Supporting Information

Ordered immobilization of serine proteases enabled by linchpin directed modification platform

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1. General information

The reagents, proteins, and enzymes were purchased from Sigma-Aldrich, Alfa Aeser, Spectrochem, and Merck. The organic solvents used were reagent grade. Aqueous buffers were prepared freshly using Millipore Grade I water (Resistivity > 5 MΩ cm, Conductivity < 0.2 µS/cm, TOC <30 ppb). Mettler Toledo (FE20) pH meter was used to adjust the final pH. The reaction mixture for the small molecules was stirred (Heidolph, 500-600 rpm). Proteins were either vortexed or incubated in Thermo Scientific MaxQ 8000 incubator shaker (350 rpm, 25-37 ºC). Amicon® Ultra-0.5 mL 3-10 kDa MWCO centrifugal filters from Merck Millipore were used to remove small molecules from protein mixture, desalting, and buffer exchange. The

resin volume is measured after letting it settle, excluding the volume of the supernatant solvent, unless specified otherwise. Organic solvents were removed by BUCHI rotavapor R-210/215, whereas aqueous samples were lyophilized by CHRiST ALPHA 2-4 LD plus lyophilizer. Circular Dichroism (CD) measurements were recorded on JASCO J-815 CD spectropolarimeter equipped with peltier temperature controller. All the spectra were measured with a scan speed of 50 nm/min, spectral band width 1 nm using 1 cm path length cuvette at 25 ^{o}C.

Chromatography: Thin-layer chromatography (TLC) was performed on silica gel coated aluminium TLC plates (Merck, TLC Silica gel 60 F254). The compounds were visualized using a UV lamp (254 nm) and stains such as iodine, ninhydrin, and 2,4-dinitrophenylhydrazine. Wherever compounds were purified by chromatography, flash column chromatography was carried out on Combiflash Rf 200, Combiflash NextGen 300+, or gravity columns using silica gel (230-400 or 100-200 mesh) from Merck.

Nuclear magnetic resonance spectra: ^{1}H, ^{13}C, ^{19}F NMR spectra were recorded on Bruker Avance III 400 and 500 MHz NMR spectrometer. ^{1}H NMR spectra were referenced to TMS (0 ppm), DMSO-d_6 (2.50 ppm), and acetone-d_6 (2.05 ppm). Whereas ^{13}C NMR spectra were referenced to $CDCl_3$ (77.16 ppm), DMSO-d_6 (39.52 ppm), and acetone-d_6 (29.84 ppm). Peak multiplicities are designated by the following abbreviations: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets. Spectra were recorded at 298 K.

Mass spectrometry: SCIEX X500B QTOF coupled with ExionLC AD UHPLC and Agilent 6130 single quad coupled with Agilent 1200 series HPLC (ESI/APCI) were used for LC-MS and protein sequencing. Poroshell 300 SB-C18 HPLC column (2.1 × 75 mm × 5 μm, flow rate 0.4 mL/min) and XB-C18 UHPLC column (2.5 x 150 mm, 1.7 μm, 100 Å, flow rate 0.3 mL/min) were used for small molecules and protein derived samples respectively. HRMS data were recorded on Bruker Daltonics MicroTOF-Q-II with electron spray ionization (ESI). Matrix assisted laser desorption/ionisation time of flight mass spectrometry was performed with Bruker Daltonics UltrafleXtreme Software-Flex control version 3.4, using sinapic acid and S3 α-cyano-4-hydroxycinnamic acid (HCCA) matrix. Data analysis was performed using SCIEX Bio-pharma view Flex, Flex analysis, and Bruker data analysis software. Peptide mass

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

Acetonitrile and H_2O were buffered with 0.01% formic acid and used as the mobile phase. Method A was used to record the LC-ESI-MS data for proteins and method B used for peptide mapping and MS/MS.

Table 1.

Method A (Column: Agilent, Poroshell 300 Å, SB-C18 5 μm 2.1 × 75 mm, flow rate 0.4 mL/minutes)

Time (minutes)	H_2O (%)	Acetonitrile
0	90	10
1	90	10
8	40	60
12	10	90
15	10	90

Table 2.

Method B (Column: Peptide mapping and MS-MS of proteins on SCIEX X500B paired with ExionLC AD UHPLC [Column: Phenomenex, bioZen, Peptide XB-C18 (2.5 x 150 mm, 1.7 μm, 100 Å) flow rate 0.3 mL/minutes. Acetonitrile and H_2O were buffered with 0.1% formic acid and used as the mobile phase.]

Time (minutes)	H_2O (%)	Acetonitrile (%)
0	95	5
2	95	5
25	50	50
26	20	80
27	20	80
28	95	5
30	95	5

2. General procedures

Protein labeling

2a. General Procedure for N-terminus transamination of serine proteases

Serine protease (5 nmol) in phosphate buffer (50 µL, 0.1 M, pH 6.5) was taken in a 2 mL microcentrifuge tube. Reagent **2a**/**2b** (250 nmol/ 1000 nmol) in phosphate buffer (50 µL, 0.1 M, pH 6.5) from a freshly prepared stock solution was added to it followed by incubating at 37 ^{o}C. After 2 h, the resulting solution was concentrated, and buffer was exchanged using 0.5 mL spin concentrators with a MWCO of 10 kDa (Amicon® Ultra-4). The buffer exchange first involved the dilution of each sample to 500 µL with 25 mM phosphate buffer (pH 6.5). Each sample was then concentrated to 100 µL, and the process was repeated 3 times. The sample was collected in phosphate buffer (190 µL, 0.1 M, pH 7.0). To this solution, O-benzylhydroxylamine (2 µmol) in DMSO (10 µL) from a freshly prepared stock solution was added. The reaction mixture was vortexed at 25 ^{o}C for 3 h to form oxime derivative. The excess of O-benzylhydroxylamine was removed by using spin concentrator and the sample was collected in water. The modification of protein was analyzed by MALDI-MS. The concentrated sample was subjected to digestion, peptide mapping, and sequencing by MS-MS.

2b. General procedure for single-site labeling of protease by LDM reagent

Serine protease (5 nmol) in phosphate buffer (200 µL, 0.1 M, pH 7.8) was taken in a 1.5 mL microcentrifuge tube. Reagent **2c-2h** (125 nmol) in DMSO (50 µL) from a freshly prepared stock solution was added to it, followed by vortexing (350 rpm) at 25 ^{o}C [Note: The reaction efficiency remains conserved in the operating temperature range of 4-25 ^{o}C]. After 6-24 h, the reaction mixture was diluted with acetonitrile: water (10:90, 1400 µL). Unreacted reagents and salts were removed by using Amicon® Ultra-4 mL 10-kDa MWCO centrifugal filters spin concentrator. The sample was further washed with acetonitrile: water (10:90) and concentrated to 200 µL. To this solution, hydroxylamine hydrochloride (10 µmol) in grade-I water (10 µL) from a freshly prepared stock solution was added. The reaction mixture was vortexed at 25 ^{o}C for 30 min. to form oxime derivative. The excess of hydroxylamine hydrochloride and salts were removed by using spin concentrator and the sample was collected in water. The modification of protein was analyzed by ESI-MS/MALDI ToF MS. The concentrated sample was subjected to digestion, peptide mapping, and sequencing by MS-MS.

2c. Protein digestion

All solutions were made freshly prior to use.

Procedure for in-solution digestion of proteases

In a 1.5 mL microcentrifuge tube, protein (0.1 mg) in 100 mM tris buffer (10 µL, pH 7.8) with urea (8 M) was incubated for 1 h at 37 °C. To this solution, reducing agent (1 µL, 0.2 M DTT in 0.1 M tris) was added, and sample was incubated for 1 h at 37 °C. Alkylating agent (4 µL,

0.2 M iodoacetamide in 0.1 M tris) was added to the solution and incubated (in dark) for 1 h at 25 °C for blocking the free sulfhydryl groups. The unreacted iodoacetamide was quenched with reducing agent (4 μL, 0.2 M DTT in 0.1 M tris) for 1 h at 25 °C. To reduce the urea concentration to 0.8 M, the sample was diluted with grade I water. To this solution, 3 μL of trypsin solution (5 μg, enzyme/protease (1:20); enzyme in water was added, and the mixture was incubated at 37 °C for 18 h. The pH of the digested solution was adjusted to < 6 (verified by pH paper) with trifluoroacetic acid (0.05%). Subsequently, the sample was used for peptide mapping by MS and sequencing by MS-MS.

2d. Immobilization of proteases

Procedure for random immobilization of proteases

The NHS ester activated resin (50 μL) was taken in a 1.5 mL microcentrifuge tube. Sodium phosphate buffer (0.1 M, pH 7.0, 3 x 1 mL) was used to wash the beads and were re-suspended (sodium phosphate buffer, 100 μL, 0.1 M, pH 7.0). Protease (20 μM) was dissolved in sodium phosphate buffer (0.1 M, pH 7.0, 400 μL) and added to the resin slurry. The mixture was vortexed at 350 rpm at room temperature for 30 minutes. Subsequently, the supernatant was collected, and the beads were washed with aqueous buffer (0.1 M, sodium phosphate buffer, pH 7.8, 3 x 1 mL) and H_2O (3 x 1 mL) to remove the adsorbed reagent from resin. The beads were further washed with the sodium phosphate buffer (0.1 M, pH 7.8, 3 x 1 mL) and re-suspended (sodium phosphate buffer, 100 μL, 0.1 M, pH 7.8). Supernatant was analyzed by UV-Vis spectroscopy for immobilization efficiency.

Procedure for ordered immobilization of proteases.

The NHS ester activated resin (50 μL) was taken in a 1.5 mL microcentrifuge tube. The resin was then transferred to 5 mL fritted polypropylene chromatography column with end tip closures. Sodium phosphate buffer (0.1 M, pH 7.0, 3 x 1 mL) was used to wash the beads and were re-suspended (sodium phosphate buffer, 100 μL, 0.1 M, pH 7.0). Simultaneously, Protease (20 μM) was modified at single site. The reaction mixture of protein modification was dissolved in sodium phosphate buffer (0.1 M, pH 7.0, 400 μL) and was added to resin slurry. The mixture was vortexed at 350 rpm at 25 ^oC for 30 minutes. Subsequently, the supernatant was collected, and the beads were washed with aqueous buffer (0.1 M, sodium phosphate buffer, pH 7.8, 3 x 1 mL) and H_2O (3 x 1 mL) to remove the adsorbed protease from resin. The beads were further washed with the sodium phosphate buffer (0.1 M, pH 7.8, 3 x 1 mL) and re-suspended (sodium phosphate buffer, 100 μL, 0.1 M, pH 7.8). Supernatant was analyzed by UV-Vis spectroscopy for immobilization efficiency.

Synthesis of hydroxylamine activated Sepharose resin

N-hydroxy succinimidyl sepharose beads (1 mL, resin loading: 23 μmol/mL) were taken in a 5 ml fritted polypropylene chromatography column with end tip closures. Sodium phosphate buffer (0.1 M, pH 7.8, 3 x 1 mL) was used to wash the beads and were re-suspended (sodium phosphate buffer, 360 μL, 0.1 M, pH 7.8). To this solution, N-Boc hydroxylamine (13.8 μM) in sodium phosphate buffer (0.1M, pH 7.8, 40 μL) from a freshly prepared stock solution was added and vortexed at 25 °C for 8 h. Subsequently, the supernatant was removed, and the beads were washed with aqueous buffer (0.1 M, sodium phosphate buffer, pH 7.8, 3 x 1 mL) and H_2O (3 x 1 mL) to remove the adsorbed reagent from resin. The beads were further washed with the sodium phosphate buffer (0.1 M, pH 7.8, 3 x 1 mL) and re-suspended in H_2O. To this solution, 100 μL TFA solution (100 μL in 900 μL H_2O) was added, and vortexed at 25 °C for 2 h. Subsequently, the supernatant was removed, and the beads were washed with aqueous buffer (0.1 M, sodium phosphate buffer, pH 7.8, 3 x 1 mL) and H_2O (3 x 1 mL) to remove the adsorbed reagent from resin. The beads were further washed with the sodium phosphate buffer (0.1 M, pH 7.8, 3 x 1 mL) and re-suspended in H_2O and stored at 4 °C until further use.

Immobilization of α-chymotrypsin and trypsin

Through linchpin directed modification free aldehyde suitable placed on α-chymotrypsin and trypsin, we proceeded with the ordered immobilization of α-chymotrypsin and trypsin. The efficiency of immobilization onto resin was quantified by change in absorbance at 280 nm. A calibration curve was formed to monitor the absorbance at 280 nm for different concentrations of α-chymotrypsin and trypsin (Figure S13). From this data, we determined the molar extinction coefficient for the enzymes from the slope of the standard curve (Figure S14). The absorbance at 280 nm was further utilized to monitor the amount of unbound proteases. The eluted fraction from the coupling reaction was analyzed to indirectly estimate the amount of protease successfully bound to the resin.

The commercially available NHS-activated sepharose resin with a binding efficiency of 16-23 μmol/mL was used. We observed approximately 50% capture efficiency (10 μmol/mL) when the protein was immobilized according to random immobilization protocol. The α-chymotrypsin was then modified by LDM reagent and reacted with the hydroxylamine modified resin. Approximately, 50% capture efficiency was observed in the case of ordered immobilization as well (Figure S14). It indicates that the ideal loading capacity of the resin is 7 μmol/mL by UV-Vis spectroscopy.

UV-Visible Spectroscopy

The spectrophotometer works on the principle of Beer-Lambert law which states that the absorbance of a material sample is directly proportional to the path length and the concentration of the material sample. Mathematically, it is given by the equation –

$$\mathbf{A = \varepsilon bc}$$

Where,

A - Absorbance of the sample

ε - Molar absorptivity co-efficient

b - Path length (length of cuvette)

c - Concentration of the sample

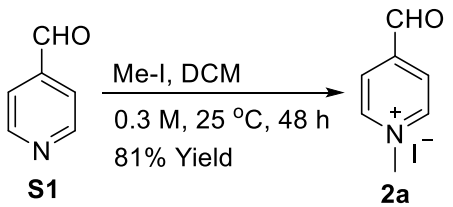
Thus, by keeping the path length constant (by using a 1 cm cuvette), concentration of the material sample can be calculated.

2e. Protein digestion using immobilized protease

In a 1.5 mL microcentrifuge tube, protein (0.1 mg) in 100 mM tris buffer (10 µL, pH 7.8) with urea (8 M) was incubated for 1 h at 37 °C. To this solution, reducing agent (1 µL, 0.2 M DTT in 0.1 M tris) was added and sample was incubated for 1 h at 37 °C. Alkylating agent (4 µL, 0.2 M iodoacetamide in 0.1 M tris) was added to the solution and incubated (in dark) for 1 h at 25 °C for blocking the free sulfhydryl groups. The unreacted iodoacetamide was quenched with reducing agent (4 µL, 0.2 M DTT in 0.1 M tris) for 1 h at 25 °C. These salts were removed using centrifugal spin concentrator (3kDa, 1.5 mL) and 100 µL sample was collected. This solution was added in 1.5 mL Microcentrifuge tube containing 20 µL immobilized α-chymotrypsin (random or ordered). The mixture was incubated at 37 °C for 18 h and subsequently, the sample was used for peptide mapping by MS and sequencing by MS-MS.

3. Synthesis and characterization data of reagents

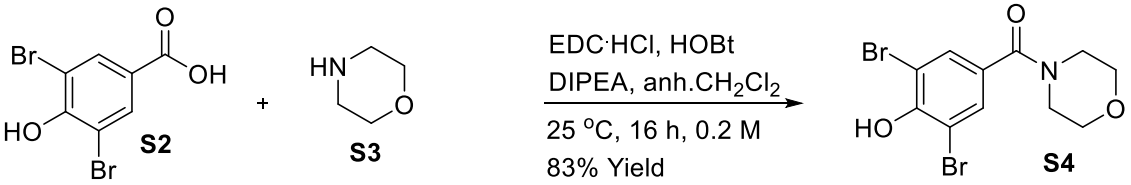
3.1 Synthesis of N-Methylpyridine-4-carboxaldehyde iodide (2a)



Scheme S1 Synthesis of N-methylpyridine-4-carboxaldehyde iodide (**2a)**

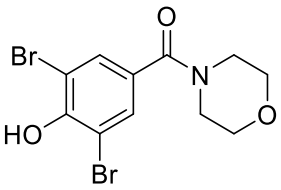
In a 25 mL round bottom flask added the pyridine-4-carboxaldehyde 19 (1 mL, 11.3 mmol) in dichloromethane (11 mL) was treated with methyl iodide (1.4 mL, 22.4 mmol) and stirred at ambient temperature for 48 h. This gave **2a** (2.4 g, 81%) filtered off as yellow/orange cubic crystals. ^{1}H NMR (500 MHz, D_2O) δ 8.83 (d, 2H), 8.16 (d, 2H), 6.25 (s, 1H), 4.43 (s, 3H) ppm. ^{13}C NMR (126 MHz, D_2O) δ 159.4, 145.4, 125.1, 87.5, 47.9. ESI-MS $[M]^+$ calcd. $C_7H_8NO^+$ 122.0, found 122.1.

3.2 Synthesis and characterization data of LDM reagents

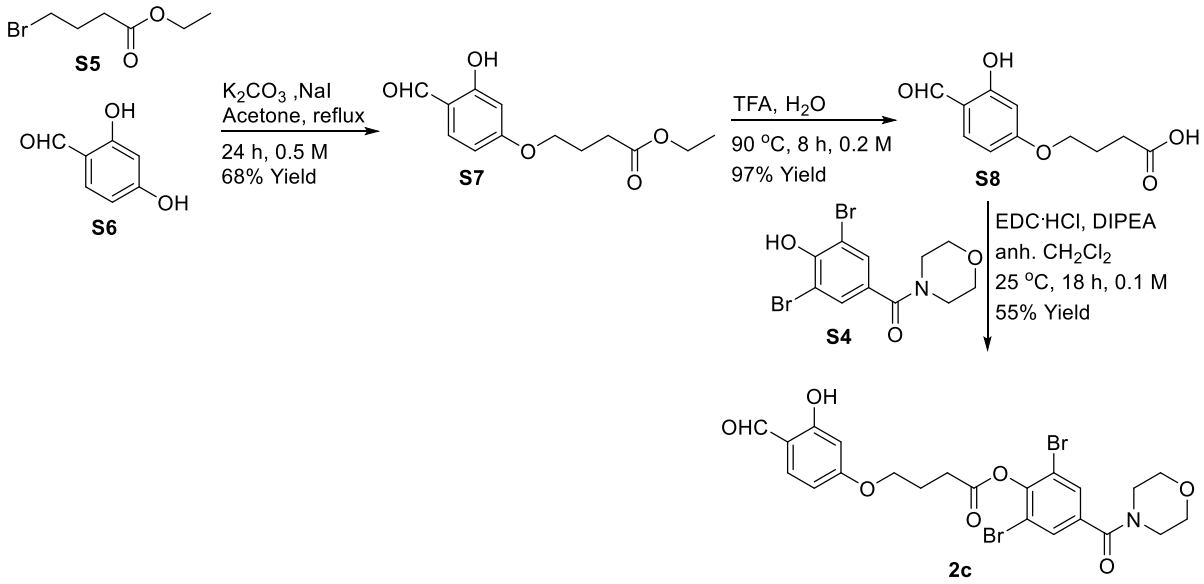


Scheme S1 Synthesis of (3,5-dibromo-4-hydroxyphenyl)(morpholino) methanone (**S4**)

(3,5-dibromo-4-hydroxyphenyl)(morpholino) methanone (S4)

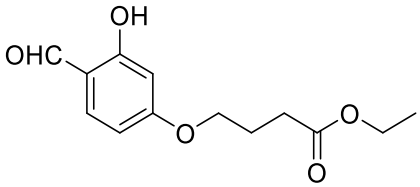


This compound was synthesized according to the procedure for synthesis of In a 10 mL round bottom flask, 3,5-dibromo-4-hydroxybenzoic acid **S2** (100 mg, 0.3 mmol), 3-(((ethylimino)methylene)amino)-N,N-dimethylpropan-1-amine (EDC·HCl, 69 mg, 0.36 mmol), and morpholine **S3** (31 mg, 0.36 mmol) were mixed in anh. CH_2Cl_2 (3 mL) at room temperature. To this solution, DIPEA (104 µL, 0.6 mmol) was added slowly and stirred for 24 h. The reaction mixture was concentrated under reduced vacuum. The purification of crude reaction mixture was performed by silica gel flash column chromatography (ethyl acetate:n-hexane, 80:20) to isolate (3,5-dibromo-4-hydroxyphenyl)(morpholino)methanone **S4** (100 mg, Yield 83%; R_f 0.60, ethyl acetate:n-hexane 80:20; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (s, 2H), 7.2 (bs, 1H), 3.90-3.25 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 151.2, 131.4, 129.7, 110.4, 66.9 (2C). HRMS (ESI) $[M+H]^+$ calcd. $C_{11}H_{12}Br_2NO_3$ 363.9184, found 363.9173.



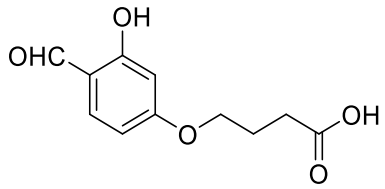
Scheme S3 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-formyl-3-hydroxyphenoxy) butanoate **2c**.

Synthesis of ethyl 4-(4-formyl-3-hydroxyphenoxy) butanoate (S7)



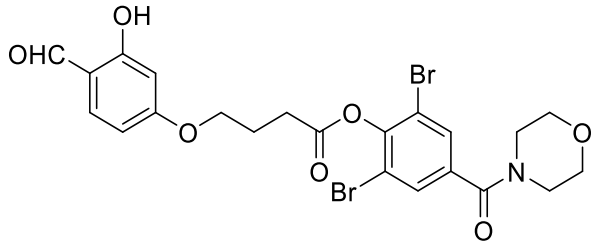
In a 25 mL round bottom flask 1 (RB1), 2,4-dihydroxybenzaldehyde **S6** (691 mg, 5 mmol) and K_2CO_3 (691 mg, 5 mmol) were dissolved in acetone (5 mL) and refluxed for 2 h. Simultaneously in another 10 ml round bottom flask (RB2), ethyl 4-bromobutyrate **S5** (716 µL, 5 mmol) and sodium iodide (825 mg, 5.5 mmol) in acetone (5 mL) were stirred at room temperature for 2 h. The solution in RB2 was transferred to RB1 and refluxed for another 24 h. Acetone was removed using rotary evaporator and the crude mixture was re-suspended in ethyl acetate and water. After solvent-solvent extraction, the combined organic layers were dried with anh. sodium sulfate, filtered, and concentrated under vacuum. The crude mixture was purified by silica gel flash column chromatography using ethyl acetate:n-hexane (3:97) to afford ethyl 4-(4-formyl-3-hydroxyphenoxy)butanoate **S7** (795 mg, 63% yield; R_f 0.44, ethyl acetate:n-hexane 10:90; white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.46 (s, 1H), 9.71 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 6.52 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.06 (t, *J* = 6.2 Hz, 2H), 2.50 (t, *J* = 7.2 Hz, 2H), 2.18-2.08 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.5, 173.0, 166.2, 164.6, 135.4, 115.3, 108.7, 101.3, 67.4, 60.7, 30.7, 24.4, 14.3 ppm. MS (ESI) $[M+H]^+$ calcd. $C_{13}H_{17}O_5$ 253.1, found 253.1.

4-(4-formyl-3-hydroxyphenoxy) butanoic acid (S8)



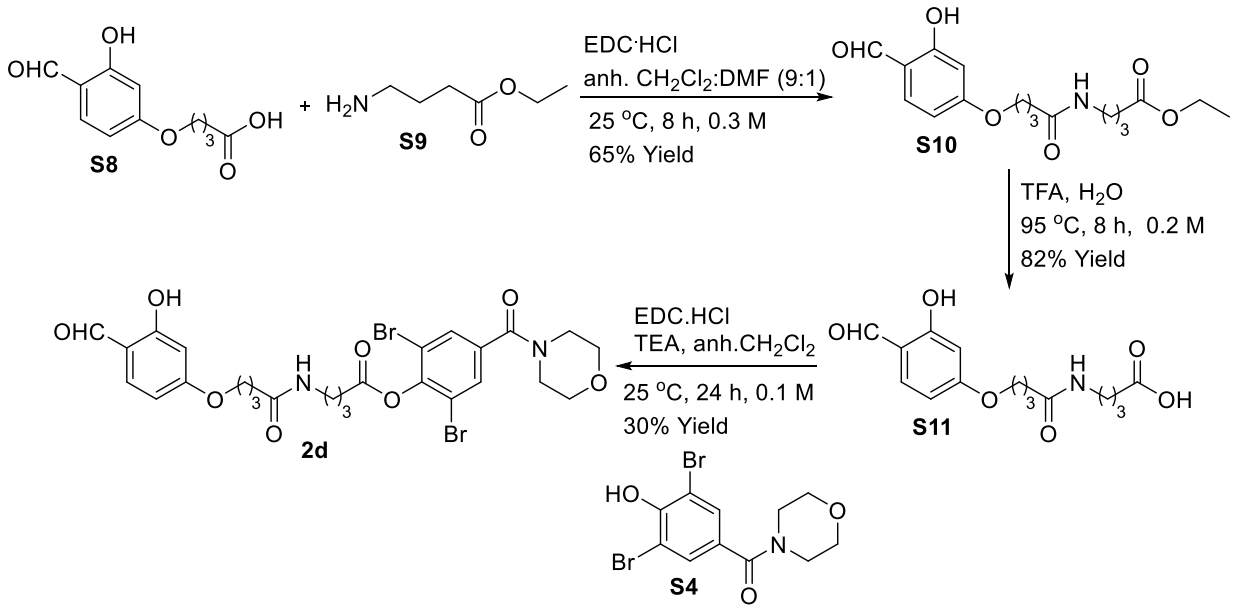
In a 25 mL round bottom flask, ethyl 4-(4-formyl-3-hydroxyphenoxy)butanoate **S7** (520 mg, 2 mmol), LiOH (145 mg, 6 mmol), were dissolved in water (6 mL), and THF (6 mL). The reaction mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated using rotary evaporator. Reaction mixture was quenched with slow addition of HCl (12 N). The compound was separated from water using ethyl acetate. The organic layer was collected and concentrated in vacuo. The purification of crude mixture was performed by silica gel column chromatography using ethyl acetate:n-hexane (30:70) to yield 4-(4-formyl-3-hydroxyphenoxy) butanoic acid **S8.** Yield 97%; R_f 0.30, ethyl acetate:n-hexane 30:70; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.46 (s, 1H), 9.72 (s, 1H), 7.43 (d, *J* = 8.7 Hz, 1H), 6.53 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.42 (d, *J* = 2.2 Hz, 1H), 4.09 (t, *J* = 6.1 Hz, 2H), 2.59 (t, *J* = 7.2 Hz, 2H), 2.21-2.10 (m, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 191.1, 174.0, 165.2, 163.1, 132.3, 116.2, 107.7, 101.2, 67.2, 30.0, 24.0 ppm. MS (ESI) $[M+H]^+$ calcd. $C_{11}H_{13}O_5$ 225.1, found 225.1.

2,6-dibromo-4-(morpholine-4-carbonyl) phenyl 4-(4-formyl-3-hydroxyphenoxy) butanoate (2c)



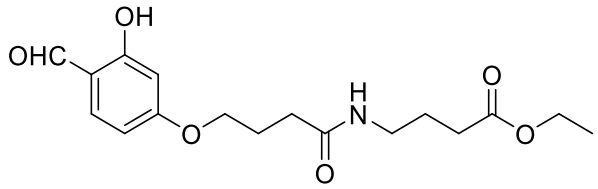
This compound was synthesized according to the procedure for synthesis of **S4**.

Yield 55%; R_f 0.47, ethyl acetate:n-hexane 25:75; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.47 (s, 1H), 9.73 (s, 1H), 7.61 (s, 2H), 7.45 (d, *J* = 8.6 Hz, 1H), 6.57 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.45 (d, *J* = 1.7 Hz, 1H), 4.18 (t, *J* = 6.0 Hz, 2H), 3.96-3.34 (m, 8H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.40-2.23 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.6, 169.2, 166.7, 166.0, 164.6, 147.5, 135.7, 135.5, 131.3, 118.3, 115.5, 108.7, 101.4, 66.9 (3C), 30.3, 24.3. HRMS (ESI) $[M+H]^+$ calcd. $C_{22}H_{22}Br_2NO_7$ 569.9763, found 569.9747.



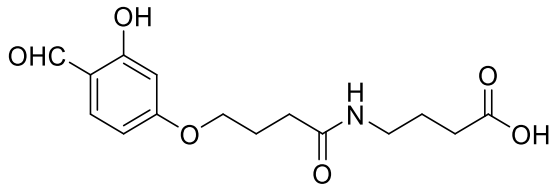
Scheme S4 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(4-formyl-3-hydroxyphenoxy) butanamido) butanoate **2d**.

Synthesis of ethyl 4-(4-(4-formyl-3-hydroxyphenoxy) butanamido) butanoate (S10)



In a 25 mL round bottom flask, 4-(4-formyl-3-hydroxyphenoxy)butanoic acid **S8** (110 mg, 0.5 mmol) in anh. CH_2Cl_2:DMF (5 mL, 9:1) and 3-(((ethylimino)methylene)amino)-N,N-dimethylpropan-1-amine (EDC·HCl, 191 mg, 1 mmol) were mixed at room temperature. After 10 minutes, ethyl 4-aminobutyrate **S9** (125 µL, 0.75 mmol) was added slowly to the reaction mixture and stirred for 8 h followed by quenching with 1N HCl. The residue was extracted with ethyl acetate, dried with anh. sodium sulphate, filtered, and concentrated under vacuum. The purification of crude reaction mixture was performed by silica gel flash column chromatography (ethyl acetate:n-hexane, 10:90) to isolate ethyl 4-(4-(4-formyl-3-hydroxyphenoxy)butanamido) butanoate **S10** (110 mg, 65% yield; R_f 0.35, ethyl acetate:n-hexane 50:50; white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 9.71 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 6.53 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.41 (d, *J* = 2.3 Hz, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 4.07 (t, *J* = 6.1 Hz, 2H), 3.34-3.26 (m, 2H), 2.41-2.31 (m, 4H), 2.20-2.10 (m, 2H), 1.88-1.78 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 173.6, 172.1, 166.2, 164.6, 135.5, 115.4, 108.6, 101.5, 67.7, 60.7, 39.2, 32.7, 31.9, 25.0, 24.7, 14.3 ppm. MS (ESI) $[M+H]^+$ calcd. $C_{17}H_{24}NO_6$ 338.2, found 338.1.

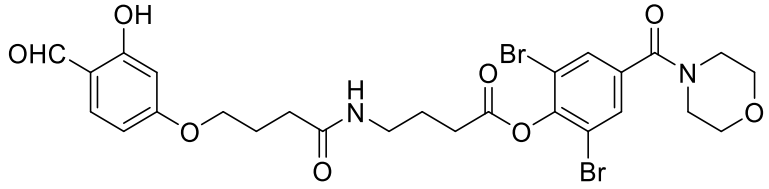
Synthesis of 4-(4-(4-formyl-3-hydroxyphenoxy) butanamido) butanoic acid (S11)



This compound was synthesized according to the procedure for synthesis of **S8**.

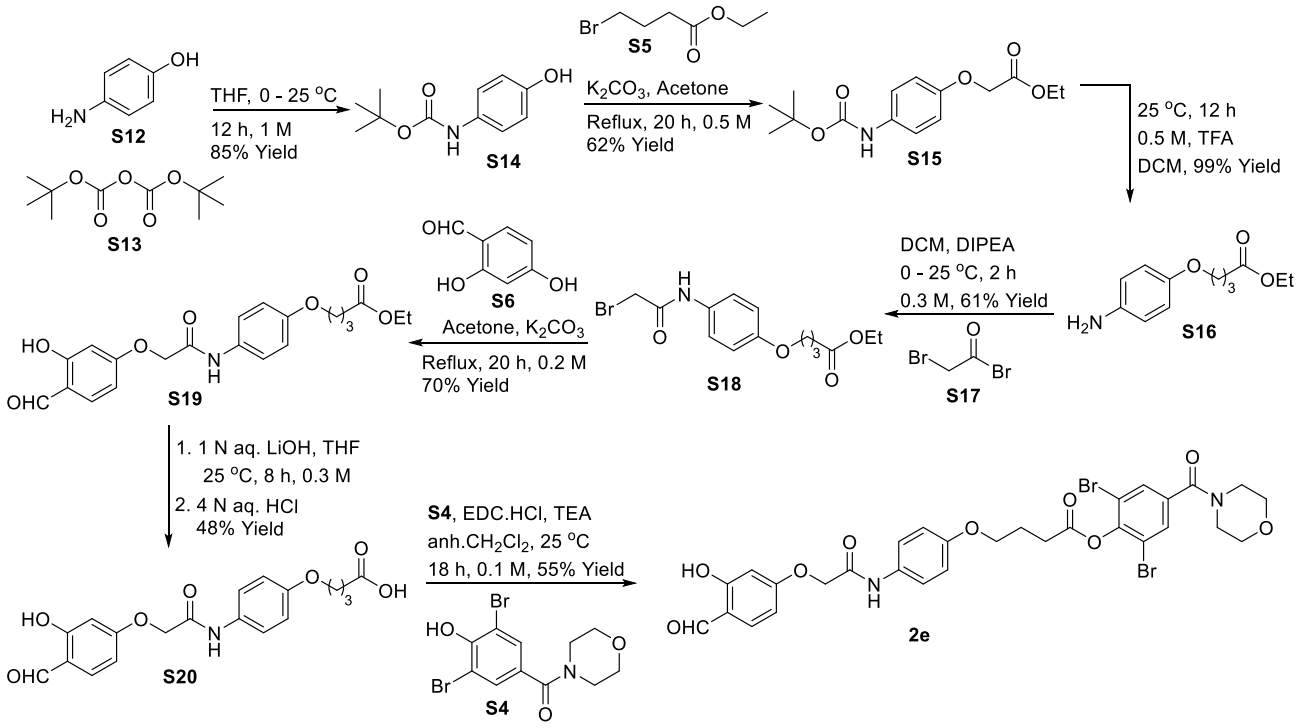
Yield 82%; R_f 0.21, ethyl acetate:n-hexane 70:30; white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 1H), 10.98 (s, 1H), 10.00 (s, 1H), 7.86 (t, *J* = 5.5 Hz, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 6.55 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.46 (d, *J* = 2.3 Hz, 1H), 4.02 (t, *J* = 6.4 Hz, 2H), 3.09-3.01 (m, 2H), 2.25-2.17 (m, 4H), 1.97-1.89 (m, 2H), 1.64-1.56 (m, 2H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.1, 174.2, 171.3, 165.3, 163.0, 132.2, 116.1, 107.7, 101.2, 67.5, 37.9, 31.5, 31.1, 24.6, 24.5 ppm. HRMS (ESI) $[M+Na]^+$ calcd. $C_{15}H_{19}NNaO_6$ 332.1110, found 332.1109.

2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(4-formyl-3-hydroxyphenoxy) butanamido) butanoate (2d)



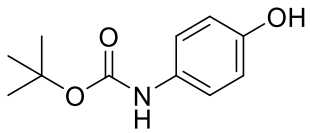
This compound was synthesized according to the procedure for synthesis of **S4**.

Yield 30%; R_f 0.79, ethyl acetate:n-hexane 60:40; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 9.70 (s, 1H), 7.60 (s, 2H), 7.42 (d, J = 8.7 Hz, 1H), 6.52 (dd, J = 8.7, 2.3 Hz, 1H), 6.41 (d, J = 2.2 Hz, 1H), 5.84 (s, 1H), 4.07 (t, J = 6.0, 2H), 3.88-3.36 (m, 10H), 2.73 (t, J = 7.3 Hz, 2H), 2.39 (t, J = 7.2 Hz, 2H), 2.24-2.11 (m, 2H), 2.09-1.96 (m, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 172.3, 169.5, 166.7, 166.2, 164.5, 147.4, 135.7, 135.6, 131.3, 118.2, 115.4, 108.5, 101.4, 67.6, 66.8, 53.6, 38.9, 32.7, 31.5, 24.9, 24.7 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{26}H_{29}Br_2N_2O_8$ 655.0291, found 655.0264.



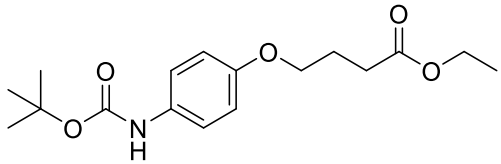
Scheme S5 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl) phenyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy) acetamido) phenoxy)butanoate **2e**.

Synthesis of tert-butyl (4-hydroxyphenyl) carbamate (S14)



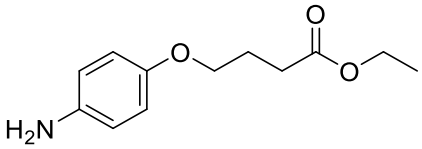
In a dry 100 mL round bottom flask, 4-aminophenol **S12** (2.182 g, 20 mmol) was dissolved in THF (10 mL). To this solution, di-tert-butyl dicarbonate **S13** (4.594 mL, 24 mmol) in THF (10 mL) was added slowly at 0 °C by using a dropping funnel. After 2 h, the reaction mixture was brought to room temperature and stirred for 10 h. The reaction mixture was concentrated in vacuo and washing of the crude solid by hexane gave the analytically pure product tert-butyl (4-hydroxyphenyl) carbamate **S14** (3.558 g, 85% yield; R_f 0.40, ethyl acetate:n-hexane 20:80; white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20-7.10 (m, 2H), 6.78-6.69 (m, 2H), 6.34 (s, 1H), 5.36 (s, 1H), 1.51 (s, 9H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.7, 152.2, 131.1, 121.6, 115.9, 80.6, 28.5 ppm. MS (ESI) $[M+Na]^+$ calcd. $C_{11}H_{15}NNaO_3$ 232.0950, found 232.0938.

Synthesis of ethyl 4-(4-((tert-butoxycarbonyl)amino) phenoxy)butanoate (S15)



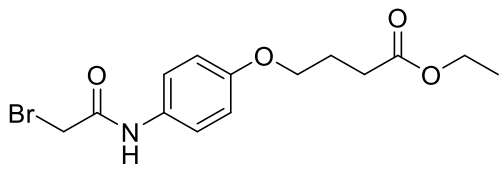
In a 100 mL round bottom flask, tert-butyl (4-hydroxyphenyl)carbamate **S14** (2.092 g, 10 mmol), ethyl 4-bromobutanoate **S5** (1.717 mL, 12 mmol), and K_2CO_3 (2.764 g, 20 mmol) were dissolved in acetone (20 mL) and refluxed for 20 h. The reaction mixture was concentrated in vacuo and portioned between ethyl acetate and water. The organic layer was separated, dried over anh. sodium sulfate and filtered. The filtrate was concentrated under reduced pressure. The crude compound was purified by silica gel flash column chromatography using ethyl acetate:n-hexane (20:80) to afford ethyl 4-(4-((tert-butoxycarbonyl)amino)phenoxy)butanoate **S15** (2.005 g, 62% yield; R_f 0.21, ethyl acetate:n-hexane 20:80; white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26-7.19 (m, 2H), 6.88-6.78 (m, 2H), 6.36 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.96 (t, *J* = 6.0 Hz, 2H), 2.50 (t, *J* = 7.3 Hz, 2H), 2.14-2.04 (m, 2H), 1.50 (s, 9H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 155.1, 153.3, 131.6, 120.7, 115.0, 80.3, 67.2, 60.6, 31.0, 28.5, 24.8, 14.4 ppm. HRMS (ESI) $[M+Na]^+$ calcd. $C_{17}H_{25}NNaO_5$ 346.1630, found 346.1649.

Synthesis of ethyl 4-(4-aminophenoxy) butanoate (S16)



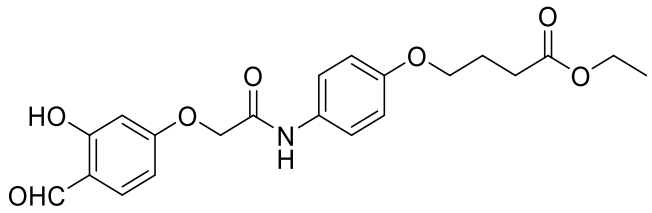
In a 100 mL round bottom flask, ethyl 4-(4-((tert-butoxycarbonyl)amino)phenoxy)butanoate **S15** (1.617 g, 5 mmol) was dissolved in CH_2Cl_2 (10 mL). To this solution, trifluoroacetic acid (1.53 ml, 20 mmol) was added slowly over 15 minutes and stirred at 25 ^{o}C for 12 h. The reaction mixture was quenched with sodium bicarbonate (slow addition), followed by addition of CH_2Cl_2. The organic layer was separated, dried with anh. sodium sulfate, filtered, and concentrated in vacuo to give ethyl 4-(4-aminophenoxy)butanoate **S16** (1.104 g, yield 99%; R_f 0.25, ethyl acetate:n-hexane 30:70; pale yellow liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.76-6.70 (m, 2H), 6.66-6.61 (m, 2H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.92 (t, *J* = 6.1 Hz, 2H), 3.41 (s, 2H), 2.49 (t, *J* = 7.3 Hz, 2H), 2.12-2.01 (m, 2H), 1.25 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.5, 152.1, 140.2, 116.5, 115.8, 67.6, 60.5, 31.0, 24.9, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{12}H_{17}NO_3$ 224.1287, found 224.1265.

Synthesis of ethyl 4-(4-(2-bromoacetamido) phenoxy) butanoate (S18)



In a 25 mL round bottom flask, ethyl 4-(4-aminophenoxy)butanoate **S16** (300 mg, 1.34 mmol) and DIPEA (494 µL, 2.68 mmol) in DCM (4 mL) were stirred at 0 ^{o}C for 5 minutes. To this solution, 2-bromoacetyl bromide **S17** (236 µL, 2 mmol) was added slowly over a period of 15 minutes. The reaction mixture was brought to room temperature and stirred for another 2 h. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated using rotary evaporator. The purification of crude reaction mixture was performed by silica gel flash column chromatography using ethyl acetate:n-hexane (10:90) to isolate ethyl 4-(4-(2-bromoacetamido)phenoxy)butanoate **S18** (281 mg, 61% yield; R_f 0.48, ethyl acetate:n-hexane 30:70; white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (s, 1H), 7.45-7.38 (m, 2H), 6.90-6.83 (m, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.02 (s, 2H), 3.99 (t, *J* = 6.1 Hz, 2H), 2.51 (t, *J* = 7.3 Hz, 2H), 2.16-2.05 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 165.3, 156.3, 130.6, 122.0, 114.9, 67.1 (2C), 60.6, 30.9, 24.7, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{14}H_{19}BrNO_4$ 344.0497, found 344.0482.

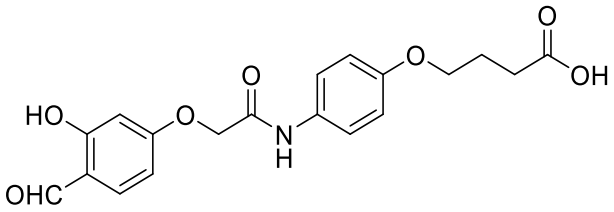
Synthesis of ethyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy)acetamido) phenoxy) butanoate (S19)



In a 25 mL round bottom flask, ethyl 4-(4-(2-bromoacetamido)phenoxy)butanoate **S18** (344 mg, 1 mmol), potassium carbonate (138 mg, 1 mmol) and 2,4-dihydroxybenzaldehyde **S6** (138 mg, 1 mmol) were dissolved in acetone (5 mL) and refluxed for 20 h. The progress of the reaction was followed by TLC. The reaction mixture was concentrated using rotary evaporator. The purification of crude reaction mixture was performed by silica gel flash column chromatography using ethyl acetate:n-hexane (10:90) to isolate ethyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy) acetamido)phenoxy) butanoate **S19** (281 mg, 70% yield; R_f 0.21, ethyl acetate:n-hexane 50:50; white solid). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.43 (s, 1H), 9.78 (s, 1H),

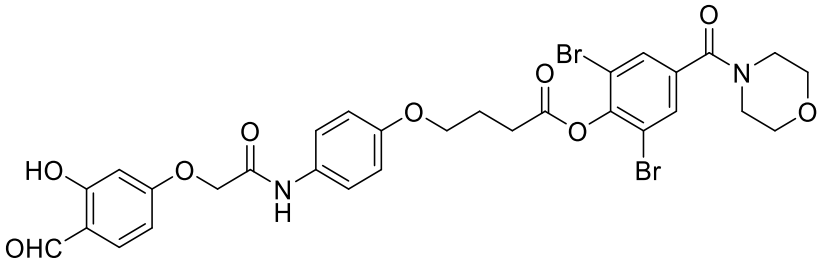
8.02 (s, 1H), 7.53 (d, *J* = 8.6 Hz, 1H), 7.50-7.42 (m, 2H), 6.92-6.84 (m, 2H), 6.65 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.54 (d, *J* = 2.4 Hz, 1H), 4.65 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.00 (t, *J* = 6.1 Hz, 2H), 2.51 (t, *J* = 7.3 Hz, 2H), 2.16-2.06 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8, 173.3, 164.8, 164.5, 163.7, 156.4, 136.0, 129.7, 122.3, 116.5, 115.1, 108.0, 102.6, 67.5, 67.2, 60.6, 30.9, 24.8, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{21}H_{24}NO_7$ 402.1553, found 402.1558.

Synthesis of 4-(4-(2-(4-formyl-3-hydroxyphenoxy)acetamido)phenoxy) butanoic acid (S20)

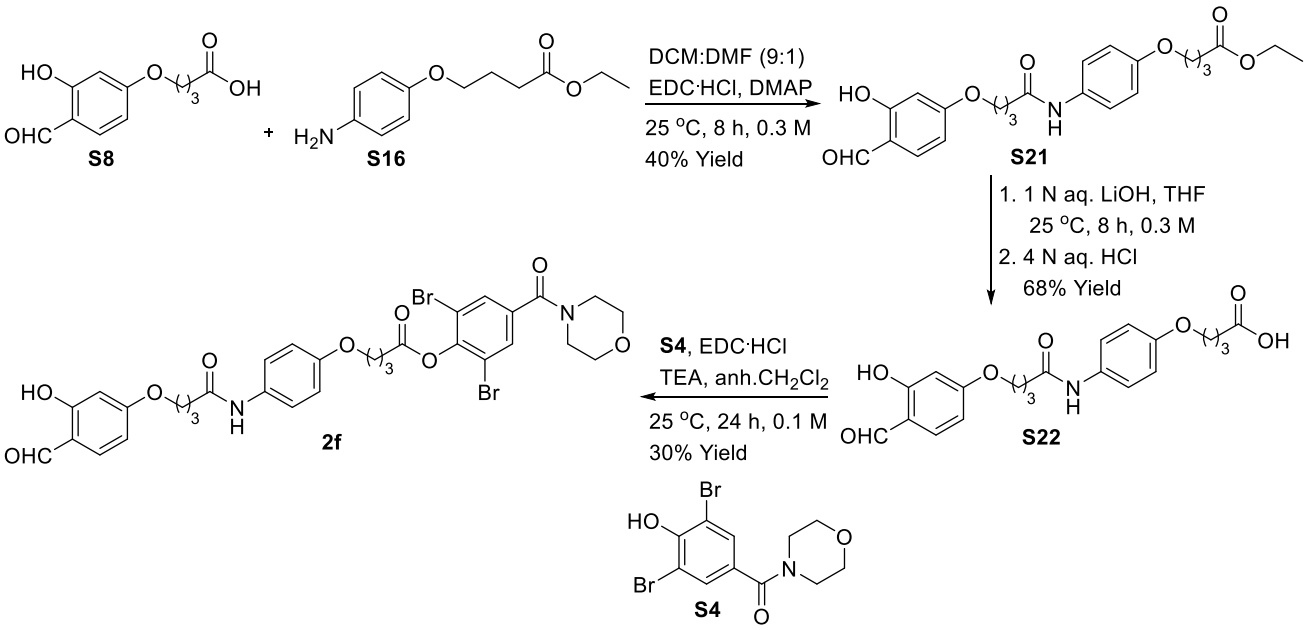


In a 10 mL round bottom flask, ethyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy) acetamido)phenoxy) butanoate **S19** (401 mg, 1 mmol) and 1 N LiOH (aq., 3 ml) were dissolved in THF (0.3 mL) and stirred at room temperature. The progress of the reaction was followed by TLC. After 8 h, the reaction mixture was quenched with 4 N HCl (aq.) and compounds were extracted with ethyl acetate. The collected organic fractions were dried over anh. sodium sulfate and concentrated in vacuo. The purification of crude reaction mixture was performed by silica gel flash column chromatography using ethyl acetate:n-hexane (70:30) to isolate 4-(4-(2-(4-formyl-3-hydroxyphenoxy)acetamido)phenoxy)butanoic acid **S20** (179 mg, 48% yield; R_f 0.30, ethyl acetate:n-hexane 80:20; pale yellow solid). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.00 (bs, 1H), 11.13 (bs, 1H), 10.04 (s, 1H), 10.00 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.54-7.49 (m, 2H), 6.94-6.85 (m, 2H), 6.63 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.52 (d, *J* = 2.2 Hz, 1H), 4.74 (s, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.97-1.86 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 190.8, 174.2, 165.3, 164.4, 162.9, 154.8, 131.9, 131.4, 121.3, 116.7, 114.5, 107.8, 101.7, 67.1, 66.7, 30.1, 24.3. HRMS (ESI) $[M+Na]^+$ calcd. $C_{19}H_{19}NO_7Na$ 396.1059, found 396.1057.

Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy) acetamido)phenoxy) butanoate (2e)

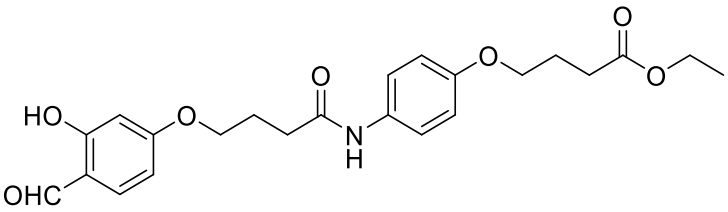


Yield 55%; R_f 0.21, ethyl acetate:n-hexane 65:25; pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.45 (s, 1H), 9.80 (s, 1H), 8.07 (s, 1H), 7.60 (s, 2H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.49 (d, *J* = 8.9 Hz, 2H), 6.93 (d, *J* = 8.9 Hz, 2H), 6.67 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.56 (d, *J* = 2.1 Hz, 1H), 4.67 (s, 2H), 4.13 (t, *J* = 6.0 Hz, 2H), 3.90-3.55 (m, 8H), 2.94 (t, *J* = 7.2 Hz, 2H), 2.36-2.24 (m, 2H) ppm. ^{13}C NMR (176 MHz, $CDCl_3$) δ 194.8, 169.4, 166.7, 164.8, 164.4, 163.6, 156.3, 147.5, 135.9, 135.5, 131.2, 129.8, 122.2, 118.3, 116.4, 115.0, 107.9, 102.5, 67.5, 66.9, 66.6, 30.4, 24.5, 14.2 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{30}H_{29}Br_2N_2O_9Na$ 741.0059, found 741.0060.



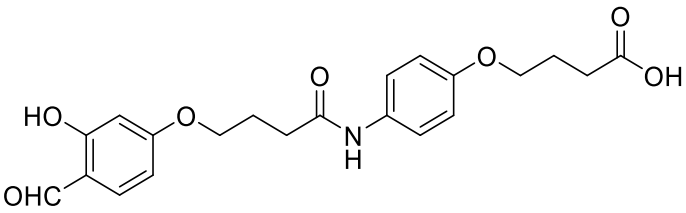
Scheme S6 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(4-(4-formyl-3-hydroxyphenoxy)butanamido) phenoxy) butanoate **2f**.

Synthesis of ethyl 4-(4-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy) butanoate (S21)



In a 25 mL round bottom flask, 4-(4-formyl-3-hydroxyphenoxy)butanoic acid **S8** (448 mg, 2 mmol), 3-(((ethylimino)methylene)amino)-N,N-dimethylpropan-1-amine (286 mg, 3 mmol), and 4-(dimethylamino)pyridine (12 mg, 0.1 mmol) were dissolved in anh. DCM:DMF (9:1, 6.6 mL). To this solution, ethyl 4-(4-aminophenoxy)butanoate **S16** (446 mg, 2 mmol) was added and stirred at 25 ^{o}C. The progress of the reaction was followed by thin layer chromatography. After 8 h, the reaction mixture was quenched with 1 N HCl (aq.) and the crude product was extracted with ethyl acetate. The collected organic fractions were dried over anh. sodium sulfate and concentrated in vacuo. The purification of crude reaction mixture was performed by silica gel flash column chromatography (ethyl acetate:n-hexane, 20:80) to isolate ethyl 4-(4-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy)butanoate **S21** (344 mg, 40% yield; R_f 0.18, ethyl acetate:n-hexane 50:50; white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.44 (s, 1H), 9.71 (s, 1H), 7.42 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 8.8 Hz, 2H), 7.21 (s, 1H), 6.83 (d, *J* = 8.9 Hz, 2H), 6.52 (dd, *J* = 8.6, 2.0 Hz, 1H), 6.42 (d, *J* = 1.8 Hz, 1H), 4.22-4.04 (m, 4H), 3.97 (t, *J* = 6.1 Hz, 2H), 2.62 (m, 4H), 2.32-2.17 (m, 2H), 2.17-2.00 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.5, 173.4, 170.1, 166.2, 164.6, 155.9, 135.5, 131.0, 121.9, 115.4, 115.0, 108.5, 101.5, 67.6, 67.2, 60.6, 33.6, 30.9, 24.9, 24.8, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{23}H_{28}NO_7$ 430.1866, found 430.1856.

4-(4-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy) butanoic acid (S22)

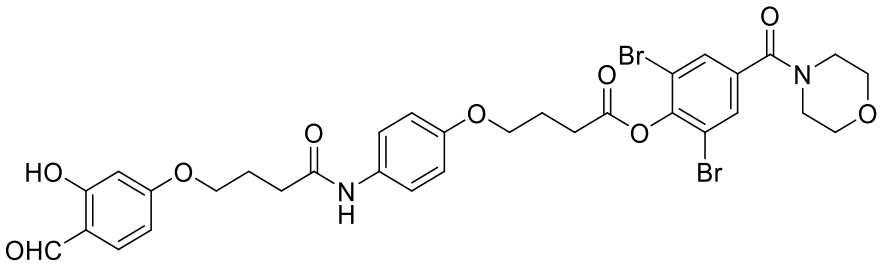


This compound was synthesized according to the procedure for synthesis of **S20**.

Yield 68%; R_f 0.17, ethyl acetate:n-hexane, 60:40; white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.07 (bs, 1H), 11.11 (bs, 1H), 10.00 (s, 1H), 9.79 (s, 1H), 7.61 (d, *J* = 8.7 Hz, 1H), 7.47

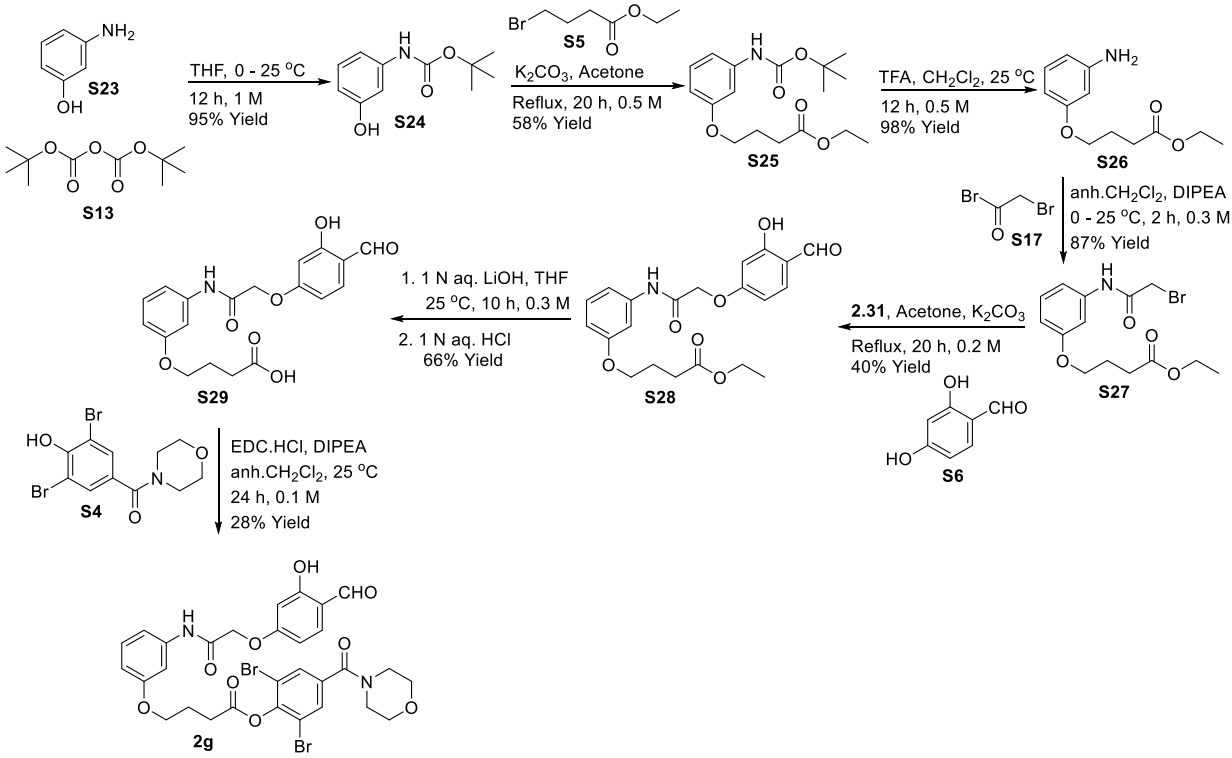
(d, *J* = 9.0 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.56 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.48 (d, *J* = 2.2 Hz, 1H), 4.08 (t, *J* = 6.3 Hz, 2H), 3.92 (t, *J* = 6.4 Hz, 2H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.36 (t, *J* = 7.3 Hz, 2H), 2.09-1.98 (m, 2H), 1.97-1.86 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 191.1, 174.1 170.0, 165.3, 163.1, 154.3, 132.5, 132.3, 120.6, 116.2, 114.4, 107.7, 101.2, 67.6, 66.7, 32.5, 30.1, 24.5, 24.3. HRMS (ESI) $[M+H]^+$ calcd. $C_{21}H_{24}NO_7$ 402.1553, found 402.1568.

2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(4-(4-formyl-3-hydroxyphenoxy) butanamido)phenoxy) butanoate (2f)



This compound was synthesized according to the procedure for synthesis of **2e**.

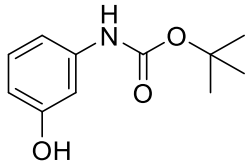
Yield 30%; R_f 0.35, ethyl acetate:n-hexane 60:40; pale yellow viscous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.45 (s, 1H), 9.71 (s, 1H), 7.60 (s, 2H), 7.43 (d, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 8.9 Hz, 2H), 7.14 (s, 1H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.53 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.43 (d, *J* = 2.1 Hz, 1H), 4.23-4.04 (m, 4H), 3.91-3,53 (m, 8H), 2.92 (t, *J* = 7.2 Hz, 2H), 2.55 (t, *J* = 7.1 Hz, 2H), 2.35-2.17 (m, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 170.2, 169.5, 166.8, 164.6, 155.8, 147.5, 135.5, 131.2, 131.1, 121.9, 118.3, 115.4, 114.9, 108.5, 101.5, 67.6, 66.9, 66.6, 33.6, 30.5, 29.8, 24.9, 24.6, 17.8, 15.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{32}H_{32}Br2N_2O_9$ 747.0553, found 747.0569.



Scheme S7 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(4-(2-(4-formyl-3-hydroxyphenoxy)acetamido)phenoxy) butanoate **2g**.

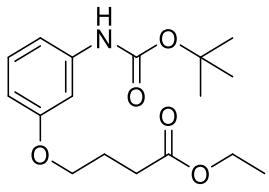
The procedure for synthesis of LDM reagent **2g** is similar to the LDM reagent **2e**.

Tert-butyl (3-hydroxyphenyl) carbamate (S24)



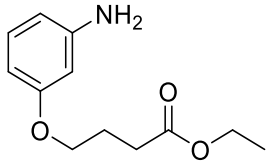
Yield 95%; R_f 0.42, ethyl acetate:n-hexane 10:90; white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18-7.06 (m, 2H), 6.72 (dd, *J* = 8.0, 1.2 Hz, 1H), 6.53 (dd, *J* = 8.1, 1.9 Hz, 1H), 6.49 (s, 1H), 5.49 (s, 1H), 1.51 (s, 9H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6, 153.0, 139.6, 130.1, 110.8, 110.3, 106.0, 81.0, 28.5 ppm. HRMS (ESI) $[M+Na]^+$ calcd. $C_{11}H_{15}NNaO_3$ 232.0950, found 232.0953.

Ethyl 4-(3-((tert-butoxycarbonyl)amino)phenoxy) butanoate (S25)



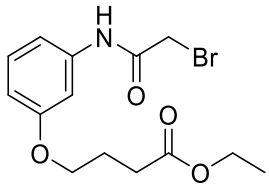
Yield 58%; R_f 0.17, ethyl acetate:n-hexane 20:80; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18-7.13 (m, 1H), 7.11 (s, 1H), 6.80 (dd, *J* = 8.0, 1.4 Hz, 1H), 6.56 (dd, *J* = 8.1, 2.2 Hz, 1H), 6.46 (s, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.00 (t, *J* = 6.1 Hz, 2H), 2.50 (t, *J* = 7.4 Hz, 2H), 2.14-2.05 (m, 2H), 1.51 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 159.7, 152.7, 139.7, 129.8, 110.9, 109.4, 104.9, 80.7, 66.8, 60.6, 31.0, 28.5, 24.8, 14.4 ppm. HRMS (ESI) $[M+Na]^+$ calcd. $C_{17}H_{25}NNaO_5$ 346.1630, found 346.1653.

Ethyl 4-(3-aminophenoxy) butanoate (S26)



Yield 98%; R_f 0.31, ethyl acetate:n-hexane 30:70; pale brown liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.07-7.01 (m, 1H), 6.34-6.26 (m, 2H), 6.25-6.21 (m, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 3.96 (t, *J* = 6.1 Hz, 2H), 3.63 (bs, 2H), 2.50 (t, *J* = 7.3 Hz, 2H), 2.13-2.02 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.4, 160.1, 147.9, 130.2, 108.1, 104.7, 101.8, 66.6, 60.5, 31.0, 24.8, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{12}H_{18}NO_3$ 224.1287, found 224.1287.

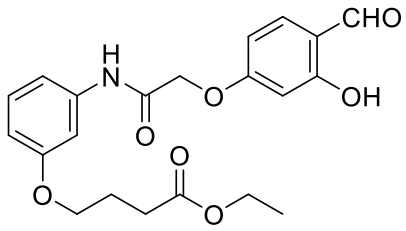
Ethyl 4-(3-(2-bromoacetamido)phenoxy) butanoate (S27)



Yield 87%; R_f 0.40, ethyl acetate:n-hexane 30:70; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.29-7.18 (m, 2H), 7.04-6.95 (m, 1H), 6.73-6.67 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.05-3.96 (m, 4H), 2.51 (t, *J* = 7.3 Hz, 2H), 2.15-2.05 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 173.3, 163.4, 159.6, 138.2, 130.0, 112.3, 111.6, 106.5,

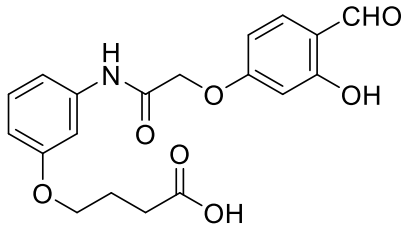
67.0, 60.6, 30.9, 29.7, 24.7, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{14}H_{19}BrNO_4$ 344.0497, found 344.0479.

Ethyl 4-(3-(2-(4-formyl-3-hydroxyphenoxy)acetamido)phenoxy) butanoate (S28)



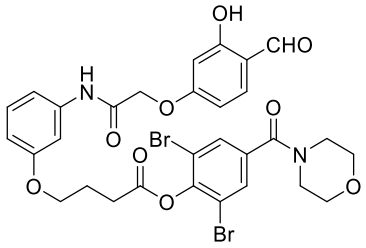
Yield 40%; R_f 0.28, ethyl acetate:n-hexane 50:50; cream solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.43 (s, 1H), 9.79 (s, 1H), 8.08 (s, 1H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.31-7.28 (m, 1H), 7.26-7.21 (m, 1H), 7.08-7.03 (m, 1H), 6.70 (dd, *J* = 8.2, 2.3 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.54 (d, *J* = 2.3 Hz, 1H), 4.65 (s, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.02 (t, *J* = 6.1 Hz, 2H), 2.51 (t, *J* = 7.3 Hz, 2H), 2.15-2.07 (m, 2H), 1.26 (t, *J* = 7.1 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8, 173.3, 164.9, 164.5, 163.6, 159.7, 137.8, 136.0, 130.0, 116.5, 112.5, 111.5, 108.0, 106.8, 102.6, 67.5, 67.0, 60.6, 30.9, 24.7, 14.4 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{21}H_{24}NO_7$ 402.1553, found 402.1530.

4-(3-(2-(4-formyl-3-hydroxyphenoxy)acetamido)phenoxy) butanoic acid (S29)

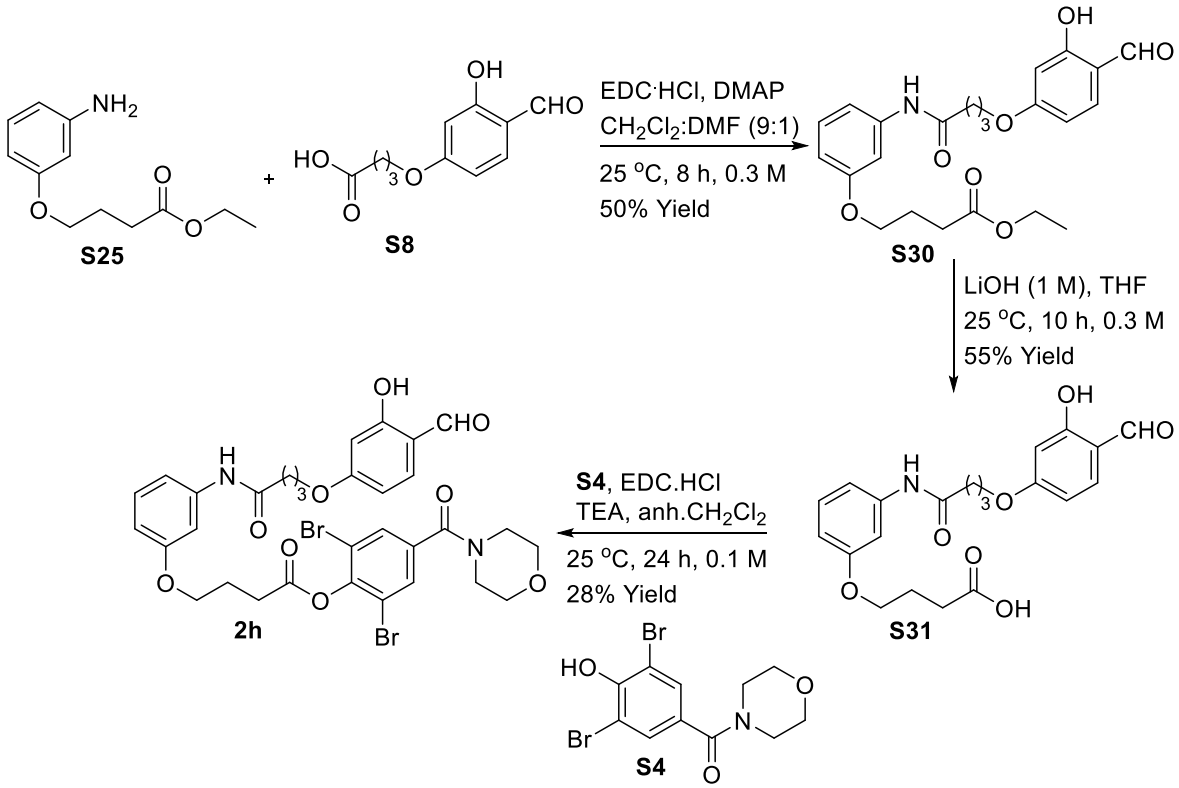


Yield 66%; R_f 0.29, MeOH:DCM 05:95; white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.11 (s, 1H), 11.01 (s, 1H), 10.12 (s, 1H), 10.04 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.34-7.29 (m, 1H), 7.24-7.19 (m, 1H), 7.18-7.13 (m, 1H), 6.66 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.63 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 4.77 (s, 2H), 3.94 (t, *J* = 6.4 Hz, 2H), 2.37 (t, *J* = 7.3 Hz, 2H), 1.99-1.87 (m, 2H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ 190.8, 174.1, 165.8, 164.4, 162.8, 158.8, 139.5, 131.9, 129.6, 116.7, 111.9, 109.8, 107.7, 105.9, 101.7, 67.1, 66.5, 30.1, 24.2 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{19}H_{20}NO_7$ 374.1240, found 374.1253.

2,6-dibromo-4-(morpholine-4-carbonyl)phenyl4-(3-(2-(4-formyl-3-hydroxyphenoxy) acetamido)phenoxy) butanoate (2g)



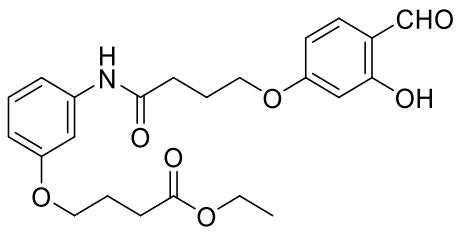
Yield 28%; R_f 0.40, ethyl acetate:n-hexane 55:45; pale yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.43 (s, 1H), 9.78 (s, 1H), 8.12 (s, 1H), 7.60 (s, 2H), 7.54 (d, *J* = 8.6 Hz, 1H), 7.37 (s, 1H), 7.31-7.24 (m, 1H), 7.04 (d, *J* = 7.6 Hz, 1H), 6.74 (dd, *J* = 8.1, 1.7 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.1 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 4.65 (s, 2H), 4.14 (t, *J* = 5.9 Hz, 2H), 3.71-3.49 (m, 8H), 2.93 (t, *J* = 7.2 Hz, 2H), 2.39-2.23 (m, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.8, 169.4, 166.8, 165.0, 164.5, 163.6, 159.6, 147.6, 137.9, 136.0, 135.6, 131.3, 130.1, 118.3, 116.5, 112.6, 111.4, 108.0, 106.9, 102.6, 67.5 (2C), 66.9, 66.5, 30.5, 24.6. HRMS (ESI) $[M+H]^+$ calcd. $C_{30}H_{29}Br_2N_2O_9$ 719.0240, found 719.0262.



Scheme S8 Synthesis of 2,6-dibromo-4-(morpholine-4-carbonyl)phenyl 4-(3-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy) butanoate **2h**.

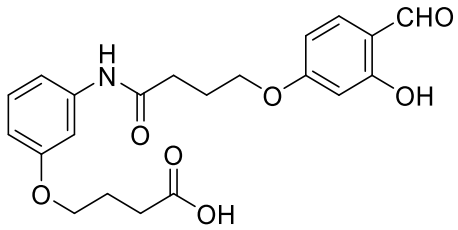
The procedure for synthesis of LDM reagent **2h** is similar to the LDM reagent **2f**.

Ethyl 4-(3-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy) butanoate (S30)



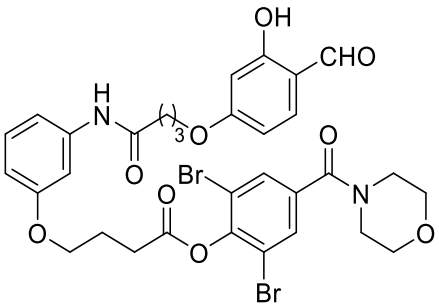
Yield 50%; R_f 0.30, ethyl acetate:n-hexane 30:70; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 9.71 (s, 1H), 7.42 (d, *J* = 8.7 Hz, 1H), 7.32 (bs, 1H), 7.28 (bs, 1H), 7.22-7.15 (m, 1H), 6.97-6.89 (m, 1H), 6.64 (dd, *J* = 8.2, 1.7 Hz, 1H), 6.53 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.43 (d, *J* = 2.2 Hz, 1H), 4.17-4.09 (m, 4H), 3.99 (t, *J* = 6.1 Hz, 2H), 2.56 (t, *J* = 7.1 Hz, 2H), 2.50 (t, *J* = 7.3 Hz, 2H), 2.28-2.19 (m, 2H), 2.14-2.05 (m, 2H), 1.26 (t, *J* = 7.1, 3.2 Hz, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 173.4, 170.3, 166.1, 164.6, 159.6, 139.1, 135.5, 129 .8, 115.4, 112.0, 110.8, 108.5, 106.3, 101.5, 67.5, 66.9, 60.6, 38.8, 33.8, 30.9, 24.7, 14.4 ppm. HRMS (ESI) $[M+Na]^+$ calcd. $C_{23}H_{27}NNaO_7$ 452.1685, found 452.1707.

4-(3-(4-(4-formyl-3-hydroxyphenoxy)butanamido)phenoxy) butanoic acid (S31)



Yield 55%; R_f 0.27, ethyl acetate:n-hexane 70:30; white solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 11.45 (s, 1H), 10.67 (bs, 1H), 9.80 (s, 1H), 9.14 (s, 1H), 7.64 (d, *J* = 8.7 Hz, 1H), 7.44 (s, 1H), 7.12.-7.09 (m, 2H), 6.62 (dd, *J* = 8.7, 2.4 Hz, 2H), 6.47 (d, *J* = 2.2 Hz, 1H), 4.20 (t, *J* = 6.3 Hz, 2H), 4.02 (t, *J* = 6.3 Hz, 2H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.50 (t, *J* = 7.3 Hz, 2H), 2.23-2.11 (m, 2H), 2.12-1.99 (m, 2H) ppm. ^{13}C NMR (126 MHz, Acetone-d_6) δ 196.1, 174.4, 171.3, 167.3, 165.2, 160.4, 141.6, 136.6, 130.3, 116.3, 112.4, 110.2, 109.3, 106.7, 102.0, 68.8, 67.6, 33.8, 33.7 30.7, 25.5 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{21}H_{24}NO_7$ 402.1553, found 402.1556.

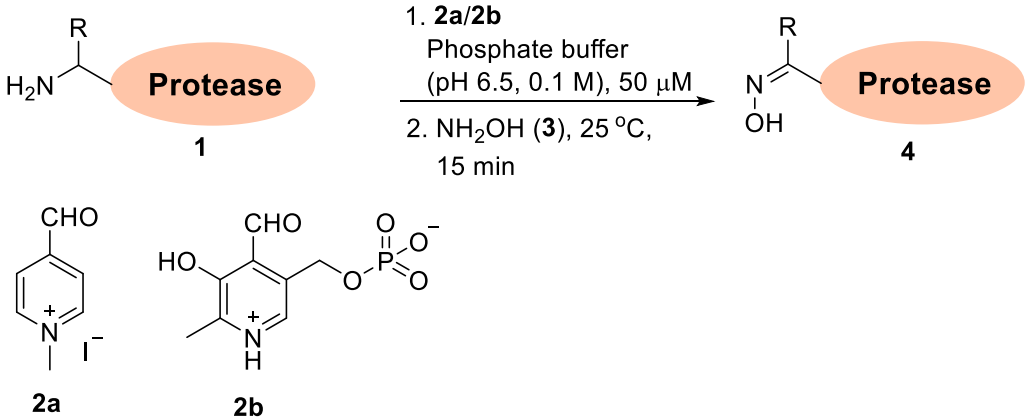
2,6-dibromo-4-(morpholine-4-carbonyl)phenyl4-(3-(4-(4-formyl-3-hydroxyphenoxy) butanamido)phenoxy) butanoate (2h)



Yield 28%; R_f 0.27, ethyl acetate:n-hexane 50:50; white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.44 (s, 1H), 9.70 (s, 1H), 7.68-7.57 (m, 3H), 7.41 (d, *J* = 8.6 Hz, 1H), 7.35 (s, 1H), 7.23-7.14 (m, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 1H), 6.51 (dd, *J* = 8.5 Hz, 1H), 6.41 (d, *J* = 1.8 Hz, 1H), 4.19-4.01 (m, 4H), 3.92-3.41 (m, 8H), 2.91 (t, *J* = 7.3 Hz, 2H), 2.55 (t, *J* = 7.0 Hz, 2H), 2.32-2.18 (m, 4H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ 194.5, 170.5, 169.4, 166.7, 166.1, 164.5, 159.4, 147.5, 139.2, 135.5, 131.4, 131.2, 129.8, 118.3, 115.4, 112.1, 110.6, 108.5, 106.4, 101.5, 67.5, 66.8, 66.3, 33.7, 30.4, 24.7, 24.5, 17.7 ppm. HRMS (ESI) $[M+H]^+$ calcd. $C_{32}H_{33}Br_2N_2O_9$ 747.0553, found 747.0581.

4. Single-site Protease Modification

4.1 N-terminus modification of proteases



Scheme S9 Biomimetic transamination reaction of proteases.

This strategy allows the installation of aldehyde on N-terminus amino acid via transamination reaction.

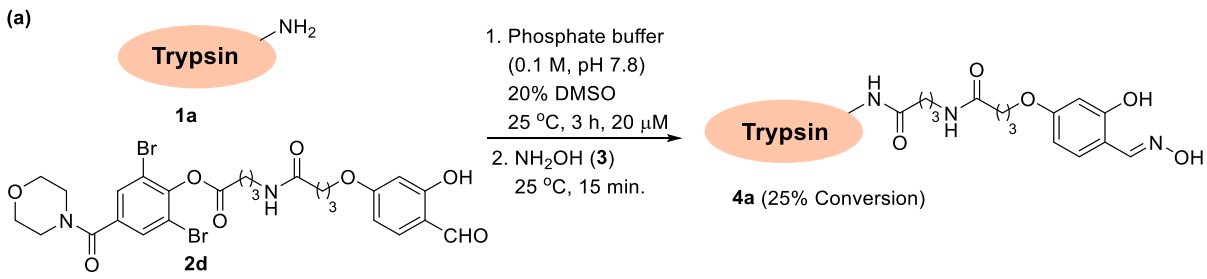
Table S3: Biomimetic N-terminal transamination of proteases

Entry	Protease	Reagent	Equivalence	% Conversion
1	α-Chymotrypsin	**2c**	50	n.d.
2	α-Chymotrypsin	**2c**	200	n.d.
3	α-Chymotrypsin	**2d**	50	n.d.
4	α-Chymotrypsin	**2d**	200	n.d.
5	Trypsin	**2c**	50	n.d.
6	Trypsin	**2c**	200	n.d.
7	Trypsin	**2d**	50	n.d.
8	Trypsin	**2d**	200	n.d.

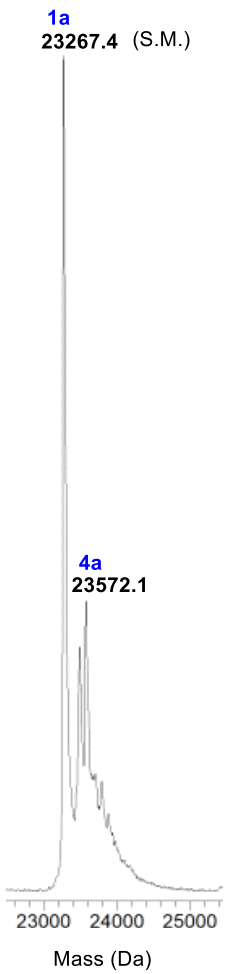
% Conversion was determined by MALDI-ToF-MS. n.d. – not detected.

In search of a methodology for single-site modification of serine proteases, we examined the biomimetic approach by Francis and coworkers to install an aldehyde tag at the N-terminus.[13] In this regard, we treated α-chymotrypsin and trypsin with N-methylpyridine-4-carboxaldehyde iodide and pyridoxal 5′-phosphate (Figure S9). We screened various reaction conditions to modify the proteases but were not successful (Table S1). On the contrary, we found unidentified species with lower masses indicating the possible degradation of proteases.

4.2 Single-site modification of proteases by LDM$_{K\text{-}K}$ reagents



(b)



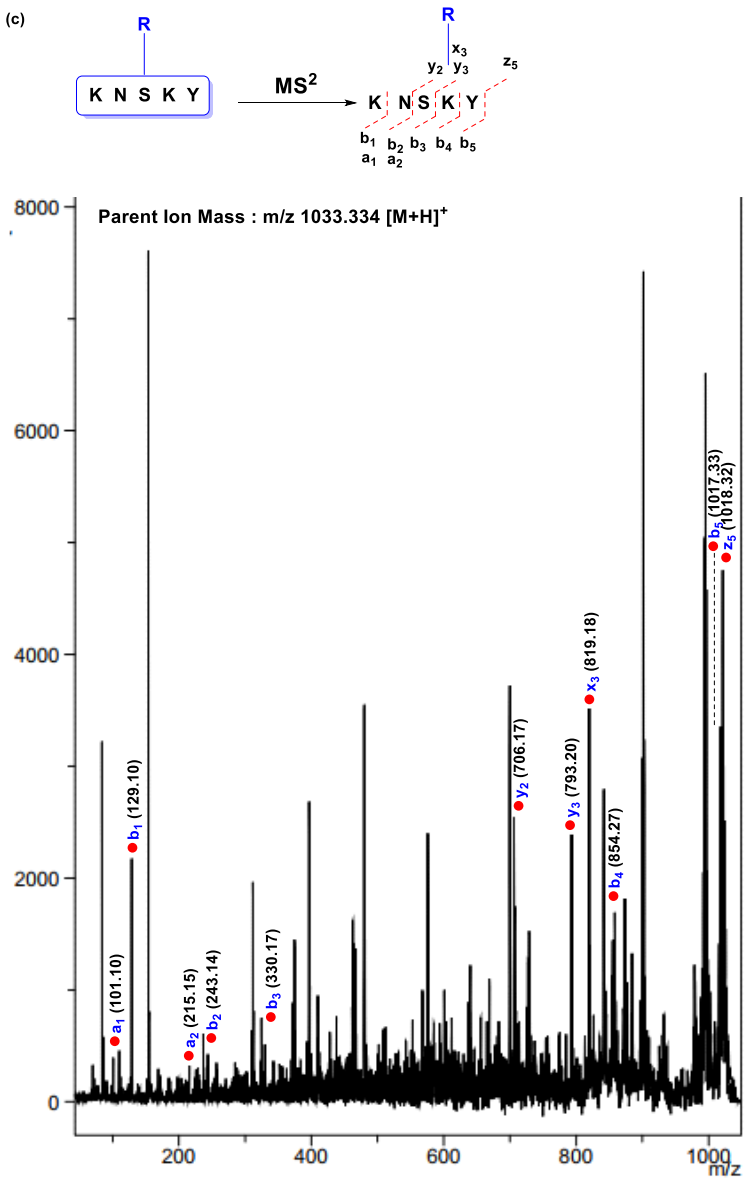
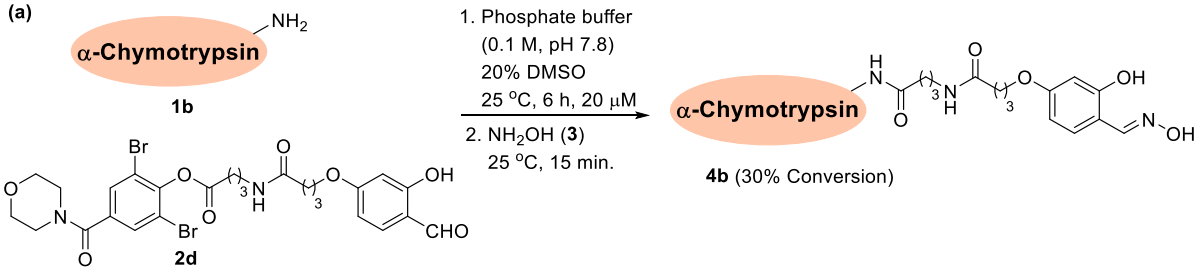
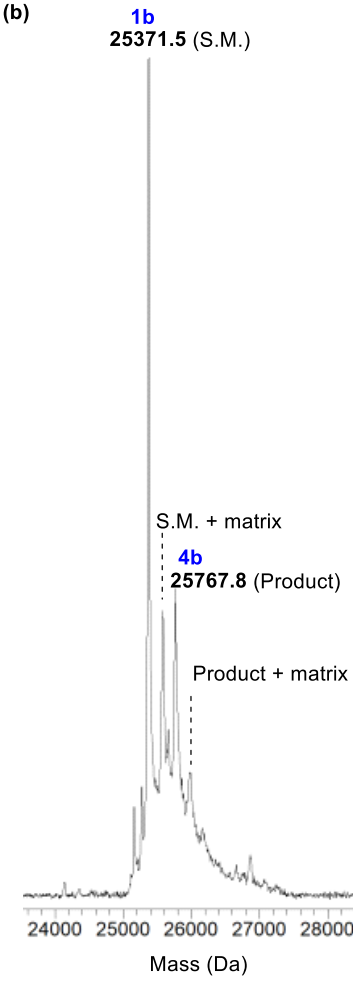


Figure S1 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2d** (25 equiv.). (b) MALDI-ToF-MS spectrum for mono-labeled trypsin **1a** after oxime **4a**. (c) MS-MS spectrum of labeled KNSKY (K88-Y92, *m/z* 1033.3 $[M+H]^+$) after the digestion of **4a** with α-chymotrypsin. The site of modification in mono-labeled trypsin C **4a** is K91.

(a)


α-Chymotrypsin
NH2
1b
Br
O
N
O
Br
2d
1. Phosphate buffer
(0.1 M, pH 7.8)
20% DMSO
25 °C, 6 h, 20 μM
2. NH2OH (3)
25 °C, 15 min.
α-Chymotrypsin
4b (30% Conversion)
OH
CHO
N
OH

(b)


1b
25371.5 (S.M.)
S.M. + matrix
4b
25767.8 (Product)
Product + matrix
24000 25000 26000 27000 28000
Mass (Da)

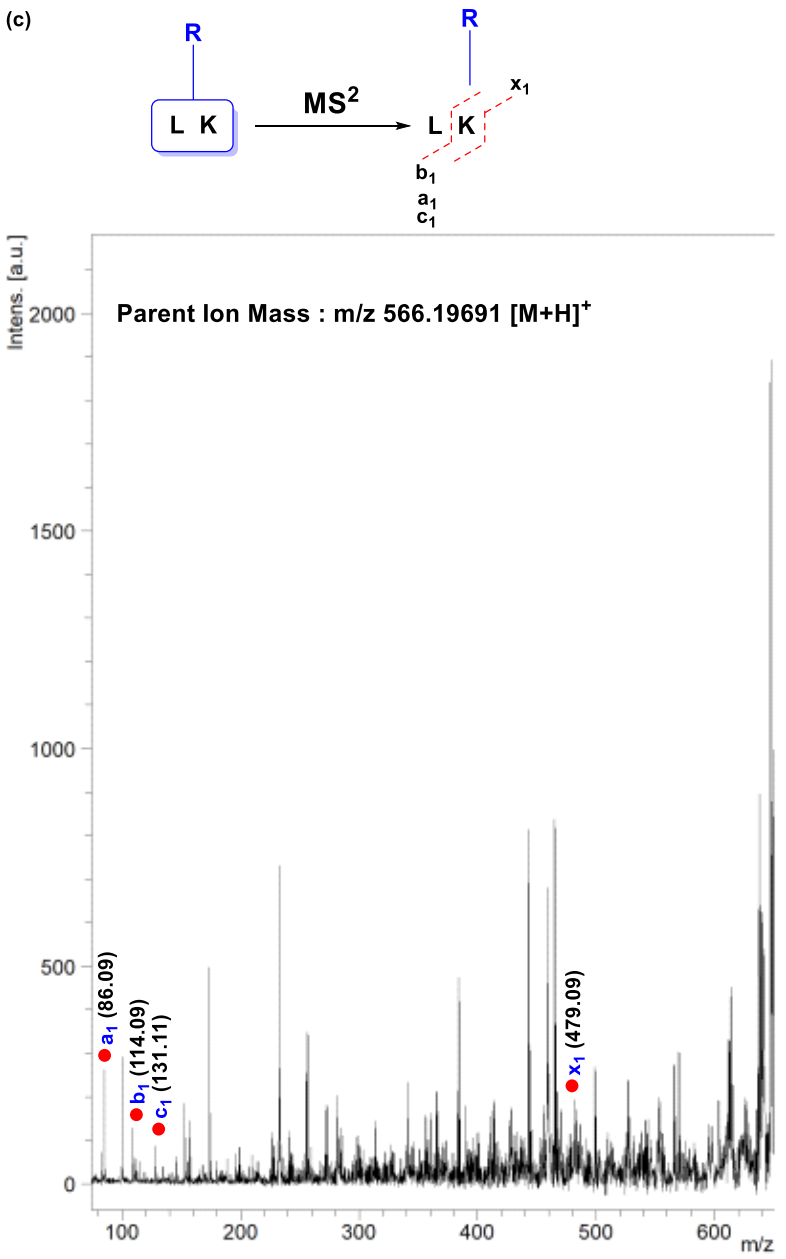


Figure S2 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2d** (25 equiv.). (b) MALDI-ToF-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **4b**. (c) MS-MS spectrum of labeled LK (L90-K91, *m/z* 566.19 $[M+H]^+$) after the digestion of **4b** with trypsin. The site of modification in mono-labeled α-chymotrypsin **4b** is K91.

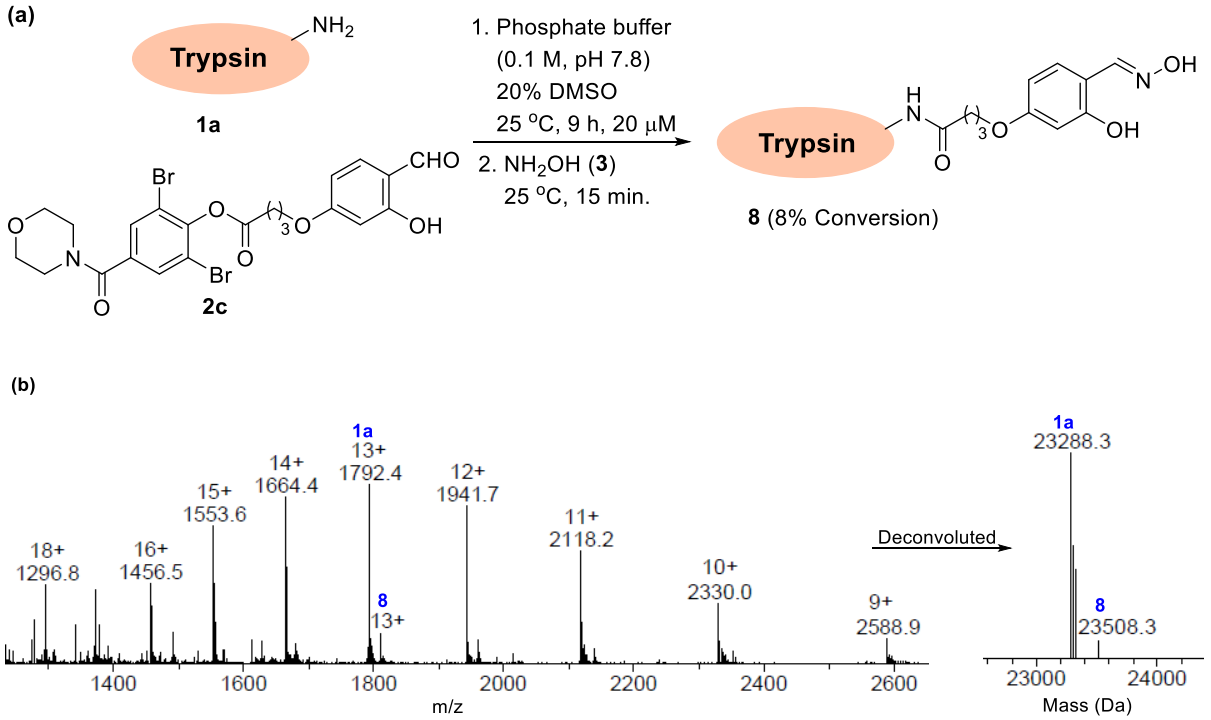


Figure S3 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2c** (25 equiv.). (b) ESI-MS spectrum for mono-labeled trypsin **1a** after oxime **8**.

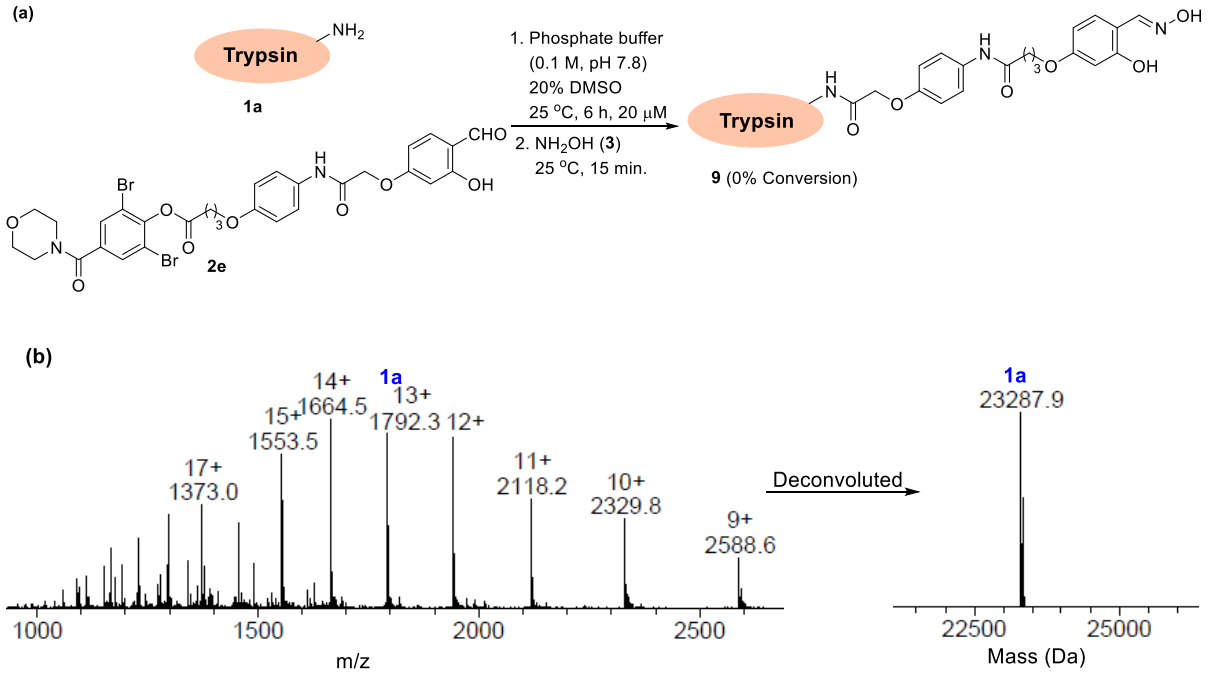


Figure S4 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2c** (25 equiv.). (b) ESI-MS spectrum for labeled trypsin **1a** after oxime **9**.

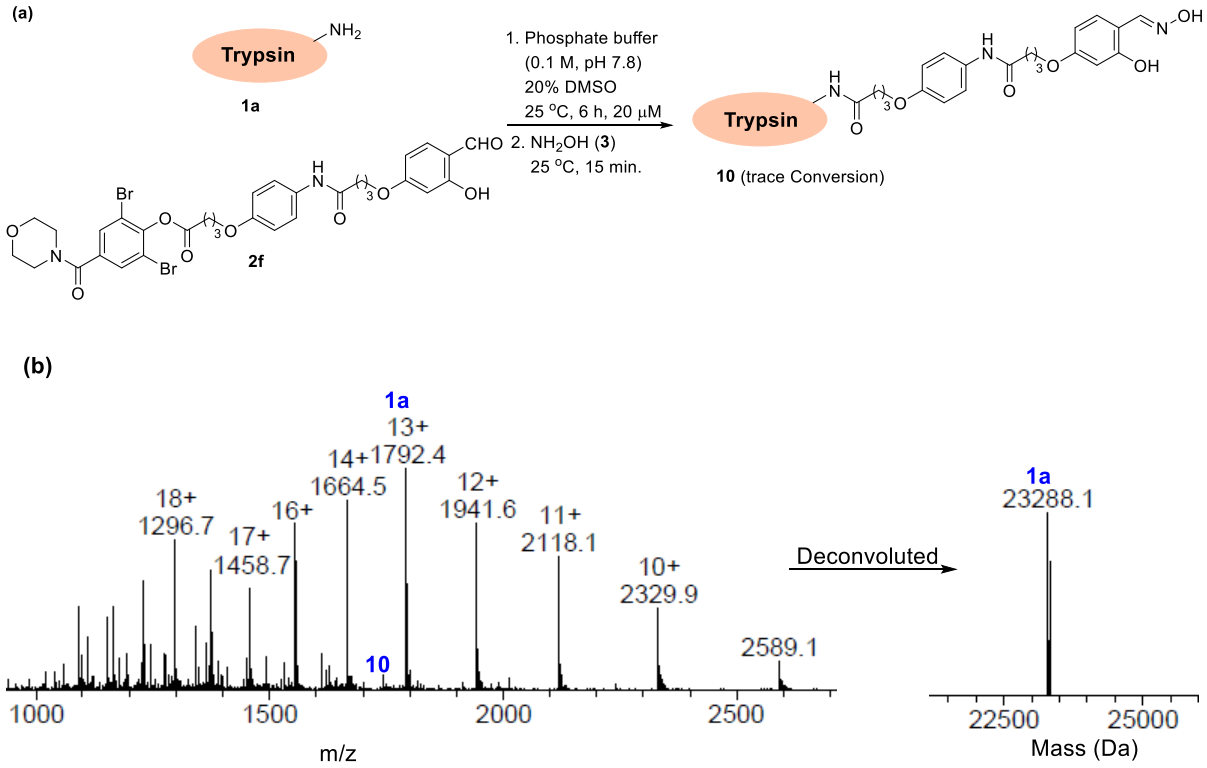


Figure S5 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2c** (25 equiv.). (b) ESI-MS spectrum for mono-labeled trypsin **1a** after oxime **10**.

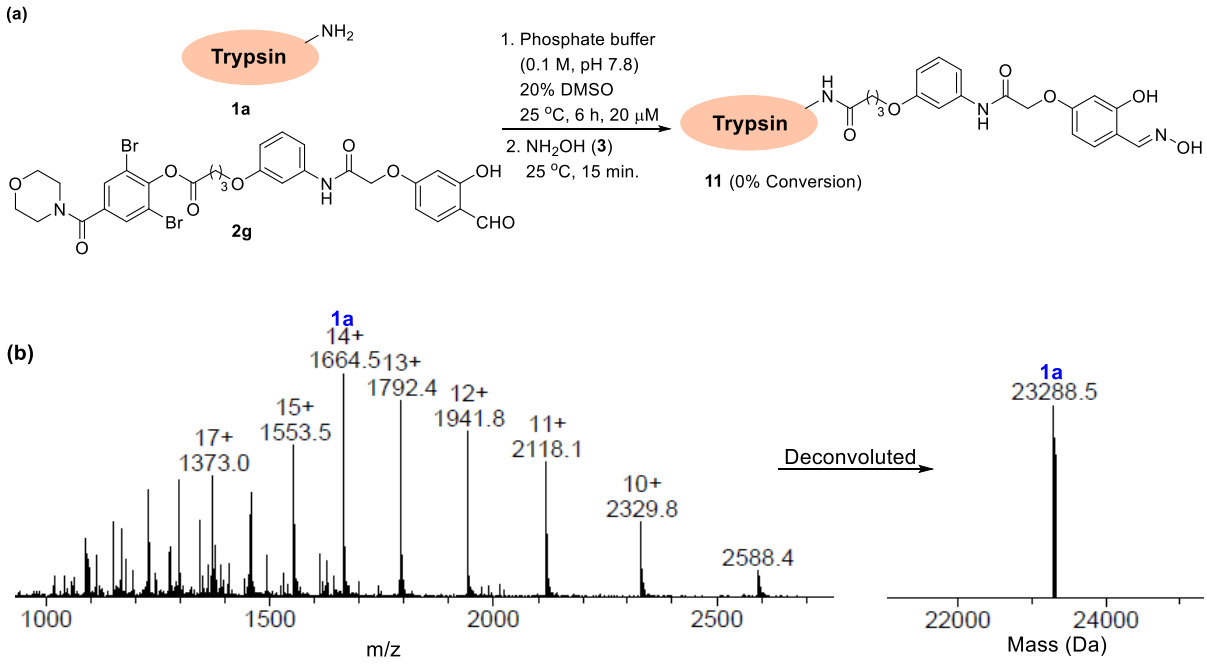


Figure S6 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2g** (25 equiv.). (b) ESI-MS spectrum for labeled trypsin **1a** after oxime **11**.

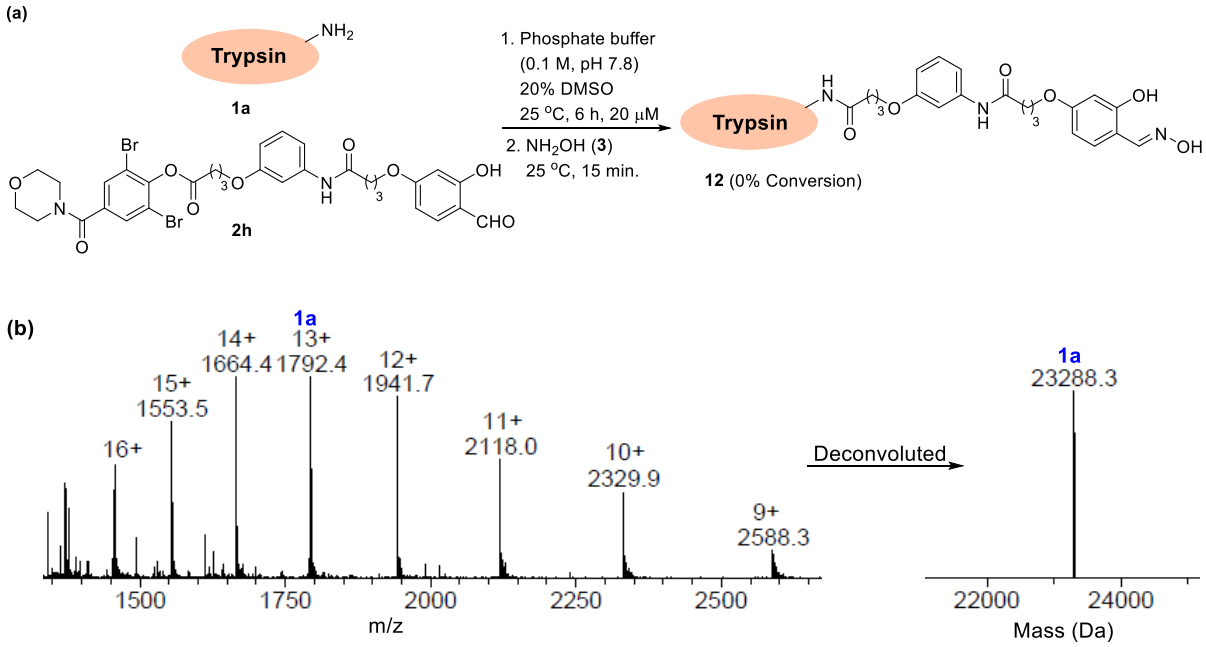


Figure S7 (a) Site-selective labeling of trypsin **1a** (1 equiv.) enabled by LDM reagent **2h** (25 equiv.). (b) ESI-MS spectrum for labeled trypsin **1a** after oxime **12**.

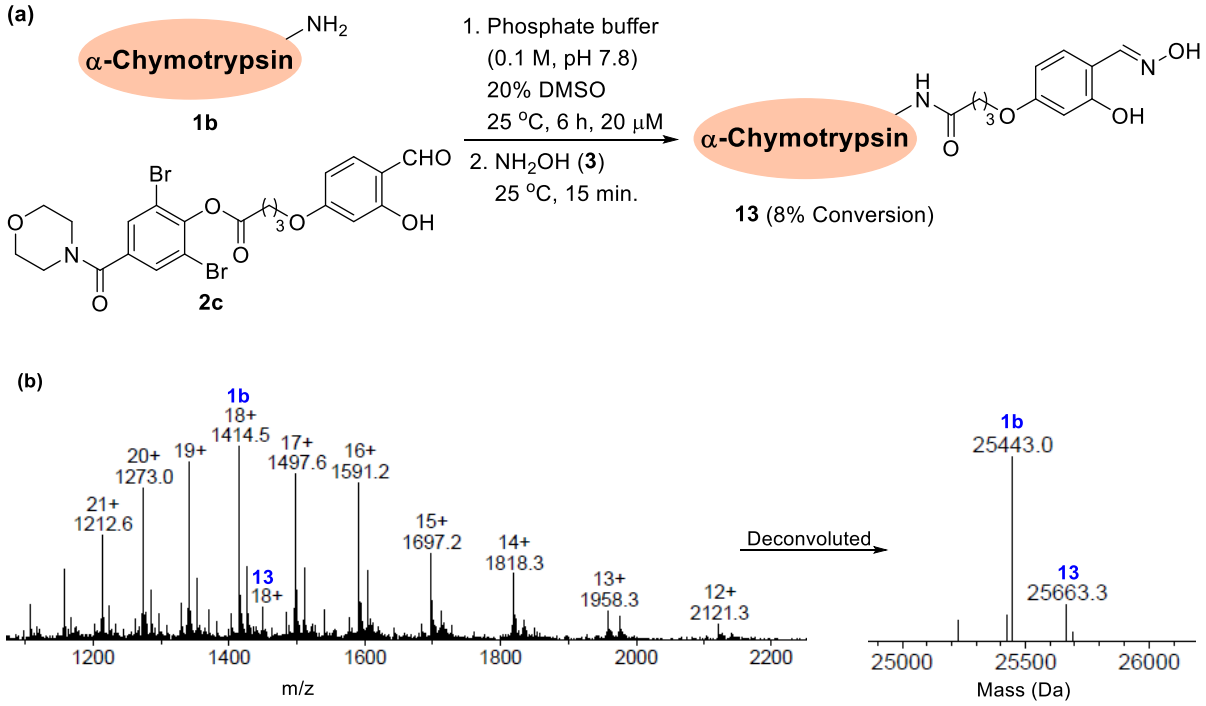


Figure S8 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2c** (25 equiv.). (b) ESI-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **13**.

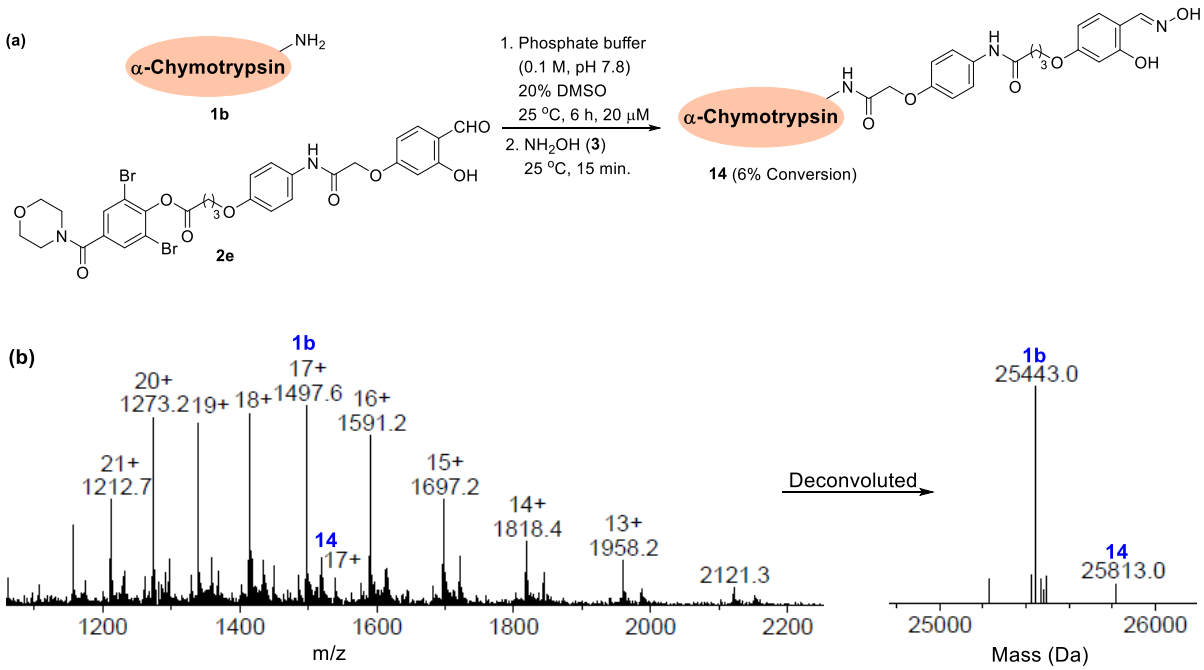


Figure S9 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2e** (25 equiv.). (b) ESI-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **14**.

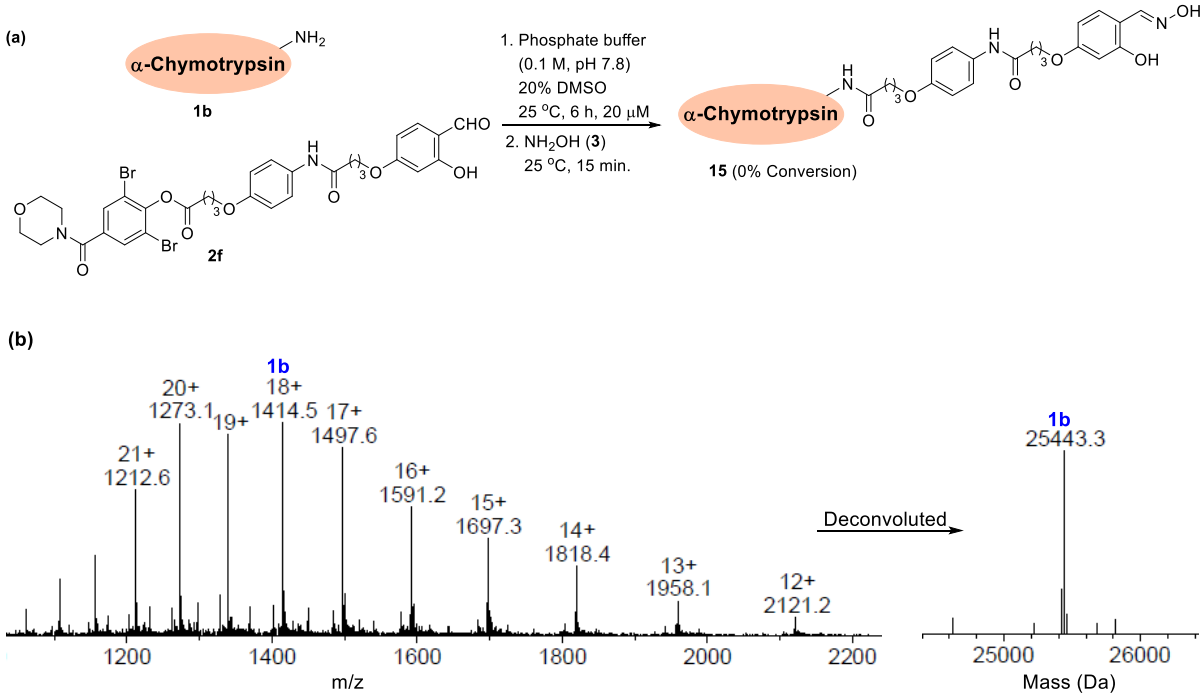


Figure S10 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2f** (25 equiv.). (b) ESI-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **15**.

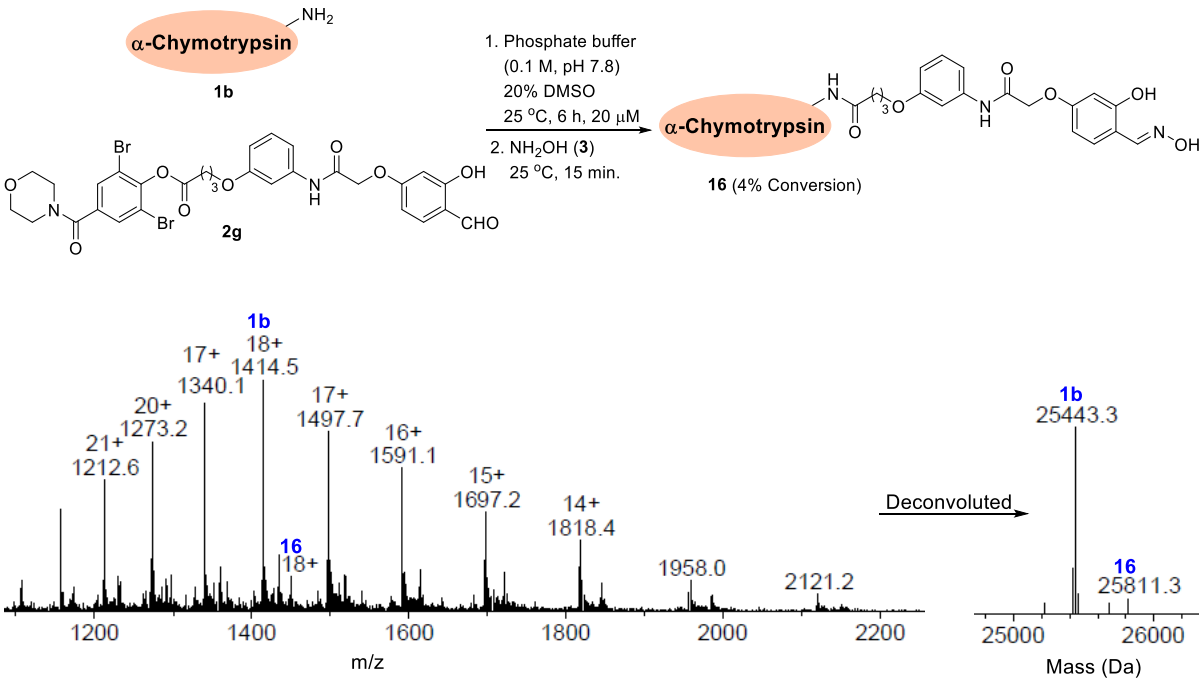


Figure S11 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2g** (25 equiv.). (b) ESI-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **16**.

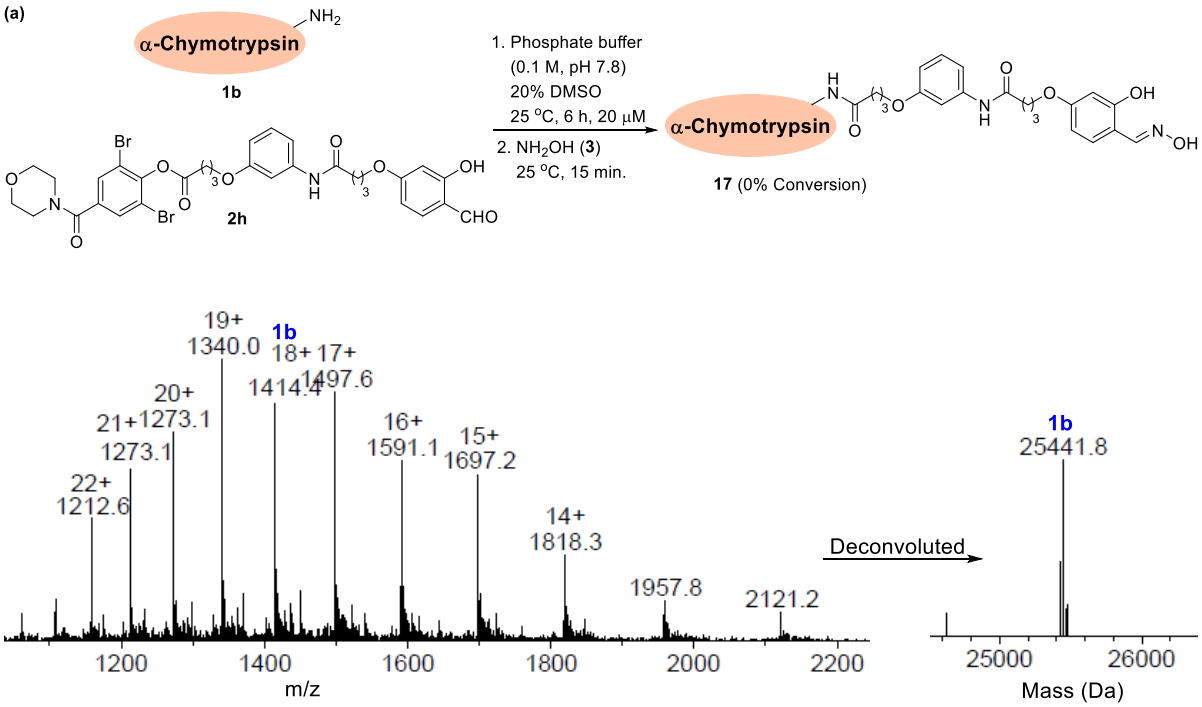


Figure S12 (a) Site-selective labeling of α-chymotrypsin **1b** (1 equiv.) enabled by LDM reagent **2h** (25 equiv.). (b) ESI-MS spectrum for mono-labeled α-chymotrypsin **1b** after oxime **17**.

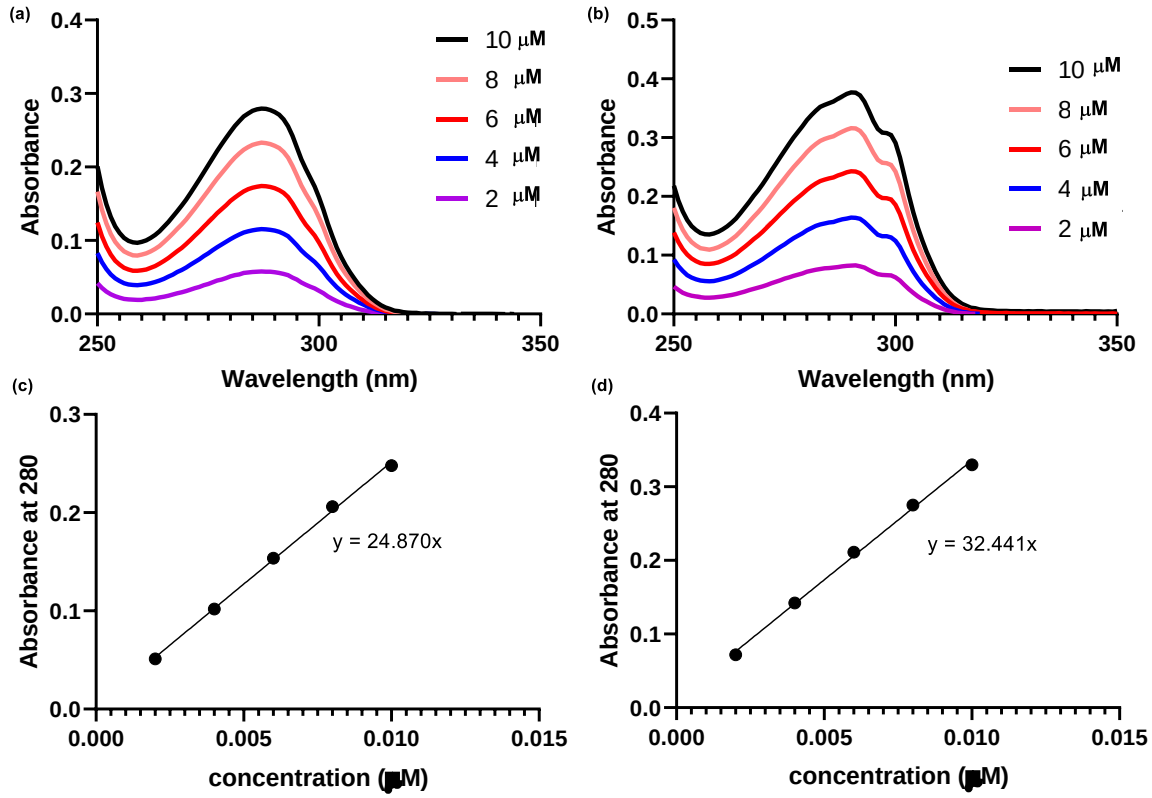


Figure S13 UV spectral calibration. (a) UV spectra of trypsin at different concentrations. (b) UV spectra of α-chymotrypsin at different concentrations. (c) absorbance monitored at 280 nm with concentration of trypsin for (a). (d) absorbance monitored at 280 nm with concentration of α-chymotrypsin for (b).

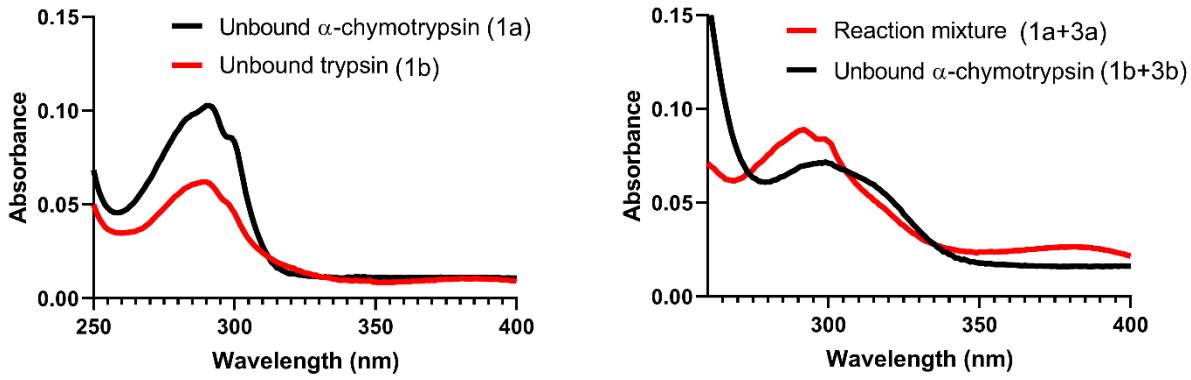
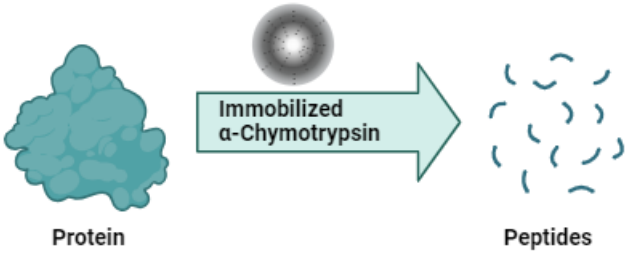


Figure S14 UV spectral analysis of immobilization. **(**a) UV spectra of unbound fractions of random immobilization of α-chymotrypsin and trypsin. (b) UV spectra of reaction mixture and unbound fraction for ordered immobilization of α-Chymotrypsin.

5. Peptide coverage using immobilized protease



Scheme S10 Schematic presentation of digestion of protein by random and ordered protease immobilized resin.

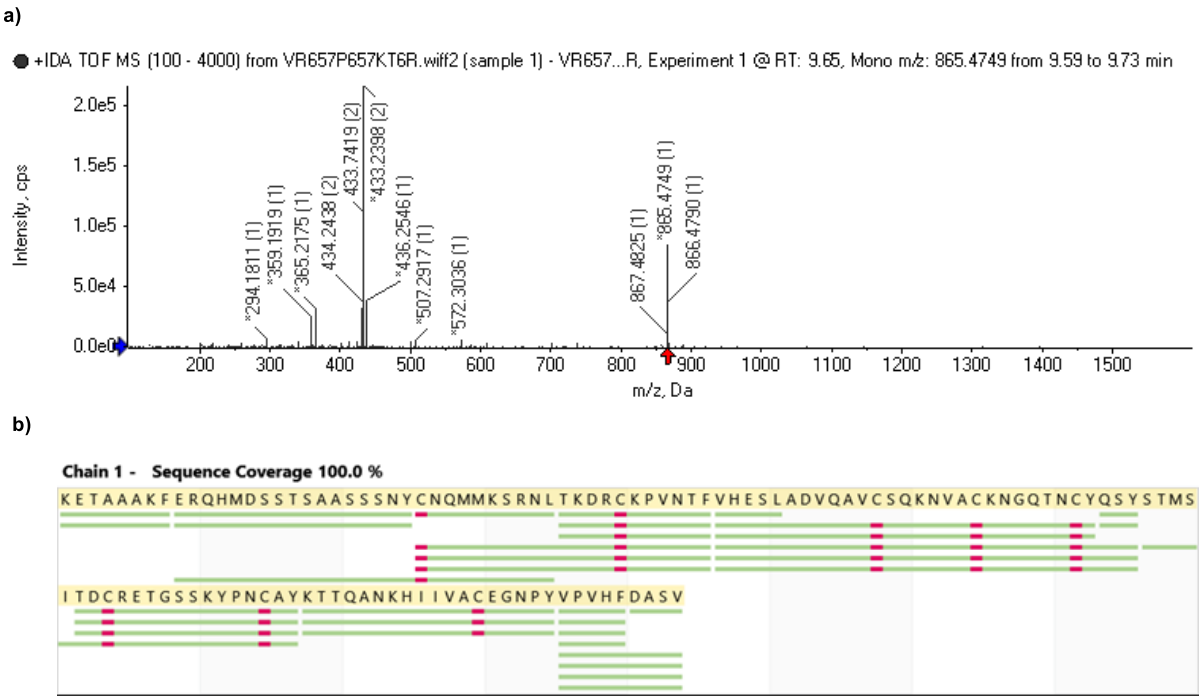


Figure S15 (a) Peptide mapping and (b) % coverage of RNase A using randomly immobilized α-chymotrypsin for cycle 1.

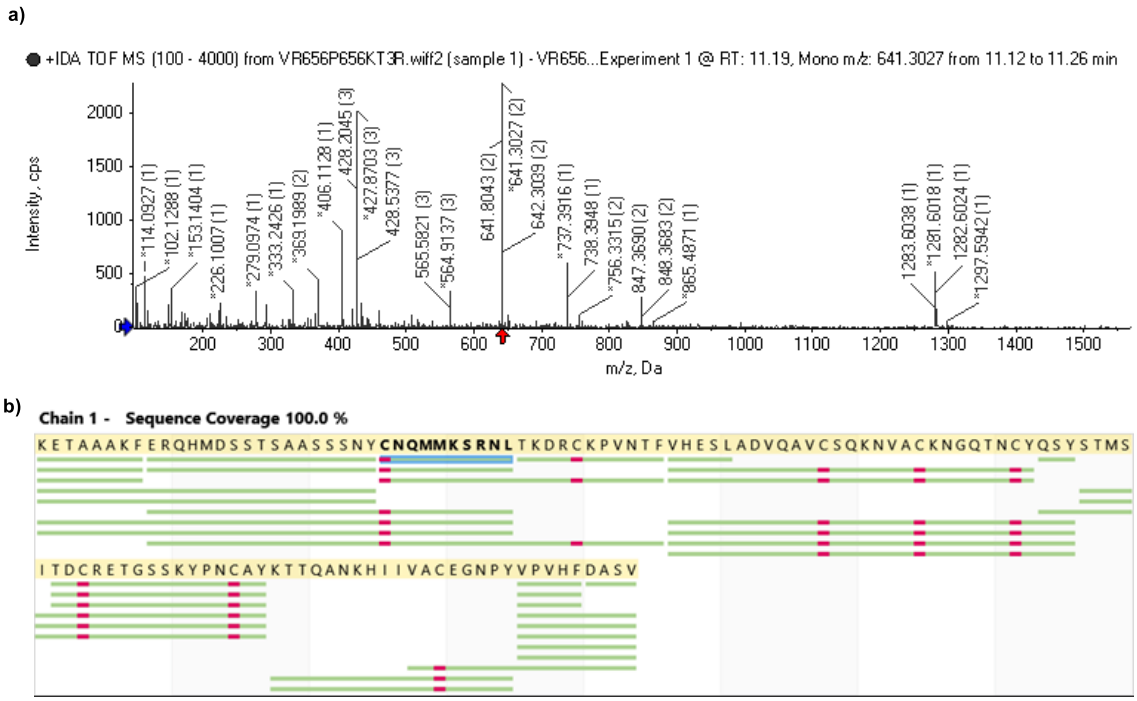


Figure S16 (a) Peptide mapping and (b) % coverage of RNase A using ordered immobilized α-chymotrypsin for cycle 1.

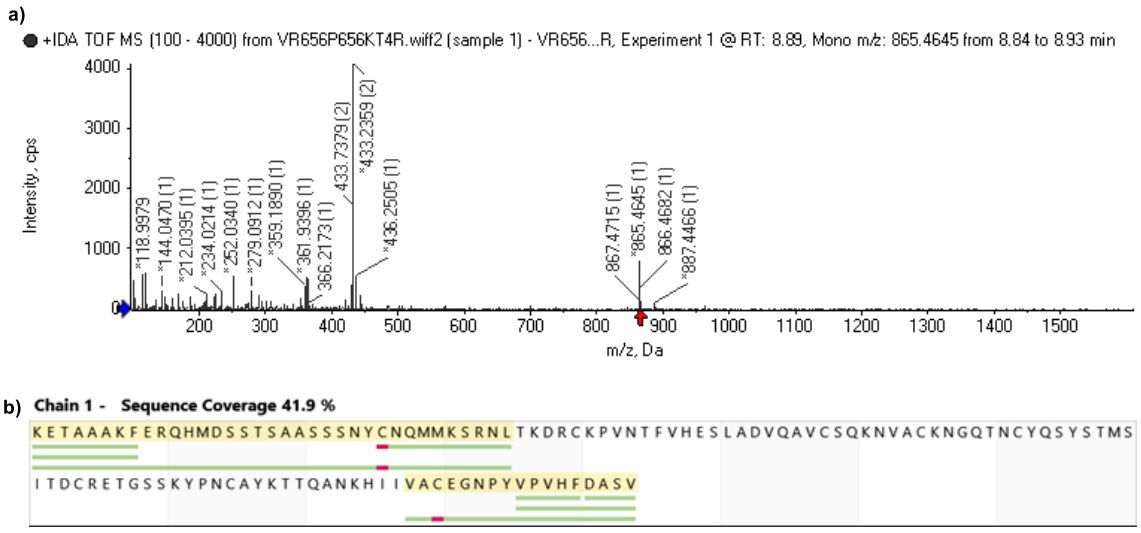


Figure S17 (a) Peptide mapping and (b) % coverage of RNase A using randomly immobilized α-chymotrypsin for cycle 2.

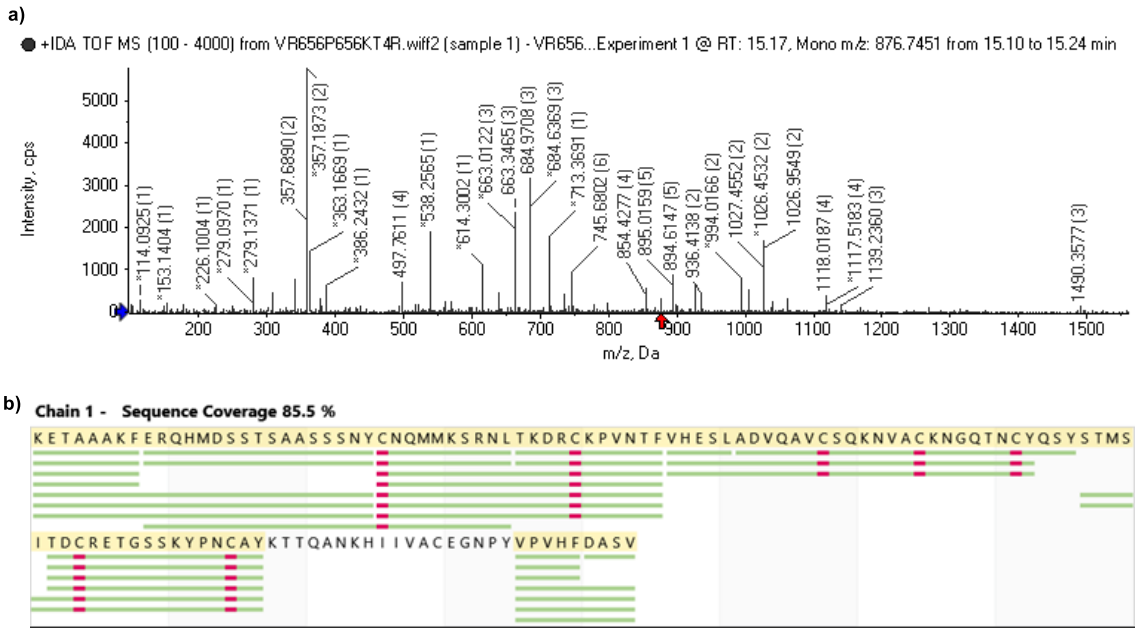


Figure S18 (a) Peptide mapping and (b) % coverage of RNase A using ordered immobilized α-chymotrypsin for cycle 2.

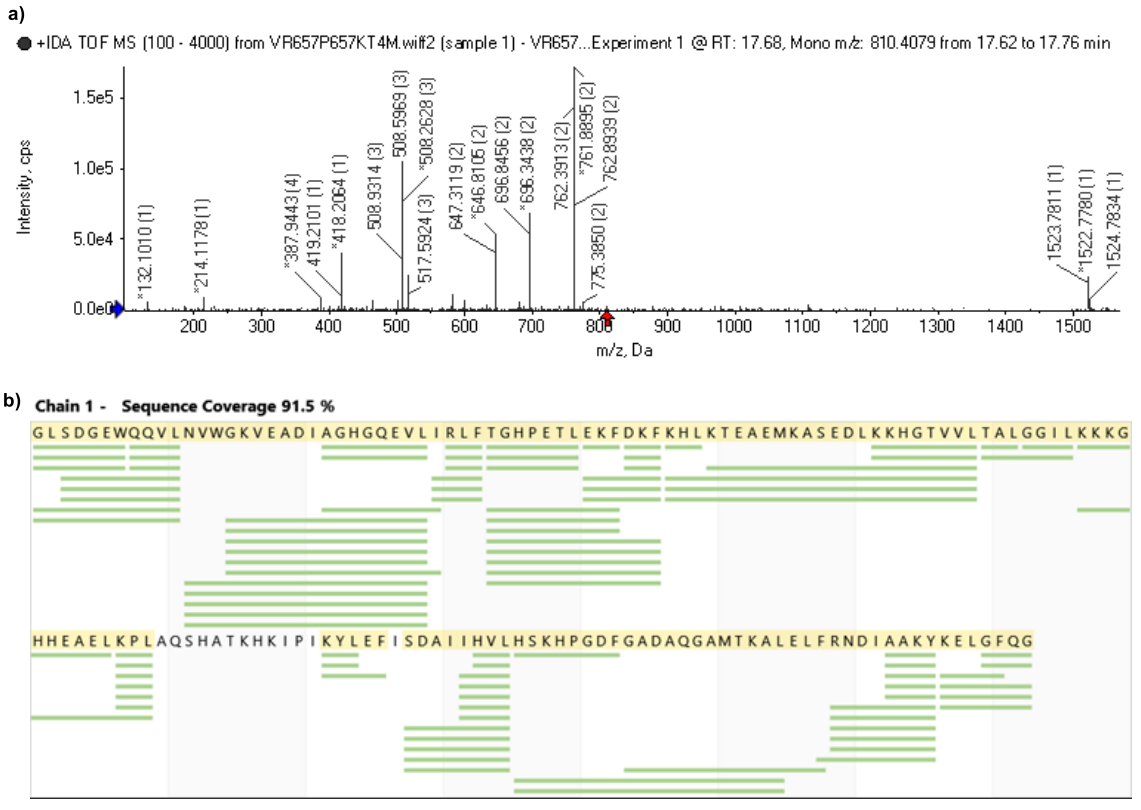


Figure S19 (a) Peptide mapping and (b) % coverage of myoglobin using randomly immobilized α-chymotrypsin for cycle 1.

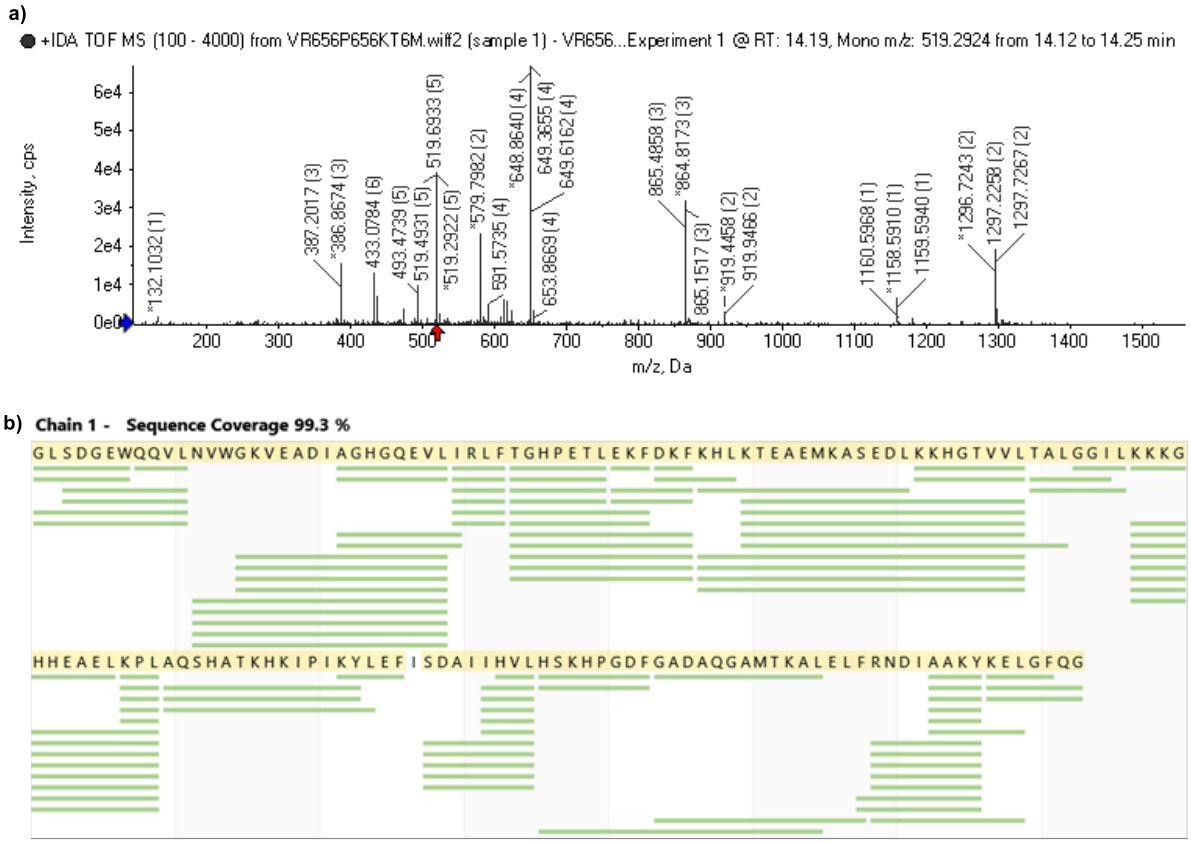


Figure S20 (a) Peptide mapping and (b) % coverage of myoglobin using ordered immobilized α-chymotrypsin for cycle 1.

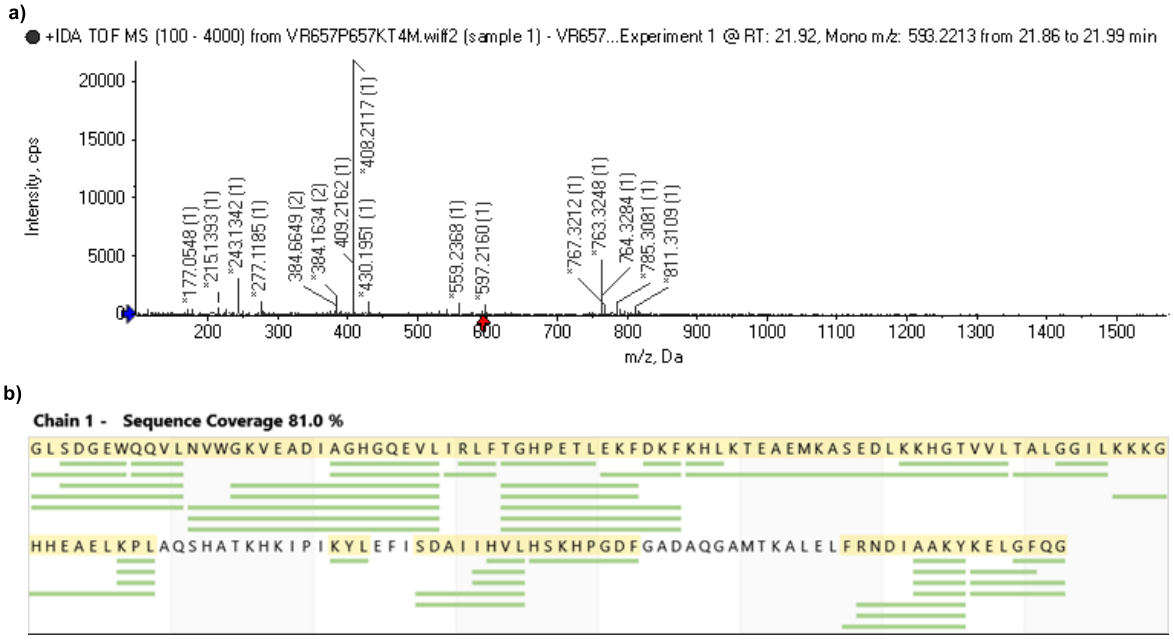


Figure S21 (a) Peptide mapping and (b) % coverage of myoglobin using randomly immobilized α-chymotrypsin for cycle 2.

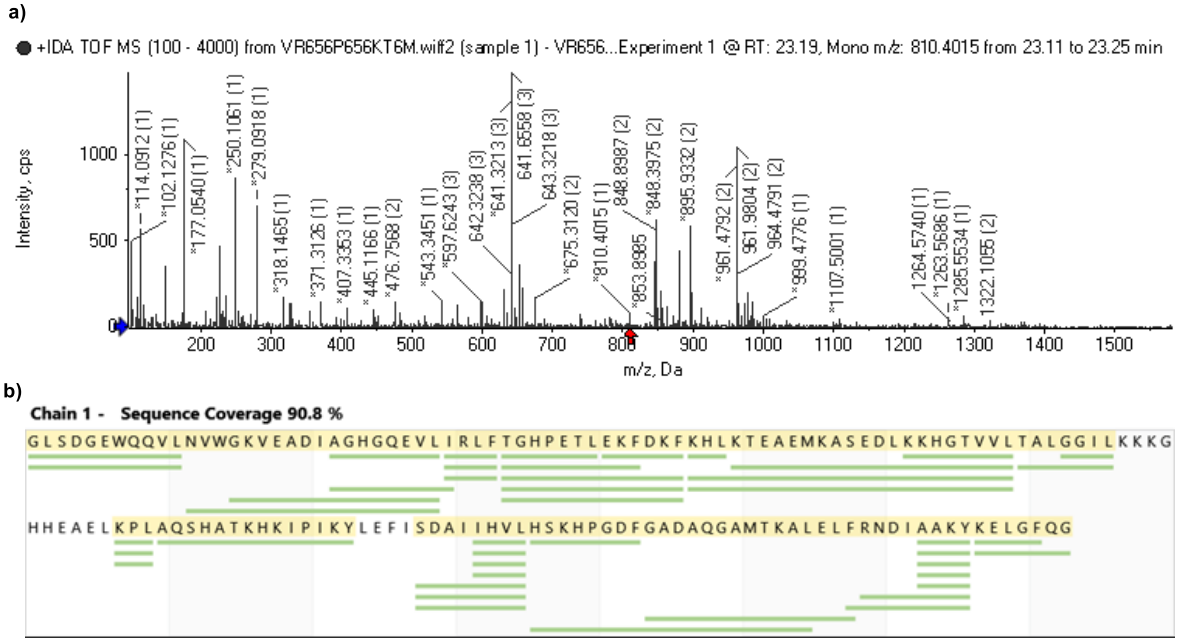


Figure S22 (a) Peptide mapping and (b) % coverage of myoglobin using ordered immobilized α-chymotrypsin for cycle 2.

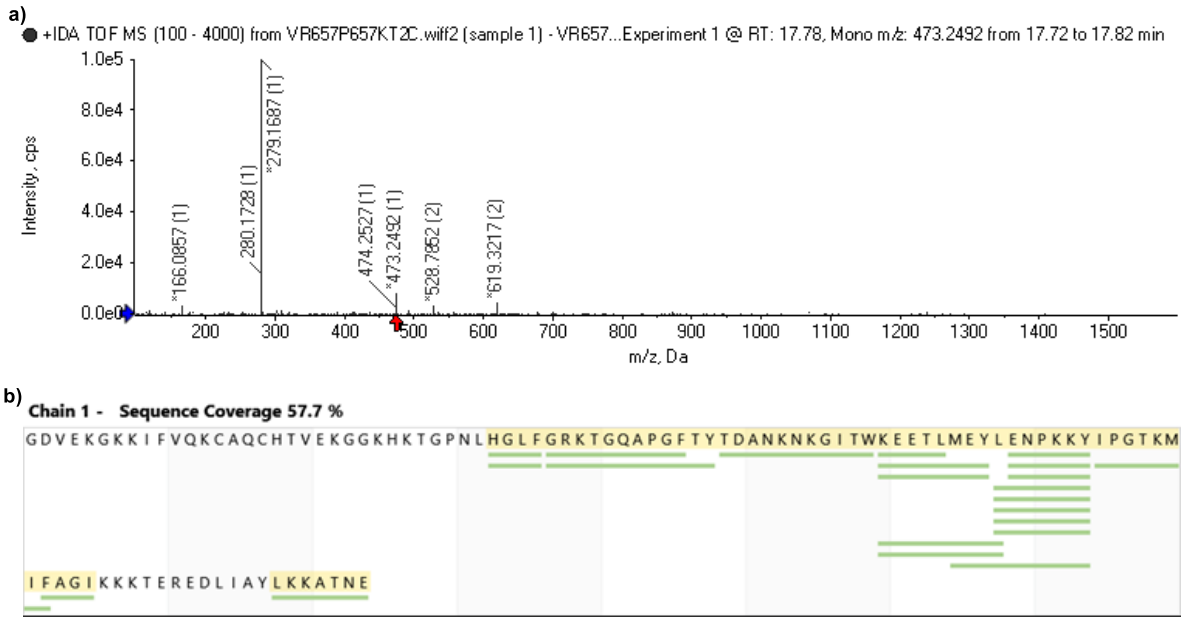


Figure S23 (a) Peptide mapping and (b) % coverage of cytochrome C using randomly immobilized α-chymotrypsin for cycle 1.

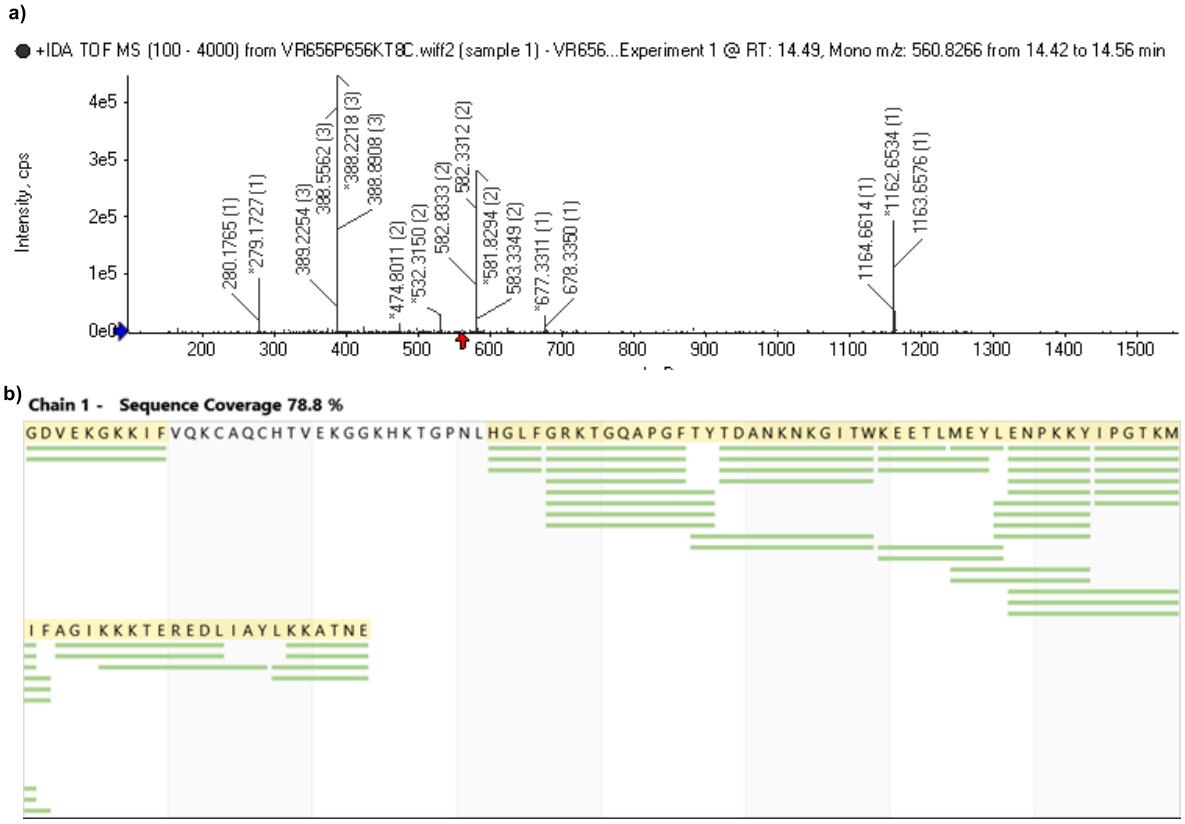


Figure S24 (a) Peptide mapping and (b) % coverage of cytochrome C using ordered immobilized α-chymotrypsin for cycle 1.

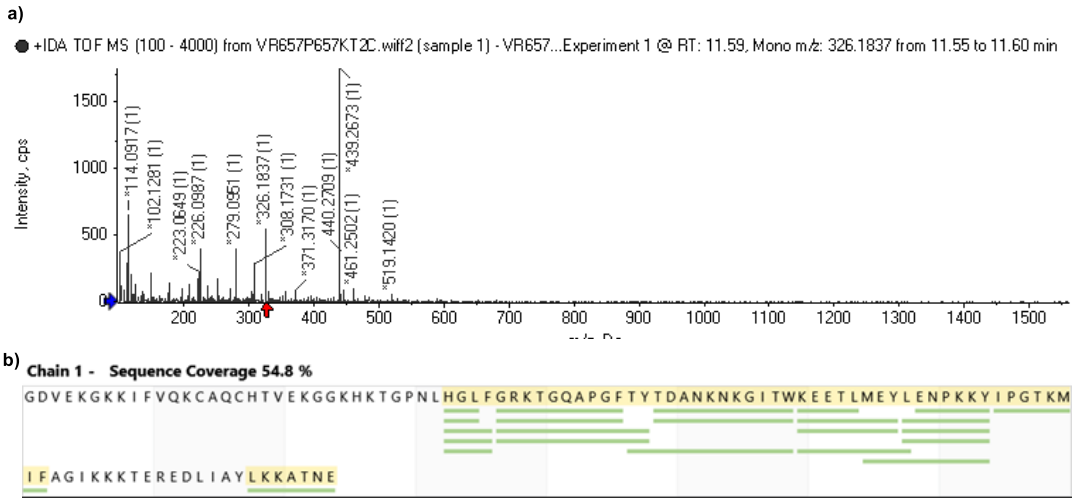


Figure S25 (a) Peptide mapping and (b) % coverage of cytochrome C using randomly immobilized α-chymotrypsin for cycle 2.

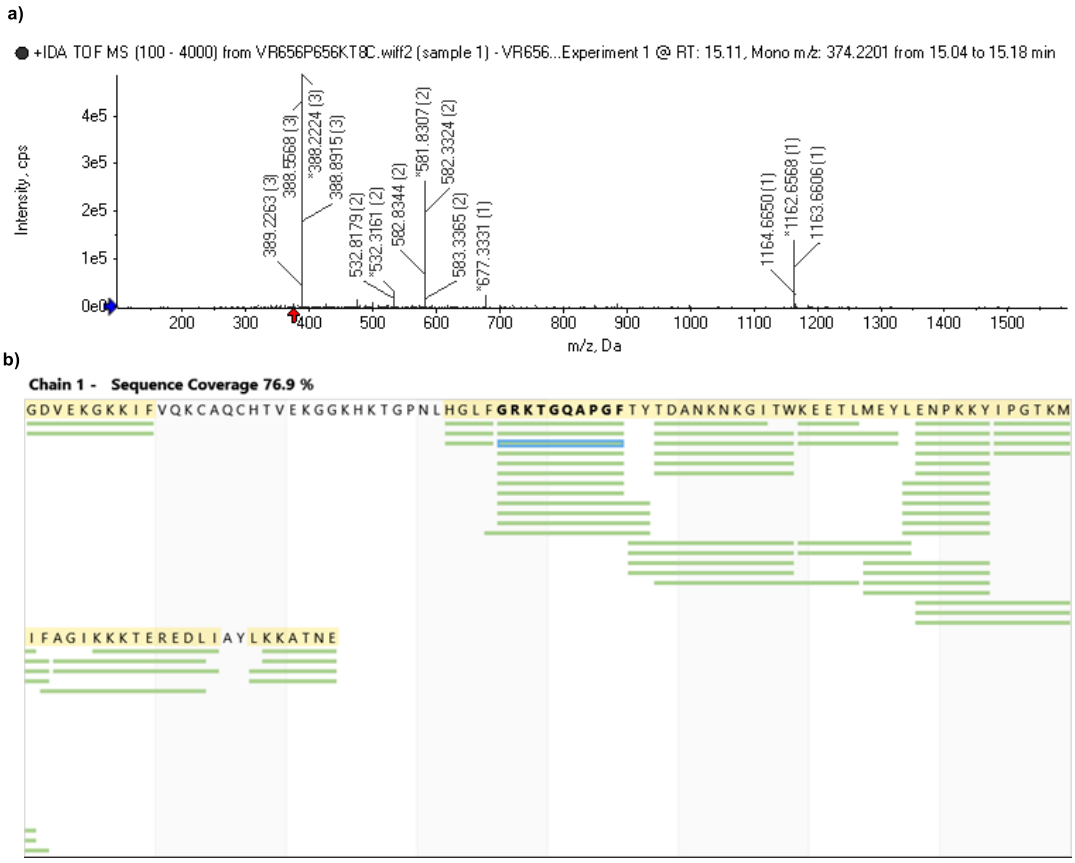


Figure S26 (a) Peptide mapping and (b) % coverage of cytochrome C using ordered immobilized α-chymotrypsin for cycle 2.

6. Protein sequence

1. Trypsin Sequence (PDB ID: 2PTC)

IVGGYTCGANTVPYQVSLNSGYHFCGGSLINSQWVVSAAHCYKSGIQVRLGEDNIN
VVEGNEQFISASKSIVHPSYNSNTLNNDIMLIKLKSAASLNSRVASISLPTSCASAGTQ
CLISGWGNTKSSGTSYPDVLKCLKAPILSDSSCKSAYPGQITSNMFCAGYLEGGKDSC
QGDSGGPVVCSGKLQGIVSWGSGCAQKNKPGVYTKVCNYVSWIKQTIASN

2. α-Chymotrypsin sequence (PDB ID: 1YPH)

Chain A – *amino acid sequence:*

CGVPAIQPVLSGL

Chain B – *amino acid sequence:*
IVNGEEAVPGSWPWQVSLQDKTGFHFCGGSLINENWVVTAAHCGVTTSDVVVAGE
FDQGSSSEKIQKLKIAKVFKNSKYNSLTINNDITLLKLSTAASFSQTVSAVCLPSASDD
FAAGTTCVTTGWGLTRY

Chain C – *amino acid sequence:*
ANTPDRLQQASLPLLSNTNCKKYWGTKIKDAMICAGASGVSSCMGDSGGPLVCKKN
GAWTLVGIVSWGSSTCSTSTPGVYARVTALVNWVQQTLAAN

3. RNase A (PDB ID: 2AAS)
amino acid sequence:
KETAAAKFERQHMDSSTSAASSSNYCNQMMKSRNLTKDRCKPVNTFVHESLADVQ
AVCSQKNVACKNGQTNCYQSYSTMSITDCRETGSSKYPNCAYKTTQANKHIIVACE
GNPYVPVHFDASV

4. Myoglobin from equine skeletal muscle (PDB ID: 1WLA)
amino acid sequence:
GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMK
ASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVL
HSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG

5. Cytochrome C from equine heart (PDB ID: 1HRC)
amino acid sequence:
AcGDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKTGQAPGFTYTDANK
NKGITWKEETLMEYLENPKKYIPGTKMIFAGIKKKTEREDLIAYLKKATNE

7. Spectral data

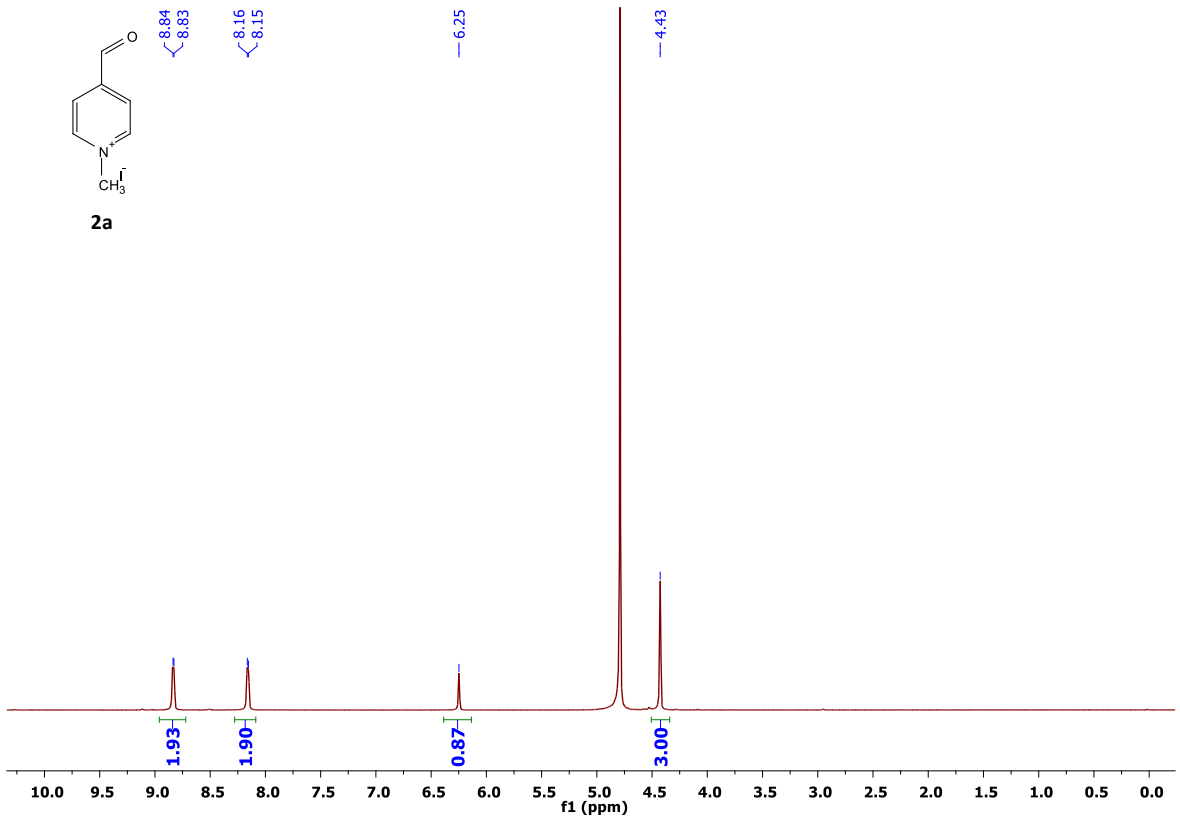


Figure S27. ^{1}H-NMR spectrum of compound **2a.**

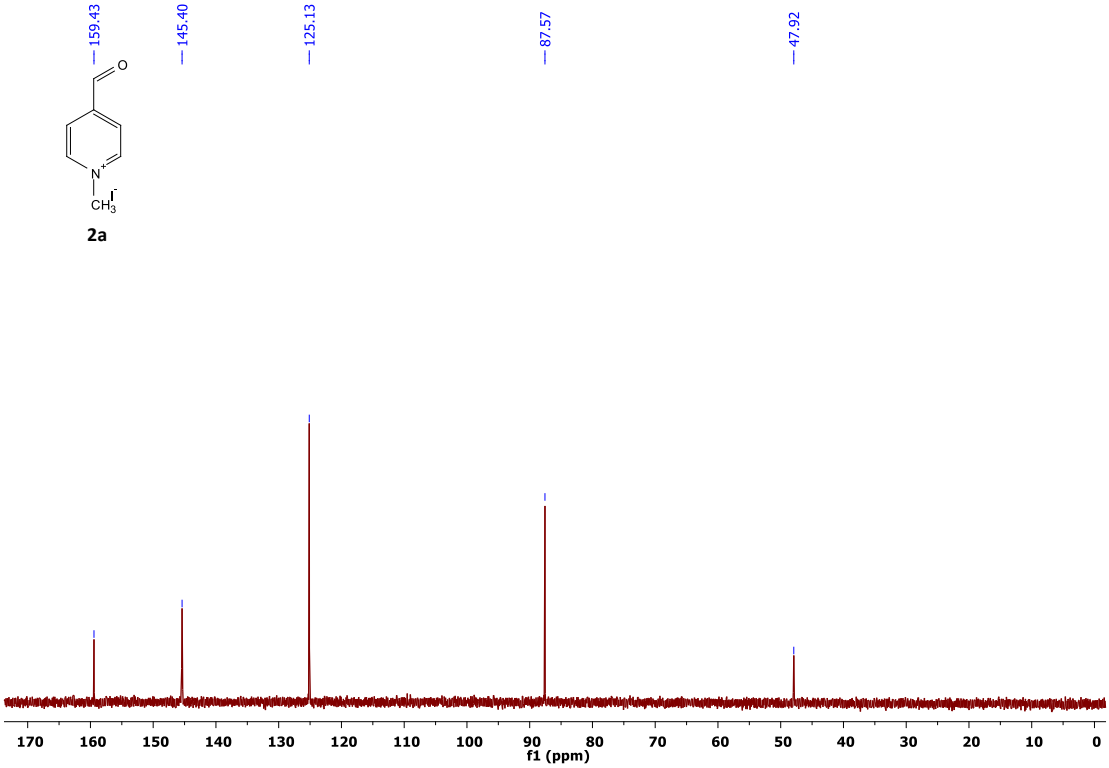


Figure S28 ^{13}C-NMR spectrum of compound **2a.**

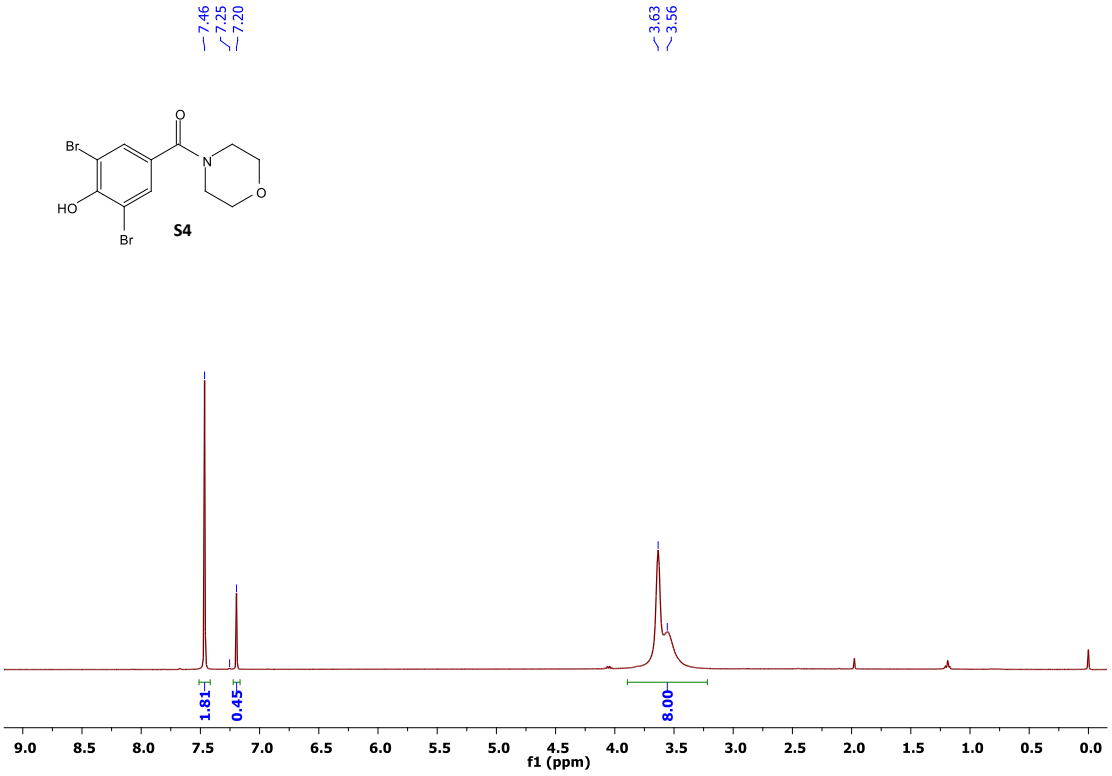


Figure S29 ^{1}H-NMR spectrum of compound **S4.**

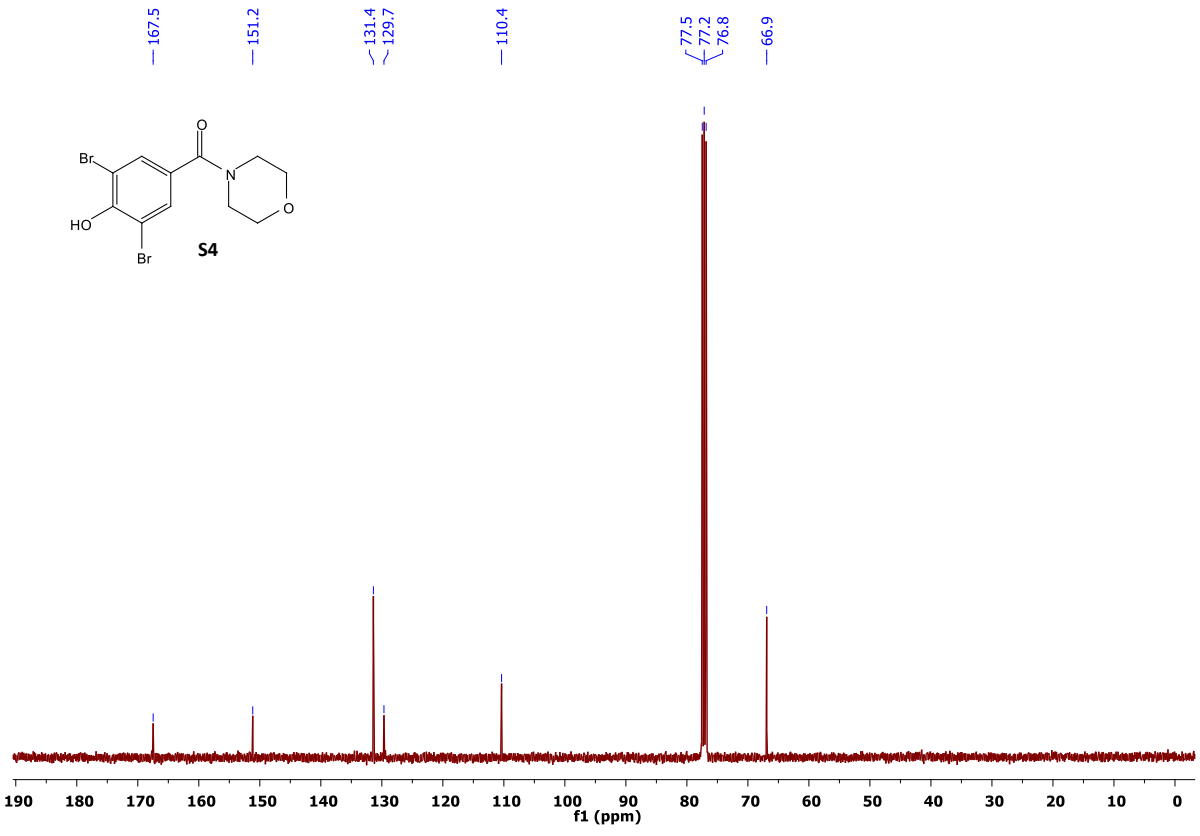


Figure S30 ^{13}C-NMR spectrum of compound **S4.**

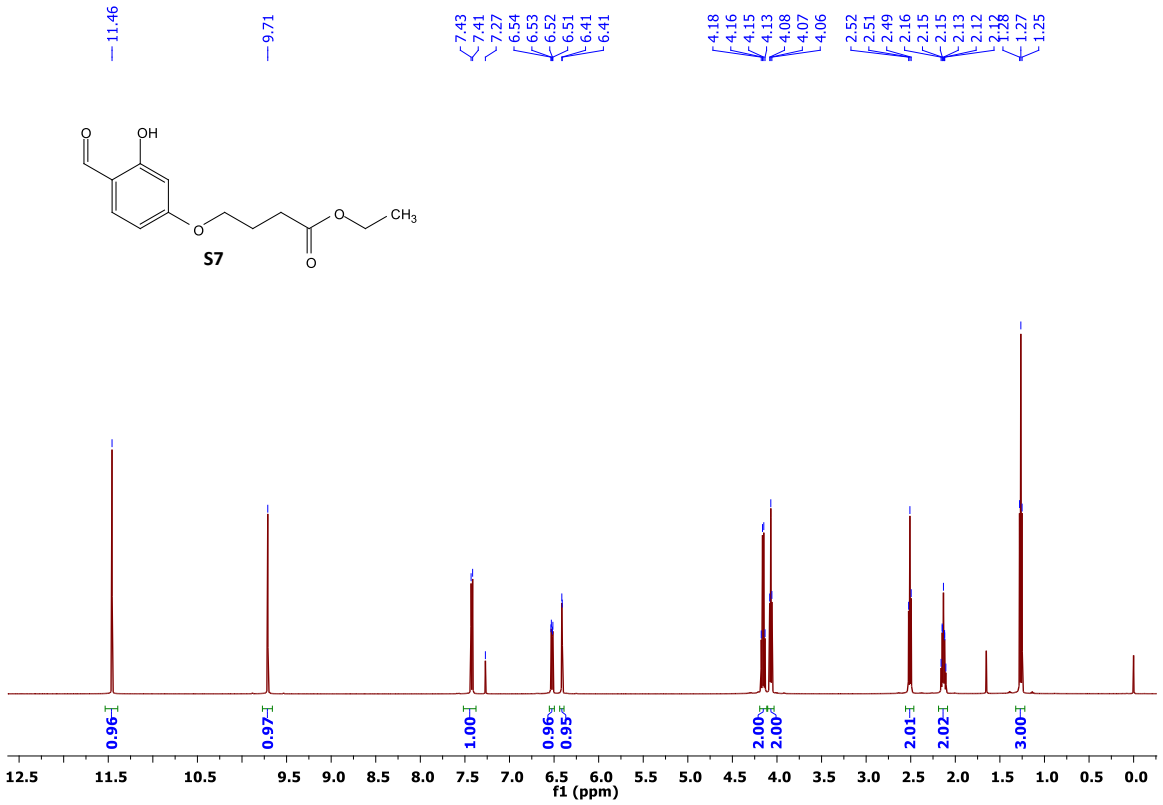


Figure S31 ^{1}H-NMR spectrum of compound **S7.**

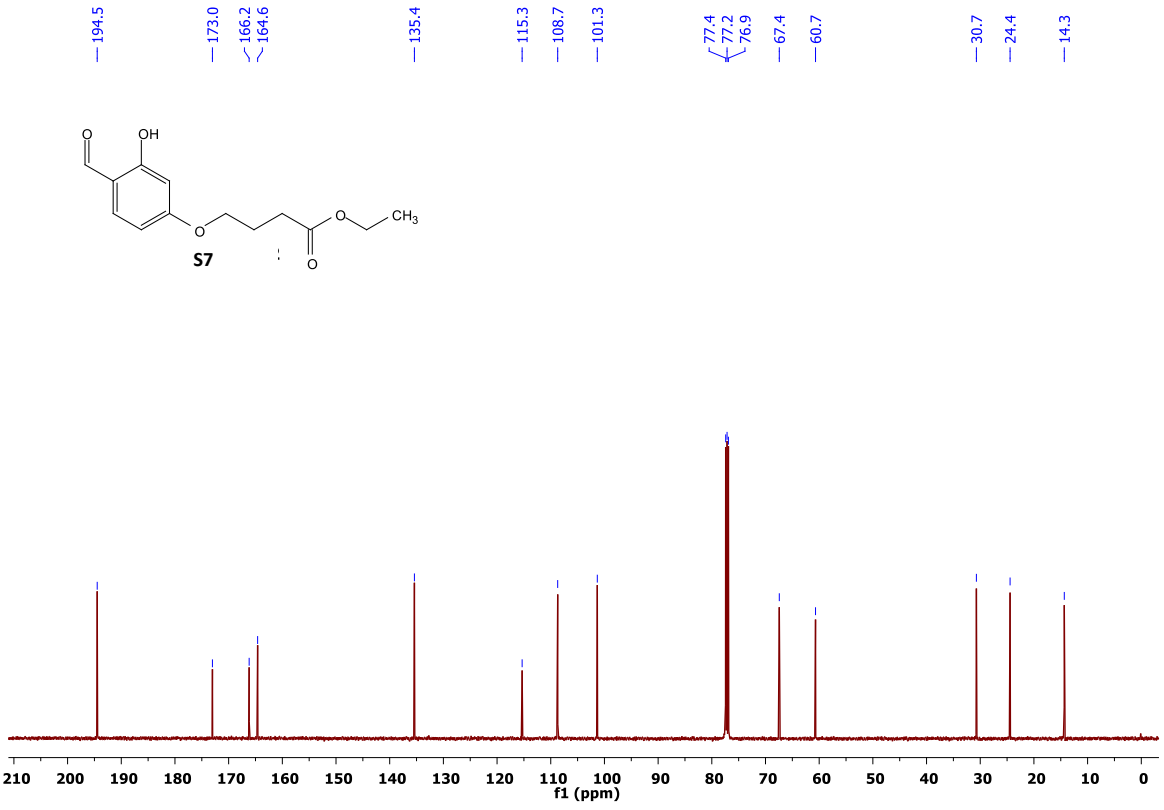


Figure S32 ^{13}C-NMR spectrum of compound **S7.**

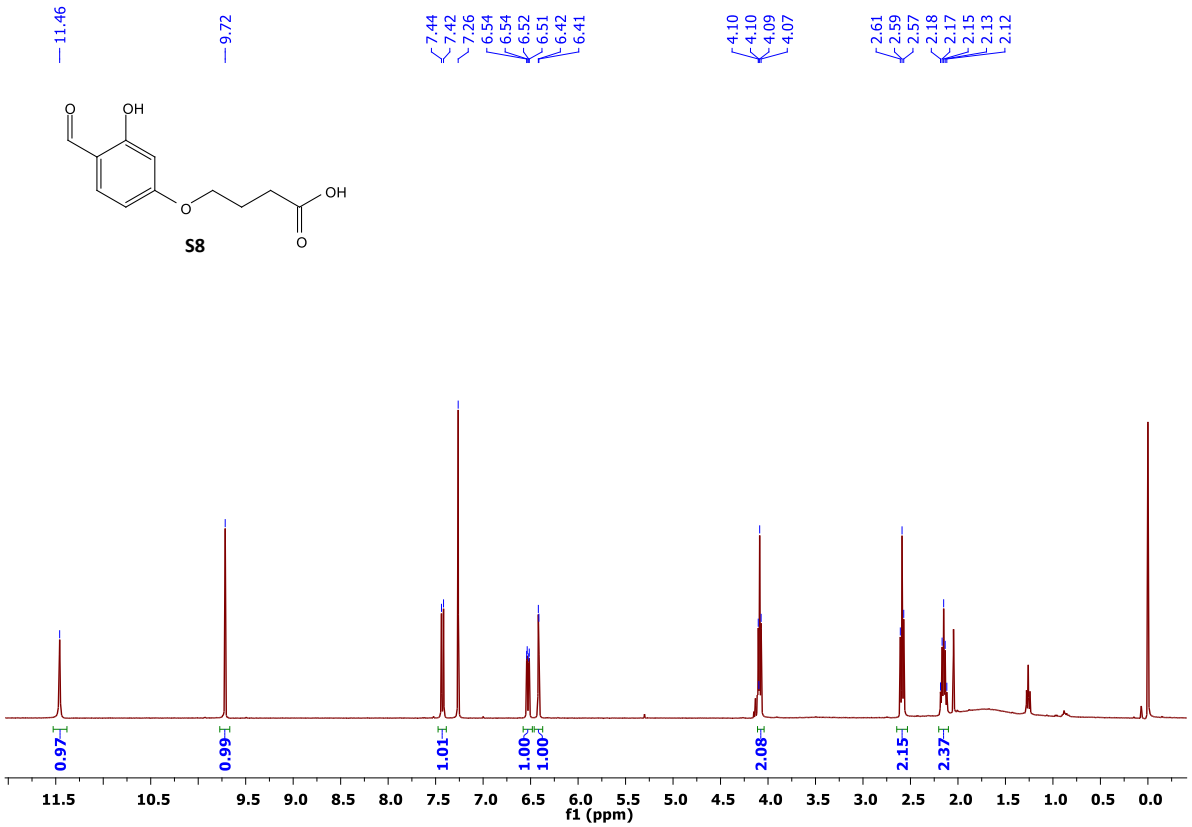


Figure S33 ^{1}H-NMR spectrum of compound **S8.**

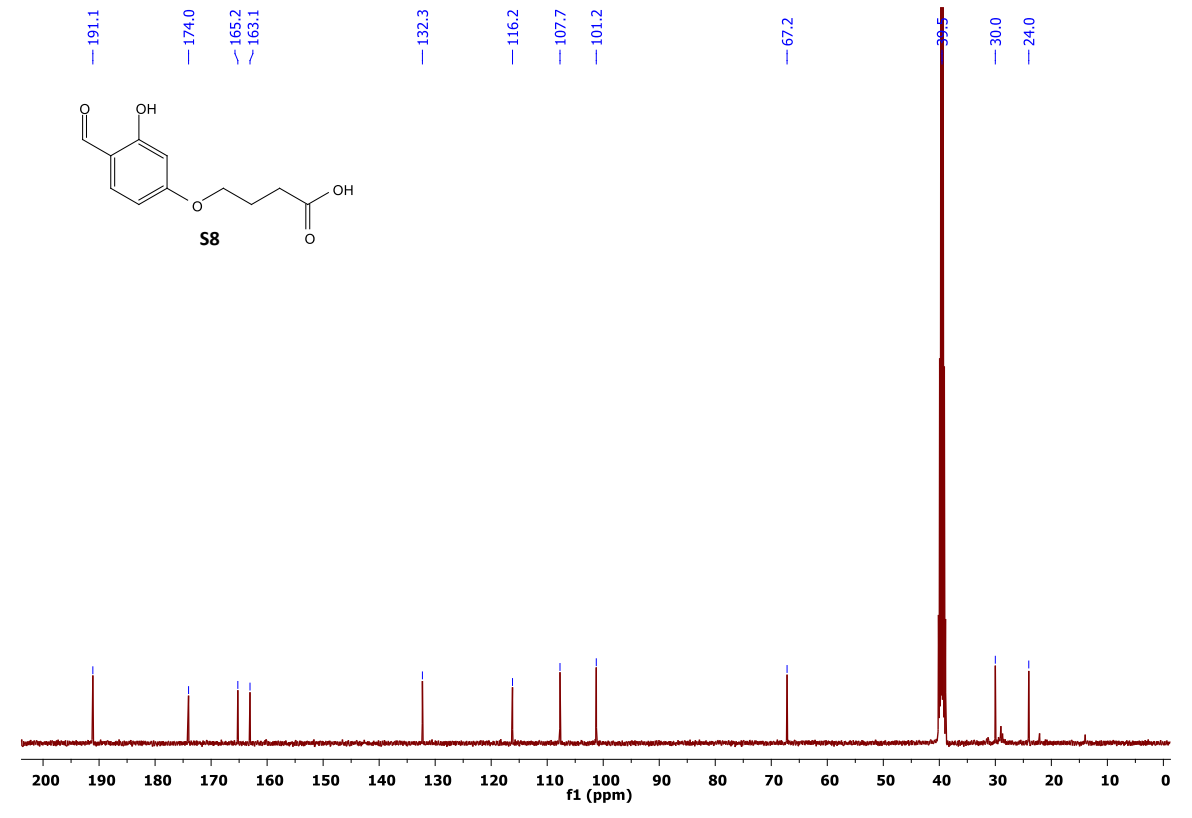


Figure S34 ^{13}C-NMR spectrum of compound **S8.**

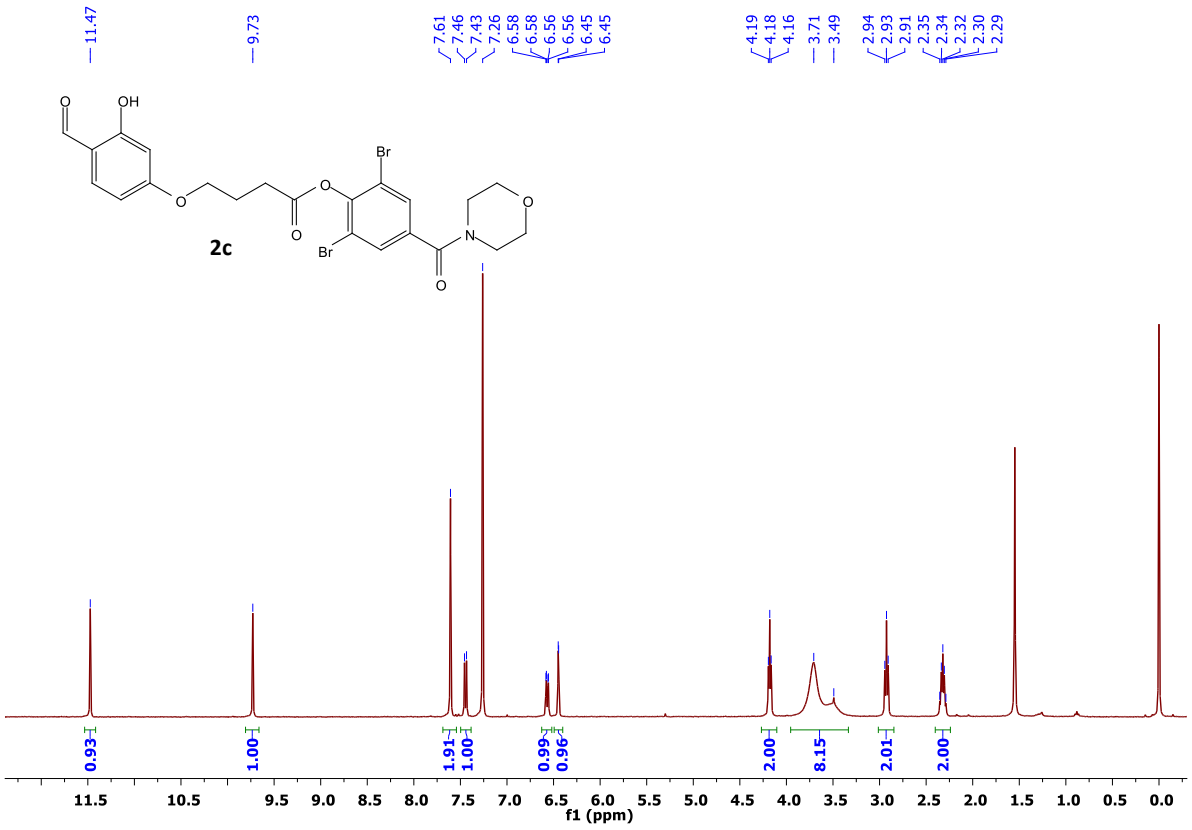


Figure S35 ^{1}H-NMR spectrum of compound **2c.**

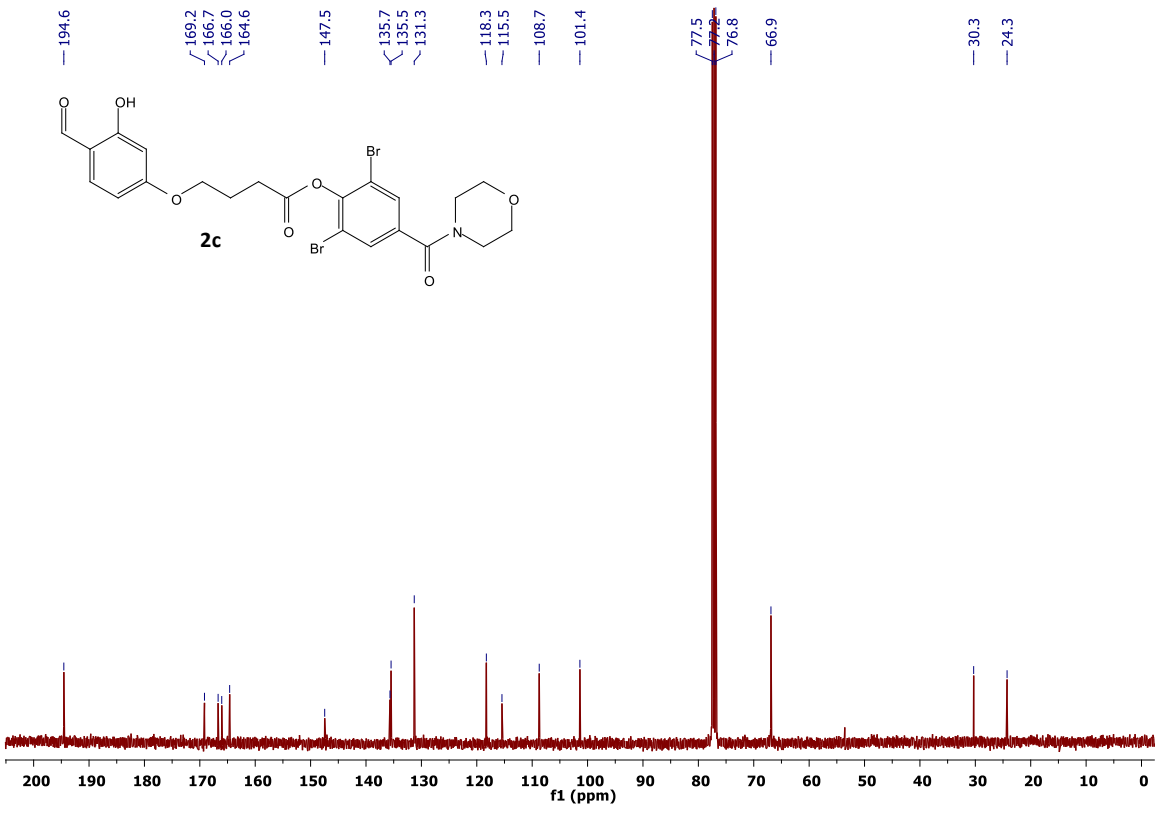


Figure S36 ^{13}C-NMR spectrum of compound **2c.**

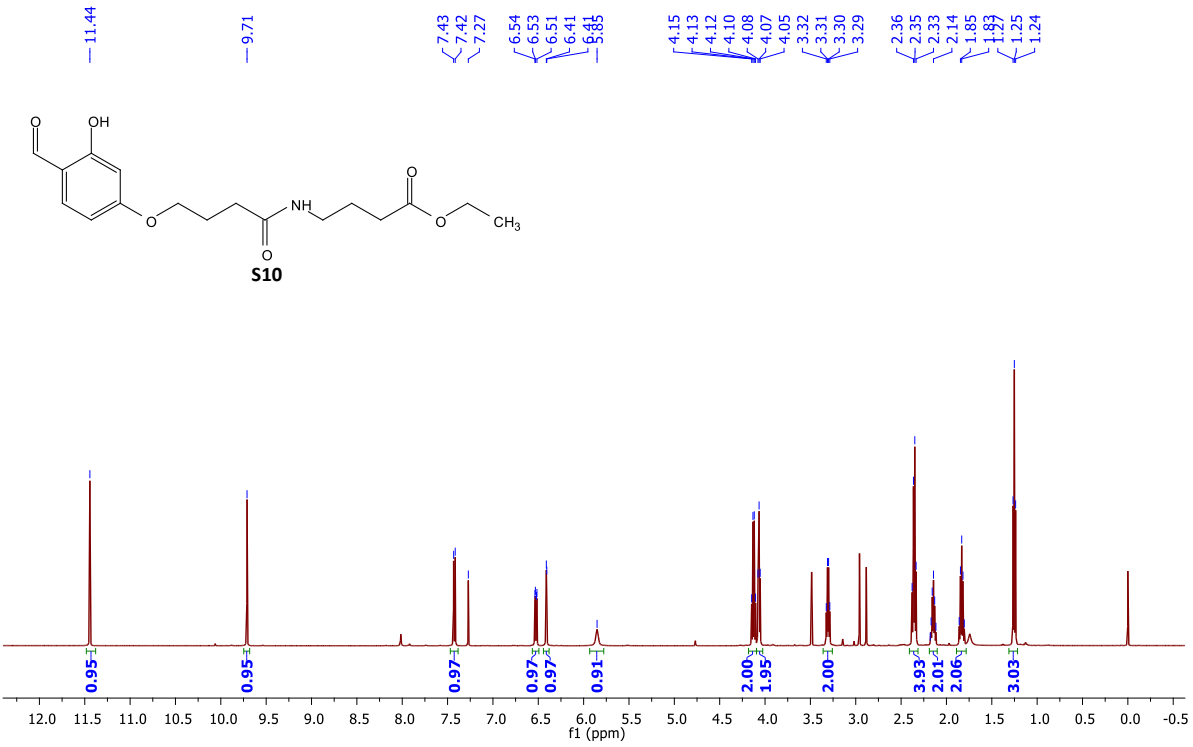


Figure S37 ^{1}H-NMR spectrum of compound **S10.**

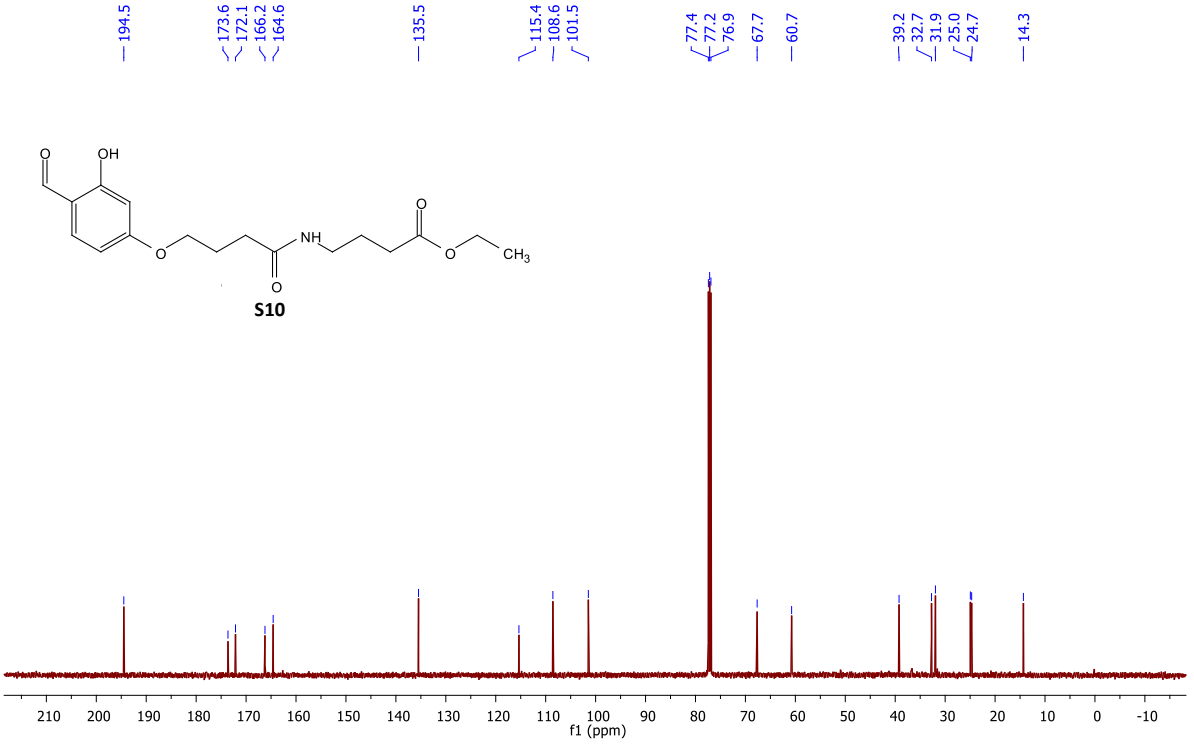


Figure S38 ^{13}C-NMR spectrum of compound **S10.**

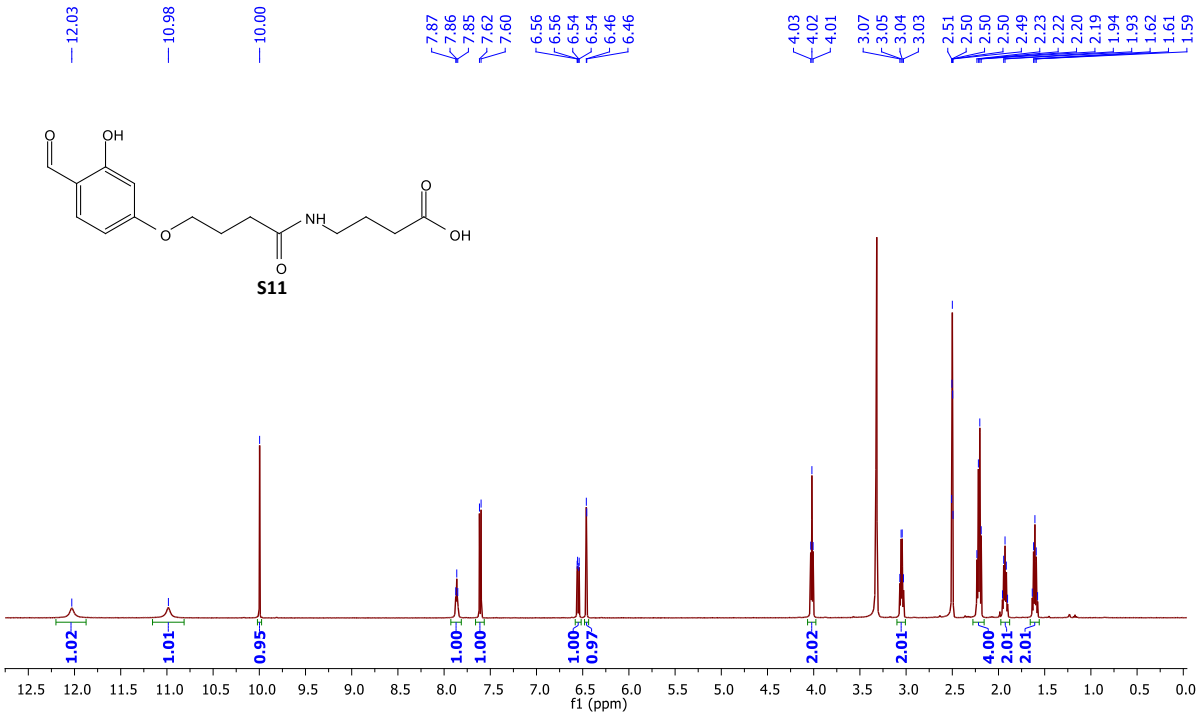


Figure S39 ^{1}H-NMR spectrum of compound **S11.**

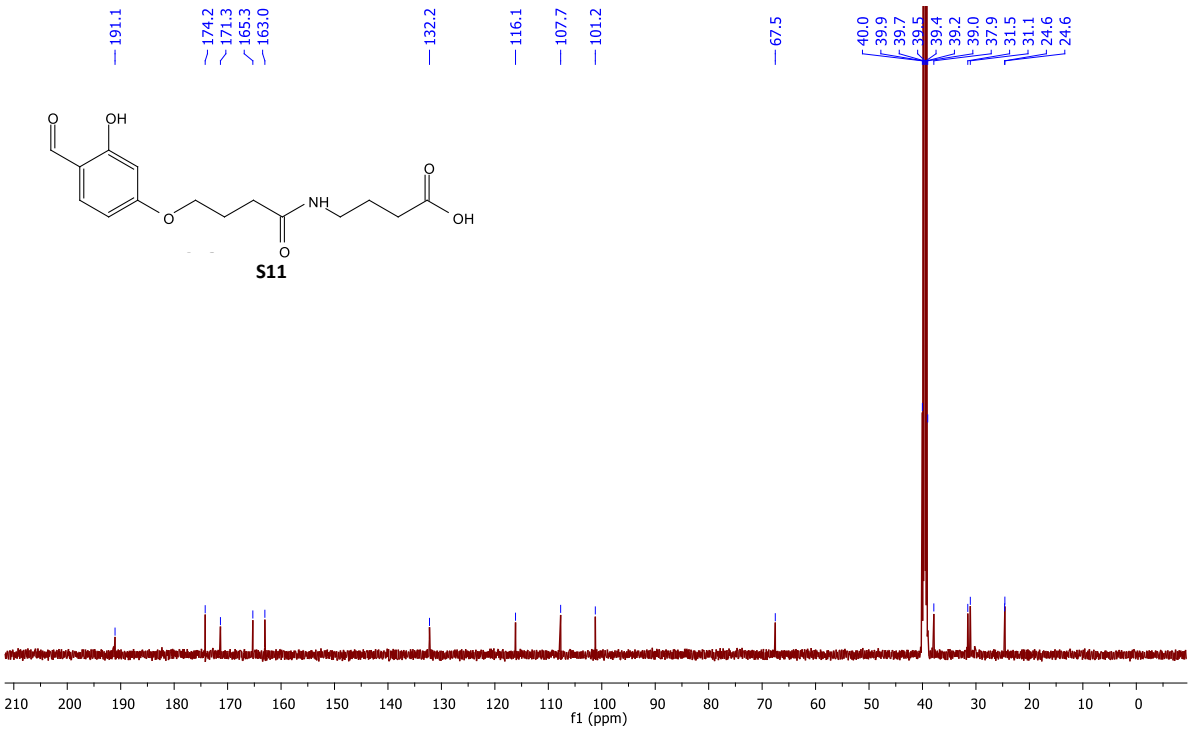


Figure S40 ^{13}C-NMR spectrum of compound **S11.**

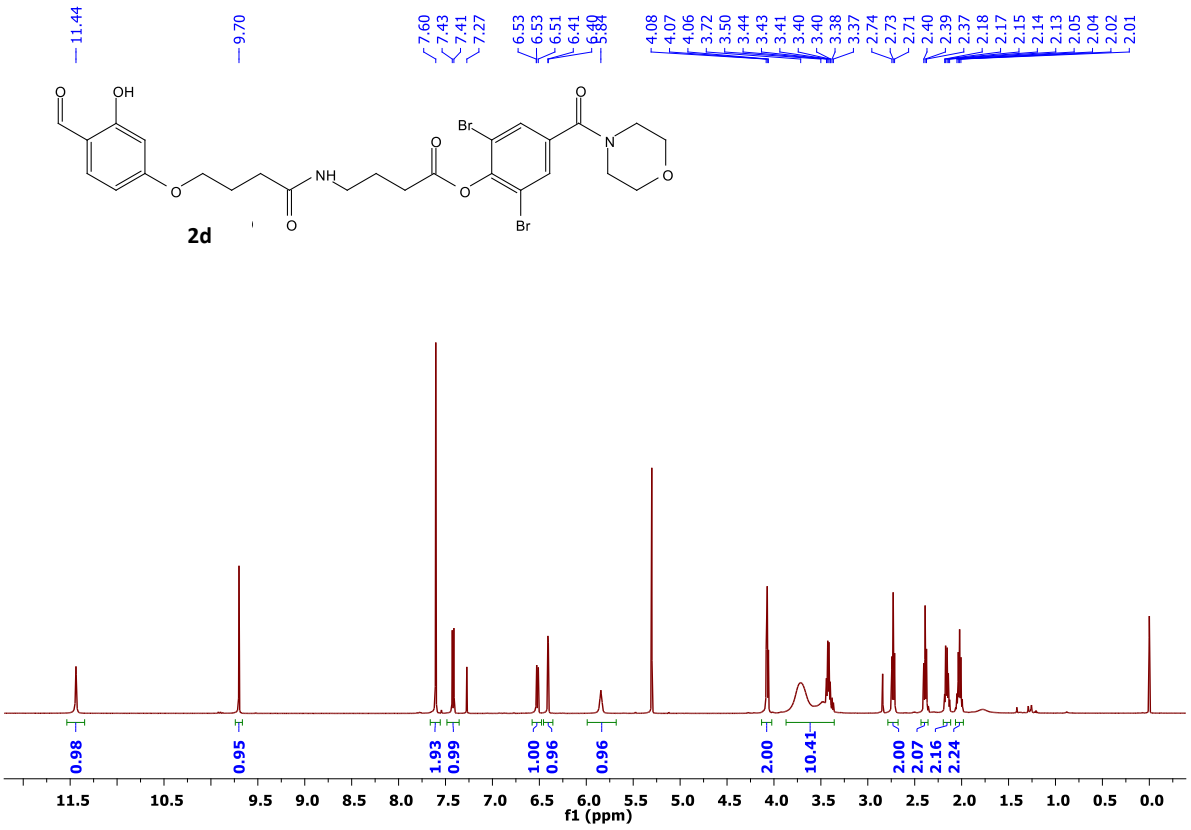


Figure S41 ^{1}H-NMR spectrum of compound **2d.**

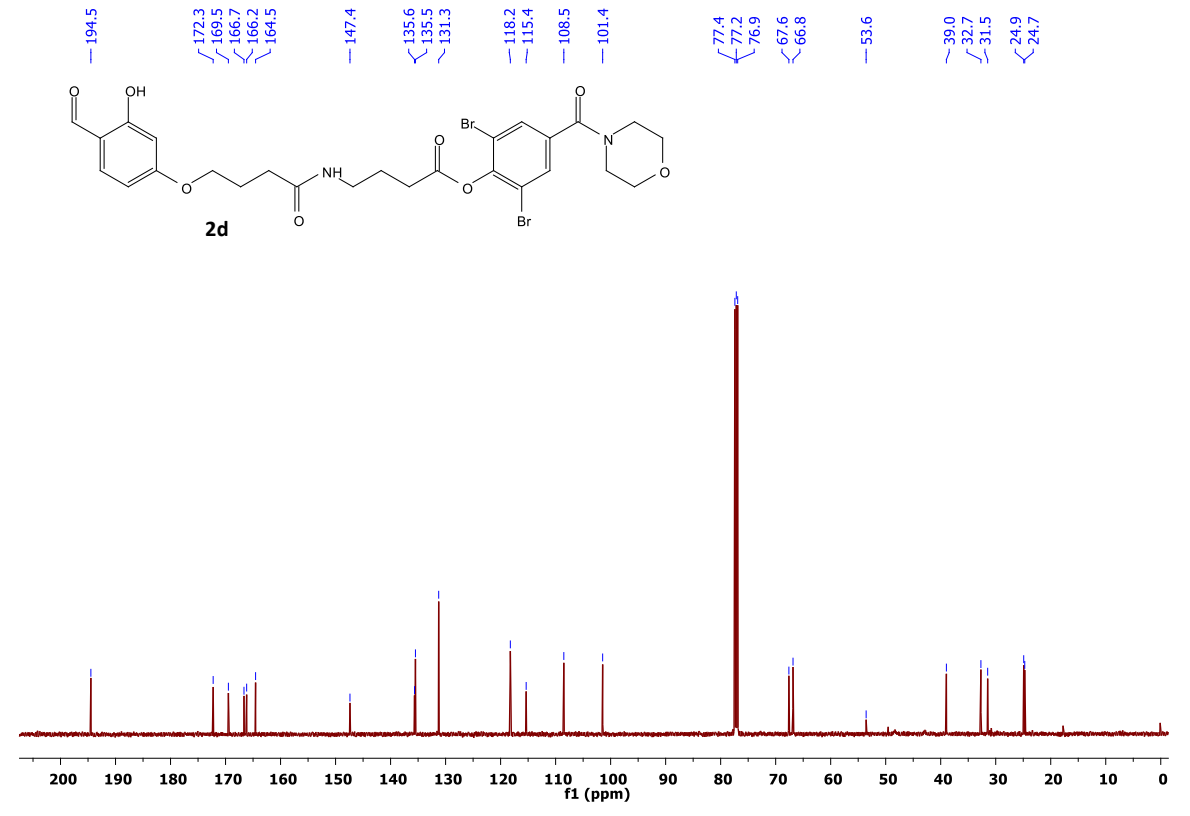


Figure S42 ^{13}C-NMR spectrum of compound **2d.**

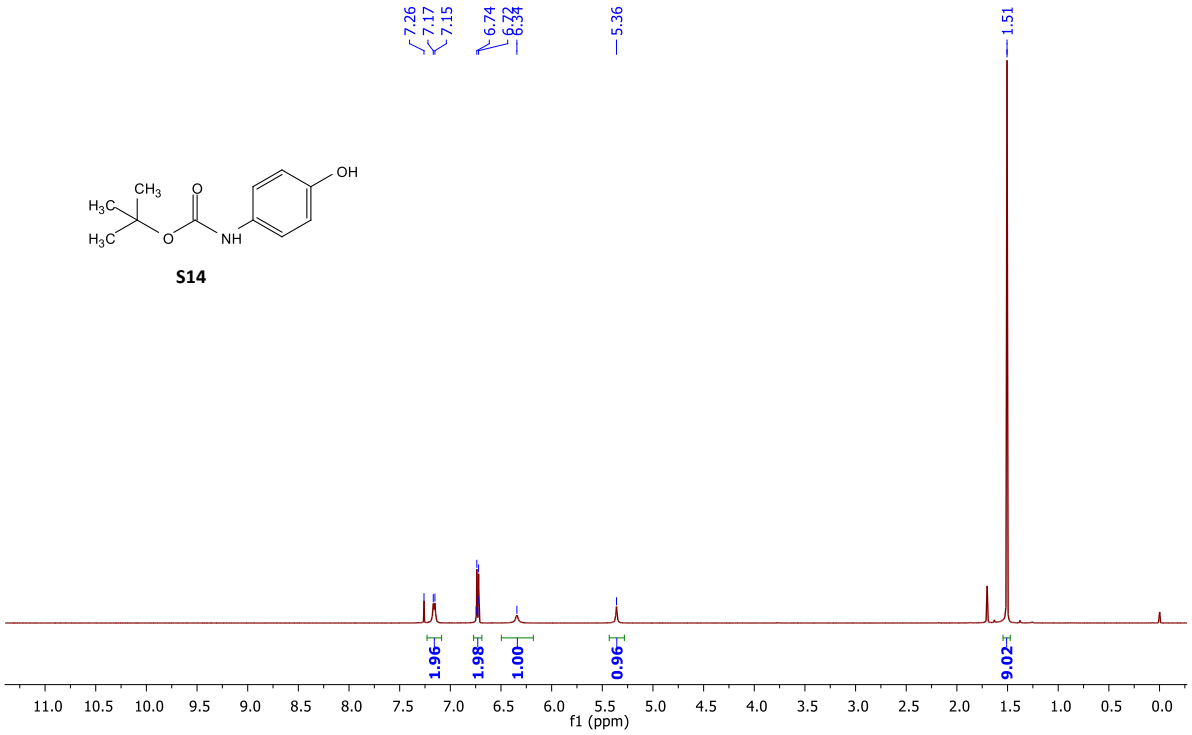


Figure S43 ^{1}H-NMR spectrum of compound **S14.**

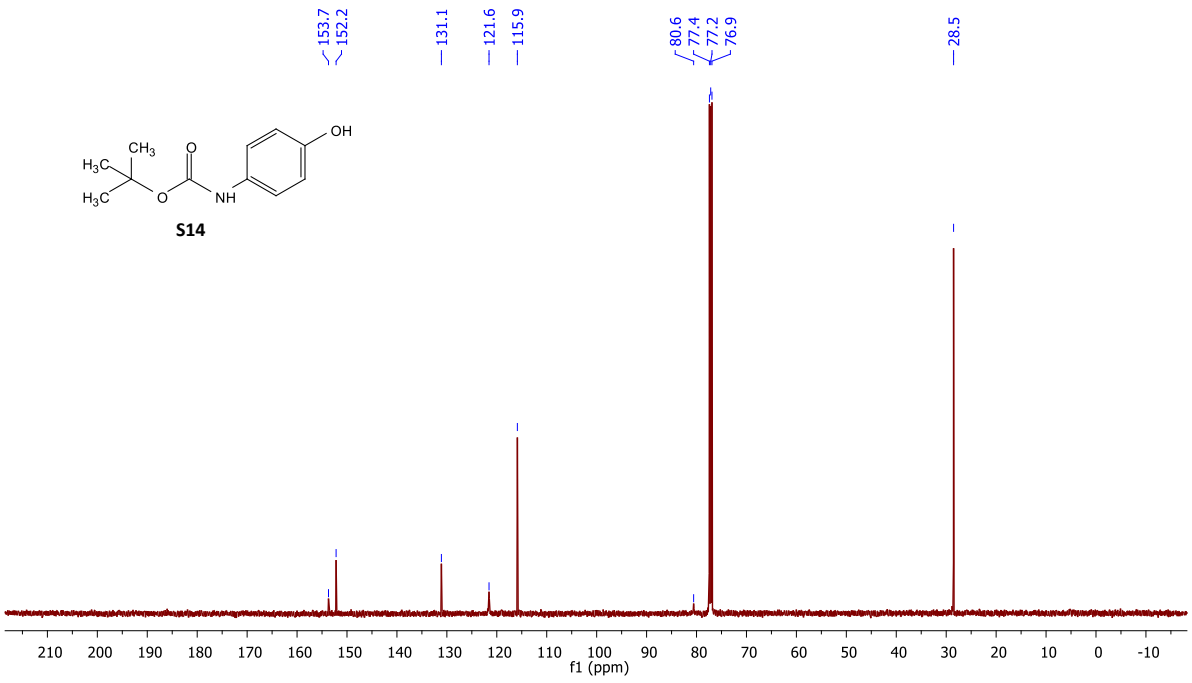


Figure S44 ^{13}C-NMR spectrum of compound **S14.**

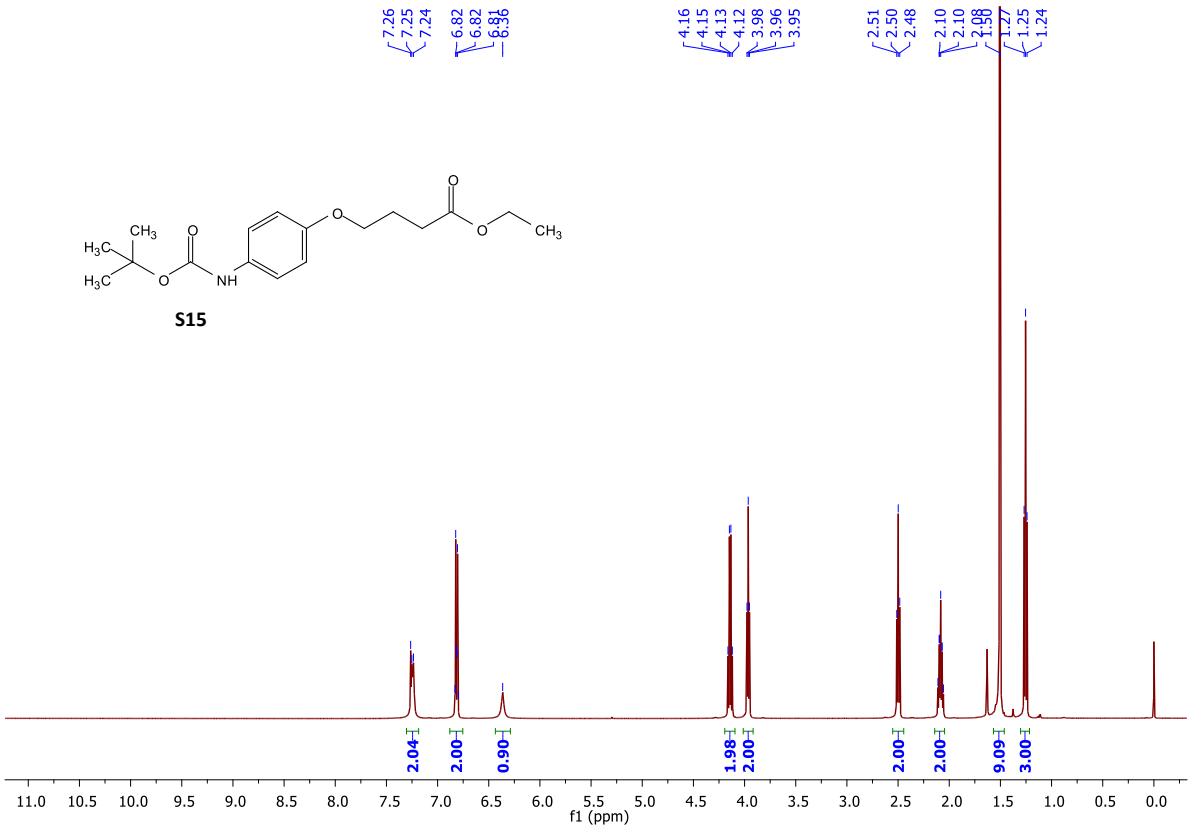


Figure S45 ^{1}H-NMR spectrum of compound **S15.**

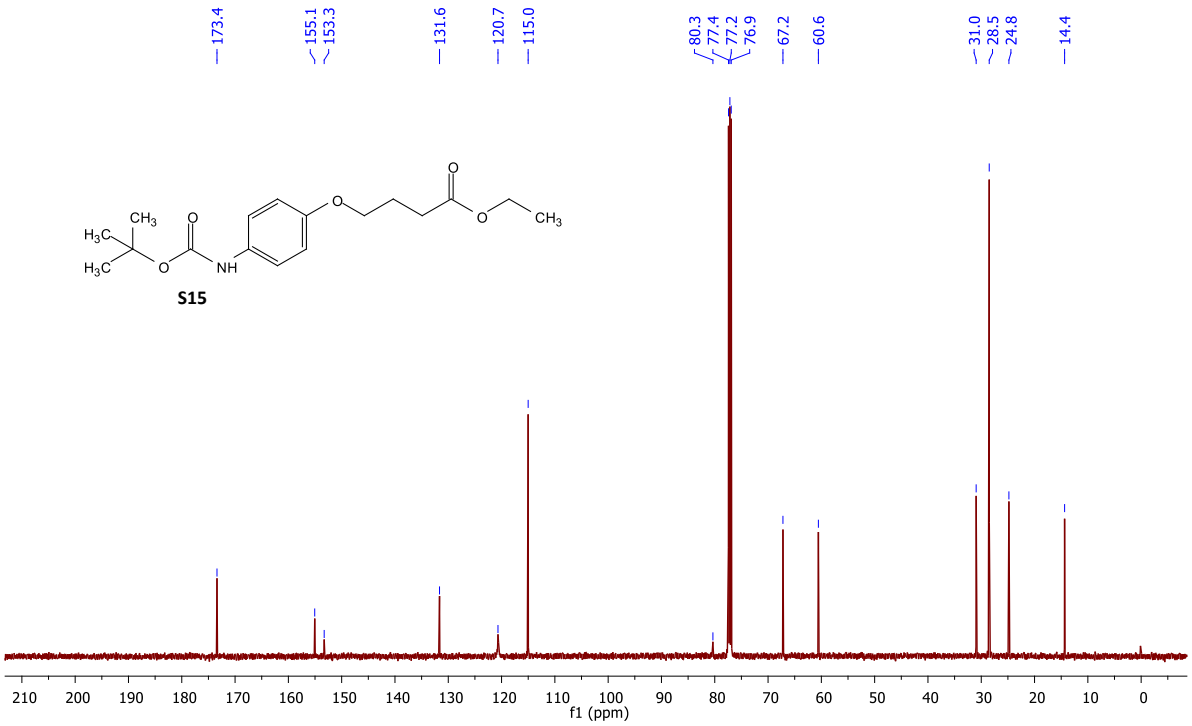


Figure S46 ^{13}C-NMR spectrum of compound **S15.**

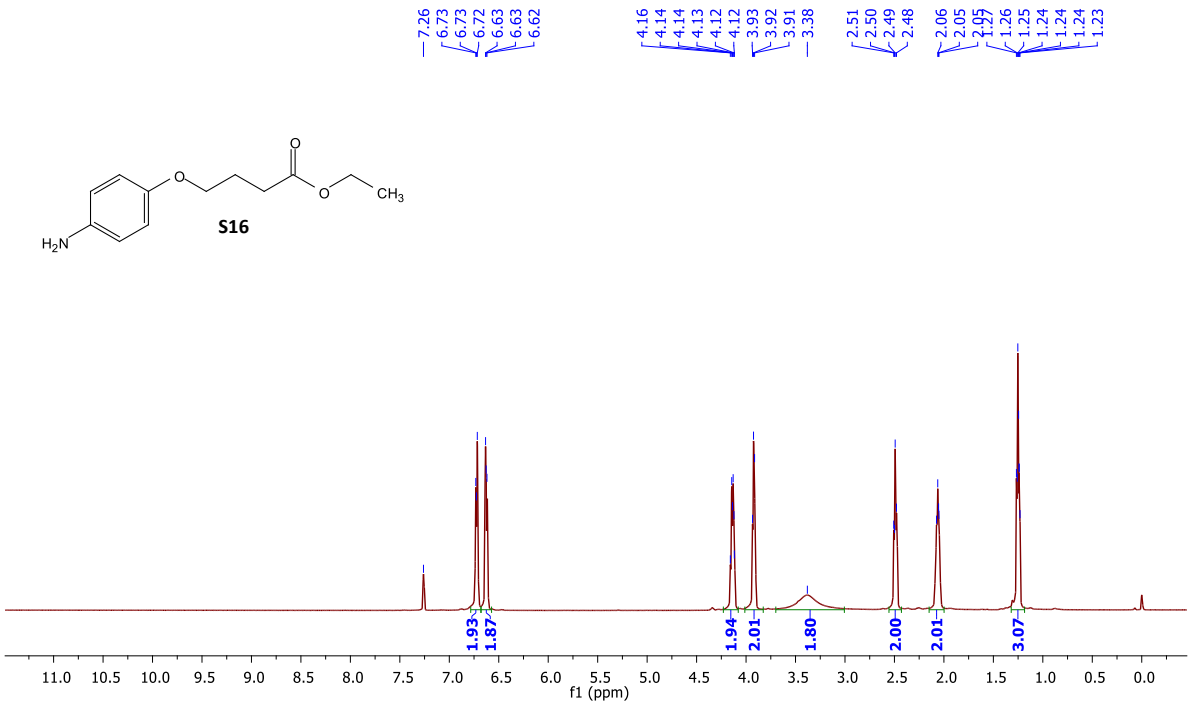


Figure S47 ^{1}H-NMR spectrum of compound **S16.**

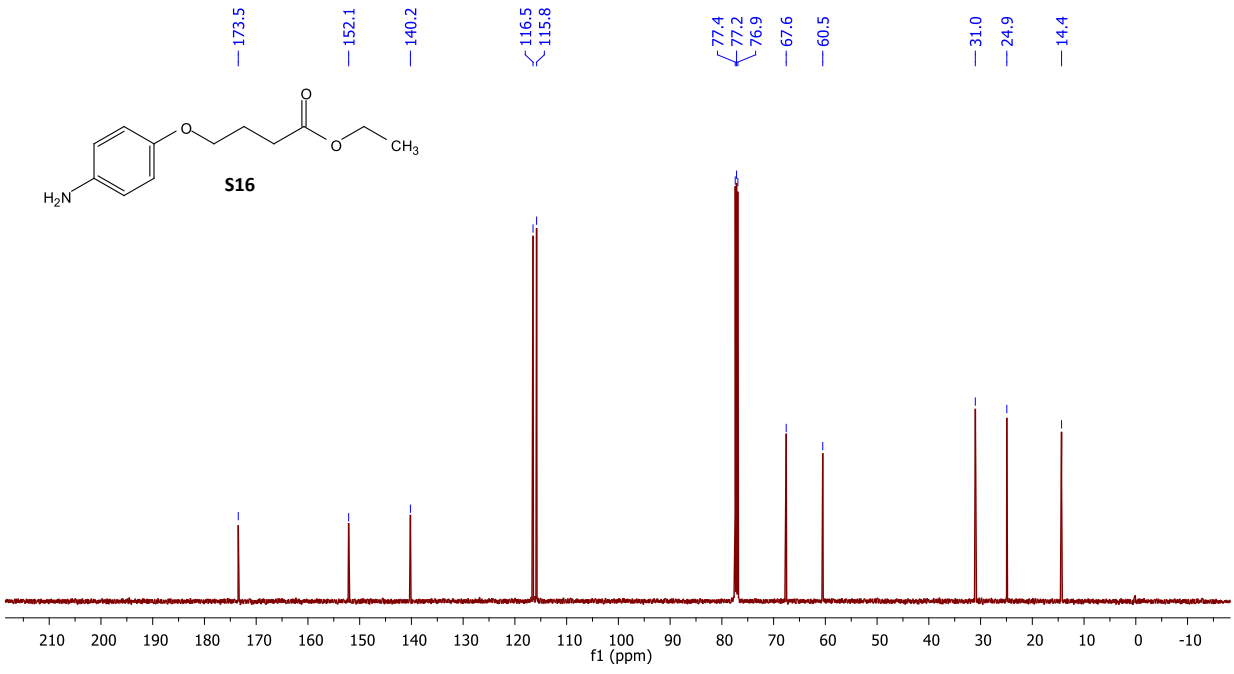


Figure S48 ^{13}C-NMR spectrum of compound **S16.**

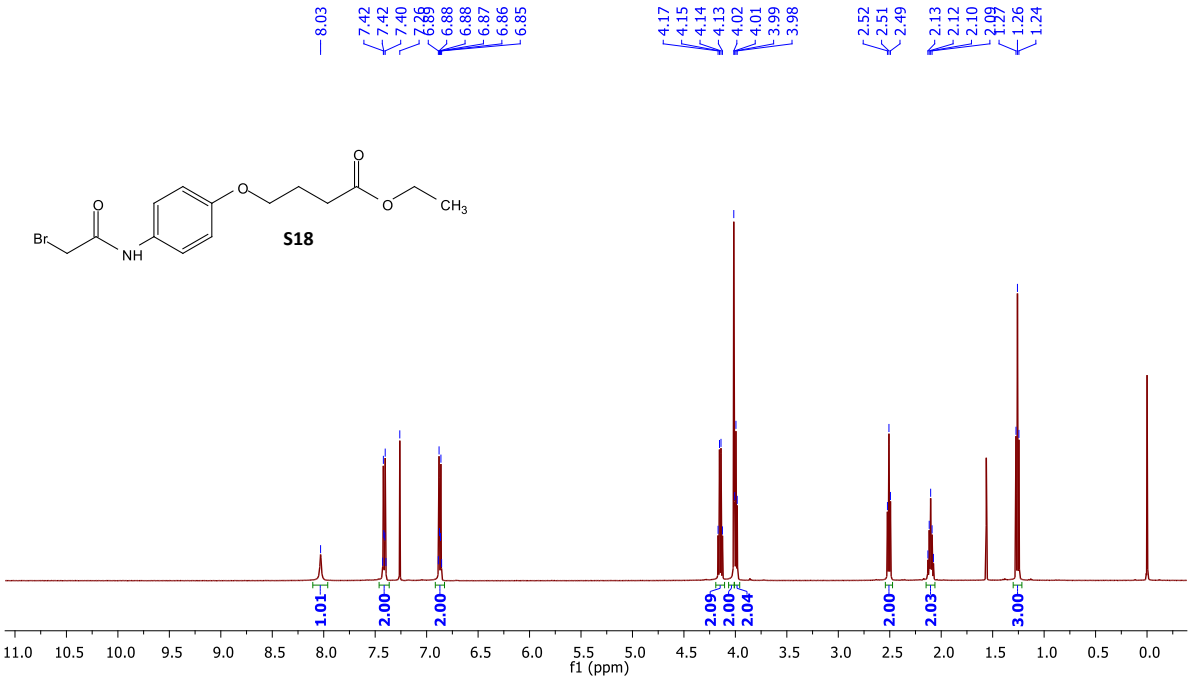


Figure S49 ^{1}H-NMR spectrum of compound **S18.**

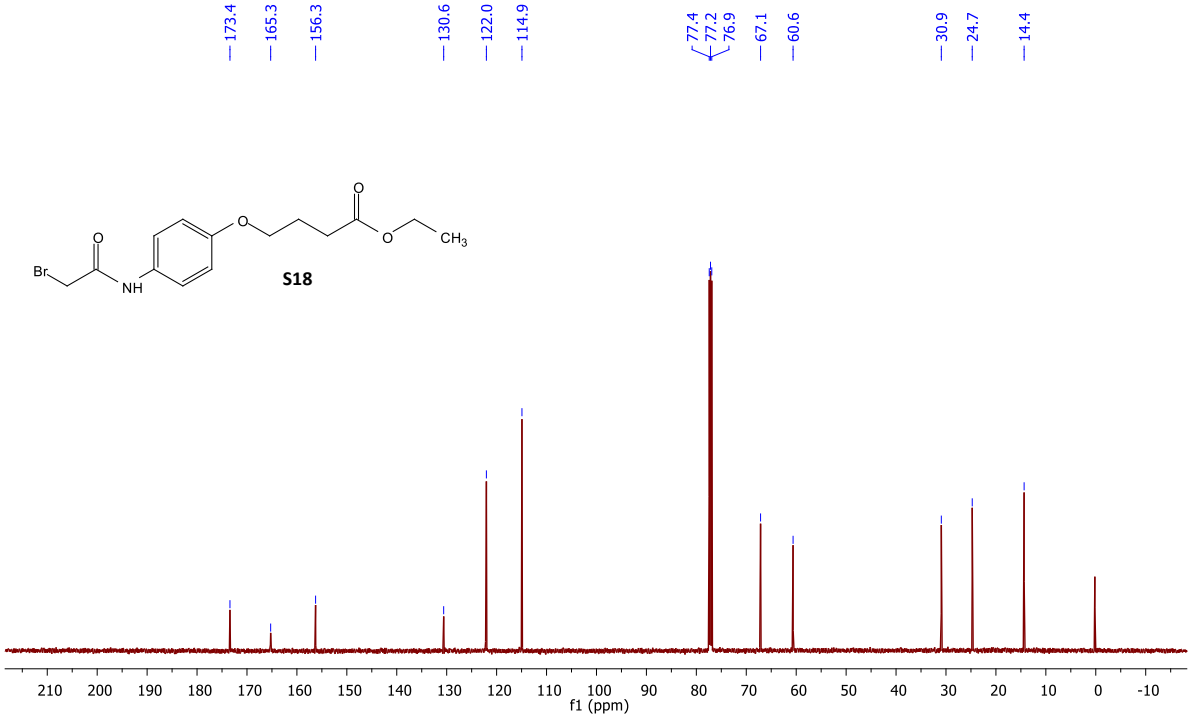


Figure S50 ^{13}C-NMR spectrum of compound **S18.**

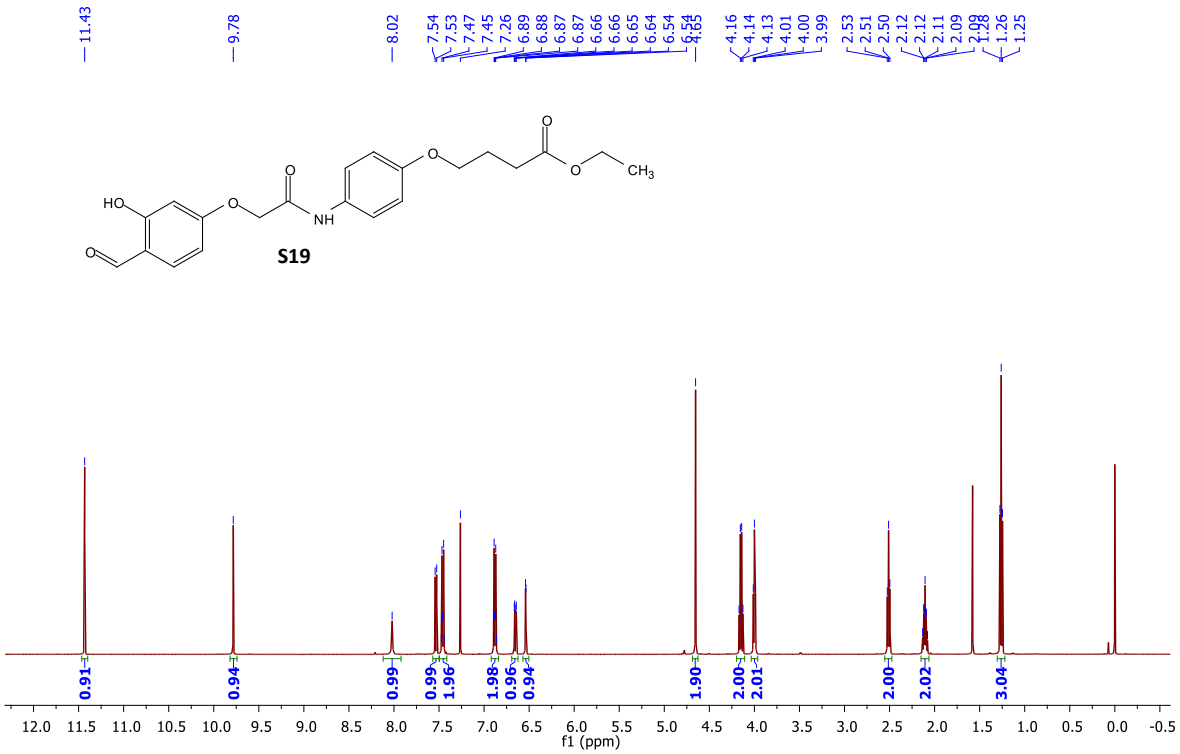


Figure S51 ^{1}H-NMR spectrum of compound **S19.**

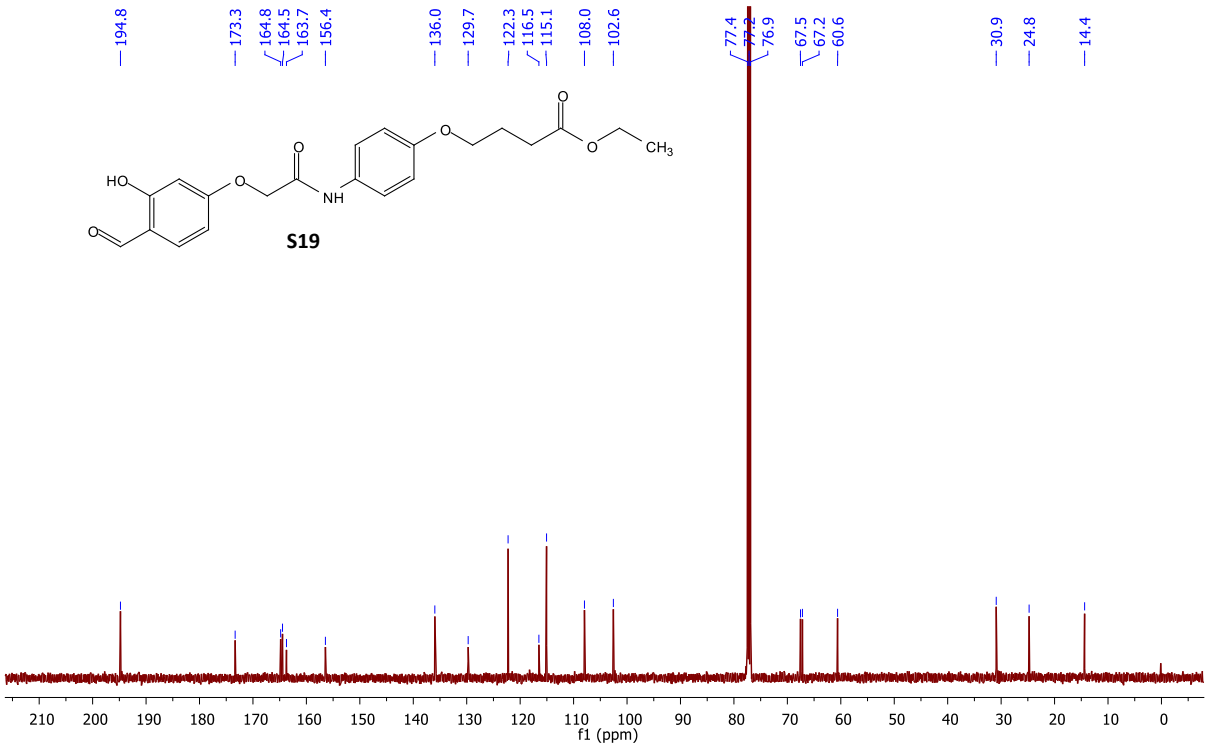


Figure S52 ^{13}C-NMR spectrum of compound **S19.**

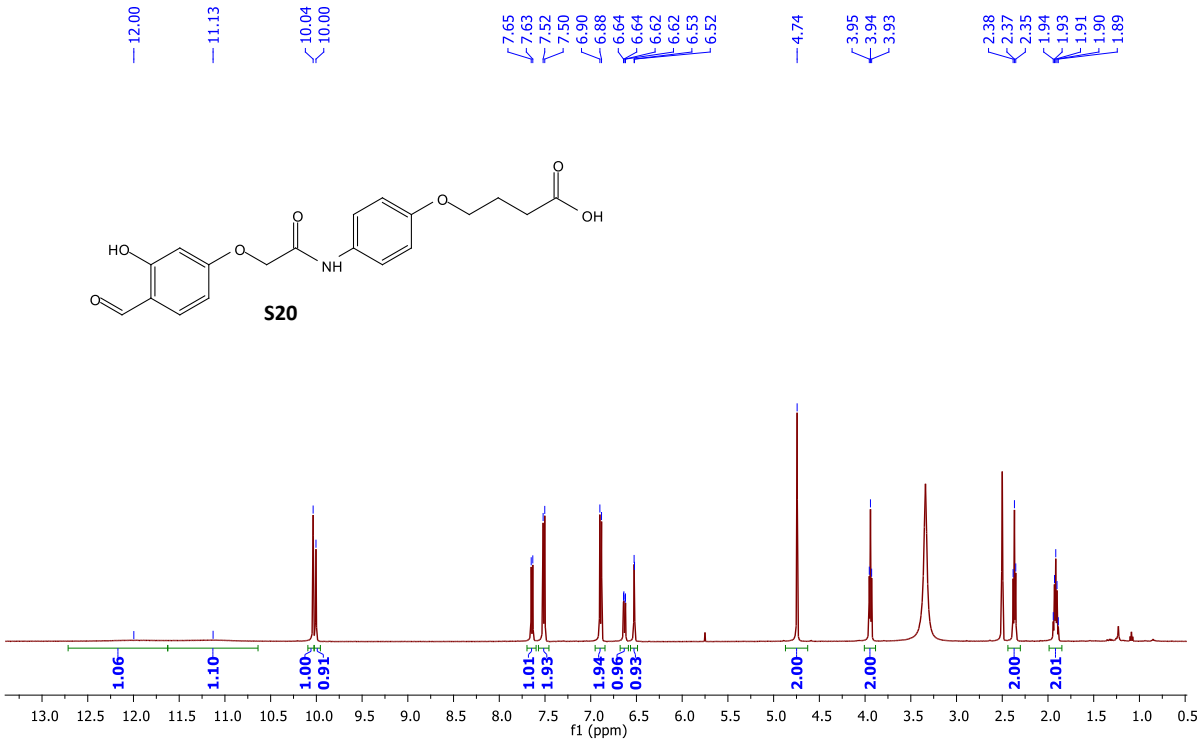


Figure S53 ^{1}H-NMR spectrum of compound **S20.**

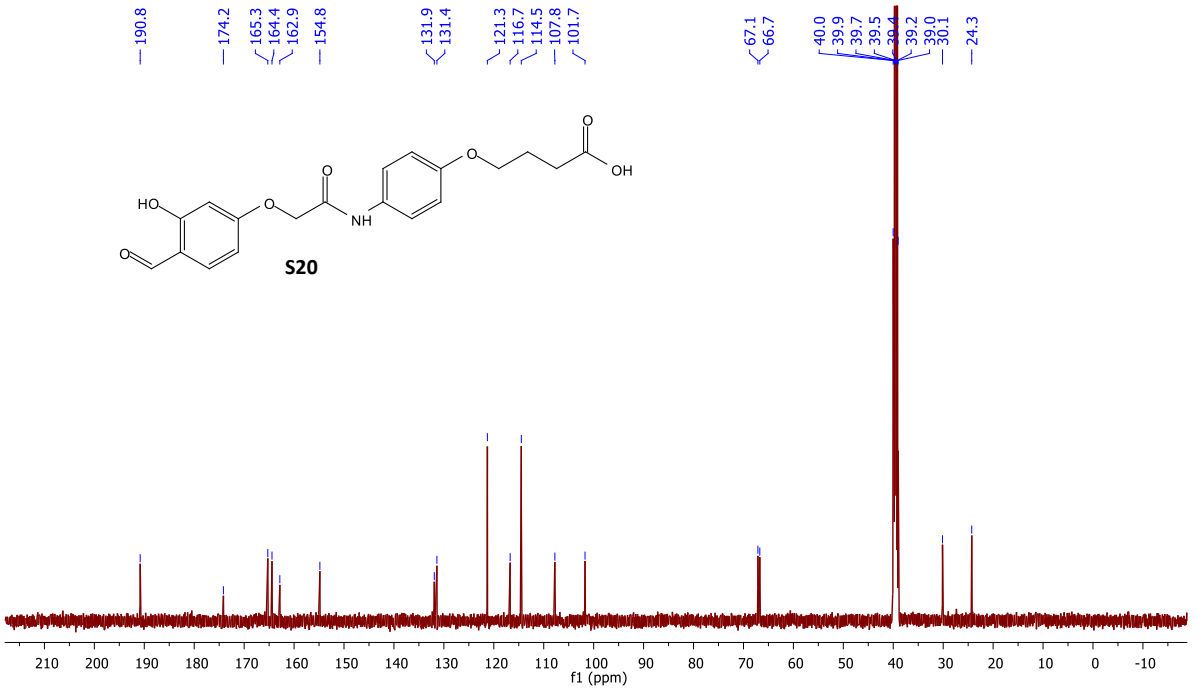


Figure S54 ^{13}C-NMR spectrum of compound **S20.**

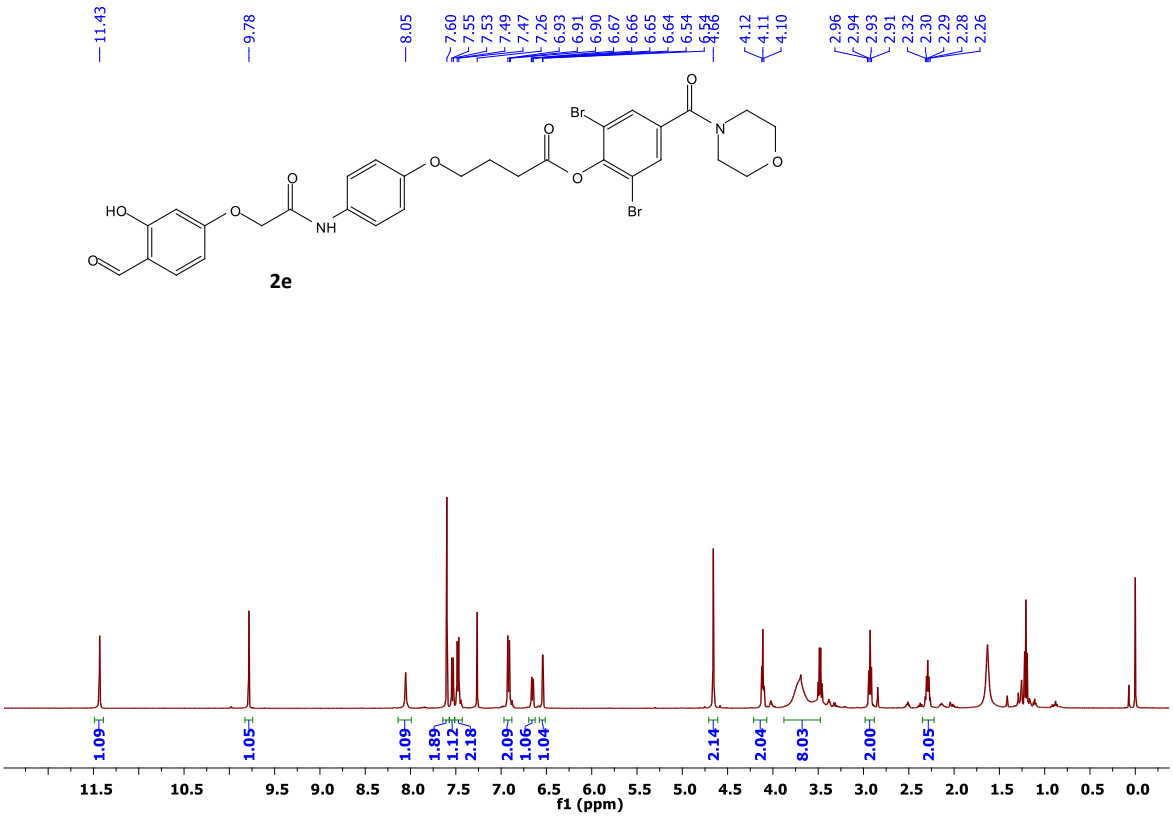


Figure S55 ^{1}H-NMR spectrum of compound **2e.**

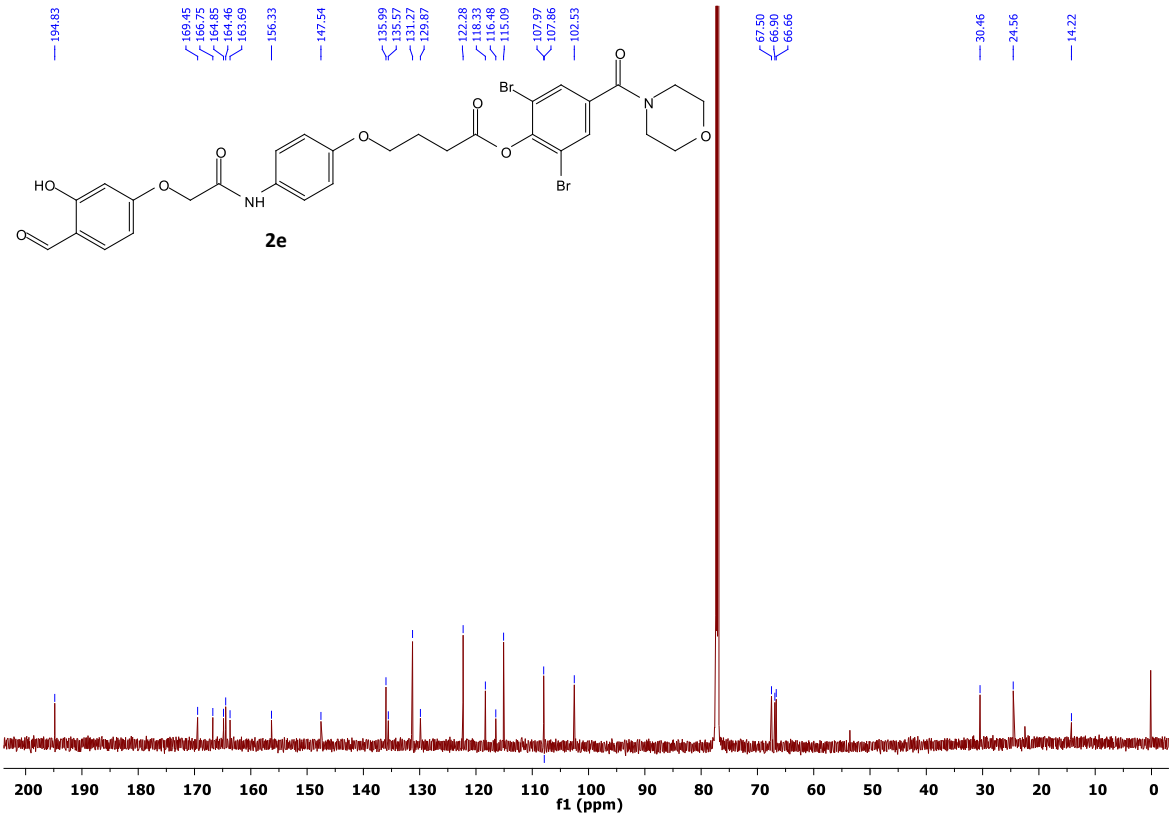


Figure S56 ^{13}C-NMR spectrum of compound **2e.**

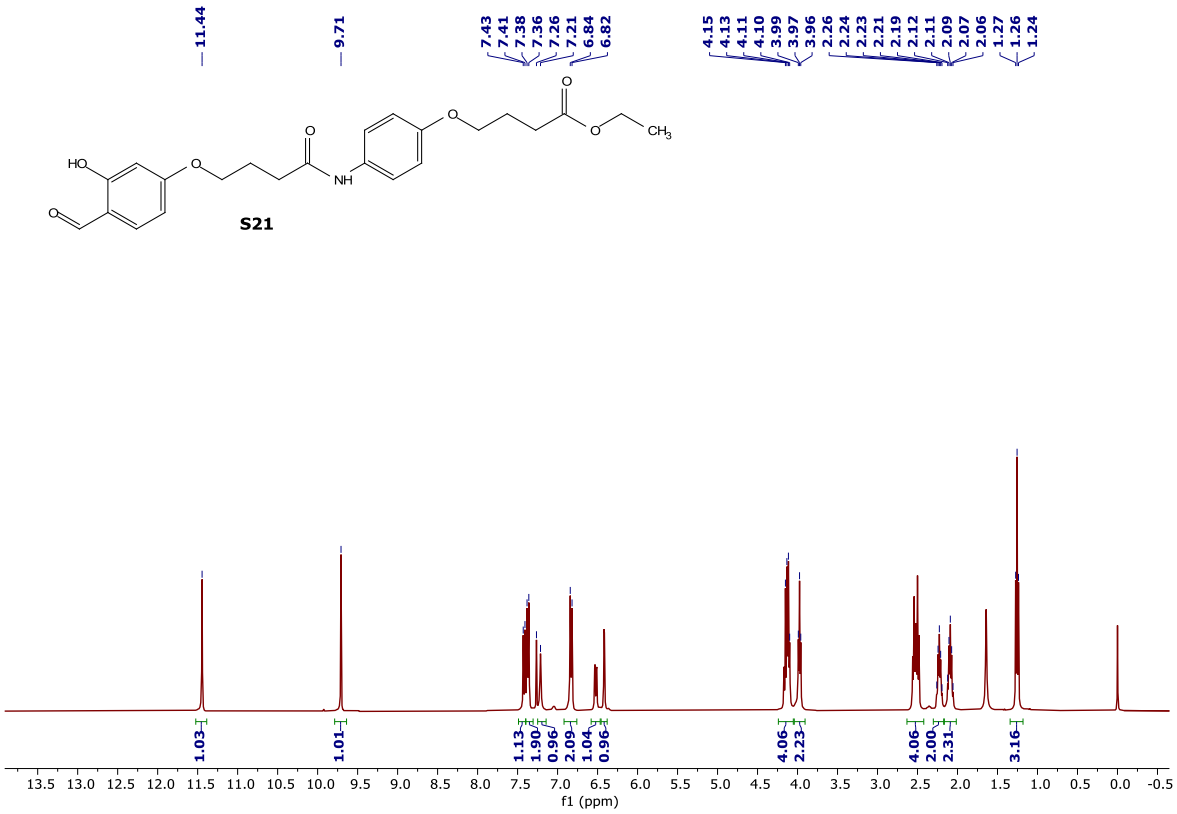


Figure S57 ^{1}H-NMR spectrum of compound **S21.**

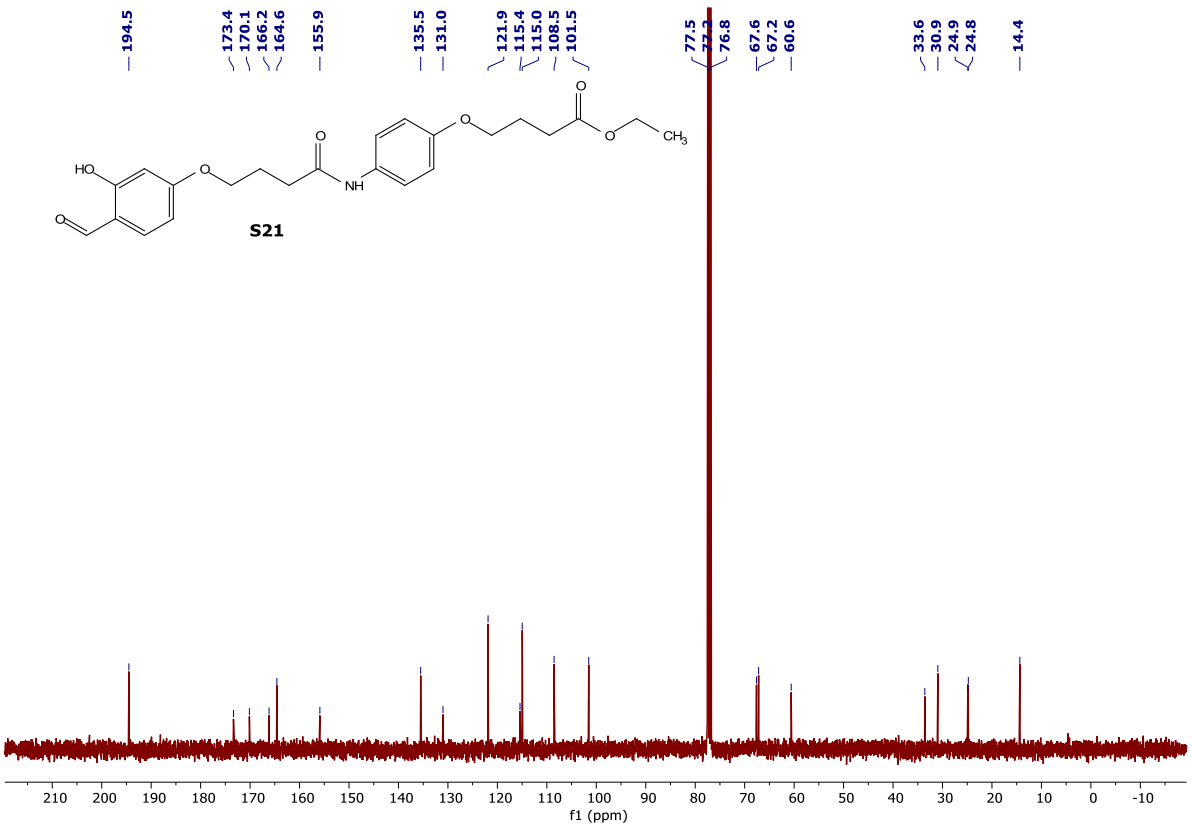


Figure S58 ^{13}C-NMR spectrum of compound **S21.**

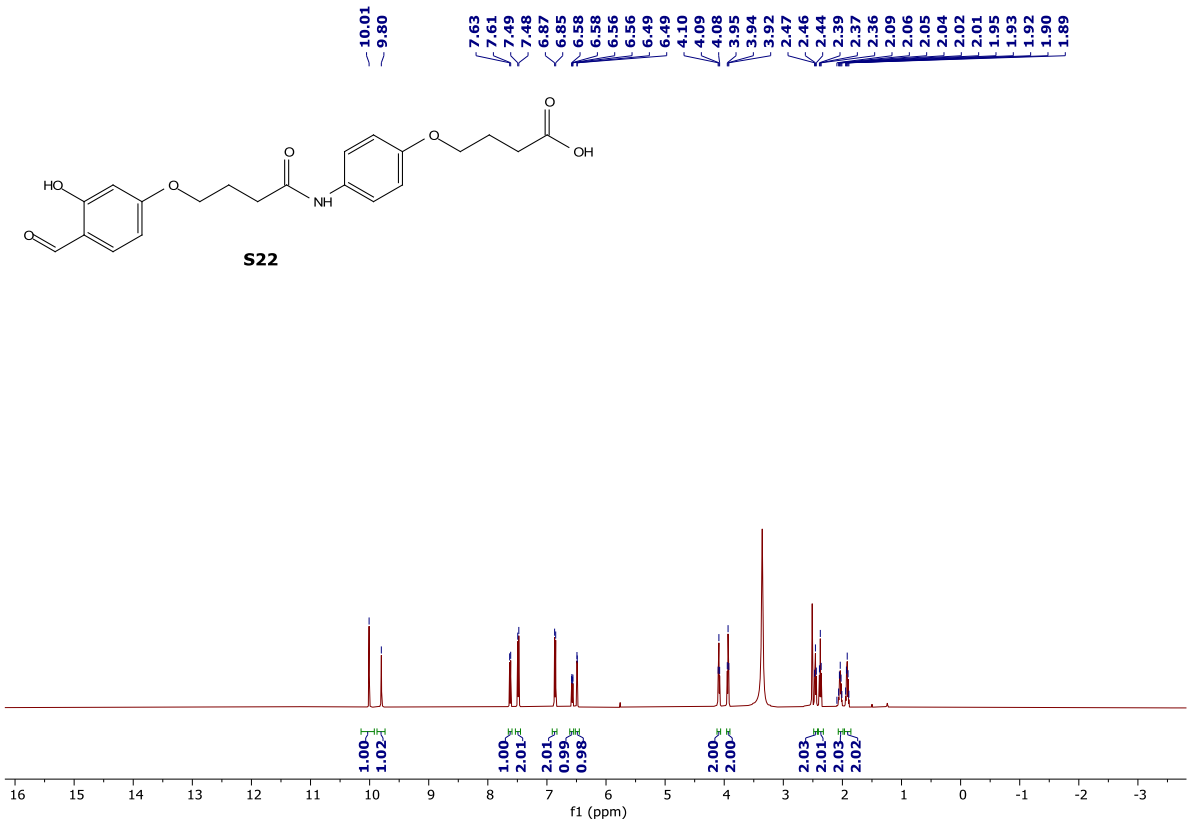


Figure S59 ^{1}H-NMR spectrum of compound **S22.**

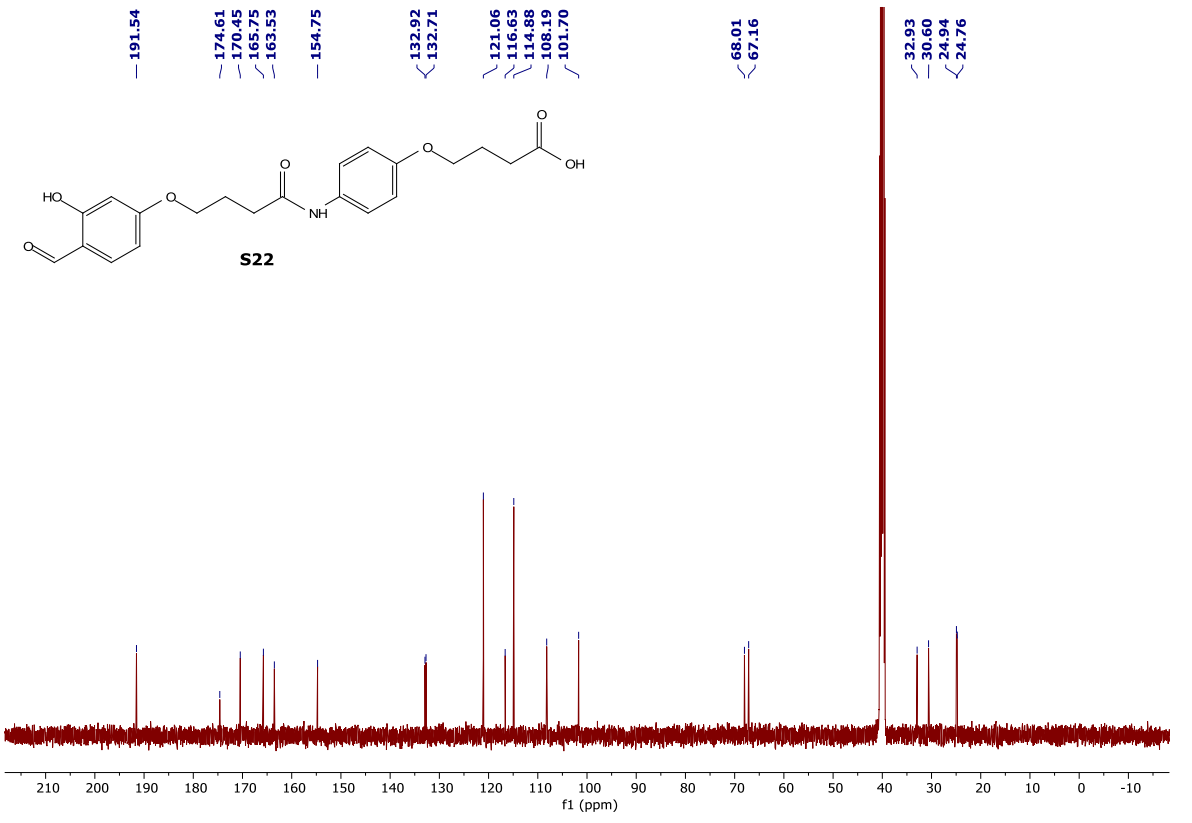


Figure S60 ^{13}C-NMR spectrum of compound **S22.**

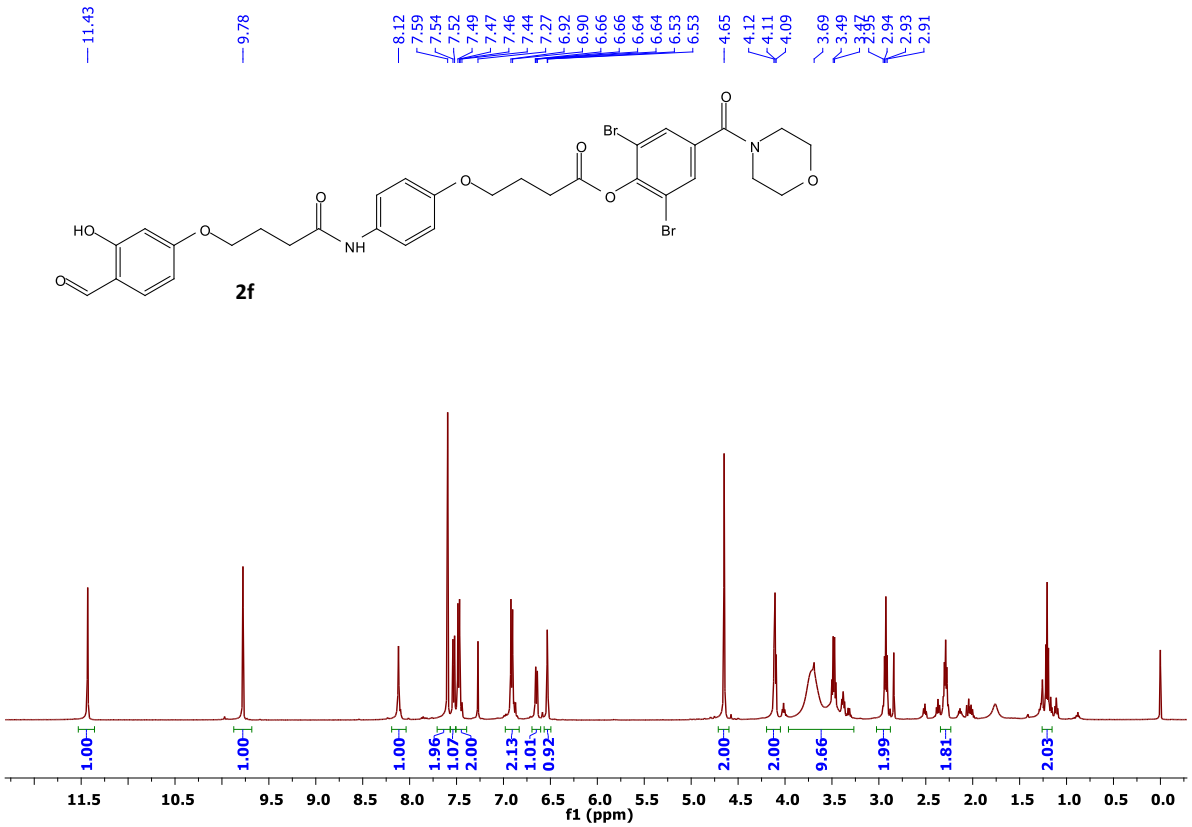


Figure S61 ^{1}H-NMR spectrum of compound **2f.**

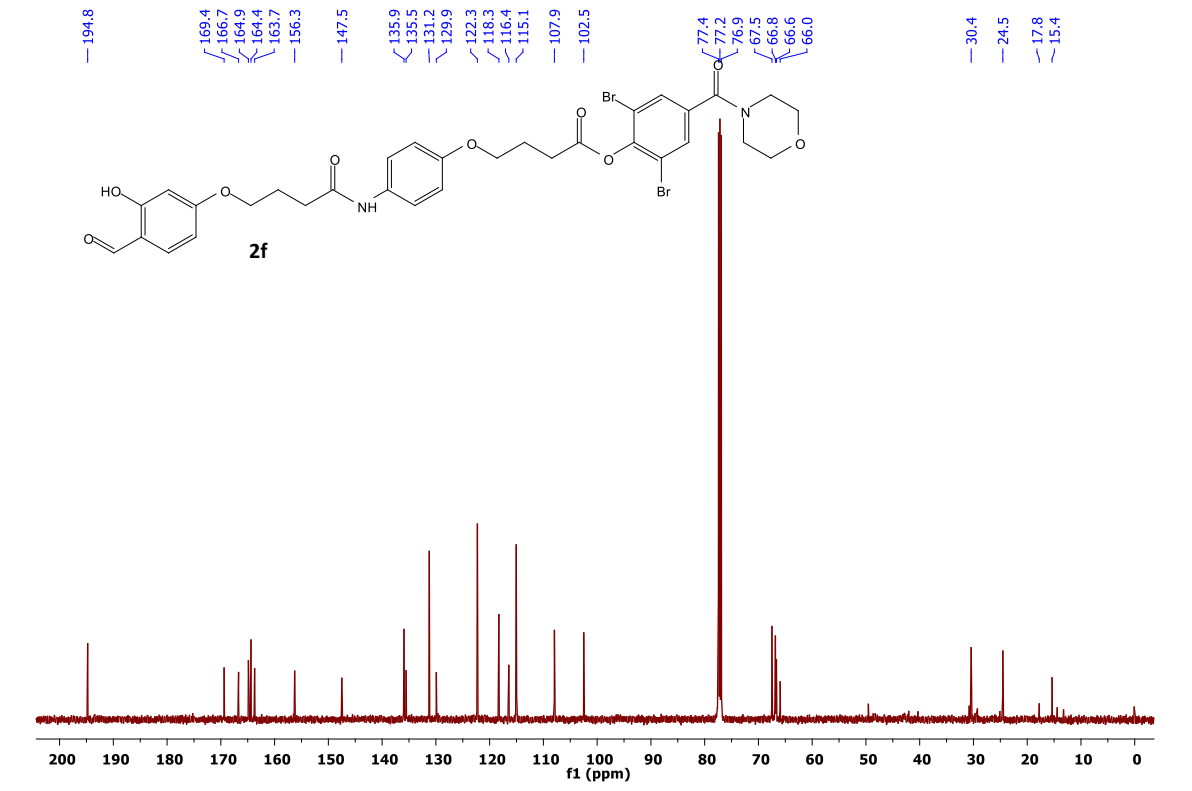


Figure S62 ^{13}C-NMR spectrum of compound **2f.**

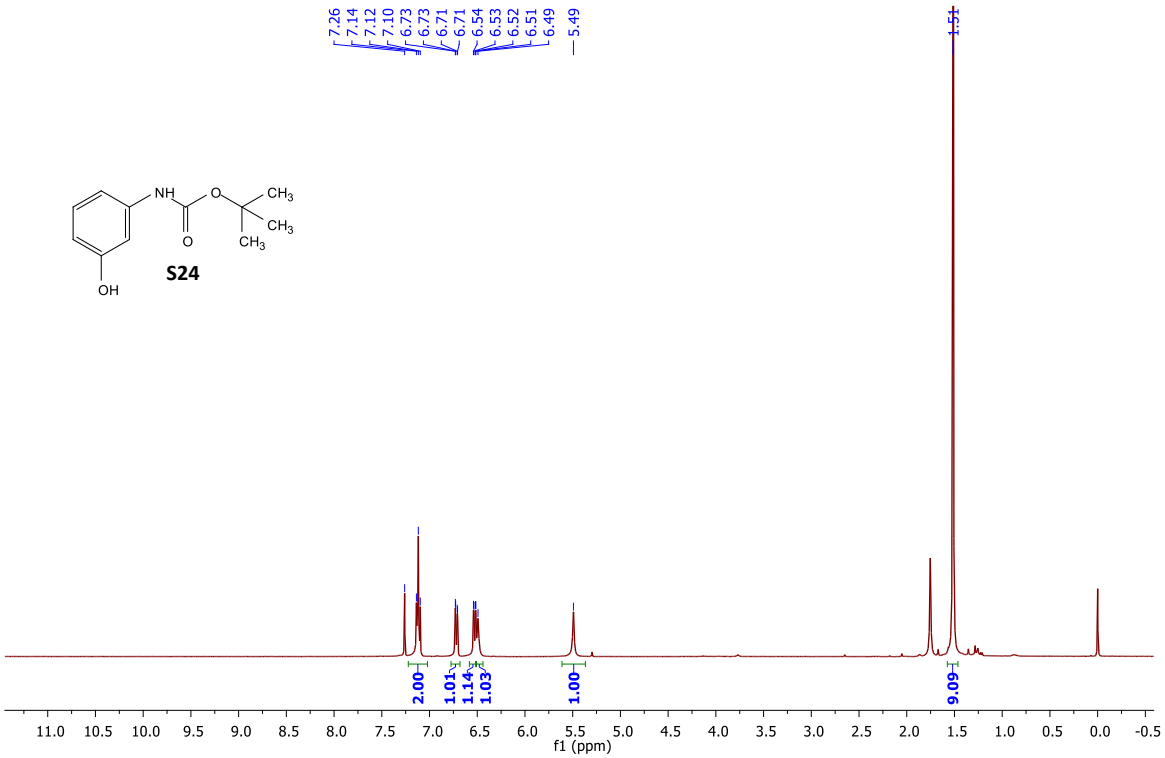


Figure S63 ^{1}H-NMR spectrum of compound **S24.**

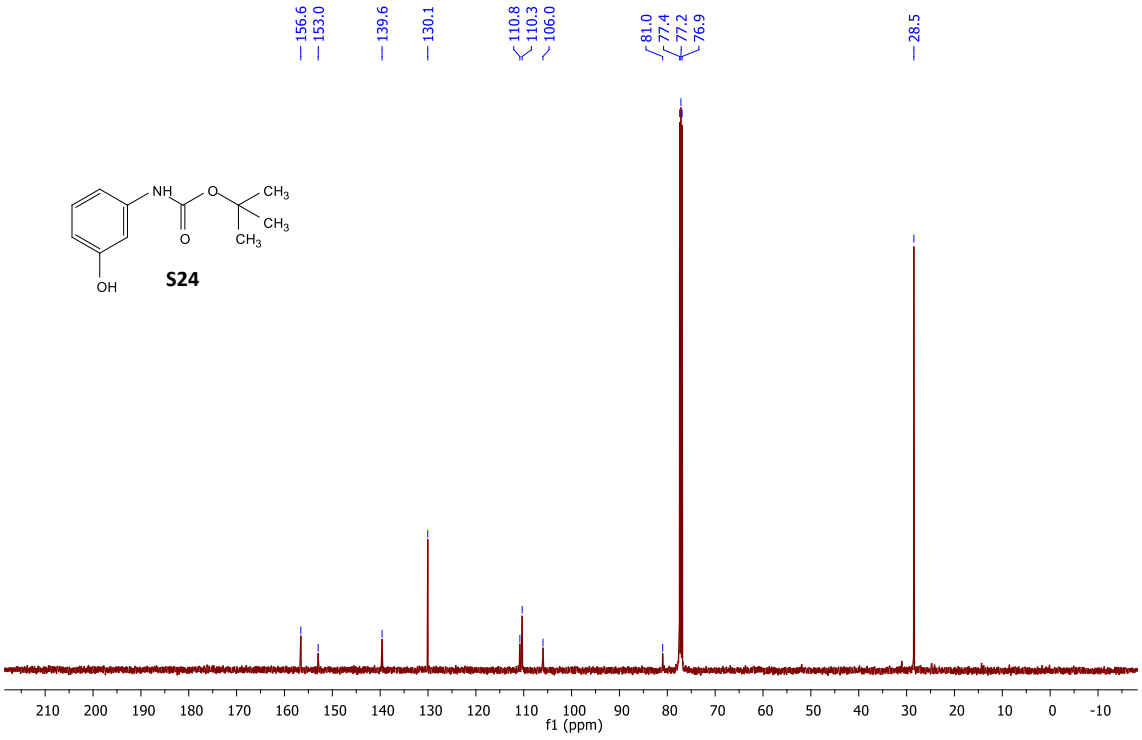


Figure S64 ^{13}C-NMR spectrum of compound **S24.**

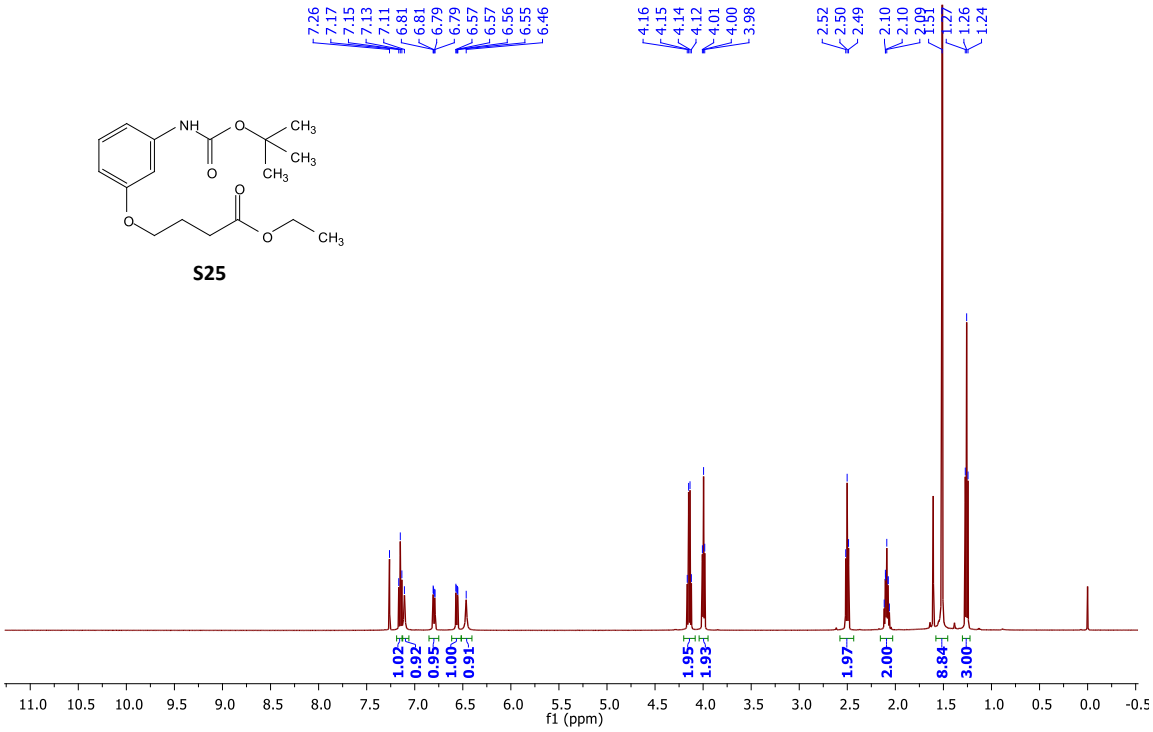


Figure S65 ^{1}H-NMR spectrum of compound **S25.**

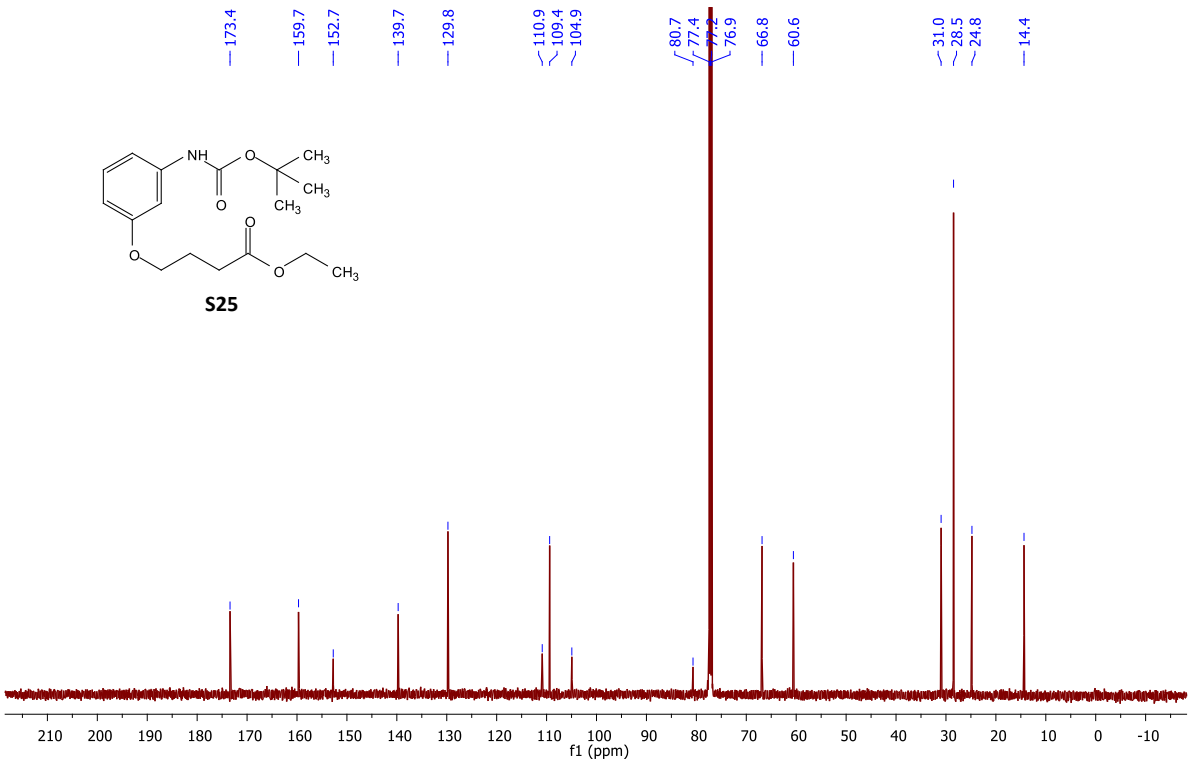


Figure S66 ^{13}C-NMR spectrum of compound **S25.**

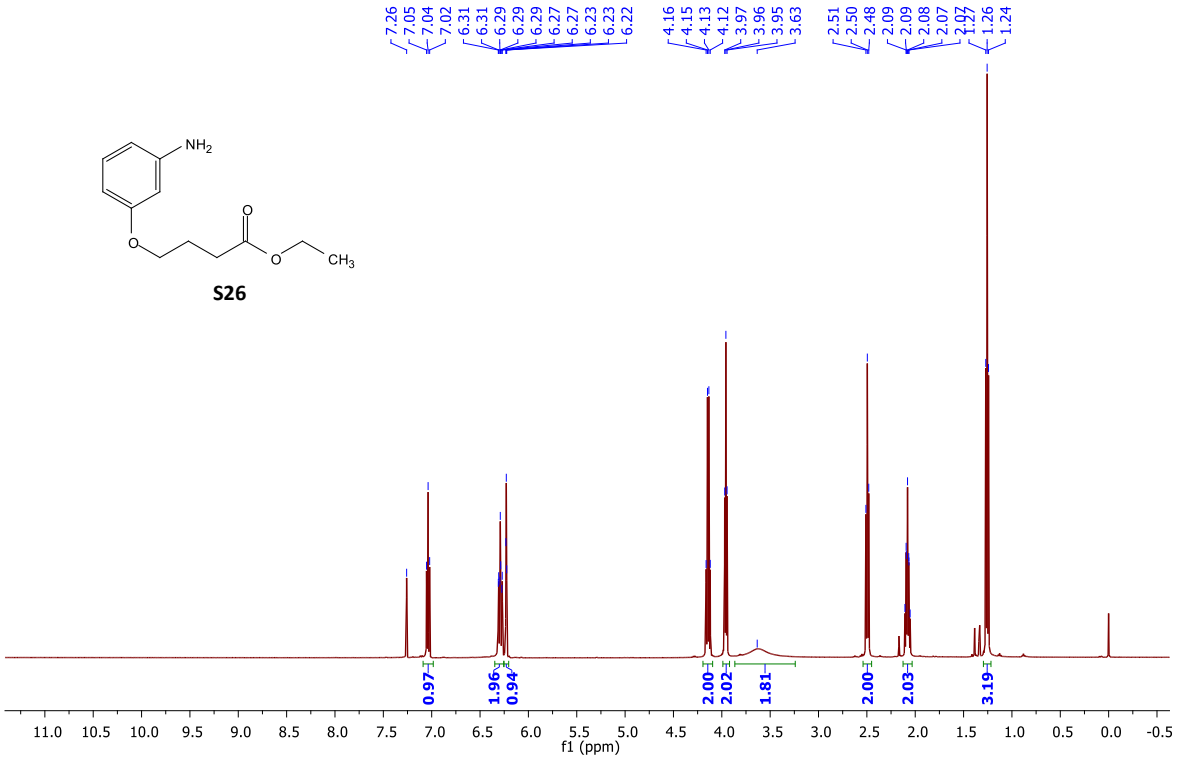


Figure S67 ^{1}H-NMR spectrum of compound **S26.**

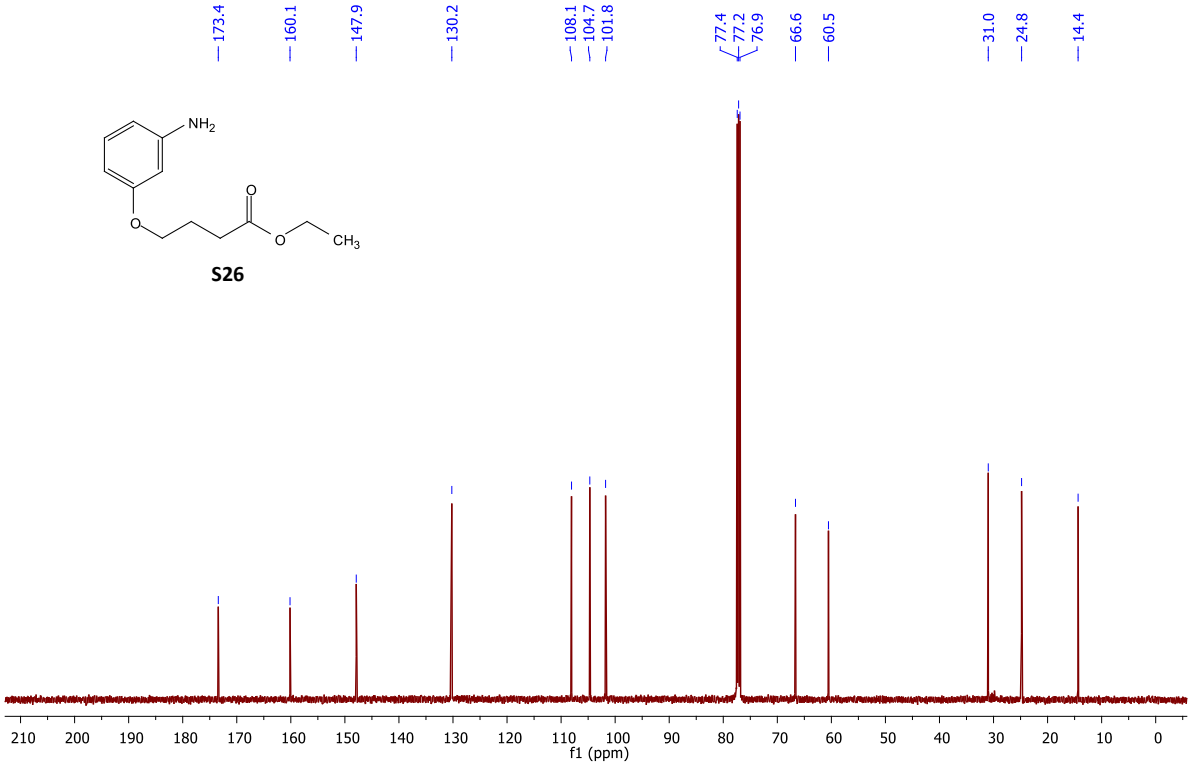


Figure S68 ^{13}C-NMR spectrum of compound **S26.**

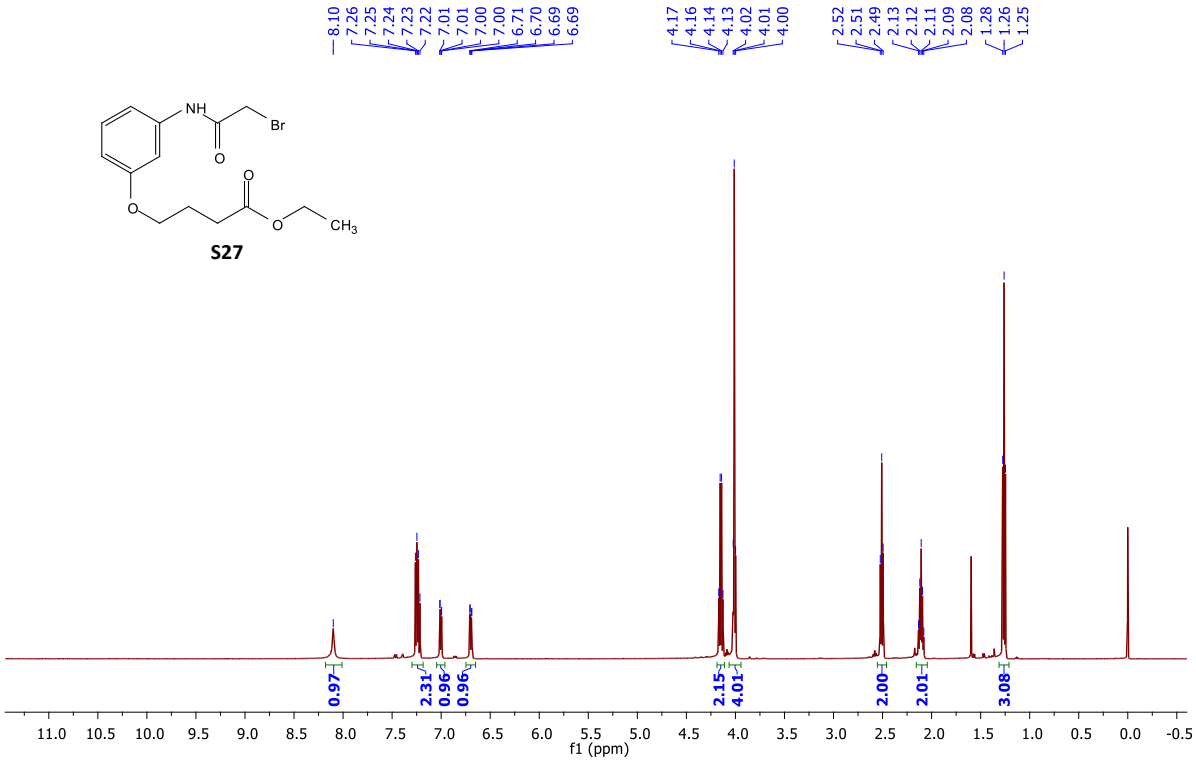


Figure S69 ^{1}H-NMR spectrum of compound **S27.**

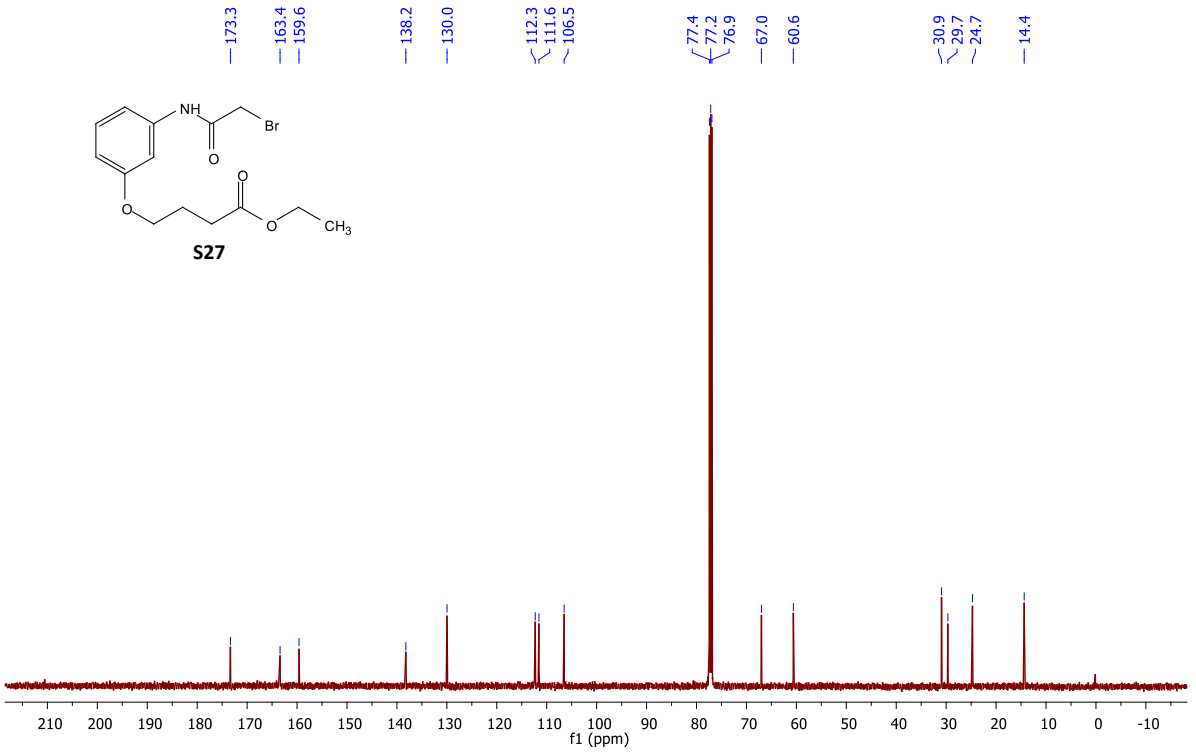


Figure S70 ^{13}C-NMR spectrum of compound **S27.**

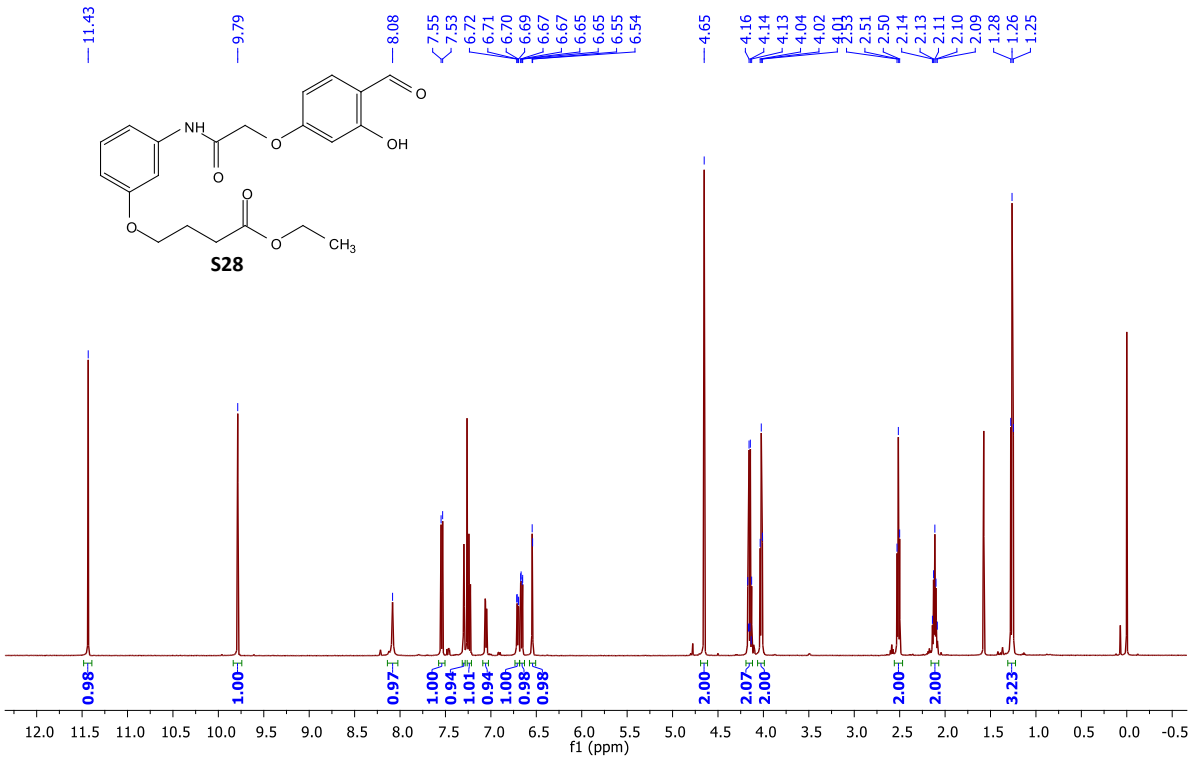


Figure S71 ^{1}H-NMR spectrum of compound **S28.**

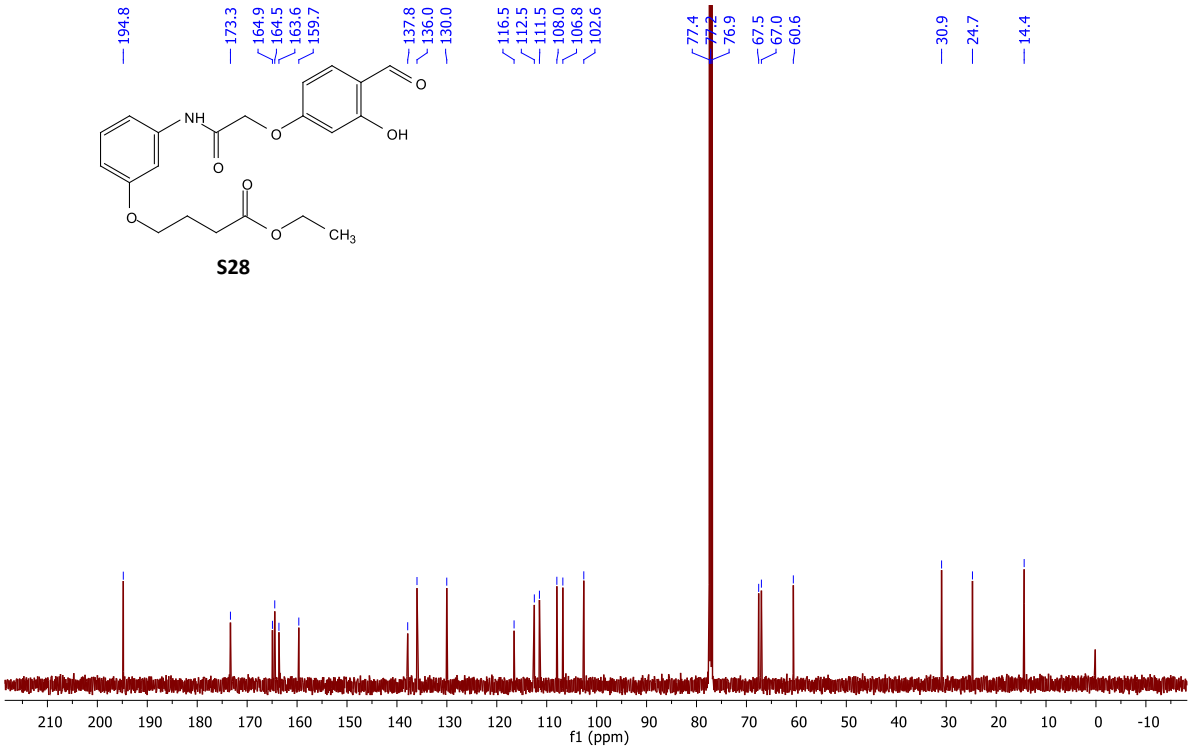


Figure S72 ^{13}C-NMR spectrum of compound **S28.**

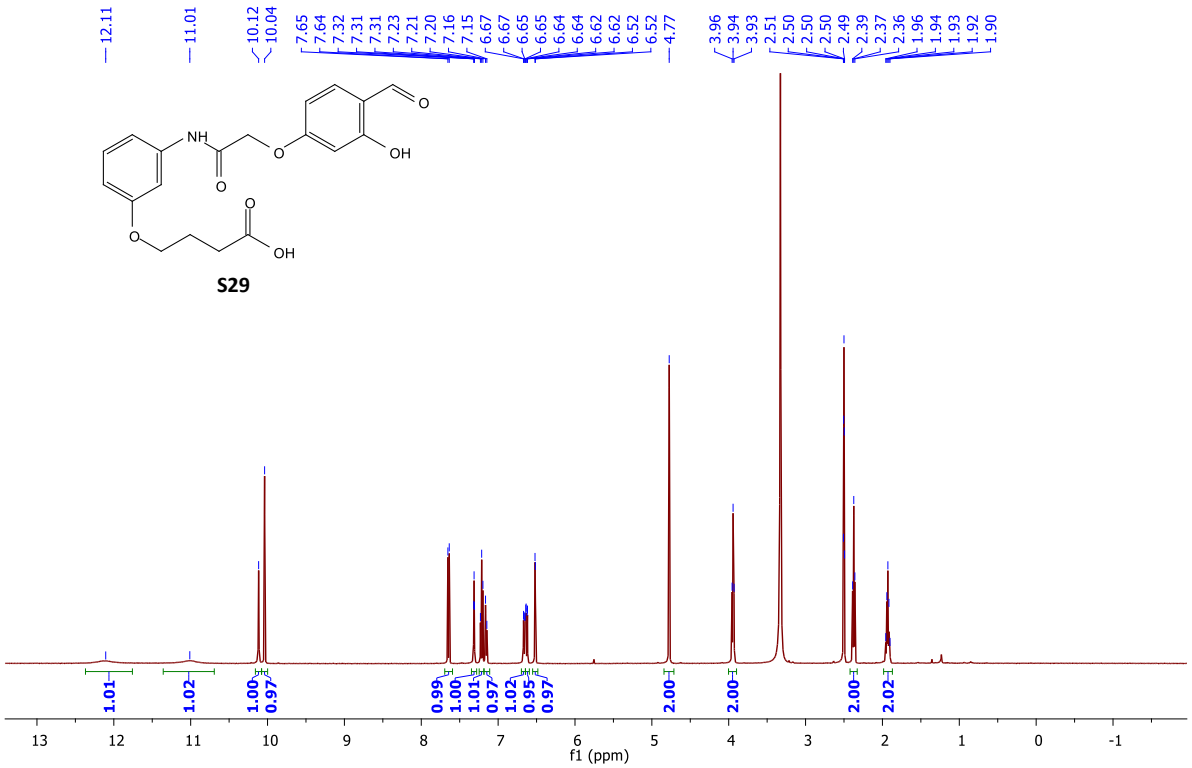


Figure S73 ^{1}H-NMR spectrum of compound **S29.**

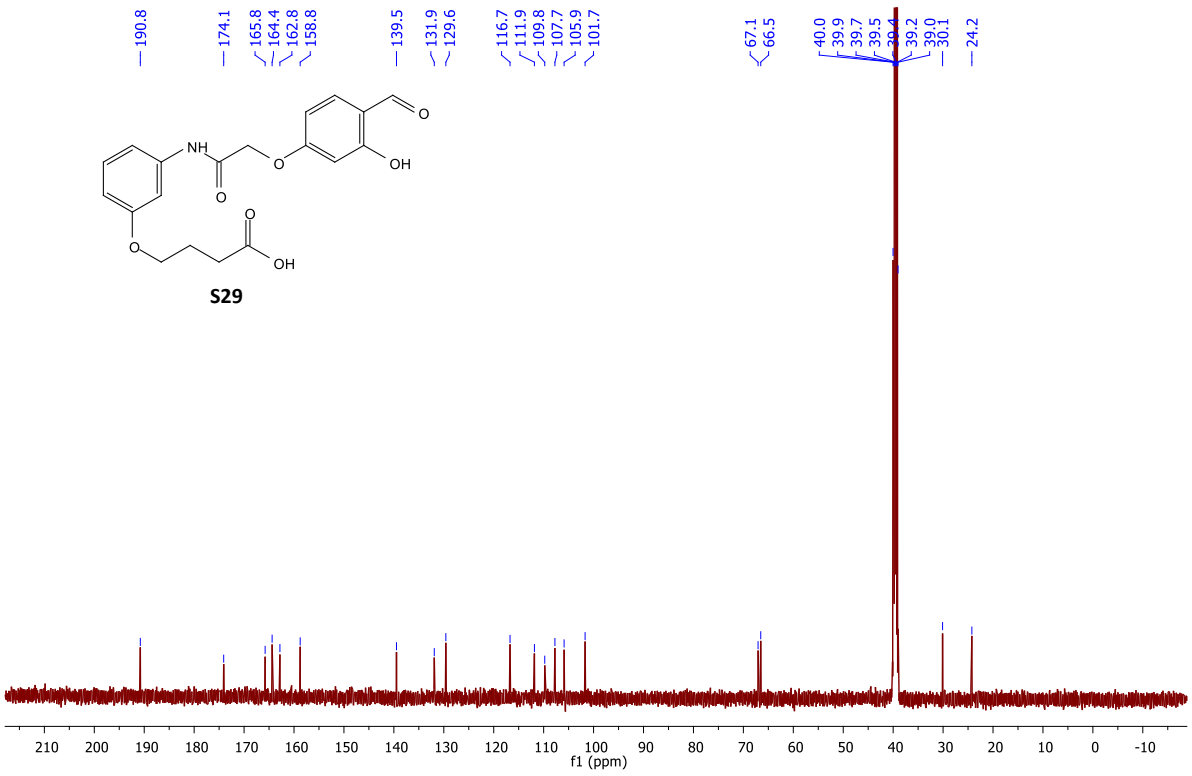


Figure S74 ^{13}C-NMR spectrum of compound **S29.**

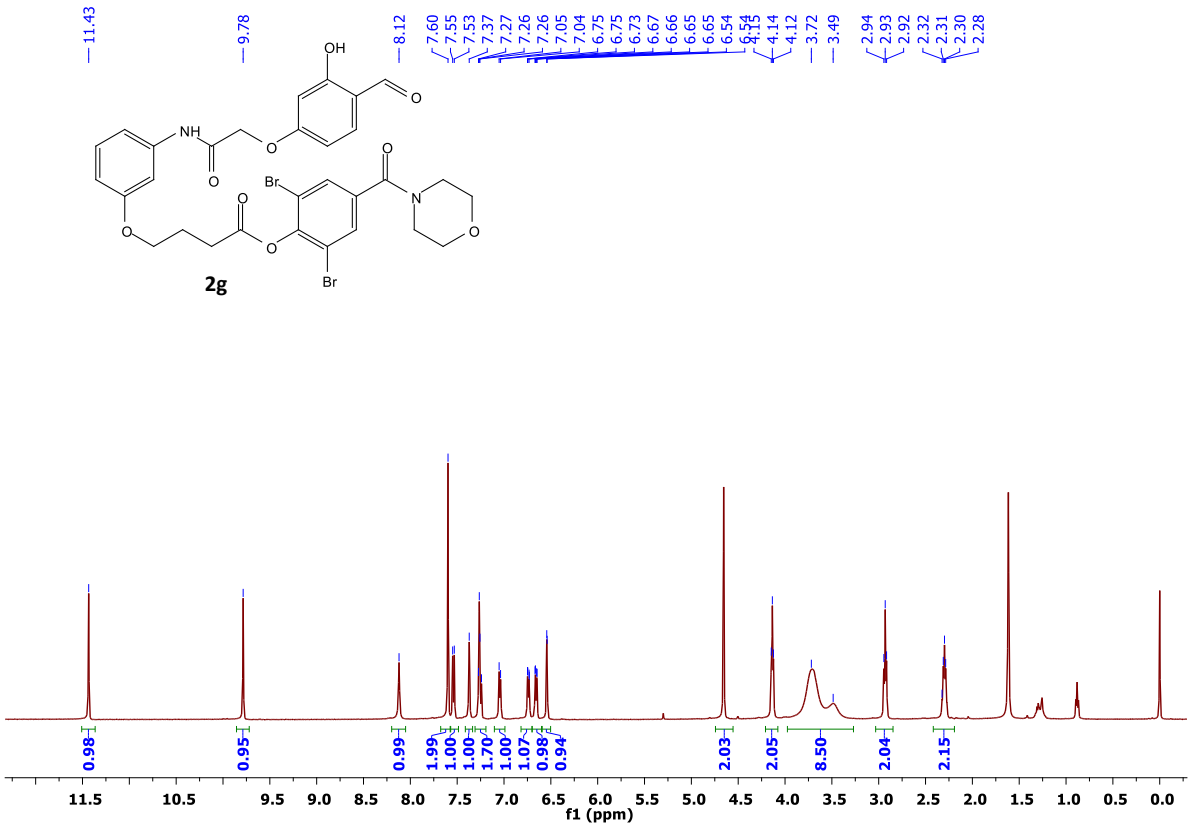


Figure S75 ^{1}H-NMR spectrum of compound **2g.**

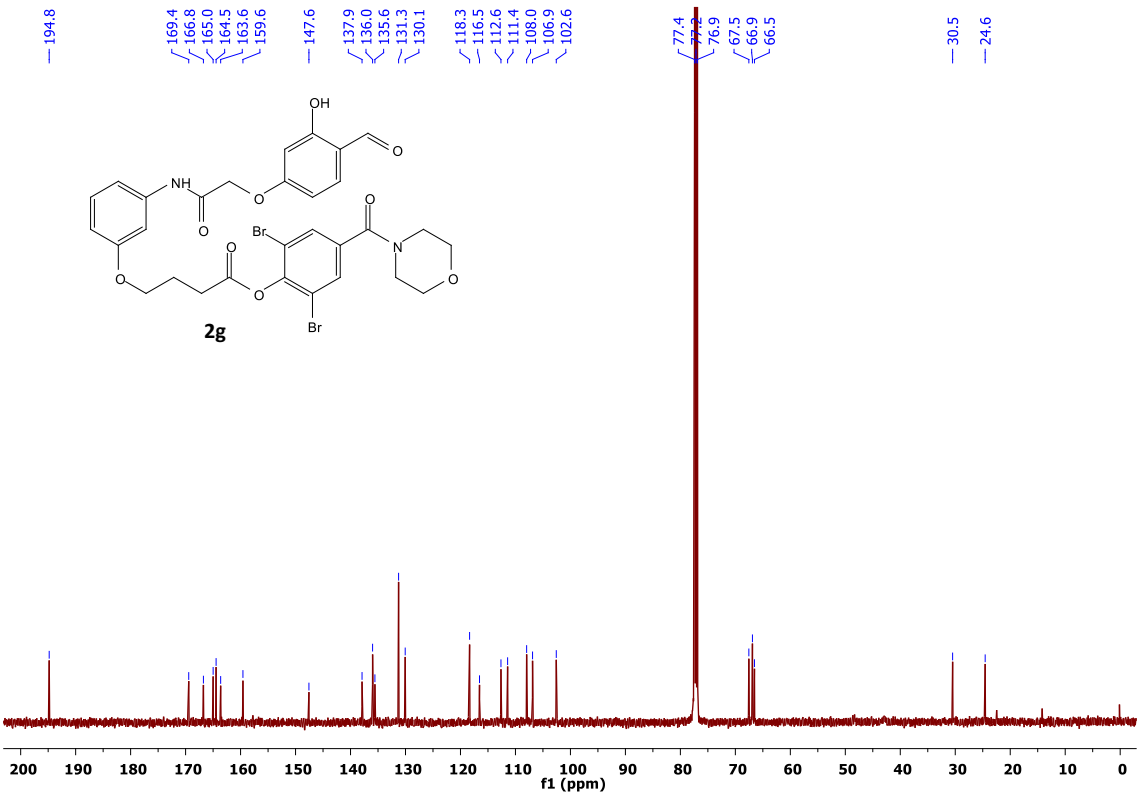


Figure S76 ^{13}C-NMR spectrum of compound **2g.**

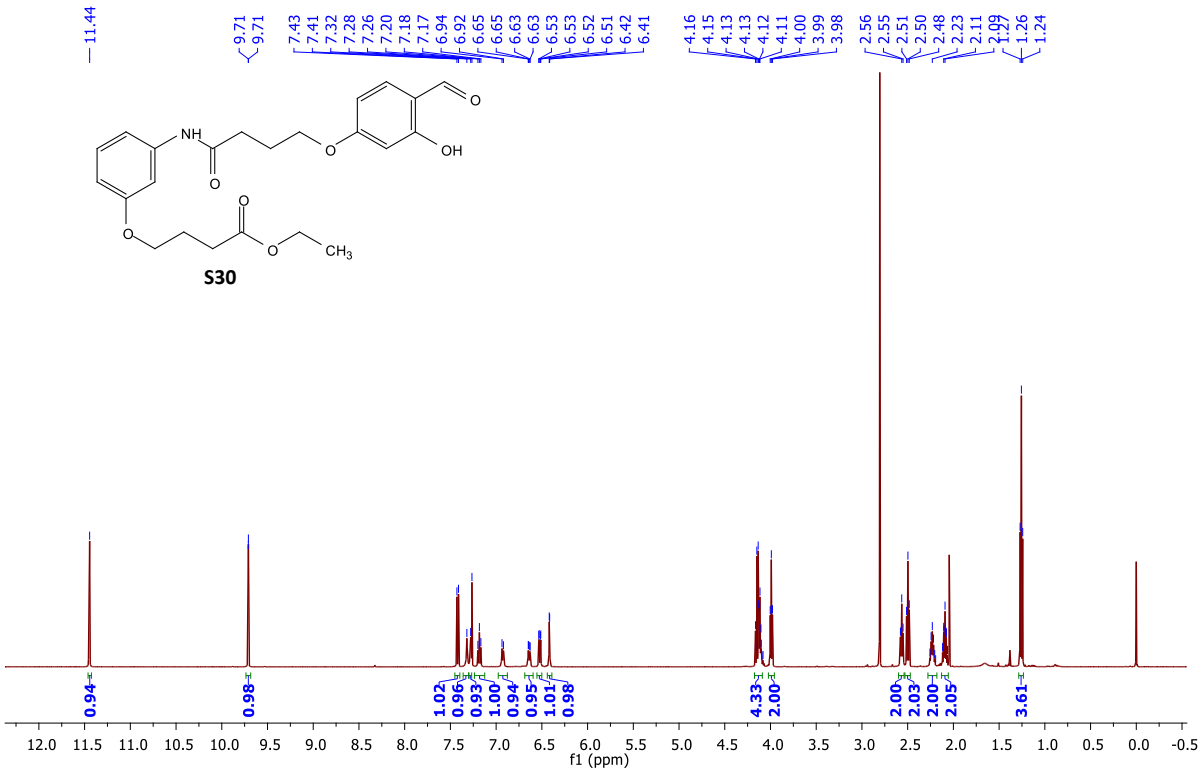


Figure S77 ^{1}H-NMR spectrum of compound **S30.**

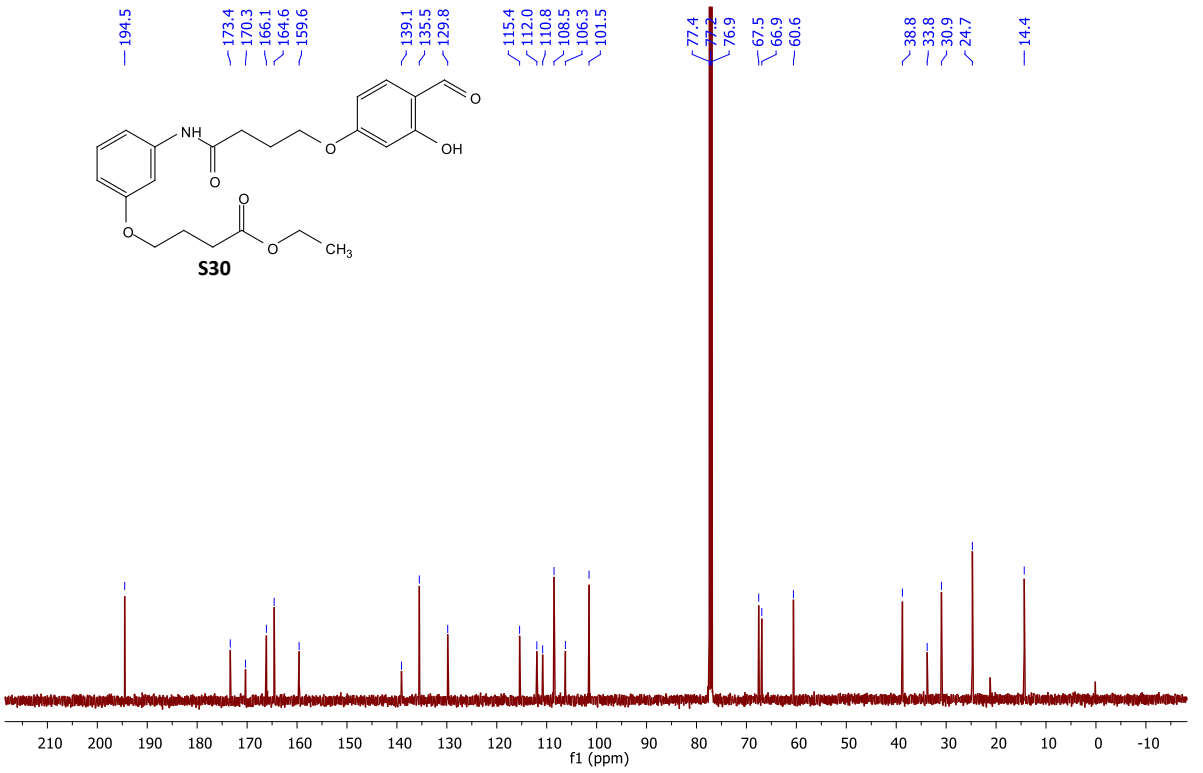


Figure S78 ^{13}C-NMR spectrum of compound **S30.**

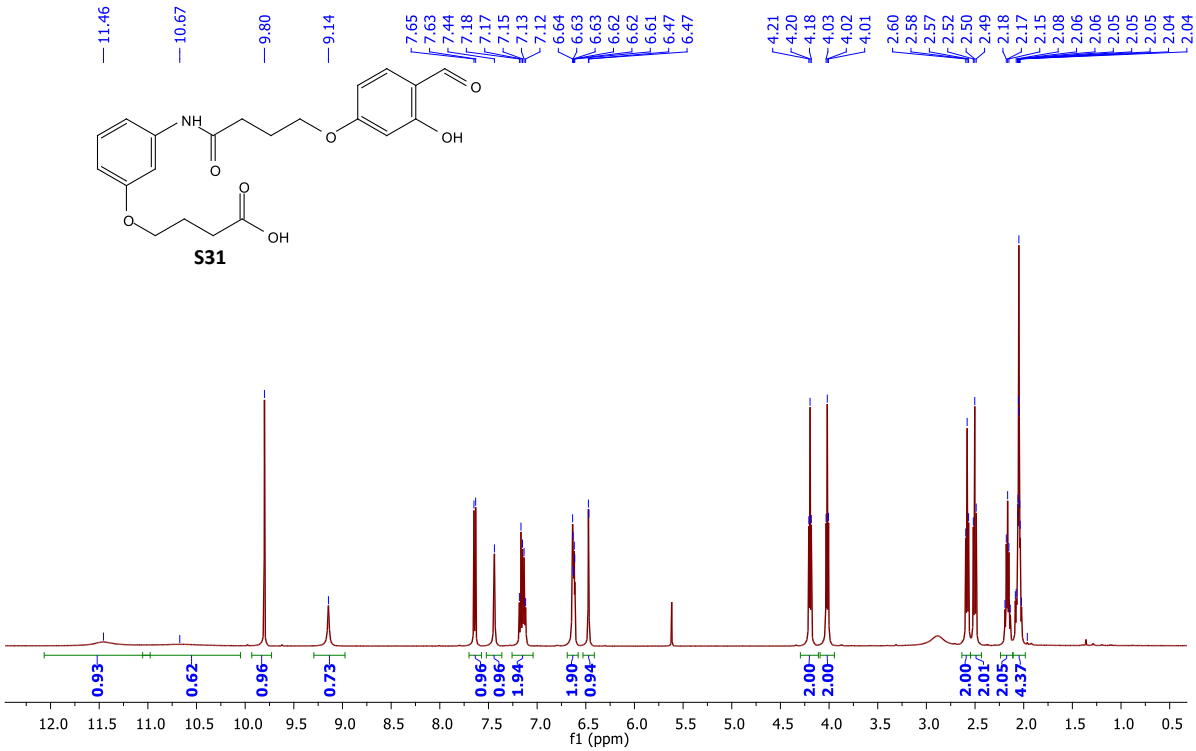


Figure S79 ^{1}H-NMR spectrum of compound **S31.**

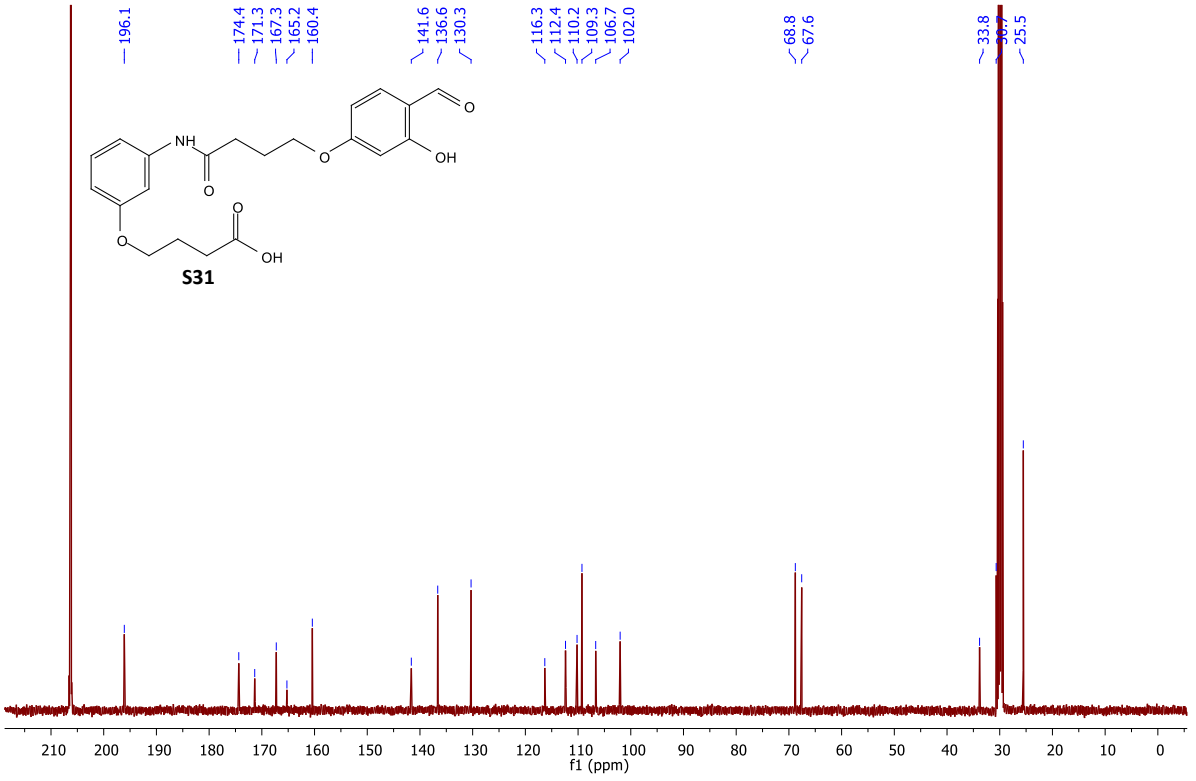


Figure S80 ^{13}C-NMR spectrum of compound **S31.**

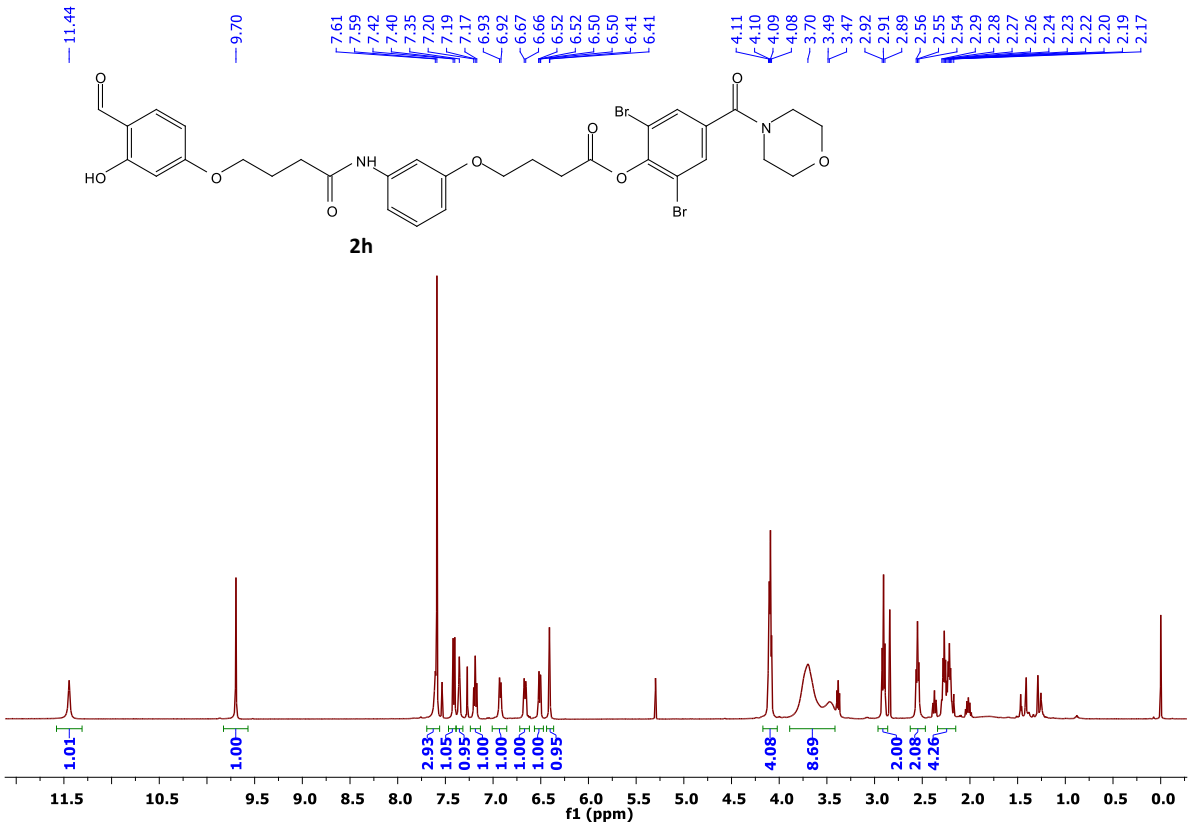


Figure S81 ^{1}H-NMR spectrum of compound **2h.**

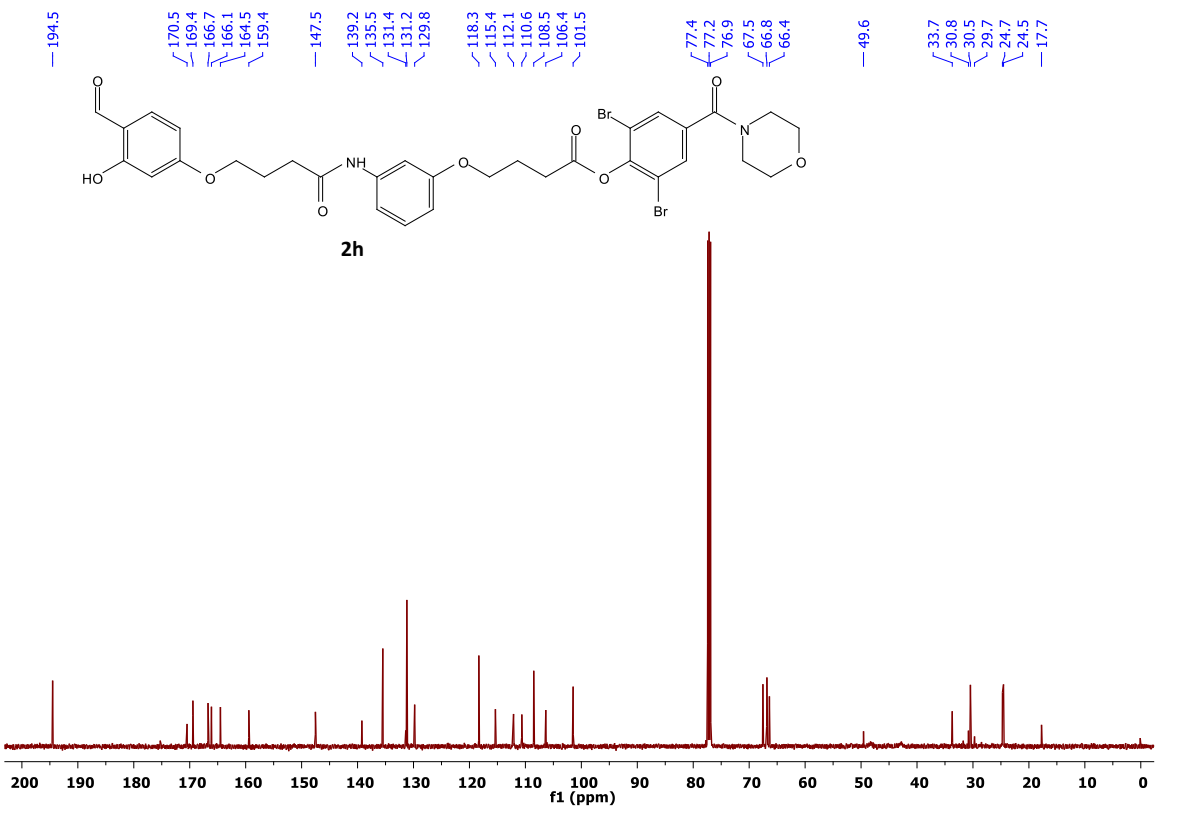


Figure S82 ^{13}C-NMR spectrum of compound **2h.**