Supporting information for

# Rationally Designed Helical Peptidomimetics Disrupt α-Synuclein Fibrillation

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## Table of contents

# 1. Synthesis

## 1.1 General Experimental

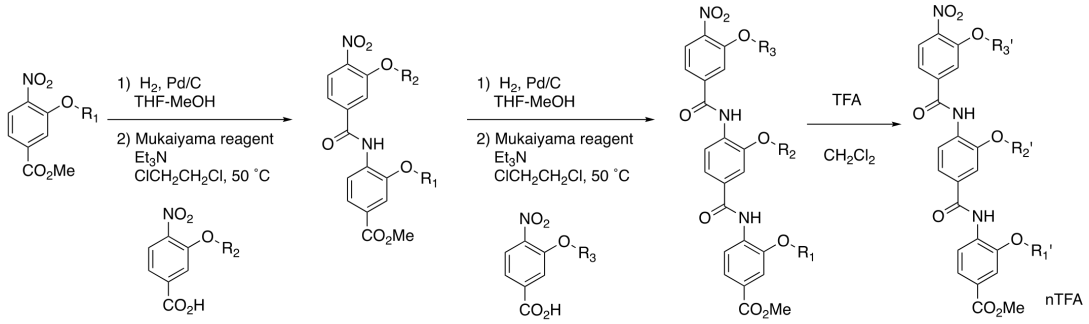
Reactions were carried out under a nitrogen or argon atmosphere in oven-dried glassware unless otherwise stated. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. Tetrahydrofuran (THF), dichloromethane (DCM), *N*,*N*'dimethylformamide (DMF) and methanol (MeOH) were anhydrous (dried on an MB-SPS-800 solvent purification system). Other solvents and reagents were used directly as received from commercial suppliers. All aqueous solutions were saturated unless specified otherwise.

Flash column chromatography was carried out using Merck 60 silica gel. Thin-layer chromatography was carried out using Merck Kieselgel 60 F254 (230-400 mesh) fluorescent treated silica, visualized under UV light (254 nm) or by staining with aqueous potassium permanganate solution.

$^{1}$H and $^{13}$C NMR spectra were recorded using a Bruker spectrometer (400, 500 or 600 MHz) running TopSpin™ software and are quoted in ppm for measurement against residual solvent peaks. These include $^{1}$H 7.26 and $^{13}$C 77.2 for chloroform; dimethyl sulfoxide $^{1}$H 2.50 and $^{13}$C 39.5. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (*J*) are given in Hertz (Hz). The $^{1}$H NMR spectra are reported as follows: δ (number of protons, multiplicity, coupling constant). Multiplicity is abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintet, m = multiplet, br = broad. IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer from a thin film deposited onto a diamond ATR module. Only selected maximum absorbances ($\nu_{max}$) of the most intense peaks are reported ($cm^{-1}$). High-resolution mass spectra were using a Bruker MicroTof (ESI) or an Agilent 7200 Accurate Mass Q-TOF GC/MS with an MSD Direct Inlet Probe (ammonia CI). Compound names are those generated by ChemBioDraw™ (CambridgeSoft) following IUPAC nomenclature.

## 1.2 General procedure for synthesis of alkoxy-substituted trimeric benzamides

### 1.2.1 General procedure (a) – reduction of nitro group followed by condensation with acid




**Scheme S1**

A mixture of nitro aromatic (1.0 eq) and palladium on carbon (10 % Pd by weight, 50 weight %) in THF-methanol (1:1, 0.4 M) was stirred under a hydrogen atmosphere (balloon pressure) for 2 h. Filtration over Celite™ and concentration *in vacuo* gave aniline, which was used in the next reaction without further purification.

To a solution of carboxylic acid (1.2 eq) and $Et_3N$ (2.4 eq) in 1,2-dichloroethane (0.5 M) was added Mukaiyama reagent (2-chloro-1-methylpyridinium iodide, 1.2 eq). The mixture was stirred at 50 ˚C for 15 min. Then a solution of the corresponding aniline (1.0 eq) in 1,2-dichloroethane (0.5 M) was added to the reaction mixture and the resulting solution was stirred at 50 ˚C for 14 h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography (EtOAc/hexane) unless otherwise stated.

### 1.2.2 General procedure (a') – reduction of nitro group with zinc followed by condensation with acid

To a mixture of nitro aromatic (1.0 eq) and zinc(0) dust (10 eq) in dichloromethane (0.4 M) was added acetic acid (one quarter the volume of dichloromethane) and the mixture was stirred at 40 ˚C for 1 h. The mixture was neutralized with aq. $NaHCO_3$ and extracted with dichloromethane twice. The organic layers were used in the next reaction without further purification.

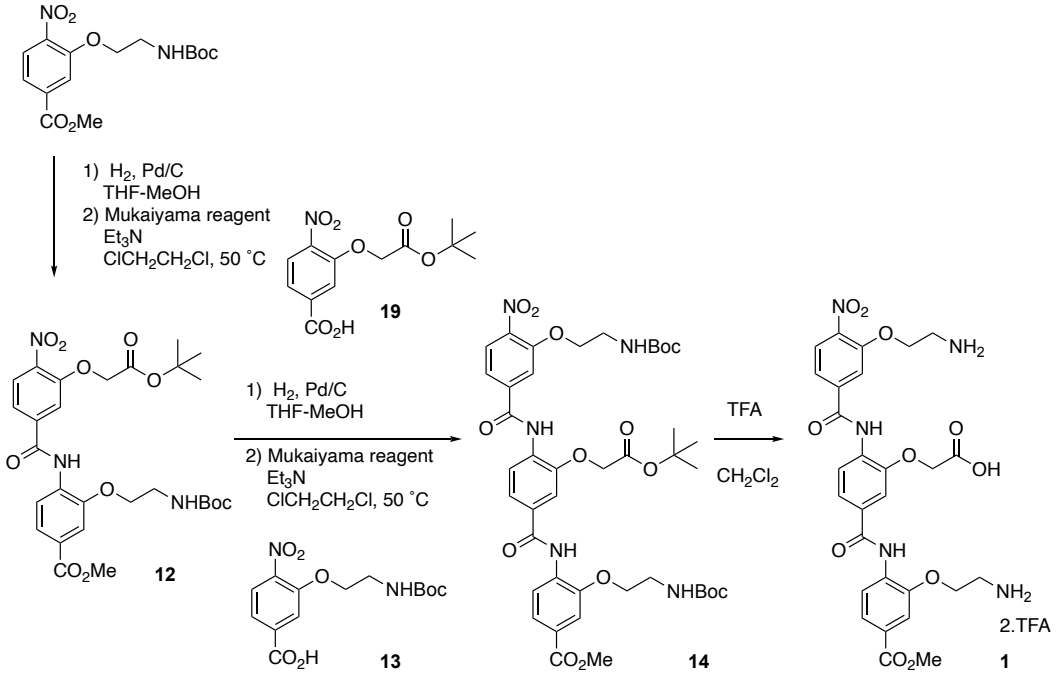
To a solution of acid (1.2 eq) and $Et_3N$ (2.4 eq) in dichloroethane (0.5 M) was added Mukaiyama reagent (2-chloro-1-methylpyridinium iodide, 1.2 eq). The mixture was stirred at 50 ˚C for 15 min. Then a solution of the corresponding aniline (1.0 eq) in dichloroethane (0.5 M) was added to the reaction mixture and the resulting solution was stirred at 50 ˚C for 14 h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography (EtOAc/hexane).

### 1.2.3 General procedure (b) – deprotection of *t*-butyl group (*N*-Boc and/or *t*-butyl ester)

To a solution of trimeric benzamide in dichloromethane (0.5 M) was added TFA (same volume as dichloromethane) and the mixture was stirred for 6 h. The reaction was concentrated *in vacuo* and the crude solid was collected and washed with diethyl ether.

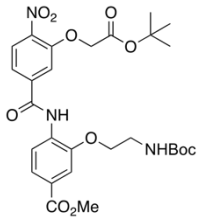
## 1.3 Targeting "E20, Q24, E28"

**Synthetic route to 2-(5-((2-(2-aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(2-aminoethoxy)-4-nitrobenzamido)phenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (1)**




**Scheme S2**

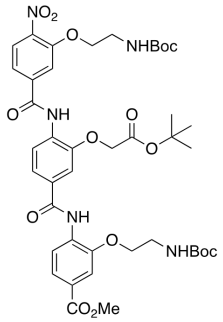
**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (12)**



According to *general procedure (a)*, methyl 3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzoate[1] (2.40 g, 7.05 mmol) was reduced to the aniline, which was then coupled with 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoic acid **19**[2][3] (2.52 g, 8.46 mmol). The residue was purified by flash column chromatography (3:7 to 1:1 EtOAc : hexane) to give *the title compound* **12** (3.00 g, 5.09 mmol, 72 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.11 (1H, brs, NH), 8.58 (1H, d, *J* 8.4, Ar-H), 7.88 (1H, d, *J* 8.4, Ar-H), 7.73 (1H, s, Ar-H), 7.68 (1H, dd, *J* 8.54, 1.2, Ar-H), 7.62 (1H, s, Ar-H), 7.46 (1H, s, Ar-H), 4.82 (1H, t, *J* 6.5, NH), 4.77 (2H, s,

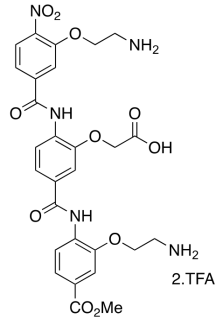
$OCH_2C{=}O$), 4.07-4.40 (2H, m, $OCH_2CH_2$), 3.85 (3H, s, $OCH_3$), 3.54-3.63 (2H, m, $CH_2N$), 1.41 (9H, s, *t*-Bu), 1.14 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 166.5, 166.5, 163.6, 156.5, 151.1, 147.2, 141.9, 139.2, 131.8, 125.7, 125.6, 123.4, 120.3, 119.1, 115.0, 111.0, 83.1, 79.7, 69.6, 66.6, 52.1, 40.9, 28.0, 28.0; IR 3397, 2976, 1694, 1424, 1086, 872, 725; HRMS (ESI) calculated for $C_{28}H_{34}N_3O_{11}$ [$(M-H)^+$]: 588.2187 found 588.2197.

**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzamido)benzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (14)**



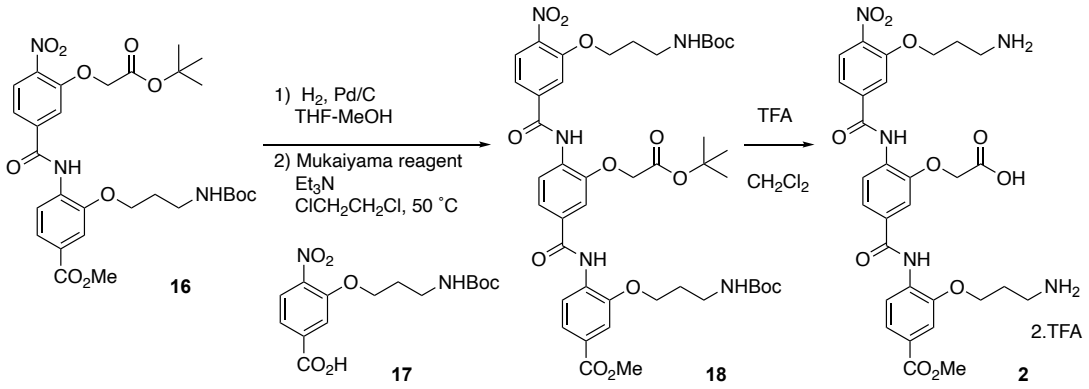

According to *general procedure (a)*, nitro compound **12** (400 mg, 0.678 mmol) was reduced to the aniline, which was coupled with 3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzoic acid[2] **13** (332 mg, 1.02 mmol). The residue was purified by flash column chromatography (2:3 to 3:2 EtOAc : hexane) to give *the title compound* **14** (428 mg, 0.493 mmol, 73 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.61 (1H, s, NH), 8.91 (1H, s, NH), 8.52-8.61 (2H, m, Ar-H), 7.92 (1H, d, *J* 8.4, Ar-H), 7.63-7.77 (5H, m, Ar-H), 7.48 (1H, s, Ar-H), 5.02-5.12 (1H, m, NHBoc), 4.74-4.80 (1H, m, NHBoc), 4.71 (2H, s, $OCH_2C{=}O$), 4.25-4.31 (2H, m, $OCH_2CH_2$), 4.10-4.18 (2H, m, $OCH_2CH_2$), 3.84-3.87 (3H, m, $OCH_3$), 3.51-3.65 (4H, m, $OCH_2CH_2$), 1.44 (9H, s, *t*-Bu), 1.38 (9H, s, *t*-Bu), 1.24 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 168.8, 166.6, 164.5, 162.9, 156.2, 155.8, 152.1, 147.8, 146.9, 141.5, 139.7, 132.5, 132.2, 130.3, 125.9, 125.1, 123.5, 122.1, 120.0, 119.6, 119.0, 114.8, 113.9, 111.2, 83.4, 79.7, 79.6, 69.5, 69.0, 68.6, 52.1, 40.0, 39.7, 28.3, 28.2, 28.1; IR 3356, 2979, 1489, 1316, 1062, 747; HRMS (ESI) calculated for $C_{42}H_{53}N_5NaO_{15}$ [$(M+Na)^+$]: 890.3430 found 890.3410.

**2-(5-((2-(2-Aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(2-aminoethoxy)-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (1)**



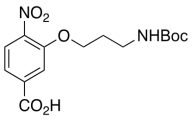
According to *general procedure (b)*, *t*-butyl compound **14** (428 mg, 0.493 mmol) was deprotected and purified by washing with dichloromethane to give *the title compound* **1** (259 mg, 0.308 mmol, 62 % yield) as a yellow solid: $\delta_H$ (400 MHz DMSO-$d_6$) 10.18 (1H, s, N-H), 9.67 (1H, s, N-H), 8.34 (1H, d, *J* 8.4, Ar-H), 7.99-8.20 (6H, m, Ar-H), 7.90 (1H, s, Ar-H), 7.66-7.82 (4H, m, $NH_2$), 7.62 (1H, s, Ar-H), 4.92 (2H, s, $OCH_2C{=}O$), 4.51 (2H, t, *J* 4.8, $OCH_2CH_2$), 4.33-4.42 (2H, m, $OCH_2CH_2$), 3.89 (3H, s, $OCH_3$), 3.21-3.48 (4H, m, $OCH_2CH_2$); $\delta_C$ (101 MHz, DMSO-$d_6$) 171.3, 166.2, 165.3, 163.9, 159.2, 158.9, 150.9, 149.7, 148.2, 141.8, 139.8, 132.3, 131.6, 131.6, 126.0, 125.8, 123.1, 123.0, 122.3, 121.8, 120.7, 115.0, 114.7, 112.2, 67.5, 66.9, 65.6, 52.6, 38.8, 38.4; IR 2963, 1674, 1598, 1198, 1129, 723; HRMS (ESI) calculated for $C_{28}H_{30}N_5O_{11}$ $[(M+H)^+]$: 612.1914 found 612.1936.

**Synthetic route to 2-(5-((2-(3-aminopropoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(3-aminopropoxy)-4-nitrobenzamido)phenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (2)**




**Scheme S3**

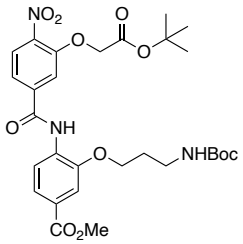
**3-(3-((*tert*-Butoxycarbonyl)amino)propoxy)-4-nitrobenzoic acid (17)**



Based on a literature procedure,[6] to a mixture of methyl 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoate **15** (2.11 g, 6.20 mmol) in THF (25 mL)-water (25 mL) was added lithium hydroxide monohydrate (391 mg, 9.30 mmol) and the mixture was stirred for 2 h. The mixture was acidified with 3 *N* HCl, extracted with dichloromethane twice. The combined organic layers were washed with brine and dried over $MgSO_4$. Filtration and concentration *in vacuo* afforded *the title compound* **17** (1.93 g, 5.67 mmol, 91 % yield) as a white solid: $\delta_H$ (400 MHz DMSO-$d_6$) 13.67 (1H, brs, COOH), 7.98 (1H, d, *J* 8.4, Ar-H), 7.76 (1H, s, Ar-H), 7.65 (1H, d, *J* 8.2, 1.4, Ar-H), 6.93 (1H, t, *J* 5.4, NH), 4.24 (2H, t, *J* 6.1, $OCH_2CH_2CH_2$), 3.09 (2H, q, *J* 6.4, $OCH_2CH_2CH_2$), 1.78-1.91 (2H, m, $OCH_2CH_2CH_2$), 1.37 (9H, m, *t*-bu); $\delta_C$ (101 MHz, DMSO-$d_6$) 166.2, 156.0, 151.4, 142.4, 136.1, 125.5, 121.6, 115.7, 77.9, 67.6, 37.0,

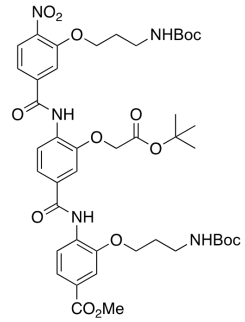
29.3, 28.6; IR 3336, 2501, 1707, 1168, 780; HRMS (ESI) calculated for $C_{15}H_{19}N_2O_7$ [(M-H)$^+$]: 339.1186 found 339.1192.

**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzoate (16)**




According to *general procedure (a)*, methyl 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoate[2][3] **15** (1.00 g, 2.94 mmol) was reduced to the aniline which was then coupled with 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoic acid **19**[4] (1.05 g, 3.53 mmol). The residue was purified by flash column chromatography (3:7 to 3:2 EtOAc : hexane) to give *the title compound* **16** (1.21 g, 2.00 mmol, 68 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.05 (1H, br, NH), 8.41 (1H, d, *J* 8.5, Ar-H), 7.95 (1H, d, *J* 8.2, Ar-H), 7.62-7.73 (3H, m, Ar-H), 7.53 (1H, s, Ar-H), 4.70 (2H, s, $OCH_2C$=O), 4.55-4.65 (1H, m, NH), 4.13 (2H, t, *J* 5.7, $OCH_2CH_2CH_2$), 3.85 (3H, s, $OCH_3$), 3.33-3.38 (2H, m, $OCH_2CH_2CH_2$), 1.90-2.03 (2H, m, $OCH_2CH_2CH_2$), 1.41 (9H, s, *t*-Bu), 1.29 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 166.5, 166.3, 163.0, 156.1, 151.4, 147.4, 141.9, 139.5, 131.5, 126.3, 126.0, 123.3, 119.9, 119.4, 114.7, 111.8, 83.2, 79.6, 66.6, 65.3, 52.1, 36.9, 29.5, 28.2, 28.0; IR 3429, 2979, 1749, 1702, 1588, 1130, 1054, 790; HRMS (ESI) calculated for $C_{29}H_{36}N_3O_{11}$ [(M-H)$^+$]: 602.2344 found 602.2353.

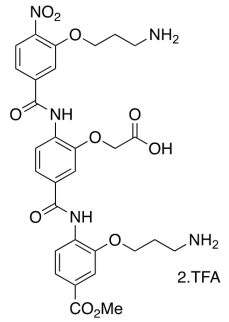
**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzamido)benzamido)-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzoate (18)**




According to *general procedure (a)*, nitro compound[1] **16** (350 mg, 0.579 mmol) was reduced to the aniline, which was coupled with 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoic acid **17** (297 mg, 0.870 mmol). The residue was purified by flash column chromatography (3:7 to 3:2 EtOAc : hexane) to give *the title compound* **18** (344 mg, 0.384 mmol, 66 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.57 (1H, brs, NH), 8.89 (1H, brs, NH),

8.58 (1H, d, *J* 8.4, Ar-H), 8.47 (1H, d, *J* 8.5, Ar-H), 7.91 (1H, d, *J* 8.4, Ar-H), 7.75 (1H, s, Ar-H), 7.48-7.72 (5H, m, Ar-H), 4.95-5.05 (1H, m, NH), 4.66 (2H, s, $OCH_2C$=O), 4.57-4.63 (1H, m, NH), 4.29 (2H, t, *J* 5.6, $OCH_2CH_2CH_2$), 4.12-4.15 (2H, m, $OCH_2CH_2CH_2$), 3.85 (3H, s, $OCH_3$), 3.28-3.41 (4H, m, $OCH_2CH_2CH_2$), 1.99-2.07 (4H, m, $OCH_2CH_2CH_2$), 1.44 (9H, s, *t*-Bu), 1.37 (9H, s, *t*-Bu), 1.32 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 168.5, 166.6, 164.1, 163.0, 156.1, 156.0, 152.2, 147.9, 147.1, 141.4, 139.5, 132.4, 132.0, 130.5, 126.0, 125.2, 123.3, 121.3, 120.0, 119.3, 119.2, 114.5, 113.6, 111.6, 83.4, 79.5, 79.1, 68.4, 68.1, 65.6, 52.1, 37.9, 37.0, 29.6, 28.9, 28.4, 28.3, 28.1; IR 3406, 2977, 1702, 1524, 1269, 1202, 764; HRMS (ESI) calculated for $C_{44}H_{58}N_5O_{15}$ [$(M+H)^+$]: 896.3923 found 896.3896.

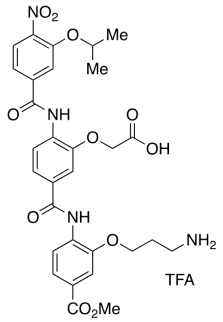
**2-(5-((2-(3-Aminopropoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(3-aminopropoxy)-4-nitrobenzamido)phenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (2)**




According to *general procedure (b)*, *t*-butyl compound **18** (344 mg, 0.384 mmol) was deprotected and purified by washing with dichloromethane to give *the title compound* **2** (201 mg, 0.232 mmol, 60 % yield) as a yellow solid: $\delta_H$ (400 MHz DMSO-$d_6$) 10.12 (1H, s, NH), 9.64 (1H, s, NH), 8.14 (1H, d, *J* 8.8, Ar-H), 8.14 (1H, d, *J* 8.8, Ar-H), 8.10 (1H, d, *J* 8.3, Ar-H), 8.03 (1H, d, *J* 8.3, Ar-H), 7.86 (1H, d, *J* 1.2, Ar-H), 7.78 (4H, brs, $NH_2$), 7.65-7.74 (3H, m, Ar-H), 7.63 (1H, d, *J* 1.5, Ar-H), 4.92 (2H, s, $OCH_2C$=O), 4.39 (2H, t, *J* 5.8, $OCH_2CH_2CH_2$), 4.25 (2H, t, *J* 6.0, $OCH_2CH_2CH_2$), 3.88 (3H, s, $OCH_3$), 2.92-3.12 (4H, m, $OCH_2CH_2CH_2$), 1.99-2.16 (4H, m, $OCH_2CH_2CH_2$); $\delta_C$ (101 MHz, DMSO-$d_6$) 171.2, 166.3, 164.7, 163.9, 158.8, 151.4, 150.1, 149.8, 141.5, 139.8, 132.1, 131.5, 131.4, 126.6, 125.7, 123.4, 123.0, 122.6, 121.8, 120.1, 118.7, 114.7, 114.3, 112.8, 67.5, 67.0, 65.9, 52.6, 36.7, 36.6, 27.2, 27.0; IR 3089, 1744, 1598, 1197, 747; HRMS (ESI) $C_{30}H_{33}N_5O_{11}$ [$(M+H)^+$]: 640.2249 found 640.2220.

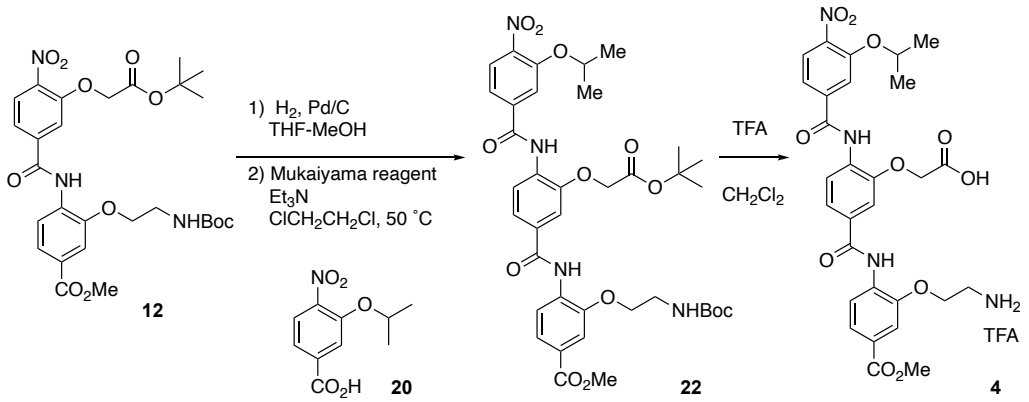
## 1.4 Targeting "E46, H50, A53"

### 2-(5-((2-(3-Aminopropoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-isopropoxy-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (3)



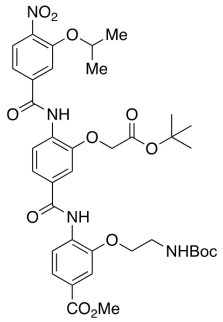
Prepared according to the procedure of Wu *et al*.[5]

### Synthetic route to 2-(5-((2-(2-aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-isopropoxy-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (4)




**Scheme S4**

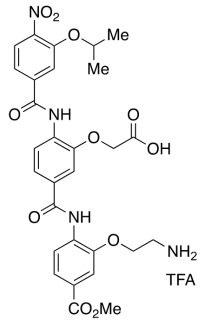
### Methyl 4-(3-(2-(tert-butoxy)-2-oxoethoxy)-4-(3-isopropoxy-4-nitrobenzamido)benzamido)-3-(2-((tert-butoxycarbonyl)amino)ethoxy)benzoate (22)



According to *general procedure (a)*, nitro compound **12** (295 mg, 0.500 mmol) was reduced to the aniline, which was then coupled with 3-isopropoxy-4-nitrobenzoic acid[5] **20** (225 mg, 1.00 mmol). The residue was purified by flash column chromatography (3:7 to 1:1 EtOAc : hexane)

to give *the title compound* **22** (204 mg, 0.266 mmol, 53 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.55 (1H, s, NH), 8.90 (1H, brs, NH), 8.58 (2H, d, *J* 8.4, Ar-H), 7.80 (1H, d, *J* 8.4, Ar-H), 7.45-7.77 (4H, m, Ar-H), 4.87 (1H, dt, *J* 12.1, 5.9, Ar-H), 4.74-4.81 (1H, m, C*H*$(CH_3)_2$), 4.71 (2H, s, OC*H*$_2$C=O), 4.10-4.17 (2H, m, OC*H*$_2$$CH_2$), 3.83-3.87 (3H, m, $OCH_3$), 3.55-3.65 (2H, m, $OCH_2$C*H*$_2$), 1.44 (9H, s, *t*-Bu), 1.31-1.39 (6H, m, CH(C*H*$_3$)$_2$), 1.24 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 168.6, 166.6, 164.5, 163.3, 156.2, 151.3, 147.7, 146.9, 142.9, 139.1, 132.5, 132.2, 130.2, 125.5, 125.0, 123.5, 122.1, 119.9, 119.0, 118.6, 115.2, 114.6, 111.2, 83.2, 79.6, 72.9, 69.0, 68.4, 52.1, 40.0, 28.2, 28.0, 21.8; IR 2977, 1747, 1400, 1004, 984, 875; HRMS (ESI) calculated for $C_{38}H_{47}N_4O_{13}$ [$(M+H)^+$]: 767.3120 found 767.3134.

**2-(5-((2-(2-Aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-isopropoxy-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (4)**

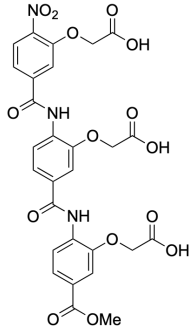



According to *general procedure (b)*, *t*-butyl compound **22** (204 mg, 0.266 mmol) was deprotected and purified by washing with dichloromethane to give *the title compound* **4** (29.2 mg, 0.0403 mmol, 15 % yield) as a yellow solid: $\delta_H$ (400 MHz DMSO-$d_6$) 13.37 (1H, brs, COOH), 10.10 (1H, s, NH), 9.65 (1H, s, NH), 8.34 (1H, d, *J* 8.4, Ar-H), 8.17 (1H, d, *J* 8.2, Ar-H), 7.95-8.09 (3H, m, Ar-H, $NH_2$), 7.87 (1H, s, Ar-H), 7.59-7.75 (5H, m, Ar-H), 4.99 (1H, sept, *J* 6.0, $CH(CH_3)_2$), 4.92 (2H, s, $OCH_2$C=O), 4.37 (2H, t, *J* 4.5, OC*H*$_2$$CH_2$), 3.89 (3H, s, $OCH_3$), 3.39 (2H, brs, $OCH_2$C*H*$_2$), 1.36 (6H, d, *J* 6.0, CH(C*H*$_3$)$_2$); $\delta_C$ (101 MHz, DMSO-$d_6$) 171.2, 166.1, 165.3, 164.1, 159.1, 158.7, 150.3, 149.6, 148.2, 142.9, 139.4, 132.3, 131.7, 131.5, 125.8, 125.4, 123.2, 122.8, 122.3, 121.7, 120.0, 115.7, 114.7, 112.3, 73.0, 67.5, 65.6, 52.6, 38.9, 22.0; IR 2359, 1717, 1519, 1345, 1204, 1109, 722; HRMS (ESI) calculated for $C_{29}H_{31}N_4O_{11}$ [$(M+H)^+$]: 611.1964 found 611.1983.

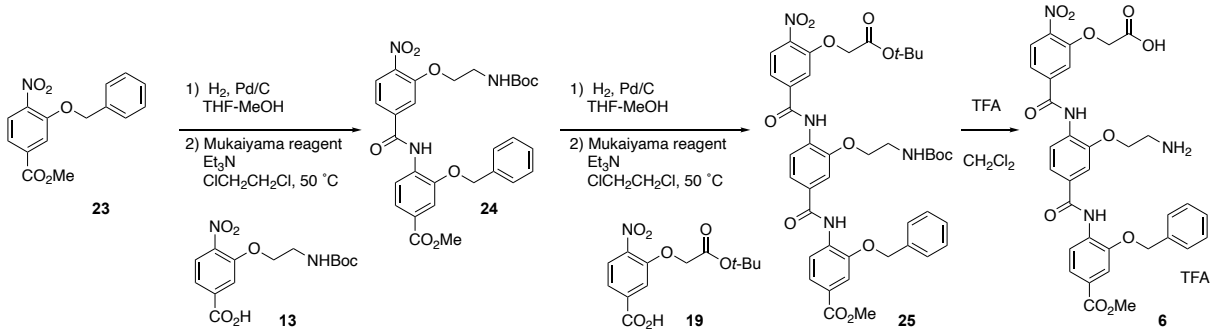
## 1.5 Negative Controls

### 2-(5-((2-(Carboxymethoxy)-4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid (5)

Prepared according to the procedure of Saraogi *et al*.[3]

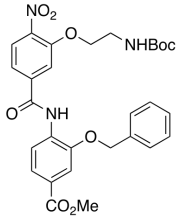


### Synthetic route to 2-(5-((2-(2-aminoethoxy)-4-((2-(benzyloxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (6)




**Scheme S5**

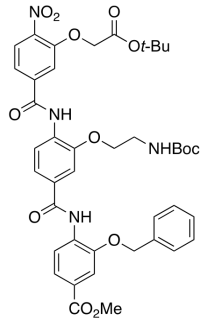
### Methyl 3-(benzyloxy)-4-(3-(2-((tert-butoxycarbonyl)amino)ethoxy)-4-nitrobenzamido)benzoate (24)



According to *general procedure (a )*, methyl 3-(benzyloxy)-4-nitrobenzoate[6] **23** (1.00 g, 3.48 mmol) was reduced to the aniline, which was coupled with 3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzoic acid[1] **13** (1.63 g, 4.99 mmol). The residue was purified by flash column chromatography (1:4 to 1:1 EtOAc : hexane) to give *the title compound* **24** (1.07 g, 1.89 mmol, 57 % yield) as a yellow solid; $\delta_H$ (400 MHz, $CDCl_3$) 8.68 (1H, s, NH), 8.53 (1H, d, *J* 8.5, Ar-H), 7.81 (1H, d, *J* 8.4, Ar-H), 7.73 (1H, d, *J* 9.6, Ar-H), 7.67 (1H, s,

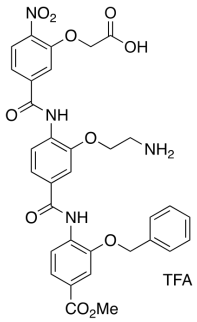
Ar-H), 7.44 (1H, s, Ar-H), 7.35-7.41 (5H, m, $OC_6H_5$), 7.28 (1H, d, *J* 8.5, Ar-H), 5.14 (2H, s, $OCH_2Ph$), 5.01 (1H, brs, NHBoc), 4.00 (2H, t, *J* 4.9, $OCH_2CH_2$), 3.87 (3H, s, $OCH_3$), 3.51 (2H, q, *J* 5.2, $OCH_2CH_2$), 1.38 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 166.4, 162.6, 155.8, 152.1, 147.0, 141.5, 139.7, 135.7, 131.6, 128.9, 128.9, 128.0, 126.0, 126.0, 123.8, 118.9, 118.5, 113.7, 112.3, 79.7, 71.6, 69.3, 52.2, 39.6, 28.3; IR 3437, 3413, 1710, 1678, 1060, 765; HRMS (ESI) calculated for $C_{29}H_{30}N_3O_9$ [$(M-H)^+$]: 564.1976 found 564.1987.

**Methyl 3-(benzyloxy)-4-(4-(3-(2-(tert-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(2-((tert-butoxycarbonyl)amino)ethoxy)benzamido)benzoate (25)**



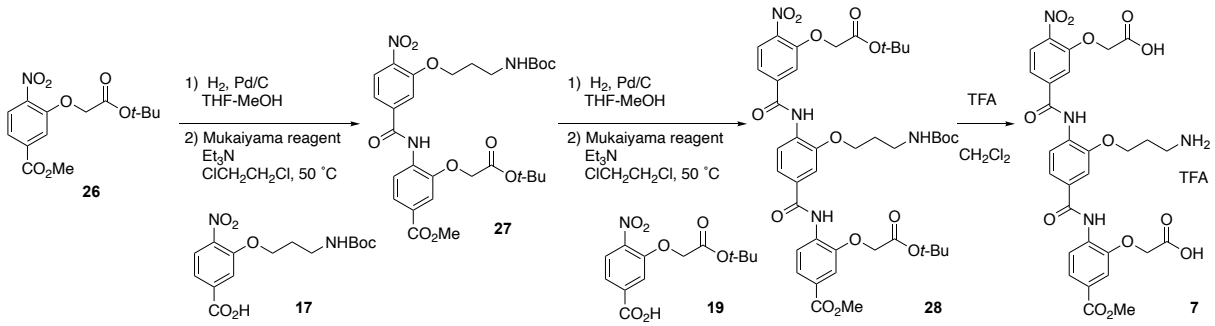

According to *general procedure (a')*, nitro compound **24** (537 mg, 1.00 mmol) was reduced to the aniline, which was coupled with 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoic acid[2][3] **19** (446 mg, 1.50 mmol). The residue was purified by flash column chromatography (1:4 to 3:2 EtOAc : hexane) to give *the title compound* **25** (450 mg, 0.552 mmol, 55 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.13 (1H, s, NH), 8.77 (1H, s, NH), 8.52-8.60 (2H, m, Ar-H), 7.88 (1H, d, *J* 8.4, Ar-H), 7.69-7.78 (2H, m, Ar-H), 7.64 (2H, d, *J* 8.4, Ar-H), 7.31-7.46 (6H, m, Ar-H), 7.26 (1H, d, *J* 8.4, Ar-H), 5.16 (2H, s, $OCH_2Ph$), 4.73-4.82 (3H, m, $OCH_2C{=}O$, NHBoc), 3.98-4.00 (2H, m, $OCH_2CH_2$), 3.86 (3H, s, $OCH_3$), 3.52-3.62 (2H, m, $OCH_2CH_2$), 1.41 (9H, s, *t*-Bu), 1.13 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 166.6, 166.5, 164.2, 163.7, 156.5, 151.1, 147.8, 146.8, 142.0, 139.0, 135.9, 132.3, 131.2, 130.0, 128.9, 128.7, 127.8, 125.7, 125.1, 123.9, 120.4, 119.4, 119.4, 118.6, 115.02, 112.3, 109.6, 83.1, 79.7, 71.4, 69.5, 66.7, 52.1, 40.0, 28.0, 28.0; IR 3426, 2982, 1716, 1603, 1009, 925, 750; HRMS (ESI) calculated for $C_{42}H_{46}N_4NaO_{13}$ [$(M+Na)^+$]: 837.2953 found 837.2936.

**2-(5-((2-(2-Aminoethoxy)-4-((2-(benzyloxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (6)**



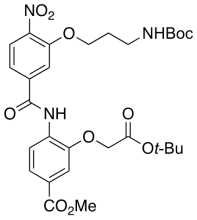
According to *general procedure (b)*, *t*-butyl compound **25** (450 mg, 0.553 mmol) was deprotected and purified by washing with dichloromethane to give *the title compound* **6** (288 mg, 0.373 mmol, 67 % yield) as a yellow solid: $\delta_H$ (400 MHz DMSO-$d_6$) 13.30 (1H, brs, COOH), 9.73 (1H, s, NH), 9.62 (1H, s, NH), 8.18 (1H, d, *J* 8.2, Ar-H), 7.88-8.02 (5H, m, Ar-H, $NH_2$), 7.70 (1H, s, Ar-H), 7.57-7.66 (5H, m, Ar-H), 7.50 (2H, d, *J* 7.3, Ar-H), 7.21-7.38 (2H, m, Ar-H), 5.24 (2H, s, $OCH_2Ph$), 4.97 (2H, s, $OCH_2C{=}O$), 4.19-4.30 (2H, m, $OCH_2CH_2$), 3.79 (3H, s, $OCH_3$), 3.18-3.44 (2H, m, $OCH_2CH_2$); $\delta_C$ (101 MHz, DMSO-$d_6$) 169.7, 166.2, 164.6, 159.2, 158.9, 150.6, 150.1, 148.7, 142.0, 139.7, 137.1, 132.4, 131.1, 130.6, 128.9, 128.4, 127.8, 126.6, 125.4, 123.4, 122.8, 122.3, 121.1, 121.0, 118.9, 115.2, 113.5, 111.4, 70.6, 66.2, 65.5, 52.6, 38.8; IR 2950, 1741, 1437, 1112, 1052, 745; HRMS (ESI) $C_{33}H_{31}N_4O_{11}$ [$(M+H)^+$]: 659.1983 found 659.1964.

**Synthetic route to 2-(5-((2-(3-aminopropoxy)-4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (7)**



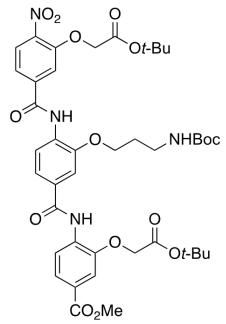

**Scheme S6**

**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzamido)benzoate (27)**



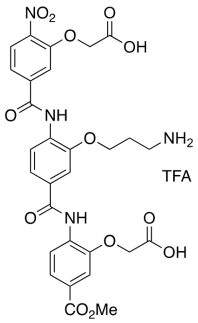
According to *general procedure (a)*, methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoate **26**[2][3] (1.00 g, 3.21 mmol) was reduced to the aniline, which was coupled with 3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzoic acid[7] **17** (1.64 g, 4.82 mmol). The residue was purified by flash column chromatography (1:4 to 3:2 EtOAc : hexane) to give *the title compound* **27** (1.00 g, 1.66 mmol, 52 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.63 (1H, s, NH), 8.59 (1H, d, *J* 8.5, Ar-H), 7.98 (1H, d, *J* 8.4, Ar-H), 7.79-7.87 (2H, m, Ar-H), 7.73 (1H, dd, *J* 8.4, 1.7, Ar-H), 7.63 (1H, d, *J* 1.7, Ar-H), 4.95-5.14 (1H, m, NHBoc), 4.69 (2H, s $OCH_2C$=O), 4.35 (2H, t, *J* 5.7, $OCH_2CH_2CH_2$), 3.93 (3H, s, $OCH_3$), 3.39 (2H, q, *J* 6.2, $OCH_2CH_2CH_2$), 1.99-2.18 (2H, m, $OCH_2CH_2CH_2$), 1.51 (9H, s, *t*-Bu), 1.45 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 168.3, 166.1, 163.0, 156.1, 152.2, 147.3, 141.3, 139.5, 133.2, 126.0, 125.9, 125.1, 119.8, 119.1, 115.5, 113.7, 83.4, 79.1, 68.4, 68.1, 52.2, 37.9, 29.0, 28.3, 28.0; IR 3372, 2980, 1758, 1711, 1479, 1227, 1102, 987, 844; HRMS (ESI) calculated for $C_{29}H_{36}N_3O_{11}$ [$(M-H)^+$]: 602.2344 found 602.2354.

**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzamido)benzoate (28)**



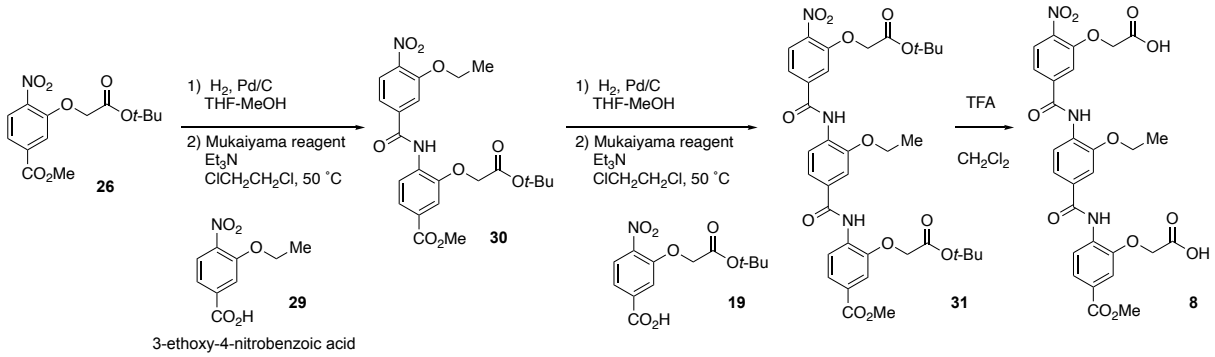

According to *general procedure (a)*, nitro compound **27** (850 mg, 1.40 mmol) was reduced to the aniline, which was coupled with 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoic acid **19**[2][3] (625 mg, 2.10 mmol). The residue was purified by flash column chromatography (1:4 to 3:2 EtOAc : hexane) to give *the title compound* **28** (357 mg, 0.419 mmol, 30 % yield) as a yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.35 (1H, s), 9.10 (1H, s, NH), 8.56 (1H, d, *J* 8.4, NH), 8.47 (1H, d, *J* 8.2, Ar-H), 7.96 (1H, d, *J* 8.4, Ar-H), 7.57-7.79 (6H, m, Ar-H), 7.51 (1H, d, *J* 1.5, Ar-H), 4.71 (1H, brs, NHBoc), 4.60 (2H, s, $OCH_2C$=O), 4.21 (2H, t, *J* 5.5, $OCH_2CH_2CH_2$), 3.85 (3H, s, $OCH_3$), 3.35-3.40 (2H, m, $OCH_2CH_2CH_2$), 1.94-2.04 (2H, m, $OCH_2CH_2CH_2$), 1.45 (9H, s, *t*-Bu), 1.42 (9H, s, *t*-Bu), 1.29 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 167.9, 167.8, 166.3, 164.4, 163.0, 156.1, 151.4, 147.9, 146.8, 141.8, 139.5, 133.5, 130.8, 130.2, 126.3, 125.1, 124.9, 120.3, 120.1, 119.5, 119.3, 114.7, 114.4, 110.4, 83.2, 83.1, 79.6, 67.7, 66.6, 65.2, 52.1, 36.9, 29.5, 28.2, 28.1, 28.0; IR 1709, 1523, 1266, 1156, 844; HRMS (ESI) calculated for $C_{42}H_{52}N_4NaO_{15}$ [$(M+Na)^+$]: 875.3321 found 875.3304.

**2-(5-((2-(3-Aminopropoxy)-4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (7)**



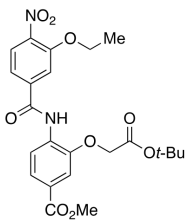
According to *general procedure (b)*, *t*-butyl compound **28** (357 mg, 0.419 mmol) was deprotected and purified by washing with dichloromethane to give *the title compound* **7** (270 mg, 0.358 mmol, 85 % yield) as a yellow solid; $\delta_H$ (400 MHz, DMSO-$d_6$) 9.94 (1H, s, NH), 9.83 (1H, s, NH), 8.27 (1H, d, *J* 8.4, Ar-H), 8.05 (1H, d, *J* 8.2, Ar-H), 7.94 (1H, d, *J* 8.7, Ar-H), 7.81 (1H, d, *J* 8.7, Ar-H), 7.69-7.79 (6H, m, Ar-H), 7.64 (1H, d, *J* 1.7, Ar-H), 5.05 (2H, s, $OCH_2C{=}O$), 4.93 (2H, s, $OCH_2C{=}O$), 4.28 (2H, t, *J* 5.8, $OCH_2CH_2CH_2$), 3.87 (3H, s, $OCH_3$), 2.97-3.10 (2H, m, $OCH_2CH_2CH_2$), 2.00-2.15 (2H, m, $OCH_2CH_2CH_2$); $\delta_C$ (101 MHz, DMSO-$d_6$) 171.2, 169.7, 166.1, 164.7, 164.0, 159.0, 158.7, 151.0, 150.7, 148.8, 141.9, 139.3, 133.4, 132.0, 130.4, 125.9, 125.5, 124.5, 123.9, 121.6, 120.8, 120.2, 115.2, 114.8, 111.9, 67.6, 66.1, 65.9, 52.6, 36.8, 27.1; IR 3104, 2361, 1678, 1521, 1419, 1197, 842; HRMS (ESI) calculated for $C_{29}H_{29}N_4O_{13}$ [$(M+H)^+$]: 641.1725 found 641.1705.

**Synthetic route to 2-(5-((4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-ethoxyphenyl)carbamoyl)-2-nitrophenoxy)acetic acid (8)**




**Scheme S7**

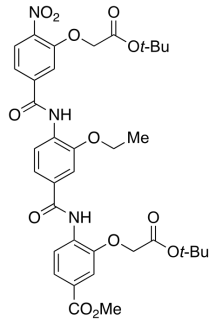
**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-ethoxy-4-nitrobenzamido)benzoate (30)**



According to *general procedure (a)*, methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoate[3,4] **26** (601 mg, 1.93 mmol) was reduced to the aniline which was then coupled

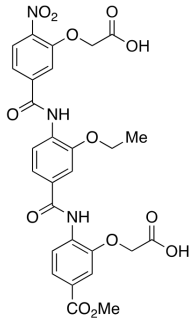
with 3-ethoxy-4-nitrobenzoic acid[8] **29** (493 mg, 2.33 mmol) at 70 ˚C. The residue was purified by flash column chromatography (1 % $Et_2O$ : DCM) to give *the title compound* **30** (774 mg, 1.63 mmol, 84 % yield over two steps) as an orange solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.57 (1H, s, NH), 8.56 (1H, d, *J* 8.6, Ar-H), 7.88 (1H, d, *J* 8.3, Ar-H), 7.80 (1H, dd, *J* 8.5, 1.7, Ar-H), 7.78 (1H, d, *J* 1.7, Ar-H), 7.68 (1H, dd, *J* 8.4, 1.7, Ar-H), 7.60 (1H, d, *J* 1.7, Ar-H), 4.67 (2H, s, $OCH_2C$=O), 4.33 (2H, q, *J* 7.0, $OCH_2CH_3$), 3.90 (3H, s, $OCH_3$), 1.50 (9H, s, *t*-Bu) 1.49 (3H, t, *J* 7.0, $OCH_2CH_3$); $\delta_C$ (101 MHz, $CDCl_3$) 168.5, 166.3, 163.4, 152.4, 147.4, 142.0, 139.4, 133.4, 126.1, 125.8, 125.3, 119.9, 118.9, 115.7, 113.9, 83.5, 68.6, 65.9, 52.3, 28.2, 14.6; IR 3400, 2986, 1744, 1709, 1600, 1523, 1503, 1350, 1228**;** HRMS (ESI) calculated for $C_{23}H_{26}N_2O_9$ [(M+Na)$^+$]: 497.1531 found 497.1533.

**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-ethoxybenzamido)benzoate (31)**



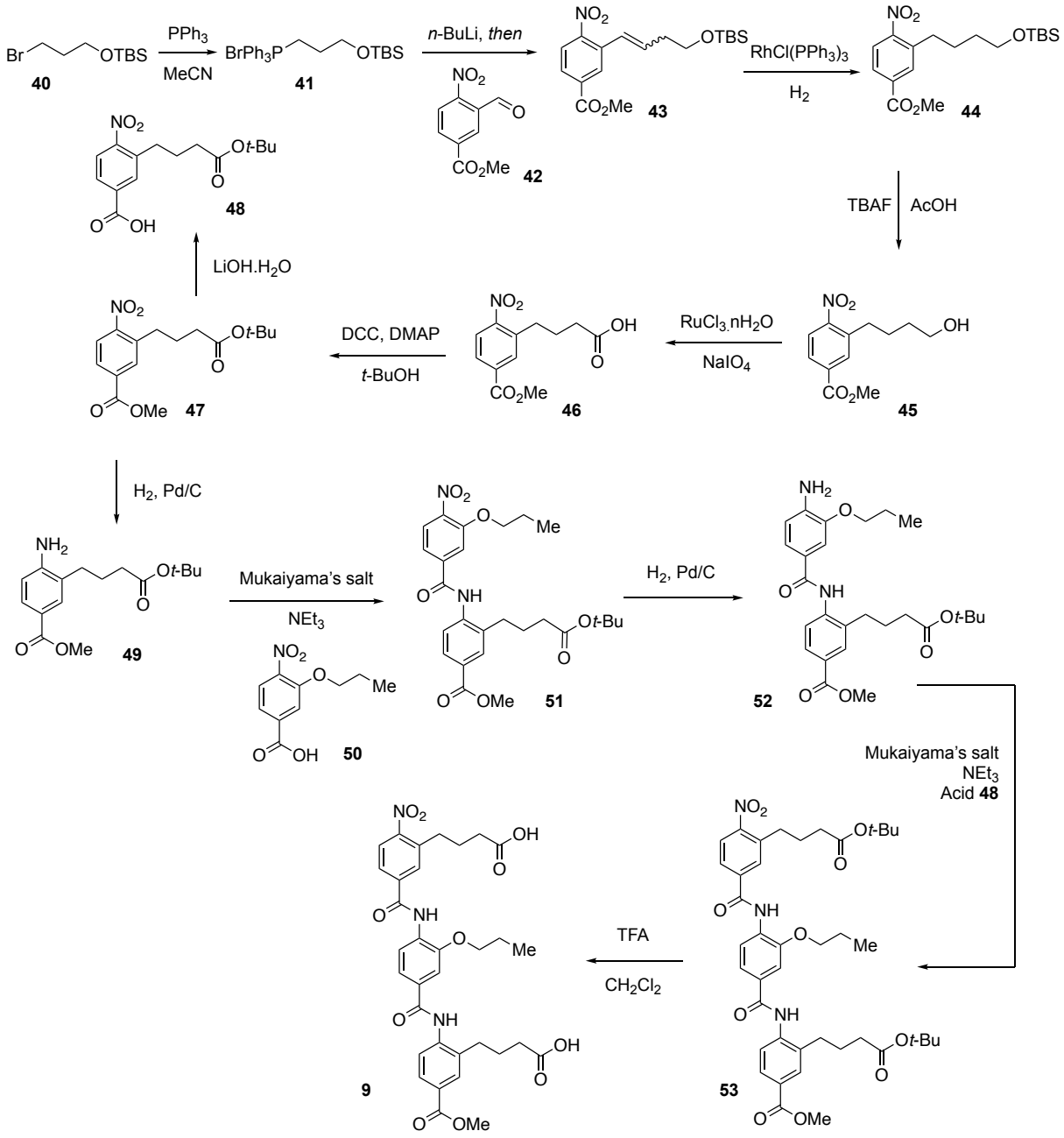

According to *general procedure (a)*, methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-ethoxy-4-nitrobenzamido)benzoate **30** (716 mg, 1.51 mmol) was reduced to the aniline which was then coupled with 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzoic acid[2][3] **19** (139 mg, 0.468 mmol). The residue was purified by flash column chromatography (5 % $Et_2O$ : DCM) to give *the title compound* **31** (160 mg, 0.221 mmol, 23 % yield) as a pale solid: $\delta_H$ (500 MHz, $CDCl_3$) 9.39 (1H, s, NH), 8.74 (1H, s, NH), 8.61 (1H, d, *J* 3.8, Ar-H), 8.60 (1H, d, *J* 3.8, Ar-H), 7.95 (1H, d, *J* 8.4, Ar-H), 7.78 (1H, dd, *J* 8.6, 1.8, Ar-H), 7.70 (1H, dd, *J* 8.4, 1.9, Ar-H), 7.64 (1H, d, *J* 1.9, Ar-H), 7.61 (1H, d, *J* 1.6, Ar-H), 7.55 (1H, d, *J* 1.8, Ar-H), 7.44 (1H, dd, *J* 8.5, 1.7, Ar-H), 4.76 (2H, s, $OCH_2C$=O), 4.67 (2H, s, $OCH_2C$=O), 4.31 (2H, q, *J* 7.0, $OCH_2CH_3$), 3.89 (3H, s, $OCH_3$), 1.53 (3H, t, *J* 7.0, $OCH_2CH_3$), 1.51 (9H, s, *t*-Bu), 1.48 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 168.1, 166.5, 166.3, 164.6, 162.8, 151.8, 147.6, 146.9, 142.1, 139.8, 133.7, 130.7, 130.3, 126.3, 125.3, 125.0, 120.4, 119.5, 119.2, 118.6, 114.9, 114.6, 110.3, 83.5, 83.2, 67.9, 66.7, 64.9, 52.2, 28.2, 28.1, 14.9; IR 3428, 2987, 2938, 1747, 1710, 1674, 1596, 1522, 1486, 1344, 1239, 1155; HRMS (ESI) calculated for $C_{36}H_{41}N_3O_{13}$ [(M+Na)$^+$]: 746.2519 found 746.2532.

**2-(5-((4-((2-(Carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-ethoxyphenyl)carbamoyl)-2-nitrophenoxy)acetic acid (8)**



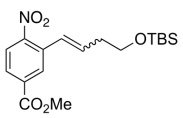
According to *general procedure (b)*, *t*-butyl compound **31** (120 mg, 0.166 mmol) was deprotected to give *the title compound* **8** (122 mg, 0.200 mmol, quant.) as a yellow solid: $\delta_H$ (400 MHz, DMSO-$d_6$) 9.80 (1H, s, NH), 9.76 (1H, s, NH), 8.29 (1H, d, *J* 8.5, Ar-H), 8.04 (1H, d, *J* 8.3, Ar-H), 8.00 (1H, d, *J* 8.8, Ar-H), 7.78 (1H, d, *J* 1.3, Ar-H), 7.65 – 7.71 (4H, m, Ar-H), 7.62 (1H, d, *J* 1.7, Ar-H), 5.05 (2H, s, $OCH_2C{=}O$), 4.91 (2H, s, $OCH_2C{=}O$), 4.24 (2H, q, *J* 6.8, $OCH_2CH_3$), 3.85 (3H, s, $OCH_3$), 1.41 (3H, t, *J* 7.0, $OCH_2C\mathit{H_3}$); $\delta_C$ (101 MHz, DMSO-$d_6$) 170.8, 169.2, 165.7, 164.3, 163.5, 150.4, 150.2, 148.3, 141.5, 139.0, 133.0, 131.3, 130.2, 125.4, 125.2, 123.6, 123.5, 121.0, 120.4, 119.7, 114.8, 114.3, 111.3, 67.2, 65.6, 64.4, 52.2, 14.5; IR 3416, 2981, 1717, 1672, 1600, 1522, 1485, 1349, 1250, 1199, 1130; HRMS (ESI) calculated for $C_{28}H_{25}N_3O_{13}$ $[(M+H)^+]$: 612.1460 found 612.1462.

## Synthetic route to 4-(5-((4-((2-(3-carboxypropyl)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-propoxyphenyl)carbamoyl)-2-nitrophenyl)butanoic acid (9)



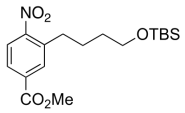

**Scheme S8**

## Methyl 3-(4-((*tert*-butyldimethylsilyl)oxy)but-1-en-1-yl)-4-nitrobenzoate (43)



Based on an adapted literature procedure,[9] *n*-butyllithium (2.5 M in hexanes, 2.42 mL, 6.05 mmol) was added dropwise to a stirred -78 °C suspension of (3-((*tert*-butyldimethylsilyl)oxy)propyl)triphenylphosphonium bromide **41** (3.11 g, 6.05 mmol) in THF (25 mL). After 40 min, the temperature was raised to -25 °C. After a further 40 min the temperature was reduced to -78 °C and a solution of methyl 3-formyl-4-nitrobenzoate **42** (1.15 g, 5.50 mmol) in THF (10 mL) was added dropwise. After 30 min the reaction mixture was warmed to 0 °C. After a further 30 min the reaction mixture was diluted with hexanes (~50 mL) and filtered over a plug of silica. The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography ($SiO_2$, 20:1 hexanes:EtOAc) to give *the title compound* **43** (1.46 g, 73% yield) as a viscous yellow oil, as an 5:2 mixture of *Z*:*E* isomers: $\delta_H$ (400 MHz, $CDCl_3$) **(*Z*)-isomer** – 8.10 (1H, d, *J* 1.7, Ar-H), 8.06 (1H, dd, *J* 8.6, 1.5, Ar-H), 8.00 (1H, d, *J* 8.3, Ar-H), 6.76 (1H, d, *J* 11.7, ArC*H*CH), 5.95 (1H, dt, *J* 11.5, 7.6, ArCHC*H*), 3.97 (3H, s, $OCH_3$), 3.68 (2H, t, *J* 6.4, $CH_2$), 2.33 (2H, app. qd, *J* 6.8, 1.7, $CH_2$), 0.88 (9H, s, *t*-BuSi), 0.04 (3H, s, $Si(CH_3)_2$); **(*E*)-isomer**[1] - 8.28 (1H, d, *J* 1.7, Ar-H), 7.98 (1H, dd, *J* 8.1, 2.2, Ar-H), 7.89 (1H, d, *J* 8.6, Ar-H), 6.86 (1H, d, *J* 15.7, ArC*H*CH), 6.40 (1H, dt, *J* 15.7, 7.1, ArCHC*H*), 3.97 (3H, s, $OCH_3$), 3.78 (2H, t, *J* 6.5, $CH_2$), 2.51 (2H, qd, *J* 6.7, 1.5, $CH_2$), 0.91 (9H, s, *t*-BuSi), 0.08 (3H, s, $Si(CH_3)_2$); $\delta_C$ ($CDCl_3$, 101 MHz)[2] 165.3, 150.9, 134.9, 133.7, 133.6, 133.1, 133.07, 132.7, 132.6, 129.8, 128.9, 128.4, 125.5, 125.4, 124.43, 124.40, 62.4, 62.3, 52.8, 36.7, 32.1, 25.9, 18.4, -5.3, -5.4; **LRMS** (ESI) calculated for $C_{18}H_{27}NO_5Si$ [$(M+H)^+$]: 366.2, found 366.1.

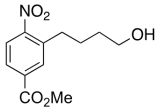
**Methyl 3-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-4-nitrobenzoate (44)**




Based on an adapted literature procedure,[10] alkene **43** (1.20 g, 3.29 mmol) was dissolved in 1:1 THF:*t*-BuOH (*v:v*, 12 mL), and the mixture was de-gassed three times by placing under vacuum until bubbles formed and back-filling with hydrogen gas (balloon pressure). Wilkinson's catalyst (122 mg, 0.11 mmol) was added, and the reaction mixture was degassed a further three times. The reaction mixture was stirred vigorously for 16 h, after which the reaction was concentrated *in vacuo* and the crude residue was purified by flash column chromatography ($SiO_2$, 20:1 pet. ether:ethyl acetate) to give the *title compound* **44** (1.17 g, 97% yield) as a pale yellow oil: $\delta_H$ (400 MHz, $CDCl_3$) 8.04 (1H, d, *J* 1.7, Ar-H), 7.98 (1H, dd, *J* 8.4, 1.8, Ar-H), 7.86 (1H, d, *J* 8.6, Ar-H), 3.96 (3H, s, $OCH_3$), 3.64 (2H, t, *J* 6.3, $OCH_2$), 2.91 (2H, t, *J* 7.8, $CH_2$), 1.58 – 1.75 (4H, m, 2 x $CH_2$), 0.88 (9H, s, *t*-BuSi), 0.04 (6H, s, $Si(CH_3)_2$); LRMS (ESI) calculated for $C_{18}H_{29}NO_5Si$ [$(M+H)^+$]: 368.2, found 368.2.

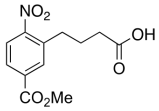
[1] Integrations scaled to integer values for minor isomer.
[2] Peaks given for both *E* and *Z* isomers.

**Methyl 3-(4-hydroxybutyl)-4-nitrobenzoate (45)**



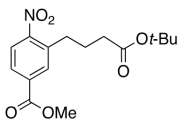

TBS protected alcohol **44** (1.05 g, 2.86 mmol) was dissolved in dichloromethane (20 mL) and TBAF (3.7 mL of 1 M in THF, 3.72 mmol) and AcOH (215 μL, 3.72 mmol) were added. After 1 h the reaction mixture was diluted with dichloromethane (50 mL), partitioned with ammonium chloride (50 mL) and the layers separated. The aqueous layer was washed sequentially with dichloromethane (2 x 50 mL) and the combined organic layers were dried (sodium sulfate) and concentrated *in vacuo*. The resultant residue was purified by flash column chromatography (2:1 → 1:1 pet. ether:ethyl acetate) to afford *the title compound* **45** (359 mg, 50% yield ) as a yellow oil: $\delta_H$ (400 MHz, $CDCl_3$) 8.04 (1H, d, *J* 1.7, Ar-H), 7.98 (1H, dd, *J* 8.6, 1.7, Ar-H), 7.88 (1H, d, *J* 8.6, Ar-H), 3.96 (3H, s,$OCH_3$), 3.69 (2H, t, *J* 6.3, HOC*H*$_2$), 2.92 (2H, t, *J* 7.8, $CH_2$), 1.63 – 1.80 (4H, m, 2 x $CH_2$); LRMS (ESI) calculated for $C_{12}H_{15}NO_5$ [$(M+Na)^+$]: 276.1, found 276.0.

**4-(5-(Methoxycarbonyl)-2-nitrophenyl)butanoic acid (46)**



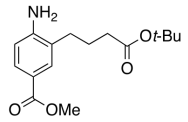

$RuCl_3$ (16 mg, 0.072 mmol) and $NaIO_4$ (1.23 g, 5.76 mmol) were added to a stirred solution of alcohol **45** (359 mg, 1.44 mmol) in 1:1 MeCN:$CCl_4$ (*v:v*, 16 mL) and water (10 mL) was added. The resulting biphasic mixture was stirred vigorously at room temperature for 16 h. The reaction mixture was diluted. The organic volatiles were removed *in vacuo* and $CH_2Cl_2$ (25 mL) and $NH_4Cl$ (sat. aq., 25 mL) were added. The layers were separated, and the aqueous phase was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by flash column chromatography ($SiO_2$, 20:1 DCM:MeOH) to give the *title compound* **46** (254 mg, 66% yield) as a pale yellow oil: $\delta_H$ (400 MHz, $CDCl_3$) 8.05 (1H, d, *J* 1.4, Ar-H), 8.00 (1H, dd, *J* 8.5, 1.6, Ar-H), 7.91 (1H, d, *J* 8.4, Ar-H), 3.96 (3H, s, $OCH_3$), 2.95 (2H, t, *J* 7.8, $CH_2$), 2.47 (2H, t, *J* 7.3, $CH_2$), 2.03 (2H, pent, *J* 7.8, $CH_2$); LRMS (ESI) calculated for $C_{12}H_{13}NO_6$ [$(M-H)^+$]: 266.1, but not labelled on spectrum.

**Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-nitrobenzoate (47)**



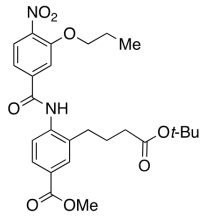
DMAP (12 mg, 0.095 mmol) and *tert*-butanol (0.45 mL, 4.8 mmol) were added to a solution of carboxylic acid **46** (254 mg, 0.95 mmol) in $CH_2Cl_2$ (1 mL) and the resulting mixture was cooled to 0 ˚C. DCC (215 mg, 1.05 mmol) was added in one portion, and after 5 min the mixture was allowed to warm to RT. After 3 h the reaction mixture was diluted with $CH_2Cl_2$ (10 mL), filtered and the filtrate was concentrated *in vacuo*. The crude residue was purified by flash column chromatography ($SiO_2$, 10:1 pet. ether:ethyl acetate) to give *the title compound* **47** (296 mg, 96 % yield) as a pale yellow oil: $\delta_H$ (400 MHz, $CDCl_3$) 8.00 (1H, d, *J* 1.8, Ar-H), 7.99 (1H, dd, *J* 8.4, 1.7, Ar-H), 7.89 (1H, d, *J* 8.5, Ar-H), 3.95 (3H, s, $OCH_3$), 2.92 (2H, t, *J* 7.8, $CH_2$), 2.30 (2H, t, *J* 7.3, $CH_2$), 1.96 (2H, pent, *J* 7.8, $CH_2$), 1.45 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 172.2, 165.3, 152.0, 136.6, 133.7, 133.2, 128.3, 124.6, 80.5, 52.7, 34.9, 31.8, 28.1, 25.9; LRMS (ESI) calculated for $C_{16}H_{21}NO_6$ [$(M+Na)^+$]: 346.1, found 346.1.

**Methyl 4-amino-3-(4-(*tert*-butoxy)-4-oxobutyl)benzoate (49)**




10% Pd/C (10 mg) was added to a solution of nitroarene **47** (101 mg, 0.309 mmol) in 1:1 THF:MeOH (*v*:*v*, 3 mL). The reaction mixture was de-gassed three times by placing under vacuum until bubbles formed and back-filling with $H_2$ gas (balloon pressure). After 1 h the reaction mixture was filtered over Celite® and concentrated *in vacuo* to give *the title compound* **49** (90 mg, 99 % yield) as a colourless oil: $\delta_H$ (400 MHz, $CDCl_3$) 7.71 – 7.73 (2H, m, Ar-H), 6.62 (1H, dd, *J* 7.3, 1.4, Ar-H), 4.47 (2H, s, $NH_2$), 3.84 (3H, s, $OCH_3$), 2.52 (2H, t, *J* 8.2, $CH_2$), 2.32 (2H, t, *J* 6.5, $CH_2$), 1.79 – 1.86 (2H, m, $CH_2$), 1.47 (9H, s, *t*-Bu); $\delta_C$ (101 MHz, $CDCl_3$) 173.6, 167.5, 149.4, 131.8, 129.7, 124.6, 119.3, 114.3, 80.8, 51.7, 34.4, 31.0, 28.3, 23.8; LRMS (ESI) calculated for $C_{16}H_{23}NO_4$ [$(M+H)^+$]: 294.2, found 294.1.

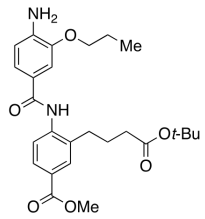
**Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-(4-nitro-3-propoxybenzamido)benzoate (51)**




Mukaiyama's reagent (81 mg, 0.32 mmol) was added to a stirred, RT solution of 4-nitro-3-propoxybenzoic acid **50**[6] (72 mg, 0.32 mmol) and triethylamine (89 µL, 0.64 mmol) in 1,2-dichloroethane (0.5 mL) and the reaction mixture was heated to 50 °C. After 15 min the solution was allowed to cool to RT and a solution of aniline **49** (78 mg, 0.27 mmol) in 1,2-dichloroethane (0.5 mL) was added. The reaction mixture was heated to 50 °C. After 3 h the reaction mixture was cooled to RT and the solvent was removed *in vacuo*. The crude residue

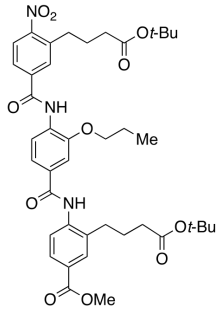
was purified by flash column chromatography ($SiO_2$, 10:1 pet. ether:ethyl acetate) to give the *title compound* **51** (119 mg, 89% yield) as a glassy yellow solid: $\delta_H$ (400 MHz, $CDCl_3$) 9.28 (1H, s, NH), 8.24 (1H, d, *J* 8.6, Ar-H), 7.96 (1H, dd, *J* 8.6, 2.1, Ar-H), 7.89 (1H, d, *J* 2.0, Ar-H), 7.84 (1H, d, *J* 8.3, Ar-H), 7.66 (1H, d, *J* 1.5, Ar-H), 7.62 (1H, dd, *J* 8.3, 1.7, Ar-H), 4.14 (2H, t, *J* 6.4, $OCH_2$), 3.91 (3H, s, $OCH_3$), 2.68 (2H, t, *J* 8.2, $CH_2$), 2.30 (2H, t, *J* 5.7, $CH_2$), 1.86 (2H, sext, *J* 6.5, $CH_2$), 1.76 – 1.83 (2H, m, $CH_2$), 1.30 (9H, s, *t*-Bu), 1.06 (3H, t, *J* 7.5, $OCH_2CH_2CH_3$); $\delta_C$ (101 MHz, $CDCl_3$) 174.4, 166.8, 165.7, 152.3, 141.7, 140.3, 140.1, 132.0, 131.4, 128.7, 126.5, 125.3, 123.0, 119.3, 114.8, 81.7, 71.4, 52.2, 33.3, 30.7, 27.9, 25.7, 22.4; LRMS (ESI) calculated for $C_{26}H_{32}N_2O_8$ [$(M+Na)^+$]: 523.2, found 523.2.

**Methyl 4-(4-amino-3-propoxybenzamido)-3-(4-(*tert*-butoxy)-4-oxobutyl)benzoate (52)**




10% Pd/C (10 mg) was added to a solution of nitroarene **51** (97 mg, 0.19 mmol) in 1:1 THF:MeOH (*v*:*v*, 2 mL). The reaction mixture was de-gassed three times by placing under vacuum until bubbles formed and back-filling with $H_2$ gas (balloon pressure). After 1 h the reaction mixture was filtered over Celite® and concentrated *in vacuo*. The crude residue was purified by flash column chromatography ($SiO_2$, 7:3 pet. ether:ethyl acetate) to give *the title compound* **52** (89 mg, 98% yield) as an off-white solid: $\delta_H$ (400 MHz, $CDCl_3$) 8.51 (1H, s, NH), 8.29 (1H, d, *J* 8.6, Ar-H), 7.93 (1H, dd, *J* 8.6, 2.2, Ar-H), 8.88 (1H, d, *J* 1.9, Ar-H), 7.49 – 7.72 (2H, m, Ar-H), 6.71 (1H, dd, *J* 7.2, 1.2, Ar-H), 4.20 (2H, s, $NH_2$), 4.06 (2H, t, *J* 6.7, $OCH_2$), 3.90 (3H, s, $OCH_3$), 2.73 (2H, t, *J* 8.0, $CH_2$), 2.30 (2H, t, *J* 6.3, $CH_2$), 1.81 – 1.91 (4H, m, $CH_2$), 1.40 (9H, s, *t*-Bu), 1.06 (3H, t, *J* 7.3, $OCH_2CH_2CH_3$); $\delta_C$ (400 MHz, $CDCl_3$) quaternary Ar in the noise. 131.3, 128.8, 122.4, 120.9, 113.2, 111.5, 70.1, 52.1, 33.9, 30.7, 28.2, 25.4, 22.7, 10.7; LRMS (ESI) calculated for $C_{26}H_{34}N_2O_6$ [$(M+H)^+$]: 471.3, found 471.3.

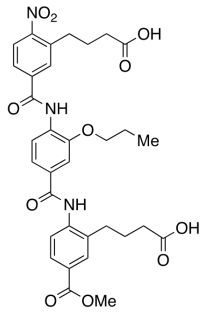
**Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-(4-(3-(4-(*tert*-butoxy)-4-oxobutyl)-4-nitrobenzamido)-3-propoxybenzamido)benzoate (53)**



Lithium hydroxide monohydrate (16 mg, 0.39 mmol) was added to a stirred RT solution of diester **47** (100 mg, 0.31 mmol) in 3:1 methanol:water (*v:v*, 1 mL). After 30 min the reaction mixture was diluted with $CH_2Cl_2$ (10 mL) and $NH_4Cl$ (sat. aq., 10 mL) was added. The layers were separated, and the aqueous phase was further extracted with $CH_2Cl_2$ (2 x 5 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. The carboxylic acid 3-(4-(*tert*-Butoxy)-4-oxobutyl)-4-nitrobenzoic acid **48** (92 mg, 96%) was obtained as a white foam of sufficient purity to be used in the next step without further purification.

Mukaiyama's reagent (49 mg, 0.19 mmol) was added to a stirred, RT solution of the acid **48** (60 mg, 0.19 mmol) and triethylamine (54 µL, 0.39 mmol) in 1,2-dichloroethane (0.5 mL) and the reaction mixture was heated to 50 °C. After 15 min the solution was allowed to cool to RT and a solution of aniline **52** (57 mg, 0.12 mmol) in 1,2-dichloroethane (0.5 mL) was added. The reaction mixture was heated to 50 °C. After 16 h the reaction mixture was cooled to RT and the solvent was removed *in vacuo*. The crude residue was purified by flash column chromatography ($SiO_2$, 10:1 pet. ether:ethyl acetate) to give the *title compound* **53** (60 mg, 64% yield) as a yellow foam: $\delta_H$ (400 MHz, $CDCl_3$) 8.92 (1H, s, NH), 8.78 (1H, s, NH), 8.63 (1H, d, *J* 8.3, Ar-H), 8.28 (1H, d, *J* 8.6, Ar-H), 8.01 (1H, d, *J* 8.4, Ar-H), 7.95 (1H, d, *J* 8.6, 2.0, Ar-H), 7.93 (1H, d, *J* 1.7, Ar-H), 7.90 (1H, d, *J* 2.0, Ar-H), 7.82 (1H, dd, *J* 8.3, 2.0, Ar-H), 7.75 (1H, dd, *J* 8.3, 1.7, Ar-H), 7.65 (1H, d, *J* 1.7, Ar-H), 4.18 (2H, t, *J* 6.6, $OCH_2$), 3.91 (3H, s, $OCH_3$), 2.99 (2H, t, *J* 7.8, $CH_2$), 2.74 (2H, t, *J* 8.1, $CH_2$), 2.30 – 2.35 (4H, m, $CH_2$), 1.83 – 2.04 (6H, m, $CH_2$), 1.45 (9H, s, *t*-Bu), 1.41 (9H, s, *t*-Bu), 1.10 (3H, t, *J* 7.4, $OCH_2CH_2CH_3$); $\delta_C$ (101 MHz, $CDCl_3$) 173.8, 172.3, 167.0, 166.2, 163.2, 147.6, 140.5, 138.9, 137.7, 131.7, 131.4, 131,2, 130.8, 130.6, 128.7, 126.1, 125.6, 125.5, 122.8, 120.6, 118.8, 111.2, 81.4, 70.6, 52.2, 35.1, 33.6, 32.3, 30.6, 28.2, 28.1, 26.1, 25.6, 22.6, 10.7; LRMS (ESI) calculated for $C_{41}H_{51}N_3O_{11}$ [$(M+H)^+$]: 762.4, found 762.3.

**4-(5-((4-((2-(3-Carboxypropyl)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-propoxyphenyl)carbamoyl)-2-nitrophenyl)butanoic acid (9)**



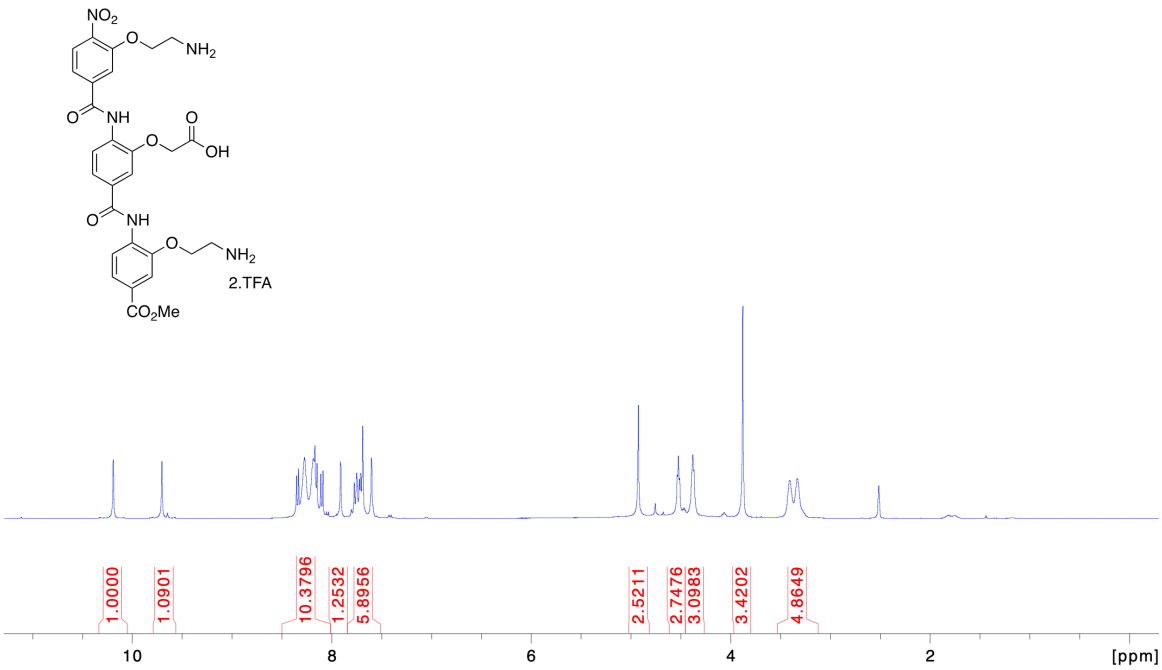

Trifluoroacetic acid (1 mL) was added to a solution of di-*tert*-butyl ester **53** (50 mg, 0.066 mmol) in $CH_2Cl_2$ (1 mL) and allowed to stand for 4 h. The volatiles were removed *in vacuo* and residual TFA was removed by co-evaporation with toluene. The solid residue was

suspended in diethyl ether, filtered under reduced pressure and washed with diethyl ether to to give *the title compound* **9** (26 mg, 61% yield) as a yellow powder: $\delta_H$ (400 MHz, MeOD-$d_4$) 8.26 (1H, d, *J* 8.8, Ar-H), 8.04 (1H, d, *J* 8.3, Ar-H), 8.03 (1H, d, *J* 2.0, Ar-H), 8.01 (1H, d, *J* 1.9, Ar-H), 7.96 (1H, dd, *J* 8.5, 2.0, Ar-H), 7.93 (1H, dd, *J* 8.3, 2.1, Ar-H), 7.67 – 7.70 (2H, m, Ar-H), 7.63 (1H, d, *J* 8.3, Ar-H), 4.19 (2H, t, *J* 6.5, $OCH_2$), 3.92 (3H, s, $OCH_3$), 2.99 (2H, t, *J* 7.8, $CH_2$), 2.81 (2H, t, *J* 7.9, $CH_2$), 2.42 (2H, t, *J* 7.3, $CH_2$), 2.34 (2H, t, *J* 7.0, $CH_2$), 1.89 – 2.06 (6H, m, $CH_2$), 1.11 (3H, t, *J* 7.5, $OCH_2CH_2C\mathit{H_3}$); LRMS (ESI) calculated for $C_{33}H_{35}N_3O_{11}$ [$(M+H)^+$]: 650.2, found 650.2.

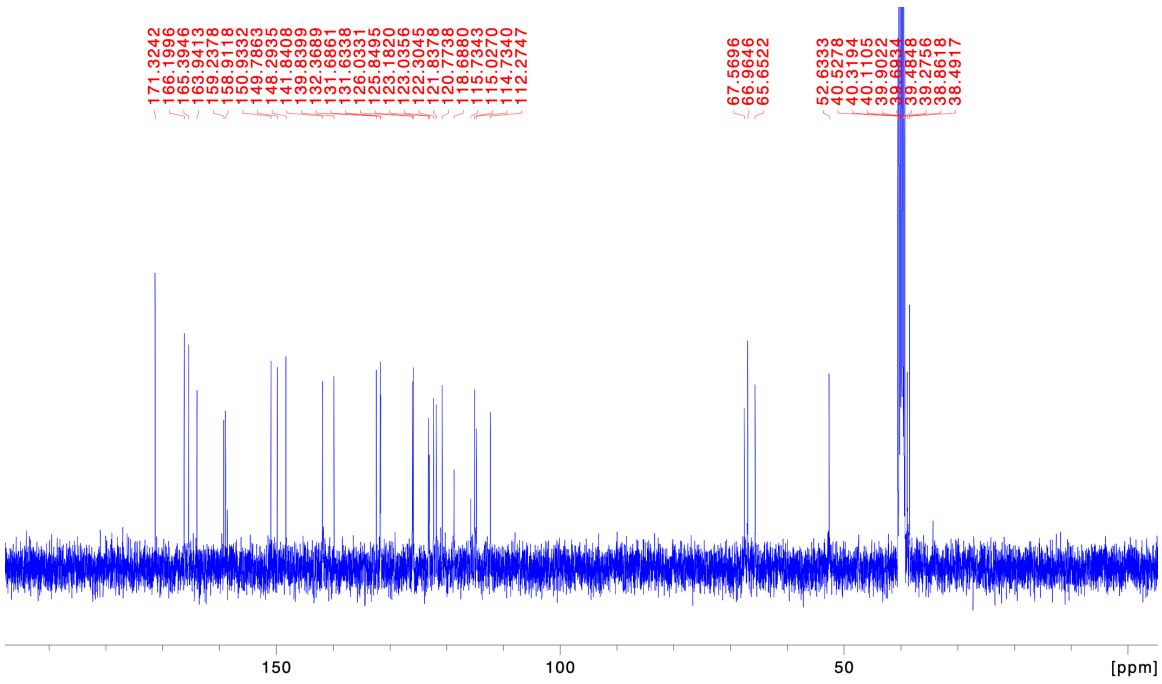
# 2. NMR Spectra of Synthetic Compounds

## 2-(5-((2-(2-Aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(2-aminoethoxy)-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (1)

### $^{1}$H, 400 MHz, $CDCl_3$

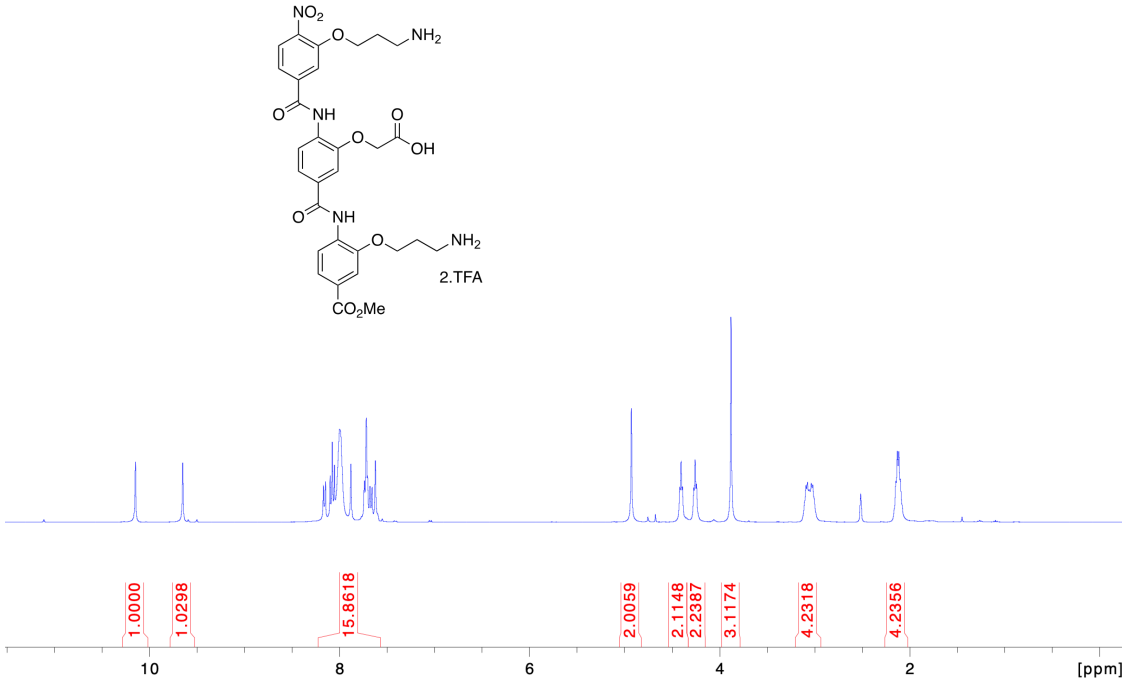



### $^{13}$C, 101 MHz, $CDCl_3$

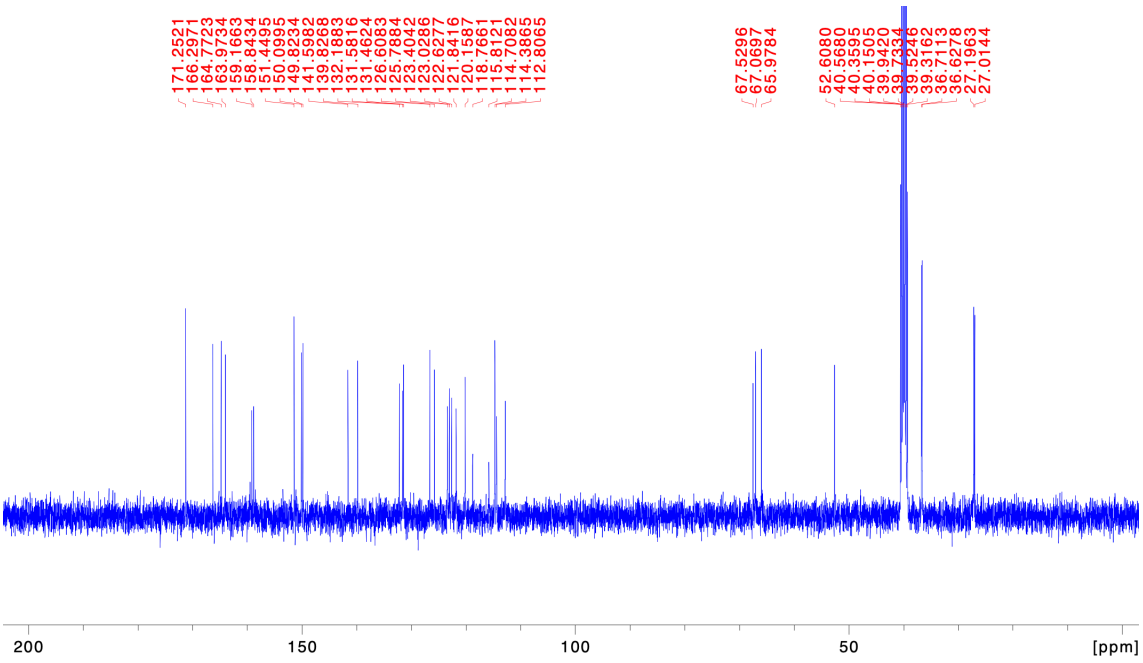


**2-(5-((2-(3-Aminopropoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-(3-aminopropoxy)-4-nitrobenzamido)phenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (2)**

**$^{1}$H, 400 MHz, DMSO-$d_6$**

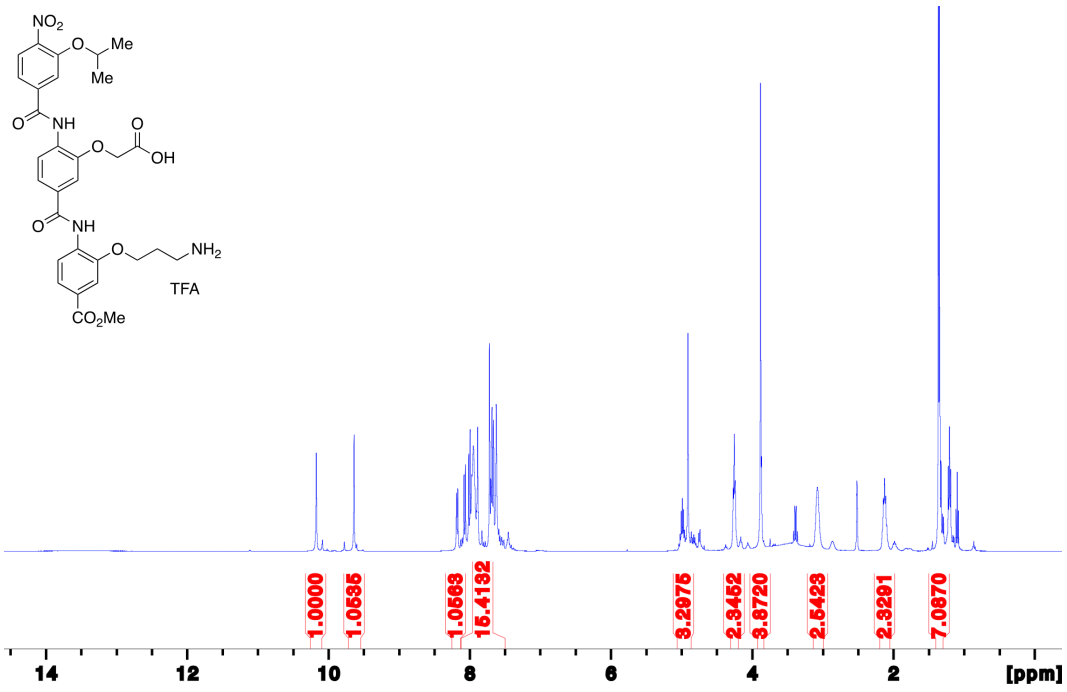



**$^{13}$C, 101 MHz, DMSO-$d_6$**

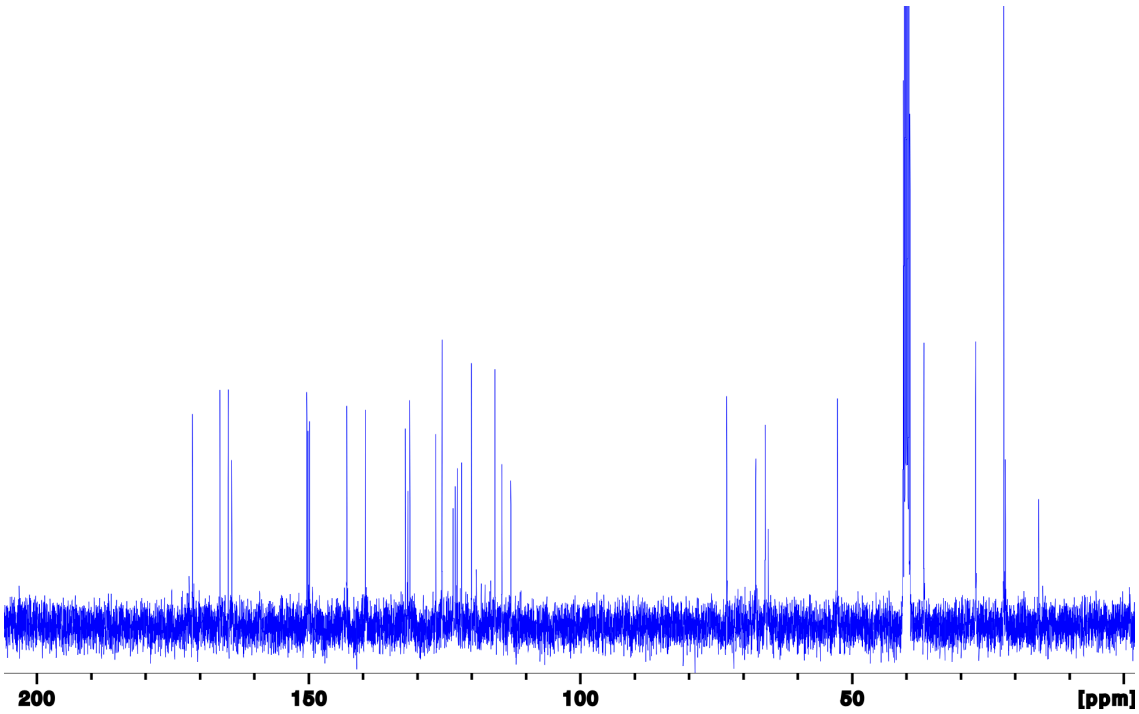


**2-(5-((2-(3-Aminopropoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-isopropoxy-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (3)**

**$^{1}$H, 400 MHz, DMSO-$d_6$**

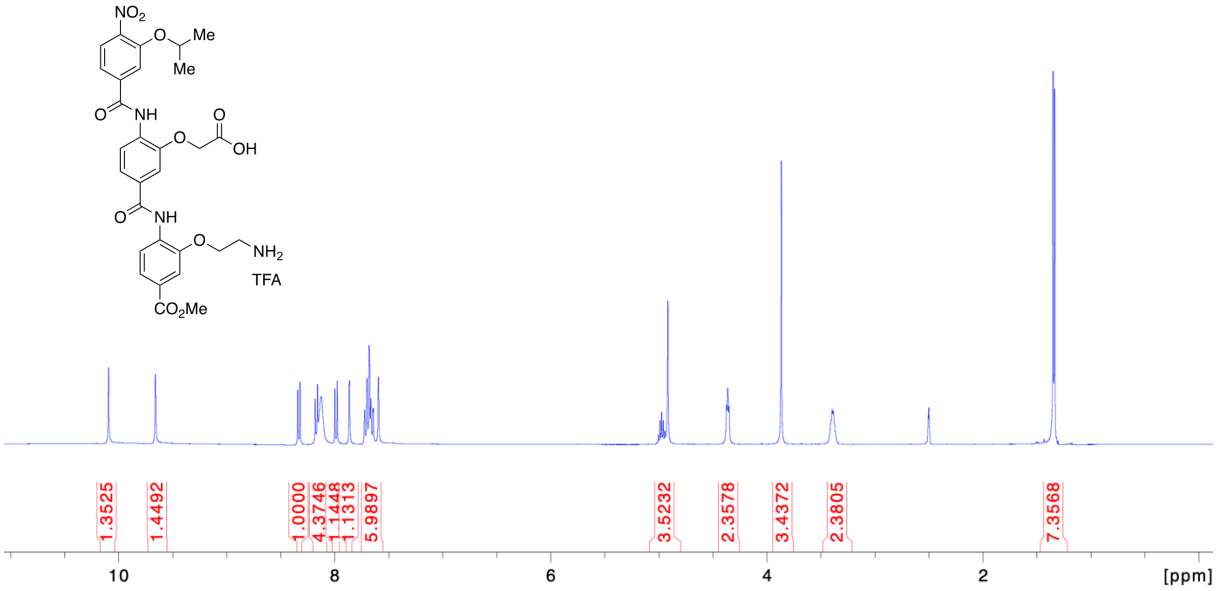



**$^{13}$C, 101 MHz, DMSO-$d_6$**

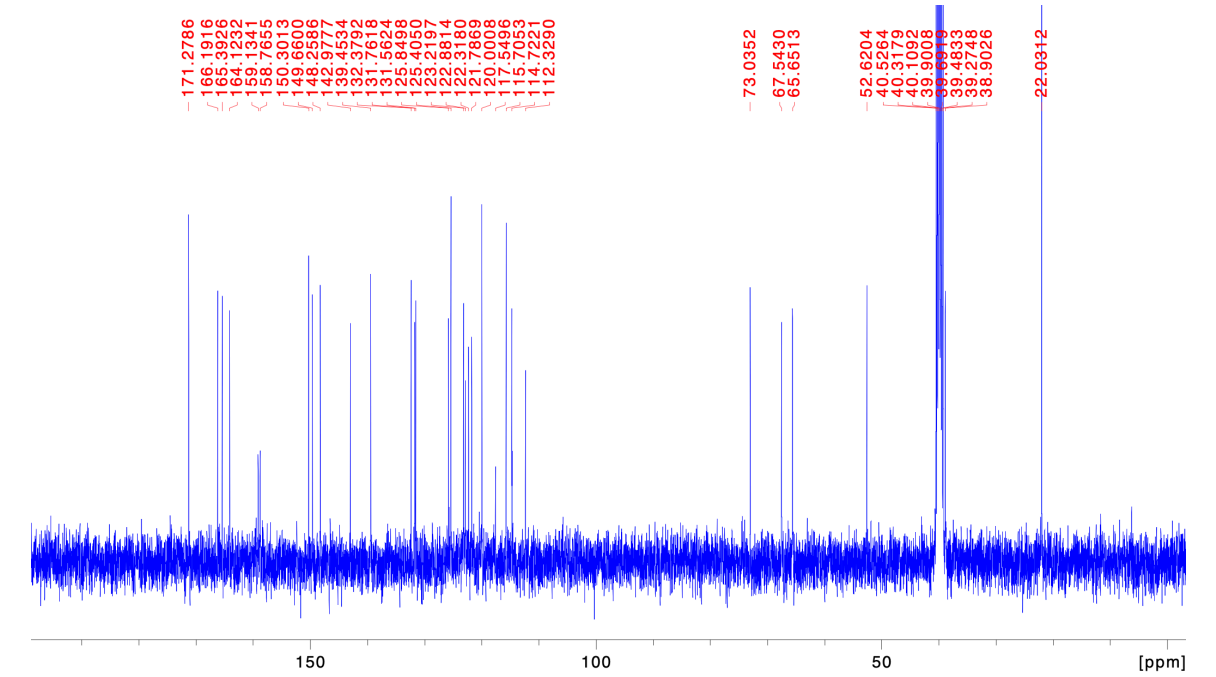


**2-(5-((2-(2-Aminoethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-(3-isopropoxy-4-nitrobenzamido)phenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (4)**

**$^{1}$H, 400 MHz, DMSO-*d*$_6$**

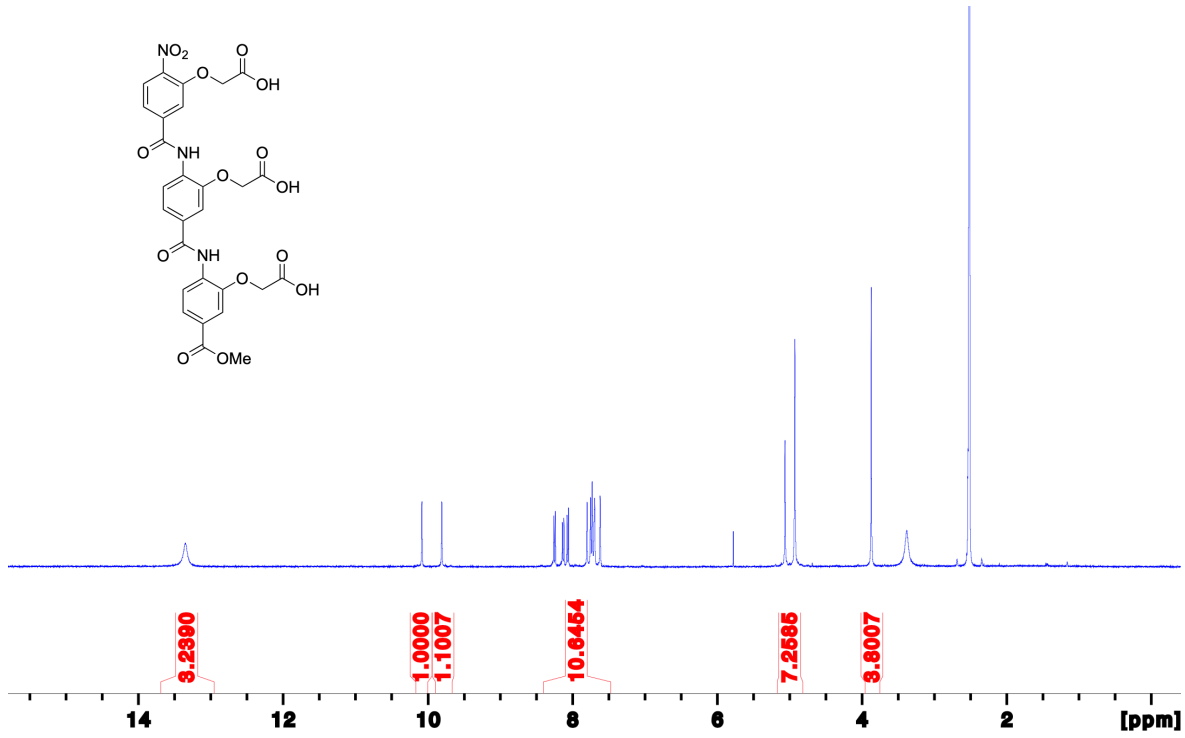



**$^{13}$C, 101 MHz, DMSO-*d*$_6$**



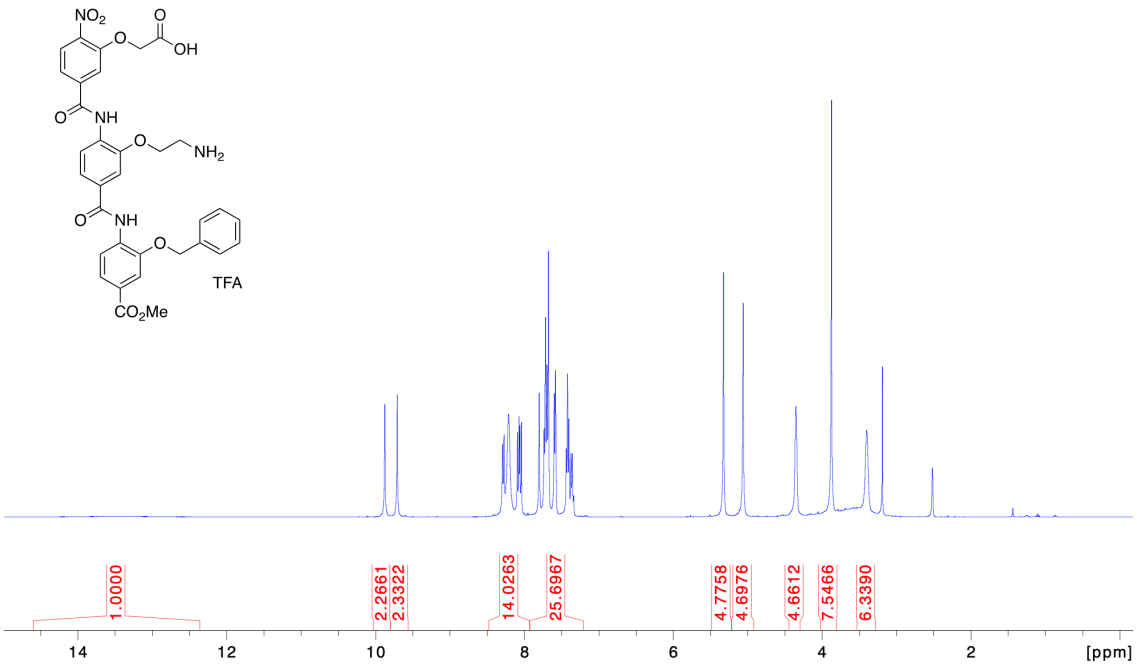
**2-(5-((2-(Carboxymethoxy)-4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid (5)**[2][3]

**$^1$H, 400 MHz, DMSO-$d_6$**

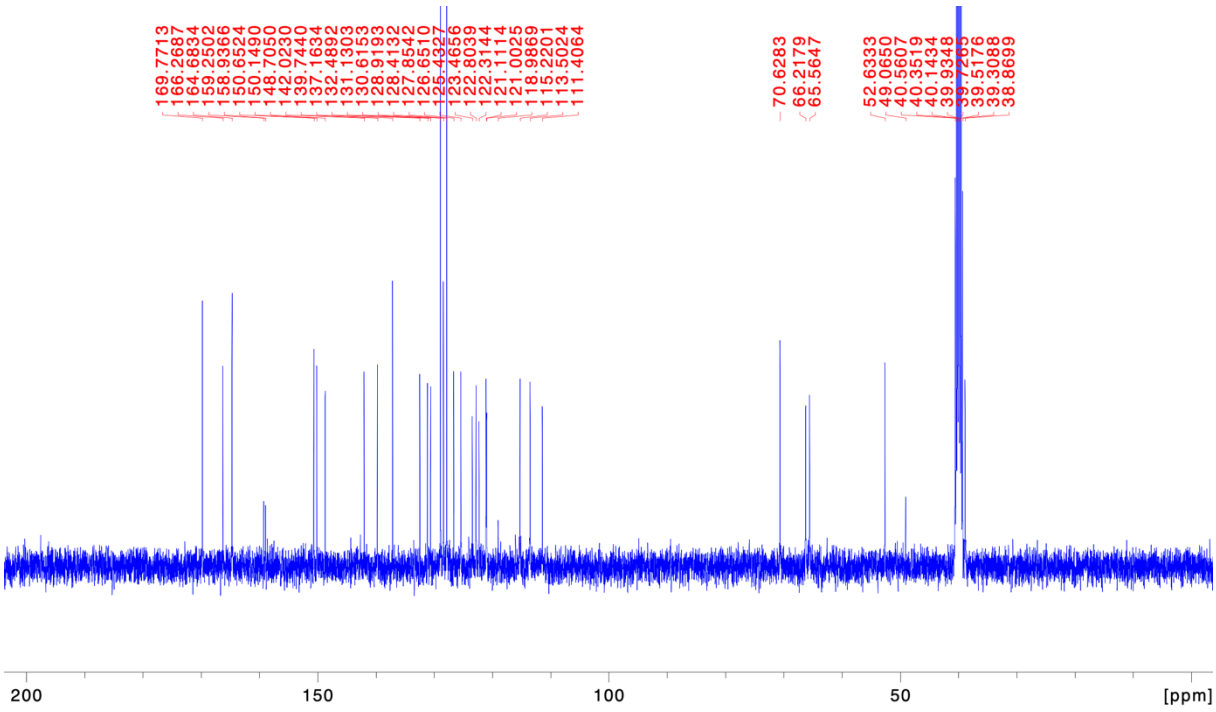


**2-(5-((2-(2-Aminoethoxy)-4-((2-(benzyloxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid di-2,2,2-trifluoroacetic acid salt (6)**

**$^{1}$H, 400 MHz, DMSO-$d_6$**

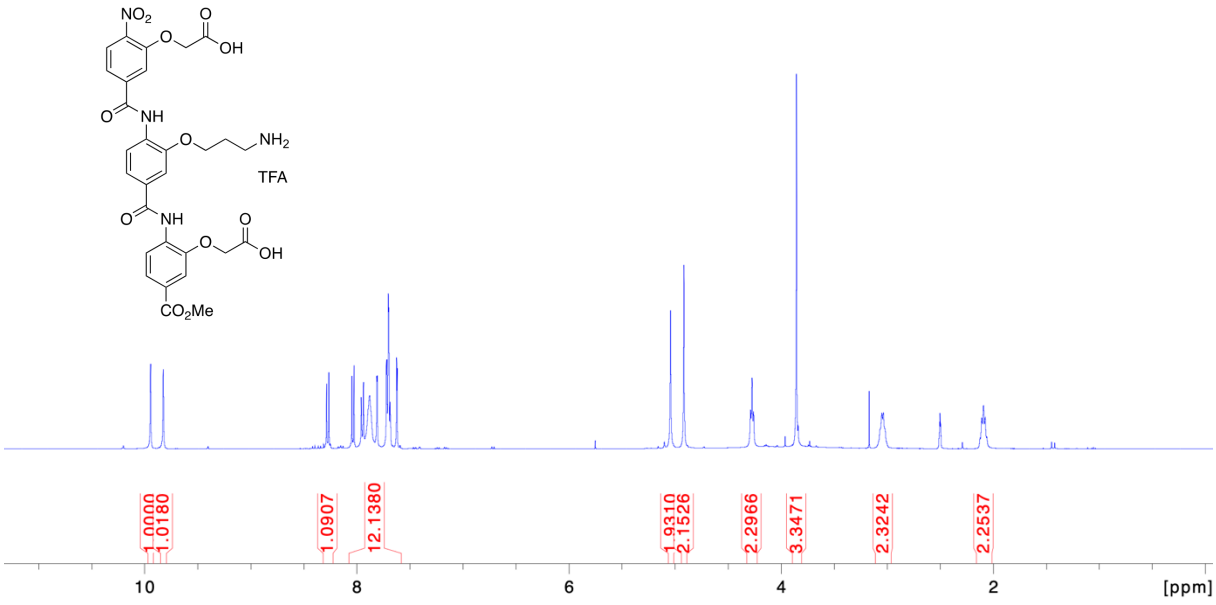



**$^{13}$C, 101 MHz, DMSO-$d_6$**

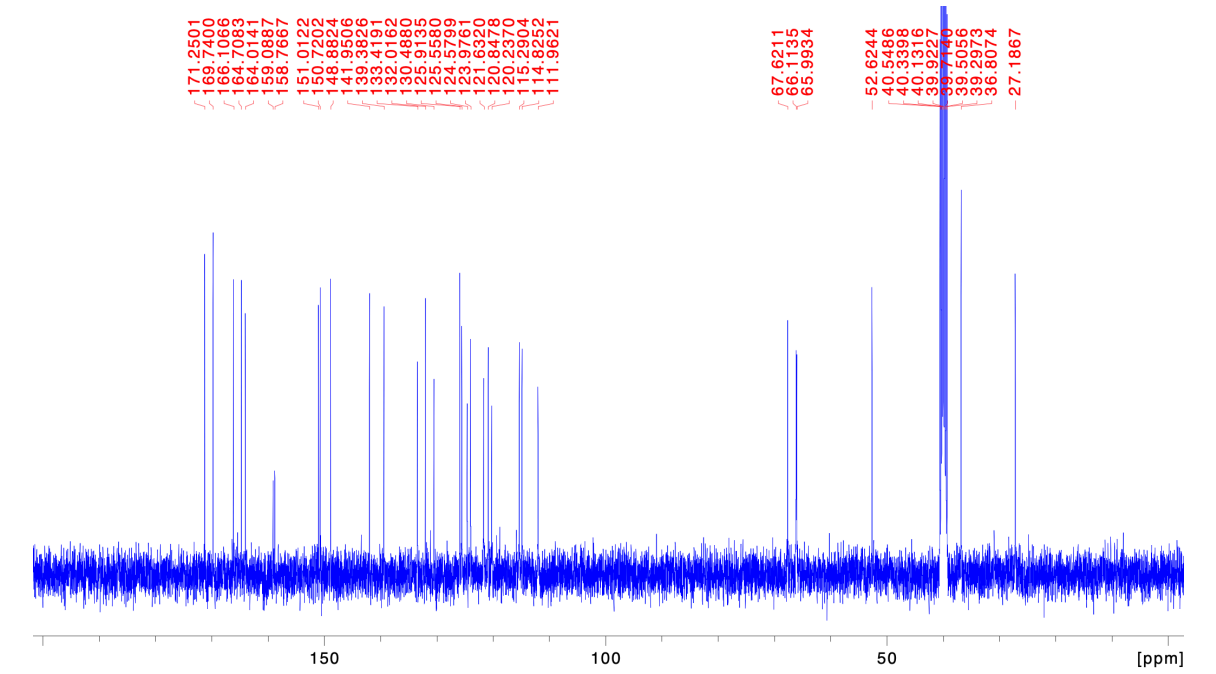


**2-(5-((2-(3-Aminopropoxy)-4-((2-(carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)phenyl)carbamoyl)-2-nitrophenoxy)acetic acid 2,2,2-trifluoroacetic acid salt (7)**

**$^{1}$H, 400 MHz, DMSO-$d_6$**

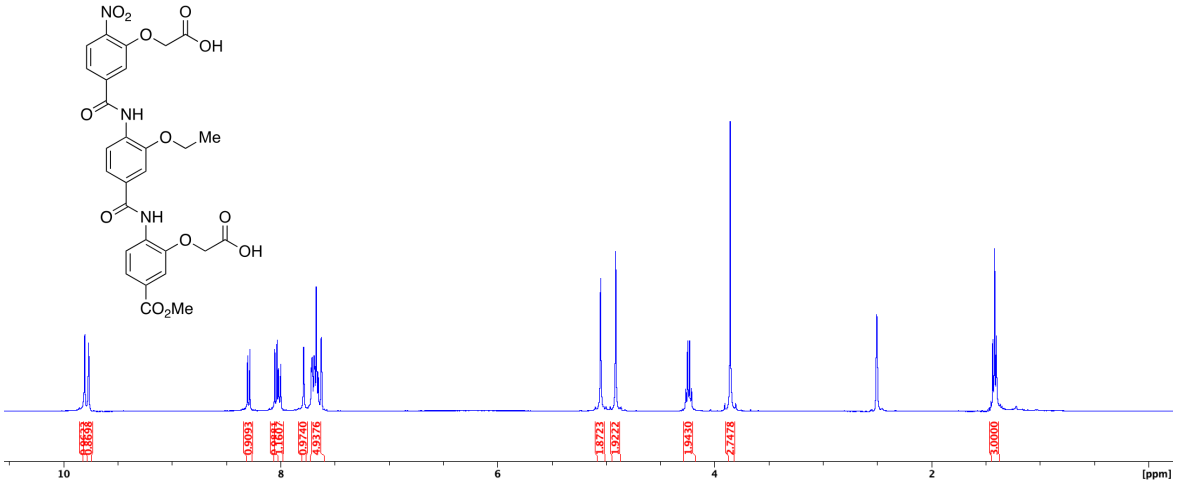



**$^{13}$C, 101 MHz, DMSO-$d_6$**

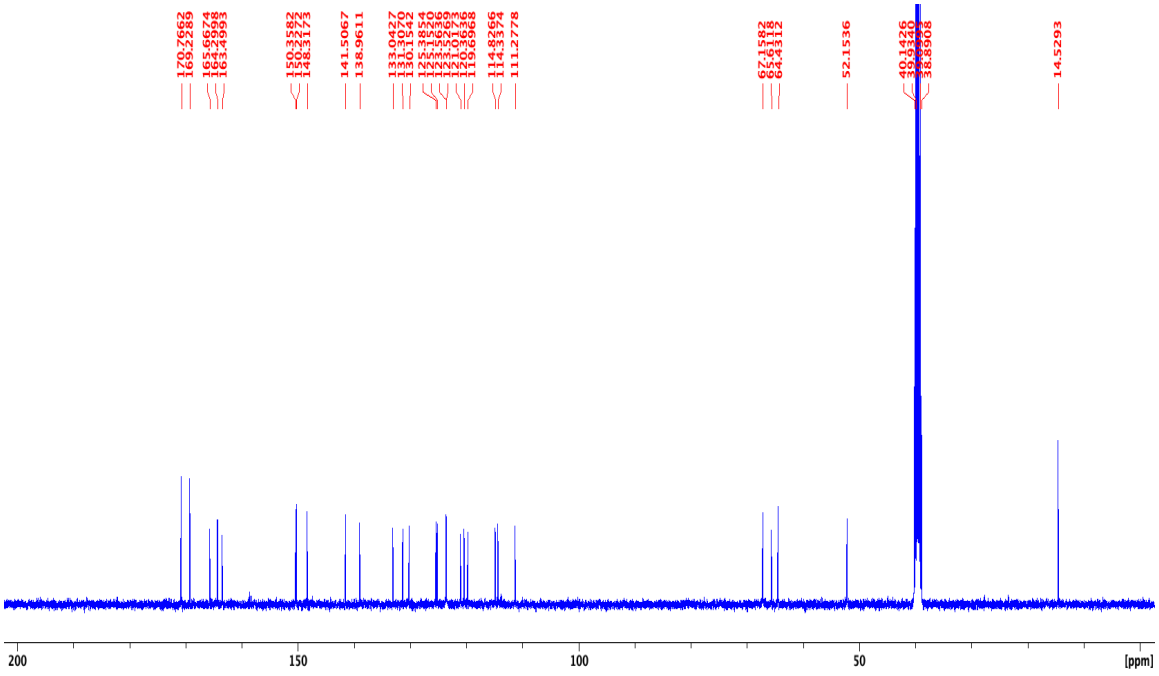


**2-(5-((4-((2-(Carboxymethoxy)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-ethoxyphenyl)carbamoyl)-2-nitrophenoxy)acetic acid (8)**

**$^{1}$H, 400 MHz, DMSO-*d*$_6$**

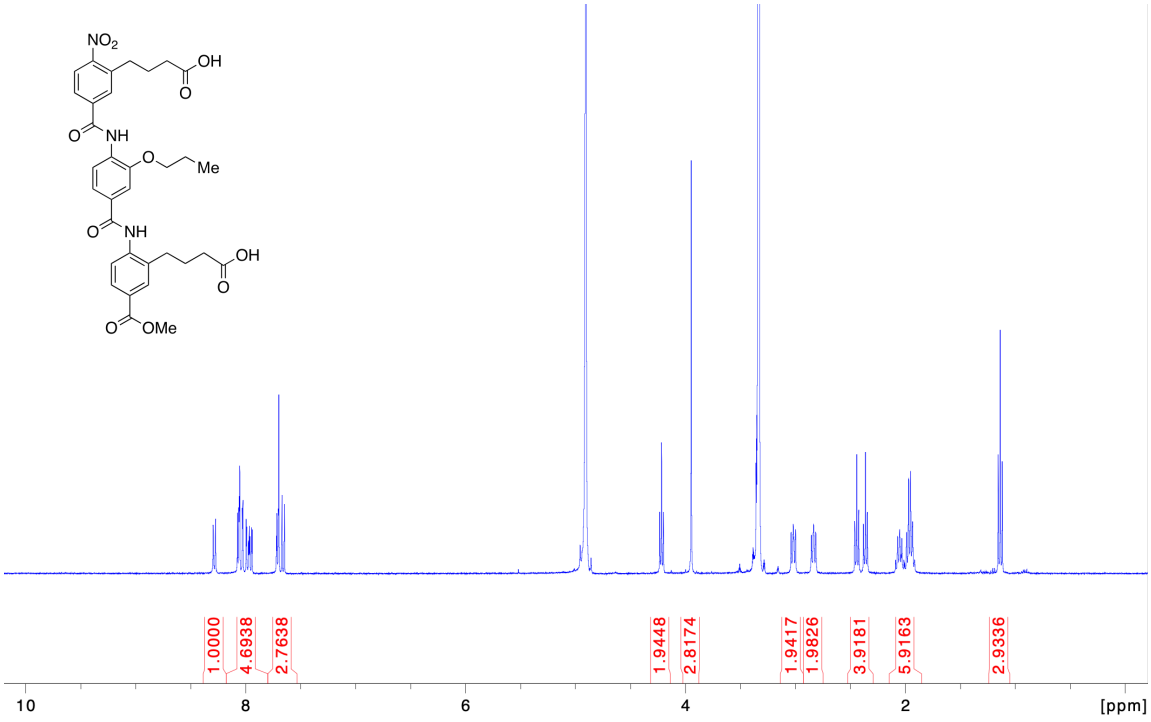



**$^{13}$C, 101 MHz, DMSO-*d*$_6$**



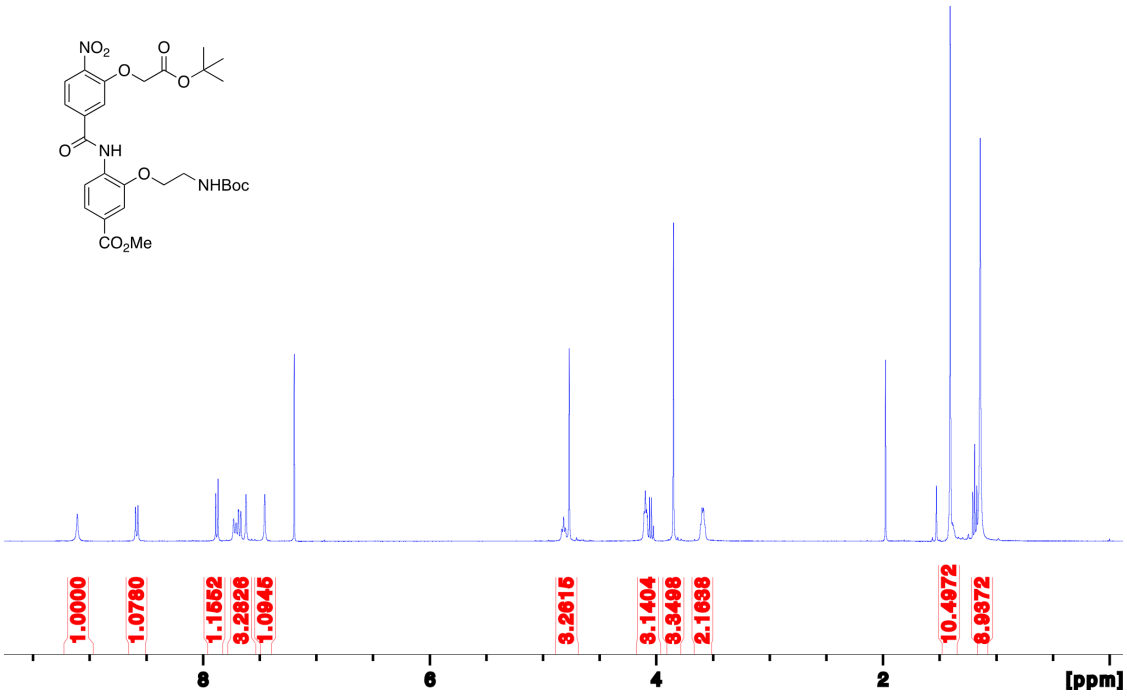
**4-(5-((4-((2-(3-Carboxypropyl)-4-(methoxycarbonyl)phenyl)carbamoyl)-2-propoxyphenyl)carbamoyl)-2-nitrophenyl)butanoic acid (9)**

**$^{1}$H, 400 MHz, $CD_3OD$**

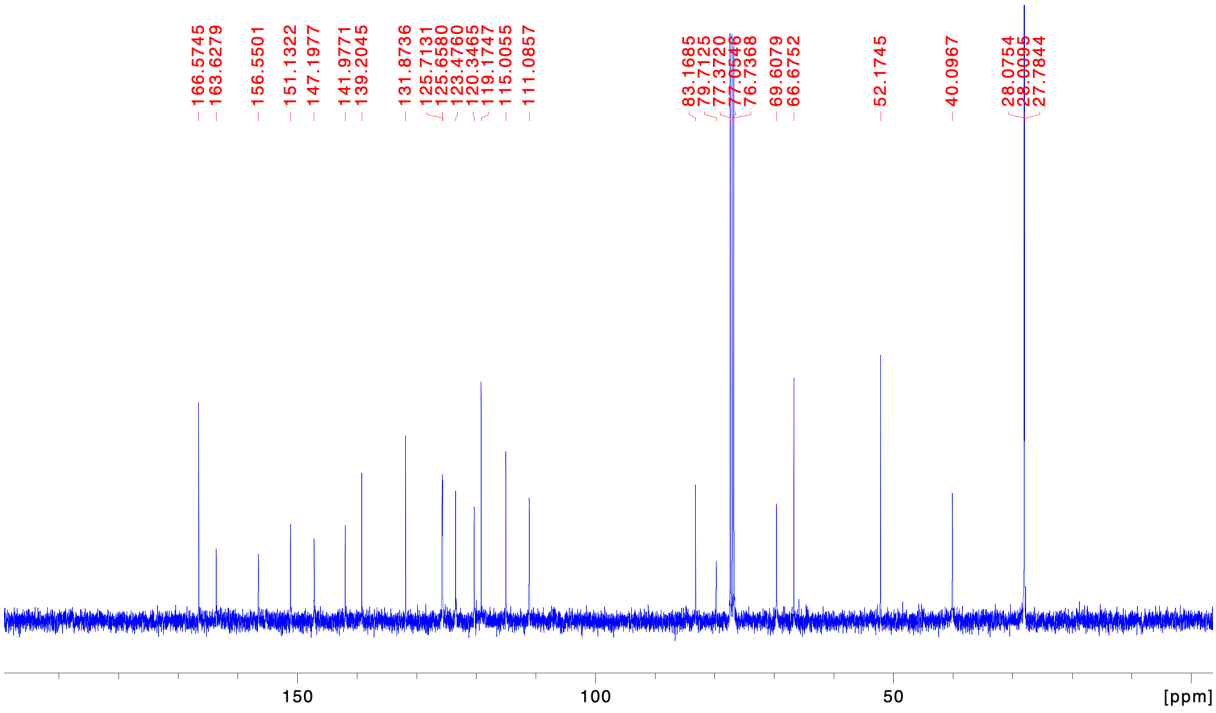


**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (12)**

**$^{1}$H, 400 MHz, $CDCl_3$**

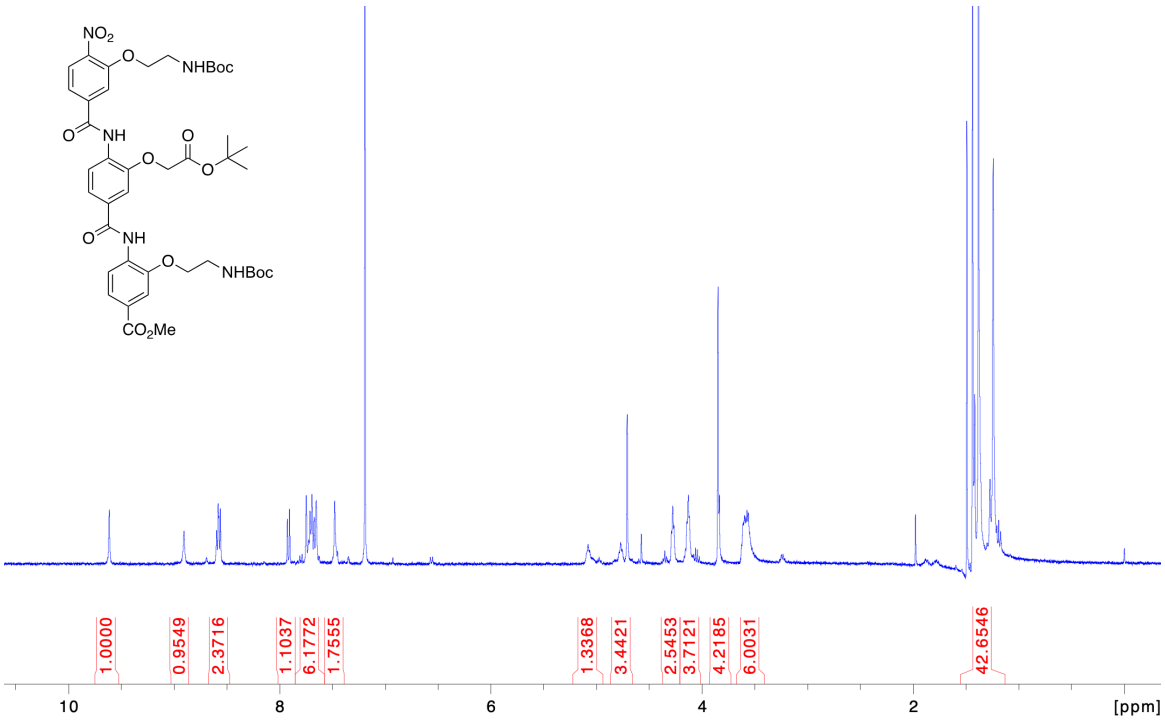



**$^{13}$C, 101 MHz, $CDCl_3$**

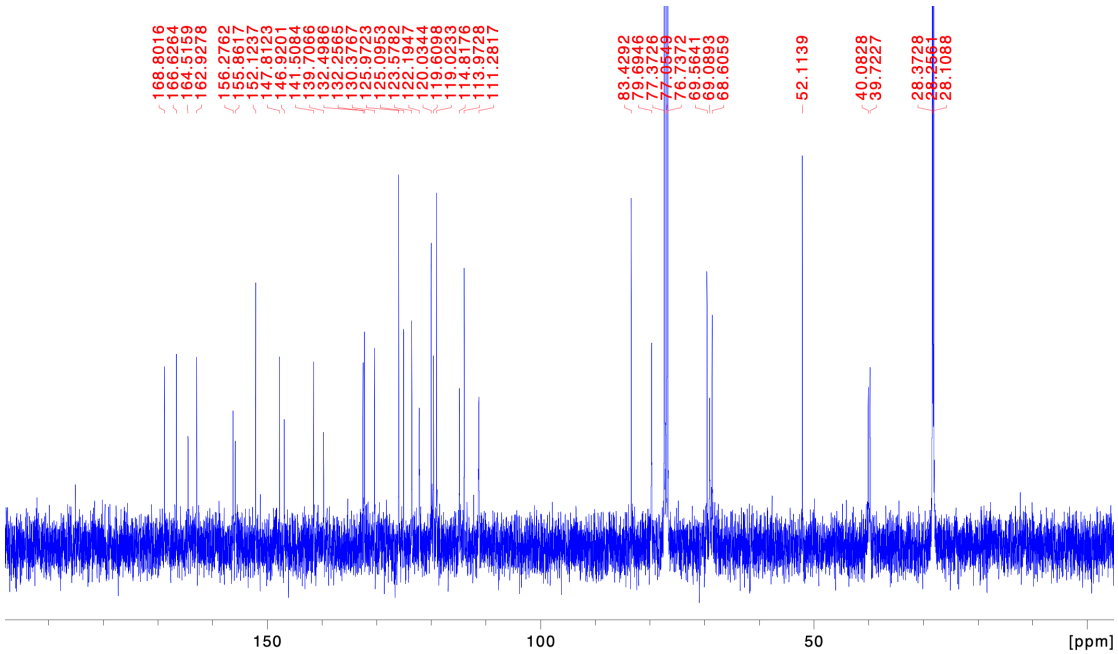


**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzamido)benzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (14)**

**$^{1}$H, 400 MHz, $CDCl_3$**

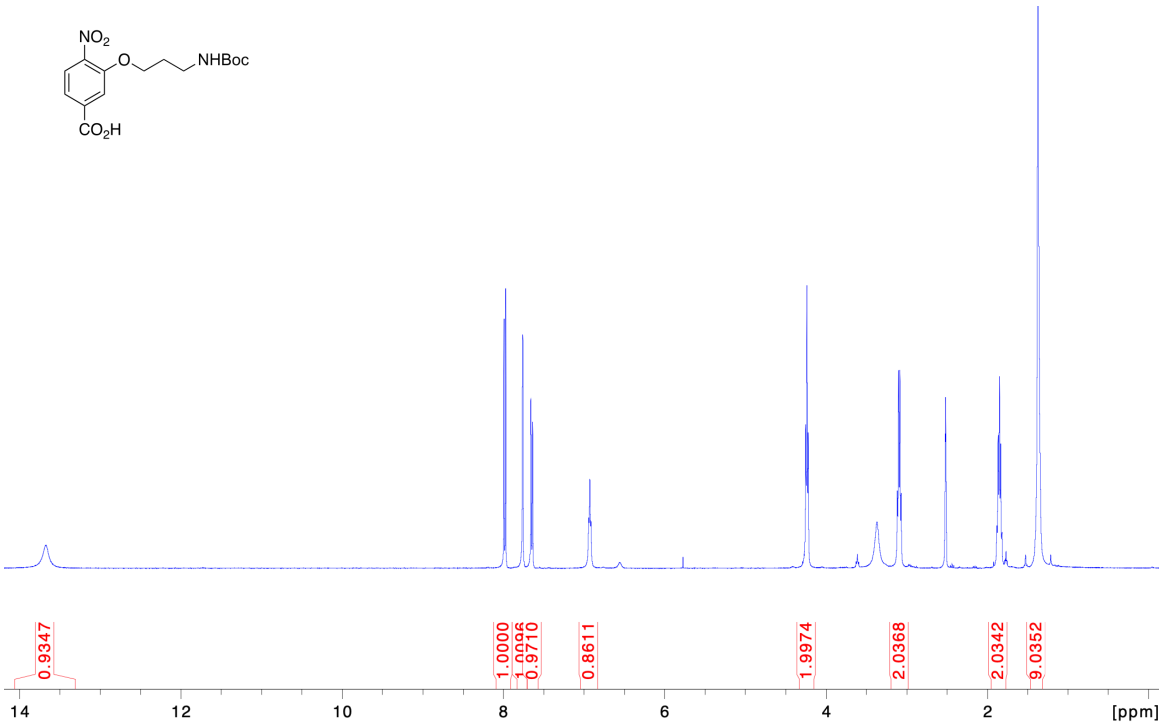



**$^{13}$C, 101 MHz, $CDCl_3$**

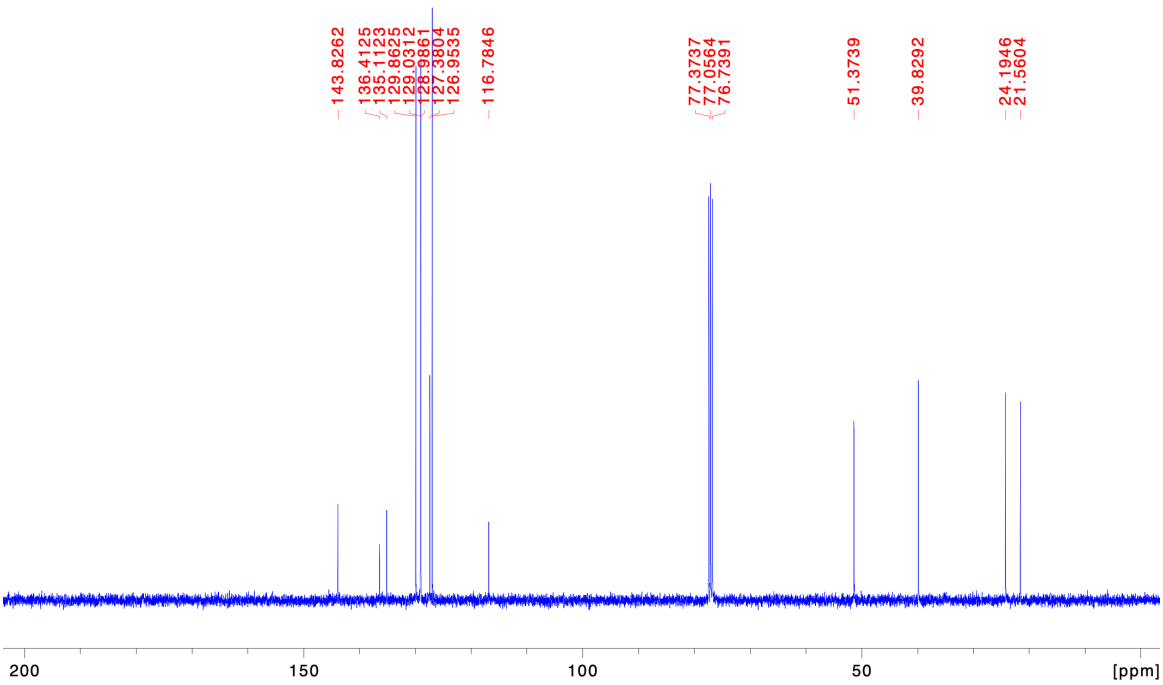


## 3-(3-((*tert*-Butoxycarbonyl)amino)propoxy)-4-nitrobenzoic acid (17)

### $^{1}$H, 400 MHz, $CDCl_3$

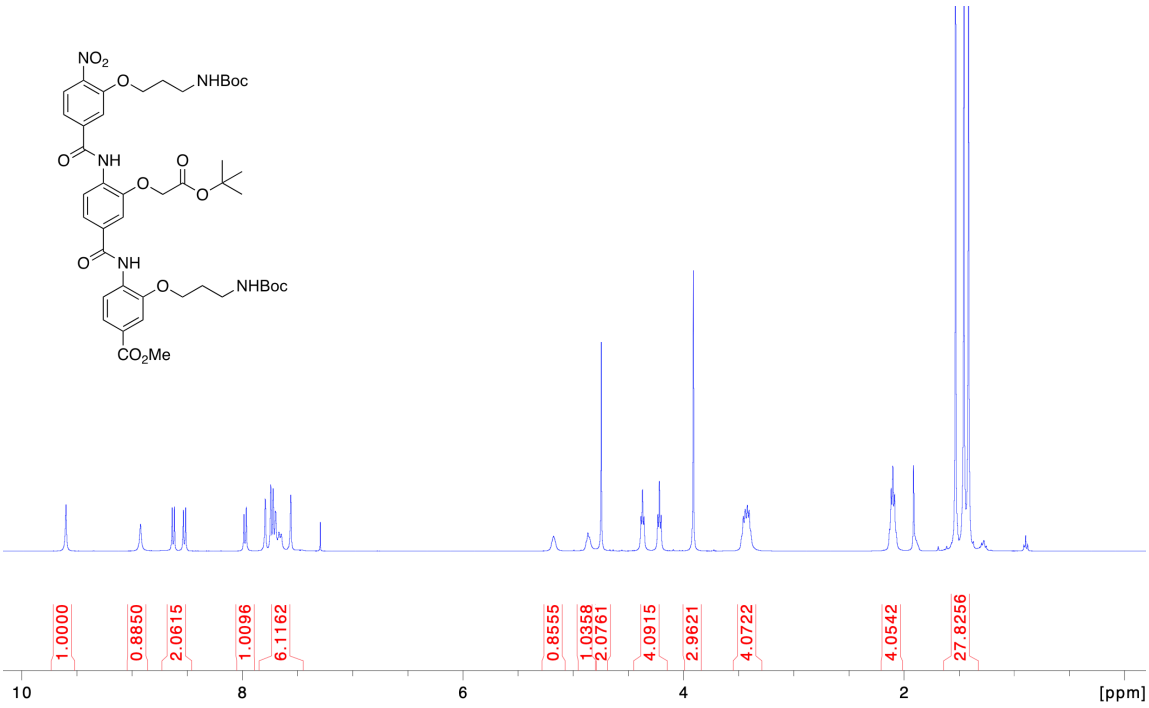



### $^{13}$C, 101 MHz, $CDCl_3$

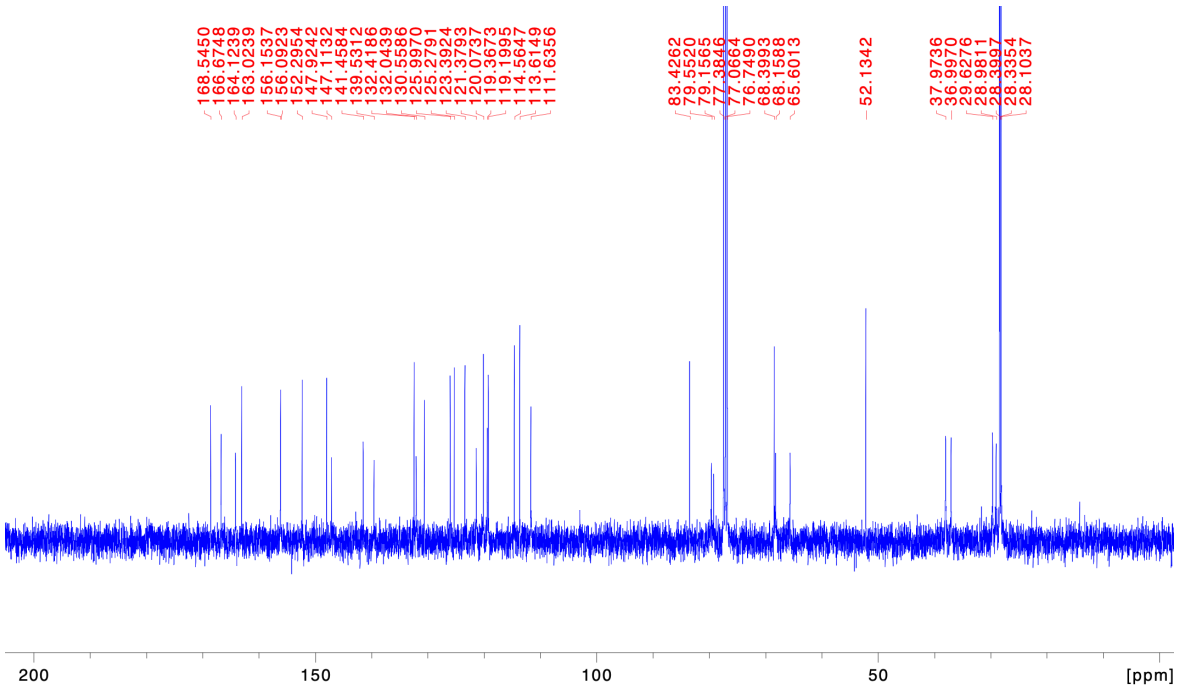


**Methyl 4-(3-(2-(tert-butoxy)-2-oxoethoxy)-4-(3-(3-((tert-butoxycarbonyl)amino)propoxy)-4-nitrobenzamido)benzamido)-3-(3-((tert-butoxycarbonyl)amino)propoxy)benzoate (18)**

**$^{1}$H, 400 MHz, $CDCl_3$**

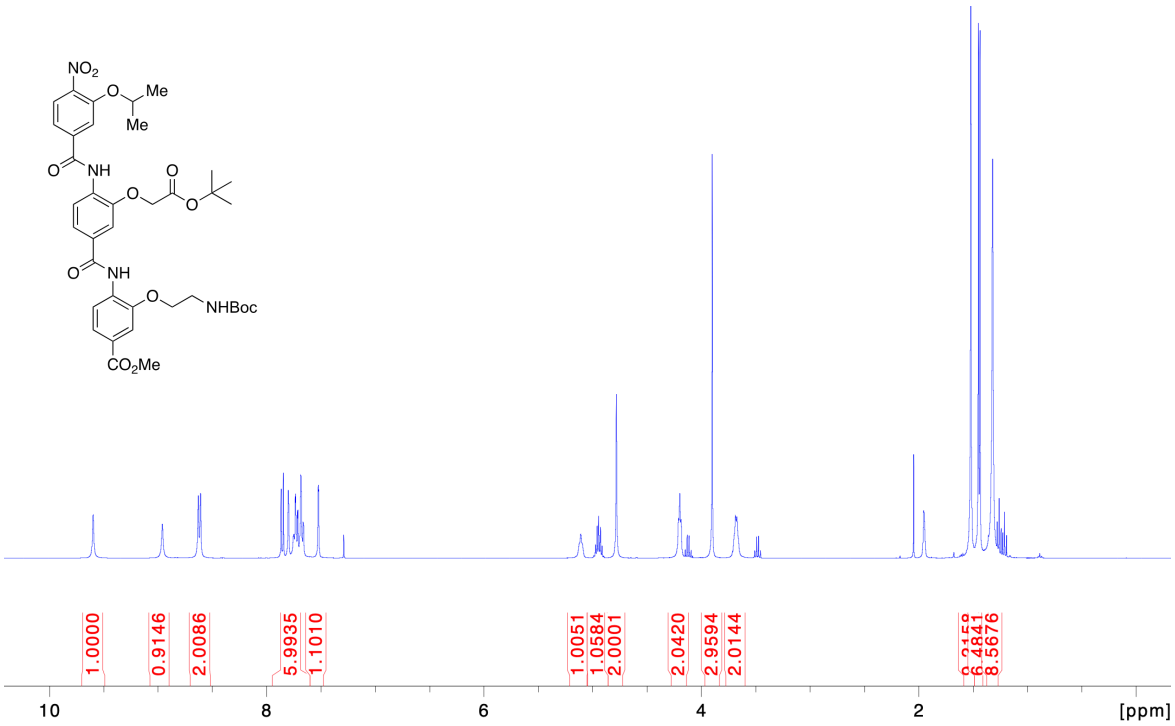



**$^{13}$C, 101 MHz, $CDCl_3$**

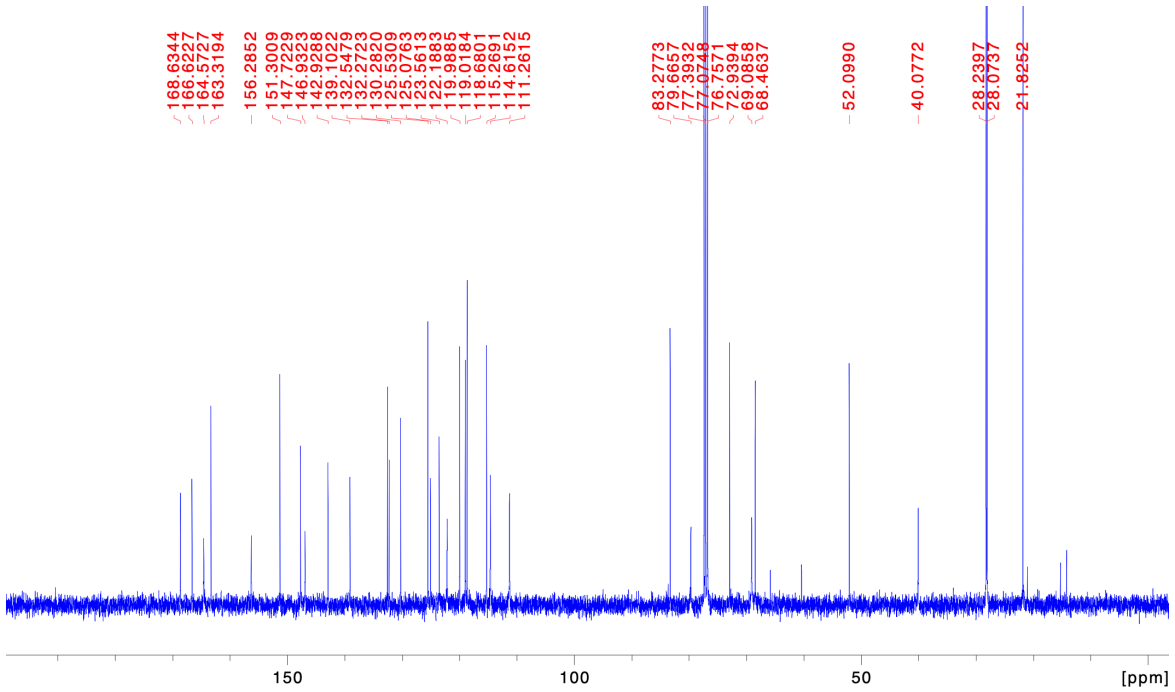


**Methyl 4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-isopropoxy-4-nitrobenzamido)benzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzoate (22)**

**$^{1}$H, 400 MHz, $CDCl_3$**

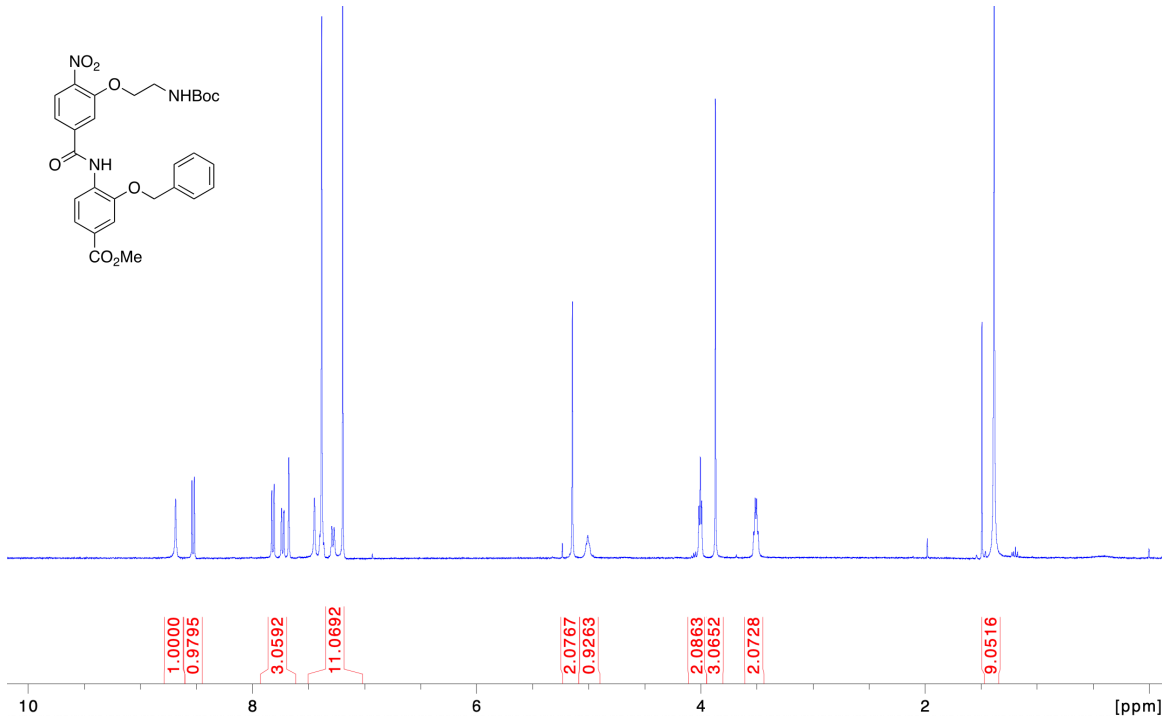



**$^{13}$C, 101 MHz, $CDCl_3$**

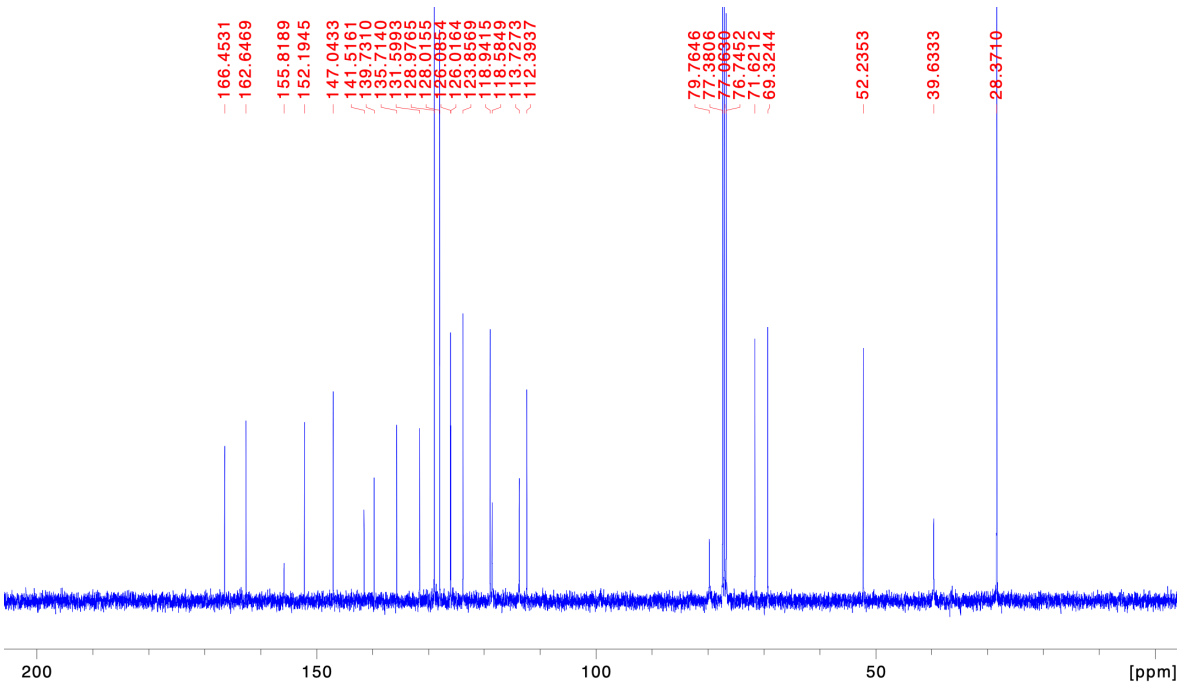


**Methyl 3-(benzyloxy)-4-(3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)-4-nitrobenzamido)benzoate (24)**

**$^{1}$H, 400 MHz, $CDCl_3$**

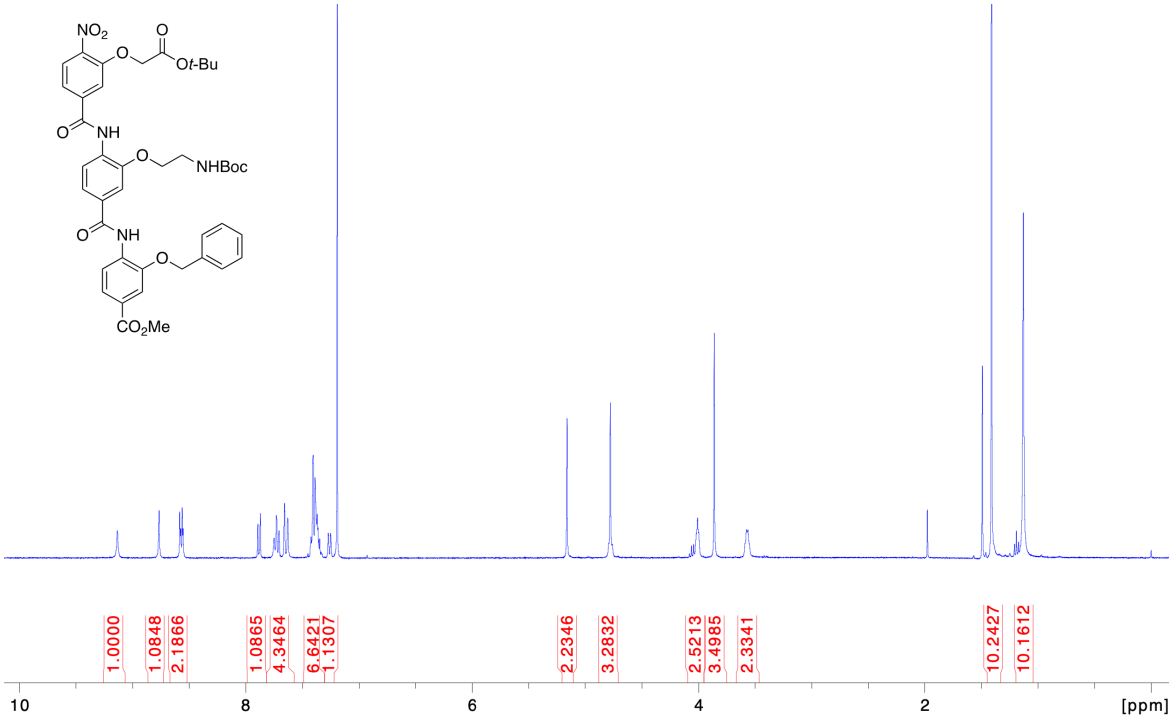



**$^{13}$C, 101 MHz, $CDCl_3$**

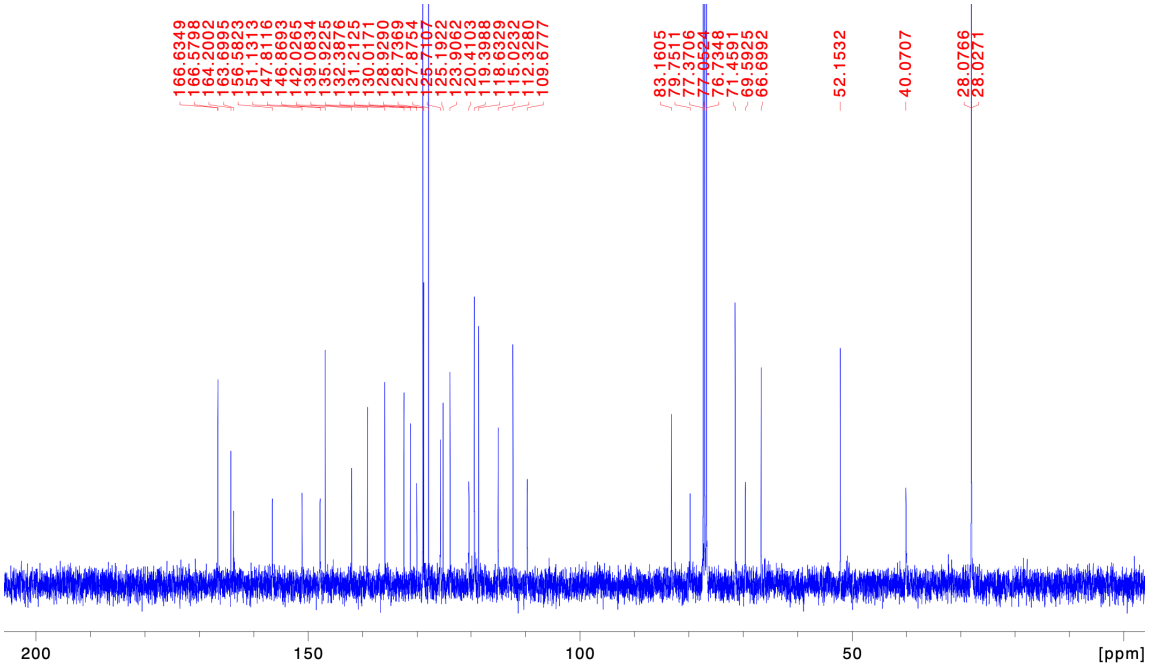


**Methyl 3-(benzyloxy)-4-(4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(2-((*tert*-butoxycarbonyl)amino)ethoxy)benzamido)benzoate (25)**

**$^{1}$H, 400 MHz, $CDCl_3$**

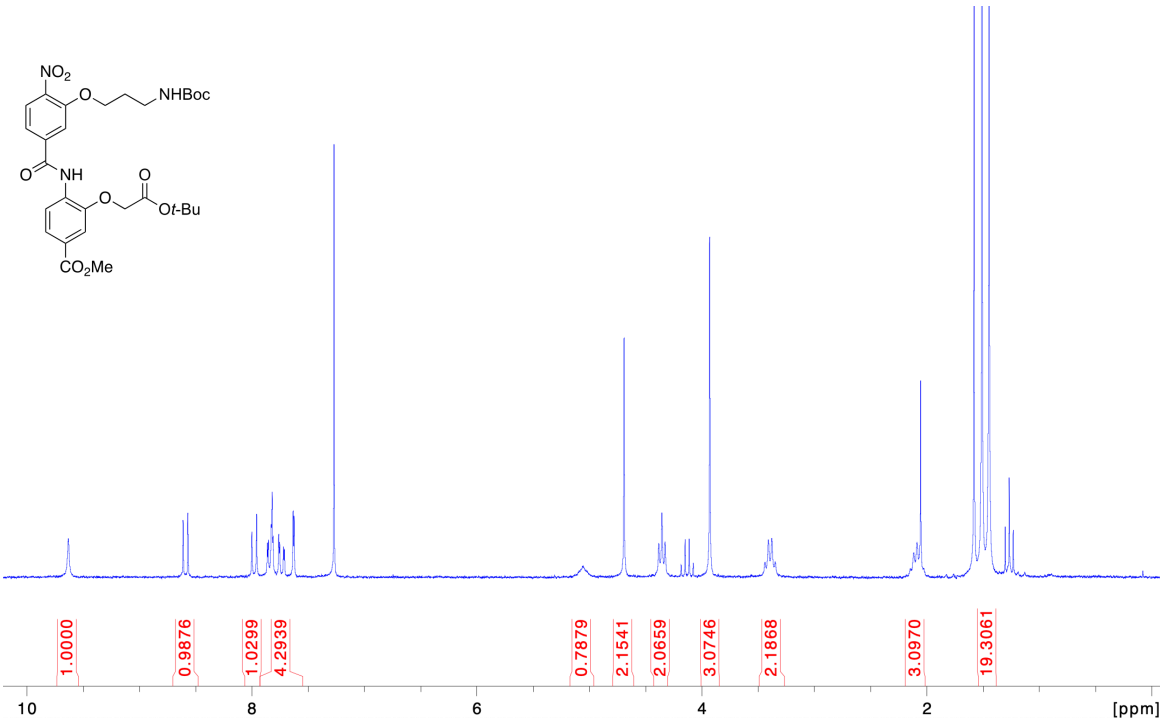



**$^{13}$C, 101 MHz, $CDCl_3$**

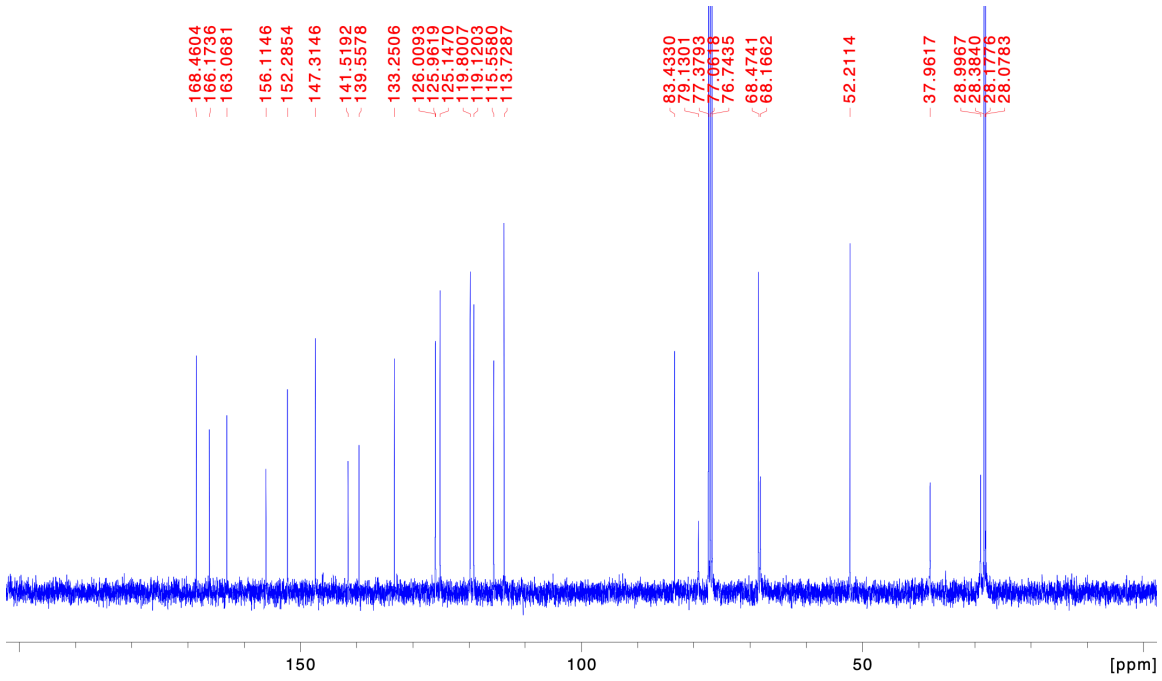


**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-(3-((*tert*-butoxycarbonyl)amino)propoxy)-4-nitrobenzamido)benzoate (27)**

**$^{1}$H, 400 MHz, $CDCl_3$**

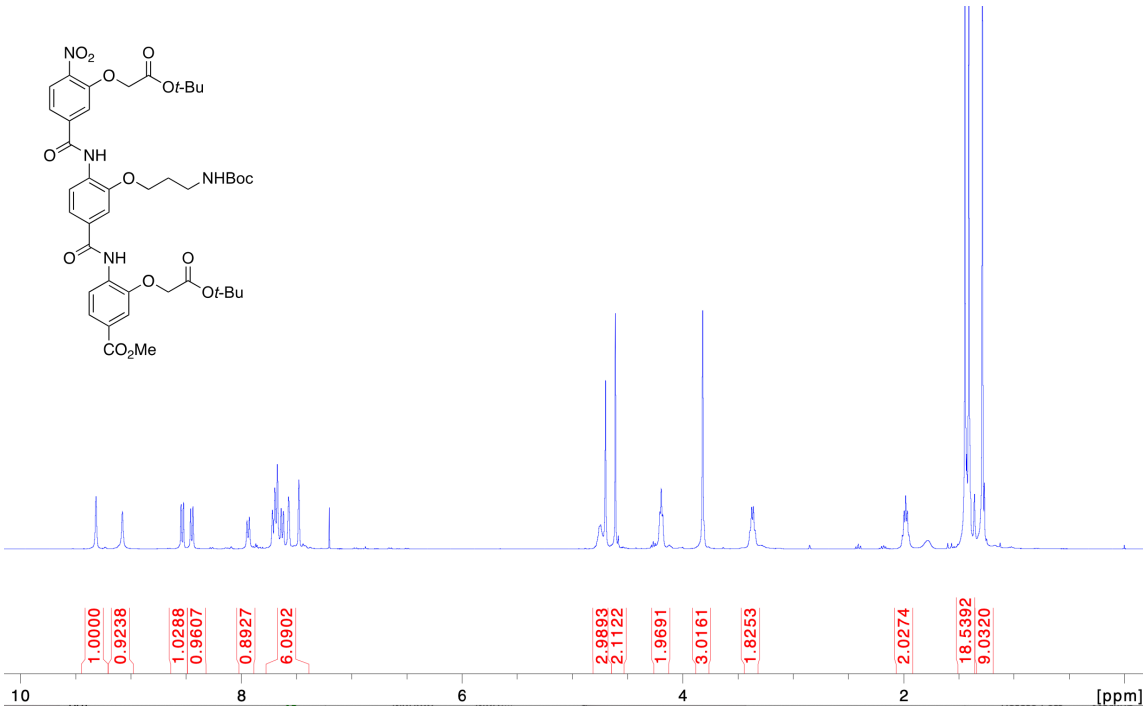



**$^{13}$C, 101 MHz, $CDCl_3$**

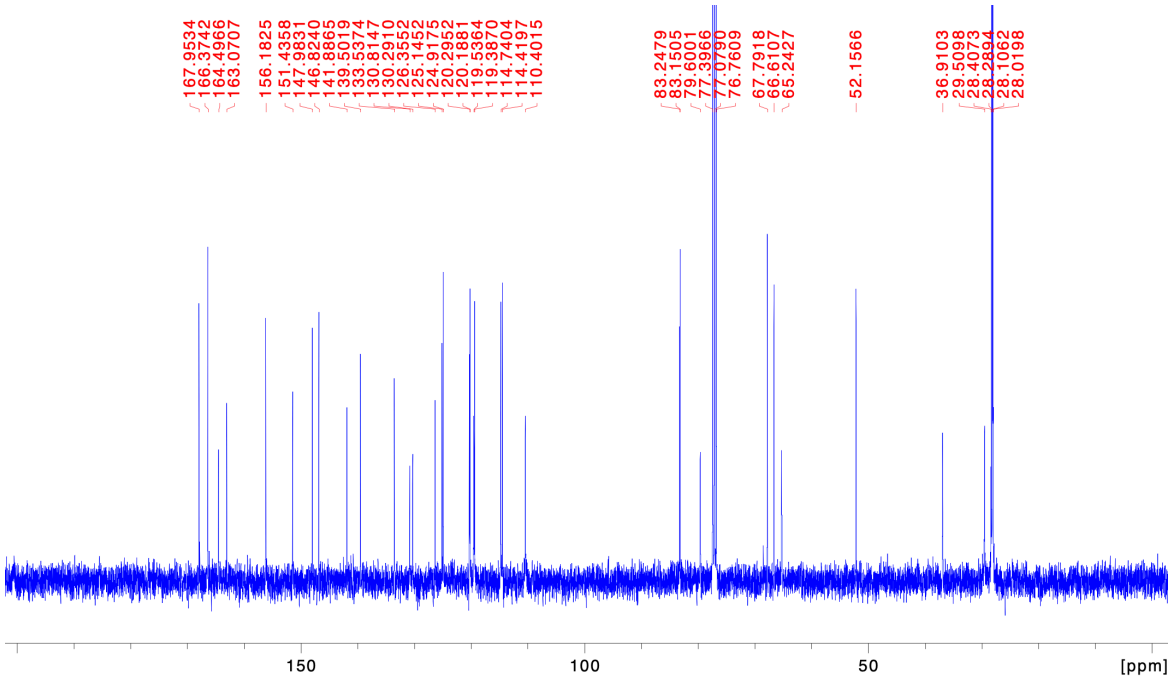


**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-(3-((*tert*-butoxycarbonyl)amino)propoxy)benzamido)benzoate (28)**

**$^{1}$H, 400 MHz, $CDCl_3$**

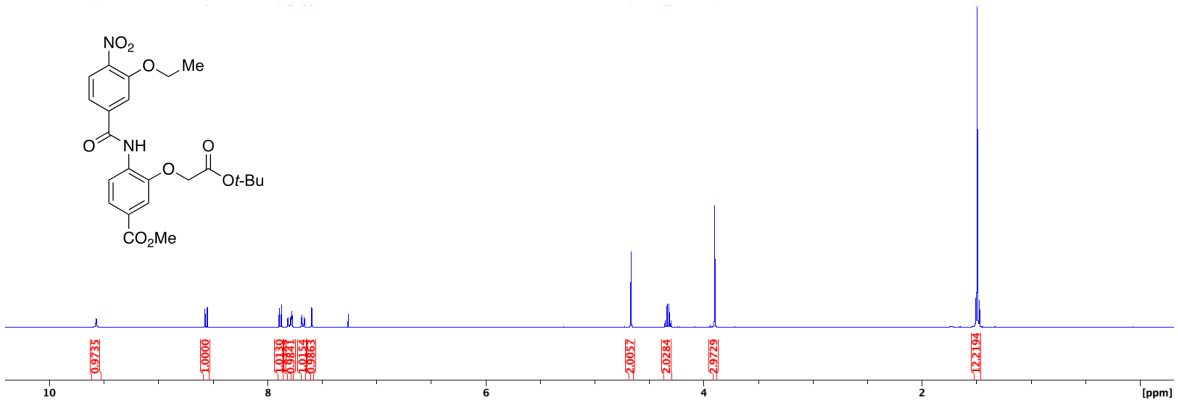



**$^{13}$C, 101 MHz, $CDCl_3$**

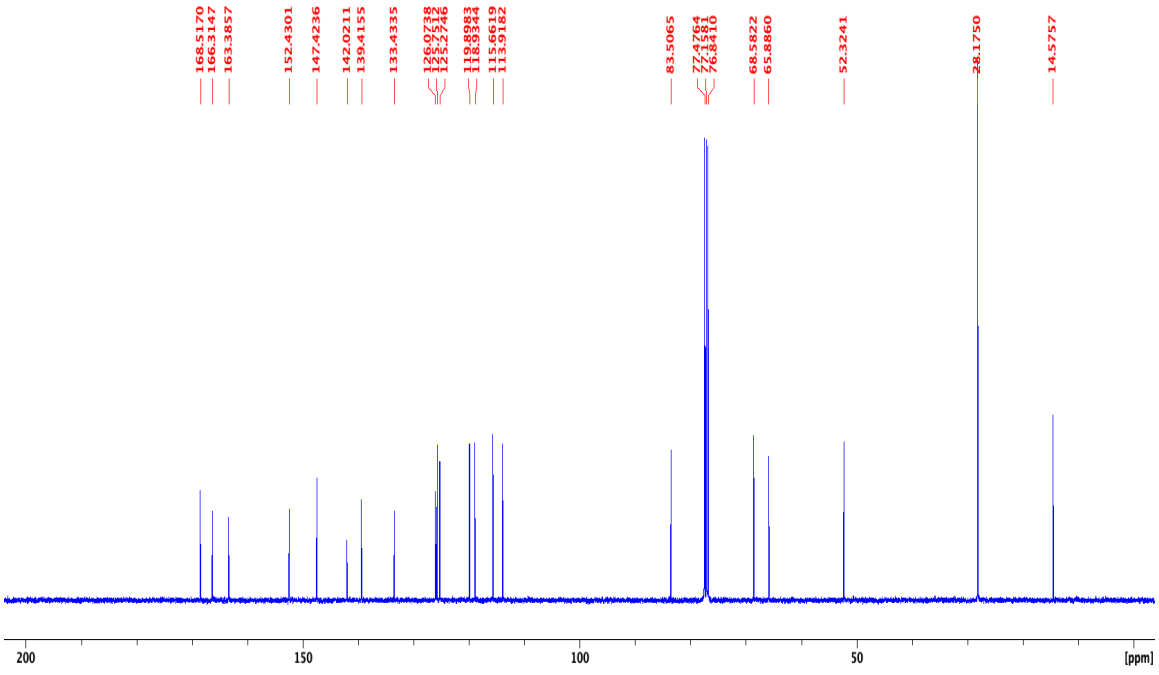


**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(3-ethoxy-4-nitrobenzamido)benzoate (30)**

**$^{1}$H, 400 MHz, $CDCl_3$**

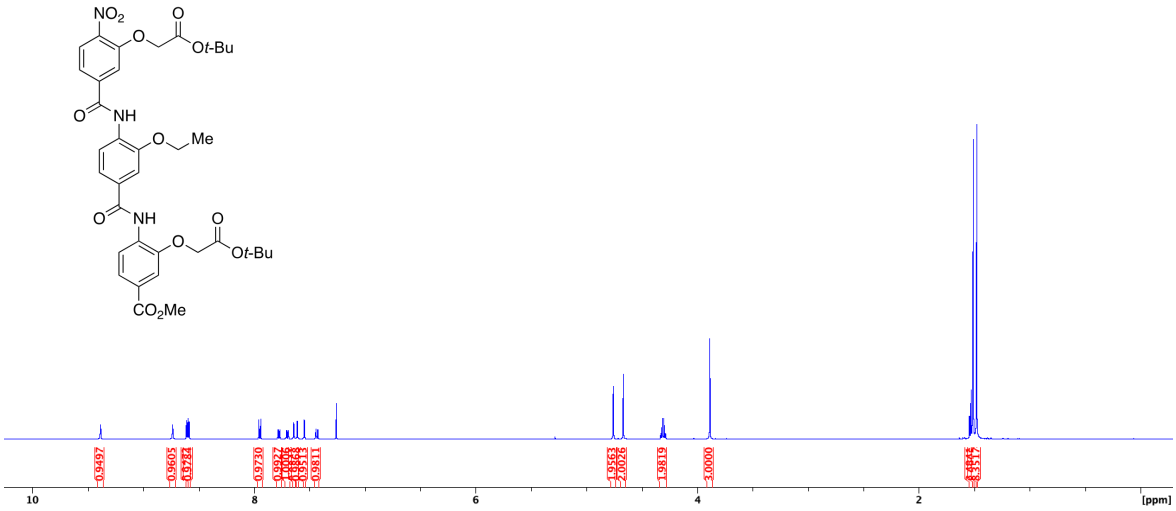



**$^{13}$C, 101 MHz, $CDCl_3$**

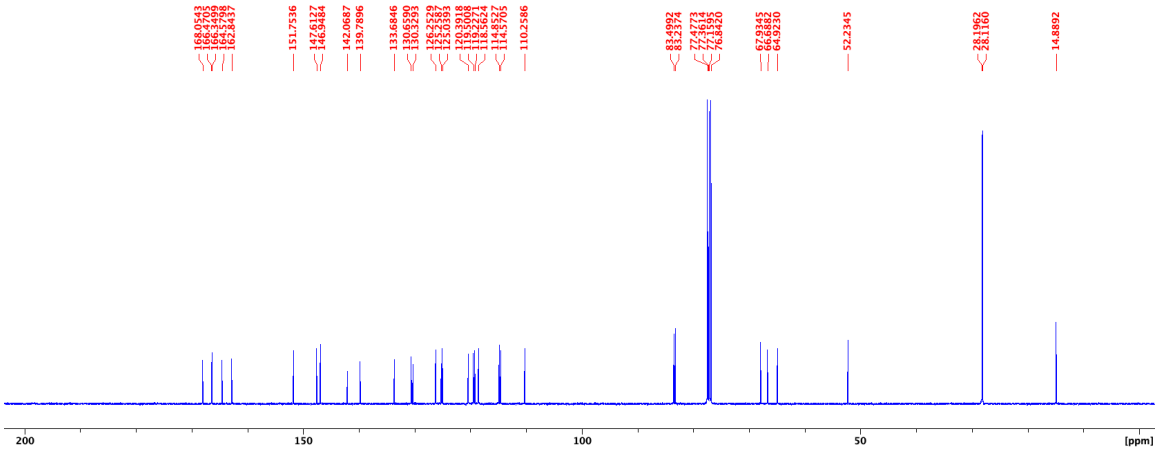


**Methyl 3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-(4-(3-(2-(*tert*-butoxy)-2-oxoethoxy)-4-nitrobenzamido)-3-ethoxybenzamido)benzoate (31)**

**$^{1}$H, 500 MHz, $CDCl_3$**

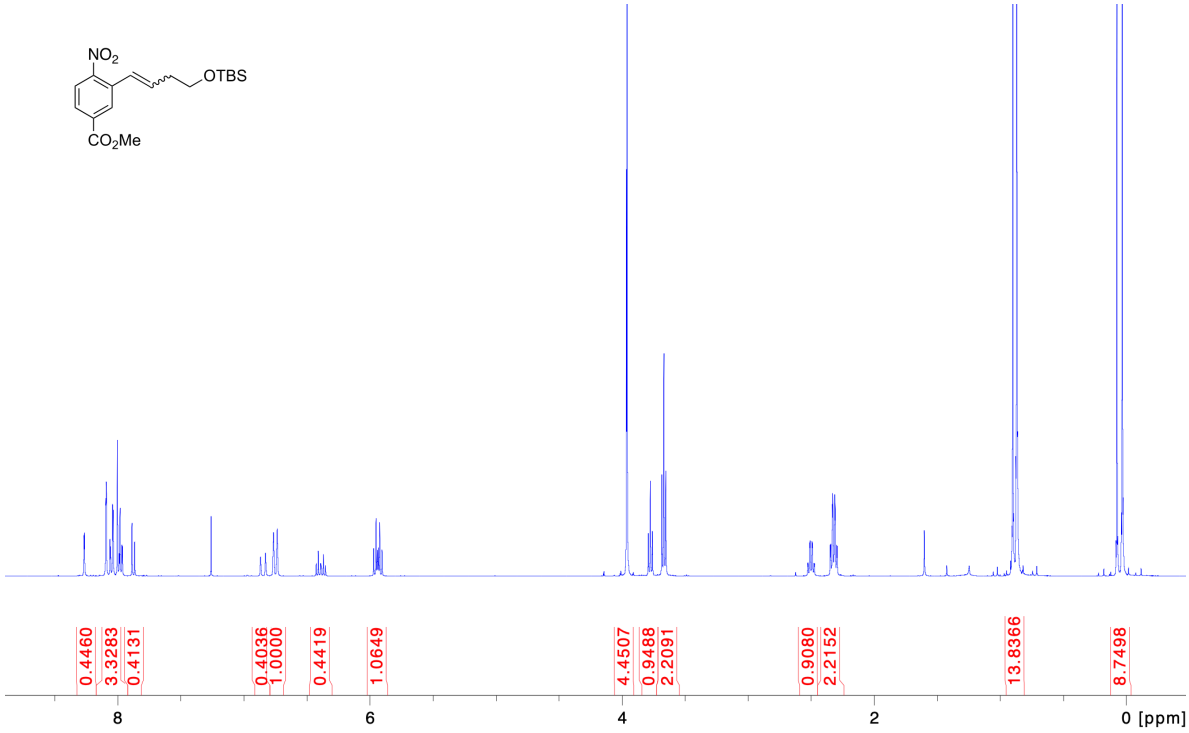



**$^{13}$C, 101 MHz, $CDCl_3$**

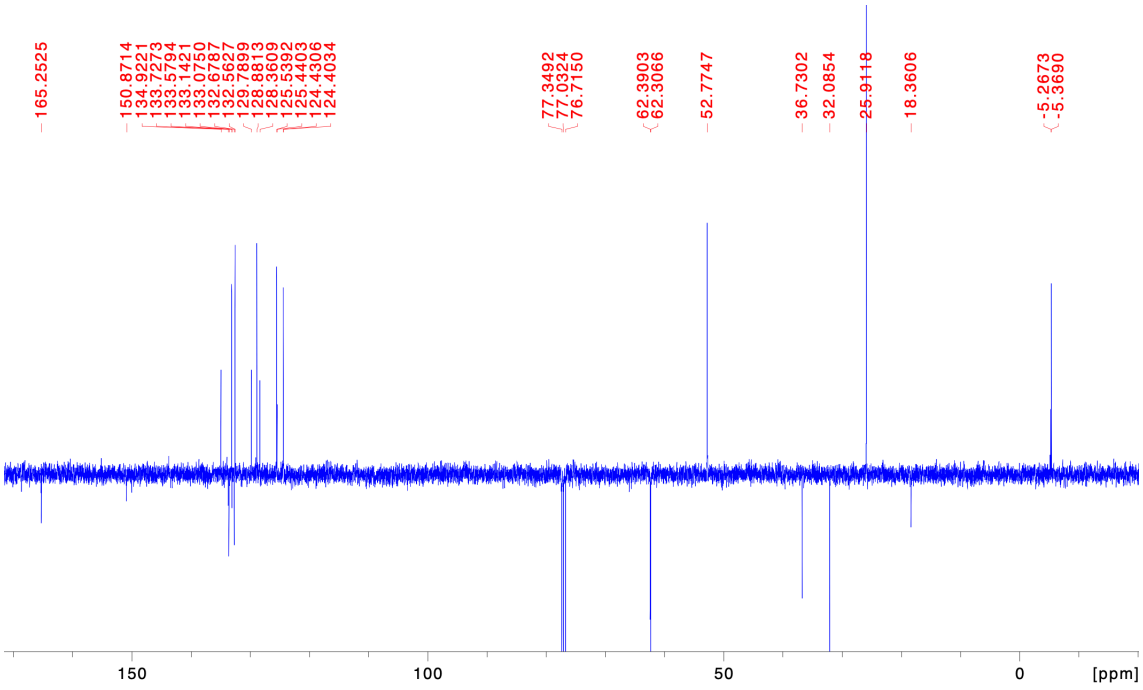


## Methyl 3-(4-((*tert*-butyldimethylsilyl)oxy)but-1-en-1-yl)-4-nitrobenzoate (43)

### $^1$H, 400 MHz, $CDCl_3$

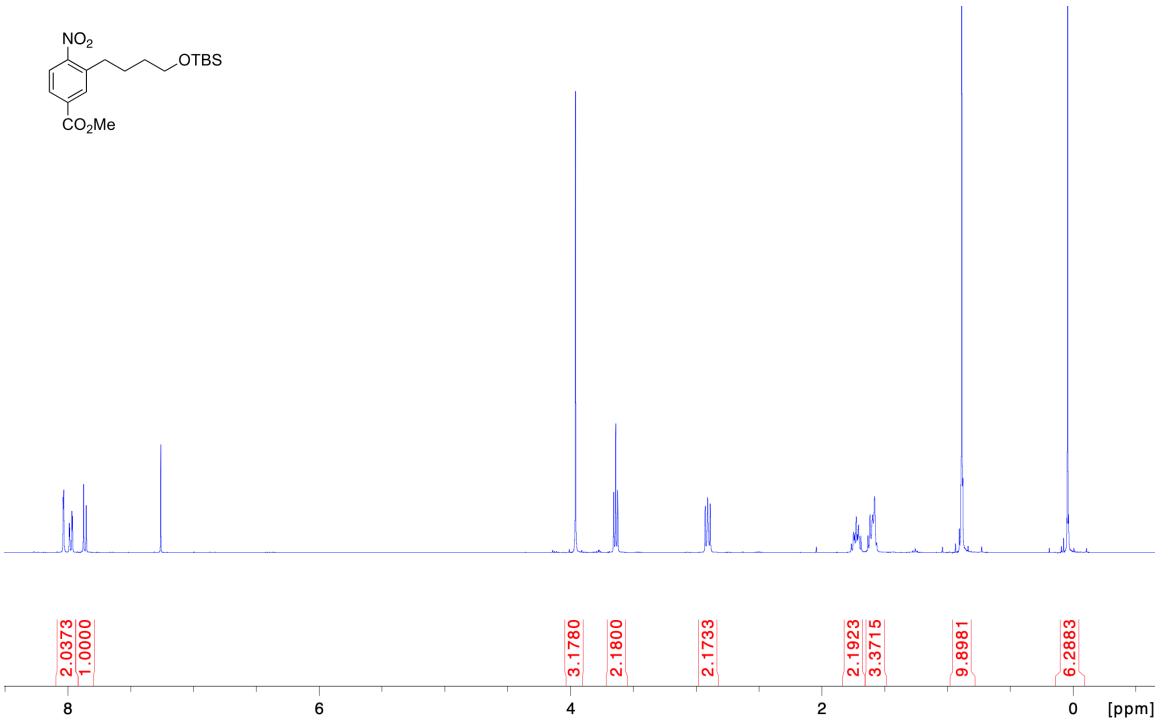



### DEPTQ, 101 MHz, $CDCl_3$



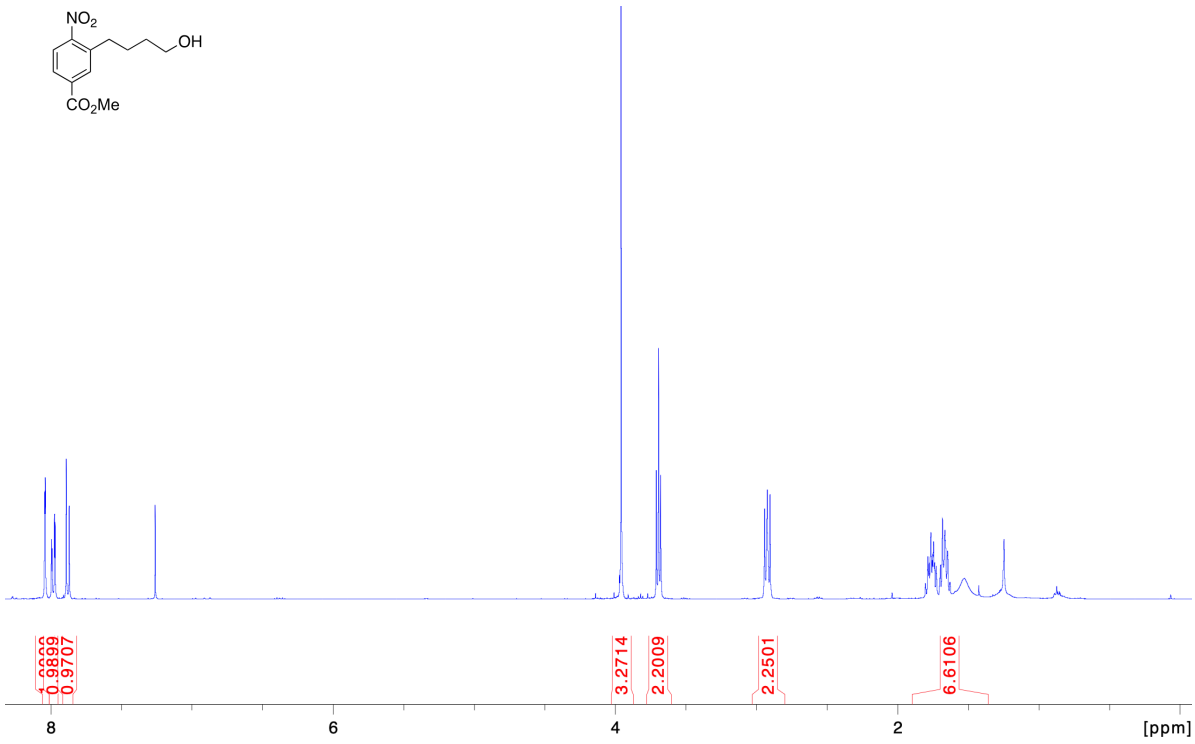
**Methyl 3-(4-((*tert*-butyldimethylsilyl)oxy)butyl)-4-nitrobenzoate (44)**

**$^1$H, 400 MHz, $CDCl_3$**



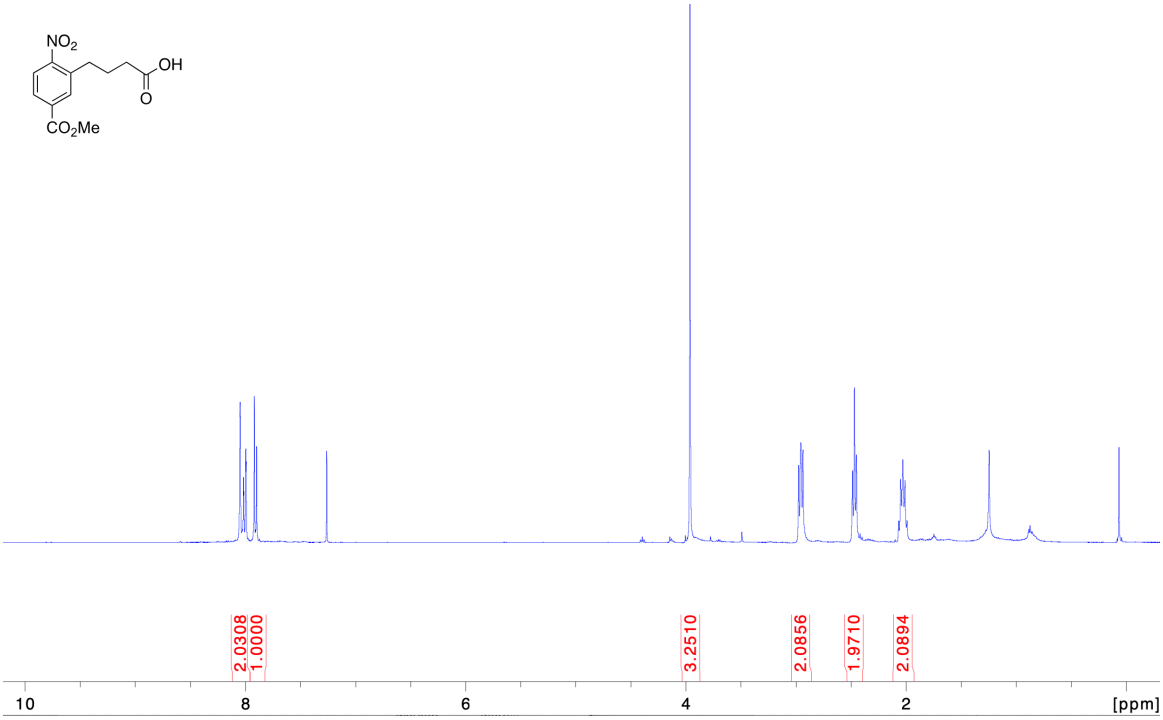
## Methyl 3-(4-hydroxybutyl)-4-nitrobenzoate (45)

### $^{1}H$, 400 MHz, $CDCl_3$



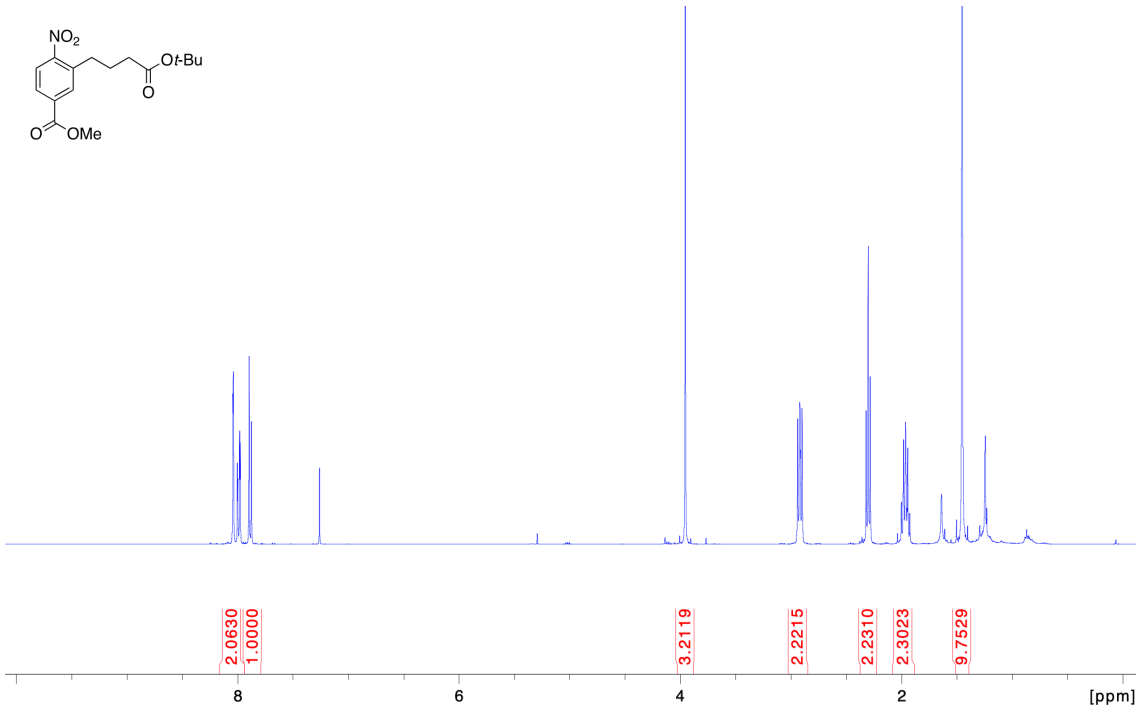
## 4-(5-(Methoxycarbonyl)-2-nitrophenyl)butanoic acid (46)

## $^{1}H$, 400 MHz, $CDCl_3$

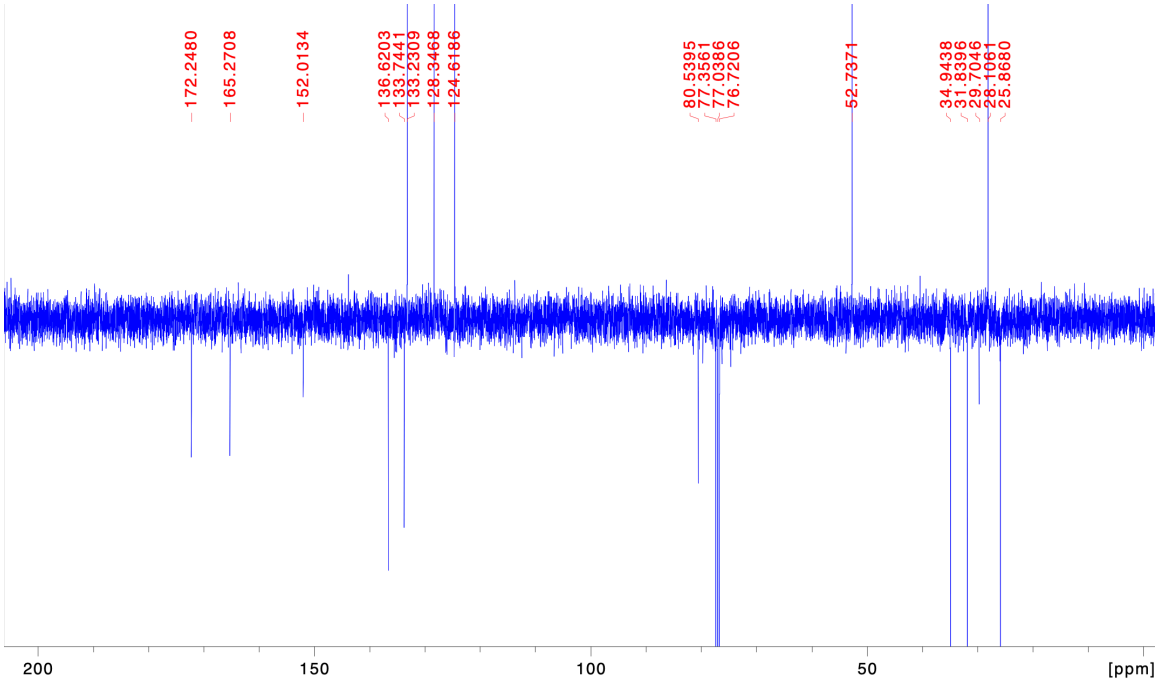


## Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-nitrobenzoate (47)

### $^1$H, 400 MHz, $CDCl_3$

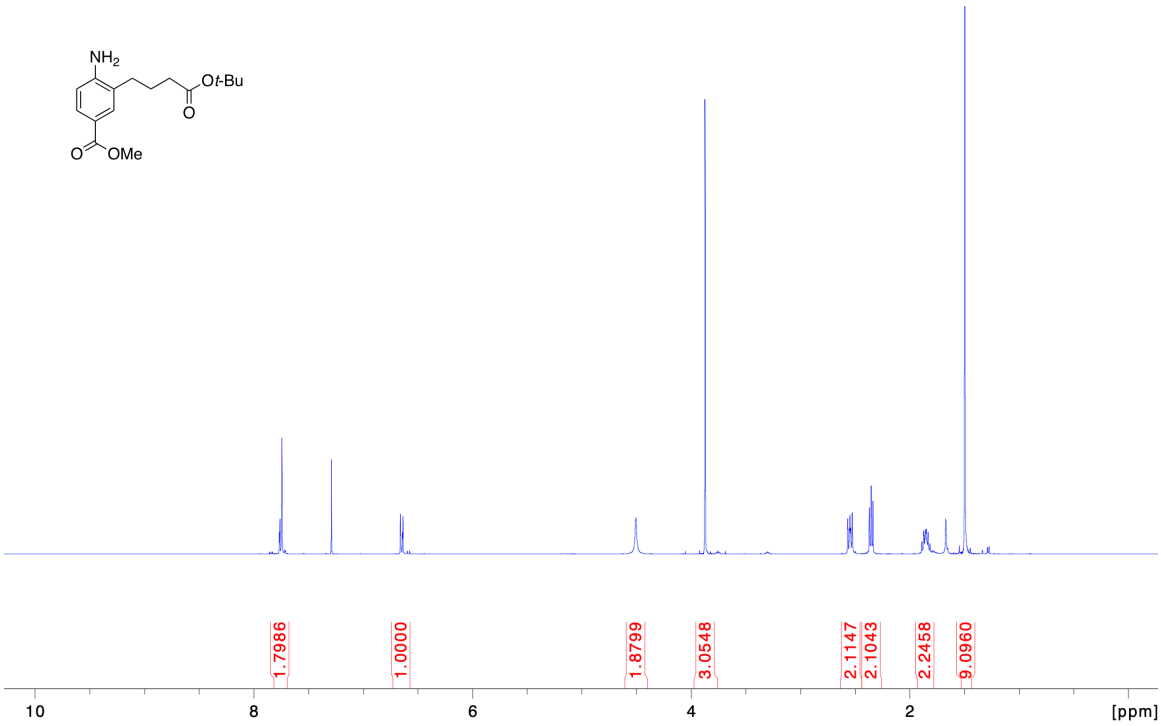



### DEPTQ, 101 MHz, $CDCl_3$

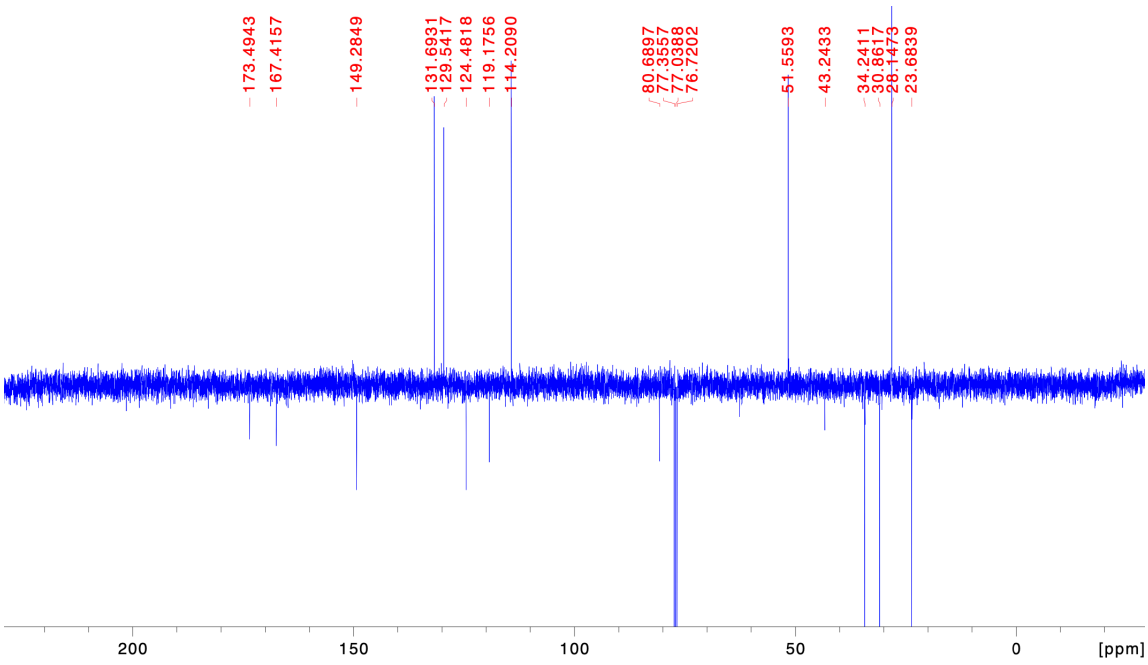


## Methyl 4-amino-3-(4-(*tert*-butoxy)-4-oxobutyl)benzoate (49)

### $^1$H, 400 MHz, $CDCl_3$

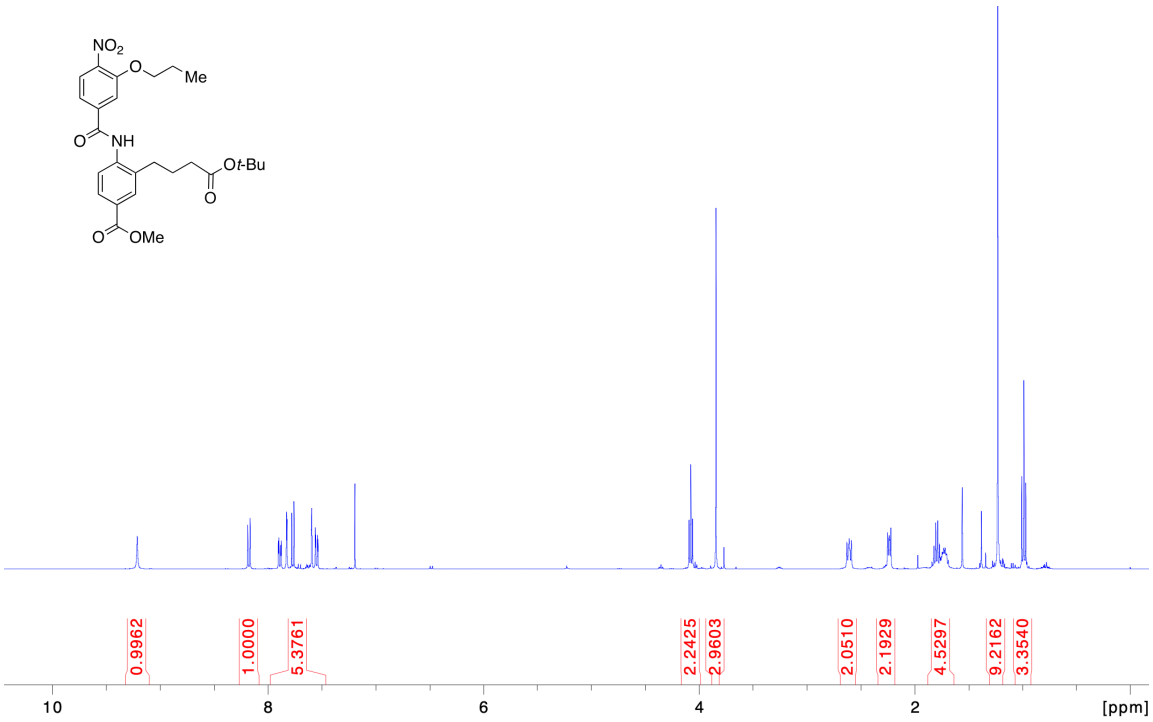



### DEPTQ, 101 MHz, $CDCl_3$

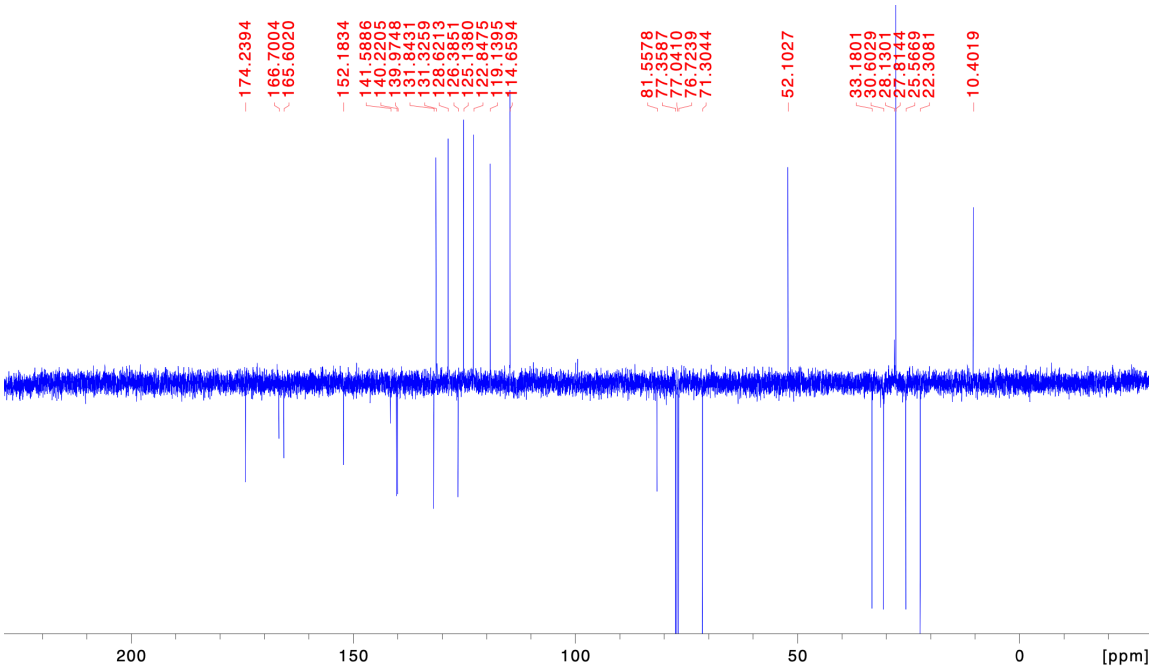


## Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-(4-nitro-3-propoxybenzamido)benzoate (51)

### $^1$H, 400 MHz, $CDCl_3$

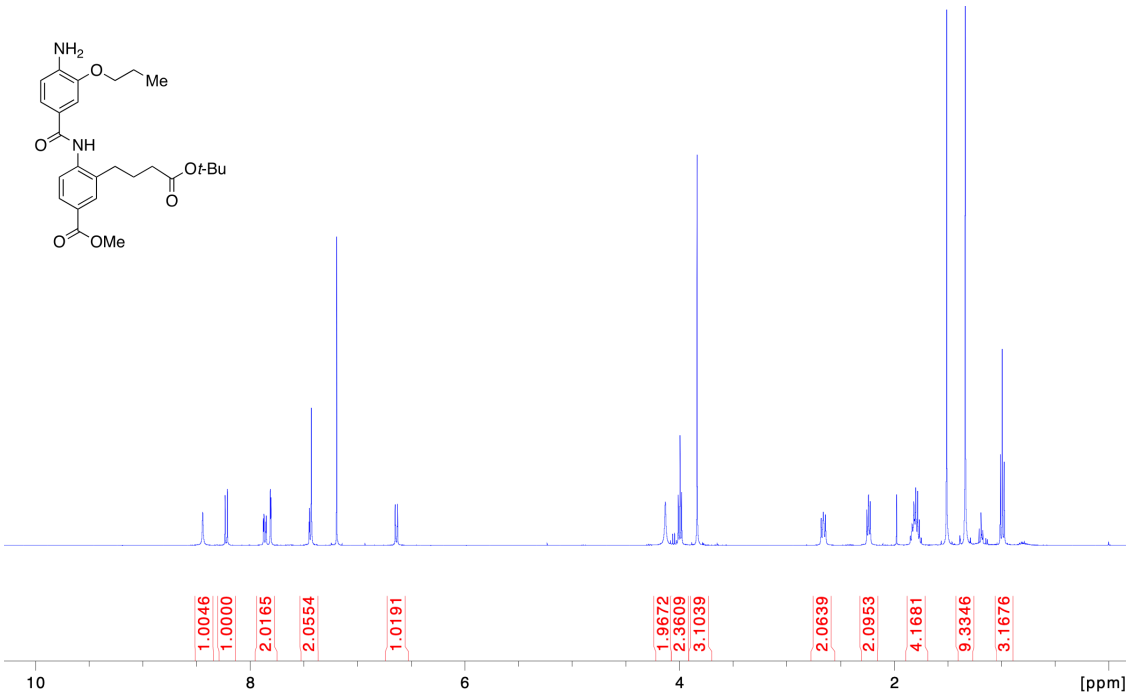



### DEPTQ, 101 MHz, $CDCl_3$

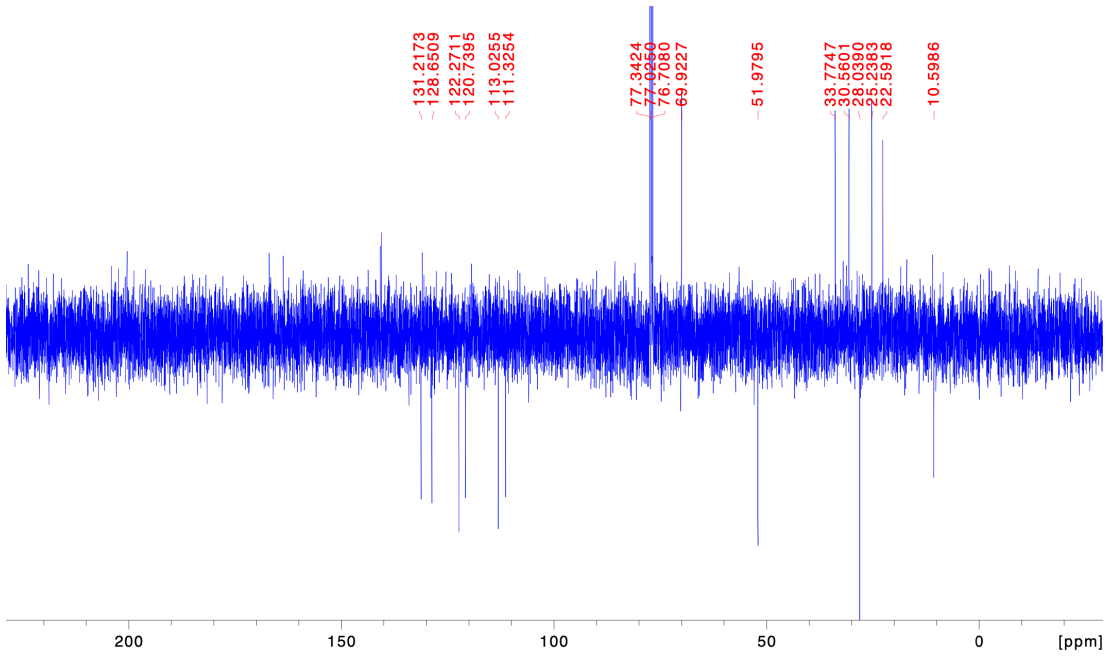


**Methyl 4-(4-amino-3-propoxybenzamido)-3-(4-(*tert*-butoxy)-4-oxobutyl)benzoate (52)**

**$^1$H, 400 MHz, $CDCl_3$**

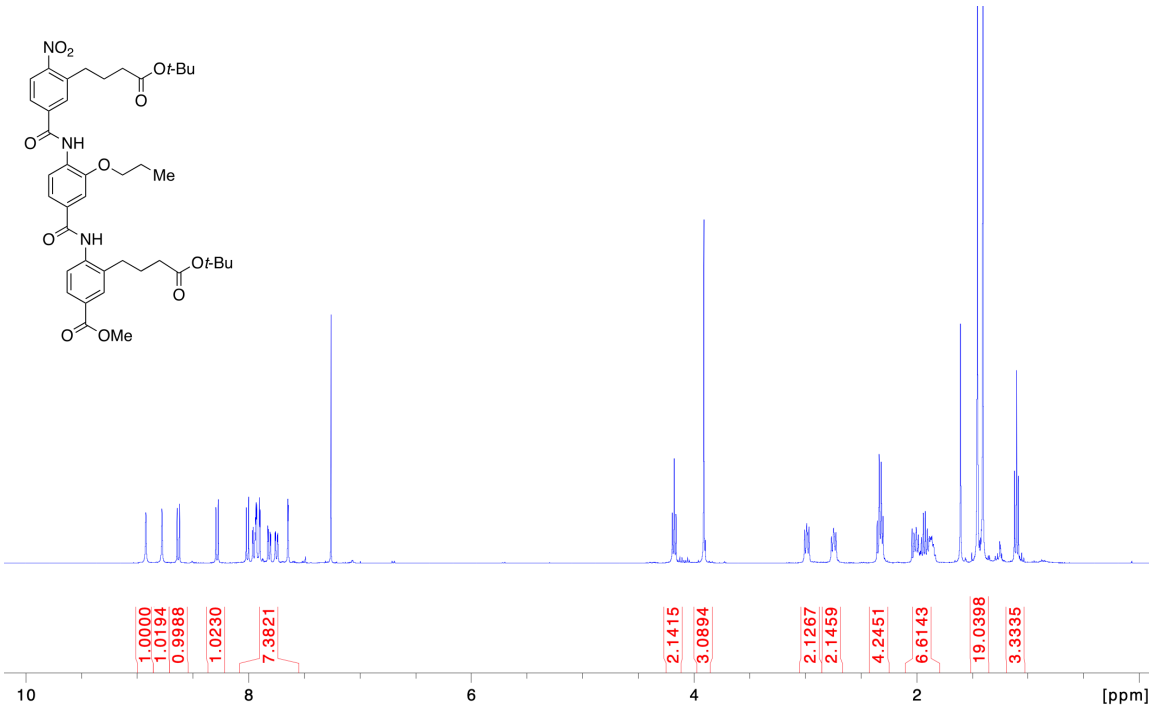



**DEPTQ, 101 MHz, $CDCl_3$**

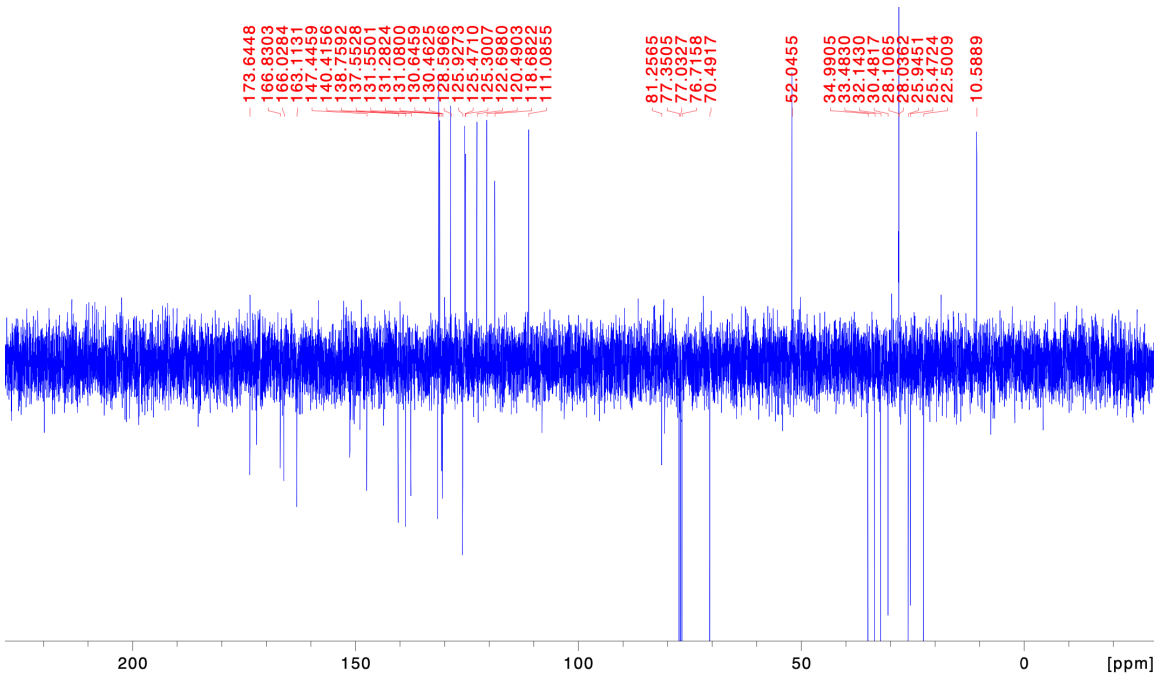


**Methyl 3-(4-(*tert*-butoxy)-4-oxobutyl)-4-(4-(3-(4-(*tert*-butoxy)-4-oxobutyl)-4-nitrobenzamido)-3-propoxybenzamido)benzoate (53)**

**$^{1}$H, 400 MHz, $CDCl_3$**

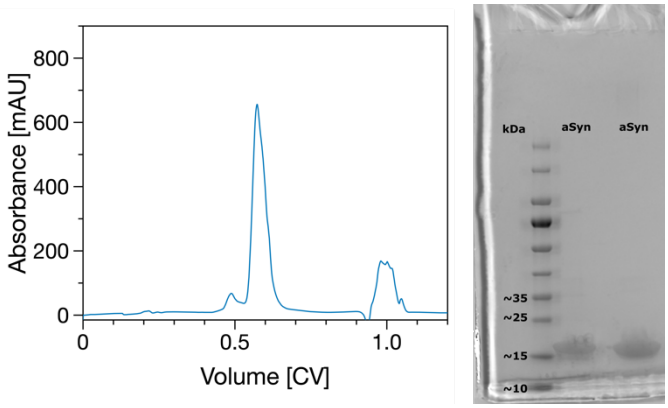



**DEPTQ, 101 MHz, $CDCl_3$**



## 3. α-Syn Expression and Purification

α-Syn was expressed in *E. Coli* BL21(DE3) and purified as described previously.[11] Briefly, the expression was conducted for 4 h at 37 ˚C in 2YT-medium and harvested. α-Syn was precipitated using ammonium sulphate after lysis of the cell pellet by heat and ultrasonication. The protein was re-suspended in 25 mM Tris-HCl pH 8, filtered (0,42 pore size) and subsequently loaded onto a HiTrapQHP anion exchange chromatography column (GE Healthcare). A gradient with 25 mM Tris-HCl, pH 8, 800 mM NaCl was run and the α-Syn containing fractions were combined, precipitated with ammonium sulphate and the pellet was stored at – 20 ˚C. The pellet was re-suspended and size exclusion chromatography was performed using a Superdex 200 Increase 10/300 GL prior to the following experiments. α-Syn concentration was determined by measuring UV-absorption at 274 nm (extinction coefficient of 5600 $M^{-1}$ $cm^{-1}$. Similarly, $^{15}N$ labeled α-Syn was expressed and purified by following the procedure above, except using different medium. The cell pellet was resuspended and grown in 250 ml M9 minimal medium containing 100 µg/ml of $^{15}NH_4Cl$ (Sigma).




*Example of a size exclusion chromatogram (left) and a SDS-PAGE (right) to confirm the purity of α-Syn*

## 4. Thioflavin-T assay

The influence of the compounds on the aggregation kinetics of α-Syn was investigated, performing Thioflavin-T (ThT) aggregation assays. 20 µM α-Syn solutions supplemented with 50 µM ThT in the presence of different compounds in the ratio of 1:0.1 were incubated at 37 ˚C in a corning 96-well half-area plate with nonbinding polystyrene surfaces. To provide a lipid environment, small unilamellar vesicles prepared from DMPS (1,2-dimyristoyl-sn-glycero-3-phospho-L-serine) were added to a final concentration of 1 mg/mL. The kinetics of amyloid formation were monitored by measuring ThT fluoresce intensity in a BMG LABTECH microplate reader. To compare the factor of the increase of the ThT fluorescence emission intensity between the samples, the ThT fluorescence intensity at the end of the experiment

was compared with the lowest emission value. The halftimes were obtained by individually fitting the curves using a generic sigmoidal equation.

## 5. Electron microscopy

The endpoint of the ThT assay were taken to prepare the TEM samples. Negative-staining method was used to prepare the samples. The grid was stained with 6 µl 2% uranyl acetate for 30 s and washed twice with 20 µl dH2O for 15 s. Images were taken on a carbon 400 mesh Cu grid under a voltage of 120 KV.

## 6. CD measurements of $\alpha$-Syn with peptidomimetics

CD spectra of α-Syn were recorded in the presence of large unilamellar vesicles (LUVs) with or without mimetic **1** or **5**. LUVs were prepared by dissolving DMPS in 97.5% chloroform, 2% methanol and 0.5% dH20 (5 mg/mL). To prepare a thin lipid film, the chloroform was evaporated using a slight stream of argon gas and incubation in a desiccator for 1 h. The lipid film was dissolved in 200 mM potassium phosphate, pH 6, and stirred for 2 h at 45˚C. The solution was then frozen and thawed 5 times (using dry ice and an Eppendorf shaker). Extrusion through 200 nm pore diameter PC membrane was done to prepare LUVs. CD samples were prepared by incubating 50 µM $\alpha$-Syn with 100 µM DMPS LUVs in 200 mM potassium phosphate, pH 6, in the presence of compound 1 and 5 in the molar ratio of 1:0.1. Far-UV CD spectra were recorded after 16 h incubation at 37˚C under quiescent conditions on a Chirascan-plus CD spectrometer using quartz cuvettes with path length of 1 mm. Three individual spectra between 250 nm and 200 nm were averaged and smoothed with a window size of 3 after subtracting the CD signal of the buffer.

## 7. References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