Electronic Supplementary Material (ESI) for ChemComm.

Sensitivity booster for mass detection enables unambiguous analysis of peptides, proteins, antibodies, and protein bioconjugates

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1. General information

The reagents, proteins, and enzymes were purchased from Sigma-Aldrich. Aqueous buffers were prepared freshly using Millipore Grade I water (Resistivity > 5 MΩ cm, Conductivity < 0.2 μS/cm, TOC <30 ppb). The final pH was adjusted using pH meter Mettler Toledo (FE20). All the solvents used in synthesis were reagent grade. The reaction mixture was stirred for small molecules (Heidolph, 500-600 rpm), whereas it was vortexed in incubator-shaker Thermo Scientific MaxQ 8000 (350 rpm) for proteins. UV spectra were recorded on Shimadzu UV-1800 UV-Vis spectrophotometer. Merck Amicon centrifugal spin concentrators (MWCO, 3 kD) were used for removal of small molecules and salts. Samples were lyophilized using CHRiST ALPHA 2-4 LD plus lyophilizer. Peptide was synthesized by SPPS using Fmoc chemistry on Biotage Syro I parallel peptide synthesis system.

Chromatography: A few samples containing non-polar solvent impurities were triturated with pentane. For reactions where chromatography was involved, flash column chromatography was carried out on Combiflash Rf 200 using 230-400 mesh silica gel. Thin-layer chromatography (TLC) was performed on Merck (TLC Silica gel 60 F_{254}) and visualized using a UV lamp (254 nm) and stains such as iodine, ninhydrin, cerium sulfate (yellow dip). Agilent Technologies 1200 series reverse phase preparative HPLC paired to a PDA and single-quad 6130 mass detector was used for purification of **2d**, **2e**, **2h**, **2i**, **2j**, and **2k.**

Nuclear magnetic resonance: ^{1}H, ^{13}C NMR spectra were recorded on Bruker Avance III 400 MHz and 500 MHz NMR spectrometer. ^{1}H NMR spectra were referenced to TMS (0 ppm), ^{13}C NMR spectra were referenced to $CDCl_3$ (77.16 ppm), D_2O (4.79 ppm) and DMSO-d_6 (39.52 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets. Spectra were recorded at 298 K.

Mass spectrometry: Low resolution mass spectra (ESI) were collected on an Agilent Technologies 1200 series HPLC paired to a single-quad 6130 mass spectrometer. Bruker Daltonics MicroTOF-Q-II with electron spray ionization (ESI) was used for the HRMS data. Matrix assisted laser desorption/ionisation time of flight mass spectrometry was performed with Bruker Daltonics UltrafleXtreme and Flex control version 3.4 software. Sinapinic acid and α-cyano-4-hydroxycinnamic acid (HCCA) matrix were used. Peptide mass[1] and fragment ion

calculator (http://db.systemsbiology.net:8080/proteomicsToolkit/FragIonServlet.html) were used for peptide mapping and sequencing.

2. Methods:

2.1. Procedure for dipeptide labeling: 2a

Gly-Phe-NH_2 **1** (2.21 mg, 0.01 mmol) was taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 0.1 ml solution of acetic anhydride. The reaction was allowed to stir at room temperature for 2 h. The reaction mixture was concentrated under the reduced pressure. The trituration with ether (1 ml) resulted in the title compound (S)-2-(2-acetamidoacetamido)-3-phenylpropanamide **2a**.

2.2. Procedure for dipeptide labeling: 2b, 2d-2f, 2h-2j

N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in a clean and dry vial (5 ml) charged with magnetic stir bar and dichloromethane (1 ml). The respective acid (**S1**, **S11**, **S14**, **S19**, **S30**, **S33**, and **S36**; 0.05 mmol) was added and allowed to stir at room temperature for 2 h. The reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted (2 × 8 ml) with water. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the crude reagent was transferred into a vial (5 ml) charged with magnetic stir bar. The mixture was resuspended in acetonitrile (200 µl) and Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.02 mmol) were added. The reaction mixture was allowed to stir at room temperature for 2 h. Further, it was diluted to 500 µl by the addition of acetonitrile and purified by preparative HPLC to render tagged Gly-Phe-NH_2 (**2b**, **2d-2f**, **2h-2j**).

2.3. Procedure for dipeptide labeling: 2c, 2g, 2k

The peptide Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) was taken in a clean and dry vial (5 ml) charged with magnetic stir bar and acetonitrile (0.2 ml). The N-hydroxysuccinimide esters of respective reagents **S6**, **S27** and **3** (0.05 mmol) and triethylamine (2.76 µl, 0.02 mmol) were added through micropipette and allowed to stir at room temperature for 2 h. The reaction mixture was diluted to 500 µl by the addition of acetonitrile and subjected to purification by preparative HPLC to render the tagged Gly-Phe-NH_2 (**2c**, **2g**, **2k**).

2.4. Procedure for checking intensity ratios of native and tagged peptides:

The peptide Gly-Phe-NH_2 **1** (1.105 mg, 0.005 mmol) was taken in a clean and dry Eppendorf tube containing acetonitrile (1 ml). The tagged Gly-Phe-NH_2 **2** (0.005 mmol) was taken in another clean and dry Eppendorf tube containing acetonitrile (1 ml). Equal volume (1 µl) of each solution were taken from the stock solution and re-diluted with acetonitrile (1 ml) in another Eppendorf. The mixture was vortexed, and 0.5 ml was transferred to the HPLC vial (3 ml) for ESI-MS. Subsequently, the intensity ratios were analyzed (ESI Fig. S1).

2.5. Procedure for peptide labeling:

The respective peptide **4** (9 nmol) was dissolved in $NaHCO_3$ buffer (0.1 M, pH 7.8, 25 µl) and was taken into Eppendorf tube (1.5 ml). To this reaction mixture, the sensitivity booster **3** (2.5 equiv. per peptide i.e. 12.8 µg, 22.5 nmol) dissolved in acetonitrile (25 µl) was added. The reaction mixture was incubated at room temperature for 2 h. Subsequently, the sample was subjected to MALDI-ToF-MS and MS-MS using α-cyano-4-hydroxycinnamic acid solution as a matrix.

2.6. Procedure for in-solution digestion of protein: (Scheme 1)

All solutions were made immediately prior to use.

1. **Denaturation:** Cytochrome C **5** solution (10 µl) containing cytochrome C (0.1 mg) in 6 M urea, 100 mM tris and 10 mM $CaCl_2$ (pH 7.8), was taken in 1.5 ml Eppendorf tube and incubated at 37 ^oC for 1 h.

2. **Spin concentration:** The mixture was diluted to 500 µl with grade I water, and the volume was reduced to 200 µl through spin concentration.

3. **Digestion:** The trypsin solution (10 µl; trypsin in 1 mM HCl dissolved in 0.1 M tris and 0.01 M $CaCl_2$) containing 1 µg of trypsin (trypsin/cytochrome C, 1:100) was added to the reaction mixture. It was incubated at 37 ^oC for 18 h, and the pH of digested solution was adjusted to < 6 (verified by pH paper) by adding 0.5% trifluoroacetic acid. Subsequently, the sample was used for MS and MS-MS.

2.7. Procedure for labeling the digested protein: (Scheme 1)

The cytochrome C **5** digest from previous step was lyophilized and re-dissolved in 50 µl of $NaHCO_3$ buffer (0.1 M, pH 7.8) in an Eppendorf tube covered with aluminium foil. From this stock solution, 12.5 µl of cytochrome C digest (0.023 mg, 1.825 nmol) taken into another 1.5 ml

Eppendorf tube covered with aluminium foil. To this reaction mixture, the sensitivity booster **3** [2.5 equiv. for each peptide (0.041 mg, 73 nmol)], dissolved in 12.5 µl of acetonitrile solution, was added. The reaction mixture was incubated at room temperature for 2 h. Reaction was subjected to MALDI-ToF-MS using α-cyano-4-hydroxycinnamic acid solution as a matrix. Reaction was analyzed and subsequently, the sample was used to MALDI MS-MS investigations.

2.8. Procedure for in-solution digestion of antibody: (Scheme 2)

All solutions were made immediately prior to use.

1. **Denaturation:** Trastuzumab (**6**) solution (100 µl) containing trastuzumab **6** (1 mg) and 6 M guanidinium hydrochloride salt (155 µl), was taken in a 1.5 ml Eppendorf tube.
2. **Disulfide reduction:** To this solution, 10 µl of reducing agent (0.2 M DTT, 0.1 M tris, and 0.01 M $CaCl_2$) was added and sample was vortexed for 1 h at 100 °C.
3. **Sulfhydryl alkylation:** To this solution, 20 µl of alkylating agent (0.2 M iodoacetamide, 0.1 M tris, and 0.01 M $CaCl_2$) was added and incubated (in dark) for 1 h at 25 ºC.
4. **Spin concentration:** The digestion mixture was diluted to 500 µl with grade I water, desalted with centrifugal spin concentration while reducing the volume to 200 µl.
5. **Trypsin:** The trypsin solution (10 µl; trypsin in 1 mM HCl dissolved in 0.1 M tris and 0.01 M $CaCl_2$) containing 100 µg of trypsin (trypsin/antibody, 1:10 w/w) was added to the above solution. The reaction mixture was incubated at 37 ºC for 18 h. The pH of digested solution was adjusted to < 6 (verified by pH paper) by adding 0.5% trifluoroacetic acid. Subsequently, the hydrolysis at C-termini of Lys and Arg resulted in the digest. The sample was used for MS.

2.9. Procedure for labeling the antibody digest:

The antibody digest from previous step was lyophilized and re-dissolved in 67 µl of $NaHCO_3$ buffer (0.1 M, pH 7.8) in an Eppendorf tube covered with aluminium foil. From this stock solution, 10 µl of antibody digest containing (0.15 mg, 1 nmol) taken into a 1.5 ml Eppendorf tube covered with aluminium foil. The sensitivity booster **3** (0.24 mg, 420 nmol; i.e. 5 equivalents per peptide) dissolved in 10 µl of acetonitrile solution was added to the reaction mixture. The reaction mixture was incubated at room temperature for 6 h. The sample was subjected to MALDI-ToF-MS using α-cyano-4-hydroxycinnamic acid as a matrix.

2.10. Procedure for protein labeling: (see, Scheme 3a)

Cytochrome C **5** (0.092 mg, 7.3 nmol) was dissolved in 80 µl of phosphate buffer (0.1 M, pH 7.0) and taken into a 1.5 ml Eppendorf tube covered with aluminium foil. 2,5-Dioxopyrrolidin-1-yl benzoate **7** (0.0032 mg, 14.6 nmol) was dissolved in acetonitrile (20 µl) and added to the reaction mixture. It was incubated at room temperature for 2 h and the reaction was followed by MALDI-ToF-MS using sinapinic acid as matrix. After 2 h, the reaction mixture was further diluted with water (0.4 ml) and lyophilized after centrifugal spin concentration to remove the unreacted reagent and salts. The sample was further utilized for digestion and sequencing.

2.11. Procedure for in-solution digestion of protein: (see, Scheme 3a)

All solutions were made immediately prior to use.

1. **Denaturation:** Cytochrome C **5a** solution (10 µl) containing cytochrome C **5a** (0.092 mg) in 6 M urea, 100 mM tris and 10 mM $CaCl_2$ (pH 7.8), was taken in 1.5 ml Eppendorf tube and incubated at 37 ^oC for 1 h.

2. **Spin concentration:** The mixture was diluted to 500 µl with grade I water, then subjected to centrifugal spin concentration for desalting of the sample. A fraction of 200 µl of digestion mixture was collected for the next step.

3. **α-Chymotrypsin:** The α-chymotrypsin solution (10 µl; α-chymotrypsin in 1 mM HCl dissolved in 0.1 M tris and 0.01 M $CaCl_2$) containing 1 µg of trypsin (α-chymotrypsin /cytochrome C, 1:100) was added to the reaction mixture. It was incubated at 37 ^oC for 18 h, and the pH of digested solution was adjusted to < 6 (verified by pH paper) by adding 0.5% trifluoroacetic acid. Subsequently, the hydrolysis at C-termini of Tyr, Phe, and Trp (partial hydrolysis with Leu and Met) resulted in the digest. Next, the sample was used for MS investigations.

2.12. Procedure for labeling the protein digest:

The cytochrome C **5a** digest from previous step was lyophilized and re-dissolved in 50 µl of $NaHCO_3$ buffer (0.1 M, pH 7.8) in an Eppendorf tube covered with aluminium foil. From this stock solution, 12.5 µl of cytochrome C digest (0.023 mg, 1.825 nmol) taken into another 1.5 ml Eppendorf tube covered with aluminium foil. To this reaction mixture, the sensitivity booster **3** [2.5 equiv. for each peptide (0.026 mg, 45 nmol)], dissolved in 12.5 µl of acetonitrile solution, was added. The reaction mixture was incubated at room temperature for 2 h. Reaction was

subjected to MALDI-ToF-MS using α-cyano-4-hydroxycinnamic acid solution as a matrix. Reaction was analyzed and subsequently, the sample was used to MALDI MS-MS investigations.

2.13. Procedure for protein labeling: 5c (see, Schemes 3b)

Under minimized light, Cytochrome C **5** (0.092 mg, 7.3 nmol) was dissolved in 80 µl of phosphate buffer (0.1 M, pH 7.0) and taken into aluminium foil covered 1.5 ml Eppendorf tube. 2,5-Dioxopyrrolidin-1-yl 4-(4-formylphenoxy)butanoate **8** (0.0022 mg, 7.3 nmol), dissolved in 20 µl of acetonitrile solution, was added to reaction mixture. Reaction mixture was incubated at room temperature for 1 h and was further diluted with water (0.4 ml). The buffer and unreacted reagent was subjected to spin concentration for desalting and concentrated by lyophilization. The sample was further utilized for digestion and sequencing.

2.14. Procedure for protein labeling: 5d (see, Schemes 3b)

Under minimized light, Cytochrome C **5** (0.092 mg, 7.3 nmol) dissolved in 80 µl of phosphate buffer (0.1 M, pH 7.0) and taken into aluminium foil covered 1.5 ml Eppendorf tube. 2,5-Dioxopyrrolidin-1-yl-4-(4-formylphenoxy)butanoate **8** (0.0022 mg, 7.3 nmol), dissolved in 20 µl of acetonitrile solution, was added to reaction mixture. Reaction mixture was incubated at room temperature for 1 h. 6-(aminooxy)-N-(bis(dimethylamino)methylene)-N-(6-phenoxyhexyl)hexan-1-aminium bromide **9** (0.71 mg, 14.6 µmol), dissolved in 100 µl of (1:1) acetonitrile and phosphate buffer (0.1 M, pH 7.0), was added and allowed to incubate at room temperature for 4 h. Reaction mixture was further diluted with water (0.4 ml). The buffer and unreacted reagent was subjected to spin concentration for desalting and concentrated by lyophilization. The sample was further utilized for digestion and sequencing.

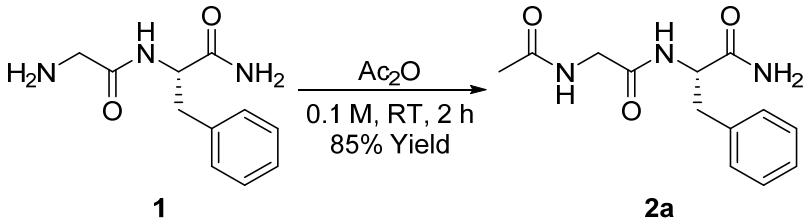
3. Procedure for synthesis of reagents

Procedure for synthesis of Gly-Phe 1

Solid phase peptide synthesis (Biotage Syro I peptide synthesizer with standard Fmoc-protecting group strategy was used for generating the required pool of peptides. Rink amide resin (loading capacity: 0.78 mmol/g) was used for synthesis of all the peptides. All Fmoc amino acids were activated by insitu HBTU/DIPEA activation procedure. The cleavage from the solid support and the simultaneous deprotection of all side chain residues were performed by suspending the fully

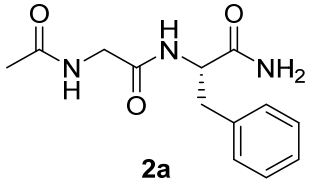
protected compound resin in TFA:H_2O:TIS (95:2.5:2.5) for 3h. The analytically pure peptides were isolated by precipitation in cold diethyl ether or by reverse phase preparative HPLC.

Procedure for synthesis of (S)-2-(2-acetamidoacetamido)-3-phenylpropanamide 2a



1 **2a**

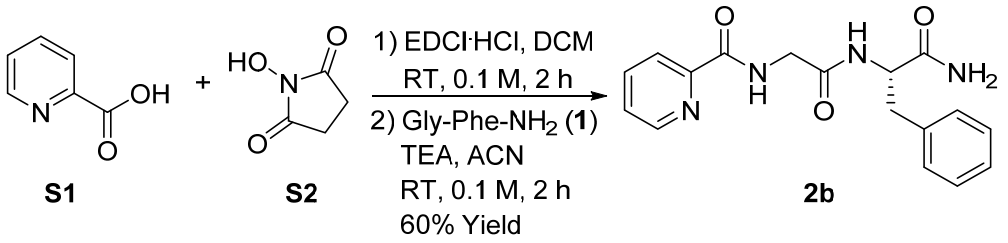
Synthesis of (S)-2-(2-acetamidoacetamido)-3-phenylpropanamide 2a



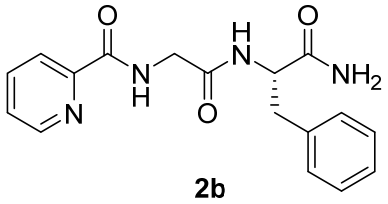
2a

Gly-Phe-NH_2 **1** (2.21 mg, 0.01 mmol) was taken in a clean and dry 5 ml vial charged with magnetic rice bead containing the 100 µl solution of acetic anhydride. Reaction mixture was allowed to stir at room temperature for 2 h and concentrated. 1 ml of ether was added to this crude reaction mixture and triturated to give the title compound (S)-2-(2-acetamidoacetamido)-3-phenylpropanamide **2a** (2.23 mg, 85% yield) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ: 7.40 (m, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 7.0 Hz, 2H), 4.63 (dd, *J* = 9.0, 5.7 Hz, 1H), 3.82 (m, 2H), 3.22 (dd, *J* = 14.0, 5.7 Hz, 1H), 3.01 (dd, *J* = 14.0, 9.0 Hz, 1H), 2.02 (s, 3H) ppm. ^{13}C NMR (126 MHz, D_2O) δ 175.8, 174.7, 171.5, 136.4, 129.1, 128.7, 127.1, 54.5, 42.4, 36.9, 21.6 ppm. HRMS (ESI) $[M+Na]^+$ calcd. for $C_{13}H_{17}N_3O_3$ 286.1162, found 286.1136.

Procedure for synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)picolinamide 2b



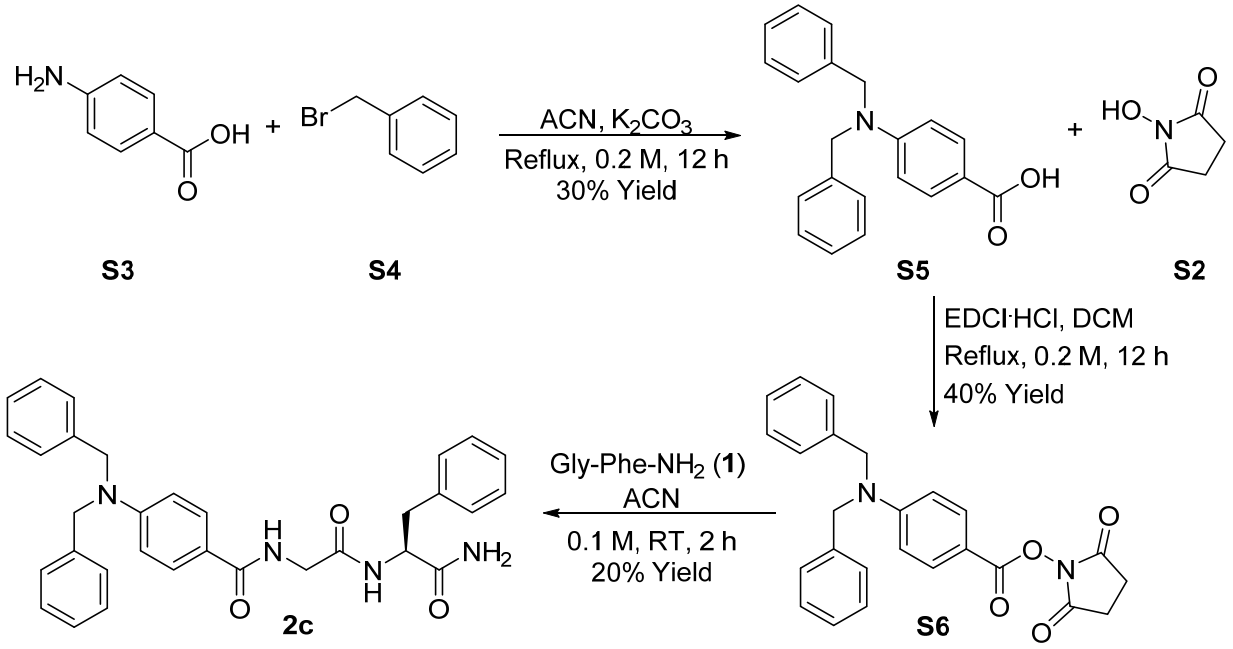
Synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)picolinamide 2b



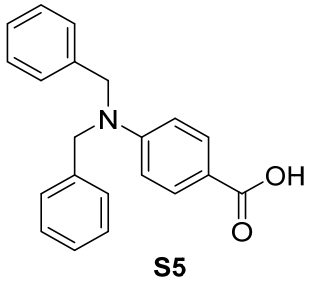
N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) was taken in a clean and dry 5 ml vial charged with magnetic stir bar containing the 1 ml solution of dichloromethane. Picolinic acid **S1** (12.3 mg, 0.1 mmol) was added and allowed to stir at room temperature for 2 h. Reaction was followed by thin layer chromatography. The reaction mixture was diluted with 9 ml of dichloromethane and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated and crude reagent was transferred into 5 ml vial charged with magnetic stir bar. 0.2 ml of acetonitrile solution was added to this crude reagent contained in the above vial. Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethylamine (2.76 µl, 0.02 mmol) was added through micro pipette and allowed to stir at room temperature for 2 h. Reaction mixture was concentrated under reduced pressure. 0.5 ml of dichloromethane was added twice to this and triturated to give the title compound (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)picolinamide **2b** (0.9 mg, 30% yield) as pale yellow solid. ^{1}H NMR (500 MHz, CD_3OD) δ 8.54 (d, *J* = 4.6 Hz, 1H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.87 (td, *J* = 7.7, 1.7 Hz, 1H), 7.47 (m, 1H), 7.14 – 7.08 (m, 4H), 7.07 – 7.02 (m, 1H), 4.55 (dd, *J* = 8.8, 5.4 Hz, 1H), 3.94 (m, 2H), 3.08 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.83 (dd, *J* = 13.9, 8.9 Hz, 1H) ppm. ^{13}C NMR (126 MHz,

CD_3OD) δ 174.6, 169.8, 165.9, 149.2, 148.5, 137.3, 136.9, 128.9, 128.0, 126.5, 126.3, 121.8, 54.3, 42.2, 37.3 ppm. HRMS (ESI) $[M+Na]^+$ calcd. for $C_{13}H_{18}N_4O_3$ 349.1271, found 349.1296.

Synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(dibenzylamino)benzamide 2c



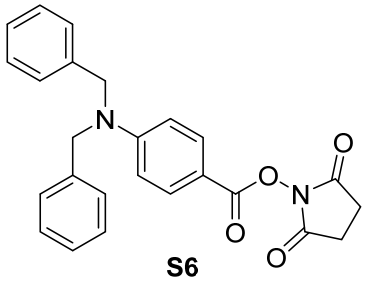
Synthesis of 4-(Dibenzylamino)benzoic Acid S5[2]



4-Amino benzoic acid **S3** (274 mg, 2 mmol) and potassium carbonate (680 mg, 4.92 mmol) were taken in a clean and dry 100 ml round bottom flask charged with magnetic stir bar containing 10 ml solution of acetonitrile. The resulting reaction mixture was refluxed for 30 min, followed by the addition of benzyl bromide **S4** (470 µl, 4 mmol). The reaction mixture was refluxed at 78 °C for 24 h. The reaction mixture was then poured into water, and the resulting precipitate was filtered off, washed with water, and dried. The resulting residue was purified by silica gel column chromatography (hexane/EtOAc, 95:5, R_f 0.25), thereby affording the desired product as

a white solid 4-(dibenzylamino)benzoic Acid **S5** (0.28 g, 36% Yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.91 (m, 2H), 7.34 (m, 4H), 7.28 (t, *J* = 7.3 Hz, 2H), 7.22 (d, *J* = 7.7 Hz, 4H), 6.73 (m, 2H), 4.72 (s, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.0, 153.2, 137.2, 132.2, 128.8, 127.3, 126.4, 116.2, 111.3, 54.0 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{21}H_{19}NO_2$ 318.1489, found 318.1484.

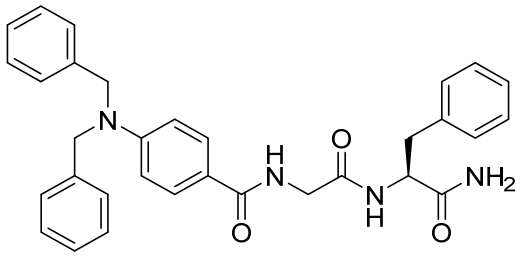
Synthesis of 2,5-dioxopyrrolidin-1-yl 4-(dibenzylamino)benzoate S6



S6

N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 1 ml solution of dichloromethane. 4-(dibenzylamino)benzoic acid **S5** (31.7 mg, 1.0 mmol) was added to this and allowed to stir at room temperature for 2 h. Reaction mixture was diluted with 9 ml of dichloromethane followed by water extraction (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure. The resulting residue was triturated with 2 ml of ether and pentane to yield white solid 2,5-dioxopyrrolidin-1-yl 4-(dibenzylamino)benzoate **S6** (27 mg, 50% yield). ^{1}H NMR (500 MHz, $CDCl_3$,): δ 7.91 (d, *J* = 9.1 Hz, 2H), 7.34 (m, 4H), 7.28 (t, *J* = 7.3 Hz, 2H), 7.19 (d, *J* = 7.0 Hz, 4H), 6.75 (d, *J* = 9.2 Hz, 2H), 4.73 (s, 4H), 2.85 (s, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 169.9, 161.8, 154.0, 136.9, 132.9, 129.1, 127.6, 126.5, 111.9, 111.7, 54.2, 25.8 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{25}H_{22}N_2O_4$ 415.1652, found 415.1672.

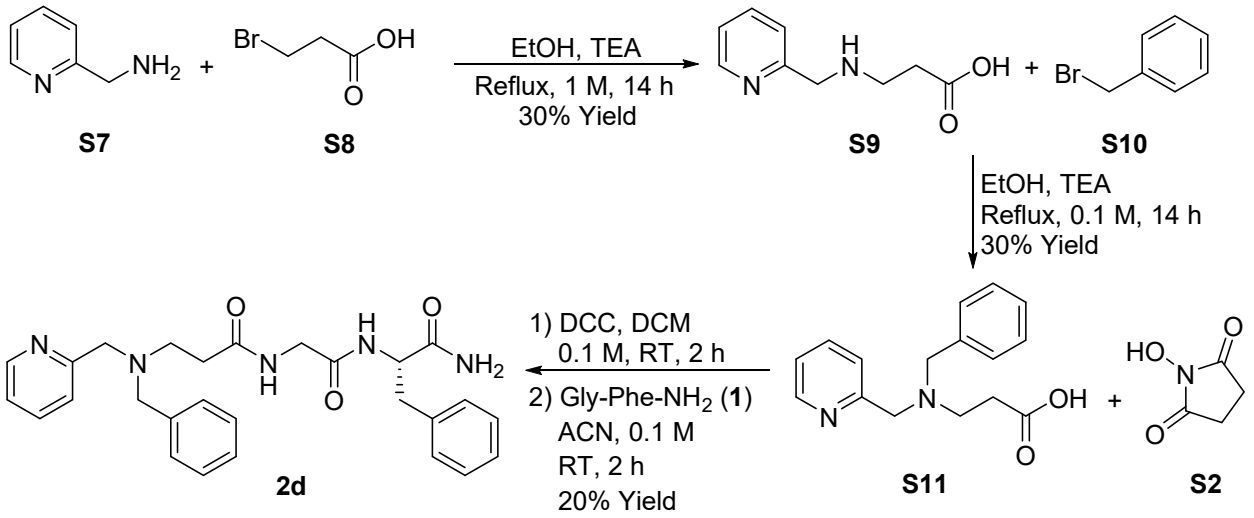
(S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(dibenzylamino) benzamide 2c



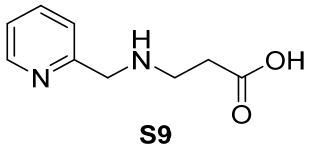
2c

Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) was taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 200 µl solution of acetonitrile. To this 2,5-dioxopyrrolidin-1-yl-4-(dibenzylamino)benzoate **S6** (16.56 mg, 0.04 mmol) and triethyl amine (2.76 µl, 0.02 mmol) were added and resulting reaction mixture was allowed to stir at room temperature for 2 h. Reaction was followed by thin layer chromatography. Reaction mixture was concentrated under reduced pressure, the resulting residue was purified via flash column chromatography on silica (DCM/MeOH, 97:2, R_f 0.1) as the eluent, thereby affording the title compound (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(dibenzylamino)benzamide **2c** (3.8 mg, 35% yield) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, *J* = 8.5 Hz, 2H), 7.34 (m, 4H), 7.29 (d, *J* = 7.1 Hz, 2H), 7.20 (m, 4H), 7.17 (m, 4H), 7.13 (m, 1H), 6.72 (d, *J* = 8.5 Hz, 2H), 4.72 (s, 4H), 4.67 (dd, *J* = 14.4, 7.1 Hz, 1H), 3.98 (m, 2H), 3.11 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 169.6, 168.1, 151.9, 137.4, 136.4, 129.2, 129.0, 128.9, 128.7, 127.3, 127.0, 126.4, 120.4, 111.6, 54.1, 44.1, 37.5, 29.7 ppm. HRMS (ESI) $[M+Na]^+$ calcd. for $C_{32}H_{32}N_4O_3$ 543.2367, found 543.2371.

Procedure for synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(benzyl(pyridin-2-ylmethyl)amino)propanamide 2d

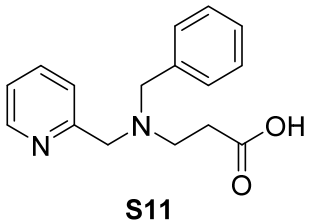


3-((pyridin-2-ylmethyl)amino)propanoic acid S9[3]



2-Aminomethylpyridine **S7** (510 µl, 5 mmol) and triethyl amine (690 µl, 5 mmol) were taken in a clean and dry 25 ml round bottom flask charged with magnetic stir bar containing the 5 ml solution of EtOH. 3-bromo propionic acid **S8** (760 mg, 5 mmol) was added and refluxed at 80 ºC for 24 h. On cooling, the resulting precipitate was filtered off, washed with small portions of acetonitrile, and dried in reduced pressure to afford 3-((pyridin-2-ylmethyl)amino)propanoic acid **S9** (360 mg, 40% yield) as colourless needles. ^{1}H NMR (500 MHz, D_2O): δ 8.59 (d, *J* = 4.6 Hz, 1H), 7.91 (m, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.47 (m, 1H), 4.38 (s, 2H), 3.30 (t, *J* = 6.6 Hz, 2H), 2.60 (t, *J* = 6.6 Hz, 2H) ppm. ^{13}C NMR (126 MHz, D_2O): δ 180.5, 152.7, 152.0, 141.0, 127.0, 126.6, 53.5, 46.9, 34.8 ppm. LRMS (ESI) $[M+H]^+$ calcd. for $C_9H_{12}N_2O_2$ 181.2, found 181.2.

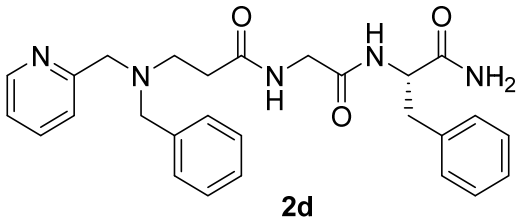
3-(benzyl(2-(pyridin-2-yl)ethyl)amino)propanoic acid S11[3]



S11

3-((pyridin-2-ylmethyl)amino)propanoic acid **S9** (180 mg, 1mmol) and triethyl amine (130 µl, 1 mmol) were taken in a clean and dry 25 ml vial charged with magnetic stir bar containing 10 ml solution of EtOH. Benzyl bromide **S10** (118 µl, 1 mmol) was added and refluxed at 80 ºC for 24 h. On cooling, the resulting precipitate was filtered off, resulting residue was purified via flash column chromatography on silica using (DCM/MeOH, 97:5, R_f 0.1) as the eluent, thereby affording the title product 3-(benzyl(pyridin-2-ylmethyl)amino)propanoic acid **S11** (108 mg, 40% yield) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.59 (m, 1H), 7.68 (m, 1H), 7.36 – 7.27 (m, 5H), 7.24 – 7.22 (m, 2H), 3.85 (s, 2H), 3.80 (s, 2H), 2.96 (t, *J* = 6.2 Hz, 2H), 2.60 (t, *J* = 6.2 Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.5, 156.4, 149.2, 137.1, 135.7, 129.5, 128.8, 128.1, 123.6, 122.8, 58.0 (unresolved doublet), 49.2, 31.1 ppm. LRMS (ESI) $[M+H]^+$ calcd. for $C_{15}H_{17}N_3O_2$ 271.3, found 271.4

(S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(benzyl(pyridin-2-ylmethyl)amino)propanamide 2d

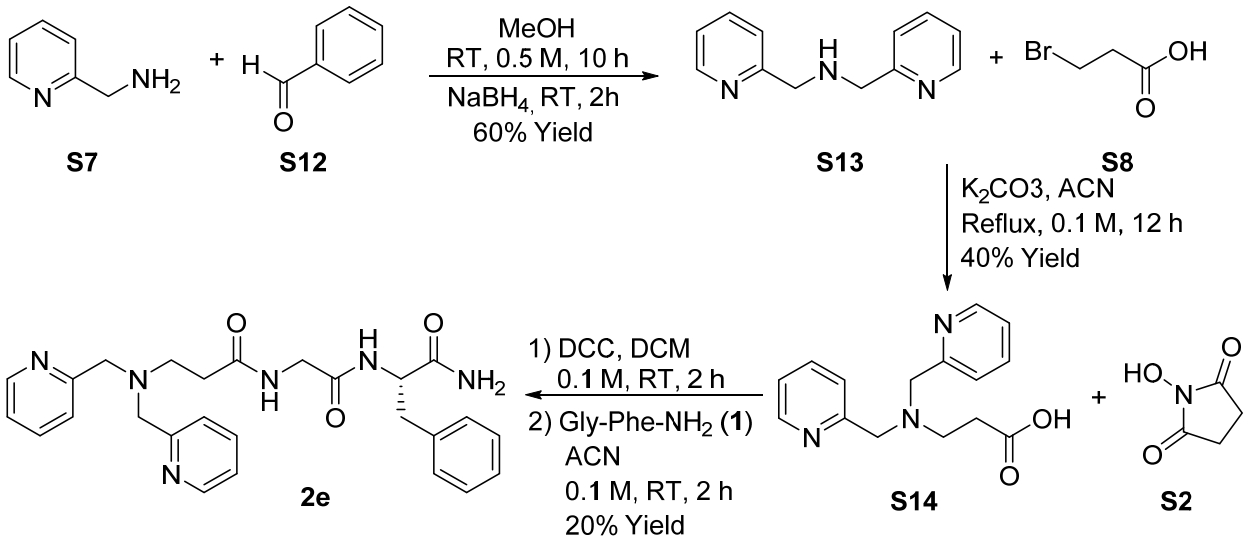


2d

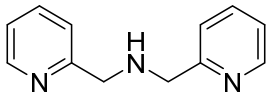
N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 1 ml solution of dichloromethane. 3-(benzyl(pyridin-2-ylmethyl)amino)propanoic acid **S11** (27 mg, 0.1 mmol) was added and allowed to stir at room temperature for 2 h. The reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted (2 × 8 ml) with water. The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the remaining

crude reagent was transferred into 5 ml vial charged with magnetic stir bar. After addition of acetonitrile (0.2 ml), Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethylamine (2.76 µl, 0.02 mmol) were added through micropipette. The reaction mixture was stirred at room temperature for 2 h and followed by ESI-MS. The reaction was diluted to 500 µl with acetonitrile and subjected to prep-HPLC for the purification to isolate (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(benzyl(pyridin-2-ylmethyl)amino)propanamide **2d** (2.82mg, 30% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (m, 1H), 7.65 (m, 1H), 7.33 – 7.25 (m, 4H), 7.24 – 7.17 (m, 6H), 7.12 – 7.09 (m, 2H), 4.70 (m, 1H), 3.88 – 3.78 (m, 2H), 3.73 (m, 2H), 3.64 (m, 2H), 3.17 (dd, *J* = 14.1, 6.7 Hz, 1H), 3.04 (dd, *J* = 14.1, 5.9 Hz, 1H), 2.75 (m, 2H), 2.49 – 2.27 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 173.7, 169.8, 157.7, 149.3, 137.1, 136.9, 136.4, 129.2, 128.6, 128.6, 127.7, 126.9, 123.7, 122.8, 122.6, 58.8, 58.1, 53.7, 49.2, 43.9, 36.9, 32.5 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{27}H_{31}N_5O_3$ 474.2500, found 474.2526.

Procedure for synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(bis(pyridin-2-ylmethyl)amino)propanamide 2e



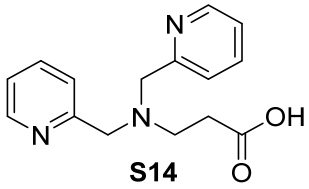
Synthesis of di-(2-picolyl)amine S13[4]



S13

To a suspension of 2-pyridinecarboxaldehyde **S12** (0.951 ml, 10 mmol) in ethanol (2 ml), a solution of 2-(aminomethyl)-pyridine **S7** (1.03 ml, 10 mmol) in ethanol (18 ml) was added dropwise at 0 °C. After 4 h, sodium borohydride (726 mg, 19.2 mmol) was added in small portions while maintaining the temperature at 0 °C. The reaction was stirred for 12 h at room temperature. Next, aqueous hydrochloric acid (5 M, 24 ml) was added dropwise and it was stirred for another 1 h. A 2 M aqueous solution of sodium hydroxide was then added until a pH of 11 was reached. The mixture was extracted with methylene chloride (6 × 100 ml), dried over sodium sulfate, and then filtered. Removal of volatiles afforded an analytically pure di-(2-picolyl)amine **S13** (1.71 g 89% yield) as a brown oil. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.56 (d, *J* = 4.7 Hz, 2H), 7.64 (m, 2H), 7.36 (d, *J* = 7.7 Hz, 2H), 7.16 (dd, *J* = 6.9, 5.4 Hz, 2H), 3.99 (s, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 159.6, 149.3, 136.5, 122.3, 122.0, 54.7 ppm. LRMS (ESI) $[M+H]^+$ calcd. for $C_{12}H_{13}N_3$ 200.1, found 200.2

Synthesis of 3-(bis(pyridin-2-ylmethyl)amino)propanoic acid S14[5]

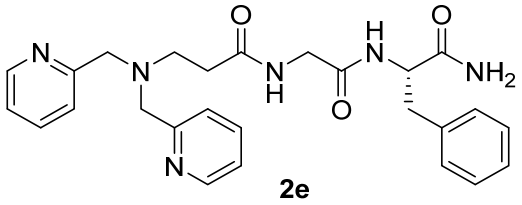


S14

Di-(2-picolyl)amine **S13** (190 mg, 1 mmol) and triethyl amine (138 µl, 1 mmol) were taken in a clean and dry 25 ml vial charged with magnetic stir bar containing 10 ml solution of EtOH. Next, 3-bromopropionic acid **S8** (152 mg, 1 mmol) was added and the reaction mixture was refluxed at 80 ºC for 24 h. The cooling to room temperature results in the formation of precipitate. It is filtered, washed with small portions of acetonitrile, and dried in vacuo to afford 3-(bis(pyridin-2-ylmethyl)amino)propanoic acid **S14** (108 mg, 40% yield) as colourless needles. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.53 (d, *J* = 4.3 Hz, 2H), 7.62 (m, 2H), 7.34 (m, 2H), 7.17 (m, 2H), 3.92 (s, 4H), 3.00 (t, *J* = 6.4 Hz, 2H), 2.60 (t, *J* = 6.4 Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): 174.2,

157.3, 148.9, 137.1, 123.6, 122.6, 59.2, 49.9, 32.4 ppm. LRMS (ESI) $[M+H]^+$ calcd. for $C_{15}H_{17}N_3O_2$ 272.1, found 272.1

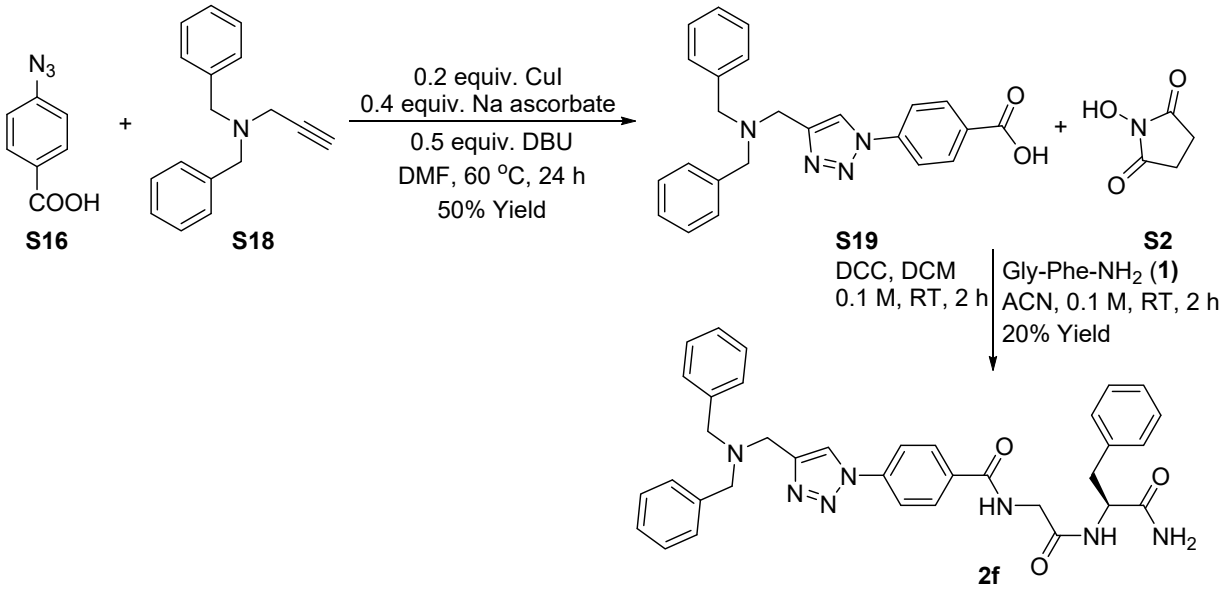
(S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(bis(pyridin-2-ylmethyl)amino)propanamide 2e



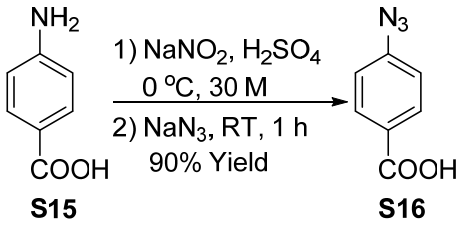
2e

N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 1 ml solution of dichloromethane. Next, 3-(bis(pyridin-2-ylmethyl)amino)propanoic acid **S14** (27.4 mg, 0.1 mmol) was added and allowed to stir at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and the remaining crude reagent was transferred into 5 ml vial charged with magnetic stir bar. It was diluted with acetonitrile (0.2 ml) and Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.02 mmol) were added through micropipette. The reaction mixture was allowed to stir at room temperature for 2 h. Subsequently, it was diluted to 500 µl with acetonitrile and subjected to prep-HPLC to isolate (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-3-(bis(pyridin-2-ylmethyl)amino)propanamide **2e** (2.82 mg, 30% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.2 Hz, 2H), 7.62 (m, 2H), 7.22 (m, 2H), 7.20 – 7.15 (m, 5H), 7.11 (m, 2H), 4.70 (m, 1H), 3.88 (m, 2H), 3.80 (m, 4H), 3.15 (dd, *J* = 14.1, 7.2 Hz, 1H), 3.07 (dd, *J* = 14.3, 5.7 Hz, 1H), 2.79 (t, *J* = 5.8 Hz, 2H), 2.44 – 2.25 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7, 170.2, 170.1, 157.9, 149.5, 136.8, 136.6, 129.2, 128.6, 126.9, 123.4, 122.6, 59.6, 53.7, 49.7, 44.2, 36.7, 32.8 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{26}H_{30}N_6O_3$ 475.2452, found 475.2478.

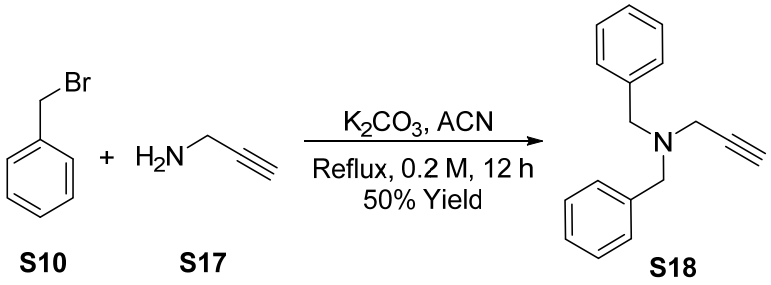
Procedure for synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(4-((dibenzylamino)methyl)-1*H*-1,2,3-triazol-1-yl)benzamide 2f



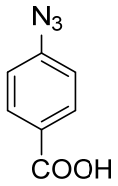
(a) Synthesis of 4-azidobenzoic acid S16



(b) Synthesis of N, N-Dibenzylpropargylamine S18



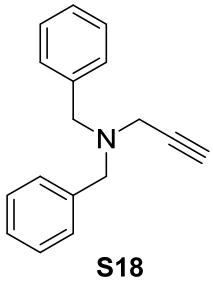
Synthesis of 4-azidobenzoic acid S16[6]



S16

To an ice-cold suspension of 4-aminobenzoic acid **S15** (137 mg, 1.0 mmol) in 6 N H_2SO_4 (2 ml), an aqueous solution of sodium nitrite (83 mg, 1.2 mmol) was added dropwise over 10 min. The resulting mixture was stirred at 0 °C for additional 30 min. An aqueous solution of sodium azide (98 mg, 1.5 mmol) was added dropwise to the above mixture. The reaction was then carried out at room temperature for 1 h. The reaction mixture was extracted with EtOAc (3 × 30 ml). The combined EtOAc phase was washed with water, brine and dried over Na_2SO_4. After removal of Na_2SO_4 via filtration, the filtrate was concentrated to dryness to afford the desired azidobenzoic acid **S16** (163 mg, 98% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$,): δ 8.11 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ 170.8, 145.8, 132.1, 125.6, 119.0 ppm.

Synthesis of N,N-Dibenzylpropargylamine S18

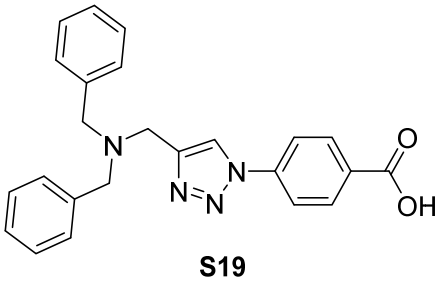


S18

A mixture of benzyl bromide **S10** (0.69 ml, 0.58 mmol), propargylamine **S17** (0.2 ml, 3 mmol), and potassium carbonate (1.232 g, 1.2 mol) were vigorously stirred in acetonitrile (15 ml) in a 100 ml round bottom flask charged with a magnetic stir bar. The reaction was refluxed for 4 h at 78 ºC and the solvent was removed under the reduced pressure. It was further re-diluted with 20 ml water and extracted with dichloromethane (3 × 15 ml). The combined organic extracts were dried over Na_2SO_4 and concentrated. The resulting residue was purified via flash column chromatography on silica (hexane/EtOAC 97:3, R_f 0.25), thereby affording N,N-

dibenzylpropargylamine **S18** (0.502 g, 71% yield) as yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (m, 4H), 7.34 – 7.28 (m, 4H), 7.24 (m, 2H), 3.68 (s, 4H), 3.25 (d, *J* = 2.4 Hz, 2H), 2.26 (t, *J* = 2.4 Hz, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.9, 129.1, 128.4, 127.2, 78.6, 73.4, 57.5, 41.2 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{17}H_{17}N$ 236.1434, found 236.1457.

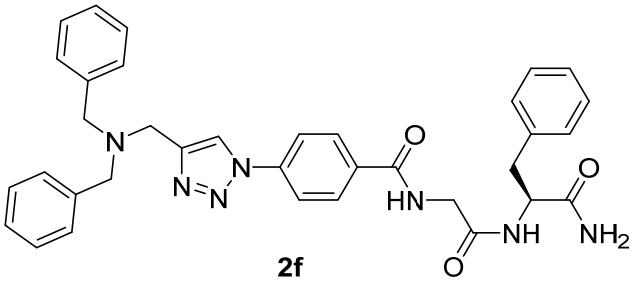
Synthesis of 4-(4-((dibenzylamino)methyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid S19[7]



S19

A mixture of CuI (1.9 mg, 0.01 mmol, 0.01 equiv.), DIPEA (2.6 mg, 0.02 mmol, 0.02 equiv.), and AcOH (1.2 mg, 0.02 mmol, 0.02 equiv.) in DMF (5 ml) was taken in a clean and dry 5 ml vial charged with magnetic stir bar. Next, N,N-dibenzylpropargyl amine **S18** (235 mg, 1 mmol) and benzyl azide **S16** (175 mg, 1.05 mmol) were added and the reaction mixture was allowed to stir at room temperature until the alkyne disappeared (~24 h). The reaction mixture was purified by a short chromatography column (DCM/MeOH, 95:5, R_f 0.2) to give 4-(4-((dibenzylamino)methyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid **S19** (199 mg, 50% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.27 (d, *J* = 8.8 Hz, 2H), 8.00 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.45 (m, 4H), 7.35 (m, 4H), 7.30 – 7.25 (m, 2H), 3.95 (s, 2H), 3.78 (s, 4H) ppm. ^{13}C NMR ($CDCl_3$, 126 MHz): δ 169.9, 146.2, 140.4, 138.1, 138.0, 131.9, 129.1, 128.5, 127.4, 120.9, 119.9, 57.7, 47.9 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{24}H_{22}N_4O_2$ 399.1816, found 399.1829.

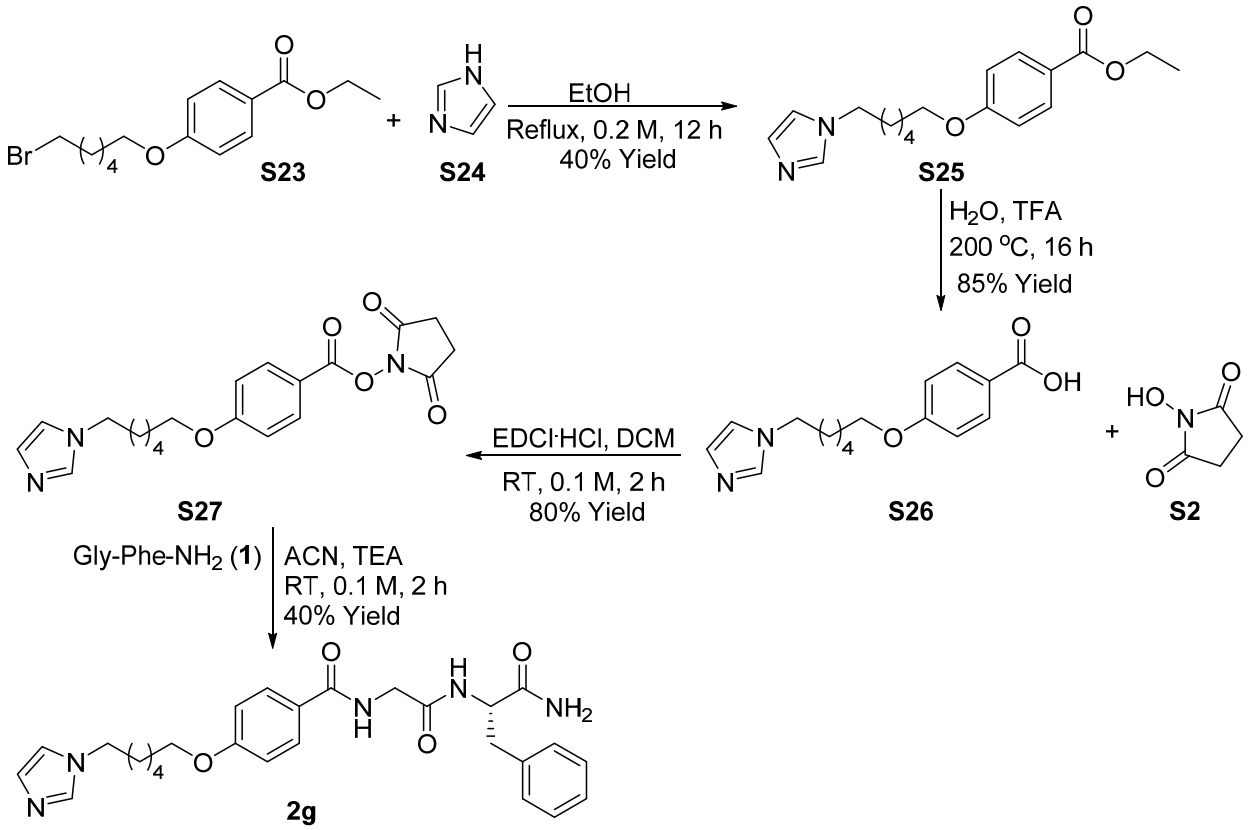
Synthesis of (S)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(4((dibenzylamino)methyl)-1*H*-1,2,3-triazol-1-yl)benzamide 2f



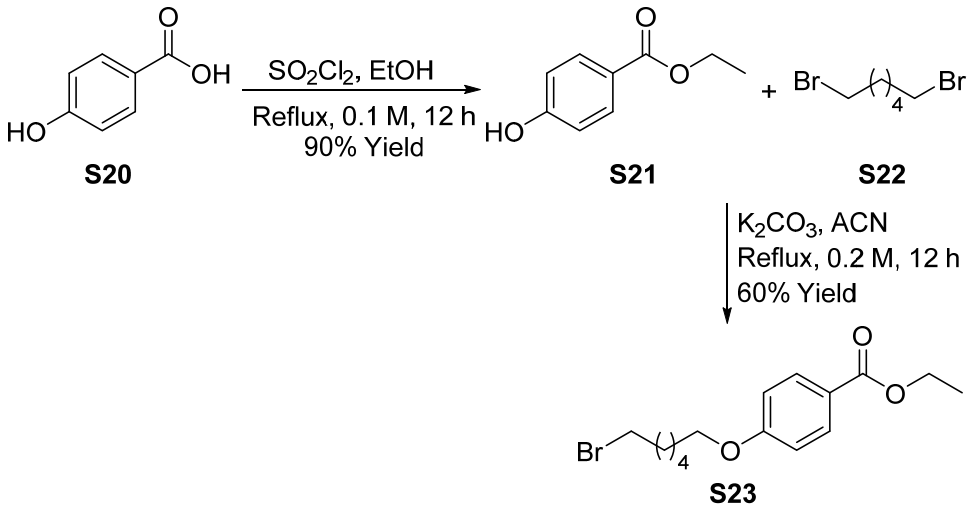
2f

N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in a clean and dry 5 ml vial charged with magnetic stir bar containing dichloromethane (1 ml). The 4-(4-((dibenzylamino)methyl)-1*H*-1,2,3-triazol-1-yl)benzoic acid **S19** (20.0 mg, 0.05 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The reaction was followed by thin layer chromatography. Next, it was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated under reduced pressure, and crude reagent was transferred into 5 ml vial charged containing acetonitrile (0.2 ml) and magnetic stir bar. Next, Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.02 mmol) were added through micropipette and the reaction mixture was stirred at room temperature for 2 h. It was further diluted to 500 µl by addition of acetonitrile and subjected to prep-HPLC to isolate pure N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)-4-(4((dibenzylamino) methyl)-1*H*-1,2,3-triazol-1-yl)benzamide **2f** (3.6 mg, 30% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, *J* = 7.9 Hz, 2H), 7.88 (s, 1H), 7.75 (d, *J* = 7.9 Hz, 2H), 7.41 (m, 4H), 7.32 (m, 4H), 7.24 (m, 2H), 7.22 – 7.11 (m, 5H), 4.71 (dd, *J* = 16.0, 10.2 Hz, 1H), 4.08 (m, 2H), 3.83 (s, 2H), 3.67 (s, 4H), 3.08 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 169.3, 166.7, 139.4, 138.9 (two peaks), 136.3, 133.1, 129.8, 129.2, 129.0, 128.8, 128.7, 128.4, 127.1, 120.5, 120.1, 57.9, 54.5, 48.2, 43.8, 38.0 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{35}H_{35}N_7O_3$ 602.2874, found 602.2884.

Synthesis of (S)-4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)benzamide 2g



a) Synthesis of ethyl 4-hydroxybenzoate S23

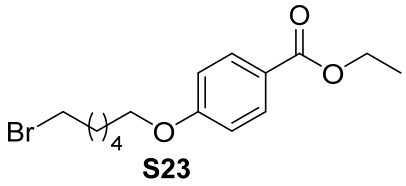


Synthesis of ethyl 4-hydroxybenzoate S21

S21

4-Hydroxybenzoic acid **S20** (138 mg, 1 mmol) was taken in a 25 ml round bottom flask containing EtOH (10 ml) and Teflon-coated magnetic stir bar. The flask was placed in an ice-water bath followed by slow addition of thionyl chloride (0.29 ml, 4 mmol). After the addition, flask was moved from ice-water bath to oil-bath and sealed by condenser having $CaCl_2$ guard tube. The reaction mixture was refluxed for 6 h before bringing it back to the room temperature. The reaction mixture was concentrated under reduced pressure to give the product ethyl 4-hydroxybenzoate **S21** (157 mg, 95% yield) as white precipitate. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.6 Hz, 2H), 6.90 (m, 2H), 4.36 (q, *J* = 7.0 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.8, 160.0, 131.9, 122.9, 115.2, 60.9, 14.4 ppm. HRMS (ESI) $[M+Na]^+$ calcd. for $C_9H_{10}O_3$ 189.0522, found 189.0536.

Synthesis of ethyl 4-((6-bromohexyl)oxy)benzoate S23

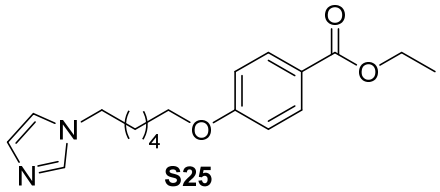


S23

4-Hydroxyethylbenzoate **S21** (165 mg, 1 mmol) and potassium carbonate (276 mg, 2 mmol) were taken in a 25 ml round bottom flask with Teflon-coated magnetic stir bar and acetonitrile (5 ml). Next, 1,6-dibromohexane **S22** (0.307 ml, 2 mmol) was added and flask was sealed with condenser. The reaction mixture was refluxed for 16 h, cooled to room temperature, and the white solid (waste of potassium carbonate) was filtered through celite. The filtrate was dried under reduced pressure to get yellow oil that was purified flash column chromatography. The desired product was eluted using (n-hexane/ethyl acetate, 98:2, Rf = 0.1) to obtain ethyl 4-(6-bromohexyloxy)benzoate **S23** (171 mg, 60% yield) as a pale-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.9 Hz, 2H), 6.90 (d, *J* = 8.9 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 4.01 (t, *J* = 6.4 Hz, 2H), 3.43 (t, *J* = 6.8 Hz, 2H), 1.94 – 1.87 (m, 2H), 1.87 – 1.77 (m, 2H), 1.55 – 1.47 (m,

4H), 1.38 (t, J = 7.1 Hz, 3H) ppm.^{13}C NMR (126 MHz, $CDCl_3$): δ 166.4, 162.8, 131.5, 122.8, 114.0, 67.9, 60.6, 33.7, 32.6, 29.0, 27.9, 25.3, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{15}H_{21}BrO_3$ 329.0747, found 329.0712.

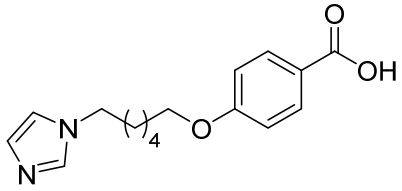
Synthesis of ethyl 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate S25



S25

Imidazole **S24** (204 mg, 3 mmol) was taken in a 25 ml round bottom flask charged with Teflon-coated magnetic stir bar containing 5 ml solution of EtOH. Ethyl 4-(6-bromohexyloxy)benzoate **S23** (329 mg, 1 mmol) was added and allowed to refluxed for 12 h. The resultant mixture was concentrated under reduced pressure and purified by a short chromatograph column [DCM/MeOH, 95:5, R_f 0.5] to afford of ethyl 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate **S25** (158.5 mg, 50% yield) as a viscous liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J = 8.9 Hz, 2H)), 7.47 (s, 1H), 7.06 (m, 1H), 6.90 (m, 1H), 6.88 (d, J = 8.9 Hz, 2H), 4.34 (q, J = 7.1 Hz, 2H), 3.99 (t, J = 6.3 Hz, 2H), 3.95 (t, J = 7.1 Hz, 2H), 1.86 – 1.75 (m, 4H), 1.55 – 1.47 (m, 2H), 1.41 – 1.34 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$, 126 MHz) δ 166.4, 162.7, 137.1, 131.5, 129.5, 122.8, 118.7, 114.0, 67.8, 60.6, 46.9, 31.0, 28.9, 26.3, 25.6, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{18}H_{24}N_2O_3$ 317.1860, found 317.1860.

4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoic acid S26

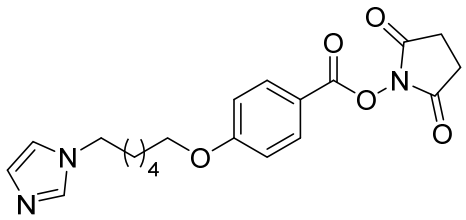


S26

ethyl 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate **S25** (316 mg, 1 mmol) was taken in a 5 ml round bottom flask charged with Teflon-coated magnetic stir bar containing 2 ml of water. Subsequently, TFA (0.918 ml, 12 mmol) was added slowly and the flask was sealed with condenser. The reaction mixture was refluxed (bath temperature of 200 ºC) for 24 h. Next, it was

cooled to room temperature and the solvent was concentrated under reduced pressure to give a yellow oil. To this, diethyl ether (3 × 3 ml) was mixed and stirred for overall 30 min at room temperature. It crashes the product as a white precipitate that is isolated and dried under reduced pressure to obtain 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoic acid **S26** (241 mg, 84% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.96 (s, 1H), 7.95 (d, *J* = 8.9 Hz, 2H), 7.72 – 7.63 (m, 1H), 7.62 – 7.51 (m, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 4.27 (t, *J* = 7.3 Hz, 2H), 4.04 (t, *J* = 6.3 Hz, 2H), 1.94 (m, 2H), 1.81 (m, 2H), 1.56 (m, 2H), 1.42 (m, 2H) ppm. ^{13}C NMR (126 MHz, CD_3OD): δ 168.4, 163.0, 134.9, 131.4, 122.5, 121.8, 119.8, 113.7, 67.6, 49.1, 29.7, 28.5, 25.6, 25.1 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{16}H_{20}N_2O_3$ 289.1547, found 289.1543.

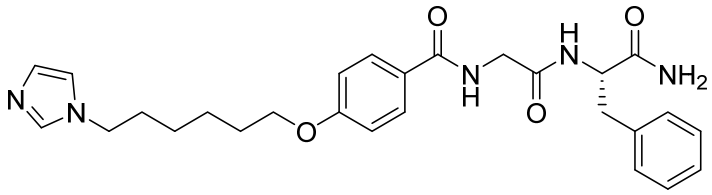
2,5-dioxopyrrolidin-1-yl-4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate S27



S27

N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (38.3mg, 0.2 mmol) were taken in a clean and dry 5 ml vial containing magnetic stir bar and dichloromethane (1 ml). Next, 4-((6-(1*H*-Imidazol-1-yl)hexyl)oxy)benzoic acid **S26** (0.028 g, 0.1 mmol) was added and reaction mixture was stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted dichloromethane (9 ml) and extracted by water (2 × 8 ml). The organic layer was dried over Na_2SO_4 and concentrated under the reduced pressure. The resulting residue was triturated with ether and pentane (2 ml each) to yield 2,5-dioxopyrrolidin-1-yl 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate **S27** (0.023 g, yield 60%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, *J* = 8.9 Hz, 2H), 7.53 (s, 1H), 7.07 (m, 1H), 6.94 (d, *J* = 9.0 Hz, 2H), 6.92 (m, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.96 (t, *J* = 7.1 Hz, 2H), 2.89 (s, 4H), 1.87 – 1.76 (m, 4H), 1.51 (m, 2H), 1.37 (m, 2H) ppm.^{13}C NMR (126 MHz, $CDCl_3$): δ 169.5, 164.4, 161.5, 137.1, 132.9, 129.2, 118.9, 117.0, 114.62, 68.2, 47.0, 31.0, 28.8, 26.3, 25.7, 25.5 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{20}H_{23}N_3O_5$ 386.1710, found 386.1700.

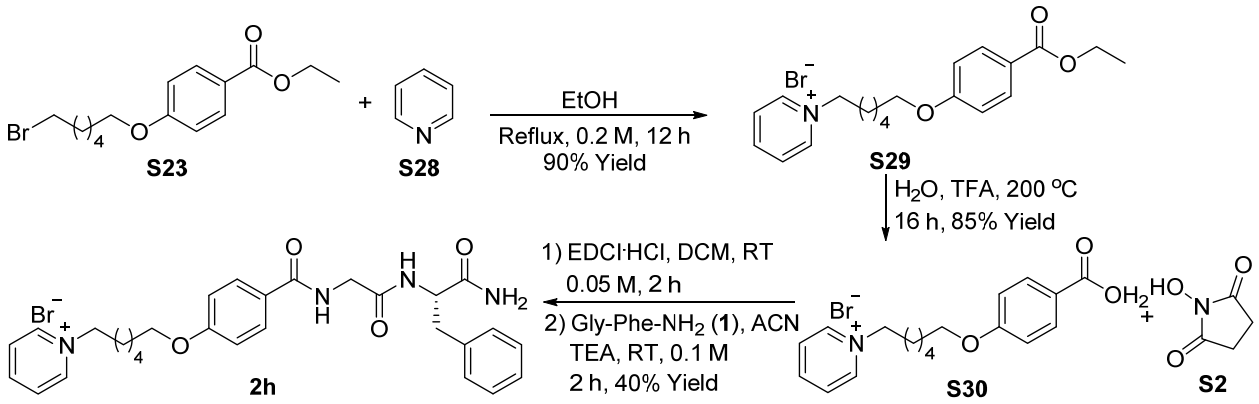
(S)-4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)benzamide 2g



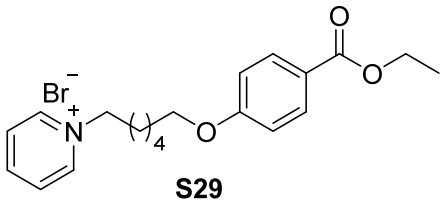
2g

Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) was taken in a clean and dry 5 ml vial charged with magnetic stir bar containing 200 µl solution of acetonitrile. To this, 2,5-dioxopyrrolidin-1-yl 4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)benzoate **S27** (15.4 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.01 mmol) were added and the resulting reaction mixture was stirred at room temperature for 2 h. The reaction was followed by thin layer chromatography and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica (DCM/MeOH, 95:5, R_f 0.1), affording the title compound (S)-4-((6-(1*H*-imidazol-1-yl)hexyl)oxy)-N-(2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)benzamide **2g** (1.9 mg, 35% yield) as yellowish white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (d, *J* = 8.7 Hz, 2H), 7.49 (s, 1H), 7.23 – 7.16 (m, 5H), 7.05 (m, 1H), 6.90 (m, 1H), 6.87 (d, *J* = 8.7 Hz, 2H), 4.69 (dd, *J* = 14.4, 6.9 Hz, 1H), 4.03 (m, 2H), 3.98 (t, *J* = 6.3 Hz, 2H), 3.95 (t, *J* = 7.1 Hz, 2H), 3.10 (m, 2H), 1.82 – 1.75 (m, 4H), 1.50 (m, 2H), 1.36 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.0, 169.4, 167.7, 162.0, 137.0, 136.4, 129.3, 129.2, 129.1, 128.7, 127.1, 125.4, 118.8, 114.3, 67.8, 54.2, 47.0, 43.9, 37.7, 30.9, 28.8, 26.2, 25.5 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{27}H_{33}N_5O_4$ 492.2605, found 492.2622.

Procedure for synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)pyridin-1-ium bromide 2h

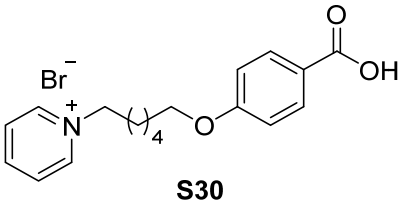


Synthesis of 1-(6-(4-(ethoxycarbonyl)phenoxy)hexyl)pyridin-1-ium bromide S29



Pyridine **S28** (0.161 ml, 2 mmol) was taken in a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar containing 5 ml of EtOH. Next, ethyl 4-((6-bromohexyl)oxy)benzoate **S23** (329 mg, 1 mmol) was added and flask was sealed with a condenser. The reaction mixture was refluxed for 12 h and concentrated under reduced pressure. The resulting residue was triturated with ether (5 ml) to give the title compound 1-(6-(4-(ethoxycarbonyl)phenoxy)hexyl)pyridin-1-ium bromide **S29** as a white solid (325 mg, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.57 (d, *J* = 5.6 Hz, 2H), 8.49 (t, *J* = 7.8 Hz, 1H), 8.11 (t, *J* = 6.9 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.08 (t, *J* = 7.3 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.99 (t, *J* = 6.2 Hz, 2H), 2.10 (m, 2H), 1.79 (m, 2H), 1.61 – 1.46 (m, 4H), 1.37 (t, *J* = 7.1 Hz, 3H) ppm.^{13}C NMR (126 MHz, $MeOD_4$) δ 162.5, 158.7, 141.3, 141.1, 127.6, 124.4, 118.8, 110.1, 63.8, 57.9, 56.7, 28.0, 24.8, 21.7, 21.5, 10.5 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{20}H_{26}NO_3$ 328.1907, found 328.1888.

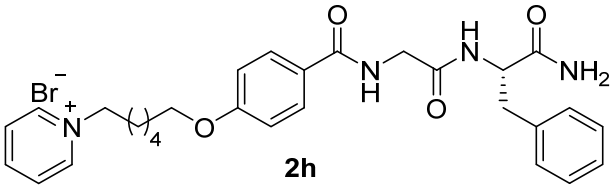
Synthesis of 1-(6-(4-carboxyphenoxy)hexyl)pyridin-1-ium bromide S30



S30

1-(6-(4-(Ethoxycarbonyl)phenoxy)hexyl)pyridin-1-ium bromide **S29** (407 mg, 1 mmol) was taken with water (2 ml) in a 5 ml round bottom flask charged with Teflon-coated magnetic stir bar. After slow addition of trifluoroacetic acid (0.918 ml, 12 mmol), the flask was sealed with condenser. The reaction mixture was refluxed (bath temperature 200 ºC) for 24 h and cooled to room temperature. The solvent was concentrated under reduced pressure delivering a yellow oil. To this, diethyl ether was added (3 × 3 ml) and stirred for overall 30 min at room temperature. The product crashes out as white precipitate which upon drying under reduced pressure delivers 1-(6-(4-carboxyphenoxy)hexyl)pyridin-1-ium bromide **S30** (265.5 mg, 81% yield) as a white precipitate. ^{1}H NMR (500 MHz, CD_3OD) δ 9.02 (d, *J* = 5.5 Hz, 2H), 8.60 (m, *J* = 7.8, 1.3 Hz, 1H), 8.12 (m, 2H), 7.95 (d, *J* = 8.9 Hz, 2H), 6.95 (d, *J* = 8.9 Hz, 2H), 4.67 (t, *J* = 7.3 Hz, 2H), 4.06 (t, *J* = 6.3 Hz, 2H), 2.08 (m, 2H), 1.83 (m, 2H), 1.59 (m, 2H), 1.49 (m, 2H) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 168.4, 163.0, 145.5, 144.6, 131.4, 128.1, 122.6, 113.7, 67.5, 61.6, 31.0, 28.5, 25.4, 25.2 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{18}H_{22}NO_3$ 300.1607, found 300.1637.

Synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)pyridin-1-ium bromide 2h

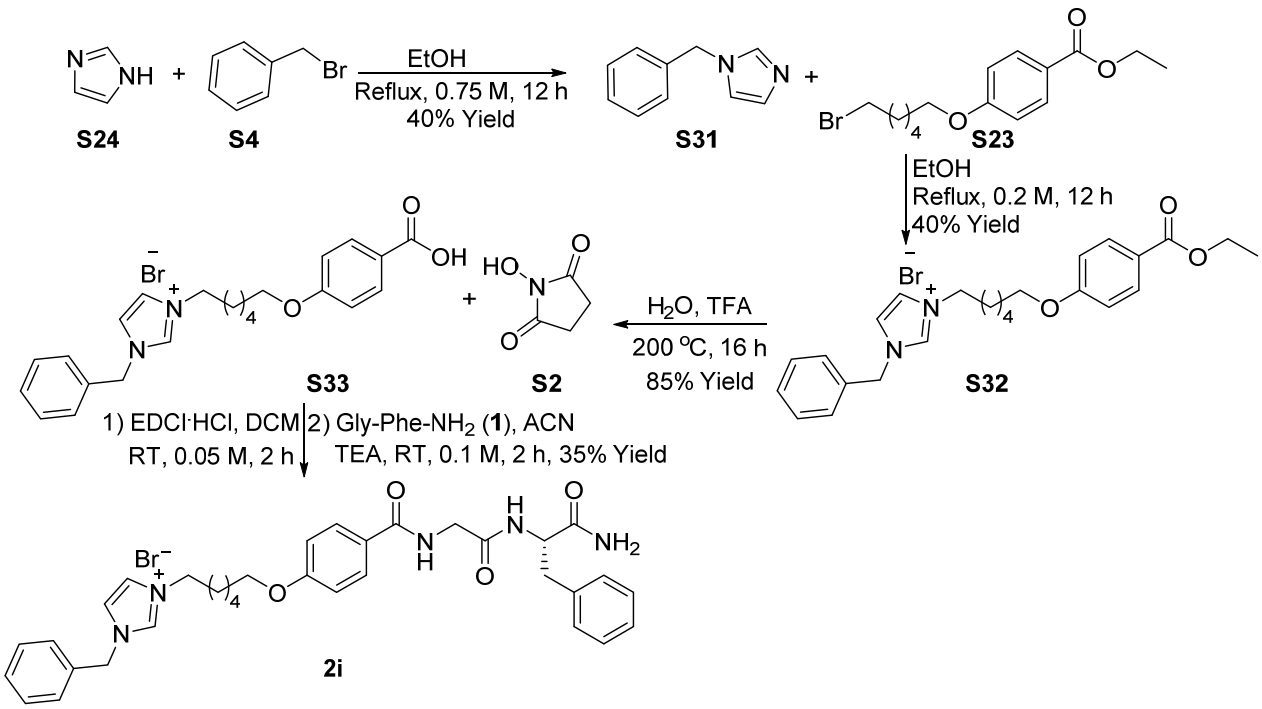


2h

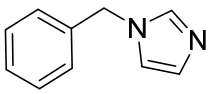
N-Hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol), N-ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (38.3 mg, 0.2 mmol), and dichloromethane (1 ml) were taken in a clean and dry 5 ml vial charged with magnetic stir bar. Next, 1-(6-(4-carboxyphenoxy) hexyl)pyridin-1-ium bromide **S30** (19.0 mg, 0.05 mmol) was added to the reaction mixture and stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted

with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated, and the crude reagent was transferred into 5 ml vial charged with magnetic stir bar. After re-diluting it in acetonitrile (0.2 ml), Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethylamine (2.76 µl, 0.02 mmol) were added through micropipette. The reaction mixture was stirred at room temperature for 2 h and followed by ESI-MS. It was further diluted to 500 µl by addition of acetonitrile and subjected to preparative HPLC to render pure (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)pyridin-1-ium bromide **2h** (3.0 mg, 30% yield) as white solid. ^{1}H NMR (500 MHz, D_2O) δ 8.82 (d, *J* = 5.8 Hz, 2H), 8.51 (t, *J* = 7.8 Hz, 1H), 8.02 (m, 2H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.41 (m, 2H), 7.36 (m, 1H), 7.33 (m, 2H), 7.04 (d, *J* = 8.7 Hz, 2H), 4.66 (dd, *J* = 8.8, 6.3 Hz, 1H), 4.62 (t, *J* = 7.2 Hz, 2H), 4.14 (t, *J* = 6.3 Hz, 2H), 3.83-3.74 (m, 2H), 3.20 (dd, *J* = 14.0, 6.5 Hz, 1H), 3.03 (dd, *J* = 14.0, 8.9 Hz, 1H), 2.04 (m, 2H), 1.80 (m, 2H), 1.53 (m, 2H), 1.39 (m, 2H) ppm. ^{13}C NMR (D_2O, 126 MHz) δ 175.8, 171.7, 171.3, 165.1, 145.4, 144.1, 136.1, 129.4, 129.2, 129.1, 128.7, 128.1, 127.1, 114.7, 68.3, 61.7, 54.3, 43.0, 36.8, 30.3, 27.7, 24.7, 24.5 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{29}H_{35}N_4O_4$ 503.2653, found 503.2632.

Procedure for synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamoyl)phenoxy)hexyl)-3-benzyl-1*H*-imidazol-3-ium bromide 2i



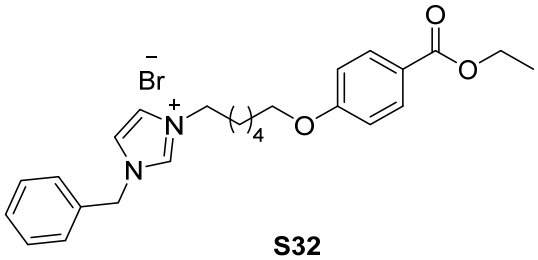
Synthesis of 1-benzyl-1*H*-imidazole S31



S31

Imidazole **S24** (100 mg, 1.5 mmol), potassium carbonate (250 mg, 1.8 mmol), and ethanol (2 ml) were taken in 5 ml round bottom flask charged with Teflon-coated magnetic stir bar. Next, benzyl bromide **S4** (0.20 ml, 1.8 mmol) was added and the reaction mixture was refluxed for 12 h. Subsequently, it was concentrated under reduced pressure and purified by a short chromatography column [DCM/MeOH, 95:5, R_f 0.5] to give the title compound 1-benzyl-1*H*-imidazole **S31** (98.4 mg, 60% yield) as greenish solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.39 – 7.30 (m, 3H), 7.18 – 7.14 (m, 2H), 7.09 (m, 1H), 6.90 (m, 1H), 5.11 (s, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 137.5, 136.2, 129.8, 129.0, 128.3, 127.3, 119.9, 50.8 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{10}H_{10}N_2$ 159.0917, found 159.0908.

Synthesis of 1-benzyl-3-(6-(4-(ethoxycarbonyl)phenoxy)hexyl)-1*H*-imidazol-3-ium bromide S32

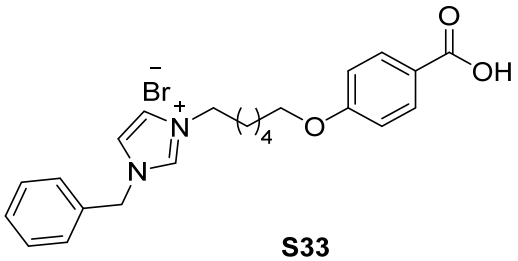


S32

In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar, 1-benzyl-1*H*-imidazole **S31** (0.158 g, 1 mmol) was taken in 5 ml of ethanol. Next, ethyl 4-((6-bromohexyl)oxy)benzoate **S23** (329 mg, 1 mmol) was added and the flask was sealed with a condenser. The reaction mixture was refluxed for 12 h and concentrated under reduced pressure. The residue was triturated with ether (5 ml) to give the title compound 1-benzyl-3-(6-(4-(ethoxycarbonyl)phenoxy)hexyl)-1*H*-imidazol-3-ium bromide **S32** (388 mg, 80% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.83 (s, 1H), 7.97 (d, J = 8.9 Hz, 2H), 7.49 (m, 2H), 7.37 (m, 2H), 7.36 (m, 1H), 7.36 (m, 1H), 7.31 (m, 1H), 6.88 (d, J = 8.9 Hz, 2H), 5.60 (s, 2H), 4.42 – 4.28 (m, 4H), 3.98 (t, J = 6.3 Hz, 2H), 1.96 (m, 2H), 1.78 (m, 2H), 1.52 (m, 2H), 1.43 (m,

2H), 1.37 (t, J = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 162.7, 137.5, 133.1, 131.5, 129.5, 129.4, 129.0, 122.8, 121.9, 121.7, 114.0, 67.7, 60.6, 53.3, 50.0, 30.1, 28.8, 25.9, 25.4, 14.4 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{25}H_{31}N_2O_3$ 407.2329, found 407.2302.

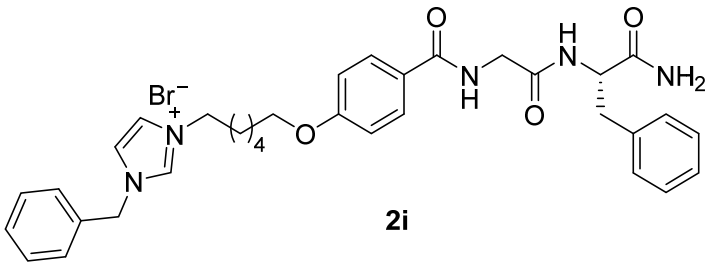
Synthesis of 1-benzyl-3-(6-(4-carboxyphenoxy)hexyl)-1*H*-imidazol-3-ium bromide S33



S33

In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar, 1-benzyl-3-(6-(4-(ethoxycarbonyl)phenoxy)hexyl)-1*H*-imidazol-3-ium bromide **S32** (487 mg, 1 mmol) was taken in 2 ml of water. Next, trifluoroacetic acid (0.918 ml, 12 mmol) was added slowly and the flask was sealed with condenser. The reaction mixture was refluxed (bath temperature 200 ºC) for 24 h and cooled to room temperature. The solvent was concentrated under reduced pressure to deliver a yellow oil. To this, diethyl ether was added (3 × 3 ml) and stirred for overall 30 min at room temperature. The crashed out white precipitate is isolated and dried under reduced pressure to deliver 1-benzyl-3-(6-(4-carboxyphenoxy)hexyl)-1*H*-imidazol-3-ium bromide **S33** (366 mg, 84% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 7.95 (d, J = 8.9 Hz, 2H), 7.67 (d, J = 2.0 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.47 – 7.38 (m, 5H), 6.95 (d, J = 8.9 Hz, 2H), 5.41 (s, 2H), 4.24 (t, J = 7.3 Hz, 2H), 4.04 (t, J = 6.3 Hz, 2H), 1.93 (m, 2H), 1.81 (m, 2H), 1.55 (m, 2H), 1.41 (m, 2H) ppm (1 exchangeable proton in addition to carboxylic acid) .^{13}C NMR (126 MHz, CD_3OD) δ 168.4, 163.0, 133.8, 131.4, 129.1, 129.0, 128.2, 122.7, 122.6, 122.5, 113.7, 67.5, 52.7, 49.5, 29.6, 28.5, 25.5, 25.1 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{23}H_{27}N_2O_3$ 379.2016, found 379.1995.

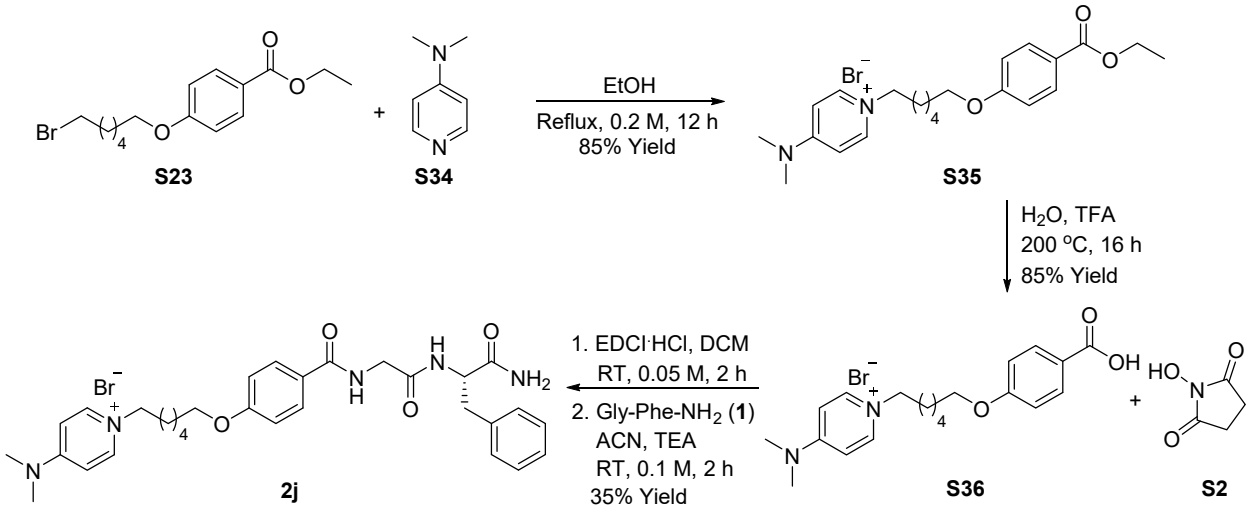
Synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)-3-benzyl-1*H*-imidazol-3-ium bromide 2i



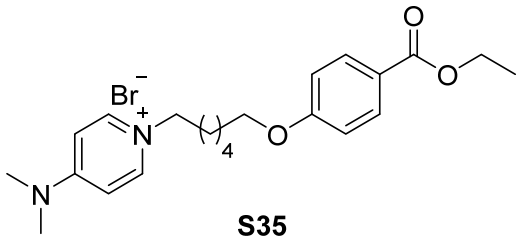
2i

In a 5 ml vial charged with magnetic stir bar, N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in 1 ml of dichloromethane. Next, 1-benzyl-3-(6-(4-carboxyphenoxy)hexyl)-1*H*-imidazol-3-ium bromide **S33** (19.0 mg, 0.05 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated, and the crude reagent was transferred into 5 ml vial charged with magnetic stir bar. It was re-diluted with acetonitrile (0.2 ml) followed by addition of Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.02 mmol). The reaction mixture was stirred at room temperature for 2 h. It was diluted to 500 µl by acetonitrile and subjected to preparative HPLC to render (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamoyl)phenoxy)hexyl)-3-benzyl-1*H*-imidazol-3-ium bromide **2i** (3.03 mg, 26% yield). ^{1}H NMR (500 MHz, D_2O) δ 8.78 (s, 1H), 7.72 (d, *J* = 8.8 Hz, 2H), 7.46 (m, 1H), 7.43 – 7.41 (m, 4H), 7.33 - 7.31 (m, 2H), 7.23 – 7.15 (m, 5H), 7.01 (d, *J* = 8.9 Hz, 2H), 5.30 (s, 2H), 4.62 (dd, *J* = 8.6, 5.6 Hz, 1H), 4.18 (t, *J* = 6.8 Hz, 2H), 4.07 (t, *J* = 6.3 Hz, 2H), 4.04 – 3.95 (m, 2H), 3.17 (dd, *J* = 14.0, 5.6 Hz, 1H), 2.99 (dd, *J* = 14.0, 8.6 Hz, 1H), 1.86 (m, 2H), 1.74 (m, 2H), 1.44 (m, 2H), 1.27 (m, 2H) ppm. ^{13}C NMR (D_2O, 126 MHz) δ 175.7, 171.6, 170.0, 161.6, 136.1, 133.6, 131.0, 129.4, 129.3, 129.2, 129.1, 128.7, 128.3, 127.1, 124.9, 122.5, 122.4, 114.6, 68.2, 54.2, 52.7, 49.5, 43.0, 36.9, 28.8, 27.7, 24.7, 24.3 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{34}H_{40}N_5O_4$ 583.3153, found 583.3123.

Procedure for synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide 2j



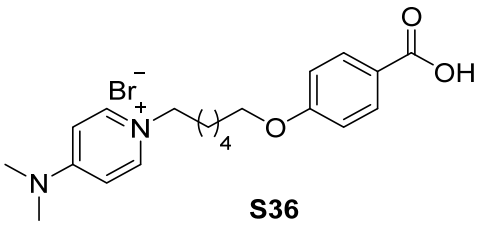
Synthesis of 1-(6-(4-(ethoxycarbonyl)phenoxy)hexyl))-4-(dimethylamino)pyridin-1-ium bromide S35



In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar, 4-(dimethylamino)pyridine **S34** (0.122 g, 1 mmol) was taken in 5 ml of ethanol. Next, ethyl 4-((6-bromohexyl)oxy)benzoate **S23** (329 mg, 1 mmol) was added, sealed by the condenser, and refluxed for 12 h. The reaction mixture was concentrated under reduced pressure and triturated with ether (5 ml) to give the title compound of 1-(6-(4-(ethoxycarbonyl)phenoxy)hexyl))-4-(dimethylamino)pyridin-1-ium bromide **S35** (360 mg, 80% yield) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (d, *J* = 7.8 Hz, 2H), 7.96 (d, *J* = 8.9 Hz, 2H), 6.98 (d, *J* = 7.8 Hz, 2H), 6.88 (d, *J* = 8.9 Hz, 2H), 4.40 (t, *J* = 7.2 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 3.99 (t, *J* = 6.2 Hz, 2H), 3.25 (s, 6H), 1.93 (m, 2H), 1.79 (m, 2H), 1.52 (m, 2H), 1.43 (m, 2H), 1.37 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 162.7, 156.2, 142.5, 131.5, 122.7, 114.0, 108.3, 67.7,

60.6, 58.0, 40.5, 31.0, 28.8, 25.7, 25.5, 14.4 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{22}H_{31}N_2O_3$ 371.2329, found 371.2355.

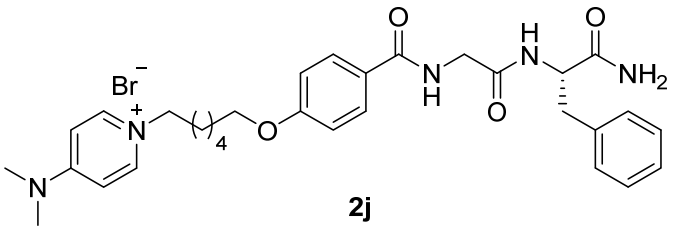
Synthesis of 1-(6-(4-carboxyphenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide S36



S36

In a 5 ml round bottom flask charged with Teflon-coated magnetic stir bar, 1-(6-(4-(ethoxycarbonyl)phenoxy)hexyl))-4-(dimethylamino)pyridin-1-ium bromide **S35** (423 mg, 1 mmol) was taken in 2 ml of water. Next, trifluoroacetic acid (0.918 ml, 12 mmol) was added slowly and the flask was sealed by condenser. The reaction mixture was refluxed (bath temperature 200 ºC) for 24 h and cooled to room temperature. The solvent was concentrated under reduced pressure delivering a yellow oil. To this, diethyl ether was added (3 × 3 ml) and stirred for overall 30 min at room temperature. The white precipitate is isolated and dried under reduced pressure to deliver 1-(6-(4-carboxyphenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide **S36** (355 mg, 84% yield). ^{1}H NMR (500 MHz, CD_3OD) δ 8.06 (d, *J* = 7.8 Hz, 2H), 7.84 (d, *J* = 8.9 Hz, 2H), 6.85 (d, *J* = 7.9 Hz, 2H), 6.85 (d, *J* = 8.9 Hz, 2H), 4.09 (t, *J* = 7.2 Hz, 2H), 3.94 (t, *J* = 6.2 Hz, 2H), 1.80 (m, 2H), 1.71 (m, 2H), 1.45 (m, 2H), 1.31 (m, 2H) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 168.5, 163.0, 156.5, 141.6, 131.4, 131.1, 113.8, 107.9, 67.5, 57.4, 38.9, 30.4, 28.5, 25.3, 25.1 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{20}H_{27}N_2O_3$ 343.2016, found 343.2003.

Synthesis of (S)-1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carbamoyl)phenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide 2j

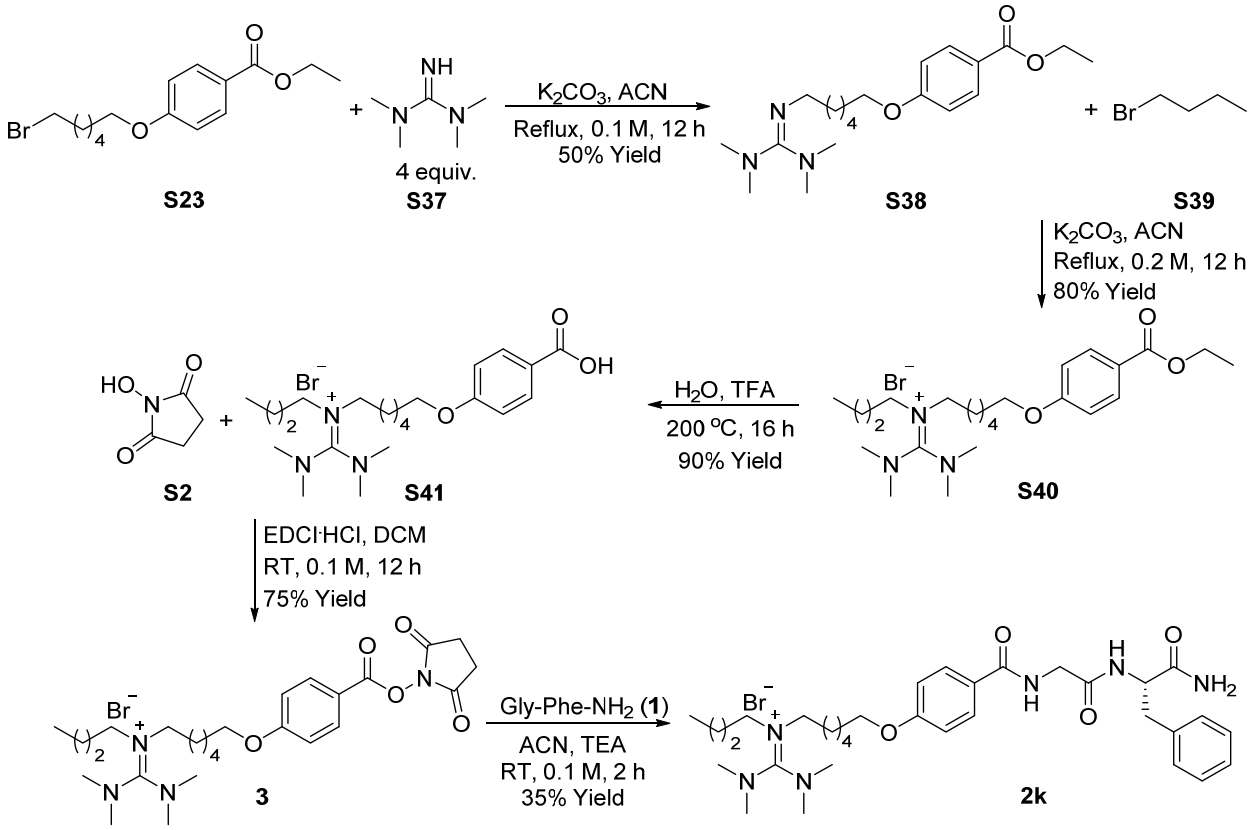


2j

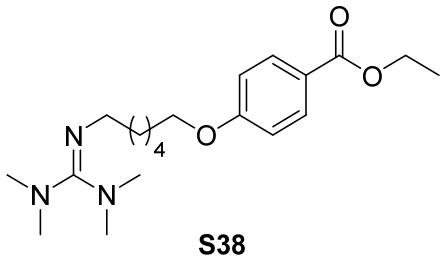
In a 5 ml vial charged with Teflon-coated magnetic stir bar, N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2

mmol) were taken in dichloromethane (1 ml). Next, 1-(6-(4-carboxyphenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide **S36** (21.1 mg, 0.05 mmol) was added and reaction mixture was stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4, concentrated, and crude reagent was transferred into 5 ml vial charged with magnetic stir bar. It was re-diluted with acetonitrile (0.2 ml) followed by the addition of Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) and triethyl amine (2.76 µl, 0.02 mmol). The mixture was stirred at room temperature for 2 h. It was diluted further to 500 µl by addition of acetonitrile and subjected to preparative HPLC to render 1-(6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamoyl)phenoxy)hexyl)-4-(dimethylamino)pyridin-1-ium bromide **2j** (2.75 mg, 25% yield). ^{1}H NMR (500 MHz, D_2O) δ 7.93 (d, *J* = 7.5 Hz, 2H), 7.74 (d, *J* = 8.7 Hz, 2H), 7.38 (m, 1H), 7.22 – 7.15 (m, 4H), 7.05 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 7.6 Hz, 2H), 4.63 (dd, *J* = 8.6, 5.6 Hz, 1H), 4.17 - 4.09 (m, 4H), 4.02 (m, 2H), 3.19 (dd, *J* = 14.0, 5.6 Hz, 1H), 3.07 (s, 6H), 3.02 (dd, *J* = 14.1, 8.4 Hz, 1H), 1.87 (m, 2H), 1.80 (m, 2H), 1.51 (m, 2H), 1.32 (m, 2H) ppm. ^{13}C (126 MHz, D_2O) δ 178.3, 174.2, 172.5, 164.1, 158.6, 143.7, 131.9, 131.7, 131.6, 131.2, 129.6, 127.4, 117.2, 109.8, 70.7, 59.9, 56.7, 45.5, 41.7, 39.4, 32.0, 30.0, 27.0, 26.9 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{31}H_{40}N_5O_4$ 546.3075, found 546.3072.

Synthesis 6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamoyl) phenoxy)-N-(bis(dimethylamino)methylene)-N-butylhexan-1-aminium bromide 2k



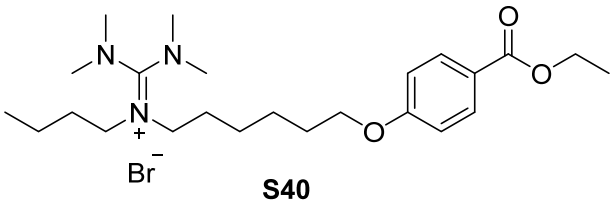
Ethyl 4-(6-((bis(dimethylamino)methylene)amino)hexyl)benzoate S38



In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar, potassium carbonate (0.68 g, 4.92 mmol) and N,N-tetramethyl guanidine **S37** (0.375 ml, 3 mmol) were taken in acetonitrile (5 ml). Next, ethyl 4-(3-bromohexyl) benzoate **S23** (0.33 g, 1 mmol) was added and the reaction mixture was refluxed for 12 h. It was concentrated under reduced pressure and the resulting residue was re-diluted with dichloromethane (10 ml). The organic layer was extracted with water (3 × 50 ml) to remove **S37** and potassium carbonate. The organic extracts were dried

over Na_2SO_4 and the solvent was concentrated under reduced pressure. The residue was suspended in diethyl ether (15 ml) and stirred for 1 h. The process was repeated twice to extract all the product in ether layer. The ether fractions were combined and concentrated under reduced pressure to afford the pure ethyl 4-(6-((bis(dimethylamino)methylene)amino)hexyl)benzoate **S38** (181.5 mg, 50% yield) as slightly yellow colour liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.34 (q, *J* = 7.1 Hz, 2H), 4.00 (t, *J* = 6.6 Hz, 2H), 3.11 (t, *J* = 6.9 Hz, 2H), 2.74 (s, 6H), 2.65 (s, 6H), 1.81 (m, 2H), 1.56 (m, 2H), 1.48 (m, 2H), 1.42 (m, 2H), 1.38 (t, *J* = 7.1 Hz, 3H) ppm.^{13}C NMR (126 MHz, $CDCl_3$) δ 166.4, 162.9, 160.1, 131.5, 122.6, 114.0, 68.2, 60.5, 49.2, 39.6, 38.9, 32.5, 29.1, 27.1, 25.8, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. for $C_{20}H_{33}N_3O_3$ 364.2595, found 364.2602.

N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(ethoxycarbonyl)phenoxy)hexan-1-aminium bromide S40

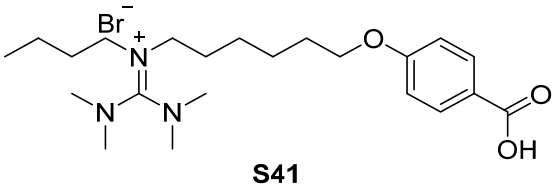


S40

In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bar, potassium carbonate (680 mg, 4.92 mmol) and n-butyl bromide **S39** (0.375 ml, 3 mmol) were taken in acetonitrile (5 ml). Next, ethyl 4-(3-((bis(dimethylamino)methylene)amino)hexyl)benzoate **S38** (363 mg, 1 mmol) was added and the reaction mixture was refluxed for 12 h. Subsequently, it was concentrated under reduced pressure and re-diluted with dichloromethane (10 ml). The organic layer was extracted with water (3 × 50 ml) to remove the base. The organic layer was dried over Na_2SO_4 and the solvent was concentrated under reduced pressure. To this, 15 ml diethyl ether was added and stirred for 1 h. The process was repeated twice to extract all the product in ether layer. The ether fractions were combined and concentrated under reduced pressure to afford the pure N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(ethoxycarbonyl)phenoxy)hexan-1-aminium bromide **S40** (400 mg, 80% yield) as slightly yellow coloured liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 4.35 (q, *J* = 7.1 Hz, 2H), 4.03 (t, *J* = 6.2 Hz, 2H), 3.35 – 3.18 (m, 4H), 3.15 (s, 6H), 3.07 (s, 6H), 1.83 (m, 2H), 1.72 – 1.44 (m, 8H), 1.40 - 1.32 (m, 5H), 0.96 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.3,

163.3, 163.1, 131.5, 122.5, 113.8, 67.6, 65.5, 49.4, 49.2, 39.2, 39.0, 29.4, 28.6, 27.3, 26.1, 25.3, 19.6, 14.0, 12.6 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{24}H_{42}N_3O_3$ 420.3221, found 420.3209.

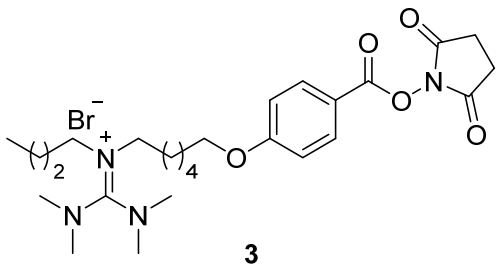
N-(bis(dimethylamino)methylene)-N-butyl-6-(4-carboxyphenoxy)hexan-1-aminium bromide S41



S41

In a 5 ml round bottom flask charged with Teflon-coated magnetic stir bar, N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(ethoxycarbonyl)phenoxy)hexan-1-aminium bromide**S40** (499 mg, 1 mmol) was taken in 2 ml of water. Next, trifluoroacetic acid (0.918 ml, 12 mmol) was added slowly and the flask was sealed with a condenser. The reaction mixture was refluxed (bath temperature 200 ºC) for 24 h and cooled to room temperature. The solvent was concentrated under reduced pressure delivering a yellow oil. To this, diethyl ether was added (3 × 3 ml) and stirred for overall 30 min at room temperature. The white precipitate from this process melts upon drying under reduced pressure, delivering N-(bis(dimethylamino)methylene)-N-butyl-6-(4-carboxyphenoxy)hexan-1-aminium bromide **S41** (376 mg, 80% yield) as a yellow coloured liquid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.96 (d, *J* = 8.9 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 4.07 (t, *J* = 6.3 Hz, 2H), 3.27 (m, 2H), 3.18 (m, 2H), 2.97 (s, 6H), 2.95 (s, 6H), 1.82 (m, 2H), 1.67 (m, 2H), 1.59 – 1.44 (m, 4H), 1.44 – 1.29 (m, 4H), 0.97 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 168.4, 163.3, 163.1, 131.5, 122.6, 113.7, 67.6, 49.4, 49.3, 39.1, 39.0, 29.4, 28.6, 27.3, 26.1, 25.3, 19.6, 12.6 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{22}H_{38}N_3O_3$ 392.2908, found 392.2933.

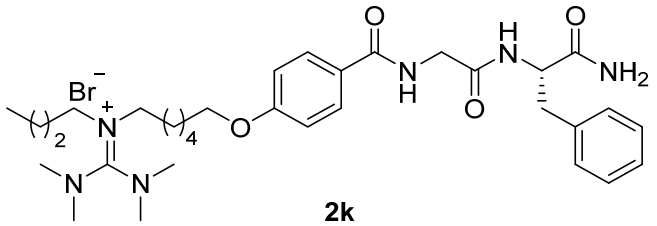
Synthesis of N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenoxy)hexan-1-aminium bromide 3



3

In a 5 ml vial charged with Teflon-coated magnetic stir bar, N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in dichloromethane (1 ml). Next, N-(bis(dimethylamino)methylene)-N-butyl-6-(4-carboxyphenoxy)hexan-1-aminium bromide **S41** (47.1 mg, 0.1 mmol) was added and the reaction mixture was stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. Next, it was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was triturated with ether and pentane (2 ml each) to yield N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenoxy)hexan-1-aminium bromide **3** (397 mg, yield 70%) as colourless liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, *J* = 8.8 Hz, 2H), 6.96 (d, *J* = 8.9 Hz, 2H), 4.06 (t, *J* = 6.1 Hz, 2H), 3.26 – 3.16 (m, 4H), 3.14 (s, 6H), 3.05 (s, 6H), 2.91 (s, 4H), 1.83 (m, 2H), 1.75 – 1.49 (m, 4H), 1.47 – 1.25 (m, 6H), 0.95 (t, *J* = 7.3 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.5, 164.4, 163.3, 161.5, 132.9, 116.9, 114.7, 68.1, 49.7, 49.3, 41.0, 40.8, 29.8, 28.8, 27.8, 26.5, 25.7, 20.0, 13.7 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{26}H_{41}N_4O_5$ 489.3071, found 489.3063.

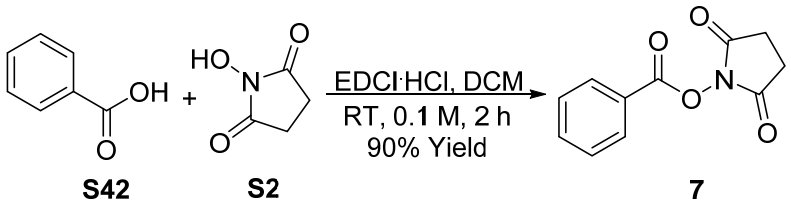
Synthesis of (S)**-6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl) carb amoyl)phenoxy)-N-(bis(dimethylamino)methylene)-N-butylhexan-1-aminium bromide 2k**



2k

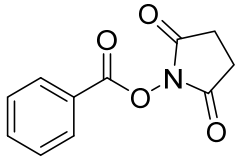
In a 5 ml vial charged with Teflon-coated magnetic stir bar, Gly-Phe-NH_2 **1** (4.42 mg, 0.02 mmol) was taken in acetonitrile (200 µl). Next, N-(bis(dimethylamino)methylene)-N-butyl-6-(4-(((2,5-dioxopyrrolidin-1-yl)oxy)carbonyl)phenoxy)hexan-1-aminium bromide **S42** (22..4 mg, 0.04 mmol) and triethyl amine (2.76 µl, 0.02 mmol) were added and the resulting reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted to 500 µl with acetonitrile and purified by preparative HPLC to give pure (S)-6-(4-((2-((1-amino-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamoyl)phenoxy)-N-(bis(dimethylamino)methylene)-N-butylhexan-1-aminium bromide **2k** (3.8 mg, 35% yield) as white solid. ^{1}H NMR (500 MHz, CD_3OD) δ 7.71 (d, *J* = 8.8 Hz, 2H), 7.13 (m, 2H), 7.12 (m, 2H), 7.06 (m, 1H), 6.87 (d, *J* = 8.9 Hz, 2H), 4.54 (dd, *J* = 8.7, 5.3 Hz, 1H), 3.97 (t, *J* = 6.2 Hz, 2H), 3.90 - 3.80 (m, 2H), 3.17 (m, 2H), 3.10 – 3.07 (m, 3H), 2.86 (s, 12H), 2.82 (m, 1H), 1.73 (m, 2H), 1.66 – 1.51 (m, 2H), 1.50 – 1.36 (m, 4H), 1.35 – 1.20 (m, 4H), 0.87 (t, *J* = 7.4 Hz, 3H) ppm. ^{13}C NMR (126 MHz, CD_3OD) δ 174.6, 170.3, 168.7, 163.3, 162.2, 137.0, 129.0, 128.9, 129.0, 126.4, 125.5, 113.8, 67.6, 54.2, 49.4, 49.2, 42.7, 39.1, 38.9, 37.4, 29.4, 28.6, 27.3, 26.1, 25.4, 19.6, 12.6 ppm. HRMS (ESI) $[M]^+$ calcd. For $C_{33}H_{51}N_6O_4$ 595.3966, found 595.3969.

Procedure for synthesis of 2,5-dioxopyrrolidin-1-yl benzoate 7 (see **Scheme 3a)**



S42 **S2** **7**

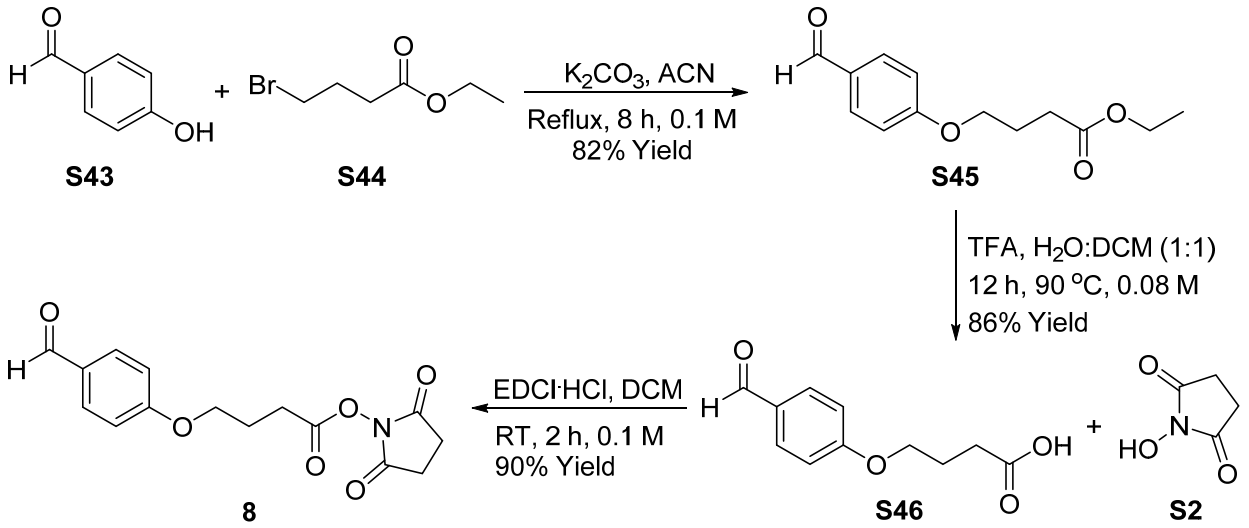
Synthesis of 2,5-dioxopyrrolidin-1-yl benzoate 7



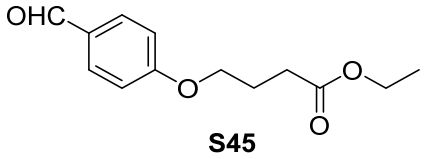
7

In a 5 ml vial charged with Teflon-coated magnetic stir bar, N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in dichloromethane (1 ml). Next, benzoic acid **S42** (0.122 g, 0.1 mmol) was added and stirred at room temperature for 2 h. The reaction was followed by thin layer chromatography. After completion, the reaction mixture was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was triturated with diethyl ether and pentane (2 ml each) to yield 2,5-dioxopyrrolidin-1-yl benzoate **7** (13.4 mg, yield 90%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (m, 2H), 7.71 (m, 1H), 7.51 (m, 2H), 2.91 (s, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.2, 161.9, 134.9, 130.6, 128.9, 125.2, 25.7. HRMS (ESI) $[M+Na]^+$ calcd. for $C_{11}H_9NO_4$ 242.0424, found 242.0428.

Procedure for synthesis of 2,5-dioxopyrrolidin-1-yl 4-(4-formylphenoxy)butanoate 8 (see, **Scheme 3b)**

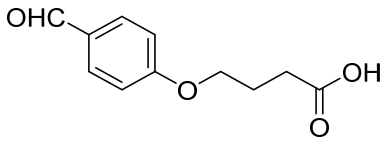


Synthesis of ethyl 4-(4-formylphenoxy)butanoate S45[8]



In a 25 ml round bottom flask, *p*-hydroxybenzaldehyde **S43** (122.1 mg, 1 mmol) was dissolved in acetonitrile (10 ml). To this aldehyde solution, K_2CO_3 (276.4 mg, 2 mmol) and ethyl 4-bromobutanoate **S44** (0.17 ml, 1.2 mmol) were added and reaction mixture was allowed to reflux for 8 h. The reaction progress was monitored by thin layer chromatography (TLC). Upon completion, the reaction mixture was filtered to remove K_2CO_3. The solution was concentrated under vacuum and the product was purified using flash column chromatography (ethylacetate:*n*-hexane 2:98) to afford ethyl 4-(4-formylphenoxy)butanoate **S45** (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 6.99 (d, *J* = 8.7 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 4.11 (t, *J* = 6.2 Hz, 2H), 2.53 (t, *J* = 7.2 Hz, 2H), 2.15 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 173.1, 164.0, 132.1, 130.0, 114.8, 67.2, 60.6, 30.7, 24.5, 14.3. MS (ESI) $[MH]^+$ calcd. for $C_{13}H_{16}O_4$ 237.1, found 237.1.

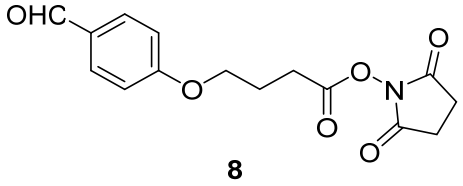
Synthesis of 4-(4-formylphenoxy)butanoic acid S46[8]



S46

The ester derivative **S45 (**194 mg, 0.82 mmol) was dissolved in water and DCM mixture (10 ml, 1:1). To this solution, trifluoroacetic acid (4 equiv.) was added and reaction temperature was elevated to 90 ºC. The reaction mixture was stirred for another 12 h and the hydrolysis of ester was monitored by TLC. Subsequently, the precipitated crude product was filtered and subjected to silica-gel flash column chromatography (ethylacetate:*n*-hexane 35:65) to afford the pure product **S46** (86% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.86 (s, 1H), 7.86 (d, *J* = 8.7 Hz, 2H), 7.12 (d, *J* = 8.7 Hz, 2H), 4.11 (t, *J* = 6.4 Hz, 2H), 2.39 (t, *J* = 7.3 Hz, 2H), 1.97 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.7, 174.4, 163.9, 132.3, 130.0, 115.3, 67.6, 30.4, 24.5. MS (ESI) $[MH]^+$ calcd. for $C_{11}H_{13}O_4$ 209.2, found 209.2.

Synthesis of 2,5-dioxopyrrolidin-1-yl 4-(4-formylphenoxy)butanoate 8

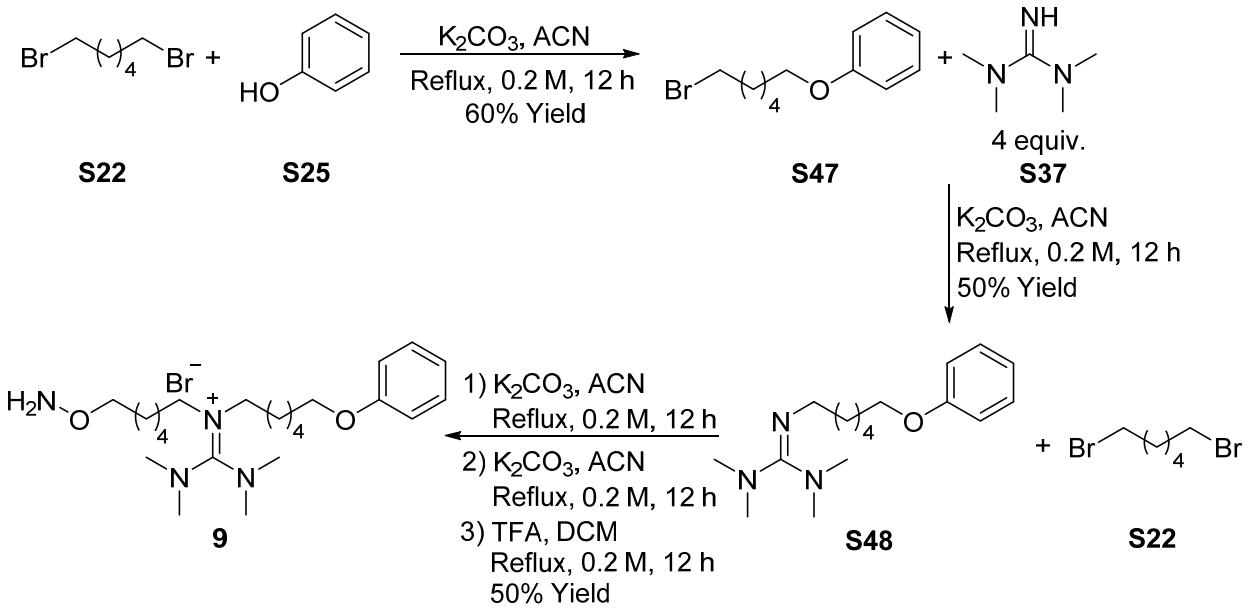


8

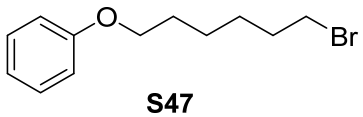
In a 5 ml vial charged with Teflon-coated magnetic stir bead, N-hydroxysuccinimide **S2** (23.0 mg, 0.2 mmol) and N-ethyl-N′-(3-dimethylaminopropyl)carbodiimide hydrochloride (38.3 mg, 0.2 mmol) were taken in dichloromethane (1 ml). Next, 4-(4-formylphenoxy)butanoic acid **S46** (20.8 g, 0.1 mmol) was added and stirred at room temperature for 2 h. The progress of reaction was followed by thin layer chromatography. The reaction mixture was diluted with dichloromethane (9 ml) and extracted with water (2 × 8 ml). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was triturated with ether and pentane (2 ml each) to yield 2,5-dioxopyrrolidin-1-yl 4-(4-formylphenoxy)butanoate **8** (27.4 mg, yield 90%) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.04 (d, *J* = 8.8 Hz, 2H), 4.18 (t, *J* = 6.0 Hz, 2H), 2.90 (t, *J* = 6.0 Hz, 2H), 2.87 (s, 4H), 2.29 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 190.8, 169.1, 168.2, 163.6, 132.0, 130.1, 114.8,

66.3, 27.7, 25.6, 24.3 ppm. HRMS (ESI) $[MH]^+$ calcd. for $C_{15}H_{15}NO_6$ 306.0972, found 306.0993.

Procedure for synthesis of 6-(aminooxy)-N-(bis(dimethylamino)methylene)-N-(6-phenoxyhexyl)hexan-1-aminium bromide 9



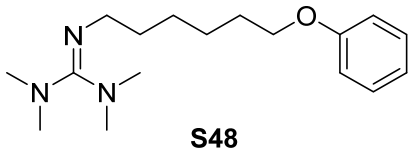
Synthesis of 1-(6-bromohexyloxy) benzene S47[9]



S47

In a 25 ml round bottom flask charged with Teflon-coated magnetic stir bar, phenol **S21** (94 mg, 1 mmol) and potassium carbonate (276 mg, 1 mmol) were taken in acetonitrile (5 ml). Next, 1, 6-dibromohexane **S22** (0.307 ml, 2 mmol) was added and the flask was sealed with condenser. The reaction mixture was refluxed for 16 h and cooled down to room temperature. The white solid was filtered through celite funnel filter. The filtrate was concentrated under reduced pressure delivering a yellow oil, which was purified by flash chromatography column (n-hexane, Rf = 0.1) to obtain 1-((6-bromohexyl)oxy)benzene **S47** (171 mg 60% yield) as a pale-yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 2H), 6.98 – 6.80 (m, 3H), 3.95 (m, 2H), 3.42 (m, 2H), 1.90 (m, 2H), 1.79 (m, 2H), 1.56 – 1.48 (m, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 159.0, 129.4, 120.6, 114.5, 67.6, 33.8, 32.7, 29.1, 27.9, 25.3 ppm. LRMS (ESI) $[MH]^+$ calcd. for $C_{12}H_{17}BrO$ 257.1, found 257.1.

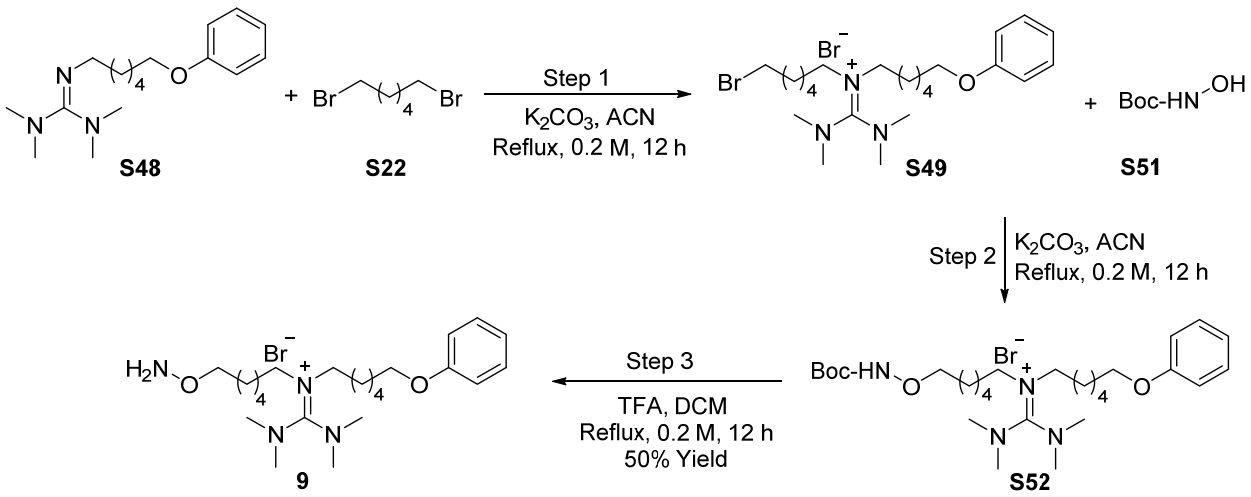
Synthesis of 1,1,3,3-tetramethyl-2-(6-phenoxyhexyl)guanidine S48



S48

In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bead, potassium carbonate (680 mg, 4.92 mmol) and N, N-tetramethyl guanidine **S37** (0.375 ml, 3 mmol) were taken in acetonitrile (5 ml). Next, 1-(6-bromohexyloxy) benzene **S23** (257 mg, 1 mmol) was added and reaction mixture was refluxed for 12 h. Subsequently, it was concentrated under reduced pressure and the residue was re-diluted with dichloromethane (10 ml). The organic layer was extracted with water (3 × 50 ml) to remove N, N-tetramethyl guanidine **S37**. The organic layer was dried over Na_2SO_4 and the solvent was concentrated under reduced pressure. The residue was mixed with ether (15 ml) and stirred for 1 h. The ether fraction was collected and the process was repeated. The combined ether fractions were concentrated under reduced pressure to afford the pure ethyl 1,1,3,3-tetramethyl-2-(6-phenoxyhexyl)guanidine **S48** (117 mg, 40% yield) as slightly yellow colour liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 2H), 6.95 (t, J = 7.3 Hz, 1H), 6.90 (d, J = 7.9 Hz, 2H), 3.97 (t, J = 6.3 Hz, 2H), 3.23 (m, 2H), 3.12 (s, 6H), 2.94 (s, 6H), 1.87-1.77 (m, 4H), 1.58 – 1.48 (m, 2H), 1.42 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.5, 159.0, 129.4, 120.6, 114.5, 67.5, 45.3, 40.6, 39.9, 29.7, 29.1, 26.6, 25.6 ppm. HRMS (ESI) $[M]^+$ calcd. for $C_{17}H_{29}N_3O$ 292.2383, found 292.2356.

Synthesis of 6-(aminooxy)-N-(bis(dimethylamino)methylene)-N-(6-phenoxyhexyl)hexan-1-aminium bromide 9



Step 1: In a 10 ml round bottom flask charged with Teflon-coated magnetic stir bead, potassium carbonate (680 mg, 4.92 mmol) and 1,1,3,3-tetramethyl-2-(6-phenoxyhexyl)guanidine **S48** (291 mg, 1 mmol) were mixed in acetonitrile (5 ml). Next, 1,6-dibromohexane **S22** (0.307 ml, 2 mmol) was added and the flask was sealed by condenser. The reaction mixture was refluxed for 16 h and concentrated under reduced pressure. The residue was re-dissolved in dichloromethane (10 ml) and the organic layer was extracted with water (3 × 50 ml) to remove potassium carbonate. The organic layer was dried over Na_2SO_4 and the solvent was concentrated under reduced pressure. To this, ether (15 ml) was added and stirred for 1 h. The semi-solid white precipitate (product) observed in the process is subjected to another round of treatment with ether (15 ml) for 1 h. The resultant white precipitate was dried under reduced pressure to get the crude N-(bis(dimethylamino)methylene)-6-bromo-N-(6-phenoxyhexyl)hexan-1-aminium bromide **S49.**

Step 2: The crude N-(bis(dimethylamino)methylene)-6-bromo-N-(6-phenoxyhexyl)hexan-1-aminium bromide **S49** was taken in another 10 ml round bottom flask in acetonitrile (5 ml). Next, tert-butyl hydroxycarbamate **S50** (266 mg, 2 mmol) and potassium carbonate (230 mg, 2 mmol) were added and the flask was sealed with condenser. The reaction mixture was refluxed for 16 h and concentrated under reduced pressure. The residue was re-dissolved in dichloromethane (10 ml) and the organic layer was extracted with water (3 × 50 ml) to remove potassium carbonate. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was mixed with ether (15 ml) and stirred for 1 h. The white

precipitate was isolated and treated with ether (15 ml) again for 1 h. Further, the white precipitate was dried under reduced pressure to render crude N-(bis(dimethylamino)methylene)-6-(((tert-butoxycarbonyl)amino)oxy)-N-(6-phenoxyhexyl)hexan-1-aminium bromide **S51**.

Step 3: The crude N-(bis(dimethylamino)methylene)-6-(((tert-butoxycarbonyl)amino)oxy)-N-(6-phenoxyhexyl)hexan-1-aminium bromide **S51** was taken in a 5 ml round bottom flask charged with feflon-coated magnetic stir bar in dichloromethane (1 ml). Next, trifluoroacetic acid (0.076 ml, 1 mmol) was added and the reaction mixture was refluxed for 12 h. The reaction mixture was concentrated under reduced pressure. The residue was re-suspended in ether (15 ml) and stirred for 1 h. The white precipitate is isolated and treated again with ether (15 ml, 1 h). The white precipitate was dried under reduced pressure to afford the 6-(aminooxy)-N-(bis(dimethylamino)methylene)-N-(6-phenoxyhexyl)hexan-1-aminium bromide **9** (218 mg, 45% yield) as a yellow colored liquid. ^{1}H NMR (500 MHz, D_2O) δ 7.41 (m, 2H), 7.05 (m, 3H), 4.10 (m, 2H), 4.02 (m, 2H), 3.4-3.15 (m, 4H), 2.90 (s, 12H), 1.78 (m, 2H), 1.65 (m, 2H), 1.46 (m, 4H), 1.40 – 1.25 (m, 8H) ppm. ^{13}C NMR (126 MHz, D_2O) δ 163.1, 158.0, 129.9, 121.5, 114.9, 75.6, 68.3, 49.1, 48.9, 39.4, 39.2, 28.0, 26.9, 26.7, 26.6, 25.7, 25.4, 24.6, 24.5. HRMS (ESI) $[M]^+$ calcd. for $C_{23}H_{43}N_4O_2$ 407.3381, found 407.3411.

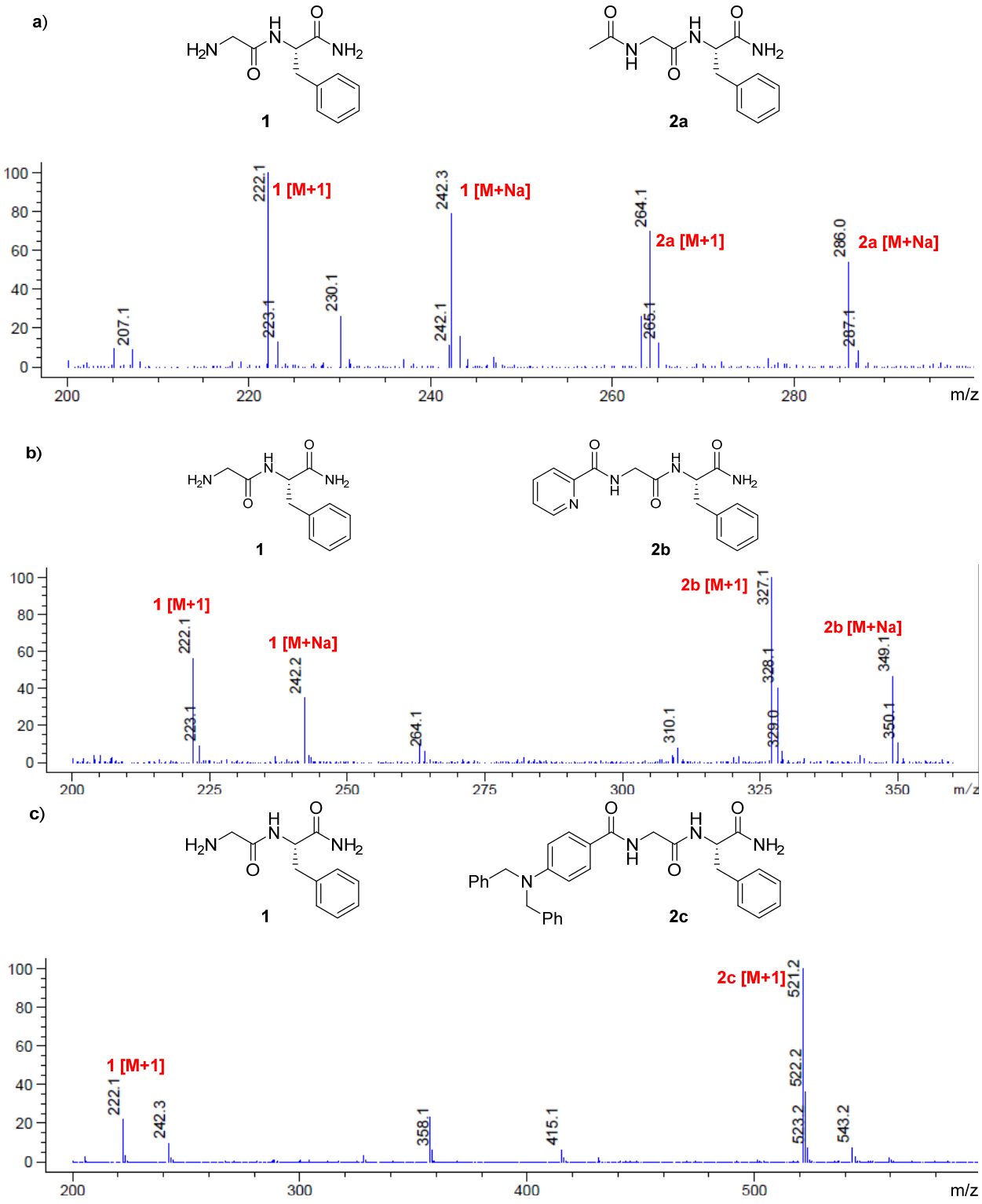
4. Reference Table S1.

S. No.	Manuscript results	Related data in ESI
1	Characterization data of synthesized compounds	Pages S6-S46
2	Table 1	Fig. S1; Pages S47-S50
3	peptides	Fig. S3-S6; Pages S53-S60
4	Scheme 1	Fig. S7; Pages S61-S69
5	Scheme 2	Fig. S8; Pages S70-S74
6	Scheme 3a	Fig. S9, S10; Pages S75-S80
7	Scheme 3b	Fig. S11; Pages S81-S83
8	Figure S12 (Scheme 1, ESI-MS)	Fig. S12; Pages S85-S86
9	Figure S13 (Scheme 3a, ESI-MS)	Fig. S13; Pages S88-S90
10	Spectral data	Pages S91-S131

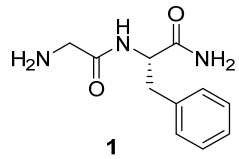
5. Experimental data

5.1 Mass data

5.1.2. Mass data for Figure 2 in the manuscript:



d)



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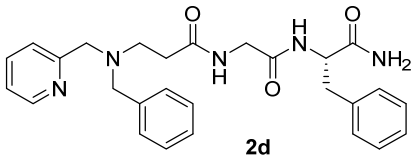

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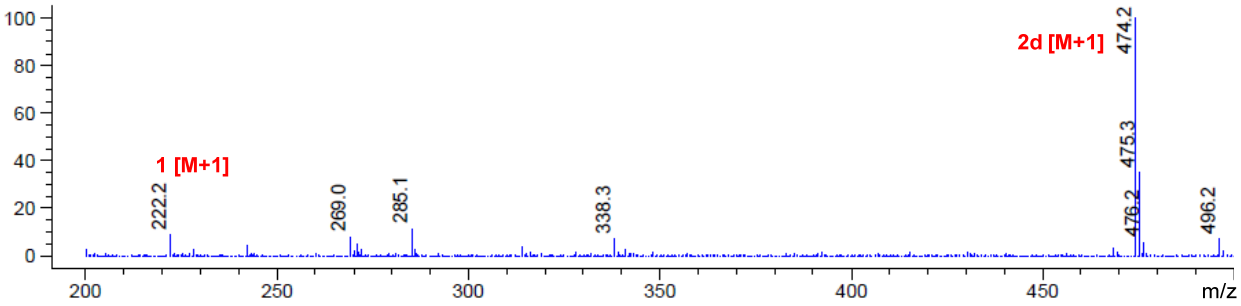
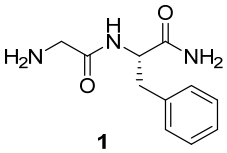


e)



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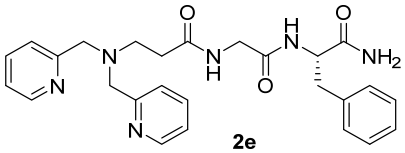

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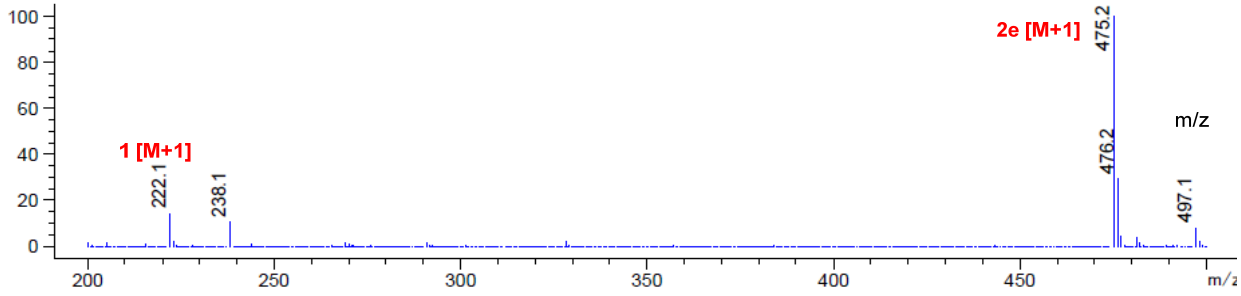

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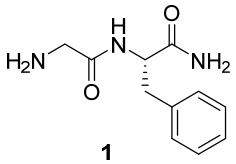

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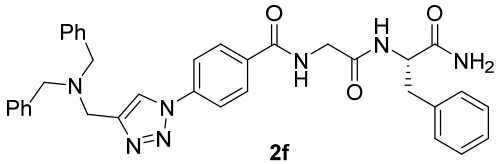

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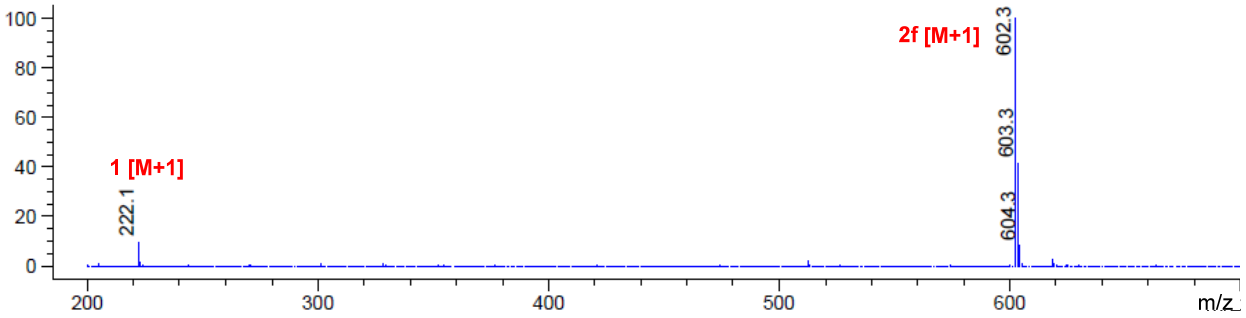
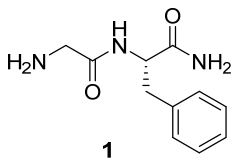


g)



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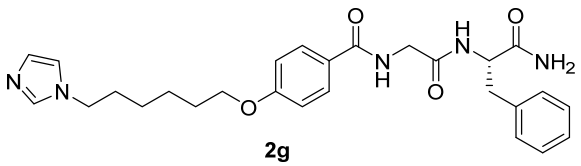

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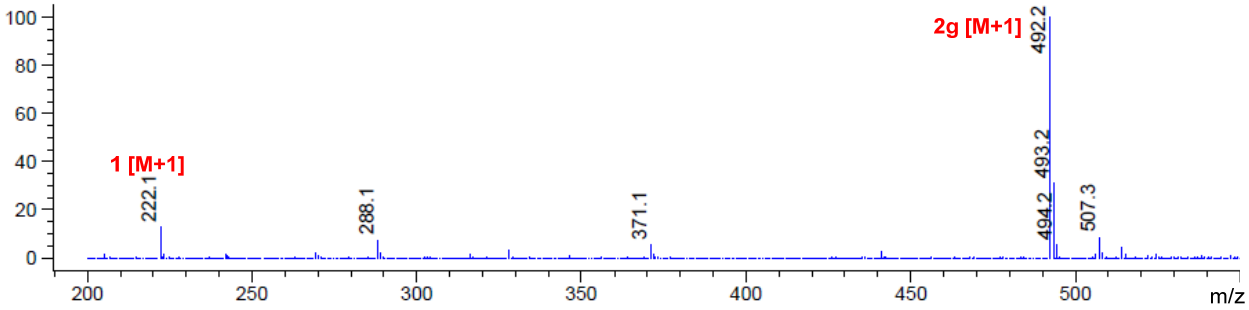
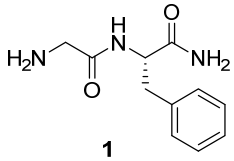


h)



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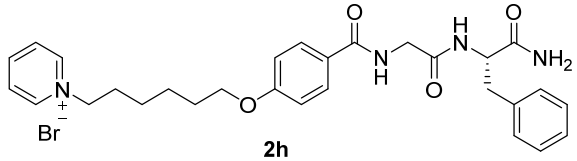

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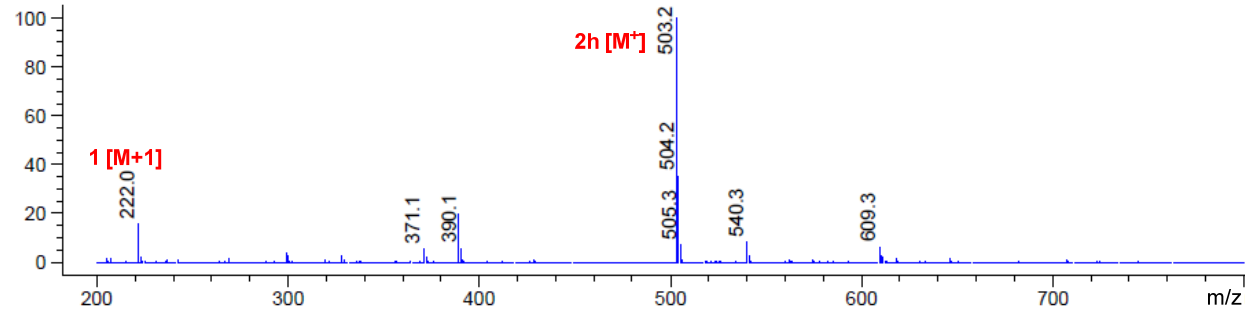
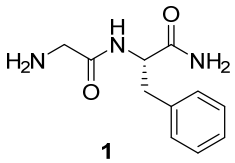
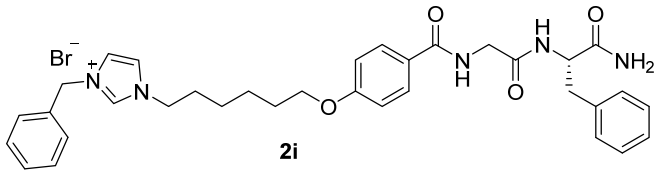


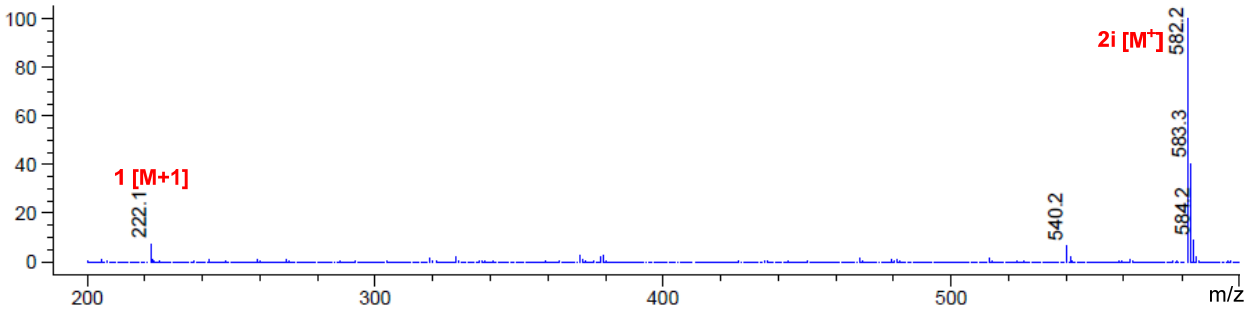
i)



1



2i



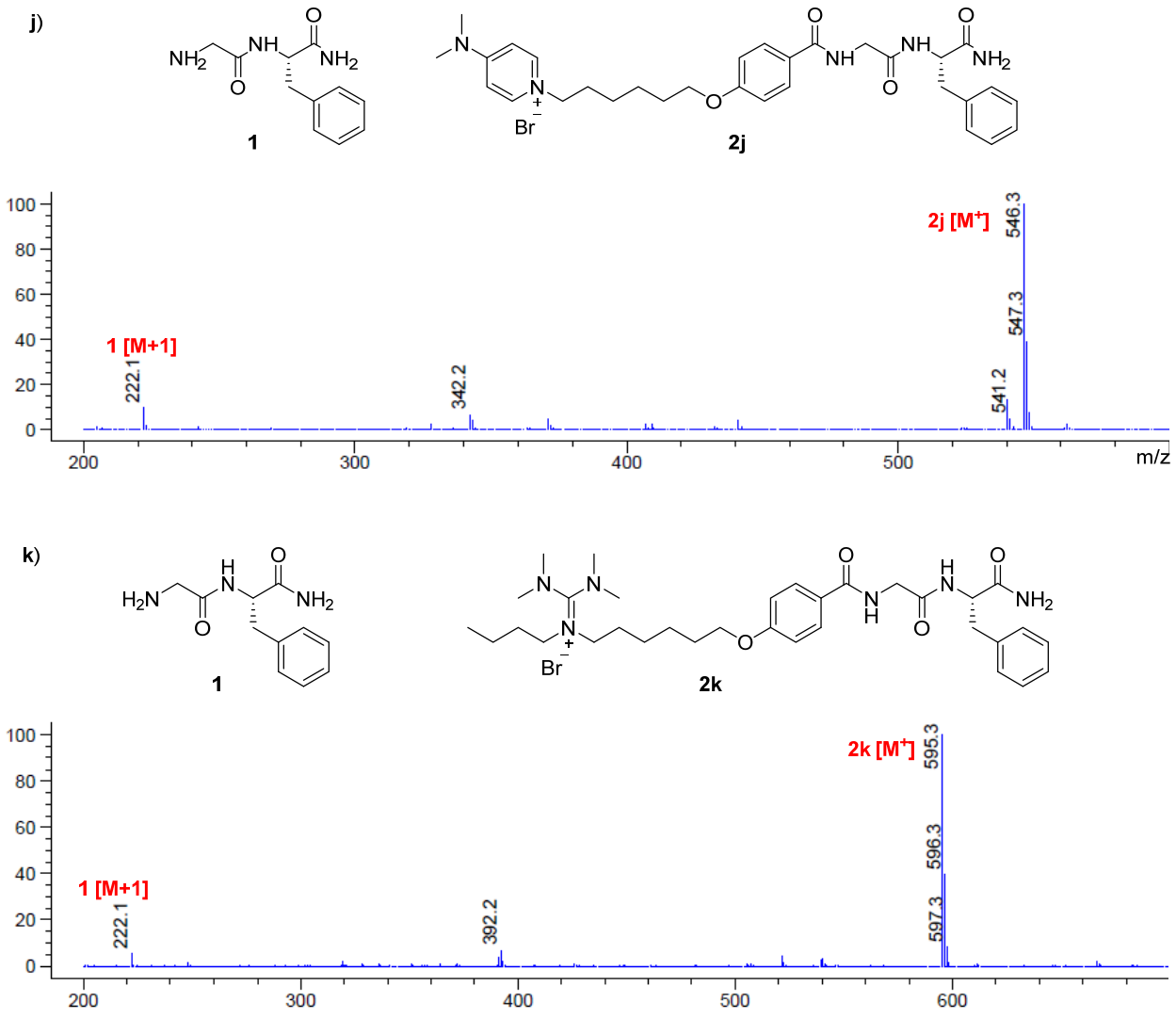


Fig S1: Relative intensity of peptides **1** and **2** (5 µM each). The ESI-MS spectrum for the peptides (1:1 ratio) (a) **1** and **2a**; (b) **1** and **2b**; (c) **1** and **2c**; (d) **1** and **2d**; (e) **1** and **2e**; (f) **1** and **2f**; (g) **1** and **2g**; (h) **1** and **2h**; (i) **1** and **2i**; (k) **1** and **2k**.

Table S2. Initial screening of reagents 2 (2a-2k) with Gly-Phe-NH_2 1

Entry	Reagents (1:1)[a]	Relative Intensity[b]
1	**1:2a**	50:50
2	**1:2b**	60:100
3	**1:2c**	22:100
4	**1:2d**	12:100
5	**1:2e**	15:100
6	**1:2f**	12:100
7	**1:2g**	13:100
8	**1:2h**	15:100
9	**1:2i**	09:100
10	**1:2j**	09:100
11	**1:2k**	06:100

[a] Concentration of each reagent is 5 μM. [b] Intensities are based on LC-ESI-MS spectrum.

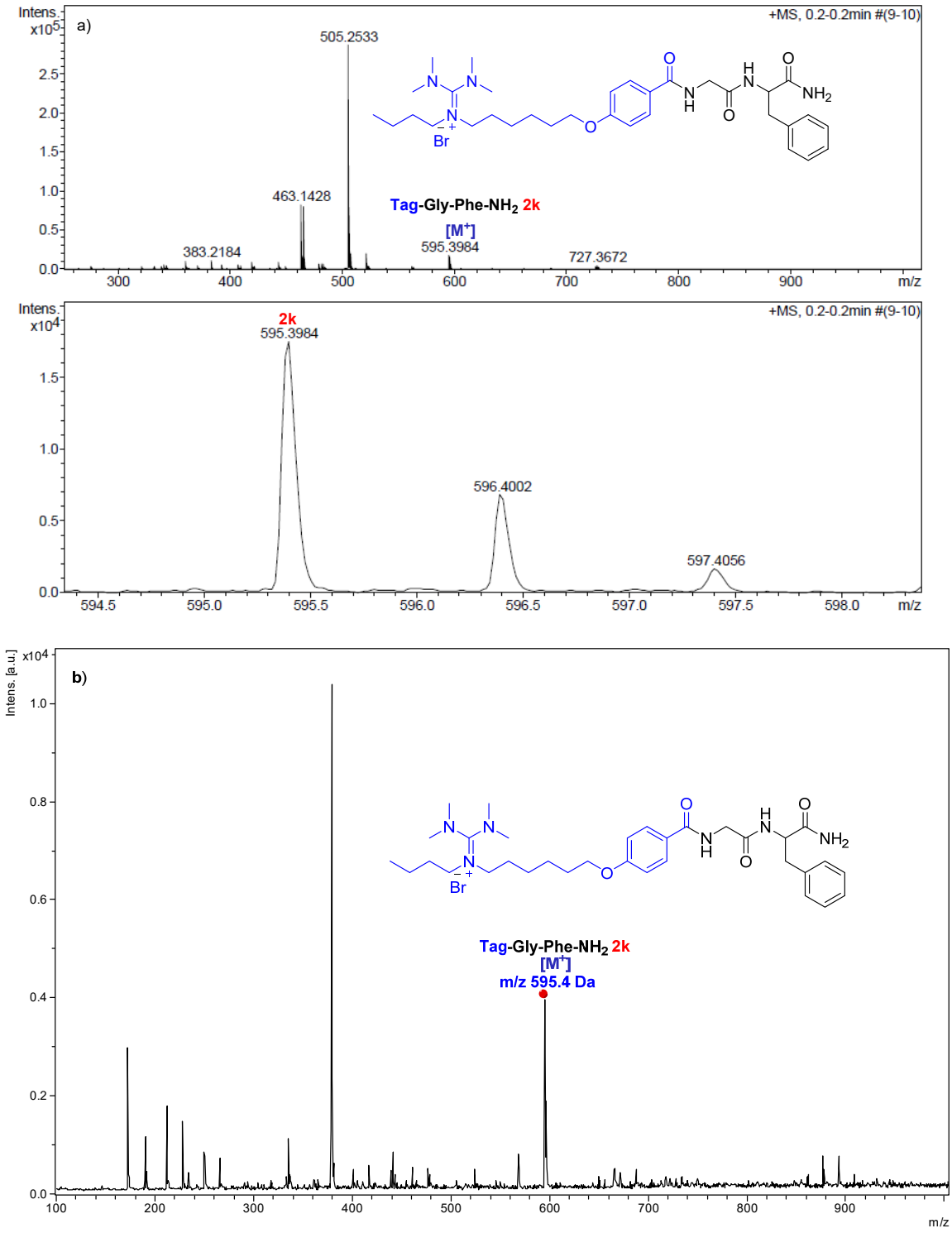
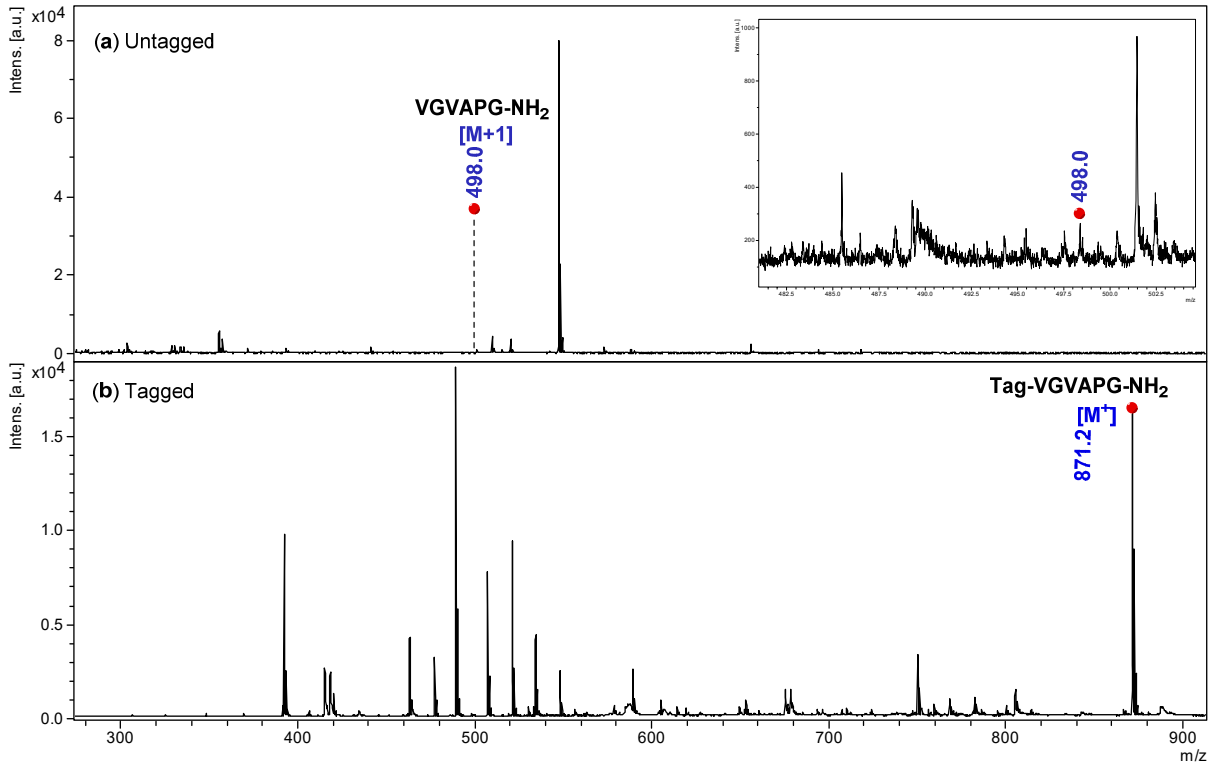
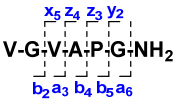
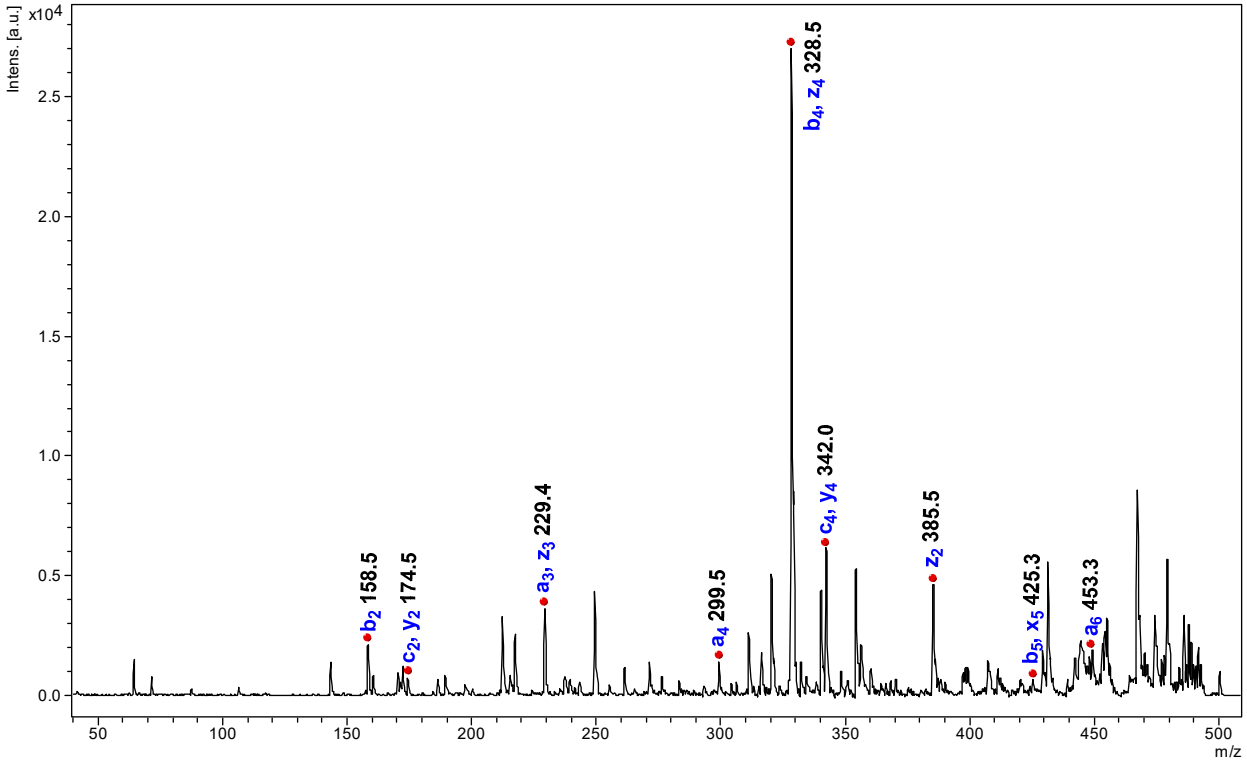


Figure S2: Detection of **Tag-GF-NH$_2$** (m/z 595.4, $[M]^+$) peptide at attomolar concentration. (a) ESI-MS spectra of **Tag-GF-NH$_2$** (m/z 595.4, $[M]^+$) at 5 aM. (b) MALDI-MS spectra of **Tag-GF-NH$_2$** (m/z 595.4, $[M]^+$) at 5 aM.


(a) Untagged
VGVAPG-NH2
[M+1]
498.0
498.0
(b) Tagged
Tag-VGVAPG-NH2
[M+]
871.2
Intens. [a.u.]
x10^4
m/z

c)


x5 z4 z3 y2
V-G-V-A-P-G-NH2
b2 a3 b4 b5 a6


b4, z4 328.5
c4, y4 342.0
z2 385.5
a3, z3 229.4
b2 158.5
c2, y2 174.5
a4 299.5
b5, x5 425.3
a6 453.3
Intens. [a.u.]
x10^4
m/z

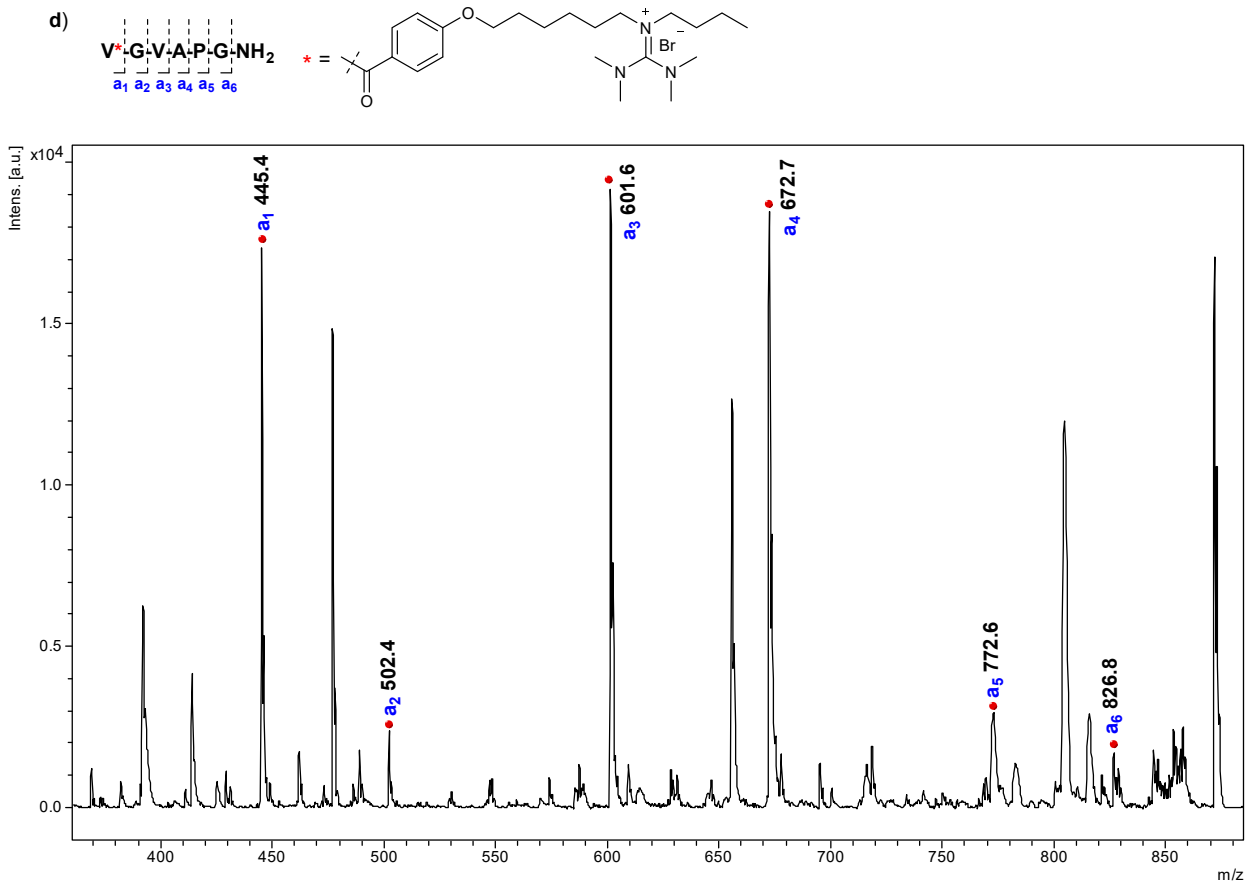
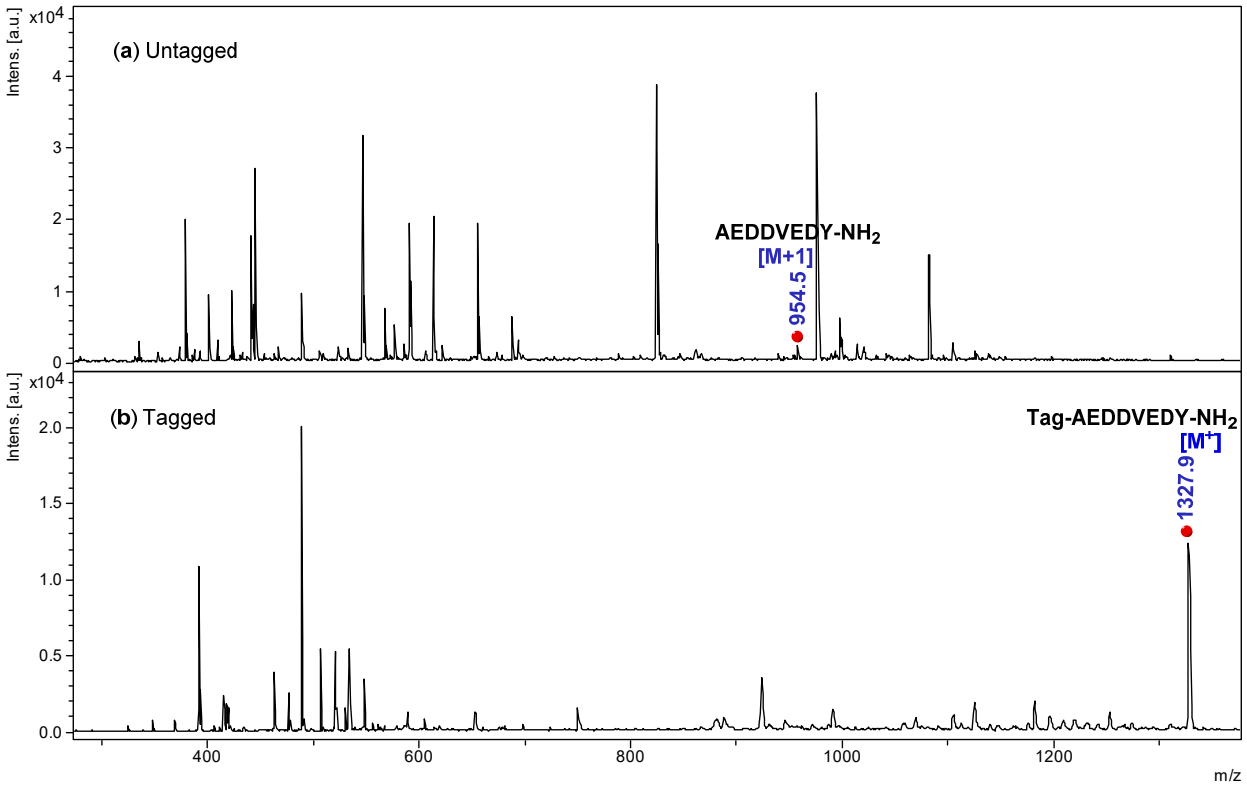
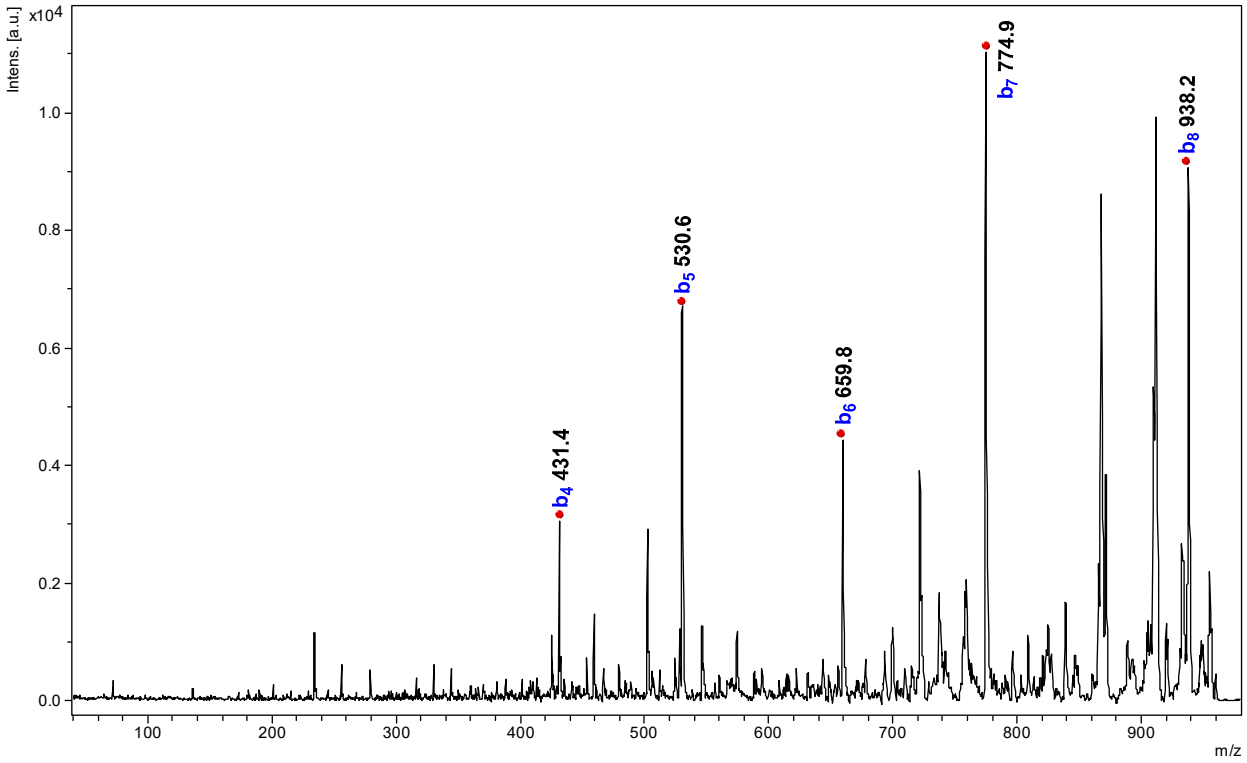


Figure S3: Screening of the peptide **VGVAPG-NH₂**. (**a**) MALDI-MS spectra of native peptide **VGVAPG-NH₂** (m/z 498.0, $[M+H]^+$), (**b**) MALDI-MS spectra of tagged peptide **Tag-VGVAPG-NH₂** (m/z 871.2, $[M]^+$). (c) MS-MS spectra of native peptide **VGVAPG-NH₂** (m/z 498.0, $[M+H]^+$). (d) MS-MS spectra of tagged peptide **Tag-VGVAPG-NH₂** (m/z 871.2, $[M]^+$).



c) A-E-D-D-V-E-D-Y-NH_2

b_4 b_5 b_6 b_7 b_8



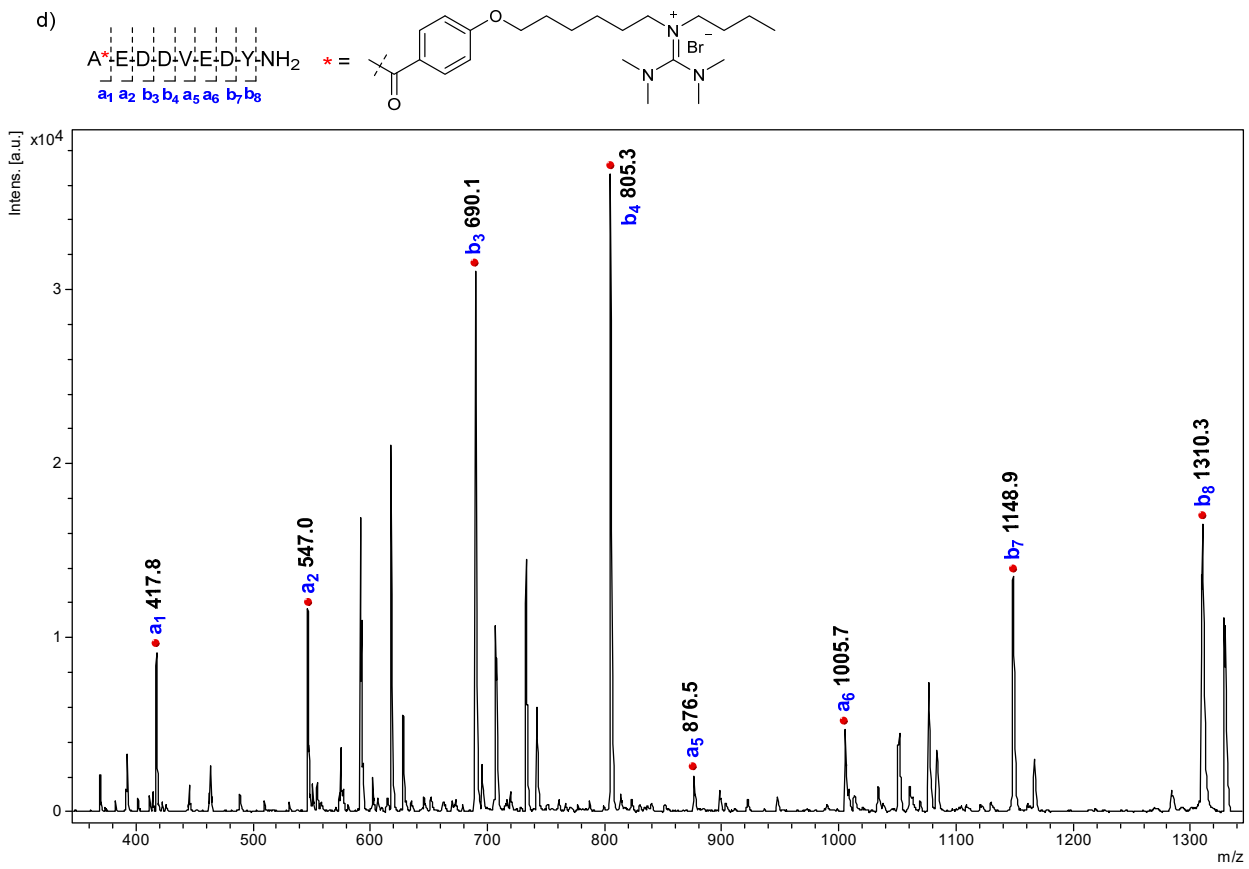
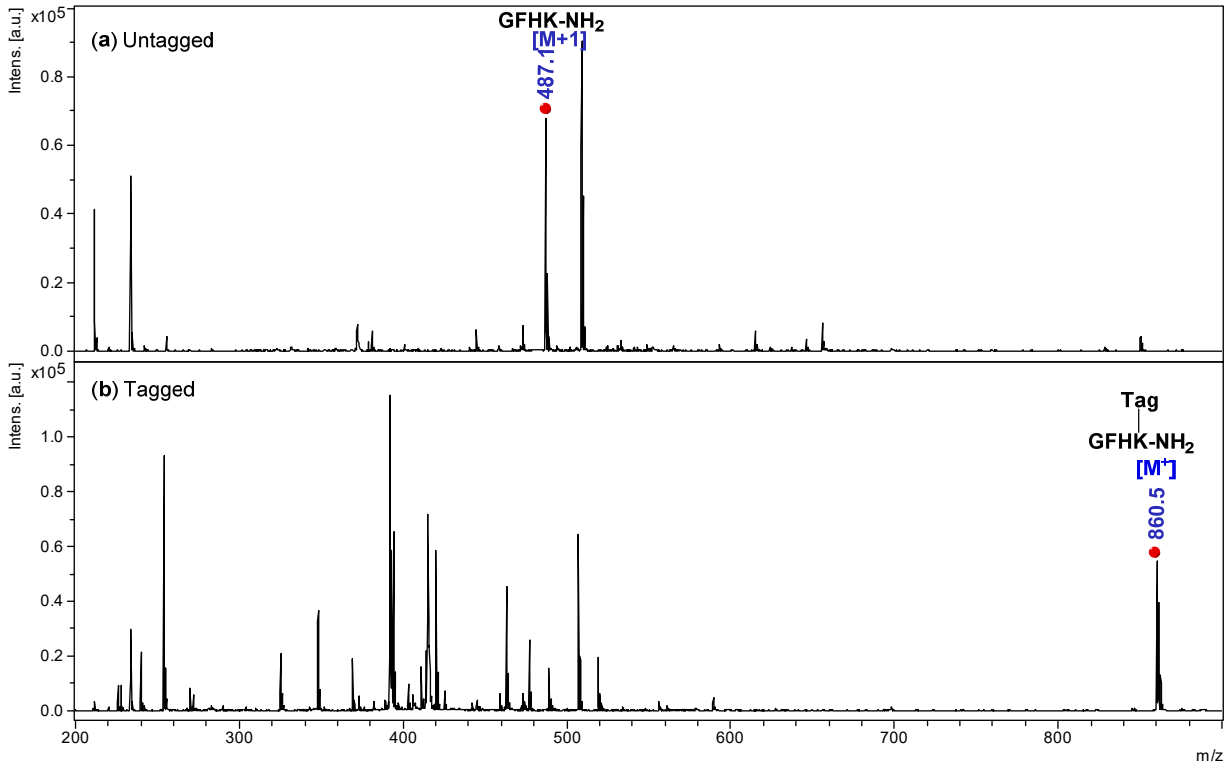
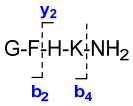
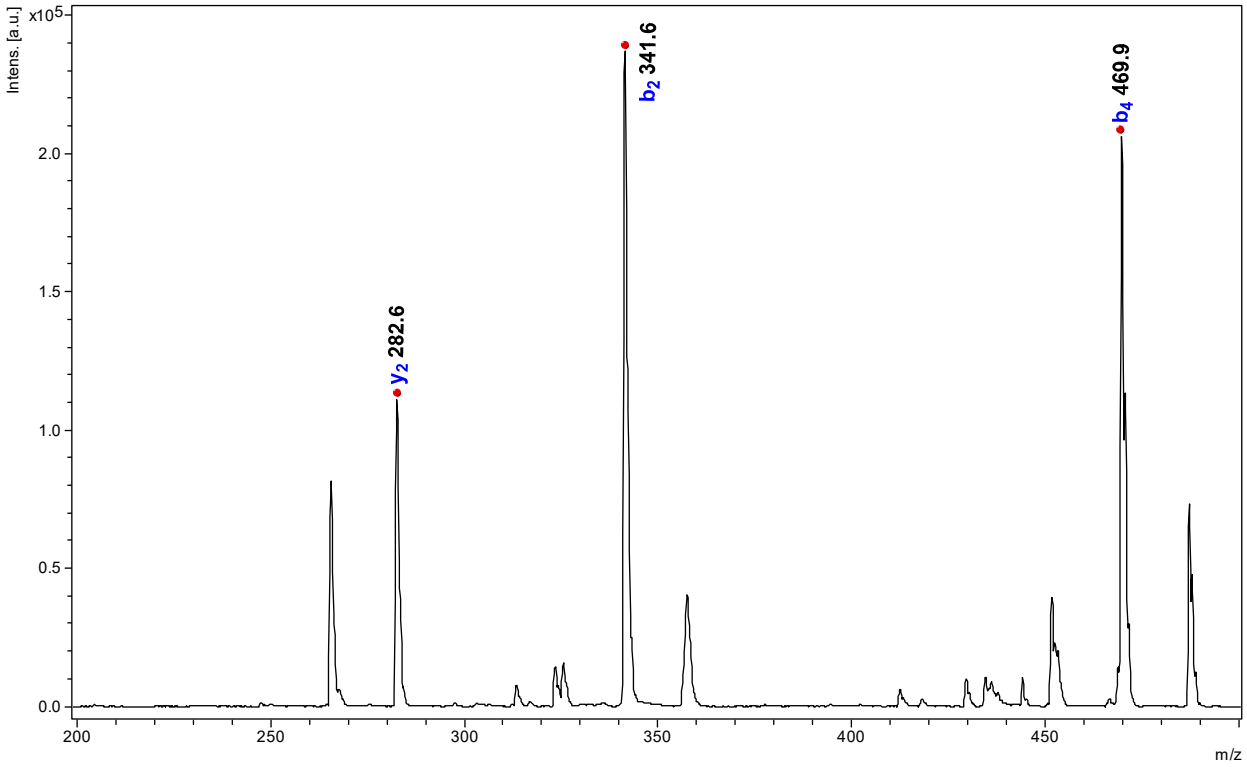


Figure S4: Screening of the peptide **AEDDVEDY-NH₂**. (a) MALDI-MS spectrum of native peptide **AEDDVEDY-NH₂** (m/z 953.5, $[M+H]^+$), (b) MALDI-MS spectrum of tagged peptide **Tag-AEDDVEDY-NH₂** (m/z 1327.9, $[M]^+$). (c) MS-MS spectrum of native peptide **AEDDVEDY-NH₂** (m/z 953.5, $[M+H]^+$). (d) MS-MS spectrum of tagged peptide **AEDDVEDY-NH₂** (m/z 1327.9, $[M]^+$).


(a) Untagged
GFHK-NH2
[M+1]
487.1
(b) Tagged
Tag
GFHK-NH2
[M+]
860.5
Intens. [a.u.]
x10^5
m/z

c)


y2
G-F-H-K-NH2
b2
b4


y2 282.6
b2 341.6
b4 469.9
Intens. [a.u.]
x10^5
m/z

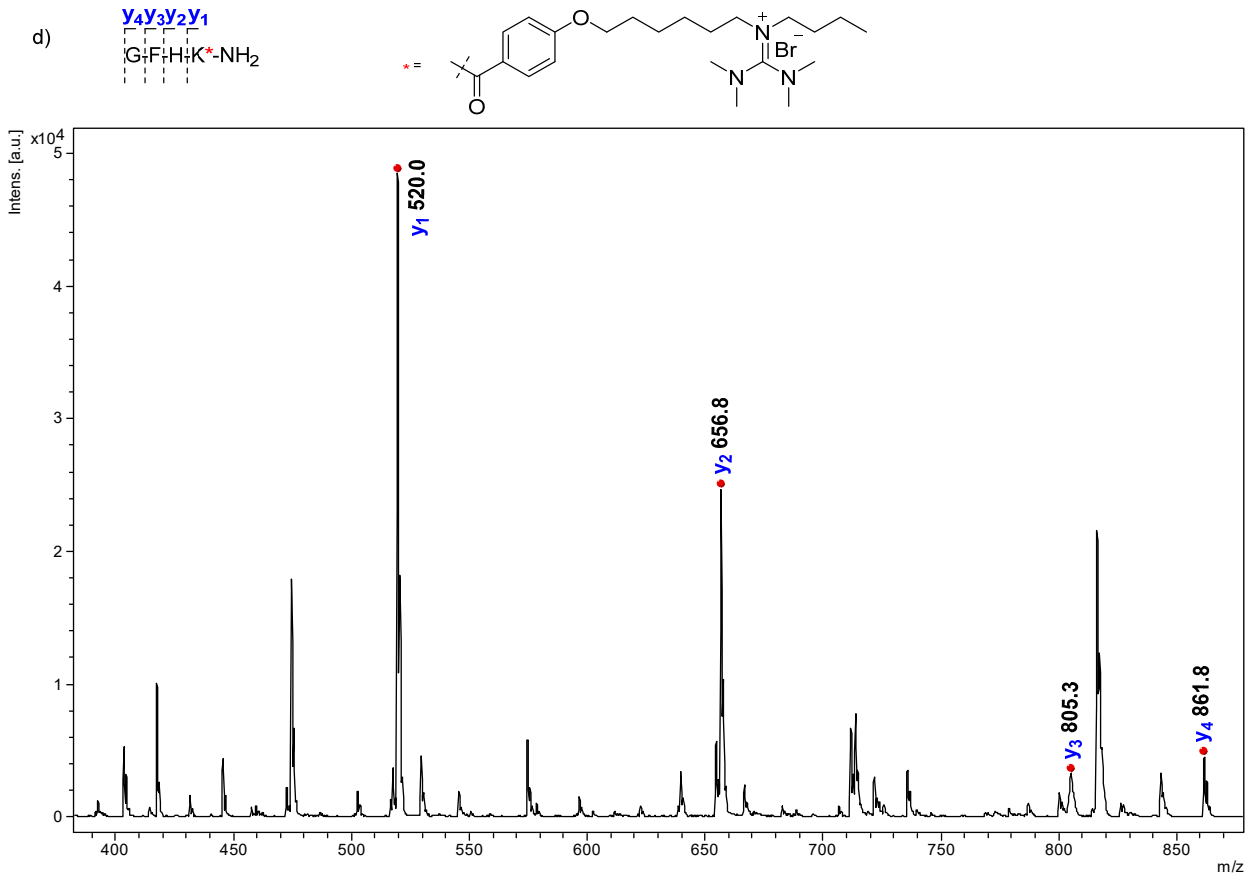
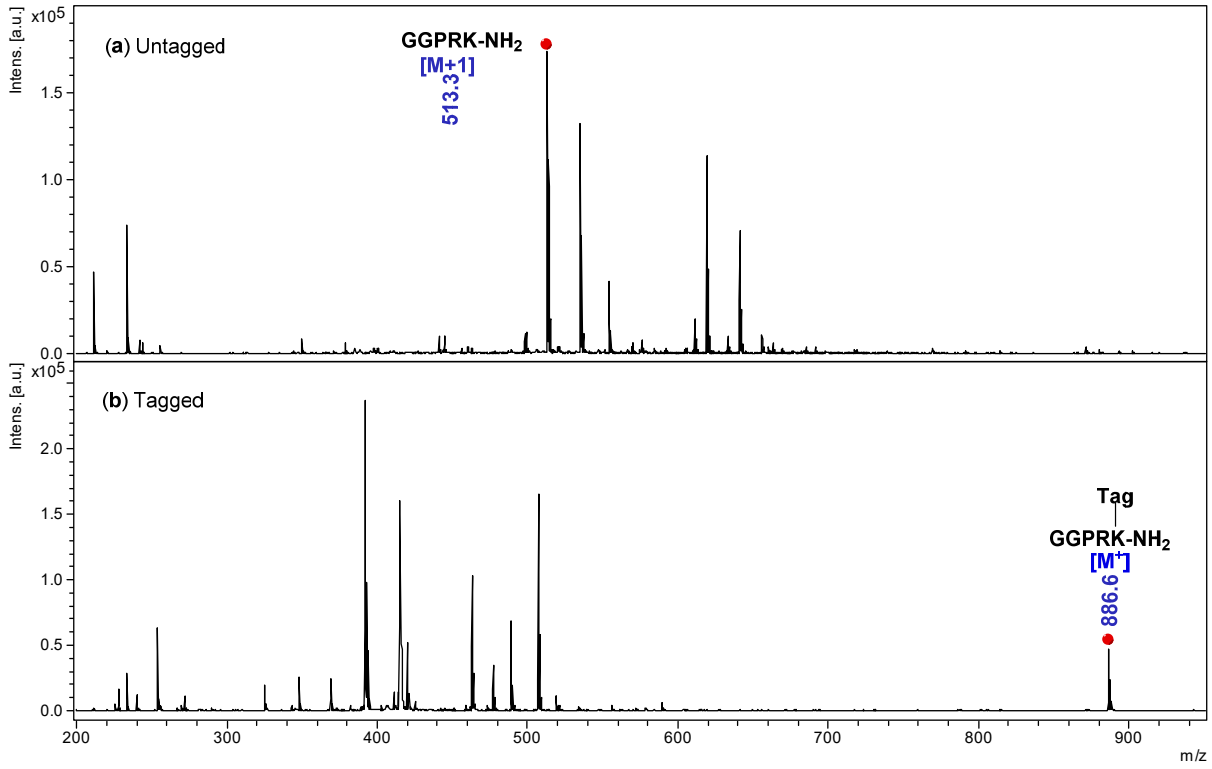
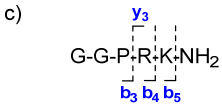
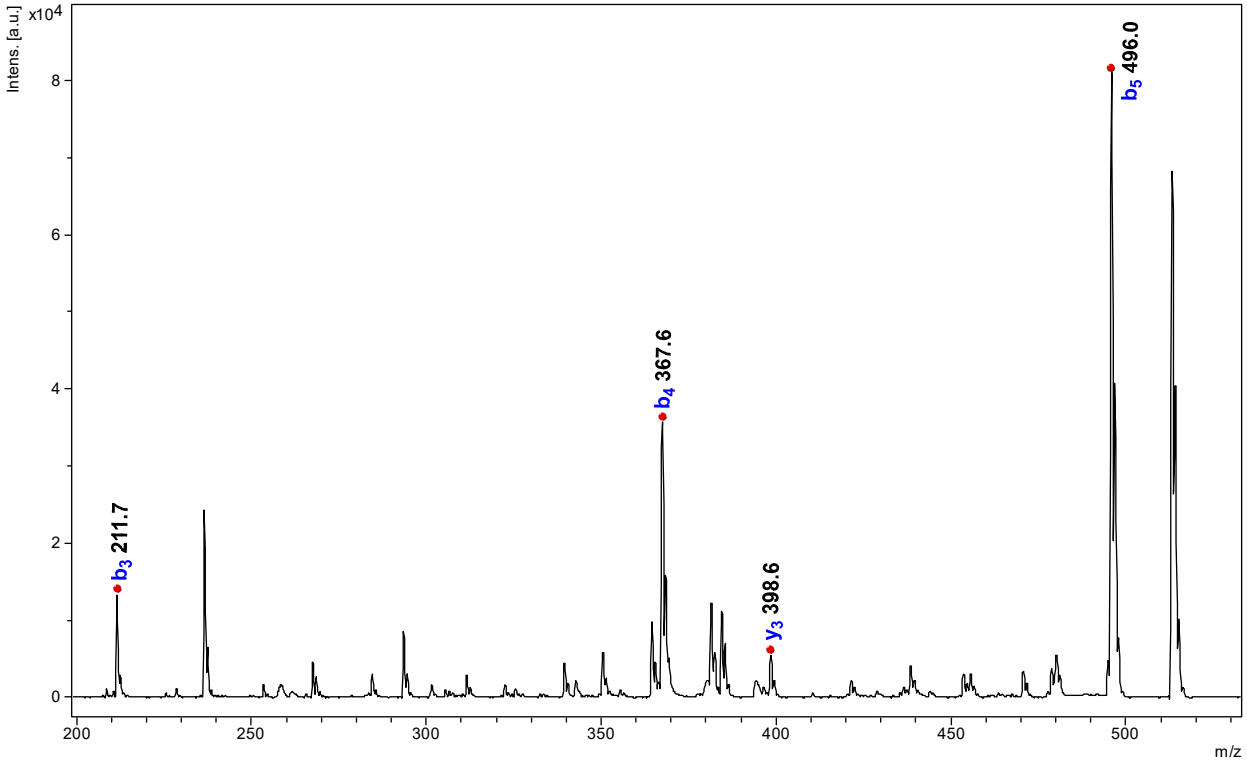


Figure S5: Screening of the peptides **GFHK-NH₂**. (**a**) MALDI-MS spectra of native peptide **GFHK-NH₂** (m/z 486.1, $[M+H]^+$), (**b**) MALDI-MS spectra of tagged peptide **Tag- GFHK-NH₂** (m/z 860.5, $[M]^+$). (c) MS-MS spectra of native peptide **GFHK-NH₂** (m/z 486.1, $[M+H]^+$). (d) MS-MS spectra of tagged peptide **Tag-GFHK-NH₂** ((m/z 860.5, $[M]^+$).


(a) Untagged
GGPRK-NH2
[M+1]
513.3
(b) Tagged
Tag
GGPRK-NH2
[M+]
886.6
Intens. [a.u.]
x10^5
m/z


c)
y3
G-G-P-R-K-NH2
b3 b4 b5


Intens. [a.u.]
x10^4
b3 211.7
b4 367.6
y3 398.6
b5 496.0
m/z

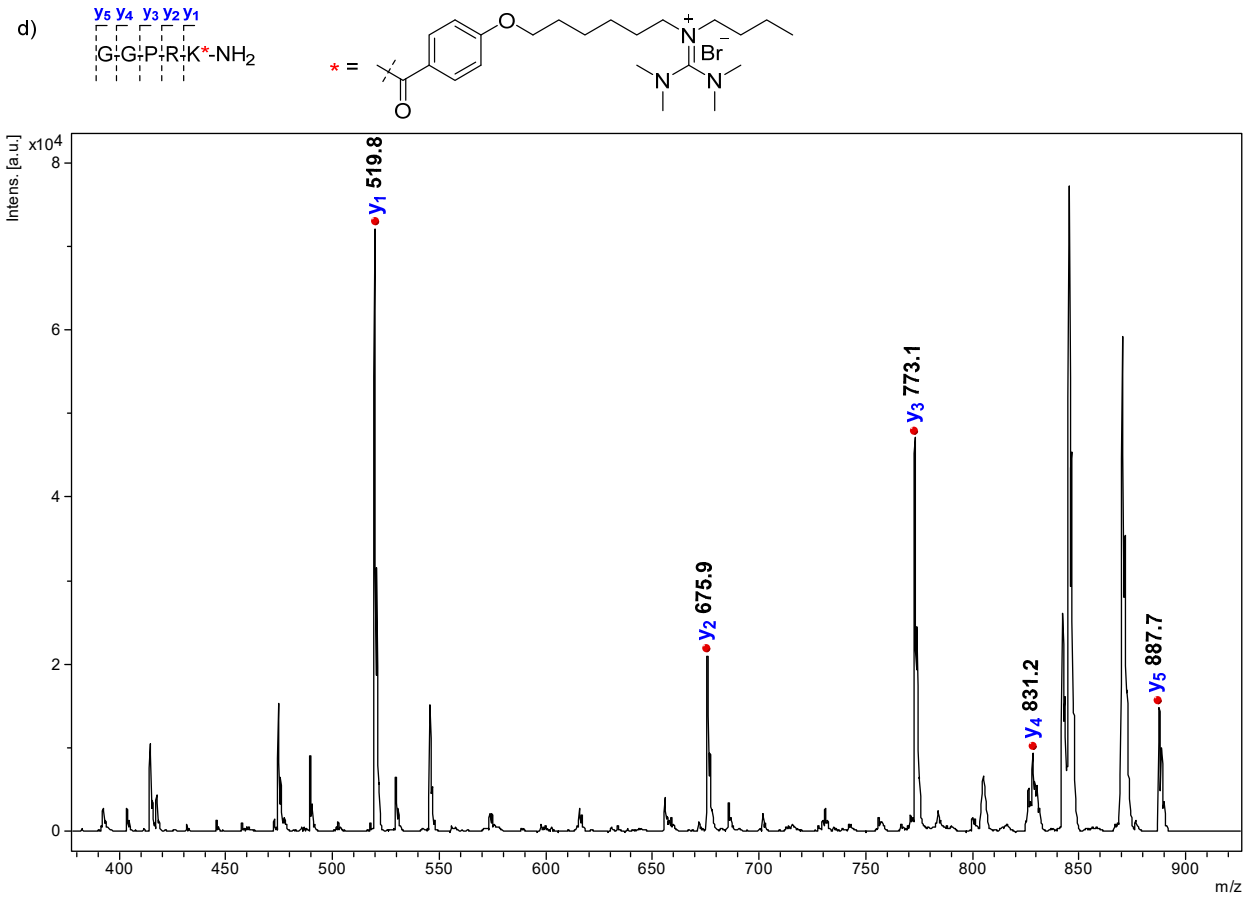
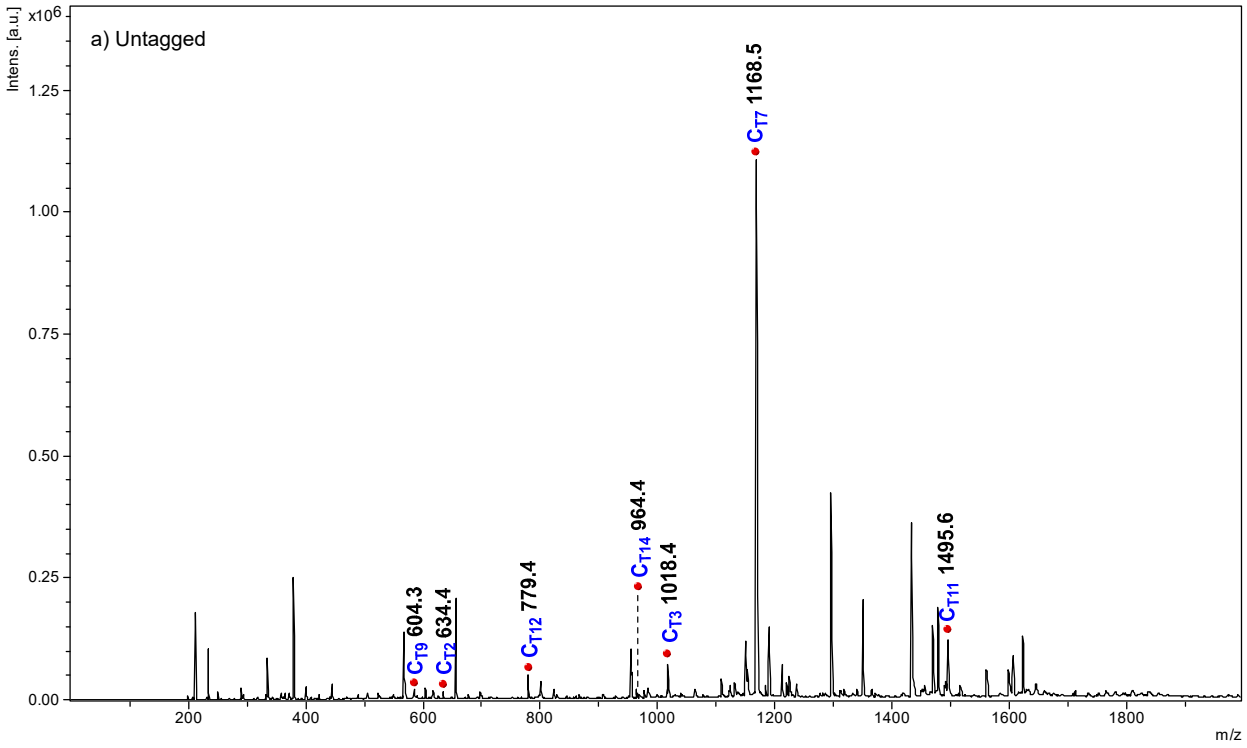
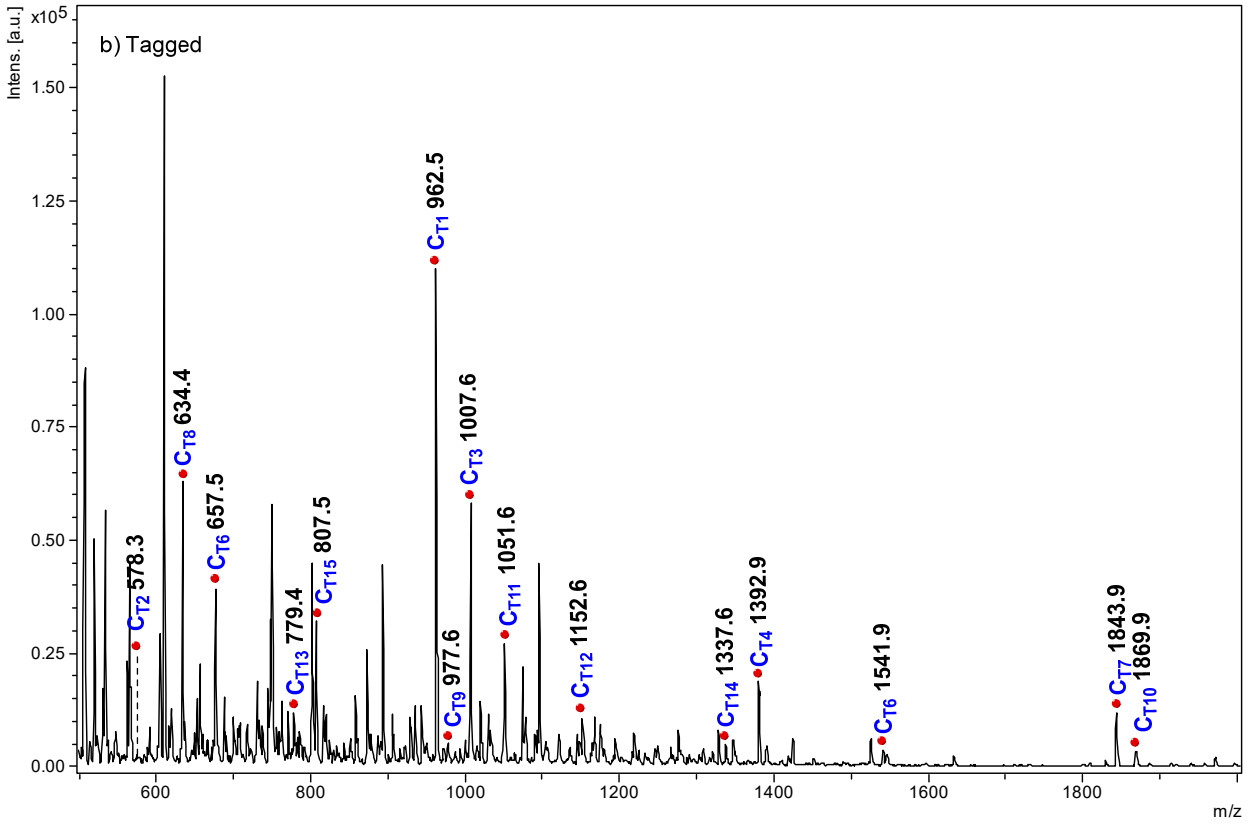
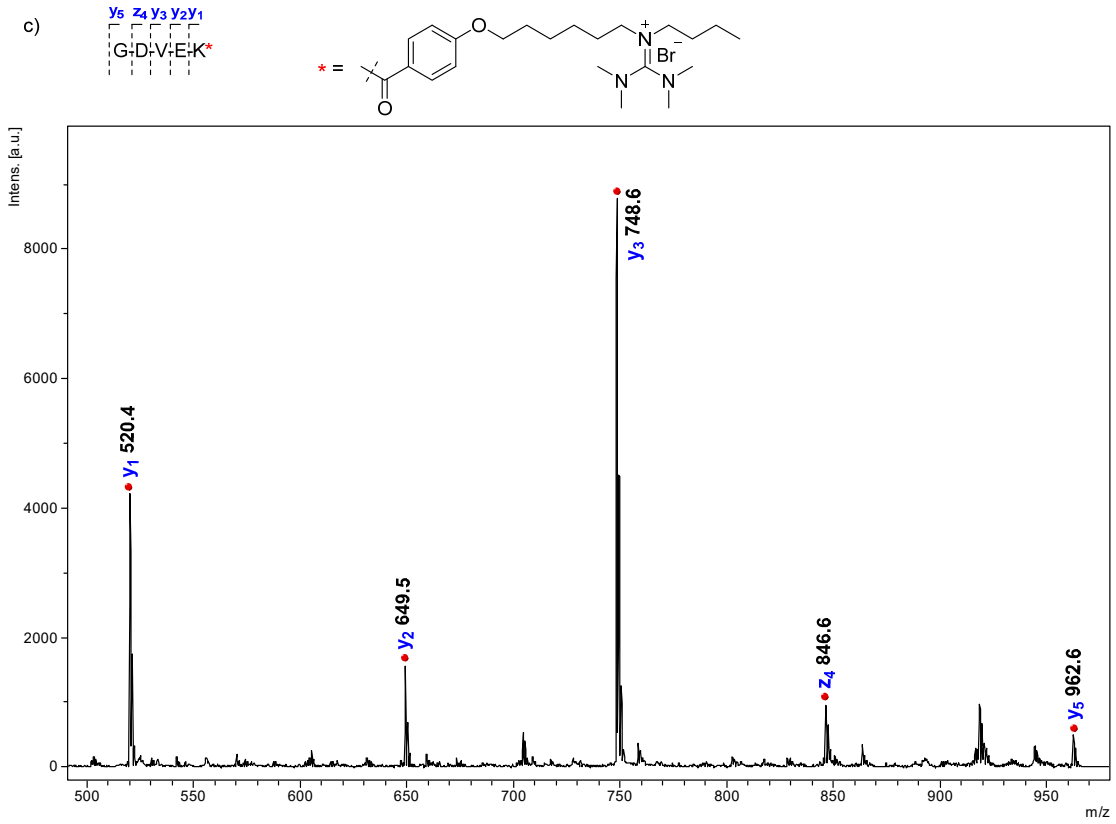
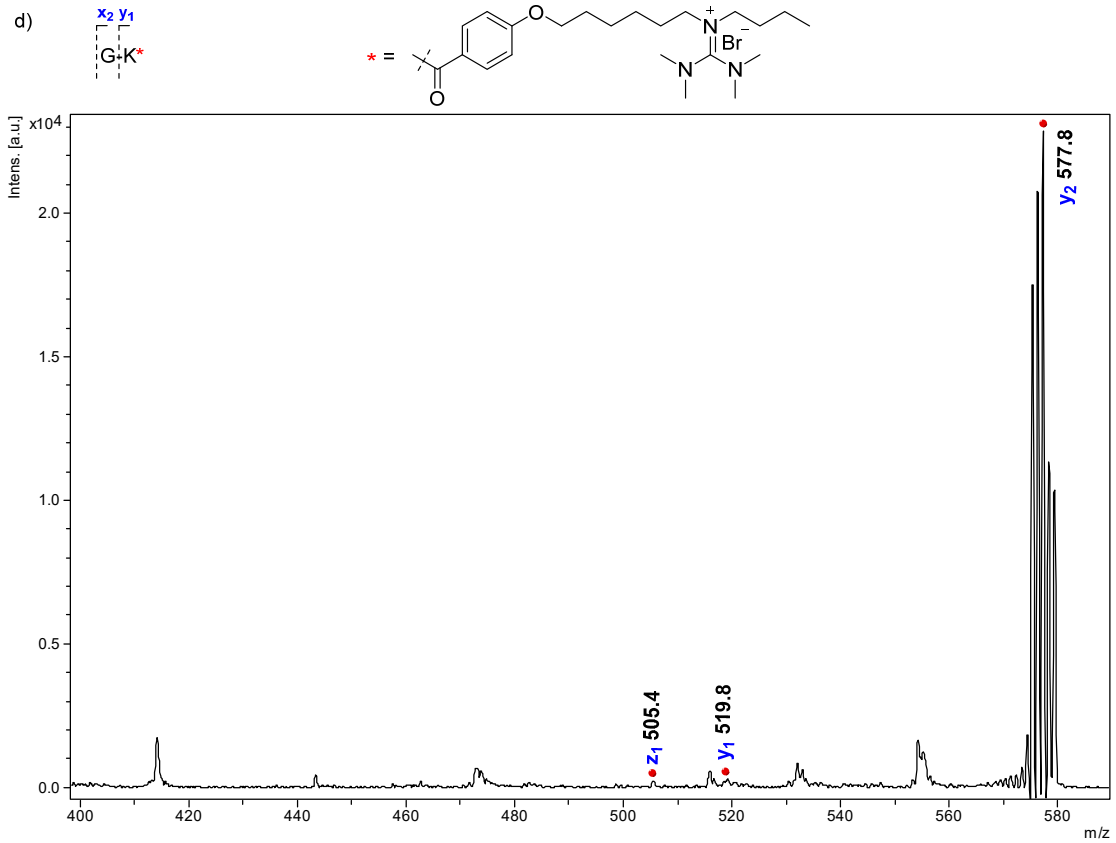


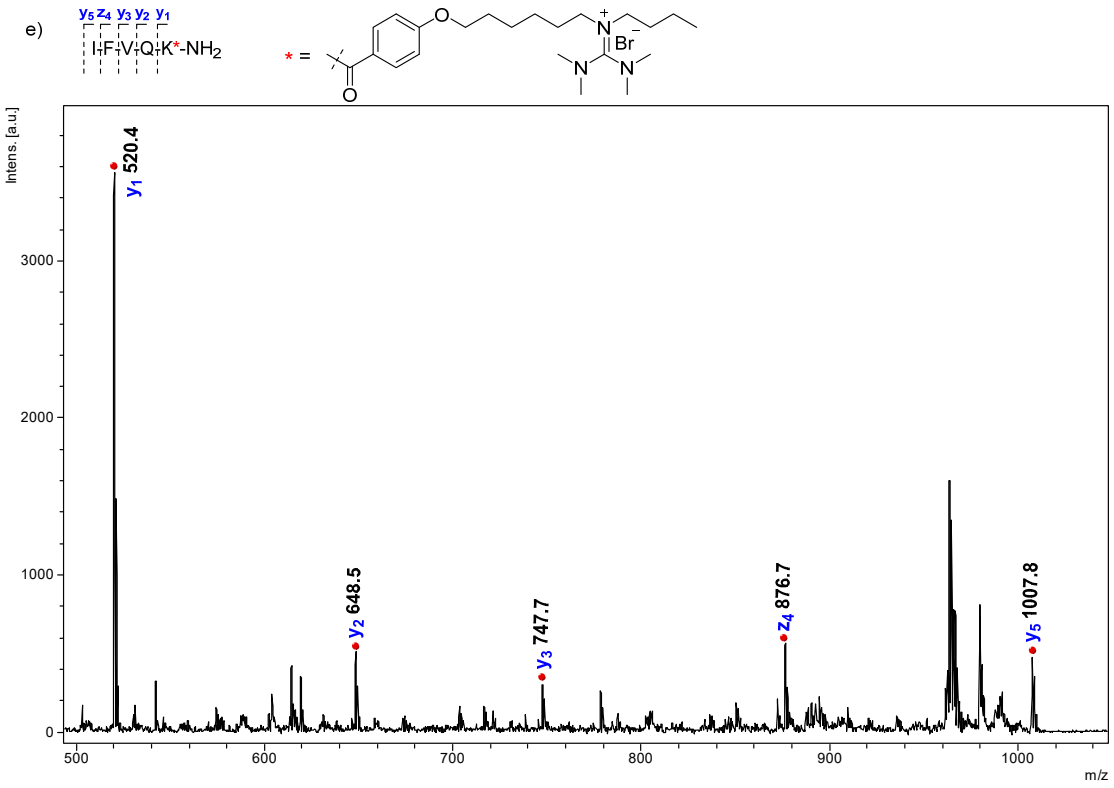
Figure S6: Screening of the peptide **GGPRK-NH₂**. (**a**) MALDI-MS spectra of native peptide **GGPRK-NH₂** (m/z 513.3, $[M+H]^+$), (**b**) MALDI-MS spectra of tagged peptide **Tag- GGPRK-NH₂** (m/z 886.6, $[M]^+$). (**c**) MS-MS spectra of native peptide **Tag-GGPRK-NH₂** (m/z 513.3, $[M+H]^+$). (**d**) MS-MS spectra of native peptide **Tag-GGPRK-NH₂** (m/z 513.3, $[M+H]^+$).

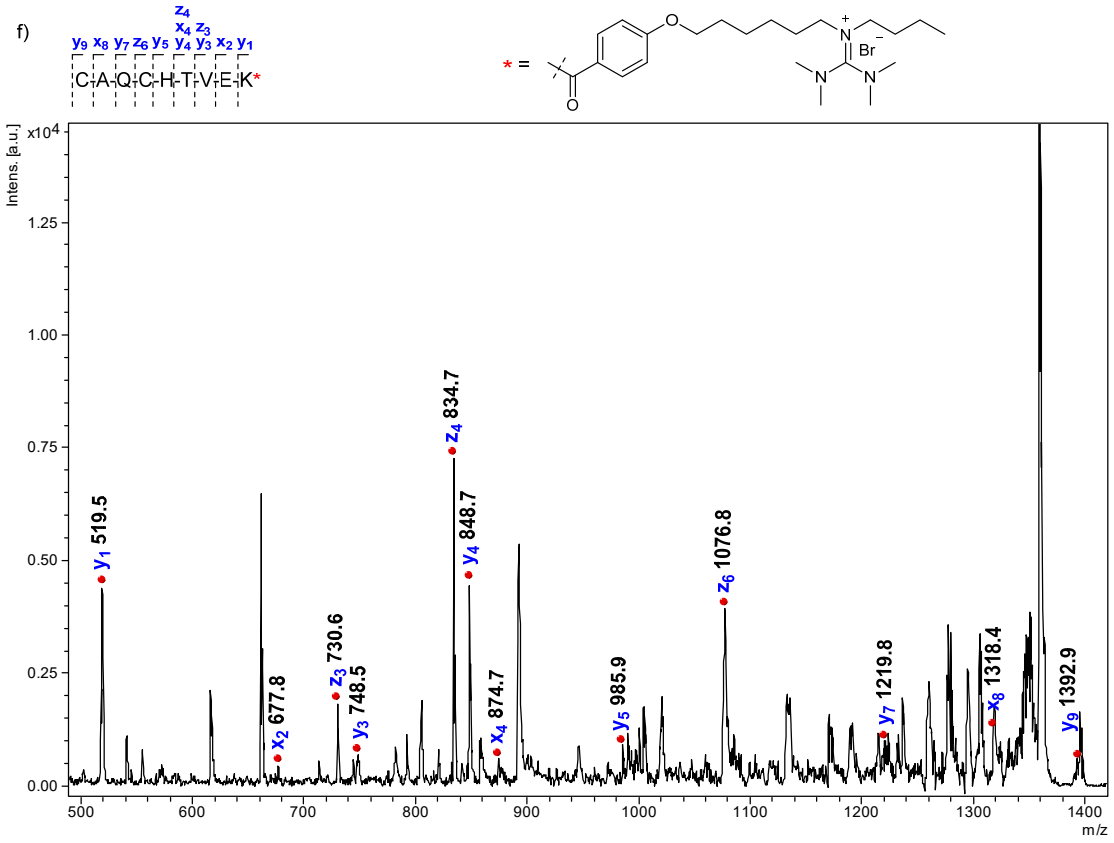

a) Untagged
Intens. [a.u.]
x10^6
1.25
1.00
0.75
0.50
0.25
0.00
200
400
600
800
1000
1200
1400
1600
1800
m/z
C_T9 604.3
C_T2 634.4
C_T12 779.4
C_T14 964.4
C_T3 1018.4
C_T7 1168.5
C_T11 1495.6


b) Tagged
Intens. [a.u.]
x10^5
1.50
1.25
1.00
0.75
0.50
0.25
0.00
600
800
1000
1200
1400
1600
1800
m/z
C_T2 578.3
C_T8 634.4
C_T6 657.5
C_T13 779.4
C_T15 807.5
C_T1 962.5
C_T9 977.6
C_T3 1007.6
C_T11 1051.6
C_T12 1152.6
C_T14 1337.6
C_T4 1392.9
C_T6 1541.9
C_T7 1843.9
C_T10 1869.9

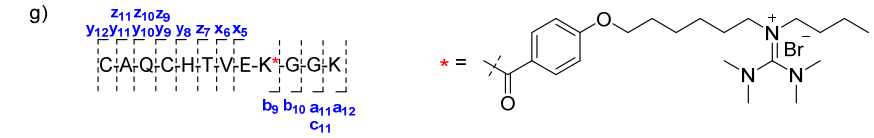

c)
y5 z4 y3 y2 y1
G D V E K*
* =
Br
Intens. [a.u.]
y1 520.4
y2 649.5
y3 748.6
z4 846.6
y5 962.6
m/z

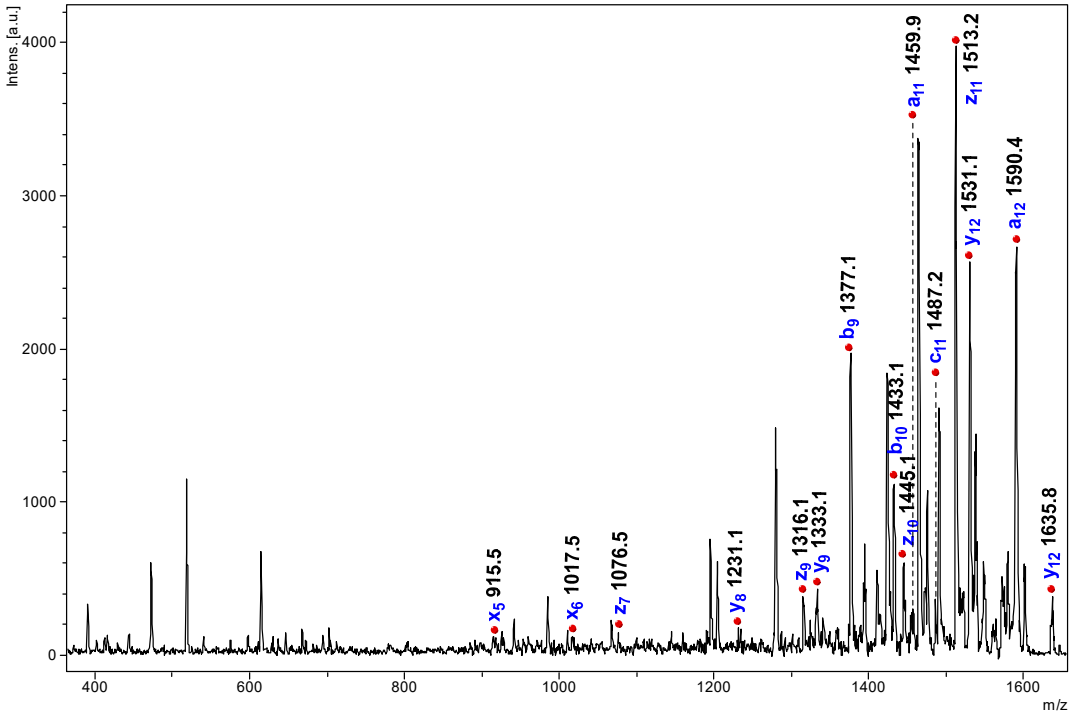

d)
x2 y1
G K*
* =
Br
Intens. [a.u.]
x10^4
z1 505.4
y1 519.8
y2 577.8
m/z


e)
y5 z4 y3 y2 y1
I-F-V-Q-K*-NH2
* =
Br
Intens. [a.u.]
y1 520.4
y2 648.5
y3 747.7
z4 876.7
y5 1007.8
m/z

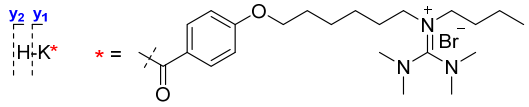

f)
z4
x4 z3
y9 x8 y7 z6 y5 y4 y3 x2 y1
C-A-Q-C-H-T-V-E-K*
* =
Br
Intens. [a.u.]
x10^4
y1 519.5
x2 677.8
z3 730.6
y3 748.5
z4 834.7
y4 848.7
x4 874.7
y5 985.9
z6 1076.8
y7 1219.8
x8 1318.4
y9 1392.9
m/z

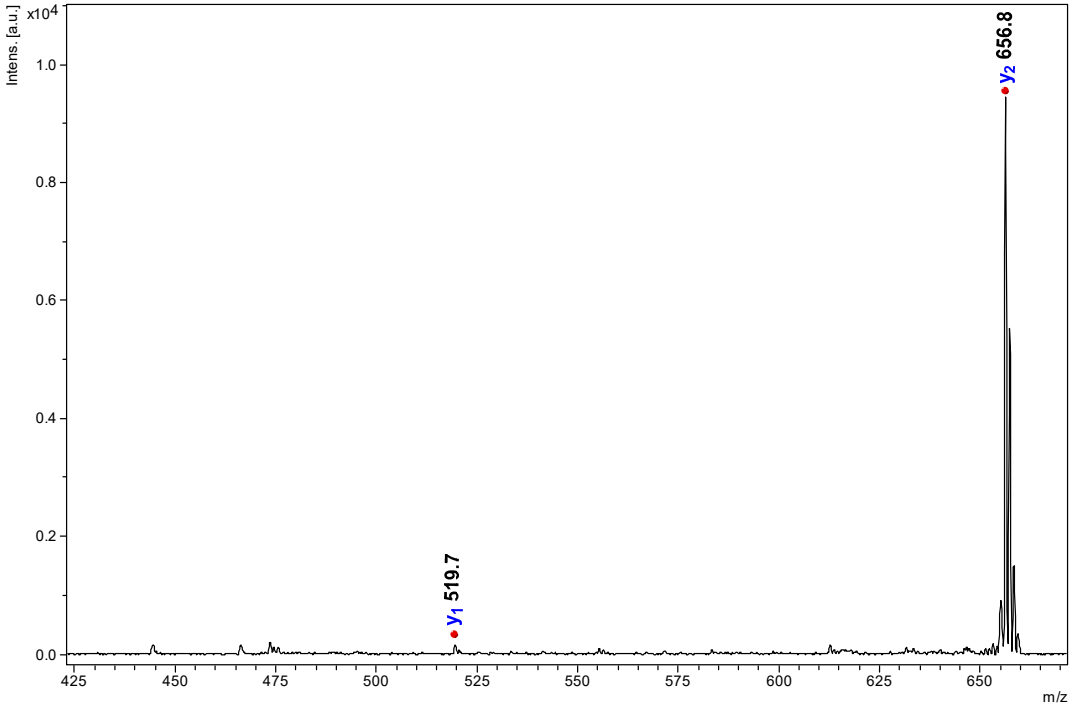
g)

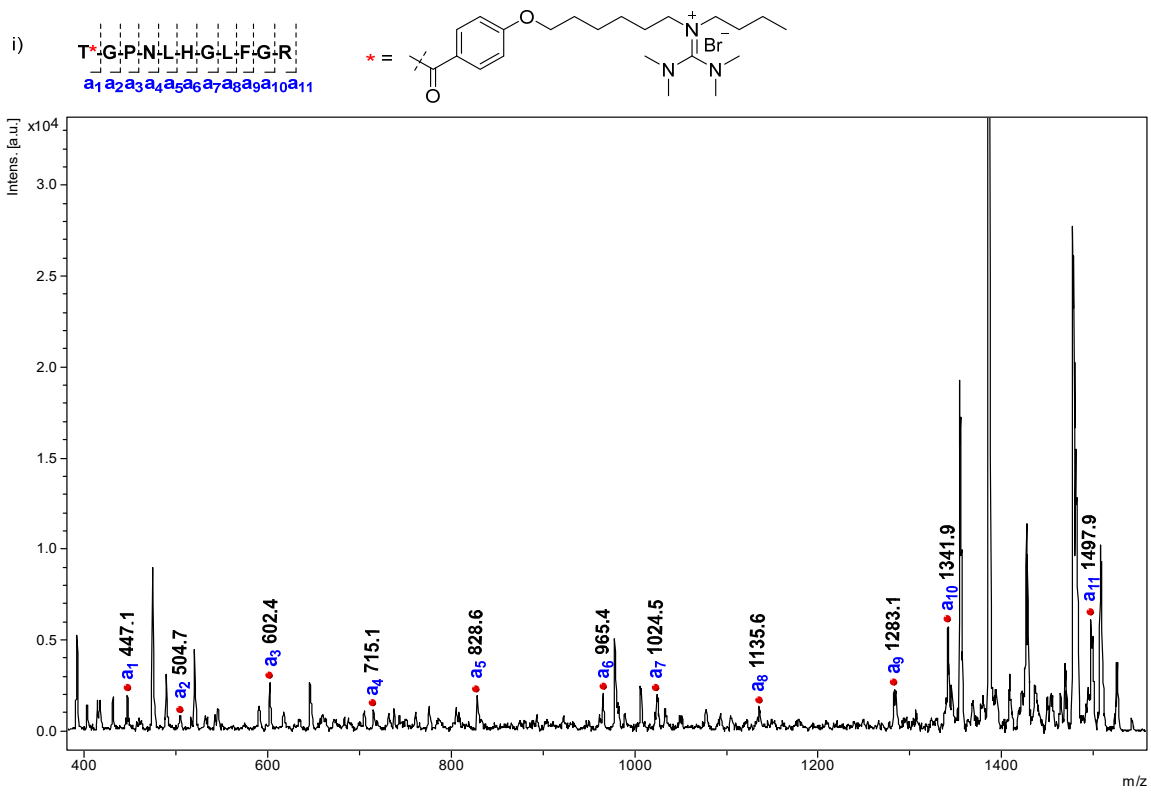

z11 z10 z9
y12 y11 y10 y9 y8 z7 x6 x5
C A Q C H T V E K* G G K
b9 b10 a11 a12
c11
* =
O
N
Br
N
N
O


Intens. [a.u.]
4000
3000
2000
1000
0
400
600
800
1000
1200
1400
1600
m/z
x5 915.5
x6 1017.5
z7 1076.5
y8 1231.1
z9 1316.1
y9 1333.1
b9 1377.1
b10 1433.1
z10 1445.1
a11 1459.9
c11 1487.2
y12 1531.1
z11 1513.2
a12 1590.4
y12 1635.8

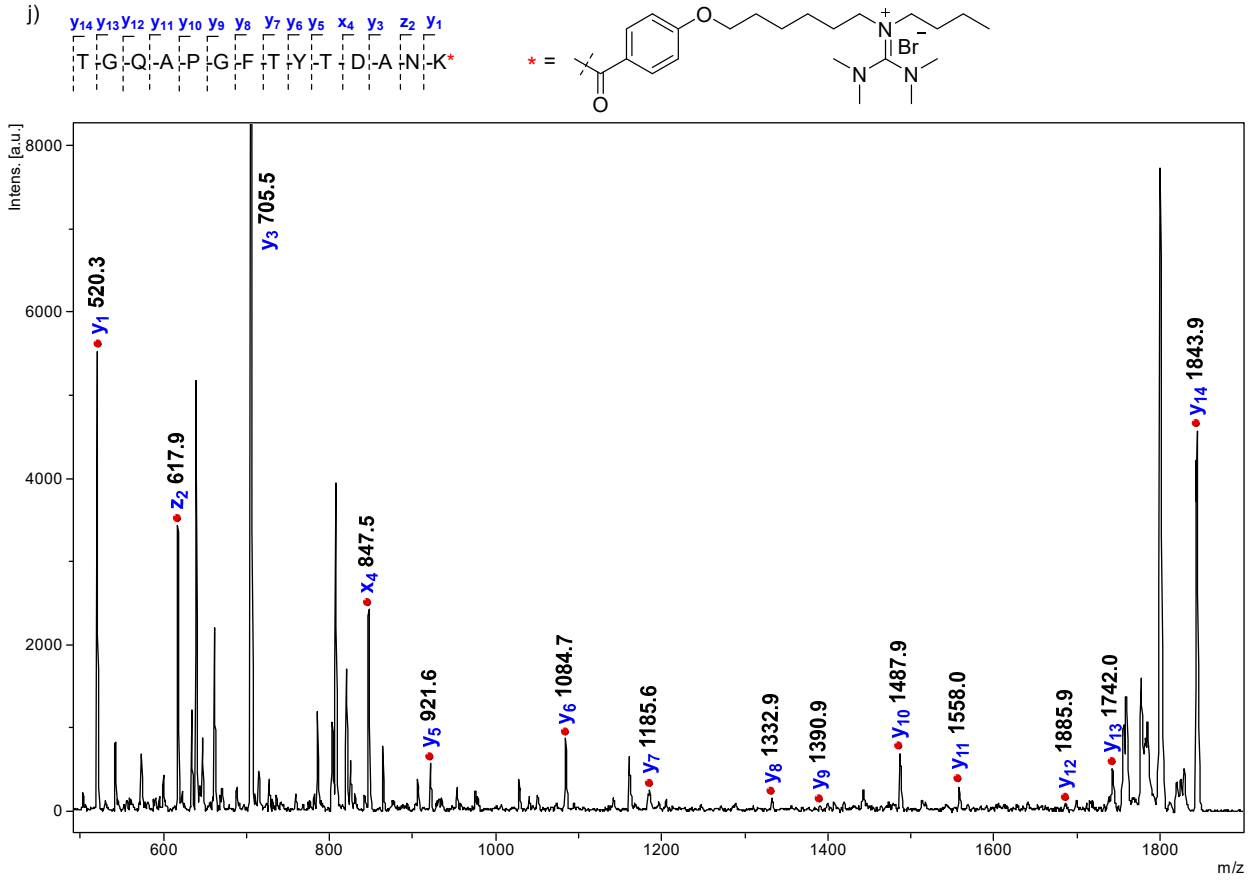
h)

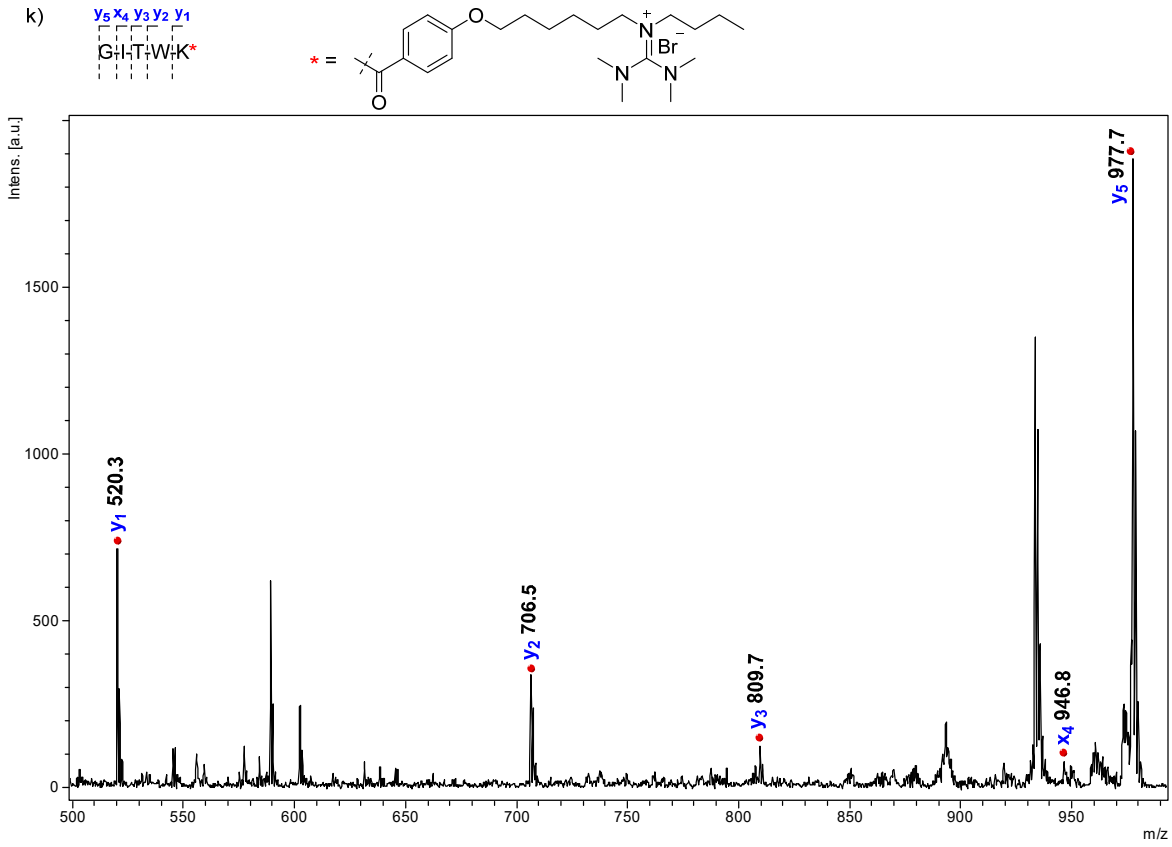

y2 y1
H K*
* =
O
N
Br
N
N
O

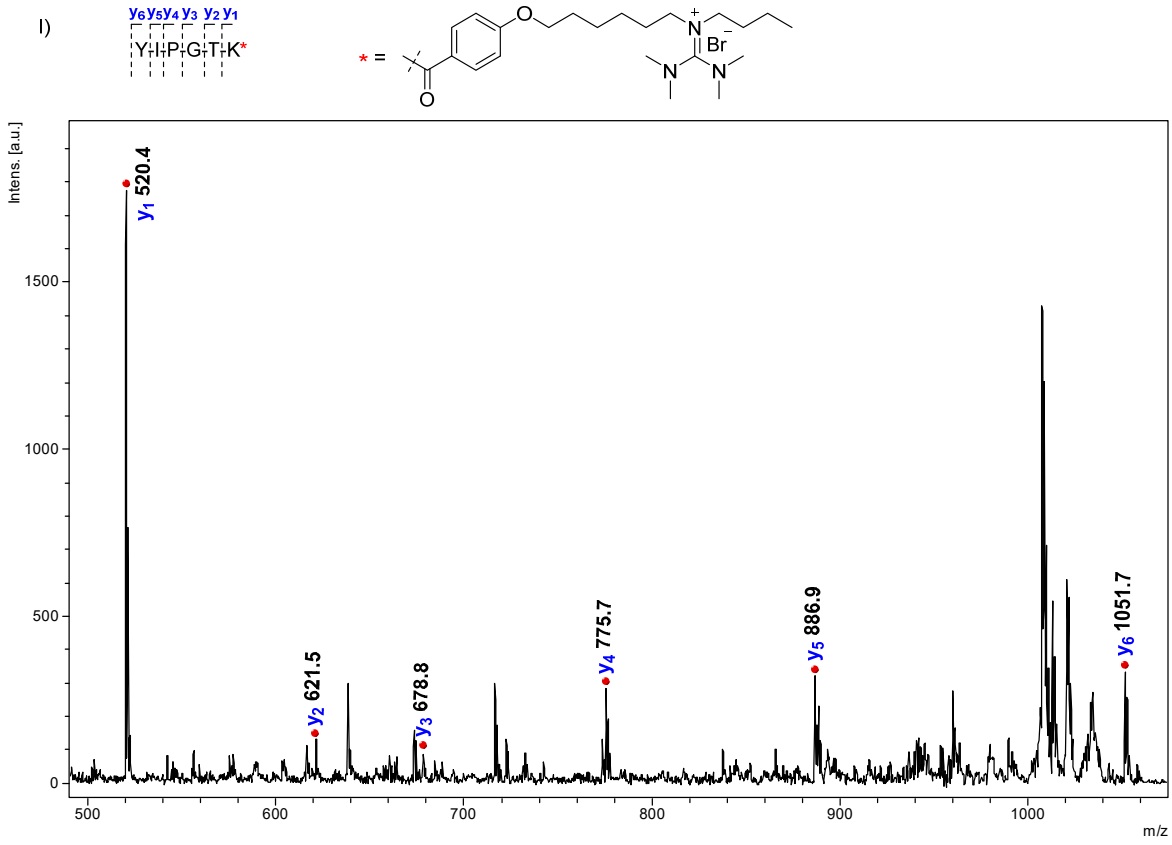

Intens. [a.u.]
x10^4
1.0
0.8
0.6
0.4
0.2
0.0
425
450
475
500
525
550
575
600
625
650
m/z
y1 519.7
y2 656.8


i)
T*-G-P-N-L-H-G-L-F-G-R
a1 a2 a3 a4 a5 a6 a7 a8 a9 a10 a11
* =
Br
N
N
N
O
O
Intens. [a.u.]
x10^4
3.0
2.5
2.0
1.5
1.0
0.5
0.0
400
600
800
1000
1200
1400
m/z
a1 447.1
a2 504.7
a3 602.4
a4 715.1
a5 828.6
a6 965.4
a7 1024.5
a8 1135.6
a9 1283.1
a10 1341.9
a11 1497.9

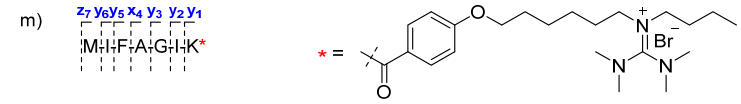
j)

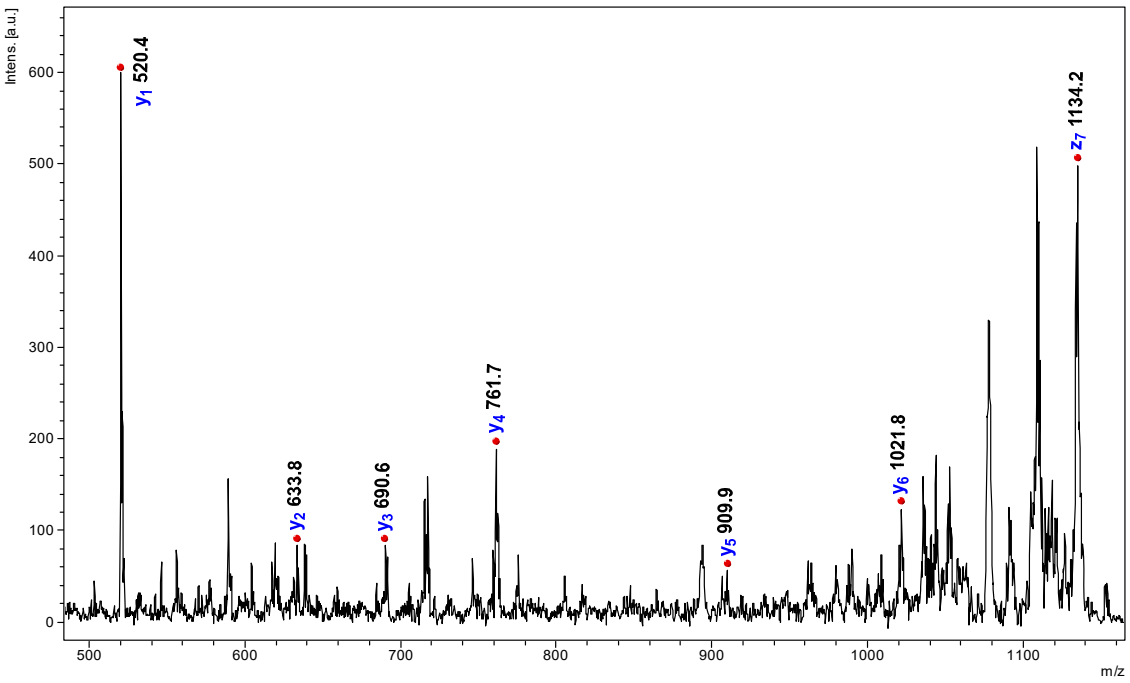

y14 y13 y12 y11 y10 y9 y8 y7 y6 y5 x4 y3 z2 y1
T G Q A P G F T Y T D A N K*
* =
O
N
Br
N
N
O
Intens. [a.u.]
8000
6000
4000
2000
0
y1 520.3
z2 617.9
y3 705.5
x4 847.5
y5 921.6
y6 1084.7
y7 1185.6
y8 1332.9
y9 1390.9
y10 1487.9
y11 1558.0
y12 1885.9
y13 1742.0
y14 1843.9
600
800
1000
1200
1400
1600
1800
m/z


k)
y5 x4 y3 y2 y1
G-I-T-W-K*
* =
Br
Intens. [a.u.]
y1 520.3
y2 706.5
y3 809.7
x4 946.8
y5 977.7
m/z

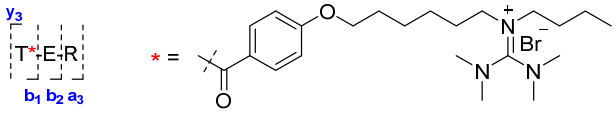

l)
y6 y5 y4 y3 y2 y1
Y-I-P-G-T-K*
* =
Br
Intens. [a.u.]
y1 520.4
y2 621.5
y3 678.8
y4 775.7
y5 886.9
y6 1051.7
m/z

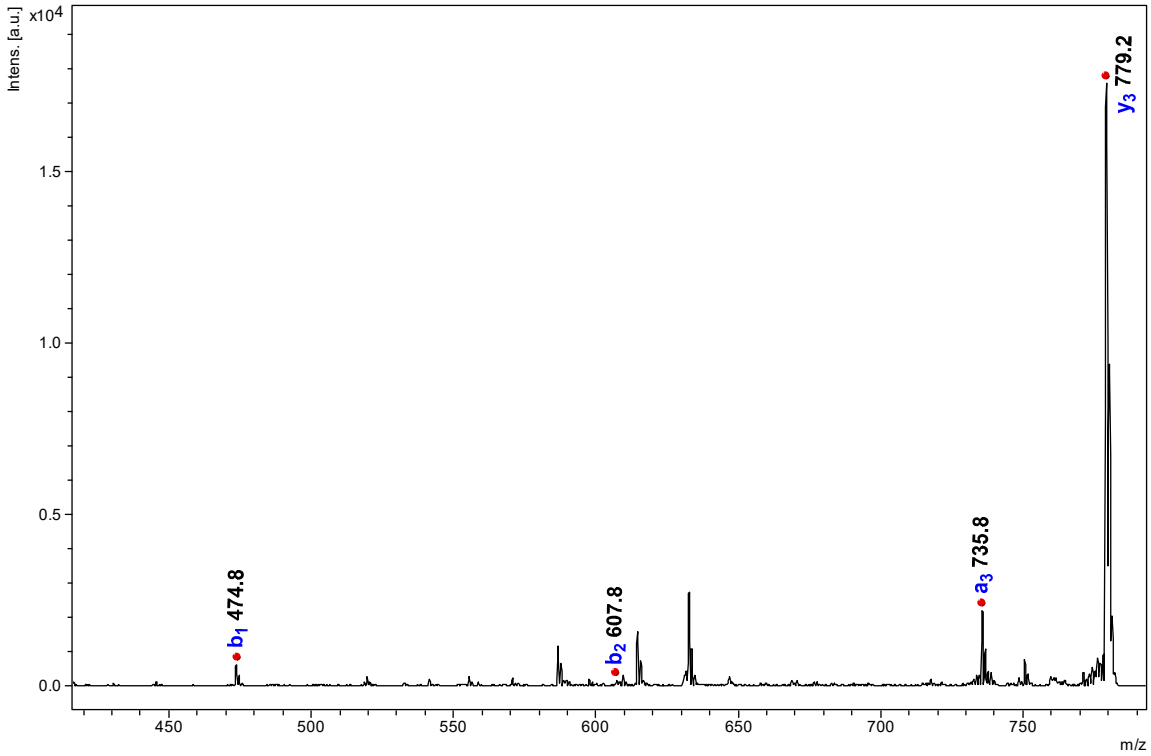
m)


z7 y6 y5 x4 y3 y2 y1
M I F A G I K*
* =
O
N
Br
N
N

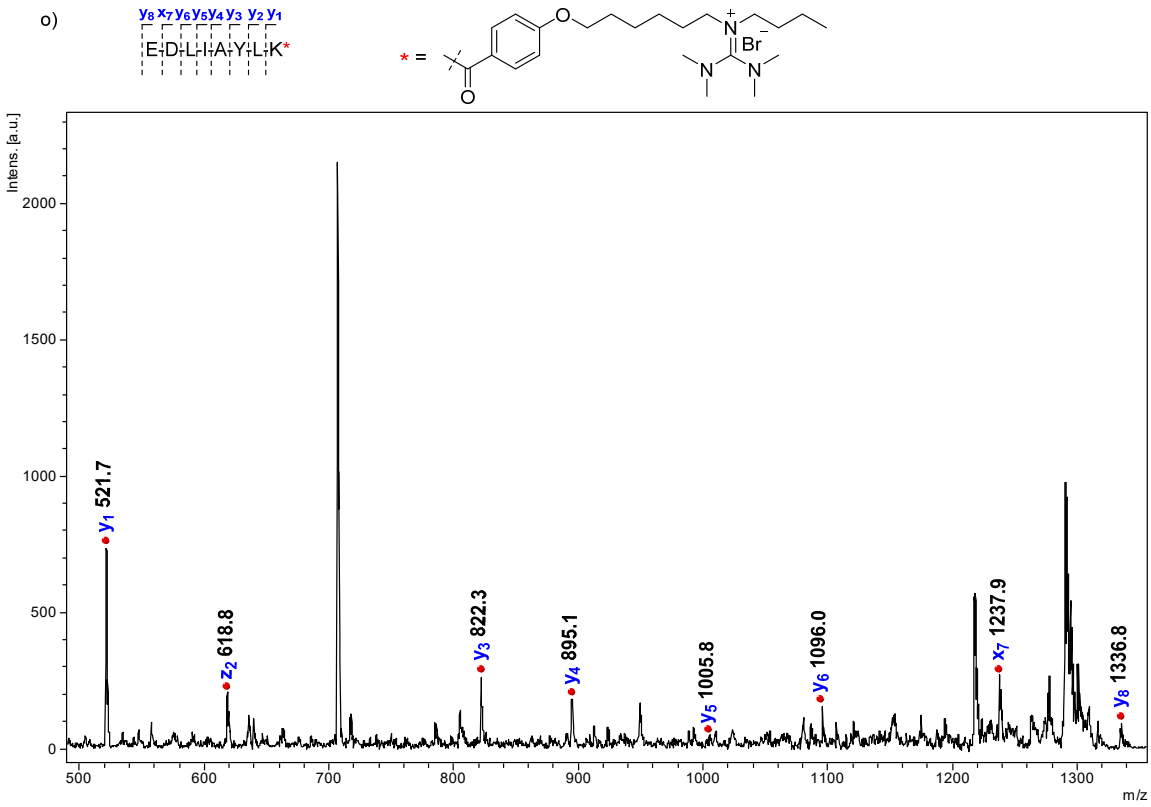

Intens. [a.u.]
600
500
400
300
200
100
0
y1 520.4
y2 633.8
y3 690.6
y4 761.7
y5 909.9
y6 1021.8
z7 1134.2
500
600
700
800
900
1000
1100
m/z

n)


y3
T* E R
b1 b2 a3
* =
O
N
Br
N
N


Intens. [a.u.]
x10^4
1.5
1.0
0.5
0.0
b1 474.8
b2 607.8
a3 735.8
y3 779.2
450
500
550
600
650
700
750
m/z

o)


y8 x7 y6 y5 y4 y3 y2 y1
E D L I A Y L K*
* =
Br
Intens. [a.u.]
y1 521.7
z2 618.8
y3 822.3
y4 895.1
y5 1005.8
y6 1096.0
x7 1237.9
y8 1336.8
m/z

p)

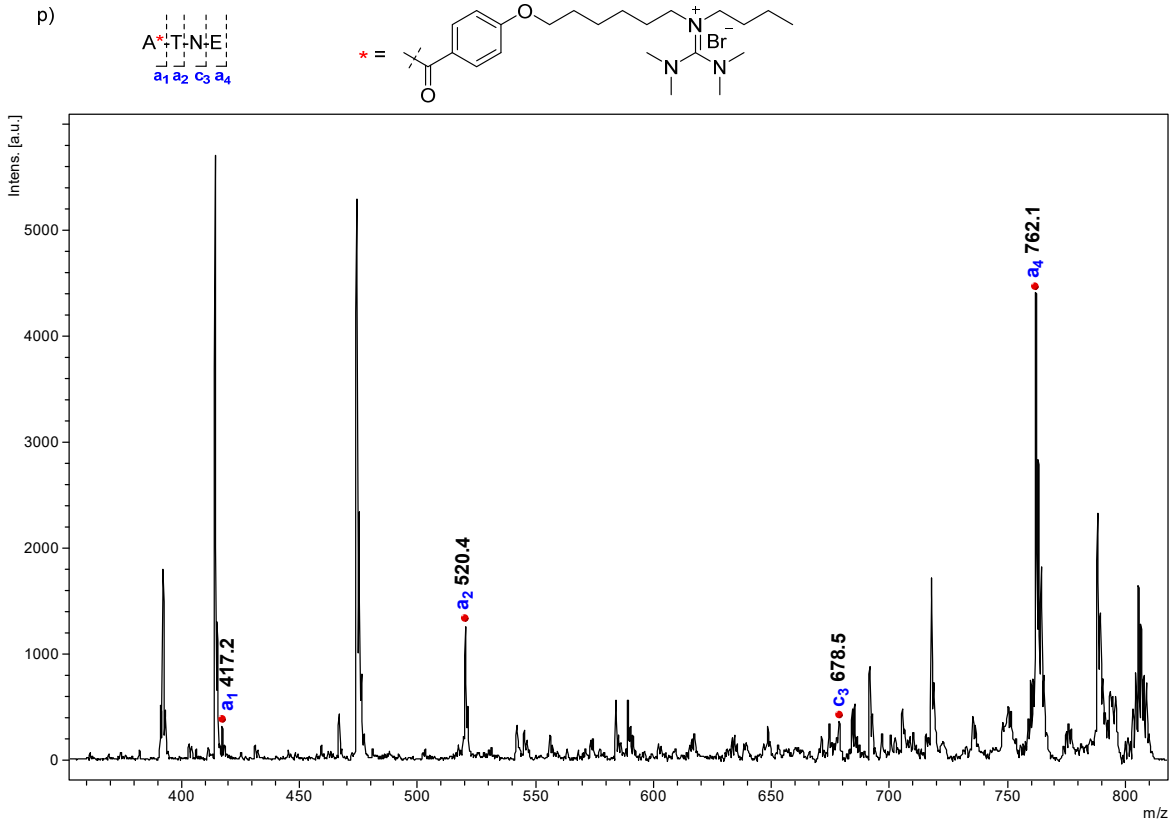
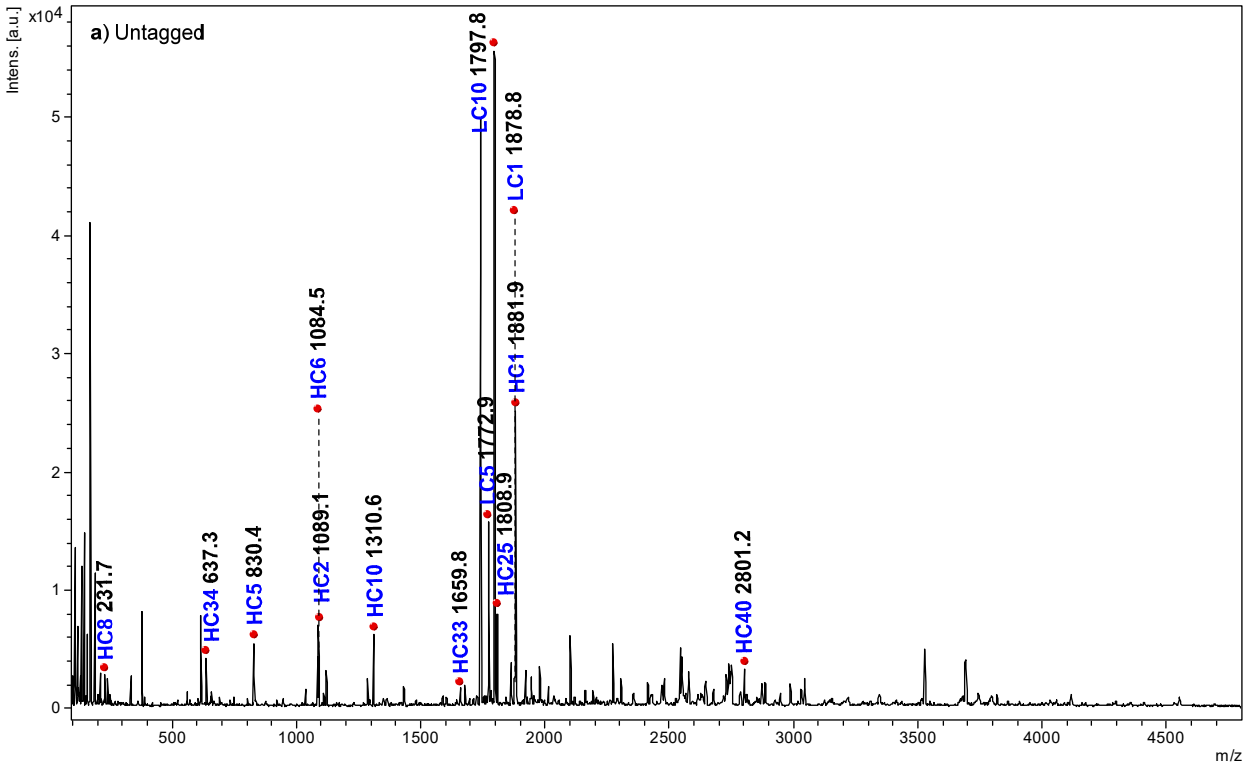
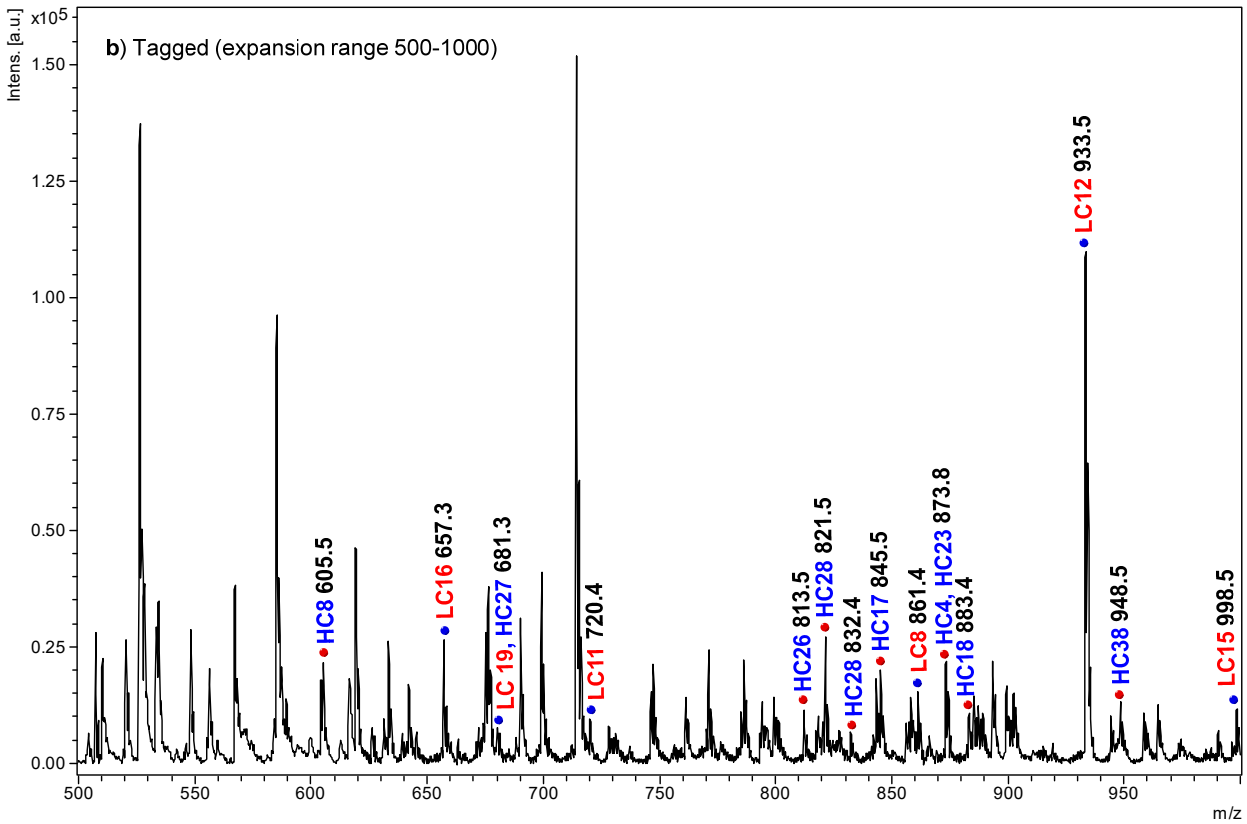
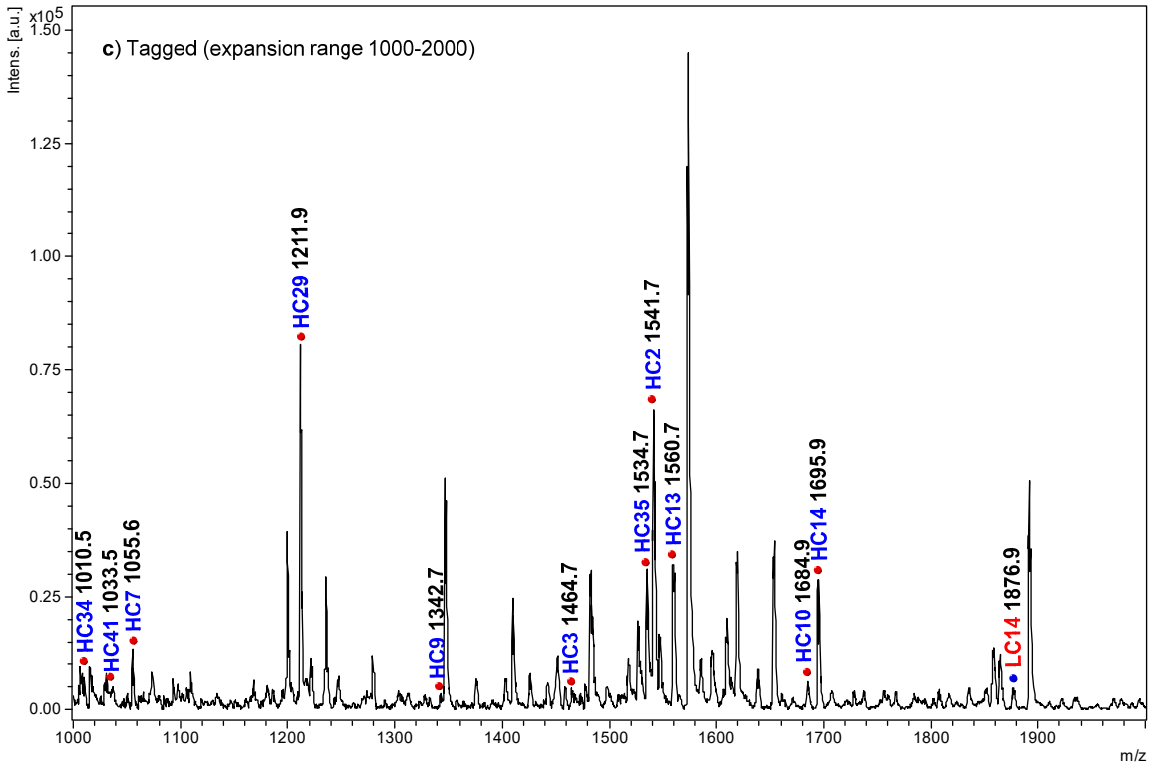
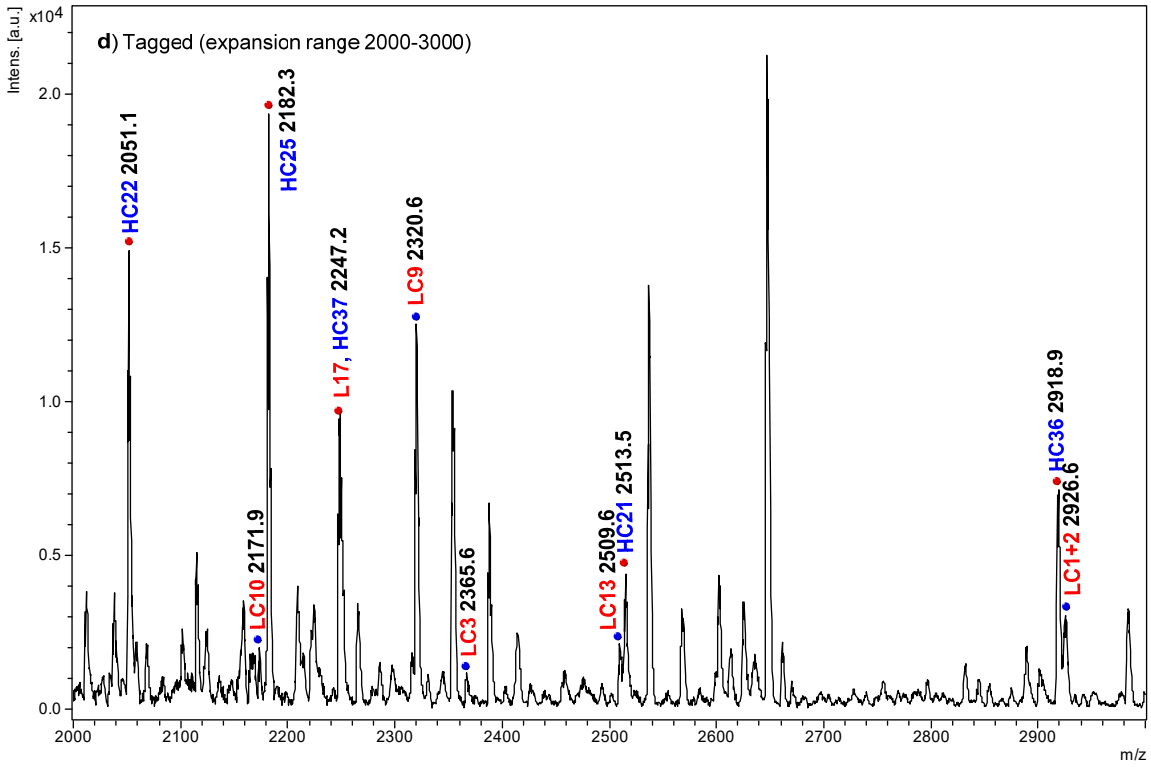

A* T N E
a1 a2 c3 a4
* =
Br
Intens. [a.u.]
a1 417.2
a2 520.4
c3 678.5
a4 762.1
m/z

Figure S7: Analyzing and sequencing the Cytochrome C (a) MALDI-MS spectra of cytochrome C digestion mixture after the digestion with trypsin. (b) MALDI-MS spectra for the tagged cytochrome C digestion mixture. (c) MS-MS of peptide sequence the XGDVEK-Tag (residues 1-5, m/z 926.6, $[M]^+$). (d) MS-MS of peptide sequence the GK-Tag (residues 6-7, m/z 578.3, $[M]^+$). (e) MS-MS of peptide sequence the IFVQK-Tag (residues 9-13, m/z 1007.6, $[M]^+$). (f) MS-MS of peptide sequence the CAQCHTVEK(Tag) (residues 14-22, m/z 1392.8, $[M]^+$) (g) MS-MS of peptide sequence the CAQCHTVEK(Tag)GGK (residues 14-25, m/z 1635.8, $[M]^+$). (h) MS-MS of peptide sequence HK-Tag (residues 26-27, m/z 656.8, $[M]^+$). (i) MS-MS of peptide sequence Tag-TGPNLHGLFGR (residues 28-38, m/z 1541.9, $[M]^+$). (j) MS-MS of peptide sequence TGQAPGFTYTDANK-Tag (residues 40-53, m/z 1843.9, $[M]^+$). (k) MS-MS of peptide sequence GITWK-Tag (residues 56-60, m/z 977.7, $[M]^+$). (l) MS-MS of peptide sequence YIPGTK-Tag (residues 74-79, m/z 1051.7, $[M]^+$). (m) MS-MS of peptide sequence the MIFAGIK-Tag (residues 80-86, m/z 1337.9, $[M]^+$). (n) MS-MS of peptide sequence MIFAGIK-Tag (residues 89-91, m/z 779.2, $[M]^+$). (o) MS-MS of peptide sequence EDLIAYLK-Tag (residues 92-99, m/z 1336.8, $[M]^+$). (p) MS-MS of peptide sequence Tag-ATNE (residues 101-104, m/z 807.9, $[M]^+$).


a) Untagged
Intens. [a.u.]
x10^4
HC8 231.7
HC34 637.3
HC5 830.4
HC6 1084.5
HC2 1089.1
HC10 1310.6
HC33 1659.8
LC10 1797.8
LC5 1772.9
HC25 1808.9
LC1 1878.8
HC1 1881.9
HC40 2801.2
m/z


b) Tagged (expansion range 500-1000)
Intens. [a.u.]
x10^5
HC8 605.5
LC16 657.3
LC 19, HC27 681.3
LC11 720.4
HC26 813.5
HC28 821.5
HC28 832.4
HC17 845.5
LC8 861.4
HC4, HC23 873.8
HC18 883.4
LC12 933.5
HC38 948.5
LC15 998.5
m/z


c) Tagged (expansion range 1000-2000)
Intens. [a.u.]
x10^5
m/z
HC34 1010.5
HC41 1033.5
HC7 1055.6
HC29 1211.9
HC9 1342.7
HC3 1464.7
HC35 1534.7
HC2 1541.7
HC13 1560.7
HC10 1684.9
HC14 1695.9
LC14 1876.9


d) Tagged (expansion range 2000-3000)
Intens. [a.u.]
x10^4
m/z
HC22 2051.1
LC10 2171.9
HC25 2182.3
L17, HC37 2247.2
LC9 2320.6
LC3 2365.6
LC13 2509.6
HC21 2513.5
HC36 2918.9
LC1+2 2926.6

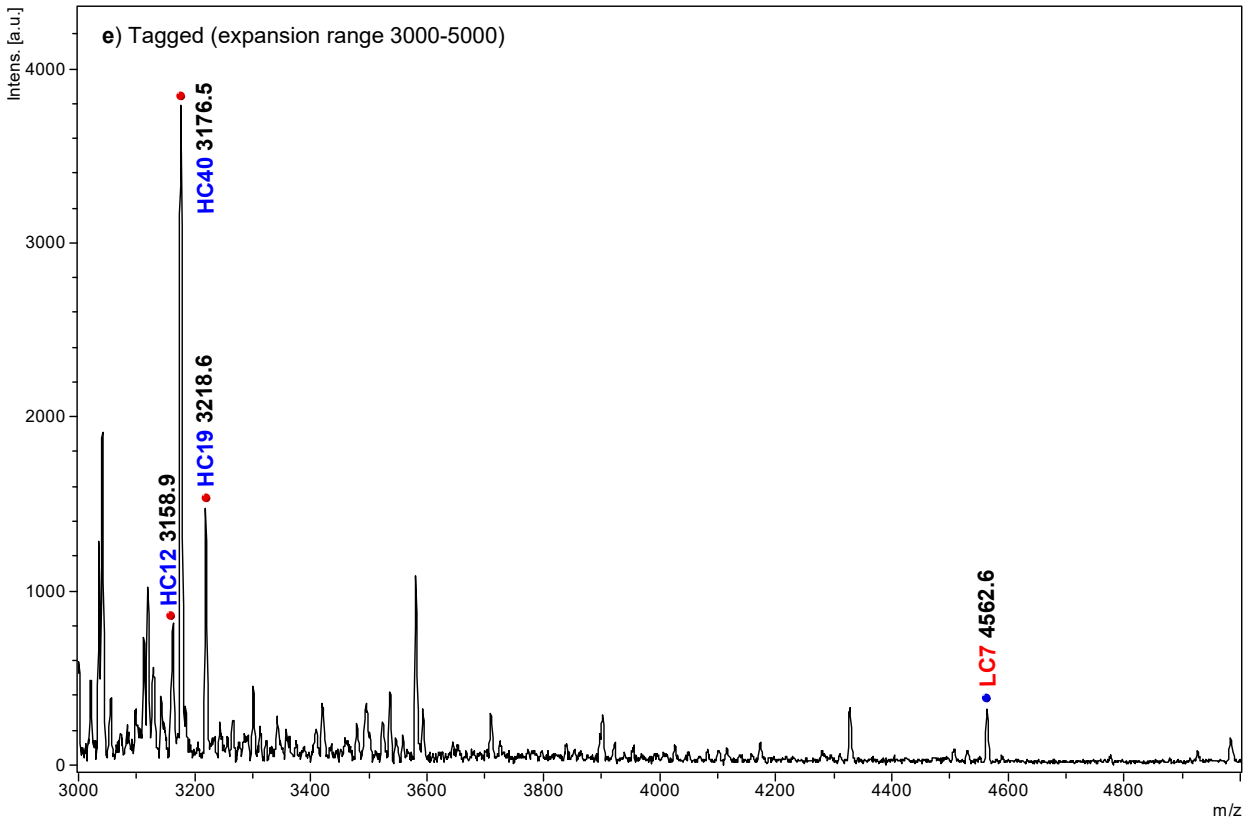


Fig S8. (**a**) Peptide mapping of untagged Trastuzumab digest. (**b**) Peptide mapping of Trastuzumab digest tagged with sensitivity booster **3**. (**b**) Expansion of mass range 500-1000. (**c**) Expansion of mass range 1000-2000. (**d**) Expansion of mass range 2000-3000. (**e**) Expansion of mass range 3000-5000.

Table S3. Sequencing of heavy chain fragment of antibody.

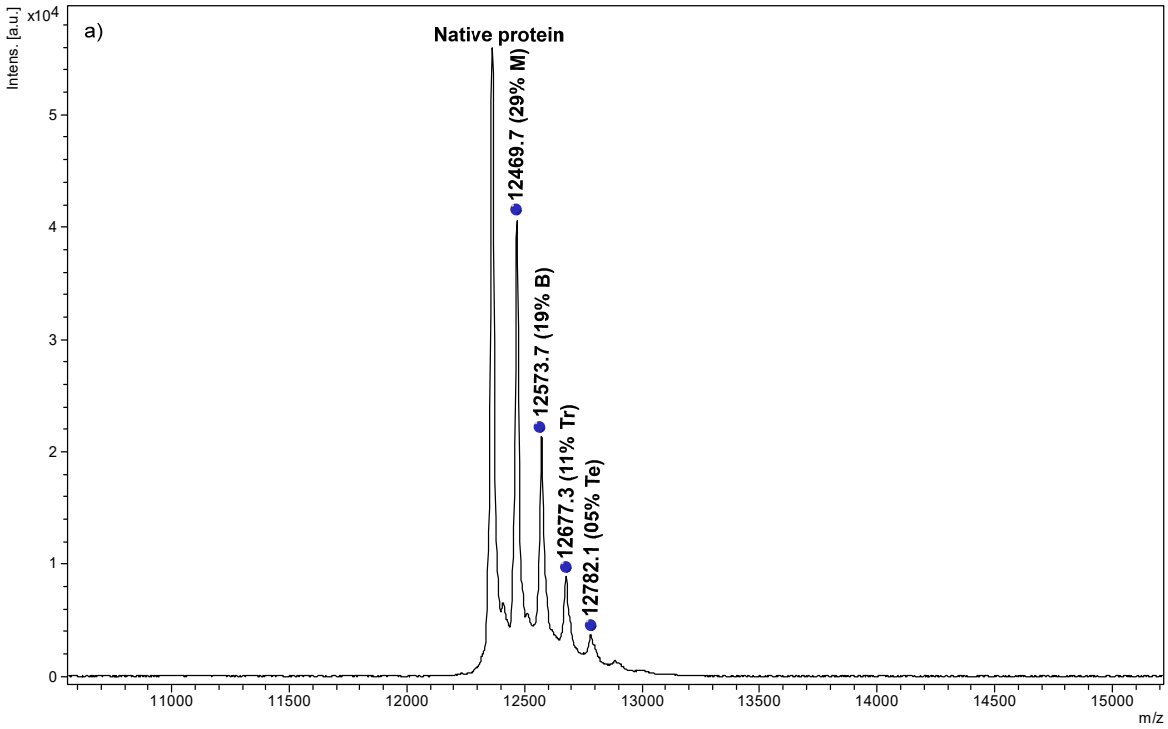
Entry	Heavy chain peptide sequence	Residues	Native	Tagged
HC1	EVQLVESGGGLVQPGGSLR	1-19	+	-
HC2	LSCAASGFNIK	20-30	+	+
HC3	DTYIHWVR	31-38	-	-
HC4	QAPGK	39-43	-	+
HC5	GLEWVAR	44-50	+	-
HC6	IYPTNGYTR	51-59	+	-
HC7	YADSVK	60-65	-	+
HC8	GR	66-67	+	+
HC9	FTISADTSK	68-76	-	-
HC10	NTAYLQMNSLR	77-87	+	+
HC11	AEDTAVYYCSR	88-98	-	-
HC12	WGGDGFYAMDYWGQGTLVTVSSASTK	99-124	-	+
HC13	GPSVFPLAPSSK	125-136	-	+
HC14	STSGGTAALGCLVK	137-150	-	+
HC15	DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVT VPSSSLGTQTYICNVNHKPSNTK	151-213	-	-
HC16	VDK	214-216	-	-
HC17	VEPK	218-221	-	-
HC18	SCDK	222-225	-	-
HC19	THTCPPCPAPELLGGPSVFLFPPKPK	226-251	-	+
HC20	DTLMISR	252-258	-	+
HC21	TPEVTCVVVDVSHEDPEVK	259-277	-	+
HC22	FNWYVDGVEVHNAK	278-291	-	+
HC23	TKPR	292-295	-	+
HC24	EEQYNSTYR	296-304	-	-
HC25	VVSVLTVLHQDWLNGK	305-320	-	+
HC26	EYK	321-323	+	+
HC27	CK	324-325	-	+
HC28	VSNK	326-329	-	+
HC29	ALPAPIEK	330-337	-	+
HC30	TISK	338-341	-	+
HC31	AK	342-343	-	+
HC32	GQPR	344-347	-	-
HC33	EPQVYTLPPSR	348-358	-	+
HC34	EEMTK	359-363	+	+
HC35	NQVSLTCLVK	364-373	+	+
HC36	GFYPSDIAVEWESNGQPENNYK	374-395	-	+
HC37	TTPPVLDSDGSFFLYSK	396-412	-	+
HC38	LTVDK	413-417	-	+
HC39	SR	418-419	-	+
HC40	WQQGNVFSCSVMHEALHNHYTQK	420-442	-	+
HC41	SLSLSPG	443-449	+	+

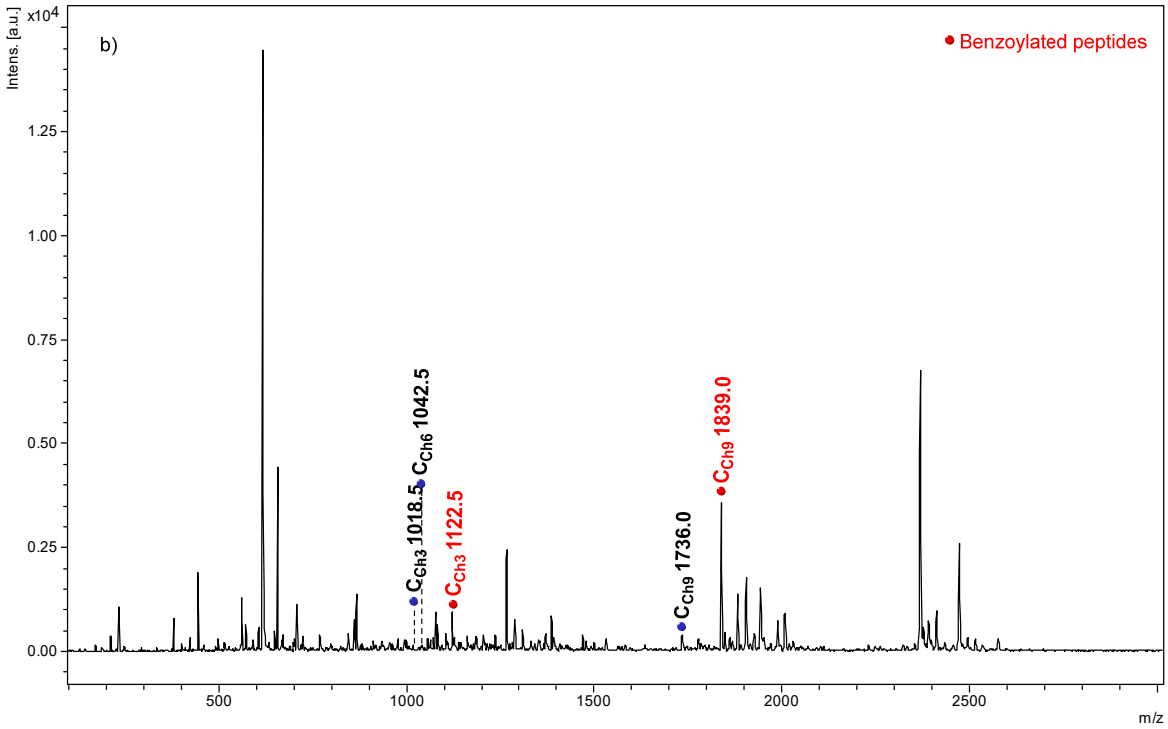
Table S4. Sequencing of light chain (LC) fragment of antibody

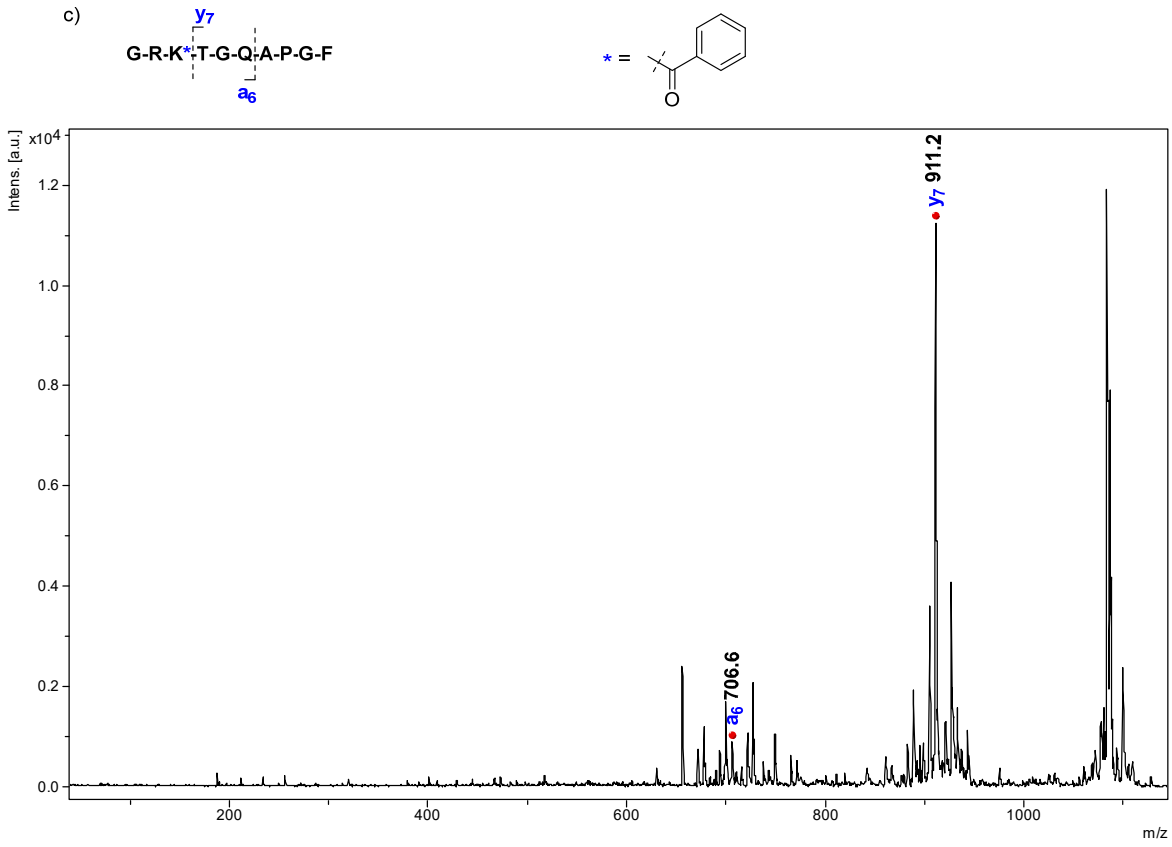
Entry	Light chain peptide sequence	Residues	Native	Tagged
LC1	DIQMTQSPSSLSASVGDR	1-18	+	+
LC2	VTITCR	19-24	-	+
LC3	ASQDVNTAVAWYQQKPGK	25-42	-	+
LC4	APK	43-45	-	-
LC5	LLIYSASFLYSGVSR	46-61	+	-
LC6	FSGSR	62-66	-	-
LC7	SGTDFTLTISSLQPEDFATY YCQQHYTTPPTFGQGTK	67-103	-	+
LC8	VEIK	104-108	-	+
LC9	TVAAPSVFIFPPSDELK	109-126	+	+
LC10	SGTASVVCLLNNFYPR	127-142	-	+
LC11	EAK	143-145	-	+
LC12	VQWK	146-149	-	+
LC13	VDNALQSGNSQESVTEQDSK	150-169	-	+
LC14	DSTYSLSSTLTLSK	170-183	-	+
LC15	ADYEK	184-188	-	+
LC16	HK	189-190	-	+
LC17	VYACEVTHQGLSSPVTK	191-207	-	+
LC18	SFNR	208-211	-	-
LC19	GEC	212-214	-	+

Table S5. Antibody - total coverage from sequencing

Entry	Heavy chain	Light chain	Total	% Coverage
Native	10/41	03/19	13/60	21%
Tagged	30/41	14/19	44/60	74%
Unmatched	3	1	48/60	80%


a)
Native protein
12469.7 (29% M)
12573.7 (19% B)
12677.3 (11% Tr)
12782.1 (05% Te)
Intens. [a.u.]
x10^4
m/z


b)
Benzoylated peptides
C Ch3 1018.5
C Ch6 1042.5
C Ch3 1122.5
C Ch9 1736.0
C Ch9 1839.0
Intens. [a.u.]
x10^4
m/z


c)
y7
G-R-K*-T-G-Q-A-P-G-F
a6
* =
O
Intens. [a.u.]
x10^4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
y7 911.2
a6 706.6
200
400
600
800
1000
m/z

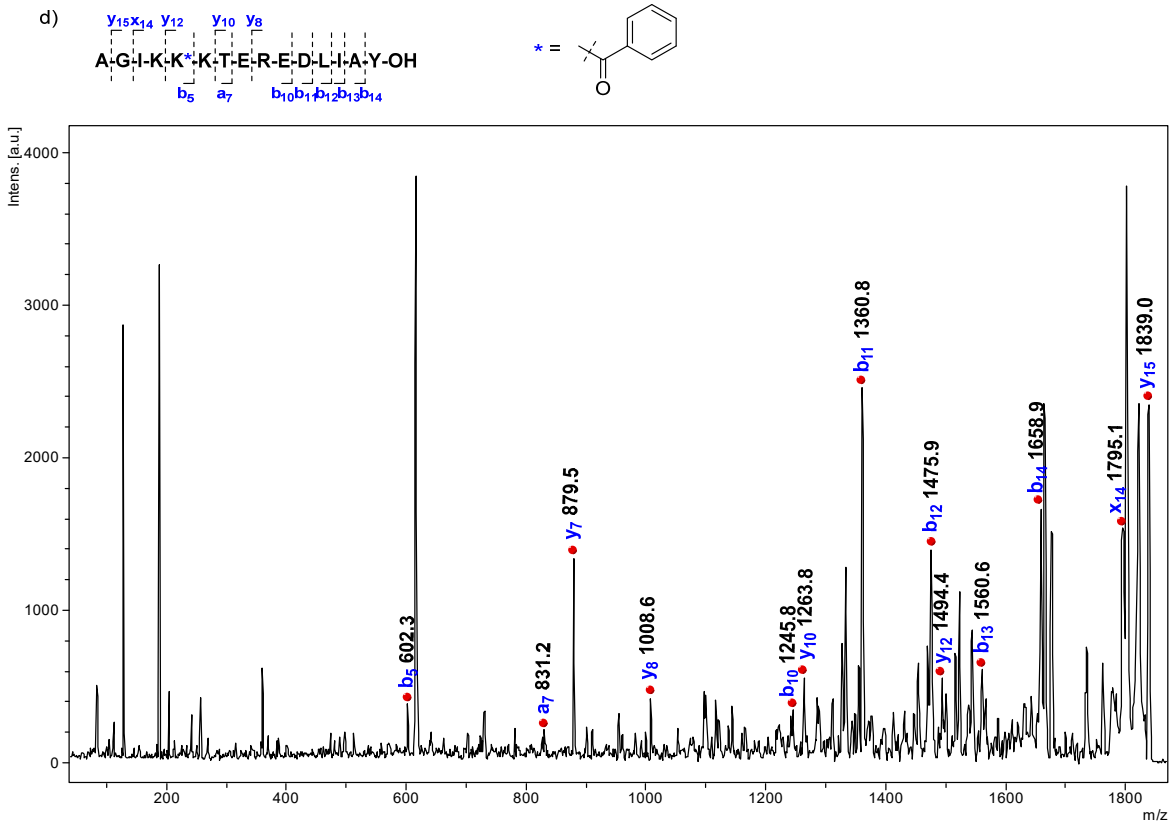
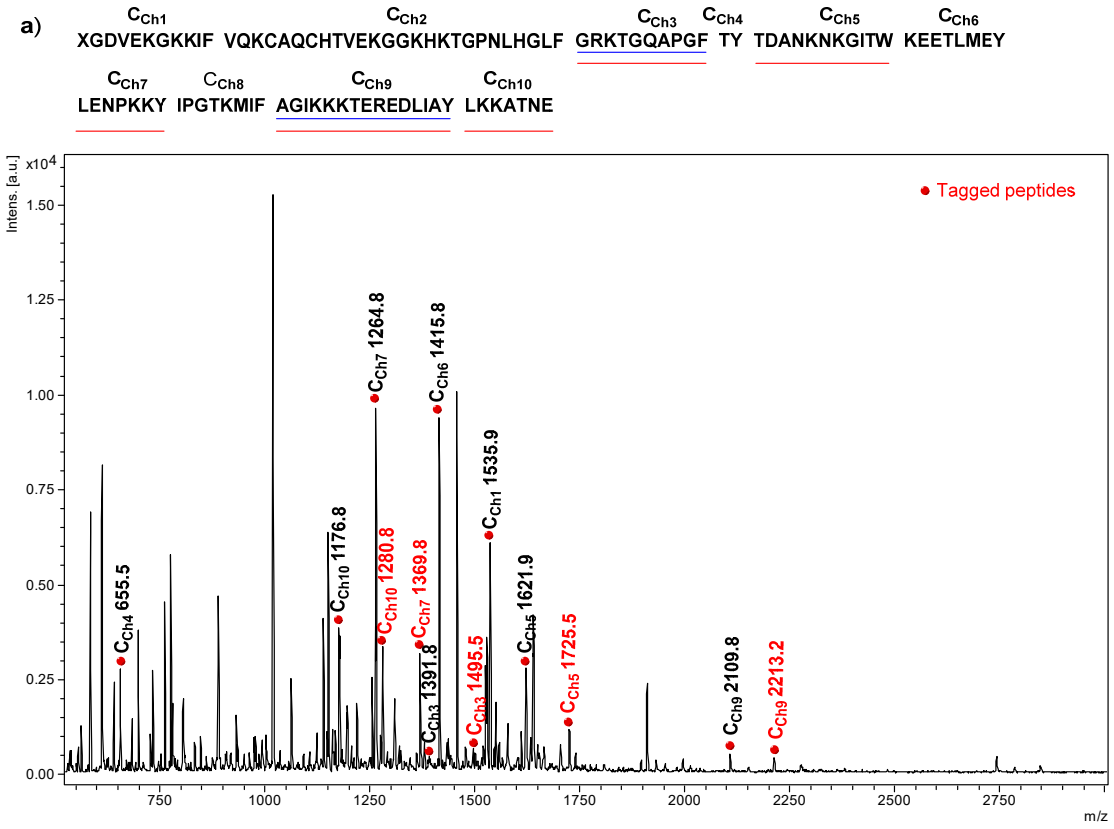
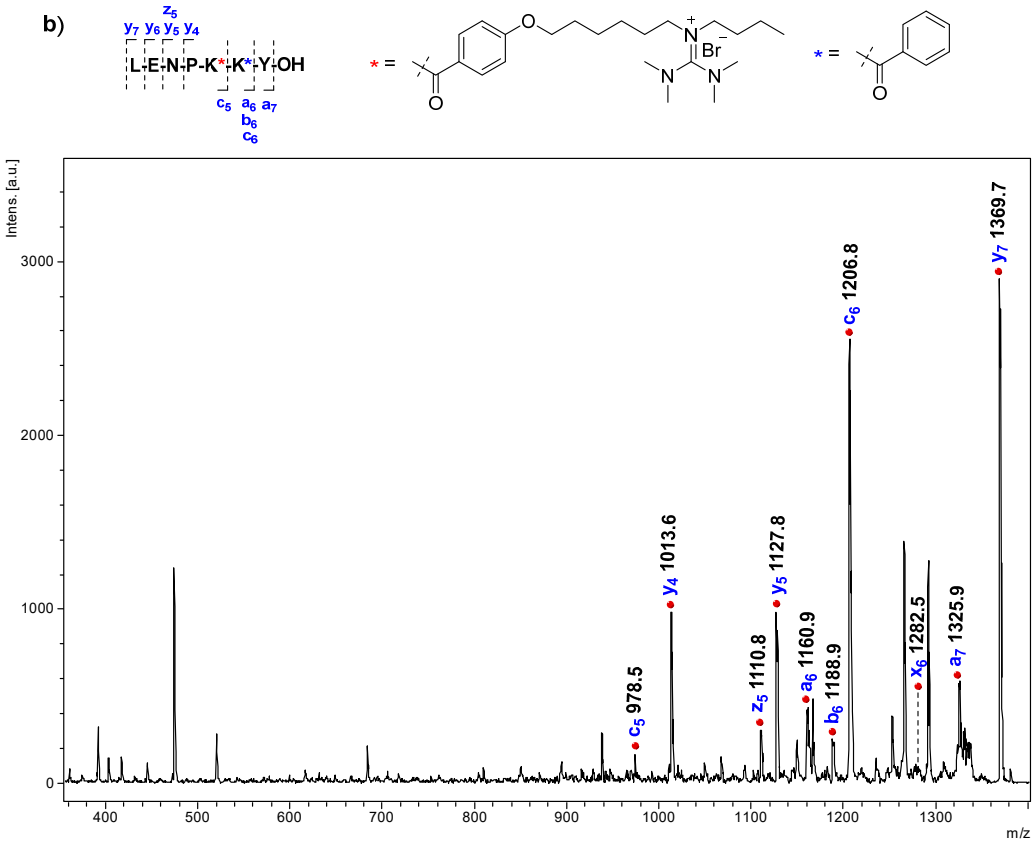
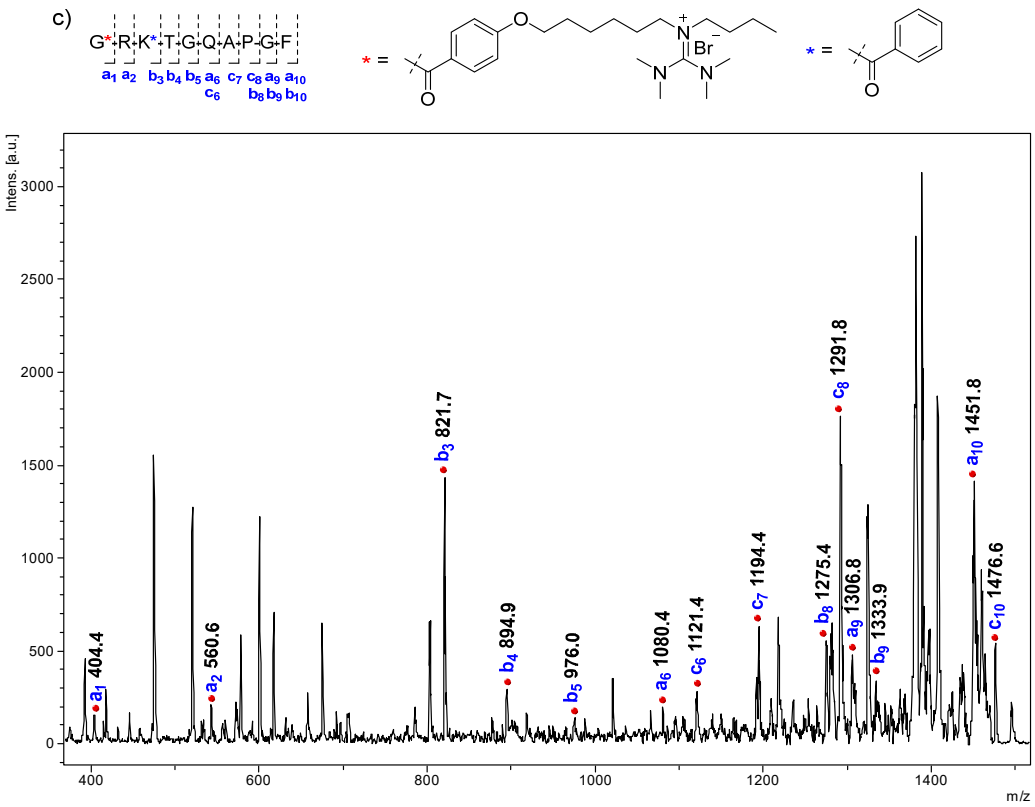

d)
y15 x14 y12 y10 y8
A-G-I-K-K*-K-T-E-R-E-D-L-I-A-Y-OH
b5 a7 b10 b11 b12 b13 b14
* =
O
Intens. [a.u.]
4000
3000
2000
1000
0
b5 602.3
a7 831.2
y7 879.5
y8 1008.6
b10 1245.8
y10 1263.8
b11 1360.8
y12 1494.4
b12 1475.9
b13 1560.6
b14 1658.9
x14 1795.1
y15 1839.0
200
400
600
800
1000
1200
1400
1600
1800
m/z

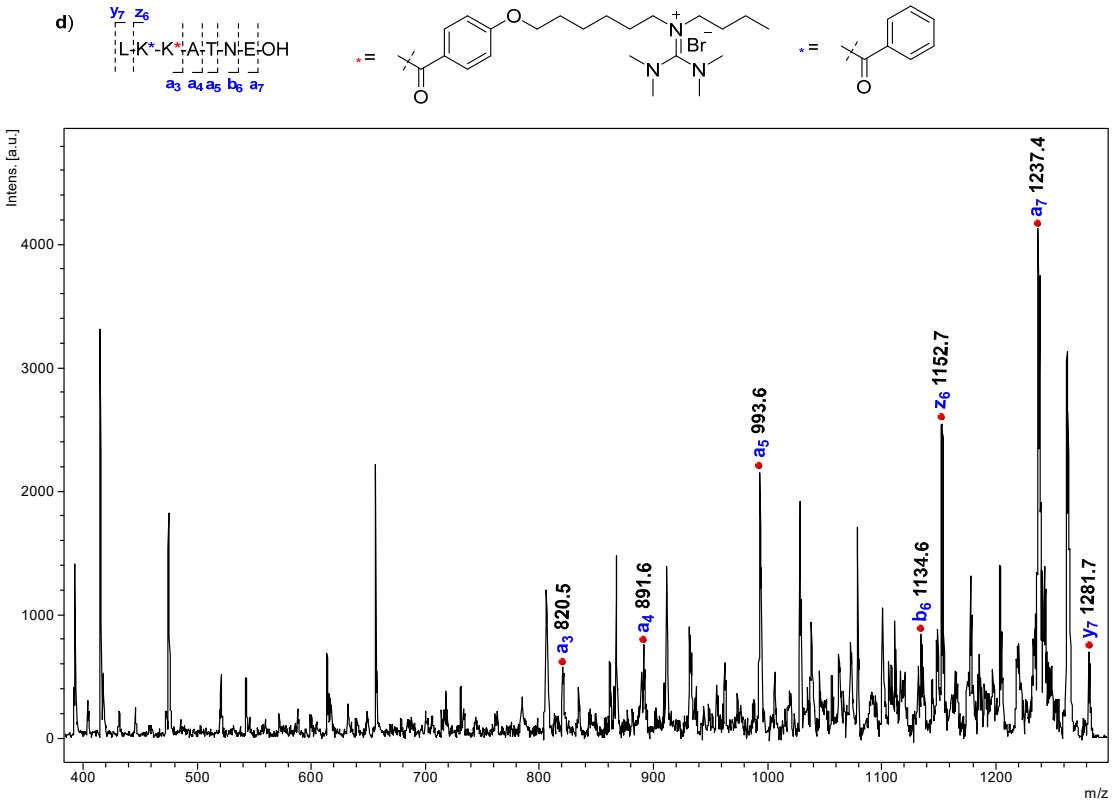
Figure S9: Heterogeneous labeling of cytochrome C **5** labeled by benzoic acid N-hydroxysuccinimide ester **7**. (a) MALDI-MS spectrum for heterogeneously labeled cytochrome C **5a**. (b) MALDI-MS spectrum of cytochrome C after the digestion of **5a** with trypsin. (c) MS-MS spectrum of labeled GRKTGQAPGF (K40, *m/z* 1125.4 $[M+H]^+$). (d) MS-MS spectrum of labeled AGIKKKTEREDLIAY (K91, *m/z* 1125.4 $[M+H]^+$).



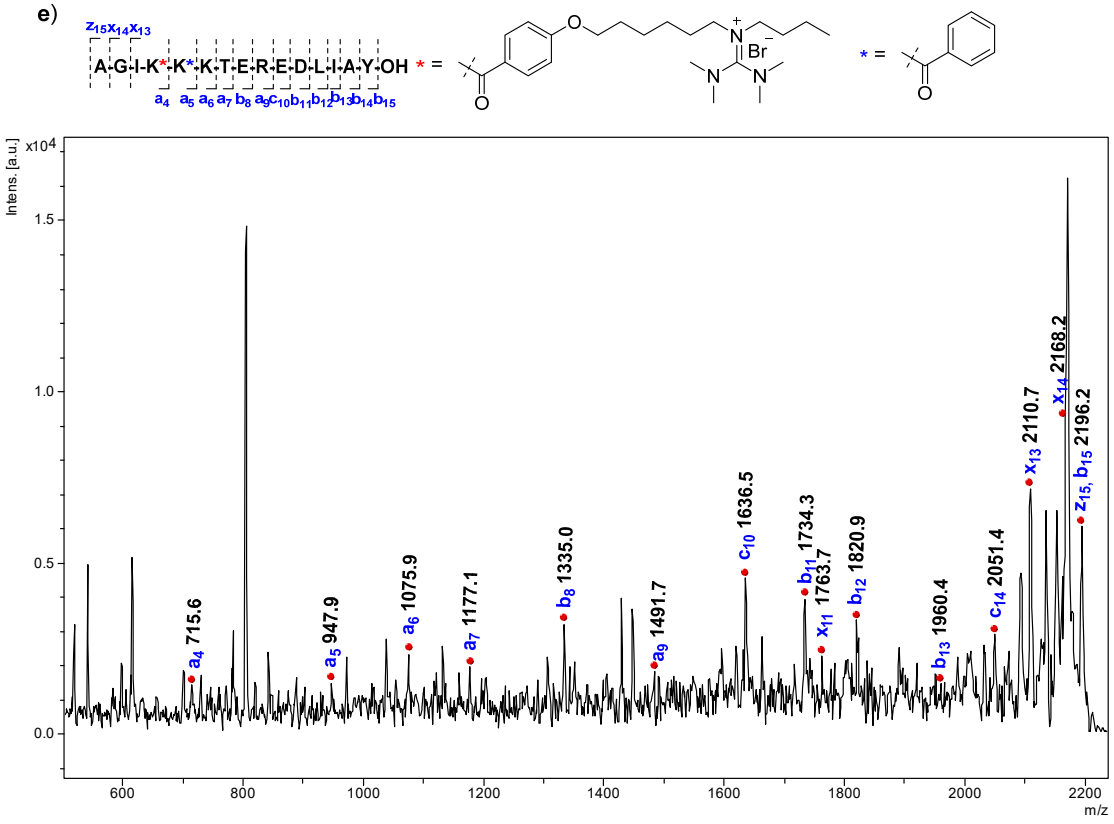

b)
L-E-N-P-K*-K*-Y-OH
* =
* =
Intens. [a.u.]
m/z
c5 978.5
y4 1013.6
z5 1110.8
y5 1127.8
a6 1160.9
b6 1188.9
x6 1282.5
a7 1325.9
c6 1206.8
y7 1369.7


c)
G*-R-K*-T-G-Q-A-P-G-F
* =
* =
Intens. [a.u.]
m/z
a1 404.4
a2 560.6
b3 821.7
b4 894.9
b5 976.0
a6 1080.4
c6 1121.4
c7 1194.4
b8 1275.4
c8 1291.8
a9 1306.8
b9 1333.9
a10 1451.8
c10 1476.6

d)


y7 z6
L-K*-K*-A-T-N-E-OH
a3 a4 a5 b6 a7
* =
Br
* =
Intens. [a.u.]
a7 1237.4
a5 993.6
z6 1152.7
b6 1134.6
a3 820.5
a4 891.6
y7 1281.7
m/z

e)


z15x14x13
A-G-I-K*-K*-K-T-E-R-E-D-L-I-A-Y-OH * =
a4 a5 a6 a7 b8 a9c10b11b12b13b14b15
Br
* =
Intens. [a.u.]
x10^4
a4 715.6
a5 947.9
a6 1075.9
a7 1177.1
b8 1335.0
a9 1491.7
c10 1636.5
b11 1734.3
x11 1763.7
b12 1820.9
b13 1960.4
c14 2051.4
x13 2110.7
x14 2168.2
z15, b15 2196.2
m/z

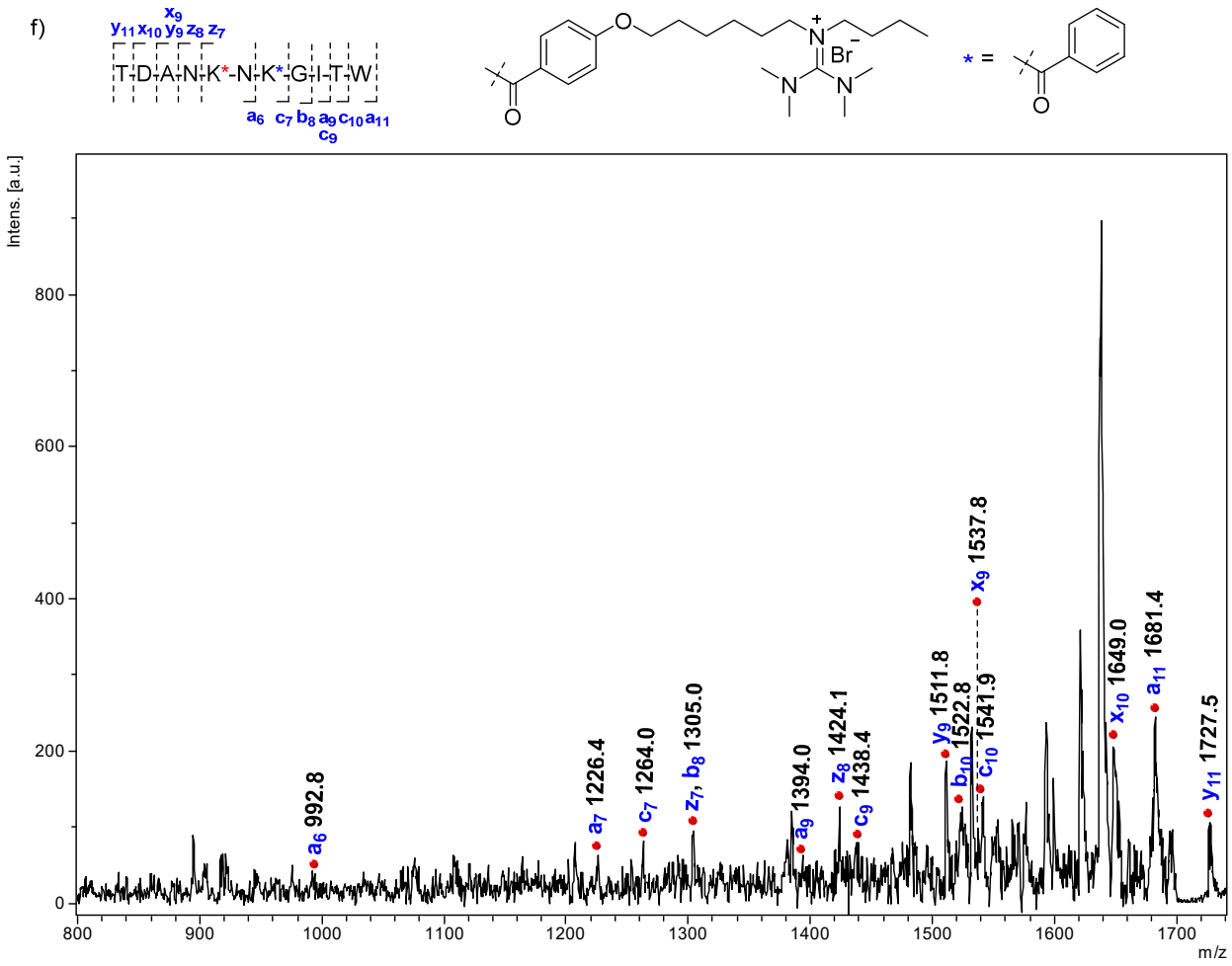
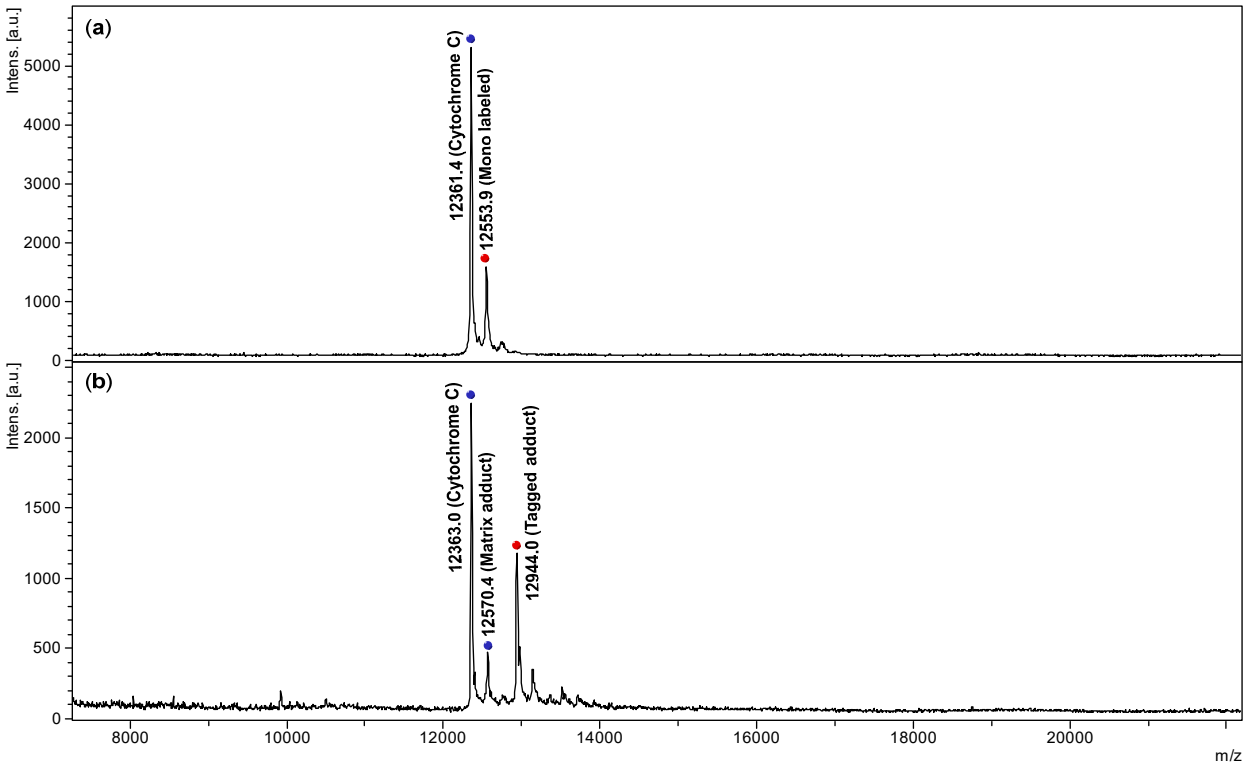
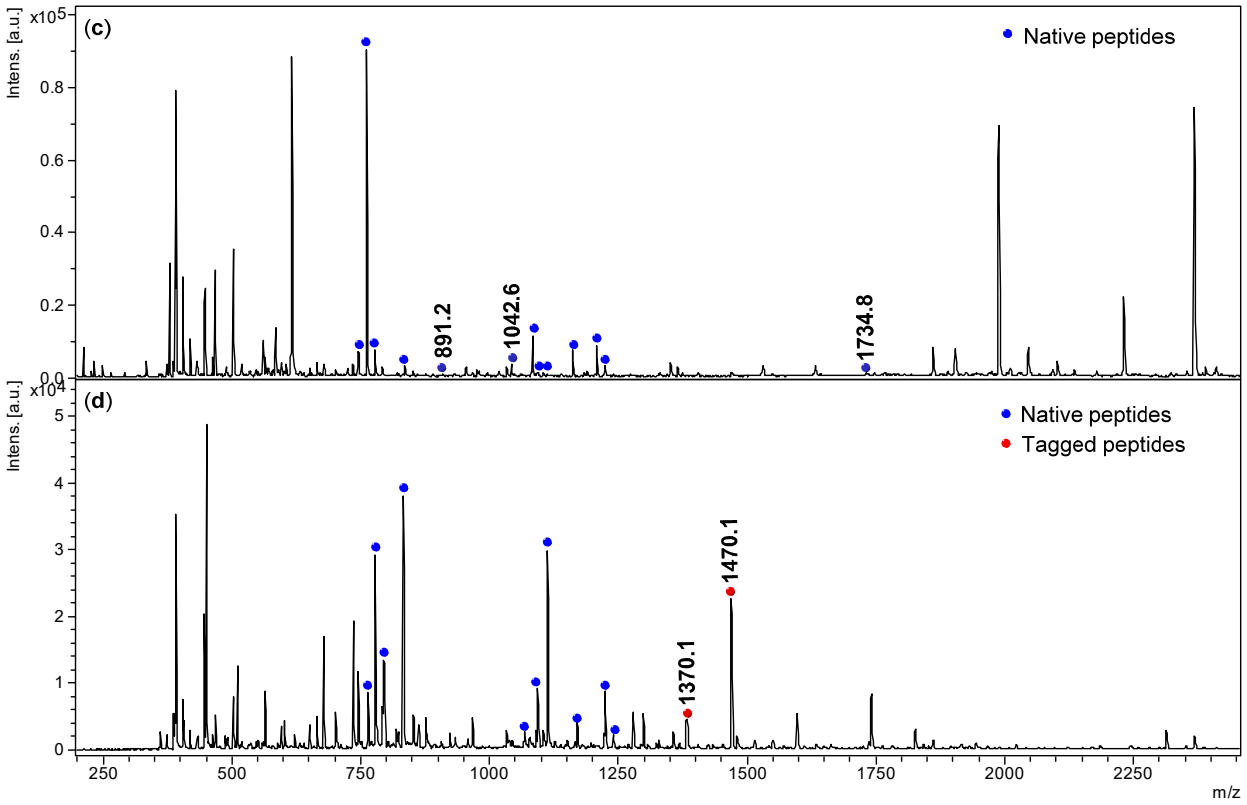
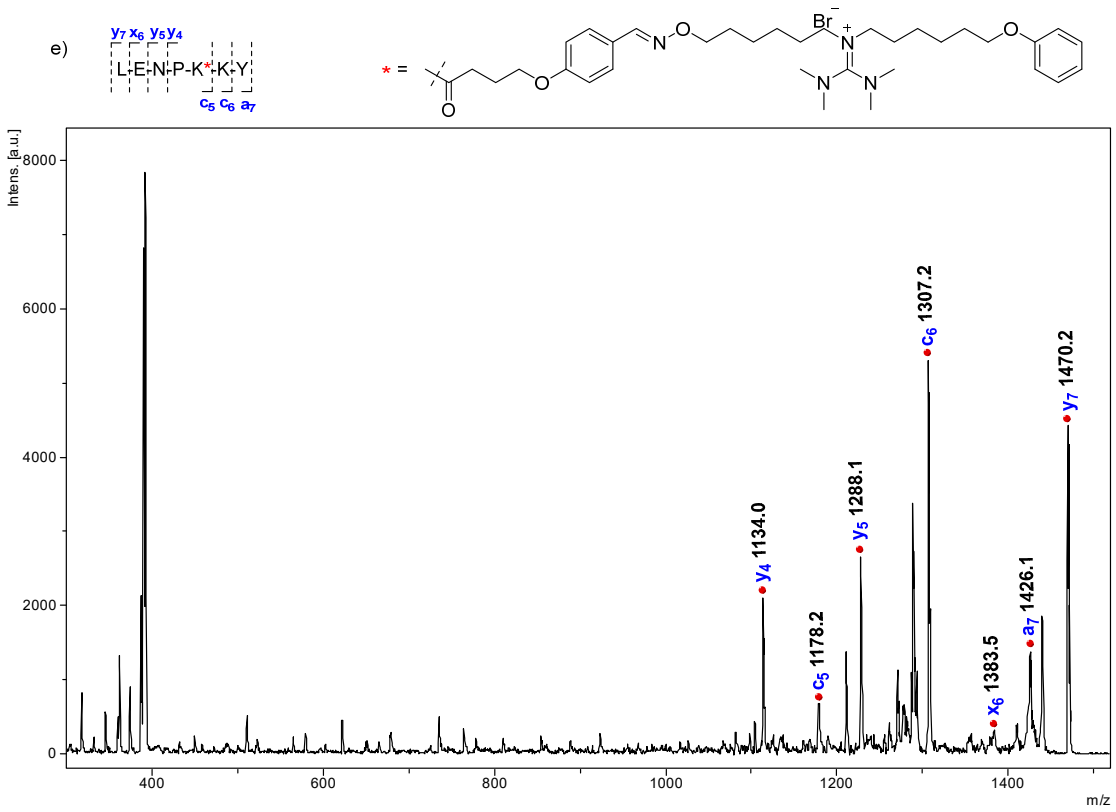


Figure S10: Labeling of cytochrome C **5a** digest with the sensitivity booster **3**. (a) MALDI-MS spectrum of tagged cytochrome C **5a** digest. (b) MS-MS spectrum of tagged peptide LENKKY (K72, m/z 1369.7 $[M]^+$). (c) MS-MS spectrum of labeled GRKTGQAPGF (K40, *m/z* 1491.7 $[M]^+$). (d) MS-MS spectrum of tagged peptide LKKATNE (K99, m/z 1281.7 $[M]^+$). (e) MS-MS spectrum of labeled AGIKKKTEREDLIAY (K87, *m/z* 2196.2 $[M]^+$). (f) MS-MS spectrum of labeled TDANKNKGITW (K54, *m/z* 1727.5 $[M]^+$).


(a)
Intens. [a.u.]
12361.4 (Cytochrome C)
12553.9 (Mono labeled)
(b)
Intens. [a.u.]
12363.0 (Cytochrome C)
12570.4 (Matrix adduct)
12944.0 (Tagged adduct)
m/z


(c)
Intens. [a.u.]
Native peptides
891.2
1042.6
1734.8
(d)
Intens. [a.u.]
Native peptides
Tagged peptides
1370.1
1470.1
m/z


e)
y7 x6 y5 y4
L-E-N-P-K*-K-Y
c5 c6 a7
* =
Br
Intens. [a.u.]
y4 1134.0
c5 1178.2
y5 1288.1
c6 1307.2
x6 1383.5
a7 1426.1
y7 1470.2
m/z

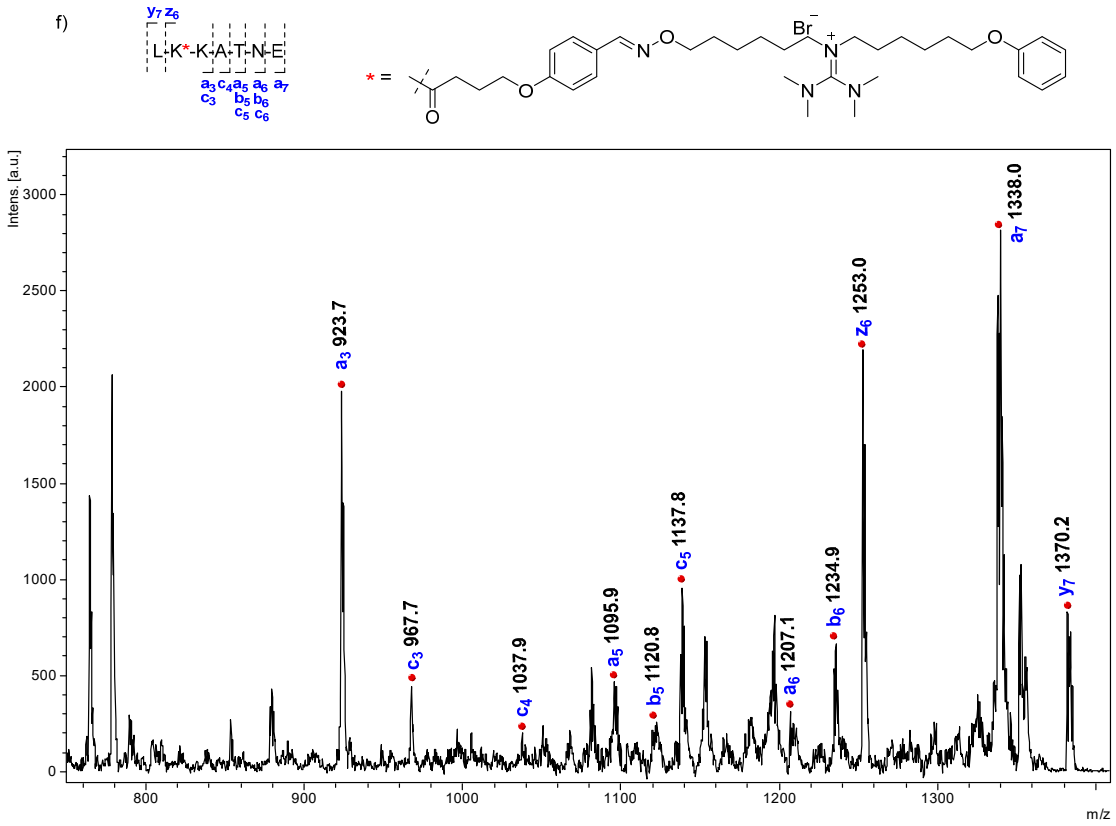
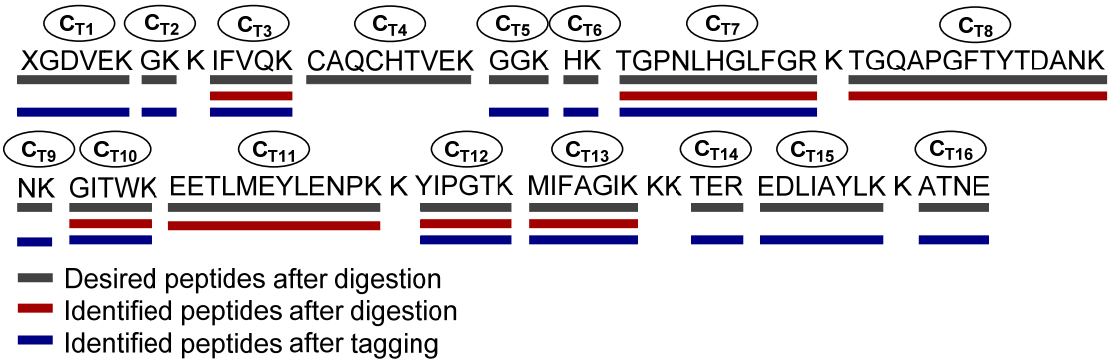
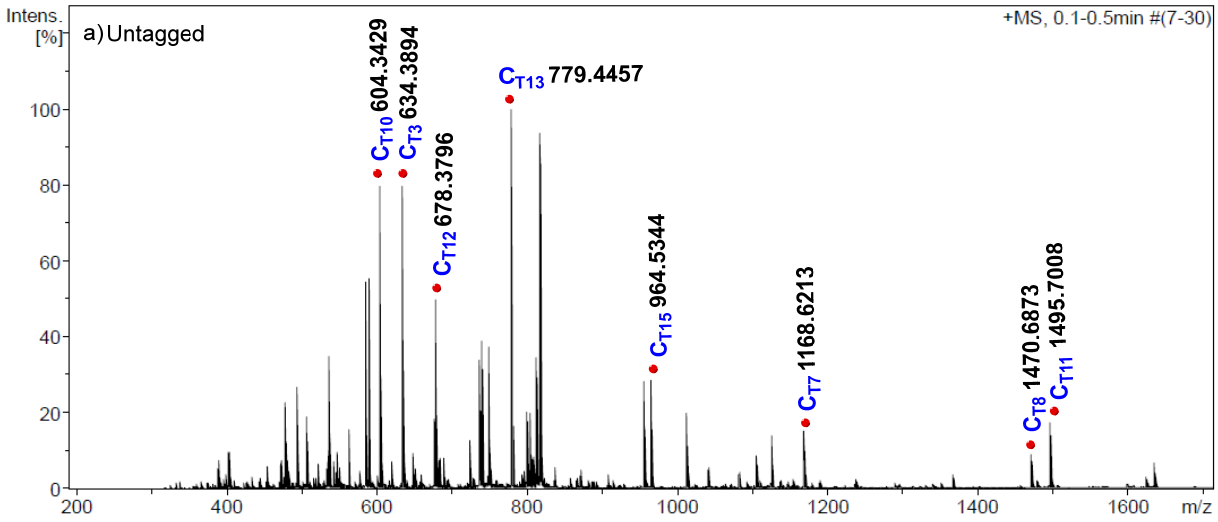

f)
y7 z6
L-K*-K-A-T-N-E
a3 c4 a5 a6 a7
c3 b5 b6
c5 c6
* =
Br
Intens. [a.u.]
a3 923.7
c3 967.7
c4 1037.9
a5 1095.9
b5 1120.8
c5 1137.8
a6 1207.1
b6 1234.9
z6 1253.0
a7 1338.0
y7 1370.2
m/z

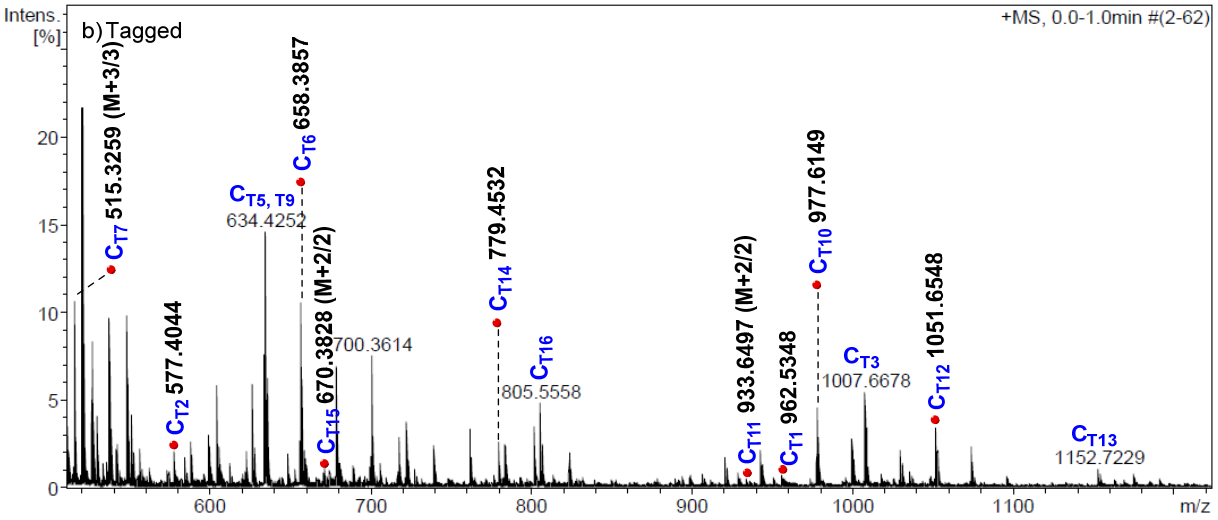
Figure S11: Single-site labeling of cytochrome C **5** enabled by aldehyde functionalised NHS ester **8**. (**a**) MALDI-MS spectrum for 20% mono-labeled cytochrome C **5c**. (**b**) MALDI-MS spectrum for 20% mono-labeled cytochrome C **5d** with alkoxyamine tag. (**c**) MALDI-MS spectrum of cytochrome C after the digestion of **5c** with trypsin. (**d**) MALDI-MS spectrum of tagged adduct of cytochrome C **5d** after the digestion with trypsin. (**e**) MALDI MS-MS spectrum for the tagged peptide **LENPKKY** (**K72**, *m/z* 1470.2 $[M]^+$). (**f**) MALDI MS-MS spectrum for the tagged peptide **LKKATNE** (**K99**, *m/z* 1370.2 $[M]^+$).

Table S6. Experimental conditions for LC-ESI-MS (Scheme 1, data in Figure S12)

Detection wavelength	214 nm
Temperature	25 °C
Mobile phase A	0.1% formic acid in 100% water
Mobile phase B	0.1% formic acid in 100% ACN
Flow rate	0.2 mL/min
Sample	Untagged and tagged tryptic digest of cytochrome C
Capillary	4500 V
Collision Cell RF	600.0 Vpp


CT1 CT2 CT3 CT4 CT5 CT6 CT7 CT8
XGDVEK GK K IFVQK CAQCHTVEK GGK HK TGPNLHGLFGR K TGQAPGFTYTDANK
CT9 CT10 CT11 CT12 CT13 CT14 CT15 CT16
NK GITWK EETLMEYLENPK K YIPGTK MIFAGIK KK TER EDLIAYLK K ATNE
Desired peptides after digestion
Identified peptides after digestion
Identified peptides after tagging


Intens. [%]
a) Untagged
+MS, 0.1-0.5min #(7-30)
CT10 604.3429
CT3 634.3894
CT12 678.3796
CT13 779.4457
CT15 964.5344
CT7 1168.6213
CT8 1470.6873
CT11 1495.7008
m/z


Intens. [%]
b) Tagged
+MS, 0.0-1.0min #(2-62)
CT7 515.3259 (M+3/3)
CT6 658.3857
CT5, T9 634.4252
CT2 577.4044
CT15 670.3828 (M+2/2)
700.3614
CT14 779.4532
CT16 805.5558
CT11 933.6497 (M+2/2)
CT1 962.5348
CT10 977.6149
CT3 1007.6678
CT12 1051.6548
CT13 1152.7229
m/z

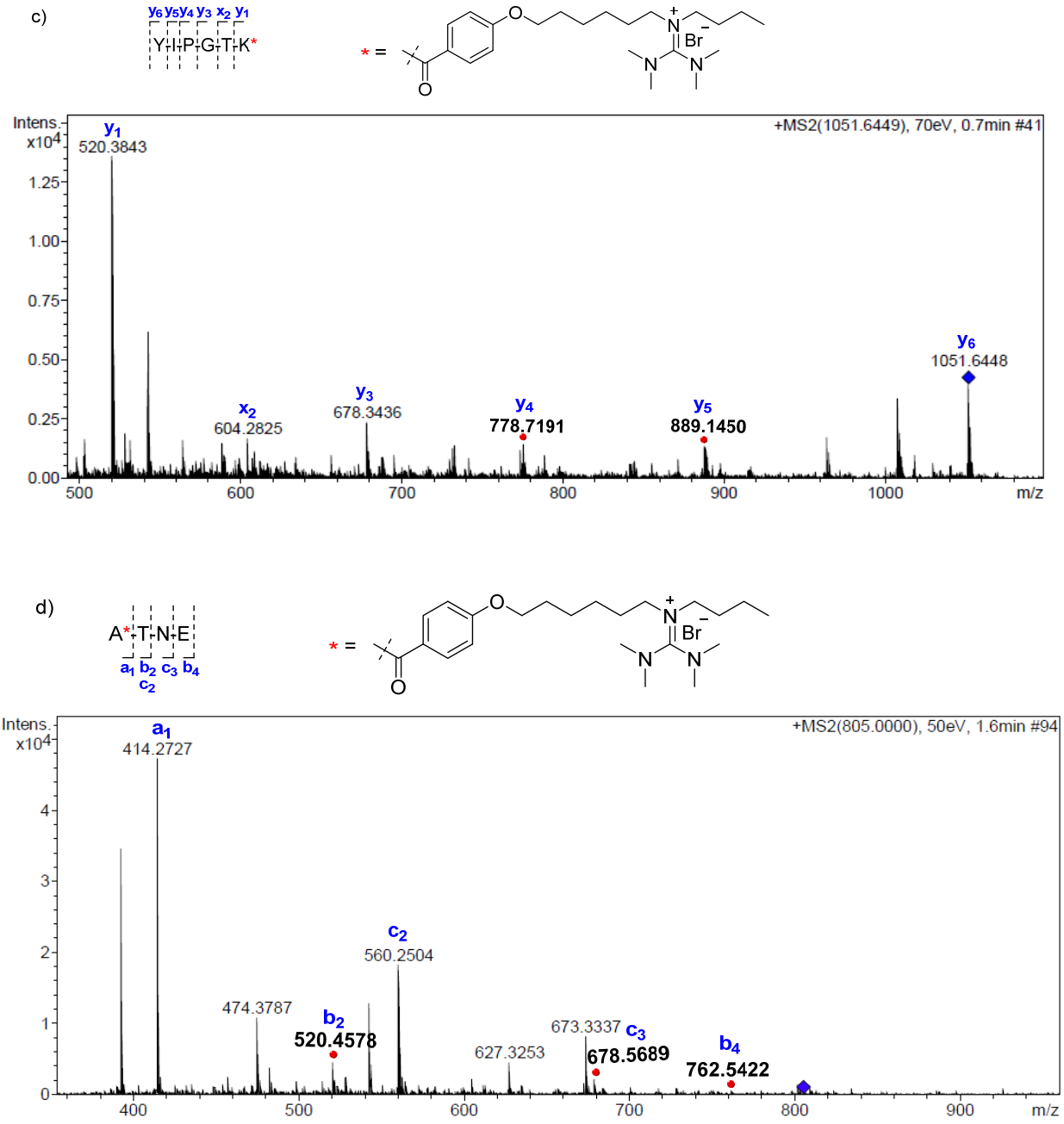


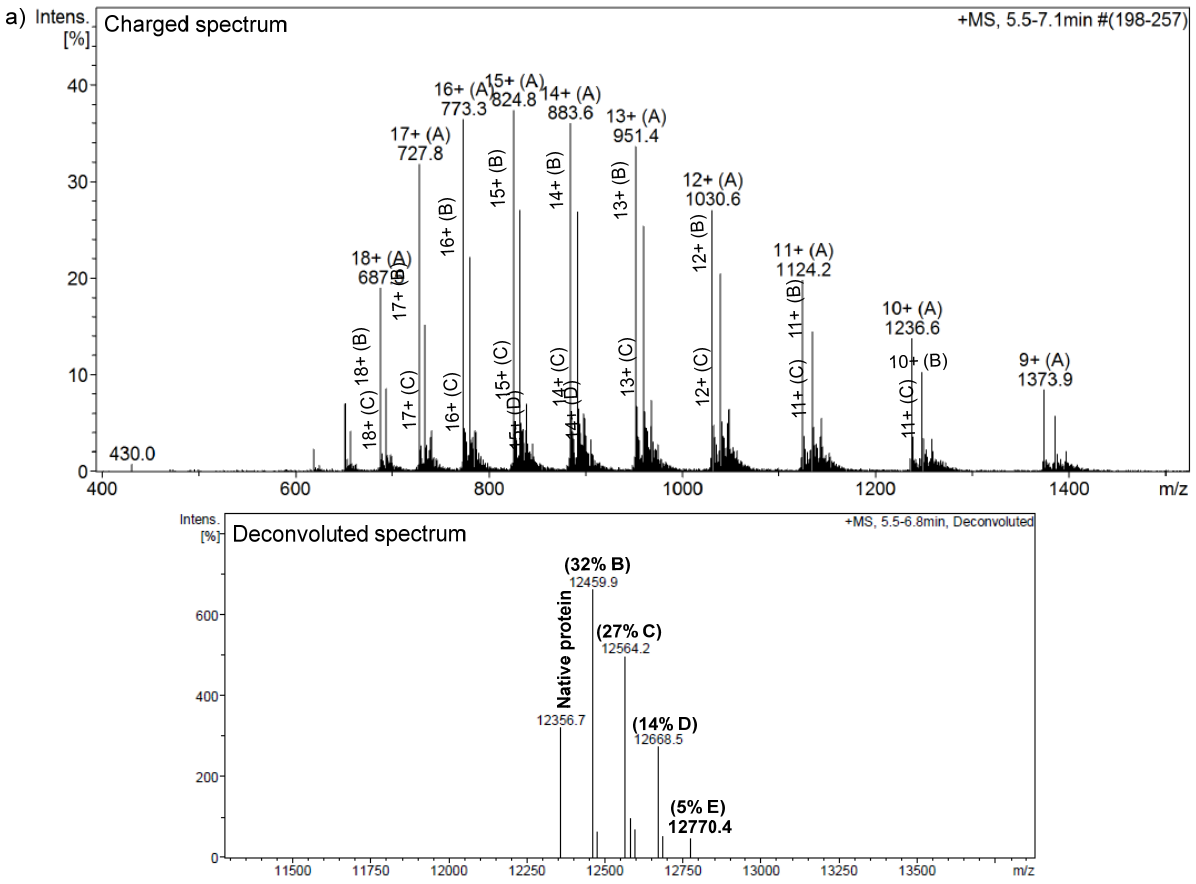
Figure S12. Analyzing and sequencing the cytochrome C (a) ESI-MS spectrum for untagged digest of cytochrome C. (b) ESI-MS spectrum for the sensitivity booster **3** tagged digest of cytochrome C. (c) ESI-MS-MS of YIPGTK-Tag (C_{T12}, residues 74-79, m/z 1051.7, $[M]^+$). (d) ESI-MS-MS of Tag-ATNE (C_{T16}, residues 101-104, m/z 805.0, $[M]^+$).

Table S7: Experimental conditions for LC-ESI-MS (Scheme 3a, data in Figure S13)

Column	AdvanceBio Peptide Map, 2.7 μm, 4.6 × 150 mm (from Agilent)
Detection wavelength	215-254 nm
Temperature	25 °C
Mobile phase A	0.01% formic acid in 100% water
Mobile phase B	0.01% formic acid in 100% ACN
Flow rate	0.3 ml/min
Sample	Tagged tetra-labeled digest of cytochrome C
Capillary Voltage	4000 V

Table S8: Gradient detail

Time	Flow (ml/min)	%A	%B
0	0.3	100	0
5	0.3	100	0
10	0.3	85	15
40	0.3	64	36
50	0.3	40	60
60	0.3	100	0



C_{Ch1} C_{Ch2} C_{Ch3} C_{Ch4} C_{Ch5} C_{Ch6}

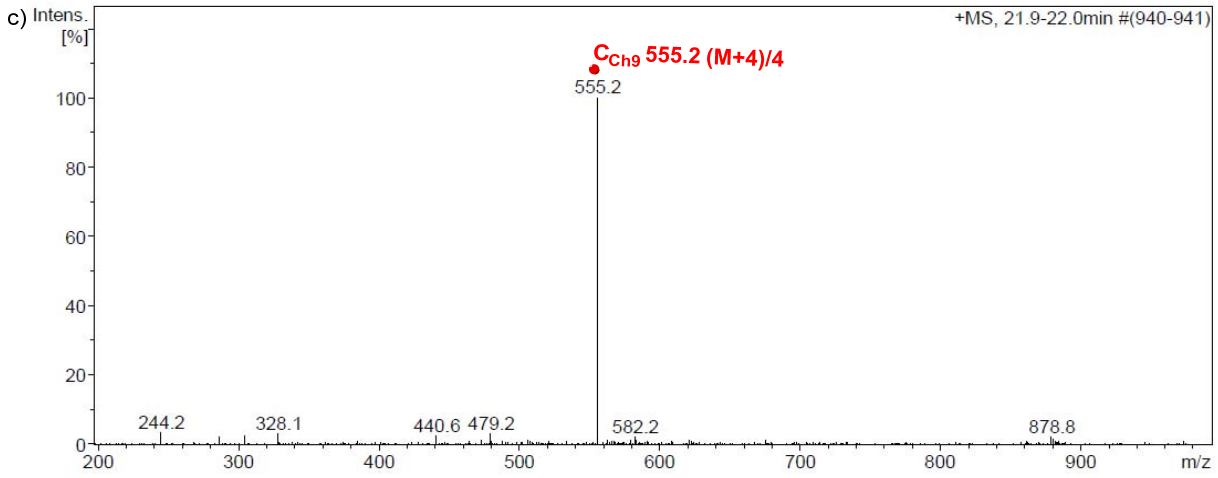
XGDVEKGKKIF VQKCAQCHTVEKGGKHKTGPNLHGLF GRKTGQAPGF TY TDANKNKGITW KEETLMEY

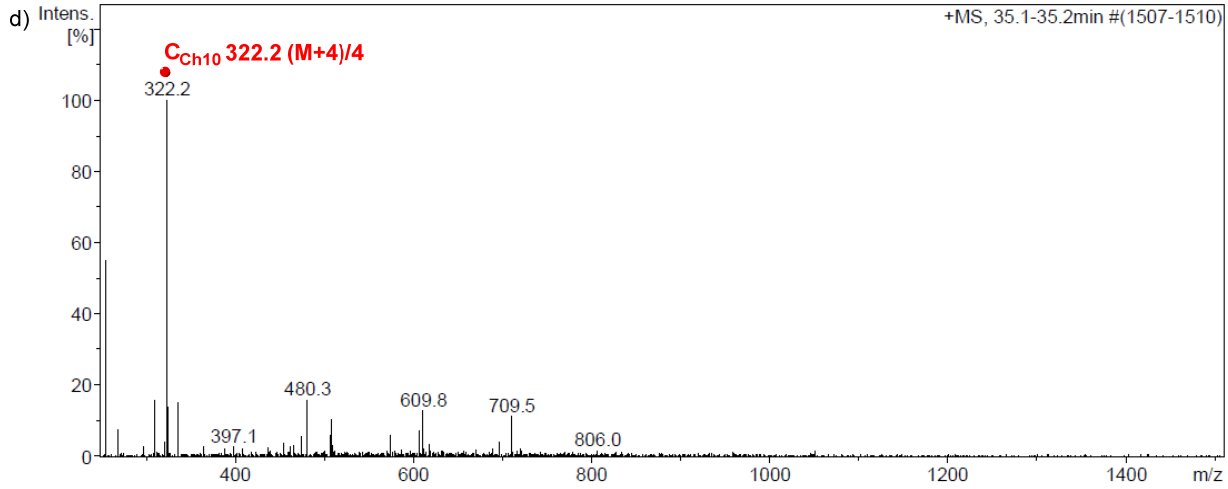
C_{Ch7} C_{Ch8} C_{Ch9} C_{Ch10}

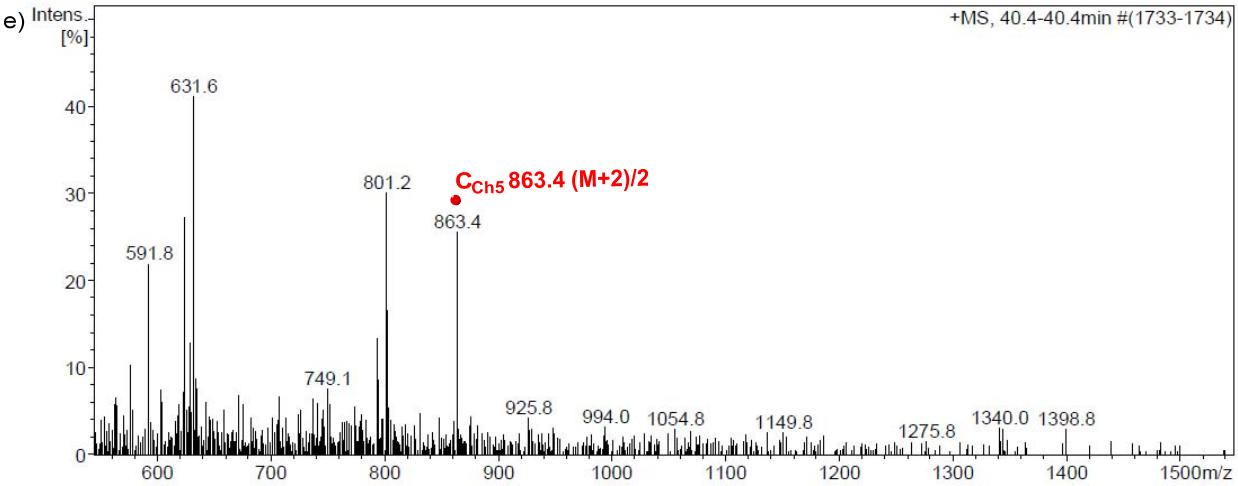
LENPKKY IPGTKMIF AGIKKKTEREDLIAY LKKATNE

Labeled peptides observed by MALDI-MS

Labeled peptides observed by ESI-MS


c) Intens.
[%]
+MS, 21.9-22.0min #(940-941)
C_{Ch9} 555.2 (M+4)/4
555.2
244.2
328.1
440.6
479.2
582.2
878.8
m/z


d) Intens.
[%]
+MS, 35.1-35.2min #(1507-1510)
C_{Ch10} 322.2 (M+4)/4
322.2
397.1
480.3
609.8
709.5
806.0
m/z


e) Intens.
[%]
+MS, 40.4-40.4min #(1733-1734)
631.6
801.2
C_{Ch5} 863.4 (M+2)/2
863.4
591.8
749.1
925.8
994.0
1054.8
1149.8
1275.8
1340.0
1398.8
m/z

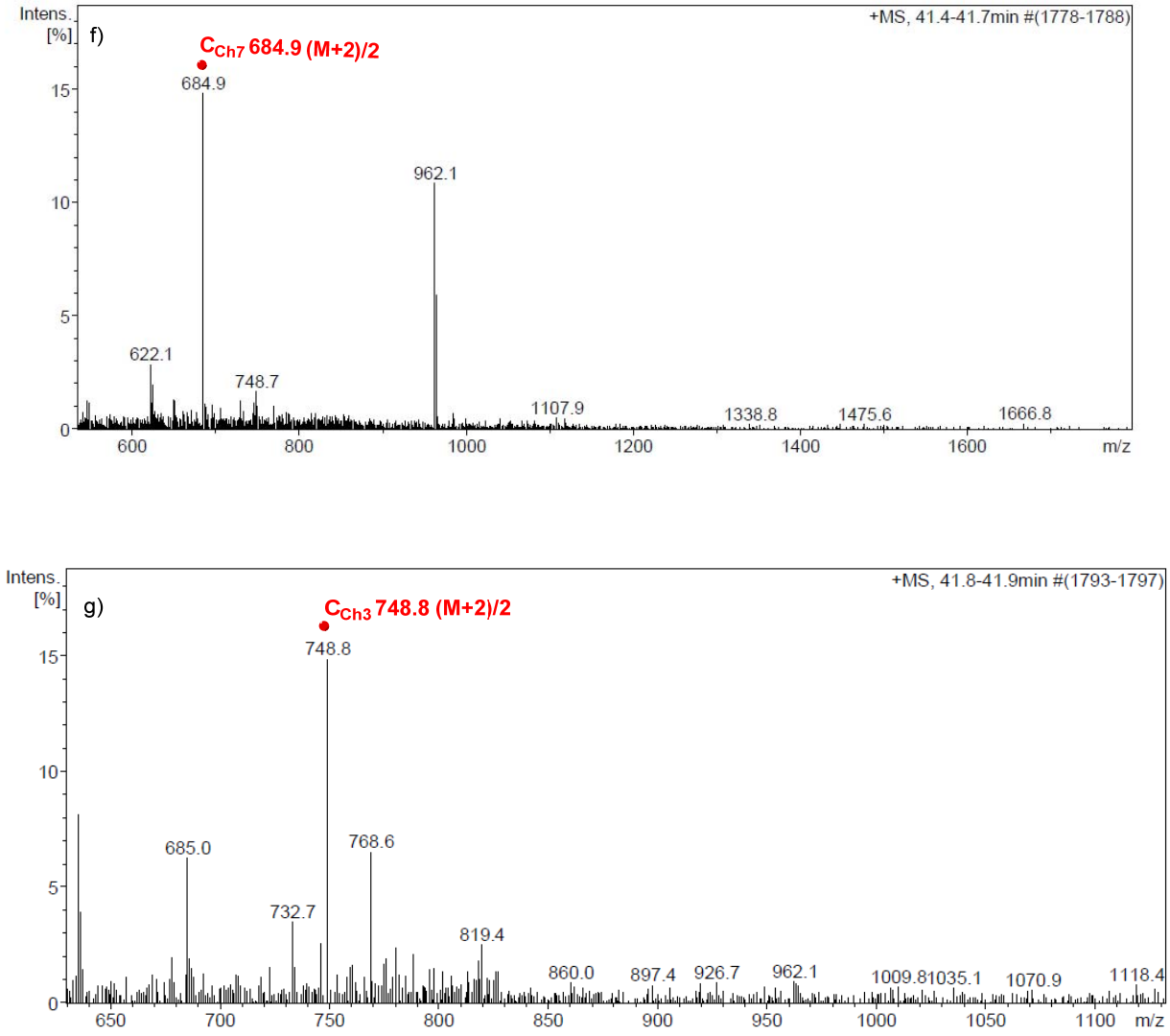
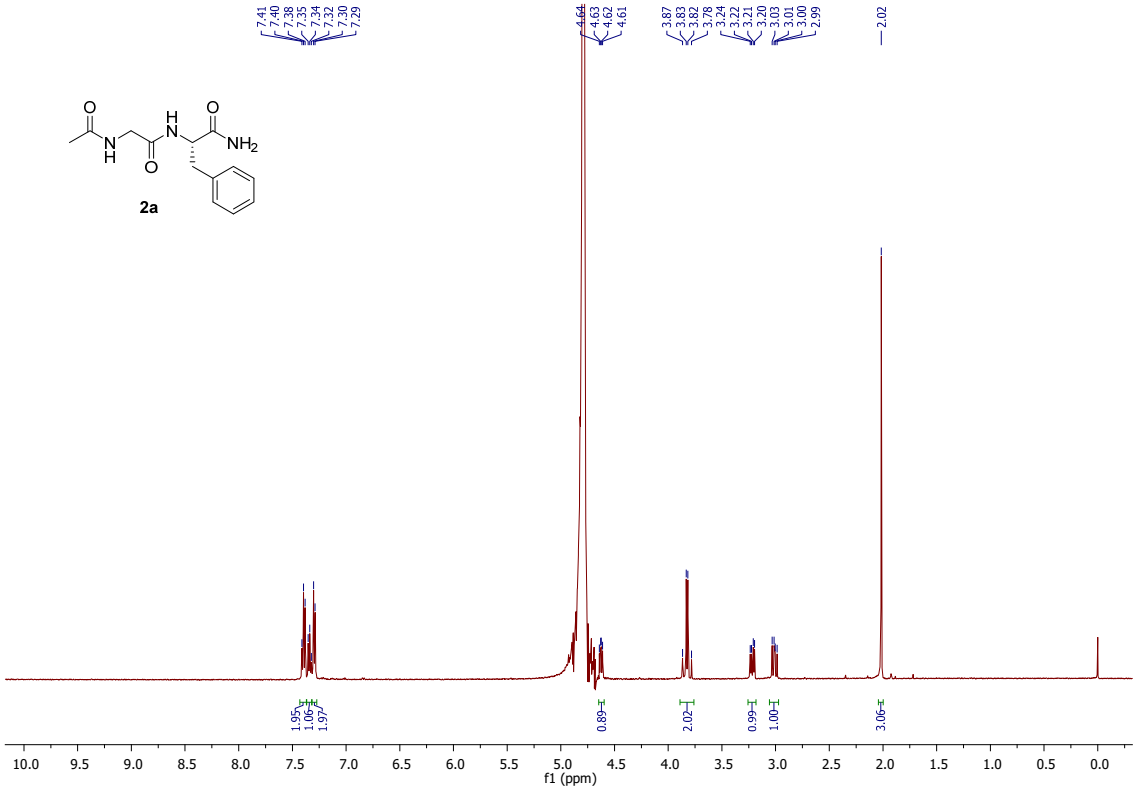
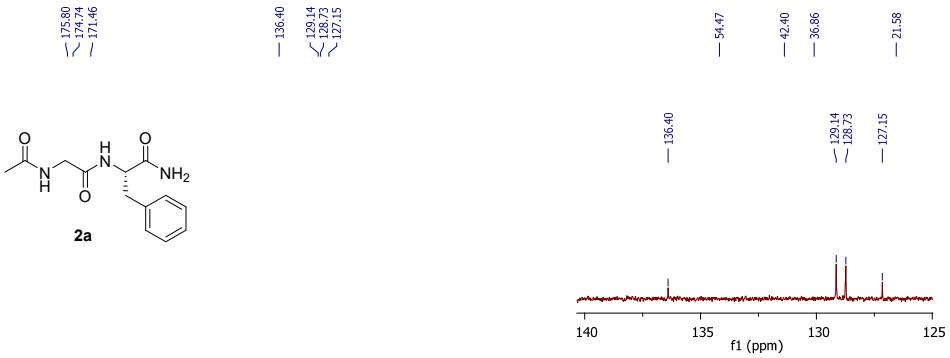


Figure S13. Analyzing and sequencing the cytochrome C (a) ESI-MS charged spectrum and deconvoluted spectrum of tetra-labeled cytochrome C in Scheme 3a; refer to Supplementary Figure S13 for MALDI data. (b) TIC spectrum of tagged tetra-labeled cytochrome C. (c) ESI-MS spectrum of AGIKKKTEREDLIAY (C_{ch9}, residues 83-97, m/z 555.2, [(M+4)/4]. (d) ESI-MS spectrum of LKKATNE (C_{ch10}, residues 98-104, m/z 322.2, [(M+4)/4]. (e) ESI-MS spectrum of TDANKNKGITW (C_{ch5}, residues 49-59, m/z 863.6, [(M+2)/2]. (f) ESI-MS spectrum of LENPKKY (C_{ch7}, residues 68-74, m/z 684.9, [(M+2)/2]. (g) ESI-MS spectrum of GRKTGQAPGF (C_{ch3}, residues 37-46, m/z 748.8, [(M+2)/2].

5.2 NMR spectra

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171.46
136.40
129.14
128.73
127.15
54.47
42.40
36.86
21.58
2a
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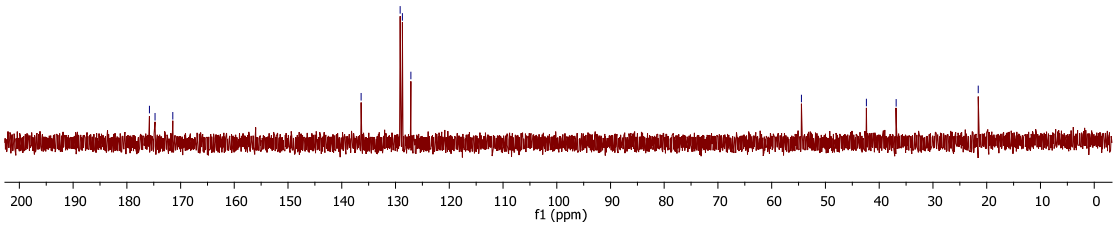
128.73
127.15
140
135
130
125
f1 (ppm)


200
190
180
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160
150
140
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120
110
100
90
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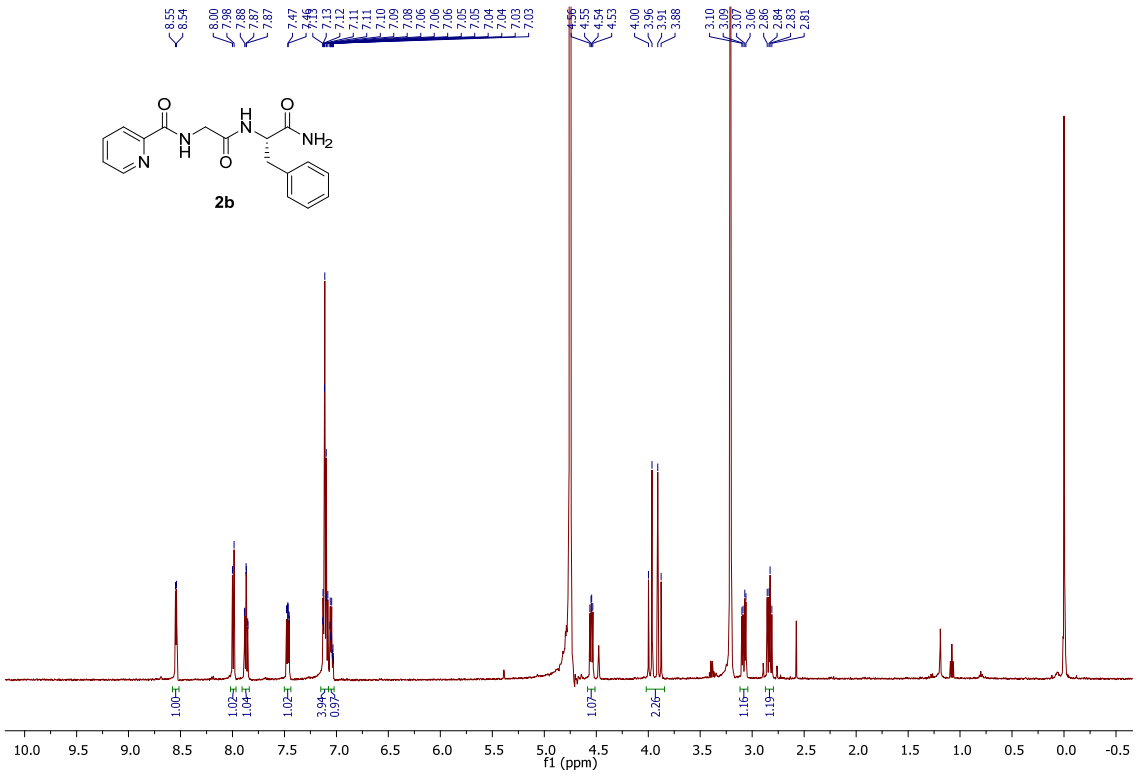
60
50
40
30
20
10
0
f1 (ppm)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7.87
7.87
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7.46
7.13
7.12
7.11
7.11
7.10
7.09
7.08
7.06
7.06
7.06
7.05
7.05
7.04
7.04
7.03
7.03
4.56
4.55
4.54
4.53
4.00
3.96
3.91
3.88
3.10
3.09
3.07
3.06
2.86
2.84
2.83
2.81
2b
1.00
1.02
1.04
1.02
3.94
0.97
1.07
2.26
1.16
1.19
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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4.0
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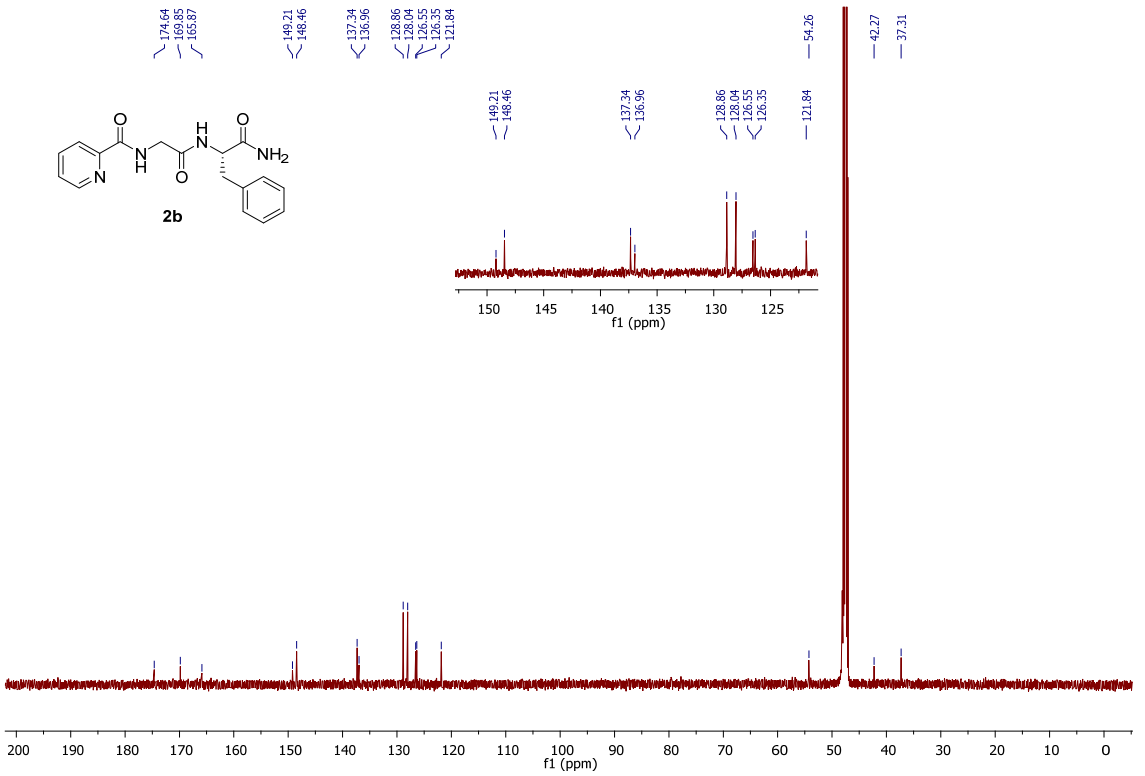
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)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128.86
128.04
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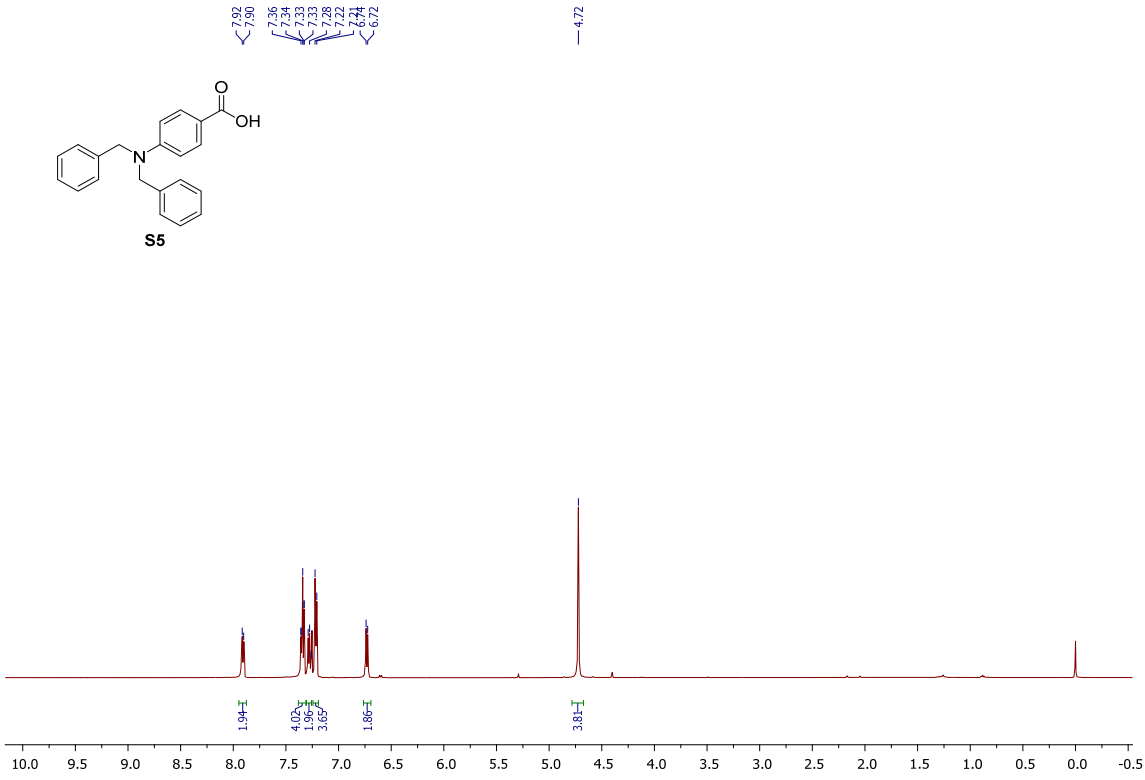
121.84
54.26
42.27
37.31
2b
f1 (ppm)
f1 (ppm)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7.22
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4.72
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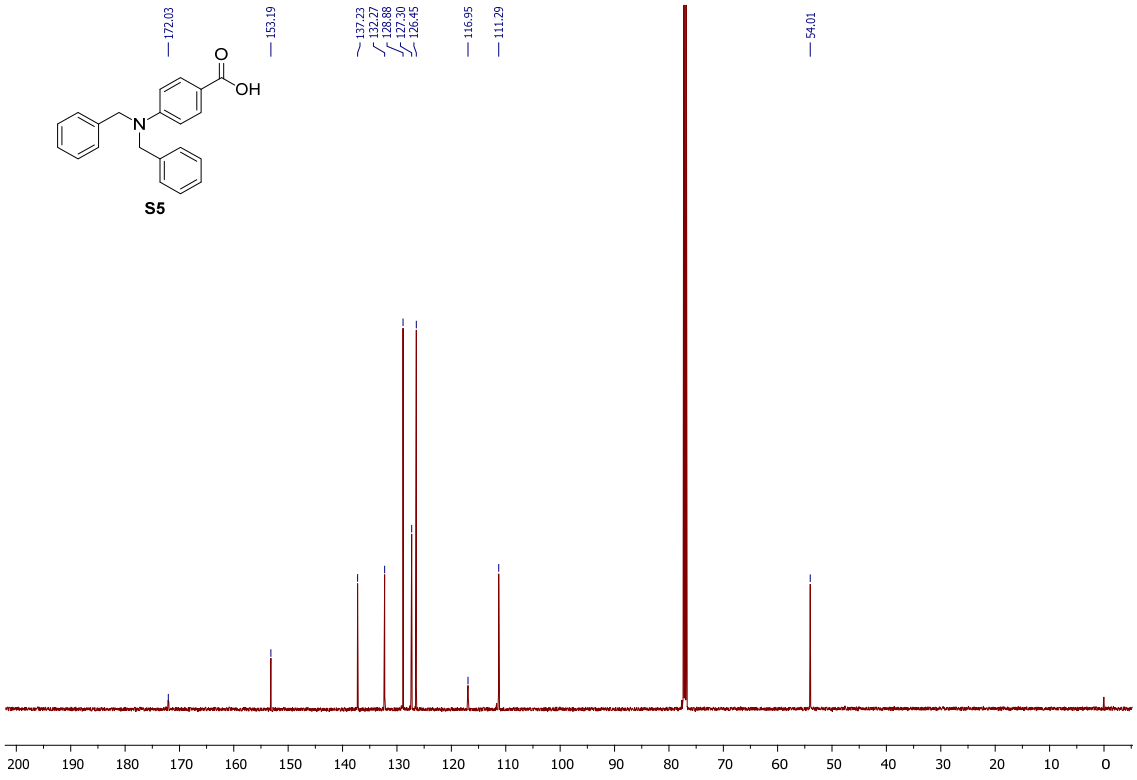
1.94
4.02
1.96
3.65
1.86
3.81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132.27
128.88
127.30
126.45
116.95
111.29
54.01
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OH
N
S5
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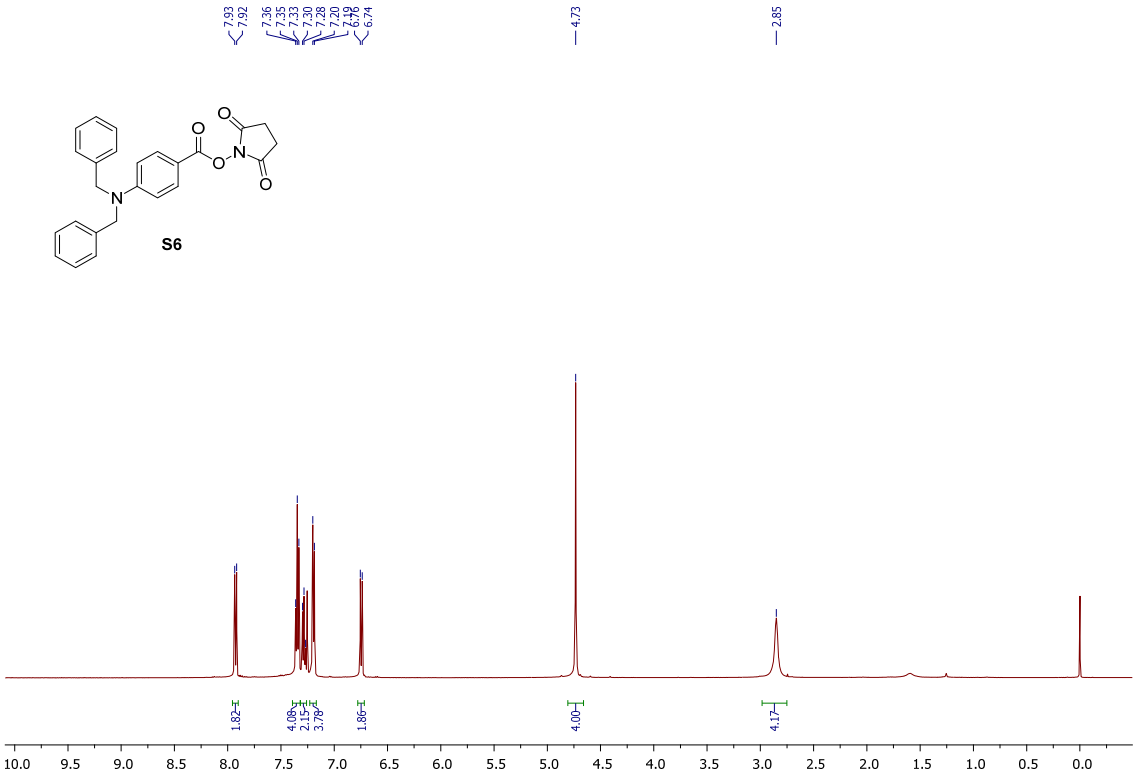
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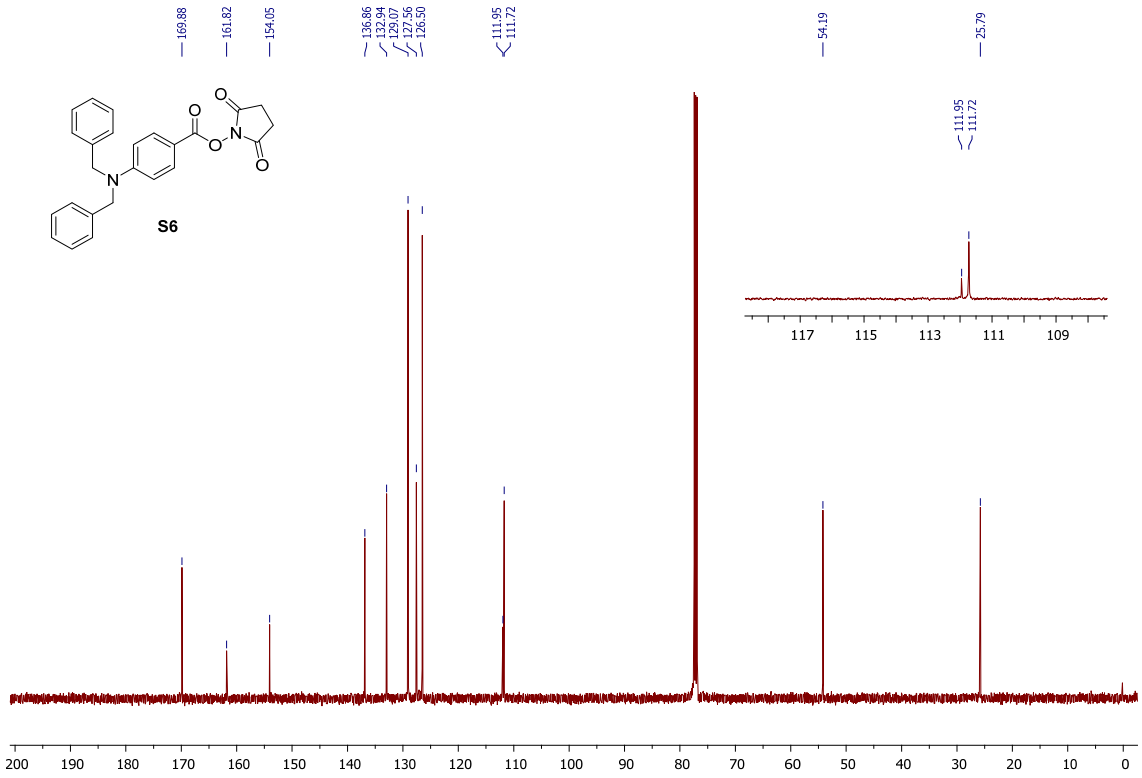
O
N
O
O
O
N
S6
1.82
4.08
2.15
3.78
1.86
4.00
4.17
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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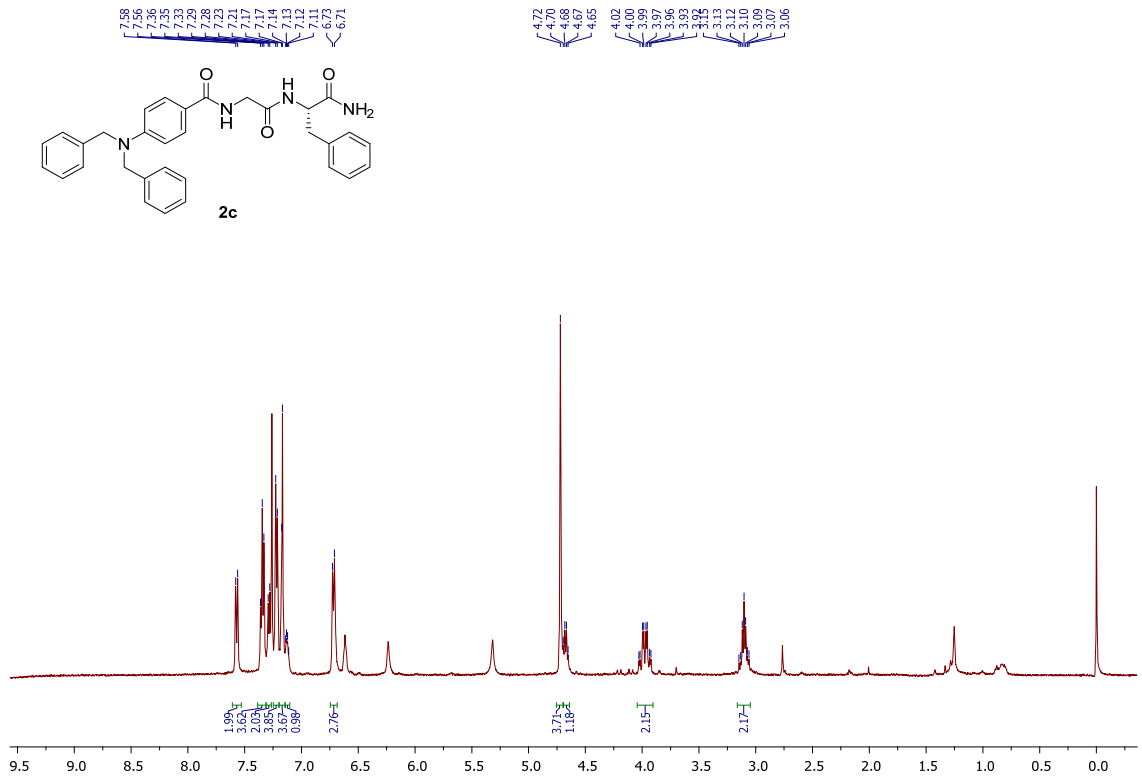
25.79
S6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7.13
7.12
7.11
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6.71
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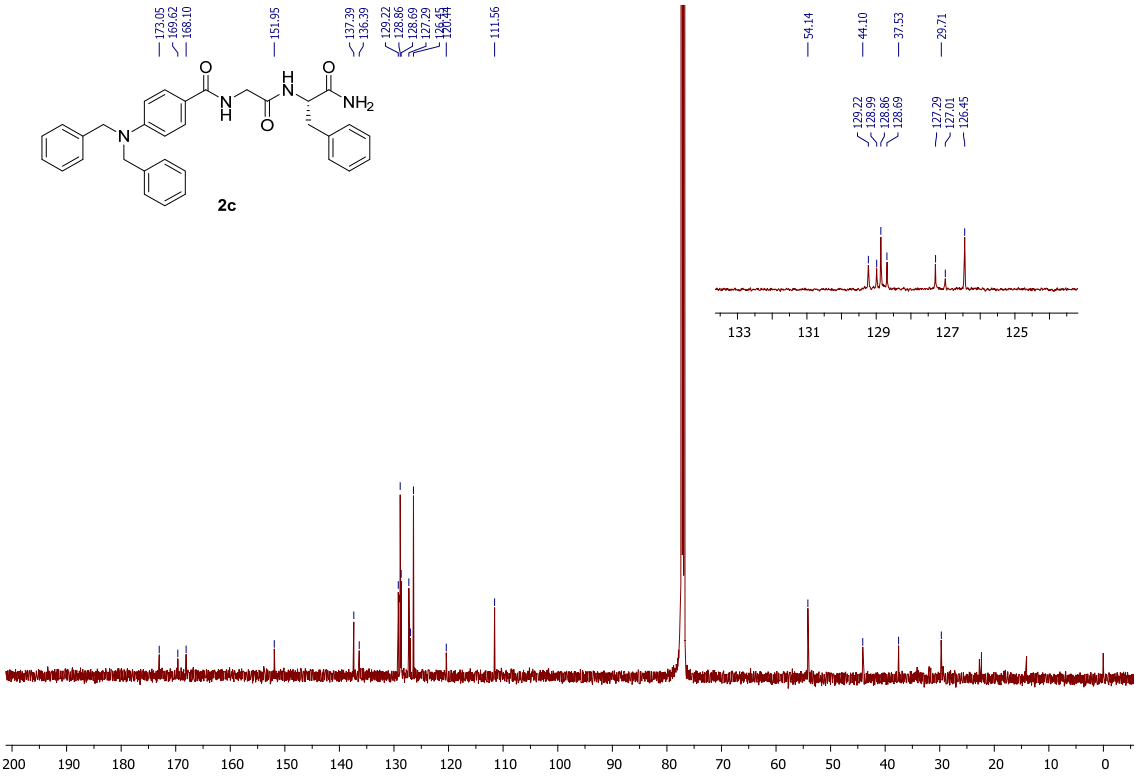
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127.29
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111.56
54.14
44.10
37.53
29.71
129.22
128.99
128.86
128.69
127.29
127.01
126.45
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131
129
127
125
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10
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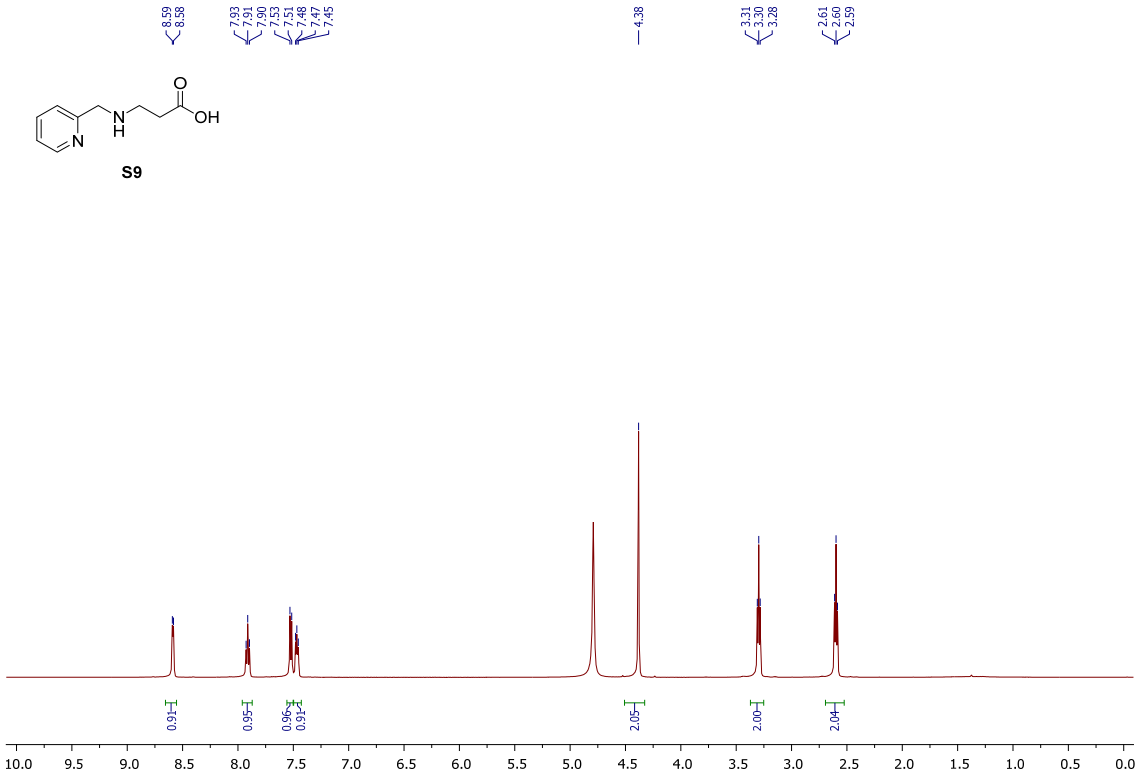
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3.28
2.61
2.60
2.59
S9
0.91
0.95
0.96
0.91
2.05
2.00
2.04
10.0
9.5
9.0
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8.0
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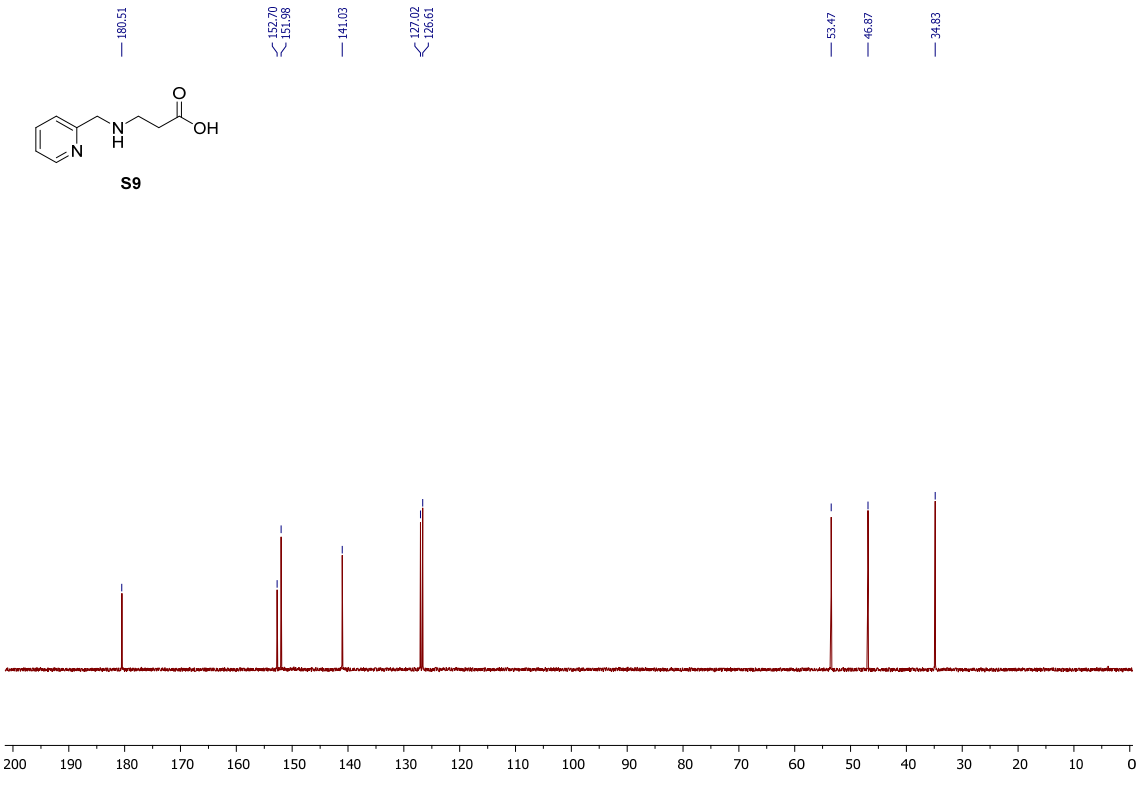
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OH
N
H
N
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8.60 8.60 8.59 8.59 8.59
7.69 7.68 7.67 7.66 7.66 7.36 7.35 7.34 7.34 7.33 7.33 7.32 7.31 7.31 7.31 7.31 7.30 7.29 7.29 7.28 7.28 7.25 7.24 7.24 7.24 7.23 7.21
3.85 3.80
2.97 2.96 2.95 2.60 2.58 2.57
O
OH
N
N
S11
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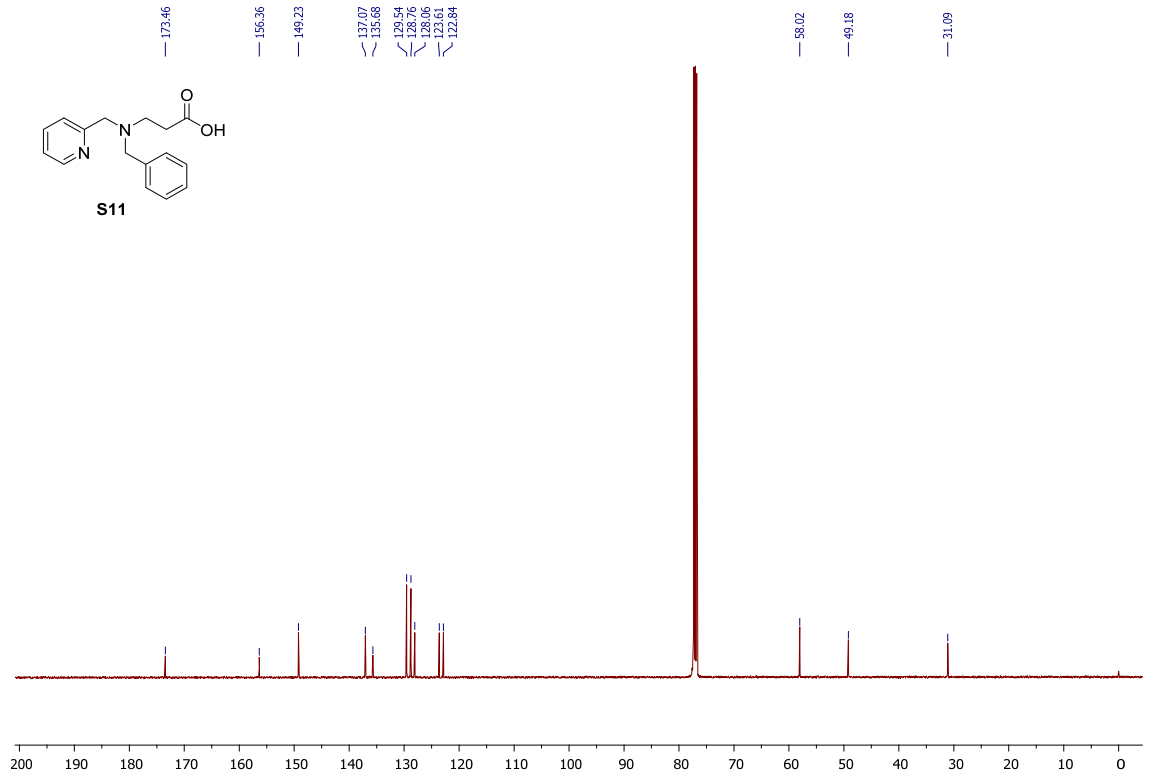
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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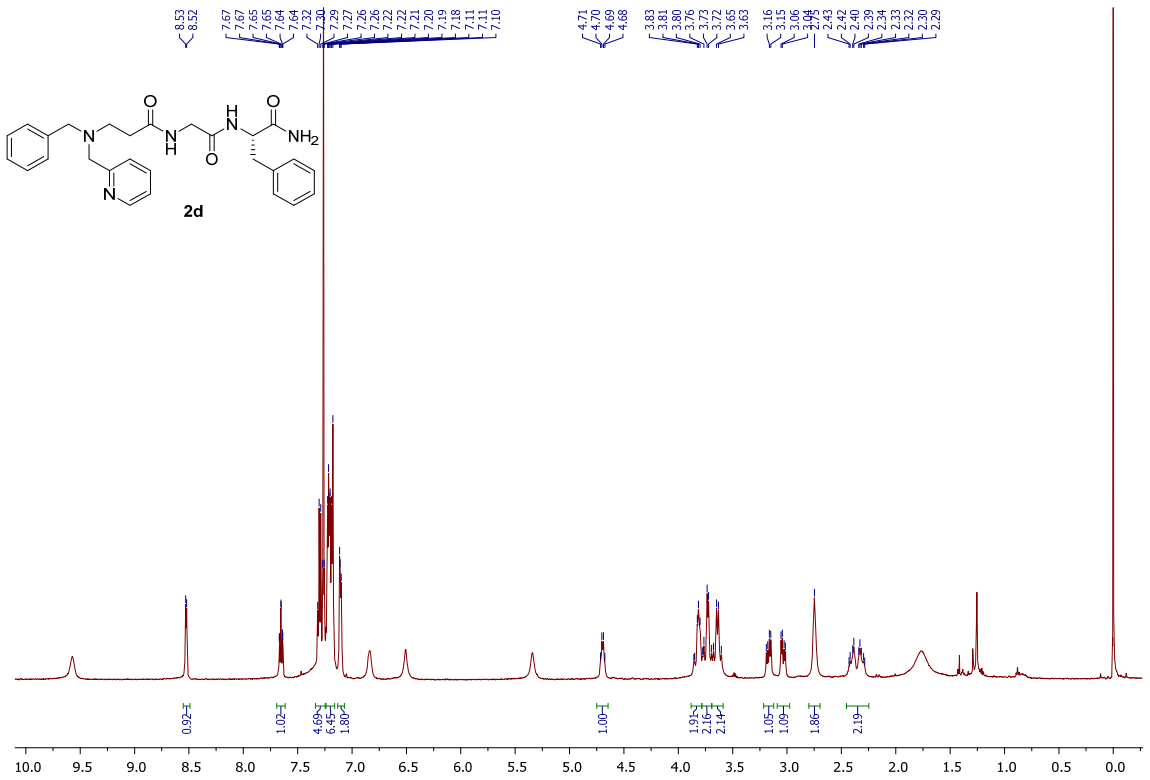
31.09
S11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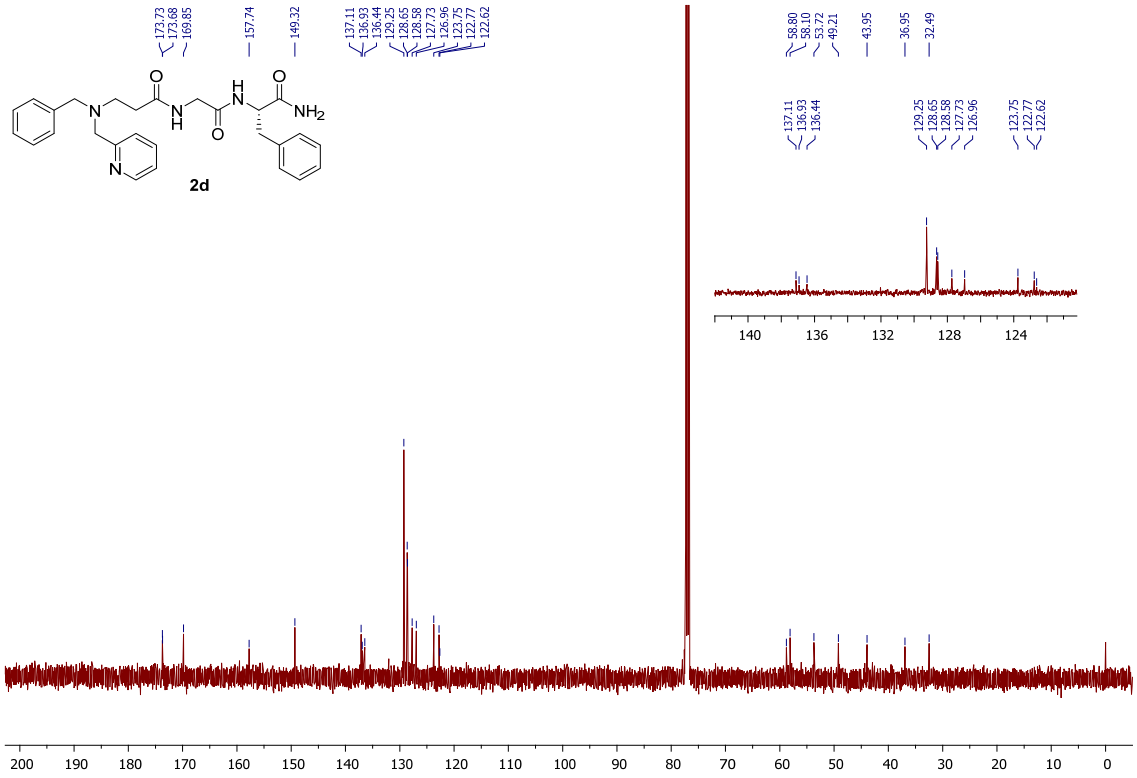
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53.72
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136
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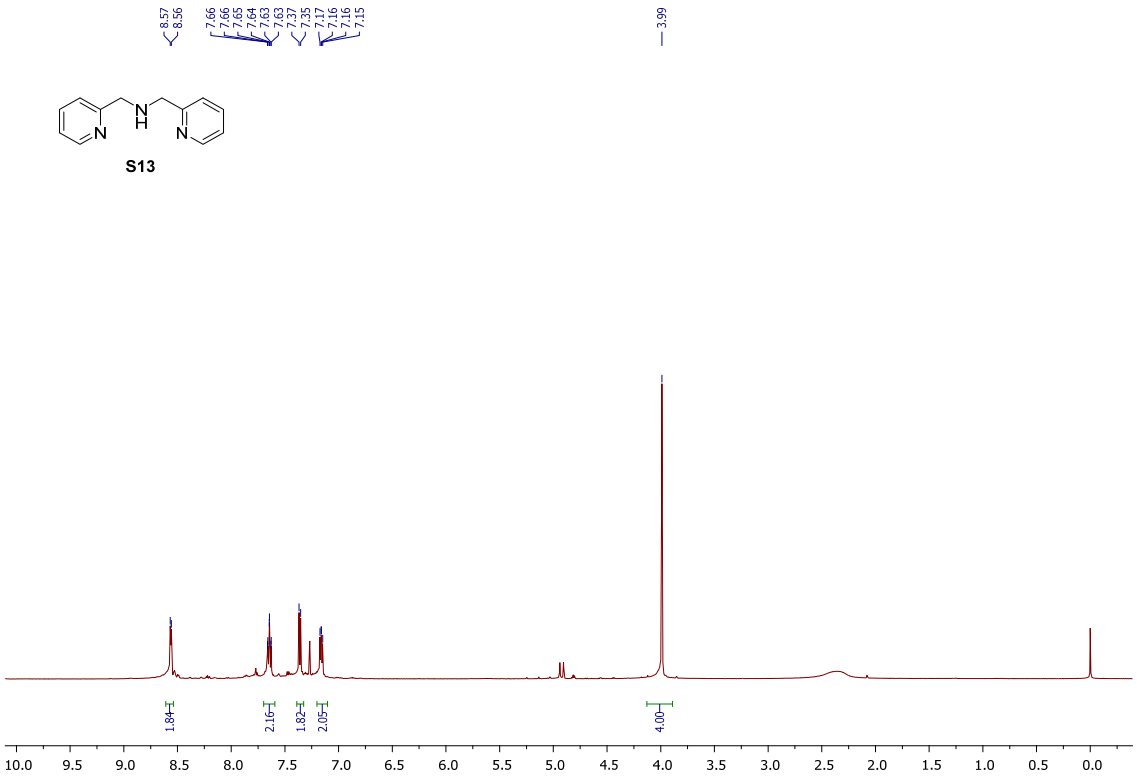
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7.15
3.99
S13
1.84
2.16
1.82
2.05
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9.0
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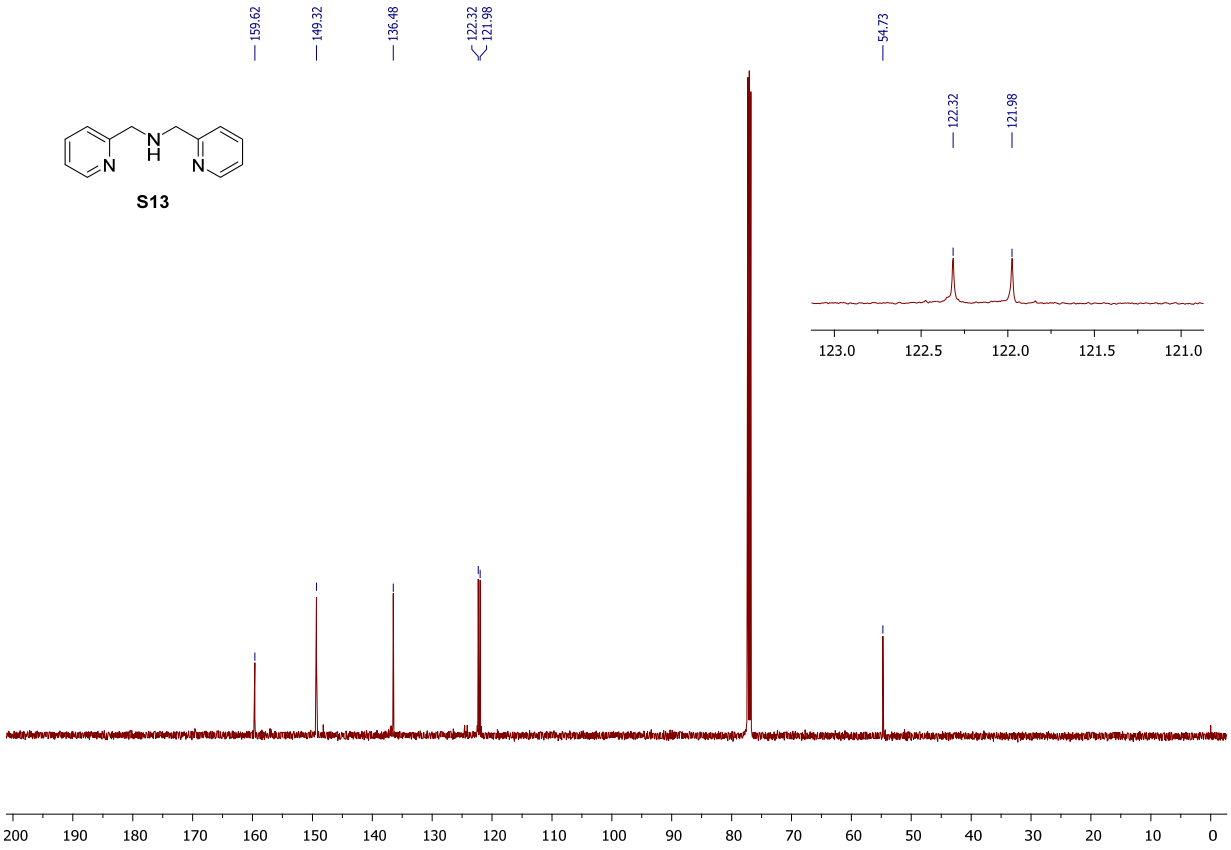
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122.32
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S13
123.0
122.5
122.0
121.5
121.0
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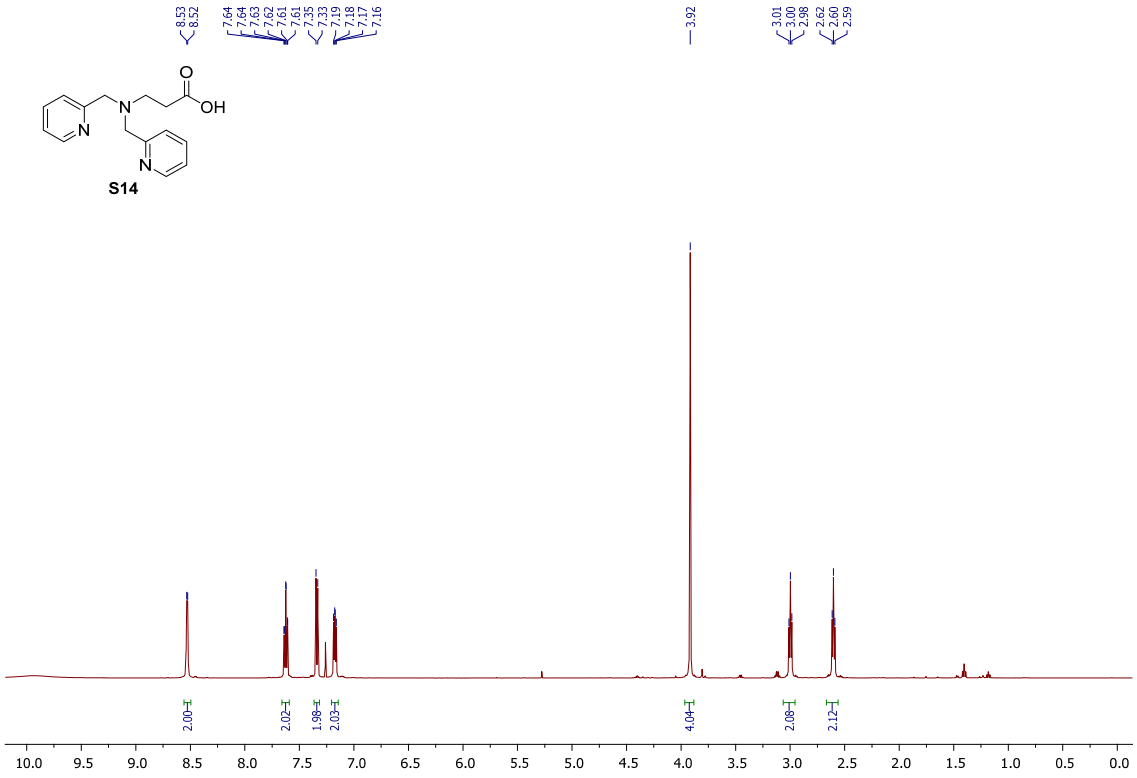
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7.61
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7.33
7.19
7.18
7.17
7.16
3.92
3.01
3.00
2.98
2.62
2.60
2.59
O
OH
N
N
N
S14
2.00
2.02
1.98
2.03
4.04
2.08
2.12
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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2.0
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1.0
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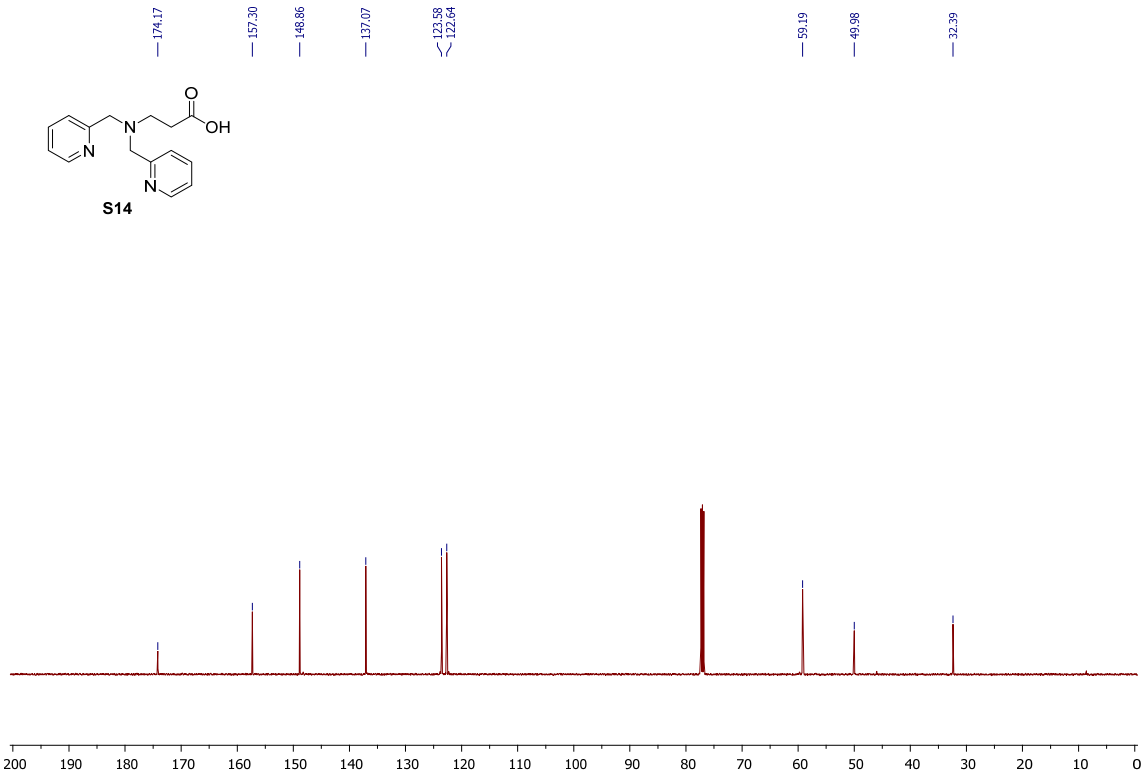
0.0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49.98
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S14
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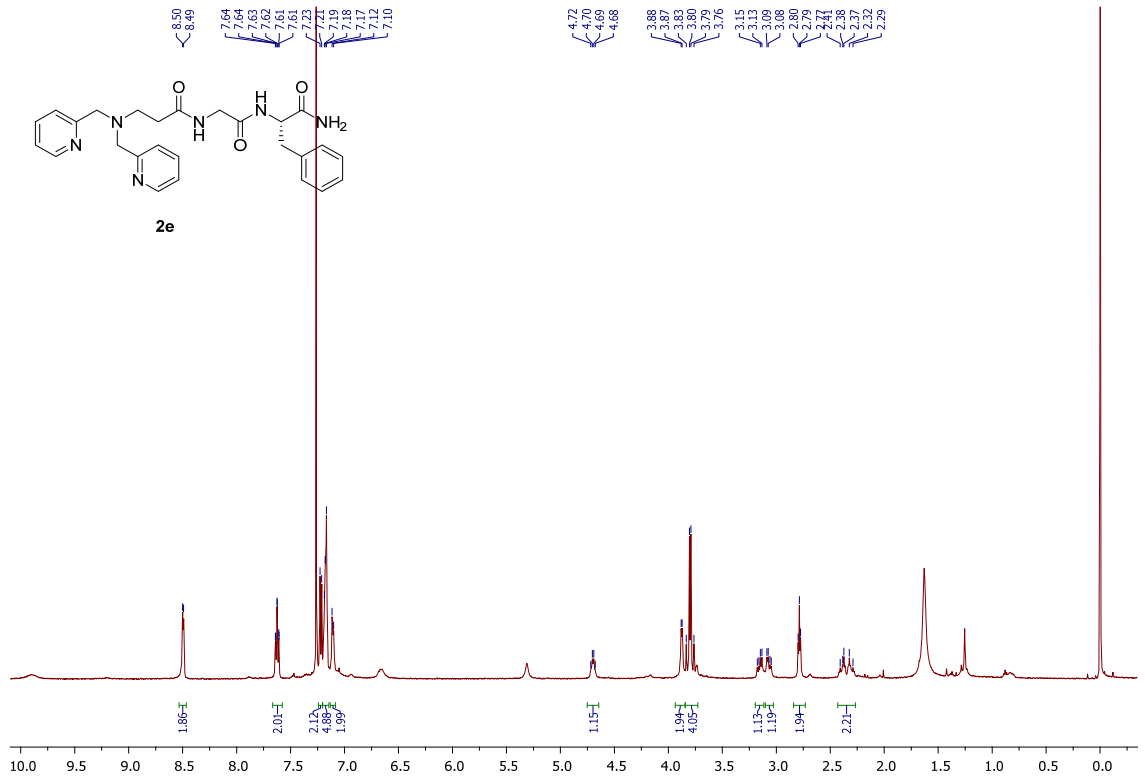
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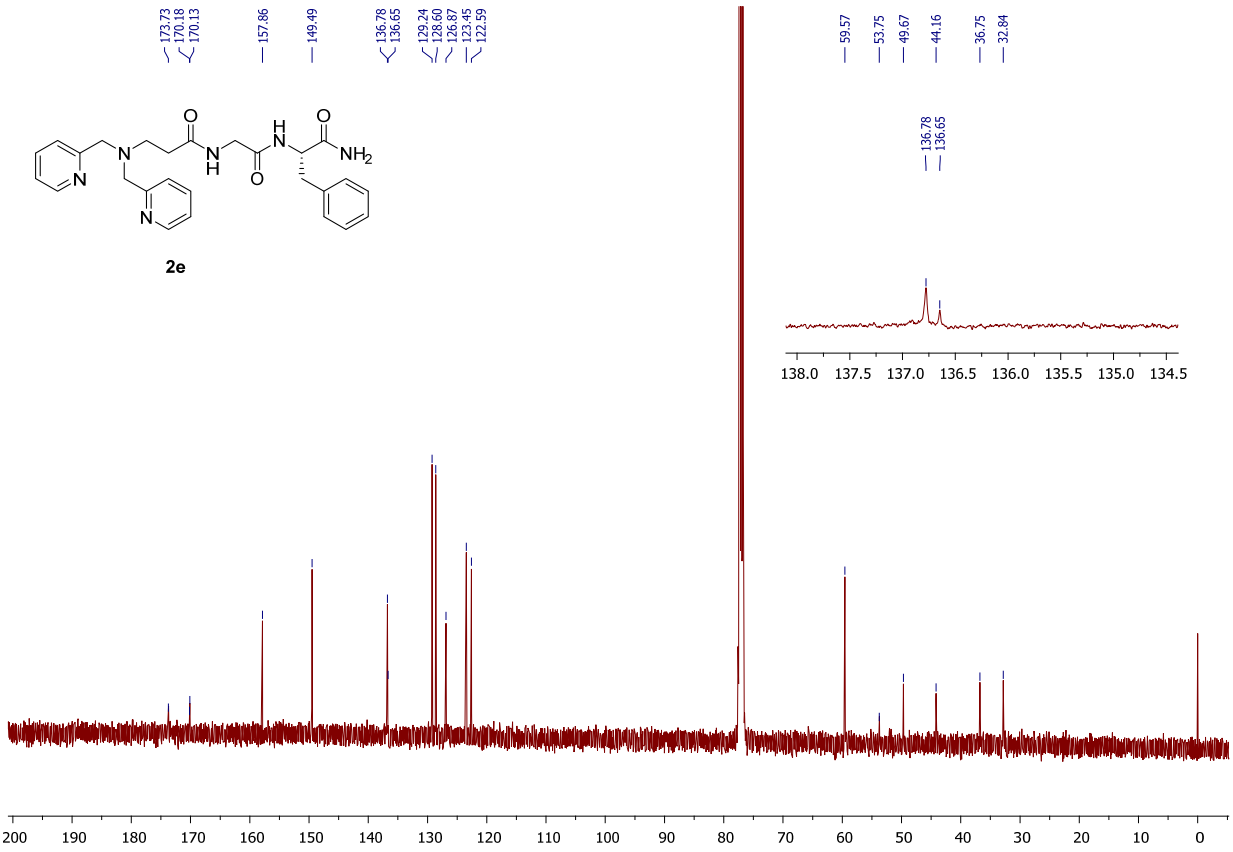
7.61
7.23
7.21
7.19
7.18
7.17
7.12
7.10
4.72
4.70
4.69
4.68
3.88
3.87
3.83
3.80
3.79
3.76
3.15
3.13
3.09
3.08
2.80
2.79
2.41
2.38
2.37
2.32
2.29
2e
1.86
2.01
2.12
4.88
1.99
1.15
1.94
4.05
1.13
1.19
1.94
2.21
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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129.24
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126.87
123.45
122.59
59.57
53.75
49.67
44.16
36.75
32.84
2e
138.0
137.5
137.0
136.5
136.0
135.5
135.0
134.5
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180
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150
140
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120
110
100
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80
70
60
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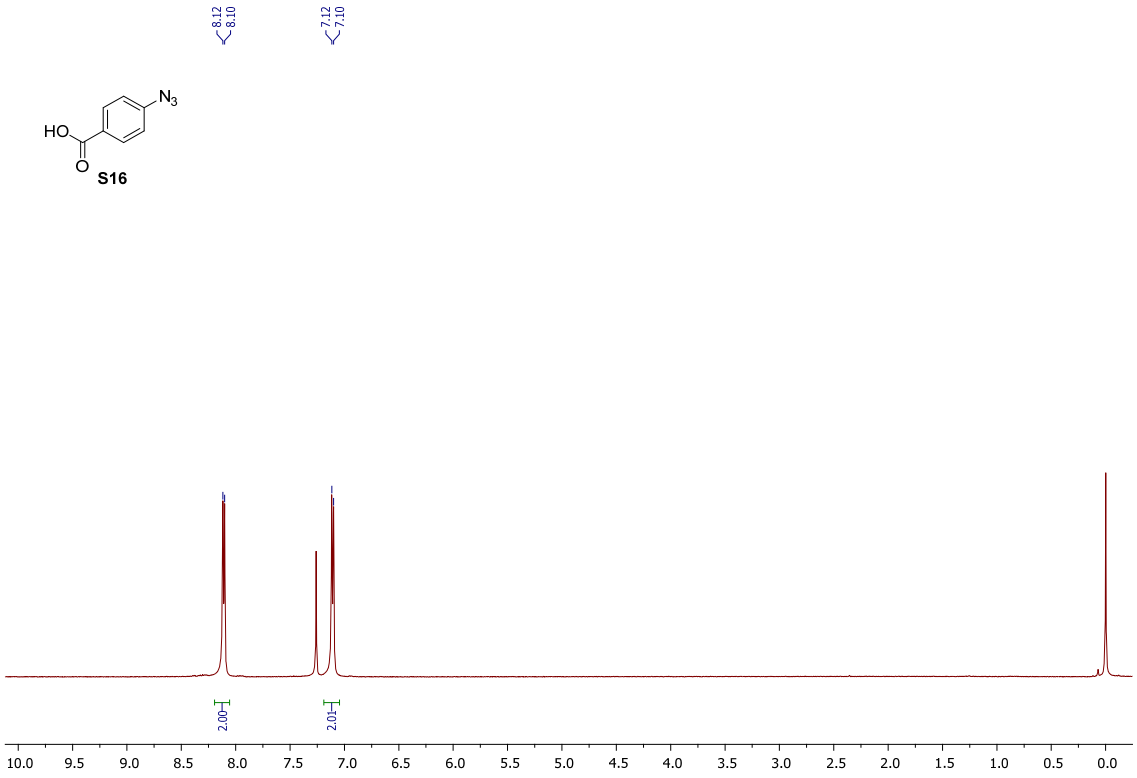
30
20
10
0


8.12
8.10
7.12
7.10
S16
2.00
2.01
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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4.0
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2.0
1.5
1.0
0.5
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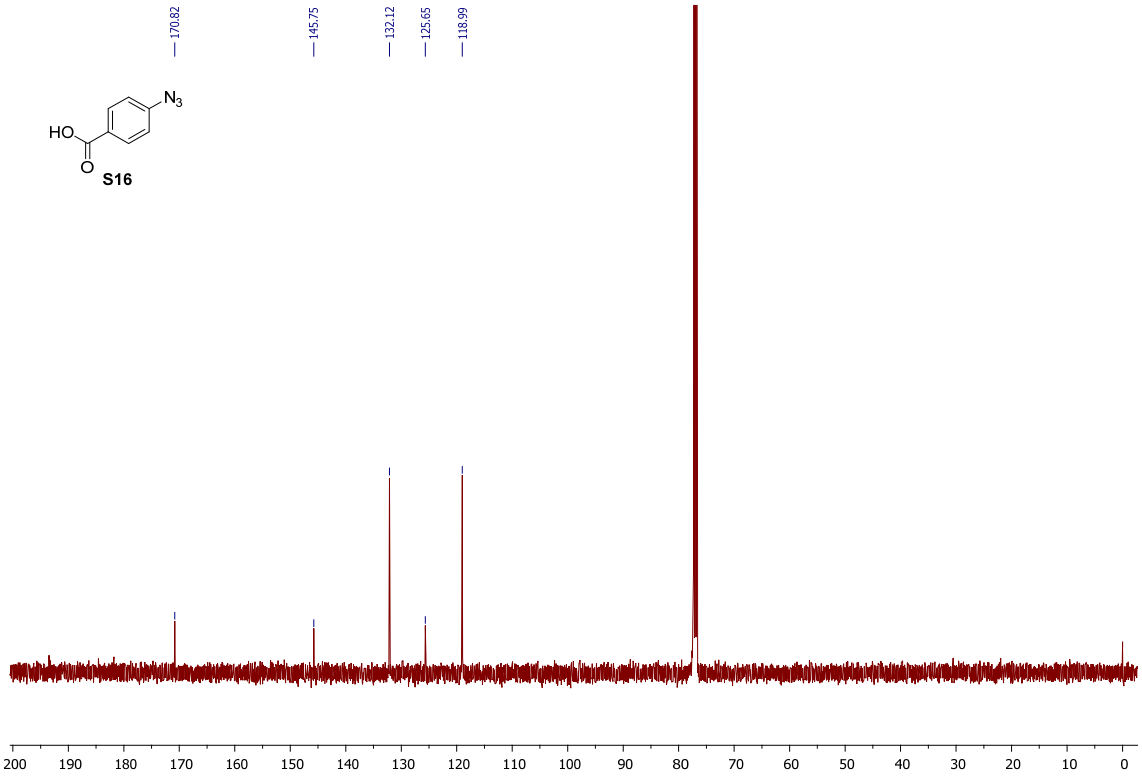
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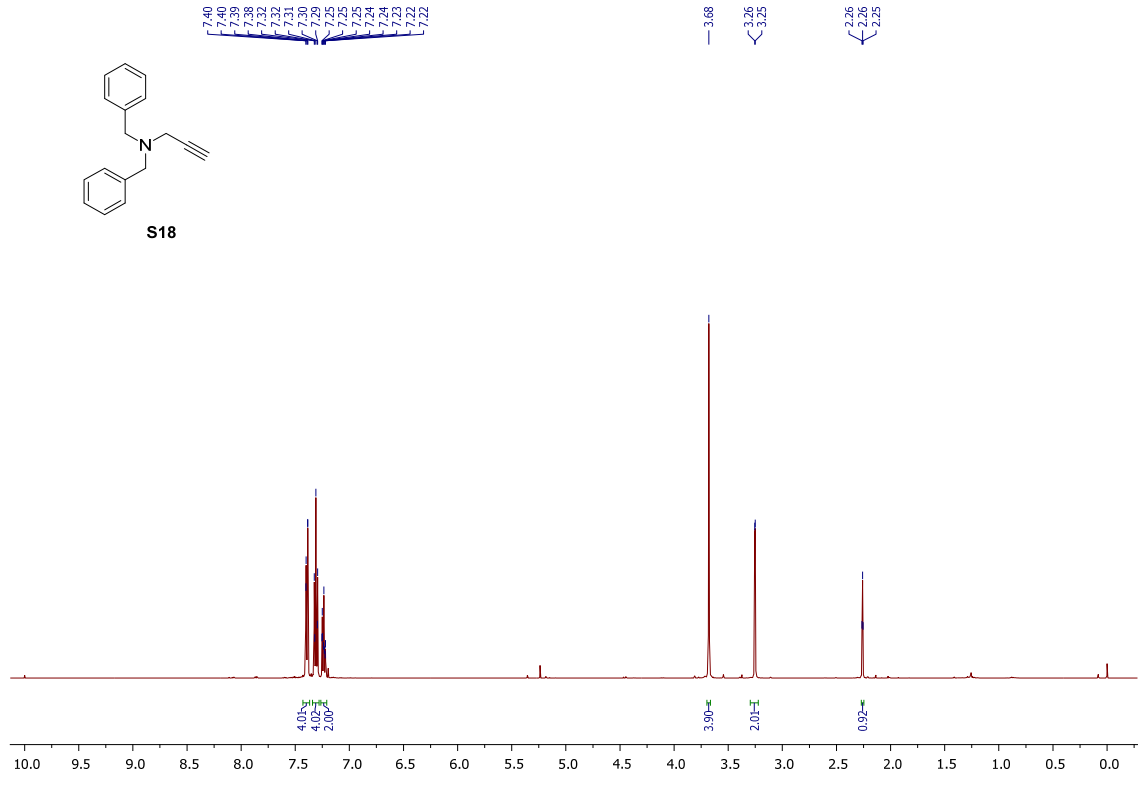
145.75
132.12
125.65
118.99
HO
O
N3
S16
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


7.40
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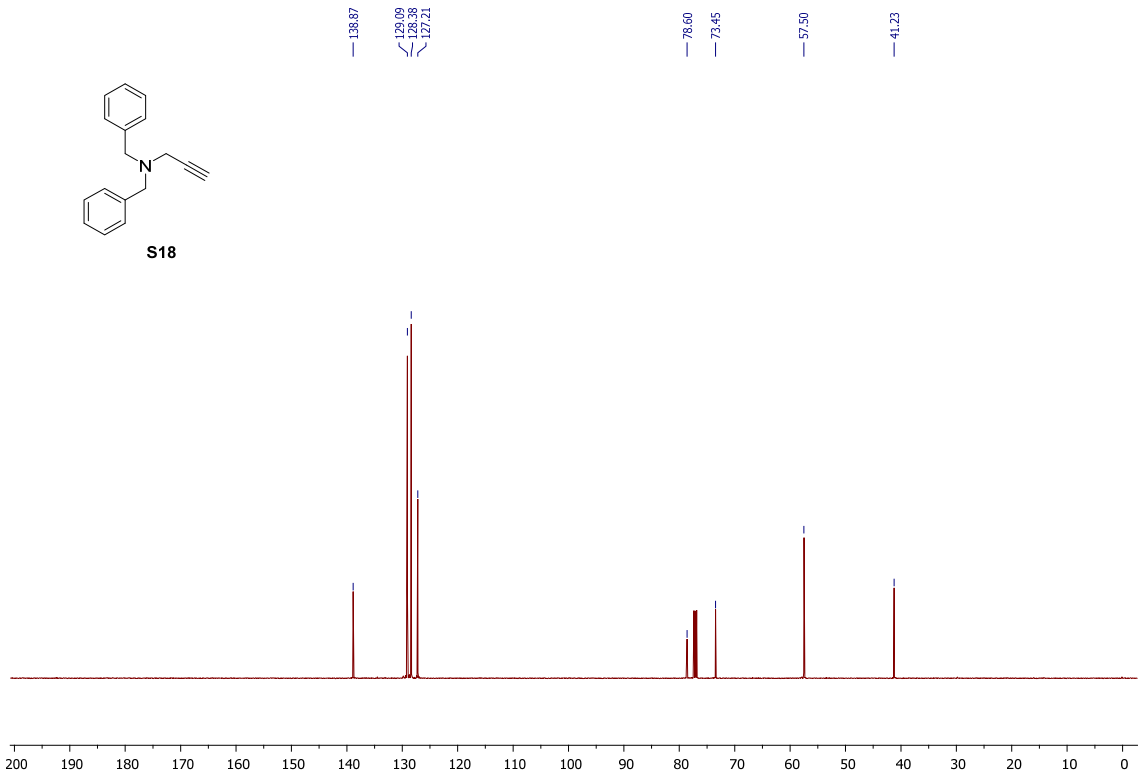
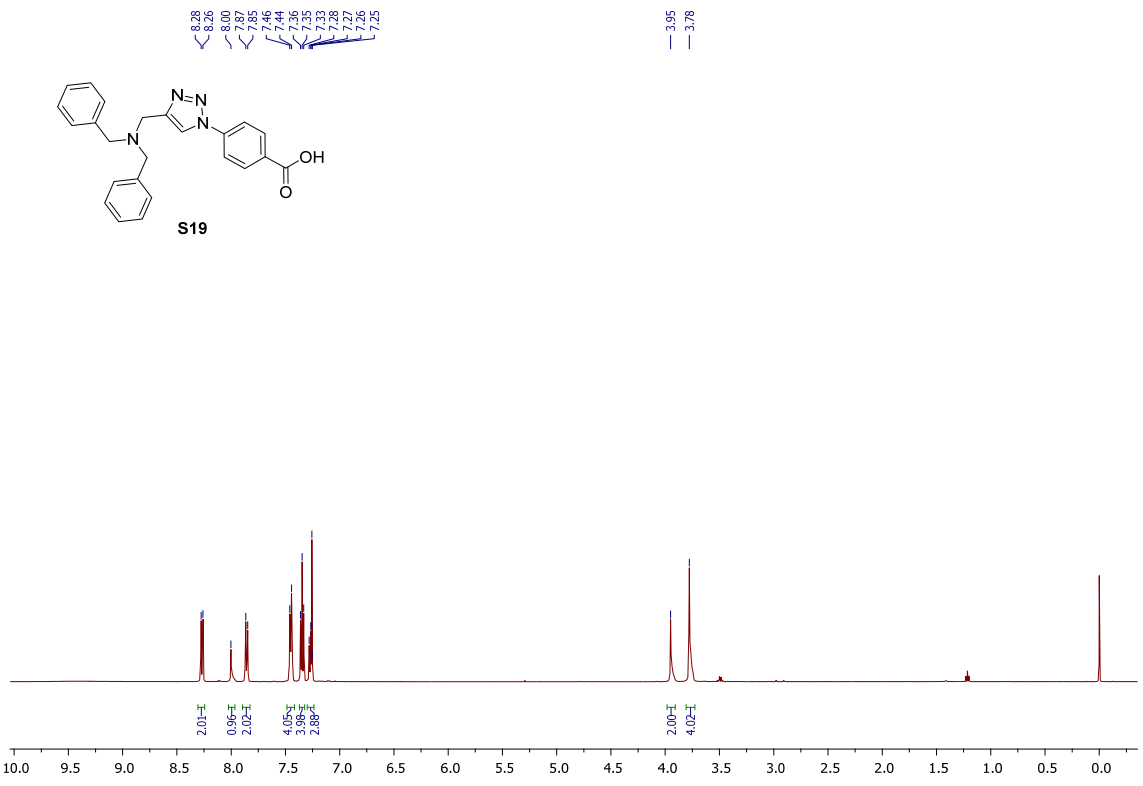
7.32
7.31
7.30
7.29
7.25
7.25
7.25
7.24
7.24
7.23
7.22
7.22
3.68
3.26
3.25
2.26
2.26
2.25
N
S18
4.01
4.02
2.00
3.90
2.01
0.92
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


138.87
129.09
128.38
127.21
78.60
73.45
57.50
41.23
N
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

S18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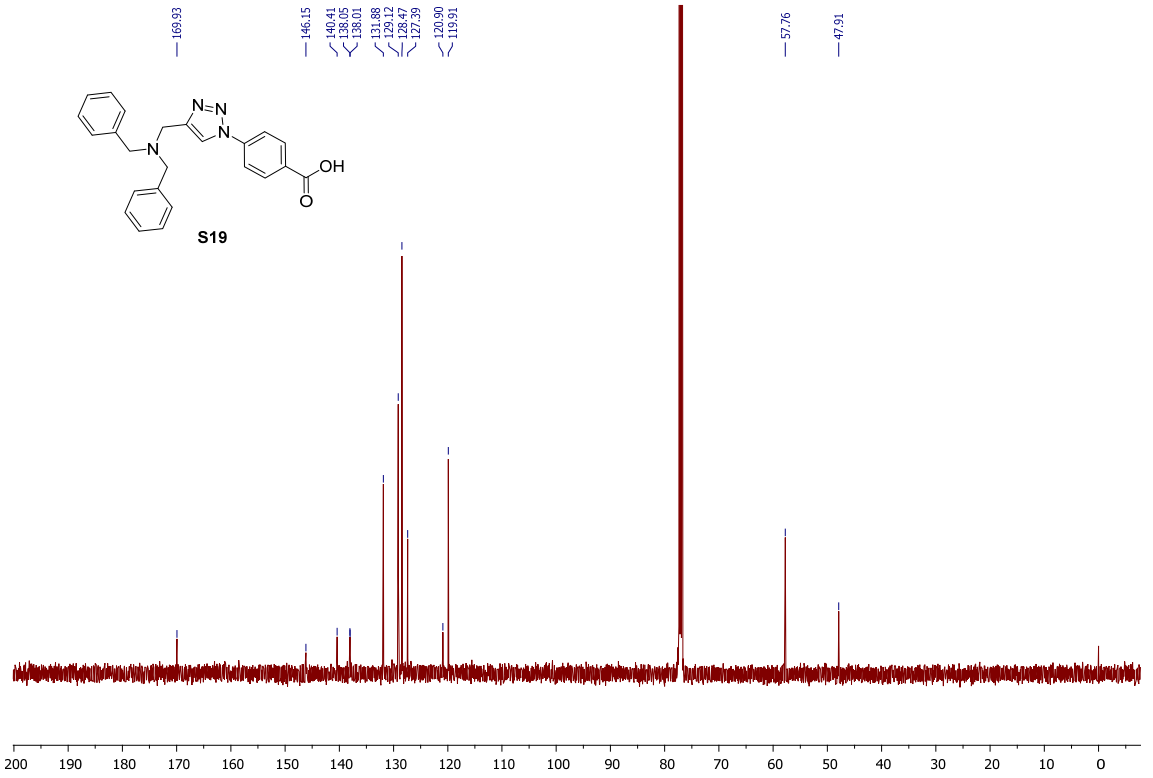
8.26
8.00
7.87
7.85
7.46
7.44
7.36
7.35
7.33
7.28
7.27
7.26
7.25
3.95
3.78
N
N
N
OH
O
2.01
0.96
2.02
4.05
3.99
2.88
2.00
4.02
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

S19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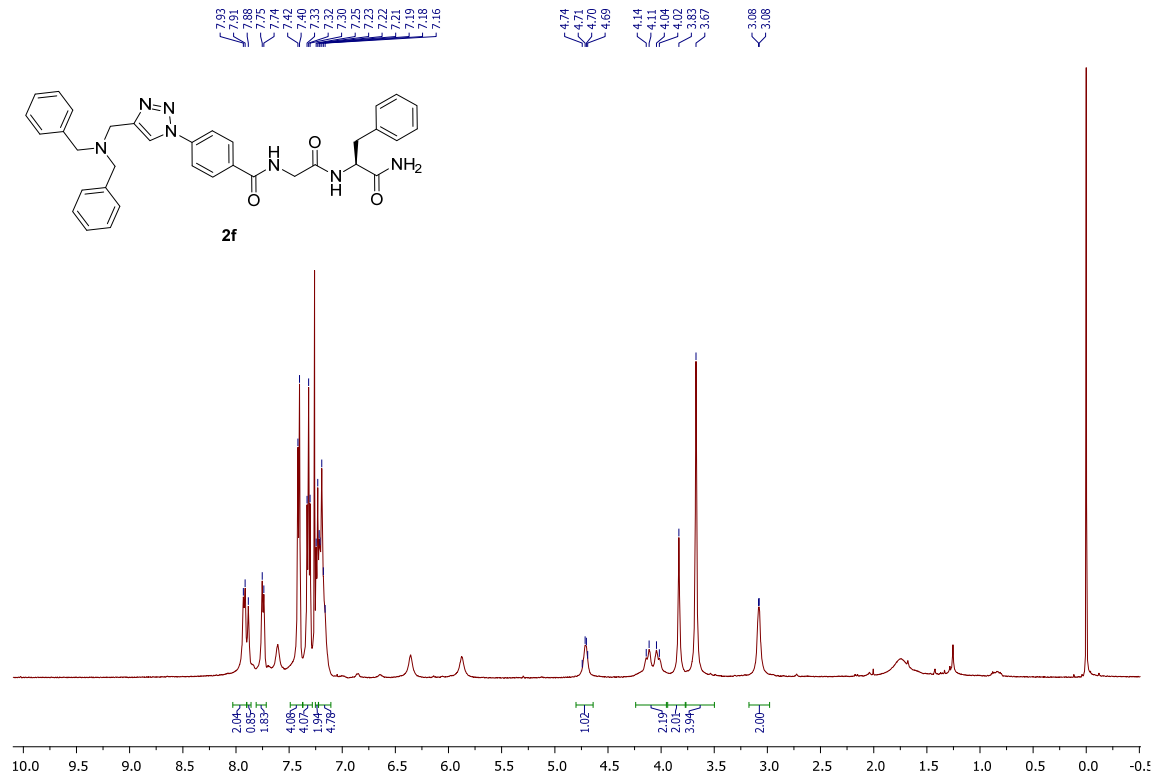
146.15
140.41
138.05
138.01
131.88
129.12
128.47
127.39
120.90
119.91
57.76
47.91
OH
O
S19
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.93
7.91
7.88
7.75
7.74
7.42
7.40
7.33
7.32
7.30
7.25
7.23
7.22
7.21
7.19
7.18
7.16
4.74
4.71
4.70
4.69
4.14
4.11
4.04
4.02
3.83
3.67
3.08
3.08
NH2
O
H
N
2f
2.04
0.85
1.83
4.08
4.07
1.94
4.78
1.02
2.19
2.01
2.04
3.94
2.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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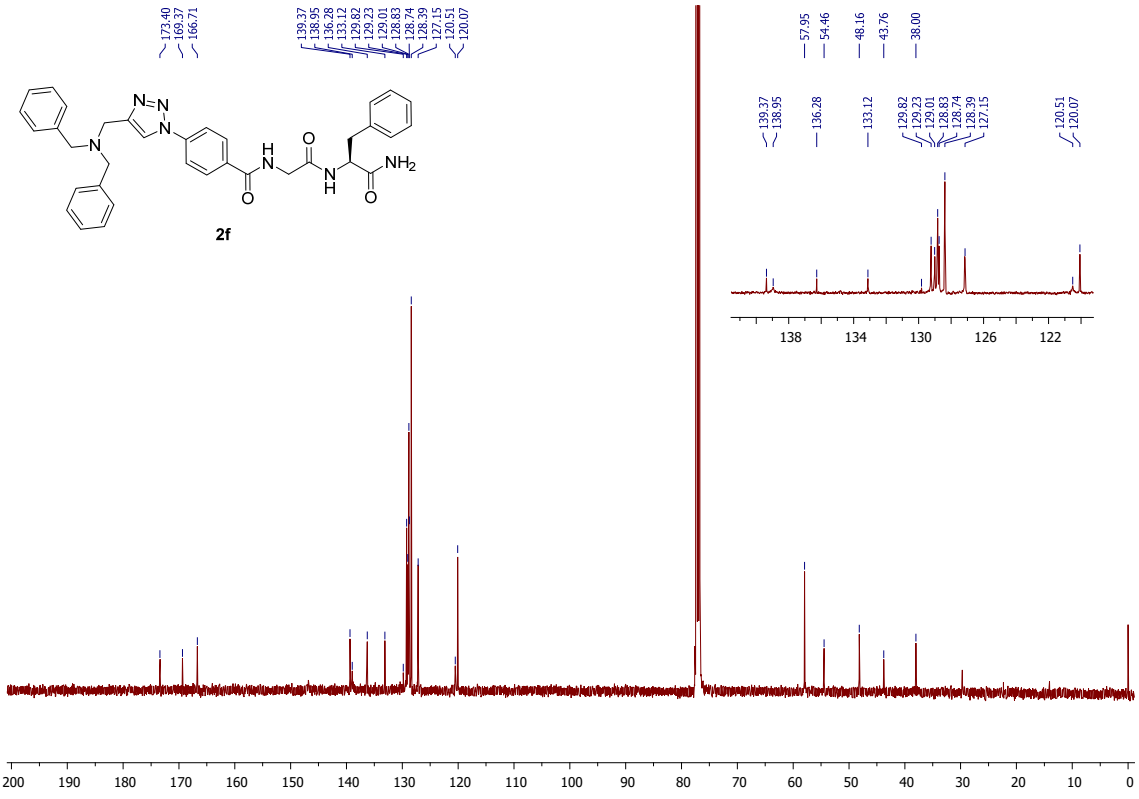
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0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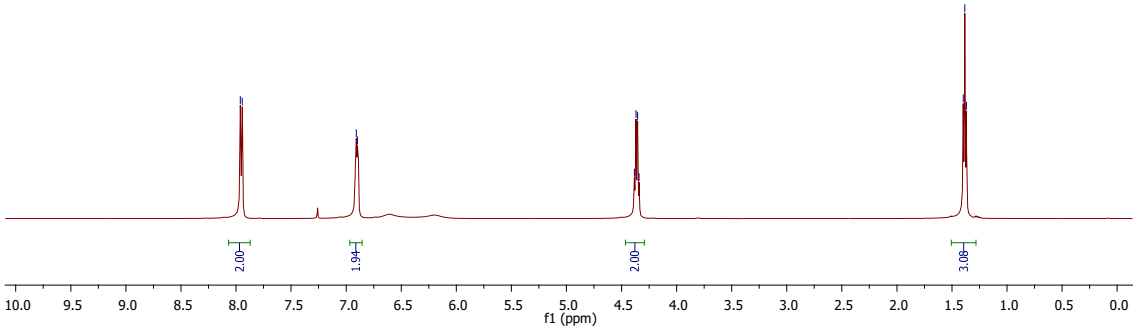
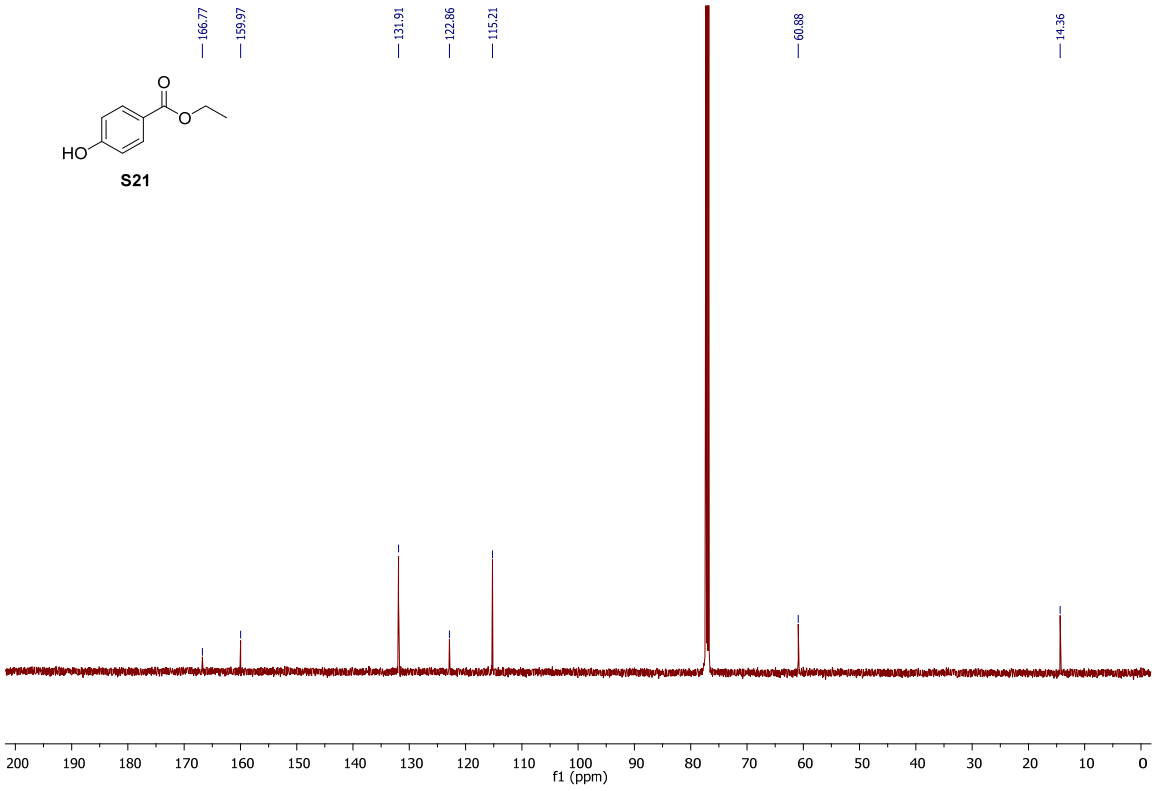
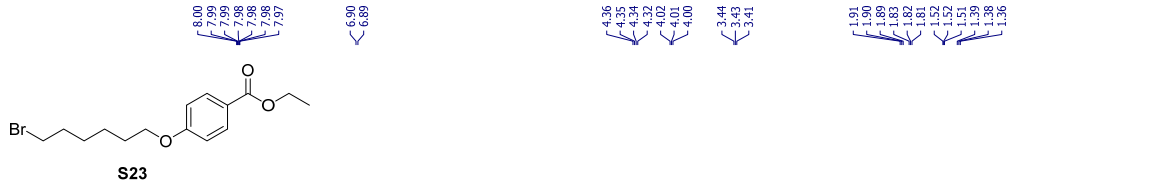
136.28
133.12
129.82
129.23
129.01
128.83
128.74
128.39
127.15
120.51
120.07
57.95
54.46
48.16
43.76
38.00
2f


7.96
7.94
6.91
6.90
4.38
4.37
4.35
4.34
1.40
1.38
1.37
S21

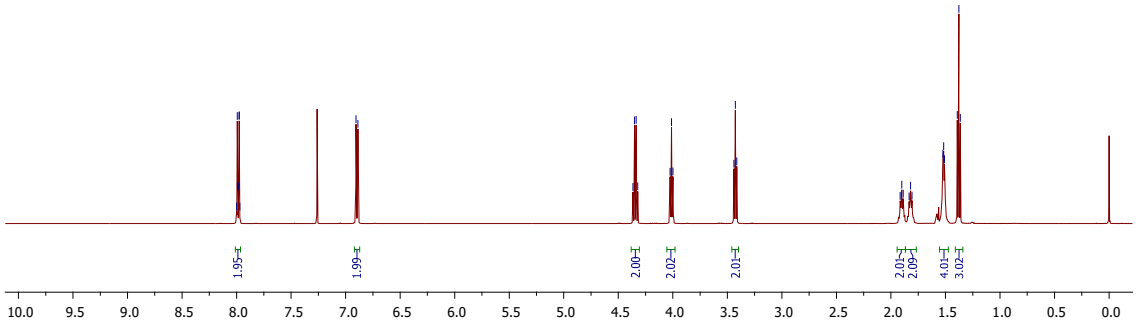

2.00
1.94
2.00
3.08
f1 (ppm)

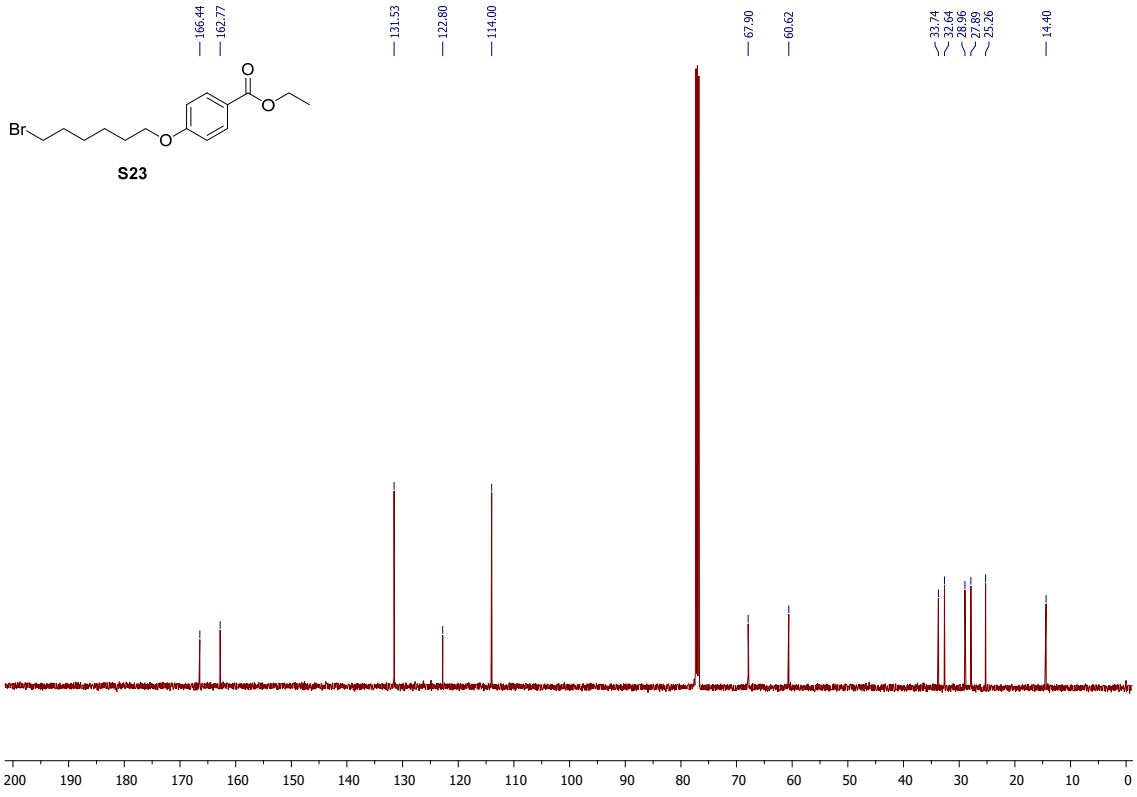

166.77
159.97
131.91
122.86
115.21
60.88
14.36
HO
O
O
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

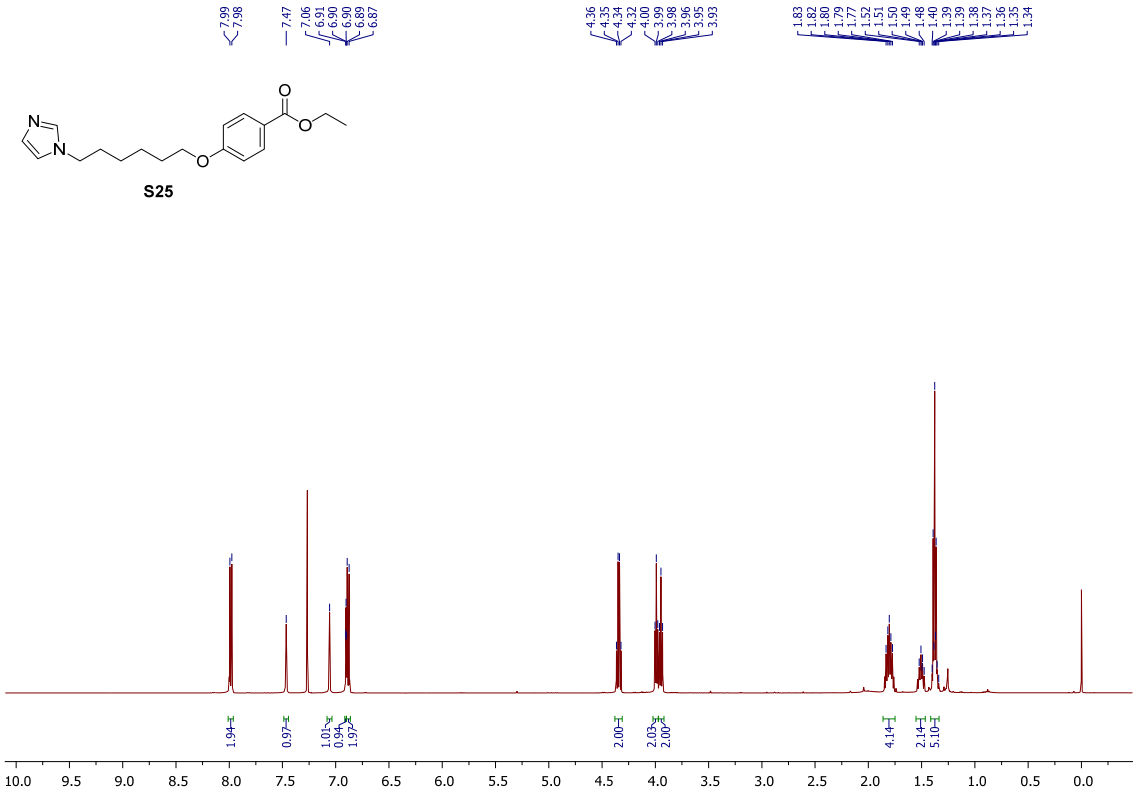
S21


8.00
7.99
7.99
7.98
7.98
7.98
7.97
6.90
6.89
4.36
4.35
4.34
4.32
4.02
4.01
4.00
3.44
3.43
3.41
1.91
1.90
1.89
1.83
1.82
1.81
1.52
1.52
1.51
1.39
1.38
1.36
Br
O
O

S23


1.95
1.99
2.00
2.02
2.01
2.01
2.09
4.01
3.02
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


166.44
162.77
131.53
122.80
114.00
67.90
60.62
33.74
32.64
28.96
27.99
25.26
14.40
Br
O
O
O
S23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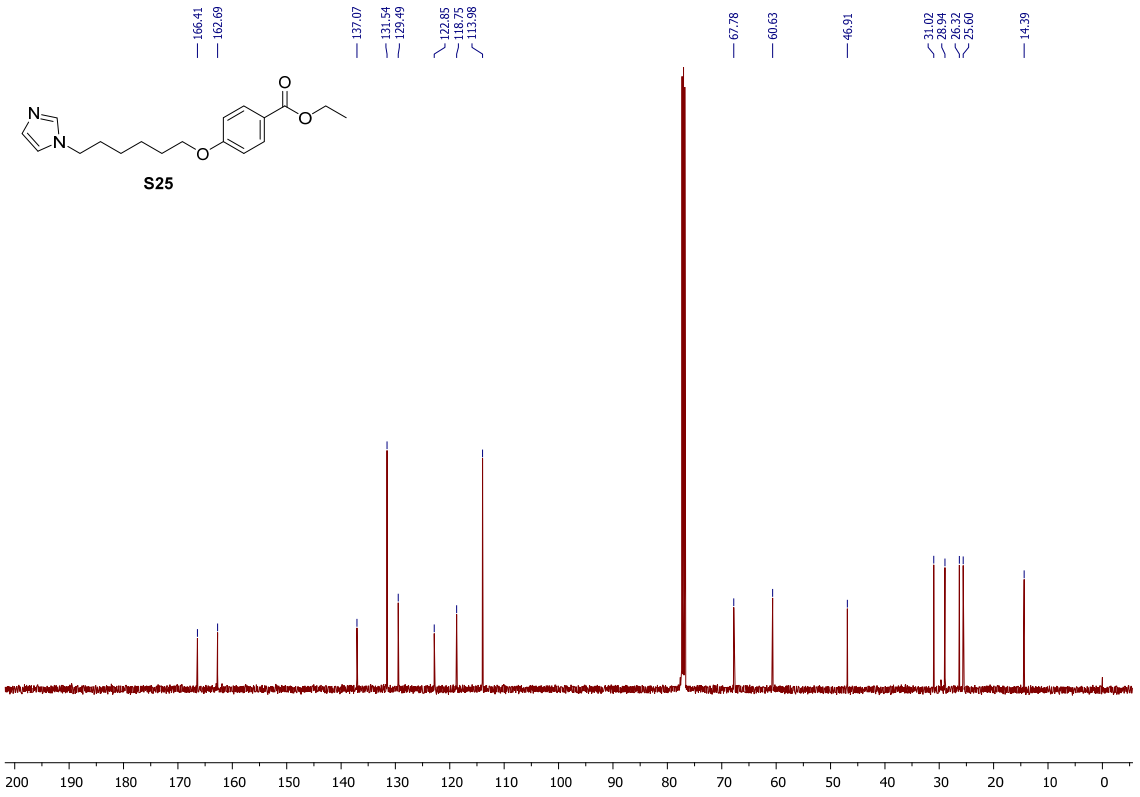
7.98
7.47
7.06
6.91
6.90
6.90
6.89
6.87
4.36
4.35
4.34
4.32
4.00
3.99
3.98
3.96
3.95
3.93
1.83
1.82
1.80
1.79
1.77
1.52
1.51
1.50
1.49
1.48
1.40
1.39
1.39
1.38
1.37
1.36
1.35
1.34
N
N
O
O
O
S25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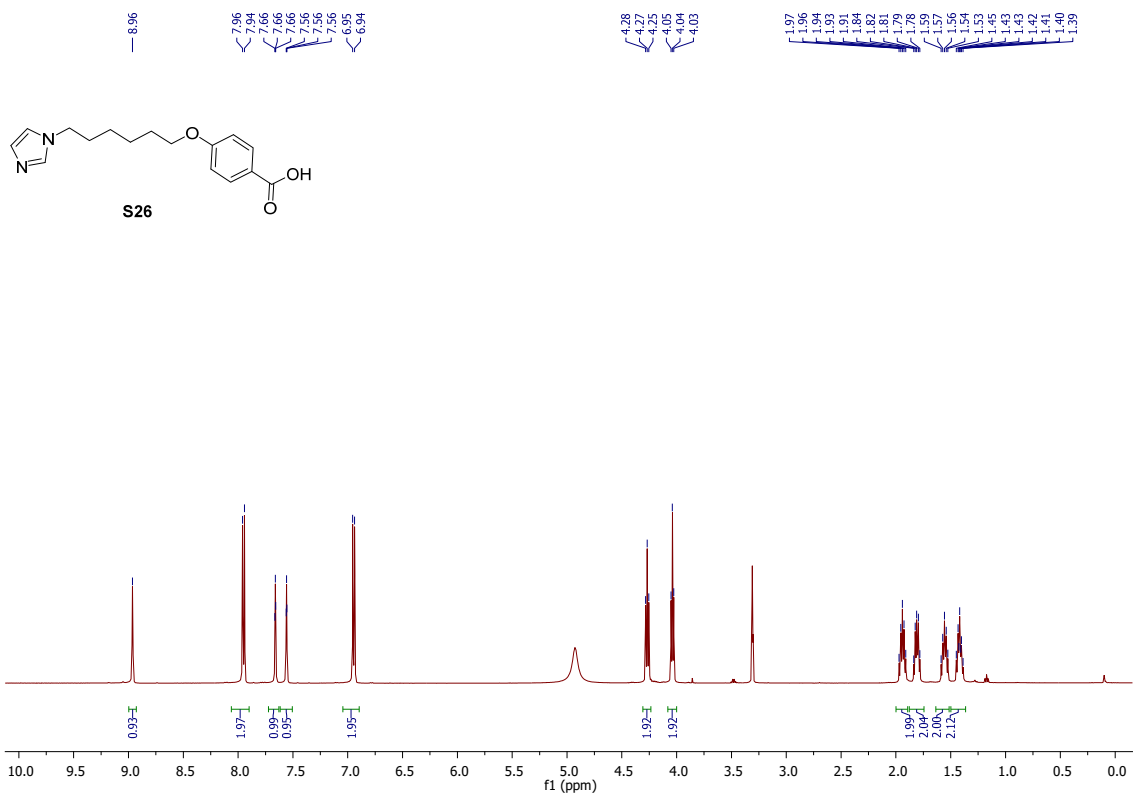
162.69
137.07
131.54
129.49
122.85
118.75
113.98
67.78
60.63
46.91
31.02
28.94
26.32
25.60
14.39
S25
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0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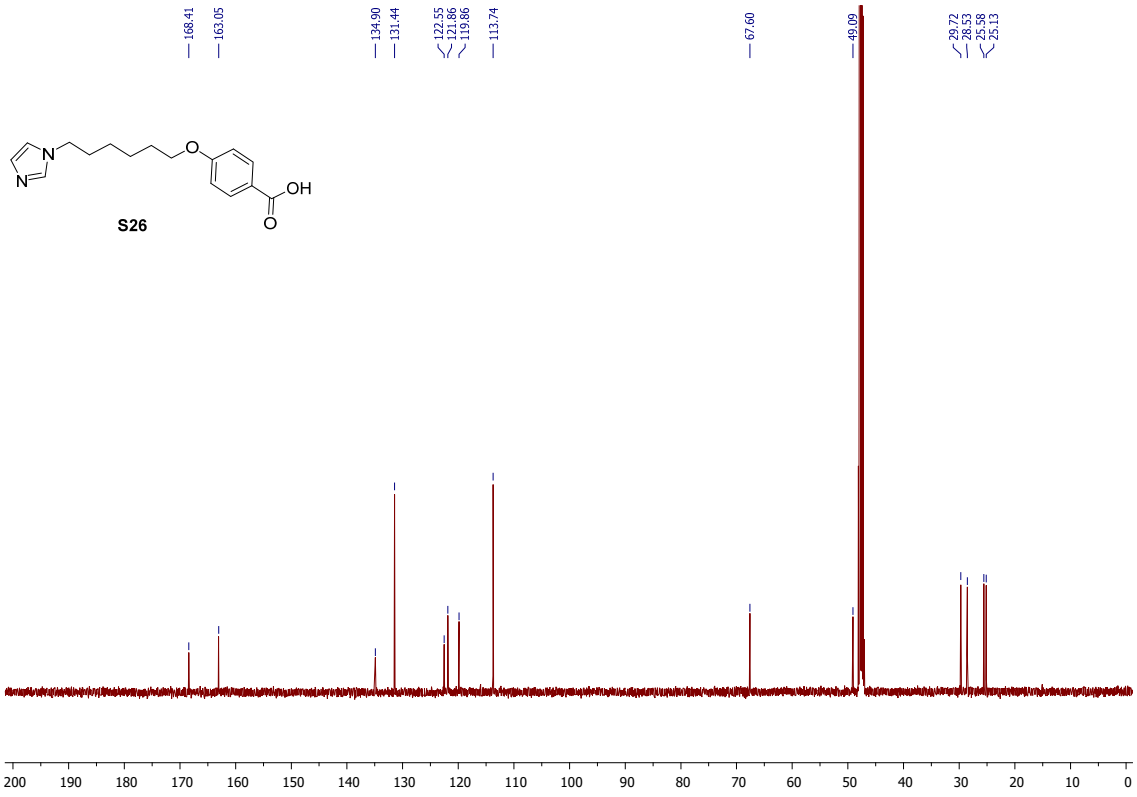
7.96
7.94
7.66
7.66
7.56
7.56
7.56
7.56
6.95
6.94
4.28
4.27
4.25
4.05
4.04
4.03
1.97
1.96
1.94
1.93
1.91
1.84
1.82
1.81
1.79
1.78
1.59
1.57
1.56
1.54
1.53
1.45
1.43
1.43
1.42
1.41
1.40
1.39
S26
0.93
1.97
0.99
0.95
1.95
1.92
1.92
1.99
2.04
2.00
2.12
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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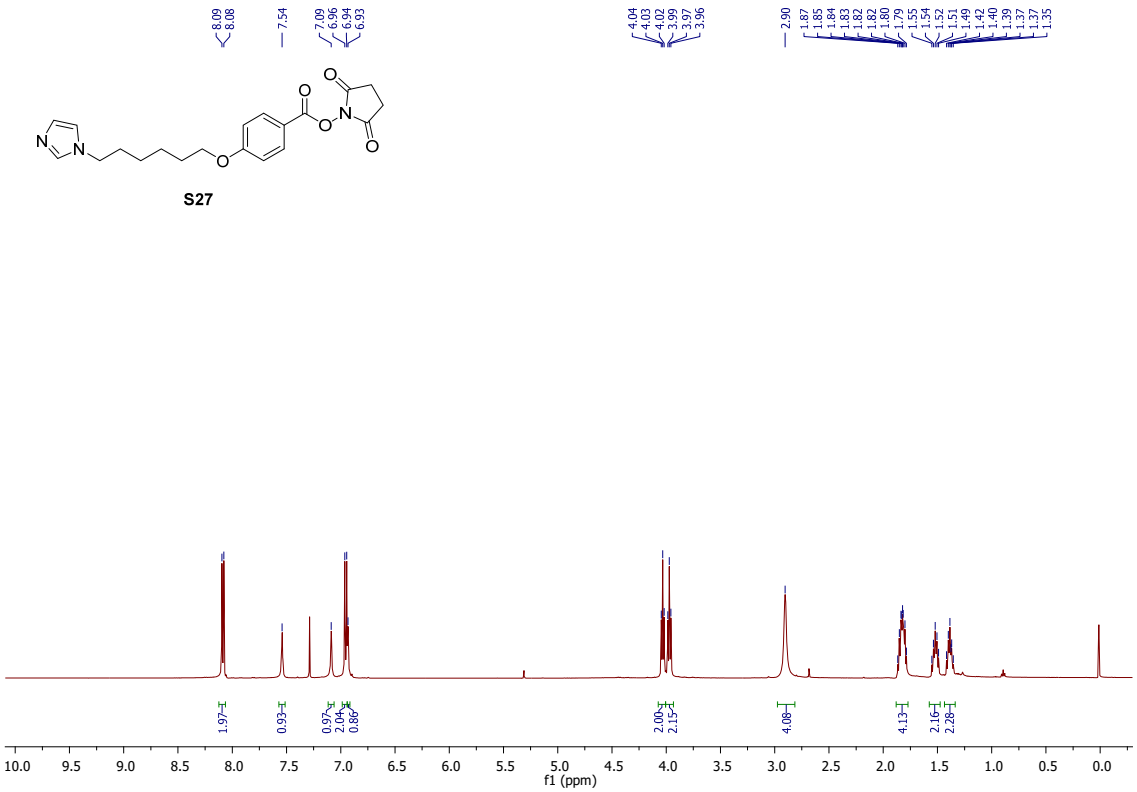
163.05
134.90
131.44
122.55
121.86
119.86
113.74
67.60
49.09
29.72
28.53
25.58
25.13
N
N
O
OH
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

S26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7.54
7.09
6.96
6.94
6.93
4.04
4.03
4.02
3.99
3.97
3.96
2.90
1.87
1.85
1.84
1.83
1.82
1.82
1.80
1.79
1.55
1.54
1.52
1.51
1.49
1.42
1.40
1.39
1.37
1.37
1.35
N
N
O
O
O
N
O
O
1.97
0.93
0.97
2.04
0.86
2.00
2.15
4.08
4.13
2.16
2.28
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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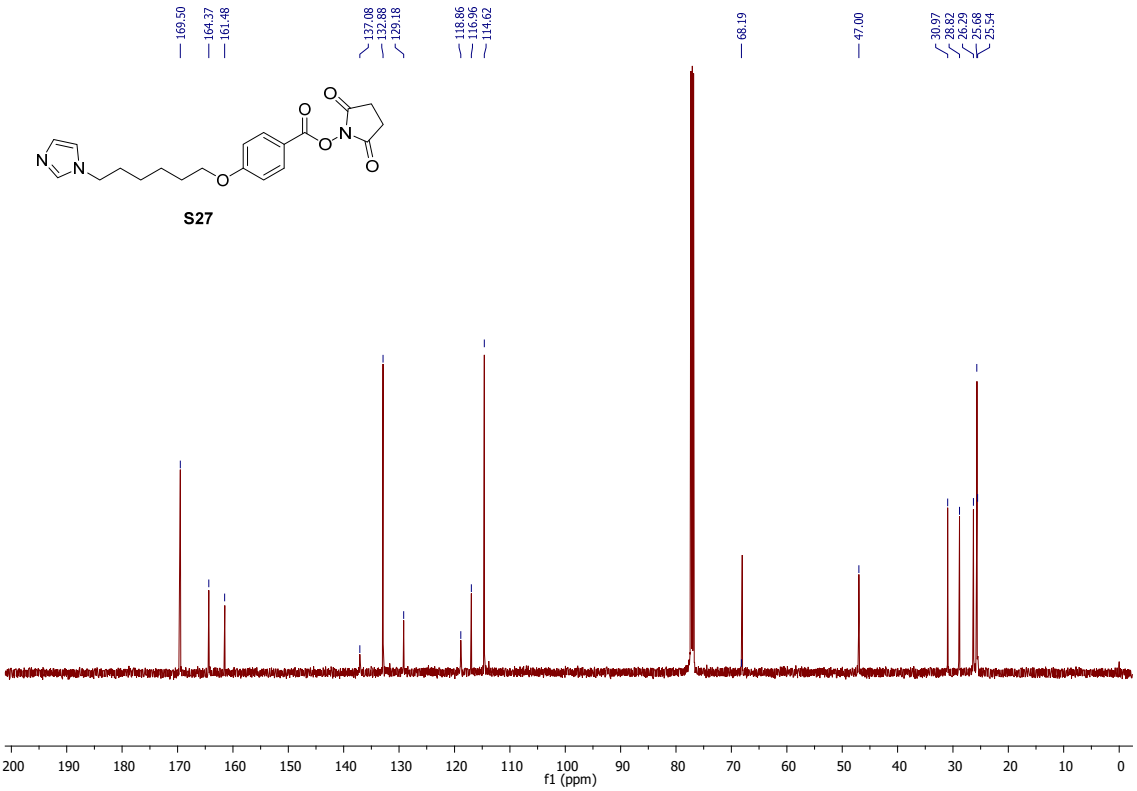
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

S27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137.08
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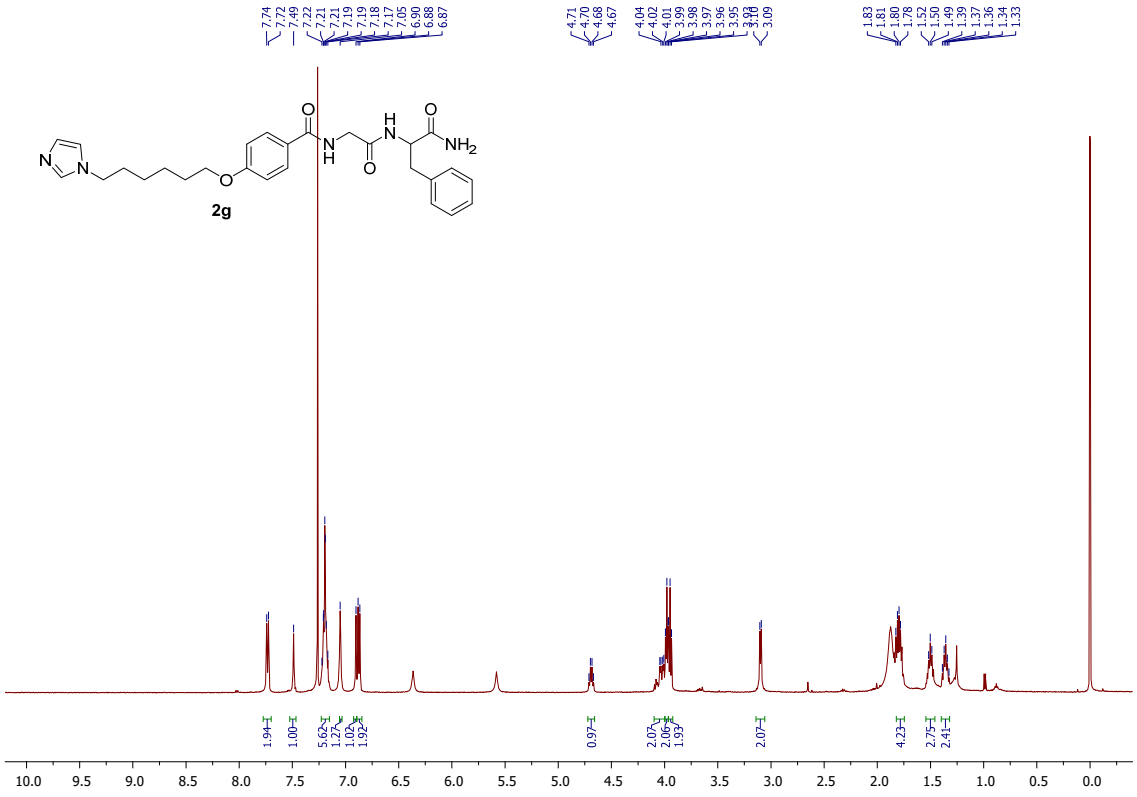
129.18
118.86
116.96
114.62
68.19
47.00
30.97
28.82
26.29
25.68
25.54
S27
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)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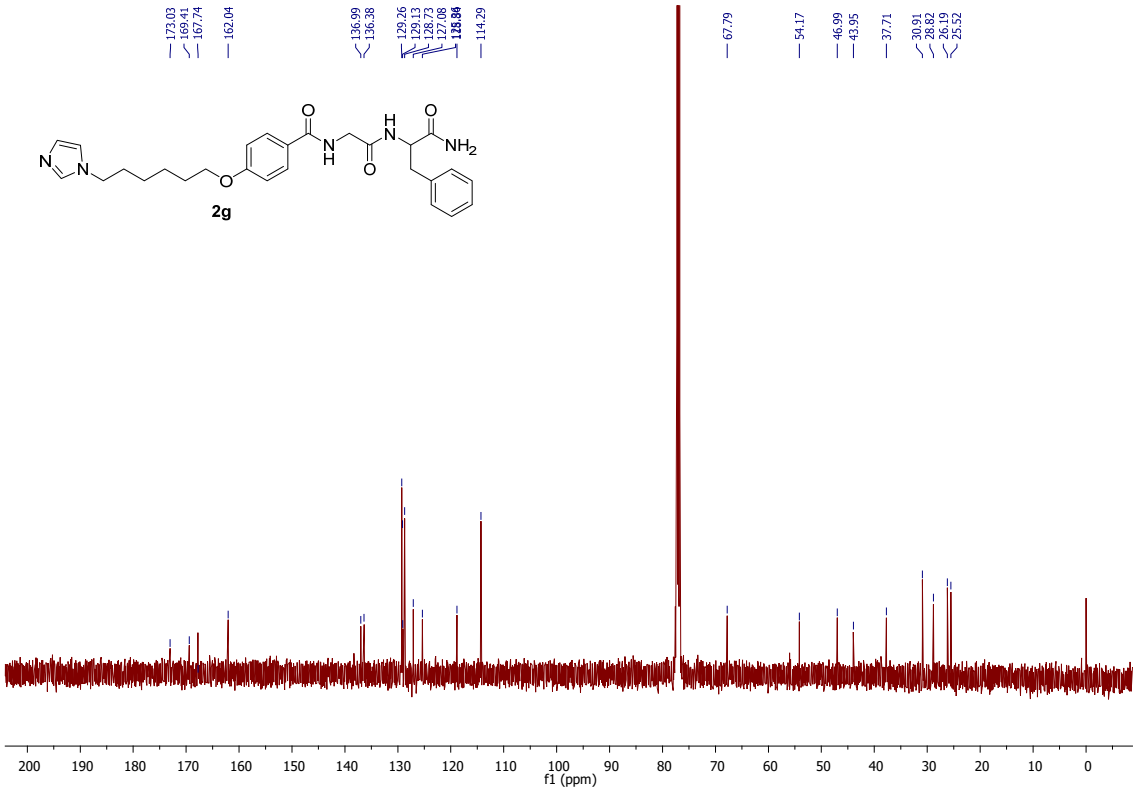
7.19
7.19
7.18
7.17
7.05
6.90
6.88
6.87
4.71
4.70
4.68
4.67
4.04
4.02
4.01
3.99
3.98
3.97
3.96
3.95
3.10
3.09
1.83
1.81
1.80
1.78
1.52
1.50
1.49
1.39
1.37
1.36
1.34
1.33
2g
1.94
1.00
5.62
1.27
1.02
1.92
0.97
2.07
2.06
1.93
2.07
4.23
2.75
2.41
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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162.04
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136.38
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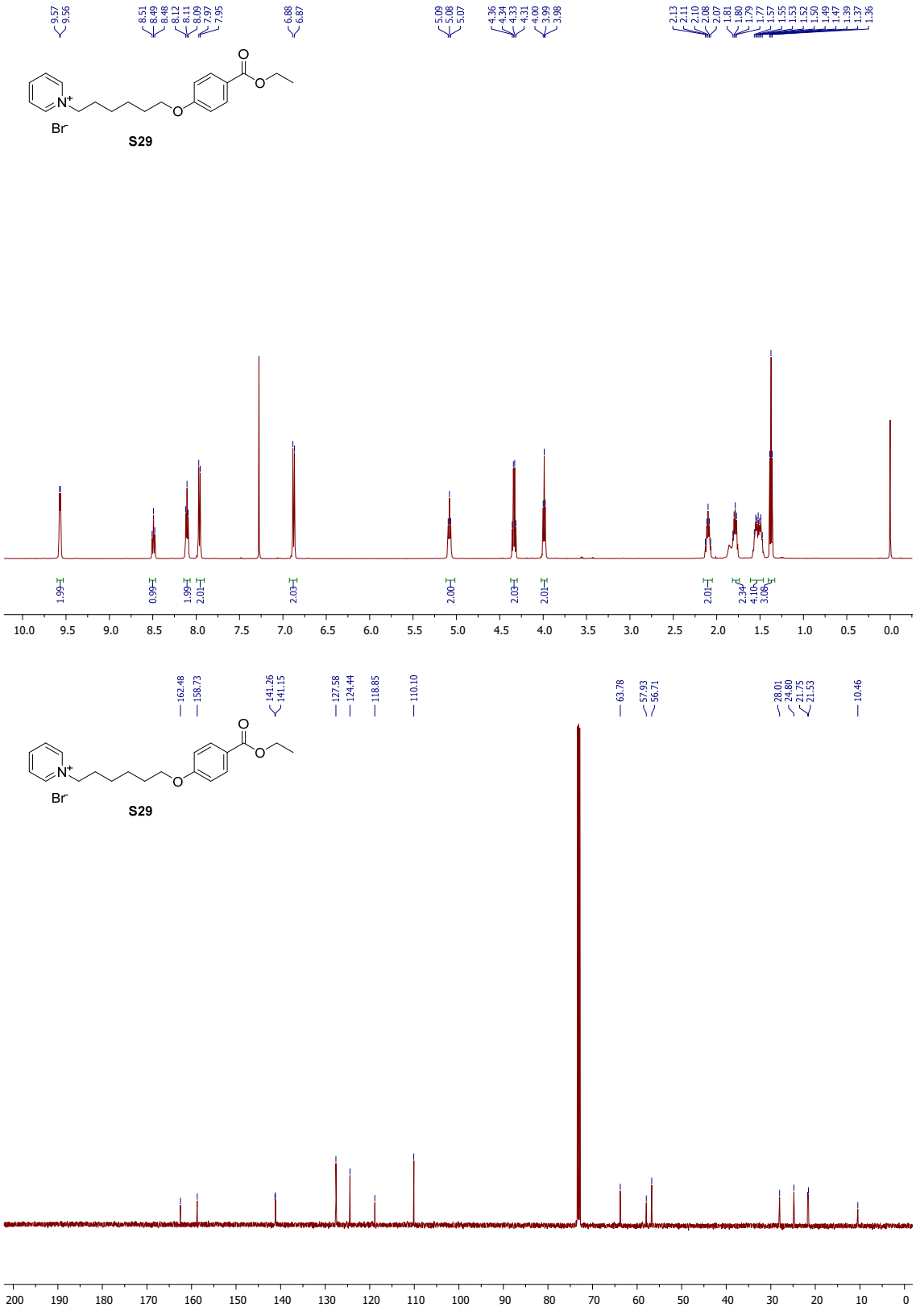
129.13
128.73
127.08
125.86
114.29
67.79
54.17
46.99
43.95
37.71
30.91
28.82
26.19
25.52
2g
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.12
8.11
8.09
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7.95
6.88
6.87
5.09
5.08
5.07
4.36
4.34
4.33
4.31
4.00
3.99
3.98
2.13
2.11
2.10
2.08
2.07
1.81
1.80
1.79
1.77
1.57
1.55
1.53
1.52
1.50
1.49
1.47
1.39
1.37
1.36
Br
S29
1.99
0.99
1.99
2.01
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2.03
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2.01
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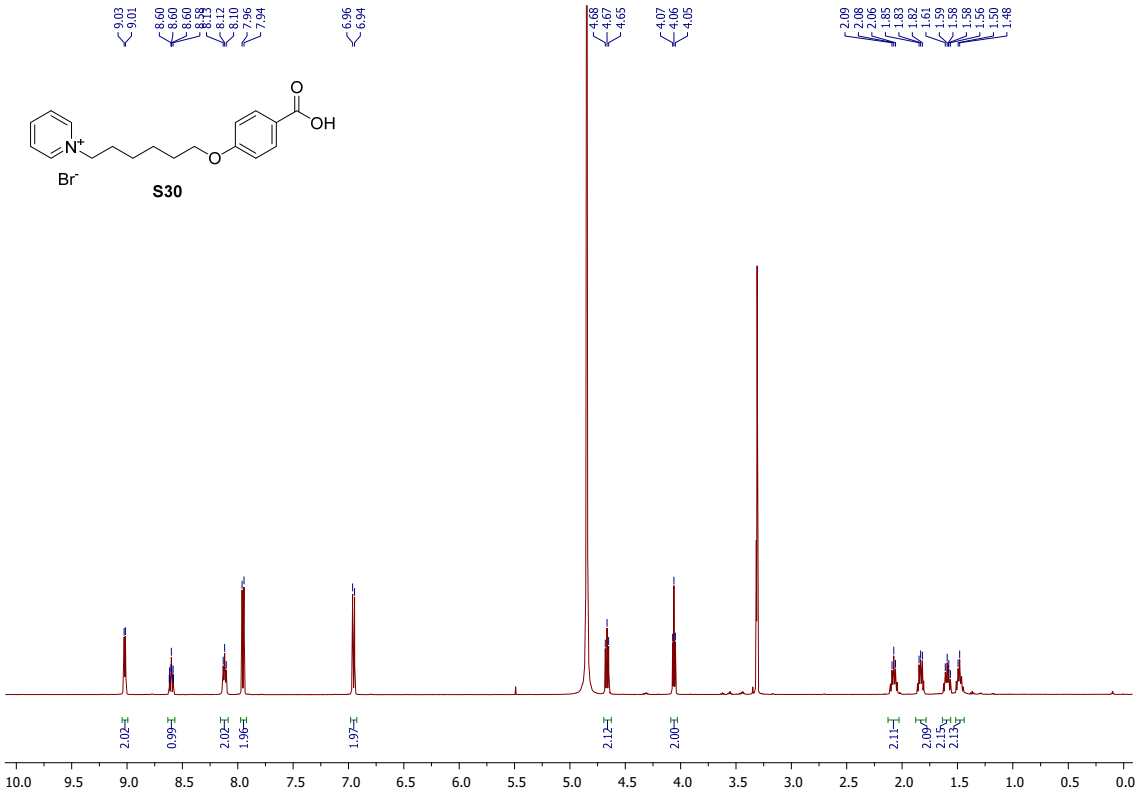
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4.05
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2.08
2.06
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1.82
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1.56
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1.48
O
OH
N+
O
Br-
S30
2.02
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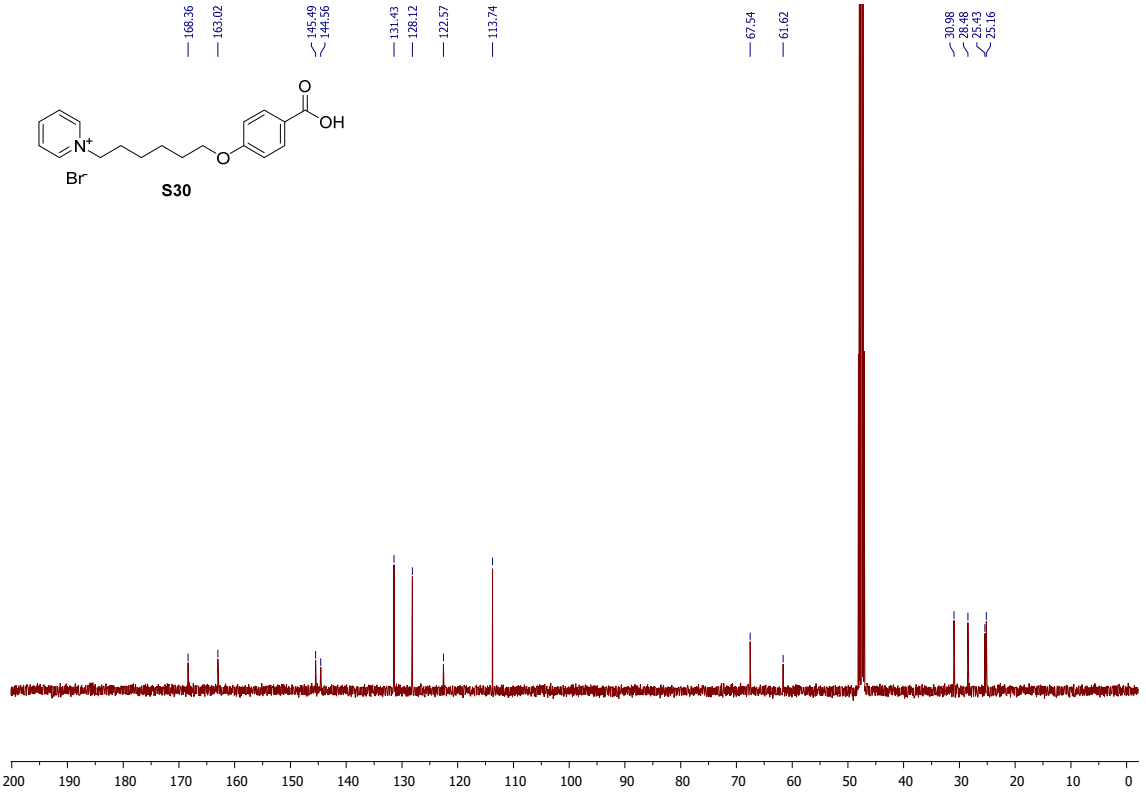
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OH
N+
O
Br-
S30
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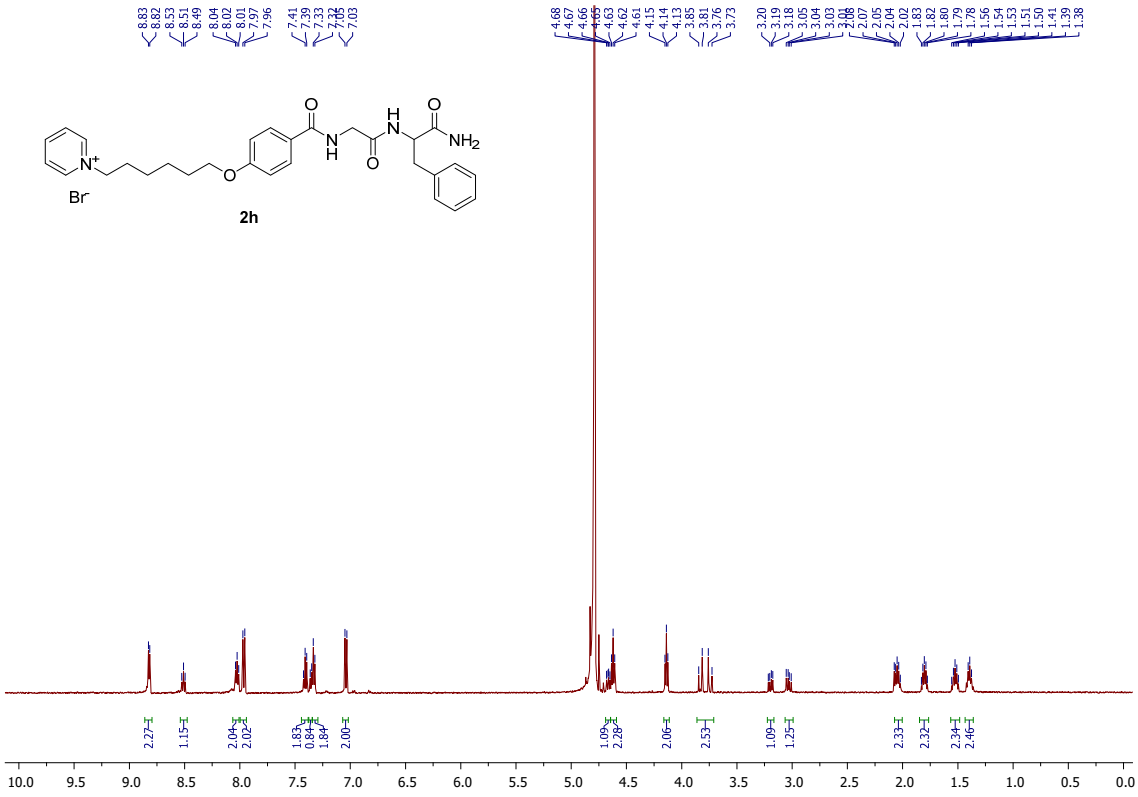
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H
O
N
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Br-
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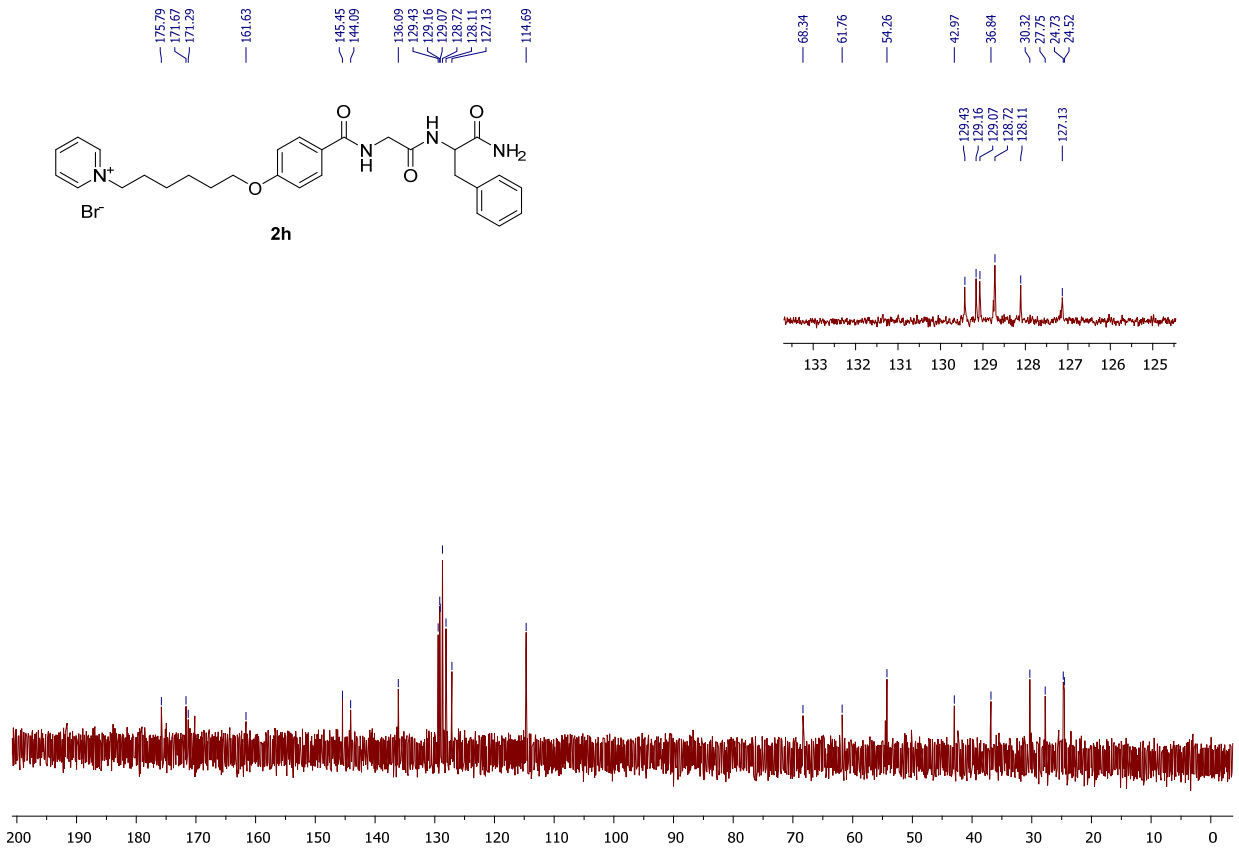
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N
H
O
H
N
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O
Br-
2h
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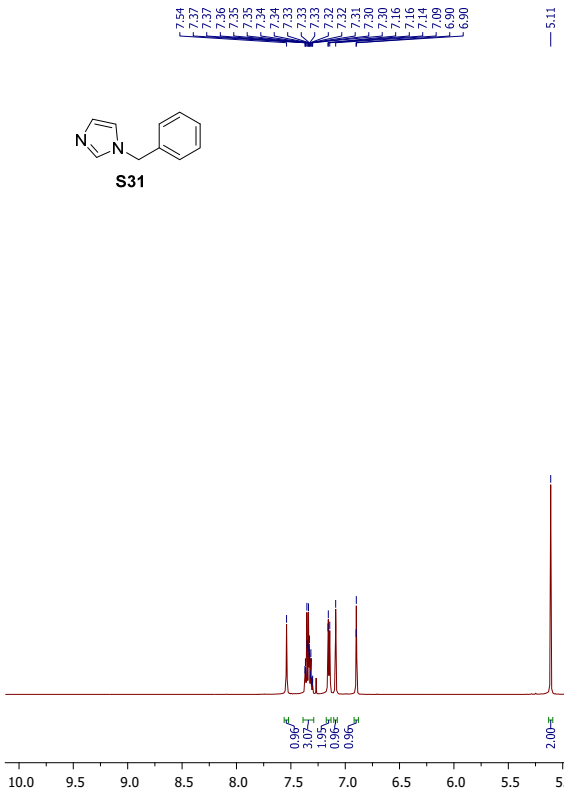
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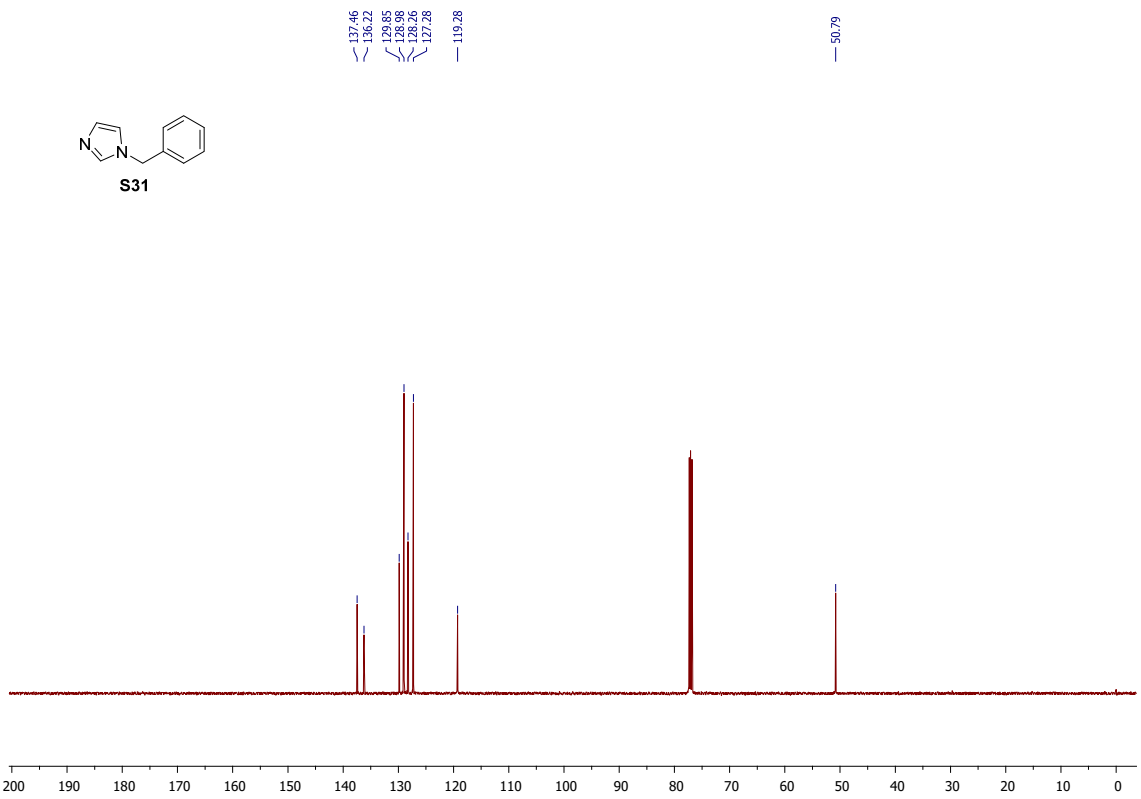
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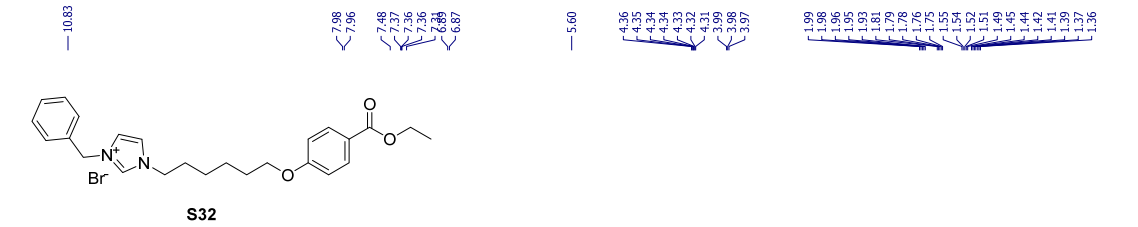
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N+
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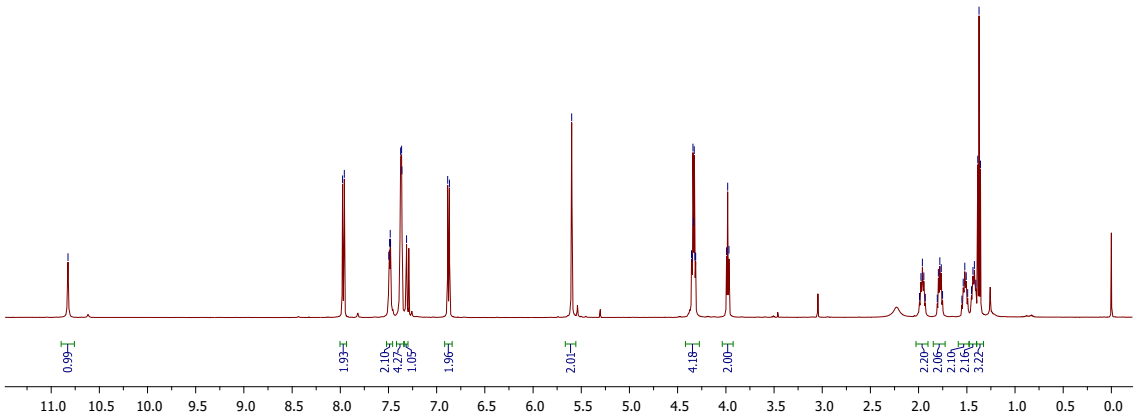
Br-
O
O
O

S32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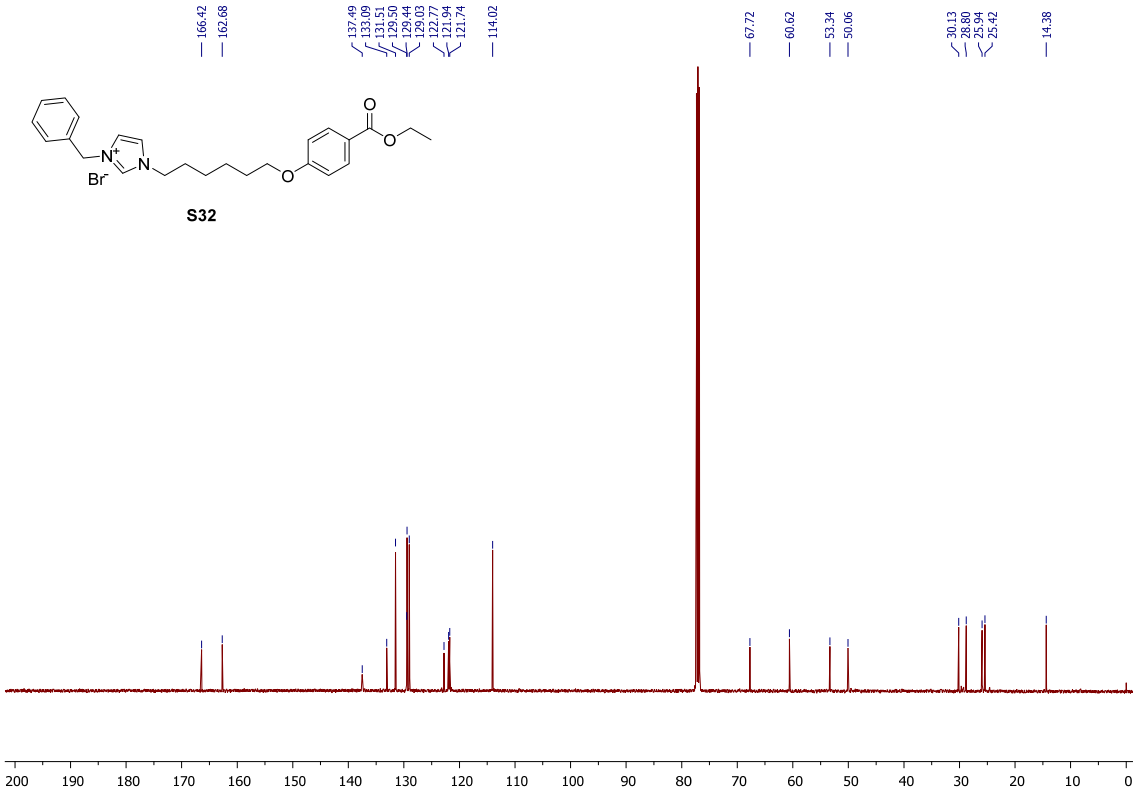
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200
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180
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150
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130
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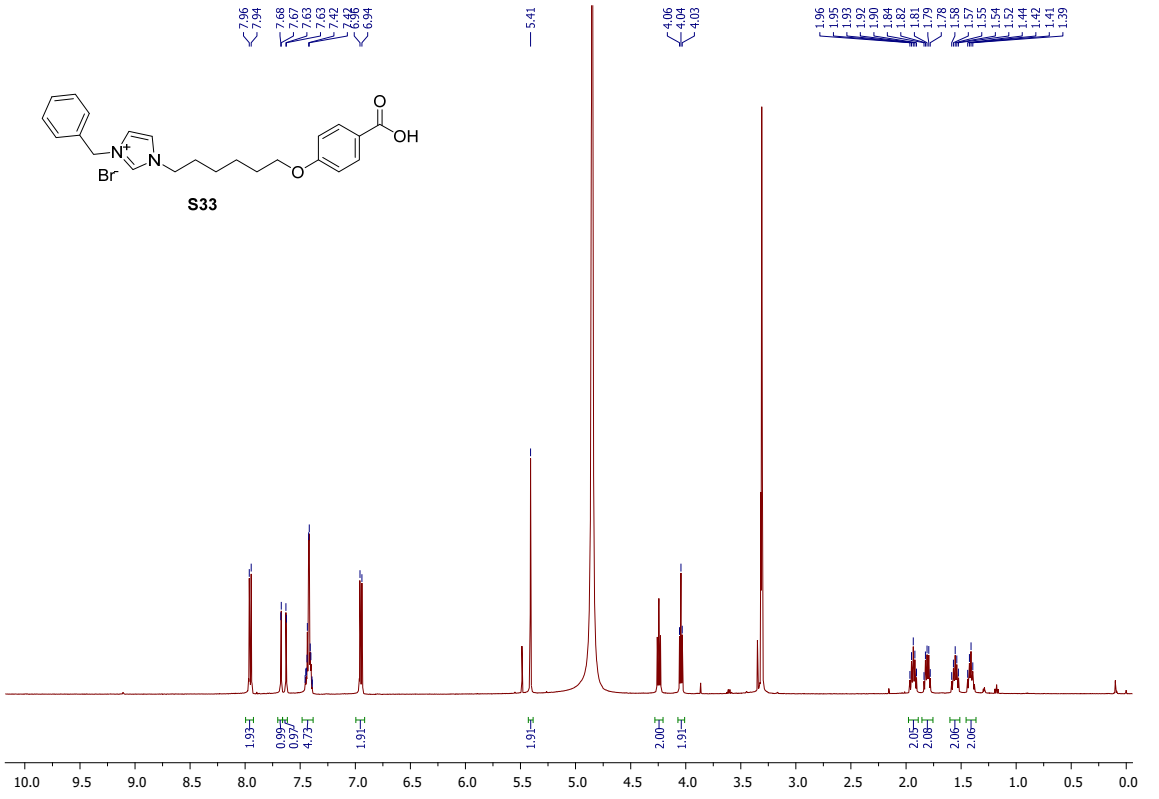
N+
N
Br-
O
O
O

S32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OH
N+
N
Br-
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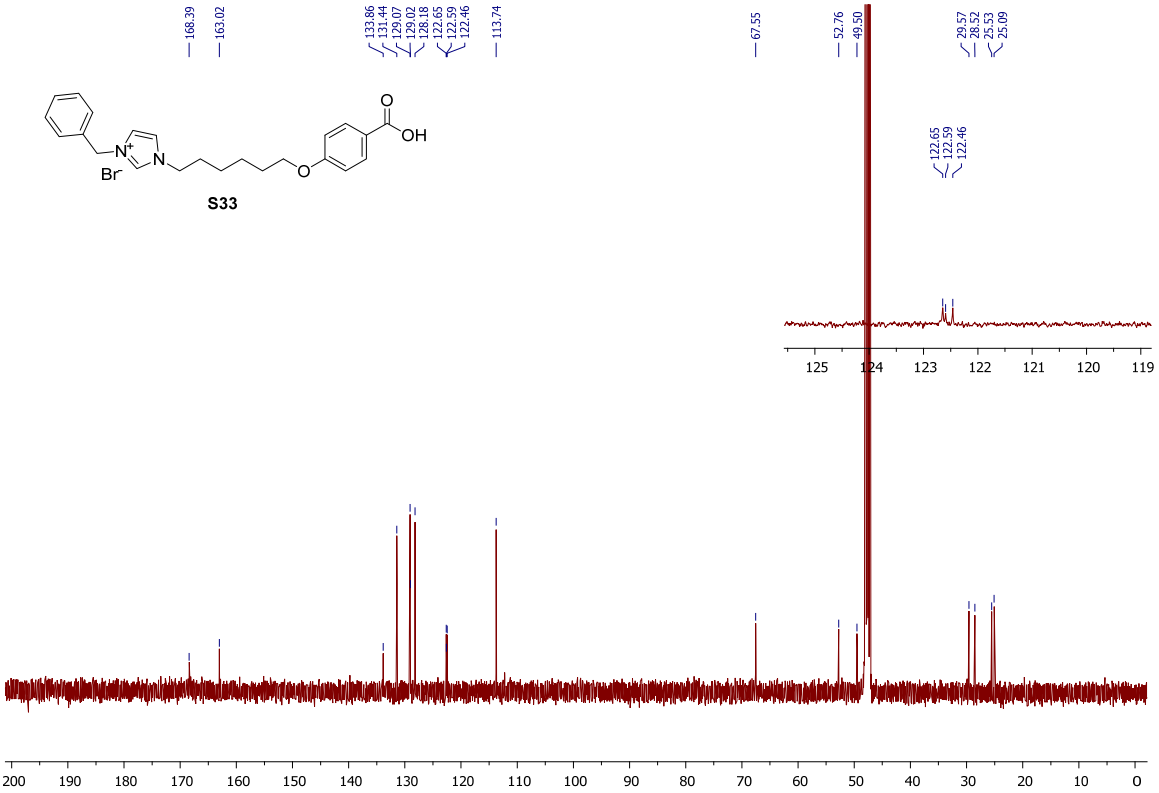
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28.52
25.53
25.09
O
OH
N+
N
Br-
O
122.65
122.59
122.46
125
124
123
122
121
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200
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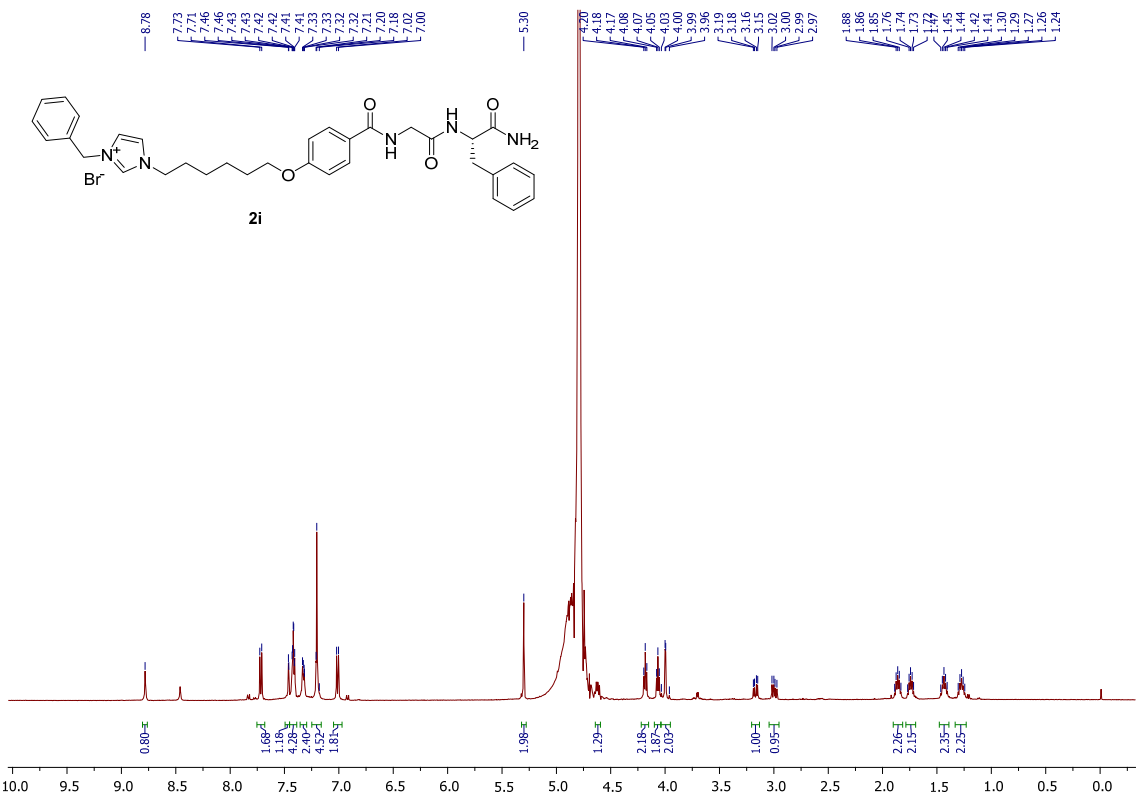
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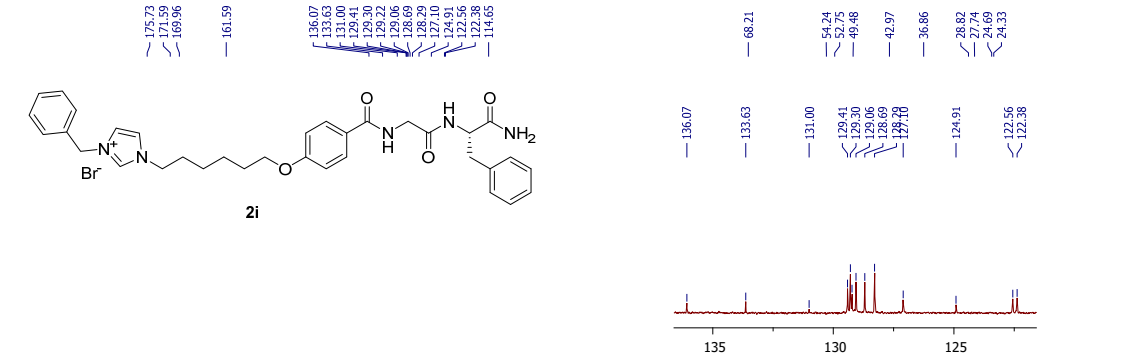
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Br-
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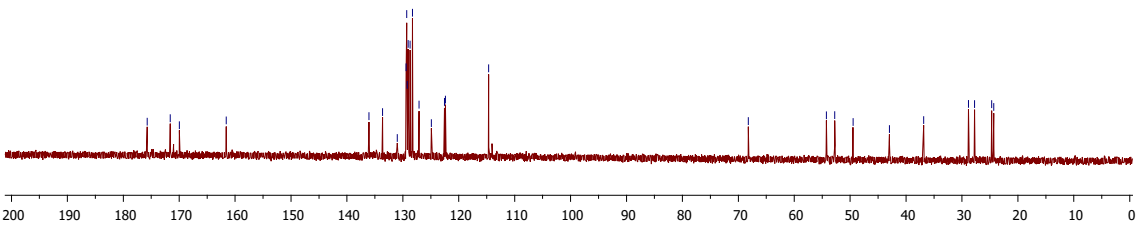
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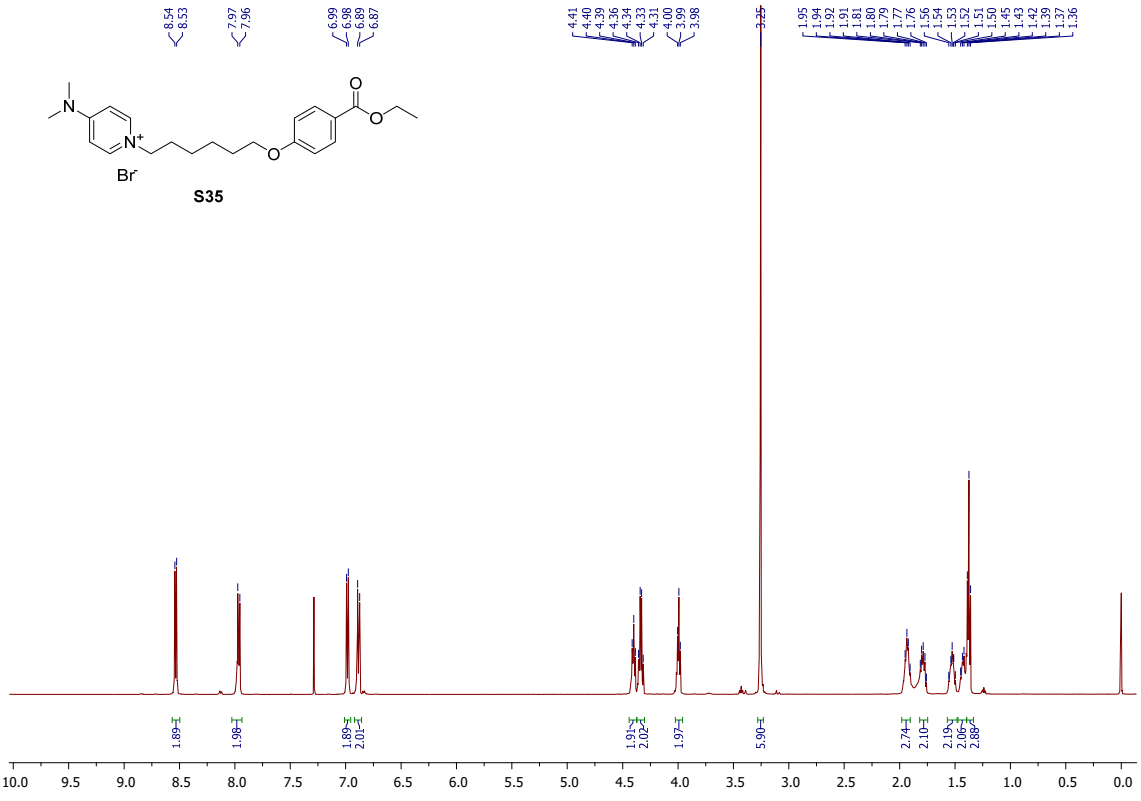
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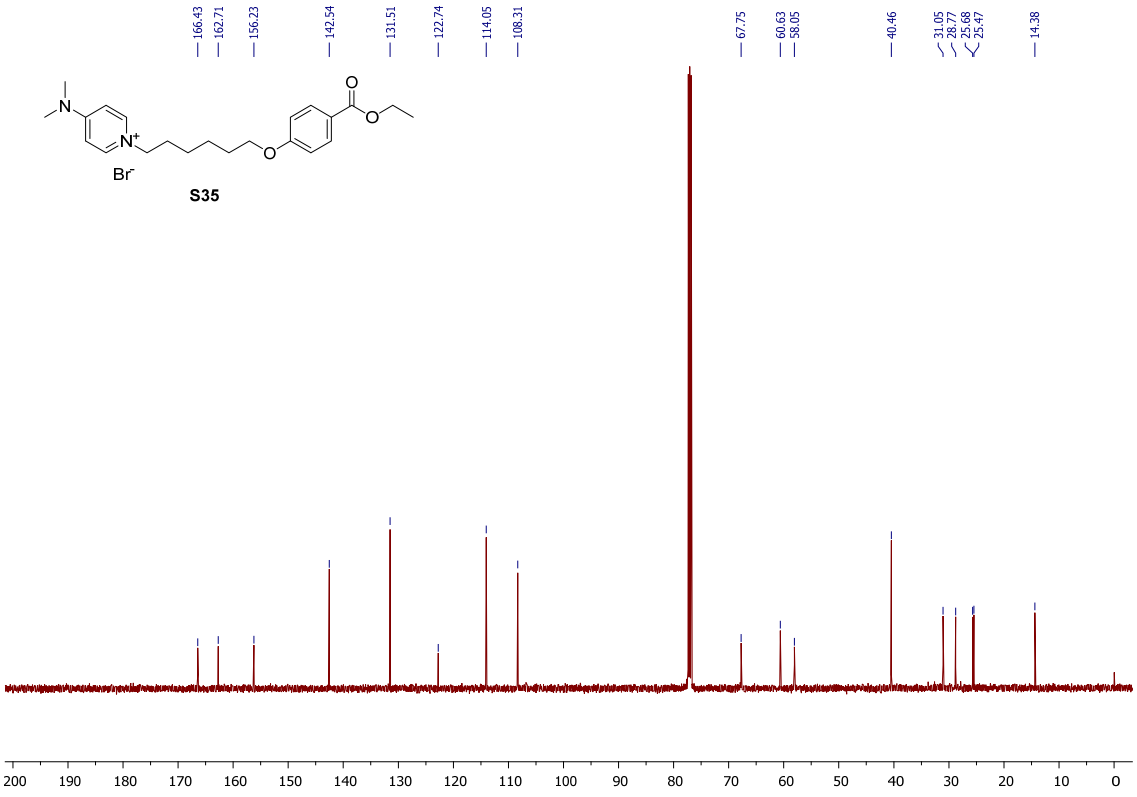
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Br-
S35
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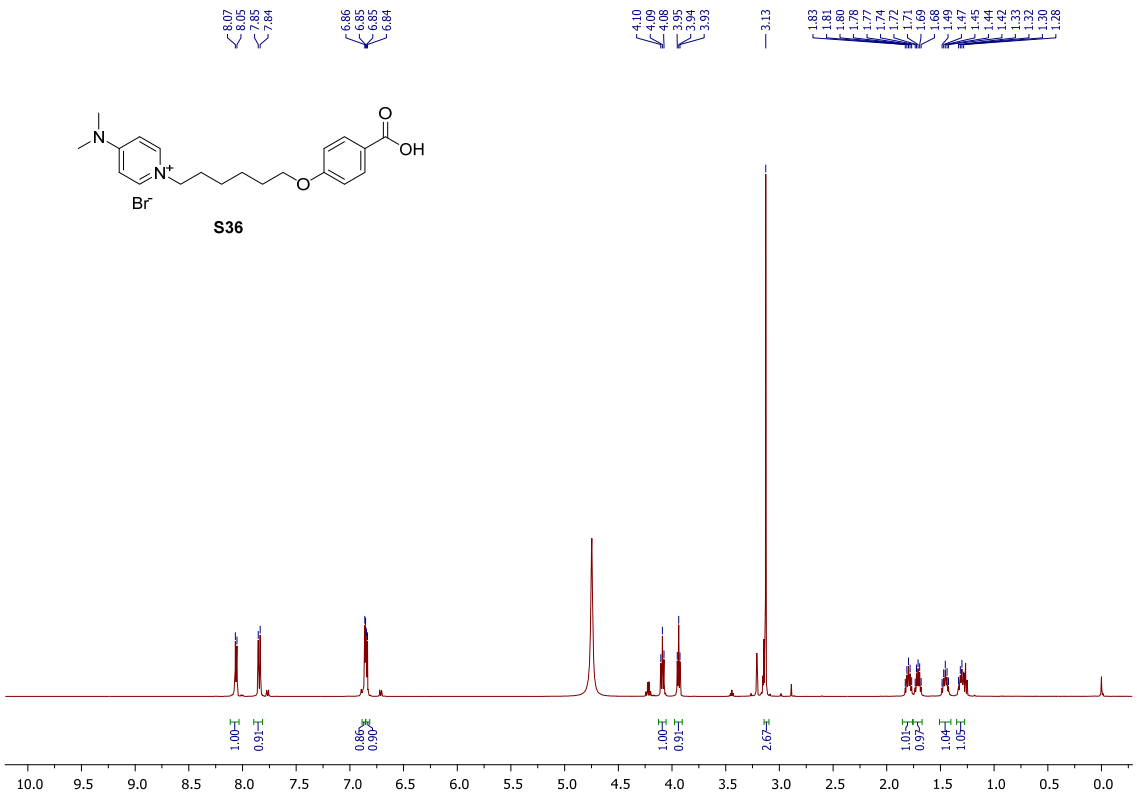
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O
Br-
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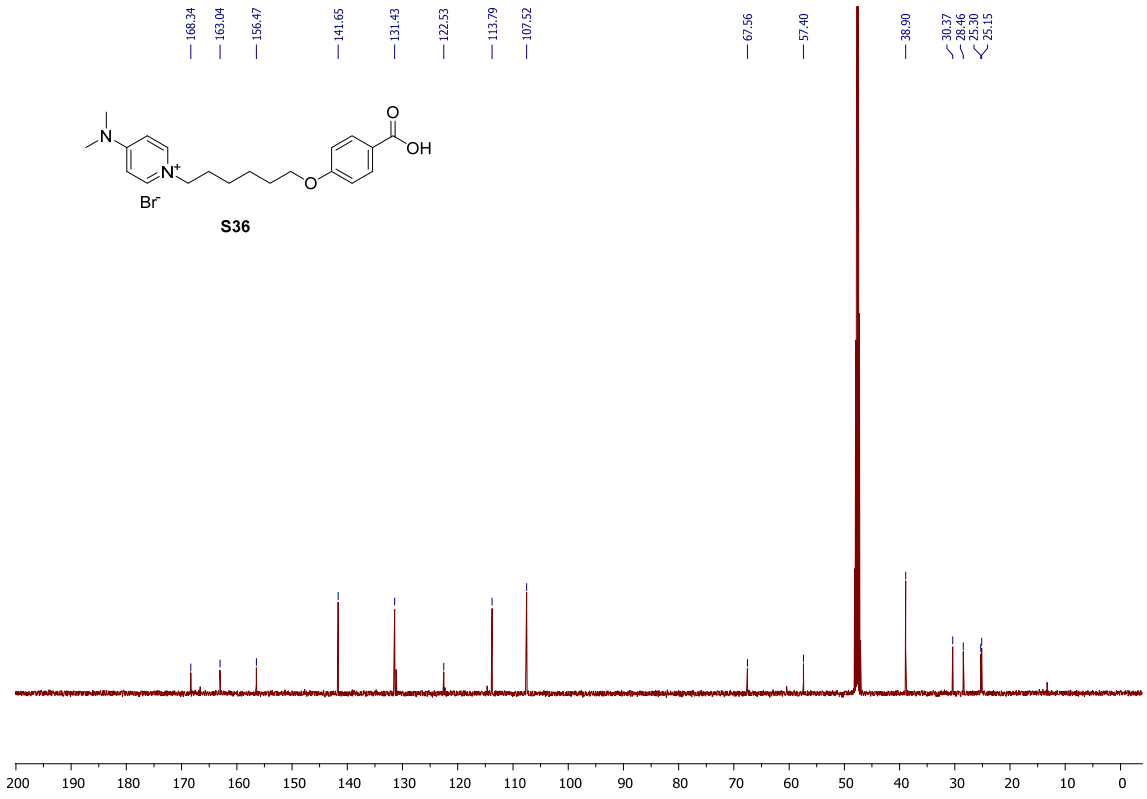
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N+
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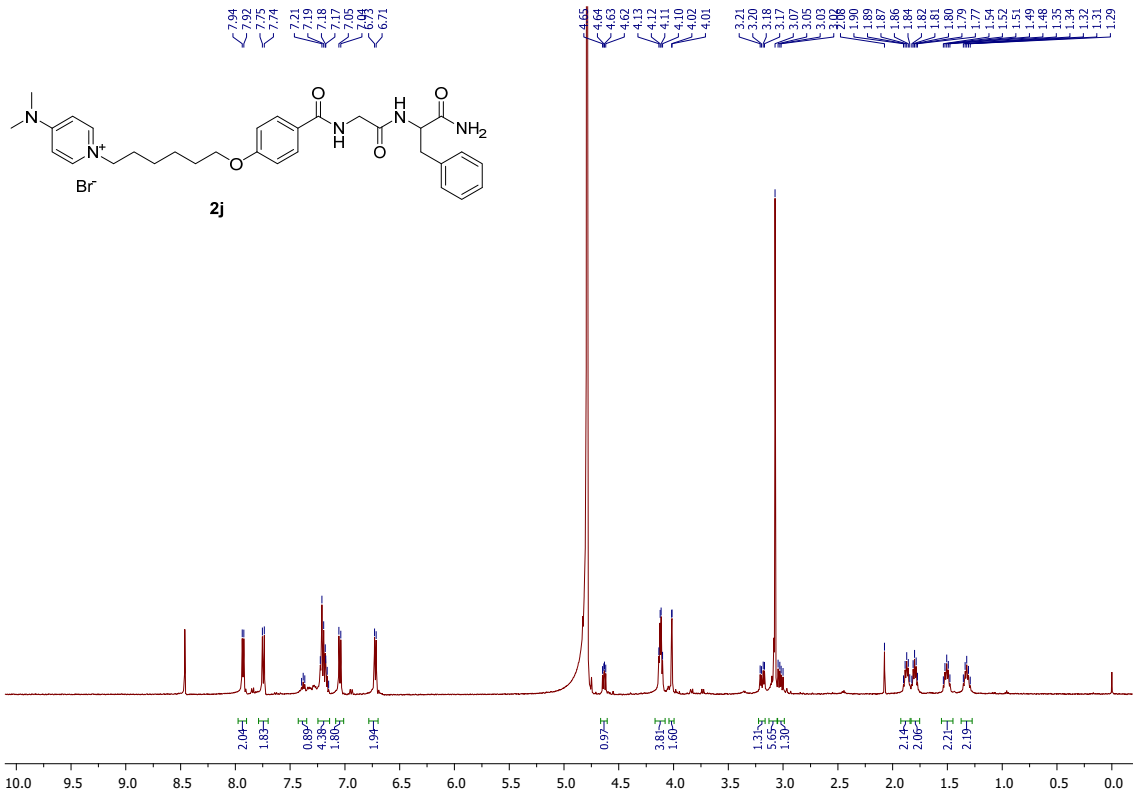
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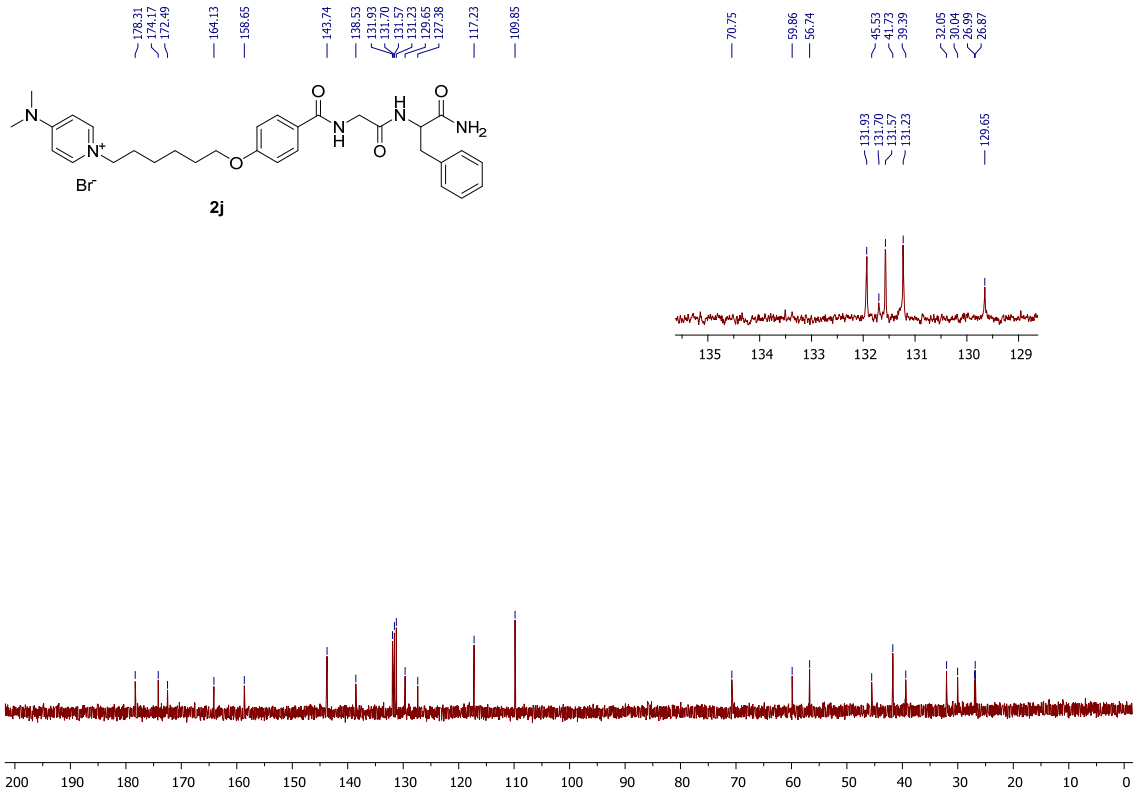
Br
2j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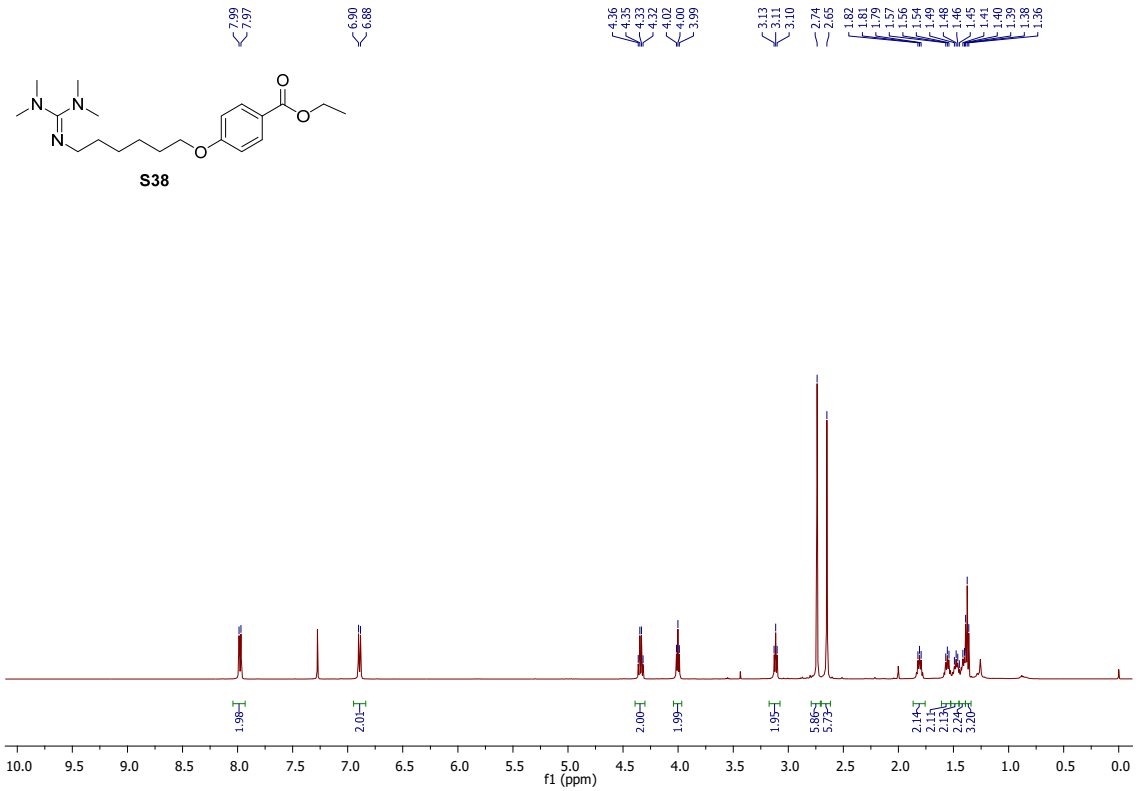
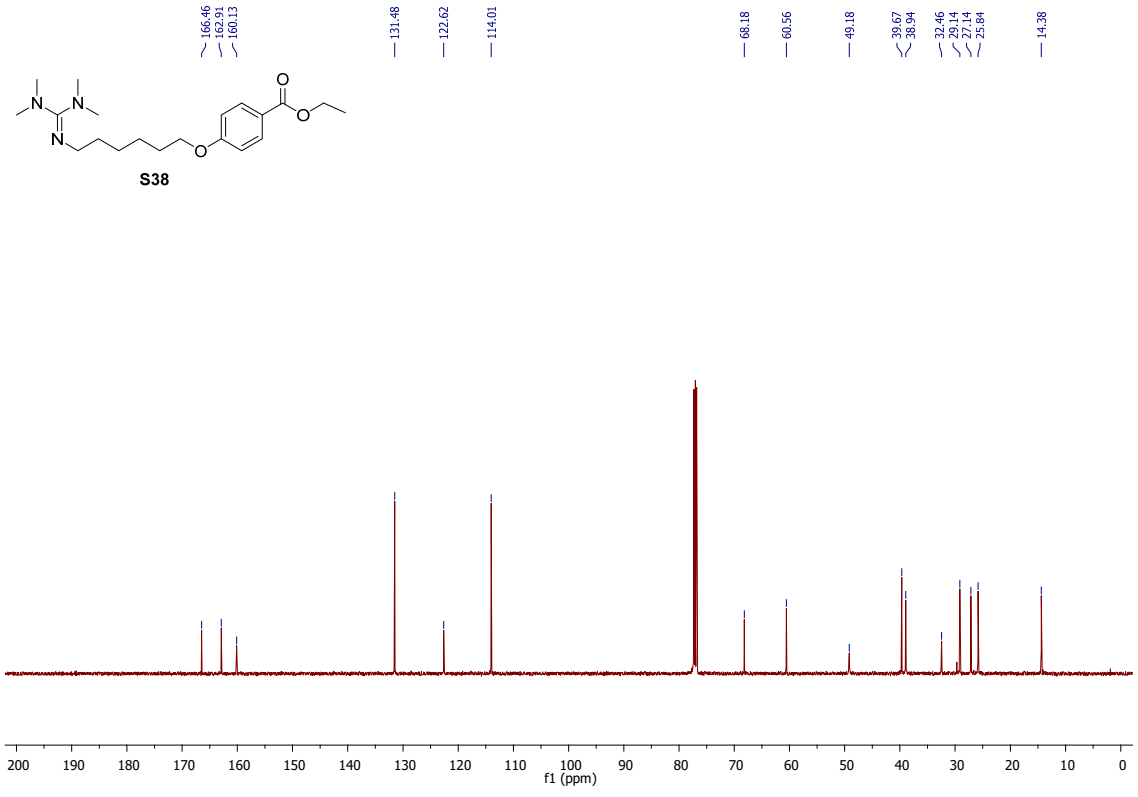
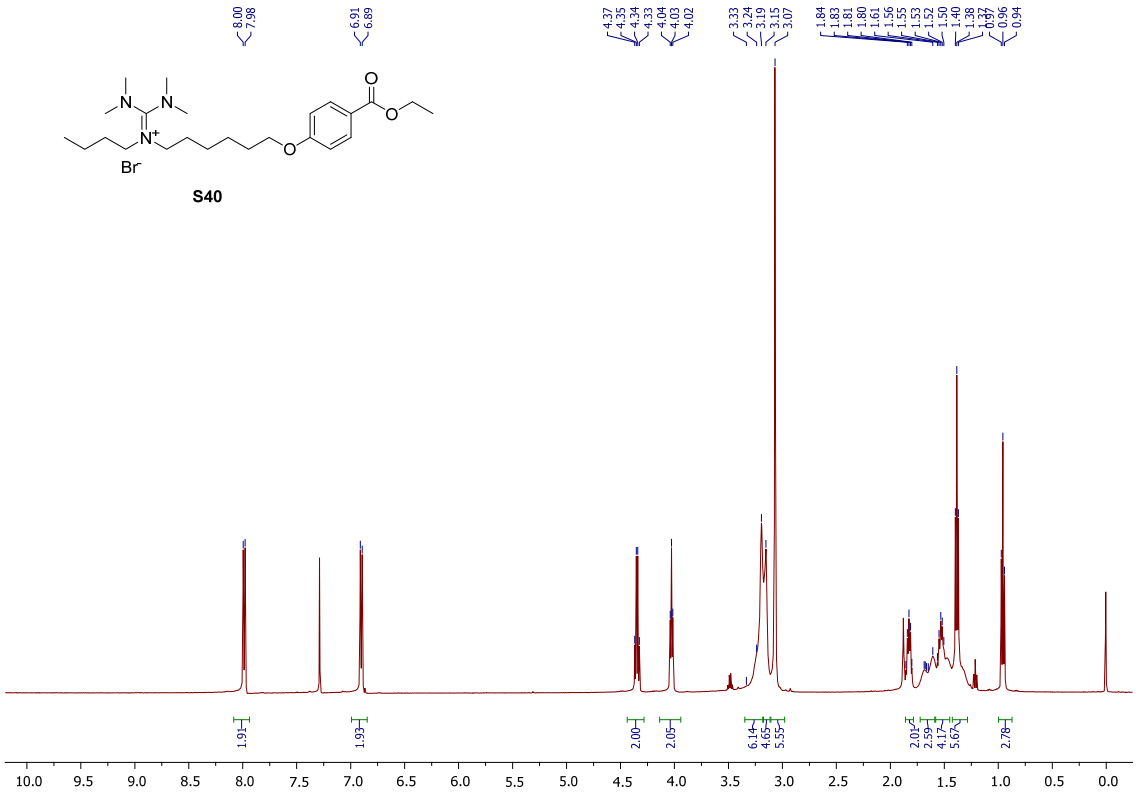
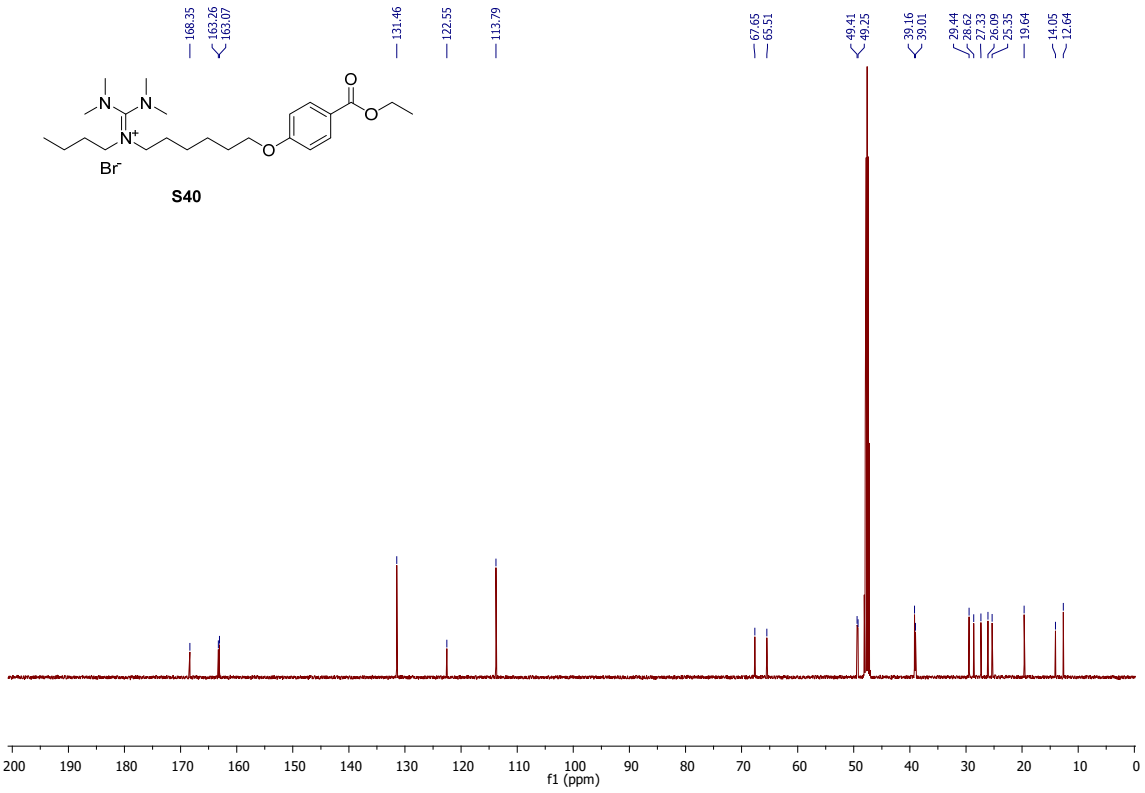
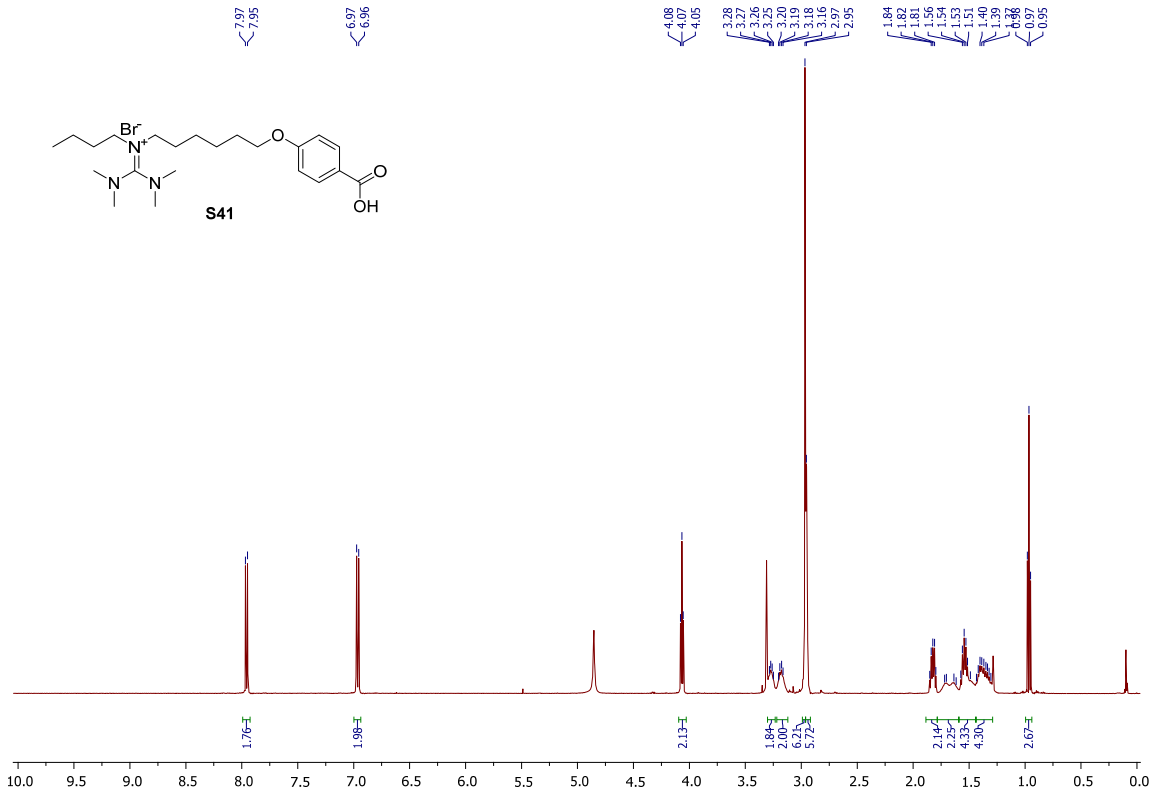
Br
2j

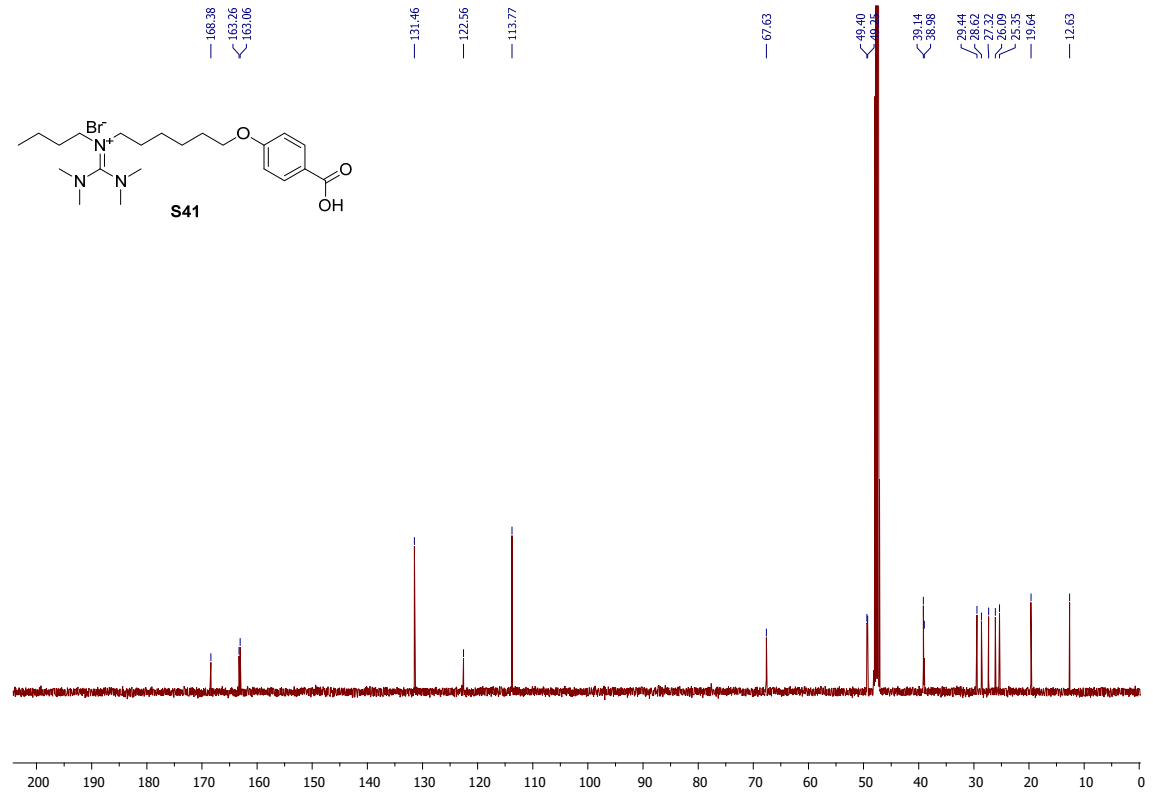

7.99
7.97
6.90
6.88
4.36
4.35
4.33
4.32
4.02
4.00
3.99
3.13
3.11
3.10
2.74
2.65
1.82
1.81
1.79
1.57
1.56
1.54
1.49
1.48
1.46
1.45
1.41
1.40
1.39
1.38
1.36
S38
1.98
2.01
2.00
1.99
1.95
5.86
5.73
2.14
2.11
2.13
2.24
3.20
f1 (ppm)

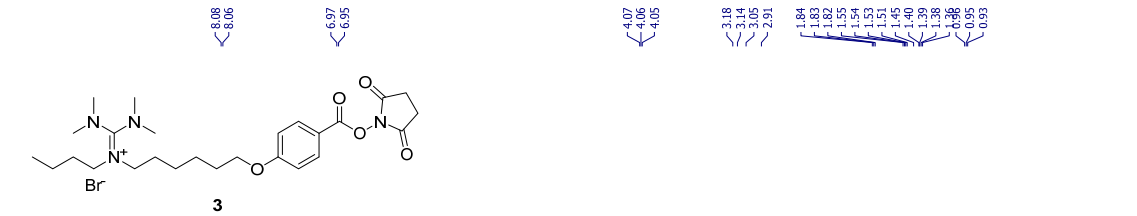

166.46
162.91
160.13
131.48
122.62
114.01
68.18
60.56
49.18
39.67
38.94
32.46
29.14
27.14
25.84
14.38
S38
f1 (ppm)


S40


S40
f1 (ppm)


S41


S41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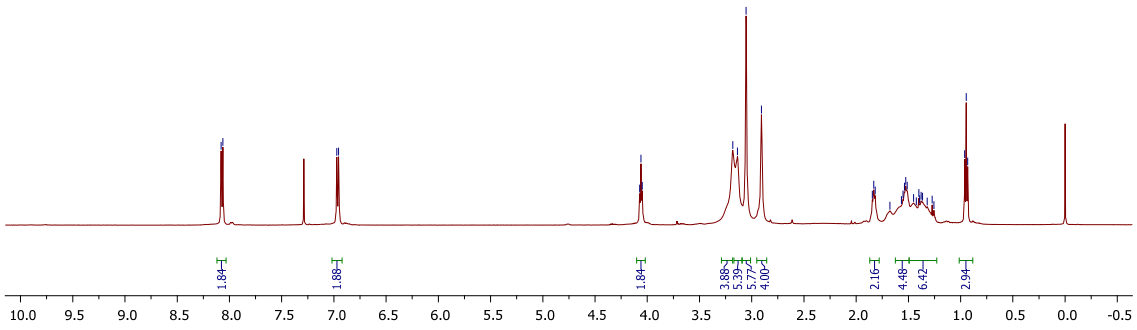
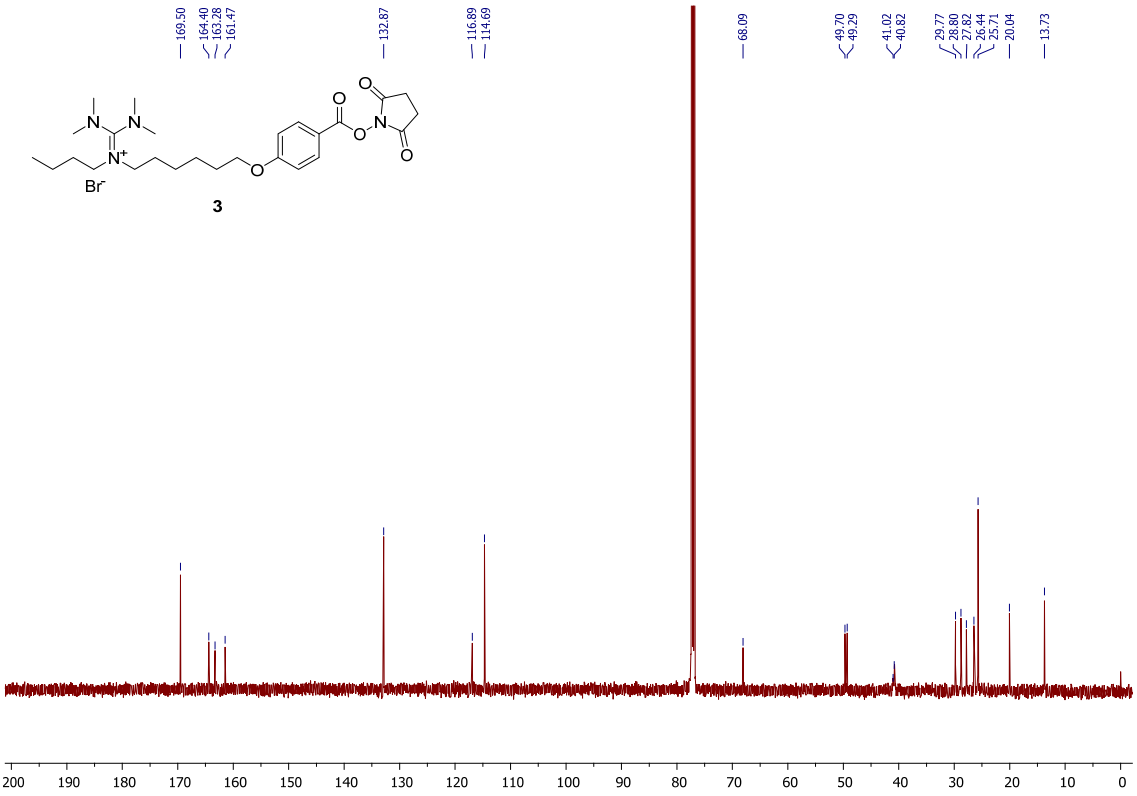
8.06
6.97
6.95
4.07
4.06
4.05
3.18
3.14
3.05
2.91
1.84
1.83
1.82
1.55
1.54
1.53
1.51
1.45
1.40
1.39
1.38
0.98
0.95
0.93
Br-
3


1.84
1.88
1.84
3.88
3.82
5.77
4.00
2.16
4.49
6.42
2.94
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5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164.40
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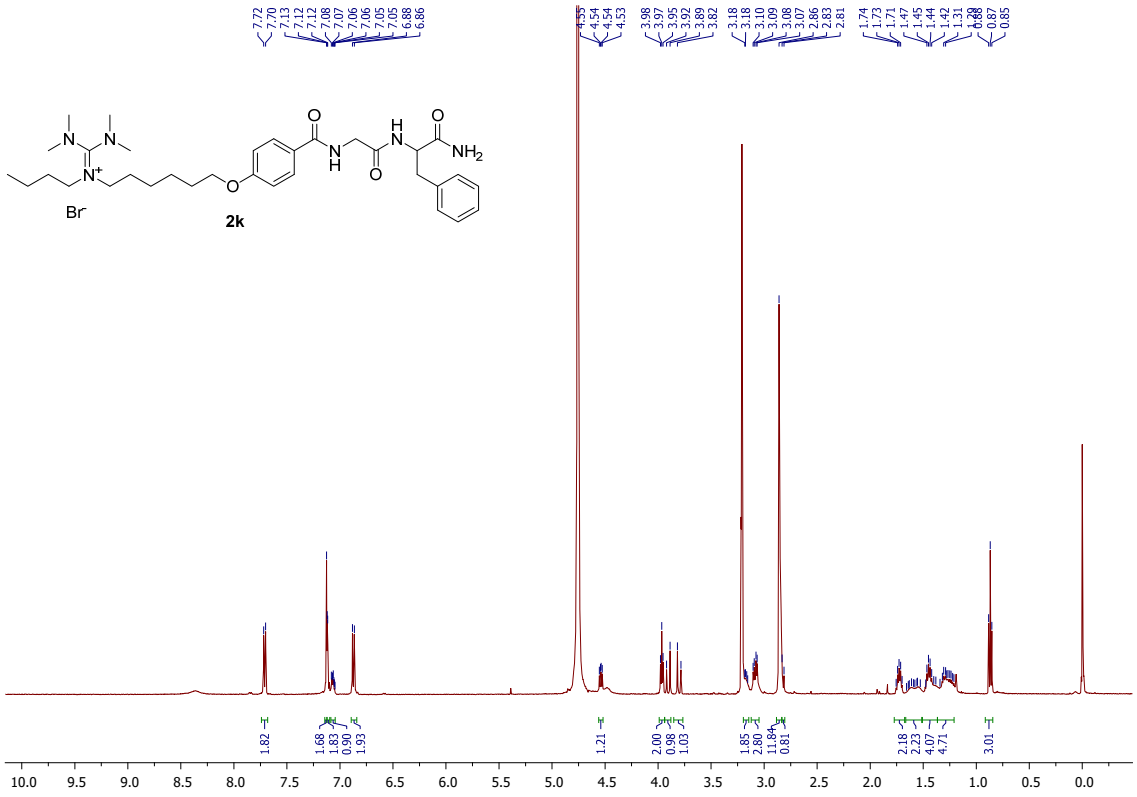
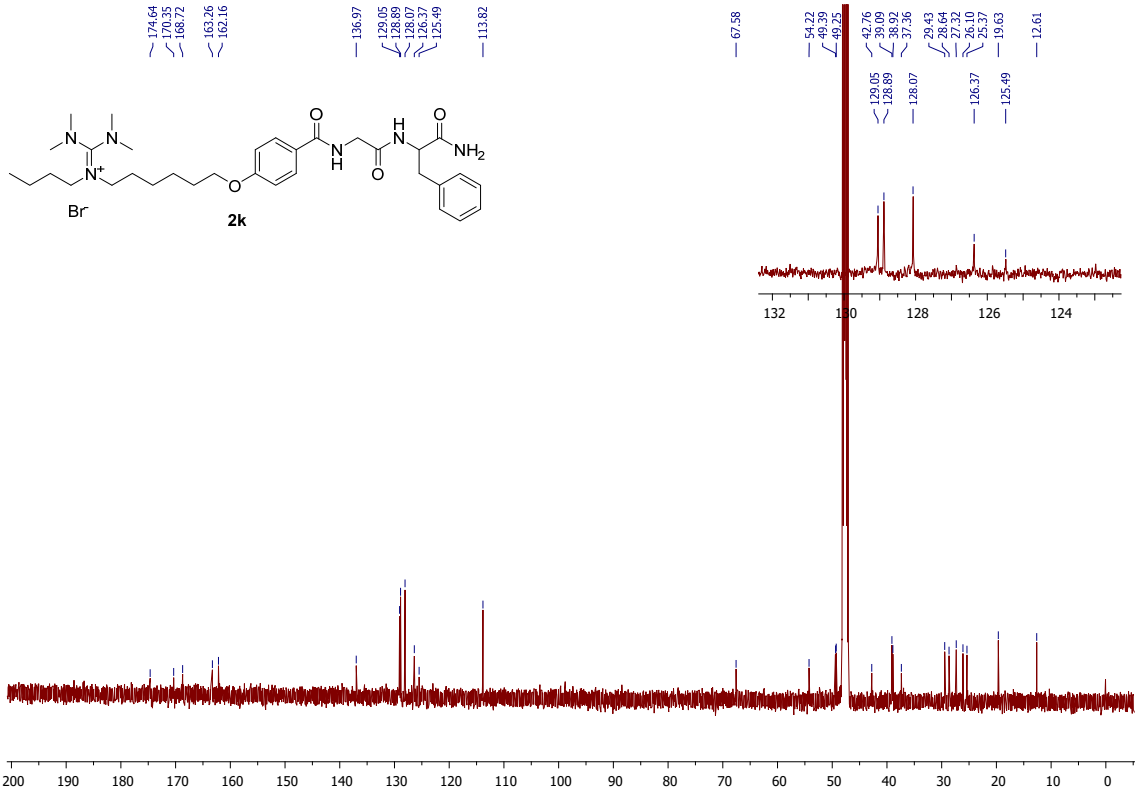
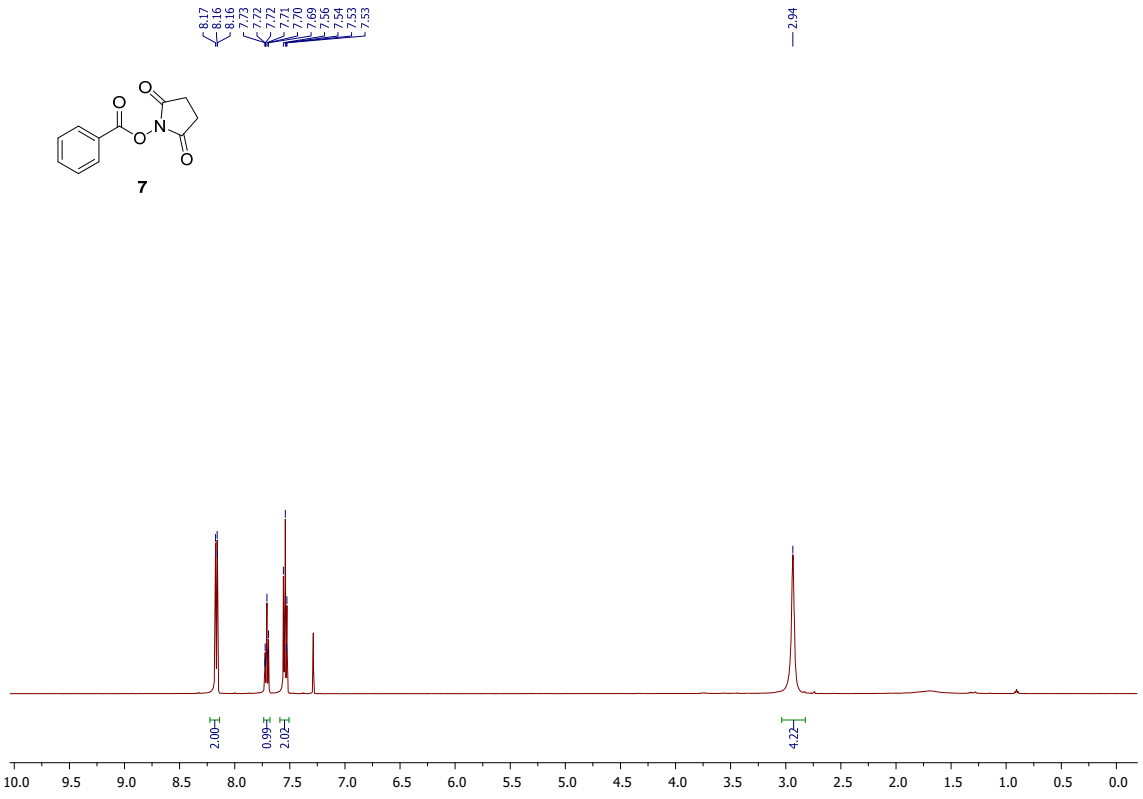
116.89
114.69
68.09
49.70
49.29
41.02
40.82
29.77
28.80
27.82
26.44
25.71
20.04
13.73
Br-
3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


Br⁻
2k


Br⁻
2k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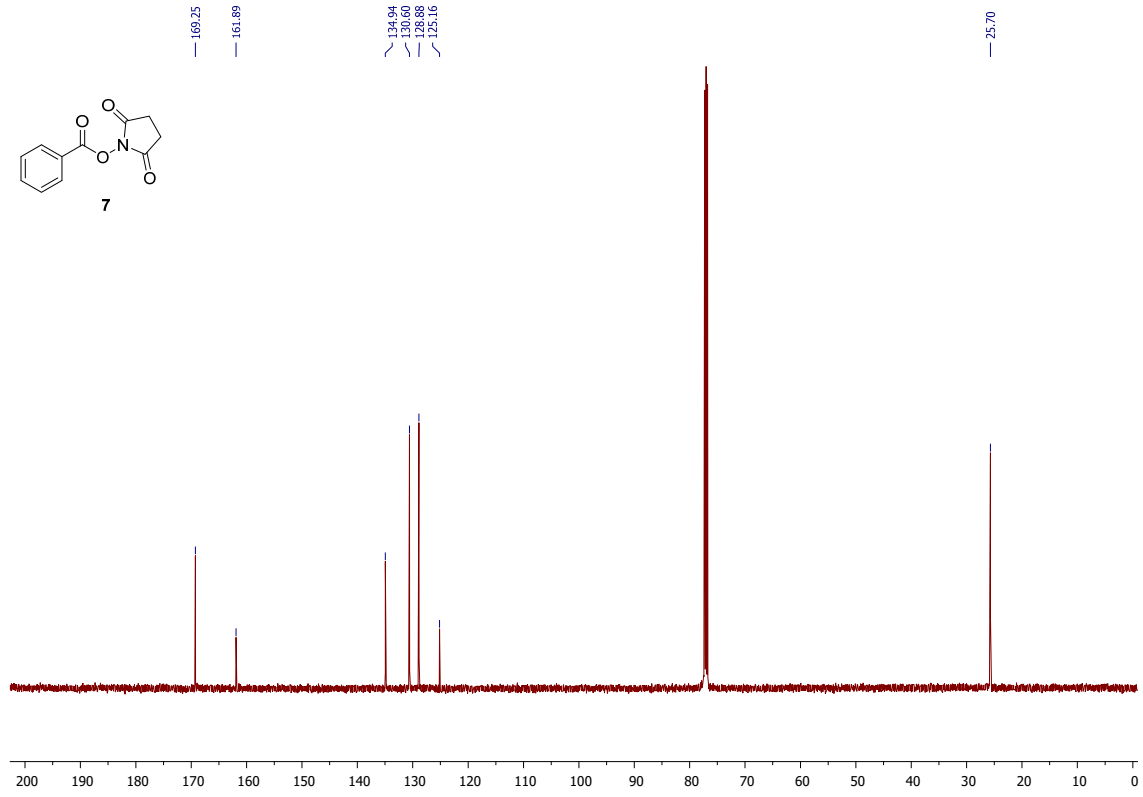
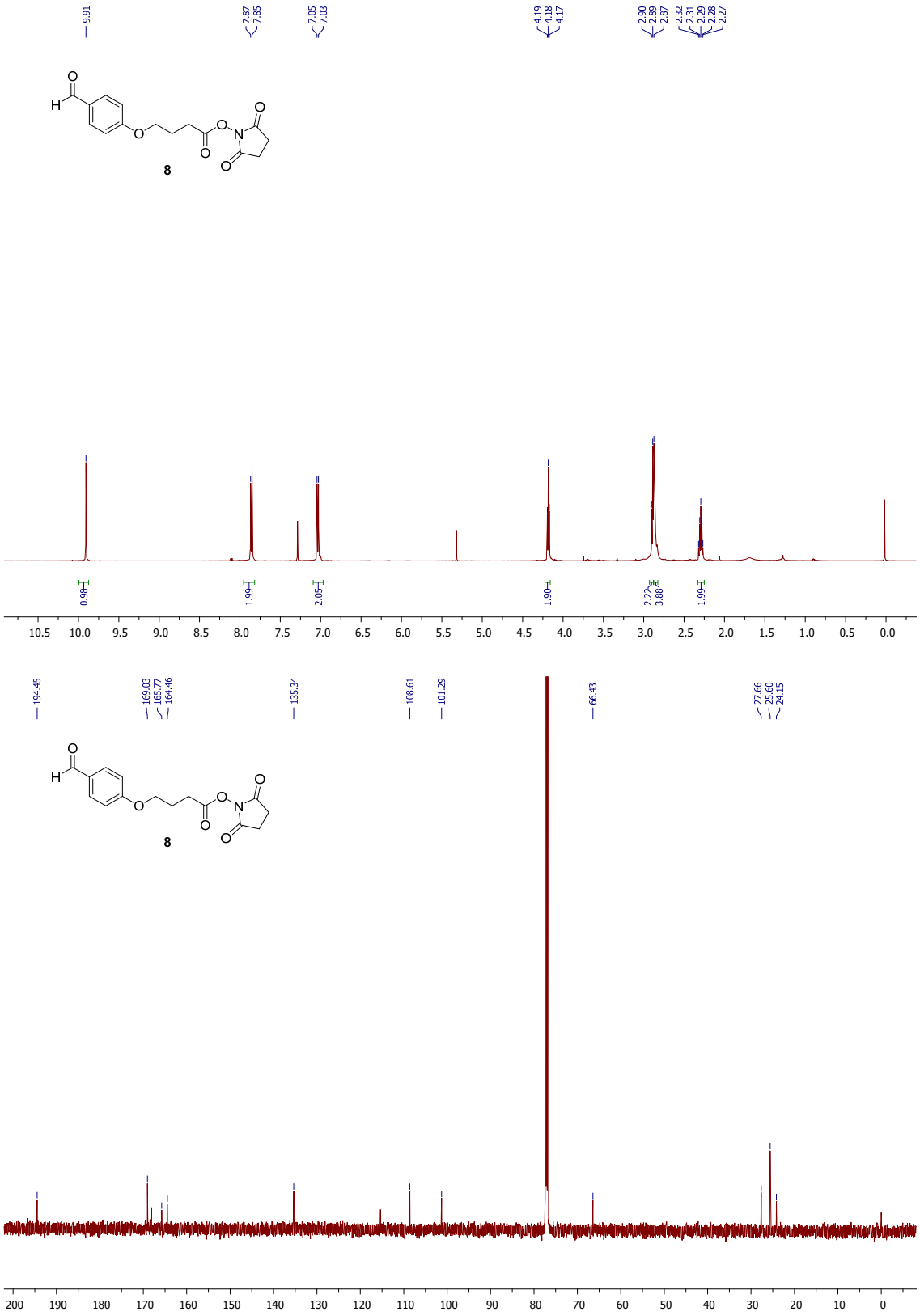
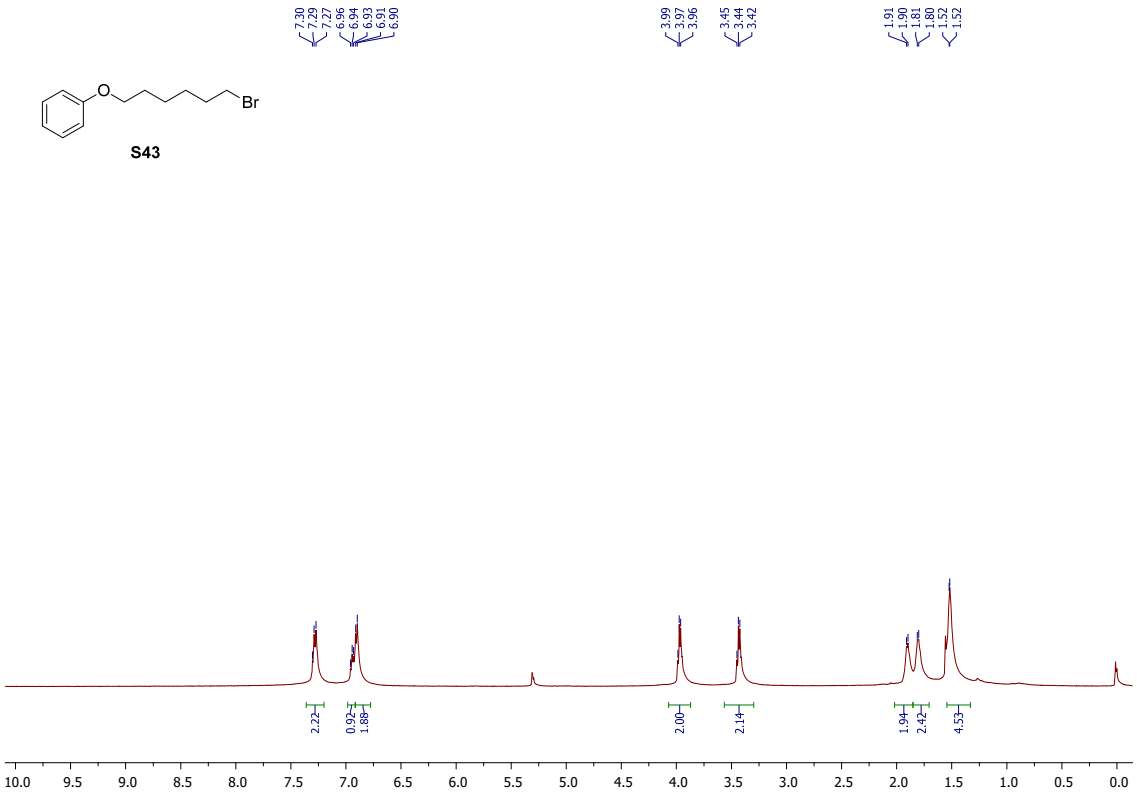
8.16
8.16
7.73
7.72
7.72
7.71
7.70
7.69
7.56
7.54
7.53
7.53
2.94
7
2.00
0.99
2.02
4.22
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


169.25
161.89
134.94
130.60
128.88
125.16
25.70
7
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


9.91
7.87
7.85
7.05
7.03
4.19
4.18
4.17
2.90
2.89
2.87
2.32
2.31
2.29
2.28
2.27
8
0.98
1.99
2.05
1.90
2.22
3.88
1.99
194.45
169.03
165.77
164.46
135.34
108.61
101.29
66.43
27.66
25.60
24.15
8


7.30
7.29
7.27
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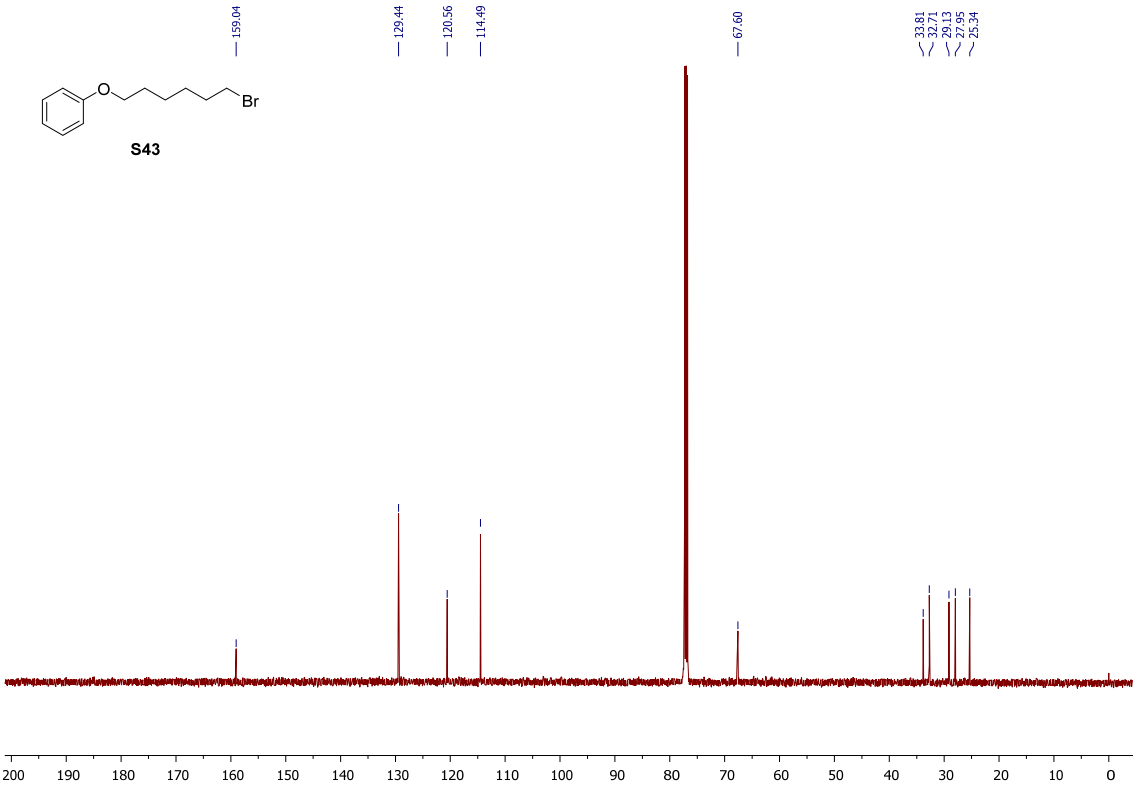
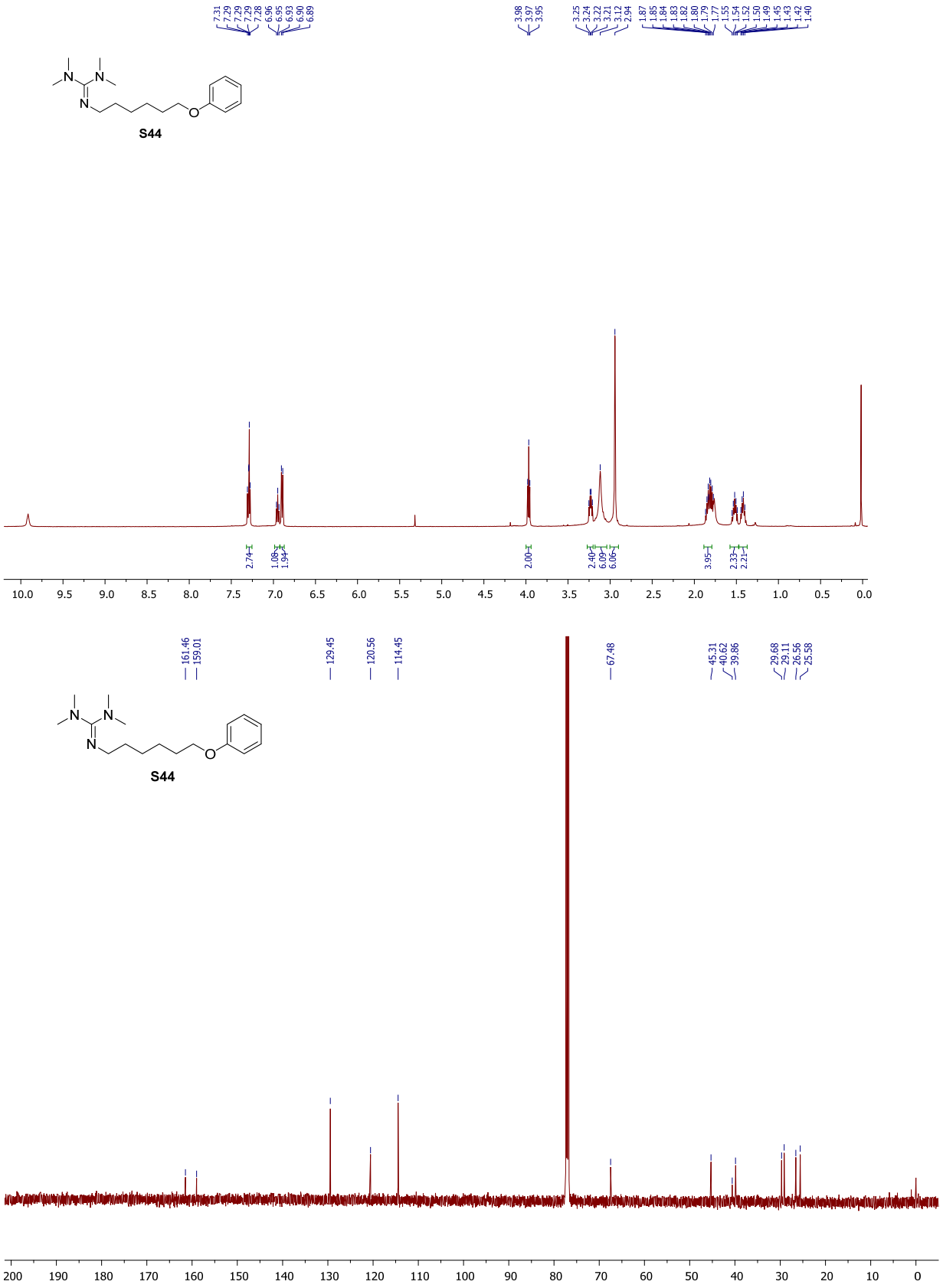
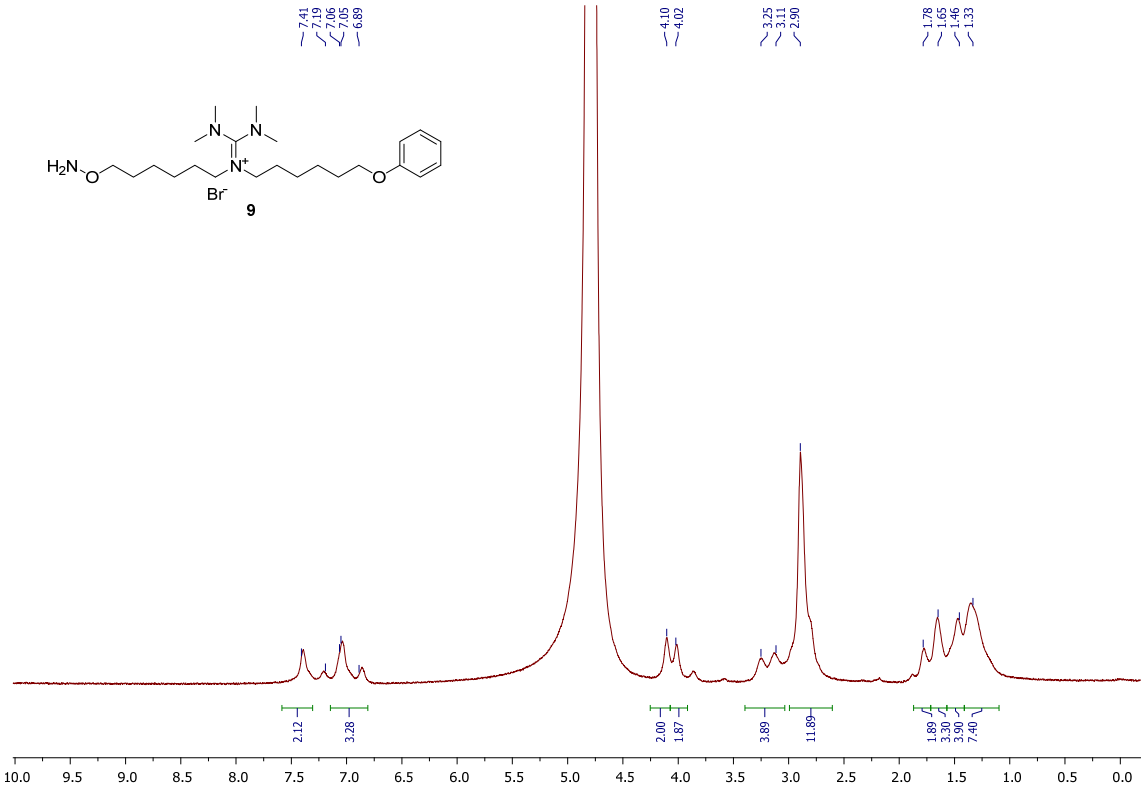
6.94
6.93
6.91
6.90
3.99
3.97
3.96
3.45
3.44
3.42
1.91
1.90
1.81
1.80
1.52
1.52
O
Br
S43
2.22
0.92
1.88
2.00
2.14
1.94
2.42
4.53
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


159.04
129.44
120.56
114.49
67.60
33.81
32.71
29.13
27.95
25.34
O
Br
S43
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


7.31
7.29
7.29
7.29
7.28
6.96
6.95
6.93
6.90
6.89
3.98
3.97
3.95
3.25
3.24
3.22
3.21
3.12
2.94
1.87
1.85
1.84
1.83
1.82
1.80
1.79
1.77
1.55
1.54
1.52
1.50
1.49
1.45
1.43
1.42
1.40
S44
2.74
1.08
1.94
2.00
2.40
6.09
6.06
3.95
2.33
2.21
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
161.46
159.01
129.45
120.56
114.45
67.48
45.31
40.62
39.86
29.68
29.11
26.56
25.58
S44
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0


7.41
7.19
7.06
7.05
6.89
4.10
4.02
3.25
3.11
2.90
1.78
1.65
1.46
1.33
2.12
3.28
2.00
1.87
3.89
11.89
1.89
3.30
3.90
7.40
10.0
9.5
9.0
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8.0
7.5
7.0
6.5
6.0
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3.5
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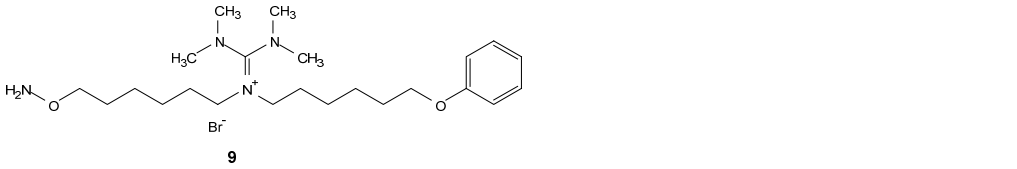
0.5
0.0


CH3
CH3
N
N
H3C
CH3
H2N
O
N+
O
Br-

9

163.10
158.02
129.90
121.46
114.89
75.58
68.27
49.11
48.93
39.36
39.25
34.95
28.00
26.91
26.75
26.63
25.66
25.44
24.66
24.52
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

6. References

[1] M. R. Wilkins, I. Lindskog, E. Gasteiger, A. Bairoch, J. C. Sanchez, D. F. Hochstrasser and R. D. Appel, *Electrophoresis,* 1997, **18**, 403.
[2] A. R. Ramya, M. L. P. Reddy, A. H. Cowley and K. V. Vasudevan, *Inorg. Chem.*, 2010, **49**, 2407.
[3] A. V. Pestov, A. E. Permyakov, P. A. Slepukhin, L. K. Neudachina and Yu. G. Yatluk*, Rus. J. Cor. Chem.,* 2010, **36**, 769.
[4] Y. Liu, R. Xiang, X. Du, Y. Ding and B. Ma, *Chem. Commun.,* 2014, **50**, 12779.
[5] C. K. Sams, F. Somoza, I. Bernal and H. Toftlund, *Inorg. Chim. Acta.,* 2001, **318**, 45.
[6] S. Raiguel, J. Thomas, K. Binnemans and W. Dehaen, *Eur. J. Org. Chem.*, 2018, **35**, 4850.
[7] M. Xu, C. Kuang, Z. Wang, Q. Yang and Y. Jiang, *Synthesis*, 2011, 223.
[8] L. Purushottam, S. R. Adusumalli, M. Chilamari and V. Rai, *Chem. Commun.,* 2017, **53**, 959
[9] L. Huang, A. Shi, F. He and X. Li, *Bioorg. Med. Chem.,* 2010, **18**, 1244.

7. Additional references related to Manuscript

[1] L. Purushottam, S. R. Adusumalli, U. Singh, V. B. Unnikrishnan, D. G. Rawale, M. Gujrati, R. K. Mishra and V. Rai, *Nat. Commun.*, 2019, **10**, 2539.
[2] X. Chen, K. Muthoosamy, A. Pfisterer, B. Neumann and T. Weil, *Bioconjugate Chem.*, 2012, **23**, 500.
[3] P. N. Joshi and V. Rai, *Chem. Commun.,* 2019, **55**, 1100.
[4] M. Chilamari, N. Kalra, S. Shukla and V. Rai, *Chem. Commun.*, 2018, **54**, 7302.
[5] M. Chilamari, L. Purushottam and V. Rai, *Chem. Eur. J.,* 2017, **23**, 3819.
[6] L. Purushottam, S. R. Adusumalli, M. Chilamari and V. Rai, *Chem. Commun.,* 2017, **53**, 959.
[7] R. Singudas, S. R. Adusumalli, P. N. Joshi and V. Rai, *Chem. Commun.,* 2015, **51**, 473.
[8] S. Verma, D. Miles, L. Gianni, I. E. Krop, M. Welslau, J. Baselga, M. Pegram, D.-Y. Oh, V. Dieras, E. Guardino, L. Fang, M. W. Lu, S. Olsen and K. Blackwell, *N. Engl. J. Med.*, 2012, **367**, 1783.
[9] A. Beck, L. Goetsch, C. Dumontet and N. Corvaïa, *Nat. Rev. Drug Discovery*, 2017, **16**, 315.
[10] M. Waliczek*,* M. Kijewska, M. Rudowska, B. Setner, P. Stefanowicz and Z. Szewczuk, *Nat. Chem.,* 2016, **6**, 1.
[11] X. Chen and Y.-W. Wu, *Org. Biomol. Chem.*, 2016, **14**, 5417.
[12] A. Bandyopadhyay, K. A. McCarthy, M. A. Kelly and J. Gao, *Nat. Commun.*, 2015, **6**, 6561.