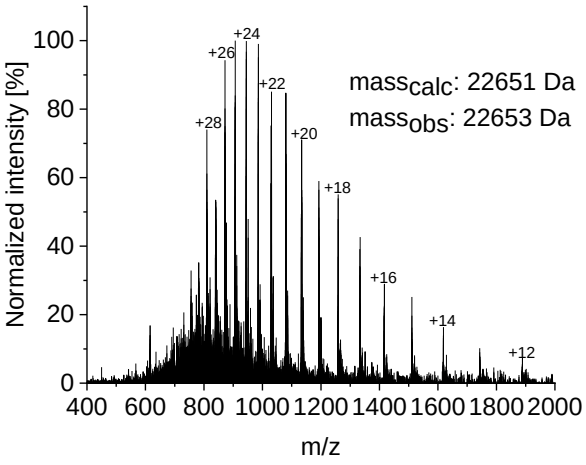




Hsp27:





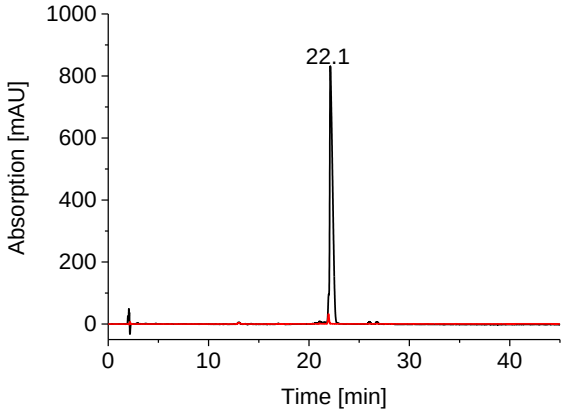
Allylation of Oligopeptides

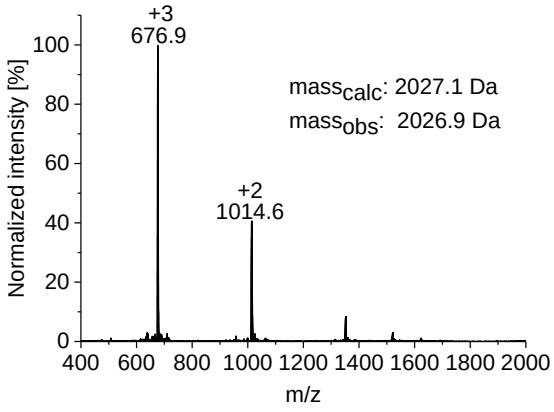
General Procedure for the Allylation of Oligopeptides

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ and BIPHEPHOS were suspended in 1.30 mL anhydrous CH_3CN and stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Afterwards, the solution was cooled to 40 °C before a solution of allylation reagent in anhydrous CH_3CN and a solution of the oligopeptide in 0.67 mL degassed H_2O were added. The resulting mixture was stirred at 40 °C until complete consumption of the starting material (reaction monitoring via HPLC-MS) was observed. The crude products were purified via analytical RP-HPLC on a C4 column (Kromasil 300-5-C4 4.6x50 mm) on a Dionex Ultimate 3000 system. The runs were performed at 1 mL/min with ddH_2O + 0.05 % TFA as buffer A and ACN + 0.05 % TFA as buffer B. For the mono-modified products a gradient from 5-25 % B in 5 min and then from 25-45 % B in 25 min was used. For the di-farnesylated products the second part of the gradient was extended to 25-75 % B in 25 min. The final analysis of all peptides was performed with a gradient from 5-65 % B in 30 min.

Geranylation of GQRNREKTGESNSCVIL (P3b)

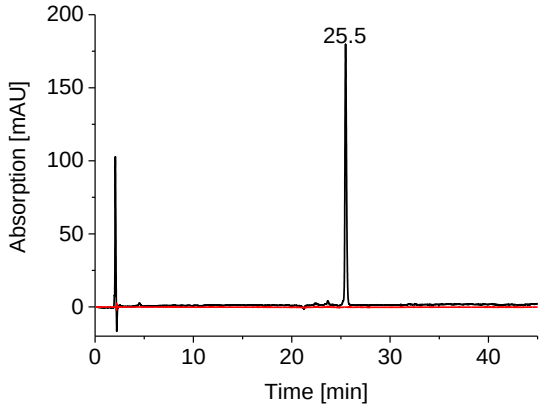
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Rb** (27.4 µL, 88 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.3 mg, 2.3 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) for 6 h. HPLC purification afforded the desired product with a purity of > 93 %.

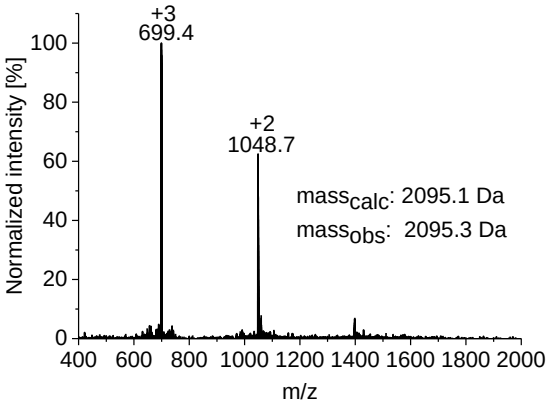




Farnesylation of GQRNREKTGESNSCVIL (P3c)

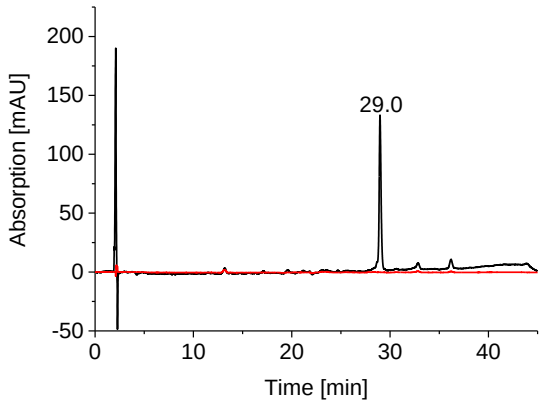
Following the general procedure, peptide (**P3**) (3.7 mg, 2.0 µmol) was reacted with **Rc** (29.0 µL, 83 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) for 5 h. HPLC purification afforded the desired product with a purity of > 96 %.

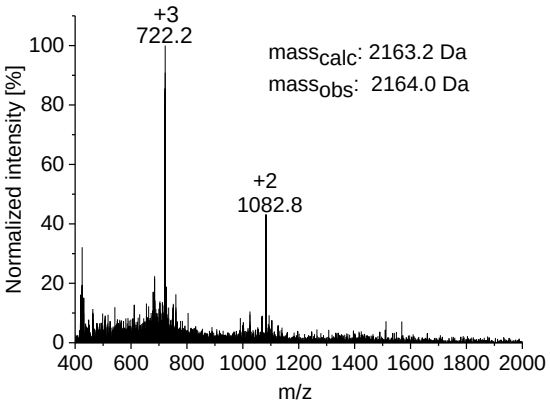




Geranylgeranylation of GQRNREKTGESNSCVIL (P3d)

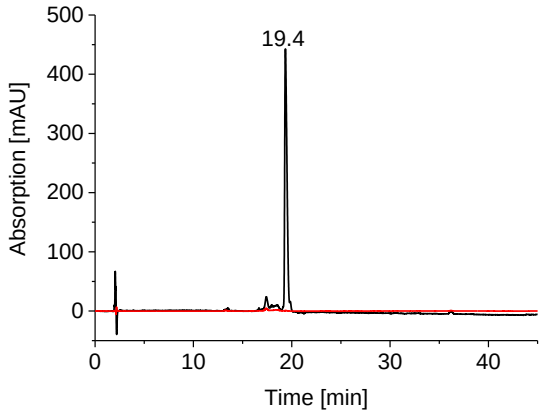
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Rd** (29.2 µL, 82 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.5 mg, 2.6 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) for 6 h. HPLC purification afforded the desired product with a purity of > 81 %.

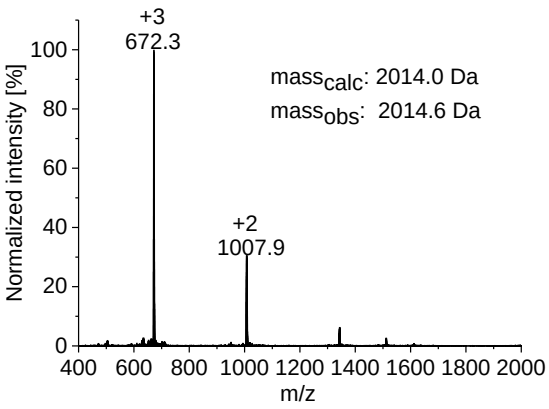




Azide-Labelling of GQRNREKTGESNSCVIL (P3e)

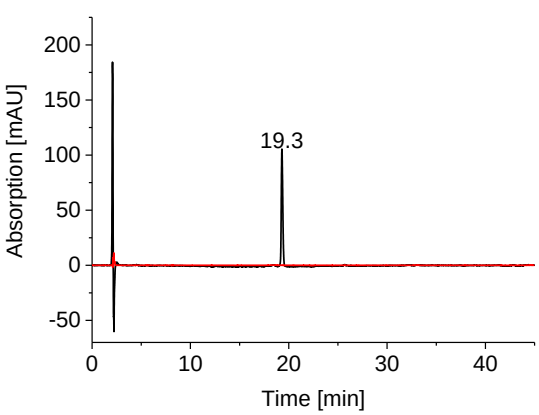
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Re** (28.8 µL, 83 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.5 mg, 2.6 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) for 3 h. HPLC purification afforded the desired product with a purity of > 92 %.

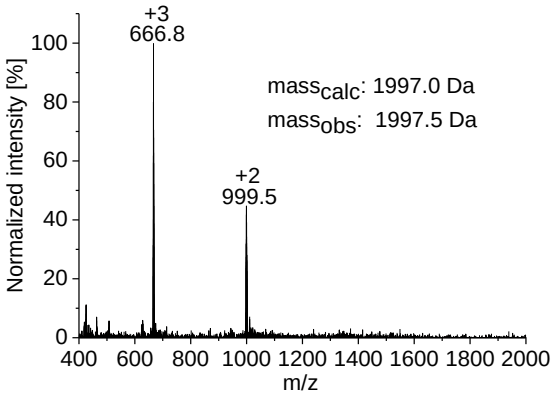




Alkyne-Labelling of GQRNREKTGESNSCVIL (P3f)

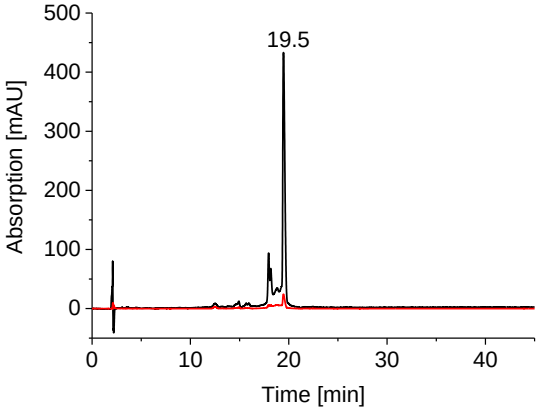
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Rf** (29.1 µL, 82 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) for 3 h. HPLC purification afforded the desired product with a purity of > 99 %.

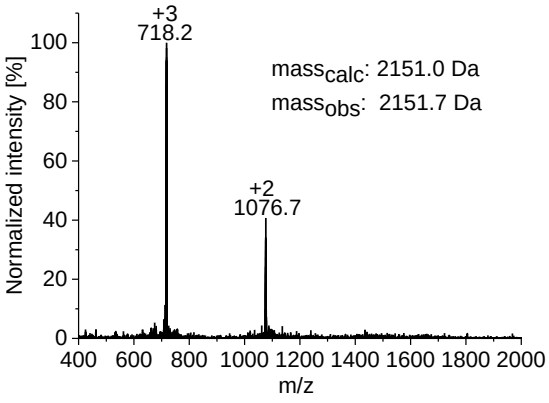




NBD-Labelling of GQRNREKTGESNSCVIL (P3g)

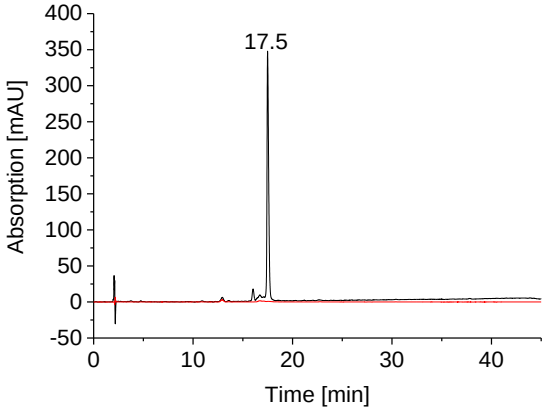
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Rg** (0.9 mg, 2.7 µmol) in presence of $Pd(dba)_2$ (1.3 mg, 2.3 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) for 3 h. HPLC purification afforded the desired product with a purity of > 77 %.

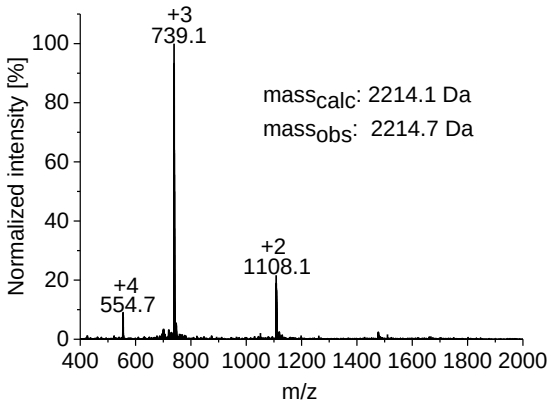




Biotin-Labelling of GQRNREKTGESNSCVIL (P3h)

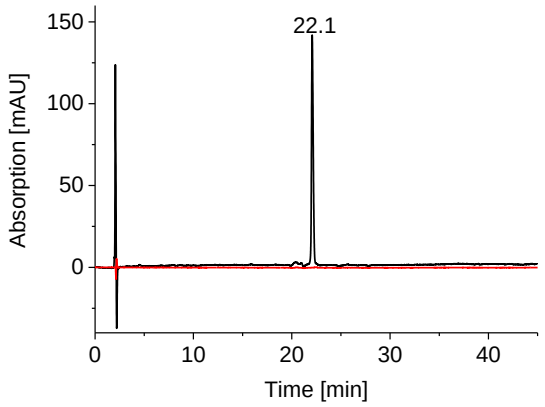
Following the general procedure, peptide (**P3**) (3.8 mg, 2.0 µmol) was reacted with **Rh** (1.0 mg, 2.5 µmol) in presence of $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) for 5 h. HPLC purification afforded the desired product with a purity of > 92 %.

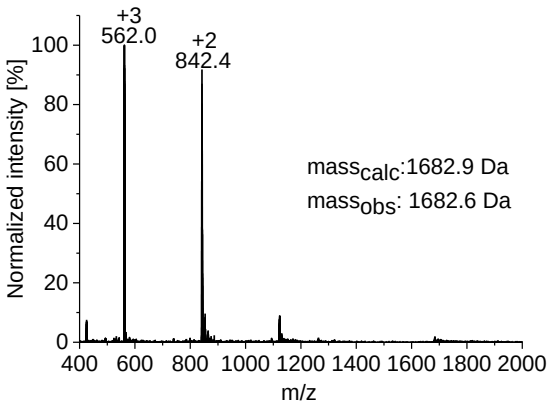




Farnesylation of GQRNREKTGESNC (P4c)

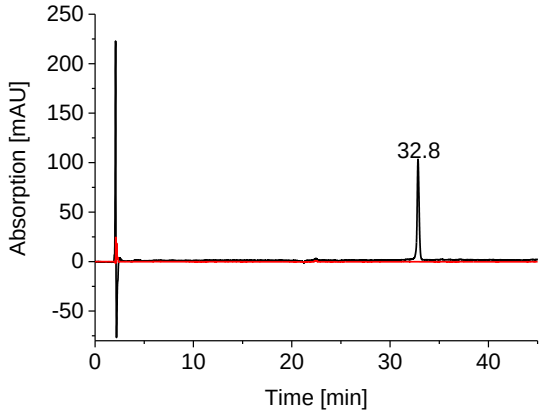
Following the general procedure, peptide (**P4**) (2.9 mg, 2.0 µmol) was reacted with **Rc** (22.4 µL, 107 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) for 18 h. HPLC purification afforded the desired product with a purity of > 99 %.

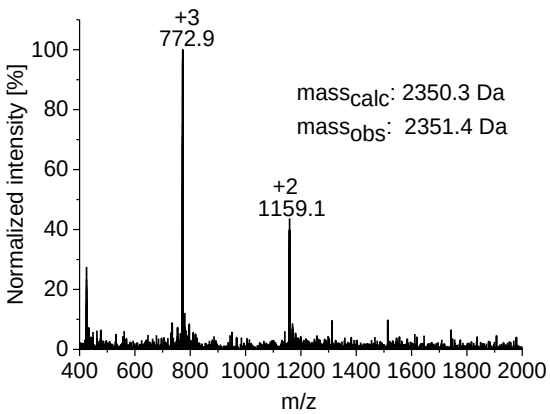




Farnesylation of GQRNREKTGESNCCVIL (P5c)

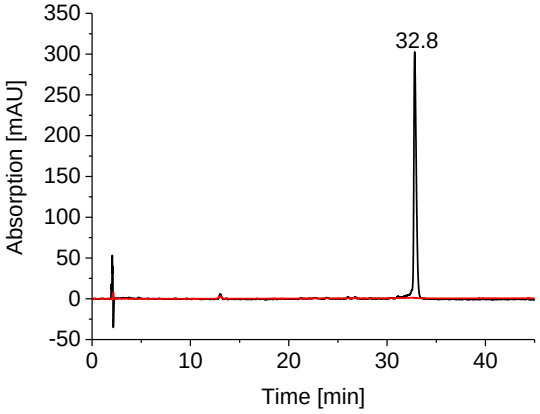
Following the general procedure, peptide (**P5**) (3.8 mg, 2.0 µmol) was reacted with **Rc** (44.9 µL, 107 mM, 4.8 µmol) in presence of $Pd(dba)_2$ (2.8 mg, 4.9 µmol) and BIPHEPHOS (3.9 mg, 4.9 µmol) for 18 h. HPLC purification afforded the desired product with a purity of > 99 %.

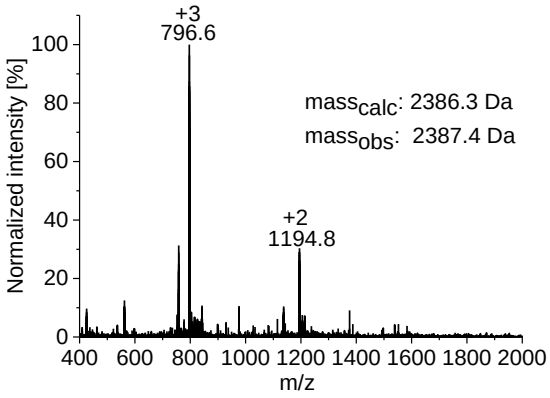




Farnesylation of GQRNREKTGESNCACVIL (P6c)

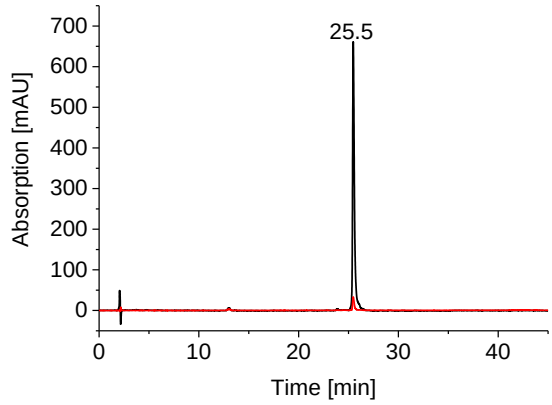
Following the general procedure, peptide (**P6**) (3.9 mg, 2.0 µmol) was reacted with **Rc** (44.9 µL, 107 mM, 4.8 µmol) in presence of $Pd(dba)_2$ (2.7 mg, 4.7 µmol) and BIPHEPHOS (3.7 mg, 4.7 µmol) for 17 h. HPLC purification afforded the desired product with a purity of > 97 %.

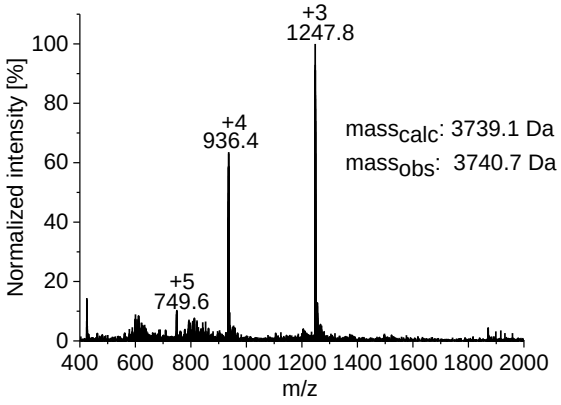




Farnesylation of MGIINTLQKYYARVRGGRCAVLSALPKEEQIG (P7c)

Following the general procedure, peptide (**P7**) (7.0 mg, 2.0 µmol) was reacted with **Rc** (22.4 µL, 107 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (2.1 mg, 2.7 µmol) for 17 h. HPLC purification afforded the desired product with a purity of > 96 %.

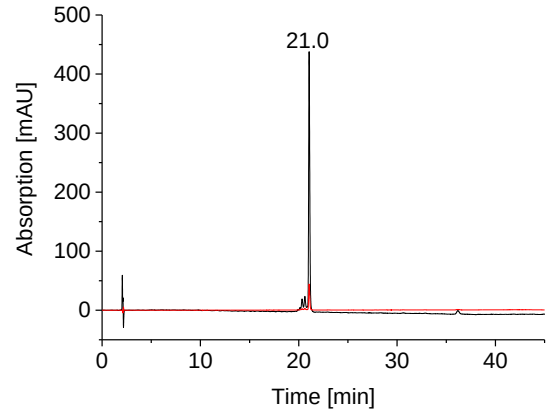


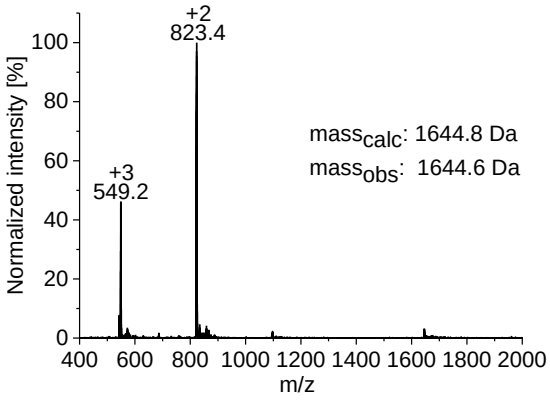


(*Z*)-Butenediyl-Stapling of EWACTAACKFLAAHA (P8i)

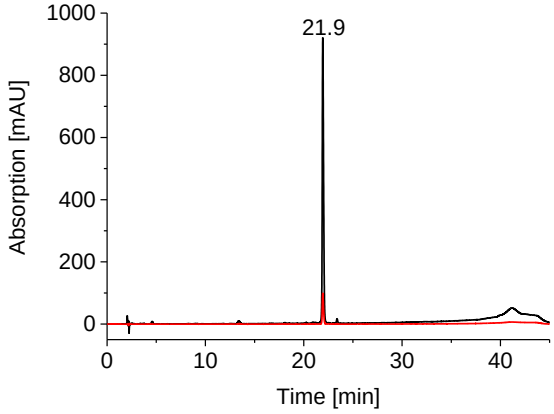
Following the general procedure, peptide (**P8**) (3.2 mg, 2.0 µmol) was reacted with **Ri** (28.5 µL, 84 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.7 mg, 4.7 µmol) and BIPHEPHOS (3.7 mg, 4.7 µmol) for 3 h. HPLC purification afforded two products with the expected mass with a purity of > 90 % and > 96 %.

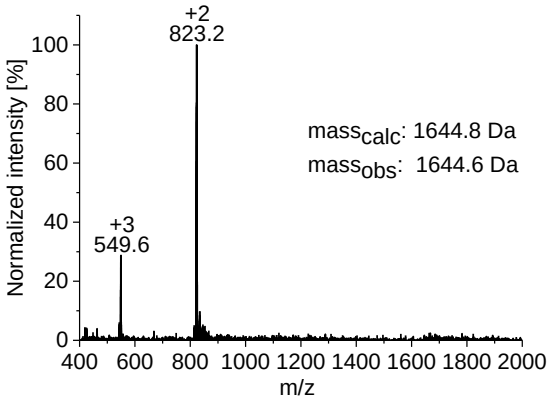
Product 1:





Product 2:

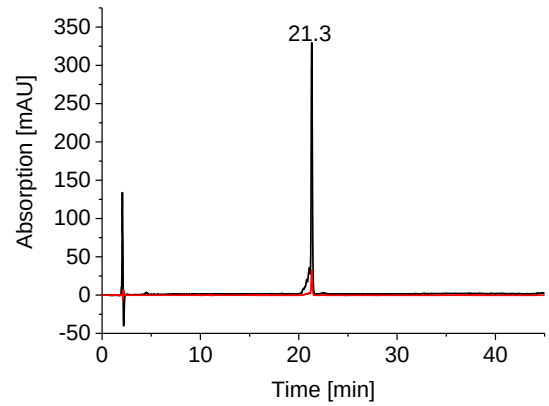


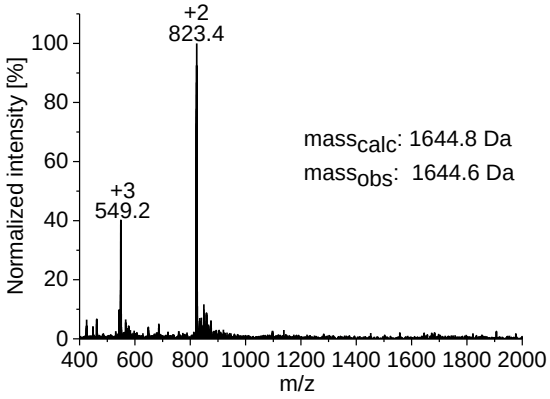


(*Z*)-Butenediyl-Stapling of EWACTACAKFLAAHA (P9i)

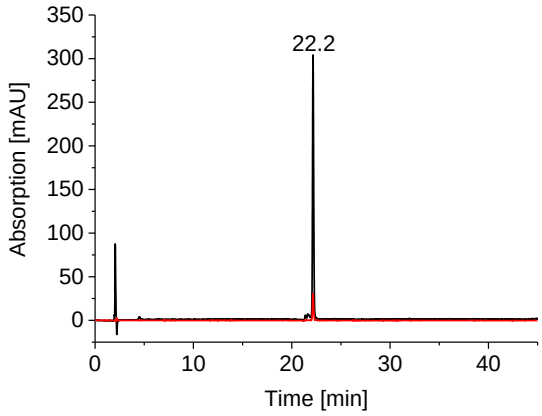
Following the general procedure, peptide (**P9**) (3.2 mg, 2.0 µmol) was reacted with **Ri** (28.5 µL, 84 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.7 mg, 4.7 µmol) and BIPHEPHOS (3.9 mg, 4.9 µmol) for 3 h. HPLC purification afforded two products with the expected mass with a purity of > 75 % and > 96 %.

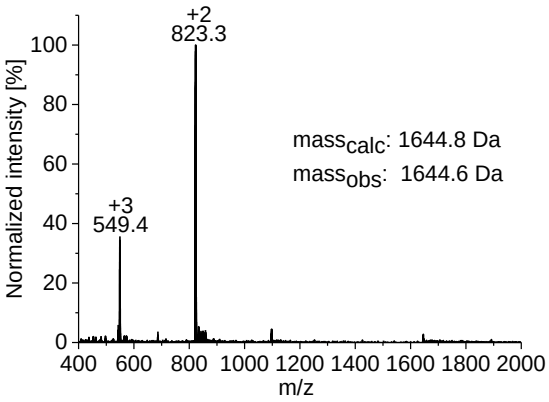
Product 1:





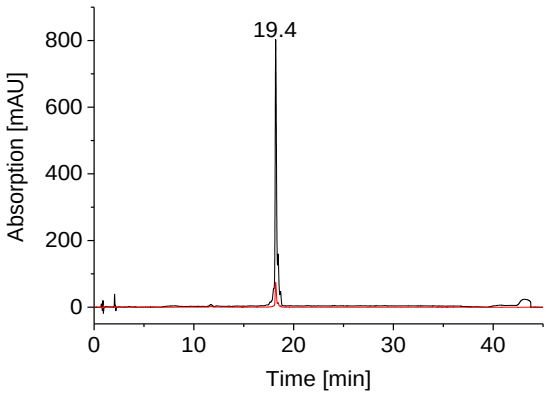
Product 2:

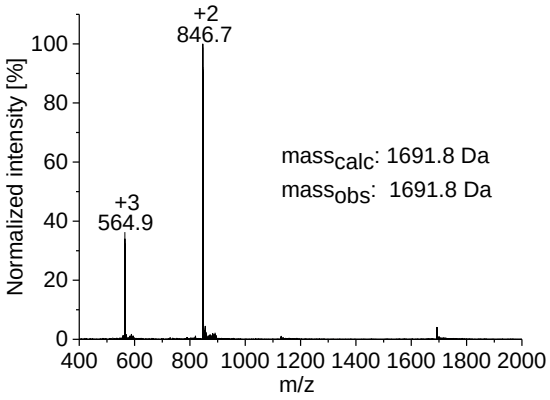




(*Z*)-Butenediyl-Stapling of AGCKNFFWKTFTSC (P10i)

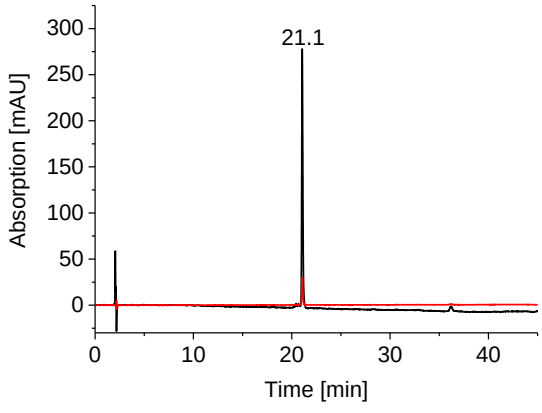
Following the general procedure, peptide (**P10**) (3.3 mg, 2.0 µmol) was reacted with **Ri** (28.5 µL, 84 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.8 mg, 4.9 µmol) and BIPHEPHOS (3.8 mg, 4.8 µmol) for 3 h. HPLC purification afforded the desired product with a purity of > 76 %.

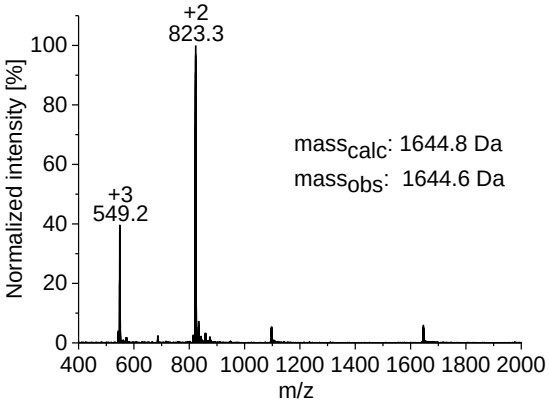




***i*-Butenediyl-Stapling of EWACTAACKFLAAHA (P8j)**

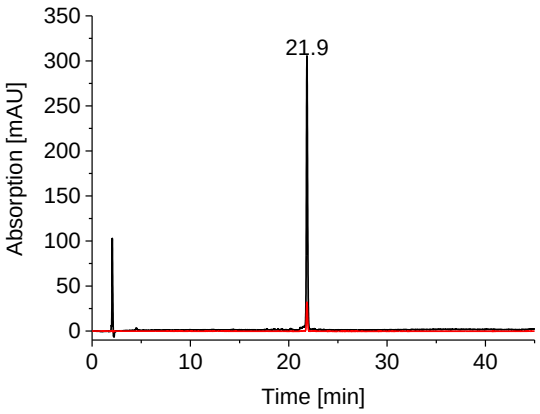
Following the general procedure, peptide (**P8**) (3.2 mg, 2.0 µmol) was reacted with **Rj** (30.6 µL, 78 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.8 mg, 4.9 µmol) and BIPHEPHOS (3.9 mg, 4.9 µmol). HPLC purification afforded the desired product with a purity of > 97 %.

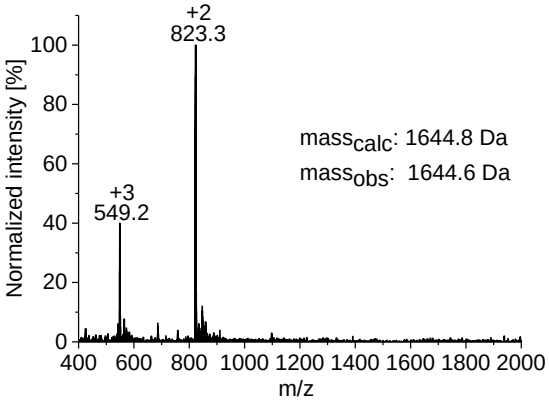




i-Butenediyl-Stapling of EWACTACAKFLAAHA (P9j)

Following the general procedure, peptide (**P9**) (3.2 mg, 2.0 µmol) was reacted with **Rj** (30.6 µL, 78 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.8 mg, 4.9 µmol) and BIPHEPHOS (3.8 mg, 4.8 µmol) for 3 h. HPLC purification afforded the desired product with a purity of > 98 %.

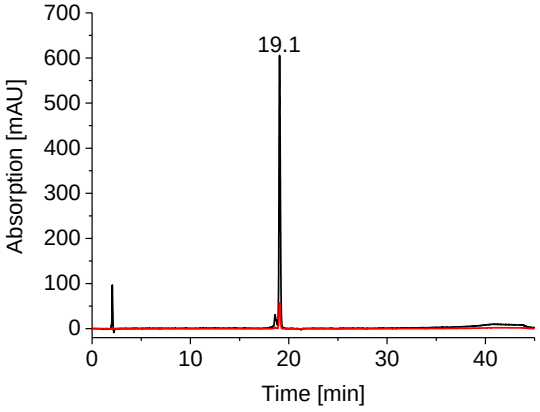


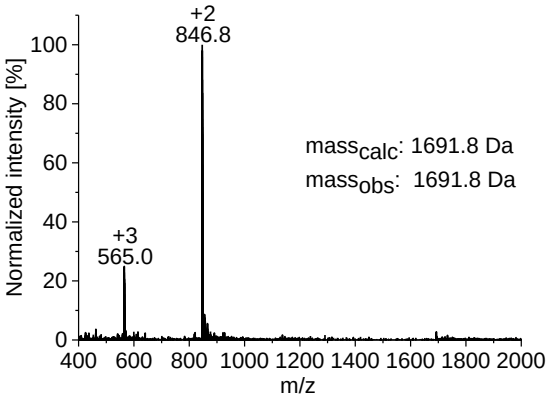


i-Butenediyl-Stapling of AGCKNFFWKTFTSC (P10j)

Following the general procedure, peptide (**P10**) (3.3 mg, 2.0 µmol) was reacted with **Rj** (30.6 µL, 78 mM, 2.4 µmol) in presence of $Pd(dba)_2$ (2.9 mg, 5.0 µmol) and BIPHEPHOS (3.8 mg, 4.8 µmol) for 3 h. HPLC purification afforded two products with the expected mass and the dimer with a purity of > 95 %, > 86 % and > 99 %.

Product 1:





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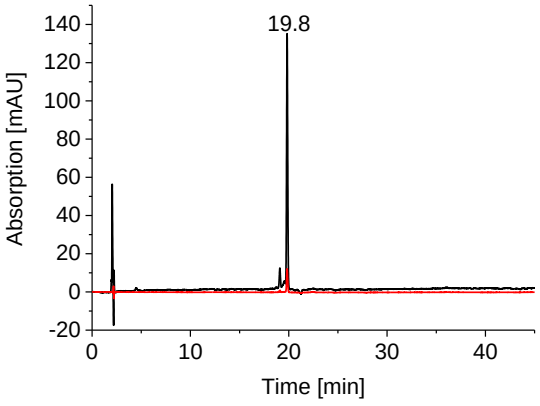
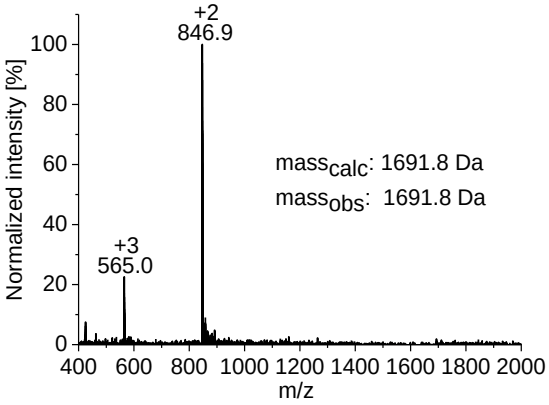




Product 3: Dimer

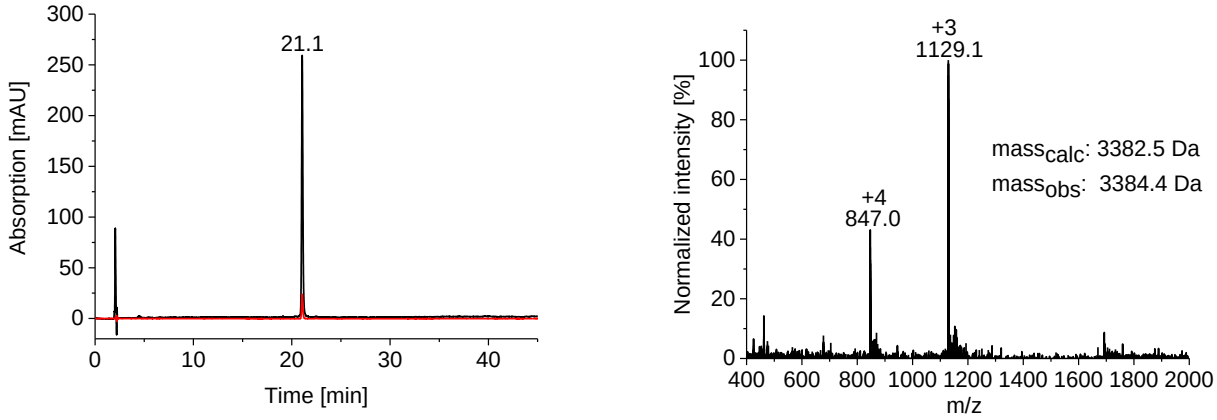


Table S4. Sequences and masses of all modified peptides and proteins.

Name	Sequence	Modification	Mass$_{calc}$:	Mass$_{obs}$:
P3b	GQRNREKTGESNSCVIL	Geranylation	2027.1 Da	2026.9 Da
P3c	GQRNREKTGESNSCVIL	Farnesylation	2095.1 Da	2095.3 Da
P3d	GQRNREKTGESNSCVIL	Geranylgeranylation	2163.2 Da	2164.0 Da
P3e	GQRNREKTGESNSCVIL	Azide	2014.0 Da	2014.6 Da
P3f	GQRNREKTGESNSCVIL	Alkyne	1997.0 Da	1997.5 Da
P3g	GQRNREKTGESNSCVIL	NBD	2151.0 Da	2151.7 Da
P3h	GQRNREKTGESNSCVIL	Biotin	2214.1 Da	2214.7 Da
P4c	GQRNREKTGESNSC	Farnesylation	1682.9 Da	1682.5 Da
P5c	GQRNREKTGESNSCCVIL	Farnesylation	2350.3 Da	2351.4 Da
P6c	GQRNREKTGESNSCACVIL	Farnesylation	2386.3 Da	2387.4 Da
P7c	MGIINTLQKYYARVRGGRC AVLSALPKEEQIG	Farnesylation	3739.1 Da	3740.7 Da
P8i	EWACTAACKFLAAHA	Staple with (*Z*)-butendiyl	1644.8 Da	1644.6 Da
P8j	EWACTAACKFLAAHA	Staple with *i*-butendiyl	1644.8 Da	1644.6 Da
P9i	EWACTACAKFLAAHA	Staple with (*Z*)-butendiyl	1644.8 Da	1644.6 Da
P9j	EWACTACAKFLAAHA	Staple with *i*-butendiyl	1644.8 Da	1644.6 Da
P10i	AGCKNFFWKTFTSC	Staple with (*Z*)-butendiyl	1691.8 Da	1691.8 Da
P10j	AGCKNFFWKTFTSC	Staple with *i*-butendiyl	1691.8 Da	1691.8 Da 3384.4 Da
UBL3-1Cys	GMSSNVPADMINLRLILVSGKTKEF LFSPNDSASDIAKHVYDNWPMDW EEEQVSSPNILRLIYQGRFLHGNVT LGALKLPFGKTTVMHLVARETLPEP NSQGQRNREKTGESNC	Geranylgeranylation	13059 Da	13059 Da
UBL3-2Cys	GMSSNVPADMINLRLILVSGKTKEF LFSPNDSASDIAKHVYDNWPMDW EEEQVSSPNILRLIYQGRFLHGNVT LGALKLPFGKTTVMHLVARETLPEP NSQGQRNREKTGESNCC	Geranylgeranylation	13436 Da	13435 Da

UBL3-1Cys	GMSSNVPADMINLRLILVSGKTKEF LFSPNDSASDIAKHVYDNWPMDW EEEQVSSPNILRLIYQGRFLHGNVT LGALKLPFGKTTVMHLVARETLPEP NSQGQRNREKTGESNC	Alkyne	12894 Da	12894 Da
Hsp27	(M)TERRVPFSLLRGPSWDPFRDW YPHSRLFDQAFGLPRLPEEWSQW LGGSSWPGYVRPLPPAAIESPAVA APAYSRALSRQLSSGVSEIRHTAD RWRVSLDVNHFAPDELTVKTKDGV VEITGKHEERQDEHGYISRCFTRKY TLPPGVDPTQVSSSLSPEGTLTVE APMPKLATQSNEITIPVTFESRAQ LGGPEAAKSDETAAK	Alkyne	(22892 Da) 22761 Da	(22893 Da) 22762 Da
Hsp27	(M)TERRVPFSLLRGPSWDPFRDW YPHSRLFDQAFGLPRLPEEWSQW LGGSSWPGYVRPLPPAAIESPAVA APAYSRALSRQLSSGVSEIRHTAD RWRVSLDVNHFAPDELTVKTKDGV VEITGKHEERQDEHGYISRCFTRKY TLPPGVDPTQVSSSLSPEGTLTVE APMPKLATQSNEITIPVTFESRAQ LGGPEAAKSDETAAK	Azide	(22908 Da) 22777 Da	(22911 Da) 22779 Da

Geranylgeranylation of Proteins

Single-Modification of UBL3-1Cys

Preparation of stock solutions: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (2.0 mg, 2.5 µmol) were suspended in 1.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution and cooled to rt (= **stock A**).

In a flame-dried and argon-flushed 10 mL Schlenk flask, **Rd** (2.5 mg, 7.2 µmol) was dissolved in 0.50 mL anhydrous CH_3CN (= **stock B**).

In an evacuated and argon-flushed 10 mL Schlenk flask, UBL3-1Cys (5.2 mg, 4.1 µmol) was dissolved in 0.20 mL degassed 3 M Gdn•HCl solution (= **stock C**).

Reaction: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, 0.10 mL of **stock A** were heated to 40 °C before 16.7 µL of **stock B** and 0.10 mL of **stock C** were added. The resulting mixture was stirred at 40 °C (reaction monitoring via HPLC-MS: 32 % conv. after 6 h). The crude product after lyophilization was purified as described for the modified peptides with a gradient from 5-25 % B in 5 min and then from 25-45 % B in 25 min.

Double-Modification of UBL3-2Cys

Preparation of stock solutions: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (3.1 mg, 5.4 µmol) and BIPHEPHOS (4.1 mg, 5.2 µmol) were suspended in 1.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution and cooled to rt (= **stock A**).

In a flame-dried and argon-flushed 10 mL Schlenk flask, **Rd** (1.9 mg, 5.5 µmol) was dissolved in 0.20 mL anhydrous CH_3CN (= **stock B**).

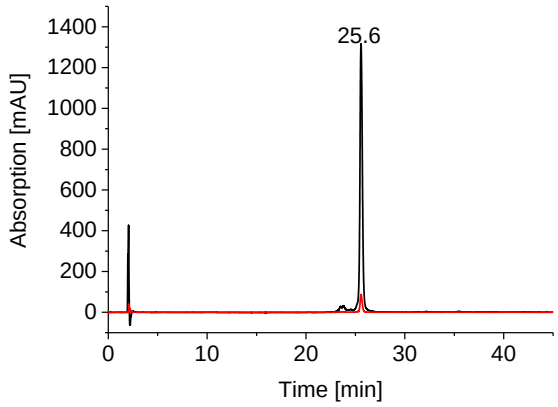
In an evacuated and argon-flushed 10 mL Schlenk flask, UBL3-2Cys (6.5 mg, 5.0 µmol) was dissolved in 0.25 mL degassed 3 M Gdn•HCl solution (= **stock C**).

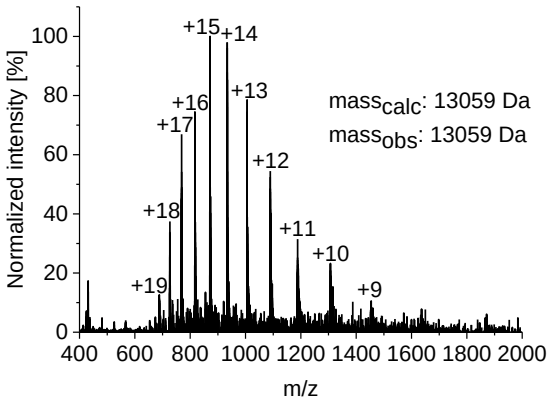
Reaction: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, 0.21 mL of **stock A** were heated to 40 °C before 44.0 µL of **stock B** and all of **stock C** were added. The resulting mixture was stirred at 40 °C (reaction monitoring via HPLC-MS: 59 % conv. after 2 h). The crude product after lyophilization was purified as described for the modified peptides with a gradient from 5-25 % B in 5 min and then from 25-65 % B in 25 min.

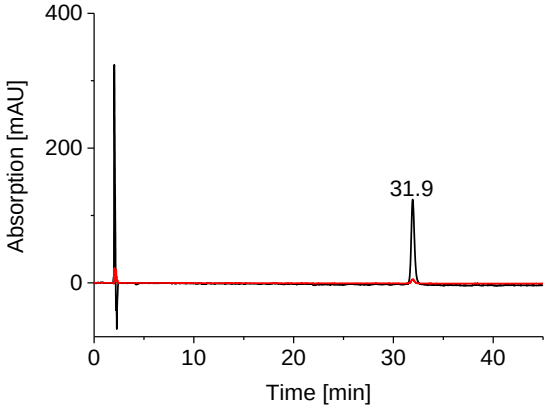
Analysis of the Modified Proteins

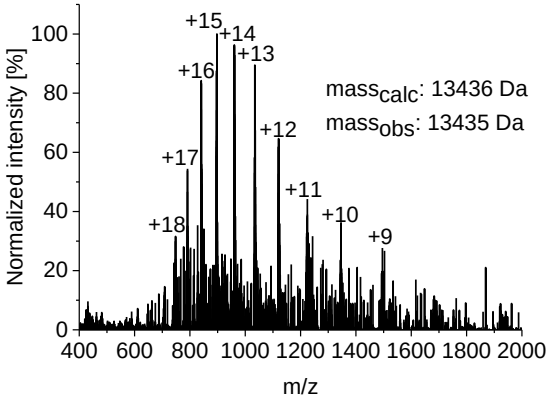
UBL3-1Cys-Gerger:





UBL3-2Cys-Gerger:





Further Modifications of Proteins

Modification of UBL3-1Cys with Alkyne-Reagent

Preparation of stock solution: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (5.5 mg, 9.6 µmol) and BIPHEPHOS (7.6 mg, 9.7 µmol) were suspended in 8.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Subsequently, **Rf** (1.9 mg, 10 µmol) was added and allowed to cool to rt (= **stock A**).

Reaction: (under oxygen-free conditions)

In an evacuated and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, UBL3-1Cys (3.0 mg, 0.23 µmol) was dissolved in 0.25 mL degassed H_2O and heated to 40 °C. Then 0.25 mL of **stock A** were added and the resulting suspension was stirred at 40 °C (reaction monitoring via HPLC-MS: full conv. after 4 h). The crude product after lyophilization was purified as described for the modified peptides with a gradient from 5-65 % B in 30 min.

Modification of Hsp27 with Alkyne-Reagent

Preparation of stock solution: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) were suspended in 2.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Subsequently, **Rf** (0.44 mg, 2.4 µmol) was added and allowed to cool to rt (= **stock B**).

Reaction: (under oxygen-free conditions)

In an evacuated and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, Hsp27 (5.7 mg, 0.25 µmol) was dissolved in 0.25 mL degassed H_2O and heated to 40 °C. Then 0.25 mL of **stock B** were added and the resulting suspension was stirred at 40 °C (reaction monitoring via HPLC-MS: full conv. after 2 h). The crude product after lyophilization was purified as described for the modified peptides with a gradient from 5-65 % B in 30 min.

Modification of Hsp27 with Azide-Reagent

Preparation of stock solution: (under oxygen-free conditions)

In a flame-dried and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, $Pd(dba)_2$ (1.4 mg, 2.4 µmol) and BIPHEPHOS (1.9 mg, 2.4 µmol) were suspended in 2.0 mL anhydrous CH_3CN, stirred in a pre-heated oil bath at 60 °C for 30 min to obtain a bright yellow solution. Subsequently, **Re** (0.48 mg, 2.4 µmol) was added and allowed to cool to rt (= **stock C**).

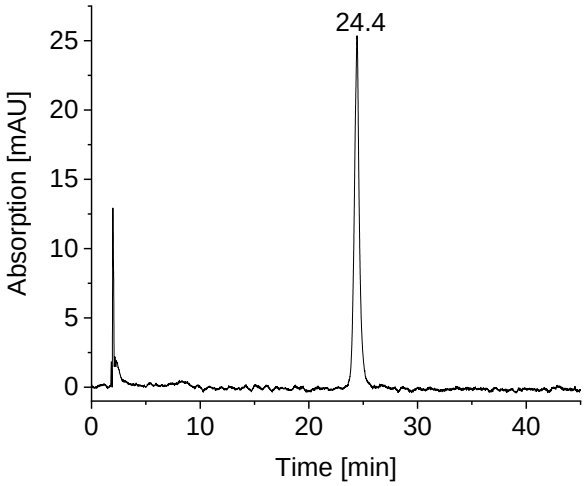
Reaction: (under oxygen-free conditions)

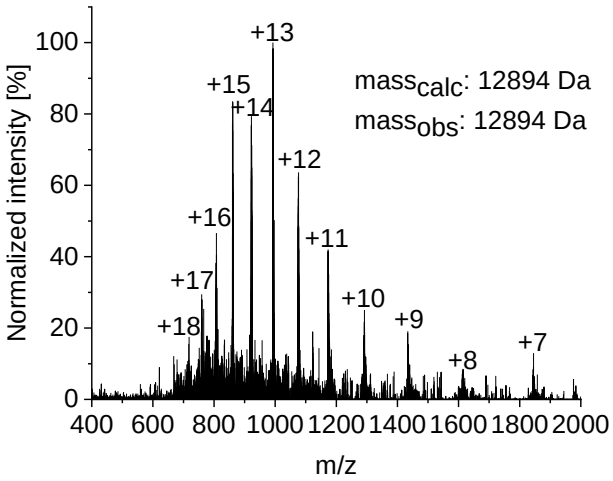
In an evacuated and argon-flushed 10 mL Schlenk flask, equipped with a Teflon-coated magnetic stirring bar, Hsp27 (5.7 mg, 0.25 µmol) was dissolved in 0.25 mL degassed H_2O and heated to 40 °C. Then 0.25 mL of **stock C** were added and the resulting suspension was stirred at 40 °C (reaction monitoring via HPLC-MS: full conv. after

2 h). The crude product after lyophilization was purified as described for the modified peptides with a gradient from 5-65 % B in 30 min.

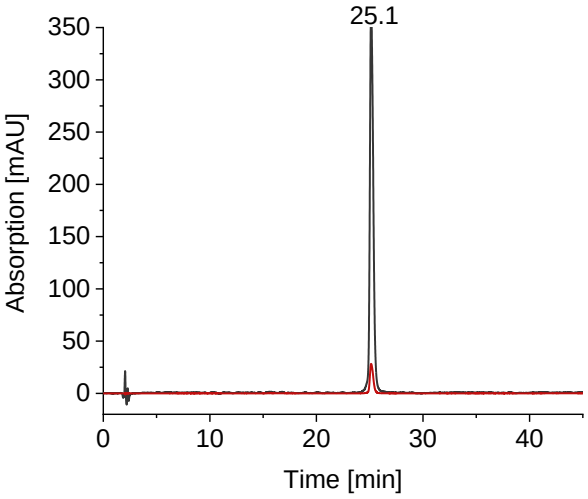
Analysis of the Modified Proteins

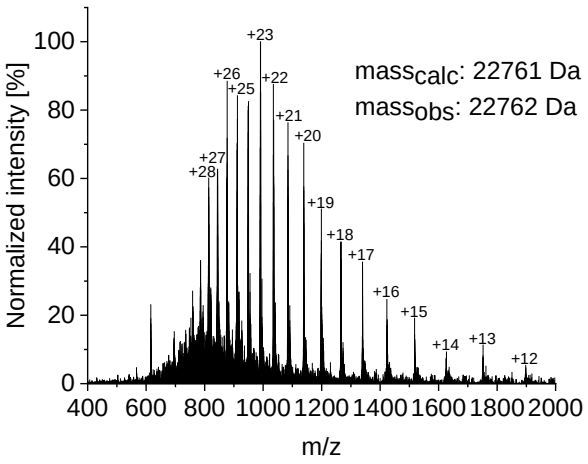
UBL3-1Cys-Alkyne:





Hsp27-Alkyne:





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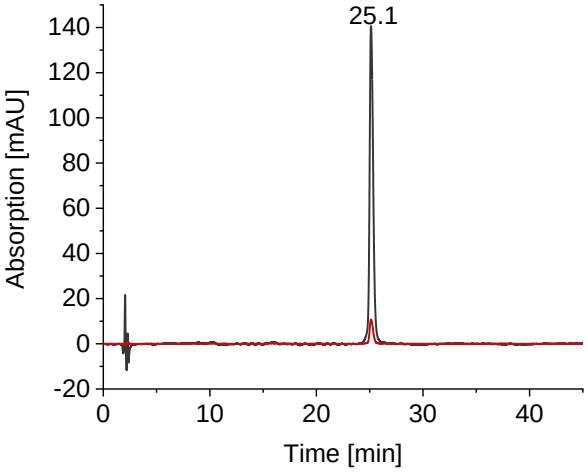
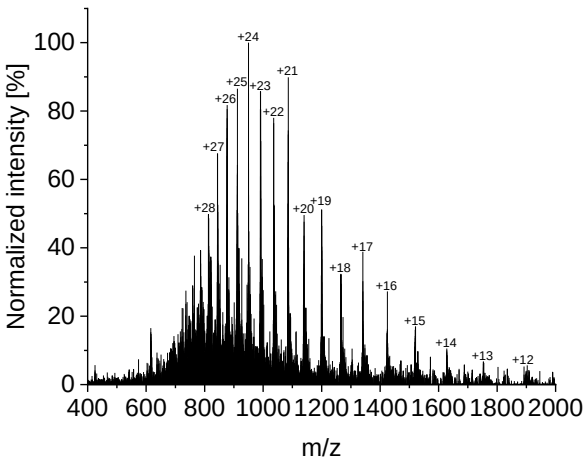
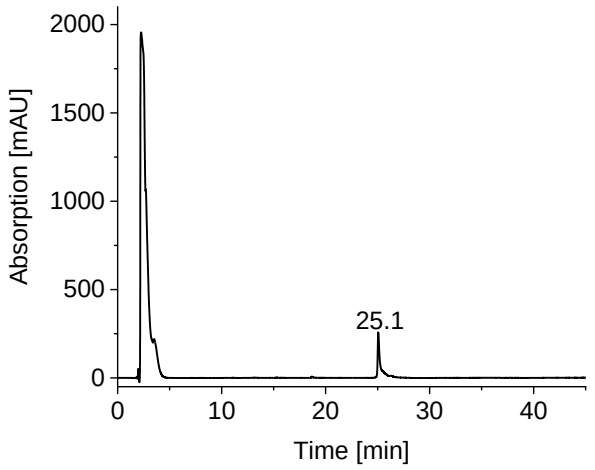
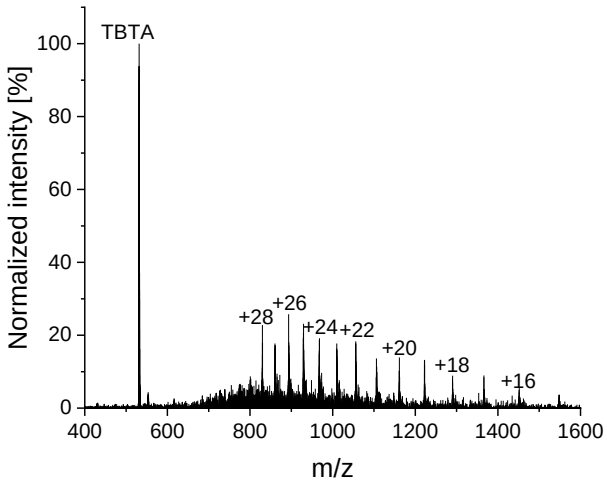


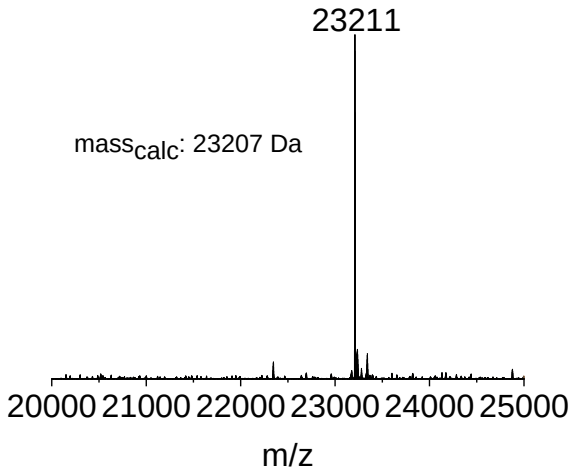


CuAAC of Hsp27-alkyne with azide-PEG3-biotin

The CuAAC was carried out in an Eppi with a reaction volume of 10 µL with final concentrations 1 mM Hsp27-alkyne, 3 mM azide-PEG3-biotin, 24 mM $CuSO_4$, 26 mM TBTA and 40 mM sodium ascorbate. 1.2 µL 200 mM $CuSO_4$ (in water) and 2.6 µL 100 mM TBTA (in DMF) were mixed and 0.8 µL 500 mM sodium ascorbate (in water) was added. To this, the mixture of Hsp27-alkyne and azide-PEG3-biotin in DMF was added. The reaction was stirred for 10 min at rt and analysis by HPLC(-MS) showed full conversion. For this, the sample was diluted with H_2O/ACN (1:1).







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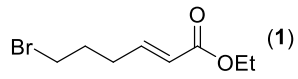
NMR-Spectra



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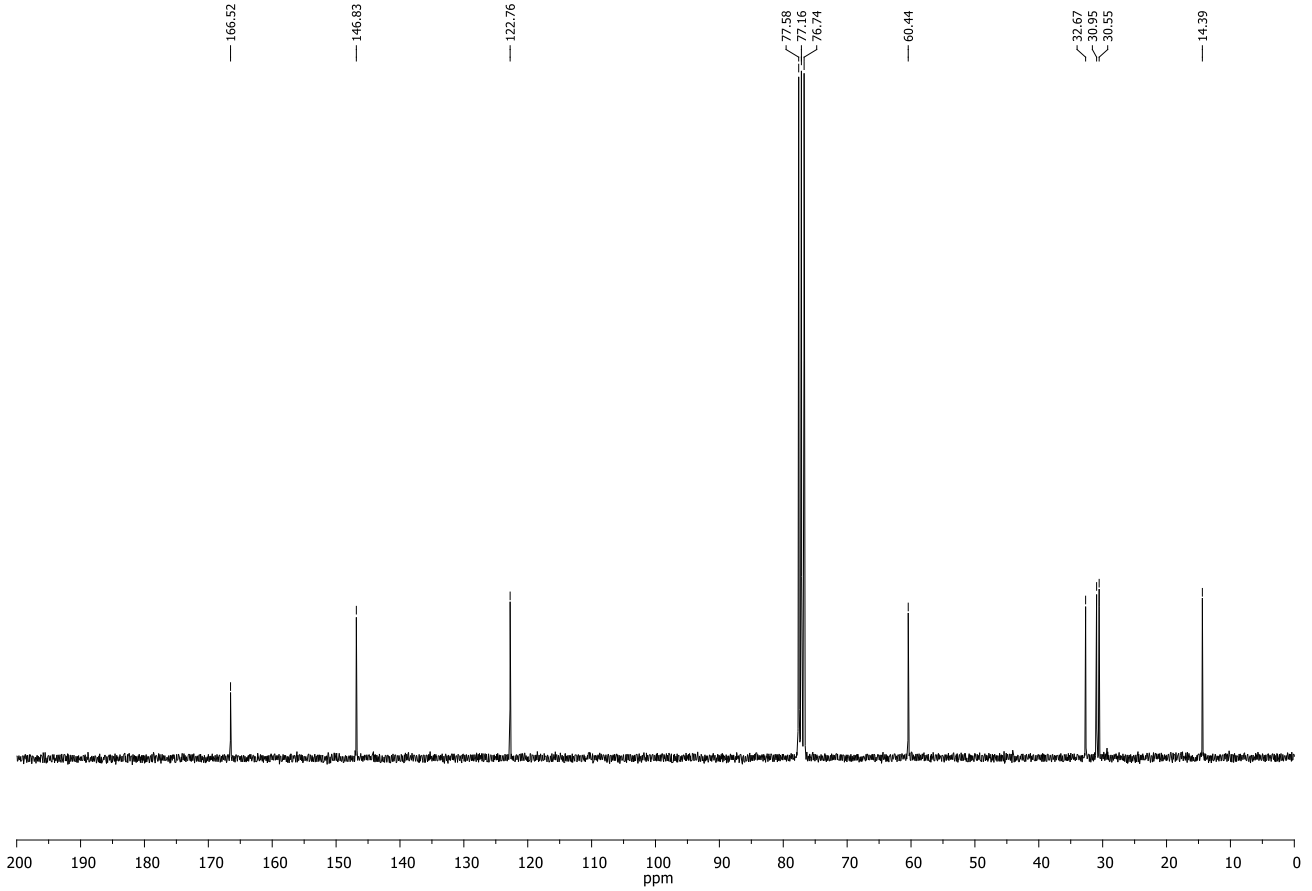
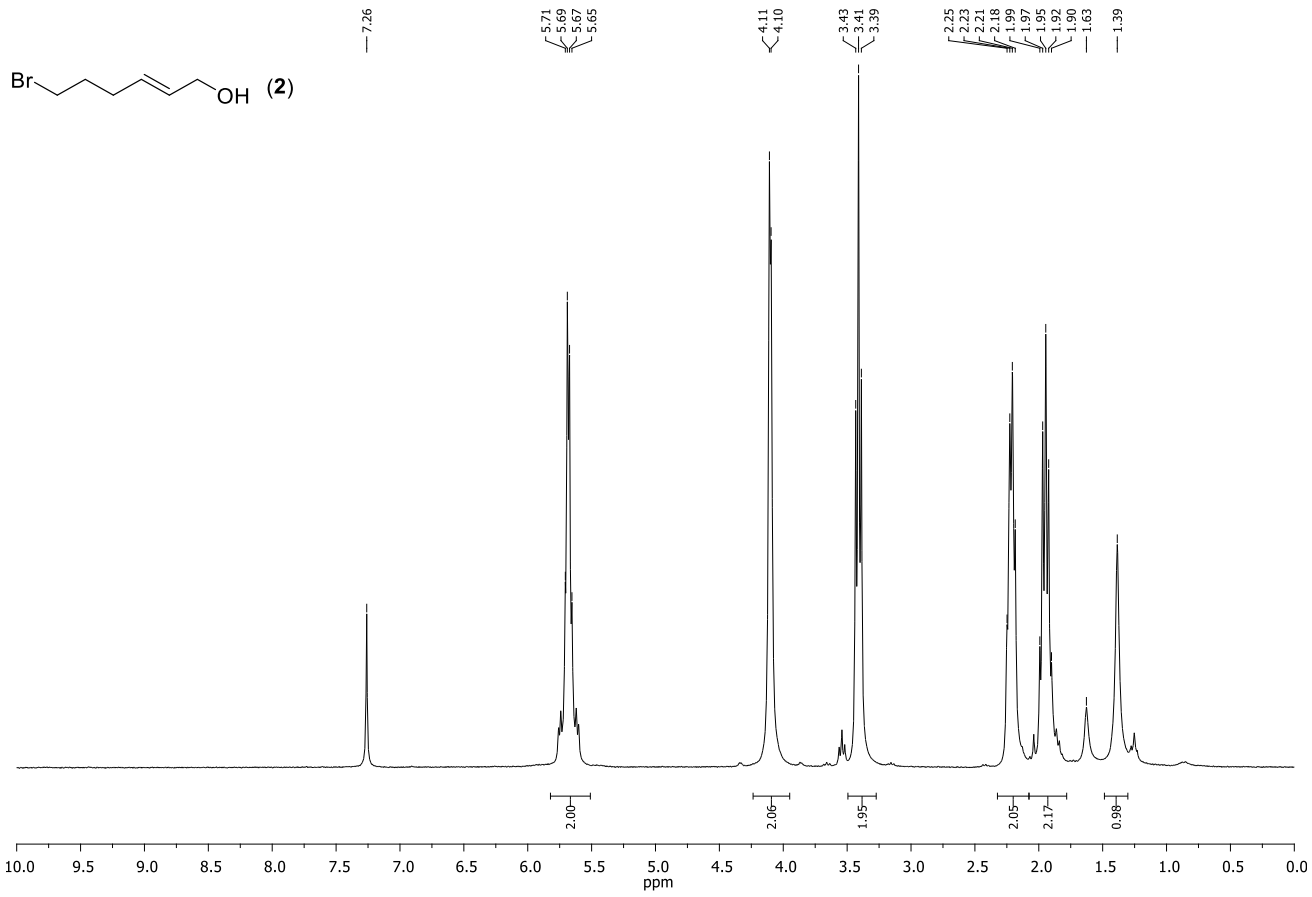
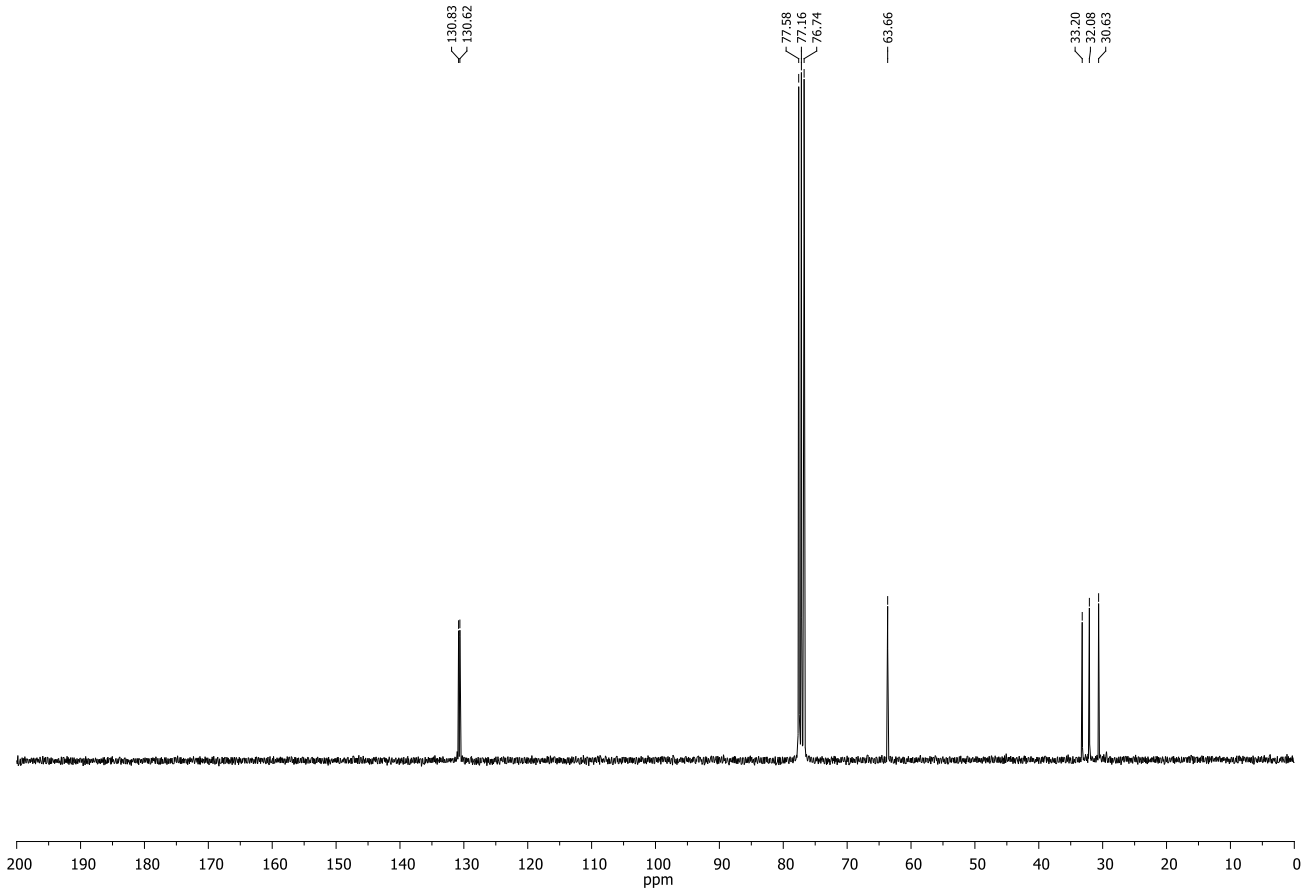


^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)





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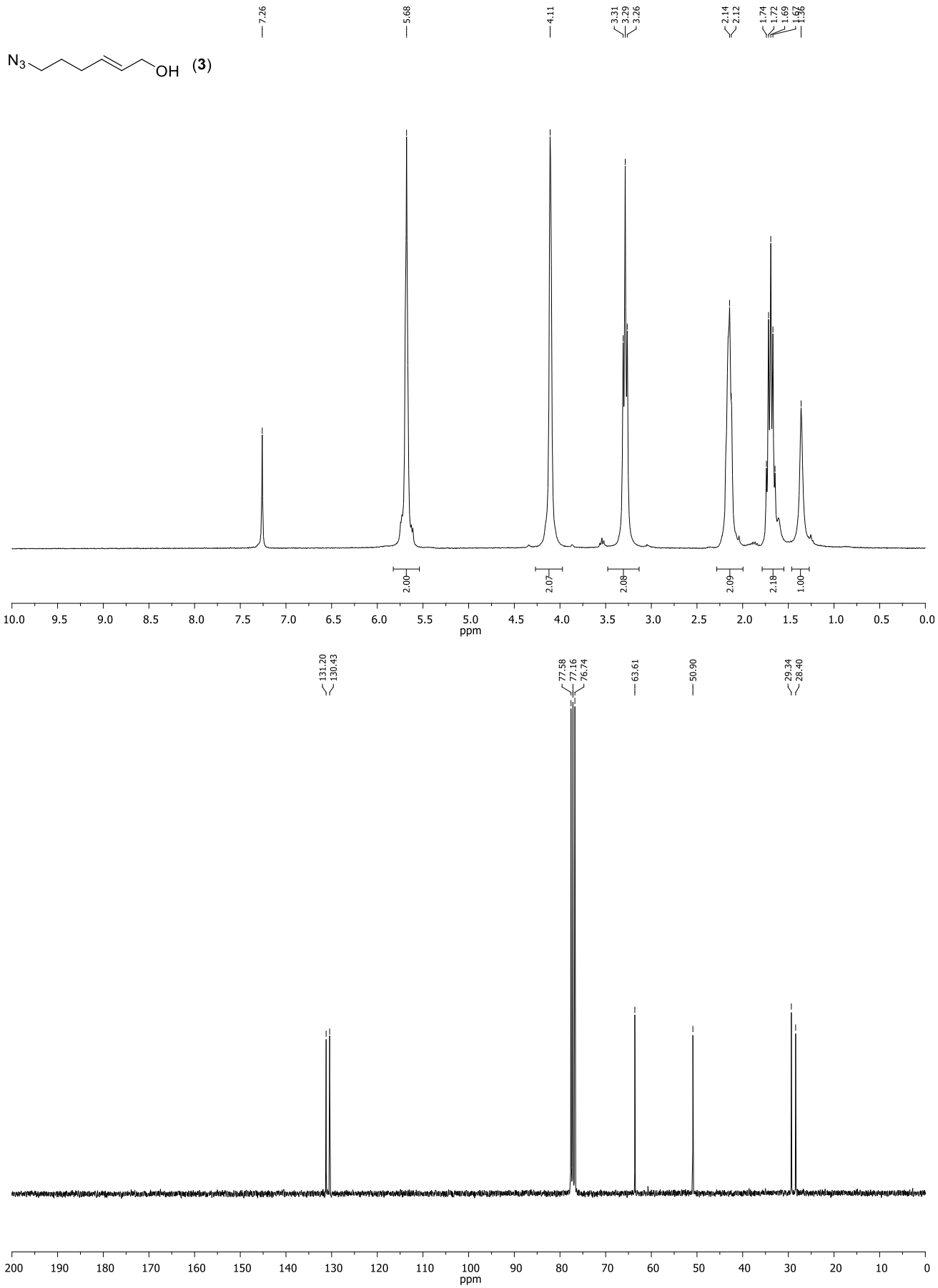
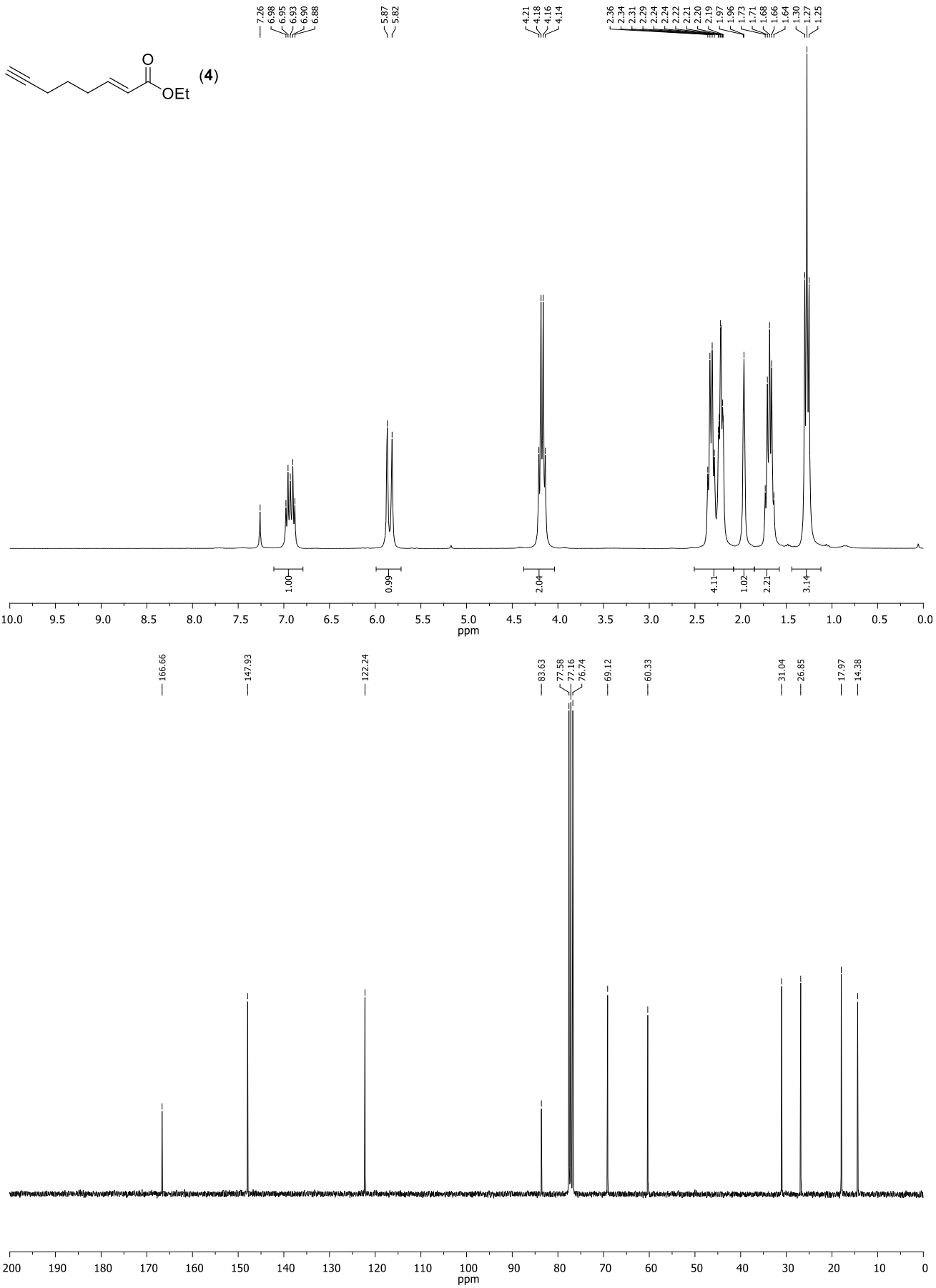
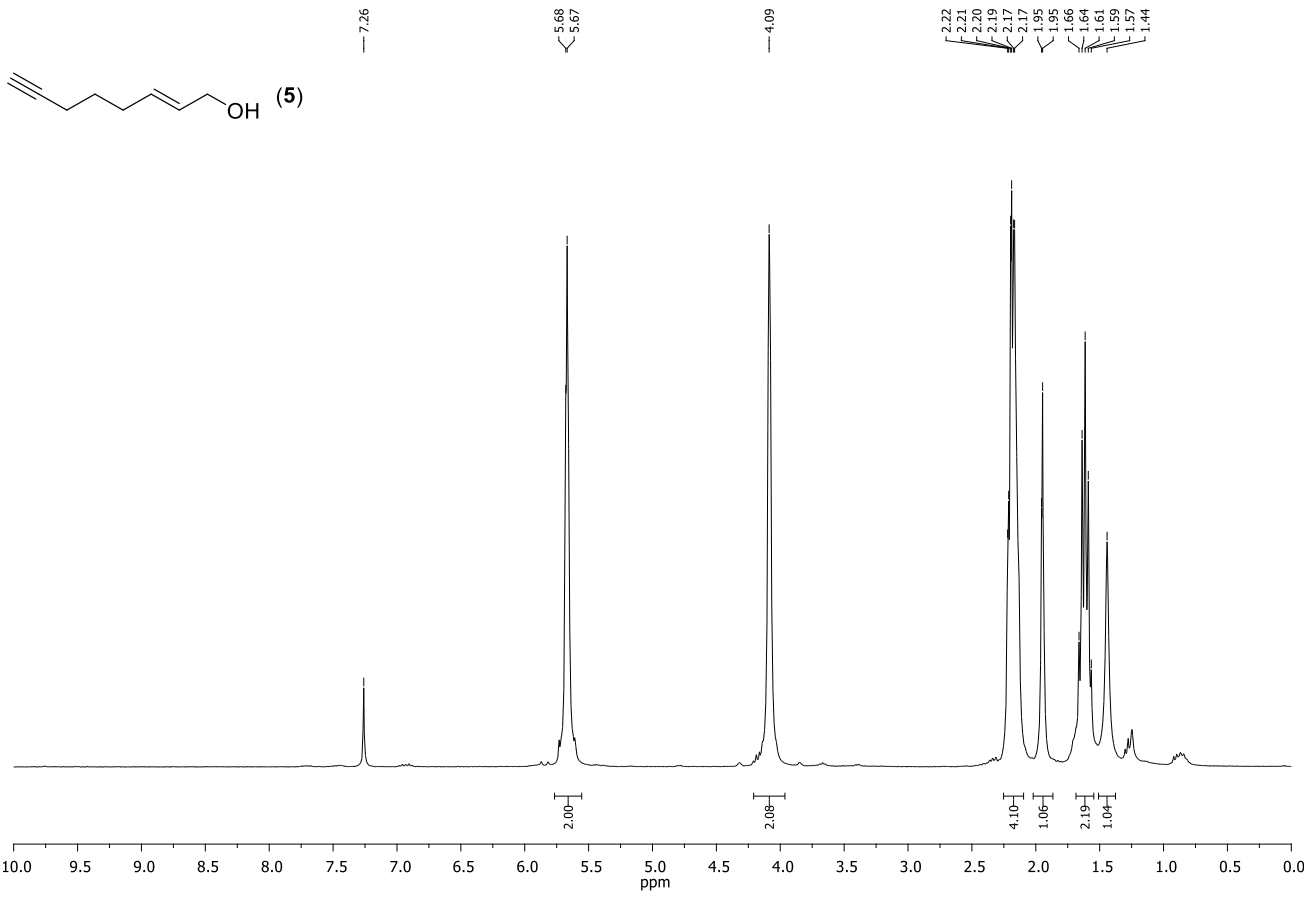


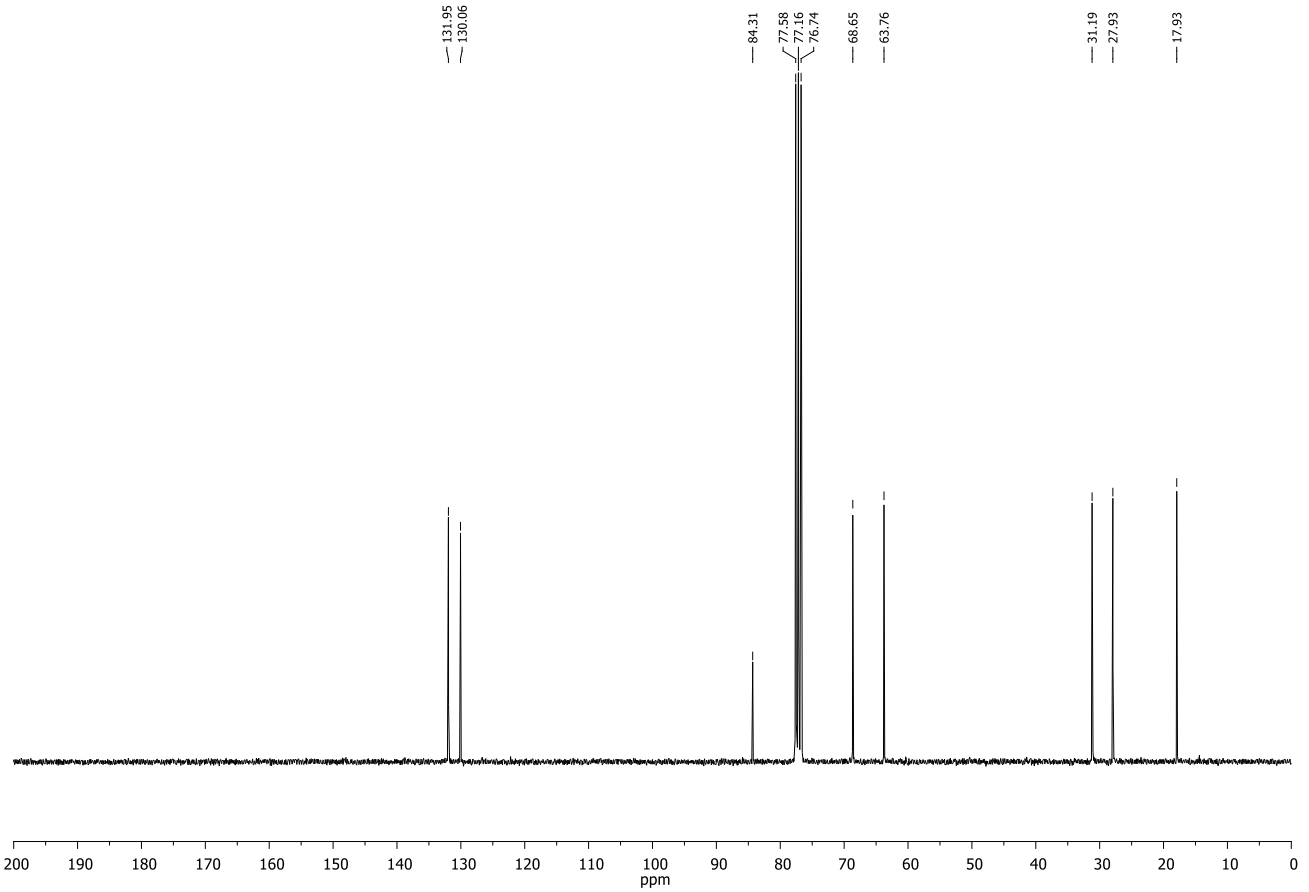
N_3 OH (**3**)

^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)





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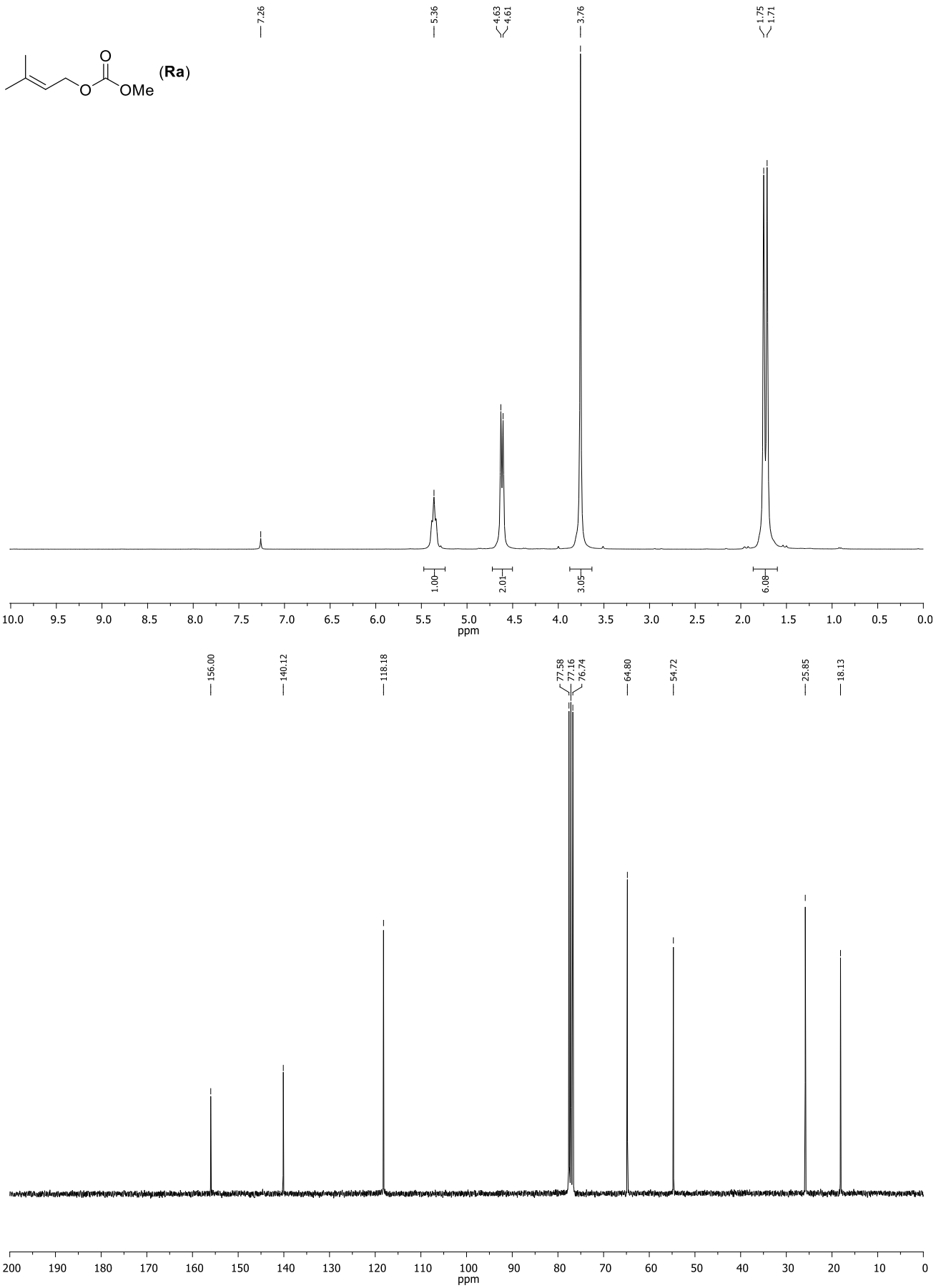

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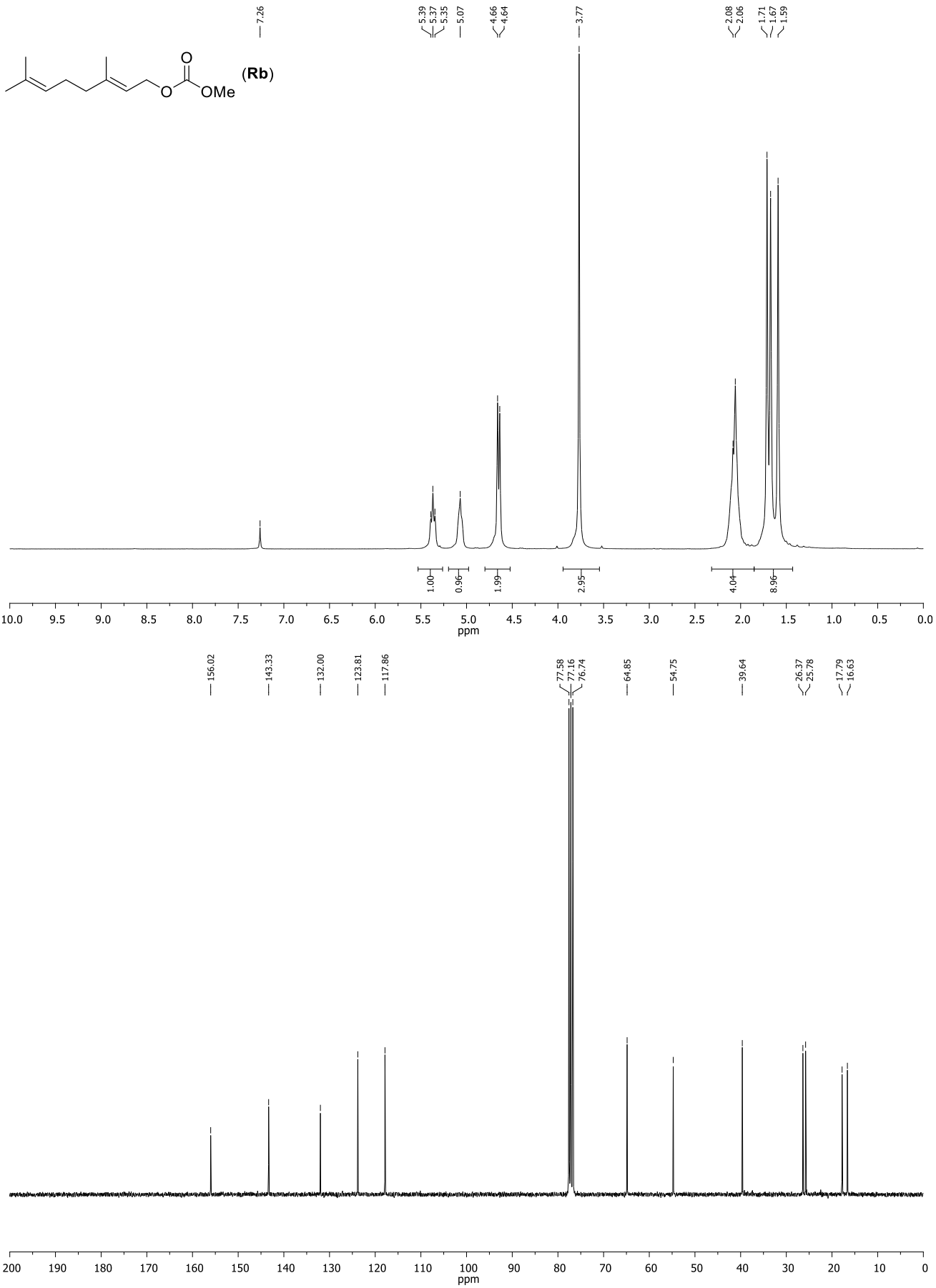

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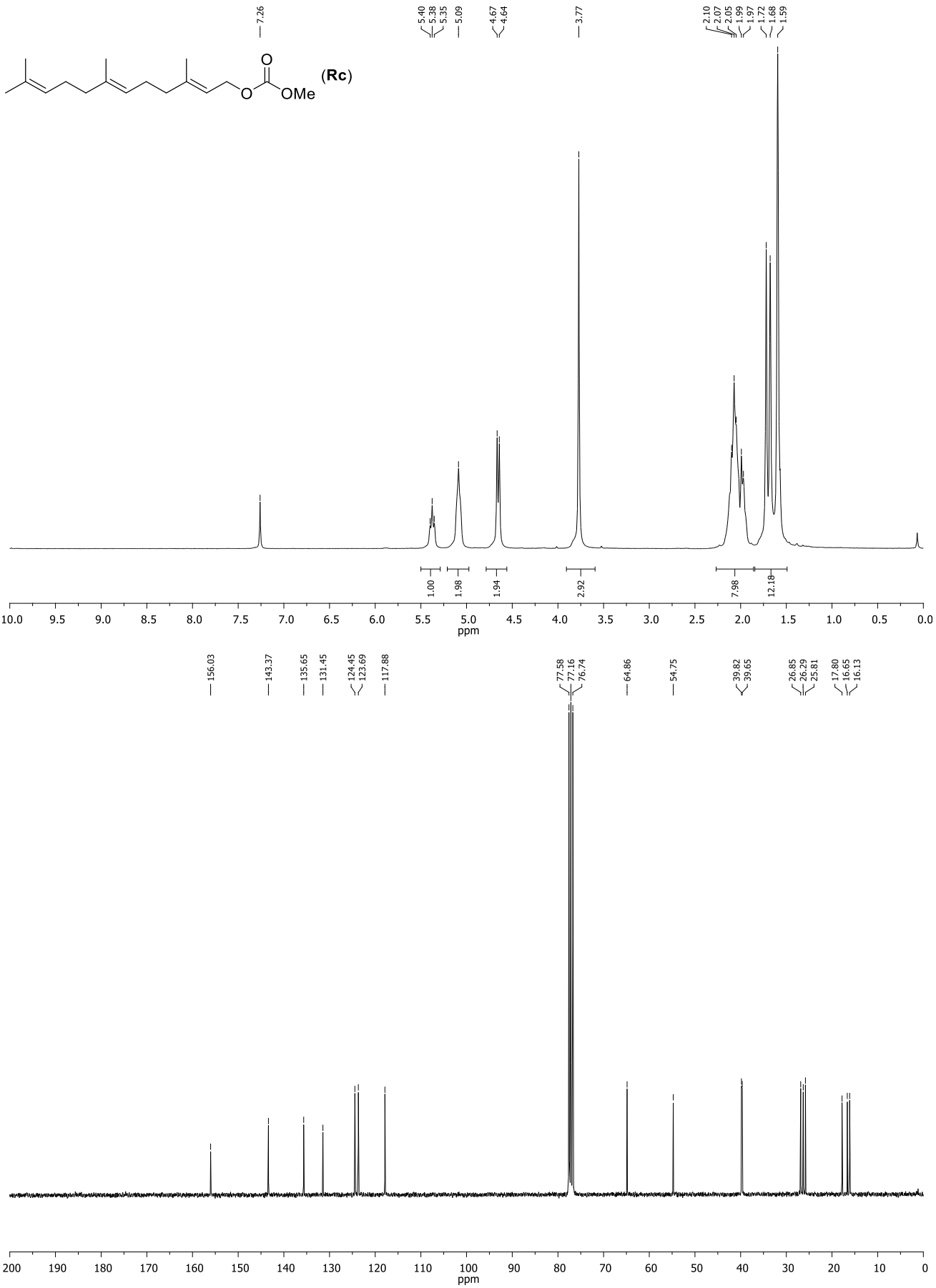

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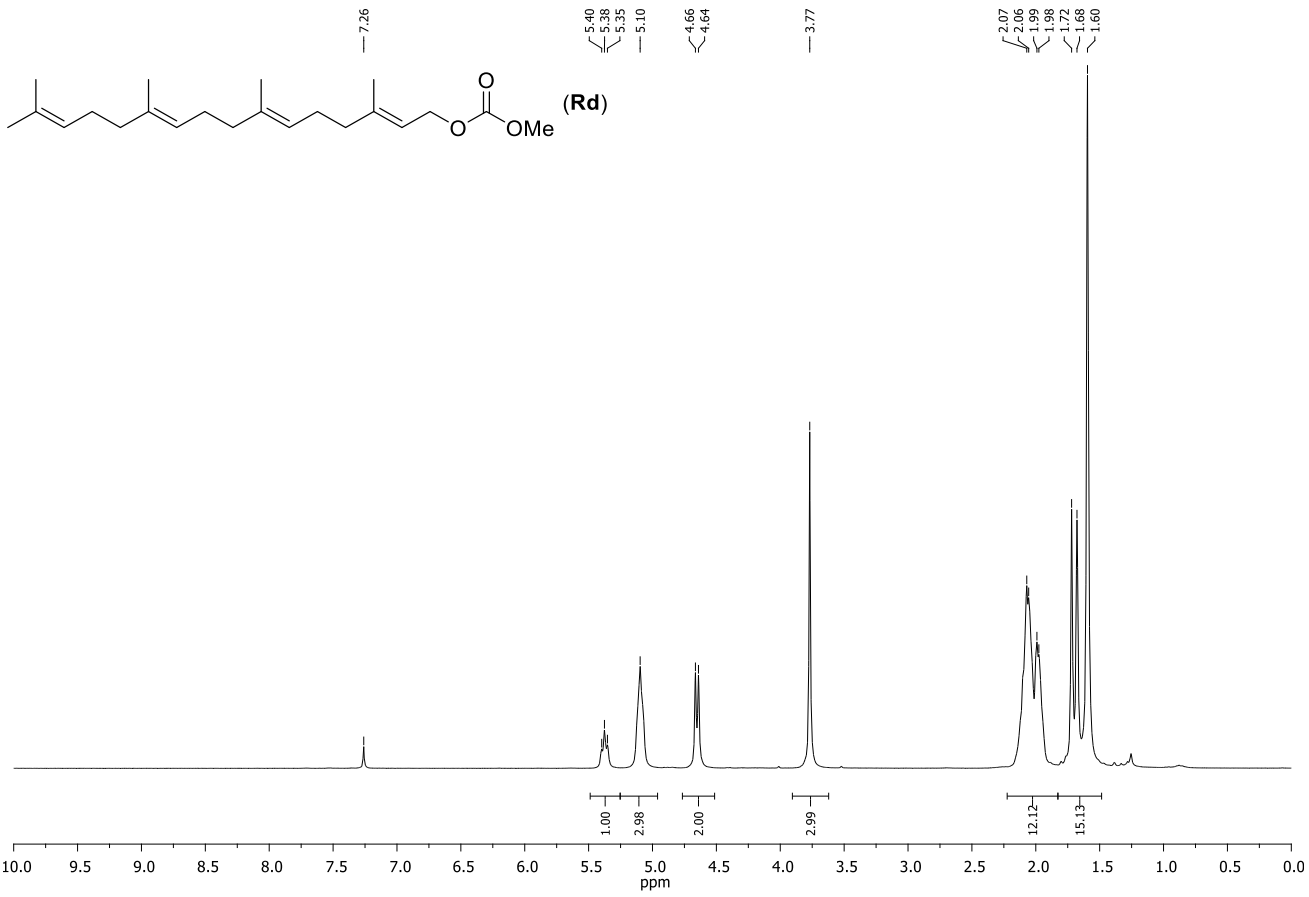

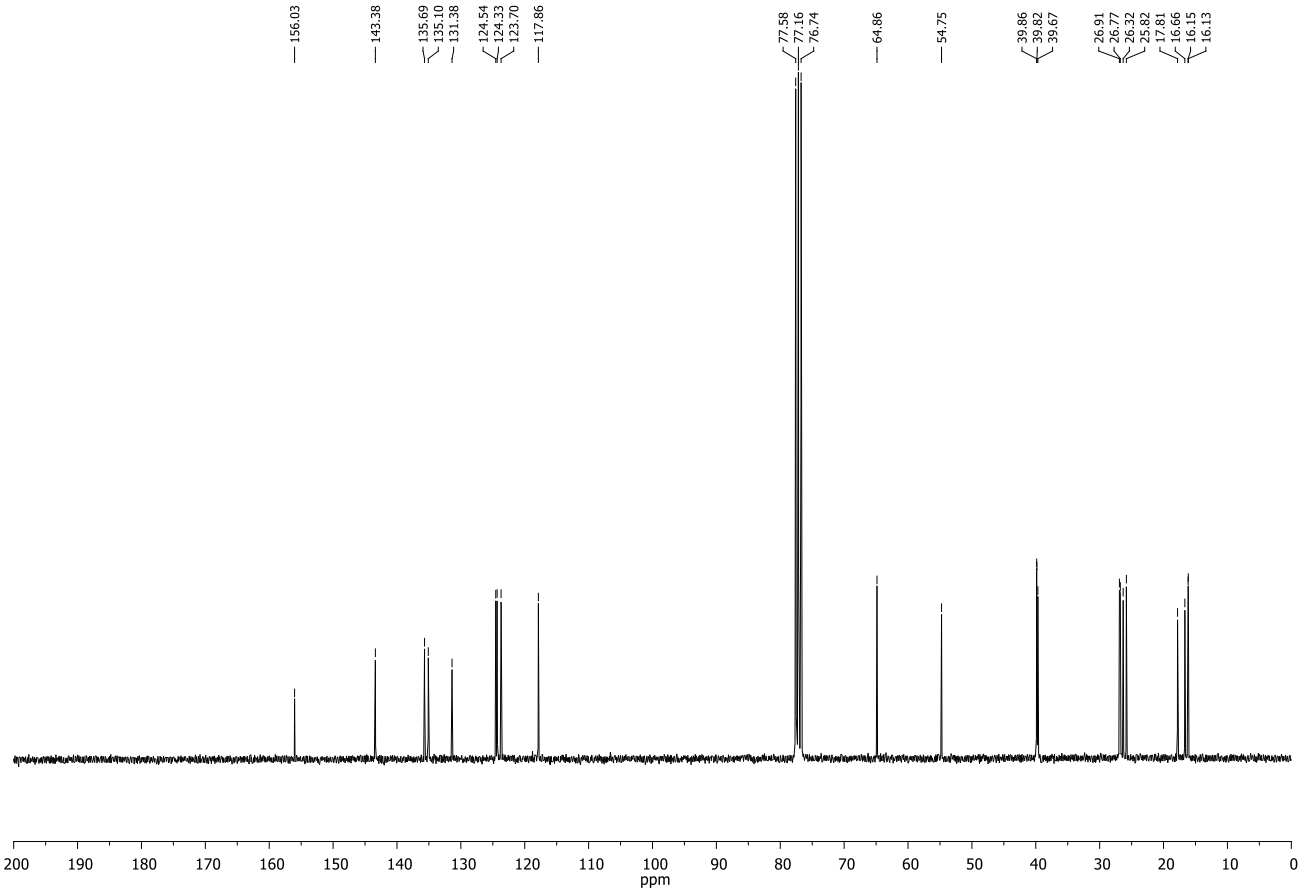

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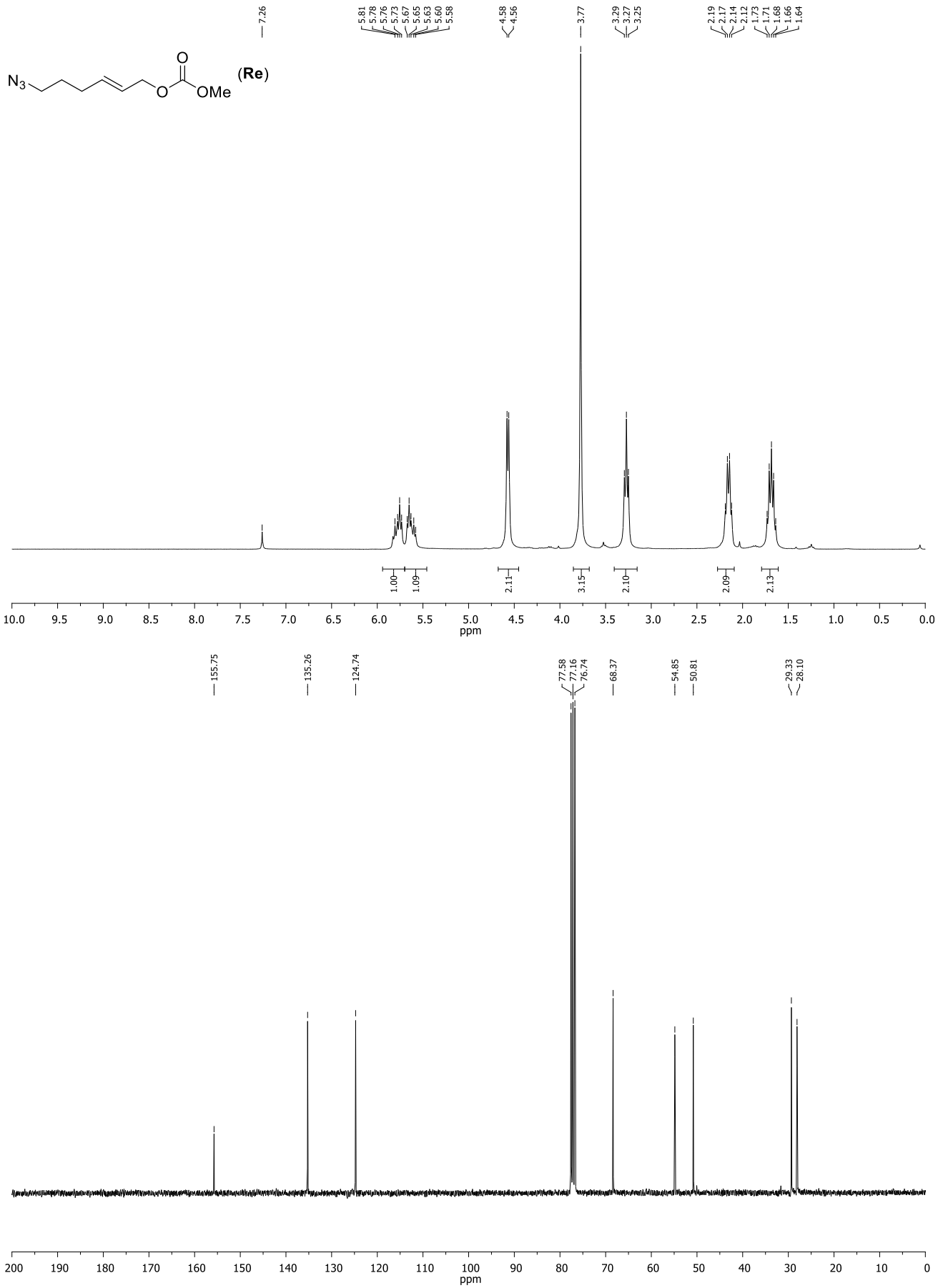
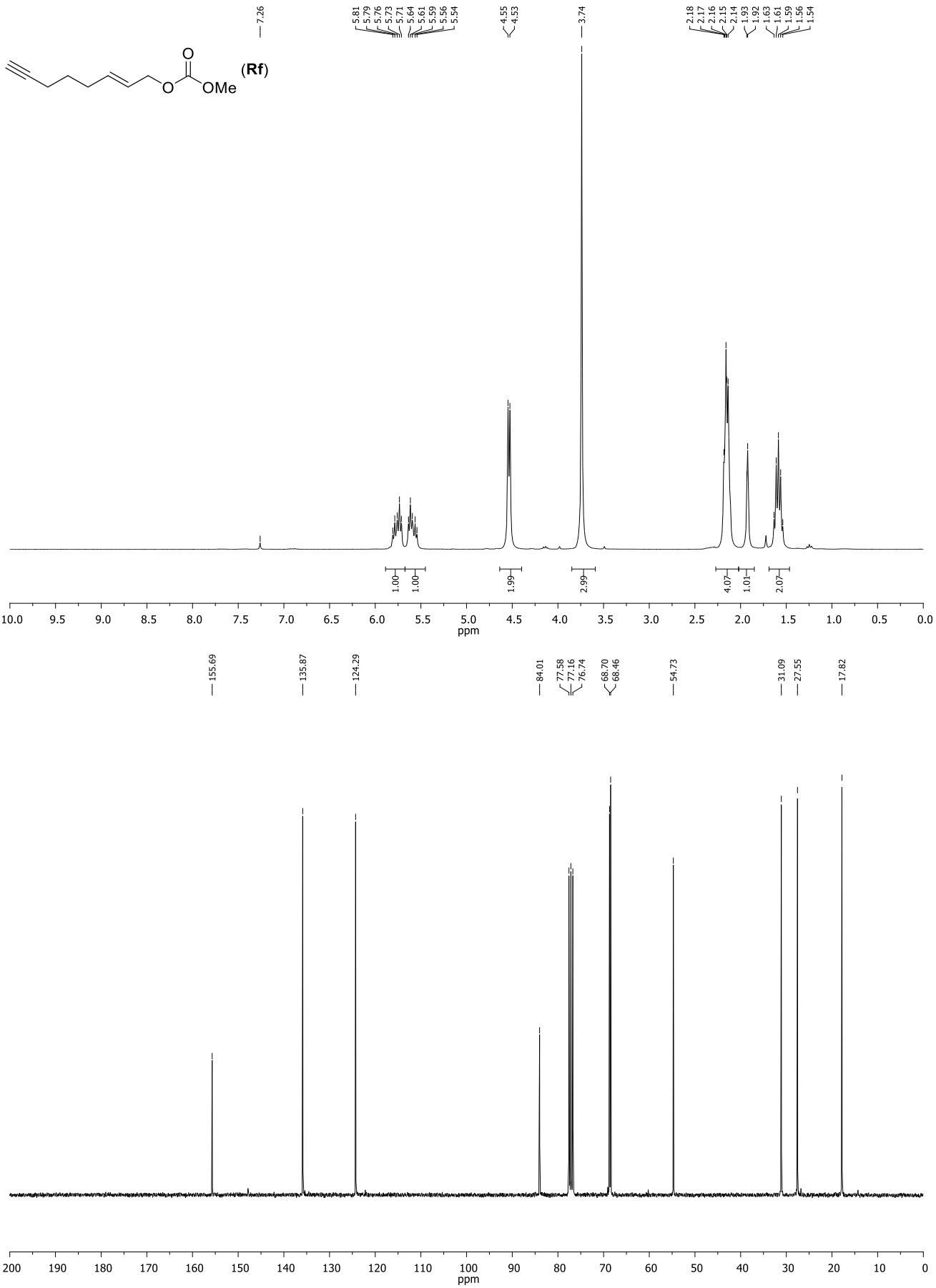


^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



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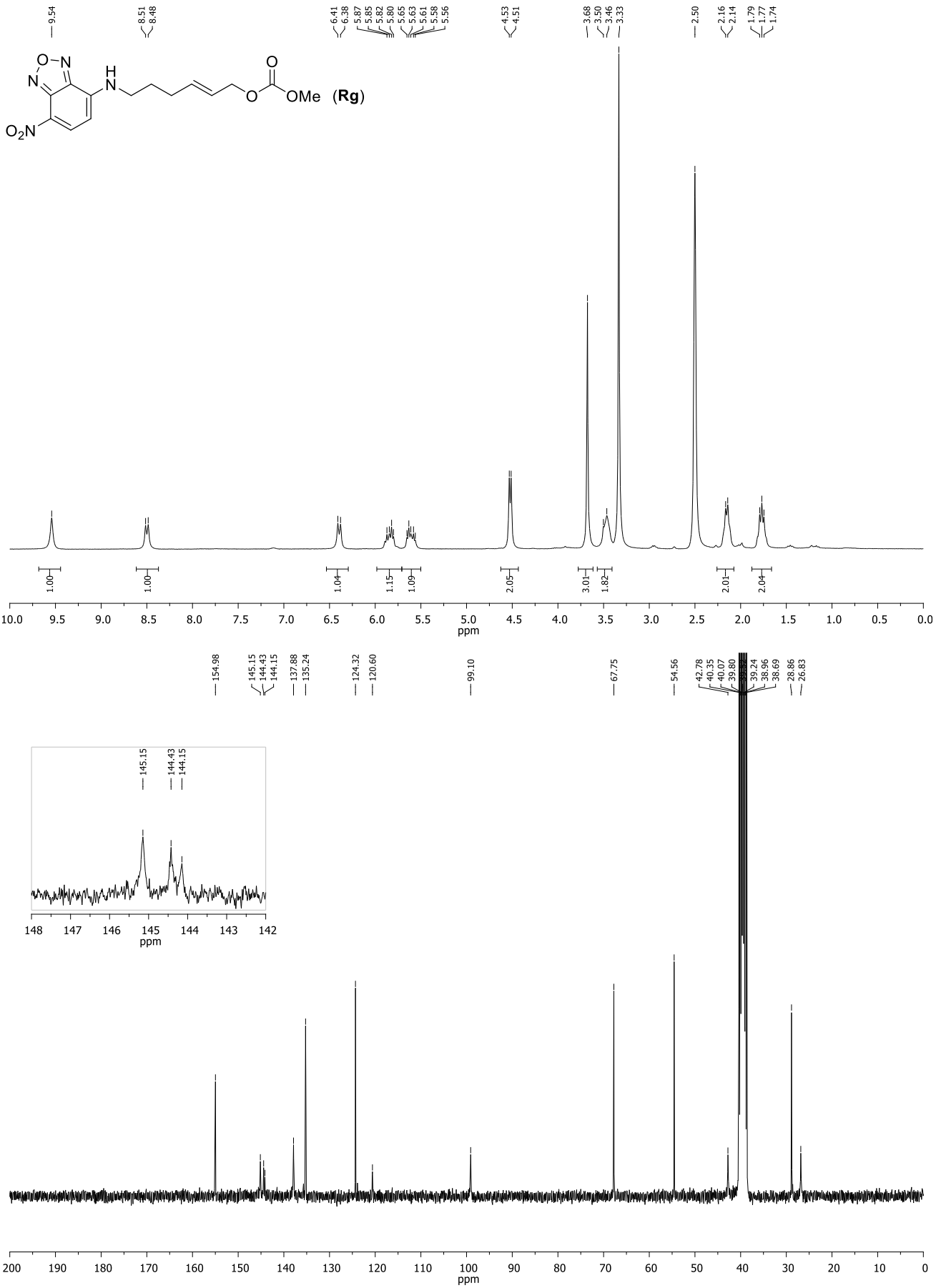
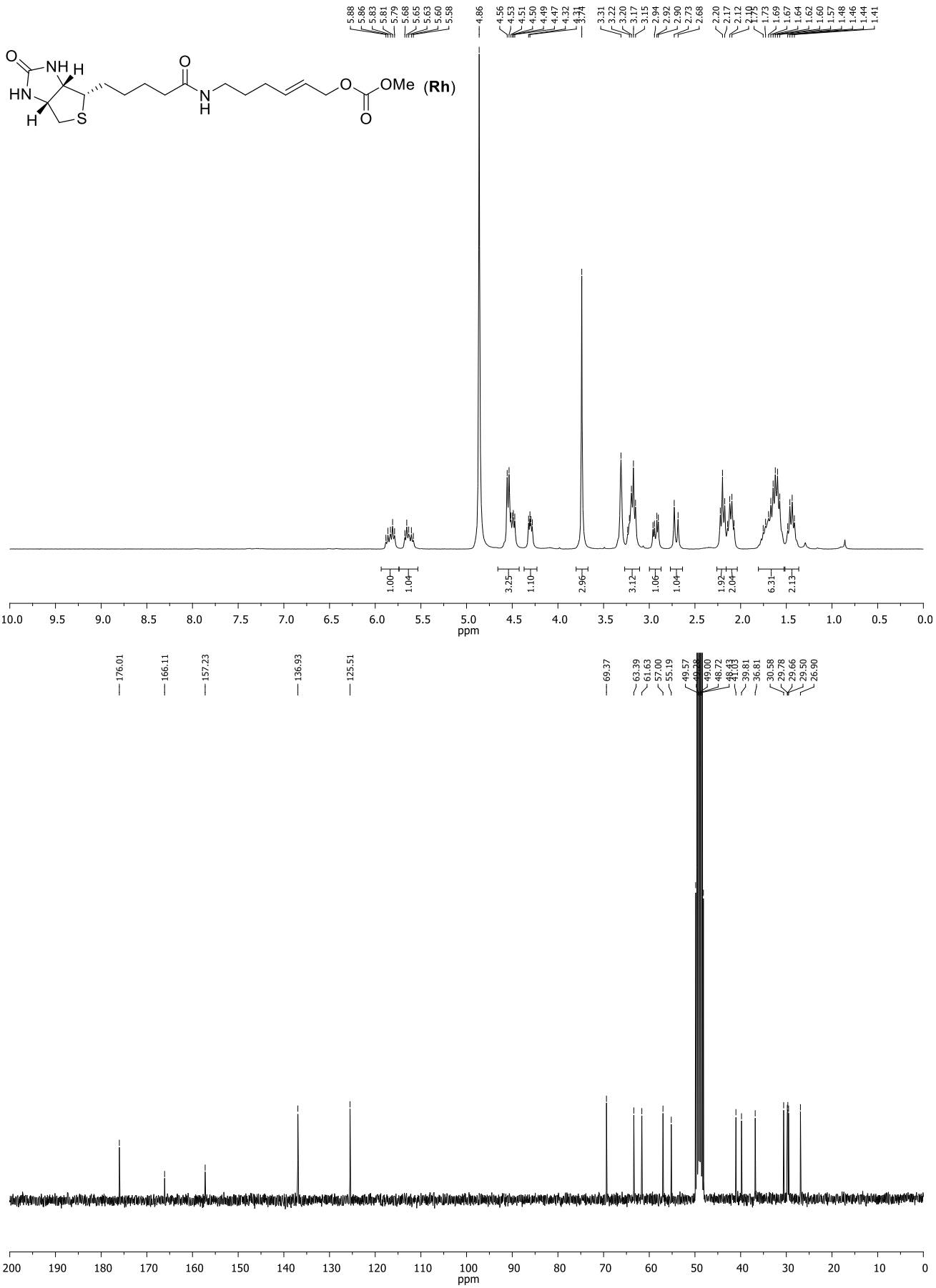
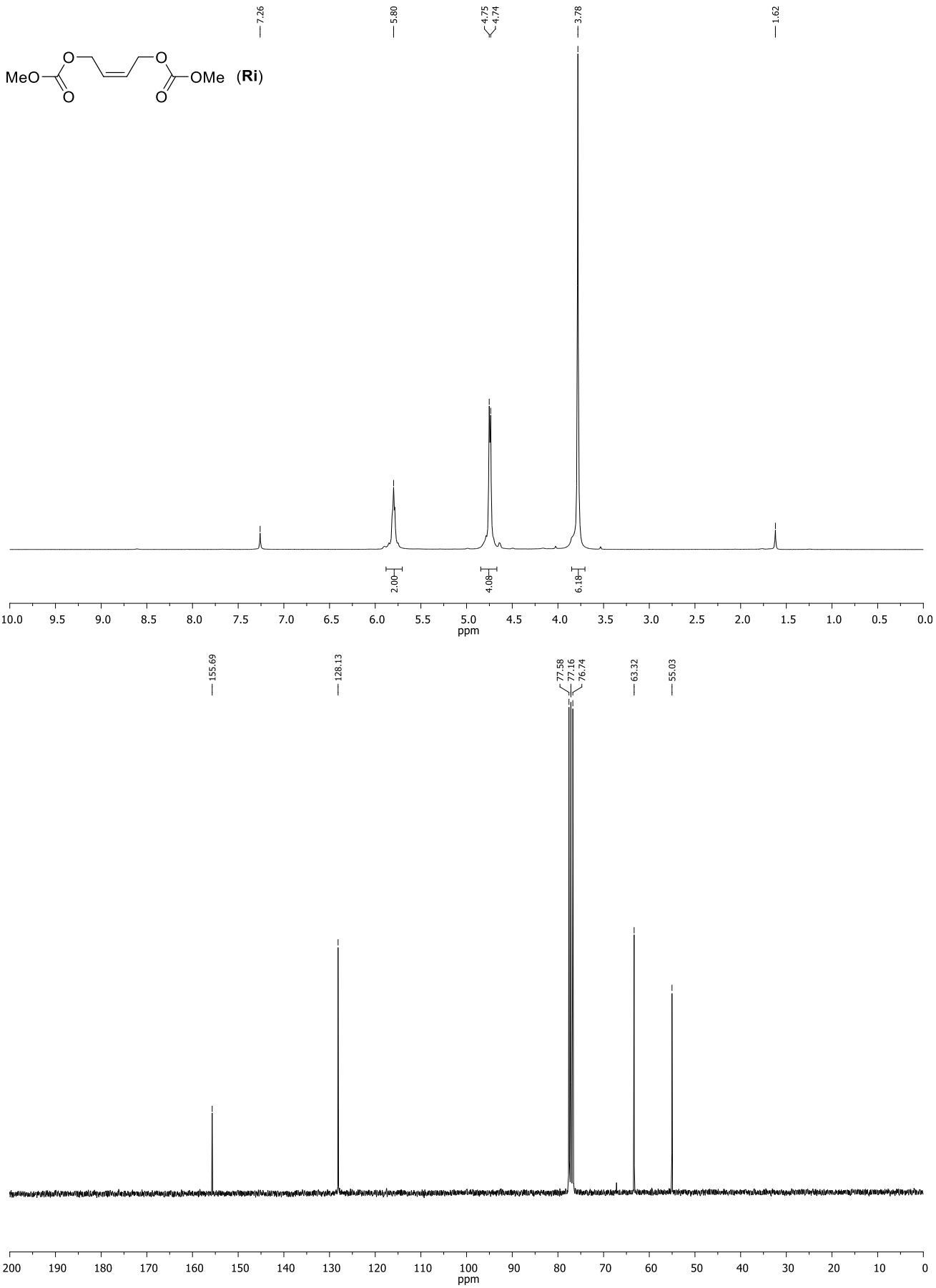


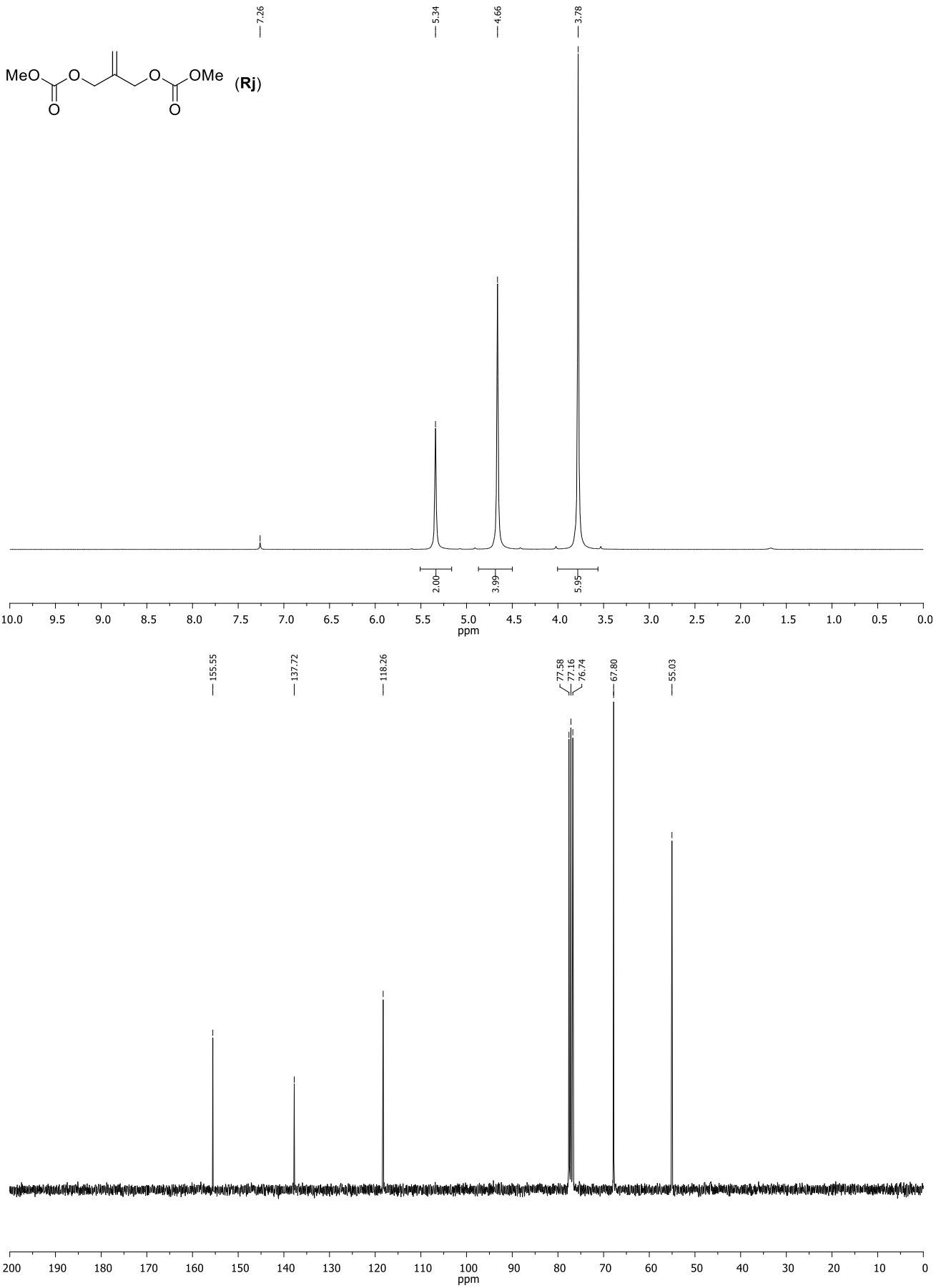
^{1}H-NMR (300.36 MHz, DMSO-d_6); ^{13}C-NMR: (75.53 MHz, DMSO-d_6)



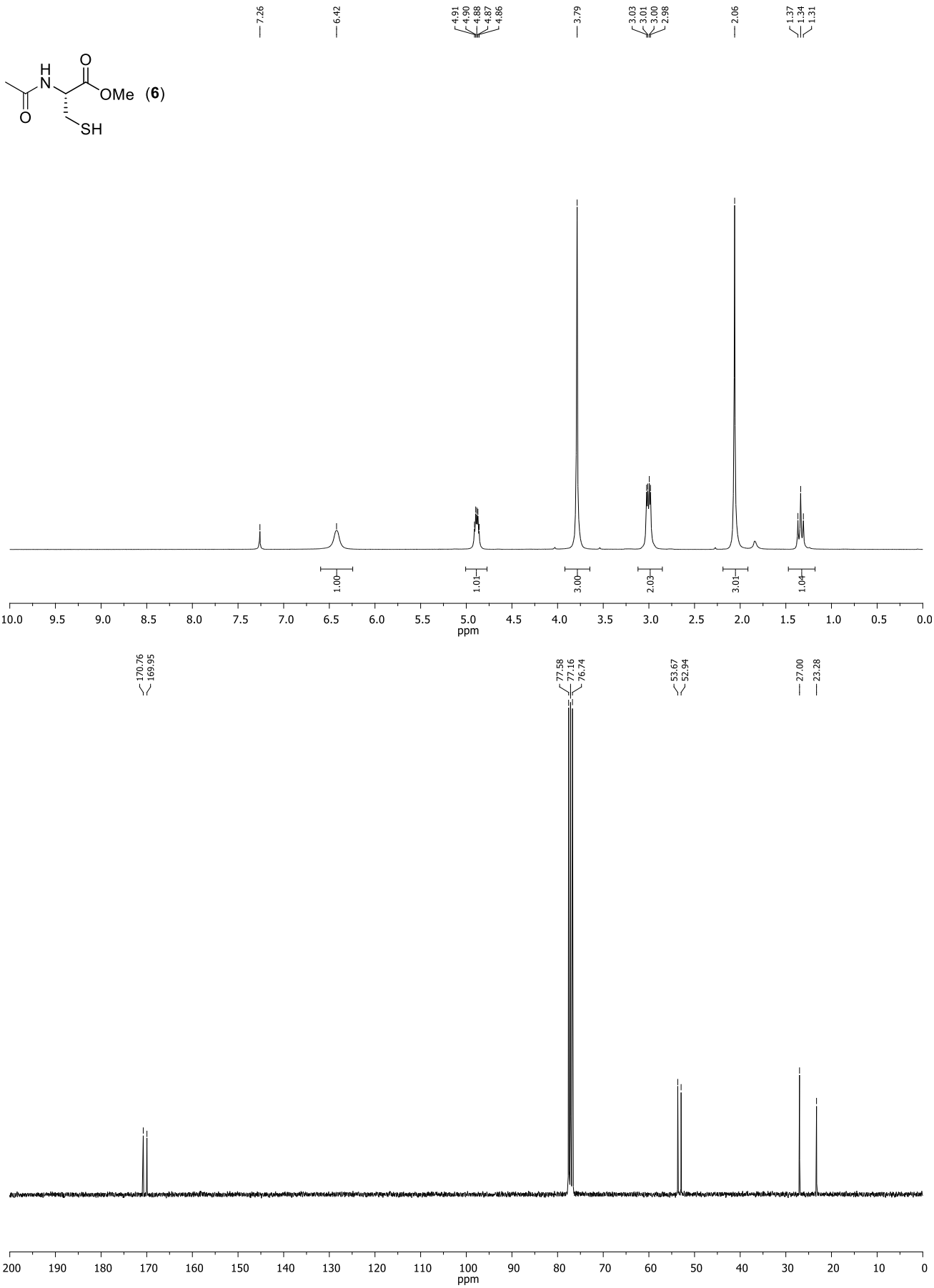
^{1}H-NMR (300.36 MHz, CD_3OD); ^{13}C-NMR: (75.53 MHz, CD_3OD)



^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



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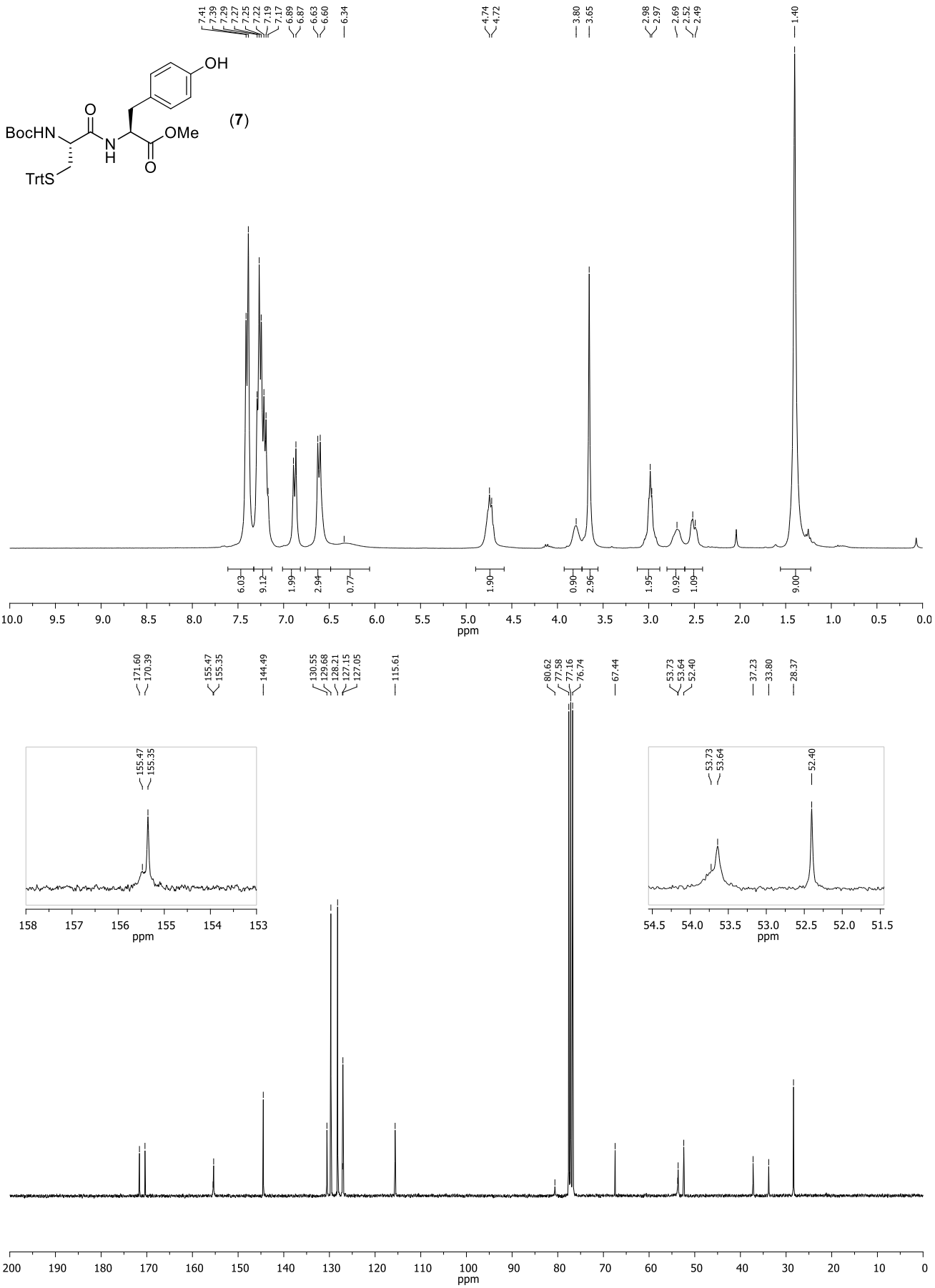

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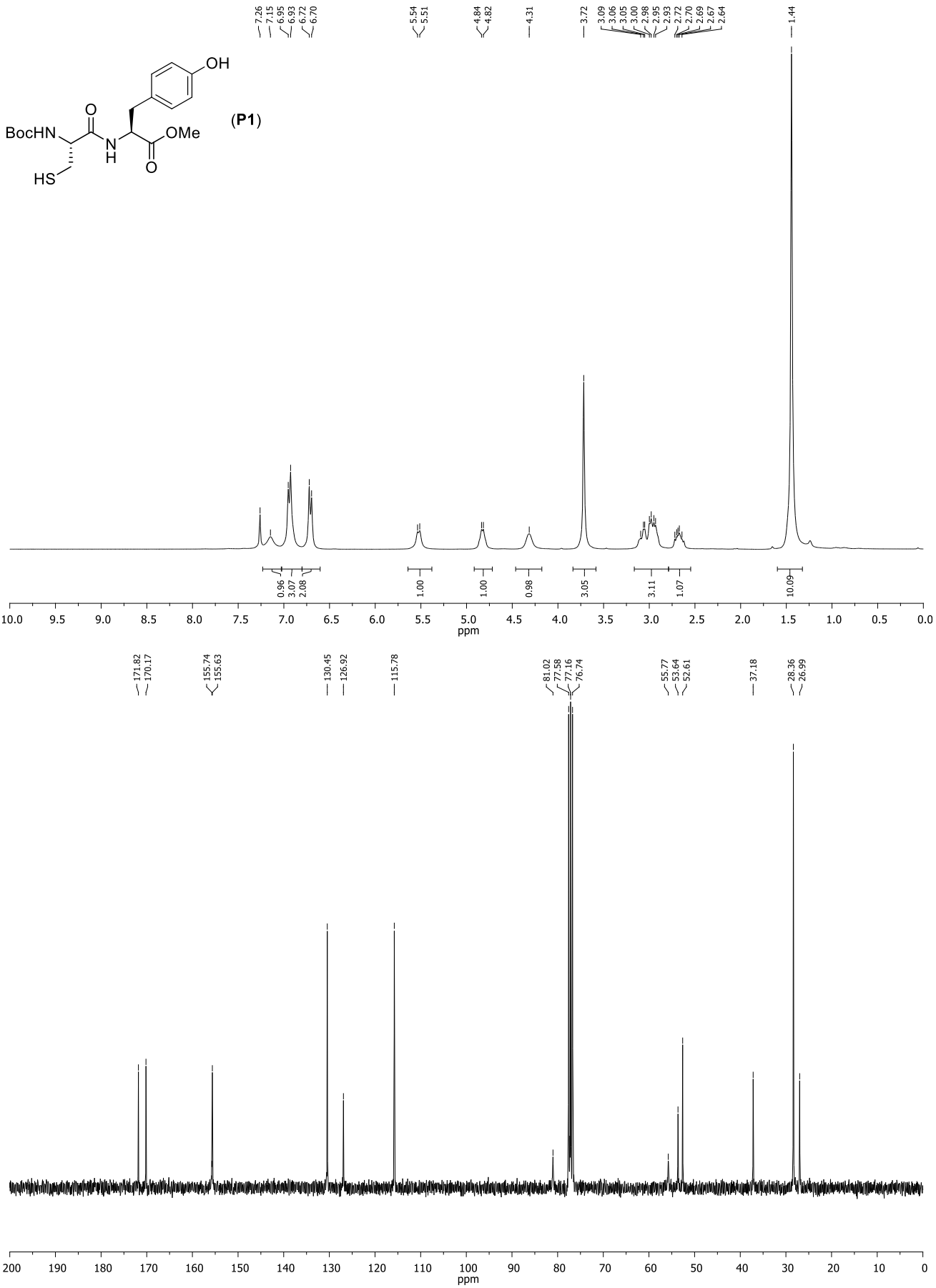

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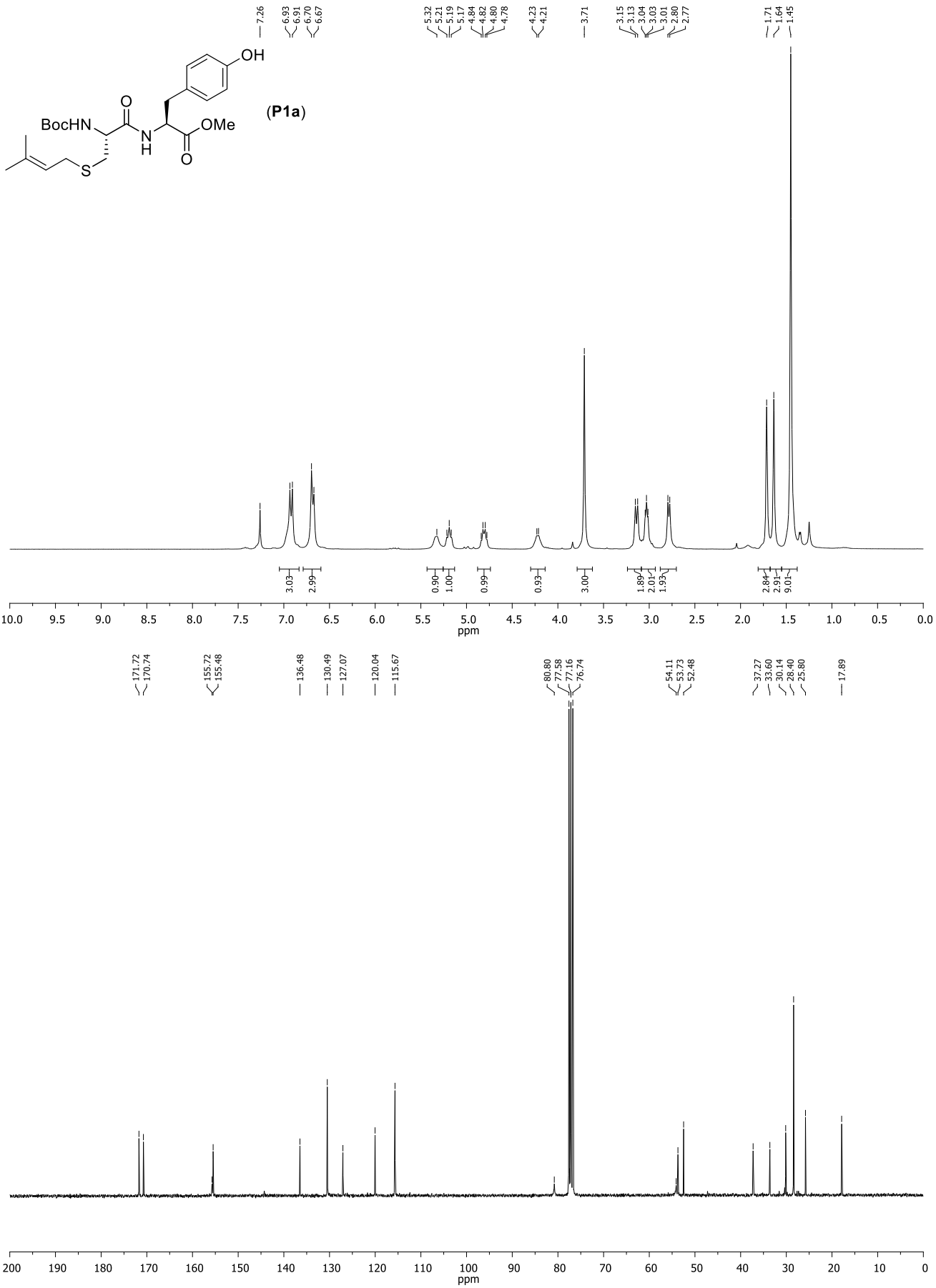
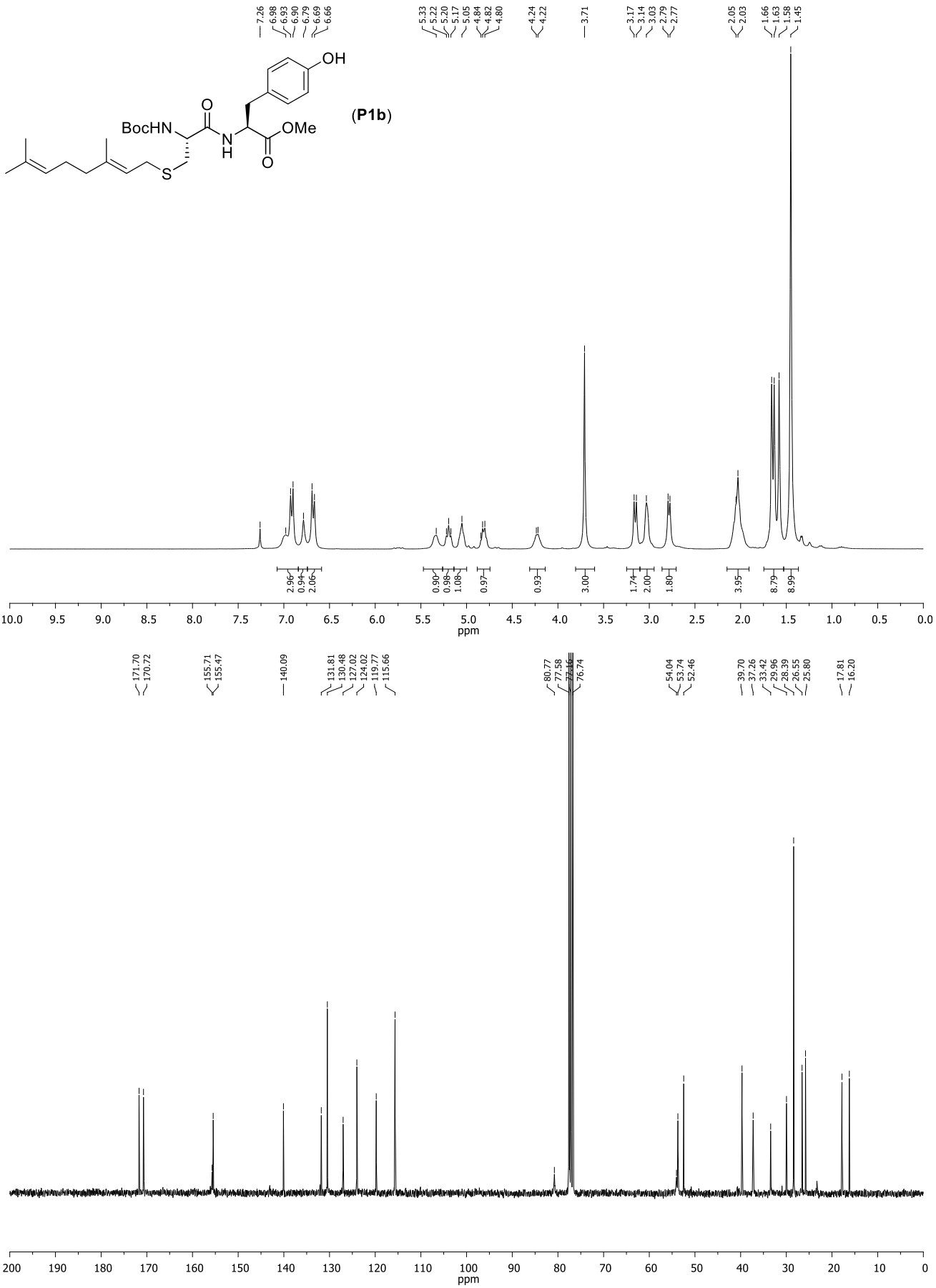
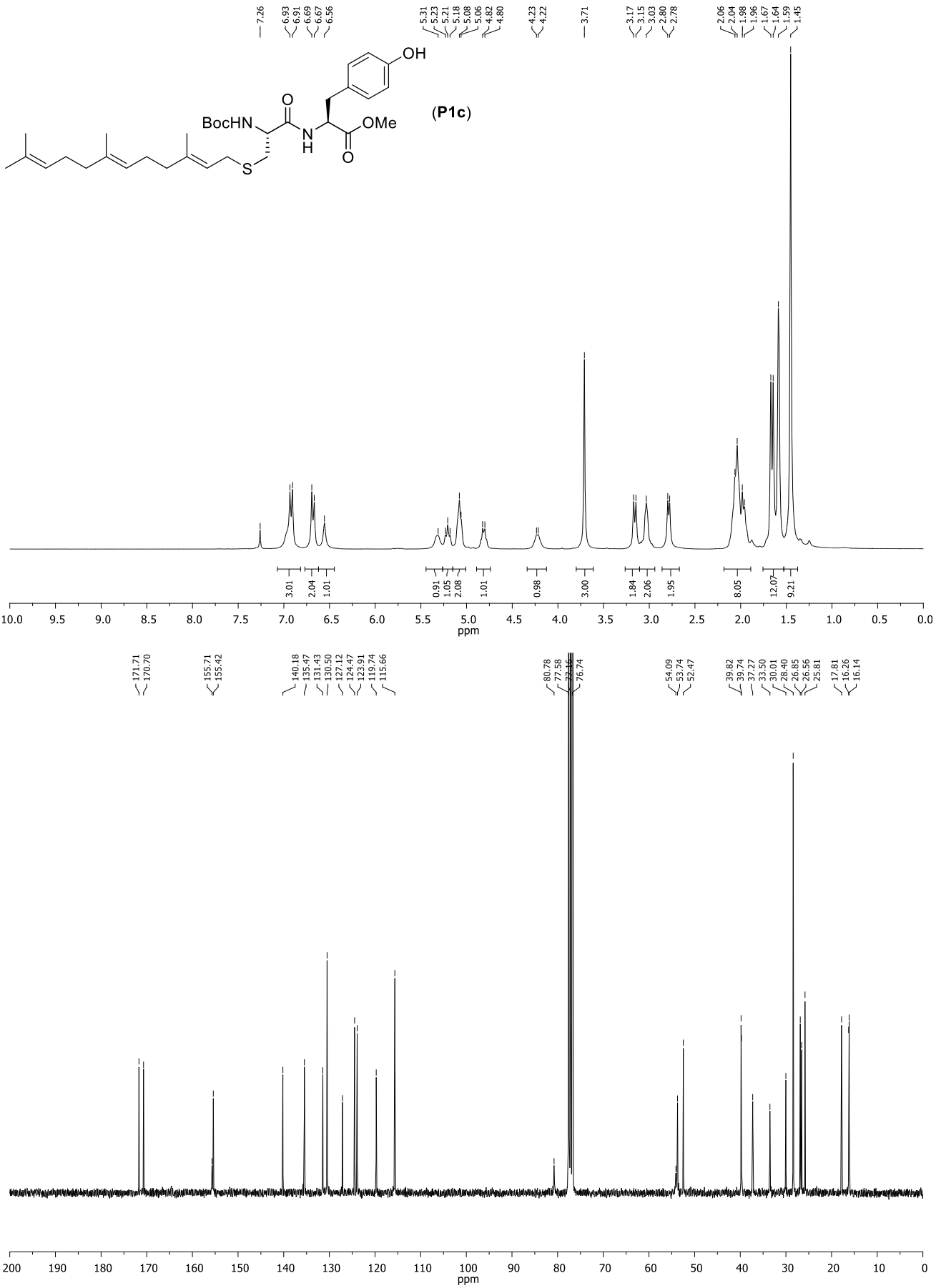


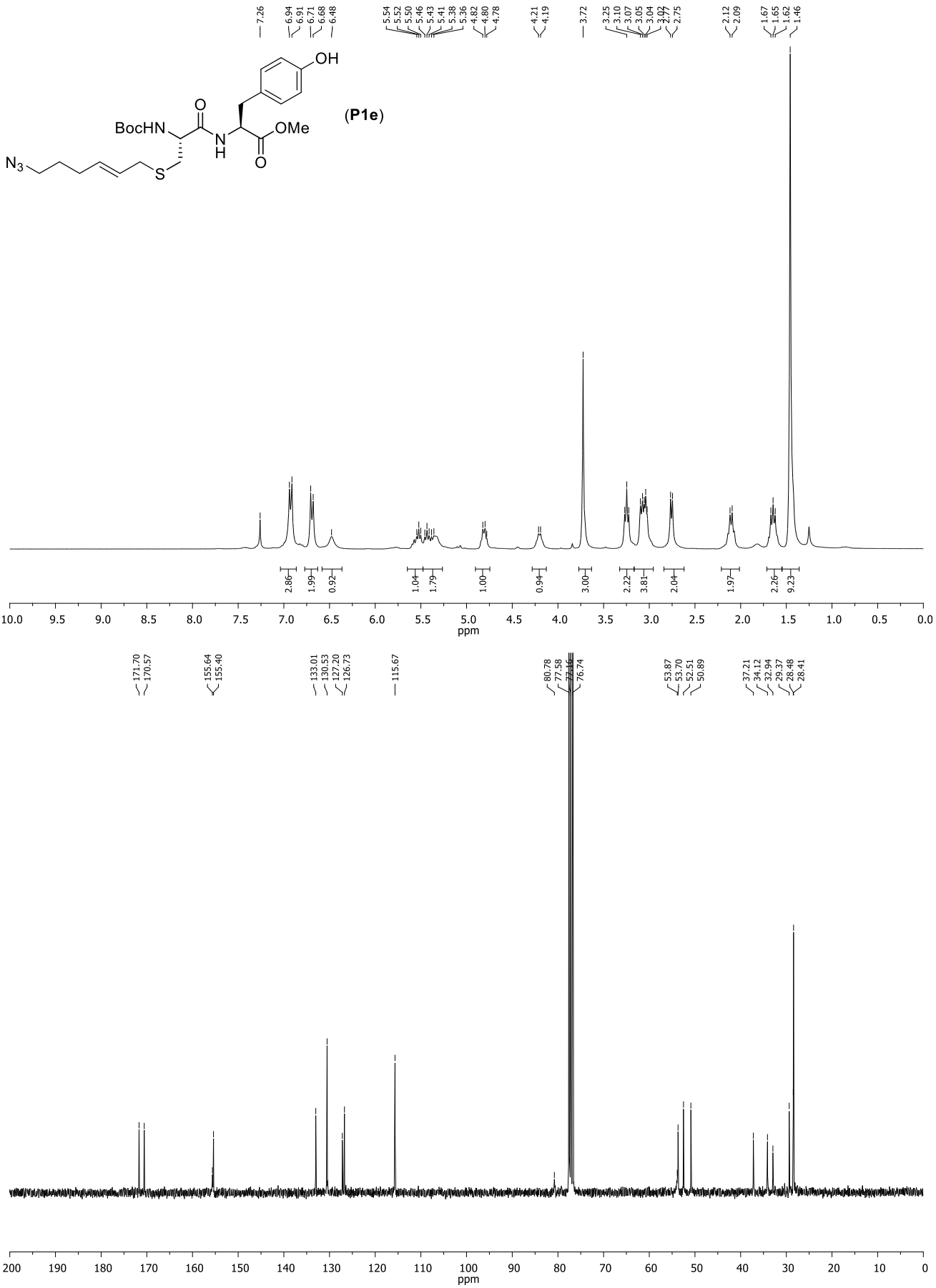
^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



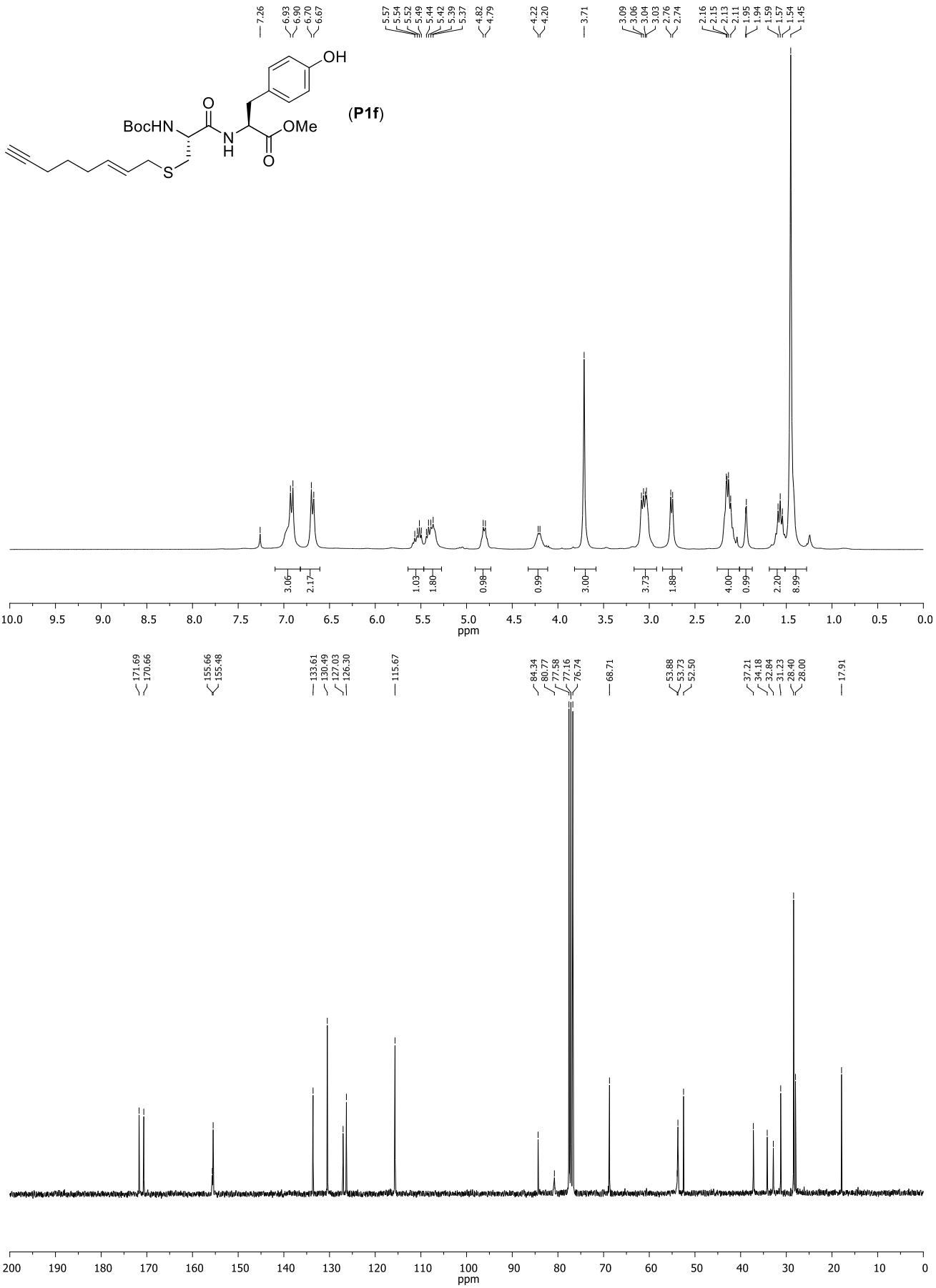
^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



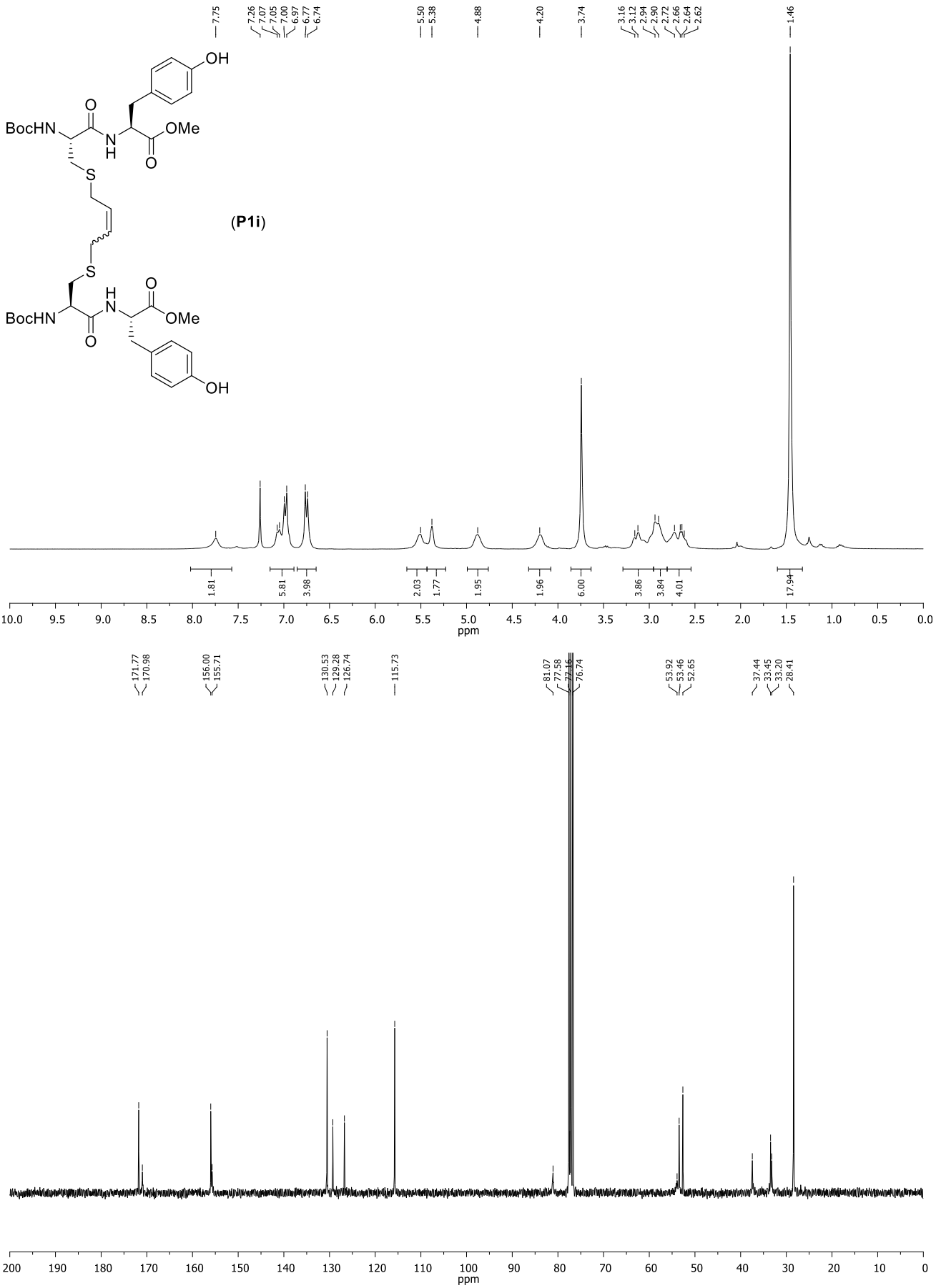
^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



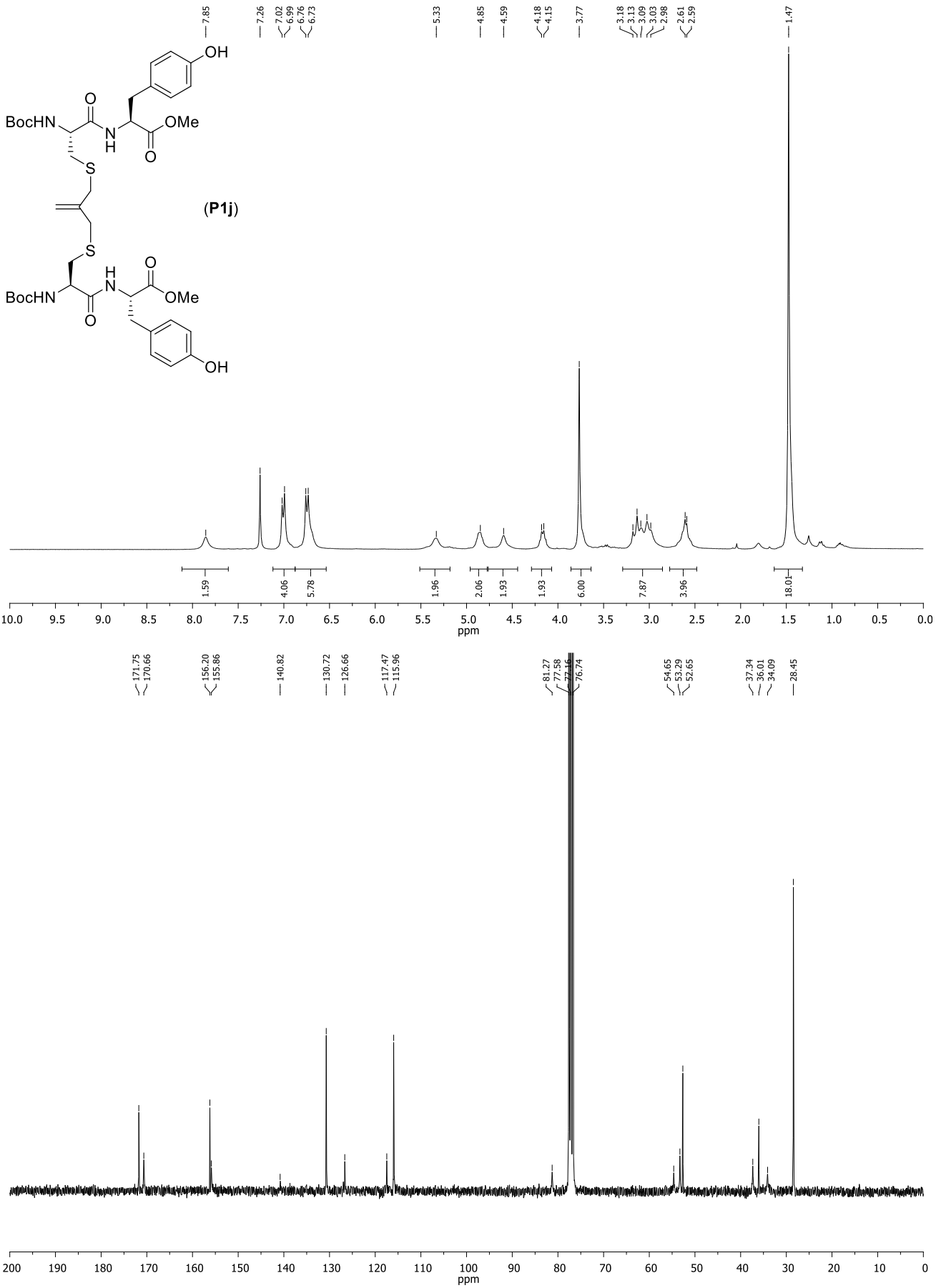
^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



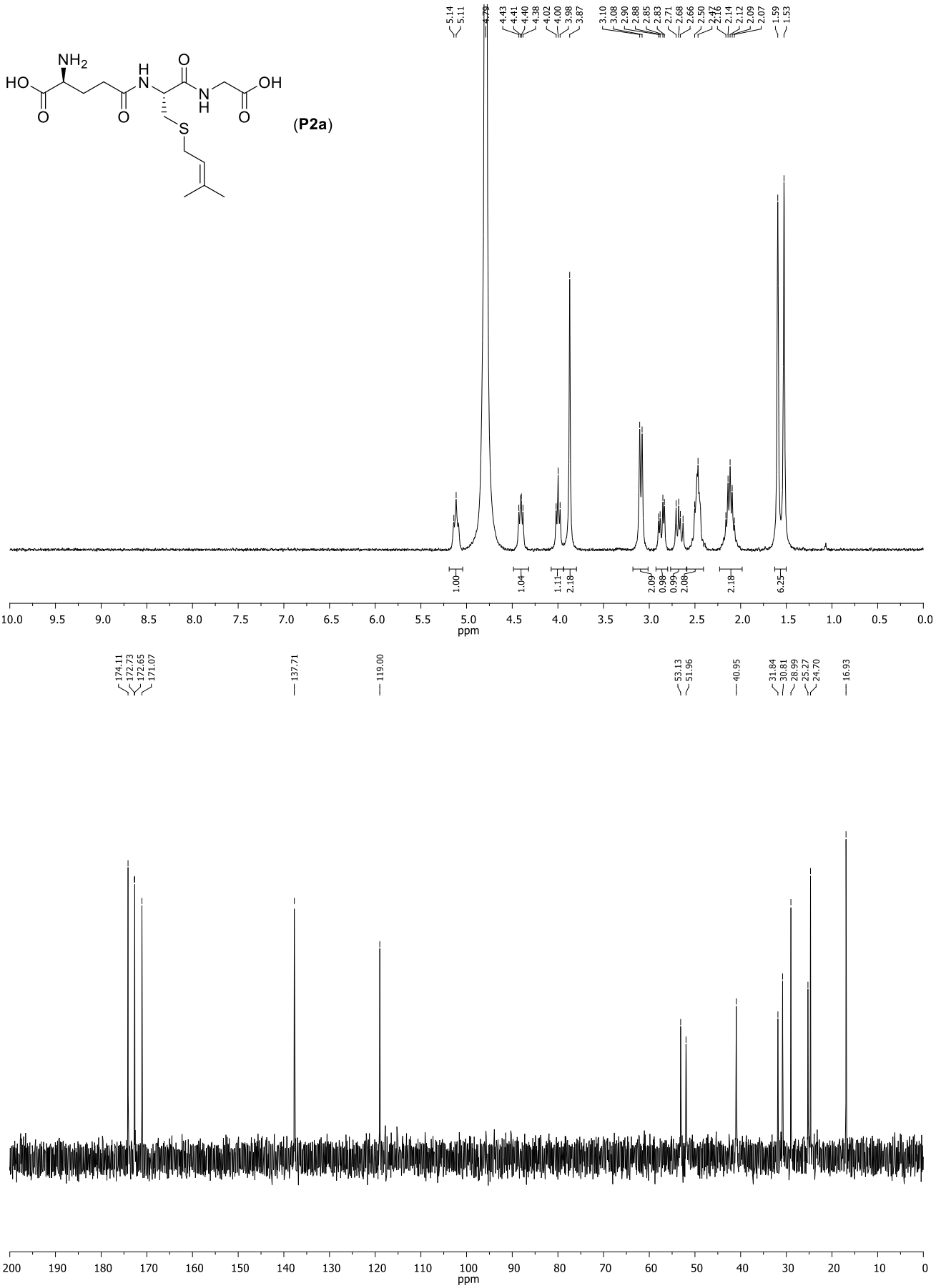
^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



^{1}H-NMR (300.36 MHz, $CDCl_3$); ^{13}C-NMR: (75.53 MHz, $CDCl_3$)



^{1}H-NMR (300.36 MHz, D_2O + DCl); ^{13}C-NMR: (75.53 MHz, D_2O + DCl)